H2B tyrosine 37-phosphorylation suppresses expression of replication dependent core histone genes

Kiran Mahajan[1], Bin Fang[2], John M. Koomen[2] and Nupam P. Mahajan[1,3]

[1]Drug Discovery, [2]Proteomics Facility, Moffitt Cancer Center

12902 Magnolia Drive, Tampa, FL 33612, USA

[3]Department of Oncologic Sciences, University of South Florida,

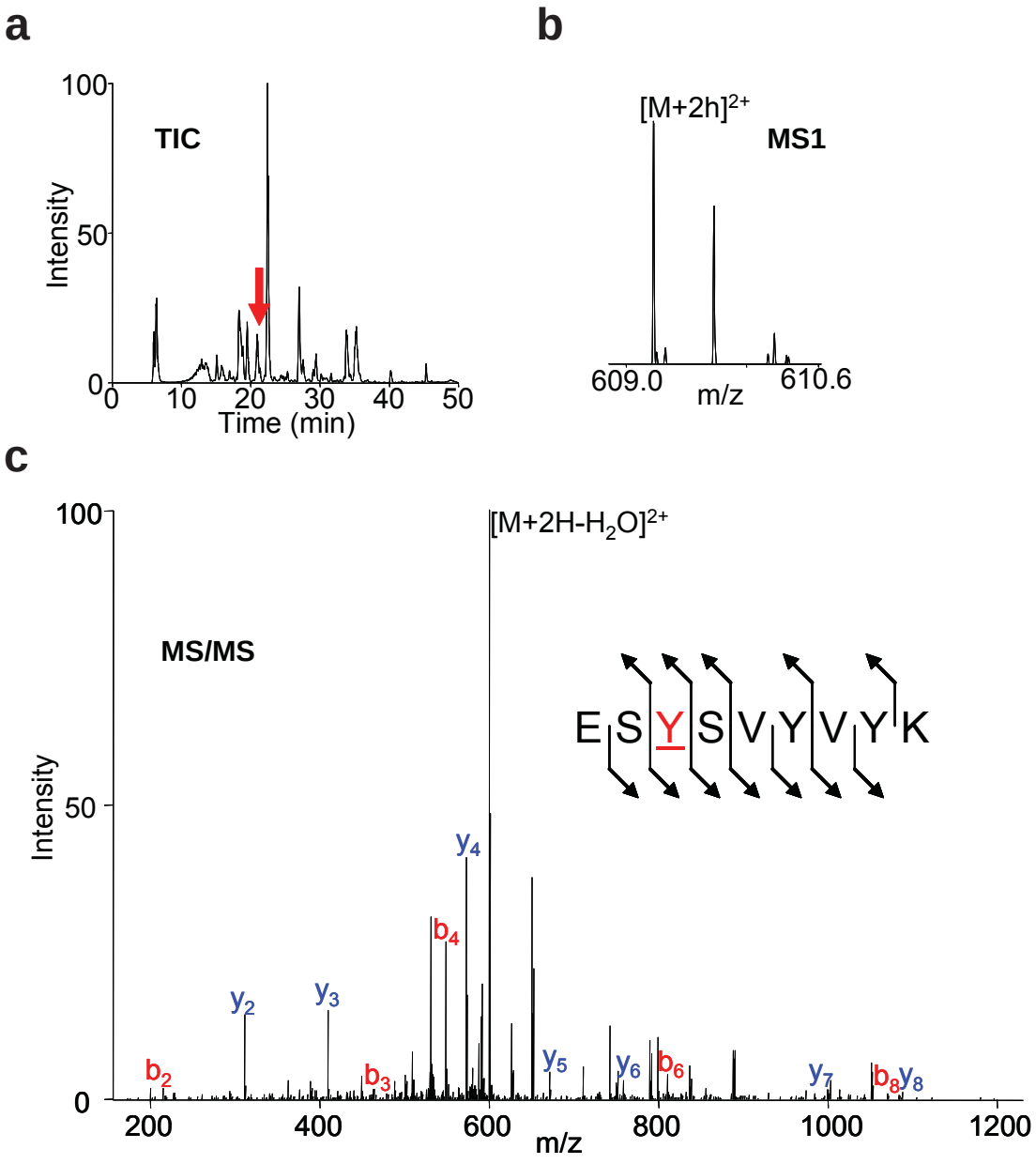
Tampa, FL 33620, USA

Correspondence and request for materials should be addressed to N.P.M. at

nupam.mahajan@moffitt.org

Nature Structural & Molecular Biology: doi:10.1038/nsmb.2356

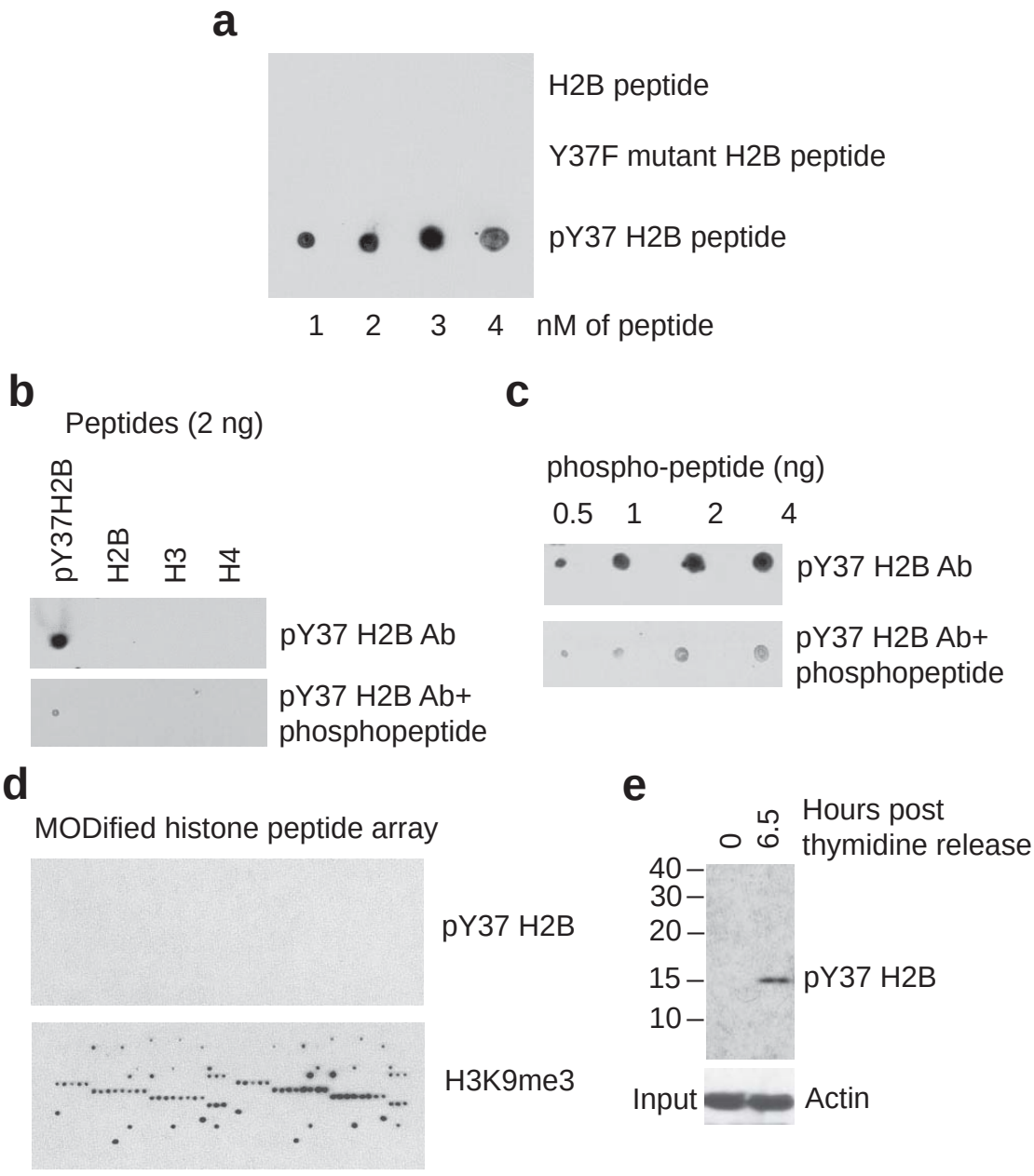
Supplementary Figure 1



Supplementary Figure 1. Identification of Histone H2B Y37-phosphorylation site. Histones were purified from HEK293 cells, followed by trypsin/chymotrypsin digestion. The peptide was detected at 21.7 mins in the total ion chromatogram (**a**) with mass-to-charge ratio 609.2303 (**b**). The tandem mass spectrum matched the sequence, ES**pY**SVYVYK indicating that the tyrosine37 was phosphorylated; the detection of the y6 and y7 is consistent with this localization (**c**). The assignment was made with Sequest with XCorr 2.34 and ΔCN: 0.27.

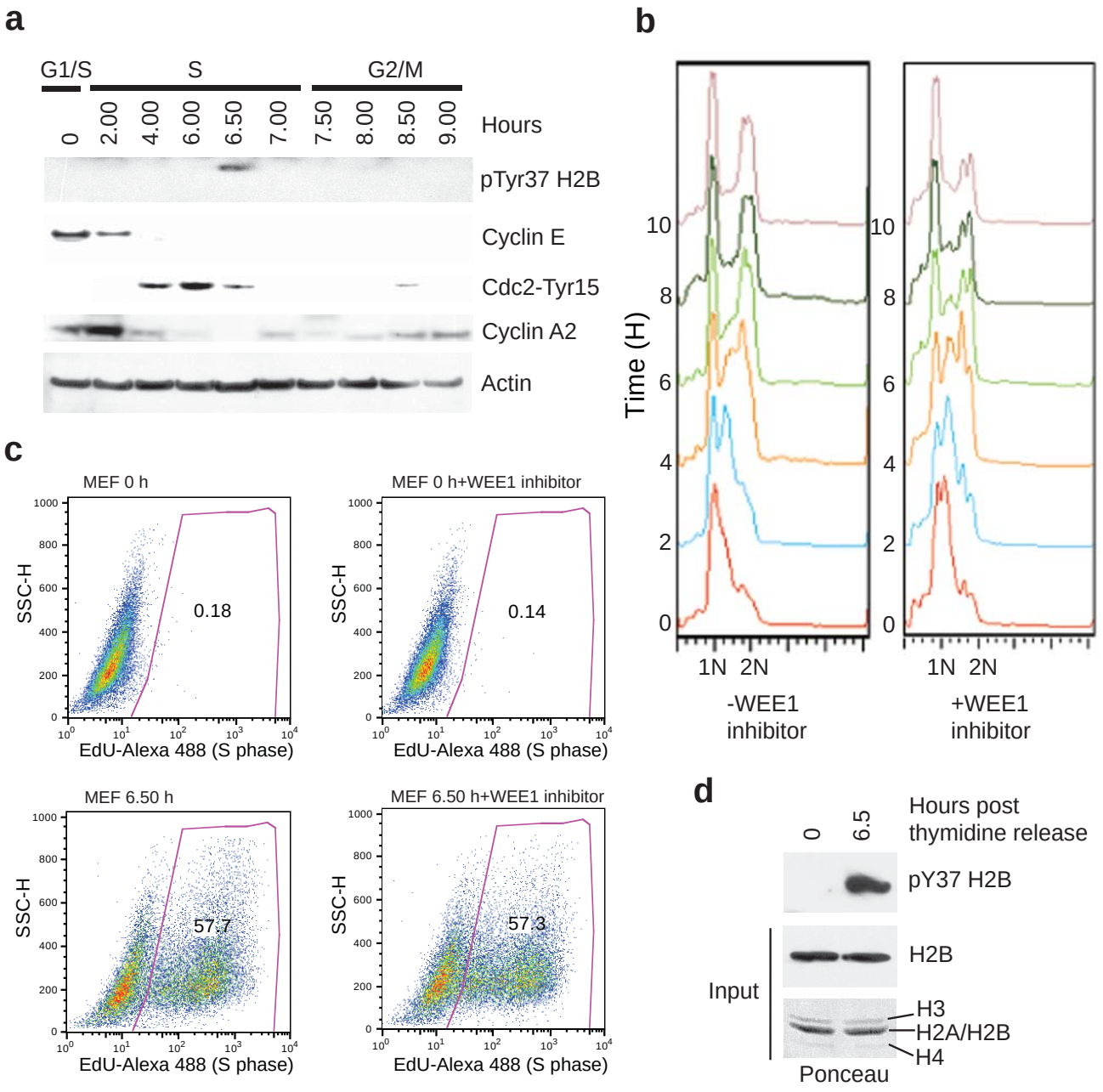
Nature Structural & Molecular Biology: doi:10.1038/nsmb.2356

Supplementary Figure 2



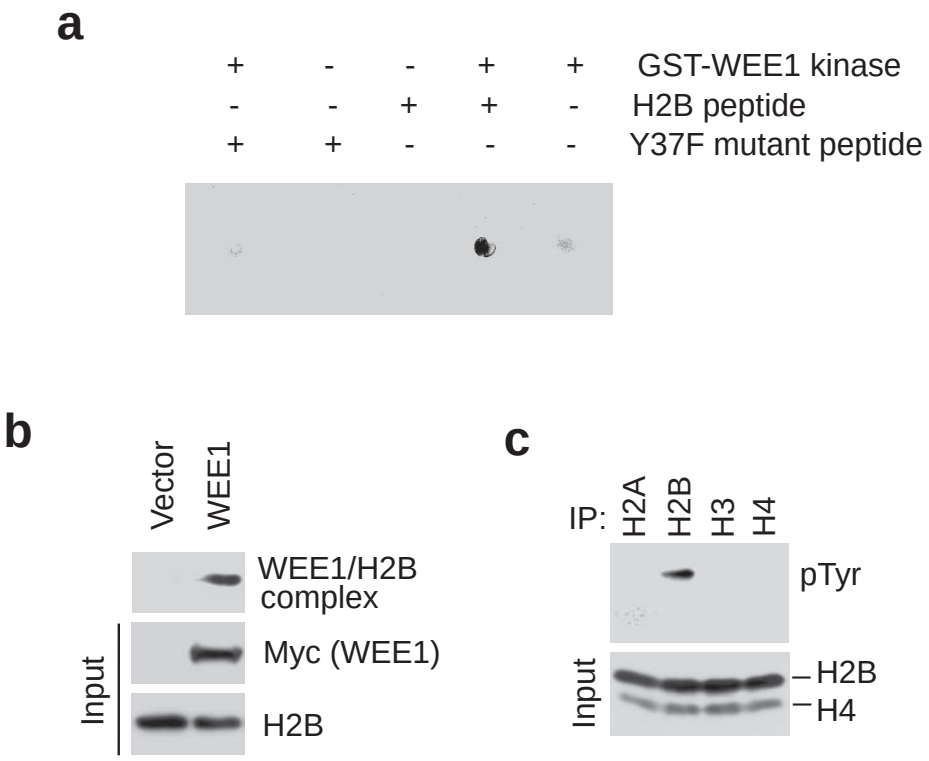
Supplementary Figure 2. Validation of pY37 H2B antibody. (**a**) Peptide spanning Y37 site, SRKES**pY**SVYVYK and identical but unmodified peptide SRKES**Y**SVYVYK and Tyr37-to-Phe mutant peptide SRKES**F**SVYVYK were spotted on nitrocellulose membrane (in increasing concentration) followed by immunoblotting with pY37 H2B antibody. (**b**) Peptides were spotted on nitrocellulose membrane. Prior to probing, the pY37 H2B antibody was pre-incubated with the phosphopeptide. (**c**) Peptide were spotted on nitrocellulose membrane in increasing concentration followed by immunoblotting with pY37 H2B antibody (top panel). Prior to probing, the pY37 H2B antibody was pre-incubated with the phosphopeptide. (**d**) The MODified Histone Peptide Array was immunoblotted with pY37 H2B (top panel) or H3K9me3 antibodies (as control). (**e**) Synchronized MEF lysates were immunoblotted with pY37 H2B antibody.

Supplementary Figure 3



Supplementary Figure 3. (**a**) Synchronized MEF lysates were immunoprecipitated with pY37 H2B antibodies followed by immunoblotting with H2B (top panel). Lysates were also immunoblotted with Cyclin E, pY15-Cdc2, Cyclin A and actin antibodies. (**b**) Synchronized MEFs treated with WEE1 inhibitor (0.62 uM) were harvested and stained with propidium iodide followed by flow cytometry for cell cycle analysis. (**c**) Synchronized MEFs treated with WEE1 inhibitor were released in fresh media. EdU was added and cells were harvested 6.50 hours post-release. Cells were fixed and `Click-it' reaction (Invitrogen) was performed followed by flow cytometry. (**d**) Synchronized MEFs were lysed and histones were isolated followed by immunoblotting with pY37 H2B antibodies.

Supplementary Figure 4

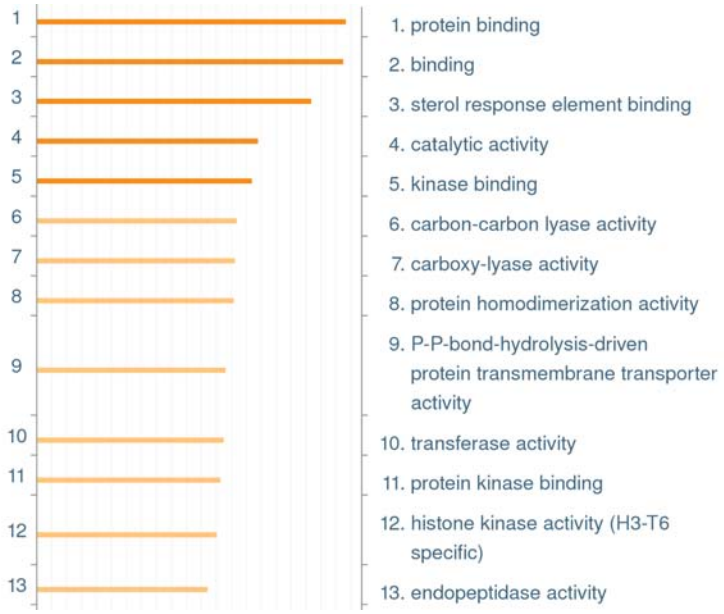


Supplementary Figure 4. (**a**) *In vitro* phosphorylation of H2B peptide by WEE1 kinase. Equimolar amounts of purified WEE1 protein and unmodified peptide SRKES**Y**SVYVYK or Tyr37-to-Phe mutant peptide SRKES**F**SVYVYK were incubated for 30 min at 30^{o}C. The reaction mix was spotted on nitrocellulose membrane followed by immunoblotting with pY37 H2B antibody. (**b**) WEE1 binds and phosphorylates endogenous H2B. HEK293T cells were transfected with myc-tagged WEE1 expressing construct or empty vector and lysates were immunoprecipitated with anti-myc antibodies followed by immunoblotting with H2B antibodies. (**c**) HEK293T cells were transfected with WEE1 expressing construct and lysates were immunoprecipitated with anti-histone H2A, H2B, H3 and H4 antibodies followed by immunoblotting with pTyr antibodies.

Supplementary Figure 5

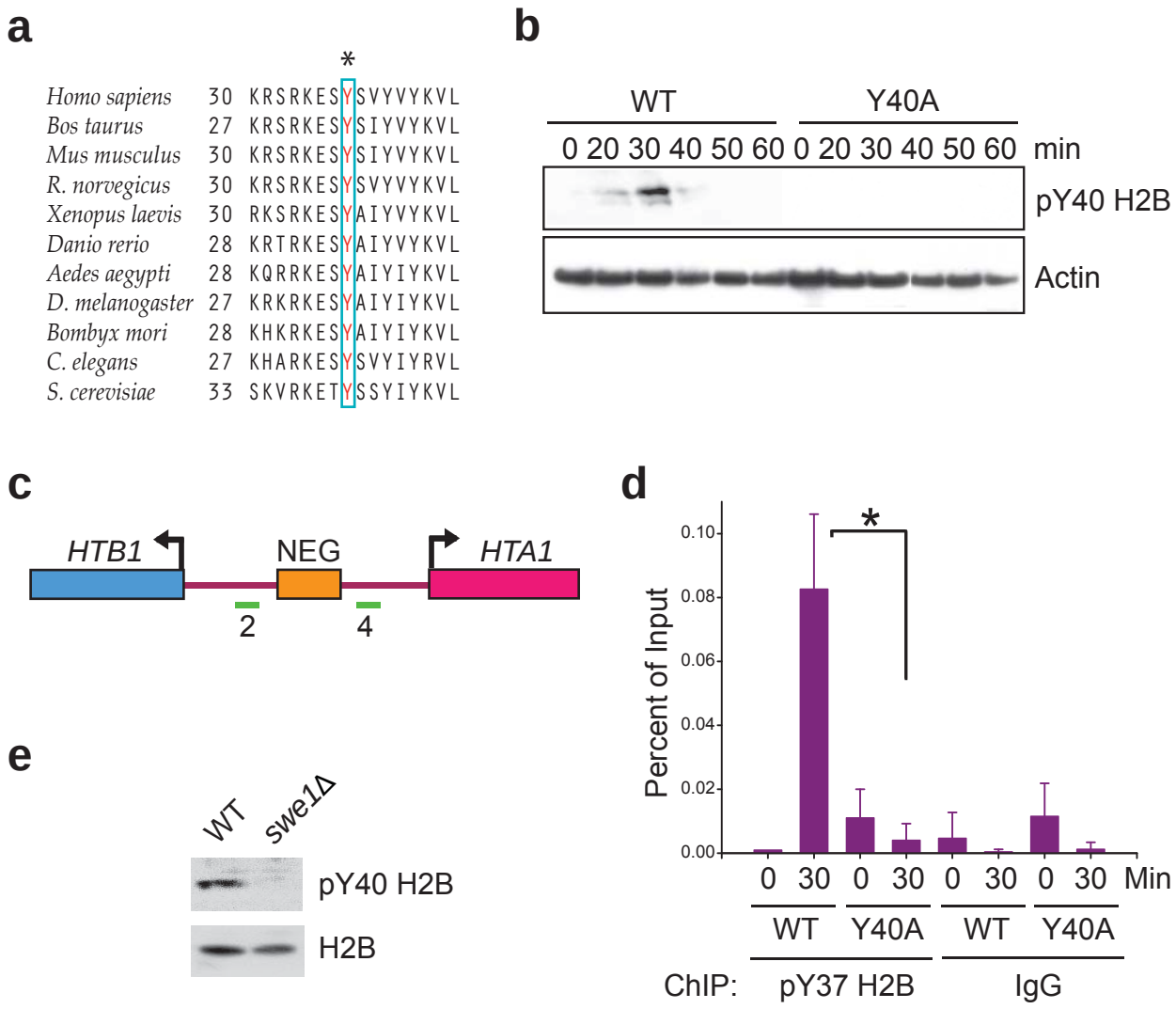
a

b



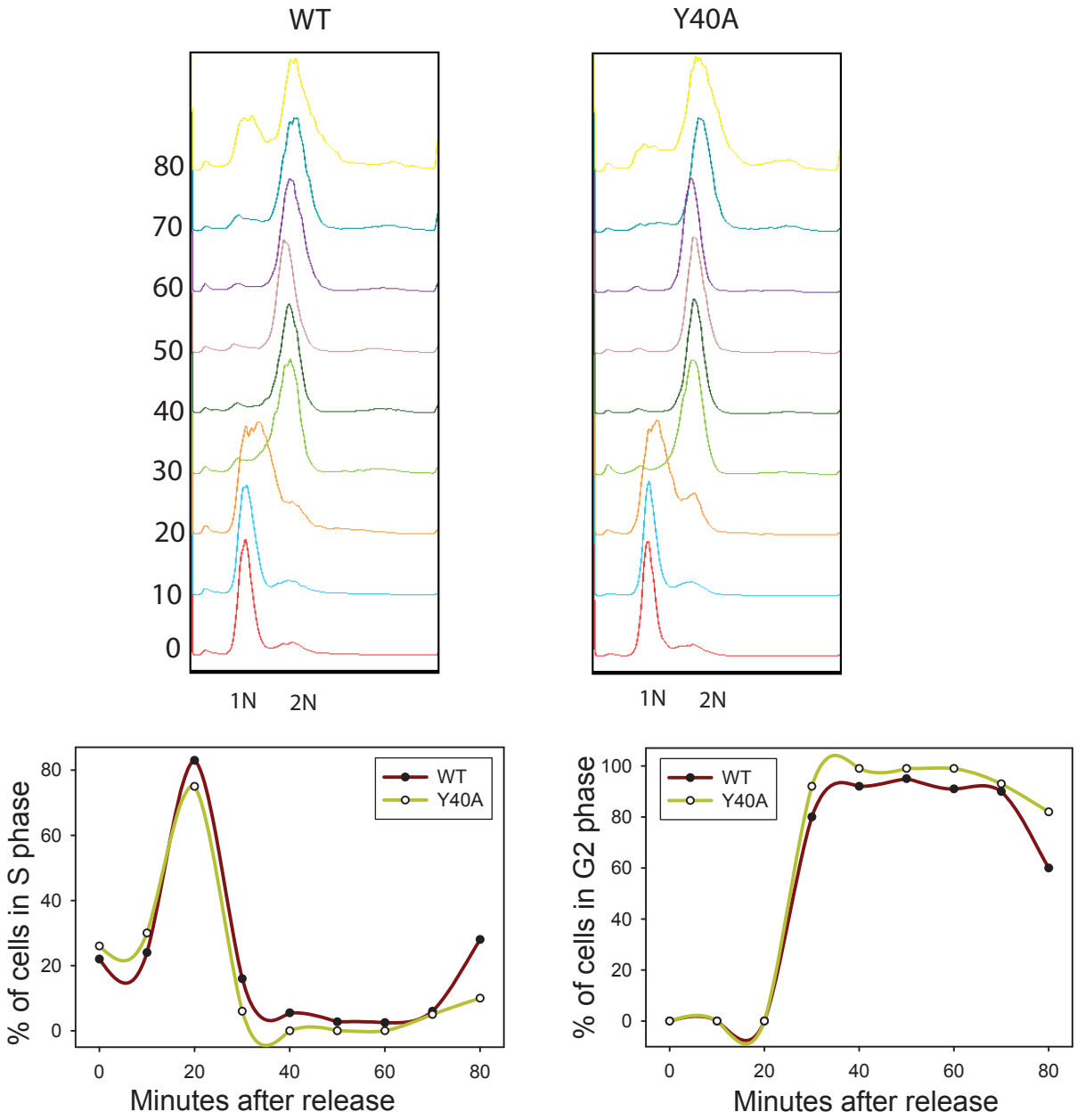
Supplementary Figure 5. (**a**) Bioinformatics analysis of pY37 H2B ChIP data. The MAST results for site 1 and 2. The location of motif 3 in site 1 and 2 are shown, red box. (**b**) This figure shows the top 13 GO terms when we used MetaCore (from GeneGo Inc) to cluster 511 genes by their GO terms in molecular function. Sorting is done based on their statistical significant in the enrichment analysis (see methods for details).

Supplementary Figure 6



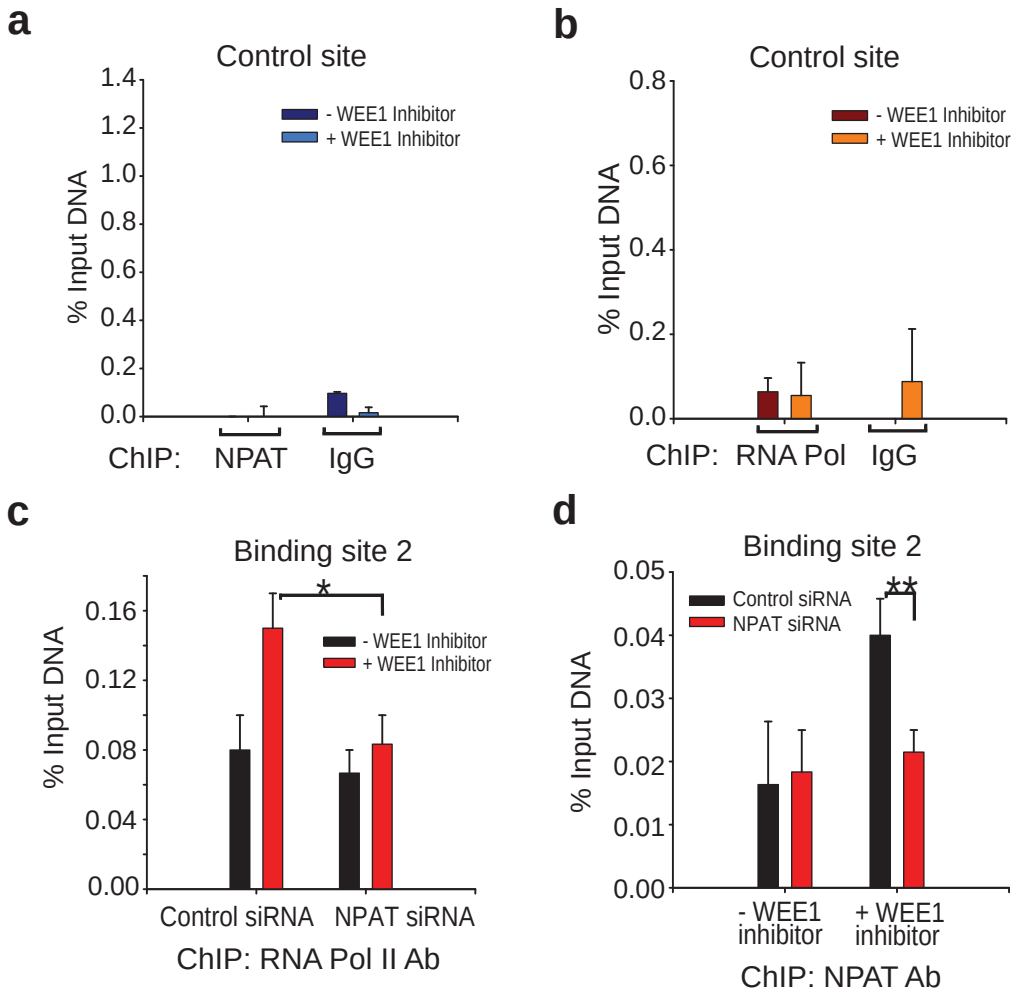
Supplementary Figure 6. Y40-phosphorylation of H2B in yeast. (**a**) Alignment of H2B protein sequences indicates that tyrosine residue at 37 is invariant from human to yeast. (**b**) Total histones prepared from α-factor synchronized WT and Y40A mutant yeast cells were immunoprecipitated with pY37 H2B antibodies followed by immunoblotting with yeast H2B antibodies (top panel). (**c**) Schematic representation of the *HTA1-HTB1* loci with approximate locations of primer sets (shown in green bars) used in ChIP-qPCR analysis. (**d**) Yeast cells synchronized using α factor were harvested at 0 and 30 min post-release. ChIP was performed using pY37 H2B or IgG antibodies followed by quantitative PCR using primers corresponding to the NEG Site. The bar graphs represents mean +/- s.d. from three experiments. **P* =0.024. (**e**) The total histones prepared from WT and *swe1*Δ mutant yeast cells were subjected to SDS-PAGE followed by immunoblotting with pY37 H2B (top panel) and H2B (bottom panel) antibodies.

Supplementary Figure 7



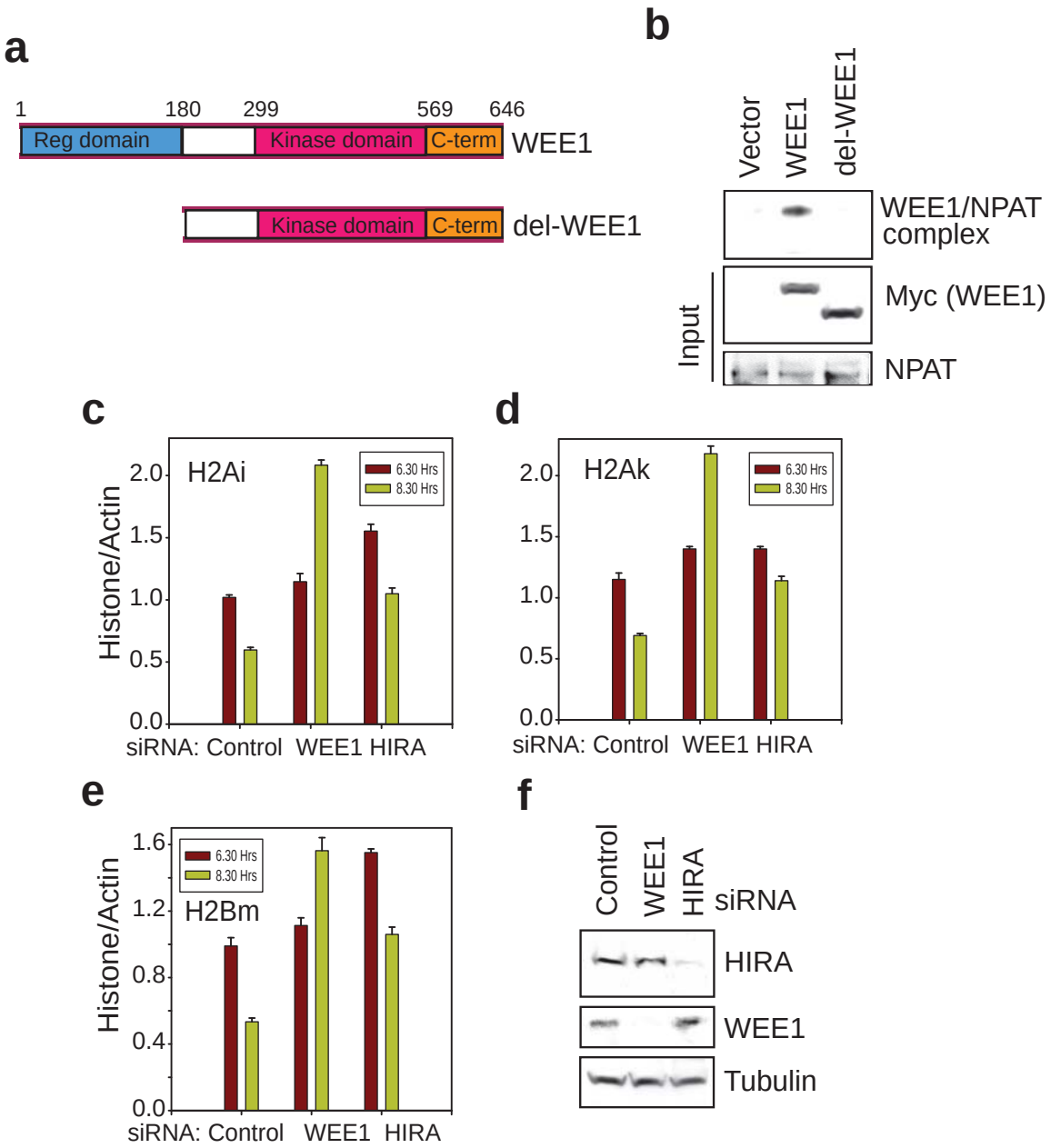
Supplementary Figure 7. Cell cycle profile of Y40A mutant is similar to WT yeast cells. The WT or Y40A mutant and WT or *swe1*Δ mutant yeast cells were synchronized with α-factor and harvested at indicated time points post-release. The cells were stained with Sytox green followed by flow cytometric analysis.

Supplementary Figure 8



Supplementary Figure 8. RNA Pol II is not present at site 2 in NPAT depleted cells. **(a, b**) The synchronized MEFs treated with WEE1 inhibitor (0.62 uM) or untreated were harvested 6.30 hours post-release. ChIP was performed using NPAT (**a**), RNA Pol II (**b**) and IgG antibodies followed by qPCR using primers corresponding to control site. (**c, d)** The synchronized MEFs were transfected with control or NPAT siRNAs, treated with WEE1 inhibitor (0.62 uM) or untreated, were harvested 6.50 hours post-release. ChIP was performed using RNA Pol II and IgG antibodies (**c**) or NPAT and IgG antibodies (**d**) followed by qPCR using primers corresponding to Site 2. *P =0.052, **P =0.04.

Supplementary Figure 9



Supplementary Figure 9. (**a**) Schematic of Myc-tagged WEE1 and deletion construct of WEE1 kinase. (**b**) HEK293 cells were transfected with Myc-tagged WEE1, del-WEE1 and vector constructs and lysates were immunoprecipitated with NPAT antibodies followed by immunoblotting with Myc antibodies. (**c-e**) MEFs were transfected with control, WEE1 and HIRA specific siRNA followed by synchronization by double thymidine block. Cells were harvested at 6.30 and 8.30 hours post thymidine-release and total RNAs were isolated followed by qRT-pCR using primers corresponding to histone H2Ai, H2Ak and H2Bm. (**f**) Lysates made from siRNA transfected MEFs were subjected to immunoblotting with respective antibodies.