Cell Reports, Volume *43*

Supplemental information

Granulocyte colony stimulating factor promotes scarless tissue regeneration

Jianhe Huang, Satish Sati, Christina Murphy, Casey A. Spencer, Emmanuel Rapp, Stephen M. Prouty, Scott Korte, Olivia Ahart, Emily Sheng, Parker Jones, Anna E. Kersh, Denis Leung, and Thomas H. Leung

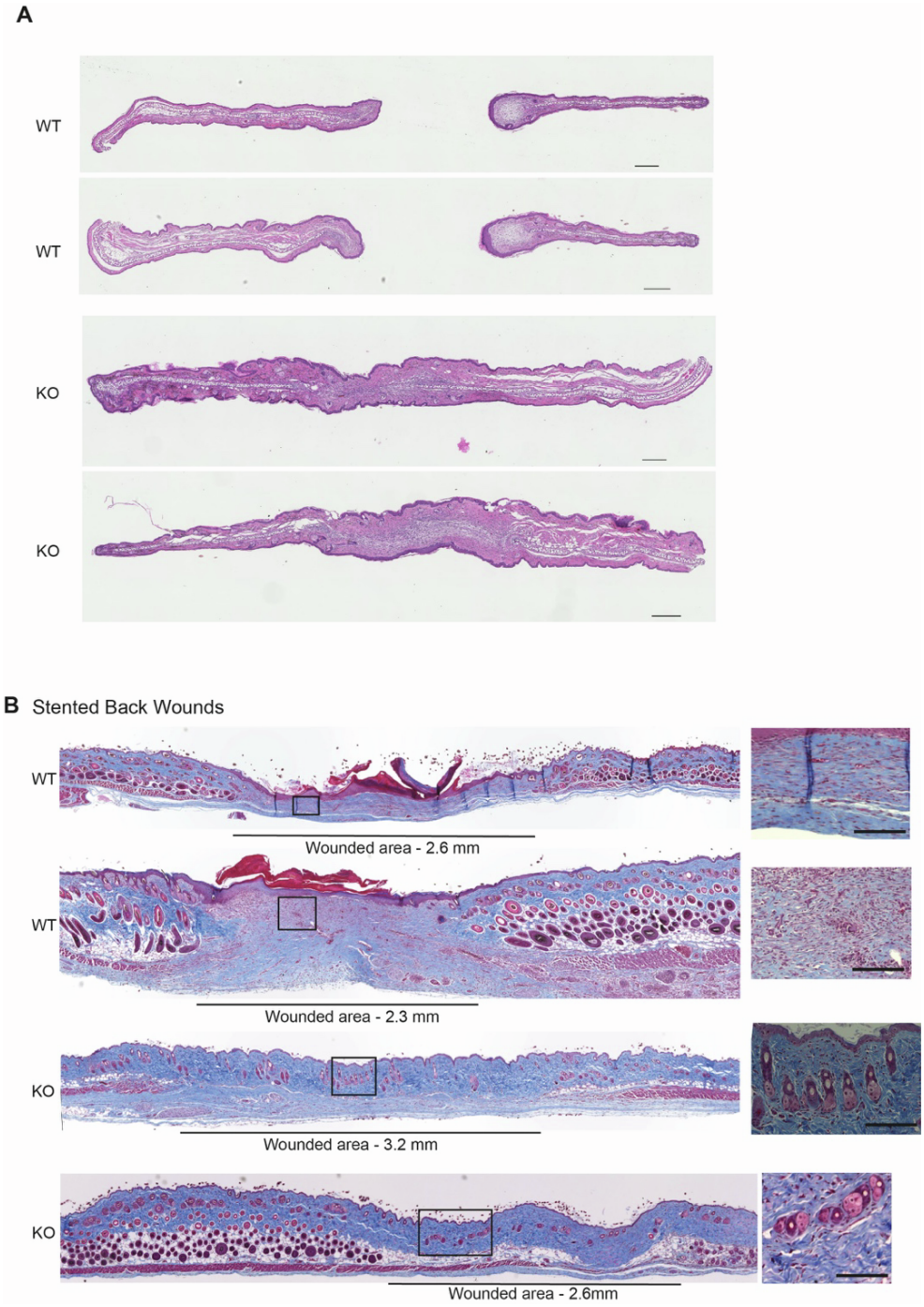

Figure S1. CXCR2-deficient mice exhibit improved wound healing, Related to Figure 1. (A) Hematoxylin & Eosin stained tissue sections from WT and CXCR2-KO ear hole wounds. Scale bars, 250μM. (B) Trichrome stained tissue sections from WT and CXCR2-KO back wounds. Injured area indicated by black line. Higher magnification images from boxed areas. Scale bars, 100μM.

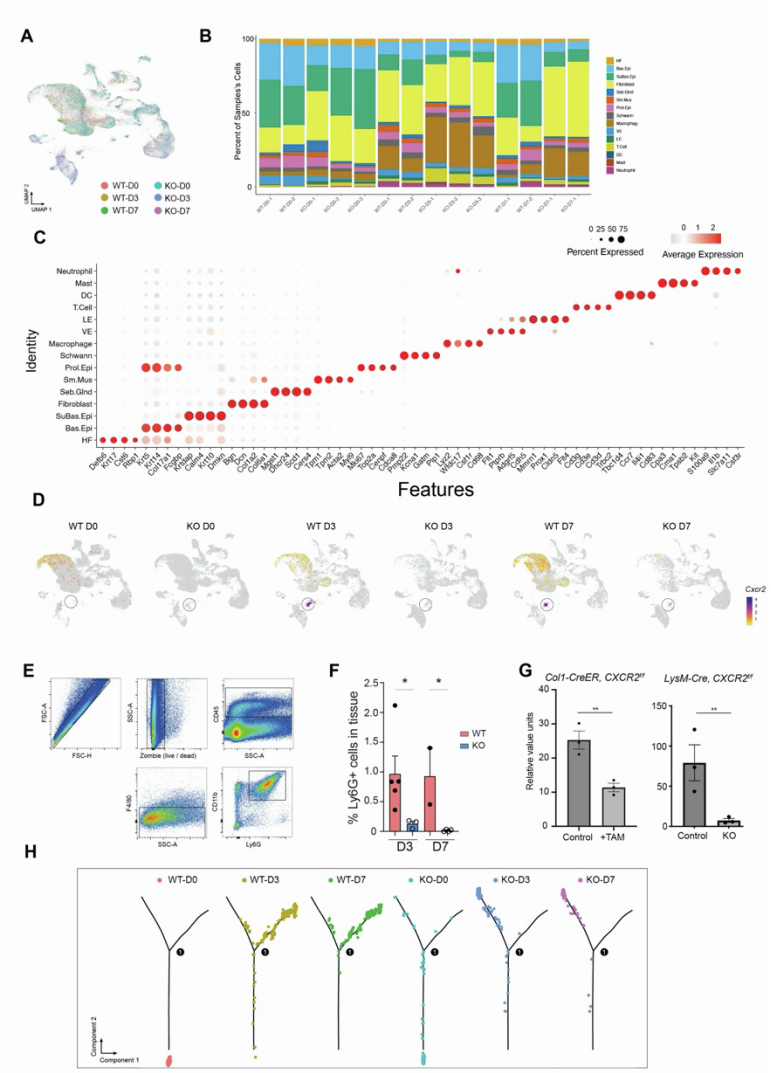

Figure S2. CXCR2+ neutrophils regulate speed of wound closure, Related to Figure 3. (**A**) UMAP plot of single-cell RNA-sequencing data of wounded skin of wild-type (WT) and CXCR2-KO mice at different time points. (**B**) Bar plot showing relative contribution of different cell types in each single-cell RNA-sequencing skin sample. VE, Vascular endothelium; LE, lymphatic endothelium; DC, Dendritic Cell; Bas.Epi, Basal Epithelial; SuBas.Epi, Suprabasal Basal Epithelial; Prol.Epi, Proliferative Epithelial; HF, Hair follicle; Sm.Mus, Smooth muscle cell; Seb.Glnd, Sebaceous gland. (**C**) Dot plot of key marker genes for each cell type. Color scale represents gene expression, and dot size represents percentage of cells expressing the marker gene. (**D**) UMAP plots depicting normalized expression of *Cxcr2* transcript in WT and CXCR2-KO wounded skin at different time points. Circle highlights neutrophils. (**E**) Flow cytometry gating strategy for mouse neutrophils. (**F**) Quantification of immunofluorescence for neutrophils (Ly6G+) in WT and CXCR2-KO wound-edge skin at day 3 (*n=5* and *3* for WT and KO respectively) and day 7 (*n=2* and *4* for WT and KO respectively) after injury. Unpaired two-tailed Student's *t*-test. *<P<0.05. (**G**) CXCR2 transcript levels in control and cell-type specific CXCR2-KO mice. n=3. Unpaired two-tailed Student's t-test. **P<0.01. (**H**) Pseudotime trajectory analysis of neutrophils from WT and CXCR2-KO mice. Each dot represents a cell. Data reported as mean ± SEM.

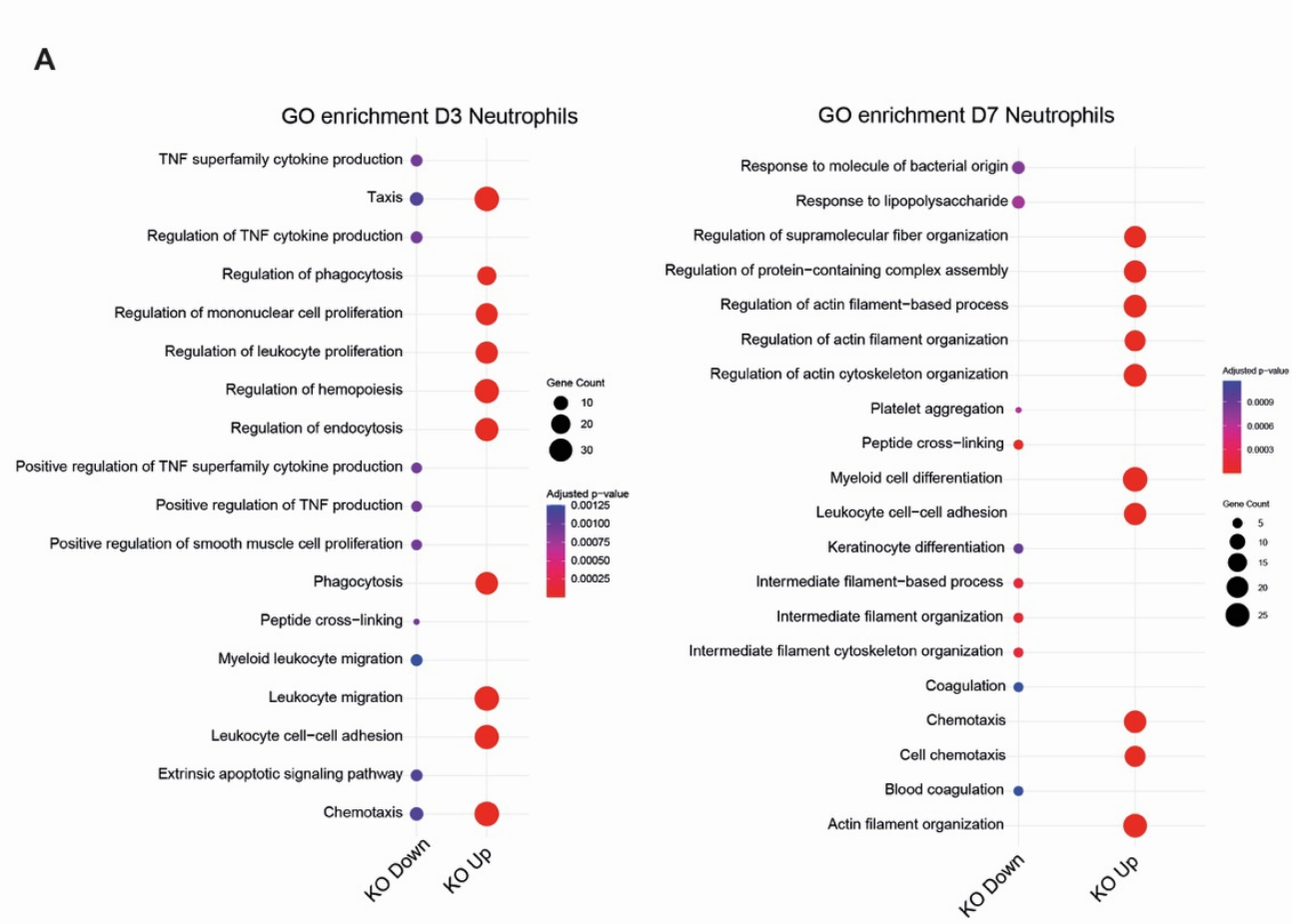

Figure S3. Gene Ontology analysis of neutrophils, Related to Figure 3. (**A**) Gene set enrichment analysis of D3 and D7 post injury neutrophils (CXCR2-KO vs WT). Dot plot of enriched pathways determined from GSEA results. Each dot demonstrates enriched pathway. The size of the dot represents gene count and the color represents the adjusted p-value.

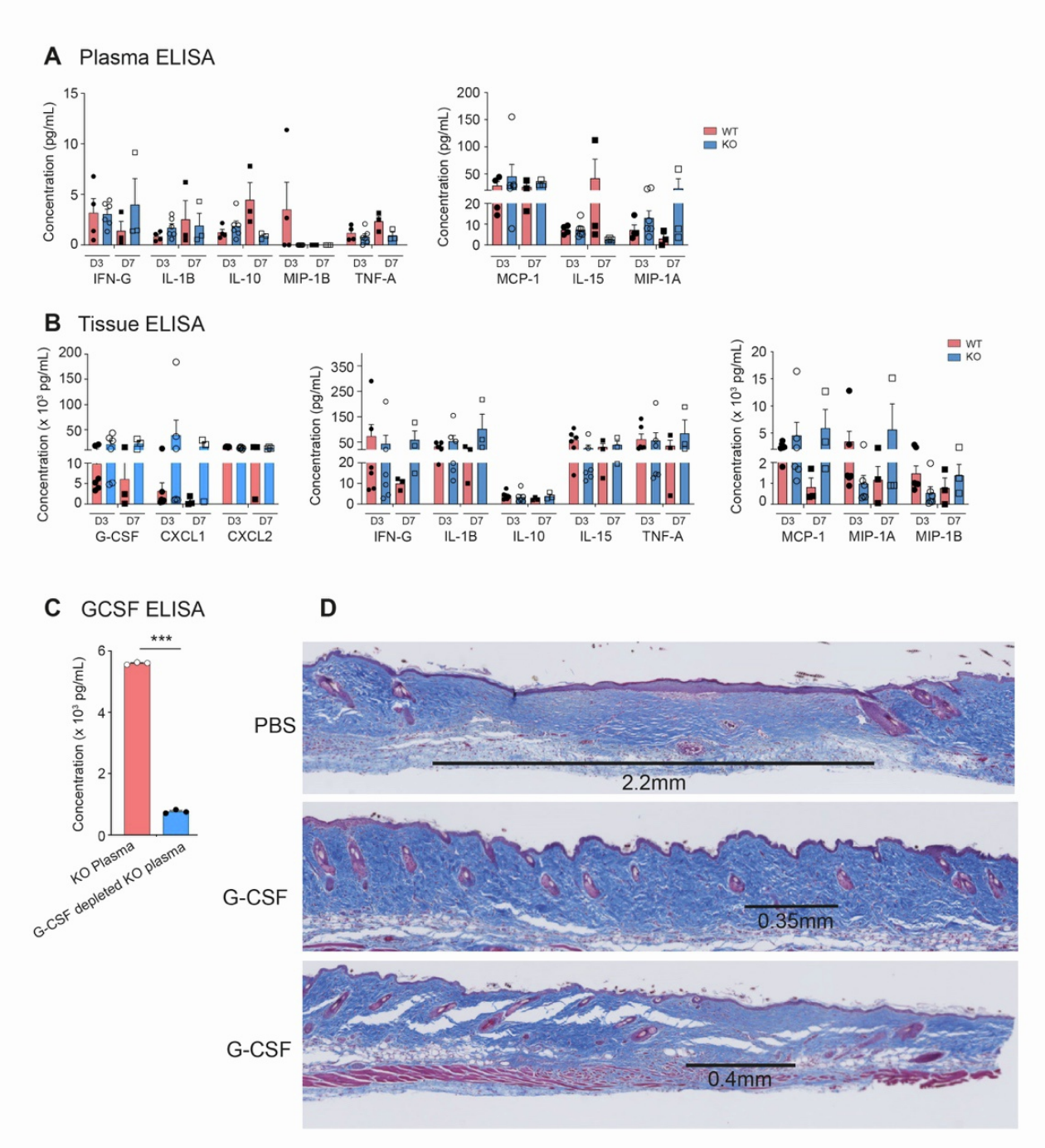

Figure S4. G-CSF is sufficient to reduce scarring and to promote tissue regeneration, Related to Figure 4. (**A**) ELISA comparing cytokine expression in injured WT (n=4 for day 3; n=3 for day 7) and CXCR2-KO (n=6 for day 3; n=3 for day 7) plasma. (**B**) ELISA comparing cytokine expression in injured WT (n=4 for day 3; n=3 for day 7) and CXCR2-KO (n=6 for day 3; n=3 for day 7) tissue. (**C**) Bar plot of G-CSF concentration in CXCR2-KO plasma (n=3) and G-CSF depleted CXCR2-KO plasma (n=3). Unpaired two-tailed Student's t-test. ***P<0.001. (**D**) Additional histological photographs of scar size of G-CSF or PBS-treated stented back wounds on WT mice at day 28 after injury. Scar size indicated on the panel.

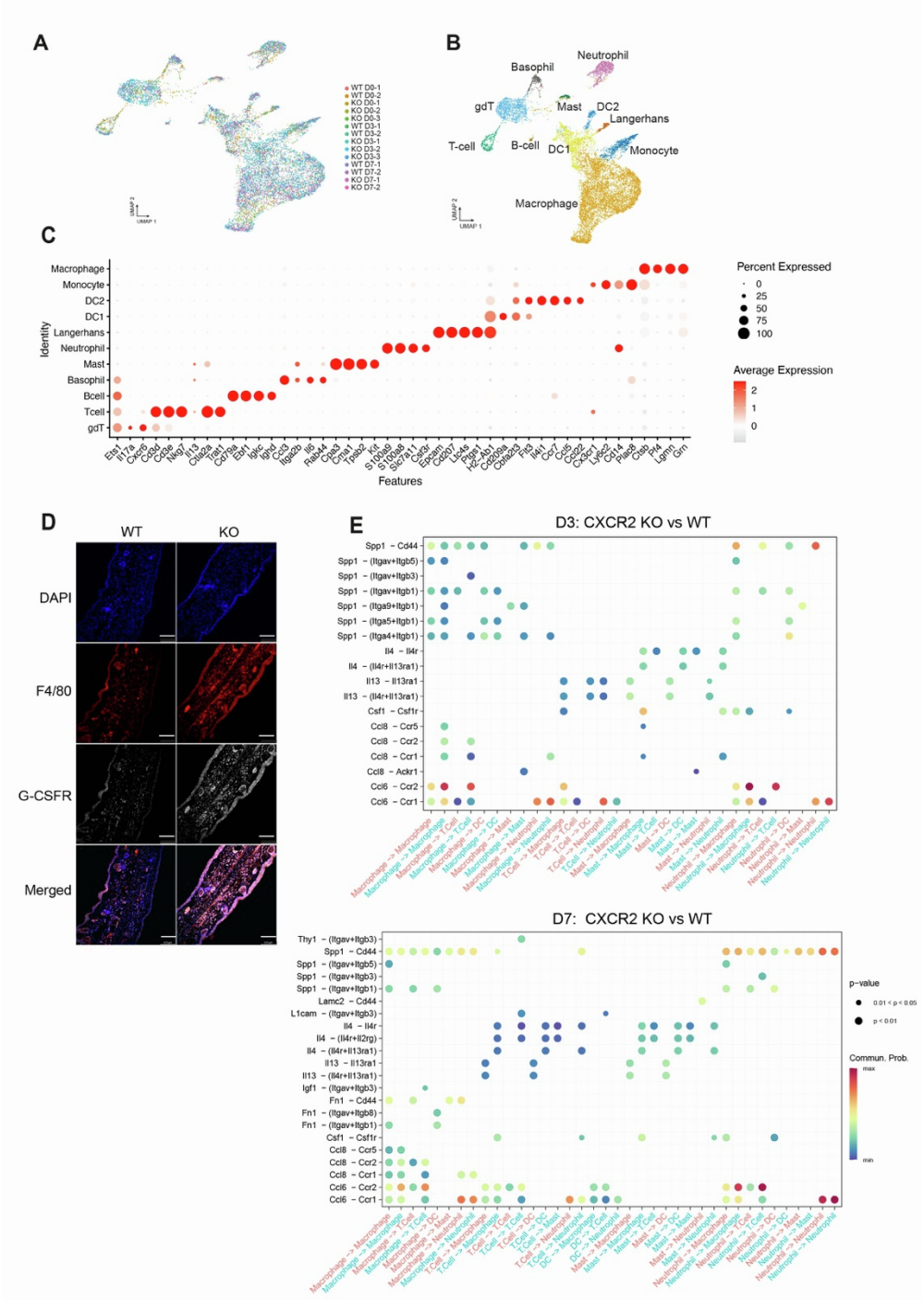

Figure S5. Altered cell-cell communication among immune cells in CXCR2-KO mice, Related to Figure 5. (**A**) UMAP plot of subclustered immune cells of wounded skin of wild-type (WT) and CXCR2-KO mice at different time points. (**B**) UMAP depicting sub-clustering of immune cells. (**C**) Dot plot of key marker genes for each cell type. Color scale represents gene expression, and dot size represents percentage of cells expressing the marker gene. (**D**) Representative images of WT and CXCR2-KO wounded skin immunostaining for F4/80 and G-CSFR (n=4). Scale bars, 100μM. (**E**) Analysis of cell-to-cell interactions between immune cells in the ear skin of WT (salmon color) and CXCR2-KO (blue color) mice.

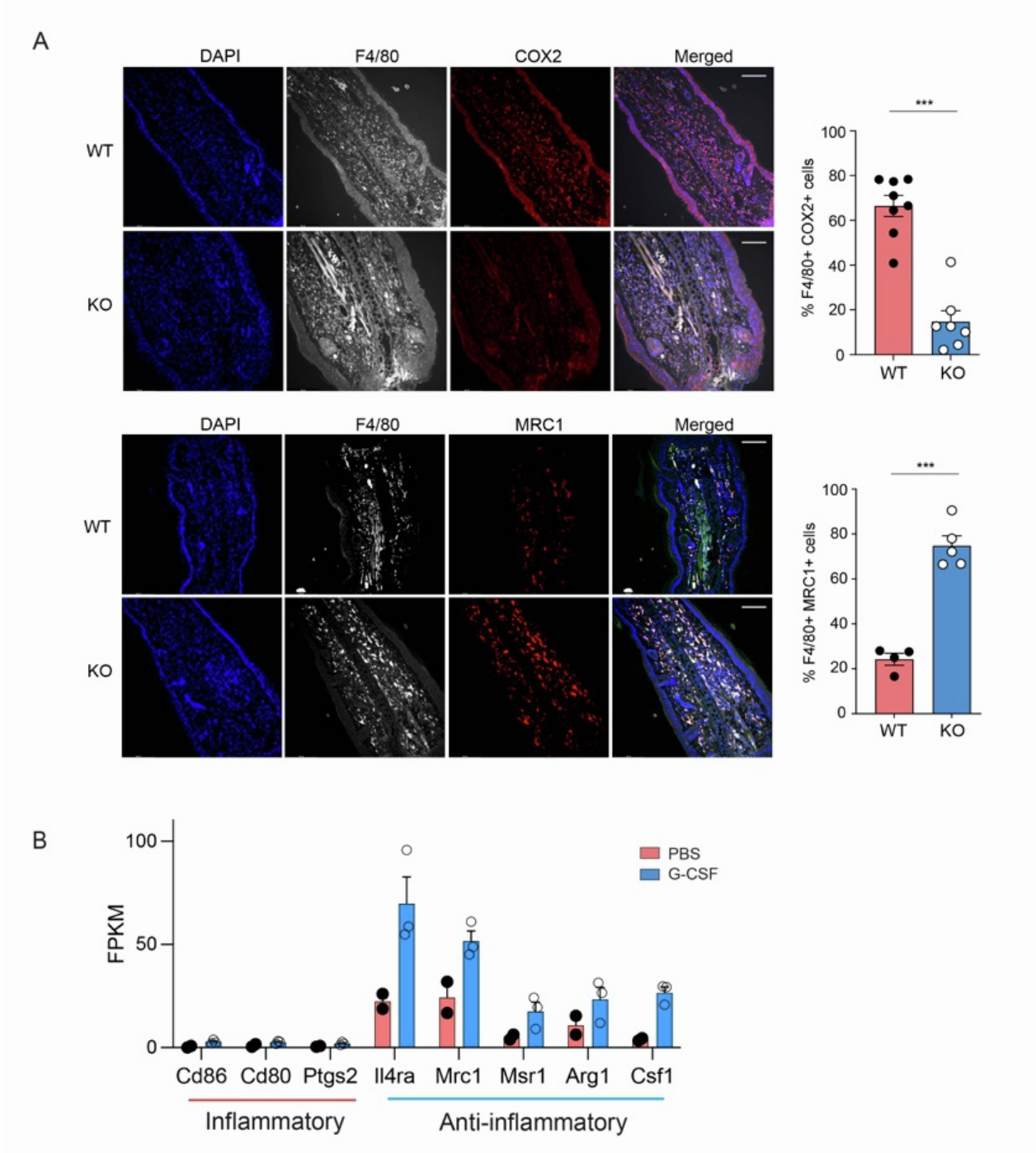

Figure S6. Increased anti-inflammatory macrophages in injured CXCR2-KO mouse skin, Related to Figure 5. (**A**) Representative images and quantification of immunofluorescence of WT and CXCR2-KO wounded ear tissue co-stained for F4/80+ and COX2+ (*n=8* for WT and *n=7* for KO) and F4/80+ and MRC1+ (*n=4* for WT and *n=5* for KO). Cell percentages are calculated with total F4/80+ cells as the denominator. Scale bars, 100μM. Unpaired two-tailed Student's *t*-test. ***P<0.01. Mean ± SEM are plotted. (**B**) Gene expression levels (FPKM) for monocytes treated with and without G-CSF (*n*=2 for PBS and *n*=3 for G-CSF).