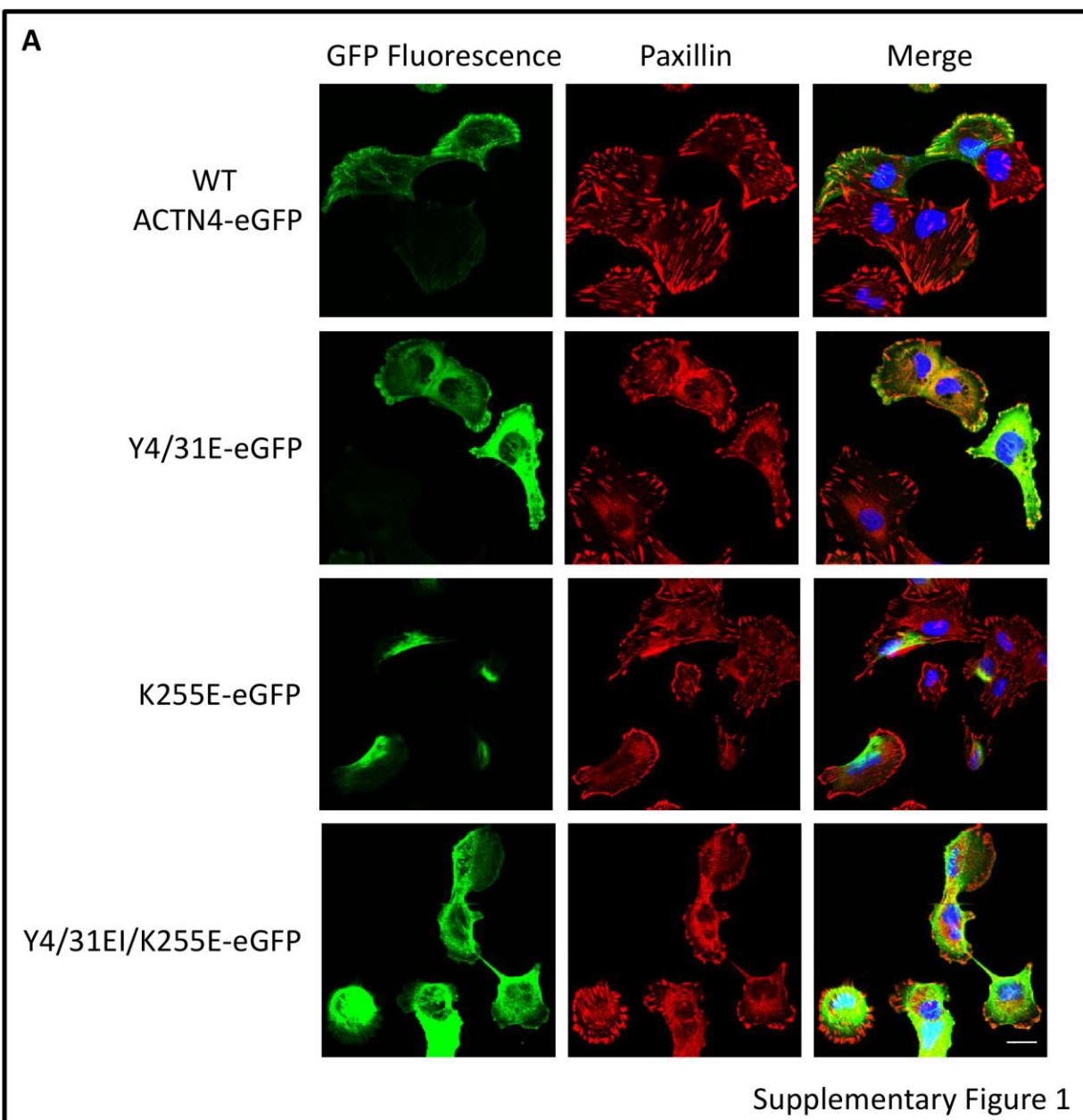




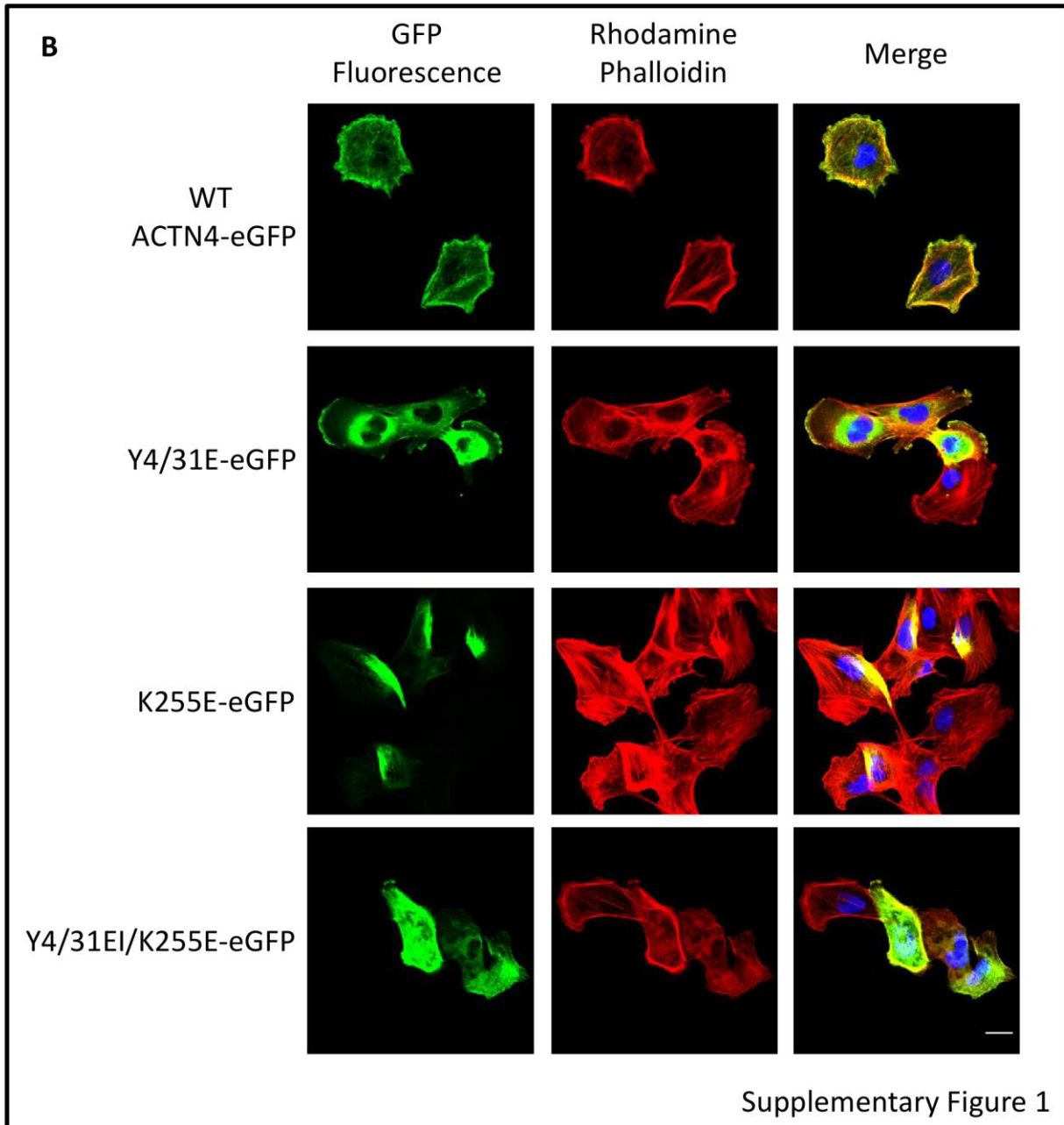


Figure S1. K255E ACTN4 does not colocalize with paxillin. **(A)** Podocytes grown in 6-well plate were transfected with indicated plasmids and then stained with paxillin antibody and DAPI. Green: fluorescence of eGFP tagged ACTN4; Red: paxillin; Blue: DAPI. **(B)** Podocytes grown in 6-well plate were transfected with indicated plasmids and then stained with rhodamine phalloidin (Red) and DAPI. Images were taken using 60x magnifications. Scale bar, 20 μ m.

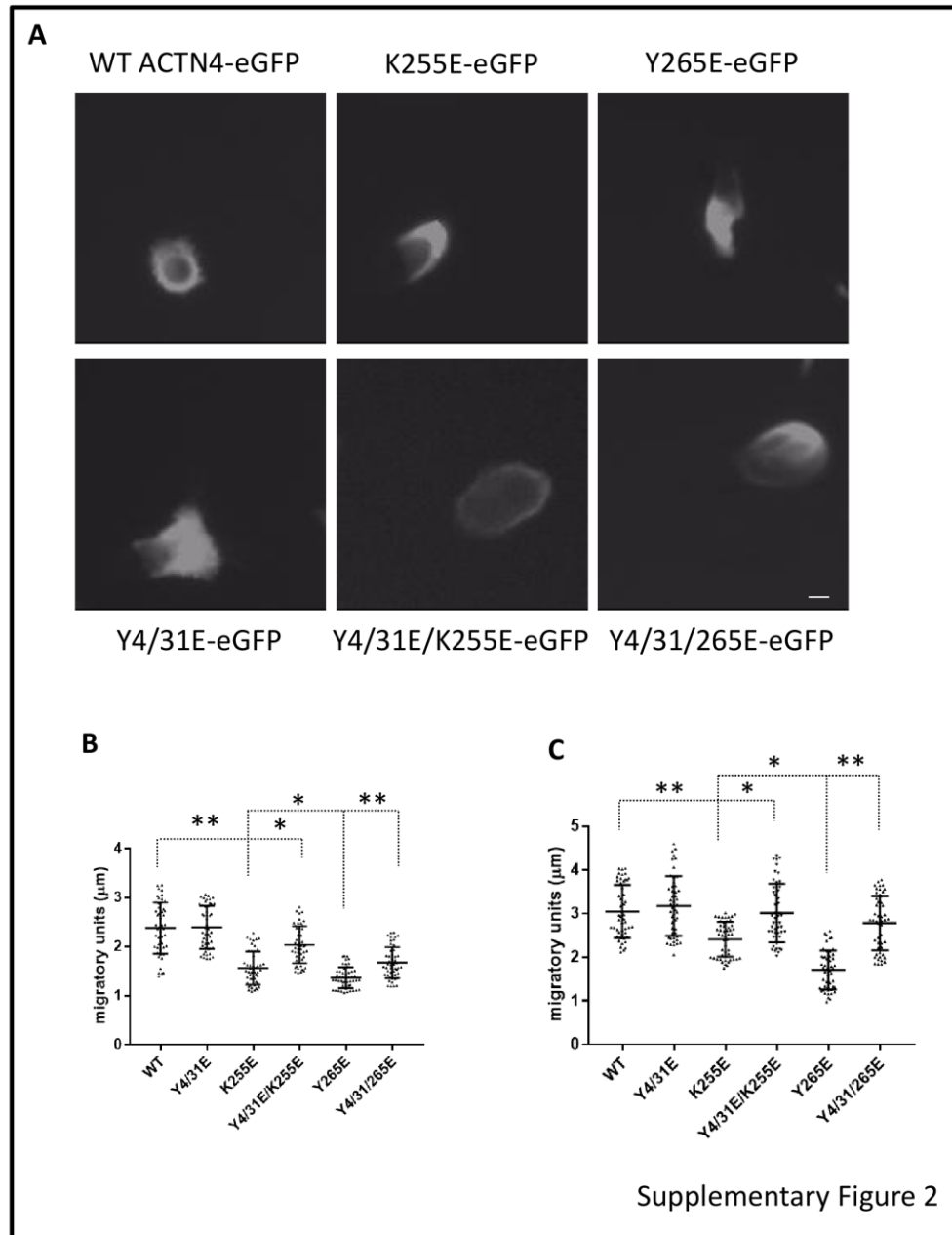


Figure S2. Y265E ACTN4 forms tight clusters during cell migration. **(A)** Representative movies of individual live migratory melanoma WM1158 cells transiently expressing GFP tagged WT or indicated ACTN4 mutants. Cells were tracked for 6 h with an interval of 10 min. Movies were created using Image J software. **(B, C)** Cell migration speed of NR6WT fibroblasts (B) and melanoma WM1158 cells (C) transiently expressing GFP tagged WT and ACTN4 mutants tracked using live microscopy and analyzed using MetaMorph software. The migratory units stand for the average distance of cells moved within 10 minutes. Cell number (N)=50. Images are a representative of three independent experiments. Data are mean of $\pm$ SD of three independent experiments. Statistical analysis was performed using Student's t-test. * $p < 0.1$, ** $p < 0.01$.

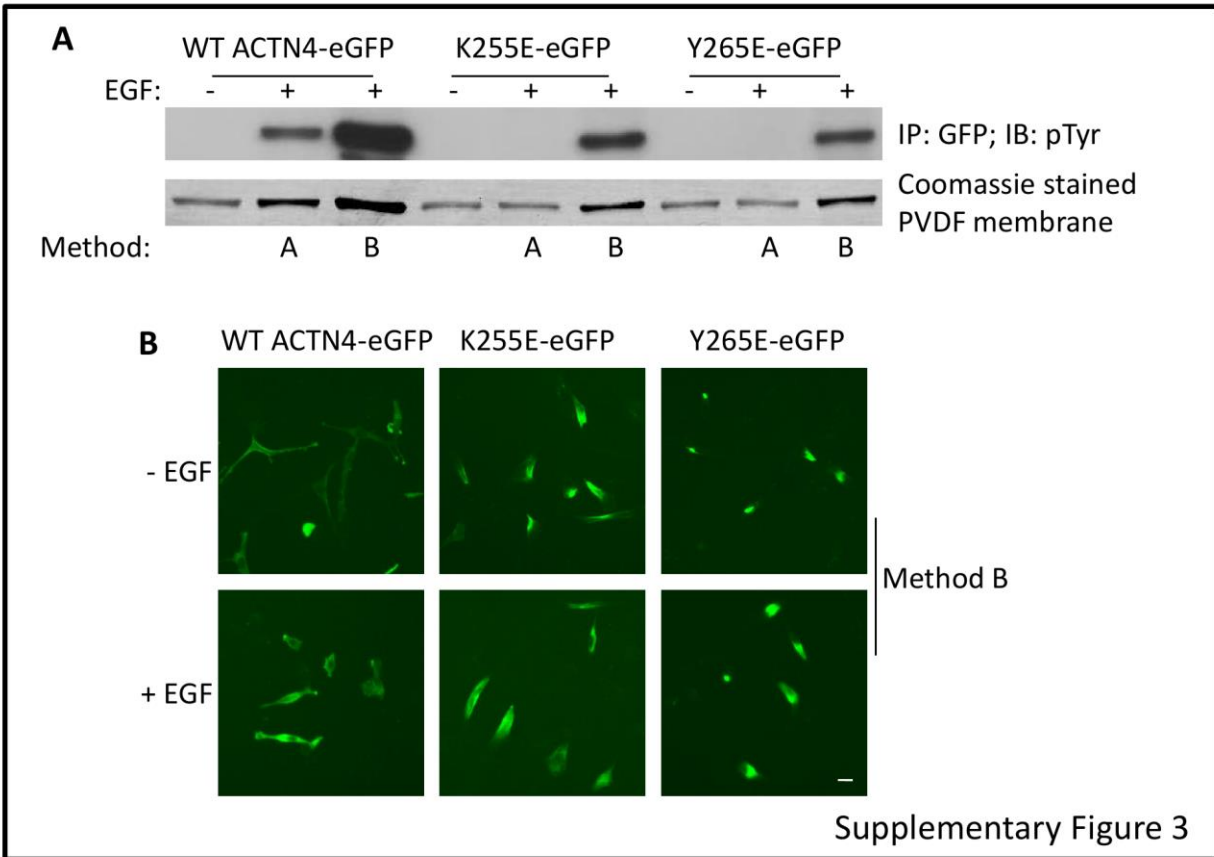


Figure S3. Continuous EGF stimulation alters the localization of both K255E and Y265E ACTN4 via phosphorylation. **(a)** Immunoblotting of immunoprecipitated GFP tagged WT, K255E and Y265E from NR6WT cells treated with EGF using method A or B. **(b)** Localization of GFP tagged WT, K255E and Y265E in NR6WT cells treated with EGF using method B. Shown are representative of three independent experiments. Scale bar, 20 μ m.

Figure S4. Original full blots of the cropped ones in the figures.

ACTN4: 0 0.5 1 2 4 8 μ M

Kd S P S P S P S P S P S P S P

WT

170
130
100
70
55
40
35
25

← ACTN4

← Actin

Y4/31E

170
130
100
70
55
40
35
25

← ACTN4

← Actin

K255E

170
130
100
70
55
40
35
25

← ACTN4

← Actin

Y4/31E/K255E

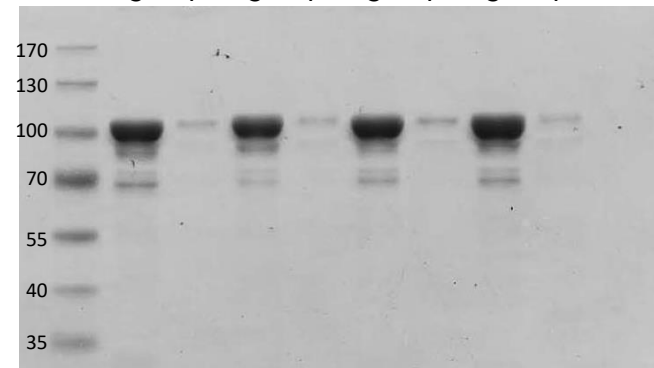
170
130
100
70
55
40
35
25

← ACTN4

← Actin

1B

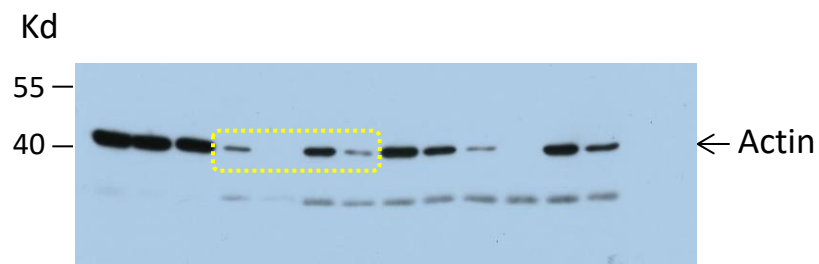
WT Y4/31E K255E Y4/31E/K255E
S P S P S P S P



← ACTN4

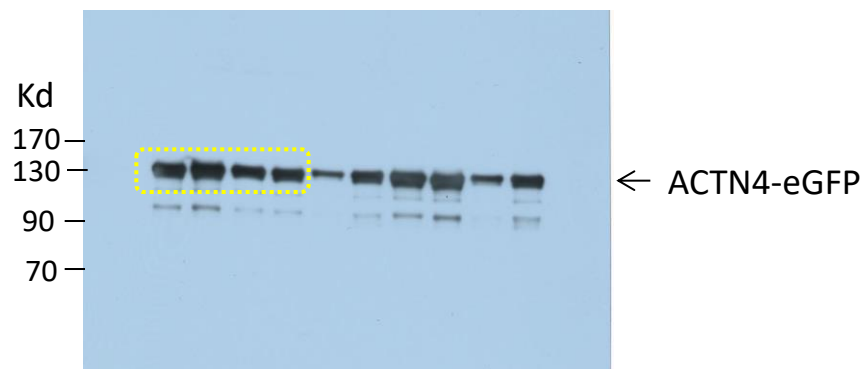
1C

Figure 1



IP: GFP
IB: Actin

Figure 2A



IP: GFP
IB: GFP

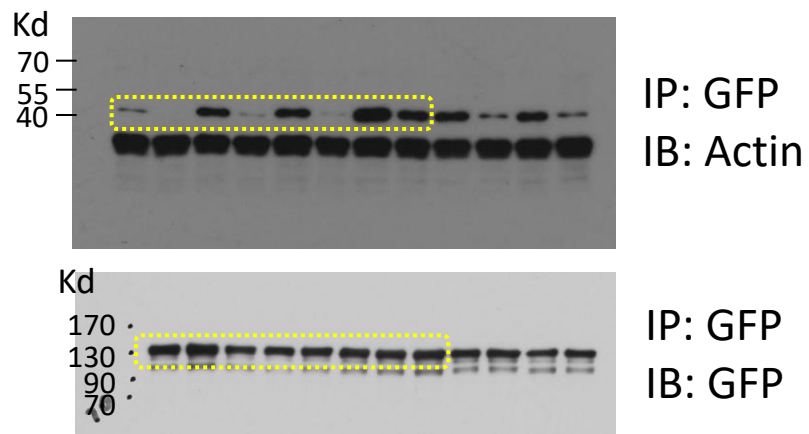


Figure 2B

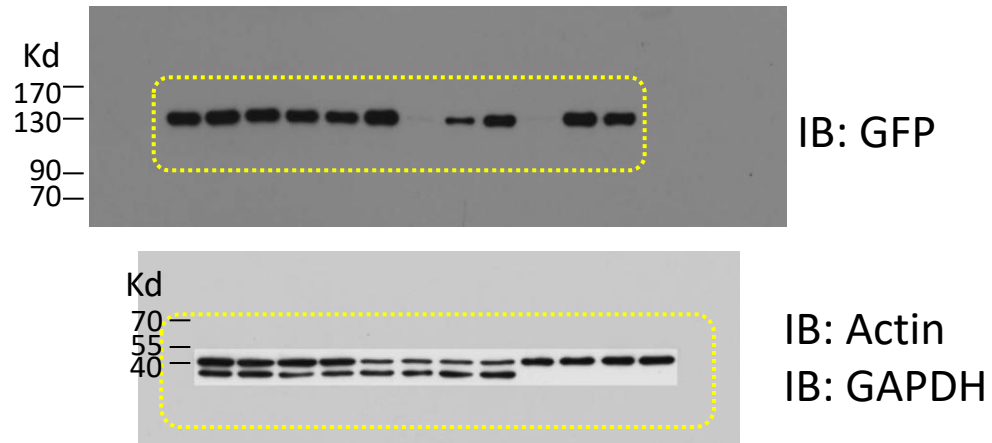


Figure 3D

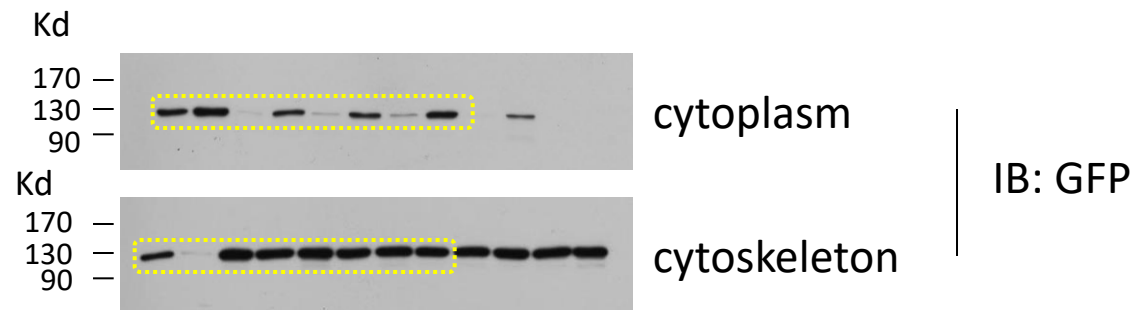


Figure 3E

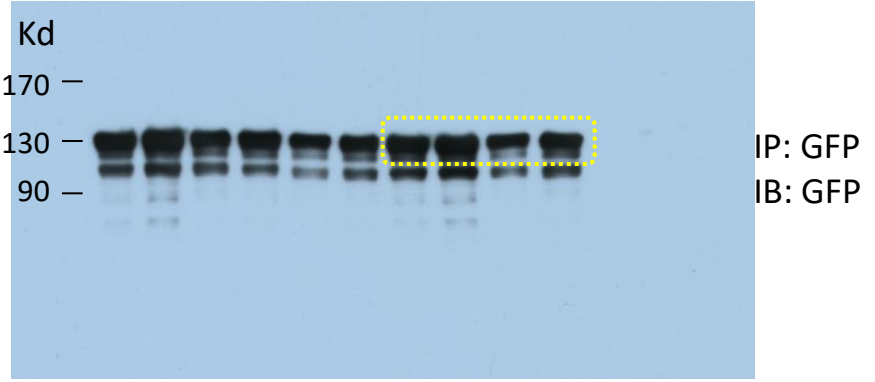
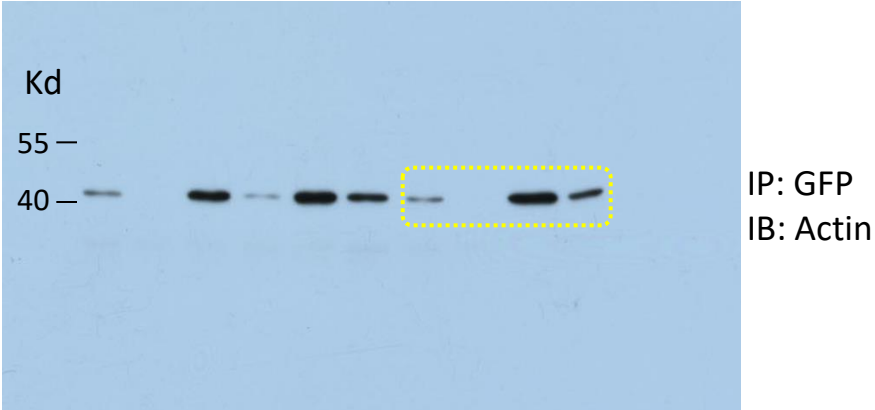


Figure 5A

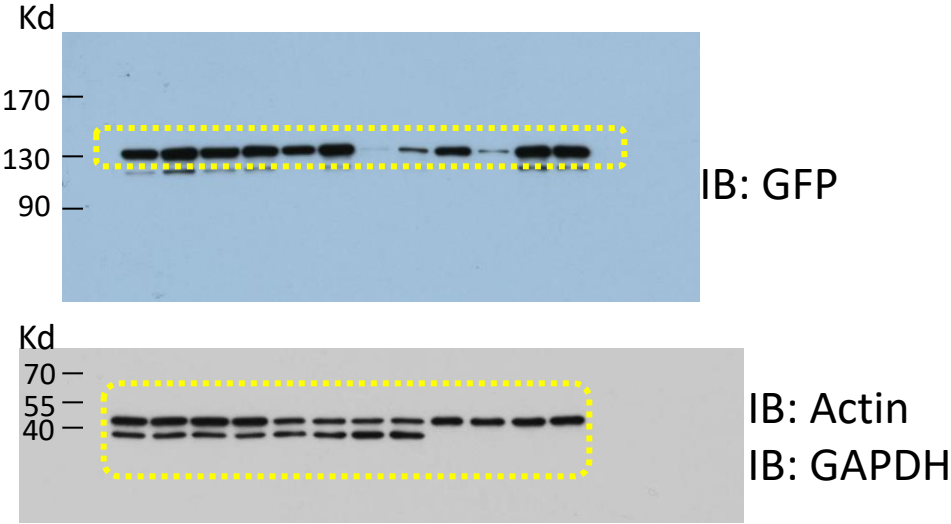


Figure 5B

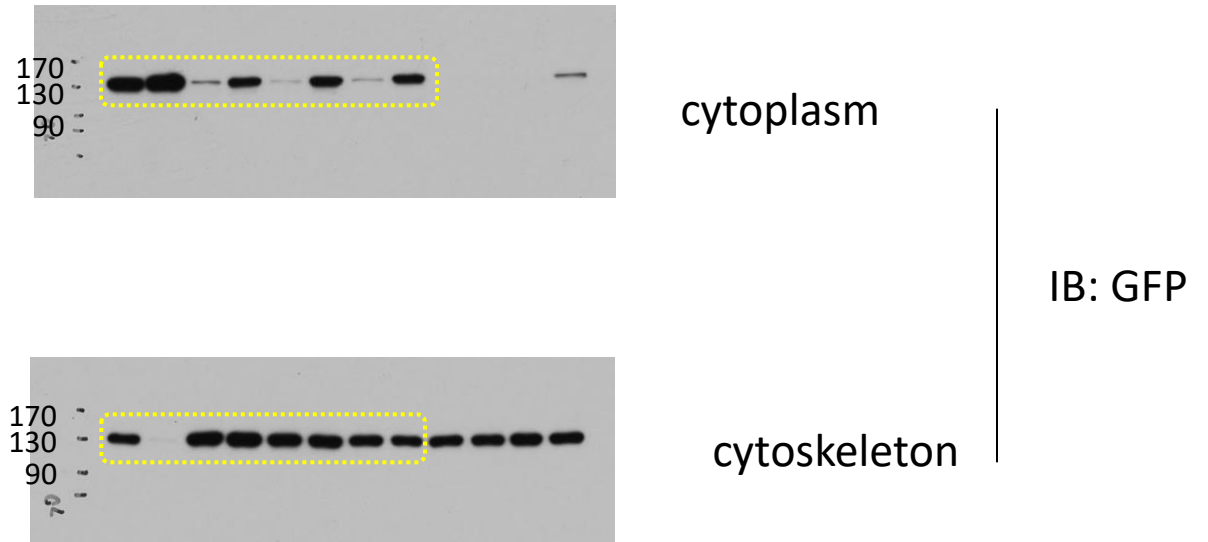


Figure 6A

Figure 7A

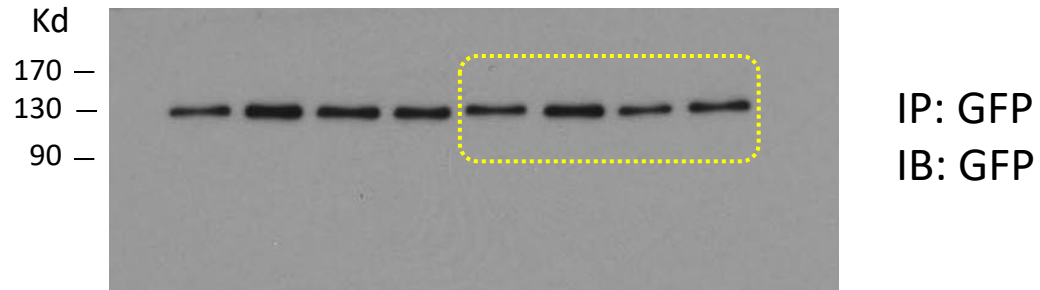
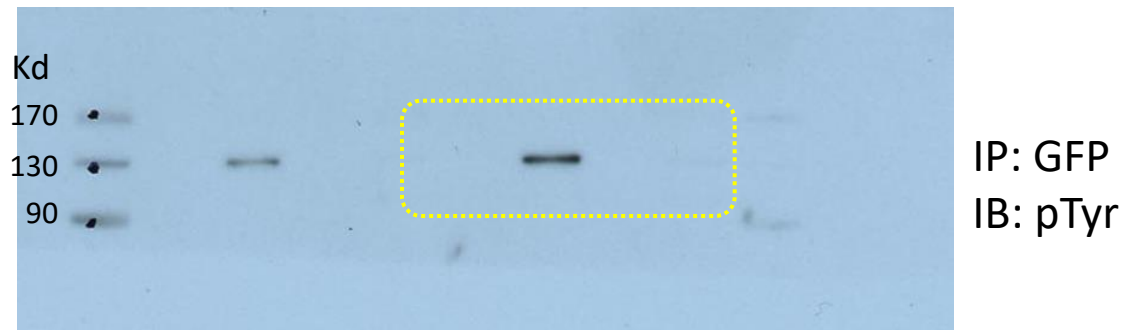


Figure 7B

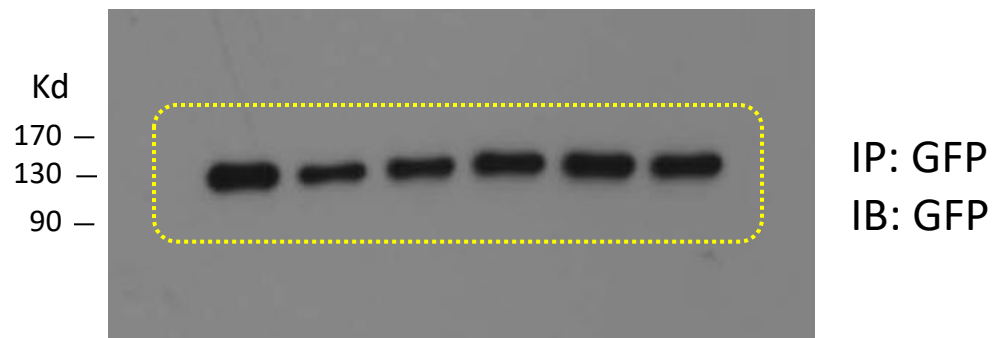
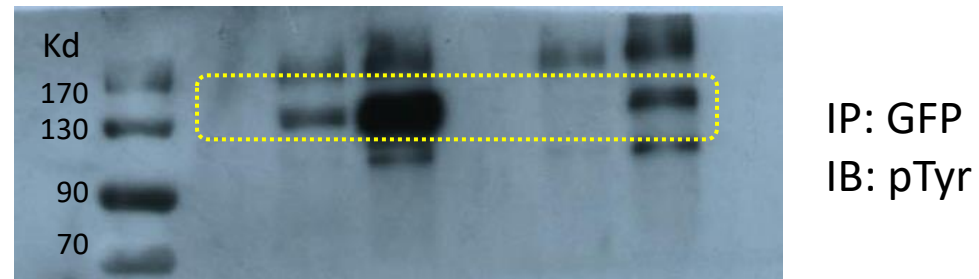
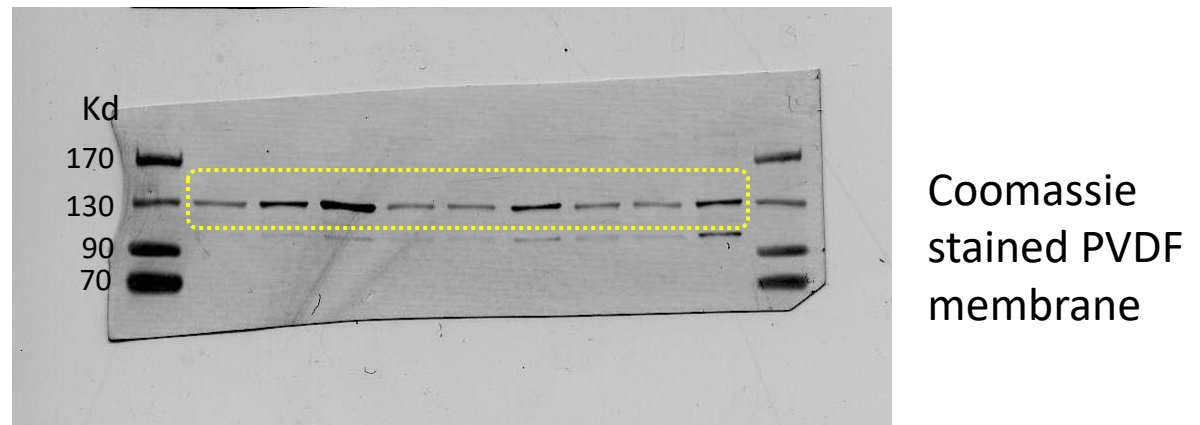
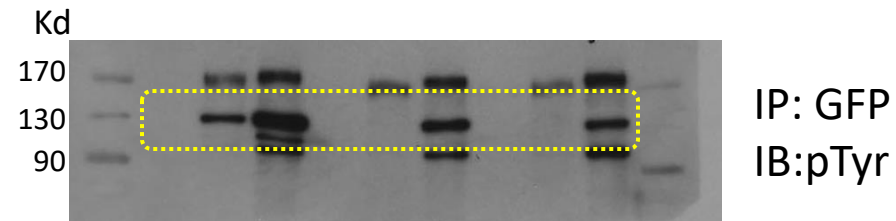


Figure 7



Supplemental Figure 3A

Supplemental Figure 3