ATP synthase K⁺- and H⁺-flux drive ATP synthesis and enable mitochondrial K⁺-uniporter function

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Supplemental Material

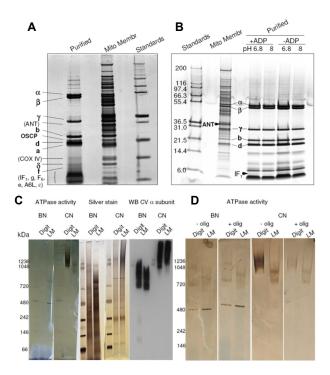


Figure 1 – figure supplement 1. Purity and protein characterization of the isolated F_1F_0 complex. F_1F_0 was purified according to manufacturer protocol (Mitosciences) and reconstituted into liposomes and planar lipid membranes. The purity of the enzyme complex was assessed by gel electrophoresis, silver staining and Western blotting (under denaturing conditions), which shows the absence of virtually all other unrelated mitochondrial proteins, including ANT, one of the most abundant inner mitochondrial membrane proteins (A,B). It has been suggested that mitochondrial ROMK potassium channel might act as the pore-forming subunit of the cytoprotective mKATP channel (Foster et al, 2012). Our immunoblotting with anti-ROMK antibody eliminated ROMK channel as a contaminant of the isolated F_1F_0 (Figure 1-figure supplement 2C). The isolated F_1F_0 showed monomers (~720 kD) and dimers (possibly even higher order multimers) when run on Blue- and clear-native gels, although the "in-gel" oligomycinsensitive ATPase activity, as seen in the clear-native gel, was largely restricted to the dimers and higher (C,D). Note that in the Blue-native gels, the charged dye Coomassie probably interferes with the in-gel ATPase assay. Also, the membrane solubilization detergents digitonin and lauryl maltoside yield different apparent ATPase activities. Importantly, the binding of the small (8 kD) IF₁ to the synthase complex is reversible and pH-dependent, with approximately half of the IF₁ being bound at pH 8 as compared to pH 6.8 (**B**). The synthase complexes during and after isolation were maintained at pH 7 to keep IF₁ binding, except in protocols for which IF₁ was intentionally removed by a short wash in alkaline (pH 10) buffer.

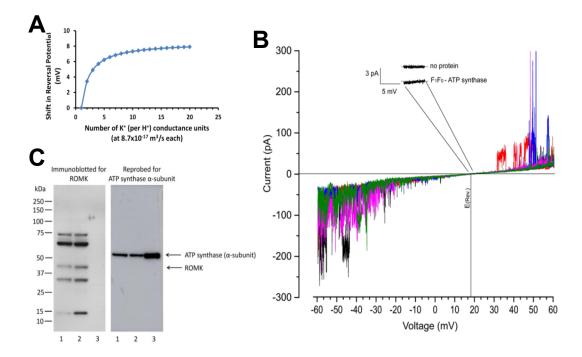


Figure 1 - figure supplement 2. Absence of functionally relevant K⁺/H⁺ antiport or independent K⁺ channel activity contaminating the F_1F_0 proteoliposome (PL) reconstitution experiments. (A) Shift in Reversal Potential (E_{rev}) as a function of a hypothetical variation in the stoichiometry of F_1F_0 H⁺ conductance units vs number of K⁺ channels ("K⁺ conductance" units). All electrophysiological data confirms the absence of coincidental coimmunoprecipitation with a contaminating K^+ channel (or a Na⁺ channel). The results of a calculation based on manuscript equation 1 show a theoretical shift in Erev as a function of a hypothetical variation on the stoichiometry of "H⁺ conductance" and "K⁺ conductance" units: note that a ratio of 1:1 yields the obtained E_{rev} in the present experiments (a ratio of 1:0 represents the absence of a "K⁺ conductance unit" and E_{rev} will equal the reversal potential for H⁺ which is set by ionic conditions to be zero; this result has never been seen in any of our experiments). A ratio of 2:1 means two "K⁺ conductance" units per "H⁺ conductance", and so on. If purified F_1F_0 complex displayed a 2:1 ratio we would observe a \sim 3.5 mV positive shift in E_{rev}, which was never observed. That E_{rev} is invariant from experiment to experiment (our resolution is < 1 mV) confirms a fixed 1:1 stoichiometry. Furthermore, the K⁺ conductance is quantitatively inhibited in parallel with that of H^+ , without any shifts in E_{rev} , by the titration with ATP (Figure 6D). Finally, the "H⁺ conductance" and "K⁺ conductance" units are all completely inhibited by both Oligo and Vent. If there was a contaminating K^+ (or Na⁺) channel co-immunoprecipitated with F_1F_0 , that channel activity would remain after these inhibitors, as we know of no K^+ (or Na⁺) channels that are coincidentally *fully* sensitive and inhibited by Oligo and Vent, but such a residual channel activity never happens. Additionally, given the apparent 1:1 stoichiometry with F₁F₀ of such a hypothetical K⁺ (or Na⁺) channel, its abundance should be similar, for example, to the γ -subunit, but there is no such unidentified band of similar abundance in silver-stained gels run on the immunoprecipitated F_1F_0 used in the present experiments (Figure 1 - figure supplement 1).

(B) Biophysical evidence ruling out contamination of different K^+ channels separate and distinct from F_1F_{o} . Currents were recorded in F_1F_o -reconstituted bilayers in the voltage-ramp mode. Five random examples of the voltageramp data are given (depicted in different colors). Inset shows the current noise amplitude around E_{rev} with reconstituted F_1F_o which remains identical to that without reconstituted proteins. In the hypothetical scenario of the presence of a separate K^+ channel distinct from F_1F_o , the noise amplitude at E_{rev} created by the continuous, independent channel-gating activities of the two putative independent channels (whose time-averaged currents exact summate to zero at E_{rev}) would be significantly larger than pure bilayer noise, and distinct from F_1F_o . In contrast, if ions entering and turning the F_1F_o c-ring are sharing the common path for both K^+ and H^+ permeation, independent gating would not occur - there would be no channel-gating activity at all at E_{rev} – therefore, the noise amplitude at E_{rev} would be equivalent to that of the plain bilayer without reconstituted proteins. The absence of any channel-gating at E_{rev} (Figure inset) rules out the possibility that K^+ is being conducted by a separate K^+ channel co-immunoprecipitated with F_1F_o . (C) Immunocaptured F_1F_0 is devoid of contamination with ATP-dependent ROMK potassium channel. Immunoblot analysis of rat heart mitochondria (10 µg; lane 1), left ventricle tissue lysate (10 µg; lane 2) and immunocapture purified rat heart F_1F_0 (~5 µg) separated on a NuPage 4-12% Bis-Tris gel prior to immunoblot assay and tested with anti-KCNJ1 (Sigma Prestige, anti-KCNJ1 Cat#:HPA026962; this antibody has been employed to identify mitochondrial ROMK channel by (Foster et al, 2012)), the blot was then re-probed with anti-ATP synthase subunit α antibody. Arrows point to location of the ROMK channel and the ATP synthase subunit α . While left ventricle and cardiac mitochondria extracts showed the expected weak but positive band ~45 kDa, there was no immunolabelling whatsoever in our F_1F_0 complex. Thus there is no ROMK contaminating our preparations to yield any functional artefact.

Supplementary file 1 Quantitative comparison of H⁺ and K⁺ current magnitudes through ATP synthase

To compute the ratio of H^+ and K^+ currents through the ATP synthase we consider the contribution of each ion as described by the Goldman-Hodgkin-Katz formalism:

$$\frac{I_{H^{+}}}{I_{K^{+}}} = \frac{P_{H}Z_{H}^{2} \frac{\Delta \Psi_{m} F^{2}}{RT} \left(\frac{[H]_{i} - [H]_{0} \exp\left(\frac{-Z_{H}\Delta \Psi_{m}F}{RT}\right)}{1 - \exp\left(\frac{-Z_{H}\Delta \Psi_{m}F}{RT}\right)} \right)}{P_{K}Z_{K}^{2} \frac{\Delta \Psi_{m}F^{2}}{RT} \left(\frac{[K]_{i} - [K]_{0} \exp\left(\frac{-Z_{K}\Delta \Psi_{m}F}{RT}\right)}{1 - \exp\left(\frac{-Z_{K}\Delta \Psi_{m}F}{RT}\right)} \right)}$$

Taking the limit of the ratio of currents when $\Delta \Psi_m$ tends to - ∞ , i.e., in the direction of ATP synthesis,

$$\lim_{\Delta \Psi_m \to -\infty} \left(\frac{I_{H^+}}{I_{K^+}} \right) = \frac{P_H Z_H^2 \left(\frac{[H]_i - [H]_0 \exp\left(\frac{Z_H \infty F}{RT}\right)}{1 - \exp\left(\frac{Z_H \infty F}{RT}\right)} \right)}{P_K Z_K^2 \left(\frac{[K]_i - [K]_0 \exp\left(\frac{Z_K \infty F}{RT}\right)}{1 - \exp\left(\frac{Z_K \infty F}{RT}\right)} \right)}$$

the linear terms ([H]_i, [K]_i and the 1 in the numerators and denominators, respectively), become negligible with respect to the exponential terms containing the dependence on $\Delta \Psi_m$ as follows:

$$\lim_{\Delta\Psi_{m}\to\infty} \left(\frac{I_{H^{+}}}{I_{K^{+}}}\right) = \frac{P_{H}Z_{H^{2}}^{2} \left(\frac{[\mathcal{W}_{I_{i}}^{-} - [H]_{0} \exp\left(\frac{Z_{H}\infty F}{RT}\right)}{\mathcal{I} - \exp\left(\frac{Z_{H}\infty F}{RT}\right)}\right)}{P_{K}Z_{K}^{2} \left(\frac{[\mathcal{K}_{I_{i}}^{-} - [K]_{0} \exp\left(\frac{Z_{K}\infty F}{RT}\right)}{\mathcal{I} - \exp\left(\frac{Z_{K}\infty F}{RT}\right)}\right)}$$

rendering

$$\lim_{\Delta \Psi_{m} \to -\infty} \left(\frac{I_{H^{+}}}{I_{K^{+}}} \right) = \frac{P_{H} Z_{H}^{2} [H^{+}]_{0}}{P_{K} Z_{K}^{2} [K^{+}]_{0}}$$

This limit will be (asymptotically) approached as the magnitude of $\Delta \Psi_m$ increases, but under realistic ionic conditions, is practically achieved for $|\Delta \Psi_m| \ge 100 \text{ mV}$. The currents ratio through ATP synthase will be proportional to the ratio of extra-mitochondrial concentrations (in mole-equivalents) of $[\text{H}^+] = 6.3 \times 10^{-8}$ over $[\text{K}^+] = 0.137$, at pH 7.2, corresponding to $\sim 5 \times 10^{-7}$: 1. This results in a ratio of H⁺: K⁺ currents $\sim 1:4$ since the ratio of permeability P_H:P_K is equal to $5.2 \pm 0.9 \times 10^{-11}$: $8.7 \pm 2.9 \times 10^{-17}$ as determined in our experiments.

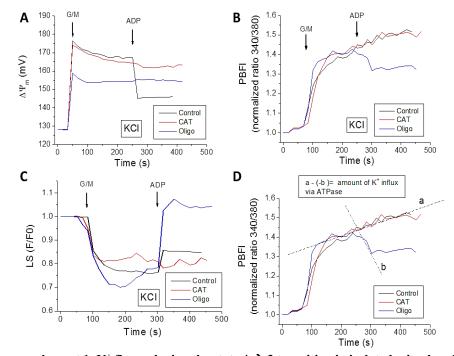


Figure 4 - figure supplement 1. K⁺ fluxes during the state 4 → 3 transition in isolated mitochondria from guinea pig heart. Freshly isolated mitochondria loaded with 20 µM PBFI-AM as described before (Aon et al, 2010) were preincubated in the absence (Control) or presence of 10 µM oligomycin (Oligo) or 10 µM carboxyatractyloside (CAT) (in the cuvette of the spectrofluorimeter, with stirring at 37°C for 3min) followed by energization with the Na⁺ salts of substrates glutamate/malate (G/M, 5 mM/5 mM) (state 4 respiration) and, subsequently, with 1mM ADP (state 3 respiration), added as indicated with arrows. Mitochondria were assayed in the same medium above described containing 137 mM KCl (instead of sucrose) and 2 mM Pi. Mitochondrial $\Delta \Psi_m$ (A), PBFI (B) and swelling (light scattering, LS) (C), were monitored simultaneously as described above. (C, D) Volume changes during mitochondrial swelling-contraction, and of the K⁺ flux sustained by the ATP synthase in state 3 respiration, were estimated in control and oligomycin-preincubated mitochondria. The relative volume changes (measured as LS) were: swelling triggered by G/M (~ 0.3 for Oligo and ~0.2 for control and carboxyatractyloside, CAT), and contraction by ADP (~ 0.1 for control and ~ 0.3 for Oligo) additions (C). On these bases, the volume change quantification can be estimated considering that 1.77 µl/mg mitochondrial protein (see (Aon et al, 2010) p.75, 2nd column, 2nd paragraph) correspond to the volume in state 4 respiration after G/M addition. A ∆volume of 30% contraction after ADP addition can be estimated equivalent to 0.5 µl/mg mito prot., thus from 1.77 to 1.24 µl/mg mito protein, sustained by a K⁺ flux equivalent to 60% of the K^+ flux at V_{max} .

(D) Estimation of the K⁺ flux sustained by the ATP synthase in state 3 respiration in the presence of Oligo: In KCl, V_{max} of K⁺ uptake rate = 172±17 nmol K⁺/min/mg prot. (see Aon et al., 2010, their Figure 2A).

Slopes (PBFI ratio/min) = Control: 0.00102; +Oligo: - 0.00169

 $dK^+/dt = influx - efflux = flux$ balance from control.

Flux balance from control + efflux = influx

0.00102 + 0.00169 = 0.00271 PBFI ratio/min = 0.0183 PBFI ratio/min/mg prot. (148 µg mitochondrial prot. per assay) K⁺ flux = 0.0183 * 1000/0.175 = 104 nmol K⁺/min/mg prot.

For a V_{max} uptake rate of K⁺ = 172 nmol/min/mg prot.; 104 nmol K⁺/min/mg prot. represents 60% of the total K⁺ flux at V_{max} .

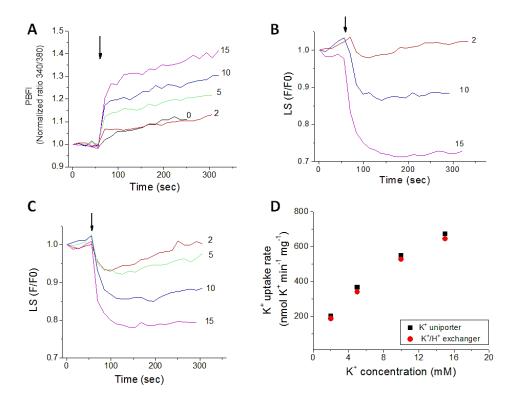


Figure 4 - figure supplement 2. K⁺ uniporter and K⁺/H⁺ exchanger fluxes in the absence or presence of quinine in isolated mitochondria from guinea pig heart.

Freshly isolated PBFI-loaded mitochondria were assayed for K⁺ fluxes in the same medium described in the legend of Figure 4 - figure supplement 1, but containing 250 mM sucrose (instead of KCl), thus enabling us to study K⁺ fluxes in a controlled fashion. After pulses of 2 to 15 mM KH₂PO₄ (indicated by arrow), K⁺ fluxes were quantified in mitochondria energized with the Na⁺ salts of G/M (5 mM/5 mM), and in the absence (Control) or presence of 50 μ M quinine, a K⁺/H⁺ exchanger inhibitor (Garlid et al, 1986; Martin et al, 1986). Mitochondrial swelling (LS: B, C) and PBFI fluorescence (A) were determined in control (B) and quinine-preincubated (A, C) mitochondria. Panel D depicts the K⁺ fluxes sustained by the K⁺ uniporter and the K⁺/H⁺ exchanger as measured in the presence of quinine or its absence, respectively. The flux through the K⁺/H⁺ exchanger was calculated as the difference between the fluxes measured in the presence of quinine (uniporter) minus in its absence (where both uniporter and exchanger are active).

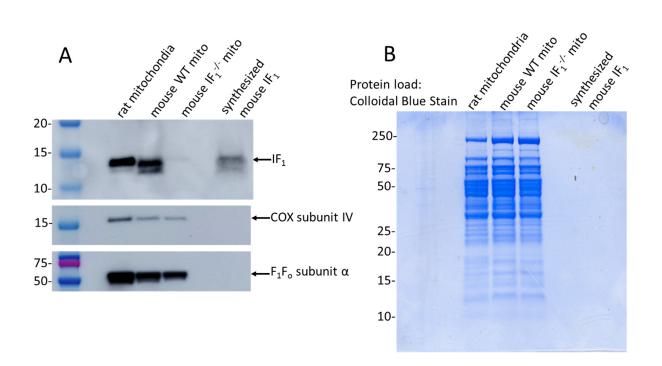


Figure 5D-figure supplement 1. Phenotype verification of IF₁ KO mouse. A. IF₁ protein expression was not detected by an anti-IF₁ antibody (Cell Signaling Technology) in IF₁^{-/-} mouse mitochondria by western blotting while the blot was positive for IF₁ protein expression in rat and mouse wild type (WT) mitochondria (15 μ g of proteins were loaded per well) and synthetized mouse IF₁ peptide (purified >98%; 50 ng). As expected, all three mitochondrial preparations showed the presence of other mitochondrial proteins (the same blot was reprobed for COX subunit IV (Cell Signaling Technology, clone 3E11) and F₁F₀ subunit alpha (Abcam). **B.** The gel was stained with Colloidal Blue Stain (Invitrogen) to demonstrate the equal total mitochondrial protein load into each well.

sp Q5RFJ9 ATIF1 PONAB	EEERY	FRAQ	SREQLAA	LKKHHE	EEIV	ннккеі	ERLQK
sp P01096 ATIF1 BOVIN	EEE <mark>RY</mark>	FRAR	A K E Q L A A	<mark>ь кк</mark> нн <mark>е</mark>	NEIS	нн <mark>аке</mark> і	ERLQK
sp Q29307 ATIF1 PIG	DEERY	FRAR	AREQLAA	LKKHHE		H H <mark>V K E I</mark>	ERLQK
sp[035143] ATIF1 MOUSE	E E D <mark>R Y</mark> E E E <mark>R Y</mark>	F R E K F R A Q	S R E Q L A A			нн <mark>ѕке</mark> ннккеі	
sp Q9UII2 ATIF1 HUMAN sp Q03344 ATIF1 RAT	EEDRY		TREQLAA			HHSKEI	ERLQK
sp A3KNL5 ATF1A DANRE	EEERY	FRQK	EREQLAA	LKNHHE	EEID	ннккеі	ERLQR
tr B5X591 B5X591 SALSA	EEERY	F R Q K	EKEQLSA	LRKHHE	EEID	HSKKEI	ERLQR
tr C3KHS5 C3KHS5 ANOFI tr C1BWJ3 C1BWJ3 ESOLU	E E E <mark>R</mark> Y E E E <mark>Y</mark> Y	F R Q K	EKEQMEA		EEIE	H H K K E I H <mark>S K K E I</mark>	ERLQK
tr C1BK85 C1BK85 OSMMO	EEEMY	FRRK	EQEQLAA	LKQHHC	EEID	ннккеі	ERLQR
sp Q1LYB6 ATF1B DANRE	EEEMY	FKRK	EQEQLSA		EEID	нн <mark>кк</mark> еі	ERLQH
sp/P37209/ATIF1 CAEEL	REEEY	FRRQ					
sp A8XZB0 ATIF2 CAEBR sp O74523 ATIF SCHPO	REDE <mark>Y</mark> KEDFF	F YKK V H Q H				H H K S Q L L H R E E L	
sp F7BK26 ATIF1 XENTR	EEERY	FRQK	E I E Q L <mark>R K</mark> E Q E Q I A S	LRKHHE	EEIR	ннкдеі	ERLQK
sp P01098 STF1 YEAST	KEDYY	ARQQ	EREQLAH	<mark>VК</mark>	EQLK	ΕΗΚΚΚΙ	ENLEN
tr Q6CL59 Q6CL59 KLULA tr Q0U9C9 Q0U9C9 PHANO	Q E D F Y S E E L Y	V K Q H	E K E Q L A Q E K A K L L A		EQLK	Q Q Q <mark>K K I</mark> Q Q <mark>R Q H I</mark>	
tr Q75A18 Q75A18 ASHGO	NEDFY		EREQLAQ		EQLQ	KQQQKI	DNLEE
tr Q0C9S3 Q0C9S3 ASPTN	QENLY	I HEK	EMEKLEA	L R	KKVN	ЕQQКН	NELDQ
tr Q6FP60 Q6FP60 CANGA	AEDYF	і к Q Н	EKEQLKN		EQLK	HHKEQL	
tr Q6CCY1 Q6CCY1 YARLI			E K E K L D A		KQLN	K L K Q D 1 E Q R K H L	TADLEK DELDK
tr Q2U6F6 Q2U6F6 ASPOR tr Q86DY6 Q86DY6 SCHJA	Q E N L Y N E E E Y	FYKL			D E H E	E Q R K H L R E V K E I	
tr A0FDQ6 A0FDQ6 BOMMO	REDEY	Г Ү К К	QKEQLAN		KEIA	FHQEQI	KRHED
tr Q17D81 Q17D81 AEDAE	HEEEY	FFKK	RQEQLHK	LKEKLI		FHEESI	KHHEE
tr Q6DJL4 Q6DJL4 XENLA sp O35147 BAD RAT		F R Q K	EQEQIAS YGRELRR	LKKHHE MS		HHKAEI GSF	ERLQK K-GLP
sp[Q61337]BAD MOUSE	<mark>L</mark> W	AAQR	YGRELRR	MS		GSF	
sp Q92934 BAD HUMAN	L W	AAQR	Y G R E L R R	MS		DSF	F K K <mark>G L</mark> P
sp Q16611 BAK HUMAN	S S	T M G Q	V G R Q L A I	<mark>G</mark>	DDIN	R R)	YDSE
tr Q91WX5 Q91WX5 MOUSE Bak1 sp O08734 BAK MOUSE	N S	I L <mark>G</mark> Q	V G R Q L A L V G R Q L A L			R R) R R)	
splQ07816 B2CL1 CHICK		A S D -	VRQALRD	A G			KRA
tr Q9JK59 Q9JK59 RAT BAK	N S	V L <mark>G</mark> Q	V G R Q L A L	<mark>G</mark> - - - -	DDIN	R R)	YDTE
sp 077737 B2CL1 PIG	VI P	MAA-	VKQALRE	A G			(R R <mark>A</mark>
sp Q07817 B2CL1 HUMAN tr Q7TS62 Q7TS62 RAT		MAA- MAA-	V K Q A L R E V K Q A L R E	AG AG			YRR <mark>A</mark> YRRA
sp P53563 B2CL1 RAT		MAA-	V K Q A L R E	A G			YRRA
sp Q64373 B2CL1 MOUSE	<mark>V I P</mark>	MAA-	V K Q A L R E	A G	DEFE	L R N	YRR <mark>A</mark>
tr A2AHX9 A2AHX9 MOUSE		M A A -	VKQALRE	A G		L R)	rrr <mark>a</mark>
tr Q9QWX2 Q9QWX2 MOUSE sp P70345 B2CL2 MOUSE	<mark>VI</mark> P <mark>G</mark> P	M A A - A A D P	V K Q A L R E	AG	DEFE DEFE		r R R A
splQ1RMX3lB2CL2 BOVIN	··· GP	AADP		A G	DEFE		
sp Q45T69 B2CL2 CANFA	<mark>G P</mark>	AADP	LHQAMRA	A G	DEFE	T R F	<mark>- R R T</mark>
sp Q92843 B2CL2 HUMAN	<mark>G P</mark>	AADP	LHQAMRA	A G	DEFE	T R F	RRT
sp Q00709 BCL2 CHICK sp O02718 BCL2 BOVIN	<mark>R P</mark> <mark>S P</mark>	A P P G	V H L A L R Q V H L T L R Q	AG			YQRD
splQ6R755 BCL2 CANFA	<mark>S P</mark>	V P P V	VHLTLRR	AG	DDFS	R R)	YRRD
sp P49950 BCL2 RAT	<mark>S P</mark>	V P P V	VHLTLRR	<mark>A G</mark> - - - -	DDFS	rr <mark>`</mark>	YRR <mark>D</mark>
sp/P10417/BCL2 MOUSE	S P	V P P V V P P V		A G	D D F S D D F S	R R)	YRRD
sp Q9JJV8 BCL2 CRIGR sp P10415 BCL2 HUMAN	<mark>S P</mark> <mark>S P</mark>	V P P V	VHLTLRR VHLTLRQ	AG AG		R R N	YRRD YRRD
tr G8GLM0 G8GLM0 CAVPO		V P P V	VHLTLRQ	A G		R R	YRQD
sp O43521 B2L11 HUMAN	<mark>M</mark> R	PEIW	IAQELRR	<mark> G</mark>		AY)	Y <mark>a r r v f</mark>
sp 088498 B2L11 RAT	<mark>L</mark> R	PEIR	IAQELRR	<mark>G</mark>		E T \	Y T R R A F
sp O54918 B2L11 MOUSE sp P59017 B2L13 MOUSE	L R					E T <mark> </mark> R V S Q D L	
sp Q9BXK5 B2L13 HUMAN	NPESS	MEDC	L L L L L A H	L G	1 1 1 E	KVSQEL	
sp P48558 BXI1 YEAST Bax	<mark>F</mark> K	Y S T V	V I S C E P I	I R	QRFM	нк <mark>\</mark>	
sp 074888 BXI1 SCHPO Bax		N T T P	VAECAKS		MAFL ROIO	RK \ QE	V <mark>Y</mark> A I L T H Q E F
sp Q9Z0F3 B2L10 MOUSE sp Q9BXH1 BBC3 HUMAN	E Q	WARE		M A			YERRRQ
sp Q80ZG6 BBC3 RAT	E E	WARE	I <mark>G</mark> AQL RR	M A	DDLN	A Q N	YERRRQ
sp Q99ML1 BBC3 MOUSE	E E	WARE	IGAQLRR	M A		Α Q Ν	Y E R R R Q
sp Q13323 BIK HUMAN sp O70337 BIK MOUSE	ME VE	G S D A G R N Q	LALRLAC VALRLAC				
splQ07813 BAX MOUSE	D A	STKK		G		S N N	
tr G5BAG6 G5BAG6 HETGA	D P	<mark>сткк</mark>		<mark> G</mark> - - - -		SN N	
splQ07812 BAX HUMAN		S T K K		G		SN N	
sp 002703 BAX BOVIN tr Q8SQ43 Q8SQ43 FELCA BAX		S T K K S T K K		G G		SN 0	И E
spj000198 HRK HUMAN		AAQL	TAARLKA				
sp P97287 MCL1 MOUSE				L G		Q R 1	ME T <mark>M</mark>
	<mark>G A</mark>	A <mark>G</mark> RR	ALETLRR	<mark>∨ G</mark> - - - -	DGVQ	R N H	ME TM HETA
splQ9Z1P3 MCL1 RAT splQ07820 MCL1 HUMAN	<mark>G</mark> A	A <mark>G</mark> RR	A L E T L R R A L E T L R R	<mark>V G</mark> - - - - <mark>V G</mark> - - - -	DGVQ DGVQ	Q <mark>R</mark> 1 R N R N R N	ME TM HETA HETA
sp Q07820 MCL1 HUMAN		A <mark>G</mark> RR T S RK	A L E T L R R A L E T L R R A L E T L R R	<mark>V G</mark> V G <mark>V G</mark>	D G V Q D G V Q D G V Q	R N H	ME M HETA HETA HETA HETA
spjQ07820jMCL1 HUMAN spjQ7YRZ9jMCL1 FELCA spjQ8HYS5jMCL1 CANFA	<mark>G A</mark> <mark>G A</mark>	A GRR T S R K A S R K A S R K	A L E T L R R A L E T L Q R	<mark>V G</mark> - - - - <mark>V G</mark> - - - -	D G V Q D G V Q D G V Q D G V Q D G V Q	R N R N R N R N R N	<mark>НЕТА</mark>
sp Q07820 MCL1 HUMAN sp Q7YRZ9 MCL1 FELCA sp Q8HYS5 MCL1 CANFA tr Q1LX52 Q1LX52 DANRE BH3	<mark>G</mark> A GA RA RA	A G R R T S R K A S R K A S R K A A R E	ALETLRR ALETLRR ALETLRR ALETLRR ALETLRR ALETLQR MAAELI	V G - - - V G - - - V G - - - V G - - - V G - - - V G - - - V G - - - V G - - - V G - - -	DGVQ DGVQ DGVQ DGVQ DGVQ DGVQ	R N + R N + R N + R N + R N + Q S +	H <mark>E</mark> T <mark>A</mark> VLSQAA
spiQ07820jMCL1 HUMAN spiQ07YRZ9jMCL1 FELCA spiQ8HYS5jMCL1 CANFA triQ1LX52]Q1LX52 DANRE BH3 triQ56VD1 Q56VD1 XENLA BH3	G A G A G A R A A R E E	A G R R T S R K A S R K A S R K A A R E L C R R		V G - - - - V G - - - - - V G - - - - - - V G - - - - - - V G - - - - - - V G - - - - - - V G - - - - - - M G - - - - - -	D G V Q D C L L E D K L E	R N	<mark>НЕТА</mark>
spjQ07820jMCL1 HUMAN spjQ77R29JMCL1 FELCA spQ8HV55JMCL1 CANFA trQ1LX52Q1LX52 DANRE BH3 trQ56VD1Q56VD1 XENLA BH3 trJA0SZK2JA0SZK2 XENTR BH3 tr/V8P747/V8P747 OPHHA BID	G A G A G A R A A R E E	A GRR T S R K A S R K A S R K A A R E L C R R L C R R	A L E T L R R A L E T L Q R A L E T L Q R A A E L I R I A T Q L A E I A A Q L A E	V G - - - V G - - - V G - - - V G - - - V G - - - V G - - - V G - - - V G - - - V G - - -	D G V Q D G V Q D G V Q D G V Q D G V Q D G V Q D K L E D K L E D K L A	R N	H <mark>E</mark> TA VLSQAA <mark>KPE</mark> VV
spi2007820jMCL1 HUMAN spi2079R29JMCL1 FELCA spi28HYS5JMCL1 CANFA trj21LX52J21LX52 DANRE BH3 trj265VD1/056VD1 XENLA BH3 trj205ZK2J20SZK2 XENTR BH3 trj287747JV8P747 OPHHA BID spi28J:05MB/BID CHICK	G A G A G A R A A R E E D R E A	A GRRK T SRK A SRK A SRK A CRR L CRR L CRR U L G V L G V L G V R T	A L E T L R R A L E E L I R A A T Q L A E I A A Q L A E I A Q L A E I A Q L A E	V G - - - V G - - - - V G - - - - V G - - - - V G - - - - I A - - - - M G - - - - I G - - - - I G - - - - I G - - - -	$\begin{array}{c} \mathbf{G} \\ $	R N	HETA VLSQAA KPEVV KPEVV EHPFV AKVV
spiQ07820jMCL1 FULMAN spiQ07R20jMCL1 FELCA spiQ08HYS5jMCL1 CANFA tr[Q1LX52]Q1LX52 DANRE BH3 tr[Q56VD1]Q56VD1 XENLA BH3 tr[Q56VD1]Q56VD1 XENLA BH3 tr[Q502K2]Q052K2 XENTR BH3 tr[Q5028]QM8JB1D CHICK tr[U6D38] NEOVI BID	G A G A G A R A A R E E E E E A E A E A E A 	A G R R K A S R K A S R K A S R K A S R K A C R R L C R R U V R T U Q D		V G - - - V G - - - V G - - - V G - - - V G - - - V G - - - M G - - - M G - - - I G - - - I G - - - I G - - -	$\begin{array}{c} \mathbf{G} \\ $	R N	H E T A V L S Q A A K P E V V K P E V V E H P F V A K V V H P G L V
spiQ07820jMCL1 HUMAN spiQ7YR29jMCL1 FELCA spiQ8HY35jMCL1 CANFA HY31LX82jQ1LX82 DANRE BH3 HY365V01265V01 XENLA BH3 HY365V01265V01 XENLA BH3 HY38F247/V8P747 OPHA BID spiQ8JGM8/BID CHICK HY19E0381/U6D381 NEOVI BID spiQ4JH50BID PIG	G A G A G A R A R A E E E E E A E A	A G R R T S R K A S R K A S R K A C R R L C R R V L G - I V Q D V I R D V I R D	A L E T L R R A L E T L R R A L E T L R R A L E T L Q R A L E T L Q R A L A Q L A I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E I A A Q L A E	V G V G	G V Q D G V Q D G V Q D G V Q D G V Q D G V Q D K L E D K L A D Q L M D D M M D R M E	R R N	HETA VLSQAA KPEVV KPEVV KPEVV HPFV AK VV HPGLV RPGLV
spiQ07820jMCL1 FUMAN spiQ07R20jMCL1 FELCA spiQ8HYS5jMCL1 CANFA trjG1LX52jQ1LX52 DANRE BH3 trjG56V01265V01 XENLA BH3 trjG56V01265V01 XENLA BH3 trjQ52K2jQ05X42 XENTR BH3 trjQ52K2jQ05X42 XENTR BH3 trjU62031(JB0381 NEOVI BH3 trjQ50K6jQ05K16 BOVIN BH3 trjQ170H510710H BH3	G A A G A A G R A R R E E E E - E E E E E E E E E E E E E E E E E E - E E E E E E E E E E E E E E E E E 	A C R R K K E R R R K K K E R R R K K K E R R R A A A A C C C C C C C C C C C C C	A L E T L R R R A L E T L R R R A L E T L R R R A L E T L R R R A L E T L R R R A L E T L R R R A L E T L R R R A L A T Q L A E I A A Q L A E I A R R L A Y I A R R L A Y I A R R L A Y I A R R L A Y I A R R L A Y I A R Q L A K V A R Q L A Q V A R Q L A Q	V G V G V G	D G V Q D C L L E D K L E D K L L D C K L D D C M D D C M E D D R L E D R L E	R N	H E T A - V L S Q A K P E V V K P E V V K P E V V H P G L V H P G M V H P G M V
spiQ07820jMCL1 HUMAN spiQ07R20jMCL1 FELCA spiQ8HYS5jMCL1 CANFA triG1LX82(LX52 DANRE BH3 triG05KVD1[D65VD1 XENLA BH3 triA052K2]A052K2 XENTR BH3 triA052K2]A052K2 XENTR BH3 triA052K2]A053K1 REOVI BH3 spiQ04JHS0jBID PIG triQ05K16JD05KIB BOVIN BH3 triQ17CH5[Q17CH5 BOVIN BH3 triQ17CH5[Q17CH5 BOVIN BH3	G A A G A A R R A R A E E E A E A E A E A E A E A E E E E E E E E A E E E A E E A 	R R K K R	A L E T L R R A L E T L R	V G		R N	Image: Constraint of the second sec
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spiQ07820jMCL1 FUMAN spiQ07R20jMCL1 FELCA spiQ8HYS5jMCL1 CANFA triQ1LX52[01.1X52 DANRE BH3 triQ58VD1[065VD1 XENLA BH3 triV8P747]V8P747 OPHHA BID spiQ8JGM8[BID CHICK triV6D381 (V6D381 NEOV) BID spiQ4JHS0[BID PHG triQ05Ki6]BOVIN BH3 triQ17CH5[Q17OH5 BOVIN BH3 triQ17CH5[Q17OH5 BOVIN BH3 triQ317CH5[D17OH5 BOVIN BH3 triQ317CH5[D17OH5 BOVIN BH3 triQ317CH5[D17OH5 BOVIN BH3 triQ317CH5[D17OH5 BOVIN BH3 triQ317CH5[D17OH5 BOVIN BH3		R R K K E R		V G - - V G - - - V G - - - V G - - - V G - - - M G - - - M G - - - I G - - - I G - - - I G - - - I G - - - I G - - - I G - - - I G - - - I G - - - I G - - - I G - - - I G - - - I A G - -		R R N	H E A - - Y L A V - V L P E V V K P P F V V K P P C V V R P P G M V H P P G M V H P P G M V H P P G M V V H P P G M V V V Q P T L V
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spiQ07820jMCL1 FULMAN spiQ07R20jMCL1 FELCA spiQ08HYS5jMCL1 CANFA tr[GLXS2]Q1LXS2 DANRE BH3 tr[GSVD1]Q56VD1 XENLA BH3 tr[GSVD1]Q56VD1 XENLA BH3 tr[GSVD1]Q56VD1 XENLA BH3 tr[GSVD1]Q5747 QFHHA BID spiQ03GM8JBID CHICK tr[GDS31]Q6D381 NEOVI BID spiQ04JHS0JBID PIG tr[GDS41G058KB BCVIN BH3 tr[G17CH5[G17CH5 BCVIN BH3 spiP70444]BID MOUSE spiQ04JLGBID RAT tr[AS030]AASAIBAASI tr[E2IV36]E2IV38 FELCA BH3 tr[E2IV36]E2IV38 FELCA BH3 tr[G2F70EJ2776 ILUMAN DID		KKKERR. KOR GSSSAC COR GL KOR GSSSAC COR GR KOR GR KOR <		V G - - V G - - - V G - - - - V G - - - - - V G - - - - - - V G - - - - - - - M G -		R R R R R R R R R R R R R R R R R R R	H E A - Y L S A - Y L S A V K P E V V K P E V V K P E V V H P G L V H P G M V H P G M V H P G M V H P G M V Q P T L V Q P T L V H P R L V H P R L V
spiQ07820jMCL1 FUMAN spiQ07R20jMCL1 FELCA spiQ08HYS5jMCL1 CANFA triQ1LX520LX52DANRE BH3 triQ56VD1[056VD1 XENLA BH3 triQ52X20502X2 XENTR BH3 triV8P747/V8P747 OPHHA BID spiQaJGMBBID CHICK triU8D381[U6D381 NEOVI BID spiQ4JHS0]BID CHICK triQ05K6[Q05KKB EOVIN BH3 triQ17CH5[Q170H5 BOVIN BH3 triQ17CH5[Q170H5 BOVIN BH3 triQ17CH5[Q170H5 BOVIN BH3 triQ17CH5[Q170H5 BOVIN BH3 triQ17CH5[Q170H5 BOVIN BH3 triQ17CH5[Q170H5 BOVIN BH3 triQ2572]V85EEV85 EHCA BH3 triQ2572[V85]E2V85 EHCA BH3 triQ2572[V86 EHCA BH3 triQ2572]V86 EHCA BH3		A G R K		V G - - V G - - - V G - - - - V G - - - - - V G - - - - - - V G - - - - - - - M G -		R R R R R R R R R R R R R R R R R R R	H I A A Y L S A Y L S C K P E V K P E V K P E V K P F V K P F V K P F V K P F C K H P G N Q P T L V Q P T L V Q P P R L Q P P R L P P R L V
spiQ07820jMCL1 HUMAN spiQ07R20jMCL1 FELCA spiQ8HYS5jMCL1 CANFA triG1LX52[011X52 DANRE BH3 triG56VD1[Q56VD1 XENLA BH3 triG56VD1[Q56VD1 XENLA BH3 triG56VD1[Q56VD1 XENLA BH3 triG56VD1[Q567A] CH1K4 triG5781[G5731 REOVI BH3 triG17CH5[G578] REOVI BH3 triG17CH5[G17CH5 BOVIN BH3 triG17CH5[G17CH5 triG17CH5[G17CH5 triG17CH5 triG17CH5[G17CH5 triG17CH5 t		A A C		V G - - V G - - - V G - - - - V G - - - - - V G - - - - - - V G - - - - - - - M G -		R N	H E A - Y K C A Y C P C Y C P P Y C P P Y C P P Y C P P Y C P P Y C P P Y C P P Y C P P Y C P P Y C P P Y C P P Q P P P Q P P P P P P P
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spiQ07820jMCL1 FUMAN spiQ07R20jMCL1 FELCA spiQ8HYS5jMCL1 CANFA triQ1LX520LX52DANRE BH3 triQ56VD1[056VD1 XENLA BH3 triQ52X2(056VD1 XENLA BH3 triQ52X2(056XC1 XENLA BH3 triQ52A7(056XC1 XENLA BH3 triQ547)405747/08P747 OPHHA BID spiQ4JH60JBID CHCK triU605K6(605K16 BOVIN BH3 triQ17QH5(Q17QH5 BOVIN BH3 triQ17QH5(Q17QH5 BOVIN BH3 triQ17QH5(Q17QH5 BOVIN BH3 triQ17QH5(Q17QH5 BOVIN BH3 triQ17QH5(Q17QH5 BOVIN BH3 triQ17QH5(Q17QH5 BOVIN BH3 triQ12V5)E2/N85 LECA BH3 triQ12V5(E2)N85 LECA BH3 triQ2V5(E2)N86 LECA BH3 triQ2V70[D22P70]D22P70 HUMAN BH3 triQ2V84[E2)N84 ELCA BH3 triQ2V84[E2)N84 PHMAN BH3 triQ2V84[E2)N84 PMAN BH3 triQ2V84[E2)N84 PMAN BH3 triQ2V84[E2]N84 PMAN BH3 triQ2V84[E2]N84 PMAN BH3 triQ2V84[E2]N84 PMAN BH3 triQ2V84[E2]N84 PMAN BH3 triQ2V84[E2]N84 PMAN BH3 triQ2V84[E2]N84 PMAN BH3 triQ2V84[E2]N84 PMAN BH3 triQ2V84[E2]N84 PMAN BH3 triQ2V84 FMAN BH3 triQ2V84 FMAN BH3 triQ2V84 FMAN BH3 TriQ2V84		A T A A A L D </td <td></td> <td>V G - - V G - - - V G - - - - V G - - - - - V G - - - - - - V G - - - - - - - M G -</td> <td></td> <td>R N</td> <td>H I A - I I A - I S Q V V I S Q V V I S Q V V I I P P I V I I P P Q I V I I P P Q I V V I I P P Q I V V I II P P Q I V V I II P P Q I V V I II P P Q I V V V I II P P Q V V V V V V V V V V V V V</td>		V G - - V G - - - V G - - - - V G - - - - - V G - - - - - - V G - - - - - - - M G -		R N	H I A - I I A - I S Q V V I S Q V V I S Q V V I I P P I V I I P P Q I V I I P P Q I V V I I P P Q I V V I II P P Q I V V I II P P Q I V V I II P P Q I V V V I II P P Q V V V V V V V V V V V V V
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spiQ07820jMCL1 FULMAN spiQ07R20jMCL1 FELCA spiQ08HYS5jMCL1 CANFA triQ1LX52[01.1X52 DANRE BH3 triQ56VD1[056VD1 XENLA BH3 triQ582VD1056VD1 XENLA BH3 triQ582V210852V2 XENTR BH3 triQ582V210852V2 XENTR BH3 triQ170H5[017QH5 BOVIN BH3 spiQ4JH50]BID PIG triQ05KIB[005KIB BOVIN BH3 triQ17QH5[017QH5 BOVIN BH3 triQ17QH5[017QH5 BOVIN BH3 triQ17QH5[017QH5 BOVIN BH3 triQ21V35]E21V85 FELCA BH3 tri221V35]E21V85 FELCA BH3 tri221V35]E21V85 FELCA BH3 tri221V35]E21V85 FELCA BH3 tri221V35]E21V85 FELCA BH3 tri221V36]E21V85 FELCA BH3 tri221V36]E21V85 FELCA BH3 tri221V37]E21V84 FELCA BH3 tri221V37]E310 HUMAN H3 spiQ4A13]BECN1 FONAB		A M A A M B A M A <td< td=""><td></td><td>Y G Y Y Y Y</td><td></td><td>RN RN R R R R R R R R R R R R R R R R R</td><td>Image: Constraint of the second sec</td></td<>		Y G Y Y Y Y		RN RN R R R R R R R R R R R R R R R R R	Image: Constraint of the second sec
spiQ07820jMCL1 FULMAN spiQ07R20jMCL1 FELCA spiQ8HYS5jMCL1 CANFA triQ1LX82(1LX82 DANRE BH3 triQ058VD1[Q56VD1 XENLA BH3 triA052K2]A05K2K2 XENTR BH3 triA052K2]A05K2K2 XENTR BH3 triA052K2]A05K16 E0V1 BH0 spiQ4JHS0jBID PIG triQ05K16J05K16 E0V1 BH3 triQ17CH5[Q17CH5 BOVIN BH3 tri21V85]E2IV85 FELCA BH3 tri21V85[E2IV85 FELCA BH3 tri21V87[E2IV86 IEMCA BH3 tri22V87[E2IV86 IEMCA BH3 tri22V87[E2IV86 IEMCA BH3 tri22V87[E2IV86 JEMCA BH3 tr		A T A A I V I <td></td> <td>Y G Y Y Y Y</td> <td></td> <td>RN RN R R R R R R R R R R R R R R R R R</td> <td>H A - H A - H A - H A - H A - H A - H A - H A - H A - H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P</td>		Y G Y Y Y Y		RN RN R R R R R R R R R R R R R R R R R	H A - H A - H A - H A - H A - H A - H A - H A - H A - H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P H P P
spiQ07820jMCL1 FULMAN spiQ07R20jMCL1 FELCA spiQ08HYS5jMCL1 CANFA triQ1LX52[01.1X52 DANRE BH3 triQ56VD1[056VD1 XENLA BH3 triQ52X2[005KV] AENLA BH3 triQ52X2[005KV] AENLA BH3 triQ52X2[005KV] BD0 spiQa1409BID PCIG triQ05XKIB[005KV] BOVIN BH3 triQ17QH5[017QH5 BOVIN BH3 triQ17QH5[017QH5 BOVIN BH3 triQ17QH5[017QH5 BOVIN BH3 triQ17QH5[017QH5 BOVIN BH3 triQ17QH5[017QH5 BOVIN BH3 triQ2V35]E21V85 FELCA BH3 triQ2V35]E21V85 triQ2V35]E21V85 FELCA BH3 triQ2V35]E21V85 FELCA BH3 triQ2V35]E21V85 FELCA BH3 triQ2V35 triQ2V35 FELCA BH3 triQ2V35 tri		A H A A L D D L D D L D D L D <td></td> <td>Y G Y Y Y Y</td> <td></td> <td>RN RN R R R R R R R R R R R R R R R R R</td> <td>Image: Constraint of the second sec</td>		Y G Y Y Y Y		RN RN R R R R R R R R R R R R R R R R R	Image: Constraint of the second sec
spiQ07820jMCL1 FULMAN spiQ07R20jMCL1 FELCA spiQ08HYS5jMCL1 CANFA triQ1LX52[01.1X52 DANRE BH3 triQ56VD1[056VD1 XENLA BH3 triQ52X2[005KV] AENLA BH3 triQ52X2[005KV] AENLA BH3 triQ52X2[005KV] BD2 spiQ3LGMBJD CHCK triU6D331[J6D34] NEOVI BH3 triQ17QH5[Q17QH5 BOVIN BH3 triQ17QH5[Q17QH5 BOVIN BH3 triQ17QH5[Q17QH5 BOVIN BH3 triQ17QH5[Q17QH5 BOVIN BH3 triQ17QH5[Q17QH5 BOVIN BH3 triQ2V35]E21V85 FELCA BH3 triQ2V35]E21V85 FELCA BH3 triQ2V35]E21V85 FELCA BH3 triQ2V35]E21V85 FELCA BH3 triQ2V35]E21V85 FELCA BH3 triQ2V36]E21V85 FELCA BH3 triQ2V37 triQ2V36]E21V85 FELCA BH3 triQ2V37 triQ2V36]E21V85 FELCA BH3 triQ2V37 triQ2V36 FELCA BH3 triQ2V36 FELCA BH3 triQ2V37 FELCA BH3 triQ2V36 FELCA BH3 triQ2V37 FELCA BH3 triQ2		A H A A L D H T A A L D H T A A L D H T A A L D H T A A L D H A A L D I <td></td> <td>V G V G V G V G V G V G V G V G V G V G V G V G V G I G I G I G I G I G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G</td> <td></td> <td>R N N N N N N N N N N N N N N N N N N N</td> <td>H A A H A A Q V Y H A Q V X Q V X P P P Q V X P P P P Q Q P P P P Q Q P P P P Q Q P P P P Q Q P P P P Q Q P P P P Q Q P P P P P P P P P P Y Y Y Y Y Y</td>		V G V G V G V G V G V G V G V G V G V G V G V G V G I G I G I G I G I G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G		R N N N N N N N N N N N N N N N N N N N	H A A H A A Q V Y H A Q V X Q V X P P P Q V X P P P P Q Q P P P P Q Q P P P P Q Q P P P P Q Q P P P P Q Q P P P P Q Q P P P P P P P P P P Y Y Y Y Y Y

Figure 8 - figure supplement 1. Multiple sequence alignment of BH3 motifs of Bcl-2 proteins and IF1. The alignment was obtained with Clustal Omega. Details of the 107 sequences corresponding to fragments of the IF1 (28 correspond to IF1 sequences) and Bcl-2 family proteins are provided in Figure 8-Table supplement 1.

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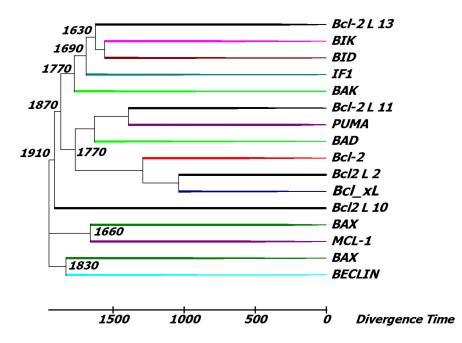


Figure 8-figure supplement 2. Phylogenetic tree of IF1 and BH3 motif containing proteins.

The tree was calculated with the Neighbor-Joining statistical method (Saitou & Nei, 1987) from the alignment performed by Clustal Omega (Figure 8-figure supplement 1). The calibration of the divergence times (in millions of years, Myr) was performed as described in Methods and to calculate the branching times the method RelTime (Tamura et al, 2012) was applied. Only positions with more than 95% site coverage were considered for the analysis, which in this case corresponds to 14 amino acids. The tree construction was performed with MEGA 6.0 (Tamura et al, 2013)

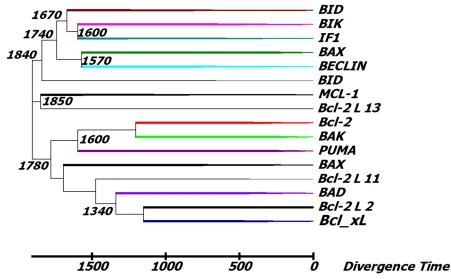


Figure 8-figure supplement 3. Phylogenetic tree of IF1 and Bcl-2 protein BH3 motif containing fragments. The tree was calculated with the Neighbor-Joining statistical method from the alignment performed by Bali-Phy with a LG substitution model (Le & Gascuel, 2008) and the Insertion/deletion model RS07 (Redelings & Suchard, 2007). The calibration of the divergence times was performed as described in methods and to calculate the branching times the method RelTime was applied. Only positions with more than 95% site coverage were considered for the analysis, which in this case corresponds to 9 residues. The tree construction was performed with MEGA 6.0. The bootstrap consensus tree obtained in Mega 6.0 was very similar to that calculated by the joint estimation of Bali-phy.

Figure 8-Table 1

Key for the input sequences used in the phylogenetic analysis

5	sp Q1LYB6 ATF1B	DANRE	ATIF1	Danio rerio
5	sp A3KNL5 ATF1A	DANRE	ATIF1	Danio rerio
5	sp P01096 ATIF1	BOVIN	ATIF1	Bos Taurus
5	sp P37209 ATIF1	CAEEL	ATIF1	Caenorhabditis elegans
5	sp Q9UII2 ATIF1	HUMAN	ATIF1	Homo sapiens
5	sp Q5RFJ9 ATIF1	PONAB	ATIF1	Pongo abelii
5	sp A8XZB0 ATIF2	CAEBR	ATIF1	Caenorhabditis briggsae
5	sp O74523 ATIF	SCHPO	ATIF1	Schizosaccharomyces pombe
5	sp Q03344 ATIF1	RAT	ATIF1	Rattus norvegicus
5	sp F7BK26 ATIF1	XENTR	ATIF1	Xenopus tropicalis
5	sp Q29307 ATIF1	PIG	ATIF1	Sus scrofa
5	sp O35143 ATIF1	MOUSE	ATIF1	Mus musculus
5	sp P01098 STF1	YEAST	Putative ATPase inhibitor	Saccharomyces cerevisiae
1	tr Q6CL59 Q6CL59	KLULA	Putative ATPase inhibitor	Kluyveromyces lactis
1	tr Q0U9C9 Q0U9C9	PHANO	Putative ATPase inhibitor	Phaeosphaeria nodorum
1	tr Q75A18 Q75A18	ASHGO	Putative ATPase inhibitor	Ashbya gossypii
1	tr Q0C9S3 Q0C9S3	ASPTN	Putative ATPase inhibitor	Aspergillus terreus
1	tr Q6FP60 Q6FP60	CANGA	Putative ATPase inhibitor	Candida glabrata
1	tr Q6CCY1 Q6CCY1	YARLI	Putative ATPase inhibitor	Yarrowia lipolytica
1	tr Q2U6F6 Q2U6F6	ASPOR	Putative ATPase inhibitor	Aspergillus oryzae
1	tr Q86DY6 Q86DY6	SCHJA	Putative ATPase inhibitor	Schistosoma japonicum
1	tr A0FDQ6 A0FDQ6	BOMMO	ATIF1	Bombyx mori
1	tr Q17D81 Q17D81	AEDAE	Putative ATPase inhibitor	Aedes aegypti
1	tr B5X591 B5X591	SALSA	ATIF1	Salmo salar
1	tr C1BWJ3 C1BWJ3	ESOLU	ATIF1	Esox Lucius
1	tr C3KHS5 C3KHS5	ANOFI	ATIF1	Anoplopoma fimbria
1	tr C1BK85 C1BK85	OSMMO	ATIF1	Osmerus mordax
1	tr Q6DJL4 Q6DJL4	XENLA	atpif1	Xenopus laevis
5	sp Q16611 BAK	HUMAN	BAK1	Homo sapiens
1	tr Q91WX5 Q91WX5	MOUSE	Bak1	Mus musculus
5	sp O08734 BAK	MOUSE	Bak1	Mus musculus
5	sp Q07816 B2CL1	CHICK	BCL2L1	Gallus gallus
1	tr Q9JK59 Q9JK59	RAT	Bak1	Rattus norvegicus
5	sp 077737 B2CL1	PIG	BCL2L1	Sus scrofa
5	sp Q07817 B2CL1	HUMAN	BCL2L1	Homo sapiens
1	tr Q7TS62 Q7TS62	RAT	Bcl211	Rattus norvegicus
5	sp P53563 B2CL1	RAT	Bcl211	Rattus norvegicus
5	sp Q64373 B2CL1	MOUSE	Bcl211	Mus musculus
1	tr A2AHX9 A2AHX9	MOUSE	Bcl211	Mus musculus
1	tr Q9QWX2 Q9QWX2	MOUSE	Bcl211	Mus musculus
5	sp P70345 B2CL2	MOUSE	Bcl2l2	Mus musculus

sp Q1RMX3 B2CL2	BOVIN	BCL2L2	Bos Taurus
sp Q45T69 B2CL2	CANFA	BCL2L2	Canis familiaris
sp Q92843 B2CL2	HUMAN	BCL2L2	Homo sapiens
sp Q00709 BCL2	CHICK	BCL2	Gallus gallus
sp O02718 BCL2	BOVIN	BCL2	Bos Taurus
sp Q6R755 BCL2	CANFA	BCL2	Canis familiaris
sp P49950 BCL2	RAT	Bcl2	Rattus norvegicus
sp P10417 BCL2	MOUSE	Bcl2	Mus musculus
sp Q9JJV8 BCL2	CRIGR	BCL2	Cricetulus griseus
sp P10415 BCL2	HUMAN	BCL2	Homo sapiens
tr G8GLM0 G8GLM0	CAVPO	BCL2	Cavia porcellus
sp O43521 B2L11	HUMAN	BCL2L11	Homo sapiens
sp O88498 B2L11	RAT	Bcl2l11	Rattus norvegicus
sp O54918 B2L11	MOUSE	Bcl2l11	Mus musculus
sp P59017 B2L13	MOUSE	Bcl2l13	Mus musculus
sp Q9BXK5 B2L13	HUMAN	BCL2L13	Homo sapiens
sp Q92934 BAD	HUMAN	BAD	Homo sapiens
sp Q61337 BAD	MOUSE	Bad	Mus musculus
sp O35147 BAD	RAT	Bad	Rattus norvegicus
sp P48558 BXI1	YEAST	Bax	Saccharomyces cerevisiae
sp O74888 BXI1	SCHPO	Bax	Schizosaccharomyces pombe
sp Q9Z0F3 B2L10	MOUSE	Bcl2110	Mus musculus
sp Q9BXH1 BBC3	HUMAN	BBC3	Homo sapiens
sp Q80ZG6 BBC3	RAT	Bbc3	Rattus norvegicus
sp Q99ML1 BBC3	MOUSE	Bbc3	Mus musculus
sp Q13323 BIK	HUMAN	BIK	Homo sapiens
sp O70337 BIK	MOUSE	Bik	Mus musculus
sp Q07813 BAX	MOUSE	BAX	Mus musculus
tr G5BAG6 G5BAG6	HETGA	BAX	Heterocephalus glaber
sp Q07812 BAX	HUMAN	BAX	Homo sapiens
sp O02703 BAX	BOVIN	BAX	Bos Taurus
tr Q8SQ43 Q8SQ43	FELCA	BAX	Felis catus
sp O00198 HRK	HUMAN	HRK	Homo sapiens
sp P97287 MCL1	MOUSE	Mcl1	Mus musculus
sp Q9Z1P3 MCL1	RAT	Mcl1	Rattus norvegicus
sp Q07820 MCL1	HUMAN	MCL1	Homo sapiens
sp Q7YRZ9 MCL1	FELCA	MCL1	Felis catus
sp Q8HYS5 MCL1	CANFA	MCL1	Canis familiaris
tr Q1LX52 Q1LX52	DANRE	BID	Danio rerio
tr Q56VD1 Q56VD1	XENLA	BID	Xenopus laevis
tr A0SZK2 A0SZK2	XENTR	BID	Xenopus tropicalis
tr V8P747 V8P747	OPHHA	BID	Ophiophagus hannah
sp Q8JGM8 BID	CHICK	BID	Gallus gallus
tr U6D381 U6D381	NEOVI	BID	Neovison vison
sp Q4JHS0 BID	PIG	BID	Sus scrofa

tr Q05KI6 Q05KI6	BOVIN	BID	Bos Taurus
tr Q17QH5 Q17QH5	BOVIN	BID	Bos Taurus
sp P70444 BID	MOUSE	BID	Mus musculus
sp Q9JLT6 BID	RAT	BID	Rattus norvegicus
tr A8ASI9 A8ASI9	RAT	BID	Rattus norvegicus
tr E2IV85 E2IV85	FELCA	BID	Felis catus
tr E2IV86 E2IV86	LEMCA	BID	Lemur catta
tr B2ZP78 B2ZP78	HUMAN	BID	Homo sapiens
sp P55957 BID	HUMAN	BID	Homo sapiens
tr A8ASI8 A8ASI8	HUMAN	BID	Homo sapiens
tr E2IV87 E2IV87	9PRIM	BID	Saimiri boliviensis
tr E2IV84 E2IV84	AOTVO	BID	Aotus vociferans
sp Q6GP52 BECN1	XENLA	Beclin-1	Xenopus laevis
sp Q4A1L3 BECN1	XENTR	Beclin-1	Xenopus tropicalis
sp Q5ZKS6 BECN1	CHICK	Beclin-1	Gallus gallus
sp Q14457 BECN1	HUMAN	Beclin-1	Homo sapiens
sp Q5R878 BECN1	PONAB	Beclin-1	Pongo abelii
sp Q91XJ1 BECN1	RAT	Beclin-1	Rattus norvegicus
sp O88597 BECN1	MOUSE	Beclin-1	Mus musculus
sp Q4A1L5 BECN1	PIG	Beclin-1	Sus scrofa
sp Q4A1L4 BECN1	BOVIN	Beclin-1	Bos Taurus

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