Heme assimilation in *Schizosaccharomyces pombe* requires cell surface-anchored protein Shu1 and vacuolar transporter Abc3.

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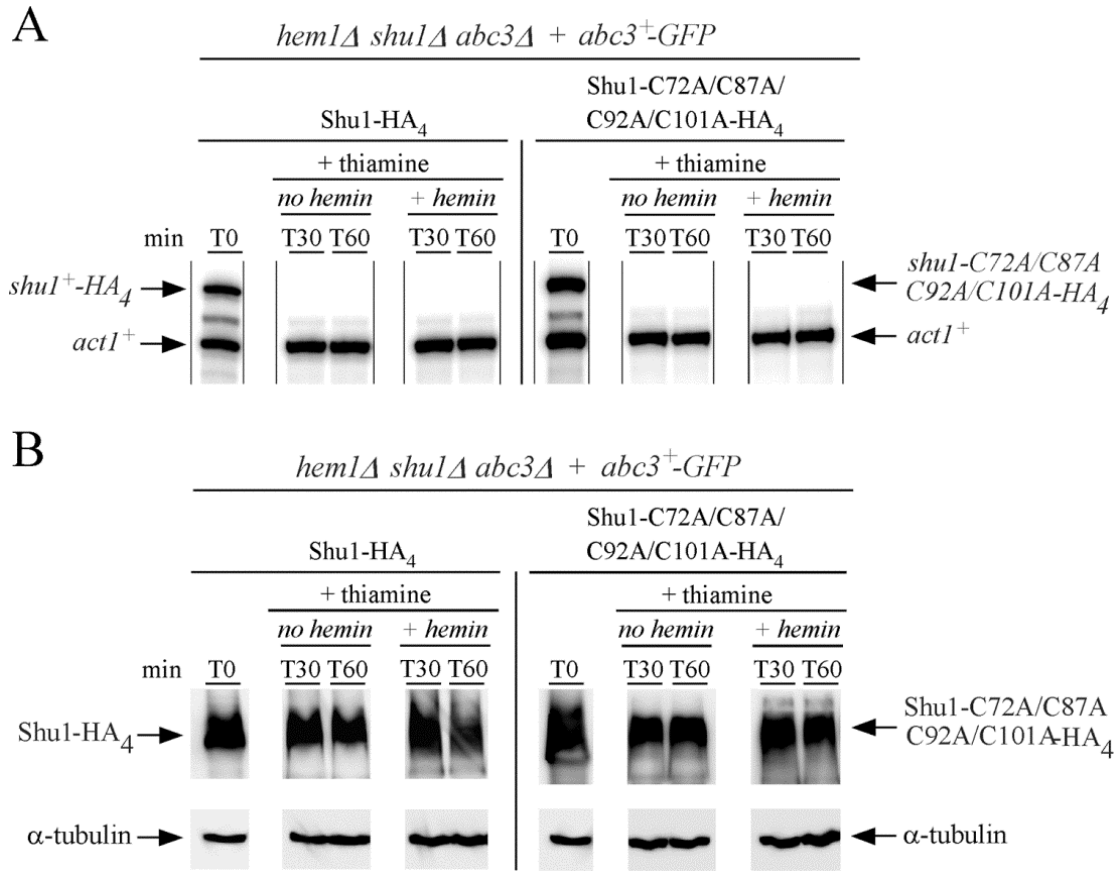

Fig. S1. *Production of cellular pools of Shu1-HA4 and Shu1-C72A/C87A/C92A/C101A-HA4 when their corresponding alleles are expressed under the control of the thiamine-regulatable nmt41x promoter. A,* Cells harboring *hem1Δ shu1Δ abc3Δ* deletions were co-transformed with pSP1*abc3*$^{+}$*-GFP* + pBP*nmt41x-shu1*$^{+}$*-HA4* or pSP1*abc3*$^{+}$*-GFP* + pBP*nmt41x-shu1C72A/C87A/C92A/C101A-HA4* plasmids and were grown in thiamine-free medium containing Dip (50 μM) for 18 h. Cultures were then transferred to ALA-free and thiamine-replete medium to repress further Shu1-HA$_4$ or Shu1-C72A/C87A/C92A/C101A-HA$_4$ protein synthesis (T0) and grown further in the presence (50 μM) or absence of hemin for 30 (T30) and 60 (T60) min. Total RNA was prepared and used in RNase protection assays to determine *shu1*$^{+}$*-HA4*, *shu1C72A/C87A/C92A/C101A-HA4* and *act1*$^{+}$ mRNA levels. Results are representative of three independent experiments. *B,* Aliquots of the cultures described in panel A were taken at the indicated time points and whole-cell extracts were analyzed by immunoblotting. Shu1-HA$_4$ and Shu1-C72A/C87A/C92A/C101A-HA$_4$ were detected using anti-HA antibody. As an internal control, total extract preparations were probed with anti-α-tubulin antibody.