SUPPLEMENTARY INFORMATION

Structure and Function of Parkin E3 Ubiquitin Ligase Reveals Aspects of RING and HECT Ligases

Riley, B.E.^{*1}, Lougheed, J.C.^{*1}, Callaway, K.¹, Velasquez, M.¹, Brecht, E.¹, Nguyen, L.¹, Shaler, T.², Walker, D.¹, Yang, Y.¹, Regnstrom, K.¹, Diep, L.¹, Zhang, Z.¹, Chiou, S.¹, Bova, M.¹, Artis, R.A.¹, Yao, N.¹, Baker, J.¹, Yednock, T.¹, Johnston, J.A.^{*1}

¹ Elan Pharmaceuticals, South San Francisco, CA, 94080, USA

² SRI International, Menlo Park, CA, 94025, USA

* These authors contributed equally to this work

* To whom correspondence should be addressed, Jennifer Johnston, jenniferjohnston@e3xbio.com



Supplementary Figure S1 Stereo ribbon diagram of Parkin R0RBR-P223 and electron density map of R0:R2. **a**, Stero ribbon diagram of Parkin R0RBR **b**, The MAD phased map of Parkin R0RBR P223 is of high quality. A representative view of the electron density, contoured at 1σ, is shown at the R0:R2 interface. For orientation, Tryptophan 462

is in the middle of the image.



Supplementary Figure S2 Comparison of Parkin R0RBR-S223 and Parkin R0RBR-P223.

a, The loop containing residue 223 is ordered in Parkin R0RBR-S223 while it is disordered in the Parkin R0RBR-P223 structure. The nearby bound Zn site is well ordered in the S223 structure while it appears to have more than one conformation in the P223 structure as indicated by the diffuse electron density and higher crystallographic B-factors for this region. The same dominant conformation was modeled in both structures. The coordination residues for the nearby bound Zn (grey spheres) are shown in sticks.



b RING 1

d



Parkin RNF144A









Supplementary Figure S3 Cartoon representations of RING domains and identification of Zn coordinating residues.

a, R0 domain is a previously unobserved fold. **b**, The R1 domains of Parkin (green) and RNF144A (pdb 1WIM, grey) are similar. **c**, The IBR domain from our crystal structure (orange) agrees with the IBR domain solved by NMR (pdb 2JMO, grey). **d**, The Parkin R2 domain (pink) is similar to the NMR structure of the IBR domain of HOIP (pdb 2CT7, grey).

b







Supplementary Figure S4 Superposition of UbcH7 on Parkin R1 domain.

a, UbcH7 (grey) was positioned on Parkin-R0RBR by superimposing R1 (green) with the RING domain in the structure of c-Cbl bound to UbcH7 (pdb 1FBV) using the pymol align command; c-Cbl is not shown for clarity. There is compatible binding between the hydrophobic grooves of Parkin R1 and UbcH7 (canonical residues involved in E2 binding, F63 and P97 of UbcH7 are labeled), similar to that previously described⁴⁷.
b, The catalytic cysteines of UbcH7 and Parkin-R0RBR are > 50 Å apart in our superposition model and the Parkin tether residues 393-395 (beige) overlap with UbcH7 in the superposition, suggesting that they adopt another conformation in E2 bound R1. Conformational changes and/or oligomerization could bring the catalytic cysteines into proximity for transthiolation. c, Electrostatic surface representation around W403 demonstrates that the inside of the pocket is hydrophobic.



#		b-ions	Seq.	Y-ions	#	
	1	115.0502	Ν			8
	2	172.0717	G	955.3617		7
	3	229.0931	G	898.3402		6
	4	524.1592	C#	841.3187		5
	5	655.1997	Μ	546.2527		4
	6	792.2586	н	415.2122		3
	7	9 <mark>23.2</mark> 991	Μ	278.1533		2
	8		К	147.1128		1

C# = Cys + Vinyl Sulfone-GlyGly

0 60 0 60 0 60 0 60 time (minutes)

22

Supplementary Figure S5 Parkin conjugation to HA-UbVS occurs on C431.

a, Mass spectrometry analysis of Parkin HA-UbVS reactions. b, Autoubiquitination of various Parkin constructs as monitored with immunoblotting with anti-Ub antibody (FK2).



Supplementary Figure S6 Parkin reactivity with HA-UbVS is specific and Parkin is not a deubiquitnating enzyme (DUB).

a, The DUB USP2 reacts with HA-UbVS and HA-Isg15VS (also shown previously⁵⁴), and was used as a positive control. Parkin reacts specifically with HA-UbVS, and to a weak extent NEDD8, and no reactivity with other Ub-like VS probes. b, Parkin or USP2 were incubated with pure UbK48 (2-7) or UbK63 (2-7) chains. Reactions were monitored by Coomassie blue or western blot with the Ub antibody FK2. For USP2, Ub chains are completely disassembled whereas Ub chains remained intact when incubated with Parkin.

⁵⁴Catic, A. et al. Screen for ISG15-crossreactive deubiquitinases. PLoS One 2, e679, doi:10.1371/journal.pone.0000679 (2007).

a	RING1	
Parkin	NSRNITCITCTDVRSP-VLVFQCNSRIVICLDOFHLYCVTRLNDRQFVHDPQLGVSLFCVAGCENSLIKELHHFRILGEEQYNRY	315
ARIH2	SHPPHHCAVCMQFVRK-ENLLSLACQ-HQFCRSQWEQHCSVLVKdGVGVGVGQQAAAQDQPLRTPEDFVFPLLPNEELREKYR	211
Dorfin	IGDF <mark>ISC</mark> PLCLLRHSKDRFPDIMTCHHRSCVDCLRCYLRIEISES-RVN <mark>ISCE</mark> ECTERFNPHDIRLILSDDVLMEK	200
HHARI	SAQDMPQQICYLNYPN-SYFTGLECG-HKFCMQCWSEYLTTKIMEEGMGQTISCFAHGCDILVDDNTVMRLITDSKVKLKYQ	259
HOIL-1	NTEPAS PVCISVLAPGZAVVLRECL-HTRCRECLQGTIRNSQEAEV3CEFIDNTYSCSGKLERZIKALLTPEDYQRFLDLG	357
HOIP	RLLAQECAVCGWALPHNRMQALTSCECTICPDOFRCHFTTALKEKHITDMVCEAOGRPDLTDDTQLLSYFSTLDIQLRESLEPDAYAL	780
RNF14	NSKLFLSSICFCEKLGSECMYFLEER-HVYCKACLKDYFEIQIRDGQVQCLNCEEPKCPSVATPGQVKELVEAELFARYD	292
RNF144A	LDPLVS_KLCLGEYPVEQMTTIAQEQCITCTLCLKQYVELLLKEGLETAISCEDAACPKQGHLQENTIECMVAABIMQRYK	94
ANKIB1	DLDTSLEDICMCSISVF2DPVDMP2G-HDFCRGOWESFLNLK_QEGEAHNIFCPAYDCF-QLVPVDIIDSVVSKEMDKRYL	405
TRIAD3	dgqliegrodygefpf-religcadaligekediryaqeavfgsg-klelsgmegscsfptsplekvipqtilykyy	586
Parkin	QQYGAERCVLQMGGVLQPRPGGAGL-LPEPDQRKVTGEGGNGLGGGEAFGREGKEAYHEG-EGSAVFEASGTTTQAYRVDER	396
ARIH2	RYLERDYVESHYQLQLQEGADQPMVIRVQEERERRVQCNRCNEVESFKQRQMYAAPTDQATIRKWITKQAD	282
Dorfin	YEEFMLRRWLVADPDCR <mark>MCEAPDC</mark> GYA <mark>Y</mark> IAFGCASCPKLTCGRECCGTEFCYHCKQIWHPNCTCDAARQERAQSLRLTIR	281
HHARI	H <mark>L</mark> ITNSF <mark>VE</mark> CNRLL <mark>K - MQEAPDC</mark> HH <mark>VV</mark> KVQYEDAKPVRCKGGRQEGFNQGENMHDPVKCKWLKKWIKKCDD	329
HOIL-1	ISIAENRSAFS <mark>Y</mark> H <mark>C</mark> KT 2DC KGWCFFEDDVNEF <mark>TCP</mark> VCFHVNGLL <mark>CX</mark> AI <mark>HB</mark> QMN <mark>CKE</mark> YQEDLALRAQNDVAAR	429
HOIP	FHKKLTSGVIMRDPKFLMCAQCSFGFIYEREQLEATOPQLEATOPQCHQTFCVRCMRQWEEQHRCRSGEDFQNWKRMNDP-E	854
RNF14	RLLLQSSLDLMADVV-YCERECQLPY-MOEEGCTMGICSSCNEAECTLCRLTYGVSPCKVTAEK_MDLRNEYLQADEAN	371
RNF144A	KLQEEREVLFDPCRTMOPASTQQAVCQLQDVGLQTPQPVQQKACRMEFQSTQXASWHPGQGQPETMPITFLPGETS	170
ANKIB1	QFDIKAFVENNPAIKMOETEGODRAVR_TKQGSNTSGSDTLSFPLLR_PAVDQGKCHLFOWEDLGEAdepCDQTWKNWLQKITEMKPEELVG-	498
TRIAD3		660
Parkin	AAEQARWEAASKETIKKTTKPOPROHVPVEKNGGCMHMKOPQPQORLEWOWNOGCEWNRVOMGDHWFDV	465
ARIH2	DSETANYISAHTKDOPKONICIEKN-GGCNHMQOS-KOKEDFOWMOLGOWKTHGSEYYEOSKYKENPDIVNQSQQA	356
Dorfin	ssisysocsgaaaddikpoppoaayiikmigsonestgaa-vocsforestwigeneststepsocsforestwigenest	356
HHARI	dsetsniiiaantkedekdhyttekd-yttekd-wydruqudkaefdwydlepwephosawyndna yneddakaardaqersr	408
HOIL-1	QTTEMLKVMLQQEAMR <mark>QRQQ</mark> QIVVQ <mark>K</mark> K-D <mark>GQ</mark> DWIR <mark>QT</mark> -V <mark>Q</mark> HT <mark>TIQW</mark> VTK <mark>S</mark> PRWGP <mark>G</mark> QPGDTSG <mark>Q</mark> RCRVNGIPCHPSCQNCH	510
HOIP	YQAQGLAMYLQEMGIDQEKGKFSYALAR-GGOMFFHQTQGRHQFCSGQYNAFYAKNKCPEPNQRVKKSLHGHHPRDCLFYLRDWT	938
RNF14	KRLLDQRYGKRVIQKALEEM <mark>E</mark> SK <mark>EWLEKNSKSQPCOGTPLEK</mark> L-D <mark>SCNKWTGT</mark> -GAMQY <mark>TGW</mark> I <mark>OMS</mark> SLSRANPYKHFNDPGSPC <mark>E</mark> NRLFYAVDV	463
RNF144A	AAFKMEEDDAPIKROKKWYIERDEGOAQMCKNCKHAFCWYCESLDDDFLLIHYDKGECEN	234
ANKIB1	VSEAYEDAANCLULINSKEDANCKSEIQKNESCNHYQDA-KOKYDEDNIGLEENKKHSSITGYYRCIEYEVIQHV	574
TRIAD3	DGAQMOYLORVSINGYDH-FCQHPRSPGAPCQECSRCSLW	733
b		
Human	TKPCPRCHVPVEKNGGCMHMKCPQPQCRLEWCWNCGCEWNRVCMGDHWFDV	
Mouse	T K PICIP RICIN V PILE K N G G C MH MKICIP Q P QICIK L E WCIWN C G C E WN R A C MG DH WF D V	
Pig	T K PICIP RICH V P V E K N G G C MH MKICIP Q P QCIQ L E WCIWNICIG WE WN R DICIMG DIHWF D V	
Green a		
NILE ti		
Fruitfl		
Zebrafi		
	service and a second second second of a left of the second s	

Supplementary Figure S7 Alignment of human RBR proteins and Parkin R2 from different species.

a, The Parkin R1, IBR, and R2 domain boundaries are indicated over the structure and conserved residues are highlighted in yellow and Zn coordination residues are boxed. The catalytic C431 of Parkin is indicated with an arrow, and H433 and E444 are indicated with black circles. HOIL-1 does not have a conserved base at the position corresponding to Parkin H433, and is not catalytic in the LUBAC complex¹. **b**, Alignment of Parkin R2 from eight different species. The catalytic C431 of Parkin is indicated with an arrow, and H433 and E444 are indicated with black circles. Conserved C-terminal residue F463 involved in the R0:R2 interface is indicated with a black box.

N



Supplementary Figure S8 Parkin's catalytic machinery.

ENP

a, Soluble levels of untagged Parkin cysteine (C) to serine (S) or alanine (A) mutants in transfected HeLa cells. GAPDH was used as the loading control. b, Immunoprecipiation (IP) of Parkin followed by western blotting (WB) with HA to demonstrate the ~8 kDa shift is HA-Ub. c, Parkininduced Tom20 loss after CCCP requires H433 but not E444. Data shown is representative of four independent experiments (standard error represents s.e.m.). The significance levels were determined using the heteroscedastic Student's t-Test with two-tailed distribution. Triple asterisk denotes P < .005, double asterisk denotes P < .01 and single asterisks denote P < .05. C431A P = .002, C431S P = .002, C431F P = .002, H433N P = .003, H433A P = .016. d. Parkin R0RBR autoubiguitination activity for various R0:R2 mutants was monitored by immunoblotting with anti-Ub antibody (FK2).

Crystal	Parkin-RORBR-P223 ^a					
Data Collection						
Space Group	C222 ₁					
Unit cell: a, b, c (Å)	86.96, 133.2, 65.39					
	HiRes	Peak (Zn)	Remote(Zn)			
Wavelength (Å)	1.1159	1.2831	1.2699			
Resolution (Å)	44-1.58(1.62-1.58) ^b	50-1.8(1.85-1.8)	50-1.8(1.85-1.8)			
Rsym	0.078(0.99)	0.107(-)	0.113(-)			
Ι/δΙ	11.0(1.6)	14.8(1.0)	15.4(1.3)			
Completeness (%)	99.5(99.8)	95.1(63.6)	97.3(70.5)			
Redundancy	6.9(6.9)	14.3(10.4)	15.6(11.6)			
Refinement						
Resolution (Å)	44-1.58					
No. reflections	52033					
Rwork/Rfree ^c	0.205/0.245					
Number of atoms						
Protein	2403					
Ligand/ion	9					
Water	267					
B-factors						
Protein	27.0					
Ligand/ion	26.7					
Water	34.5					
R.m.s. deviations						
Bond lengths (Å)	0.025					
Bond angles	2.26°					

^a One crystal was used for all data sets. The Peak and Remote data sets were used to determine

experimental phases and the final structure was refined against a high resolution (HiRes) data set. ^b Values in parentheses indicate the highest resolution shell

 $^{\rm c}R_{\rm free}$ was calculated with a randomly chosen 5% of the data for the P223 structure and this same set was used for the S223 structure

Supplementary Table S1 Data collection and refinement from the crystallography studies of Parkin R0RBR P223.

Crystal	Parkin-RORBR-S223 ^a			
Data Collection				
Space Group	C222 ₁			
Unit cell: a, b, c (Å)	87.11, 133.9, 66.21			
Resolution (Å)	17-2.00(2.12-2.00) ^b			
Rsym	0.111(0.398)			
l/σl	12.1(3.6)			
Completeness (%)	82.3(36.3) ^c			
Redundancy	6.1(5.5)			
Refinement				
Resolution (Å)	17-2.00			
No. reflections	21783			
Rwork/Rfree ^d	0.181/0.215			
Number of atoms				
Protein	2405			
Ligand/ion	8			
Water	263			
B-factors				
Protein	20.6			
Ligand/ion	17.7			
Water	23.3			
R.m.s. deviations				
Bond lengths (Å)	0.019			
Bond angles	1.84°			

^a One crystal was used

^b Values in parentheses indicate the highest resolution shell

^cCompleteness was 95.6% to 2.24Å at the edge of the detector

 $^{d}R_{free}$ was calculated with a randomly chosen 5% of the data for the P223 structure and this same set was used for the S223 structure

Supplementary Table S2 Data collection and refinement from the crystallography studies of Parkin R0RBR S223.