**Figure S1. Surface plasmon resonance assay of 26-aa Bak BH3 peptide binding to Bcl-2, Mcl-1 and Bcl-$x_L$ *in vitro*. A-C,** the 26-aa Bak BH3 peptide was immobilized on a CM5 chip. Responses to different concentrations of Mcl-1 (A), Bcl-$x_L$ (B) and Bcl-2 variant 1 (C) are shown. **D,** responses to Bak BH3 peptide chip are compared between Bcl-2, Bcl-$x_L$, Mcl-1 and GST at a concentration of 600 nM. The results shown are representative of 3 independent experiments using different chips and different protein batches.

**Figure S2. Surface plasmon resonance assay of Bak protein binding to Bcl-2, Mcl-1 and Bcl-$x_L$ *in vitro*. A,** purified BakΔTM was immobilized on a CM5 chip. Responses to different concentrations of Bcl-$x_L$ are shown. **B,** responses to Bak chip were compared between Bcl-2 (variant 1), Bcl-$x_L$, Mcl-1 and GST at a concentration of 600 nM.

**Figure S3. Bak N85A/I86A binds to Bcl-2 (variant 2) *in vitro*. A,** GST-Bcl-2ΔTM was immobilized on a CM5 chip. Responses to different concentrations of Bak N85A/I86A are shown. **B,** responses of the Bcl-2 (variant 2) chip to Bak and Bak N85A/I86A at a concentration of 2 μM were compared. Results shown are representative of 2 independent experiments using different chips and different protein batches. **C,** based on the surface plasmon resonance assay, the affinities of Bak and Bak N85A/I86A for Bcl-2 (variant 2) were determined. Results are mean and range of 2 independent experiments using different chips and different protein batches.

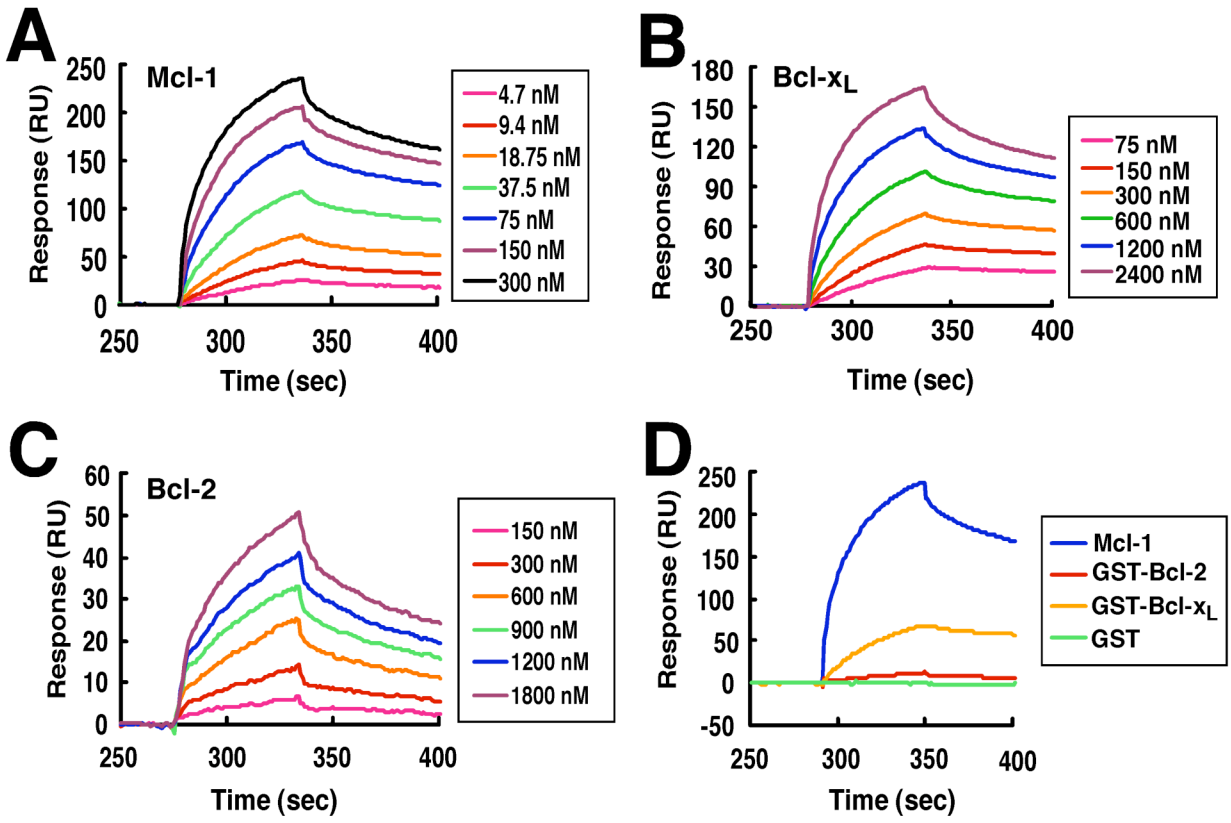
**Figure S4. Bcl-2 knockdown and Mcl-1 knockdown cause Bak activation in Jurkat cells.** 48 h after transfection with pCMS5A (empty vector), pCMS5A/Bcl-2 shRNA, or pCMS5A/Mcl-1 shRNA, Ab-1 was used to immunoprecipitate active Bak from CHAPS lysates. Recovered proteins and lysates were blotted for Bak. Heavy chain is shown here as loading control for the immunoprecipitates.

**Figure S5. Different Bcl-2 variants exhibit different abilities to protect against Mcl-1 knockdown-induced apoptosis in Jurkat cells.** 48 h after co-transfection with pCMS5C (EBFP vector, panels A and B) or pCMS5C-Mcl-1-shRNA (Mcl-1 sh, panels C-F) and pCMS5A (EGFP vector, panels A-C), pCMS5A-Bcl-2 variant 1 (panel D), pCMS5A-Bcl-2 variant 2 (panel E) or pCMS5A-Bcl-2 variant 3 (panel F), Jurkat cells were collected and stained with APC-annexin V. Panel A illustrates gate (R1) used to identify EBFP-histone $H2B^+$ cells (indicating Mcl-1 knockdown), which were then analyzed for EGFP-histone H2B expression (indicating successful transfection of vector or Bcl-2 variants) and absence or presence of annexin V binding in panels B-F. The percentage of vector- or Bcl-2 variant-transfected cells that were positive for annexin V binding (upper right panel over lower right panel) is indicated to the right of each dot plot in panels B-F. These values are summarized in Fig. 5B of the main text.

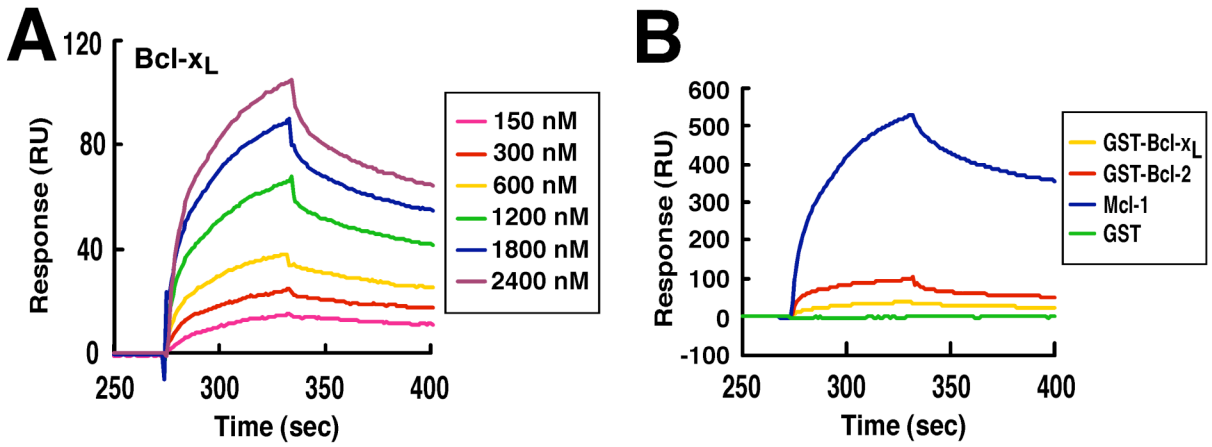
**Figure S6. Different Bcl-2 variants have different abilities to protect against cycloheximide-induced apoptosis in Jurkat cells.** 24 h after transfection with pCMS5A (panels A and B), pCMS5A-Bcl-2 variant 1 (panel C), pCMS5A-Bcl-2 variant 2 (panel D) or pCMS5A-Bcl-2 variant 3 (panel E), Jurkat cells were treated with diluent (0.1% DMSO, panel A) or 3.5 μM cycloheximide (panels B-E) for another 24 h, then stained with APC-annexin V. The percentage of vector- or Bcl-2 variant-transfected cells that were positive for annexin V binding (upper right panel over lower right panel) is indicated to the right of each dot plot. Additional samples (not shown) transfected with the Bcl-2 variants were treated with diluent for 24 h and stained with APC-annexin V. These values are summarized in Fig. 5D of the main text.

**Figure S7. Further quantitation of Bcl-2 and Bax in Jurkat, Molt3, CEM and RL cells. A,** whole cell lysates of $3 \times 10^5$ for Jurkat, Molt3, CEM and Daudi cells were subjected to western blot for Bcl-2.

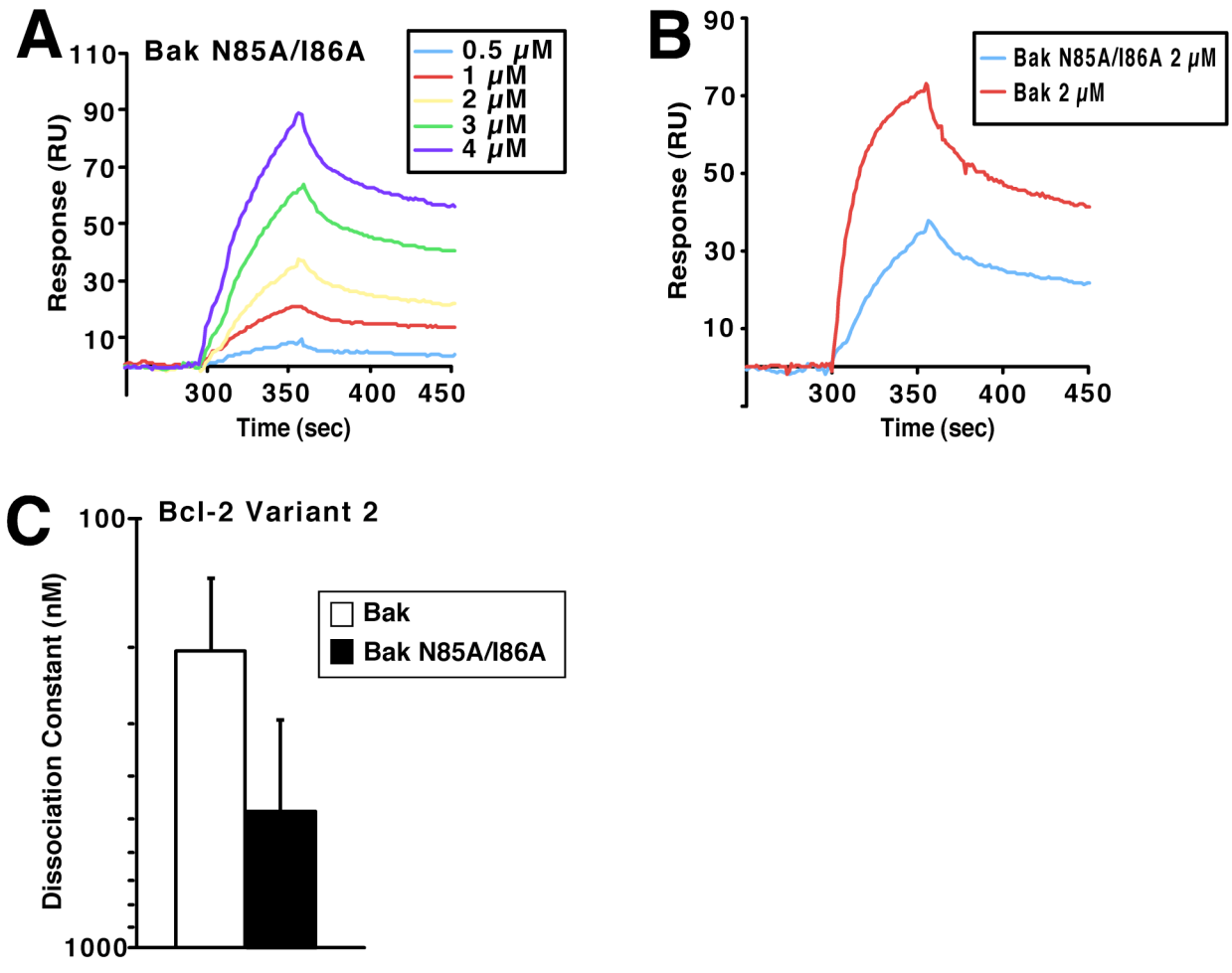
Purified GST-Bcl-2ΔTM (0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 ng) is shown for comparison. **B,** whole cell lysates of $3 \times 10^5$ for Jurkat, Molt3, CEM, Daudi and RL cells were subjected to western blot for Bax. Purified GST-BaxΔTM (0.15, 0.3, 0.6, 1.2, 2.4 ng) is shown for comparison.



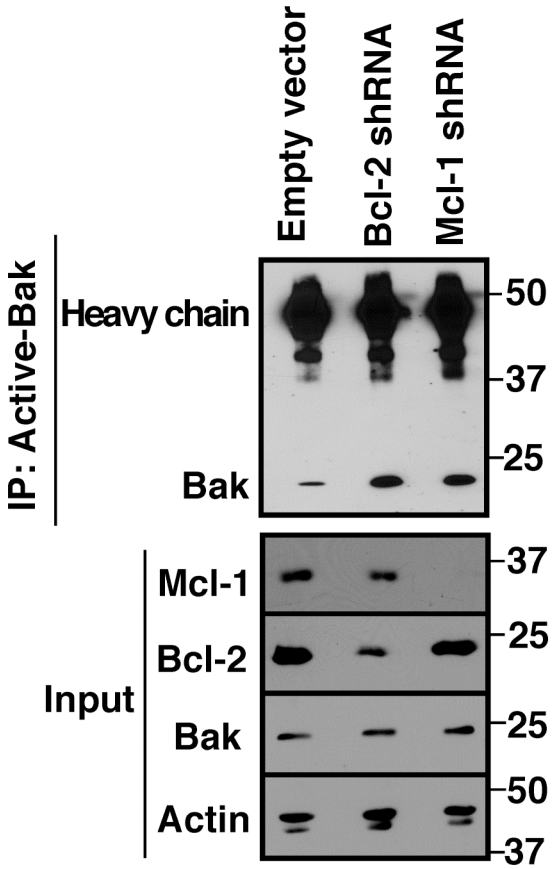

**Dai et al., Fig. S1**



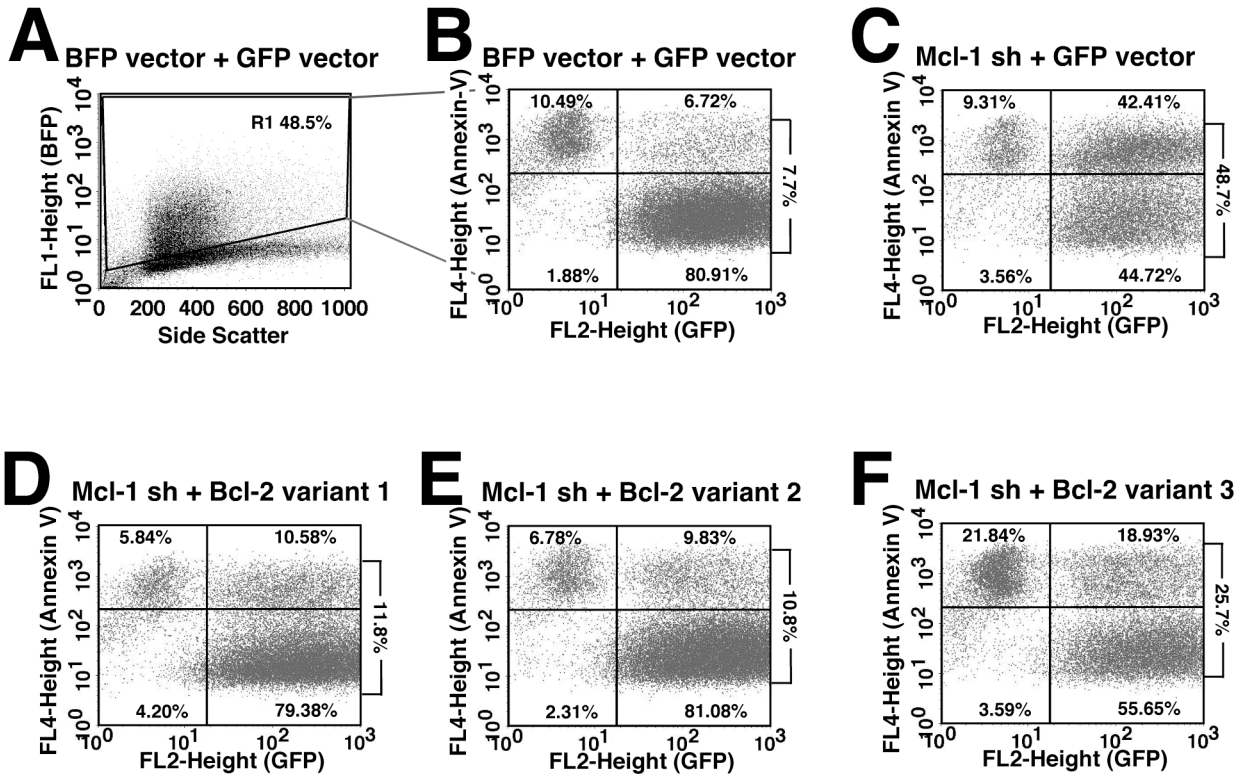

**Dai et al., Fig. S2**



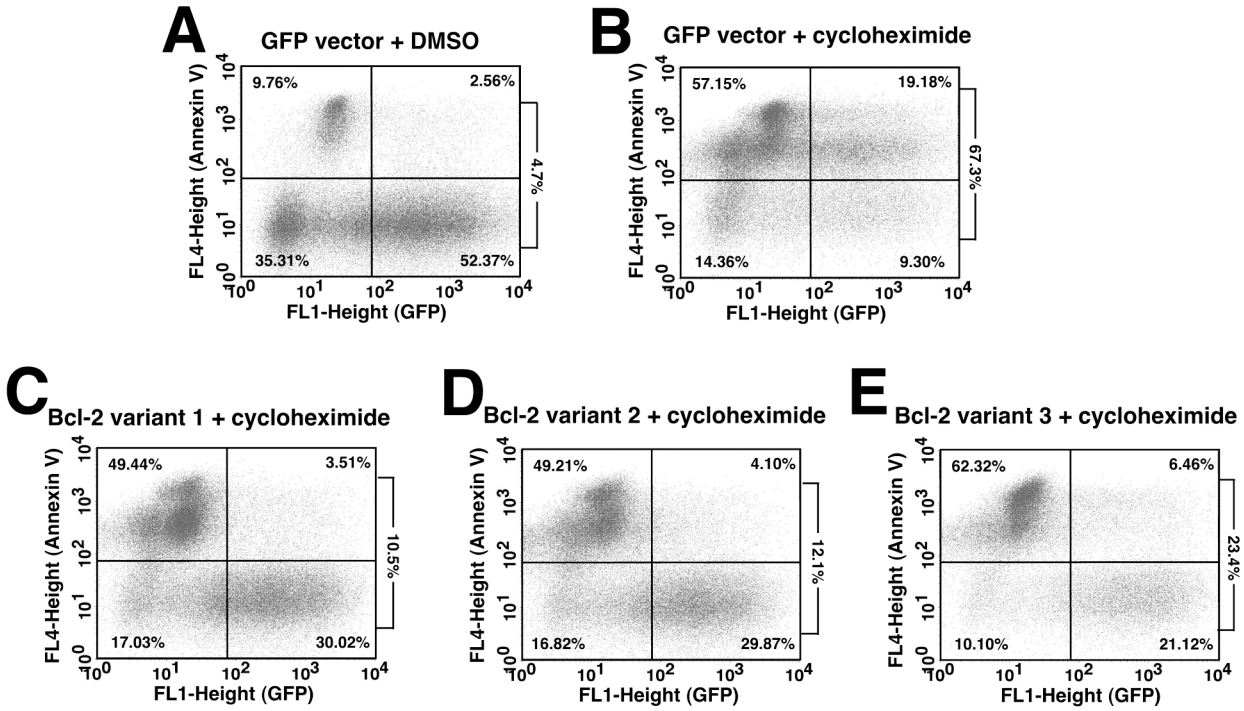

**Dai et al., Fig. S3**



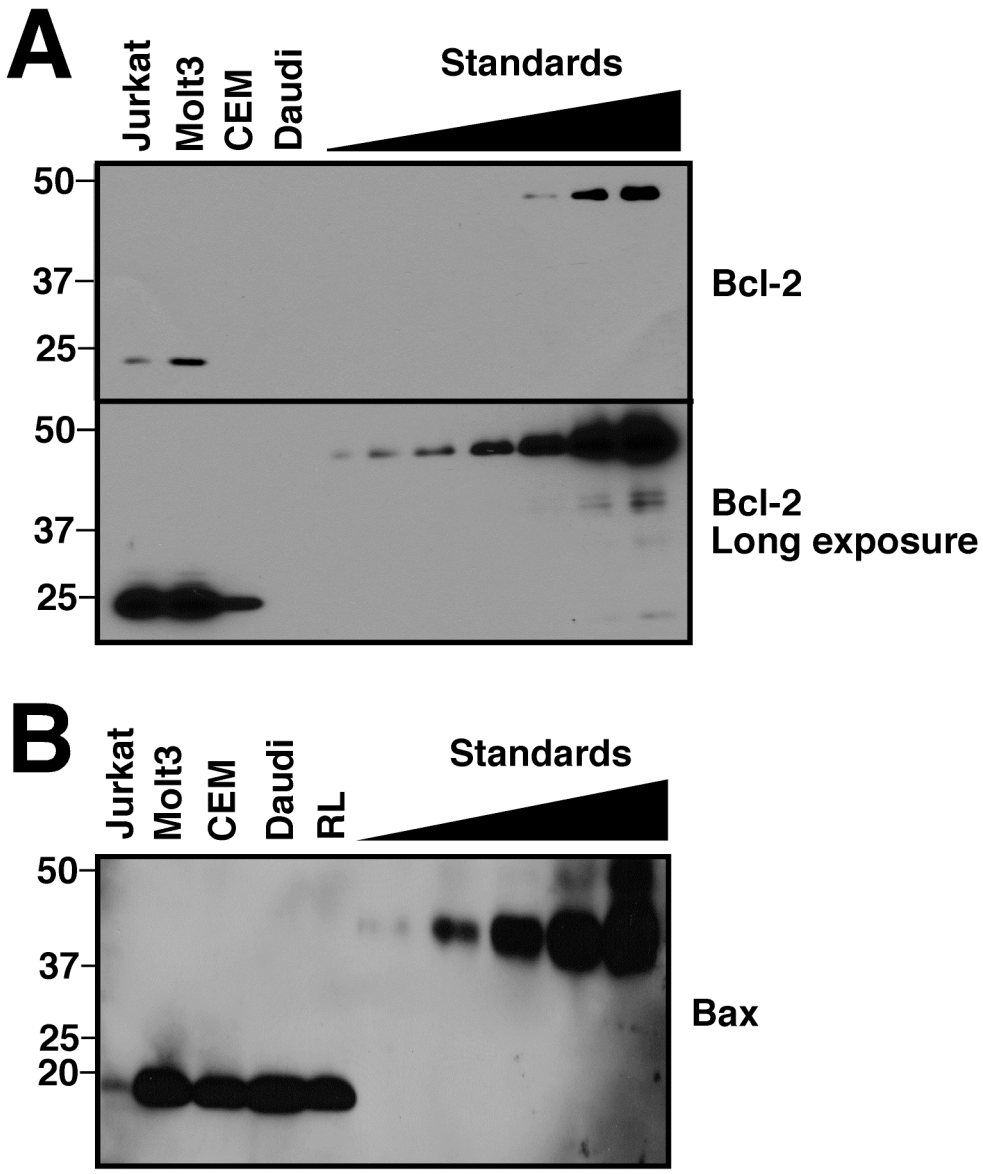

**Dai et al., Fig. S4**




**Dai et al., Fig. S5**




**Dai et al., Fig. S6**




Dai et al., Fig. S7