

Supplemental Data

MLi-2, a potent, selective and centrally active compound for exploring the therapeutic potential and safety of LRRK2 kinase inhibition

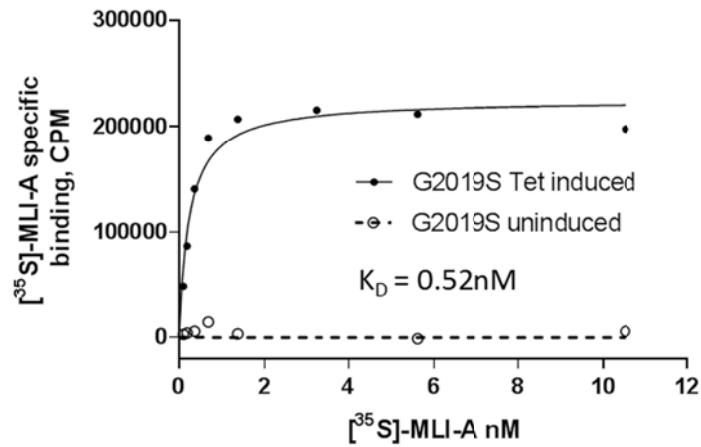
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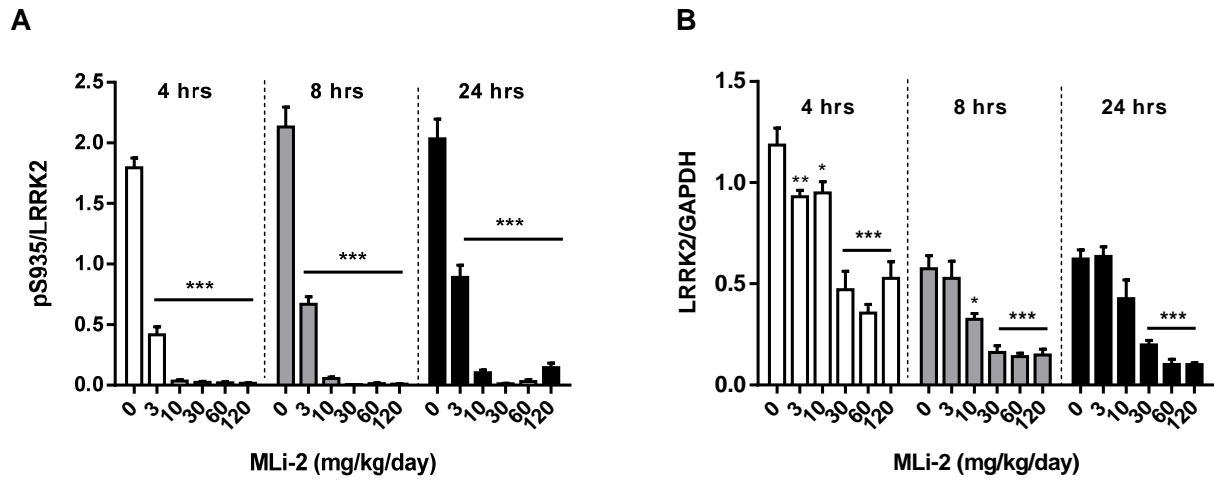
(JPET #227587)

Compound	[³⁵ S]-MLi-A Binding IC ₅₀ (nM)	pSer935 LRRK2 Western IC ₅₀ (nM)
MLi-1	13.3	63.2
MLi-2	3.4	1.4
MLi-3	26.8	60.6
MLi-4	12.4	10
LRRK2-IN-1	145.5	1172

Supplementary Table 1. Comparative Potency of LRRK2 inhibitors in the whole cell radioligand binding assay versus cellular potency in LRRK2 pSer935 LRRK2 dephosphorylation assay. Both assays were performed with WT LRRK2 expressing SH-SY5Y cells.

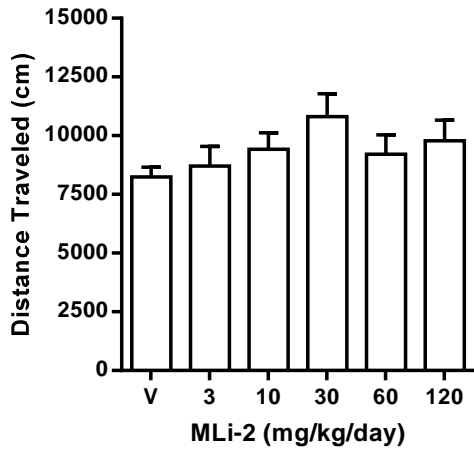


Supplementary Figure 1. Whole-cell saturation binding with increasing concentrations of [³⁵S]-MLi-A in tet-inducible G2019S LRRK2 expressing SHSY5Y cells. Binding was performed in the absence of induction (non-induced) or where expression was induced for 48hr in the presence of tetracycline (TET-induced). The results are presented as specific binding and are averages of triplicate reactions. The K_D value, in nM, is derived from the graph.

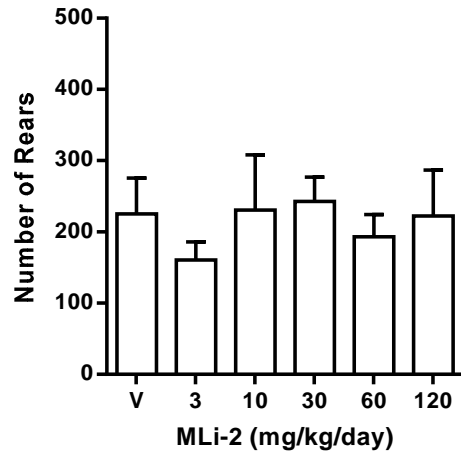


Supplementary Figure 2. Effect of MLI-2 (3 – 120 mg/kg/day) on (a) pSer935 (ratio of pSer935/total LRRK2) and (b) total LRRK2 (total LRRK2/GAPDH) in kidney of mice after 11 days of in-diet dosing. Mice were euthanized at 4, 8 and 24 hours after the start of the dark cycle on day 11 of dosing. Data are mean \pm SEM; n = 5 per treatment group. *, $P < 0.001$ compared with the vehicle diet treated animals.

A



B



Supplementary Figure 3. Effect of MLi-2 (3 – 120 mg/kg/day) on (a) distance travelled and (b) number of rears in mice after 11 days of in-diet dosing. Locomotor activity and rearing behavior were measured over a 60 minute period. Data are mean \pm SEM; n = 5 per treatment group.