Supplementary Figure 1

MyosinVa is expressed in brain, but not in the liver. Homogenate of 3 individual E15.5 mouse cerebral cortices and livers were analysed by western blotting using indicated antibodies.

Supplementary Figure 2

Normal morphology of cortical and hippocampal neurons in dilute lethal mice. a, b, Brains of wild type (wt) and dilute lethal (dilute) littermates were harvested at P16 and processed for Golgi staining[7]. The overall morphology of neurons in the polymorphic layer of the hippocampus, a, in addition to neurons in the cerebral cortex, b, is normal. c, Vibratome sections of the cortex of P12 wt and dilute lethal mice were labelled with Topro (blue) and anti-NeuN antibody (green) to visualize equal neuronal soma sizes.

Supplementary Figure 3

All members of the MyosinV class, MyosinVa, MyosinVb, and MyosinVc, interact with PTEN. HEK293 cells were co-transfected with GFP-PTEN and flag-MVag, flag-MVbg or flag-MVcg. Following anti-flag pull-down, precipitates were analysed with anti-PTEN and anti-flag antibodies (for MyosinV globular domains, MVg). Empty flag vector (-) was used in control experiments.

Supplementary Figure 4

MyosinVa, MyosinVb, MyosinVc and PTEN expression in dilute lethal mice. a, Cortices from E15.5 wild type (wt) or dilute lethal (dilute) mice were isolated for real time quantitative PCR. Each data point represents the mean mRNA level normalized to three control genes (*Gapdh, Hprt* and *Prgk1*) $\pm$ sem. Values are standardized to the expression of the gene in wt mice, which was set to 1. n=12 mice in each group; no significant changes were detected (Student's t-test). b, Expression of

MyosinVb in wt brains at E15.5 is significantly lower compared to MyosinVa. Bar graph show the average cycle threshold ± sem; n=12 in each group. *p<0.0001. MyosinVc expression was undetectable.

Supplementary Figure 5

Loss of MyosinVb in dilute lethal mice increases neuronal soma size. a, Validation of MyosinVb siRNA. HEK293 cells were transfected by nucleofection and cultured for 48 hours before analysis by western blotting using indicated antibodies. Expression of Flag-MyosinVb globular domain (MVbg) with MyosinVb siRNA leads to substantial knockdown of MVbg (densitrometry measurements below blot reflect anti-flag/loading). B, Hippocampal neurons from E15.5 wild type (wt) or dilute lethal (dilute) mice were cultured for 7 days *in vitro* (DIV) and co-transfected with IresGFP and unspecific siRNA (0.5 pM), or with IresGFP and MyosinVb siRNA (0.5 pM) before fixation at 14 DIV. Each data point represents the relative neuronal soma area compared to control (IresGFP) expressing neurons ± sem, n=3 independent experiments, in each experiment at least 80 neurons were assessed. *p<0.005.

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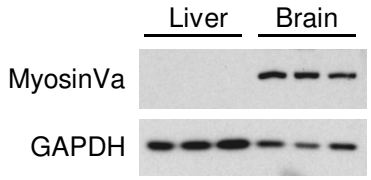
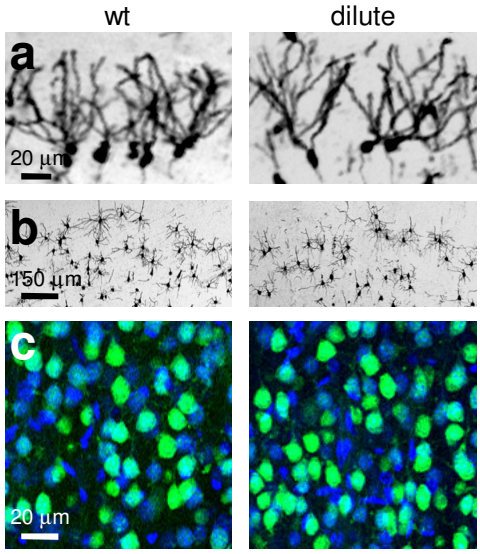
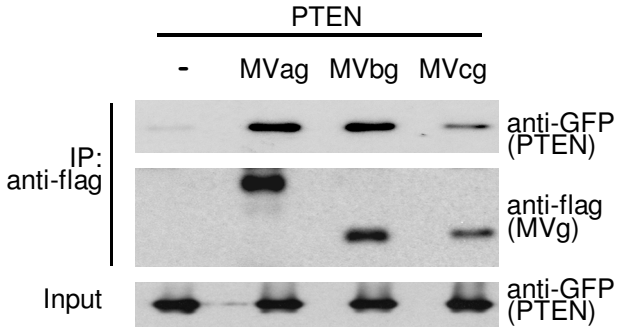
Supplementary Figure 1 – Eickholt : MyosinVa is expressed in brain, but not in the liver.



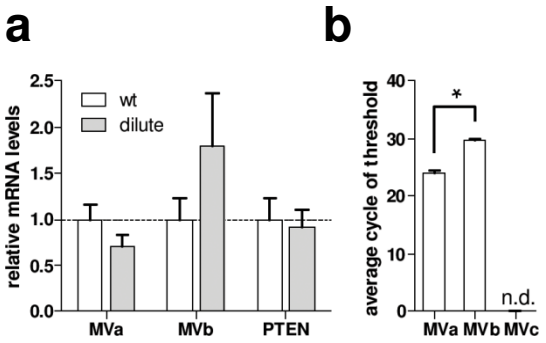
Supplementary Figure 2 – Eickholt : Normal neuronal morphology in dilute lethal mice.



Supplementary Figure 3 – Eickholt : All members of the MyosinV class, MyosinVa, MyosinVb, and MyosinVc, interact with PTEN.



Supplementary Figure 4 – Eickholt : MyosinVa, MyosinVb, MyosinVc and PTEN expression in wild type and dilute lethal mice.



Supplementary Figure 5 – Eickholt : Loss of MyosinVb in dilute lethal mice increases neuronal soma size.