Inventory of Supplemental Information

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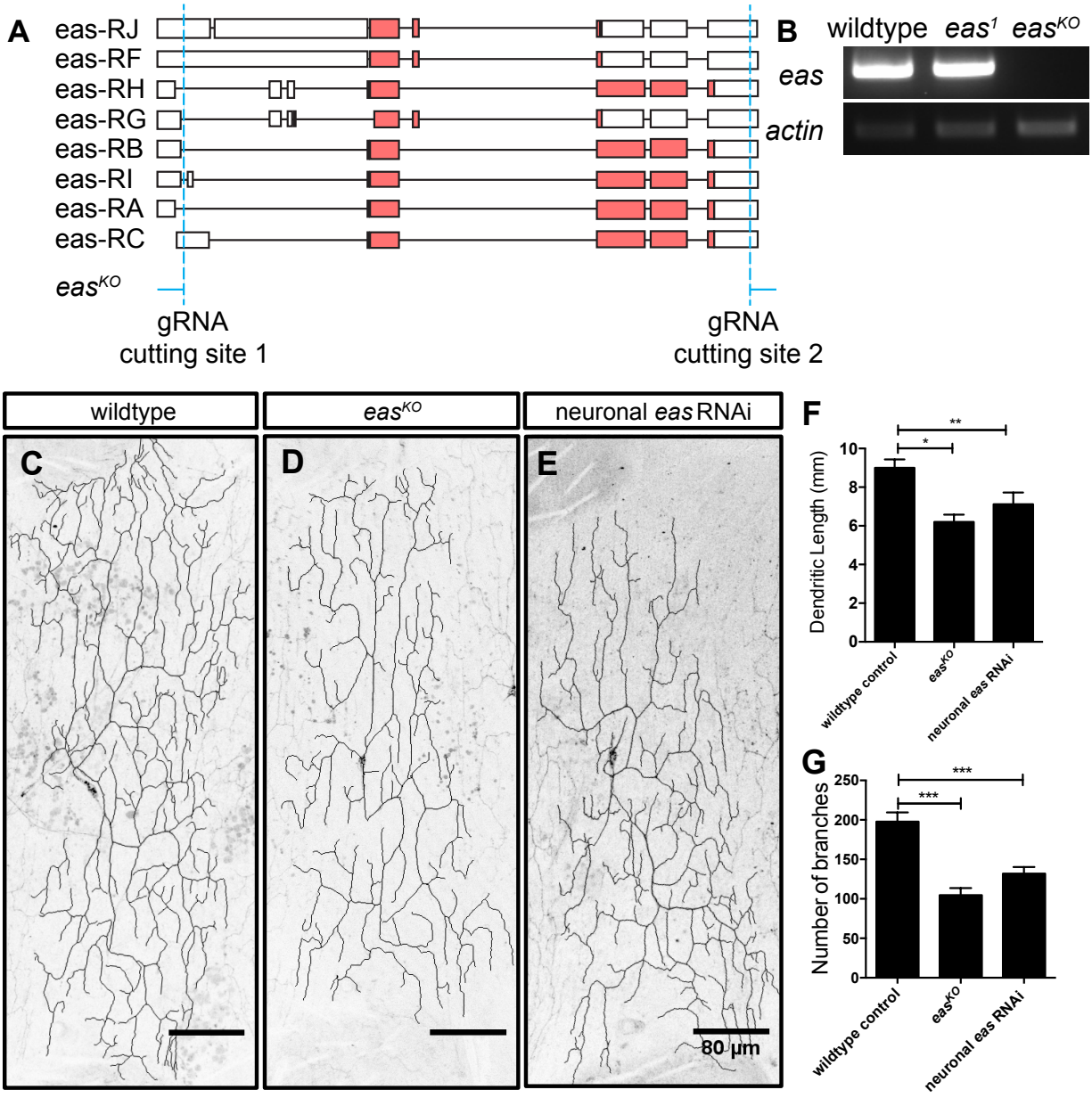
Figure S1. Related to Figure 1. Generation of *eas*^{KO} mutants



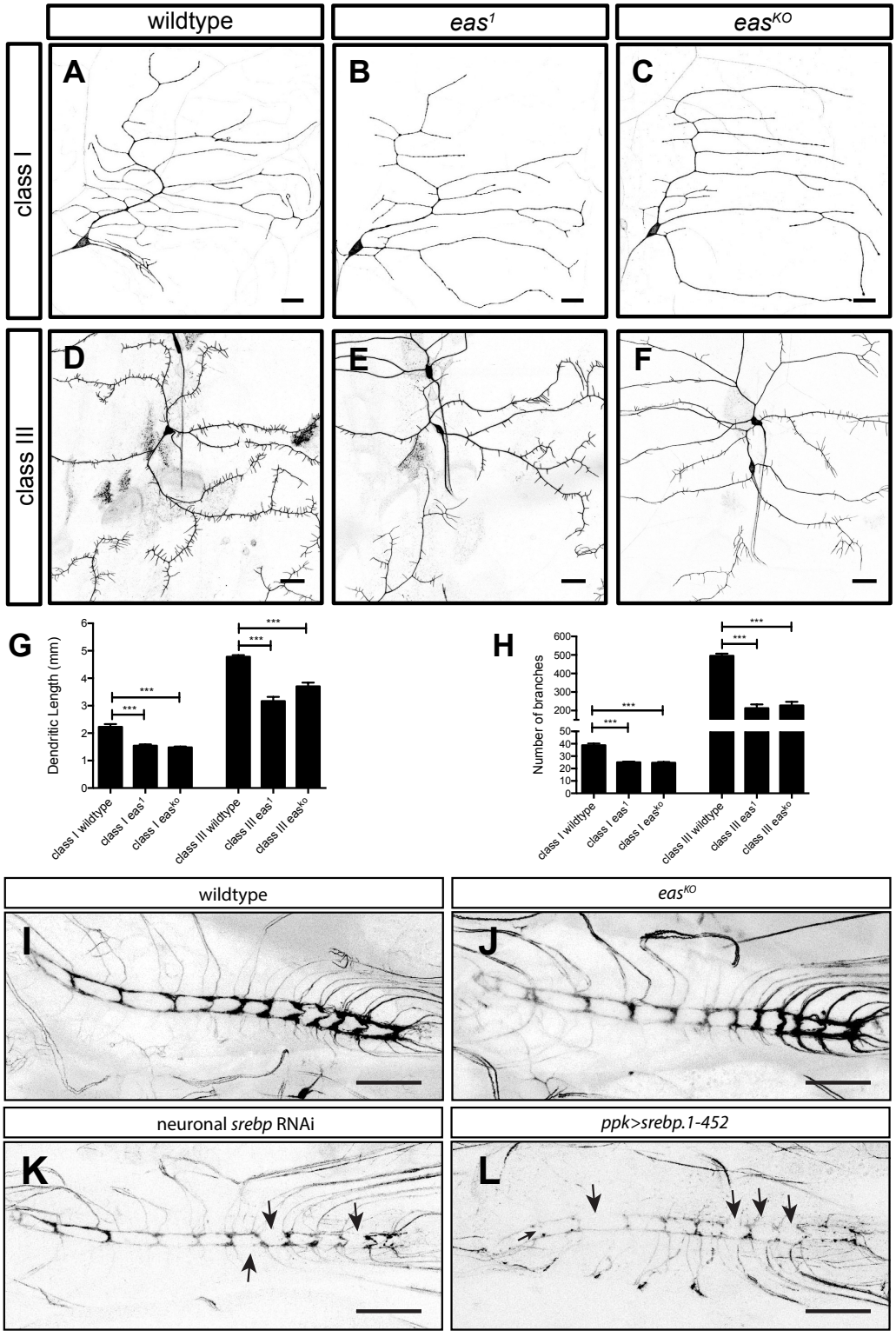
(A) The *Drosophila eas* gene structure. Exons are represented by filled boxes, 5′- and 3′-UTRs by open boxes, and splicing events by straight lines. All predicted alternative splicing products are shown. Two guideRNA(gRNA) cutting sites are marked by dotted lines (deleted region indicated by gap).

(B) RT-PCR showing the complete absence of mRNA transcript in homozygous *eas*^{KO} mutants, while the homozygous *eas*^{1} mutants still express truncated EAS proteins.

(C-E) Sample images showing dendrite morphology for the indicated genotypes.

(F and G) Quantification of total dendritic length (F) and number of branches (G) in wildtype, eas^{KO} and *eas* RNAi neurons in 1 day old adults. n = 6 per genotype. Scale bars represent 80 μm.

Figure S2. Related to Figure 1. Loss of eas also leads to reduced dendritic growth in class I and III da neurons.



(A-C) Sample images showing dendrite morphology of class I da neurons (labeled by *Gal4*$^{2\text{-}21}$*, UAS-tdTomato*) for

the indicated genotypes.

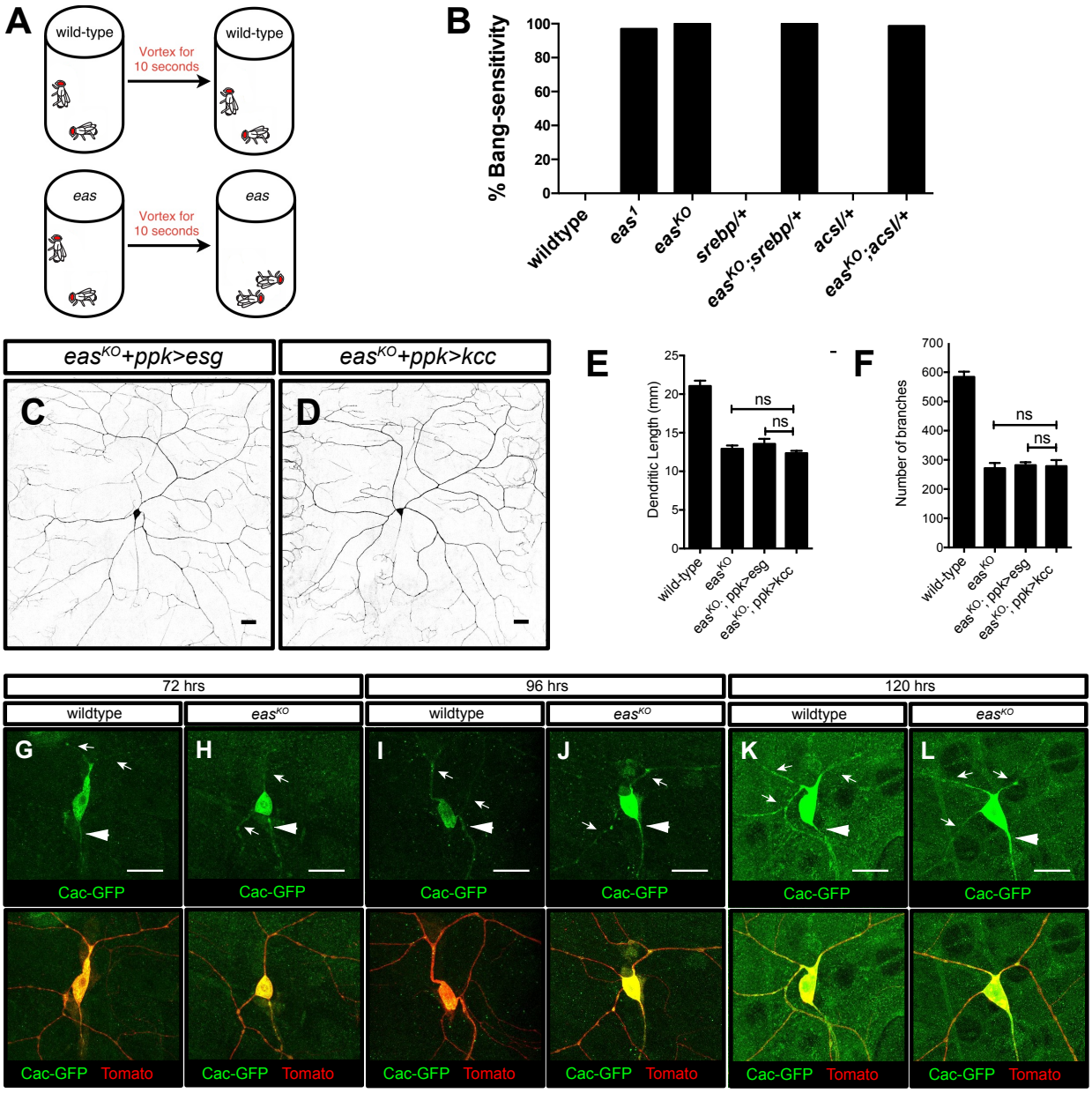
(D-F) Sample images showing dendrite morphology of class III da neurons (labeled by *Gal4*$^{19\text{-}12}$*, UAS-tdTomato*) for the indicated genotypes.

(G and H) Quantification of total dendritic length (G) and number of branches (H) in wildtype, *eas*^{KO} and *eas*^{1} neurons.

Scale bars represent 30 μm. n = 6 per genotype. Data are mean ± SEM for all figures. ***p < 0.001 by one-way ANOVA tests.

(I-L) Sample images showing the morphology of axonal projections in the ventral nerve cord for the indicated genotypes. Scale bars represent 80 μm.

Figure S3. Related to Figure 4. Seizure-like phenotype in *eas* mutants is not suppressed by reducing SREBP signaling



(A) Diagram showing seizure assay. Briefly, adult flies were vortexed at maximum speed for 10 seconds. Wildtype flies do not exhibit any bang sensitivity, while eas^{1} and eas^{KO} mutants display paralysis.

(B) Behavioral paralysis in eas^{KO} mutants is not suppressed by mutations that ameliorated the dendrite morphogenesis phenotype in class IV da neurons (n = 50 per genotype).

(C and D) Sample images showing dendrite morphology for the indicated genotypes.

(E and F) Quantification of total dendritic length (E) and number of branches (F) for the indicated genotypes. Scale bars represent 30 μm. n = 10, 9, 4, and 4 for wild-type, eas^{KO}, eas^{KO}*; ppk>esg*, and eas^{KO}*; ppk>kcc*, respectively. Data are mean ± SEM for all figures. One-way ANOVA tests were used. ns, not significant.
(G-L) Sample images showing the distributions of Cac-GFP in the class IV da neurons for wildtype and eas^{KO} animals. Axons and dendrites are marked by arrowheads and arrows, respectively. Scale bars represent 20 μm.

2. Supplemental Experimental Procedures

Fly stocks

These following mutant alleles were used: eas^{1}, provided by Dr. Mark Tanouye (University of California at Berkeley), eas^{KO} (generated in this study), and $srebp^{189}$ (Kunte et al., 2006), $acs^{MI12066}$ (BL56480), $acsl^{KO}$ (BL32331). Other transgenic flies used in this study were: *UAS-bbc-RNAi* (VDRC7989)*, UAS-pect-RNAi* (BL63710)*, UAS-srebp-RNAi* (BL25975) (Song et al., 2014), *UAS-srebp* (BL8236)*, UAS-srebp.1-452* (BL41017)*, UAS-fas-RNAi* (BL29349), *UAS-eas* (provided by Dr. Mark Tanouye, Sanford Burnham Medical Research Institute), *UAS-acc-RNAi* (VDRC8105)*, UAS-cac-RNAi* (Saras and Tanouye, 2016)*, UAS-esg* (Hekmat-Scafe et al., 2005)*, UAS-kcc* (Hekmat-Scafe et al., 2010), $Gal4^{21\text{-}7}$ (Song et al., 2007), $Gal4^{19\text{-}12}$ (Xiang et al., 2010), $Gal4^{2\text{-}21}$ (Parrish et al., 2006), *ppk-Gal4* (Grueber et al., 2003), *ppk-CD4-tdTomato* (Han et al., 2011), *UAS-tdTomato* (Han et al., 2011)*, UAS-dcr-2* (Dietzl et al., 2007). Animals were reared at 25°C in density-controlled vials or at 29°C for *bbc* and *pect* RNAi experiments. To image *eas* mutants, homozygous *eas* female flies were crossed with male flies carrying *ppk-CD4-tdTomato.* Male larvae from the next generation were imaged 5-7 days after the crosses were made.

Western Blotting

To analyze SREBP protein levels in vivo, brains were removed from wandering third instar larvae and homogenized in HKT lysis buffer (10mM HEPES pH7.2, 100mM KCl, 1% TritonX-100, 2mM DTT, PMSF, and protease inhibitors from Roche) at 4 °C. Sample buffer was added to the lysis buffer, and samples were boiled for 10 min. Equal numbers of brains (3–5) from control and experimental groups were separated by 4-12% gradient Bis-Tris precast gels (Novex) using Mops-SDS running buffer (Novex), and analyzed by Western blot. The primary antibodies used were mouse monoclonal anti-SREBP (1:1000, Cat# 557036, BD Biosciences, RRID: AB_384985) and mouse monoclonal anti-tubulin (1:1000, Cat# T9026, Sigma, RRID: AB_477593). Quantification of the intensity of the protein bands obtained in Western blots was performed using Image Studio™ Lite Software. The

percentages of the mature SREBP proteins in each genotype were calculated by dividing the level of the mature SREBP protein by the total level of SREBP proteins (both full length and mature forms of SREBP).

Generating *eas* knockout flies

Prior to generating *eas* knockout flies, the *eas* gene in vasa-Cas9 flies was sequenced to confirm the presence of guideRNA sites. For the generation of *eas* knockout flies, annealed oligos were ligated with BbsI-digested pU6-BbsI-gRNA, in order to generate targeting guideRNAs. The sequences of the guideRNAs used are: 5'-GCGGAATTTAGGTCGTGCGAAGG-3' and 5'-TCCCAATGCGCTGAGTGGCCGGG-3'. GuideRNAs were then injected into vasa-Cas9 fly embryos. *eas* knockout animals were identified with genomic PCR and further confirmed by sequencing.

Immunohistochemistry

Larvae were collected at 72, 96, and 120 hrs after egg laying (AEL), dissected in PBS, fixed in 4% PFA for 20 min, blocked with 5% normal goat serum. Body wall fillets were stained with chicken anti-GFP (1:500, Aves Labs) and rat anti-tdTomato [16D7] (1:500, KeraFAST) antibodies in a 0.3% Triton X-100 solution overnight at 4°C, and subsequently with goat anti-chicken (1:500, Invitrogen A11039) and goat anti-rat (1:500, Invitrogen A21434) antibodies in a 0.3% Triton X-100 solution for 3 hours at room temperature.

Bang-sensitivity assay

Bang-sensitivity tests were performed as previously described (Pavlidis et al., 1994). Before testing, flies were raised and aged for at least 3 days at 25 °C. For testing, each group of flies was vortexed at maximum speed for 10 seconds. The number of flies that exhibit temporary paralysis and total number of flies tested were scored for quantification.

3. Supplemental References

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