

Med, Volume 6

Supplemental information

Diagnosing recipient- vs. donor-derived posttransplant myelodysplastic neoplasm via targeted single-cell mutational profiling

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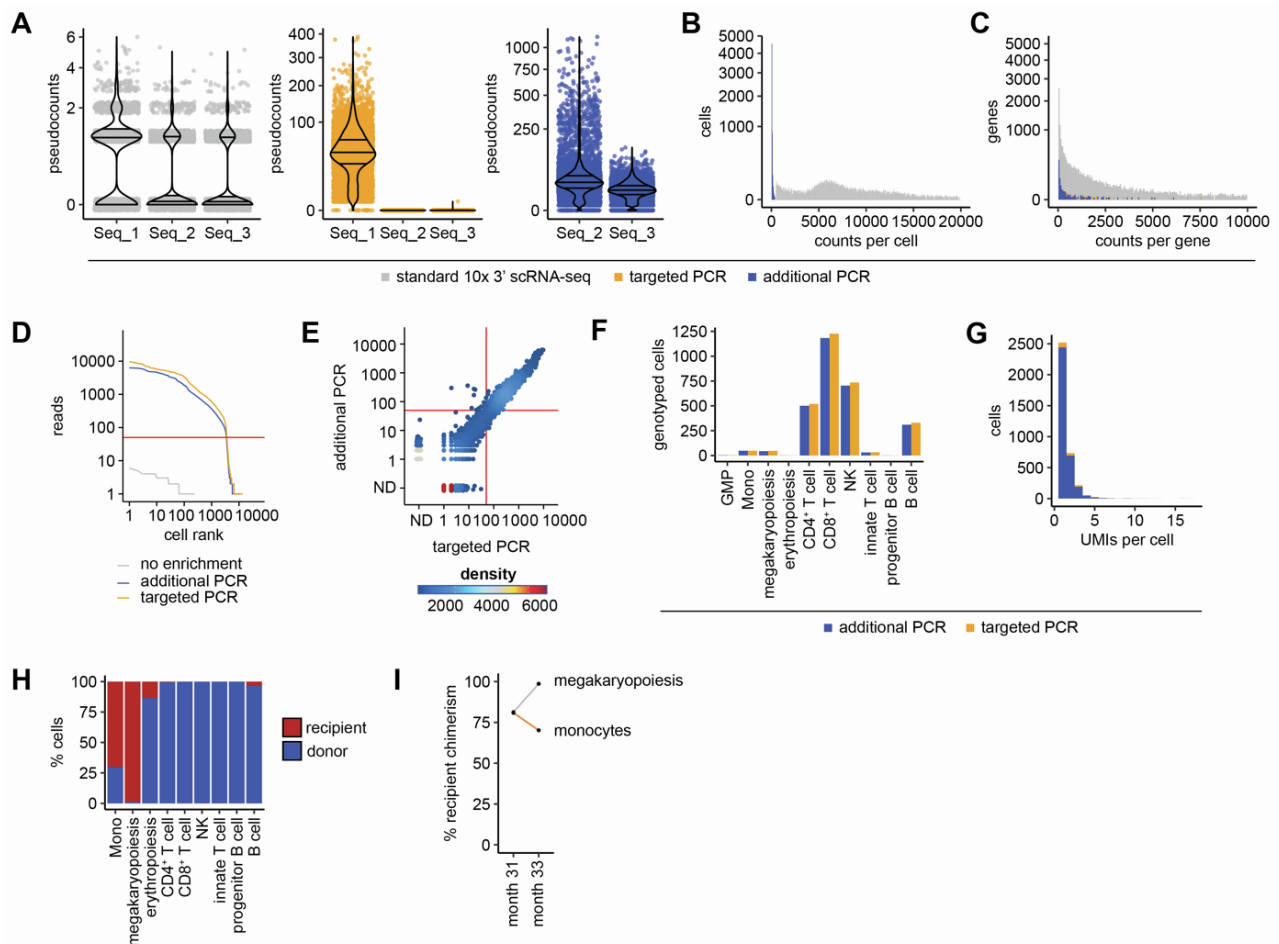


Figure S1. Deconvolution of recipient- and donor-derived cells, related to Figure 4. (A) Pseudocounts of overhang sequences used for construction of targeted (Seq_1) and additional PCR (Seq_2 and Seq_3) libraries quantified in native 3' scRNA-seq (grey), targeted (yellow) and additional PCR (blue) amplicon libraries. **(B, C)** Distribution of non-*U2AF1* counts per cell (B) and counts per non-*U2AF1* gene (C) in each library. **(D)** Knee plot demonstrating sequencing coverage of *U2AF1*^{S34Y} locus across single cell profiles for native 3' scRNA-seq data (grey) and amplicon data after targeted (yellow) or additional (blue) PCR amplification strategy. Red line at sequencing depth of 50 reads indicates cut-off for cells considered high-quality and utilized for downstream analyses of amplicon data. **(E)** Sequencing depth of *U2AF1*^{S34Y} locus for both amplicon libraries across all detected cell barcodes. The color indicates the density of cell barcodes. ND: not detectable. **(F, G)** Number of genotyped cells for *U2AF1*^{S34Y} locus using both PCR amplification strategies (yellow: targeted, blue: additional PCR) across major cell types (F) and number of UMIs detected for molecules covering the *U2AF1*^{S34Y} locus per cell (G). **(H)** Percentage of recipient- (red) and donor-derived (blue) cells across major cell types at 33 months post AA diagnosis. **(I)** Percentage of recipient-derived chimerism in monocytes and megakaryopoietic cells at month 31 and 33 following diagnosis of AA and onset of MDS.

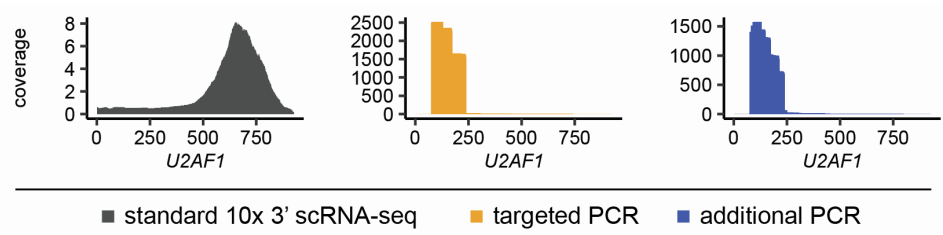


Figure S2. Pseudobulk coverage of reads mapping to *U2AF1*, related to Figure 4. The targeted (yellow) and additional PCR (blue) amplicon libraries show high sequence specificity and enrichment at the *U2AF1*^{S34Y}-specific locus compared to the native 3' scRNA-seq library (grey). Note that the coverage indicates the number of raw sequence reads (x1000) mapping to genomic regions of coding exons + 3' UTR of the *U2AF1* gene (in bp from 5' to 3').

FLOW CYTOMETRY

Parameter	Cutoff	Value	Points
CD34+ myeloblast-related cluster size	$\geq 2\%$	0.69%	0
B-progenitor-related cluster size	$\leq 5\%$	56.30%	0
Myeloblast CD45 expression (ratio)	≤ 4	6.87	0
Granulocyte side scatter value (ratio)	≤ 6	8.20	0
Total Della Porta Score			0



MOLECULAR ASSESSMENT

- CD34+ donor chimerism: 100%
- Mutational status: WT

Figure S3. Remission of MDS following second allo-HSCT, related to Figure 1. Flow cytometry monitoring shows normal results for all four components of the Della-Porta scoring system²⁰, suggesting complete resolution of previously detected MDS-associated clinical parameters. Mutational status shows absence of formerly present MDS-typical mutations.