Supplementary Materials

Supplementary Methods

DNA plasmids

For the mCherry-conjugated MT1-MMP construct, the cDNA encoding MT1-MMP was first amplified by PCR with a sense primer containing a *Hind*III site, and a reverse primer containing a gene sequence encoding GGS as a linker and an *EcoR*I site. The PCR product of cDNA encoding mCherry was then fused to the C-terminus of the MT1-MMP-GGS fragment before this whole piece of recombinant gene product was cloned into pcDNA3.1 with *Hind*III/*Xho*I sites. The GFP-fused Src construct is a gift from Margaret C. Frame at the Beatson Institute for Cancer Research (1).

Expression, purification and refolding of the MT1-MMP's catalytic domain

The cDNA sequence encoding for the peptide sequence of the catalytic domain of MT1-MMP (MT1-CAT) was expressed in the pET21 plasmid. The resulting recombinant plasmid was used to transform *E. coli* BL21 (DE3) Codon Plus cells (Stratagene, San Diego, CA). The expression of the MT1-CAT construct was induced for 6 h at 37°C using 1 mM isopropyl β-D-thiogalactoside. The cells were collected by centrifugation and disrupted by sonication on ice in 10 mM Tris-HCl buffer, pH 8.0, containing 1 M NaCl, 1 mM phenylmethylsulfonyl fluoride and lysozyme (5 mg/ml). The inclusion bodies were collected by centrifugation (15,000xg; 20 min). The pellet (10 mg total protein) was washed three times using 10 mM Tris-HCl, pH 8.0, containing 1 M NaCl and 1% Triton X-100. The resulting pellet was dissolved in 10 mM Tris-HCl, pH 8.0,

containing 6 M guanidine hydrochloride and 10 mM 2-mercaptoethanol. The soluble sample was refolded using a 100-fold dilution in 100 mM Tris-HCl, pH 8.0, supplemented with 1 mM $CaCl_2$, 10 μM $ZnCl_2$, 500 mM L-arginine monohydrochloride and 20% glycerol. The refolded MT1-CAT was concentrated using a 10 kDa-cutoff concentrator (Millipore, Billerica, MA) and dialyzed against 50 mM HEPES, pH 7.5, containing 1 mM $CaCl_2$, 10 μM $ZnCl_2$ and 0.005% Brij35.

The measurement of cleavage efficiency

The cleaved substrate proteins of MT1-MMP were subjected to 10% SDS-PAGE gels followed by Coomassie Blue staining. After de-staining with 50% (v/v) methanol in water with 10% acetic acid, the cleaved or intact fragments of biosensor proteins were visualized on the gel and recorded by a digital camera (Olympus). GM6001 (5 μM), where indicated, was added to inhibit MT1-MMP activity.

Expression and purification of Golli-MBP J37

The construction of the pET22B-J37 plasmid expressing Golli-MBP-J37 (MBP-J37; GenBank #AAA37720) was reported earlier (2, 3). The MBP-J37 construct was C-terminally tagged with a 6xHis tag. *E. coli* BL21 (DE3)-Codon Plus cells were transformed using the plasmid. The expression of the MBP-J37 construct was induced by 1 mM isopropyl β-D-thiogalactoside for 6 h at 37°C. The MBP-J37 protein was isolated from the soluble fraction of *E. coli* cells on a HiTrap Co^{2+}-chelating Sepharose FastFlow column. The MBP-J37 protein was eluted using a 0-500 mM gradient of imidazole concentrations. The peak fractions were combined and concentrated using a 5 kDa cut-off concentrator (Millipore), and dialyzed against 10 mM Tris-HCl, pH 8.0, containing 200 mM NaCl.

References:

1. Sandilands, E, Cans, C, Fincham, VJ, et al. RhoB and actin polymerization coordinate Src activation with endosome-mediated delivery to the membrane. Dev Cell 2004; 7*:*855-69.
2. Feng, JM, Fernandes, AO, Campagnoni, CW, Hu, YH, and Campagnoni, AT The golli-myelin basic protein negatively regulates signal transduction in T lymphocytes. J Neuroimmunol 2004; 152*:*57-66.
3. Shiryaev, SA, Savinov, AY, Cieplak, P, et al. Matrix metalloproteinase proteolysis of the myelin basic protein isoforms is a source of immunogenic peptides in autoimmune multiple sclerosis. PLoS One 2009; 4*:*e4952.

Legends for Supplementary Figures, Table, and Movies

Supplementary Figure 1. (**A**) The protein solutions of the CFP/YFP-based Src biosensor (left) and mOrange2/mCherry-based MT1-MMP biosensor (right). (**B**) The PAGE gel showing the sizes of mOrange2/mCherry-based MT1-MMP biosensor with or without the incubation of MT1-CAT for 8 hrs.

Supplementary Figure 2. The fluorescence intensity images of mOrange2 and mCherry with the mOrange2 excitation in HeLa cells transfected with the mOrange2/mCherry-based MT1-MMP biosensor. The scar bar represents the length of 30 μm.

Supplementary Figure 3. MT1-MMP fused with mCherry and Src fused with EGFP were co-transfected into HeLa cells which were subsequently stimulated by EGF. The images of MT1-MMP-mCherry and Src-EGFP were displayed and overlaid to demonstrate their co-localization at the cell periphery upon EGF stimulation. The scar bar represents the length of 30 μm.

Supplementary Figure 4. *In vitro* characterization of the 'AHLR' MT1-MMP biosensor. (**A**) Emission spectra of the 'AHLR' MT1-MMP biosensor. (excited at 437 nm) before and after incubation with the catalytic domain of MT1-MMP (CAT) for various periods of time as indicated. The broken lines highlight the regions encompassing emission maxima of ECFP (blue) and YPet (yellow). (**B**) PAGE gel showing the sizes of 'AHLR' MT1-MMP biosensor with or without the incubation of CAT after 2 hrs.

Supplementary Table 1. The parameters of dichroic mirrors, excitation and emission filters for FRET and different fluorescence proteins.

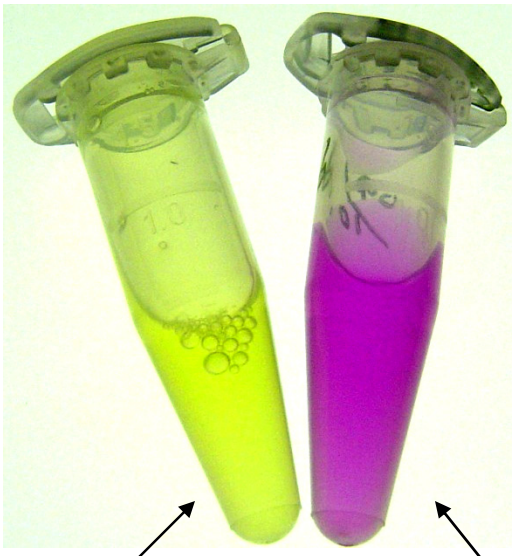
Movie 1: EGF-induced FRET response in HeLa cells expressing MT1-MMP and the mOrange2/mCherry-based MT1-MMP biosensor.

Movie 2: EGF-induced FRET response in HeLa cells expressing the mOrange2/mCherry-based KRas-Src biosensor.

Movie 3: EGF-induced FRET response of both ECFP/Citrine-based KRas-Src biosensor (left) and mOrange2/mCherry-based MT1-MMP biosensor (right) in a single live HeLa cell.

Movie 4: The inhibition of Src by PP1 blocked the EGF-induced FRET response of both ECFP/Citrine-based KRas-Src biosensor (left) and mOrange2/mCherry-based MT1-MMP biosensor (right) in a single live HeLa cell.

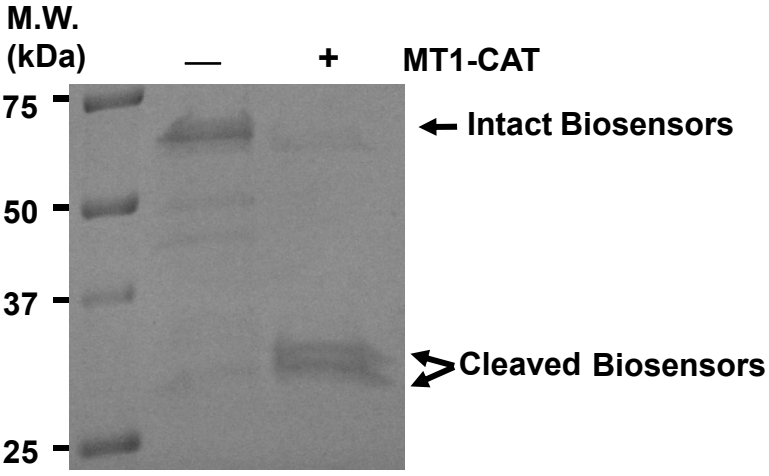
A



Purified Src Biosensor
(ECFP/Citrine)

Purified MT1-MMP Biosensor
(mOrange2/mCherry)

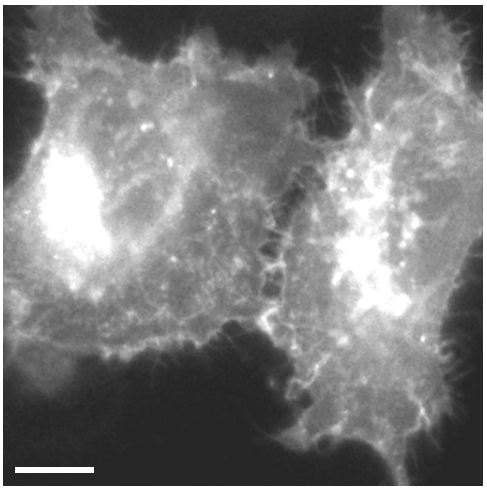
B



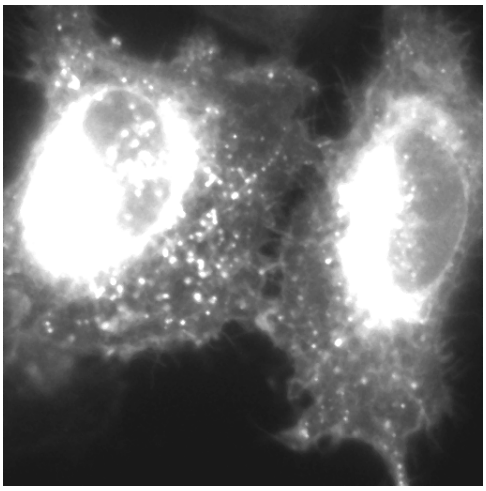
Supplementary Figure 1

MT1-MMP biosensor

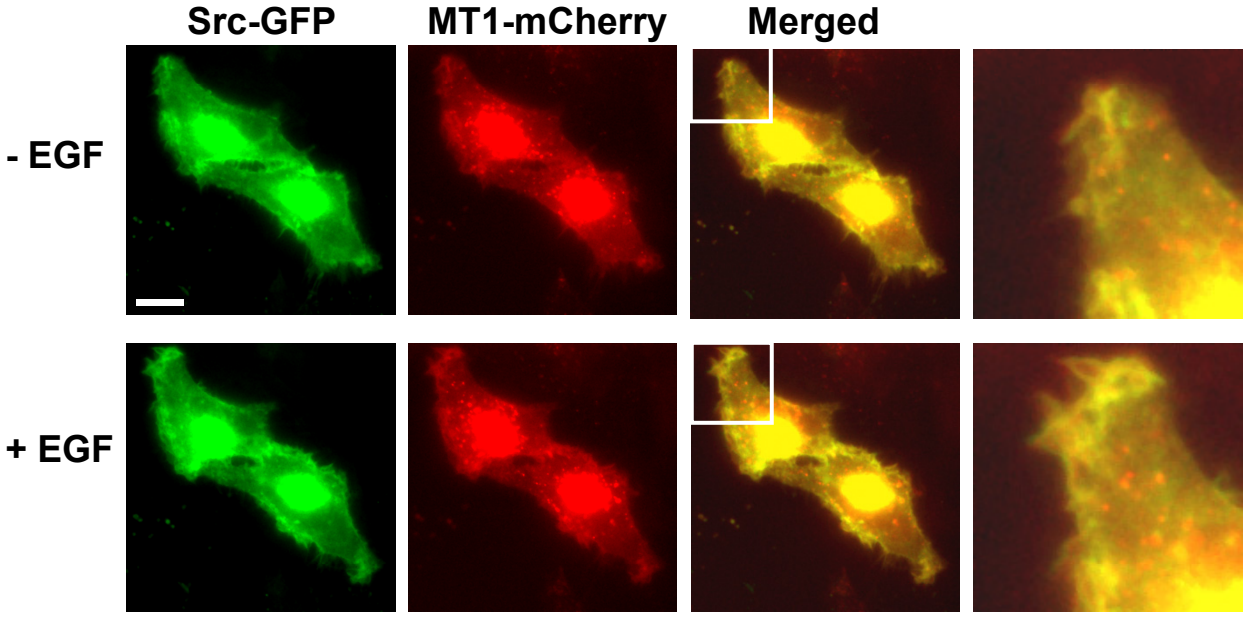
mOrange2



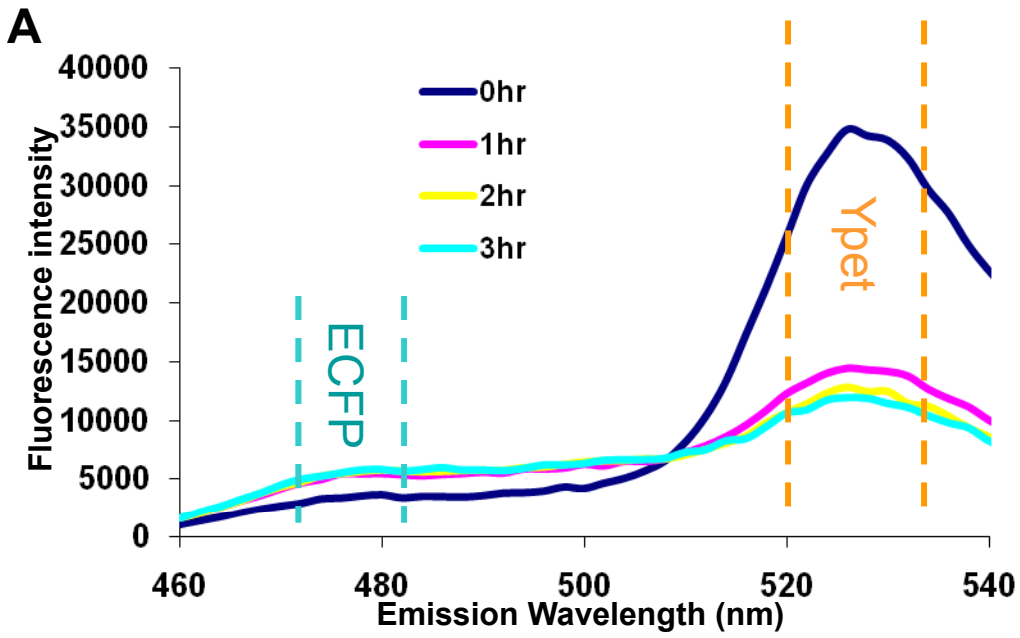
mCherry

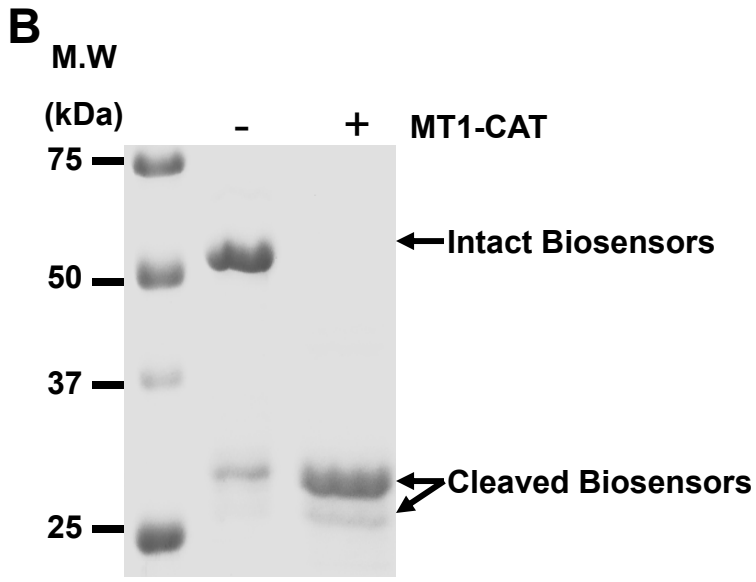


Supplementary Figure 2



Supplementary Figure 3





Supplementary Figure 4

Supplementary Table 1. The settings of filters for fluorescence imaging

	Excitation Filters (nm)	Dichroic Mirrors (long pass; nm)	Emission Filters (nm)
CFP	420/20	450	475/40
YFP (FRET)			535/25
mOrange2	548/20	560	572/20
mCherry (FRET)			630/20
GFP	495/10	515	535/25
mCherry	560/40	595	653/95