Supplemental Figure Legends

**Fig. S1. Subcellular localization of miniBax and its truncation mutants**, related to Fig. 2. Δ(SR) cells carrying miniBax and its truncation mutants in pS105 vector were grown and thermally induced as in Fig. 2B. Cell Fractionation was carried out as described in Supplemental Experimental Procedures, and examined by western blot with an α-Flag antibody. T: the total protein. M: the membrane fraction. S: the supernatant.

**Fig. S2. Subcellular localization of miniBax and its BH3 mutants**, related to Fig. 3. (A) Lysis curves of Δ(SR) cells carrying pS-miniBax/R−, pS-miniBaxL63E/R−, or pS-F-miniBaxL63P/R− were carried out as in Fig. 2B. (B) At 45 min, cultures were collected for cell fractionation as in Fig. S1. T: the total protein. M: the membrane fraction. S: the supernatant.

**Fig. S3. Expression of S105 or Bax/Bak mutants from lysogens**, related to Fig. 5. (A) Lysis curves of MC4100ΔtonA cells or MC4100ΔtonA lysogens carrying λS105-Flag, λF-miniBaxH3A, λF-BaxΔH6, or λF-miniBak prophages were carried out as in Fig. 2B. A550 of each culture was measured at a 15 min. interval. (B) The expression of S105 or Bax/Bak mutants by western blot with α-Flag antibody at 75 min.

**Fig. S4. Expression of relevant proteins in the R-LacZ background**, related to Fig. 6. (A) At 15 min, cultures carrying the indicated plasmids in Fig. 6B were examined for the expression of R-LacZ and its truncation mutants by western blot with an α-c-Myc antibody. (B) Expression of miniBax and miniBaxH3A at 15 min in Fig. 6B by western blot with α-Flag antibody. (C) Expression of Bax mutants at 30 min in Fig. 6D.

**Fig. S5. Differential lysis activity of full-length Bax and a miniBax mutant under R and R-LacZ background**, related to Fig. 6. (A) Lysis curves of Δ(SR) cells carrying indicated plasmids were carried out as in Fig. 2B. (B) Expression of Bax and miniBaxY115A,F116A at 30 min in (A) was examined by western blot with an α-Flag antibody.
**Fig. S6. Suppression of homo-oligomerization of miniBaxH3A by Bcl-xL.** At 60 min., cultures of F-miniBaxH3A lysogens carrying the indicated plasmids in Fig. 7A were collected and subjected to BMH crosslinking assay as described in the Experimental Procedures followed by SDS PAGE and western blot analysis with an α-Flag antibody.

**Fig. S7. Amino acid sequences of miniBax and holin S105.**

**Movie S1. Lysis of Δ(SR) cells carrying pS-F-miniBax plasmid.**

**Movie S2. Lysis of Δ(SR) cells carrying pS-F-miniBaxH3A plasmid.**

**Table S1. Plaque formation by Bax/Bak mutants,** related to Fig. 4. Titers of chimeric λ phages from supernatant of cultures at 75 min in Fig. 4B were measured by plaque forming assay as described in Experimental Procedures.

---

**Extended Experimental Procedures**

**Reagents and Antibodies**


**Plasmid Construction**

*Input text here.*
Pang et al.

S105 is replaced by an N-terminal Flag tagged miniBax by QuickChange. There is an XhoI site between the flag tag and miniBax. N-terminal flag tagged Bax, BaxΔH6, Bak, miniBak, Bim, Bcl-xL and C-terminal flag tagged tBid were cloned into pS105 vector using the same strategy. For convenience of cloning other Bcl-2 family protein genes into pS105 vector, pS-F-miniBax was modified by a silent mutation (nt -215 C to G, which ablates the internal EcoRI site of pS105) and an insertion of an EcoRI site between the stop codon of miniBax and A93 of S105. The modified plasmid pS-F-miniBax was referred as pS-Fxe-miniBax, and used for cloning other Bcl-2 family protein genes or their mutants into pS105 vector by ligation using XhoI and EcoRI sites. Nonfunctional R in pS105 vector was achieved by introducing two consecutive stop codons (sequence: TAATAACGAATTC) between G38 and G39 of R gene. pS-GFP/R−, pS-Bcl-xL/R−, and pS-Bcl-xLmt8/R− were generated using similar strategies as described above based on pS-Fxe-miniBax. However, the N-terminal Flag tag was replaced by a His9 tag in these three constructs. N-terminal Flag tagged miniBax, miniBaxH3A, BaxΔH6 were cloned into pS105/R-LacZ by the same strategy of constructing pS-F-miniBax. The plasmids pS105/R-LacZt1 and pS105/R-LacZt2 are isogenic to pS105/R-LacZ except that they carry nonsense mutations at L8 and C498 of LacZ respectively. All Bcl-2 family protein cDNAs except for Bax are from human. Sequences of miniBax, miniBaxΔH2, miniBaxH3A, miniBaxH4A, miniBaxΔH5, miniBaxΔH9 are identical to BaxH(2-5)-CT, BaxH(3-5)-CT, BaxH(2,4,5)-CT, BaxH(2,3,5)-CT, BaxH(2-4)-CT, BaxH(2-5) in our previous study (George et al., 2007, George et al., 2010). BaxΔH6 was constructed by deleting the amino acids V129-E146 of Bax (George et al., 2010). miniBak was generated by fusing S68-G146 of human Bak and R209-K233 of Bcl-xL with amino acids GP in between. BakΔH1 is generated by deleting the first 67 amino acids from Bak. miniBcl-xL was generated by fusing V80-E158 with R209-K233 of Bcl-xL, with amino acids GTPT in between. Bcl-xLmt8 is Bcl-xL G138E/R139L/I140N (Cheng et al., 1996).

**Cell Fractionation**

Δ(SR) cells carrying indicated plasmids were grown and thermo induced as described before. An aliquot of each culture was collected before lysis time, and passed through EmulsiFlex-C5 twice at the pressure of 16,000 psi. Cultures were collected at 45 min Fig. S3. 1 mL of the pressate was saved for the Total protein sample (T). 5 mL of the pressate was centrifuged at 100,000g for 1 hr to pellet the membrane. After the centrifugation, the supernatant was collected and was the
Supernatant protein sample (S). The membrane pellet was resuspended in 5 mL of LB and was the Membrane protein sample (M). 1 mL of all samples (T, S, and M) was TCA precipitated, and resuspended in sample loading buffer. Samples were balanced according $A_{550}$ units collected, loaded to SDS-PAGE and western blotted.

**TCA precipitation**

1 mL culture aliquot was added to 250 μl of ice cold, 50% TCA and 12.5 μl 2% DOC, and then kept on ice for 20 min. The precipitate was collected by centrifugation at 22,000g at 4°C for 20 min, and washed once with acetone. Pellets were then air dried and resuspended in SDS sample buffer.
Fig. S1. Localization of miniBax and its truncation mutants.
Fig. S2. Sub-cellular fractionation of miniBax and its BH3 mutants.
Fig. S3. Expression and lysis activities of S105 or Bax/Bak mutants from lysogens.
Fig. S4. Expression of relevant proteins in the $R$-lacZ background.
Fig. S5. Differential lysis activity of full length Bax and a miniBax mutant under \( R \) and \( R\text{-}lacZ \) background.
Fig. S6. Suppression of active Bax-mediated bacterial lysis by Bcl-xL.
Fig. S7. Amino acid sequences of miniBax and holin S105.
<table>
<thead>
<tr>
<th>Plasmid name</th>
<th>PFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>pS105-Flag</td>
<td>$5.8 \times 10^5$</td>
</tr>
<tr>
<td>pS-F-miniBax</td>
<td>0</td>
</tr>
<tr>
<td>pS-F-miniBaxH3A</td>
<td>$3.5 \times 10^5$</td>
</tr>
<tr>
<td>pS-F-BaxΔH6</td>
<td>$3.4 \times 10^5$</td>
</tr>
<tr>
<td>pS-F-miniBak</td>
<td>$3.2 \times 10^5$</td>
</tr>
</tbody>
</table>

Table S1. Plaque formation by Bax and Bak mutants.