

1 **Figure S1. Generation of *Trappc9* deficient animal models.**

2 (A), Schematic illustration of two *zTrappc9* morpholino knock-down constructs. MO1 targets  
3 within exon 2, and MO2 targets between exon 3 and subsequent introns. Both morpholinos  
4 modify mRNA splicing.

5 (B-C), RT-qPCR analysis of the knock-down efficiency for two morpholinos in 24 hpf  
6 zebrafish (B) and time course of MO2 (C).  $n=3$  experiments.

7 (D), Schematic illustration of CRISPR/Cas9-mediated *zTrappc9* gene editing. There were 5  
8 mutants identified in Target 1 (located within exon 3), with mutant 5 leading to the most  
9 significant change in *zTrappc9* mRNA sequence, labeled as *zTrappc9<sup>m/m</sup>*.

10 (E), Sequencing results of the five different mutants after testing in zebrafish.

11 (F), The majority of mutant F3 eggs did not divide and remained in the 1-cell stage.  $n=20$  pairs  
12 of F2 zebrafish, and 19 pairs of AB zebrafish.

13 (G), Schematic illustration of Cre-loxP-mediated *mTrappc9* conditional knock-out (KO) at  
14 exon 2 to exon 5, resulting in functional deficiency of isoform II with initial ATG at exon 2  
15 (1139 aa, predicted molecular weight 125.4 kDa) and isoform I with initial ATG at exon 3 (960  
16 aa, 106 kDa), while the downstream transcript may be translated into a truncated protein (787  
17 aa, 87.4 kDa) due to the alternative ATG site at exon 7.

18 (H), Identification of *mTrappc9* deletion by PCR genotyping using two different primer pairs.  
19 The presence of a band via PCR by T5/T8 (intron 1 to exon 2) indicates a wildtype  
20 (*mTrappc9<sup>+/+</sup>*) allele; the presence of a band via PCR by T5/T16 (intron 1 to intron 5,  
21 downstream of the deletion site) indicates a knockout (KO, *mTrappc9<sup>m/m</sup>*) allele. If both bands  
22 are present, this indicates a heterozygous genotype (*mTrappc9<sup>+/m</sup>*).

23 (I), Body weight increase in *mTrappc9<sup>m/m</sup>* mice as compared with *mTrappc9<sup>+/+</sup>* mice starting  
24 at 7 weeks old.  $n=6$  mice/group.

25 (J), Absence of full-length mTrappc9 protein in the brain of *mTrappc9<sup>m/m</sup>* mice detected by  
26 western blot analysis.  $n=3$  mice/group.

27 (K), Co-immunoprecipitation and western blot analysis with a mTrappc9 C-terminal antibody,  
28 which showed the absence of full-length mTrappc9 protein (~130 kDa), but very minor  
29 expression of a truncated mTrappc9 (~90 kDa) in *mTrappc9<sup>m/m</sup>* mice. Meanwhile, mTrappc9  
30 can pull down mTrappc10 in *mTrappc9<sup>m/m</sup>* mice, indicating that the truncated mTrappc9 has  
31 potential for assembling a TRAPP II-like complex.  $n=10$  experiments

32 (L), RT-qPCR analysis of mRNA expression between exons 2-5 (KO) and after exon 6 (new  
33 start primer at exon 13-15). The results showed that there was mRNA expression in the C-  
34 terminal sequence of mTrappc9.  $n=3$  experiments.

35 Data are means  $\pm$  SEM; *t*-tests (F); One-way ANOVA (B); Two-way ANOVA (C, I, L);  
36 \*\*\*\* $P \leq 0.0001$ ; \*\* $P \leq 0.01$ ; ns, not significant ( $P > 0.05$ ).

37  
38 **Figure S2. Phenotypes of zebrafish and mice after *Trappc9* deficiency.**

39 (A-B), The embryonic brain sizes of *zTrappc9* mutant and morphant zebrafish were smaller  
40 than AB (wildtype) zebrafish. Representative images from  $n=60-200$  embryos/group in 11  
41 experiments (A);  $n=10$  embryos (B).

42 (C-D), The adult brain size of *zTrappc9* mutant zebrafish was significantly smaller than AB  
43 (wildtype) zebrafish. Representative images from  $n=3$  zebrafish/group.

44 (E-H), There was no significant difference in the brain size of P1 *mTrappc9<sup>m/m</sup>* mice (E, F),

45 but a significant decrease in the brain size of P20 *mTrappc9<sup>m/m</sup>* mice was observed as compared  
46 with corresponding littermates (**G, H**). Representative images from *n*=3 mice/group.  
47 (**I-L**), The brain and spinal cord (thoracic) of adult *mTrappc9<sup>m/m</sup>* mice were significantly  
48 smaller than WT (C57BL/6 wildtype) and *mTrappc9<sup>+/+</sup>* mice. Representative images from *n*=3  
49 mice/group (**I, J**), *n*=10 mice/group (**K, L**).  
50 Data are means ± SEM; *t*-tests (**D, F, H, J, L**); Two-way ANOVA (**B**); \*\*\*\**P*≤0.0001;  
51 \*\**P*≤0.01; ns, not significant (*P*>0.05). Scale bars: 100 μm (**A**); 1 mm (**C**); 2 mm (**E, G, I, K**).  
52

53 **Figure S3. Behavioral tests of adult *mTrappc9<sup>+/+</sup>* and *mTrappc9<sup>m/m</sup>* mice.**

54 (**A-B**), Open field test. Both the total moving distance (**A**) and the average moving speed (**B**)  
55 of *mTrappc9<sup>m/m</sup>* mice were reduced compared to *mTrappc9<sup>+/+</sup>* mice, indicating reduced  
56 spontaneous exploration activity of *mTrappc9<sup>m/m</sup>* mice. *n*=10 mice/group.  
57 (**C**), Novel object recognition test. *mTrappc9<sup>m/m</sup>* mice spent less time exploring novel objects  
58 compared to *mTrappc9<sup>+/+</sup>* mice. *n*=10 mice/group.  
59 (**D-E**), Barnes maze test. The escape latency across 4 training sessions on the first day of  
60 experiments was significantly longer in *mTrappc9<sup>m/m</sup>* mice than *mTrappc9<sup>+/+</sup>* mice, indicating  
61 decreased learning ability (**D**). In the probe test, the short-term memory of *mTrappc9<sup>m/m</sup>* mice  
62 was not affected, but the long-term memory ability decreased significantly as compared to  
63 *mTrappc9<sup>+/+</sup>* mice (**E**). *n*=11-12 mice/group.  
64 (**F**), Contextual fear conditioning test. Freezing time is decreased in *mTrappc9<sup>m/m</sup>* mice,  
65 indicating decreased contextual fear response and impaired learning. *n*= 10 mice/group.  
66 Data are means ± SEM; *t*-tests (**A, B, C, F**); Two-way ANOVA (**D, E**); \*\*\*\**P*≤0.0001;  
67 \*\*\**P*≤0.001; \**P*≤0.05; ns, not significant (*P*>0.05).  
68

69 **Figure S4. The number and type of neural cells in the brains of *Trappc9* deficient zebrafish  
70 and mice do not show significant change.**

71 (**A-B**), RNA *in situ* hybridization and RT-qPCR analysis of *sox2* mRNA expression in zebrafish  
72 embryos identified no significant difference among the three groups. Representative images  
73 from *n*=20-26 embryos/group in 10 experiments (**A**); *n*=3 experiments (**B**).  
74 (**C-E**), SOX2 immunostaining of E13.5 mouse cerebral cortex failed to detect any significant  
75 difference in SOX2<sup>+</sup> cell number (**D**) or thickness of ventricular and subventricular layers in  
76 *mTrappc9<sup>m/m</sup>* mice (**E**). Representative images from *n*=3 mice/group (**C**); *n*=5-8 sections (**D**,  
77 **E**).  
78 (**F-H**), PAX6 and TBR2 immunostaining of E15.5 mouse cerebral cortex. PAX6 labels NSCs  
79 of the subventricular zone (SVZ), and TBR2 labels NPCs. Cell number (**G**) and thickness (**H**)  
80 of PAX6<sup>+</sup>/TBR2<sup>+</sup> cell layers did not show significant difference in *mTrappc9<sup>m/m</sup>* mice.  
81 Representative images from *n*=3 mice/group (**F**); *n*=4-6 sections (**G, H**).  
82 (**I-L**), NeuN (neurons) and OLIG2 (oligodendrocytes) immunostaining of adult mouse cerebral  
83 cortex and thoracic spinal cord (**K**)/(**L**) and corresponding cell number statistics of (**I**)/(**J**). The  
84 number of neurons and oligodendrocytes did not show significant difference in *mTrappc9<sup>m/m</sup>*  
85 mice compared to control groups. Representative images from *n*=3 mice/group (**I, J**); *n*=7  
86 sections (**K**); *n*=5 sections (**L**).  
87 (**M, N**), Western blot analysis of adult mouse cerebral cortex, and integrated density of protein  
88 bands ratio to GAPDH (**N**). There was no significant difference in the protein expression of

89 GFAP, NeuN, OLIG2 (oligodendrocytes), or TMEM119 (microglia) in *mTrappc9<sup>m/m</sup>* mice. *n*=3  
90 mice/group.  
91 **(O-R)**, GFAP immunostaining of adult mouse corpus callosum and thoracic spinal cord to  
92 visualize astrocytes **(Q)/(R)** and the corresponding area ratio of GFAP fluorescence **(O)/(S)**.  
93 No significant difference was detected among the three groups. Representative images from  
94 *n*=3 mice/group **(O, P)**; *n*=10 sections **(Q)**; *n*=5 sections **(R)**.  
95 **(S-V)**, TMEM119 immunostaining of adult mouse corpus callosum and thoracic spinal cord to  
96 visualize microglia **(U)/(V)** and the corresponding area ratio of TMEM119 fluorescence **(S)/(T)**.  
97 No significant difference was detected among the three groups. Representative images from  
98 *n*=3 mice/group **(S, T)**; *n*=18 sections **(U)**; *n*=5 sections **(V)**.  
99 Data are means ± SEM; *t*-tests **(D, E)**; One-way ANOVA **(Q, R, U, V)**; Two-way ANOVA **(B,**  
100 **G, H, K, L, N)**; ns, not significant (*P*>0.05). Scale bars: 25 μm **(F)**; 50 μm **(A, C, O, S)**; 150  
101 μm **(I, J, P, T)**.

102

103 **Figure S5. Synapses are reduced after *Trappc9* knock-out.**

104 **(A, B)**, SYN labeled pan-synapse of adult mouse cerebral cortex **(A)** and corresponding area  
105 percentage of SYN fluorescence **(B)**. The area of SYN fluorescence is reduced in *mTrappc9<sup>m/m</sup>*  
106 mice, indicating reduction of synapses. Representative images from *n*=3 mice/group **(A)**; *n*=8  
107 sections **(B)**.

108 **(C, D)**, SYP labeled pre-synapse of adult mouse cerebral cortex **(C)** and corresponding  
109 quantification of SYP<sup>+</sup> puncta per area **(D)**. The number of puncta is reduced in *mTrappc9<sup>m/m</sup>*  
110 mice, indicating fewer number of synapses. Representative images from *n*=3 mice/group **(C)**;  
111 *n*=10 sections **(D)**.

112 **(E, F)**, PSD95 labeled post-synapse of adult mouse cerebral cortex **(E)** and corresponding area  
113 percentage of PSD95 fluorescence **(F)**. The area of PSD-95 fluorescence is reduced in  
114 *mTrappc9<sup>m/m</sup>* mice, indicating reduced expression of excitatory post-synaptic receptors that  
115 may correspond to a decrease in the number of synapses. Representative images from *n*=3  
116 mice/group **(E)**; *n*=8 sections **(F)**.

117 **(G, H)**, SYT2 labeled post-synapse of adult mouse cerebral cortex **(G)** and corresponding area  
118 percentage of SYT2 fluorescence **(H)**. The area of SYT2 fluorescence is reduced in  
119 *mTrappc9<sup>m/m</sup>* mice, indicating reduced expression of post-synaptic receptors that may  
120 correspond to a decrease in the number of synapses. Representative images from *n*=3  
121 mice/group **(G)**; *n*=9 sections **(H)**.

122 Data are means ± SEM; One-way ANOVA **(B, D, F, H)**; \*\*\*\**P*≤0.0001; \**P*≤0.05. Scale bars:  
123 20 μm **(C)**; 50 μm **(A, E)**; 150 μm **(G)**.

124

125 **Figure S6. Altered expression of genes and proteins related to neurites projection in**  
126 ***Trappc9* deficient zebrafish and mice.**

127 **(A, B)**, Single cell RNA sequencing of P1 mouse cerebral cortex. **(A)** t-SNE map of  
128 *mTrappc9<sup>+/+</sup>* (*n*=34407 cells) and *mTrappc9<sup>m/m</sup>* (*n*=34504 cells). Single cells are colored based  
129 on different neural cell types. **(B)** Proportion of each cluster in two groups. There was no  
130 significant difference in the proportion of neural cell types between *mTrappc9<sup>+/+</sup>* and  
131 *mTrappc9<sup>m/m</sup>* mice.

132 **(C, D)**, Significantly enriched pathways of single cell RNA sequencing. Many of the down-

133 regulated pathways (C) and up-regulated pathways (D) were related to axonal and synaptic  
134 functions.

135 (E), RT-qPCR analysis of NF- $\kappa$ B downstream genes in 24 hpf zebrafish embryos. Only 3 genes  
136 had statistically significant changes and may contribute to neurite hypoplasia.  $n=3$  experiments.

137 (F), Volcano map of differential proteins detected by proteomics: blue and red dots represent  
138 downregulation and upregulation, respectively. Many TRAPP II complex subunits were  
139 significantly downregulated.  $n=3$  mice/group.

140 (G, H), Significantly enriched pathways of quantitative proteomics, displaying down-regulated  
141 (G) and up-regulated (H) pathways. Many pathways were related to axon development,  
142 synapses, and vesicles.

143 Data are means  $\pm$  SEM; Two-way ANOVA (E); \* $P \leq 0.05$ ; ns, not significant ( $P > 0.05$ ).

144

145 **Figure S7. The correlation of TRAPP II components with Golgi apparatus, COPI, and**  
146 **endosomes is not affected by *Trappc9* deficiency in neuronal cultures.**

147 (A, B), *Trappc9/Trappc10* and GM130 (*cis*-Golgi) immunostaining of NSC-differentiated  
148 neurons (A) and corresponding area of GM130 positivity in the cell body (B).  $n=4$   
149 coverslips/group (A);  $n=13$  neurons (B).

150 (C, D), *Trappc9/Trappc10* and COPI ( $\gamma$ -COPI) immunostaining of primary cultured neurons  
151 (C) and corresponding expression area of COPI in the cell body (D).  $n=5$  coverslips/group (C);  
152  $n=15$  neurons (D).

153 (E-I), *Trappc9/Trappc10* and RAB5 (early endosomes) immunostaining of NSC-differentiated  
154 neurons. Due to the spatial overlap, it's difficult to evaluate the co-localization between  
155 *Trappc9/Trappc10* and RAB5 by simple quantification. Instead, we used a co-localization  
156 coefficient between the two fluorescent channels, with the results showing that there were no  
157 significant differences between *mTrappc9*<sup>+/+</sup> and *mTrappc9*<sup>m/m</sup> neurons.  $n=7$  coverslips/group  
158 (E);  $n=16$  growth cones (F-I).

159 Data are means  $\pm$  SEM; Two-way ANOVA (B, D, F, G, H); ns, not significant ( $P > 0.05$ ); Scale  
160 bars: 10  $\mu$ m (A, C, E).

161

162 **Figure S8. The distribution of *Trappc9* is reduced in *Trappc9* deficient neurons.**

163 (A, B), *Trappc9/Trappc10* immunostaining of neurons differentiated from NSCs of E13.5  
164 mouse cerebral cortex cultured for 6 days. While there was a decrease in *mTrappc9* in the  
165 neuronal cell body, there was no significant changes in the intensity of *mTrappc10*.  
166 Representative images from  $n=8$  coverslips/group (A);  $n=22$  neurons (B).

167 (C-F), *Trappc9* and F-actin/ $\alpha$ -tubulin immunostaining of mouse NSC-differentiated neurons  
168 cultured for 6 days showing the number of *mTrappc9* puncta per micron of nascent neurites  
169 decreased significantly. Arrowheads indicated several newborn neurites, which showed the  
170 reduction in the distribution of *Trappc9* in *mTrappc9*<sup>m/m</sup> neurons compared to *mTrappc9*<sup>+/+</sup>.  
171 Representative images from  $n=8$  coverslips/group (C, E);  $n=33$  nascent neurites (D, F).

172 Data are means  $\pm$  SEM; *t*-tests (B, D, F); \*\*\*\* $P \leq 0.0001$ ; Scale bars: 10  $\mu$ m (A, C, E).

173

174 **Figure S9. The puncta distribution of *Trappc9* and *Trappc10* along**  
175 **microfilaments/microtubules is reduced in neurons treated with Latrunculin and**

176 **Vinblastine.**

177 **(A)**, Latrunculin and vinblastine treated *mTrappc9<sup>+/+</sup>* neurons labeled by F-actin and  $\alpha$ -tubulin.  
178 NSC-differentiated neurons at day 6 were treated with latrunculin (5  $\mu$ M, 2h) or vinblastine (5  
179  $\mu$ M, 4h). Latrunculin and Vinblastine treatments impair the growth of microfilaments and  
180 microtubules respectively. Representative images from  $n=3$  coverslips/group.

181 **(B, D)**, Trappc10 and F-actin (microfilaments) immunostaining. The number of Trappc10<sup>+</sup>  
182 puncta along microfilaments was reduced in neurons after latrunculin treatment.  
183 Representative images from  $n=3$  coverslips/group **(B)**;  $n=18$  neurons **(D)**.

184 **(C, E)**, Trappc10 and  $\alpha$ -tubulin (microtubules) immunostaining. The number of Trappc10<sup>+</sup>  
185 puncta along microtubules was reduced in neurons after vinblastine treatment. Representative  
186 images from  $n=3$  coverslips/group **(C)**;  $n=18$  neurons **(E)**.

187 **(F, G)**, Trappc9 and F-actin immunostaining. The number of Trappc9<sup>+</sup> puncta distributed along  
188 the microfilament in neurons was decreased after treatment with latrunculin. Representative  
189 images from  $n=3$  coverslips/group **(F)**;  $n=18$  neurons **(G)**.

190 **(H, I)**, Trappc9 and  $\alpha$ -tubulin immunostaining. The number of Trappc9<sup>+</sup> puncta distributed  
191 along the microtubules in neurons was decreased after treatment with Vinblastine.  
192 Representative images from  $n=3$  coverslips/group **(H)**;  $n=18$  neurons **(I)**.

193 Data are means  $\pm$  SEM;  $t$ -tests **(D, E, G, I)**; \*\*\*\* $P \leq 0.0001$ ; Scale bars: 10  $\mu$ m **(A, B, C, F, H)**.

194

195 **Figure S10. Sperm cell development is inhibited in *mTrappc9<sup>m/m</sup>* mice.**

196 **(A, B)**, Immunostaining of  $\alpha$ -tubulin to label the tail of sperm cells in testicular sections of  
197 mice at 20 weeks old, showing a reduction in sperm cell number in *mTrappc9<sup>m/m</sup>* mice  
198 compared to *mTrappc9<sup>+/+</sup>* mice. Representative images from  $n=3$  mice/group **(A)**;  $n=7$  sections  
199 **(B)**.

200 **(C)**, Epididymis section of mice at 10 weeks old. The red arrowheads point out the cross section  
201 of several sperm tails showing the normal “9+2” structure in the tail of *mTrappc9<sup>m/m</sup>* sperm as  
202 compared to tail of *mTrappc9<sup>+/+</sup>* sperm. Representative images from  $n=3$  mice/group.

203 Data are means  $\pm$  SEM;  $t$ -tests **(B)**; \*\*\*\* $P \leq 0.0001$ ; Scale bars: 50  $\mu$ m **(A)**; 500 nm **(C)**.



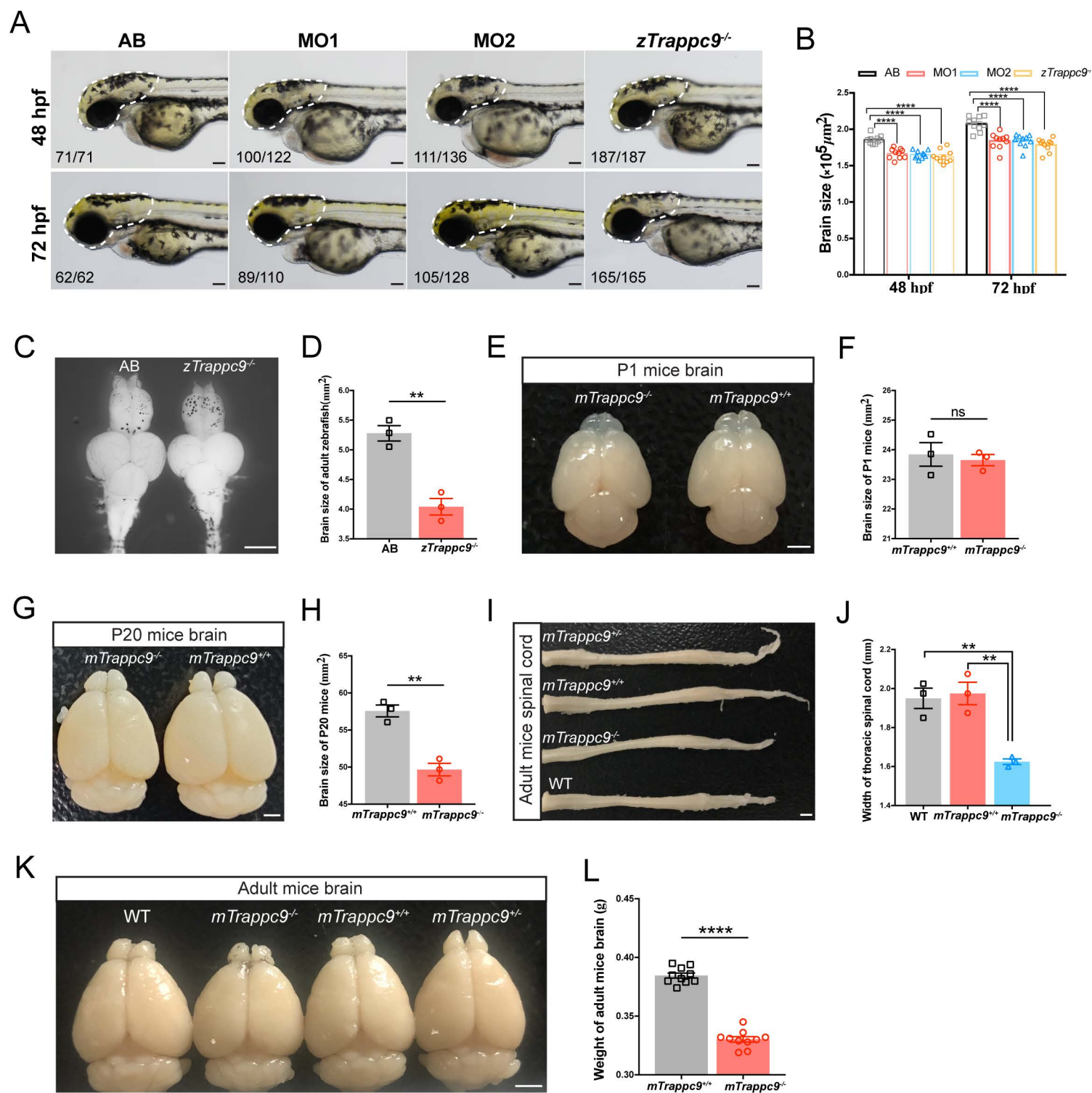
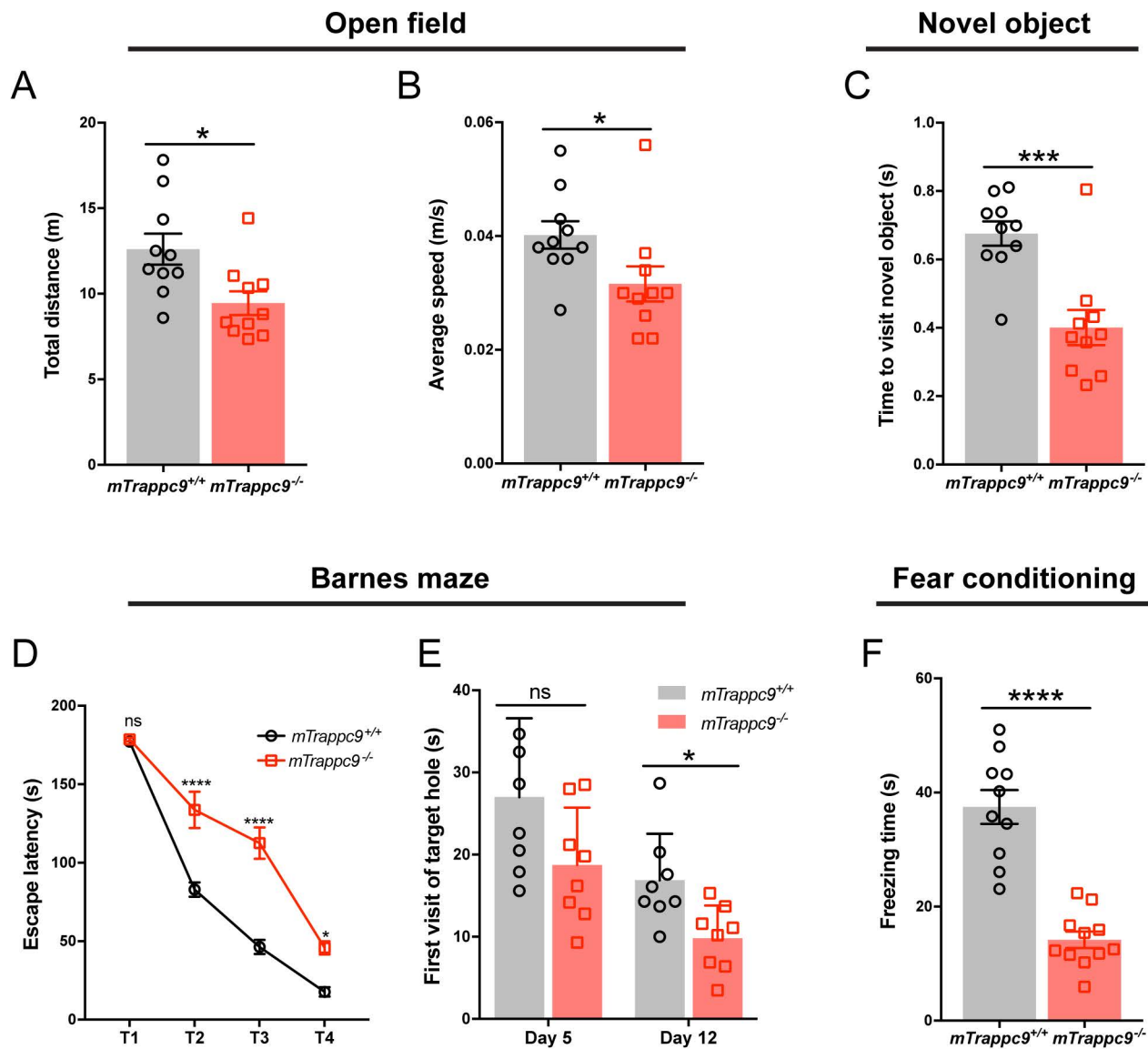


Figure S3





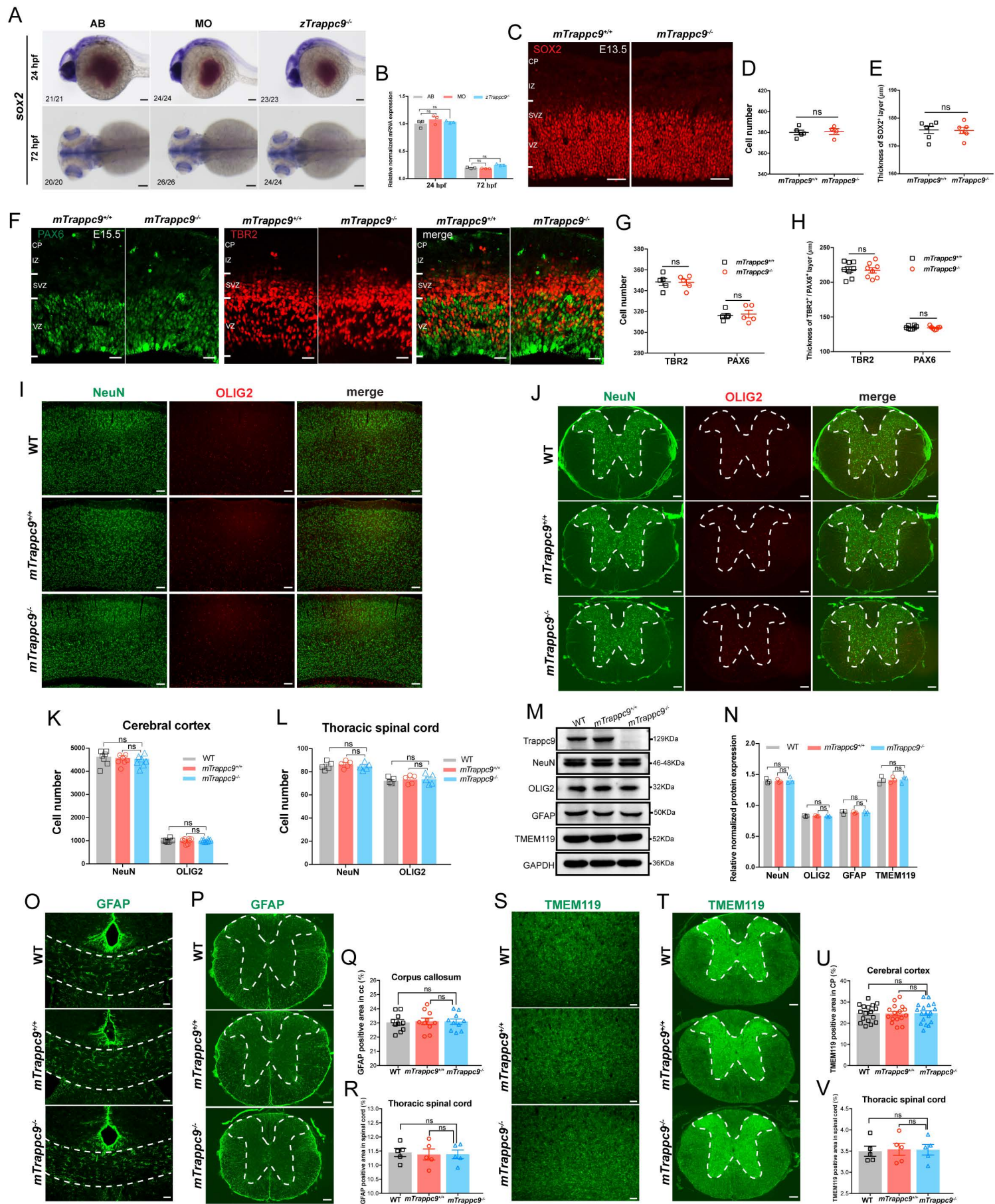
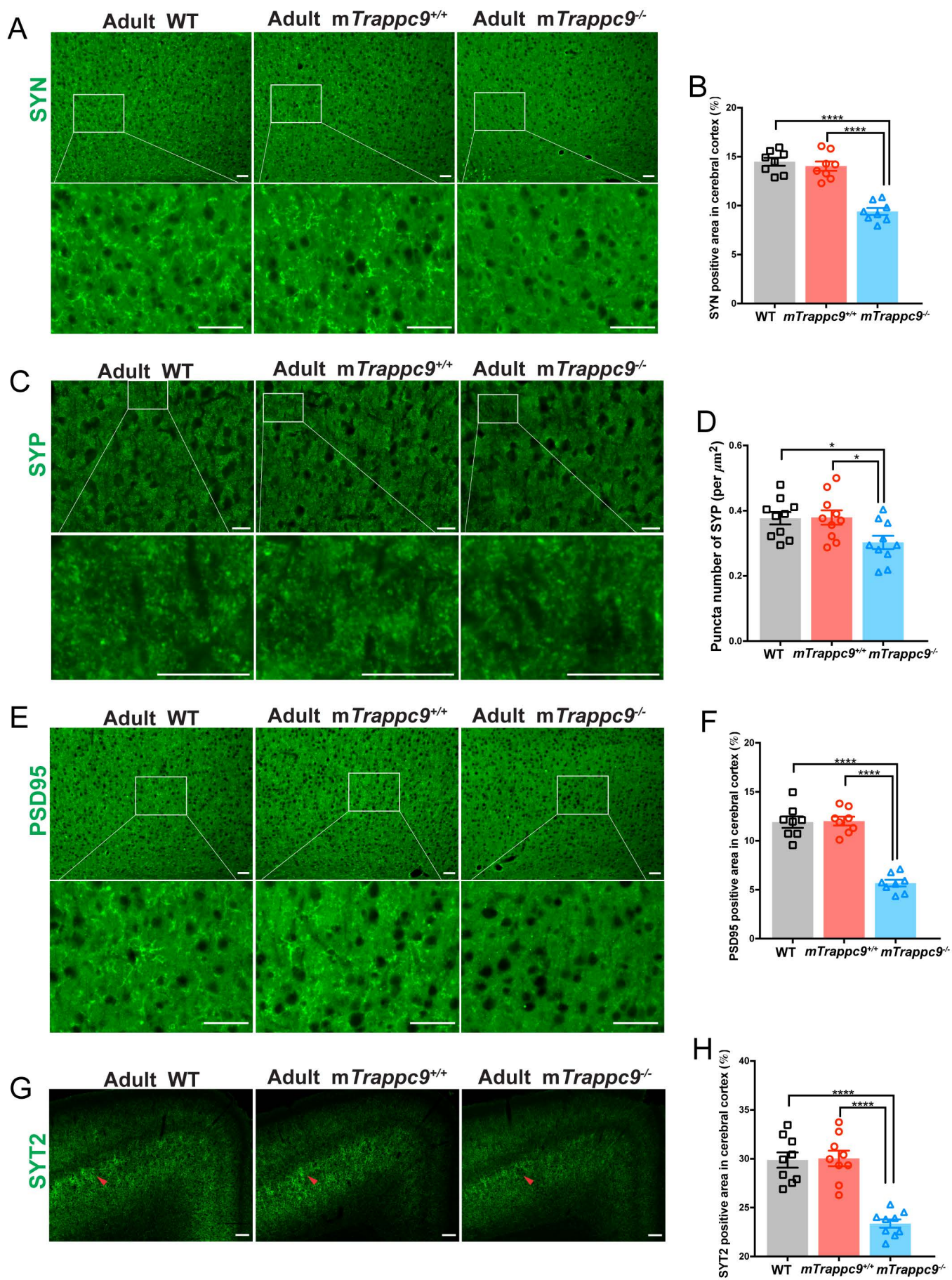
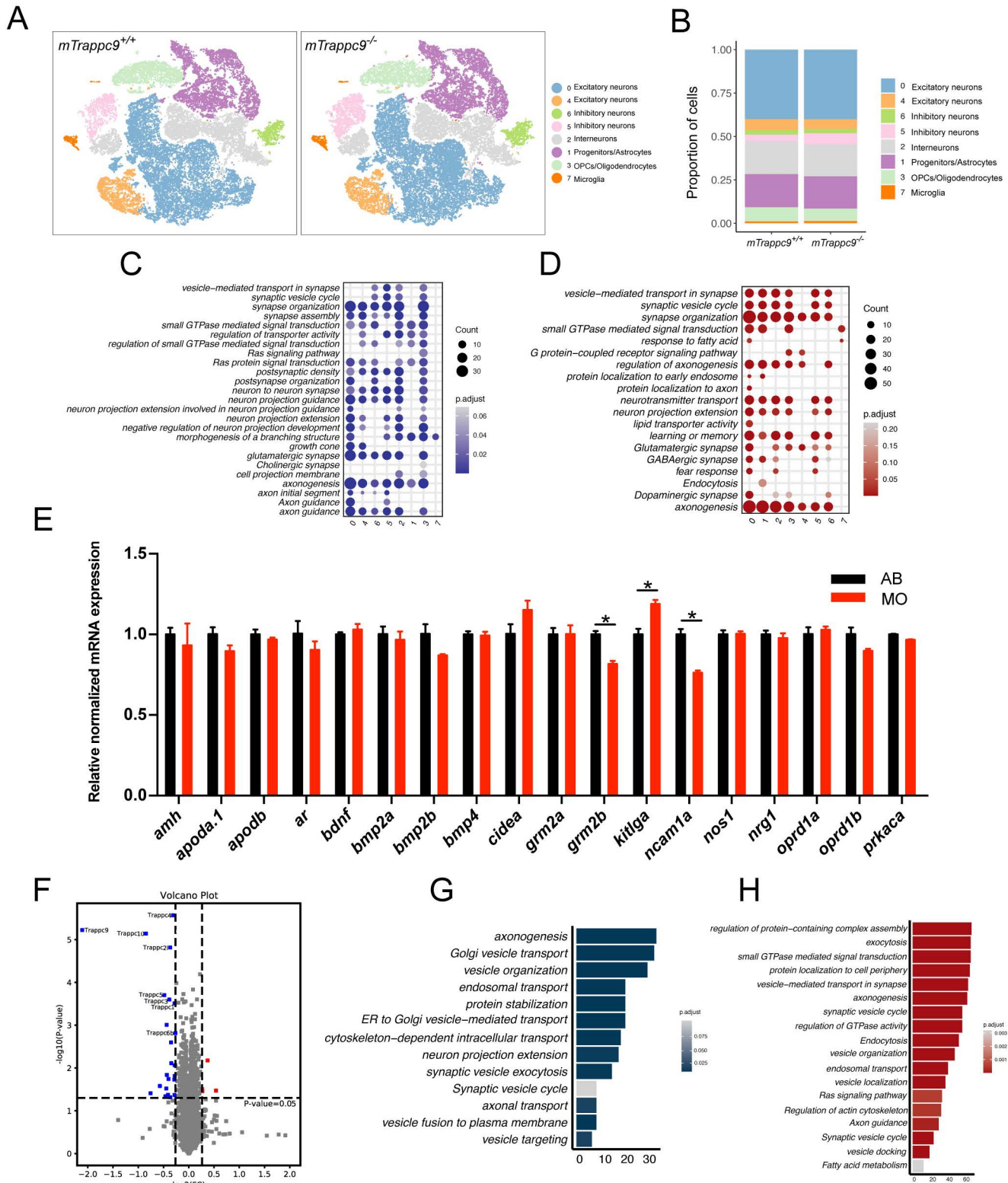


Figure S5





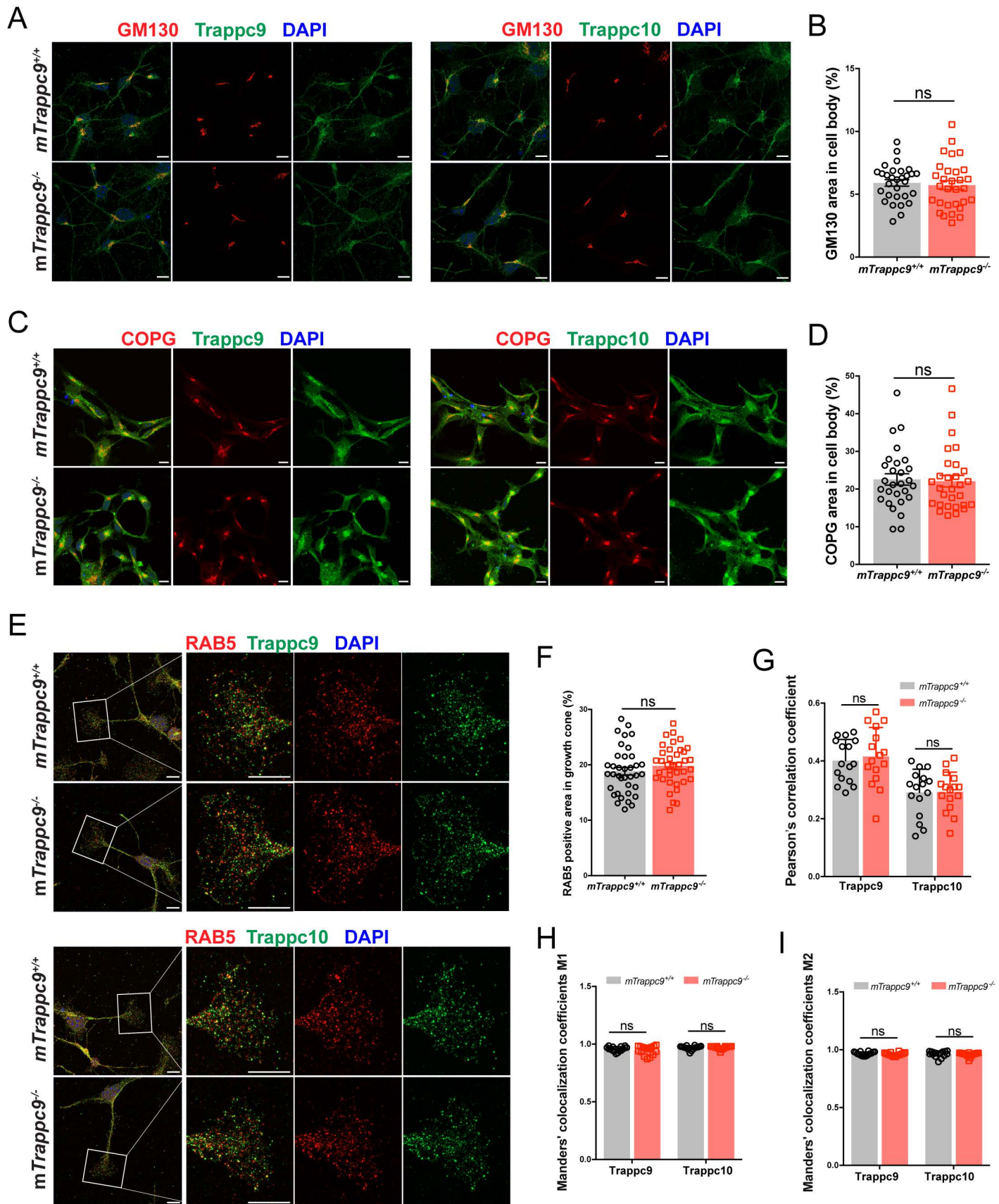


Figure S8

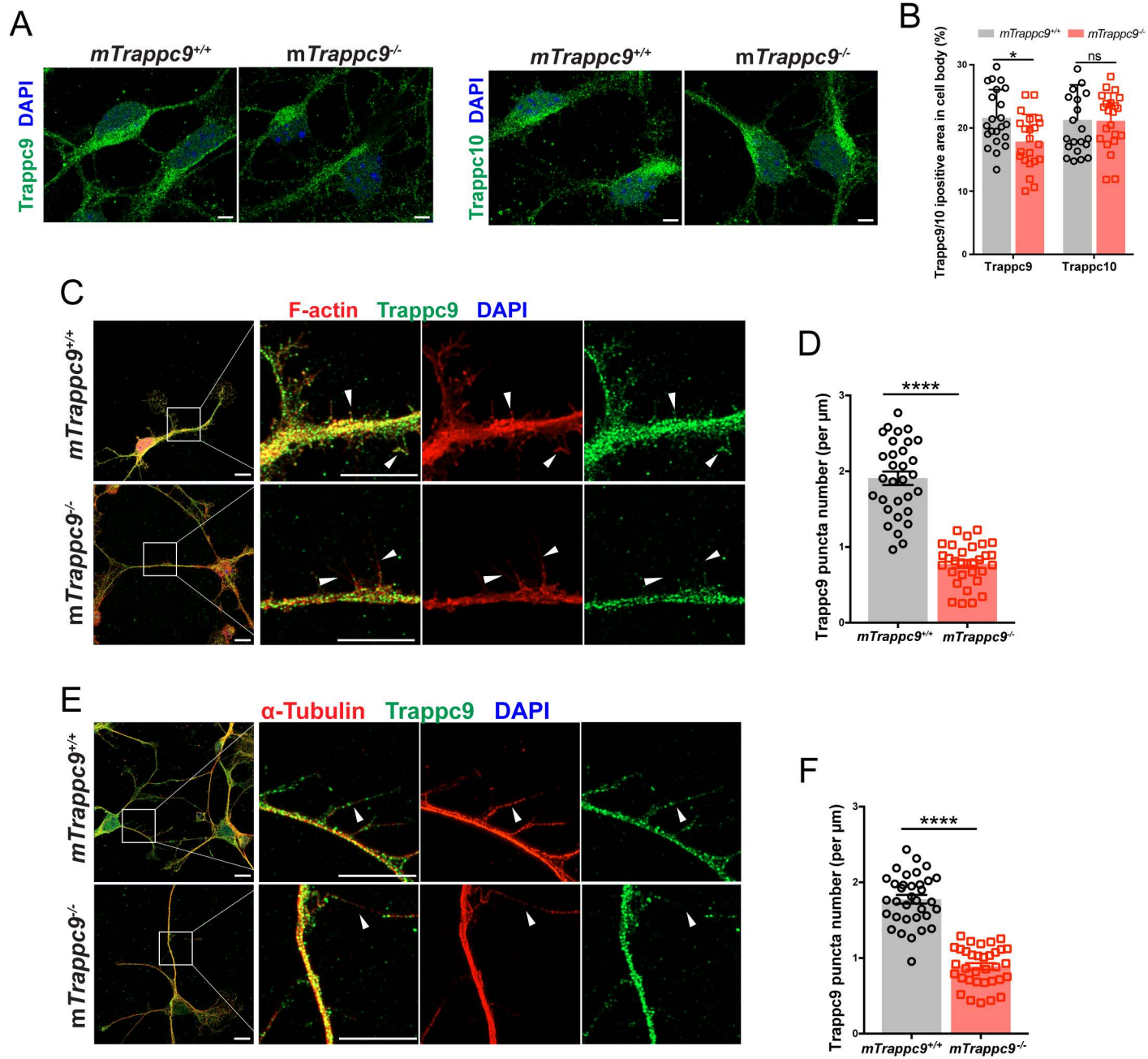


Figure S9

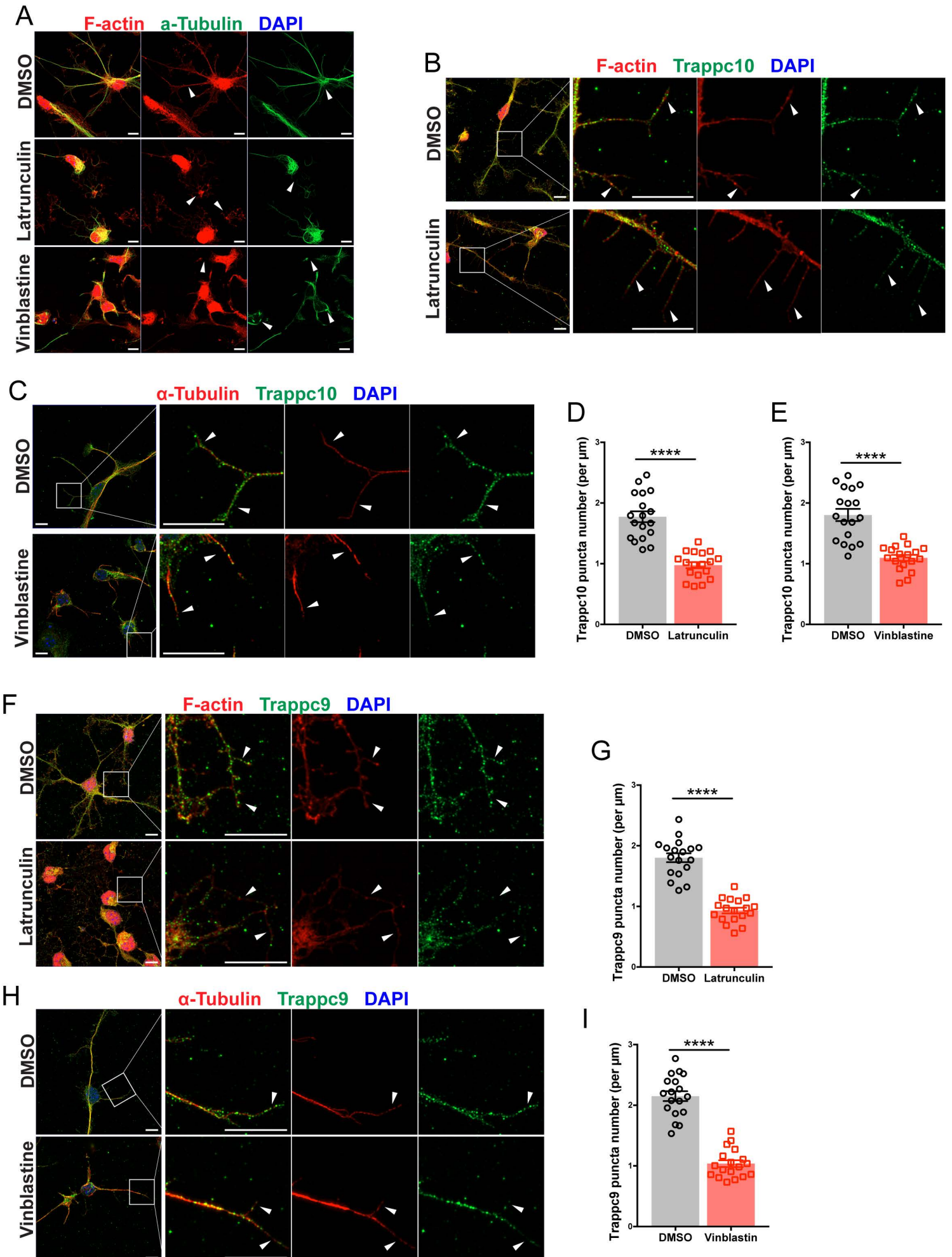


Figure S10

