Supplementary material for

Naturally Occurring Hyperactive Variants of Human Parkin

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TABLE S1: Details of the natural variants included in this study

Variant	MDSGene classification	Age at onset[1]	Type of mutation	Allele frequency (gnomAD)	Population database (dpSNP)	Disease database (ClinVar)	Findings in this study
P37L	Possibly pathogenic	30/36	Homozygous	4.09e-4	Yes	Yes (Conflicting reports of pathogenicity)	Activity similar to WT
V224A	--			4.15e-5	Yes	No	Activity similar to WT
R234Q	Possibly pathogenic	35	Compound heterozygous	1.26e-4	Yes	Yes (Uncertain pathogenicity)	Increased activity when unphosphorylated
R256C	Probably pathogenic	37/56	Heterozygous	6.03e-4	Yes	Yes (Uncertain pathogenicity)	Increased activity when unphosphorylated
M458L	--			1.76e-4	Yes	Yes (Uncertain pathogenicity)	Increased activity when unphosphorylated

[1]Age of onset and type of mutation are from the first reported case of the variant (as per MDSGene and ClinVar). For variants with two ages of onset, multiple patients were tested, and the mutation appeared in two different individuals.

Table S2. Primers used to generate mutants of human parkin

Purpose	Template used	Primer used
To mutate P37L	Human wildtype full length parkin	GTTGCGAAACGTCAGGGTGTTTTAGCA GACCAGCTGCGTGTTATC
To mutate V224A	Human wildtype full length parkin	CCTCTGACAAAGAAACCTCTGCTGCTC TGCACCTGATCGCTACC
To mutate R243Q	Human wildtype full length parkin	CCTGATCGCTACCAACTCCCAGAACAT CACCTGCATCACCTGC
To mutate R256C	Human wildtype full length parkin	CTGGTTTTTCAGTGCAACTCTTGTCACG TTATCTGCCTGGATTG
To mutate M458L	Human wildtype full length parkin	GCGAATGGAACCGTGTTTGCCTGGGTG ACCACTGGTTCGAC
To add stop codon to full length and generate P37L (Ubl)	Human P37L Full length parkin (stop codon added)	TCAGCGTCCGTGGCGTAAATAACAGGA AATGAACGCGACTGG
To mutate R104A	Human wildtype full length parkin	GTGAACCGCAGTCTCTGACAGCTGTTG ACCTGTCTTCCTCTGT
To mutate L102K	Human wildtype full length parkin	GTGAACGTGAACCGCAGTCTAAAACA CGTGTTGACCTGTCTTC
To mutate T103A	Human wildtype full length parkin	ACGTGAACCGCAGTCTCTGGCACGTGT TGACCTGTCTTCC
To mutate V105K	Human wildtype full length parkin	AACCGCAGTCTCTGACACGTAAAGACC TGTCTTCCTCTGTTCTG
To mutate D106S	Human wildtype full length parkin	GCAGTCTCTGACACGTGTTGCCCTGTC TTCCTCTGTTCTGC
To mutate L107L	Human wildtype full length parkin	CAGTCTCTGACACGTGTTGACAAGTCT TCCTCTGTTCTGCCGG
To mutate K161N	Human wildtype full length parkin	AGCGTGTACAGCCGGGTAACCTGCGTG TTCAGTGTTCCAC
To delete residues 101-109	Human wildtype full length parkin	GGTTGTGAACGTGAACCGCAGTCTGTT CTGCCGGGTGACTCC

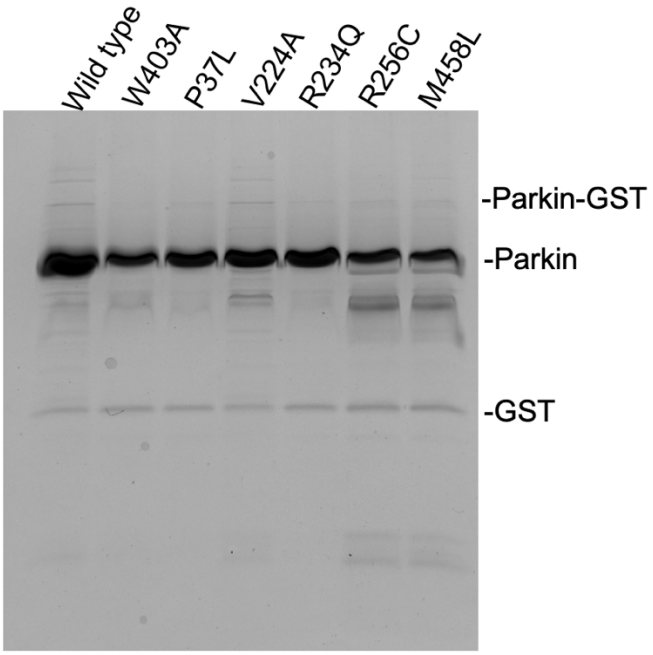

Figure S1: SDS-PAGE analysis of purified proteins.

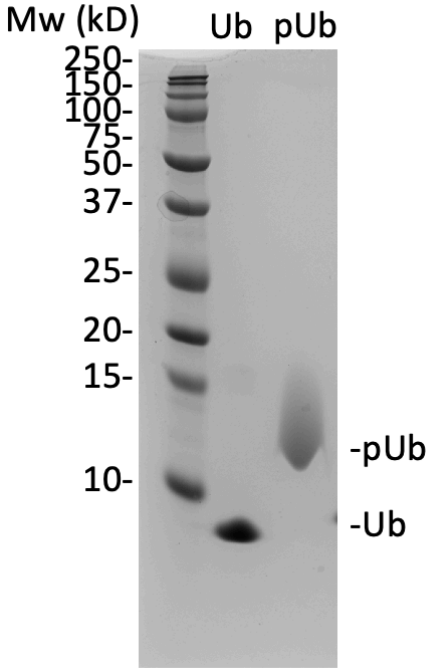

Figure S2: Phos-tag gel analysis of ubiquitin phosphorylation.

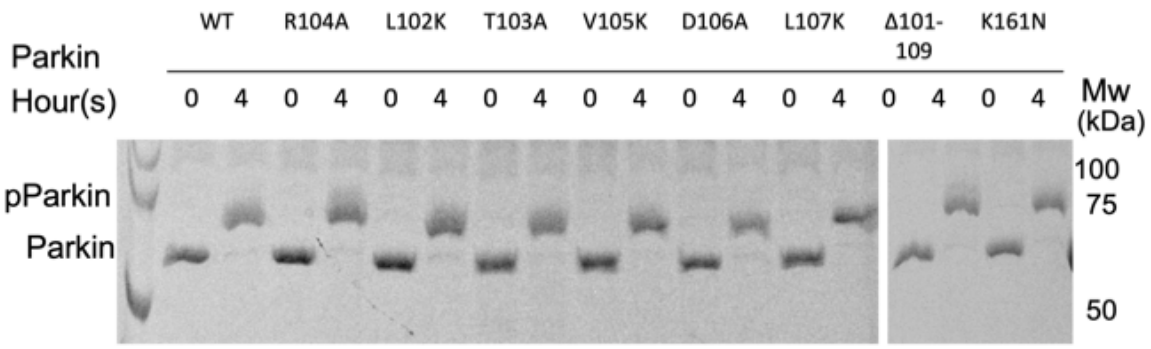

Figure S3: Representative phos-tag gel of parkin ACT mutants to confirm phosphorylation by PINK1 before autoubiquitination assays. Two replicates were performed (n=2), once before each autoubiquitination assay with the ACT mutants.

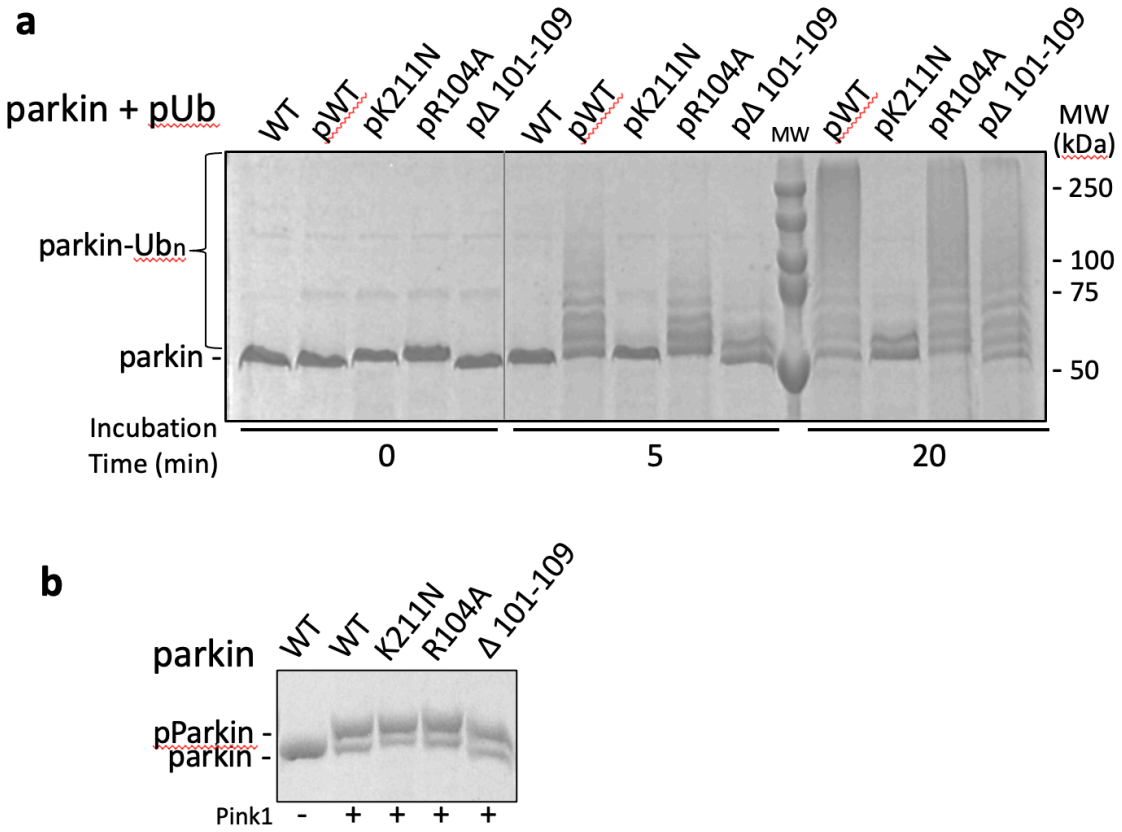

Figure S4: Preliminary experiment showing autoubiquitination activity of ACT variants. **a** Time course of the autoubiquitination assay of the two ACT mutants, compared to unphosphorylated wild-type (WT), phosphorylated wild-type (pWT), and phosphorylated K211N parkin (pK211N). The phosphorylated R104A mutant had activity similar to phosphorylated wild-type while the phosphorylated truncation Δ101-109 had decreased activity. **b** Phos-tag gel confirming that the proteins used in the experiment were equally phosphorylated. The experiment was performed once (n=1).

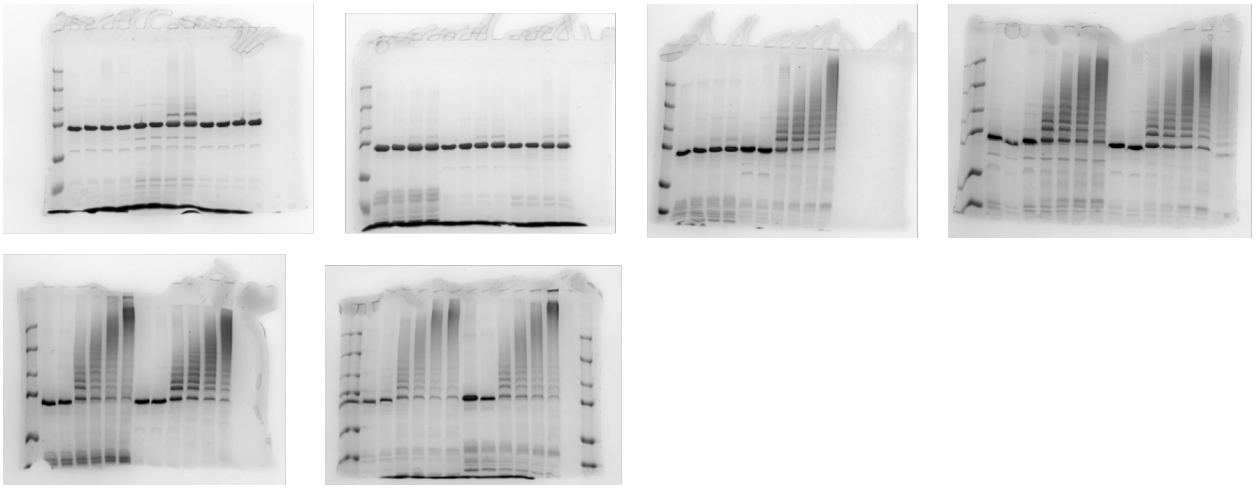

Figure S5: Uncropped gels for autoubiquitination assays done on unphosphorylated and phosphorylated. These gels were used in figures 1c and 2a.

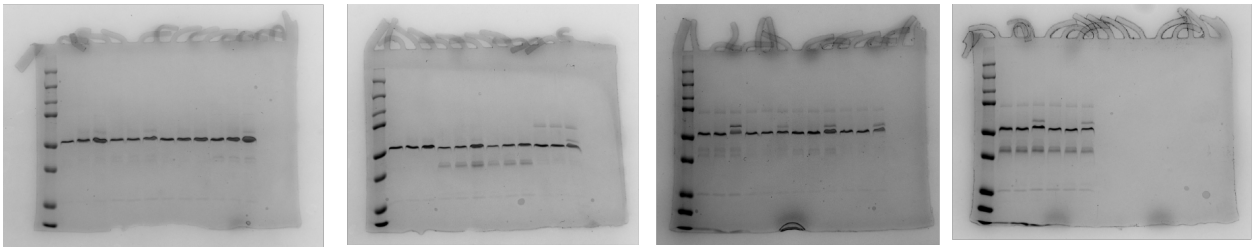

Figure S6: Uncropped gels for UbVS assays done on unphosphorylated and phosphorylated parkin. These gels were used in figure 3a and 3b.

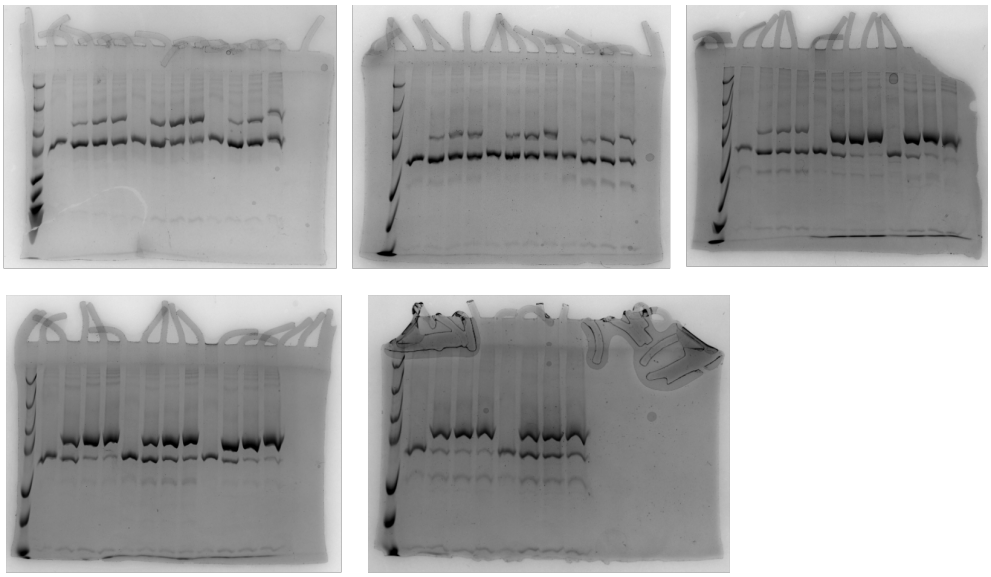

Figure S7: Uncropped gels for kinase assays done on full-length parkin in the absence and presence of pUb. There gels were used in figure 4a and 4b.

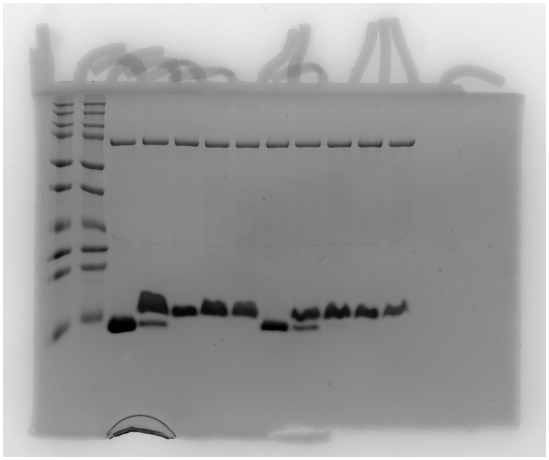

Figure S8: Uncropped gel for kinase assays done on the isolated wild-type and P37L mutant Ubl domain only. This gel was used in figure 6b.

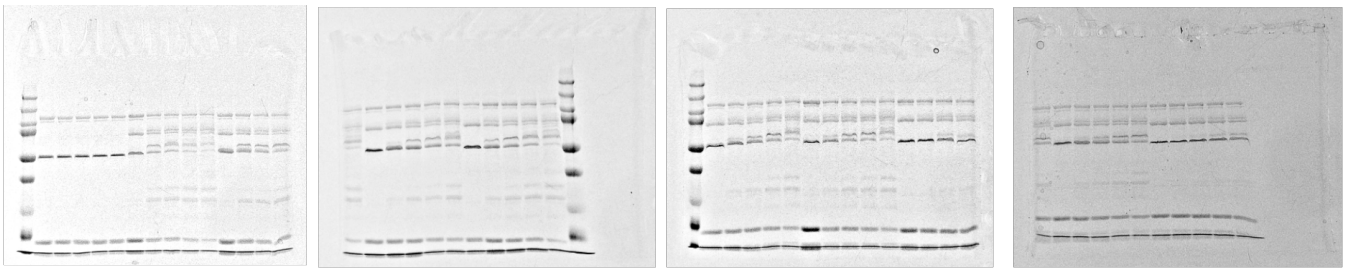

Figure S9: Uncropped gels for autoubiquitination assays done on the phosphorylated ACT mutants. These gels were used in figure 7a.