Expanded View Figures

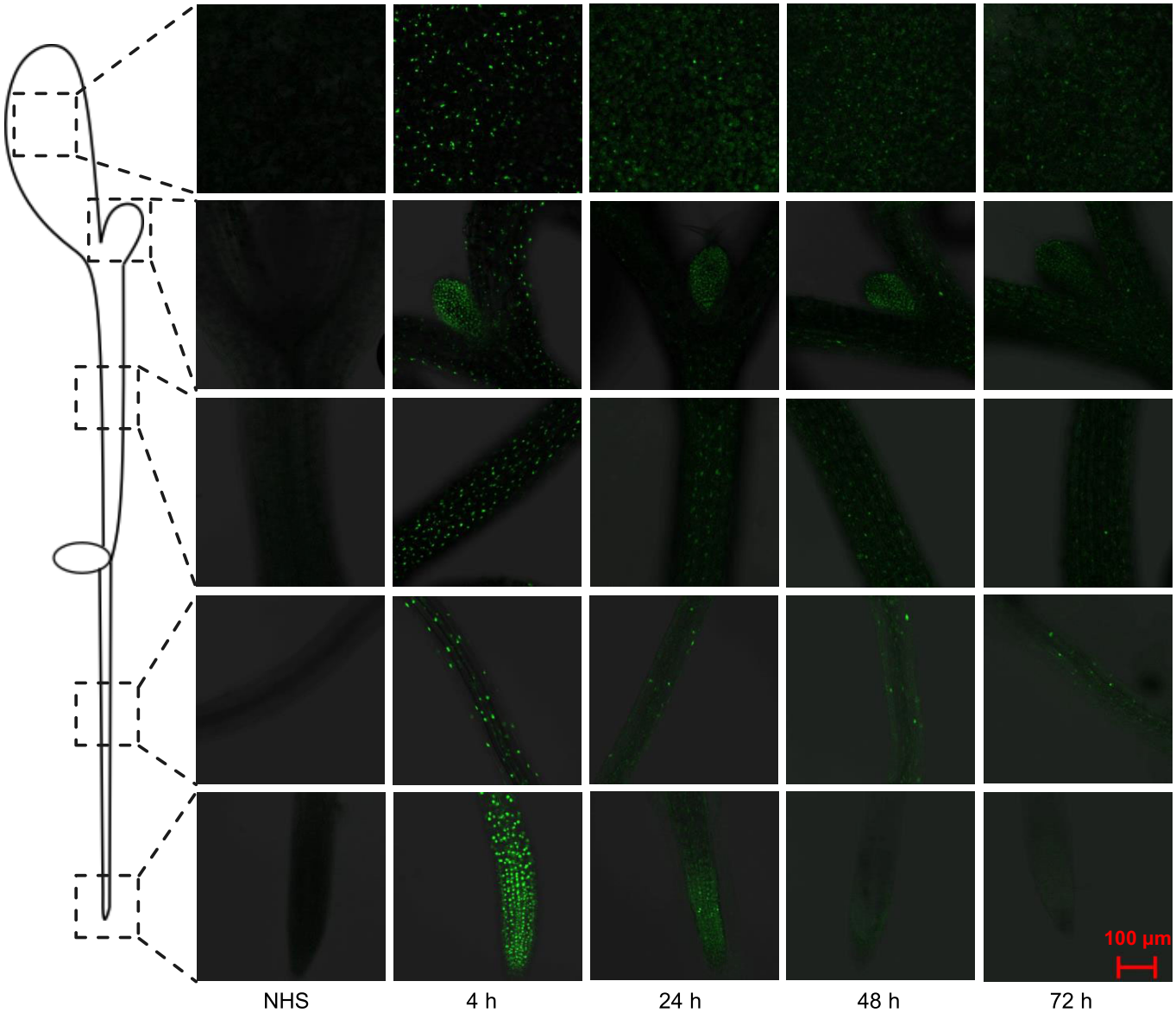
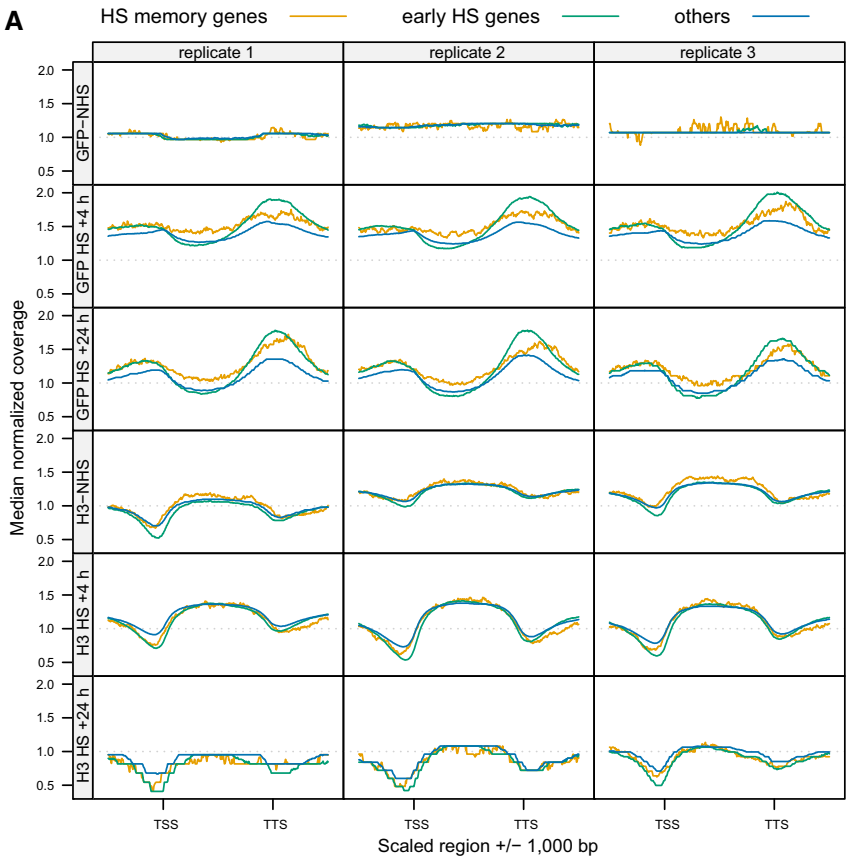


Figure EV1. Accumulation of H3.3-GFP in different organs after HS.

H3.3-GFP localizes to nuclei throughout the seedling after HS. The signal is strongest at 4 h after HS and declines thereafter. In highly dividing cells such as those in the root tip, the signal disappears more rapidly and is only present up to 24 h after HS. In non-dividing cells such as those in the elongation zone, the signal is still present at 72 h after HS. All micrographs were taken with the same settings and magnification. Size bar: 0.1 mm.



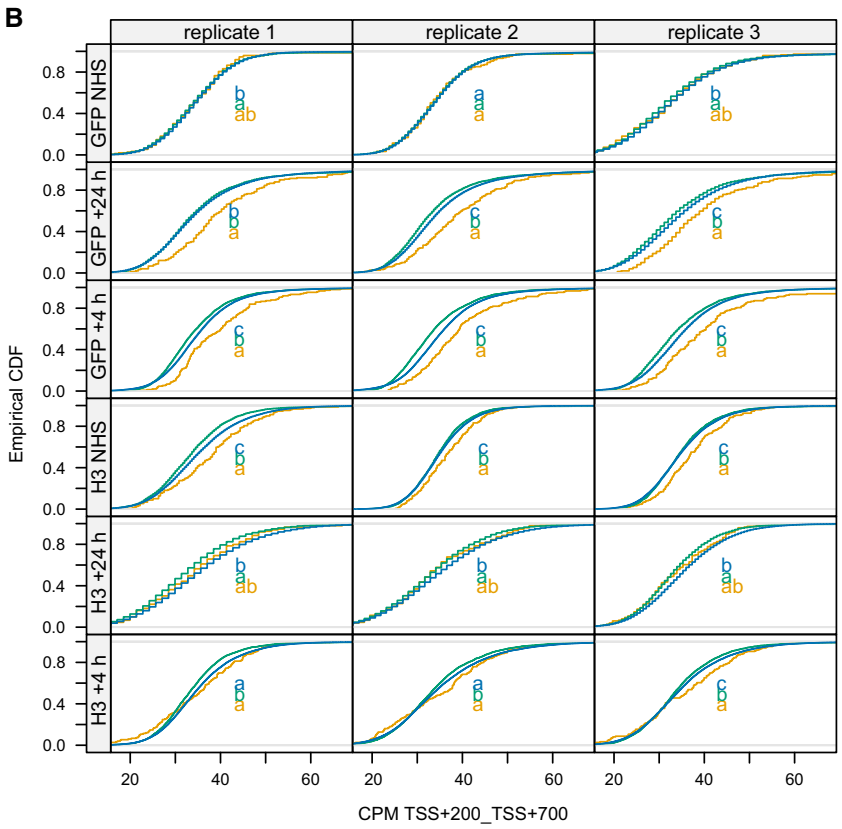


Figure EV2. Accumulation of H3.3-GFP and H3 within HS memory and non-memory genes after HS for individual replicates (cf. Fig 3).

A The global distribution of H3.3-GFP during the memory phase was determined by ChIP-seq on H3.3-GFP seedlings without HS (NHS) or 4 or 24 h after HS with anti-H3 or anti-GFP, respectively. The mean normalized coverage was plotted per replicate for H3.3-GFP (retained histones) or H3 (global pool of histones) between TSS and TTS ± 1 kb for different gene groups (HS memory genes, early HS genes, and all other genes). H3.3-GFP signal in the gene body is higher in HS memory genes 24 h after HS, but not NHS, suggesting that H3.3-GFP histones are retained during HS memory. Data are from individual replicates.

B Empirical cumulative distribution function displaying the coverage (counts per million, cpm) for the indicated groups and samples in the region TSS + 200 bp to TSS + 700 bp. Different letters indicate significant differences ($P < 0.05$, KS test). Data from three individual replicates are plotted.

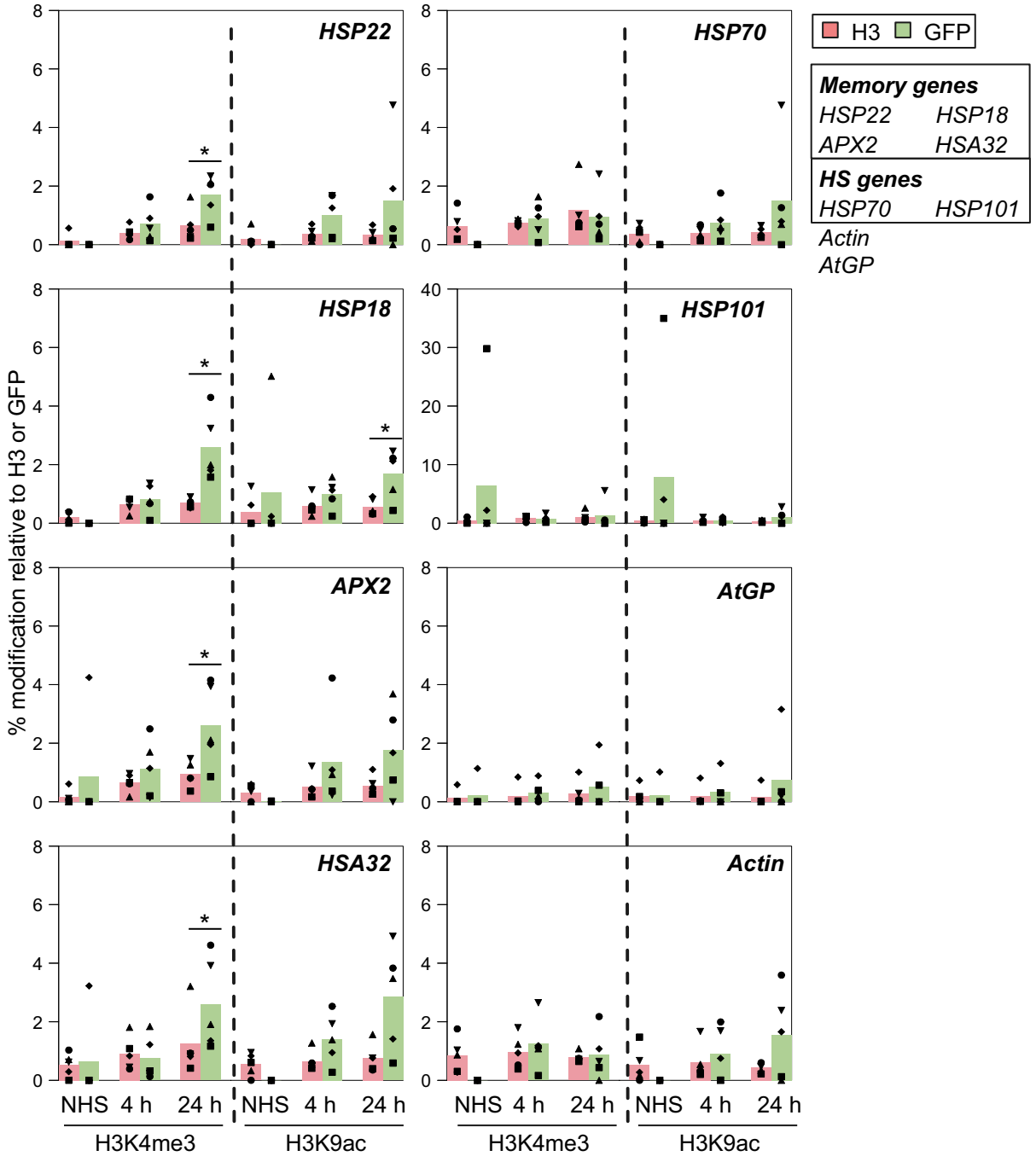


Figure EV3. HS-induced enrichment of H3K4me3 and H3K9ac on retained H3.3-GFP compared to the total pool of H3, 4 and 24 h after HS (cf. Fig 4).

Enrichment of H3K4me3 and H3K9ac on H3 incorporated during the HS response (GFP, green), and on the total histone H3 (red), respectively, at the indicated loci as determined by sequential ChIP-qPCR on H3.3-GFP seedlings without HS (NHS), or 4 and 24 h after HS, respectively. Chromatin was immunoprecipitated with anti-H3 or anti-GFP antibodies followed by anti-H3K4me3, anti-H3K9ac, or IgG (negative control) antibodies. The percentage of histone modifications among H3 or GFP was calculated and normalized on the average signal of each replicate. To control for variation in ChIP efficiency between replicates, each replicate was normalized to the average signal from that replicate (overall measured amplicons). The relative enrichment of either modification is higher in retained histones than in the total pool of histones. Data shown are means and individual data points of five biological replicates. Asterisks indicate significant differences between H3 and GFP ($P < 0.05$, Wilcoxon Mann–Whitney test).

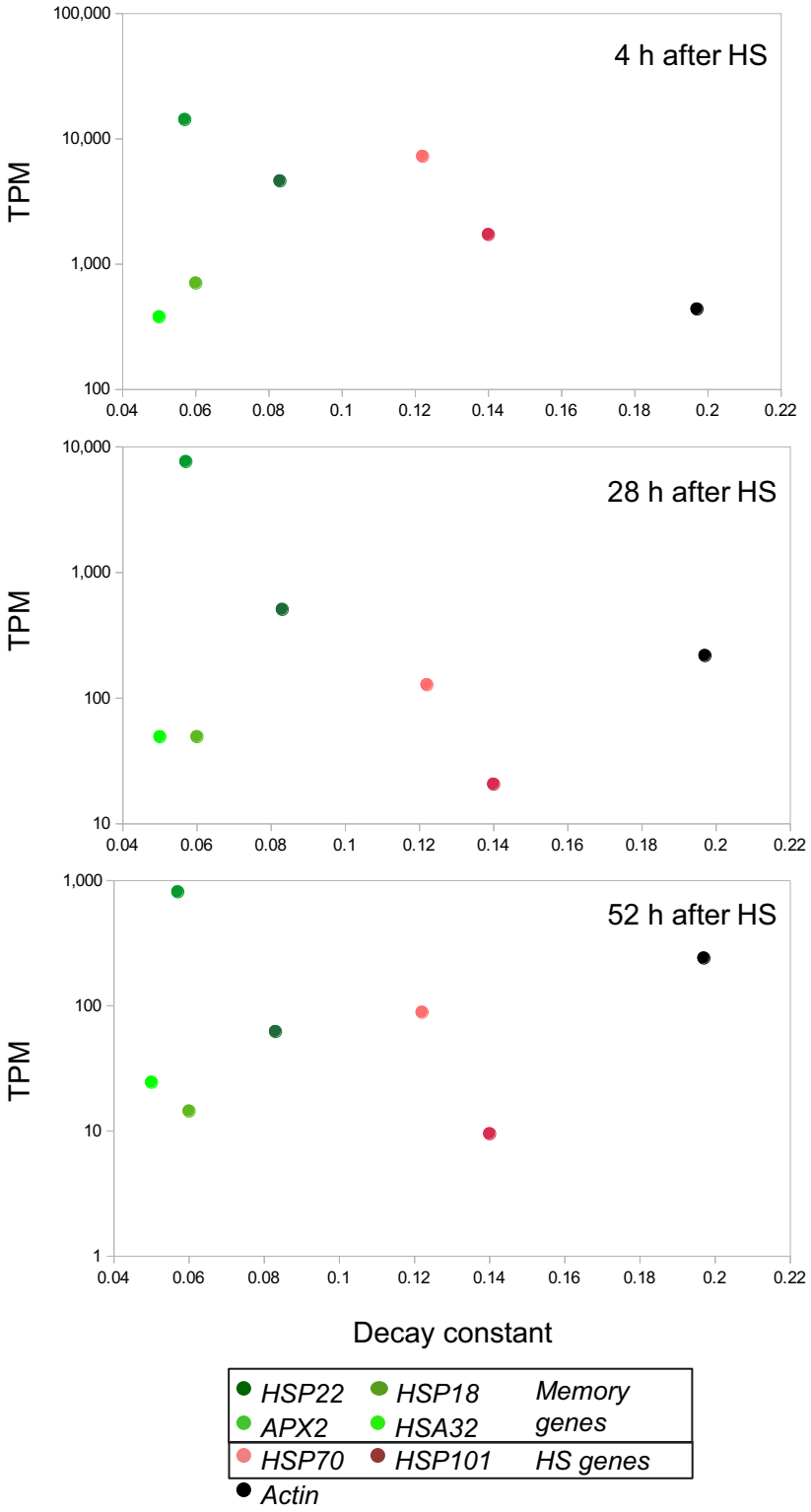


Figure EV4. Histone decay does not correlate with gene expression level after HS.

Expression values of tested genes at 4, 28, and 52 h after HS were taken from Friedrich et al (2021) and plotted against their histone decay constants. No correlation was found between histone decay and gene expression after HS.

Figure EV5. Histone retention in memory genes is independent of *HSFA2*.

H3.3-GFP association and dissociation after HS at the indicated loci as determined by ChIP-qPCR on H3.3-GFP seedlings in Col wild type or hsfa2 without HS (NHS), and 4, 12, 24, 36, 48, and 72 h after HS.

A Dynamics of H3.3-GFP enrichment relative to total H3. Data shown are means ± s.e.m. of three biological replicates.

B–D Dynamics of H3.3-GFP dissociation were evaluated by determining the percentage of GFP/H3 retained for each locus between wild type (top) and *hsfa2* (bottom). For each locus, GFP/H3 values were normalized to the 4 h value (B). The data were used to fit an exponential decay function (C), and derive dissociation constants for each locus as an estimate of histone turnover (D). Differences in histone decay between memory genes (i.e., *HSP18*$^+$, *APX2*$^+$, and *HSA32*) versus non-memory genes (i.e., *ACT*, *HSP101*, and *HSP70*) were significant in both wild type and *hsfa2*. Data shown are means of three biological replicates ± 95% confidence interval (CI). For statistical testing, 100 samples were drawn randomly from a normal distribution with mean and standard deviation of the decay constant for each gene. These data were used to perform a two-sample *t*-test.

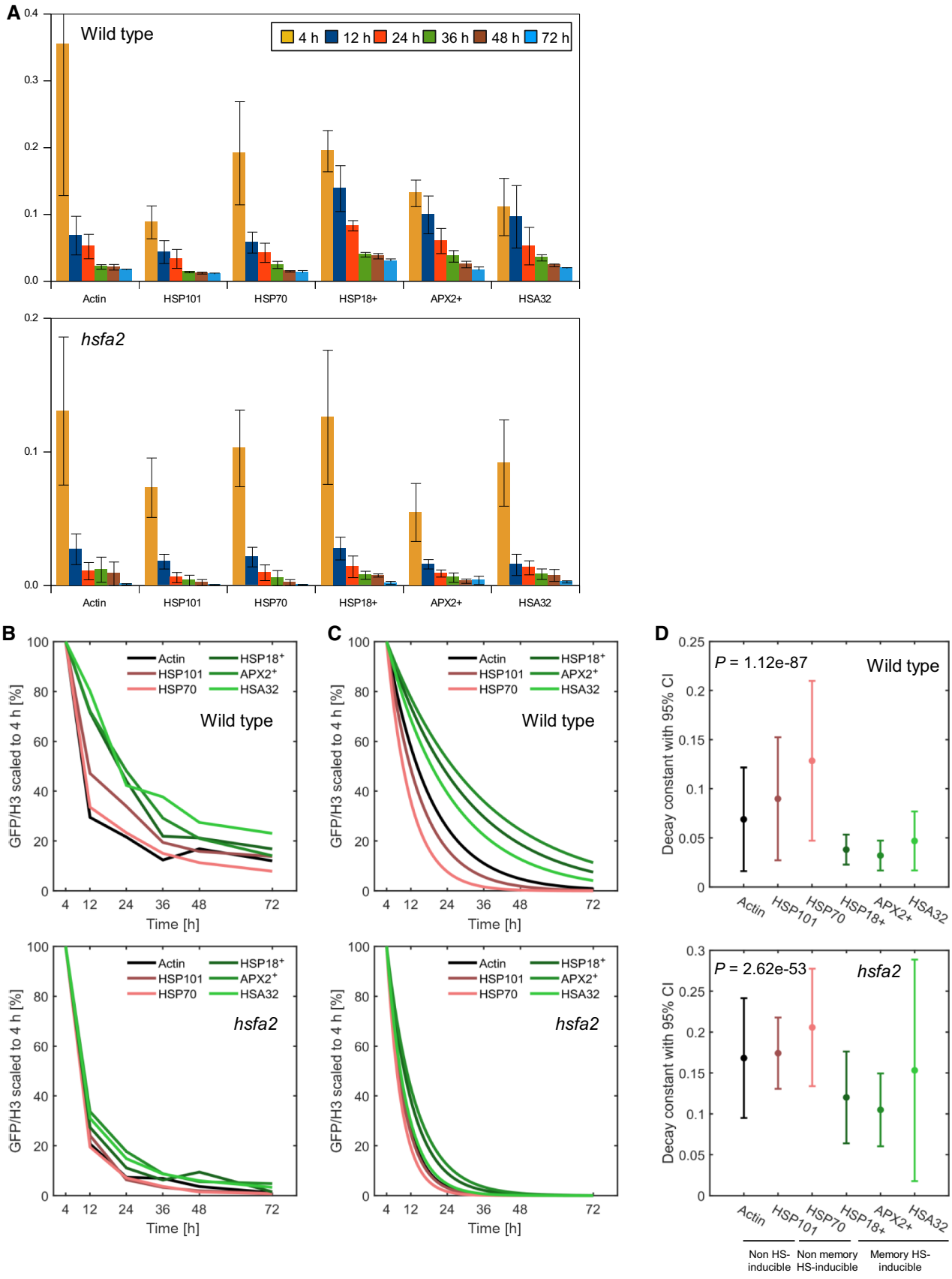

A
Wild type
4 h 12 h 24 h 36 h 48 h 72 h
hsfa2
Actin HSP101 HSP70 HSP18+ APX2+ HSA32
B
C
GFP/H3 scaled to 4 h [%]
Time [h]
Actin HSP101 HSP70 HSP18+ APX2+ HSA32
Wild type
hsfa2
D
P = 1.12e-87
P = 2.62e-53
Decay constant with 95% CI
Non HS-inducible
Non memory HS-inducible
Memory HS-inducible

Figure EV5.