

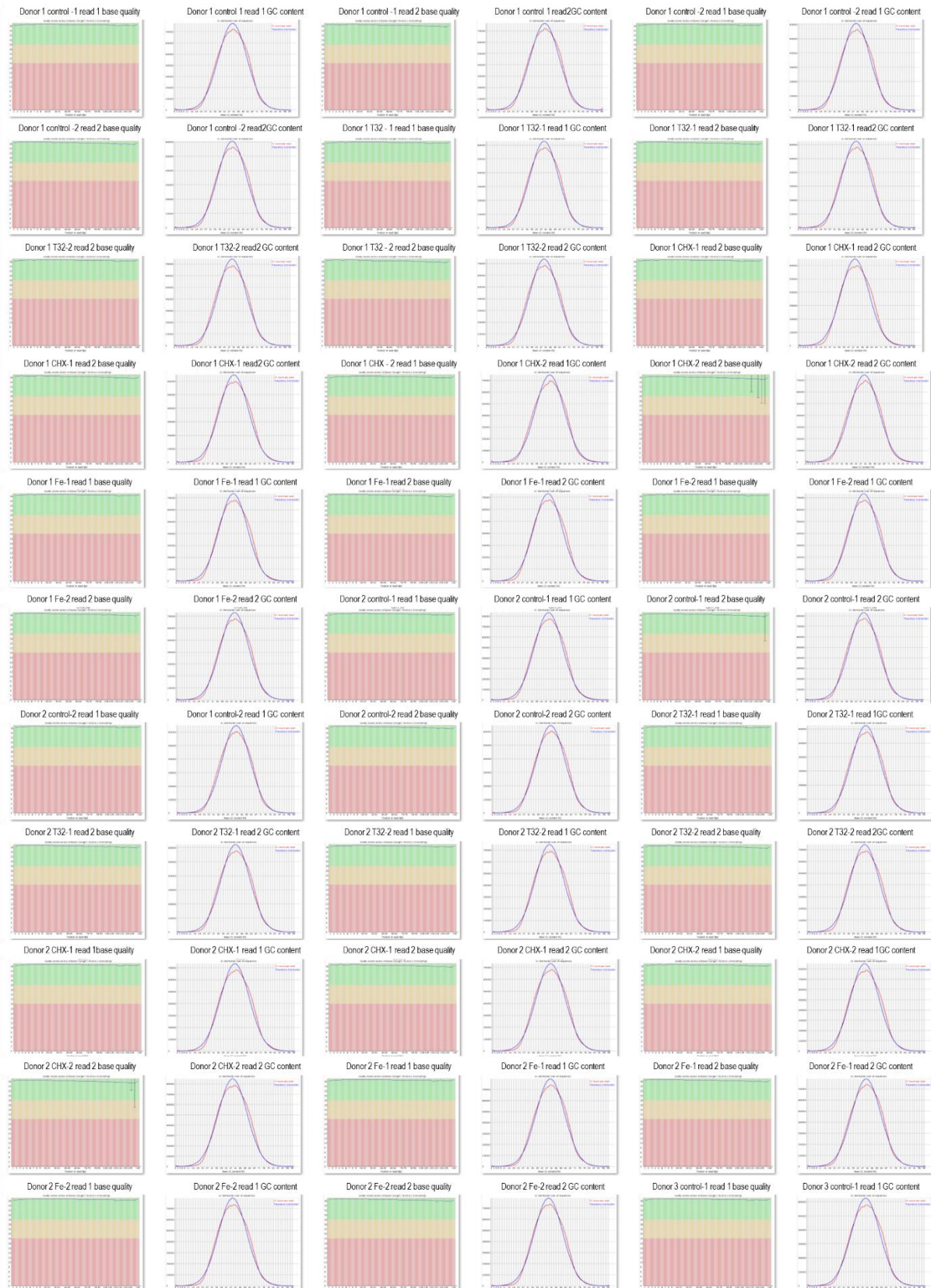
Endogenous plasma resuspension of peripheral blood mononuclear cells prevents preparative-associated stress that modifies polyA-enriched RNA responses to subsequent acute stressors

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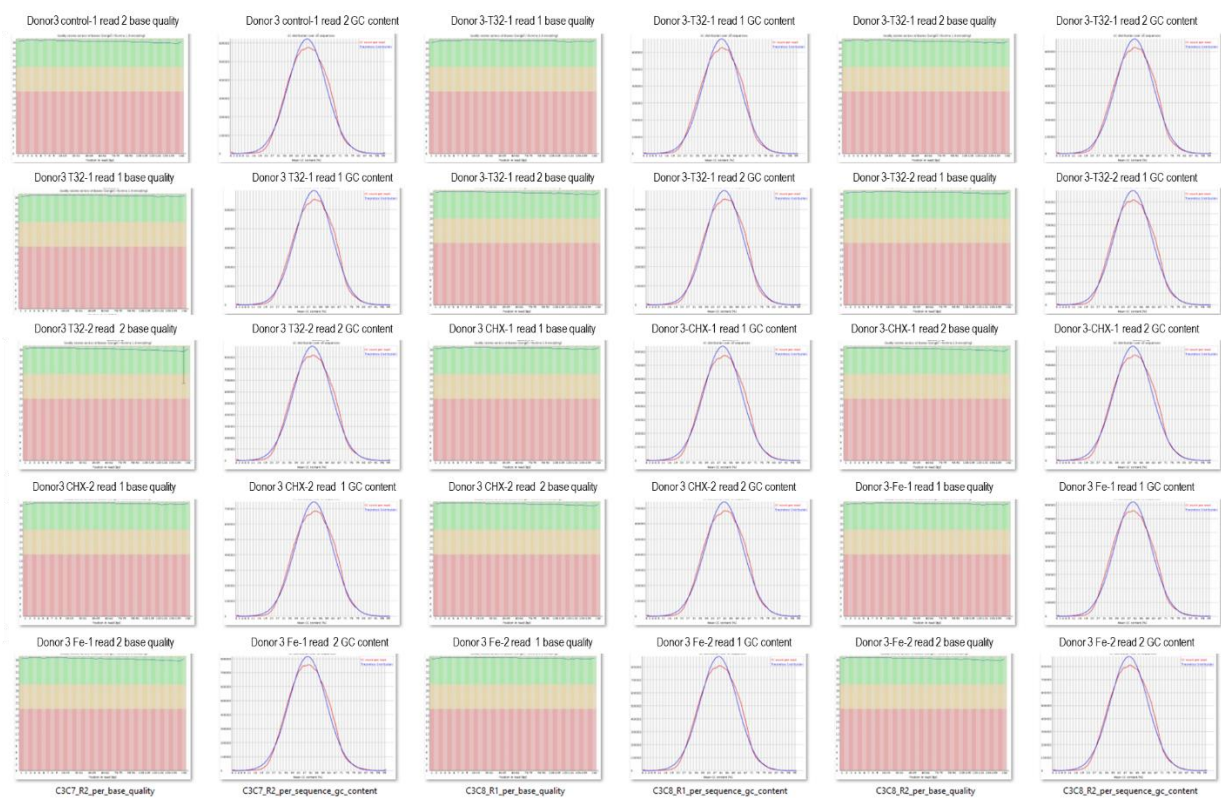
DATA SUPPLEMENT

Supplementary Tables S1-S11	<i>See separate Excel file</i>
Supplementary Figure S1	Quality scores
Supplementary Figure S2	Gene ontology (GO) enrichment analyses of genes differentially expressed in plasma-resuspended PBMCs from donors 1-3 after 1h treatment with 100µg/ml cycloheximide - -
Supplementary Figure S3	Preparation methods for PBMCs.
Supplementary Figure S4	Gene ontology (GO) enrichment analyses of genes differentially expressed in PBMCs from donors 4-6 after Wash 1 (Ficoll method) compared to plasma-resuspended PBMCs
Supplementary Figure S5	Gene ontology (GO) enrichment analyses of genes differentially expressed in PBMCs from donors 7-9 after Wash 2 (CPT™ method) compared to plasma-resuspended PBMCs
Supplementary Figure S6	Gene ontology (GO) enrichment analyses of genes differentially expressed in washed PBMCs from donors 4-9 after 10µmol/L ferric citrate for 1h
Supplementary Figure S7	KEGG terms for genes differentially expressed in washed PBMCs from donors 4-9 after 10µmol/L ferric citrate for 1h
Supplementary References	

Supplementary Figure S1: Quality scores



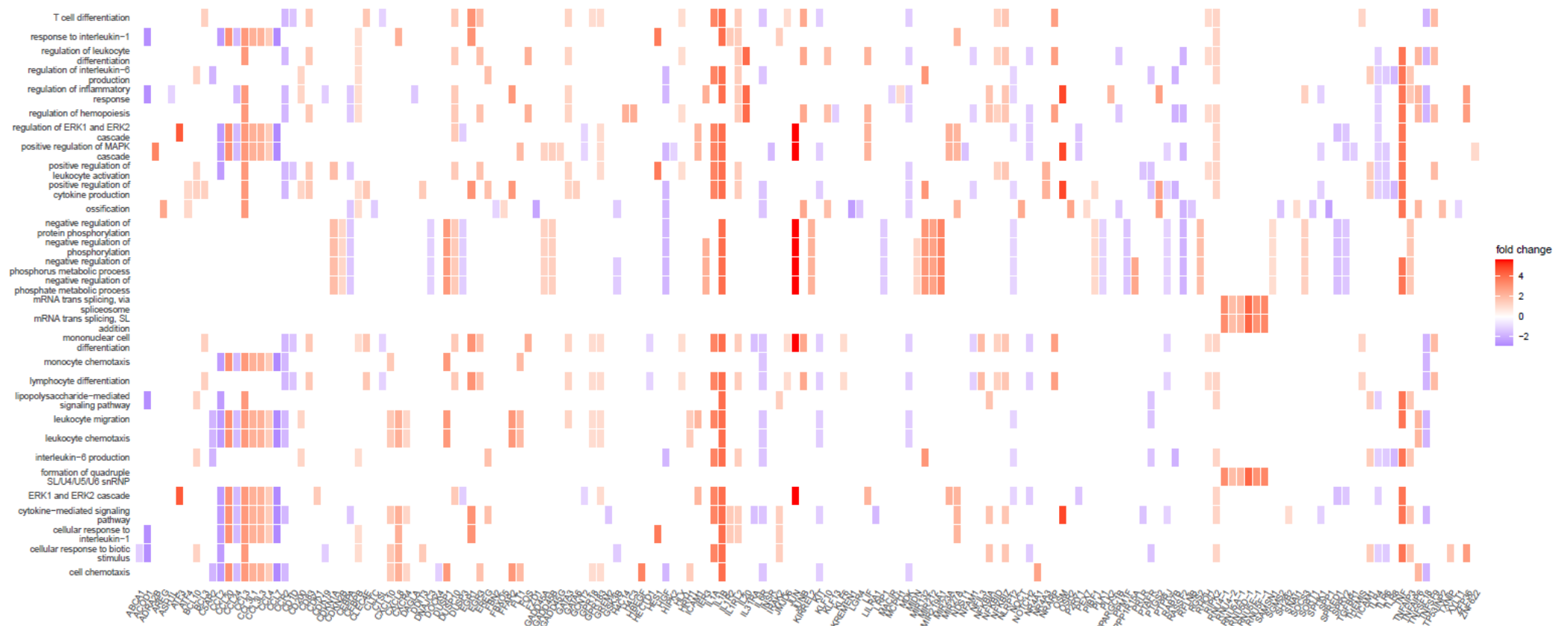
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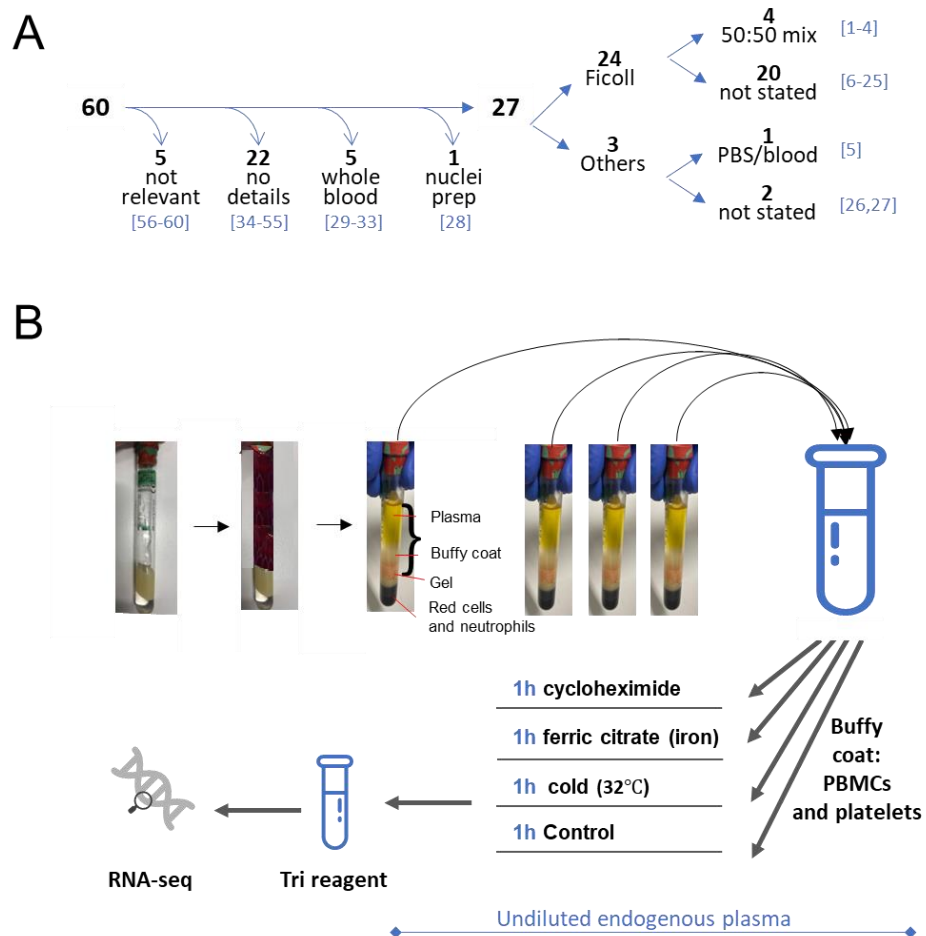
Quality scores highlighted by green, yellow and red thresholds of acceptability, and GC content (blue: theoretical distribution, red observed GC count per read) for bidirectional reads in all libraries derived from donors 1-3 peripheral blood mononuclear cells (PBMCs).

Data from all 166 genes where the adjusted p-value was $<1.5 \times 10^{-8}$ in plasma-resuspended PBMCs from donors 1-3 after 1h treatment with 100 μ g/ml cycloheximide.

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Supplementary Figure S3: Preparation methods for PBMCs.

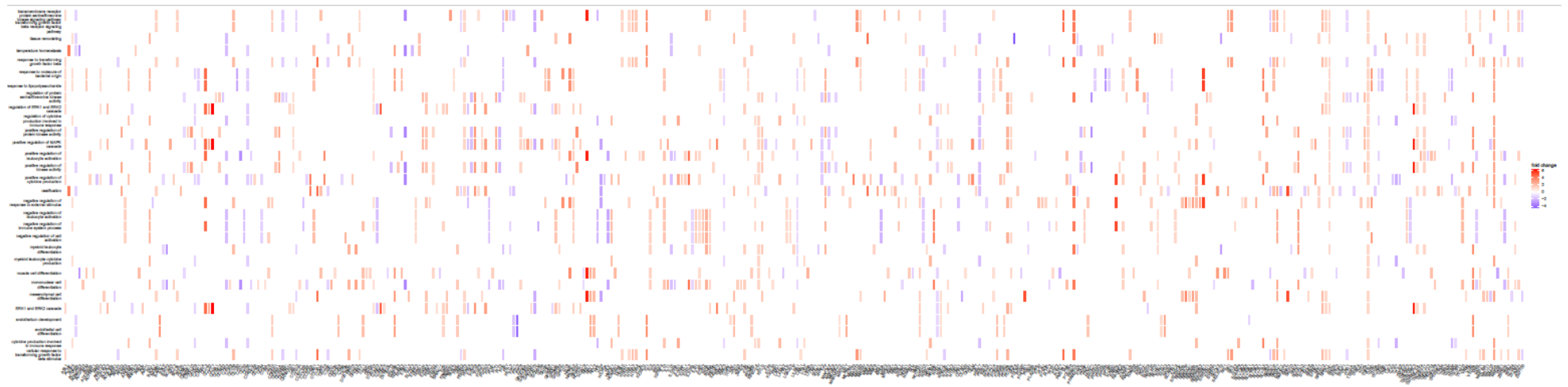


A) Flow chart indicating described methods in the 60 PubMed-listed manuscripts retrieved by “PBMC” and “RNASeq” search terms (April 2022-June 2023)[1-60]. PBS: phosphate buffered saline. **B)** Pictorial representation of protocol where PBMCs are treated in their endogenous plasma. In the reported experimental design to evaluate 4 treatments in donors 1-3, following pooling, the buffy coats were distributed equally to experimental tubes for 10 minutes prewarm, 1hr experimental treatments, centrifugation and TRI reagent lysis of the cell pellet to fix nucleic acid species.[61]

Supplementary Figure S4: Gene ontology (GO) enrichment analyses of genes differentially expressed in PBMCs from donors 4-6 after Wash 1 (Ficoll method) compared to plasma-resuspended PBMCs

Data from all 205 genes where the adjusted p-value was $<1.5 \times 10^{-8}$

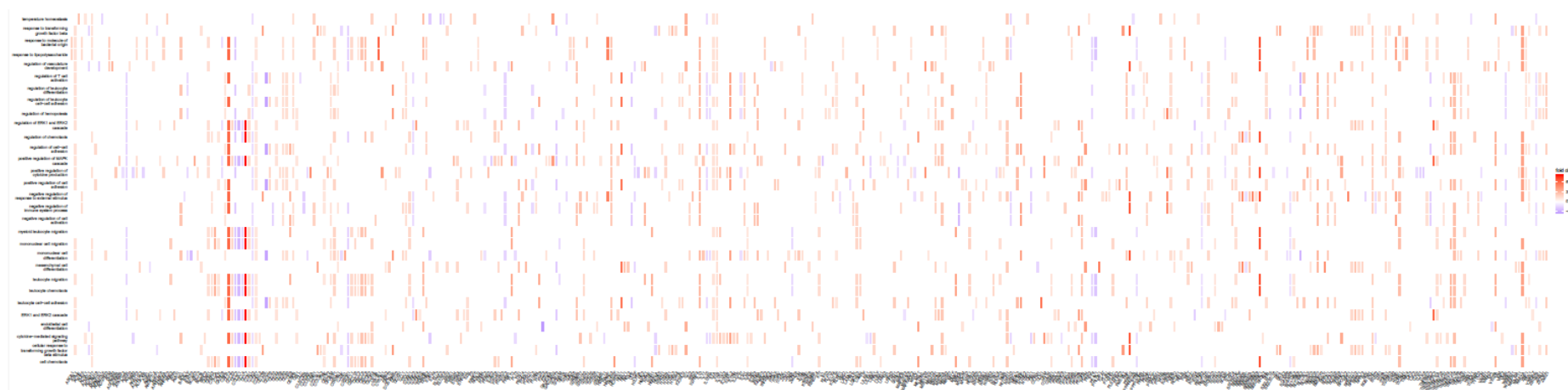
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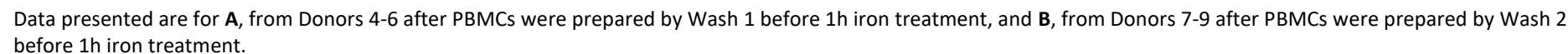
Supplementary Figure S5: Gene ontology (GO) enrichment analyses of genes differentially expressed in PBMCs from donors 7-9 after Wash 2 (CPT™ method) compared to plasma-resuspended PBMCs

Data from all 754 genes where the adjusted p-value was $<1.5 \times 10^{-8}$

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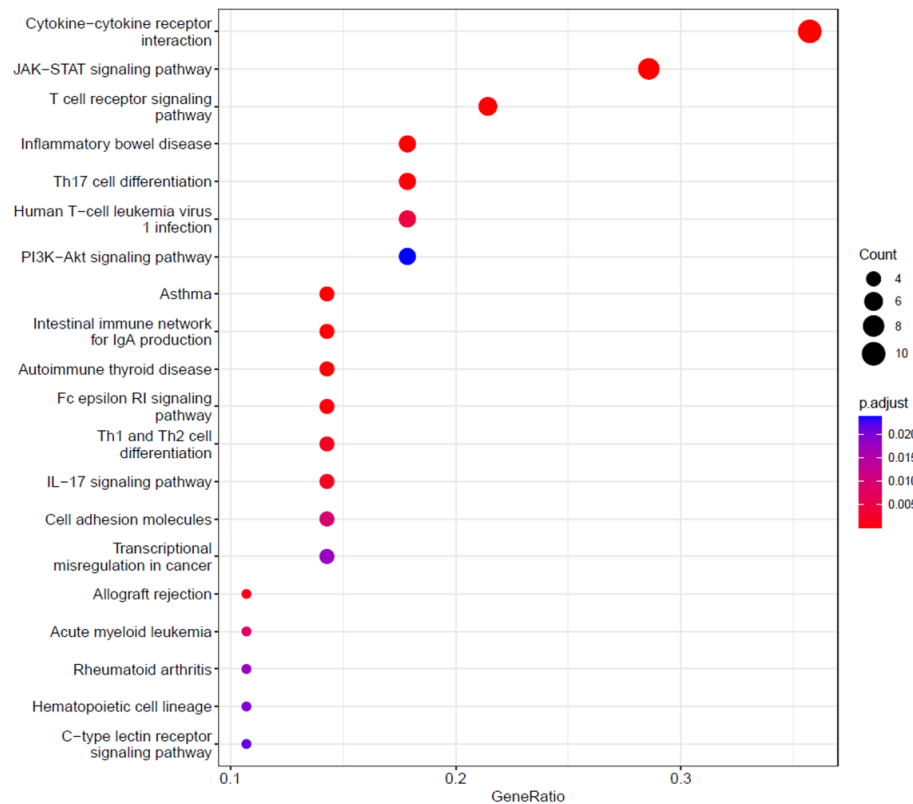
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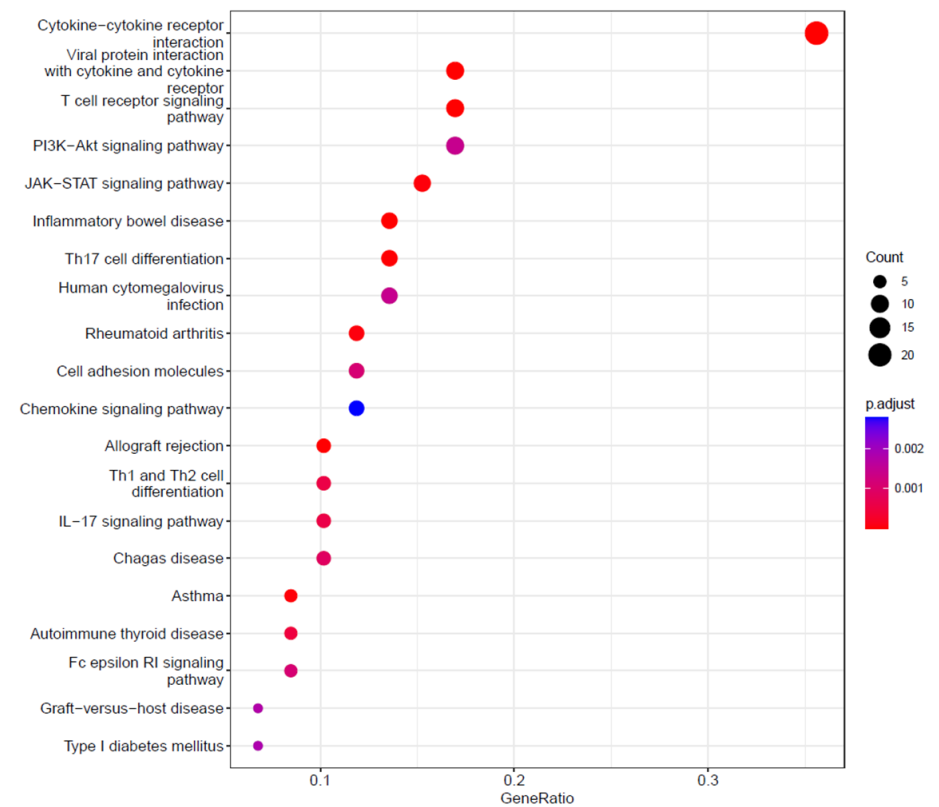
Supplementary Figure S7: KEGG terms for genes differentially expressed in washed PBMCs from donors 4-9 after 10 μ mol/L ferric citrate for 1h

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A



B



Data presented are for **A**, from Donors 4-6 after PBMCs were prepared by Wash 1 before 1h iron treatment, and **B**, from Donors 7-9 after PBMCs were prepared by Wash 2 before 1h iron treatment.

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