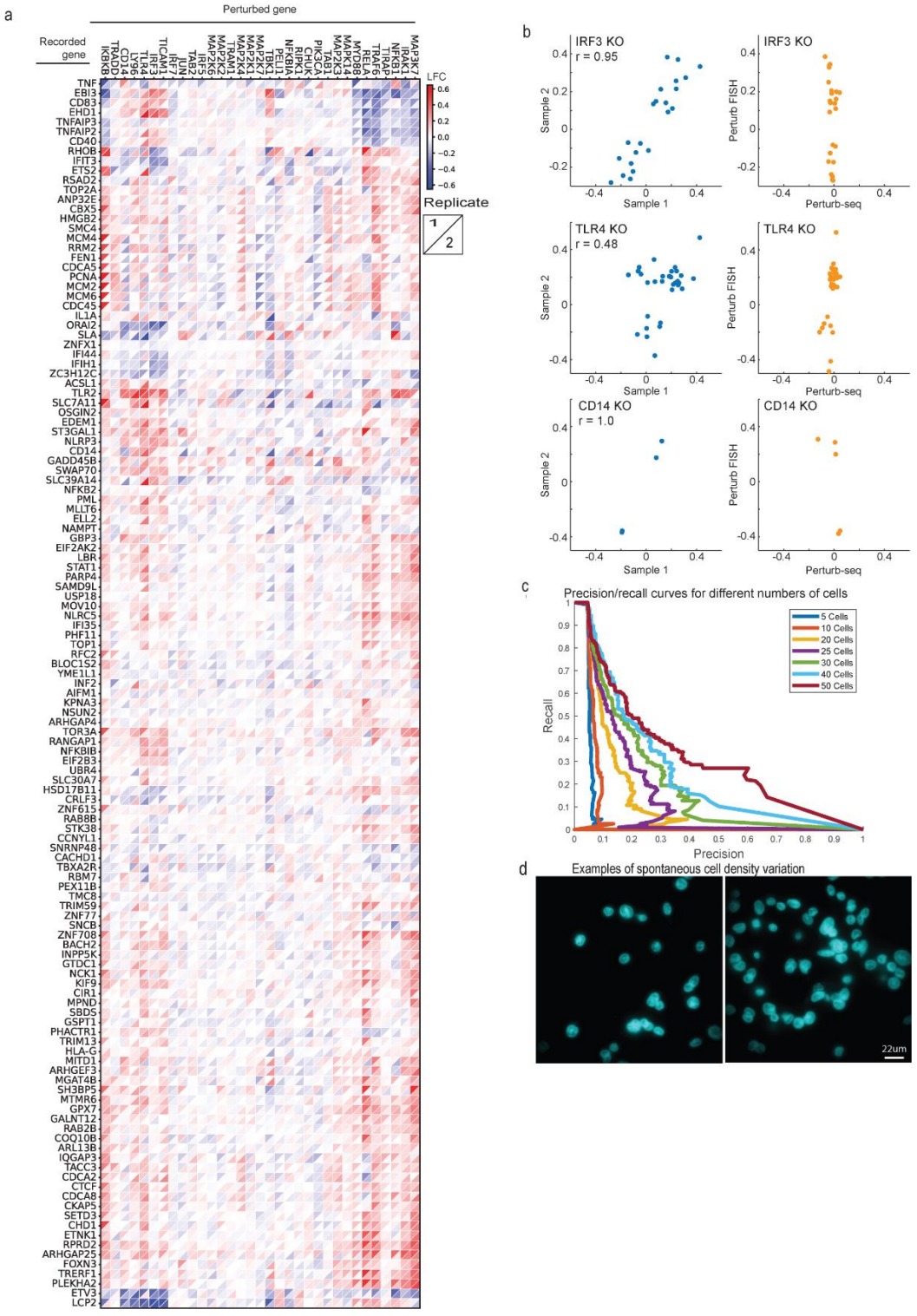
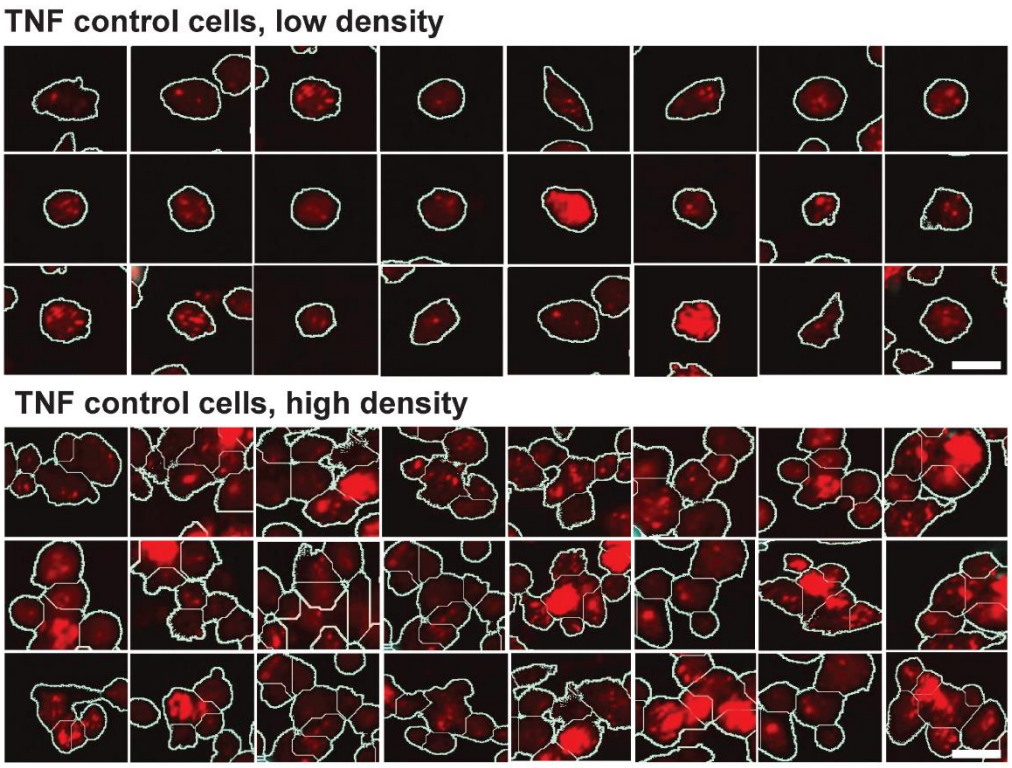
Supplementary Figures

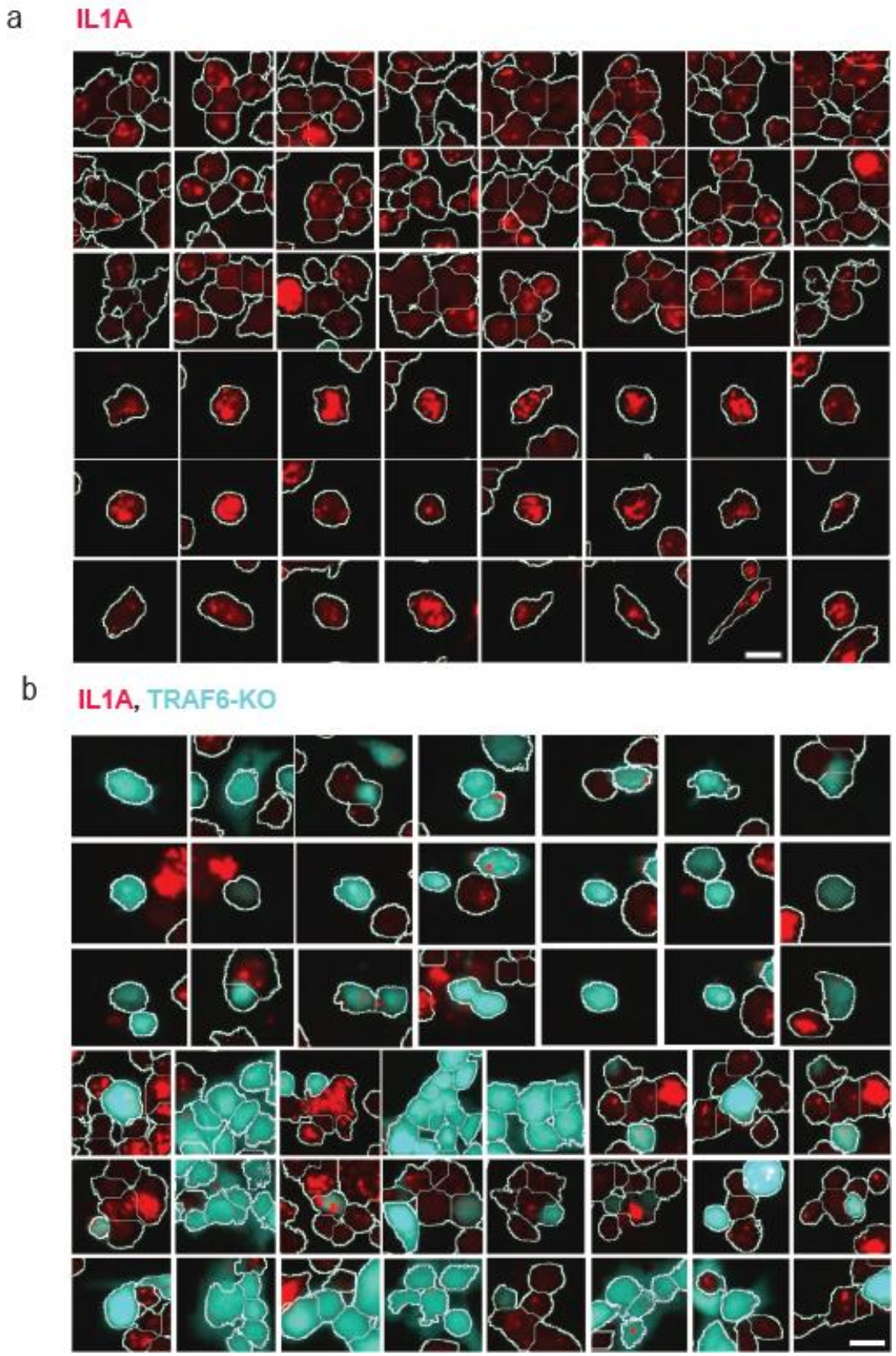


Supplementary figure 1. A) Heatmap of LFC effect sizes (colorbar) between Perturb-FISH replicate 1 (upper left triangle of each square) and replicate 2 (lower right triangle). Rows and columns are clustered based on UPGMA clustering of sample1 data. B) Left: scatterplots of significant effect sizes (q<0.1) determined in Perturb-seq (x-axis) and combined Perturb-FISH (y-axis). Right: scatterplots of significant effect sizes

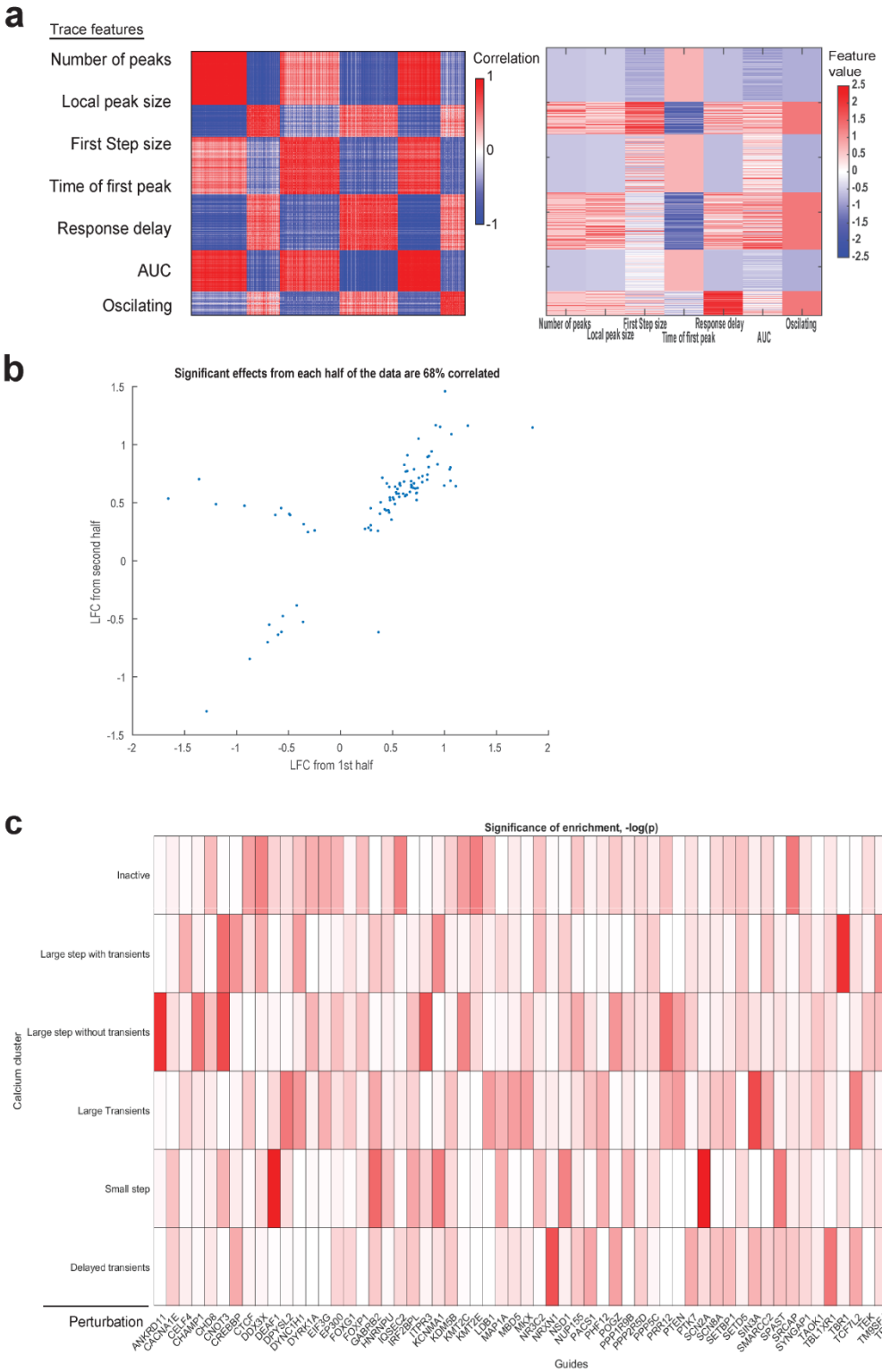
(q<0.1) determined in Perturb-FISH replicate 2 (x-axis) and replicate 1 (y-axis). Shown are the effects from IRF3, TLR4 and CD14, which have the lowest correlation between Perturb-FISH and Perturb-seq. Effects represent log-fold changes (LFC; natural log base) in expression relative to control cells. C) Precision recall curves for effects learned in downsampled Perturb-FISH data using increasing numbers of cells. D) Example images (DAPI) of spontaneously occurring areas of varied densities.



Supplementary figure 2. RNAscope images showing TNF mRNAs (in red) in THP1s cells at low and high density. Blue cells received a guide against NFKB1, other cells received a non-targeting guide. Scale bar: 30µm.

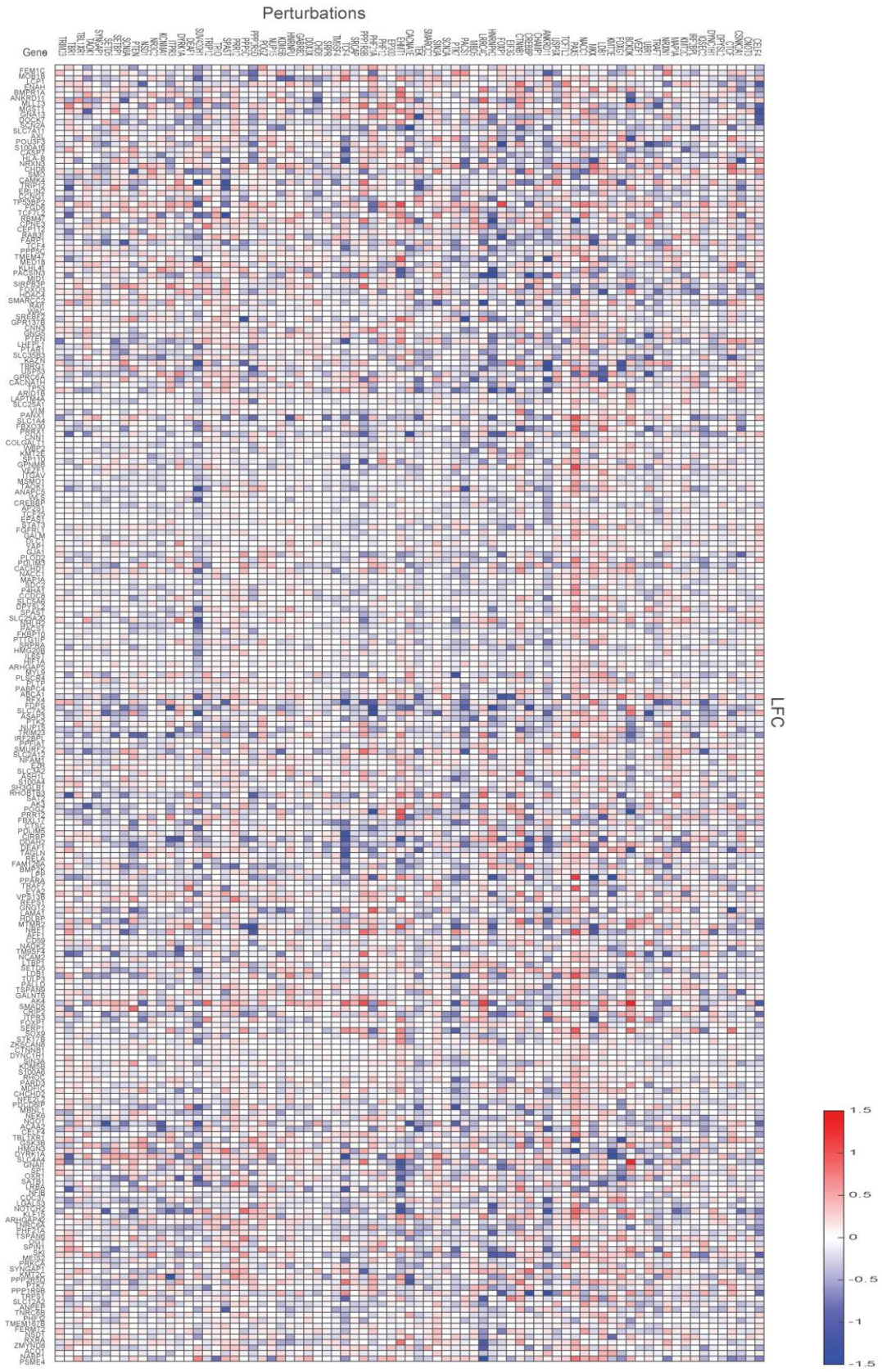


Supplementary figure 3. RNAscope images showing IL1a mRNAs (in red) in THP1s at low and high density. Blue cells received a guide against TRAF6, other cells received a non-targeting guide. Scale bar: 30µm.



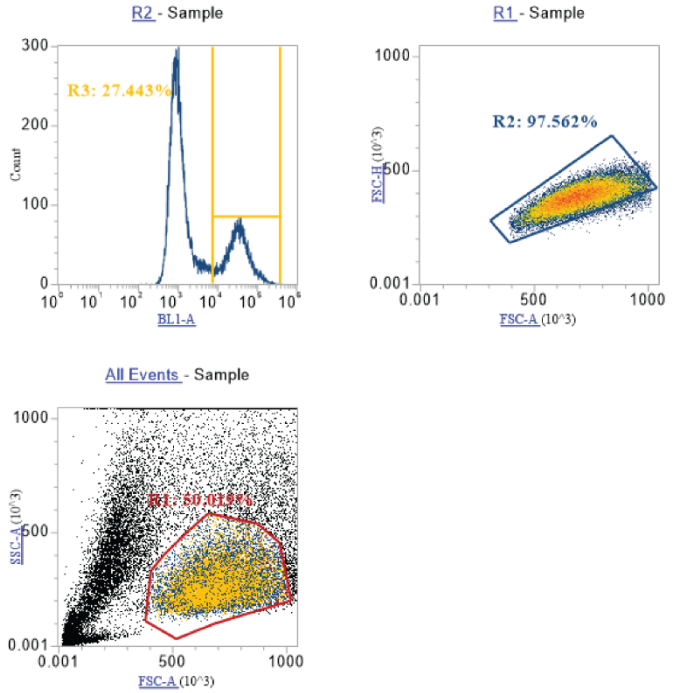
Supplementary figure 4. A) Heatmap of cell-to-cell correlation (left) and definition of calcium features (right). Cells are clustered using k-means clustering. B) Scatter plot of significant effects from one half of the data (x-axis) versus the

other half (y-axis). C) Heatmap of the significance of the guide (x-axis) enrichment in each cluster. Significance is shown as -log(p-value), using a natural log base. (scale bar).

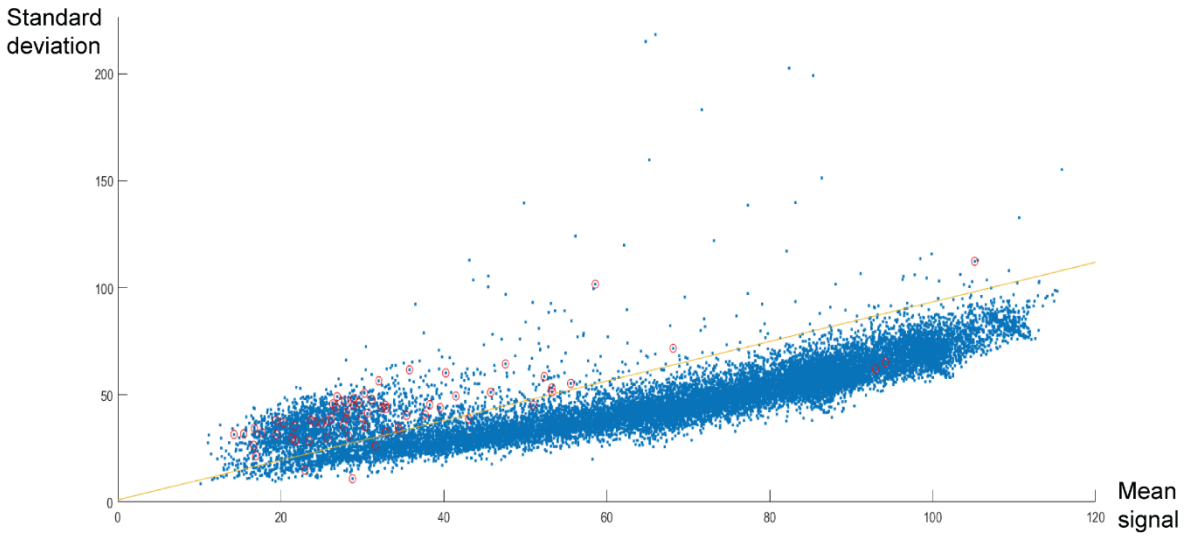


Supplementary figure 5. Heatmap showing LFC effects of perturbations (x-axis) on gene expression (y-axis). Effects represent log-fold changes (LFC; natural log base) in expression relative to control cells.

a



b



Supplementary figure 6. A) Evaluation of Cas9 activity: flow cytometry report of THP1 cells infected with a vector containing both a GFP sequence and a guide against GFP. 73% of the cells are GFP negative (top left). B) Scatter plot of decoded guides showing standard deviation (y-axis) as a function of mean signal across all 15 images (x-axis). Dots circled in red indicate false positives (blank barcodes that do not actually correspond to any guide in the library). The line indicates the applied cut off used during quality filtering of the data. Only decoded guides below the cutoff line were used in downstream analysis.