Supporting Information

Edenberg et al. 10.1073/pnas.1315325111

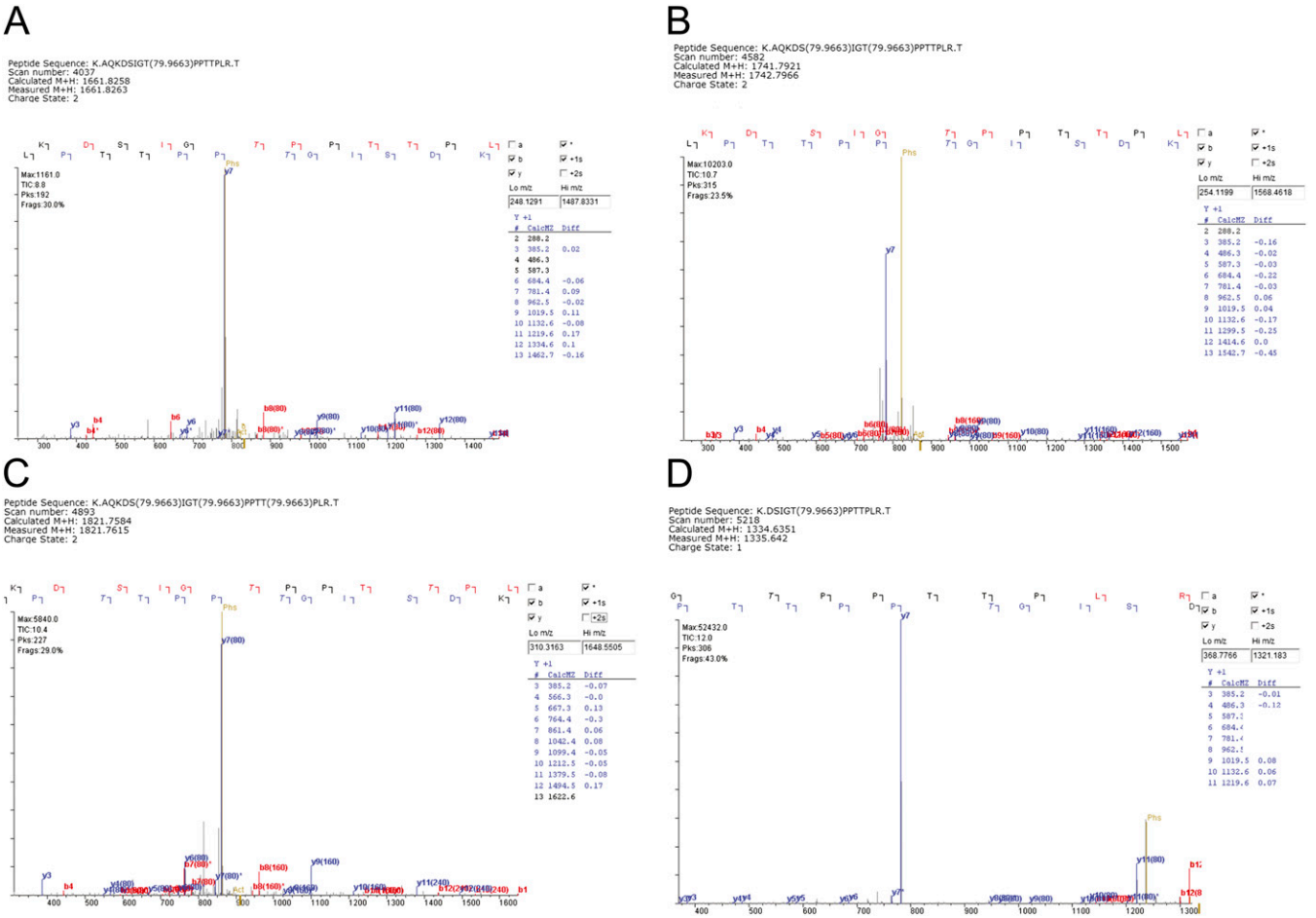

Fig. S1. (Continued)

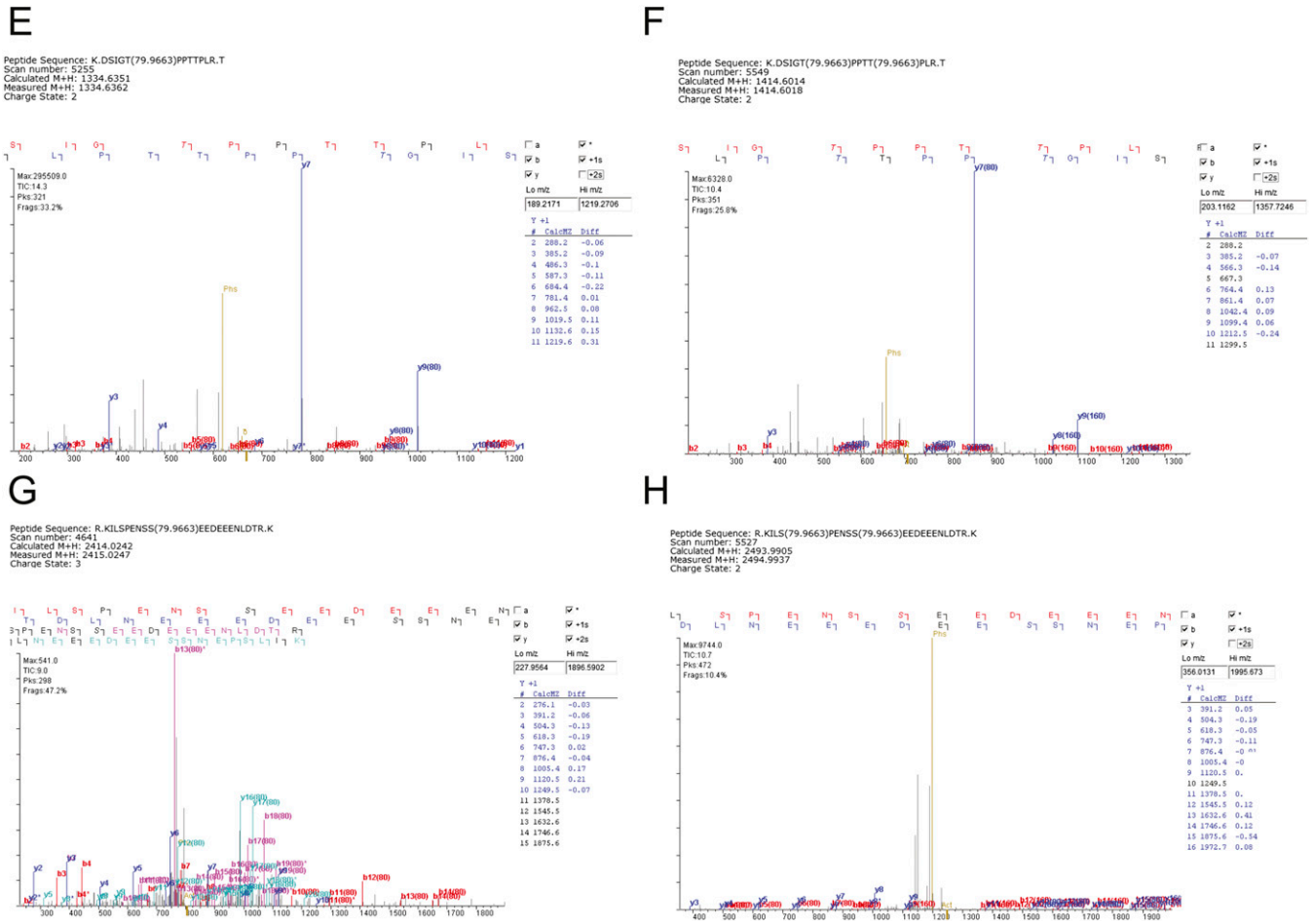

Fig. S1. (Continued)

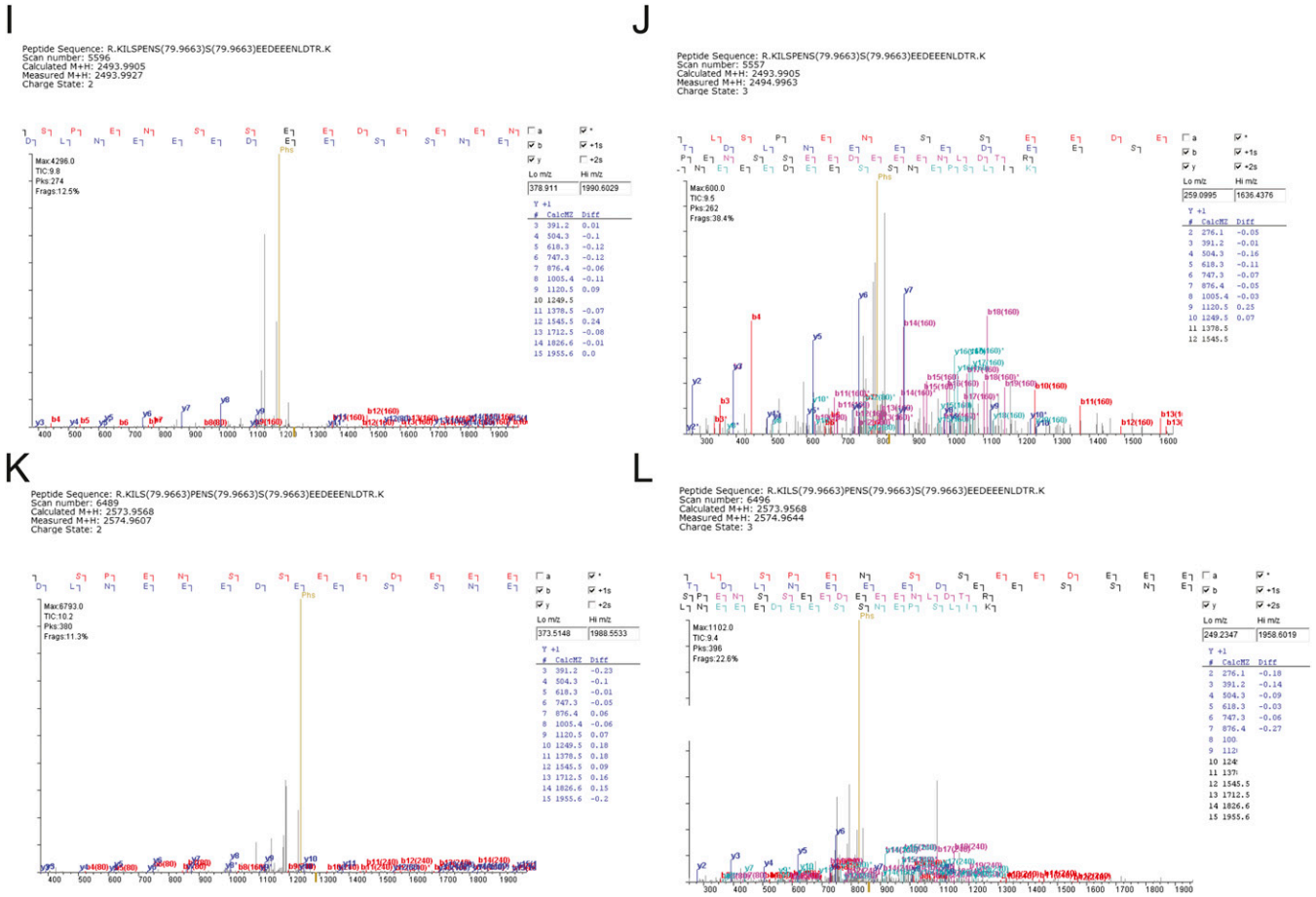

Fig. S1. (Continued)

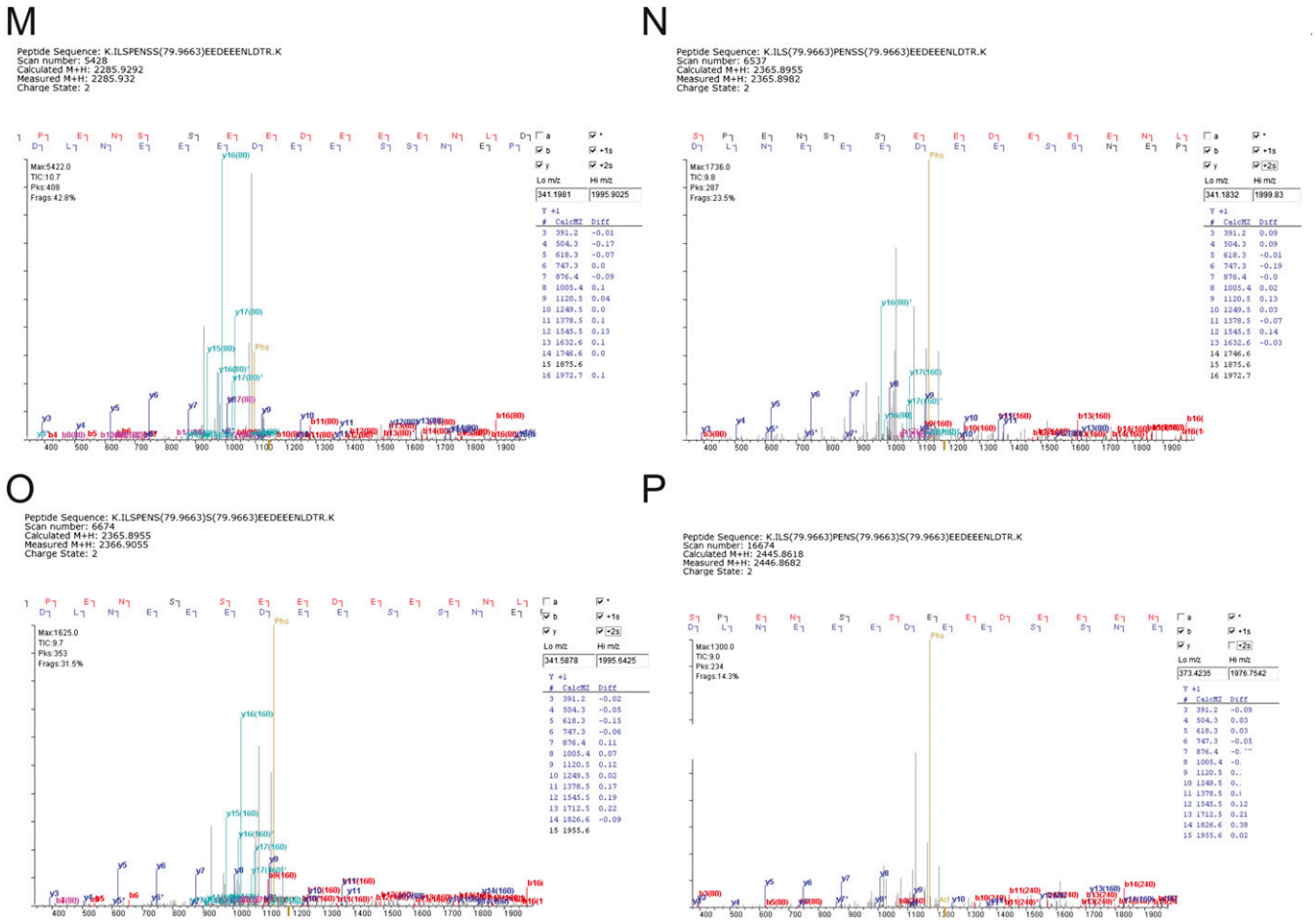

Fig. S1. (Continued)

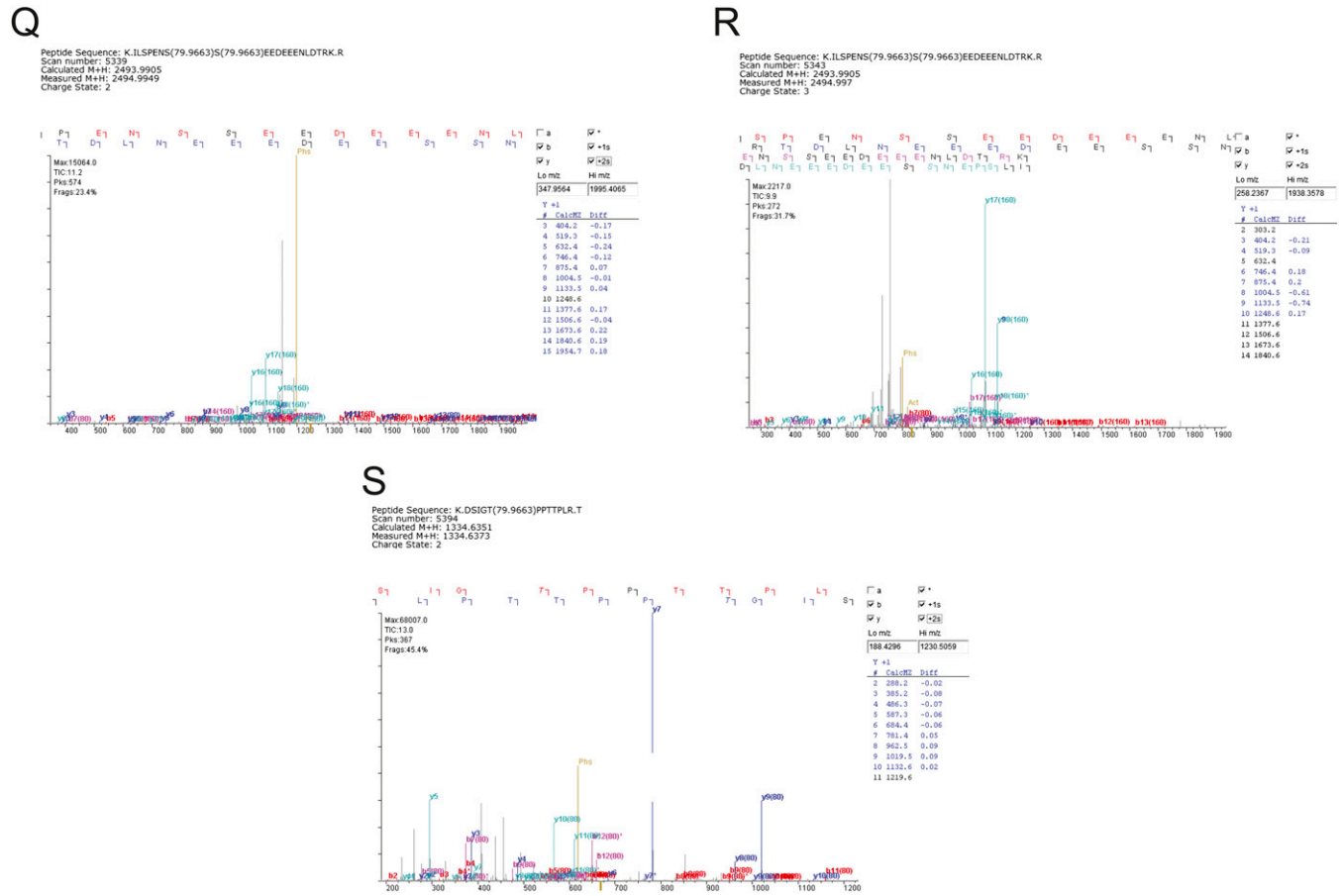

Fig. S1. Spectra from mass spectrometry analysis of phospho-sites identified within degron 1 (D1) and degron 2 (D2). (*A*–*R*) Spectra from purification in the presence of methyl methane sulfonate (MMS). (*S*) Spectra from purification from untreated cells. All sites were identified in more than one peptide.

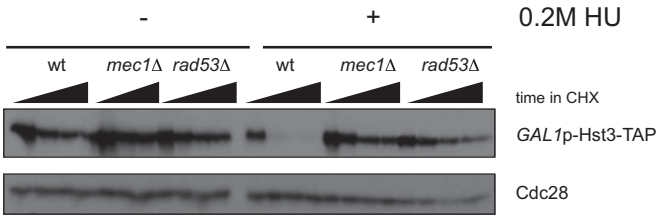

Fig. S2. The increase in Hst3 turnover in response to DNA damage is *MEC1*- and *RAD53*-dependent and is independent of the GFP tag. Hst3-TAP is under the control of the *GAL1* promoter. The experiment was performed as in Fig. 3*A*. The black triangle represents the time after the addition of cycloheximide (CHX) (t = 0, 15, 30, and 45 min except for *mec1*Δ undamaged turnover, which has time points t = 0, 15, and 30 min only).

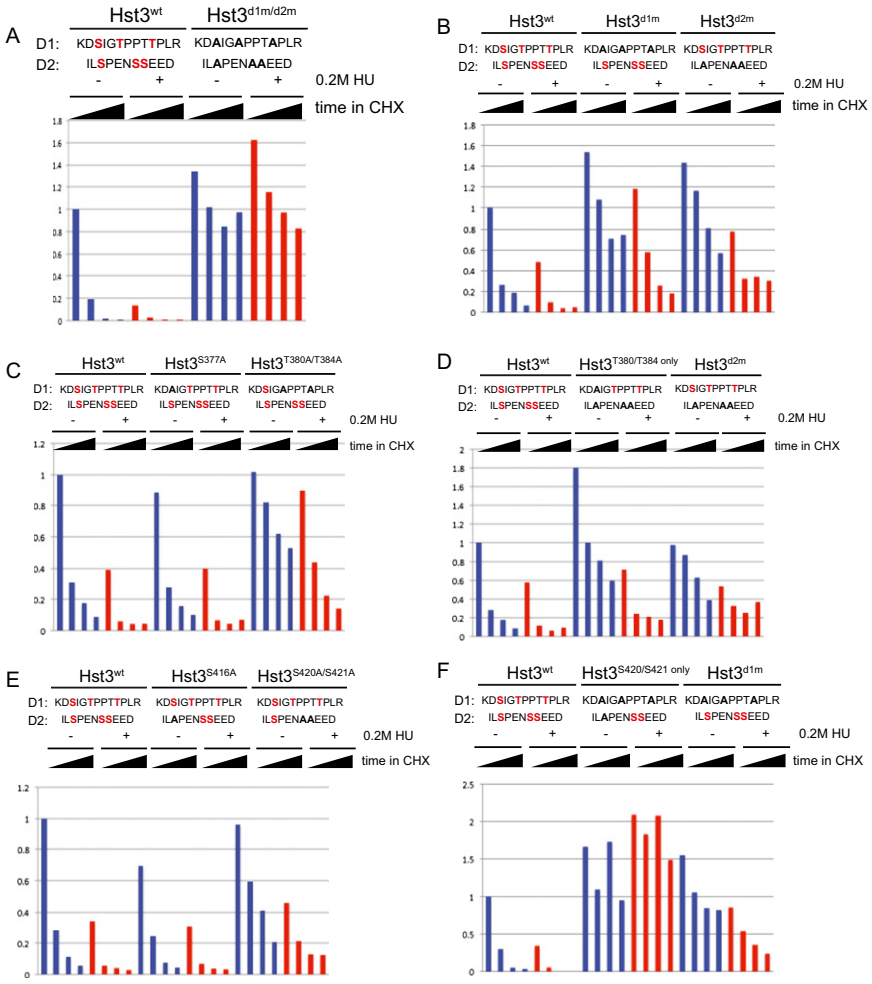

Fig. S3. Quantitative analysis of Hst3 turnover (gels shown in Fig. 4). (*A–F*) Turnover of Hst3 wild-type and site mutants, as noted, was quantified. Wild-type phosphorylated residues are in red, and mutations are in black. Within each graph, the starting level of untreated wild-type is set to 1. Blue bars represent untreated samples; red bars represent HU-treated samples.

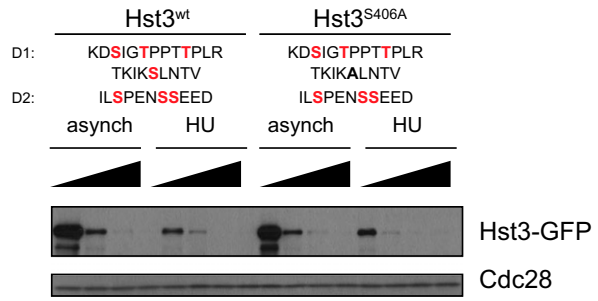

Fig. S4. Hst3 turnover is unaffected by mutation of S406. Wild-type phosphorylated residues are in red, and mutations are in black. The experiment was performed as in Fig. 4, with Hst3 expressed under the control of the *GAL1* promoter and tagged with GFP. The black triangle indicates the time after the addition of cycloheximide (t = 0, 15, 30, and 45 min).

Table S1. Details of mass spectrometry analysis on Hst3

Sites	Part of degron?
Site in untreated cells	
T380	D1
Sites in MMS-treated cells	
T172	
S173	
T377	D1
T380	D1
T384	D1
S406	
S416	D2
S420	D2
S421	D2

Table S2. Strains

Strain name	Strain background	Genotype	Used in figure
yERE10	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3-3Flag-KanMx	Figs. 1 *A* and *B* and 5 *B* and *C*
yERE22	S288C	*MAT*a his3Δ1 leu2Δ0 ura3Δ0 URA3-*GAL1*p-Hst3-3Flag-KanMx cdc53-1	Fig. 1 *A* and *B*
yERE23	S288C	*MAT*a his3Δ1 leu2Δ0 ura3Δ0 URA3-*GAL1*p-Hst3-3Flag-KanMx cdc4-1-HYGMx	Figs. 1 *A* and *B* and 5 *B* and *C*
yERE26	S288C	*MAT*a his3Δ1 leu2Δ0 ura3Δ0 URA3-*GAL1*p-HST3-3Flag-KanMx cdc4-1-HYGMx	Figs. 1*C* and 5 *B* and *C*; same as yERE23
yERE477	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KanMx-*GAL1*p-HST3-9myc-Nat^{R} cdc4-1-HYG^{R} with pYES2-GST-URA	Fig. 1*D*
yERE478	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KanMx-*GAL1*p-HST3-9myc-Nat^{R} cdc4-1-HYG^{R} with pYES2-GST-Cdc4ΔF-Flag-URA	Fig. 1*D*
yERE479	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KanMx-*GAL1*p-HST3$^{d1m/d2m}$-9myc-Nat^{R} cdc4-1-HYG^{R} with pYES2-GST-URA	Fig. 1*D*
yERE480	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KanMx-*GAL1*p-HST3$^{d1m/d2m}$-9myc-Nat^{R} cdc4-1-HYG^{R} with pYES2-GST-Cdc4ΔF-Flag-URA	Fig. 1*D*
yERE114	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-HST3-GFP-His3Mx	Fig. 2*A* and *B*
yERE102	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-HST3Δ97-GFP-His3Mx	Fig. 2*A*
yERE315	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KanMx-*GAL1*p-HST3-GFP-His3Mx	Figs. 2 *B* and 3*D*
yERE331	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KanMx-*GAL1*p-HST3^{C97}-GFP-His3Mx	Fig. 2*B*
yERE332	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KanMx-*GAL1*p-GFP-His3Mx	Fig. 2*B*
yERE328	S288C	*MAT*a his3Δ1 leu2Δ0 ura3Δ0 KanMx-*GAL1*p-HST3-GFP-His3Mx sml1Δ::LEU2	Fig. 3*A*
yERE329	S288C	*MAT*a his3Δ1 leu2Δ0 ura3Δ0 KanMx-*GAL1*p-HST3-GFP-His3Mx sml1Δ::LEU2 rad53Δ::KanMx	Fig. 3*A*
yERE221	S288C	his3Δ1 leu2Δ0 ura3Δ0 sml1Δ::LEU2 HygMx-*TEF1*p-Hst3-GFP-His3Mx	Fig. 3*B*
yERE222	S288C	his3Δ1 leu2Δ0 ura3Δ0 sml1Δ::LEU2 mec1Δ::KanMx HygMx-*TEF1*p-Hst3-GFP-His3Mx	Fig. 3*B*
yERE223	S288C	his3Δ1 leu2Δ0 ura3Δ0 sml1Δ::LEU2 rad53Δ::KanMx HygMx-*TEF1*p-Hst3-GFP-His3Mx	Fig. 3*B*
yERE210	S288C	URA-*GAL1*p-Hst3-3Flag-KanMx his3Δ1 leu2Δ0 ura3Δ0	Fig. 3*C*
yERE211	S288C	URA-*GAL1*p-Hst3-3Flag-KanMx his3Δ1 leu2Δ0 ura3Δ0 dun1Δ::KanMx	Fig. 3*C*
yERE471	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KanMx-*GAL1*p-HST3-GFP-His3Mx mck1Δ::KanMx	Fig. 3*D*
yNLM5	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3-TAP-His3Mx	Fig. S2 (1)
yERE21	S288C	*MAT*a his3Δ1 leu2Δ0 ura3Δ0 sml1Δ::LEU2 mec1Δ::KanMx URA3-*GAL1*p-Hst3-TAP-His3Mx	Fig. S2
yERE27	S288C	*MAT*a his3Δ1 leu2Δ0 ura3Δ0 sml1Δ::LEU2 rad53Δ::KanMx URA3-*GAL1*p-Hst3-TAP-His3Mx	Fig. S2
yBEN1	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3^{wt}-GFP-His3Mx	Fig. 4 and Fig. S4
yBEN19	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3$^{d1m/d2m}$-GFP-His3Mx	Fig. 4*A*
yBEN5	S288C	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3^{S406A}-GFP-His3Mx	Fig. S4
yBEN4	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3^{d1m}-GFP-His3Mx	Fig. 4 *B* and *F*
yBEN11	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3^{d2m}-GFP-His3Mx	Fig. 4 *B* and *D*
yBEN2	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3^{S377A}-GFP-His3Mx	Fig. 4*C*
yBEN3	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3$^{T380A/T384A}$-GFP-His3Mx	Fig. 4*C*
yBEN13	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3$^{T380/T384\ only}$-GFP-His3Mx	Fig. 4*D*
yBEN7	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3^{S416A}-GFP-His3Mx	Fig. 4*E*
yBEN9	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3$^{S420A/S421A}$-GFP-His3Mx	Fig. 4*E*
yBEN18	S288c	*MAT*a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 URA3-*GAL1*p-Hst3$^{S420/S421\ only}$-GFP-His3Mx	Fig. 4*F*
yBRT9-2B	A364A	*MAT*a ade2 ade3 leu2 trp1 ura3 cyh2 sic1Δ::LEU2	Fig. 5*A*
yBRT9-1A	A364A	*MAT*a ade2 ade3 leu2 trp1 ura3 cyh2 sic1Δ::LEU2 cdc4-1	Fig. 5*A*
yMCB4-1	S288c	*MAT*α his3Δ1 leu2Δ0 ura3Δ0 lys2Δ cdc4-1-HYGMx	Fig. 5 *B* and ***C***

1. Maas NL, Miller KM, DeFazio LG, Toczyski DP (2006) Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4. *Mol Cell* 23(1):109–119.