Supplementary Fig. 1. Insertion of HA and Myc tags did not affect MMP-9 secretion. Effect of domain swapping, TIMP-1 co-precipitation, and specificity of peptides on MMP-9 induced cell migration.

A) Expression of wild type, HA- and Myc-tagged MMP-9 in COS-1 cells transfected with the cDNAs. The conditioned medium from COS-1 cells transfected with cDNAs was examined by gelatin zymography.

B) Expression of MMP-9 and HA- or Myc- tagged MMP-9 in COS-1 cells examined by Western blotting. The conditioned medium from transfected COS-1 cells was precipitated followed by Western blotting.

C) Insertion of HA or Myc tag in MMP-9 cDNA does not affect antibodies to precipitate MMP-9 chimera. The conditioned medium and cell lysate from COS-1 cells transfected with cDNAs as indicated were immunoprecipitated with anti-HA antibody (upper panel) and anti-Myc antibody (lower panel) followed by Western blotting using the same antibodies.

D) Mutant MMP-9, generated by swapping the PEX domain with that of MMP-2 (MMP-9/PEX_{MMP2}), expresses comparable levels of proteins with wild type MMP-9, as examined by Western blotting (upper panel) and by gelatin zymography (lower panel).

E) TIMP-1 co-precipitated with MMP-9 in both the lysate and the conditioned medium of transfected COS-1 cells examined by a co-immunoprecipitation assay. The conditioned medium and cell lysate were immunoprecipitated by anti-TIMP-1 antibody followed by anti-MMP-9 antibody for Western blotting. MMP-9 and a/b tubulin were used as controls for protein expression in the conditioned medium and cell lysate, respectively.

F) Specificity of IVS4 peptide on inhibition of MMP-9-induced cell migration. COS-1 cells transfected with MMP-9 or MT1-MMP cDNAs were pre-treated with IVS4 peptide and IVS4 scrambled peptide for 30 min followed by transwell migration assay. Each data point was performed in triplicate and the experiment was repeated three times (*$P < 0.05$).

Supplementary Fig. 2. Silencing of CD44 in COS-1 cells using a shRNA approach.

A) The outermost of blade I of the MMP-9 PEX domain interacts with CD44. COS-1 cells co-transfected with cDNAs as indicated were immunoprecipitated with anti-CD44 antibody followed by Western blotting using MMP-9 antibody. An aliquot of the conditioned medium was examined by Western blotting for MMP-9 to monitor expression level of MMP-9 in each transfection.

B) Silencing of CD44 in COS-1 cells by a shRNA approach. Expression of CD44 in COS-1 cells stably infected with retrovirus containing shRNA luciferase (a-c) or CD44 shRNA (d-f) was analyzed by immunofluorescence staining using anti-CD44 antibodies. Nuclei were counterstained with DAPI (blue).

C) Expression of CD44 in silenced COS-1 cells examined by flow cytometry. COS-1 cells expressing shRNAs for luciferase or CD44 were examined by flow cytometry using anti-CD44 antibody.

Supplementary Fig. 3. CD44 activates EGFR to regulate MMP-9-enhanced cell migration.

A) COS-1 cells transfected with vector or MMP-9 cDNAs were pre-treated with 8 different inhibitors targeting distinct receptor tyrosine kinase pathways (AG1024, PD173074, PHA665752, Genistein, PP2, AG490, AG1478 or AG1296) for 30 min followed by a transwell migration assay. Each data point was performed in triplicate and the experiment was repeated three times (*$P < 0.05$).

B) Densitometric analysis of the ratio of phosphorylation of pERK, pAKT, pFAK and pEGFR to corresponding pan antibodies.

Supplementary Fig. 4. Ratio of homodimer versus monomer of MMP-9 in COS-1 cells.

A) Densitometric analysis of the percent homodimer and monomer in the conditioned media and cell lysates of MMP-9 transfected COS-1 cells examined by gelatin zymography (upper left

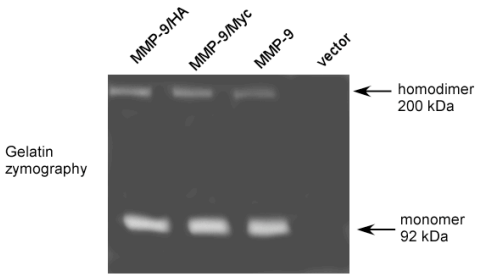
panel). MMP-9 transfected COS-1 cell lysates were further separated by ultracentrifugation showing membrane-bound and cytosolic MMP-9 (upper right panel). Densitometric analysis of monomer vs. dimer in each sample is shown in the chart.

B) Densitometric analysis of the percent of dimer and monomer in the conditioned media and cell lysates of MMP-9 in HT-1080 cells (left) and MDA-MB-435 (right) examined by gelatin zymography. Densitometric analysis of monomer vs. dimer in each sample is shown in the chart.

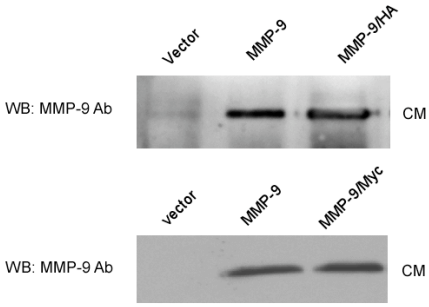
C) Recombinant proMMP-9 purified from COS-1 cells transfected with MMP-9 cDNA was subjected to FPLC gel filtration. The elution fractions from #38 to #70 (10 μl) were analyzed by gelatin zymography. Molecular weight marker proteins (200, 150 and 66 kDa) were used to calibrate the column for molecular mass.

Supplementary Figure 1

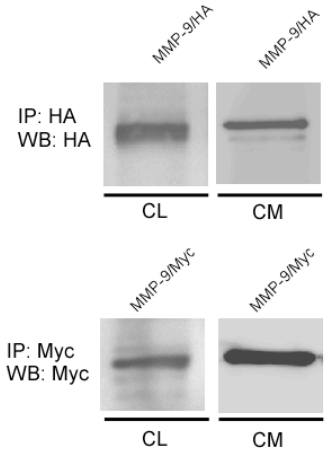
A



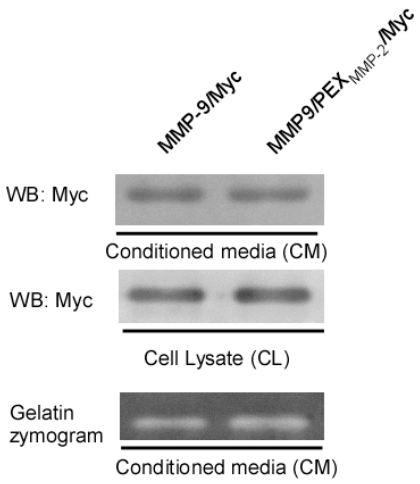
B



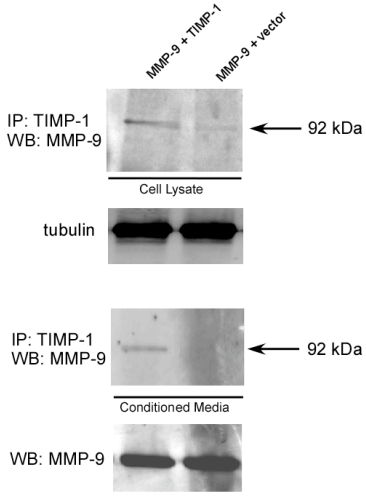
C



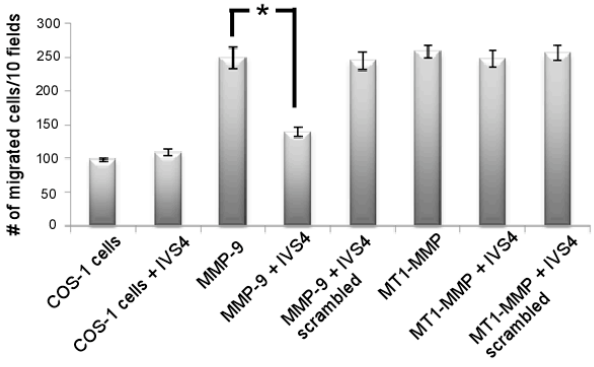
D



E

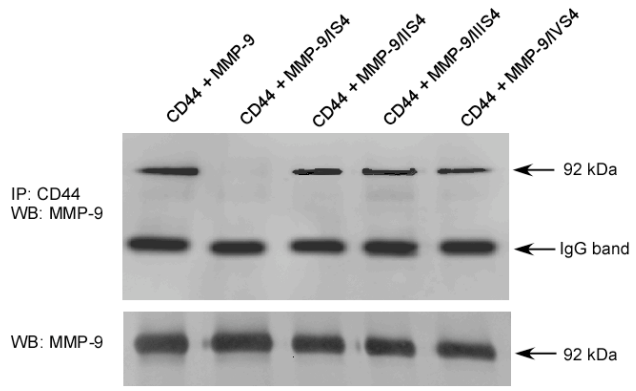


F

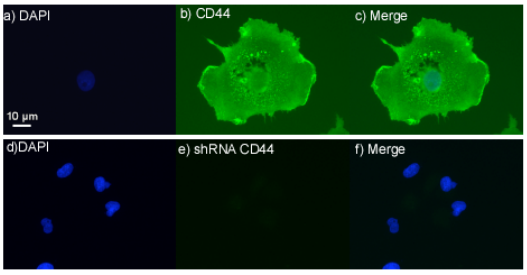


Supplementary Figure 2

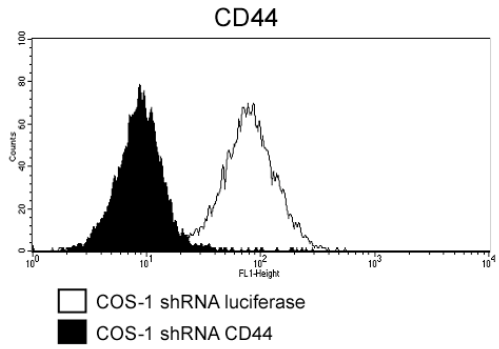
A



B

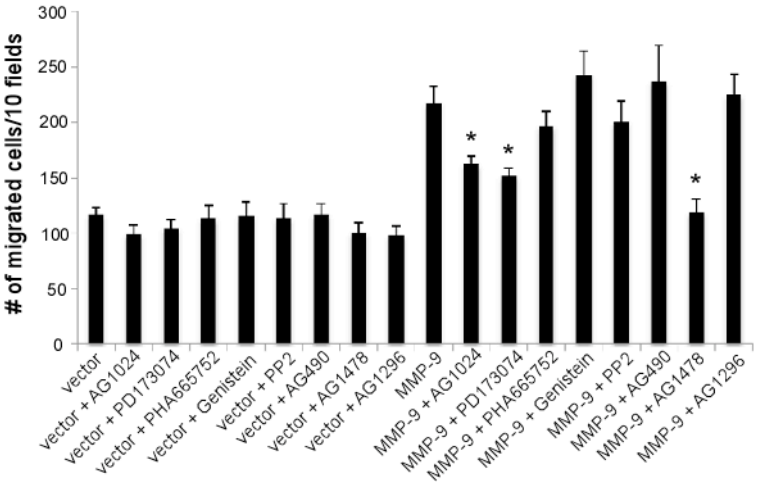


C

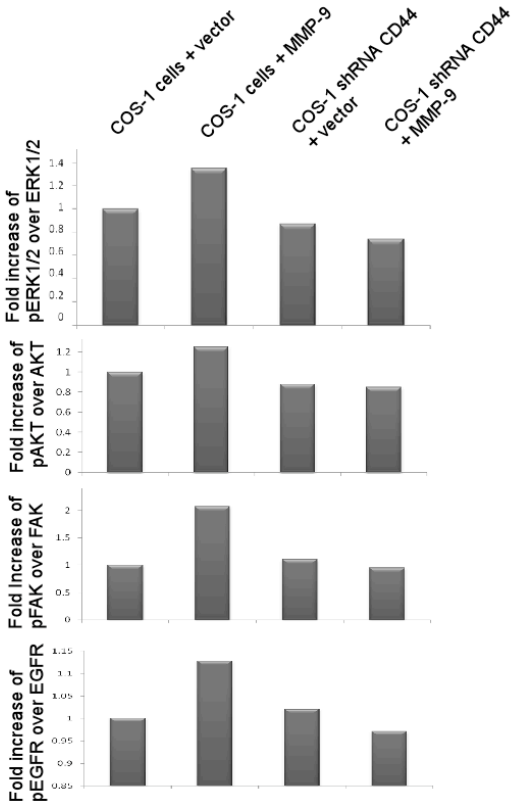


Supplementary Figure 3

A

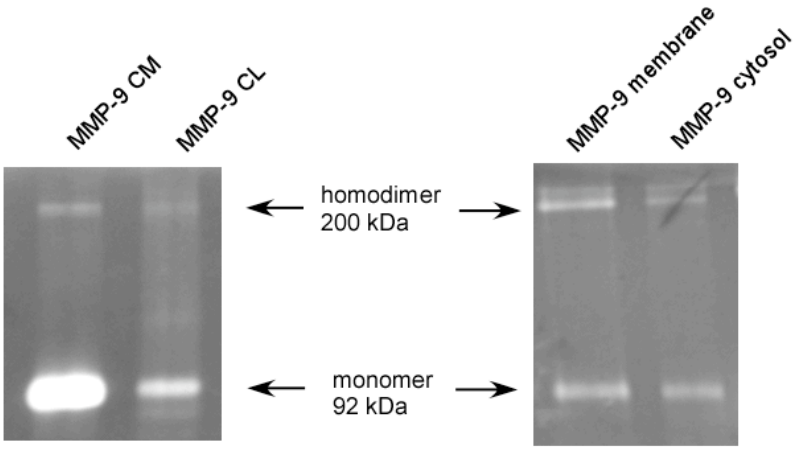


B



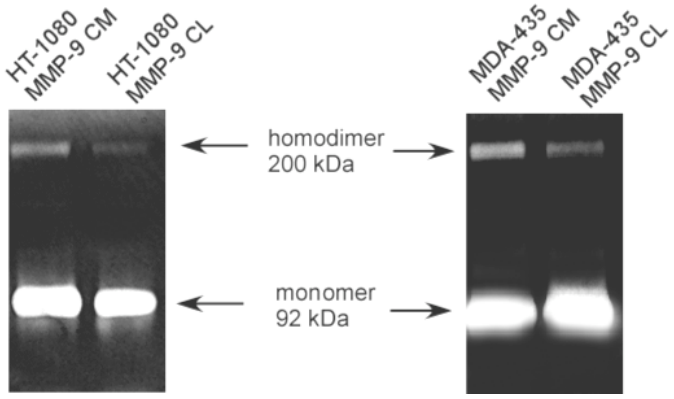
Supplementary Figure 4

A



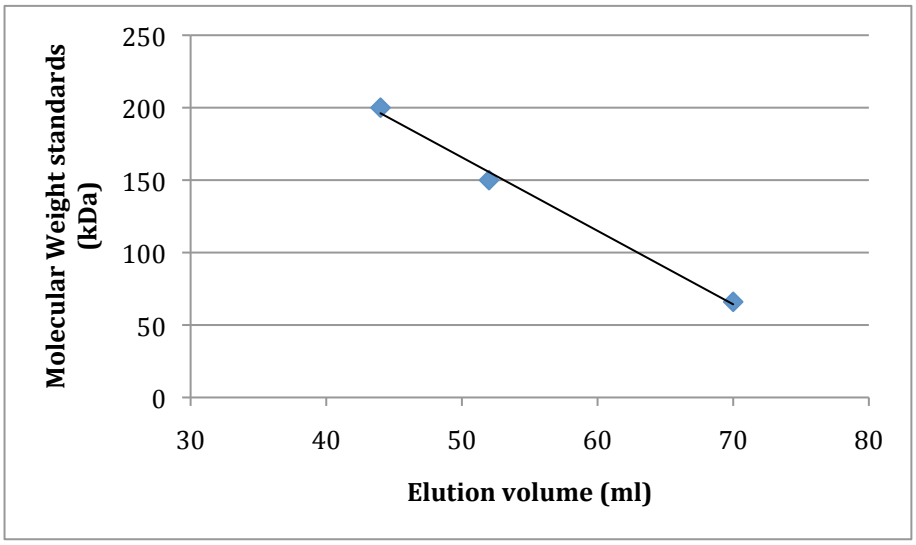
	Densitometry analysis (% of total)	
CM MMP-9 homodimer (200 kDa)	8.1%	
CM MMP-9 monomer (92 kDa)	65.9%	100 %
CL MMP-9 homodimer (200 kDa)	7.5%	
CL MMP-9 monomer (92 kDa)	18.5%	
Total homodimer CM + CL (200 kDa)	15.6%	100 %
Total monomer CM + CL (92 kDa)	84.4%	
Membrane MMP-9 homodimer (200 kDa)	27.4%	
Membrane MMP-9 monomer (92 kDa)	35.9%	100 %
Cytosolic homodimer (200 kDa)	9.3%	
Cytosolic monomer (92 kDa)	27.4%	

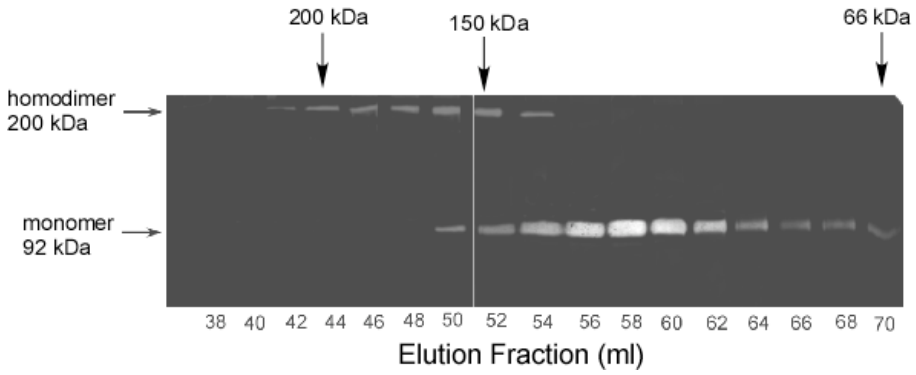
B



	Densitometry analysis (% of total)	
HT-1080 CM MMP-9 homodimer (200 kDa)	20.7%	
HT-1080 CM MMP-9 monomer (92 kDa)	79.3%	100%
HT-1080 CL MMP-9 homodimer (200 kDa)	9.9%	
HT-1080 CL MMP-9 monomer (92 kDa)	90.1%	
Total HT-1080 homodimer CM + CL (200 kDa)	18.5%	100%
Total HT-1080 monomer CM + CL (92 kDa)	81.5%	
MDA-435 CM MMP-9 homodimer (200 kDa)	13.9%	
MDA-435 CM MMP-9 monomer (92 kDa)	86.1%	100%
MDA-435 CL MMP-9 homodimer (200 kDa)	4.3%	
MDA-435 CL MMP-9 monomer (92 kDa)	95.7%	
Total MDA-435 homodimer CM + CL (200 kDa)	9.1%	100%
Total MDA-435 monomer CM + CL (92 kDa)	90.9%	

C


Molecular Weight standards (kDa)
250
200
150
100
50
0
30
40
50
60
70
80
Elution volume (ml)


200 kDa
150 kDa
66 kDa
homodimer
200 kDa
monomer
92 kDa
38 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70
Elution Fraction (ml)