SUPPLEMENTARY INFORMATION

Telomere damage promotes vascular smooth muscle cell senescence and immune cell recruitment after vessel injury

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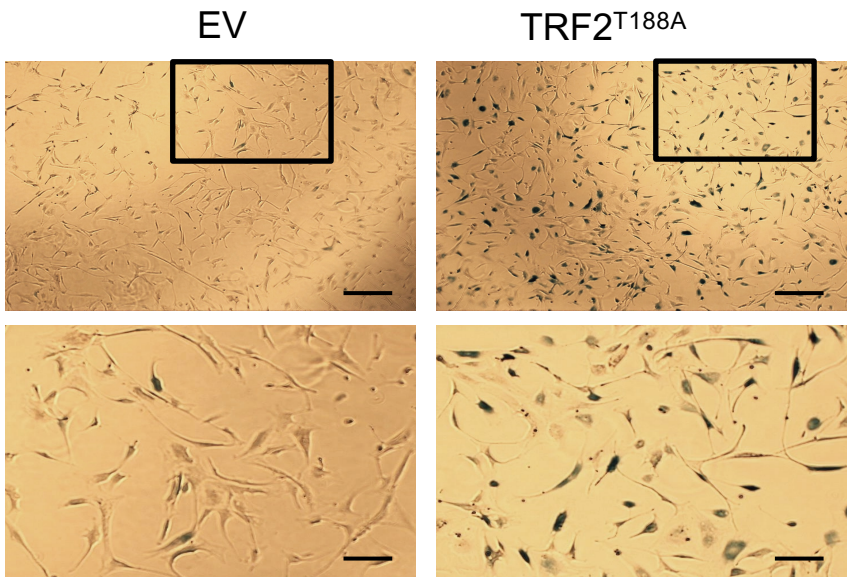
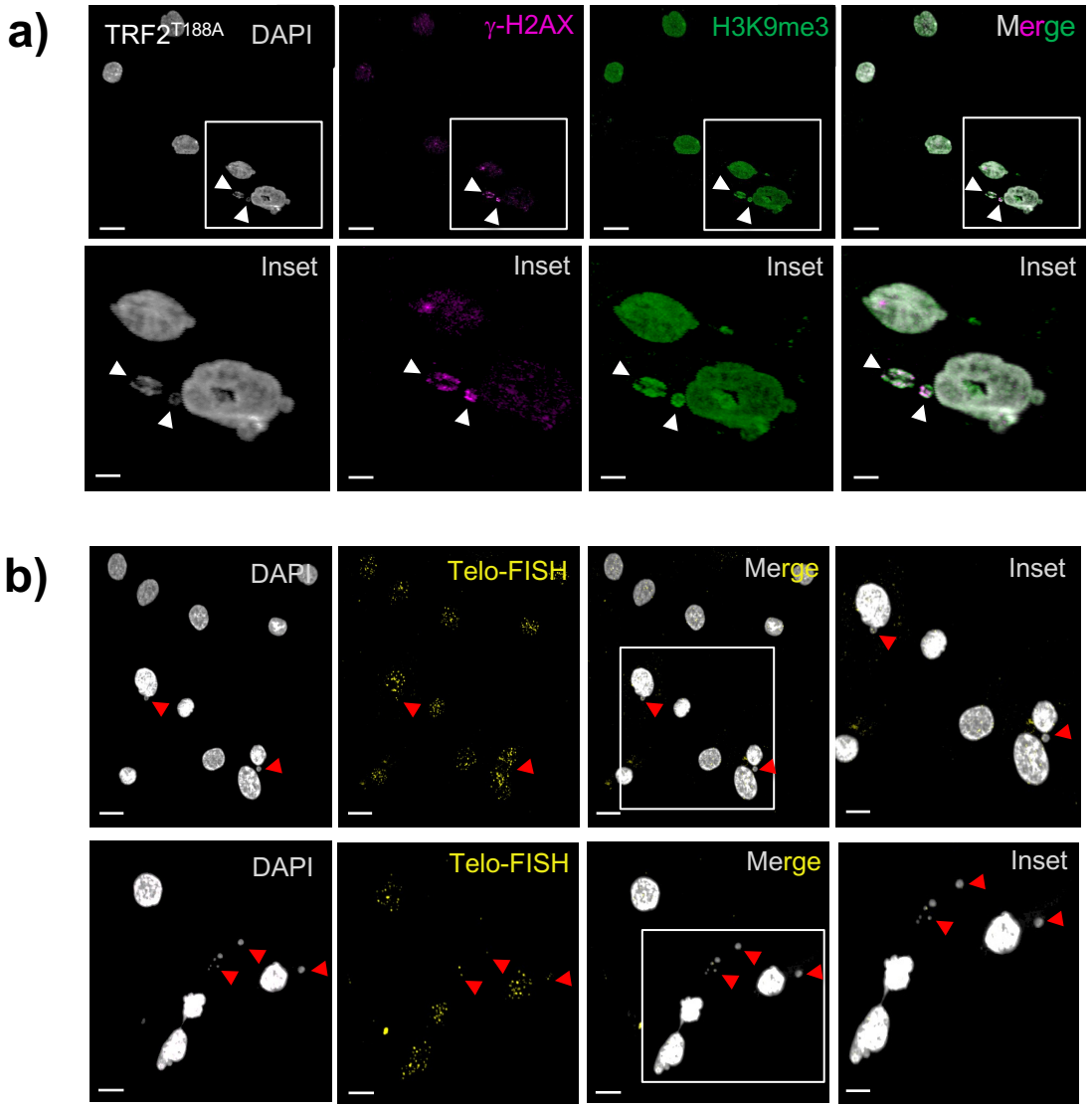
Supplementary Figure 1



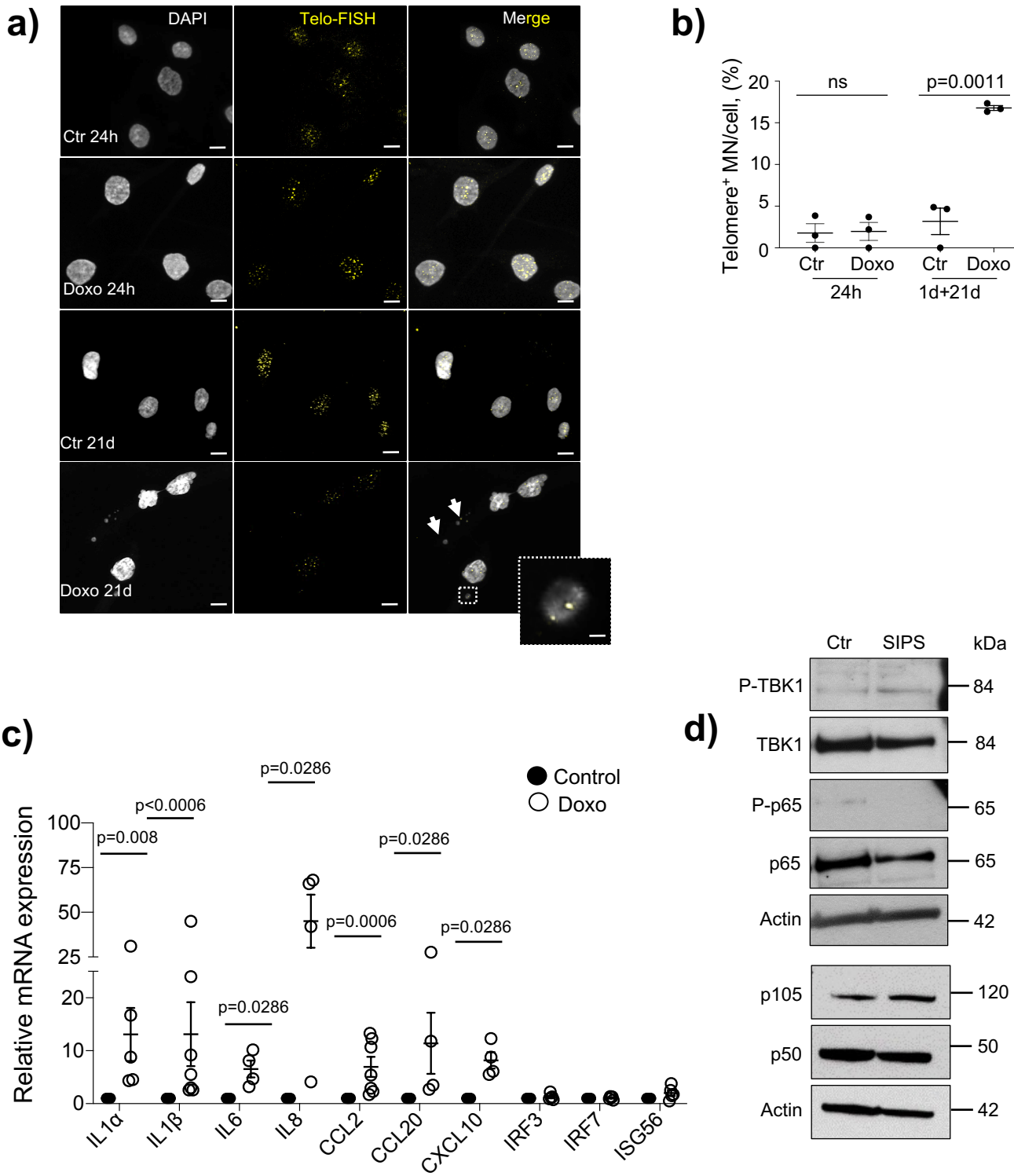
Supplementary Figure 1. SAβG activity in human VSMCs expressing an empty vector or TRF2^{T188A} Human VSMCs infected with an empty vector (EV) or lentivirus expressing TRF2^{T188A}, and analysed for the senescence marker SAβG. Low-power (top, scale bar=150μm) and high-power (bottom, scale bar= 50μm) representative images of SAβG-positive cells.

Supplementary Figure 2



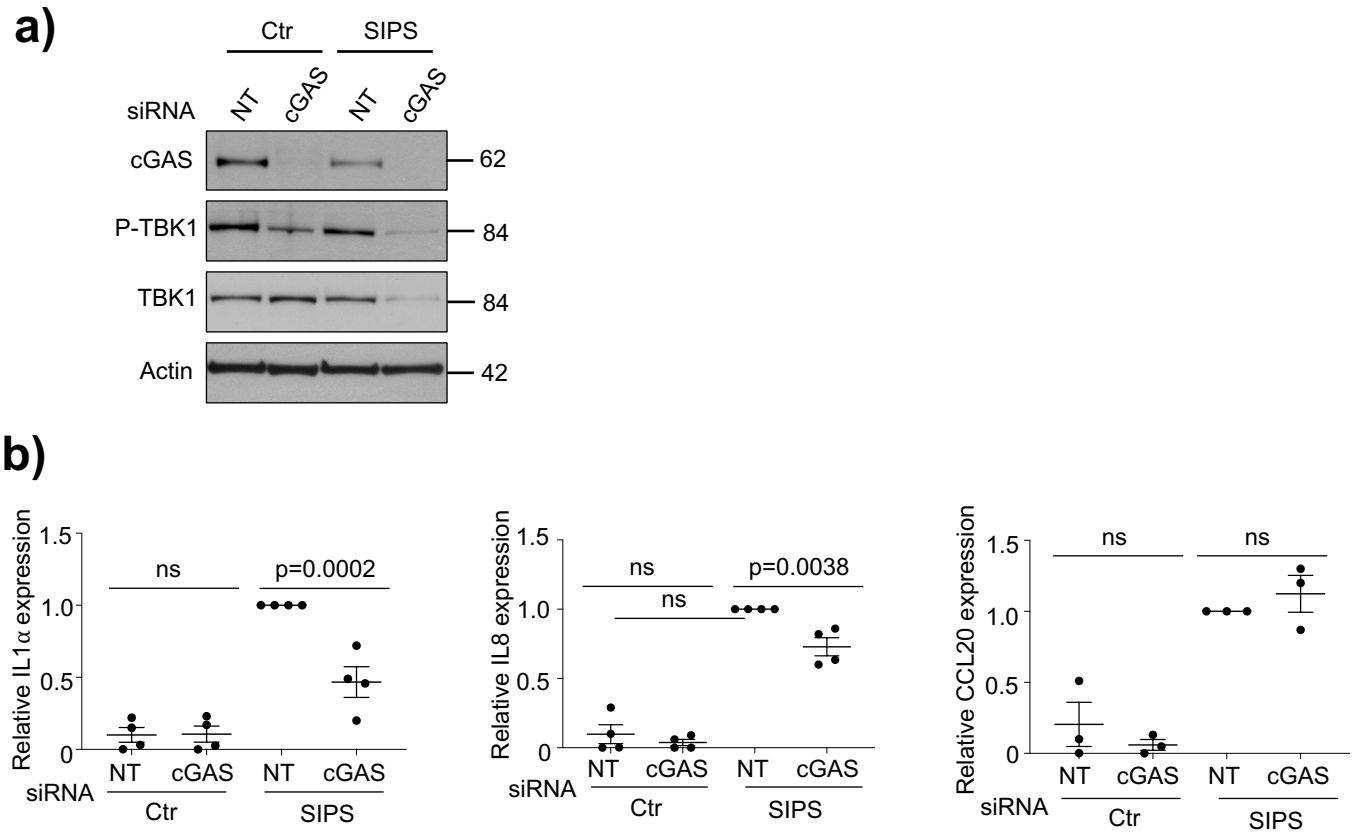
Supplementary Figure 2. Micronuclei in TRF2^{T188A} VSMCs
a) Examples of micronuclei (white arrowheads) seen at low power (upper panels) or high power view of inset image (lower panels) in TRF2^{T188A} VSMCs. Scale bars=20μm and 7.5μm (insets).
b) Examples of micronuclei containing telomere signals by TELO-FISH in TRF2^{T188A} VSMCs (red arrowheads) . High power view is shown on the right. Scale bars=20μm and 12.5μm (insets).

Supplementary Figure 3



Supplementary Figure 3. Doxorubicin-induced premature senescence promotes SASP
a) Telo-FISH and **b)** quantification of Telomere+ micronuclei (MN)/cell in control cells or those undergoing doxo24h treatment or doxo-induced SIPs (Doxo1d+21d). DAPI (white) and telo-FISH (yellow). Arrows indicate MN. Scale bars=10μm and 1.25μm (inset). **c)** Relative mRNA expression of selected cytokines in control hVSMCs or those undergoing doxo-induced SIPS (Doxo1d+21d). n=4-7 independent experiments. **d)** Western blot for TBK1, p65 NF-κB and their phosphorylated forms and p50 and p105 NF-κB members in control hVSMCs or those undergoing doxo-induced SIPS. Data shown represent Means±SEM, n=3-5 independent experiments, ns-non significant (p>0.05), unpaired, two-tailed Student's t-test or Mann-Whitney U test.

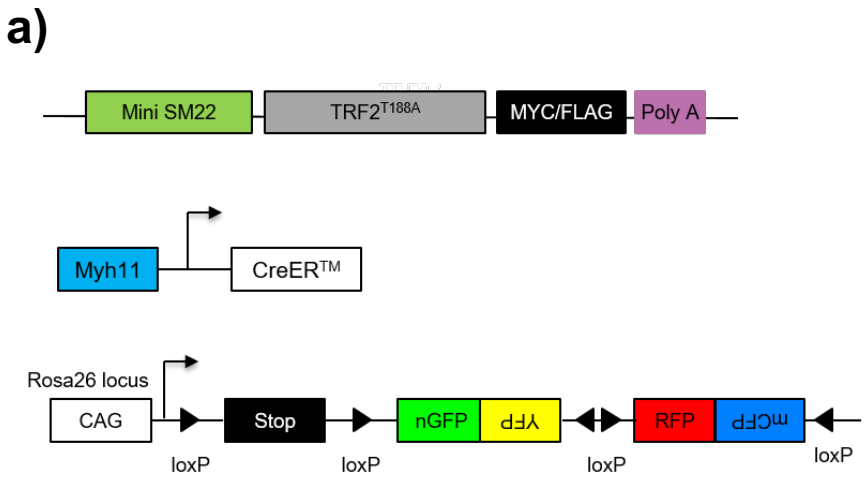
Supplementary Figure 4

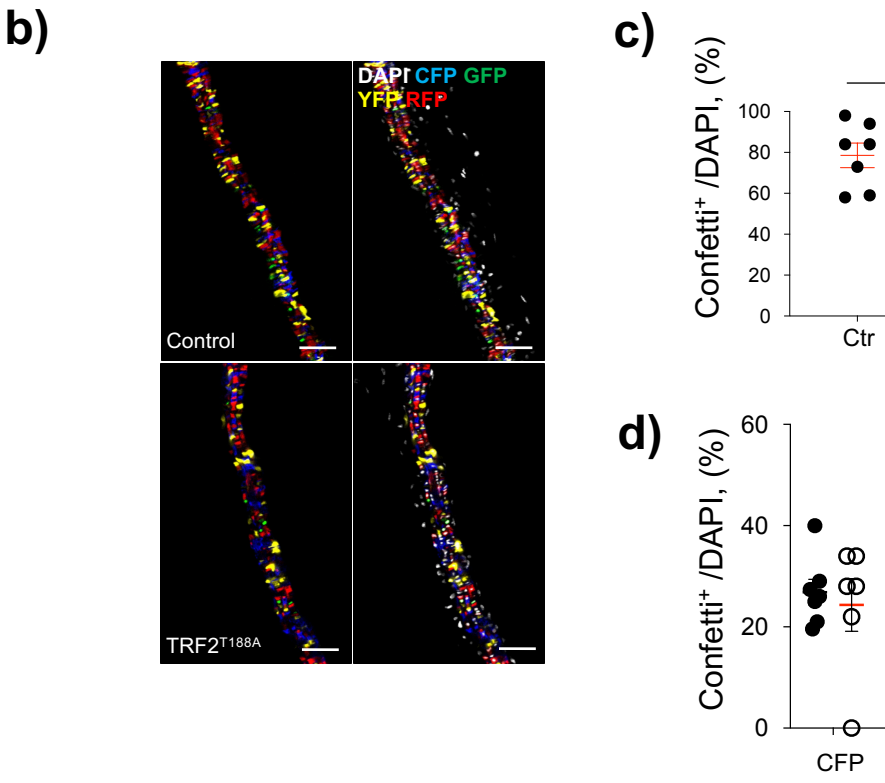


Supplementary Figure 4. Doxorubicin-induced premature senescence promotes SASP through cGAS-STING-TBK1 pathway

a) Western blot for cGAS, P-TBK1 and TBK1 in control hVSMCs or those undergoing doxo-induced SIPS transfected with non-targeting (NT)- or *cGAS*-siRNA. **b)** Relative mRNA expression of IL1α, IL8 and CCL20 in control hVSMCs or those undergoing doxo-induced SIPS transfected with non-targeting (NT)- or *cGAS-siRNA*. Data shown represent Means±SEM, n=3-5 independent experiments, ns-non significant (p>0.05), 1-way ANOVA with Bonferroni multiple corrections.

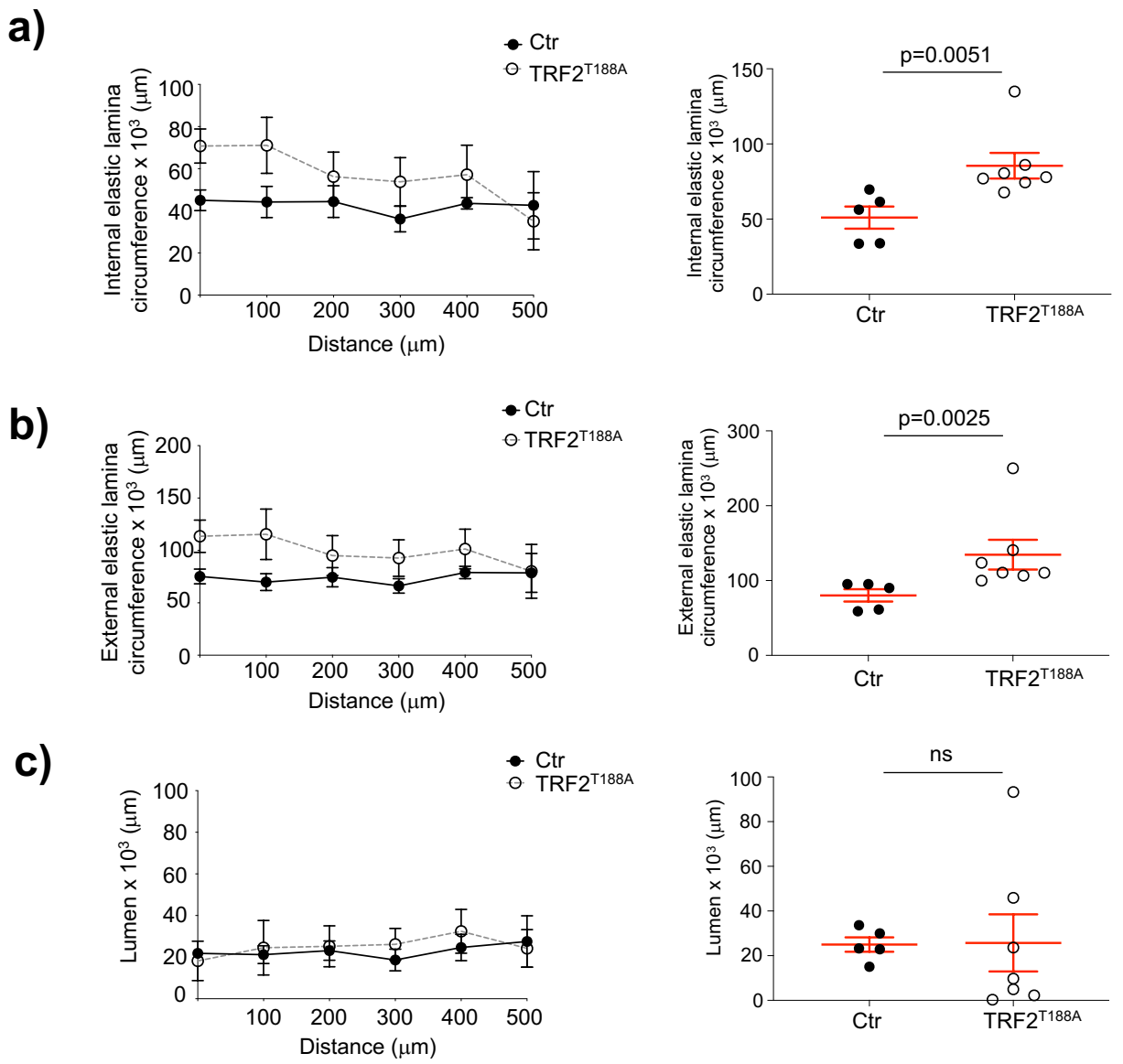
Supplementary Figure 5





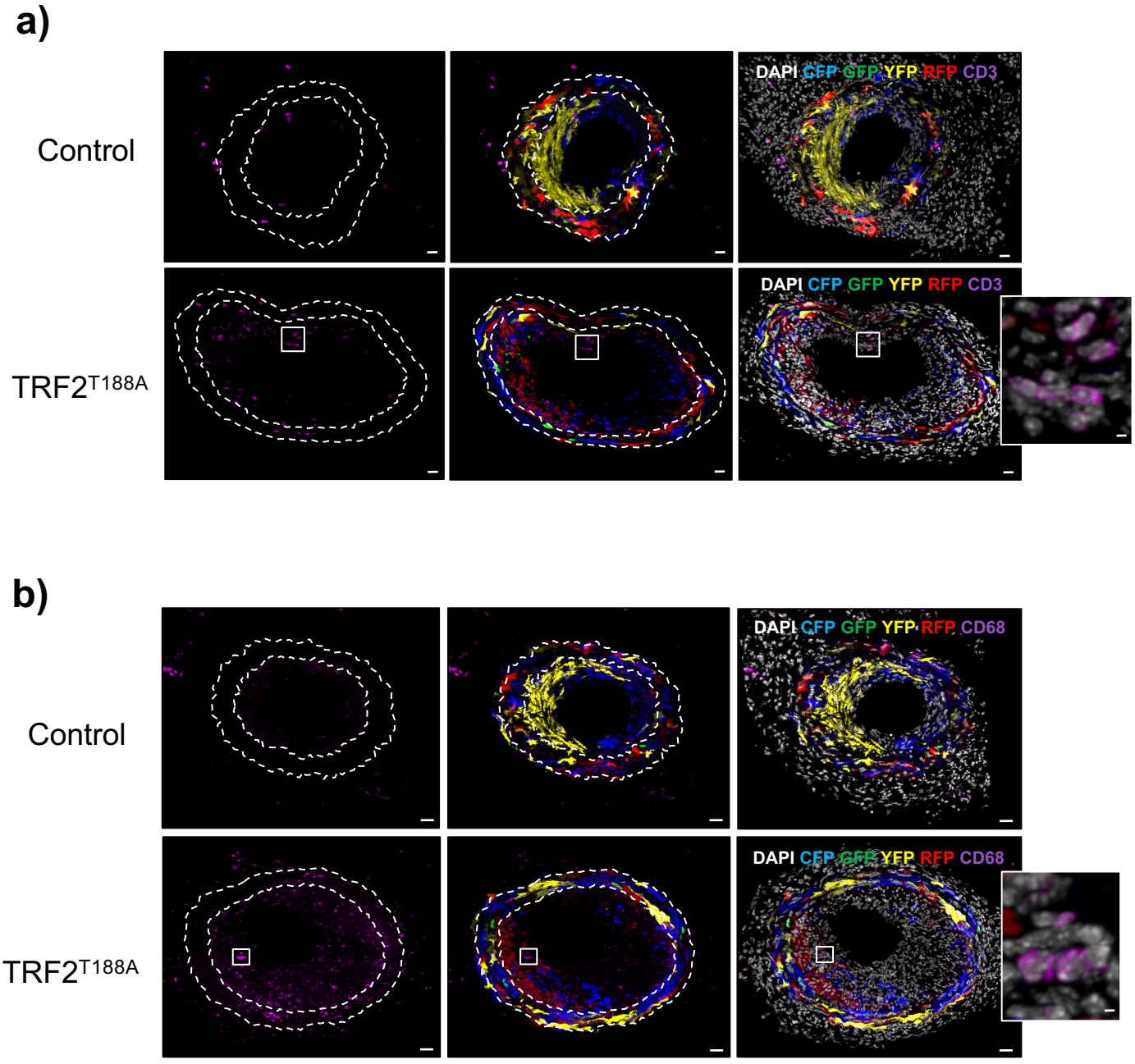
Supplementary Figure 5. VSMC labeling efficiency in TRF2^{T188A} mice and littermate controls. **a)** Schematic of *SM22α-TRF2^{T188A}/Myh11-Cre^{ERTM}* and *Rosa26-Confetti* transgenic constructs. **b)** Right common carotid artery (RCCA) sections derived from *TRF2^{T188A}* mice and littermate controls. Scale bar=200μm. **c)** Percentage of total confetti$^+$ cells and **d)** each confetti reporter color quantified on longitudinal sections of unligated RCCA of *TRF2^{T188A}* animals and littermate controls. Data shown in **(c-d)** represent Means±SEM, n≥6 mice for each group, ns-non significant (p>0.05), Mann-Whitney U test.

Supplementary Figure 6



Supplementary Figure 6. TRF2^{T188A} promotes outward remodeling in response to injury
a) Internal elastic lamina, **b)** external elastic lamina and **c)** lumen area of Control (Ctr) or *TRF2^{T188A}* mice using six serial sections 100μm apart (left panels). Right panels represent maximum area. Data represent Means±SEM, n≥5 mice for group, ns-non significant (p>0.05), Mann-Whitney U test.

Supplementary Figure 7

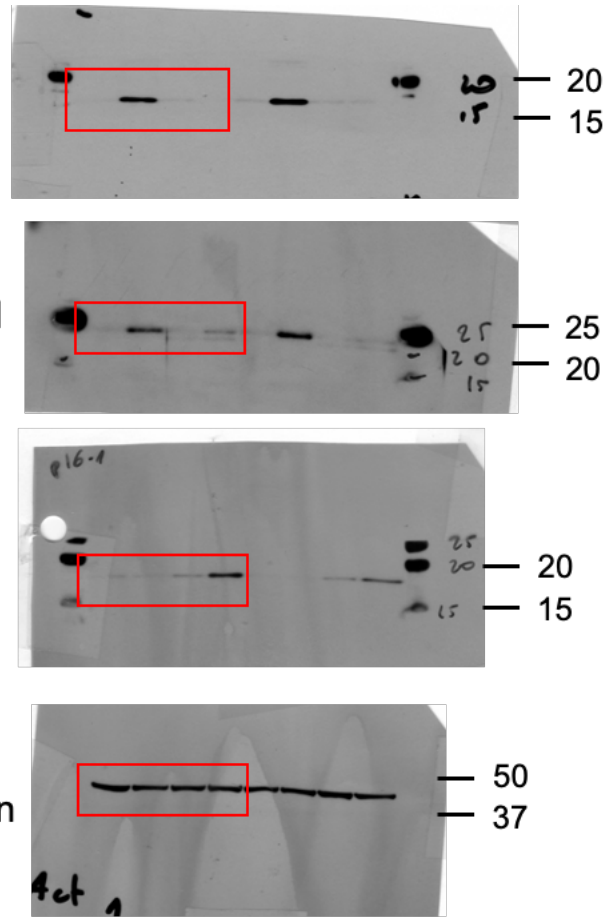


Supplementary Figure 7. Enhanced infiltration of lymphocytes and macrophages in TRF2^{T188A} mice.
LCCA cryosections of *TRF2^{T188A}* and control mice 28d following carotid ligation stained with **a)** CD3 (magenta) or **b)** CD68 (magenta) and counterstained with DAPI (white). Outlined regions show cluster of cells that are CD3^{+} or CD68^{+}. Scale bars=30μm and 4.8μm (inset).

Supplementary Figure 8

Uncropped gels

Fig 1g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15
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25
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p16
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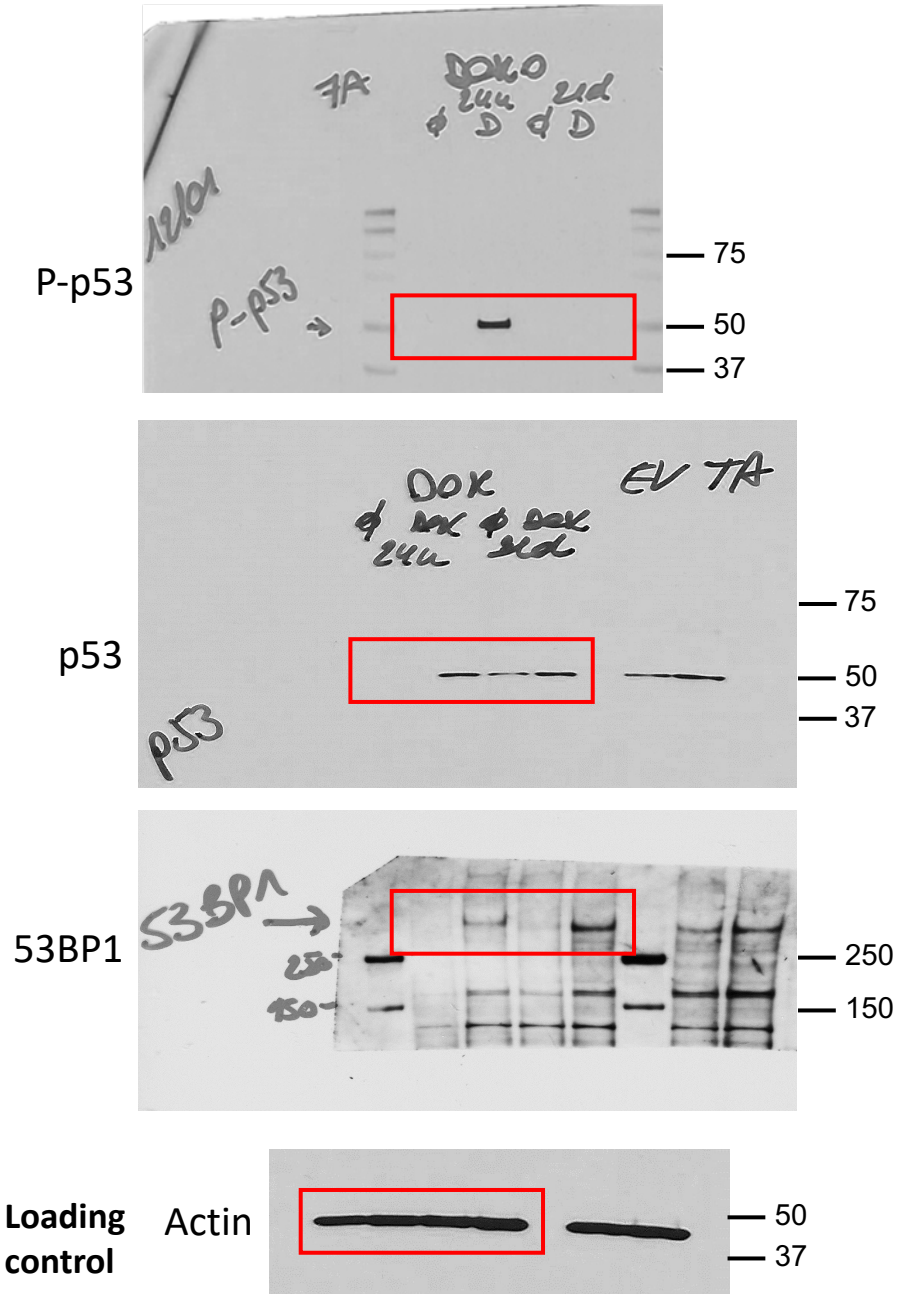
Actin
50
37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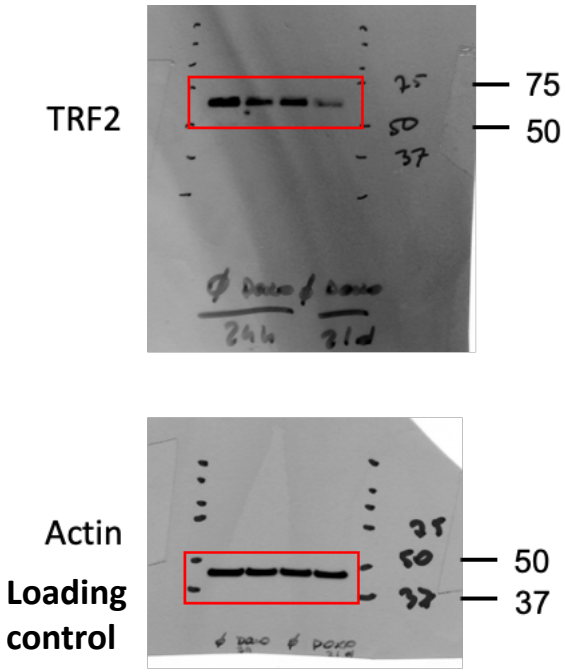
Actin
50
37

Fig 1j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Actin
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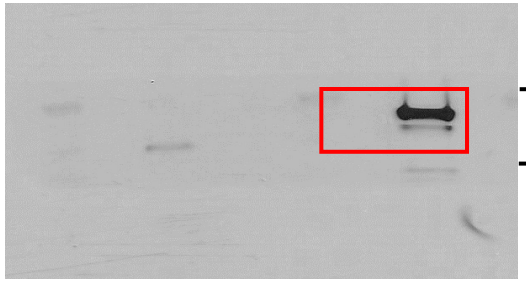
37

Fig 2d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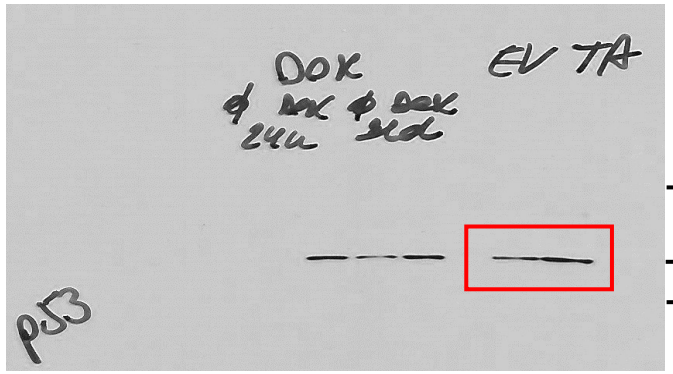
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EV TA
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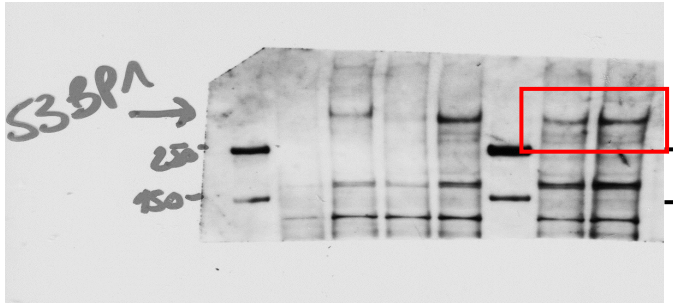
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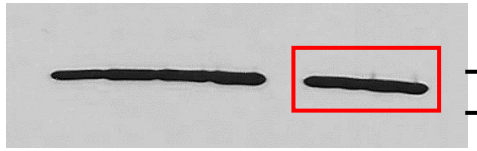
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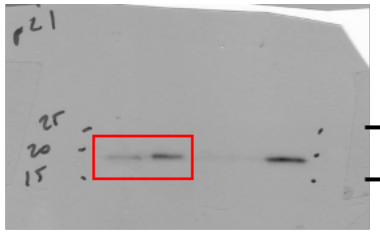
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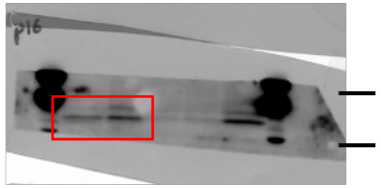
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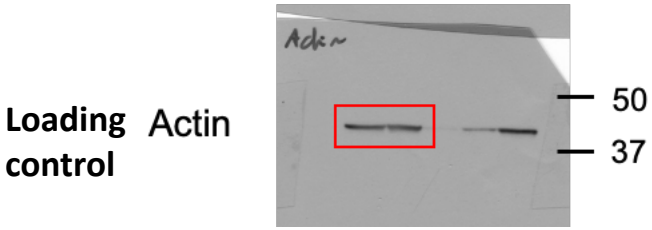
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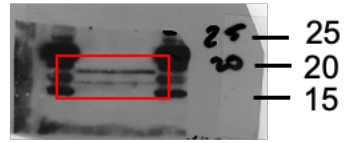
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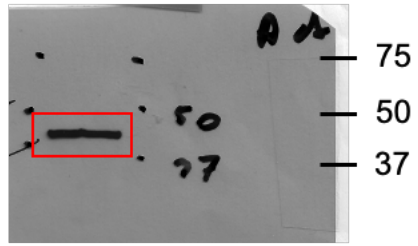
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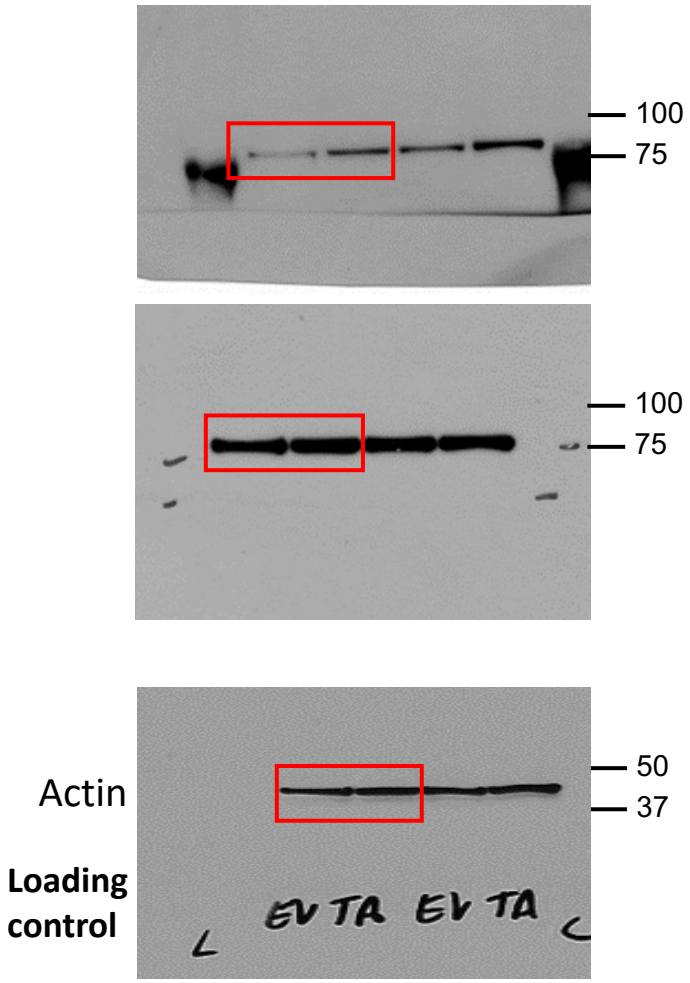
Fig 4d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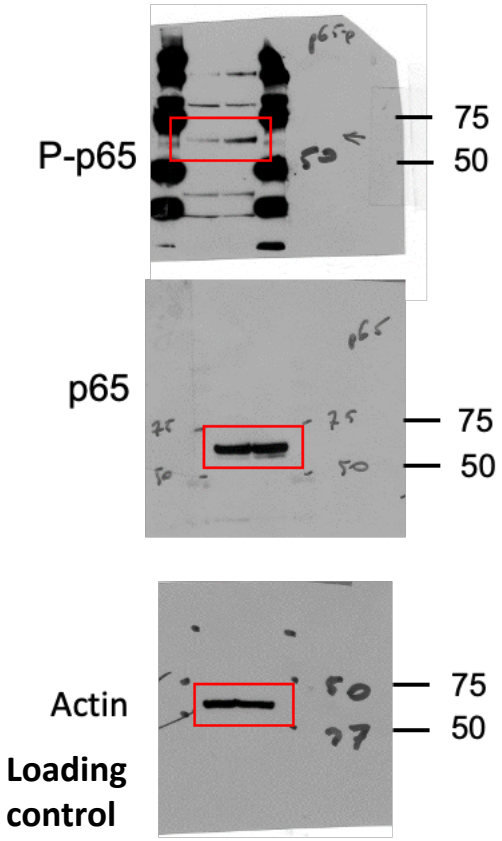
EV TA EV TA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Actin
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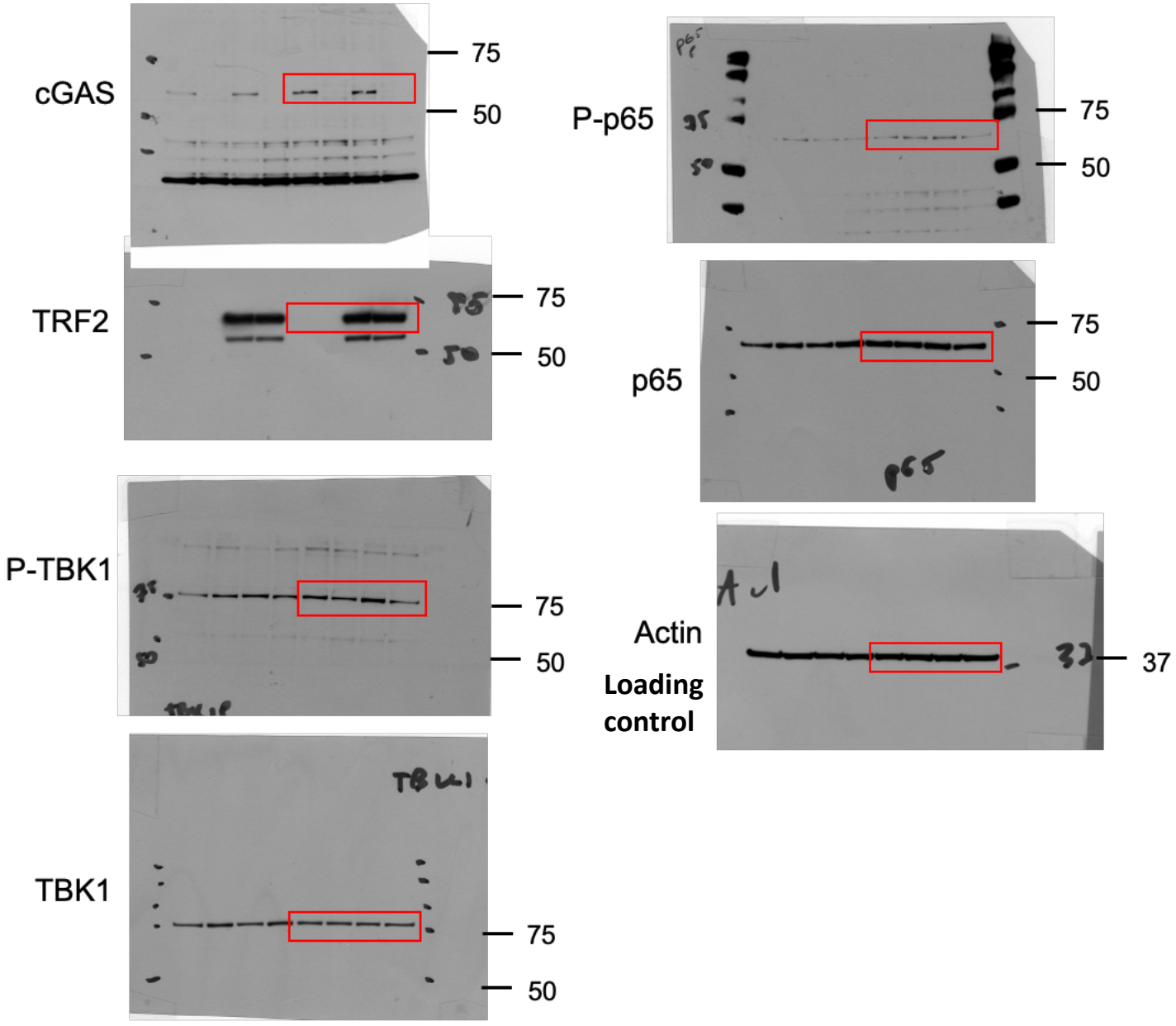
Fig 4f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Actin
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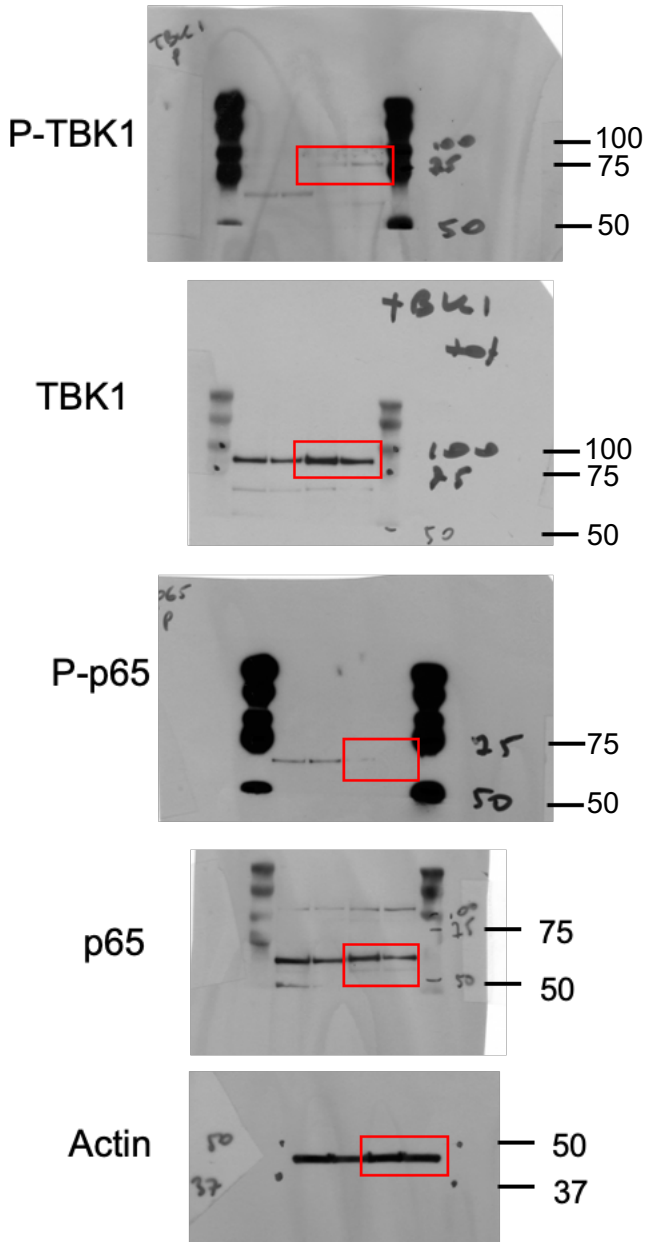
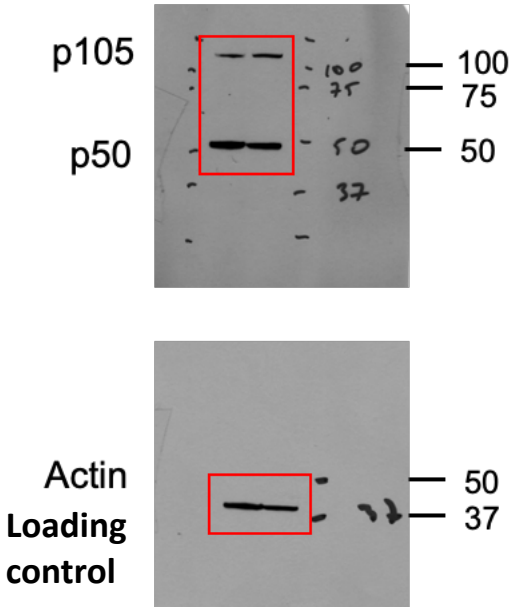
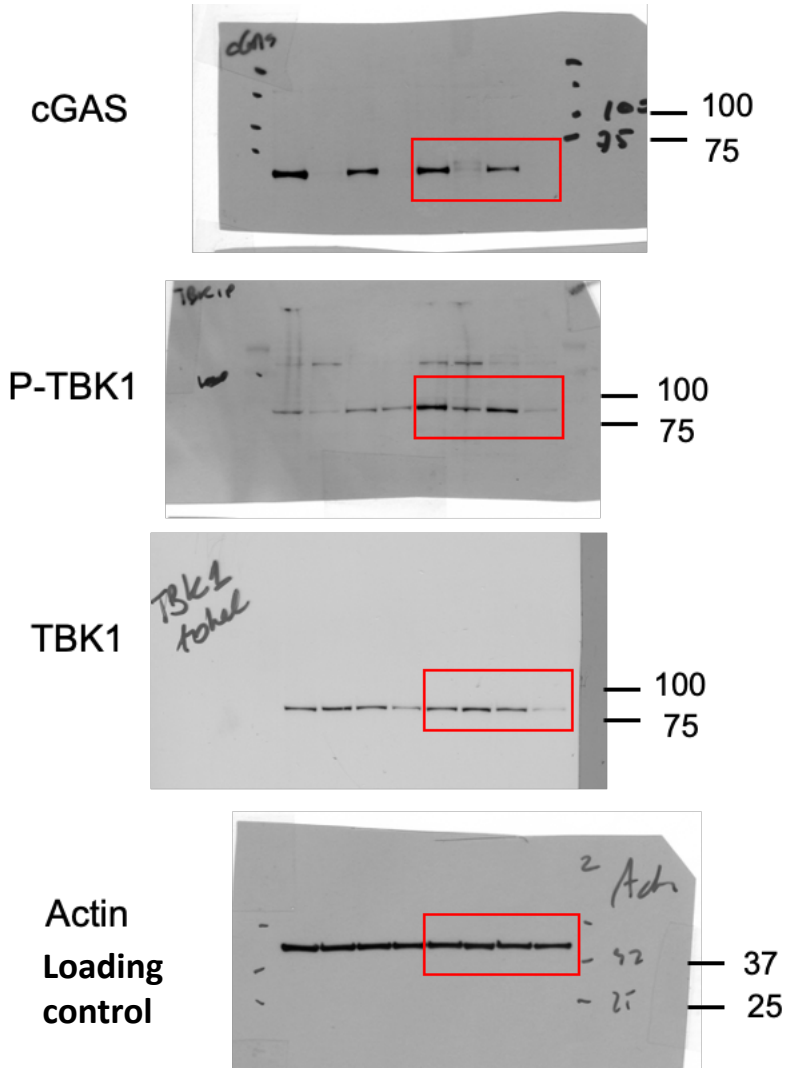
37

Supplementary Fig 3d





Supplementary Fig 4a



Human DNA primers	
IL1α	Fw 5'- ACTGCCCAAGATGAAGACCA -3' Rv 5'- TGGTCTCACTACCTGTGATGG -3'
IL1β	Fw 5'- TCGCCAGTGAAATGATGGCT -3' Rv 5'- TGGAAGGAGCACTTCATCTGTT -3'
IL8	Fw 5'- AGAGAGCTCTGTCTGGACCC -3' Rv 5'- CTCAGCCCTCTTCAAAAACTTCT -3'
IL6	Fw 5'- CATCCTCGACGGCATCTCAG -3' Rv 5'- TCACCAGGCAAGTCTCCTCA -3'
CCL2	Fw 5'- CTCAGCCAGATGCAATCAATG -3' Rv 5'- CTTCTTTGGGACACTTGCTGC -3'
CCL20	Fw 5'- AACCATGTGCTGTACCAAGAGT -3' Rv 5'- AAGTTGCTTGCTTCTGATTCGC -3'
CXCL10	Fw 5'- CCAGAATCGAAGGCCATCAA -3' Rv 5'- CATTTCCTTGCTAACTGCTTTCAG -3'
IRF3	Fw 5'- TCGTGATGGTCAAGGTTGT-3' Rv 5'- AGGTCCACAGTATTCTCCAG-3'
IRF7	Fw 5'- CCTCTCCAGATGCCAGTCCC -3' Rv 5'- AAGGAGCCACTCTCCGAACA -3'
ISG56	Fw 5'- CAAAGGGCAAAACGAGGCAG -3' Rv 5'- CCCAGGCATAGTTTCCCCAG -3'
p16	Fw 5'- CATAGATGCCGCGGAAGGT -3' Rv 5'- AAGTTTCCCGAGGTTTCTCAGA -3'
p21	Fw 5'- GACTCTCAGGGTCGAAAACG -3' Rv 5'- GGATTAGGGCTTCCTCTTGG -3'
RPL13A	Fw 5'- CGAGGTTGGCTGGAAGTACC -3' Rv 5'- CCGTAGCCTCATGAGCTGTT -3'
TeloC (for RT)	Rv 5'- CCCTAACCCTAACCCTAA -3'
TeloG (for RT)	Rv 5'- TAGGGTTAGGGTTAGGG -3'
Telo (for qPCR)	Fw 5'- CGGTTTGTTTGGGTTTGGGTTTGCCTTTGCCTTTGGGTT -3' Rv 5'- GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT -3'
RPP0	Fw 5'- TTCATTGTGGGAGCAGAC -3'
RPP0	Rv 5'- CAGCAGTTTCTCCAGAGC -3'

Supplementary Table 1

List of QPCR primers