Expanded View Figures

Figure EV1. Depletion of API5 sensitizes cells to PFT-mediated apoptosis.

A, B HeLa cells were transfected with either control or API5 siRNAs for 1 day and then treated with 300 ng/ml of PFT for 24 h. The cells were harvested and the dead cells were measured by FACS analysis after Annexin V/PI staining as detailed in the Materials and Methods. Shown in (B) are data from three (n = 3; left panel) or four (n = 4, middle panel) independent experiments. The dead cells include Annexin V-positive early apoptotic as well as Annexin V/PI double-positive cells, indicating the late apoptotic/secondary necrotic populations as analysed by flow cytometry. The efficiency of the knockdown with siRNA#1 was monitored by immunoblots (B, right panel).

C Microscopy analysis of API-5 depleted cells upon α-toxin treatment. HeLa cells were transfected with siRNA and treated with PFT as mentioned before. The cells were treated with *in situ* caspase-3/7 substrate (green) for 30 min as mentioned in the Materials and Methods. The images were acquired after 6 h post-toxin treatment. Scale bar, 300 μm.

Source data are available online for this figure.

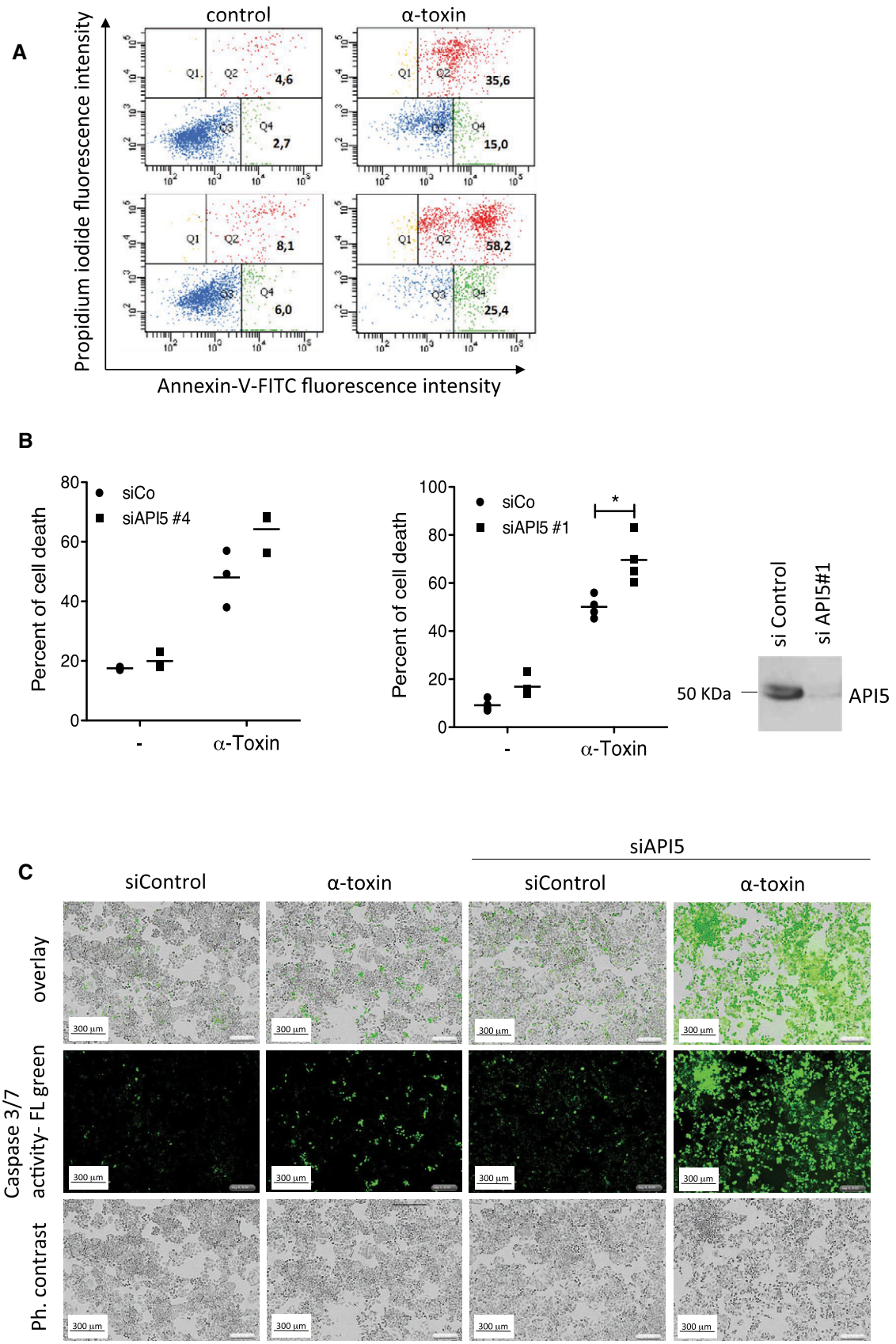


Figure EV1.

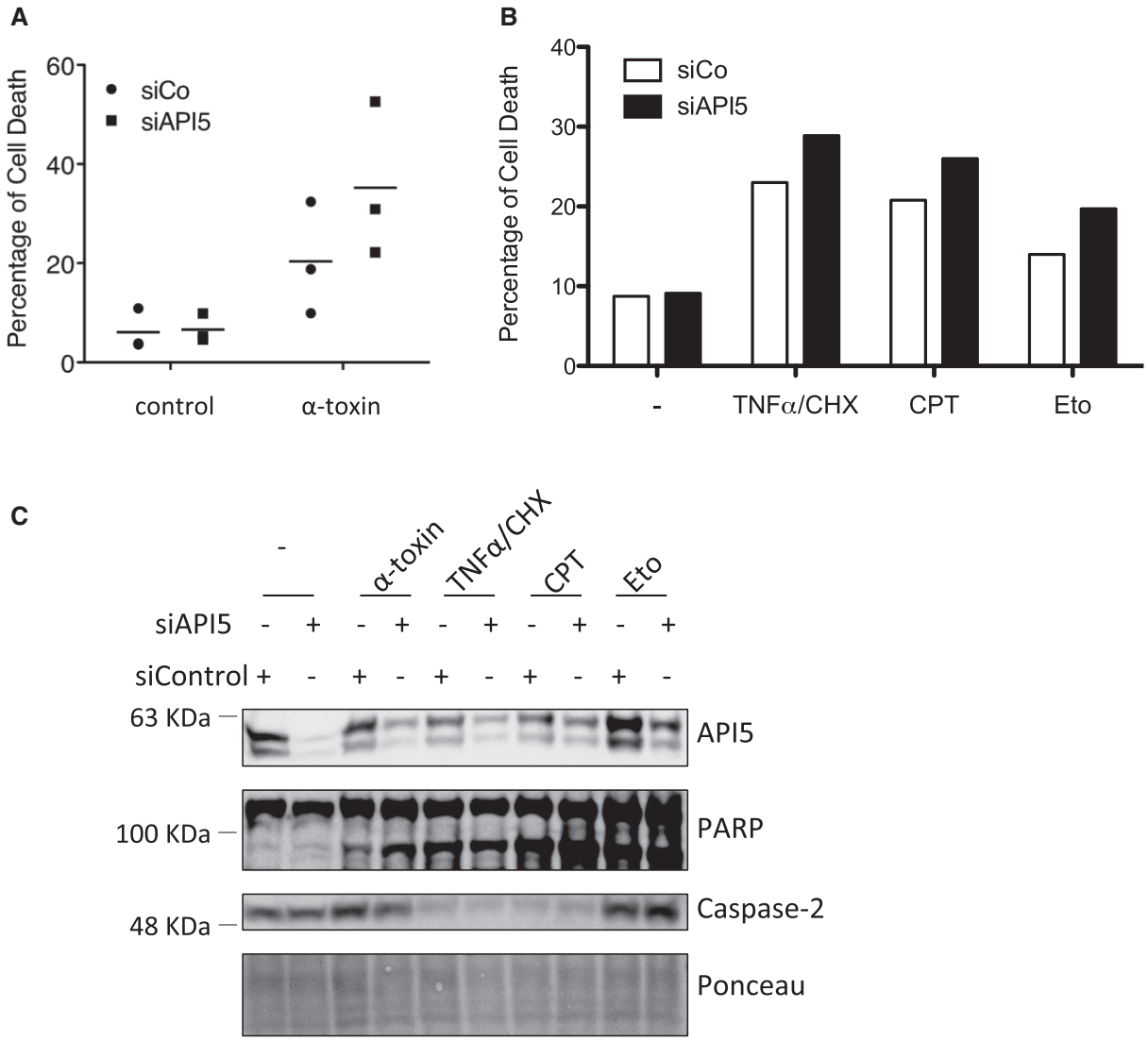


Figure EV2. Depletion of API5 sensitizes NCI-H1650 cells to PFT-mediated apoptosis.

A–C NCI-H1650 cells (lung adenocarcinoma) cells were transfected with control or API5 siRNAs employing Saint-Red reagent. After 24 h, the cells were treated with α-toxin (150 ng/ml), TNF-α (20 ng/ml)/CHX, camptothecin (4 μM) or etoposide (50 μM) for 24 h. Cells were harvested and labelled with propidium iodide for analysing cell death by (A, B) FACS analysis (n = 3 in A, n = 1 in B) or for (C) Western blot analysis.

Source data are available online for this figure.

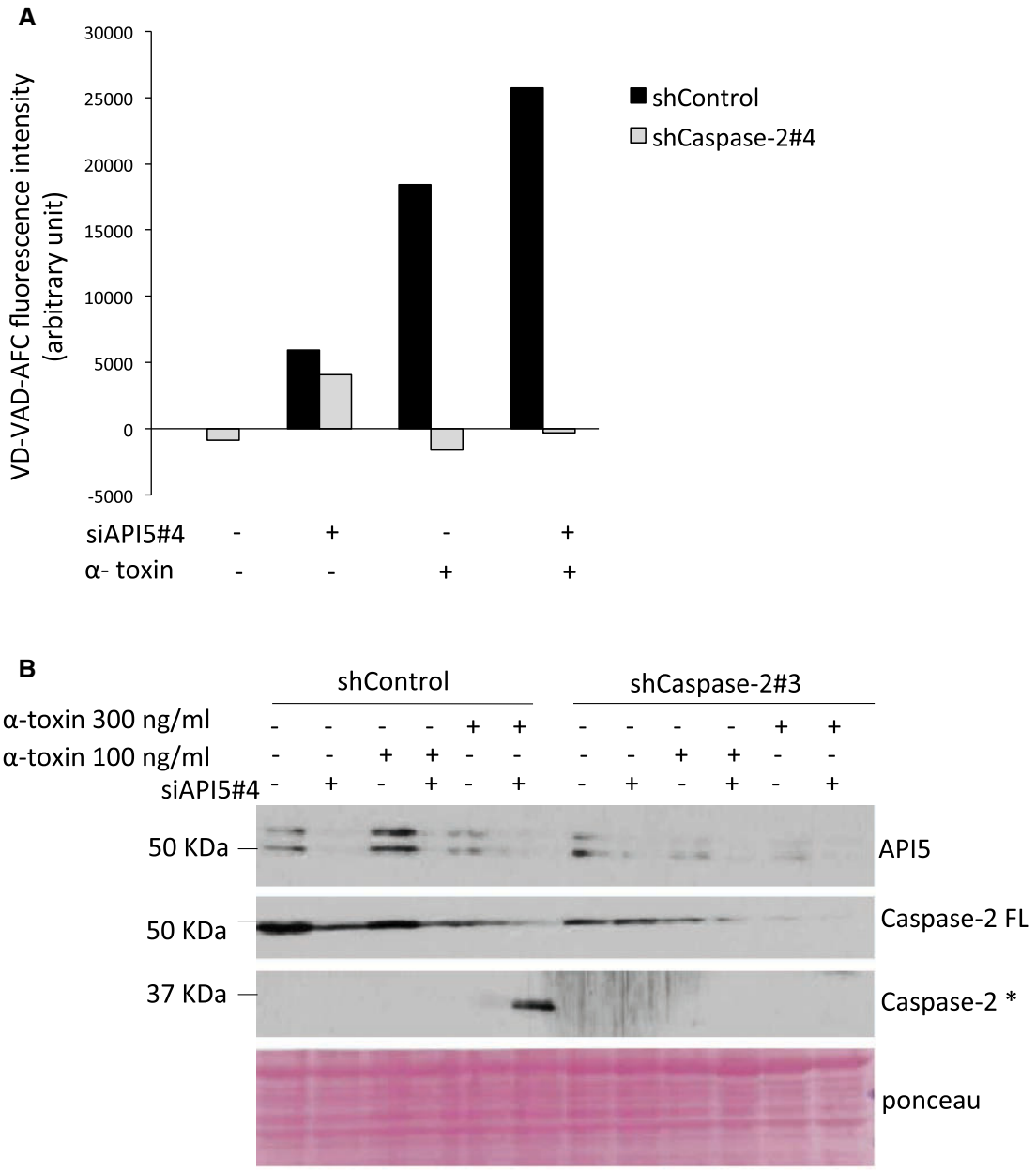


Figure EV3. Depletion of API5 enhances caspase-2 activation and processing.

A Measuring caspase-2 activity. ShControl and shCaspase-2 HeLa cells were transfected with siRNAs, and 24 h later, they were challenged with α-toxin (300 ng/ml); 24 h post-treatment, the samples were subjected to *in vitro* caspase-2 activity measurement as indicated in the Materials and Methods and following manufacturer's instructions.

B The cells were treated as above and 24 h later were subjected to Western blot analysis. FL: full length, *: processed form.

Source data are available online for this figure.

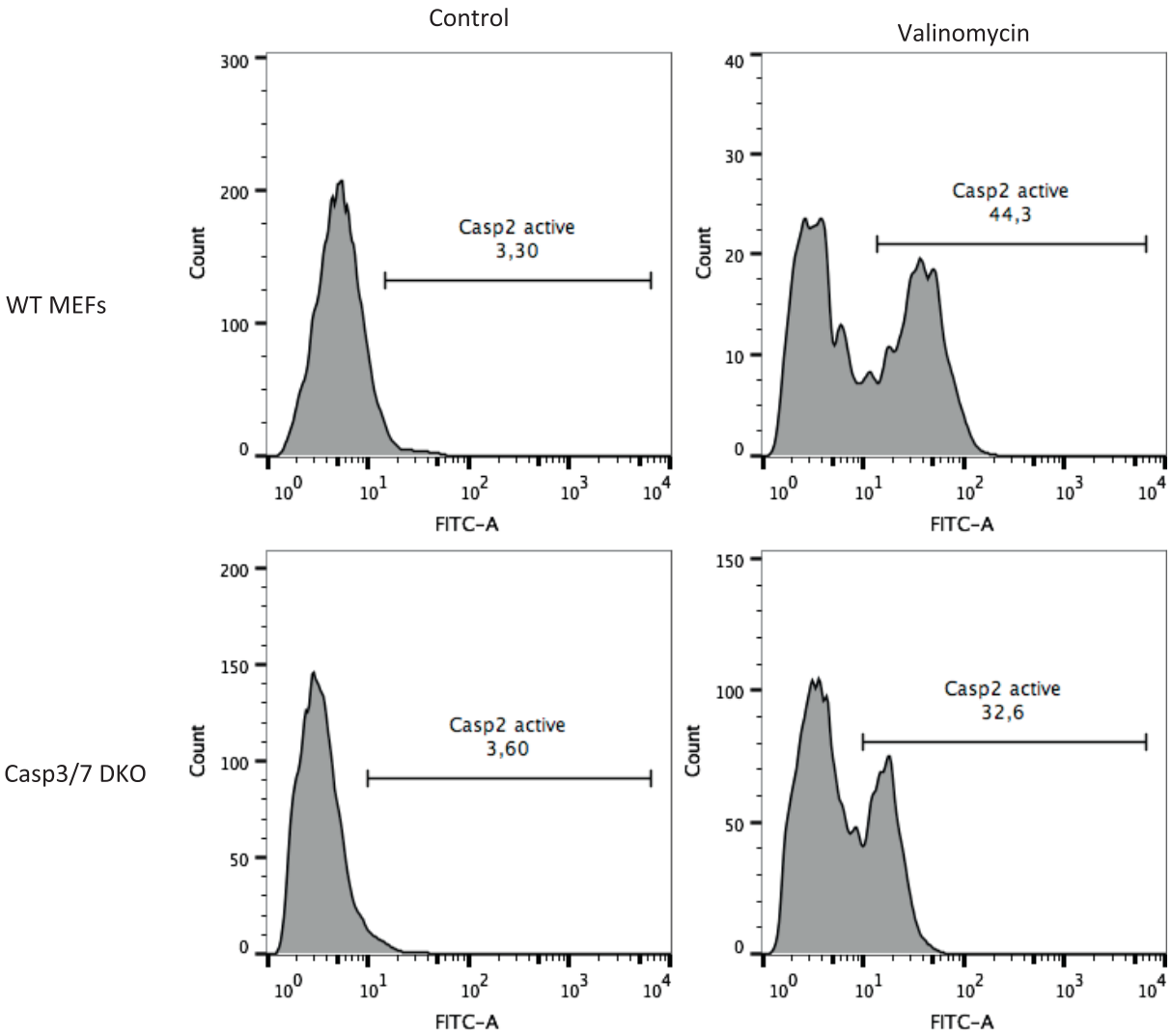
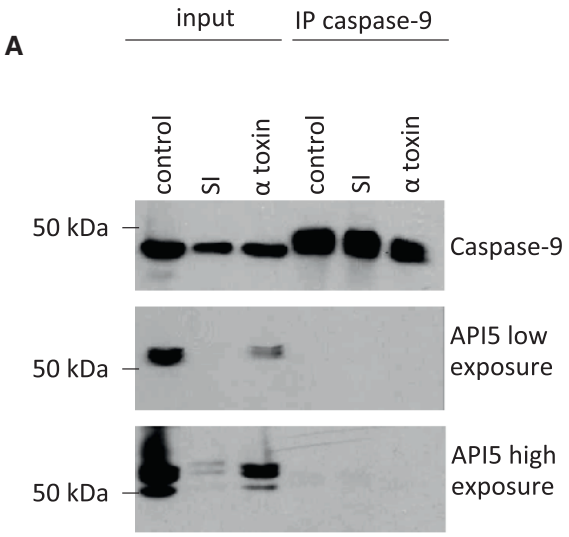


Figure EV4. Caspase-2 is activated in response to potassium ion depletion in a caspase-3/7-independent manner.
Wild-type or caspase-3/7 double KO MEFs were treated with valinomycin (30 μM) for 24 h. Cells were then collected and treated with FAM-VDVAD-FMK-FLICA reagent. Caspase-2 activity was measured by FACS analysis following the manufacturer's protocol (ImmunoChemistry). Shown are data from a single representative experiment.



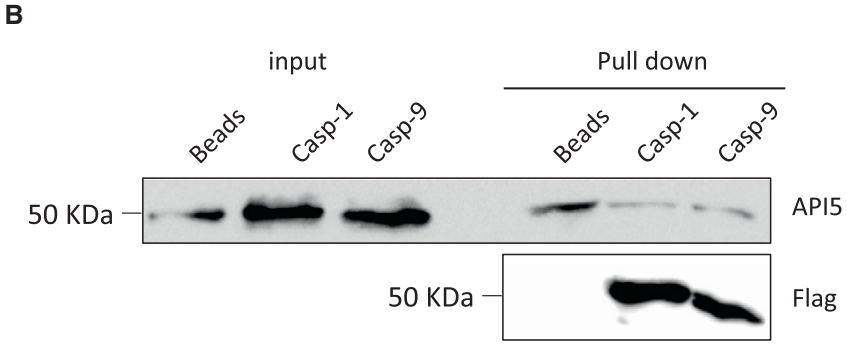


Figure EV5. API5 did not interact with caspase-9 and caspase-1.

A HeLa cells were incubated in serum-free EBSS (SI, starvation-induced) media or treated with α-toxin, and the samples were lysed and subjected to caspase-9 immunoprecipitation (IP) (see Materials and Methods) 24 h post-treatment. The total lysates and the immunoprecipitated-eluted samples were analysed by Western blot.

B 293T cells were transfected with pCMV2-Flag-Caspase-1 or pCMV3-Flag-Caspase-9 for 48 h. The Flag-tagged caspases were precipitated by anti-Flag M2 Magnetic Beads. The anti-Flag M2 Magnetic Beads were washed 5 times and then incubated overnight at 4°C with recombinant API5. After washing (3×), the beads were re-suspended in SDS buffer and subjected to Western blot analysis.

Source data are available online for this figure.