Molecular Cell

Supplemental Information

Heptad-Specific Phosphorylation of RNA Polymerase II CTD

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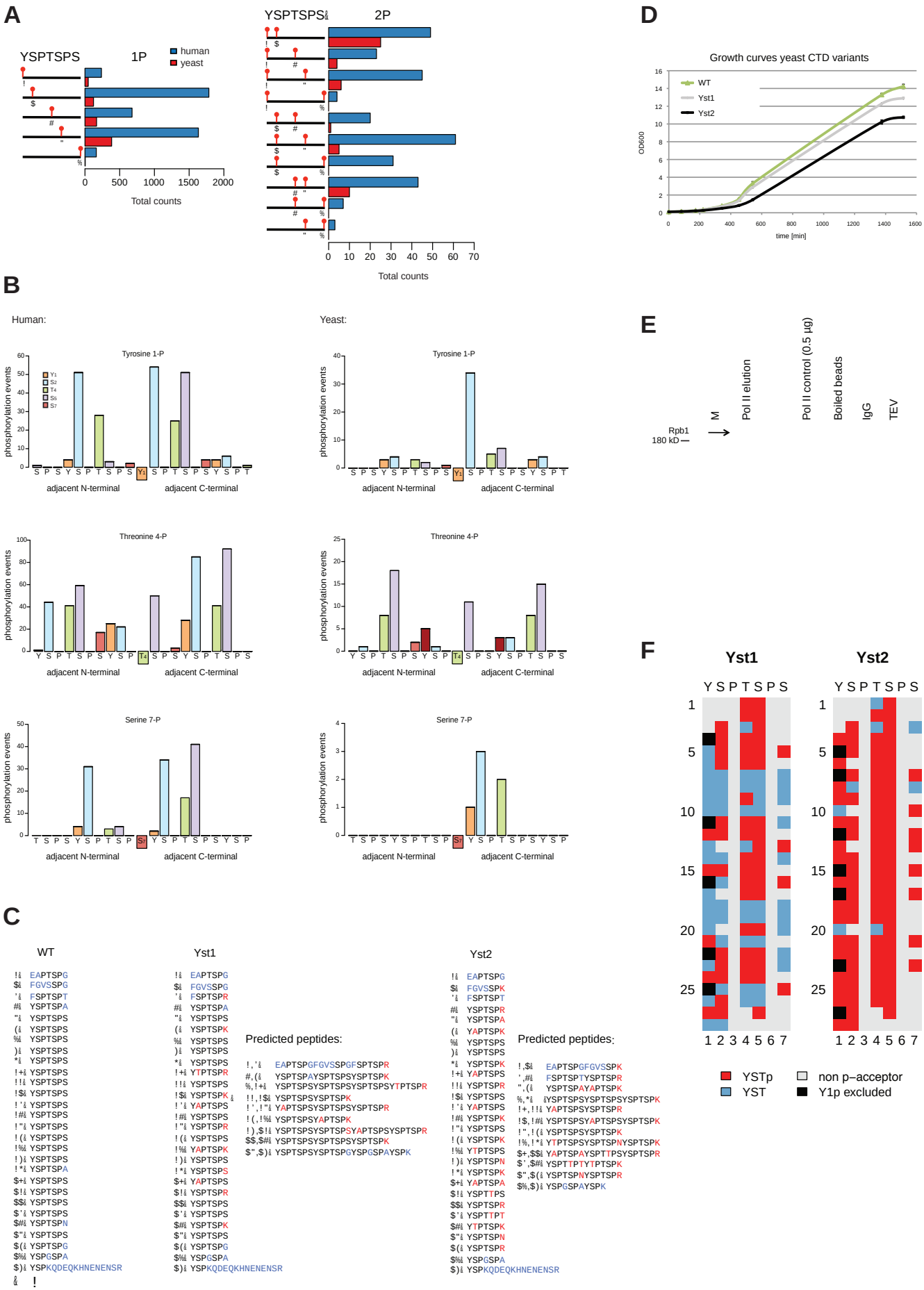
A
YSPTSPS 1P
human
yeast
Total counts
YSPTSPS¿ 2P
Total counts
D
Growth curves yeast CTD variants
WT
Yst1
Yst2
OD600
time [min]
B
Human:
Yeast:
Tyrosine 1-P
Threonine 4-P
Serine 7-P
phosphorylation events
adjacent N-terminal
adjacent C-terminal
E
Rpb1
180 kD
M
Pol II elution
Pol II control (0.5 µg)
Boiled beads
IgG
TEV
F
Yst1
Yst2
YSPTSPS
non p–acceptor
YSTp
YST
Y1p excluded
C
WT
Yst1
Yst2
Predicted peptides:
Predicted peptides:

Figure S3

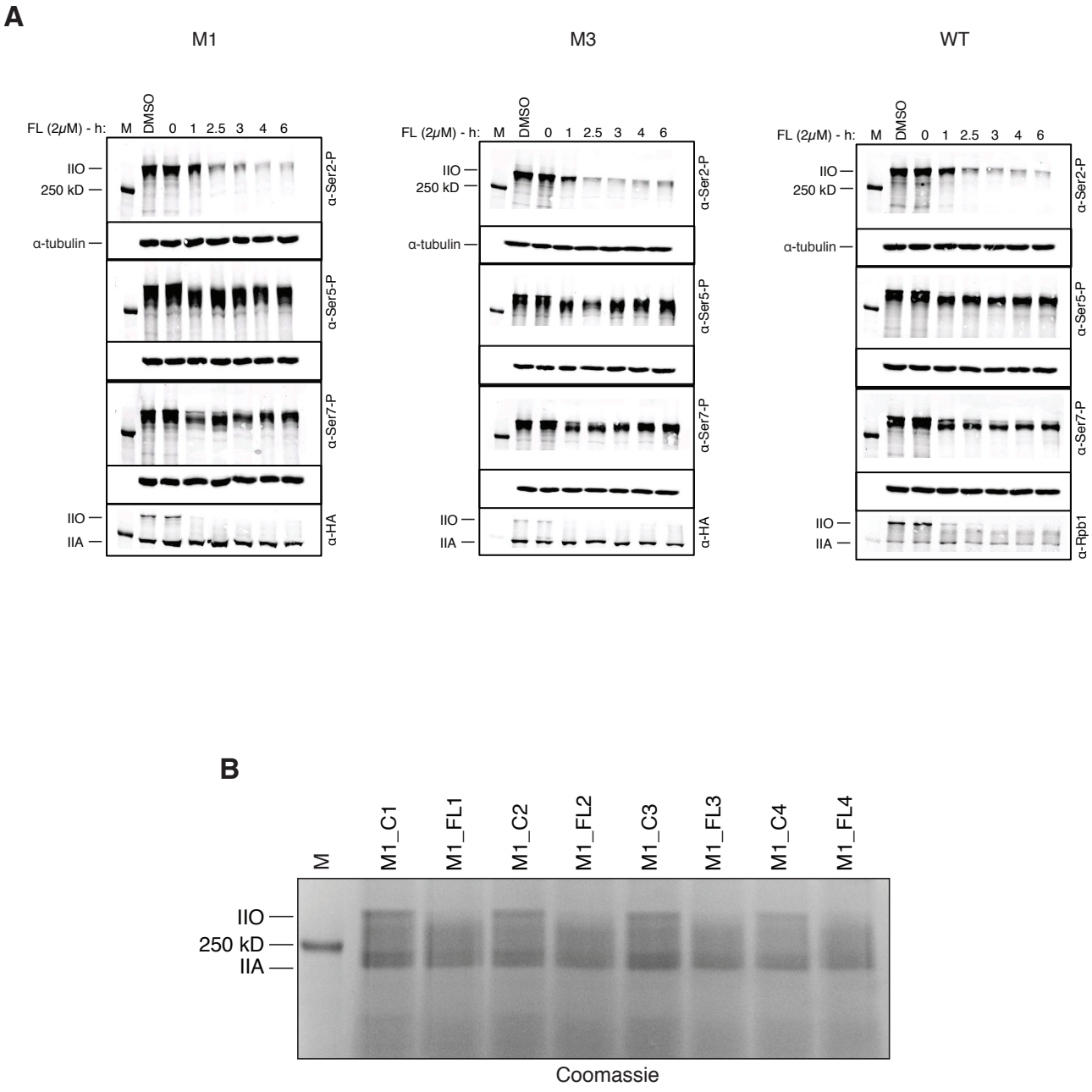
A
M1
M3
WT
FL (2μM) - h: M DMSO 0 1 2.5 3 4 6
IIO
250 kD
IIA
α-tubulin
α-Ser2-P
α-Ser5-P
α-Ser7-P
α-HA
α-Rpb1
B
M
M1_C1
M1_FL1
M1_C2
M1_FL2
M1_C3
M1_FL3
M1_C4
M1_FL4
IIO
250 kD
IIA
Coomassie

Figure S4

Supplementary Figures

Figure S1, related to Figure 1. (A) (Left) Sequence of mammalian WT-CTD. Blue letters: non-consensus residues. (Right) List of tryptic CTD peptides comprising CTD repeats 32-52. Numbers indicate CTD repeats covered by the corresponding peptide. Peptides 39 and 40 as well as peptides 46-47 and 48-49 have identical amino acid compositions. (B) List of tryptic CTD peptides of CTD variants M1, M2, M4 and M5. Red letters: amino acid substitution or addition. Blue letters: non-consensus residues. Numbers indicate CTD repeats covered by the corresponding peptide. (C) Western blot analysis showing the stable expression of the five mammalian CTD variants, M1-M5, after α-amanitin treatment for 2 weeks, using α-HA (3F10/Roche) for detection of the two main Rpb1-forms, IIO and IIA. No α-HA signal was detected in WT-Raji cells (WT). M: marker. (D) Comparison of growth rate (top) and cell viability (bottom) between WT and CTD variant M1, which carries the highest number of mutations of all five newly established CTD variants in this study. Daily measurements were performed within a time window of five days. (E) Western blot analysis of WTrec, M1, M3 and WT (endogenous Pol II) using specific antibodies against the various CTD phospho-residues (α-Ser2-P, Ser5-P, Ser7-P, Tyr1-P and Thr4-P) and total Rpb1 (α-Rpb1 and α-HA). IIO and IIA designate the hyperphosphorylated and hypophosphorylated forms of the large subunit Rpb1 of Pol II. α-tubulin was used as a loading control.

Figure S2, related to Figure 2. Scheme of mapped phosphosites within the CTD variants M2, M4 and M5. CTD residues are shown by squares. Red: identified phosphoresidue; blue: residue covered in analysis; grey: non p-acceptor residue; black: excluded phosphoresidue due to false positives; yellow: residue not covered in analysis.

Figure S3, related to Figure 3. (A) (Left) Total counts of the five different CTD phosphosites (1P) within mono-heptads in human (blue) and yeast (red). (Right) Total counts of all 10 possible double-phosphosite combinations (2P) within mono-heptads in human (blue) and yeast (red). All repeats with the sequence YSPTSPX (X = S, T, K, R, N, E or G) were included in the final data set. (B) Next neighbor phosphorylation study of Y_1-P, T_4-P and S_7-P in human (Left) and yeast (Right). Only double phosphorylated CTD peptides were taken into account and the defined sequence area comprised -10 to +10 CTD residues. Y_1-P and S_7-P are underrepresented due to data exclusion from Y_1-P-false positive biased peptides and high number of S_7 replacements within the CTD variants, respectively. (C) CTD sequences of yeast WT (Left) and yeast CTD variants Yst1 and Yst2 are shown. Tryptic peptides of corresponding CTD variants Yst1 and Yst2 are listed. Red letters: amino acid substitution. Blue letters: non-consensus residues. Numbers indicate CTD repeats covered by the corresponding peptide. (D) Comparison of growth rates between yeast WT and yeast CTD variants, Yst1 and Yst2. Measurements were performed within a time window of 1,600 minutes.

(E) Coomassie stained PAA-gel of TAP-purified yeast Rpb1 of CTD variant Yst1 after elution. From left to right: Marker; Yst1-Rpb1 eluat; Rpb1-mass control (0.5 μg); boiled beads only (control); IgG-control; TEV-control; M: marker. (F) Scheme of mapped phosphosites within the two yeast CTD variants Yst1 and Yst2. CTD residues are shown by squares. Red: identified phosphoresidue; blue: residue covered in analysis; grey: non p-acceptor residue; black: excluded phosphoresidue due to false positives; yellow: residue not covered in analysis.

Figure S4, related to Figure 5. (A) Western blot analysis of Ser2-P, Ser5-P and Ser7-P after treatment of CTD variant M1, M3 and WT with 2 μM flavopiridol (FL) at different time points (0h, 1h, 2.5h, 3h, 4h and 6h). In addition a DMSO control was added and total Rpb1-protein was detected by either α-HA (M1 and M3) or α- Rpb1 (WT). α-tubulin was used as a loading control. M: marker. (B) Coomassie stained PAA-gel. Four replicates of both, purified M1-untreated (M1_C1-4) and -flavopiridol-treated (M1_FL1-4/2.5h /2 μM) samples are shown in alternate order from the left to the right. M: marker.