

## Supplemental Data

### Figure S1

The number-labeled cells (shown at  $t = 0$ ) were used to produce the quantified data in Figure 1B and Figure S2. In all experiments in this study, cells that divided, died, or moved into/out of the view during the course of imaging were excluded from quantification (see Methods). Movie S1 corresponds to the complete time course imaging data after TNF- $\alpha$  treatment. The expressed fluorescent proteins were p65:EGFP (green) and pIL-8:mCherry (red).

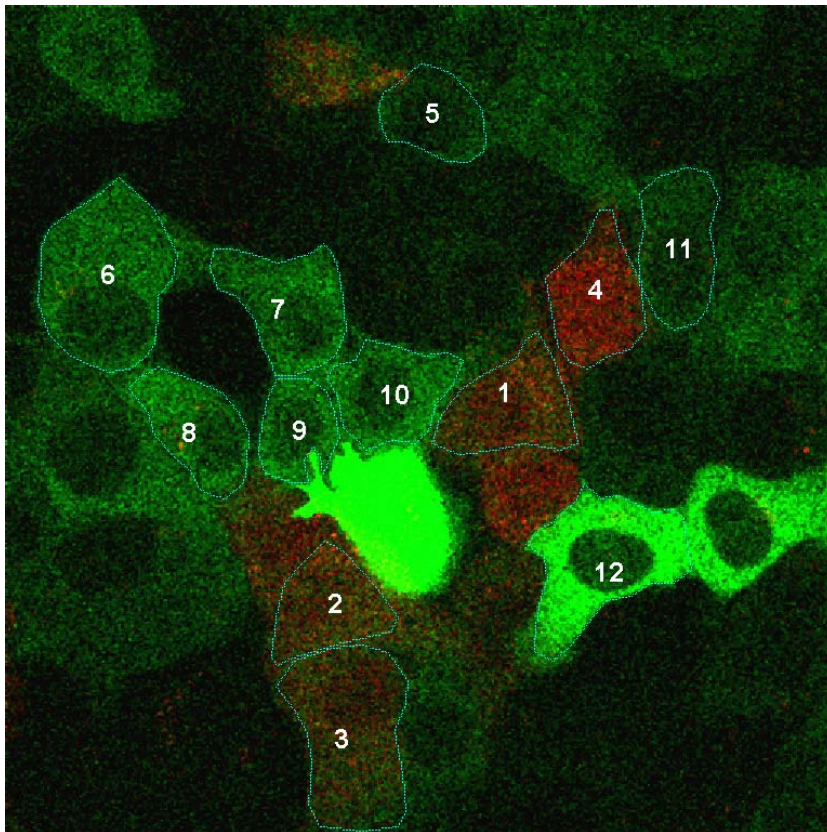
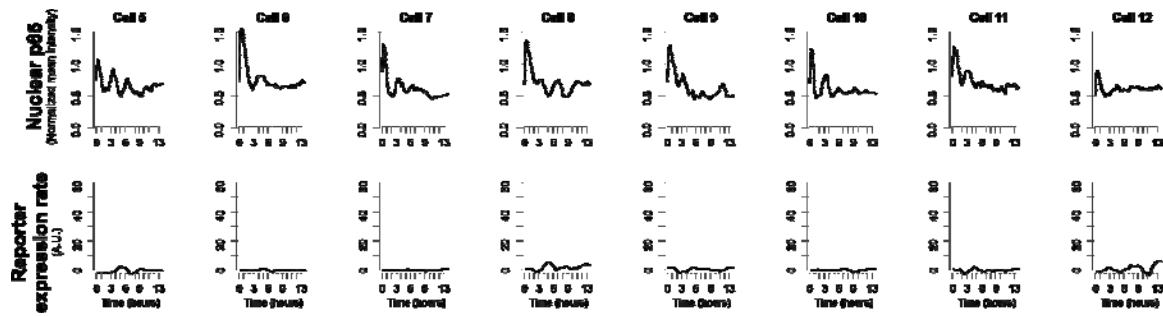


Figure S2

Quantification of cells 5 to 12 shown in Figure S1 in the same format as in Figure 1B.

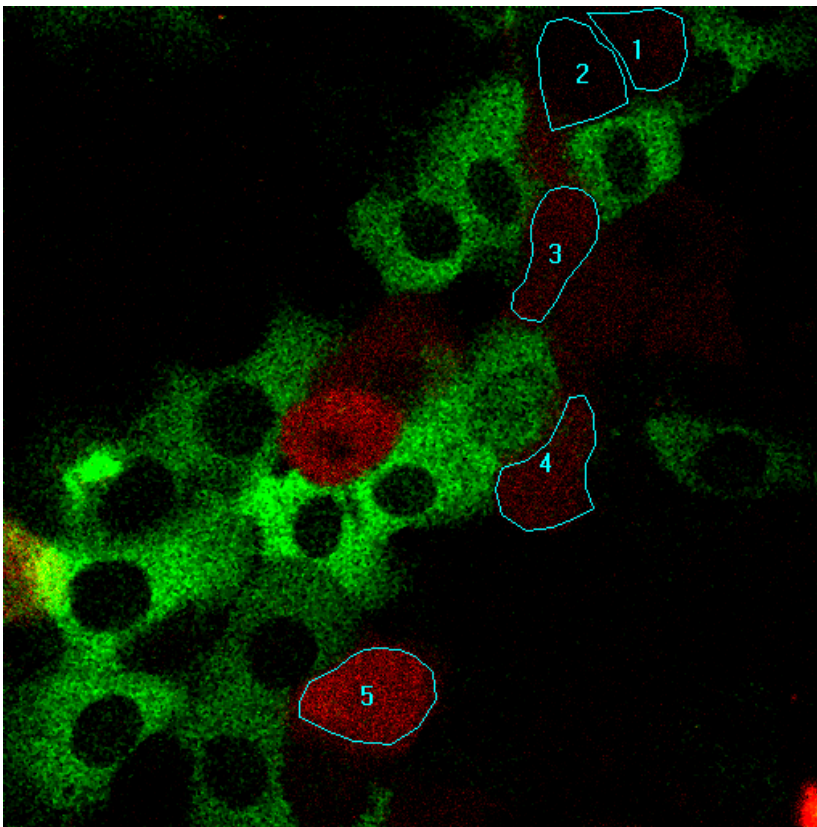


**Figure S3**

The labeled cells (shown at  $t = 0$ ) were used to produce the quantified data in Figure 3B.

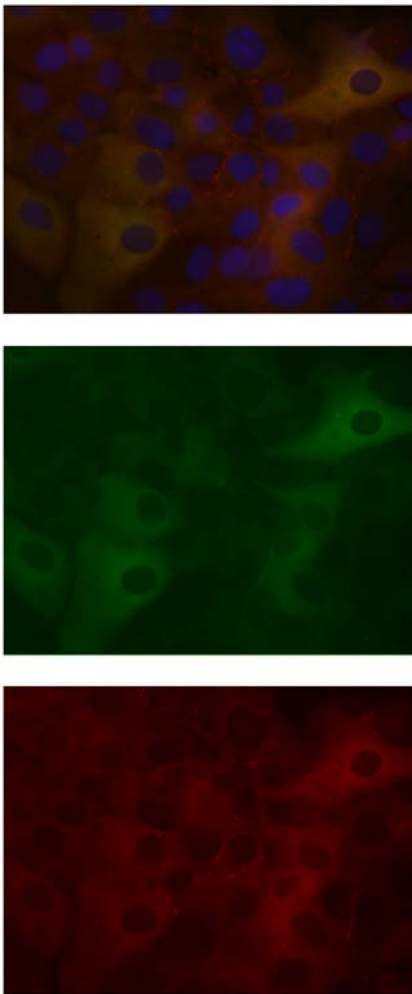
Many cells in this time course experiment were excluded from quantification due to overlapping and/or movement into and out of the field of imaging (see Methods). Movie S2 corresponds to the complete time course imaging data after bortezomib treatment.

p65:EGFP, green; pIL-8:mCherry, red.



**Figure S4**

Immunofluorescence to measure the p65-EGFP fusion level in comparison to the total p65. The red channel (bottom panel) shows the staining by the antibody that labels both the endogenous p65 and the fusion protein. The green channel (middle panel) represents the level of p65-EGFP. The top panel is the merge of both channels with DAPI (blue) staining the nuclei.



**Movie S1**

Time course of cells expressing p65:EGFP (green) and pIL-8:mCherry (red) after TNF- $\alpha$  treatment, quantified in Figure 1B.

**Movie S2**

Time course of TNF- $\alpha$  pre-treated cells expressing p65:EGFP (green) and pIL-8:mCherry (red) after bortezomib treatment, quantified in Figure 3B.

**Movie S3**

Time course of a single living cell expressing p65:EGFP after TNF- $\alpha$  treatment, represented by both the grayscale time lapse images and the corresponding quantification of nuclear:total ratio from EGFP intensities. The time lapse image acquisition started 15 minutes after TNF- $\alpha$  treatment. The graph in the plot tracks the nuclear p65 level of the cell on the left side of the field of view.