Supplementary Figures

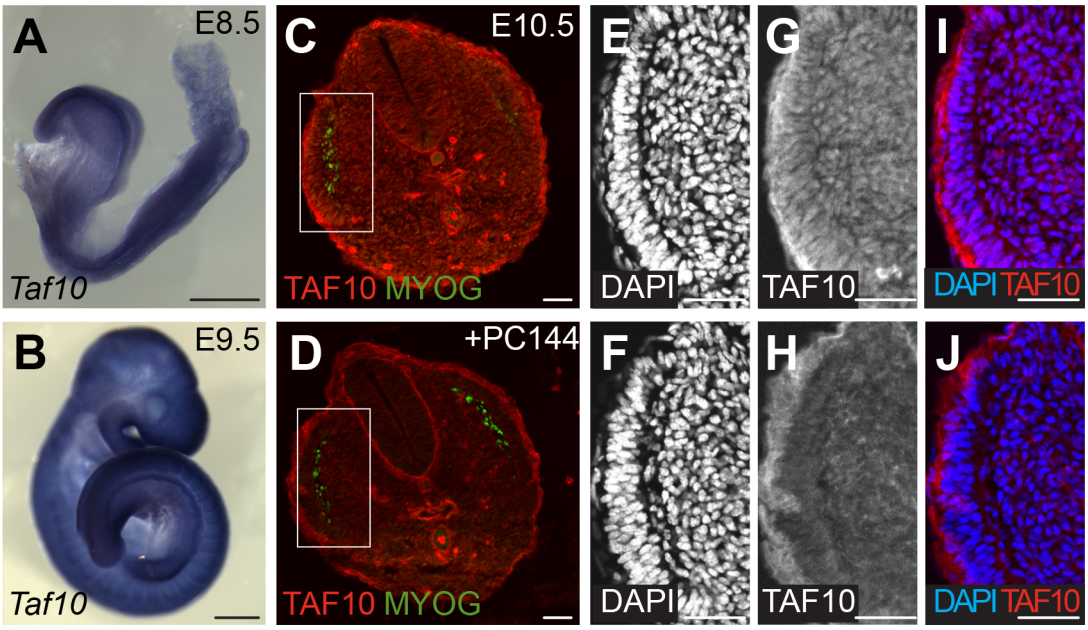

Fig. S1. TAF10 is expressed in the nuclei of the embryo. (A-B) Whole-mount *in situ* hybridization of *Taf10* at E8.5 (A) and E9.5 (B). (C-J) Co-immunolocalization of TAF10, Myogenin and DAPI in E10.5 tail transverse sections. (C-D) Colocalization of TAF10 (red) and Myogenin (MYOG, green). (E-J) DAPI (E,F), TAF10 (G,H) and merge (I,J) magnifications corresponding to the boxes indicated in C and D. (D,F,H,J) Competition with the PC144 peptide used to raise the anti-TAF10 antibody. Nuclear signal of TAF10 (D,H,J) is abolished without affecting the Myogenin signal (D). The non nuclear signal that persists after peptide competition is not specific. Scale bars in A-B and C-J represent 500 μm and 50 μm, respectively.

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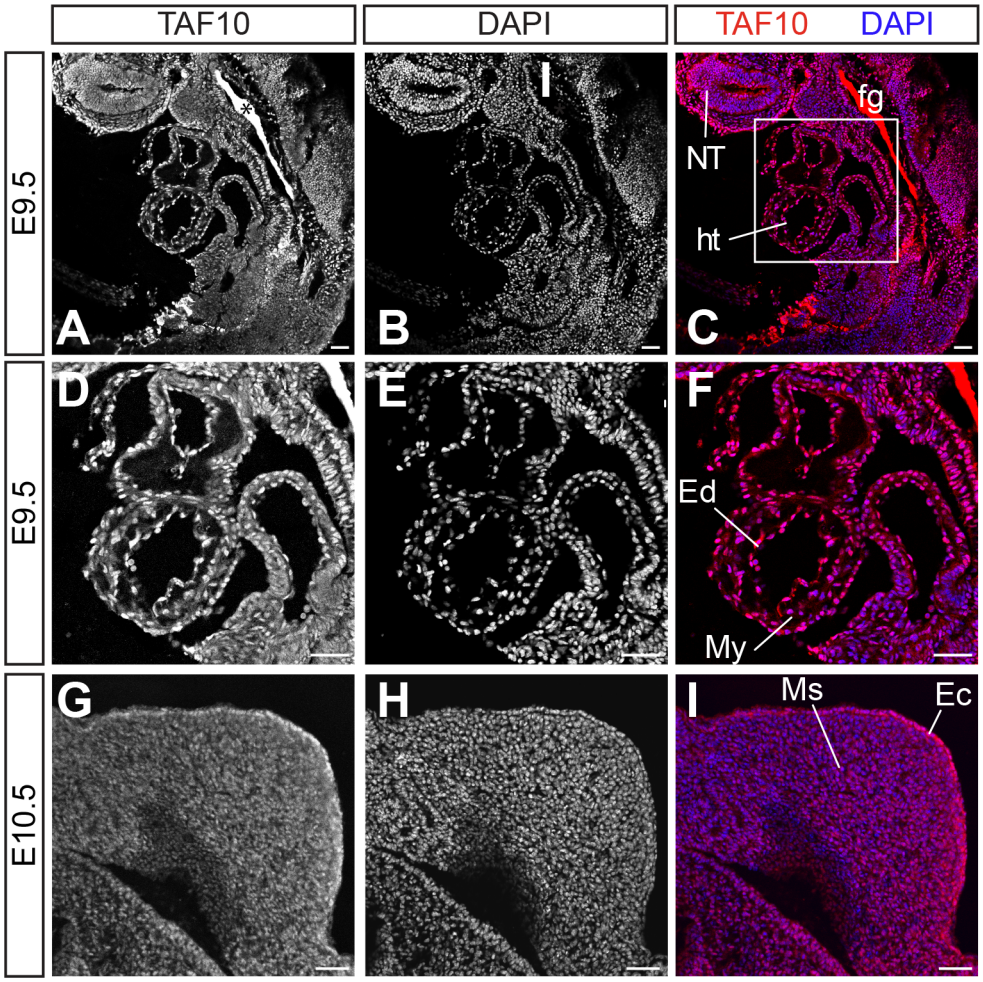

Fig. S2. TAF10 is ubiquitously expressed in the embryo. (A-I) Immunolocalization of TAF10 (A,D,G) and DAPI (B,E,H) on wild type embryo sections at E9.5 (A-F) and E10.5 (G-I). (A-C) Sagittal section at the level of the anterior part of the embryo. The asterisk marks the trapping of the secondary antibody in the foregut pocket. (D-F) is a magnification of the region indicated in C, focusing on the heart. (G-I) is a section at the level of the limb bud. NT; neural tube, ht; heart, fg; foregut; Ed; endocardium, My; myocardium, Ms; mesenchyme, Ec; ectoderm. Scale bars represent 50 μm.

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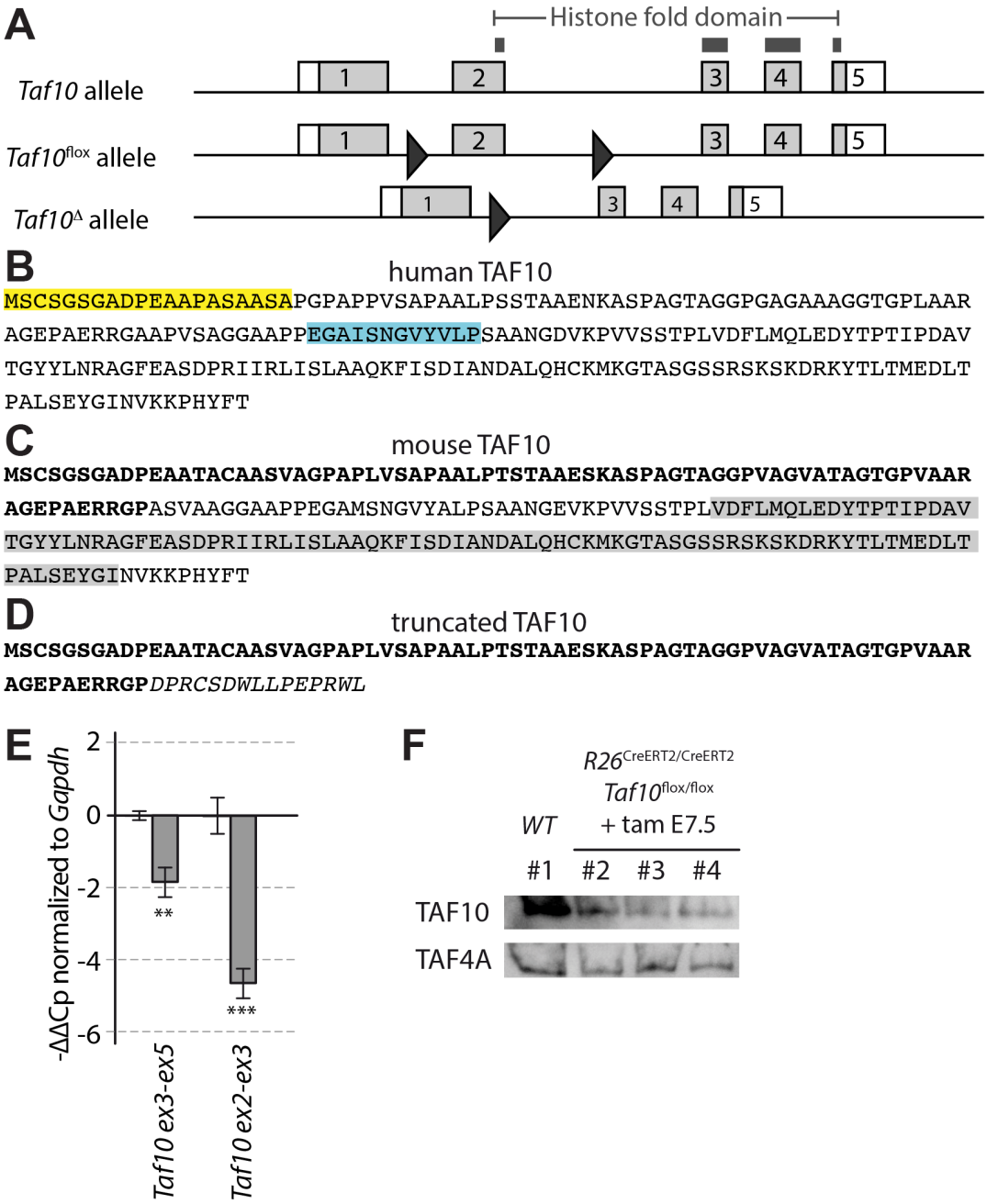

Fig. S3. Deletion of *Taf10*. (A) Strategy of the generation of the *Taf10* deletion using the *Taf10*^{flox} allele (Mohan et al., 2003). The exon 2 is deleted upon Cre expression. The coding sequences of the Histone Fold Domain (HFD) is highlighted by dark grey bars. The deleted allele can theoretically produce a truncated protein that does not contain the HFD, coded by exon 1 and but exon 3 that is out of frame. (B) Protein sequence of the human TAF10: the sequence of the peptides used to raise the 23TA1H8 (yellow) and 6TA2B11 (blue) anti-TAF10 antibodies (C,D) Protein sequence of the murine wild type TAF10 (C) and of the truncated protein (D) potentially present after deletion. Coding sequences of exon 1 are indicated in bold characters. The new extra 15 amino-acids encoded by exon 3 are indicated in italics. The HFD is highlighted in grey. (E) RT-qPCR analysis from tail tips of E9.25 control (white) and *TCre*/+;*Taf10*$^{flox/flox}$ mutant (grey) tail tips. *Taf10 ex3-ex5* amplifies a sequence that is shared by the wild type and the deleted transcripts whereas *Taf10 ex2-ex3* amplifies a sequence only present in wild type transcript. -ΔΔCp are normalized to *Gapdh*. **; p-value <0.01, ***; p-value <0.001 (n=4 for *Taf10 ex2-ex3* and n=2 for *Taf10 ex3-ex5*, Aspin Welch corrected Student's t-test). The error bars indicate s.e.m. (F) Anti-TAF10 and anti-TAF4 western blot analysis of whole cell extract from E8.5 *R26*$^{CreERT2/CreERT2}$;*Taf10*$^{flox/flox}$ embryos, induced at E7.5 by tamoxifen injection at E7.5.

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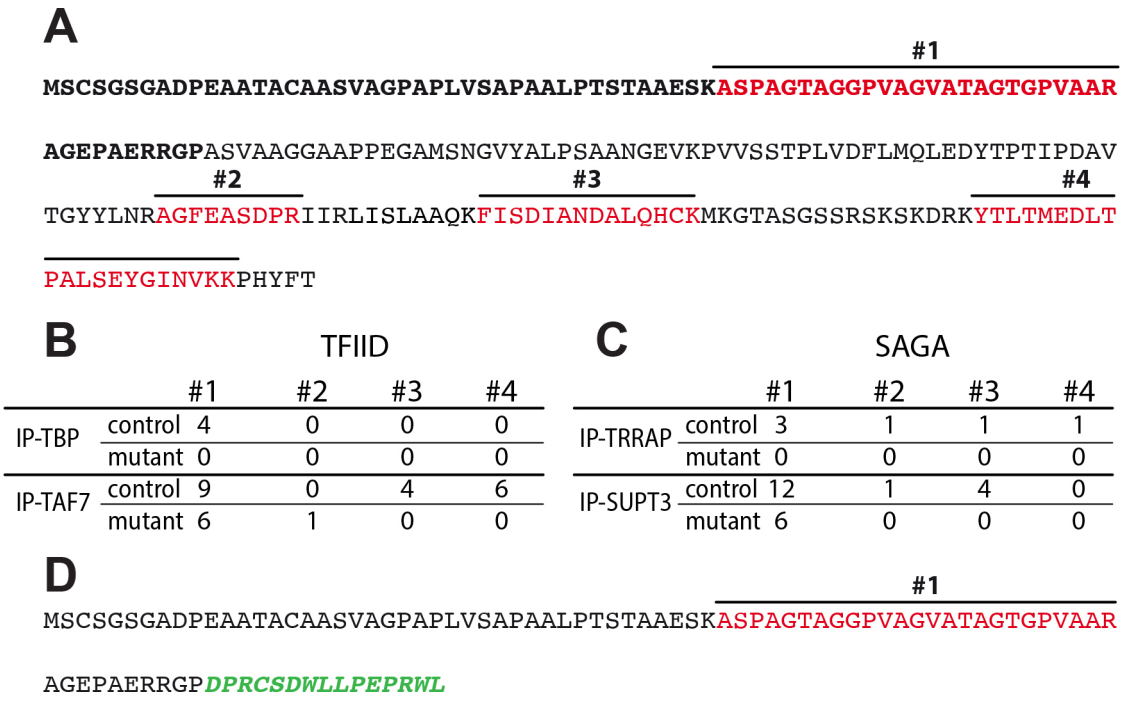

Fig. S4. Abnormal distribution of the TAF10 MS peptides detected in *R26Cre;Taf10* mutant embryos. (A) Localization of the MS peptides (#1 to #4) on the sequence of the full-length TAF10 protein. The peptides are indicated in red letters. The coding sequence corresponding to the first exon is highlighted in bold. (B-C) Number of TAF10 detected MS peptides in control and *R26Cre;Taf10* mutant embryos in each TFIID (B) and SAGA (C) IPs. (D) Localization of the MS peptide #1 (red) on the sequence of putative TAF10 truncated protein. The 15 extra amino-acids coded by exon 3 (not in frame) are indicated in green italics.

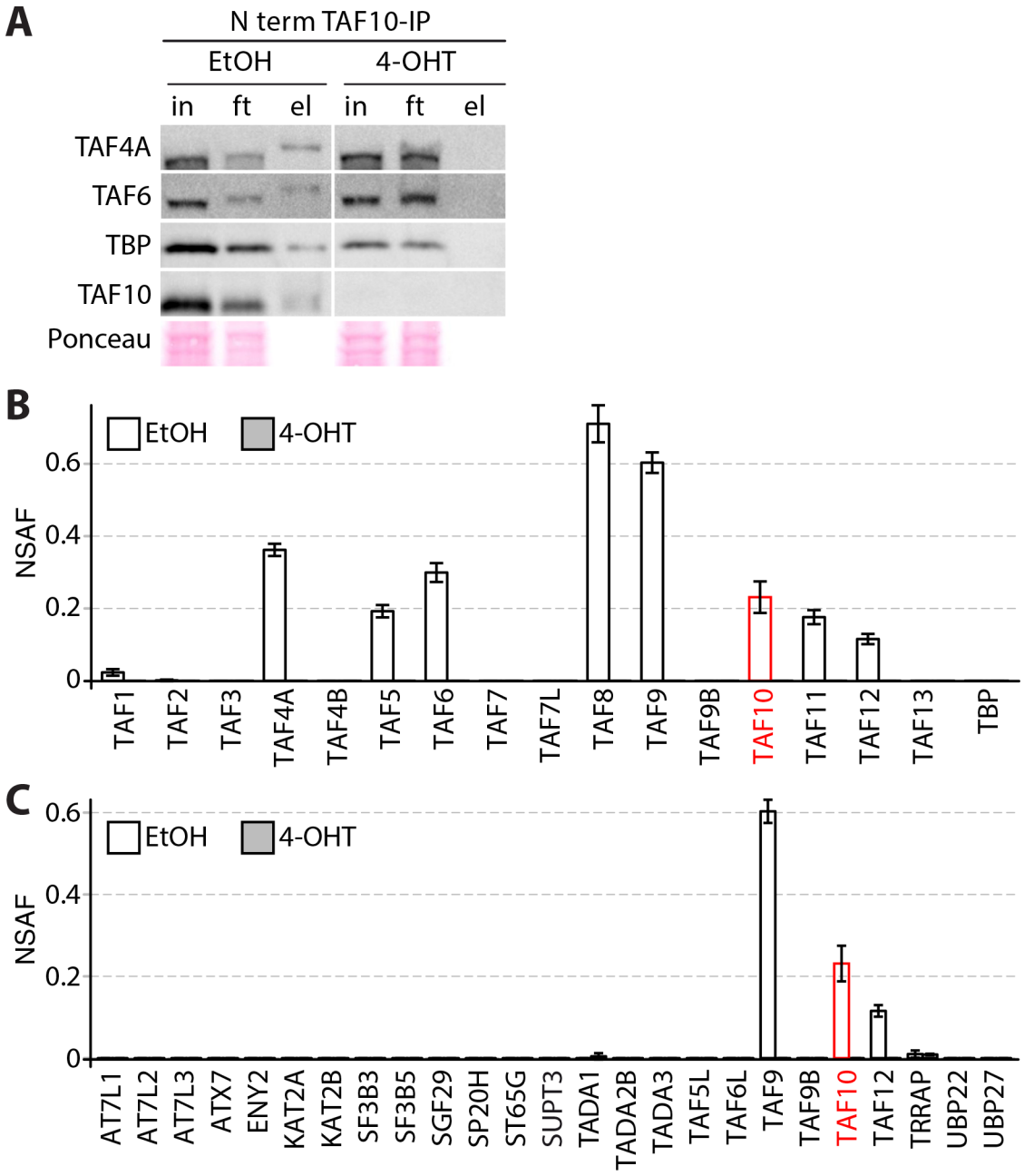

Fig. S5. The potential truncated TAF10 peptide is not able to form a TFIID complex in *Taf10* mutant ES cells (A) Western blot analysis of TBP, TAF4A, TAF6, TBP and full length TAF10 protein of input (in), flowthrough (ft) and elution (el) from Nterm-TAF10-IP from control (EtOH) and mutant (4-OHT) *Taf10* conditional mutant ES cells. (B,C) NSAF values for TFIID (B) and SAGA (C) complexes subunits of Nterm-TAF10-IP from control (EtOH) and Taf10 mutant (4-OHT) ES whole cell extracts. The control (EtOH) and mutant (4OHT) conditions are indicated in white and grey, respectively. 4-OHT; 4-hydroxy-tamoxifen.

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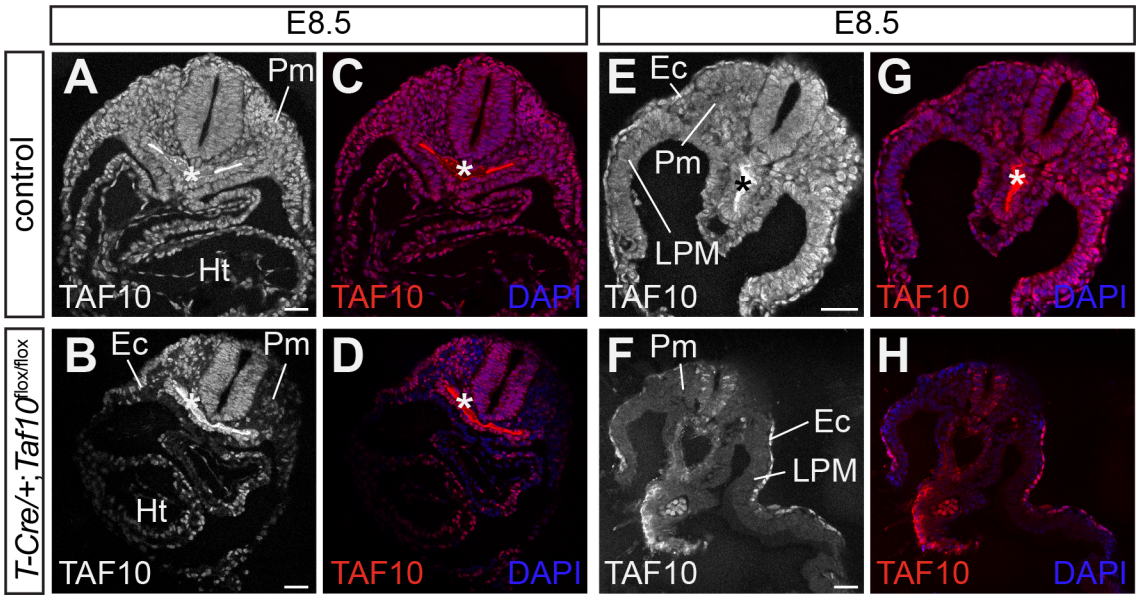

Fig. S6. TAF10 depletion is already effective in the LPM and in the paraxial mesoderm at E8.5. (A-H) TAF10 immunocolocalization on E8.5 transverse wild type (10 somites; A,C,E,G) and *T-Cre;Taf10*$^{flox/flox}$ mutant (8 somites; B,D,F,H) embryos, at the heart (A-D) and at the posterior somites (E-H) levels. Pm; paraxial mesoderm, Ec; ectoderm, LPM; lateral plate mesoderm, Ht; heart. The asterisk (*) indicates background due to secondary antibody trapping in the endoderm. Scales bars represent 50 μm.

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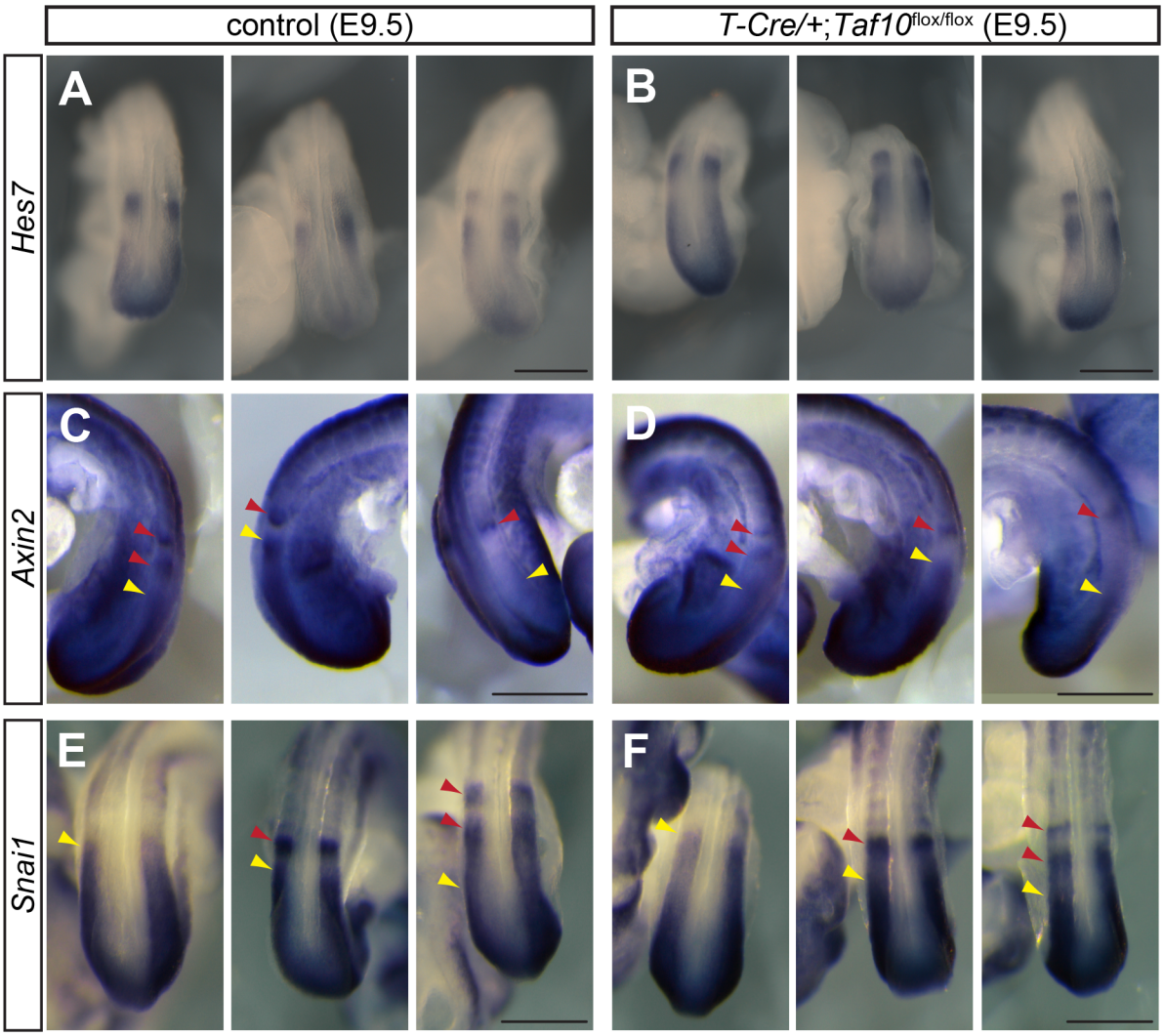

Fig. S7. Expression of the cyclic genes in the absence of TAF10. (A-F) Whole-mount *in situ* hybridization of E9.5 control (A,C,E,) and *T-Cre*/+;*Taf10*$^{flox/flox}$ mutant (B,D,F) embryos using *Hes7* (A,B), *Axin2* (C,D) and *Snai1* (E,F). For each probe, 3 different phases expression pattern are displayed, bands are highlighted by red arrows and the anterior limit of the posterior domain yellow arrows in C-F. The absence of TAF10 in the PSM does not affect the cyclic expression of Notch pathway (*Hes7*), Wnt (*Axin2*) or FGF (*Snai1*) pathways. Scale bars represent 500 μm.

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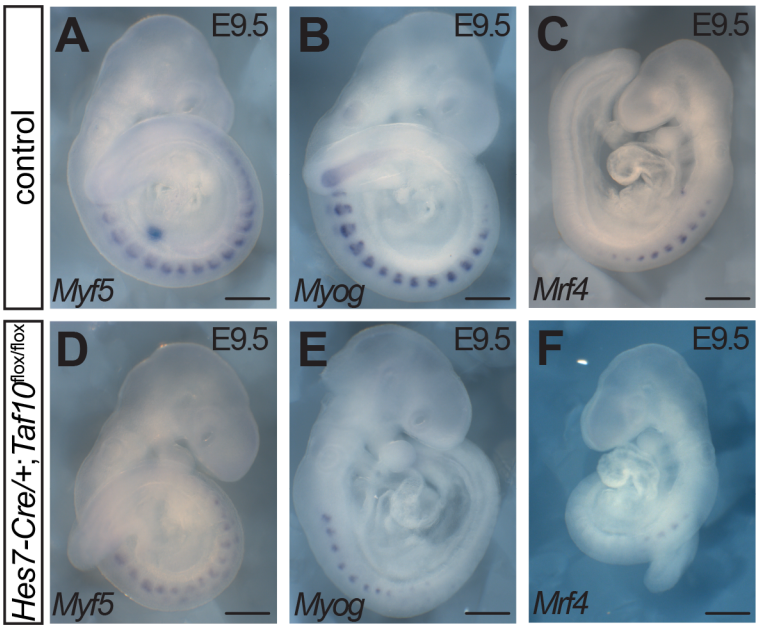

Fig. S8. Delayed myogenesis in *Hes7-Cre*/+;*Taf10*$^{flox/flox}$ mutant embryos. (A-F) Whole-mount *in situ* hybridization of E9.5 control (A-C) and *Hes7-Cre*/+;*Taf10*$^{flox/flox}$ mutant (D-F) embryos using *Myf5* (A,D), *Myog* (B,E) and *Mrf4* (C,F) showing decreased expression of these myogenic markers in the absence of TAF10. Scale bars represent 500 μm.

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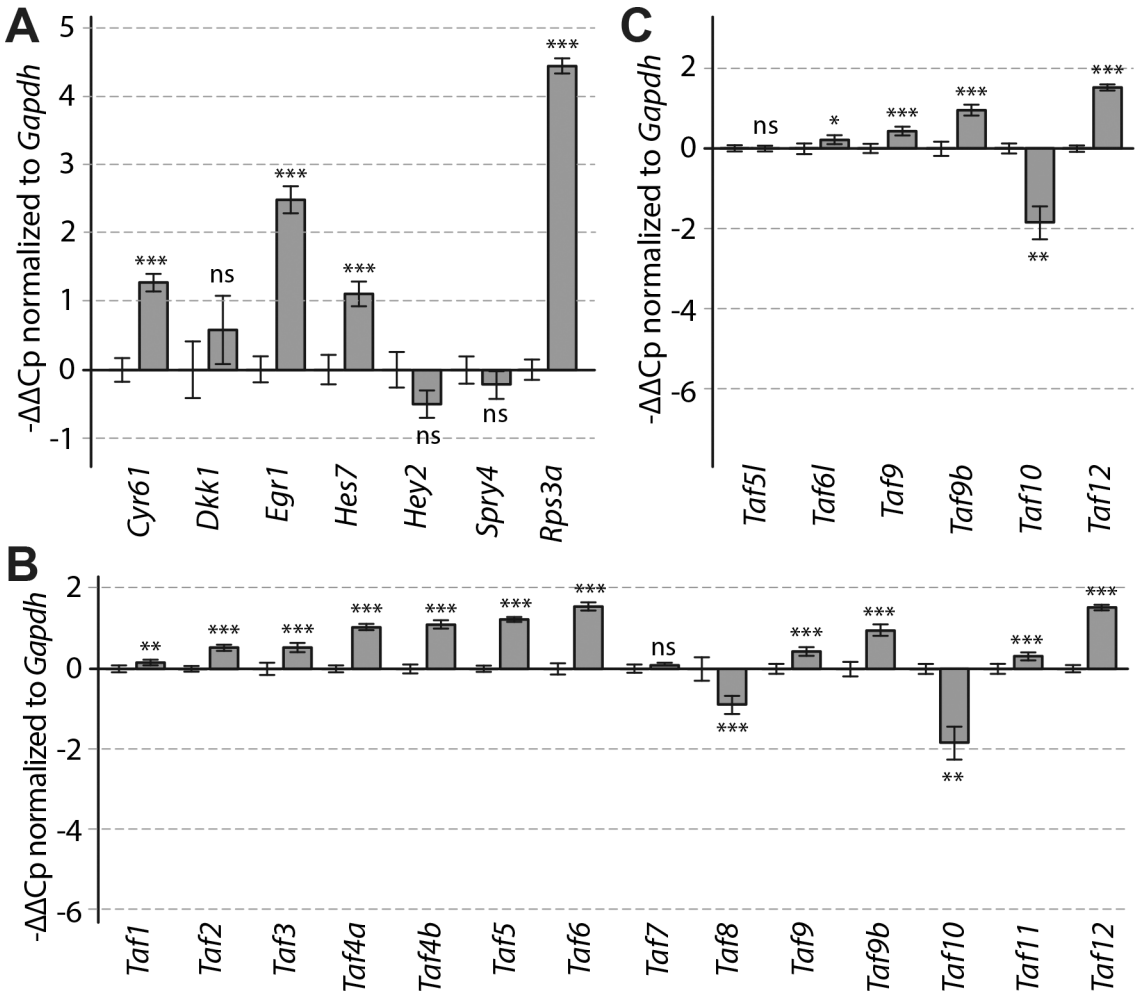

Fig. S9. Validation of the microarray analysis. (A-C) RT-qPCR analysis for cyclic genes (A), TFIID-related TAFs (B) and SAGA-related TAFs (C) from tail tips of E9.25 control (white) and *TCre*/+;*Taf10*$^{flox/flox}$ mutant (grey) tail tips. -ΔΔCp are normalized to *Gapdh*. ns; non significant, *; p-value <0.05, **; p-value <0.01, ***; p-value <0.001 (n=4 except for *Taf10* where n=2, Aspin Welch corrected Student's t-test). The error bars indicate s.e.m.

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	control			
	IP TBP	***IP TAF7***	***IP SUPT3***	***IP TRRAP***
PSM* bait	10/6/7	40/37/33	19/18/20	62/65/71
SAF bait	0.02421859	0.107611082	0.046817713	0.025712615
total SAF	3.235144528	4.882771593	3.73445435	7.255567912
total protein detected	1185	1401	1213	1670

	mutant			
	IP TBP	***IP TAF7***	***IP SUPT3***	***IP TRRAP***
PSM* bait	7/7/6	32/24/29	8/15/9	67/67/52
SAF bait	0.021105319	0.082964463	0.026096693	0.02418102
total SAF	7.205885749	3.567467454	4.777603295	8.991233196
total protein detected	1525	1137	1054	1613

Table S1. Mass spectrometry results for the different IPs.
PSM*; peptide spectrum match, SAF; spectral abundance factor

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Table S2. Differentially expressed genes in PSM of E9.5 *T-Cre;Taf10* mutant versus control embryos

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antibody	*type*	*reference*
anti-H3	rabbit polyclonal	Abcam 1791
anti-TAF10	mouse monoclonal	6TA2B11 (Mohan et al., 2003)
antiNterm-TAF10	mouse monoclonal	23TA1H8 (Wieczorek et al., 1998)
anti-TBP	mouse monoclonal	3TF13G3 (Brou et al., 1993)
anti-TAF4	mouse monoclonal	32TA2B9 (Perletti et al., 2001)
anti-TAF5	mouse monoclonal	1TA1C2 (Jacq et al., 1994)
anti-TAF6	mouse monoclonal	22TA2A1 (Wieczorek et al., 1998)
anti-TRRAP	mouse monoclonal	2TRA1B3 (Nagy et al., 2010)
anti-GST	mouse monoclonal	15TF21D10 (Nagy et al., 2010)
anti-Myogenin	rabbit polyclonal	SC-576 Santa Cruz
anti-Rabbit IgG, Alexa Fluor® 488 conjugate	goat polyclonal	Molecular Probes A-11008
anti-Mouse IgG Alexa Fluor® 546 conjugate	goat polyclonal	Molecular Probes A-11018
anti-Rabbit IgG Peroxydase conjugate	goat polyclonal	Jackson ImmunoResearch 111-035-144
anti-Mouse IgG Peroxydase conjugate	goat polyclonal	Jackson ImmunoResearch 111-036-071

Table S3. List of antibodies.

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Table S4. Primer sequences.

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Supplementary Material and Methods

Mouse lines

Tg(*T-Cre*) (Perantoni, 2005), Tg(*Hes7-Cre*) (Niwa et al., 2007), Tg(*Luvelu*) (Aulehla et al., 2008), *R26*^{CreERT2} (Ventura et al., 2007), *R26*^{R} (Soriano, 1999) and *Taf10*^{flox} (Mohan et al., 2003) lines have already been described.

Generation of antibodies

The rabbit polyclonal anti-SUPT3 (3118), anti-TAF7 (3475) and anti-TAF8 (3478) have been generated at the IGBMC antibody facility, with purified proteins. The first 285 amino-acids of human SUPT3 fused to a His tag were produced in BL21DE3 bacteria and purified with Ni-NTA beads (Qiagen). Whole protein cDNAs for human TAF7 and mouse TAF8 were produced via baculovirus in SF9 cells. For TAF8, infected SF9 cell pellet was boiled and resolved on a 10% SDS PAGE gel, then the TAF8 corresponding band was cut, frozen in liquid nitrogen and crushed. Resulting powder was directly injected into rabbits. For SUPT3 and TAF7, the purified proteins were injected into rabbits directly. The resulting sera were then purified using Affigel (Biorad) coupling followed by Poly-Prep columns (Biorad) purification against the TAF7 protein, the first 285 amino-acids SUPT3 or TAF8-TAF10 coupled protein to purify anti-TAF7, anti-SUPT3 and anti-TAF8 antibodies, respectively.

Immunoprecipitations from *R26*$^{CreERT2/R}$;*Taf10*$^{flox/flox}$ embryos

Pooled lysates from control and mutant embryos, respectively, were split in 4. IPs were performed in two series: first, anti-GST, anti-TBP, anti-TAF7 and anti-TRRAP (IP mock, IP TBP #1, IP TAF7 #1 and IP TRRAP #1). For the second series, flow through (FT) was collected after an overnight incubation, and used as inputs for the second IPs of the other complexes with fresh Dynabeads coated with fresh antibodies overnight at 4°C (IP TAF7 #2 from FT GST #1, IP TBP #2 from FT TRRAP #1, IP SUPT3 #2 from FT TBP #1 and IP TRRAP #2 from FT TAF7 #1). IP

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SUPT3 #2, IP TRRAP #1, IP TAF7 #2 and IP TBP #1 that yielded the highest number of peptides for the bait were conserved for the study.

Immunoprecipitations from *R26*$^{CreERT2/R}$;*Taf10*$^{flox/flox}$ mouse ES cells

R26$^{CreERT2/R}$;*Taf10*$^{flox/flox}$ mouse ES cells (mES) were derived from *R26*$^{CreERT2/R}$;*Taf10*$^{flox/flox}$ E3.5 blastocysts (Vincent SD, unpublished data), maintained on gelatin 0.1% in PBS-coated (PAN BIOTECH) feeder-free culture plates at 37 °C in 5% CO2, in a maintenance medium composed of DMEM supplemented with 15% fetal bovine serum (FBS, Millipore), penicillin, streptomycin, 2 mM L-glutamine, 0.1 mM non essential amino acids, 0.1% β- mercaptoethanol, 1,500 U/mL LIF and 2i inhibitors (Ying et al., 2008) (CHIR99021 3μM and PD0325901 1μM (Axon medchem)). Mouse ES cells were treated with 0.01% EtOH (control) or 100nM 4-OHT (SIGMA) (mutant) for 4 days.

After 2 PBS washes at 4°C, the cells were then scrapped, collected after 20817 rcf centrifugation for 15 min at 4°C and lysed in 10% glycerol, 20 mM Hepes (pH7), 0.35 M NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.1% Triton X-100 with protease inhibitor cocktail (PIC, Roche) on ice. Lysates were treated 3 times with pestle stroke followed by 3 liquid nitrogen freezing-thaw cycles. Lysates were centrifuged at 20817 rcf for 15 min at 4°C and the supernatants were used directly for IPs or stored at -80°C for western blots.

Four mg inputs were incubated with Protein G Sepharose beads (SIGMA) coated with the anti N terminal TAF10 (23TA1H8) mouse monoclonal antibody (Wieczorek et al., 1998) overnight at 4°C. Immunoprecipitated proteins were washed twice 5 min with 500 mM KCl buffer (25 mM Tris-HCl HCl (pH7), 5 mM MgCl2, 10% glycerol, 0.1% NP40, 2 mM DTT, 100 mM KCl and PIC (Roche)) and eluted with 0.1 M glycine (pH2.8) for 5 min three times. Elution fractions were neutralized with 1.5 M Tris-HCl (pH8.8).

Mass spectrometry analyses and NSAF calculations

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Samples were TCA precipitated, reduced, alkylated and digested with LysC and Trypsin at 37°C overnight. After C18 desalting, samples were analyzed using an Ultimate 3000 nano-RSLC (Thermo Scientific, San Jose, California) coupled in line with an linear trap Quadrupole (LTQ)-Orbitrap ELITE mass spectrometer via a nano-electrospray ionization source (Thermo Scientific). Peptide mixtures were loaded on a C18 Acclaim PepMap100 trap column (75 µm inner diameter × 2 cm, 3 µm, 100 Å; Thermo Fisher Scientific) for 3.5 min at 5 µl/min with 2% acetonitrile (ACN), 0.1% formic acid in H_2O and then separated on a C18 Accucore nano-column (75 µm inner diameter × 50 cm, 2.6 µm, 150 Å; Thermo Fisher Scientific) with a 240-min linear gradient from 5% to 50% buffer B (A: 0.1% FA in H_2O; B: 80% ACN, 0.08% FA in H_2O) followed with 10 min at 99% B. The total duration was set to 280 min at a flow rate of 200 nL/min. Peptides were analyzed by high resolution full MS scan (R240K, from 300 to 1650 m/z range) followed by 20 MS/MS events using data-dependent CID (collision induced dissociation) acquisition.

Proteins were identified by database searching using SequestHT (Thermo Fisher Scientific) with Proteome Discoverer 1.4 software (Thermo Fisher Scientific) a combined Mus musculus database (Swissprot, release 2015_11, 16730 entries) where 5 sequences of protein of interest (TrEMBL entries) were added. Precursor and fragment mass tolerances were set at 7 ppm and 0.5 Da respectively, and up to 2 missed cleavages were allowed. Oxidation (M) was set as variable modification, and carbamidomethylation (C) as fixed modification. Peptides were filtered with a false discovery rate (FDR) and rank 1: FDR at 5 %, rank 1 and proteins were identified with 1 unique peptide.

Normalized spectral abundance factor (NSAF) (Zybailov et al., 2006) normalized to the bait ($NSAF_{bait}$) were obtained as followed (PSM*; peptide spectrum match, SAF; spectral abundance factor, x; protein of interest):

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$$SAF_{(x)} = \frac{PSM^{*}_{x(IP)} - PSM^{*}_{x(IPmock)}}{length(x)}$$

$$NSAF_{(x)} = \frac{SAF_{(x)}}{\sum_{i=1}^{n} SAF_{(xi)}} \times 100$$

$$NSAF_{bait(x)} = \frac{NSAF_{(x)}}{NSAF_{(bait)}}$$

For the Nterm-TAF10 IP analyses, only the NSAF was calculated since the bait was not detected in the mutant conditions.

Microarrays and statistical analysis

Total RNA was prepared from 3 replicates (control and mutant), following the recommendations of the manufacturer. Biotinylated single strand cDNA targets were prepared, starting from 150 ng of total RNA, using the Ambion WT Expression Kit (Cat # 4411974) and the Affymetrix GeneChip® WT Terminal Labeling Kit (Cat # 900671) according to Affymetrix recommendations. Following fragmentation and end-labeling, 1.9 µg of cDNAs were hybridized for 16 hours at 45ºC on GeneChip® Mouse Gene 1.0 ST arrays (Affymetrix). The chips were washed and stained in the GeneChip® Fluidics Station 450 (Affymetrix) and scanned with the GeneChip® Scanner 3000 7G (Affymetrix) at a resolution of 0,7 μm. Raw data (.CEL Intensity files) were extracted from the scanned images using the Affymetrix GeneChip® Command Console (AGCC) version 3.2.

Background correction, quantile normalization and summarization by median polish were performed using RMA (Bioconductor package version 2.14 (R version 3.1.0)). Data were filtered automatically by estimating the 100th lowest value of the data series and setting up a background threshold to 3 times this value and by removing manually all the pseudogenes and the expressed sequences. After filtration, 18064 out of 34760 probesets (51.9%) remained. Statistical analysis was performed using the FCROS package version 1.1 (R version 3.1.0) (Dembélé and Kastner,

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2014) that calculates a f value. Differences are considered significant for f value below 0.025 or above 0.0975. Scatter plot and vulcano plots were performed using R software version 3.1.0.

RT-qPCR and statistical analyses

Unless specified, primers (Table S4) were designed using Primer3 (www.ncbi.nlm.nih.gov/tools/primer-blast) and validated.

To compare RNA polymerases I and II transcriptions, each Cp values were normalised by dividing each Cp to the mean of all Cp (mutants and controls) for one set of primers. Data were analysed using a Student's *t*-test with an Aspin Welch correction.

For the gene expression analyses from tail tips, -ΔΔCp values were calculated first by normalizing each Cp to the mean of the Cps for *Gapdh*, then by subtracting each ΔCp of the different controls from the ΔCp of the sample of interest for a given gene of interest, therefore generating 2x16 -ΔΔCp values for mutants and controls, for one given gene. Data were analysed using a Student's *t*-test with an Aspin Welch correction. Calculations and graphs were obtained using R (3.1.0).

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