SUPPLEMENTARY

Online supplemental material is available at xxx

EXPERIMENTAL PROCEDURES

Crosslinking

1 µg/µl of subcellular fractionated sample was prepared in MSH buffer. 5 mM BMH dissolved in DMSO or a vehicle control was added to the sample (thereby the DMSO concentration should not exceed 10% final volume). The reaction was allowed to proceed for 30 min at room temperature. Samples were pelleted at full speed for 15 min, and the reaction was quenched by adding DTT containing SDS sample buffer. Denaturation was at RT for 30 min and the samples were stored at 4°C until they were loaded on a gel and detected by Western Blot analysis.

List of p23- and control shRNAs from SIGMA.

No.	Product name
1	NM_019766-**335s1c1**
2	NM_019766-**1215s1c1**
3	NM_019766-**420s1c**
4	NM_019766-**668s1c1**
5	NM_019766-**605s1c1**
C	**SHC002; Mission non-Target shRNA control vector**

FIGURE LEGENDS

Suppl. Figure 1: BMH-crosslinking of FDM extracts reveals a crosslinked Bax band of ca. 43 kD.

Anti-Bax Western blot analysis of BMH-crosslinked (5 mM) cytosolic and mitochondrial fractions of FDM wt cells deprived of IL-3 for 0 and 16 h. DMSO was used as a negative crosslink control. h: hours; Cyto: cytosol; Mito: mitochondrial fraction. Note that apart from the Bax monomer, a crosslinked band at 43 kD appears in both healthy and IL-3 deprived cytosol (black arrow). In the healthy mitochondria fraction this band is slightly lower than a Bax dimer (red arrow) and is converted into multiple, oligomeric bands upon IL-3 deprivation. Asteriks: unspecific bands also seen in the DMSO control.

Suppl. Figure 2: Reported potential Bax binding partners elute differently from gel filtration chromatography than cytosolic Bax complexes from MEFs and liver cells.

Anti-Bax, anti-Mcl-1, anti-Bcl-x_L, anti-14-3-3-θ, anti-Pin-1 or anti-p23 Western blot analysis of gel filtration chromatography of the cytosol of MEF (A) or mouse liver cells (B). 250 µl of 1 mg cytosol in IB_C-buffer was loaded onto the column and separated into 500 µl fractions. Note that whereas the elution profile of Bcl-x_L, Mcl-1 partially overlaps with that of Bax, no such overlap is seen with 14-3-3-θ and Pin-1 (red rectangle). In liver cells, also p23 does not overlap with Bax, although this is clearly seen in FDM and MEF (see Fig. 6A).

Suppl. Figure 3: Generation and testing of novel anti-N and anti-C Bax antibodies.

A, The murine Bax sequence of 192 aa is shown. The new, self-made anti-Bax antibodies are directed against the very N-terminal amino acids 2-14 (α-N, red) or the very C-terminal amino acids 184-192 (α-C, green) of murine Bax. The blue and grey bars encompass the epitopes of the commercially available, conformation-specific anti-Bax antibodies α-NT and α-6A7. While α-C also recognizes human Bax, α-N antibody is mouse specific. *B*, Anti-NT, anti-N and anti-C-Bax IPs of cytosolic and mitochondrial CHAPS (1%) extracts from healthy and IL-3 deprived FDMs (16 h). The inputs, IPs and the supernatants after IP (SN) were loaded on a 15% SDS gel and immunoblotted with anti-NT-

Bax antibody. Note that while anti-NT-Bax is partially conformation specific, i.e. immunoprecipitating only active Bax from IL-3 deprived mitochondria, anti-N- and anti-C-Bax recognize native Bax in its inactive and active states.

Suppl. Figure 4: Efficiency of anti-C Bax IPs for mass spectrometry analysis and Bax peptides identified by Nanoflow-HPLC-MS/MS LTQ-FT.
A, Silver stain of anti-C-Bax IPs either using unpurified or affinity purified (pur) anti-C antibodies. Note that the anti-C_{pur} lane contains fewer heavy and light chains, but endogenous Bax is not detectable as compared to rec. Bax. *B*, Bax peptides identified by MS/MS. The colored peptides were found in different mass spectrometric analysis. Yellow: BN (2.-wC6); Green (2 adjoining peptides) IP (2.-w6); Red: (2 adjoining peptides) BN (1.-wC3 and 2.-wC6), IP (2.-w6); Blue: BN (1.-wC3) (see Suppl. Tables S1 and S3)

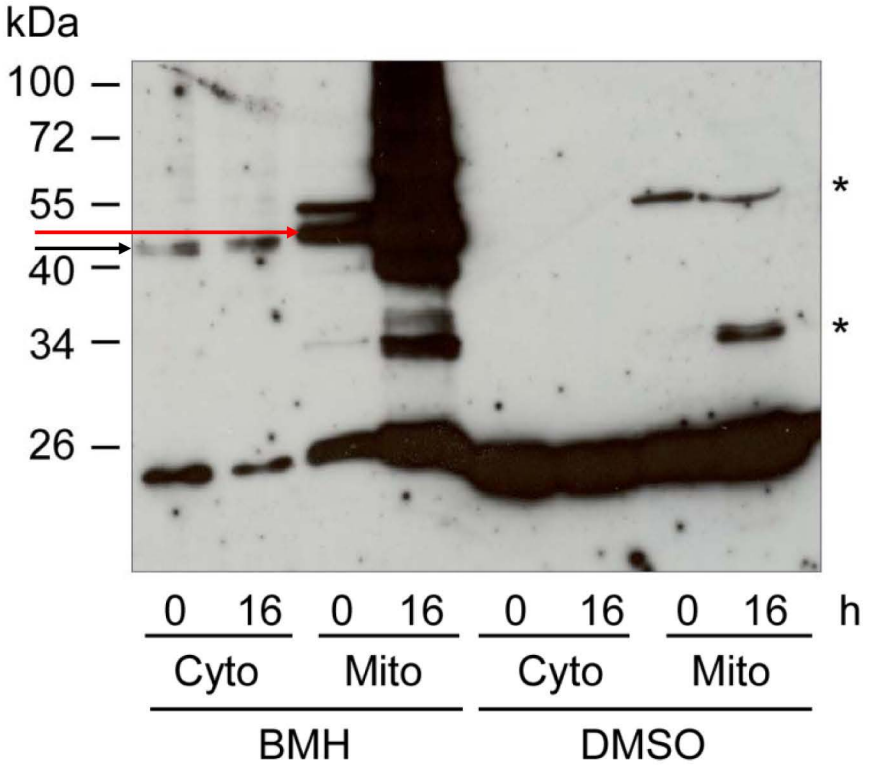
Suppl. Figure 5: p23 hsp90 co-chaperone does not co-immunoprecipitate with Bak, Bcl-x_L or Bcl-2
600 µg of total extracts from FLAG-hBcl-x_L/p23-V5 co-transfected (A), FLAG-hBcl-x_L transfected (B), His-Bak transfected (C) or FLAG-hBcl-2 or FLAG-hBax transfected HEK293T cells (D) were incubated with either anti-FLAG M2 or Ni^{2+}-NTA agarose beads for 2 h. The beads were washed (10-20 mM imidazole for His-Bak) and eluted with either 3xFLAG peptide (A,B,D) or direct boiling in sample buffer (C). Input (before IP), the eluate (IP) and the remaining supernatant after IP (SN) were loaded on 15% SDS PAGE and immunoblotted with anti-FLAG (A,D), anti-V5 (A), anti-Bcl-x (B), anti-Bak NT (C) or anti-p23 (B,C,D) antibodies. C: vector control (pcDNA3.1); V5: p23-V5; Fx_L: FLAG-hBcl-x_L; HBak: His-hBak; FBc: FLAG-hBcl-2; FBx: FLAG-hBax. Note that all the immunoprecipitations worked effectively (except for His-Bak where some His-Bak still remained in the supernatant after IP). However, p23 was only co-immunoprecipitated with FLAG-Bax (D, lane FBx), not with FLAG-Bcl-2 (D, lane FBc), FLAG-Bcl-x_L (A,B, lanes Fx_L) or His-Bak (lane HBak) (C).

Suppl. Figure 6: Cytosolic Bax is not recognized by the conformation specific antibody 6A7.
Anti-Bax Western blot analysis of an anti-Bax 6A7 immunoprecipitation from a MEF cytosol or recombinant human Bax in the presence or absence of Triton X-100 (TX). As control IP an IgG antibody is used. Note that cytosolic endogenous Bax is not in a N-terminally open conformation detectable by 6A7 (except in the presence of TX which provokes this conformational change). In the absence of TX, recombinant Bax sticks to the beads

Suppl. Table 1: Peptides from the gel filtration/BN/SDS-PAGE purification method identified by Nanoflow-HPLC-MS/MS LTQ-FT.
Results from two independent purifications are shown (1., 2.). In both we identified 3 different peptides of Bax. Additionally, 4 peptides of hemoglobin and 3 peptides of Sjogren's syndrome nuclear autoantigen were found in the first analysis, while 2 peptides of coiled-coil domain containing 43 and 3 peptides of LUC7-like 2 were identified in the 2nd analysis. The number behind the „w" (wild type) indicates the gel slice number. All found peptides are shown, the significant ones are marked in bold (peptide score).

Suppl. Table 2: p23 hsp90 co-chaperone peptides from gel filtration/BN/SDS-PAGE purification and anti-Bax IP identified by Nanoflow-HPLC-MS/MS LTQ-FT.
Results from two independent gel filtration/BN/SDS-PAGE (BN) purifications and two independent anti-Bax immunoprecipitations (IP) are shown (1., 2.). For each analysis at least 3 specific p23 peptides were detected. However two peptides were also seen in the Ig control (IP-wI7) indicating that p23 may also non-specifically bind to Ig or beads. The number behind the „w" or „A" (wild type) indicates the gel slice number. All found peptides are shown, the significant ones are marked in bold (peptide score).

Suppl. Table 3: Peptides from anti-Bax IPs identified by Nanoflow-HPLC-MS/MS LTQ-FT.
From the anti-Bax IPs, 4 specific peptides were detected for Bax and 2 peptides for cytosolic 5',3'-nucleotidase. The number behind the „w" (wild type) indicates the gel slice number. All found peptides are shown, the significant ones are marked in bold (peptide score)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Cyto
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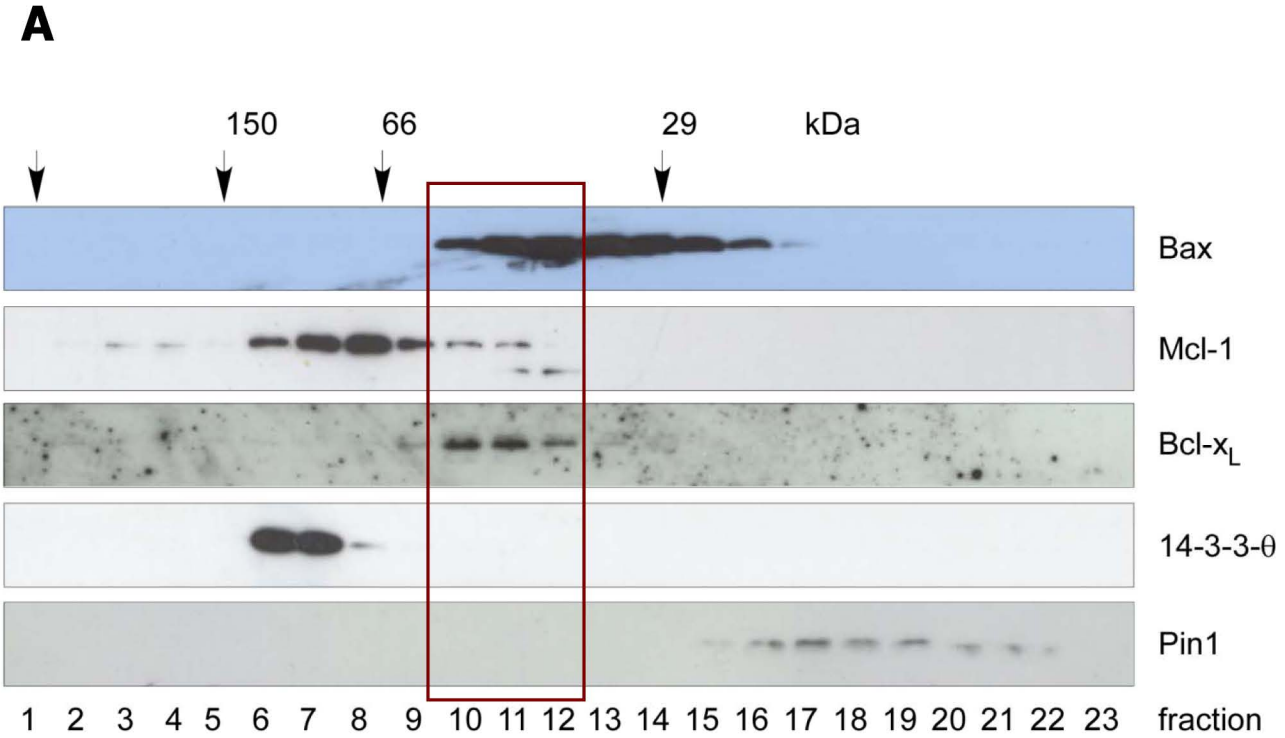
Cyto
Mito
BMH
DMSO

Fig. S1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kDa
Bax
Mcl-1
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14-3-3-θ
Pin1
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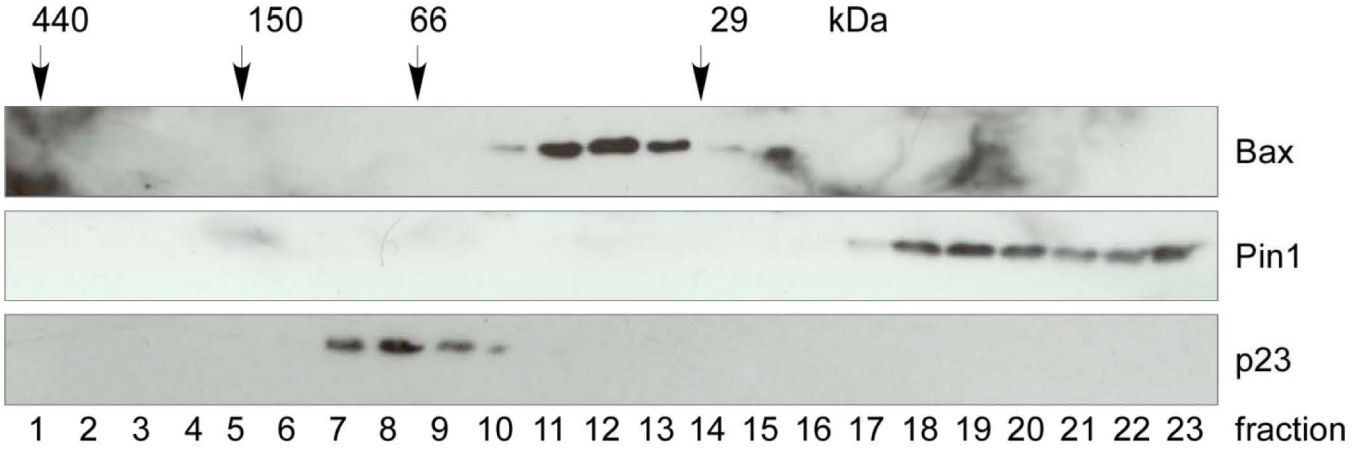
fraction

Fig. S2

B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150
66
29
kDa
Bax
Pin1
p23
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
fraction

Fig. S2

A

MDGSGEQLGSGGPTSSEQIMKTGAFLLQGFIQDRAGRMAGETPELTLEQP 50

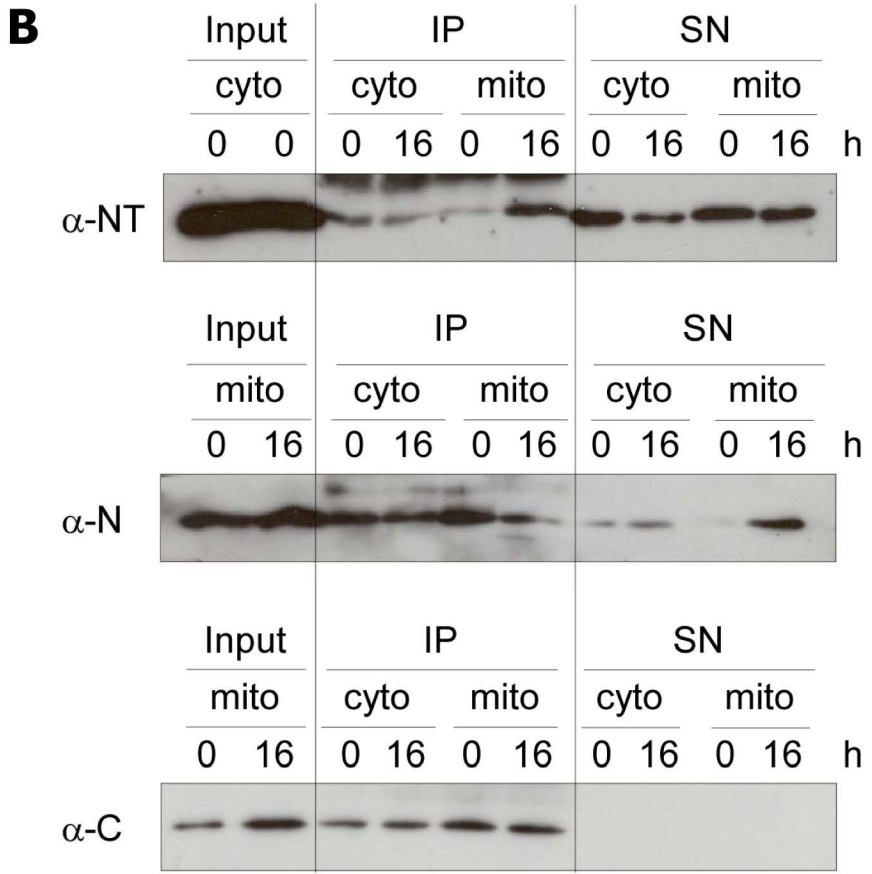
PQDASTKKLSECLRRIGDELDSNMELQRMIADVDTDSPREVFFRVAADMF 100

ADGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMGWTLDFLRERLL 150

VWIQDGGWEGLLSYFGTPTWQTVTIFVAGVLTASLTIWKKMG 192

α-NT (1-21): conformation specific
α-6A7 (12-24): conformation specific
α-N (2-14): N-terminal selfmade
α-C (184-192): C-terminal selfmade

Fig. S3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cyto
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cyto
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α-C

Fig. S3

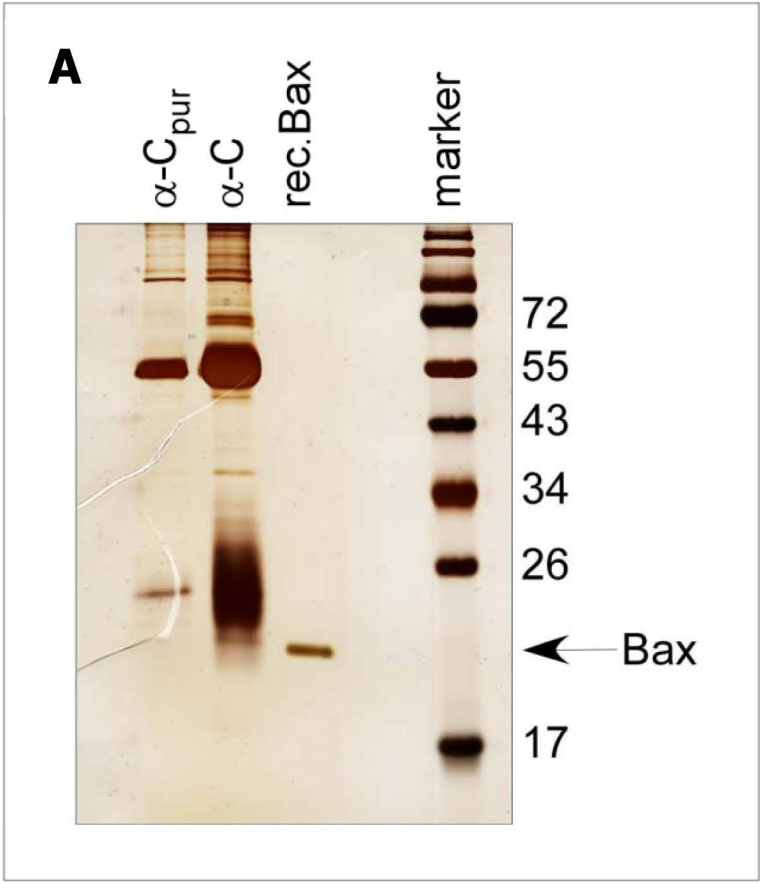

Fig. S4

B

MDGSGEQLGSGGPTSSEQIMKTGAFLLQGFIQDRAGRMAGETPEL
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FFRVAADMFADGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMG
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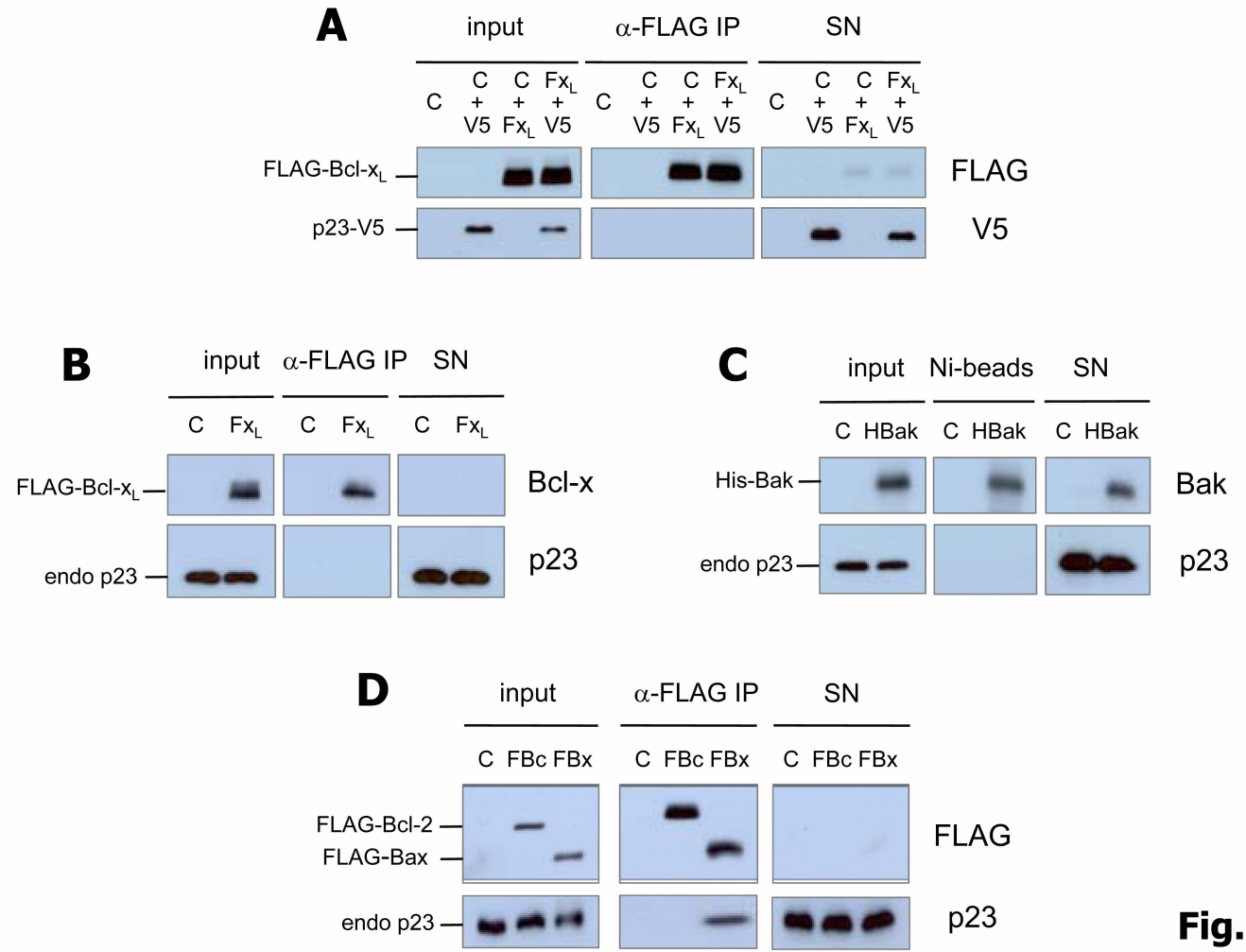
LTIWKKMG

Fig. S4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SN
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FxL
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p23
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HBak
His-Bak
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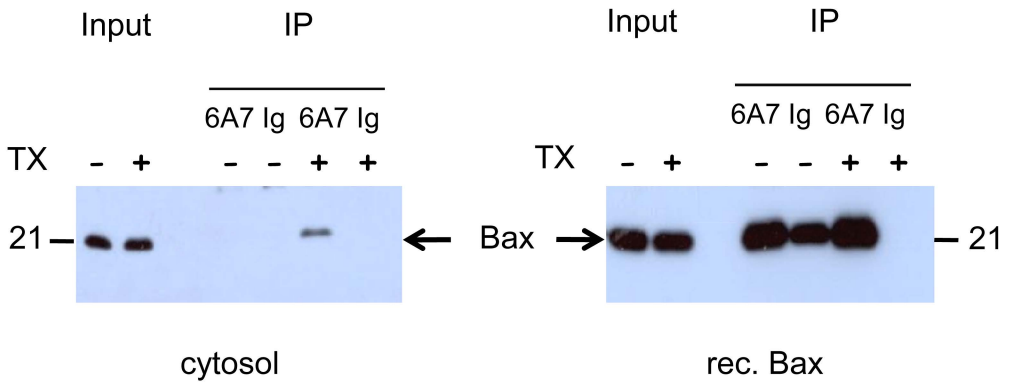
Fig. S5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6A7 Ig 6A7 Ig
TX - + - - + +
21
Bax
cytosol
Input
IP
6A7 Ig 6A7 Ig
TX - + - - + +
21
rec. Bax

Fig. S6

Gel spot	Protein mass (Dalton)	Protein name	gi number	Ratio	Peptide score	Peptide sequence
1.-wC3	21337	BAX	2493273	0.37	39	R.VVALFYFASK.L
				1.80	45	R.MIADVDTDSPR.E
				2.17	40	R.MIADVDTDSPR.E + Oxidation (M)
				3.14	**63**	R.MIADVDTDSPR.E + Oxidation (M)
				6.34	**60**	R.IGDELDSNMELQR.M + Oxidation (M)
2.-wC1	16046	Hemoglobin	62901559	-0.12	19	K.LHVDPENFR.L
				-0.51	**43**	R.LLVVYPWTQR.F
				0.80	**96**	K.VNVEEVGGEALGR.L
				-11.60	**60**	K.VNVWEVGGEALGR.L
				-0.44	56	K.EFTPQVQAAYQK.V
2.-wC1	13549	Sjogren's syndrome nuclear autoantigen1	12963687	0.18	**49**	R.TIAETEAAYLK.I
				-3.06	24	R.QIQQEEDEKQR.L + Gln->pyro-Glu (N-term Q)
				-3.44	**42**	K.ILESSQTLLSVLK.R
2.-wC6	21337	Bax	2493273	0.06	**63**	R.MIADVDTDSPR.E
				1.72	**67**	K.TGAFLLQGFIQDR.A
				-1.94	59	R.IGDELDSNMELQR.M
				-4.73	**72**	R.IGDELDSNMELQR.M
2.-wC10	25034	coiled-coil domain containing 43	13385406	0.63	**58**	R.NTNVEDVLNAR.K
				2.15	**63**	K.EDEVQAIATLIEK.Q
2.-wC13	45667	LUC7-like 2	4929587	-0.92	39	R.VHELNEEIGK.L
				-1.69	**90**	R.LAETQEEISAEVAAK.A
				-1.90	**102**	K.VEQLGAEGNVEESQK.V

Suppl. Table 1

Gel spot	gi number	Ratio	Peptide score	Peptide sequence
1.BN-A8	5081800	2.46	24	R.SILCCLR.K + 2 Carbamidomethyl(C)
		2.38	**45**	K.DVNVNFEK.S
		6.76	**69**	K.LTFSCLGGSDNFK.H + Carbamidomethyl (C)
1.BN-A9	5081800	3.55	23	R.SILCCLR.K + 2 Carbamidomethyl(C)
		4.45	**51**	K.DVNVNFEK.S
		5.65	**85**	K.LTFSCLGGSDNFK.H + Carbamidomethyl (C)
2.BN-w8	9790017	0.29	30	R.SILCCLR.K + 2 Carbamidomethyl(C)
		-0.65	38	K.GESGQSWPR.L
		0.25	**82**	K.LTFSCLGGSDNFK.H + Carbamidomethyl (C)
1.IP-wC4	1362727	-3.43	25	K.DVNVNFEK.S
		0.95	**52**	K.LTFSCLGGSDNFK.H + Carbamidomethyl (C)
2.IP-wC6	9790017	1.37	31	R.SILCCLR.K
		0.67	24	R.SILCCLR.K
		-4.68	42	K.DVNVNFEK.S
		-0.06	22	K.GESGQSWPR.L
		-2.24	**76**	K.LTFSCLGGSDNFK.H
		-1.69	31	K.LTFSCLGGSDNFK.H
2.IP-wI7	5081800	5.16	18	R.SILCCLR.K
		1.92	**79**	K.LTFSCLGGSDNFK.H

Suppl.Table 2

Gel spot	protein mass (dalton)	Protein name	gi number	Ratio	Peptide score	Peptide sequence
2.-w6	21337	Bax	2493273	0.76	38	K.LSECLR.R + Carbamidomethyl (C)
				1.47	**64**	R.MIADVDTDSPR.E
				-0.10	62	R.MIADVDTDSPR.E + Oxidation (M)
				1.39	**78**	R.IGDELDSNMELQR.M + Oxidation (M)
				0.65	53	R.MAGETPELTLEQPPQ DASTK.K + Oxidation (M)
				0.69	**102**	R.MAGETPELTLEQPPQ DASTK.K + Oxidation (M)
				1.38	**79**	R.MAGETPELTLEQPPQ DASTKK.L + Oxidation (M)
2.-w10	23062	5',3'-nucleotidase (cytosolic)	7657031	6.65	**60**	R.WVEQNLGPEFVER.I
				4.10	**47**	R.GFLANEQYGALRPDL AEK.V

Suppl. Table 3