Expanded View Figures

Figure EV1. Related to Figs 1 and 2. NMR spectra of Bax (α2–α5) in bicelles and solution with assignments.

A 2D ^{1}H–^{15}N TROSY-HSQC spectrum of the uniformly [^{15}N, ^{13}C, ^{2}H]-labeled Bax (α2–α5) protein in DMPC/DHPC bicelles (q = 0.5) recorded at ^{1}H frequency of 600 MHz. The backbone amide resonances were assigned to the indicated residues.

B 2D ^{1}H–^{15}N TROSY-HSQC spectrum of the uniformly [^{15}N, ^{13}C, ^{2}H]-labeled soluble Bax (α2–α5) protein recorded at ^{1}H frequency of 600 MHz. The backbone amide resonances were assigned to the indicated residues.

C NMR chemical shift changes of Bax (α2–α5) upon the addition of the bicelles. The plot of chemical shift changes between the spectra of bicelle-bound Bax (α2–α5) and soluble Bax (α2–α5). The combined chemical shift changes (Δδ) were calculated by using the equation: $\Delta\delta = \sqrt{(\omega_H \Delta\delta_H)^2 + (\omega_N \Delta\delta_N)^2}$ where $\Delta\delta_H$ and $\Delta\delta_N$ are chemical shift changes (in ppm) in the ^{1}H and ^{15}N dimensions, respectively, and ω_H and ω_N are normalization factors ($\omega_H = 1.00$, $\omega_N = 0.15$).

D The chemical shift changes of Bax (α2–α5) were mapped to the structure of the bicelle-bound Bax (α2–α5), colored in one chain according to the scale of chemical shift changes shown below.

Source data are available online for this figure.

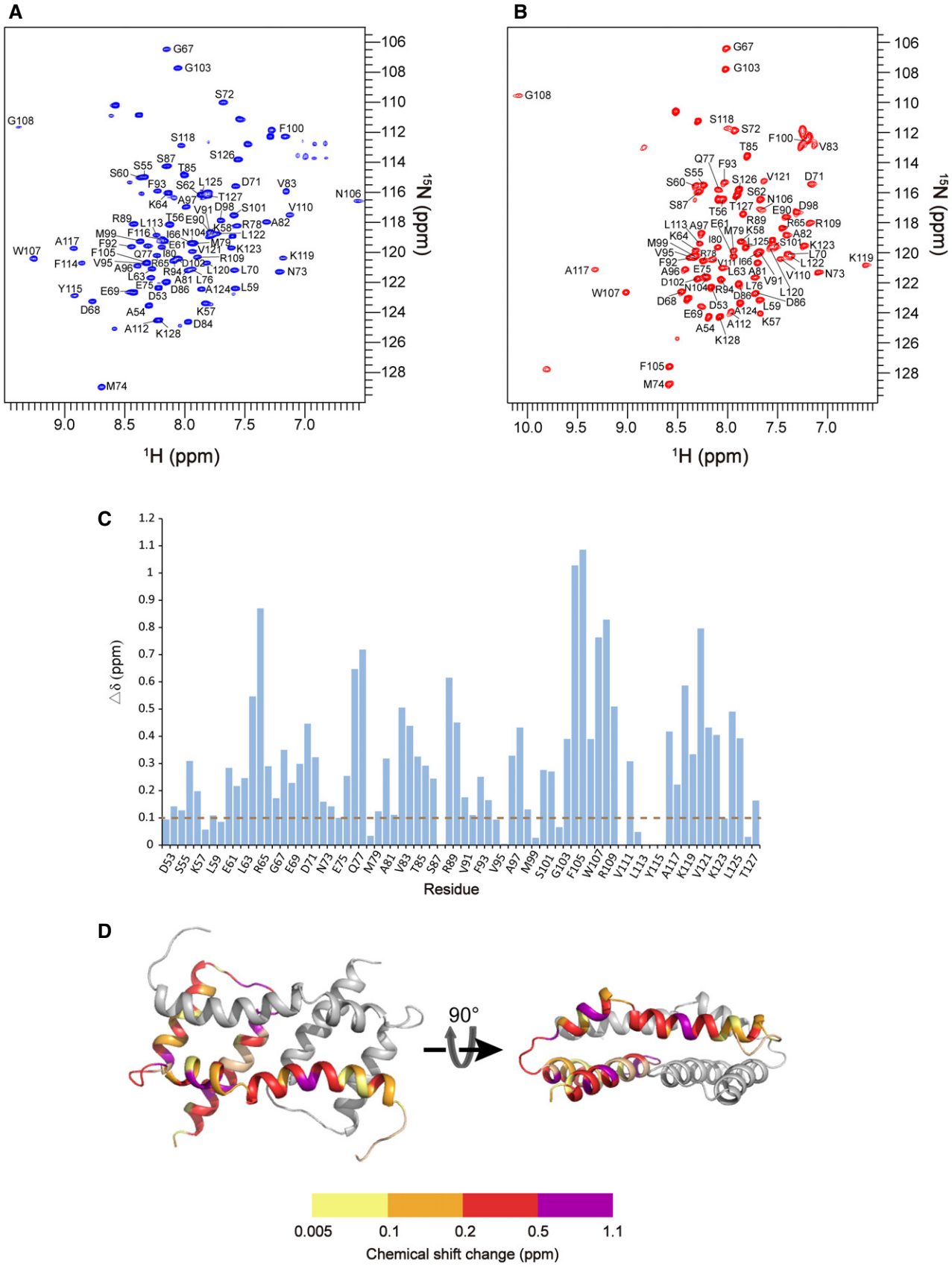

Figure EV1.

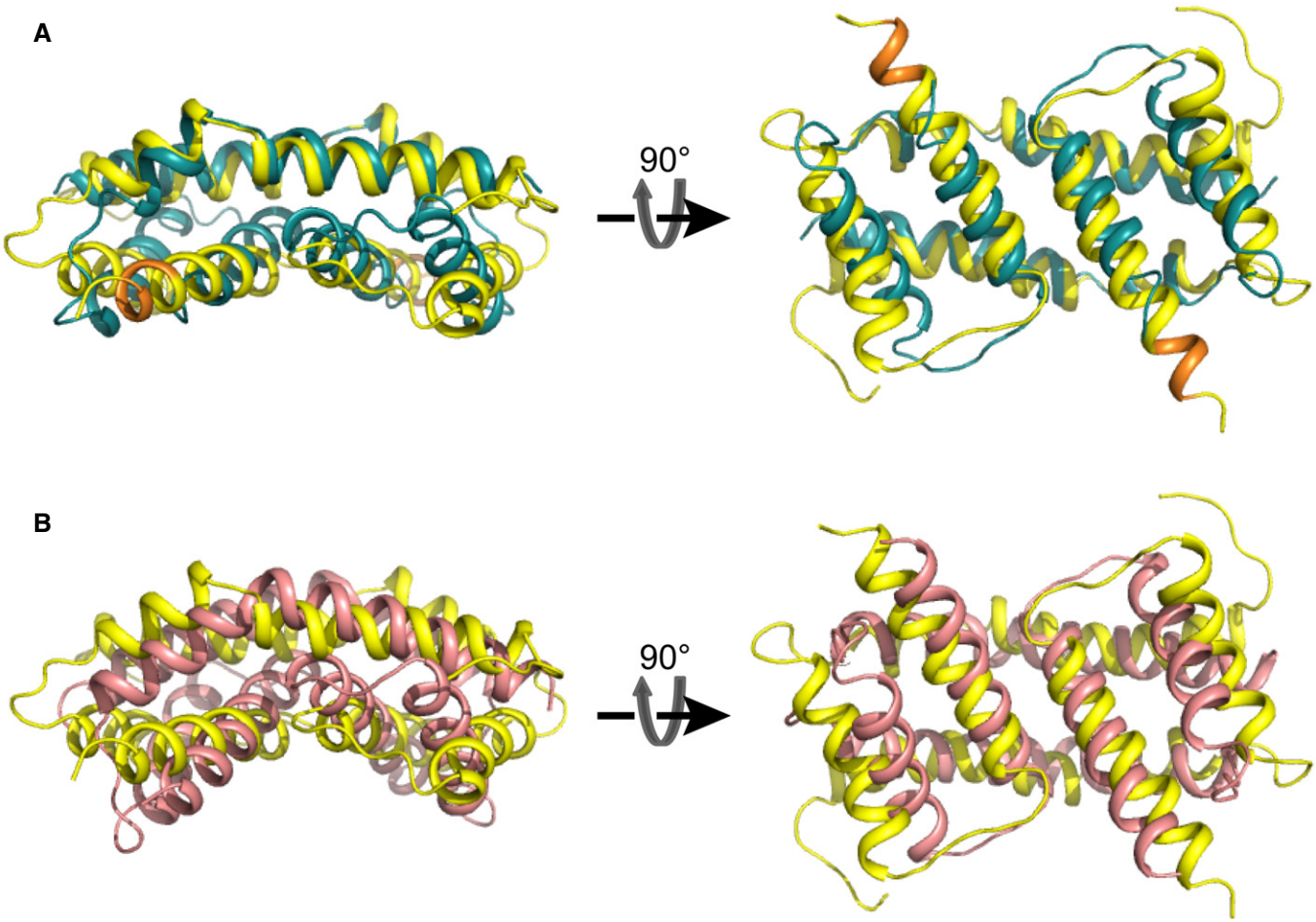

Figure EV2. Related to Fig 2. Comparison of Bax (α2–α5) dimer structures in bicelles (yellow) to crystal structures of Bax (α2–α5) (cyan) and Bak (α2–α5) (salmon) dimers.

A Three-dimensional structure superposition of the structure of Bax (α2–α5) in bicelles determined by NMR (yellow; PDB code: 6L8V) and the structure determined by crystallography in the absence of membranes (cyan; PDB code: 4BDU).The RMSD value between the superimposed protein backbones is 3.878 Å. Note that the residues from K123 to S126 form an extra α helical turn (orange) in the NMR structure.

B Three-dimensional structure superposition of the bicelle-bound structure of Bax (α2–α5) (yellow; PDB code: 6L8V) and the Bak (α2–α5) dimer structure determined by crystallography in the absence of membranes (salmon; PDB code: 4U2V). The RMSD value between the superimposed protein backbones is 5.177 Å.

Figure EV3. Interactions of Bax (α2–α5) mutants with lipid bicelles.

A–D Superimposed 2D ^{1}H–^{15}N TROSY-HSQC spectra of (A) bicelle-bound (green) and soluble (red) Bax (α2–α5) S118D, (B) bicelle-bound (teal) and soluble (magenta) Bax (α2–α5) R89E, (C) bicelle-bound (yellow) and soluble (black) Bax (α2–α5) R89A, and (D) bicelle-bound (blue) and soluble (purple) Bax (α2–α5) F93E recorded at 600 MHz.

E Spectral peak intensity decay (I/I_0) for A97 and F100 caused by PRE agent 16-DSA, determined from a series of ^{1}H–^{15}NTROSY-HSQC spectra of bicelle-bound Bax (α2–α5) (black) or the R89E (green), R89A (red) and F93E (magenta) mutants recorded at different [16-DSA]. I/I_0 = the peak intensity in the presence 16-DSA/that in the absence of 16-DSA.

Source data are available online for this figure.

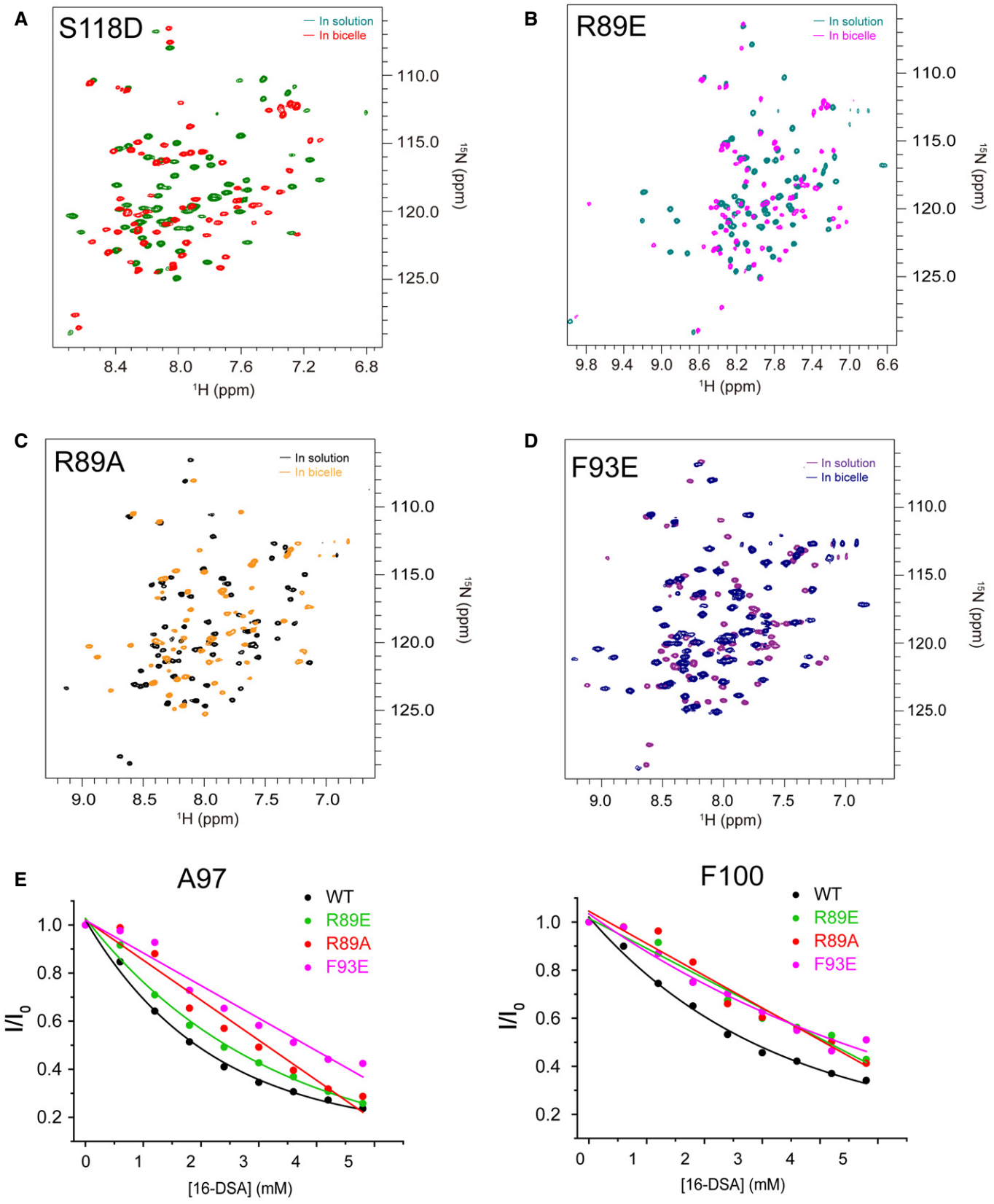

Figure EV3.

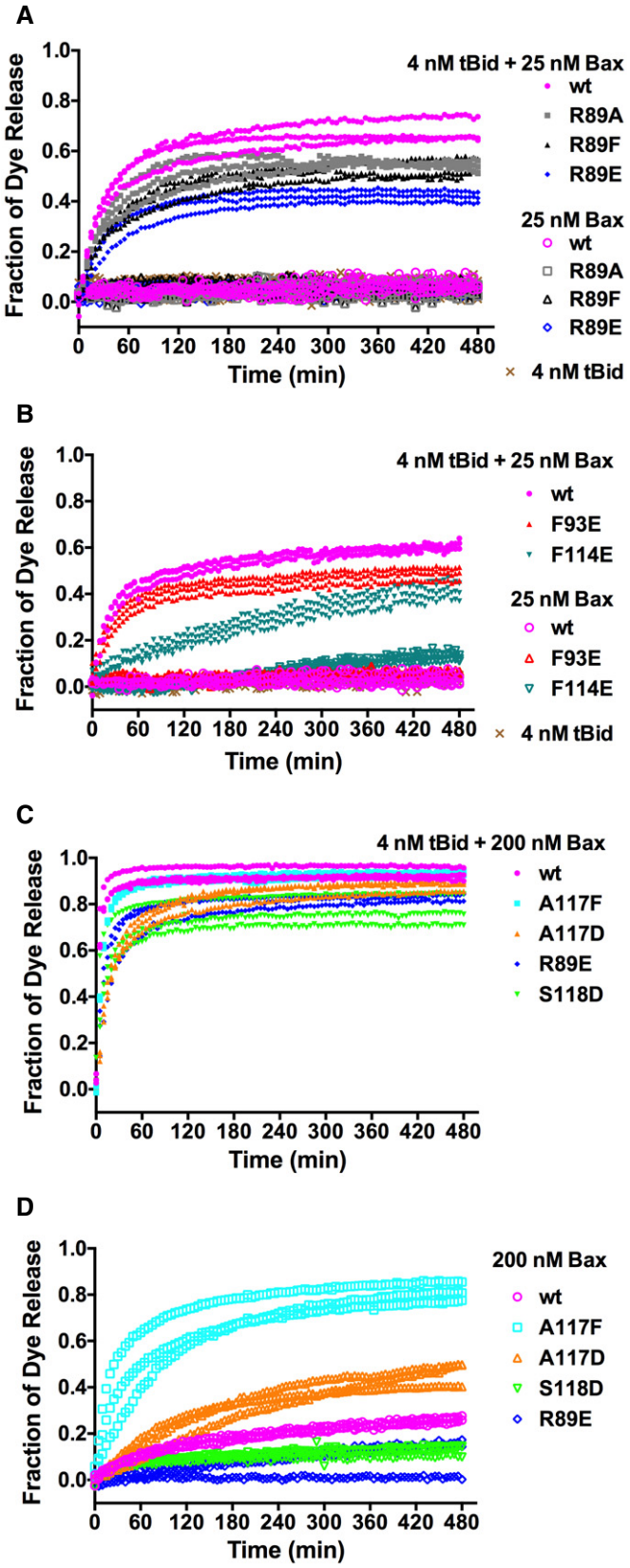

Figure EV4. Related to Fig 4. Pore-forming activity of Bax mutants at low or high concentration.

A–D The fluorescent dye release from mitochondrion-mimic liposomes by 25 or 200 nM wt or mutant Bax in the presence or absence of 4 nM tBid as indicated was measured during a time course by fluorescence quenching. The fraction of dye release was normalized to that by detergent. The data shown were obtained from $n = 3$ independent replicates using the same preparations of the proteins and liposomes.

Source data are available online for this figure.

Figure EV5. Related to Fig 4. Effect of membrane-binding defective mutations on the Bax core dimerization.

A A ribbon diagram of the NMR structure of bicelle-bound Bax (α2–α5) dimer. One protomer is colored with the helices labeled. The C_α carbons of the residue pairs that were replaced by Cys pairs for disulfide crosslinking are shown as spheres linked by dashed lines. The R89E, A117D, and S118D mutations are represented by sticks.

B–E [^{35}S]Met-labeled Bax proteins with two Cys at position 59 and 79 (B, D) or one Cys at 69 (C, E) and an additional mutation, if indicated, to disrupt the α2–α5 core dimer interaction with membranes were synthesized, activated by Bax BH3 peptide, and targeted to the mitochondria lacking endogenous Bax and Bak proteins. The mitochondria-bound proteins were oxidized by CuPhe for 30 min to induce disulfide crosslinking of the two protomers via the Cys pair(s) in the core dimer interface as shown in (A). The radioactive crosslinked Bax dimer was then separated from the monomer by non-reducing SDS–PAGE and detected by phosphor-imaging. The representative data from two to four independent experiments are shown. The relative dimer:monomer ratio shown at the bottom of the phosphor-images was determined by the intensities of the dimer and monomer bands of the Cys mutant with the additional mutation, normalized to that of the corresponding Cys mutant without the additional mutation. The mitochondrial association efficiency of each Bax mutant (E) was determined by the summed intensity of the monomer and dimer bands in the mitochondrial fraction that was divided and treated with CuPhe for 0 or 30 min (I_M), the intensity of the monomer band in the corresponding soluble fraction that was divided by 0.015 to adjust the inequivalent loading between the mitochondrial (1 equivalent (eq)) and soluble (0.015 eq) fractions (I_S), and the following equation, $E = I_M/(I_M + I_S)$. The relative mitochondrial association efficiency of each Bax Cys mutant with the additional mutation (E_R) to that of the Bax Cys mutant without the additional mutation was obtained by dividing the E of the former with the E of the latter and shown at the bottom of the phosphor-images.

Source data are available online for this figure.

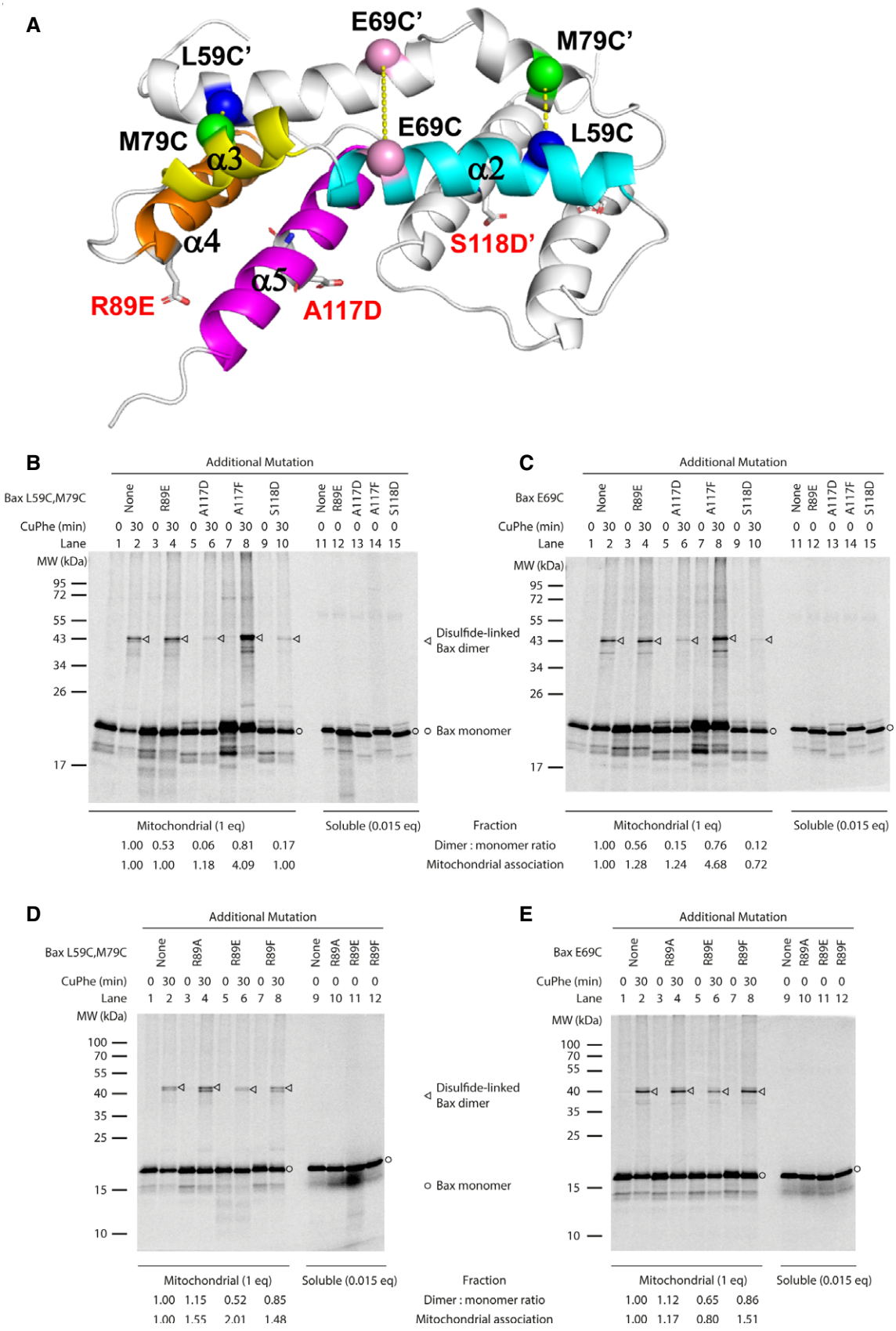
A
E69C'
L59C'
M79C'
M79C
E69C
L59C
α3
α2
α4
S118D'
α5
R89E
A117D
B
Additional Mutation
Bax L59C,M79C
None R89E A117D A117F S118D None R89E A117D A117F S118D
CuPhe (min) 0 30 0 30 0 30 0 30 0 30 0 0 0 0 0
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
MW (kDa)
95 72 55 43 34 26 17
Disulfide-linked Bax dimer
Bax monomer
Mitochondrial (1 eq)
Soluble (0.015 eq)
1.00 0.53 0.06 0.81 0.17
1.00 1.00 1.18 4.09 1.00
C
Additional Mutation
Bax E69C
None R89E A117D A117F S118D None R89E A117D A117F S118D
CuPhe (min) 0 30 0 30 0 30 0 30 0 30 0 0 0 0 0
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
MW (kDa)
95 72 55 43 34 26 17
Fraction
Mitochondrial (1 eq)
Soluble (0.015 eq)
Dimer : monomer ratio 1.00 0.56 0.15 0.76 0.12
Mitochondrial association 1.00 1.28 1.24 4.68 0.72
D
Additional Mutation
Bax L59C,M79C
None R89A R89E R89F None R89A R89E R89F
CuPhe (min) 0 30 0 30 0 30 0 30 0 0 0 0
Lane 1 2 3 4 5 6 7 8 9 10 11 12
MW (kDa)
100 70 55 40 35 25 15 10
Disulfide-linked Bax dimer
Bax monomer
Mitochondrial (1 eq)
Soluble (0.015 eq)
1.00 1.15 0.52 0.85
1.00 1.55 2.01 1.48
E
Additional Mutation
Bax E69C
None R89A R89E R89F None R89A R89E R89F
CuPhe (min) 0 30 0 30 0 30 0 30 0 0 0 0
Lane 1 2 3 4 5 6 7 8 9 10 11 12
MW (kDa)
100 70 55 40 35 25 15 10
Fraction
Mitochondrial (1 eq)
Soluble (0.015 eq)
Dimer : monomer ratio 1.00 1.12 0.65 0.86
Mitochondrial association 1.00 1.17 0.80 1.51

Figure EV5.