SUPPLEMENTARY TABLES

Supplemental Table 1. Yeast genotypes used in this study.

Strain Number	**Genotype**
MBY623-638 and 662-669	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
MBY703-706, 713-714, and 799	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*
MBY710, 711, and 715	*MATaa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*

Supplemental Table 2. List of yeast strains used in this study.

Number	Name	Description
MBY623	*ura3::NatMX pESC-URA*	Empty vector control in a wild-type background
MBY624	*rpd3::NatMX pESC-URA*	Empty vector control in an *rpd3Δ* background
MBY625	*hda1::NatMX pESC-URA*	Empty vector control in an *hda1Δ* background
MBY626	*hda2::NatMX pESC-URA*	Empty vector control in an *hda2Δ* background
MBY627	*hda3::NatMX pESC-URA*	Empty vector control in an *hda3Δ* background
MBY628	*hos1::NatMX pESC-URA*	Empty vector control in a *hos1Δ* background
MBY629	*hos2::NatMX pESC-URA*	Empty vector control in a *hos2Δ* background
MBY630	*hos3::NatMX pESC-URA*	Empty vector control in a *hos3Δ* background
MBY631	*ura3::NatMX pESC-URA-PIF1*	Pif1 overexpression in a wild-type background
MBY632	*rpd3::NatMX pESC-URA-PIF1*	Pif1 overexpression in an *rpd3Δ* background
MBY633	*hda1::NatMX pESC-URA-PIF1*	Pif1 overexpression in an *hda1Δ* background
MBY634	*hda2::NatMX pESC-URA-PIF1*	Pif1 overexpression in an *hda2Δ* background
MBY635	*hda3::NatMX pESC-URA-PIF1*	Pif1 overexpression in an *hda3Δ* background
MBY636	*hos1::NatMX pESC-URA-PIF1*	Pif1 overexpression in a *hos1Δ* background
MBY637	*hos2::NatMX pESC-URA-PIF1*	Pif1 overexpression in a *hos2Δ* background

MBY638	*hos3::NatMX pESC-URA-PIF1*	Pif1 overexpression in a *hos3Δ* background
MBY662	*ura3::NatMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in a *rpd3Δ* background
MBY663	*rpd3::NatMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in an *hda1Δ* background
MBY664	*hda1::NatMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in an *hda2Δ* background
MBY665	*hda2::NatMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in an *hda3Δ* background
MBY666	*hda3::NatMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in a *hos1Δ* background
MBY667	*hos1::NatMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in a *hos2Δ* background
MBY668	*hos2::NatMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in a *hos3Δ* background
MBY669	*hos3::NatMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in a *rpd3Δ* background
MBY693	*esa1-414 pESC-URA*	Empty vector control in an *esa1-414* background
MBY694	*esa1-414 pESC-URA-PIF1*	Pif1 overexpression in an *esa1-414* background
MBY703	*gcn5Δ::KanMX pESC-URA empty vector*	Empty vector control in a *gcn5Δ* background
MBY704	*gcn5Δ::KanMX pESC-URA-PIF1*	Pif1 overexpression in a *gcn5Δ* background
MBY705	*rtt109Δ::KanMX pESC-URA empty vector*	Empty vector control in an *rtt109Δ* background
MBY706	*rtt109Δ::KanMX pESC-URA-PIF1*	Pif1 overexpression in an *rtt109Δ* background
MBY710	*WT MBY580 pESC-URA empty vector*	Empty vector control in a wild-type background
MBY711	*WT MBY580 pESC-URA-PIF1*	Pif1 overexpression in a wild-type background

MBY713	*gcn5*Δ*::KanMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in a *gcn5*Δ background
MBY714	*rtt109*Δ*::KanMX pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in an *rtt109*Δ background
MBY715	*WT MBY580 pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in a wild-type background
MBY799	*esa1-414 pESC-URA-PIF1ΔN*	Pif1ΔN overexpression in an *esa1-414* background

Supplemental Table 3. Plasmids used in this study.

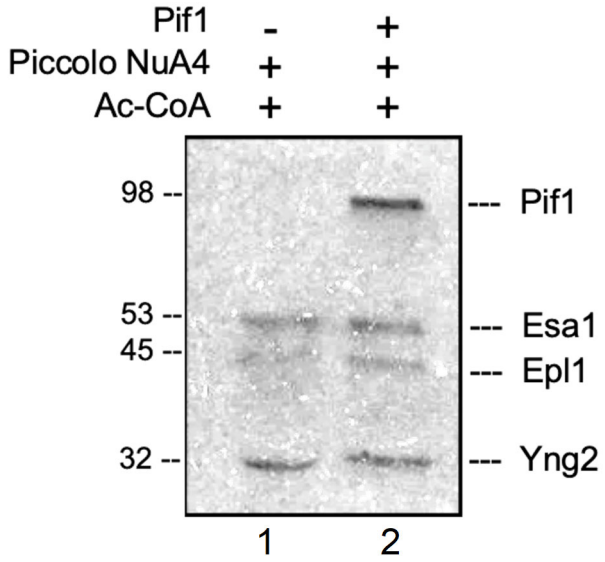
Number	Name	Description
pMB524	pESC-URA	Multi-copy vector enabling epitope tagging of genes cloned under the control of the bidirectional *GAL1,10* promoter
pMB526	pESC-URA-Pif1	Pif1 cloned into pESC-URA, enabling galactose induction and C-terminal FLAG tagging
pMB540	pESC-URA-Pif1ΔN	Pif1ΔN cloned into pESC-URA, enabling galactose induction and C-terminal FLAG tagging
pMB472	pSUMO-Pif1	Nuclear isoform of Pif1 cloned into the pSUMO vector for over-expression in *Escherichia coli*
pMB562	pSUMO-Pif1ΔN	Pif1ΔN cloned into the pSUMO vector for over-expression in *E. coli*
pST44	P6XHis-Epl1/Yng2/Esa1	Wild-type Esa1, Yng2 (2-18), and 6X His-Epl1 (51-380) cloned into the pST44 vector for over-expression in *E. coli*

Supplemental Table 4: **Oligonucleotides used in this study.** Capital letters = dNTP, small letters = NTP, and Bio - biotinylated.

Oligo name and length (nt)	**Sequence (5' – 3')**
T1 (45)	TTTTTTTTTTTTTTTTTTTTTTTTTGTGTCACTCACATAGCGTTC-Bio
D1 (45)	GAACGCTATGTGAGTGACACTTTTTTTTTTTTTTTTTTTTTTTTT
D2 (45)	gaacgcuaugugagugacacuuuuuuuuuuuuuuuuuuuuuuuu(O-methyl)u
D3 (20)	GAACGCTATGTGAGTGACAC
D4 (20)	GAACGCTATGugagugaca(O-methyl)c
D5 (65)	ACGTCATTGGTCTGGGGAGGGTGGGGAGGGTGGGGAAGGTTTTTCACTAATAAGTTCCGCGGCGG–Bio
T2 (21)	CCGCCGCGGAACTTATTAGTG
T50 (50)	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

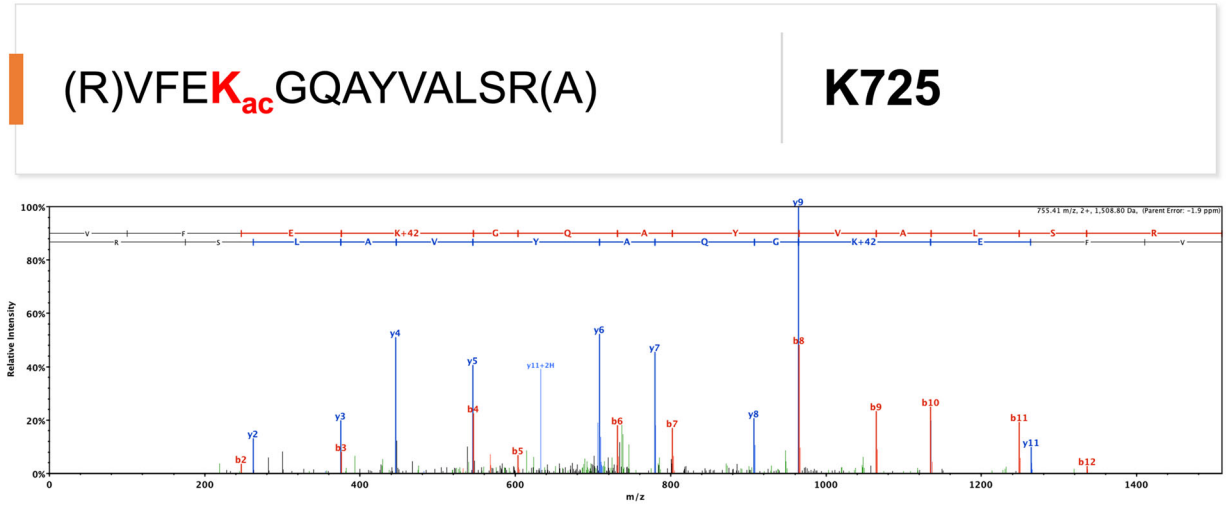
SUPPLEMENTARY FIGURES

Supplementary Figure 1:



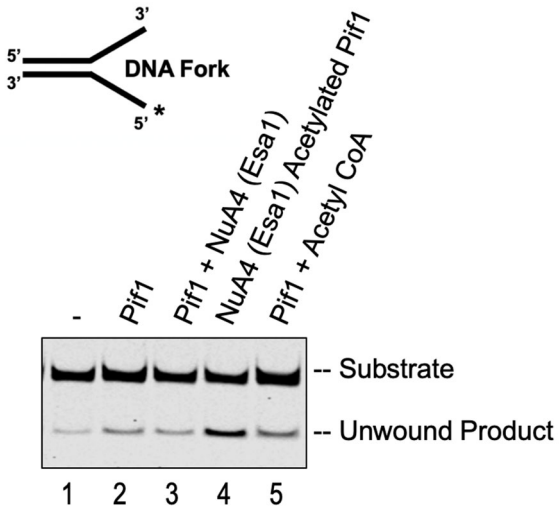
Piccolo NuA4 (Esa1) *in vitro*-acetylates Pif1: Recombinant NuA4 and full-length Pif1 were incubated along with ^{14}C labelled-acetyl CoA as described in the Materials and Methods. The reaction products were separated on a 4-15% SDS-PAGE gel and subsequently subjected to autoradiography. Piccolo NuA4 (Esa1) was capable of robustly acetylating Pif1 *in vitro* (lane 2). The Esa1 subunit also underwent autoacetylation and acetylated the other subunits (Epl1 and Yng2) of the NuA4 complex (lanes 1 and 2).

Supplementary Figure 2:



Spectra for Acetylated Pif1 Lysine 725: Representative spectra for lysine acetylation sites on Pif1 annotated on Scaffold (Proteome Software, Portland OR). The b-ions are labeled in red, and y-ions are labeled in blue. Neutral loss and other parent ion fragments are shown in green. The sequence of the acetylated peptide is denoted above the spectra with the acetylated lysine (K) highlighted in bold red font.

Supplementary Figure 3:



Stimulation of helicase activity is dependent on Pif1 acetylation alone. Helicase assays was performed using an IR labeled DNA fork in the presence of one nanomolar of either UM-Pif1 (lane 2), Pif1 + Piccolo NuA4 (Esa1) (lane 3), AC-Pif1 (lane 4) or Pif1 + Acetyl CoA lithium salt as described in Materials and Methods.