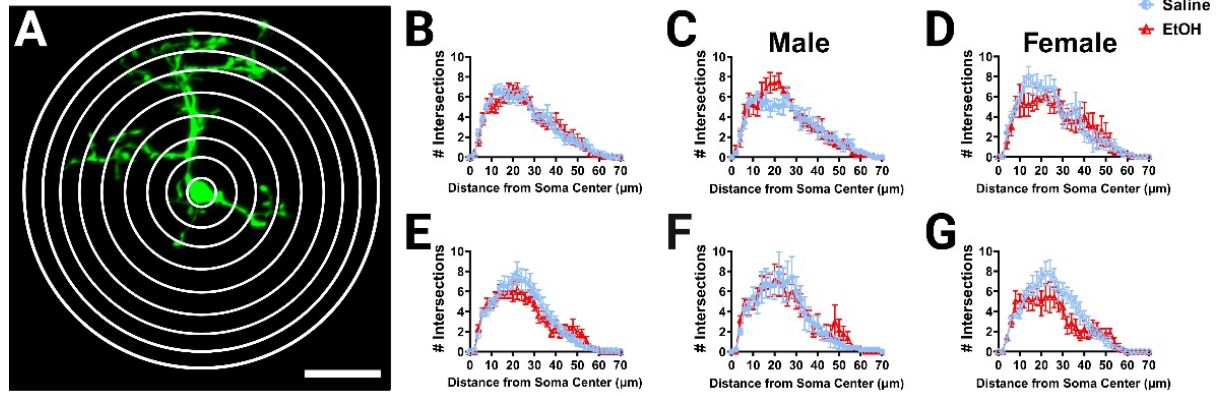
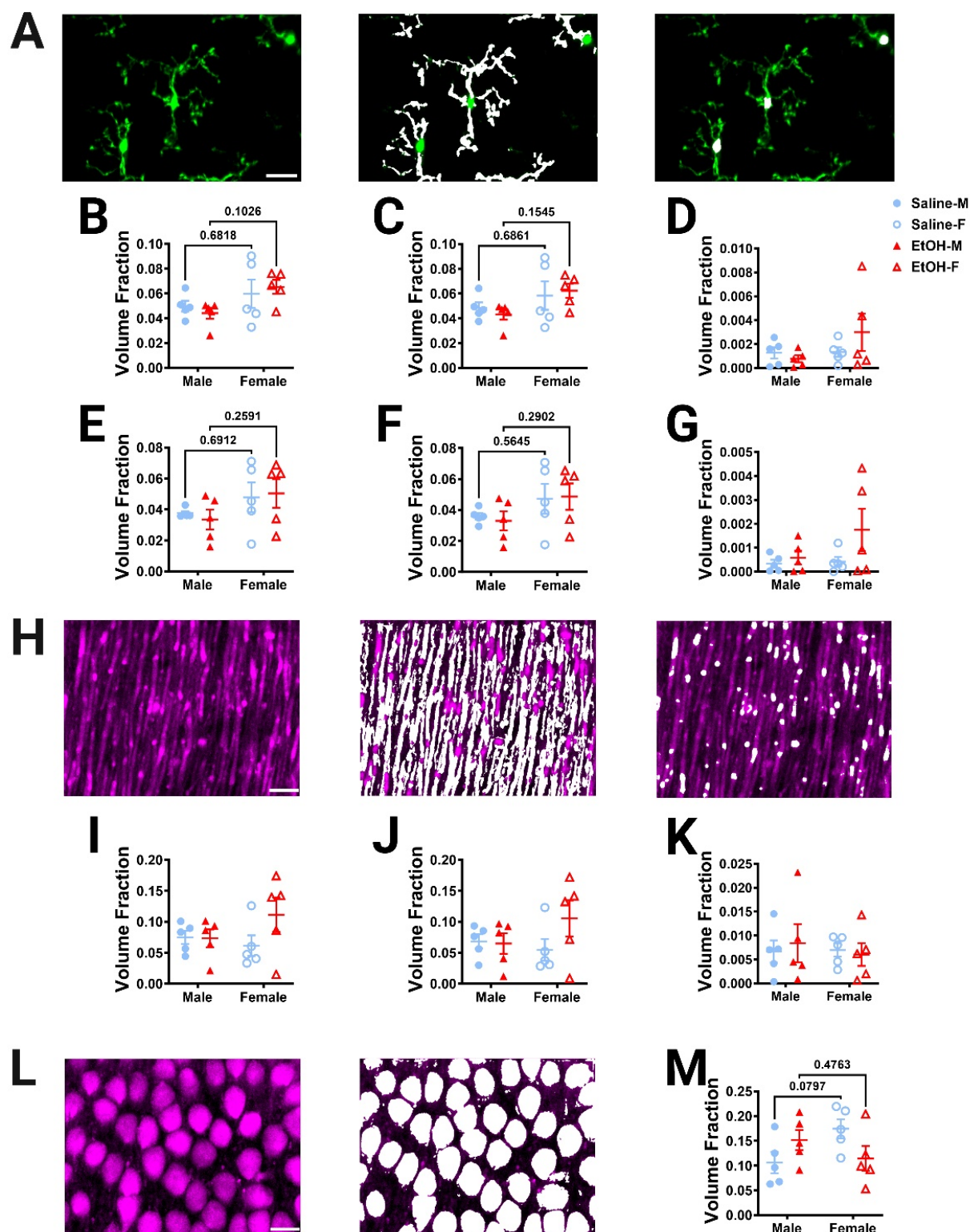


**Figure S1: Cerebellar microglia morphology in each layer of lobule IV/V in fixed tissue.** (A-C, E-G, I-K, M-O) Sholl curves for the ML (A-C), PCL (E-G), GL (I-K), and WM (M-O). For each layer, sexes were combined (A, E, I, M) or separated into males (B, F, J, N) and females (C, G, K, O). There were no differences in ramification due to treatment or sex in any layer. (D, H, L, P) Individual Sholl curves were fit using a hierarchical Bayesian approach to capture variation at each level of the experimental hierarchy. 95% credible intervals for effects on each parameter from (Figure 3B) were calculated across treatments and sexes in the ML (D), PCL (H), GL (L), and WM (P). (A-L) Each datapoint represents a treatment group and sex. Data are presented as the mean  $\pm$  SEM.

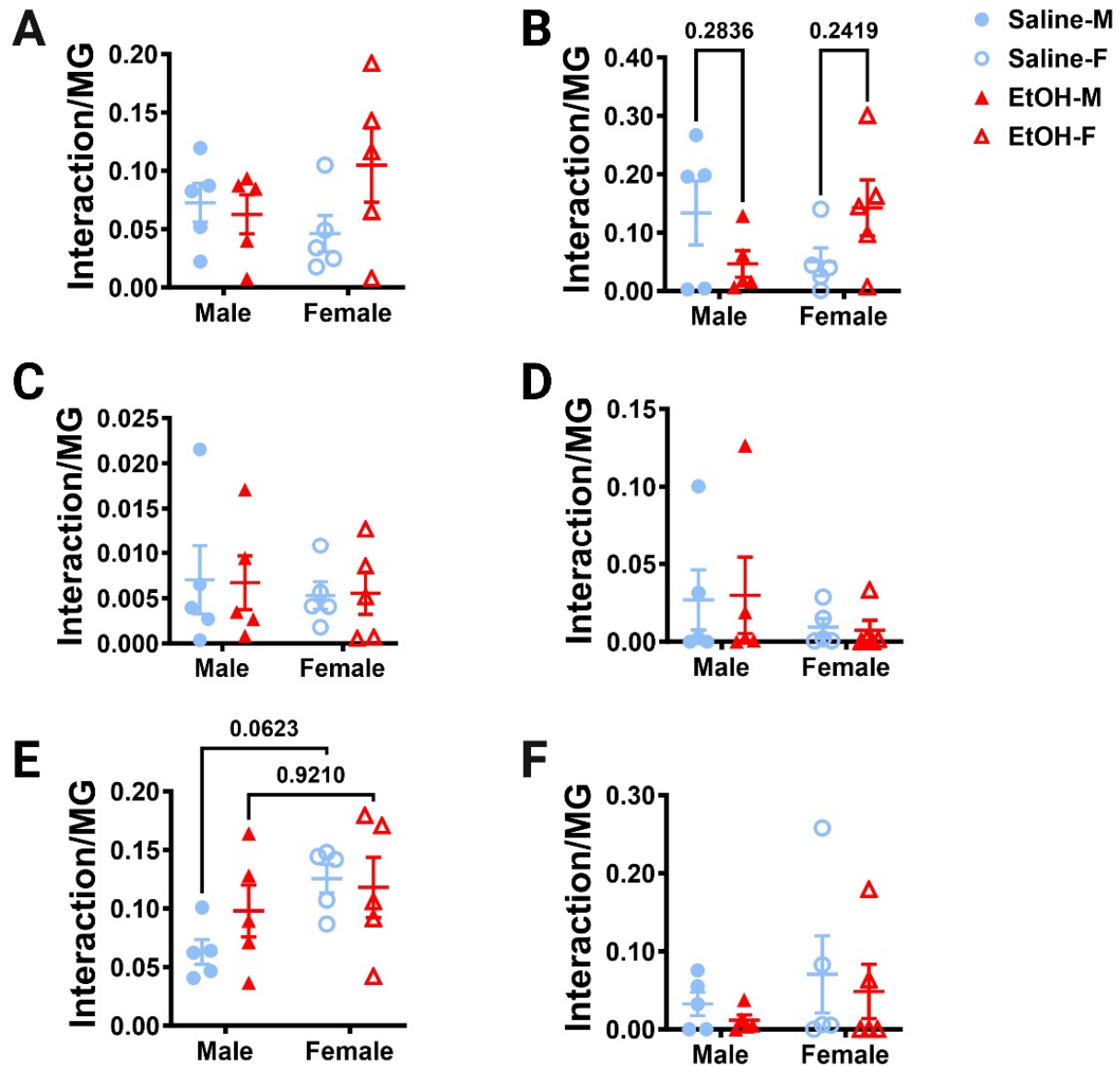


**Figure S2: Cerebellar microglia morphology *in vivo*.** (A) *In vivo* two-photon image of cerebellar microglia. Concentric rings were drawn around each microglia to examine process ramification. (B-G) Sholl curves for microglia in the ML (B-D) and PCL (E-G) when sexes were combined (B, E) or separated into males (C, F) or females (D, G). (B-G) Each datapoint represents a treatment group and sex. Data are presented as the mean  $\pm$  SEM. Scale bar= 25 $\mu$ m



**Figure S3. Cerebellar microglia and Purkinje cell subcomponents *in vivo*.** (A, H, L) *In vivo* two-photon images showing microglia (A), Purkinje cell dendrites and branch points in the ML (H), and Purkinje cell somas in the PCL (L). White overlay indicates subcomponents for each cell type. (B-G) Whole microglia (B, E) were divided into processes (C, F) and somas (D, G) in either the ML (B-D) or PCL (E-G). (B) ML whole microglia pixel numbers had no main effect for treatment ( $F(1, 16) = 5.406e-006$ ,  $p = 0.9982$ ) and no

treatment-sex interaction effect ( $F(1, 16) = 0.6330$ ,  $p = 0.4379$ ), but female mice had significantly more microglia pixels than male mice ( $F(1, 16) = 4.769$ ,  $p = 0.0442$ ), with a trend in the ethanol-dosed group ( $p = 0.1026$ ). **(C)** ML microglia process pixel numbers had no main effect for treatment ( $F(1, 16) = 0.007151$ ,  $p = 0.9337$ ) and no treatment-sex interaction effect ( $F(1, 16) = 0.4152$ ,  $p = 0.5285$ ), but female mice had a trend towards more microglia pixels than male mice ( $F(1, 16) = 4.106$ ,  $p = 0.0597$ ). **(D)** ML microglia soma pixel numbers had no main effect for treatment ( $F(1, 16) = 0.4740$ ,  $p = 0.5010$ ), or sex ( $F(1, 16) = 1.790$ ,  $p = 0.1996$ ), and no treatment-sex interaction effect ( $F(1, 16) = 1.621$ ,  $p = 0.2211$ ). **(E)** PCL whole microglia pixel numbers had no main effect for treatment ( $F(1, 16) = 0.009064$ ,  $p = 0.9253$ ) and no treatment-sex interaction effect ( $F(1, 16) = 0.1963$ ,  $p = 0.6637$ ), but female mice had a trend towards more microglia pixels than male mice ( $F(1, 16) = 3.303$ ,  $p = 0.0879$ ). **(F)** PCL microglia process pixel numbers had no main effect for treatment ( $F(1, 16) = 0.01486$ ,  $p = 0.9045$ ) and no treatment-sex interaction effect ( $F(1, 16) = 0.08783$ ,  $p = 0.7708$ ), but female mice had a trend towards more microglia pixels than male mice ( $F(1, 16) = 3.497$ ,  $p = 0.0799$ ). **(G)** PCL microglia soma pixel numbers had no main effect for treatment ( $F(1, 16) = 2.652$ ,  $p = 0.1230$ ), or sex ( $F(1, 16) = 1.644$ ,  $p = 0.2180$ ), and no treatment-sex interaction effect ( $F(1, 16) = 1.254$ ,  $p = 0.2793$ ). **(I-K)** Whole Purkinje cells in the ML **(I)** were divided into dendrites **(J)** and branchpoints **(K)**. **(I)** ML whole Purkinje cell pixel numbers had no main effect for treatment ( $F(1, 16) = 1.695$ ,  $p = 0.2113$ ), or sex ( $F(1, 16) = 0.4414$ ,  $p = 0.5159$ ), and no treatment-sex interaction effect ( $F(1, 16) = 1.938$ ,  $p = 0.1829$ ). **(J)** ML Purkinje cell dendrite pixel numbers had no main effect for treatment ( $F(1, 16) = 1.445$ ,  $p = 0.2467$ ), or sex ( $F(1, 16) = 0.4560$ ,  $p = 0.5091$ ), and no treatment-sex interaction effect ( $F(1, 16) = 1.887$ ,  $p = 0.1884$ ). **(K)** ML Purkinje cell branch pixel numbers had no main effect for treatment ( $F(1, 16) = 0.02213$ ,  $p = 0.8836$ ), or sex ( $F(1, 16) = 0.1447$ ,  $p = 0.7087$ ), and no treatment-sex interaction effect ( $F(1, 16) = 0.2422$ ,  $p = 0.6293$ ). **(M)** Purkinje cell somas were identified in the PCL. PCL Purkinje cell soma pixel numbers had no main effect for treatment ( $F(1, 16) = 0.1150$ ,  $p = 0.7389$ ), or sex ( $F(1, 16) = 0.5121$ ,  $p = 0.4845$ ), but there was a significant treatment-sex interaction effect ( $F(1, 16) = 5.996$ ,  $p = 0.0262$ ) with a post hoc trend of saline females having more pixels than saline males ( $p = 0.0797$ ). **(B-G, I-K, M)** Each datapoint represents an individual animal. Data are presented as the mean  $\pm$  SEM. Two-way ANOVA with Bonferroni post hoc comparisons. Scale bar = 25  $\mu$ m



**Figure S4. Cerebellar microglia-Purkinje cell subcomponent interactions *in vivo*.** (A-F) The overlap between the microglia and Purkinje cell subcomponent pixels were normalized to the number of the microglia pixels to determine microglia-Purkinje cell interaction in the ML (A-D) and PCL (E-F). (A) ML microglia process x Purkinje cell dendrite interactions had no main effect for treatment ( $F(1, 16) = 1.317$ ,  $p = 0.2679$ ), or sex ( $F(1, 16) = 0.1365$ ,  $p = 0.7167$ ), and no treatment-sex interaction effect ( $F(1, 16) = 2.615$ ,  $p = 0.1254$ ). (B) ML microglia soma x Purkinje cell dendrite interactions had no main effect for treatment ( $F(1, 16) = 0.004292$ ,  $p = 0.9486$ ), or sex ( $F(1, 16) = 0.02414$ ,  $p = 0.8785$ ), but there was a significant treatment-sex interaction effect ( $F(1, 16) = 5.067$ ,  $p = 0.0388$ ). (C) Microglia process x Purkinje cell branch interactions had no main effect for treatment ( $F(1, 16) = 0.0001596$ ,  $p = 0.9901$ ), or sex ( $F(1, 16) = 0.2689$ ,  $p = 0.6112$ ), and no treatment-sex interaction effect ( $F(1, 16) = 0.009910$ ,  $p = 0.9219$ ). (D) ML microglia soma x Purkinje cell branch interactions had no main effect for treatment ( $F(1, 16) = 0.0009207$ ,  $p = 0.9762$ ), or sex ( $F(1, 16) = 1.521$ ,  $p = 0.2352$ ), and no treatment-sex interaction effect ( $F(1, 16) = 0.02347$ ,  $p = 0.8802$ ). (E) PCL microglia process x Purkinje cell soma interactions had no main effect for treatment ( $F(1, 16) = 0.5339$ ,  $p = 0.4756$ ) and no treatment-sex interaction effect ( $F(1, 16) = 1.290$ ,  $p = 0.2727$ ), but female mice had significantly more interactions than male mice ( $F(1, 16) = 4.863$ ,  $p = 0.0424$ ), with a trend apparent in saline-dosed mice.

( $p=0.0623$ ). **(F)** PCL microglia soma x Purkinje cell soma interactions had no main effect for treatment ( $F(1, 16) = 0.4601$ ,  $p = 0.5073$ ), or sex ( $F(1, 16) = 1.418$ ,  $p = 0.2511$ ), and no treatment-sex interaction effect ( $F(1, 16) = 0.0003324$ ,  $p = 0.9857$ ). **(A-F)** Each datapoint represents an individual animal. Data are presented as the mean  $\pm$  SEM. Two-way ANOVA with Bonferroni post hoc comparisons.