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Ubiquitin-specific proximity labeling for the identification of E3 ligase substrates

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Supplementary Table 1: Plasmids

Name	Source
pRK5-HA-Ubiquitin-WT[1]	pRK5-HA-Ubiquitin-WT was a gift from Ted Dawson (Addgene plasmid # 17608 ; http://n2t.net/addgene:17608 ; RRID:Addgene_17608)
pJW1512[2]	pJW1512 was a gift from Jonathan Weissman (Addgene plasmid # 62366 ; http://n2t.net/addgene:62366 ; RRID:Addgene_62366)
pMD2.G	pMD2.G was a gift from Didier Trono (Addgene plasmid # 12259 ; http://n2t.net/addgene:12259 ; RRID:Addgene_12259)
psPAX2	psPAX2 was a gift from Didier Trono (Addgene plasmid # 12260 ; http://n2t.net/addgene:12260 ; RRID:Addgene_12260)
pCMV-8xHis-Ub[3]	pCMV-8xHis-Ub was a gift from William Kaelin (Addgene plasmid # 107392 ; http://n2t.net/addgene:107392 ; RRID:Addgene_107392)
PX330	kindly provided by D.B.Wheeler and M.L.Meyerson
pRK5-puroR	kindly provided by D.B.Wheeler and M.L.Meyerson
pLX305	Broad GPP
pLX307	Broad GPP
pDONR223	Broad GPP
pRK5-GW	Generated by Gibson assembly using the following three fragments: Backbone 1: PCR-amplified from pRK5-puroR using HH01-pRK5-Cterm-F and HH02-ampR-R. Backbone 2: PCR-amplified from pRK5-HA-Ubiquitin-WT using HH02-ampR-F and HH03-pRK5-CMV-R. Insert: PCR-amplified from pLX305 using HH03-pRK5-CMV-attR1-F and HH01-pRK5-Cterm-attR2-R.
PX330-sgCRBN-2	Generated by ligating annealed oligonucleotides containing CRBN-targeting sequences (sgCRBN-2F/R) with BbsI-digested PX330 vector.
pENTR223-VHL	Broad GPP
pENTR223-3F-BirA	Generated by BP cloning using pDONR223 and an attB-flanked amplicon of 3F-BirA (made by PCR amplification of pRK5-BirA-3F-mCherry using attB-3F-F and attB-BirA-R)
pENTR223-BirA	Generated by site directed mutagenesis of pENTR223-3F-BirA using BirA-F and pENTR223-ATG-R
pENTR223-BirA-3F	Generated by site directed mutagenesis of pENTR223-BirA using pENTR223-3F-attL-F and BirA-3F-R
pENTR223-CRBN-3F-BirA	Generated by Gibson assembly using the following two fragments: Backbone: PCR-amplified from pENTR223-3F-BirA using 3F-F and pENTR223-ATG-R. Insert: PCR-amplified from an ORF of CRBN using pENTR223-ATG-CRBN-F and CRBN-GS-3F-R
pENTR223-BirA-3F-CRBN	Generated by Gibson assembly using the following two fragments: Backbone: PCR-amplified from pENTR223-BirA-3F using pENTR223-STOP-attL2-F and 3F-R. Insert: PCR-amplified from an ORF of CRBN using 3F-GS-CRBN-F and pENTR223-CRBN-attL2-R
pENTR223-Avi-HA-Ubiquitin	Generated by BP cloning using pDONR223 and an attB-flanked amplicon of Avi-HA-ubiquitin (made by PCR amplification of pRK5-Avi-HA-ubiquitin using attB-Avi-F and attB-UBC-R)
pENTR223-Avi-HA-IKZF1	Generated by Gibson assembly using the following two fragments: Backbone: PCR-amplified from pENTR223-Avi-HA-Ubiquitin using pENTR223-STOP-attL2-F and HA-GS-R. Insert: PCR-amplified from an ORF of IKZF1 using HA-GS-IKZF1-F and pENTR223-IKZF1-attL2-R
pENTR223-VHL-3F-BirA	Generated by Gibson assembly using the following two fragments: Backbone: PCR-amplified from pENTR223-3F-BirA using 3F-F and pENTR223-ATG-R. Insert: PCR-amplified from pENTR223-VHL using pENTR223-ATG-VHL-F and VHL-GS-3F-R
pENTR223-CRBN(D249Y)-3F-BirA	Generated by site-directed mutagenesis of pENTR223-CRBN-3F-BirA using D249Y-F and D249Y-R
pENTR223-CRBN(W386A)-3F-BirA	Generated by site-directed mutagenesis of pENTR223-CRBN-3F-BirA using W386A-F and W386A-R
pENTR223-VHL(Y98H)-3F-BirA	Generated by site-directed mutagenesis of pENTR223-VHL-3F-BirA using Y98H-F and Y98H-R
pENTR223-VHL(C162F)-3F-BirA	Generated by site-directed mutagenesis of pENTR223-VHL-3F-BirA using C162F-F and C162F-R
pENTR223-CRBN-3F	Generated by site directed mutagenesis of pENTR223-CRBN-3F-BirA using pENTR223-STOP-attL2-F and 3F-R

pRK5-Avi-ubiquitin	Generated by site-directed mutagenesis of pRK5-HA-Ubiquitin-WT using AviTag-Ub-F and pRK5-AviTag-R
pRK5-Avi-HA-ubiquitin	Generated by site-directed mutagenesis of pRK5-Avi-ubiquitin using HA-Ub-F and AviTag-HA-R
pRK5-BirA-3F-mCherry	Generated by Gibson assembly using the following three fragments: Backbone 1: PCR-amplified from pRK5-puroR using HH01-pRK5-Cterm-F and HH02-ampR-R. Backbone 2: PCR-amplified from pRK5-puroR using HH02-ampR-F and HH03-pRK5-Nterm-R. Insert: PCR-amplified from pJW1512 using HH03-pRK5-Nterm-3F-F and HH01-pRK5-Cterm-mCherry-R
pRK5-CRBN-3F-BirA	Generated by LR cloning from pENTR223-CRBN-3F-BirA and pRK5-GW
pLX307-CRBN-3F-BirA	Generated by LR cloning from pENTR223-CRBN-3F-BirA and pLX307
pLX307-BirA-3F-BirA	Generated by LR cloning from pENTR223-BirA-3F-CRBN and pLX307
pRK5-Avi-HA-IKZF1	Generated by LR cloning from pENTR223-Avi-HA-IKZF1 and pRK5-GW
pRK5-1A-HA-IKZF1	Generated by site-directed mutagenesis of pRK5-Avi-HA-IKZF1 using (-1)Avi-F and pENTR223-ATG-R
pRK5-2A-HA-IKZF1	Generated by site-directed mutagenesis of pRK5-Avi-HA-IKZF1 using (-2)Avi-F and pENTR223-ATG-R
pRK5-3A-HA-IKZF1	Generated by site-directed mutagenesis of pRK5-Avi-HA-IKZF1 using (-3)Avi-F and pENTR223-ATG-R
pRK5-A1-HA-IKZF1	Generated by site-directed mutagenesis of pRK5-Avi-HA-IKZF1 using Avi(-1)-F and Avi-R
pRK5-A2-HA-IKZF1	Generated by site-directed mutagenesis of pRK5-Avi-HA-IKZF1 using Avi(-2)-F and Avi-R
pRK5-A3-HA-IKZF1	Generated by site-directed mutagenesis of pRK5-Avi-HA-IKZF1 using Avi(-3)-F and Avi-R
pRK5-V5-IKZF1	Generated by site-directed mutagenesis of pRK5-Avi-HA-IKZF1 using V5-IKZF1-F and pENTR223-V5-R
pRK5-3A-HA-ubiquitin	Generated by site-directed mutagenesis of pRK5-Avi-HA-ubiquitin using (-3)Avi-F and pRK5-ATG-R
pRK5-A3-HA-ubiquitin	Generated by site-directed mutagenesis of pRK5-Avi-HA-ubiquitin using Avi(-3)-F and Avi-R
pLX307-VHL-3F-BirA	Generated by LR cloning from pENTR223-VHL-3F-BirA and pLX307
pLX307-DCAF15-3F-BirA	Synthesized by Genscript
pLX307-CRBN(D249Y)-3F-BirA	Generated by LR cloning from pENTR223-CRBN(D249Y)-3F-BirA and pLX307
pLX307-CRBN(W386A)-3F-BirA	Generated by LR cloning from pENTR223-CRBN(W386A)-3F-BirA and pLX307
pLX307-VHL(Y98H)-3F-BirA	Generated by LR cloning from pENTR223-VHL(Y98H)-3F-BirA and pLX307
pLX307-VHL(C162F)-3F-BirA	Generated by LR cloning from pENTR223-VHL(C162F)-3F-BirA and pLX307
pLX307-CRBN-3F	Generated by LR cloning from pENTR223-CRBN-3F and pLX307
pLX307-GSPT1-V5	Synthesized by Genscript
pLX307-VCL-V5	Synthesized by Genscript

Supplementary Table 2: Oligos and Primers

Name	Sequence
sgCRBN-2F	CACCGTAAACAGACATGGCCGGCGA
sgCRBN-2R	AAACTCGCCGGCCATGTCTGTTTAC
CRBN-Ex1-F2	gcaggcctgtaattgtccctcc
CRBN-Ex1-R2	gtaaccgctgtgaatctgacccg
HH01-pRK5-Cterm-F	tgagcggccgctaagtaagtaaggatcccc
HH02-ampR-R	gtatgcggcgaccgagttgctcttgc
HH02-ampR-F	gcaagagcaactcggtcgccgcatac
HH03-pRK5-CMV-R	aacgagctctgcttatatagacctcccacc
HH03-pRK5-CMV-attR1-F	ggtgggaggtctatataagcagagctcgttGGCTAGCATCGATTGATC
HH01-pRK5-Cterm-attR2-R	ggggatccttacttacttagcggccgctcaCATTACTAACCGGTAACCAC
attB-3F-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAgccaccatggactacaaagacc
attB-BirA-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTtcatttttctgcactacgcaggga
BirA-F	AAGGATAACACCGTGCCAC
pENTR223-ATG-R	catggtggcTAAGCCTGC
BirA-3F-R	atctttataatcaccgtcatggtctttgtagtcTTTTTCTGCACTACGCAG
pENTR223-3F-attL-F	catgacatcgattacaaggatgacgatgacaagTGAAACCCAGCTTTCTTG
3F-F	gactacaaagaccatgacggtg
pENTR223-ATG-CRBN-F	AAGCAGGCTTAgccaccatggccggcgaaggagatc
CRBN-GS-3F-R	ccgtcatggtctttgtagtcagaacccaagcaaagtattactttgtctggac
pENTR223-STOP-attL2-F	TGAAACCCAGCTTTCTTGtac
3F-R	CTTGTCATCGTCATCCTTGTAATCG
3F-GS-CRBN-F	ACAAGGATGACGATGACAAGggttctgccggcgaaggagatc
pENTR223-CRBN-attL2-R	taCAAGAAAGCTGGGTTTCAcaagcaaagtattactttgtctgg
attB-Avi-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAgccaccatgggactgaacg
attB-UBC-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTtcaaccacctctgagacggaggaccag
HA-GS-R	ggatcccgcatagtccg
HA-GS-IKZF1-F	tgccggactatgcgggatccgatgctgatgagggtcaagac
pENTR223-IKZF1-attL2-R	TACAAGAAAGCTGGGTTtcagctcatgtggaagcggtg
pENTR223-ATG-VHL-F	AAGCAGGCTTAgccaccatgCCCCGGAGG
VHL-GS-3F-R	ccgtcatggtctttgtagtcagaaccATCTCCCATCCGTTGATGTGC
D249Y-F	TTCCTTATATtATGCTGAGACCTTAATGGACAG
D249Y-R	TACAGCCAGCGAGGC
W386A-F	TGGGTATGCCgcGACTGTTGCCCAG
W386A-R	GGAAACCAGCTGTGTTCTG
Y98H-F	GCAGCCCcACCCAACGCTG
Y98H-R	GGCTCGCCGTCGAAG
C162F-F	AAAGAGCGATtCCTCCAGGTTGTC
C162F-R	CAGAGTATACACTGGCAGTG
AviTag-Ub-F	gaaaatcgaatggcacgaaggatCCATGCAGATCTTCGTGAAGACCC
pRK5-AviTag-R	tgagcctcgaagatgtcgttcagtCCCATGGTGGCGCGAATG
HA-Ub-F	tgtgccggactatgcgGGATCCATGCAGATCTTC
AviTag-HA-R	tcgtacggatatgaaccTTCGTGCCATTCGATTTTC
HH03-pRK5-Nterm-R	ggtggcgcgaatggccttggtgaag
HH03-pRK5-Nterm-3F-F	cttcaccaaggccattcgcgccaccatggactacaaagac
HH01-pRK5-Cterm-mCherry-R	ggggatccttacttacttagcggccgctcacttgtacagctcgtccatgccg
(-1)Avi-F	ctgaacgacatcttcgaggc
(-2)Avi-F	aacgacatcttcgaggctc
(-3)Avi-F	gacatcttcgaggctcagaaaatc
Avi(-1)-F	CGAATGGCACggaGGTTCATATC
Avi(-2)-F	CGAATGGagcggaGGTTCATATCCGTACG
Avi(-3)-F	CGAAgGGagcggaGGTTCATATCCGTACGATG
Avi-R	ATTTTCTGAGCCTCGAAGATG
V5-IKZF1-F	CTCCTCGGTCTCGATTCTACGggatccgatgctgatg
pENTR223-V5-R	AGGGTTAGGGATAGGCTTACCcatggtggcTAAGCC
pRK5-ATG-R	catggtggcgcgaatg

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