Suppl. Fig. Legends:

Suppl. Fig. 1. Dose dependent inhibition of MMP-14-mediated cell migration by IS4 peptides: COS-1 cells transfected with GFP control and MMP-14 cDNAs were pretreated with IS4 scrambled-control peptide (100 μM) or different concentrations of IS4 peptides followed by a Transwell chamber migration assay. Each construct was assayed in triplicate and the experiments were repeated three times. Two-tailed Student's t-test employed. * P <0.05, ** P < 0.01, and *** P < 0.001.

Suppl. Fig. 2. Expression of MMP-14 correlates with invasive ability of cancer cells: A-B) Total RNA and cell lysates in human breast (MCF-7 and MDA-MB-231) and prostate (LNCaP and Du145) cancer cells were examined by real time RT-PCR and Western blotting, respectively, for evaluation of endogenous MMP-14 in the cells. HKGs: house-keeping genes including HPRT and GAPDH. β-actin antibody was employed as a loading control. C) Hormone-independent MDA-MB-231 and Du145 cells, but not hormone-dependent MCF-7 and LNCaP cells display scattering growth pattern in 3D type I collagen. The cancer cells were mixed with type I collagen and cultured at 37 °C. Cell growth pattern was monitored by phase contrast microscopy daily. The picture presented shows the 6th day image. Bar: 50 μm.

Suppl. Fig. 3. Decrease of cell adhesion in COS-1 cells expressing MMP-14:

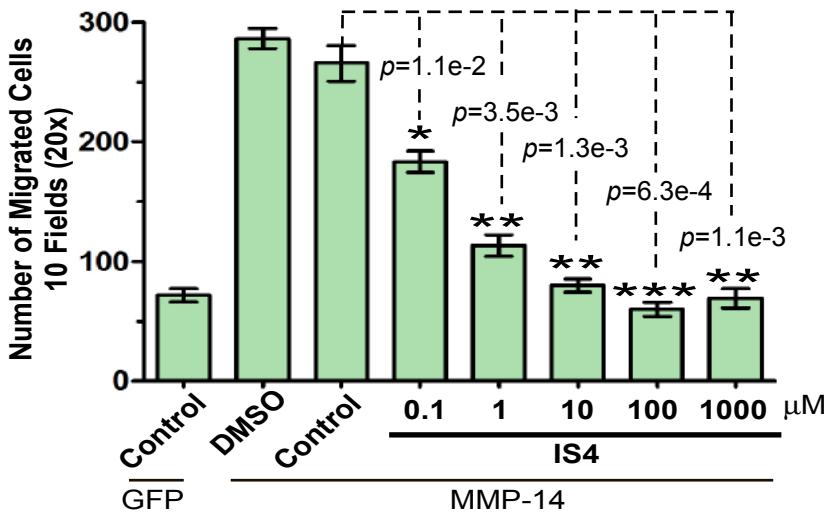
Hyaluronic Acid Adhesion Assay: 96-well tissue culture plates were coated with 3 mg/ml hyaluronic acid in PBS or 2% BSA as a control overnight at 4°C. Nonspecific sites were blocked with 2% BSA for 10 minutes at room temperature. The wells were washed with PBS and seeded with COS-1 transfected cells (45,000 cells/well) previously incubated with 2.5mM calcein in phenol-free DMEM. Adhesion was allowed to proceed at 37°C for 1h. The wells were then washed two times with PBS and plate was read on a Molecular Devices Gemini EM (excitation 494, emission 517).

A) COS-1 cells transfected with cDNAs as indicated were assessed for adhesion to a hyaluronic acid substrate (3 mg/ml). BSA-coated plates were used as a control. Adhesion was assessed by comparing fluorescence readings taken before and after washing cells from the plates. Adhesion percentage was determined by comparing fluorescence after PBS washes to total fluorescence of each sample. Two-tailed Student's t-test employed. * P <0.05, ** P < 0.01, and *** P < 0.001.

B) Representative images of the adhesion assay.

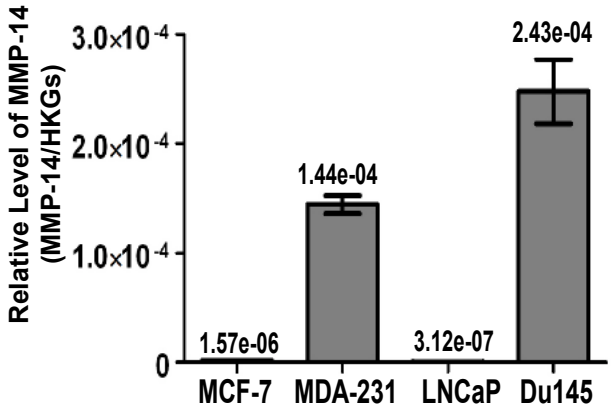
Suppl. Fig. 4. Inhibition of cell migration by recombinant MMP-14 PEX protein: The MMP-14 PEX domain was cloned into pET16b E. coli expression vector with Histag at the C-terminus. pET16b and $MMP14_{PEX}$/pET16b were expressed in BL21 cells and recombinant protein was purified from inclusion bodies. COS-1 cells transfected with GFP control and MMP-14 cDNAs were treated with control preparation and $rMMP14_{PEX}$ (10 μg/ml) followed by a Transwell chamber migration assay. Each construct was assayed in triplicate and the experiments were repeated three times. Two-tailed Student's t-test employed. * P < 0.05.

Suppl. Fig. 1



Suppl. Fig. 2

A



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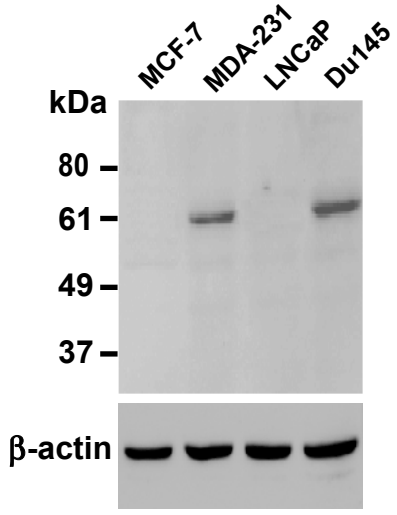

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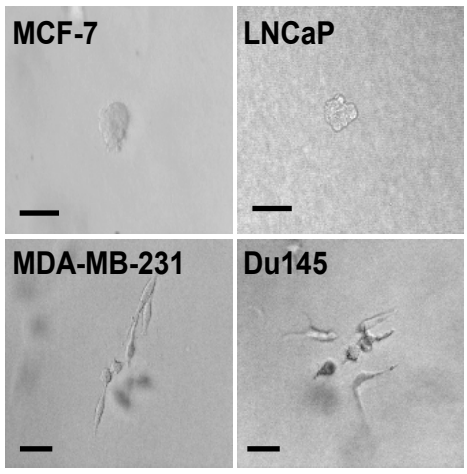
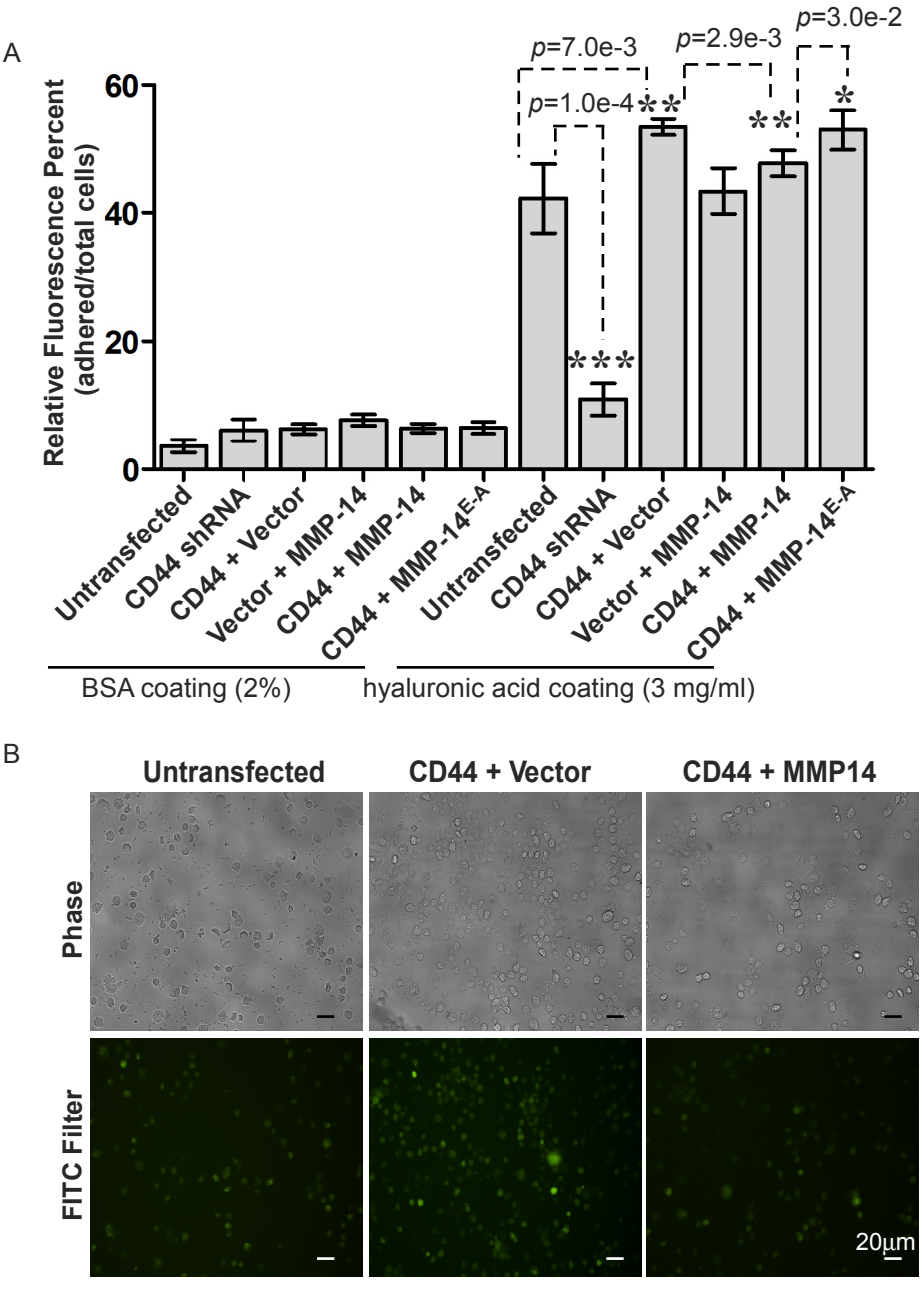
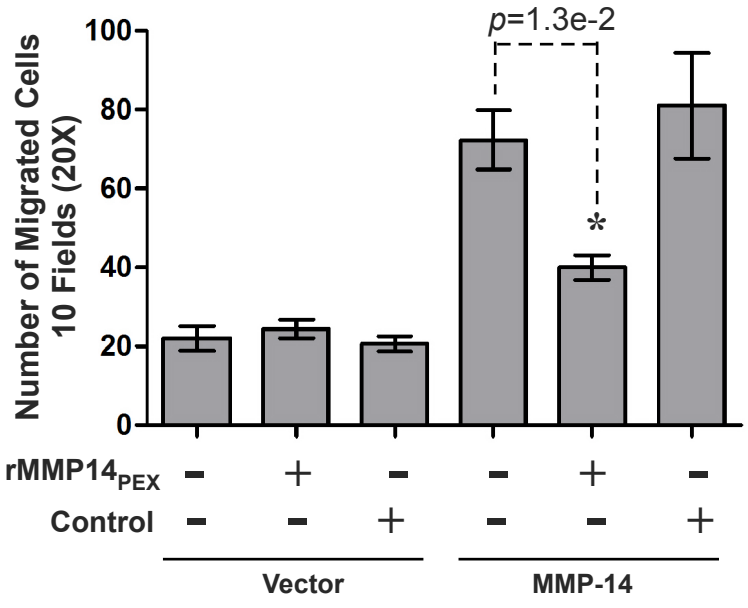


Suppl. Fig. 3



Suppl. Fig. 4



Supple Table 1: Primer List (shaded letters indicated sequence of MMP14 replaced with that of MMP1)

$MMP14_{HA}$	Fragment A	F-1563	5′ CACGAATTCCGGACCATGTCTCCCGCCCCAAGA 3′
	Fragment A	R-1006	5′ GGCGTAGTCGGGCACGTCGTAGGGGTAGCGCTTCCTTCGAACATTGGC 3′
	Fragment B	F-1863	5′ TACCCCTACGACGTGCCCGACTACGCCTACGCCATCCAGGGTCTCAAATGG 3′
	Fragment B	R-1564	5′ CACGAATTCTCAGACCTTGTCCAGCAGGGAAC 3′
$MMP14_{HA}$-$MMP1_{IS4}$	Fragment A	F-1563	5′ CACGAATTCCGGACCATGTCTCCCGCCCCAAGA 3′
	Fragment A	R-1237	5′ CTCAACTTCCGGGTAGAAGGGATTCCTCACCCGCCAGAACC 3′
	Fragment B	F-1236	5′AATCCCTTCTACCCGGAAGTTGAGCTCAATTTCATTTCTGTTTTCTGGCGGGGCCTGCCTGCG3′
	Fragment B	R-1564	5′ CACGAATTCTCAGACCTTGTCCAGCAGGGAAC 3′
$MMP14_{HA}$-$MMP1_{IIS4}$	Fragment A	F-1563	5′ CACGAATTCCGGACCATGTCTCCCGCCCCAAGA 3′
	Fragment A	R-1244	5′ TCCGTGTAGCACATTCTGTCCATCAAACACCCAATGCTTGTC 3′
	Fragment B	F-1243	5′GGACAGAATGTGCTACACGGATACCCCAAGGACATCTACGAGCTGGGCCGAGGGCTGCCTA 3′
	Fragment B	R-1564	5′ CACGAATTCTCAGACCTTGTCCAGCAGGGAAC 3′
$MMP14_{HA}$-$MMP1_{IIIS4}$	Fragment A	F-1563	5′ CACGAATTCCGGACCATGTCTCCCGCCCCAAGA 3′
	Fragment A	R-1246	5′ TGGATCCATAGATCGTTTATATTCGTTGAAACGGTAGTACTT 3′
	Fragment B	F-1245	5′TATAAACGATCTATGGATCCAGGTTATCCCAAAATGATAGCAGTCTGGGAAGGGATCCCTGAG 3′
	Fragment B	R-1564	5′ CACGAATTCTCAGACCTTGTCCAGCAGGGAAC 3′
$MMP14_{HA}$-$MMP1_{IVS4}$	Fragment A	F-1563	5′ CACGAATTCCGGACCATGTCTCCCGCCCCAAGA 3′
	Fragment A	R-1248	5′ AGTCAAAATTCTCTTCGTTTTCAGCTTCTGGTTGTTGAATTT 3′
	Fragment B	F-1247	5′ AAAACGAAGAGAATTTTGACTCTCCAGAAAGCTAGGGACTGGATGGGCTGCCCA 3′
	Fragment B	R-1564	5′ CACGAATTCTCAGACCTTGTCCAGCAGGGAAC 3′

Suppl. Table 2

Motif	MMPs	Amino Acid Sequence
$MMP14_{HA}$-$MMP1_{IS4}$	MMP-1	^{241}PFYPEVELNFISV^{253}
	MMP-14	^{347}NQVMDGYPMPIGQ^{359}
$MMP14_{HA}$-$MMP1_{IIS4}$	MMP-1	^{287}GQNVLHGYPKDIY^{299}
	MMP-14	^{392}EASLEPGYPKHIK^{404}
$MMP14_{HA}$-$MMP1_{IIIS4}$	MMP-1	^{337}YKRSMDPGYPKMIA^{350}
	MMP-14	^{441}ELRAVDSEYPKNIK^{454}
$MMP14_{HA}$-$MMP1_{IVS4}$	MMP-1	^{385}TKRILTLQKA^{394}
	MMP-14	^{493}VEPGYPKSAL^{502}

Suppl. Table 3

Name	Sequence
IS4	Ac-VMDGYPMP-NH_2
IS4 Scrambled	Ac-MVPDPMYG-NH_2
IIS4	Ac-FDEASLEP-NH_2
IIIS4	Ac-ELRAVDSE-NH_2
IVS4	Ac-GYPKSALR-NH_2
IVS4 Scrambled	Ac-KGYPSLRA-NH_2
Generic Scrambled	Ac-ALAIGAFA-NH_2