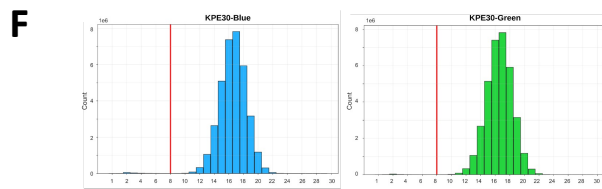
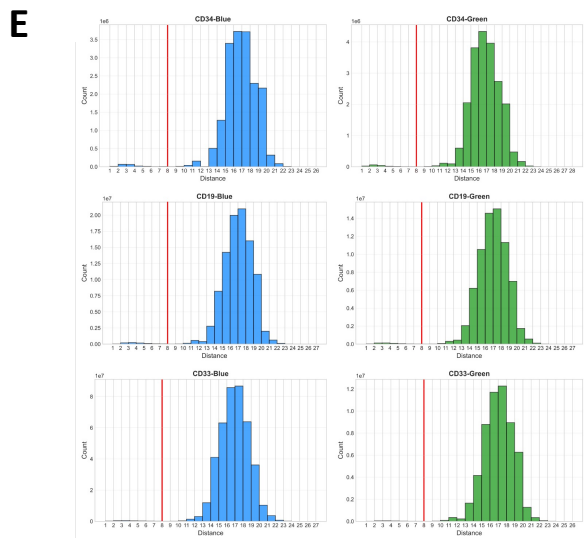
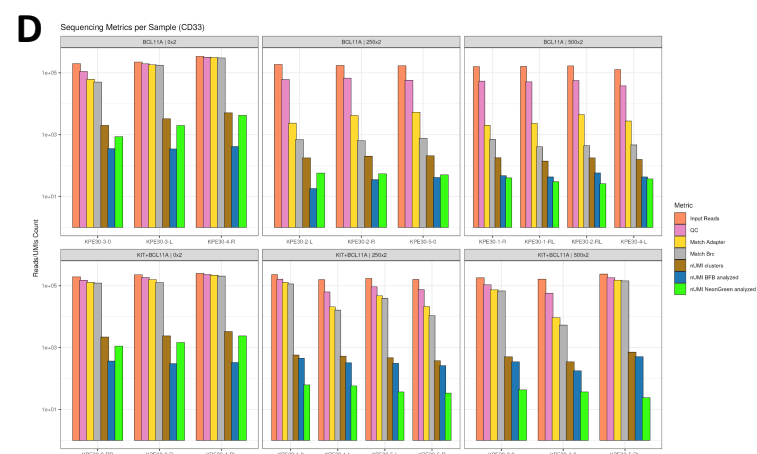
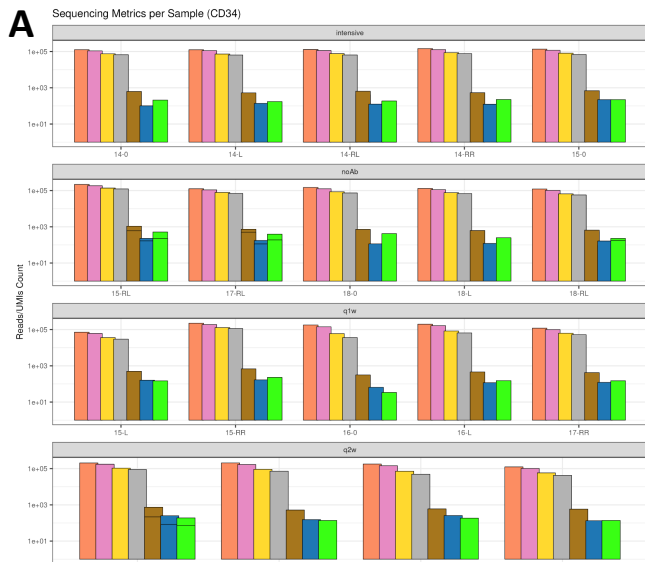

Supplementary information

Non-genotoxic transplantation and in vivo selection through epitope editing

In the format provided by the
authors and unedited

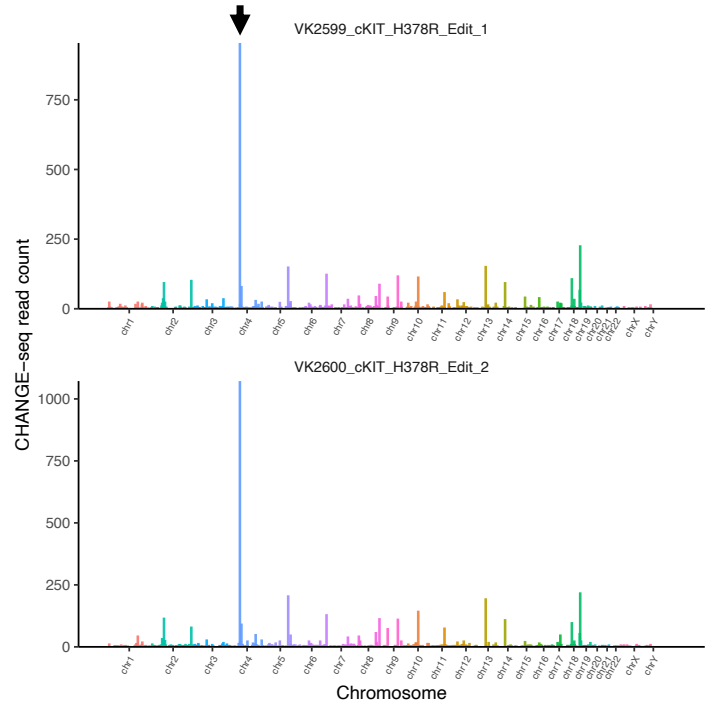
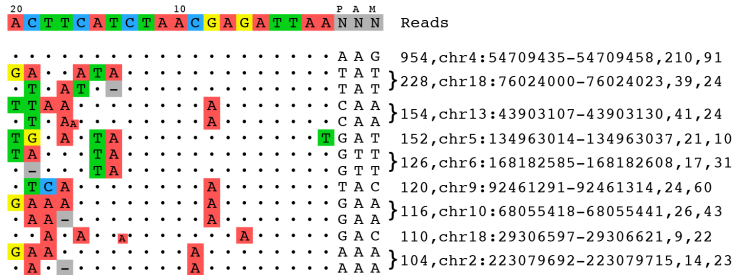


Supplementary figure 1. Bioinformatic processing and quality control of genomic barcodes

(A-D) Read retention across sequential filtering and annotation steps for sorted BM cells. Bar plots report the number of reads retained at each stage of the preprocessing and filtering pipeline. Shown are the total input reads (Input Reads), reads passing initial quality control (QC), reads matching the forward primer adapter (Match Adapter), reads with a valid barcode assignment (Match Brc), and the number of UMI clusters after distance-based clustering (nUMI cluster). For fluorescence-resolved experiments, the number of UMI clusters supported by more than one read and retained for downstream analysis is shown separately for mTagBFP-labeled and mNeonGreen-labeled populations. Plots report data for (A) lin-CD34⁺ sorted cells from the experiment described in Fig.2C; (C) CD19⁺ sorted cells from the experiment described in Fig.2C; (B) CD33⁺ sorted cells from the experiment described in Fig.2C; (D) Bulk BM cells from the experiment described in Fig.5A. **(E,F)** Pairwise UMI distance distributions used to define clustering thresholds. Histograms show the distribution of pairwise Levenshtein distances between UMI sequences prior to clustering. A clear bimodal structure is observed, with a first mode corresponding to closely related UMIs likely arising from sequencing or PCR errors and a second mode reflecting genuinely distinct barcode sequences. The vertical dashed line indicates the selected distance threshold (Levenshtein distance = 8) used for DBSCAN-based UMI clustering. (E) Data relative to the sorted cells from the experiment described in Fig.2C; (F) Data relative to bulk BM cells from the experiment described in Fig.5A.

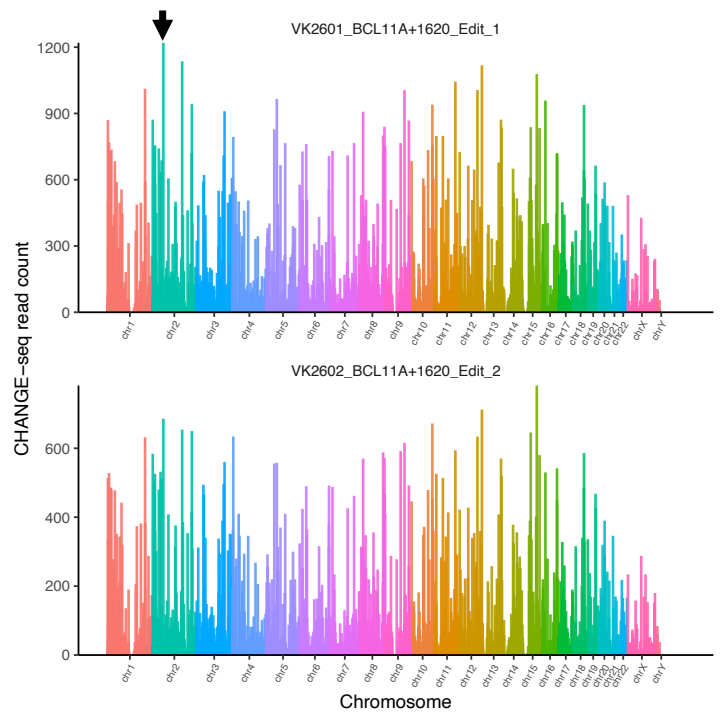
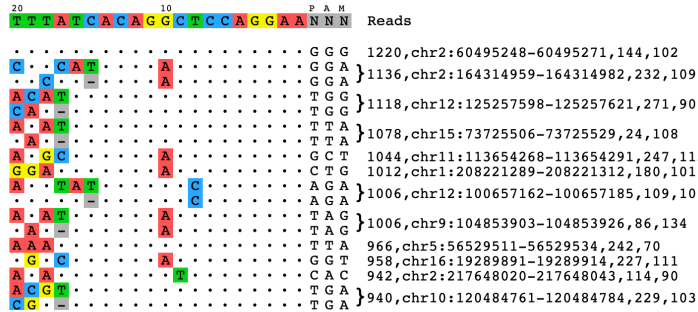
A CHANGE-seq-BE - KIT^{H387R} BE (KIT-Y sgRNA)

VK2599_cKIT_H378R_Edit_1

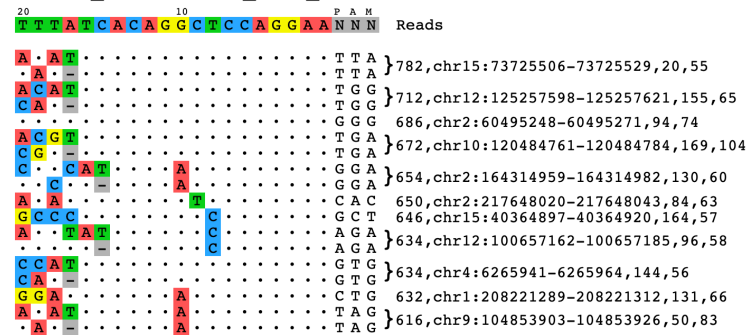


B CHANGE-seq-BE - BCL11A⁺⁵⁸ BE (1620 sgRNA)

VK2601_BCL11A+1620_Edit_1



VK2602_BCL11A+1620_Edit_2

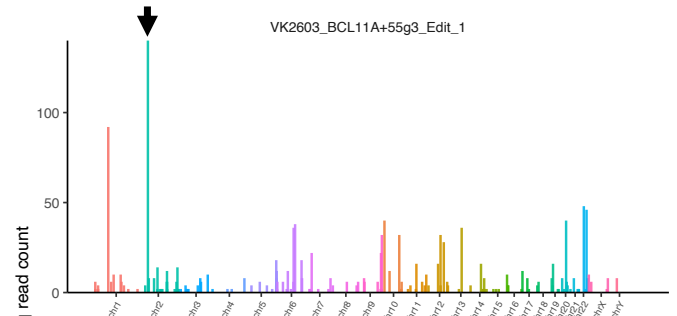
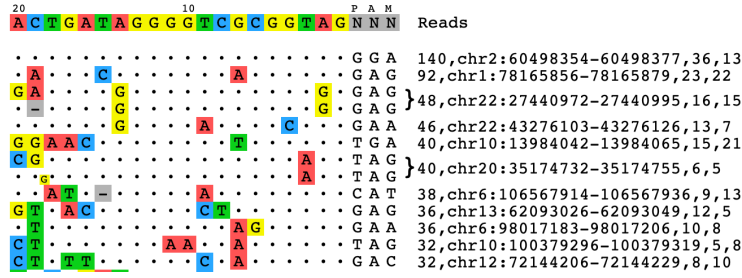


Supplementary figure 2. CHANGE-Seq-BE and CHANGE-Seq nominate potential off-target sites of KIT and BCL11A editing

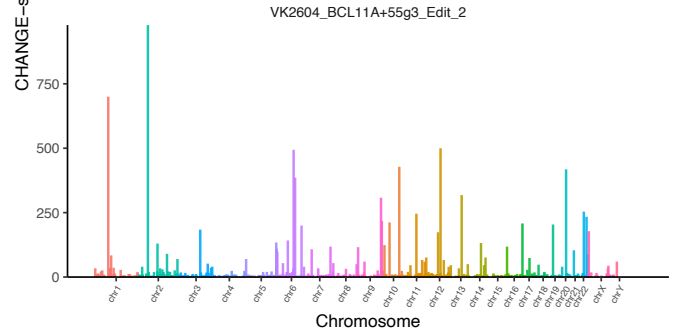
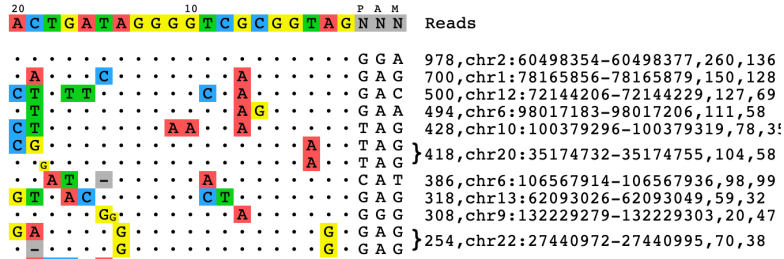
(A) Top off-targets nominated by CHANGE-Seq-BE for KITH378R sgRNA-Y in combination with SpRY-ABE8e. (B) Top off-targets nominated by CHANGE-Seq-BE for BCL11A+58 sgRNA-1620 in combination with SpRY-ABE8e. For all conditions, results from N=2 replicates are reported.

A CHANGE-seq-BE - BCL11A⁺⁵⁵ BE (sg3 sgRNA)

VK2603_BCL11A+55g3_Edit_1

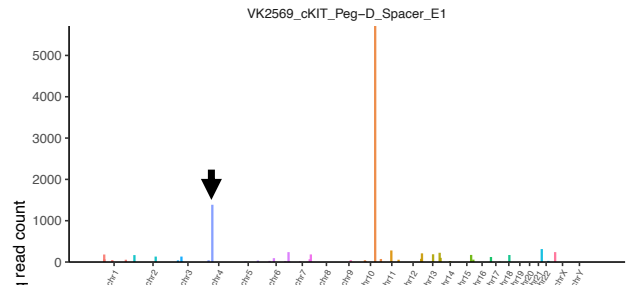
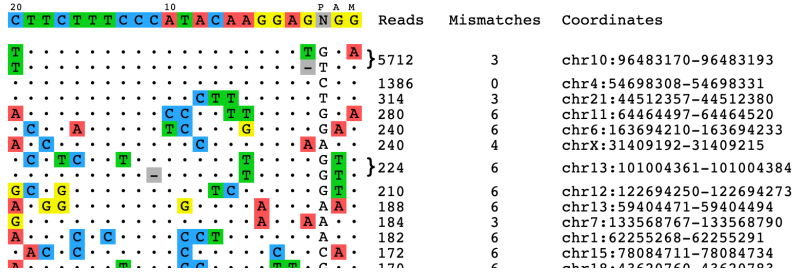


VK2604_BCL11A+55g3_Edit_2

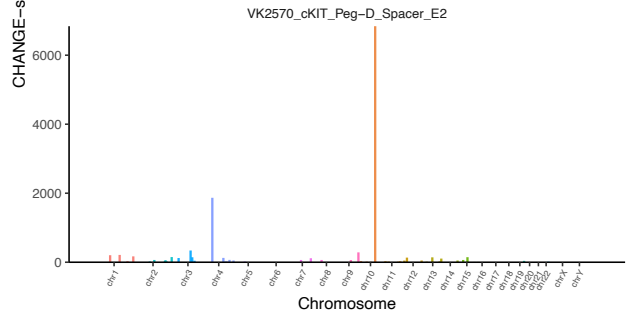
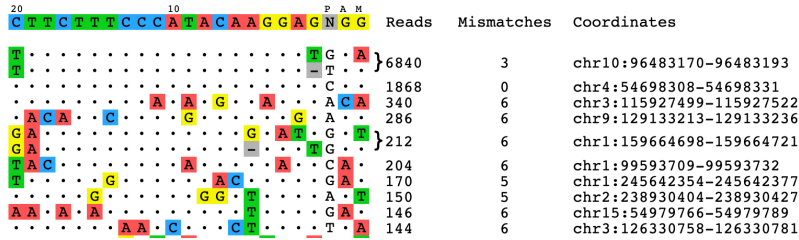


B CHANGE-seq - KIT^{D121L} peg-D spacer

VK2569_cKIT_Peg-D_Spacer_E1



VK2570_cKIT_Peg-D_Spacer_E2

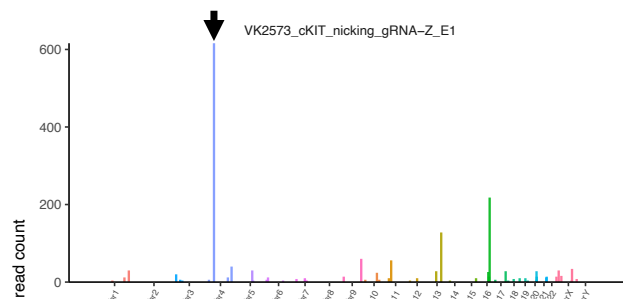
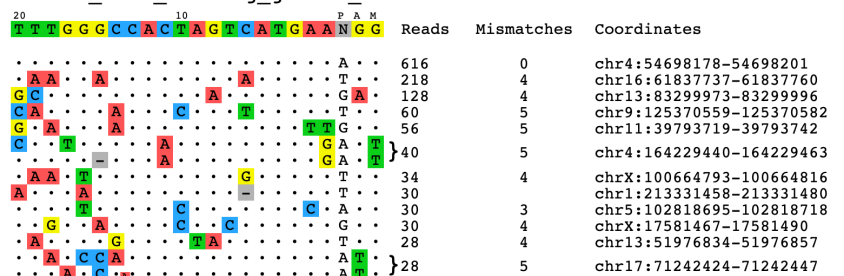


Supplementary figure 3. CHANGE-Seq-BE and CHANGE-Seq nominate potential off-target sites of KIT and BCL11A editing

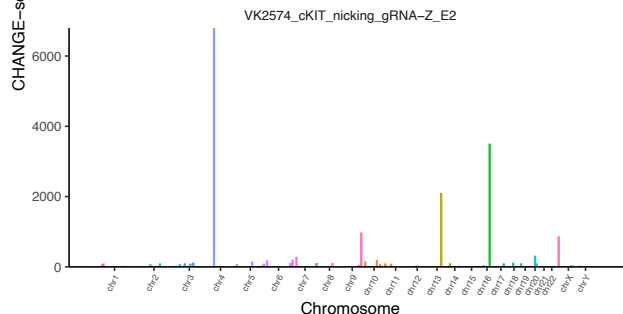
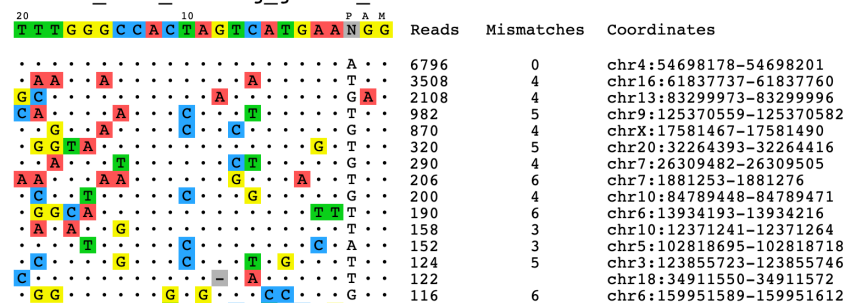
(A) Top off-targets nominated by CHANGE-Seq-BE for BCL11A+55 sgRNA-3 in combination with SpRY-ABE8e. (B) Top off-targets nominated by CHANGE-Seq for KITD121L pegRNA-D spacer. For all conditions, results from N=2 replicates are reported.

CHANGE-seq - KIT^{D121L} nicking guide-Z

VK2573 cKIT nicking gRNA-Z E1



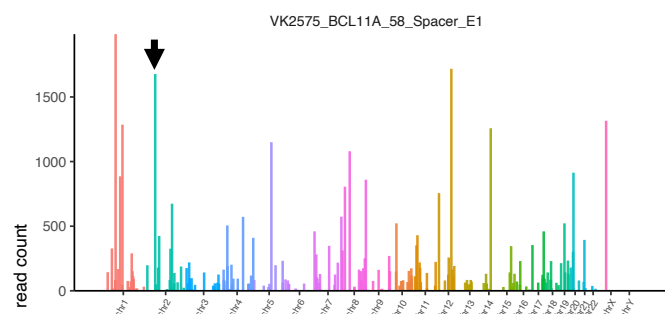
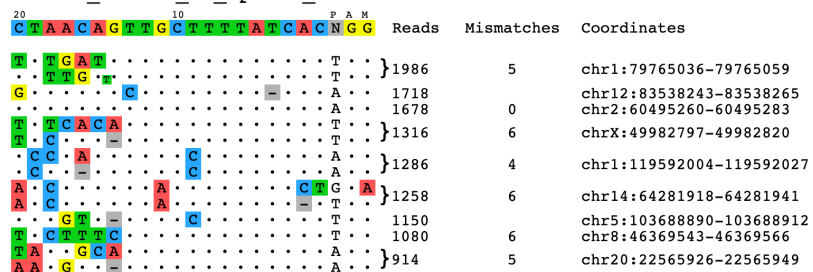
VK2574 cKIT nicking gRNA-Z E2



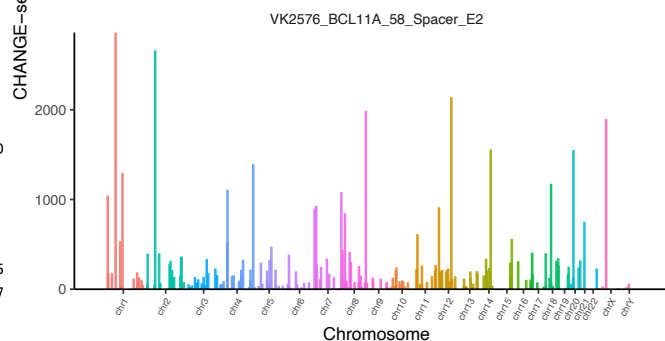
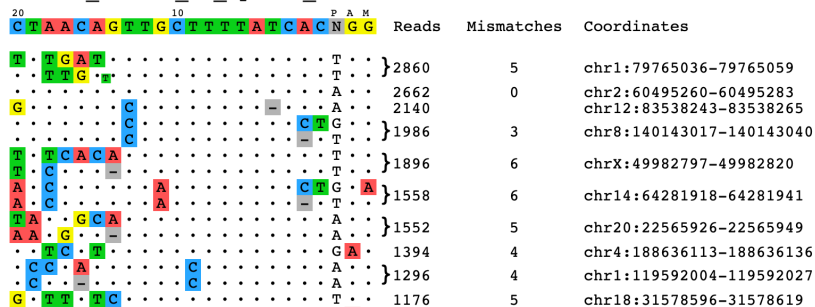
B

CHANGE-seq - BCL11A⁺⁵⁸ pegRNA spacer

VK2575 BCL11A 58 Spacer E1



VK2576_BCL11A_58_Spacer_E2



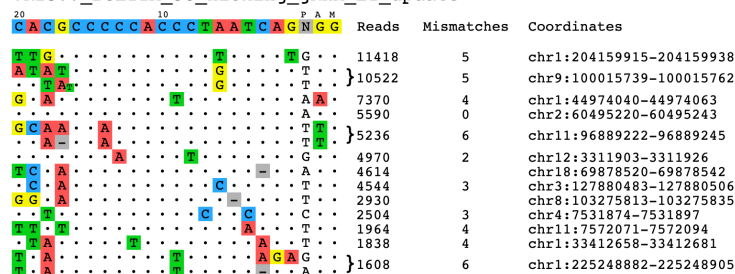
Supplementary figure 4. CHANGE-Seq-BE and CHANGE-Seq nominate potential off-target sites of KIT and BCL11A editing

(A) Top off-targets nominated by CHANGE-Seq for KIT nicking gRNA-Z. (B) Top off-targets nominated by CHANGE-Seq for BCL11A+58 pegRNA spacer. For all conditions, results from N=2 replicates are reported.

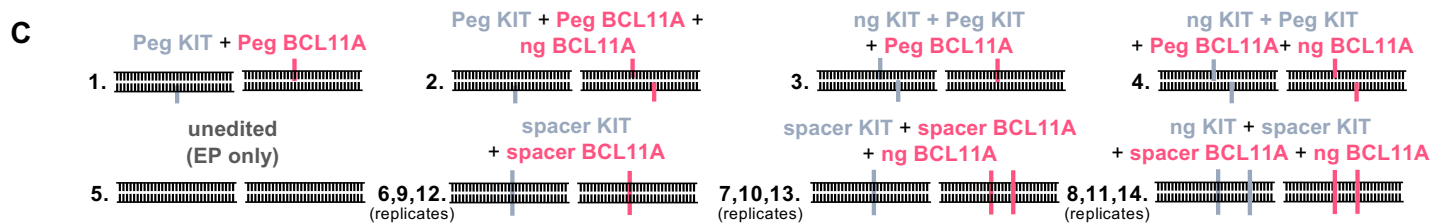
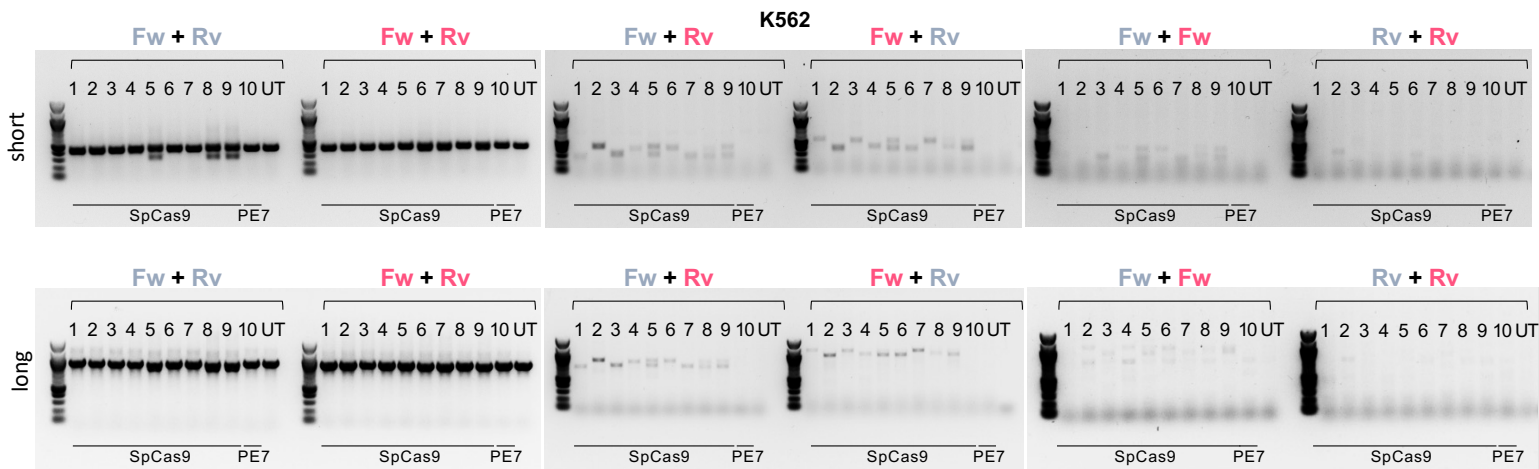
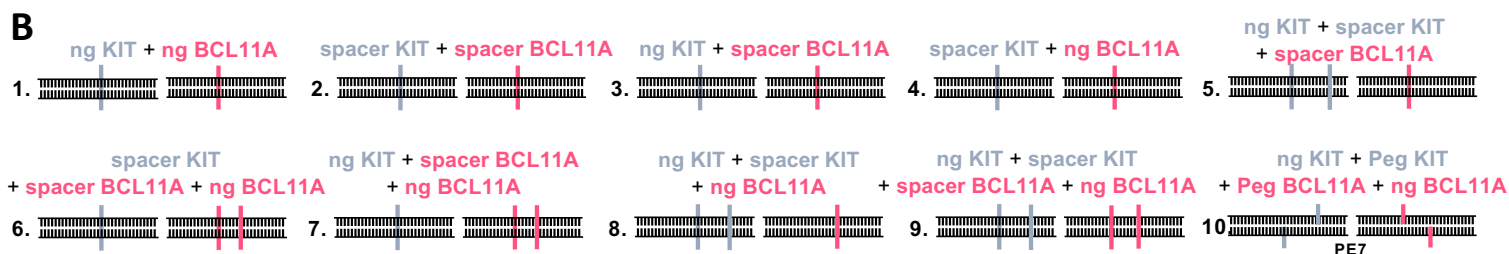
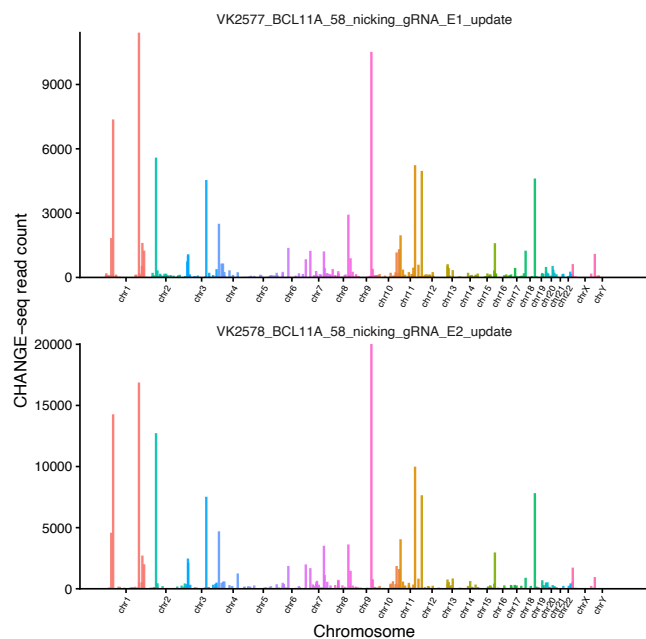
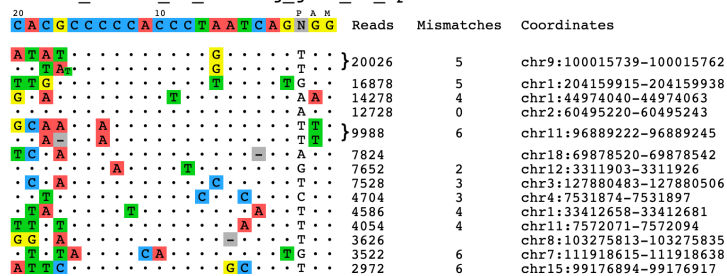
A

CHANGE-seq - BCL11A⁺⁵⁸ nicking guide

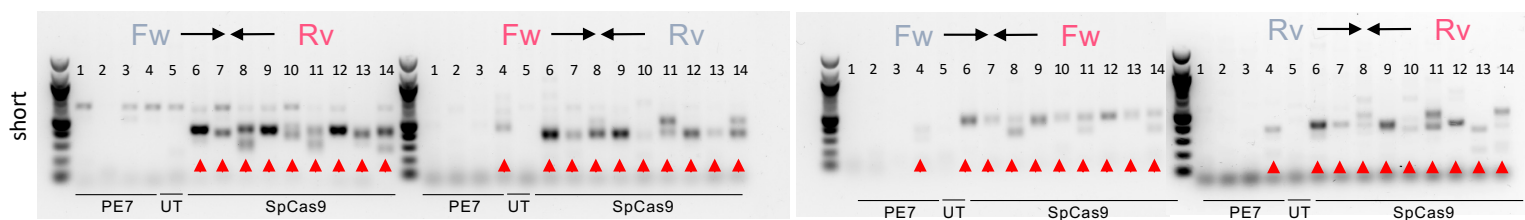
VK2577_BCL11A_58_nicking_gRNA_E1_update



VK2578_BCL11A_58_nicking_gRNA_E2_update



CD34+ HSPCs



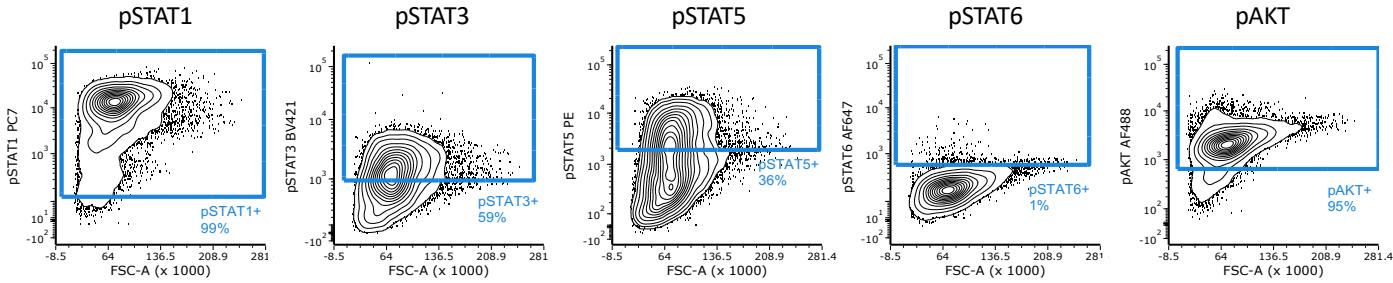
Supplementary figure 5. CHANGE-Seq nominates potential off-target sites for BCL11A prime editing and Uncropped agarose gel images for PCR-mediated detection of PE-induced translocations

(A) Top off-targets nominated by CHANGE-Seq for BCL11A+58 nicking gRNA. Results from N=2 replicates are reported.

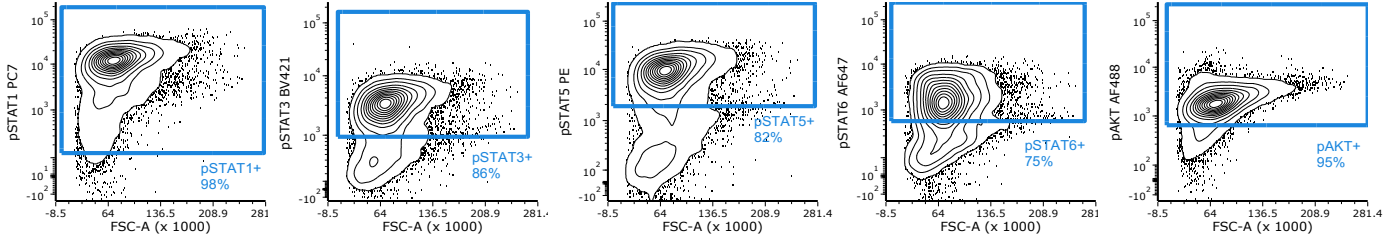
(B-C) Uncropped agarose gel images for PCR-mediated detection of PE-induced translocations (compare Extended Figure 6D-G). (B) Editing conditions (gRNA combinations) evaluated on K562 cells to assess detection of chromosomal translocations between KIT and BCL11A loci. Uncropped agarose gel electrophoresis of PCR products generated using short- and long- amplicon primer combinations (top and bottom, respectively) (C) Editing conditions evaluated in edited CD34⁺ HSPCs to assess detection of KIT–BCL11A translocations in primary cells. Uncropped agarose gel electrophoresis of PCR products generated using short-amplicon primer pairs.

KIT-D PE3
donor 1

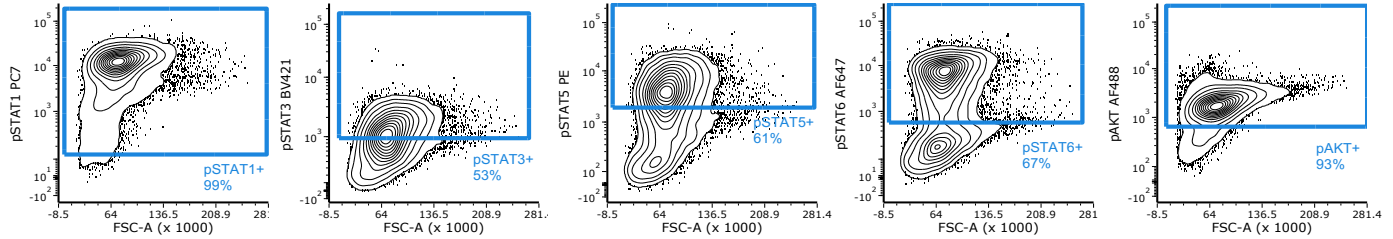
unstim.



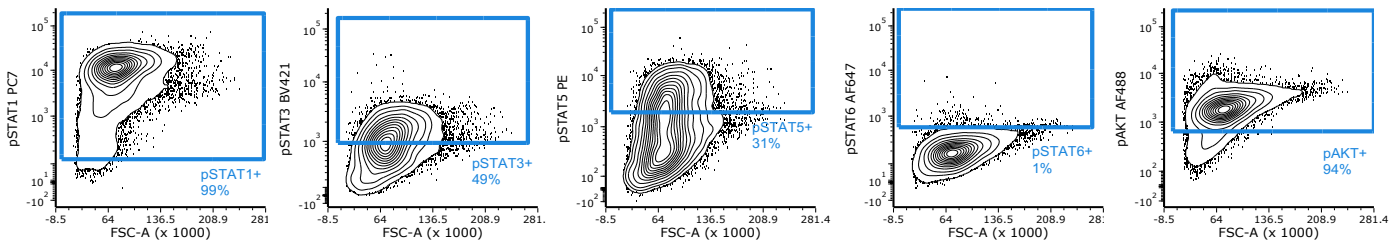
IFN β



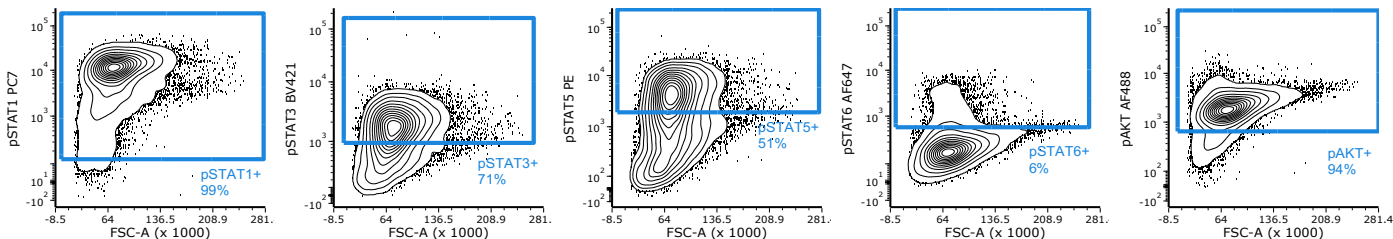
IL-4



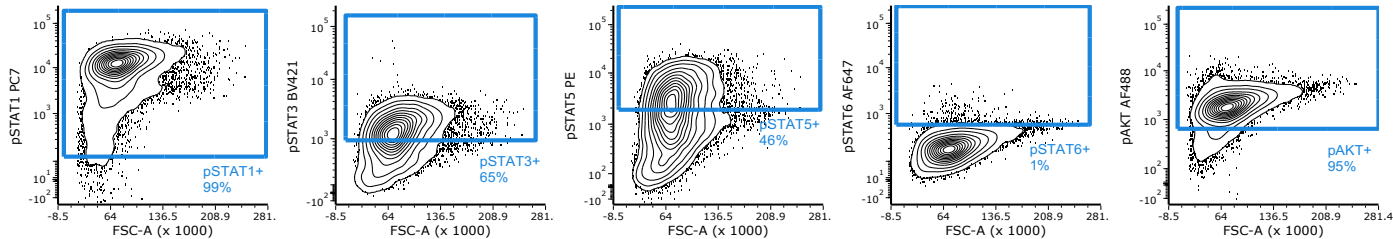
SCF



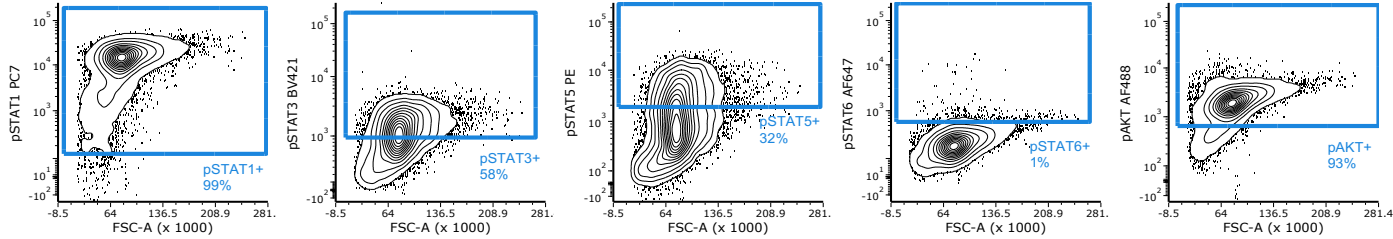
G-CSF



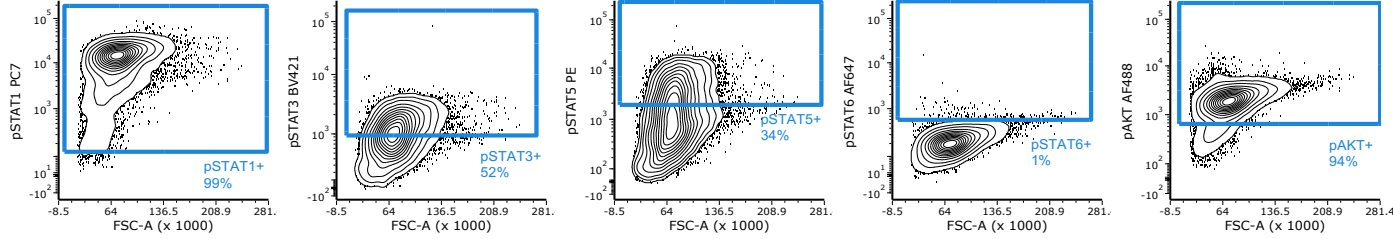
IFN γ



PMA/
iono



LPS

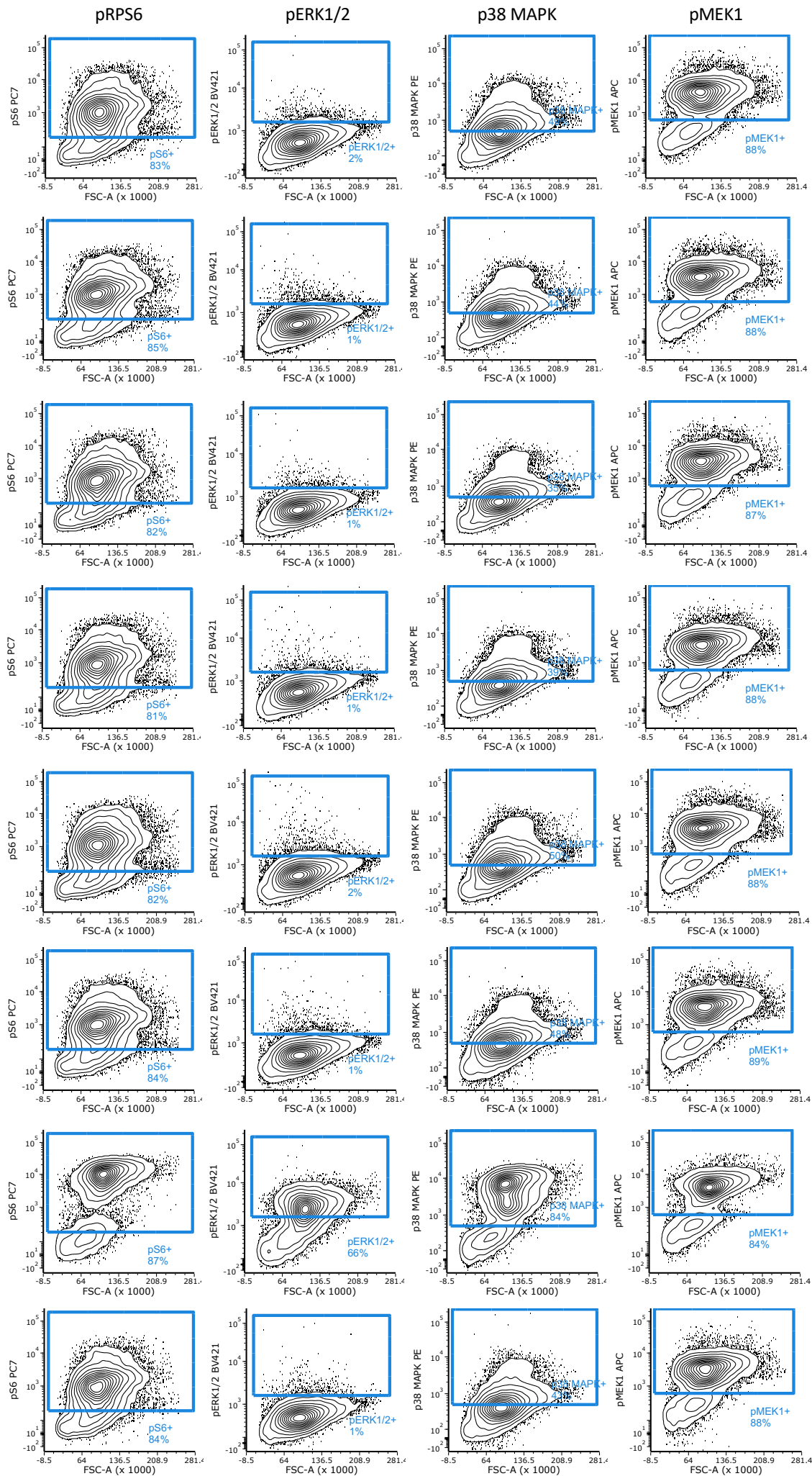


Supplementary figure 6. Phosphoflow analysis of differentiated myeloid cells derived from edited CD34+ HSPCs – Flow panel 1

Representative flow cytometry plots of the phosphoflow analysis of differentiated myeloid cells derived from edited CD34+ HSPCs prime edited for KITD121L PE or electroporation only reported in Extended Figure 4L. The plots for KITD121L PE3 condition of healthy donor 1 are reported here. Rows represent different stimuli, while columns are individual intracellular phospho-proteins, as labeled above each column.

KIT-D PE3
donor 1

unstim.



Supplementary figure 7. Phosphoflow analysis of differentiated myeloid cells derived from edited CD34+ HSPCs – Flow panel 2

Representative flow cytometry plots of the phosphoflow analysis of differentiated myeloid cells derived from edited CD34+ HSPCs prime edited for KITD121L PE or electroporation only reported in Extended Figure 4L. The plots for KITD121L PE3 condition of healthy donor 1 are reported here. Rows represent different stimuli, while columns are individual intracellular phospho-proteins, as labeled above each column.