Weber et al., http://www.jcb.org/cgi/content/full/jcb.200610148/DC1

Supplemental materials and methods

Measurement of apoptosis

To test for the presence of active caspase-3, cells were washed in PBS before fixation in 4% formaldehyde. Cells were washed in PBS and incubated in the presence of monoclonal anti–active-caspase-3 antibody (BD Biosciences) in permeabilization buffer (0.5% [wt/vol] BSA and 0.5% [wt/vol] saponin in PBS). Cells were washed in permeabilization buffer and incubated with species-specific Cy3-conjugated secondary antibody (Dianova). Flow cytometry was performed using a FACSCalibur system (Becton Dickinson).

For assessment of nuclear morphology, cells were stained with Hoechst dye (Sigma-Aldrich), removed from the plate by vigorous pipetting, and scored in UV light under a fluorescence microscope. Assays were done at least in duplicate as indicated, and a minimum of 200 cells per sample was counted. Analyses were performed by three investigators in independent experiments, who obtained very similar results.