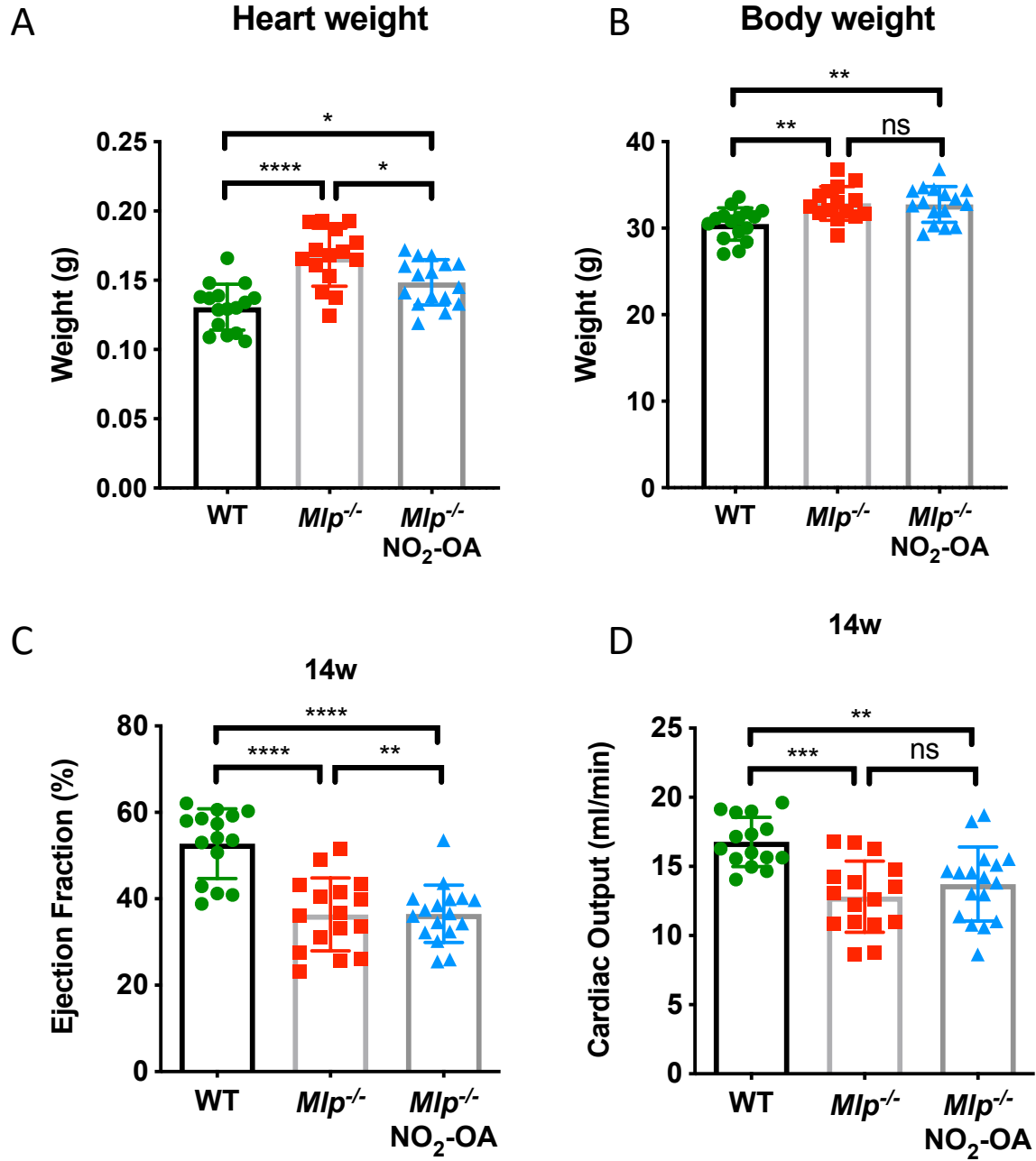


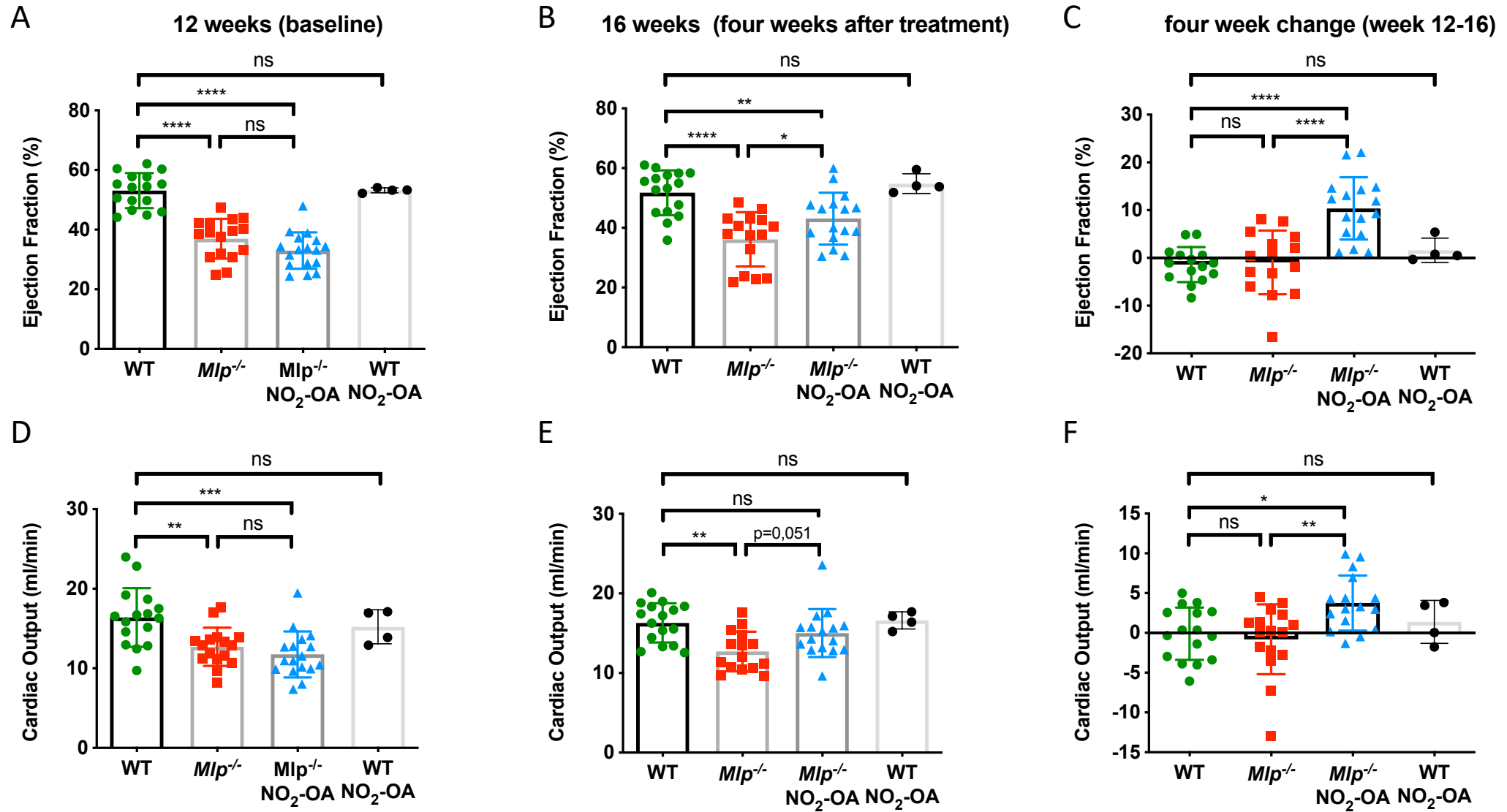
Nitro-oleic acid (NO₂-OA) improves systolic function in dilated cardiomyopathy by attenuating myocardial fibrosis

Supplemental Material

Supplemental Figure S1



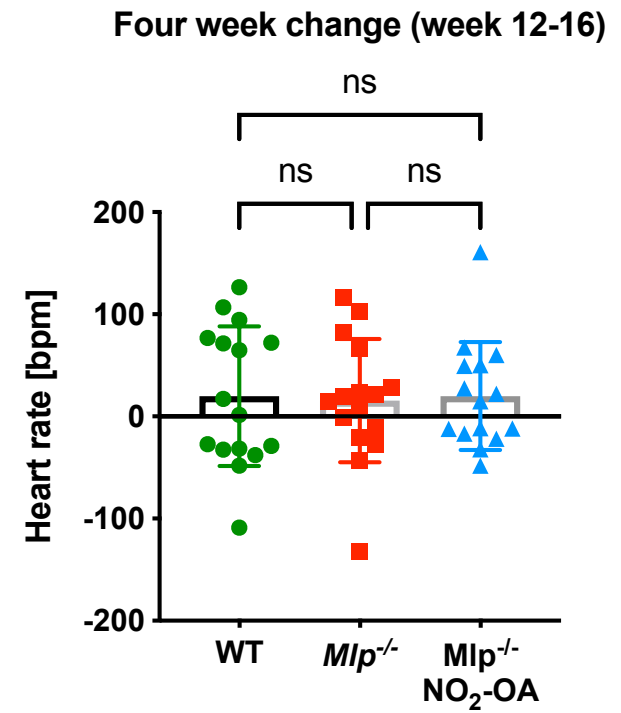
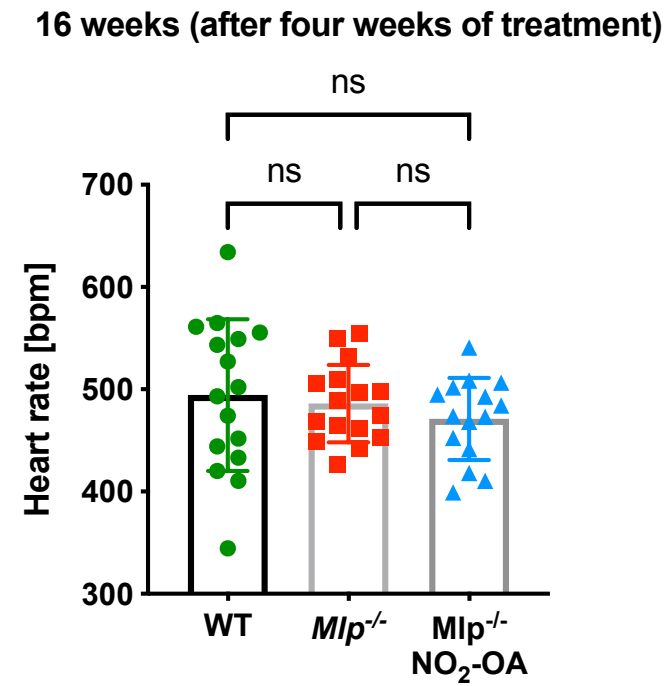
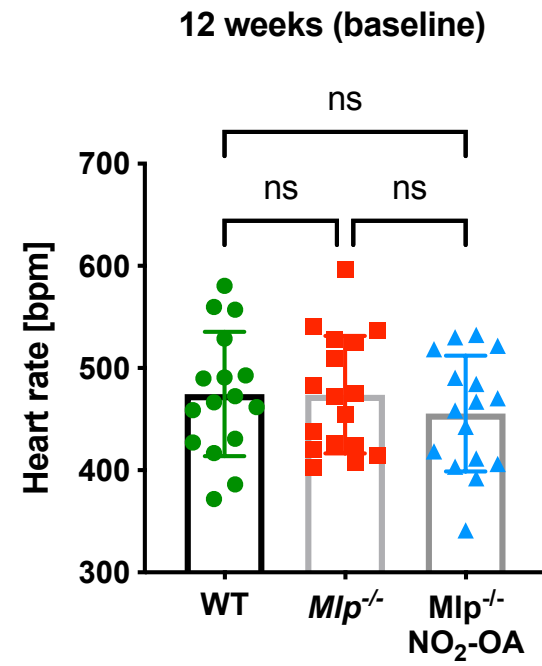
Supplemental Figure S2



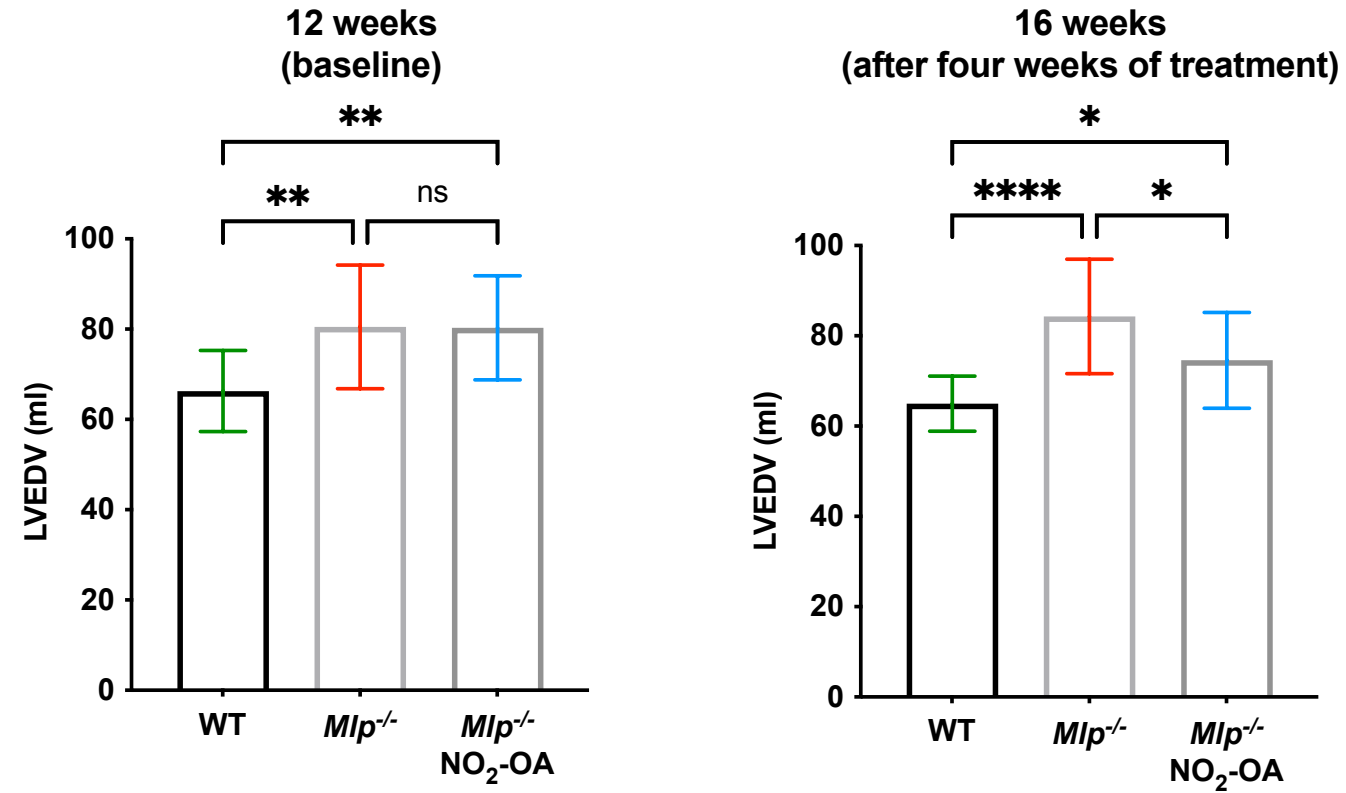
Supplemental Figure S3



Supplemental Figure S4



Supplemental Figure S5

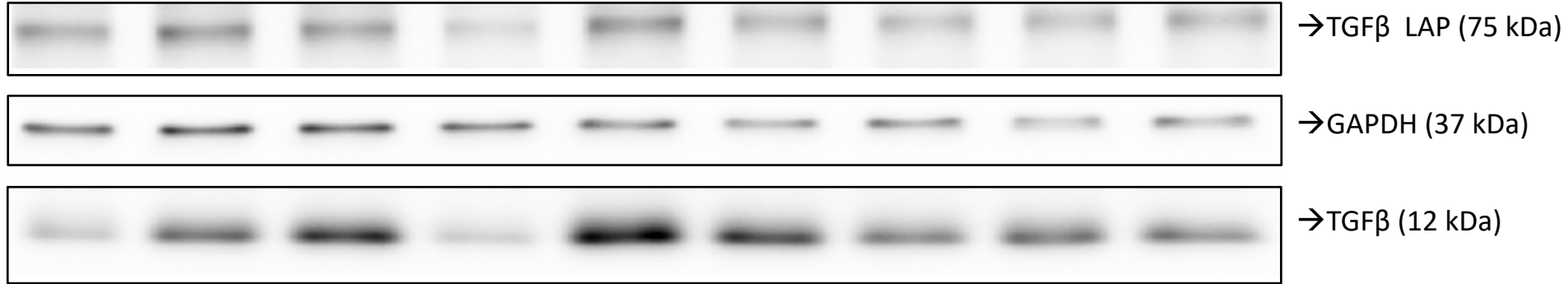


Uncropped Western Blot – as used in Figure 3

C= unstimulated control

T= stimulated with TGFb 10ng/ml

TN= TGFb + NO₂OA



Conditions:

12,5 µg sample / pocket on 12% Gel

Blot on NC at 200 mA for 2 h

Block 1h in 5% BSA

1st AB:

aSMA 1:5000 at 4°C o/n

2nd AB: anti-Rb

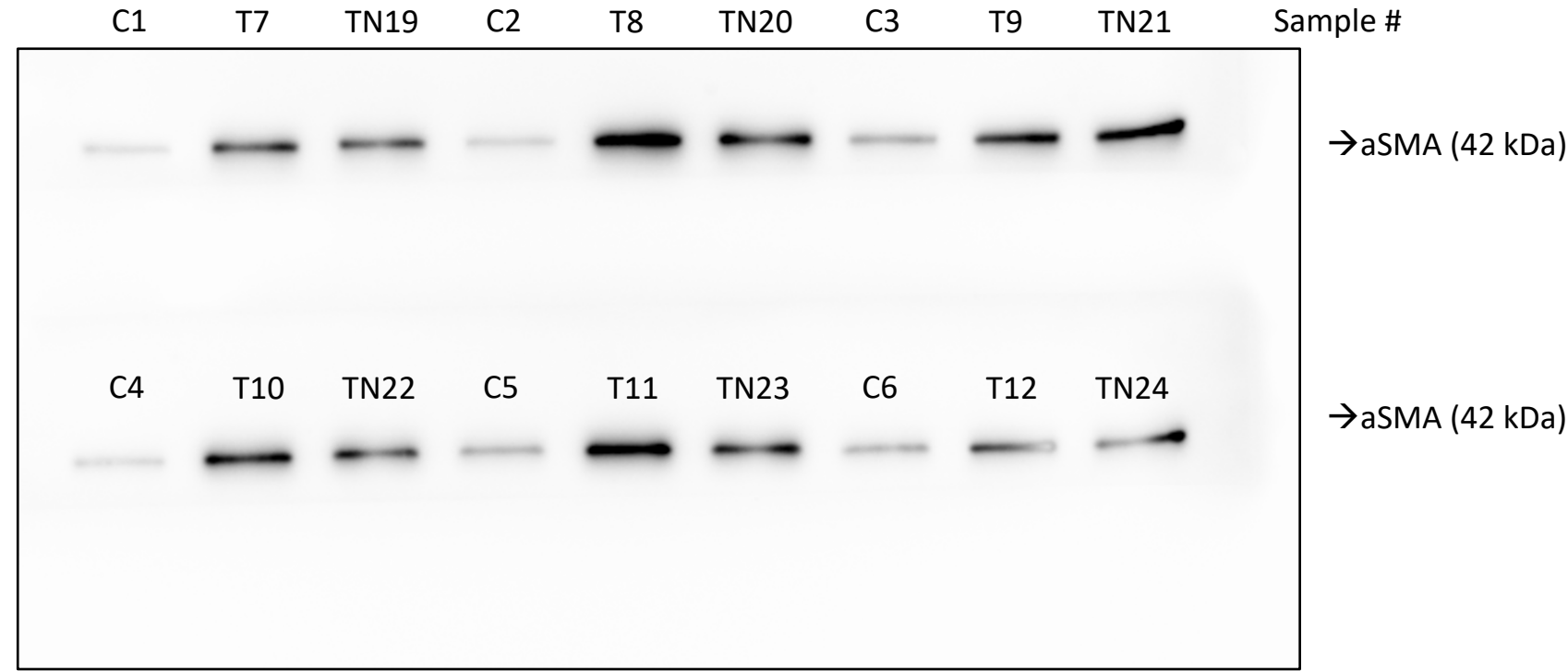
In 5% BSA at RT for 2h

Detection: ECL/Femto 4 to 1

Detection time aSMA 10 s

Uncropped Western Blot – Focus on aSMA (as used in Figure 4A)

C= unstimulated control
T= stimulated with TGFb 10ng/ml
TN= TGFb + NO₂OA



Conditions:
12,5 µg sample / pocket on 12% Gel
Blot on NC at 200 mA for 2 h
Block 1h in 5% BSA

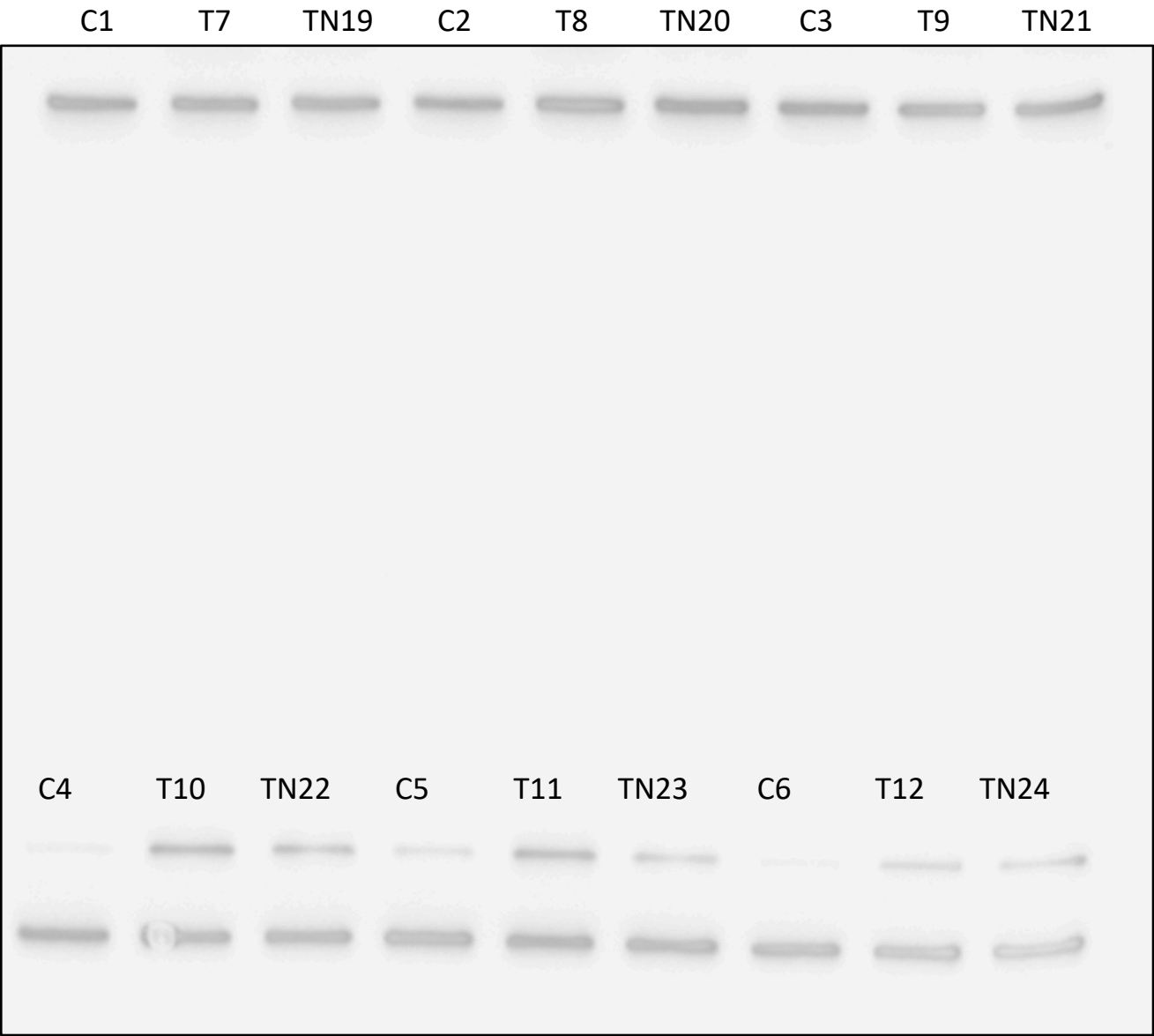
1st AB:
aSMA 1:5000 at 4°C o/n

2nd AB: anti-Rb
In 5% BSA at RT for 2h

Detection: ECL/Femto 4 to 1
Detection time aSMA 10 s

Uncropped Western Blot – Focus on GAPDH (as used in Figure 4A)

C= unstimulated control
T= stimulated with TGFb 10ng/ml
TN= TGFb + NO₂OA



Sample #

→GAPDH (37 kDa)

→aSMA (42 kDa) see previous slide

→GAPDH (37 kDa)

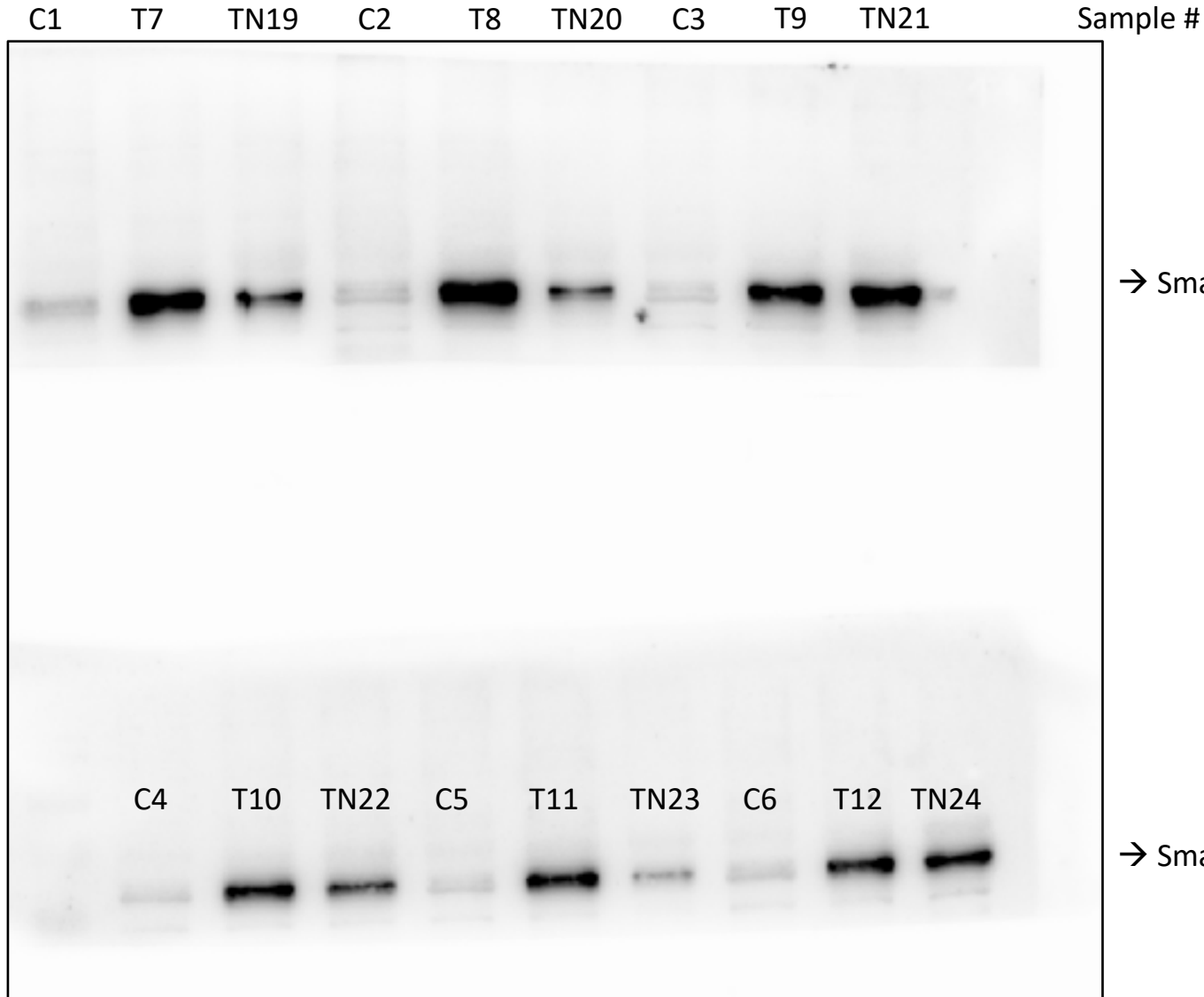
Conditions:
12,5 µg sample / pocket on 12% Gel
Blot on NC at 200 mA for 2 h
Block 1h in 5% BSA

1st AB:
GAPDH 1:7500 at 4°C o/n

2nd AB: anti-Rb
In 5% BSA at RT for 2h

Detection: ECL/Femto 4 to 1
Detection time GAPDH <1 s

Uncropped Western Blot – Focus on pSmad 2 pSer 465/467 (as used in Figure 4C)



C= unstimulated control

T= stimulated with TGFb 10ng/ml

TN= TGFb + NO₂OA

→ Smad 2 pSer465/467 (~ 58 kDa)

Conditions:

12,5 µg sample / pocket on 12% Gel

Blot on NC at 200 mA for 2 h

Block 1h in 5% BSA

1st AB:

Smad 2 pSer465/467 1:1000 at 4°C o/n

2nd AB: anti-Rb

In 5% BSA at RT for 2h

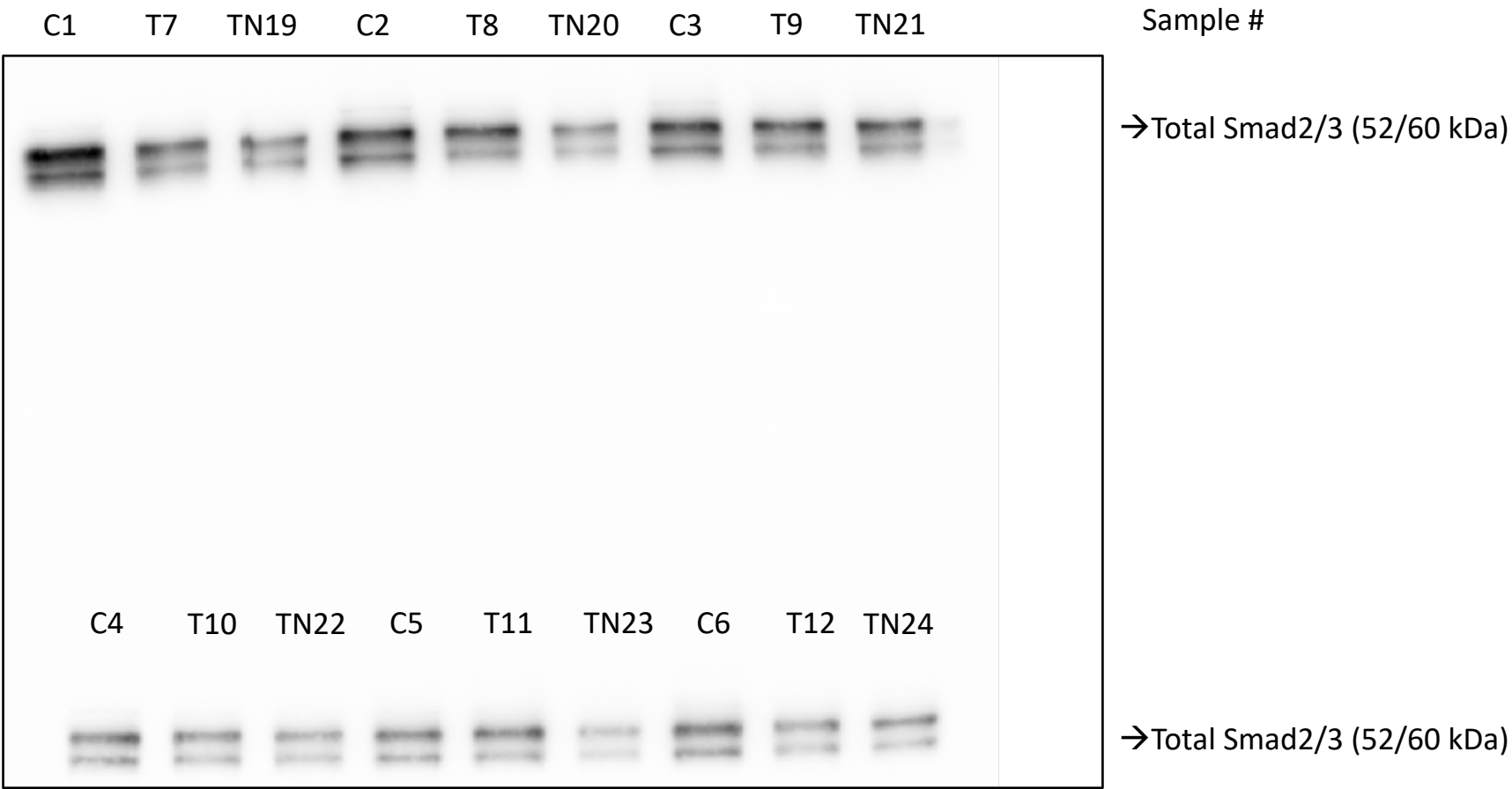
Detection: ECL/Femto 4 to 1

Detection time Smad 2 pSer465/467 30 s

→ Smad 2 pSer465/467 (~ 58 kDa)

C= unstimulated control
T= stimulated with TGFb 10ng/ml
TN= TGFb + NO₂OA

Uncropped Western Blot – Focus on Total Smad 2/3 (as used in Figure 4C)



Conditions:
12,5 µg sample / pocket on 12% Gel
Blot on NC at 200 mA for 2 h
Block 1h in 5% BSA

1st AB:
After stripping with 5M NaOH for 5 min
Total Smad 2/3 1:1000 at 4°C o/n

2nd AB: anti-Rb
In 5% BSA at RT for 2h

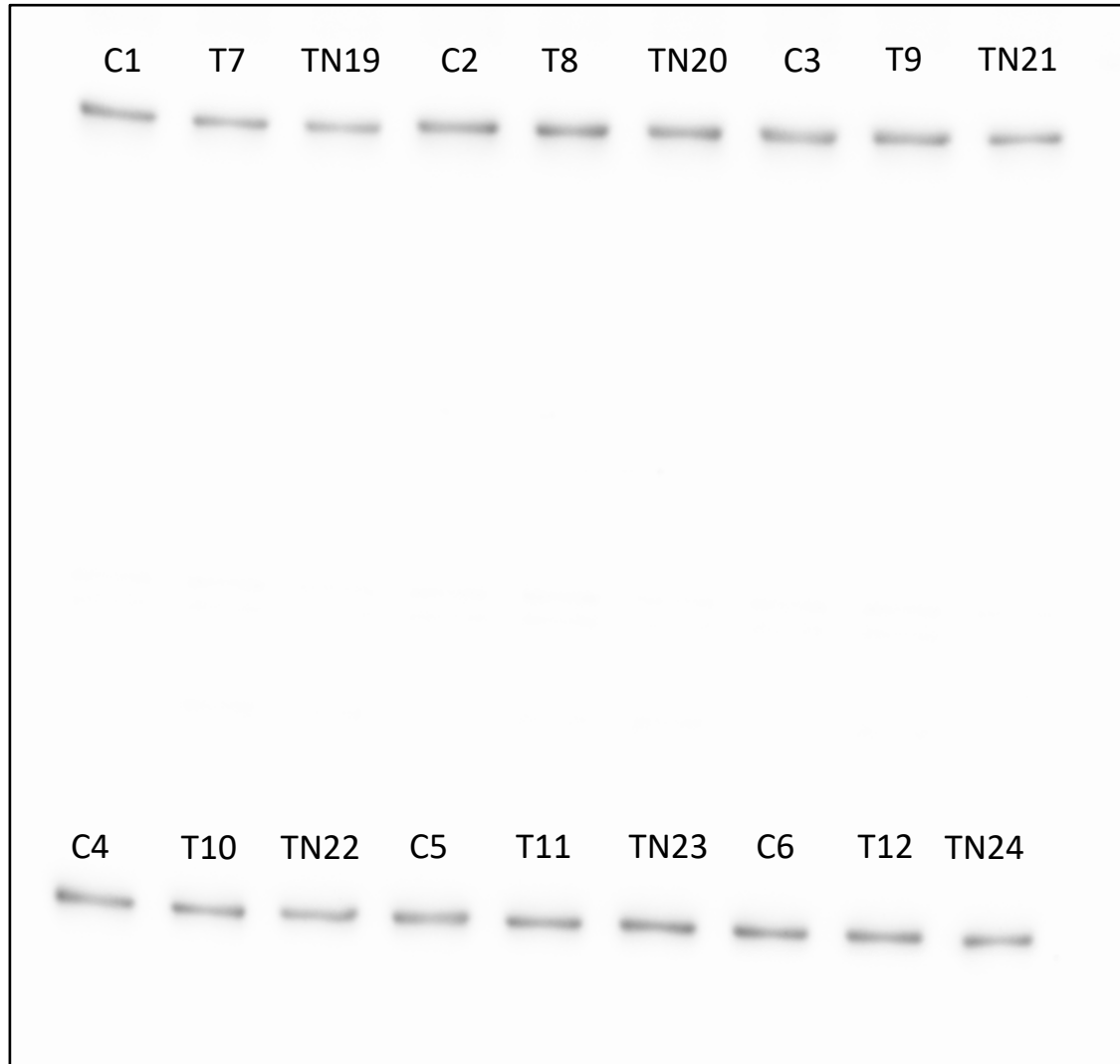
Detection: ECL/Femto 2 to 1
Detection time Smad 2/3 60s

Uncropped Western Blot – Focus on GAPDH (as used in Figure 4C)

C= unstimulated control

T= stimulated with TGFb 10ng/ml

TN= TGFb + NO₂OA



Sample #

→GAPDH (37 kDa)

→GAPDH (37 kDa)

Conditions:

12,5 µg sample / pocket on 12% Gel

Blot on NC at 200 mA for 2 h

Block 1h in 5% BSA

1st AB:

GAPDH 1:7500 at 4°C o/n

2nd AB: anti-Rb

In 5% BSA at RT for 2h

Detection: ECL/Femto 4 to 1

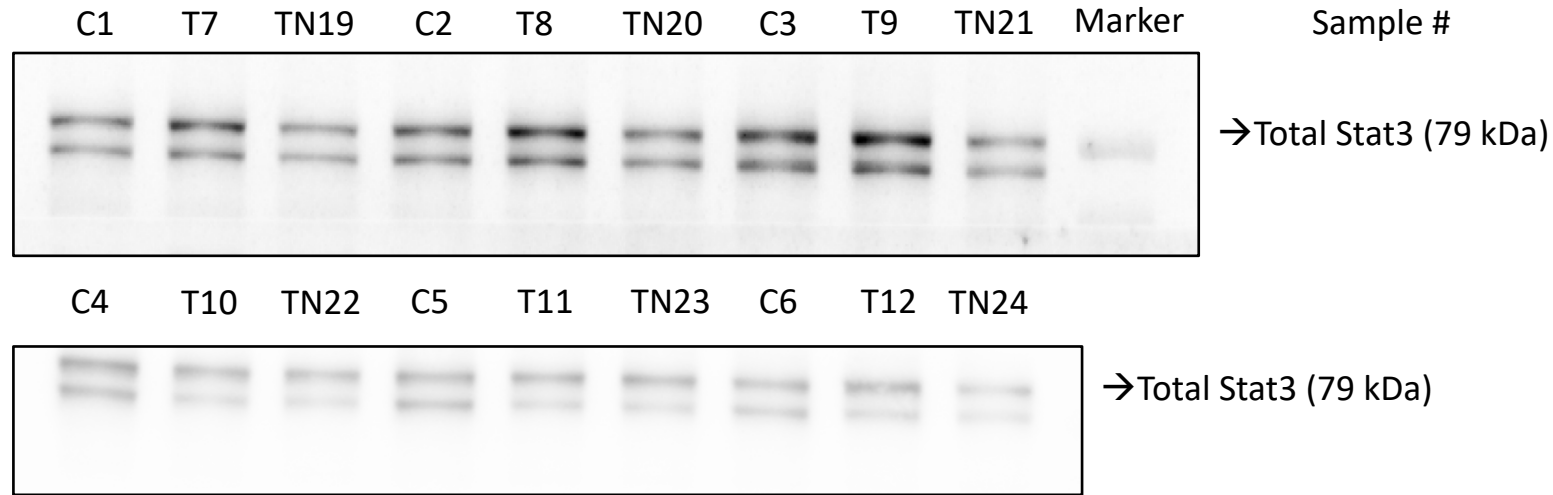
Detection time GAPDH 1,5 s

Uncropped Western Blot – Focus on STAT3 (as used in Figure 4D)

C= unstimulated control

T= stimulated with TGFb 10ng/ml

TN= TGFb + NO₂OA



Conditions:

12,5 µg sample / pocket on 12% Gel

Blot on NC at 200 mA for 2 h

Block 1h in 5% BSA

1st AB:

After stripping with 5M NaOH for 5 min

Total Stat3 1:1000 at 4°C o/n

2nd AB: anti-Rb

In 5% BSA at RT for 2h

Detection: ECL/Femto 2 to 1

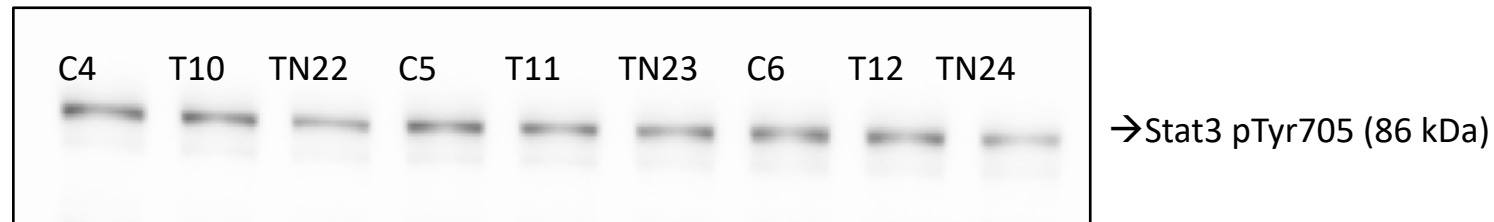
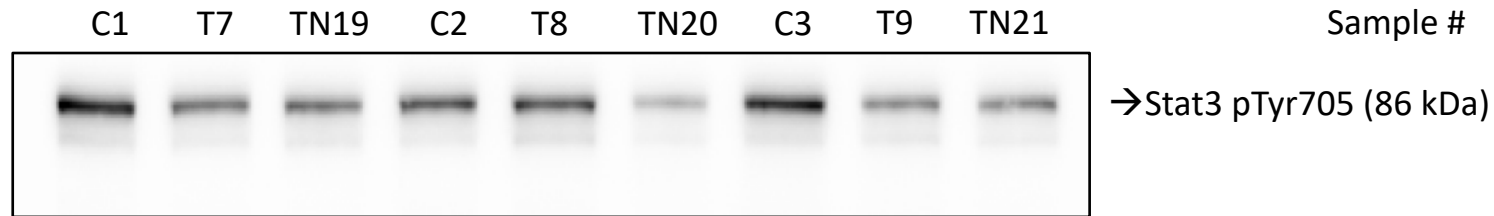
Detection time total Stat 3 60 s

Uncropped Western Blot – Focus on STAT3 (as used in Figure 4D)

C= unstimulated control

T= stimulated with TGFb 10ng/ml

TN= TGFb + NO₂OA



Conditions:

12,5 µg sample / pocket on 12% Gel

Blot on NC at 200 mA for 2 h

Block 1h in 5% BSA

1st AB:

Stat3 pTyr705 1:1000 at 4°C o/n

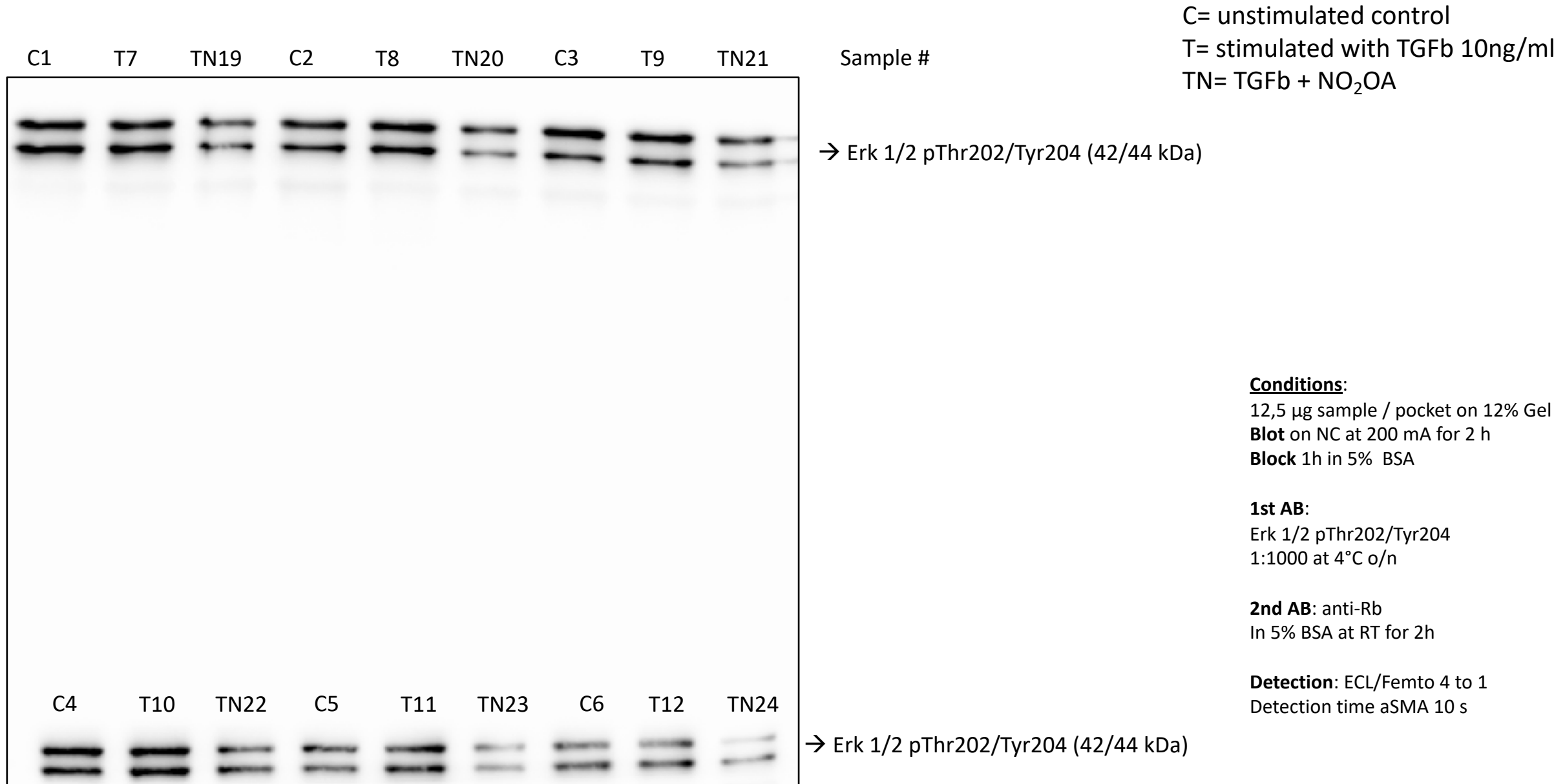
2nd AB: anti-

In 5% BSA at RT for 2h

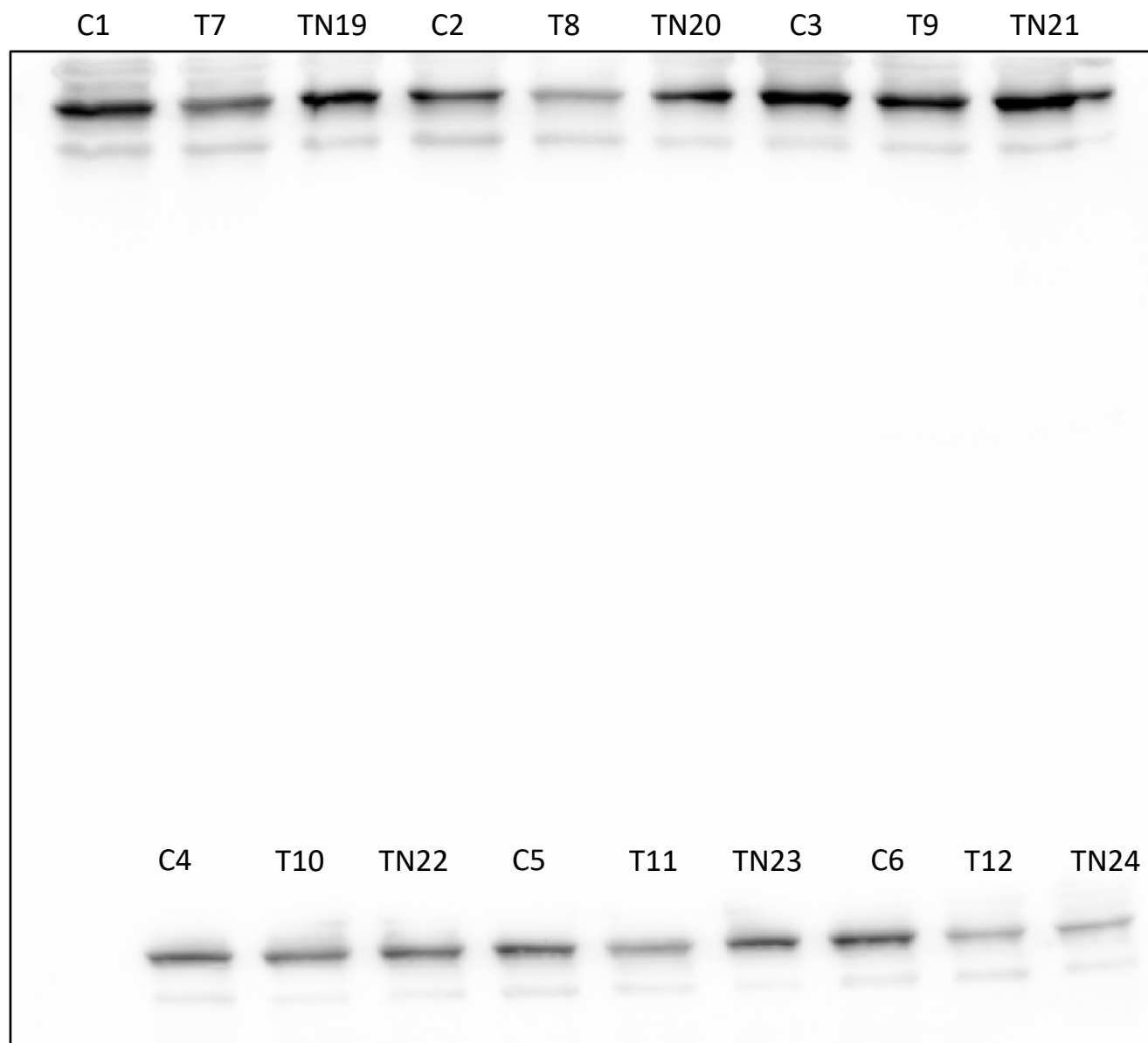
Detection: ECL/Femto 1 to 1

Detection time Stat3 pTyr705 30 s

Uncropped Western Blot – Focus on Erk 1/2 pThr202/Tyr204 (as used in Figure 4E)



Uncropped Western Blot – Focus on Total Erk 1/2 (as used in Figure 4E)



Sample #

→ Total Erk 1/2 (42/44 kDa)

C= unstimulated control

T= stimulated with TGFb 10ng/ml

TN= TGFb + NO₂OA

Conditions:

12,5 µg sample / pocket on 12% Gel

Blot on NC at 200 mA for 2 h

Block 1h in 5% BSA

1st AB:

Total Erk 1/2

1:1000 at 4°C o/n

2nd AB: anti-Rb

In 5% BSA at RT for 2h

Detection: ECL/Femto 4 to 1

Detection time aSMA 10 s

→ Total Erk 1/2 (42/44 kDa)

Gene	Forward Primer	Reverse Primer
Actin β	GCTGTATTCCCCTCCATCG	GGGGTGTTGAAGGTCTCAAA
TGF β	AGCCCGAAGCGGACTACTAT	ACGCCAGGAATTGTTGCTAT
TGF β R1	TGGTCCAGTCTGCTTCGTCT	GTGGTGCCCTCTGAAATGAA

Supplementary Table S1: Primers used for qPCR