Supplemental Materials

Molecular Biology of the Cell

Mani et al.

Developmentally regulated GTP-binding protein 2 coordinates Rab5 activity and transferrin recycling.

Muralidharan Mani[1], Unn Hwa Lee[1], Nal Ae Yoon[1], Hyo Jeong Kim[1], Myoung Seok Ko[2], Wongi Seol[3], Yeonsoo Joe[1], Hun Taeg Chung[1], Byung Ju Lee[1], Chang Hoon Moon[4], Wha Ja Cho[4], Jeong Woo Park[1]

[1]Department of Biological Sciences, University of Ulsan, Ulsan 680-749, Korea

[2]Department of Medical Science, University of Ulsan College of Medicine, Seoul 138-736, Korea

[3]Inam Institute for Brain Science, Wonkwang University Sanbon Hospital, Gunpo 435-040, Korea

[4]Biomedical Research Center, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan 682-060, Republic of Korea

Running title: DRG2 coordinates Rab5 activity

Address correspondence to: Jeong Woo Park, E-mail: jwpark@ulsan.ac.kr

Table S1, Related to Materials and Methods. Oligonucleotide sequences used for real-time and semi-quantitative PCR and generation of plasmid constructs

Gene or construct name	Primer Sequence(5' → 3')
pmRFP-hDRG2	CGGGATCCACCATGGGGATCTTAGAGAAG, CCGCTCGAGTACTTCTTCACGATCTGGAT
pEGFP-hDRG2	CGCGGATCCACTATGGGGATCTTGGA, CCGCTCGAGCACTTCTTCACAATCTG
pEGFPC1-RabGAP5	GGAATTCGATGTCAGGAAGCCATACA CGGGATCCTCACCCGTCCACATCCCA
pEGFPN1-shRNAR-DRG2	CGGGATCCACCATGGGGATCTTAGAGAAG, CCGCTCGAGCTTCTTCACGATCTGGAT, GCCCTGTTTGTACGTTTATAA, TTATAAACGTACAAACAGGGC
DRG2	CTCAACAGTCACACTGACAC, TACCGCAACTGATAACTACA
EEA1	GATGGGTTGGTGACTGATTCA ACTCCTTAGCTGGCTTATTGT
DRG2-g*	GAGCGGAGGTGATGAGAGTCAAGAG, ACTCTGGCAACTCTCTGCAACAACA
PGK-neo-g*	AGCACGTACTCGGATGGAAGCCGGTC, ACTCTGGCAACTCTCTGCAACAACA
GAPDH	ATGACAACTTTGGCATTGTG,

	CATACTTGGCAGGTTTCTCC

*Primers for DRG2 KO mice genotyping

Restriction sites used for subcloning are underlined.

Supporting Figures

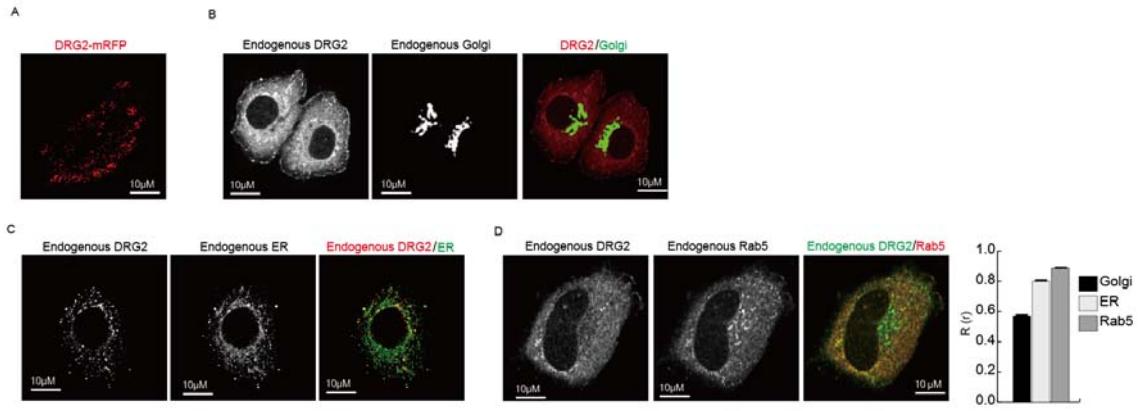

Figure S1, Related to Figure 1. Subcellular localization of DRG2. (A) MCF7 cells were transfected with mRFP-DRG2. (B-D) MCF7 cells were stained for endogenous DRG2 and (B) Golgi apparatus, (C) endoplasmic reticulum, or (D) Rab5. Representative confocal images are displayed. Graph represents Pearson's co-localization coefficient (R(r)) between DRG2 and organelle markers. Values are mean ± SD from three separate experiments, with 10 different cells per group per experiment.

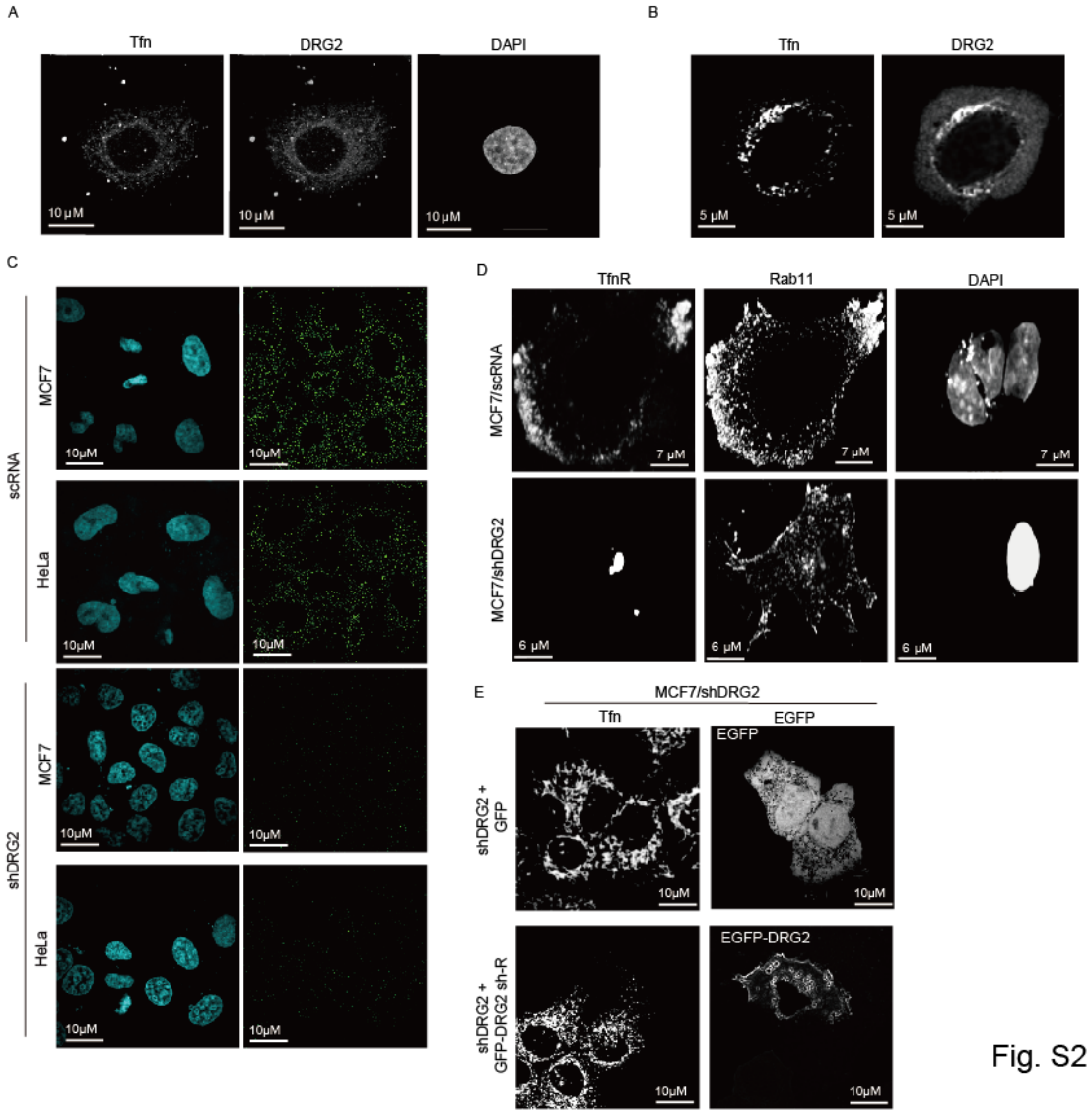

Fig. S2

Figure S2, Related to Figure 3. Cells are treated as described in the legend of Figure 3. (A) Single channel confocal images of DRG2, Tfn, and nucleus (DAPI) in MCF7 cells. (B) Single channel confocal images of DRG2 and Tfn receptor (TfnR) in HeLa cells. (C) Single channel confocal images of endogenous DRG2 and nucleus (DAPI) in control and DRG2-depleted MCF7 and HeLa cells. (D) Single channel confocal images of TfnR and Rab11 in control and DRG2-depleted MCF7 cells. (E) Single channel confocal images of GFP and Tfn.

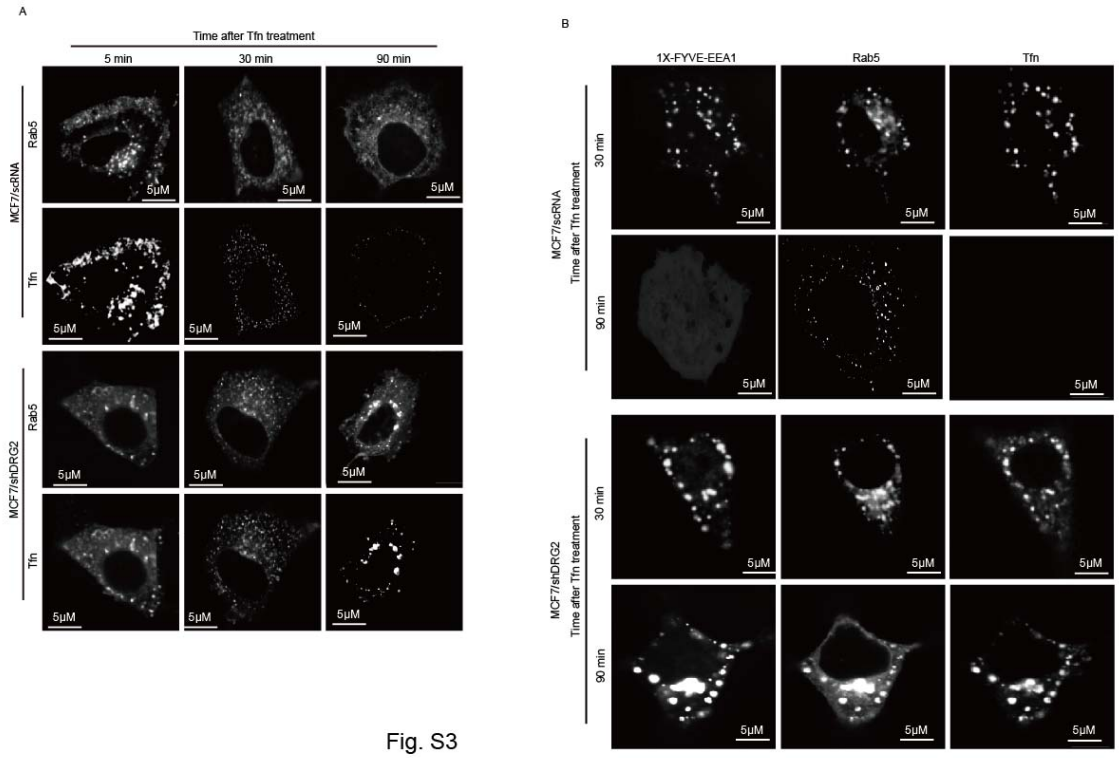

Fig. S3

Figure S3, Related to Figure 4. Cells are treated as described in the legend of Figure 4. (A) Single channel confocal images of Rab5 and Tfn in control and DRG2-depleted MCF7 cells. (B) Single channel confocal images of EEA1-FYVE, Rab5 and Tfn in control and DRG2-depleted MCF7 cells.

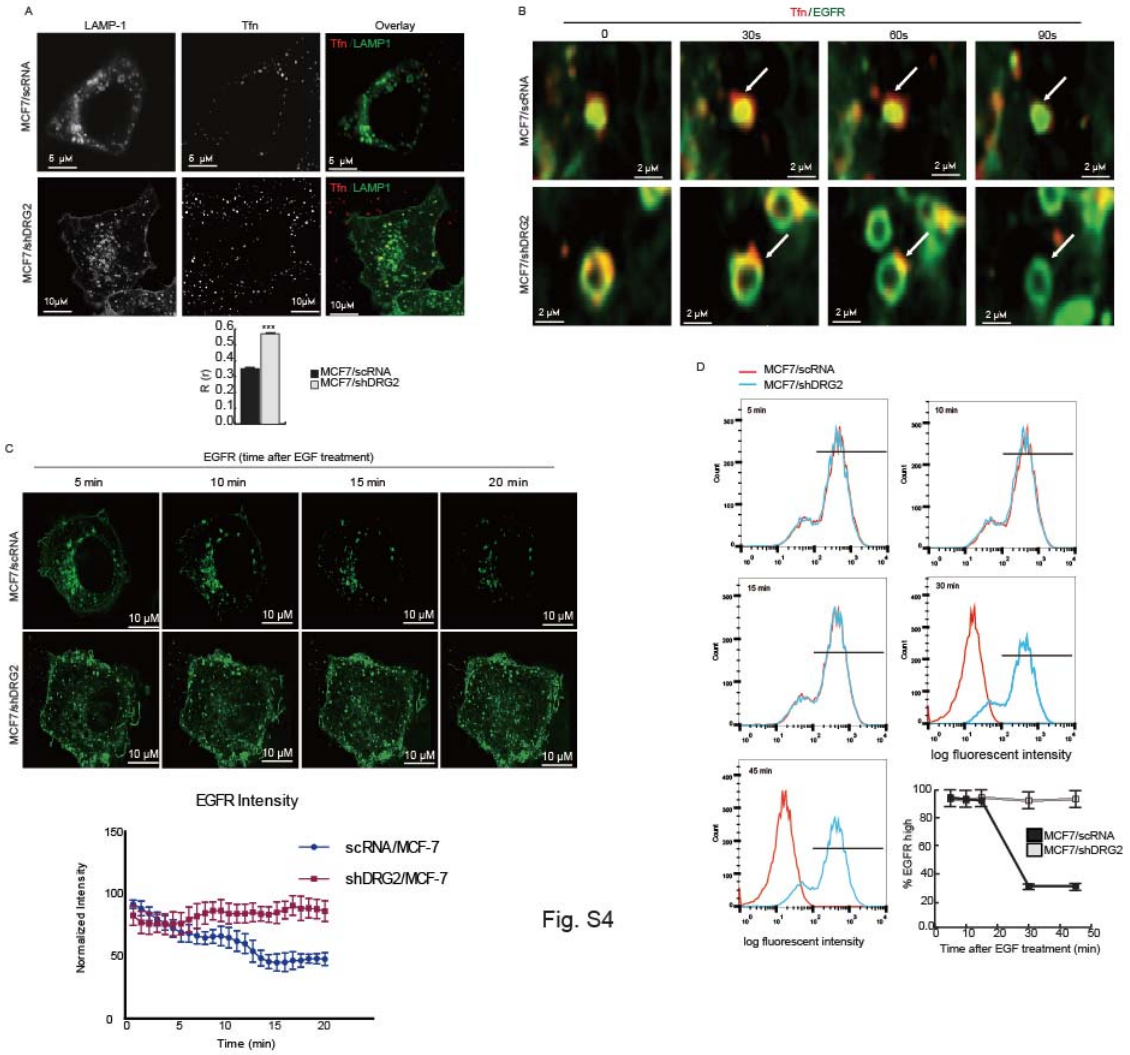

Fig. S4

Figure S4, Related to Figure 4. DRG2 depletion does not affect sorting of Tfn and EGFR into different endosomes but inhibits EGFR degradation. (A) DRG2 depletion increases localization of Tfn to LAMP1-containing endosomes. Control and DRG2 depleted MCF7 cells were transfected with mGFP-LAMP1 and incubated with Alexa 594-conjugated Tfn. Representative confocal images are displayed. Graph shows Pearson's co-localization coefficient (R(r)) between LAMP1 and Tfn. (B) DRG2 depletion does not affect the sorting of Tfn and EGFR. Control and DRG2-depleted MCF7 cells were transfected with GFP-EGFR and incubated with Alexa 594-conjugated Tfn and EGF and imaged at 30 s intervals by time-lapse confocal microscopy after incubation with Tfn for 20 min. (C

and D) DRG2 depletion inhibits EGFR degradation. Control and DRG2-depleted MCF7 cells were transfected with GFP-EGFR and incubated with 100 ng/mL EGF for the indicated times. (C) Representative confocal images are displayed. Graph represents the normalized fluorescent intensities in arbitrary units (AU). All values are mean ± SD from three separate experiments, with 5 different cells per group per experiment. $p^{***}<0.001$. (D) Flow cytometry histogram of control and DRG2-depleted MCF7 cells. Graph represents the mean ± SD from three independent experiments.

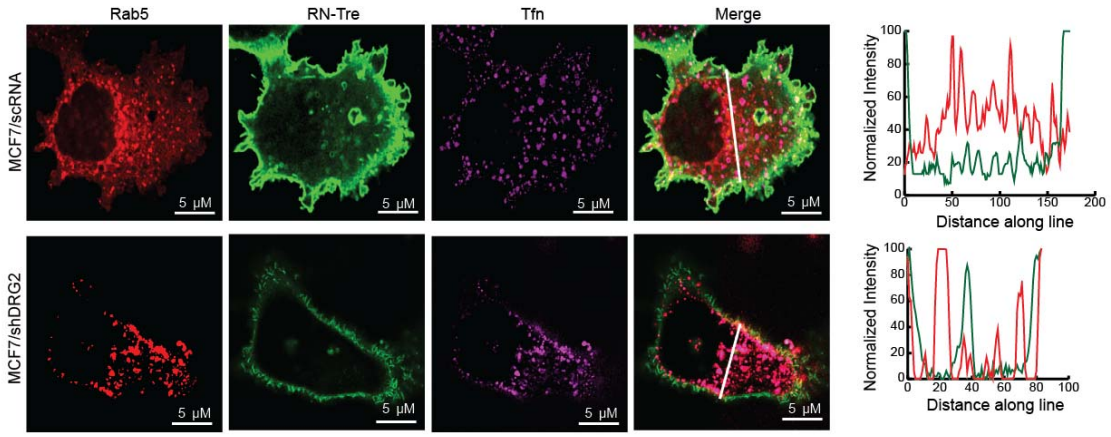

Fig. S5

Figure S5, Related to Figure 5. RN-Tre does not localizes on Rab5 endosomes. Control and DRG2-depleted MCF7 cells were cotransfected with mRFP-Rab5 and EGFP-RN-Tre and incubated with Tfn-Alexa 647 for 20 min. Representative confocal images are displayed. Graphs represent linear pixel values across cells.

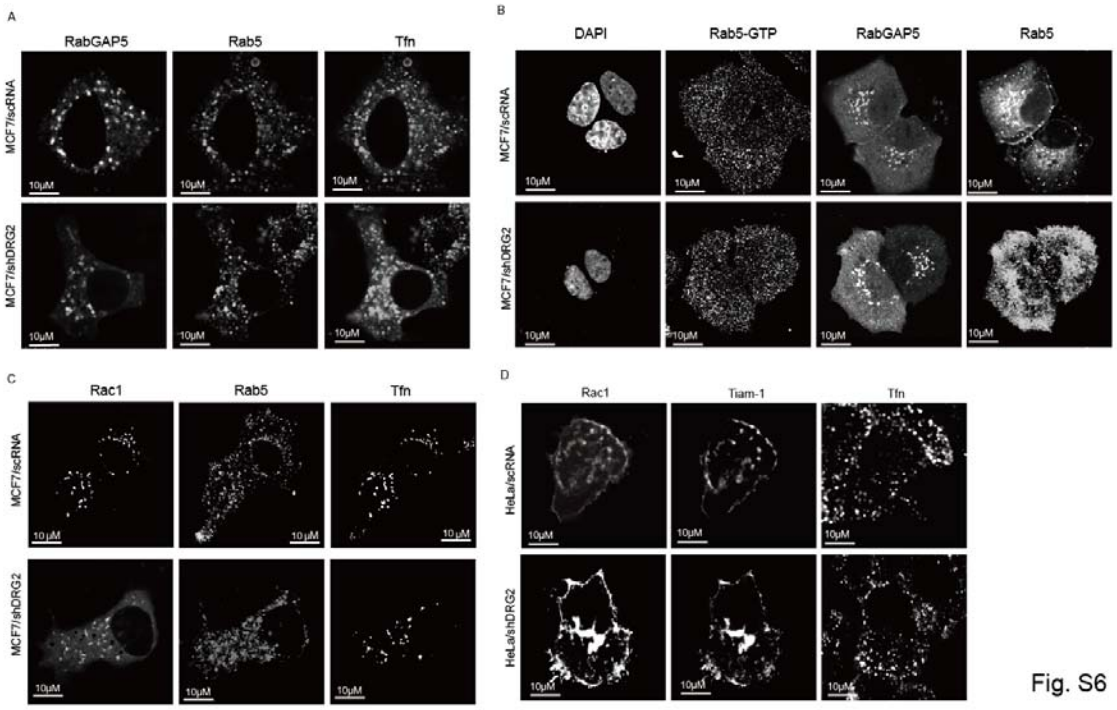

Figure S6, Related to Figure 5. Cells are treated as described in the legend of Figure 5. (A) Single channel confocal images of Rab GAP5, Rab5, and Tfn in control and DRG2-depleted MCF7 cells. (B) Single channel confocal images of Rab5-GTP, Rab GAP5, Rab5, and nucleus (DAPI) in control and DRG2-depleted MCF7 cells. (C) Single channel confocal images of Rac1, Rab5, and Tfn in control and DRG2-depleted MCF7 cells. (D) Single channel confocal images of Rac1, TIAM-1, and Tfn in control and DRG2-depleted MCF7 cells.

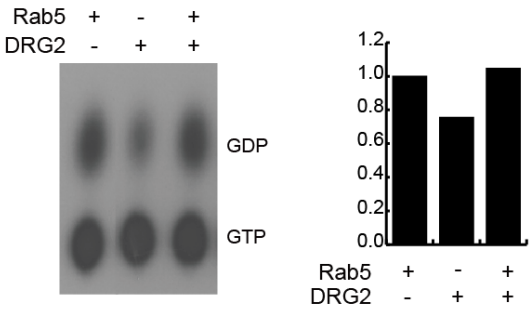

Figure S7, Related to Figure 6. DRG2 does not display GAP activity towards Rab5. Bound nucleotides were visualized by autoradiography. A representative result is displayed. Graph shows quantification of the Rab5 GTP-to-GDP ratio.