

Online supplement to:

Transglutaminase 2: a new player in bronchopulmonary dysplasia?

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This online supplement consists of one table, six figures, and the associated six figure legends:

Table S1. Primers employed in this study (by convention, mouse genes are indicated in lower case, and human genes in upper case).

Gene	Forward primer	Reverse primer
Mouse primers		
<i>tgm1</i>	5'-ACACCCCAGGAGACCAGCAGT-3'	5'-ATCATCCTGCCGCTGCCAGT-3'
<i>tgm2</i>	5'-GGCCGTGATGACCGGGAGGA-3'	5'-GATGCGCATGGCCACCCCTG-3'
<i>tgm3</i>	5'-GCACCAAGGCAGTAGGCAGC-3'	5'-TGTTGCGCCGAAAGATGCGT-3'
<i>tgm4</i>	5'-TGGAGACTGCCAGCATCGGC-3'	5'-ACCAGAAGCCTTCGCCATGACC-3'
<i>tgm5</i>	5'-TCAGCACCAAGAGCATCCAGAGTGA-3'	5'-GCTTGGTGGAGGCCCTTGAGACC-3'
<i>tgm6</i>	5'-ACCAGGAGGACCCAGCCACC-3'	5'-TTGCCCTGCCACTGGCCTTG-3'
<i>tgm7</i>	5'-AGGGTCAGATGAGCGCCTGGA-3'	5'-GCGTTCCTTCTCGGTTCCAGCA-3'
<i>hprt</i>	5'-GATGATCTCTCAACTTTA-3'	5'-AGTCTGGCCTGTATCCAA-3'
<i>18S rRNA</i>	5'-AGGGGAGAGCGGGTAAGAGA-3'	5'-GGACAGGACTAGGCGBAACA-3'
Human primers		
<i>TGM1</i>	5'-GCACCACACAGACGAGTATGA-3'	5'-GGTGTATGCGATCAGAGGATTC-3'
<i>TGM2</i>	5'-GAGGAGCTGGTCTTAGAGAGG-3'	5'-CGGTCACGACACTGAAGGTG-3'
<i>TGM3</i>	5'-ATGGCTGCTCTAGGAGTCCAG-3'	5'-GTTTTGGCCTCTCCGCAAGAT-3'
<i>TGM4</i>	5'-TGAATCAGGACAACGCCGTTT-3'	5'-GTGGTAGGATTGTAGGGGCTG-3'
<i>TGM5</i>	5'-AGCTGCTAGACAAGAGCCTG-3'	5'-CCACTCTGCTGACGTAGACG-3'
<i>TGM6</i>	5'-TCAGGCTTTCCTCTCACCG-3'	5'-CTGAGCACGTACTCCTGTCTC-3'
<i>TGM7</i>	5'-TTGCGGCTTGAGTCTGTGCG-3'	5'-GTGATGTGGTCTGTTCTGGGAC-3'
<i>HPRT</i>	5'-AAGGACCCACGAAGTGTG-3'	5'-GGCTTTGTATTTTGCTTTTCCA-3'

FIGURE S1. Densitometric analysis of immunoblots of Tgm1 and Tgm2 in developing mouse lungs. The pixel values were assessed for the a) single Tgm1 band, or b) for the two Tgm2 bands, and normalized for the pixel values for the α -tubulin band, to create relative values. The relative pixel values for three independent experiments ($n = 3$, per group) were combined and averaged, and the data reflect the mean \pm S.D. All associations were tested for statistical significance by assessment of p values (indicated above the horizontal line), which were calculated by one-way ANOVA with Tukey's *post hoc* test.

FIGURE S2. Provision of the raw C_t values for mouse real-time RT-PCR analyses. The C_t values are illustrated for the *Tgm1*, *Tgm2*, and *Tgm3* genes, using the primers listed in table S1. Each point represents the mean value of two (duplicate) C_t values for a single animal. *Author note:* this is a response to a reviewer request.

FIGURE S3. Regulation of transglutaminase mRNA levels by TGF- β_1 in the human A549 cell-line. The influence of TGF- β_1 on transglutaminase gene expression was assessed in the human A549 lung epithelial cell-line. The *HPRT* gene was employed as a reference gene. Primer sequences are provided in Table 2. The numbers below the brackets indicate the p values, which were assessed by unpaired Student's *t*-test ($n = 3$; per group), and compare vehicle- *versus* TGF- β_1 -treated groups. Only p values <0.05 are indicated. Note: data for *TGM1* and *TGM2* can be found in fig. 4a and fig. 4e in the manuscript proper.

FIGURE S4. Regulation of transglutaminase mRNA levels by TGF- β_1 in primary human lung vascular endothelial cells and primary human pulmonary artery smooth muscle cells. The influence of TGF- β_1 on transglutaminase gene expression was assessed by real-time RT-PCR using the primer sequences provided in Table 2, with the *HPRT* gene serving as reference. All associations were tested for statistical significance by assessment of p values, which were calculated by unpaired Student's *t*-test ($n = 3$; per group), and which compared vehicle- *versus* TGF- β_1 -treated groups. The numbers below the brackets indicate the p values, which were assessed by unpaired Student's *t*-test ($n = 3$; per group), and compare vehicle- *versus* TGF- β_1 -treated groups. Only p values <0.05 are indicated.

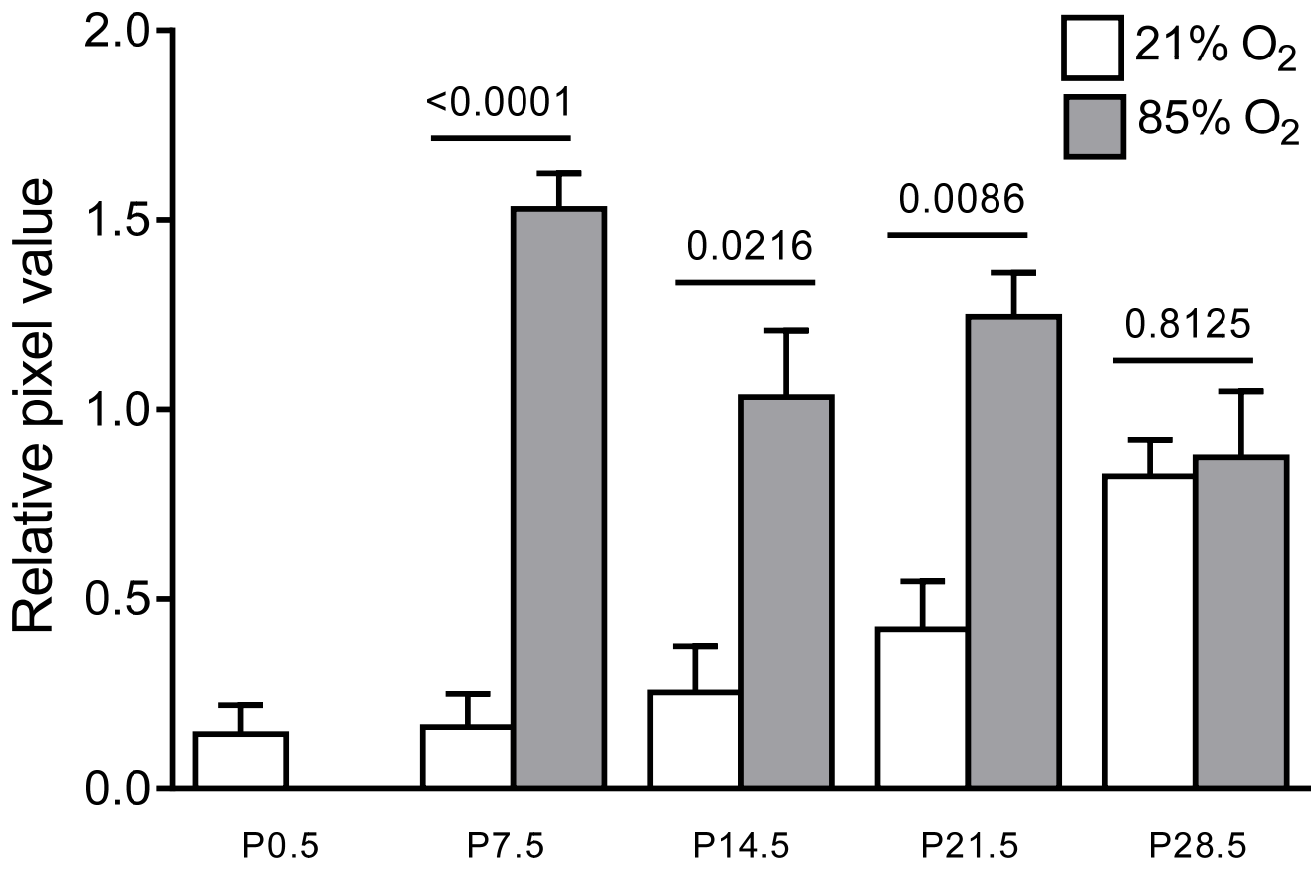
FIGURE S5. Regulation of transglutaminase mRNA levels by TGF- β_1 in mouse primary alveolar type II cells and mouse primary lung fibroblasts. The influence of TGF- β_1 on

transglutaminase gene expression was assessed by real-time RT-PCR using the primer sequences provided in Table 2, with the *hprt* gene serving as reference. All associations were tested for statistical significance by assessment of p values, which were calculated by unpaired Student's *t*-test ($n = 3$; per group), and which compared vehicle- *versus* TGF- β_1 -treated groups. No statistical associations with a p value below 0.05 were detected, and therefore, no p values are indicated. Note: data for *tgm1* and *tgm2* can be found in Figure 4b and Figure 4f for alveolar type II cells, and in fig. 4d and fig. 4h for mouse fibroblasts, in the manuscript proper.

FIGURE S6. Assessment of Tgm2 localisation in the lungs of mice exposed to a) and b) 21% O₂ or c) and d) 85% O₂ that received either a) and c) control antibody (MOPC21) or b) and d) anti-TGF- β neutralising antibody (1D11), sacrificed at day P10.5. The patterns documented are representative of the patterns observed in at least two other series'.

Figure S1

a) Tgm1



b) Tgm2

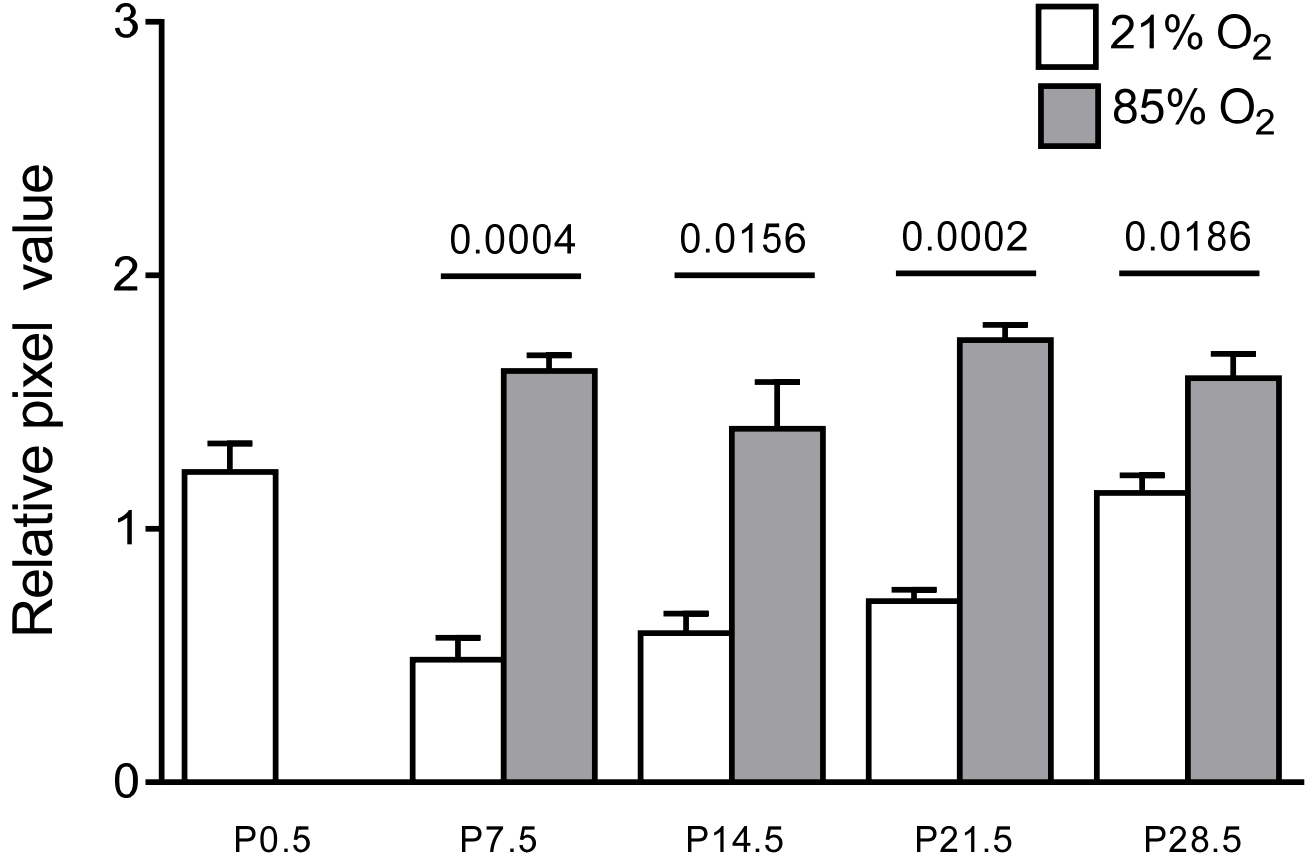


Figure S2

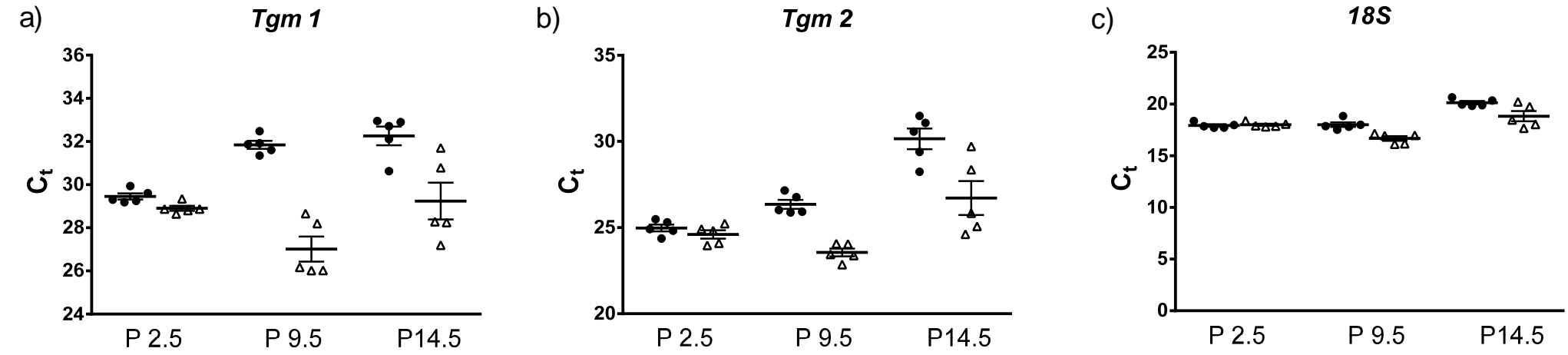


Figure S3

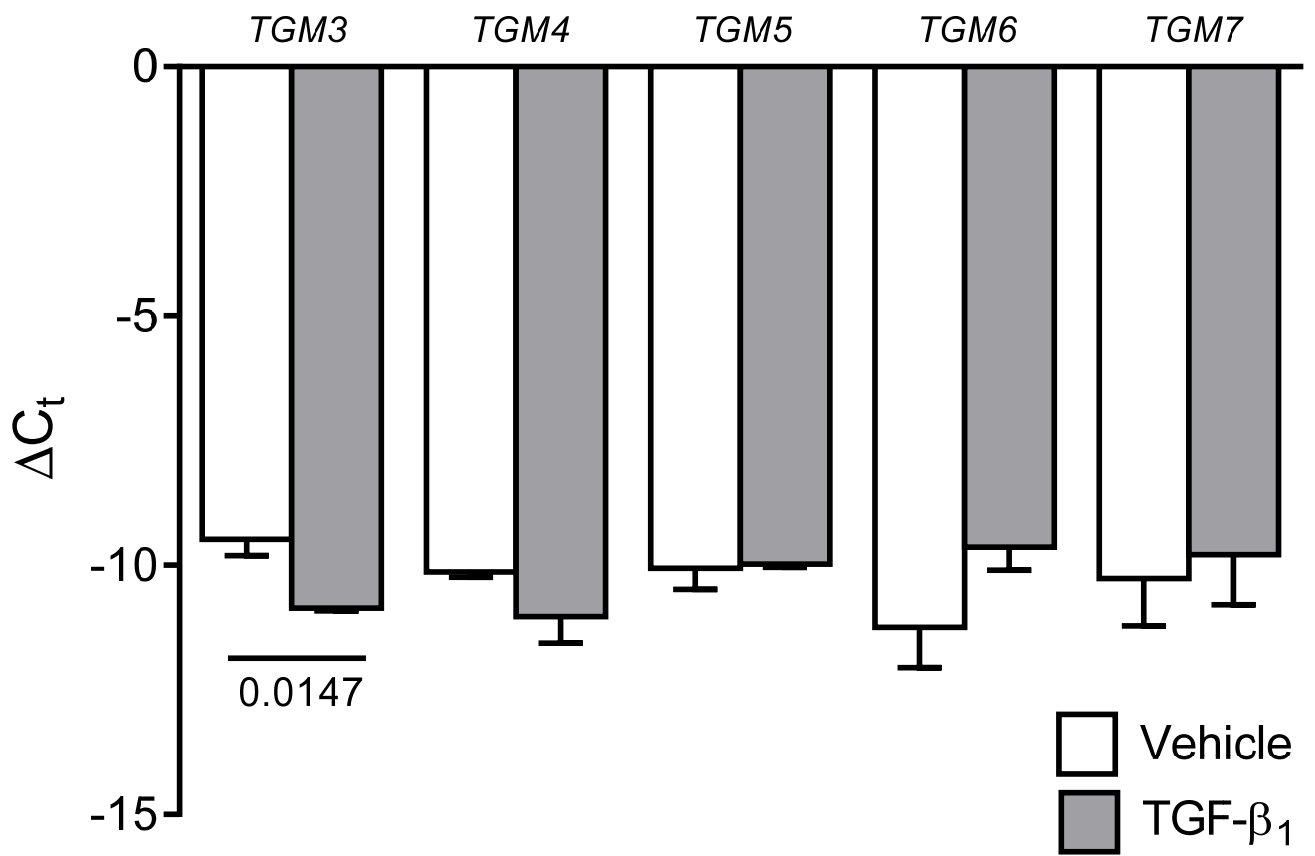
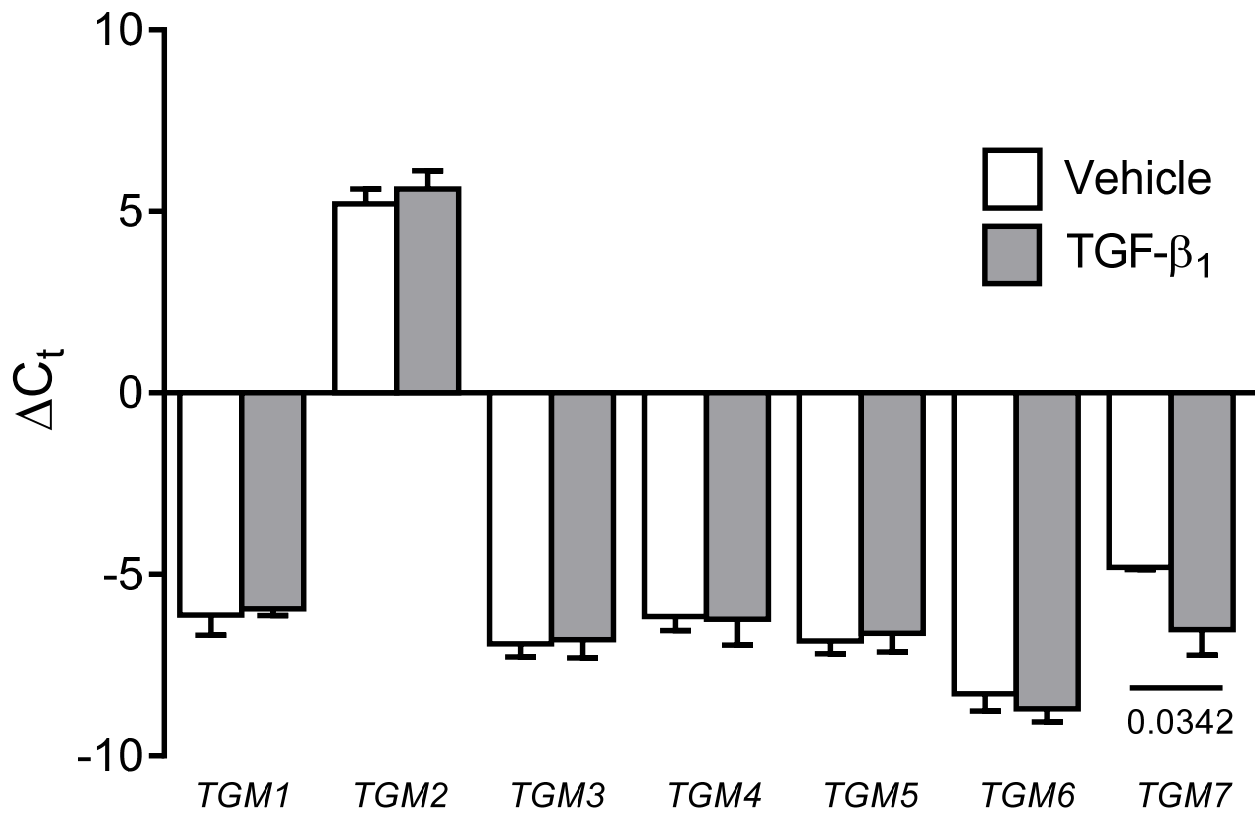


Figure S4

a) primary human lung vascular endothelial cells



b) primary human pulmonary artery smooth muscle cells

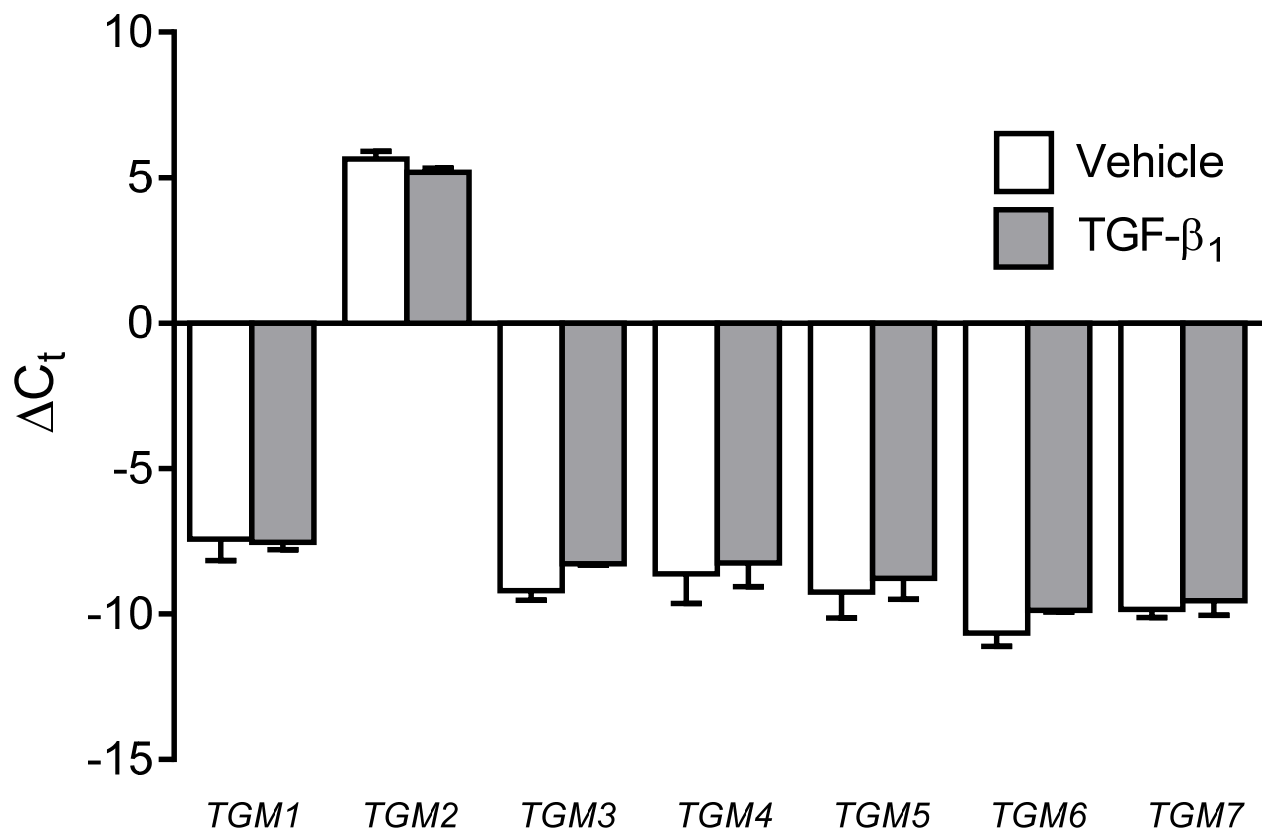
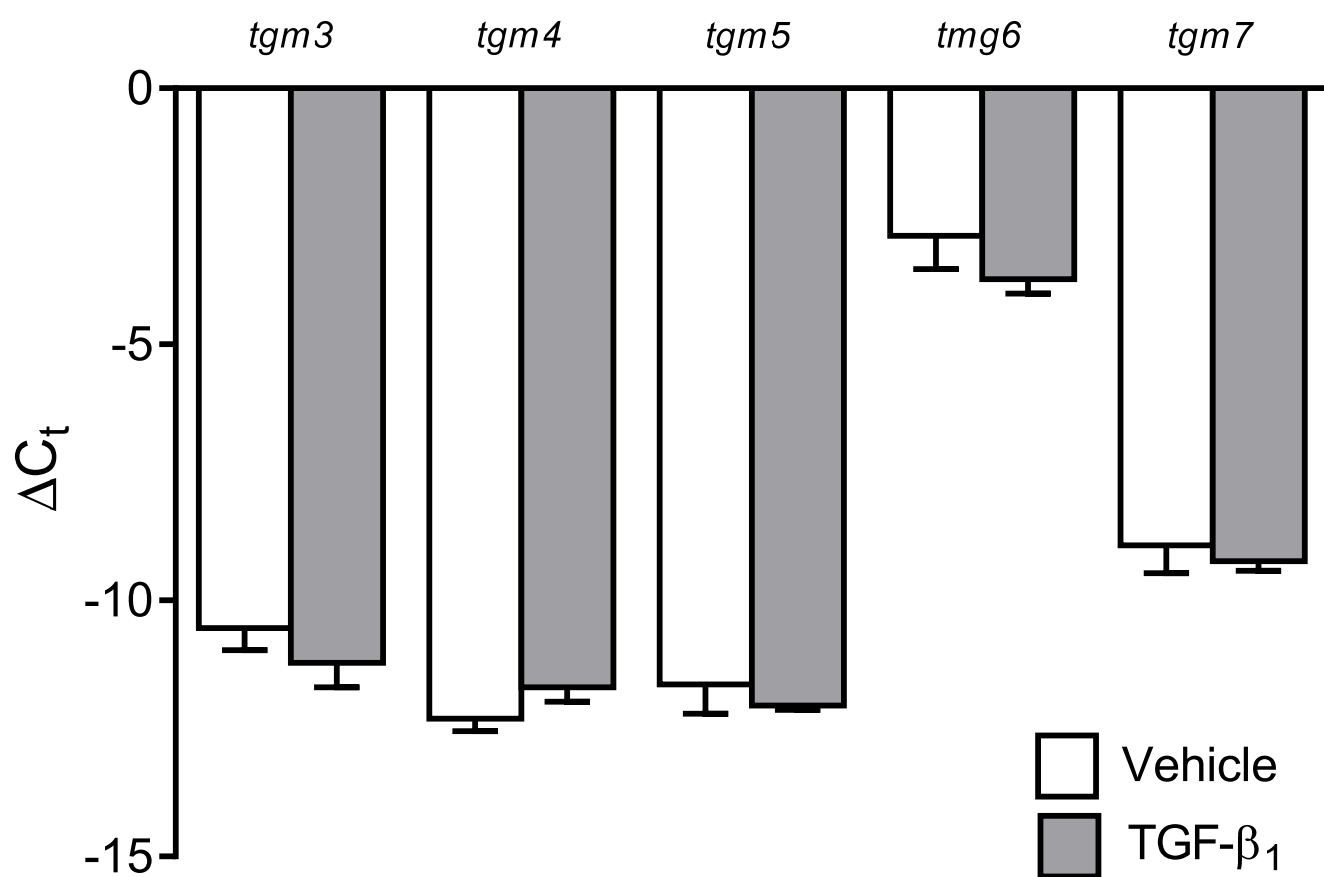


Figure S5

a) primary mouse alveolar type II cells



b) primary mouse lung fibroblasts

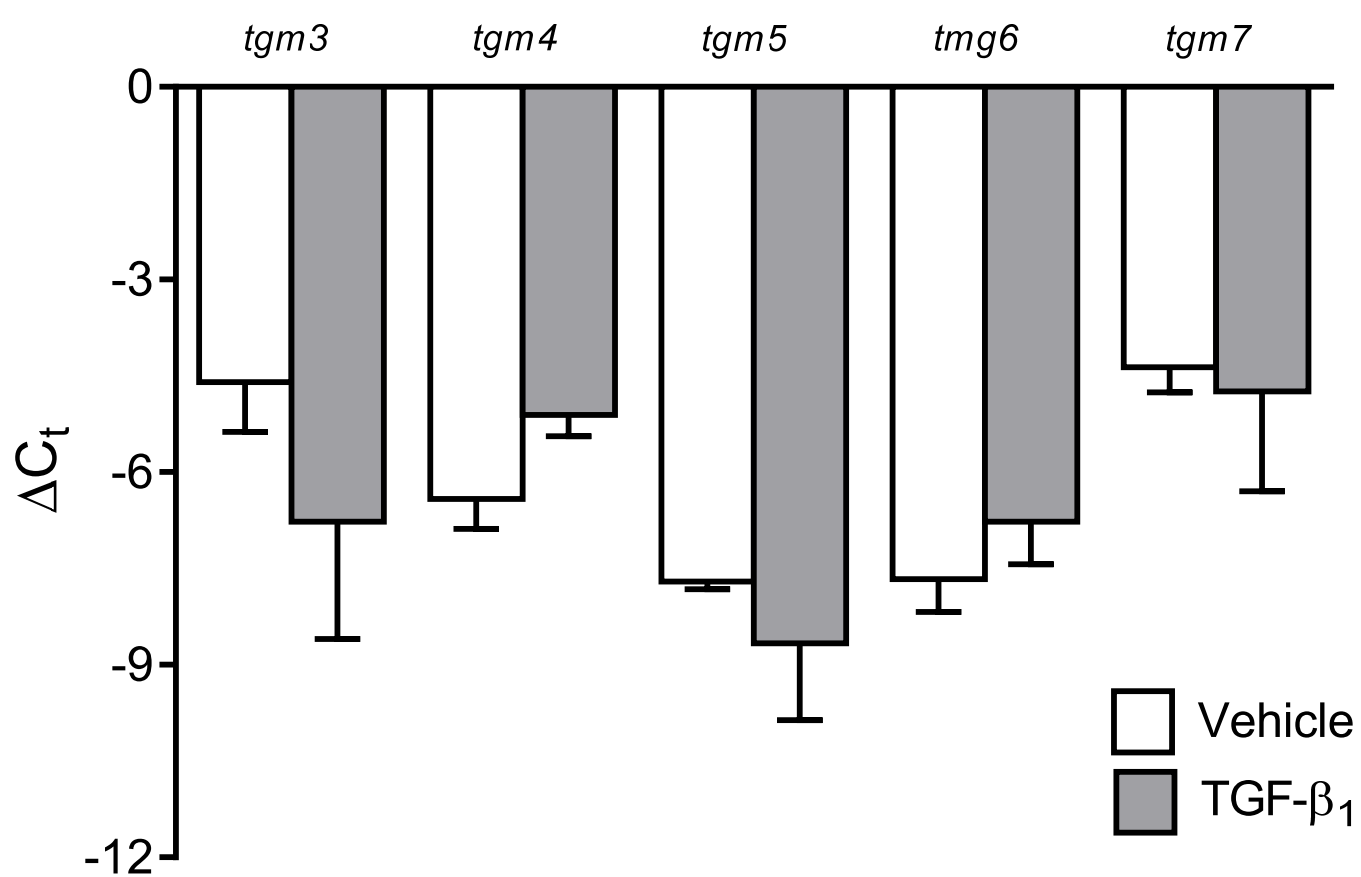


Figure S6

