## **Supplemental Materials**

## The intrinsically disordered C-terminal domain triggers nucleolar localization and function

## switch of PARN in response to DNA damage

Tian-Li Duan <sup>†</sup>, Guang-Jun He <sup>†</sup>, Li-Dan Hu and Yong-Bin Yan <sup>\*</sup>

State Key Laboratory of Membrane Biology, School of Life Sciences, Tsinghua University, Beijing 100084, China

<sup>†</sup> These authors contributed equally to this work.

\* Correspondence: Dr. Yong-Bin Yan, School of Life Sciences, Tsinghua University, Beijing 100084,

China; Phone: +86-10-6278-3477; Fax: +86-10-6277-2245; E-mail: ybyan@tsinghua.edu.cn.

Running Title: Role of C-terminal domain in PARN function



**Figure S1. Effect of temperature (A and B), pH (C and D) and K<sup>+</sup> (E and F) on PARN-CTD structural features monitored by far-UV CD (A, C and E) and Trp fluorescence excited at 295 nm (B, D and F).** Similar results were also obtained for Trp and Tyr fluorescence excited at 280 nm (data not shown). The factors did not influence PARN-CTD secondary and tertiary structures. Similar results were obtained for factors including divalent metals (Mg<sup>2+</sup>, Mn<sup>2+</sup> and Ca<sup>2+</sup>), low concentration of ureal and macromolecular crowding reagent including dextran-70 and PEG-20000 (data not shown).



Figure S2.  $K^+$  enhances PARN-CTD Trp fluorescence in a concentration-dependent manner, implying that there is specific binding of  $K^+$  with PARN-CTD.

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	Anopheles	SSSAGPGEDAGV	
	Ciona	· SALPTATNGRQSSPFRPSS·····ATKSFISIV	· · · EAAQ   TD T 566
	Bombyx	·KTAEAANNDCKKTEKTPVR························	· · · Q INATATT 542
	Harpegnathos	ASGVCCEDRKRKL - PVGN ALDDALAGKSGNGATPTSST	ISLPSLSS 550
	Camponotus	··· TDVSLEDRKRKLSSPENT···· AV······ KGNGKSTTN····	
	Branchiostoma	SLPETFAPWNGEGKRLKVTS	SDVGRRVS 426
	Danio	·SSRKWASDGWADTSYPSVAMT······TASGYSHTD	NRHQAVKRSIS 560
	Gasterosteus	• TPKSWGEDGWLKPHYTSAS • • • • • • • • • • • • • • • • • •	HKDAPD 556
	Tetraodon	• TAKPWGEDGWVKSPYT • • • • • • • • • • • • • • • • • • •	SLRKRSIS 561
	Takifugu	• TTKAWGEDGWVKSHYT••••••••••••••••••••••••••••••••••••	S <mark>LRKRSI</mark> S 555
	Xenopus	• TKRKWAEDGWKDLERKRLKTQYNSY · IPQTPVFYGNCFVAPS	YAVKRSMS 569
	Anolis	PAKRKWAEDGWKEAESKRLKTQAAPYGTVQGRYYNPNSFTASS	AAGKRS <mark>M</mark> S 573
	Gallus	• AKRKCTEDSWKEMERKRLKTQGSPY• ISQSRYYCINSFTGTS•	<mark>IV</mark> GKRSMS 509
	Was	• VKRKWTEDSWKEVDRKRPHMQGPCY•••••• HSNSFTAAG•	••• <mark>vl</mark> gkrtls 556
	Monodelphis	• NKRKWTENSWKEVERKRLKTQCTSY• SPQSRYYCVNSFTATS•	···A <mark>V</mark> GKRN <mark>M</mark> S 570
	Bos	• MKRKWTEDSWKEVEPKRLNTQCGSY• SLQNHHYHANSLTATS•	•••• T <mark>V</mark> GKRNLS 570
	Homo	• IKRKWTEDSWKEADSKRLNPQCIPY•TLQNHYYRNNSFTAPS•	•••• T <mark>V</mark> GKRN <mark>L</mark> S 570
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	)		
	Anopheles	AAKRKO <mark>m</mark> deatppt <mark>m</mark> adkdataakddehdndednoggwr 616	
	Anopheles Ciona	AAKRKO <mark>m</mark> deatppt <mark>m</mark> adkdataakddehdndednogogwr 616 Shasrspdepe <mark>l</mark> kkoks <mark>i</mark> edcattttnkoeete <mark>w</mark> kottt 627	
	Anopheles Ciona Bombyx	AAKRKO <mark>m</mark> deatppt <mark>m</mark> adkdataakddehdndedngggwr 616 Shasrspdepel Kkoksiedcattttnkoeete <mark>v</mark> kottt 627 SSGGFEvnel Esptkk <mark>l</mark> kgasetkkr <mark>i</mark> dek <mark>iid - RFDSE 612</mark>	
	Anopheles Ciona Bombyx Harpegnathos	AAKRKO <mark>mdeatpptmadkdataakddehdndedngggwr 616</mark> Shasrspdepelkkoksiedcattttnkoeetewkottt 627 SSGGFEwnelesptkklkgasetkkr <mark>i</mark> dekiid - RFDSE 612 NDGWE <mark>I</mark> ATGKRRKKRREON <mark>V</mark> E 586	
-	Anopheles Ciona Bombyx Harpegnathos Camponotus	AAKRKOMDEATPPTMADKDATAAKDDEHDNDEDNGGGWR 616 Shasrspdepelkkoksiedcattttnkoeetevkottt 627 SSGGFEVNELESPTKKLKGASETKKRIDEKIID-RFDSE 612 	
	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma	AAKRKOMDEATPPTMADKDATAAKDDEHDNDEDNGGGWR 616 Shasrspdepelkkoksiedcattttnkoeetevkottt 627 SSGGFEVNELESPTKKLKGASETKKRIDEKIID-RFDSE 612 	
_	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio	AAKRKOMDEATPPTMADKDATAAKDDEHDNDEDNGGGWR 616 SHASRSPDEPELKKQKSIEDCATTTTNKQEETEWKQTTT 627 SSGGFEWNELESPTKKLKGASETKKRIDEKIID-RFDSE 612 	
_	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus	AAKRKOMDEATPPTMADKDATAAKDDEHDNDEDNGGGWR 616 SHASRSPDEPELKKQKSIEDCATTTTNKQEETEWKQTTT 627 SSGGFEWNELESPTKKLKGASETKKRIDEKIID-RFDSE 612 	
_	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus Tetraodon	AAKRKOMDEATPPTMADKDATAAKDDEHDNDEDNGGGWR 616 SHASRSPDEPELKKQKSIEDCATTTTNKQEETEWKQTTT 627 SSGGFEWNELESPTKKLKGASETKKRIDEKIID-RFDSE 612 	
_	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus Tetraodon Takifugu	AAKRKOMDEATPPTMADKDATAAKDDEHDNDEDNGGGWR 616 SHASRSPDEPELKKOKSIEDCATTTTNKOEETEWKOTTT 627 SSGGFEWNELESPTKKLKGASETKKRIDEKIID-RFDSE 612 	
-	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus Tetraodon Takifugu Xenopus	AAKRKOMDEATPPTMADKDATAAKDDEHDNDEDNGGGWR 616 SHASRSPDEPELKKOKSIEDCATTTTNKOEETEWKOTTT 627 SSGGFEWNELESPTKKLKGASETKKRIDEKIID-RFDSE 612 	
-	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus Tetraodon Takifugu Xenopus Anolis	AAKRKOMDEATPPTMADKDATAAKDDEHDNDEDNGGGWR 616 SHASRSPDEPELKKOKSIEDCATTTTNKOEETEWKOTTT 627 SSGGFEWNELESPTKKLKGASETKKRIDEKIID-RFDSE 612 	
-	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus Tetraodon Takifugu Xenopus Anolis Gallus	A K K K G M D E A T P P T M A D K D A T A A K D D E H D N D E D N G G G W R 616 S H A S R S P D E P E K K Q K S I E D C A T T T N K Q E E T E V K Q T T T 627 S G G F E V N E E S P T K K L K G A S T K K R I D E K I I D • R F D S E 612 • • • • • • • • • • • • • • • • • • •	
-	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus Tetraodon Takifugu Xenopus Anolis Gallus Wus	A K K K G M D E A T P P T M A D K D A T A A K D D E H D N D E D N G G G W R 616 S H A S R S P D E P E K K Q K S I E D C A T T T N K Q E E T E V K Q T T T 627 S G G F E V N E L E S P T K K L K G A S T K K R I D E K I I D • R F D S E 612 • • • • • • • • • • • • • • • • • • •	
_	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus Tetraodon Tetraodon Takifugu Xenopus Anolis Gallus Wus Wonodelphis	A K K K G M D E A T P P T M A D K D A T A A K D D E H D N D E D N G G G W R 616 S H A S R S P D E P E K K Q K S I E D C A T T T T N K Q E E T E V K Q T T T 627 S G G F E V N E L E S P T K K L K G A S T K K R I D E K I I D • R F D S E 612 • • • • • • • • • • D G W Q A T G K R K K K R E Q N V E • • • • • • 586 • • • • • • • • • E D G W Q A T G K R K K K R E Q N V E • • • • • • 586 • • • • • • • • E D G W Q A T G K R K K K K D Q A A G G • • • • • 557 A G G E G E E H P E D G D E D E N G D E M K E K S E • • • • • • • • 476 A E E A E L D E F S A N Q S Q • • • G K R S K K H K K K S D A • S E • • • 647 • • • • • • • Q Q A P A V H G R K G Q K K K K Q Q K P K G • A S A T Q 608 • • • • • • • • E R A T A T • • K K G P K K K K K S E 6 • A E • • 615 / L D E K E N D D D D E D E A T • • H E S T E K K K K S E 6 • G E • • 644 • E V R A Q E D D P S N P G A T E Q G K K P K N H K R Q K T D S • T P P E T 617 E E E E E E E E E E E E Q D S Q E G K K K A K R L K K A K M E P G S P S E D 627 / P E • • • D Q T N S Y A E S P P D V K K R A K R F K K I K S E Q • V S A G G 559 / S D S E L E Q T D S C T D P L P E G R K K S K K L K R M K K E L • S L A G S 609 S D C E L D Q T D S C A E S L T E G K K K A K K C K M K K E L • T P A G 523	
_	Anopheles Ciona Bombyx Harpegnathos Camponotus Branchiostoma Danio Gasterosteus Tetraodon Takifugu Xenopus Anolis Gallus Wus Wonodelphis Bos	A K R K G M D E A T P P T M A D K D A T A A K D D E H D N D E D N G G G W R 616 S H A S R S P D E P E K K Q K S I E D C A T T T T N K Q E E T E V K Q T T T 627 S G G F E V N E E S P T K K K G A S T K K R I D E K I I D • R F D S E 612 • • • • • • • • • • • • • • • • • • •	

Figure S3. Sequence alignment of the predicted NLS (A) and NoLS (B) performed by the online software MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/). The sequences from bottom to top used for alignment are: *Homo sapiens* (NP\_002573.1), *Bos taurus* (NP\_001094588.1), *Monodelphis domestica* (XP\_001375777.1), *Mus musculus* (NP\_083037.1), *Gallus gallus* (NP\_001025800.1), *Anolis carolinensis* (XP\_003228705.1), *Xenopus (Silurana) tropicalis* (NP\_001184102.1), *Takifugu rubripes* (XP\_003972409.1), *Tetraodon nigroviridis* (CAF98128.1), *Gasterosteus aculeatus*, *Danio rerio* (NP\_957382.1), *Branchiostoma floridae* (XP\_002606119.1), *Camponotus floridanus* (EFN61018.1), *Harpegnathos saltator* (EFN75773.1), *Bombyx mori* (NP\_001153677.1), *Ciona intestinalis* (XP\_002129059.1), *Anopheles gambiae str. PEST* (XP\_308433.4).



**Figure S4. Representative profiles of flow cytometry analysis of cell apoptosis determined by Annexin V-FITC binding (horizontal) and PI exclusion (vertical).** The HEK-293T cells were transfected by the empty vector (MOCK), Flag-PARN or Flag-PARN(1-540).



Figure S5. Western blot analysis of the protein levels of CstF-50 and p53 in untreated or UV-treated HEK-293T cells transfected with plasmids containing the full length or truncated PARN. NPM1 and actin were used as the controls.