Supplementary Information

Activation of Hsp70 reduces neurotoxicity by promoting polyglutamine protein degradation

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Supplementary Results

Supplementary Table 1

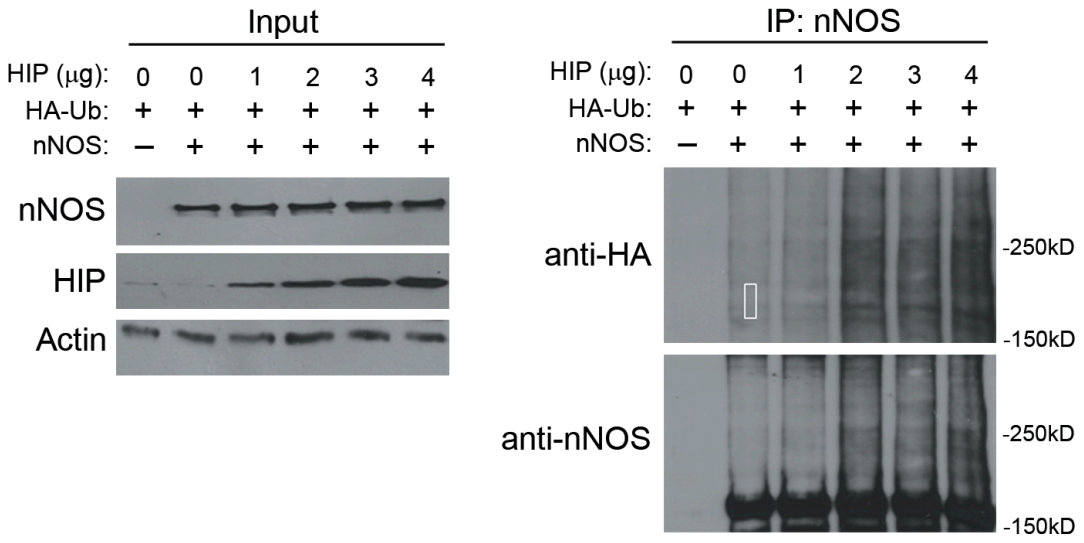
Table 1. HSPA8 residues in contact with the five MKT-077 docking families shown in the context of the human HSPA paralogs

	11	*12*	*13*	*14*	*15*				*69*	*70*	*71*	*72*	*73*	*74*	*75*	***76***		*80*	*81*	*82*	*83*	*84*	
HSPA8	L	G	T	T	Y				D	A	K	R	L	I	G	***R***		D	A	V	V	Q	
HspA1A	L	G	T	T	Y				D	A	K	R	L	I	G	***R***		D	P	V	V	Q	
HspA1L	L	G	T	T	Y				D	A	K	R	L	I	G	***R***		D	P	V	V	Q	
Hspa2	L	G	T	T	Y				D	A	K	R	L	I	G	***R***		D	A	T	V	Q	
HSPA5	L	G	T	T	Y				D	A	K	R	L	I	G	***R***		D	P	S	V	Q	
HSPA6	L	G	T	T	Y				D	A	K	R	L	I	G	***R***		D	T	T	V	Q	
HSPA9	L	G	T	T	N				A	T	K	R	L	I	G	***R***		D	P	E	V	Q	
HSP12A	F	G	T	T	S				Y	A	A	R	D	F	Y	***H***		P	N	E	A	K	
HSPA12b	F	G	T	T	S				Y	T	A	R	D	Y	Y	***H***		P	E	E	A	R	
HSPA13	L	G	T	T	Y				D	A	K	R	F	I	G	***K***		A	E	E	L	E	
HSPA14	L	G	C	T	S				K	V	K	Q	I	L	G	***R***		D	P	Q	A	Q	
	145	146	147	**148**	**149**	**150**	151	152	153	154	155	156		*173*	*174*	*175*	*176*	*177*					
HSPA8	T	V	P	***A***	***Y***	***F***	N	D	S	Q	R	Q		I	N	E	P	T					
HspA1A	T	V	P	***A***	***Y***	***F***	N	D	S	Q	R	Q		I	N	E	P	T					
HspA1L	T	V	P	***A***	***Y***	***F***	N	D	S	Q	R	Q		I	N	E	P	T					
Hspa2	T	V	P	***A***	***Y***	***F***	N	D	S	Q	R	Q		I	N	E	P	T					
HSPA5	T	V	P	***A***	***Y***	***F***	N	D	S	Q	R	Q		I	N	E	P	T					
HSPA6	T	V	P	***A***	***Y***	***F***	N	D	S	Q	R	Q		I	N	E	P	T					
HSPA9	T	V	P	***A***	***Y***	***F***	N	D	S	Q	R	Q		I	N	E	P	T					
HSP12A	T	V	P	***A***	***I***	***W***	K	Q	P	A	K	Q		A	L	E	P	E					
HSPA12b	T	V	P	***A***	***I***	***W***	K	Q	P	A	K	Q		A	L	E	P	E					
HSPA13	S	V	P	***A***	***E***	***F***	D	L	K	Q	R	N		I	N	E	P	T					
HSPA14	T	V	P	***F***	***D***	***F***	G	E	K	Q	K	N		I	H	E	P	S					
	202	*203*	*204*	*205*	*206*	*207*	*208*		*220*	*221*	***222***	***223***	*224*	***225***	***226***	***227***	***228***	*229*	*230*		*318*	*319*	*320*
HSPA8	G	G	T	F	D	V	S		K	S	***T***	***A***	G	***D***	***T***	***H***	***L***	G	G		E	K	A
HspA1A	G	G	T	F	D	V	S		K	A	***T***	***A***	G	***D***	***T***	***H***	***L***	G	G		E	K	A
HspA1L	G	G	T	F	D	V	S		K	A	***T***	***A***	G	***D***	***T***	***H***	***L***	G	G		E	K	A
Hspa2	G	G	T	F	D	V	S		K	S	***T***	***A***	G	***D***	***T***	***H***	***L***	G	G		E	K	A
HSPA5	G	G	T	F	D	V	S		K	A	***T***	***N***	G	***D***	***T***	***H***	***L***	G	G		Q	K	V
HSPA6	G	G	T	F	D	V	S		K	A	***T***	***A***	G	***D***	***T***	***H***	***L***	G	G		E	K	A
HSPA9	G	G	T	F	D	I	S		K	S	***T***	***N***	G	***D***	***T***	***F***	***L***	G	G		Q	K	A
HSP12A	G	G	T	V	D	L	T		K	A	***T***	***G***	G	***P***	***Y***	***G***	***S***	L	G		R	D	L
HSPA12b	G	G	T	V	D	L	T		K	A	***S***	***G***	G	***P***	***Y***	***G***	***A***	V	G		E	A	L
HSPA13	G	G	T	L	D	V	S		R	A	***M***	***S***	G	***N***	***N***	***K***	***L***	G	G		Q	Q	V
HSPA14	G	T	S	L	S	L	S		L	S	***T***	***N***	T	***D***	***D***	***N***	***I***	G	G		R	G	L

Red, contacts with four or five families; orange, contacts with two families; yellow, contacts with one family. These residues are also shown in Fig. 11.
The residues that showed chemical shifts are shown in italics. The numbering is for HSPA8.
The alignment was carried out in BLAST-P.

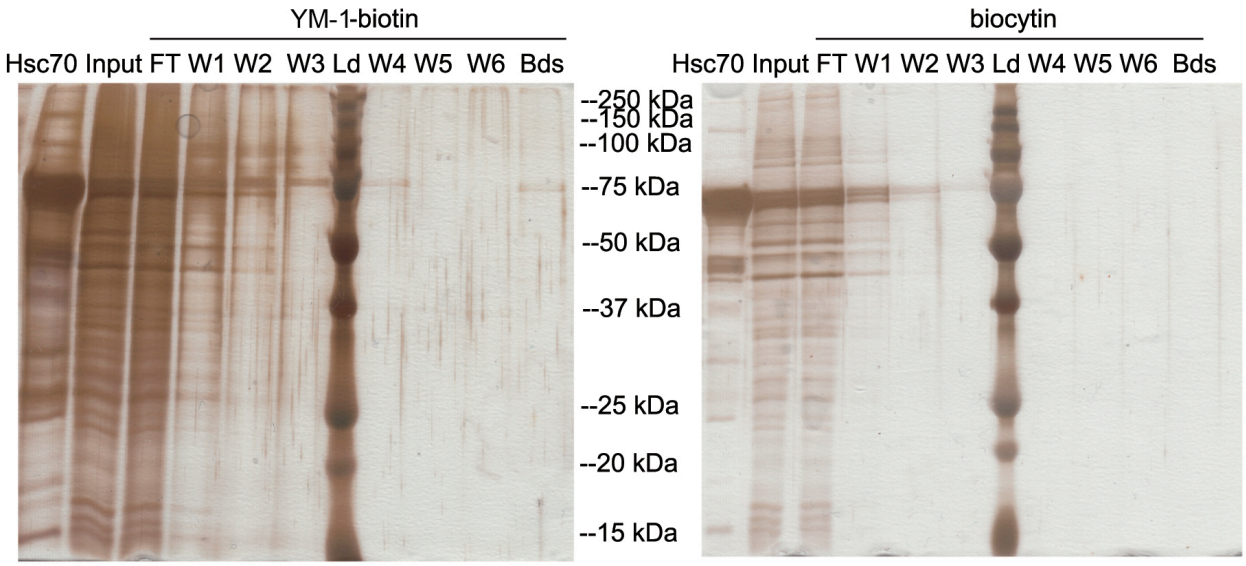
Supplementary Figure 1. Hip promotes nNOS ubiquitination.

HEK293T cells transiently expressing nNOS, HA-ubiquitin (HA-Ub) and increasing amounts of Hip were treated with lactacystin (24 hr). nNOS was immunoprecipitated (IP) from cytosolic lysates (input, on left; IP on right), and western blots were probed as indicated. Region quantified in Fig 1a is highlighted by the rectangle in the right panel.



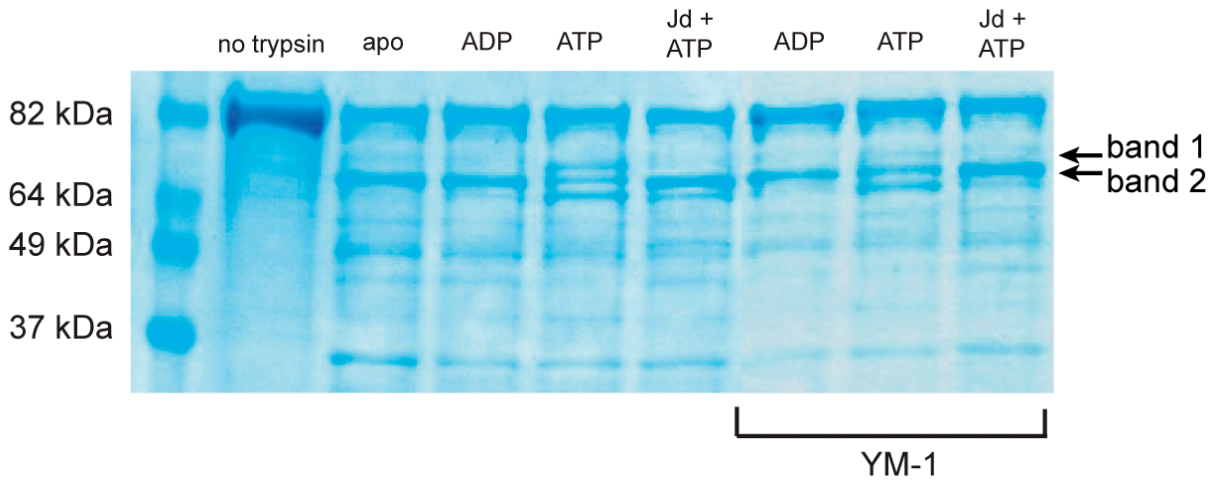
Supplementary Figure 2. YM-1-biotin selectively pulls down Hsp70 from HeLa cell lysates.

Cell lysates (input) were incubated with YM-1-biotin or control (biocytin) overnight and washed six times (W1-W6) with buffer. Remaining proteins were eluted by boiling the beads (Bds). Samples were separated by SDS-PAGE and silver stained. Recombinant Hsc70 is shown in the first lane as a control.



Supplementary Figure 3. Partial trypsin proteolysis shows that YM-1 favors an ADP-like conformation.

Full length human Hsp70 was treated with ADP, ATP or ATP and a J domain (Jd). While proteolysis of the ADP-treated sample produces a single characteristic band (band 2), addition of ATP yields an additional fragment (band 1). Co-incubation with YM-1 (200 μM) partially suppresses the appearance of band 1 and favors an ADP-like conformation.

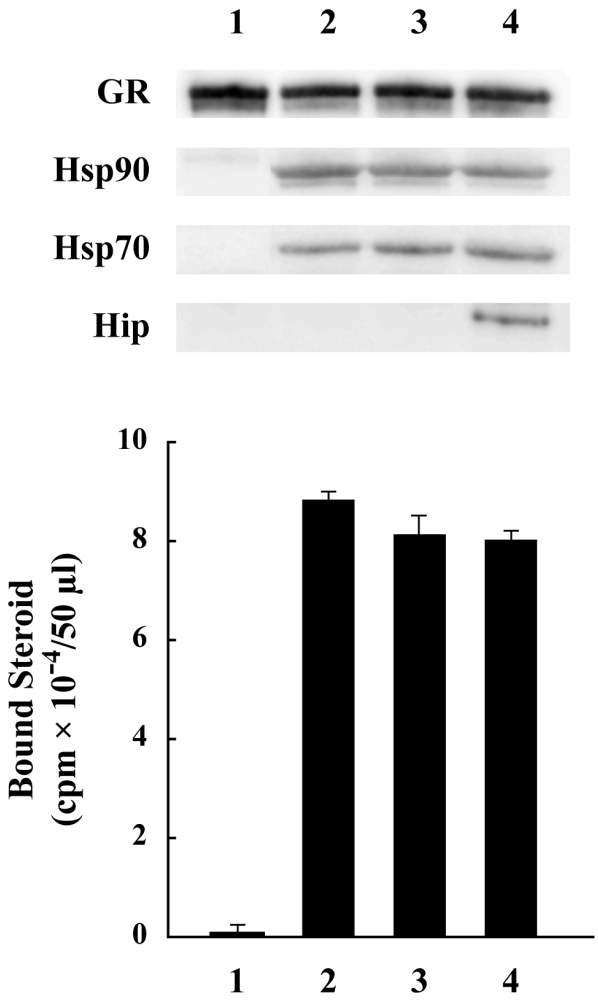


Supplementary Figure 4. YM-1 decreases insoluble AR112Q.

PC12 cells expressing tet-regulated AR112 were treated with R1881 (10 nM) and YM-1 for 16 hours. Immunoblot analysis shows soluble and pelleted (15,000 *g*) AR112Q.

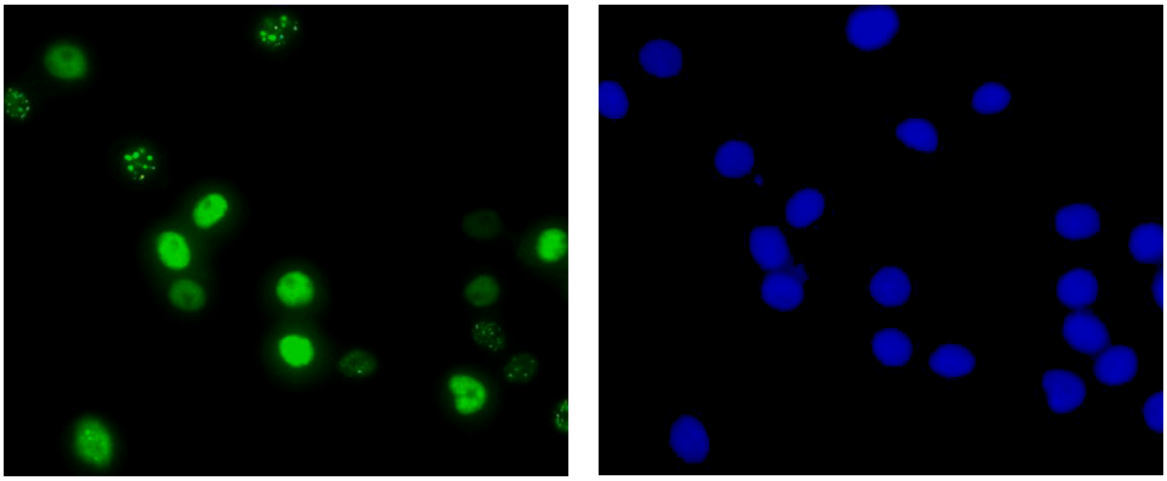
Supplementary Figure 5. YM-1 does not affect GR-Hsp90 heterocomplex assembly.

Aliquots of immunoadsorbed glucocorticoid receptor (GR) were stripped of chaperones with NaCl and incubated with rabbit reticulocyte lysate (RL). After washing, the immune pellets were assayed by immunoblotting for GR and associated proteins or bound with [^{3}H] dexamethasone for assay of steroid binding activity. Lane 1, stripped GR; lanes 2-4, stripped GR incubated with 50 μl of RL alone (lane 2), RL plus 1 μM YM-1 (lane 3), or RL plus 6.9 μM Hip (lane 4). Hip is added at a roughly 1:1 ratio with reticulocyte Hsp70.



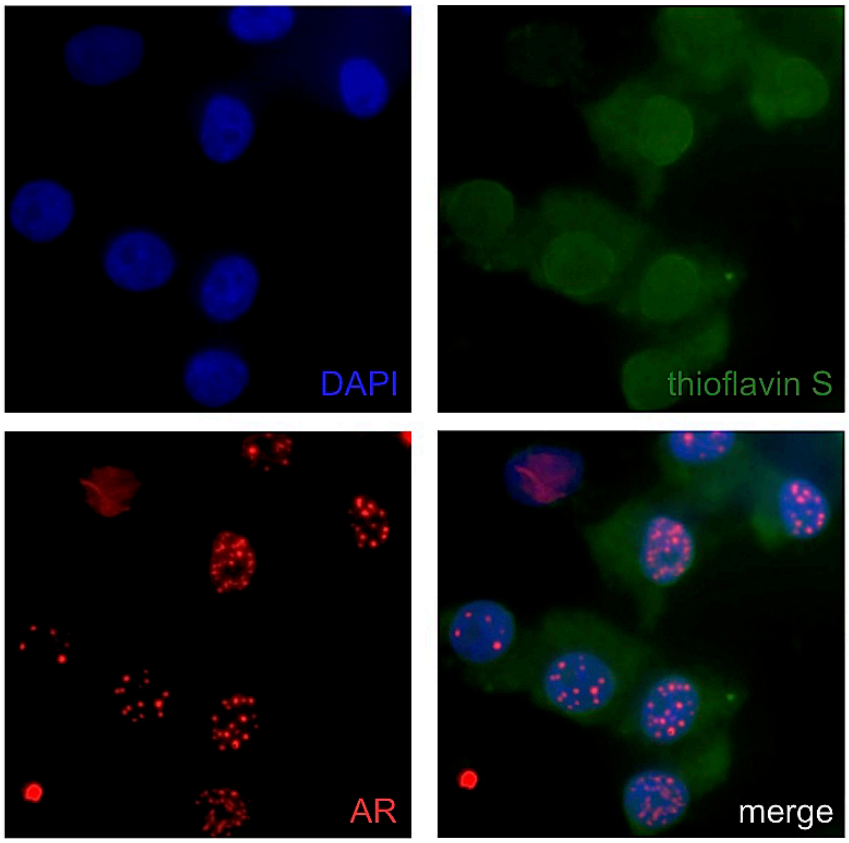
Supplementary Figure 6. AR112Q expression in PC12 cells after 48hr induction in the presence of 10 nM R1881.

Cells were stained for AR (left panel). Nuclei were stained with DAPI (right panel).



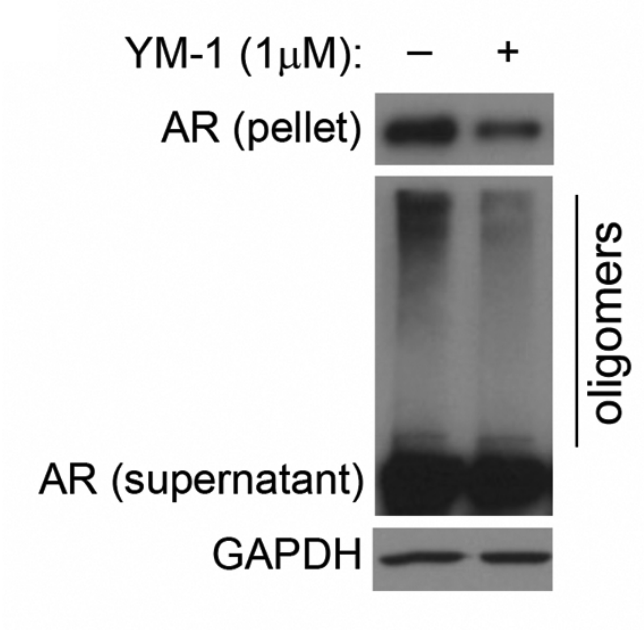
Supplementary Figure 7. Polyglutamine AR intranuclear inclusions do not stain with thioflavin S.

PC12 cells expressing tet-regulated AR112Q were treated with R1881 (10 mM) for 72 hr, and then stained for AR (red, lower left panel) and thioflavin S (green, upper right panel). Nuclei were stained with DAPI (blue, upper left panel).



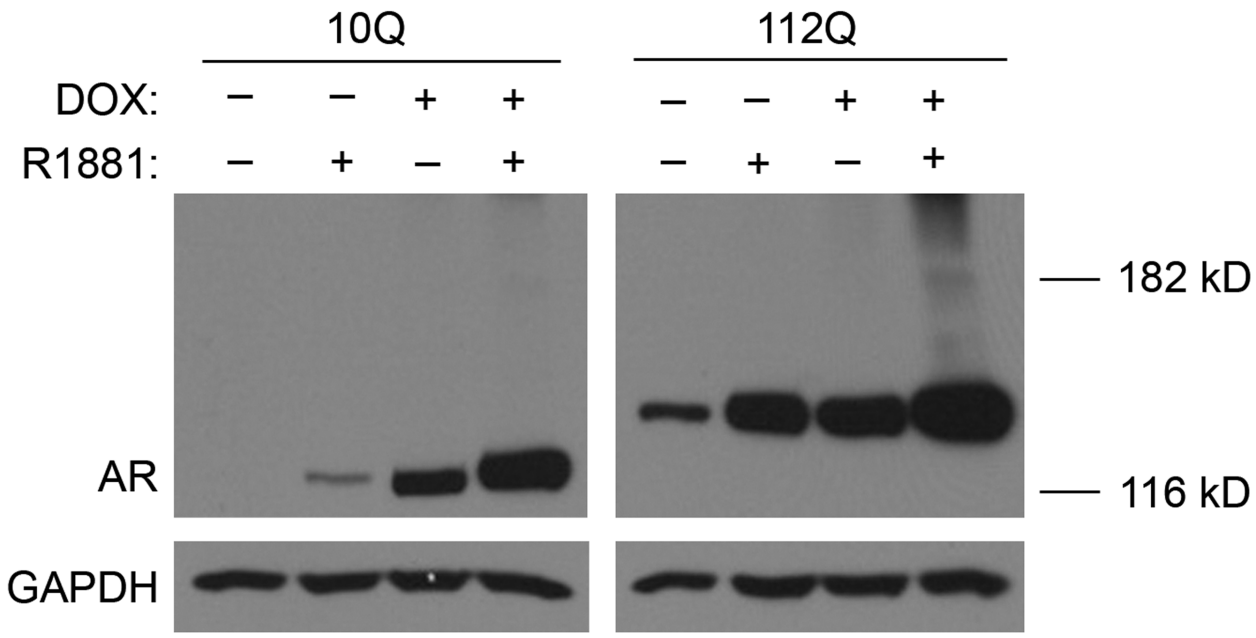
Supplementary Figure 8. YM-1 promotes clearance of insoluble and oligomeric AR112Q.

Lysates were prepared from cells treated as in Fig. 4a. Immunoblot shows decreased AR112Q monomer in the 15,000 *g* pellet and diminished high MW oligomers in the soluble fraction after ultracentrifugation.



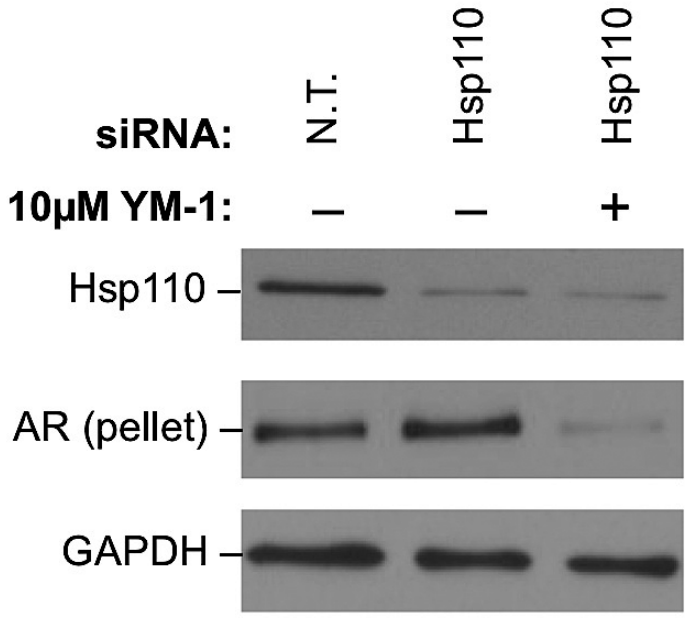
Supplementary Figure 9. Hormone and glutamine length-dependent polyQ AR oligomerization in Tet-ON PC12 cells.

PC12 cells were induced to express AR10Q (left panel) or AR112Q (right panel) with doxycycline (0.5 μg/μl) and treated with R1881 (10 nM) for 48 hours. Lysates were analyzed after ultracentrifugation at 100,000 *g* for 30 min. High MW AR112Q oligomers are seen by western blot in the presence of ligand.



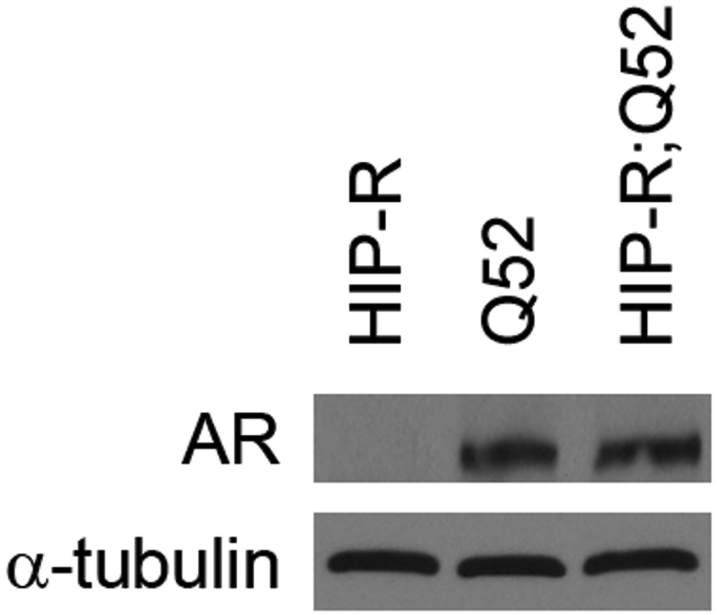
Supplementary Figure 10. Hsp110 knockdown does not affect the response to YM-1.

PC12 cells expressing tet-regulated AR112Q were transfected with siRNAs targeted at Agp-2, a constitutive Hsp110, or non-targeted control (N.T.). After 24 hrs, cells were treated with R1881 (10 nM) plus YM- or vehicle. Effects of YM-1 on pelleted AR112Q were analyzed by western blot.



Supplementary Figure 11. UAS-hAR52Q expression in *Drosophila.*

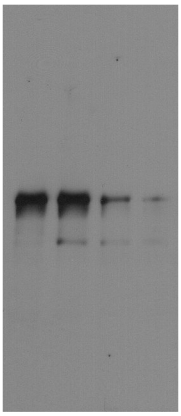
Immunoblot analysis of AR52Q expression in adult fly heads under control of the OK371-Gal4 driver. Expression of AR52Q (middle lane) is not diminished when expressed in conjunction with HIP-R (right lane) in flies reared on food supplemented with vehicle.



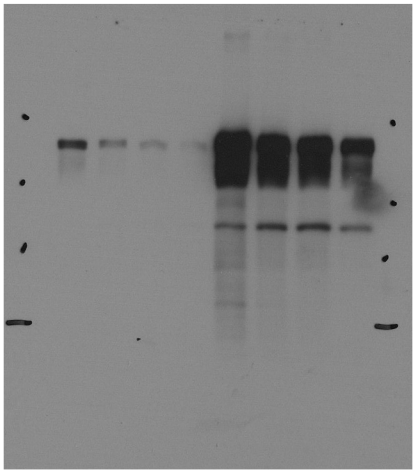
Supplementary Figure 12. Uncropped western blots for figures 1b, 1c, 3a, and 4c – f.

Note that membranes probed in Fig 4 were cut into horizontal strips, separately probed with primary and secondary antibodies, and then developed as shown.

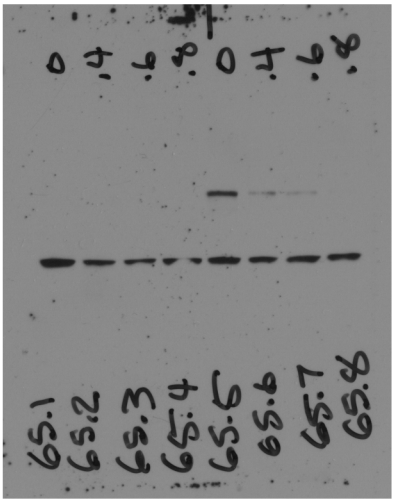
Fig 1b



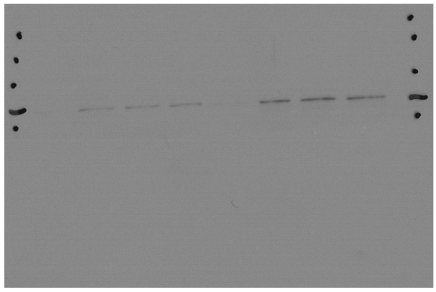
AR Pellet



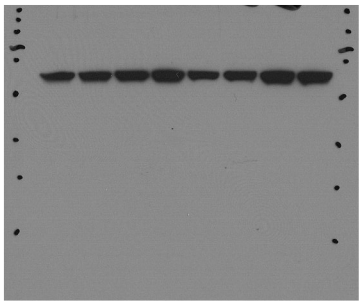
AR Sup



Hsp70

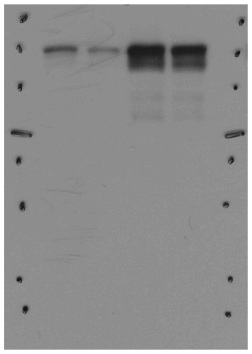


Hip

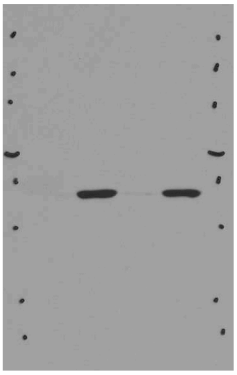


GAPDH

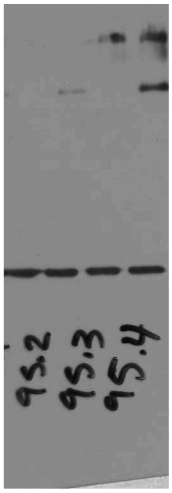
Fig 1c



AR112Q

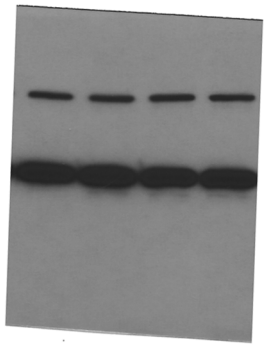


Hip

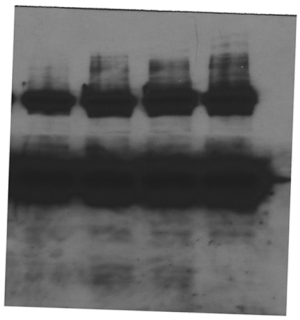


GAPDH

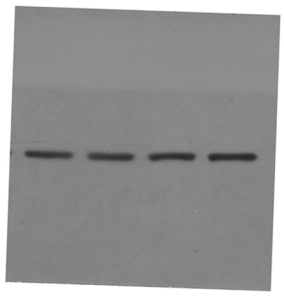
Fig 3a, left



nNOS
(2 sec)

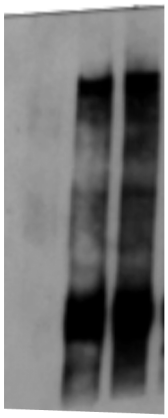


nNOS
(90 sec)

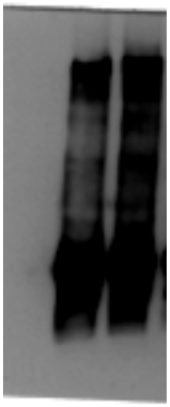


Hsp70

Fig 3a, right



WB:
Ub



WB:
nNOS

Fig 4

Fig 4c

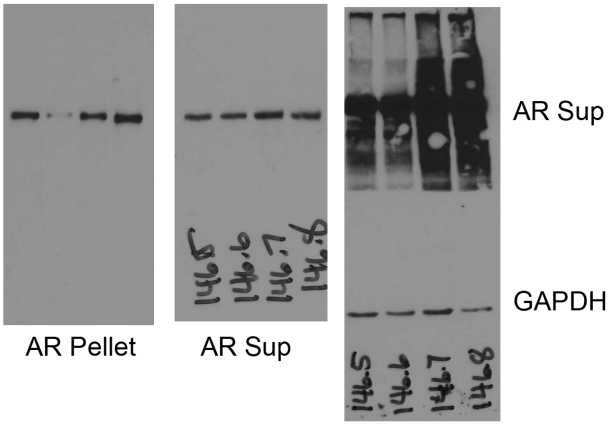


Fig 4d

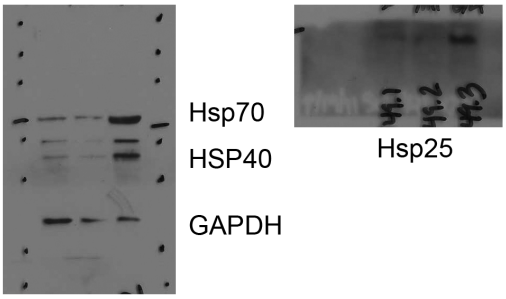


Fig 4e

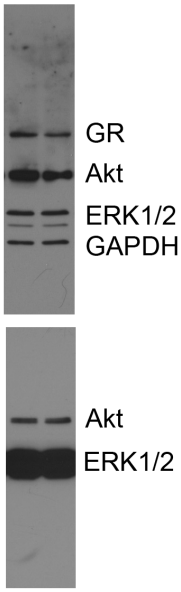


Fig 4f