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(54) **METHOD OF DETECTING HUMAN MATRIX METALLOPROTEINASE 9 USING ANTIBODIES**

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(58) **Field of Classification Search**  
CPC ..... *G01N 33/573*; *G01N 2333/96494*  
See application file for complete search history.

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(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 207 days.

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(51) **Int. Cl.**

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***A61K 39/00*** (2006.01)

(57) **ABSTRACT**

The present disclosure provides compositions and methods of use involving binding proteins, e.g., antibodies and antigen-binding fragments thereof, that bind to the matrix metalloproteinase-9 (MMP9) protein (MMP9 is also known as gelatinase-B), such as where the binding proteins comprise an immunoglobulin (Ig) heavy chain (or functional fragment thereof) and an Ig light chain (or functional fragment thereof).

(52) **U.S. Cl.**

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**9 Claims, 4 Drawing Sheets**

FIGURE 1

Anti-MMP9 humanized heavy chains

AB0041	QVQLKESGPG LVAPSQSLSI TCTVSGFSLL SYGVHWVRQP FKGLEWLGV
VH1	QVQLQESGPG LVKPSEILSL TCTVSGFSLL SYGVHWVRQP FKGLEWLGV
VH2	QVQLQESGPG LVKPSEILSL TCTVSGFSLL SYGVHWVRQP FKGLEWLGV
VH3	QVQLQESGPG LVKPSEILSL TCTVSGFSLL SYGVHWVRQP FKGLEWLGV
VH4	QVQLQESGPG LVKPSEILSL TCTVSGFSLL SYGVHWVRQP FKGLEWLGV
AB0041	IWIIGGTTNIN SALMRLSIS KDDSKSQVFL KMNSLQDDEI AIYYCARYYY
VH1	IWIIGGTTNIN SALMRLTIS KDDSKSTIVYL KMNSLKTEDI AIYYCARYYY
VH2	IWIIGGTTNIN SALMRLTIS KDDSKNTIVYL KMNSLKTEDI AIYYCARYYY
VH3	IWIIGGTTNIN SALMREFTIS KDDSKNTIVYL KMNSLKTEDI AIYYCARYYY
VH4	IWIIGGTTNIN SALMREFTIS KDDSKNIVYL KMNSLKTEDI AIYYCARYYY
AB0041	GNDYWGQGTIS VTVSS (SEQ ID NO:3)
VH1	GNDYWGQGTIS VTVSS (SEQ ID NO:5)
VH2	GNDYWGQGTIL VTVSS (SEQ ID NO:6)
VH3	GNDYWGQGTIL VTVSS (SEQ ID NO:7)
VH4	GNDYWGQGTIL VTVSS (SEQ ID NO:8)

FIGURE 2

Anti-MMP9 humanized light chains

AB0041	DI <sup>1</sup> VM <sup>2</sup> IQ <sup>3</sup> SH <sup>4</sup> K <sup>5</sup> F	M <sup>6</sup> ST <sup>7</sup> SV <sup>8</sup> G <sup>9</sup> DR <sup>10</sup> VS	I <sup>11</sup> T <sup>12</sup> C <sup>13</sup> K <sup>14</sup> A <sup>15</sup> S <sup>16</sup> Q <sup>17</sup> D <sup>18</sup> VR	N <sup>19</sup> T <sup>20</sup> V <sup>21</sup> A <sup>22</sup> W <sup>23</sup> Y <sup>24</sup> Q <sup>25</sup> Q <sup>26</sup> T	G <sup>27</sup> Q <sup>28</sup> S <sup>29</sup> P <sup>30</sup> K <sup>31</sup> L <sup>32</sup> L <sup>33</sup> L <sup>34</sup> L <sup>35</sup> Y <sup>36</sup> S
Vk1	DI <sup>1</sup> VM <sup>2</sup> IQ <sup>3</sup> SP <sup>4</sup> S <sup>5</sup> F	LS <sup>6</sup> AS <sup>7</sup> VG <sup>8</sup> DR <sup>9</sup> VI	I <sup>11</sup> T <sup>12</sup> C <sup>13</sup> K <sup>14</sup> A <sup>15</sup> S <sup>16</sup> Q <sup>17</sup> D <sup>18</sup> VR	N <sup>19</sup> T <sup>20</sup> V <sup>21</sup> A <sup>22</sup> W <sup>23</sup> Y <sup>24</sup> Q <sup>25</sup> Q <sup>26</sup> T	G <sup>27</sup> K <sup>28</sup> A <sup>29</sup> P <sup>30</sup> K <sup>31</sup> L <sup>32</sup> L <sup>33</sup> L <sup>34</sup> Y <sup>35</sup> S
Vk2	DI <sup>1</sup> VM <sup>2</sup> IQ <sup>3</sup> SP <sup>4</sup> SS	LS <sup>6</sup> AS <sup>7</sup> VG <sup>8</sup> DR <sup>9</sup> VI	I <sup>11</sup> T <sup>12</sup> C <sup>13</sup> K <sup>14</sup> A <sup>15</sup> S <sup>16</sup> Q <sup>17</sup> D <sup>18</sup> VR	N <sup>19</sup> T <sup>20</sup> V <sup>21</sup> A <sup>22</sup> W <sup>23</sup> Y <sup>24</sup> Q <sup>25</sup> Q <sup>26</sup> K <sup>27</sup> E	G <sup>28</sup> K <sup>29</sup> A <sup>30</sup> P <sup>31</sup> K <sup>32</sup> L <sup>33</sup> L <sup>34</sup> Y <sup>35</sup> S
Vk3	DI <sup>1</sup> Q <sup>2</sup> MT <sup>3</sup> IQ <sup>4</sup> SP <sup>5</sup> SS	LS <sup>6</sup> AS <sup>7</sup> VG <sup>8</sup> DR <sup>9</sup> VI	I <sup>11</sup> T <sup>12</sup> C <sup>13</sup> K <sup>14</sup> A <sup>15</sup> S <sup>16</sup> Q <sup>17</sup> D <sup>18</sup> VR	N <sup>19</sup> T <sup>20</sup> V <sup>21</sup> A <sup>22</sup> W <sup>23</sup> Y <sup>24</sup> Q <sup>25</sup> Q <sup>26</sup> K <sup>27</sup> E	G <sup>28</sup> K <sup>29</sup> A <sup>30</sup> P <sup>31</sup> K <sup>32</sup> L <sup>33</sup> L <sup>34</sup> Y <sup>35</sup> S
Vk4	DI <sup>1</sup> Q <sup>2</sup> MT <sup>3</sup> IQ <sup>4</sup> SP <sup>5</sup> SS	LS <sup>6</sup> AS <sup>7</sup> VG <sup>8</sup> DR <sup>9</sup> VI	I <sup>11</sup> T <sup>12</sup> C <sup>13</sup> K <sup>14</sup> A <sup>15</sup> S <sup>16</sup> Q <sup>17</sup> D <sup>18</sup> VR	N <sup>19</sup> T <sup>20</sup> V <sup>21</sup> A <sup>22</sup> W <sup>23</sup> Y <sup>24</sup> Q <sup>25</sup> Q <sup>26</sup> K <sup>27</sup> E	G <sup>28</sup> K <sup>29</sup> A <sup>30</sup> P <sup>31</sup> E <sup>32</sup> L <sup>33</sup> L <sup>34</sup> L <sup>35</sup> Y <sup>36</sup> S
AB0041	SS <sup>1</sup> Y <sup>2</sup> R <sup>3</sup> N <sup>4</sup> T <sup>5</sup> G <sup>6</sup> V <sup>7</sup> P <sup>8</sup> D	R <sup>9</sup> F <sup>10</sup> T <sup>11</sup> G <sup>12</sup> S <sup>13</sup> G <sup>14</sup> S <sup>15</sup> G <sup>16</sup> I <sup>17</sup> D	F <sup>18</sup> T <sup>19</sup> I <sup>20</sup> SS <sup>21</sup> V <sup>22</sup> Q <sup>23</sup> A	E <sup>24</sup> D <sup>25</sup> L <sup>26</sup> A <sup>27</sup> V <sup>28</sup> Y <sup>29</sup> F <sup>30</sup> C <sup>31</sup> Q <sup>32</sup>	H <sup>33</sup> Y <sup>34</sup> I <sup>35</sup> T <sup>36</sup> P <sup>37</sup> Y <sup>38</sup> T <sup>39</sup> F <sup>40</sup> T <sup>41</sup> F <sup>42</sup> E <sup>43</sup> G
Vk1	SS <sup>1</sup> Y <sup>2</sup> R <sup>3</sup> N <sup>4</sup> T <sup>5</sup> G <sup>6</sup> V <sup>7</sup> P <sup>8</sup> D	R <sup>9</sup> F <sup>10</sup> T <sup>11</sup> G <sup>12</sup> S <sup>13</sup> G <sup>14</sup> S <sup>15</sup> G <sup>16</sup> I <sup>17</sup> D	F <sup>18</sup> I <sup>19</sup> L <sup>20</sup> I <sup>21</sup> SS <sup>22</sup> L <sup>23</sup> Q <sup>24</sup> A	E <sup>25</sup> D <sup>26</sup> V <sup>27</sup> A <sup>28</sup> V <sup>29</sup> Y <sup>30</sup> F <sup>31</sup> C <sup>32</sup> Q <sup>33</sup>	H <sup>34</sup> Y <sup>35</sup> I <sup>36</sup> T <sup>37</sup> F <sup>38</sup> Y <sup>39</sup> T <sup>40</sup> F <sup>41</sup> E <sup>42</sup> G
Vk2	SS <sup>1</sup> Y <sup>2</sup> R <sup>3</sup> N <sup>4</sup> T <sup>5</sup> G <sup>6</sup> V <sup>7</sup> P <sup>8</sup> D	R <sup>9</sup> F <sup>10</sup> T <sup>11</sup> G <sup>12</sup> S <sup>13</sup> G <sup>14</sup> S <sup>15</sup> G <sup>16</sup> I <sup>17</sup> D	F <sup>18</sup> I <sup>19</sup> L <sup>20</sup> I <sup>21</sup> SS <sup>22</sup> L <sup>23</sup> Q <sup>24</sup> A	E <sup>25</sup> D <sup>26</sup> V <sup>27</sup> A <sup>28</sup> V <sup>29</sup> Y <sup>30</sup> F <sup>31</sup> C <sup>32</sup> Q <sup>33</sup>	H <sup>34</sup> Y <sup>35</sup> I <sup>36</sup> T <sup>37</sup> F <sup>38</sup> Y <sup>39</sup> T <sup>40</sup> F <sup>41</sup> E <sup>42</sup> G
Vk3	SS <sup>1</sup> Y <sup>2</sup> R <sup>3</sup> N <sup>4</sup> T <sup>5</sup> G <sup>6</sup> V <sup>7</sup> P <sup>8</sup> D	R <sup>9</sup> F <sup>10</sup> T <sup>11</sup> G <sup>12</sup> S <sup>13</sup> G <sup>14</sup> S <sup>15</sup> G <sup>16</sup> I <sup>17</sup> D	F <sup>18</sup> I <sup>19</sup> L <sup>20</sup> I <sup>21</sup> SS <sup>22</sup> L <sup>23</sup> Q <sup>24</sup> A	E <sup>25</sup> D <sup>26</sup> V <sup>27</sup> A <sup>28</sup> V <sup>29</sup> Y <sup>30</sup> F <sup>31</sup> C <sup>32</sup> Q <sup>33</sup>	H <sup>34</sup> Y <sup>35</sup> I <sup>36</sup> T <sup>37</sup> F <sup>38</sup> Y <sup>39</sup> T <sup>40</sup> F <sup>41</sup> E <sup>42</sup> G
Vk4	SS <sup>1</sup> Y <sup>2</sup> R <sup>3</sup> N <sup>4</sup> T <sup>5</sup> G <sup>6</sup> V <sup>7</sup> P <sup>8</sup> D	R <sup>9</sup> F <sup>10</sup> T <sup>11</sup> G <sup>12</sup> S <sup>13</sup> G <sup>14</sup> S <sup>15</sup> G <sup>16</sup> I <sup>17</sup> D	F <sup>18</sup> I <sup>19</sup> L <sup>20</sup> I <sup>21</sup> SS <sup>22</sup> L <sup>23</sup> Q <sup>24</sup> A	E <sup>25</sup> D <sup>26</sup> V <sup>27</sup> A <sup>28</sup> V <sup>29</sup> Y <sup>30</sup> F <sup>31</sup> C <sup>32</sup> Q <sup>33</sup>	H <sup>34</sup> Y <sup>35</sup> I <sup>36</sup> T <sup>37</sup> F <sup>38</sup> Y <sup>39</sup> T <sup>40</sup> F <sup>41</sup> E <sup>42</sup> G
AB0041	G <sup>1</sup> T <sup>2</sup> K <sup>3</sup> L <sup>4</sup> E <sup>5</sup> I <sup>6</sup> K	(SEQ ID NO:4)			
Vk1	G <sup>1</sup> T <sup>2</sup> K <sup>3</sup> V <sup>4</sup> E <sup>5</sup> I <sup>6</sup> K	(SEQ ID NO:9)			
Vk2	G <sup>1</sup> T <sup>2</sup> K <sup>3</sup> V <sup>4</sup> E <sup>5</sup> I <sup>6</sup> K	(SEQ ID NO:10)			
Vk3	G <sup>1</sup> T <sup>2</sup> K <sup>3</sup> V <sup>4</sup> E <sup>5</sup> I <sup>6</sup> K	(SEQ ID NO:11)			
Vk4	G <sup>1</sup> T <sup>2</sup> K <sup>3</sup> V <sup>4</sup> E <sup>5</sup> I <sup>6</sup> K	(SEQ ID NO:12)			

FIGURE 3

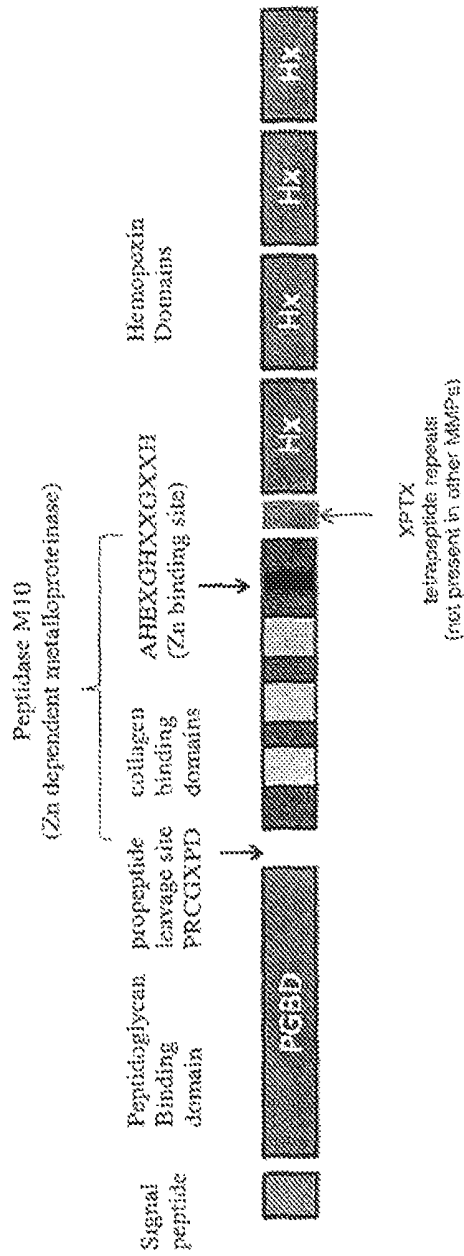


Figure 4: Comparison between AB0041, M4, and M12 heavy and light chains

Light chains	
	Signal Peptide
M4	.....CDRL1 MESQI QV F V F V F L W L S Q V D G D I V M T O S H K F M F T S V G D R V S I T C K A S Q D V R N T V A W Q Q K T G Q S P K L I Y S A S Y R N T G V P D
AB0041	.....CDRL2 MESQI QV F V F V F L W L S Q V D G D I V M T O S H K F M S T S V G D R V S I T C K A S Q D V R N T V A W Q Q K T G Q S P K L I Y S S S Y R N T G V P D
M12	.....CDRL2 - - - - QV F V Y M L L W L S Q V D G D I V M T O S Q K F M S T S V G D R V S V T C K A S Q A V G T N V A W Q Q K P G Q S P K A L I Y S A S Y R F S I G V P D
M4	.....kappa constant R F T O S I S Q T D F T I S S V Q A E D L A L Y C Q Q H Y S I P Y T F G Q Q T K L E V N R A D A A P T V S I F P P S T R D P P R A N
AB0041	.....kappa constant R F T O S G S T D F T I S S V Q A E D L A V F C Q Q H Y I T P Y T F G Q Q T K L E I K R A D A A P T V S I F P P S T R D P P R A N
M12	.....kappa constant R F T O S G S T D F T I S N W G S E D L A E Y F C Q Q Y N S Y P Y T F G Q Q T K L E I K R A D A A P T V S I F P P S T R D P P R A N
Light chains	
	Signal Peptide
M4	.....CDRL1 M A V L V L F L C L V A F P S C V L S Q V Q L K E S O P O L V A P S O S L S I T C T V S Q F S L L S Y G V H W R Q P P G K G L E W L G V I W F G G S T N Y N S
AB0041	.....CDRL1 M A V L V L F L C L V A F P S C V L S Q V Q L K E S O P O L V A P S O S L S I T C T V S Q F S L L S Y G V H W R Q P P G K G L E W L G V I W F G G T T N Y N S
M4	.....CDRL3 A L M S R L S I S K D D S K S Q V F L K M N S L Q T D D T A M Y C A R Y Y A M D Y W G Q G T S V T V S S A K T T P P S V Y F L A P G C C D T I G S S V T L G
AB0041	.....CDRL3 A L M S R L S I S K D D S K S Q V F L K M N S L Q T D D T A I Y C A R Y Y G M D Y W G Q G T S V T V S S A K T T P P S V Y F L A P G C C D T I G S S V T L G
M4	.....igg2b constant C L V K G Y F P E S V T V T W S G S L
AB0041	.....igg2b constant C L V K G Y F P E S V T V T W S G S L

# METHOD OF DETECTING HUMAN MATRIX METALLOPROTEINASE 9 USING ANTIBODIES

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a National Phase filing under 35 U.S.C. §371 of International Application No. PCT/US2012/027160 having an international filing date of Feb. 29, 2012, the contents of which are incorporated here by reference in their entirety.

## REFERENCE TO SEQUENCE LISTING SUBMITTED VIA EFS-WEB

The entire content of the following electronic submission of the sequence listing via the USPTO EFS-WEB server, as authorized and set forth in MPEP §1730 II.B.2(a)(C), is incorporated herein by reference in its entirety for all purposes. The sequence listing is identified on the electronically filed text file as follows:

File Name	Date of Creation	Size (bytes)
246102008540Seqlist	Feb. 29, 2012	65,102 bytes

## FIELD

This disclosure is in the field of extracellular enzymes, extracellular matrix enzymes, proteases and immunology.

## BACKGROUND

Matrix metalloproteinases (MMPs) belong to a family of extracellular enzymes involved in forming and remodeling the extracellular matrix. These enzymes contain a conserved catalytic domain in which a zinc atom is coordinated by three histidine residues. Over 20 members of this family are known, organized into a number of groups including collagenases, gelatinases, stromelysins, matrilysins, enamelysins and membrane MMPs.

MMP2 and MMP9 belong to the gelatinase group of matrix metalloproteinases. Besides containing signal peptide, propeptide, catalytic, zinc-binding and heamopexin-like domains common to most MMPs, the gelatinases also contain a plurality of fibronectin-like domains and an O-glycosylated domain.

MMPs are associated with a number of diseases. However, available inhibitors of MMPs have been unsuccessful, in part due to toxicity and lack of efficacy. Therefore, there is a need for specific and effective MMP inhibitors.

## SUMMARY

The present disclosure provides compositions and methods of use involving binding proteins, e.g., antibodies and antigen-binding fragments thereof, that bind to matrix metalloproteinase-9 (MMP9) protein (also known as gelatinase-B). The binding proteins typically are antibodies or fragments (e.g., antigen-binding fragments) thereof and typically contain an immunoglobulin (Ig) heavy chain (or functional fragment thereof) and an Ig light chain (or functional fragment thereof). The heavy chain is typically an IgG, typically a human IgG, such as an IgG1 or IgG4, or

other IgG such as an IgG2, or modified version thereof. The light chain typically is a kappa chain.

Among the MMP9 binding proteins, e.g., antibodies, are those that bind specifically to MMP9 and not to other matrix metalloproteinases. Such MMP9 binding proteins find use in applications in which it is necessary or desirable to obtain specific modulation (e.g., inhibition) of MMP9, e.g., without directly affecting the activity of other matrix metalloproteinases. Thus, in certain embodiments of the present disclosure an anti-MMP9 antibody or fragment thereof is a specific inhibitor of the activity of MMP9. In some aspects, the MMP9 binding proteins disclosed herein will be useful for inhibition of MMP9 while allowing normal function of other, related matrix metalloproteinases.

The antibodies and fragments can be described with reference to their amino acid sequences or portions thereof, and/or various functions such as binding specificity to MMP9 or particular epitopes thereof or the ability to compete for binding to epitopes on MMP9 with particular antibodies, and/or activity, such as the ability to inhibit MMP9, e.g., non-competitively.

The antibodies and fragments include those having a heavy chain variable (VH) region having a heavy chain complementary determining region (CDR) with an amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15; those having a light chain variable (VL) region having a light chain complementary determining region (CDR) with an amino acid sequence of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18. Exemplary antibodies and fragments include those having a heavy chain CDR1 with the amino acid sequence of SEQ ID NO: 13, a heavy chain CDR2 with the amino acid sequence of SEQ ID NO: 14, and a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 15, and those having a heavy chain CDR3 of SEQ ID NO: 15. Exemplary antibodies and fragments further include those with a light chain CDR1 with the amino acid sequence of SEQ ID NO: 16, a light chain CDR2 with the amino acid sequence of SEQ ID NO: 17, and a light chain CDR3 with the amino acid sequence of SEQ ID NO: 18, and those having a light chain CDR3 with the amino acid sequence of SEQ ID NO: 18, as well as those having heavy chain CDRs of SEQ ID NOs: 13, 14, and 15, and light chain CDRs of SEQ ID NOs: 16, 17, and 18.

Exemplary antibodies and fragments further include those having a heavy chain CDR1 with the amino acid sequence of SEQ ID NO: 34, a heavy chain CDR2 with the amino acid sequence of SEQ ID NO: 35, and a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 36, those with a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 36, those with a light chain CDR1 with the amino acid sequence of SEQ ID NO: 37, a light chain CDR2 with the amino acid sequence of SEQ ID NO: 38, and a light chain CDR3 with the amino acid sequence of SEQ ID NO: 39, those with a light chain CDR3 with the amino acid sequence of SEQ ID NO: 39, as well as those having heavy chain CDRs of SEQ ID NOs: 34, 35, and 36, and light chain CDRs of SEQ ID NOs: 37, 38, and 39.

Exemplary antibodies and fragments further include those having a light chain CDR1 with the amino acid sequence of SEQ ID NO: 42, a light chain CDR2 with the amino acid sequence of SEQ ID NO: 43, and a light chain CDR3 with the amino acid sequence of SEQ ID NO: 44, and those with a light chain CDR3 with the amino acid sequence of SEQ ID NO: 44.

The antibodies and fragments further include those having a VH region with an amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7,

or SEQ ID NO: 8, and those having a VL region with an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, as well as antibodies and fragments having a VH region with an amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8 and a VL region with an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12. In a particular example, the antibodies or fragments have a VH region of SEQ ID NO: 7 and a VL region of SEQ ID NO: 12, or at least at or about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with such sequences. They further include those having a VH region with an amino acid sequence set forth in SEQ ID NO: 32 or 47, and those with a VL region with an amino acid sequence set forth in SEQ ID NO: 33 or in SEQ ID NO: 41 or in SEQ ID NO: 48, and combinations thereof, and sequence having at least at or about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with such sequences.

The antibodies and fragments further include those having a VH region with an amino acid sequence set forth in SEQ ID NO: 1, and/or having a VL region with an amino acid sequence set forth in SEQ ID NO: 2, and/or a VH or VL region having at least at or about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with such sequences.

In some cases, the heavy chain is encoded by a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 19-22 and the light chain is encoded by a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 23-26.

In some embodiments, the antibodies or fragments thereof inhibit the enzymatic activity of MMP9, such as by non-competitive inhibition.

The antibodies and fragments further include those that specifically bind to a given epitope of MMP9. In some cases, the epitope is an epitope specifically bound by any of the above-described antibodies. In one example, the epitope contains an amino acid residue (i.e., one or more amino acid residue(s)) outside of cysteine-switch active pocket of SEQ ID NO: 27. In certain examples, the epitope includes an amino acid residue (i.e., one or more amino acid residue(s)) within a given region of MMP9, for example, where the region is residues 104-202 of SEQ ID NO: 27. In some examples, the epitope includes an amino acid residue (i.e., one or more amino acid residue(s)) within a given region of MMP9, for example, where the region is residues 104-119, residues 159-166, or residues 191-202 of SEQ ID NO: 27. In one example, the epitope includes an amino acid residue (i.e., one or more amino acid residue) within a region of MMP9 that is residues 104-119 of SEQ ID NO: 27, an amino acid residue within a region of MMP9 that is residues 159-166 of SEQ ID NO: 27, and an amino acid residue within a region of MMP9 that is residues 191-202 of SEQ ID NO: 27. In some cases, the epitope includes E111, D113, R162, or I198 of SEQ ID NO: 27. In some cases, it includes R162 of SEQ ID NO: 27. In some cases, it includes E111, D113, R162, and I198 of SEQ ID NO: 27.

In some cases, the antibody or fragment is human or is humanized.

In some examples, the antibodies and fragments specifically bind to human MMP9 with a dissociation constant ( $K_d$ ) equal to or lower than 100 nM, optionally lower than 10 nM, optionally lower than 1 nM, optionally lower than 0.5 nM, optionally lower than 0.1 nM, optionally lower than 0.01

nM, or optionally lower than 0.005 nM, in certain examples, between 0.1 and 0.2 nM, or between 0.1 and 10 pM, e.g., between 0.4 and 9 pM, such as between 0.4 and 8.8 pM, in the form of monoclonal antibody, scFv, Fab, or other form of antibody measured at a temperature of about 4° C., 25° C., 37° C. or 42° C.

Also among the provided antibodies and fragments are those having at least at or about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with any of the above-described antibodies or containing various portions with at least at or about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the respective portions of the antibodies described above, such as having a VH region with such identity with SEQ ID NO: 7 and a VL region with such identity with SEQ ID NO: 12. Also provided are antibodies that compete for binding to MMP9 with any of the above-described antibodies, such as those that compete for binding to MMP9 with an antibody having a VH region with the amino acid sequence set forth in SEQ ID NO: 7 and a VL region with the amino acid sequence set forth in SEQ ID NO: 12.

Also provided are isolated nucleic acids encoding the antibodies and fragments, such as nucleic acids including a coding sequence for any of the above-described antibodies and fragments. Among the provided nucleic acids are those containing a nucleotide sequence encoding a heavy chain polypeptide comprising CDRs with the amino acid sequences set forth in SEQ ID NOs: 13-15, and/or a light chain polypeptide comprising CDRs with the amino acid sequences set forth in SEQ ID NOs: 16-18. In one example, the nucleotide sequence encodes the heavy chain polypeptide, which has an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, and 5-8. In another example, the nucleotide sequence encodes the light chain polypeptide, which has an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, and 9-12. In one example, the nucleotide sequence includes a sequence selected from the group consisting of SEQ ID NOs: 19-26, such as SEQ ID NO: 21, SEQ ID NO: 26, or SEQ ID NOS: 21 and 26. Also provided are vectors containing such nucleic acids and cells including the same, such as host cells.

Also provided are pharmaceutical compositions including the antibodies, fragments, nucleic acids, vectors, and cells. In some examples, the pharmaceutical compositions further include a carrier or excipient, such as a pharmaceutically acceptable or biologically acceptable carrier or excipient. In some cases, the pharmaceutical compositions are used in the provided therapeutic methods and uses.

Also provided are methods and uses of the antibodies, fragments, nucleic acids, vectors, cells, and compositions, for example in therapeutics, such as inhibiting MMP9 in a subject, and diagnostics, such as for detecting MMP9 in the subject.

For example, provided are diagnostic and prognostic methods involving detection of MMP9, and agents (such as any of the above-described anti-MMP9 antibodies and other MMP9 binding proteins) for use in such methods. In some cases, the diagnostic method detects MMP9 expression in a test sample from a subject. Such methods can be carried out, for example, by contacting the test sample with an antibody or fragment as described herein (such as any of the above-described antibodies or fragments) and detecting binding of the antibody or fragment to protein in the sample, thereby detecting the presence of MMP9. In some cases, a sample is first obtained or provided. In some examples, the methods include comparing the amount or level of MMP9 detected to

a control level or amount, such as by comparing the amount of binding detected in the test sample with an amount of binding of the antibody or fragment to a control sample. In some cases, the methods involve simply comparing a test level and a control level of MMP9. In some cases, a higher test level (as compared to the control level) is indicative of the disease or condition.

In some cases, the MMP9 detected by the method indicates the presence of a disease or condition in the subject, such as an MMP9-associated disease or condition. In some cases, the methods further include treating the subject or adjusting (i.e., altering or discontinuing) treatment of the subject based on the results of the method, e.g., based on the levels of MMP9 detected in the sample. Among the biological samples are tissue, cells isolated from such tissues, and the like. In some cases, the methods are performed on liquid samples, such as blood, plasma, serum, whole blood, saliva, urine, or semen. Tissue samples include, for example, formalin-fixed or frozen tissue sections.

Also provided are methods of inhibiting MMP9 activity in a subject and/or treating a disease or condition in the subject, for example, using an agent that non-competitively inhibits MMP9, and agents (such as any of the above-described anti-MMP9 antibodies and other MMP9 binding proteins) for use in such methods. The methods generally are carried out by administering to the subject an MMP9 binding protein, such as an MMP9-binding antibody or fragment thereof as provided herein, e.g., in an effective amount. The antibody or fragment generally specifically binds to and non-competitively inhibits MMP9, for example, such that MMP9 activity is inhibited in the subject. In some cases, the antibody or fragment is one that binds MMP9 outside of the cysteine-switch active pocket, such as in one of the epitopes described above. In some cases, the antibody or fragment does not substantially bind to an MMP protein other than MMP9 and/or does not substantially bind to MMP2.

The subject generally is one with a disease or condition, typically one associated with increased or decreased MMP9 expression and/or activity. In certain cases, the subject with a disease or condition associated with increased MMP9 expression and/or activity. In other cases, the subject with a disease or condition associated with decreased MMP9 expression and/or activity.

Also provided are MMP9 polypeptides, including mutant MMP9 polypeptides, such as those containing residues 111-198 of SEQ ID NO: 27, and those having an amino acid sequence containing residues 111-198 of SEQ ID NO: 27 with an amino acid substitution at residue 111, 113, 162, or 198 of SEQ ID NO 27, or with an amino acid substitution at all such residues.

Also provided are uses of any of the above-described antibodies, nucleic acids, vectors, cells, and compositions, in the therapeutic and diagnostic methods described above.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the amino acid sequence of the heavy chain variable region of a mouse monoclonal anti-MMP9 antibody (AB0041), along with the amino acid sequences of humanized variants of heavy chain (VH1-VH4), aligned to show differences in framework amino acid sequence resulting from humanization. CDRs are shown in italics, and amino acids that are different in the humanized variants, compared to the parent mouse monoclonal, are underlined.

FIG. 2 shows the amino acid sequence of the light chain variable region of a mouse monoclonal anti-MMP9 antibody (AB0041), along with the amino acid sequences of human-

ized variants of this light chain (VH1-VH4), aligned to show differences in framework amino acid sequence resulting from humanization. CDRs are shown in italics, and amino acids that are different in the humanized variants, compared to the parent mouse monoclonal, are underlined.

FIG. 3 shows a schematic diagram of the MMP9 protein.

FIG. 4 shows a comparison between the amino acid sequences of the heavy and light chains of antibodies designated AB0041, M4, and M12.

#### DETAILED DESCRIPTION

Practice of the present disclosure employs, unless otherwise indicated, standard methods and conventional techniques in the fields of cell biology, toxicology, molecular biology, biochemistry, cell culture, immunology, oncology, recombinant DNA and related fields as are within the skill of the art. Such techniques are described in the literature and thereby available to those of skill in the art. See, for example, Alberts, B. et al., "Molecular Biology of the Cell," 5<sup>th</sup> edition, Garland Science, New York, N.Y., 2008; Voet, D. et al. "Fundamentals of Biochemistry: Life at the Molecular Level," 3<sup>rd</sup> edition, John Wiley & Sons, Hoboken, N.J., 2008; Sambrook, J. et al., "Molecular Cloning: A Laboratory Manual," 3<sup>rd</sup> edition, Cold Spring Harbor Laboratory Press, 2001; Ausubel, F. et al., "Current Protocols in Molecular Biology," John Wiley & Sons, New York, 1987 and periodic updates; Freshney, R. I., "Culture of Animal Cells: A Manual of Basic Technique," 4<sup>th</sup> edition, John Wiley & Sons, Somerset, N.J., 2000; and the series "Methods in Enzymology," Academic Press, San Diego, Calif. See also, for example, "Current Protocols in Immunology," (R. Coico, series editor), Wiley, last updated August 2010.

Certain MMPs play roles in tumor growth, metastasis, inflammation, autoimmunity, and vascular disease. See, for example, Hu et al. (2007) *Nature Reviews: Drug Discovery* 6:480-498. Thus, it is desirable to inhibit the activity of one or more particular MMPs in certain therapeutic settings. While sharing significant homology at a sequence level, the expression and functional roles of the two gelatinases MMP9 and MMP2 vary significantly. MMP9 expression is induced by a number of disease associated cytokines and growth factors. Also, the MMP9 knockout mouse is protected in a variety of disease models, whereas MMP2 is more constitutively expressed and the MMP2 knockout animals tend toward little protection. Some studies have shown that MMP2 knockout mouse exhibited worse disease in challenge models. For some diseases or disorders, the activity of more than one MMPs is inhibited. In clinical studies, the inhibitors to more than one MMPs have caused adverse effects, such as toxicity or lack of efficacy, that are not desired. It has been shown that the activity of certain MMPs, e.g., MMP2, is often required for normal tissue homeostasis and/or is protective against disease. Certain available MMP inhibitors have caused side effects.

Among the provided embodiments are agents, including therapeutic reagents, such as antibodies and antigen-binding fragments thereof, that specifically inhibit the catalytic activity of a single MMP or a select plurality of MMPs, such as MMP9 and that do not react with or inhibit certain other MMPs or any other MMPs. Also among the provided embodiments are methods and uses of the same for treatment of various diseases.

#### MMP9 Binding Proteins

The present disclosure provides binding proteins, e.g., antibodies and fragments (e.g., antigen-binding fragments) thereof, that bind to the matrix metalloproteinase-9 (MMP9)

protein (MMP9 is also known as gelatinase-B), e.g., human MMP9, such as the human MMP9 having an amino acid sequence set forth in SEQ ID NO: 27 or SEQ ID NO: 28. The binding proteins of the present disclosure generally comprise an immunoglobulin (Ig) heavy chain (or functional fragment thereof) and an Ig light chain (or functional fragment thereof).

The disclosure further provides MMP9 binding proteins that bind specifically to MMP9 and not to other matrix metalloproteinases such as MMP1, MMP2, MMP3, MMP7, MMP9, MMP10, MMP12, and MMP13. Such specific MMP9 binding proteins are thus generally not significantly or detectably crossreactive with non-MMP9 matrix metalloproteinases. MMP9 binding proteins that specifically bind MMP9 find use in applications in which it is necessary or desirable to obtain specific modulation (e.g., inhibition) of MMP9, e.g., without directly affecting the activity of other matrix metalloproteinases.

In certain embodiments of the present disclosure, an anti-MMP9 antibody is an inhibitor of the activity of MMP9, and can be a specific inhibitor of MMP9. In one embodiment, the MMP9 binding proteins disclosed herein is useful for inhibition of MMP9 while not affecting other matrix metalloproteinases. "An inhibitor of MMP" or "inhibitor of MMP9 activity" can be an antibody or an antigen binding fragment thereof that directly or indirectly inhibits activity of MMP9, including but not limited to enzymatic processing, inhibiting action of MMP9 on its substrate (e.g., by inhibiting substrate binding, substrate cleavage, and the like), and the like.

The present disclosure also provides MMP9 binding proteins that specifically bind to non-mouse MMP9, such as human MMP9, Cynomolgus monkey MMP9, and rat MMP9.

The present disclosure also provides MMP9 binding proteins (e.g., anti-MMP9 antibodies and functional fragments thereof) that act as non-competitive inhibitors. A "non-competitive inhibitor" refers to an inhibitor binds at site away from substrate binding site of an enzyme, and thus can bind the enzyme and effect inhibitory activity regardless of whether or not the enzyme is bound to its substrate. The non-competitive or allosteric inhibition is generally independent of substrate association or concentration. Such non-competitive inhibitors can, for example, provide for a level of inhibition that can be substantially independent of substrate concentration.

MMP9 binding proteins (e.g., antibodies and functional fragments thereof) of the present disclosure include those that bind MMP9, particularly human MMP9, and having a heavy chain polypeptide (or functional fragment thereof) that has at least about 80%, 85%, 90%, 95% or more amino acid sequence identity to a heavy chain polypeptide disclosed herein.

MMP9 binding proteins (e.g., antibodies and functional fragments thereof) of the present disclosure include those that bind MMP9, particularly human MMP9, and having a light polypeptide (or functional fragment thereof) that has at least about 80%, 85%, 90%, 95% or more amino acid sequence identity to a heavy chain polypeptide disclosed herein.

MMP9 binding proteins (e.g., antibodies and functional fragments thereof) of the present disclosure include those that bind MMP9, particularly human MMP9, and have a heavy chain polypeptide (or functional fragment thereof) having the complementarity determining regions ("CDRs")

of heavy chain polypeptide and the CDRs of a light chain polypeptide (or functional fragment thereof) as disclosed herein.

"Homology" or "identity" or "similarity" as used herein in the context of nucleic acids and polypeptides refers to the relationship between two polypeptides or two nucleic acid molecules based on an alignment of the amino acid sequences or nucleic acid sequences, respectively. Homology and identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology/similarity or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. In comparing two sequences, the absence of residues (amino acids or nucleic acids) or presence of extra residues also decreases the identity and homology/similarity.

As used herein, "identity" means the percentage of identical nucleotide or amino acid residues at corresponding positions in two or more sequences when the sequences are aligned to maximize sequence matching, i.e., taking into account gaps and insertions. Sequences are generally aligned for maximum correspondence over a designated region, e.g., a region at least about 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or more amino acids or nucleotides in length, and can be up to the full-length of the reference amino acid or nucleotide. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer program, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Examples of algorithms that are suitable for determining percent sequence identity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) *J. Mol. Biol.* 215: 403-410 and Altschul et al. (1977) *Nucleic Acids Res.* 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). Further exemplary algorithms include ClustalW (Higgins D., et al. (1994) *Nucleic Acids Res* 22: 4673-4680), available at [www.ebi.ac.uk/Tools/clustalw/index.html](http://www.ebi.ac.uk/Tools/clustalw/index.html).

Residue positions which are not identical can differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine.

Sequence identity between two nucleic acids can also be described in terms of hybridization of two molecules to each other under stringent conditions. The hybridization conditions are selected following standard methods in the art (see, for example, Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, (1989) Cold Spring Harbor, N.Y.). An example of stringent hybridization conditions is hybridization at 50° C. or higher and 0.1×SSC (15 mM sodium chloride/1.5 mM sodium citrate). Another example of stringent hybridization conditions is overnight incubation at 42° C. in a solution: 50% formamide, 5×SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1×SSC at about 65° C. Stringent hybridization conditions are hybridization conditions that are at least as stringent as the above representative conditions, where conditions are considered to be at least as stringent if they are at least about 80% as stringent, typically at least 90% as stringent as the above specific stringent conditions.

Accordingly, the present disclosure provides, for example, antibodies or antigen binding fragments thereof, comprising a heavy chain variable region polypeptide having at least 80%, 85%, 90%, 95%, or greater amino acid sequence identity to an amino acid sequence of a heavy chain variable region described herein (e.g., SEQ ID NOS:1 or 5-8), and a variable light chain polypeptide having at least 80%, 85%, 90%, 95%, or greater amino acid sequence identity to an amino acid sequence of a light chain polypeptide as set forth herein (e.g., SEQ ID NOS:2 or 9-12).

Examples of anti-MMP9 antibodies of the present disclosure are described in more detail below.

#### Antibodies

The MMP9 binding proteins include antibodies and functional fragments thereof, such as those that specifically bind to MMP9. As used herein, the term "antibody" means an isolated or recombinant polypeptide binding agent that comprises peptide sequences (e.g., variable region sequences) that specifically bind an antigenic epitope. The term is used in its broadest sense and specifically covers monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, nanobodies, diabodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments including but not limited to Fv, scFv, Fab, Fab' F(ab')<sub>2</sub> and Fab<sub>2</sub>, so long as they exhibit the desired biological activity. The term "human antibody" refers to antibodies containing sequences of human origin, except for possible non-human CDR regions, and does not imply that the full structure of an immunoglobulin molecule be present, only that the antibody has minimal immunogenic effect in a human (i.e., does not induce the production of antibodies to itself).

An "antibody fragment" comprises a portion of a full-length antibody, for example, the antigen binding or variable region of a full-length antibody. Such antibody fragments may also be referred to herein as "functional fragments" or "antigen-binding fragments". Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (Zapata et al. (1995) *Protein Eng.* 8(10):1057-1062); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pep-

sin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen combining sites and is still capable of cross-linking antigen.

"Fv" is a minimum antibody fragment containing a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three complementarity-determining regions (CDRs) of each variable domain interact to define an antigen-binding site on the surface of the V<sub>H</sub>-V<sub>L</sub> dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or an isolated V<sub>H</sub> or V<sub>L</sub> region comprising only three of the six CDRs specific for an antigen) has the ability to recognize and bind antigen, although generally at a lower affinity than does the entire F<sub>v</sub> fragment.

The "F<sub>ab</sub>" fragment also contains, in addition to heavy and light chain variable regions, the constant domain of the light chain and the first constant domain (CHO of the heavy chain. Fab fragments were originally observed following papain digestion of an antibody. Fab' fragments differ from Fab fragments in that F(ab') fragments contain several additional residues at the carboxy terminus of the heavy chain CH<sub>1</sub> domain, including one or more cysteines from the antibody hinge region. F(ab')<sub>2</sub> fragments contain two Fab fragments joined, near the hinge region, by disulfide bonds, and were originally observed following pepsin digestion of an antibody. Fab'-SH is the designation herein for Fab' fragments in which the cysteine residue(s) of the constant domains bear a free thiol group. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to five major classes: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

"Single-chain Fv" or "sFv" or "scFv" antibody fragments comprise the V<sub>H</sub> and V<sub>L</sub> domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains, which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113 (Rosenburg and Moore eds.) Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) in the same polypeptide chain (V<sub>H</sub>-V<sub>L</sub>). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain, thereby creating two antigen-binding sites. Diabodies are additionally described, for example, in EP 404,097; WO 93/11161 and Hollinger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448.

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Components of its natural environment may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an isolated antibody is purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, for example,

more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence, e.g., by use of a spinning cup sequenator, or (3) to homogeneity by gel electrophoresis (e.g., SDS-PAGE) under reducing or nonreducing conditions, with detection by Coomassie blue or silver stain. The term "isolated antibody" includes an antibody in situ within recombinant cells, since at least one component of the antibody's natural environment will not be present. In certain embodiments, isolated antibody is prepared by at least one purification step.

As used herein, "immunoreactive" refers to antibodies or fragments thereof that are specific to a sequence of amino acid residues ("binding site" or "epitope"), yet if are cross-reactive to other peptides/proteins, are not toxic at the levels at which they are formulated for administration to human use. "Epitope" refers to that portion of an antigen capable of forming a binding interaction with an antibody or antigen binding fragment thereof. An epitope can be a linear peptide sequence (i.e., "continuous") or can be composed of non-contiguous amino acid sequences (i.e., "conformational" or "discontinuous"). The term "preferentially binds" means that the binding agent binds to the binding site with greater affinity than it binds unrelated amino acid sequences.

Anti-MMP9 antibodies can be described in terms of the CDRs of the heavy and light chains. As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat et al., *J. Biol. Chem.* 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); by Chothia et al., *J. Mol. Biol.* 196:901-917 (1987); and MacCallum et al., *J. Mol. Biol.* 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison.

TABLE 1

	CDR Definitions		
	Kabat <sup>1</sup>	Chothia <sup>2</sup>	MacCallum <sup>3</sup>
V <sub>H</sub> CDR1	31-35	26-32	30-35
V <sub>H</sub> CDR2	50-65	53-55	47-58
V <sub>H</sub> CDR3	95-102	96-101	93-101
V <sub>L</sub> CDR1	24-34	26-32	30-36
V <sub>L</sub> CDR2	50-56	50-52	46-55
V <sub>L</sub> CDR3	89-97	91-96	89-96

<sup>1</sup>Residue numbering follows the nomenclature of Kabat et al., supra<sup>2</sup>Residue numbering follows the nomenclature of Chothia et al., supra<sup>3</sup>Residue numbering follows the nomenclature of MacCallum et al., supra

As used herein, the term "framework" when used in reference to an antibody variable region is intended to mean all amino acid residues outside the CDR regions within the variable region of an antibody. A variable region framework is generally a discontinuous amino acid sequence between about 100-120 amino acids in length but is intended to reference only those amino acids outside of the CDRs. As used herein, the term "framework region" is intended to mean each domain of the framework that is separated by the CDRs.

In some embodiments, an antibody is a humanized antibody or a human antibody. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. Thus, humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins which contain minimal sequence derived from non-human immunoglobulin. The non-human sequences are located primarily in the variable regions, particularly in the complementarity-determining regions (CDRs). In some embodiments, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In certain embodiments, a humanized antibody comprises substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. For the purposes of the present disclosure, humanized antibodies can also include immunoglobulin fragments, such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies.

The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. See, for example, Jones et al. (1986) *Nature* 321:522-525; Riechmann et al. (1988) *Nature* 332:323-329; and Presta (1992) *Curr. Op. Struct. Biol.* 2:593-596.

Methods for humanizing non-human antibodies are known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" or "donor" residues, which are typically obtained from an "import" or "donor" variable domain. For example, humanization can be performed essentially according to the method of Winter and co-workers, by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. See, for example, Jones et al., supra; Riechmann et al., supra and Verhoeyen et al. (1988) *Science* 239:1534-1536. Accordingly, such "humanized" antibodies include chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In certain embodiments, humanized antibodies are human antibodies in which some CDR residues and optionally some framework region residues are substituted by residues from analogous sites in rodent antibodies (e.g., murine monoclonal antibodies).

Human antibodies can also be produced, for example, by using phage display libraries. Hoogenboom et al. (1991) *J. Mol. Biol.* 227:381; Marks et al. (1991) *J. Mol. Biol.* 222:581. Other methods for preparing human monoclonal antibodies are described by Cole et al. (1985) "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, p. 77 and Boerner et al. (1991) *J. Immunol.* 147:86-95.

Human antibodies can be made by introducing human immunoglobulin loci into transgenic animals (e.g., mice) in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon immunological challenge, human antibody production is observed, which closely resembles that seen in humans in all respects,

including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al. (1992) *Bio/Technology* 10:779-783 (1992); Lonberg et al. (1994) *Nature* 368: 856-859; Morrison (1994) *Nature* 368:812-813; Fishwald et al. (1996) *Nature Biotechnology* 14:845-851; Neuberger (1996) *Nature Biotechnology* 14:826; and Lonberg et al. (1995) *Intern. Rev. Immunol.* 13:65-93.

Antibodies can be affinity matured using known selection and/or mutagenesis methods as described above. In some embodiments, affinity matured antibodies have an affinity which is five times or more, ten times or more, twenty times or more, or thirty times or more than that of the starting antibody (generally murine, rabbit, chicken, humanized or human) from which the matured antibody is prepared.

An antibody can also be a bispecific antibody. Bispecific antibodies are monoclonal, and may be human or humanized antibodies that have binding specificities for at least two different antigens. In the present case, the two different binding specificities can be directed to two different MMPs, or to two different epitopes on a single MMP (e.g., MMP9).

An antibody as disclosed herein can also be an immunoconjugate. Such immunoconjugates comprise an antibody (e.g., to MMP9) conjugated to a second molecule, such as a reporter. An immunoconjugate can also comprise an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

An antibody that "specifically binds to" or is "specific for" a particular polypeptide or an epitope refers to the selective binding of the antibody to the target antigen or epitope; these terms, and methods for determining specific binding, are well understood in the art. An antibody exhibits "specific binding" for a particular target antigen or epitope if it binds with greater affinity, avidity, more readily, and/or with greater duration to that target antigen or epitope than it does with other substances. In some embodiments, the antibody that specifically binds to the polypeptide or epitope is one that binds to that particular polypeptide or epitope without substantially binding to any other polypeptide or polypeptide epitope.

In some embodiments, the provided antibodies specifically bind to human MMP9 with a dissociation constant ( $K_d$ ) equal to or lower than 100 nM, optionally lower than 10 nM, optionally lower than 1 nM, optionally lower than 0.5 nM, optionally lower than 0.1 nM, optionally lower than 0.01 nM, or optionally lower than 0.005 nM, in certain examples, between 0.1 and 0.2 nM, or between 0.1 and 10 pM, e.g., between 0.4 and 9 pM, such as between 0.4 and 8.8 pM, in the form of monoclonal antibody, scFv, Fab, or other form of antibody measured at a temperature of about 4° C., 25° C., 37° C. or 42° C.

In certain embodiments, an antibody of the present disclosure binds to one or more processing sites (e.g., sites of proteolytic cleavage) in MMP9, thereby effectively blocking processing of the proenzyme or preproenzyme to the catalytically active enzyme, and thus reducing the proteolytic activity of the MMP9.

In certain embodiments, an antibody according to the present disclosure binds to MMP9 with an affinity at least 2 times, at least 5 times, at least 10 times, at least 25 times, at least 50 times, at least 100 times, at least 500 times, or at least 1000 times greater than its binding affinity for another MMP. Binding affinity can be measured by any method

known in the art and can be expressed as, for example, on-rate, off-rate, dissociation constant ( $K_d$ ), equilibrium constant ( $K_{eq}$ ) or any term in the art.

In certain embodiments, an antibody according to the present disclosure is one that inhibits the enzymatic (i.e., catalytic) activity of MMP9, such as a non-competitive inhibitor of the catalytic activity of MMP9. In certain embodiments, an antibody according to the present disclosure binds within the catalytic domain of MMP9. In additional embodiments, an antibody according to the present disclosure binds outside the catalytic domain of MMP9.

Also provided are antibodies or antigen binding fragments thereof that compete with any one or more of the anti-MMP9 antibodies or antigen binding fragments thereof described herein for binding to MMP9. Thus, the present disclosure contemplates anti-MMP9 antibodies, and functional fragments thereof, that compete for binding with, for example, an antibody having a heavy chain polypeptide of any of SEQ ID NOS: 1 or 5-8, a light chain polypeptide of SEQ ID NOS: 2 or 9-12, or combinations thereof. In one embodiment, the anti-MMP9 antibody, or functional fragment thereof, competes for binding to human MMP9 with the antibody described herein as AB0041.

#### Epitope Binding

Also provided are antibodies and fragments thereof that bind to the same epitope, e.g., MMP9 epitope as any one or more of the antibodies described herein. Also provided are antibodies and fragments that specifically bind to an epitope of MMP9, where the epitope includes an amino acid residue within a particular region of MMP9 or multiple regions of MMP9. Such regions can include, for example, structural loops and/or other structural domains of MMP9, such as those shown to be important for binding to exemplary antibodies described herein. Typically, the regions are defined according to amino acid residue positions on the full-length MMP9 sequence, e.g., SEQ ID NO: 27. In some examples, the epitope is outside of cysteine-switch active pocket of SEQ ID NO: 27. In some example, the epitope contains an amino acid residue (i.e., one or more amino acid residue(s)) within a region that is residues 104-202 of SEQ ID NO: 27. In one example, the epitope contains an amino acid residue (i.e., one or more amino acid residue(s)) within a region that is residues 104-119, residues 159-166, or residues 191-202 of SEQ ID NO: 27. In some aspects, the epitope includes an amino acid residue (i.e., one or more amino acid residue(s)) within a region of MMP9 that is residues 104-119 of SEQ ID NO: 27, an amino acid residue within a region of MMP9 that is residues 159-166 of SEQ ID NO: 27, and an amino acid residue within a region of MMP9 that is residues 191-202 of SEQ ID NO: 27. In some cases, the epitope includes E111, D113, R162, or 1198 of SEQ ID NO: 27. In some cases, it includes R162 of SEQ ID NO: 27. In some cases, it includes E111, D113, R162, and 1198 of SEQ ID NO: 27.

#### MMP9 Sequence

The amino acid sequence of human MMP9 protein is as follows:

		(SEQ ID NO: 27)	
60	MSLWQPLVLV LVLGCCFAA PRQRQSTLVL FPGDLRNLTLT	50	
	DRQLAEEYLY		
	RYGYTRVAEM RGESKSLGPA LLLLQKQLSL PETGELDSAT	100	
	LKAMRTPRCG		
65	VPDLGRFQTF EGDWKWHHNN ITYWIQNYSE DLPRAVIDDA	150	
	FARAFALWSA		

-continued

VTPLTFTRVY SRDADIVIQF GVAEHGDGYP FDGKDGLLAH 200  
 AFPPGPGIQG  
 DAHFDDDELW SLGKGVVVPT RFGNADGAAC HPPFIFEGRS 250 5  
 YSACTTDGRS  
 DGLPWCSTTA NYDTDDRFGF CPSERLYTRD GNADGKPCQF 300  
 PFIFQGQSYS  
 ACTTDGRSDG YRWCAATANY DRDKLFGFCP TRADSTVMGG 350 10  
 NSAGELCVFP  
 FTFLGKEYST CTSEGRGDGR LWCATTSNFD SDKKWGFCPD 400  
 QGYSLFLVAA  
 HEFGHALGLD HSSVPEALMY PMYRFTEGPP LHKDDVNGIR 450 15  
 HLYGPRPEPE  
 PRPPTTTTPQ PTAPPTVCPT GPPTVHPSEK PTAGPTGPPS 500  
 AGPTGPPTAG  
 PSTATTVPLS PVDDACNVNI FDAIAEIGNQ LYLFDKGKYW 550  
 RFSEGRGSRP  
 QGPFLIADKW PALPRKLDV FEEPLSKKLF FFSGRQVWVY 600  
 TGASVLGPRR  
 LDKLGLGADV AQTGALRSR RGKMLLFSGR RLWRFDVKAQ 650  
 MVDPRSASEV  
 DRMPFGVPLD THDVFQYREK AYFCQDRFYW RVSSRSELNQ 700  
 VDQVGYVTYD  
 ILQCPED

Protein domains are shown schematically in FIG. 3 and are indicated below:

Amino Acid #	Feature
1-19	Signal Peptide
38-98	Peptidoglycan Binding Domain
R98/C99	Cysteine-switch active pocket
112-445	Zn dependent metalloproteinase domain
223-271	Fibronectin type II domain (gelatin binding domain)
281-329	Fibronectin type II domain (gelatin binding domain)
340-388	Fibronectin type II domain (gelatin binding domain)
400-411	Zn binding region
521-565	Hemopexin-like domain
567-608	Hemopexin-like domain
613-659	Hemopexin-like domain
661-704	Hemopexin-like domain

The amino acid sequence of mature full-length human MMP9 (which is the amino acid sequence of the propoly-peptide of SEQ ID NO:27 without the signal peptide) is:

(SEQ ID NO: 28)  
 APRQRQSTLVL FPGDLRTNLT DRQLAEEYLY RYGYTRVAEM  
 RGESKSLGPA LLLLQKQLSL PETGELDSAT LKAMRTPRCG  
 VPDLGRFQTF EGDWKWHHHN ITYWIQNYSE DLPRVIDDA  
 FARAFALWSA VTPLTFTRVY SRDADIVIQF GVAEHGDGYP  
 FDGKDGLLAH AFPPGPGIQG DAHFDDDELW SLGKGVVVPT  
 RFGNADGAAC HPPFIFEGRS YSACTTDGRS DGLPWCSTTA  
 NYDTDDRFGF CPSERLYTRD GNADGKPCQF PFIFQGQSYS  
 ACTTDGRSDG YRWCAATANY DRDKLFGFCP TRADSTVMGG  
 NSAGELCVFP FTFLGKEYST CTSEGRGDGR LWCATTSNFD

-continued

SDKKWGFCPD QGYSLFLVAA HEFGHALGLD HSSVPEALMY  
 PMYRFTEGPP LHKDDVNGIR HLYGPRPEPE PRPPTTTTPQ  
 PTAPPTVCPT GPPTVHPSEK PTAGPTGPPS AGPTGPPTAG  
 PSTATTVPLS PVDDACNVNI FDAIAEIGNQ LYLFDKGKYW  
 RFSEGRGSRP QGPFLIADKW PALPRKLDV FEEPLSKKLF  
 FFSGRQVWVY TGASVLGPRR LDKLGLGADV AQTGALRSR  
 RGKMLLFSGR RLWRFDVKAQ MVDPRSASEV DRMPFGVPLD  
 THDVFQYREK AYFCQDRFYW RVSSRSELNQ VDQVGYVTYD  
 ILQCPED

The amino acid sequence of the signal peptide is MSLWQPLVLV LVLGCCFA (SEQ ID NO:29).

Also provided are MMP9 polypeptides, including mutant MMP9 polypeptides. Such peptides are useful, for example, in generating and selecting antibodies and fragments as provided herein. Exemplary polypeptides include those having an amino acid sequence containing residues 104-202 of SEQ ID NO: 27, and those having an amino acid sequence of SEQ ID NO: 27 with an amino acid substitution at residue 111, 113, 162, or 198 of SEQ ID NO 27 or with an amino acid substitution at all such residues. Other exemplary polypeptides include those having an amino acid sequence containing residues 111-198 of SEQ ID NO: 27, and those having an amino acid sequence containing residues 111-198 of SEQ ID NO: 27 with an amino acid substitution at residue 111, 113, 162, or 198 of SEQ ID NO 27 or with an amino acid substitution at all such residues. Such polypeptides find use, for example, in selecting antibodies that bind to epitopes containing such residues and/or for which such residues of MMP9 are important for binding, such as those described herein.

The present disclosure contemplates MMP9 binding proteins that bind any portion of MMP9, e.g., human MMP9, with MMP9 binding proteins that preferentially bind MMP9 relative to other MMPs being of particular interest.

Anti-MMP9 antibodies, and functional fragments thereof, can be generated accordingly to methods well known in the art. Exemplary anti-MMP9 antibodies are provided below. Mouse Monoclonal Anti-MMP9 Antibodies

A mouse monoclonal antibody to human MMP9 was obtained as described in Example 1. This antibody contains a mouse IgG2b heavy chain and a mouse kappa light chain, and is denoted AB0041.

The amino acid sequence of the AB0041 heavy chain is as follows:

(SEQ ID NO: 1)  
 MAVLVFLCLVAFPSCVLSQVQLKESGPGLVAPSQSLSI TCTVSGFS  
 LLSYGVHVRPPGKGLEWLVGIWTGTTNYSALMSRLSISIKDDSK  
 SQVFLKMSLQTDITAIYYCARYYYGMDYWGQTSVTVSSAKTTTPS  
 VYPLAPCGDITGSSVTLGCLVKGYFPEPVTVTVNSGSLSSVHTFP  
 ALLQSGLYTMSSVTVPSSTWPSQTVTCSVAHPASSTTVDKKLEPSG  
 PISTINPCPPCKECKCPAPNLEGGPSVFI FPPNIKDLVMI SLTPKV  
 TCVVVDVSEDDPDVRI SWFVNNEVHTAQTQTHREDYNS TIRVVSAL  
 PIQHQDWMMSGKEFKCKVNNKDLPSPIERTISKIKGLVRAPQVYILPP

-continued

*PAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDS*

*DGSYFIYSKLDIKTSKWEKTDSPFCNVRHEGLKNYYLKKTISRSPGK*

The signal sequence is underlined, and the sequence of the IgG2b constant region is presented italics.

The amino acid sequence of the AB0041 light chain is as follows:

(SEQ ID NO: 2)  
MESQIQVFVFLWLSGVDGDIVMTQSHKFMSTSVGDRVSITCKASQDV  
 RNTVAWYQKQTGQSPKLLIYSSYRNTGVPDRFTGSGSGTDFTFITISSV  
 QAEDLAVYFCQHYITPYTFGGGKLEIKRADAAPTVSIFPPSSEQLT  
SGASVVCFLNFPYKIDINVKWKIDGSEKQNGVLNSWTDQDSKDYSTYSMS  
STLTLTKDEYERHNSYTCEATHKTSPIVKSFNREK

The signal sequence is underlined, and the sequence of the kappa constant region is presented in italics.

The following amino acid sequence comprises the framework regions and complementarity-determining regions (CDRs) of the variable region of the IgG2b heavy chain of AB0041 (with CDRs underlined):

(SEQ ID NO: 3)  
QVQLKESGPGLVAPSQSLSITCTVSGFSLLSYGVHWRQPPGKLEWLG  
IWTGGTTNYSALMSRLSISKDDSKSQVFLKMNSLQTDDTAIYYCARY  
YGMIDYWGQGTSLVTVSS

The following amino acid sequence comprises the framework regions and complementarity-determining regions (CDRs) of the variable region of the kappa light chain of AB0041 (with CDRs underlined):

(SEQ ID NO: 4)  
 DIVMTQSHKFMSTSVGDRVSITCKASQDVRNTVAWYQKQTGQSPKLLIY  
SSYRNTGVPDRFTGSGSGTDFTFITISSVQAEDLAVYFCQHYITPYTF  
GGGKLEIK

Other exemplary mouse anti-human MMP9 antibodies (e.g., M4 and M12) are described in Example 1B. An exemplary anti-mouse MMP9 antibody (AB0046) is described in Example 1C. Other exemplary mouse anti-human MMP9 antibodies include antibodies comprise the variable regions having the sequence of SEQ ID NO: 3, and the constant regions having 95% similarity as the sequences of the IgG2b constant regions. In addition, the exemplary mouse anti-human MMP9 antibodies include antibodies comprise the variable regions having the sequence of SEQ ID NO: 4, and the constant regions having 95% similarity as the sequences of the IgG2b constant regions. Other exemplary mouse anti-human MMP9 antibodies include antibodies comprise the variable regions having the sequences of SEQ ID NOS: 3 and 4, and the constant regions having 95% similarity as the sequences of the IgG2b constant regions. Such anti-mouse antibodies are suitable for testing and assessing the MMP9-inhibition methods.

Heavy-Chain Variants

The amino acid sequences of the variable regions of the AB0041 heavy and light chains were separately modified, by altering framework region sequences in the heavy and light chain variable regions. The effect of these sequence

alterations was to deplete the antibody of human T-cell epitopes, thereby reducing or abolishing its immunogenicity in humans.

Four heavy-chain variants were constructed, in a human IgG4 heavy chain background containing a S241P amino acid change that stabilizes the hinge domain (Angal et al. (1993) *Molec. Immunol.* 30:105-108), and are denoted VH1, VH2, VH3 and VH4. The amino acid sequences of their framework regions and CDRs are as follows:

VH1 (SEQ ID NO: 5)  
QVQLQESGPGLVKPSSETLSLTCTVSGFSLLSYGVHWRQPPGKLEWLG  
 VIWTGGTTNYSALMSRLTISKDDSKSTVYLKMNSLKTEDTAIYYCARY  
 YGMDYWGQGTSLVTVSS

VH2 (SEQ ID NO: 6)  
QVQLQESGPGLVKPSSETLSLTCTVSGFSLLSYGVHWRQPPGKLEWLG  
 VIWTGGTTNYSALMSRLTISKDDSKNTVYLKMNSLKTEDTAIYYCARY  
 YGMDYWGQGTSLVTVSS

VH3 (SEQ ID NO: 7)  
QVQLQESGPGLVKPSSETLSLTCTVSGFSLLSYGVHWRQPPGKLEWLG  
 VIWTGGTTNYSALMSRFTISKDDSKNTVYLKMNSLKTEDTAIYYCARY  
 YGMDYWGQGTSLVTVSS

VH4 (SEQ ID NO: 8)  
QVQLQESGPGLVKPSSETLSLTCTVSGFSLLSYGVHWRQPPGKLEWLG  
 VIWTGGTTNYSALMSRFTISKDDSKNTLYLKMNSLKTEDTAIYYCARY  
 YGMDYWGQGTSLVTVSS

FIG. 1 shows an alignment of the amino acid sequences of the variable regions of the humanized heavy chains and indicates the differences in amino acid sequences in the framework regions among the four variants.

Light-Chain Variants

Four light-chain variants were constructed, in a human kappa chain background, and are denoted Vk1, Vk2, Vk3 and Vk4. The amino acid sequences of their framework regions and CDRs are as follows:

Vk1 (SEQ ID NO: 9)  
 DIVMTQSPSFLSASVGDRVTITCKASQDVRNTVAWYQKQTGKAPKLLIYS  
 SSYRNTGVPDRFTGSGSGTDFTLTISLQAEDVAVYFCQHYITPYTFGGG  
 TKVEIK

Vk2 (SEQ ID NO: 10)  
 DIVMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQKPGKAPKLLIYS  
 SSYRNTGVPDRFTGSGSGTDFTLTISLQAEDVAVYFCQHYITPYTFGGG  
 TKVEIK

Vk3 (SEQ ID NO: 11)  
 DIQMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQKPGKAPKLLIYS  
 SSYRNTGVPDRFSGSGSGTDFTLTISLQAEDVAVYFCQHYITPYTFGGG  
 TKVEIK

-continued

Vk4  
 (SEQ ID NO: 12)  
 DIQMTQSPSSLSASVGRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYS  
 5  
 SSSYRNTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYITPYPFGG  
 10  
 GTKVEIK

FIG. 2 shows an alignment of the amino acid sequences of the variable regions of the humanized light chains and indicates the differences in amino acid sequences in the framework regions among the four variants.

The humanized heavy and light chains are combined in all possible pair-wise combinations to generate a number of functional humanized anti-MMP9 antibodies. For example, provided are antibodies with a heavy chain variable (VH) region having the amino acid sequence set forth in any of SEQ ID NOs: 3, 5, 6, 7, and 8; antibodies having a light chain variable (VL) region having the amino acid sequence set forth in any of SEQ ID NOs: 4, 9, 10, 11, and 12; and antibodies with a heavy chain variable (VH) region having the amino acid sequence set forth in any of SEQ ID NOs: 3, 5, 6, 7, and 8 and a light chain variable (VL) region having the amino acid sequence set forth in any of SEQ ID NOs: 4, 9, 10, 11, and 12, as well as antibodies that compete for binding to MMP9 with such antibodies and antibodies having at least at or about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with such antibodies. In one example, the antibody has a VH region with an amino acid sequence having at least at or about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with SEQ ID NO: 7 and a VL region with an amino acid sequence having at least at or about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with SEQ ID NO: 12, or a VH region of SEQ ID NO: 7 and a VL region of SEQ ID NO: 12.

Additional heavy chain variable region amino acid sequences having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to the heavy chain variable region sequences disclosed herein are also provided. Furthermore, additional light chain variable region amino acid sequences having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to the light chain variable region sequences disclosed herein are also provided.

Additional heavy chain variable region amino acid sequences having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more sequence identity to the heavy chain variable region sequences disclosed herein are also provided. Furthermore, additional light chain variable region amino acid sequences having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more sequence identity to the light chain variable region sequences disclosed herein are also provided.

Complementarity-Determining Regions (CDRs)

In some embodiments, the CDRs of the heavy chain of exemplary provided anti-MMP9 antibodies as disclosed herein have the following amino acid sequences:

CDR1 : (SEQ ID NO: 13)  
 GFSLLSYGVH  
 CDR2 : (SEQ ID NO: 14)  
 VIWTGGTTNYSALMS

-continued

CDR3 : (SEQ ID NO: 15)  
 YYYGMDY

Thus, among the provided anti-MMP9 antibodies are antibodies having a heavy chain CDR1 region with an amino acid sequence as set forth in SEQ ID NO: 13, antibodies having a heavy chain CDR2 region with an amino acid sequence set forth in SEQ ID NO: 14, and antibodies having a heavy chain CDR3 region with an amino acid sequence as set forth in SEQ ID NO: 15, and antibodies that compete for binding with or bind to the same epitope on MMP9 as such antibodies. In some cases, the antibodies contain VH CDRs having the sequences set forth in SEQ ID NO: 13, 14, and 15.

In some embodiments, the CDRs of the light chain of exemplary anti-MMP9 antibodies as disclosed herein have the following amino acid sequences:

CDR1 : (SEQ ID NO: 16)  
 KASQDVRNTVA  
 CDR2 : (SEQ ID NO: 17)  
 SSSYRNT  
 CDR3 : (SEQ ID NO: 18)  
 QQHYITPYT

Thus, among the provided anti-MMP9 antibodies are antibodies having a light chain CDR1 region with an amino acid sequence as set forth in SEQ ID NO: 16, antibodies having a light chain CDR2 region with an amino acid sequence set forth in SEQ ID NO: 17, and antibodies having a light chain CDR3 region with an amino acid sequence as set forth in SEQ ID NO: 18, and antibodies that compete for binding with or bind to the same epitope on MMP9 as such antibodies. In some cases, the antibodies contain VL CDRs having the sequences set forth in SEQ ID NO: 16, 17, and 18.

Nucleic Acids Encoding Anti-MMP9 Antibodies

The present disclosure provides nucleic acids encoding anti-MMP9 antibodies and functional fragments thereof. Accordingly, the present disclosure provides an isolated polynucleotide (nucleic acid) encoding an antibody or antigen-binding fragment as described herein, vectors containing such polynucleotides, and host cells and expression systems for transcribing and translating such polynucleotides into polypeptides.

The present disclosure also contemplates constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise at least one polynucleotide as above.

The present disclosure also provides a recombinant host cell which comprises one or more constructs as above, as well as methods of production of the antibody or antigen-binding fragments thereof described herein which method comprises expression of nucleic acid encoding a heavy chain polypeptide and a light chain polypeptide (in the same or different host cells, and from the same or different constructs) in a recombination host cell. Expression can be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression, an antibody or antigen-binding fragment can be isolated and/or purified using any suitable technique, then used as appropriate.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells and many others. A common bacterial host is *E. coli*.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including operably linked promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and/or other sequences as appropriate. Vectors can be plasmids, viral e.g. phage, or phagemid, as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*, 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference in their entirety.

The nucleic acid encoding a polypeptide of interest is integrated into the genome of the host cell or can be maintained as a stable or transient episomal element.

Any of a wide variety of expression control sequences—sequences that control the expression of a DNA sequence operatively linked to it—can be used in these vectors to express the DNA sequences. For example, a nucleic acid encoding a polypeptide of interest can be operably linked to a promoter, and provided in an expression construct for use in methods of production of recombinant MMP9 proteins or portions thereof.

Those of skill in the art are aware that nucleic acids encoding the antibody chains disclosed herein can be synthesized using standard knowledge and procedures in molecular biology.

Examples of nucleotide sequences encoding the heavy and light chain amino acid sequences disclosed herein, are as follows:

VH1:

(SEQ ID NO: 19)  
 CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC  
 CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT  
 CTCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT  
 CCAGGGAAGG GCCTGGAATG GCTGGGCGTG ATCTGGACCG  
 GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGCT  
 GACCATCTCC AAGGACGACT CCAAGTCCAC CGTGTACCTG  
 AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT  
 ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA  
 GGGCACCTCC GTGACCGTGT CCTCA

VH2:

(SEQ ID NO: 20)  
 CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC  
 CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT

-continued

CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT  
 CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG  
 GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGCT  
 GACCATCTCC AAGGACGACT CCAAGAACAC CGTGTACCTG  
 AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT  
 ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA  
 GGGCACCCCTG GTCACCGTGT CCTCA

VH3:

(SEQ ID NO: 21)  
 CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAG  
 CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT  
 CTCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT  
 CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG  
 GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGTT  
 CACCATCTCC AAGGACGACT CCAAGAACAC CGTGTACCTG  
 AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT  
 ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA  
 GGGCACCCCTG GTCACCGTGT CCTCA

VH4:

(SEQ ID NO: 22)  
 CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC  
 CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT  
 CTCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT  
 CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG  
 GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGTT  
 CACCATCTCC AAGGACGACT CCAAGAACAC CCTGTACCTG  
 AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT  
 ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA  
 GGGCACCCCTG GTCACCGTGT CCTCA

Vk1:

(SEQ ID NO: 23)  
 GACATCGTGA TGACCCAGTC CCCAGCTTC CTGTCCGCCCT  
 CCGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA  
 GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAGCCC  
 GGCAAGGCC CCAAGCTGCT GATCTACTCC TCCTCTTACC  
 GGAACACCGG CGTGCCCGAC CGGTTTACCG GCTCTGGCTC  
 CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC  
 GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA  
 CCCCCTACAC CTTCCGGCGGA GGCACCAAGG TGGAAATAAA

A

Vk2:

(SEQ ID NO: 24)  
 GACATCGTGA TGACCCAGTC CCCCTCCAGC CTGTCCGCC  
 CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA  
 GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAGCCC

-continued

GGCAAGGCC CCAAGCTGCT GATCTACTCC TCCTCTTACC  
 GGAACACCGG CGTGCCCGAC CGGTTTACCG GCTCTGGCTC  
 CGGCACCGAC TTTACCTGA CCATCAGCTC CCTGCAGGCC  
 GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA  
 CCCCCTACAC CTTGCGCGGA GGCACCAAGG TGGAAATAAA  
 A  
 Vk3 :  
 (SEQ ID NO: 25)  
 GACATCCAGA TGACCCAGTC CCCCTCCAGC CTGTCCGCC  
 CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCCCA  
 GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAGCCC  
 GGCAAGGCC CCAAGCTGCT GATCTACTCC TCCTCTTACC  
 GGAACACCGG CGTGCCCGAC CGGTTCTCTG GCTCTGGAAG  
 CGGCACCGAC TTTACCTGA CCATCAGCTC CCTGCAGGCC  
 GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA  
 CCCCCTACAC CTTGCGCGGA GGCACCAAGG TGGAAATAAA  
 A  
 Vk4 :  
 (SEQ ID NO: 26)  
 GACATCCAGA TGACCCAGTC CCCCTCCAGC CTGTCCGCC  
 CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA  
 GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAGCCC  
 GGCAAGGCC CCAAGCTGCT GATCTACTCC TCCTCTTACC  
 GGAACACCGG CGTGCCCGAC CGGTTCTCTG GCTCTGGAAG  
 CGGCACCGAC TTTACCTGA CCATCAGCTC CCTGCAGGCC  
 GAGGACGTGG CCGTGTACTA CTGCCAGCAG CACTACATCA  
 CCCCCTACAC CTTGCGCGGA GGCACCAAGG TGGAAATAAA  
 A

Because the structure of antibodies, including the juxta-  
 position of CDRs and framework regions in the variable  
 region, the structure of framework regions and the structure  
 of heavy- and light-chain constant regions, is well-known in  
 the art; it is well within the skill of the art to obtain related  
 nucleic acids that encode anti-MMP-9 antibodies. Accord-  
 ingly, polynucleotides comprising nucleic acid sequences  
 having at least 75%, at least 80%, at least 85%, at least 90%,  
 at least 95%, at least 98% and at least 99% homology to any  
 of the nucleotide sequences disclosed herein are also pro-  
 vided. Accordingly, polynucleotides comprising nucleic acid  
 sequences having at least 75%, at least 80%, at least 85%,  
 at least 90%, at least 95%, at least 98% and at least 99%  
 identity to any of the nucleotide sequences disclosed herein  
 are also provided. In one example, the polynucleotide con-  
 tains at least at or about 75%, 80%, 85%, 90%, 91%, 92%,  
 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence  
 identity with SEQ ID NO: 21 or includes or is SEQ ID NO:  
 21 and/or contains at least at or about 75%, 80%, 85%, 90%,  
 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more  
 sequence identity with SEQ ID NO: 26 or includes or is SEQ  
 ID NO: 26.

## Pharmaceutical Compositions

MMP9 binding proteins, as well as nucleic acid (e.g.,  
 DNA or RNA) encoding MMP9 binding proteins, can be  
 provided as a pharmaceutical composition, e.g., combined  
 with a pharmaceutically acceptable carrier or excipient.  
 Such pharmaceutical compositions are useful for, for  
 example, administration to a subject in vivo or ex vivo, and  
 for diagnosing and/or treating a subject with the MMP9  
 binding proteins, such as in any of the therapeutic or  
 diagnostic methods provided herein.

Pharmaceutically acceptable carriers are physiologically  
 acceptable to the administered patient and retain the thera-  
 peutic properties of the antibodies or peptides with which it  
 is administered. Pharmaceutically-acceptable carriers and  
 their formulations are and generally described in, for  
 example, Remington's pharmaceutical Sciences (18th Edi-  
 tion, ed. A. Gennaro, Mack Publishing Co., Easton, Pa.  
 1990). One exemplary pharmaceutical carrier is physiologi-  
 cal saline. Each carrier is "pharmaceutically acceptable"  
 in the sense of being compatible with the other ingredients of  
 the formulation and not substantially injurious to the patient.

Pharmaceutical compositions can be formulated to be  
 compatible with a particular route of administration, sys-  
 temic or local. Thus, pharmaceutical compositions include  
 carriers, diluents, or excipients suitable for administration by  
 various routes.

Pharmaceutical compositions can include pharmaceuti-  
 cally acceptable additives. Examples of additives include,  
 but are not limited to, a sugar such as mannitol, sorbitol,  
 glucose, xylitol, trehalose, sorbose, sucrose, galactose, dex-  
 tran, dextrose, fructose, lactose and mixtures thereof. Phar-  
 maceutically acceptable additives can be combined with  
 pharmaceutically acceptable carriers and/or excipients such  
 as dextrose. Additives also include surfactants such as  
 polysorbate 20 or polysorbate 80.

The formulation and delivery methods will generally be  
 adapted according to the site and the disease to be treated.  
 Exemplary formulations include, but are not limited to,  
 those suitable for parenteral administration, e.g., intrave-  
 nous, intra-arterial, intramuscular, or subcutaneous admin-  
 istration, or oral administration.

Pharmaceutical compositions for parenteral delivery  
 include, for example, water, saline, phosphate buffered  
 saline, Hank's solution, Ringer's solution, dextrose/saline,  
 and glucose solutions. The formulations can contain auxil-  
 iary substances to approximate physiological conditions,  
 such as buffering agents, tonicity adjusting agents, wetting  
 agents, detergents and the like. Additives can also include  
 additional active ingredients such as bactericidal agents, or  
 stabilizers. For example, the solution can contain sodium  
 acetate, sodium lactate, sodium chloride, potassium chlo-  
 ride, calcium chloride, sorbitan monolaurate or trietha-  
 nolamine oleate. Additional parenteral formulations and  
 methods are described in Bai (1997) J. Neuroimmunol.  
 80:65 75; Warren (1997) J. Neurol. Sci. 152:31 38; and  
 Tonegawa (1997) J. Exp. Med. 186:507 515. The parenteral  
 preparation can be enclosed in ampules, disposable syringes  
 or multiple dose vials made of glass or plastic.

Pharmaceutical compositions for intradermal or subcuta-  
 neous administration can include a sterile diluent, such as  
 water, saline solution, fixed oils, polyethylene glycols, glyce-  
 rine, propylene glycol or other synthetic solvents; antibac-  
 terial agents such as benzyl alcohol or methyl parabens;  
 antioxidants such as ascorbic acid, glutathione or sodium  
 bisulfite; chelating agents such as ethylenediaminetet-  
 raacetic acid; buffers such as acetates, citrates or phosphates  
 and agents for the adjustment of tonicity such as sodium  
 chloride or dextrose.

Pharmaceutical compositions for injection include aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Antibacterial and antifungal agents include, for example, parabens, chlorobutanol, phenol, ascorbic acid and thimerosal. Isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride may be included in the composition. The resulting solutions can be packaged for use as is, or lyophilized; the lyophilized preparation can later be combined with a sterile solution prior to administration.

Pharmaceutically acceptable carriers can contain a compound that stabilizes, increases, or delays absorption or clearance. Such compounds include, for example, carbohydrates, such as glucose, sucrose, or dextrans; low molecular weight proteins; compositions that reduce the clearance or hydrolysis of peptides; or excipients or other stabilizers and/or buffers. Agents that delay absorption include, for example, aluminum monostearate and gelatin. Detergents can also be used to stabilize or to increase or decrease the absorption of the pharmaceutical composition, including liposomal carriers. To protect from digestion the compound can be complexed with a composition to render it resistant to acidic and enzymatic hydrolysis, or the compound can be complexed in an appropriately resistant carrier such as a liposome. Means of protecting compounds from digestion are known in the art (see, e.g., Fix (1996) *Pharm Res.* 13:1760 1764; Samanen (1996) *J. Pharm. Pharmacol.* 48:119 135; and U.S. Pat. No. 5,391,377, describing lipid compositions for oral delivery of therapeutic agents).

Compositions of the present invention can be combined with other therapeutic moieties or imaging/diagnostic moieties as provided herein. Therapeutic moieties and/or imaging moieties can be provided as a separate composition, or as a conjugated moiety present on an MMP9 binding protein.

Formulations for in vivo administration are generally sterile. In one embodiment, the pharmaceutical compositions are formulated to be free of pyrogens such that they are acceptable for administration to human patients.

Various other pharmaceutical compositions and techniques for their preparation and use will be known to those of skill in the art in light of the present disclosure. For a detailed listing of suitable pharmacological compositions and associated administrative techniques one can refer to the detailed teachings herein, which can be further supplemented by texts such as Remington: *The Science and Practice of Pharmacy* 20th Ed. (Lippincott, Williams & Wilkins 2003).

Pharmaceutical compositions can be formulated based on the physical characteristics of the patient/subject needing treatment, the route of administration, and the like. Such can be packaged in a suitable pharmaceutical package with appropriate labels for the distribution to hospitals and clinics wherein the label is for the indication of treating a disorder as described herein in a subject. Medicaments can be packaged as a single or multiple units. Instructions for the dosage

and administration of the pharmaceutical compositions of the present invention can be included with the pharmaceutical packages and kits described below.

#### Methods of Use

The MMP9 binding proteins, including anti-MMP9 antibodies and fragments thereof, of the present disclosure can be used, for example, in therapeutic and diagnostic methods, such as methods of detection of MMP9 in a sample, methods of treatment (e.g., as in methods of inhibition of angiogenesis), and methods of diagnosis and prognosis. Thus, provided are diagnostic and therapeutic methods and uses of the anti-MMP9 antibodies. Examples of methods of use are described below.

#### Methods of Treatment

Provided herein are methods of treatment, including methods of treating diseases and disorders associated with MMP9 expression and/or activity, as well as uses of the provided antibodies and compositions in such methods. The diseases and disorders include, but are not limited to cancer, e.g., tumors (e.g., primary or metastatic tumors), such as those that express or are disposed in a tissue which expresses MMP9, and inflammatory diseases, such as inflammatory bowel diseases, rheumatoid arthritis and inflammatory myopathies.

As used herein, “treat” or “treatment” means stasis or a postponement of development of one or more symptoms associated with a disease or disorder described herein, or ameliorating existing uncontrolled or unwanted symptoms, preventing additional symptoms, or ameliorating or preventing the underlying metabolic causes of symptoms. Thus, the terms denote that a beneficial result has been conferred on a mammalian subject with a disease or symptom, or with the potential to develop such disease or symptom. A response is achieved when the patient experiences partial or total alleviation, or reduction of signs or symptoms of illness, and can include, without limitation, prolongation of survival. The expected progression-free survival times can be measured in months to years, depending on prognostic factors including the number of relapses, stage of disease, and other factors.

Also provided are pharmaceutical compositions for use in connection with such methods, such as those containing any of the antibodies or fragments thereof described herein. Compositions can be suitable for administration locally or systemically by any suitable route.

In general, MMP9 binding proteins are administered in a therapeutically effective amount, e.g., in an amount to effect inhibition of tumor growth in a subject, to inhibit metastasis, to inhibit inflammation, to inhibit tissue destruction, to inhibit MMP9 activity, or to treat the particular disease or condition associated with MMP9.

As used herein, unless otherwise specified, the term “therapeutically effective amount” or “effective amount” refers to an amount of an agent or compound or composition that when administered (either alone or in combination with another therapeutic agent, as may be specified) to a subject is effective to prevent or ameliorate the disease condition or the progression of the disease, or result in amelioration of symptoms, e.g., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. In one example, when in vivo administration of an

anti-MMP9 antibody is employed, normal dosage amounts can vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 µg/kg/day to 50 mg/kg/day, optionally about 100 µg/kg/day to 20 mg/kg/day, 500 µg/kg/day to 10 mg/kg/day, or 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration. In one embodiment, intravenous dosage range from about 1 mg/kg to about 30 mg/kg. In some embodiments, intravenous dosages range from at or about 1 mg/kg to at or about 14 mg/kg, such as from at or about 2 mg/kg to at or about 14 mg/kg, q14d, once every 14 days. In other embodiments, subcutaneous dosages range from at or about 1 mg/kg to at or about 28 mg/kg, such as from at or about 2 mg/kg to at or about 28 mg/kg, q14d, once every 14 days. In some embodiments, the effective amount of dosage is administered once every 7 to 28 days. In one embodiment, the effective amount of dosage is administered once every 7 days. In another embodiment, the effective amount of dosage is administered once every 28 days.

The selected dosage regimen will depend upon a variety of factors including the activity of the MMP9 binding protein, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular composition employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A clinician having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian can start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In some cases, the methods of treatment include parenteral administration, e.g., intravenous, intra-arterial, intramuscular, or subcutaneous administration, or oral administration of the agent, e.g., anti-MMP9 antibody or composition containing the same.

As used herein, the term "subject" means a mammalian subject. Exemplary subjects include, but are not limited to humans, monkeys, dogs, cats, mice, rats, cows, horses, goats and sheep. In some embodiments, the subject has cancer, an inflammatory disease or condition, or an autoimmune disease or condition, and can be treated with the agent of the present invention as described below.

If needed, for treatments, methods can further include additional therapies, such as in the case of cancer, surgical removal of the cancer and/or administration of an anti-cancer agent or treatment in addition to an MMP9 binding protein. Administration of such an anti-cancer agent or treatment can be concurrent with administration of the compositions disclosed herein.

#### Methods of Detection of MMP9

The present disclosure also contemplates methods of detecting MMP9 in a subject, e.g., to detect tumor or tumor-associated tissue expressing MMP9, or tissue or fluid or other biological sample associated with a disease as described herein, such as autoimmune or inflammatory disease. Thus, methods of diagnosing, monitoring, staging or detecting a tumor having MMP9 activity are provided.

Samples (e.g., test biological samples) from a subject (e.g., an individual suspected of having or known to have a tumor associated with MMP9 expression, or suspected of

having or known to have another disease or condition), can be analyzed for MMP9 presence, absence, expression, and/or levels. For example, such samples can be collected and analyzed by detecting the presence or absence of binding of an MMP9 binding protein, such as an antibody or fragment as described herein, to substance (e.g., protein) in the sample. In some examples, the methods further include comparing the amount of binding detected to an amount of binding to a control sample, or comparing the detected level of MMP9 to a control level of MMP9. In some cases, the methods indicate the presence, absence, or severity of an MMP9-associated disease or condition, such as one described herein.

This analysis can be performed prior to the initiation of treatment using an MMP9 binding protein as described herein, or can be done as part of monitoring of progress of cancer treatment. In some embodiments, provided are methods of treatment, carried out by performing the detection assays and initiating, altering, or discontinuing treatment of the subject, for example, based on the results of the diagnostic assay. Such diagnostic analysis can be performed using any sample, including but not limited to tissue, cells isolated from such tissues, and the like. In some cases, the methods are performed on liquid samples, such as blood, plasma, serum, whole blood, saliva, urine, or semen. Tissue samples include, for example, formalin-fixed or frozen tissue sections.

Any suitable method for detection and analysis of MMP9 can be employed. Various diagnostic assay techniques known in the art can be adapted for such purpose, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases.

MMP9 binding proteins for use in detection methods can be labeled with a detectable moiety. The detectable moiety directly or indirectly produces a detectable signal. For example, the detectable moiety can be any of those described herein such as, for example, a radioisotope, such as <sup>3</sup>H, <sup>14</sup>C, <sup>32</sup>P, <sup>35</sup>S, or <sup>125</sup>I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate (FITC), Texas red, cyanin, photocyran, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, β-galactosidase or horseradish peroxidase.

Detection can be accomplished by contacting a sample under conditions suitable for MMP9 binding protein binding to MMP9, and assessing the presence (e.g., level) or absence of MMP9 binding protein-MMP9 complexes. A level of MMP9 in the sample in comparison with a level of a reference sample can indicate the presence of a tumor or tumor-associated tissues having MMP9 activity. The reference sample can be a sample taken from the subject at an earlier time point or a sample from another individual.

Various aspects of the invention are further described and illustrated by way of the several examples which follow, none of which are intended to limit the scope of the invention.

## EXAMPLES

### Example 1A

#### Preparation of Antibodies to Human MMP-9

The full-length human MMP9 protein without a signal peptide (SEQ ID NO. 28) was used to immunize mice. Spleen cells from immunized mice were fused with myeloma cells to generate a hybridoma library. Monoclonal

cultures were prepared and screened to identify cultures expressing an anti-MMP9 monoclonal antibody.

An antibody (AB0041) was purified from one of the cultures and characterized. This antibody contained an IgG2b heavy chain and a kappa light chain. Characterization included testing for the binding of AB0041 to other human MMPs and to MMP9 proteins from other species, including cynomolgus monkey, rat and mouse. As shown in Table 2, the AB0041 antibody had greater affinity to human and cynomolgus MMP9, that it had lower affinity to rat MMP9. In addition, the AB0041 antibody did not bind to murine MMP9 or to many human non-MMP matrix metalloproteinases.

TABLE 2

Cross reactivity of AB0041 and AB0045		
MMP Tested	Dissociation constant (Kd)	
	AB0045	AB0041
Human MMP1	>100 nM	>100 nM
Human MMP2	>100 nM	>100 nM
Mouse MMP2	>100 nM	>100 nM
Human MMP3	>100 nM	>100 nM
Human MMP7	>100 nM	>100 nM
Human MMP8	>100 nM	>100 nM
Human MMP9	0.168 ± 0.117 nM	0.133 ± 0.030 nM
Cynomolgus monkey MMP9	0.082 ± 0.022 nM	0.145 ± 0.16 nM
Mouse MMP9	>100 nM	>100 nM
Rat MMP9	0.311 ± 0.017 nM	0.332 ± 0.022 nM
Human MMP10	>100 nM	>100 nM
Human MMP12	>100 nM	>100 nM
Human MMP13	>100 nM	>100 nM

Additional characterization included assaying the binding of AB0041 to mutant mouse and human MMP9 proteins. Non-identical residues in the catalytic domain of mouse and human MMP9 proteins were identified, and forty-six non-identical amino acid residues were selected for mutagenesis. Most mutations were generated in mouse MMP9: the mouse amino acid residues were mutated to match those of human MMP9. Other mutations were generated in human MMP9: the human amino acid residues were mutated to match those of mouse MMP9. The mutated mouse or human MMP9 proteins were used in an ELISA assay.

In the ELISA assay, the AB0041 antibody was used as the primary antibody and a goat anti-mouse IgG antibody conjugated to horseradish peroxidase was used to detect the binding. The wild-type human MMP9 was used a positive control and the wild-type mouse MMP9 was used as a negative control. The results of the ELISA assay showed an arginine residue at position 162 of the MMP9 amino acid sequence (R162) as important for the MMP9 binding of the AB0041 antibody. The results also showed the amino acid residues E111, D113, and 1198 were important for the MMP9 binding of the AB0041 antibody. Based on the crystal structure of MMP9, E111, D113, R162, and 1198 are grouped near each other around a Ca<sup>2+</sup> ion binding pocket of MMP9. In this study, the AB0041 antibody was shown to specifically bind to an epitope containing amino acid residues within regions of MMP9 containing amino acid residues 104-119, 159-166, and 191-202.

In an enzymatic assay for MMP9, the AB0041 antibody was found to act as a non-competitive inhibitor of MMP9.

Example 1B

Preparation of Additional Antibodies to Human MMP-9

Additional hybridomas were generated, which produced antibodies having variable regions that shared identity with AB0041. One such hybridoma, designated M4, expressed an antibody containing the heavy chain (IgG2b) sequence:

(SEQ ID NO: 30)

MAVLVLFCLCLVAFPSCVLSQVQLKESGPGLVAPSQSLSTCTVSGFSLLS  
 YGVHWRQPPGKGLEWLGVIWTTGGSTNYNSALMSRLSISKDDSKSQVFLK  
 MNSLQTDDTAMYYCARYYYAMDYWGQGTSTVTVSSAKTTPPSVYPLAPGCC  
 DTTGSSVTLGCLVKGYFPESTVTWNSGSLSSVHTFPALLQSGLYTMSS  
 SVTVPSSTWPSQTVTCSVAHPASSTTVDKKLEPSGPISTINPCPKCKECH  
 KCPAPNLEGGPSVFIAPPNIKDVLMI SLTPKVTCVVVDVSEDDPDVRI SW  
 FVNNVEVHTAQTQTHREDYNSTIRVVSALPIQHQDWMMSGKEFKCKVNNKD  
 LPSPIERTISKIKGLVRAPQVYI LPPPAEQLSRKDVSLTCLVGFNPGDI  
 SVEWTSNGHTEENYKDTAPVLDS DGSFYIYSKL DIKTSKWEKTD SFCNV  
 RHEGLKNYYLKKTISRSPGK

(signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics), and the light chain (kappa) sequence:

(SEQ ID NO: 31)

MESQIQVFVFLNLSGVDGDIVMTQSHKFMFTSVGDRVSITCKASQD  
 VRNTVAWYQKKTGQSPKLLIYSASYRNTGVPDRFTGSI SGTDFTFITIS  
 SVQAEDLALYYCQOHYSTPYTEGGGKLEVKRADAAPT VSI FPPSSEQ  
 LTTGGASVVCFLNNFYPKDINVWKIDGSE RQNGVLSWTDQD SKDST  
 YSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSENRENC.

(signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics)

The M4 antibody had a variable heavy chain with an amino acid sequence:

(SEQ ID NO: 32)

QVQLKESGPGLVAPSQSLSTCTVSGFSLLSYGVHWRQPPGKGLEWLG  
 VIWTTGGSTNYNSALMSRLSISKDDSKSQVFLKMNSLQTDDTAMYYCARY  
 YYAMDYWGQGTSTVTVSS

(CDRs 1, 2, and 3 (SEQ ID NOs 34, 35, and 36, respectively) underlined)

and a variable light chain with the amino acid sequence

(SEQ ID NO: 33)

DIVMTQSHKFMFTSVGDRVSITCKASQDVRNTVAWYQKKTGQSPKLL  
 IYSASYRNTGVPDRFTGSI SGTDFTFITISSVQAEDLALYYCQOHYST  
 PYTFGGGKLEVK.

(CDRs 1, 2, and 3 (SEQ ID NOs 37, 38, and 39, respectively) underlined)

Another such hybridoma, designated M12, expressed only a kappa chain, having the sequence:

(SEQ ID NO: 40)  
QVFVYMLLWLSGVDGDIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVA  
 WYQQKPGQSPKALIYSASYRFSQVDRPTGSGSGTDFTLTISNVQSEDL  
 AEYFCQQYNSYPYTFGGGKLEIKRADAAPT~~VSIFPPSSEQLTSSGASV~~  
 VCFLN~~NFYPKDINVKWKIDG~~SERQNGVLNSWTDQDSKSTYSMSSTLTL  
 TKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC.

(signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics)

The M12 antibody had a variable light chain with the amino acid sequence

(SEQ ID NO: 41)  
 DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKALI  
 YSASYRFSQVDRPTGSGSGTDFTLTISNVQSEDLAEYFCQQYNSYPY  
 TFGGGKLEIK.

(CDRs 1, 2, and 3 (SEQ ID NOs 42, 43, and 44, respectively) underlined)

A sequence comparison, showing differences between the M4 and M12 heavy and light chains as compared with AB0041 antibody is shown in FIG. 4.

An enzymatic assay was carried out. The results demonstrated that the antibodies produced by the M4 and M12 hybridomas acted as non-competitive inhibitors of MMP9 (data not shown).

Example 1C

Preparation of Antibodies to Mouse MMP-9

Another mouse antibody, AB0046, was generated. Using a process similar to that described in Example 1A, the MMP9-knockout mice (strain B6.FVB (Cg)-M9<sup>tmlTvu/J</sup>) was immunized using targeted domains of the pro/catalytic domain fragment of murine MMP9. The AB0046 antibody had a kappa light chain with an amino acid sequence

(SEQ ID NO: 45)  
MSSAQFLGLLLCFQGT~~RCDIQMTQTSSLSASL~~GDRVTISCSASQGI  
 SNYLNWYQQKPDGTFKLLIYYTSLHSGVPSRFSQSGSGTDYSLTISN  
 LEPEDIATYYCQQYGLWLPRTFGGGKLEIKRADAAPT~~VSIFPPSSEQL~~  
 TSGGASVVCFLN~~NFYPKDINVKWKIDG~~SERQNGVLNSWTDQDSKSTY  
 SMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

(signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics) and an IgG1 heavy chain with an amino acid sequence

(SEQ ID NO: 46)  
MGWSSIILFLVATATGVHSQVQLQPGSVLVRPGASVKLSCTASGYTF  
 TSYWMNWKQRPQGLEWIGEIYPISGRTNYNEKFKVKATLTVDTSSS  
 TAYMDLNSLTSEDSAVYYCARSRANWDDYWGQTTTLTVSSAKTTPPSV  
 YPLAPGSAQAQNSMVTLGCLVKGYFPEPVTVTVNSGSLSSGVHTFPAV  
 LQSDLYTLSSSVTVPSSTWVPESETVTCNVAHPASSTKVDKIKIVPRDCGC

-continued

KPCICTVPEVSSVFI~~FPKPKDVLTI~~TLTPKVTCVVVDISKDDPEVQF  
 SWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRV  
 NSAAFPAPIEKTI~~SKTKGRPKAPQVYTI~~PPPKEQMAKDKVSLT~~CMITD~~  
 FFPEDITVEWQWNGQPAENYKNTQPI~~MDT~~DGSYFVYSKLNVQKSNWEA  
 GNTFTCSVLHEGLHNHHT~~TEKLSL~~HSPGK

(signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics).

The following amino acid sequence comprises the framework regions and complementarity-determining regions (CDRs) of the variable region of the IgG1 heavy chain of AB0046 (with CDRs underlined):

(SEQ ID No: 47)  
 QVQLQQPGSVLVRPGASVKLSCTASGYTFTSYWMNWKQRPQGLEW  
 IGEIYPISGRTNYNEKFKVKATLTVDTSSSTAYMDLNSLTSEDSAVY  
 YCARSRANWDDYWGQTTTLTVSS.

The following amino acid sequence comprises the framework regions and complementarity-determining regions (CDRs) of the variable region of the kappa light chain of AB0046 (with CDRs underlined):

(SEQ ID No: 48)  
 DIQMTQTSSLSASLGD~~RVTISCSASQGISNYLNWY~~QQKPDGTFK  
 LLIYYTSLHSGVPSRFSQSGSGTDYSLTISNLEPEDIATYYCQQ  
 YGLWLPRTFGGGKLEIK

Additional characterizations showed that the AB0046 antibody bound to mouse MMP9 non-competitively or its binding was not dependant on the concentration of mouse MMP9. The AB0046 antibody did not bind to human MMP9 or MMP2, mouse MMP2, 3, 7, 8, or 12. Using epitope analysis as described in Example 1A, it was shown that the proline residue at position 162 of the mouse MMP9 amino acid sequence (P162) (corresponding to R162 of human MMP9) was important for the MMP9 binding of the AB0046 antibody. The results suggested that the AB0046 antibody specifically bound to an epitope containing a residue within a portion of mouse MMP9 corresponding to the portion containing amino acids 159-166 of human MMP9. Thus, the AB0046 antibody was an inhibitory antibody specific to mouse MMP9 and had similar kinetics of binding and inhibition as those of AB0041. Because AB0046 is specific to mouse MMP9 and binds to an epitope as AB0041/AB0045, AB0046 is suitable for assays which uses either AB0041 or AB0045.

Further characterization showed that the AB0046 antibody was a murine IgG1 isotype, having a limited effector function in mouse.

Three other mouse anti-MMP9 antibodies were generated using similar methods, which were non-inhibitory and for which P162 was important for binding.

Example 2

Humanization of Antibodies to Human MMP9

The amino acid sequences of the heavy chain and light chain of the mouse AB0041 antibody were altered at certain locations in the framework (i.e., non-CDR) portion of their

variable regions to generate proteins that are less immunogenic in humans. These amino acid sequence changes were shown in FIGS. 1 and 2. The cross-reactivity of one humanized antibody, referred to as AB0045, is shown in Table 2A above.

The humanized variant anti-MMP9 antibody, AB0045 (humanized, modified IgG4 (S241P); see Example 2, above) contained the humanized AB0041 heavy chain variant VH3 (having the sequence set forth in SEQ ID NO: 7

(QVQLQESGPGLVKPSSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLG  
WLGVIWTGGTTNYSALMSRFTISKDDSKNTVYVKMNSLKTEDTAI  
YYCARYYYGMDYWGQGLTVTVSS)

and the humanized AB0041 light chain variant VH4 (having the light chain sequence set forth in Vk4 (having the sequence set forth in SEQ ID NO: 12

(DIQMTQSPSSLSASVGDVITITCKASQDVRNTVAWYQQKPKAPKLL  
IYSSSYRNTGVPDRFSGSGSGTDFTLTITSSSLQAEDVAVYYCQOHYITP  
YTFGGGTKVEIK) ) .

The heavy chain of the AB0045 antibody has the sequence set forth in SEQ ID NO: 49

(MGWSLILLFLVAVATRVHSQVQLQESGPGLVKPSSETLSLTCTVSGFSL  
LSYGVHWVRQPPGKLEWLGVIWTGGTTNYSALMSRFTISKDDSKNTV  
YVKMNSLKTEDTAIYYCARYYYGMDYWGQGLTVTVSSASTKGFPSVFPLA  
PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSVHTFPAVLQSSG  
LYSLSSVTVTPSSSLGKTYITCNVDHKPSNTKVDKRVESKYGPPCPPCP  
APEFLGGPSVFLFPPPKDITLMI SRTPEVTCVVVDVSDPEVQFNWYV  
DGEVHNHAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL  
PSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI  
AVEWESNGQPENNYKTPPVLDSDGFFLYSRLTVDKSRWQEGNVFSCS  
VMHEALHNHYTQKLSLSLGLK

(signal sequence underlined; sequence of the constant region presented italics)); the light chain of the AB0045 antibody has the sequence set forth in SEQ ID NO: 50

(MRVPAQLLGLLLWLPGARCDIQMTQSPSSLSASVGDVITITCKAS  
QDVRNTVAWYQQKPKAPKLLIYSSSYRNTGVPDRFSGSGSGTDFTL  
TITSSSLQAEDVAVYYCQOHYITPYTFGGGTKVEIKRTVAAPSVFIFPP  
SDEQLKSGTASVVCCLNNFYPREAKVQWVKDNLQSGNSQESVTEQD  
SKDSTYSLSSITLTLKADYKHKVYACEVTHQGLSSPVTKSFNRGEC

(signal sequence underlined; sequence of the constant region presented italics)). The antibody contains 1312 amino acids in length, is composed of two heavy chains and two light chains, and has a theoretical pI of about 7.90, extinction coefficient of about 1.50 AU/cm at 280 nm for 1 g/L, a molecular weight of about 144 kDa, and density of about 1 g/mL in formulation buffer (50-100 mg/mL product concentration).

Further characterization of this antibody is described in Example 3, below.

### Example 3

#### Characterization of Variant MMP9 Antibody AB0045 and Comparison to AB0041 and AB0046

As described above, AB0045 and AB0041 antibodies are non-competitive inhibitors of MMP9. Thus, both antibodies inhibit MMP9 enzymatic activity independently of substrate concentration. The AB0045 antibody binds to the same MMP9 epitope as the AB0041 antibody with an affinity in the  $1 \times 10^{-12}$  molar range, as shown by direct binding and surface plasmon resonance (SPR) assays. Both antibodies are specific for MMP9, with no significant non-specific binding observed against other purified protein targets including purified domains and full length forms of MMP enzymes. Both AB0045 and AB0041 antibodies are cross-reactive with native and recombinant human and recombinant rat and cynomolgus monkey MMP9.

The in vitro binding affinity, inhibition characteristics, and the specificity of the antibodies of AB0045, AB0041 and AB0046 for MMP9 of human and non-human origin were determined using Enzyme-Linked Immunosorbent Assay (ELISA) and an MMP9 enzymatic assay. SPR analysis was also used to generate dissociation constants ( $K_d$ ) of AB0045 and AB0041.

In the ELISA assay, the  $K_d$  value of AB0045 and AB0041 antibodies for human, cynomolgus monkey, and rat MMP9 derived from ELISA were all found to be  $<400$  pM. The ELISA data illustrated that both AB0045 and AB0041 antibodies cross-react with MMP9 from all the relevant toxicology species tested. The AB0046 antibody was shown to be specific to mouse MMP9 and therefore could be used as a surrogate antibody in mouse efficacy models. The results showed that the  $K_d$  value of the AB0045 antibodies for human MMP9 was  $0.168 \pm 0.117$  nM and the  $K_d$  value of the AB0041 antibody was  $0.133 \pm 0.030$  nM. The results on the AB0046 antibodies showed it bound to mouse MMP9 with the  $K_d$  value of  $0.218 \pm 0.097$  nM. In the SPR analysis, the results showed that the  $K_d$  values of AB0045 and AB0041 antibodies for human MMP9 were 8.8 pM and 0.4 pM, respectively.

The enzymatic inhibitory activities of AB0045, AB0041, and AB0046 antibodies were evaluated in an assay assessing MMP9-mediated cleavage of a fluorogenic peptide substrate Mca-PLGL-Dpa-AR-NH<sub>2</sub>. All three antibodies inhibited MMP9 enzyme activity. The IC<sub>50</sub> values of AB0045 ( $0.691 \pm 0.097$  nM) and AB0041 ( $0.569 \pm 0.185$  nM) for human MMP9 were not statistically different. The IC<sub>50</sub> value for the AB0046 inhibition of mouse MMP9 was  $0.352 \pm 0.03$  nM. The value was not adjusted for the concentration of active enzyme that was generated during the preparation. Additional MMP9 enzymatic assay under steady-state conditions was used to determine IC<sub>50</sub> and mode of inhibition. In this assay, the IC<sub>50</sub> values of AB0045 ranged from 0.148 nM to 0.161 nM in a 20-fold range of substrate concentration, and in one example is 0.158 nM. The results showed that the MMP9 inhibitory activity of AB0045 was non-competitive.

TABLE 2B

Binding and Inhibitory Properties of AB0045, AB0041, and surrogate mouse antibody AB0046			
	AB0045	AB0041	AB0046
ELISA			
Human MMP9	0.168 ± 0.117 nM	0.133 ± 0.030 nM	>100 nM
Dissociation constant			
Cynomolgus monkey	0.082 ± 0.022 nM	0.145 ± 0.16 nM	>100 nM
MMP9 Dissociation constant			
Mouse MMP9	>100 nM	>100 nM	0.218 ± 0.097 nM
Dissociation constant			
Rat MMP9	0.311 ± 0.017 nM	0.332 ± 0.022 nM	>100 nM
Dissociation constant			
SPR			
Human MMP9	8.8 pM	0.4 pM	ND
Dissociation constant			
Activity Assay			
Human MMP9 IC <sub>50</sub>	0.691 ± 0.097 nM	0.569 ± 0.185 nM	>100 nM
Cynomolgus monkey	0.194 ± 0.048 nM*	0.189 ± 0.019 nM*	>100 nM
MMP9 IC <sub>50</sub>			
Rat MMP9 IC <sub>50</sub>	8.23 ± 1.24 nM*	2.78 ± 1.17 nM*	>100 nM
Mouse MMP9 IC <sub>50</sub>	>100 nM	>100 nM	0.352 ± 0.03 nM*

The results confirmed that AB0045 and AB0041 have equivalent binding and inhibitory properties and that

AB0046 can serve as a relevant mouse surrogate antibody, for example, in mouse models of human disease.

## SEQUENCE LISTING

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<160> NUMBER OF SEQ ID NOS: 50

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<222> LOCATION: (1)...(470)
<223> OTHER INFORMATION: AB0041 heavy chain
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)...(19)
<223> OTHER INFORMATION: signal peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (135)...(470)
<223> OTHER INFORMATION: IgG2b constant region

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Met Ala Val Leu Val Leu Phe Leu Cys Leu Val Ala Phe Pro Ser Cys
 1             5             10            15

Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala
 20            25            30

Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu
 35            40            45

Leu Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu
 50            55            60

Glu Trp Leu Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser
 65            70            75            80

Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asp Ser Lys Ser Gln
 85            90            95

Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr
 100           105           110

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Tyr Cys Ala Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
   115                               120           125

Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro
   130                               135           140

Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly
  145                               150           155           160

Cys Leu Val Lys Gly Tyr Phe Pro Glu Ser Val Thr Val Thr Trp Asn
   165                               170           175

Ser Gly Ser Leu Ser Ser Ser Val His Thr Phe Pro Ala Leu Leu Gln
   180                               185           190

Ser Gly Leu Tyr Thr Met Ser Ser Ser Val Thr Val Pro Ser Ser Thr
   195                               200           205

Trp Pro Ser Gln Thr Val Thr Cys Ser Val Ala His Pro Ala Ser Ser
   210                               215           220

Thr Thr Val Asp Lys Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr Ile
  225                               230           235           240

Asn Pro Cys Pro Pro Cys Lys Glu Cys His Lys Cys Pro Ala Pro Asn
   245                               250           255

Leu Glu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Asn Ile Lys Asp
   260                               265           270

Val Leu Met Ile Ser Leu Thr Pro Lys Val Thr Cys Val Val Val Asp
   275                               280           285

Val Ser Glu Asp Asp Pro Asp Val Arg Ile Ser Trp Phe Val Asn Asn
   290                               295           300

Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn
  305                               310           315           320

Ser Thr Ile Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp
   325                               330           335

Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro
   340                               345           350

Ser Pro Ile Glu Arg Thr Ile Ser Lys Ile Lys Gly Leu Val Arg Ala
   355                               360           365

Pro Gln Val Tyr Ile Leu Pro Pro Pro Ala Glu Gln Leu Ser Arg Lys
   370                               375           380

Asp Val Ser Leu Thr Cys Leu Val Val Gly Phe Asn Pro Gly Asp Ile
  385                               390           395           400

Ser Val Glu Trp Thr Ser Asn Gly His Thr Glu Glu Asn Tyr Lys Asp
   405                               410           415

Thr Ala Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Ile Tyr Ser Lys
   420                               425           430

Leu Asp Ile Lys Thr Ser Lys Trp Glu Lys Thr Asp Ser Phe Ser Cys
   435                               440           445

Asn Val Arg His Glu Gly Leu Lys Asn Tyr Tyr Leu Lys Lys Thr Ile
   450                               455           460

Ser Arg Ser Pro Gly Lys
  465                               470

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<210> SEQ ID NO 2
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<213> ORGANISM: Mus musculus
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<222> LOCATION: (1)...(234)
<223> OTHER INFORMATION: AB0041 light chain
<220> FEATURE:

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<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)...(20)
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<220> FEATURE:
<221> NAME/KEY: misc_feature
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<223> OTHER INFORMATION: kappa constant region

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Met Glu Ser Gln Ile Gln Val Phe Val Phe Val Phe Leu Trp Leu Ser
 1             5             10             15

Gly Val Asp Gly Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser
 20             25             30

Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp
 35             40             45

Val Arg Asn Thr Val Ala Trp Tyr Gln Gln Lys Thr Gly Gln Ser Pro
 50             55             60

Lys Leu Leu Ile Tyr Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp
 65             70             75             80

Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser
 85             90             95

Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln His Tyr
 100            105            110

Ile Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 115            120            125

Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
 130            135            140

Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
 145            150            155            160

Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
 165            170            175

Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
 180            185            190

Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
 195            200            205

His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
 210            215            220

Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 225            230

<210> SEQ ID NO 3
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<212> TYPE: PRT
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<222> LOCATION: (1)...(115)
<223> OTHER INFORMATION: variable region of the IgG2b heavy chain of
AB0041
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (26)...(35)
<223> OTHER INFORMATION: complementarity-determining region (CDR)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (50)...(65)
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Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln  
 1 5 10 15  
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr  
 20 25 30  
 Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu  
 35 40 45  
 Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met  
 50 55 60  
 Ser Arg Leu Ser Ile Ser Lys Asp Asp Ser Lys Ser Gln Val Phe Leu  
 65 70 75 80  
 Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala  
 85 90 95  
 Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr  
 100 105 110  
 Val Ser Ser  
 115

<210> SEQ ID NO 4  
 <211> LENGTH: 107  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus  
 <220> FEATURE:  
 <221> NAME/KEY: CHAIN  
 <222> LOCATION: (1)...(107)  
 <223> OTHER INFORMATION: variable region of the kappa light chain of  
 AB0041  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (24)...(34)  
 <223> OTHER INFORMATION: complementarity-determining region (CDR)  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (50)...(56)  
 <223> OTHER INFORMATION: complementarity-determining region (CDR)  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (89)...(97)  
 <223> OTHER INFORMATION: complementarity-determining region (CDR)  
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Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr  
 20 25 30  
 Val Ala Trp Tyr Gln Gln Lys Thr Gly Gln Ser Pro Lys Leu Leu Ile  
 35 40 45  
 Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Thr Gly  
 50 55 60  
 Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala  
 65 70 75 80  
 Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln His Tyr Ile Thr Pro Tyr  
 85 90 95  
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> SEQ ID NO 5  
 <211> LENGTH: 115  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
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<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(115)
<223> OTHER INFORMATION: VH1 heavy chain variant

<400> SEQUENCE: 5

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1           5           10           15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr
           20           25           30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
           35           40           45
Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met
           50           55           60
Ser Arg Leu Thr Ile Ser Lys Asp Asp Ser Lys Ser Thr Val Tyr Leu
           65           70           75           80
Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
           85           90           95
Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
           100           105           110

Val Ser Ser
           115

```

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<210> SEQ ID NO 6
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(115)
<223> OTHER INFORMATION: VH2 heavy chain variant

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<400> SEQUENCE: 6

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1           5           10           15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr
           20           25           30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
           35           40           45
Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met
           50           55           60
Ser Arg Leu Thr Ile Ser Lys Asp Asp Ser Lys Asn Thr Val Tyr Leu
           65           70           75           80
Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
           85           90           95
Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
           100           105           110

Val Ser Ser
           115

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<210> SEQ ID NO 7
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
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<222> LOCATION: (1)...(115)
<223> OTHER INFORMATION: VH3 heavy chain variant

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&lt;400&gt; SEQUENCE: 7

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu  
 1 5 10 15  
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr  
 20 25 30  
 Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu  
 35 40 45  
 Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met  
 50 55 60  
 Ser Arg Phe Thr Ile Ser Lys Asp Asp Ser Lys Asn Thr Val Tyr Leu  
 65 70 75 80  
 Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala  
 85 90 95  
 Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr  
 100 105 110  
 Val Ser Ser  
 115

&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 115

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: synthetic construct

&lt;220&gt; FEATURE:

&lt;221&gt; NAME/KEY: VARIANT

&lt;222&gt; LOCATION: (1)...(115)

&lt;223&gt; OTHER INFORMATION: VH4 heavy chain variant

&lt;400&gt; SEQUENCE: 8

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu  
 1 5 10 15  
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr  
 20 25 30  
 Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu  
 35 40 45  
 Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met  
 50 55 60  
 Ser Arg Phe Thr Ile Ser Lys Asp Asp Ser Lys Asn Thr Leu Tyr Leu  
 65 70 75 80  
 Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala  
 85 90 95  
 Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr  
 100 105 110  
 Val Ser Ser  
 115

&lt;210&gt; SEQ ID NO 9

&lt;211&gt; LENGTH: 107

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: synthetic construct

&lt;220&gt; FEATURE:

&lt;221&gt; NAME/KEY: VARIANT

&lt;222&gt; LOCATION: (1)...(107)

&lt;223&gt; OTHER INFORMATION: Vk1 light chain variant

&lt;400&gt; SEQUENCE: 9

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Asp Ile Val Met Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
 1           5           10           15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
           20           25           30
Val Ala Trp Tyr Gln Gln Lys Thr Gly Lys Ala Pro Lys Leu Leu Ile
           35           40           45
Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Thr Gly
           50           55           60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
65           70           75           80
Glu Asp Val Ala Val Tyr Phe Cys Gln Gln His Tyr Ile Thr Pro Tyr
           85           90           95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
           100           105

```

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<210> SEQ ID NO 10
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<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(107)
<223> OTHER INFORMATION: Vk2 light chain variant

<400> SEQUENCE: 10

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Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1           5           10           15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
           20           25           30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
           35           40           45
Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Thr Gly
           50           55           60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
65           70           75           80
Glu Asp Val Ala Val Tyr Phe Cys Gln Gln His Tyr Ile Thr Pro Tyr
           85           90           95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
           100           105

```

```

<210> SEQ ID NO 11
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(107)
<223> OTHER INFORMATION: Vk3 light chain variant

<400> SEQUENCE: 11

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 1           5           10           15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
           20           25           30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
           35           40           45

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Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Ser Gly
 50                               55                               60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
65                               70                               75                               80

Glu Asp Val Ala Val Tyr Phe Cys Gln Gln His Tyr Ile Thr Pro Tyr
                               85                               90                               95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
      100                               105

```

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<210> SEQ ID NO 12
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(107)
<223> OTHER INFORMATION: Vk4 light chain variant

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<400> SEQUENCE: 12

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1                               5                               10                               15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
      20                               25                               30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
      35                               40                               45

Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Ser Gly
 50                               55                               60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
65                               70                               75                               80

Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln His Tyr Ile Thr Pro Tyr
      85                               90                               95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
      100                               105

```

```

<210> SEQ ID NO 13
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(10)
<223> OTHER INFORMATION: complementarity-determining region (CDR1) of
heavy chain of anti-MMP9 antibody

```

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<400> SEQUENCE: 13

```

```

Gly Phe Ser Leu Leu Ser Tyr Gly Val His
 1                               5                               10

```

```

<210> SEQ ID NO 14
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(16)
<223> OTHER INFORMATION: complementarity-determining region (CDR2) of
heavy chain of anti-MMP9 antibody

```

```

<400> SEQUENCE: 14

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Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met Ser  
 1 5 10 15

<210> SEQ ID NO 15  
 <211> LENGTH: 7  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (1)...(7)  
 <223> OTHER INFORMATION: complementarity-determining region (CDR3) of  
 heavy chain of anti-MMP9 antibody

<400> SEQUENCE: 15

Tyr Tyr Tyr Gly Met Asp Tyr  
 1 5

<210> SEQ ID NO 16  
 <211> LENGTH: 11  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (1)...(11)  
 <223> OTHER INFORMATION: complementarity-determining region (CDR1) of  
 light chain of anti-MMP9 antibody

<400> SEQUENCE: 16

Lys Ala Ser Gln Asp Val Arg Asn Thr Val Ala  
 1 5 10

<210> SEQ ID NO 17  
 <211> LENGTH: 7  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (1)...(7)  
 <223> OTHER INFORMATION: complementarity-determining region (CDR2) of  
 light chain of anti-MMP9 antibody

<400> SEQUENCE: 17

Ser Ser Ser Tyr Arg Asn Thr  
 1 5

<210> SEQ ID NO 18  
 <211> LENGTH: 9  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (1)...(9)  
 <223> OTHER INFORMATION: complementarity-determining region (CDR3) of  
 light chain of anti-MMP9 antibody

<400> SEQUENCE: 18

Gln Gln His Tyr Ile Thr Pro Tyr Thr  
 1 5

<210> SEQ ID NO 19  
 <211> LENGTH: 345

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(345)
<223> OTHER INFORMATION: nucleotide sequence encoding VH1 heavy chain
    amino acid sequence

<400> SEQUENCE: 19

caggtgcagc tgcaggaatc eggccctggc ctggtaaac cctccgagac actgtccctg    60
acctgcaccg tgtccggctt ctccctgctg tcttacggcg tgcactgggt cgcacagcct    120
ccagggaagg gcttgaatg gctggcgctg atctggaccg gggcaccac caactacaac    180
tccgccctga tgtcccggtt gaccatctcc aaggacgact ccaagtccac cgtgtacctg    240
aagatgaact cctgaaaac cgaggacacc gccatctact actgcgccg gtactactac    300
ggcatggact actggggcca gggcacctcc gtgaccgtgt cctca                    345

<210> SEQ ID NO 20
<211> LENGTH: 345
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(345)
<223> OTHER INFORMATION: nucleotide sequence encoding VH2 heavy chain
    amino acid sequence

<400> SEQUENCE: 20

caggtgcagc tgcaggaatc eggccctggc ctggtaaac cctccgagac actgtccctg    60
acctgcaccg tgtccggctt ctccctgctg tcttacggcg tgcactgggt cgcacagcct    120
ccaggcaaag gcttgaatg gctggcgctg atctggaccg gggcaccac caactacaac    180
tccgccctga tgtcccggtt gaccatctcc aaggacgact ccaagaacac cgtgtacctg    240
aagatgaact cctgaaaac cgaggacacc gccatctact actgcgccg gtactactac    300
ggcatggact actggggcca gggcacctcg gtcaccgtgt cctca                    345

<210> SEQ ID NO 21
<211> LENGTH: 345
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(345)
<223> OTHER INFORMATION: nucleotide sequence encoding VH3 heavy chain
    amino acid sequence

<400> SEQUENCE: 21

caggtgcagc tgcaggaatc eggccctggc ctggtaaac cctccgagac actgtccctg    60
acctgcaccg tgtccggctt ctccctgctg tcttacggcg tgcactgggt cgcacagcct    120
ccaggcaaag gcttgaatg gctggcgctg atctggaccg gggcaccac caactacaac    180
tccgccctga tgtcccggtt caccatctcc aaggacgact ccaagaacac cgtgtacctg    240
aagatgaact cctgaaaac cgaggacacc gccatctact actgcgccg gtactactac    300
ggcatggact actggggcca gggcacctcg gtcaccgtgt cctca                    345

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<210> SEQ ID NO 22  
 <211> LENGTH: 345  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (1)...(345)  
 <223> OTHER INFORMATION: nucleotide sequence encoding VH4 heavy chain  
 amino acid sequence

<400> SEQUENCE: 22

```
caggtgcagc tgcaggaatc cggccctggc ctggtcaagc cctccgagac actgtccctg    60
acctgcaccg tgtccggctt ctccctgctg tctacggcg tgcactgggt cgcacagcct    120
ccaggcaaag gcttgaatg gctggcgctg atctggaccg gcggcaccac caactacaac    180
tccgccctga tgtccggctt caccatctcc aaggacgact ccaagaacac cctgtacctg    240
aagatgaact ccttgaaac cgaggacacc gccatctact actgcgccg gtactactac    300
ggcatggact actggggcca gggcacctgt gtcaccgtgt cctca                      345
```

<210> SEQ ID NO 23  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (1)...(321)  
 <223> OTHER INFORMATION: nucleotide sequence encoding Vk1 light chain  
 amino acid sequence

<400> SEQUENCE: 23

```
gacatcgtga tgaccagtc cccagcttc ctgtccgct cgtgggcca cagagtgacc    60
atcacatgca aggcctctca ggacgtgcg aacaccgtgg cctggatca gcagaaaacc    120
ggcaaggccc ccaagctgct gatctactcc tctctctacc ggaacaccgg cgtgcccgac    180
cggtttaccg gctctggctc cggcaccgac ttaccctga ccatcagctc cctgcaggcc    240
gaggacgtgg cctgtactt ctgccagcag cactacatca cccctacac cttcggcgga    300
ggcaccaagg tggaaataaa a                                          321
```

<210> SEQ ID NO 24  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (1)...(321)  
 <223> OTHER INFORMATION: nucleotide sequence encoding Vk2 light chain  
 amino acid sequence

<400> SEQUENCE: 24

```
gacatcgtga tgaccagtc cccctccagc ctgtccgct ctgtgggcca cagagtgacc    60
atcacatgca aggcctctca ggacgtgcg aacaccgtgg cctggatca gcagaagccc    120
ggcaaggccc ccaagctgct gatctactcc tctctctacc ggaacaccgg cgtgcccgac    180
cggtttaccg gctctggctc cggcaccgac ttaccctga ccatcagctc cctgcaggcc    240
gaggacgtgg cctgtactt ctgccagcag cactacatca cccctacac cttcggcgga    300
ggcaccaagg tggaaataaa a                                          321
```

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<210> SEQ ID NO 25
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(321)
<223> OTHER INFORMATION: nucleotide sequence encoding Vk3 light chain
        amino acid sequence

<400> SEQUENCE: 25

gacatccaga tgaccagtc cccctccagc ctgtccgcct ctgtgggcca cagagtgacc      60
atcacatgca aggcctccca ggacgtgagg aacaccgtgg cctggatca gcagaagccc      120
ggcaaggccc ccaagctgct gatctactcc tcctcctacc ggaacaccgg cgtgcccagc      180
cggttctctg gctctggaag eggcaccgac ttaccctga ccatcagctc cctgcaggcc      240
gaggacgtgg ccgtgtactt ctgccagcag cactacatca cccctacac cttcggcgga      300
ggcaccaagg tggaaataaa a                                             321

```

```

<210> SEQ ID NO 26
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(321)
<223> OTHER INFORMATION: nucleotide sequence encoding Vk4 light chain
        amino acid sequence

<400> SEQUENCE: 26

gacatccaga tgaccagtc cccctccagc ctgtccgcct ctgtgggcca cagagtgacc      60
atcacatgca aggcctctca ggacgtgagg aacaccgtgg cctggatca gcagaagccc      120
ggcaaggccc ccaagctgct gatctactcc tcctcctacc ggaacaccgg cgtgcccagc      180
cggttctctg gctctggaag eggcaccgac ttaccctga ccatcagctc cctgcaggcc      240
gaggacgtgg ccgtgtacta ctgccagcag cactacatca cccctacac cttcggcgga      300
ggcaccaagg tggaaataaa a                                             321

```

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<210> SEQ ID NO 27
<211> LENGTH: 707
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(707)
<223> OTHER INFORMATION: matrix metalloproteinase 9 (MMP9)
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)...(19)
<223> OTHER INFORMATION: signal peptide
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (38)...(98)
<223> OTHER INFORMATION: peptidoglycan binding domain
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (98)...(99)
<223> OTHER INFORMATION: propeptide cleavage site
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (112)...(445)

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<223> OTHER INFORMATION: Zn dependent metalloproteinase domain
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (223)...(271)
<223> OTHER INFORMATION: fibronectin type II domain (gelatin binding
domain)
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (281)...(329)
<223> OTHER INFORMATION: fibronectin type II domain (gelatin binding
domain)
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (340)...(388)
<223> OTHER INFORMATION: fibronectin type II domain (gelatin binding
domain)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (400)...(411)
<223> OTHER INFORMATION: Zn binding region
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (521)...(565)
<223> OTHER INFORMATION: hemopexin-like domain
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (567)...(608)
<223> OTHER INFORMATION: hemopexin-like domain
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (613)...(659)
<223> OTHER INFORMATION: hemopexin-like domain
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (661)...(704)
<223> OTHER INFORMATION: hemopexin-like domain

<400> SEQUENCE: 27

Met Ser Leu Trp Gln Pro Leu Val Leu Val Leu Leu Val Leu Gly Cys
 1             5             10            15

Cys Phe Ala Ala Pro Arg Gln Arg Gln Ser Thr Leu Val Leu Phe Pro
      20             25             30

Gly Asp Leu Arg Thr Asn Leu Thr Asp Arg Gln Leu Ala Glu Glu Tyr
      35             40             45

Leu Tyr Arg Tyr Gly Tyr Thr Arg Val Ala Glu Met Arg Gly Glu Ser
      50             55             60

Lys Ser Leu Gly Pro Ala Leu Leu Leu Leu Gln Lys Gln Leu Ser Leu
      65             70             75             80

Pro Glu Thr Gly Glu Leu Asp Ser Ala Thr Leu Lys Ala Met Arg Thr
      85             90             95

Pro Arg Cys Gly Val Pro Asp Leu Gly Arg Phe Gln Thr Phe Glu Gly
      100            105            110

Asp Leu Lys Trp His His His Asn Ile Thr Tyr Trp Ile Gln Asn Tyr
      115            120            125

Ser Glu Asp Leu Pro Arg Ala Val Ile Asp Asp Ala Phe Ala Arg Ala
      130            135            140

Phe Ala Leu Trp Ser Ala Val Thr Pro Leu Thr Phe Thr Arg Val Tyr
      145            150            155            160

Ser Arg Asp Ala Asp Ile Val Ile Gln Phe Gly Val Ala Glu His Gly
      165            170            175

Asp Gly Tyr Pro Phe Asp Gly Lys Asp Gly Leu Leu Ala His Ala Phe
      180            185            190

Pro Pro Gly Pro Gly Ile Gln Gly Asp Ala His Phe Asp Asp Asp Glu
      195            200            205

Leu Trp Ser Leu Gly Lys Gly Val Val Val Pro Thr Arg Phe Gly Asn

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210		215		220											
Ala	Asp	Gly	Ala	Ala	Cys	His	Phe	Pro	Phe	Ile	Phe	Glu	Gly	Arg	Ser
225					230					235					240
Tyr	Ser	Ala	Cys	Thr	Thr	Asp	Gly	Arg	Ser	Asp	Gly	Leu	Pro	Trp	Cys
			245						250					255	
Ser	Thr	Thr	Ala	Asn	Tyr	Asp	Thr	Asp	Asp	Arg	Phe	Gly	Phe	Cys	Pro
			260					265					270		
Ser	Glu	Arg	Leu	Tyr	Thr	Arg	Asp	Gly	Asn	Ala	Asp	Gly	Lys	Pro	Cys
		275					280					285			
Gln	Phe	Pro	Phe	Ile	Phe	Gln	Gly	Gln	Ser	Tyr	Ser	Ala	Cys	Thr	Thr
		290				295						300			
Asp	Gly	Arg	Ser	Asp	Gly	Tyr	Arg	Trp	Cys	Ala	Thr	Thr	Ala	Asn	Tyr
305					310					315					320
Asp	Arg	Asp	Lys	Leu	Phe	Gly	Phe	Cys	Pro	Thr	Arg	Ala	Asp	Ser	Thr
			325						330					335	
Val	Met	Gly	Gly	Asn	Ser	Ala	Gly	Glu	Leu	Cys	Val	Phe	Pro	Phe	Thr
			340					345					350		
Phe	Leu	Gly	Lys	Glu	Tyr	Ser	Thr	Cys	Thr	Ser	Glu	Gly	Arg	Gly	Asp
		355					360					365			
Gly	Arg	Leu	Trp	Cys	Ala	Thr	Thr	Ser	Asn	Phe	Asp	Ser	Asp	Lys	Lys
		370				375					380				
Trp	Gly	Phe	Cys	Pro	Asp	Gln	Gly	Tyr	Ser	Leu	Phe	Leu	Val	Ala	Ala
385					390					395					400
His	Glu	Phe	Gly	His	Ala	Leu	Gly	Leu	Asp	His	Ser	Ser	Val	Pro	Glu
			405						410					415	
Ala	Leu	Met	Tyr	Pro	Met	Tyr	Arg	Phe	Thr	Glu	Gly	Pro	Pro	Leu	His
			420					425					430		
Lys	Asp	Asp	Val	Asn	Gly	Ile	Arg	His	Leu	Tyr	Gly	Pro	Arg	Pro	Glu
		435					440					445			
Pro	Glu	Pro	Arg	Pro	Pro	Thr	Thr	Thr	Thr	Pro	Gln	Pro	Thr	Ala	Pro
		450				455					460				
Pro	Thr	Val	Cys	Pro	Thr	Gly	Pro	Pro	Thr	Val	His	Pro	Ser	Glu	Arg
465					470					475					480
Pro	Thr	Ala	Gly	Pro	Thr	Gly	Pro	Pro	Ser	Ala	Gly	Pro	Thr	Gly	Pro
			485						490					495	
Pro	Thr	Ala	Gly	Pro	Ser	Thr	Ala	Thr	Thr	Val	Pro	Leu	Ser	Pro	Val
			500					505					510		
Asp	Asp	Ala	Cys	Asn	Val	Asn	Ile	Phe	Asp	Ala	Ile	Ala	Glu	Ile	Gly
		515					520					525			
Asn	Gln	Leu	Tyr	Leu	Phe	Lys	Asp	Gly	Lys	Tyr	Trp	Arg	Phe	Ser	Glu
530						535					540				
Gly	Arg	Gly	Ser	Arg	Pro	Gln	Gly	Pro	Phe	Leu	Ile	Ala	Asp	Lys	Trp
545					550					555					560
Pro	Ala	Leu	Pro	Arg	Lys	Leu	Asp	Ser	Val	Phe	Glu	Glu	Pro	Leu	Ser
				565					570					575	
Lys	Lys	Leu	Phe	Phe	Phe	Ser	Gly	Arg	Gln	Val	Trp	Val	Tyr	Thr	Gly
			580					585					590		
Ala	Ser	Val	Leu	Gly	Pro	Arg	Arg	Leu	Asp	Lys	Leu	Gly	Leu	Gly	Ala
		595						600				605			
Asp	Val	Ala	Gln	Val	Thr	Gly	Ala	Leu	Arg	Ser	Gly	Arg	Gly	Lys	Met
610						615					620				
Leu	Leu	Phe	Ser	Gly	Arg	Arg	Leu	Trp	Arg	Phe	Asp	Val	Lys	Ala	Gln
625					630					635					640

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Met Val Asp Pro Arg Ser Ala Ser Glu Val Asp Arg Met Phe Pro Gly  
645 650 655

Val Pro Leu Asp Thr His Asp Val Phe Gln Tyr Arg Glu Lys Ala Tyr  
660 665 670

Phe Cys Gln Asp Arg Phe Tyr Trp Arg Val Ser Ser Arg Ser Glu Leu  
675 680 685

Asn Gln Val Asp Gln Val Gly Tyr Val Thr Tyr Asp Ile Leu Gln Cys  
690 695 700

Pro Glu Asp  
705

<210> SEQ ID NO 28  
<211> LENGTH: 688  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (1)...(688)  
<223> OTHER INFORMATION: mature full-length matrix metalloproteinase 9  
(MMP9)

<400> SEQUENCE: 28

Ala Pro Arg Gln Arg Gln Ser Thr Leu Val Leu Phe Pro Gly Asp Leu  
1 5 10 15

Arg Thr Asn Leu Thr Asp Arg Gln Leu Ala Glu Glu Tyr Leu Tyr Arg  
20 25 30

Tyr Gly Tyr Thr Arg Val Ala Glu Met Arg Gly Glu Ser Lys Ser Leu  
35 40 45

Gly Pro Ala Leu Leu Leu Leu Gln Lys Gln Leu Ser Leu Pro Glu Thr  
50 55 60

Gly Glu Leu Asp Ser Ala Thr Leu Lys Ala Met Arg Thr Pro Arg Cys  
65 70 75 80

Gly Val Pro Asp Leu Gly Arg Phe Gln Thr Phe Glu Gly Asp Leu Lys  
85 90 95

Trp His His His Asn Ile Thr Tyr Trp Ile Gln Asn Tyr Ser Glu Asp  
100 105 110

Leu Pro Arg Ala Val Ile Asp Asp Ala Phe Ala Arg Ala Phe Ala Leu  
115 120 125

Trp Ser Ala Val Thr Pro Leu Thr Phe Thr Arg Val Tyr Ser Arg Asp  
130 135 140

Ala Asp Ile Val Ile Gln Phe Gly Val Ala Glu His Gly Asp Gly Tyr  
145 150 155 160

Pro Phe Asp Gly Lys Asp Gly Leu Leu Ala His Ala Phe Pro Pro Gly  
165 170 175

Pro Gly Ile Gln Gly Asp Ala His Phe Asp Asp Asp Glu Leu Trp Ser  
180 185 190

Leu Gly Lys Gly Val Val Val Pro Thr Arg Phe Gly Asn Ala Asp Gly  
195 200 205

Ala Ala Cys His Phe Pro Phe Ile Phe Glu Gly Arg Ser Tyr Ser Ala  
210 215 220

Cys Thr Thr Asp Gly Arg Ser Asp Gly Leu Pro Trp Cys Ser Thr Thr  
225 230 235 240

Ala Asn Tyr Asp Thr Asp Asp Arg Phe Gly Phe Cys Pro Ser Glu Arg  
245 250 255

Leu Tyr Thr Arg Asp Gly Asn Ala Asp Gly Lys Pro Cys Gln Phe Pro  
260 265 270

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Phe Ile Phe Gln Gly Gln Ser Tyr Ser Ala Cys Thr Thr Asp Gly Arg  
 275 280 285  
 Ser Asp Gly Tyr Arg Trp Cys Ala Thr Thr Ala Asn Tyr Asp Arg Asp  
 290 295 300  
 Lys Leu Phe Gly Phe Cys Pro Thr Arg Ala Asp Ser Thr Val Met Gly  
 305 310 315 320  
 Gly Asn Ser Ala Gly Glu Leu Cys Val Phe Pro Phe Thr Phe Leu Gly  
 325 330 335  
 Lys Glu Tyr Ser Thr Cys Thr Ser Glu Gly Arg Gly Asp Gly Arg Leu  
 340 345 350  
 Trp Cys Ala Thr Thr Ser Asn Phe Asp Ser Asp Lys Lys Trp Gly Phe  
 355 360 365  
 Cys Pro Asp Gln Gly Tyr Ser Leu Phe Leu Val Ala Ala His Glu Phe  
 370 375 380  
 Gly His Ala Leu Gly Leu Asp His Ser Ser Val Pro Glu Ala Leu Met  
 385 390 395 400  
 Tyr Pro Met Tyr Arg Phe Thr Glu Gly Pro Pro Leu His Lys Asp Asp  
 405 410 415  
 Val Asn Gly Ile Arg His Leu Tyr Gly Pro Arg Pro Glu Pro Glu Pro  
 420 425 430  
 Arg Pro Pro Thr Thr Thr Thr Pro Gln Pro Thr Ala Pro Pro Thr Val  
 435 440 445  
 Cys Pro Thr Gly Pro Pro Thr Val His Pro Ser Glu Arg Pro Thr Ala  
 450 455 460  
 Gly Pro Thr Gly Pro Pro Ser Ala Gly Pro Thr Gly Pro Pro Thr Ala  
 465 470 475 480  
 Gly Pro Ser Thr Ala Thr Thr Val Pro Leu Ser Pro Val Asp Asp Ala  
 485 490 495  
 Cys Asn Val Asn Ile Phe Asp Ala Ile Ala Glu Ile Gly Asn Gln Leu  
 500 505 510  
 Tyr Leu Phe Lys Asp Gly Lys Tyr Trp Arg Phe Ser Glu Gly Arg Gly  
 515 520 525  
 Ser Arg Pro Gln Gly Pro Phe Leu Ile Ala Asp Lys Trp Pro Ala Leu  
 530 535 540  
 Pro Arg Lys Leu Asp Ser Val Phe Glu Glu Pro Leu Ser Lys Lys Leu  
 545 550 555 560  
 Phe Phe Phe Ser Gly Arg Gln Val Trp Val Tyr Thr Gly Ala Ser Val  
 565 570 575  
 Leu Gly Pro Arg Arg Leu Asp Lys Leu Gly Leu Gly Ala Asp Val Ala  
 580 585 590  
 Gln Val Thr Gly Ala Leu Arg Ser Gly Arg Gly Lys Met Leu Leu Phe  
 595 600 605  
 Ser Gly Arg Arg Leu Trp Arg Phe Asp Val Lys Ala Gln Met Val Asp  
 610 615 620  
 Pro Arg Ser Ala Ser Glu Val Asp Arg Met Phe Pro Gly Val Pro Leu  
 625 630 635 640  
 Asp Thr His Asp Val Phe Gln Tyr Arg Glu Lys Ala Tyr Phe Cys Gln  
 645 650 655  
 Asp Arg Phe Tyr Trp Arg Val Ser Ser Arg Ser Glu Leu Asn Gln Val  
 660 665 670  
 Asp Gln Val Gly Tyr Val Thr Tyr Asp Ile Leu Gln Cys Pro Glu Asp  
 675 680 685

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<210> SEQ ID NO 29  
 <211> LENGTH: 19  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

Met Ser Leu Trp Gln Pro Leu Val Leu Val Leu Leu Val Leu Gly Cys  
 1                   5                   10                   15  
 Cys Phe Ala

<210> SEQ ID NO 30  
 <211> LENGTH: 470  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus  
 <220> FEATURE:  
 <221> NAME/KEY: CHAIN  
 <222> LOCATION: (1)...(470)  
 <223> OTHER INFORMATION: M4 heavy chain (IgG2b)

<400> SEQUENCE: 30

Met Ala Val Leu Val Leu Phe Leu Cys Leu Val Ala Phe Pro Ser Cys  
 1                   5                   10                   15  
 Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala  
 20                   25                   30  
 Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu  
 35                   40                   45  
 Leu Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu  
 50                   55                   60  
 Glu Trp Leu Gly Val Ile Trp Thr Gly Gly Ser Thr Asn Tyr Asn Ser  
 65                   70                   75                   80  
 Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asp Ser Lys Ser Gln  
 85                   90                   95  
 Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr  
 100                   105                   110  
 Tyr Cys Ala Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr  
 115                   120                   125  
 Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro  
 130                   135                   140  
 Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly  
 145                   150                   155                   160  
 Cys Leu Val Lys Gly Tyr Phe Pro Glu Ser Val Thr Val Thr Trp Asn  
 165                   170                   175  
 Ser Gly Ser Leu Ser Ser Ser Val His Thr Phe Pro Ala Leu Leu Gln  
 180                   185                   190  
 Ser Gly Leu Tyr Thr Met Ser Ser Ser Val Thr Val Pro Ser Ser Thr  
 195                   200                   205  
 Trp Pro Ser Gln Thr Val Thr Cys Ser Val Ala His Pro Ala Ser Ser  
 210                   215                   220  
 Thr Thr Val Asp Lys Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr Ile  
 225                   230                   235                   240  
 Asn Pro Cys Pro Pro Cys Lys Glu Cys His Lys Cys Pro Ala Pro Asn  
 245                   250                   255  
 Leu Glu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Asn Ile Lys Asp  
 260                   265                   270  
 Val Leu Met Ile Ser Leu Thr Pro Lys Val Thr Cys Val Val Val Asp  
 275                   280                   285

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Val Ser Glu Asp Asp Pro Asp Val Arg Ile Ser Trp Phe Val Asn Asn  
290 295 300

Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn  
305 310 315 320

Ser Thr Ile Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp  
325 330 335

Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro  
340 345 350

Ser Pro Ile Glu Arg Thr Ile Ser Lys Ile Lys Gly Leu Val Arg Ala  
355 360 365

Pro Gln Val Tyr Ile Leu Pro Pro Pro Ala Glu Gln Leu Ser Arg Lys  
370 375 380

Asp Val Ser Leu Thr Cys Leu Val Val Gly Phe Asn Pro Gly Asp Ile  
385 390 395 400

Ser Val Glu Trp Thr Ser Asn Gly His Thr Glu Glu Asn Tyr Lys Asp  
405 410 415

Thr Ala Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Ile Tyr Ser Lys  
420 425 430

Leu Asp Ile Lys Thr Ser Lys Trp Glu Lys Thr Asp Ser Phe Ser Cys  
435 440 445

Asn Val Arg His Glu Gly Leu Lys Asn Tyr Tyr Leu Lys Lys Thr Ile  
450 455 460

Ser Arg Ser Pro Gly Lys  
465 470

<210> SEQ ID NO 31  
 <211> LENGTH: 234  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus  
 <220> FEATURE:  
 <221> NAME/KEY: CHAIN  
 <222> LOCATION: (1)...(234)  
 <223> OTHER INFORMATION: M4 light chain (kappa)

<400> SEQUENCE: 31

Met Glu Ser Gln Ile Gln Val Phe Val Phe Val Phe Leu Trp Leu Ser  
1 5 10 15

Gly Val Asp Gly Asp Ile Val Met Thr Gln Ser His Lys Phe Met Phe  
20 25 30

Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp  
35 40 45

Val Arg Asn Thr Val Ala Trp Tyr Gln Gln Lys Thr Gly Gln Ser Pro  
50 55 60

Lys Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Asn Thr Gly Val Pro Asp  
65 70 75 80

Arg Phe Thr Gly Ser Ile Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser  
85 90 95

Ser Val Gln Ala Glu Asp Leu Ala Leu Tyr Tyr Cys Gln Gln His Tyr  
100 105 110

Ser Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Val Lys Arg  
115 120 125

Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln  
130 135 140

Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr  
145 150 155 160

Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln

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165	170	175	
Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr	180	185	190
Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg	195	200	205
His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro	210	215	220
Ile Val Lys Ser Phe Asn Arg Asn Glu Cys	225	230	

<210> SEQ ID NO 32  
 <211> LENGTH: 115  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 32

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln	1	5	10	15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr	20	25	30	
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu	35	40	45	
Gly Val Ile Trp Thr Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met	50	55	60	
Ser Arg Leu Ser Ile Ser Lys Asp Asp Ser Lys Ser Gln Val Phe Leu	65	70	75	80
Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala	85	90	95	
Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr	100	105	110	
Val Ser Ser	115			

<210> SEQ ID NO 33  
 <211> LENGTH: 107  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 33

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Phe Thr Ser Val Gly	1	5	10	15
Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr	20	25	30	
Val Ala Trp Tyr Gln Gln Lys Thr Gly Gln Ser Pro Lys Leu Leu Ile	35	40	45	
Tyr Ser Ala Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Thr Gly	50	55	60	
Ser Ile Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala	65	70	75	80
Glu Asp Leu Ala Leu Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Tyr	85	90	95	
Thr Phe Gly Gly Gly Thr Lys Leu Glu Val Lys	100	105		

<210> SEQ ID NO 34  
 <211> LENGTH: 10  
 <212> TYPE: PRT

-continued

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<213> ORGANISM: Mus musculus

<400> SEQUENCE: 34

Gly Phe Ser Leu Leu Ser Tyr Gly Val His  
 1                    5                    10

<210> SEQ ID NO 35

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 35

Val Ile Trp Thr Gly Gly Ser Thr Asn Tyr  
 1                    5                    10

<210> SEQ ID NO 36

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 36

Tyr Tyr Tyr Ala Met Asp Tyr  
 1                    5

<210> SEQ ID NO 37

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 37

Lys Ala Ser Gln Asp Val Arg Asn Thr Val Ala  
 1                    5                    10

<210> SEQ ID NO 38

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 38

Ser Ala Ser Tyr Arg Asn Thr  
 1                    5

<210> SEQ ID NO 39

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 39

Gln Gln His Tyr Ser Thr Pro Tyr Thr  
 1                    5

<210> SEQ ID NO 40

<211> LENGTH: 229

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<220> FEATURE:

<221> NAME/KEY: CHAIN

<222> LOCATION: (1) ... (229)

<223> OTHER INFORMATION: M12 kappa chain

<400> SEQUENCE: 40

Gln Val Phe Val Tyr Met Leu Leu Trp Leu Ser Gly Val Asp Gly Asp  
 1                    5                    10                    15

Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly Asp  
 20                    25                    30



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<210> SEQ ID NO 43  
 <211> LENGTH: 7  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 43

Ser Ala Ser Tyr Arg Phe Ser  
 1 5

<210> SEQ ID NO 44  
 <211> LENGTH: 9  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 44

Gln Gln Tyr Asn Ser Tyr Pro Tyr Thr  
 1 5

<210> SEQ ID NO 45  
 <211> LENGTH: 233  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus  
 <220> FEATURE:  
 <221> NAME/KEY: CHAIN  
 <222> LOCATION: (1)...(233)  
 <223> OTHER INFORMATION: AB0046 kappa light chain

<400> SEQUENCE: 45

Met Ser Ser Ala Gln Phe Leu Gly Leu Leu Leu Leu Cys Phe Gln Gly  
 1 5 10 15  
 Thr Arg Cys Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala  
 20 25 30  
 Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Ser Ala Ser Gln Gly Ile  
 35 40 45  
 Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Phe Lys  
 50 55 60  
 Leu Leu Ile Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg  
 65 70 75 80  
 Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn  
 85 90 95  
 Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Gly Trp  
 100 105 110  
 Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala  
 115 120 125  
 Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu  
 130 135 140  
 Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro  
 145 150 155 160  
 Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn  
 165 170 175  
 Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr  
 180 185 190  
 Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His  
 195 200 205  
 Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile  
 210 215 220  
 Val Lys Ser Phe Asn Arg Asn Glu Cys  
 225 230

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<210> SEQ ID NO 46  
 <211> LENGTH: 460  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus  
 <220> FEATURE:  
 <221> NAME/KEY: CHAIN  
 <222> LOCATION: (1)...(460)  
 <223> OTHER INFORMATION: AB0046 IgG1 heavy chain  
 <400> SEQUENCE: 46

Met Gly Trp Ser Ser Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15  
 Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ser Val Leu Val Arg  
 20 25 30  
 Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Tyr Thr Phe  
 35 40 45  
 Thr Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu  
 50 55 60  
 Glu Trp Ile Gly Glu Ile Tyr Pro Ile Ser Gly Arg Thr Asn Tyr Asn  
 65 70 75 80  
 Glu Lys Phe Lys Val Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser  
 85 90 95  
 Thr Ala Tyr Met Asp Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val  
 100 105 110  
 Tyr Tyr Cys Ala Arg Ser Arg Ala Asn Trp Asp Asp Tyr Trp Gly Gln  
 115 120 125  
 Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val  
 130 135 140  
 Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr  
 145 150 155 160  
 Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr  
 165 170 175  
 Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val  
 180 185 190  
 Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser  
 195 200 205  
 Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala  
 210 215 220  
 Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys  
 225 230 235 240  
 Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe  
 245 250 255  
 Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val  
 260 265 270  
 Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe  
 275 280 285  
 Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro  
 290 295 300  
 Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro  
 305 310 315 320  
 Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val  
 325 330 335  
 Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr  
 340 345 350

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Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys  
 355 360 365

Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp  
 370 375 380

Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro  
 385 390 395 400

Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser  
 405 410 415

Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala  
 420 425 430

Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His  
 435 440 445

His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys  
 450 455 460

<210> SEQ ID NO 47  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 47

Gln Val Gln Leu Gln Gln Pro Gly Ser Val Leu Val Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Tyr Thr Phe Thr Ser Tyr  
 20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Glu Ile Tyr Pro Ile Ser Gly Arg Thr Asn Tyr Asn Glu Lys Phe  
 50 55 60

Lys Val Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Asp Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Ser Arg Ala Asn Trp Asp Asp Tyr Trp Gly Gln Gly Thr Thr  
 100 105 110

Leu Thr Val Ser Ser  
 115

<210> SEQ ID NO 48  
 <211> LENGTH: 107  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 48

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly  
 1 5 10 15

Asp Arg Val Thr Ile Ser Cys Ser Ala Ser Gln Gly Ile Ser Asn Tyr  
 20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Phe Lys Leu Leu Ile  
 35 40 45

Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Pro  
 65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Gly Trp Leu Pro Arg  
 85 90 95

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Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> SEQ ID NO 49  
 <211> LENGTH: 461  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: CHAIN  
 <222> LOCATION: (1)...(461)  
 <223> OTHER INFORMATION: AB0045 heavy chain

<400> SEQUENCE: 49

Met Gly Trp Ser Leu Ile Leu Leu Phe Leu Val Ala Val Ala Thr Arg  
 1 5 10 15

Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys  
 20 25 30

Pro Ser Glu Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu  
 35 40 45

Leu Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu  
 50 55 60

Glu Trp Leu Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser  
 65 70 75 80

Ala Leu Met Ser Arg Phe Thr Ile Ser Lys Asp Asp Ser Lys Asn Thr  
 85 90 95

Val Tyr Leu Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr  
 100 105 110

Tyr Cys Ala Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr  
 115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
 130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly  
 145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
 165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
 180 185 190

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
 195 200 205

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser  
 210 215 220

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys  
 225 230 235 240

Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu  
 245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu  
 260 265 270

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln  
 275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys  
 290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu  
 305 310 315 320

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys  
 325 330 335

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Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys  
 340 345 350

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
 355 360 365

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys  
 370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
 385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly  
 405 410 415

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln  
 420 425 430

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn  
 435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
 450 455 460

<210> SEQ ID NO 50  
 <211> LENGTH: 234  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct  
 <220> FEATURE:  
 <221> NAME/KEY: CHAIN  
 <222> LOCATION: (1)...(234)  
 <223> OTHER INFORMATION: AB0045 light chain

<400> SEQUENCE: 50

Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Trp Leu Pro  
 1 5 10 15

Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser  
 20 25 30

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp  
 35 40 45

Val Arg Asn Thr Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro  
 50 55 60

Lys Leu Leu Ile Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp  
 65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 85 90 95

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln His Tyr  
 100 105 110

Ile Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg  
 115 120 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
 130 135 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
 145 150 155 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
 165 170 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
 180 185 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
 195 200 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro

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210	215	220
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys		
225	230	

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What is claimed is:

1. A method of detecting human MMP9 in a sample, 10  
comprising:
  - a) contacting the sample with an antibody or antigen-binding fragment thereof comprising a heavy chain variable ( $V_H$ ) region and a light chain variable ( $V_L$ ) region wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 34, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 35, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 36; wherein the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 37, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and, 15
  - b) detecting binding of the antibody or antigen-binding fragment thereof to human MMP9 in the sample, thereby detecting the presence or absence of the human MMP9. 20
2. The method of claim 1, further comprising the step of comparing the amount of binding detected in the sample to an amount of binding to a control sample.
3. The method of claim 1, wherein the sample is a tissue or fluid sample from a subject.
4. The method of claim 3, wherein the fluid sample is a blood, plasma, serum, whole blood, saliva, urine, or semen sample.
5. The method of claim 3, wherein the tissue sample is a formalin-fixed sample or a frozen sample.
6. The method of claim 1, wherein the antibody or antigen-binding fragment thereof is labeled with a detectable moiety.
7. The method of claim 6, wherein the label moiety is radioisotope, a fluorescent or chemiluminescent compound, or an enzyme.
8. The method of claim 1, wherein the heavy chain variable ( $V_H$ ) region comprises the amino acid sequence set forth in SEQ ID NO: 32.
9. The method of claim 1, wherein the light chain variable ( $V_L$ ) region comprises the amino acid sequence set forth in SEQ ID NO: 33.

\* \* \* \* \*

专利名称(译)	使用抗体检测人基质金属蛋白酶9的方法		
公开(公告)号	<a href="#">US9550836</a>	公开(公告)日	2017-01-24
申请号	US14/382301	申请日	2012-02-29
[标]申请(专利权)人(译)	SMITH VICTORIA 麦考利SCOTT		
申请(专利权)人(译)	SMITH, VICTORIA 麦考利, SCOTT		
当前申请(专利权)人(译)	GILEAD生物制剂, INC.		
[标]发明人	SMITH VICTORIA MCCAULEY SCOTT		
发明人	SMITH, VICTORIA MCCAULEY, SCOTT		
IPC分类号	G01N33/53 G01N33/573 C12N9/64 C07K16/40 A61K39/00		
CPC分类号	C07K16/40 C12N9/6491 C12Y304/24035 G01N33/573 A61K2039/505 A61K2039/54 A61K2039/545 C07K2317/34 C07K2317/56 C07K2317/565 C07K2317/76 C07K2317/92 G01N2333/96494 A61P43/00 C07K2317/24		
其他公开文献	US20150140580A1		
外部链接	<a href="#">Espacenet</a> <a href="#">USPTO</a>		

摘要(译)

本公开提供了涉及结合蛋白质例如结合于基质金属蛋白酶-9 (MMP9) 蛋白 (MMP9也称为明胶酶-B) 的结合蛋白, 例如抗体及其抗原结合片段的组合物和使用方法, 例如其中结合蛋白包含免疫球蛋白 (Ig) 重链 (或其功能片段) 和Ig轻链 (或其功能片段)。

FIGURE 1

Anti-MMP9 humanized heavy chains

```

AB041  QVQLRISGQGLVAPRSQISL TCTSGSRLI SYGSHWGP PGCEMLGY
VH1  QVQLRISGQ LVPKRSLSL TCTSGSRLI SYGSHWGP PGCEMLGY
VH2  QVQLRISGQ LVPKRSLSL TCTSGSRLI SYGSHWGP PGCEMLGY
VH3  QVQLRISGQ LVPKRSLSL TCTSGSRLI SYGSHWGP PGCEMLGY
VH4  QVQLRISGQ LVPKRSLSL TCTSGSRLI SYGSHWGP PGCEMLGY

AB041  IWGGTAA SALARSLIS FDSKRFEL KMSLSDT AYICRHYI
VH1  IWGGTAA SALARSLIS FDSKRFEL KMSLSDT AYICRHYI
VH2  IWGGTAA SALARSLIS KDSKRFEL KMSLSDT AYICRHYI
VH4  IWGGTAA SALARSLIS KDSKRFEL KMSLSDT AYICRHYI

AB041  GSDWQNTS VTSS (SEQ ID NO:3)
VH1  GSDWQNTS VTSS (SEQ ID NO:5)
VH2  GSDWQNTL VTSS (SEQ ID NO:6)
VH3  GSDWQNTL VTSS (SEQ ID NO:7)
VH4  GSDWQNTL VTSS (SEQ ID NO:8)
    
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