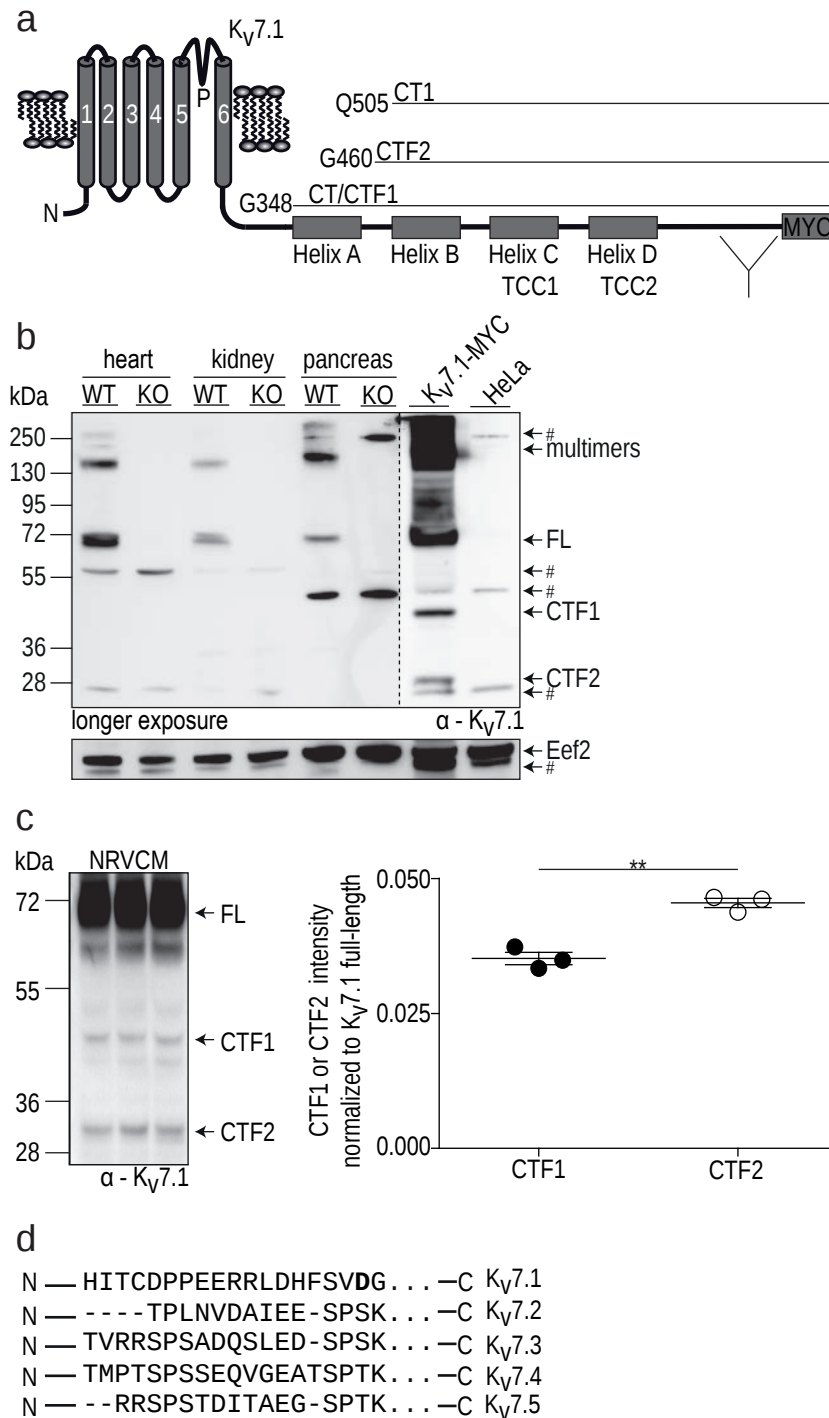


Supplementary Information for

Doxorubicin induces caspase-mediated proteolysis of K_v7.1

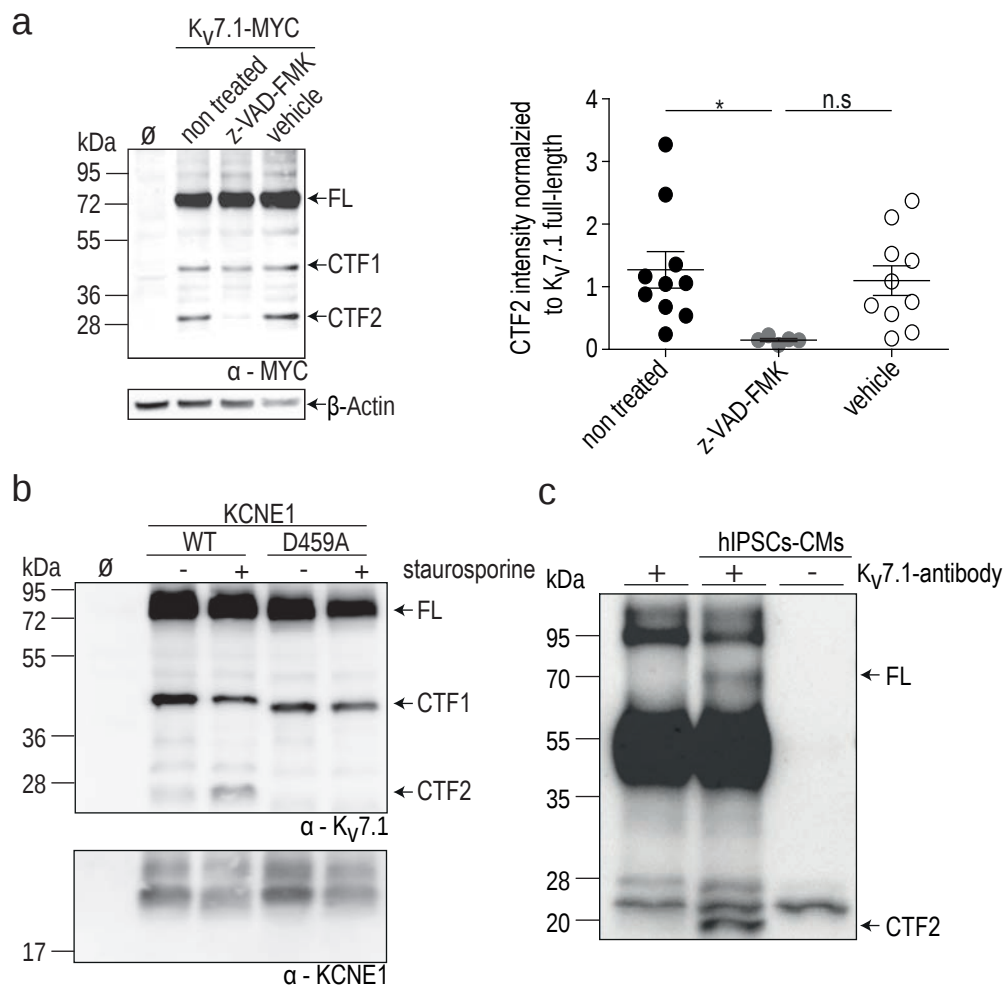
Anne Strigli, Christian Raab, Sabine Hessler, Tobias Huth, Adam J. T. Schuldt,
Christian Alzheimer, Thomas Friedrich, Paul W. Burridge,
Mark Luedde and Michael Schwake



Supplementary Figure 1: **a**, Schematic illustration of the topology of Kv7.1. Voltage Sensing Domain (S1 – S4) and Pore Domain (S5 – S6) are shown within the membrane. The C-terminus contains four helices. The third (helix C) and fourth (helix D) helices are also known as tetramerization coiled-coil 1 and 2 for their high probability to form amphipathic helical structures. Wye indicates binding site of the C-terminal anti-Kv7.1 antibody to the last eleven amino acids. Assumed starting points of CT- and CT1-construct as well as CTF1 and CTF2 are highlighted. **b**, Western blot analysis of indicated mouse organs. Untransfected (Ø) and HeLa cells overexpressing Kv7.1 as well as Kv7.1 deficient tissue lysates served as negative controls. # indicates nonspecific binding of the antibody. **c**, Lysates of NRVM were analyzed by immunoblotting. Densitometric analysis of 3 independent experiments of the CTF1 or CTF2 band intensity normalized to the Kv7.1 full-length band intensity. Statistics were tested with two-tailed unpaired student's t-test. **d**, Alignment of Kv7.1 – Kv7.5. The D459 in Kv7.1 is highlighted in bold. **(b,c)** anti-Kv7.1 antibody, anti-Eef2 antibody. All dot blots are shown as mean and error bars as SEM.

Species	Amino acid sequence																					
Homo sapiens	H	I	T	C	D	P	-	P	-	-	E	E	R	R	L	D	H	F	S	V	D	G
Chelonia mydas	Q	I	T	Y	D	H	V	M	-	-	D	D	R	K	S	D	-	F	N	I	D	A
Rattus norvegicus	H	I	T	C	D	P	-	P	-	-	E	D	R	R	P	D	H	F	S	I	D	G
Heterocephalus glaber	H	I	T	C	D	P	-	P	-	-	E	E	R	R	P	D	H	F	S	V	D	G
Canis familiaris	H	I	T	C	E	P	V	S	-	-	E	E	R	R	P	D	H	F	S	V	D	N
Felis catus	H	I	T	C	E	P	V	S	-	-	E	K	R	R	P	D	H	F	S	V	D	T
Squalus acanthias	N	I	T	Y	D	H	V	V	D	K	D	D	R	K	F	E	N	S	N	I	D	G
Loxodonta africana	H	I	T	Y	D	P	V	G	-	-	D	D	R	R	A	D	R	L	S	L	D	G
Anolis carolinensis	Q	I	T	F	D	H	A	V	-	-	D	E	R	K	S	D	N	F	H	L	D	P
Chrysemys picta bellii	H	I	T	Y	D	H	V	M	-	-	D	D	R	K	S	D	-	F	N	I	D	A
Pelodiscus sinensis	H	I	T	Y	D	H	V	V	-	-	D	D	R	K	S	D	-	F	S	I	D	A
Ovis aries	H	I	T	C	E	P	V	L	-	-	E	D	R	R	P	E	P	I	S	V	D	G
Mus musculus	H	I	T	Y	D	P	-	P	-	-	E	D	R	R	P	D	H	F	S	I	D	G
Sorex araneus	H	I	T	C	E	P	P	A	-	-	E	G	R	R	P	V	H	F	S	V	D	G
Dasyus novemcinctus	H	I	T	C	E	P	A	P	-	-	E	E	R	R	P	D	H	A	S	L	D	G
Meleagris gallopavo	H	I	T	Y	D	H	V	T	-	-	D	D	K	K	P	D	-	F	C	F	D	T
Panthalops hodgsonii	H	I	T	C	E	P	V	L	-	-	X	D	R	R	P	E	P	V	S	V	D	G
Vicugna pacos	H	I	T	C	D	L	A	S	-	-	E	E	R	R	P	D	H	F	S	V	D	G
Bos taurus	H	I	T	C	D	P	V	A	-	-	E	D	R	R	P	E	P	F	S	V	D	G
Pongo abelii	H	I	T	C	D	P	-	P	-	-	E	E	R	R	L	D	H	F	S	V	D	G
Tursiops truncatus	H	I	T	C	D	P	T	S	-	-	E	E	R	R	P	E	H	F	S	V	D	G
Trichechus manatus	H	I	T	C	D	P	A	G	-	-	E	D	R	R	A	D	G	L	S	L	D	G
Papio anubis	H	I	T	C	D	P	-	P	-	-	E	E	R	R	L	D	H	F	S	V	D	G
Camelus ferus	H	I	T	C	D	L	A	A	-	-	E	E	R	R	P	D	H	F	S	V	D	G
Gallus gallus	H	I	T	Y	D	H	V	T	-	-	D	D	K	K	P	D	-	F	C	F	D	T
Sarcophilus harrisii	H	I	T	Y	D	H	I	A	-	-	E	D	R	K	S	D	Q	F	A	M	D	G
Echinops telfairi	Q	I	T	C	D	A	A	C	-	-	E	E	R	R	P	D	H	S	A	L	D	G
Ochotona princeps	H	I	T	C	E	P	-	P	-	-	E	E	R	R	P	D	H	F	S	T	D	S
Alligator mississippiensis	H	I	T	Y	D	H	V	M	-	-	D	D	R	K	S	D	-	F	S	I	D	A
Latimeria chalumnae	H	I	T	Y	D	H	V	M	-	-	N	D	P	K	S	E	G	L	S	M	D	G
Cricetulus griseus	H	I	T	Y	D	P	-	P	-	-	E	D	R	R	P	D	H	F	S	I	D	G
Macaca mulatta	H	I	T	C	D	P	-	P	-	-	E	E	R	R	L	D	H	F	S	V	D	G
Mustela putorius furo	H	I	T	C	E	P	V	S	-	-	E	D	R	K	P	D	H	F	S	V	D	S
Pteropus alecto	H	I	T	C	D	H	V	P	-	-	D	E	Q	R	T	D	P	F	S	V	D	C
Chinchilla lanigera	H	I	T	C	D	P	-	P	-	-	E	E	R	R	P	D	H	F	S	V	D	G

Supplementary Figure 2: Alignment of Kv7.1 from 35 different species. The D459 is highlighted in red.



Supplementary Figure 3: a, Western blot analysis of Cos7 cells expressing Kv7.1-MYC treated with 50 – 100 μ mol per L z-VAD-FMK for 12 hours. Untransfected ($\emptyset$), non-treated and vehicle-treated cells served as negative controls. Densitometric analysis of 5 – 10 independent experiments of CTF2 band intensity normalized to Kv7.1 full-length band intensity. Statistics were tested with One-Way Anova followed by Bonferroni's Multiple Comparison Test. **b**, Western blot analysis of cells used for patch-clamp recordings. **c**, Immunoprecipitation study analyzed by western blots of human induced pluripotent stem cell derived cardiomyocytes with a Kv7.1 antibody. Lysate without antibody and antibody control served as negative controls. **(a)** anti-myc antibody, anti- β -actin antibody. **(b)** anti-KCNE1 antibody. **(b,c)** anti-Kv7.1 antibody. All dot blots are shown as mean and error bars as SEM.

Supplementary Information - Uncropped Blots

Figure 1a - α -K_v7.1

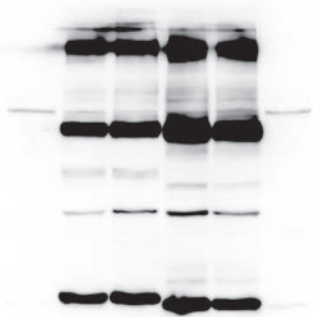


Figure 1b - α -MYC

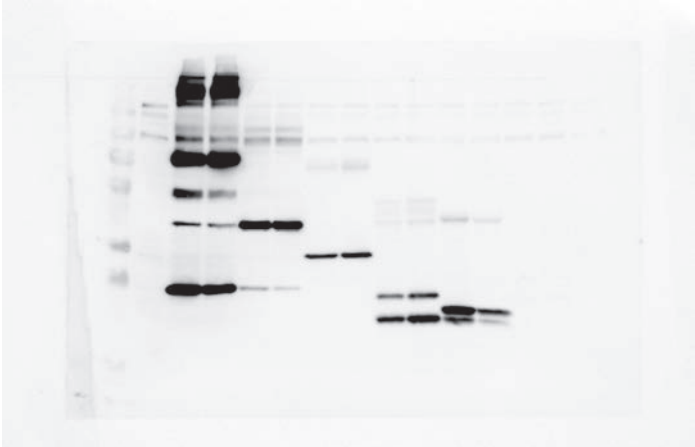


Figure 1c - α -MYC

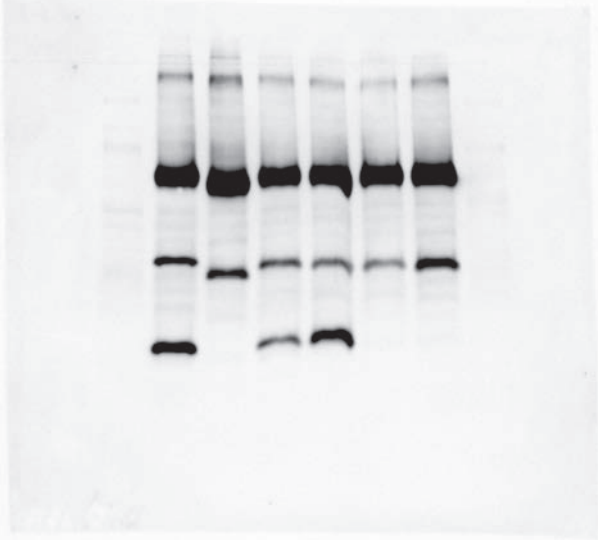


Figure 1d - α -MYC

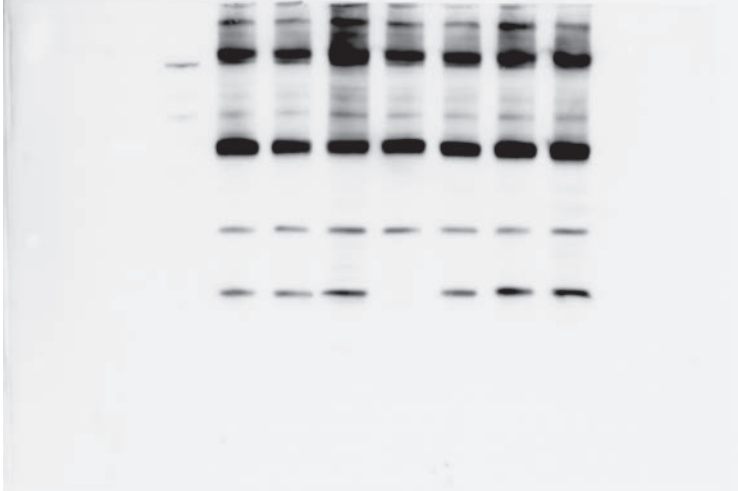


Figure 1d - α -actin



Figure 2a - α - MYC

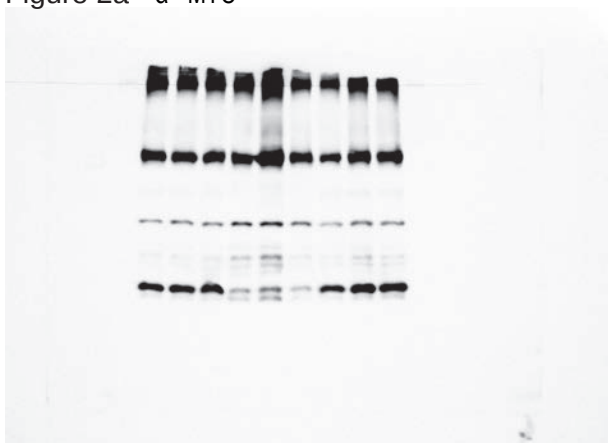


Figure 2b - α - MYC

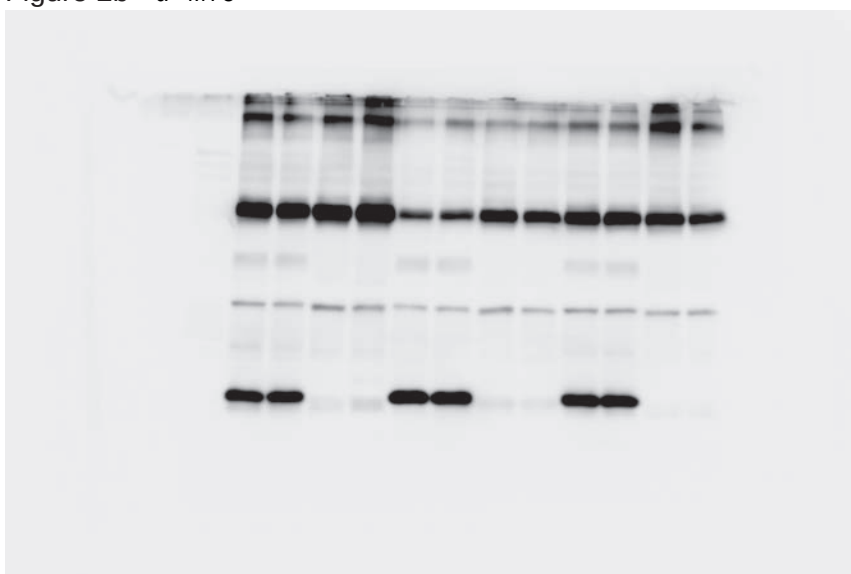


Figure 2c - α - MYC

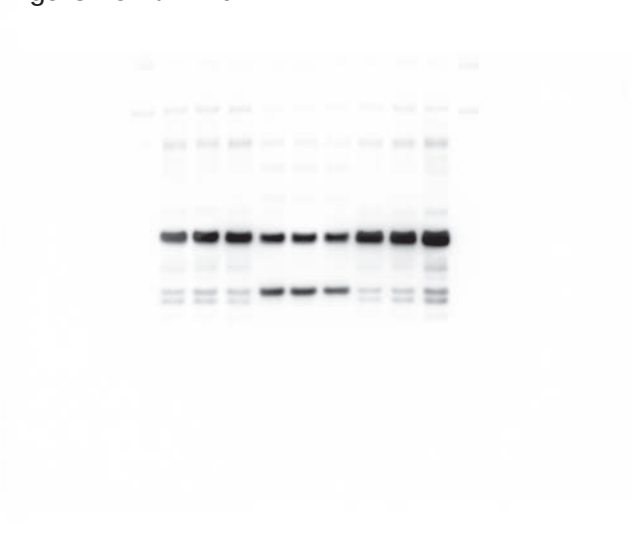


Figure 2c - α - actin



Figure 3a - α - MYC

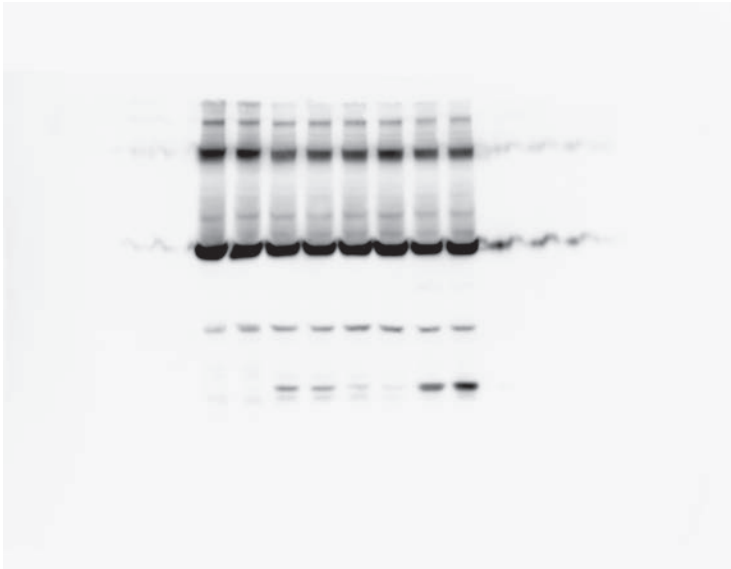


Figure 3a - α - caspase-3

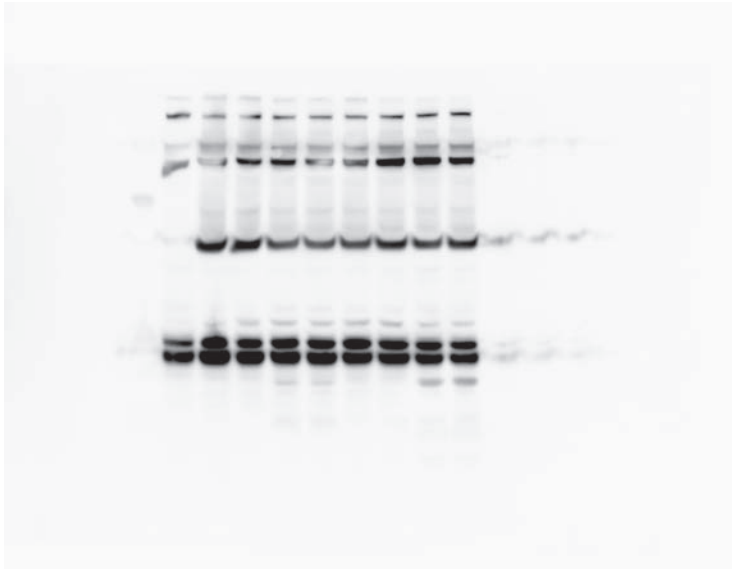


Figure 3a - α - actin



Figure 3b - α - MYC

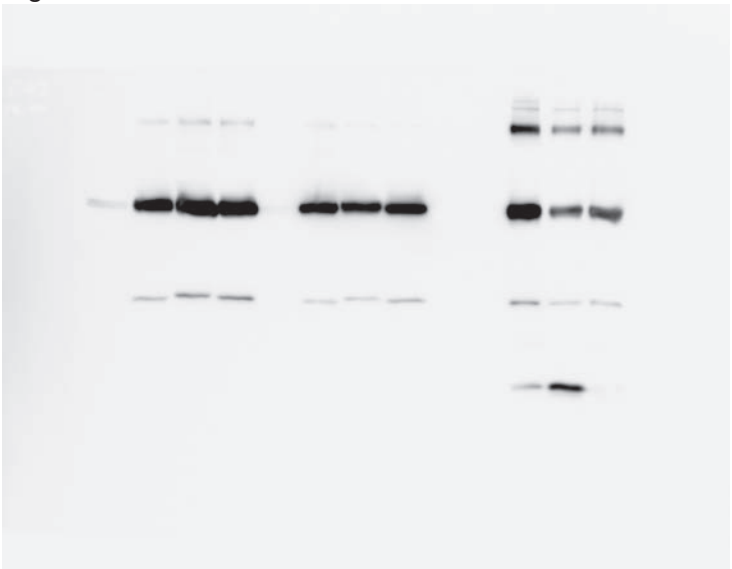


Figure 3b - α - caspase-3

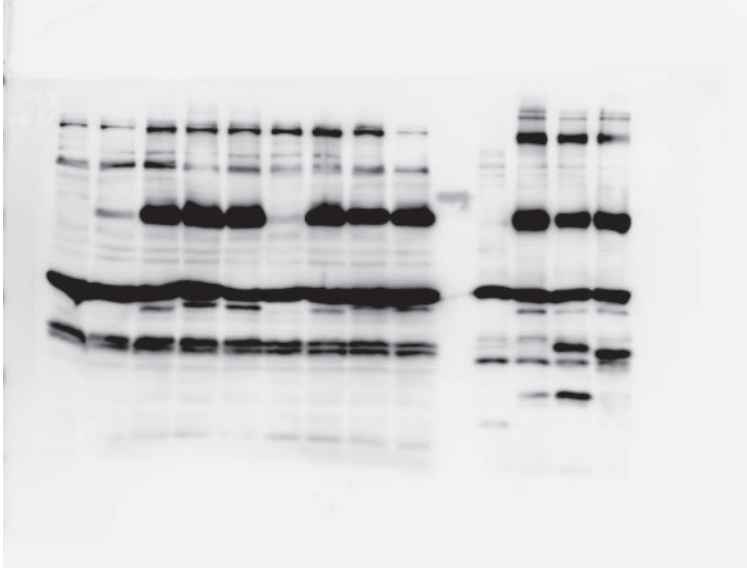


Figure 3b - α - actin

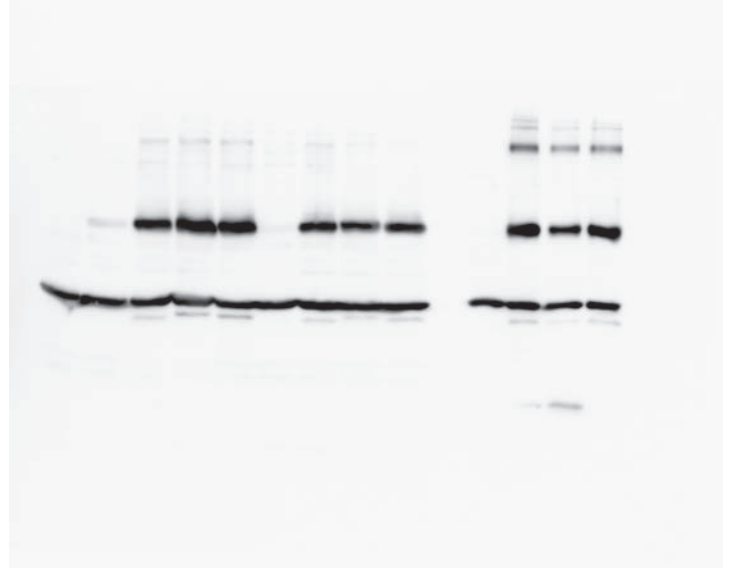


Figure 3c - α - MYC

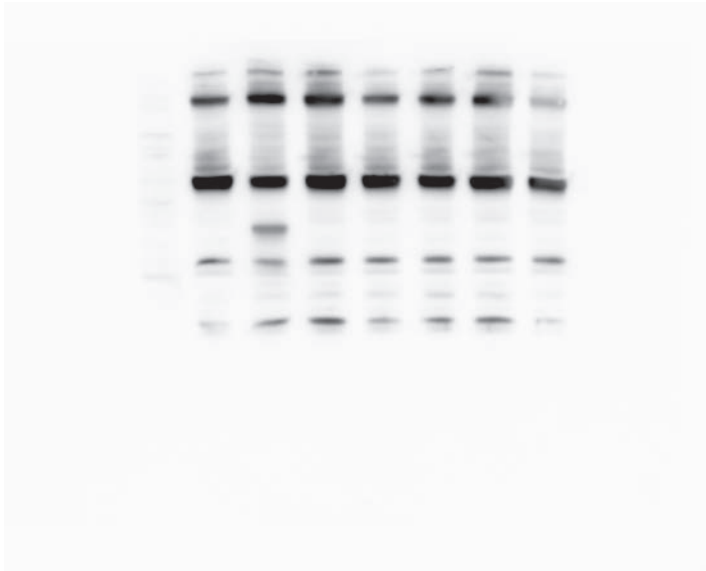


Figure 3c - α - actin

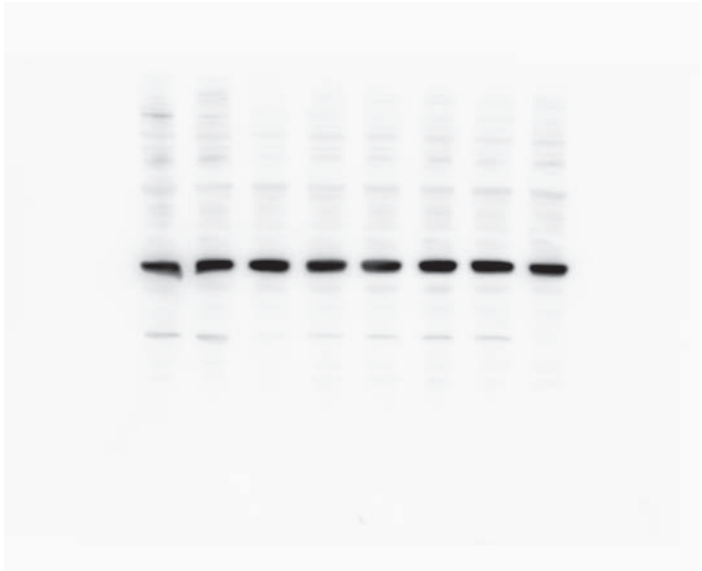


Figure 3d - α - K_v7.1

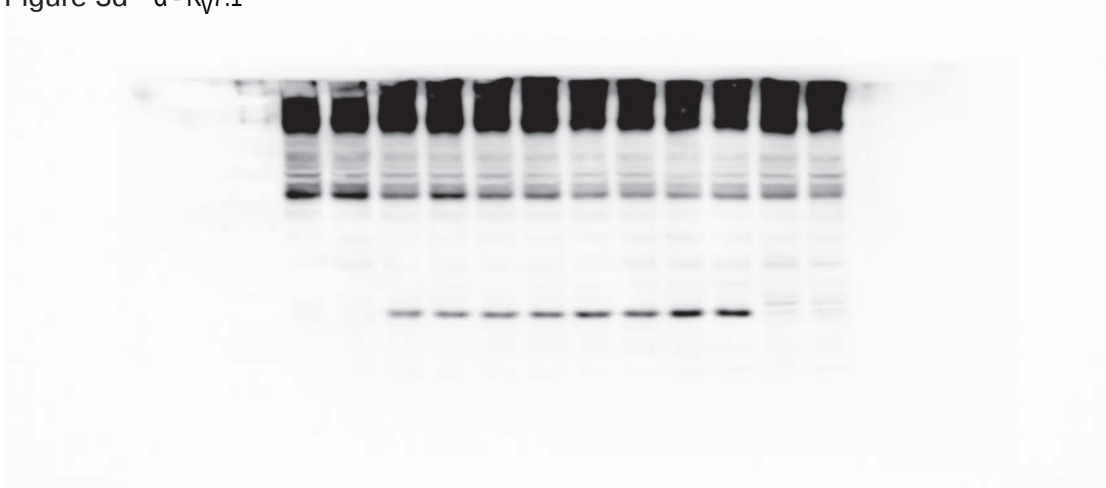


Figure 3d - α - caspase-3

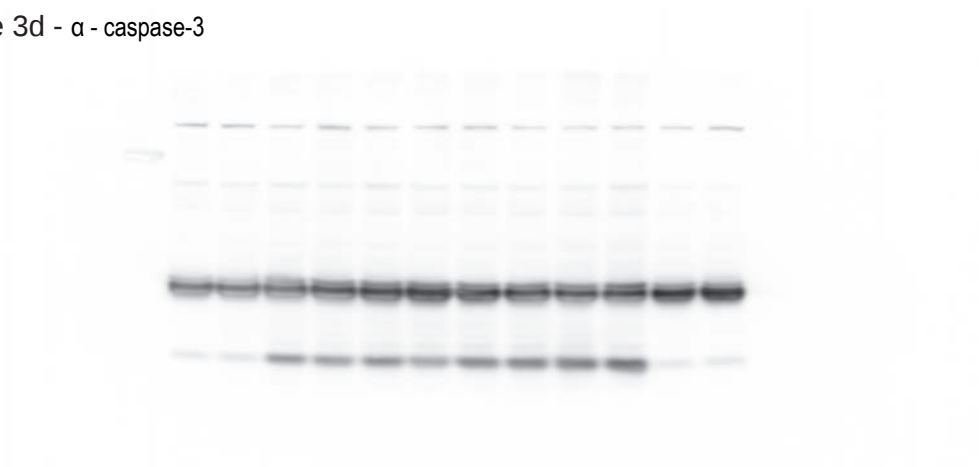


Figure 4c - α - K_v7.1

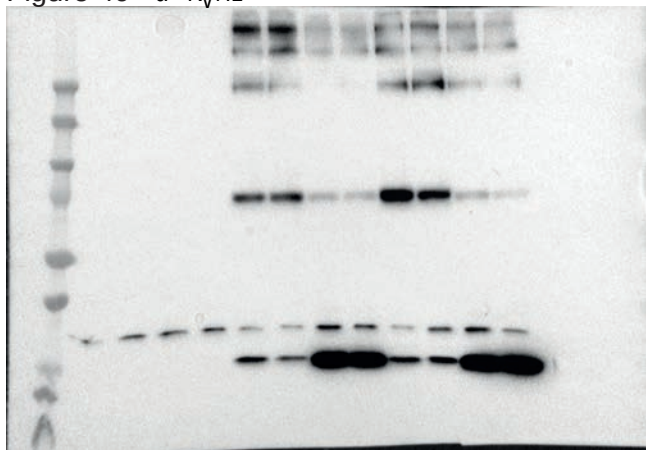


Figure 4c - α - KCNE1

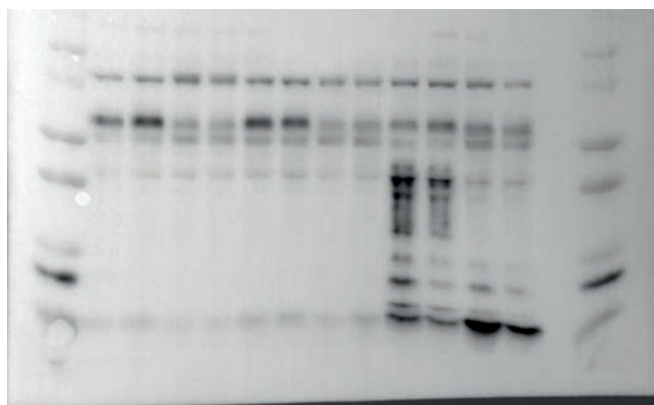


Figure 4c - α - GAPDH

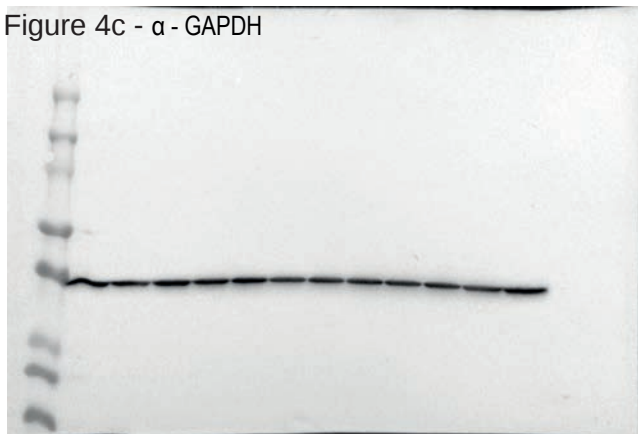


Figure 4c - α - caspase-3

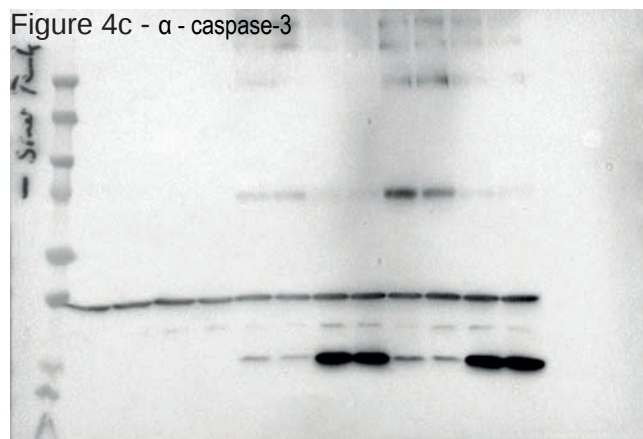


Figure 4d - α - K_v7.1 IP

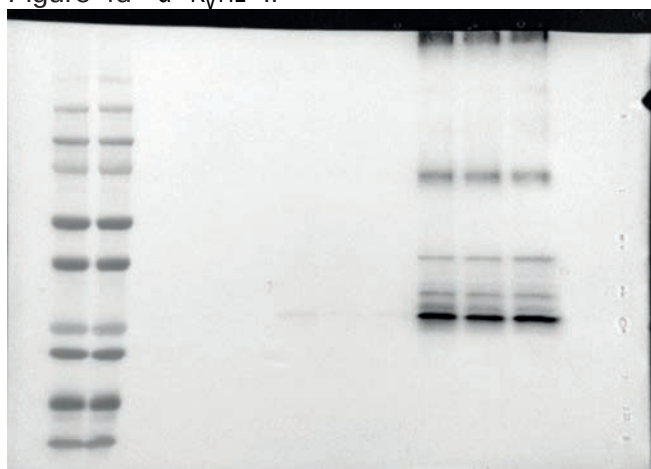


Figure 4d - α - K_v7.1 TL

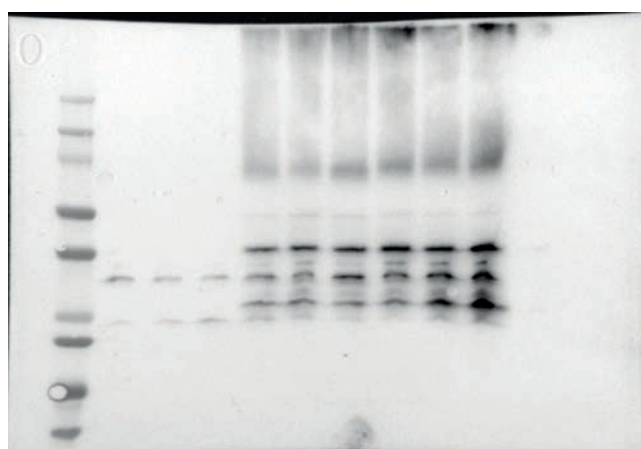


Figure 4d - α - GAPDH IP

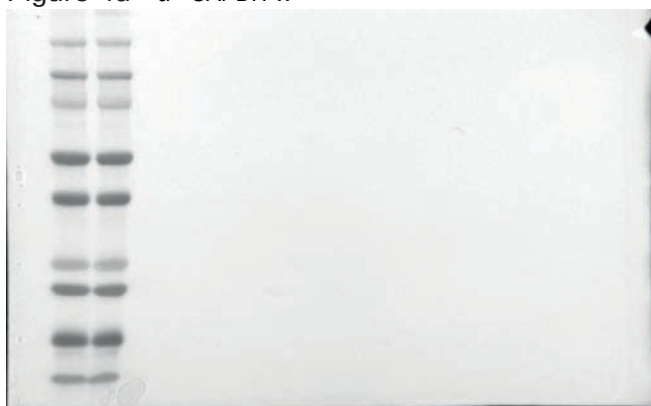


Figure 4d - α - GAPDH TL

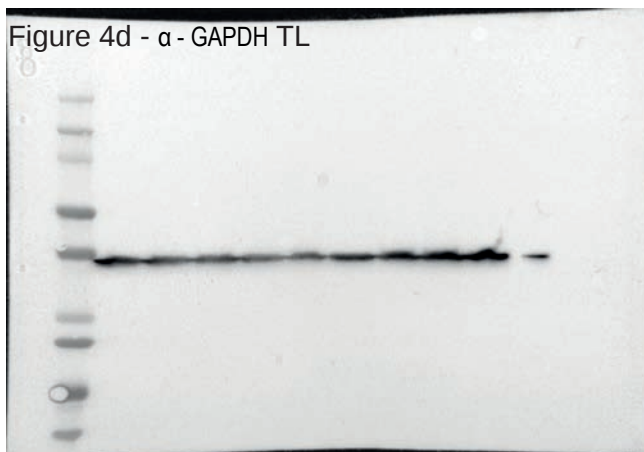


Figure 4e - α - MYC

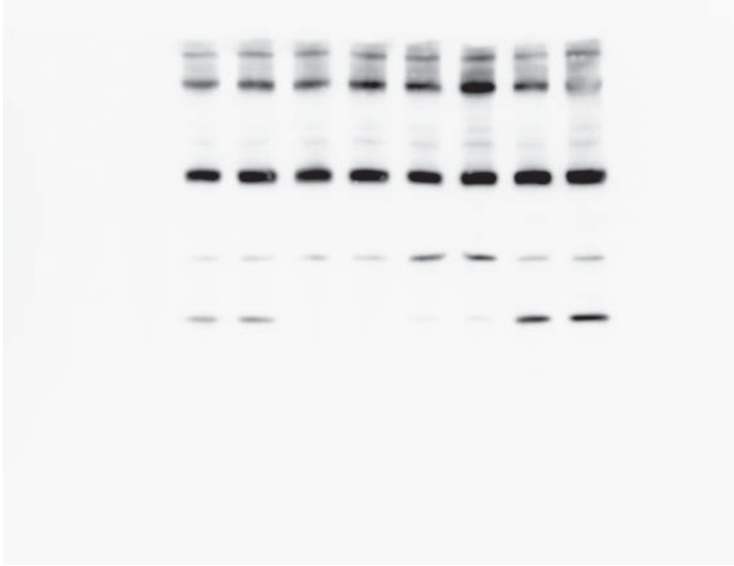


Figure 4e - α - actin

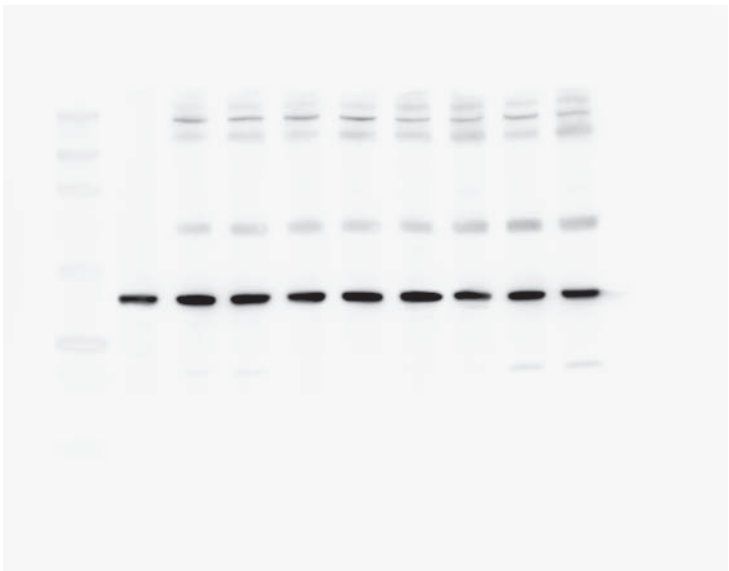


Figure 4f - α - MYC (right IP; left TL)

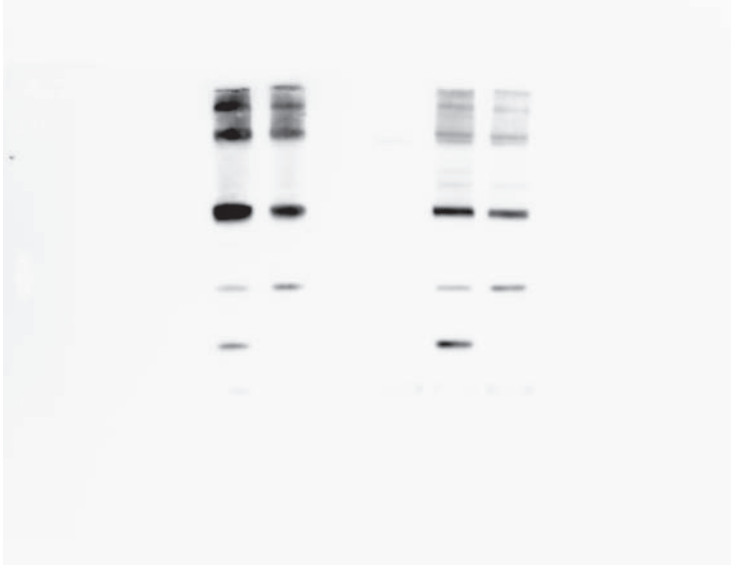


Figure 4f - α - calmodulin (right IP; left TL)

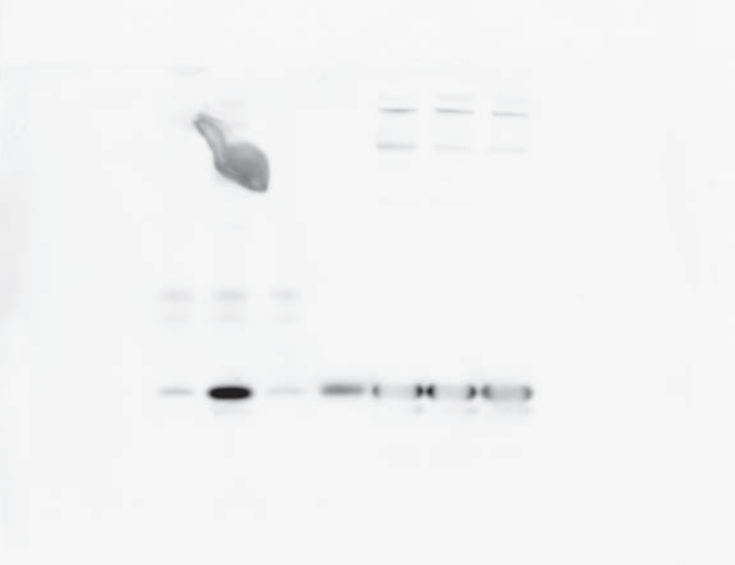


Figure 5a - α - K_v7.1

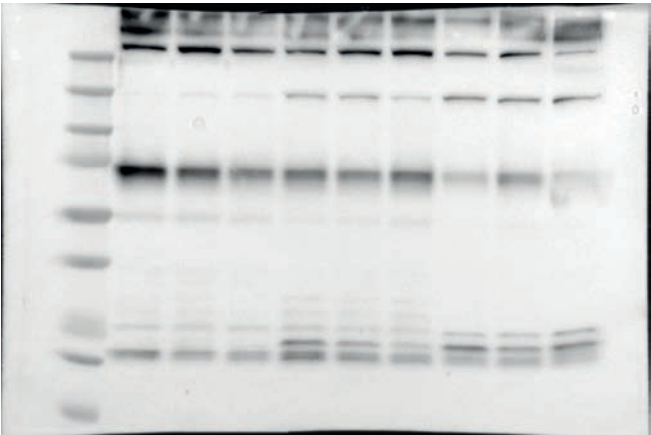


Figure 5a - α - GAPDH

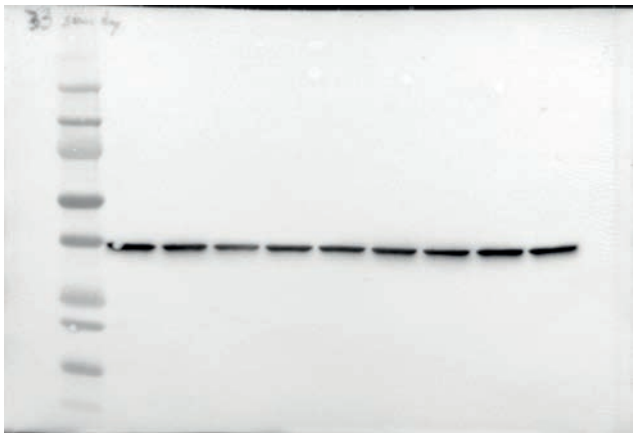
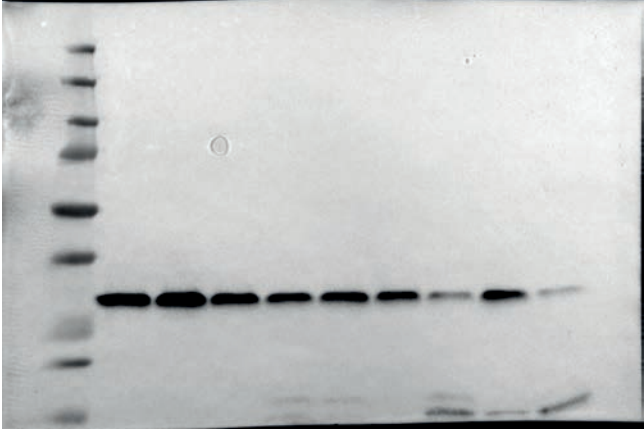
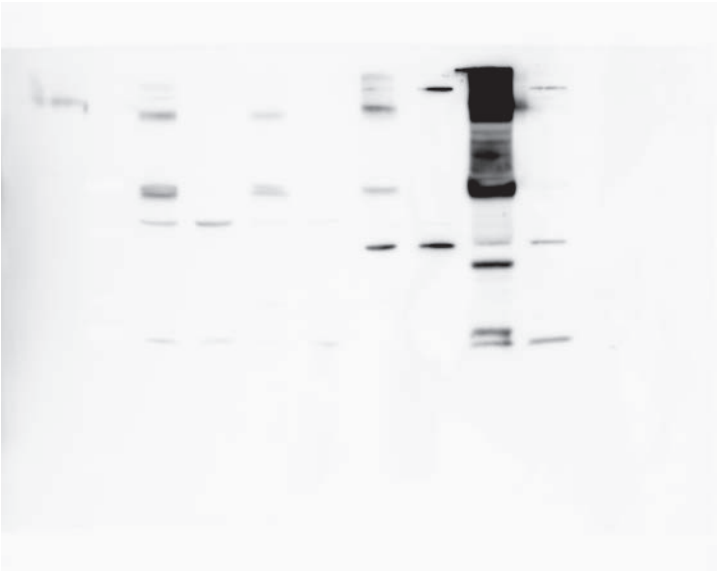


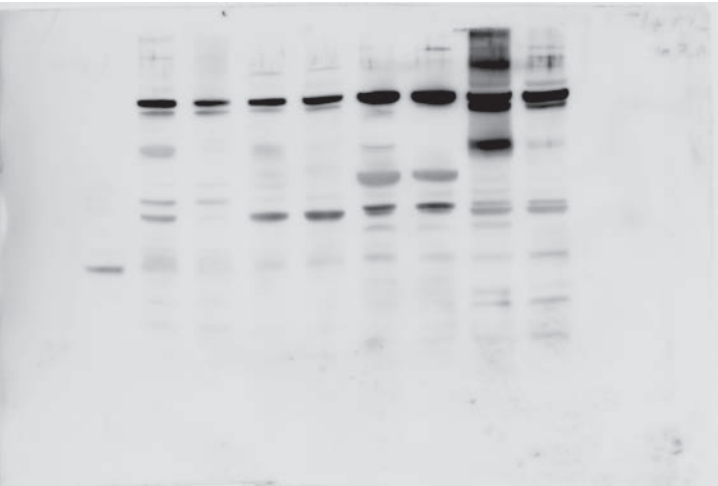
Figure 5a - α - caspase-3



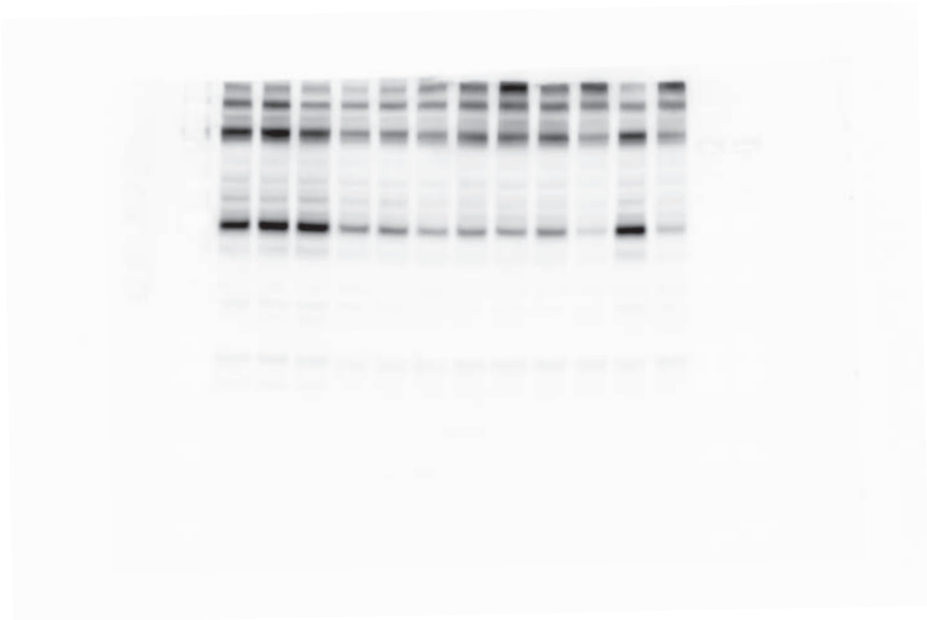
Supplementary Figure 1b - α - K γ 7.1



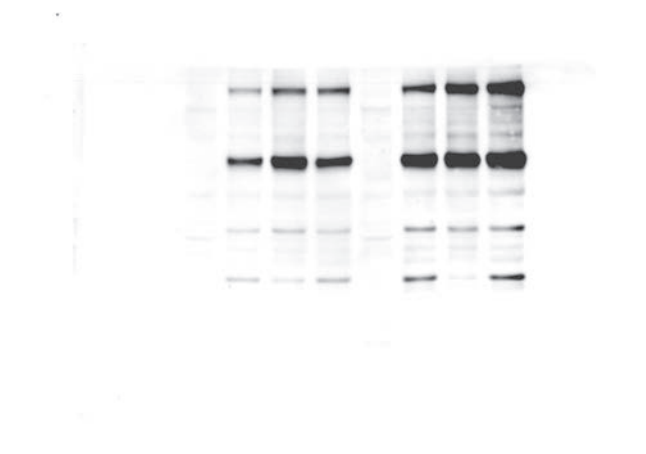
Supplementary Figure 1b - α - Eef2



Supplementary Figure 1c - α - K γ 7.1



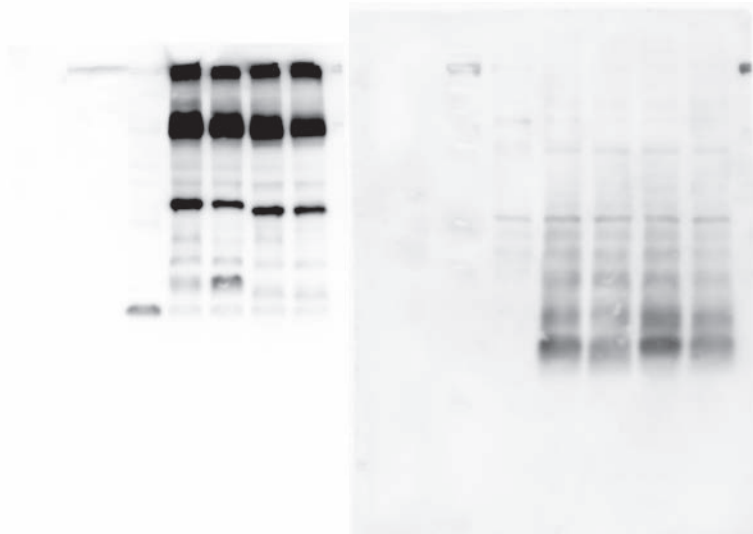
Supplementary Figure 3a - α - MYC



Supplementary Figure 3a - α - actin



Supplementary Figure 3b - α - K_v7.1 (left) ; α - KCNE1 (right)



Supplementary Figure 3c - α - K_v7.1

