

Supplementary Materials for

Computer vision reveals hidden variables underlying NF- κ B activation in single cells

Parthiv Patel, Nir Drayman, Ping Liu, Mustafa Bilgic, Savaş Tay*

*Corresponding author. Email: tays@uchicago.edu

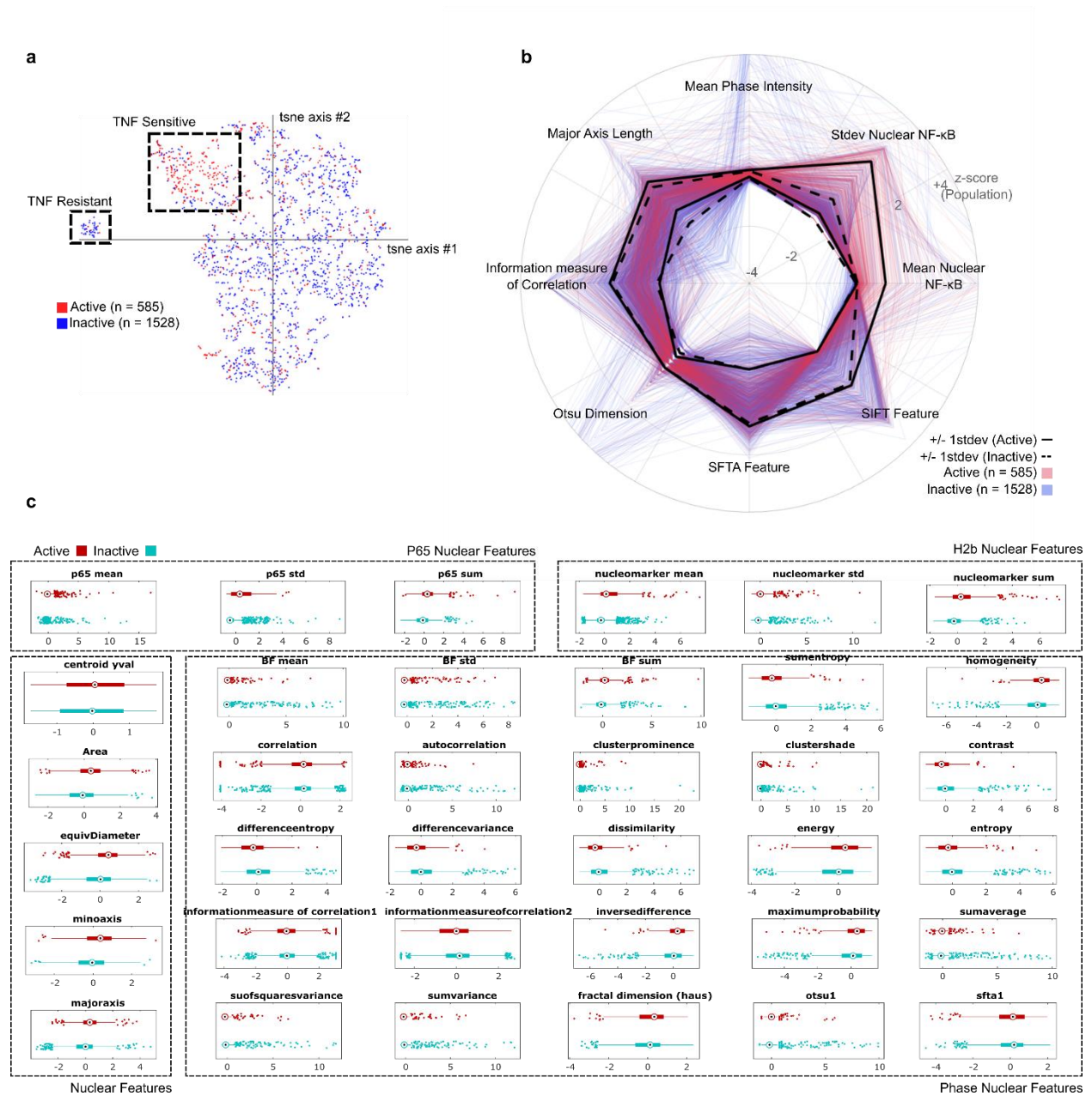
Published 22 October 2021, *Sci. Adv.* 7, eabg4135 (2021)
DOI: 10.1126/sciadv.abg4135

The PDF file includes:

Figs. S1 to S9
Legends for tables S1 to S5

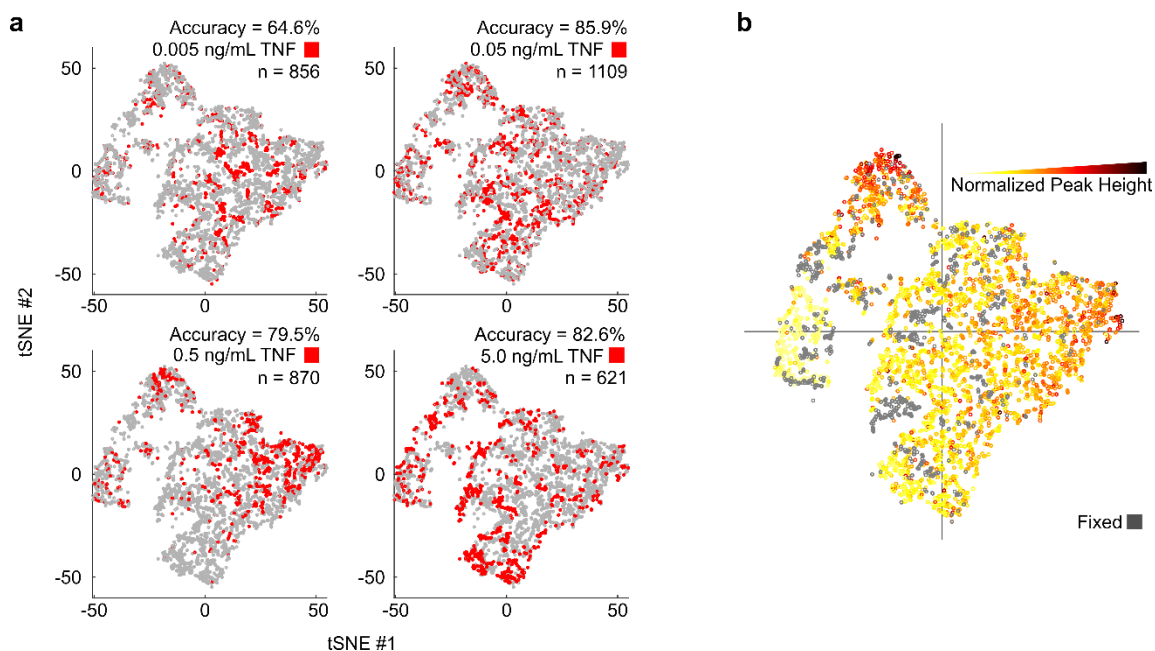
Other Supplementary Material for this manuscript includes the following:

Tables S1 to S5

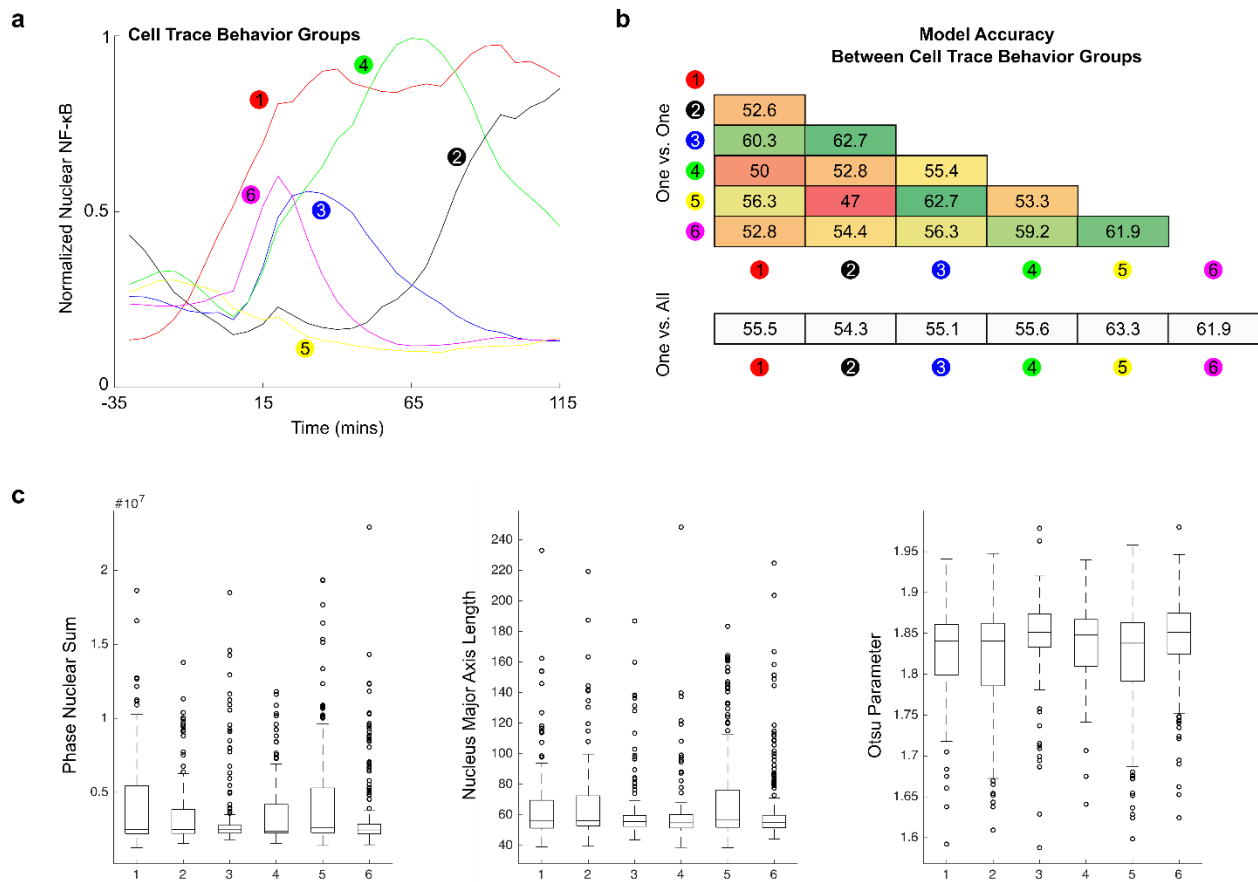


Supplementary Figure 1. Independent Repeat of single dose prediction for TNF activation

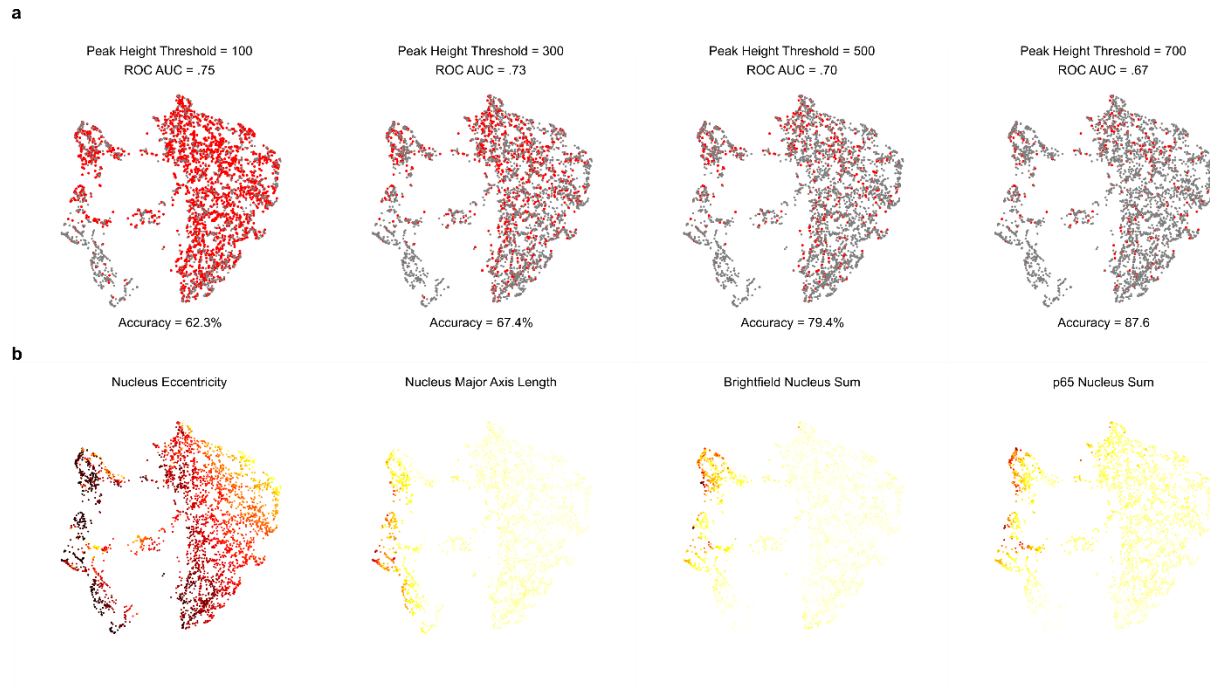
(a) tSNE of unstimulated cells at 0.1ng/mL TNF. (b) Highly predictive features for each cell (c) Standardized descriptors for unstimulated fibroblasts are shown in boxplots with active cells (red) and inactive cells (blue)



Supplementary Figure 2. Single cell population distribution in tSNE space (a) tSNE of different TNF dose stimulations (0.005, 0.05, 0.5, 5.0 ng/mL TNF) from t=0 descriptive variables. Cells from a dose are indicated in red and individual SVM model accuracies are listed. **(b)** tSNE visualization for NF-κB peak heights with overlay of fixed cells.



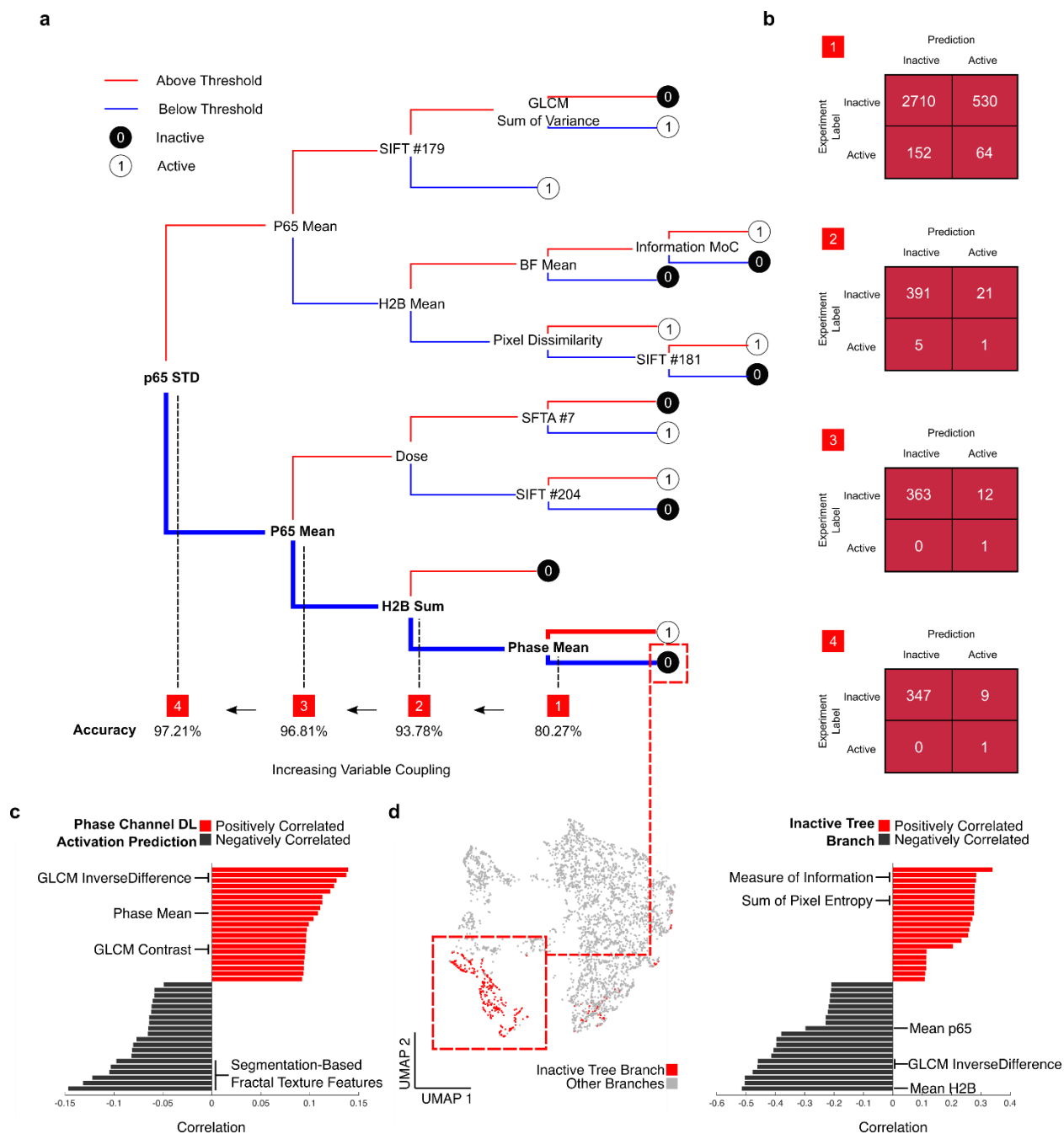
Supplementary Figure 3. Cell trace dynamics activation criteria for prediction shows modest prediction results (a) Single cell traces were clustered using k-means clustering (k=6). Median traces for each group is shown. (b) Classifier accuracies show how well individual groupings compare to other individual groupings and to all other groupings. (c) Some features, including phase nuclear sum, nucleus major axis length, and otsu parameter discriminate between trace behavior types.



Supplementary Figure 4. Activation level thresholding shows minimal differences in ROC

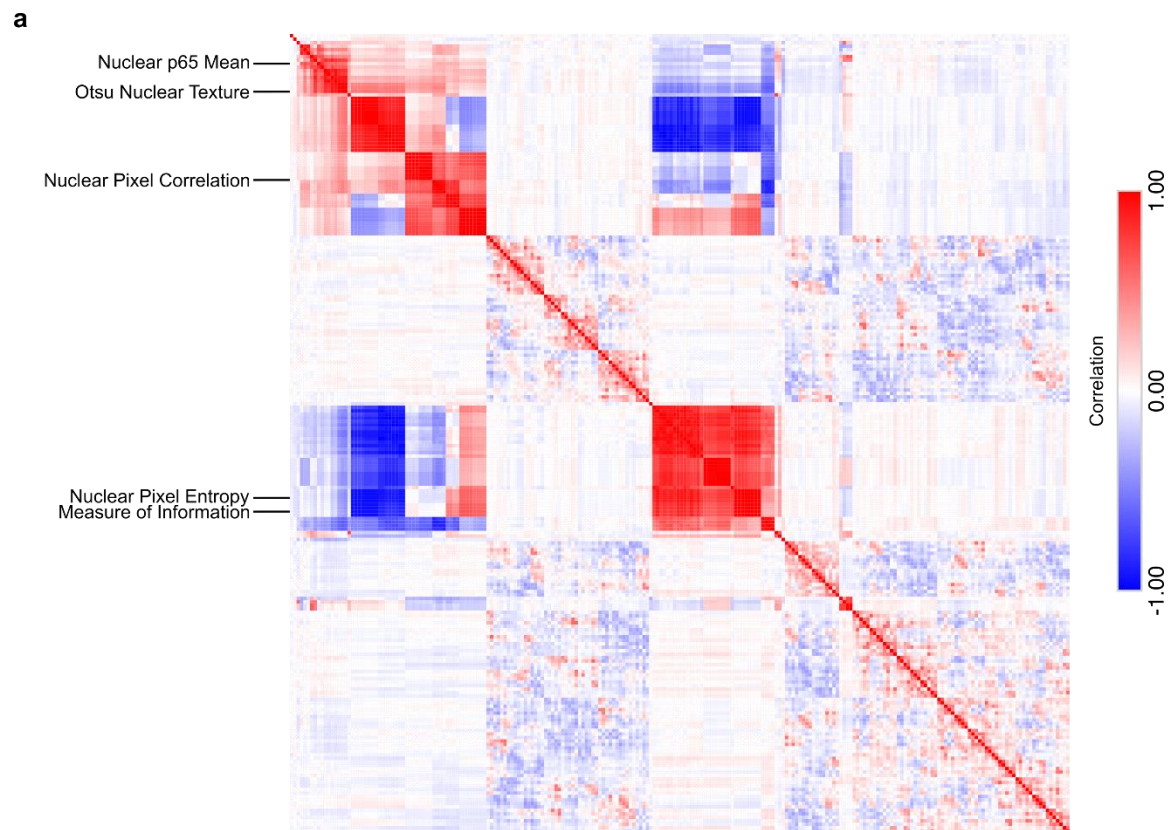
AUC (a) Cell label assignment using different mean nuclear peak height threshold values.

Analysis throughout the manuscript uses peak height threshold of 500. **(b)** Cell features that track with the divide between clusters include nuclear size metrics like nuclear eccentricity and major axis length. Similarly, protein levels of p65 and total brightfield sum also track with this divide.

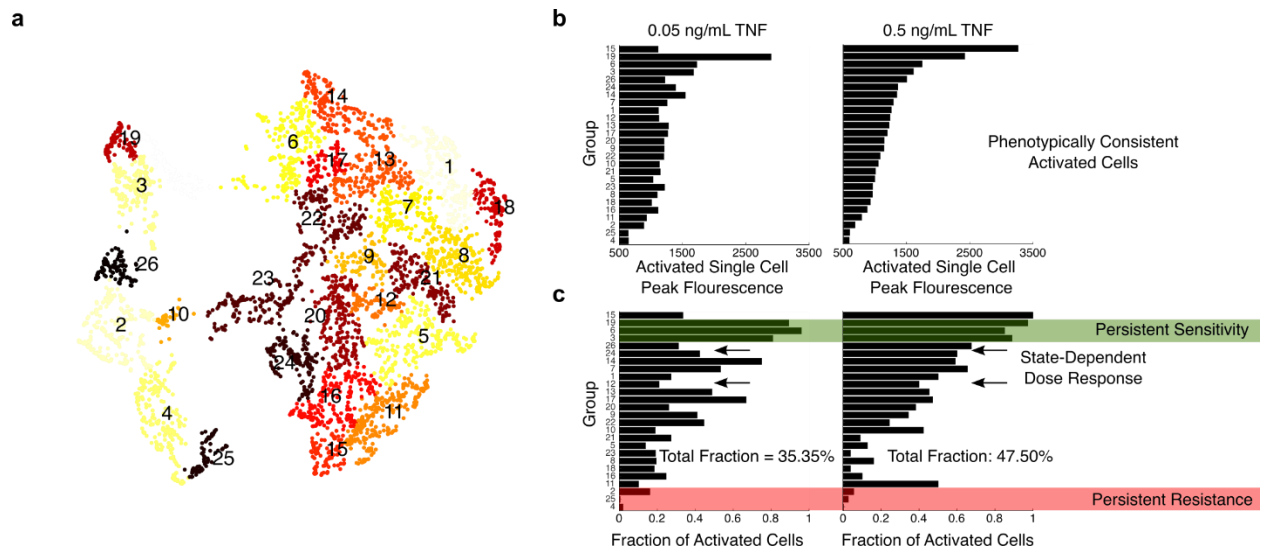


Supplementary Figure 5. Multiple variable coupling leads to improved prediction (a) A single tree model in an ensemble tree classifier is shown with thresholded decision boundaries for different variables (blue, under threshold; red, above threshold). An individual tree branch is analyzed for the power of variable coupling and accuracy is shown with the addition of addition

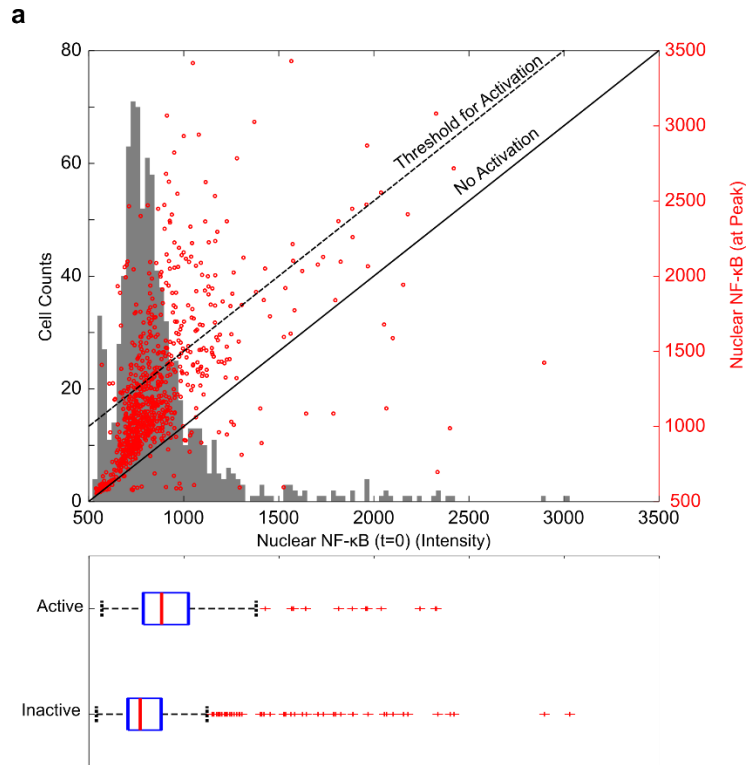
features **(b)** Individual classification accuracy is shown in each stage in the analyzed tree branch. **(c)** Correlation between the phase classifier and feature variables is shown with no correlation between prediction labels and p65 or H2b features. **(d)** Cell mapping in the UMAP projection is shown for the group of analyzed cells in the individual tree branch. Correlation found for features in this grouping of cells is shown. Individual features have a higher correlation when predicting this individual grouping of cells.



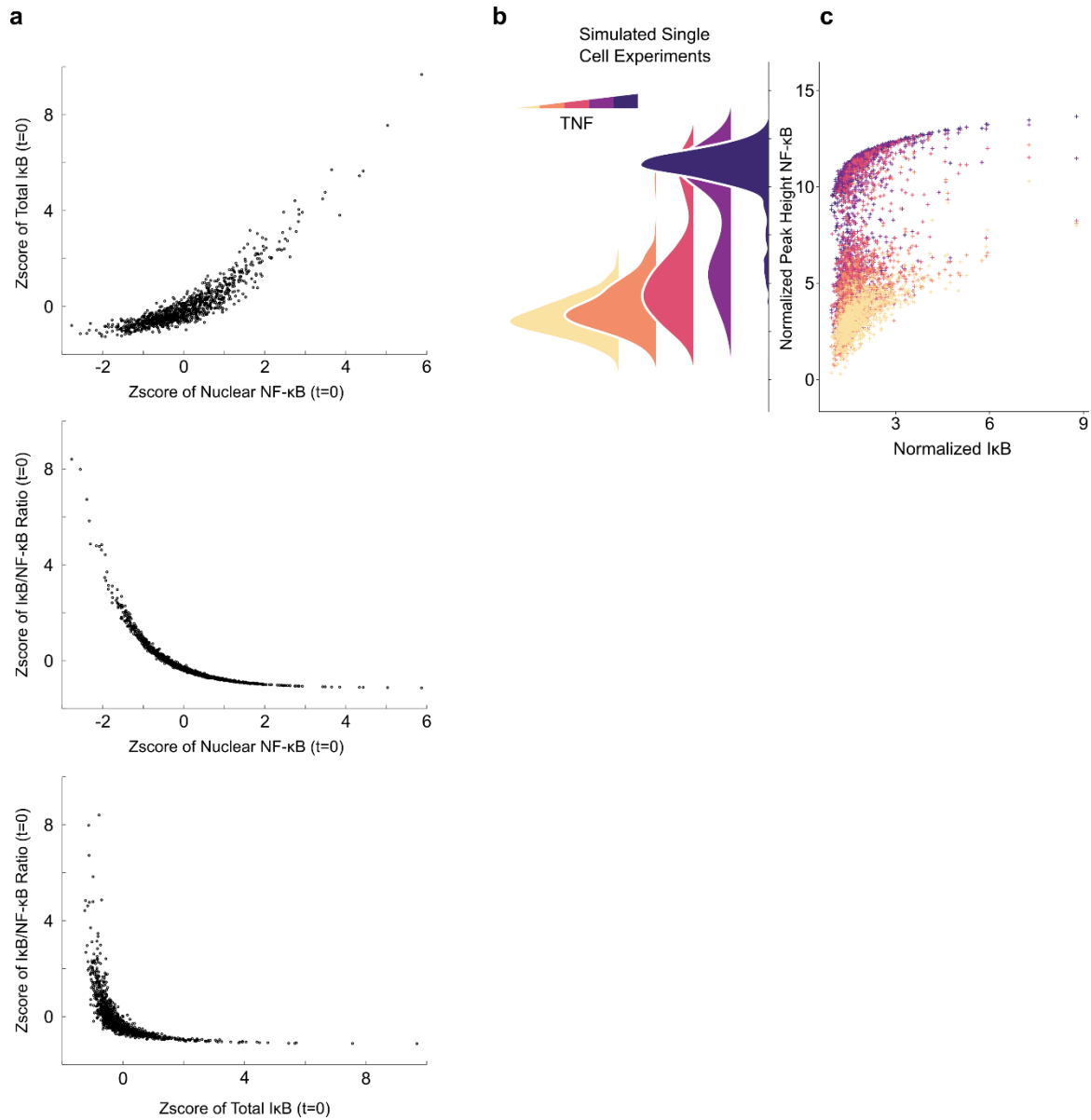
Supplementary Figure 6. Image feature covariance matrix (a) Covariance matrix for features colored by Correlation (red =1, blue =-1).



Supplementary Figure 7. Phenotypic analysis of cell state reveal both persistent and dose regulated states. (a) We use single cells across the UMAP space, clustered by local adjacency into communities to look for phenotypic differences in state by dosage (b) Activated single cells show consistent phenotype across different doses of TNF (0.05 ng/mL and 0.5ng/mL, left and right) (c) but show differences in the fraction of activated cells based on state. There are consistently sensitive and resistant groups of cell phenotypes, but also cellular states that have dose dependent differences in activation fraction



Supplementary Figure 8. p65 levels in unstimulated cells (a) Baseline levels of nuclear p65 is shown in the histogram. Cells that are classified as activated must reach a threshold of >500 peak-height. Boxplot is shown below comparing active and inactive classifications.



Supplementary Figure 9. Simulation comparisons between total IκB, IκB:NFκB, and Nuclear NFκB (a) Simulation comparisons of key features: total IκB, IκB:NFκB, and Nuclear NFκB. **(b)** Mathematical modeling simulations show single cell nuclear NF-κB peak heights increase with increasing TNF dose. **(c)** High IκB levels in cells require a smaller TNF dose to achieve NF-κB activation.

Supplementary Table 1: Model Comparison

Supplementary Table 2: Texture Features

Supplementary Table 3: Prestimulation Simulation Parameters

Supplementary Table 4: Abbreviations

Supplementary Table 5: Simulation Parameters