Supplementary Material for:

DJ-1 is not a deglycase and makes a modest contribution to cellular defense against methylglyoxal damage in neurons

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Supplementary Fig. S1. Efficiency of shRNA DJ-1 knockdown in M17 cells. Stable M17 cell lines transduced with control shRNA or DJ-1 shRNA were blotted for DJ-1 (upper panel, arrow) and β -actin (lower panel, arrowhead) and imaged on an LI-COR Odyssey imager. From left, lanes 1-6 show control shRNA cells seeded at 5,000 (lands 1-3) or 10,000 (lanes 4-6) cells per well and lanes 7-12 show DJ-1 shRNA cells seeded at 5,000 (lanes 7-9) or 10,000 cells per well (lanes 10-12). Markers on the right of the blot are in kilodaltons.

Supplemental Figure S2. **HPLC analysis of the effect of DJ-1 on dG glycation in vitro.** Raw HPLC elution profiles with peaks labeled by species are shown for all conditions in Fig 2A,B. In (E), "+DJ-1 after" is the result of adding DJ-1 after preincubation of MG and dG. In (F), "+DJ-1 before" is the result of adding DJ-1 at the same time and MG and dG.

Supplemental Figure S3. Aldehyde scavengers reduce glycation products similarly to DJ-1. (A) HPLC elution profile of dG glycation by MG in the absence of DNPH. (B) Addition of DNPH with MG results in predominantly unmodified dG, similar to the effect of DJ-1 in Fig. 2A,B and Supplemental Fig. S1F. (C) Incubation of DNPH with MG creates several hydrazones (peaks 5,6, and 7) that absorb at 510 nm as described in (Gilbert & Brandt 1975). (D) Aminoguanidine has similar effects to DNPH (B) and DJ-1 (Fig. 2A,B and Supplemental Fig. S2F) on preventing dG glycation.

Supplemental Figure S4. Proposed mechanism for DJ-1 glyoxalase activity. Electron flow is shown with curved arrows, with MG in red and water in blue. Direction of the reaction is shown with straight arrows, with some steps presumed reversible shown in double arrows. We note that the identities of the general base B and the general acid HA are not known but may represent a protonation/deprotonation cycle of Glu18, although this is speculative.

Supplemental Figure S5. DJ-1 slightly decreases cellular concentrations of irreversible glycation products in whole mouse brain. In all panels, isotope-dilution mass spectrometry was used to obtain relative concentrations of modified vs. unmodified dG, G, or Lys. Two-way ANOVA with multiple comparisons was used for statistical analysis using Šídák's test with p-values shown. Detailed two-way ANOVA values are show in Supplemental Table S9. Small but consistent elevations in glycated products were observed in whole brains from DJ-1^{-/-} mice compared to WT controls. Each measurement is shown as a circle with standard error of the mean shown in error bars.

We performed post hoc power calculations using the R package pwr2 with the function pwr.2way. Alpha was set at 0.05 and values of Cohen's f were derived from sum of squares in the respective two-way ANOVA calculated in Prism (GraphPad Software).

Figure (sample)	# groups Factor A	# groups, Factor B	per n group A	per n group в	f, factor A	f, factor в	Power
5A (M17)	3	$\overline{2}$	$3-6$	$3-6$	1.41	0.65	1,0.965
5B (iPSC)	3	$\overline{2}$	$3-6$	$3-6$	0.609	0.275	0.887, 0.359
5C (neurons)	3	$\overline{2}$	$3 - 4$	$3 - 4$	0.893	0.474	0.96,0.6
6A (CEdG)	2	$\overline{2}$	4	4	0.828	0.695	0.929,0.899
6B (CEG)	$\overline{2}$	$\overline{2}$	4	4	20.18	28.0	1,1
6C (CEL)	$\overline{2}$	$\overline{2}$	4	4	0.868	0.966	0.95,0.994
S ₅ (mouse brain)	3	$\overline{2}$	$5-6$	$5-6$	0.673	1.17	0.941,0.999

Table S1. Power Analysis for Data in Figures 5, 6, and S5

We performed sample size stimulation using the R package pwr2 with the function ss.2way. Alpha was set at 0.05, beta as 0.2 (hence power = 0.8) and values of Cohen's f were derived from sum of squares in the respective two-way ANOVA calculated in Prism (GraphPad Software).

Table S3. Two-way ANOVA details for Figure 5A (CEdG, CEG, CEL in M17 cells)

Table S5: Two-way ANOVA details for Figure 5C (CEdG, CEG, CEL in mouse primary neurons)

Table S6: Two-way ANOVA details for Figure 6A (CEdG in M17 cells, BSO vs. vehicle)

Table S7: Two-way ANOVA details for Figure 6B (CEG in M17 cells, BSO vs. vehicle)

Table S8: Two-way ANOVA details for Figure 6C (CEL in M17 cells, BSO vs. vehicle)

Table S9: Two-way ANOVA details for Figure S5 (whole mouse brain)

Supplemental References

Gilbert, R. P. and Brandt, R. B. (1975) Spectrometric Determination of Methylglyoxal with 2,4-dinitrophenylhydrazine. *Analytical Chemistry* **47,** 2418-2422.