



**Supplemental Figure S4** (related to Fig. 4). **p73-dependent gene expression during differentiation of airway epithelium in organotypic cultures**

**(A and B)** Principal component analysis (PCA) of murine RNA-seq and small RNA-seq data. PCA data of normalized log transformed gene counts of murine MTEC RNA-seq **(A)** and small RNA-seq **(B)** data for different time points (ALI D0, 4, 7 and 14) and conditions (p73KO and WT).

**(A)** RNA-seq samples show a clear separation between ALI D0 and ALI D4-14 on the x-axis (1<sup>st</sup> principal component), and a clear separation between the conditions KO vs. WT on the y-axis (2<sup>nd</sup> principal component).

**(B)** Small RNA-seq samples cluster into ALI D0 and ALI D4-14 on the x-axis (1<sup>st</sup> principal component). At early time points the separation into WT and KO conditions is not as pronounced as for the RNA-seq data, but becomes visible for the later differentiation time points (especially ALI D7-14) on the y-axis (2<sup>nd</sup> principal component).

Overall, the clustering into time points of differentiation and genotypes suggests good reproducibility and high quality of the data.

**(C and D)** Hierarchical clustering and barplots of differentially expressed genes (DEGs) from RNA-seq **(C)** and small RNA-seq **(D)** analyses. *Left panels*, Hierarchical clustering of differentially expressed genes comparing wildtype (WT green) to p73 knockout (KO blue) samples for four time points during MTEC differentiation (ALI D0, 4, 7, 14). Expression differences are shown as color-coded Z-scores (blue to red). In addition, the bottom line plot in panel **C** shows Z-score normalized WT expression values for *TP73* (blue), *FoxJ1* (red), *Mcidas*

(purple), *Rfx2* (olive), and *Rfx3* (cyan) in WT MTECs during differentiation stages at ALI D0, 4, 7 and 14. *Right panels*, Barplots for each DEG displaying the **maximum** log2 expression differences between WT and KO conditions. DE genes are shown in grey (DE), DE genes that contain a TP73 peak are displayed in yellow (& Peak), and DE genes that contain a TP73 peak and TP73-binding motif are shown in red (& Motif). A large proportion of differentially expressed genes (panel **C**) are bound and contain a TP73-binding motif (red lines), suggesting a direct regulation of target genes by TP73. Whereas the absence of *TP73* functionality during early differentiation stages is accompanied by upregulation of potential target genes (panel **C**, ALI D0 and D4), later stages of differentiation show prominent downregulation of genes (panel **C**, ALI D7 and D14). Small RNAs (**D**) are differentially expressed primarily during the later stages of MTEC differentiation, and fewer miRNA species show TP73 binding and motifs. The latter might simply be due to our current lack of knowledge about the genome coordinates of primary miRNAs and their promoters.