A

GFAP IBA1 GALC

Merge

Glial-specific marker

Hoechst

Phase

B

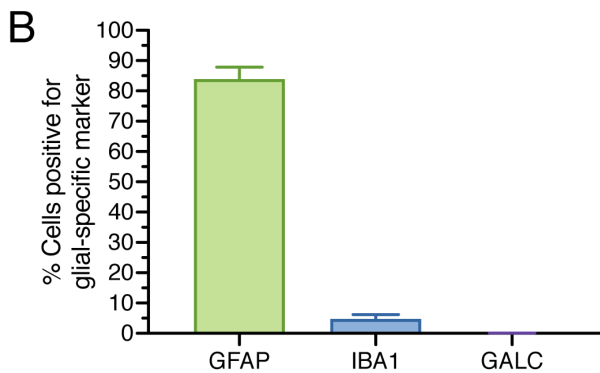

Figure S1. Primary cortical glial preparations are enriched for astrocytes. (**A**) Representative images of primary mouse non-transgenic cortical glia immunostained for GFAP to label astrocytes, IBA1 to label microglia, and GALC to label oligodendrocytes (images for GALC are maximum projections generated from a Z-stack). Hoechst labels total cells in the field. Bar: 50 µm. (**B**) Corresponding quantification of the percent cells positive for each glial-specific marker out of total cells labeled with Hoechst. Mean ± SEM; n=2 independent experiments (3-7 DIV).

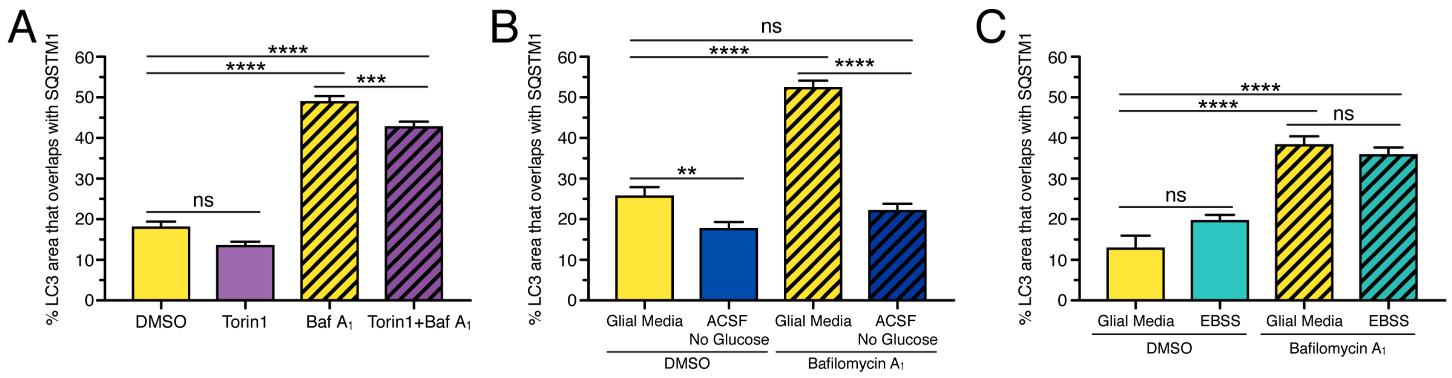

Figure S2. Starvation in ACSF dampens selective autophagy in primary astrocytes. Quantification of the percentage of GFP-LC3 puncta area that overlaps with SQSTM1 puncta after 4 h in (**A**) torin1 (mean ± SEM; one-way ANOVA with Tukey's post hoc test; n=53-82 cells from 3 independent experiments, 5-7 DIV), (**B**) ACSF without glucose (mean ± SEM; one-way ANOVA with Tukey's post hoc test; n=38-53 cells from 3 independent experiments, 5-6 DIV) and (**C**) EBSS (mean ± SEM; one-way ANOVA with Tukey's post hoc test; n=25-39 cells from 3 independent experiments, 4-5 DIV). Baf A_1, bafilomycin A_1.

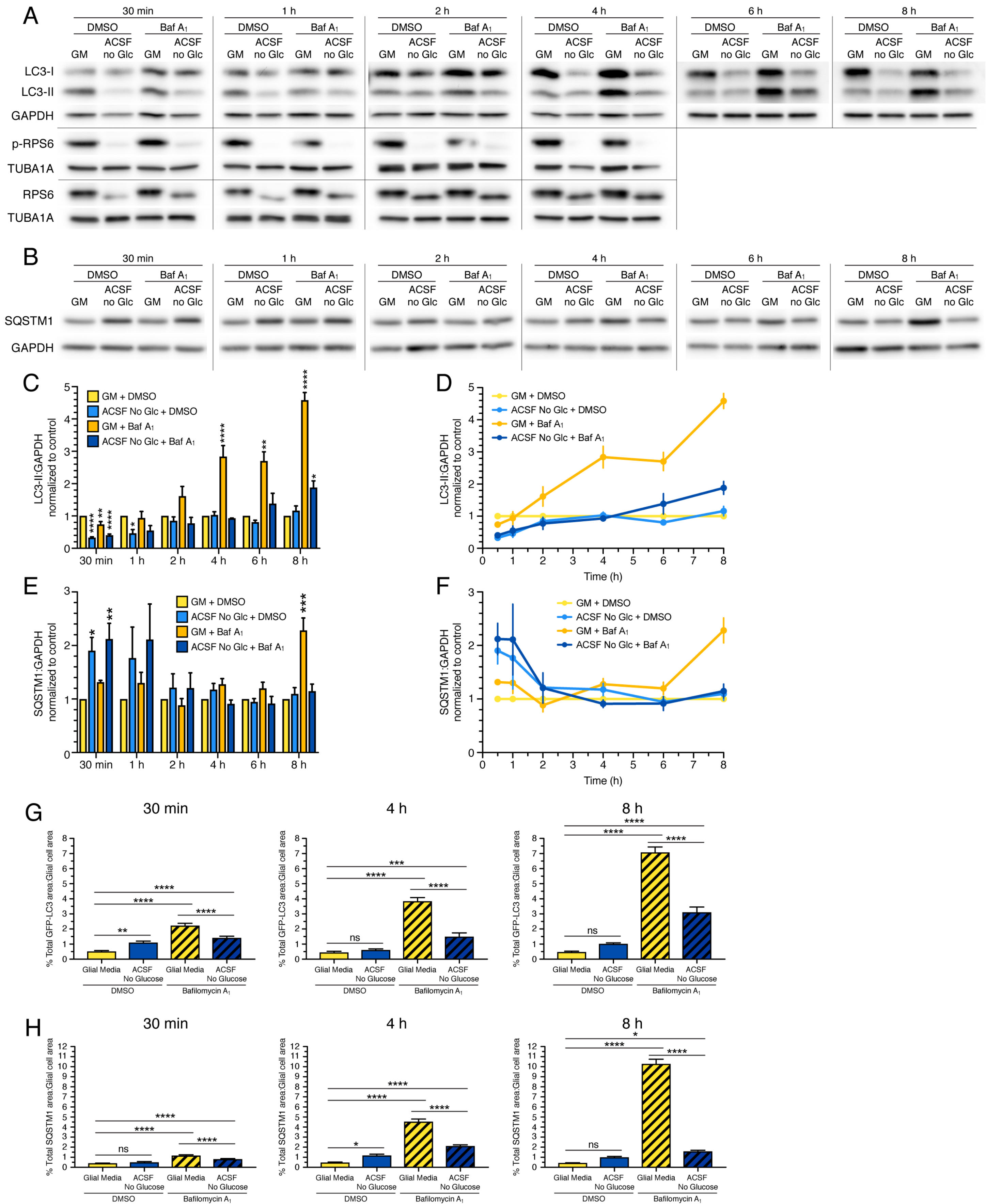

Figure S3. Starvation time course in ACSF without glucose dampens autophagy in primary astrocytes. (**A** and **B**) Time course of glia starved in ACSF without glucose and analyzed by immunoblotting. GAPDH and TUBA1A/α-tubulin serve as

loading controls; horizontal lines designate individual blots. (**C-F**) Corresponding quantification of immunoblot analysis in A and B displayed as either bar graphs (**C** and **E**) or line plots (**D** and **F**). (**C** and **D**) LC3-II levels were normalized to GAPDH (mean ± SEM; one-way ANOVA with Dunnett's post hoc test; statistical tests were performed independently for each time point; bars not designated are ns; n=3-4 independent experiments, 3-7 DIV). (**E** and **F**) SQSTM1 levels were normalized to GAPDH (mean ± SEM; one-way ANOVA with Dunnett's post hoc test; statistical tests were performed independently for each time point; bars not designated are ns; n=3-4 independent experiments, 3-7 DIV). (**G**) Quantification of total GFP-LC3-puncta area normalized to cell area of astrocytes starved in ACSF without glucose for the indicated times (mean ± SEM; one-way ANOVA with Tukey's post hoc test; 30 min, n=73-137 cells from 4 independent experiments; 4 h, n=58-68 cells from 4 independent experiments; 8 h, n=50-85 cells from 3 independent experiments, 3-6 DIV). The data for 4 h are the same as the data shown in Figure 2B. (**H**) Quantification of total SQSTM1 puncta area normalized to cell area of astrocytes starved in ACSF without glucose for the indicated times (mean ± SEM; one-way ANOVA with Tukey's post hoc test; 30 min, n=87-178 cells from 4 independent experiments; 4 h, n=58-91 cells from 4 independent experiments, 8 h, n=62-106 cells from 3 independent experiments, 3-6 DIV). The data for 4 h are the same as the data shown in Figure 2C. Baf A_1, bafilomycin A_1; Glc, glucose; GM, glial media.

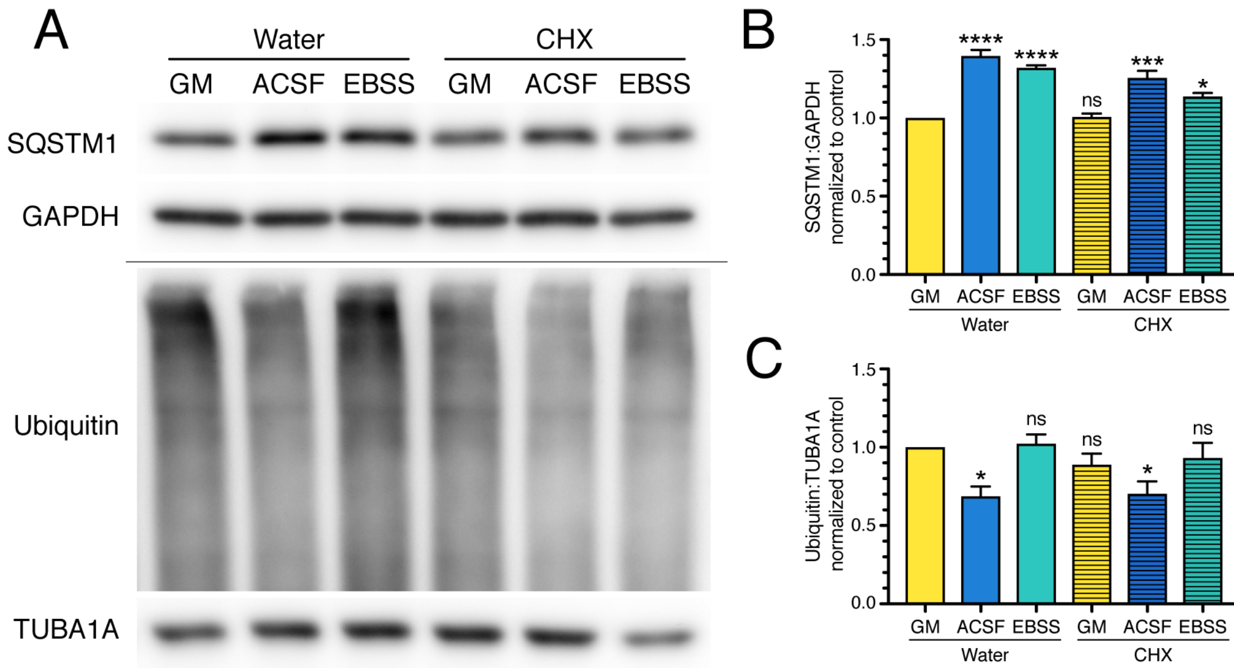

Figure S4. Starvation-induced increase in SQSTM1 is partially due to new protein synthesis. (**A**) Immunoblot analysis of glia starved for 30 min in either ACSF or EBSS ± cycloheximide (CHX) and analyzed by immunoblotting. GAPDH and TUBA1A/α-tubulin serve as loading controls; horizontal lines designate individual blots. (**B** and **C**) Corresponding quantification of immunoblot analysis in A. (**B**) Mean ± SEM; one-way ANOVA with Dunnett's post hoc test; n=3 independent experiments; 6-7 DIV. (**C**) Mean ± SEM; one-way ANOVA with Dunnett's post hoc test; n=5 independent experiments; 6-7 DIV.

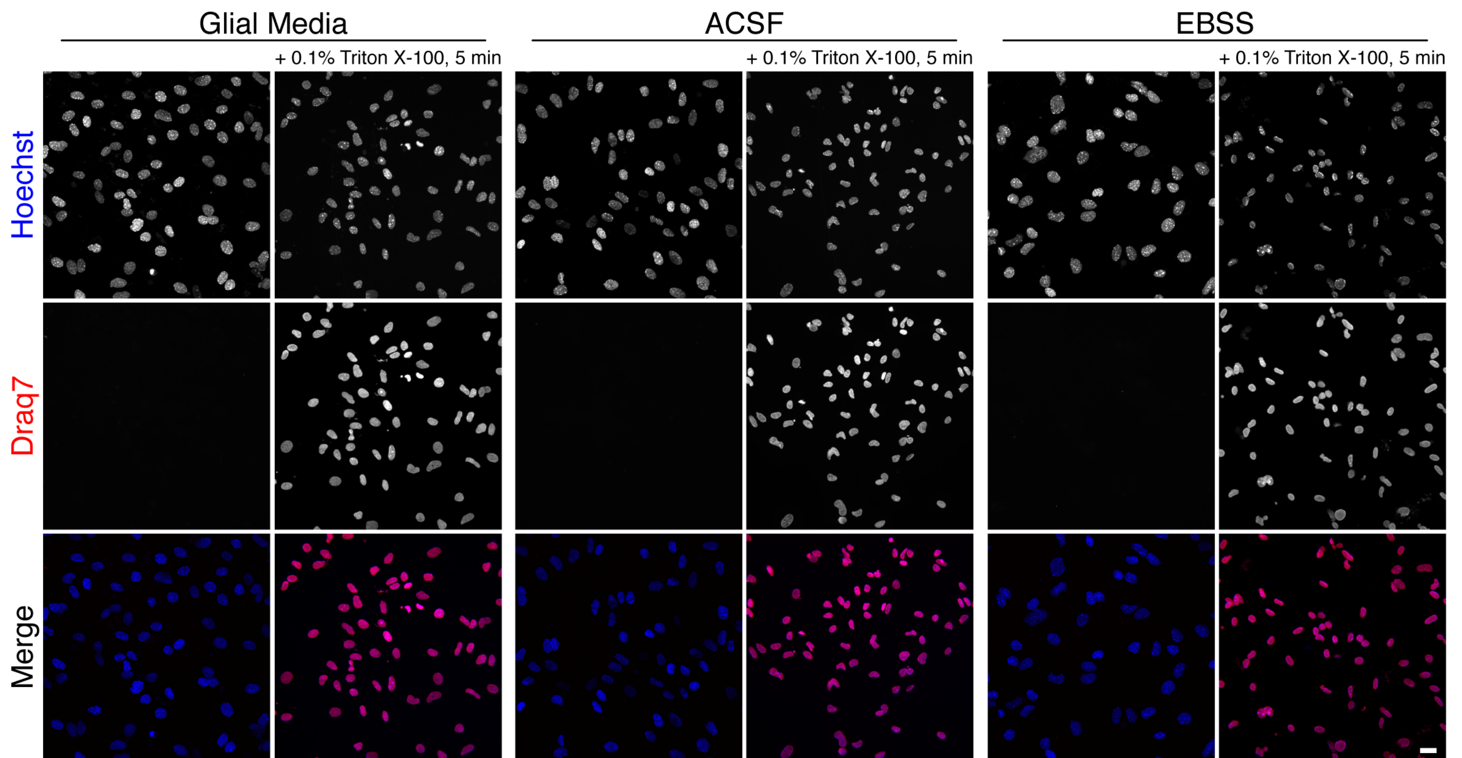

Figure S5. Starvation of glia in ACSF or EBSS does not cause cytotoxicity. Maximum projections of Z-stacks of Hoechst and Draq7 labeling of glia incubated for 4 h in either normal glial media, ACSF, or EBSS (Bar: 20 µm). Hoechst is a membrane permeable DNA dye that labels total cells, and Draq7 is a membrane impermeable DNA dye that will label only dead and membrane-compromised cells. In all treatment conditions, comparing fed controls (in glial media) to ACSF to EBSS, only ~1% of total Hoechst-positive cells were co-positive for Draq7, indicating very low levels of cytotoxicity. As a positive control, following each treatment, cells were permeabilized with 0.1% Triton X-100 to allow accessibility of the Draq7 dye. Under these conditions, 100% of Hoechst-positive cells were co-positive for Draq7. Thus, the starvation treatments are not causing cytotoxicity within the 4 h tested.

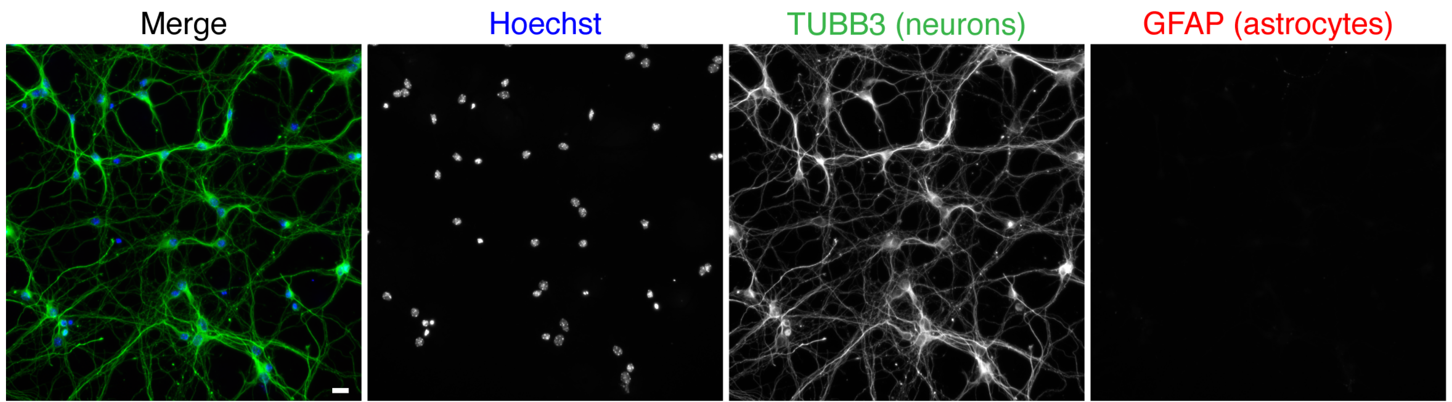

Figure S6. Primary hippocampal neuron preparations are highly pure (~96% of cells are neurons). Representative images of primary hippocampal neuron preparations at 8 DIV labeled for Hoechst (labels total cells), TUBB3/β3 tubulin (neuron-specific marker), and GFAP (astrocyte-specific marker). Bar: 20 μm.