Supplementary Figure 1

Overview of the HSF2 DBD two-site HSE structure.

a) Crystals of HSF2 DBD bound to DNA grown overnight at room temperature. Arrow indicates the crystal used for data collection. b) Stereo image of the 2F_o-F_c electron density map of the sequence-specific interaction of HSF2 Arg63 and the guanine of the nGAAn motif contoured at 2.0 σ. c) Alignment of human HSF2 DBD (blue) and K. lactis DBD (gold). d) Illustration of the interaction of HSF2 Lys72, analogous to HSF1 Lys80, interacting with the phosphate backbone through the epsilon amino group in the side chain as well as the peptide backbone
Supplementary Figure 2

The HSF2 wing domain does not contact DNA.

Stereo image of an $F_\text{O} - F_\text{C}$ simulated annealing omit map contoured at 2.0 $\sigma$ (green) overlaid with the final $2F_\text{O} - F_\text{C}$ electron density map contoured at 0.8 $\sigma$ (blue). These electron density maps confirm the topology of the wing domain and support the conclusion that the wing domain does not engage in DNA contacts.
**Supplementary Figure 3**

**HSF2 DBD C-terminal contacts with DNA.**

a) Lys110 reaches toward the phosphate backbone to engage in water-mediated hydrogen bond network to contact the phosphate of A7, which is located in between the two nGAAn motifs. b) Arg109 utilizes three water molecules to create a hydrogen bond network with the phosphate of G1 and the bases of G2 and T3 of the 2-site HSE sequence. Arg109 also makes a direct contact to the base of G2. c) Sequence alignment of HSFs. The sequence encoding for the carboxyl terminal helix shown in purple in Fig 3 is underlined. Specific residues discussed in Fig 3 are indicated by an asterisk. d) Crystals of HSF2 bound to a 3-site HSE DNA sequence grew in 3-4 days at room temperature. Arrow indicates the crystal that was used for data collection. e) Stereo image of 2Fo-Fc electron density maps for the sequence-specific Arg63 interaction with guanine of the nGAAn motif contoured at 2.0 σ. All four monomers in the asymmetric unit utilize this interaction.
Supplementary Figure 4

In vitro analysis of HSF DBD wing-domain chimeras.

a) Immunoblot analysis of in vitro SUMOylated 6xHis-tagged HSF1 DBD and HSF2 DBD using anti-His tag antibody. HSF1 DBD is not modified by SUMO1 or SUMO2, whereas HSF2 DBD is efficiently modified with the SUMO E3 ligases RANBP2ΔFG or PIAS1, and also in the absence of E3 ligase (UBC9 Only). b) Wing domain sequences of HSF1, HSF2 and HSF4. Underlined sequence delineates the wing domain and the sequences that were used to create the HSF1 and HSF2 DBD chimeras. Shown in red are putative SUMOylation sites. c) In vitro DNA binding analysis of the HSF wing chimera DBDs. DBD protein was titrated into 1 nM FITC-labeled HSE DNA and changes in relative polarization of the sample were measured and converted to binding Kd values. All DBD variants exhibit similar affinity for HSE DNA in vitro.

Nature Structural & Molecular Biology: doi:10.1038/nsmb.3150
HSF1 and HSF2 directly interact and are cross-linked in a complex.

a) Illustration of the dual cassette used for HSF1 and HSF2 co-expression in E. coli. A bi-directional, lac operated, IPTG-inducible promoter was used to simultaneously express StreptII-tagged HSF1 and 6xHis-tagged HSF2 in the pET15b vector. b) Coomassie staining of size exclusion fractions containing HSF1/2 hetero-complexes from Fig. 7b. c) Coomassie staining of HSF1/2 hetero-complexes following DSS crosslinking. Increasing concentrations of DSS result in the appearance of higher molecular weight species. Numbered boxes indicate bands that were excised for mass spectrometry analysis for the data described in Supplementary Table 1. d) Immunoblot analysis of HSF1 in the purification of HSF1/2 heterocomplexes. Co-expression of WT HSF1 and WT HSF2 results in elution of HSF1 following tandem streptactin and NiNTA affinity chromatography (NE, DUAL). Expression of HSF1ΔLZ1-3 and WT HSF2 does not result in elution of HSF1 following tandem affinity chromatography (NE, DUALΔ1). However, HSF1 is observed in the Streptactin Elution suggesting that HSF1 is lost during NiNTA purification since it is not interacting with 6xHis-HSF2.