**Supplementary Appendix to**

**BMP7 Signaling in *TGFBR2*-deficient Stromal Cells Provokes Epithelial Carcinogenesis**

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**Supplementary Materials and Methods**

**Cytokine and growth factor array**

The Mouse Angiogenesis Antibody Array (R&D, #ARY015) was used according to the manufacturer’s protocol for the analysis of HGF and other potential growth factors in conditioned media from wt and *TGFBR2cKO* primary forestomach fibroblasts. Fibroblasts were starved with DMEM with 0.1% FBS overnight, before further starving with serum-free medium 24 hours prior to protein lysate harvest as described for western blots in main Materials and Methods section. For quantification, densitometry analysis was performed using Image J software. The integrated density was measured for each spot and a background measurement using the same selection was subtracted from the measured value. The average was calculated from measurements of both spots for each cytokine/growth factor. Lastly, the ratio of the TGFBR2cKO average intensities to the wt average intensities was calculated.

**RT-PCR analyses**

RT-PCR analyses were performed as described in the main Materials and Methods section. The following primer sets were used in addition for supplementary figures:

CCL5 forward: TCACCATCATCCTCACTGCAGC

CCL5 reverse: TCTCTGGGTTGGCACACACTTG

CTGF forward: ATGTCAGTGCGCAGCCGAAGCAG

CTGF reverse: AGTCTGCAGAAGGTATTGTCATTG

EGF forward: AAGCAAGGCGATTTGGATAGCC

EGF reverse: TTCGCAGTACTTCCGGTCTCGG

FGF2 forward: ACTACAACTCCAAGCAGAAGAGAG

FGF2 reverse: TCAGCTCTTAGCAGACATTGGAAG

FGF10 forward: ATGTGGAAATGGATACTGACAC

FGF10 reverse: TCATGGCTAAGTAATAGTTGCTG

GM-CSF forward: ATTGTGGTCTACAGCCTCTCAGC

GM-CSF reverse: AGGTGGTAACTTGTGTTTCACAGTC

HB-EGF forward: ACTGGATCCACAAACCAGCTGC

HB-EGF reverse: TGAGAAGTCCCACGATGACAAG

IGF1 forward: AGATACACATCATGTCGTCTTCAC

IGF1 reverse: TTCTGAGTCTTGGGCATGTCAG

IGF2 forward: AAGTCGATGTTGGTGCTTCTC

IGF2 reverse: TCACTGATGGTTGCTAGACATC

IL-6 forward: ACTGATGCTGGTGACAACCACG

IL-6 reverse: AGCTTATCTGTTAGGAGAGC

KGF forward: ATACTGACACGGATCCTGCCAAC

KGF reverse: TGCATAGAGTTTCCCTTCCTTG

NGF forward: TGGATGGCATGCTGGACCCAAGC

NGF reverse: ATGAACCTCCAGGCAGCCTGC

SDF1α forward: AGTCAGCCTGAGCTACCGATG

SDF1α reverse: TAAAGCTTTCTCCAGGTACTC

SFRP1 forward: AGCGAGTACGACTACGTGAGC

SFRP1 reverse: ACCGTTCTTCAGGAACAGCACAAG

TNC forward: TGTCCCCTCCCAAAGACCTTATTG

TNC reverse: TCGGGTGTTGTTTTTCACAATGTGC

TGFα forward: ACAGCTCGCTCTGCTAGCGCTG

TGFα reverse: TTCTCATGTCTGCAGACGAGG

TGFβ1 forward: ACTCTCCACCTGCAAGACCATCGAC

TGFβ1 reverse: TGGTAGAGTTCCACATGTTGCTCC

Wnt1 forward: TCTACTACGTTGCTACTGGCAC

Wnt1 reverse: TTGCACTCTTGGCGCATCTCAGAG

Wnt3 forward: TCCTCGCTGGCTACCCAATTTG

Wnt3 reverse: GTGCTTGTTCATAGCTGAGCGG

**Supplementary figure legends**

**Supplementary figure 1. (A)** Immunolabeling for phosphorylated p63 (p-p63), CK5, CK20 and Ki67 in forestomach sections of approximately 6-week-old mice with the listed genotypes. DAB (brown) was used as color substrate for the immunohistochemistry, nuclear hematoxylin counterstaining was omitted for p-p63 and Ki67. S: stroma. Arrowhead points to a Ki67+ stromal cell. Scale bar: 20 μm. **(B)** Visualization of FSP1-GFP and αSMA-RFP fluorescent gene product in sections of the listed tissues of FSP1-GFP;αSMA-RFP double transgenic mice. L: lumen, E: epithelium, S: stroma, M: smooth muscle, dotted line: outlines the boundaries of the esophageal lumen. DAPI (blue): nuclei. Scale bar: 100 μm. Arrows point to FSP1-GFP+ stroma. **(C)** Immunolabeling for FSP1 and CD45 in sections of the forestomach of approximately 6-week-old mice with the listed genotypes. White arrow: co-localization of FSP1 and CD45. \* depicts CD45+ cells within a vessel. S: stroma. E: epithelium. Scale bar: 50 μm. **(D)** H&E staining of a section of the forestomach of a Tgfbr2floxE2; αSMA-Cre mouse. Scale bar: 50 μm. S: stroma. E: epithelium. L: lumen in the forestomach. (**E**) Western blots analyses for HGF protein level in 2-week-old wt and *TGFBR2cKO* forestomach whole tissue lysates, 30 μg of protein loaded per lane, each lane represents a distinct mouse. (**F**) Densitometry analyses of western blots shown in Figure 2E. (**G**) Densitometry analyses of western blots shown in Figure 2G.

**Supplementary figure 2.** Individual channels microscopy imaging for the data presented in Figure 2F. Immunolabeling for FSP1 and Ki67 in sections of the forestomach of approximately 6-week-old mice with the listed genotypes. L: lumen. S: stroma. E: epithelium (for TGFBR2cKO: squamous cell carcinoma). Scale bar: 60 μm.

**Supplementary figure 3.** **(A)** RT-PCR electrophoretic product for the listed genes in the forestomach fibroblasts from approximately 6-week-old mice with the listed genotypes. Buffer: no template control. (**B-C**) Mouse angiogenesis antibody array and upregulated (red) or downregulated (green) growth factors/cytokines from the conditioned media of fibroblasts isolated from approximately 6-week-old mice with the listed genotypes. The graph (C) depicts the relative densitometry intensity ratio (*TGFBR2cKO* over wt); wherein a value of 1 depicts no change, a value > 1 shows increase in the cytokine (see also red arrows), and a value < 1 shows decrease in the cytokine (see also green arrow). **(D)** Densitometry analyses of western blots shown in Figure 3A.

**Supplementary figure 4.** **(A)** RT-PCR analyses for HGF in wt and *TGFBR2cKO* forestomach fibroblasts with or without Activin A (50 ng.ml-1). **(B)** Densitometry analyses of western blots shown in Figure 3D. **(C)** Immunolabeling for ALK6, ALK2 and ALK3 in sections of the forestomach of a *TGFBR2cKO* mouse. DAB (brown) was used as color substrate for the immunohistochemistry. Scale bar: 100 μm. **(D)** Western blots and densitometry analyses of wt, *TGFBR2cKO*, and *TGFBR2/SMAD4cKO* forestomach fibroblast lysates for the listed proteins, 30 μg of protein loaded per lane, each lane represents a distinct mouse. Cells were exposed to BMP7 (100 ng.ml-1) for 0, 24 or 48 hours prior to protein harvest. One-way ANOVA comparison to the control group (DMEM with 0.1% FBS). The data is presented as the mean ± SEM. See accompanying source data. **(E)** Densitometry analyses of western blots shown in Figure 4C.

**Supplementary figure 5.** Uncropped western blots