Expanded View Figures

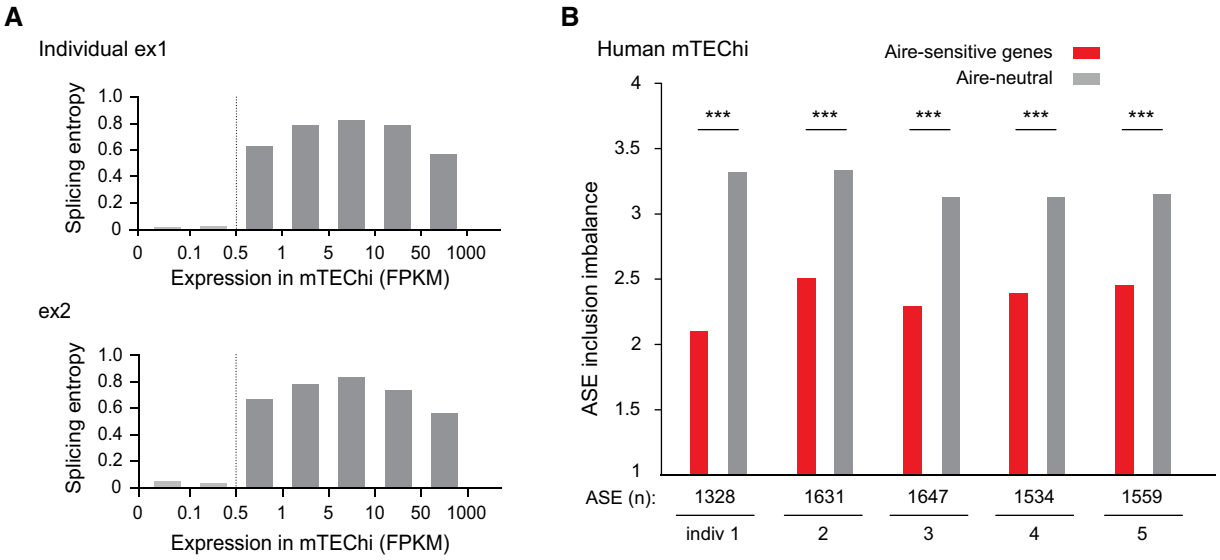

Figure EV1. Low levels of ASE inclusion imbalance for Aire-sensitive genes in human mTEChi.

A Median splicing entropy of genes binned according to their expression values. FPKM of 0.5 corresponds to the threshold over which the transcript isoform diversity can be accurately characterized in our RNA-seq dataset. Example of two individuals is shown.

B Levels of ASE inclusion imbalance for Aire-sensitive and neutral genes in mTEChi of five different individuals, *** $P < 10^{-4}$ (chi-squared test).

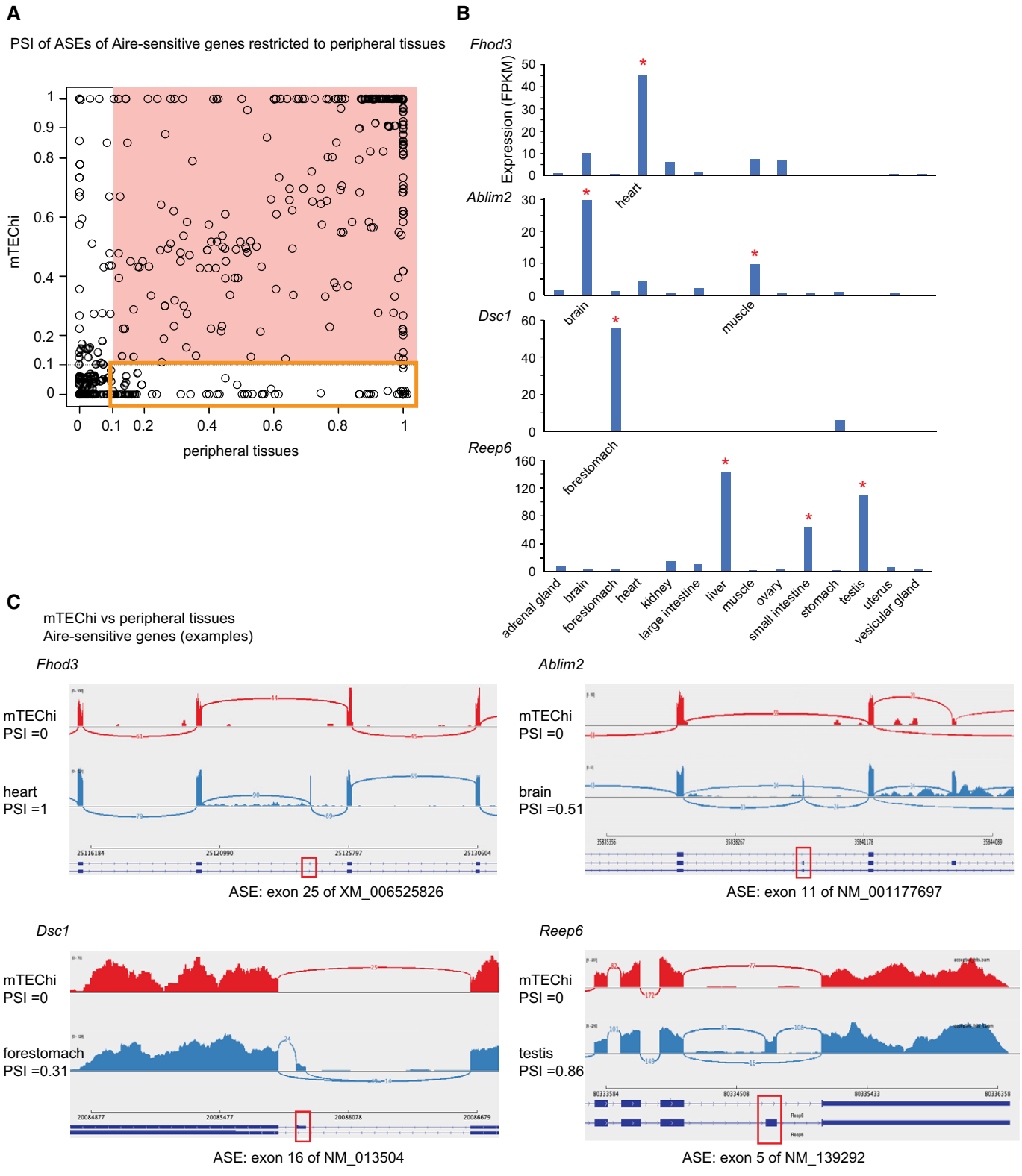

Figure EV2.

Figure EV2. PSI values of ASEs of Aire-sensitive genes in mTEChi and in their tissues of expression.

A Each ASE is represented by a circle. ASEs excluded in mTEChi or in the periphery (PSI < 0.1) are shown on a white background, otherwise on a salmon-colored background. The ASEs present in the periphery (PSI > 0.1) and excluded in mTEChi (PSI < 0.1) are framed by an orange square.

B Level of expression of *Fhod3*, *Ablim2*, *Dsc1*, and *Reep6* (taken as examples of Aire-sensitive genes with a specific or selective peripheral expression) from RNA-seq data of mTEChi and 14 mouse tissues. Asterisks indicate the tissue(s) of specific/selective expression: *Fhod3* is specific to the heart, *Dsc1* to the forestomach; *Ablim2* is selective to the brain and muscle, *Reep6* to the liver, small intestine, and testis.

C Shashimi plots of the above genes show ASE exclusion (PSI < 0.1) in mTEChi (red) and some level of inclusion (PSI > 0.1) in the tissues of expression (blue). Arcs representing splice junctions connect exons. Red boxes indicate alternative spliced exons present or absent in the transcript isoforms of *Fhod3*, *Ablim2*, *Dsc1*, and *Reep6*.

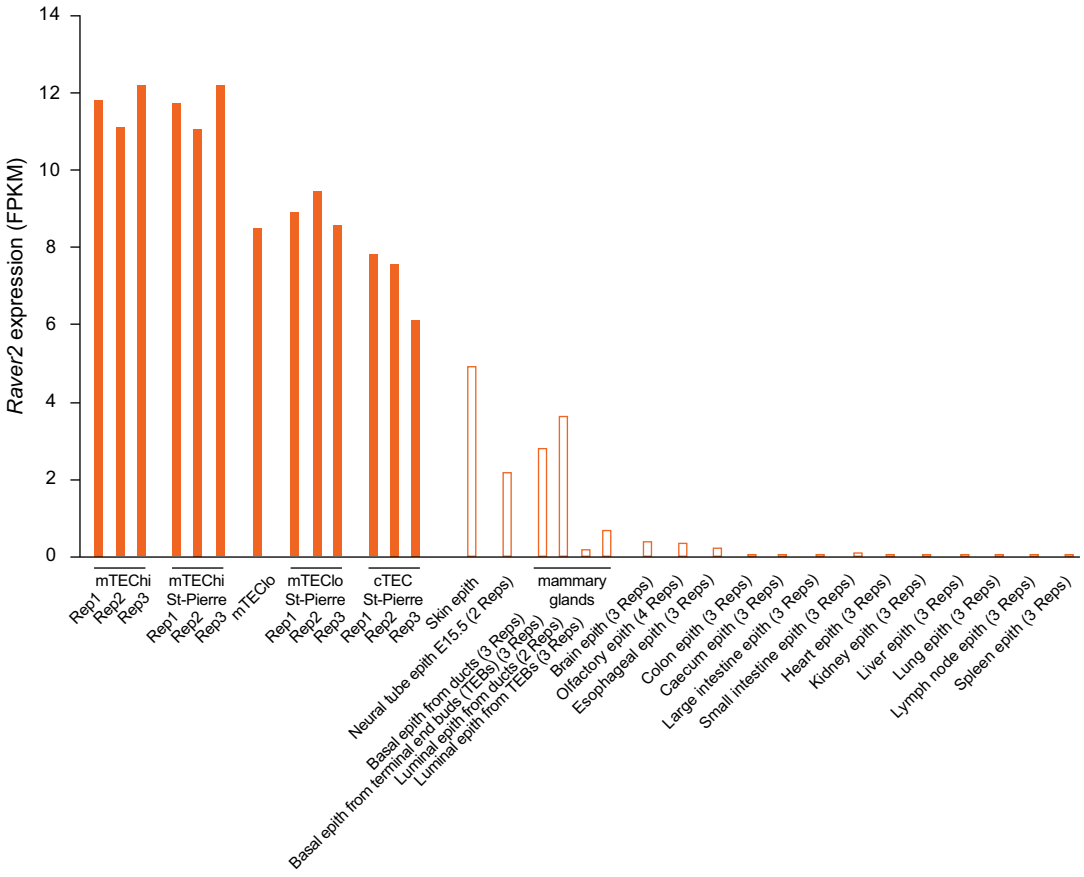

Figure EV3. Comparison of *Raver2* expression levels across TECs and epithelial cells from a variety of peripheral tissues.

Closed bars are for TECs (mTEChi, mTEClo, and cTECs) including our dataset and St-Pierre dataset for replication. Open bars correspond to *Raver2* expression calculated by re-analysis of public datasets of epithelial cells isolated from peripheral tissues.

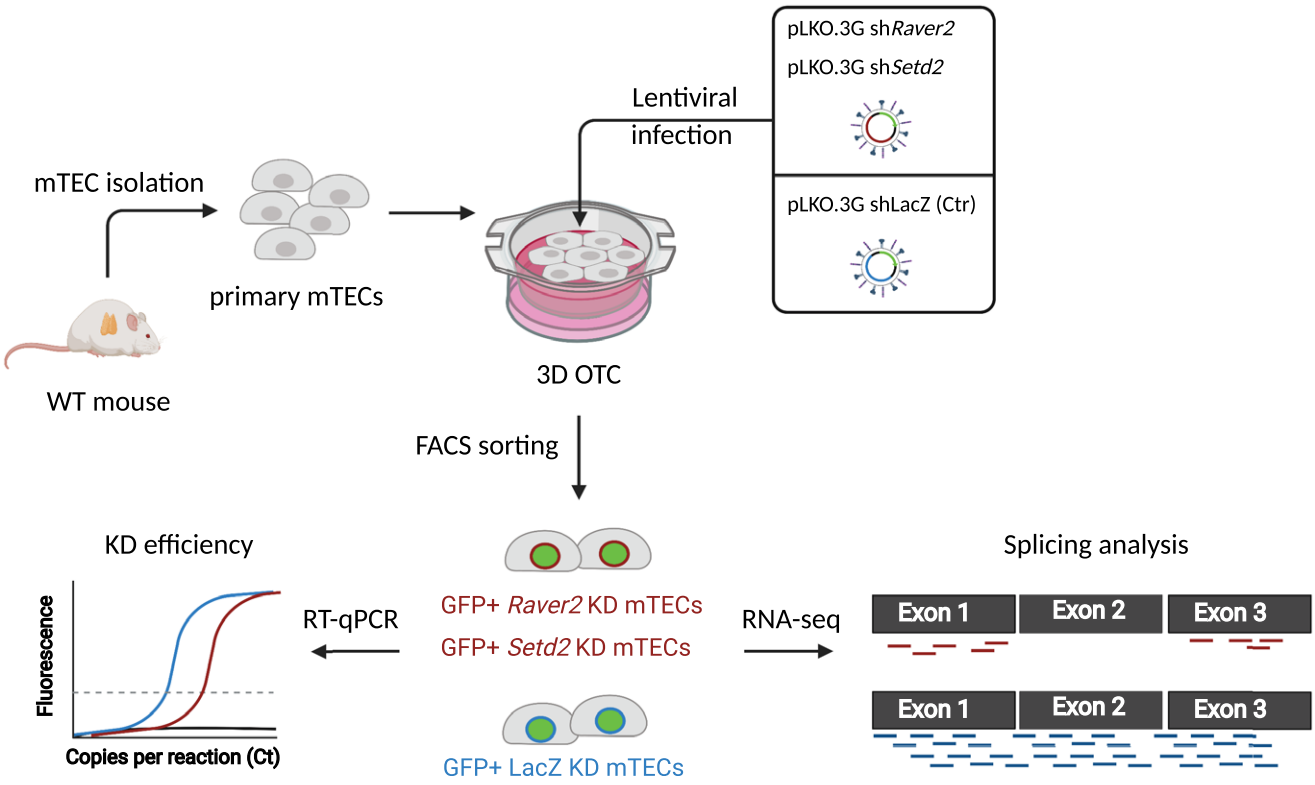

Figure EV4. Schematic of the knockdown strategy of primary mTECs *ex vivo*.

Primary mTECs were isolated from thymi of WT mice and seeded along with shRNA-containing lentiviruses on a 3D organotypic culture system (3D OTC). Three days or 5 days after infection, GFP^+ mTECs expressing the lentigenes were isolated for knockdown efficiency by qPCR or for splicing analyses by RNA-seq experiments. This figure was created with BioRender (biorender.com).

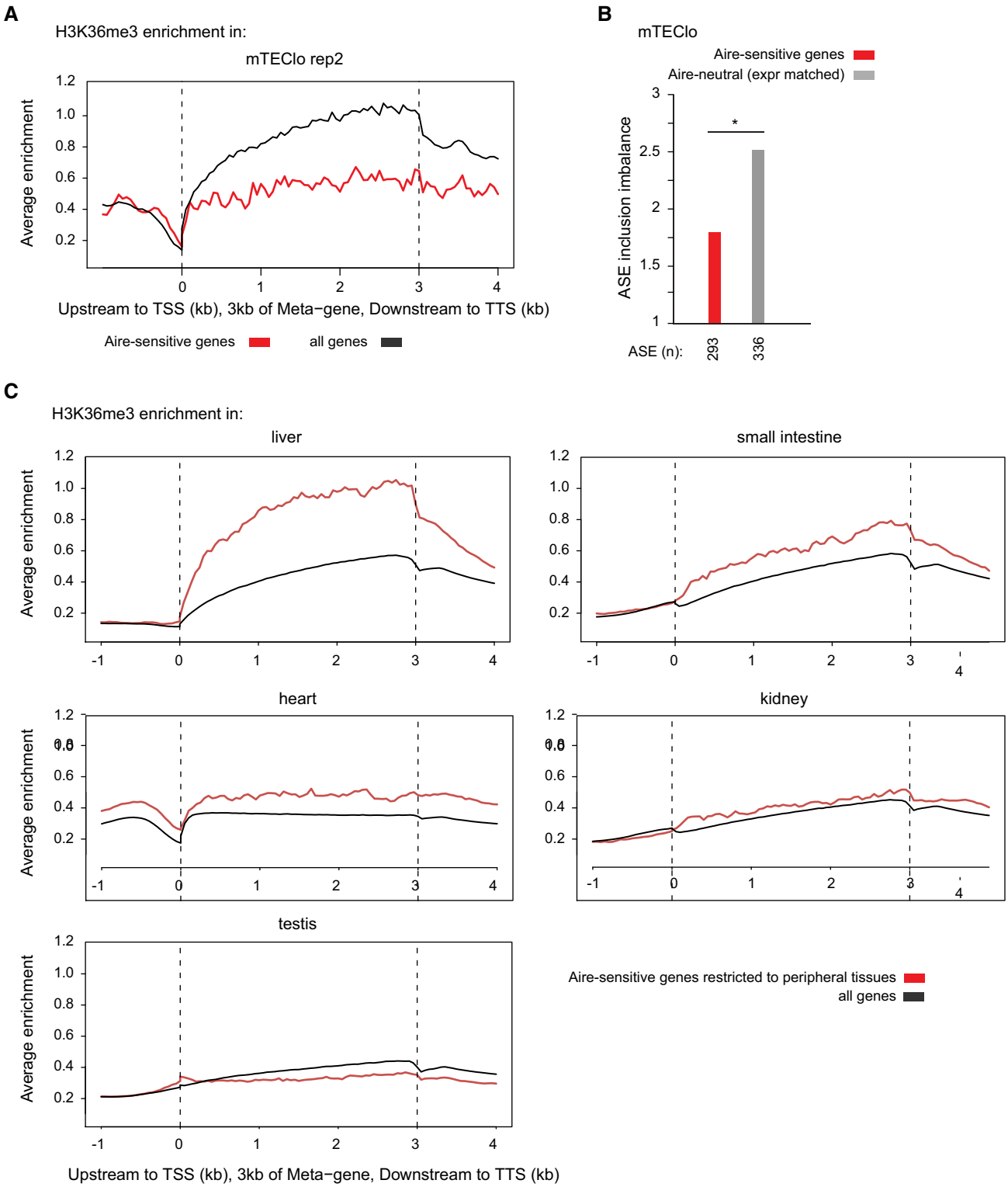

Figure EV5. Reduced H3K36me3 deposition at Aire-sensitive genes in mTEClo in comparison to their tissues of expression.

A Metagene profiles of the average normalized enrichment of H3K36me3 for Aire-sensitive genes (red) in mTEClo; all genes are shown in (black).

B Levels of ASE inclusion imbalance for Aire-sensitive genes and neutral genes (expression matched), *$P < 0.05$ (chi-squared test performed in one mTEClo dataset).

C Metagene profiles of the average normalized enrichment of H3K36me3 for Aire-sensitive genes (red) in their tissues of expression; all genes are shown in (black).