G3 Genes | Genomes | Genetics

The yeast Ess1 prolyl isomerase controls Swi6 and Whi5 nuclear localization

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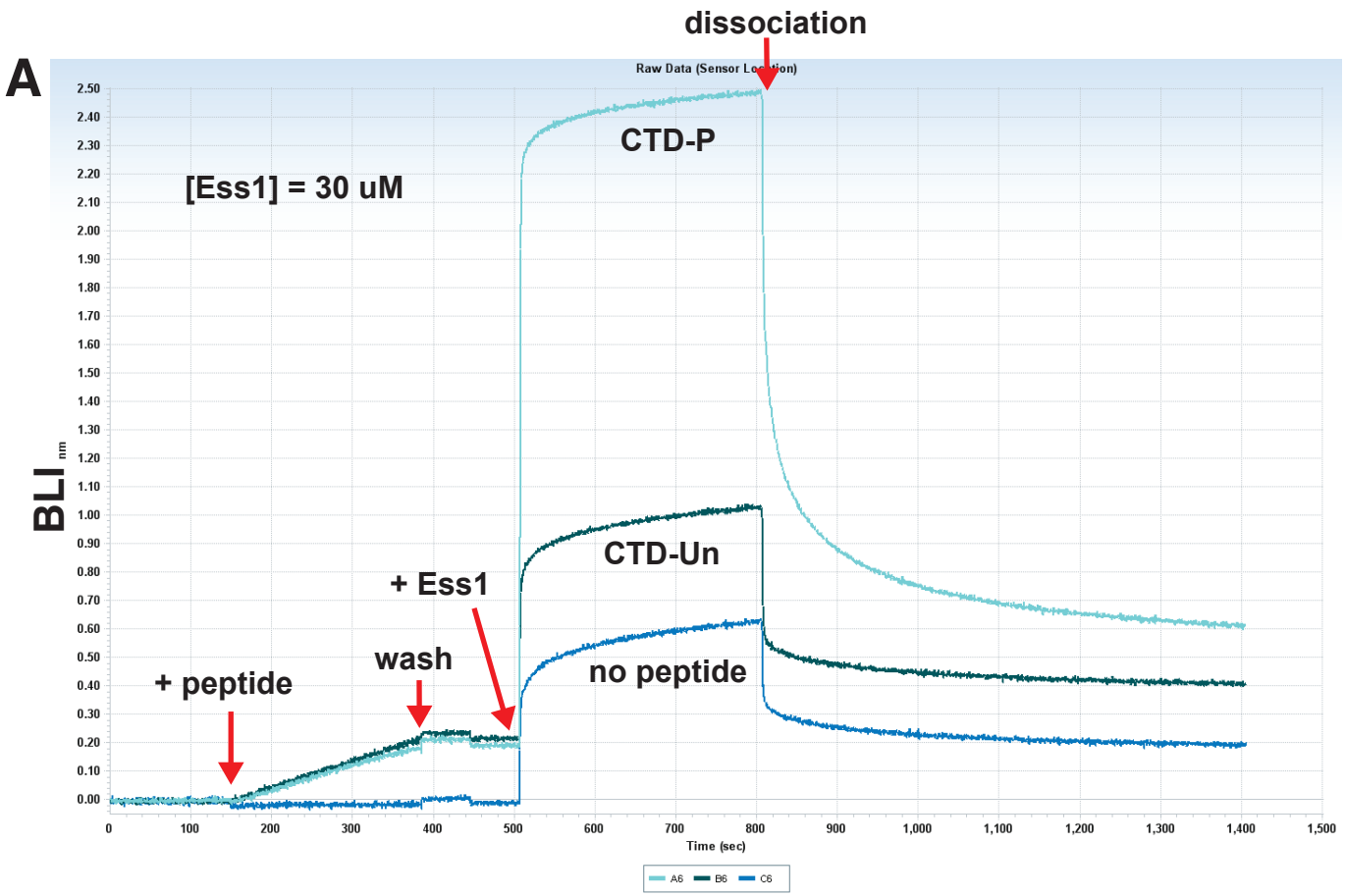
A
dissociation
Raw Data (Sensor Location)
CTD-P
[Ess1] = 30 uM
CTD-Un
+ Ess1
wash
+ peptide
no peptide
BLI nm
Time (sec)
A6 B6 C6

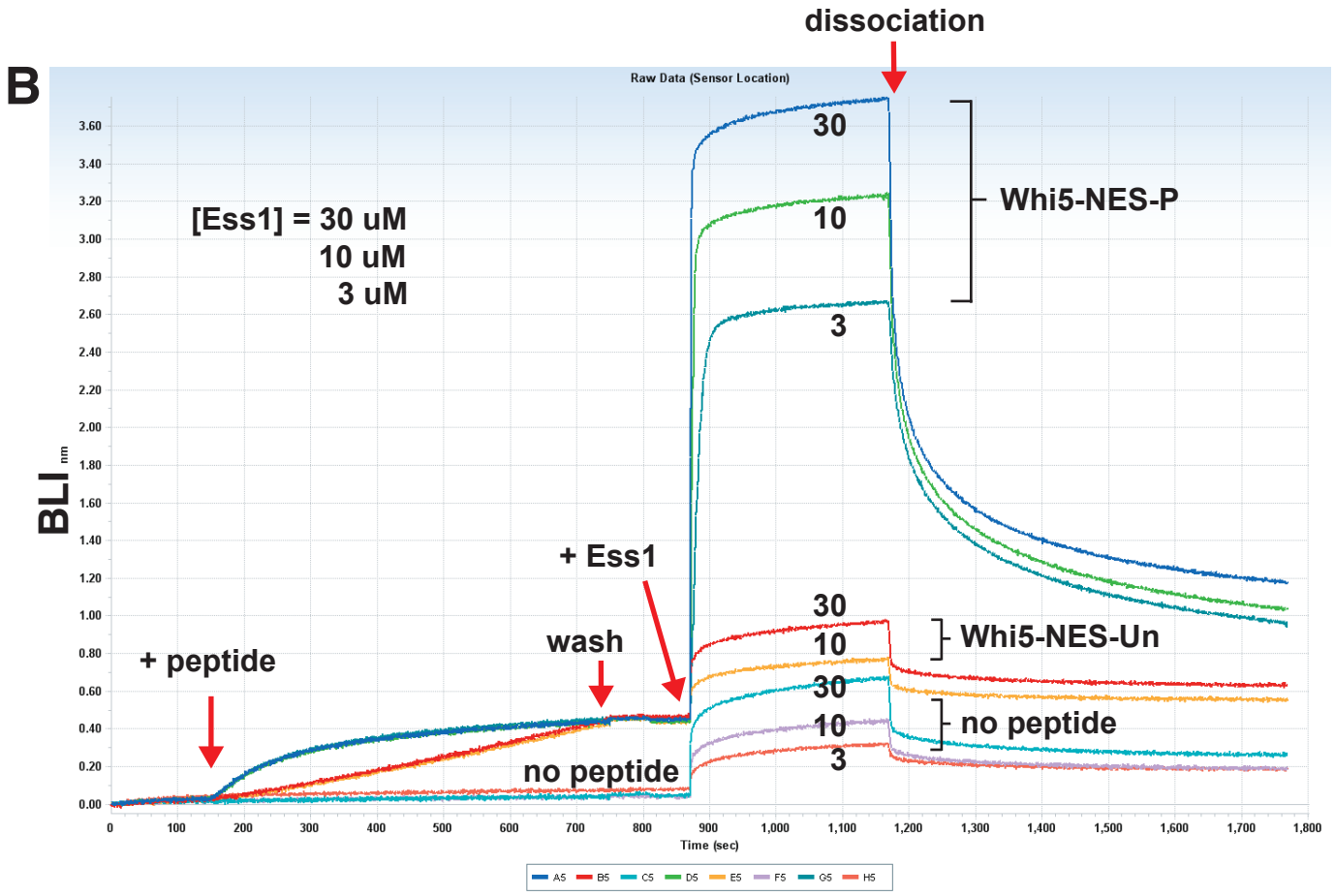
B
dissociation
Raw Data (Sensor Location)
30
10
3
Whi5-NES-P
[Ess1] = 30 uM
10 uM
3 uM
+ Ess1
30
10
Whi5-NES-Un
wash
+ peptide
30
10
3
no peptide
no peptide
BLI nm
Time (sec)
A5 B5 C5 D5 E5 F5 G5 H5

Figure S1 Representative BLI experiments. (**A**) Raw data for binding of purified Ess1 protein (30 μM) to control peptides from RNA polymerase II CTD. Time of addition of biotinylated peptides (or no-peptide controls) to streptavidin-coated sensors is indicated by the arrow **(+ peptide**). After peptide binding, sensors were washed in buffer alone prior to transfer into buffer with Ess1 protein (**+Ess1**). Sensors with peptide and bound Ess1 were placed into fresh buffer (**dissociation**). (**B**) Example of raw data using peptides representing the nuclear export sequence (NES) of Whi5 at three concentrations of Ess1 as indicated. Samples A5, D5, and G5 are the Whi5-NES-P peptide with 30, 10, 3 μM Ess1 protein, respectively. Samples B5 and E5 are Whi5-NES-UN peptide with 30 and 10 μM Ess1 protein, respectively. Samples C5, F5, and H5 are "no-peptide-added" controls with 30, 10, 3 μM Ess1 protein, respectively. Note that the no-peptide controls show increased (background) signal with higher Ess1 protein concentrations, as expected**.** For both panels **A** and **B,** the (**P**) indicates phospho-Ser peptides, (**Un**) indicates unphosphorylated peptides. Full peptide sequences are given in the main text (**Table 5).**

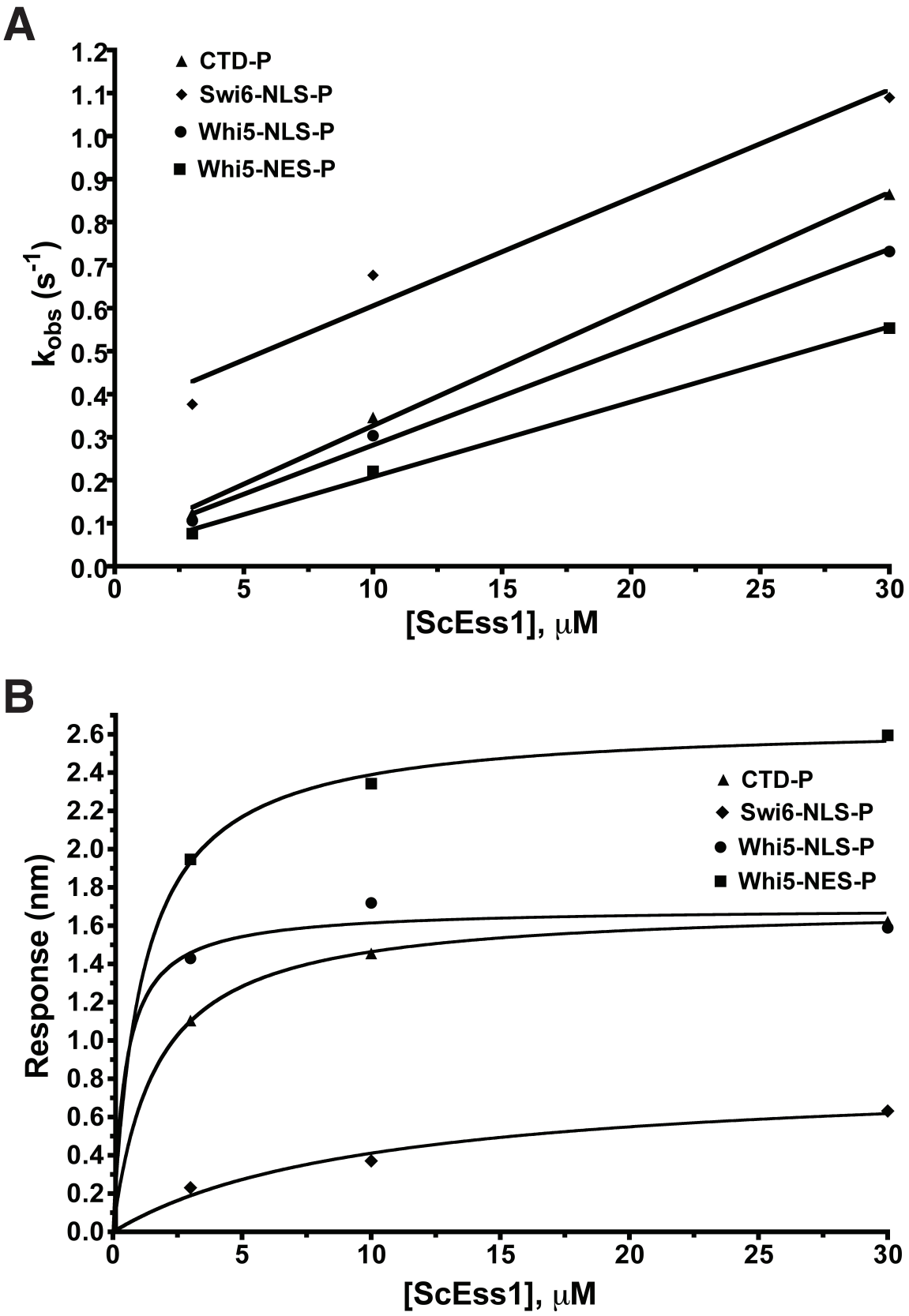
A
CTD-P
Swi6-NLS-P
Whi5-NLS-P
Whi5-NES-P
k_{obs} (s^{-1})
[ScEss1], μM
B
CTD-P
Swi6-NLS-P
Whi5-NLS-P
Whi5-NES-P
Response (nm)
[ScEss1], μM

Figure S2 Estimation of apparent K_d values for binding of Ess1 to peptides. From the BLI kinetic assays of Ess1 binding with each peptide (Figure 6), regression analyses were used to determine the apparent dissociation constants (K_{app}) for binding of Ess1 to each of the query peptides. (A) For each phosphorylated peptide, observed rates (k_{obs}) for Ess1 association were plotted *vs.* the concentration of Ess1, and the slope provides an estimate of the 2nd-order association rate (k_a, $M^{-1}s^{-1}$), which is listed in Table S4. The kinetic estimate for K_D (K_{appK}, Table 5) was calculated as the ratio of k_d/k_a values from Table S4. (B) The plateau value for binding at each Ess1 concentration (Figure 6) was assumed to represent the amount of Ess1/peptide complex at equilibrium. For each phosphorylated peptide, these equilibrium binding responses were plotted *vs.* Ess1 concentration and fit to a hyperbolic binding isotherm to obtain the equilibrium estimate for K_D (K_{appEq}, Table 5).

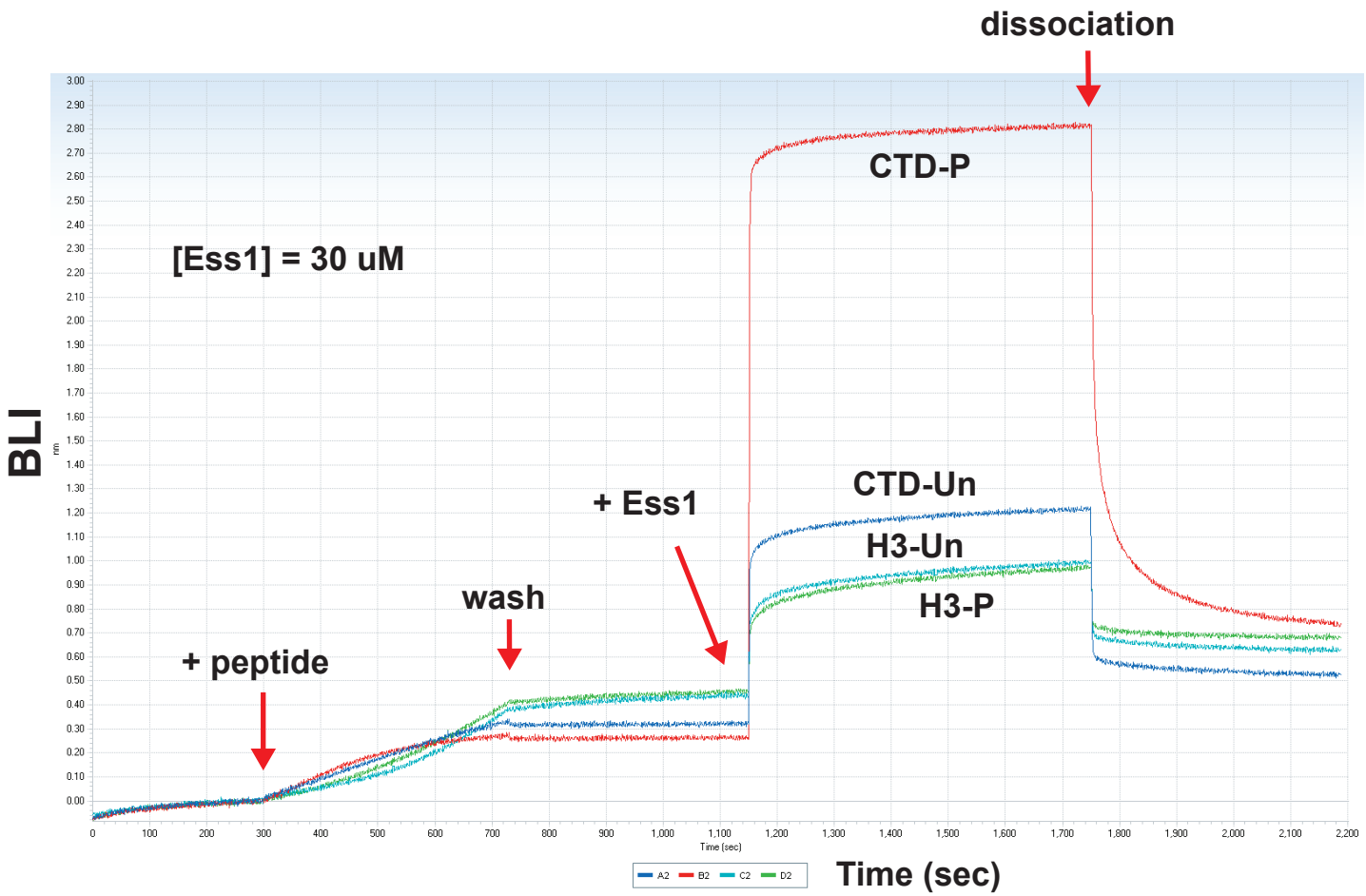

Figure S3 BLI experiment testing Ess1 binding to non-substrate a phospho-peptide. Raw data for binding of purified Ess1 protein (30 μM) to control peptides from the RNA polymerase II CTD (as in Figure S1), and to non-substrate control peptides from Histone H3 residues 1-21. **H3-Un** is unphosphorylated, **H3-P** is phosphorylated at serine 10. Experiment is carried out as described in Legend to Figure S1. Histone peptides were from Millipore (cat # 12-403; 12-427) The sequences are ARTKQTARK**S**TGGKAPRKQLA-GGK-Biotin, with the Ser residue that is phosphorylated in peptide H3-P highlighted. Levels of Ess1 binding to both H3 peptides is essentially identical to binding to no-peptide added control samples (data not shown).

Table S1 Oligonucleotides used in this study

Oligo Name	Sequence	Use	Gene	reference
OW38	CATAACCGACTACGGCAC	strain construction	ESS1 5'-F	Ma et al., 2012
OW989	TGTTTACAAAAAAATACAAGAATCCGTTACTAAAGATTCAGTATAGCGACC AGCATTCAC	strain construction	NAT-R	Ma et al., 2012
OW1534	TTCGCGAAGTAACCCTTCGTGGA	RT-qPCR control	SNR6-F	Ma et al., 2012
OW1535	GTAAAACGGTTCATCCTTATGCAG	RT-qPCR control	SNR6-R	Ma *et al.,* 2012
UP007	CAAGGTCACACTCCACTCCA	RT-qPCR	SWI4-F	This study
UP008	GTAGTCCTGCTGACCCAAGC	RT-qPCR	SWI4-R	This study
UP009	ACGACCGAGGTGAGTTATGG	RT-qPCR	SWI6-F	This study
UP010	GCTTGCCTCTGGCAGTATTC	RT-qPCR	SWI6-R	This study
UP011	GGATGGACGGACAAGACACT	RT-qPCR	WHI5-F	This study
UP012	TCCACTTCGGTATCCGACTC	RT-qPCR	WHI5-R	This study
UP013	CCACGTCCGTATCTTCCTCT	RT-qPCR	MBP1-F	This study
UP014	ATATAGGGAGCGCTGTGTGG	RT-qPCR	MBP1-R	This study
UP015	TTTACCCTCCGACGCTACTG	RT-qPCR	WHI3-F	This study
UP016	CCTCCTCTTCTGCTGTTTGG	RT-qPCR	WHI3-R	This study
UP027	TGACGATCCACTAGCGACTTC	RT-qPCR	CLN1-2-F	This study
UP028	CTCGACCTGGCACCATTC	RT-qPCR	CLN1-2-R	This study
UP096	ATTCGACTGCCTTGAGCTGT	RT-qPCR	CLB1-F	This study
UP097	CCCATTCGTACTCCTCCAGA	RT-qPCR	CLB1-R	This study
UP094	ACACCCAAGATGAGGCATTC	RT-qPCR	HCM1-F	This study
UP095	GGGTGCAGAGGACTTTCTTG	RT-qPCR	HCM1-R	This study
UP098	TTGCCTGTTCATTGCCTGTA	RT-qPCR	CLB6-F	This study
UP099	GAATTTGGCCATGTTCCTTG	RT-qPCR	CLB6-R	This study
UP100	CCCATCATTGCGATAAAAGGT	RT-qPCR	POL1-F	This study
UP101	ACGCTTACCAAAAACGGAGA	RT-qPCR	POL1-R	This study
UP102	TCAAGGAGGTCATGGAGGTC	RT-qPCR	SCJ1-F	This study
UP103	CATCTGCAGAGCCACTACCA	RT-qPCR	SCJ1-R	This study
UP104	GTCTATGAAGCAGCCCAAGC	RT-qPCR	HPR1-F	This study
UP105	CATCCAACGCTTCCACTTTT	RT-qPCR	HPR1-R	This study
UP082	TACATGGTCCATCGGCTGTA	RT-qPCR	CDC28-F	This study
P083	TAGGTCTTTTCTGCGCCATT	RT-qPCR	CDC28-R	This study
P084	TTTCTCCACCATTTGCCTTC	RT-qPCR	CLN3-F	This study
UP085	TGAATTTTGTGGGATGCTCA	RT-qPCR	CLN3-R	This study

UP086	CCAACCTTCTACGGCGAATA	RT-qPCR	CDC14-F	This study
UP087	TATCCTGCATGGATTCGTCA	RT-qPCR	CDC14-R	This study
UP088	AGAAAAGAACGTCGGGACTG	RT-qPCR	NRM1-F	This study
UP089	ATACCATCCTGGCATGAGGA	RT-qPCR	NRM1-R	This study
UP090	GTTTTCAGCCGGAATATGGA	RT-qPCR	CLN2-F	This study
UP091	CATTGGTTGCGTTATTGCTG	RT-qPCR	CLN2-R	This study
UP092	CGAAGGATACCGCTATGGAA	RT-qPCR	HSL1-F	This study
UP093	AGCATATGCGCATCATCAAA	RT-qPCR	HSL1-R	This study
UP108	TATCGTTGAATCAGGAAGCGGTGTTCGTGTGATCAAGCGGGTAGGTGGTCG ACGGATCCCCGGG	strain construction	ess1^{H164R} + YFP-HIS3 F	This study
OW318	GGGAATAAGAACATAAAAAGGTGAGGTGGAGAAGCAAATGCCAACCATC GATGAATTCGAGCTCG	strain construction	ess1^{H164R} + YFP-HIS3 R	This study

F = forward; R = reverse

Tables S2-S3
Available for download at http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.113.008763/-/DC1

Table S2 Complete list of SGA interactions with *ess1^{H164R}*. 429 genetic interactions were identified after setting a calibrated P-value of between +0.2 to +1.0 (aggravating interactions), or – 0.2 to -1.0 (alleviating interactions).

Table S3 Results of SlimMapper Gene Ontology (GO) analysis. List shows functional categories of genes that interact with *ESS1* derived from the SGA using a calibrated P-value cutoff value of 0.25. Analysis was done using SlimMapper http://www.yeastgenome.org/cgi-bin/GO/goSlimMapper.pl and yeast GO-Slim:Process was used to sort genes into indicated functional categories.

Table S4 Fitting Results for BLI Kinetic Assays

Peptide	k_a ($M^{-1}s^{-1}$) ±SD	k_d (s^{-1}) ±SD	%Rmax ±SD
CTD-P	27100 ±1220	0.0923 ±0.0274	71 ±1
Swi6-NLS-P	25100 ±4570	0.2537 ±0.0881	100
Whi5-NLS-P	22800 ±1420	0.0722 ±0.0384	72 ±1
Whi5-NES-P	17500 ±830	0.0503 ±0.0144	64 ±2

Summary of results for fitting curves of BLI kinetic data (**Fig. 6**). The dissociation rate (k_d) is an average (±standard deviation (SD)) for fits at 3 concentrations of each peptide (3, 10, 30 μM). With the exception of the Swi6-NLS-P peptide, dissociation appeared biphasic, and the largest portion of signal for bound Ess1 (%Rmax) dissociated with the k_d value is shown; a smaller portion appeared to dissociate at a slower rate, which might have been due to re-binding as the concentration of free peptide increased. For Swi6-NLS-P, dissociation was faster and monophasic; all signal for bound Ess1 dissociated with the k_d value shown. The k_a value for each peptide (±SD) was obtained from the slope in supplemental **Fig. S2** (the dependence of association kinetics (k_{obs}) on the peptide concentration).