Figure S1. Analysis of genomic and cDNA sequences of the targeted regions in WT-KI and indicated mutant KI cells, with WT and corresponding mutant sequences underlined. (A) WT-KI cells; **(B)** K21E-KI cells; **(C)** D33A-KI cells; **(D)** D68R-KI cells; **(E)** L70A/D71A-KI cells; **(F)** S184V-KI cells.

Figure S2. Analysis of apoptosis in knock-in cells by annexin V/propidium iodide (PI) staining. HCT116 cells with indicated *BAX* genotypes were treated with 120 μM sulindac for 36 hours, or 10 ng/ml TRAIL for 24 hours. After treatment, cells were stained with annexin V/PI and analyzed by flow cytometry. Cells in the two right quadrants represent annexin V-positive apoptotic cells, and their percentages are indicated.

Figure S3. Expression of Bcl-2 family members in cells with or without sulindac or TRAIL treatment. (A) HCT116 cells were treated with 120 μM sulindac or 10 ng/ml TRAIL for 24 hours. BH3-only Bcl-2 family members, including Bim, PUMA, Bid and Bad, were analyzed by Western blotting. **(B)** HCT116 cells transfected with GFP-Bax for 24 hours, along with untreated HCT116, WT-KI and MEF cells, were analyzed for Bax expression by Western blotting. **(C)** Whole cell lysate, cytosolic and mitochondrial fractions isolated from HCT116 cells treated with 120 μM sulindac for 36 hours or 10 ng/ml TRAIL for 24 hours were probed for Bak expression. Cox IV and β-actin were used as control for loading and fractionation.

Figure S4. Interactions of WT and mutant Bax with other Bcl-2 family members in cells lysed with different detergents. *BAX*-KO cells were co-transfected with GFP-tagged WT Bax and HA-tagged Bim, HA-tagged PUMA, or V5-tagged Bcl-X_L. Cells were lysed with a buffer containing either 0.5% NP-40 or 0.5% CHAPS as detergent. IP was performed with anti-GFP antibody to pull down Bax. Bax bound to the co-transfected proteins and the pull-down efficiency were analyzed by Western blotting of GFP and HA/V5, respectively.

Figure S5. Suppression of sulindac-induced apoptosis by Bcl-X_L. (A) Parental and indicated Bax knock-in HCT116 cells were transfected with V5-tagged Bcl-X_L and treated with 120 μM sulindac for 36 hours. Expression of the transfected Bcl-X_L was analyzed by Western blotting. **(B)** Apoptosis in cells treated as in (A) was determined by nuclear staining. ***: $P<0.001$.

Figure S6. Indomethacin-induced apoptosis in WT-KI and L70A/D71A-KI cells. Parental and indicated Bax knock-in HCT116 cells treated with 500 μM indomethacin were analyzed for apoptosis induction. **(A)** Apoptosis in cells treated for 48 hours was analyzed by counting cells with condensed and fragmented nuclei after nuclear staining with Hoechst 33258. **(B)** Apoptosis in cells treated for 36 hours was analyzed by annexin V/PI staining and flow cytometry. Cells in the two right quadrants represent annexin V-positive apoptotic cells.

Figure S1

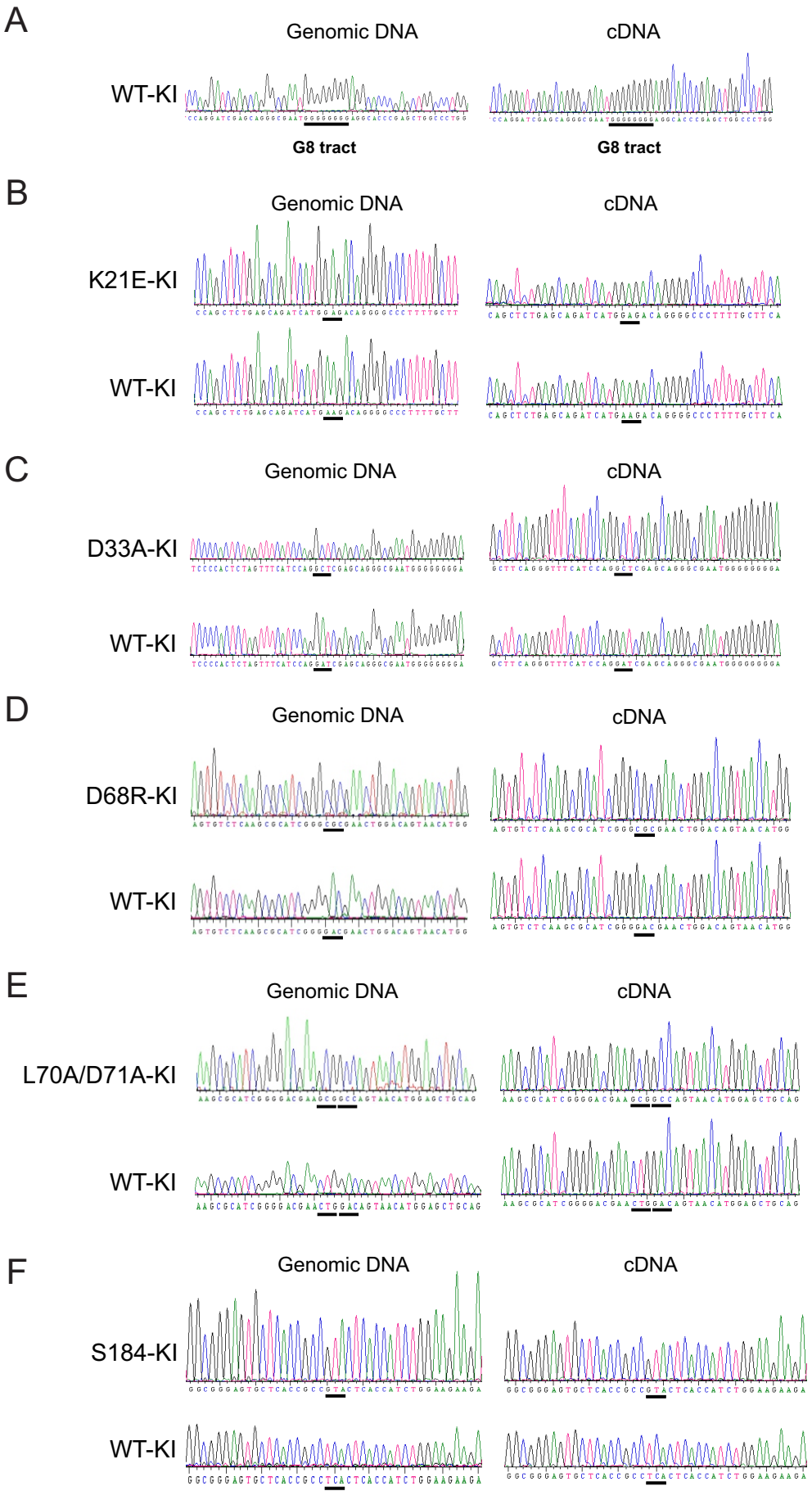
A
Genomic DNA
cDNA
WT-KI
G8 tract
G8 tract
B
Genomic DNA
cDNA
K21E-KI
WT-KI
C
Genomic DNA
cDNA
D33A-KI
WT-KI
D
Genomic DNA
cDNA
D68R-KI
WT-KI
E
Genomic DNA
cDNA
L70A/D71A-KI
WT-KI
F
Genomic DNA
cDNA
S184-KI
WT-KI

Figure S2

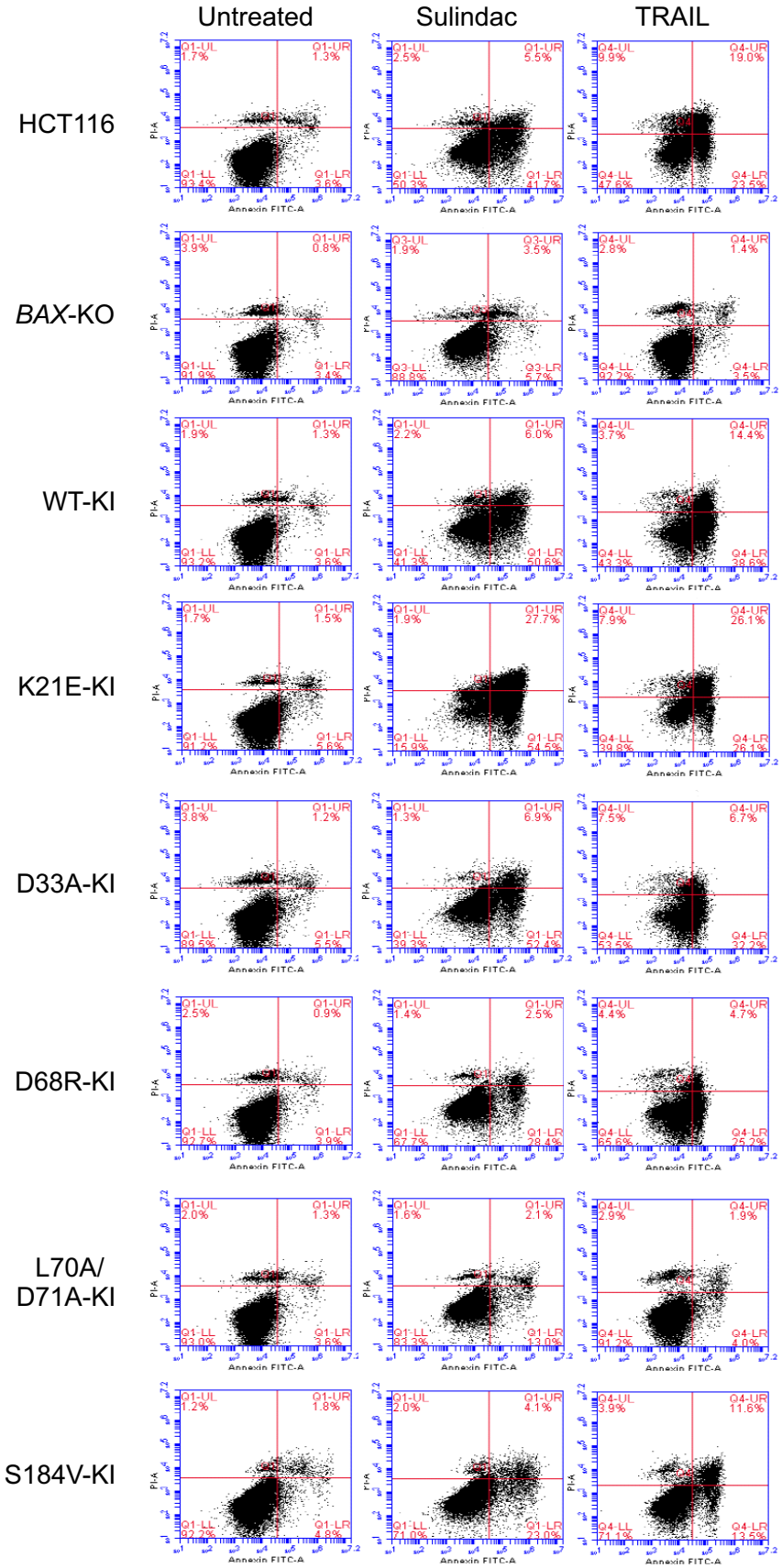
Untreated
Sulindac
TRAIL
HCT116
BAX-KO
WT-KI
K21E-KI
D33A-KI
D68R-KI
L70A/
D71A-KI
S184V-KI

Figure S3

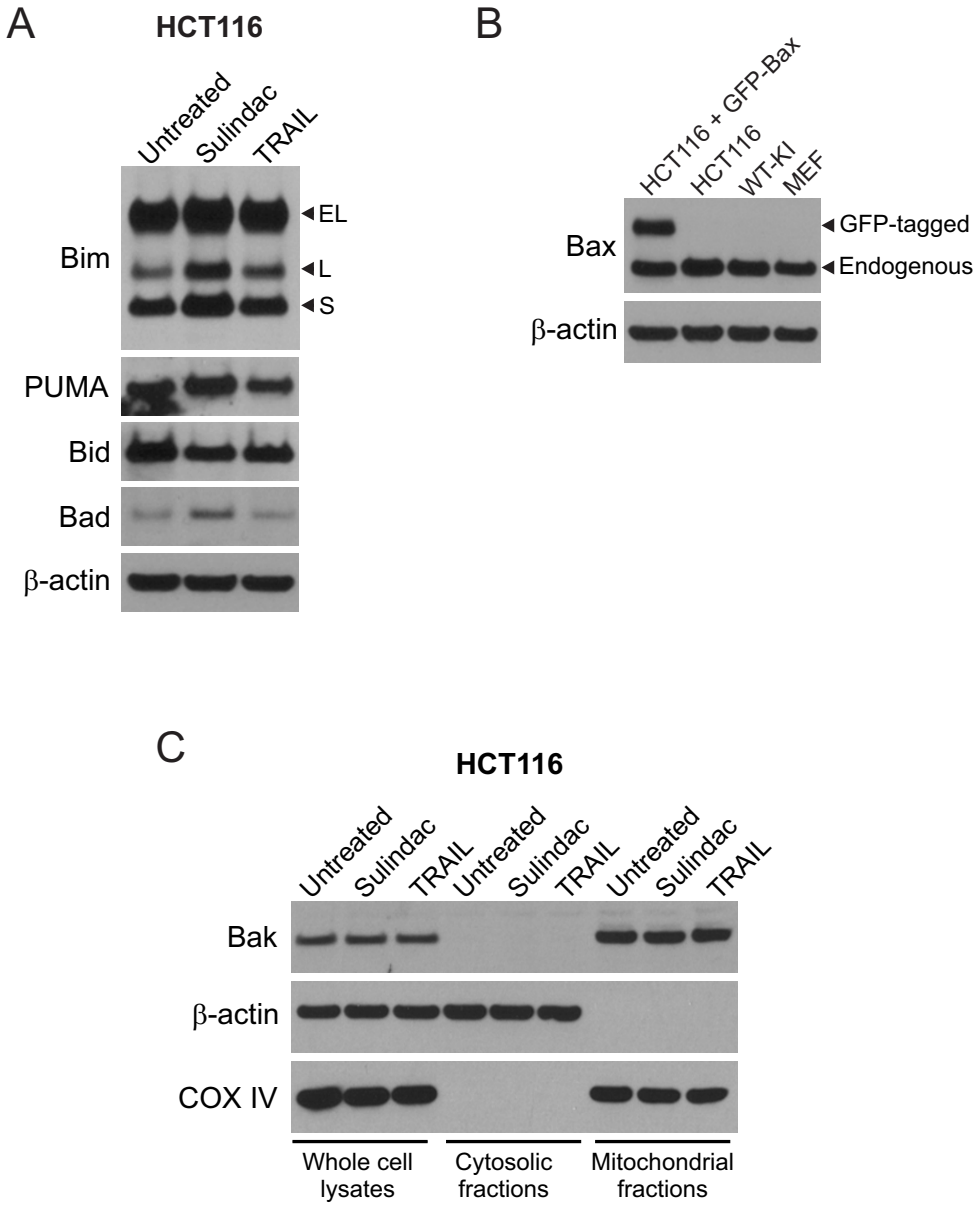
A
HCT116
Untreated
Sulindac
TRAIL
Bim
EL
L
S
PUMA
Bid
Bad
β-actin
B
HCT116 + GFP-Bax
HCT116
WT-KI
MEF
Bax
GFP-tagged
Endogenous
β-actin
C
HCT116
Untreated
Sulindac
TRAIL
Untreated
Sulindac
TRAIL
Untreated
Sulindac
TRAIL
Bak
β-actin
COX IV
Whole cell lysates
Cytosolic fractions
Mitochondrial fractions

Figure S4

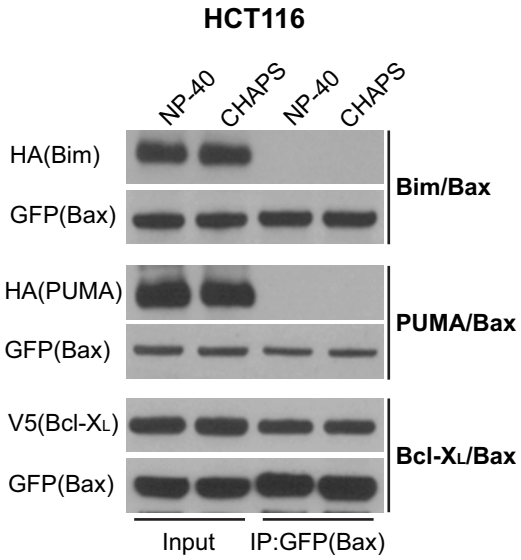
HCT116
NP-40
CHAPS
NP-40
CHAPS
HA(Bim)
GFP(Bax)
Bim/Bax
HA(PUMA)
GFP(Bax)
PUMA/Bax
V5(Bcl-XL)
GFP(Bax)
Bcl-XL/Bax
Input
IP:GFP(Bax)

Figure S5

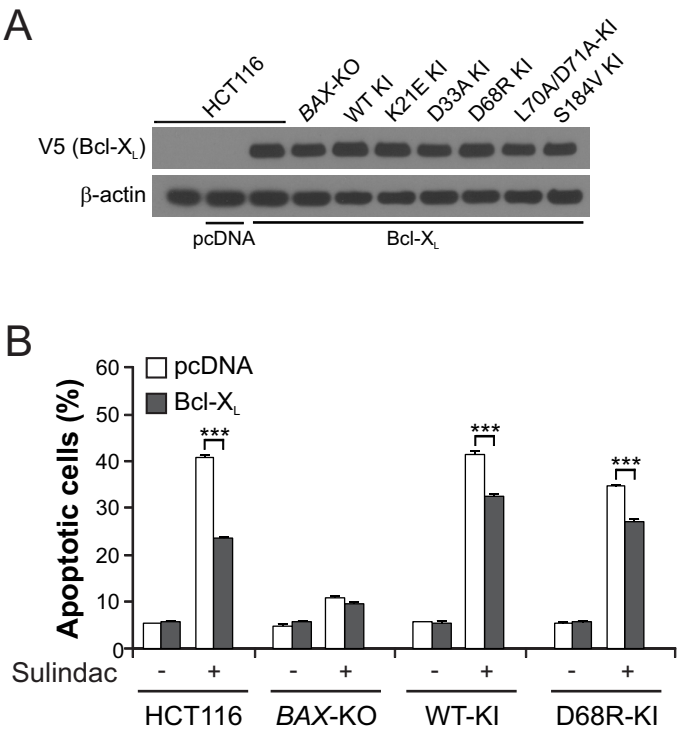
A
HCT116
BAX-KO
WT KI
K21E KI
D33A KI
D68R KI
L70A/D71A-KI
S184V KI
V5 (Bcl-X$_L$)
β-actin
pcDNA
Bcl-X$_L$
B
pcDNA
Bcl-X$_L$
Apoptotic cells (%)
60
50
40
30
20
10
0
***
***
***
Sulindac
-
+
-
+
-
+
-
+
HCT116
BAX-KO
WT-KI
D68R-KI

Figure S6

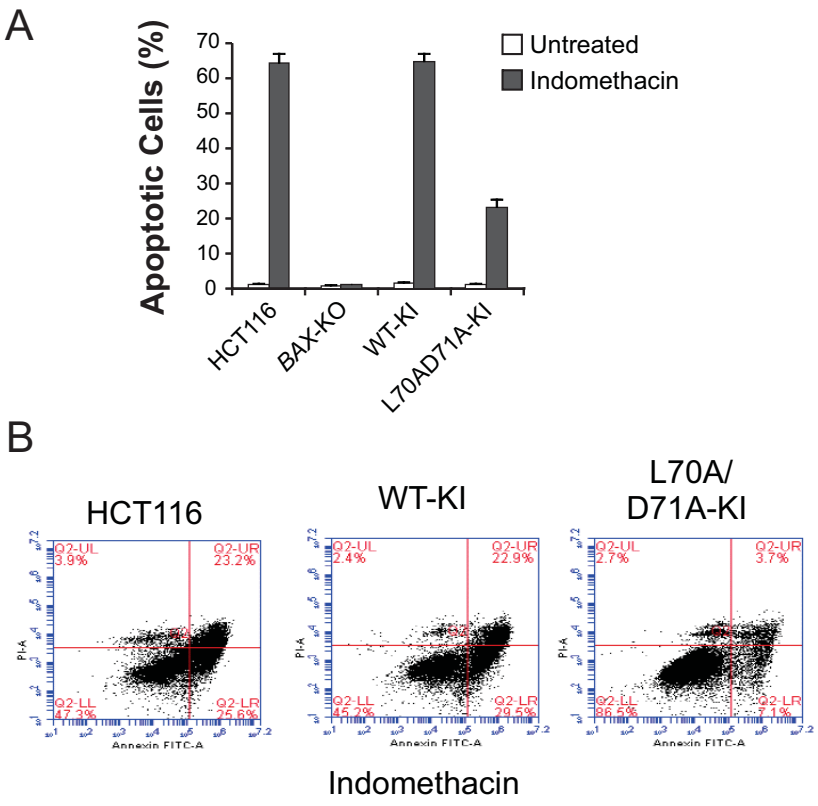
A
Apoptotic Cells (%)
70
60
50
40
30
20
10
0
Untreated
Indomethacin
HCT116
BAX-KO
WT-KI
L70AD71A-KI
B
HCT116
WT-KI
L70A/
D71A-KI
Q2-UL
3.9%
Q2-UR
23.2%
Q2-LL
47.3%
Q2-LR
25.6%
Q2-UL
2.4%
Q2-UR
22.9%
Q2-LL
45.2%
Q2-LR
29.5%
Q2-UL
2.7%
Q2-UR
3.7%
Q2-LL
86.5%
Q2-LR
7.1%
PI-A
Annexin FITC-A
Indomethacin

Table S1. Primers for constructing Bax WT, K21E, D33A, D68R, and L70AD71A knock-in vectors and PCR screen.

Purpose	Orientation	Sequence
Vector construction		
Left homologous arm	Forward	5'-GGG AAA GUg tta tct ctt ggg ctc aca agt tag a-3'
	Reverse	5'-GGA GAC AUt ggg acc cta gca gat aaa gtg ac-3'
Right homologous arm	Forward	5'-GGT CCC Aug aag tcc agg gtc ccc agc t-3'
	Reverse	5'-GGC ATA GUc agg tcc agt aaa tgc ttg ttg a-3'
PCR screen		
Left arm	Forward	5'-tca gca cag att agt ttc tgc ca-3'
	Neo reverse	5'-ttg tgc cca gtc ata gcc g-3'
Right arm	*Neo* forward	5'-tct tga cga gtt ctt ctt ag-3'
	Reverse	5'-cct gac cac tct gct aaa tca c-3'
PCR screen (after Cre)		
Left arm	Forward	5'-tca gca cag att agt ttc tgc ca-3'
	LoxP reverse	5'-gac ctc agc gat atc cc tag-3'

Capital letters represent incorporated restriction enzyme site sequences.

Table S2. Primers for constructing Bax S184V knock-in vector and PCR screen.

Purpose	Orientation	Sequence
Vector construction		
Left homologous arm	Forward	5’-GGG AAA GU gaga cgg att ctt gct cta ttg tc-3’
	Reverse	5’-GGA GAC AUc agg gga ct gaga tga acg g-3’
Right homologous arm	Forward	5’-GGT CCC Aug ccc gca atc ctg cct tct-3’
	Reverse	5’-GGC ATA GUc ctg tga ct gaga acc cac atc-3’
PCR screen		
Left arm	Forward	5’-gag gtc agg cca aag cct gca c-3’
	Neo reverse	5’-ttg tgc cca gtc ata gcc g-3’
Right arm	*Neo* forward	5’-tct tga cga gtt ctt ctt ag-3’
	Reverse	5’-caa agg tgg ggt ggg aac aga g-3’
PCR screen for S184V targeting		
	Exon4 forward	5’-tgg cag ctg aca tgt ttt ctg ac-3’
	Neo reverse	5’-ttg tgc cca gtc ata gcc g-3’
PCR screen (after Cre)		
Left arm	Forward	5’-gag gtc agg cca aag cct gca c-3’
	LoxP reverse	5’-gac ctc agc gat atc cc tag-3’

Capital letters represent incorporated restriction enzyme site sequences.