Supplemental information

Regulation of DENND3, the exchange factor for the small GTPase Rab12 through an intramolecular interaction

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Running title: Regulation of DENND3

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Figure legends

SUPPLEMENTAL FIGURE 1. Secondary structure prediction for two regions of DENND3.
Region surrounding Y940 of DENND3, underlined, is predicted as a loop. (from the PSIPRED protein structure prediction server http://bioinf.cs.ucl.ac.uk/psipred/)

SUPPLEMENTAL FIGURE 2. Full-length DENND3 does not bind to the linker *in trans*.
HEK-293T cells were transfected with Flag-tagged wild-type DENND3, its Y940D/Y940F mutant, or Flag-tagged Ext-DENN. Lysates were incubated with 5 µg GST or GST-linker coupled to glutathione-Sepharose beads for 1 hour at 4 °C. Proteins specifically bound to the beads were processed for Western blot with anti-Flag antibody. An aliquot of the lysate (starting material; SM) equal to 5% of that added to the beads was analyzed in parallel.

SUPPLEMENTAL FIGURE 3. There is no cross talk between the phosphorylation of tyrosine 940 and ULK-mediated phosphorylation.

A, HEK-293T cells were transfected with Flag-DENND3 wild-type, S554A, Y940D or Y940F mutant. Lysates were incubated with GST, GST-14-3-3ε wild-type or K50E mutant coupled to glutathione-Sepharose beads. Protein specifically bound to the beads was processed for Western blot with anti-Flag antibody. An aliquot of the lysate (starting material; SM) equal to 10% of that added to the beads was analyzed in parallel.

B, HEK-293T cells were transfected with Flag-DENND3 wild-type, Y940D or Y940F. Lysates were processed for Western blot with anti-Flag antibody or anti-pS554 antibody.

SUPPLEMENTAL FIGURE 4. Phosphorylation of Y940 in the linker alters the conformation of DENND3.

A, B and C, Cells were transfected with Flag-DENND3 wild-type (A), Y940D (B) or Y940F (C) mutants. Lysates were subjected to glycerol gradient centrifugation. The fractions were collected from the top of gradient and then analyzed by Western blot with the indicated antibodies.

SUPPLEMENTAL FIGURE 5. DENND3 oligomerization is not kept by the interaction between the linker and Ext-DENN.

A, A potential model showing a possible organization of DENND3 oligomer.

B, Lysates from HEK-293T cells co-transfected with Flag- and HA-DENND3, Flag- and HA-DENND3 Y940F, or Flag- and HA-DENND3 Y940D were incubated with protein G beads alone or protein G beads coupled to anti-Flag antibody (IP-Flag). Proteins bound specifically to the beads were processed for Western blot with anti-Flag or anti-HA antibody.

SUPPLEMENTAL FIGURE 6. The intramolecular interaction blocks access of Rab12 to Ext-DENN.

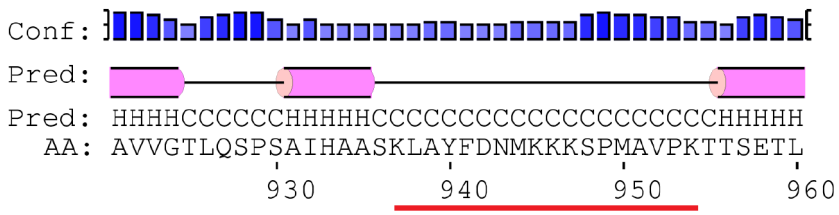
Cells were transfected with Flag tagged full-length Ext-DENN or Flag-Ext-DENN with deletion from residue 21 to 30. With presence of 5 mM EDTA, lysates were incubated with 10 μg GST or GST-Rab12 coupled to glutathione-Sepharose beads with or without addition of 20 μg purified linker. Proteins specifically bound to the beads were processed for Western blot with anti-Flag antibody. An aliquot of the lysate (starting material; SM) equal to 1% of that added to the beads was analyzed in parallel.

SUPPLEMENTAL FIGURE 7. DENND3 subcellular localization is not affected by Y940 mutation.

A, HeLa cells transfected with wild-type DENND3 with Flag tag at N-/C- terminal or Flag-DENND3 Y940D/Y904F/S554A were processed for immunocytochemistry. In the left column of panels the cells were transfected 16 hours. In the right column the cells were transfected only 10 hours. The scale bar represents 5 μM.

B, Lysates from HEK-293T cells transfected with Flag-tagged wild-type DENND3 or its Y940D/Y940F mutant were spun at 800 g for 10 min at 4 °C. The supernatant was then centrifuged at 200,000 g for 30 min at 4 °C, the resulting supernatant (S) and pellet (P) was processed for SDS-PAGE and Western blot with anti-RME-8, anti-Flag, anti-Na^+K^+-ATPase or anti-GAPDH antibody.

C, HeLa cells co-transfected with GFP-Rab12 and Flag-tagged wild-type DENND3 or its Y940D/Y940F mutant were processed for pre-permeabilization with saponin and subsequent immunocytochemistry. The scale bar represents 5 μM.



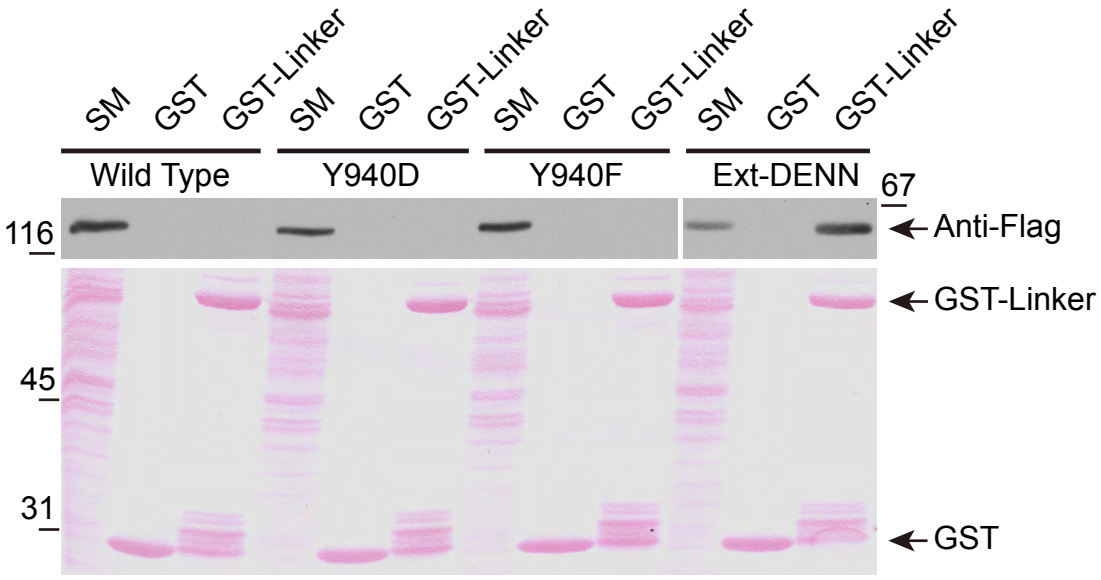
Legend:

= helix Conf: = confidence of prediction

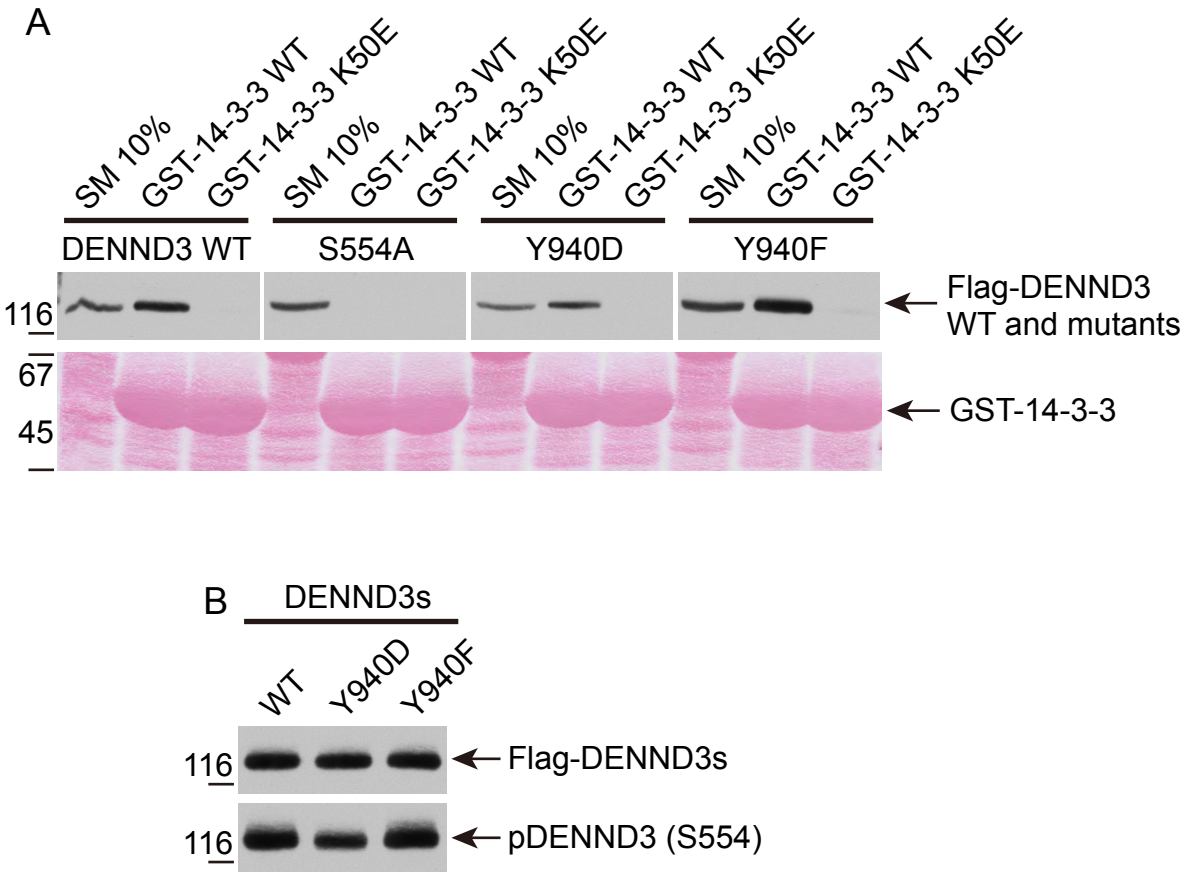
= coil AA: target sequence

Pred: predicted secondary structure

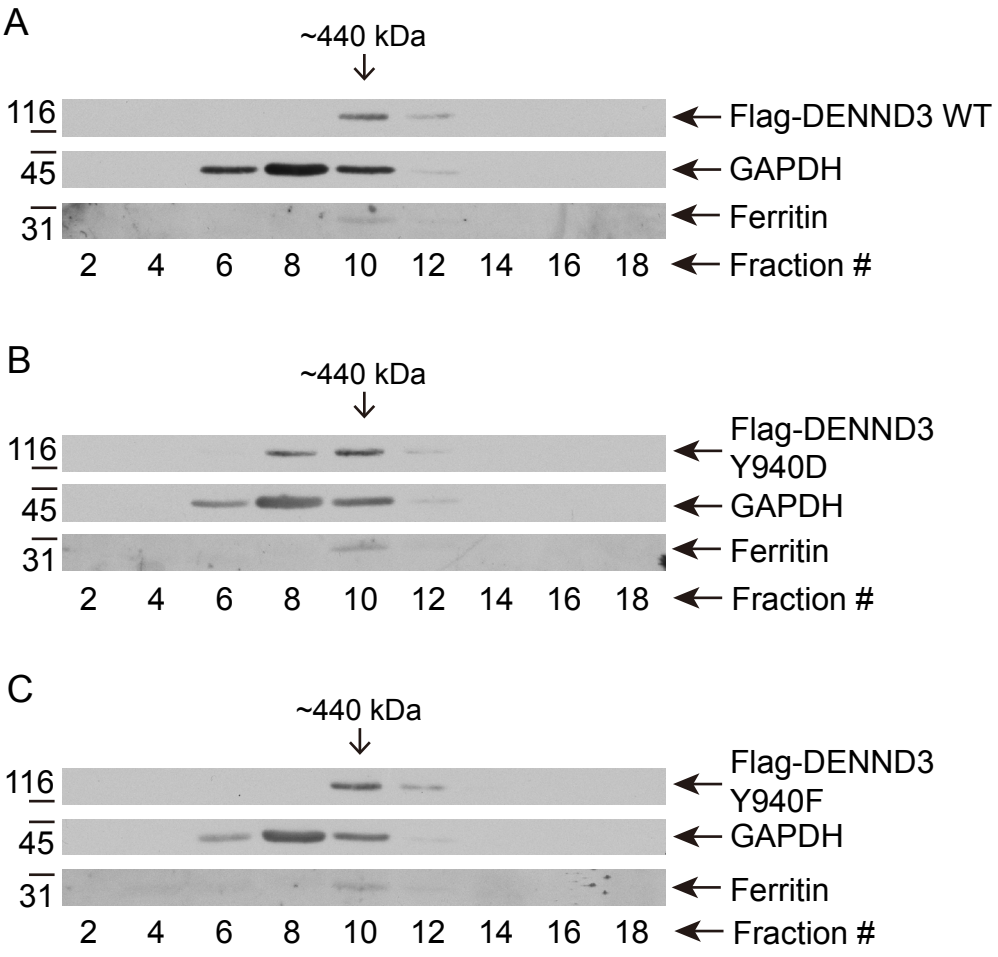
Xu et al., Supplemental Figure 1



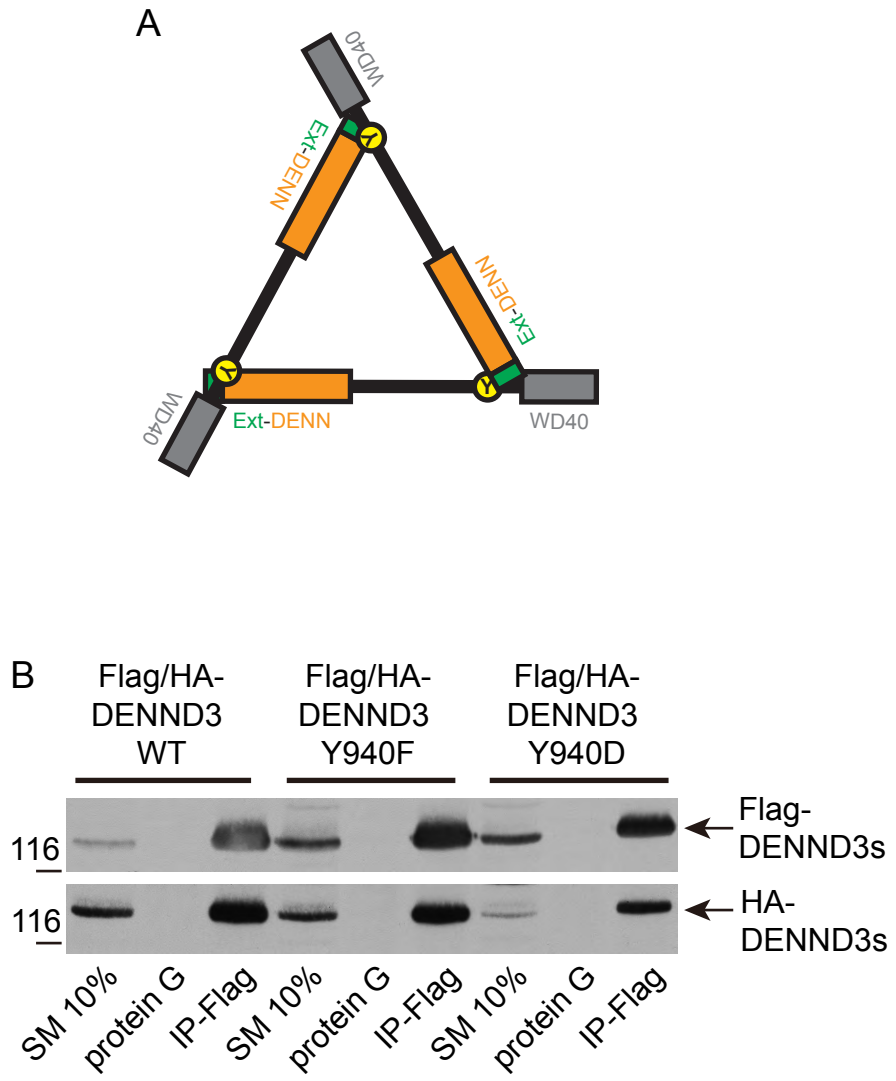
Xu et al., Supplemental Figure 2



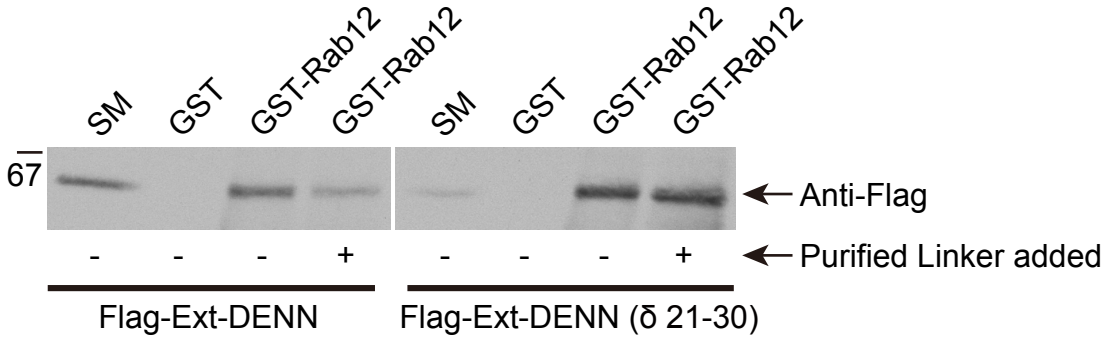
Xu et al., Supplemental Figure 3



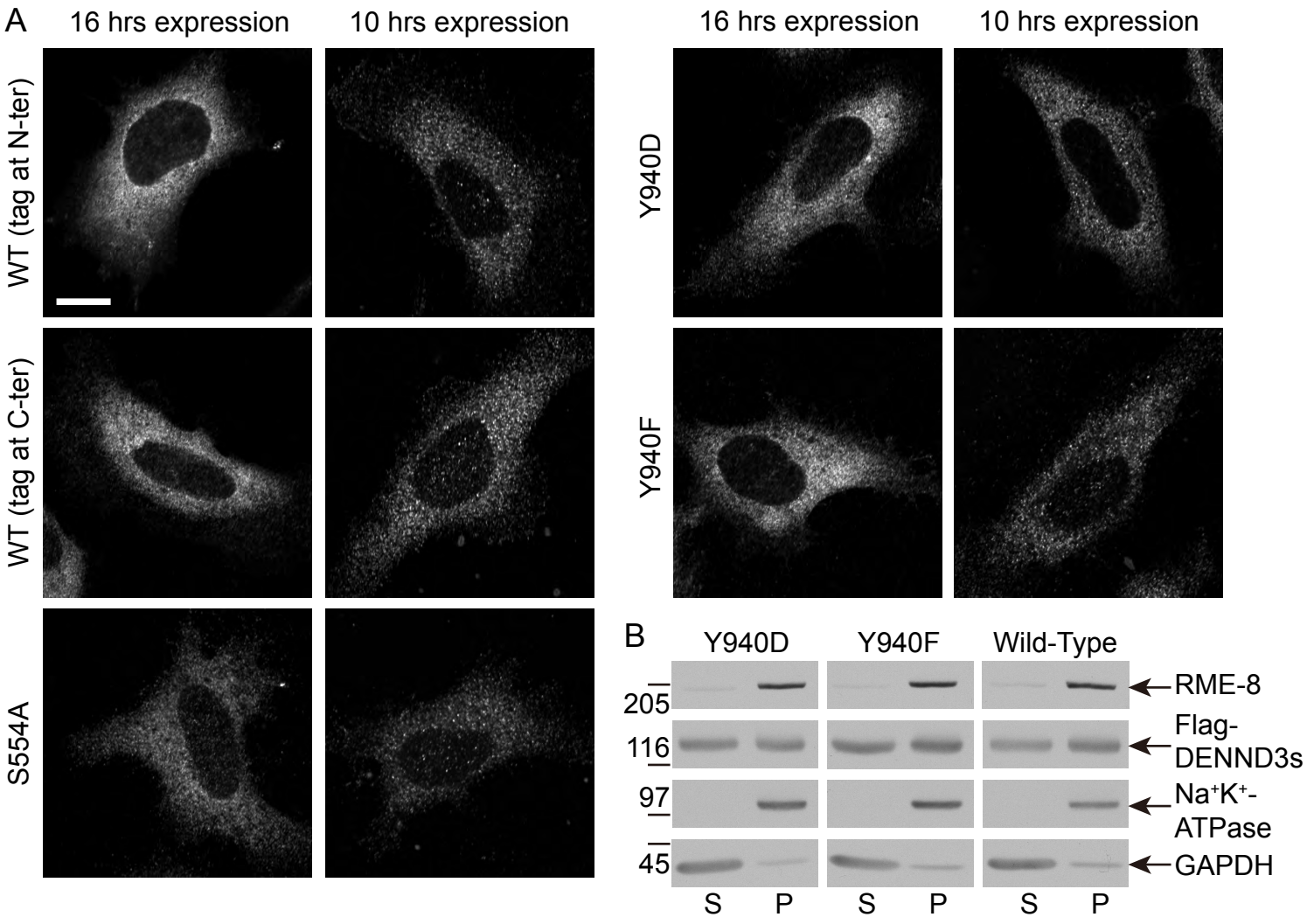
Xu et al., Supplemental Figure 4

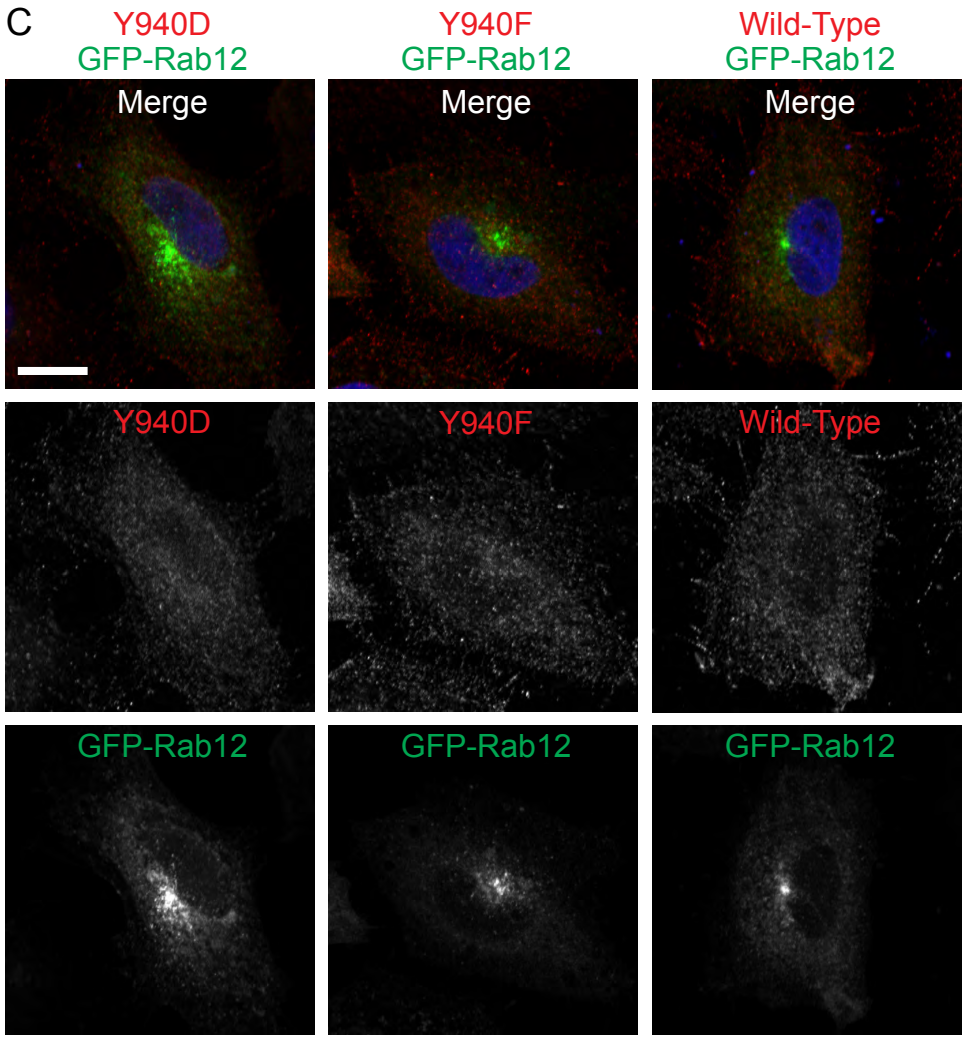


Xu et al., Supplemental Figure 5



Xu et al., Supplemental Figure 6





Xu et al., Supplemental Figure 7