Figure S1

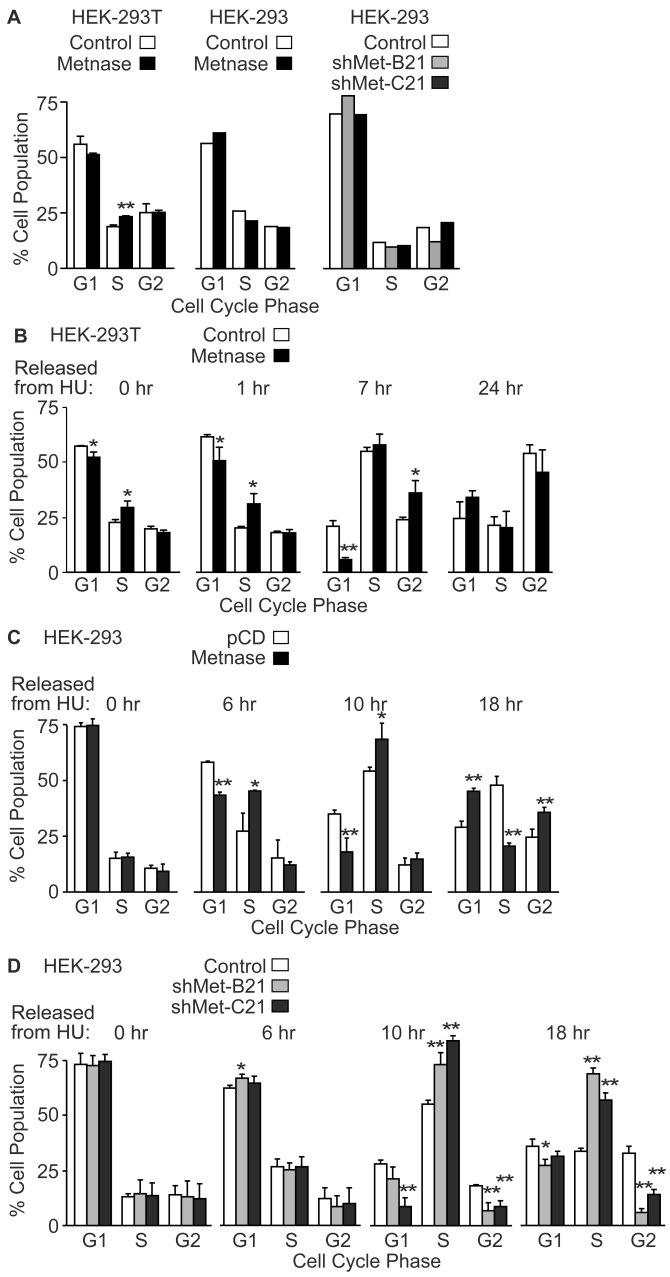

Figure S1. Metnase promotes cell cycle progression after replication stress. A) Cell cycle distributions of log phase cultures of HEK-293T, HEK-293 cells stably transfected with empty or Metnase overexpression vectors, and HEK-293 cells stably transfected with empty or Metnase knockdown vectors. Values in left graph are averages (±SD) from three experiments; other graphs show data from single experiments. B, C) Cell cycle distributions of HEK-293T or HEK-293 cells, with or with Metnase overexpression, after 18 h treatment with 5 mM HU and release into normal growth medium for indicated times. Values are averages (±SD) of three experiments; * indicates $P < 0.05$, ** indicates $P < 0.01$. D) Cell cycle distributions of HEK-293 cells, with or without Metnase knockdown following HU release. Data presented as in panels B and C.

Figure S2

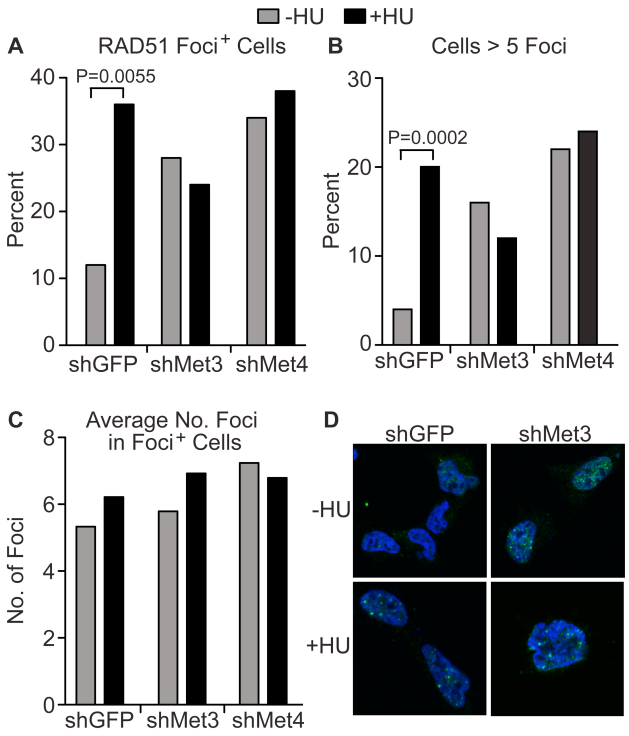

Figure S2. HU-induced RAD51 focus formation in control and Metnase knockdown cells. HEK-293 control (shGFP) and two Metnase knockdown (shMet3/4) cell lines were treated with 10 mM HU for 4 hr or mock treated and prepared for immunofluorescence microscopy to detect RAD51. RAD51 foci were scored in 50 cells per cell line per treatment. (A) Percentage of cells with at least one RAD51 focus. (B) Percentage of cells with >5 foci. (C) Average number of RAD51 foci per cell in cells with at least one focus. (D) Representative images of RAD51 foci (green) and DAPI-stained nuclei (blue).

Table S1. Conserved PIP boxes in Metnase and other human DNA repair/metabolism proteins.

Protein	Function(s)	PIP box*	Reference
Metnase	NHEJ, decatenation, fork restart	(119) VVQKGLQ-FH	(1)
PARP-1	DNA repair, fork restart	(668) PVQDLIKMIF	(2)
DNMT1	DNA methyltransferase	(162) TRQTTITSHF	(3)
DNA Polβ	DNA repair polymerase	(215) VEQLQKV-HF	(4)
p66	DNA polδ subunit	(454) NRQVSITGFF	(5)
MYH	BER glycosylase	(521) MGQQVLDNFF	(6)
UNG2	BER glycosylase	(2) IGQKTLYSFF	(7)
APE2	BER endonuclease	(288) RGQKNLKSYF	(8)
XPB	NER endonuclease	(988) QTQLRIDSFF	(9)
BLM	DNA repair helicase	(81) TNQQRVKDFF	(10)
RECQL5β	DNA repair helicase	(962) EAQN-LIRHF	(10)
p15 PAF	Cell growth promotion	(60) KWQKGIGEFF	(11)
ING1b	Apoptosis	(7) GEQLHLVNY	(12)
MDM2	E3 ubiquitin ligase	(481) PIQMIVLTYF	(13)
WSTF	Chromatin remodeling	(662) LLQDEIAEDY (1024) RYQDIIHSIH (1099) ALQASVIKKF (1432) TEQCLVALLH	(14)
	Consensus PIP box:	[K-A]Qxx-I/L/Vxx(F/Y/H/W)$_2$	(15)

*All proteins have the core PIP Q-I/L/V motif, and nearly all (including Metnase) have the C-terminal pair of F/Y/H residues (shown in red). Numbers in parentheses indicate amino acid sequence numbers for indicated peptides. Many PIP boxes have upstream K and/or A residues; Metnase and PARP-1 have conservative substitutions (V for A) at this position, indicated in green.

Supplemental References

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