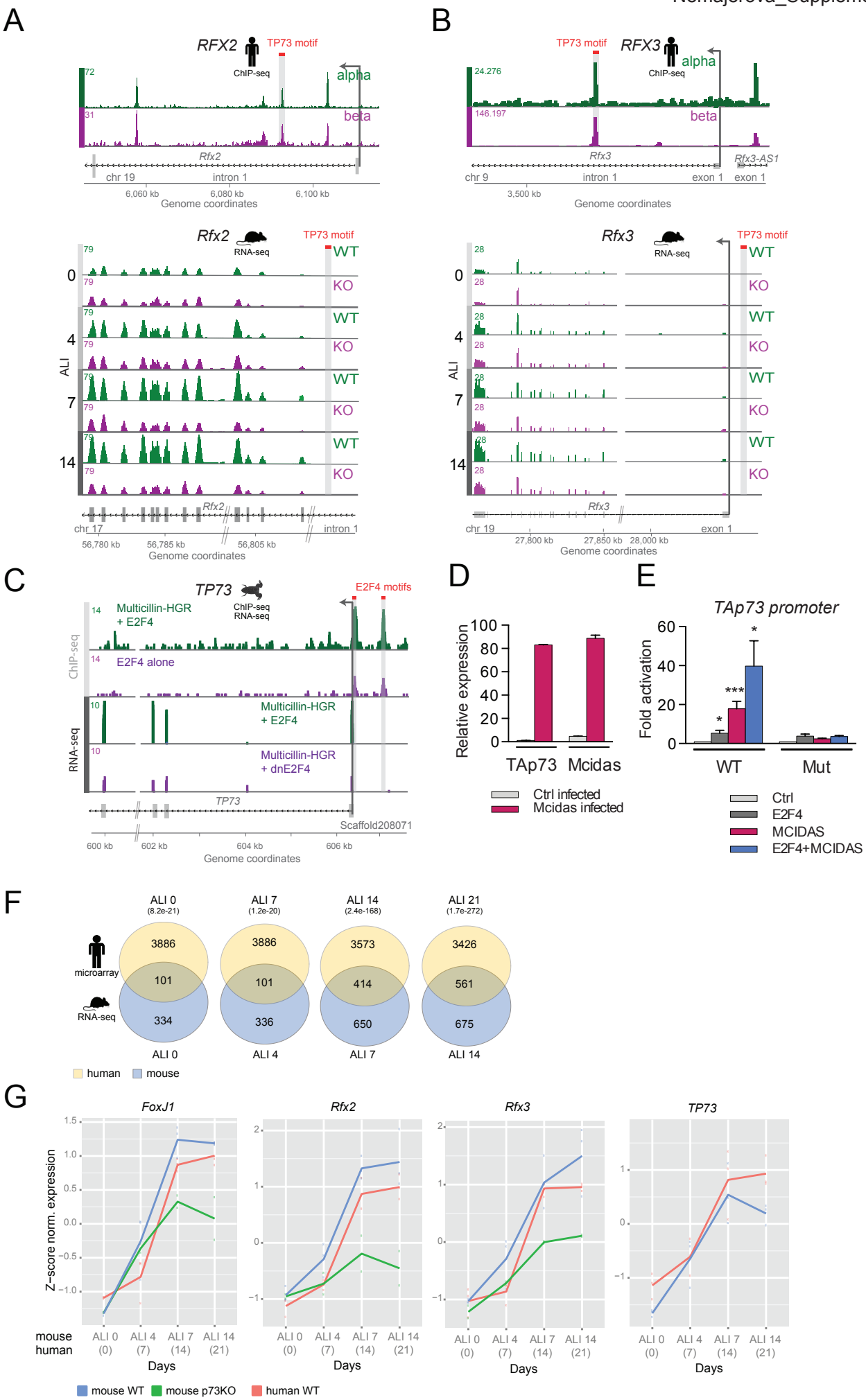
A
RFX2
TP73 motif
ChIP-seq
alpha
beta
Rfx2
chr 19
intron 1
6,060 kb
6,080 kb
6,100 kb
Genome coordinates
Rfx2
RNA-seq
TP73 motif
WT
KO
ALI
0
4
7
14
chr 17
intron 1
56,780 kb
56,785 kb
56,805 kb
Genome coordinates
B
TP73 motif
RFX3
ChIP-seq
alpha
beta
24.276
146.197
Rfx3
Rfx3-AS1
chr 9
intron 1
exon 1
3,500 kb
Genome coordinates
Rfx3
RNA-seq
TP73 motif
chr 19
exon 1
27,800 kb
27,850 kb
28,000 kb
Genome coordinates
C
TP73
ChIP-seq
RNA-seq
E2F4 motifs
Multicillin-HGR + E2F4
E2F4 alone
Multicillin-HGR + E2F4
Multicillin-HGR + dnE2F4
ChIP-seq
RNA-seq
TP73
Scaffold208071
600 kb
602 kb
604 kb
606 kb
Genome coordinates
D
Relative expression
TAp73
Mcidas
Ctrl infected
Mcidas infected
E
TAp73 promoter
Fold activation
WT
Mut
Ctrl
E2F4
MCIDAS
E2F4+MCIDAS
F
ALI 0
(8.2e-21)
ALI 7
(1.2e-20)
ALI 14
(2.4e-168)
ALI 21
(1.7e-272)
microarray
RNA-seq
3886
101
334
3886
101
336
3573
414
650
3426
561
675
ALI 0
ALI 4
ALI 7
ALI 14
human
mouse
G
FoxJ1
Rfx2
Rfx3
TP73
Z-score norm. expression
mouse
human
ALI 0 (0)
ALI 4 (7)
ALI 7 (14)
ALI 14 (21)
Days
mouse WT
mouse p73KO
human WT

Supplemental Figure S6 (related to Fig. 4)**. Differential expression and potential regulation of *Rfx2*, *Rfx3* and *TP73* during multiciliogenesis. The TP73-centered ciliogenesis network appears conserved between human and mouse.**

(**A**) TAp73 ChIP-seq genome tracks of the human *RFX2* locus (GSE15780) and RNA-seq tracks of the average gene expression of *Rfx2* in mouse. *Top* Merged ChIP-seq tracks of TAp73α (alpha, green) and TAp73β (beta, purple). TP73-binding motifs are highlighted in red and the transcriptional start site is marked with an arrow. *Bottom* Average *Rfx2* expression data for the different stages of murine MTEC differentiation (ALI D0, 4, 7, 14) for WT (green) and p73 KO (purple) samples. Predicted TP73-binding motifs in Intron 1 of the human and mouse *Rfx2* gene are highlighted in red (TP73 motif).

(**B**) TAp73 ChIP-seq genome tracks of the human *RFX3* locus (GSE15780) and RNA-seq tracks of the average gene expression of *Rfx3* in mouse. *Top* and *Bottom* panels as in (**A**). Of note, the predicted murine TP73 motifs lie upstream of the transcriptional start site in the promoter region of the *Rfx3* gene, and the TP73 motifs in human lie within the first intron of the gene, further supporting conserved TP73-dependent gene regulation between human and mouse.

(**C**) Genome tracks of the regulation of *TP73* gene expression, one of only 7 deregulated transcription factors, by transcriptional co-activator Multicilin (*aka* Mcidas) and E2F4 in *X. laevis* animal cups (GSE59309). *Top* ChIP-seq tracks display differential E2F4 binding (average reads) to *TP73* with co-expression (green, Multicilin-HGR + E2F4) *versus* without co-expression of Multicilin-HGR (purple, E2F4 alone). *Bottom* RNA-seq data tracks display the average expression of the *TP73* gene in *X. laevis* animal cups expressing Multicilin-HGR with E2F4 (green, Multicilin-HGR + E2F4) or without E2F4 (purple, Multicilin-HGR + dominant-negative dnE2F4).

Importantly, E2F4 can bind to the *TP73* gene only in the presence of Multicilin (Mcidas). Moreover, *TP73* gene expression is decreased by co-expression of a dominant negative form of E2F4. This data strongly suggests that the Multicilin-E2F4 transcription factor complex directly binds and regulates *TP73* gene expression during multiciliogenesis. Predicted E2F4-binding motifs in the *TP73* gene are highlighted in red (E2F4 motifs).

(**D**) WT MTECs at ALI D0 infected with lentiviral mouse Mcidas induce TAp73 expression. qRT-PCR for TAp73 and Mcidas from MTECs 72h after infection. Error bars represent standard deviation of the mean (SD) of 3 technical replicas.

(**E**) MCIDAS/E2F4-dependent direct responsiveness of the human TP73 gene. Luciferase reporter assays in Saos2 cells transfected with empty plasmid, human E2F4 alone, MCIDAS alone, or E2F4 and MCIDAS combined. 'WT' reporter contains the putative E2F4-binding motifs within the cognate TP73 DNA region. 'Mut' control reporter contains the same cognate DNA region but lack the E2F4-binding motifs. See also Supplemental Table S5 for DNA sequences. Data derived from 4 independent experiments. Error bars represent standard error of the mean (SEM).

(**F and G**) **Comparative analysis of murine and human airway differentiation.** (**F**) Venn diagrams comparing the total set of differentially expressed genes from WT *versus* p73KO mice for each of the four investigated time points during MTEC differentiation (blue circles, ALI D0, 4, 7, 14) with the total set of differentially expressed genes during regeneration of human airway mucociliary epithelium from proliferating HAECs (human airway epithelial cells) *versus* differentiating HAECS (yellow circles, GSE22142). Significantly enriched gene set overlap for each differentiation time point was calculated using a two-sided Fisher's exact test (p-values in

parenthesis). The highly significant overlap especially during ALI D7 and D14 suggests a core gene regulatory network during airway multiciliogenesis conserved between human and mouse. (**G**) Z-score normalized expression of *FOXJ1*, *RFX2*, *RFX3* and *TP73* during human mucociliary airway (red; ALI D0, 7, 14, 21) and murine wildtype MTEC (blue; ALI D0, 4, 7, 14) differentiation. Green lines indicate the expression of genes in murine MTEC cells that lack all functional *TP73* gene products (p73KO). The data nicely illustrates the highly conserved expression patterns of the core multiciliogenesis transcription factors *FoxJ1*, *Rfx2*, *Rfx3* in human and mouse and conversely, the strongly reduced induction of these factors in the absence of *TP73* expression.