

Supplemental Materials

Molecular Biology of the Cell

Hughey et al.

Figure S1, related to Figure 1. (A) Representative traces of single-cell NF- κ B activation dynamics for different types and concentrations of stimuli. Intensity represents the relative nuclear localization of p65-dsRed fusion protein, calculated as mean nuclear intensity divided by initial mean cytoplasmic intensity. (B) Interpeak times for the data presented in Figure 1B. (C) Interpeak times for the data presented in Figure 3.

Figure S2, related to Figure 2. Histogram of mean interpeak time for all the cells depicted in Fig. 2.

Figure S3. Sensitivity of the mean and coefficient of variation (CV) of the interpeak time to each parameter in the computational model.

Figure S4, related to Figure 4. The clones show an intermediate amount of intercellular variability. The blue curve is the expected distribution of the F-statistic, if there is no intercellular variability for that clone. The orange curve is the expected distribution of the F-statistic, if that clone is identical to the original polyclonal cells shown in Figure 3 (given the number of cells observed and the number of interpeak times for each cell of that clone). Both expected distributions were calculated by bootstrapping. The black vertical line is the observed F-statistic for that clone.

Interactive Figure. Please visit <http://archive.simtk.org/livecellnfkb/hughey2014/interactive/>. The interactive figure is based on the same data as presented in Figure 2, namely 20-hour time-courses of stimulation with 10 ng/mL TNF.

The figure allows deeper exploration of the data than is possible with a traditional static figure. You can interact with the figure in several ways to focus on one or more cells.

On the left are two plots, one showing the mean and standard deviation for each cell, the other showing the CV of the peak amplitude for each cell. You can zoom in on these plots by using the zoom slider below the two plots. To focus on a particular cell, simply hover its data-point in one of the plots on the left. You can also select a range of cells, by clicking and dragging. The dynamics of p65-dsRed nuclear localization for that cell (or those cells) will appear in green (or orange, for a group of cells) at the bottom axes of the figure. The plots on the right side of the figure will change according to which cells are selected.

For the plots on the right, there are two possible views: Population Statistics and Cell Details. To switch between these two views, use the tabs at the top right of the figure.

Population Statistics shows a splom (scatterplot matrix) of the mean interpeak time, CV interpeak time, and CV peak amplitude. Histograms for each of the three features are plotted on the diagonal of the splom. You can click and drag to select a range of cells in any one of the scatterplots, and those cells will be highlighted in the other scatterplots, as well as in the other plots in the figure.

Cell Details shows the interpeak time and peak amplitude over time, and also shows scatterplots of consecutive interpeak times and peak amplitudes.

Additional options for viewing the figure are available by clicking on “Options” at the top of the figure. Here, you can choose how to sort the cells in the plots on the left, whether to color cells according to field of view (location) in the experiments, and whether to only show cells from particular fields of view.

Figure S1

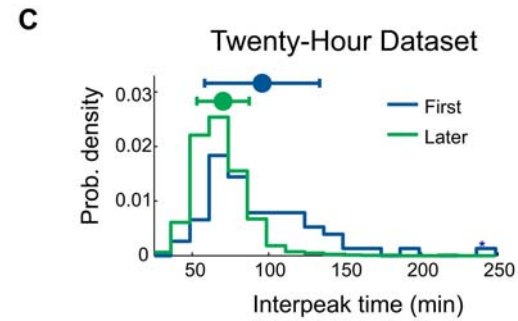
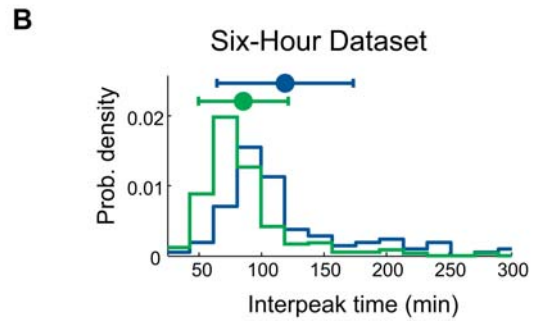
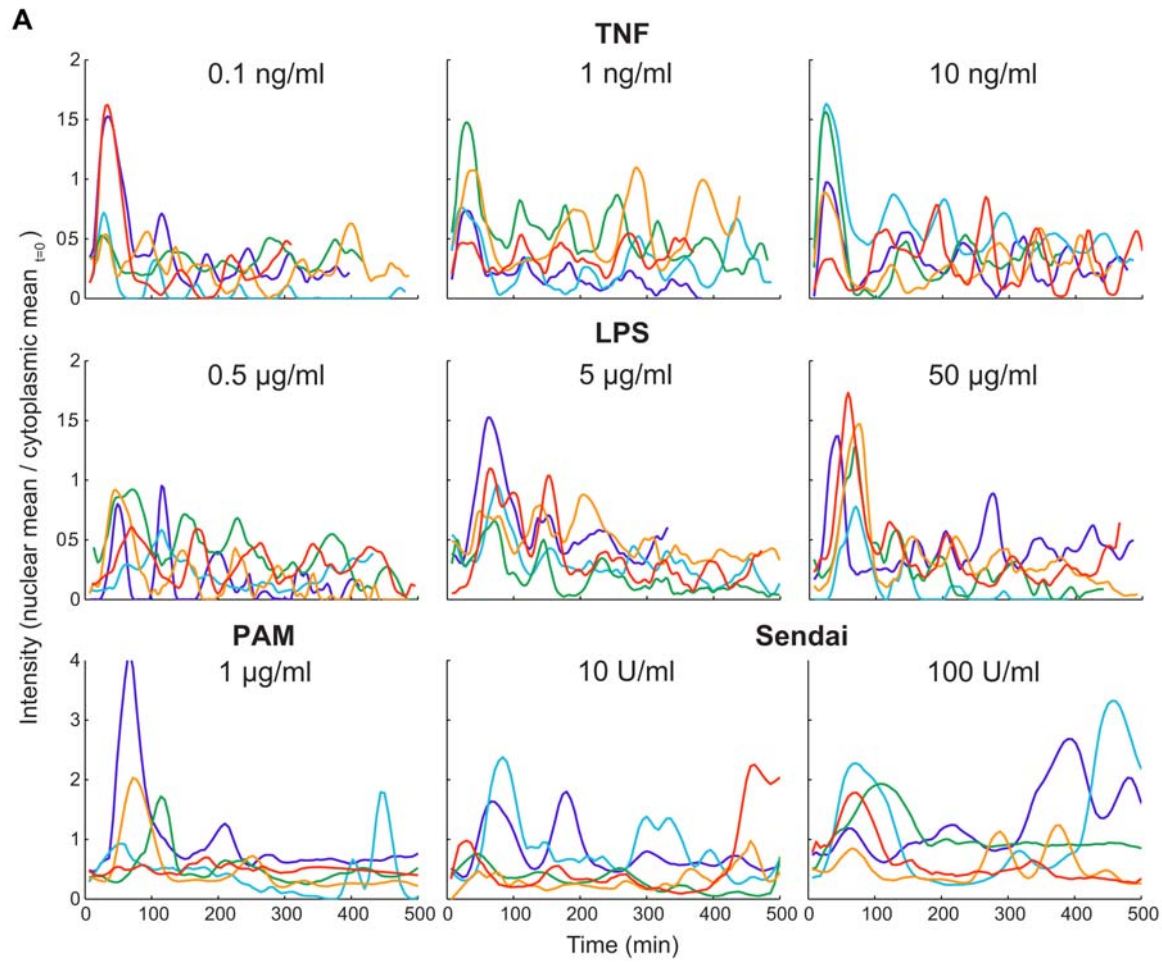


Figure S2

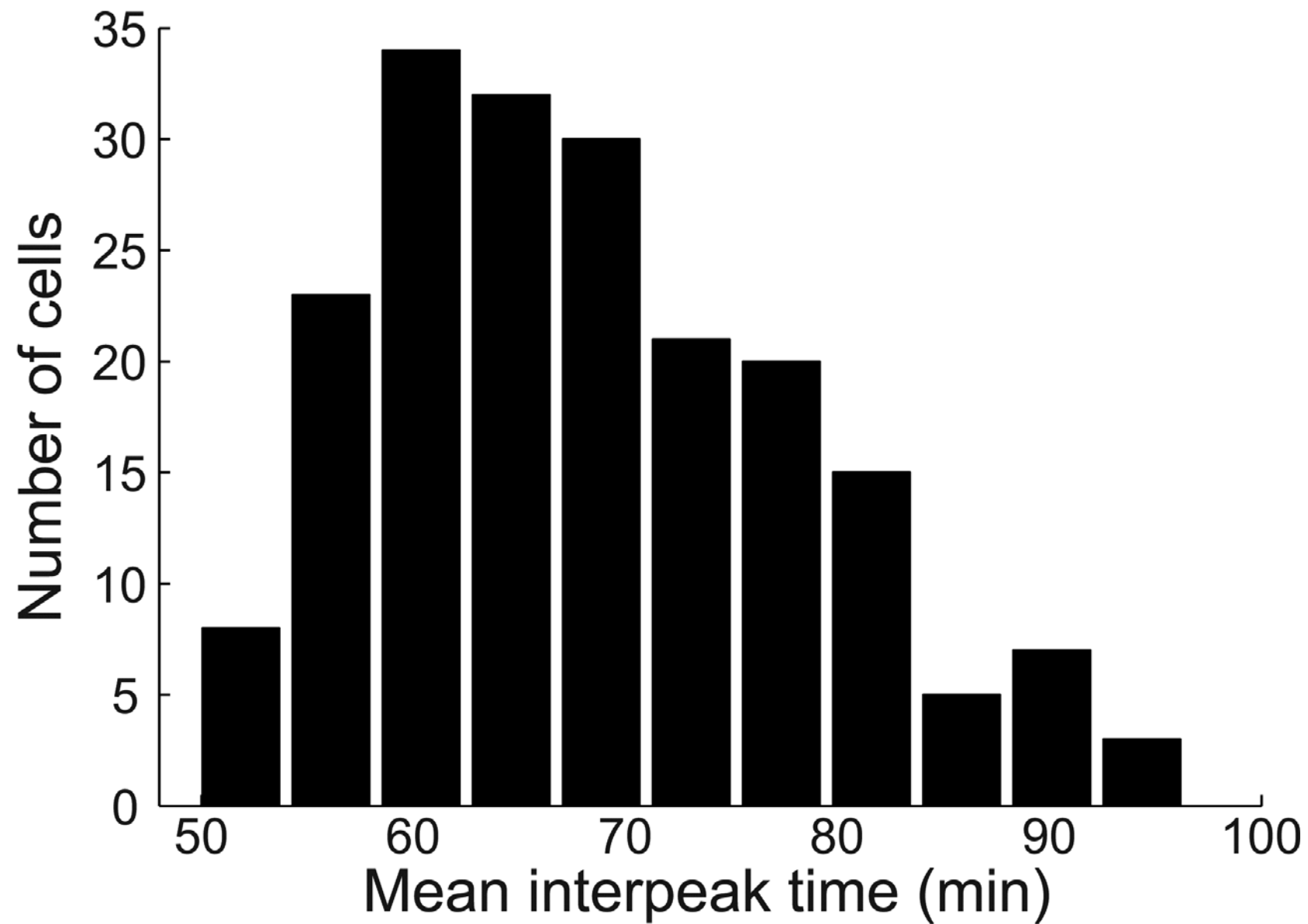


Figure S3

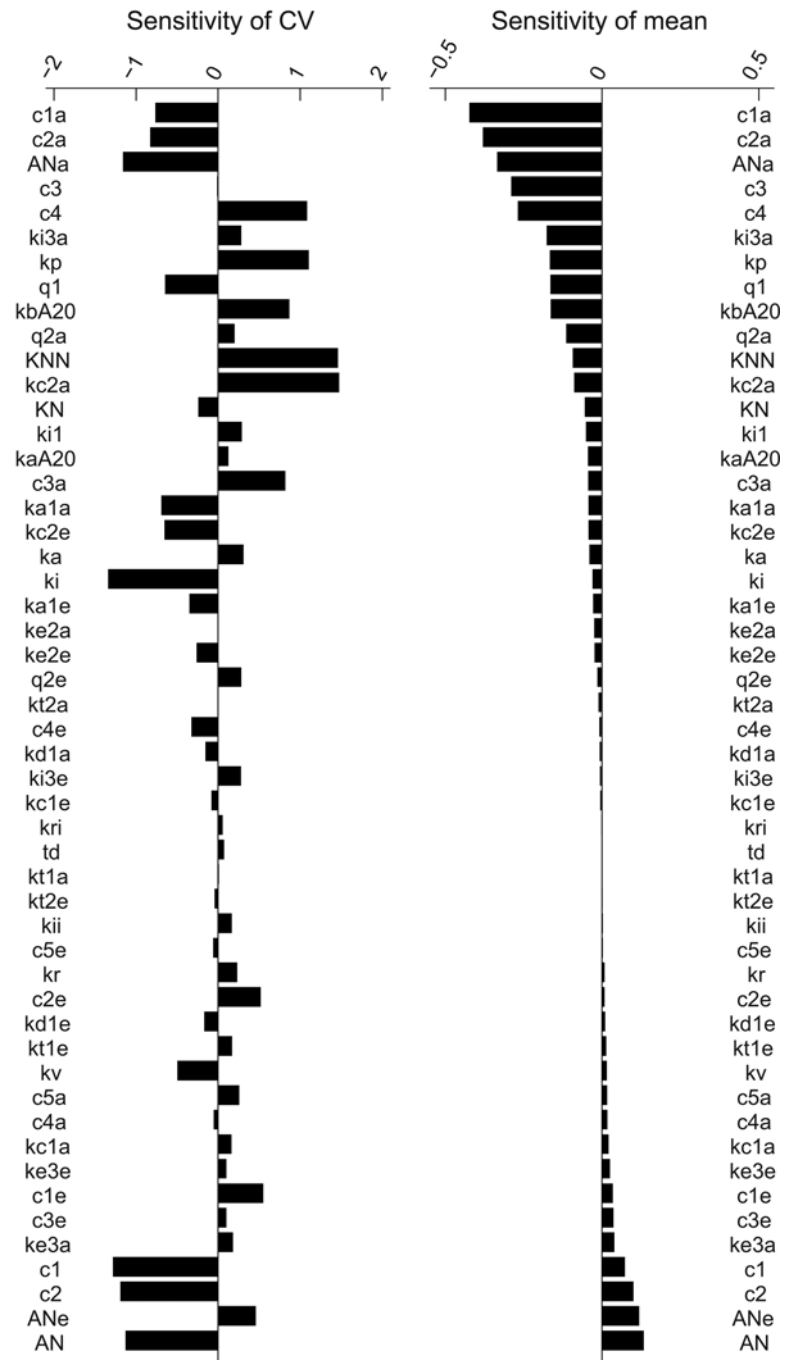
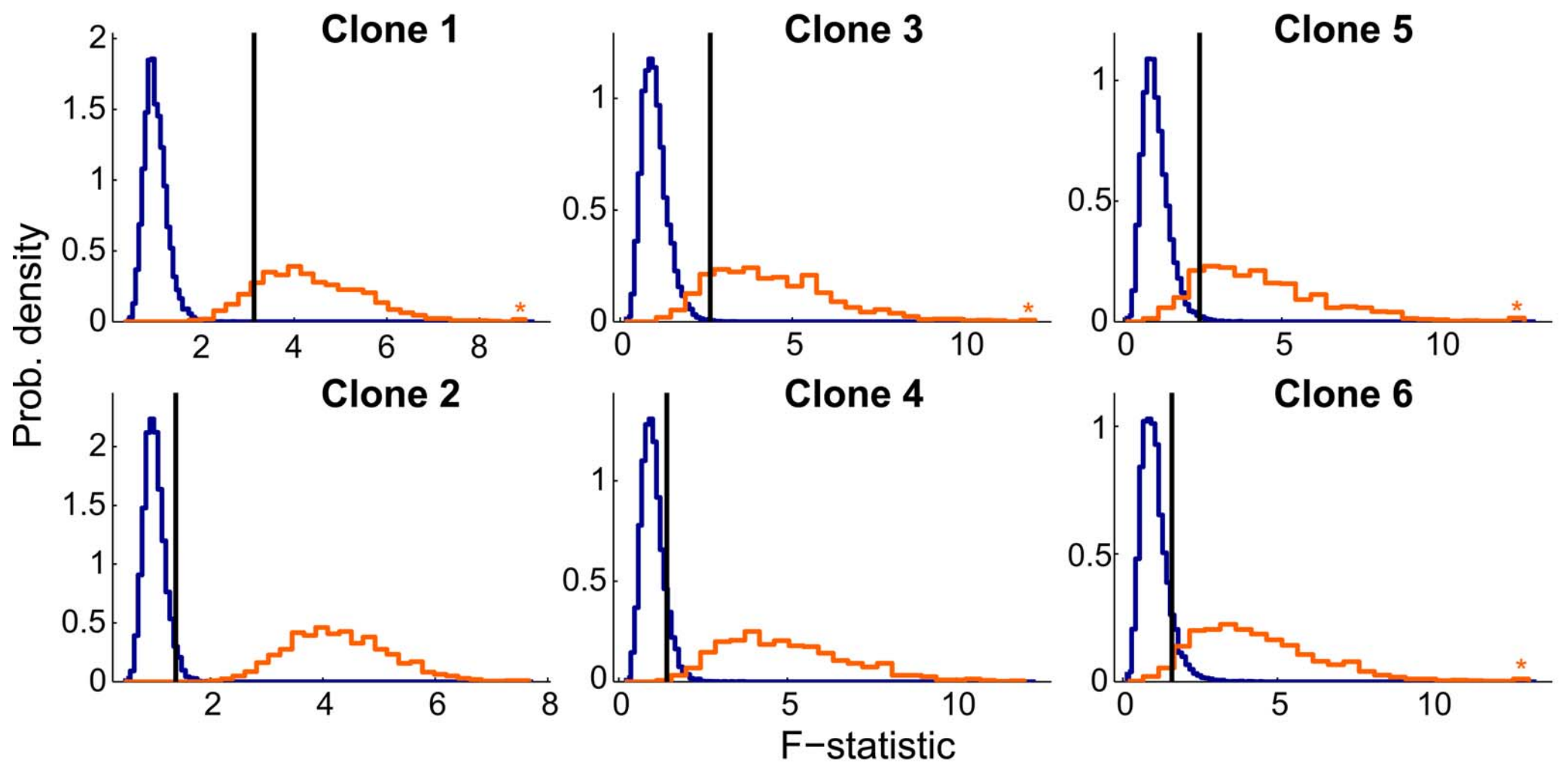


Figure S4



— No intercellular variability
— Same intercellular variability as polyclonal cells