Supporting Information

Damodaren et al. 10.1073/pnas.1707955114

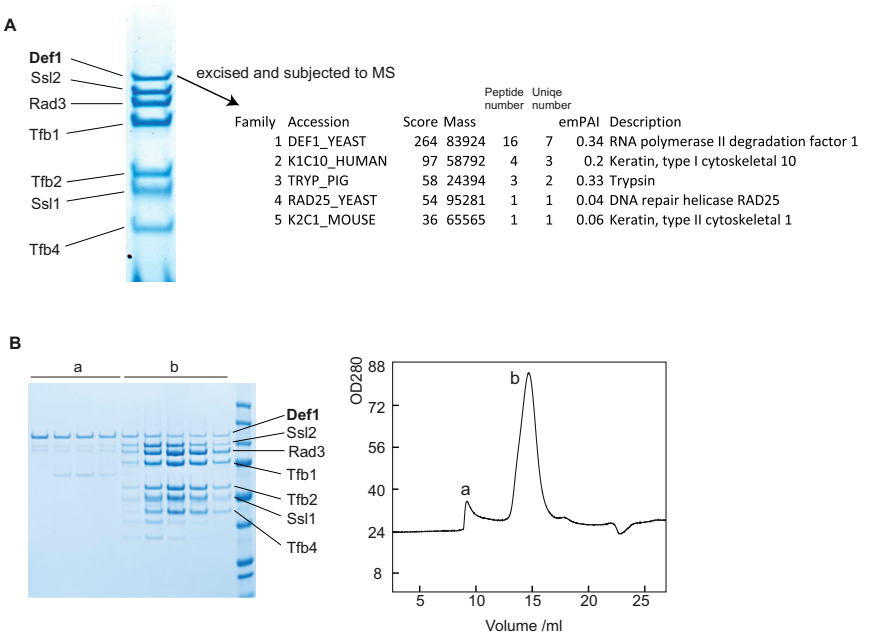
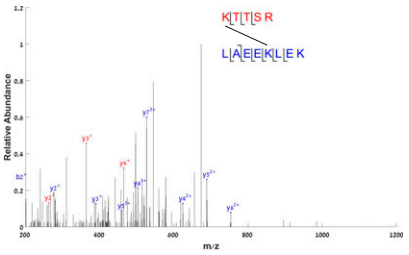
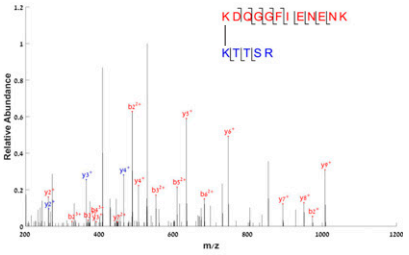


Fig. S1. Def1-TFIIH from yeast cell extract. (*A*) TFIIH-Def1 was obtained after extensive washing with high salt during the TAP affinity purification. The 100-kDa band was excised from the gel and subjected to MS analysis, which confirmed that the 100-kDa band corresponds to Def1. No other yeast proteins were detected except Rad25 (Ssl2, a 90-KDa subunit of TFIIH). (*B*) Peak a in Fig. 1*A* was subjected to gel filtration through a Superose 6 column (*Right*) and analyzed by SDS/PAGE (*Left*). More than 90% of Def1 dissociated from TFIIH (peak a) in the void volume. The remaining ~20% of Def1 remained bound to TFIIH (peak b).

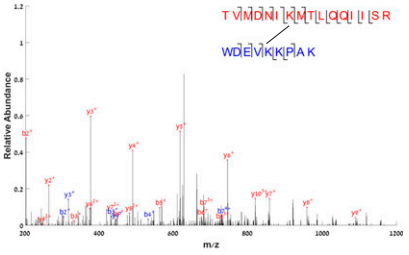
Def1 K229 - Tfb2 K415



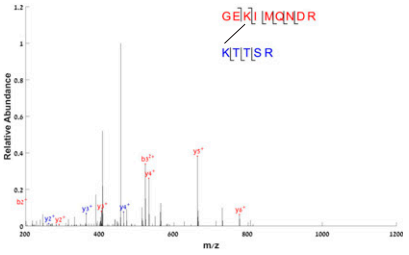
Rad3 K752 - Def1 K229



Tfb1 K101 - Def1 K70



Tfb1 K300 - Def1 K229



Tfb1 K438 - Def1 K229

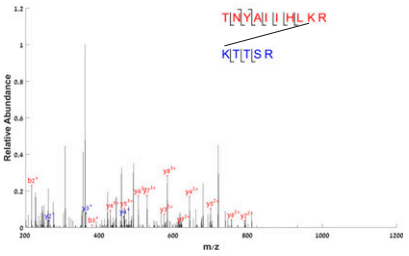


Fig. S2. High-resolution fragmentation spectra of cross-linked peptides between TFIIH and Def1.

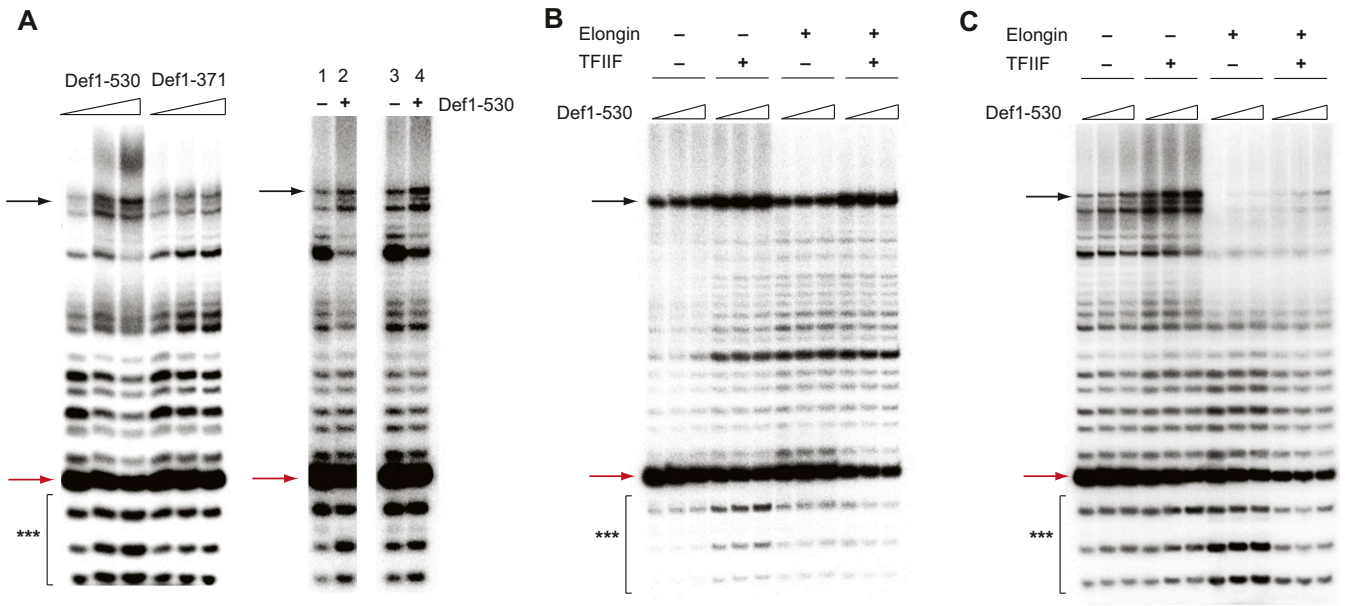


Fig. S3. Def1-530, but not Def1-371, stimulates transcription restart from TFIIS-induced cleavage of arrested pol II transcribing complexes. (*A*, *Left*) Pol II transcribing complexes were formed on a DNA-RNA hybrid containing 9-nt radiolabeled nascent transcript and supplemented with TFIIS (6 pmol) and increasing amounts of Def1-530 (0, 4, and 8 pmol) or Def1-371 (0, 4, and 8 pmol). Elongation of the 9-nt RNA was initiated by the addition of ATP, GTP, and CTP, and the reaction was stopped after 1 min. TFIIS-induced small RNA cleavage products (6–8 nt) are indicated by ***. (*A*, *Right*) Lanes 1 and 2: pol II transcribing complex was preincubated with TFIIS (6 pmol) and Def1 (4 pmol) and was elongated for 1 min as in *A*. Lanes 3 and 4: pol II transcribing complexes were elongated for 1 min in the presence of TFIIS (6 pmol) and stalled by the T-stop at +32. Then 4 pmol of Def1-530 (*Right*) or buffer 300 as a vehicle control (*Left*) was added into the reaction, followed by incubation for another 1 min. (*B*) Same as in *A*, *Left* in the absence of TFIIS. Pol II transcribing complexes were supplemented with Elongin (6 pmol), TFIIF (6 pmol), and increasing amounts of Def1 (0, 4, and 8 pmol), as indicated over the lanes. (*C*) Same as *B* except with the addition of 9 pmol of TFIIS.

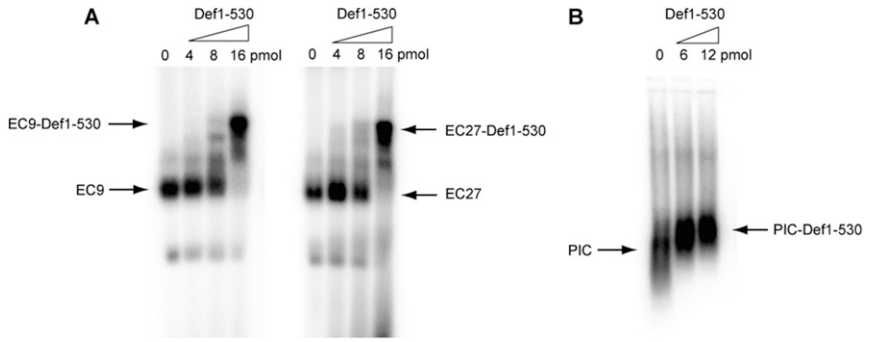


Fig. S4. Native gel analysis of the interaction of Def1 with pol II elongation complexes (*A*) or the PIC (*B*). (*A*) Pol II transcribing complexes were formed on templates containing 9-nt and 27-nt radiolabeled nascent transcripts. Approximately 3.75 pmol of pol II transcribing complexes was mixed with increasing amounts of GST-Def1-530. (*B*) 1.2 pmol of PIC was formed on a radiolabeled SNR20 promoter fragment, and was mixed with increasing amounts of GST-Def1-530.

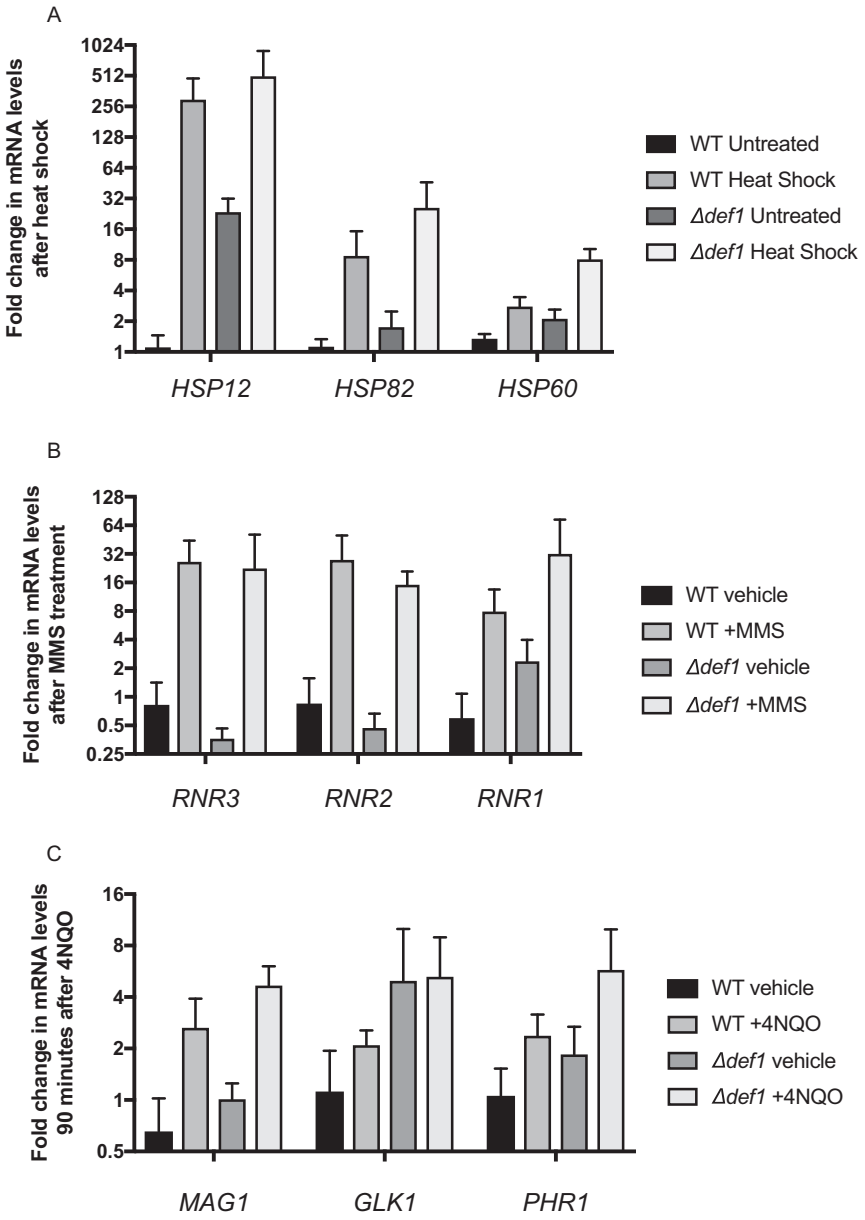


Fig. S5. qPCR analysis of mRNA levels of stress-response genes induced by cellular stimuli. WT and Δdef1 strains were subjected to heat shock at 37 °C (*A*), 0.03% MMS (*B*), and 8 μg/mL 4-NQO (*C*). After 90 min, cells were harvested, and RNA was extracted with TRizol. mRNA levels of each gene indicated were measured by qPCR. Mean ± SEM ΔΔCt values (relative to WT mRNA levels before stress induction) are plotted ($n = 3$).

Dataset S1. MS-based semiquantitative proteomics analysis of Def1-TFIIH. Possible cross-linked candidates generated from sequences of the 50 most abundant proteins were searched against cross-links under matching criteria described previously (15, 21)

Dataset S1

Dataset S2. Nonredundant compilation of all cross-links identified for Def1-TFIIH. Cross-links with high MS/MS fragmentation scores were assigned automatically. The estimated false-positive rate for the cross-links that were assigned automatically is <2.5%. Score 1 is defined as 2 for cross-links in which each peptide has at least four observed fragments from the b- and y-series, 1 for cross-links in which one of the peptides has only three observed fragments from the b- and y-series, and 0 otherwise. Score 2 is defined as {number of matching b and y fragments}/{number of residues in both peptides}

Dataset S2