Supporting Information

Development of a Protease Biosensor Based on a Dimerization-Dependent Red Fluorescent Protein

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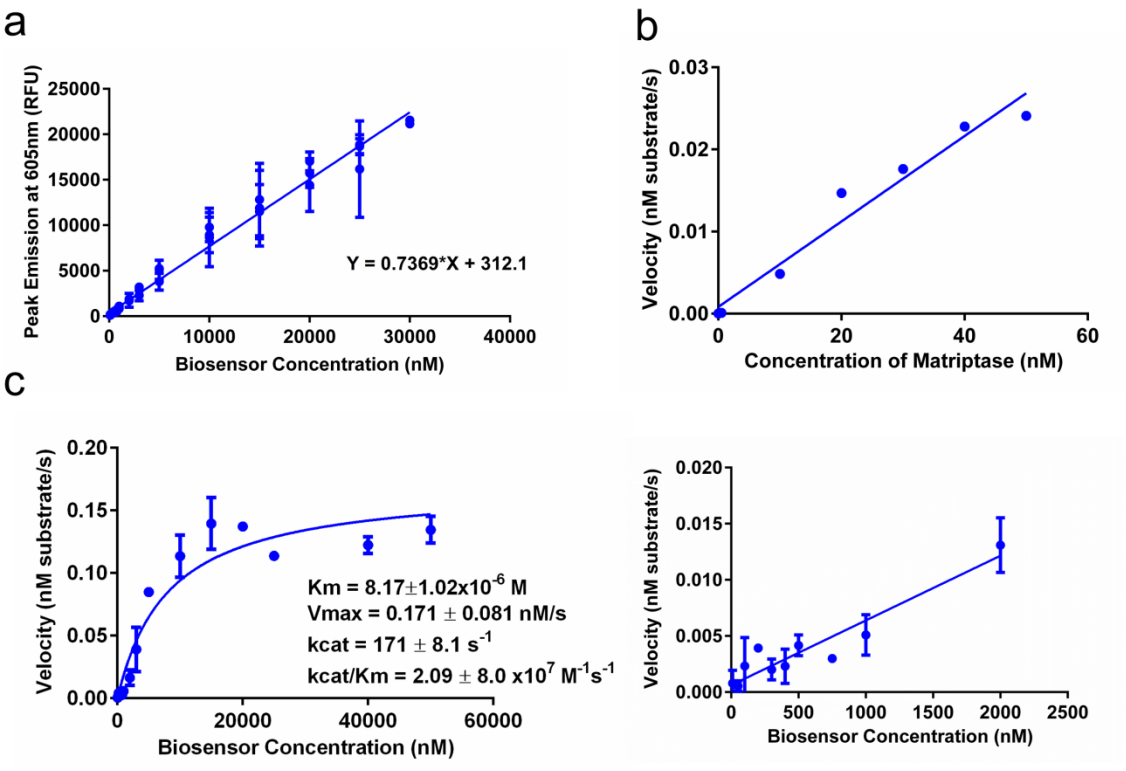
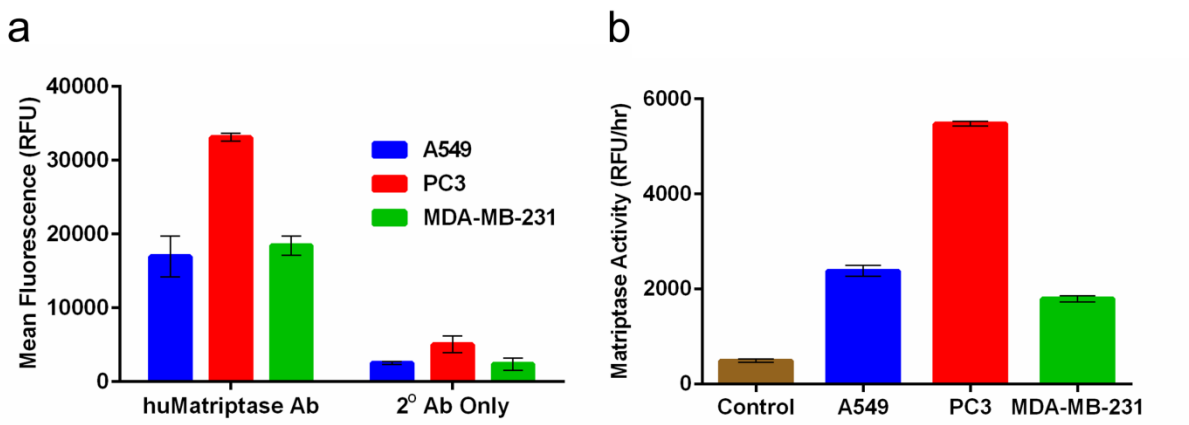
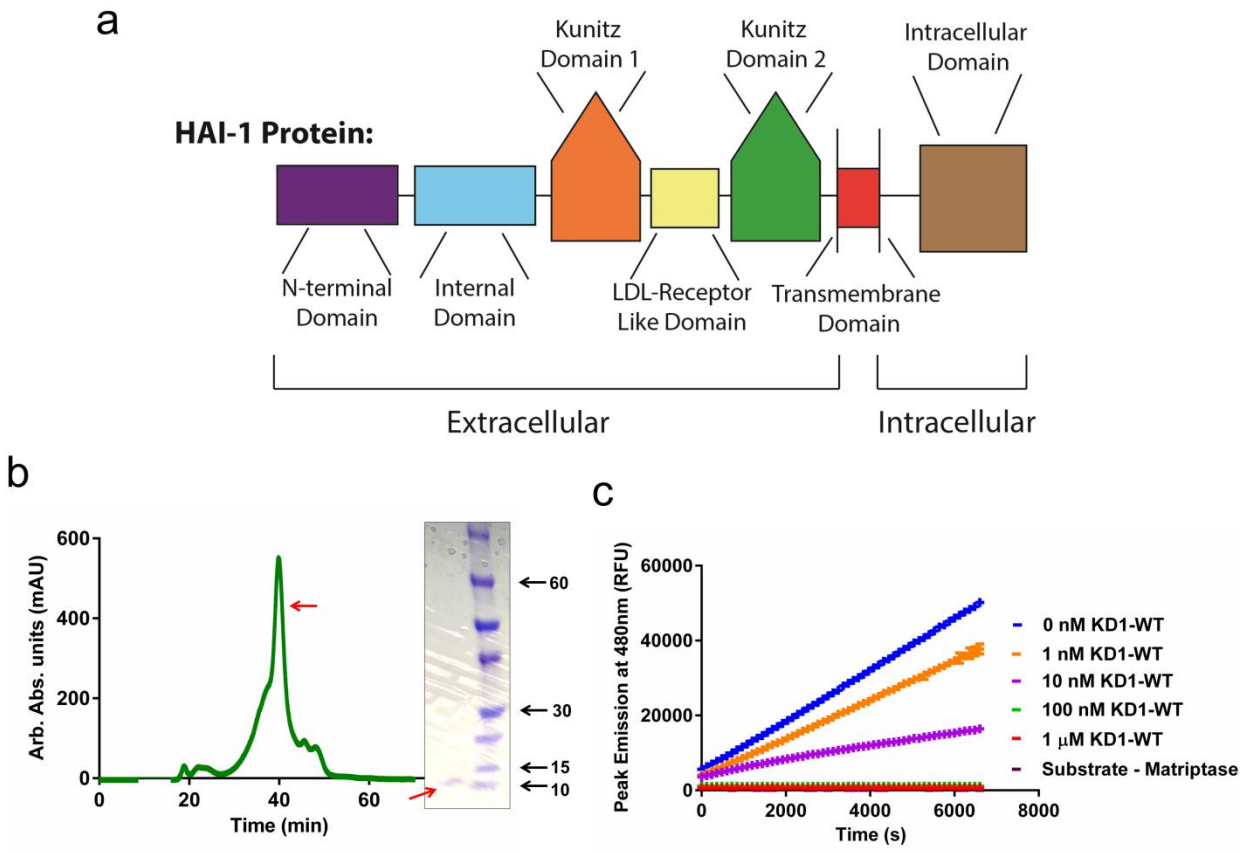


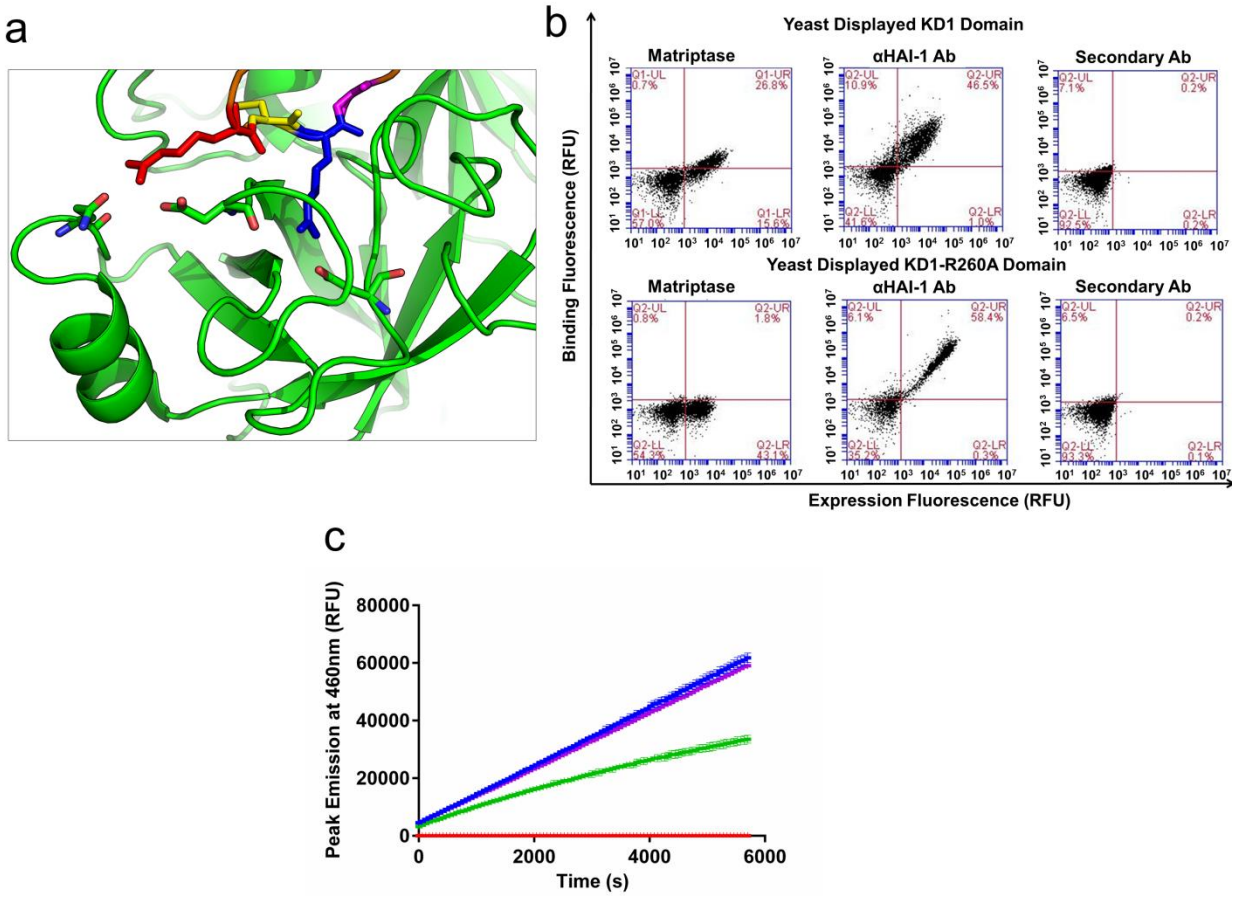
Supporting Information Figure 1. Biosensor 4 was characterized in additional assays including: (a) standard curve for biosensor concentration vs fluorescent emission, (b) matriptase titration response, and (c) Michaelis-Menten curve and kinetic parameters (right panel, initial k_{cat} regime). All plots were fit and analyzed using GraphPad Prism Software. Data are representative of at least three independent experiments. Mean values are reported, with error as standard deviation.



Supporting Information Figure 2. Human cancer cell lines A549 lung (blue), PC3 prostate (red), and MDA-MB-231 breast (green) were investigated for expression of active matriptase. (**a**) Cell anchored matriptase expression on tumor cells was measured by flow cytometry using an anti-human matriptase antibody. Secondary antibody only control is also shown. (**b**) Cell anchored matriptase activity was measured with a commercial matriptase substrate. A microtiter plate reader was used to monitor fluorescent emission over time, compared to media alone conditions (brown). A549 (blue), PC3 (red), and MDA-MB-231 (green) cells.



Supporting Information Figure 3. (a) The human Kunitz Domain 1 (KD1) protein from the extracellular domain of HAI-1 was cloned and expressed in yeast. **(b)** KD1 (red arrow) was purified using size exclusion chromatography (left). Chromatogram represents protein absorbance at 280nm over time and is representative of at least 4 individual production and purification events; SDS-PAGE also shown (right). (**c**) Time course measuring matriptase activation of a commercial substrate in the presence of increasing concentration of soluble KD1 monomer (KD1). Plot was generated using GraphPad Prism software. Data are representative of at least three independent experiments. Mean values are reported, with error as standard deviation.



Supporting Information Figure 4. (**a**) Crystal structure of matriptase (green) in complex with KD1.[1] Image generated using Pymol (PDB 4ISN). Primary KD1 binding interface comprised of Arg258 (red), Cys259 (yellow), Arg260 (blue), and Gly261 (magenta) enables specific and high affinity interaction with matriptase active site side chains. **(b)** Scatter plots of yeast displayed KD1 and KD1-R260A measuring matriptase binding (domain function), anti-human HAI-1 antibody (Ab) binding (expression), and secondary Ab binding (negative control). (**c**) Time course plot of commercial substrate activation by matriptase with yeast-displayed KD1 (green), KD1-R260A (magenta), or non-induced yeast controls with (blue) or without (red) matriptase.

References:

(1) Zhao, B., Yuan, C., Li, R., Qu, D., Huang, M., and Ngo, J. C. K. (2013) Crystal structures of matriptase in complex with its inhibitor hepatocyte growth factor activator inhibitor-1. *J. Biol. Chem. 288*, 11155–11164.