

Figure 1. A “reusable” single cell for epigenomic analysis (RscEpi-seq). **a**, Generating a “reusable” single cell. **b**, Acquiring locational information of individual antibodies on the genome.

Figure 1

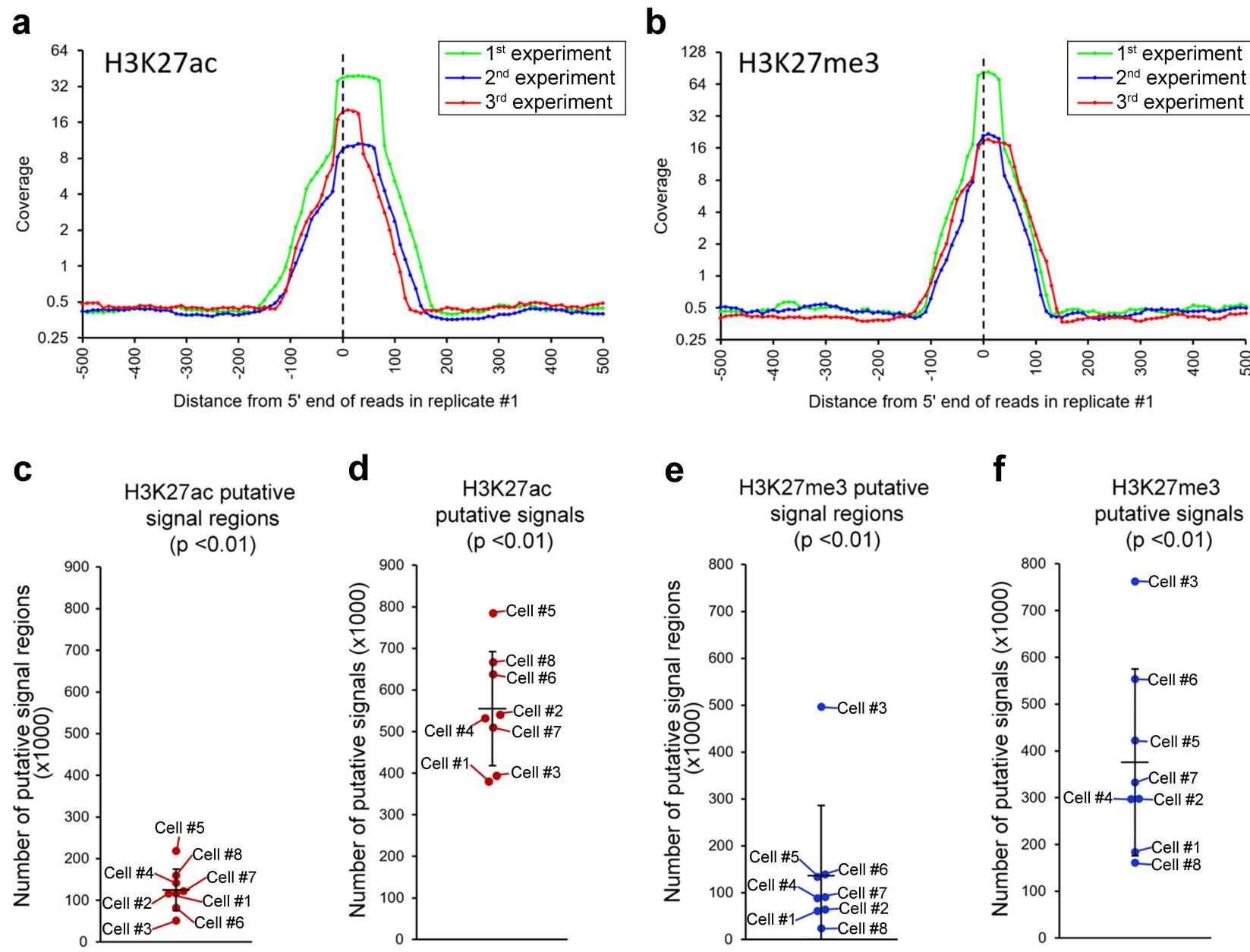


Figure 2. Repeating the same experiments using the same single cell. **a** and **b**, Locations of H3K27ac (**a**) and H3K27me3 (**b**) are preserved over repeated experiments in “reusable” single cells. Eight K562 single cells were used. Genomic regions, where signals were detected in the first experiments in 8 single cells, were analyzed in the same 8 single cells. Signal distributions in the first (green line), second (blue line) and third (red line) experiments are shown. **c** and **e**, Signal enriched genomic regions were identified by a statistical test, bootstrap test in every single cell without data aggregation of other single cells ($p < 0.01$). Signal enriched regions were determined by 2 criteria. First is signal enrichment over control IgG in a genomic region (500 bp bin size). Second is statistical significance compare to random controls of antibody and control IgG (See Online Method for the detail). **d** and **f**, Numbers of putative signals per cell derived from anti-H3K27ac (**d**) and anti-H3K27me3 (**f**). Numbers of putative signals in the signal enriched genomic regions ($p < 0.01$) were counted in every single cell.

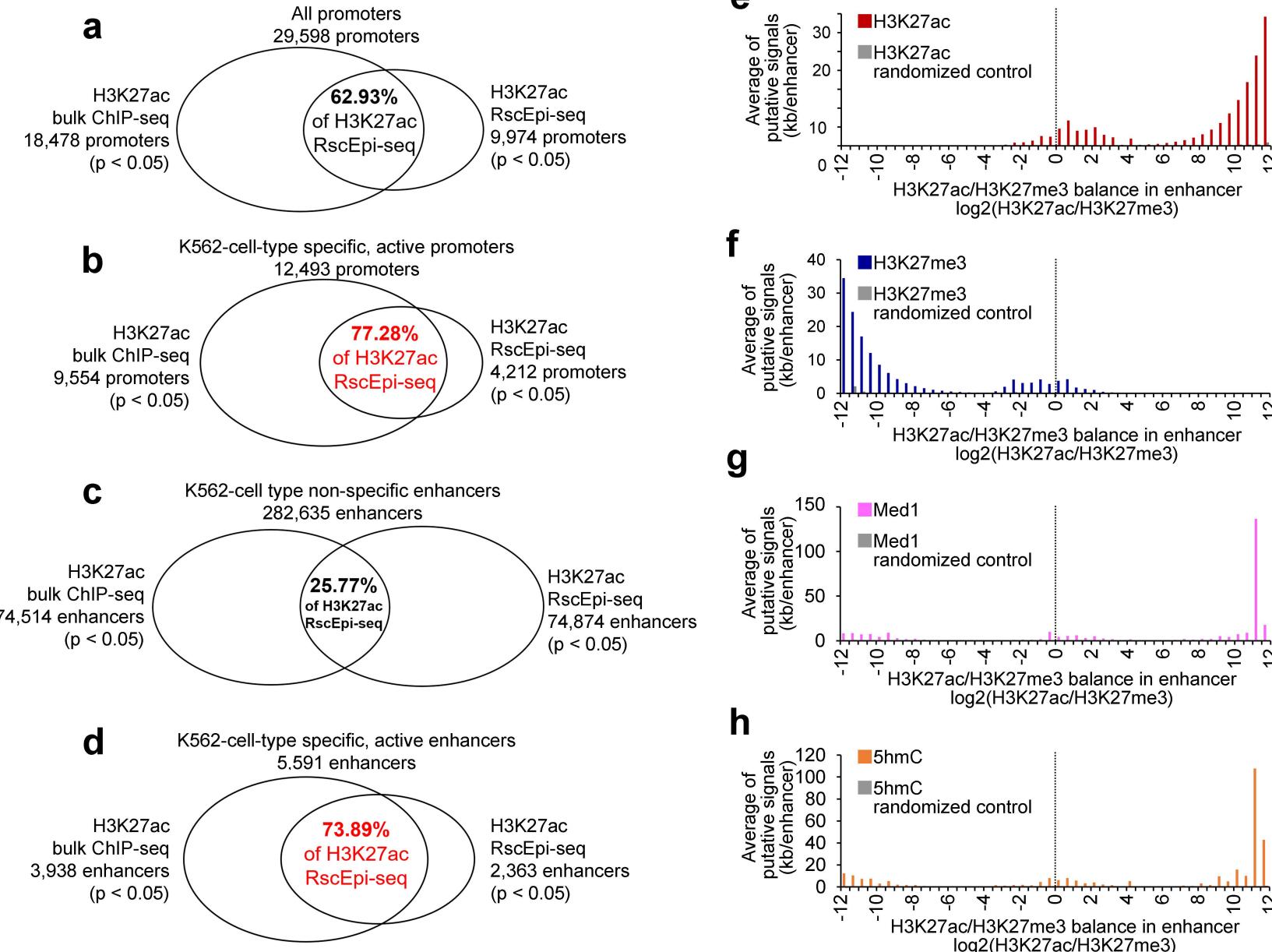


Figure 3. Evaluation of the putative signals by bulk ChIP-seq (a-d) and by enhancer classification with H3K27ac (e), H3K27me3 (f), mediator complex subunit 1 (g, Med1) and 5-hydroxymethylcytosine (h, 5hm). a, H3K27ac signal enrichment in all promoters. A statistical test, bootstrap test calculated the significance of H3K27ac signal enrichment compared to randomized control of H3K27ac signals in each promoter ($p < 0.05$). Numbers of enriched promoters in bulk ChIP-seq and RscEpi-seq were shown as Venn diagram (see Online Methods for the detail). b, H3K27ac signal enrichment in known K562-cell active promoters. c, H3K27ac signal enrichment in atypical active enhancers of K562 cells. d, H3K27ac signal enrichment in typical active enhancers of K562 cells. e-h, Enhancer classification by relative ratio $\log_2(\text{H3K27ac}/\text{H3K27me3})$ and evaluation by Med1 and 5hmC. e, Signal counts of H3K27ac in the classified enhancers. f, Signal counts of H3K27me3 in the classified enhancers. g, Signal counts of Med1 in the classified enhancers. h, Signal counts of 5hmC in the classified enhancers.

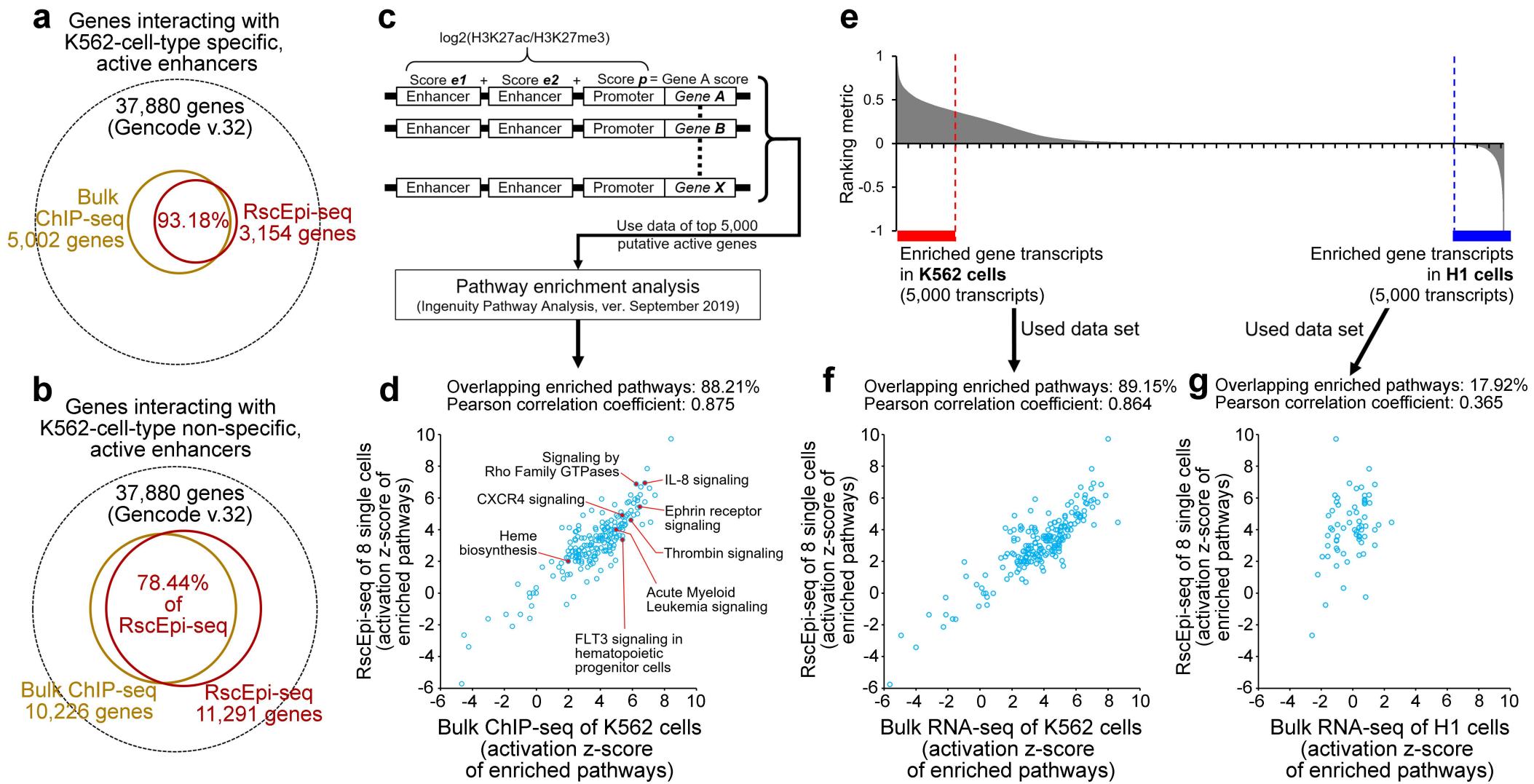


Figure 4. Signal evaluation at levels of target genes and target pathways. **a**, Interacting genes with K562-cell typical active enhancers detected by bulk ChIP-seq and RscEpi-seq. Active enhancers were determined by relative ratio $\log_2(\text{H3K27ac}/\text{H3K27me3})$ in bulk ChIP-seq and RscEpi-seq ($p < 0.05$, bootstrap test). Interacting genes of the determined active enhancers were inferred using experimentally validated interaction of enhancer-gene in K562 cells (see Online Methods for the detail). **b**, Interacting genes with atypical active enhancers detected by bulk ChIP-seq and RscEpi-seq. Statistical significance of active enhancers were calculated by bootstrap test ($p < 0.05$) compared to H3K27ac and H3K27me3 randomized controls. **c**, Workflow to infer biological functions of active enhancers and promoters. **d**, Significantly enriched canonical pathways in bulk ChIP-seq and RscEpi-seq. Activation z-score of bulk ChIP-seq (x-axis) and RscEpi-seq (y-axis) were shown, and Pearson correlation coefficient was calculated. **e**, Differential gene expression in bulk RNA-seq of K562 cells and H1 cells (embryonic stem cell line). Top 5,000 transcripts in K562 cells and H1 cells were used as input data in subsequent pathway enrichment analysis. **f**, Significantly enriched canonical pathways in bulk RNA-seq of K562 cells and RscEpi-seq of K562 single cells (8 cells). **g**, Significantly enriched canonical pathways in bulk RNA-seq of H1 cells and RscEpi-seq of K562 single cells (8 cells).

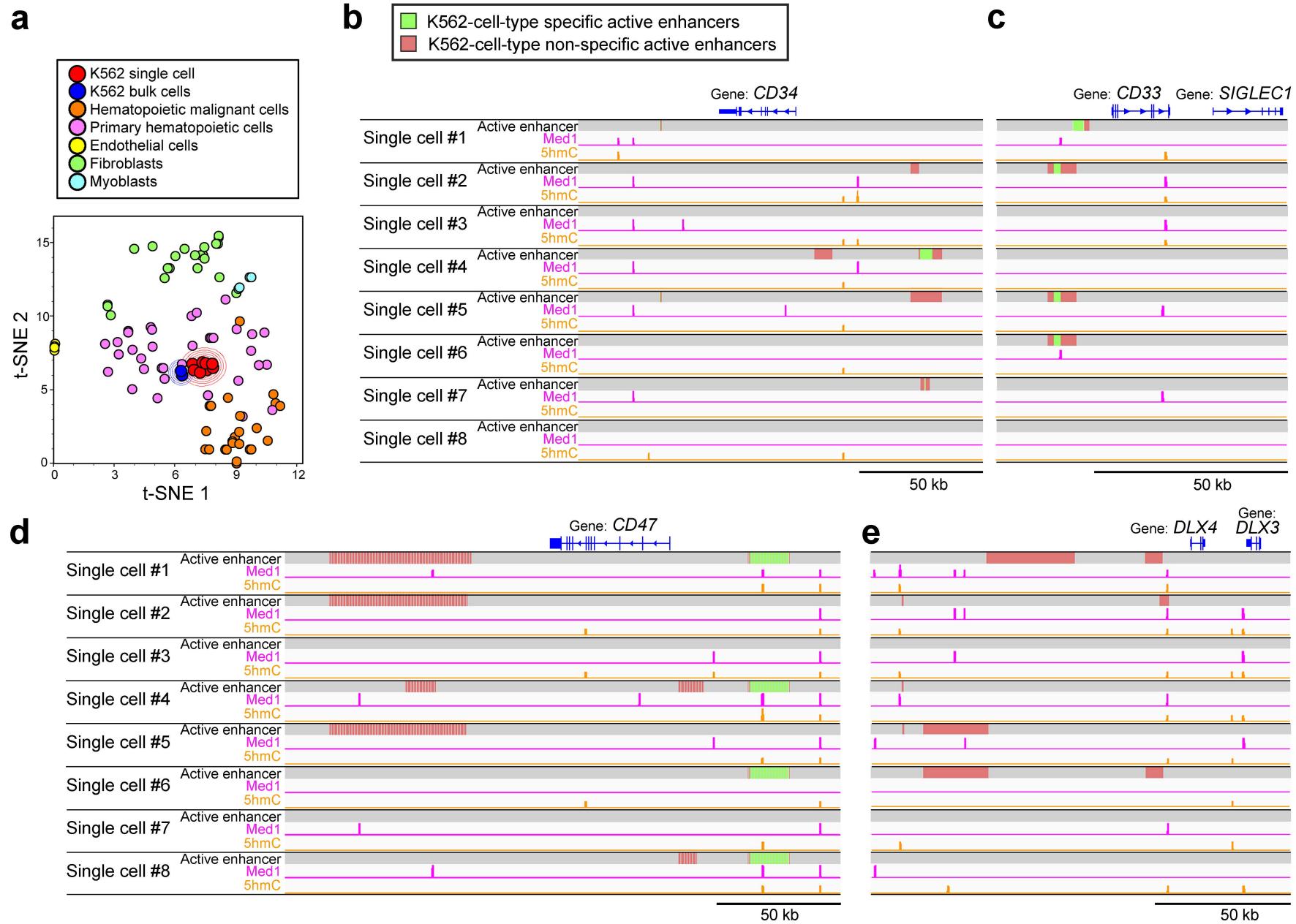
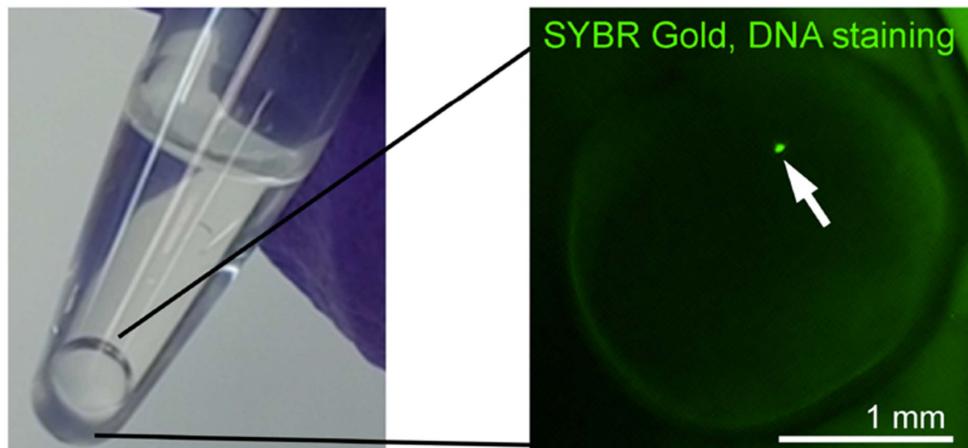
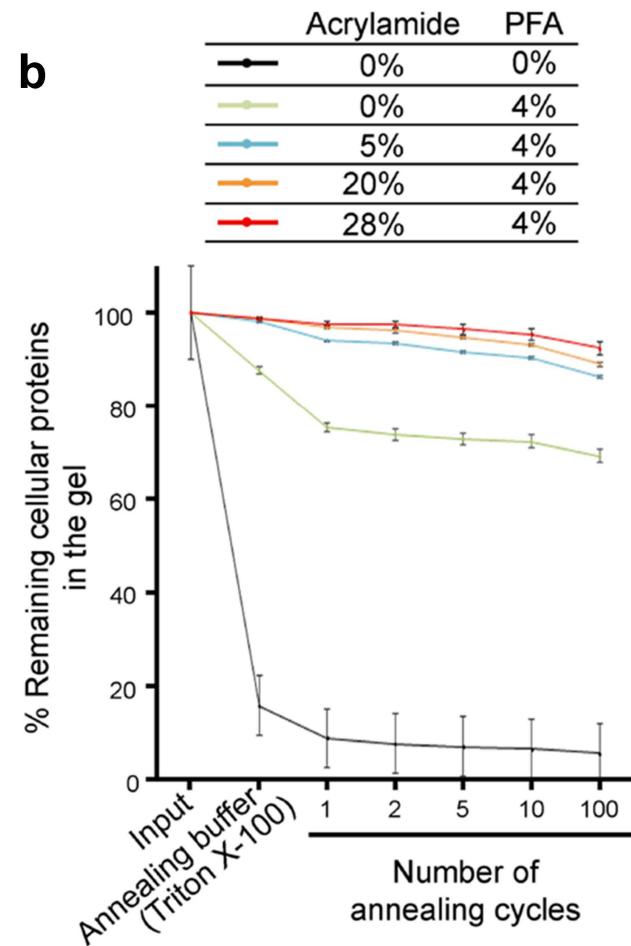
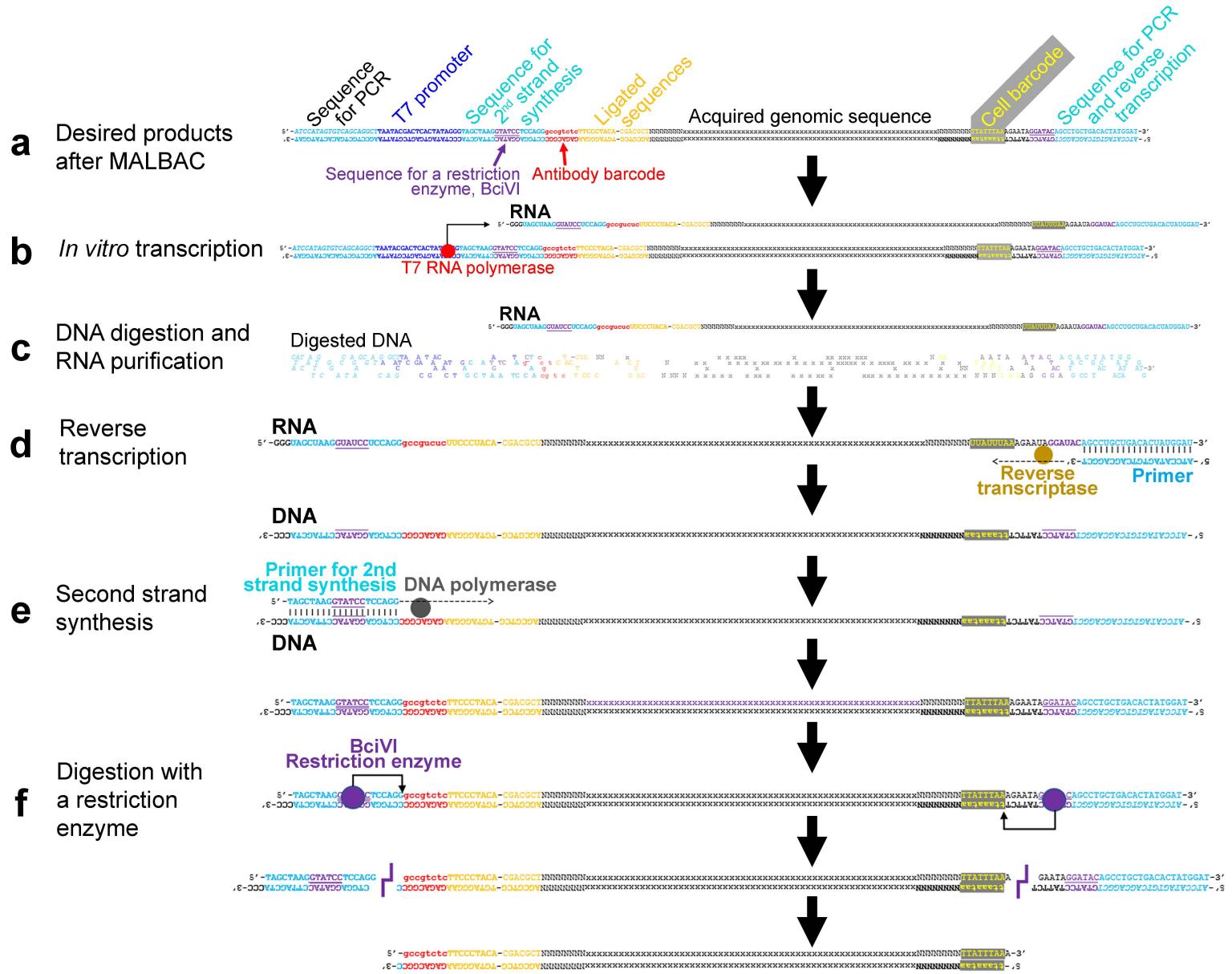


Figure 5. RscEpi-seq reveals cell identity and epigenetic heterogeneity. **a**, Cell identity recognition by epigenetic status of enhancers and promoters. Epigenetic status [Log₂(H3K27ac/H3K27me3)] of enhancers and promoters were calculated using data sets of various cells derived from bulk ChIP-seq and 8 K562 single cells derived from RscEpi-seq. Data dimensions of cells were compressed by t-Stochastic Neighbor Embedding (t-SNE). **b-e**, Detected active enhancers, Med1 and 5hmC in K562 single cells at loci of *CD34* (**b**), *CD33* (**c**), *CD47* (**d**) and *DLX4* (**e**). Signals of H3K27ac, H3K27me3, Med1 and 5hmC ($p < 0.01$) were used as input data. Active enhancers were identified based on significant H3K27ac dominance in relative ratio Log₂(H3K27ac/H3K27me3) compared to randomized controls of H3K27ac and H3K27me3 ($p < 0.05$, bootstrap test). Known typical active enhancers of K562 cells (green) are active enhancers identified by bulk cell analysis. Atypical active enhancers are active enhancers identified in other cells, but not in bulk K562 cells. (See also Supplementary Figure 5).

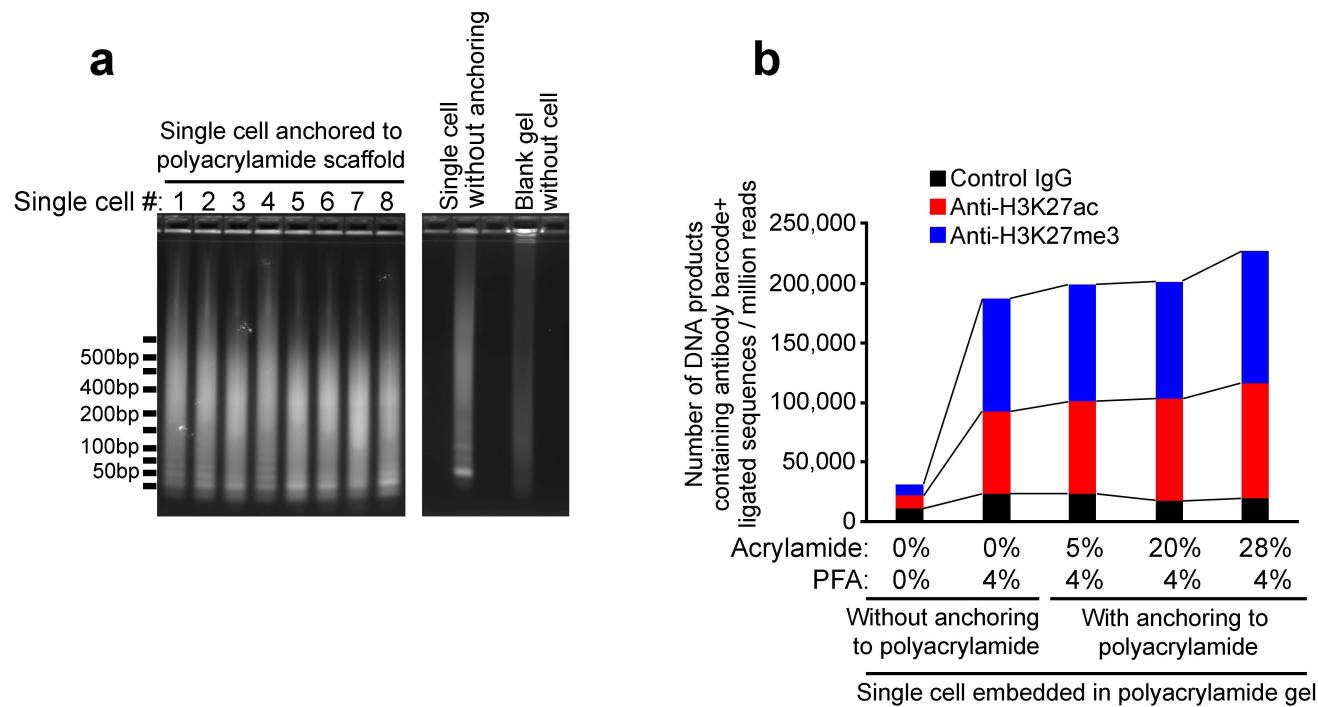
Figure 5

a**b**

Supplementary Figure 1. Generating “reusable” single cells. **a**, A “reusable” single cell in a PCR tube. A single cell was embedded and anchored to a polyacrylamide (4%) scaffold. DNA in the cell nucleus was visualized by SYBR Gold. **b**, Preservation of cellular proteins during heating and cooling cycles. Preservation of cellular proteins in non-fixed (0% acrylamide and 0% PFA), PFA-only-fixed (0% acrylamide and 4% PFA) and reusable (5-28% acrylamide and 4% PFA) single cells after heating and cooling cycles. The cells (3.33×10^5 cells/tube) were mixed with PBS containing 3.88% acrylamide, 0.12% bis-acrylamide and 1% ammonium persulfate. The cells were embedded into polyacrylamide by adding mineral oil containing 0.2% N,N,N',N'-tetramethylethane-1,2-diamine. Eluted proteins were measured by Micro BCA after the heating and cooling cycles (94 °C for 3 min and 4 °C for 10 min). Inputs are total proteins from 3.33×10^5 cells. Each data point represents the mean of triplicate experiments. Error bars reflect standard deviations.

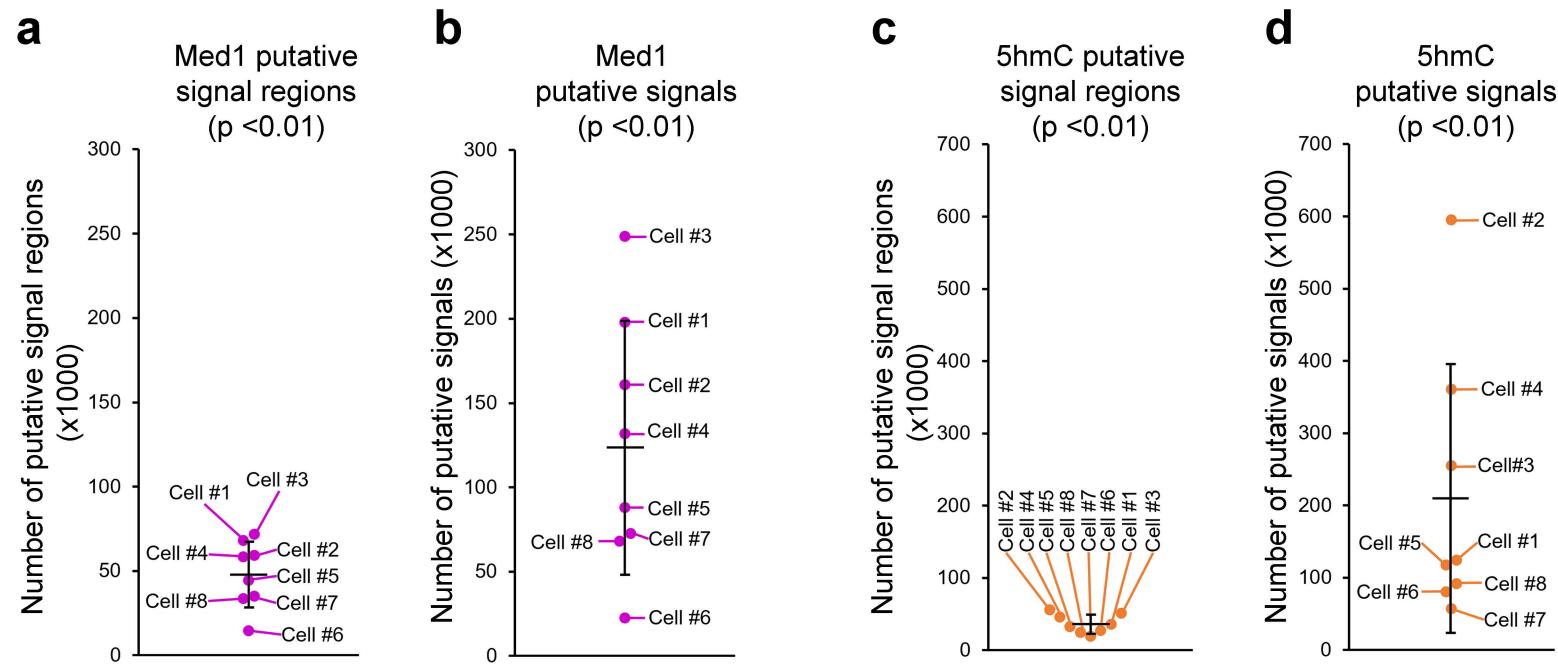


Supplementary Figure 2. Representation of a method to reduce genome-derived byproducts generated by MALBAC in the method. **a**, Desired products from MALBAC at the end of Figure 1b. **b**, Conversion of the desired products into RNA by *in vitro* transcription using T7 promoter in the antibody probe. **c**, Digestion of DNA including genome-derived byproducts of MALBAC, and RNA purification by phase separation. **d**, Reverse transcription of the purified RNA. **e**, Second strand synthesis using a specific primer. **f**, Removal of extra sequences by digestion with the restriction enzyme BciVI.

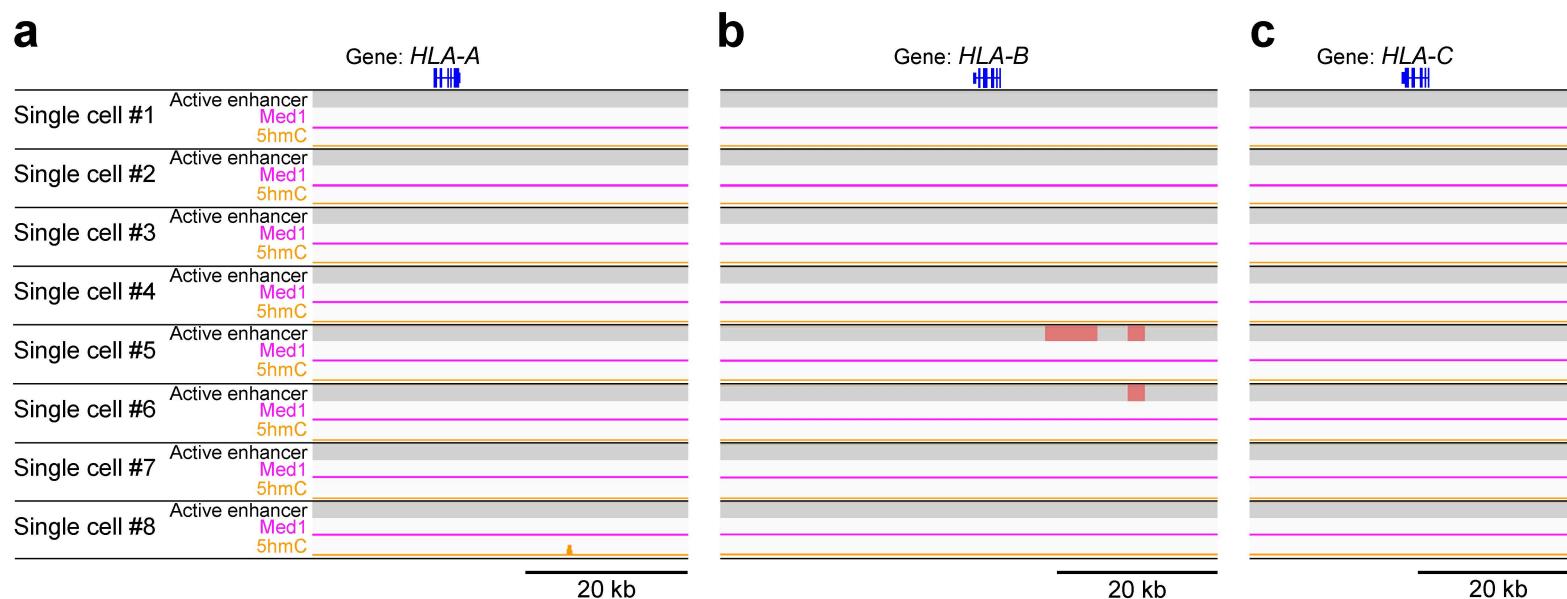


Supplementary Figure 3. DNA products from single cells embedded in a polyacrylamide gel.

a, Gel images of products at the end of the series of reactions. DNA was visualized with SYBR Gold in 2% agarose gels. **b**, Numbers of DNA products containing sequences of the antibody probe and the first random primer. Single cells were embedded in a 4% polyacrylamide gel bead with or without anchoring the proteins to the polyacrylamide scaffold.



Supplementary Figure 4. Reanalyzing the same single cells with different antibodies. **a** and **c**, Signal enriched genomic regions of mediator complex subunit 1 (Med1, **a**) and 5-hydroxymethylcytosine (5hmC, **c**) in a cell. Signal enriched regions were determined by 2 criteria. First is signal enrichment over control IgG in a genomic region (500 bp bin size). Second is statistical significance compare to random controls of antibody and control IgG (see Online Method for the detail). Genomic regions have p -values less than 0.01 were considered as putative signal regions. **b** and **d**, Numbers of putative signals per cell from Med1 (**b**) and 5hmC (**d**). Numbers of putative signals in the signal enriched genomic regions were counted in every single cell.



Supplementary Figure 5. Epigenetic satatus of known inactive loci, HLA-A, HLA-B and HLA-C in K562 single cells.
a-c, Active enhancers [$\text{Log}_2(\text{H3K27ac}/\text{H3K27me3})$], Med1 and 5hmC in known inactive loci, HLA-A, HLA-B and HLA-C. Singlas of H3K27ac, H3K27me3, Med1 and 5hmC ($p < 0.01$) were used as input data. Active enhancers were identified based on significant H3K27ac dominance in relative ratio $\text{Log}_2(\text{H3K27ac}/\text{H3K27me3})$ compared to randomized controls of H3K27ac and H3K27me3 ($p < 0.05$, bootstrap test). Known typical active enhancers of K562 cells were not detected. Atypical active enhancers (red) are active enhancers identified in other cells, but not in bulk K562 cells.