Molecular Basis of Pirh2-mediated p53 Ubiquitylation

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Supplementary Information

Supplementary figure 1

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Supplementary figure 1. NMR-generated structure models of the three Pirh2 domains. The ensembles of top 20 NMR models from the final calculations are illustrated as wires for (a) the Pirh2 NTD (residues 15-137), (b) the Pirh2 RING (residues 142-187) and (c) the Pirh2 CTD (residues 215-255). For clarity, the disordered regions are not shown.



Supplementary figure 2. The solution structure of the Pirh2 RING domain. (a) Superposition of the Pirh2 RING domain (red) with the RING domains from RBX1 (PDB: 1LDJ-B), BRCA1 (PDB: 1JM7-A) and EL5 (PDB: 1IYM-A), colored in blue. (b)

Transparent surface representation of the Pirh2 RING domain with the surface hydrophobic residues colored in yellow.



¹⁵N Pirh2 NTD + p53 DBD

Supplementary figure 3. NMR titrations of the Pirh2 NTD with the p53 DBD. Overlay of the HSQC spectra for the ¹⁵N -labeled Pirh2 NTD (blue) titrated with the p53 DBD (red).

Residues H70, A71, Q72 and E76 are highlighted to demonstrate the peak shifts observed in the HSQC spectra of the Pirh2 NTD when it is bound by the p53 DBD.

Supplementary figure 4



Supplementary figure 4. NMR titrations of the Pirh2 CTD with the p53 TET domain proteins. (a) Overlay of the HSQC spectra for the ¹⁵N -labeled Pirh2 CTD (blue) titrated

with the p53 TET (red). Backbone resonances correspond to the residues A249, A251, R255 are shifted upon binding to the p53 TET (red). (b) and (c) Overlay of the HSQC spectra of the ¹⁵N-labeled Pirh2 CTD (blue) titrated with p53 dimeric mutant (p53 DM) and p53 monomeric mutant (p53 MM) shown in red.



Supplementary figure 5. Autoubiquitylation and ubiquitylation of p53 by Pirh2 are dependent on the presence of ATP, Ub, E1, E2 and Pirh2. Full length Pirh2 protein was employed in an *in vitro* ubiquitylation assay in the presence and absence of ATP, E1, E2 (UBE2D2/UbcH5b), 6XHis-tagged ubiquitin and p53 as indicated. The reaction products were resolved on a 7.5% SDS-PAGE gel and detected by Western blot. Pirh2 autoubiquitylation (lanes 1-6, left panel) was detected by blotting with a monoclonal antibody against GST. Ubiquitylation of p53 was detected by blotting with a monoclonal antibody against p53 (PAB 1801) (lanes 7-12, right panel).

Supplementary figure 6

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CXXCX(7-12)HCXXCX	XCX(9)HCXXCXXCX(8)HXC
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* 20	* 40	*	60 *	80	* 100
XP 0014506 :ATYFCSICNLYEDD-PK-MD	VINCLECKNORREIKEC	NFHCKVCGI	LNKKNQ-DSHKCIDQ	-IADTNCPIC-LKNI	(ISTTYIAQ : 81
XP 0014462 :AKYFCSICKLYDDD-PN-KD	ITHCDOONMORREVKEN	NFHCNTCGI	LSKSHQ-NSHKCINO	-AAENDCPIC-LONL	(ASTSYINQ : 81
XP 0010225 :GKYFODICKLFNND-TT-SS	MYHCDKCKMCRMCTKES	NFHCDVCDI	MSKTLE-KNHKCIEK	-KTEQDCPIC-LENL	ISTKLWQQ : 81
XP 0014241 :ARYYCEICKLYQDD-EK-SN	OF HCEKONMORLGIKEE	YFHCETCDV	LSLLIK-STHICIQK	-AFEONCAVC-ODYL	NSTQLVQQ : 81
NP 197938. :GKYFCKVCKLYDDD-TS-KK	OYHCDGCGICRICGREN	FFHCYKCGC	YSILLK-NGHPCVEG	-AMHHDCPIC-FEFLF	ESRNDVTV : 81
CAN68688.1 :GKYFCETCKLFDDD-TS-KK	QYHCNGCGICRIGGREN	IFF HC YK <mark>C</mark> RC	YSVLLK-NSHPCVEG	-AMHHDCPVC-FEYLF	ESTDDVTV : 81
AAS87371.1 :GKYFCEVCKLFDDD-VS-KQ	OYHCNGCGI CRIGGKEN	FFHCSKCGC	YSIVEK-NSHACVEG	-AMHHDCPIC-FEYLF	ESTNDVSV : 81
ABF13303.1 :F	RYHCSGCGICRTEGSEN	IFF HC YK <mark>C</mark> GC	YSTLLK-NSHPCVEG	-AMHHDCPVC-FEYLF	ESRNDVTVMPCGHTIHKSCL- : 76
NP 0010489 :GKYECAKONFYDDD-VS-KN	QFHCDGCGICRTEGAEN	IFF HC DK <mark>C</mark> GC	YSYVLK-DSHHCVER	-AMHHNCPVC-FEYLF	DSTKDISA : 81
NP_0010647 :GKYFCEKONFFDDD-VS-KN	QYHCDGCGICRTCGVDF	FFHCDKCGC	YSNVER-DSHHCVEG	-AMHHNCPVC-FEYLE	DSTKDISV : 81
AAM65683.1 :GKYFCSKCKFFDDD-LS-KK	QYHCDECGICRT EGEEN	IFF HC KRCRC	YSKIME-DKHQCVEG	-AMHHNCPVC-FEYLE	DSTRDITV : 81
AAD02556.1 :GKYYCSKONFFDDD-VS-KD	QYHCDKCGICRT CGNSN	FFHCNRCEC	YSNMMK-ESHICVER	-AMHHNCPVC-FEYVE	PDTTKNITV : 81
NP_0010669 :GEYFCDVCKFYDDD-TE-KG	QF HC YD <mark>C</mark> GICRVEGKEN	YF HC AKCGS	YAVALR-DNHQCVEN	-SMRQNCPIC-YEYLE	DSLKGTRV : 81
NP_197366. :GEYFCSICIFYDDD-TE-KQ	QFHCDDCGICRVCGREN	FFHCKKCGS	YAVGLR-NNHRCVEN	-SMRHHCPIC-YEYLE	PDSLKDTNV : 81
ABF95920.1 :GEYFCSACKFFDDD-VD-RE	HF HCQDCGI CRVEGKDN	FFHCEKCGS	YSVSIR-DKHCCIEN	-SMKNNCPIC-YEYLE	DSLRETSV : 81
AAP21226.1 :GEYFCDICKFFDDD-IS-KE	QFHCDDCGICRVCGRDF	FFHCQNCGA	YGMGIR-DKHSCIEN	-STKNSCPVC-YEYL	7DsVKAAHV : 81
XP_0016233 :FGFYFCALCRLYDDAD-KG	QF HC DM <mark>C</mark> GICRVCGKDF	FFHCAKCDI	IPITER-SSHKCIEK	-SSRSNCPIC-LEDL	HTSRVPCQV : 81
XP_0016226 :FGRYFCEICRLFDDQE-KG	QFHCDACGICRICGRKN	FY <mark>HC</mark> PRCDI	LGVGLK-DSHKCVDK	-SARNDCPVC-LEDI	HTSRISANM : 81
EAT42854.1 :FGRYTELVENLFDDED-RN	QY HC DG <mark>C</mark> GICRVEGRGF	FFHCKVCNM	LPMQLQVDGHRCVEN	-VSRSNCPVC-LDDI	HTSRIPCH : 81
XP_320050. :FGRYTCLVONLFDDED-RN	QYHCDGCGICRVCGRGF	FFHCEVCNM	LPLQLKYDGHRCVEN	-VSRSNCPVC-LDDI	HTSRIPCH : 81
NP_609030. :FGKYTeLIeNLFDDAD-MQ	QYHCHGCGICRICGAEN	IFF HC EV <mark>C</mark> NM	LPIQLKIDGHRCVEN	-ISRSHCPVC-LGDI	HTSRIPCH : 81
XP_0013572 :FGKYTCLICNLFDDAD-KQ	QYHCHGCGICRICGADN	IFF HC EV <mark>C</mark> NM	LPIQUKIDGHRCVEN	-ISRSHCPVC-LGDI	HTSRIPCH : 81
XP_974078. :FGKYTCLECNLFDDED-KN	QFHCSGCGICRICGSDF	FFHCEKCNM	LPIQEK-NGHKCVEN	-VSRANCPVC-LEDIH	HTSRIPCHI : 81
XP_392132. :FGKYT_LEONLFDDED-N	QYHCDGCGICRVEGRDF	FFHCAKCNM	LPVQLQ-NGHTCVEN	-VSHANCPVC-LEDIF	HTSRIPCHI : 81
AAH78283.1 :KFGEYYCDICHLFDKD-KK	QYHCQPCGICRICPREF	YFHCTKCNLO	LGTELK-DKHKCVEN	-VSRQNCPVC-MEDIF	HTSRVGAHV : 81
NP_997765. :KFGEYYCDICHLFDKD-KK	QYHCQPCGICRIEPREF	YFHCTKCNL	LGTELK-DKHKCVEN	-VSRQNCPVC-MEDIF	HTFRVGAHV : 81
CAG02458.1 :QFGEYYONICHLFDKD-KK	QYHCQPCGICRICPREF	YFHCEKCNLO	LAQDLH-GNHKCVEN	-VSRQNCPVC-MEDIF	HTSRIEAKV : 81
NP_0010887 :VFGDYYONICHLFDKD-RK	QYHCDGCGICRICPKEE	FEHCTKONL	LPLSER-GNHKCIEN	-VSRQDCPIC-LEDIH	HTSRVGARV : 81
NP_0010743 :LFGEYYCDVCHLFDRD-KK	QYHCDECGICRICPKEI	FFHCPKCNL	LSLSER-GKHKCHEN	-VSRQDCPIC-LEDIH	HTSRVEARV : 81
AAH47393.1 :LFGEYYCDICHLFDKD-RK	QYHCENCGICRIEPKEI	FFHCLKCNL	LAMNIQ-GRHKCIEN	-VSQQNCPIC-LEDIH	HISRVVAHV : 81
NP_056251. :LFGEYYCDICHLFDKD-KK	QNHCENOGICRICPKEI	FFHCLKCNL	LAMNIQ-GRHKOHEN	-VSRQNCPIC-LEDIH	ITSRVVAHV: 81
NP_0010772 :LFGEYYCSVCHLFDKD-KK	QYHCENCGICRICPKEI	FFHCLKCNL	LAMNIQ-GKHKCHEN	-VSRQNCPIC-LEDIH	ITSRIAAQV : 81
NP_0010909 :LFGEYYesICHLFDKD-KK	QYHCENOGUCRICPKEI	FFHCLKCNL	LAMNIQ-GKHKOHEN	-VSRQNCPIC-LEDIF	ITSHIVAHV: 81
EDL05285.1 :LFGEYNCSICHLFDKD-NR	QANCESCGICRICPKEI	FFHCLKCNL	LTTNI R-GKHKOHEN	-VSRQNCPIC-LEDIH	ITSRVVAHV : 81
AAH23138.1 :LFGEYWeSICHLFDKD-WR	QMICESOGICRICPKEI	FFICLKONS	LTTNER-GKEKOEEN	-VSRQNOPHO-LEDH	ITSRVVAHV: 81
EDL88593.1 :LFGEYWeSICHLFDKD-WK	OVICESOGICRICPREI	FFHCLKCNL	LAMTICR-GKIIKOIIEN	-VSRQNOPIC-LEDIF	ITSRVVAHV: 81
XP_UU13754 :VFGEYWONIOHLFDKD-WK	QMECEKCGICRIEPKEI	FFRCLKCNL	LSLSIQ-GHEKOTEN	-VSRQNOPHO-LEDH	HTSRVVAHV: 81
XP_UUISIU6 :LFGEYWOHIOHLFDKD-RK	OM#CERCETCRIEPKEF	FFHOSKONLO	LALSIQ-GKIIKOIEN	-VSRQNXLAP-LQDH	HTSRVVAHV: 81
XP_640522. :ARYFCDVCKFYDDS-PT-RK	IMHODKOGICRVESRDI	YIHODKONG	LNKSGF-NSHKOTEN	KFSNCPIC-MEDILE	SSREPSTPF : 81
XP_666118. :KYR SKONLWDDNGIE-EN	VERCORCELORAGOES	TEHCKVCCM	YPLS K-ETHKOLEN	-SCREPCERC-LEDIE	YISIKTVSI: 81
NP_586340. :VSKYP SK NLWDSS-D-DQ	TEHODGONVORREDPKS	SEHEDICOT	LVTRGP-RDUTHVEN	-TASGNOPTO-AEE	SEGMEVLVL: 81
ABI15584.1 :ICODICHTLCMIGPEVMP	AMECHSCGWCRVELOHS	SVHCERCDS	FDKNFV-DEHVOIE-	PCVCEVC-QQNUF	CDALTPEFSLKC: 81
XP_770592. :TRONIONTLCMLGPEMAP	TTHEOHONSCHVELOEP	IVRHOSTORT.	ISREDY-DTHVODC-	DNMCEVC-MIPFA	ADNVMSEIMLPC: 81
XP_769594. :ShipKONYISLISPEVAP	FRHCESCTFORVERQGE	HKHODICKQ	YKKOFF-DTEKEDAT	DAACCHE-ORREIT	(TELYDHTEIG: 81
AP_/00//0. :RTELICGMGREGRE	SHARARGETCHQELPGY	SENCERCOT	HTVANG-QVMACAPD	FONGALE-TDOLE	CONTINITATATA
VD 0012267MARDTONSSG-SLOGLCSV	CENCOTODKOPCEEGD1	SERCHQUED	SICINCPVICETV	-COBCCATDATSCRU	(QRVNTFL: 80
AF_0013307 :MAEDIQHSSG-SEGLCSV	CENCOTODKOPCEEGD1	SERCHQUED	SICINCPVICETV	-CÖRGCATDATS(281)	(PRVNTEL: 80
BAD42043.1 : HEDDDDDDDDHHEG-SIGSLCAH	CENCUSCUKCPCKKGDF	ISENCOFCOD	GREIWCP-VETV	-CVEGELADRARGEN	(NEVADV: 81
BAD42044.1 : HEDDDDDDEWHEG-SICSLCAH	CENCOSCOROPCERGDE	SEREDFODD	GREIWEP-VETV	-CREGEVDSVSGS	(NHVADV: 81
VD 071272ATVIEDSTRACT	CENCOGONOPCEEGDI	CHARGEDDOKM	TLVPDHUNDR	-CIFGGELDEFTGS	INDVADVEDD: 01
AF_9/13/2. :AIVLEDISIRGRELPTAM	NC C Cr d	NC C	T PUKKDERMENDEL	SCCTORUMERTETAL	(FINE AL #1 : 01
			- n c	Cp C	

b

CXXCX(9)HCXXCXXCX(9)HCXXCXXCX(6)HCXXCXXCX(4-5)HXC

						Co	opy 1			Co	py 2		(Сору	3							
		*		20		*		40		*		- 6	0 -	.,	*	-	80		*	100	*	
XP_0011785	:	TSEGYRLC	КЕ	CHRYVA	AE	NIHCS	KCQE	CPSK	NGTT	THCE	ICNK	CVKPS	FSHC	KCN-1	м-С	EIAT-S	DC	GKVQAGOH	IVCGALDH			: 81
AAN35181.1	:	SSDDFWFC	к1	CNRFSS	SE	NKHCN	KCNQ	CTSK	SGHT	VHCN(жк	CVKPN	RSHC	vck-	T-C	ELPD-H	RCGF	EKTETTC	HICGET			: 81
XP_970334.	:	ANEGYKFC	QE	C <mark>N</mark> KWVS	NE	NKHCP	ICQK	CTSK	DGRT	THCPI	ICA R	CVKPS	M OHCI	KCG-:	L-F	PPI						: 61
AAI07394.1	:	SEEGYRFC	3L	CQRYVS	RE	NOHCV	HCNS	CTSK	DGRK	is <mark>hc</mark> fi	LCKK	CVKPS	WIHCI	JTCN-I	R-C	ALPD-H	sc	LGPKDGCF	TCGALDH			: 81
XP_341225.	:	SEEGYRFC	sv	CORYVS	RE	NOHCV	HCNS	стяк	DGRK	is <mark>hc</mark> fi	FCKK	CVKPS	WIHCI	ITCN-I	R-C	AI PD-8	sc	LGPKDGCF	TCGSLDH			: 81
BAB15524.1	:	TEEGYRFC	sP	CQRYVS	LE	NOHCE	HCNS	CTSK	DG <mark>RK</mark> I	IN <mark>HC</mark> F I	LCKK	CVKPS	WIHC:	SICN-D	H−C	AVPD-H	sc	EGPKHGCE	FICGELDH			: 81
NP_079212.	:	TEEGYRFC	sp	CORYVS	LE	NOHCE	LCNS	стяк	DGRK	IN <mark>HC</mark> F I	lckk	CVKPS	WIHC:	SICN-I	H−©	AVPD-8	sc	EGPKHGCF	ICGELDH			: 81
XP_0010823	:	TEEGYRFC	3L	CQRYVS	LE	NOHCE	HCNS	CTSK	DGRK	IN <mark>HC</mark> FI	LCKK	CVKPS	WIHC:	BICN-D	H−C	AVPD-H	s					: 65
XP_0010825	:	TEEGYRFC	3L	CQRYVS	LE	NOHCE	HCNS	CTSK	DGRK	IN <mark>HC</mark> FI	lckk	CVKPS	WIHC:	SICN-I	H−©	AVPD-0	sc	EGPKDGCE	TCGELDH			: 81
XP_0014996	:	IEEGYRFC	PI	CQRYVS	LE	NOHCE	HCNS	CTSK	DG <mark>RK</mark> I	IN <mark>HC</mark> FI	FCKK	CVKPS	WIHC:	SICN-I	H−©	ALPD-H	sc	EGPKDGCF	VCGELDH			: 81
XP_536271.	:	SEEGYRFC	PI	CQRYIS	LE	NOHCE	HCNS	CTSK	DGRK	IN <mark>HC</mark> FI	FCKK	CVKPS	WIHC:	ICG-I	H-C	AI PD-0	sc	GGPKDGCE	TCGELDH			: 81
XP_588725.	:	IEEGYRFC	PI	CQRYVS	LE	NOHCE	HCNS	CTSK	DG <mark>RK</mark> I	IN <mark>HC</mark> FI	LCKK	CVKPS	WIHC:	SICN-I	H-C	ALPD-H	sc	KGPKDGCF	ICGELDH			: 81
XP_0013673	:	IEEGYRFC	TM	CORYVS	LE	NOHCE	ICNS	CTSK	DGRR	IK HC LI	lckk	CVKPS	WIHC:	SICN-1	Y−C	AIPN-0	sc	AGAKDGCE	TOGEADH			: 81
XP_0015138	:	AEEGYRFC	sV	CORYVS	LE	NOHCE	ICNS	CTSK	DG <mark>RR</mark> I	JHHCF]	lckk	CVKPS	WTHC	SICD-	Q-C	ALPN-H	AC	QHAEGGOF	TCGEADH			: 81
XP_420756.	:	AEEGYRFC	AI	CQRYVS	3G	NHHCE	ICNS	CTSK	DGRR	IK HC AJ	LCER	CVKPS	WFHCI	IKCN-	c-c	AI ES-B	sc	EKTNVGCE	VCGKAGH			: 81
NP_0010871	:	ASEGYRFC	sV	CERFVC	SE	NKHCD	ICNR	CTSK	DGRS	KHCSO	CKK	CVKPS	W SHC	BACN-I	H−C	ALPG-H	PC	GTAGDGCE	LCGGKGH			: 81
NP_0010081	:				G	NKHCN	ICNC	CTSK	DGRP	IKHCT(2CNK	CVKPS	WTHC	BACN-I	H−©	ALPD-8	PC	GTAGRGCE	LCGGKDH	KRRGCPHQSVS/	AHGKRM-	: 81
XP_692840.	:	EAEGYRFC	sV	CERYVS	AA	NKHCP	KCDM	CPSK	DGRE]	IR HC DI	ECGR	CVKPS	10 RHC	scc-i	R-C	ALPD-H	PC	GQRQTGCE	NGSQKH			: 81
NP_610973.	:	PEEGYKYC	QK	CDC TA	KE	NLHCN	RCGK	CPSV	NG <mark>QT</mark>	KHCE:	3CDA	CVKPN	VVHC :	DCR-1	R−C	TQKEG	NCSF	YQTKQH	MCGQ			: 81
EAT44163.1	:	ANEGYKWC	3E	CQRSVH	RT	NLHCR	VCRK	CPSK	NGST	(R HC KI	KCNW	CVKPN	YVHC:	TCG-I	R-C	TQVQG	NCSS	YRKQLNCF	RICLK			: 81
CAE59579.1	:	KVDGYKFC	ES	CDRYVT	KN	NIHCE	RCDA	CISV	EQGK	IN <mark>HC</mark> DO	2CDK	CVKPR	.⊻VHC.	ECE-I	R−C	H YG	RC	IQKY				: 69
NP_496368.	:	NVAGYKFC	EV	CDRYVT	ER	NVHCD	RCQA	CISV	EQGK	INHCEI	KCDK	CVKPR	YVHC.	QCA-I	R-C	H YG	RC	IQKHDE				: 71
		eg c		c		N HC	C (CtSk	g 5	5 HC	С	CVKP	HC	С	0	: h	C	c	C			

Supplementary figure 6. Sequence alignment of the zinc β -spiral motif in the Pirh2 NTD. (a) The protein sequences contain two copies of HCxxCxxC sequence element. (b) The sequences contain three copies of HCxxCxxC sequence element. The consensus sequence patterns for the zinc β -spiral motif are indicated.