**SUPLLEMENTAL MATERIAL LEGENDS**

***Supplemental Files:***

**Supplementary File 1:** Differential Gene Expression Analysis of all genotype and developmental stage comparisons using EdgeR. Cut off: logF.C. ≥0.5/≤-0.5; Adj. p-value ≤0.05.

**Supplementary File 2:** DAVID Functional Annotation to identify clusters of enriched Gene Ontology (GO) and KEGG pathway terms across multiple genotype comparisons and developmental stages.

**Supplementary File 3:** EnrichmentMap network output built using the DAVID Functional Annotations (File 2) between the [M vs W] and [M vs R] genotype comparisons at each developmental stage.

**Supplementary File 4:** Results of the ICA module analysis. Includes statistical enrichments and a description of the co-expressed genes and GO terms for each ICA module.

***Supplemental Figures:***

**Supplementary Figure 1, Related to Figure 1. Validation of the Asp-fTRG line and developmental & cell type specific Asp expression.** (A) Cartoon representation of the Asp-fTRG fosmid construct, consisting of the *asp* genomic region (includes all endogenous regulatory elements) and the FlyFos tag, which carries a superfolder GFP tag (among others) which allows for visualization of the protein using α-GFP antibodies. (B) PCR validation of the Asp-fTRG line, using primers at the positions denoted in (A). Expected band is marked with an asterisk; non-specific bands are labeled *ns.* (C) A central brain neuroblast in prophase (top) and metaphase (bottom) labeled with α-GFP to visualize Asp-fTRG and α-Dlg to label cell outlines. Yellow arrowheads mark the expected Asp signal at astral microtubules and spindle poles. (D) Asp-fTRG expression in the entire CNS from 3rd instar larva, P7 pupa, and 3-day old adults. Note strong expression in the larval brain, which becomes much weaker but detectable in the pupal and adult brain (white outlined regions). (E) Quantification of pixel intensities from the cell body (regions outlined in (D) show the relative area the measurements were taken). All values were normalized to the average pixel intensity value of the adult. n=>5 brains. (F) An entire larval optic lobe stained with α-GFP to label Asp-fTRG and α-Dpn (deadpan) to mark asymmetrically dividing neuroblasts (magenta). (G) An entire larval optic lobe stained with α-GFP to label Asp-fTRG and α-Repo to visualize glial cells. Two z-planes are shown to highlight the different glial cell populations (D-V; Dorsal-Ventral). (H) Close up view of the Transition Zone (TZ) region, marked by α-L’Sc. Asp-fTRG is labeled with α-GFP. Note cytoplasmic localization for Asp in interphase TZ cells; one cell (white outline) is in metaphase of mitosis and shows strong Asp-fTRG signal at the astral microtubules and spindle poles (yellow arrowheads). Unpaired t-test, \*\*\*P≤0.0005; \*\*\*\*P≤0.0001. Error bars represent standard deviation. Scale bars: 3 μm (C); 50 μm (D); 10 μm (F-H).

**Supplementary Figure 2, Related to Figure 2. Brain size measurements across developmental stages and for the genetic rescue experiments** (A) Cartoon of the larval and pupal/adult brain. The optic lobes of the larval brain consists of two neuroblast (NBs) regions that will give rise to distinct neuron and glial populations in the adult brain. Central brain NBs (green) make neurons and glia for the central brain of the adult, optic lobe NBs (also known as medulla neuroblasts) and neuroepithelial cells (red) generate the neurons and glia of the adult optic lobe. For μ-CT measurements, the ‘entire brain’ consists of both larval optic lobes (blue outline) segmented as a whole, while the pupal and adult entire brain consists of both optic lobe regions plus the central brain. ‘Optic lobe’ measurements were taken from individually segmented optic lobes of the pupa and adult. The larval ventral nerve cord was not included in the size analysis, although it was included in the flow cytometry analysis due to the difficulty in accurately separating it from the optic lobes during dissection. (B) μ-CT measurements of *asp* heterozygous control (WT, *aspT25/+*) and *asp* mutant (*aspT25/aspDf*) entire brain volume from 3–5-day old females (F) and males (M). Males show a larger percent decrease in overall brain size (14% vs 27%) but are difficult to obtain due to significantly higher lethality compared to females. Thus, females were used for all subsequent measurements. (C) μ-CT measurements of *asp* heterozygous control (WT, *aspT25/+*) and *asp* mutant (*aspT25/aspDf*) volume from larva entire brain. Same measurements as described in (C) but for (D) pupa entire brain, (E) pupa optic lobe, (F) adult entire brain, (G) adult optic lobe. (H) μ-CT measurements of *asp rescue control* (*ubi-GFP::aspMF/+; aspT25/+*) and *asp rescue* (*ubi-GFP::aspMF/+; aspT25/aspDf*) volume from larval entire brain, (I) adult entire brain, and (J) adult optic lobes. (K) Genetic rescue experiments using the UAS/Gal4 system with the *asp rescue* fragment. *Insc-Gal4, Elav-Gal4,* and *Repo-Gal4* were used to express the fragment in most neuroblasts, neurons, and glia, respectively. Entire brain measurements are shown. Genotyped matched controls were used for all comparisons except for *Repo-Gal4*, which used a constitutively expressed *asp rescue* fragment strain as WT. These were stained and imaged during the same experiment under identical conditions. Also note no difference in overall brain size between the *Repo-Gal4* rescue strain and the *asp* mutant alone (orange plots). (L) Same genetic rescue assay outlined in (K) but measuring optic lobe volume only for the indicated drivers. Data are represented as the T-ratio (brain volume normalized to overall body size) and each dot represents a single brain. These values were used to generate the ‘normalized’ graphs in Figure 2. For violin plots, solid red line represents the median, and the dashed lines denote interquartile range (IQR). n≥5 brains; Welch’s t-test. ns, P>0.05; \*\*P≤0.01; \*\*\*P≤0.001; \*\*\*\*P≤0.0001.

**Supplementary Figure 3, Related to Figure 2. Validation of flow cytometry for neuronal cell counts from single brains.** (A) Forward scatter (FSC-A) vs. Side scatter (SSC-A) plot from a single dissociated adult brain. Boxed area shows the first gate of events used for later analysis. (B) DyeCycle Violet™ vs SSC-H of the gated area in (A) from an unlabeled (-DyeCycle Violet) brain, which serves as a negative control to establish the fluorescence threshold of a positive event (labeled nuclei). (C) DyeCycle Violet™ vs SSC-H of a labeled (+DyeCycle Violet) brain. The gate labeled ‘a’ represent intact nuclei, as judged by FACS sorting and later immunostaining of this population using an anti-lamin antibody (Panel F, top panels) and was used to determine the number of cells (events) shown in Figure 1E-H. The gate labeled ‘b’ represents debris and was not included in the analysis (Panel F, bottom panel). A second validation was performed using an Actin-Gal4/UAS-H2Av::RFP strain that was either not co-labeled (D) or was co-labeled with DyeCycle Violet™ (E). Events displaying a high intensity in both the RFP and DyeCycle violet channels (V+, R+) were intact nuclei, and correspond to the ‘a’ gate shown in (C). (F) Microscopy images of the events sorted from the ‘a’ and ‘b’ gates in panel (C). Yellow arrowheads denote intact nuclei as judged by intact nuclear lamin staining, which was not observed in the ‘b’ population. Scale bars= 5 μm.

**Supplementary Figure 4, Related to Figure 2. G-TRACE Analysis of *Insc-Gal4, Elav-Gal4,* and *Repo-Gal4.*** The G-Trace system allows for both real-time and lineage tracing of Gal4 driver activity (Evans *et al.* 2009). (A) *Insc-Gal4* real time expression pattern (RFP) in the larval brain, which is localized to all neuroblast populations in the central brain and optic lobe. Dlg was used to mark cell outlines and identify relevant cell populations (magenta). We did not observe real time or lineage expression (not shown) in the OPC, IPC, or LPCs, as reported elsewhere (Wang *et al.* 2011). (B) Same brain shown in (A) but from a different focal plane to highlight the lack of Gal4 activity in the OPC and LPC. (C) *Elav-Gal4* expression pattern (green). Note strong overlap with α-Elav antibody staining (magenta), confirming strong expression in neurons. (D) *Repo-Gal4* expression pattern (green). Note strong overlap with α-Repo antibody staining (magenta), confirming strong expression in glia. Scale bars: 20 μm.

**Supplementary Figure 5, Related to Figure 3. Sample variability between RNA-Seq datasets.** (A) Sample-to-Sample distances for each genotype (heterozygous control (Wildtype), *asp* mutant and *asp* rescue) and all developmental stages (larva, LB; pupa, PB; adult, AB), calculated using edgeR. (B) PCA analysis of each genotype and developmental stage. (C) Sample-to-Sample distances for each developmental stage and all genotypes. (D) PCA analysis of each developmental stage and genotype. PC1 & PC2 describe the majority (>50%) of the variability in the datasets.

**Supplementary Figure 6, Related to Figure 3. Transcription trends across development.** Heat map representation of all DES across all developmental stages (larvae, pupae, adults) for the (A) *asp* mutant vs. WT, (B) *asp* mutant vs. *asp* rescue, and (C) *asp* rescue vs. WT comparisons, colored by logF.C. (red=>.5, blue =<-.5). logCPM vs logF.C. plots for the *asp* rescue vs. WT comparisons from (D) larvae, (E) pupae, and (F) adult brains. WT=wildtype (*asp* heterozygous control).

**Supplementary Figure 7, Related to Figure 6. ICA module analysis to identify co-expressed gene signatures in *asp* mutant brains.**

Plot of logF.C. vs. gene for the (A) larval M146-, (B) adult M393+, and (C) larval M113- modules. Z-score enrichment is shown in green font for each genotype comparison. Each dot is colored based on the mutant vs wildtype (Mut vs WT, blue) and mutant vs rescue (Mut vs Res, red) value. The red dotted line shows the 0.5/-0.5 logF.C. position. Not all gene names are included on the x-axis for clarity. Relevant genes (*doa, E(bx), faf, e(spl)mγ-HLH*) are highlighted. (D) Scatterplot showing significantly enriched co-expression modules found through ICA analysis of the [M vs W] and [M vs R] DGE datasets from pupal brains. Each module is plotted as a point based on its Z-score enrichment from each analysis. Only significant modules with a Z-score of >3/<-3 are shown. Co-expressed modules having at least one immune system-related GO term are colored red, with M115+ outlined with a red box. A subset of the more significantly enriched (Z-score ≥10) modules are also labeled in gray font. The Pearson’s Correlation Coefficient (*r)* is shown in blue font (two-tailed P<0.0001).

**Supplementary Figure 8, Related to Figure 7. Characterization of the fly CNS immune response** (A) Cartoon diagram of the insect immune response. Toll and IMD receptors respond to distinct stimuli (classified as PAMPs and DAMPs) and rely on the NF-ΚB factors Relish, Dorsal, and Dif to activate downstream effectors of the antimicrobial family of peptides (Bomanins, Attacins, etc). (B) AMP expression (qPCR) during pupal stages in *asp* mutant and *asp* rescue animals. Data is shown as the relative expression ratio (RER), red dotted line shows the RER value for wildtype, normalized to 1. Expression in *asp* mutant brains is shown in blue, *asp* rescue in red. (C) qPCR analysis of the upstream Toll and IMD pathway components throughout development in *asp* mutants from larva (blue), pupa (red), and adults (green). Data is shown as the relative expression ratio (RER), red dotted line shows the RER value for wildtype, normalized to 1. Confocal imaging of P7 pupal brains labelled with α-brp (nc82) to visualize the neuropil in (D) *asp* heterozygous control (wildtype) (*aspT25/+*) and (E) *asp* mutants (*aspT25/aspDf*). Red arrowheads point out disrupted neuropil boundaries. High Resolution μ-CT tomograms of *dif1; relE38, asp* triple mutant showing an (F) XY plane and (G) YZ plane highlighting severe optic lobe neuropil disorganization identical to the *asp* mutant (Schoborg *et al.* 2019) (not shown). Multiple attempts were made to image triple mutant brains using confocal microscopy, but the ‘stickiness’ of these brains caused aggregation in the tubes and prevented proper mounting. Medulla (Me) and Lobula (Lo) neuropil regions are labeled. Body axes in each μ-CT view denoted as D, dorsal; L, left; A, anterior. Welch’s t-test. ns, P>0.05; \*\*P≤0.01; \*\*\*P≤0.001; \*\*\*\*P≤0.0001. Error bars represent standard deviation. Scale bars = 50 μm (D, E) and 100 μm (F, G).

**Supplementary Figure 9, Related to Figure 8. Brain size measurements after genetically inhibiting the immune response and apoptosis**. Individual adult (A) entire CNS volume and (B) optic lobe values (T-Ratio) used to generate the bar graphs in Figure 8A, 8B from the double and triple mutant genetic analysis. (C) qPCR analysis of *Dif* mRNA levels in the brain following knockdown with either *Repo-Gal4* or *Elav-Gal4*. >95% reduction was observed compared to the control. (D) Total number of cleaved death caspase-1 (Dcp-1) positive foci in WT and *asp* mutant third instar larval brains. (E) Individual adult entire CNS volume and (F) optic lobe values (T-Ratio) used to generate the bar graphs in Figure 8F, 8G from the UAS-P35 apoptosis inhibition experiments. Numbers represent the percent decrease from the indicated genotype comparisons. Data are represented as the T-ratio (brain volume normalized to overall body size) and each dot is a single brain. For violin plots, solid line is the median, and the dashed lines denote interquartile range (IQR). n≥5 brains; Welch’s t-test. ns, P>0.05; \*\*P≤0.01; \*\*\*P≤0.001; \*\*\*\*P≤0.0001.