

SUPPORTING INFORMATION

Supplemental Figure 1. Validation of valosin-containing protein (VCP) as a nonspecifically cross-reacting protein. VCP-directed IP from wild-type and DJ-1 knockout mice followed by Western blotting with a DJ-1-directed antibody unequivocally establishes nonspecific crossreactivity of the DJ-1-directed antibody with VCP. A VCP-directed polyclonal antibody was used to capture VCP side-by-side from tissue extracts of DJ-1^{-/-} mice and wild-type mice which had been subjected to the tcTPC procedure. A subsequent Western blotting analysis in which the blotting membrane was probed with the DJ-1 antibody we had used for the DJ-1 interactome investigation (i) established that the DJ-1 antibody can cross-react with a 97 kDa band present in both DJ-1 knockout and wild-type mice, (ii) documented that this cross-reactive band is strongly enriched in the VCP directed immunoprecipitation eluate, and (iii) showed complete absence of DJ-1 in the same VCP immunoprecipitation eluate. Thus, the presence of DJ-1 in the interactome list could be explained by an apparent cross-reactivity of the commercial DJ-1 antibody we had employed for the generation of the interactome dataset. Extracts before co-IP (lanes 1 and 4), unbound co-IP material (lanes 2 and 5) and co-IP eluate fractions (lanes 3 and 6). Please note the increase in DJ-1 antibody signal for a band of ~95 kDa (VCP has apparent MW of 97kDa) following VCP directed co-IP from DJ-1 knockout mouse extracts (lanes 4 and 6).

Supplemental Figure 2. Determination of specific candidate DJ-1 interactors based on iTRAQ ratio comparisons. The WT:KO iTRAQ ratios of brain (115:114) (A) and liver (117:116) (B) DJ-1 interactomes and the HZ:KO iTRAQ ratios obtained for the DJ-1 interactome analysis in mouse embryonic stem cell lines (115:114) (C) are depicted graphically. A solid line indicates a baseline iTRAQ ratio of 1.0 (assuming no DJ-1-specific enrichment in a comparison of specific versus control) and dashed lines

represent thresholds of one and two standard deviations (SD) above the baseline calculated from the iTRAQ ratios observed in the individual subdatasets. Note that in addition to the bait protein (DJ-1), the iTRAQ ratios of two outliers in each graph were removed from the calculation of the standard deviations. Proteins which possessed ratios above the threshold of two SD are bolded.

Supplemental Figure 3. Significant correlation of brain and liver DJ-1 interactome data.

(A) Graph depicting the correlation between brain and liver iTRAQ ratios including the line of best fit (solid line) and 95% confidence intervals (dashed lines). A Pearson correlation coefficient of 0.857 was significant at a two-tailed $P = 0.01$ level (SPSS ver. 18, IBM Corporation). (B) The theoretical relation between sample size and the Pearson correlation coefficient at six different powers and at a two-sided 0.01 significance level is depicted based on R statistical modeling source code (Quick-R, Robert I. Kabacoff, and 'pwr' code library, Stéphane Champely). As demonstrated by the intersection of the dashed lines, the brain and liver iTRAQ ratio sample size of 37 at a correlation coefficient of 0.857 is appropriate.

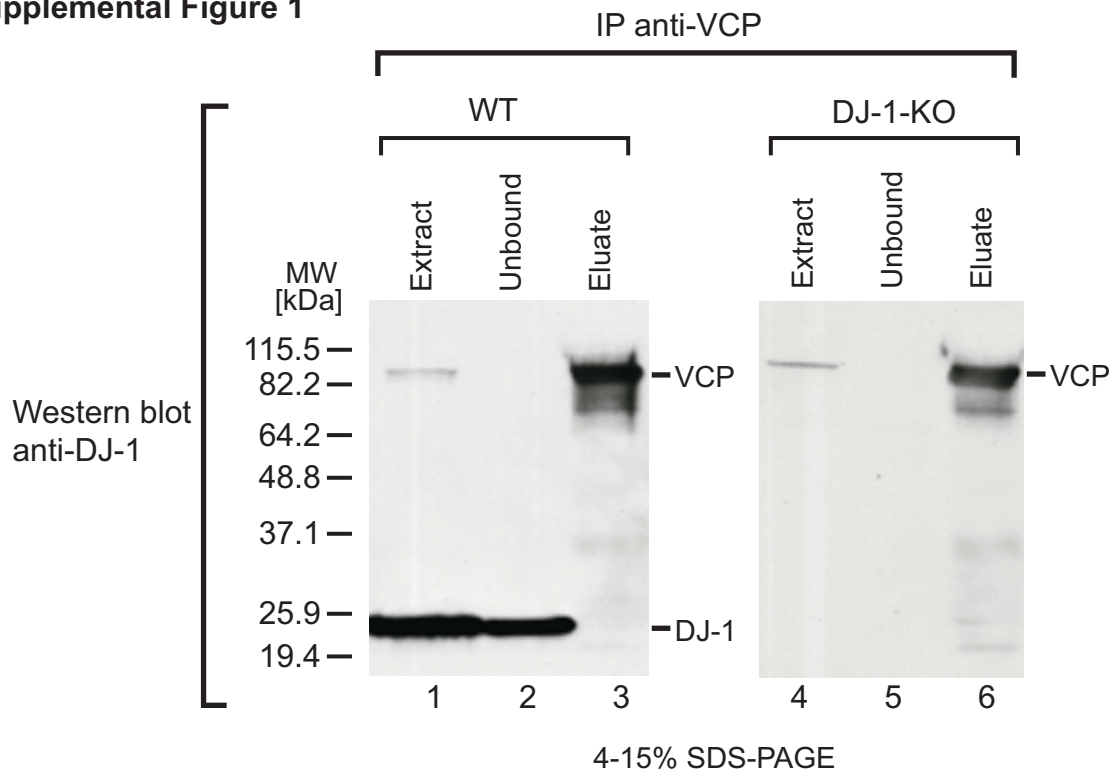
Supplemental Tables. Please refer to **Supplemental Tables 1, 2 or 3** for details of analyses summarized in **Tables 1, 2 or 3**, respectively. Depicted in these Supplemental Tables are information on proteins and peptides observed, including information on masses, percent sequence coverage, confidence scores, iTRAQ ratios, the calculation of average reporter ion intensities and the corresponding standard deviations of this calculation. Note that **Supplemental Tables 1, 2 or 3** depict analyses conducted with ProteinPilot, ProteinProspector and Mascot, respectively, and therefore the formats of the tables (and confidence scores) reflect the different designs of these search algorithms.

Supplemental Table 1. Details of DJ-1 candidate interactors in spectral counting-based comparison of formaldehyde crosslinked DJ-1 KO, HZ or WT ES cells +/- H₂O₂.

Supplemental Table 2. DJ-1 candidate interactors in spectral counting-based comparison of formaldehyde crosslinked DJ-1 KO, HZ or WT ES cells +/- H₂O₂.

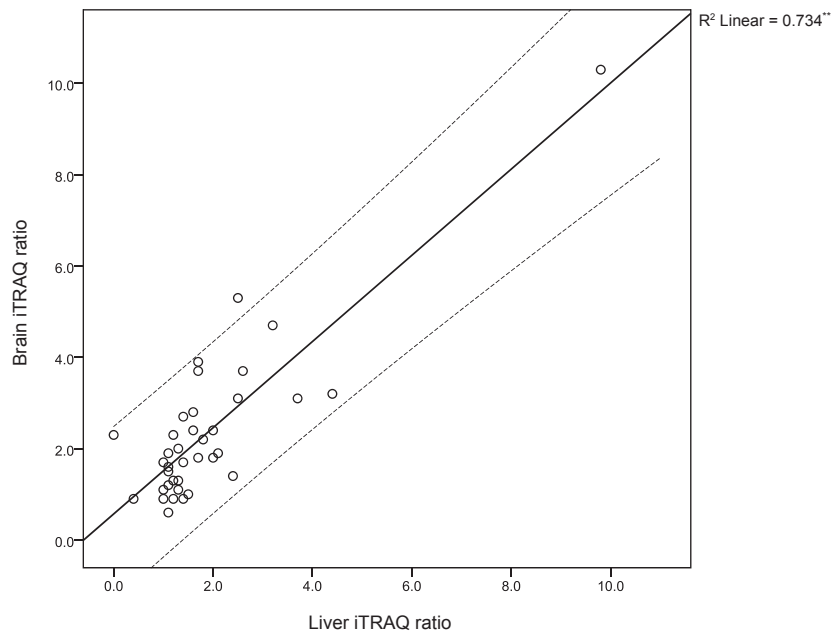
Supplemental Table 3. DJ-1 candidate interactors in four-plex iTRAQ-based comparison of formaldehyde crosslinked DJ-1 KO, HZ or WT ES cells +/- H₂O₂.

Supplemental Figure 1



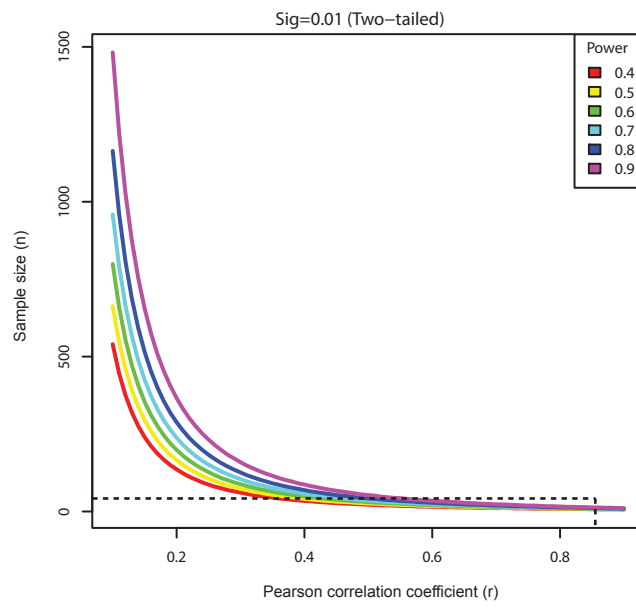
Supplemental Figure 3

A



** Pearson correlation coefficient = 0.857 (significant at the 0.01 level, 2-tailed)

B



	2	0.02	1219.85	610.33	1218.63	610.32 EATTEGQLR	I:TRAQ		99	0.0	0.0	48.5	51.5					
	2	-0.02	1087.87	534.79	1087.69	534.80 QNVKAEK	I:TRAQ	I:TRAQ(K)@7	98	0.8	2.7	43.3	53.2					
	2	0.02	1151.60	576.81	1151.58	576.80 EYWNLRK	I:TRAQ		94	1.8	3.9	41.6	52.7					
	2	0.06	1118.61	593.31	1118.55	593.29 DAAELWQK	I:TRAQ	I:TRAQ-Lys-Gln@9	90	3.7	7.5	26.4	52.4					
									Average	2.1	4.8	43.0	50.7					
									STDEV	2.1	4.4	4.7	3.1					
Enolase, alpha	IP00756027.1	4	28.6	3	-0.93	2247.19	750.07	2248.12	750.38 DATNVVDEGGFAPNLK	I:TRAQ	I:TRAQ(K)@19	99	12.8	52.4	8.4	26.4		
												98	12.0	45.2	9.8	33.0		
												98	14.6	41.7	14.0	28.8		
												95	11.7	47.2	11.2	27.6		
												Average	12.8	47.2	11.2	27.6		
												STDEV	1.3	4.7	2.8	2.8		
HspA8	IP00480560.1	6	28.3	3	-0.33	1936.96	646.66	1936.99	646.67 NZVMRPFNTYVDK	I:TRAQ	I:TRAQ(K)@15	99	12.7	42.0	9.5	35.8		
												95	13.4	41.7	5.7	39.2		
												94	17.9	62.6	0.0	19.5		
												90	10.4	41.0	10.2	38.3		
												89	10.6	46.1	4.6	38.4		
												84	20.5	30.9	23.4	25.2		
												Average	14.2	44.1	8.5	32.8		
												STDEV	4.1	10.4	8.0	8.3		
Superoxide dismutase SOD1	IP00130589.7	2	28.3	2	0.02	1317.68	659.85	1317.66	659.84 DGVANVSEDR	I:TRAQ		99	9.4	14.3	31.8	44.5		
												99	5.8	22.0	25.0	47.2		
												Average	7.6	19.1	28.4	46.8		
												STDEV	2.5	5.4	4.8	1.9		
Peroxiredoxin-1	IP00848536.1	18,927	4	26.8	2	0.00	1037.53	519.77	1037.53	519.77 ADEGISFR	I:TRAQ		99	7.1	17.5	12.9	62.4	
												99	6.1	27.2	12.4	84.1		
												98	6.1	12.7	15.4	66.1		
												Average	6.0	19.1	13.9	61.0		
												STDEV	1.4	7.4	2.2	6.3		
Carbonic anhydrase 3	IP00221890.6	4	28.2	3	0.02	1625.88	542.87	1625.86	542.86 QDNQSPLENTK	I:TRAQ	I:TRAQ(K)@12	99	23.9	32.8	11.4	32.8		
												99	12.7	33.9	12.3	41.1		
												97	9.9	5.0	19.6	80.4		
												Average	13.9	22.2	14.4	51.2		
												STDEV	11.5	19.3	4.5	25.4		
HspA5	IP00319992.1	3	26.0	3	0.01	2124.14	709.06	2124.13	709.05 SDFSTASDNGQPTVTK	I:TRAQ	I:TRAQ(K)@17	99	13.6	7.7	39.2	39.5		
												93	10.6	42.4	26.8	11.1		
												93	10.6	18.6	34.0	45.2		
												Average	14.5	16.2	30.6	38.7		
												STDEV	11.1	8.4	10.8	7.2		
Glutamate dehydrogenase 1	IP00114209.1	3	25.6	3	-0.01	1634.81	545.84	1634.82	545.85 DVHSGGLATYSR	I:TRAQ		99	12.1	20.2	28.3	39.5		
												97	19.9	32.6	21.0	26.5		
												95	4.8	21.9	20.9	42.3		
												Average	12.1	29.7	26.1	36.1		
												STDEV	7.5	6.7	5.2	8.4		
Syntaxin-binding protein 1	IP00415403.1	4	22.7	3	-0.01	1519.70	507.87	1519.71	507.88 ADQPTAKDQPK	I:TRAQ	I:TRAQ(K)@12	99	18.8	55.6	9.3	16.3		
												99	17.8	70.6	6.3	5.3		
												98	20.5	71.9	2.4	5.2		
												74	15.5	71.3	3.4	9.8		
												Average	18.2	67.3	5.4	9.1		
												STDEV	2.1	7.8	3.1	5.2		
Lactate dehydrogenase	IP00869393.1	38,368	2	22.7	2	0.01	1287.69	644.85	1287.68	644.85 VTLTFREEAR	I:TRAQ		99	6.7	13.4	28.6	51.3	
												95	13.2	20.2	28.6	40.8		
												Average	7.6	16.6	17.5	46.1		
												STDEV	4.1	4.8	1.4	7.4		
Ketoadid dehydrogenase E1	IP00331555.2	50,774	2	21.7	3	0.02	1799.95	600.99	1799.93	600.98 VDDNDYFAVYNATK	I:TRAQ	I:TRAQ(K)@14	99	19.5	20.2	25.1	35.3	
												99	15.3	37.4	12.0	19.4		
												Average	17.4	26.8	18.5	27.3		
												STDEV	11.2	9.3	9.3	11.2		
Creatine kinase B-type	IP00136703.1	2	21.0	2	-0.03	1174.62	588.32	1174.65	588.33 LLMEMQR	I:TRAQ		99	24.6	68.8	2.5	0.0		
												97	18.6	69.4	0.0	0.0		
												Average	29.6	69.1	1.3	0.0		
												STDEV	1.4	0.4	1.8	0.0		
Ig gamma 2b	IP00754836.2	8	19.3	3	0.03	1793.98	599.00	1793.96	599.99 VVSTLPHQDQWM	I:TRAQ	I:TRAQ(K)@5; Deamidated(Q)@10; Dethiomethyl(M)@13	cleaved M-S@C-term	99	25.2	17.8	24.1	32.9	
												I:TRAQ	99	16.6	43.5	28.8	11.1	
												I:TRAQ	99	24.2	25.1	20.9	29.8	
												I:TRAQ	99	27.1	30.2	21.5	21.2	
												No I:TRAQ; Deamidated(Q)@4; I:TRAQ(K)@8; Phospho(T)@8; I:TRAQ(K)@23	cleaved H-T@N-term	97	30.2	29.2	19.1	21.5
												I:TRAQ	99	23.5	20.1	28.6	17.6	
												I:TRAQ	99	27.3	26.8	22.8	23.0	
												I:TRAQ	98	20.8	15.2	28.9	37.1	
												Average	30.0	27.2	23.5	24.3		
												STDEV	3.5	8.6	2.9	8.5		
Phosphoglycerate kinase 1	IP00555069.3	3	19.2	3	0.02	1777.91	593.64	1777.89	593.64 LGDYYNDAFGTAHR	I:TRAQ		99	12.5	11.6	31.3	44.6		
												92	8.5	33.9	24.1	33.5		
												81	9.3	36.4	25.0	30.3		
												Average	10.1	27.3	26.5	36.1		
												STDEV	2.1	13.6	4.2	7.5		
Adenosylhomocysteinase	IP00505533.1	3	19.0	3	-0.01	1938.00	646.34	1938.01	646.35 GIBSEETTDVHNLKY	I:TRAQ	I:TRAQ(K)@15	99	8.8	16.2	24.5	49.5		
												96	0.0	0.0	34.7	65.3		
												85	39.9	13.4	32.9	49.8		
												Average	14.6	9.9	30.7	54.9		
												STDEV	4.9	8.7	5.4	9.1		
Catalase	IP00869393.1	3	16.9	3	0.00	1534.73	512.58	1534.73	512.58 NFDVHPDVGAR	I:TRAQ		99	18.9	15.1	18.0	48.0		
												88	6.0	16.5	25.4	21.1		
												79	3.5	8.2	25.6	63.8		
												Average	9.5	13.3	22.5	54.9		
												STDEV	9.3	4.5	4.0	9.2		
Argininosuccinate lyase	IP00214788.5	2	13.4	2	0.03	1660.94	554.65	1660.91	554.64 AEMQQLGDLK	I:TRAQ	I:TRAQ(K)@12	99	5.1	8.4	26.0	60.5		
												98	1.2	6.6	33.2	59.1		
												Average	3.2	7.5	29.6	59.8		
												STDEV	2.8	1.2	5.1	1.0		
Carbamoyl-phosphate synthase	IP00111908.8	168,495	4	12.9	2	0.02	1233.71	617.86	1233.68	617.85 VPAHYQVTR	I:TRAQ		97	4.3	9.7	24.3	61.7	
												96	0.0	0.0	31.0	69.0		
												93	4.7	7.0	37.6	60.7		

