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Supplemental Information

GM-CSF Calibrates Macrophage Defense and Wound Healing Programs during Intestinal Infection and Inflammation

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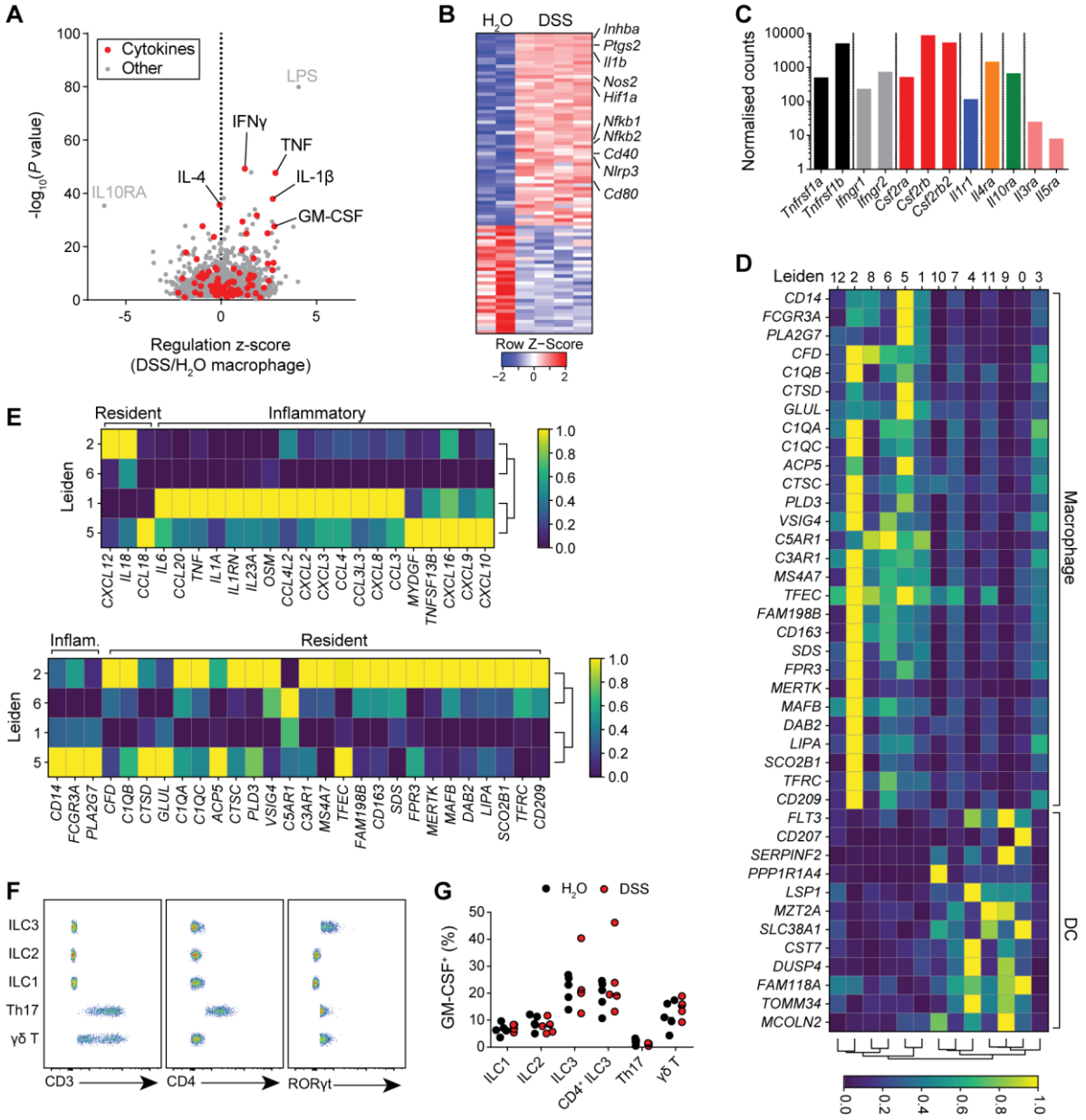

Figure S1. GM-CSF is an upstream regulator of inflammatory macrophage function in the intestine. Related to Figure 1. (**A**) Plot of z-score and *P* value for predicted regulators of DSS vs H_2O macrophage activation, determined by IPA. Data derived from GEO: GSE109040. (**B**) Heatmap of predicted GM-CSF regulated genes in DSS macrophages from dataset in **A**. (**C**) Normalised expression counts of candidate cytokine receptor genes in colonic macrophages from **A**. (**D, E**) Heatmaps of marker genes and cytokines distinguishing (**D**) macrophages from dendritic cells and (**E**) resident from inflammatory macrophages amongst all MNPs in human mucosal biopsies from patients with ileal Crohn's disease, determined by scRNA-seq. Data derived from GEO: GSE134809. (**F**) Surface marker and RORγt expression by colonic ILC and T cell subsets. (**G**) GM-CSF$^+$ cells as a proportion of each immune cell subset from mice treated with H_2O or 2 % DSS for 6 days. $N = 5$ per group. Medians indicated. Data (**F, G**) representative of two independent experiments. *P* values were calculated in IPA software (**A**).

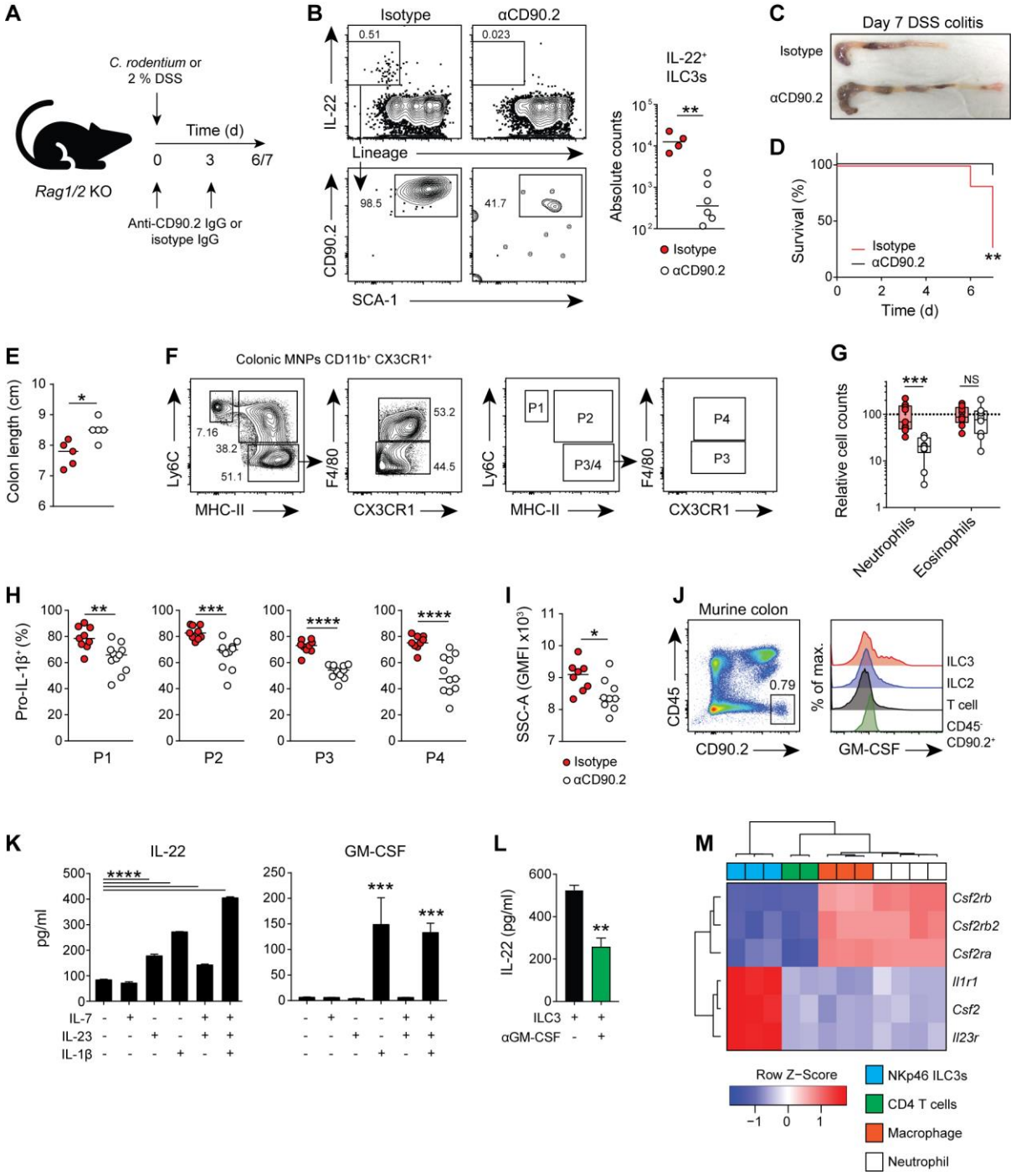

Figure S2. ILCs and innate GM-CSF augment intestinal macrophage activation during enteropathic infection and colitis. Related to Figure 2. (**A**) Schematic of ILC depletion strategy. (**B**) Colonic lineage$^-$ CD90.2$^+$ SCA-1$^+$ IL-22$^+$ ILC3 quantification in 2 % DSS-treated *Rag2*$^{-/-}$ mice, as in **A** (n = 4-6 per group). Medians indicated. (**C**) Colon length in *Rag2*$^{-/-}$ mice treated as in **A** at day 7 after 2 % DSS administration. (**D**) Survival of mice treated as in **C** (exceeding 20 % weight loss). N = 10-11 per group. (**E**) Colon length in mice treated as in **A**, infected with *C. rodentium* at 7 dpi. N = 5 per group. Medians indicated. (**F**) Representative MNP waterfall gating strategy in murine colon. (**G**) Colonic neutrophil and eosinophil relative cell count in *Rag2*$^{-/-}$ mice treated with 2 % DSS ± isotype or anti-CD90.2 IgG for 6 days. N = 9-11 per group. Medians indicated. (**H**) Pro-IL-1β expression by colonic MNP subsets (P1-P4) in mice treated as in **B**. Medians indicated. (**I**) SSC of colonic Ly6C^{lo} MHC-II$^+$ MNPs from mice treated as in **A** with *C. rodentium*. Medians indicated. (**J**) GM-CSF expression by non-haematopoietic CD90.2$^+$ CD45.2$^-$ cells in murine colon. Mice treated as in **A**. (**K**) IL-22 and GM-CSF production by flow-sorted intestinal ILC3s cultured with indicated cytokines for 16h. Mean ± SEM indicated. (**L**) IL-22 production in ILC3-BMDM co-cultures ± isotype or anti-GM-CSF IgG after 16h. Mean ± SEM indicated. (**M**) Cytokine and receptor expression by intestinal NKp46$^+$ ILC3s, lymph node CD4$^+$

T cells, lung macrophages and blood neutrophils from the Immgen Consortium Phase 2 (GEO: GSE37448). Data are representative of three independent experiments (**B-I, K, L**), with single (**B, C, E-G, I, K, L**) or two pooled experiments (**D, G, H**) shown. *P* values were calculated using Mann-Whitney U test (**B, E, G-I**), Gehan-Breslow-Wilcoxon test (**D**), a one-way ANOVA with Tukey's multiple comparisons test (**K**), and Student's *t* test (**L**). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

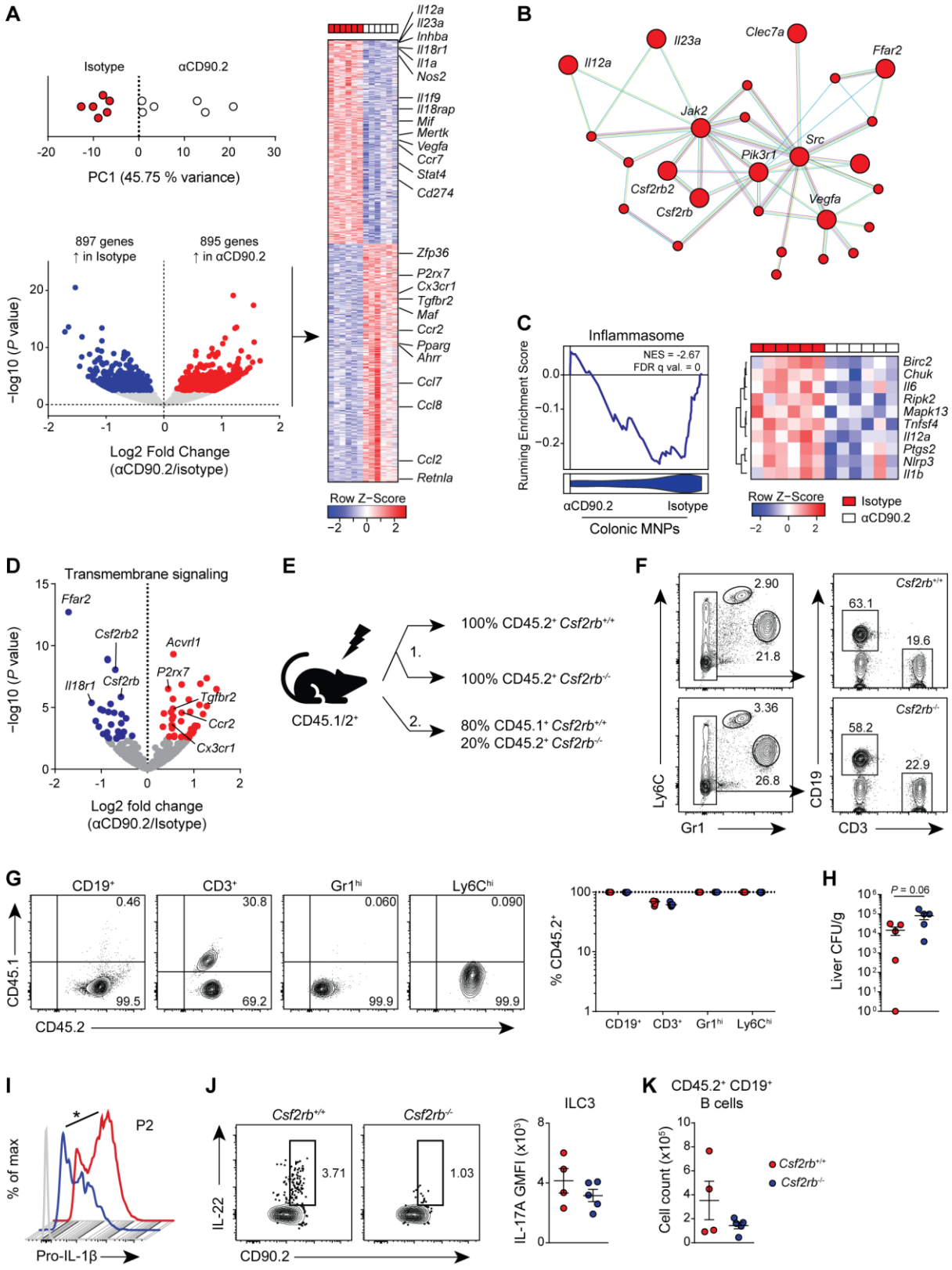

Figure S3. GM-CSF drives inflammatory macrophage function and type 17 immunity *in vivo*. Related to Figure 3. (**A**) Principal component 1 of RNAseq dataset of colonic Ly6C^{lo} MHC-II^{+} macrophages from *Rag2*$^{-/-}$ mice treated with 2 % DSS ± isotype or anti-CD90.2 IgG antibodies (top), and volcano plot and heatmap of all DEGs (adj. *P* val. < 0.05). Selected genes of interest are highlighted. *N* = 6 per group. (**B**) STRING analysis of gene networks (highest confidence = 0.900; no interactors) among top 200 down-regulated differentially expressed genes (DEGs = adj. *P* value < 0.05) from colonic macrophages in **A**. (**C**) RNAseq gene set enrichment analysis (GSEA) of inflammasome genes in colonic Ly6C^{lo} MHC-II^{+} macrophages in mice treated

as in **c**. *N* = 6 per group. Top 10 leading edge genes shown in heatmap. (**D**) Volcano plot of transmembrane signalling genes (GO:0004888) in colonic macrophages from **A**. (**E**) Bone marrow chimera generation strategy. (**F, G**) Blood reconstitution analysis in 100 % bone marrow chimeric mice at 8 weeks post-irradiation. *N* = 5 per group. Medians indicated. (**H**) Liver CFU in 100 % bone marrow chimeras at 10 dpi. *N* = 5 per group. Mean ± SEM indicated. (**I**) Colonic P2 MNP pro-IL-1β expression in 100 % chimeric mice shown in **F**. Mean ± SEM indicated. (**J**) IL-22^{+} gating and IL-17A quantification of donor colonic CD3ϵ^{-} CD4^{-} ILCs in 100 % chimeric mice at 10 dpi. Mean ± SEM indicated. (**K**) Colonic donor CD19^{+} B cell quantification from mice in **J**. Data representative of two independent experiments. *P* values were calculated using the standard DESeq 2 method with multiple comparisons correction using the BH procedure (**A, D**), Student's t test (**H**), and nonparametric Mann-Whitney U test (**I**). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

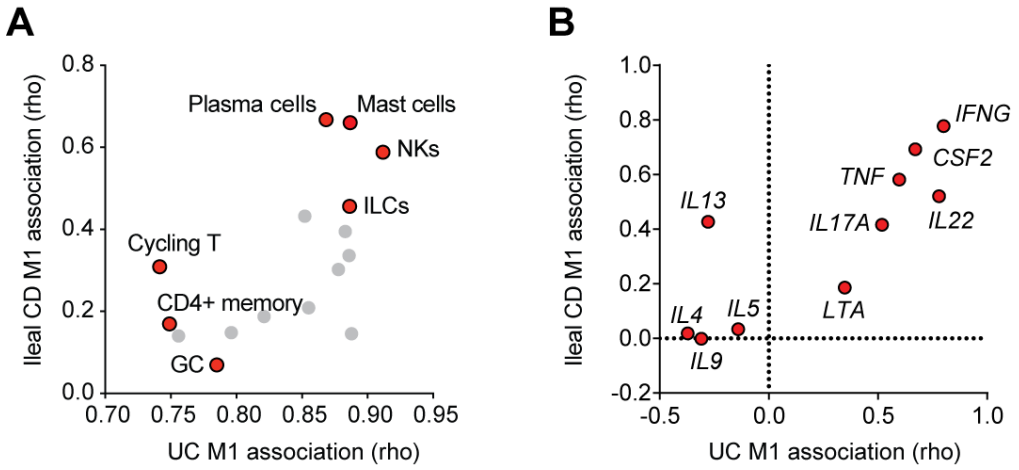

Figure S4. ILCs and GM-CSF regulate intestinal macrophage polarisation. Related to Figure 4. (**A, B**) Immune cell signature (**A**) and cytokine expression (**B**) rho values for correlation with sLPS M1 signature in transcriptomics datasets of UC (GEO: GSE59071) and ileal CD (GEO: GSE93624) mucosal biopsies.

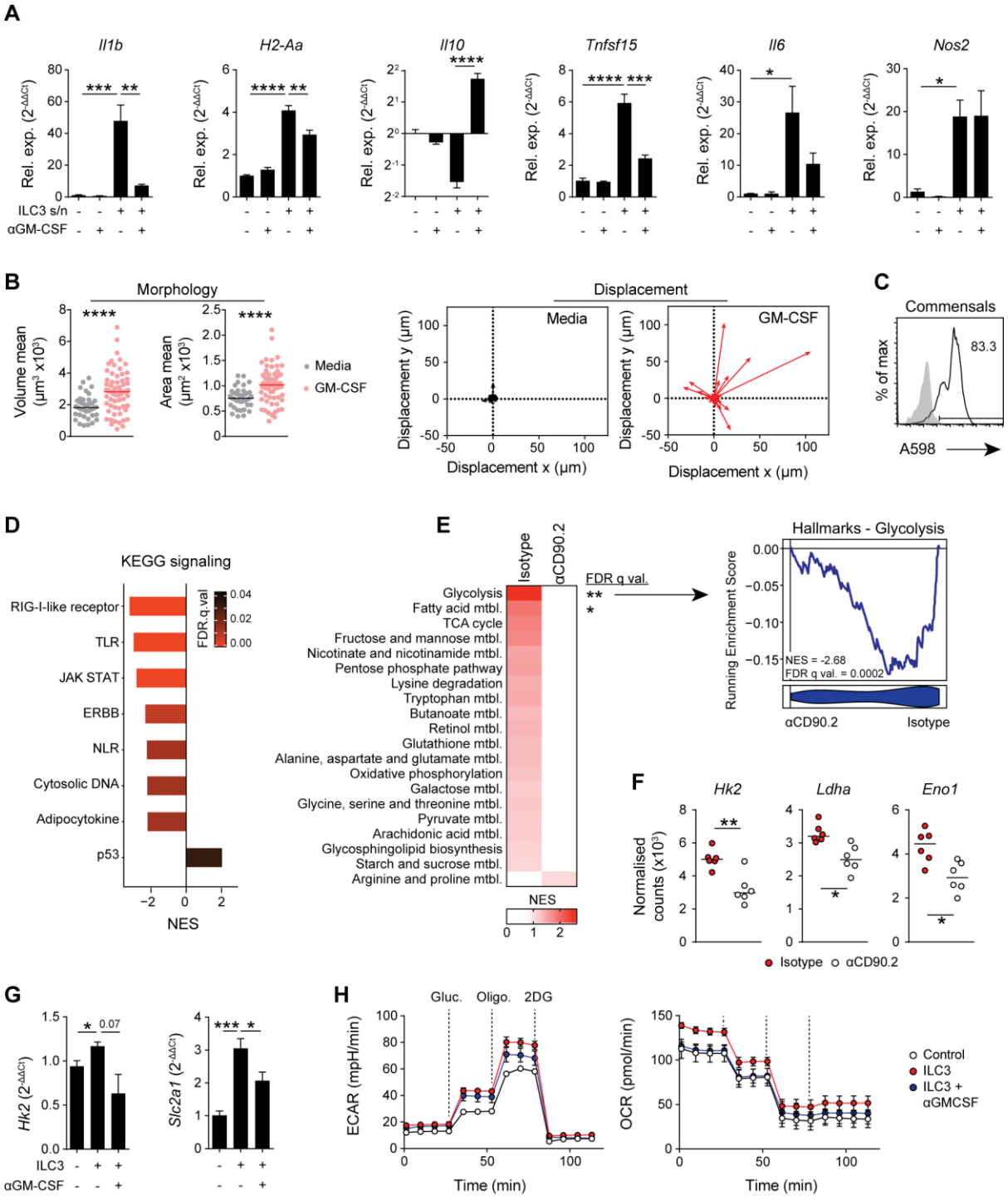

Figure S5. ILC3-derived GM-CSF regulates MNP microbicidal activity and metabolism. Related to Figure 5. (**A**) Cytokine, MHC-II, and NOS2 expression in BMDMs treated with ILC3-conditioned media ± anti-GM-CSF IgG for 16 h. Mean ± SEM indicated. (**B**) Morphology and displacement analysis of control or GM-CSF-primed BMDMs. Medians are indicated. (**C**) Fluorescent labelling of murine intestinal commensals. (**D**) KEGG signalling pathways in colonic macrophages ± anti-CD90.2 IgG administration after 6 days of DSS by RNAseq. (**E**) RNAseq GSEA of Kyoto Encyclopaedia of Genes and Genomes (KEGG) metabolic pathways in colonic Ly6C^{lo} MHC-II^{+} macrophages from *Rag2*$^{-/-}$ mice treated with 2 % DSS ± isotype or anti-CD90.2 IgG for 6 days (n = 6 per group). Medians indicated. (**F**) Expression of key glycolysis genes from macrophages in **E**. Medians indicated. (**G**) HK2 and GLUT-1 expression in BMDMs ± ILC3-conditioned supernatant for 16h ± anti-GM-CSF IgG. Mean ± SEM indicated. (**H**) ECAR and OCR trace in BMDMs ± ILC3-conditioned supernatant ± anti-GM-CSF IgG for 16 h. Mean ± SEM indicated. Data are representative of two (**B, H**) or three (**A, G**) independent experiments. *P* values calculated using a one-way ANOVA (**A, G**), Mann-Whitney U test (**B**). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

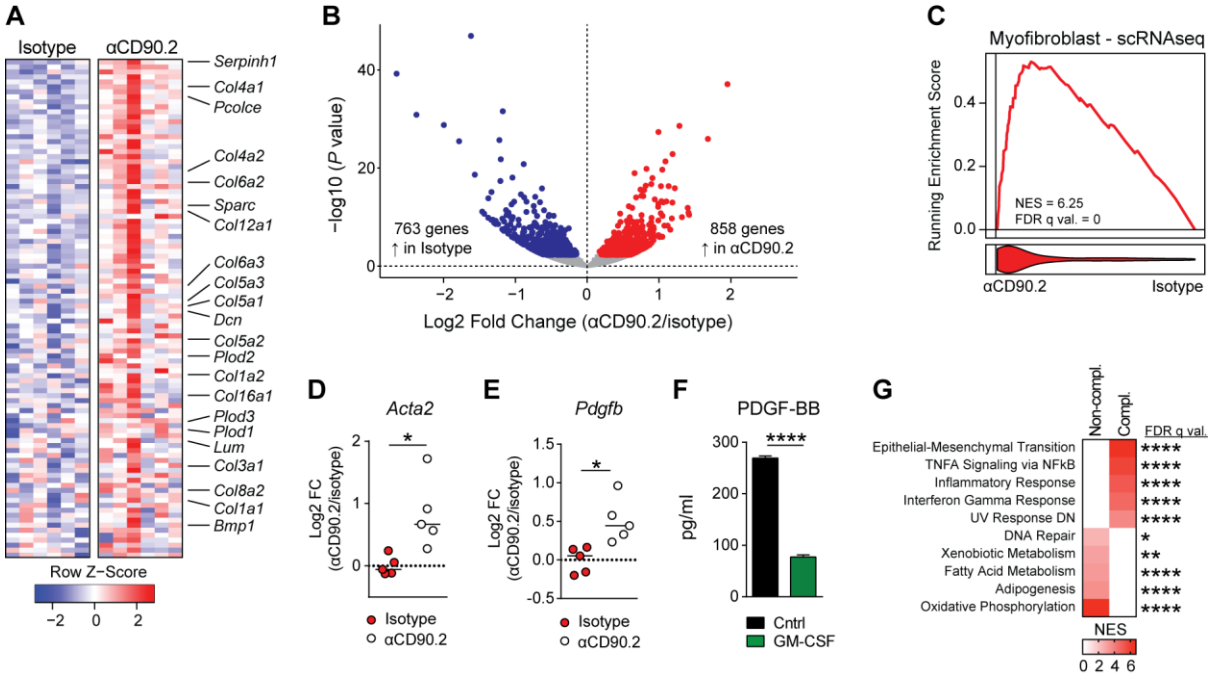

Figure S6. ILCs suppress a pro-repair macrophage phenotype. Related to Figure 6. (**A**) Heatmap of *Epithelial-Mesenchymal Transition* leading edge genes showing enrichment in colonic Ly6C^{lo} MHC-II^{+} macrophages from *Rag2*$^{-/-}$ mice treated with 2 % DSS ± anti-CD90.2 IgG for 6 days (n = 6 per group). Annotated genes are involved in collagen formation or interaction. (**B**) Volcano plot of DEGs in whole colonic tissue from mice treated as in **A**. (**C**) Myofibroblast gene signature GSEA in whole colonic tissue from mice treated as in **A**. (**D**) *Acta2* expression in whole colonic tissue from mice in **A**. Medians are indicated. (**E**) *Pdgfb* expression in whole colon tissue determined by RNAseq in mice treated as in **A**. Medians indicated. (**F**) PDGF-BB ELISA on supernatants from BMDMs treated with control or GM-CSF-supplemented media for 24 h. Mean ± SEM indicated. (**G**) GSEA of Hallmarks pathways in a publicly available transcriptomics dataset of complicated (n = 27) versus non-complicated (n = 183) ileal Crohn's disease biopsies, derived from GEO: GSE93624. Data are representative of three independent experiments (**F**). P values were calculated by DESeq 2 method with multiple comparisons correction using the BH procedure (**A, B, D, E**), and Student's t test (**F**).* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.