Fortian and Sorkin J. Cell Science

SUPPLEMENTAL MATERIALS

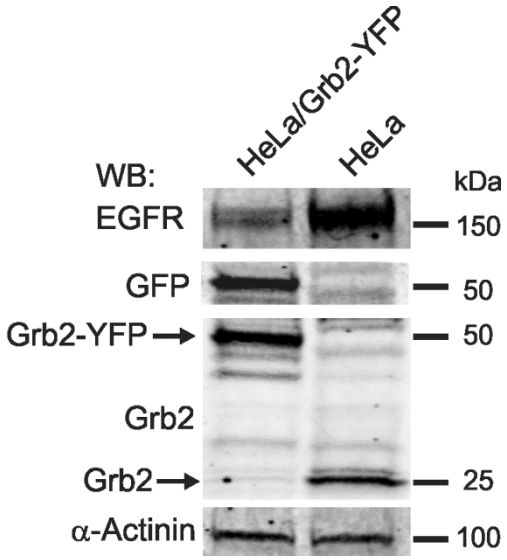
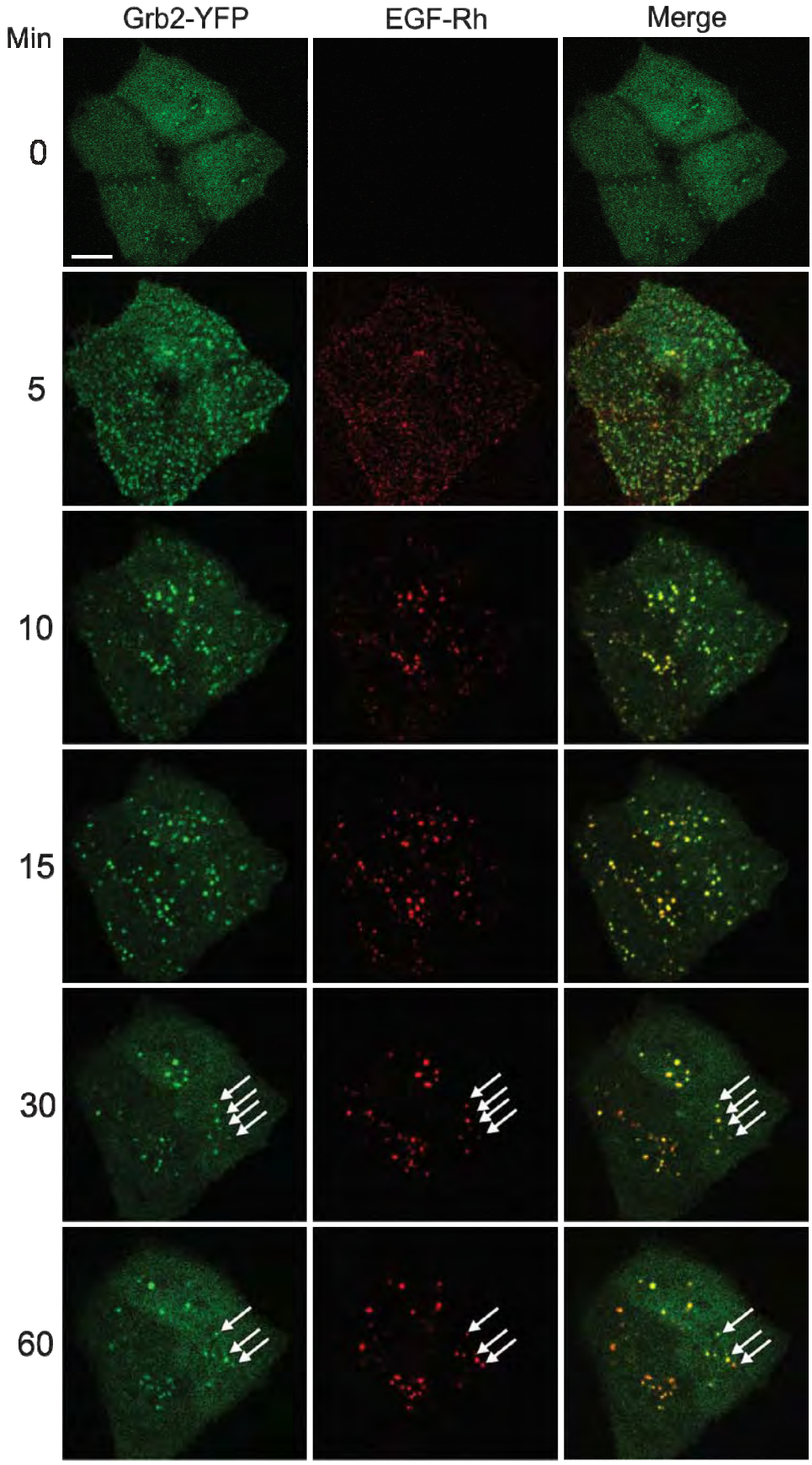


Figure S1. Western blot analysis of Grb2-YFP and Grb2 expression in parental HeLa and HeLa/Grb2-YFP cells.

Equal amounts of lysates of HeLa and HeLa/Grb2-YFP cells were resolved by SDS-PAGE, and probed by western blotting with antibodies to Grb2, GFP, EGFR and α-actinin (loading control). The amount of Grb2 immunoreactivity was 2.13-fold higher in HeLa/Grb2-YFP than in parental HeLa cells.

Figure S2. Time-lapse imaging of Grb2-YFP in cells stimulated with 20 ng/ml EGF-Rh (0-60 min) (Next page)

3D time-lapse imaging of HeLa/Grb2-YFP cells was performed during the first hour after addition of 20 ng/ml EGF-Rh to cells at 37°C with 5-min intervals between frames as described in "Methods". Selected time lapse x-y and x-z images are presented. Arrows point on examples of co-localization of EGF-Rh and Grb2-YFP in endosomes. Scale bar, 10 μm.


Min
Grb2-YFP
EGF-Rh
Merge
0
5
10
15
30
60

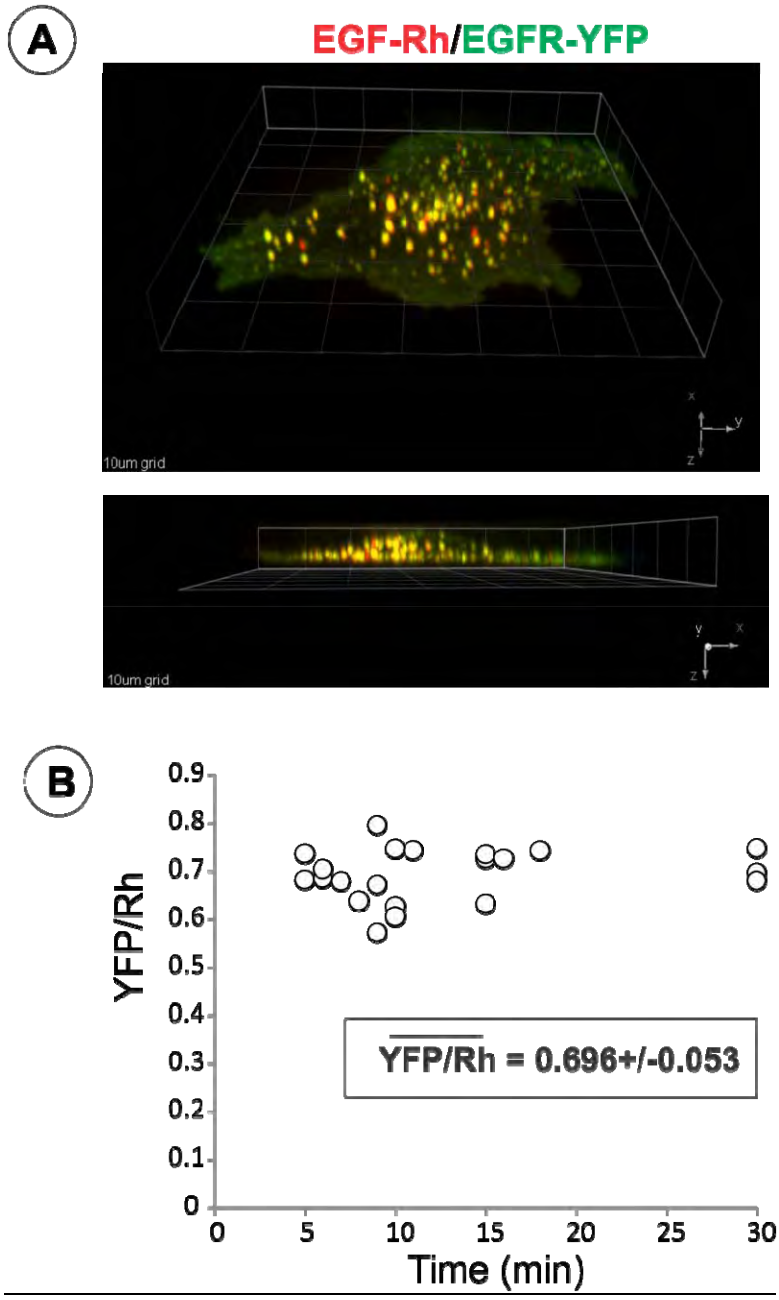


Figure S3. Apparent fluorescence intensity ratio of YFP and rhodamine in equimolar EGFR-YFP/EGF-Rh complexes.

(**A**) PAE cells transiently expressing EGFR-YFP were incubated with 100 ng/ml of EGF-Rh for 5-30 min at 37^{o}C. Live-cell 3-D imaging was performed through 515 and 561 channels as described in Figs. 1 and 2. An example of a 3-D image (two different views) of a cell incubated with EGF-Rh for 30 min is presented. Grid, 10 μm.

(**B**) Calculations of the ratio of YFP to rhodamine fluorescence (YFP/Rh) in 3-D images of cells exemplified in (***A***) were performed as described in "Methods". Each data point represents an individual cell.

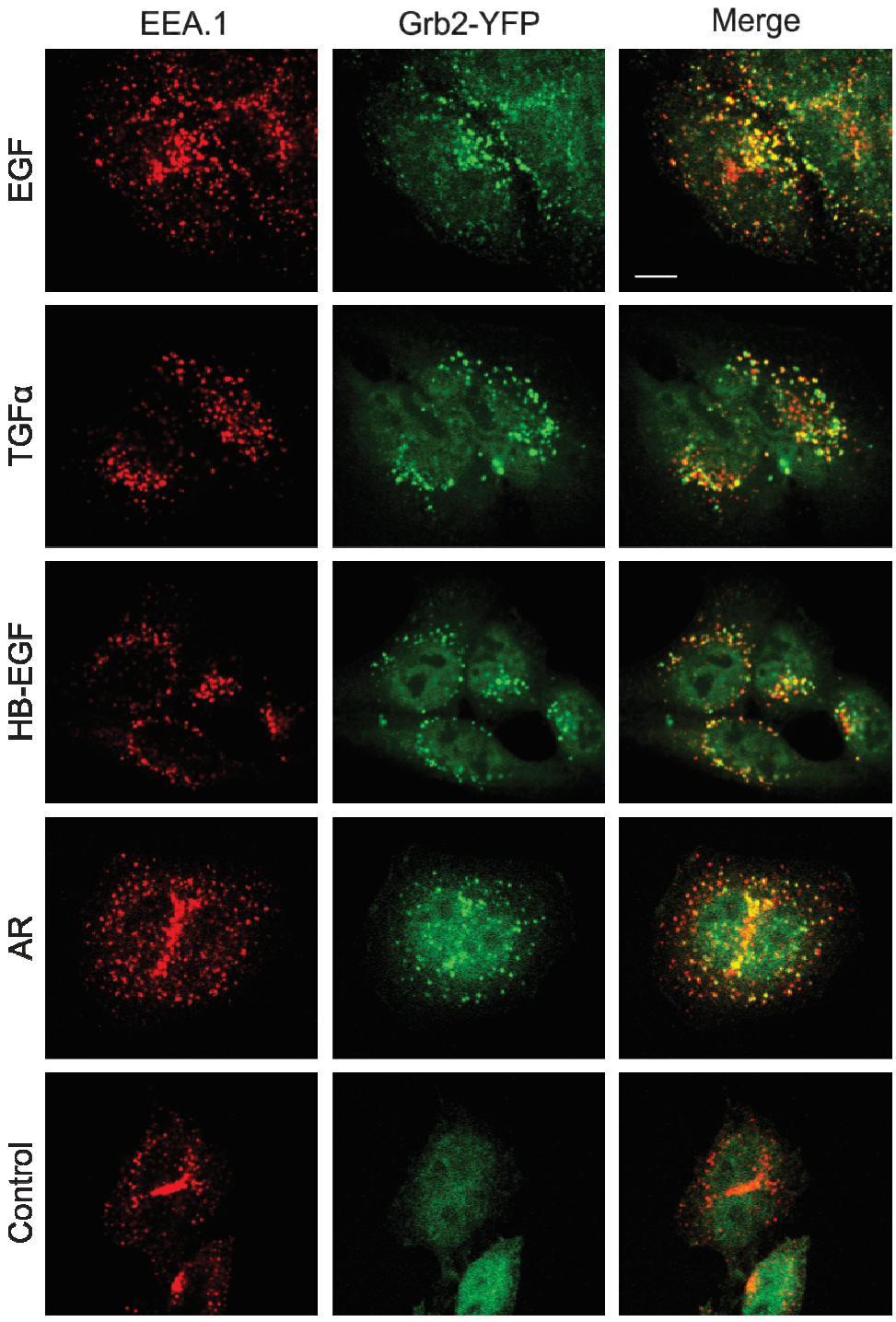
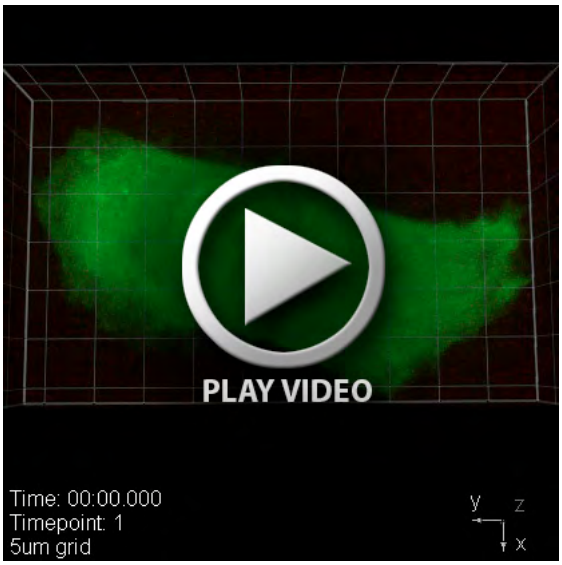
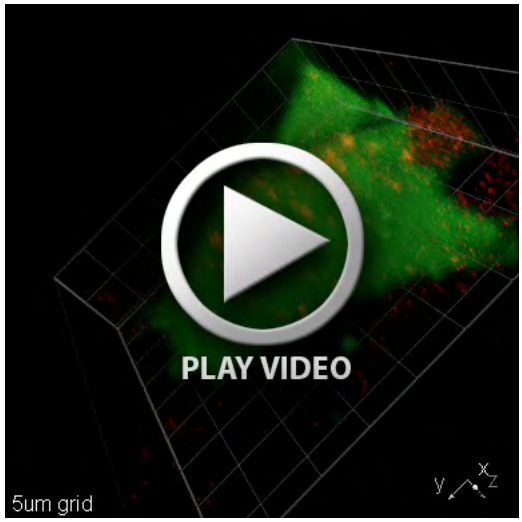


Figure S4. Colocalization of Grb2-YFP with EEA.1 in HeLa/Grb2-YFP cells treated with various unlabeled EGFR ligands.

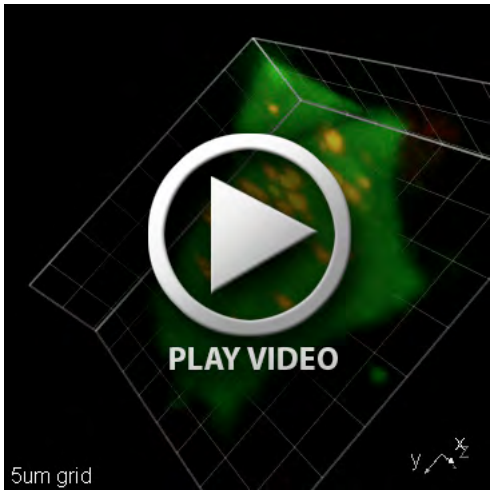
Cells grown on glass coverslips were left untreated (***Control***) or treated with EGF (2 ng/ml), TGFα (2 ng/ml), HB-EGF (2 ng/ml) or AR (400 ng/ml) at 37°C for 30 min, fixed and stained with mouse monoclonal antibody to EEA.1 followed by the secondary antibody conjugated with Cy3. Scale bar, 10 μm.



Movie 1. 3D time-lapse movie sequence of HeLa/Grb2-YFP cells treated with EGF-Rh. x-y-z view of the 3D time-lapse image sequence presented in Figure 1. The cells were incubated with 2 ng/ml EGF-Rh for 0-10 min. Merged Rhodamine (red) and YFP (green) images are shown.



Movie 2. HeLa/Grb2-YFP cells incubated with EGF-Rh for 5 min. 160 degrees rotational 3D-view of a cell treated with 2 ng/ml EGF-Rh for 5 min from the time-lapse imaging sequence presented in Figure 2. Merged Rhodamine (red) and YFP (green) image is shown.



Movie 3. HeLa/Grb2-YFP cells incubated with EGF-Rh for 30 min. 160 degrees rotational 3D-view of a cell treated with 2 ng/ml EGF-Rh for 30 min from the time-lapse imaging sequence presented in Figure 2. Merged Rhodamine (red) and YFP (green) image is shown.