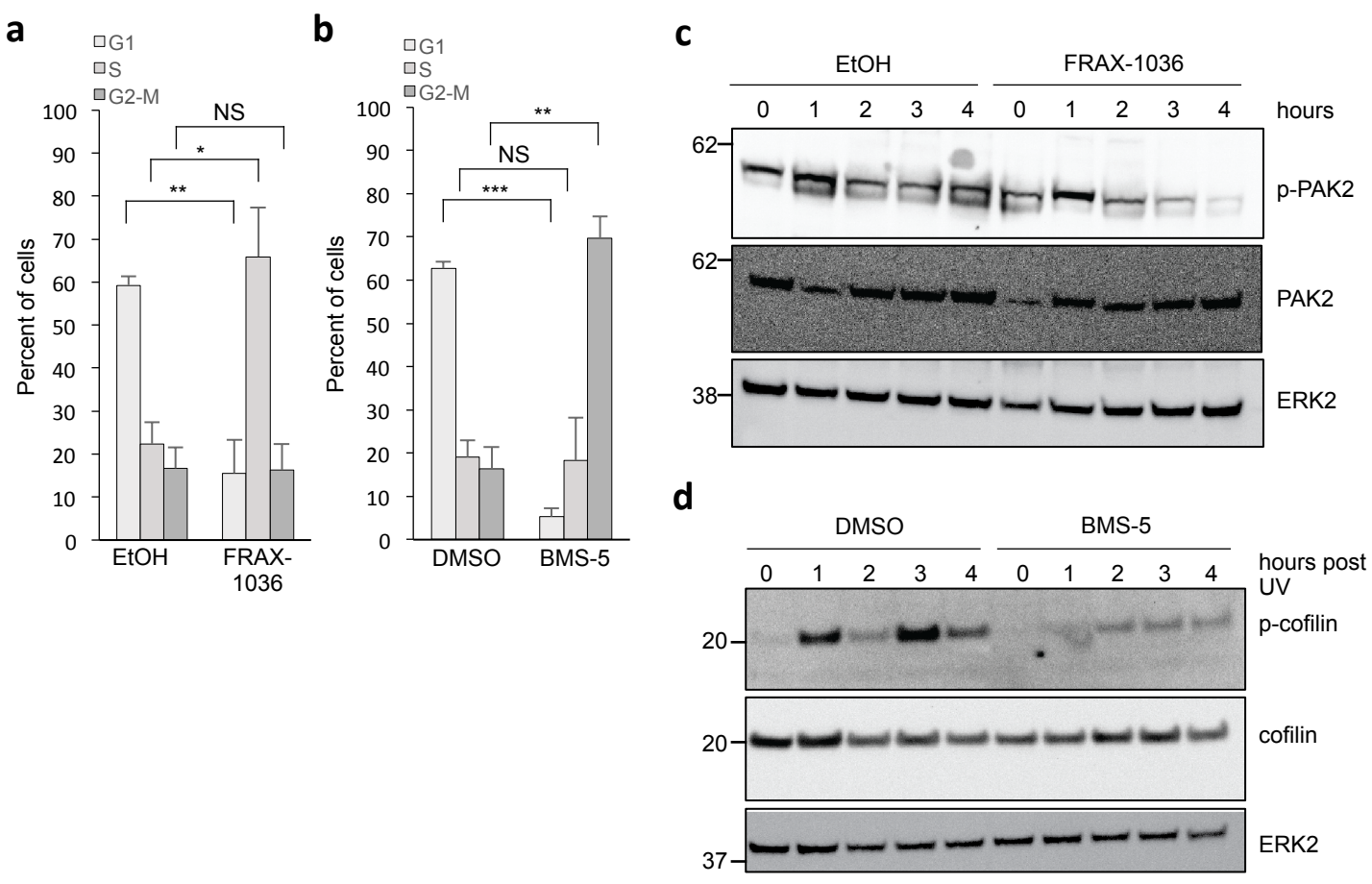
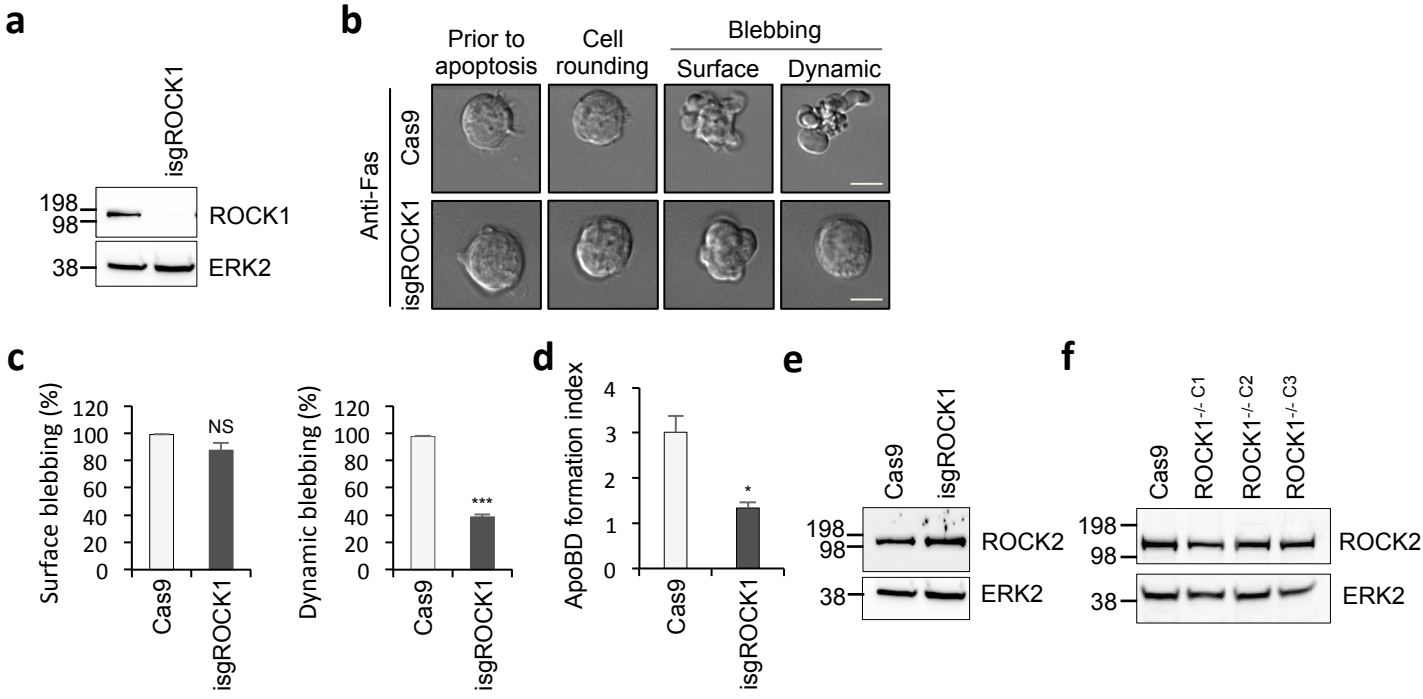
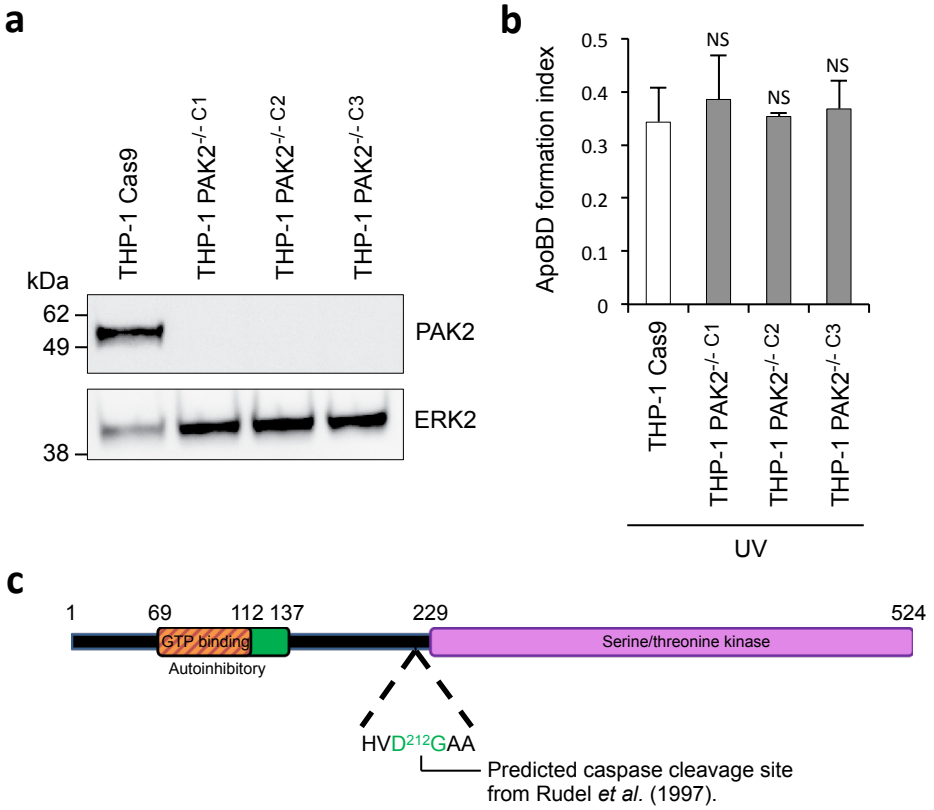


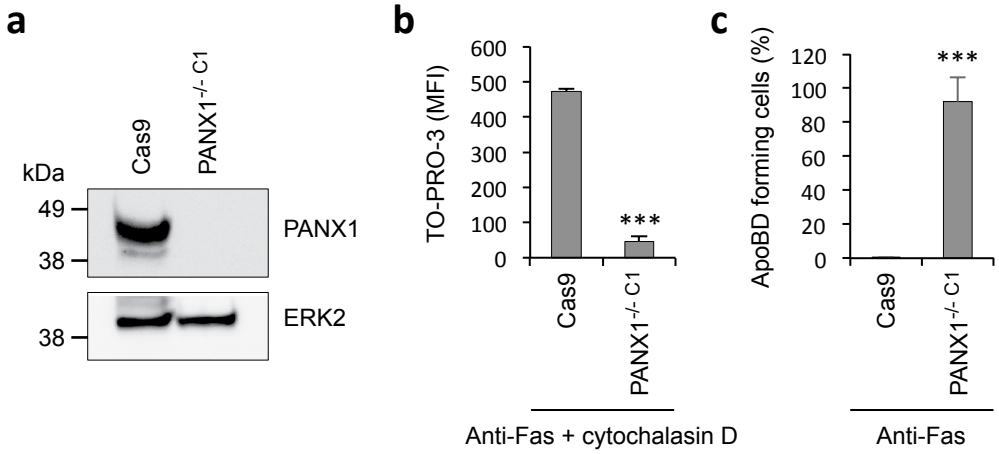
Supplementary Figure 1. Treatment with PAK2 inhibitor FRAX1036 and LIMK1 inhibitor BMS-5 arrests cell cycle progression. (a) Jurkat T cells treated with FRAX-1036 (2.5 μM) for 24 h showed an increase in cells arrested in S-phase as determined by propidium iodide staining monitored by flow cytometry. **(b)** Jurkat T cells treated with BMS-5 (10 μM) for 24 h showed an increase in cells arrested in G2-M phase as determined by propidium iodide staining monitored by flow cytometry. **(c)** Level of phosphorylated PAK2 (p-PAK2) was determined by immunoblot analysis of Jurkat T cells treated with vehicle (EtOH) or FRAX-1036 (2.5 μM) for 0 to 4 h. **(d)** Level of phosphorylated cofilin (p-cofilin) was determined by immunoblot analysis of UV-irradiated (150 mJ/cm^2) Jurkat T cells treated with vehicle (DMSO) or BMS-5 (10 μM) for 0 to 4 h. Error bars represent s.e.m. Data represents three independent experiments. * P<0.05, ** P<0.01, *** P<0.001, NS = P>0.05, unpaired Student's two-tailed t-test.



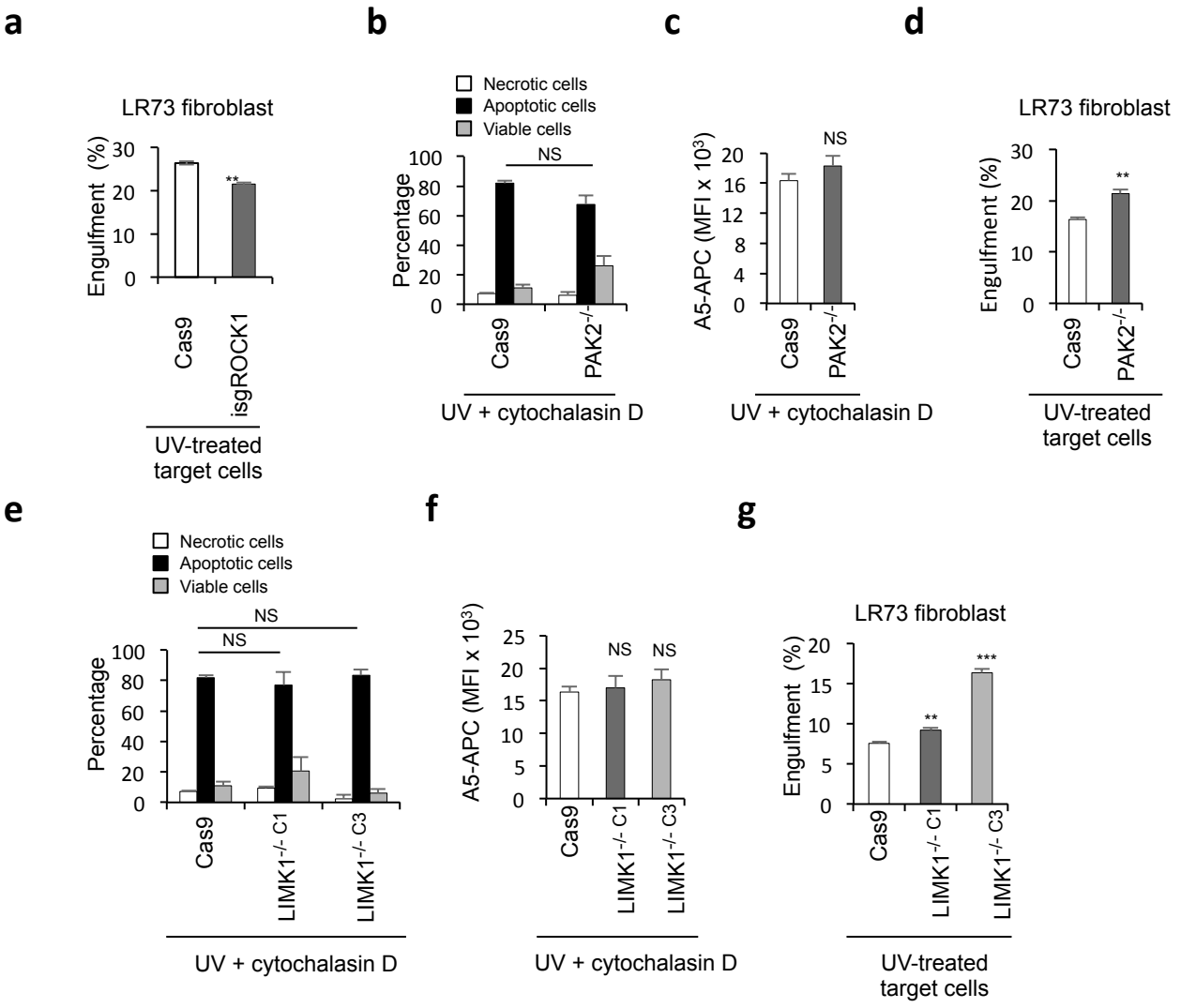
Supplementary Figure 2. Loss of ROCK1 expression in Jurkat T cells impairs apoptotic membrane blebbing and ApoBD formation. (a) Loss of ROCK1 protein expression with CRISPR/Cas9-mediated *ROCK1* gene disruption in doxycycline (dox, 1 μg/ml) treated isgROCK1 Jurkat T cells was validated by immunoblot analysis. **(b)** Representative time-lapse DIC imaging of dox-treated Cas9 and isgROCK1 Jurkat T cells over 4 h post anti-Fas (0.5 μg/ml) treatment showing apoptotic morphology. **(c)** Quantification of apoptotic isgROCK1 cells that underwent surface and dynamic blebbing (*n*=3). **(d)** Formation of ApoBDs from apoptotic isgROCK1 cells treated with anti-Fas (0.5 μg/ml) and trovafloxacin (40 μM), as measured by flow cytometry (*n*=3). **(e)** Expression of ROCK2 in dox-treated Cas9 and isgROCK1 Jurkat cells and (**f**) ROCK1 knockout clonal cells**,** was monitored by immunoblot analysis. Error bars represent s.e.m. Scale bar, 10 μm. Data represents three independent experiments. * P<0.05, *** P<0.001, NS = P>0.05, unpaired Student's two-tailed t-test.



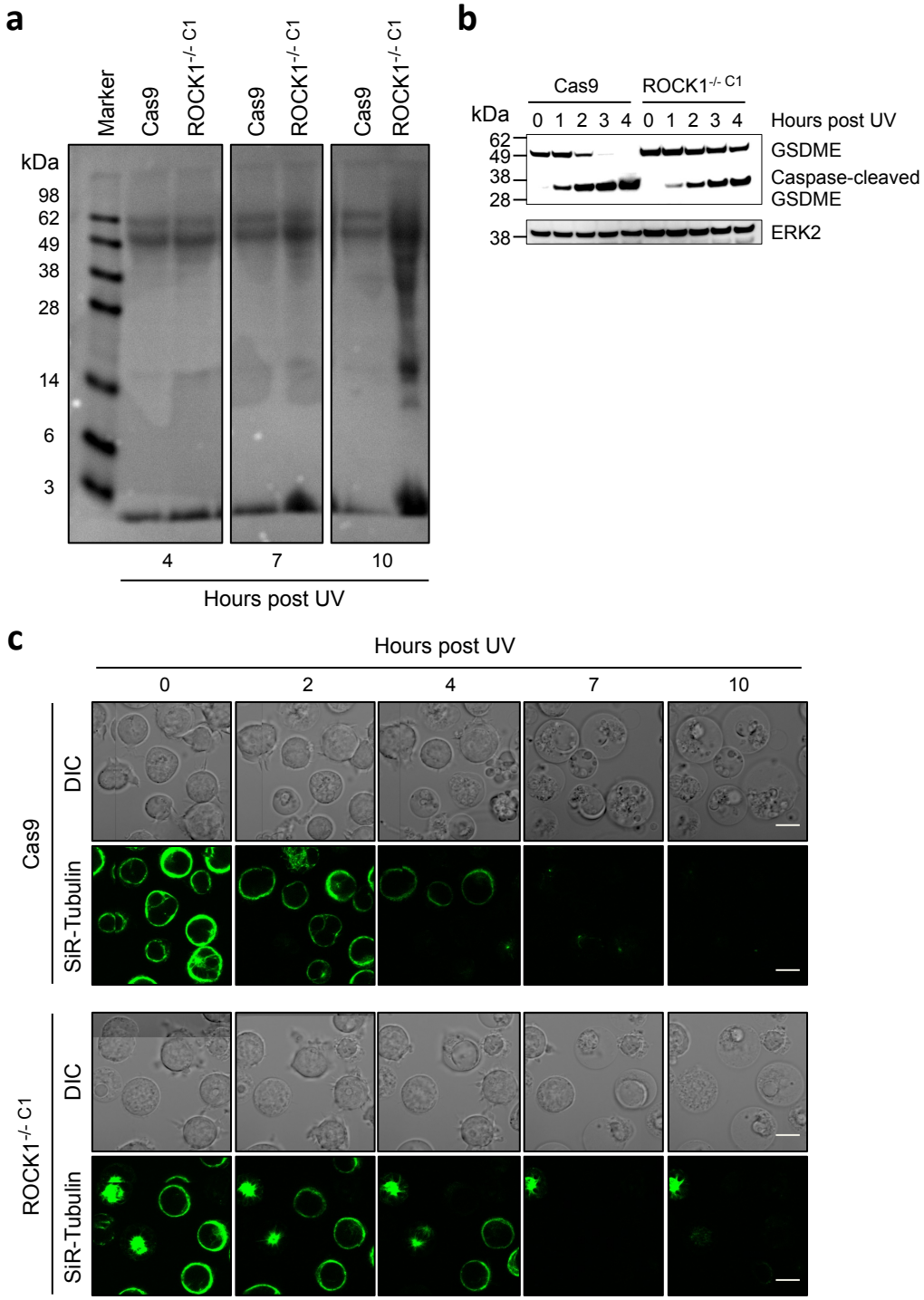
Supplementary Figure 3. Loss of PAK2 in THP-1 monocytes does not impair ApoBD formation. (a) Loss of PAK2 protein expression with CRISPR/Cas9-mediated PAK2 gene disruption in three THP-1 cell clonal populations (THP-1 PAK2$^{-/-\ C1}$, THP-1 PAK2$^{-/-\ C2}$, THP-1 PAK2$^{-/-\ C3}$) was validated by immunoblot analysis. **(b)** Formation of ApoBDs from UV irradiated THP-1 PAK2$^{-/-}$ cells, as monitored by flow cytometry (n=3). **(c)** Schematic representation of human PAK2. Key functional domains and the predicted caspase cleavage site from Rudel et al. (1997) shown. THP-1 Cas9, THP-1 cells expressing Cas9 only was used as a control. (b) Error bars represent s.e.m. Data are representative of three independent experiments. NS = P>0.05, unpaired Student's two-tailed t-test.



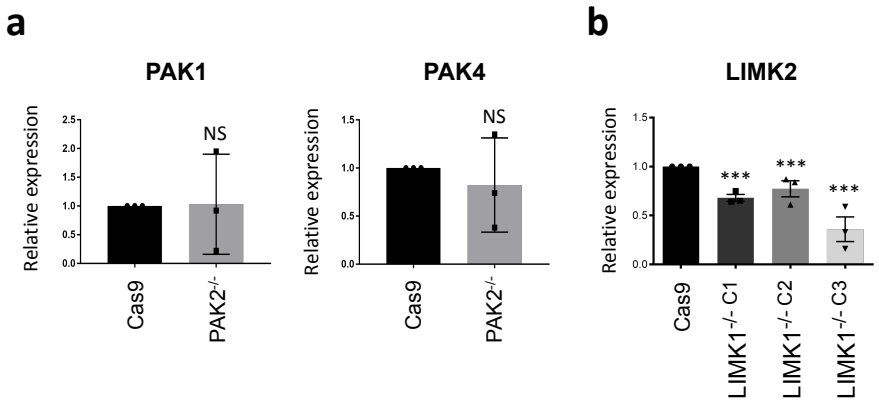
Supplementary Figure 4. Loss of PANX1 expression enhances ApoBD formation. (a) Loss of PANX1 protein expression with CRISPR/Cas9-mediated PANX1 gene disruption in Jurkat T cell clonal population (PANX1$^{-/-\ C1}$) was validated by immunoblot analysis. **(b)** Reduction in TO-PRO-3 uptake (a nucleic acid dye that enters apoptotic Jurkat T cells via caspase-activated PANX1 channels) in apoptotic PANX1$^{-/-\ C1}$ cells, as measured by flow cytometry. Cells were treated with anti-Fas (0.5 μg/ml) to induce apoptosis and cytochalasin D (10 μM) to prevent the disassembly of apoptotic cells in all samples to ensure fair comparison between cell lines (n=3). (c) Formation of ApoBDs from PANX1$^{-/-\ C1}$ cells treated with anti-Fas (0.5 μg/ml) to induce apoptosis, as measured by flow cytometry (n=3). Cas9, Jurkat T cells expressing Cas9 only was used as a control. (b and c) Error bars represent s.e.m. Data are representative of at least three independent experiments. *** P<0.001, unpaired Student's two-tailed *t*-test.



Supplementary Figure 6. Loss of PAK2 and LIMK1 does not leads to a defect in efferocytosis by fibroblast cells (a) Quantitation of the uptake of UV-treated CypHer5E-stained non-clonal Cas9, isgROCK1 cells by CellTrace Violet-stained hamster LR73 fibroblast cells as determined by flow cytometry. **(b)** Level of necrotic, apoptotic and viable cells were determined in UV-treated PAK2-/- cells in the presence of cytochalasin D (10 μM) by flow cytometry (n=3), and **(c)** PtdSer exposure on apoptotic PAK2$^{-/-}$ based on A5-PE staining as determined by flow cytometry (n=3) , **(d)** Quantitation of the uptake of UV-treated CypHer5E-stained Cas9, PAK2$^{-/-}$ cells by CellTrace Violet-stained hamster LR73 fibroblast cells as determined by flow cytometry. **(e)** Level of necrotic, apoptotic and viable cells were determined in UV irradiated LIMK1$^{-/-\ C1}$ and LIMK1$^{-/-\ C3}$ cells in the presence of cytochalasin D (10 μM) by flow cytometry (n=3), and **(f)** PtdSer exposure on apoptotic LIMK1$^{-/-\ C1}$ and LIMK1$^{-/-\ C3}$ based on A5-PE staining as determined by flow cytometry (n=3) , **(g)** Quantitation of the uptake of UV-irradiated CypHer5E-stained Cas9, LIMK1$^{-/-\ C1}$ and LIMK1$^{-/-\ C3}$ cells by CellTrace Violet-stained hamster LR73 fibroblast cells as determined by flow cytometry. Data are representative of at least three independent experiments. ** P<0.01, *** P<0.001, NS = P>0.05, unpaired Student's two-tailed t-test.



Supplementary Figure 6. Early onset of secondary necrosis in ROCK1$^{-/-}$ Jurkat T cells is independent of GSDME and microtubule network function in apoptosis. (a) Ponceau stain of western-transferred precipitated supernatant proteins of Cas9 and ROCK1$^{-/-\ C1}$ Jurkat T cells at 4, 7 and 10 h post UV (150 mJ/cm^2) induced apoptosis. **(b)** Immunoblot analysis of GSDME expression and cleavage in UV-treated (150 mJ/cm^2) Cas9 and ROCK1$^{-/-\ C1}$ Jurkat T cells. **(c)** Representative time-lapse confocal microscopy images of UV-treated (150 mJ/cm^2) Cas9 and ROCK1$^{-/-\ C1}$ Jurkat T cells stained with SiR-Tubulin to monitor microtubules during the progression of apoptosis. Scale bar, 10 µm. Data are representative of three independent experiments.



Supplementary Figure 7. PAK2 and LIMK1 knockout Jurkat T cells express corresponding isoforms. (a) Quantitative PCR (qPCR) analysis of PAK2$^{-/-}$ cells showed no significant change in PAK1 and PAK4 expression as compared to Cas9 control (*n*=3). **(b)** LIMK1$^{-/-}$ cells showed a reduction in the expression of LIMK2 mRNA as determined by qPCR analysis (*n*=3). ΔΔCT method was used to analyze qPCR data. Error bars represent s.e.m. Data represents three independent experiments. *** *P*<0.001, NS = *P*>0.05, unpaired Student's two-tailed *t*-test.