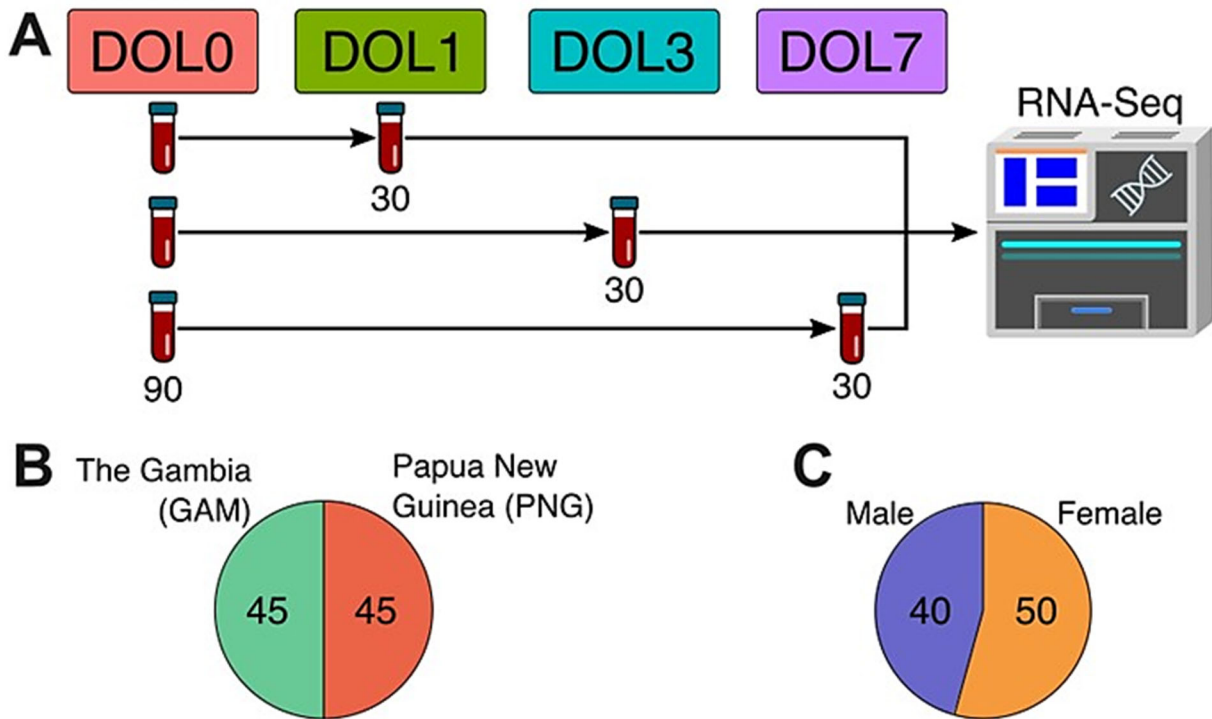


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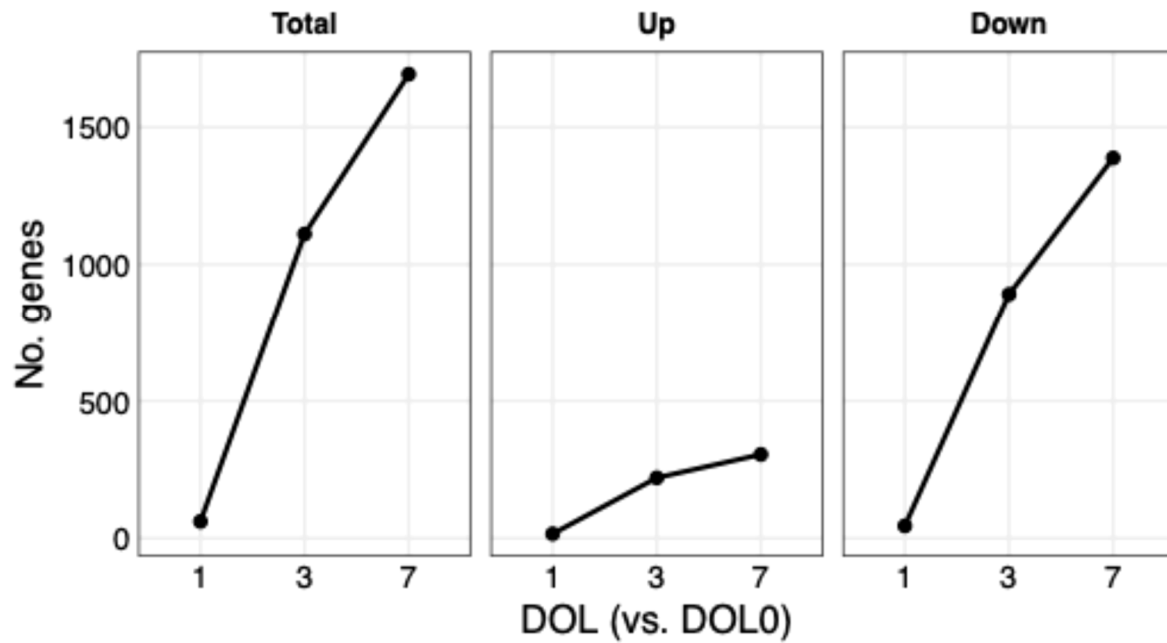
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S. Table 9	Significant results of Wilcox test comparing cell types on flow cytometry myeloid panel between GAM & PNG at each DOL.

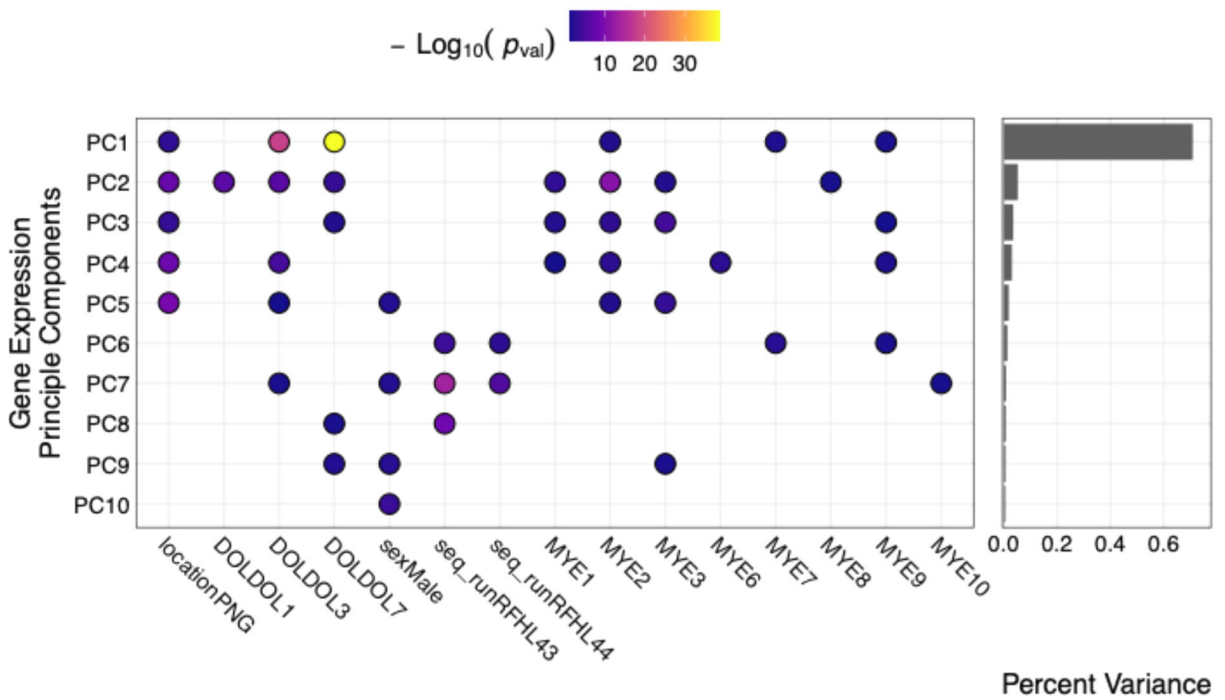
SUPPLEMENTARY FIGURES



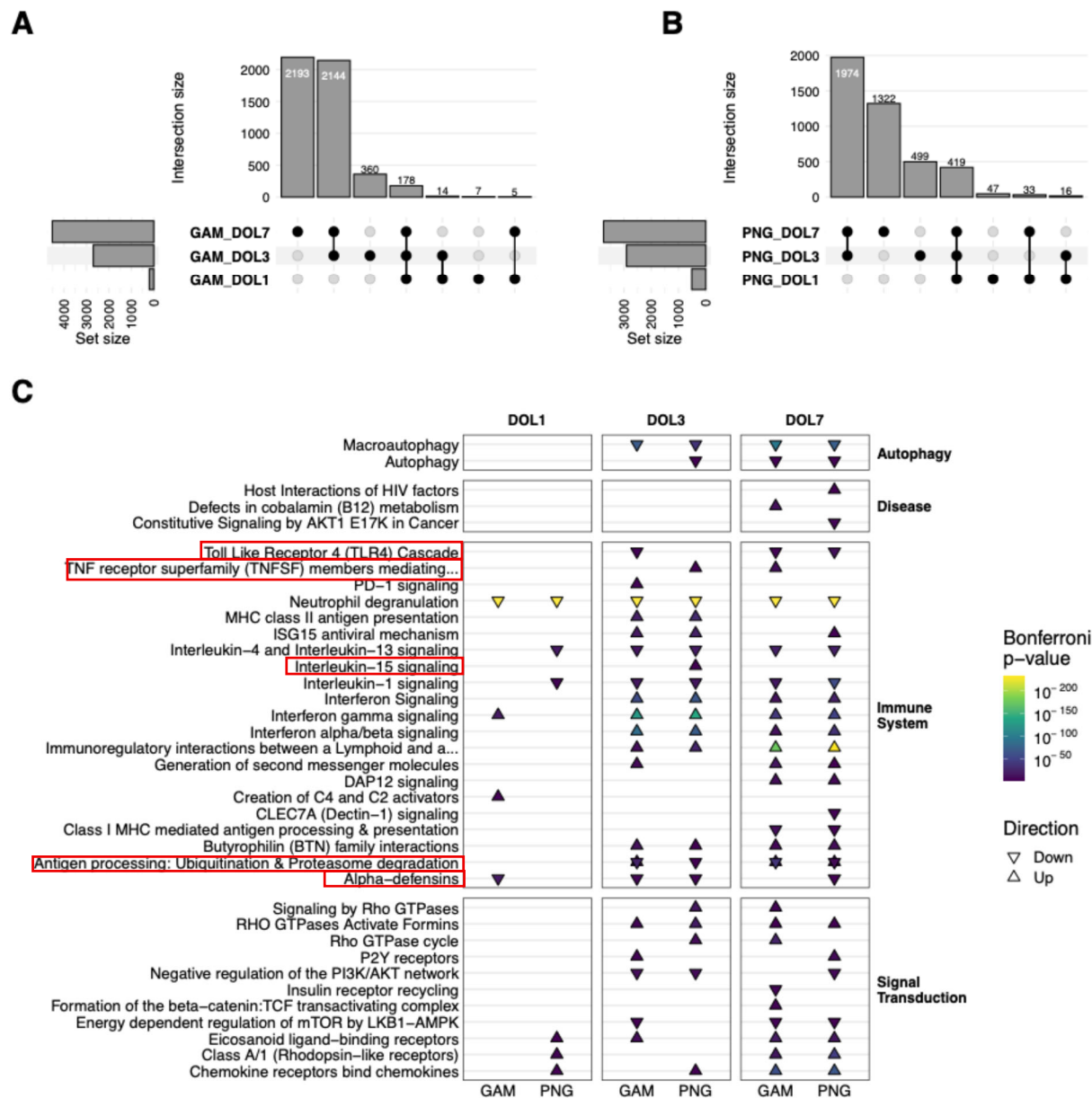
Supplementary Figure 1: Study design/workflow and participant demographics. **A.** Blood samples from neonates were collected at DOL0, followed by another blood draw at either DOL1, 3, or 7 based on a computer-generated block randomization strategy. **B.** An equal number of individuals were enrolled from both locations, The Gambia (GAM) and Papua New Guinea (PNG). **C.** The study included a near-equal number of male and female participants, to allow for mitigation of any potential sex differences.



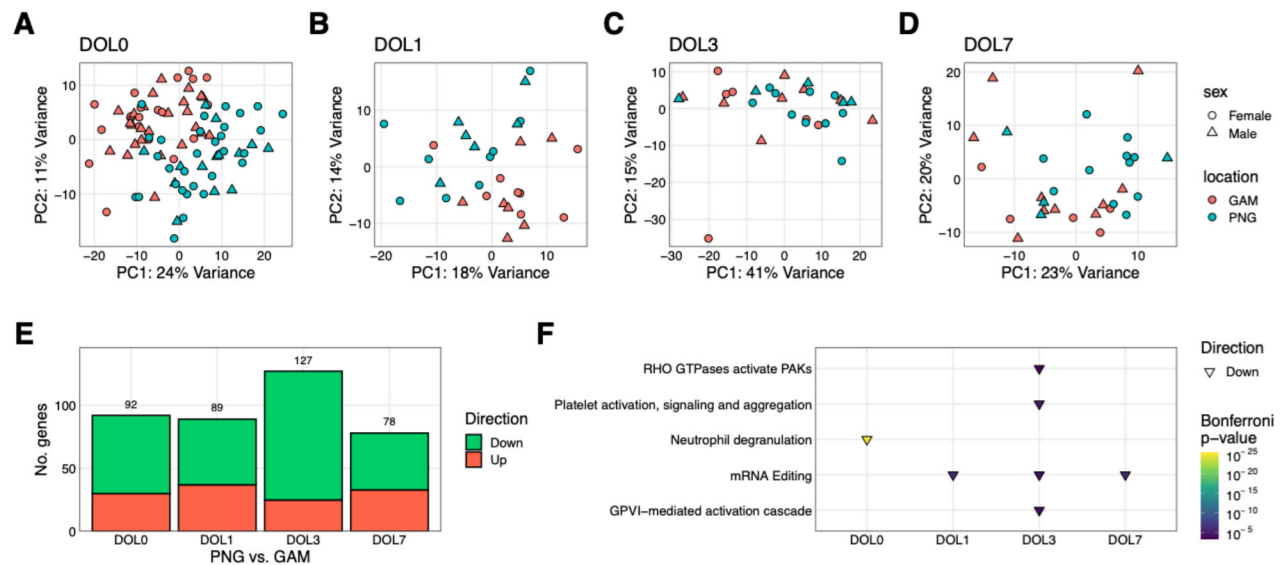
Supplementary Figure 2. Summary of DE genes identified in overall ontogeny model. In this DESeq2 analysis, an overall analysis of changes over the first week of life was completed by combining all samples from both locations (paired, indexed to DOL0) without considering location. The total number of DE genes increased over time, and was primarily driven by down-regulated genes.



Supplementary Figure 3. Contributions from biological and experimental variables to transcriptomic variation. A linear model was used to assess the contribution of different variables to the principal components derived from the gene expression data. Ten principal components derived from flow cytometry measurements of various myeloid cell-types were also investigated (dimensionality reduction used to reduce to components MYE1-MYE10) for their contributions to the gene expression variance. Location and day of life (DOL) were observed as the major contributors to transcriptomic variance, followed by differences in cell type proportions, sequencing batch, and sex, which played more minor roles (PC with a lower percent variance, see panel to right) in influencing gene expression in this dataset.



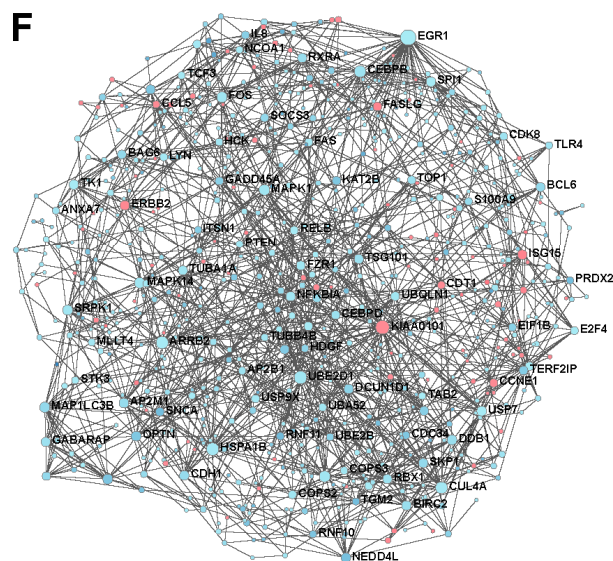
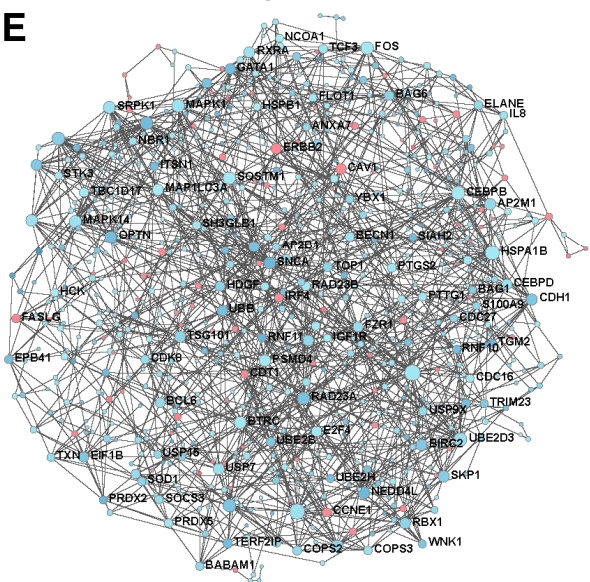
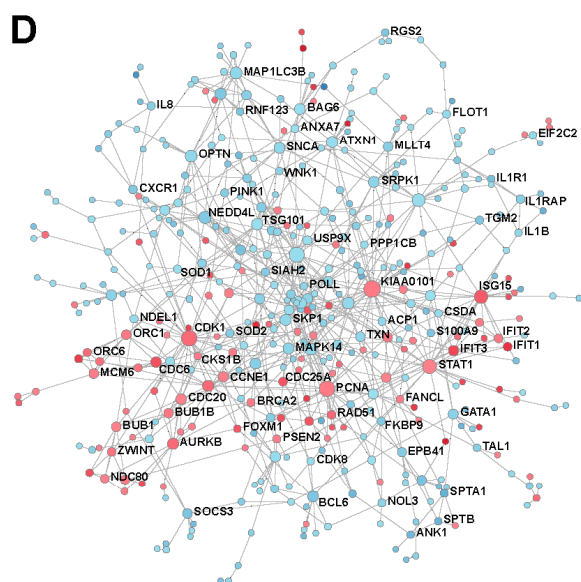
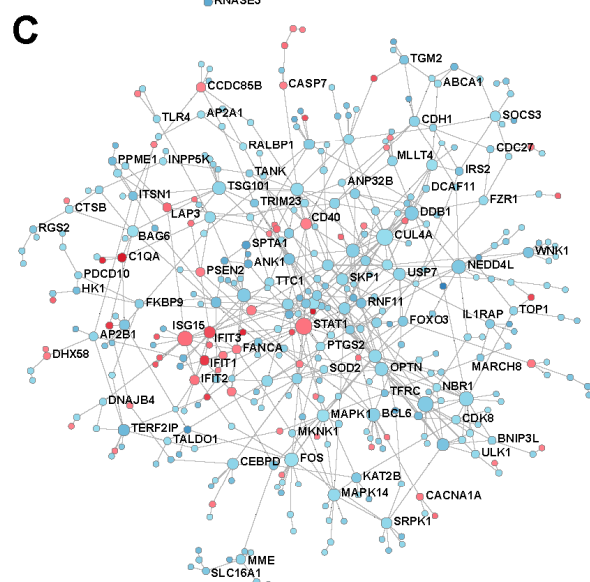
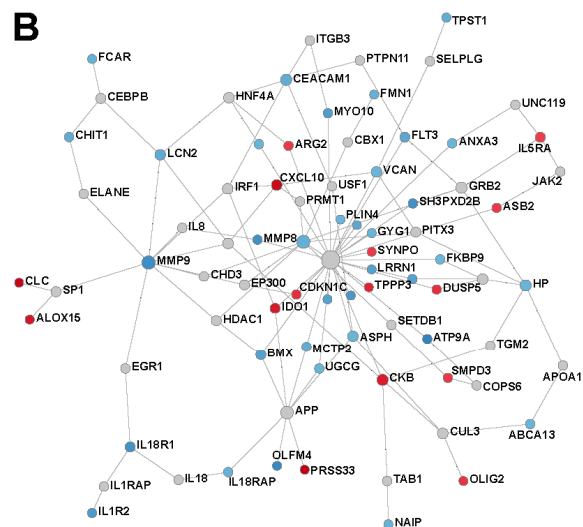
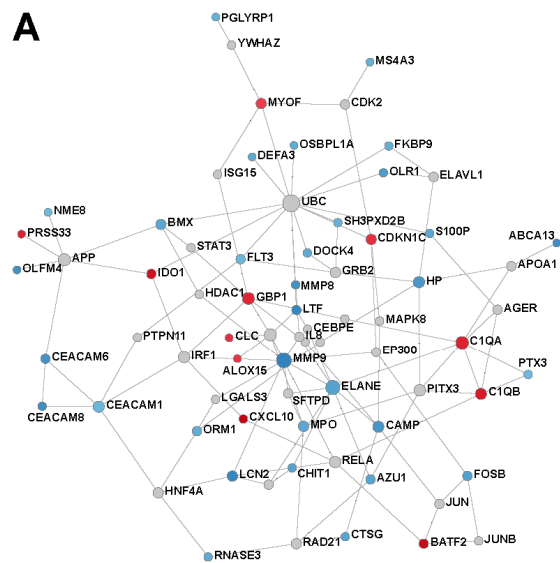
Supplementary Figure 4. Influence of location on population-specific DE genes using lower cut-offs (adjusted p-value ≤ 0.05 and absolute fold-change ≥ 1.5). DE genes were identified using a paired DESeq2 model (DOL1/3/7 indexed to DOL0) also separating by location). **A.** Upset plot depicting numbers of unique and shared DE genes identified in GAM neonates over DOL1/3/7. **B.** Upset plot depicting numbers of unique and shared DE genes identified in PNG neonates over DOL1/3/7. **C.** Reactome pathways of selected categories found to be statistically significantly enriched (adjusted p-value < 0.01) based on the DE genes identified in both **A.** and **B.** Directionality of arrow indicates direction of up- or down-regulated genes, color indicates level of significance. Interesting pathways are highlighted with red boxes.



Supplementary Figure 5: Directly comparing each DOL between GAM and PNG throughout the first week of life revealed some transcriptomic differences. PCA plots show that the difference between populations is largest at DOL0/1, and decreases throughout the first week of life, (panels A-D). DE genes identified when directly comparing each DOL from GAM and PNG in an unpaired model across the first week of life (panel E). Most significant genes were down-regulated in neonates from PNG relative to those from GAM. Reactome pathways found to be statistically significantly enriched (adjusted p-value < 0.01) based on the DE genes identified in E (panel F).

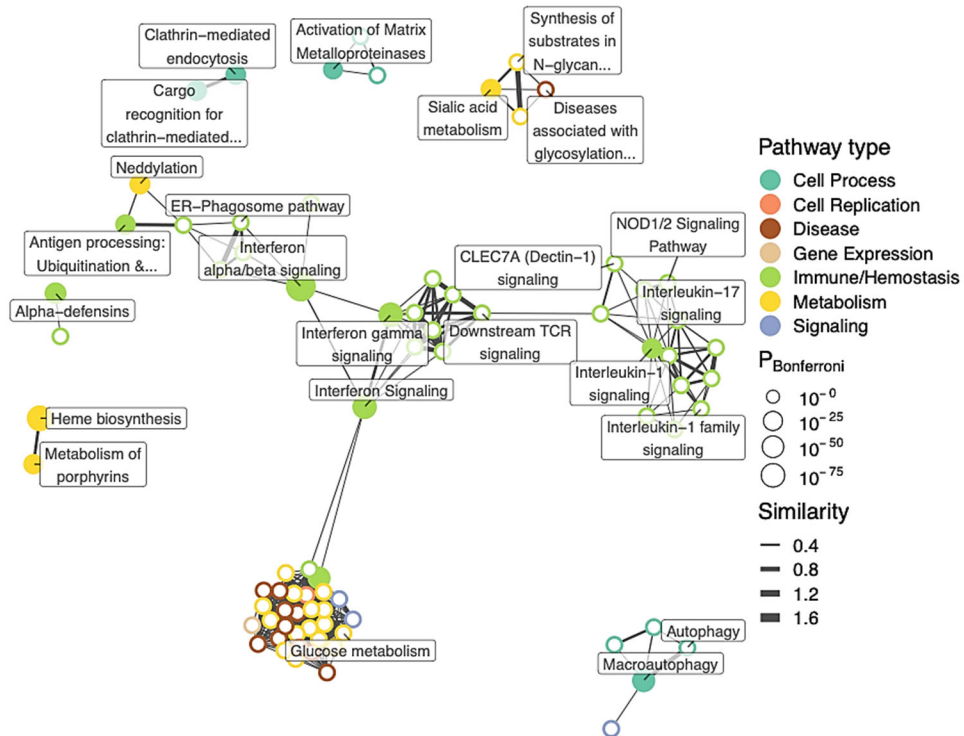


Supplementary Figure 6. Summary of significantly enriched pathways comparing overall ontogeny to population-specific ontogeny. This figure is an expanded version of Figure 2C with all pathways that were significantly enriched while Figure 2C includes only selected high-level categories.

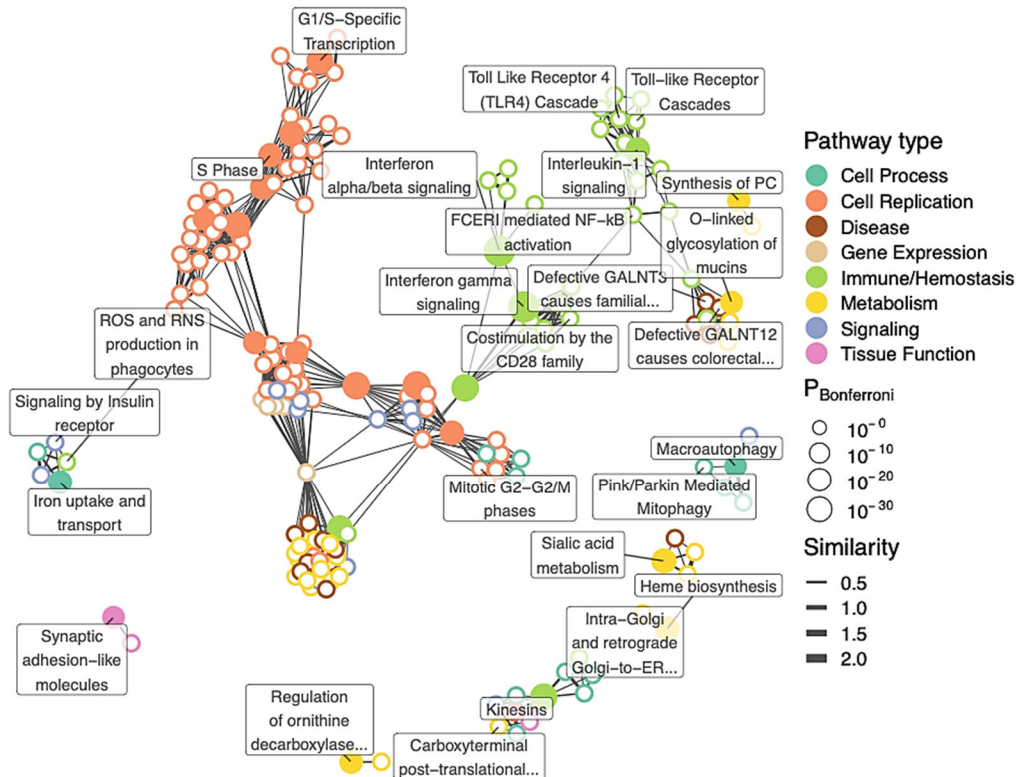


Supplementary Figure 7. Protein-protein interaction (PPI) networks highlighting differences in immune development between GAM and PNG in the first week of life, consistent with pathway rewiring. DE genes identified at DOL1 for (A) GAM and (B) PNG, DOL3 for (C) GAM and (D) PNG and DOL7 for (E) GAM and (F) PNG. Networks are built using NetworkAnalyst from DE genes obtained when comparing latter days to DOL0 for the two locations. Nodes are colored by direction of fold-change (red are up-regulated; blue are down-regulated).

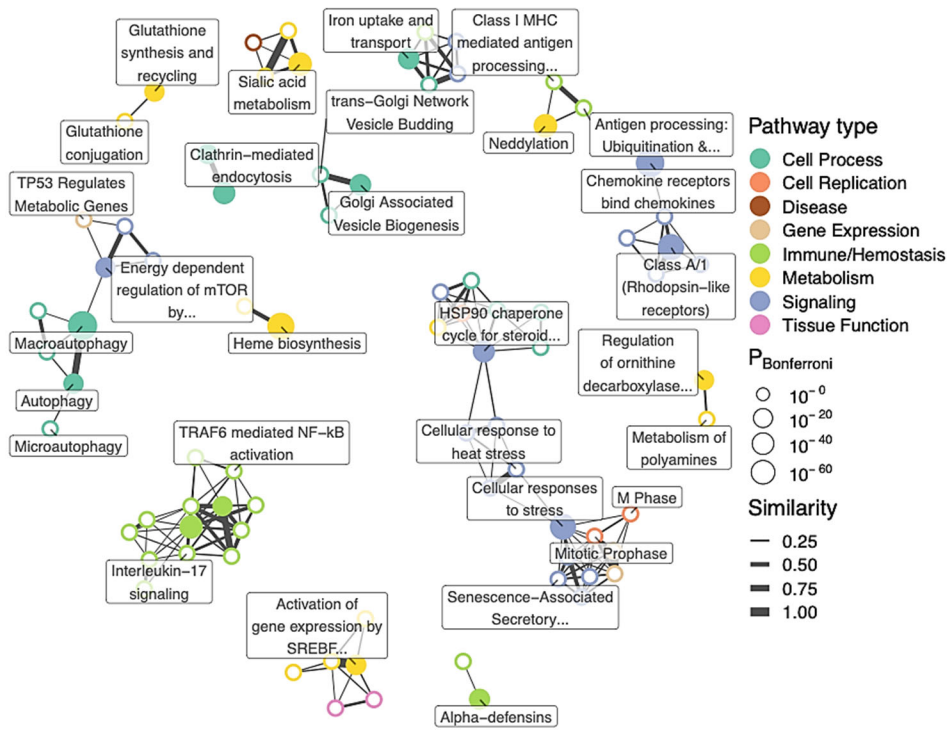
A



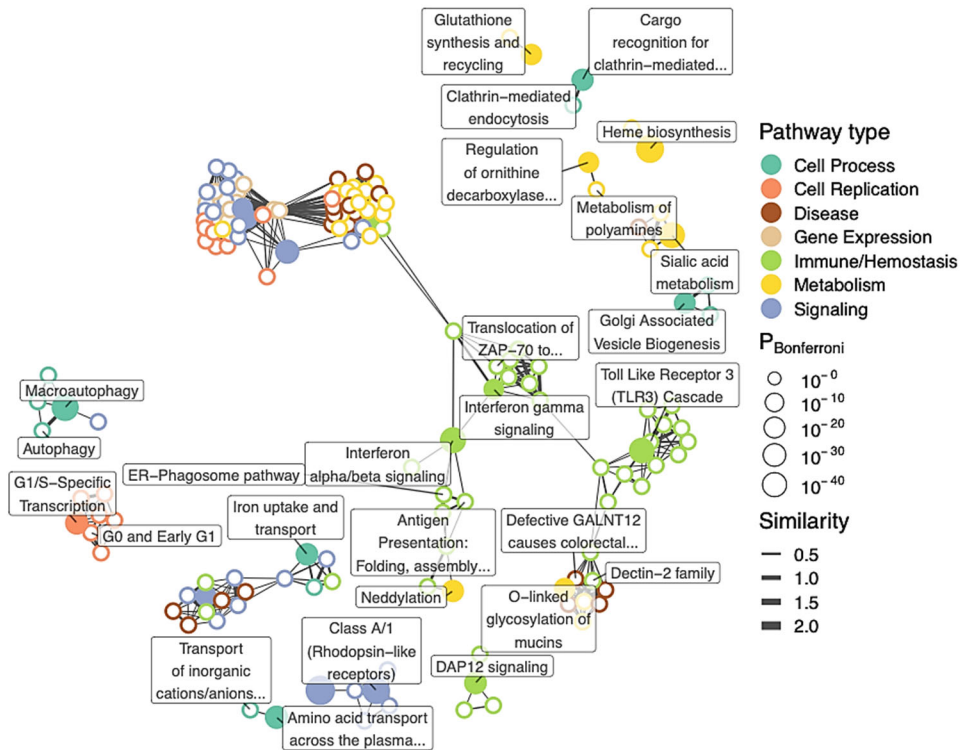
B



C

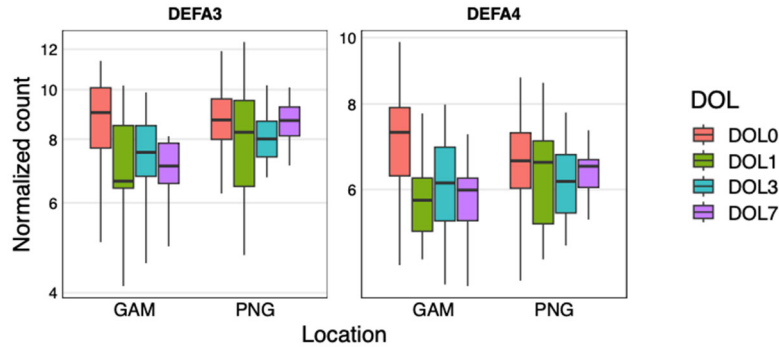


D

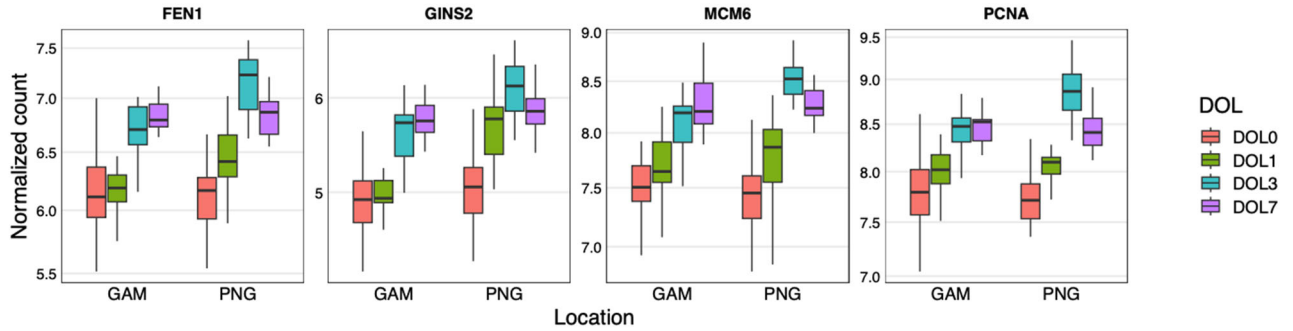


E

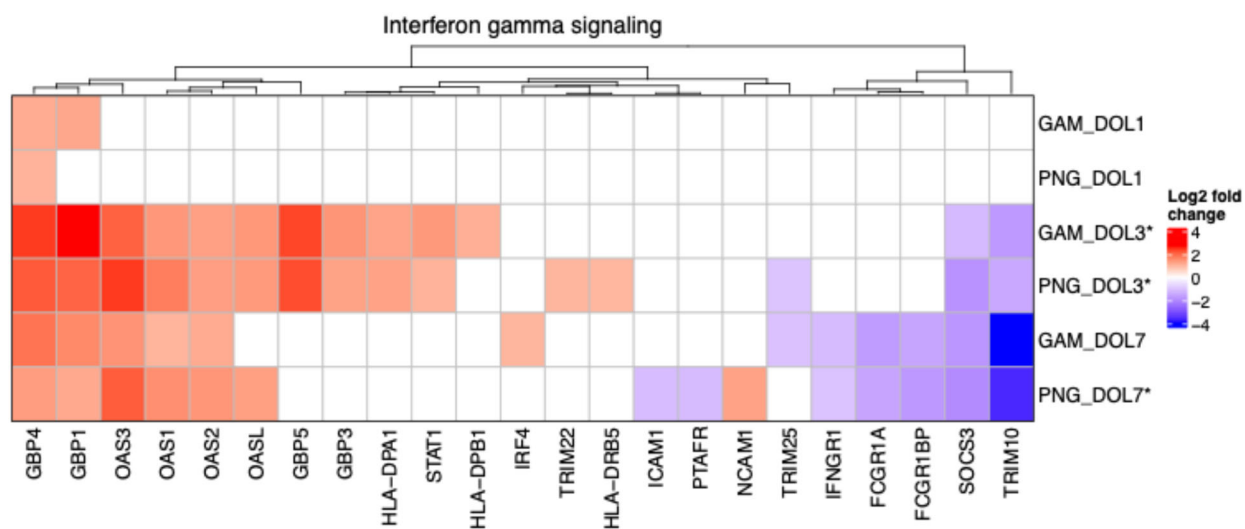
GAM: Alpha defensins

**F**

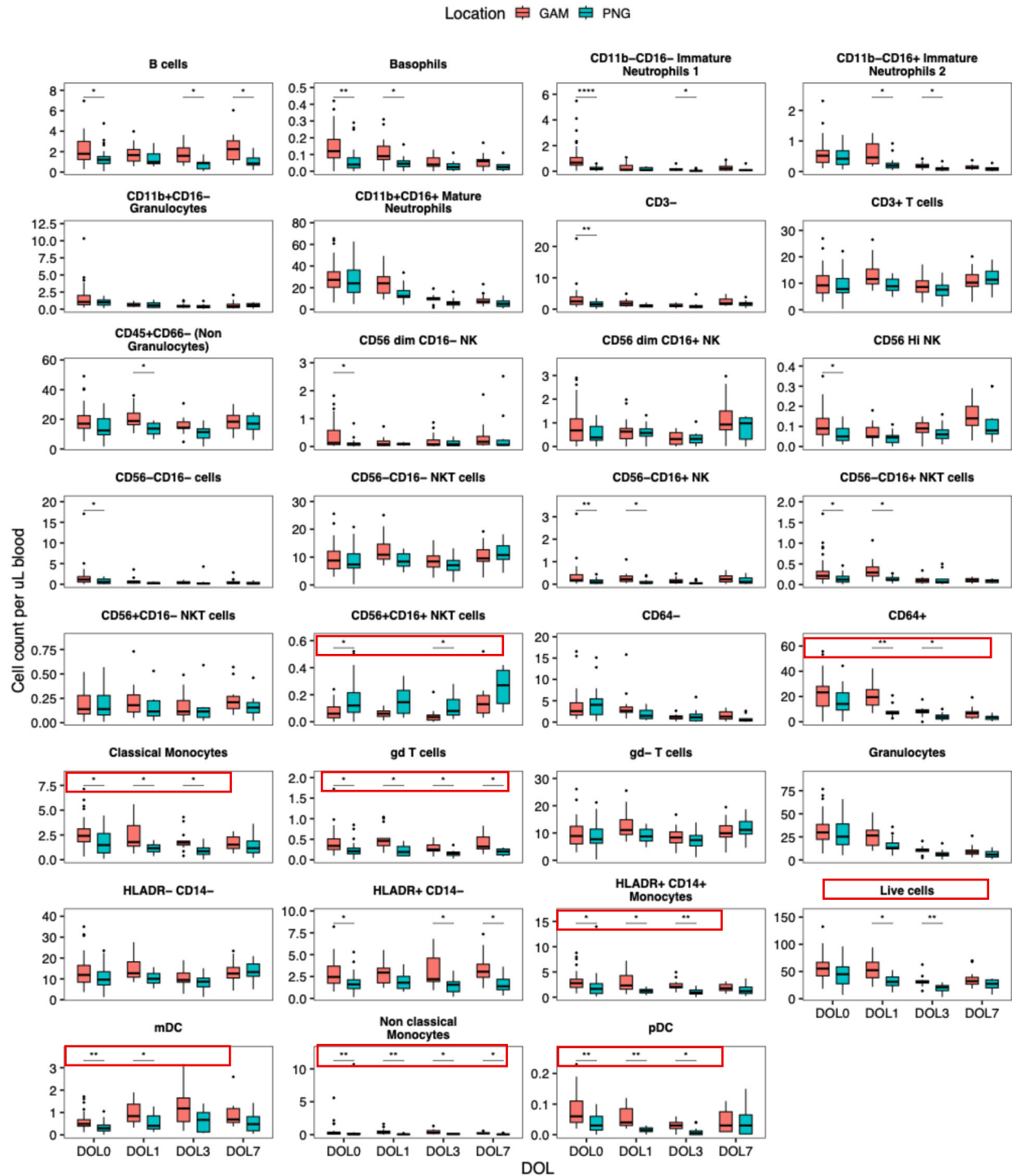
PNG: S Phase

**Supplementary Figure 8. Pathway-based networks reveal population-specific trends.**

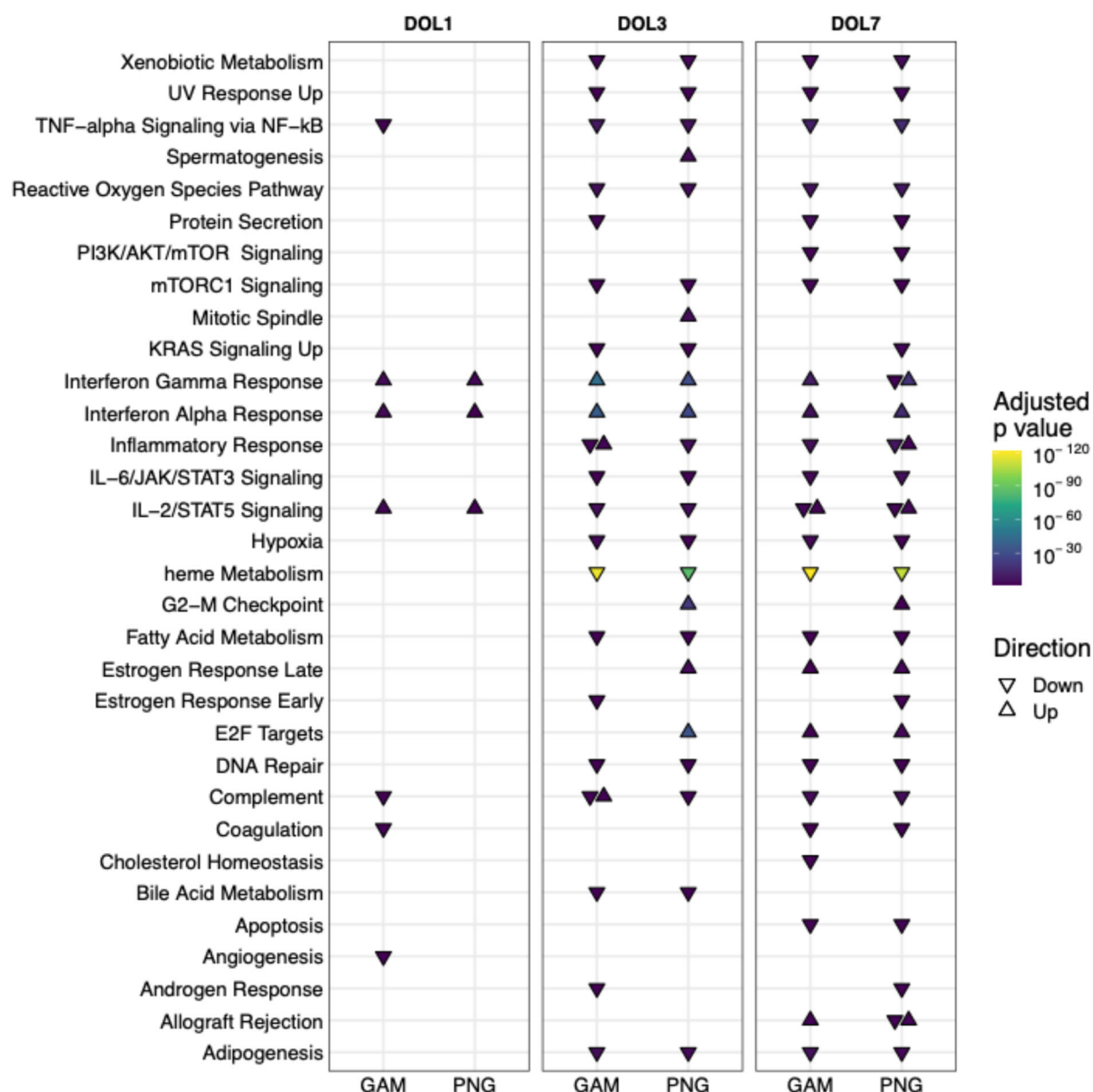
Pathway enrichment was performed in pathlinkR (using the Sigora method) for DOL3 in GAM (A) and PNG (B), and DOL7 in GAM (C) and PNG (D). Each enriched pathway is represented as a node, the size of the node represents significance of enrichment. The edges connecting pathways represent similarity in constituent genes in the pathway (measured by Jaccard index). (E) and (F) show particular signature genes in the alpha defensin and S phase pathways respectively.



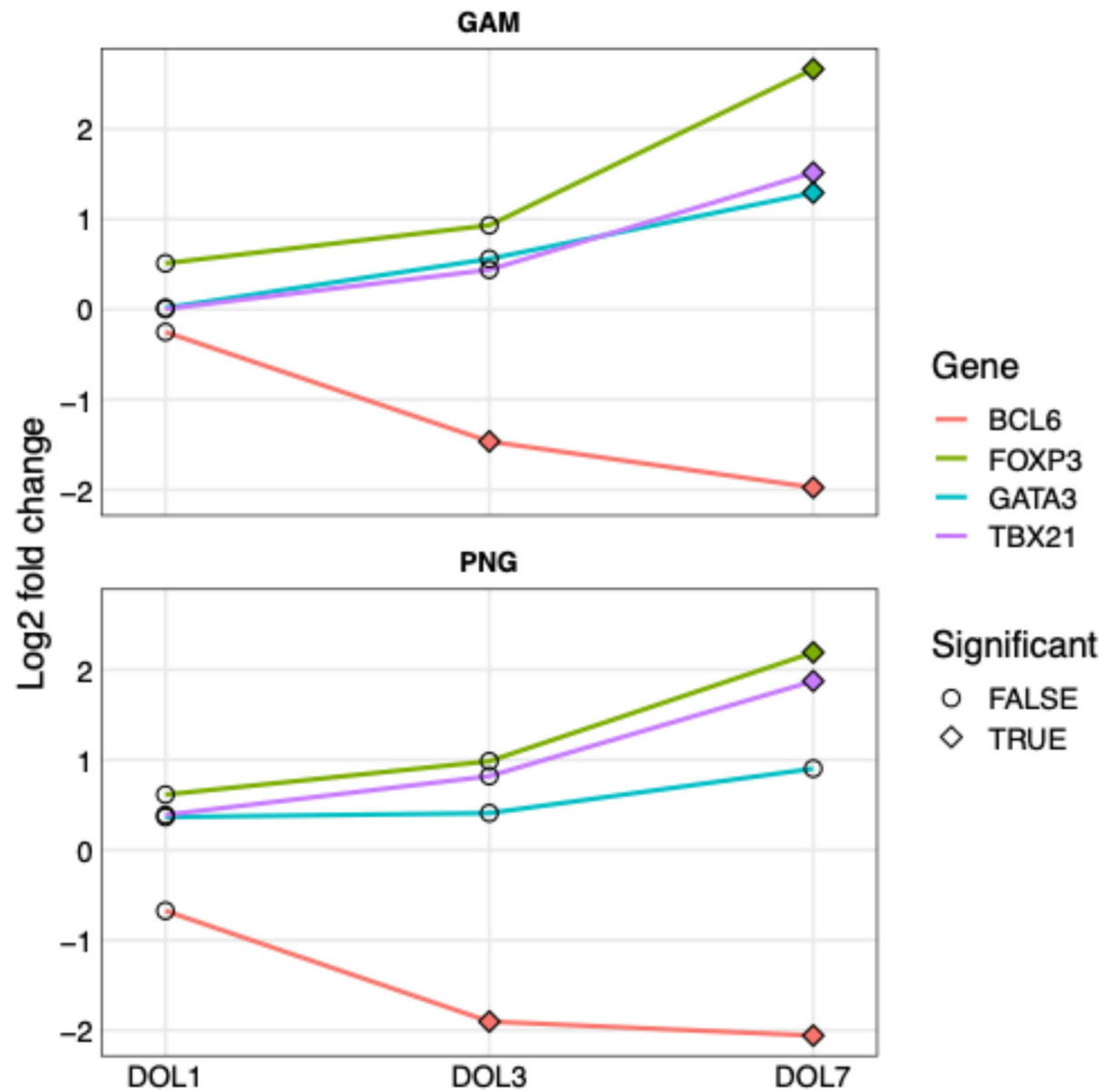
Supplementary Figure 9: Heatmap depicting fold changes of genes from the “Interferon gamma signaling” pathway. Each row represents a differentially-expressed (DE) comparison, with “*” denoting those comparisons for which the pathway was significantly enriched (corrected p-value < 0.001). Empty cells indicate genes in the pathway which were not significantly DE for a particular comparison.



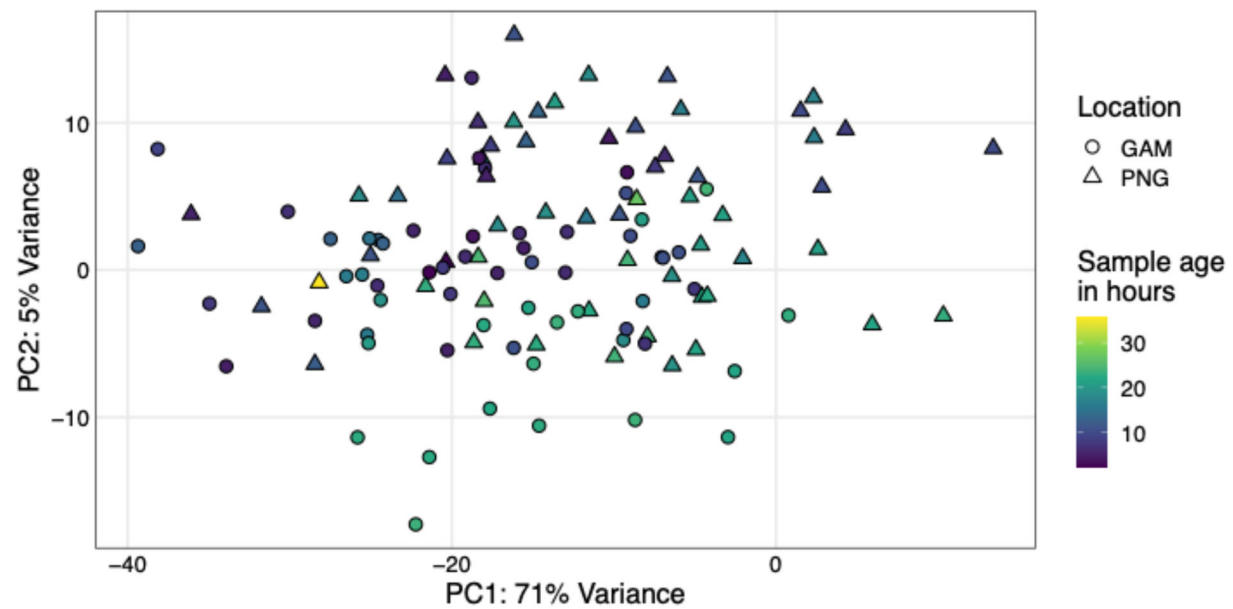
Supplementary Figure 10: Changes in myeloid cell types in the blood throughout the first week of life as assessed by Flow cytometry. Significant differences in absolute counts of cells/ μL of blood between the two locations (GAM and PNG) were determined via a Wilcoxon ranked sum test (unpaired) and p-values were adjusted using the Benjamini-Hochberg correction. Cells with population specific differences are shown by red boxes around the statistical analysis and generally reflect minor cell populations.



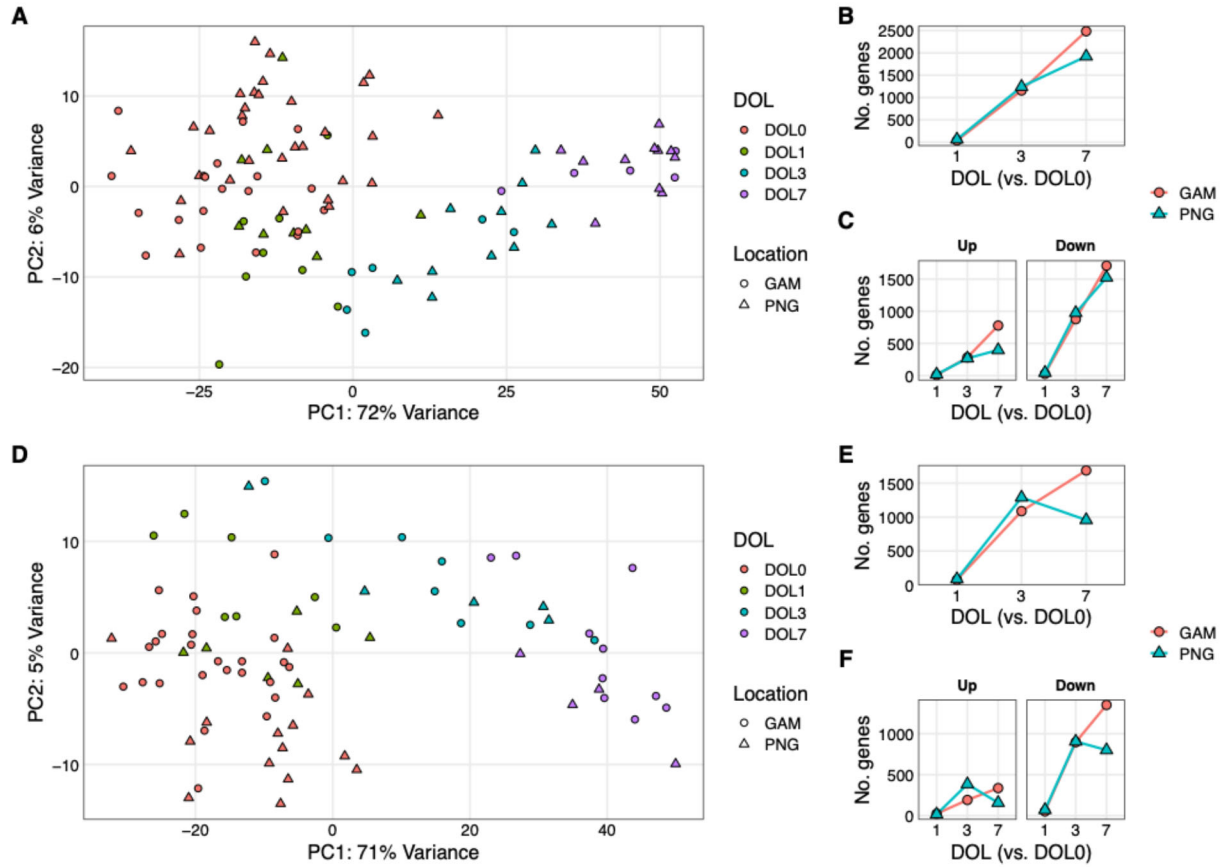
Supplementary Figure 11: Enrichment of MSigDB terms during the first week of life. enrichR was used to identify enriched terms from the MSigDB Hallmark Gene Sets. The number of significantly enriched terms (adjusted p-value < 0.05) increases from DOL1 to DOL3, then remains relatively constant moving to DOL7. Directionality of arrows signifies direction of differential-expression, wherein arrows pointing up signify up-regulation and down signify down-regulation compared to expression at DOL0. Arrows pointing both up and down for any given pathway signify a mixed response with a significant enrichment of sets of genes that were both up and down regulated.



Supplementary Figure 12: Comparing differential expression of T cell marker genes. Fold changes of four transcriptional regulators of major effector T cell subsets, BCL6 (T_{FH}), FOXP3 (T_{reg}), GATA3 (T_H2), and TBX21 (T_H1) were visualized across the first week of life in each population.



Supplementary Figure 13. Principal component analysis of samples collected at birth (DOL0). Shape of the points represents the location, while the color represents the age of the sample (in hours).



Supplementary Figure 14. Patterns of sex-specific gene expression changes across first week of life: females (A-C) and males (D-F). Principal component analysis in (A) female samples only and (D) male samples only, where location is represented by shape and color represents DOL. Total number of differentially expressed (DE) genes when comparing DOL 1/3/7 to DOL0 in (B) females only or (E) males only (shape and color indicate location). DE genes were also evaluated separately by directionality of differential expression (up or down) in (C) females only and (F) males only.