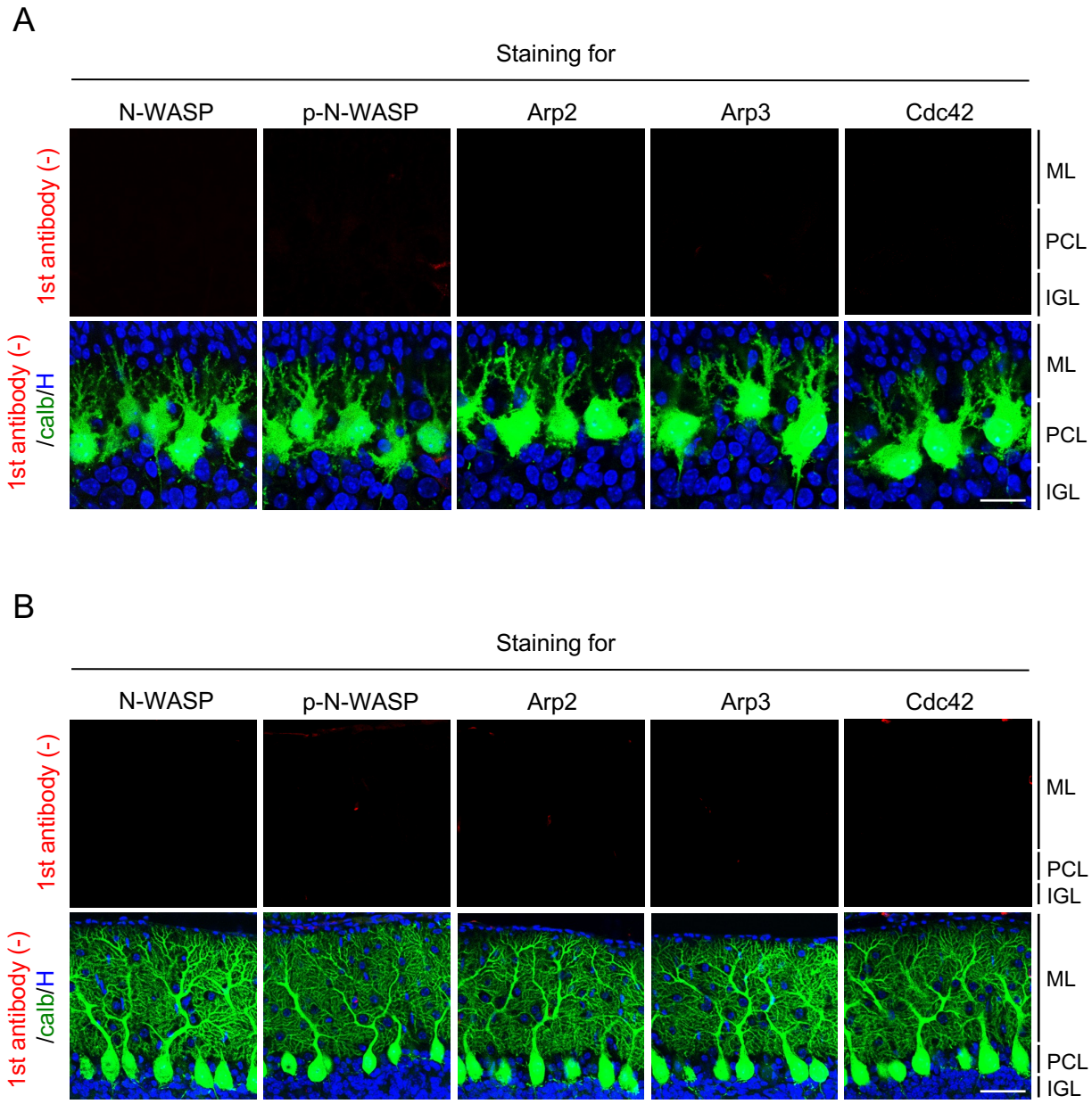


Fig. S1. Validation of the specificity of antibodies used in Fig. 1

For validation of immunosignals for N-WASP, phospho-N-WASP, Arp2, Arp3, and Cdc42 in Fig.1, sections of P6 (A) and P14(B) cerebella were immunostained without primary antibodies except for anti-calbindin (calb, a PC marker). Note that no signal was detected by Alexa594-conjugated fluorescent secondary antibodies alone (upper panels). H: Hoechst, ML: molecular layer, PCL: Purkinje cell layer, IGL: internal granule cell layer. Scale bars represent 20 μm (A) or 50 μm (B).

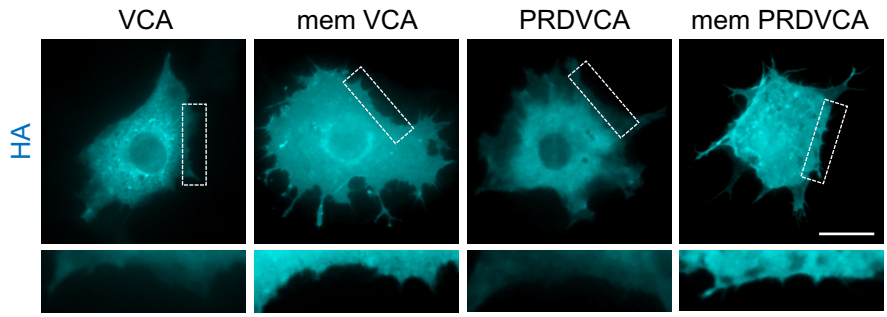


Fig. S2. Cellular localization of N-WASP mutants

HA-tagged N-WASP VCA, mem VCA, PRDVCA, and mem PRDVCA were expressed in NIH3T3 cells. Cells were immunostained with antibody against HA. The dotted boxes indicate the positions of the high-magnification images shown in the bottom panels. Note that N-WASP VCA and PRDVCA were not abundantly localized at the juxtamembrane region, while N-WASP mem VCA and mem PRDVCA were clearly localized to the cell rim. Scale bar represents 10 μm.

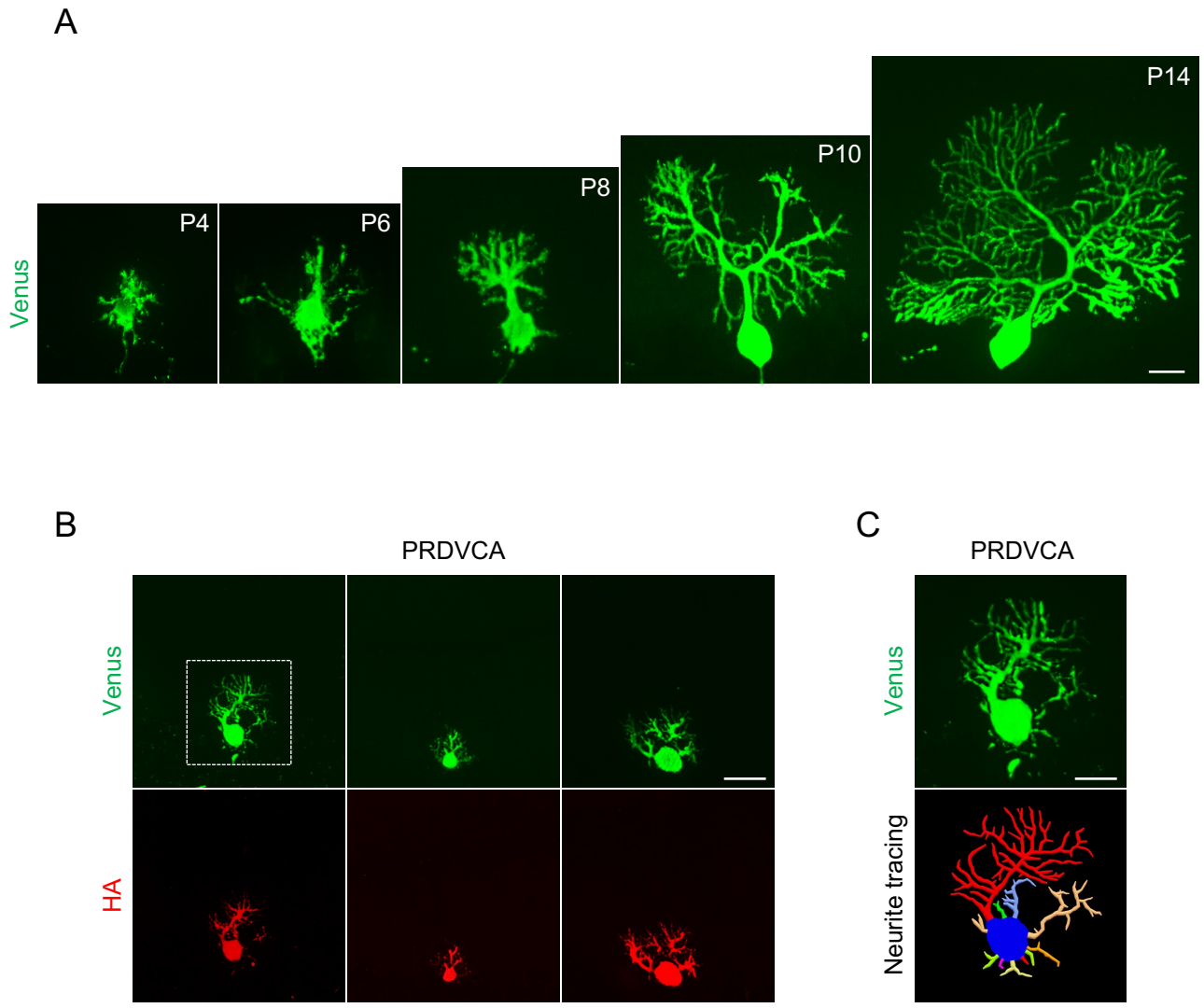


Fig. S3. Developing PC dendrites show drastic morphological change during early postnatal stage

(A) Z sections of Venus-expressing PCs at P4-P14. Venus was expressed in PCs through *in utero* electroporation, and sections were immunostained with antibody against GFP. Note that PCs rapidly transit from the "stellate cell" shape with multidirectional perisomatic dendrites at P4 to the "young PC" shape with a single stem dendrite at P10. (B) Z sections of N-WASP PRDVCA-expressing PCs at P35. HA-tagged N-WASP PRDVCA was specifically expressed in PCs through *in utero* electroporation. Venus was coexpressed in PCs to visualize dendrite morphology. Sections were immunostained with antibodies against GFP (for Venus) and HA. Dotted box indicates the position of the high-magnification image shown in (C). (C) High-magnification image of PC in (B). Bottom panel represents neurite reconstruction of a PC using Neurolucida. Scale bars represent 20 μm (A) and (C), or 50 μm (B).

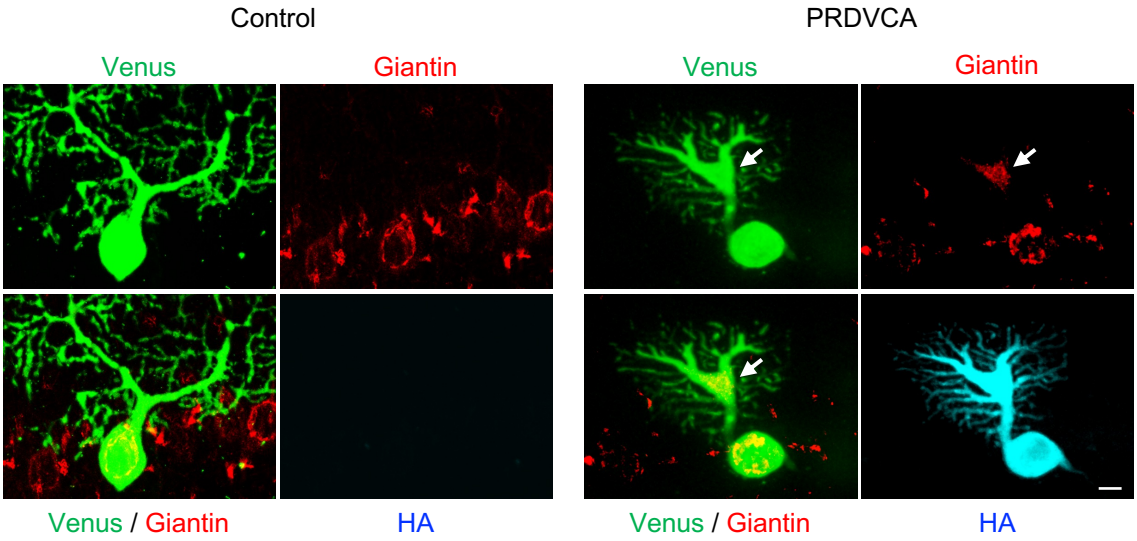


Fig. S4. Inhibition of Arp2/3 causes aberrant hypertrophic structures in the stem-like dendrites in PCs

Z sections of Venus-expressing PCs (control) and N-WASP PRDVCA and Venus-coexpressing PCs (PRDVCA) at P21. HA-tagged N-WASP PRDVCA and Venus were expressed in PCs using *in utero* electroporation. Sections were immunostained with antibodies against GFP (for Venus), HA, and giantin (a marker for the Golgi apparatus). Representative images of PC expressing N-WASP PRDVCA with stem-like dendrite are shown. Arrowheads indicate the hypertrophic structure in the stem-like dendrite. Note that the giantin-labeled golgi apparatuses are highly accumulated in the hypertrophic structure. Scale bar represents 10 μm.

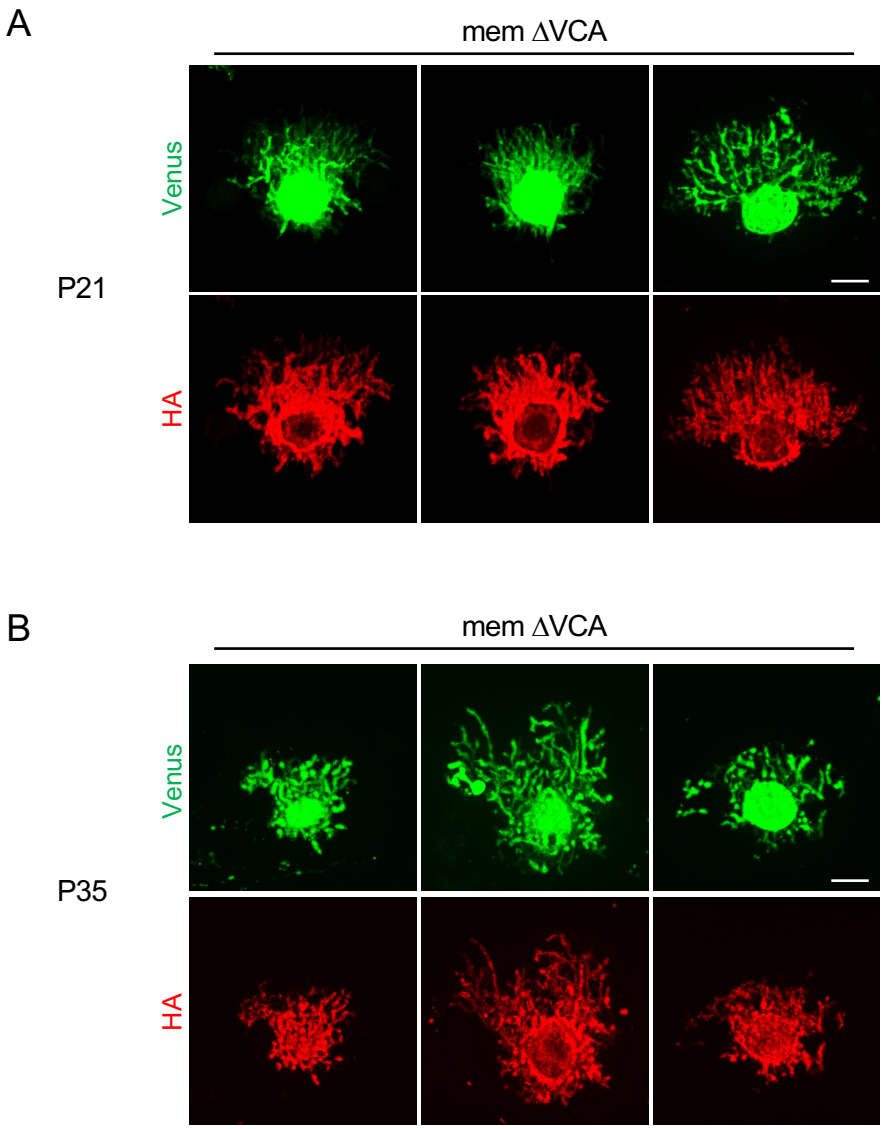


Fig. S5. Inhibition of N-WASP severely impairs maturation of PC dendrites

Examples of N-WASP mem ΔVCA-expressing PCs. Sections of N-WASP mem ΔVCA and Venus-expressing PCs at P21 (A) and P35 (B). HA-tagged N-WASP mem ΔVCA and Venus were coexpressed in PCs by *in utero* electroporation. Sections were immunostained with antibodies against GFP (for Venus) and HA. Three examples of N-WASP mem ΔVCA-expressing PCs are shown. Scale bars represent 20 μm.

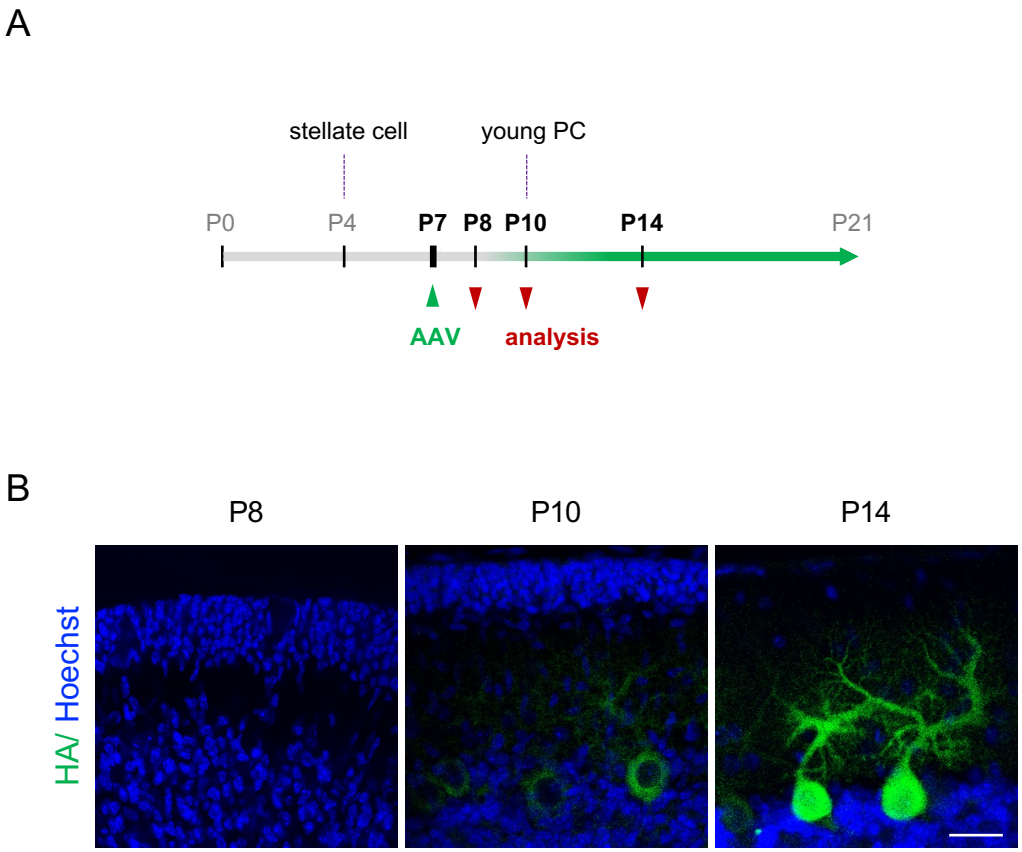


Fig. S6. Time course of AAV expression in PCs

(A) Scheme of analysis for temporal expression of AAV-L7-6-HA-N-WASP PRDVCA in PCs. AAV was intravenously injected at P7, and analyzed at P8, P10, and P14. (B) Sections were immunostained with antibody against HA. Note that N-WASP PRDVCA expression was detected from P10 when all PCs enter the young PC stage. Scale bar represents 20 μm.

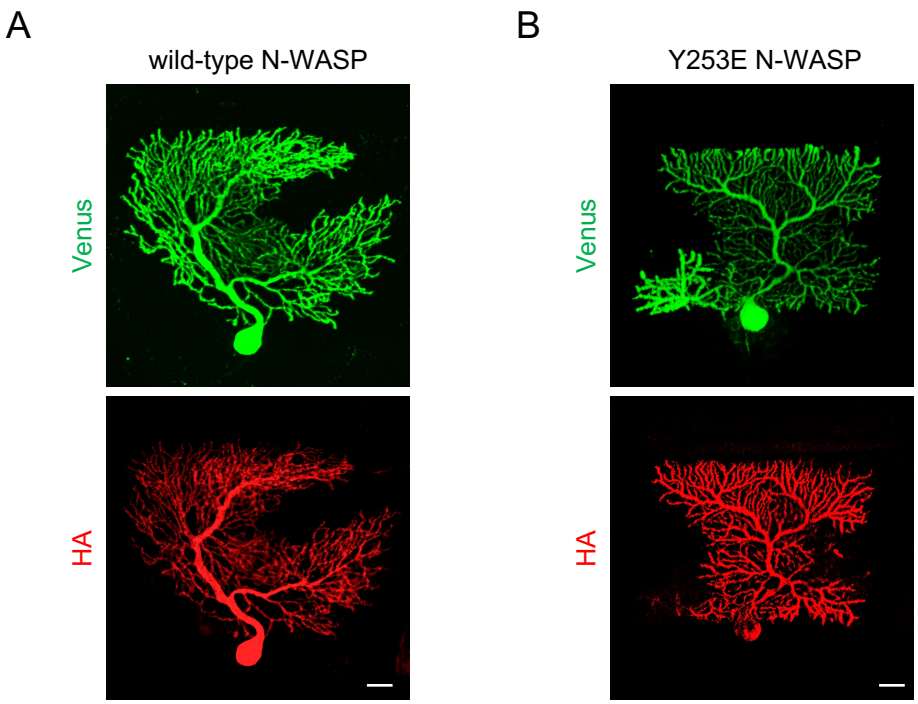


Fig. S7. Overexpression of wild-type N-WASP or N-WASP Y253E do not impair maturation of PC dendrites

Z sections of wild-type N-WASP (A) or N-WASP-Y253E (B)-expressing PCs at P21. HA-tagged wild-type N-WASP and Venus were coexpressed in PCs by *in utero* electroporation. Sections were immunostained with antibodies against GFP (for Venus) and HA. Note that wild-type N-WASP or N-WASP-Y253E-expressing PCs normally develop their dendrites. Scale bars represent 20 μm.

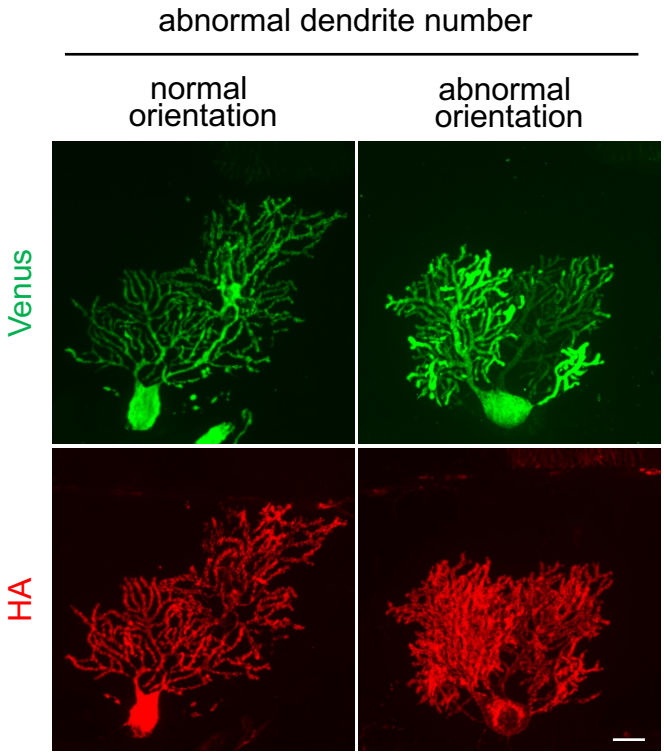
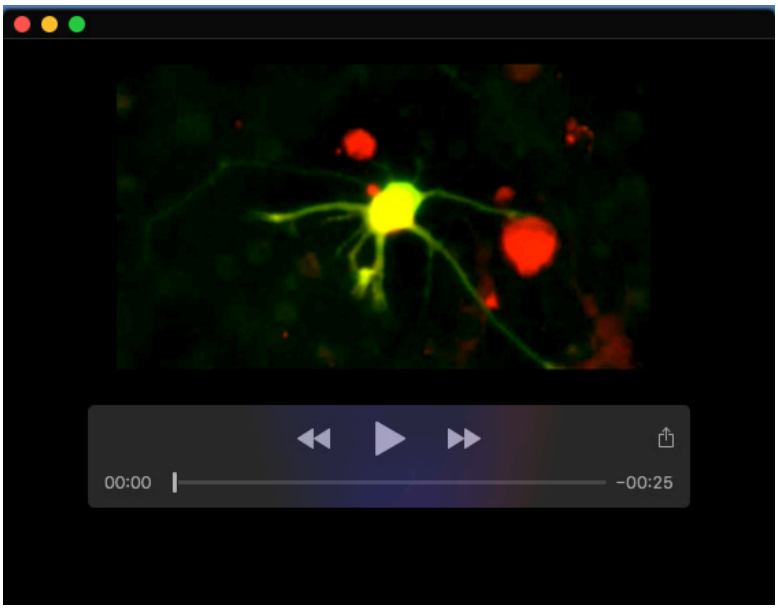
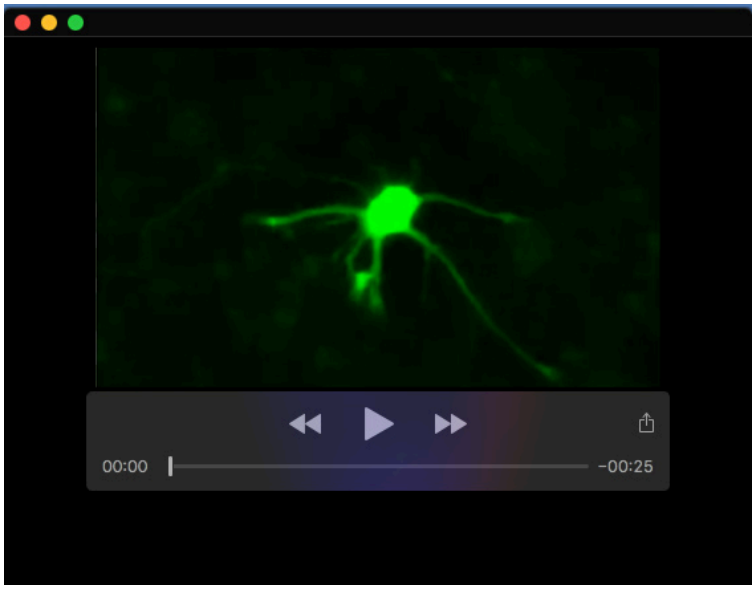


Fig. S8. Abnormalities of mem N-WASP Y253E-expressing PCs

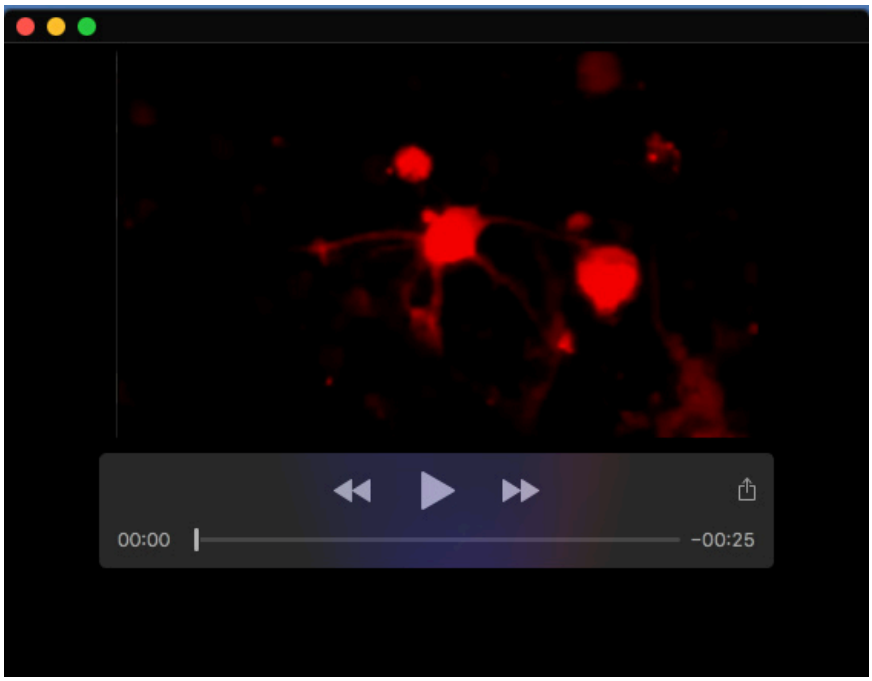
Z sections of membrane-anchored N-WASP Y253E (mem Y253E)-expressing PCs at P21. HA-tagged N-WASP mem Y253E was specifically expressed in PCs by *in utero* electroporation. Venus was expressed in PCs to visualize dendrite morphology. Sections were coimmunostained with antibodies against GFP (for Venus) and HA. Two PCs with multiple dendrites expressing N-WASP mem Y253E are shown. Note that the PC in the left panels has normal dendritic orientation (i.e., apical), while the PC in the right panels has abnormal orientation (i.e., apical and lateral). Scale bar represents 20 μm.



Movie 1. Live cell imaging for dendrite branching and Arp3-mCherry localization in cultured PCs at 5DIV. The movie is a superposition of GFP and Arp3-mCherry. See also Fig. 2A-C.



Movie 2. Live cell imaging for dendrite branching and Arp3-mCherry localization in cultured PCs at 5DIV. The movie is GFP alone. See also Fig. 2A-C.



Movie 3. Live cell imaging for dendrite branching and Arp3-mCherry localization in cultured PCs at 5DIV. The movie is Arp3-mCherry alone. See also Fig. 2A-C.