

Additional material

Figure S1

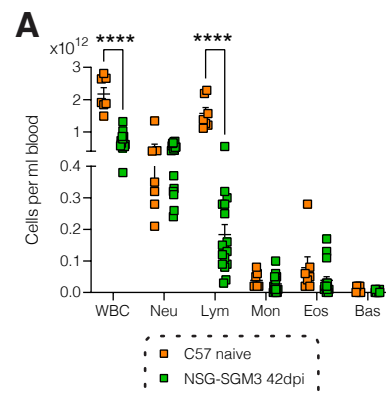


Figure S1. Blood profile of non-humanised NSG-SGM3 mice. A) NSG-SGM3 mice without human immune reconstitution are lymphopenic and have fewer white blood cells (WBCs) compared to wild type C57BL6/J mice. Neu: neutrophils, Lym: lymphocytes, Mon: monocytes, Eos: eosinophils, Bas: basophils. Data points represent blood counts from individual animals, with the mean and standard error of the mean (SEM) also indicated. Two-way ANOVA with Sidak's post hoc; ****, $p < 0.0001$.

Figure S2

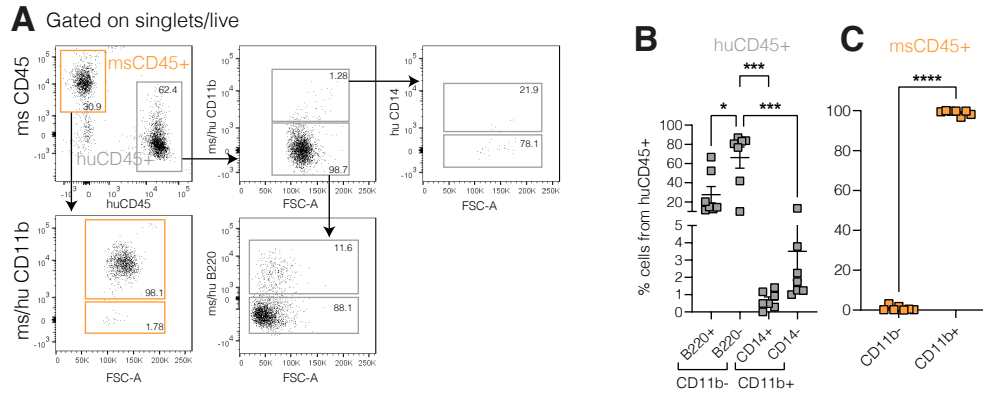


Figure S2. Blood profile of humanised NSG-SGM3 mice. A) Representative flow cytometry plots and gating strategy for human and mouse immune cell populations. B and C) Composition of human (B) and mouse (C) CD45+ cells in the blood at 16 weeks post-engraftment (42 days post-injury). Data points in (B) and (C) represent blood counts from individual animals, with the mean and standard error of the mean (SEM) also indicated. Statistical analysis was performed using two-way ANOVA with Tukey's post-hoc (B) or unpaired student's t-test (C); $n=7/\text{group}$; *, $p<0.05$; ***, $p<0.001$; ****, $p<0.0001$.

Figure S3

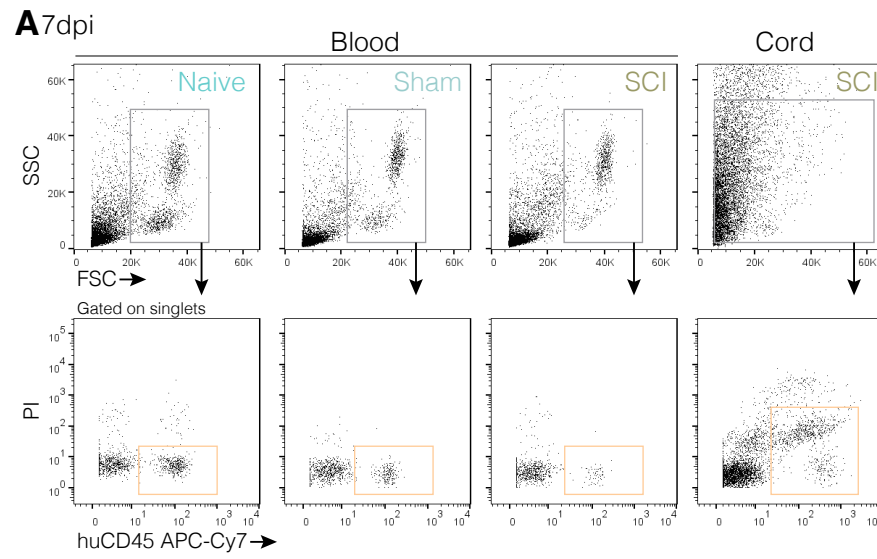


Figure S3. Gating strategy for the isolation of human immune cells. A) Representative FACS plots for blood (left) and spinal cord (right) and gating strategy used for the sorting of human immune cells (i.e., huCD45⁺; orange gate) from blood and spinal cord samples.

Figure S4

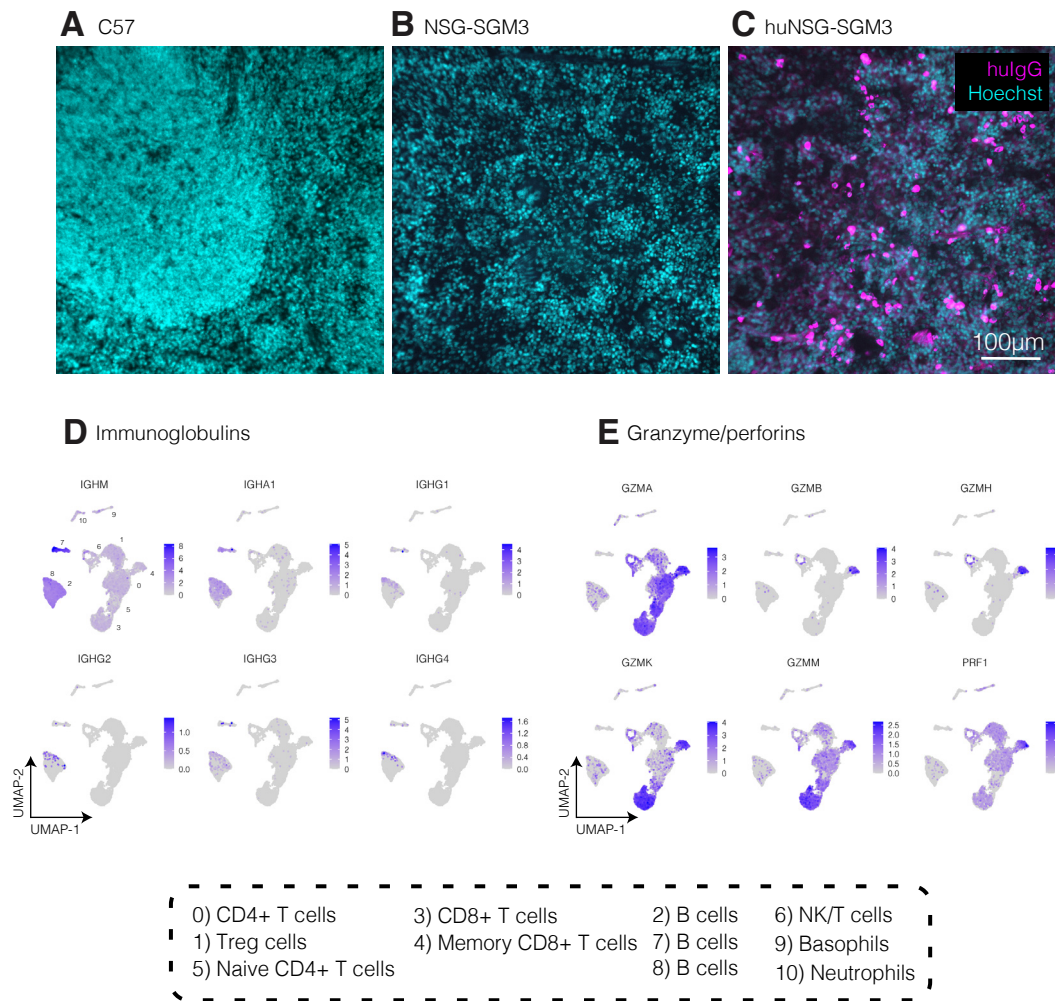


Figure S4. Evidence for immunoglobulin (Ig) class switching, granzyme and perforin expression by human lymphocytes. A to C) Human IgG (huIgG; magenta) staining, or the appropriate lack thereof, in representative spleen sections from C57BL6/J (A), NSG-SGM3 (B) and humanised (hu) NSG-SGM3 mice (C); Hoechst+ cell nuclei are shown in cyan. Scale bar is 100µm. D and E) UMAP plots showing gene expression levels of select immunoglobulin genes (D) and granzyme/perforin genes (E) with cluster annotation within the huMice single-cell RNAseq atlas. Colour represents z-score expression level.

Figure S5

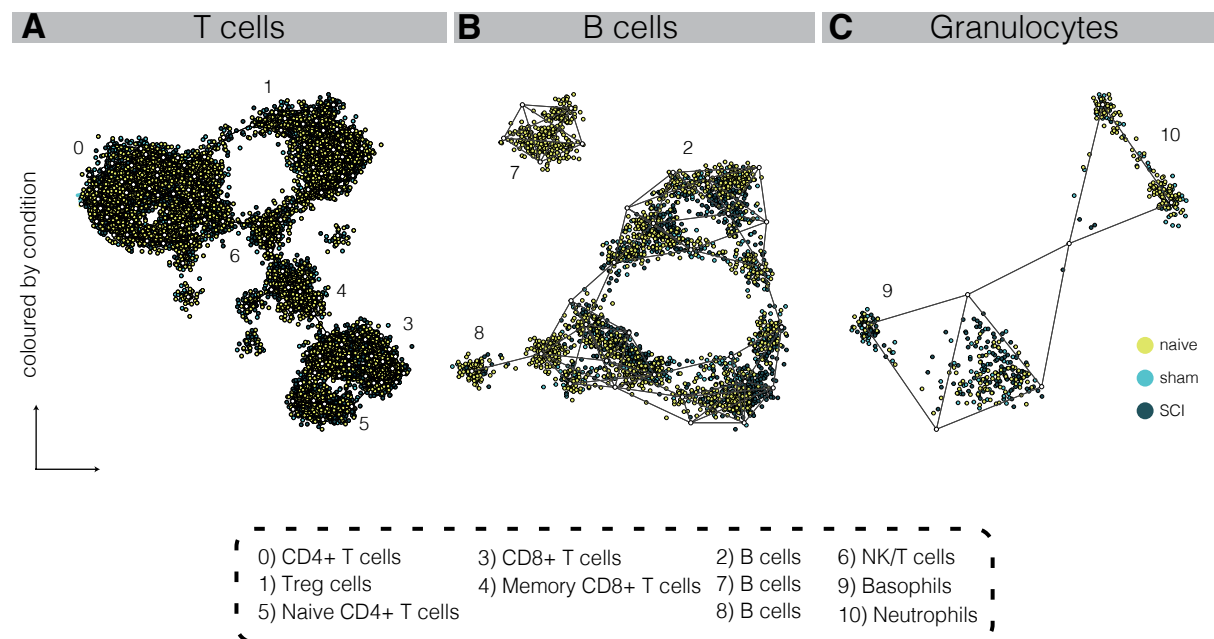
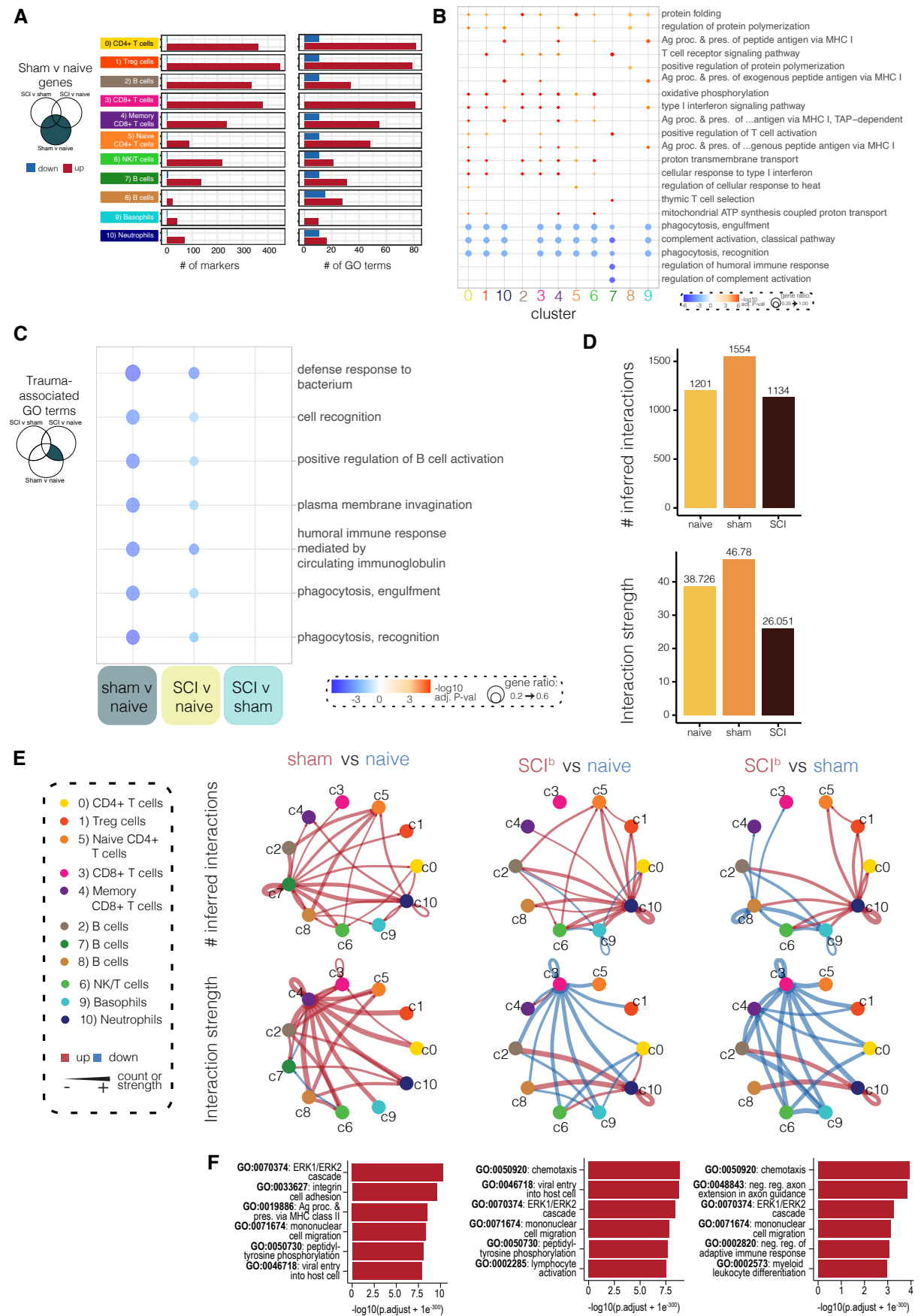


Figure S5. Clustering and/or colocalisation of human immune cells relative to the experimental condition. A to C) MetaCell networks for circulating leukocytes, split into major cell types and subsets (see cluster numbers), for T cells (A), B cells (B), and granulocytes (C). Cells are colour-coded by experimental condition, i.e., naïve (yellow), sham (light blue) and spinal cord injury (SCI; dark blue) to visualise their (co-) location within the MetaCell network diagram.

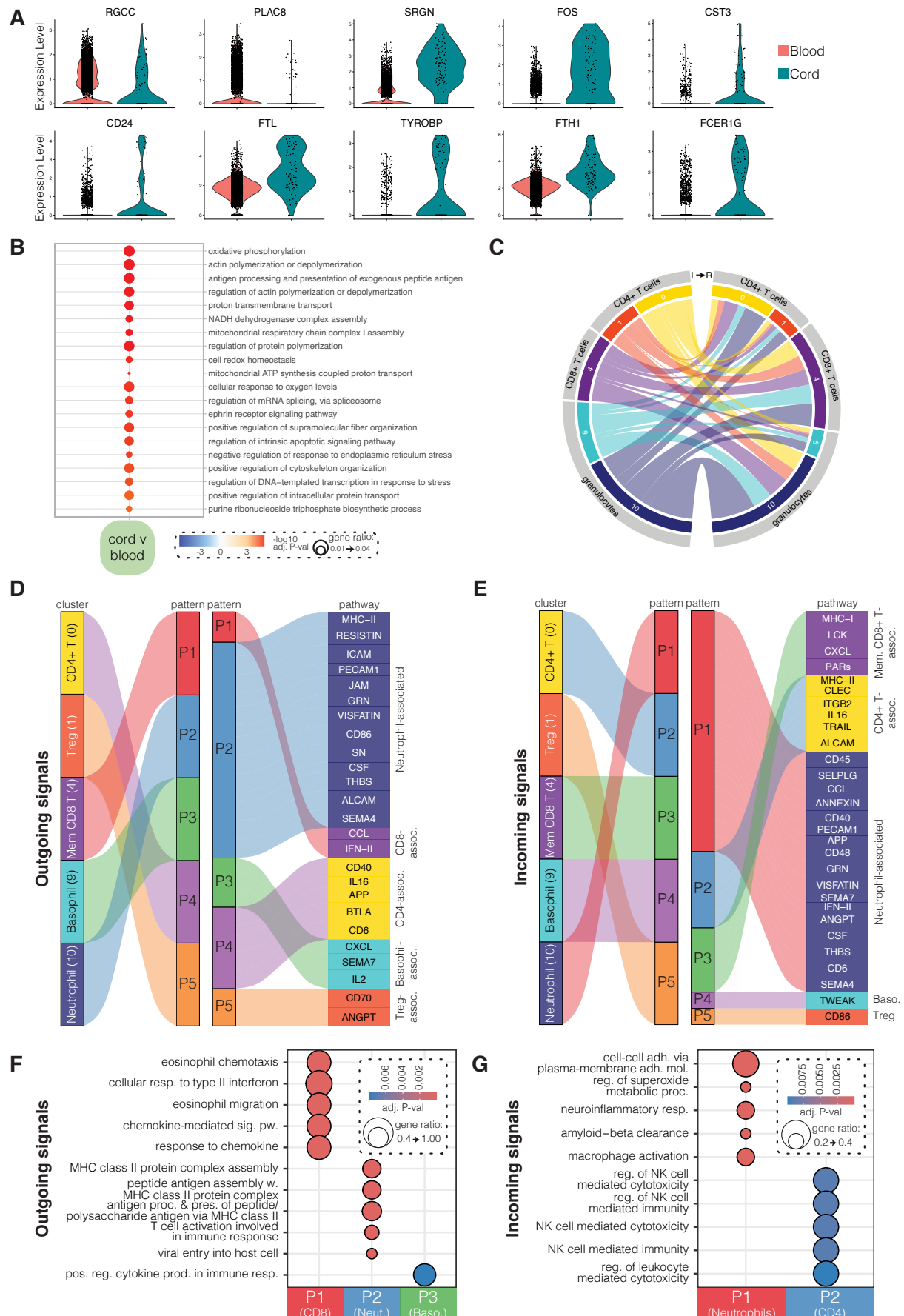
Figure S6



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Figure S6. Non-neurological trauma mostly increases inflammation and interactions between circulating human immune cells whilst SCI decreases this. A) Number of differentially expressed genes (DEGs) (i.e., markers; left) and corresponding significant gene ontology (GO) terms (right), as identified for each cluster in response to tissue injury (i.e., between sham and naïve; coloured part of Venn Diagram). B) Top regulated GO terms relating to DEGs between sham versus (v) naïve conditions, for each classified cell cluster. C) GO terms regulated in the same direction in both ‘sham v naïve’ and ‘SCI v naïve’ comparisons (i.e. reflecting trauma-associated rather than SCI-specific changes in GO terms; coloured part of Venn Diagram). For GO plots in B and C, blue colour represents the $\log_{10}(\text{adjusted } p\text{-value})$ for downregulated genes/terms while red colour would represent $-\log_{10}(\text{adjusted } p\text{-value})$ for upregulated genes/terms; dot size represents ratio of genes contributing to the GO term. (D) Number (top) and strength (bottom) of cell-to-cell interaction (CCI) events predicted to occur in blood samples from naïve, sham and SCI mice, based on known ligand-receptor (LR) pairs. (E) Network diagrams showing differential communication between pairs of cell types (clusters) under the specified conditions. Red lines indicate greater connectivity in the first-named sample compared to the second; blue lines indicate a downregulation, as in a greater connectivity in the second-named sample compared to the first. Only the top 20% of connections are shown, and the relative number and/or strength of predicted CCIs is indicated by the line weight. (F) Top 6 enriched GO terms associated with CCI events predicted to be upregulated for comparisons shown in E (bottom plots, interaction strength); LR pairs that were filtered to remove pairs enriched in the reference (i.e. blue) condition were removed prior to GO analysis

Figure S7



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Figure S7. Transcriptome comparison and communication between infiltrated human immune cells. A) Violin plots showing log expression of the top regulated genes in huCD45+ cells isolated from the spinal cord (green) and blood (pink). B) Top gene ontology (GO) terms enriched in human immune cells isolated from the injured spinal cord compared to those in the circulation (i.e., cord v blood) across whole samples. Red colour represents $-\log_{10}(\text{adjusted } p\text{-value})$ for upregulated genes/terms; dot size represents ratio of genes contributing to the GO term. C) Predicted cell-to-cell interactions (CCIs) between identified human immune cell types in the injured spinal cord, based on known interactions between ligands (L; left) and receptors (R; right). Coloured segments around each half of the plot represent cell clusters, grouped into main immune cell types as per the annotation in the grey bars/segments. Connecting line widths indicate the number of predicted ligand-receptor (LR) events between the connected cell types; line colours indicate sender cell identity. D) Outgoing communication patterns for named cell clusters (left). Unique signalling patterns (P) were predicted for each, with a total of five outgoing patterns detected, respectively (middle; P1-P5). Signalling pathways (encompassing one or more LR pairs) associated with each pattern are also shown (right). E) As for D, but now showing unique incoming communication patterns (i.e., signals received) for each cell cluster. F) Top 5 significant GO terms associated with the outgoing signalling patterns shown in D. Ligands predicted to transmit the outgoing signals were used as input for GO enrichment analysis; no significant hits were found for P4 and P5. G) As for F, but using receptors for incoming signals (highlighted in E) instead for GO enrichment analysis.

Figure S8

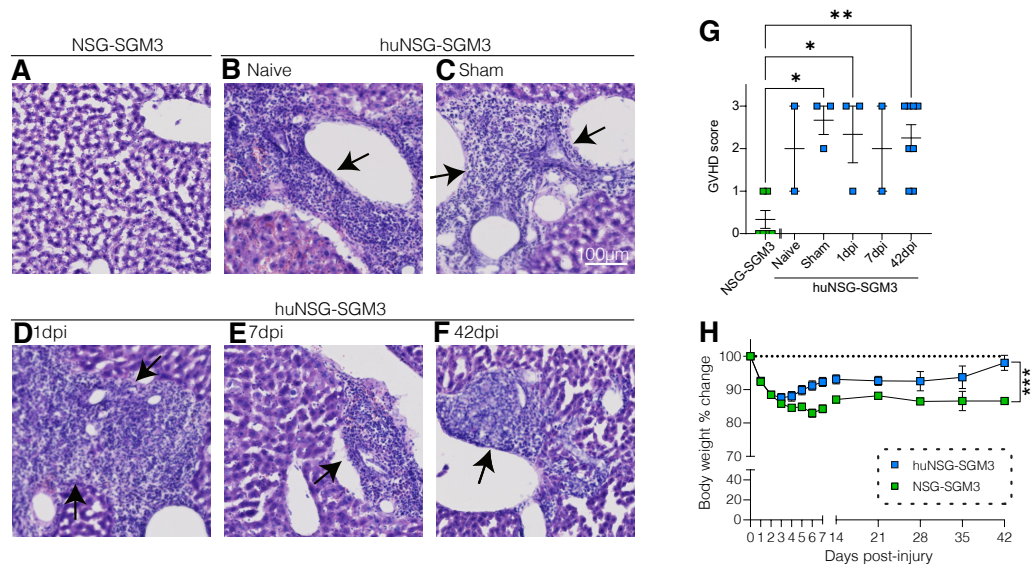


Figure S8. Histological evidence of graft-versus-host disease in humanised NSG-SGM3 mice. A to F) Representative images of H&E-stained livers showing perivascular immune infiltrate in humanized (hu) NSG-SGM3 mice (denoted by arrows), characteristic of graft-versus-host disease (GVHD). Scale bar is 100µm. G) GVHD scores for livers of naïve, sham and SCI (1, 7 and 42 days post-injury; dpi); non-humanised NSG-SGM3 mice were included as an additional control. A score of ‘0’ (no visible pathology) is visually represented in (A) and a score of ‘3’ (sporadic perivascular infiltration with some additional spread into the parenchyma) in (B-F). H) Bodyweight of NSG-SGM3 and huNSG-SGM3 mice following SCI (normalised to pre-surgery weight). Statistical analyses were performed using one-way ANOVA with Tukey’s post hoc (G), or a mixed effects model with Sidak’s multiple comparisons test (H); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.