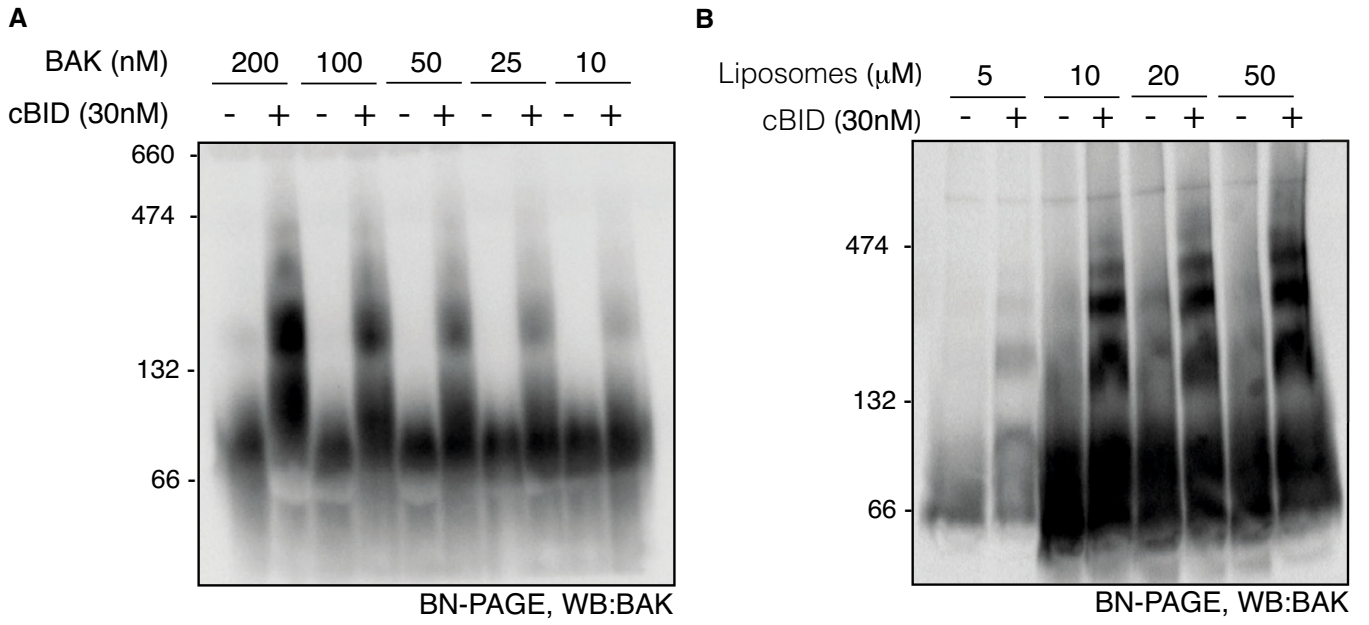
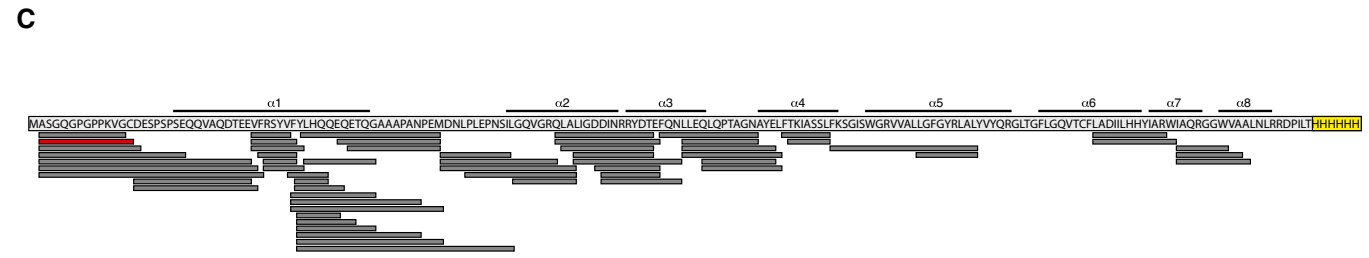
Expanded View Figures

Figure EV1. HDX-MS analysis of BAK-6H during oligomerisation on liposomes.

A, B Liposomes were incubated with BAK-6H and cBID at the indicated concentration prior to the analysis of BAK oligomerisation on BN-PAGE.
C BAK peptide coverage following combined proteolysis with pepsin and *Aspergillus* Type XIII protease.
D Example of time-dependent deuteration of BAK peptide. The increase in *m/z* of a BAK peptide (shown in red in C) due to incorporation with deuterium over time.

Source data are available online for this figure.





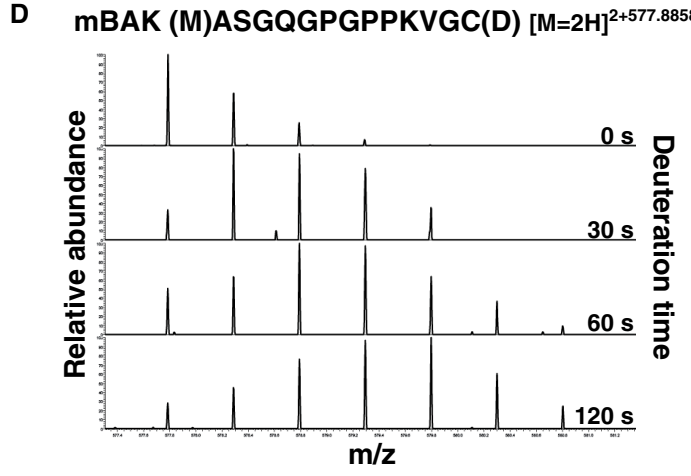


Figure EV1.

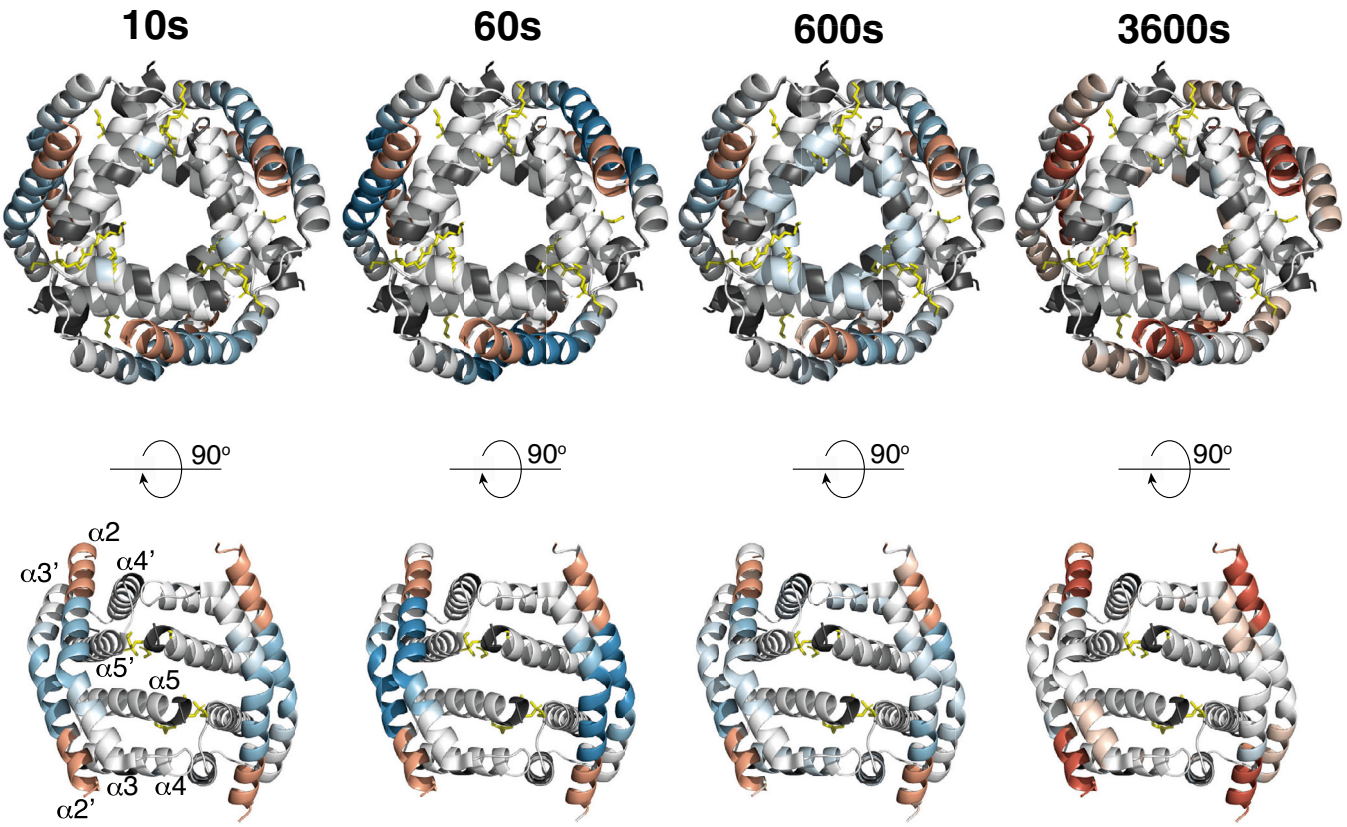


Figure EV2. HDX-MS mapped onto hexameric BAK.

Change in deuteration of BAK compared to inactive BAK is mapped onto the structure of hexameric BAK core dimers (trimer of α2-α5 homodimers) with bound *E. coli* lipids (*yellow stick*) (PDB:6UXM, Cowan *et al*, 2020). Colour coding as in Fig 2B. Lower panel is a 90° rotated view of the hexamer showing the uppermost 2 dimers only.

Figure EV3. BAK BH4 mutants adopt an activated conformation without exogenous stimulus.

Bax$^{-/-}$*Bak*$^{-/-}$ MEFs expressing BAK, BAK(ΔCys) or BAK(ΔCys) with mutations in the BH4 domain were incubated with doxycycline (3 h) to induce BAK expression followed by incubation with BH3 mimetics where indicated (for 2 h) and conformation change of BAK was assessed by intracellular flow cytometry with an antibody that recognises activated BAK (G3172). FACS profiles from three independent experiments are shown.

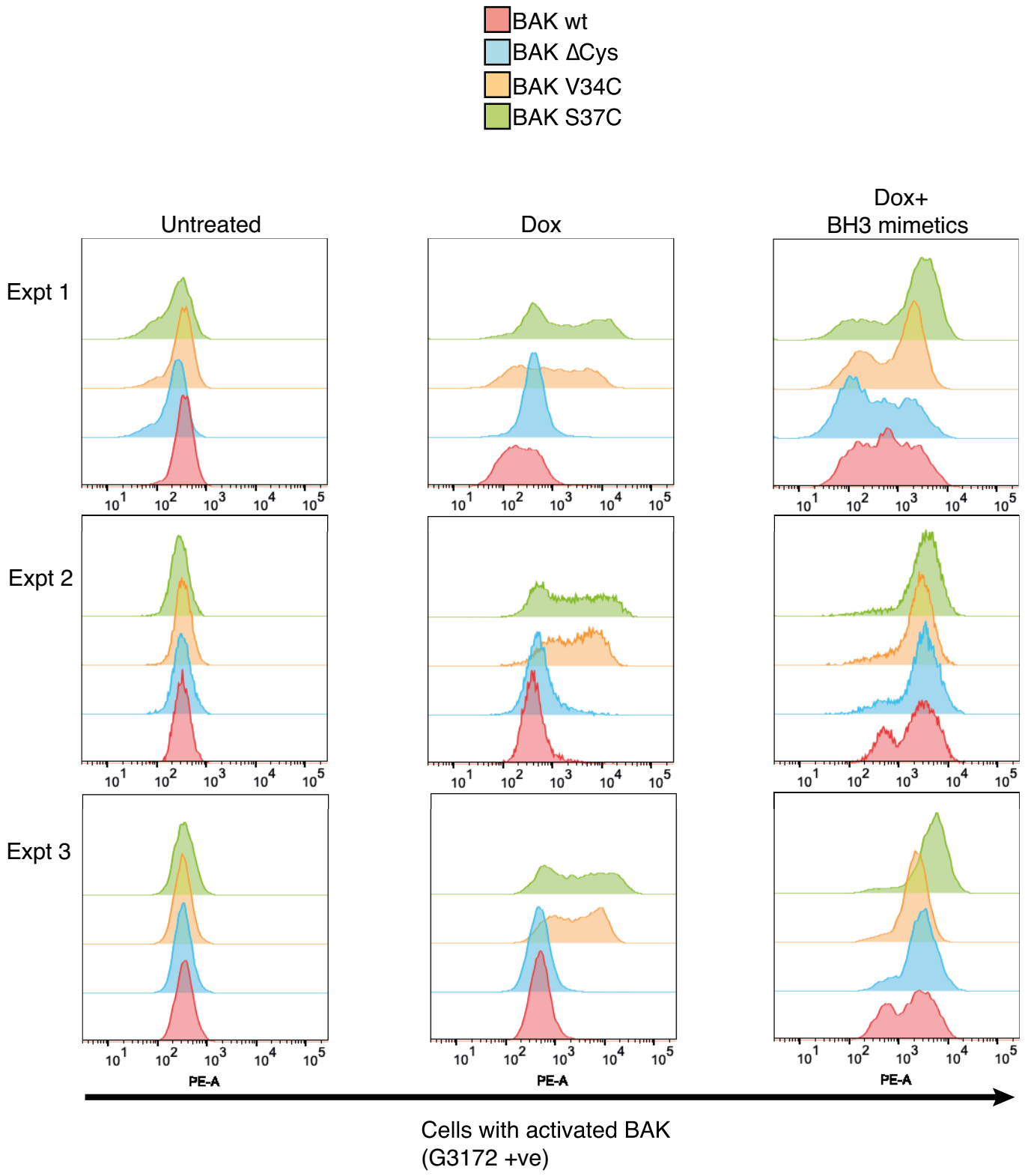
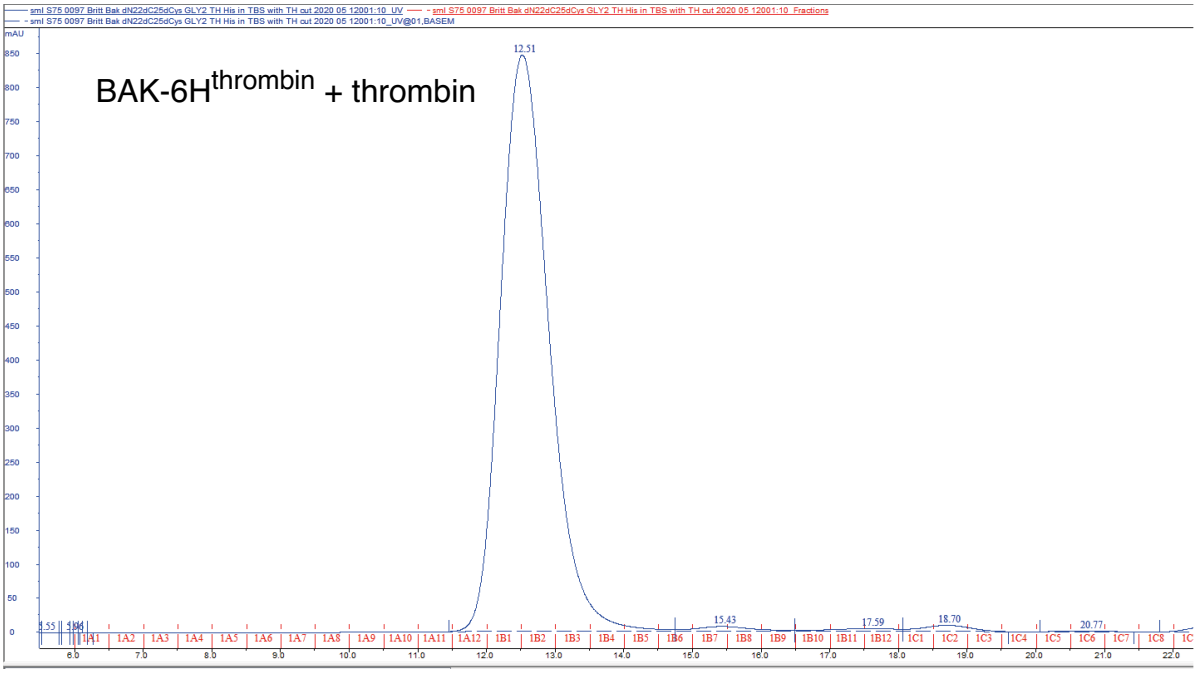


Figure EV3.



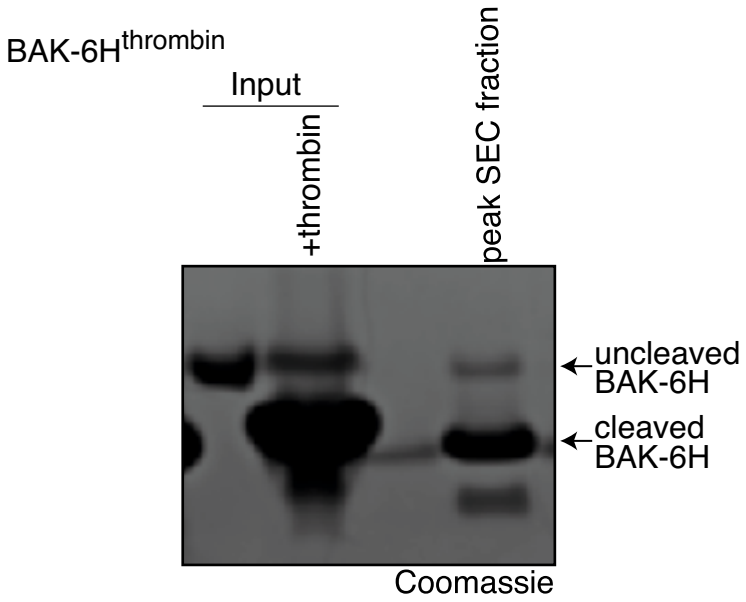


Figure EV4. α1-2 loop cleavage in recombinant BAK.

Recombinant BAK-6H^{thrombin} was cleaved with thrombin prior to size exclusion chromatography. Coomassie-stained SDS–PAGE of input and the peak fraction reveals efficient loop cleavage.

Source data are available online for this figure.

Figure EV5. BAK α1-2 loop cleavage does not inhibit cytochrome *c* release.

A FLAG-BAK or FLAG-BAK^{thrombin} were stably expressed in *Bax*$^{-/-}$*Bak*$^{-/-}$ MEFs and assessed by immunoblotting.

B Cells from A were treated with combined BH3 mimetic drugs for 24 h and cell death was analysed by PI uptake. Data are mean ± SD of three independent experiments.

C Mitochondria-enriched fractions isolated from cells in A were incubated with cBID (10 nM) in the presence or absence of thrombin for the indicated times and membrane pellet (P) and supernatant (S) were separated and immunoblotted for cytochrome *c* or BAK (4B5). Data from two independent experiments are shown, third experiment is shown in Fig 5E.

Source data are available online for this figure.

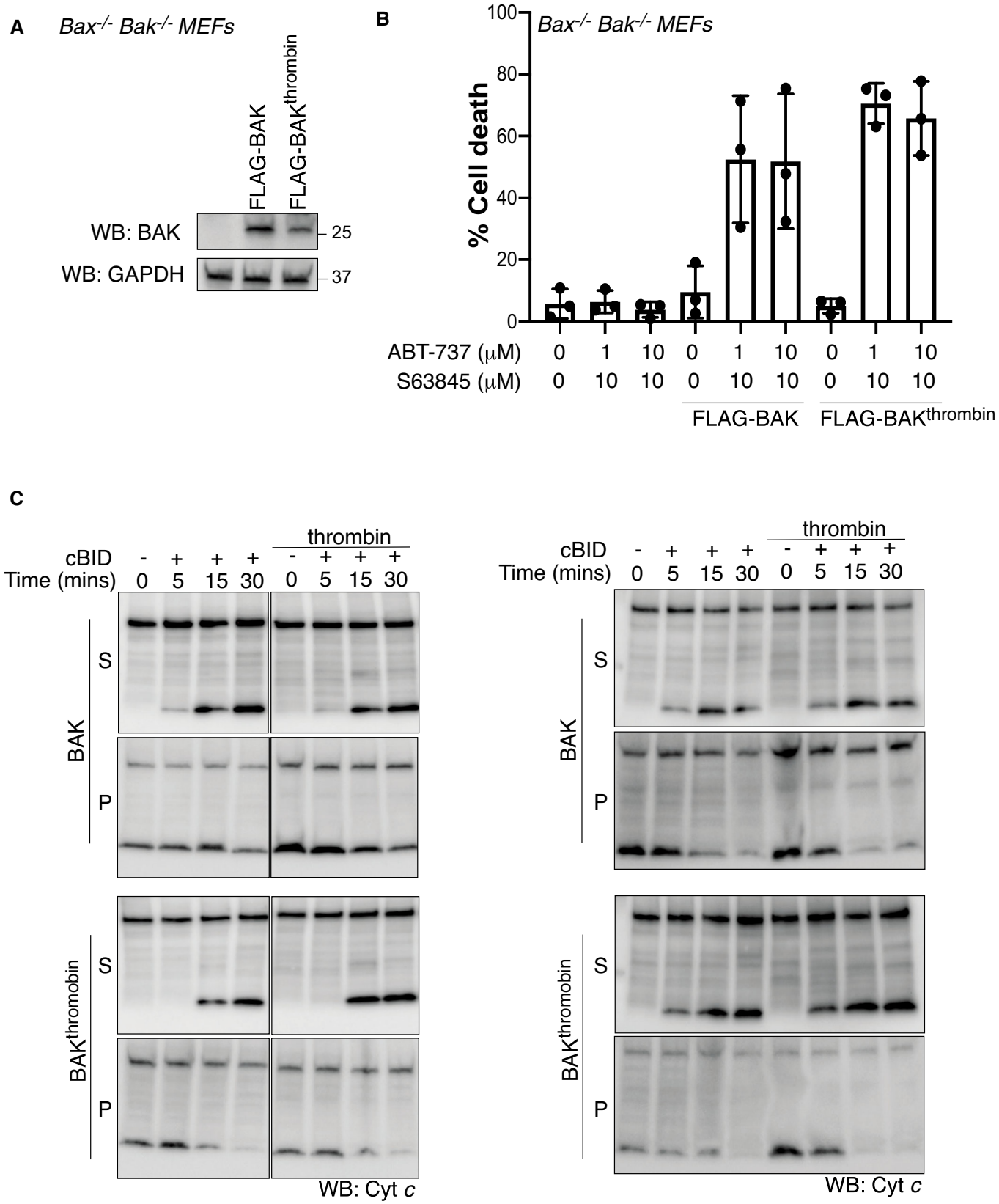

A
Bax-/- Bak-/- MEFs
FLAG-BAK
FLAG-BAKthrombin
WB: BAK
WB: GAPDH
25
37
B
Bax-/- Bak-/- MEFs
% Cell death
100
80
60
40
20
0
ABT-737 (μM) 0 1 10 0 1 10 0 1 10
S63845 (μM) 0 10 10 0 10 10 0 10 10
FLAG-BAK
FLAG-BAKthrombin
C
thrombin
cBID - + + + - + + +
Time (mins) 0 5 15 30 0 5 15 30
BAK
S
P
BAKthromobin
WB: Cyt c

Figure EV5.