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Supplemental information

Discovery of small-molecule positive allosteric modulators of Parkin E3 ligase

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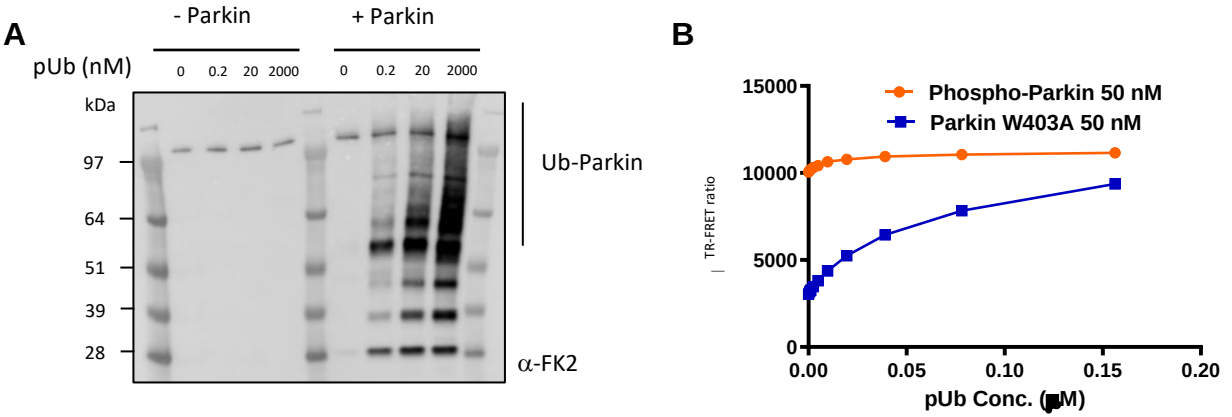


Figure S1. Primary assay development – Related to Figure 1. A) FK2 antibody detects Parkin autoubiquitination as measured by Western blot. Ubiquitin chains with increasing length are generated with increasing levels of pUb in an *in vitro* reaction. B) The TR-FRET signal vs. pUb concentration for W403A-Parkin and phospho-Parkin. W403A-Parkin is pUb-dependent and is not as active as phospho-Parkin, which is pUb-independent. These curves represent n=1 technical and biological replicate.

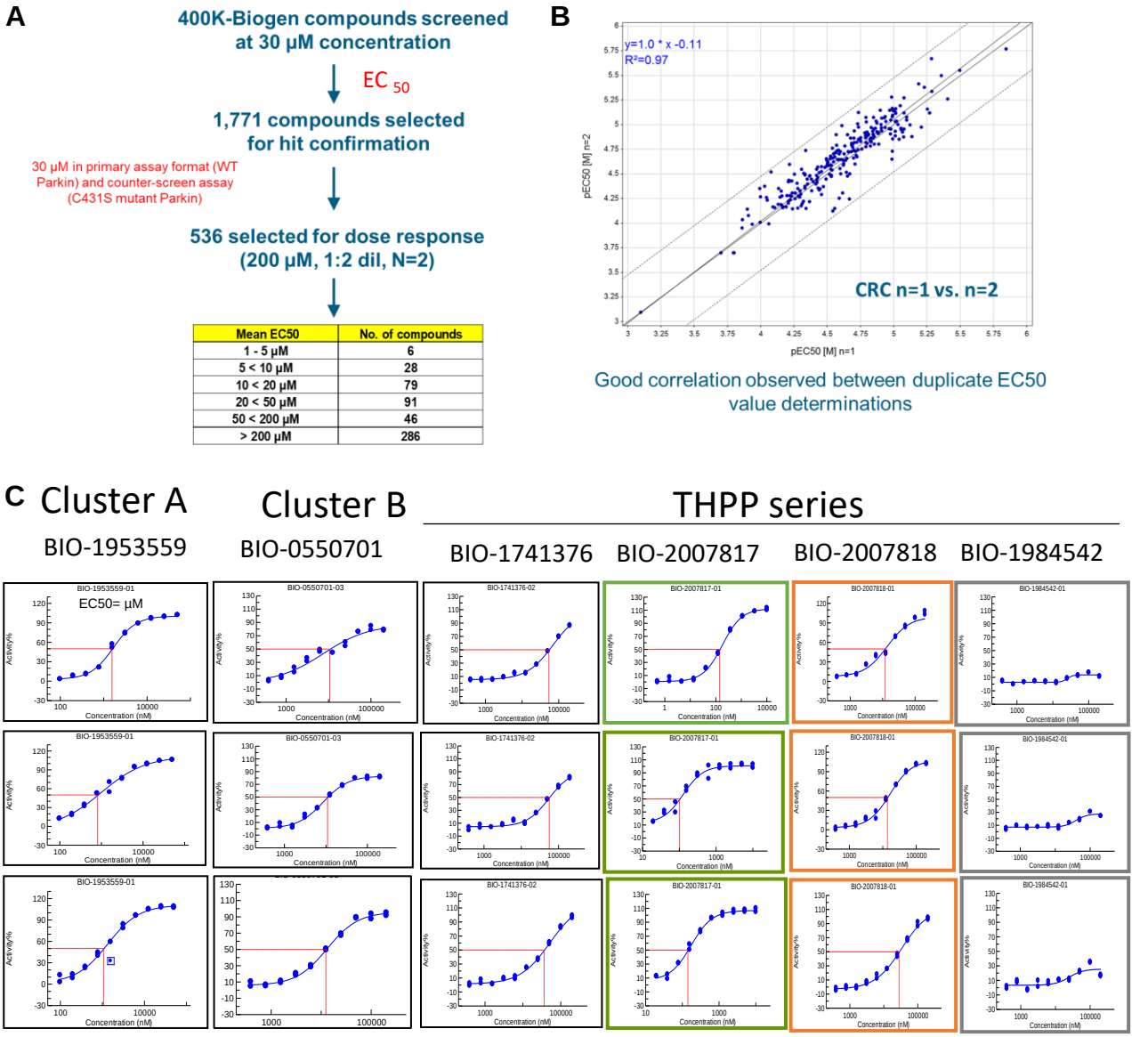


Figure S2. HTS screening platform and data analysis – Related to Figure 2. A) Hit-triage scheme for HTS. B) Correlation between duplicate EC50 value determinations for 536 selected compounds. C) Measurements of concentration response curves for key compounds in Cluster A (BIO1953559 is the single active enantiomer of BIO-0030130 racemate), Cluster B (BIO-0050701), original singleton THPP compound hit BIO-1741376, and representative THPP compounds (BIO-2007817, BIO-2007818 and BIO-1984542). Panel C represents n=3 biological replicates and n=2 technical replicates.

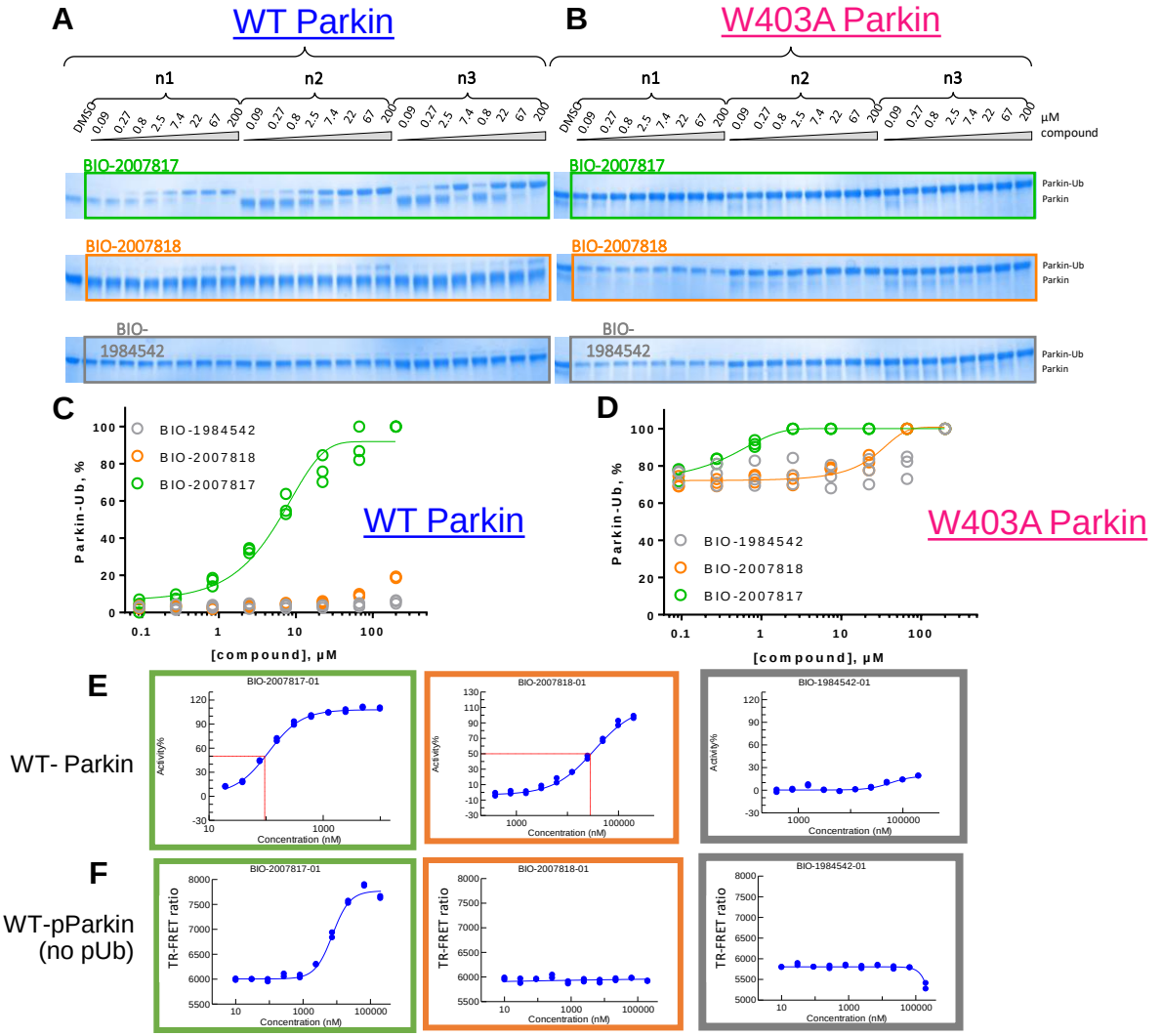
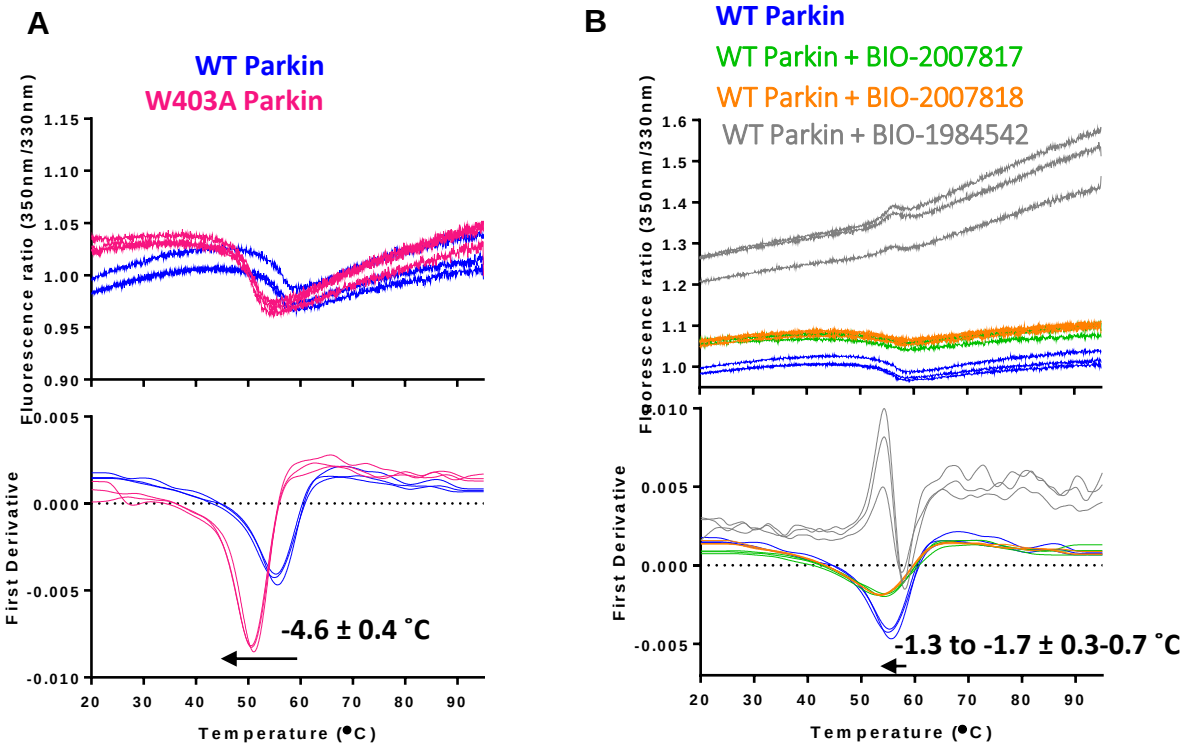


Figure S3. Compounds accelerate Ub-charging regardless for various Parkin activation states – Related to Figure 4. Gel cutouts demonstrating the % charging for A) WT- and B) W403A-Parkin with respect to compound concentrations, n=3 biological replicates. Note that there is a gel loading mistake in n=3 in which 2.5 and 7.4uM compound lanes were swapped, but the quantitation is corrected in the curve in Fig. S3C. C-D) Quantitation of the charging % is shown in C for WT-Parkin and D for W403A-Parkin. TR-FRET autoubiquitination activity assay comparing the 3 THPP compounds in ability to activate WT-Parkin with pUb (Normalized to W403A Parkin in E) and pParkin without pUb (TR-FRET ration in F) demonstrating that BIO-2007817 activating compound (green) is able to activate both forms of Parkin. Curves in E and F represent n=2 technical replicates and n=1 biological replicates.



C

	T_m (˚C), n=1	T_m (˚C), n=2	T_m (˚C), n=3	ΔT_m (˚C)	st. error on ΔT_m (˚C)
WT	55.6	55.0	55.2	-	-
W403A	50.5	50.6	51.0	-4.6	0.4
WT + BIO-2007817	54.4	53.2	54.2	-1.3	0.7
WT + BIO-2007818	53.4	53.6	53.6	-1.7	0.3
WT + BIO-1984542	54.0	53.7	54.0	-1.3	0.3

Figure S4. Compounds alone do not recapitulate the extent of destabilization on Parkin structure imparted by activating W403A-Parkin mutant – Related to Figure 4. A) Activating W403A-Parkin mutant is 4.6 ˚C less thermally stable than WT-Parkin whereas B) compounds only impart 1.3-1.7 ˚C destabilization which is not consistent with their ranked potency. C) Data summary. Experiments were conducted in triplicates. ΔTm is reported on change in average values and standard error is calculated as the sum of square errors of averaged Tm.

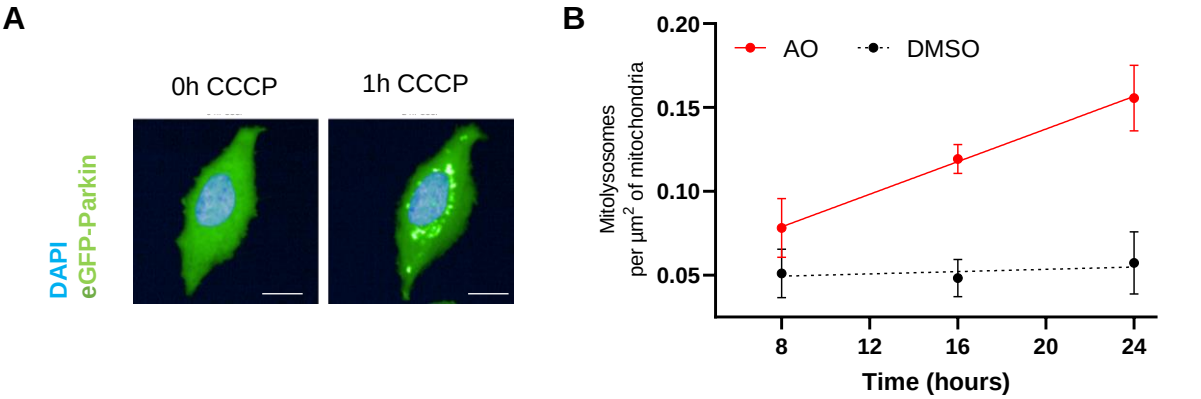


Figure S5. Compounds do not affect the rate of Parkin translocation to mitochondria – Related to Figure 5. A) eGFP-Parkin stable HELA cell line showing perinuclear clustering of GFP signal upon CCCP activation consistent with mitochondrial localization. B) Time-course of MitoQC signal accumulation in stable SH-SY5Y cells upon treatment with 5 µM Antimycin A + 10 µM Oligomycin. Data averaged from 6 wells, error bars represent standard deviation.

Figure 4A

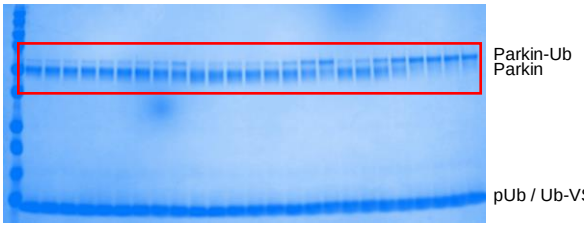


Figure 4C

Figure 4E

Figure 4F

Figure 4G

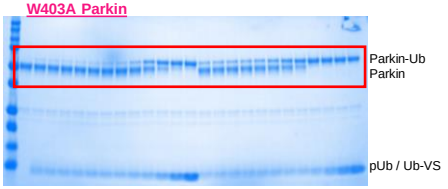


Figure S3A

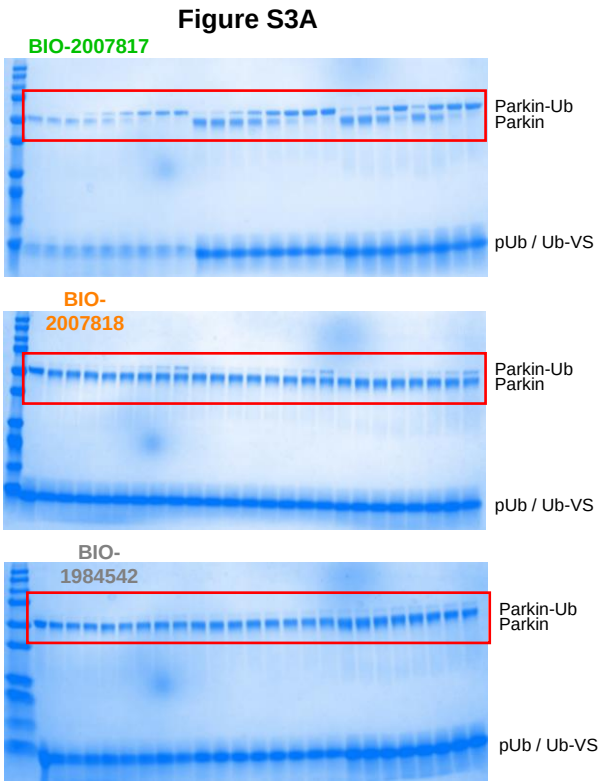


Figure S3B

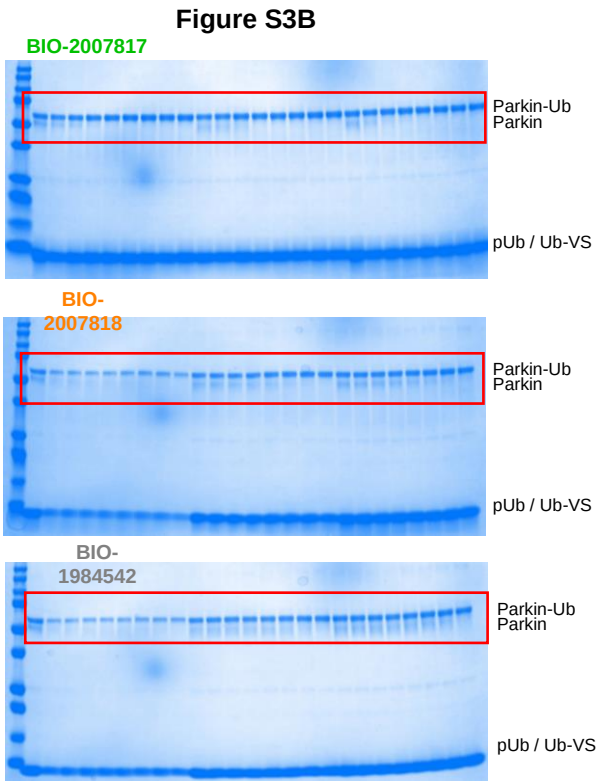


Figure S6. Full gels used Figure 4 and Figure S4 with cut-out area shown in red rectangles – Related to Figure 4.

Table S1 Technical details of the high-throughput screen for Parkin small molecule activators – Related to Figure 2 and STAR Methods.

Category	Parameter	Description
Assay	In vitro	TR-FRET
	Target	20 nM E1, 400 nM E2, 50 nM E3, 380 nM F-UB, 25 µM Ub, 5 µM pUB, 1 mM ATP, 7.5 nM Tb-labeled FK2 ab, 20 mM EDTA
	Detection of TR-FRET	Excitation 340 nm, Emission-520 nm & 490 nm
	Controls	Positive control 20 nM W403A-Parkin Negative control 20 nM C431S -Parkin
	Assay time and temperature	2 hrs ; 37^{o}C
	Ec_{50} pUb	
Library	400,000 compounds	
	Biogen	
Screen	1536 well plates	Oct 24, 2018 – Nov 26, 2018
	10mM compounds with X % DMSO	Plate acceptance criteria: Robust Z' value > 0.5, assay window > 2, track consistency of Ec_{100} pUb control wells, no obvious plate effects.
	C431S & W403A Parkin control	
	Reagent/compound dispensing system	
	Envision 2103 Multilabel Readers, Perkin Elmer	
	Z'=0.84 Assay window 2.8 $\pm$ 0.6	
	Activation for compound median + 3 SD gives Hit cutoff rate 15% of control, 536 hits, 0.13% hit rate	
Post-HTS analysis	Repetition and confirmation at 10 µM concentration response	
	536 compounds (Hit rate = 0.13%) n=2	
	474 compounds passed CRC and QC	
	Repurchased hits to confirm purity	