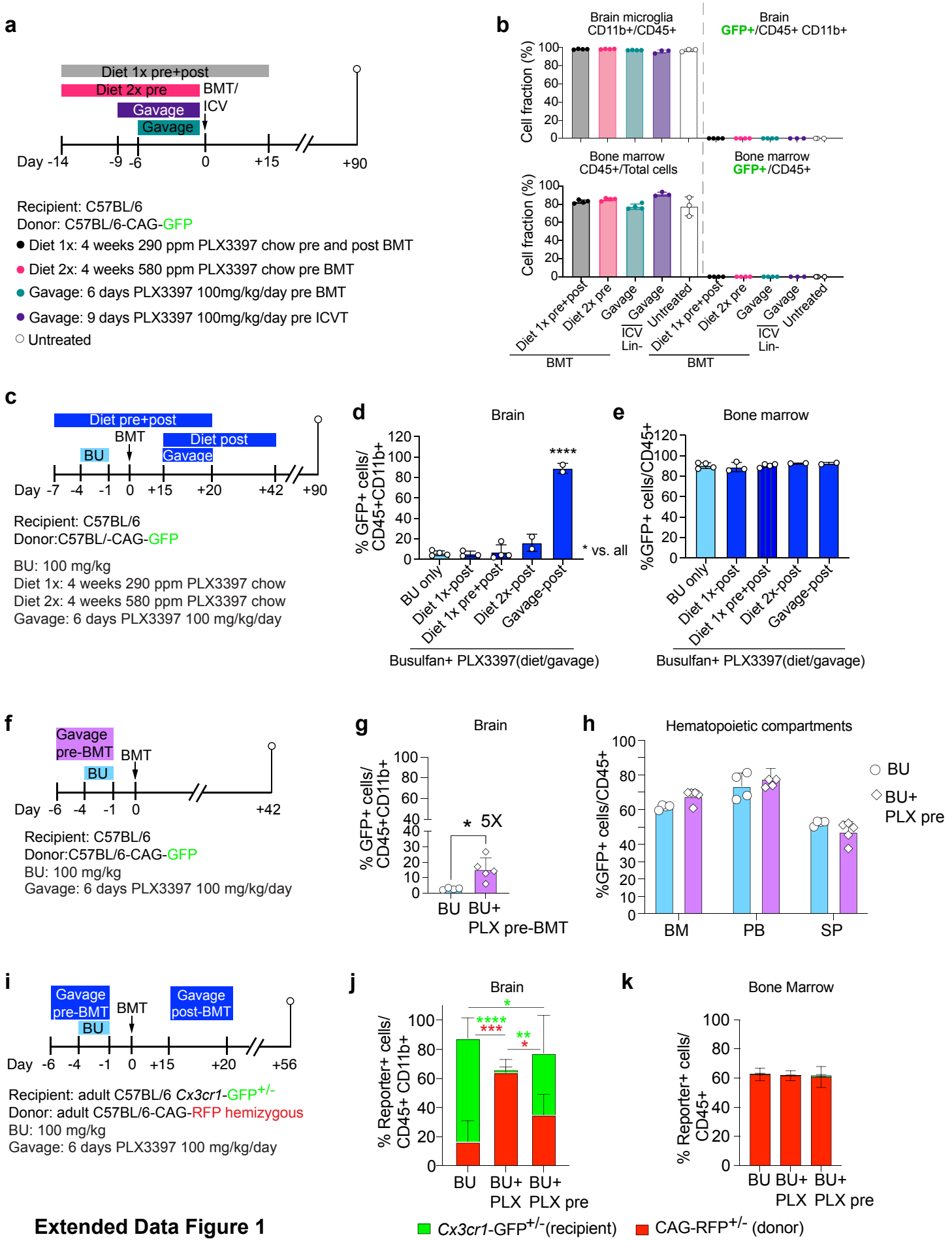
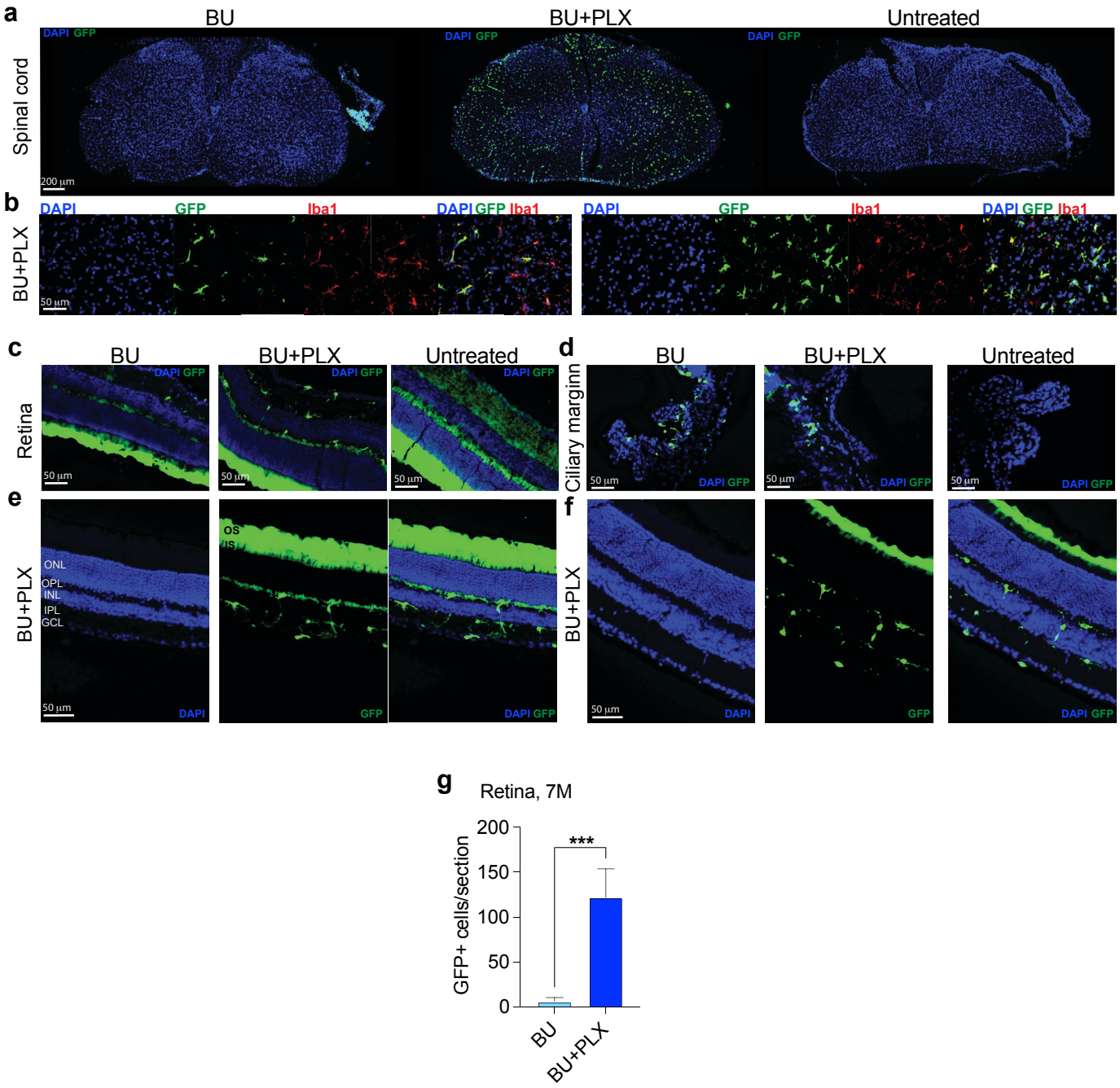


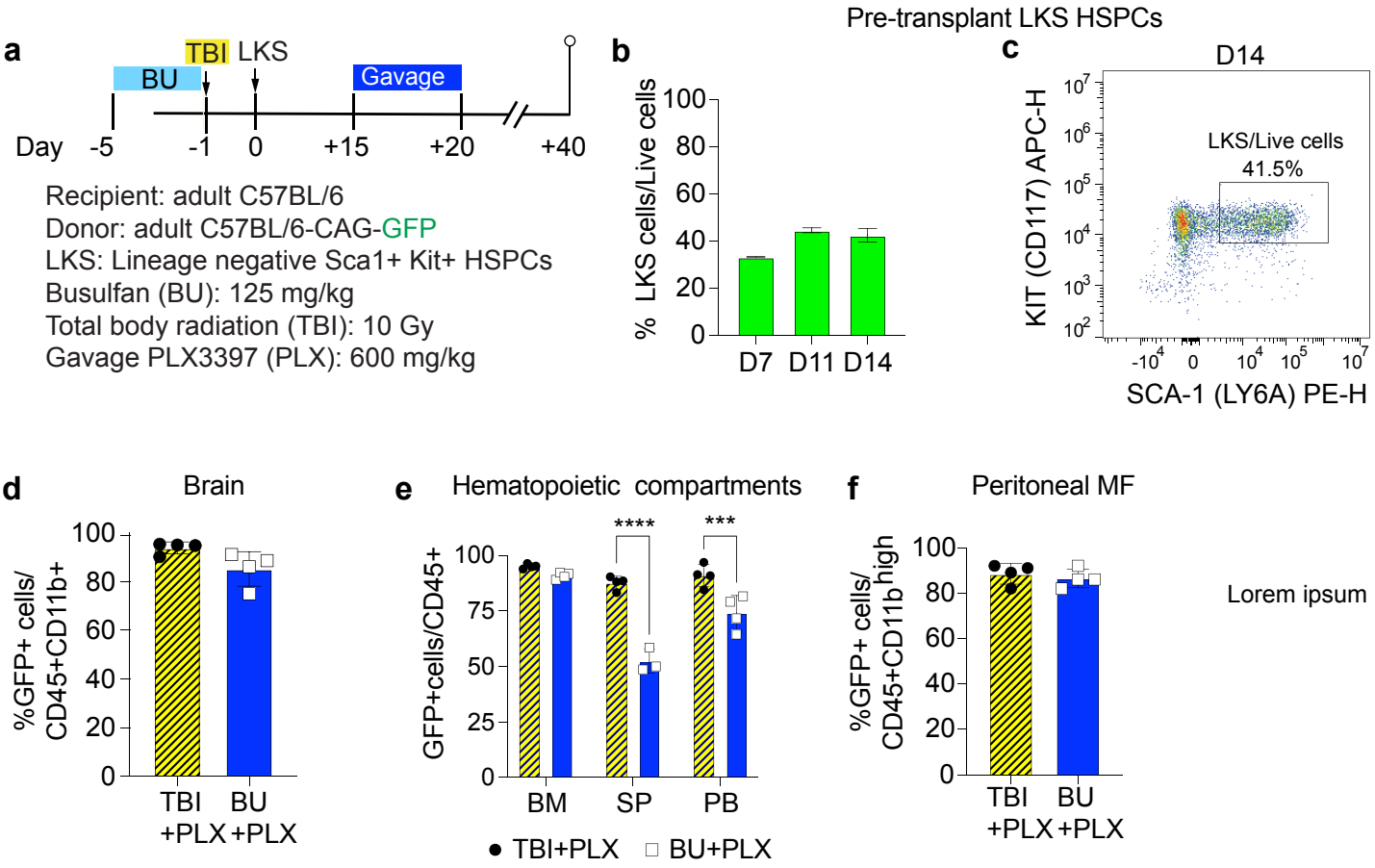
Extended Data Figure 1

Extended data Figure 1. Optimization of the PLX3397 regimen.
a-h. Analysis of donor cell engraftment in brain and hematopoietic compartments of adult C57BL/6 mice treated with Busulfan (BU) and various PLX3397 (PLX) regimens**. a,** Experimental design, and timeline. The mice conditioned with PLX, received intravenous whole bone marrow (BMT) or intracerebroventricular (ICV) injection of Lineage negative (Lin-) bone marrow cells, isolated from adult homozygous C57BL/6-CAG-GFP mice. Age-matched untreated mice were used as controls. PLX was administered by either oral gavage (100 mg/kg/day for either 6 or 9 days) or using drug-complexed chows at a concentration of either 290 ppm (diet 1x) or 580 ppm (diet 2X). **b,** Analysis of donor cell engraftment in brain and bone marrow of adult C57BL/6 mice treated with PLX alone (right panels) and measured by flow cytometry in the brain (top panel) and bone marrow (bottom panel) at study end. The left panel shows the relative frequency of CD11b+/ CD45+ cells in the brain and CD45+ hematopoietic cells in the bone marrow at the same time point. BMT: n=4; ICV Lin- n=3, Untreated n=3. **c**, Experimental timeline showing the myeloablation of mice with BU (100 mg/kg) and PLX administered using different regimens. BU (100 mg/kg, 25 mg/kg/day) was administered prior to BMT from adult homozygous C57BL/6-CAG-GFP mice. PLX was administered by either oral gavage for 6 days (600 mg/kg, 100 mg/kg/ day) or using drug- complexed chows. Follow up was 3 months after BMT. **d-e**, Donor chimerism as measured by flow cytometry in brain (**d**) and bone marrow (**e**) at study end. BU only n=4, Diet-1x-post n=3, Diet-1x pre + post n=4, Diet-2x-post n=2, gavage-post n=2. **f-h,** Analysis of donor cell engraftment in brain and bone marrow of adult C57BL/6 mice treated with BU and oral gavage of PLX pre-transplant. **f,** Experimental timeline. Follow up was 1.5 months after BMT. **g-h,** Donor chimerism (fraction of GFP+ CD45+CD11b+ cells) measured by flow cytometry in brain (**g**) and hematopoietic compartments [fraction of GFP+ CD45+ cells bone marrow (BM), peripheral blood (PB), and spleen (SP), **h**] at study end. BU n=4, BU + PLX pre-BMT n=5**. i-k,** Analysis of donor cell engraftment in brain and bone marrow of *Cx3cr1*-GFP$^{+/-}$ mice treated with BU and PLX either pre- or post-BMT. **i,** Experimental timeline. Follow up was 2 months after BMT. **j-k,** fraction of transplant-derived RFP+ and recipient *Cx3cr1*-GFP$^{+/-}$ cells measured in the brain (**j**) and bone marrow (**k**) by flow cytometry. BU n=4, BU + PLX n=4, BU + PLX pre-BMT n=3. **b, d-e, g-h, j-k,** Data are reported as Mean ± SD. Statistical analysis: **b, d-e, h, j-k,** one-way ANOVA with Tukey post-hoc; **g,** two-tailed unpaired t-test.



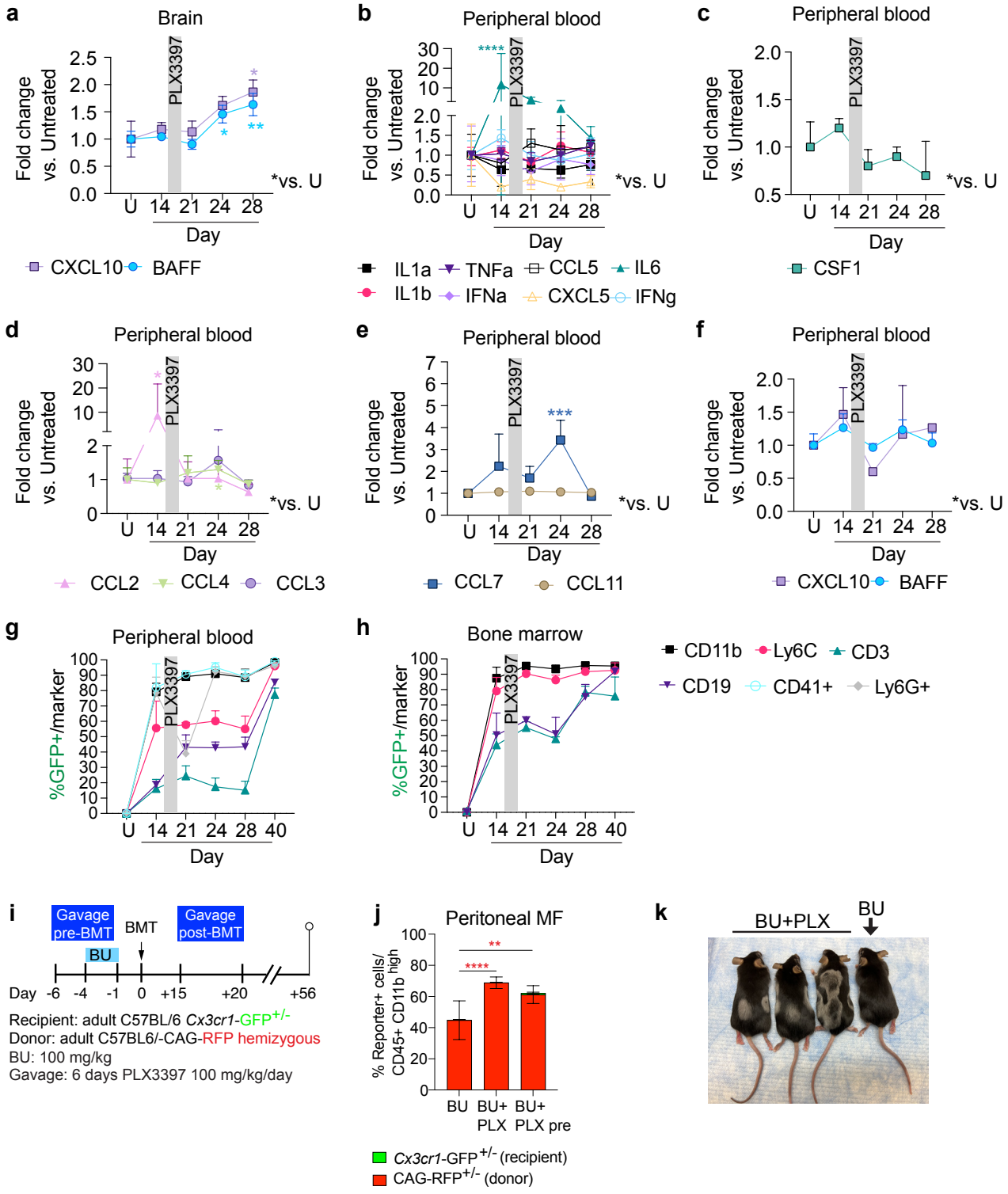
Extended data Figure 2. Bone-marrow-derived MGLCs efficiently replace microglia in spinal cord and retina in mice conditioned with BU + PLX.

Representative images of the spinal cord (**a-b**) and retina (**c-g**) of adult C57BL/6 mice conditioned with BU or BU + PLX, 7 months after total bone marrow transplant from C57BL/6-CAG-GFP mice and conditioned as depicted in **Fig. 1a;** age-matched, untreated mice were used as controls. **a,** Engraftment of transplant derived GFP+ cells in the spinal cords. Green signal is the natural GFP fluorescence of transplant-derived cells. **b**, Immunostaining of the spinal cord with the myeloid-microglia markers Iba1 showing its expression in donor-derived GFP+ MGCLs. **c-g,** Engraftment of transplant-derived GFP+ cells in the retina. **c-d**, In mice treated with BU alone the BM-derived GFP+ cells were mostly integrated in the ciliary margin, with very few cells integrated in the neuroretina. **c-f,** In BU + PLX -treated mice the GFP+ cells perfectly integrated in the neuroretina occupying the physiological microglia niches [inner and outer plexiform layer (IPL, OPL, respectively)], ciliary margin (CM) and, to a minor extent, ganglion cell layer (GCL) with no disruption of the photoreceptor outer nuclear layer (ONL)**;** OS: outer segments; IS: inner segments. **g,** Quantification of GFP+ cells/retinal section in BU- and BU + PLX -treated mice. Data are reported as Mean ± SD. Statistical analysis: two-tailed unpaired t test, p=0.0007.

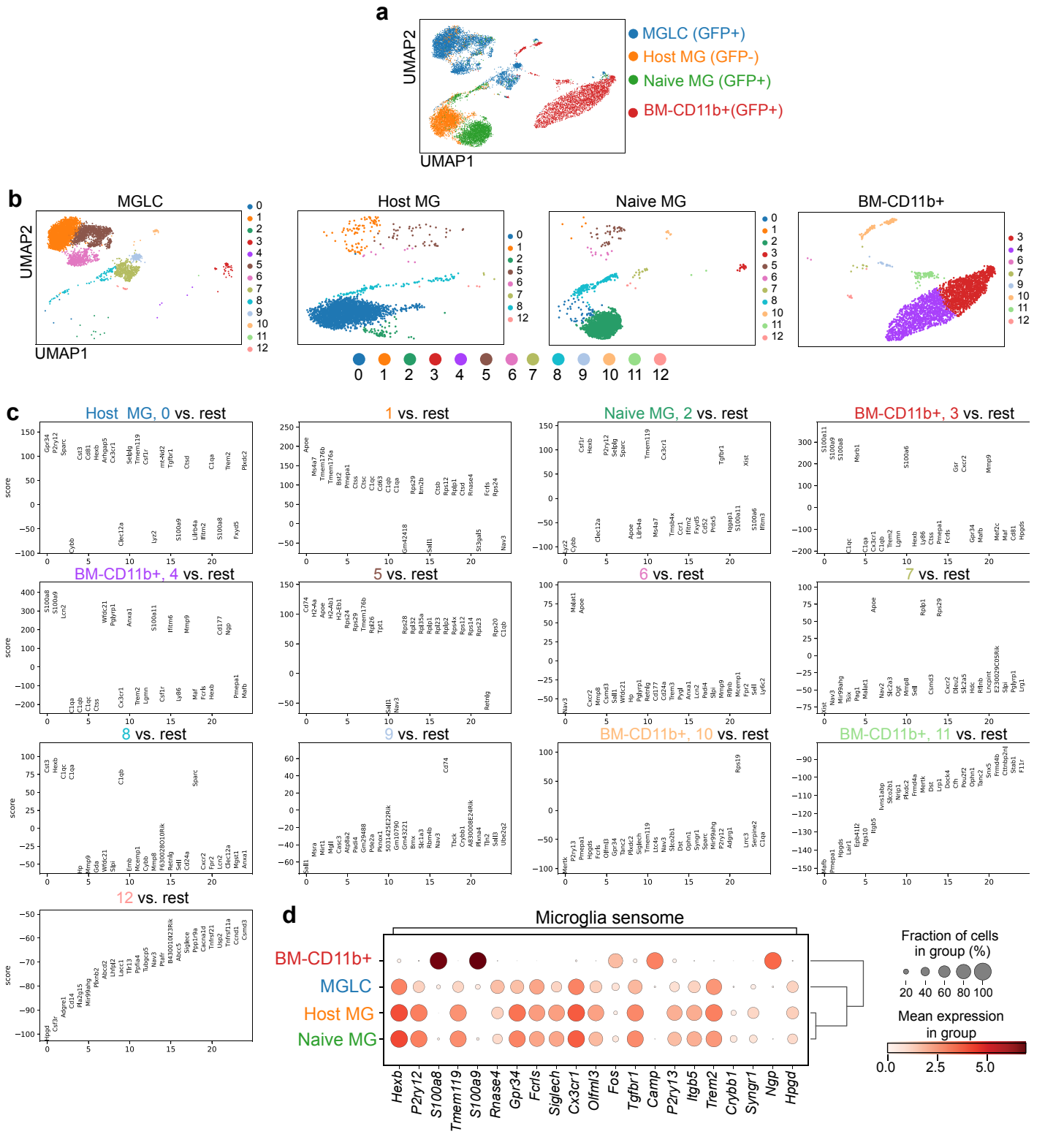


Extended data Figure 3. Kinetics of LKS HSPC engraftment using the BU+ PLX conditioning.

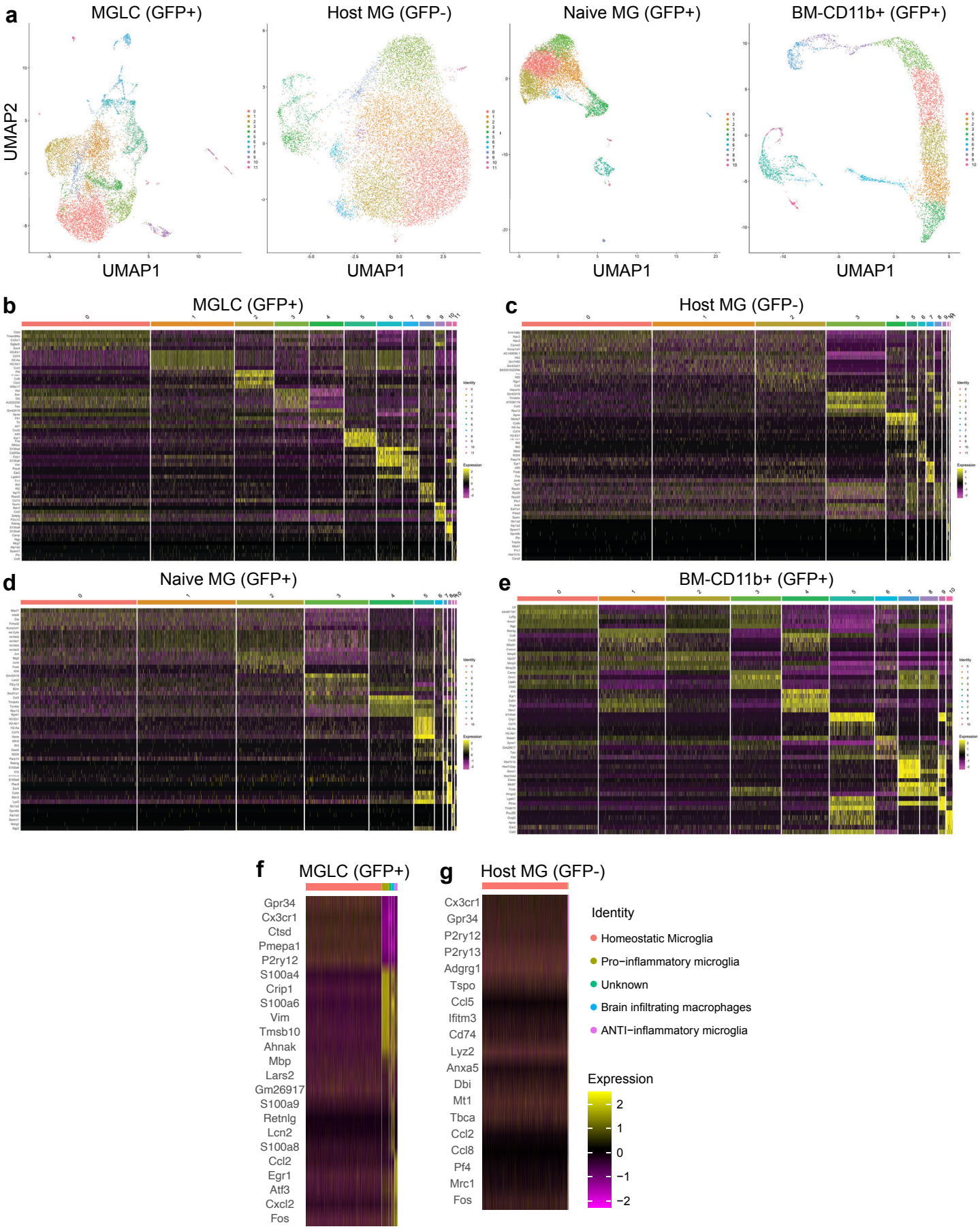
a, Experimental timeline showing conditioning and transplant of C57BL/6 mice with LKS HSPCs isolated from adult homozygous C57BL/6-CAG-GFP mice and expanded in culture for 14 days. Follow up was 40 days after transplant. Myeloablation was performed using either Total body irradiation (TBI, 10 Gy) or Busulfan (BU, 125 mg/kg, 25 mg/kg/day). **b,** Fraction of lineage negative, LKS HPSCs out of the total live cells quantified by flow cytometry using stemness markers KIT (CD117) and SCA-1. **c,** representative flow plot of LKS in culture at culture day 14. **d-f,** Engraftment of transplant-derived GFP+ cells in brain (**d**), hematopoietic compartments [bone marrow (BM), spleen (SP) and peripheral blood (PB)] (**e**) and peritoneum (**f**) at study end. **b, d-f,** Data are Mean ± SD. n=4 mice/cohort. **d-f,** TBI+PLX n=4, BU + PLX n=4, except in SP where BU + PLX n=3. Statistical analysis: **b, e,** one-way ANOVA Tukey post-hoc; **d, f,** two-tailed unpaired t test.



Extended data Figure 4. Kinetics of cytokine production and cell engraftment using the BU+ PLX conditioning. a-f, Cytokine analysis performed by 48-plex Luminex assay in brain (**a**) and peripheral blood (**b-f**) at the indicated time points. All cytokine quantifications and statistics are relative to untreated mice (U). **g-h,** Kinetics of hematopoietic lineage reconstitution in peripheral blood (**g**), bone marrow (**h**) of adult C57BL/6 mice before and after BU + PLX conditioning and up to 40 days post-transplant. The fraction of GFP+ myeloid cells (CD11b+, Ly6C+), platelets (CD41+), granulocytes (Ly6G+), T cells (CD3+) and B cells (CD19+) measured by flow cytometry are shown. **i-j,** Analysis of CD45+ CD11b^{high} macrophage (MF) engraftment in the peritoneum of *Cx3cr1*-GFP$^{+/-}$ mice treated with Busulfan and PLX either pre- or post-BMT. **i,** Experimental timeline. Follow up was 2 months after BMT. **j,** fraction of transplant-derived RFP+ and recipient *Cx3cr1*-GFP$^{+/-}$ MF measured by flow cytometry; BU n=4, BU + PLX n=4, BU + PLX pre-BMT n=3. **k,** Representative image showing the patches of hair discoloration observed in adult C57BL/6 mice conditioned with BU + PLX but not the ones conditioned with BU alone. Time point of the analyses is 7 months after transplant. The picture is representative of n=10/cohort. **a-j,** Data are Mean ± SD. Statistical analysis: **a-f,** one-way ANOVA vs. Untreated with Dunnett post-hoc; **g-h**, two-way ANOVA Tukey post-hoc; **j,** one-way ANOVA with Tukey post-hoc.



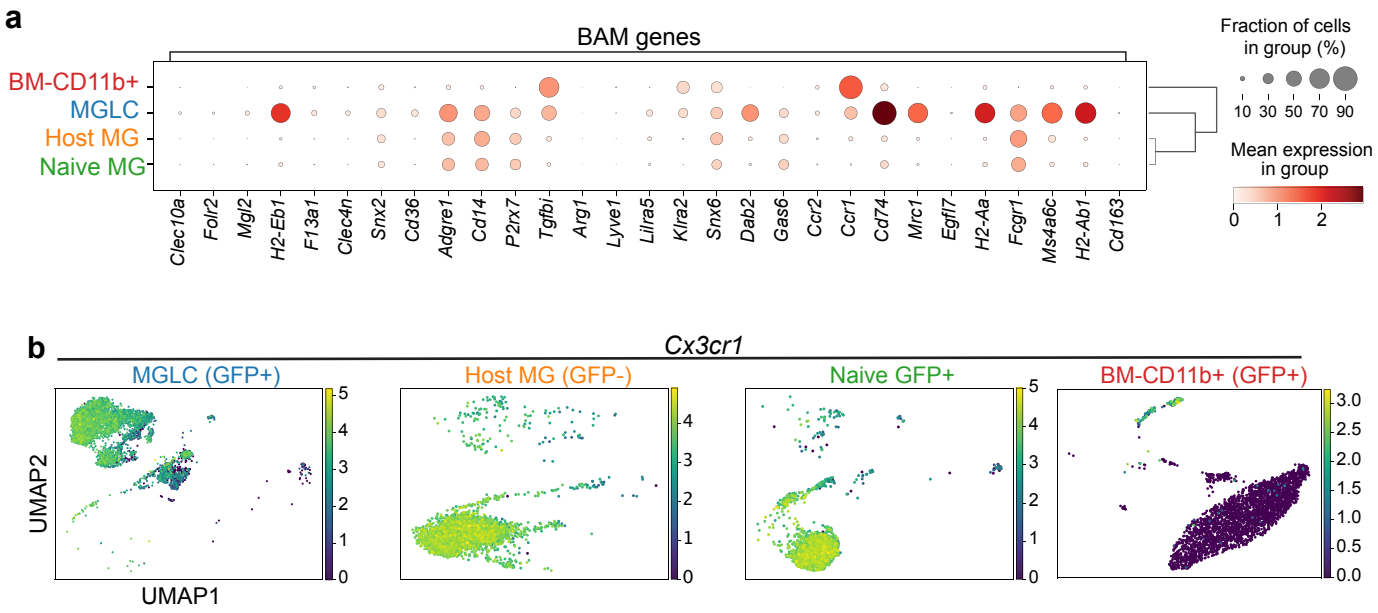
Extended Data Figure 5. Cluster analysis and top 25 differentially expressed genes in MGLCs, host MG, naïve MG and BM-CD11b+ cells combined in a single scRNA-seq dataset. a-d, Analyses of single-cell RNA sequencing (scRNA-seq) data performed on FACS-sorted CD45+CD11b+ cells isolated from mice that underwent BU + PLX conditioning and total bone marrow transplant (BMT) as depicted in **Fig. 1a and Fig. 3a**. The time point of the analyses is 4 months after BMT. **a-b,** UMAP of 1) GFP+ CD45+ CD11b+ (**MGLC**), 2) GFP- CD45+ CD11b+ (host conditioned MG or **host-MG**), 3) GFP+ CD45+ CD11b+ from BM (**BM-CD11b+),** and 4) GFP+ CD45+ CD11b+ MG from untreated age-matched donor mice (**naïve MG**) analyzed as bulk (**a**) or separated in 13 clusters based on differential gene expression (**b**). **c,** Top 25 differentially expressed genes per cluster. The cluster color matches the one depicted in panel b. The fraction of cells contained in each cluster is depicted in **Fig. 3d.** The score indicates the t-statistic. **d,** Dot plot showing the differential gene expression of "microglia sensome" signature genes[1] in MGLCs (GFP+), host-MG (GFP-), naïve MG (GFP+) and BM-CD11b+ (GFP+). The dot size represents the percentage of cells expressing the gene in each sample and the color represents the gene average expression as depicted in the legend (right panel).



Extended Data Figure 6

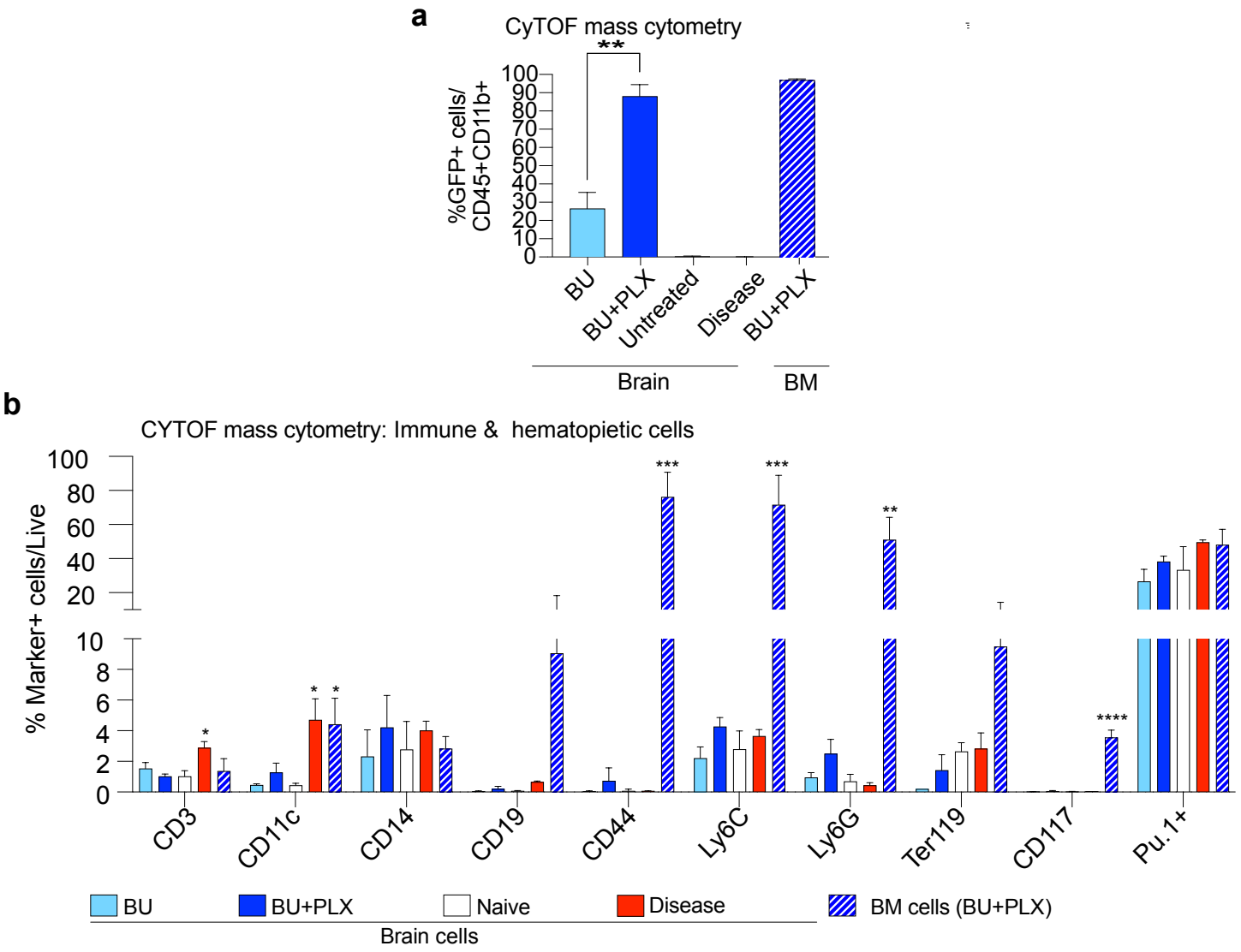
Extended data Figure 6. Single cell heterogeneity and cluster analysis of MGLCs, host MG, naïve MG and BM-CD11b+ cells analyzed as distinct scRNA-seq datasets.

a-f, Analyses of single-cell RNA sequencing (scRNA-seq) data performed of FACS-sorted CD45+CD11b+ cells isolated from mice that underwent BU + PLX conditioning and total bone marrow transplant (BMT) as depicted in **Fig. 1a and Fig. 3a**. The time point of the analyses is 4 months after BMT. **a,** UMAPs of 1) GFP+ CD45+ CD11b+ (**MGLC**), 2) GFP- CD45+ CD11b+ (host conditioned MG or **host-MG**), 3) GFP+ CD45+ CD11b+ from BM (**BM-CD11b+),** and 4) GFP+ CD45+ CD11b+ MG from untreated age-matched donor mice (**naïve MG**). The analyses were performed analyzing the 4 datasets separately. **a,** UMAPs showing the single sample clustering. **b,** Heatmap showing the top 5 differentially expressed genes per cluster for MGLCs (**b**), host-MG (**c**), naïve MG (d) and BM-CD11b+ (**e**). Some genes are included in the top set of multiple clusters. The differential expression range is from -2 to 2. Each column in the heat map is the expression of a single cell. The colored bar indicates the cluster of the cells underneath it. **f-g,** Heatmap showing the top 5 differentially expressed genes per each signature (homeostatic microglia, pro-inflammatory microglia, brain infiltrating macrophages, anti-inflammatory microglia, unknown) for MGLCs (**f**) and host-MG (**g**). Some genes are included in the top set of multiple cell types. The differential expression range is from -2 to 2. Each column in the heat map is the expression of a single cell. The colored bar indicates the cell type of the cells underneath it. Within the MGLC population 83% of cells showed a “homeostatic microglia” transcriptional signature. The 99% of cells in the host MG samples showed a “homeostatic microglia” transcriptional signature.



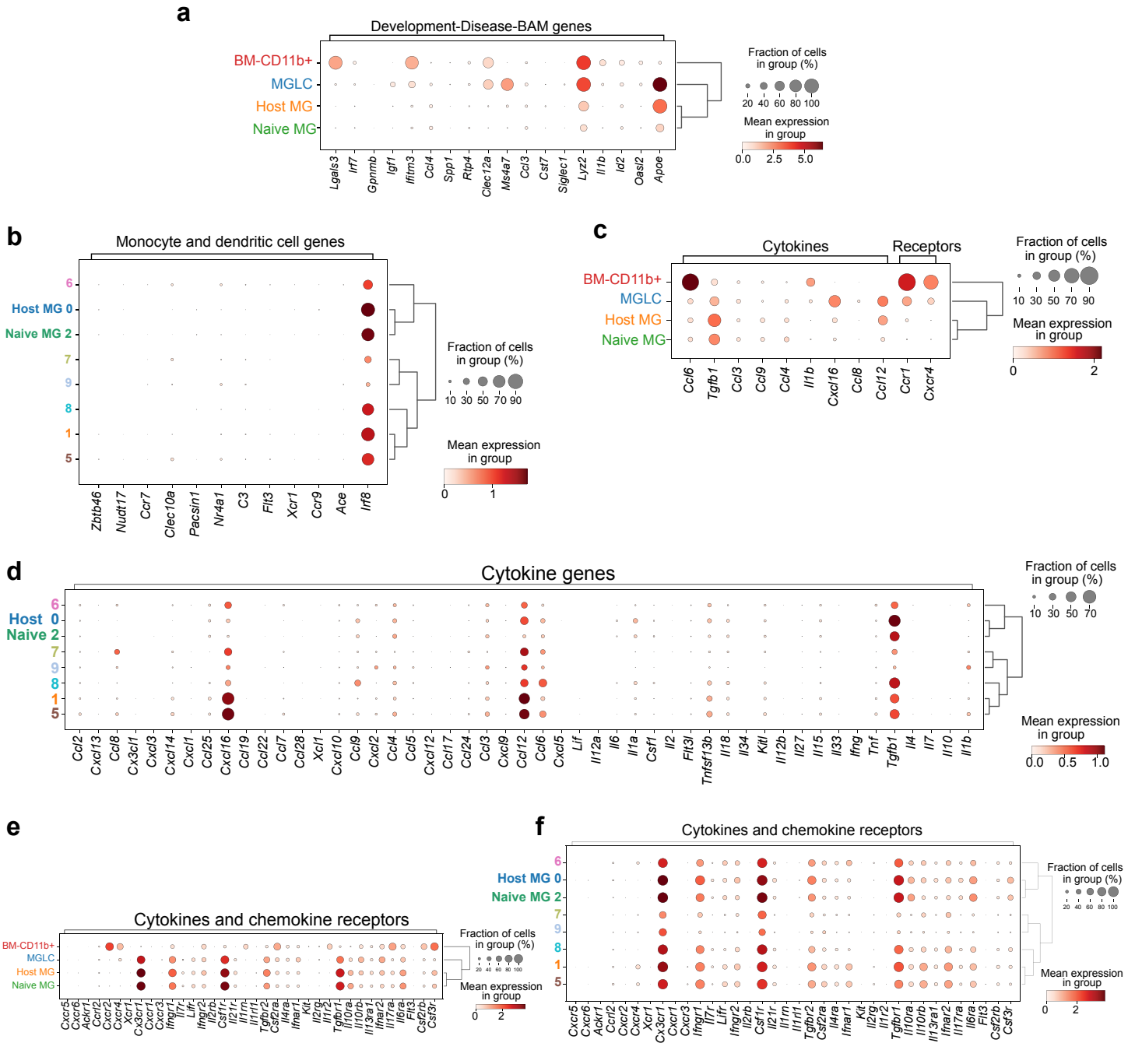
Extended Data Figure 7. MGLCs upregulate genes characteristic of microglia and brain border-associated macrophages (BAM).

a-b, Analyses of single-cell RNA sequencing (scRNA-seq) data performed on FACS-sorted CD45+CD11b+ cells isolated from mice that underwent BU + PLX conditioning and total bone marrow transplant (BMT) as depicted in **Fig. 1a and Fig. 3a**. The time point of the analyses is 4 months after BMT. We isolated and compared four samples: 1) GFP+ CD45+ CD11b+ (**MGLC**), 2) GFP- CD45+ CD11b+ (host conditioned MG or **host-MG**), 3) GFP+ CD45+ CD11b+ from BM (**BM-CD11b+),** and 4) GFP+ CD45+ CD11b+ MG from untreated age-matched donor mice (**naïve MG**). **a**, The dot plot shows the differential gene expression of brain border-associated macrophages (BAM) signature genes in MGLCs (GFP+), host-MG (GFP-), naïve MG (GFP+) and BM-CD11b+ (GFP+). The dot size represents the percentage of cells expressing the gene in each sample and the color represents the gene average expression as depicted in the legend (right panel). **b,** UMAPs showing the expression of the microglia/myeloid-specific *Cx3cr1* gene in MGLC (GFP+), host-MG (GFP-), naïve MG (GFP+) and BM-CD11b+ (GFP+) clusters.



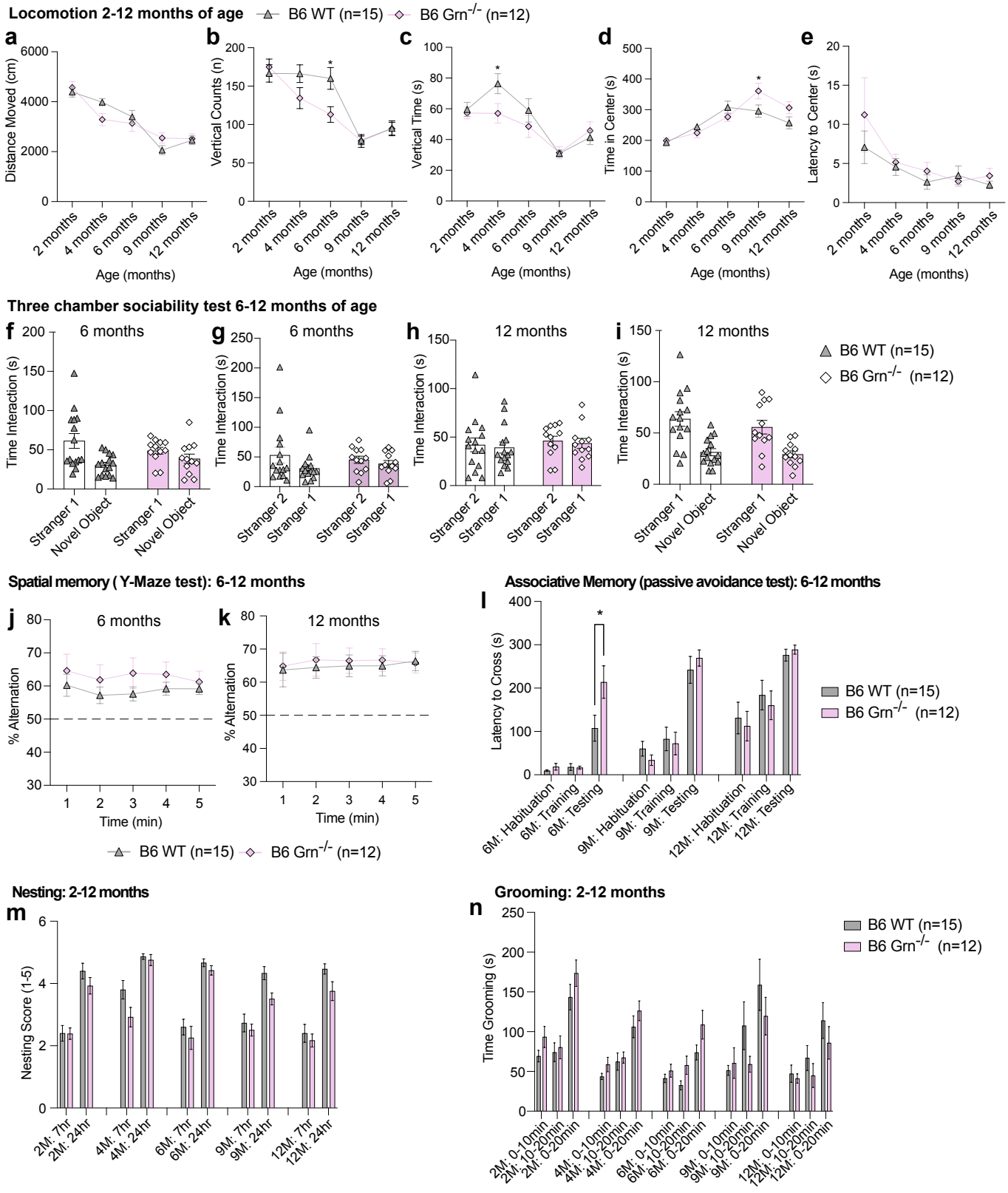
Extended Data Figure 8. Engraftment of GFP+ MGLCs and analyses of immune cell markers measured by High-dimensional CyTOF mass cytometry.

a-b, Analyses of marker expression by high-dimensional CyTOF mass cytometry in cells isolated from the brain or bone marrow of C57BL/6 mice conditioned with either BU (n=2) or BU + PLX, (n=2) as depicted in **Fig. 1a**. The time point of the analyses is 7 months after BMT. Cells isolated from untreated age-matched C57BL/6 mice (Naive, n=2) and GFP- microglia isolated from an age-matched mouse model of Mucopolysaccharidosis type 1 with abnormal microglia (Disease, n=2) were used as comparison (Disease, n=2). **a**, Percentage of transplant derived GFP+/CD45+CD11b+ as measured by anti-GFP intracellular staining by CYTOF. **b,** Percentage of marker positive cells out of the total live cells in each experimental cohort. The individual markers are depicted on the x axis. **a-b,** Data are reported as Mean ± SD. Statistical analyses:**a**, **b**, one-way ANOVA vs. WT untreated with Dunnet's post-hoc.



Extended Data Figure 9. Differential expression of development-disease-border-associated macrophage genes, monocyte and dendritic cell genes, cytokine, and cytokine receptors genes.

a-h, Analyses of single-cell RNA sequencing (scRNA-seq) data performed on FACS-sorted CD45+CD11b+ cells isolated from mice that underwent BU + PLX conditioning and total bone marrow transplant (BMT) as depicted in **Fig. 1a and Fig. 3a**. The time point of the analyses is 4 months after BMT. We isolated and compared four samples: 1) GFP+ CD45+ CD11b+ (**MGLC**), 2) GFP- CD45+ CD11b+ (host conditioned MG or **host-MG**), 3) GFP+ CD45+ CD11b+ from BM (**BM-CD11b+),** and 4) GFP+ CD45+ CD11b+ MG from untreated age-matched donor mice (**naïve MG**). **a, c, e,** Dot plot showing the differential gene expression of signature genes in MGLCs (GFP+), host-MG (GFP-), naïve MG (GFP+) and BM-CD11b+ (GFP+). The dot size represents the percentage of cells expressing the gene in each sample and the color represents the gene average expression as depicted in the legend (right panel). **b, d, f,** Dot plots showing the differential gene expression in MGLCs (GFP+), host-MG (GFP-), naïve MG (GFP+) and BM-CD11b+ (GFP+) in the different clusters (depicted in **Fig. 3 c-d)**. The dot size represents the percentage of cells expressing the gene in each sample and the color represents the gene average expression as depicted in the legend (right panel).



Extended Data Figure 10. Serial Neurobehavioral phenotyping of Grn$^{-/-}$ mice.
a-n, Neurobehavioral analysis of wild-type (n=15) and *Grn*$^{-/-}$ (n=12) mice from 2 to 12 months of age. **a-e**, Analyses of mouse locomotion and exploratory behavior at 2, 4, 6, 9 and 12 months of age. **f-i**, Analyses of mouse sociability evaluated using the 3-chamber sociability test at 6 and 12 months of age. **j-k**, Analyses of mouse spatial memory using the Y-maze spontaneous alternation test at 6 and 12 months of age. **l,** Analyses of mouse associative memory using the passive avoidance test, at 6, 9 and 12 months of age. **m,** Analyses of mouse cognitive deficit evaluated by their nesting ability at 2, 4, 6, 9 and 12 months of age. **n,** Analyses of mouse obsessive-compulsive behavior, reflected by their grooming activity at 2, 4, 6, 9 and 12 months of age. All data are reported as Mean ± SD. Statistical analyses: **a-n**, two-way ANOVA with Sidak post-hoc.