

## Supplementary Information

**Table S1.** PCR Primers used for site-directed mutagenesis<sup>a</sup>.

XnC-BamA	Amino Acid Substitution	PCR Primers <sup>b</sup>
R572C-BamA	R573C	Forward: 5'-CCCGACAGATGGTTCA <b>t</b> GTGTCAACCTGACCG-3' Reverse: 5'-CGGTCAGGTTGACACA <b>Ca</b> TGAACCATCTGTCTGGG-3'
V573C-BamA	V573C	Forward: 5'-CGACAGATGGTTCACGT <b>tg</b> CAACCTGACCGGTAAAG-3' Reverse: 5'-CTTTACCGGTCAGGTT <b>Gca</b> ACGTGAACCATCTGTCTCG-3'
N574C-BamA	N574C	Forward: 5'-CAGATGGTTCACGTGTCT <b>tg</b> CCTGACCGGTAAAGTGACC-3' Reverse: 5'-GGTCACTTTACCGGTCAG <b>Gca</b> GACACGTGAACCATCTG-3'
L575C-BamA	L575C	Forward: 5'-GGTTCACGTGTCAAC <b>tg</b> cACCGGTAAAGTGACCATTCC-3' Reverse: 5'-GGAATGGTCACTTTACCGGT <b>gca</b> GTTGACACGTGAACC-3'
T576C -BamA	T576C	Forward: 5'-CACGTGTCAACCTGT <b>g</b> CGGTAAAGTGACCATTTCCTG-3' Reverse: 5'-CAGGAATGGTCACTTTACCG <b>ca</b> CAGGTTGACACGTG-3'
G577C-BamA	G577C	Forward: 5'-GTGTCAACCTGAC <b>Ct</b> GTAAAGTGACCATTTCCTGGATC-3' Reverse: 5'-GATCCAGGAATGGTCACTTTACA <b>Ca</b> GGTCAGGTTGACAC-3'
K578C-BamA	K578C	Forward: 5'-GTCAACCTGACCGGT <b>tgt</b> GTGACCATTTCCTGGATCG-3' Reverse: 5'-CGATCCAGGAATGGTCAC <b>aca</b> ACCGGTCAGGTTGAC-3'
V579C-BamA	V579C	Forward: 5'-GTCAACCTGACCGGTAA <b>At</b> gcACCATTCCTGGATCGG-3' Reverse: 5'-CCGATCCAGGAATGGT <b>gca</b> TTTACCGGTCAGGTTGAC-3'

<sup>a</sup>Mismatched nucleotides for mutagenesis are in bold face.

<sup>b</sup>“Forward” denotes the primer complementary to the 5'–3' DNA strand, “Reverse” the primer complementary to the 3'–5' DNA strand.

Table S2. Analysis of the CD spectra of XnC-BamA mutants in lipid bilayers of *di*C<sub>12</sub>PC<sup>a</sup>

BamA	Algorithm	Set	$\alpha$ -helix (%)	$\beta$ -sheet (%)	$\beta$ -turn (%)	Random coil (%)	NRMSD
wt-BamA	CONTIN	4	16	31	22	32	0.11
	CONTIN	7	16	30	20	34	0.11
	CDSSTR	4	13	33	24	31	0.01
	CDSSTR	7	15	29	21	34	0.02
R572C-BamA	CONTIN	4	16	29	22	32	0.17
	CONTIN	7	16	27	20	37	0.17
	CDSSTR	4	12	34	24	30	0.02
	CDSSTR	7	17	25	22	36	0.02
V573C-BamA	CONTIN	4	17	30	21	31	0.06
	CONTIN	7	17	30	21	33	0.06
	CDSSTR	4	15	32	24	30	0.01
	CDSSTR	7	17	29	21	33	0.02
N574C-BamA	CONTIN	4	16.4	31.5	20.8	31.3	0.103
	CONTIN	7	16.4	31.5	20.8	31.3	0.103
	CDSSTR	4	13	32	24	31	0.015
	CDSSTR	7	16	31	22	32	0.018
L575C-BamA	CONTIN	4	18	30	21	31	0.07
	CONTIN	7	18	30	21	32	0.07
	CDSSTR	4	14	30	24	31	0.02
	CDSSTR	7	17	28	22	33	0.02
T576C-BamA	CONTIN	4	17	31	21	31	0.08
	CONTIN	7	17	30	20	34	0.08
	CDSSTR	4	14	30	23	31	0.01
	CDSSTR	7	17	29	20	34	0.01
G577C-BamA	CONTIN	4	16	32	20	32	0.11
	CONTIN	7	16	33	20	31	0.11
	CDSSTR	4	12	32	24	31	0.02
	CDSSTR	7	16	30	21	32	0.03
K578C -BamA	CONTIN	4	16	32	21	32	0.07
	CONTIN	7	16	31	20	33	0.07
	CDSSTR	4	13	31	24	32	0.02
	CDSSTR	7	16	29	21	34	0.02
V579C -BamA	CONTIN	4	15	33	20	32	0.11
	CONTIN	7	15	32	20	33	0.11
	CDSSTR	4	12	33	24	32	0.02
	CDSSTR	7	16	30	22	33	0.02

<sup>a</sup>After folding into bilayers of *di*C<sub>12</sub>PC, the compositions of the secondary structures of wt-BamA and  $\chi_n$ C-BamA mutants were analyzed from CD spectra using DICHROWEB (see *ref.* [43] and references therein). For analysis, the deconvolution algorithms, CONTIN [41] and CDSSTR [42] were used. These algorithms and the two sets of reference spectra resulted in very similar compositions of the secondary structures of BamA and  $\chi_n$ C-BamA mutants.