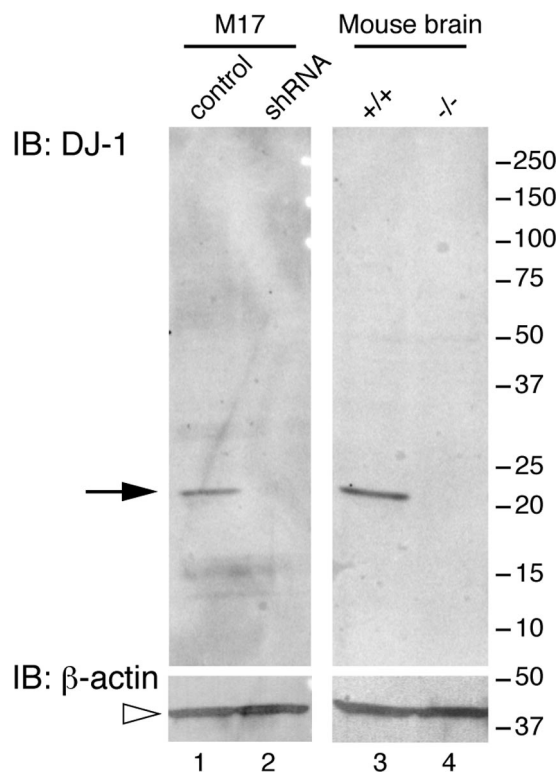# Supporting Information

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Fig. S1. Quality control for DJ-1 antibody. The antibody to DJ-1 used for immunoprecipitation was validated by Western blotting in both human cell lines (M17 neuroblastoma; lanes 1 and 2) and mouse brain (lanes 3 and 4). Negative controls were shRNA cell lines for the M17 cells (lane 2) and knockout mouse brain (lane 4). In both human and mouse, a single band of ≈22 kDa is seen that is greatly decreased or absent after knockdown or knockout of DJ-1, respectively. The same blots were reprobed with a mAb to β-actin to confirm equal loading. Molecular mass markers on the right of the blots are in kDa.
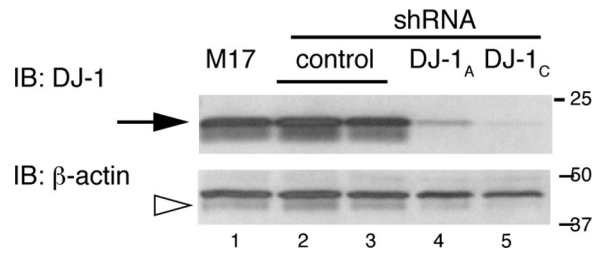
Fig. S2. Quality control for DJ-1 shRNA lines. We used lentiviral constructs to make stable cell lines expressing either a nonsense shRNA (lanes 2 and 3) or two distinct shRNA sequences to DJ-1 (lanes 4 and 5). The nonsense shRNA did not affect DJ-1 expression levels compared with parental cells (lane 1) but the shRNA constructs decreased expression by >85 and >95%, respectively. The blot was reprobed with β-actin to confirm equal loading.

Fig. S3. DJ-1 affects steady-state protein but not RNA levels. (*a*) M17 cells expressing a control shRNA (lanes 1 and 2) or an shRNA to DJ-1 (lanes 3 and 4) were blotted for GPx4 or β-actin. Lanes 1 and 2 are under basal conditions, where GPx4 levels were higher in DJ-1 knockdown cells, and lanes 3 and 4 show that after PQ GPx4 is induced in control cells but is decreased in knockdown cells. Vertical bar indicates that replicate samples were removed for display purposes. (*b* and *c*) Bar graphs show quantification of protein (*b*) and RNA (*c*) levels under the four conditions averaged from two independent cell lines. (*d* and *e*) A similar pattern of higher basal levels but a failure of induction of MAPK8IP1 protein was seen, without increases in RNA levels (*f*). (*g*) In WT mice, aging is associated with accumulation of acidic DJ-1 isoforms. pI markers are shown above the blots. (*h*) Quantification of DJ-1 immunoreactivity from $n = 3$ animals per group. (*i*) Higher MAPK8IP3 protein levels were seen in young (<1 month) knockout mice compared with WT controls. With aging (24 months), MAPK8IP3 levels were increased in WT animals but decreased in DJ-1 knockout animals. (*j* and *k*) Quantitation for MAPK8IP1. (*j*) and MAPK8IP3 (*k*) is shown for protein level, from $n = 3$ animals per time point. Differences between the two age groups and the interaction between age and genotype are significant by two-way ANOVA.

Fig. S4. DJ-1 deficient flies are preferentially sensitive to GSH synthesis inhibitors. Isogenic controls in black (■), mutants for DJ-1b (DJ1b KO; ▲) in blue and for both DJ-1a and DJ-1b (DKO, double knockout; ▼) in red were exposed to 5% BSO + 1 mM Na_2SeO_3 (*a*) or 1 mM PQ + 1 mM Na_2SeO_3 (*b*). All were chemicals given by addition to the agar. Percent of flies alive is presented as mean ± SEM; 150 flies total (0–3 days old) were examined for each condition. Differences between strains were significant when using two-way ANOVA for exposure and strain on either the BSO or PQ datasets ($P < 0.001$).

Table S2. Selenoprotein and selenium metabolism enzyme transcripts associated with DJ-1

Symbol	GenBank accession no.	Definition	Fold enrichment	Diff_ score
SEPW1*	NM_003009.2	Selenoprotein W, 1	12.1	371
GPX4*†	NM_002085.1	GSH peroxidase 4 (phospholipid hydroperoxidase)	9.2	371
SELH*	NM_170746.1	Selenoprotein H	5.3	115
SEPX1*	NM_016332.2	Selenoprotein X, 1	3.6	231
SEPHS2*†	NM_012248.2	Selenophosphate synthetase 2	2.6	213
SELT*	NM_016275.3	Selenoprotein T	2.4	203
GPX3*	NM_002084.2	GSH peroxidase 3	2.2	79.5

*Confirmed by RT-PCR in M17 cells.
†Confirmed in mouse brain

Table S3. Nuclear and mitochondrial encoded mitochondrial transcripts associated with DJ-1

Symbol	GenBank accession no.	Definition	Fold Enrichment	Diff_ score
MTND4*	NM_173711.1	NADH dehydrogenase 4	120	356
MTND2*	NM_173709.1	NADH dehydrogenase 2	83	82.8
MTATP6	NM_173702.1	ATP synthase 6	54	371
MTND5*	NM_173713.1	NADH dehydrogenase 5	46	50.0
MTND1*†	NM_173708.1	NADH dehydrogenase 1	39	371
MTCO2*	NM_173705.1	Cytochrome *c* oxidase II	7.5	109
MTCO1*	NM_173704.1	Cytochrome *c* oxidase I	4.0	359
NDUFB1	NM_004545.3	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 1.7 kDa	3.9	329

  * Confirmed by RT-PCR.
†Confirmed in mouse brain.

Table S4. PTEN/Akt pathway transcripts associated with DJ-1

Symbol	GenBank accession no.	Definition	Fold enrichment	Diff_ score
JUND*	NM_005354.2	Jun D protooncogene	9.0	371
RPS6KB2	NM_003952.1	Ribosomal protein S6 kinase, 70 kDa, polypeptide 2	5.4	39.8
PPP2R2C*	NM_181876.1	Protein phosphatase 2, regulatory subunit B, γ isoform, variant 2	4.0	41.2
BCL2L1[†]	NM_001191.2	BCL2-like 1, transcript variant 2	3.8	309
RASL10B	NM_033315.2	RAS-like, family 10, member B (RASL10B)	3.5	128
MAPK8IP1*[†]	NM_005456.2	MAPK 8-interacting protein 1 (MAPK8IP1)	3.4	103
EIF4EBP1	NM_004095.2	Eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1)	3.2	58.7

* Confirmed by RT-PCR.
[†]Confirmed in mouse brain.

Table S5. Additional RNA sequences retrieved from CLIP cloning experiments.

Gene	GenBank accession no.	Sequence
Additional candidate interactors based on BLAST from recovered sequences		
NUP98	NM_016320.3	taccaaagatggatagccaaggagcgtttccaatcca
PPFIA3	NM_003660.2	gagccgtttcaggcacataatcccataa
RBM5	NM_005778.1	ctgcctttggctcctaggccagctccctttagcgaactt
LZTR1	NM_006767.2	acgaactccacgtcgcagaactggcgg
CDC14	NM_003671.2	gccagaagcgagagcccctcgg
PPP1R16B	NM_015568.2	tgggcaccttctccagcagctgcagctccgtca
CDC14	NM_003671.2	cgaggggctctcgcttctggcgccaagcgcccg
SLC35E1	NM_024881.3	ggaggcaaaggagaagagagaagtttcccttccatctggatctgg
ELAVL3	NM_032281.2	gacgggaaaggagtgtggggctgggccttt
EIF4B	NM_001417.3	accggccaccatatcgatccat
EXOC7	NM_001013839.1	tcccatctagaccatgctgggaatactgtttcagaaggttct
MYCN	NM_005378.4	ccggggctgcgggcacgagcgctgg
LOC728937	NM_001093731.2	ggcatcgaagacgctcgcttcagaaatgtccct
Short sequences that could not be identified		
		tcgtcggactgtagaactctgaacctgtcggtggtcgccgtatcattaatcccgcggcca
		cagtcttagcaggaccgcctcttcgaccgacttgagtgct
		atttgggtggggacagagttccaaaccatcacata
		cgatcggtcgcgcagctcagtaggataga
		gattaatgatactgcgaccaccg
		attggccttgaaggactg
		gccgggacgttagcac
		ccccaagacct
		atcaccagggtga
		cgctccctcgctg
		gggcaggcacatgt
		ccggaacccaaa
		gcacggccagggaa
		gtagtggttcat
		taccgctgg
		ggcctttggata
		agtgattctggga
		ggcgatcatt

Other Supporting Information Files

Table S1 (XLS)