Supporting information for

Golgi-resident TRIO regulates membrane trafficking during neurite outgrowth

Tao Tao[1†], Jie Sun[1†], Yajing Peng[2], Yeqiong Li[1], Pei Wang[1], Xin Chen[1], Wei Zhao[1], Yan-Yan Zheng[1], Lisha Wei[1], Wei Wang[1], Yuwei Zhou[1], Jianghuai Liu[1], Yun Stone Shi[1], and Min-Sheng Zhu[1]*

From the [1]Model Animal Research Center and MOE Key Laboratory of Model Animal for Disease Study, Nanjing University, Nanjing 210061, China; [2]Department of Medicine, University of Wisconsin-Madison, Madison, WI 53706

Running title: *TRIO regulates membrane trafficking*

[†]These authors contributed equally to this work
*To whom correspondence should be addressed: Min-Sheng Zhu: Model Animal Research Center, Nanjing University, 12 Xuefu Road, Pukou District, Nanjing 210061, Chia;
zhums@nju.edu.cn; Tel.86-25-58641529; Fax.86-25-58641500.

Materials included

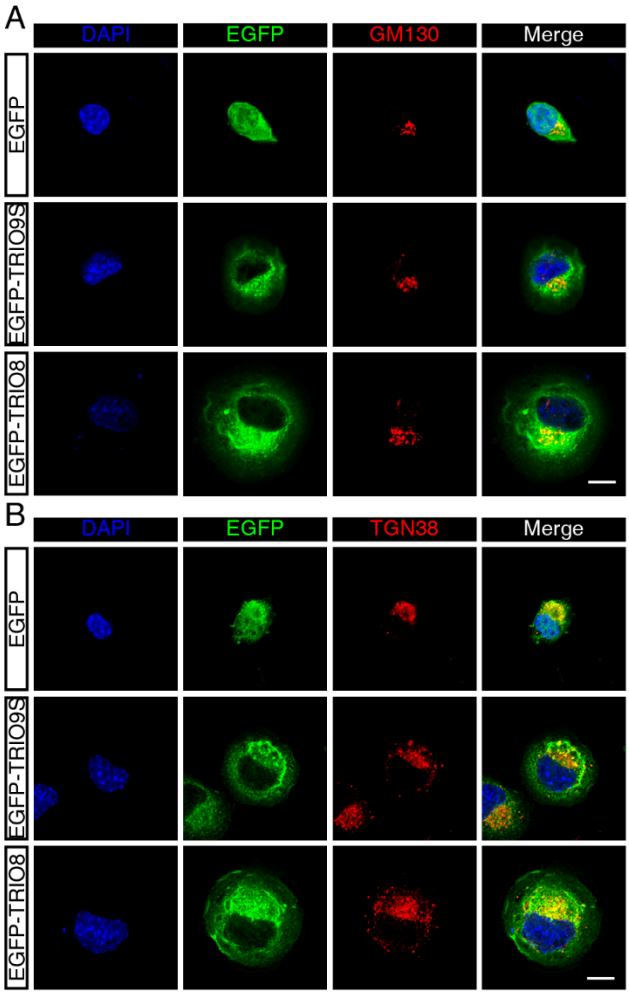

Figure S1. Exogenous expressed TRIO isoforms enrich in Golgi apparatus. (**A**) Neuro-2a cells that transfected with pEGFP-C3, pEGFP-TRIO9S or pEGFP-TRIO8 were stained with the Golgi marker GM130. The scale bar represents 10 μm. (**B**) Neuro-2a cells that transfected with pEGFP-C3, pEGFP-TRIO9S and pEGFP-TRIO8 were stained with the trans-Golgi network marker TGN38. The scale bar represents 10 μm.

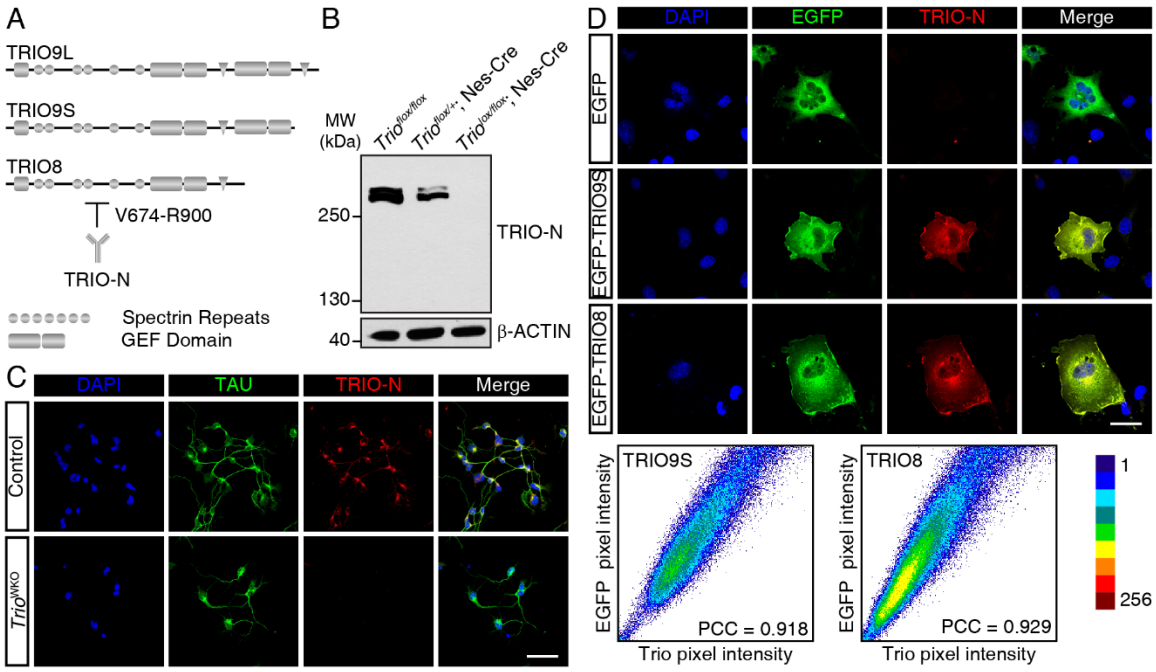

Figure S2. Characterization of TRIO-N antiserum. (**A**) Schematic diagram of the TRIO isoforms expressed in the cerebellum and the antigen sequence used for generation of TRIO-N antiserum. TRIO-N antiserum targets V674-R900 in the spectrin repeats. (**B**) Cerebella isolated from P0.5 mice of the indicated genotypes were lysed and subjected to Western blotting using the homemade TRIO-N antiserum to determine the specificity of this antiserum. TRIO-N did not recognize an unspecific protein at a molecular mass greater than 130 kDa. (**C**) CGNs isolated from *Trio*^{WKO} mice and their control littermates were cultured for 2 DIV and stained with the TRIO-N antiserum and the Tau antibody as a neuronal marker. TRIO immunofluorescence was not observed in *Trio*^{WKO} CGNs, confirming the specificity of this antiserum for immunofluorescence staining. The scale bar represents 40 µm. (**D**) COS-7 cells transfected with either pEGFP-C3, pEGFP-TRIO9S, or pEGFP-TRIO8 were fixed, stained with the TRIO-N antiserum and colocalization with EGFP fluorescence was observed. Scatter plot and PCC were shown in the bottom. TRIO fluorescence strongly correlated with EGFP, suggesting the efficacy of this antiserum. The scale bar represents 40 µm.

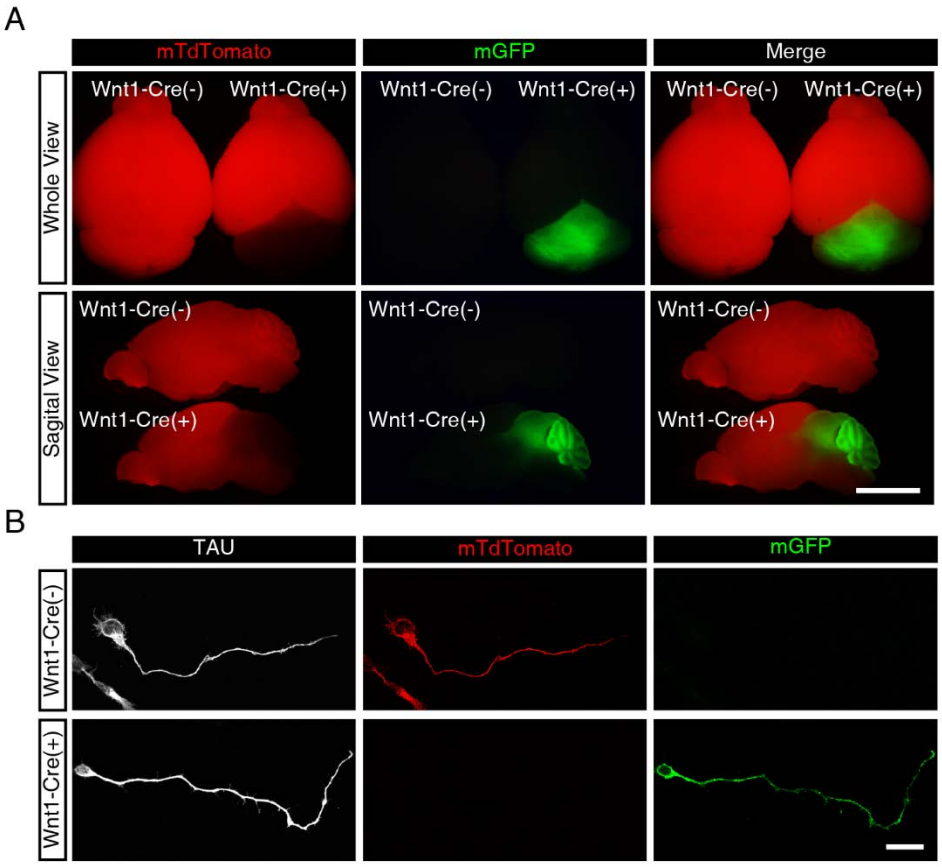

Figure S3. Wnt1-Cre is expressed in the cerebellum and cultured CGNs. (**A**) Wnt1-Cre mice were crossed with Rosa-mTmG mice to detect the Wnt1-Cre expression pattern. Whole brains isolated from Wnt1-Cre-positive or -negative mice were fixed and subjected to observation under the DsRed and EGFP channels using a Leica MZ16F fluorescence stereomicroscope equipped with a digital camera. The scale bar represents 5 mm. (**B**) CGNs were isolated from Rosa-mTmG; Wnt1-Cre-positive or negative mice, cultured for 2 DIV, and fixed for DAPI staining. Wnt1-Cre-negative CGNs only expressed mTdTomato, the red fluorescent protein, but Wnt1-Cre-positive CGNs expressed GFP, suggesting Wnt1-Cre was expressed in cultured CGNs. The scale bar represents 20 μm.

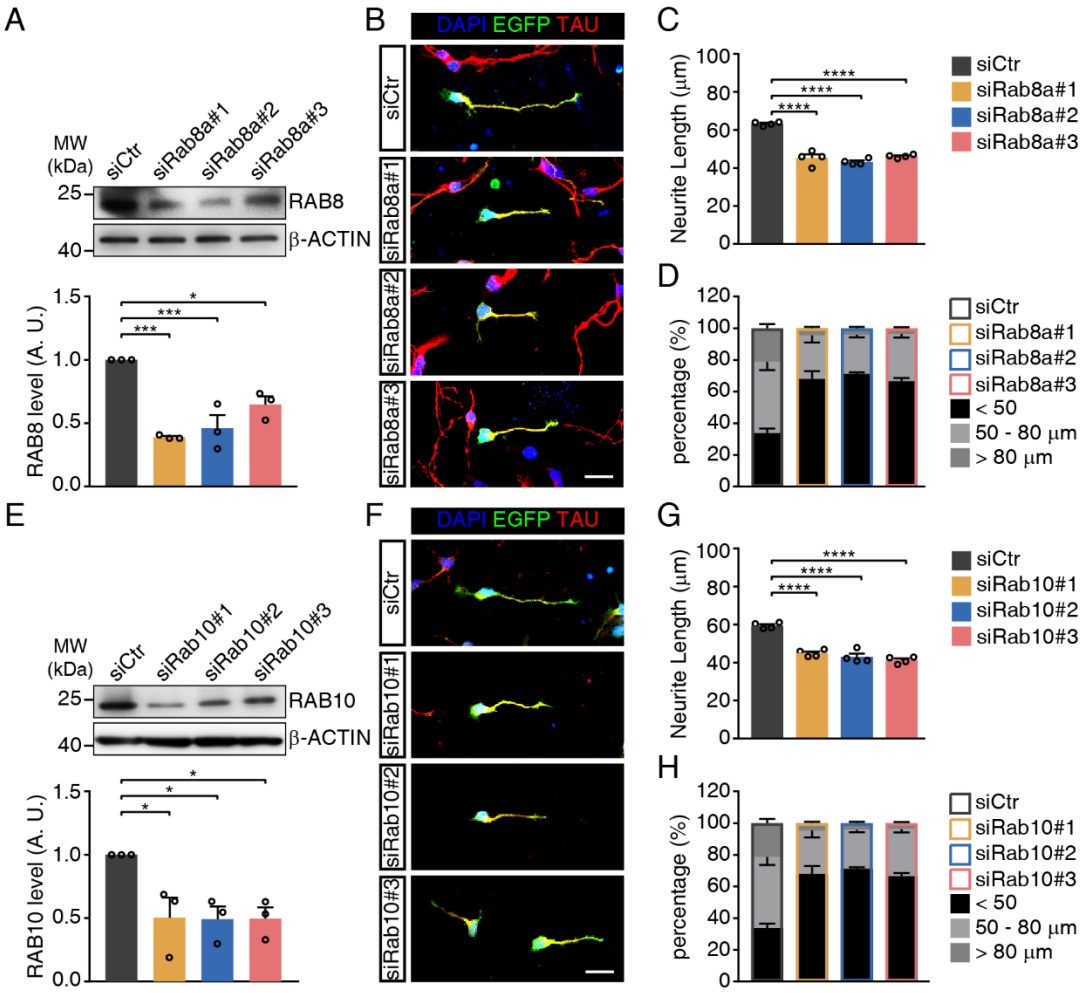

Figure S4. Knockdown of RAB8A and RAB10 inhibits neurite outgrowth in CGNs. (**A**) Knockdown effect of siRNA to endogenous RAB8A in cultured CGNs. Error bar, SEM; one-way ANOVA with Bonferroni's test; **P* < 0.05, ****P* < 0.001; *n* = 3; A.U., arbitrary units. (**B**) CGNs isolated from wild-type mice were nucleofected with either control siRNA or one of the *Rab8a* specific siRNAs, and cultured for 2 DIV, followed by staining with DAPI, EGFP, and Tau. The scale bar represents 20 μm. (**C**) Quantification of the neurite length in the images shown in (B). Error bar, SEM; one-way ANOVA with Bonferroni's test; *****P* < 0.0001; *n* = 4. Numbers of measured neurons are indicated in the columns. (**D**) Quantification of neurite length distribution in (C). Error bar, SEM; *n* = 4. (**E**) Knockdown effect of siRNA to ectopic-expressed FLAG-RAB10 in COS-7 cells. Error bar, SEM; one-way ANOVA with Bonferroni's test; **P* < 0.05; *n* = 3; A.U., arbitrary units. (**F**) CGNs isolated from wild-type mice were nucleofected with either control siRNA or one of the *Rab10* specific siRNAs, and cultured for 2 DIV, followed by staining with DAPI, EGFP, and Tau. The scale bar represents 20 μm. (**C**) Quantification of the neurite length in the images shown in (F). Error bar, SEM; one-way ANOVA with Bonferroni's test; *****P* < 0.0001; *n* = 4. Numbers of measured neurons are indicated in the columns. (**H**) Quantification of neurite length distribution in (G). Error bar, SEM; *n* = 4.

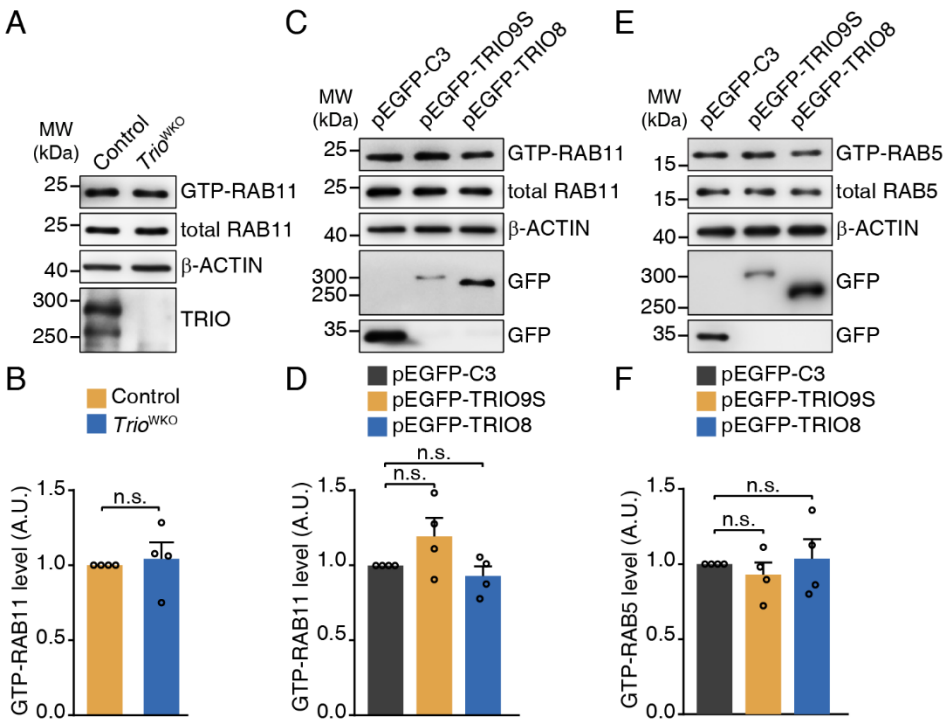

Figure S5. TRIO deletion or overexpression do not affect RAB11 or RAB5 activity. (**A**) GST-FIP3-RBD11 pulldown assay to determine GTP-RAB11 level in cerebella isolated from P10 *Trio*^{WKO} mice and littermate control mice. (**B**) Quantification of GTP-RAB11 levels in (A), Error bar, SEM; Student's t-test; n.s., not significant; *n* = 4. A.U., arbitrary units. (**C**) GST-FIP3-RBD11 pulldown assay to determine GTP-RAB11 levels in Neuro-2a cells transfected with either the pEGFP-TRIO9S, pEGFP-TRIO8, or pEGFP-C3 plasmid. (**D**) Quantification of GTP-RAB11 levels in (C). Error bar, SEM; one-way ANOVA with Bonferroni's test; n.s., not significant; *n* = 4. A.U., arbitrary units. (**E**) GST-RABAPTIN5-R5BD pulldown assay to determine GTP-RAB5 levels in Neuro-2a cells transfected with either the pEGFP-TRIO9S, pEGFP-TRIO8, or pEGFP-C3 plasmid. (**F**) Quantification of GTP-RAB11 levels in (E). Error bar, SEM; one-way ANOVA with Bonferroni's test; n.s., not significant; *n* = 4. A.U., arbitrary units.

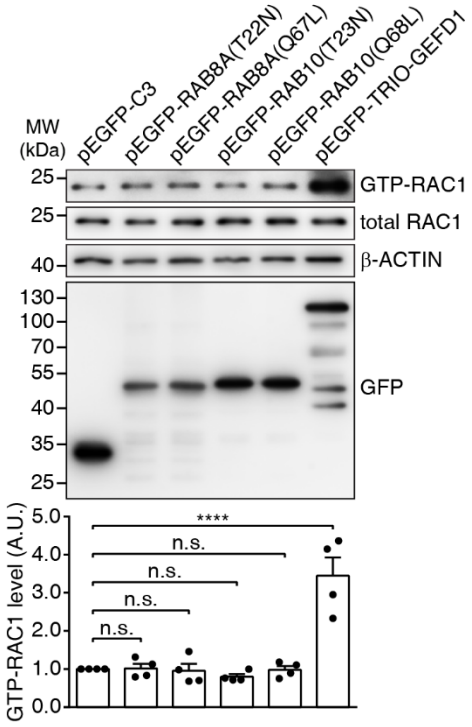

Figure S6. RAB8 or RAB10 mutants do not affect RAC1 activity in Neuro-2a cells. GST-PAK1-GBD pulldown assay to determine GTP-RAC1 levels in Neuro-2a cells transfected with either pEGFP-C3, pEGFP-RAB8A(T22N), pEGFP-RAB8A(Q67L), pEGFP-RAB10(T22N), pEGFP-RAB10(Q68L), or pEGFP-TRIO-GEFD1. pEGFP-TRIO-GEFD1 acts as a positive control for RAC1 activation. Quantification of GTP-RAC1 in each condition was shown in the bar graph and scatter plot at the bottom. Error bar, SEM; one-way ANOVA with Bonferroni's test; ****$P < 0.0001$; n.s., not significant; $n = 4$. A.U., arbitrary units.

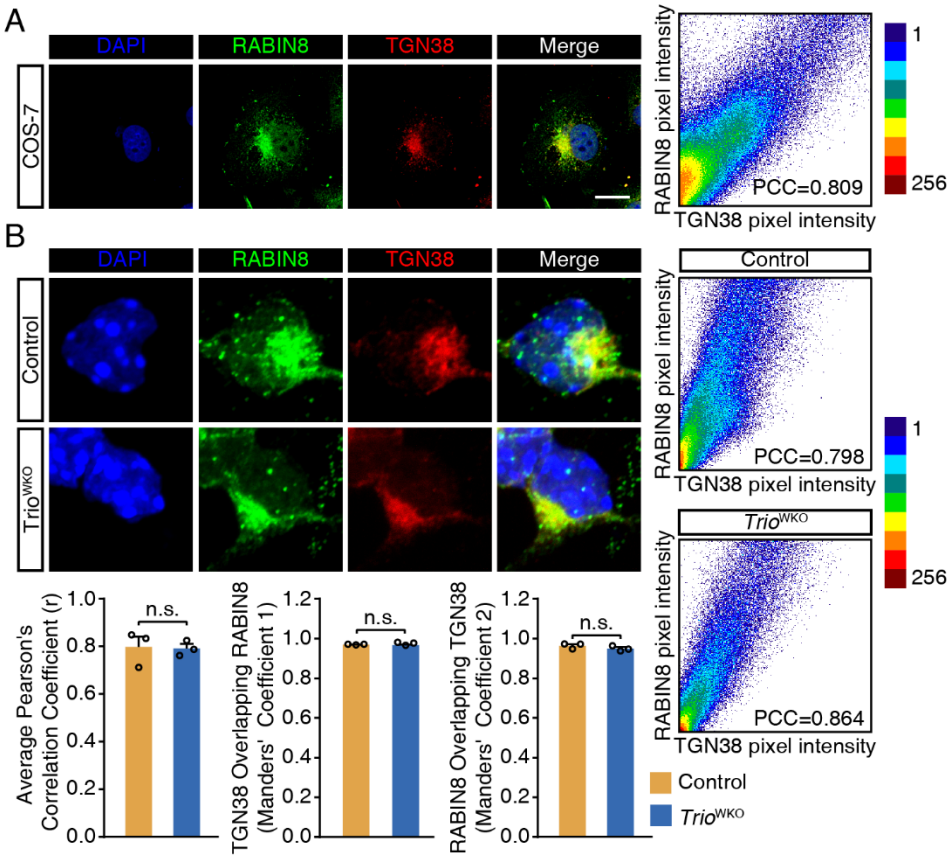

Figure S7. TRIO deletion did not affect RABIN8 localization in TGN. (**A**) COS-7 cells were stained with TGN-38 and RABIN8 antibodies. RABIN8 was mainly distributed in TGN-38-positive regions. The scale bar represents 20 μm. Scatter plots and PCC of fluorescence intensities of both channels were shown in the right. (**B**) Colocalization analysis of RABIN8 and TGN in CGNs. Scale bar represents 5 μm. Scatter plots and PCC of fluorescence intensities of both channels were shown in the right. Error bar, SEM; Student's t-test; n.s., not significant; each group comprised 3 mice (n = 3) and 18 neurons per group.

Movie S1. Time-lapse imaging of tdTomato-RAB8A-positive vesicles in control CGNs.

Movie S2. Time-lapse imaging of tdTomato-RAB8A-positive vesicles in *Trio*^{WKO} CGNs.

Movie S3. Time-lapse imaging of tdTomato-RAB10-positive vesicles in control CGNs.

Movie S4. Time-lapse imaging of tdTomato-RAB10-positive vesicles in *Trio*^{WKO} CGNs.