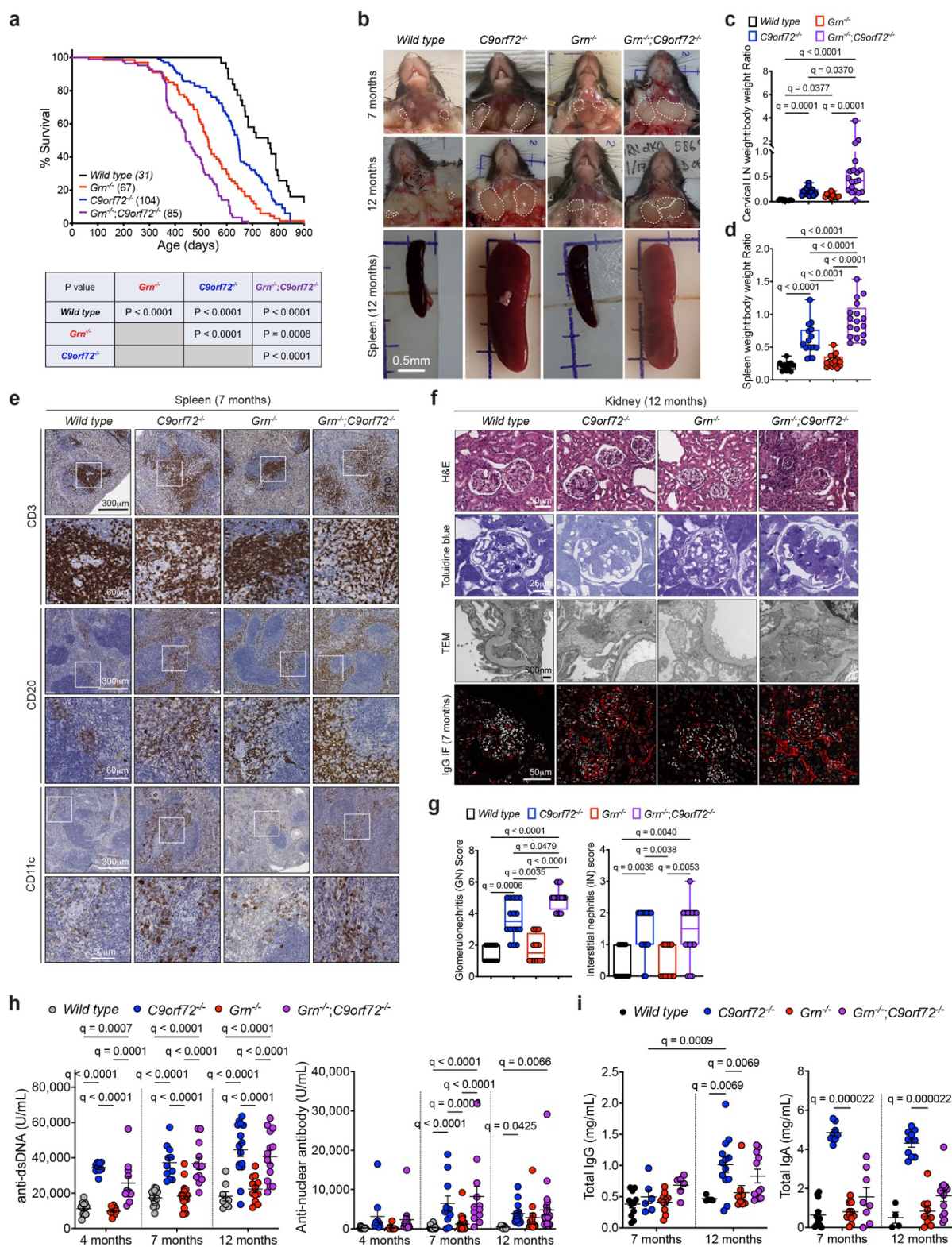
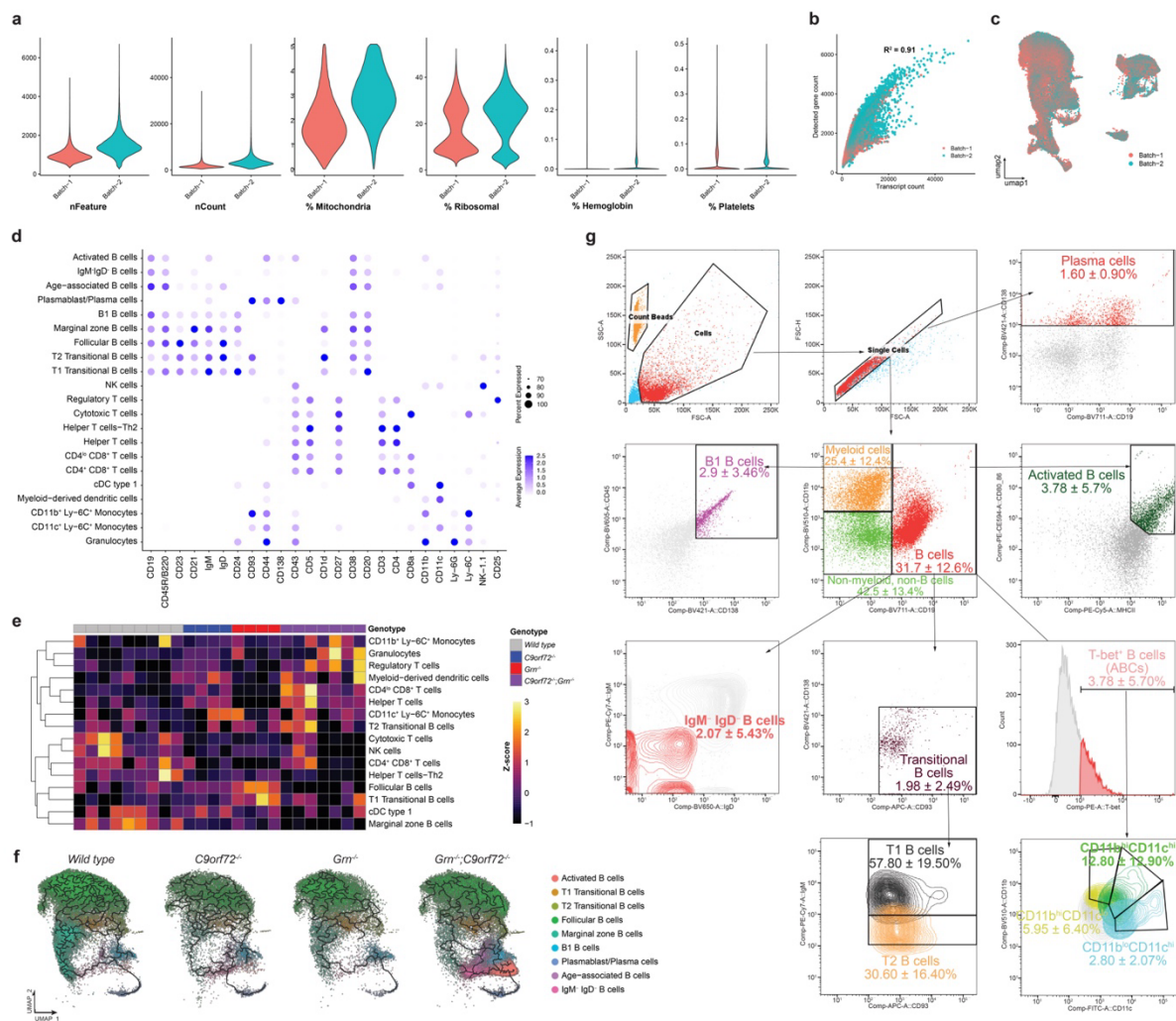




Extended Data Fig. 1 | See next page for caption.

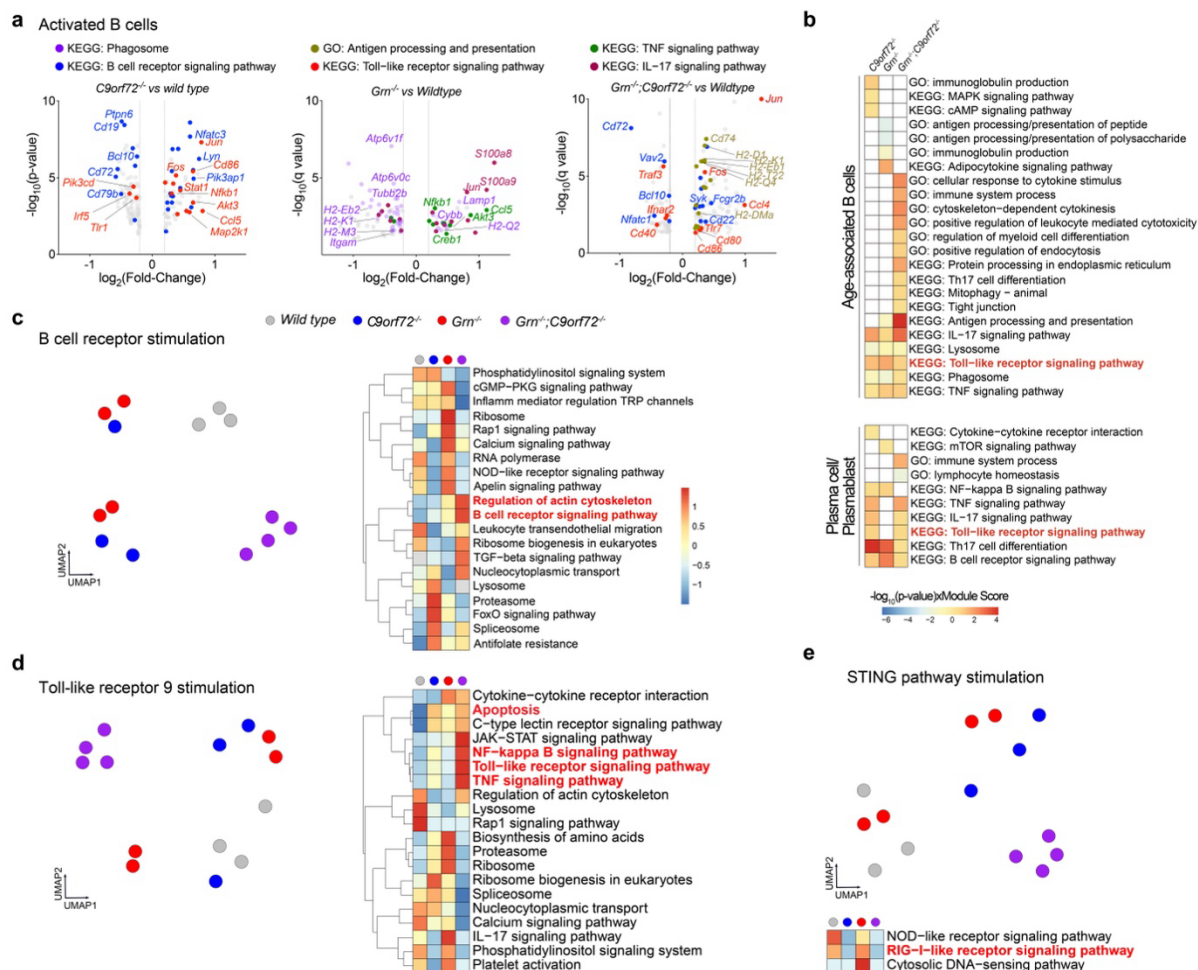
Extended Data Fig. 1 | Loss of PGRN and C9orf72 causes early lethality with severe autoimmune dysfunction. (a) Kaplan-Meier curves demonstrating the early lethality in *Grn*^{-/-}; *C9orf72*^{-/-} mice compared to *wild type*, *C9orf72*^{-/-}, and *Grn*^{-/-} mice. Statistics used Log-rank (Mantel-Cox) test. (b) *C9orf72*^{-/-}, *Grn*^{-/-}, and *Grn*^{-/-}; *C9orf72*^{-/-} mice showed cervical lymphadenopathy and splenomegaly compared to *wild type* at 7 and 12-month-old. (c-d) Quantification of cervical lymph node weight and spleen weight to body weight ratio. Number of 12-month-old mice used for quantification: cervical lymph node weight: *wild type* (12), *C9orf72*^{-/-} (14), *Grn*^{-/-} (12), *Grn*^{-/-}; *C9orf72*^{-/-} (19); spleen weight: *wild type* (13), *C9orf72*^{-/-} (16), *Grn*^{-/-} (16), *Grn*^{-/-}; *C9orf72*^{-/-} (18). (e) Immunohistochemical stains for CD3 (T cells), CD20 (B cells), and CD11c (myeloid cells) showed prominent effacement of splenic architecture in 7-month-old *Grn*^{-/-}; *C9orf72*^{-/-} mice. (f) At 12-month-old, *C9orf72*^{-/-} and *Grn*^{-/-}; *C9orf72*^{-/-} mice showed severe glomerulonephropathy, characterized by

numerous sclerotic glomeruli (H&E and toluidine blue stains), effacement of the foot processes in podocytes and the presence of electron-dense granular deposits in the interstitium (transmission electron microscopy (TEM)), and deposits of IgG in the glomeruli and tubules (immunofluorescent confocal images at 7-month-old). (g) Quantification of glomerulonephropathy (GN) and interstitial nephritis (IN) scores in *wild type* (13), *C9orf72*^{-/-} (16), *Grn*^{-/-} (12), *Grn*^{-/-}; *C9orf72*^{-/-} (16). Statistics used Kruskal-Wallis test with multiple comparisons and data were mean ± s.e.m. (h-i) Enzyme-linked immunosorbent assay (ELISA) showed an age-dependent increase in anti-dsDNA antibodies and anti-nuclear autoantibodies titer that was detected as early as 4 months (h), and a significant increase in total IgG titer in *C9orf72*^{-/-} and *Grn*^{-/-}; *C9orf72*^{-/-} mice at 12-month-old (i), but total IgA levels were only increased in *C9orf72*^{-/-} mice. Statistics used 2-way ANOVA with multiple comparisons, data were mean ± s.e.m.



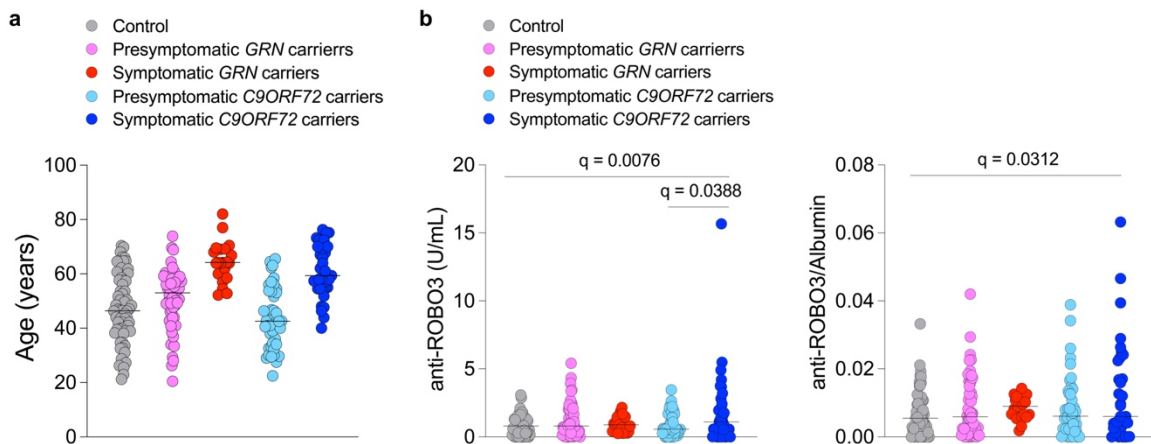
Extended Data Fig. 2 | CITE-seq and multi-parameter flow cytometry validate aberrant expansion of B cells in *Grn*^{-/-}; *C9orf72*^{-/-} mice. (a-c) Quality control metrics of two batches of CITE-seq samples that were calculated to be acceptable for single-cell sequencing-based analyses (a), the batch effects were found to be minimal (b), and the unsupervised clustering can be visualized on the UMAP (c). (d) Detailed classifications of each cellular

cluster in CITE-seq analysis are displayed using feature plots of cluster-specific genes. (e) The overall cellular frequencies analysis of each cell type identified on CITE-seq visualized using a heatmap showed a modest expansion of peripheral myeloid clusters in addition to B cells. (f) B cell-focused pseudotime analysis demonstrated the B cell maturation trajectory among different genotypes. (g) Gating strategy for multi-parameter flow cytometry analysis.



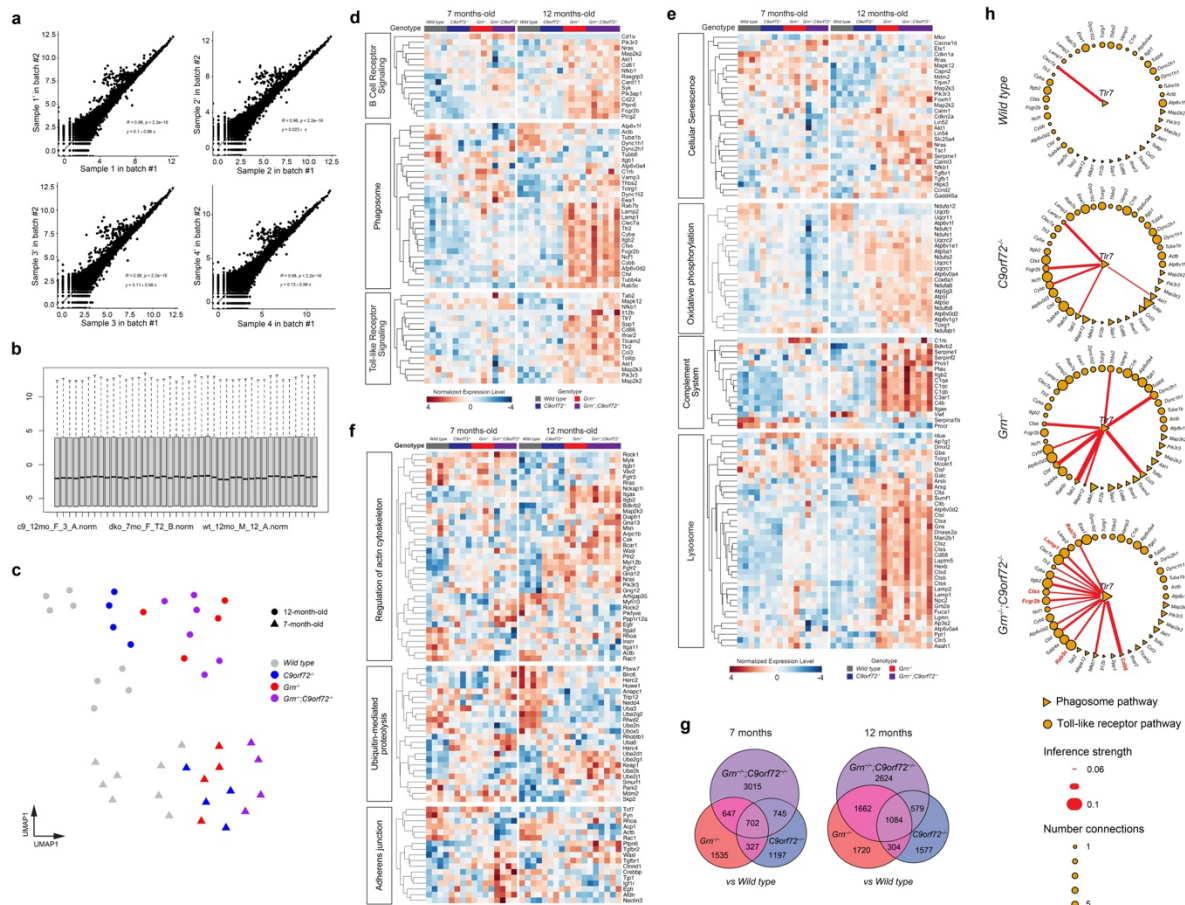
Extended Data Fig. 3 | GSEA analysis of differentially expressed genes in activated B cells, age-associated B cells, and plasma cells/plasmablasts and *in vitro* stimulation of splenic B cells isolated from *wild type*, *C9orf72*^{-/-}, *Grn*^{-/-} and *Grn*^{-/-};C9orf72^{-/-} mice. (a) Volcano plots of differentially expressed genes (DEGs) in *Grn*^{-/-};C9orf72^{-/-} activated B cells, compared to *wild type*, *C9orf72*^{-/-}, and *Grn*^{-/-} activated B cells. **(b)** GSEA analysis of *Grn*^{-/-};C9orf72^{-/-} age-associated and plasma cells/plasmablasts using GO and KEGG databases. **(c-e)** UMAPs and GSEA analyses

of splenic B cells from *wild type* (gray), *C9orf72*^{-/-} (blue), *Grn*^{-/-} (red), and *Grn*^{-/-};C9orf72^{-/-} (purple) mice after stimulation by B cell receptor ligation **(c)**, toll-like receptor 9 activation **(d)**, and activation of STING pathway **(e)** showed distinct transcriptomic profiles in B cells from *Grn*^{-/-};C9orf72^{-/-} mice compared to those from *C9orf72*^{-/-} and *Grn*^{-/-} mice. The bolded enriched pathways in each individual stimulation condition in the GSEA analyses are those shared with activated splenic B cells from CITE-seq analysis.



Extended Data Fig. 4 | Anti-ROBO3 antibody titers in CSF samples from control, presymptomatic or symptomatic *GRN* carriers, and presymptomatic or symptomatic *C9orf72* carriers. (a) Age distribution of control and *GRN* or *C9orf72* carriers at presymptomatic or symptomatic stage of their disease progression. **(b)** ELISA

assays reveal significantly higher titers of anti-ROBO3 autoantibodies in CSF samples from symptomatic *C9orf72* carriers than presymptomatic *C9orf72* carriers and presymptomatic and symptomatic *GRN* carriers. Statistics used one-way ANOVA with Tukey's multiple comparison test.

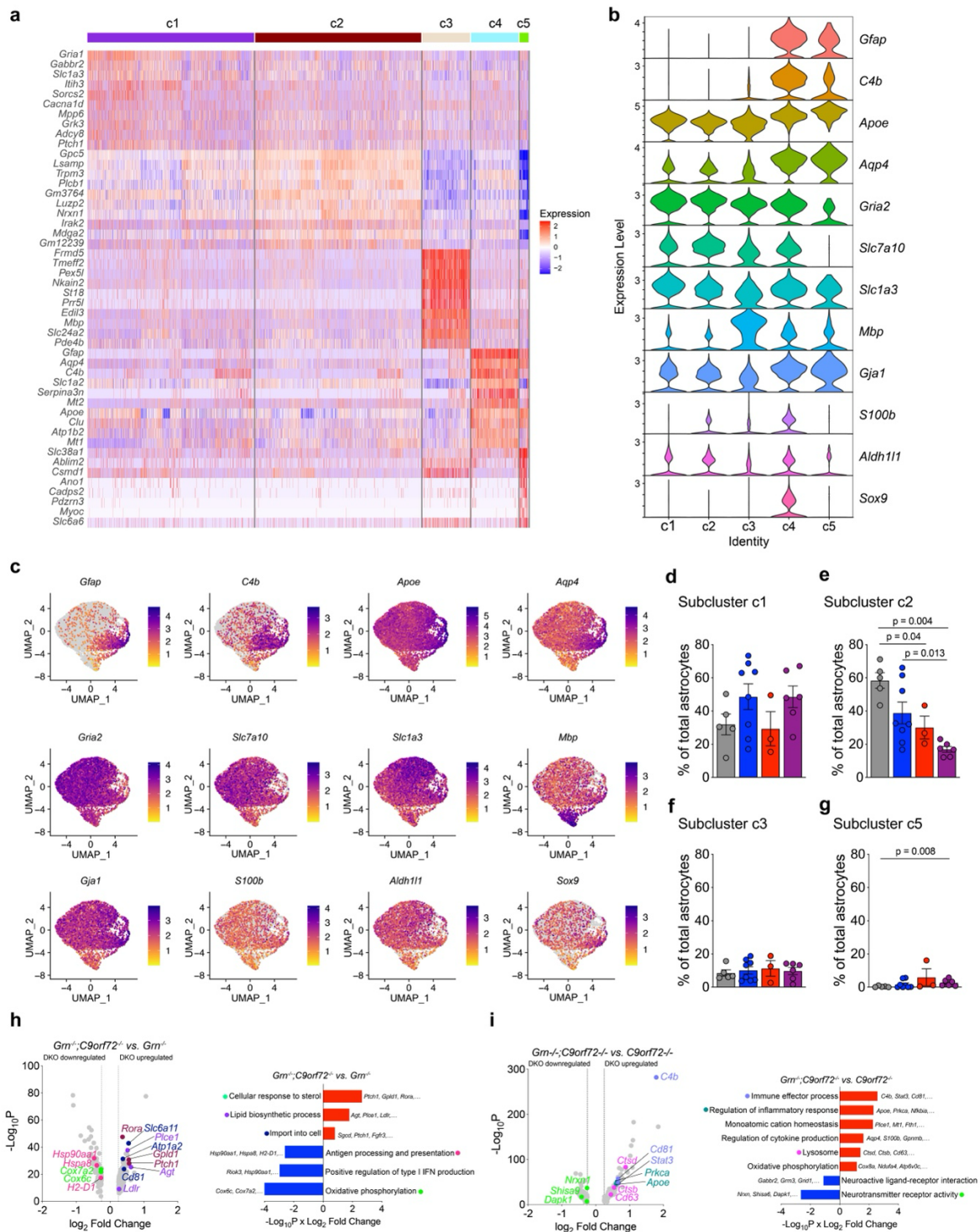


Extended Data Fig. 5 | Age-dependent transcriptomic changes in 7 and 12-month-old wild type, C9orf72^{-/-}, Grn^{-/-} and Grn^{-/-};C9orf72^{-/-} mice. (a) Correlation analysis of samples from different batches of bulk RNA-seq using hemibrains from 7 and 12-month-old wild type, C9orf72^{-/-}, Grn^{-/-} and Grn^{-/-};C9orf72^{-/-} mice. (b) Post-normalization algorithms visualized using box plots showed minimal batch-to-batch effect in brain hemisphere bulk RNA-seq data. (c) UMAP of the samples from bulk RNA-seq. (d-e) Heatmaps of bulk RNA-seq show age-dependent, genotype-specific transcriptomic changes in pathways related to B cell receptor signaling, phagosome, toll-like receptor signaling, cellular senescence, oxidative phosphorylation, complement, and

lysosome. (f) GO terms and KEGG pathways enriched in 7 and 12-month-old Grn^{-/-};C9orf72^{-/-} mice include regulation of actin cytoskeleton, ubiquitin-mediated proteolysis, and adhere junction pathways. (g) Venn diagrams showing the overlap in the differential expressed genes (DEGs) between C9orf72^{-/-}, Grn^{-/-} and Grn^{-/-};C9orf72^{-/-} mouse brains at 7- and 12-months of age-old. (h) Gene regulatory network analysis based on bulk RNA-seq data from 12-month-old mice between Tlr7 and key genes in phagosome and toll-like receptor pathways. The red lines indicate Tlr7 as a regulatory gene of toll-like receptor and phagosome pathway related genes. Node size reflects the number of connections the gene has as a regulatory or target gene in the network.

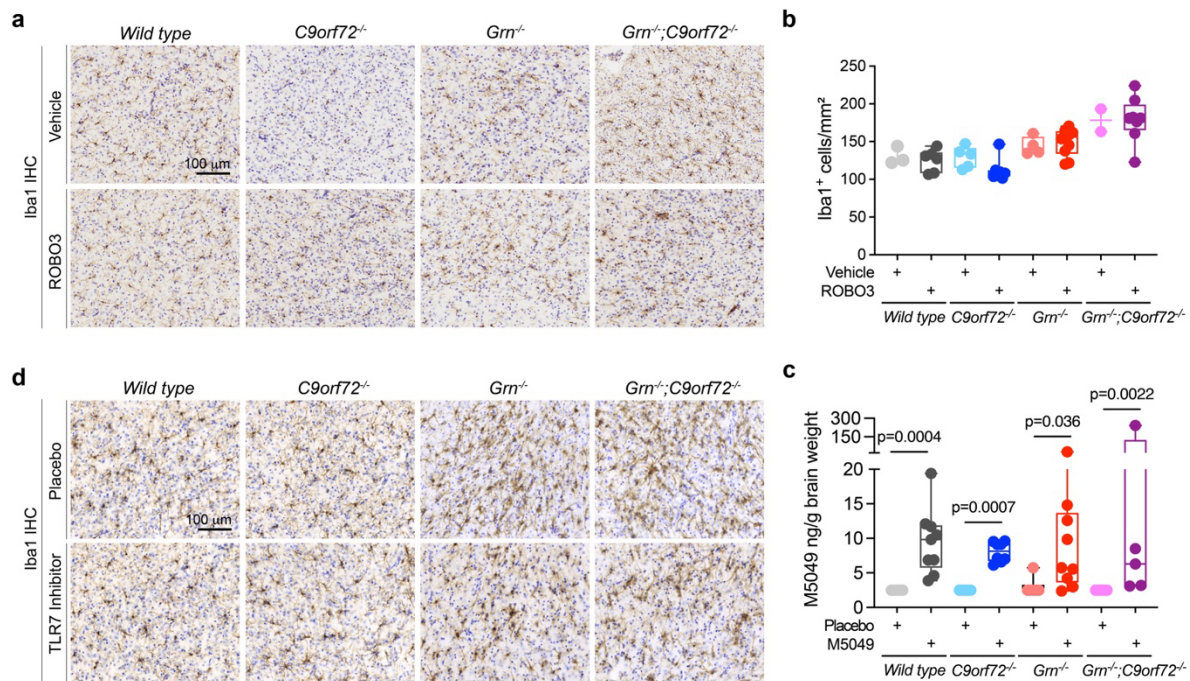
Extended Data Fig. 6. Quality control for single nuclear RNA-sequencing (snRNA-seq) of thalami from 12-month-old *wild type*, *C9orf72*^{-/-}, *Grn*^{-/-} and *Grn*^{-/-};*C9orf72*^{-/-} mice. (a) Combined UMAPs of snRNA-seq data from *wild type*, *C9orf72*^{-/-}, *Grn*^{-/-} and *Grn*^{-/-};*C9orf72*^{-/-} mice mouse thalami showing the batch-to-batch differences (left) and the removal of doublets using DoubletFinder. (b) Distribution of the transcriptomic activity of the identified cell clusters as shown by the histogram of UMI counts per nuclei. (c) Graphs showing the filtered UMIs per cell, number of

genes per cell, and percent of mitochondrial genes for each sample. (d) Pearson correlation coefficient of 0.96 comparing UMIs to the number of genes detected for each sample using a scatter plot. (e) A table showing the number of biological replicates used for snRNA-seq per genotype. (f) Feature plot showing cell type-specific markers used to determine each cell type clusters. (g) Heatmap of the top 10 markers unique to each cluster as identified through Seurat's FindAllMarkers function (method of test used is MAST).



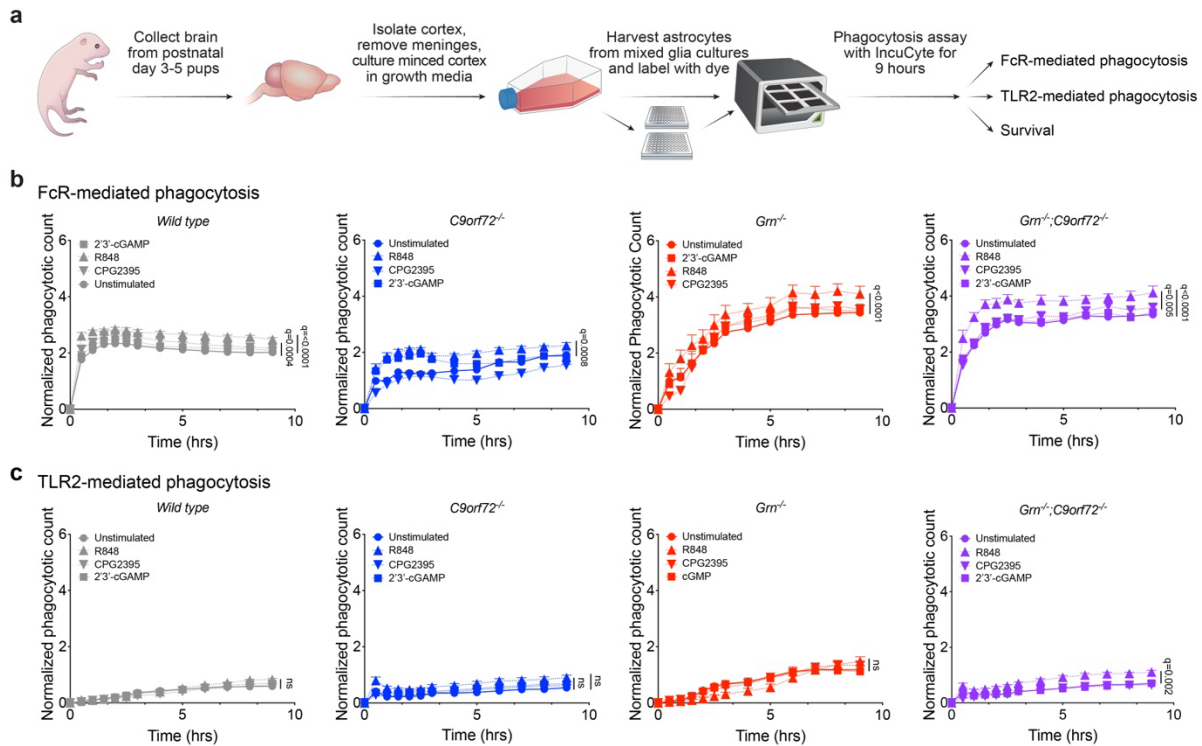
Extended Data Fig. 7. Subclustering and bioinformatic analysis of astrocyte clusters in wild type, *Grn*^{-/-}, and *C9orf72*^{-/-} and *Grn*^{-/-};*C9orf72*^{-/-} mice. (a-c) Astrocyte subcluster analysis using heatmap of top 10 genes for each subcluster (a), violin plot of representative genes that are enriched in each cluster (b), and (c) feature plot of representative genes, including *Gfap*, *C4b*, *Apoe*, *Aqp4*, *Gria2*, *Slc7a10*, *Slc1a3*, *Mbp*, *Gja1*, *S100b*, *Aldh1l1*,

and *Sox9*. (d-g) Astrocyte subcluster composition in each genotype. Statistics were performed using nonparametric Mann-Whitney U test. (h-i) Volcano plots and GSEA analyses highlighting the differentially expressed genes (DEGs) and the GO terms and KEGG pathways that is defined between *Grn*^{-/-};*C9orf72*^{-/-} astrocytes and *Grn*^{-/-} astrocytes (h) and between *Grn*^{-/-};*C9orf72*^{-/-} astrocytes and *C9orf72*^{-/-} astrocytes (i).



Extended Data Fig. 8 | Immunohistochemical stains for microglia in Robo3 ECD-immunized and TLR7 antagonist M5049-treated mouse brain and detection of M5049 in the brain of *wild type*, *Grn*^{-/-}, and *C9orf72*^{-/-} and *Grn*^{-/-};*C9orf72*^{-/-} mouse brain. (a) Immunohistochemical stain of Iba1⁺ microglia in *wild type*, *Grn*^{-/-}, and *C9orf72*^{-/-} and *Grn*^{-/-};*C9orf72*^{-/-} mice after Robo3 ECD immunization. (b) Quantification of Iba1⁺ microglia in mice after Robo3 ECD

immunization show no significant differences between vehicle and Robo3 ECD immunized mice in each genotype. (c) Concentration of M5049 in brain tissues of *wild type*, *Grn*^{-/-}, and *C9orf72*^{-/-} and *Grn*^{-/-};*C9orf72*^{-/-} mice after 8 weeks of daily oral gavage. Statistics were performed using nonparametric Mann-Whitney U test. (d) Immunohistochemical stains of Iba1⁺ microglia in *wild type*, *Grn*^{-/-}, and *C9orf72*^{-/-} and *Grn*^{-/-};*C9orf72*^{-/-} mice after daily oral gavage of TLR7 antagonist M5049 at 10-months-old.



Extended Data Fig. 9. Determination of phagocytosis and apoptosis in *wild type*, *C9orf72*^{-/-}, *Grn*^{-/-} and *Grn*^{-/-};*C9orf72*^{-/-} astrocytes using IncuCyte live cell imaging system. (a) A schematic diagram showing the experimental procedures for primary astrocyte cultures using neonatal mouse brain and live imaging for FcR-mediated and TLR2-mediated phagocytosis using IncuCyte. **(b)** FcR-mediated phagocytosis can be detected in in *wild type*, *C9orf72*^{-/-}, *Grn*^{-/-}, and *Grn*^{-/-};*C9orf72*^{-/-} astrocytes under basal media (unstimulated) or treated with TLR7 agonist R848, TLR9 agonist

CPG2395, or STING agonist 2'3'-cGAMP. **(c)** Minimal phagocytosis of pHrodo-Zymosan was detected in *wild type*, *C9orf72*^{-/-}, *Grn*^{-/-}, and *Grn*^{-/-};*C9orf72*^{-/-} astrocytes under basal media (unstimulated) or treated with TLR7 agonist R848, TLR9 agonist CPG2395, or STING agonist. Statistics for panels **b-c** used Friedman's test and non-parametric test with Benjamani-Hochberg multiple testing correction using all time points. Data were mean $\pm$ s.e.m. from 8 independent biological replicates.