Molecular Cell

Supplemental Information

Heptad-Specific Phosphorylation of RNA Polymerase II CTD

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A

Mammalian WT-CTD:

1	YSPTSPA	27	YTPTSPN
2	YEPRSPGG	28	YSPTSPS
3	YTPQSPS	29	YSPTSPS
4	YSPTSPS	30	YSPTSPS
5	YSPTSPS	31	YSPSSPR
6	YSPTSPN	32	YTPQSPT
7	YSPTSPS	33	YTPSSPS
8	YSPTSPS	34	YSPSSPS
9	YSPTSPS	35	YSPTSPK
10	YSPTSPS	36	YTPTSPS
11	YSPTSPS	37	YSPSSPE
12	YSPTSPS	38	YTPTSPK
13	YSPTSPS	39	YSPTSPK
14	YSPTSPS	40	YSPTSPK
15	YSPTSPS	41	YSPTSPT
16	YSPTSPS	42	YSPTTPK
17	YSPTSPS	43	YSPTSPT
18	YSPTSPS	44	YSPTSPV
19	YSPTSPS	45	YTPTSPK
20	YSPTSPS	46	YSPTSPT
21	YSPTSPS	47	YSPTSPK
22	YSPTSPN	48	YSPTSPT
23	YSPTSPN	49	YSPTSPKGST
24	YTPTSPS	50	YSPTSPG
25	YSPTSPS	51	YSPTSPT
26	YSPTSPN	52	YSLTSPAISPDDSDEEN

Trypsin →

Predicted peptides containing heptads 32-52:

32-35	YTPQSPTYTPSSPSYSPSSPSYSPTSPK
36-38	YTPTSPSYSPSSPEYTPTSPK
39	YSPTSPK
40	YSPTSPK
41-42	YSPTSPTYSPTTPK
43-45	YSPTSPTYSPTSPVYTPTSPK
46-47	YSPTSPTYSPTSPK
48-49	YSPTSPTYSPTSPK
50-52	GSTYSPTSPGYSPTSPTYSLTSPAISPDDSDEEN

B

M2

1-2	YSPTSPAYEPR
2-4	SPGGYTPQSPSYSPTSPR
5-7	YSPTSPSYSPTSPNYSPTSPK
8-10	YSPTSPSYSPTSPSYSPTSPK
11-13	YSPTSPSYSPTSPSYSPASPK
14-16	YSPTSPSYSPTSPSYSPSSPK
17-19	YSPTSPSYSPTSPSYSPLSPK
20-22	AYSPTSPSYSPTSPSYSPLSPR
23-25	YSPTSPNYTPTSPSYSPTSPK
26-28	YSPTSPNYTPTSPNYSPTSPK
29-31	YAPTSPSYSPASPSYAPSSPR
32-33	YTPQSPTYTPSSPK
34-35	YSPSSPSYSPTSPK
36-38	YTPTSPSYSPSSPEYTPTSPK
39-40	YSPTSPSYSPTSPK
41-42	YSPTSPTYSPTTPK
43-45	YSPTSPTYSPTSPVYTPTSPR
46-47	YSPTSPTYSPTSPK
48-49	YSPTSPTYSPTSPR
50-51	GSTYSPTSPGYSPTSPK
52	YSLTSPAISPDDSDEEN

M4

1-2	YSPTSPAYEPR
2-4	SPGGYTPQSPSYSPTSPK
5-7	YSPTSPSYSPTSPNYSPTSPK
8-9	YSPTSPSYSPTSPK
10-12	YSPTSPSYSPTSPSYSPTSPK
13-15	YSPTSPSYSPTSPSYSPASPK
16-18	YSPTSPSYSPTSPSYSPSSPK
19-21	AYSPTSPSYSPTSPSYSPLSPR
22-24	YSPTSPNYSPTSPNYTPTSPK
25-28	YSPTSPSYSPTSPNYTPTSPNYSPTSPK
29-31	YSPTSPSYSPTSPSYSPSSPR
32-35	YTPQSPTYTPSSPSYSPSSPSYSPTSPK
36-38	YTPTSPSYSPSSPEYTPASPR
39	YSPTSPK
40	YSPTSPR
41-42	YSPTSPTYSPTTPK
43-45	YSPTSPTYSPTSPVYTPTSPK
46-47	YSPTSPTYSPTSPR
48-49	YSPTSPTYSPTSPK
50-52	GSTYSPTSPGYSPTSPTYSLTSPAISPDDSDEEN

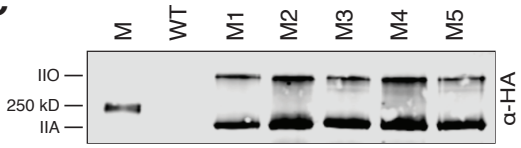
M3

1-2	YSPTSPAYEPR
2-4	SPGGYTPQSPSYSPTSPR
5-7	YSPTSPSYSPTSPNYSPTSPK
8-10	YSPTSPSYSPTSPSYSPTSPK
11-12	AYSPTSPSYSPTSPK
13-15	AYSPTSPSYSPTSPSYSPTSPK
16-17	YSPTSPSYSPTSPK
18-20	AAYSPTSPSYSPTSPSYSPTSPK
21-23	AYSPTSPSYSPTSPNYSPTSPK
24-26	YTPTSPSYSPTSPSYSPTSPK
27-29	AYTPTSPNYSPTSPSYSPTSPK
30-31	YSPTSPSYSPASPK
32-33	YTPQSPTYTPSSPK
34-35	YSPSSPSYSPTSPK
36-38	YTPTSPSYSPSSPEYTPASPR
39-40	AAYSPTSPSYSPTSPK
41-42	YSPTSPTYSPTTPK
43-45	YSPTSPTYSPTSPVYTPTSPK
46-47	AYSPTSPTYSPTSPK
48-49	YSPTSPTYSPTSPK
50-51	GSTYSPTSPGYSPTSPK
52	YSLTSPAISPDDSDEEN

M5

1-2	YSPTSPAYEPR
2-4	SPGGYTPQSPSYSPTSPK
5-8	YSPTSPSYSPTSPNYSPTSPSYSPTSPK
9-11	YSPTSPSYSPTSPSYSPTSPK
12-14	YSPTSPSYSPTSPSYSPASPK
15-18	YSPTSPSYSPTSPSYSPTSPSYSPTSPK
19-22	YSPTSPSYSPTSPSYSPASPSYSPTSPK
23-25	AYSPTSPNYTPTSPSYSPTSPK
26-28	YSPTSPNYTPTSPNYSPTSPK
29-31	YSPTSPSYSPTSPSYSPSSPR
32-33	YTPQSPTYTPSSPK
34-35	YSPSSPSYSPTSPK
36-38	YTPTSPSYSPSSPEYTPASPK
39	YSPTSPR
40	YSPTSPK
41-42	YSPTSPTYSPTTPK
43-45	YSPTSPTYSPTSPVYTPTSPK
46-47	YSPTSPTYSPTSPR
48-49	YSPTSPTYSPTSPK
50-52	GSTYSPTSPGYSPTSPTYSLTSPAISPDDSDEEN

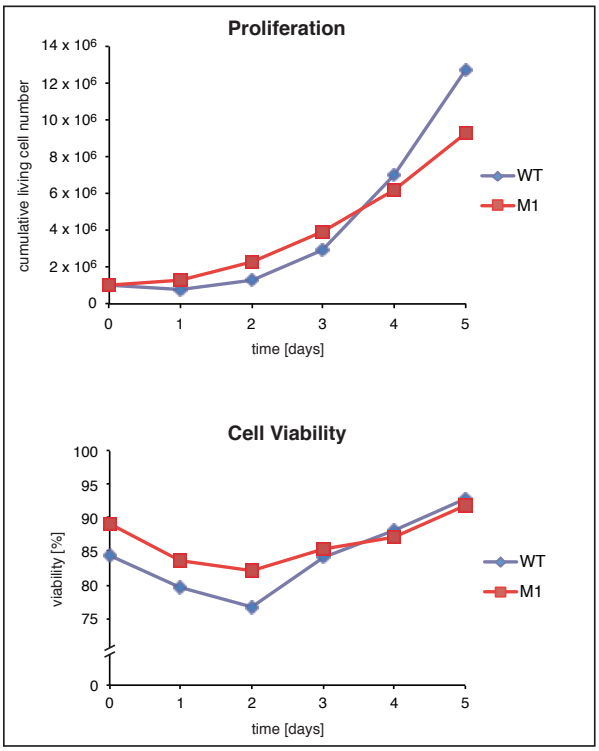
C



Western blot

D

Proliferation and cell viability of WT and M1



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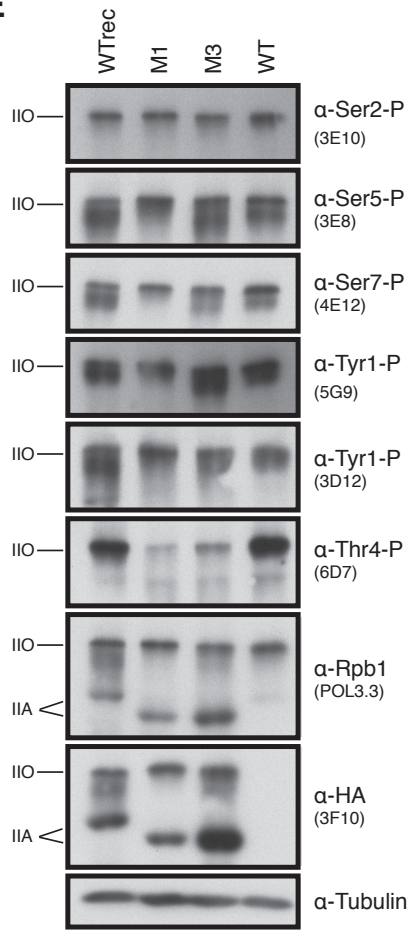


Figure S1

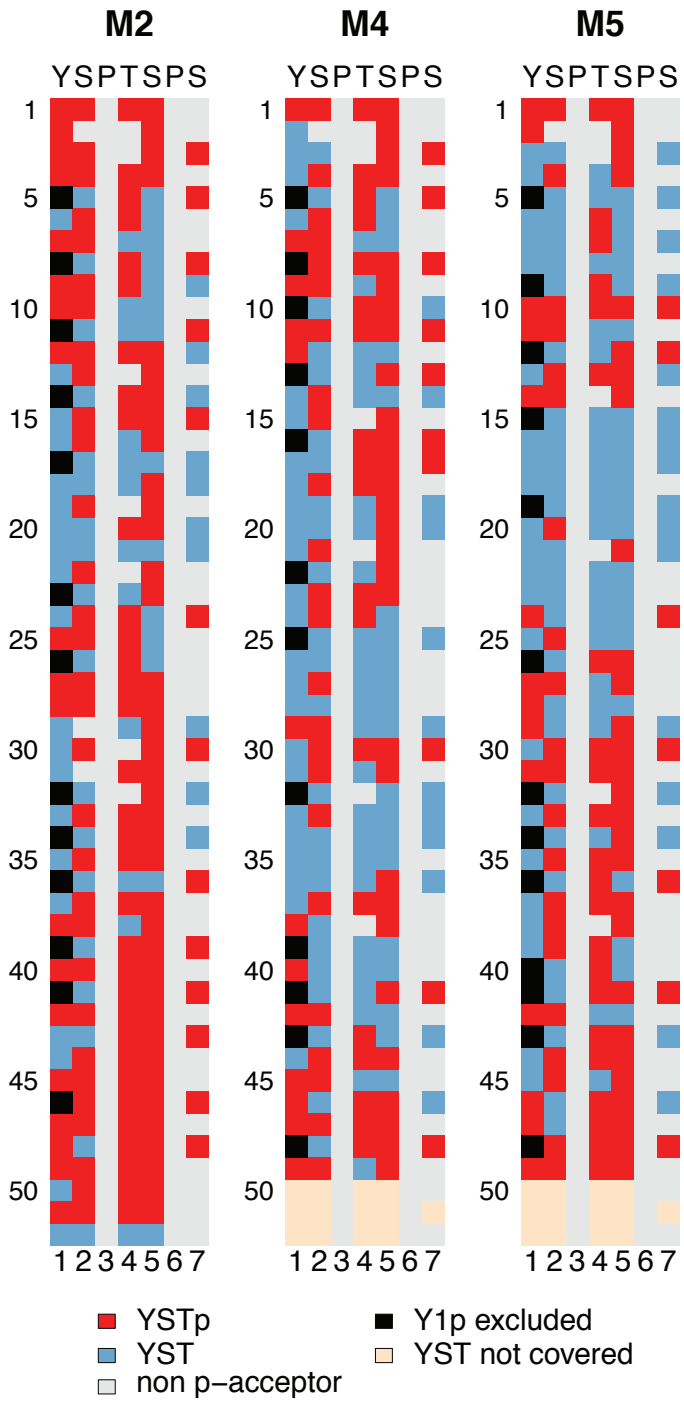
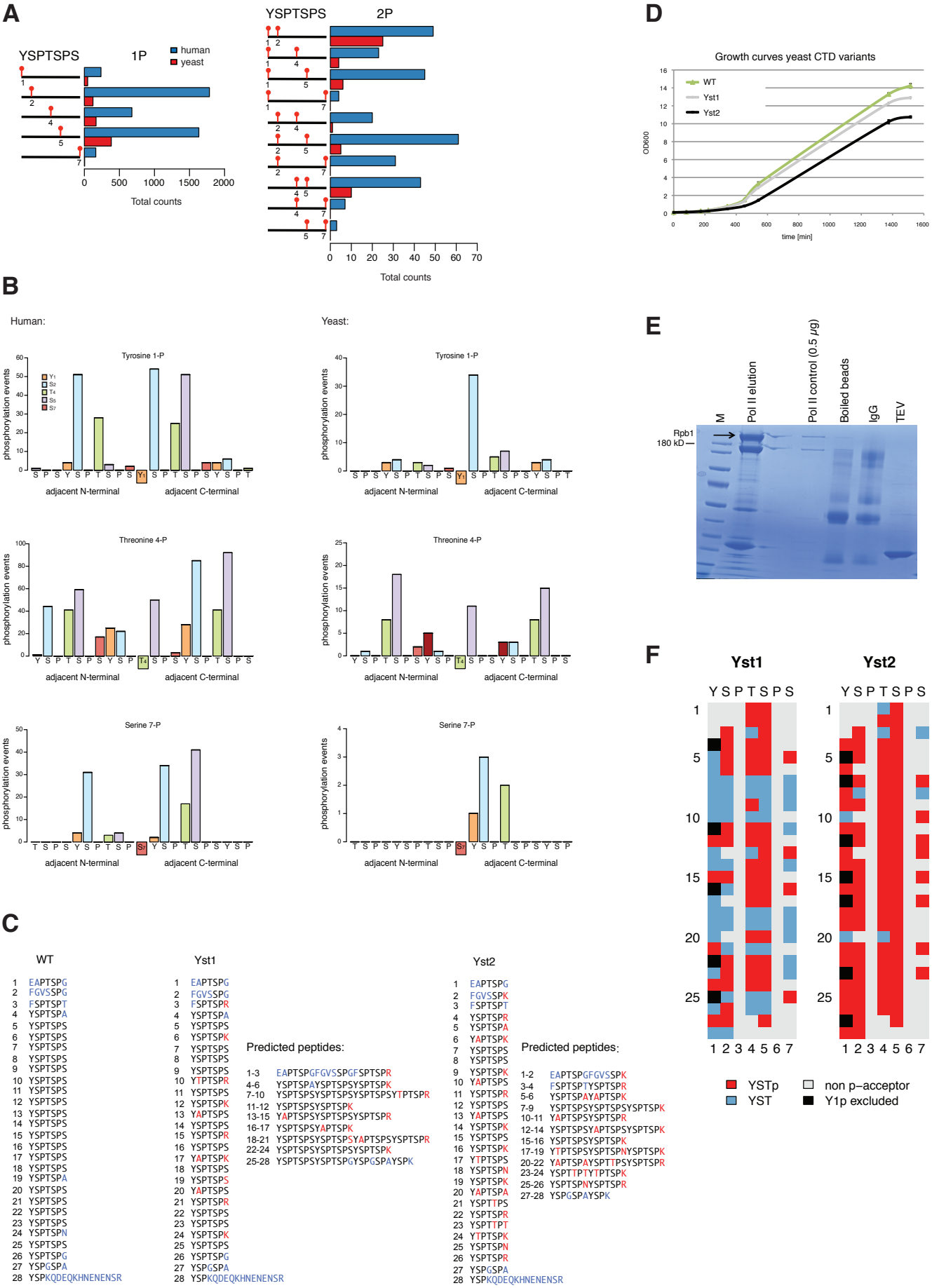

M2
M4
M5
Y S P T S P S
1
5
10
15
20
25
30
35
40
45
50
1 2 3 4 5 6 7
YSTp
YST
non p−acceptor
Y1p excluded
YST not covered

Figure S2


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
M
Pol II elution
Pol II control (0.5 μg)
Boiled beads
IgG
TEV
Rpb1
180 kD
F
Yst1
Yst2
YSPTSPS
YSTp
YST
non p-acceptor
Y1p excluded
C
WT
1 EAPTSPG
2 FGVSSPG
3 FSPTSPT
4 YSPTSPA
5 YSPTSPS
6 YSPTSPS
7 YSPTSPS
8 YSPTSPS
9 YSPTSPS
10 YSPTSPS
11 YSPTSPS
12 YSPTSPS
13 YSPTSPS
14 YSPTSPS
15 YSPTSPS
16 YSPTSPS
17 YSPTSPS
18 YSPTSPS
19 YSPTSPA
20 YSPTSPS
21 YSPTSPS
22 YSPTSPS
23 YSPTSPS
24 YSPTSPN
25 YSPTSPS
26 YSPTSPG
27 YSPGSPA
28 YSPKQDEQKHNENENSR
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1 EAPTSPG
2 FGVSSPG
3 FSPTSPR
4 YSPTSPA
5 YSPTSPS
6 YSPTSPK
7 YSPTSPS
8 YSPTSPS
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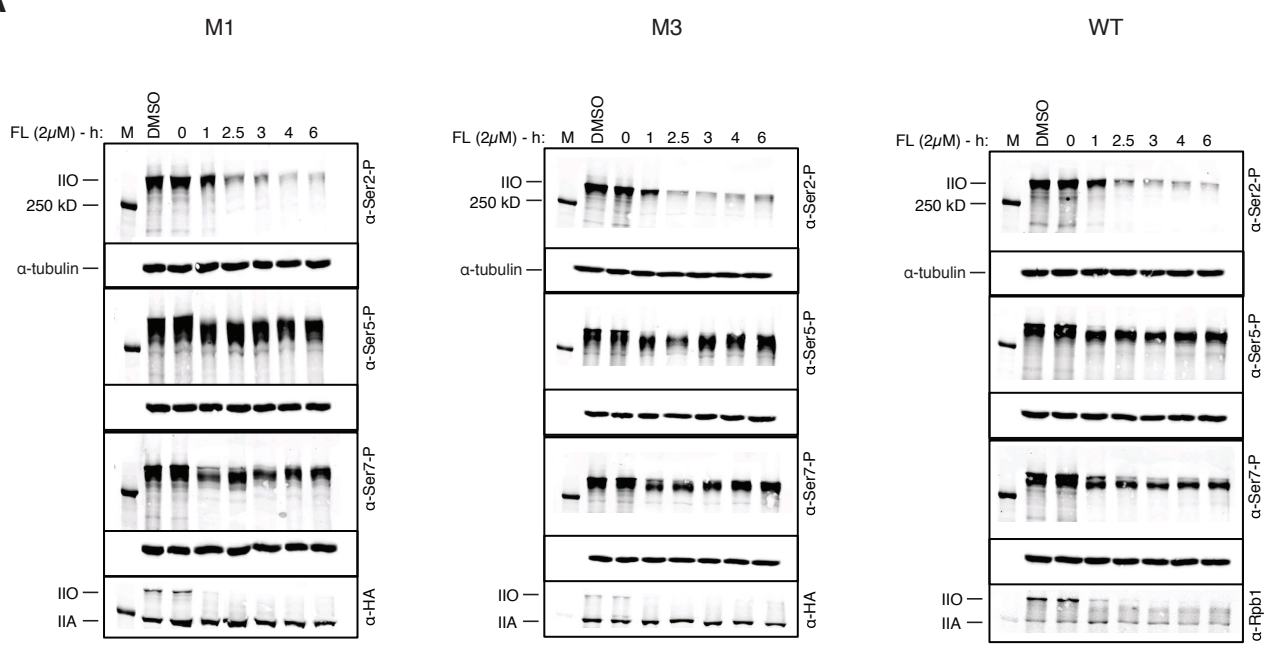
21 YSPTSPR
22 YSPTSPS
23 YSPTSPS
24 YSPTSPK
25 YSPTSPS
26 YSPTSPG
27 YSPGSPA
28 YSPKQDEQKHNENENSR
Predicted peptides:
1-3 EAPTSPGFGVSSPGFSPTSPR
4-6 YSPTSPAYSPTSPSYSPTSPK
7-10 YSPTSPSYSPTSPSYSPTSPSYTPTSPR
11-12 YSPTSPSYSPTSPK
13-15 YAPTSPSYSPTSPSYSPTSPR
16-17 YSPTSPSYAPTSPK
18-21 YSPTSPSYSPTSPSYAPTSPSYSPTSPR
22-24 YSPTSPSYSPTSPSYSPTSPK
25-28 YSPTSPSYSPTSPGYSPGSPAYSPK
Yst2
1 EAPTSPG
2 FGVSSPK
3 FSPTSPT
4 YSPTSPR
5 YSPTSPA
6 YAPTSPK
7 YSPTSPS
8 YSPTSPS
9 YSPTSPK
10 YAPTSPS
11 YSPTSPR
12 YSPTSPS
13 YAPTSPS
14 YSPTSPK
15 YSPTSPS
16 YSPTSPK
17 YTPTSPS
18 YSPTSPN
19 YSPTSPK
20 YAPTSPA
21 YSPTTPS
22 YSPTSPR
23 YSPTTPT
24 YTPTSPK
25 YSPTSPN
26 YSPTSPR
27 YSPGSPA
28 YSPKQDEQKHNENENSR
Predicted peptides:
1-2 EAPTSPGFGVSSPK
3-4 FSPTSPTYSPTSPR
5-6 YSPTSPAYAPTSPK
7-9 YSPTSPSYSPTSPSYSPTSPK
10-11 YAPTSPSYSPTSPR
12-14 YSPTSPSYAPTSPSYSPTSPK
15-16 YSPTSPSYSPTSPK
17-19 YTPTSPSYSPTSPNYSPTSPK
20-22 YAPTSPAYSPTTPSYSPTSPR
23-24 YSPTTPTYTPTSPK
25-26 YSPTSPNYSPTSPR
27-28 YSPGSPAYSPK

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

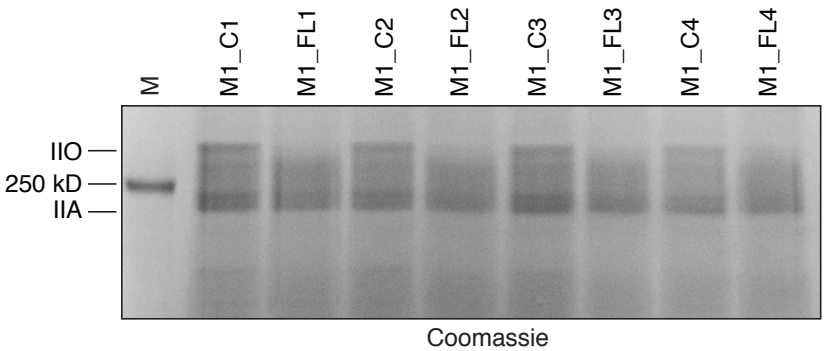

M
M1_C1
M1_FL1
M1_C2
M1_FL2
M1_C3
M1_FL3
M1_C4
M1_FL4
IIO
250 kD
IIA
Coomassie

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.