

Supporting Information

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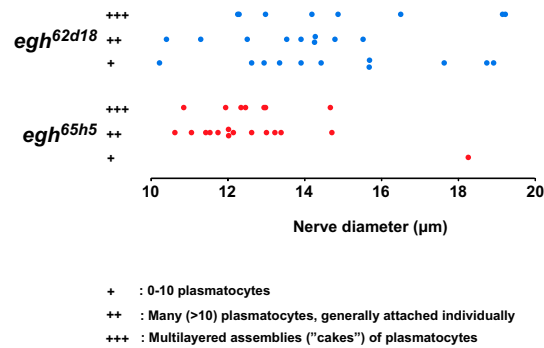


Fig. S1. Relationship between nerve diameter and plasmacyte accumulation in *egh* mutants. Semiquantitative analysis of the number of plasmatocytes accumulated on the A9 nerves in the two *egh* mutant strains 62d18 and 65h5, displayed in relation to nerve diameter.

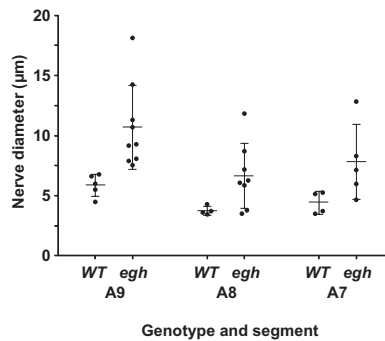


Fig. S2. Enlargement of nerves extending from abdominal segment 9 through 7 in *egh* mutants. Scatter plot showing the diameter of peripheral wild-type and *egh* nerves extending from abdominal segments A9, A8, and A7, as measured on transmission electron microscopy micrographs. Brackets indicate mean \pm SD. A two-factor ANOVA following logarithmic transformation of the data indicated a significantly increased diameter of *egh* nerves ($P = 0.0004$). The nerve diameter generally depended on the identity of the segment ($P = 0.0204$), whereas the effect of genotype on the diameter was independent of the segment (no interaction between genotype and segment; $P = 0.6406$). Accordingly, the *egh*:WT ratios of mean diameters were similar (1.82 for A9; 1.77 for both A8 and A7).

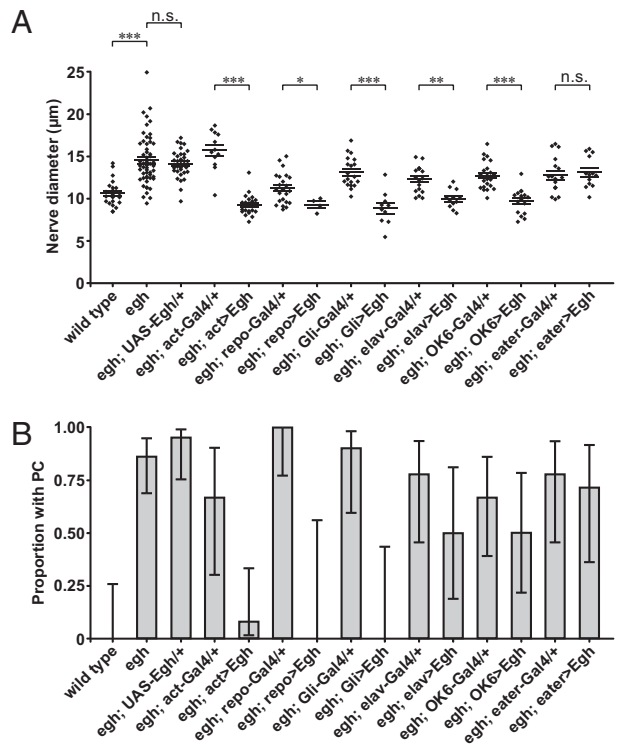


Fig. S3. Egh is required in the subperineurial glia to regulate growth and prevent attachment of immune cells. Tissue requirement of Egh for suppression of nerve overgrowth (A) and attachment of plasmacytes (PC) to peripheral nerves (B). The UAS/Gal4 system was used to express transgenic Egh in the *egh*^{62d18} background, either ubiquitously (*act-Gal4*), in all glia (*repo-Gal4*), subperineurial glia (*Gli-Gal4*), all neurons (*elav-Gal4*), motor neurons (*OK6-Gal4*), or plasmacytes (*eater-Gal4*). (A) Scatter plot of the A9 nerve diameter, measured 48 µm posterior to the ventral nerve cord. Mean and ± SEM are indicated; **P* < 0.05, ***P* < 0.001, ****P* < 0.0001. n.s., not significant. (B) The proportions of larvae with plasmacytes attached to A9 nerves. Error bars mark 95% confidence intervals.

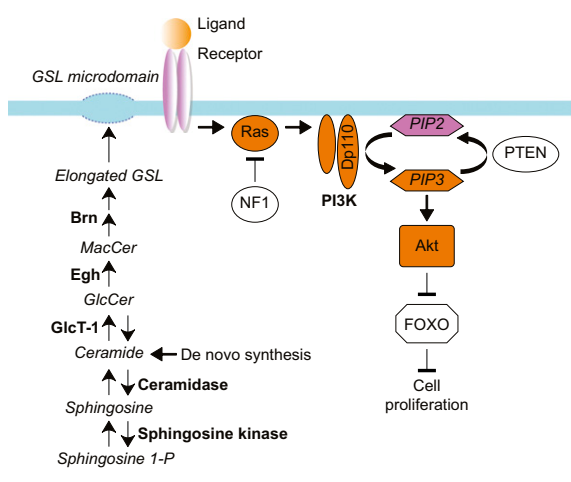


Fig. S4. Schematic representation of GSL metabolism and PI3K signaling in *Drosophila*. De novo synthesis of sphingolipids is a multistep process that results in the production of ceramide from palmitoyl-CoA and serine. Ceramide can be glycosylated by UDP-glucose:glucosylceramide synthase (GlcT-1) to generate GlcCer. GlcCer is converted to MacCer by Egh, and MacCer is further elongated by Brn.

Table S1. Nerve diameter and plasmotocyte attachment

Genotype	Nerve diameter (μm), mean \pm SEM	<i>n</i> *	Larvae with plasmatocytes on nerves (%)	95% CI (%)	<i>n</i>
Wild type (<i>Oregon-R</i>)	10.62 \pm 0.34	20	0	0–25.9	11
<i>egh</i> ^{62d18}	14.55 \pm 0.40	55	86	68.5–94.3	28
<i>egh</i> ^{65h5}	12.69 \pm 0.33	40	95	77.3–99.2	21
<i>egh</i> ; <i>UAS-Egh</i> /+	14.14 \pm 0.28	34	95	75.4–99.1	19
<i>egh</i> ; <i>act5c-Gal4</i> /+	15.72 \pm 0.67	12	67	30.0–90.3	6
<i>egh</i> ; <i>act5c>Egh</i>	9.26 \pm 0.22	26	8	1.4–33.3	13
<i>egh</i> ; <i>repo-Gal4</i> /+	11.22 \pm 0.36	24	100	77.2–100	13
<i>egh</i> ; <i>repo>Egh</i>	9.29 \pm 0.40	4	0	0–56.2	3
<i>egh</i> ; <i>Gli-Gal4</i> /+	13.13 \pm 0.41	19	90	59.6–98.2	10
<i>egh</i> ; <i>Gli>GFPnls</i>	13.10 \pm 0.35	20	100	72.3–100	10
<i>Gli>GFPnls</i>	9.98 \pm 0.32	9	0	0–43.5	5
<i>egh</i> ; <i>Gli>Egh</i>	8.88 \pm 0.63	10	0	0–43.5	5
<i>egh</i> ; <i>elav-Gal4</i> /+	12.36 \pm 0.37	18	78	45.3–93.7	9
<i>egh</i> ; <i>elav>Egh</i>	9.99 \pm 0.32	12	50	18.8–81.2	6
<i>egh</i> ; <i>OK6-Gal4</i> /+	12.73 \pm 0.33	23	67	39.1–86.2	12
<i>egh</i> ; <i>OK6>Egh</i>	9.70 \pm 0.37	16	50	21.5–78.5	8
<i>egh</i> ; <i>eater-Gal4</i> /+	12.78 \pm 0.51	17	78	45.3–93.7	9
<i>egh</i> ; <i>eater-Gal4eater-Gal4</i>	13.65 \pm 0.69	10	83	43.7–97.0	6
<i>egh</i> ; <i>eater>Egh</i>	13.13 \pm 0.51	13	71	35.9–91.2	7
<i>Gli>Ras</i> ^{V12}	14.49 \pm 1.08	14	0	0–35.4	7
<i>egh</i> ; <i>UAS-Ras</i> ^{V12} /+	12.45 \pm 0.78	8	75	30.1–95.4	4
<i>egh</i> ; <i>Gli>Ras</i> ^{V12}	18.73 \pm 1.23	17	78	45.3–93.7	9
<i>Gli>Ras</i> ^{N17}	9.58 \pm 0.21	10	0	0–43.5	5
<i>Gli>Dp110</i> ^{CAAX}	17.62 \pm 1.48	16	67	35.4–87.9	9
<i>egh</i> ; <i>UAS-Dp110</i> ^{D954A} /+	13.21 \pm 0.47	14	49	48.7–97.4	7
<i>egh</i> ; <i>Gli>Dp110</i> ^{D954A}	10.47 \pm 0.27	25	47	24.8–69.9	15
<i>Gli>Dp110</i> ^{D954A}	9.70 \pm 0.42	13	0	0–35.4	7
<i>egh</i> ; <i>UAS-PTEN</i> /+	12.36 \pm 0.56	12	100	61.0–100	6
<i>egh</i> ; <i>Gli>PTEN</i>	8.94 \pm 0.19	24	25	8.9–53.2	12
<i>egh</i> ; <i>Akt1</i> ⁰⁴²²⁶ /+	9.84 \pm 0.71	6	100	43.9–100	3
<i>egh</i> ; <i>UAS-FOXO</i> /+	13.43 \pm 0.32	8	75	30.1–95.4	4
<i>egh</i> ; <i>Gli>FOXO</i>	10.19 \pm 0.37	11	29	8.2–64.1	7
<i>Gli>FOXO</i>	9.67 \pm 0.21	16	0	0–29.9	9
<i>brn</i> ^{1.6P6}	11.07 \pm 0.57	10	0	0–39.0	6
<i>egh</i> ; <i>UAS-β4GalT6</i> /+	14.35 \pm 1.11	7	100	56.6–100	5
<i>egh</i> ; <i>Gli>β4GalT6</i>	9.73 \pm 0.40	11	33	9.7–70.0	6

Summary of the diameter of A9 peripheral nerves, measured 48 μm from the ventral nerve cord exit point, and the percentage of larvae with plasmatocytes attached to the A9 nerves. CI, confidence interval.

*Number of nerves.