Supplemental Materials

**The HSA domain binds nuclear actin-related proteins to regulate chromatin-remodeling ATPases**

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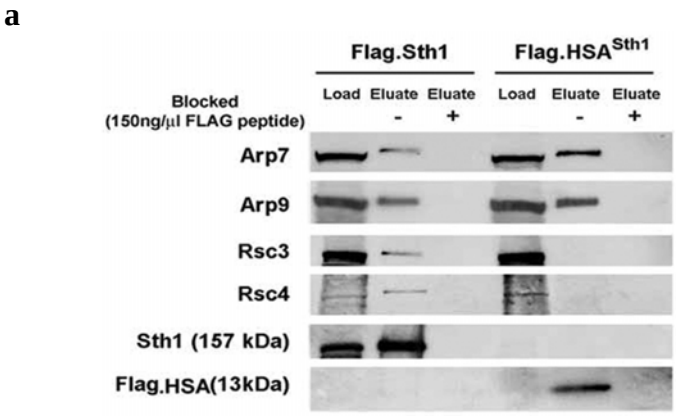
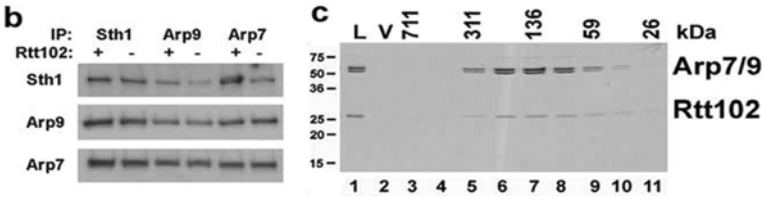
**Supplementary Figure 1**

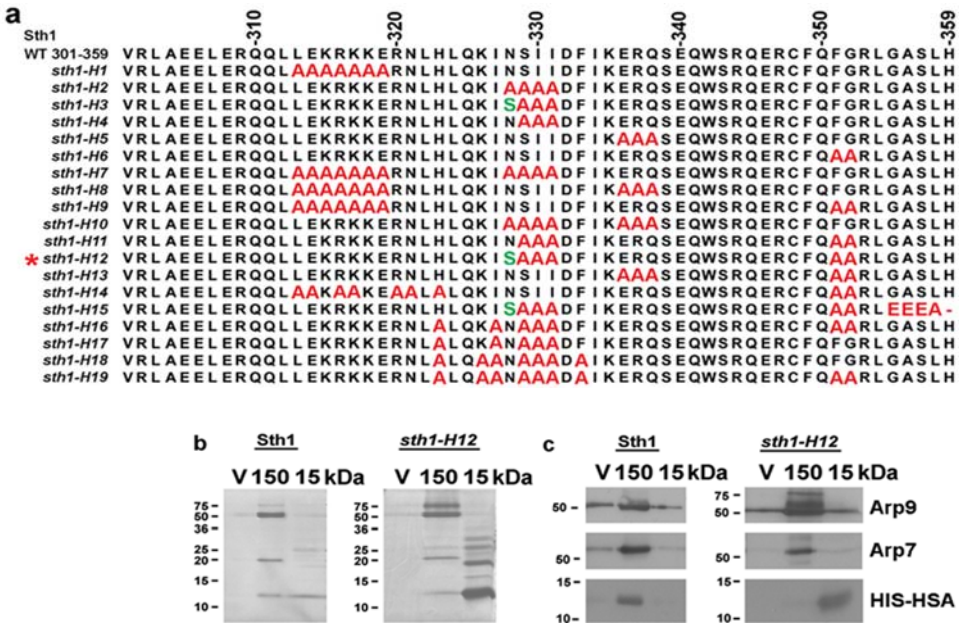



**Supplementary Figure 1a. Sth1-HSA domain is sufficient to bind Arp7/9.**
FLAG-tagged Sth1 and Sth1-HSA domain were overexpressed under control of a MET25-inducible promoter. Cellular extracts were subjected to co-immunoprecipitation and western analysis, revealing the specific association of the HSA domain with Arp7 and Arp9.

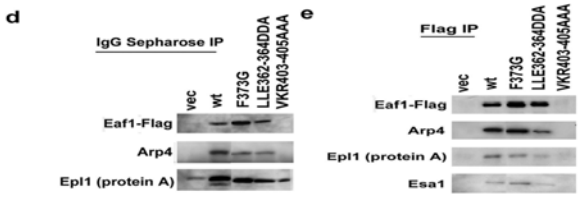



**Supplementary Figure 1b. Rtt102 is not required for ARP associaton with the HSA domain.** Sth1, Arp7 and Arp9 were immunoprecipitated from cells either in the presence (+) or absence (–) of Rtt102. **1c. Rtt102 binds Arp7/9.** Recombinant V5-Rtt102 was co-expressed with Arp7 and Arp9, and purified using anti-V5 antibodies. The purified proteins were analyzed by gel filtration. Arp7, Arp9 and Rtt102 elute from the column at a size corresponding to a trimer of these proteins, at around 130 kDa. L, Load; V, Void.

## Supplementary Figure 2




**Supplementary Figure 2. Multiple amino acid sequences within the Sth1 HSA domain determine Arp7/9 binding.** A series of point mutations were constructed within the Sth1-HSA domain amino acids (aa) 301-372 (**Suppl. Fig. 2a** shows the sequence of aa 301-359) and expressed in bacteria along with Arp7, Arp9 and Rtt102. This HIS-tagged HSA construct was purified and, by gel filtration, analyzed for binding to the ARPs. Only one construct, *sth1-H12*, showed a decrease in ARP binding along with a purification of the unbound HSA domain, shown here by Coomassie staining (**Suppl. Fig. 2b**) and western analysis (**Suppl. Fig. 2c**) of the insoluble void fraction (**Suppl. Fig. 2b,c**; V), the intact HSA-ARP-Rtt102 fraction (**Suppl. Fig. 2b,c**; 150 kDa), and the unbound HSA containing fraction (**Suppl. Fig. 2b,c**; 15 kDa). We note that a construct that contained the *H12* mutation, and also a frameshift mutation at aa 359 (*sth–H15*), yielded very low levels of protein. We find that this derivative binds ARPs weakly, and when it releases ARPs, it is unstable and aggregates, and thus is not observed at 15 kDa.




Mutations to highly conserved amino acid sequences were made within the FLAG-tagged NuA4 Eaf1 HSA domain. These constructs were expressed in an Epl1-TAP-tagged stain and immunoprecipitated using either IgG beads (specific for Epl1) (**Suppl. Fig. 2d**) or anti-FLAG beads (specific for Eaf1) (**Suppl. Fig. 2e**), then assayed for the association of NuA4 complex members by western analysis. Two mutated constructs (F373G and LLE362-364DDA) retained Arp4. An additional construct, VKR403-405AAA, did not produce detectable levels of protein. Thus, we were unable to uncouple Arp4 release and Eaf1 protein instability.

## Supplementary Table 1

| | *arp* allele | *sth1* allele | Sth1 domain | Growth Conditions 28C | 35C | 38C | 0.03%MMS | 5mM caffeine | 1.5% formamide |
|---|---|---|---|---|---|---|---|---|---|
| | | *STH1* | | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| | *arp9Δ* | *STH1* | | ---+ | - | - | ---+ | - | - |
| Group I | *arp9Δ* | *sth1N384K* | Post-HSA | +++ | ++ | --+ | ++++ | ++ | + |
| | *arp9Δ* | *sth1D385K* | Post-HSA | +++ | + | - | +++ | + | + |
| Group II | *arp7/9Δ* | *sth1L680M* | Snf2-like | +++ | ++ | - | +++ | + | --+ |
| | *arp9Δ* | *sth1L681F* | Snf2-like | ++ | + | - | +++ | --+ | --+ |
| Group III | *arp9Δ* | *sth1T373P* | Post-HSA | +++ | + | - | + | ++ | + |
| | *arp9Δ* | *sth1L392V* | Post-HSA | +++ | ++ | - | - | ++ | ++ |
| | *arp9Δ* | *sth1L680V* | Snf2-like | +++ | ++ | - | + | ++ | ++ |
| | *arp9Δ* | *sth1E676Q* | Snf2-like | +++ | + | - | + | ++ | + |
| Group IV | *arp9Δ* | *sth1K382N* | Post-HSA | ++ | --+ | - | - | ---+ | ---+ |
| | *arp9Δ* | *sth1K688T* | Snf2-like | ++ | - | - | - | - | ---+ |

| | *arp* allele | *sth1* allele | Sth1 domain | Growth Conditions 50mM HU | glycerol | 2% ETOH | raffinose | galactose | 1.2M NaCl |
|---|---|---|---|---|---|---|---|---|---|
| | | *STH1* | | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| | *arp9Δ* | *STH1* | | - | - | - | - | - | - |
| Group I | *arp9Δ* | *sth1N384K* | Post-HSA | ++ | ++++ | +++ | +++ | ++ | --+ |
| | *arp9Δ* | *sth1D385K* | Post-HSA | ++ | ++++ | +++ | ++++ | ++ | - |
| Group II | *arp7/9Δ* | *sth1L680M* | Snf2-like | + | ++++ | ++ | ++++ | ++ | - |
| | *arp9Δ* | *sth1L681F* | Snf2-like | + | ++++ | ++ | +++ | ++ | - |
| Group III | *arp9Δ* | *sth1T373P* | Post-HSA | + | ++++ | +++ | +++ | + | - |
| | *arp9Δ* | *sth1L392V* | Post-HSA | + | ++++ | +++ | ++++ | + | - |
| | *arp9Δ* | *sth1L680V* | Snf2-like | + | ++++ | +++ | +++ | + | - |
| | *arp9Δ* | *sth1E676Q* | Snf2-like | + | ++++ | +++ | +++ | + | - |
| Group IV | *arp9Δ* | *sth1K382N* | Post-HSA | ---+ | + | + | + | ---+ | - |
| | *arp9Δ* | *sth1K688T* | Snf2-like | - | ++ | + | - | ---+ | - |

**Supplementary Table 1. *mra* mutations of Sth1 rescue some but not all phenotypes associated with *arpΔ*.** An array of plate phenotypes were tested on cells expressing *mra* mutaions of Sth1 in an *arpΔ* background. Some but not all phenotypes were rescued. Scoring is based on ability to grow. +, growth. –/+, some growth. –, no growth.

**Supplementary Table 2** ***Saccharomyces cerevisiae*** **strain genotypes**

| Strain | Mating type | Genotype |
|---|---|---|
| YBC86 | *MAT a* | *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 arp9Δ::LEU2 [Yep24-SWP59]* |
| BCY211 | *MAT a* | *Rsc2-TAP::TRP pep4::HIS3 prb1::LEU2 prc1::HISG can1 ade2 trp1 ura3 his3 leu2-3,112* |
| BCY251 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1 arp9Δ::LEU2 mra1 MFA1::HIS3* |
| BCY252 | *MAT α* | *STH1-TAP::TRP pep4::HIS3 can1 ade2 trp1 ura3 his3 leu2 arp9Δ::LEU2* [*p1522 mra1-1 URA3]* |
| BCY255 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1arp9Δ::LEU2[p111] sth1L392V* |
| BCY256 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1arp9Δ::LEU2[p111] sth1T373P* |
| BCY257 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1arp9Δ::LEU2[p111] sth1K688T* |
| BCY258 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1arp9Δ::LEU2[p111] sth1L681F* |
| BCY259 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1arp9Δ::LEU2[p111] sth1E676Q* |
| BCY260 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1arp9Δ::LEU2[p111] sth1K382N* |
| BCY261 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1arp9Δ::LEU2[p111] sth1L680V* |
| BCY342 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1 arp9Δ::LEU2 mra2* |
| BCY352 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1Δ arp9::LEU2* |
| BCY395 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1 arp7Δ::TRP1 arp9Δ::LEU2 mra1* |
| BCY404 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1* |
| BCY405 | *MAT a* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1* |
| BCY425 | *MAT a* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1 swp59Δ::LEU2 mra1* |
| BCY426 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1 arp9Δ::LEU2 mra1* |
| BCY433 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1 arp7Δ::LEU2 mra3* |
| BCY483 | *MAT α* | *ade2-1 trp1-1 can1-100 leu2-3,112 his3-15 ura3-1arp9Δ::LEU2[p111]* |
| YBC605 | *MAT a* | *his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0* |
| YBC928 | *MAT a* | *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 pep4D::Kanmx* |
| YBC943 | *MAT a* | *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 sth1Δ::HIS3 [STH1 PBL50(URA3)]* |
| YBC3068 | *MAT a* | *EPL1-TAP::TRP eaf1D::clonat ura3 lys2 met15 leu2 his3* |

BCY strains and YBC strains are derivatives of the W303 and S288C genetic backgrounds, respectively.