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Supplementary information

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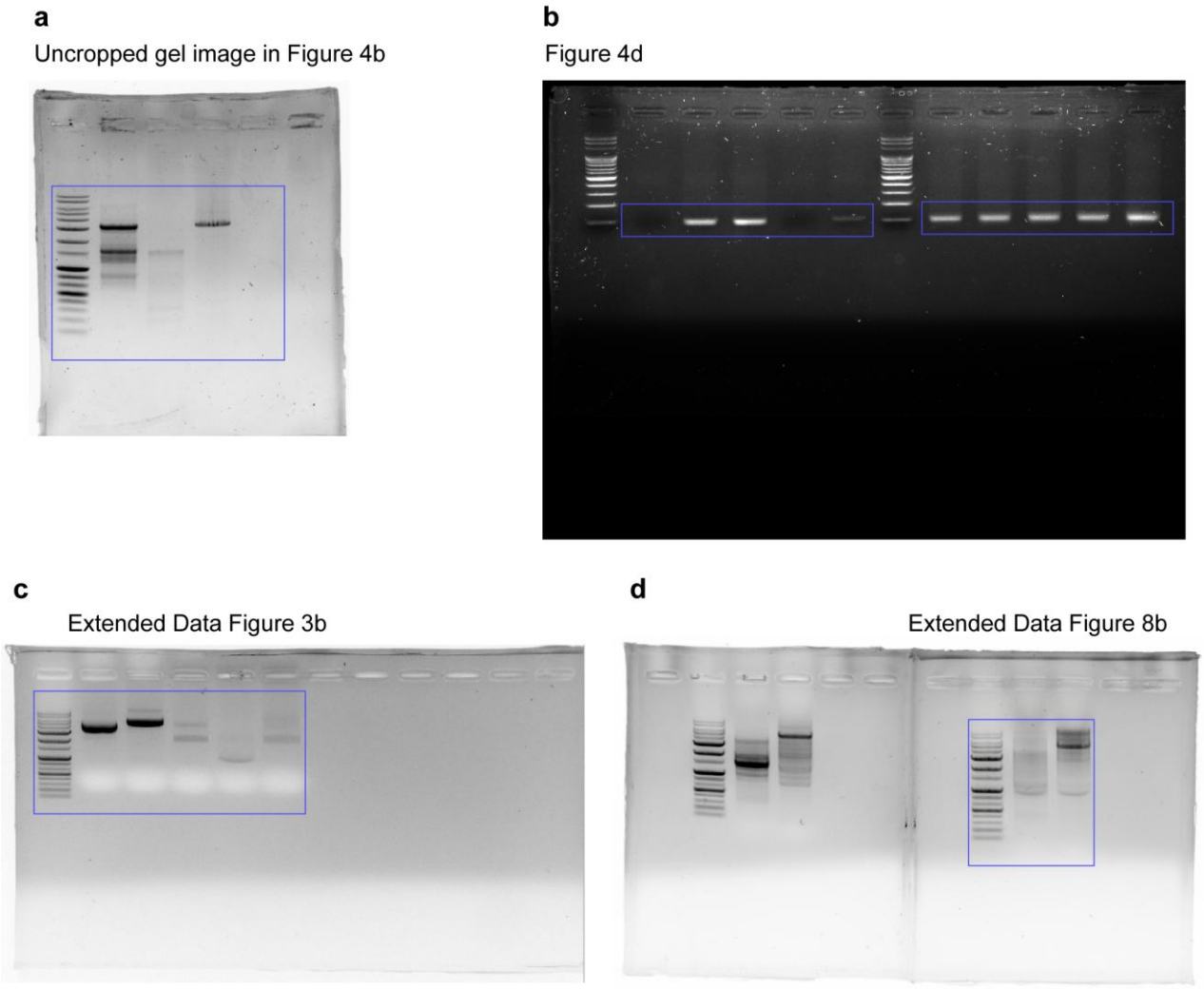
# Precise genomic integration of large DNA fragments by donor-directed annealing using prime editing

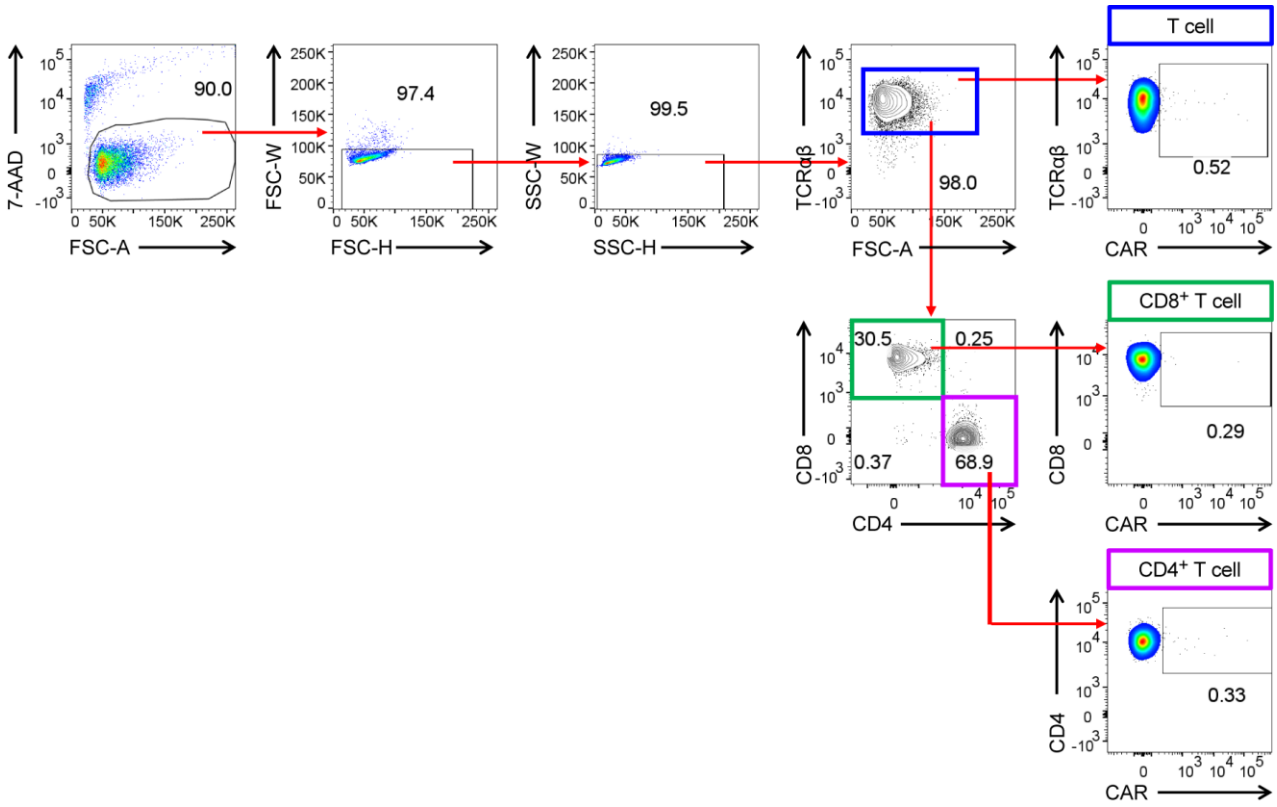
In the format provided by the authors and unedited

**List of Supplementary Information:**

Supplementary Figure 1 to 2.




**Supplementary Figure 1. Uncropped gel images for main and supplementary figures. a.** Uncropped gel for **Figure 4a** (long-range PCR confirming 2.9 kb insertion with 100 kb or 1 Mb deletions under DF-PA); cropped regions are boxed. **b.** Uncropped gel for **Figure 4d** showing cDNA junction PCR for F9 CDS (exons 2–8) and *GAPDH* control; boxed areas correspond to the main figure. **c.** Uncropped gel for **Extended Data Figure 3b** showing cssDNA confirmation; cropped regions are boxed. **d. Extended Data Figure 8b** showing chromosomal translocation confirmation; cropped regions are boxed.




**Supplementary Figure 2. Flow-cytometric gating strategy for quantification of CAR-expressing human T cells**. Representative gating strategy used to analyze CAR expression in engineered primary human T cells shown in **Figure 5c**. Viable cells were first identified based on forward scatter area (FSC-A) and exclusion of 7-AAD-positive cells. Cell doublets and aggregates were sequentially excluded using FSC-H versus FSC-W and SSC-H versus SSC-W. T cells were then identified as TCRαβ-positive cells. Within the TCRαβ-positive population, total T cells were analyzed directly for CAR expression, while $CD8^+$ and $CD4^+$ T-cell subsets were defined according to CD8 and CD4 expression and subsequently analyzed for CAR positivity. Numbers indicate the percentage of cells within the respective parent gate. Representative plots are shown.