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Dendrite morphogenesis depends on relative levels of NT-3/TrkC signaling

William Joo,1,2 Simon Hippenmeyer,1 Liqun Luo1,2,*

Neurotrophins regulate diverse aspects of neuronal development and plasticity, but their precise in vivo functions during neural circuit assembly in the central brain remain unclear. We show that the neurotrophin receptor tropomyosin-related kinase C (TrkC) is required for dendritic growth and branching of mouse cerebellar Purkinje cells. Sparse TrkC knockout reduced dendrite complexity, but global Purkinje cell knockou had no effect. Removal of the TrkC ligand neurotrophin-3 (NT-3) from cerebellar granule cells, which provide majorafferent input to developing Purkinje cell dendrites, rescued the dendrite defects caused by sparse TrkC disruption in Purkinje cells. Our data demonstrate that NT-3 from presynaptic neurons (granule cells) is required for TrkC-dependent competitive dendrite morphogenesis in postsynaptic neurons (Purkinje cells)—a previously unknown mechanism of neural circuit development.

Neurotrophins regulate the survival, differentiation, and plasticity of peripheral and central neurons (1–3). The mammalian neurotrophin family signals through three tropomyosin-related kinase (Trk) receptors, as well as the p75 neurotrophin receptor (p75NTR). Whereas brain-derived neurotrophic factor (BDNF) has been intensely studied, much less is known about neurotrophin-3 (NT-3) and its receptor, TrkC, despite their widespread expression in the developing and adult brain (4, 5). The lack of central brain cell death in mice lacking NT-3 and TrkC contrasts starkly with the severe reductions in sensory and sympathetic neurons and suggests survival-independent functions (6, 7). NT-3 functions in dendrite morphogenesis in brain slice culture and in propoioceptive axon patterning (8–10). However, evaluating the roles of NT-3/TrkC signaling in the central brain in vivo has been hindered by the early postnatal lethality of NT-3 or TrkC knockout mice and the limited cellular resolution of phenotypic analyses (7, 11).

To study the cell-autonomous function of TrkC in mouse neural development, we used mosaic analysis with double markers (MADM) (12), which with a null allele of trkC that removes all isoforms of the receptor (14) and a pan-neural Nestin-Cre line (15) to drive recombination throughout the brain. Thus, in an otherwise heterozygous animal (trkC+/-), CreloxP-mediated mitotic recombination between homologous chromosomes sparsely labels wild-type (trkC+/+) and homozygous mutant (trkC-/-) cells in distinct colors (figs. S1A and S2). In these animals, sparse trkC-/- cells were labeled with green fluorescent protein (GFP) (green), trkC+/- cells with tdTomato (red), and trkC+/+ cells with both GFP and tdTomato (yellow). Cells without recombination, which remained colorless, were all trkC+/-.

Although survival of central brain cells was not apparently affected by sparse trkC removal (figs. S2 and S3A), consistent with previous observations (6, 7), we observed a distinctive Purkinje cell dendrite phenotype (Fig. 1).

Both trkC+/- and trkC-/- Purkinje cells extended complex dendritic arborsthat spanned the entire molecular layer of the cerebellum (Fig. 1A, left and middle). In contrast, arbors from trkC-/- cells failed to reach the pial surface (65 out of 72 cells; Fig. 1A, right). These stunted arbors exhibited normal dendritic spine density (fig. S3B), but spanned only ~75% of the molecular layer (Fig. 1B). To determine when the trkC-/- dendrite phenotype emerges, we compared trkC+/- and trkC-/- cells between postnatal day 7 (P7) and P21, when Purkinje cells normally elaborate their dendrites and begin to form synapses (fig. 1C and fig. S4). Although indistinguishable from control cells at P7 and P10, trkC-/- cells exhibited reduced dendritic arbor height, branch number, and total dendrite length compared to trkC+/- cells by day 14 (Fig. 1, C and D, and fig. S4). Growth and branching phenotypes persisted in 3-month-old animals (Fig. 1, C and D, and fig. S4), indicating that loss of TrkC caused a morphogenesis defect rather than a developmental delay. Furthermore, the most distal dendritic branches were preferentially lost in trkC-/- cells (fig. S3C). Uniparental disomy (13) (fig. S5) and fluorescent markers (fig. S6) did not affect Purkinje cell dendrite phenotypes. Thus, our mosaic analysis suggested that TrkC is required cell-autonomously for proper Purkinje cell dendrite growth and branching.

Although Trk signaling normally requires the tyrosine kinase domain (16), TrkC also has a kinase-independent role in synaptogenesis (17). To determine whether dendritic arborization relies on kinase activity, we examined MADM mice harboring a conditional allele in which loxP sites flank an exon encoding part of the TrkC kinase domain (18). Here, Nestin-Cre mediated interchromosomal recombination within the MADM cassettes, and also excised this exon to generate a “knase-dead” allele (trkCKD; fig. S1B). At P21, Purkinje cells homozygous for this allele (trkCKD/+) exhibited dendrite height, branch number, and total dendritic length phenotypes apparently affected by sparse trkC removal (figs. S2 and S3A), consistent with previous observations (6, 7), we observed a distinctive Purkinje cell dendrite phenotype (Fig. 1).
comparable to those of trkC<sup>−/−</sup> cells (Fig. 2). Thus, proper dendritic arborization requires TrkC kinase activity.

Because perturbing TrkC in a sparse population (0.5 to 1%) of Purkinje cells disrupted their dendritic arbors, we examined how TrkC ablation from all Purkinje cells affects dendrite morphology. Calbindin immunostaining labels Purkinje cells and their dendrites, which normally span the entire molecular layer of the cerebellum (Fig. 3A). trkC conditional knockout using Purkinje cell-specific pcp2-Cre (19) (fig. S1C) did not reduce Purkinje cell dendrite height compared to controls (Fig. 3, A and B). To analyze dendrite branching in more detail, we used MADM cassettes to sparsely label Purkinje cells in the Purkinje cell–specific trkC conditional knockout background. In this context, pcp2-Cre removed trkC from all Purkinje cells, but GFP and tdTomato were reconstituted only in a sparse subset (5 to 10%) of Purkinje cells through interchromosomal recombination.

**Fig. 1.** TrkC is cell-autonomously required for Purkinje cell dendritic arborization. (A) Postnatal day 21 (P21) red trkC<sup>+/+</sup> and yellow trkC<sup>+/−</sup> Purkinje cell dendritic arbors span the entire molecular layer of the cerebellar cortex. In contrast, trkC<sup>−/−</sup> Purkinje cell dendritic arbors are shorter and fail to reach the pial surface (dashed white lines). Short (a) and long (b) arrows indicate distance from pial surface to top of arbor and molecular layer span, respectively. Scale bar, 50 μm. (B) Quantifications of height deficiency index (a/b) for WT MADM and trkC MADM. Here and in subsequent figures, all values are means ± SEM. ***P < 0.001, one-way analysis of variance (ANOVA) with Tukey’s multiple comparisons test. (C) Traces of Purkinje cell dendritic arbors between P7 and P90. Dashed lines indicate the external granule layer margin in P7–P10, or the pial surface in P14–90. Dots indicate primary branch start point. (D) Quantification of dendrite branch number (left) and cumulative dendrite length (right). Red, trkC<sup>+/+</sup>; green, trkC<sup>−/−</sup>. **P < 0.01, ***P < 0.001, two-way ANOVA with Bonferroni’s multiple comparisons test. See table S1 for additional information including N for each experiment.

**Fig. 2.** Dendritic arborization requires TrkC kinase activity. (A) Analogous to trkC<sup>−/−</sup> arbors (Fig. 1), trkC<sup>KD/+</sup> arbors are shorter than those of trkC<sup>+/+</sup> and trkC<sup>KD/+</sup> cells at P21 and fail to reach the pial surface. (B) Quantifications of height deficiency index (***P < 0.001, one-way ANOVA with Tukey’s multiple comparisons test), branch number, and cumulative dendrite length (**P < 0.01, ***P < 0.001, unpaired t test) as in Fig. 1. Also see table S1.
Fig. 3. Normal dendrite morphogenesis when all Purkinje cells lack TrkC. (A) Calbindin staining labels Purkinje cells and their dendrites (green). Removing trkC from all Purkinje cells (right) does not cause global reductions in dendrite height relative to controls (left) at P21. (B) Molecular-layer thickness across multiple lobules was similar for control pcp2-Cre+/− (white) and pcp2-Cre+/−;trkC KD<sup>fl</sup>ox/fox (black) mice. No significant differences, two-way ANOVA with Tukey’s multiple comparisons test. (C) trkC<sup>KD</sup>/− Purkinje cells do not exhibit dendrite height or branching defects relative to trkC<sup>+/+</sup> cells. (D) Quantifications of height deficiency index, branch number, and cumulative length as in Fig. 1 (no significant differences, unpaired t test). Also see table S1. DAPI, 4′,6-diamidino-2-phenylindole.

Fig. 4. Testing NT-3/TrkC-dependent competition. (A) β-Gal staining reports NT-3 expression patterns in a NT-3<sup>lacZneo/+</sup> knock-in mouse (green). Left: coronal section of the mouse medulla at P7. FoxP2 staining labels inferior olive neurons (red). β-Gal is present in a subset of neurons in the inferior olive principal nucleus (IOPr), but not in the temporal or dorsal nuclei (IOM, IOD). Middle and right: At P7, β-Gal is enriched in granule cells of posterior folia. By P10, β-Gal is present in all folia. No signal was detectable in the external granular layer (EGL, bracket), which contains granule cell progenitors. Likewise, no signal was detectable in Purkinje cells or the deep cerebellar nuclei (DCN, dashed line), the postsynaptic targets of Purkinje cells. (B) Schematic of a viral approach to test relative NT-3/TrkC signaling in dendrite morphogenesis. Viruses encoding either tdTomato (AAV-tdT) or a dominant-negative TrkC together with GFP (AAV-trkCDN-2A-GFP) were coinjected into math1-Cre;NT-3<sup>flox/flox</sup>, math1-Cre;NT-3<sup>flox/+</sup>, or control math1-Cre mice at P1. tdTomato expressing Purkinje cells were analyzed 21 to 24 days after injection. (C) Dendritic arbors of tdTomato expressing Purkinje cells. In math1-Cre;NT-3<sup>flox/+</sup> or math1-Cre;NT-3<sup>flox/flox</sup> mice, TrkC<sup>DN</sup> expressing cells exhibited decreased arbor complexity much like that of MADM trkC<sup>−/−</sup> cells (Fig. 1). However, in math1-Cre;NT-3<sup>flox/flox</sup> animals, dendrite defects were rescued to wild-type levels. (D) Quantification of height deficiency index. *P < 0.05, one-way ANOVA with Tukey’s multiple comparisons test. (E) Quantification of tdTomato and GFP/TrkC<sup>DN</sup> dendritic branch number (top) and cumulative length (bottom). Boxes indicate the mean (middle line) and 25 to 75% range, while whiskers indicate maximum and minimum values. ***P < 0.001, two-way ANOVA with Sidak’s multiple comparisons test. Also see table S1.
Purkinje cells exhibited normal dendrite height, branching, and length (Fig. 3, C and D). Thus, in contrast to sparse MADM-based knockout of TrkC, TrkC ablation from all Purkinje cells did not disrupt dendritic arborization.

Differences in the timing of trkC removal are unlikely to account for the distinct outcomes of global and sparse knockout (Figs. 1 to 3). 

pep2-Cre mediated recombination in nearly all Purkinje cells by P7 and markedly reduced trkC mRNA levels in Purkinje cells by P10 (Fig. S7), well before dendrite phenotypes emerge at P14. A more likely interpretation is that the observed dendrite defects depend on the sparseness of trkC deletion. This raised the possibility of a competitive mechanism (20), in which dendrite morphogenesis depends on relative differences in trkC signaling between neighboring Purkinje cells.

We next investigated the expression pattern of NT-3, the ligand for TrkC (16), using a lacZ knock-in reporter in the NT-3 locus (21). The lacZ product β-galactosidase (β-Gal) was transiently expressed at P7 (but not at P14) in a small subset of inferior olive neurons, which extend climbing fibers to Purkinje cell dendrites (Fig. 4A, left). β-Gal was also expressed in cerebellar granule cells, which send parallel fibers to provide major inputs to Purkinje cell dendrites, but was undetectable in the external granular layer, which contains granule cell progenitors (Fig. 4A, middle and right). This suggests that postmitotic granule cells express NT-3 after migrating to the internal granular layer. Although restricted to granule cells in posterior folia at P7 (Fig. 4A, middle), β-Gal was expressed in all folia by P10 (Fig. 4A, right), coinciding with the time window of TrkC-dependent dendritic development (Fig. 1, C and D). Purkinje cell dendrite morphogenesis likely relies on NT-3 produced around or after P10, as dendrite phenotypes of sparse trkC/−/− Purkinje cells were essentially equal in anterior and posterior folia (fig. S8). Given that β-Gal was undetectable in the deep cerebellar nuclei, the postnatal targets of Purkinje cells (Fig. 4A), presynaptic granule cells are the best candidate cellular source of NT-3.

Conditional knockout of NT-3 from granule cell progenitors using math1-Cre (22) did not affect Purkinje cell dendrite height (fig. S9), branch number, or total length (Fig. 4, B to E). This is consistent with the absence of dendrite phenotypes in Purkinje cell–specific trkC knockout (Fig. 3). We next devised a method to investigate NT-3/TrkC signaling in a competitive context (Fig. 4B). Adeno-associated virus serotype 8 (AAV8) preferentially transduces Purkinje cells when injected into neonatal mice (23). We exploited this tropism to cotransduce two AAV vectors, the first expressing tdTomato as a control, and the second expressing a dominant-negative TrkC construct (fig. S10) together with GFP (TrkCΔDN, 2A-GFP). We achieved sparse labeling (~0.5%) by controlling the titer and volume of neonatal injections (fig. S11). In P21 mice, TrkCΔDN-expressing GFP-positive Purkinje cells exhibited reduced dendrite height, branch number, and total length relative to control, tdTomato-expressing Purkinje cells (Fig. 4, C to E, left columns). This viral approach thus corroborated results from MADM-based sparse knockout.

To test sparse TrkC loss-of-function in the absence of NT-3, we neonatally transduced AAV viruses into conditional knockout mice in which math1-Cre removes NT-3 from all granule cells (Fig. 4B). In herozygous (NT-3+/−) conditional knockout animals, TrkCΔDN-expressing GFP-positive Purkinje cells still exhibited reduced branching number and length compared to tdTomato-positive control cells (Fig. 4, C to E, middle columns). However, in homozygous (NT-3−/−) conditional knockout animals, the dendrite phenotypes of TrkCΔDN-expressing cells were completely suppressed (Fig. 4, C to E, right columns). Thus, granule cell–derived NT-3 is necessary for TrkC-dependent competitive dendrite morphogenesis in Purkinje cells.

In summary, although Purkinje cell dendrite development can proceed in the absence of NT-3 or TrkC, our data indicate that relative intercellular differences in NT-3/TrkC signaling can profoundly modulate dendrite morphogenesis. Specifically, Purkinje cells with lower TrkC levels relative to their neighbors exhibit reduced dendritic arbor complexity. Future studies should elucidate how Purkinje cells compare signals during competitive axon stabilization (24). For example, heterogeneous TrkC activation during development may diversify Purkinje cell dendrite complexity, or homeostatically adjust dendrite branching rates to ultimately equalize Purkinje cell participation in the cerebellar circuit. Furthermore, NT-3/TrkC may cooperate with other signals to regulate dendrite development. Although p75NTR likely acts as a receptor for paracrine signals during competitive axon stabilization and pruning (24, 25), we found that sparse trkC knockout phenotypes persisted in p75NTR−/− mice (fig. S12), suggesting alternative mechanisms. Indeed, TrkC/NT-3 signaling may mediate competition by enhancing neuronal activity or synaptogenesis, both of which are known to modulate dendritic arborization (26, 27). In one scenario, differential TrkC/NT-3 signaling may drive competitive parallel fiber synapse formation and locally stabilize Purkinje cell dendritic branches. Consistent with this idea, each parallel fiber forms synapses with only a subset of Purkinje cells along its trajectory (28), whereas TrkC can mediate postsynaptic differentiation (17) and NT-3 can be anterogradely transported and released from central neuron presynaptic terminals (29).

According to the classic neurotrophic theory, developing axons compete for limiting amounts of neurotrophic factors from their target tissues, which signal retrogradely to support neuronal survival and axon growth (30, 31). Our findings expand this paradigm and suggest that growing dendrites analogously require anterograde NT-3 from their presynaptic partners during competitive dendrite growth.
Supplementary Materials for

Dendrite morphogenesis depends on relative levels of NT-3/TrkC signaling

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Materials and Methods
Figs. S1 to S12
Table S1
References
Materials and Methods

Mouse lines
Mouse lines with chromosome 7 MADM cassettes (13), trkC null allele (14), p75 null allele (39), trkC KD$^{\text{lox}}$ (18), NT-3$^{\text{lox}}$ (40), Nestin-Cre (15), Pcp2-Cre (19), Math1-Cre (22), NT-3lacZ/neo$^{\text{const}}$ (21), and the AI14 Cre reporter (32) have been described previously. All analyses were carried out in a mixed C57/BL6, CD1 genetic background. All animal procedures were carried out according to animal care guidelines approved by Stanford University’s Administrative Panel on Laboratory Animal Care (APLAC).

Dendrite arbor quantifications
Phenotypic analyses included only Purkinje cells residing on the flat banks of folia 3, 4-5, 6, 8, and 9. Purkinje cells lying within sulci (folds between adjacent folia) were excluded. For height deficiency index, we measured the distance between the pial surface and the top of the Purkinje cell dendritic arbor, and divided this distance by the total span of the molecular layer, to account for variations between folium thickness. Dendritic arbors were traced using Imaris software and Simple Neurite Tracer from ImageJ/FIJI. Arbor tracing for AAV-labeled cells in NT-3 conditional knockout backgrounds (Fig. 4C-E) was performed blind to genotype.

Virus vector construction
Full-length TrkC coding sequence was amplified from a mouse whole-brain cDNA library and inserted into a TOPO vector. To assemble the TrkC$^{\text{DN}}$ construct, a TrkC fragment containing the extracellular and transmembrane domains, as well as a short cytoplasmic domain (amino acids 1-486; see fig. S10) was amplified using primers atggatgtctctctttgc and atggttgatgtgatgcagtg, fused to 2A peptide followed by GFP, and subcloned into an AAV production vector together with the CMV promoter, the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), and the bovine growth hormone polyadenylation signal (bGH polyA).

Virus injections
Recombinant AAVs (constructs: pCMV-tdTomato-WPRE-hGH polyA, pCMV-trkCDN-2AGFP-WPRE-hGH polyA) were produced by the Stanford Gene Vector and Virus Core and injected into the cerebellum according to procedures modified from (41). Approximate dilutions: AAV-tdTomato, 5.5x10$^7$ IU/mL, AAV-trkC$^{\text{DN}}$-2A-GFP, 2.4x10$^7$ IU/mL. Postnatal day 1 pups were subjected to cold anesthesia, disinfected, and injected with 750-1000nL AAV solution using a stereotaxic apparatus. Pups were then resuscitated over a heat pad, returned to mothers, and analyzed 2-3 weeks post-injection.

Immunostaining, antibodies, and imaging
Mice were perfused with 4% paraformaldehyde before brain tissue was removed, equilibrated in 30% sucrose/PBS solution for ~48h, embedded in cryo-sectioning medium, and stored at −80°C. 40μm floating sections were stained at 4°C for ~48h in primary antibody according to the following dilutions: chicken anti-GFP (1:500, Aves Labs), rabbit anti-DsRed (1:500, Clontech), mouse anti-Calbindin-D-28K (1:1000, Sigma), rabbit anti-β-Gal (1:250, MP Biomedicals), goat anti-FoxP2 (1:500, AbCam). Secondary antibodies were raised in donkey against mouse, rabbit, chicken, or goat antisera (Jackson Immunoresearch), and conjugated to Alexa 488, FITC, Cy3, or Alexa 647. Confocal images were collected with a Zeiss LSM 510 or 780, and processed with Zeiss LSM software, ImageJ, or Adobe Photoshop.
Fig. S1. Summary of genetic manipulations.
(A) Schematic representation of MADM, used in Fig. 1. Two reciprocally chimeric cassettes, each consisting of split GFP and tdTomato with intervening intronic loxP sites, were knocked into homologous centromeric loci on chromosome 7. A null allele of trkC was placed distal to one cassette. G2 recombination generates either one red trkC+/+ cell and one green trkC–/– cell (top branch), or one yellow trkC+/– cell and an unlabeled trkC+/– cell (bottom branch). (B) Schematic of MADM using a conditional allele of trkC (trkC K/D flox), used in Fig. 2. Nestin-Cre mediates interchromosomal trans-recombination.
between MADM cassettes, as well as *cis*-recombination between the *loxP* sites of the conditional allele. Here, green cells are *trkC*<sup>+/+</sup> and red cells are *trkC<sup>KD/KD</sup>*. (C) Schematic of *trkC* conditional knockout from Purkinje cells, with sparse MADM-based labeling, used in Fig. 3. Mice with MADM cassettes are transheterozygous for the *trkC* null allele and the conditional allele. *pcp2-Cre* excises the conditional allele to render all Purkinje cells *trkC<sup>KD</sup>*. While most cells remain unlabeled (top branch), *pcp2-Cre* also mediates recombination between MADM cassettes in a sparse subset of postmitotic Purkinje neurons, labeling them with both GFP and tdT (yellow, bottom branch). (D) Low magnification of the cerebellum at P21 from the genetic scheme in (C).
Fig. S2. Summary of trkC MADM analyses in the central brain.
(A) Mid-sagittal section of a trkC MADM mouse brain at postnatal day 21. Green trkC/– cells are present in all brain regions examined. (B) Representative images of the olfactory bulb (left), the CA1 region of the hippocampus (middle), and the dentate gyrus (right). TrkC removal does not cause gross defects at the level of cell positioning or dendrite morphology. (C) Example of trkC MADM-labeled cells in the cerebellum. Calbindin staining labels Purkinje cells and their dendrites. Bergmann glia and granule cells are labeled more densely relative to Purkinje cells or deep cerebellar nuclei neurons. (D) A yellow trkC+/- cell immediately adjacent to a green trkC+/– cell, which exhibits stunted dendrite morphology (top left). trkC+/- Bergmann glia do not exhibit gross morphological phenotypes (top right). trkC+/- cerebellar granule cells migrate properly to the internal granular layer of the cerebellum (bottom left). Calbindin-positive, green axon boutons cluster around the soma of a deep cerebellar nuclei neuron (*), suggesting that trkC+/- Purkinje cells project axons properly to their targets (bottom right). Scale bars: 1 mm (A), 100 μm (B, D), 500 μm (C), 25 μm (D, lower right).
Fig. S3. Quantitative analysis of Purkinje cell survival, Sholl analysis of dendritic branching, and dendritic spine density in WT and trkC MADM.
(A) Green/red cell number ratios for Purkinje cells at postnatal day 21 or 90. Individual data points represent ratios of Purkinje cell counts per cerebellar hemisphere, and lines represent their geometric means. All red and green Purkinje cells in each cerebellar hemisphere were counted, and data was pooled from all P21 and P90 WT or trkC MADM animals (see Table S1 for N). Loss of trkC did not affect Purkinje cell survival. (B) Despite their dendrite arborization phenotypes, remaining dendrite branches of trkC<sup>−/−</sup> cells do not exhibit gross deficits in spine density compared to trkC<sup>+/+</sup> cells. Scale bar, 10 μm. Spine density analyzed at P21 and quantified (per 10 μm of dendrite) on the right. (C) Sholl analysis of trkC<sup>+/+</sup> and trkC<sup>−/−</sup> Purkinje cell dendrite arbors at P21 (left) and P90 (right). At both timepoints, dendrite complexity is preferentially reduced in distal regions of trkC<sup>−/−</sup> Purkinje cell dendrite arbors. Analyses were performed using the ImageJ Sholl analysis plugin (Start radius 10 μm, ending radius 200 μm, step size 10 μm, radius span 5 μm, median span type). * p<0.05, *** p<0.001, two-way ANOVA with Tukey’s multiple comparisons test.
Fig. S4: Time course of Purkinje cell dendrite development between P7 and P90.

(A-B) At P7 and P10, Purkinje cells possess relatively rudimentary dendritic arbors that extend to the edge of the external granular layer (EGL), which contains granule cell progenitors. trkC+/+ and trkC−/− Purkinje cells are indistinguishable at these timepoints. (C) By P14, granule cell migration to the internal granule cell layer is nearly complete. trkC+/+ and trkC+/− Purkinje cell dendrites start to contact the pial surface (dashed white lines). In contrast, trkC−/− dendritic arbors extend only partially through the molecular layer. (D) trkC−/− dendrite phenotypes remain severe at P90. These phenotypes are illustrated and quantified in Fig. 1B-D.
Fig. S5: Uniparental disomy does not affect Purkinje cell dendrite arborization.

(A) Left: trkC MADM schematic according to maternal or paternal disomy. Recombination between loxP sites results in near-complete uniparental disomy because MADM cassettes are knocked in close to the centromeres on chromosome 7. Thus, in this mating scheme, green trkC−/− cells are disomic for the maternal chromosome 7 (MM), while red trkC+/− cells are disomic for paternal chromosome 7 (PP). Right: In the reverse-gender mating scheme, trkC−/− cells are PP while trkC+/− cells are MM. Previous qualitative observations suggest that imprinting does not grossly affect Purkinje cell dendrite morphogenesis (13). Here, we test this quantitatively. (B) P21 MM and PP trkC+/− Purkinje cells do not exhibit dendrite phenotypes or disomy-specific morphological differences. Likewise, trkC−/− cells exhibit dendrite phenotypes regardless of maternal or paternal disomy. (C) Left: Quantification of height deficiency index for WT MADM. Uniparental disomy alone does not affect Purkinje cell dendrite height. Right: In trkC MADM, MM and PP trkC−/− cells exhibit equally severe height phenotypes. (D) Quantification of dendrite branch number and cumulative length for trkC MADM. Together, these data indicate that imprinting does not affect Purkinje cell dendrite morphogenesis, and does not genetically interact with trkC loss-of-function. ** p<0.01, one-way ANOVA with Tukey’s multiple comparisons test.
Fig. S6: Reversing the fluorescent marker scheme does not affect MADM analyses. 

(A) Left: when the trkC null allele is distal to the TG cassette, green cells are trkC−/− and red cells are trkC+/- (as in Fig. 1). Right: if the null allele is placed distal to the GT cassette, the MADM labeling scheme is reversed, such that red cells are trkC−/− while green cells are trkC+/- . See also Fig. 2. 

(B-C) Red trkC−/− cells exhibit dendrite height phenotypes comparable to those of green trkC−/− cells (Fig. 1C), indicating that Purkinje cell dendrite morphogenesis is unaffected by the type of expressed fluorescent protein.
**pcp2-Cre<sup>+/−</sup>; R<sub>26</sub>CAG-loxP-STOP-loxP-tdTomato/+**

**A**

**B**

**C**

trk<sub>C</sub> KD<sup>lox</sup>

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Exon 13 Exon 14 Exon 15

---

mRNA

---

Probe

**D**

pcp2:Cre; trk<sup>C</sup><sup>+/+</sup>

**E**

pcp2-Cre; trk<sub>C</sub>KD/KD

**F**

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Fig. S7: Onset of pcp2-Cre activity as indicated by a Cre reporter and trkC mRNA expression

(A) pcp2-Cre mice were crossed to a Cre-dependent reporter line, which expresses tdTomato upon excision of a floxed stop cassette (32). By P7, pcp2-Cre mediates recombination in nearly all Purkinje cells, although isolated cells remain blank in certain lobules (right panel). ML, molecular layer. EGL, external granular layers of both lobules. Dashed lines show approximate position of the EGL border for each folium. (B) Recombination in Purkinje cells is complete by P14. (C) Schematic of the trkC KD^lox locus. Exon 14 encodes a crucial region of the TrkC kinase domain (18), and is flanked by loxP sites. A ~550 bp DIG-labeled probe spanning exons 13-15 was transcribed, hybridized to P10 cerebellum, and visualized with sheep AP-anti-DIG (Roche) and FastRed substrate. Slices were also co-stained with calbindin to label Purkinje cells. (D) At P10, trkC mRNA signal appeared as puncta in Purkinje cells and in the granule cell layer (GCL, border indicated by the dashed lines) in control pcp2-Cre^{+/−};trkC^{+/−} cerebellum. Purkinje cell bodies are outlined in white. (E) trkC mRNA puncta were largely absent in Purkinje cells of pcp2-Cre^{+/−};trkC^{KD/KD} animals. (F) pcp2-Cre^{+/−};trkC^{KD/KD} animals exhibited drastically reduced trkC mRNA puncta number in Purkinje cells relative to control animals (left, N=41 and 46 cells for trkC^{+/−} vs. trkC^{KD/KD}, respectively). Thus, pcp-Cre likely disrupts TrkC function by P10 or earlier. In contrast, pcp-Cre did not affect the density of TrkC puncta in the GCL (right). *** p<0.001, unpaired t-test. Weak binding of the probe to exons 13 or 15 of mRNAs lacking exon 14 may contribute to the residual signals. Residual Purkinje cell signal thus likely represents an overestimate of exon 14-containing trkC mRNA.
Fig. S8: Purkinje cell dendrite phenotypes are equally severe in anterior and posterior lobules. (A) NT-3 enrichment in posterior lobules at P7 (Fig. 4A, middle panel) raises the possibility of lobule-specific dendrite phenotypes. However, trkC−/− Purkinje cells in anterior vs. posterior lobules did not exhibit differences in dendrite phenotype severity at P21. (B) Height deficiency index for Purkinje cells in lobules 3-4/5 vs. lobules 8-9. These data suggest that if granule cell-derived NT-3 acts as a TrkC ligand, it likely affects Purkinje cell dendrite growth around or after P10, when NT-3 is uniformly distributed across all lobules (Fig. 4A, right panel).
Fig. S9: NT-3 removal from granule cells does not cause dendrite height defects.

(A) Removing NT-3 from granule cells using math1-Cre (right panel) does not cause global reductions in dendrite height relative to controls (left panel), as examined using calbindin staining at P21. (B)

Quantification of molecular layer thickness across multiple cerebellar lobules for control Math1-Cre<sup>+/−</sup> (white) or Math1-Cre<sup>+/−</sup>; NT-3<sup>flox/flox</sup> (black) mice. No significant differences by one-way ANOVA with Tukey’s multiple comparisons test.
Fig. S10: Validating dominant negative TrkC.

(A) Schematic of TrkC protein variants. Full-length TrkC contains a cysteine-rich cluster, three leucine-repeat-rich regions (LRR1-3), and two Ig-like domains (Ig1-2), followed by a single transmembrane domain and a cytoplasmic tyrosine kinase domain. The \textit{trkC} gene also encodes several shorter isoforms that lack the kinase domain and possess a distinct C-terminal region (TrkC\textsubscript{TK–}, middle) (33). We designed a putative dominant negative construct (TrkC\textsubscript{DN}, right) containing the extracellular and transmembrane regions, and a short cytoplasmic region lacking the C-terminal domains of either TrkC or TrkC\textsubscript{TK–}. Analogous kinase domain-lacking isoforms of TrkB have been shown to exert dominant negative effects (34-36). (B) TrkC expression in HEK293T (left panel) or Neuro2A cells (right panel) causes cell death in a fraction of transfected cells (compare 1\textsuperscript{st} and 2\textsuperscript{nd} white columns, see ref. 37). Exogenous NT-3 application suppresses cell death in TrkC expressing cells, but not in cells co-expressing TrkC and TrkC\textsubscript{DN} (compare black columns), supporting the notion that TrkC\textsubscript{DN} acts in a dominant negative manner. Cell death is assayed by the percentage of trypan blue positive cells. * \(p<0.05\), *** \(p<0.001\), one-way ANOVA with Tukey’s multiple comparisons test.
Fig. S11: A viral approach for sparse TrkC loss-of-function.

(A) Schematic of the virus injection protocol similar to Fig. 4B except that analyses were performed at P15-16. (B) AAV co-injection can achieve sparse (~0.5%) Purkinje cell labeling. Left panels: Examples of red tdTomato-labeled control Purkinje cells at P15. Right panels: example of a GFP-labeled TrkC^{DN}-expressing Purkinje cell. An antibody against the TrkC extracellular domain could not detect endogenous TrkC protein, but detected overexpressed TrkC^{DN} in green Purkinje cells (bottom row). (C) Example dendritic arbor traces from tdT (top) and TrkC^{DN}-expressing cells (bottom). 7/18 TrkC^{DN}-expressing cells exhibited a dendrite height phenotype. (D) TrkC^{DN} expression causes a significant decrease in dendrite height, branch number, and cumulative length relative to control tdT-expressing cells. Our virus-based loss-of-function approach thus corroborates MADM analyses, although dendrite phenotypes were not as quantitatively severe as in MADM (Fig. 1C-E and Table S1). ***p<0.001, **p<0.01, unpaired t-test.
Fig. S12: 

(A) p75 is highly expressed in Purkinje cells at P7 (38), and is known to mediate competition-dependent axonal branch elimination in sympathetic and olfactory receptor neurons (24, 25). However, MADM- labeled trkC+/+ and trkC+/– Purkinje cells exhibit normal dendritic arbors in a whole-animal p75 homozygous mutant background (p75−/−; left and middle panels). (B) Quantification of height deficiency index for trkC MADM in whole-animal p75+/+, p75+/−, or p75−/− backgrounds. These data indicate that p75 is not required for Purkinje cell dendrite development. Furthermore, p75 removal did not rescue the dendrite phenotype of trkC+/− cells, suggesting that p75-independent signals regulate competition between Purkinje cells.
Table S1: Raw data from dendrite analyses, Fig. 1-4.

**Figure 1. Mean ± SEM (# cells examined)**

<table>
<thead>
<tr>
<th>P21 Height deficiency index (Fig. 1B)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT MADM, 4 animals</strong></td>
</tr>
<tr>
<td>0.019±0.014 (14)</td>
</tr>
</tbody>
</table>

**Dendrite branch number (Fig. 1D, left)**

<table>
<thead>
<tr>
<th>P7, 4 animals</th>
<th>P10, 3 animals</th>
<th>P14, 2 animals</th>
<th>P21, 5 animals</th>
<th>P90, 3 animals</th>
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<tbody>
<tr>
<td>42.91±6.098 (11)</td>
<td>116.14±9.334 (7)</td>
<td>531.3±39.332 (6)</td>
<td>920.9±86.97 (7)</td>
<td>1133±109.5 (7)</td>
</tr>
<tr>
<td>46.93±4.495 (14)</td>
<td>122.0±12.03 (6)</td>
<td>293.8±31.88 (6)</td>
<td>374.6±39.45 (10)</td>
<td>545.7±69.23 (7)</td>
</tr>
</tbody>
</table>

**Cumulative dendrite length, μm (Fig. 1D, right)**

<table>
<thead>
<tr>
<th>Cumulative dendrite length, μm (Fig. 1D, right)</th>
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<tbody>
<tr>
<td>0.019±0.011 (21)</td>
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**Figure 2. Mean ± SEM (# cells examined), 3 animals**

<table>
<thead>
<tr>
<th>Height deficiency index (Fig. 2B, left)</th>
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<tbody>
<tr>
<td>**trakC&lt;sup&gt;Cre&lt;sup&gt;-/+&lt;/sup&gt;&lt;/sup&gt; 972.5±72.75 (5)</td>
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**Cumulative dendrite length, μm (Fig. 2B, right)**

<table>
<thead>
<tr>
<th>Cumulative dendrite length, μm (Fig. 2B, right)</th>
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<tr>
<td>5519±508.3 (5)</td>
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**Figure 3. Mean ± SEM (# cells examined)**

<table>
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<th>Height deficiency index (Fig. 3D, left)</th>
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<tbody>
<tr>
<td>**trakC&lt;sup&gt;-/+&lt;/sup&gt; 0.015±0.010 (15)</td>
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</table>

**Dendrite branch number (Fig. 3D, middle)**

<table>
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<tbody>
<tr>
<td>**trakC&lt;sup&gt;-/+&lt;/sup&gt; 972.7±70.48 (3)</td>
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**Cumulative dendrite length, μm (Fig. 3D, right)**

<table>
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<th>Cumulative dendrite length, μm (Fig. 3D, right)</th>
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<tbody>
<tr>
<td>**trakC&lt;sup&gt;-/+&lt;/sup&gt; 5529±193.4 (3)</td>
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</table>

**Figure 4. Mean ± SEM (# cells examined)**

<table>
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<tr>
<th>Height deficiency index (Fig. 4D)</th>
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<tbody>
<tr>
<td>**math1-Cre&lt;sup&gt;–/+&lt;/sup&gt;; NT-3&lt;sup&gt;/+&lt;/sup&gt; 0.009±0.005 (18)</td>
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**Dendrite branch number (Fig. 4E, top)**

<table>
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<th>Dendrite branch number (Fig. 4E, top)</th>
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<tbody>
<tr>
<td>**math1-Cre&lt;sup&gt;-/+&lt;/sup&gt;; NT-3&lt;sup&gt;/+&lt;/sup&gt; 1020±77.02 (8)</td>
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**Cumulative dendrite length, μm (Fig. 4E, bottom)**

<table>
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<th>Cumulative dendrite length, μm (Fig. 4E, bottom)</th>
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<tbody>
<tr>
<td>**math1-Cre&lt;sup&gt;-/+&lt;/sup&gt;; NT-3&lt;sup&gt;/+&lt;/sup&gt; 5656±238.1 (8)</td>
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</table>

<table>
<thead>
<tr>
<th><strong>trakC&lt;sup&gt;-/+&lt;/sup&gt; MADM, 5 animals</strong></th>
<th><strong>trakC&lt;sup&gt;KD/KD&lt;/sup&gt; MADM, 5 animals</strong></th>
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</thead>
<tbody>
<tr>
<td>0.019±0.014 (14)</td>
<td>0.019±0.011 (21)</td>
</tr>
<tr>
<td>WT MADM, 4 animals</td>
<td>P7, 4 animals</td>
</tr>
<tr>
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<table>
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</table>
References and Notes


14. L. Tessarollo, P. Tsoulfas, M. J. Donovan, M. E. Palko, J. Blair-Flynn, B. L. Hempstead, L. F. Parada, Targeted deletion of all isoforms of the trkC gene suggests the use of alternate receptors by its ligand neurotrophin-3 in neuronal development and implicates trkC in normal


