

Figure 2e

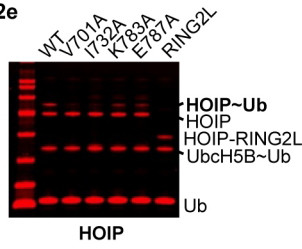


Figure 3d

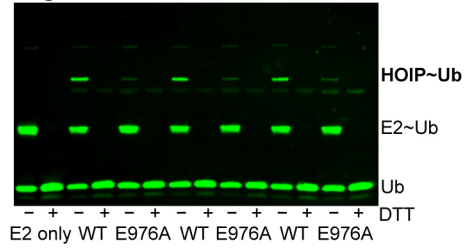
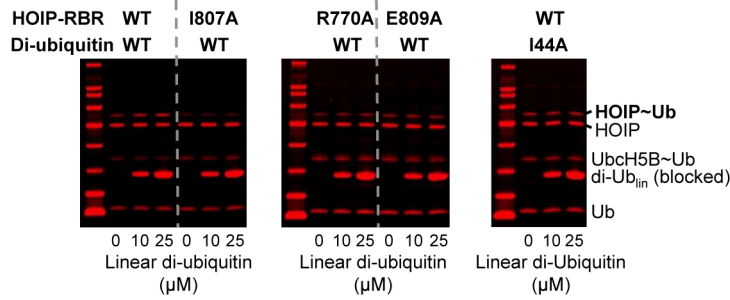
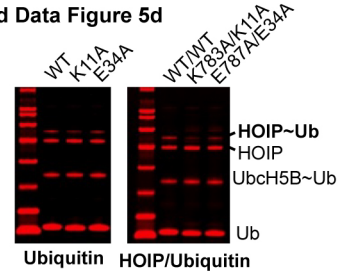


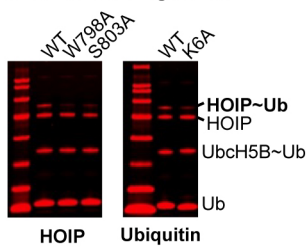
Figure 4b / Extended Data Figure 9d



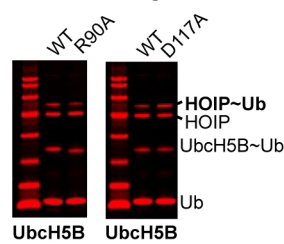
Extended Data Figure 5d



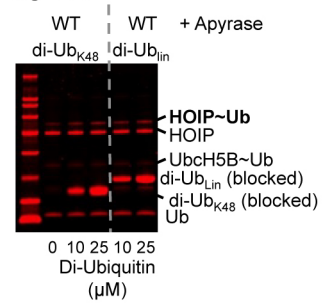
Extended Data Figure 5f



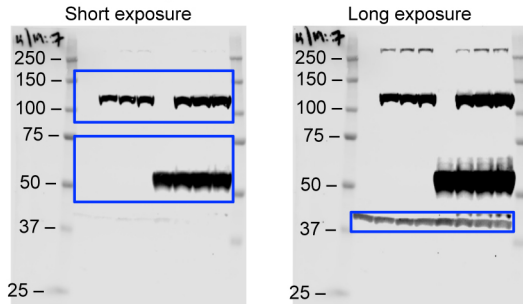
Extended Data Figure 7b



Extended Data Figure 9e



Extended Data Figure 9f



Supplementary Figure 1 | Uncropped blots and representative gels/blots of quantitative HOIP~Ub thioester transfer assays. Representative SDS-PAGE gels for each quantitative HOIP~Ub thioester transfer experiment displayed in the figures and Extended Data figures and uncropped blots of immunoblotting experiments.

RING1

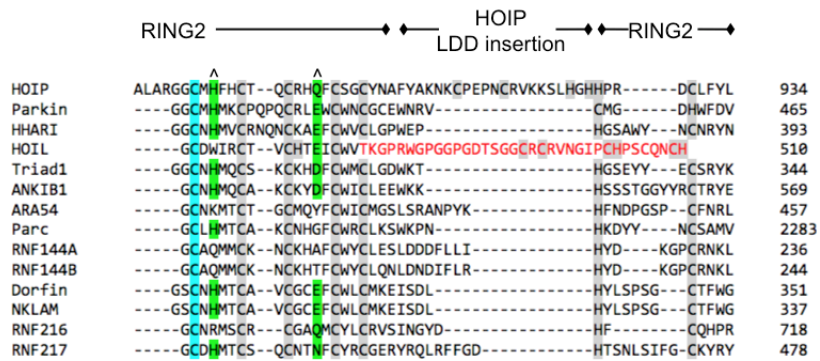
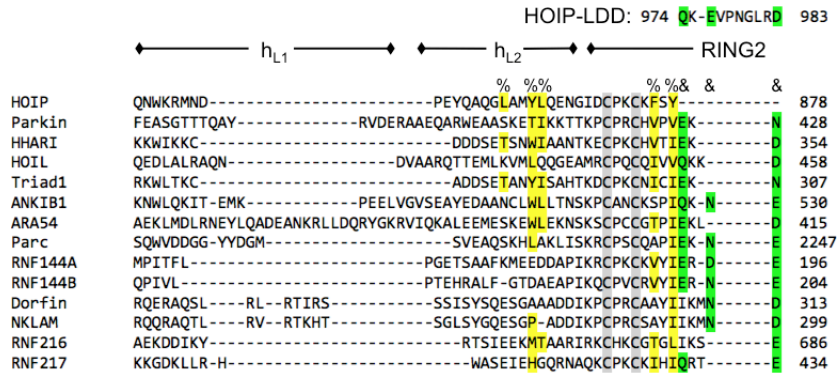
	\$	\$ \$	\$	
HOIP	CAVCGWALPHNRMQALTSCE--C--TICPDCFRQHTIAL-KEKHIT-----DMVCPA---	746		
Parkin	CIITCDVRS---PVLVFQCNSRH--VICLDCFHLYCVTRLNDRQFVHDPQLGYSLPCVA-G-	292		
HHARI	CQICYLNYV-NSYFTGLECG--H--KFCMQCWSEYLTIKIMEEGMGQ-----TISCPAHG-	235		
HOIL	CPVCYSVLAPGEAVVRECL--H--TFCRECLQGTIRN----SQEA-----EVSCPFDIN	328		
Triad1	CAVCMQFVR-KENLLSLACQ--H--QFCRSCWEQHCSVLV-KDGVGV-----GVSCMAQD-	187		
ANKIB1	CDICMCSISVFEDPVDMPG--H--DFCRGCWESFLNLKI-QEGEAH-----NIFCPAYD-	382		
ARA54	CSICFCEKLGSECMYFLSCR--H--VYCKACLKDYFEIQI-RDGGVQ-----CLNCPPEPK-	269		
Parc	CPVCVSPLGDDDLPSLCCM--H--YCKSCWNEYLTTRI-EQNLVL-----NCTCPIAD-	2119		
RNF144A	CKLCLGEYPVEQMTTIAQCQ--C--IFCTLCLKQVVELLI-KEGLET-----AISCDA--	69		
RNF144B	CKLCLCEQSLDKMTTLQEQ--C--IFCTACLKQVQLAI-REGCGS-----PITCPDMV-	79		
Dorf1n	CPCLLRHSHKDRFPDITMCH--H--RSCVDCLRQYLRLEI-SESRVN-----ISCP--	178		
NKLAM	CPCLLVRLLPPERAPRLSCP--H--RSCRDCLRHYLRLEI-SESRVP-----ISCP--	165		
RNF216	CRCCYGEFP--FEELTQCADAH--LFCKECLIRYAQEAVFSGKLE-----LSCMEGS--	563		
RNF217	CRVCLEDKPIKP----LPC--CKKAVCEECLKVYLSAQV-QLGQVE-----IKCPITE-	308		

RING1 → ← h_{E1} ← h_{E2} ← IBR

	* * ## # *	
HOIP	---CGRPDLTDDTQLLSYFSTLDIQLRESLEPDAYAL-FHKKLTGVLMRDPKFLW-CAQ---	801
Parkin	---CPNSLIKE-----LHH-FR-ILGEEQYNR-YQYGAEECV-LQMGGVL-CPRPG-	336
HHARI	---CDILVDD--NTV-----MRLITDSKVKLK-YQHLLTNSFVFCNRLKW-CPAPD-	280
HOIL	TYSKSGKLE--REI-----KAL-LTPEDYQR-FLDLGISIAENRSFASYH-CKTPD-	375
Triad1	---CPLRTP--DFV-----FPLLPNEELREK-YRRYLFDRDYVESHVQLQL-CPGAD-	232
ANKIB1	---CFQLVPV--DII-----ESVV-SKEMDKR-YLQFDIKAFVENNPAIKW-CPTPG-	426
ARA54	---CPSVATP--GQV-----K-ELVEAELFAR-YDRLLQSSLDLMADVYV-CPRPC-	313
Parc	---CPAQPFG--AFI-----RAIVSSPEVISK-YEKALLRGVYVESCNSLNTW-CTNPQG	2165
RNF144A	---CPKQGHQENEI-----E-CMVAAEIMQR-YKLLQFREVLFDPDPCRTW-CPAST-	115
RNF144B	---CLNHGTQAEI-----A-CLVVDQFQL-YQRLKFRREVHLDPRYRW-CPVAD-	125
Dorf1n	---CTERFNP--HDI-----RLILSDDVLMKEYEYFMLRRNLVADPDCRW-CPAPD-	223
NKLAM	---CSERLNP--HDI-----RLLLADPPLMHKYEYFMLRRYLASDPDCRW-CPAPD-	210
RNF216	---CTCSFPT--SEL-----EKVLPQTILYKY-YERKAEVEVAAYADELVRCPSP---	607
RNF217	---CFEFL--TTV-----VYNLT-HEDSIK-Y-KYFLE-LGRIDSSTKX-CPQ---	348

IBR →

	# #	
HOIP	CSFGFIYREQ-----LEATCPQ----CHQTFVCRCKRQWEEQHRGRSCEDF	844
Parkin	CGAGLPPDPQRK-----VTCEGNGLGCGFACRECKEAY--HEGE-CSAV	380
HHARI	CHHVVKVQYDP-----AKPVRC-----KCGRFCCNCGENW--HDPVKCKWL	320
HOIL	CKGWCFFDDVN-----EFTCPV----CFHVNCLLCKA-I--HEQMCKEY	414
Triad1	CPMVIRVQEP-----RARRVQCNR----CNEVFCFKCRQMY--HAPTDCAI	273
ANKIB1	CDRAVRLTKQGSNTSGSDTLSFPLLRAPAVDC--GKGLHF--CWEC--LGEAHEPCDQTW	481
ARA54	CQLPVMQEP-----GCTMGICSS-----CNFAFCTLRLTY--HGVSCKVT	353
Parc	CDRILCRGG-----LGCSTTCSK----CGWASCFNCS--FPEAHYPASCGHM	2206
RNF144A	CQAVCQLQDVGLQ-----TPQPVQCKA----CRMEFCSTCKASW--HPGQGCPT	159
RNF144B	CQTVCPVASSDPG-----QPVLVECPG-----CHLKFCSCCKDAW--HAEVSCRDS	169
Dorf1n	CGYAVIAFGCASC-----PKLTCGREG--CGTEFCYHCKQIW--HPNQCDAA	267
NKLAM	CGYAVIAYGCASC-----PKLTCGREG--CGTEFCYHCKQIW--HPNQCDMA	254
RNF216	CSFPALLSDV-----KRFSCPDPH--CRKETCRKCOGLWKE-HNGLTCEEL	651
RNF217	CKHFTYFKKKGHIPTP-----SRSESKYKIQCP-----CQFVWCFKCHSPW--HEGVNCKEY	399



Supplementary Data 1 | Sequence alignment of all human RBR E3 ligases. Domains and regions are annotated above the alignment. Functionally important sites are indicated with symbols: E2 binding site: \$, UBR1: *, UBR2: %, UBR3: #, contact conduit 1: &, and contact conduit 2: ^.

Conserved hydrophobic and charged/polar residues in these sites are highlighted with yellow or green background, respectively. Zinc coordinating Cys and His residues are highlighted with grey background; the active site Cys residues are highlighted with cyan background. HOIP-LDD residues 974-983 containing contact conduit 1 (see also Extended Data Fig. 7) are included as a separate sequence aligned above the functionally corresponding residues of the RING2 of the other RBRs. The C-terminal part of HOIL-RING2 also contains an insertion, which has not been aligned with the other RBRs (red).