SUPPLEMENTARY INFORMATION

An assay to image neuronal microtubule dynamics in mice

Tatjana Kleele[1], Petar Marinković[2,3], Philip R. Williams[1], Sina Stern[4], Emily E. Weigand[5], Peter Engerer[1], Ronald Naumann[6], Jana Hartmann[7], Rosa M. Karl[7], Frank Bradke[4], Derron Bishop[5], Jochen Herms[2,3,8], Arthur Konnerth[7,8,9], Martin Kerschensteiner[8,10], Leanne Godinho[1] and Thomas Misgeld[1,3,8,9]

[1] Institute of Neuronal Cell Biology, Technische Universität München, Munich, Germany

[2] Center for Neuropathology and Prion Research, Ludwig-Maximilians-Universität München, Munich, Germany

[3] German Center for Neurodegenerative Diseases (DZNE), Munich, Germany

[4] Axon Growth and Regeneration, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

[5] Department of Physiology, Indiana University School of Medicine–Muncie, Muncie, Indiana, USA

[6] Transgenic Core Facility, Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany

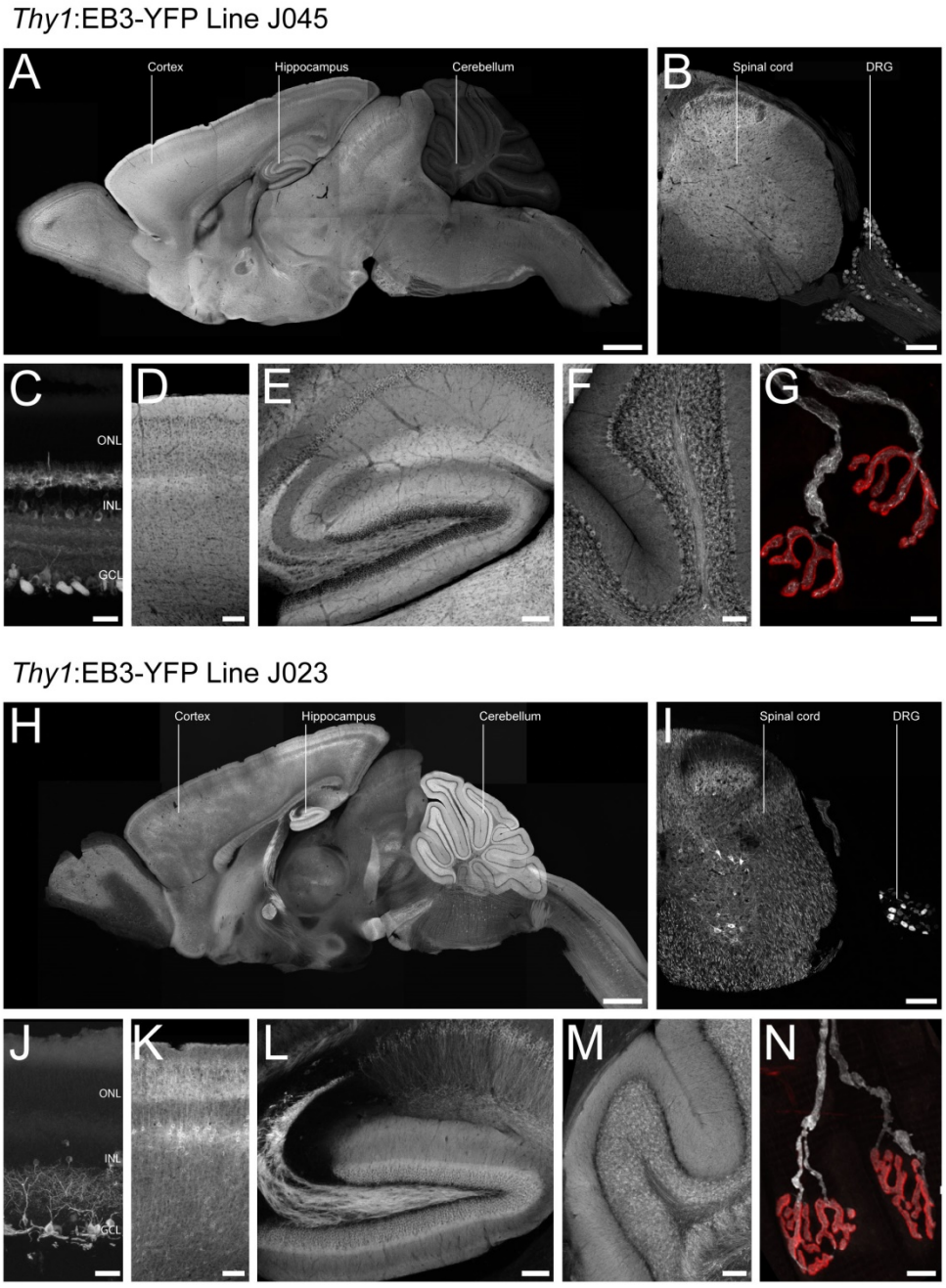
[7] Institute of Neuroscience, Technische Universität München, Munich, Germany

[8] Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

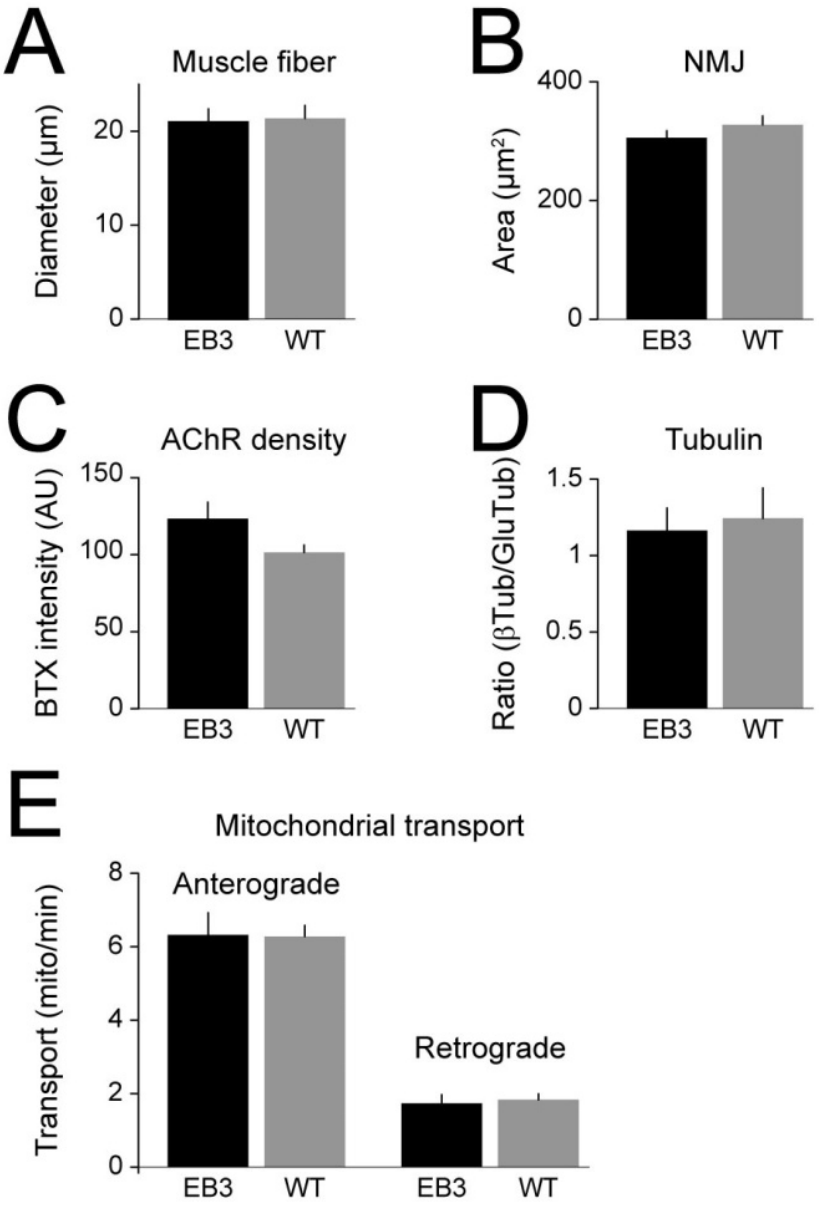
[9] Center of Integrated Protein Science (CIPS^{M}), Munich, Germany

[10] Institute of Clinical Neuroimmunology, Ludwig-Maximilians Universität München, Munich, Germany

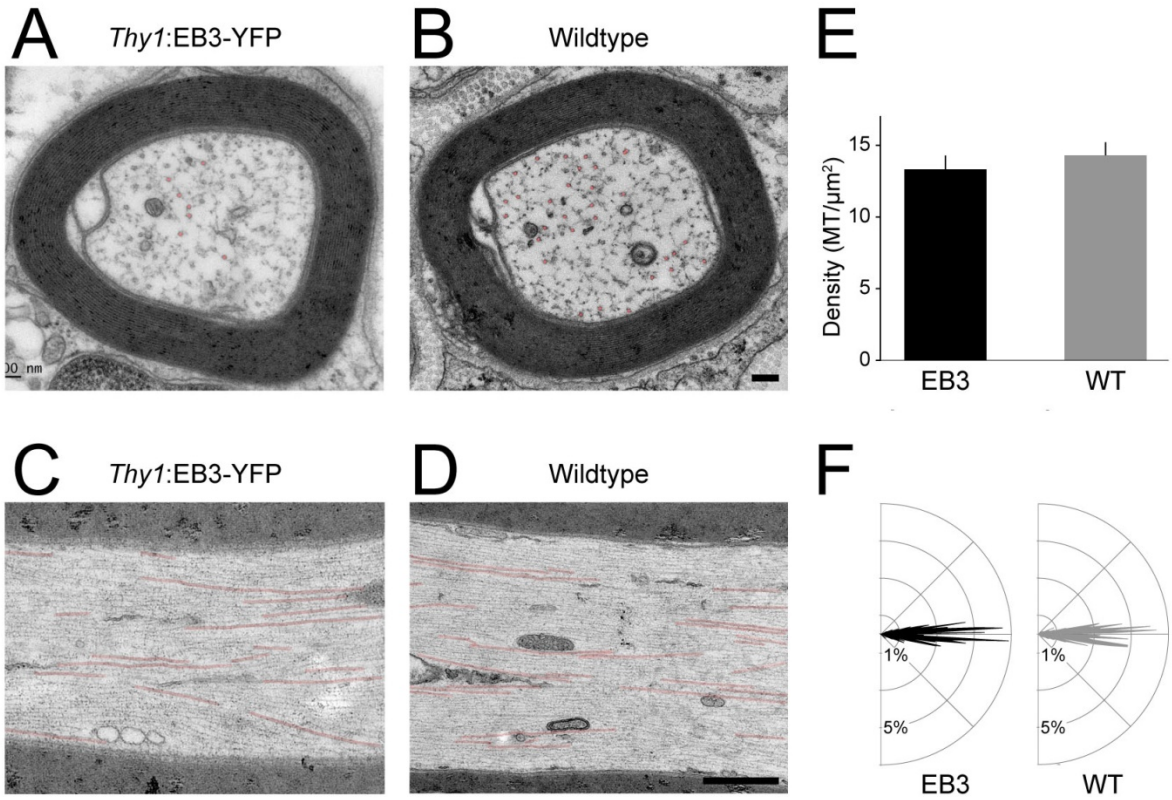
SUPPLEMENTARY FIGURES



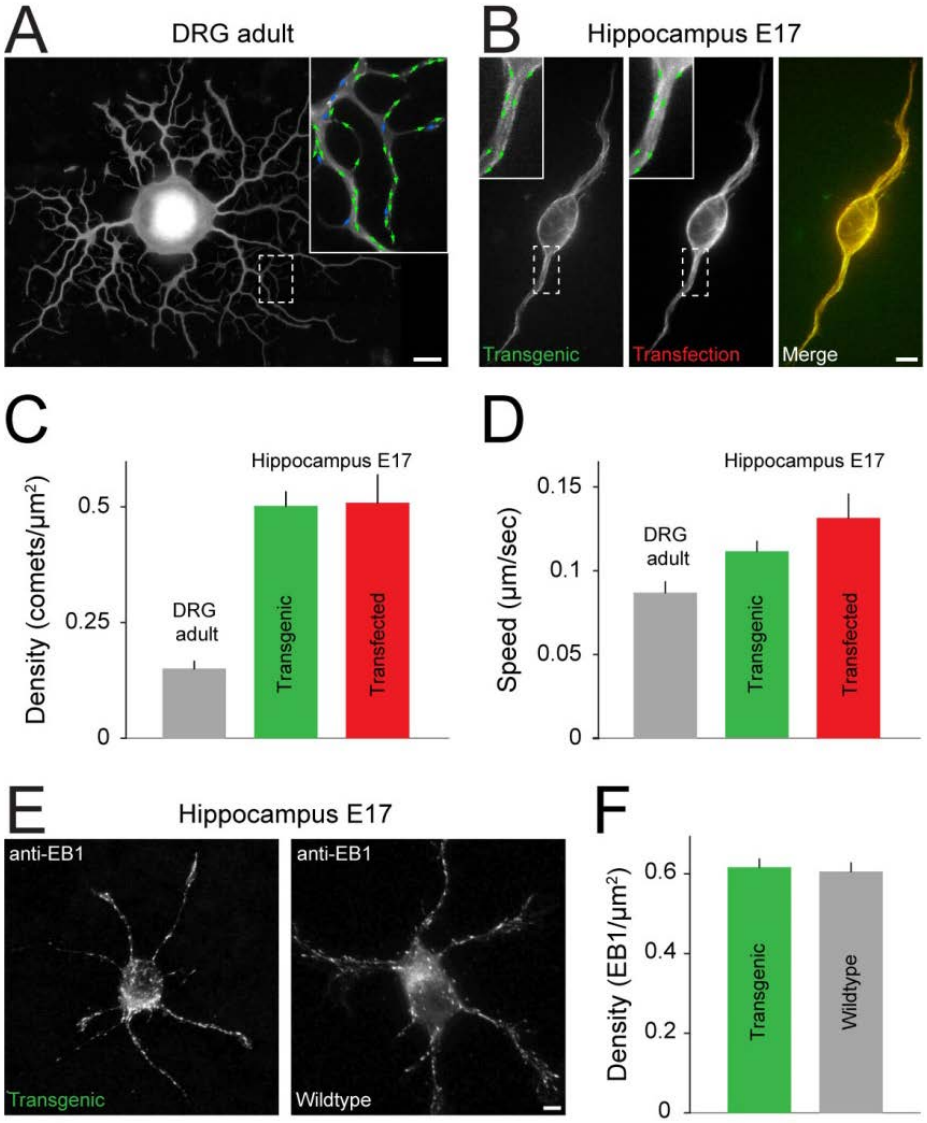
Supplementary Figure 1│ **Expression pattern in two different *Thy1*:EB3-YFP transgenic mouse lines.** The EB3-YFP transgene is expressed widely in both transgenic lines (**A-G**: EB3J045; **H-N**: EB3J023) in the CNS (**A** and **H**, sagittal section of the brain; **B** and **I**, horizontal section of the spinal cord; **C** and **J**, retina; **D** and **K**, cortex; **E** and **L**, hippocampus; **F** and **M**, cerebellum) and the PNS (**G** and **N**, neuromuscular junctions, postsynaptic receptors stained with bungarotoxin in **red**; but also note expression in dorsal root ganglia in **B** and **I**). Scale bar in **A** and **H** 1 mm; in **B** and **I** 200 µm; in **C** and **J** 100 µm; in **D**, **E**, **F**, **K**, **L** and **M** 50 µm and in **G** and **N** 5 µm.



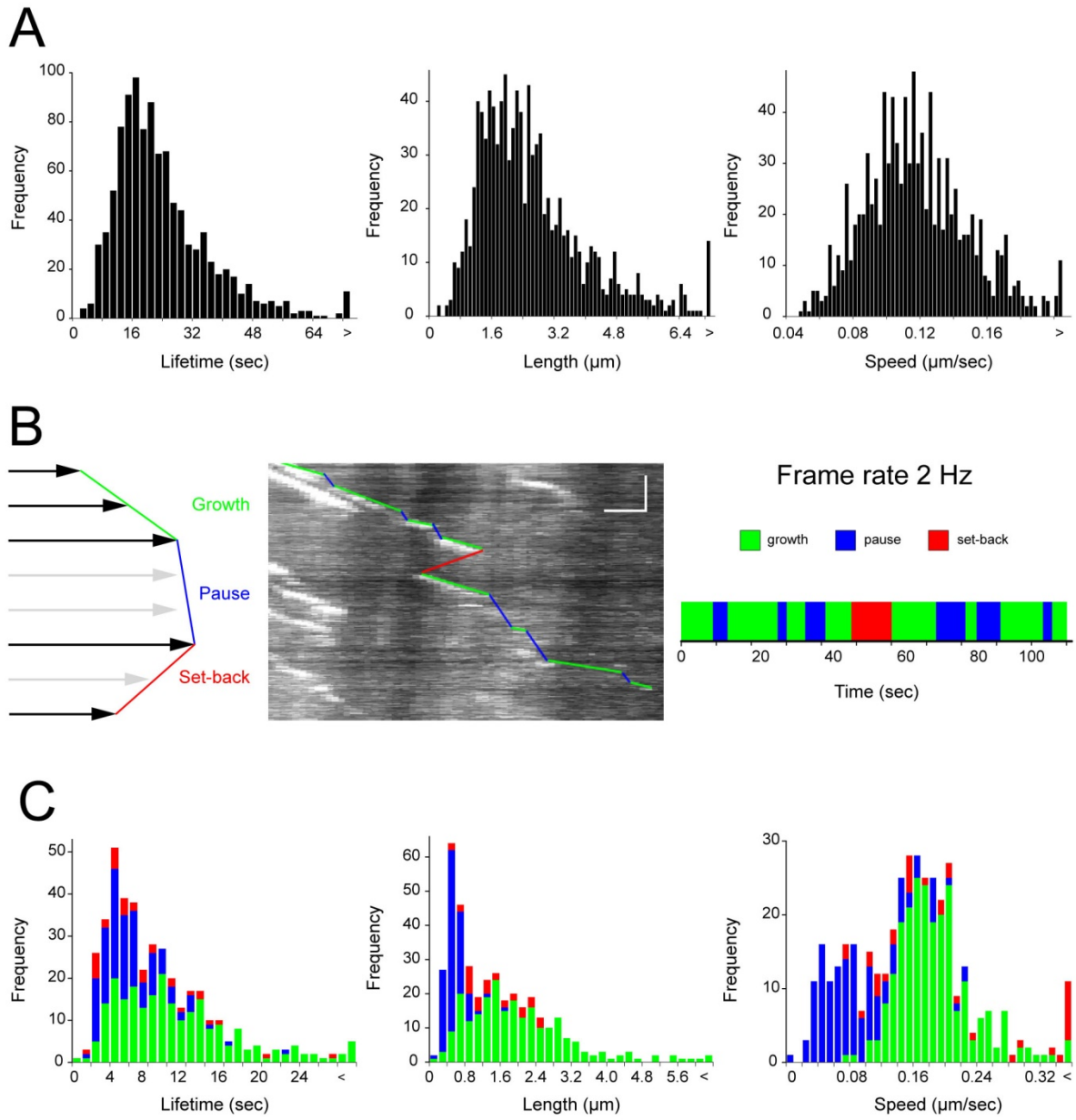
Supplementary Figure 2 | No abnormalities were found in the neuromuscular system of *Thy1*:EB3-YFP mice. (**A-C**) *Thy1*:EB3-YFP mice (line J045; "EB3") have normal muscle fiber diameters (**A**), as well as normal postsynaptic areas (**B**) and receptor densities (**C**) at neuromuscular junctions (compared to non-transgenic litter-mates, "WT"; n > 240 muscle fibers or n > 50 synapses from 3 animals per genotype). (**D-E**) The ratio of all neuronal tubulin ("βTub") to detyrosinated tubulin ("GluTub"; measured by double-immunostainings; **D**; n > 40 axons from 3 mice per genotype) and the axonal transport flux of mitochondria (measured in *MitoMice* crossed into *Thy1*:EB3-YFP mice, **E**) is the same between transgenic and non-transgenic litter-mates (n > 25 axons from 3 animals per genotype).



Supplementary Figure 3 | PNS axons and microtubules are ultrastructurally normal in *Thy1*:EB3-YFP mice. (**A-D**) Electron micrographs of perpendicular (**A, B**) and longitudinal (**C, D**) sections of axons in intramuscular fascicles of *Thy1*:EB3-YFP mice (line J045; **A, C**) and in non-transgenic litter-mates (**B, D**) are indistinguishable. (**E, F**) Quantification of microtubule (MT) density (**E**) and orientation in relation to axon axis (polar plots, **F**). Microtubules are pseudo-colored red; n ≥ 3 axons from 2 mice. Scale bar in **B** 0.1 µm (also for **A**); in **D** 0.5 µm (also for **C**).



Supplementary Figure 4 | Comparison of endogenous, transfection- and transgene-based comet labeling in cultured neurons. (A) Adult DRG neuron from *Thy1*:EB3-YFP transgenic mice. Inset shows a higher magnification of DRG neurites with EB3 comets represented as in **Fig. 1**. (**B**) E17 hippocampal neurons show a 100% co-localization (insets) of the EB3-YFP transgene and transfected EB3-mCherry comets. (**C, D**) Quantification of density and speed from adult DRG and E17 hippocampal neurons cultured from *Thy1*:EB3-YFP mice and EB3-mCherry transfected wildtype hippocampal neurons. Transgenic and transfected hippocampal neurons do no differ significantly in density (**C**) or speed (**D**; n = 7 neurites from ≥ 3 cells for each group). (**E, F**) Immunostainings of EB1 in *Thy1*:EB3-YFP transgenic and wildtype E17 hippocampal neurons show a similar pattern and density of speckled staining (**E**). Quantification of EB1 speckle density shows no significant difference between those two groups (**F**; n = 32 neurites from ≥ 9 cells for each group). Scale bar in **A** 20µm and in **B, E** 5µm.



Supplementary Figure 5 | Analysis of comet tracks. (**A**) Histograms showing the distribution of lifetime, length and speed of the comets in distal motor axons in the triangularis sterni explant, which were also used to generate plots in **Fig. 1**. (**B**) Analysis of track characteristics derived from 2 Hz imaging of comets in intercostal axons of *Thy1*:EB3-YFP triangularis explants. Schematic of movement behaviors (**left**), kymograph of an axon segment with classified track segments indicated for one comet (**middle**), analysis as "event classification" along the time axis (**right**). (**C**) Frequency distribution of track segments color coded for respective behavior (n = 69 tracks from 2 animals). Scale bars in **B** 2 µm, horizontal bar; 10 sec, vertical bar.

SUPPLEMENTARY TABLE

Line	Spinal cord		Retina			Cortex	Cerebellum			Hippocampus				Expression level
	MN	DRG	RG	AC	BC		GC	MF	PC	DG	CA1	CA2	CA3	
J045	+	+	+	+	-	+	+	+	Subset	+	+	+	+	Dim*
J023	+	+	Subset	Subset	-	L2/3; L5	+	+	-	+	+	+	-	Bright**
J030	+	+	+	Subset	Subset	L5	+	Subset	-	+	+	+	-	Very bright***

+: expression in most cells; subset: expression in < 80% of cells
AC: amacrine cells; BC: bipolar cells; CA: cornu ammonis; DG: dentate gyrus; DRG: dorsal root ganglion; GC: granule cells; L: cortical layer; MF: mossy fibers; MN: motor neurons; PC: Purkinje cells; RG: retinal ganglion cells

* used in most experiments reported here

**used in cortical *in vivo* imaging

*** not used as labeling pattern is filamentous rather than comet-like *in vivo*

Supplementary Table 1 | Summary of expression patterns in three *Thy1*:EB3-YFP mouse lines.