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Resource

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Single-cell analysis of human MAIT cell transcriptional, functional and clonal diversity

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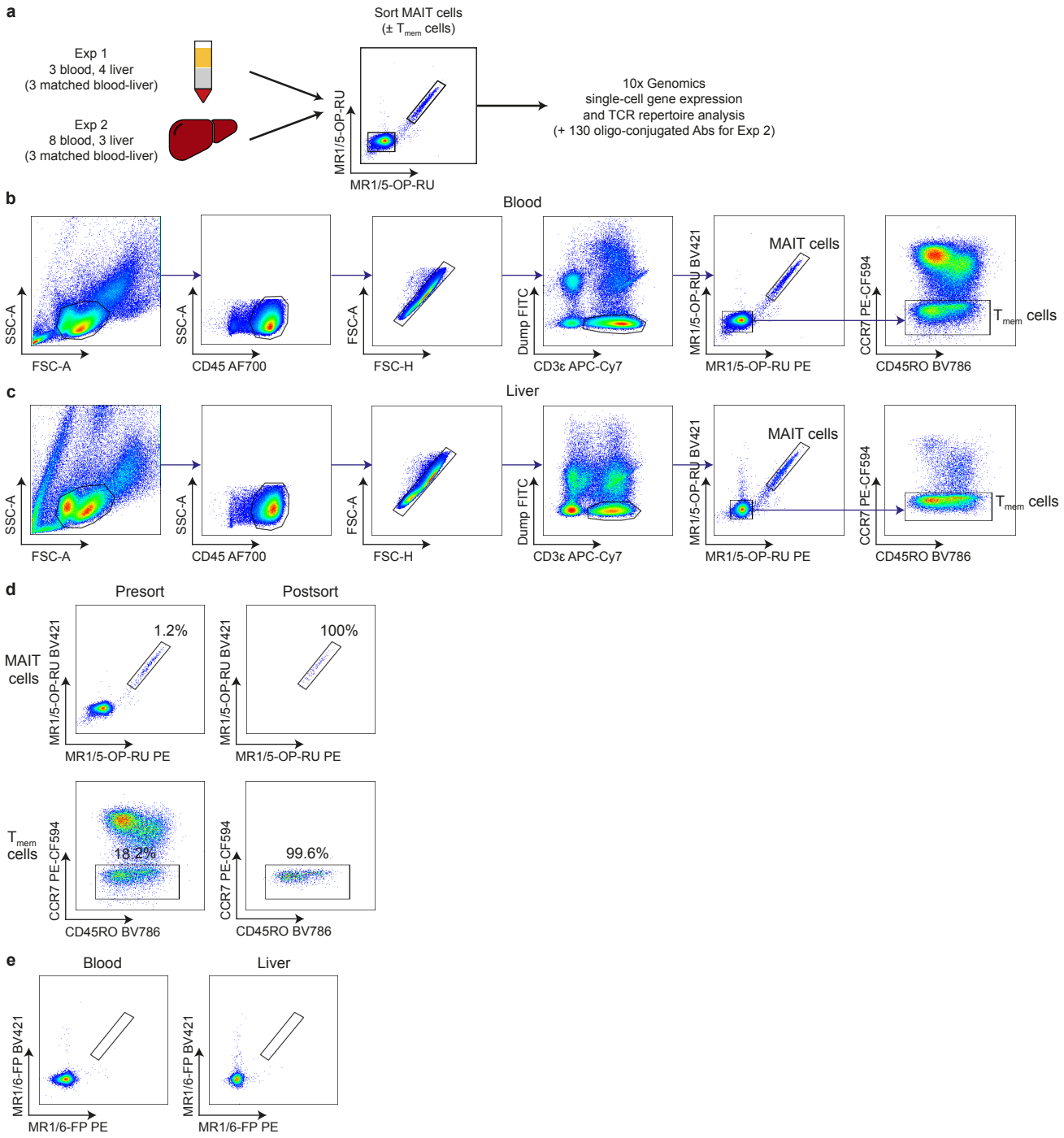
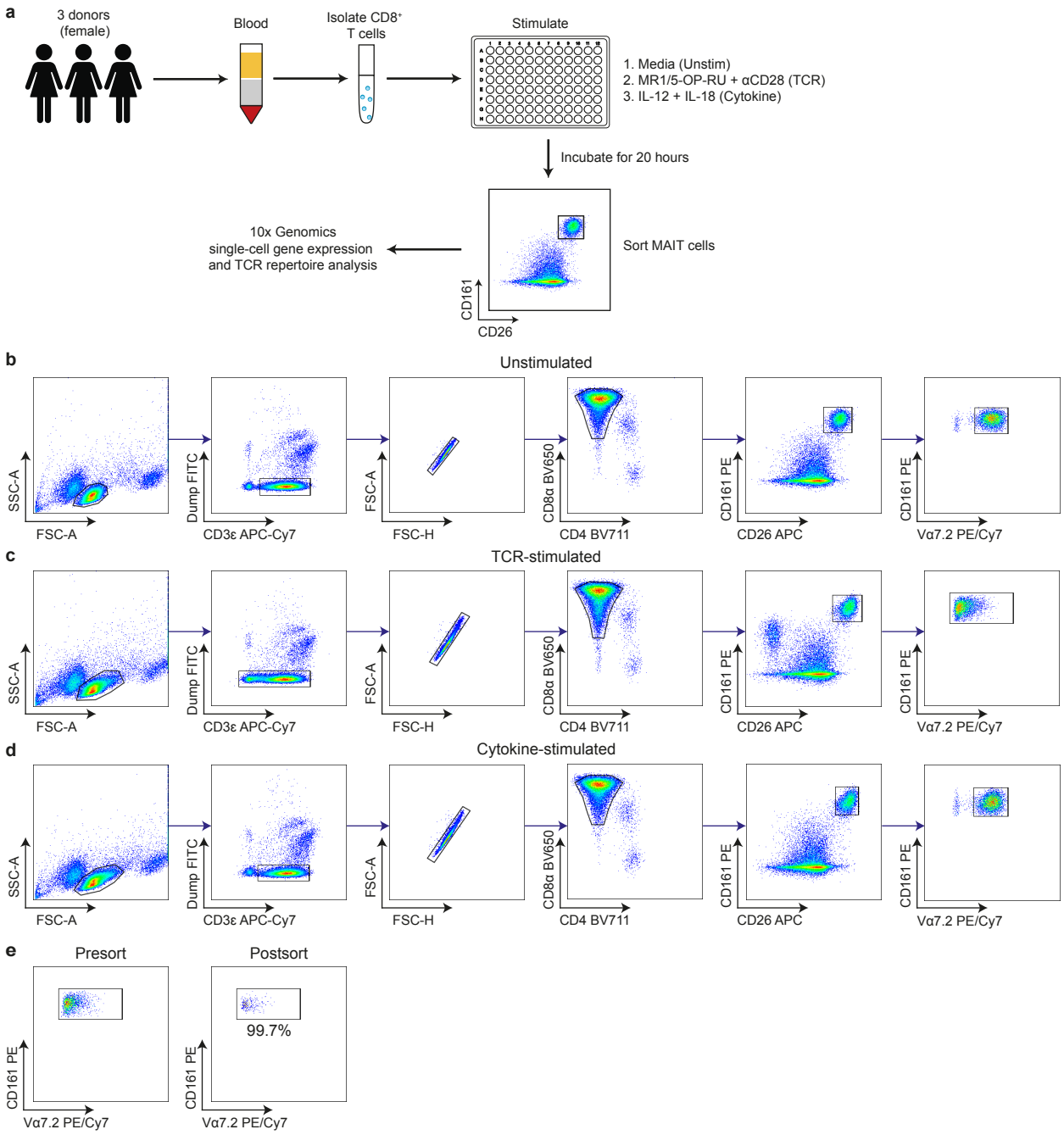
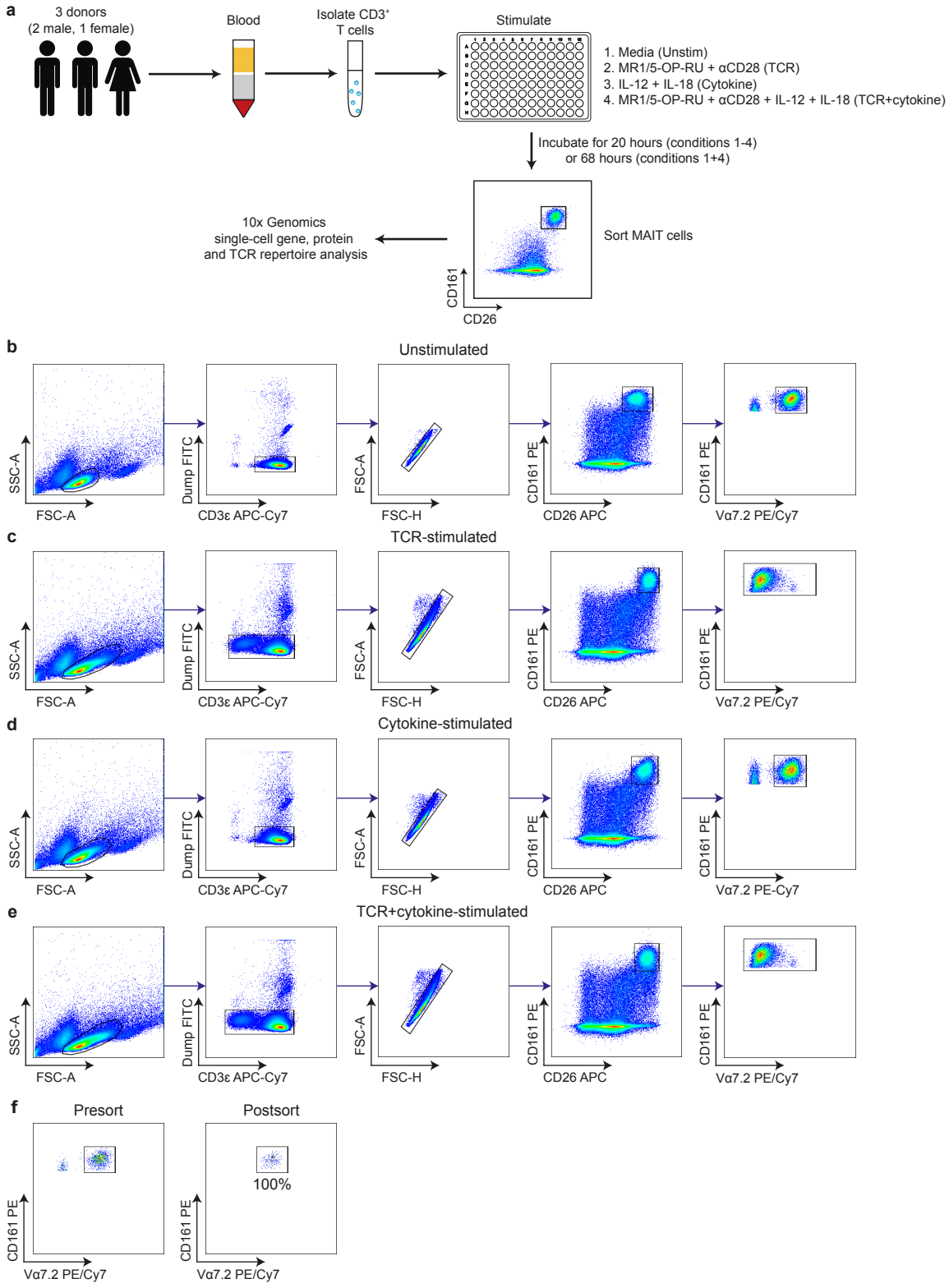


Supplementary Fig. 1 | Blood-liver experiments schematic and gating strategy. a, Schematic illustrating the experimental protocol for Exp 1 and Exp 2. **b**,**c**, Gating strategy for sorting MAIT cells ($CD3^+MR1/5\text{-}OP\text{-}RU^+$) and conventional memory T (T_{mem}) cells ($CD3^+MR1/5\text{-}OP\text{-}RU^-CCR7^-$) from matched human blood (**b**) and liver (**c**). Dump channel contained antibodies to CD14, CD19, TCR γδ, TCR Vα24-Jα18, and TCR Vδ2, plus SYTOX Green Nucleic Acid Stain. **d**, FACS plots for a representative sample showing MAIT and T_{mem} cells presort and postsort. Sort purity was > 99%. **e**, FACS plots for representative blood and liver samples stained with the negative control MR1/6-FP tetramer.



Supplementary Fig. 2 | Exp 3 schematic and gating strategy. a, Schematic illustrating the experimental protocol for Exp 3. **b**-**d**, Gating strategy for sorting MAIT cells ($CD3^+CD8^+CD26^+CD161^{hi}V\alpha7.2^+$) from unstimulated (**b**), TCR-stimulated (**c**), and cytokine-stimulated (**d**) isolated $CD8^+$ cells. Due to TCR downregulation within the TCR stimulation condition (**c**), MAIT cells were sorted as $CD8^+CD26^+CD161^{hi}$ lymphocytes. The $CD26^-CD161^{int}$ lymphocytes are $CD3^-CD8^{low}$ and are likely to be NK cells. Dump channel contained antibodies to CD14, CD19, TCR γδ, TCR Vα24-Jα18, and TCR Vδ2, plus SYTOX Green Nucleic Acid Stain. **e**, FACS plots for a representative sample (TCR stimulation condition) showing MAIT cells presort and postsort. Sort purity was > 99%.



Supplementary Fig. 3 | Exp 4 schematic and gating strategy. a, Schematic illustrating the experimental protocol for Exp 4. **b**-**e**, Gating strategy for sorting MAIT cells ($CD3^+CD26^+CD161^{hi}V\alpha7.2^+$) from unstimulated (**b**), TCR-stimulated (**c**), cytokine-stimulated (**d**), and TCR+cytokine-stimulated (**e**) isolated $CD3^+$ cells. Due to TCR downregulation within the TCR and TCR+cytokine stimulation conditions (**c**, **e**), MAIT cells were sorted as $CD26^+CD161^{hi}$ lymphocytes. Dump channel contained antibodies to CD14, CD19, TCR γδ, TCR Vα24-Jα18, and TCR Vδ2, plus SYTOX Green Nucleic Acid Stain. **f**, FACS plots for a representative sample (unstimulated condition) showing MAIT cells presort and postsort. Sort purity was > 99%.