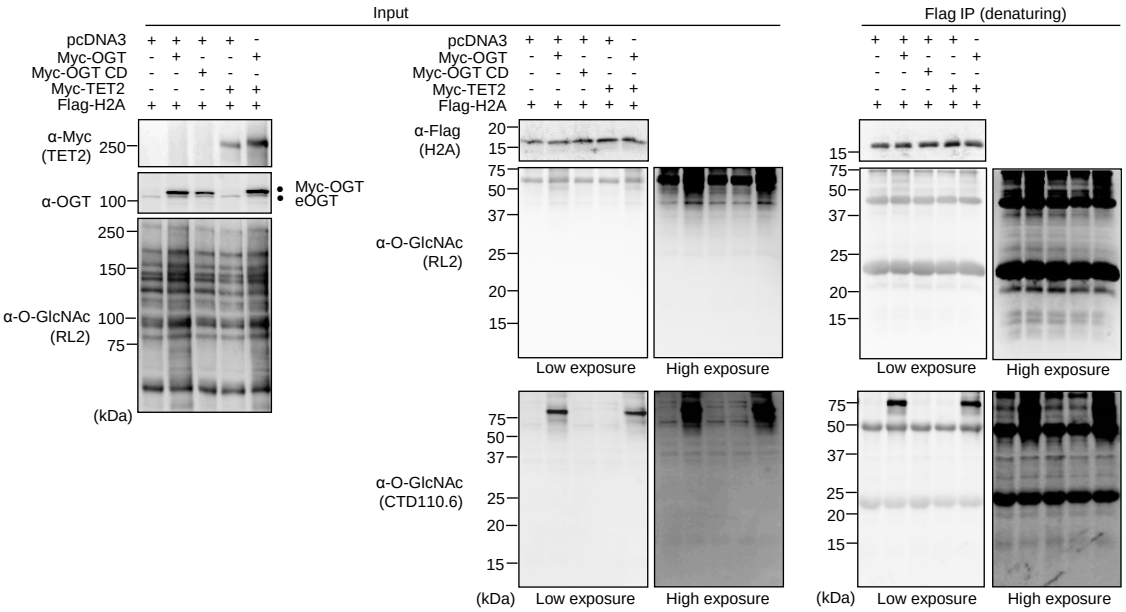
Supplemental Figure 1

A

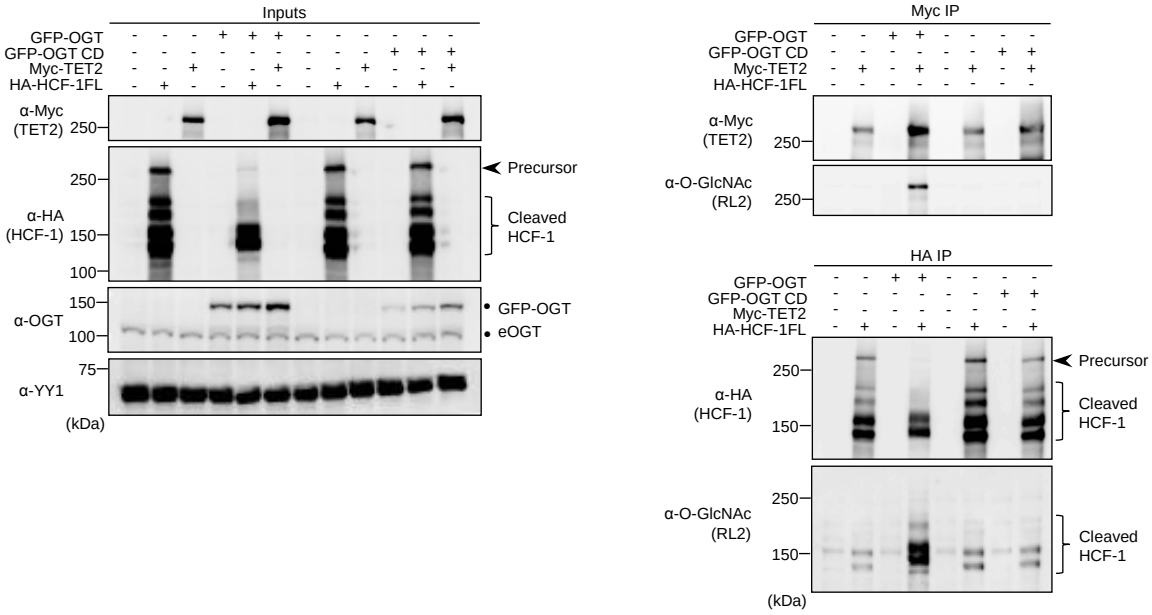


Supplemental Fig.1. Undetectable O-GlcNAcylation of histone H2A.

HEK293T cells were transfected with Flag-H2A along with either pcDNA3 empty vector, Myc-OGT, Myc-OGT catalytic dead (MYC-OGT CD) or Myc-TET2, as well as the combination of Myc-OGT with Myc-TET2. Three days post-transfection, cells were harvested and analysed for Flag-H2A O-GlcNAcylation as conducted for Flag-H2B (see Fig.1). Flag immunoprecipitation (Flag-IP) of exogenous H2A was conducted following sample denaturation. Dots indicate Myc-OGT and endogenous OGT (eOGT). kDa; Molecular weight marker in Kilodalton.

Supplemental Figure 2

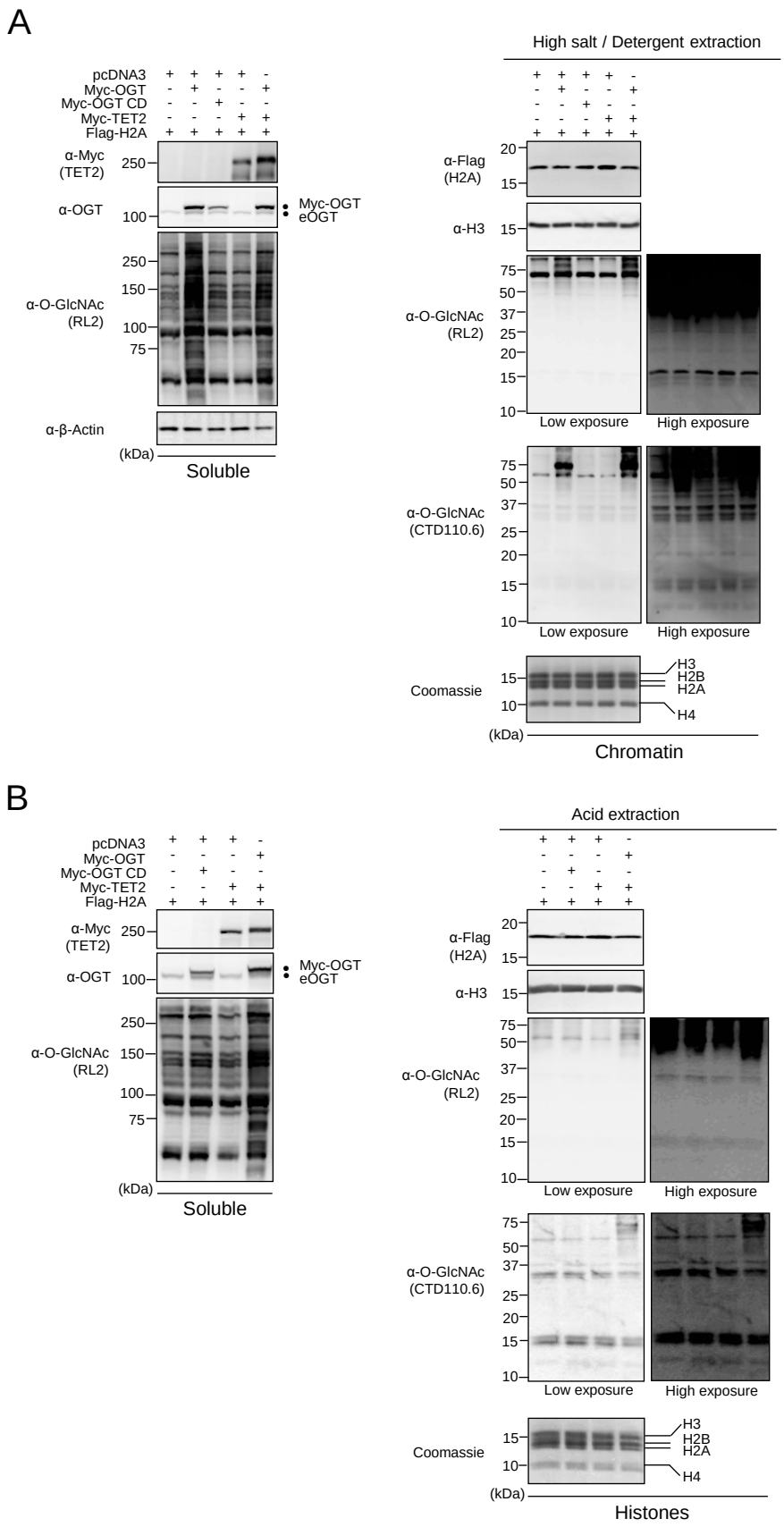
B



Supplemental Fig.2. OGT O-GlcNAcylates both TET2 and HCF-1 and modulates HCF-1 cleavage.

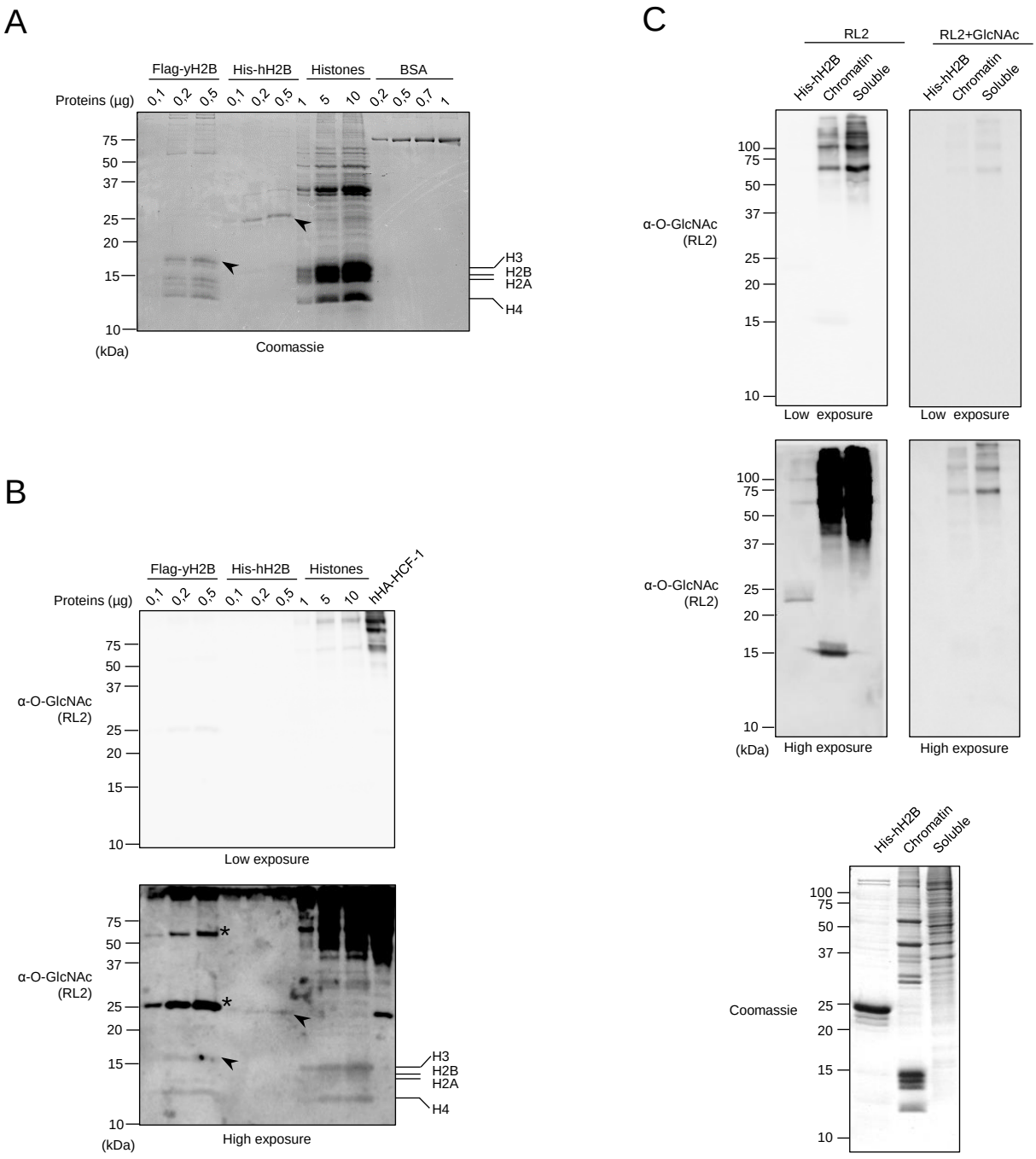
HEK293T cells were transfected with either GFP-OGT or GFP-OGT catalytic dead (CD) in the presence of HA-HCF-1 full length (FL) or Myc-TET2 expression vectors. Three days post-transfection, cells were harvested and total cell lysates were subjected to immunoprecipitation (IP), following sample denaturation, using anti-Myc or anti-HA antibodies to purify TET2 and HCF-1 respectively. Total cell lysates (Input fractions) as well as immunopurifications were subjected to western blotting analysis using the indicated antibodies. Arrow indicates the full length (precursor) form of HCF-1 and brace indicates the cleaved forms of HCF-1. Dots indicate GFP-OGT and endogenous OGT (eOGT). kDa; Molecular weight marker in Kilodalton.

Supplemental Figure 3



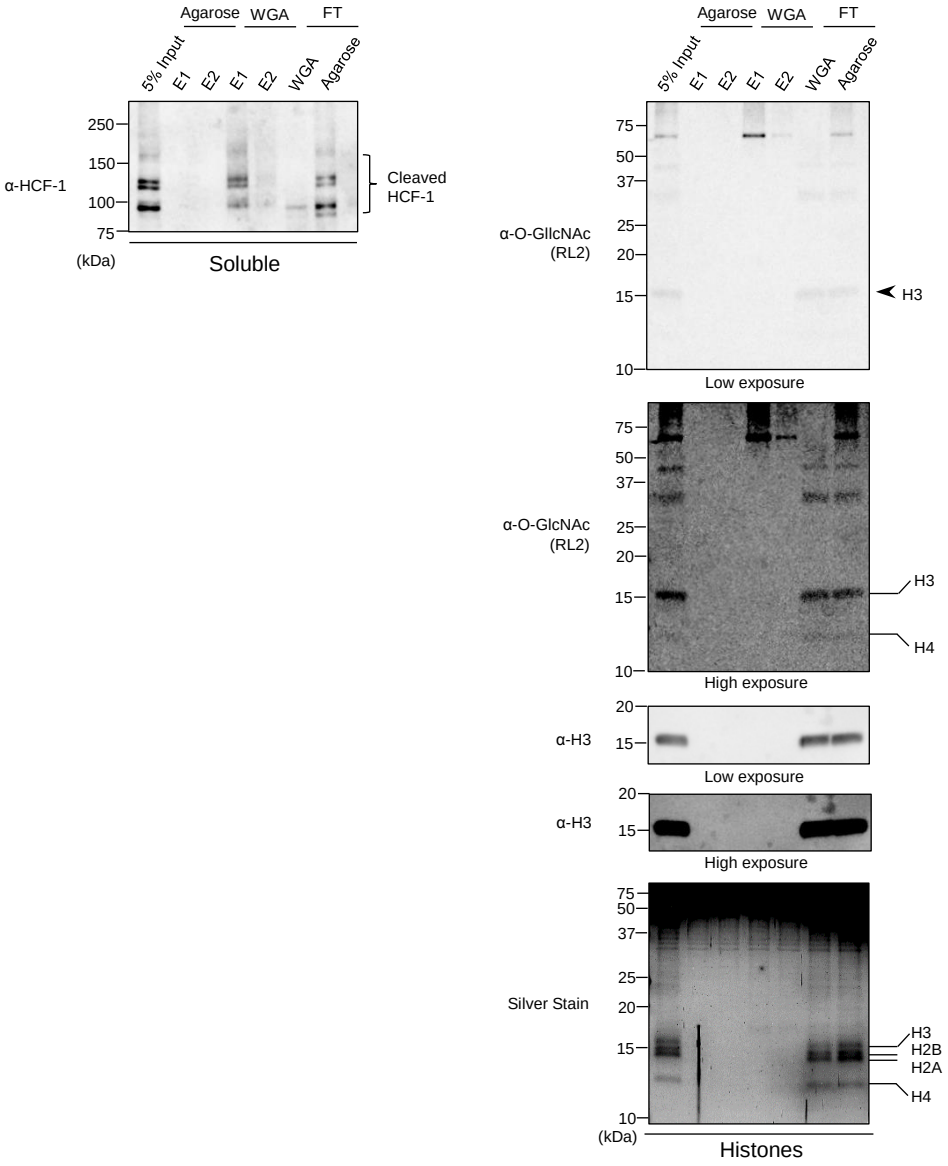
Supplemental Fig.3. Undetectable O-GlcNAcylation of endogenous histones. HEK293T cells were transfected as in Supplemental Fig.1. Three days post-transfection, cells pellets were collected for subsequent high salt /detergent extraction as well as histones acid extraction and cellular extracts were analysed by western blotting with the indicated antibodies. (**A**) Chromatin high salt extraction. (Left) Soluble fraction showing global increase of O-GlcNAcylation following OGT overexpression. (Right) Detection of O-GlcNAcylation using RL2 and CTD110.6 antibodies on chromatin fraction. (**B**) Histone acid extraction showing (Left) the soluble fraction and global O-GlcNAcylation levels and (Right) the histone fraction detected with both O-GlcNAc antibodies. β-Actin and histone H3 were used as loading controls. Coomassie Brilliant Blue staining indicates abundance of histones loaded. Dots indicate Myc-OGT and endogenous OGT (eOGT). kDa; Molecular weight marker in Kilodalton.

Supplemental Figure 4



Supplemental Fig.4. Detection of background signals by anti-O-GlcNAc RL2 antibody following immunoblotting of mammalian, yeast or recombinant histones. (**A**) Coomassie Brilliant Blue staining showing molecular weight and relative quantification of purified recombinant yeast Flag-H2B (Flag-yH2B), human His-H2B (His-hH2B) and acid extracted histones purified from HeLa cells relative to bovine serum albumin (BSA). (**B**) Increasing amounts of recombinant Flag-yH2B, His-hH2B and acid extracted histones from HeLa cells quantified in (**A**) were analysed by western blot using α-O-GlcNAc (RL2) antibody. Purified human HCF-1(hHA-HCF-1) from HEK293T cells was used as a control for RL2 detection. Lines indicate purified endogenous histones from HeLa cells and Flag-yH2B. Arrows indicate Flag-yH2B and His-hH2B position. Asterisks indicate non-specific signal from the heavy and light chains of Flag antibody respectively. (**C**) N-Acetyl-D-Glucosamine competition with RL2 antibody. RL2 antibody was incubated with 1 M of N-acetylglucosamine (GlcNAc) for 1 hour. Antibody mixture was then used to immunoblot recombinant human His-hH2B, chromatin and soluble fractions from U2OS cells. Coomassie Brilliant Blue staining was used as a loading control. kDa; Molecular weight marker in Kilodalton.

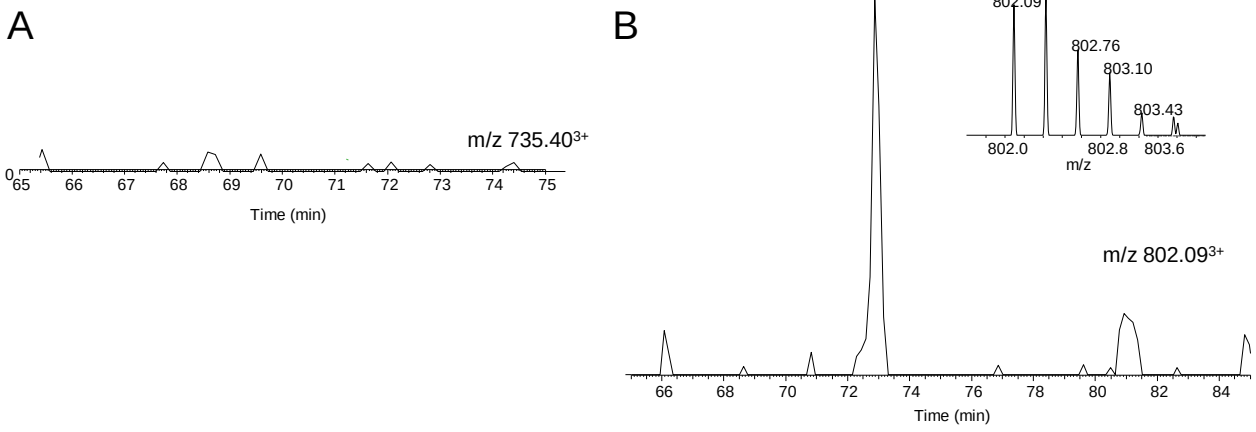
Supplemental Figure 5

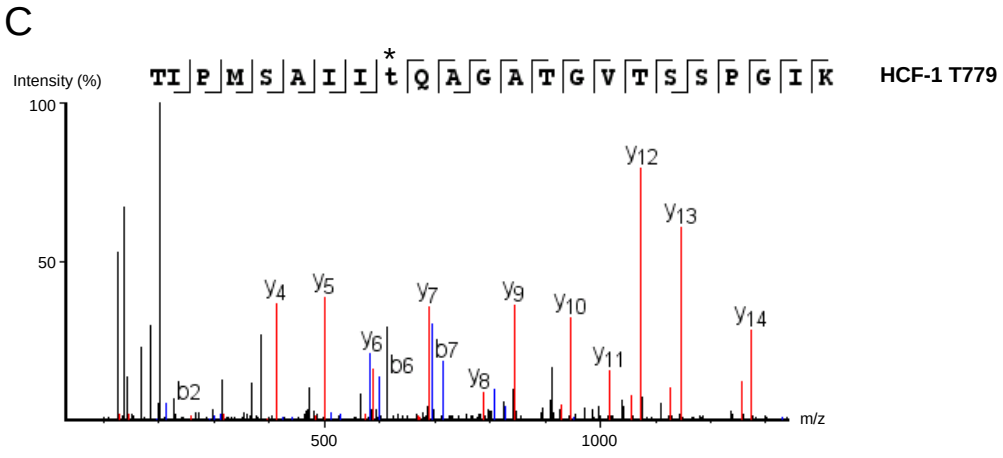


Supplemental Fig.5. The core histones are not enriched by WGA lectin resin in conditions that ensure complete HCF-1 depletion from extracts.

HeLa cells were harvested and acid extraction of histones was performed. The indicated soluble (Left panel) and histone fractions (Right panel) were incubated overnight with the agarose bound WGA lectin resin or with the agarose resin to control for non-specific binding. The flow through (FT) was kept and the proteins were eluted from the resins (E1 and E2) with N-acetylglucosamine (GlcNAc). Soluble fraction showing depletion of HCF-1 on the WGA lectin resin (Left panel) . Western blot and silver stain analysis of the collected elutions revealed no interaction between the core histones and the WGA lectin resin (Right panel). Arrows and lines indicate histones molecular weight. kDa; Molecular marker in Kilodalton.

Supplemental Figure 6



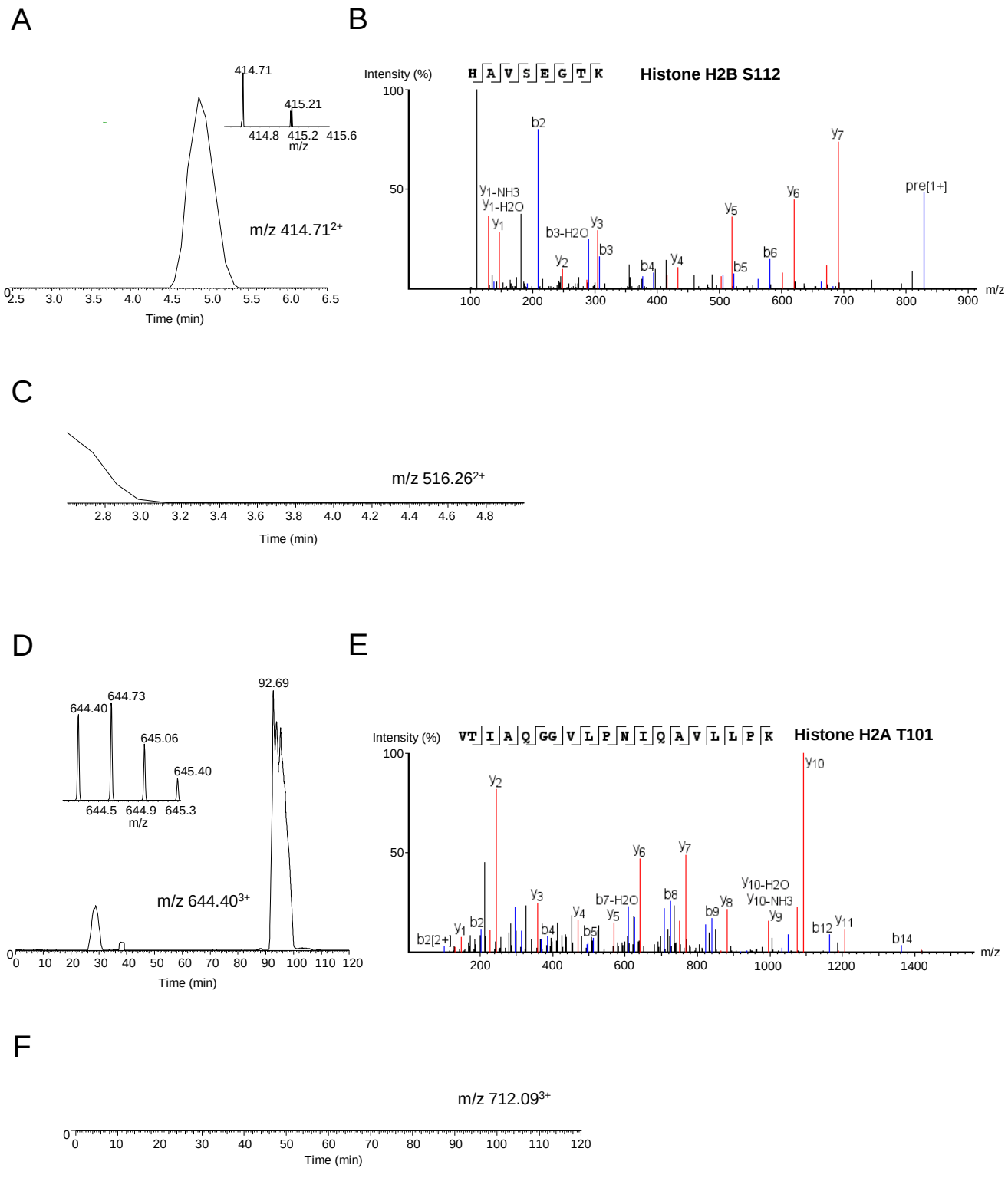


Supplemental Fig.6. HCD MS/MS spectra for HCF-1 O-GlcNAcylated peptide containing T779 modification site. (**A**) Extracted ion chromatogram for the HCF-1 peptide TIPMSAIITQAGATGVTSSPGIK at m/z 735.40^{2+} showing that the peptide was not detected. (**B**) Extracted ion chromatogram for the HCF-1 peptide TIPMSAIIT(GlcNAc)QAGATGVTSSPGIK at m/z 802.09^{2+} with the corresponding MS spectrum at 72.87 min. (**C**) MS/MS spectrum showing that the Thr9 indicated with the star is modified with the GlcNAc moiety.

Supplemental Table 1. Identification of O-GlcNAcylation sites on HCF-1

m/z	PTM sites of HCF-1	Peptide Sequence	References	ppm
927.77	T575	**lPPSSAPTVLSVPAGTtlVKTMAVTPGTTTLPATVK**	Novel	1.07
896.47	T588	**TMAVTPGTTtLPATVK**	Novel	-2.9
751.89	S620?/S622	**TAAAQVGTsVsSATNTSTRPIITVHK**	Ref. 47, 48, 50	-0.88
1,002.19	T625?/S628?	**TAAAQVGTSVSSAtNTsTRPIITVHK**	Ref. 51	0.12
846.12	T640?/T642	**SGtVtVAQQAQVVTTVVGGVTK**	Novel	0.3
778.42	T651	**SGTVTVAQQAQVVtTVVGGVTK**	Novel	0.67
1,084.85	T694	**VMSVVQTKPVQtSAVTGQASTGPVTQIIQTKGPLPAG TILK**	Novel	-1.9
847.70	T698	**VMSVVQTKPVQTSAVtGQASTGPVTQIIQTKGPLPAG TILK**	Novel	-2.9
1,001.94	T738?/S742	**LVTSADGKPTTIITtTQAsGAGTKPTILGISSVSPSTTK PGTTTIIK**	Novel	0.04
802.09	T771	**tIPMSAIITQAGATGVTSSPGIK**	Ref. 26, 27	1.0
1,187.97	S775?	**TIPMsAIItQAGATGVTSSPGIKSPITIITTK**	Novel	-3.7
802.09	T779	**TIPMSAIItQAGATGVTSSPGIK**	Ref. 26, 27, 47, 48, 50	0.32
1,141.36	T784	**TIPMSAIItQAGAtGVTSSPGIKSPITIITTKVMTSGTGA PAK**	Novel	1.2
726.40	T800	**SPITIItTKVMTSGTGAPAK**	Ref. 26, 27	-0.2
1,089.09	T801	**SPITIITtKVMTSGTGAPAK**	Ref. 47, 48, 49, 50	-0.2
742.45	T858	**LVtPVTVSAVKPAVTTLVVK**	Novel	0.11

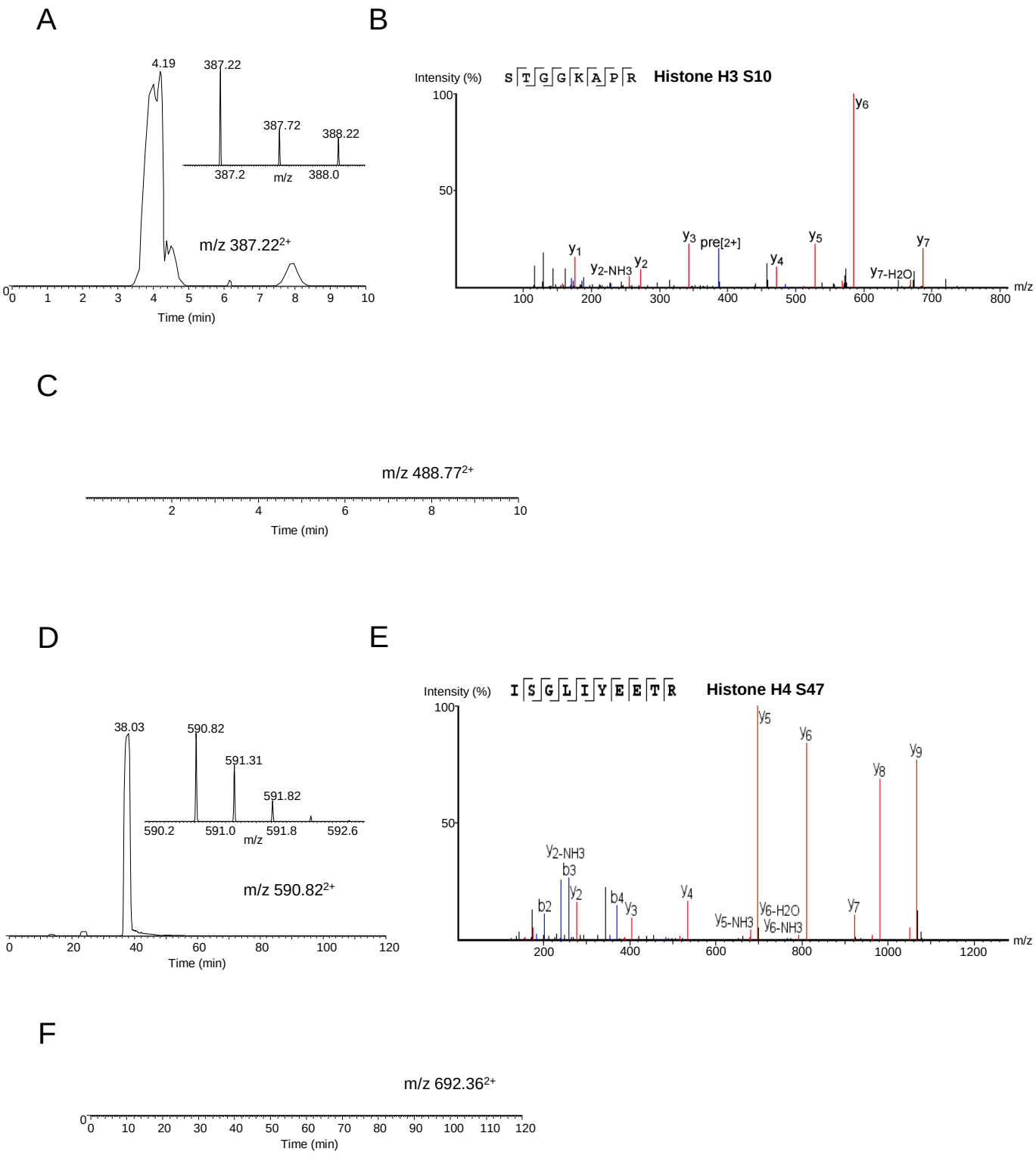
Supplemental Figure 7



Supplemental Fig.7. HCD MS/MS spectra for H2B and H2A peptides containing Ser112 and Thr101 respectively.

(**A-C**) HCD MS/MS spectra for H2B. Extracted ion chromatogram for the H2B peptide HAVSEGTK at m/z 414.71^{2+} showing that the peptide was detected (**A**) along with the corresponding MS spectrum at 4.9 min and its MS/MS spectrum (**B**). (**C**) Extracted ion chromatogram for m/z $516,26^{2+}$ corresponding to the expected peptide HAVSEGTK with a GlcNAC moiety. The absence of signal at m/z $516,26^{2+}$ indicates that the corresponding glycopeptide was not detected. (**D-F**) HCD MS/MS spectra for H2A. Extracted ion chromatogram for the H2A peptide VTIAQGGVLPNIQAVLLPK at m/z 644.40^{3+} showing that the peptide was detected (**D**) along with the corresponding MS spectrum at 92.69 min and its MS/MS spectrum (**E**). (**F**) Extracted ion chromatogram at m/z 712.09^{3+} corresponding to the expected peptide VTIAQGGVLPNIQAVLLPK bearing a GlcNAC moiety. The absence of a signal at m/z 712.09^{3+} indicates that the corresponding glycopeptide was not detected.

Supplemental Figure 8



Supplemental Fig.8. HCD MS/MS spectra for H3 and H4 peptides containing Ser10 and Ser47 respectively.

(**A-C**) HCD MS/MS spectra for H3. Extracted ion chromatogram for the H3 peptide STGGKAPR at m/z 387.22^{2+} showing that the peptide was detected as evidenced from its (**A**) corresponding MS spectrum at 4.01 min and its MS/MS spectrum (**B**). (**C**) Extracted ion chromatogram for the expected peptide STGGKAPR bearing a GlcNAC moiety at m/z 488.77^{3+}, the absence of a signal indicates that the corresponding glycopeptide was not detected. (**D-F**) HCD MS/MS spectra for H4. Extracted ion chromatogram for the H4 peptide ISGLIYEETR at m/z 590.82^{2+} showing that the peptide was detected as evidenced from the corresponding (**D**) MS spectrum at 39.03 min and its MS/MS spectrum (**E**). (**F**) Extracted ion chromatogram for the expected peptide ISGLIYEETR bearing a GlcNAC moiety at m/z 692.36^{2+}, the absence of signal indicates that the corresponding glycopeptide was not detected.