

Supplementary information for

Nanopore mediated protein delivery enabling 3-color single molecule tracking in living cells

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Supplementary Movies

Movie 1 EGF stimulated recruitment of intracellularly delivered Grb2-AF647 onto plasma membrane in HeLa cells. Scale bar is 5 μm for all movies.

Movie 2 Grb2-AF488 single molecule imaging in HeLa cells

Movie 3 SOS-HaloTag-JF549 single molecule imaging in HeLa cells

Movie 4 Simultaneously single molecule imaging of Grb2-AF647 and SOS-HaloTag-JF549 in HeLa cells

Movie 5 Simultaneously single molecule imaging of Grb2-AF647 and Src-HaloTag-JF549 in HeLa cells

Movie 6 RBD-AF647 single molecule imaging in HeLa cells

Movie 7 RBD-R59A-AF647 single molecule imaging in HeLa cells

Movie 8 Simultaneously 3-color single molecule imaging of Grb2-AF488, SOS-HaloTag-JF549 and RBD-AF647 in HeLa cells

Movie 9 Bulk delivery of RBD-AF647 in mouse T cells

Movie 10 Single molecule delivery of RBD-AF647 in mouse T cells

Figure S1

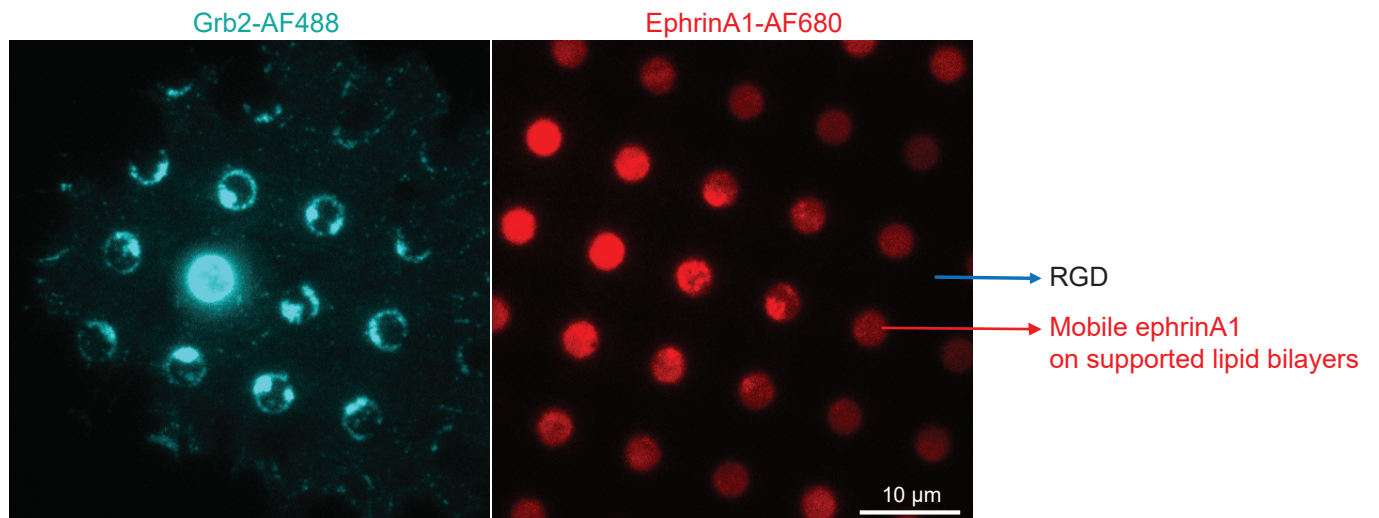


Figure S1. Grb2-AF488 delivered COS7 cells spread on ephrinA1-SLBs and RGD substrate

Figure S2

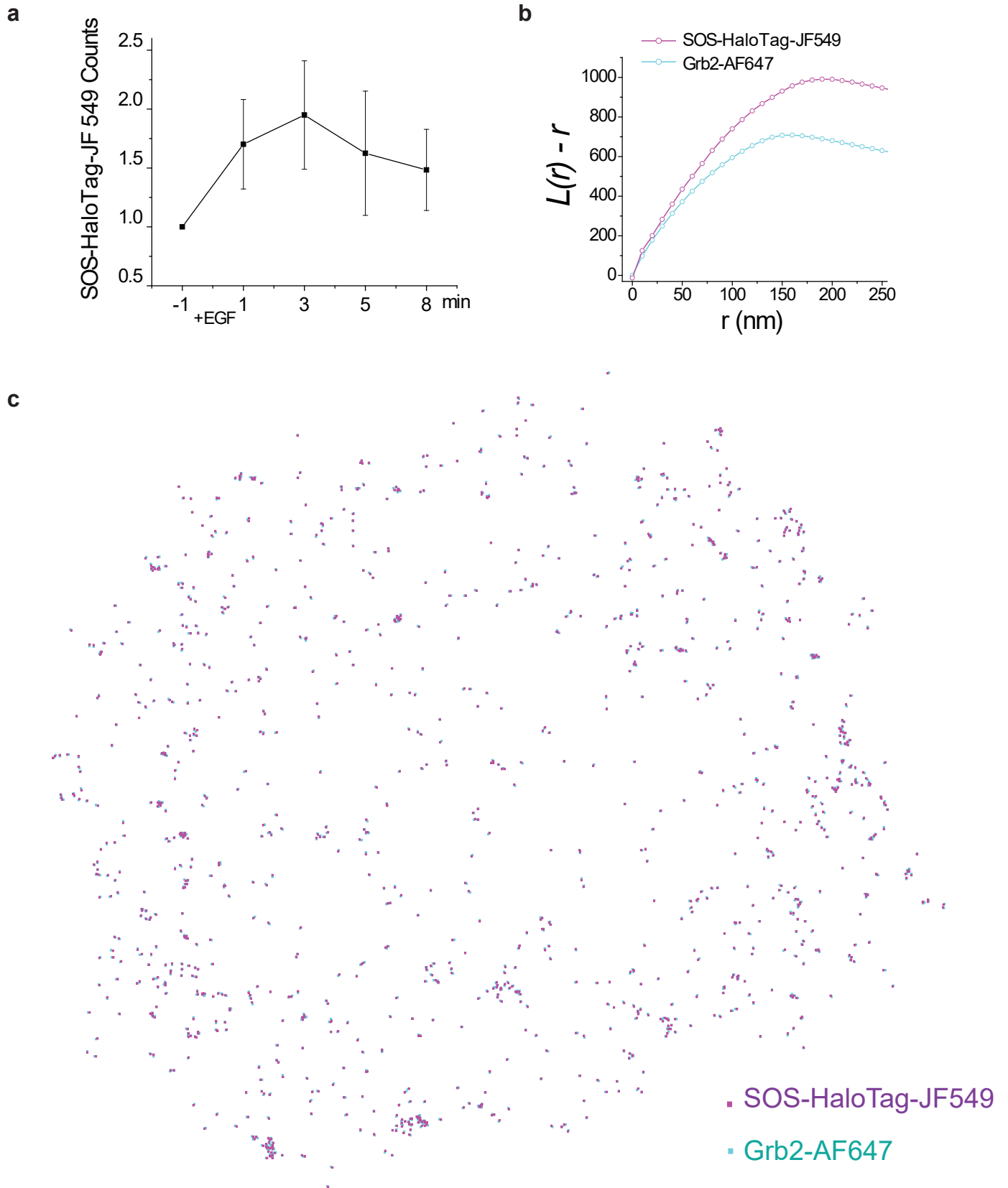


Figure S2. (a) Counts of SOS-HaloTag-JF549 molecule appearances in each 1.5 sec recording period as response to EGF stimulation. N=6 cells, error bar shown as standard deviation. (b) Cluster analysis of Grb2-AF647 and SOS-HaloTag-JF549. (c) Isolation of Grb2-AF647 and SOS-HaloTag-JF549 doublets.

Figure S3

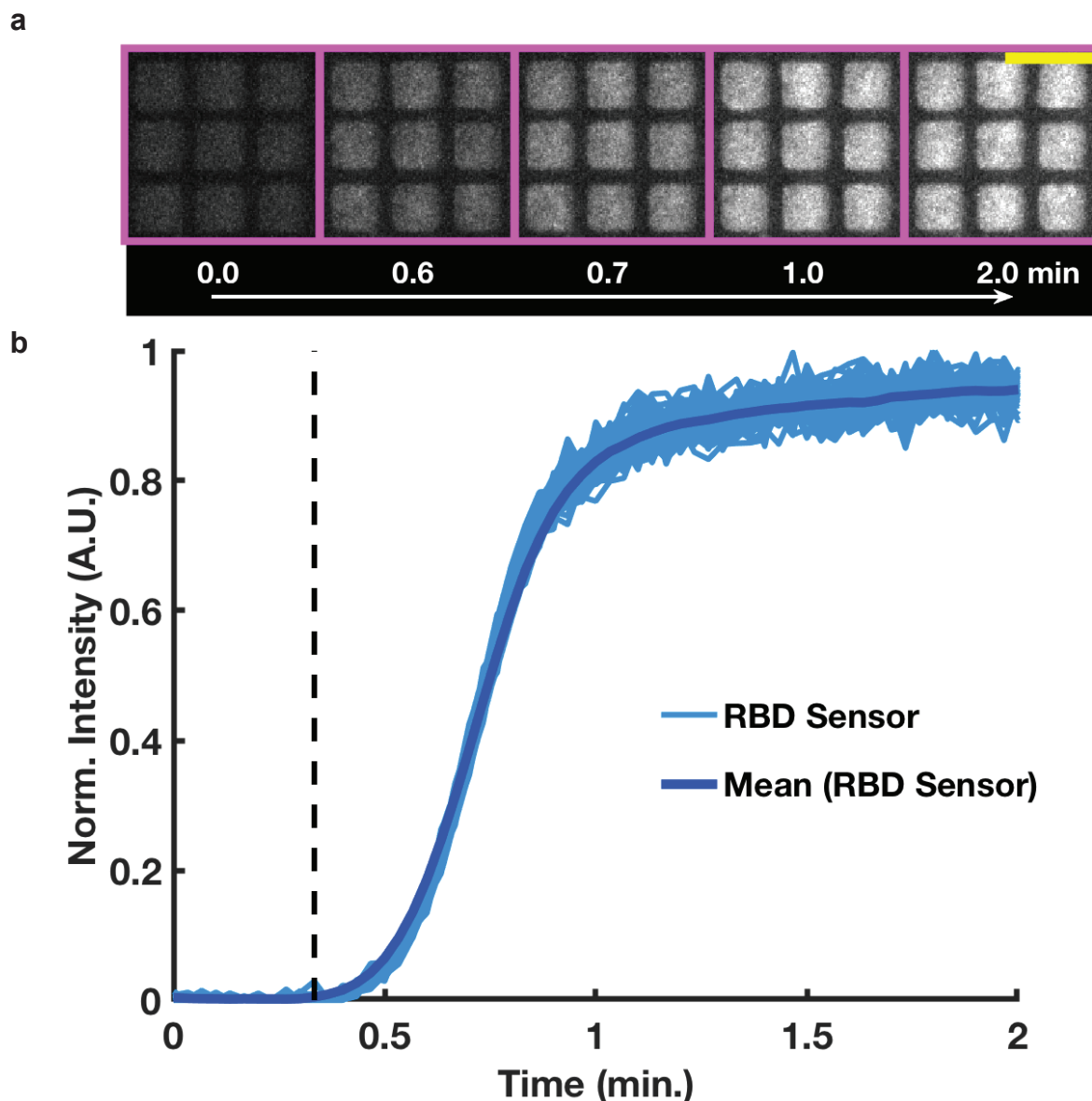
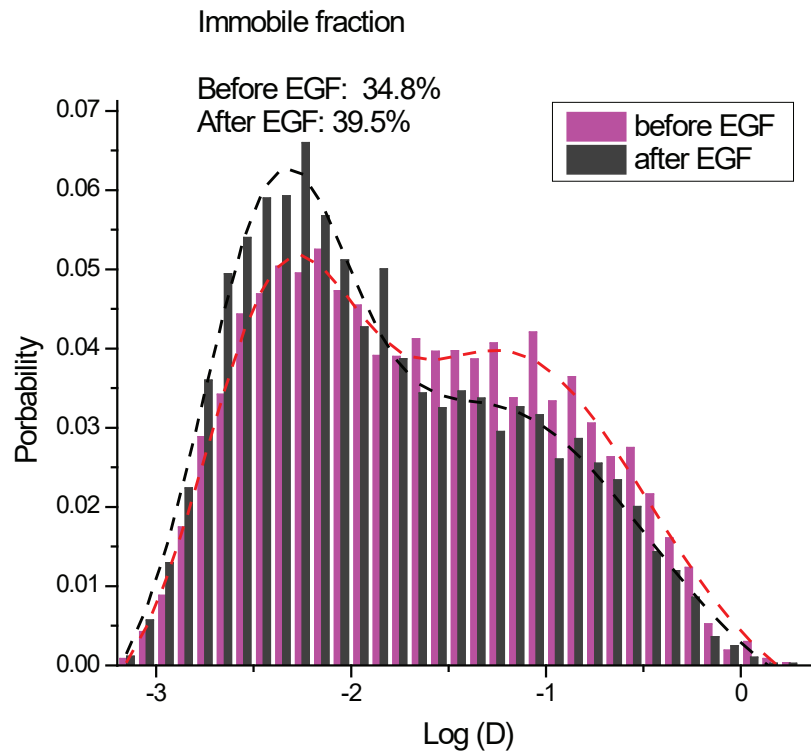


Figure S3. NHS ester labeled RBD sensor is sensitive to Ras activation state. GEF-mediated activation of H-Ras reconstituted on micropatterned supported membranes and monitored with RBD labeled via NHS ester chemistry. A micropatterned membrane functionalized with GDP-bound H-Ras was exposed to 40nM of SOS-Cat (the minimal catalytic core of the GEF Son of Sevenless) and 1 mM GTP to initiate the reaction. (a) Montage of TIRF images monitoring binding of AF647 labeled RBD to active Ras and (b) corresponding reaction trajectories ($n=55$). Scalebar on (a) is 10 μ m.

Figure S4

a



b

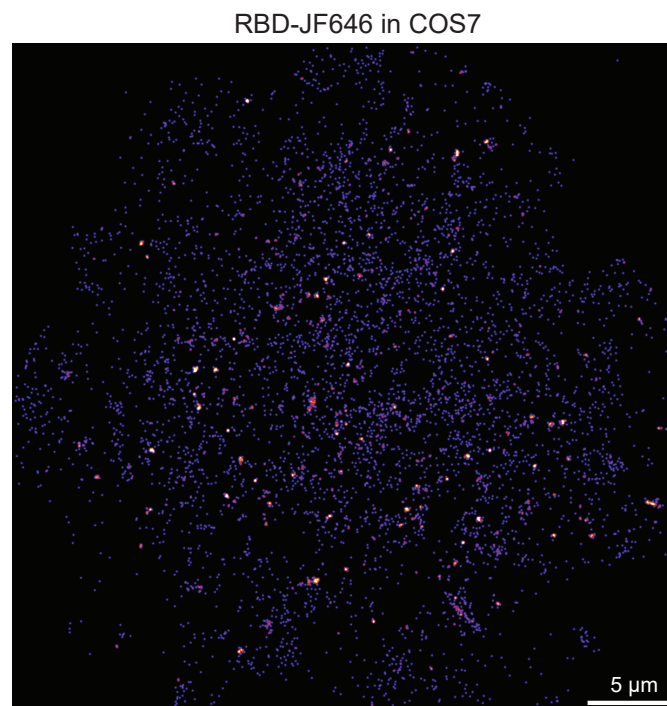


Figure S4. (a) Distribution of RBD-AF647 diffusion coefficient (D) from each molecular trajectory before or after EGF stimulation. N=10 cells. (b) Reconstructed frequency image of RBD-JF646 single molecule recruitment after EGF stimulation in COS7 cells.

Figure S5

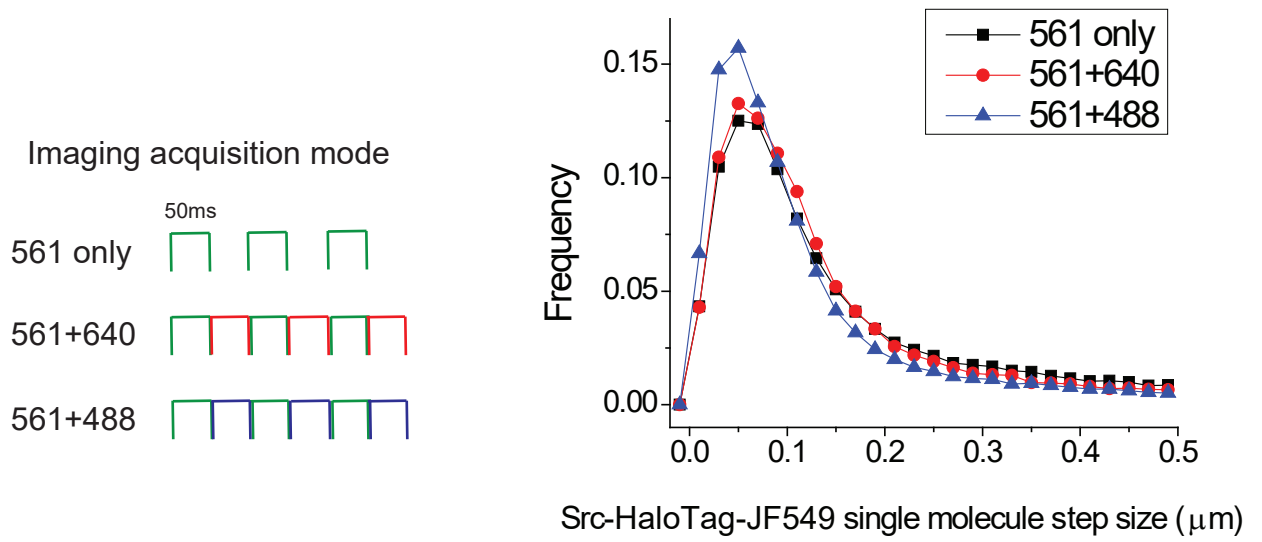


Figure S5. Src-HaloTag-JF549 single molecule step size analysis with different acquisition mode.