# Supporting Information

# Activatable and Cell-Penetrable Multiplex FRET Nanosensor for Profiling MT1-MMP Activity in Single Cancer Cells

Eddie Y. Chung[1], Christopher J. Ochs[2], Yi Wang[3], Lei Lei[1,3], Qin Qin[1], Andrew M. Smith[3], Alex Y. Strongin[4], Roger Kamm[2], Ying-Xin Qi[5,*], Shaoying Lu[1,3,*], Yingxiao Wang[1,3,*]

[1]Department of Bioengineering & Institute of Engineering in Medicine, University of California, San Diego, California, USA; [2]Singapore-MIT Alliance for Research and Technology, BioSystems and Micromechanics, Singapore; [3]Department of Bioengineering, University of Illinois, Urbana-Champaign, Illinois, USA; [4]Sanford Burnham Medical Research Institute, La Jolla, California, USA; [5]Institute of Mechanobiology and Medical Engineering, Shanghai Jiao Tong University, Shanghai, China.

[*]Correspondence: yiw015@ucsd.edu; kalu@ucsd.edu; qiyx@sjtu.edu.cn

**Supplementary Figure Legends**

**Figure S1.** The effect of binding competitor imidazole on the spectra of the FRET nanosensors. The spectra of the nanosensors with (blue line) or without (red line) addition of imidazole (100 mM). The spectra were obtained after incubation for 3 min.

**Figure S2.** Visualize MT1-MMP activity in breast cancer cells with GM wash-out assay. The left panel show the DIC images of the cells. The middle and right panels show the QD/FRET ratio images of the cells from t = 0 min (middle) to t = 5 min (right) after GM washout. **(a)** The cancer cells were incubated with nanosensors for 1 hour. **(b)** The cancer cells were incubated with nanosensors for >6 hours.

**Figure S3.** The effect of the RGD motif. **(a)** Normalized QD Intensity is compared in cells with the QD nanosensor (with the RGD motif, n = 22) and those with QD only (no RGD motif, n = 19). The QD intensity of the QD only group was close to zeros. * indicates statistically significant difference, $p<0.001$. Error bar: SEM. **(b)** shows the donor fluorescence intensity and DIC images of the cells. **Top panels**: The MT1-MMP biosensor with the RGD motif showed clear donor fluorescence intensity in all cells. **Lower panels:** the MT1-MMP biosensors without the RGD motif showed no donor fluorescence intensity at the location of cells.

**Figure S4.** The QD/FRET ratio of the nanosensor with the varying ratios of peptide-Cy3:QD during the assembly. There is no significant difference in QD/FRET ratio when the ratio of peptide-Cy3:QD is at 31:1, 62:1, or 93:1.

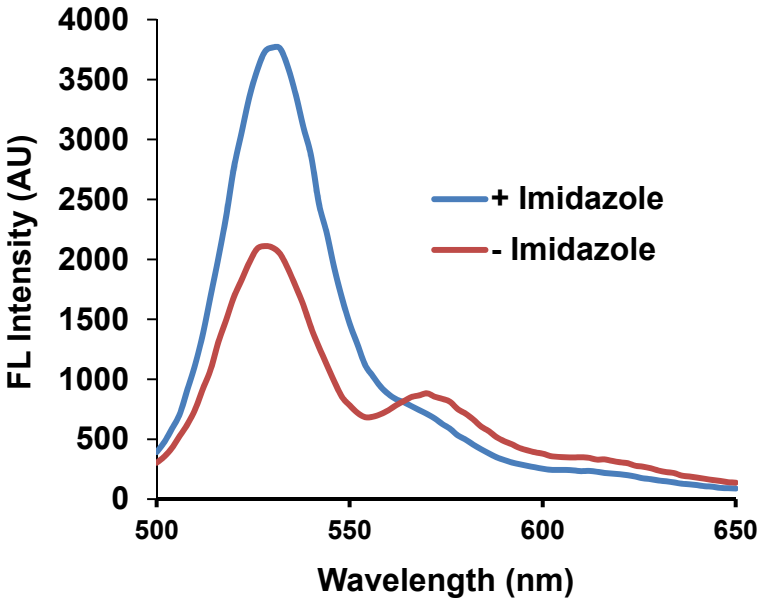
4000
3500
3000
2500
2000
1500
1000
500
0
FL Intensity (AU)
500
550
600
650
Wavelength (nm)
+ Imidazole
- Imidazole

**Figure S1**

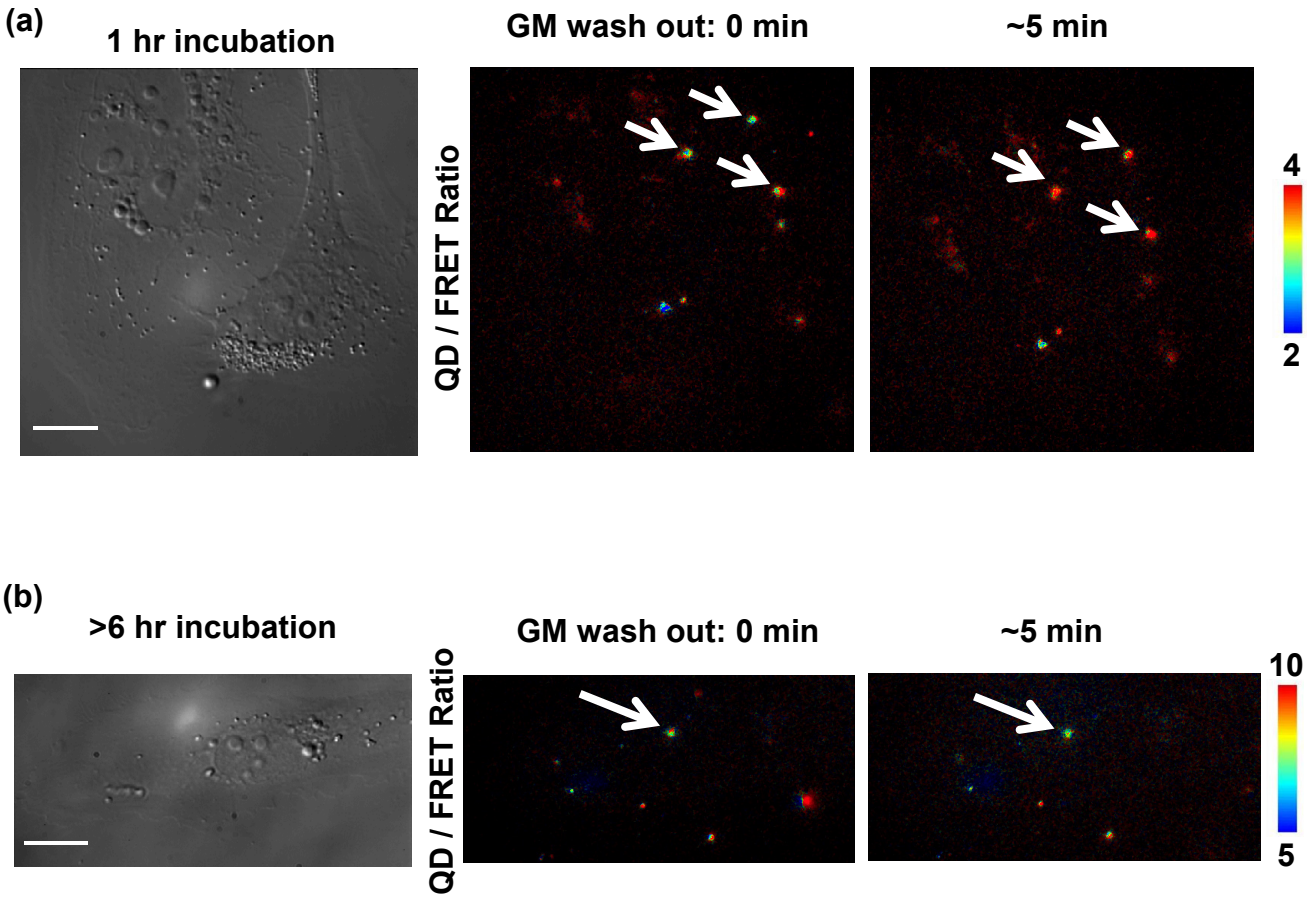
(a)
1 hr incubation
GM wash out: 0 min
~5 min
QD / FRET Ratio
4
2
(b)
>6 hr incubation
GM wash out: 0 min
~5 min
QD / FRET Ratio
10
5

Figure S2

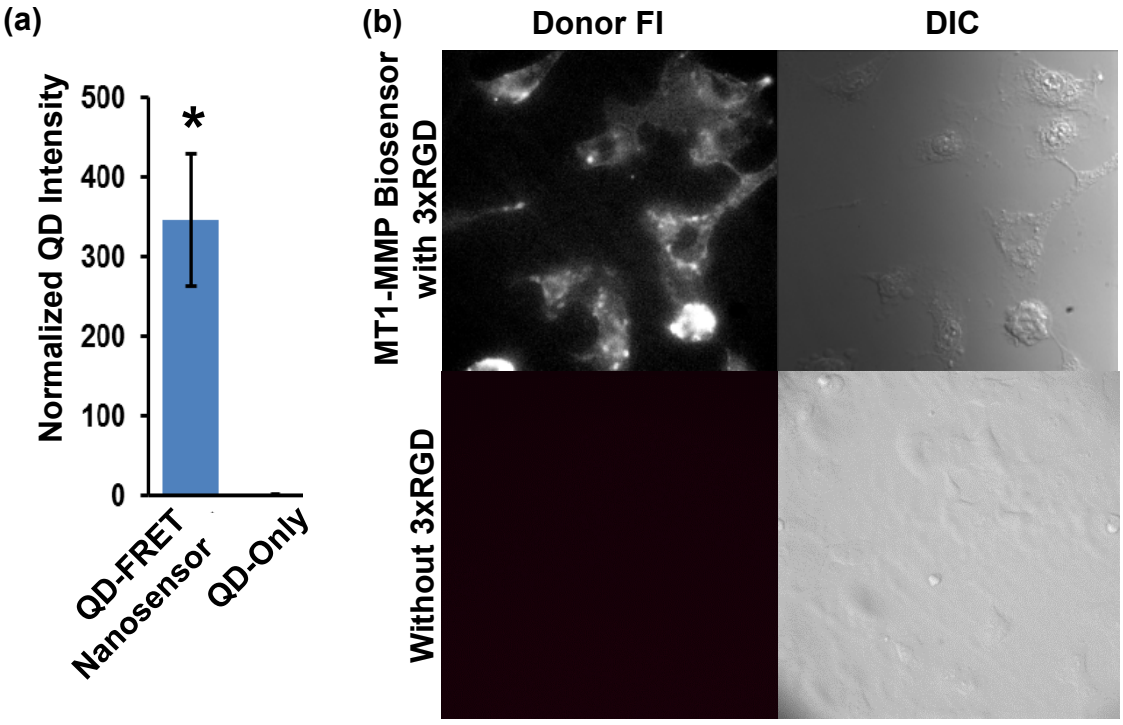
(a)
Normalized QD Intensity
500
400
300
200
100
0
*
QD-FRET Nanosensor
QD-Only
(b)
Donor FI
DIC
MT1-MMP Biosensor with 3xRGD
Without 3xRGD

Figure S3

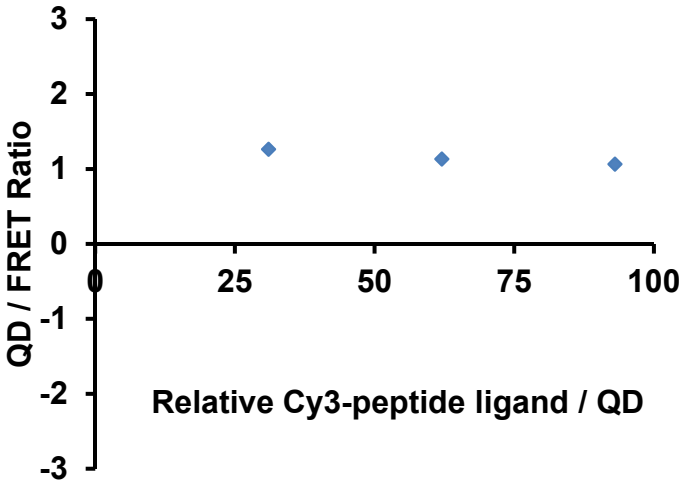
3
2
1
0
-1
-2
-3
QD / FRET Ratio
0
25
50
75
100
Relative Cy3-peptide ligand / QD

**Figure S4**