Supplemental Material

Genetic interactions and transcriptomics implicate fission yeast CTD prolyl isomerase Pin1 as an agent of RNA 3' processing and transcription termination that functions via its effects on CTD phosphatase Ssu72

Ana M. Sanchez, Angad Garg, Stewart Shuman, and Beate Schwer

Supplemental Figures S1, S2, S3, S4, S5, S6, and S7

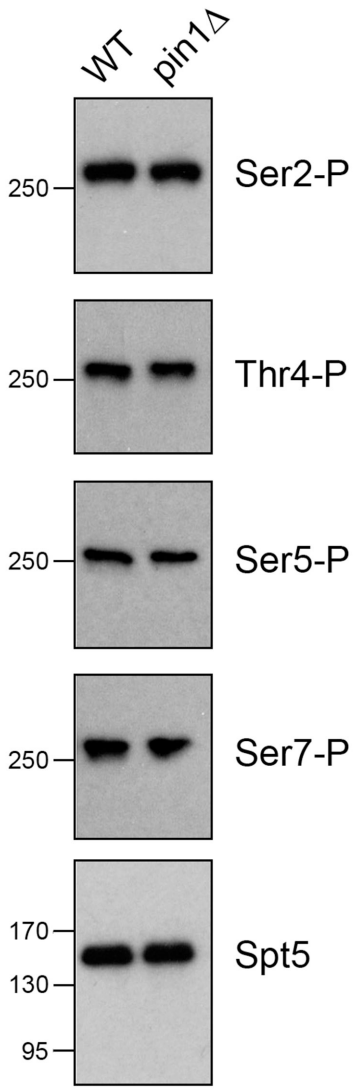

Figure S1. Ablation of Pin1 does not affect CTD serine/threonine phosphorylation globally. Whole-cell extracts from *pin1*$^{+}$ (WT) and *pin1*Δ strains growing logarithmically at 30˚C were resolved by SDS-PAGE and subjected to Western blotting with the indicated polyclonal CTD phospho-specific antibodies and with a polyclonal antibody against transcription factor Spt5 (as a loading control). The positions and sizes (in kilodaltons) of marker polypeptides are indicated at left.

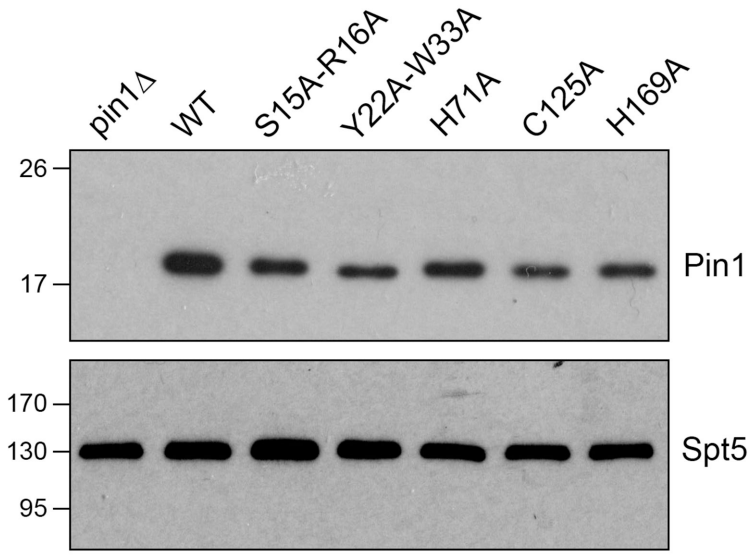

Figure S2. Western blot of Pin1 mutants. Whole-cell extracts from *pin1*Δ, *pin1*-WT, and the indicated *pin1-Ala* strains growing logarithmically at 30˚C were resolved by SDS-PAGE and subjected to Western blotting with polyclonal Pin1 and Spt5 antibodies. The positions and sizes (in kilodaltons) of marker polypeptides are indicated at left.

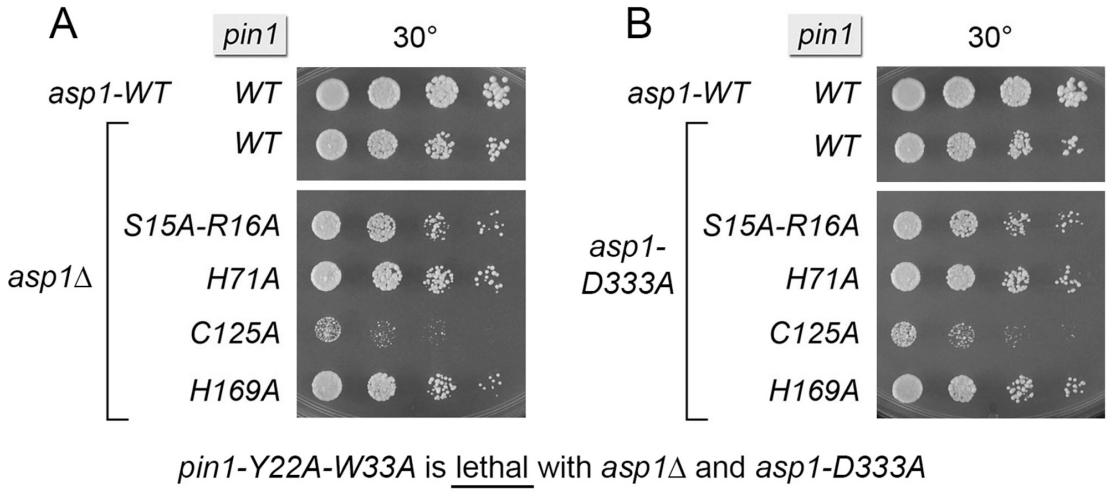

Figure S3. Activity of Pin1-Ala mutants in *asp1*Δ and *asp1-D333A* backgrounds. *S. pombe* strains bearing the indicated *pin1* alleles in combination with *asp1*Δ (panel A) or *asp1-D333A* (panel B) were spot-tested for growth on YES agar at 30˚C. As noted at bottom, *pin1-Y22A-W33A* was lethal in the *asp1*Δ and *asp1-D333A* backgrounds.

Sample	Total Paired Reads	Mapped Reads
WT (1)	21,449,706	20,980,488 (98%)
WT (2)	23,888,710	23,365,656 (98%)
WT (3)	22,670,827	22,083,962 (97%)
*pin1*Δ (1)	21,813,736	21,296,390 (98%)
*pin1*Δ (2)	22,583,876	22,028,306 (98%)
*pin1*Δ (3)	21,273,120	20,762,723 (98%)

Figure S4. RNA-seq read counts for triplicate biological replicates.

Sample Pairs	Pearson Coefficient
WT (1) vs (2)	0.976
WT (1) vs (3)	0.978
WT (2) vs (3)	0.987
*pin1*Δ (1) vs (2)	0.984
*pin1*Δ (1) vs (3)	0.984
*pin1*Δ (2) vs (3)	0.984

Figure S5. RNA-seq data reproducibility between biological replicates.

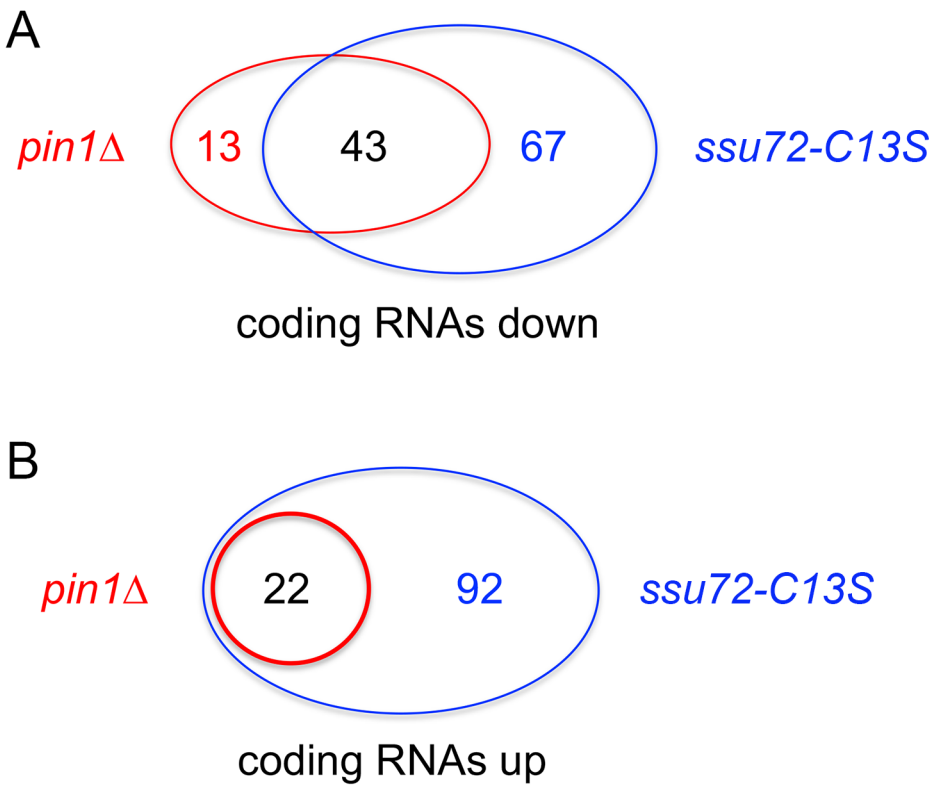

Figure S6. Overlapping transcriptional signatures of *pin1*Δ and *ssu72-C13S*. Venn diagrams specify the numbers of protein-coding poly(A)$^+$ transcripts that were significantly down-regulated by at least twofold (A) or significantly up-regulated by at least twofold (B) in the *pin1*Δ and *ssu72-C13S* strains.

gene	log2 change
pho1	−4.37
pho84	−3.83
mei2	−2.89
ecl3	−2.21
grt1	−1.91
dak2	−1.67
mfm2	−1.47
spk1	−1.45
map1	−1.44
isp4	−1.38
SPAC56F8.15	−1.34
SPAPJ695.02	−1.32
SPAC18G6.12c	−1.29
tspO	−1.21
ste11	−1.14
nde2	−1.13
abp2	−1.11
anc1	−1.10
mam2	−1.10
wdr83	−1.05
SPAC2H10.01	−1.03
SPAC23H3.15c	4.24
SPAC750.01	3.56
str1	3.46
frp1	2.63
srx1	2.59
SPAC11D3.01c	2.27
SPCC794.03	2.18
fio1	2.03
SPAC27D7.09c	1.89
gnr1	1.80
fip1	1.55
pof14	1.48
SPCC569.09	1.45
SPAC25B8.12c	1.23
bfr1	1.03

Figure S7. Overlapping transcriptional signatures of *pin1*Δ and *asp1-D333A*. Lists of: (i) 21 annotated protein-coding genes that were down-regulated at least two-fold in *asp1-D333A* cells that were also down-regulated in *pin1*Δ cells; and (ii) 15 protein-coding genes that were up-regulated at least two-fold in *asp1-D333A* cells that were also up-regulated in *pin1*Δ cells. The log2 fold changes in *asp1-D333A* versus wild-type are shown. The gene functions are listed in Figures 10 and 11.