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		
tgm1	5´-ACACCCCAGGAGACCAGCAGT-3´	5´-ATCATCCTGCCGCTGCCAGT-3´
tgm2	5´-GGCCGTGATGACCGGGAGGA-3´	5´-GATGCGCATGGCCACCCCTG-3´
tgm3	5´-GCACCAAGGCAGTAGGCAGC-3´	5´-TGTTGCGCCGAAAGATGCGT-3´
tgm4	5´-TGGAGACTGCCAGCATCGGC-3´	5´-ACCAGAAGCCTTCGCCATGACC-3´
tgm5	5´-TCAGCACCAAGAGCATCCAGAGTGA-3´	5´-GCTTGGTGAGGCCCTTGAGACC-3´
tgm6	5´-ACCAGGAGGACCCAGCCACC-3´	5´-TTGCCCTGCCACTGGCCTTG-3´
tgm7	5´-AGGGTCAGATGAGCGCCTGGA-3´	5´-GCGTTCCTTCTCGGTTCCAGCA-3´
hprt	5´-GATGATCTCTCAACTTTA-3´	5´-AGTCTGGCCTGTATCCAA-3´
18S rRNA	5´-AGGGGAGAGCGGGTAAGAGA-3´	5´-GGACAGGACTAGGCGGAACA-3´
Human primers		
TGM1	5´-GCACCACACAGACGAGTATGA-3´	5´-GGTGATGCGATCAGAGGATTC-3´
TGM2	5´-GAGGAGCTGGTCTTAGAGAGG-3´	5´-CGGTCACGACACTGAAGGTG-3´
TGM3	5´-ATGGCTGCTCTAGGAGTCCAG-3´	5´-GTTTTGGCCTCTCCGCAAGAT-3´
TGM4	5´-TGAATCAGGACAACGCCGTTT-3´	5´-GTGGTAGGATTGTAGGGGCTG-3´
TGM5	5´-AGCTGCTAGACAAGAGCCTG-3´	5´-CCACTCTGCTGACGTAGACG-3´
TGM6	5´-TCAGGCTTTCCTCTCACCG-3´	5´-CTGAGCACGTACTCCTGTCTC-3´
TGM7	5´-TTGCGGCTTGAGTCTGTCG-3´	5´-GTGATGTGGTCGTTCTGGGAC-3´
HPRT	5´-AAGGACCCCACGAAGTGTTG-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 ± 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* and 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.

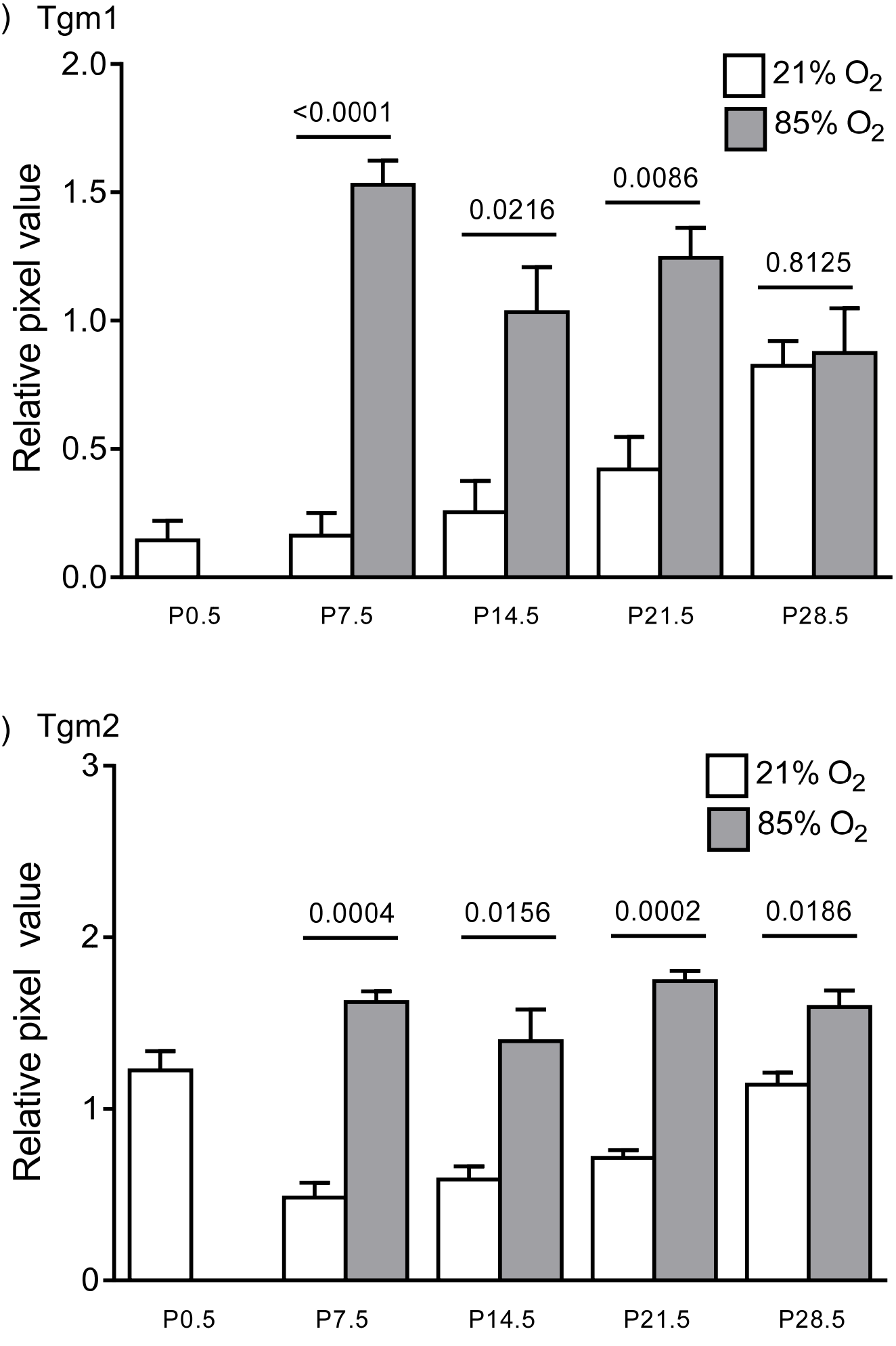
FIGURE S4. Regulation of transglutaminase mRNA levels by TGF-β_1 in primary human lung vascular endothelial cells and primary human pulmonary artery smooth muscle cells. The influence of TGF-β_1 on transglutaminase gene expression was assessed by real-time RT-PCR using the primer sequences provided in 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.

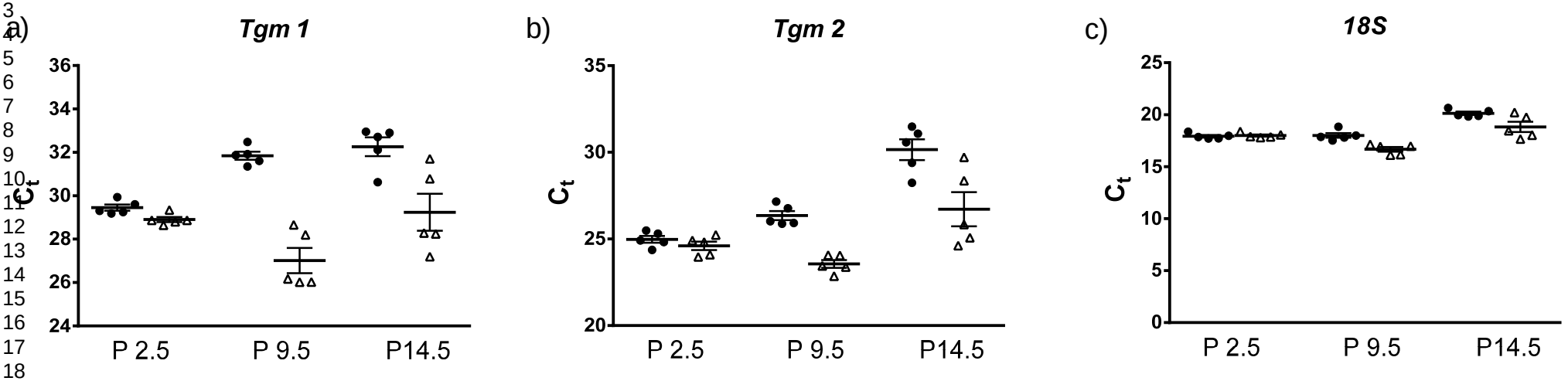
FIGURE S5. Regulation of transglutaminase mRNA levels by TGF-β_1 in mouse primary alveolar type II cells and mouse primary lung fibroblasts. The influence of TGF-β_1 on

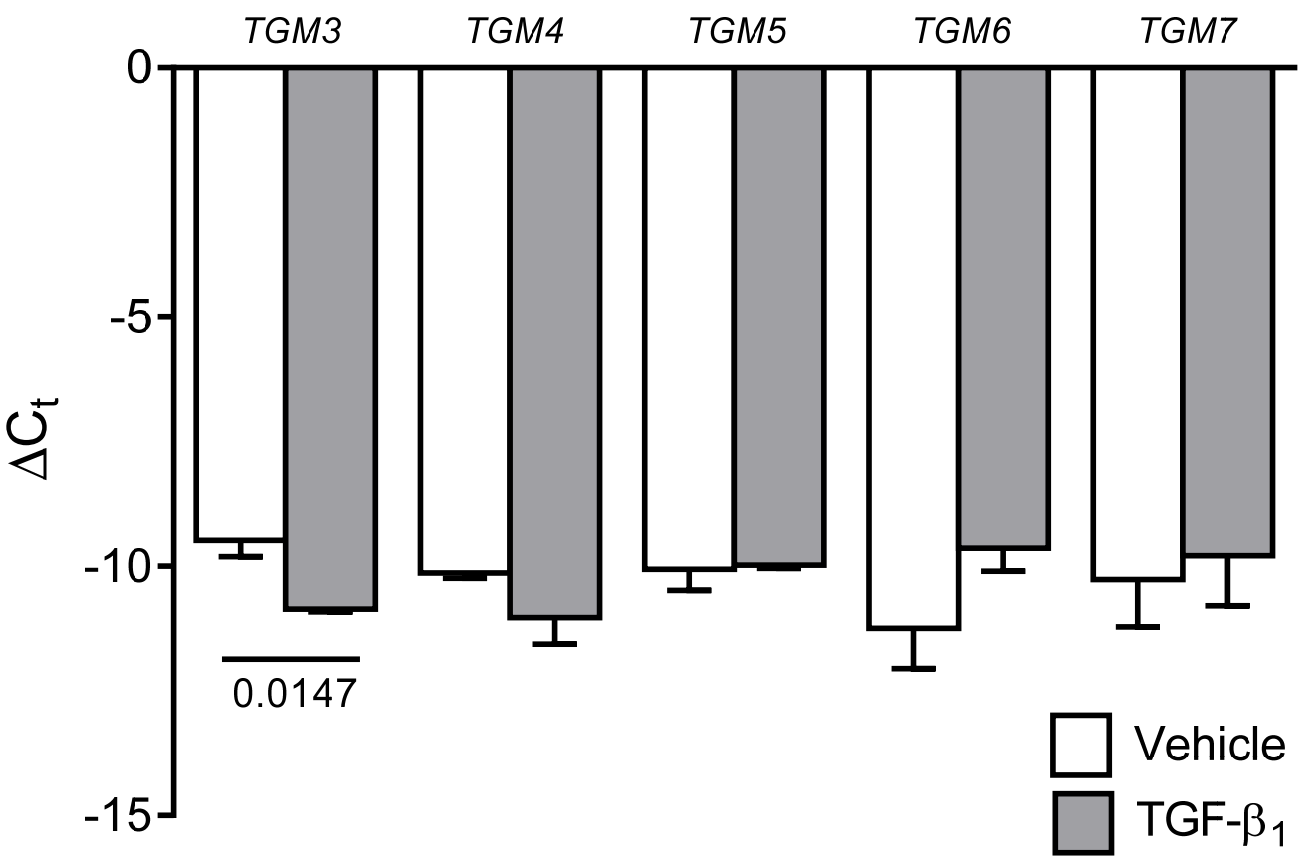
transglutaminase gene expression was assessed by real-time RT-PCR using the primer sequences provided in Table 2, with the *hprt* gene serving as reference. All associations were tested for statistical significance by assessment of p values, which were calculated by unpaired Student's *t*-test (n = 3; 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% O_2 or c) and d) 85% O_2 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 oberved in at least two other series'.

Figure S1


a) Tgm1
Relative pixel value
2.0
1.5
1.0
0.5
0.0
21% O2
85% O2
<0.0001
0.0216
0.0086
0.8125
P0.5
P7.5
P14.5
P21.5
P28.5
b) Tgm2
Relative pixel value
3
2
1
0
21% O2
85% O2
0.0004
0.0156
0.0002
0.0186
P0.5
P7.5
P14.5
P21.5
P28.5


a)
Tgm 1
C_t
36
34
32
30
28
26
24
P 2.5
P 9.5
P14.5
b)
Tgm 2
C_t
35
30
25
20
P 2.5
P 9.5
P14.5
c)
18S
C_t
25
20
15
10
5
0
P 2.5
P 9.5
P14.5


TGM3
TGM4
TGM5
TGM6
TGM7
0
-5
-10
-15
ΔCt
0.0147
Vehicle
TGF-β1

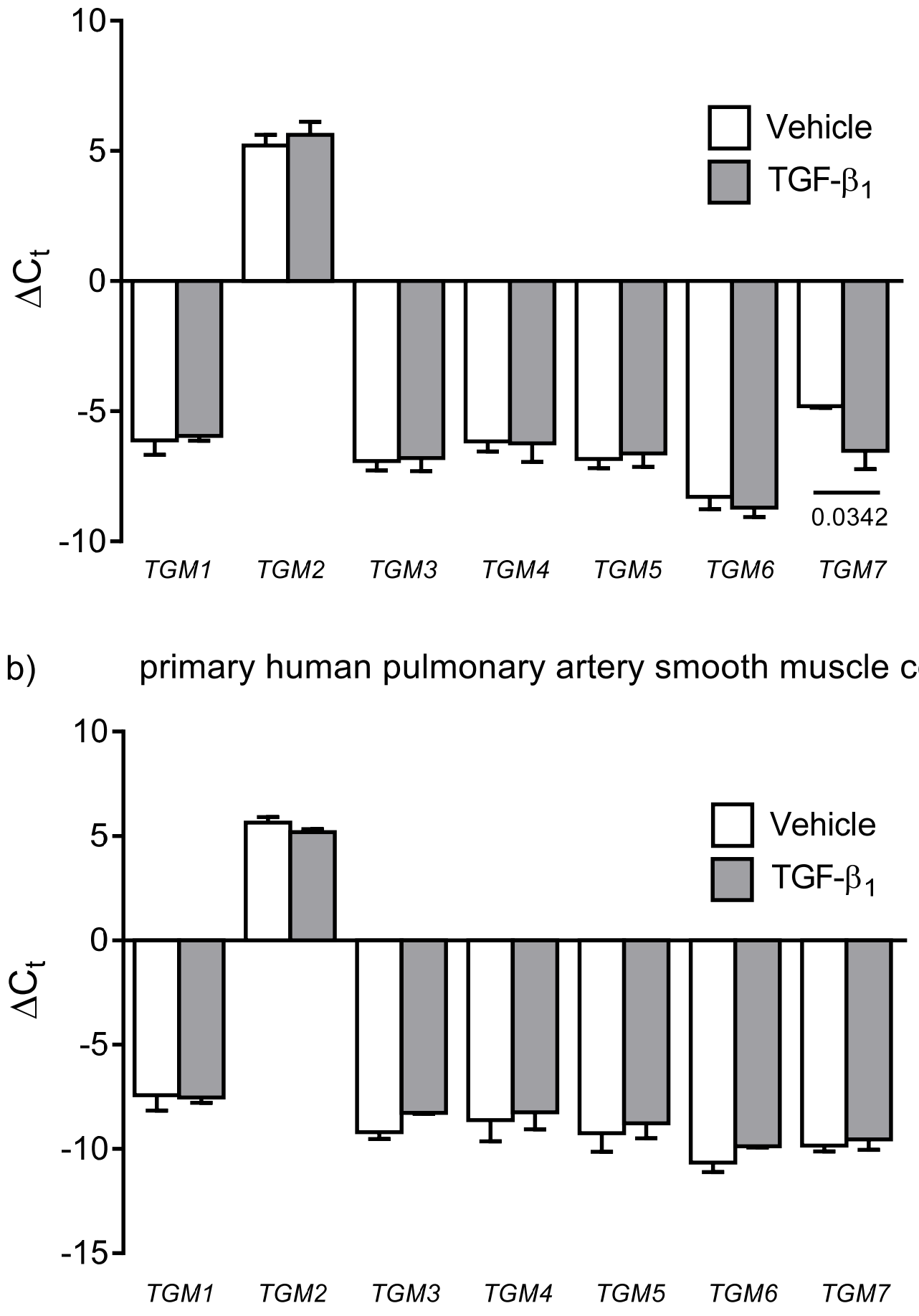

a)
primary human lung vascular endothelial cells
10
5
0
-5
-10
ΔCt
Vehicle
TGF-β1
0.0342
TGM1
TGM2
TGM3
TGM4
TGM5
TGM6
TGM7
b)
primary human pulmonary artery smooth muscle cells
10
5
0
-5
-10
-15
ΔCt
Vehicle
TGF-β1
TGM1
TGM2
TGM3
TGM4
TGM5
TGM6
TGM7

Figure S5

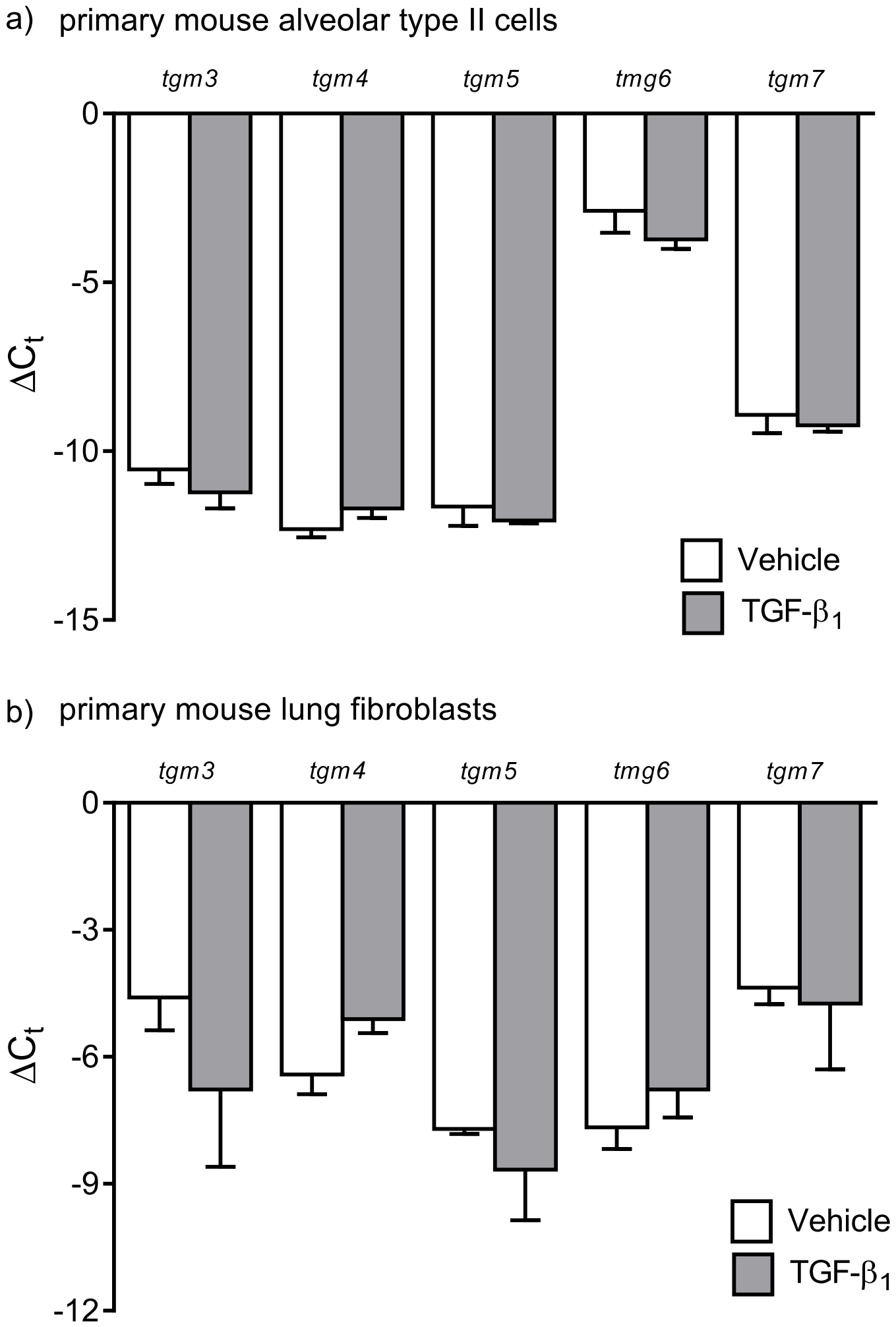

a) primary mouse alveolar type II cells
tgm3
tgm4
tgm5
tmg6
tgm7
0
-5
-10
-15
ΔCt
Vehicle
TGF-β1
b) primary mouse lung fibroblasts
tgm3
tgm4
tgm5
tmg6
tgm7
0
-3
-6
-9
-12
ΔCt
Vehicle
TGF-β1

Figure S6