b





С



Supplementary Figure 1: Binding analysis with ICE1 deletion mutants and ICE2 or ELL.

(a) C-terminal fragment of ICE1 binds to ICE2 *in vitro*. HA-immunopurified complexes from baculovirus-infected Sf9 cells expressing the indicated combinations of FLAG-tagged ICE2 and HA-tagged full-length ICE1 (FL), N-terminal fragment (NL: 1-1190), or C-terminal fragment (CL: 1191-2266) analyzed by Western blotting. (b) ICE1 N-terminal and C-terminal fragments bind to ELL *in vitro*. HA-immunopurified complexes from baculovirus-infected insect cells expressing the indicated combinations of HA-tagged ICE1 FL, NL or CL and FLAG-tagged ELL were analyzed by Western blotting. (c) Western blotting for FLAG-immunopurified complexes from parental 293FRT cells and 293FRT cells stably expressing FLAG-tagged BTBD19 (as a control) and FLAG-tagged ICE1-CL.



Supplementary Figure 2: MED26 is required for the occupancy of little elongation complex at a subset of *small nuclear RNA* genes.

(**a**, **b** and **c**) Depletion of MED26 decreases the occupancy of ICE1 at a subset of Pol II-transcribed *snRNA* genes. Genome browser track examples showing the effect of MED26 depletion on ICE1 occupancy at a subset of *snRNA* genes.



Supplementary Figure 3: Colocalization of ICE1 (red) with coilin (green) in Cajal bodies. HeLa cells were fixed by methanol and stained with anti-MED26 and anti-Coilin antibodies. Scale bars: 2 µm.



Supplementary Figure 4: Generation of mouse embryonic fibroblast (MEF) cells from MED26 gene trap homozygous embryos.

(a) Western blotting showing MED26 and GAPDH expression 48 hr after transfection of non-targeting siRNA or each of three different siRNAs targeting MED26. (b) Western blotting for Mediator subunits MED26 and MED18 and GAPDH of MEF cells from wild-type embryos and MED26 gene trap homozygous embryos. (c) HA-tagged MED26 wild type (wt) or point mutant (R61A, K62A) was stably expressed in MEF cells generated from MED26 gene trap homozygous mice embryos. Western blotting was performed with antibodies against HA, MED26, MED1, Rpb1 and Hsp90.



Supplementary Figure 5: TAF1 interacts with MED26 NTD in the presence of TAF7.

(a) TAF7 directly interacts with MED26 NTD. Recombinant proteins of FLAG-tagged MED26-NTD derivatives (wt: wild type, mut: R61A, K62A) and HA-tagged TAF7 were bacterially expressed. Recombinant proteins of FLAG-tagged MED26-NTD derivatives were immobilized on anti-FLAG M2 agarose and were then incubated with recombinant protein of HA-tagged TAF7. After washing, bound proteins were eluted and analyzed by Western blotting. (b) TAF1 interacts with MED26 NTD in the presence of TAF7. Wild type (wt) or point mutant (mut) versions of FLAG-tagged MED26 NTD were immobilized on anti-FLAG M2 agarose. The M2 agarose complex was incubated with HA-tagged TAF1 or HA-tagged TAF1/His-tagged TAF7 heterodimer. After washing, bound proteins were eluted and analyzed by Western blotting. All proteins were expressed in and purified from baculovirus-infected insect cells. (c) Recombinant TAF7 proteins do not bind to each other. HA-tagged MED26 NTD and HA-tagged TAF7 were immobilized on anti-HA agarose. The anti-HA agarose complex was incubated with FLAG-tagged TAF7. After washing, bound proteins were eluted and analyzed by Western blotting. All proteins were expressed in and purified from baculovirus-infected insect cells.



Supplementary Figure 6: TAF7 occupancy at Pol II-transcribed *snRNA* genes.

(**a**, **b** and **c**) TAF7 and ICE1 are present at a subset of *snRNA* genes. Comparison of the genome browser tracks of ICE1 and TAF7 ChIP-sequence analysis. ChIP-sequence tracks of ICE1 (pink) are from Supplementary Figure 2a-c.



Supplementary Figure 7: Immobilized oligo(dC)-tailed template assay.

(a) Schematic representation of the immobilized oligo(dC)-tailed template assay. Oligo(dC)-tailed templates immobilized on avidin beads were incubated with purified recombinant protein EAF1 in the presence or absence of Mediator containing FLAG-tagged MED26 wild type (wt) or R61A, K62A (mut). After washing, bead-bound proteins were detected by Western blotting. (b) Mediator containing MED26 R61A, K62A (mut) failed to recruit EAF1 to the template. (c) TAF7 knockdown did not affect the occupancy of Mediator at a subset of snRNA genes. Ct values of each ChIP were normalized to that of input. Each value is the average of three independent experiments; error bars show standard deviation (s.d.). The P values for the indicated comparisons were determined by Student' s t test (*, P < 0.05; **, P < 0.01).

а

b



Supplementary Figure 8: Original immunoblot data for Fig. 1a









Supplementary Figure 10: Original immunoblot data for Fig. 1c

Original immunoblot data for Fig. 2a







Supplementary Figure 12: Original immunoblot data for Fig. 3e



Supplementary Figure 13: Original immunoblot data for Fig. 6b and e



Supplementary Figure 14: Original immunoblot data for Fig. 6f

Original immunoblot data for Fig. 7b



Original immunoblot data for Fig. 7c



Original immunoblot data for Fig. 7d



Supplementary Figure 15: Original immunoblot data for Fig. 7b, c and d



Supplementary Figure 16: Original immunoblot data for Fig. 7f

Original immunoblot data for Fig. 9a



Original immunoblot data for Fig. 9d



Supplementary Figure 17: Original immunoblot data for Fig. 9a and d

Supplementary Methods

Primers used for qPCR analysis

Human U1 snRNA

5'-GGGAGATACCATGATCACGAAGGT-3' (forward)

5'-ATGCAGTCGAGTTTCCCACA-3' (reverse)

+1kb of human U1 snRNA

5'-TCAGGTTGAGAGAGAGAGCTAAGT-3' (forward)

5'-CCCAAGAAGCCCTTTCCTATT-3' (reverse)

Human U2 snRNA

5'- GTTTAATATCTGATACGTCCTCTATCC-3' (forward)

5'- TCGATGCGTGGAGTGGAC-3' (reverse)

Human U4-1 snRNA

5'-TAGGCCCTAAACTCACCTTTGCGA-3' (forward)

5'-AGCAATAATCGCGCCTCGGATAGA-3' (reverse)

Human U4-2 snRNA

5'-ATGAGGTTTATCCGAGGCGCGATT-3' (forward)

5'-CGACTATATTTCAAGTCGTCATGGCGGG-3' (reverse)

-5kb of human U4 snRNA

5'-AAATTAGCTGGGGCATGGTGGTGTG-3' (forward)

5'-AATCACGGCTCAGTGCAGCCTAAA-3' (reverse)

Human U5A snRNA

5'-ACTCTGGTTTCTCTTCAGATCGCA-3' (forward)

5'-CTTGCCAAAGCAAGGCCTCAAA-3' (reverse)

Human U5B snRNA

5'-ACTCTGGTTTCTCTTCAGATCGT-3' (forward)

5'-CTTGTCGGAACAAGGCCTCAAA-3' (reverse)

+16kb of human U5 snRNA

5'-TGGTGCCACTCAGAGCCTATTTCA-3' (forward)

5'-TTCCCAGGCATGTTCTCTCA-3' (reverse)

Human U6 snRNA

5'-GCTCGCTTCGGCAGCACATATACTAA-3' (forward)

5'-ACGAATTTGCGTGTCATCCTTGCG-3' (reverse)

Human U11 snRNA

5'-TTCTGTCGTGAGTGGCACACGTA-3' (forward)

5'-AACGATCACCAGCTGCCCAAATAC-3' (reverse)

Human GAPDH

5'-TCGACAGTCAGCCGCATCTTCTTT-3' (forward)

5'-GCCCAATACGACCAAATCCGTTGA-3' (reverse)

Human SNORD118

5'-GAGGGCAGATTAGAACATGATGA-3' (forward)

5'-GCAATCAGGGTGTTGCAAGT-3' (reverse)

Human CELF3

5'-CTCCAGCTCTCATCTCAACTC-3' (forward)

5'-AAGACCTGGAGGGTTAGGT-3' (reverse)

-3kb of human c-Myc

5'-AACCTCCACTGCCAGAAGTCCTTA-3' (forward)

5'-GAAATTTACCTGGCACGTGTCCCT-3' (reverse)

Promoter region of human c-Myc

5'-TTCTCAGAGGCTTGGCGGGAAA-3' (forward)

5'-CTGCCTCTCGCTGGAATTACTACA-3' (reverse)

Mouse U1A1 snRNA

5'-GGAGATACCATGATCACGAAGG-3' (forward)

5'-AGTCGAGTTTCCCGCATTT-3' (reverse)

Mouse U2 snRNA

5'-TGGCTAAGATCAAGTGTAGTATCTG-3' (forward)

5'-GCTCCTATTCCAACTCCTACTTC-3' (reverse)

Mouse U3B snRNA

5'-TGTGTAGAGCACCCGAAAC-3' (forward)

5'-GGAGGGAAGAACGATCATCAA-3' (reverse)

Mouse U5G snRNA

5'-CTCTGGTTTCTCTTCAGATCGTATAA-3' (forward)

5'-TGTCAAGACAAGGCCTCAAA-3' (reverse)

Mouse U6 snRNA

5'-CGAGTCCTCACTGCTTATGTG-3' (forward)

5'-CAGGCATCCCGCAAAGTA-3' (reverse)

Mouse U11 snRNA

5'-AACTCGATTGCTGTGCGT-3' (forward)

5'-GATCACCAGCTGCCCAATTA-3' (reverse)