

Supplemental Material

Research Articles | Cellular/Molecular

MTCL2 is essential for the bipolar-to-multipolar transition in the dendrite extension of cerebellar granule neurons.

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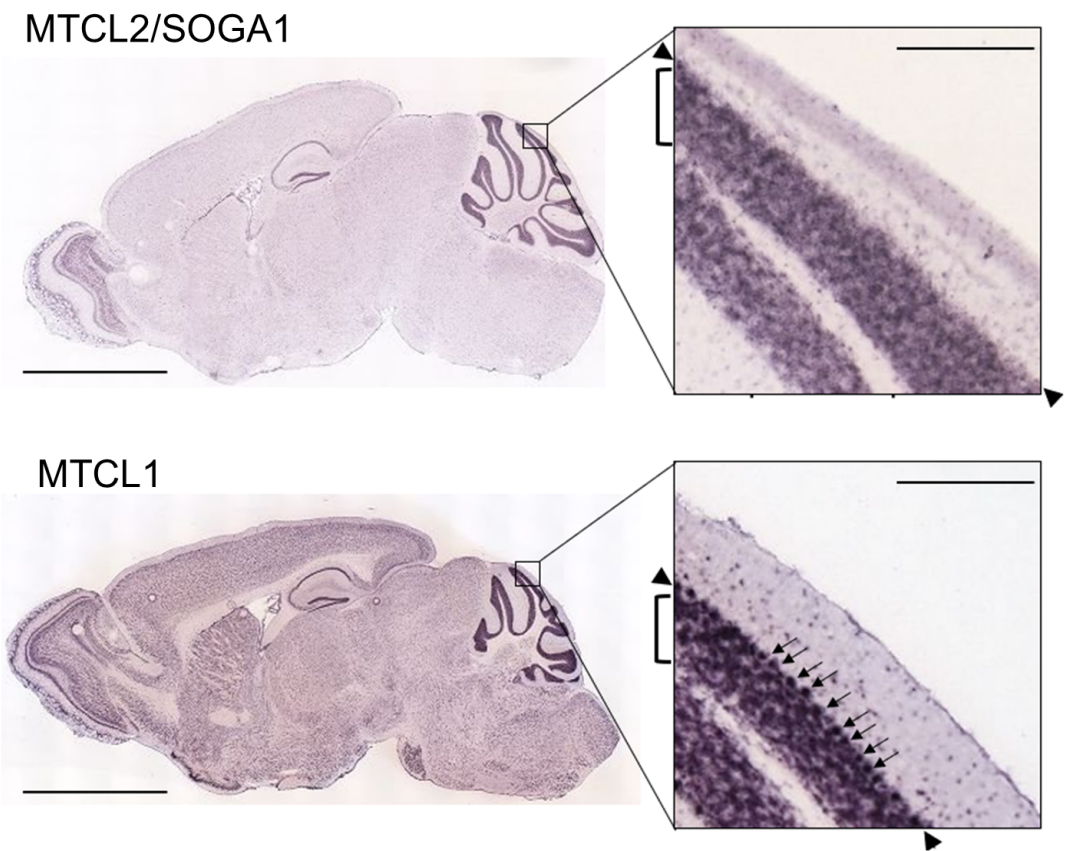
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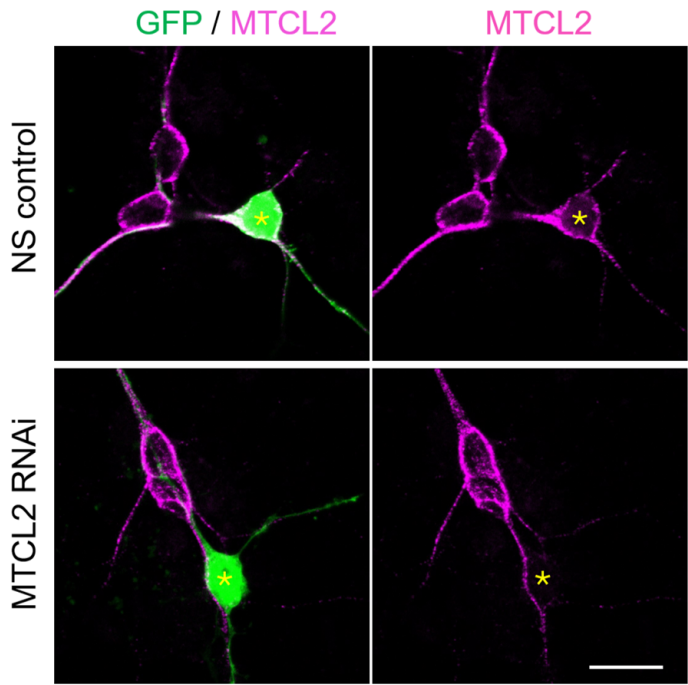
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Figure S1

A



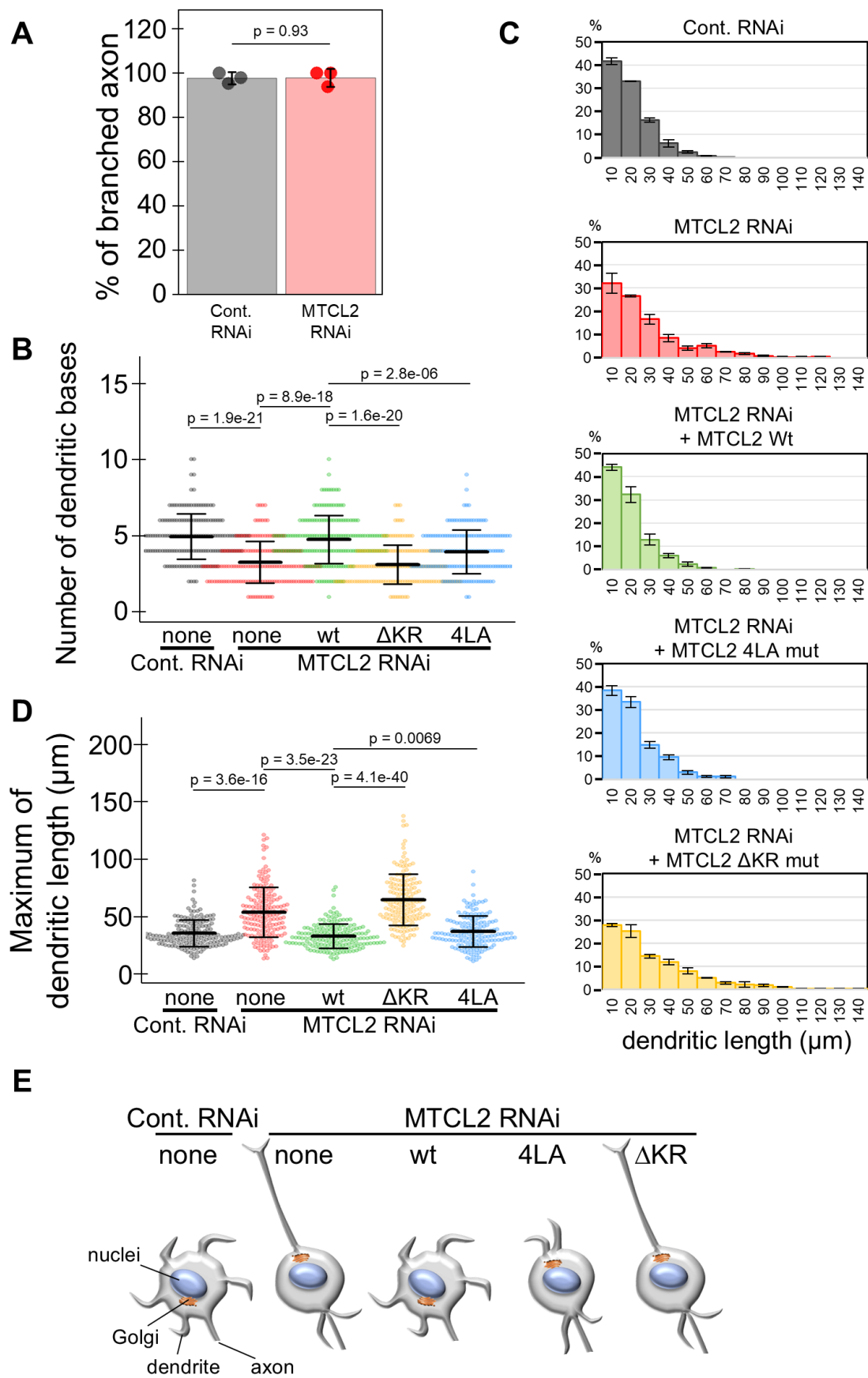
B



Supplementary Figure S1: MTCL2 expression in cerebellar granule neurons

(A) *In situ* hybridization data of MTCL2/SOGA1 (top) and MTCL1 (bottom) published in the Allen Mouse Brain Atlas (<https://mouse.brain-map.org/>). The corresponding single sagittal sections of adult male mice at 56 weeks of age are shown. Arrowheads and square brackets in the enlarged images (right panels) indicate the positions of the Purkinje cell layer and molecular layer, respectively. Arrows in the bottom enlarged image indicate the Purkinje cells. Note that MTCL1, but not MTCL2, was detected in the Purkinje cells. Scale bars: 3.356 mm (left panels) 0.209 mm (right enlarged panels). (B) Expression of MTCL2 in cultured CGNs. CGNs transfected with the indicated shRNA vector were fixed at DIV6 and stained for MTCL2. Projected views of the z-stack images are shown. Asterisks indicate GFP-positive cells in which the indicated shRNA vectors were introduced. NS: nonsilencing. Scale bar: 10 μ m.

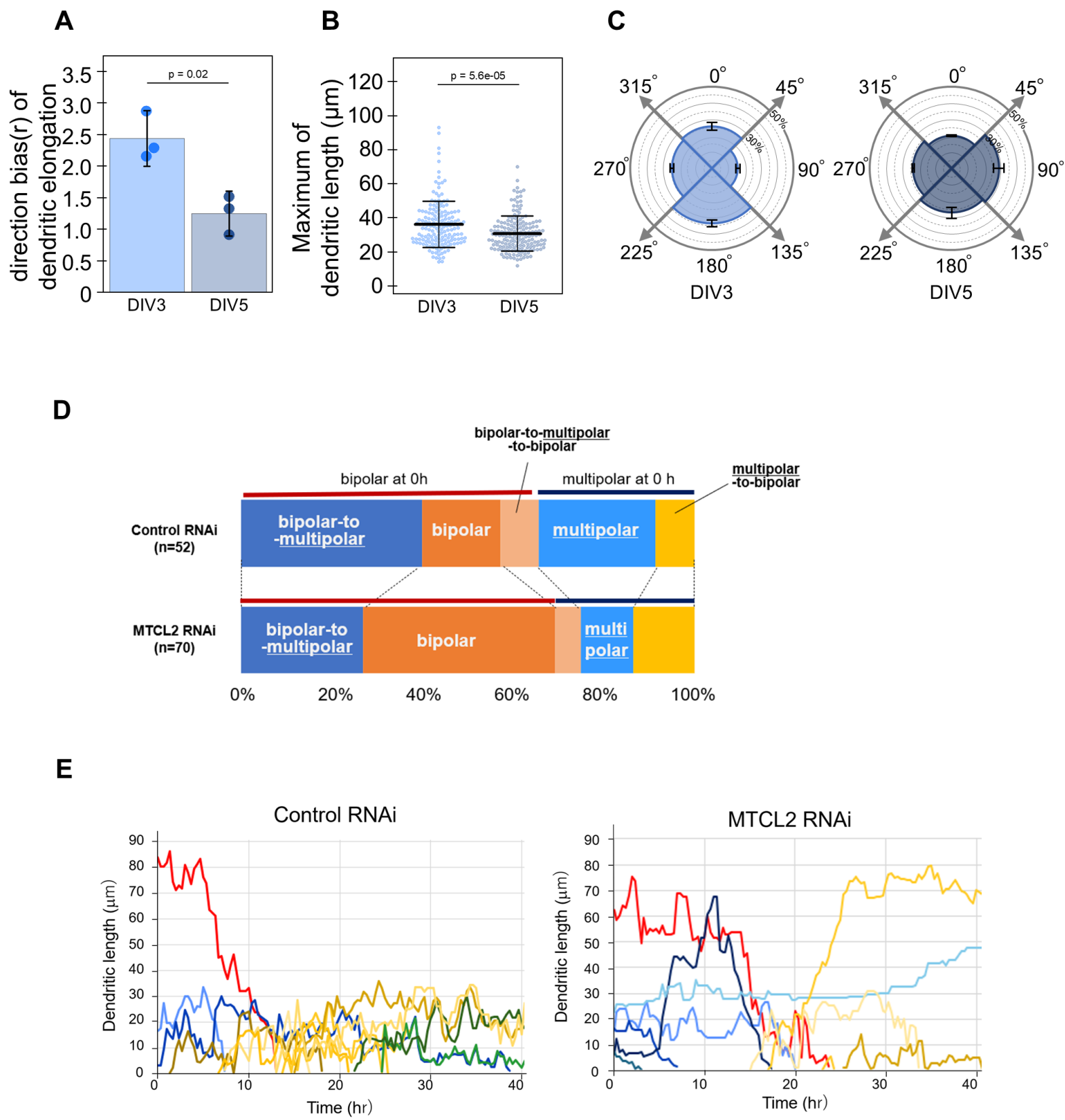
Figure S2



Supplementary Figure S2: Effects of MTCL2 knockdown on axonal and dendritic growth of cultured CGNs.

(A) The percentage of cells with branched axons in the CGNs analyzed in Fig. 2B. Each data point represents the average value from each of the 3 independent experiments. The means $\pm$ S.E. are shown. (B) Distribution of dendritic base numbers among the CGNs analyzed in Fig. 2D. Each data point represents the result of one neuron. The means $\pm$ S.D. are indicated for each scatter plot. (C) Length distribution of all dendrites analyzed in Fig. 2D. Histograms of the average values from each of the 3 independent experiments are shown. The means $\pm$ S.E. of 3 are indicated. (D) Maximum dendrite length distribution in the CGNs analyzed in Fig. 2D. Each data point represents the result of one neuron. The means $\pm$ S.D. are indicated for each scatter plot. In (B) and (D), the Kruskal–Wallis test was employed in conjunction with the Holm-corrected Mann-Whitney U test to ascertain statistical significance. (E) Dendritic phenotypes of CGNs subjected to the indicated conditions are illustrated. Note that MTCL2 Δ KR did not rescue any defects in the dendritic extension of MTCL2, whereas MTCL2 4LA showed partial rescue activity against the shortening of preexisting bipolar dendrites, similar to wild-type MTCL2.

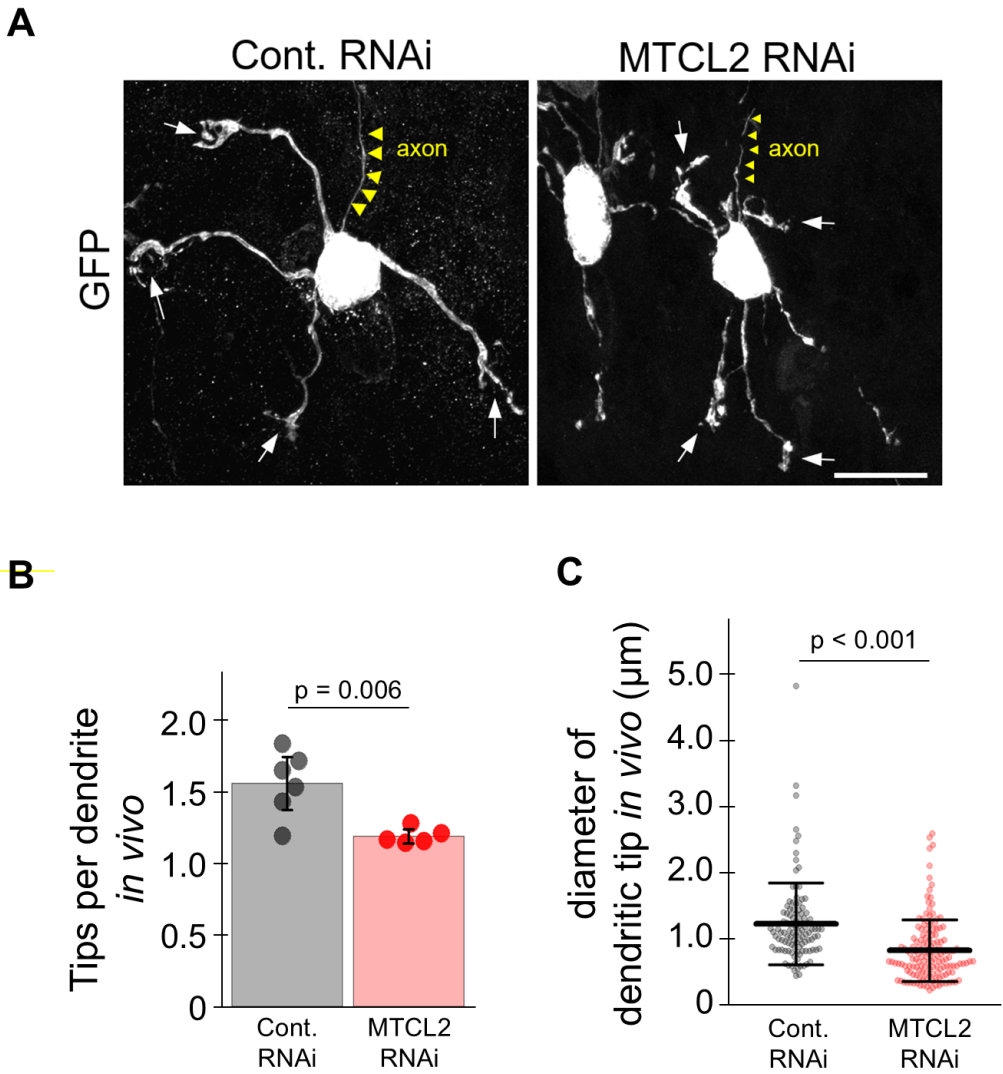
Figure S3



Supplementary Figure S3: Dendrite extensions of GCNs exhibit bipolar-to-multipolar transitions after DIV3.

(A) Comparison of the dendritic direction bias (r) between the CGNs at DIV3 and 5 analyzed in Figs. 4B. Each data point represents the average value from each of the 3 independent experiments. The means $\pm$ S.E. are shown. (B) Maximum dendrite length distributions of the CGNs at DIV3 and 5 analyzed in Figs. 4B. Each data point represents the result of one neuron. The means $\pm$ S.D. are indicated for each scatter plot. (C) θ distribution of the Golgi position in the CGNs at DIV3 and DIV5 analyzed in Fig. 4D. The polar histograms of the average values from each of the 3 independent experiments are shown. The means $\pm$ S.E. are shown. Welch's two-sample t-test and the Wilcoxon rank-sum test were used to examine the statistical significance of (A) and (B), respectively. (D) Classification of live imaging data from DIV3 to DIV4. The thick red and dark blue lines indicate the proportion of cells exhibiting bipolar and multipolar morphologies, respectively, at the start of recording. (E) Dynamic dendrite length changes in the CGNs analyzed in Fig. 4E and F. Data for different dendrites were colored differently. Note that in both cases, the initial long bipolar dendrites exhibited shrinkage. However, the MTCL2-depleted CGN did not consistently form short dendrites with dynamic features. Instead, the cell re-elongates long dendrites.

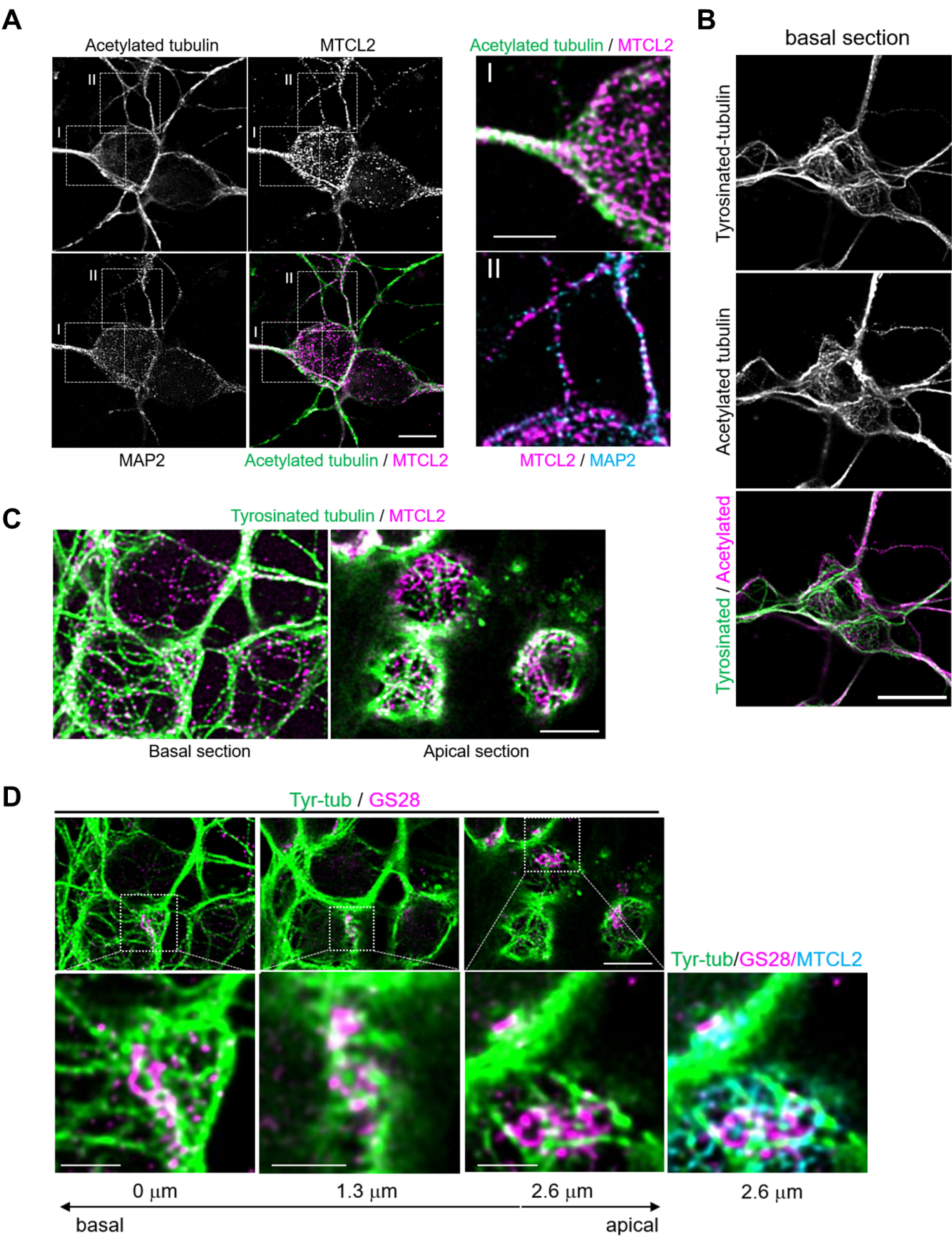
Figure S4



Supplementary Figure S4: Effects of MTCL2 knockdown on the glomerular development in CGNs.

(A) Representative images of a single CGN detected in the IGL after the *in vivo* electroporation of either the control or MTCL2 knockdown vector. Cell morphologies were visualized using the GFP signal. Single images are presented, which are projected from confocal Z-stack images covering the entire cell body of individual CGNs using maximum projection. The corresponding 3D stereoscopic images are provided in Movies 3 and 4. The white arrows indicate the tips of branched dendrites, indicating the development of glomerular structures. Scale bar: 10 μm . (B) The number of branching tips within 5 μm from the distal end of the dendrite. More than 100 dendrites each from 25 control and 29 MTCL2 knockdown CGNs (collected from at least five mice each) were analyzed. Each data point represents the average for each mouse subjected to the indicated RNAi. The means $\pm$ S.E. are shown. Welch's two-sample t-test was used to examine statistical significance. (C) Distribution of dendritic tip diameter. Each data point represents the result of one dendritic tip in CGNs analyzed in (B). The means $\pm$ S.D. are shown. The Wilcoxon rank-sum test with continuity correction was used to examine statistical significance.

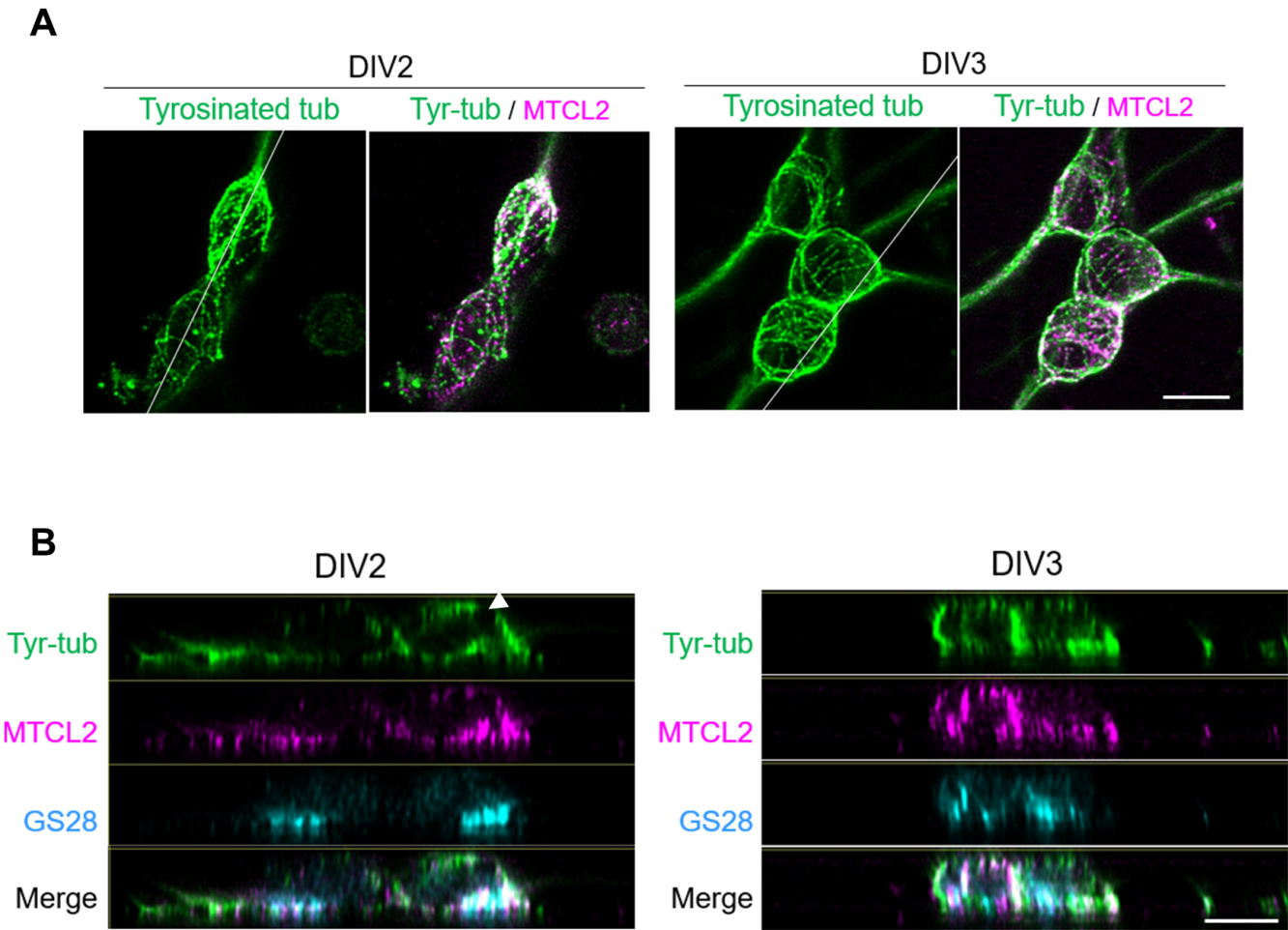
Figure S5



Supplementary Figure S5: Colocalization of MTCL2 with MTs in polarized CGNs.

(A) High-resolution confocal images of CGNs fixed at DIV5 and stained for acetylated tubulin, MTCL2, and MAP2. A single confocal image of the basal plane is shown with various staining combinations. The regions indicated by dotted squares (I) and (II) in the left panels are enlarged in the right. Note that, in contrast to MAP2, MTCL2 granules were detected not only on MTs but also in cell bodies. Scale bars: 5 and 2.5 μm in the left and right panels, respectively. (B) High-resolution confocal images of CGNs fixed at DIV5 and stained for tyrosinated and acetylated tubulin. A single confocal image of the basal plane is shown. Note that tyrosinated tubulin is more enriched in the MT network within the cell body than acetylated tubulin. Scale bar: 10 μm . (C) Enlarged merged images of the basal (0 μm) and apical (2.6 μm) sections of the CGNs shown in Fig. 6A. Scale bar: 5 μm . (D) The upper panels present the same confocal images shown in Figure 6A as different merged images combining MT and Golgi apparatus staining as indicated. The bottom panels are the enlarged views of the regions indicated by white rectangles in the upper panels. Only for the enlarged view of the most apical section is also presented as a merged image combining MT, Golgi apparatus and MTCL2 staining as indicated. Scale bars: 5 and 2 μm in the top and bottom panels, respectively.

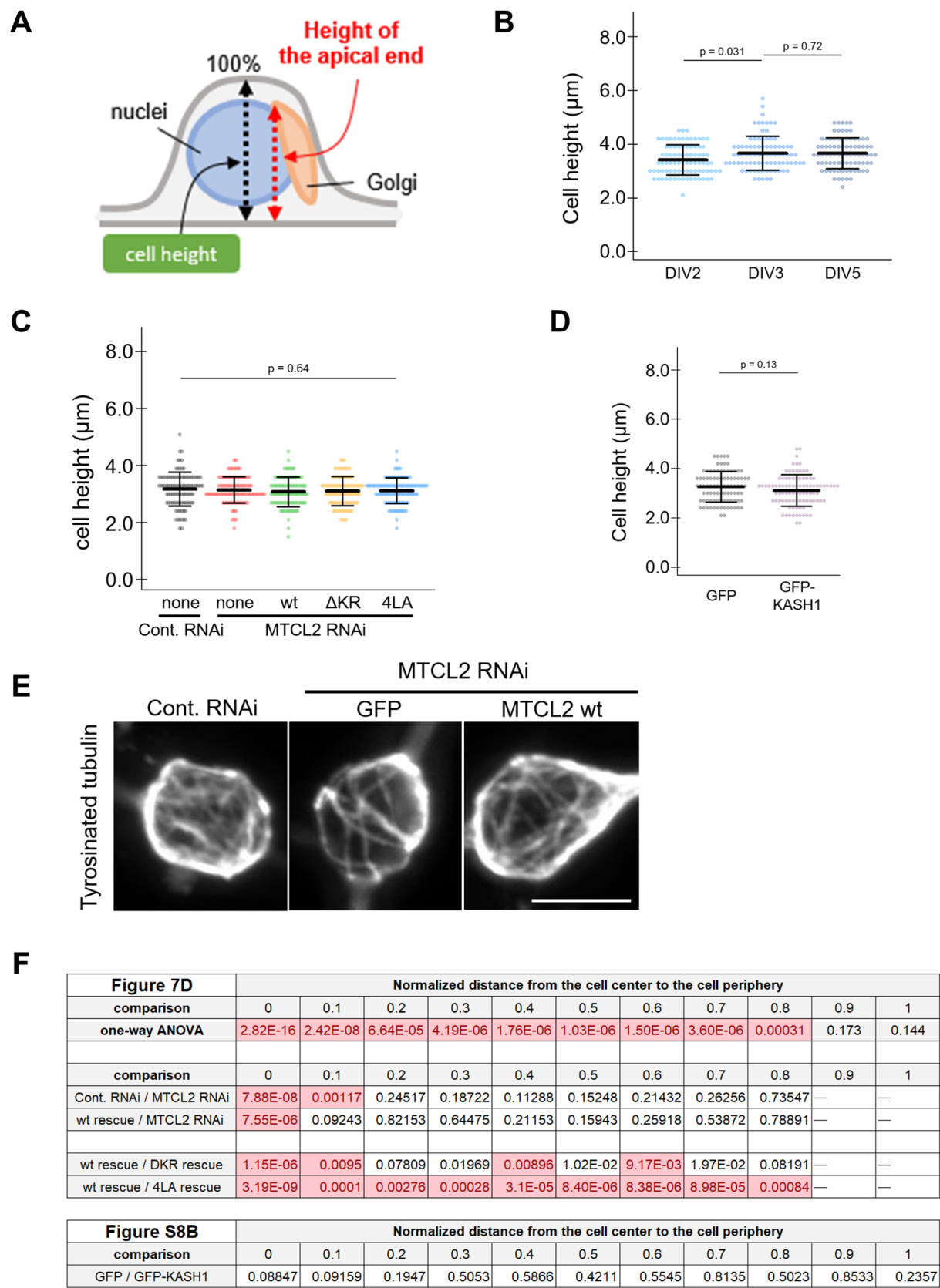
Figure S6



Supplementary Figure S6: Colocalization of MTCL2 with MTs in immature CGNs.

(A) Projection views of the z-stack images of the CGNs analyzed in Figure 6D. Note that at DIV2, the apical MTs tended to align with the bipolar axis without being organized into the MT cage. At DIV3, MT cages were detected on which the MTCL2 granules were colocalized. Scale bar: 5 μ m. (B) Cross-sectional views of the z-stack images shown in Figure 6D. The white lines in (A) indicate the position at which each view is created. Scale bar: 5 μ m.

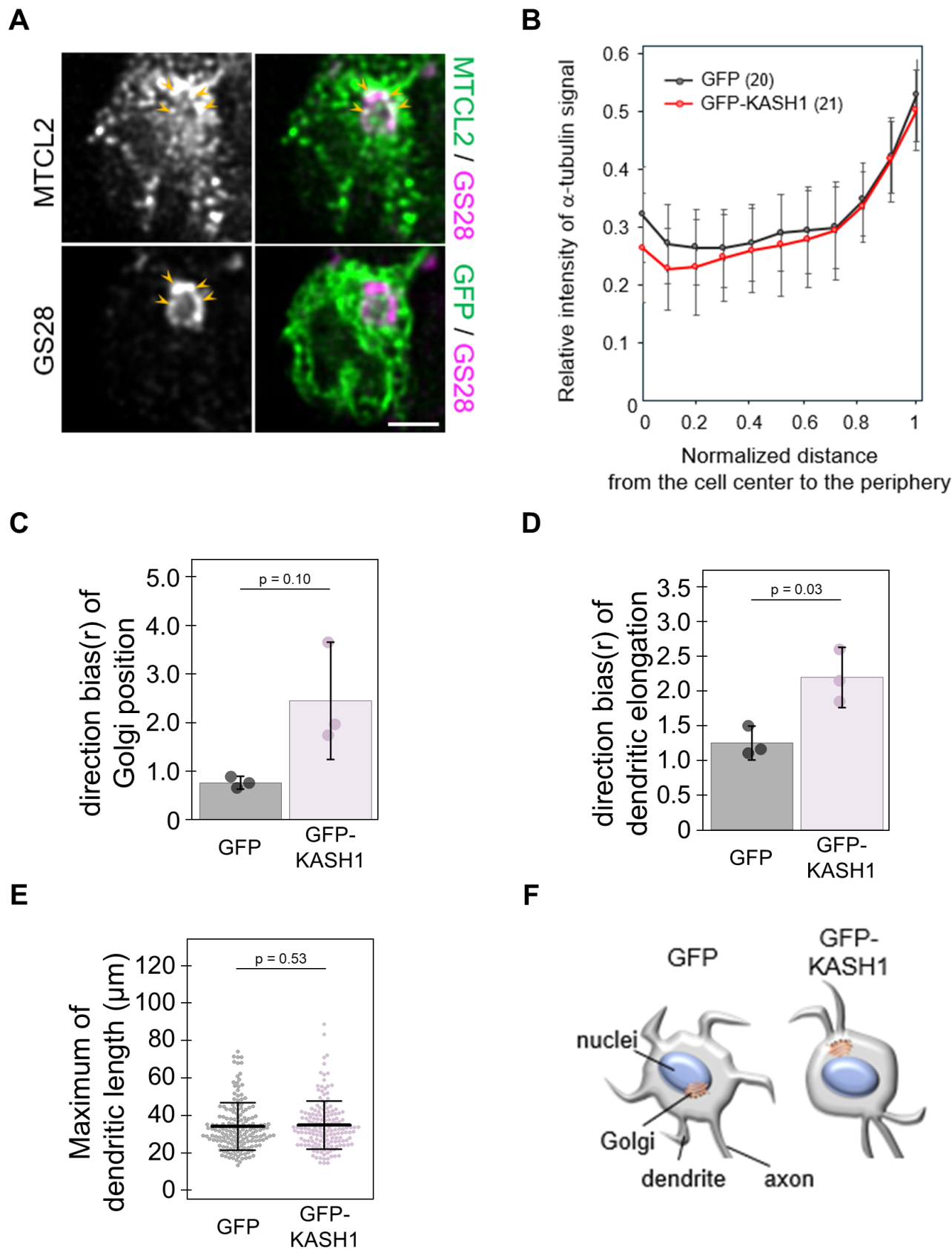
Figure S7



Supplementary Figure S7: CGN heights and MT densities at various maturation stages and experimental conditions.

(A) Schematic representation of how cell height and the height of the apical edge of the Golgi apparatus were defined in this study. (B-D) Distribution of CGN height at the indicated DIV (B), under the indicated RNAi/rescue conditions (C), and when expressing the indicated GFP proteins (D). Each data point represents the result of one neuron observed in 3 independent experiments. The means $\pm$ S.D. are indicated for each scatter plot. In both (B) and (C), the Kruskal-Wallis test was employed to assess differences among groups, and Holm-corrected Mann-Whitney U tests were performed as post-hoc pairwise comparisons to evaluate statistical significance. In (D), the Wilcoxon rank-sum test was used to estimate statistical significance. (E) Representative images of the MT cage of CGNs subjected to the indicated MTCL2 knockdown and rescue. Projection views of confocal z-stack images covering the entire cell body of CGNs. Scale bar: 5 μ m. (F) Statistical significance of changes in MT cage density caused by MTCL2 knockdown and rescue (Figure 7D) and GFP-KASH1 overexpression (Figure S8B). For Figure 7D, one-way ANOVA was performed, followed by Holm-corrected Welch's two-sample t-tests as post-hoc comparisons. For Figure S8B, the Wilcoxon rank-sum test was used. In both cases, *p* values were calculated for the indicated comparisons at each relative distance. Pink indicates *p* values less than 0.01.

Figure S8



Supplementary Figure S8: Inhibition of LINC complex activity mimicked most phenotypes induced by MTCL2 knockdown.

(A) A basal confocal section of the GFP-KASH1-expressing cell shown in Figure 8B. The focus plane is indicated by the dotted lines in Figure 8B. Orange arrowheads indicate the sites where MTCL2 associates with the Golgi apparatus. Scale bar: 2 μ m. (B) Comparison of MT cage density in CGNs subjected to GFP or GFP-KASH1 overexpression. The number of cells (n) analyzed for each condition is indicated in parentheses. The means $\pm$ S.D. are shown. *P* values estimated using the Wilcoxon test are listed in Figure S7E. (C and D) Comparison of the direction bias (*r*) of dendrites and the Golgi position between the GFP- and GFP-KASH1-expressing CGNs analyzed in Figure 8D and Figure 8F, respectively. Each data point represents the average value from each of the 3 independent experiments. The means $\pm$ S.E. are shown. (E) Distribution of the maximum dendrite lengths of the CGNs analyzed in Fig. 8E. Each data point represents the result of a single neuron. The means $\pm$ S.D. are indicated for each scatter plot. Welch's two-sample *t*-test was used to verify statistical significance in (C) and (D), and the Wilcoxon rank-sum test was used for (E). (F) Schematic representation of the dendritic phenotypes of CGNs expressing GFP and GFP-KASH1.