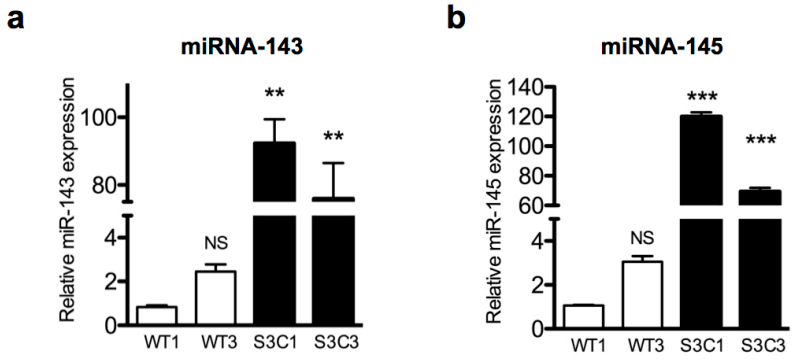
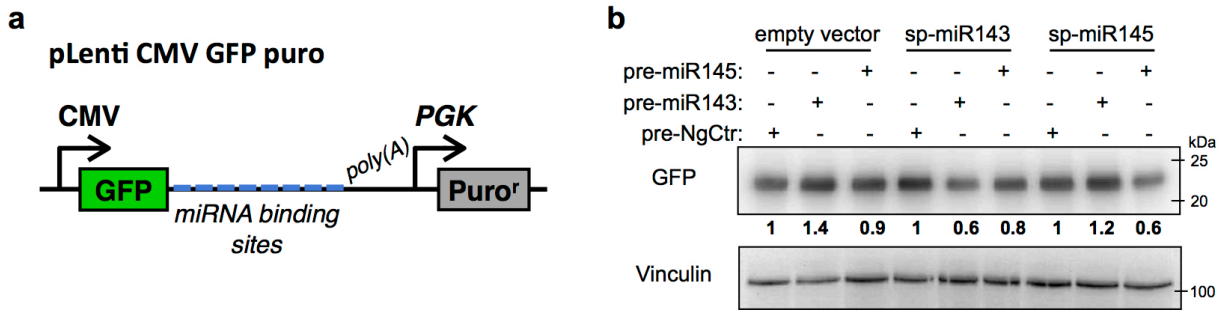
SUPPLEMENTARY INFORMATION

Supplementary Figure S1



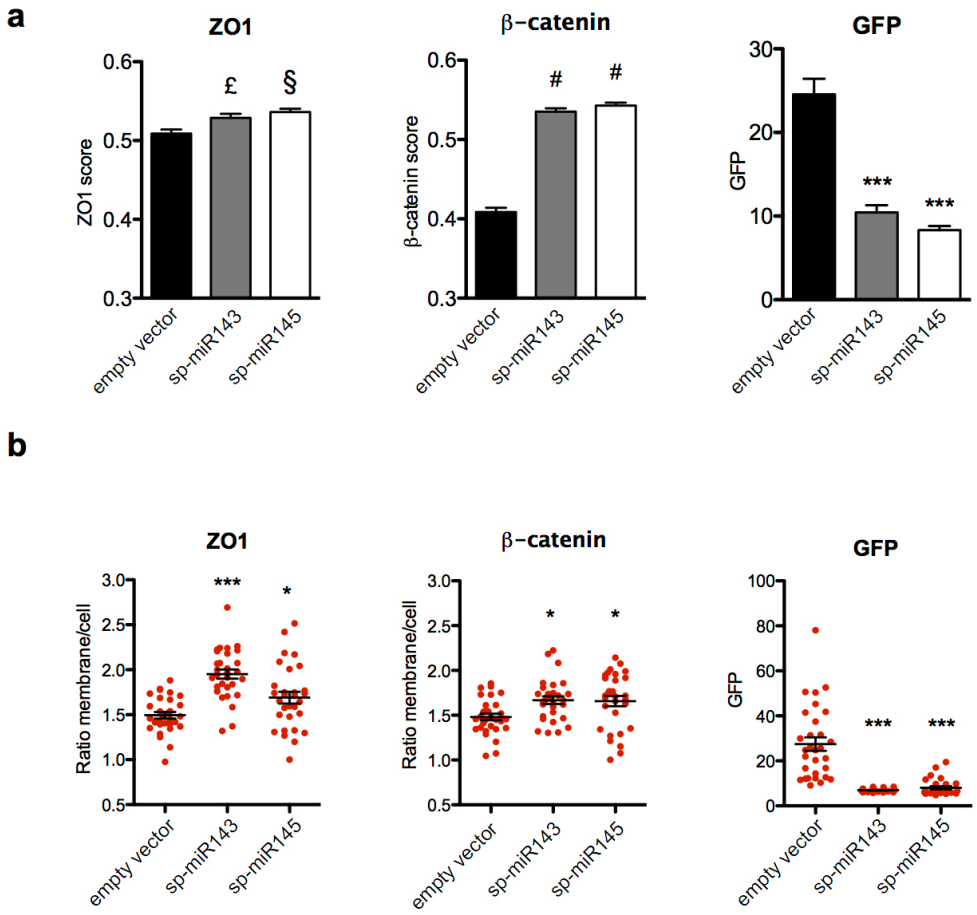
Supplementary Figure S1. miRs-143 and -145 expression in WT and S3C NeuT derived cell lines. (a,b) Expression of the indicated miRNA was measured by qRT-PCR in two independent cell lines derived from WT and S3C NeuT tumors (WT1, WT3, S3C1, S3C3). Data are mean ± S.E.M. of expression values relative to WT1 cells, n=3.

Supplementary Figure S2



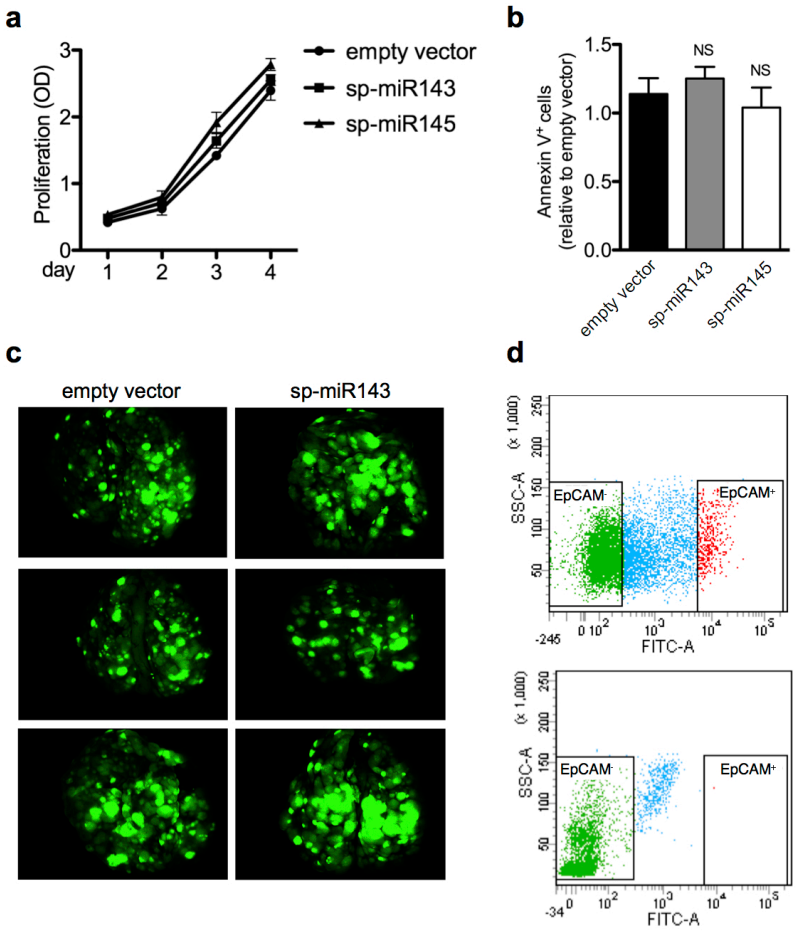
Supplementary Figure S2. Generation and testing of miRNAs sponge vectors. (a) Schematic representation of pLenti CMV GFP Puro construct carrying multimerized miR-143 or miR-145 binding sites in the 3'UTR of the GFP cassette. **(b)** HEK293 cells were transiently transfected with sponge vectors specific for miR-143 (sp-miR143), miR-145 (sp-miR145) or with the empty vector, together with the indicated miRNA precursors or with a negative control (pre-NgCtr). GFP expression levels were measured by Western blot on total cell lysates. Numbers indicate signal intensity, normalized for loading and relative to each pre-NgCtr condition.

Supplementary Figure S3



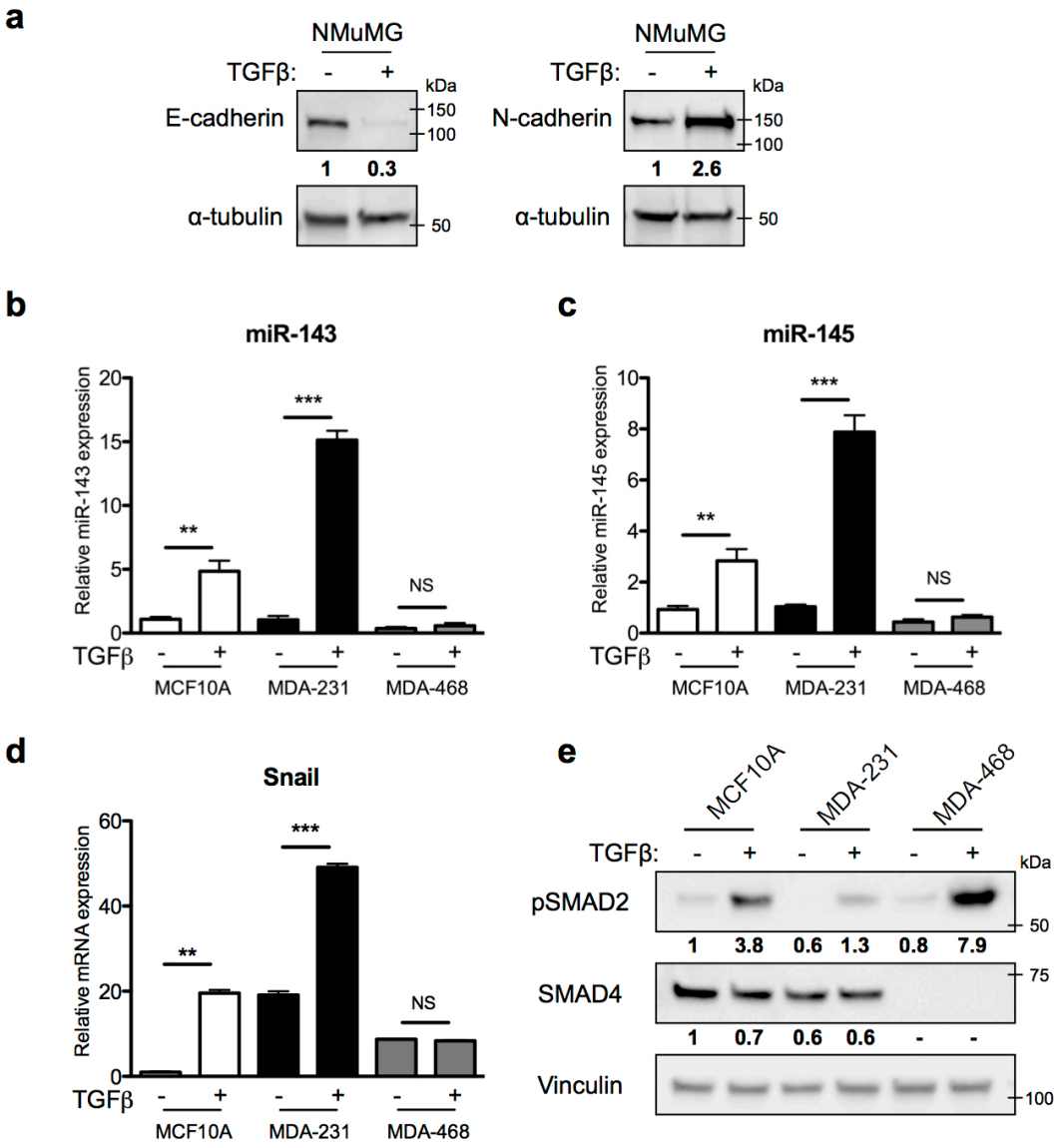
Supplementary Figure S3. Quantification of the distribution of ZO1 and β-catenin at the cell contacts. miR-143 and -145 sponged S3C cells were stained for ZO1 and β-catenin, followed by confocal analysis as described for main Figure 1c. Quantification was obtained by two different approaches based on either total image **(a)**, or single cell **(b)** analysis. **(a)** Total image approach. The score for ZO1 or β-catenin distribution was obtained considering regularly alternating DAPI and ZO1/β-catenin signal peaks across 79 horizontal equally spaced lines traced on the Z-stack projection, as described in the Material and Methods section. Results are shown as mean score ± S.E.M. of 79 lines across eight independent confocal images per each marker. P-values: £, $p<0.005957$; §, $p<3.406e^{-05}$; #, $p<2.2e^{-16}$; n=632. The total intensity of the GFP signal in the same images (mean ± S.E.M., n=8) is also shown. **(b)** Single cell approach. 30 single cells for each group were randomly chosen, and the mean intensity of the signal (ZO1 or β-catenin) was measured both along the membrane and in the whole cell. Shown is the ratio between the two values, representing the relative distribution of the signal, with a high ratio corresponding to higher membrane signal. Mean score ± S.E.M., n=30. The total intensity of the GFP signal in the same cells is also shown.

Supplementary Figure S4



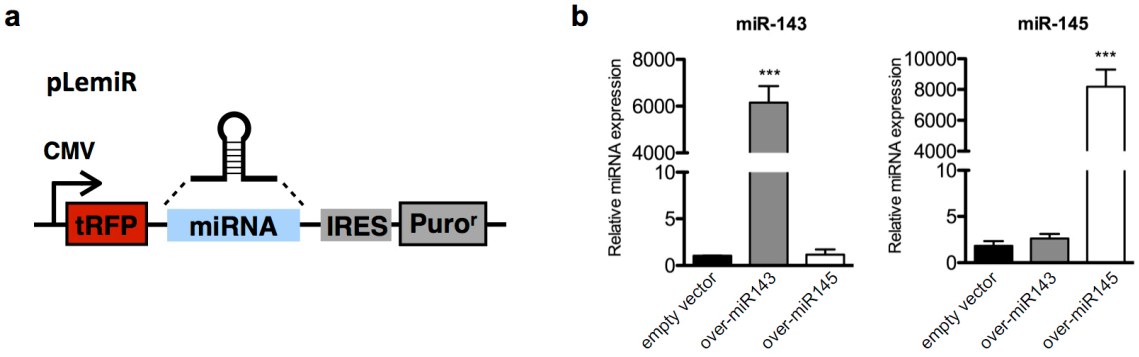
Supplementary Figure S4. Characterization of sponged S3C cells. (a) Proliferation of the indicated sponged or control S3C cells was assessed by measuring the Optical Density (OD) of Crystal violet-stained cells. Results are shown as mean OD ± S.E.M. of three independent experiments performed in triplicate. **(b)** Annexin V positive cells were measured by flow-cytometry upon 24h of plating on poly-HEMA treated dishes. Results are expressed as mean ± S.E.M. of Annexin V^+ cells relative to empty vector control cells, n=3. **(c)** lungs from mice injected i.v. with sp-miR143 or empty vector cells were collected after three weeks and analyzed for GFP expression in the metastases (freshly isolated whole lungs). **(d)** Representative scatter plot of sorted cells. Lungs from mice injected with empty vector S3C cells were enzymatically disaggregated three weeks after the injection. The resulting cells were stained with an EpCAM-FITC antibody and sorted in $EpCAM^+$ and $EpCAM^-$ fractions (upper panel). Note that almost no EpCAM+ cells were detected in control lungs from non injected mice (lower panel).

Supplementary Figure S5



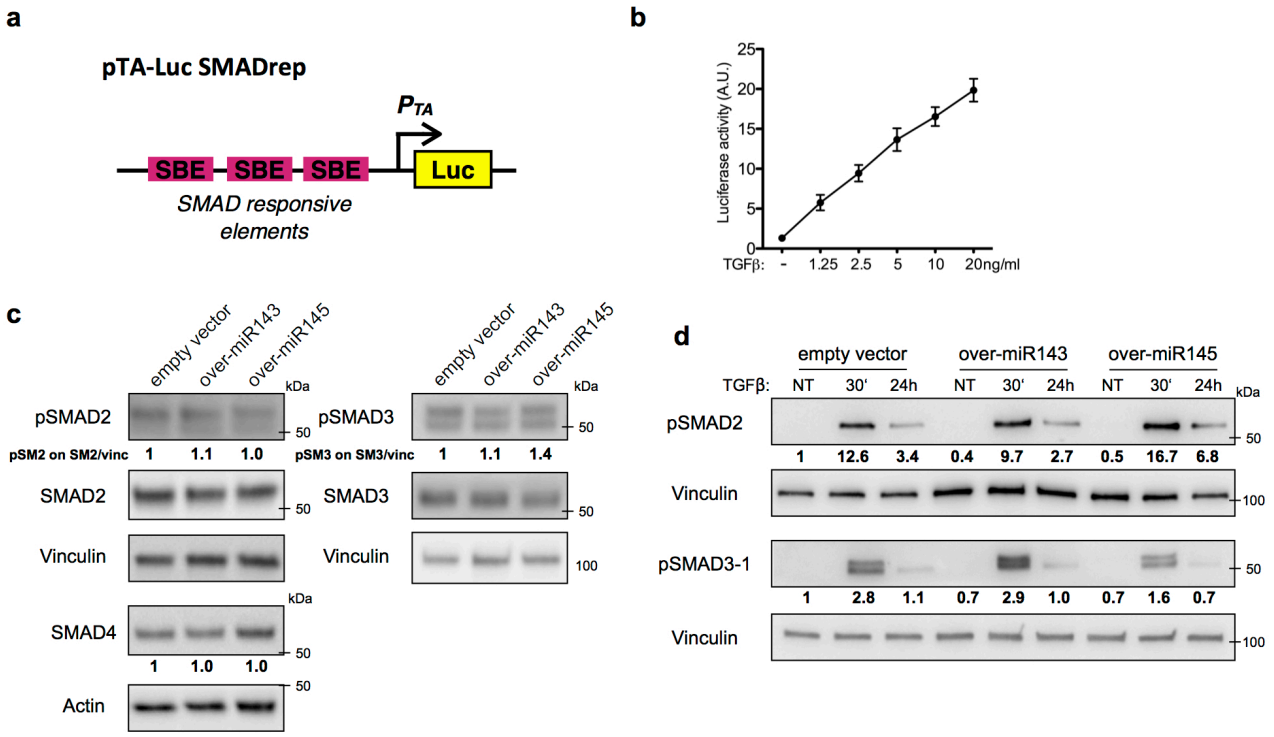
Supplementary Figure S5. TGF-β responsiveness and miRNAs regulation in multiple cell models. **(a)** NMuMG cells were treated or not with TGF-β for 5 days and analysed by Western blot for the expression of E-cadherin and N-cadherin. Numbers indicate signal intensity, normalized for loading and relative to the control sample. **(b-e)** The human mammary cell lines MCF10A, MDA-MB-231 (MDA-231) and MDA-MB-468 (MDA-468) were treated or not with TGF-β for 24 hours followed by analysis of miRNAs expression levels **(b, c)**, Snail mRNA expression **(d),** SMAD2 phosphorylation and SMAD4 expression levels **(e)**. Data are mean ± S.E.M. of expression values relative to MCF10A untreated cells, n=3.

Supplementary Figure S6



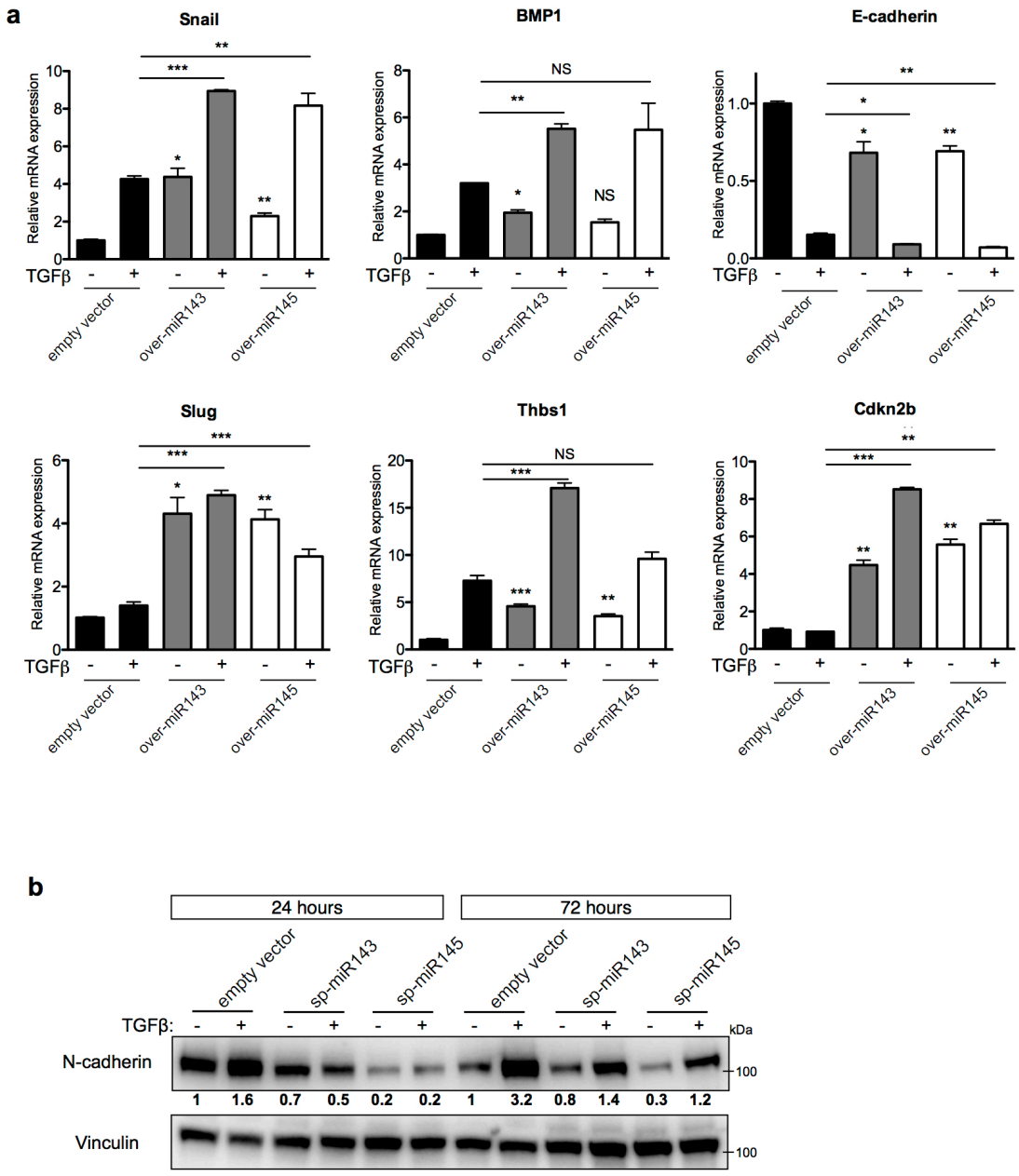
Supplementary Figure S6. Generation and testing of miRNAs overexpressing vectors. (a) Schematic representation of the lentiviral vector pLemir, engineered to express the precursor sequences of either miR-143 or miR-145. **(b)** Expression levels of the indicated miRNAs were evaluated by qRT-PCR in NMuMG cells transduced with the indicated overexpressing vectors or with the empty control vector. Results are shown as mean ± S.E.M. of expression values relative to empty vector control, n=3.

Supplementary Figure S7



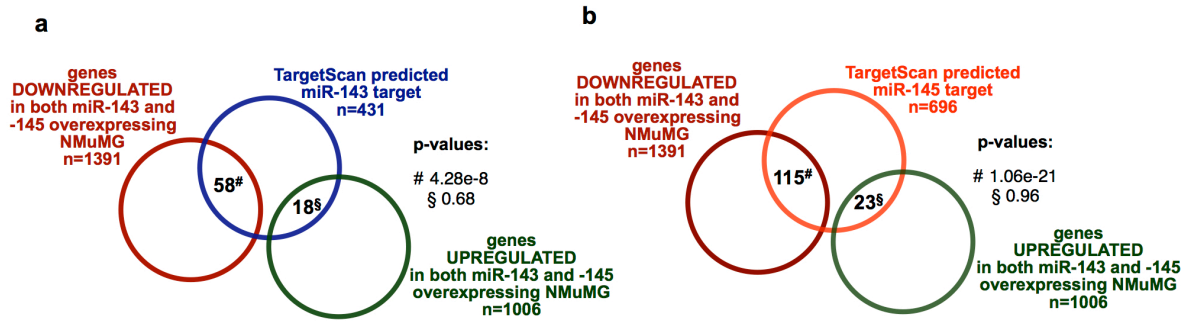
Supplementary Figure S7. Generation of the SMAD reporter vector and SMADs activity in NMuMG overexpressing cells. (a) To generate the SMAD-reporter vector, three SMAD binding elements (SBE) were inserted upstream of the minimal TK promoter in the pTA-Luciferase vector. **(b)** Luciferase activity of NMuMG cells transfected with the SMAD-reporter vector and stimulated for 24h with the indicated doses of TGF-β. Results are shown as mean ± S.E.M. of luciferase values after normalization to Renilla luciferase and relative to untreated cells. **(c,d)** Western blot analysis assessing phosphorylated and total protein levels of the indicated SMAD proteins in NMuMG overexpressing cells under basal conditions **(c)** or upon treatment with TGF-β for the indicated times **(d)**.

Supplementary Figure S8



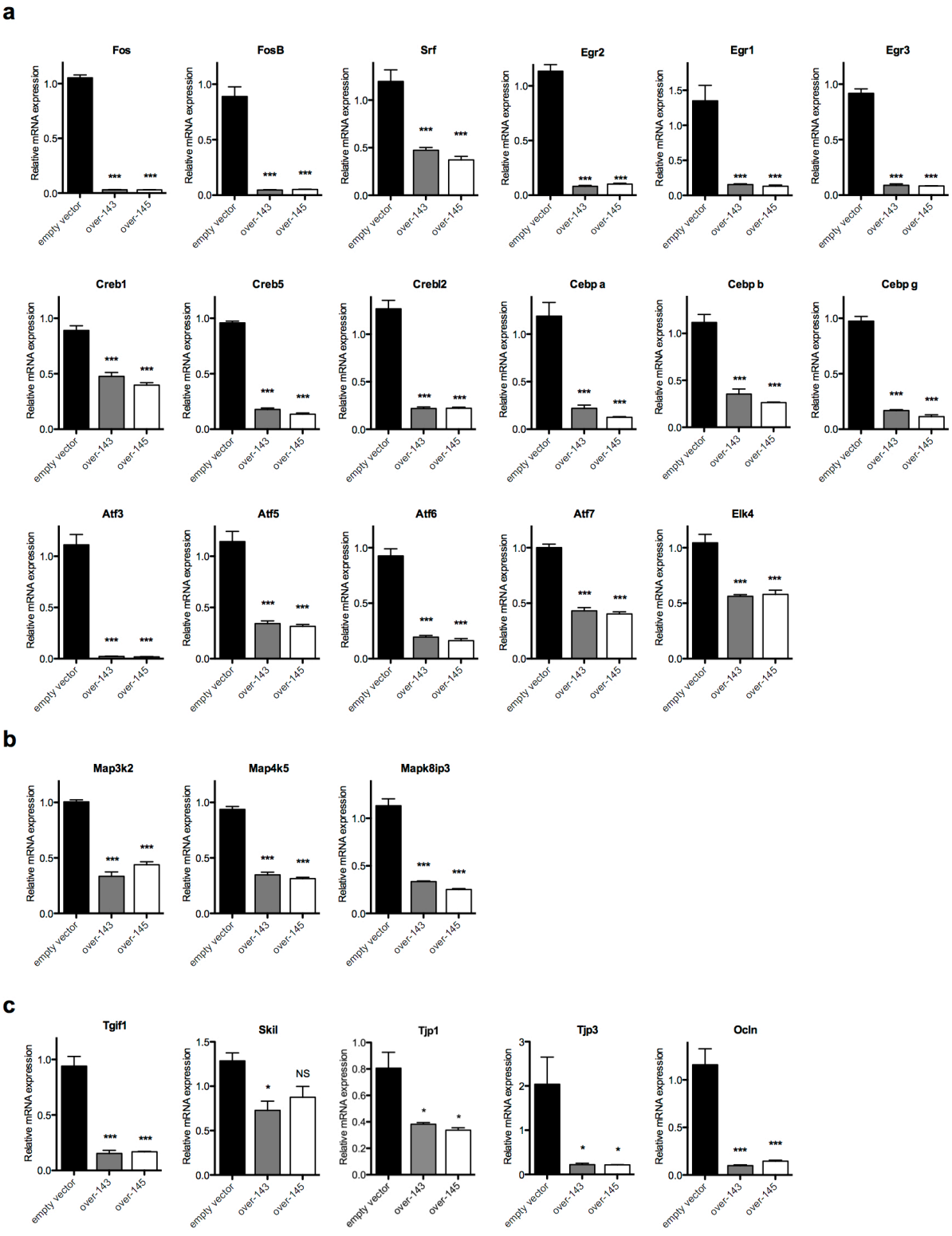
Supplementary Figure S8. Expression of TGF-β-regulated genes upon miRNAs modulation in NMuMG cells. (a) NMuMG cells overexpressing miR-143 or miR-145 were treated with TGF-β for 24 hours and analyzed for the expression of the indicated mRNAs (mean ± S.E.M. of expression values relative to the untreated empty vector control, n=3). **(b)** Sponged NMuMG cells were treated with TGF-β for the indicated times and analyzed for N-cadherin expression. Numbers indicate signal intensity, normalized for loading and relative to the specific untreated control sample.

Supplementary Figure S9



Supplementary Figure S9. TargetScan predicted miRNAs targets are significantly enriched in dowregulated mRNAs. (a, b) Venn diagrams representing the overlap between TargetScan predicted targets of miR-143 **(a)** or miR-145 **(b)**, and the genes commonly modulated by the overexpressed miRNAs in NMuMG cells. Note statistically significant enrichment only among downregulated genes.

Supplementary Figure S10



Supplementary Figure S10. qRT-PCR validation of RNA sequencing data. The expression of a subset of mRNAs identified as down-modulated by RNA sequencing was validated by qRT-PCR. The indicated mRNAs were measured from total RNA of the indicated NMuMG cells. The mRNA encoding for **(a)** Transcription factors, **(b)** MAP kinases, **(c)** TGFβ inhibitors and tight junction proteins were analyzed. Results are shown as mean ± S.E.M. of expression values relative to empty vector, after normalization to beta-actin RNA. *, $p<0.05$, **, $p<0.01$;***, $p<0.001$, n=4.