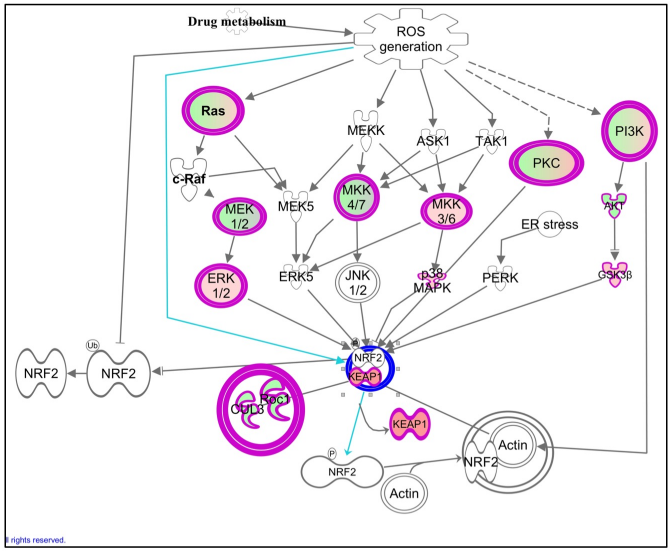
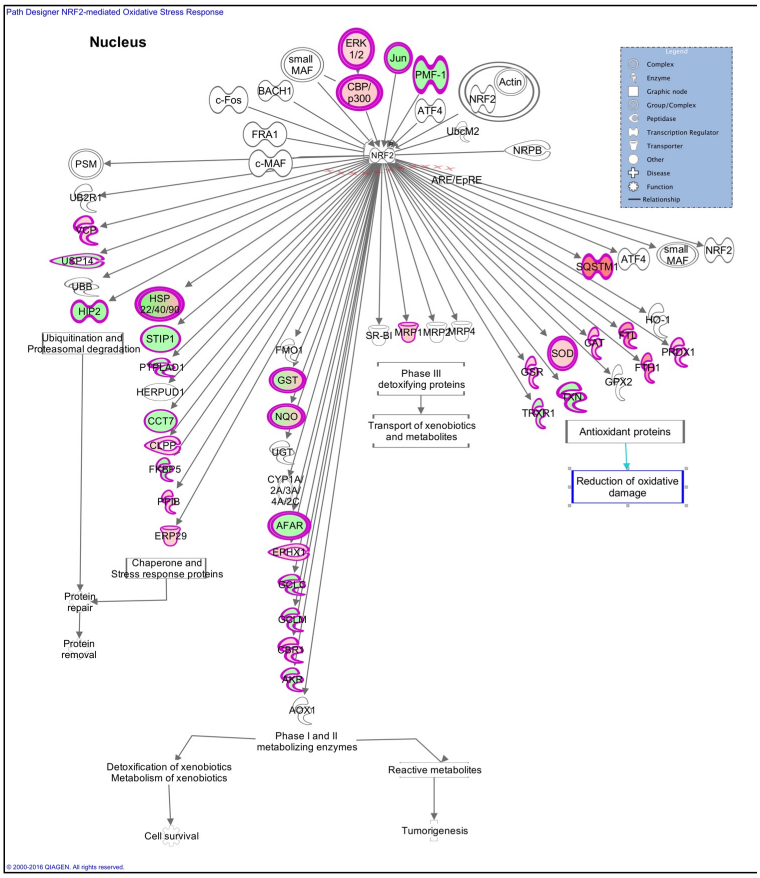
a

Cytoplasm

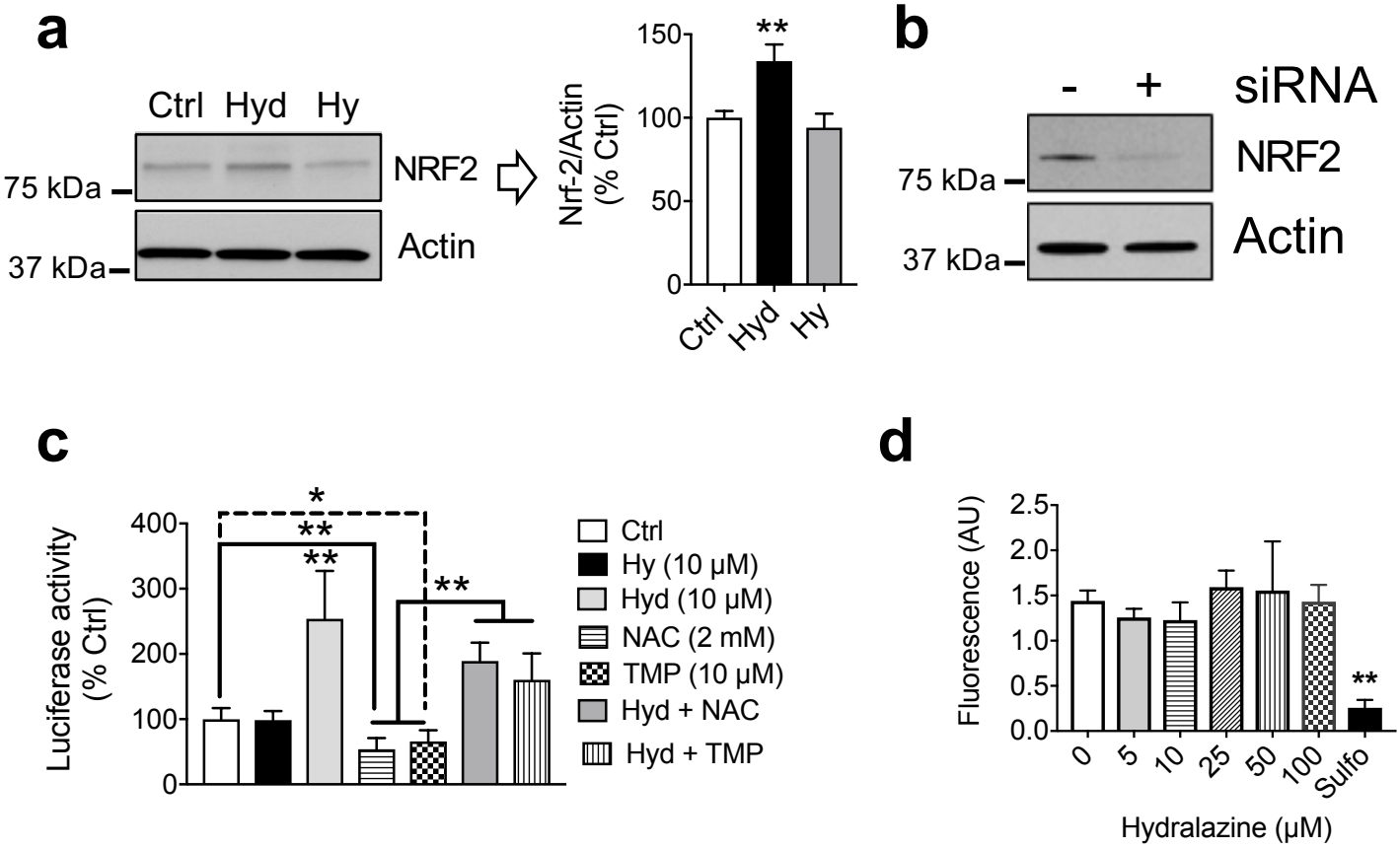


b

Nucleus

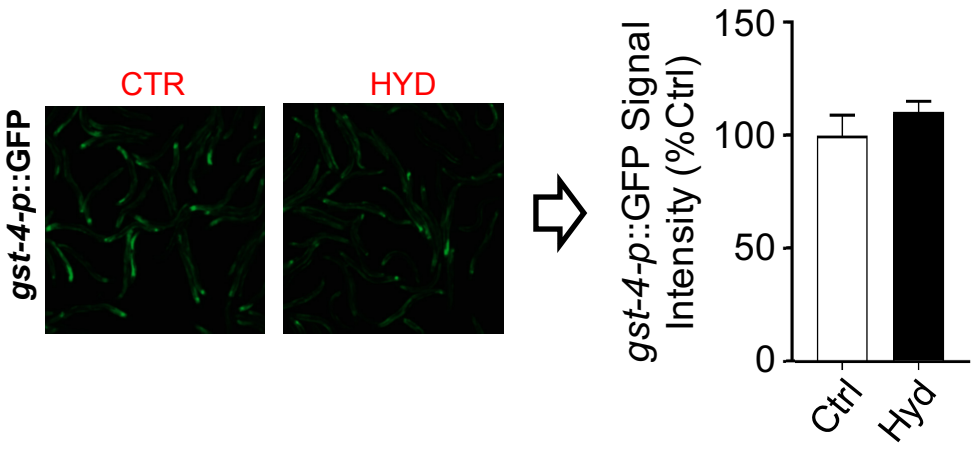


Supplementary Figure 1. NRF2 pathway activation identified with IPA using protein ratios determined using shotgun analysis of SH-SY5Y cells grown in SILAC media and treated with hydralazine. (a) NRF2 pathway activation in the cytoplasm. **(b)** NRF2 pathway activation in the nucleus. For shape and color codes follow this link. (http://ingenuity.force.com/ipa/articles/Feature_Description/Legend).



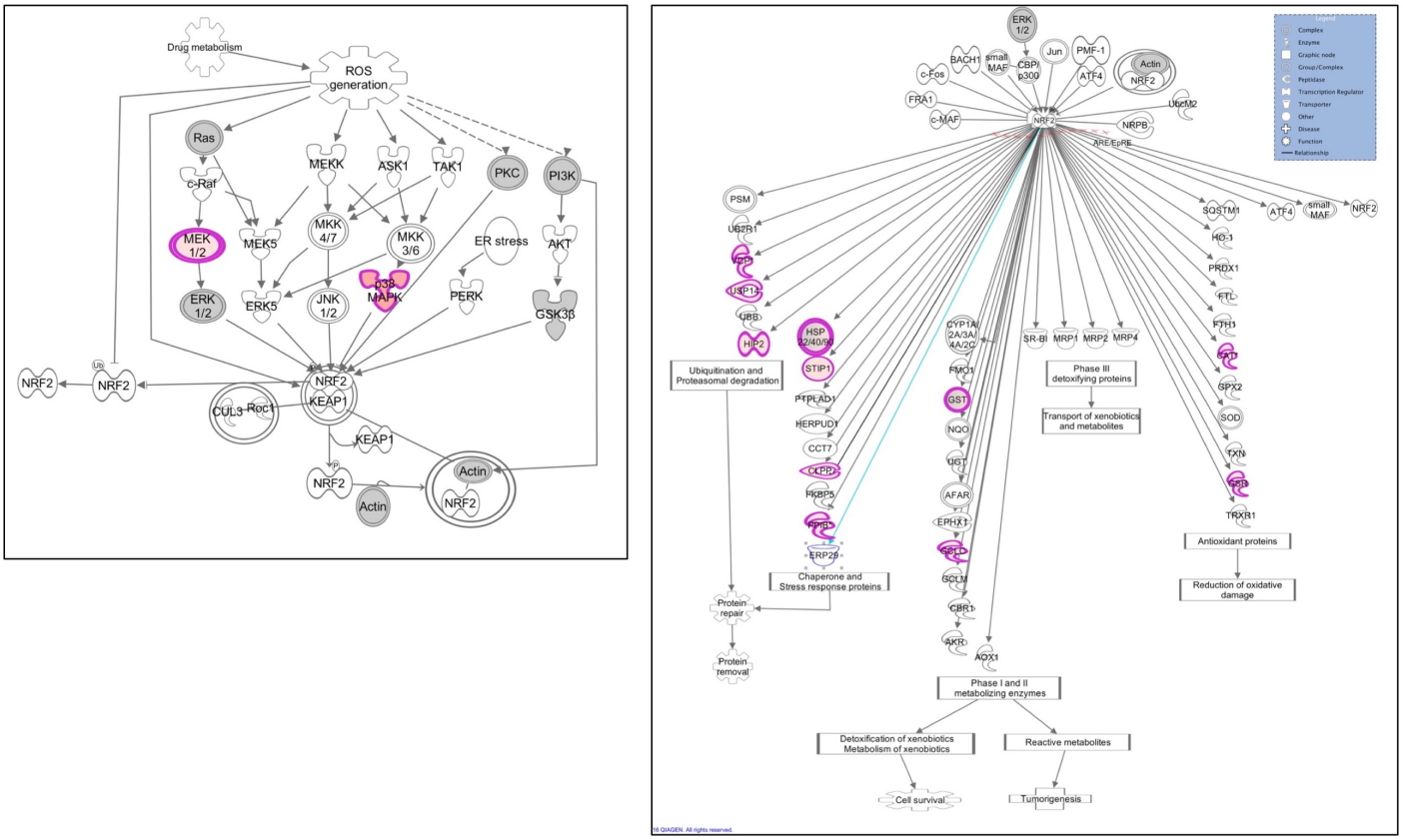
Supplementary Figure 2. Validating NRF2 activation and exploring its mechanism of action

(a) Hydrazine used as negative control for hydralazine did not increase NRF2 protein measured by Western blot analysis. Both hydralazine and hydrazine were used at 5 μM concentration. **(b)** Confirming the specificity of the antibody used for NRF2 Western blot analysis using NRF2 knockdown SH-SY5Y cells. **(c)** The ARE-driven luciferase activity was decreased in SH-SY5Y cells treated with antioxidant compounds N-acetyl cysteine (NAC, 2 mM) and Tempol (TMP, 10 μM) and increased when cells were treated with the antioxidant compounds and hydralazine (10 μM) indicating that hydralazine-mediated NRF2 activation was ROS independent, *p<0.05 and **p<0.01, student t-test, n=3, mean ± SD. **(d)** KEAP1-NRF2 Inhibitor Screening Assay showed that hydralazine dose not directly interrupt the interaction between NRF2 and KEAP1. **p<0.01 student t-test, n=3, mean ± SD. Sulforaphane (5 μM) was used as a positive control (inhibitor), **p<0.01, student t-test, n=3, mean ± SD.

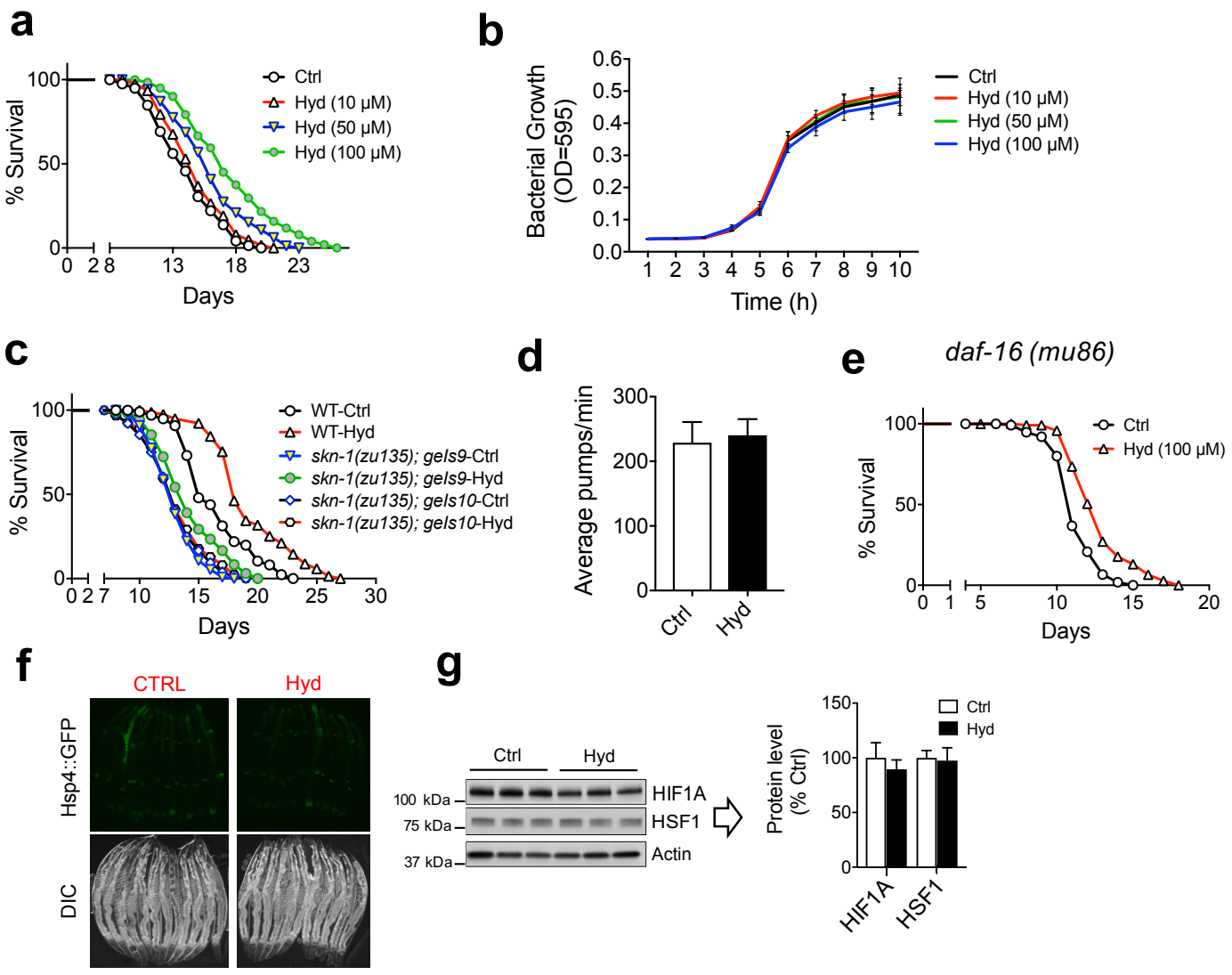


Supplementary Figure 3. Testing a downstream target of SKN-1 in a mutant strain lacking functional SKN-1C. Hydralazine treatment did not induce *gst-4p*::GFP expression in transgenic worms (*dvIs19*) lacking a functional SKN-1 isoform C in their intestine in a mutant background *skn-1(zu67).* p>0.05 student t-test, n=50 two independent trials, mean ± SD.

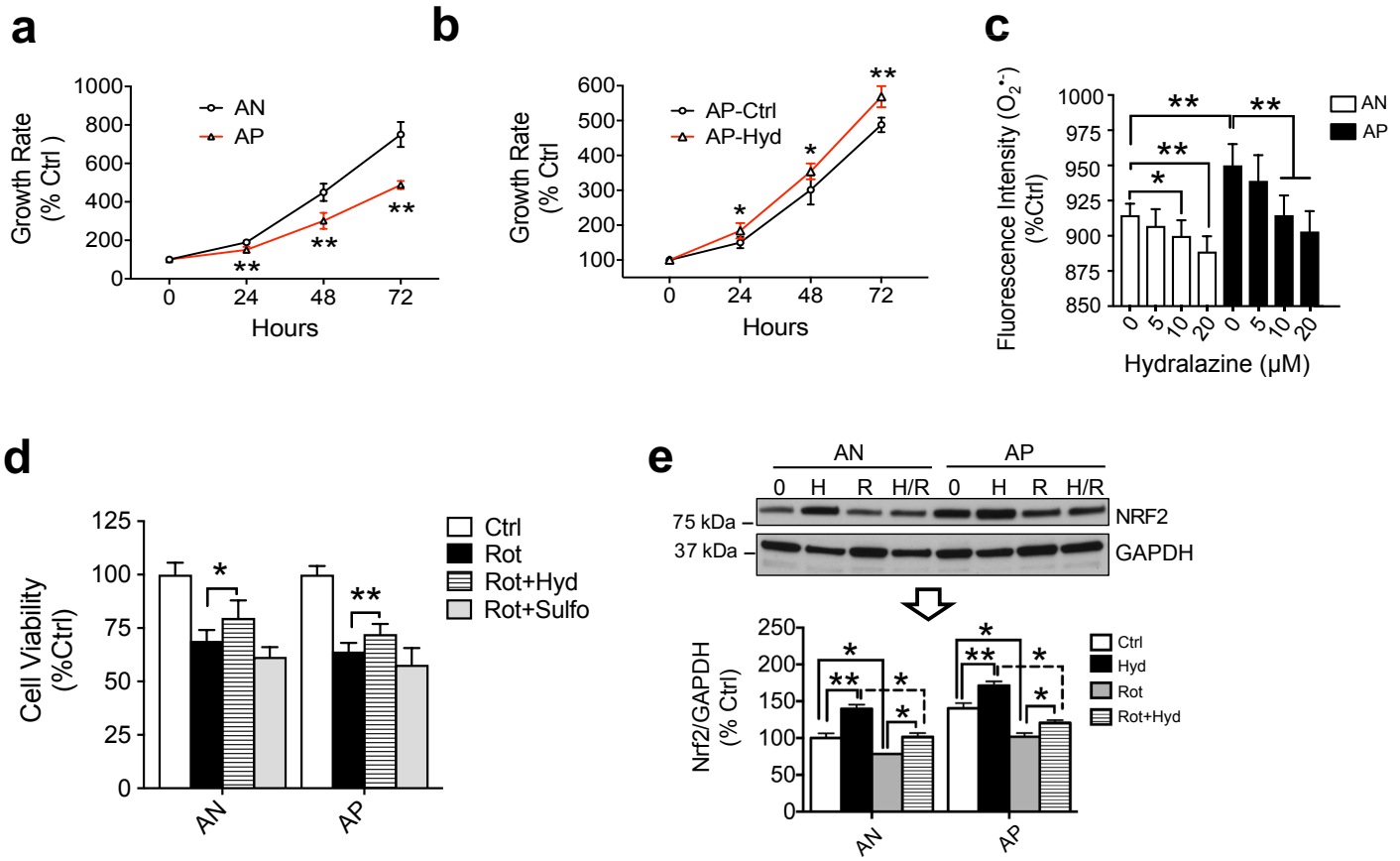
a **b**



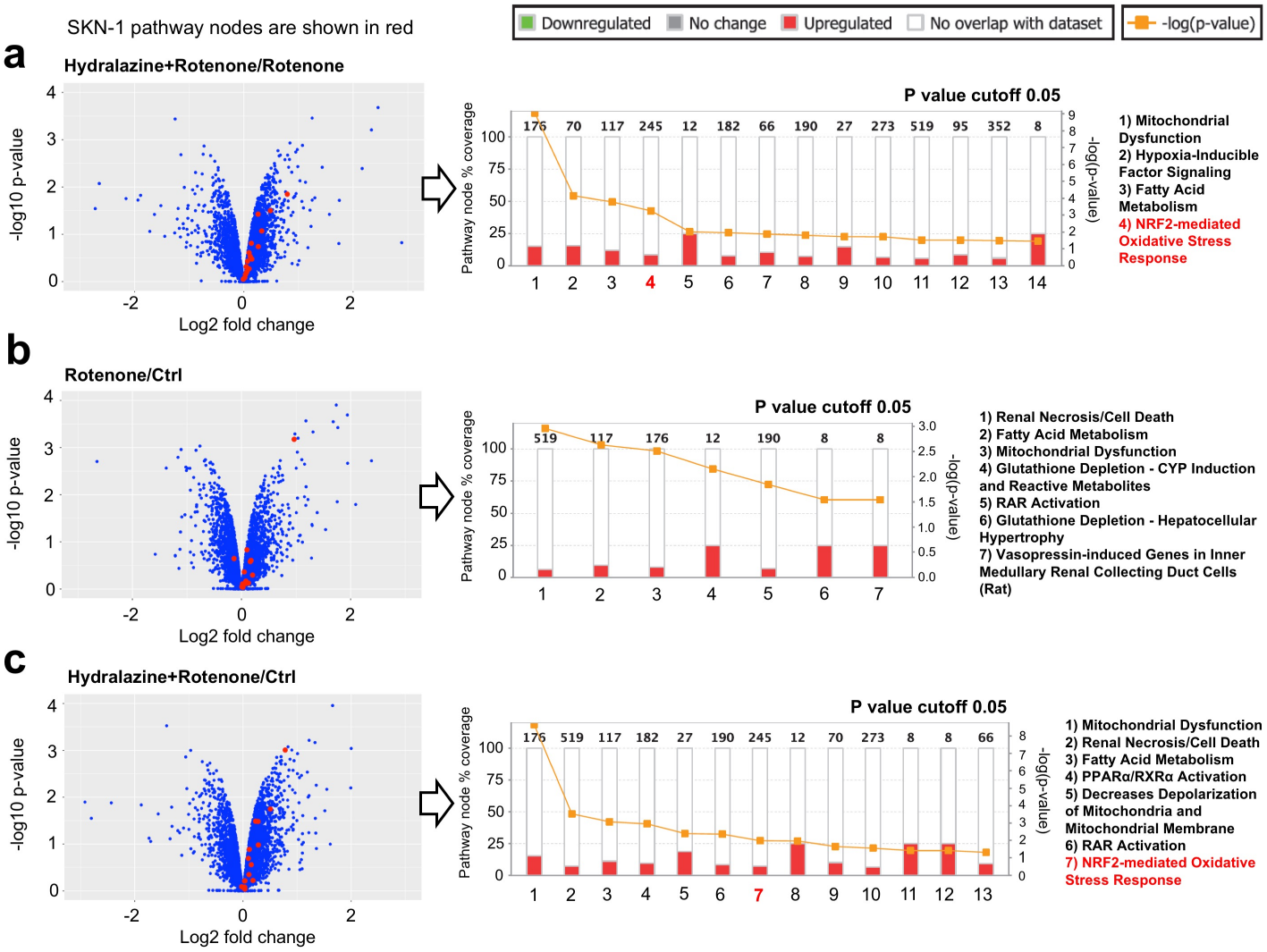
Supplementary Figure 4. SKN-1/NRF2 pathway activation identified with IPA using protein ratios determined using label free analysis of wild type *C. elegans* treated with hydralazine or vehicle. (a) SKN-1/NRF2 pathway activation in the cytoplasm. **(b)** SKN-1/NRF2 pathway activation in the nucleus. For shape and color codes follow this link (http://ingenuity.force.com/ipa/articles/Feature_Description/Legend).



Supplementary Figure 5. Hydralazine extends lifespan in *C. elegans.* For all lifespan statistics, see Supplementary Table 3. (a) Hydralazine treatment increased *C. elegans* lifespan in a dose-dependent manner. **(b)** HB101 bacteria in liquid LB treated with different concentrations of hydralazine did not show growth retardation, two-tailed student t-test, n=6. **(c)** Expression of *skn-1* isoforms *b* by transgene *gels9* partially restores longevity benefits of hydralazine (100 μM) while expression of isoform *c* in transgenic *gels10* did not, signifying the role of *skn-1* isoforms *b* in hydralazine-mediated lifespan extension. **(d)** Pharyngeal pumping rate of wild type young (day 4) *C. elegans* treated with 100 μM hydralazine was not significantly altered, rulings out the possibility of hydralazine interfering with food uptake mimicking calorie restriction, two-tailed student t-test, n=32, mean ± SD. **(e)** Hydralazine mediated lifespan extension was not significantly attenuated in mutant *daf-16 C. elegans* ruling out the possibility of daf-2 insulin/IGF-1 signaling pathway involvement in prolongevity effects of hydralazine. **(f)** Fluorescence microscopic images tracing *hsp-4p::GFP* protein, a reporter of UPR^{ER} activation, indicated that hydralazine does not induce ER stress. **(g)** Two other important regulators of aging paradigm, HIF1A and HSF1, did not change in SH-SY5Y cells treated with 10 μM hydralazine. Two tailed student t-test, n=3, mean ± SD.



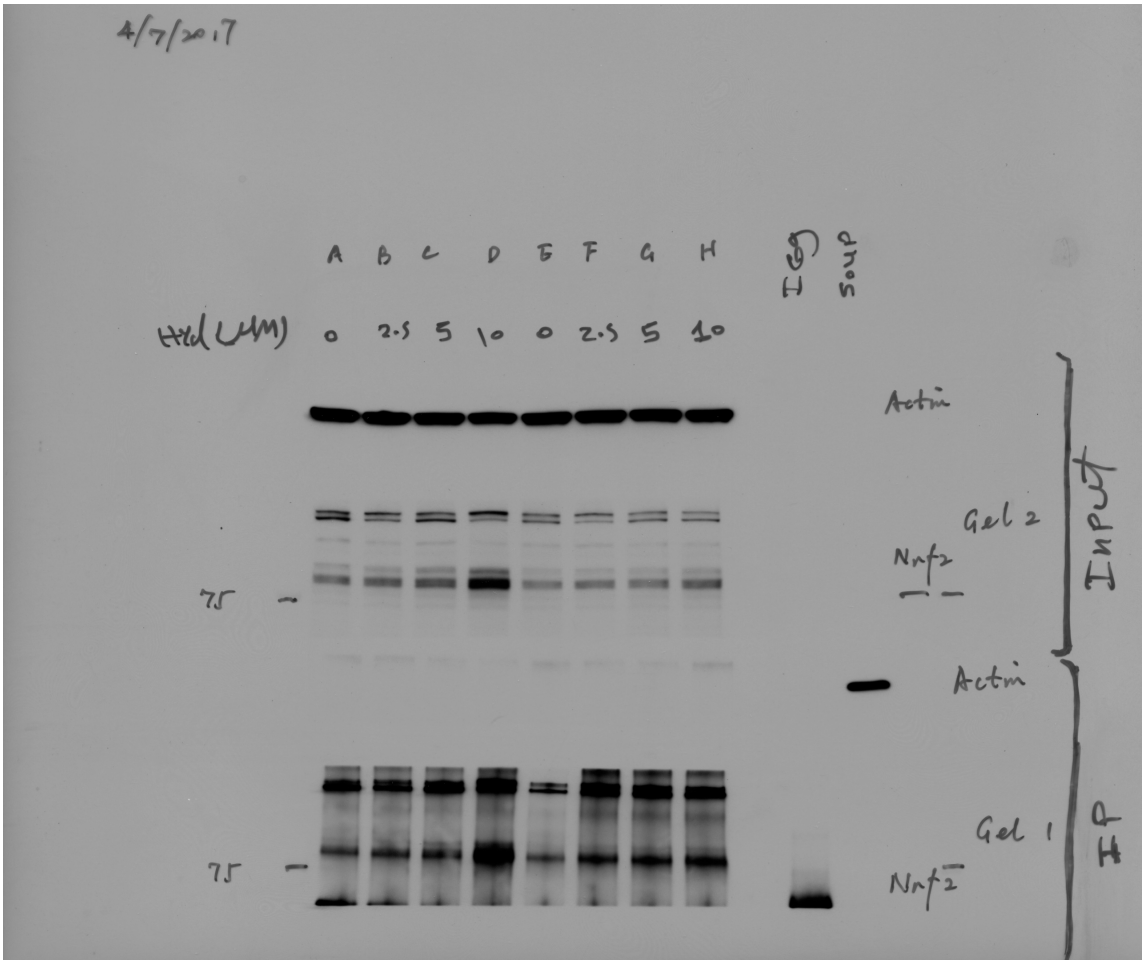
Supplementary Figure 6. Hydralazine protects tauopathy model cells from exogenous and endogenous stressors presenting a therapeutic potential for the treatments of neurodegenerative diseases. (a) Cell growth analysis showed a slower growth rate for aggregate-positive (AP) cells compared to control aggregate-negative (AN) cells. **p<0.01 student t-test, n=8, mean ± SD. **(b)** Hydralazine treatment (5 μM) improves the growth rate of aggregate-positive cells. *p<0.05 and **p<0.01, student t-test, n=8, mean ± SD. **(c)** Superoxide fluorescence signal intensity was higher in aggregate-positive cells compared to the control cells and decreases with hydralazine treatment in a dose dependent-manner in both cell models. *p<0.05 and **p<0.01, student t-test, n=6, mean ± SD. **(d)** Hydralazine (10 μM) improved the viability of both aggregate-positive and negative cells that are under rotenone stress (1 μM). Sulforaphane (5 μM) was used as positive control. **p<0.01, student t-test, n=6, mean ± SD. **(e)** Hydralazine (10 μM) reversed rotenone-mediated (1 μM) reduction in NRF2 protein signal intensity measured by Western blot. *p<0.05 and **p<0.01, student t-test, n=3, mean ± SD.



Supplementary Figure 7. Hydralazine activates SKN-1/NRF2 pathway in worms treated with rotenone. **(a)** Volcano plot showing the ratio (hydralazine+rotenone/rotenone) distribution of proteins quantified by label-free mass spectrometry. Proteins ratios obtained by label free mass spectrometry were uploaded for IPA analysis. SKN-1/NRF2 was number four in the top five activated stress response pathways (p-value cutoff of 0.05, right-tailed Fisher Exact Test.). **(b)** Volcano plot showing the ratio (rotenone/Ctrl) distribution of proteins quantified by label-free mass spectrometry. The results of IPA analysis for worms treated with rotenone compared to control. SKN-1/NRF2 was not among activated pathways (p-value cutoff of 0.05, right-tailed Fisher Exact Test.). **(c)** Volcano plot showing the hydralazine+rotenone/Ctrl ratio distribution of proteins quantified by label-free mass spectrometry. SKN-1/NRF2 pathway was among activated pathways when worms treated with rotenone and hydralazine were compared to control worms (p-value cutoff of 0.05, right-tailed Fisher Exact Test.). We showed our results pathways based on the p-value simply because this is a statistical figure of-merit. From a statistical point of view, a p-value means that the probability of getting the results obtained with a set null hypothesis (in our case was whether the results are statistically significantly different or not) is true. This is an indication for reproducibility. Further, these results were confirmed with the volcano plot based on Tukey Honestly Significant difference Test.

Supplementary Figure 8:

Figure 3a.



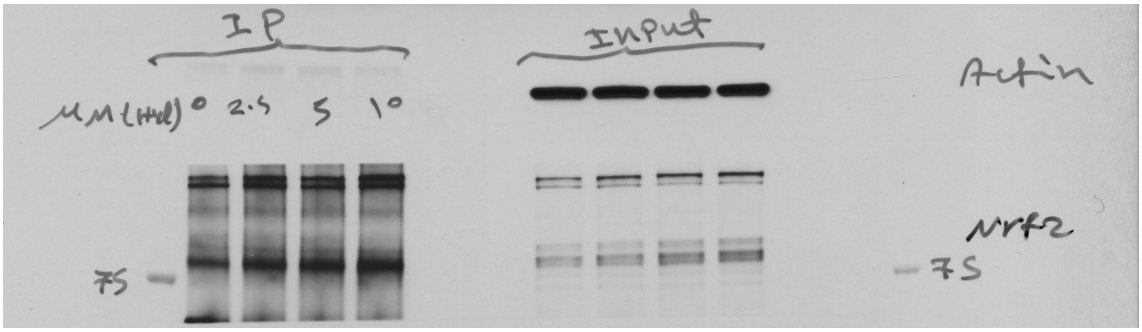


Figure 3c.

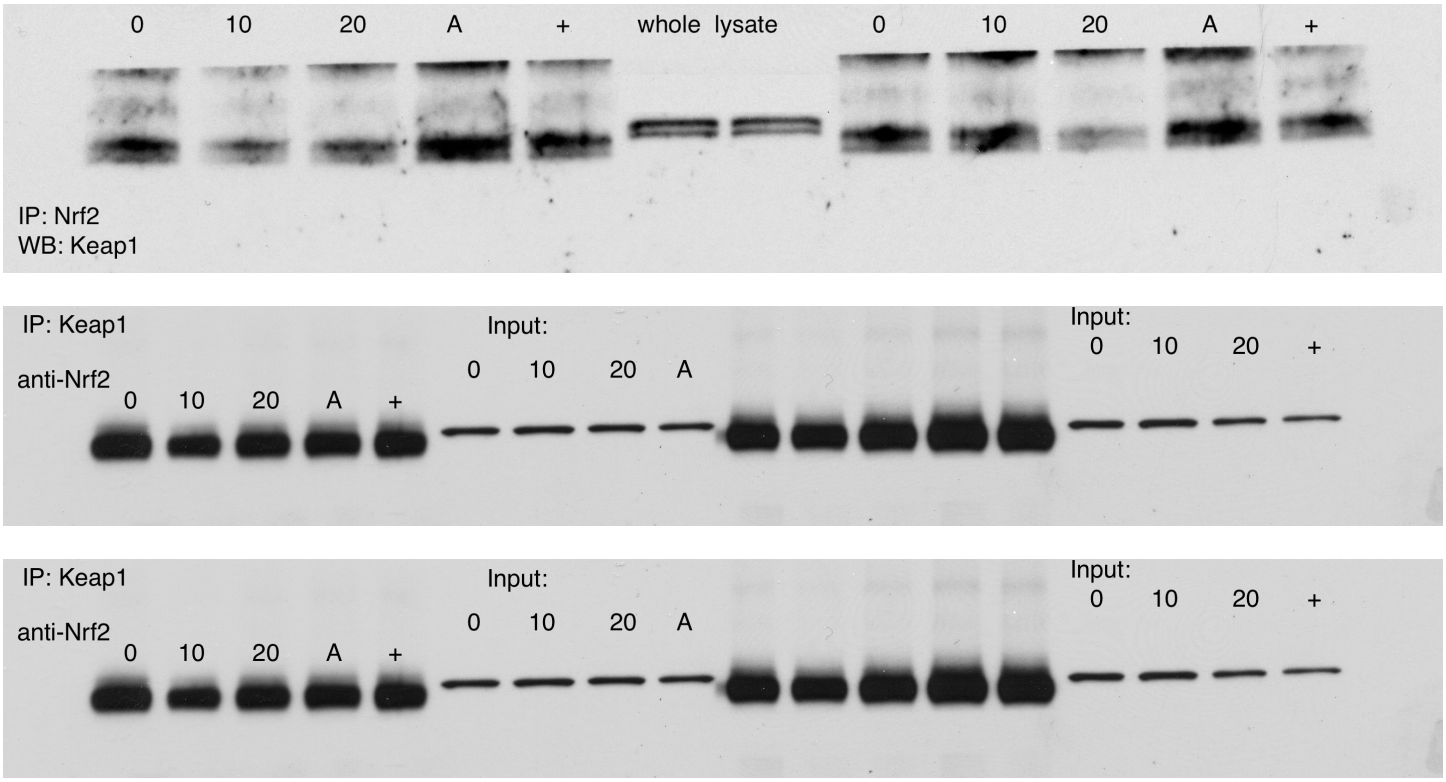

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Figure 3d.

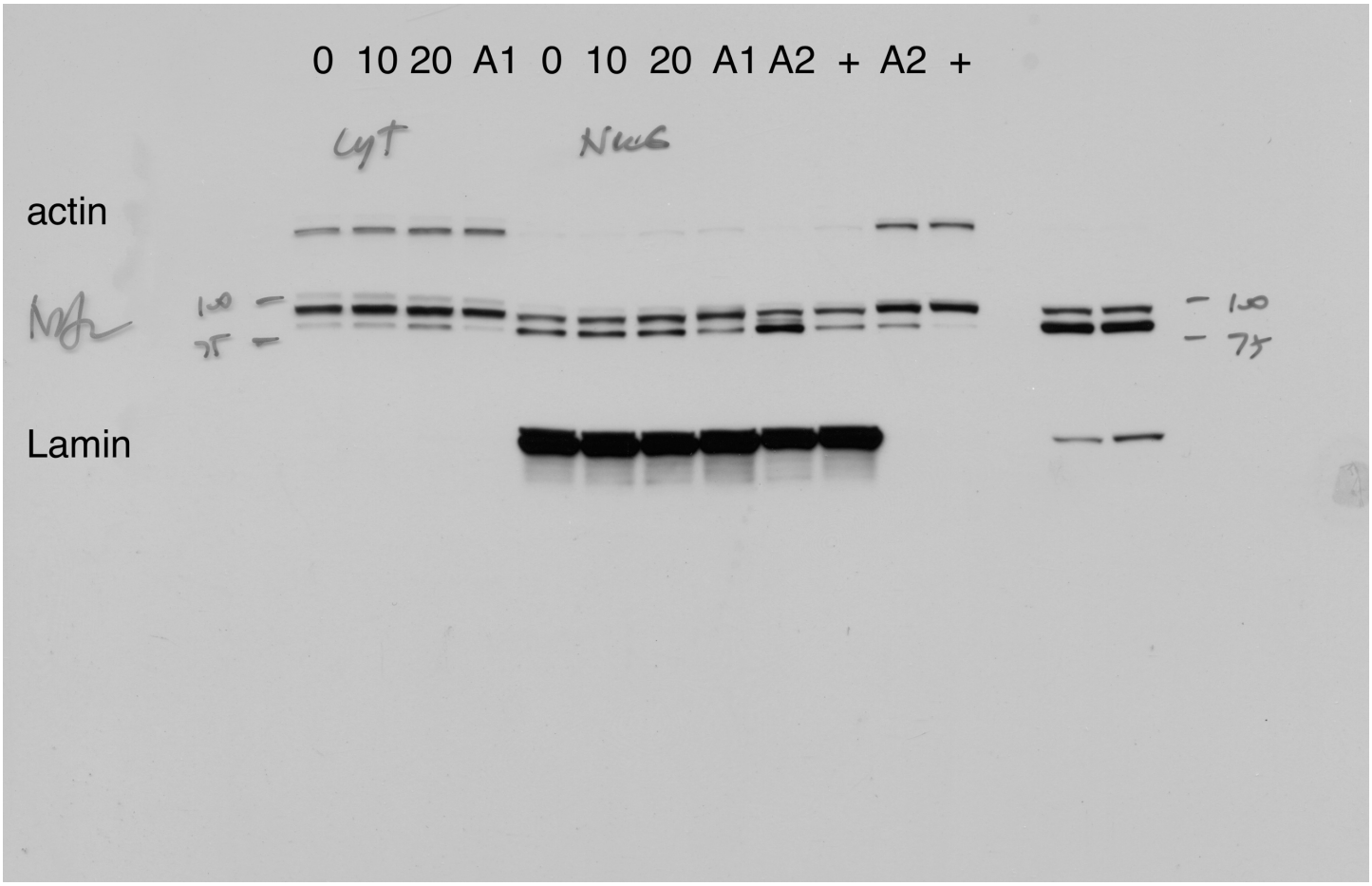
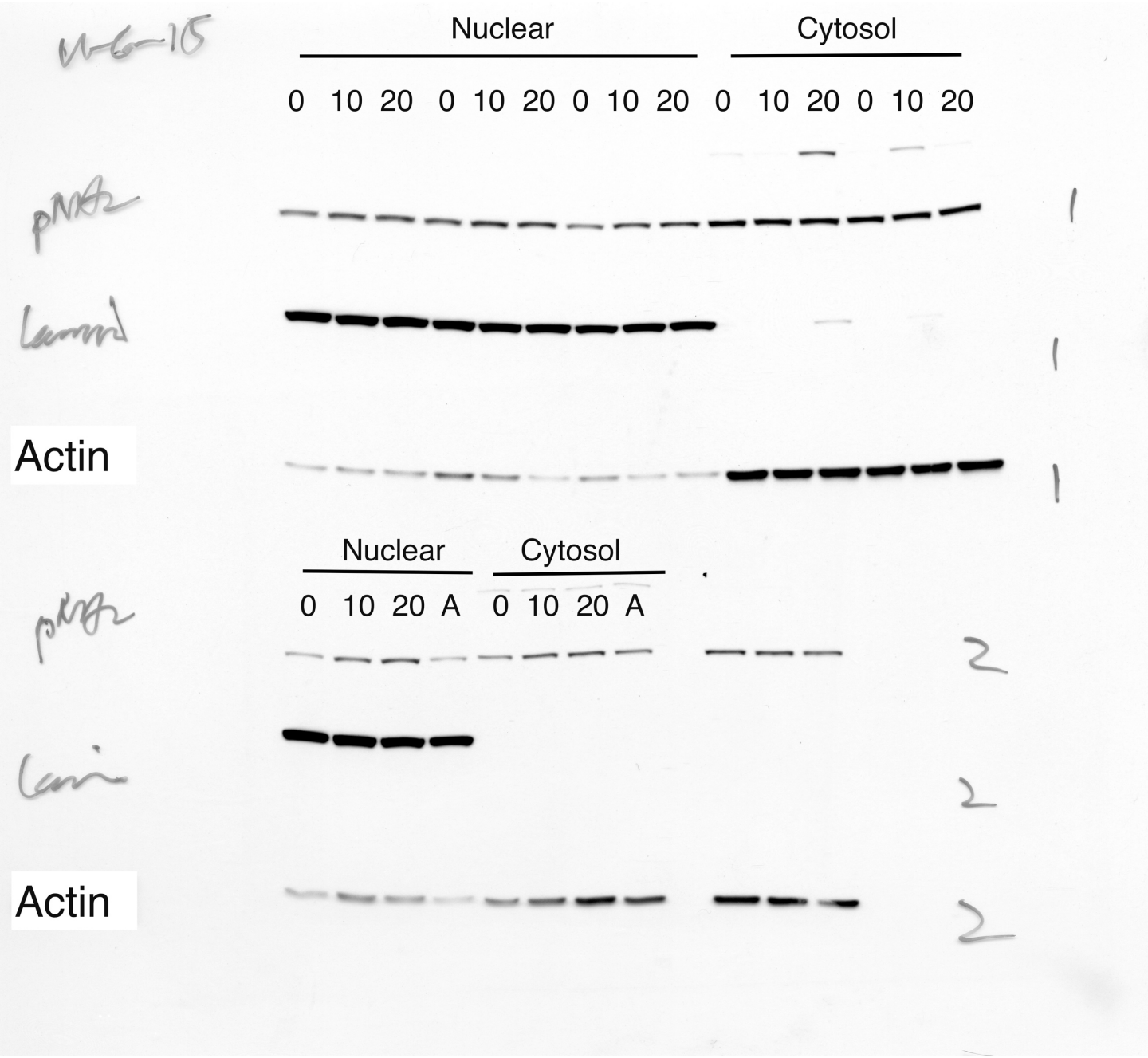


Figure 3e.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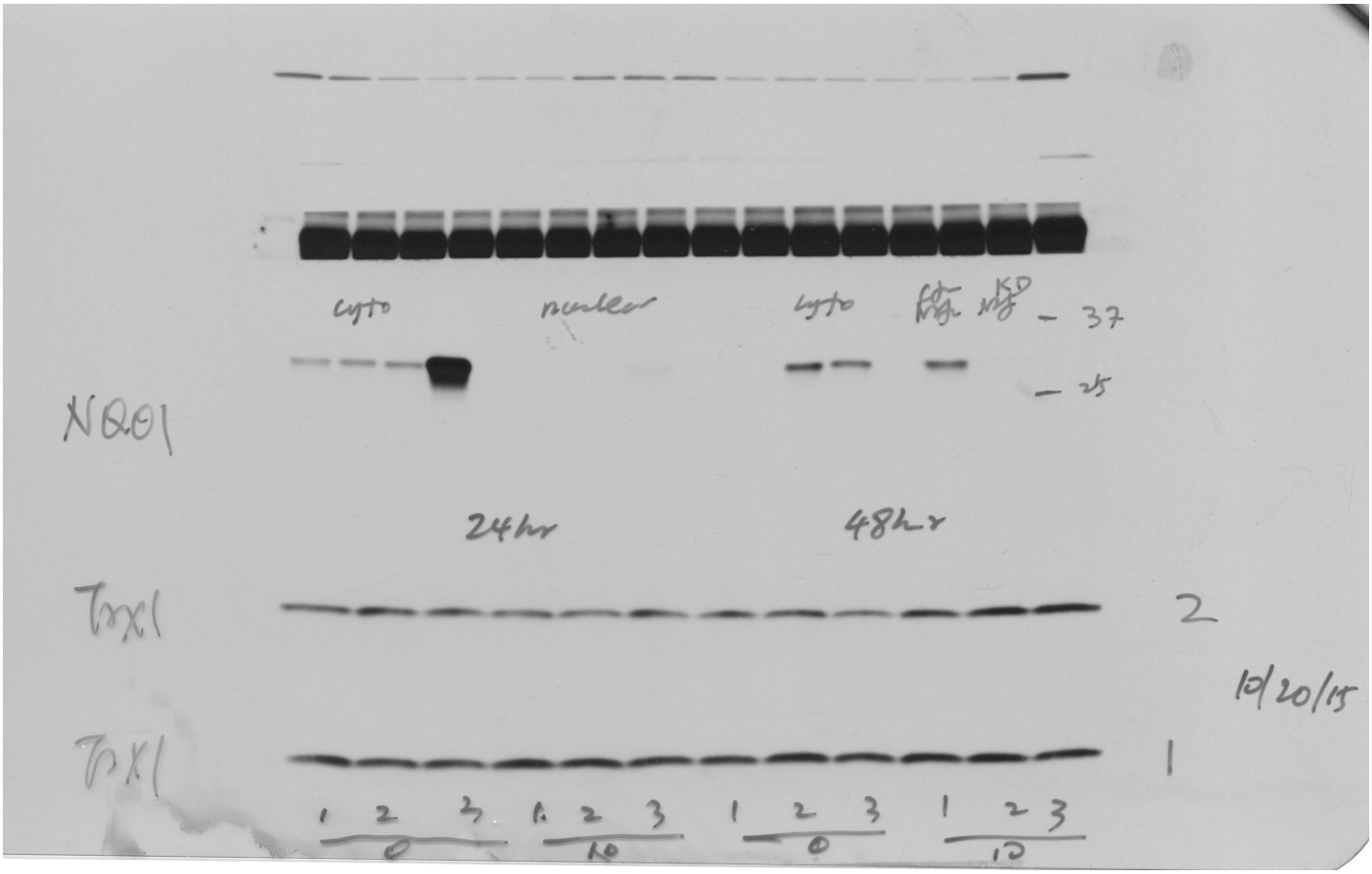
Actin
Nuclear
Cytosol
0 10 20 A 0 10 20 A
Actin

Figure 3f.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- 25
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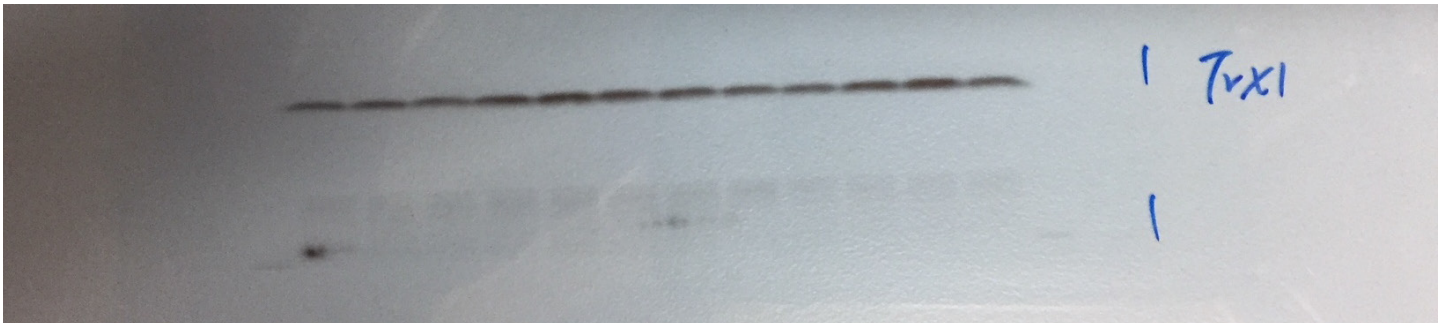
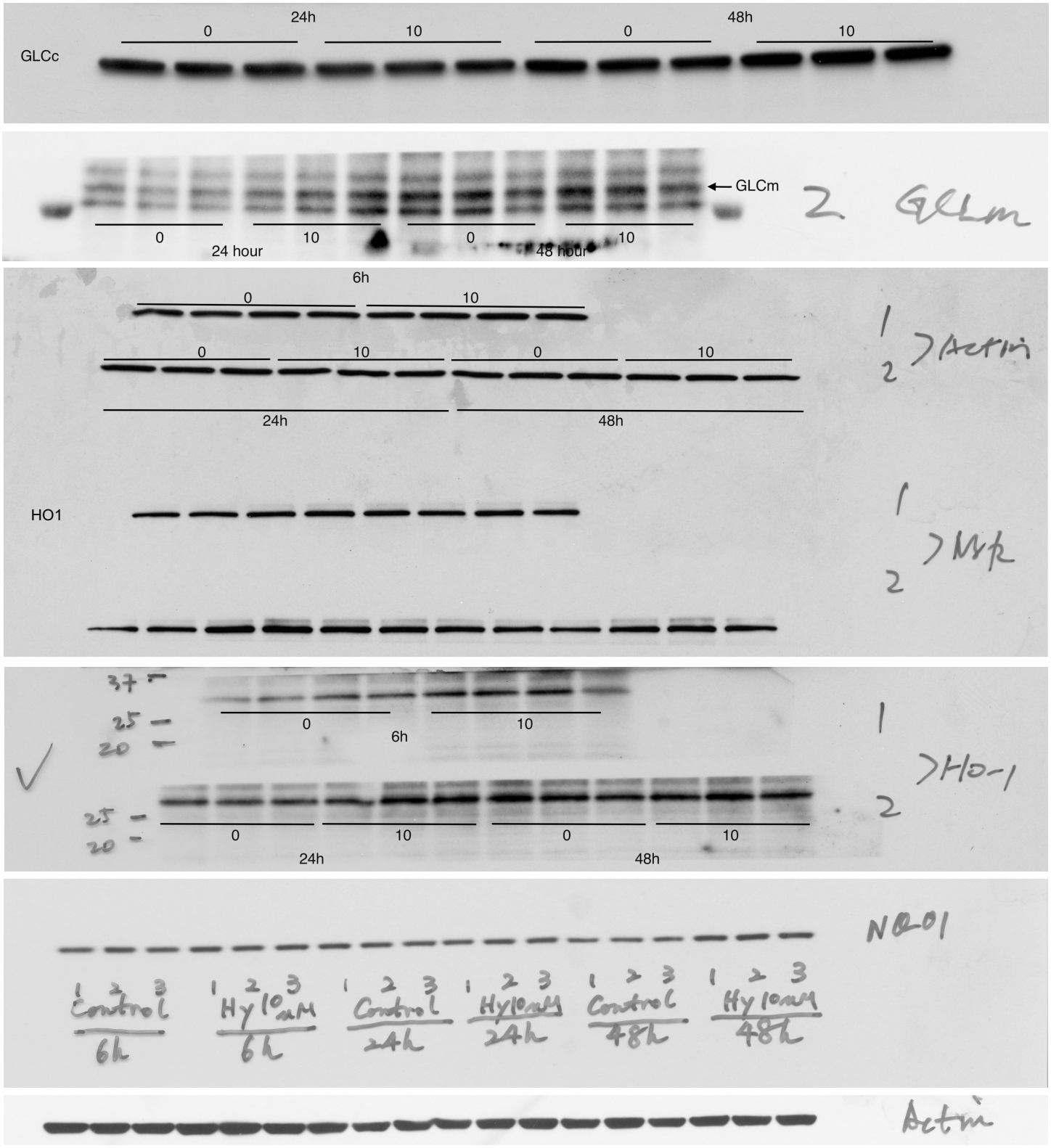

1 TrX1
1

Figure 3i.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Actin
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HO1
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10
24h
48h
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1 2 3
Control
6h
1 2 3
Hy 10 uM
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Control
24h
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Hy 10uM
24h
1 2 3
Control
48h
1 2 3
Hy 10uM
48h
Actin

Supplementary Table 1. Sequences of primers used for quantitative real-time PCR analysis of NRF2 pathway gene expression.

Gene name	Forward (5'-3')	Reverse (5'-3')	Annealing temperature (°C)
NRF2	**AACCACCCTGAAAGCACAGC**	**TGAAATGCCGGAGTCAGAATC**	60
NQO1	**CGCAGACCTTGTGATATTCCAG**	**CGTTTCTTCCATCCTTCCAGG**	60
HMOX1	**TCTCTTGGCTGGCTTCCTTAC**	**GCTTTTGGAGGTTTGAGACA**	60
GST4	**GAGAACCCTGATTGACATGTA**	**GCTGATTACCAACAAGAAAGC**	60
GSTP1	**TCCCTCATCTACACCAACTATGAG**	**GGTCTTGCCTCCCTGGTT**	60
GCLC	**ATGGAGGTGCAATTAACAGAC**	**ACTGCATTGCCACCTTTGCA**	60
GCLM	**GCTGTATCAGTGGGCACAG**	**CGCTTGAATGTCAGGAATGC**	60
β-actin	**GCCGGGACCTGACTGACTAC**	**TTCTCCTTAATGTCACGCACGAT**	60

Supplementary Table 2. Statistical data for lifespan studies.

Corresponding Figure	Strain, treatment	Median Lifespan (days)		% difference	P-values	N (number of animals)	
		Vehicle	Treatment			Vehicle	Treatment
Fig. 5a and Suppl. Fig 5a	N2, 10 µM Hyd	14	15	+07.14	0.3494	105	108
	N2, 50 µM Hyd	13	15	+15.38	<0.0001	86	98
		14	16	+14.28	<0.0001	105	112
	N2, 100 µM Hyd	14	17	+21.50	<0.0001	105	109
		13	16	+23.07	<0.0001	86	88
		15	18	+20.00	<0.0001	75	84
		14	18	+28.60	<0.0001	108	112
Fig. 5b	N2-heat-inactivated 100 µM Hyd	18	22	+22.22	<0.0001	117	120
		17	21	+23.52	<0.0001	102	95
	N2- 100 µM Hyd pretreated HB101	15	15	-	02696	99	90
Fig. 5c	N2,100 µM Hyd	15	18	+20.00	<0.0001	130	112
		14	16	+14.28	<0.0001	117	101
	N2,100 µM Curcumin	15	16	+06.25	0.0815	130	122
	N2,20 mM Metformin	15	17	+13.30	<0.0006	130	99
		14	16	+14.28	<0.0001	117	120
Fig. 5d	N2- Scr. *RNAi*,100 µM Hyd	13	16	+23.07	<0.0001	79	80
		14	17	+21.50	<0.0001	87	84
	N2- *skn-1 RNAi*, 100 µM Hyd	10	09	-10.00	0.0199	89	84
		12	12	-	0.9684	94	102
Fig. 5e	EU31, 100 µM Hyd	10	10	-	0.3257	80	95
		11	11	-	0.4184	112	108
	EU1,100 µM Hyd	11	12	+09.09	0.0141	91	105
		13	15	+16.16	<0.0001	103	97
Fig. 5g	DA1113,100 µM Hyd	22	21	-04.54	0.4118	70	67
		24	24	-	0.2646	82	83
Suppl. Fig. 5c	LG357,100 µM Hyd	13	13	-	0.3820	90	95
		11	11	-	0.3628	108	115
		12	13	+08.33	0.1946	94	93
	LG348,100 µM Hyd	14	16	+14.28	<0.0001	108	101
		12	13	+08.33	0.0868	68	69
		11	12	+09.09	0.0304	77	75
Suppl. Fig. 5e	CF1038,100 µM Hyd	10	12	+20.00	<0.0001	56	60
		11	13	+18.18	<0.0001	64	64
Fig. 6d	BR6516, 100 µM Hyd	13	17	+30.76	<0.0006	64	60
	BR5270,100 µM Hyd	08	11	+37.50	<0.0001	106	105
		10	13	+30.00	<0.0001	71	75
		11	13	+18.00	<0.0001	114	112