**SUPPLEMENTARY FIGURE LEGENDS**

**Suppl. Fig. 1. a** A cartoon showing the partial vector map of full-length vinculin tagged to GFP sequence at the 5’ terminus and driven by CAG promoter. **b** Cartoon depicting different mutant clones of *vinculin* constructed and tested. Red vertical lines indicate the positions of point mutations.

**Suppl. Fig. 2. Vinculin tail domain expression increases neurite branching.** **a** Quantification of GFP fluorescence intensity in the cell soma of neurons expressing either control plasmid, Vcl-FL or Vcl-T. The expression levels of Vcl-T was comparable to that of control plasmid and Vcl-FL (n=25 cells). **b** Neocortical neurons from P0.5 pups were electroporated with Vcl-T and control GFP plasmid and grown for 4 DIV. Cells were fixed and immunostained for GFP (to label transfected neurons) and Tau (to label axons). Vcl-T expression increased both the number of primary and secondary neurites but did not affect polarity as seen by only one Tau+ neurite. Non-transfected neurons (white arrows) and dead cells (yellow arrows). Scale, 100 µm. **c** Quantification of number of Tau+ neurites and number of primary and secondary neurites from (a) (n=100 cells). \*\*\**p*<0.001, \*\*\*\**p*<0.0001; ns, non-significant. Two-tailed Student’s *t* test.

**Suppl. Fig. 3. Vcl-T increases branching early in development *in vitro*.** Cortical neurons from neonatal mice were transfected with control plasmid and Vcl-T. **a-d** Cells were fixed at 24 h **(a)** and 48 h **(c)** and visualized for GFP expression. Vcl-T expressing neurons had a greater number of both primary and secondary neurites even at 24 h post-plating. Quantification of the length of the axon, number of primary and secondary neurites at 24 h **(b)** and 48 h **(d)** (n=100 cells). Scale, 100 µm (a), (c) \*\*\*\**p*<0.0001; ns, non-significant. Two-tailed Student’s *t* test (b), (d).

**Suppl. Fig. 4. Expression of Vcl-T does not affect focal adhesions (FA).** **a, c** Representative images of neurons transfected with either control GFP vector or Vcl-T construct and immunostained for GFP and focal adhesion kinase (FAK) showing cell soma (**a**) and growth cone (**c**). Scale, 10µm. **b, d** Quantification of FAK density in the cell soma (**b**) and growth cone (**d**) in control and Vcl-T expressing neurons (n=10-12 cells). ns, non-significant.

**Suppl. Fig. 5. Expression of Vcl-T does not affect cofilin distribution.** **a, c** Representative images of neurons transfected with control GFP vector or Vcl-T construct and immunostained for GFP and Cofilin. Expression of Vcl-T does not affect cofilin distribution in the cell soma (**a**) or growth cone (**c**) (Scale, 10µm). **b** Quantification of cofilin density in cell soma (**c**) and growth cone (**d**) in control and Vcl-T expressing neurons (n=10-12 cells). ns, non-significant.

**Suppl. Fig. 6. Abolishing actin-Vcl-T interaction does not affect cell soma but collapses growth cone. a** Representative images of neocortical neuronstransfected with control vector, Vcl-T and Vcl-TI997A constructs and stained for F-actin using rhodamine-conjugated phalloidin (Rho-Pha). Abolishing interaction between Vcl-T and actin (Vcl-TI997A) does not affect the cell soma area. **b** Quantification of the cell soma area in control, Vcl-T and Vcl-TI997A expressing neurons shown in (a) (n=100 cells). **c** Representative images from live imaging of control Vcl-T and Vcl-TI977A expressing neurons co-transfected with LifeAct-mCherry to visualize actin. Abolishing actin-Vcl-T interaction collapses growth cone similar to Vcl-T. **d** Quantification of the growth cone area in control, Vcl-T and Vcl-TI997A expressing neurons shown in (c) (n=25 cells). **e** Representative images of neurons electroporated with either empty GFP (control) vector or vinculin full-length defective in actin-binding (Vcl-FLI997A). **f** Quantification of axon length and number of primary and secondary neurites from neurons shown in (e) (n=100 cells). Scale, 50 µm (a), 10 µm (c), 100 µm (e). \*\*\*\**p*<0.0001; ns, non-significant. One-way ANOVA (b), (d), Two-tailed Student’s *t* test (f)