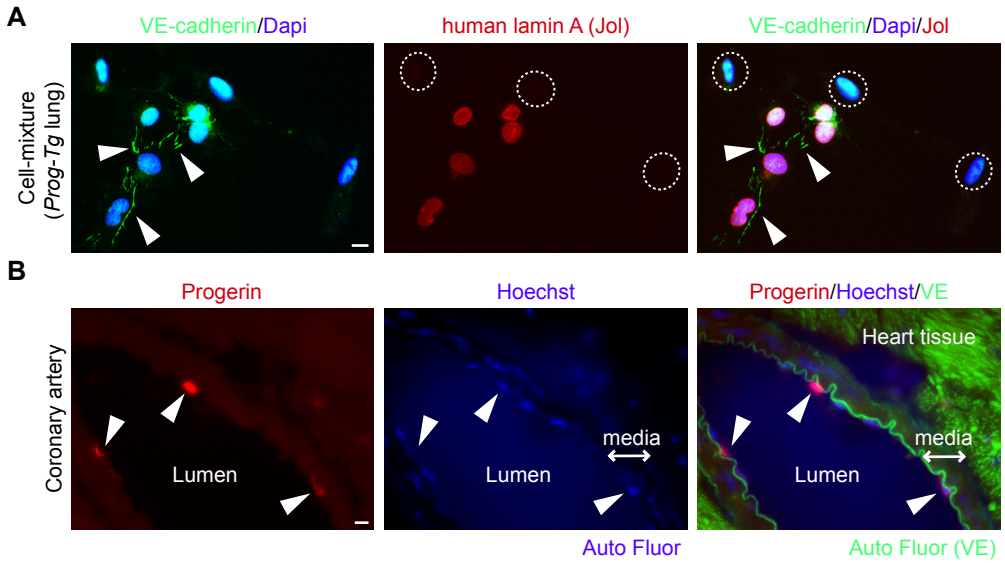
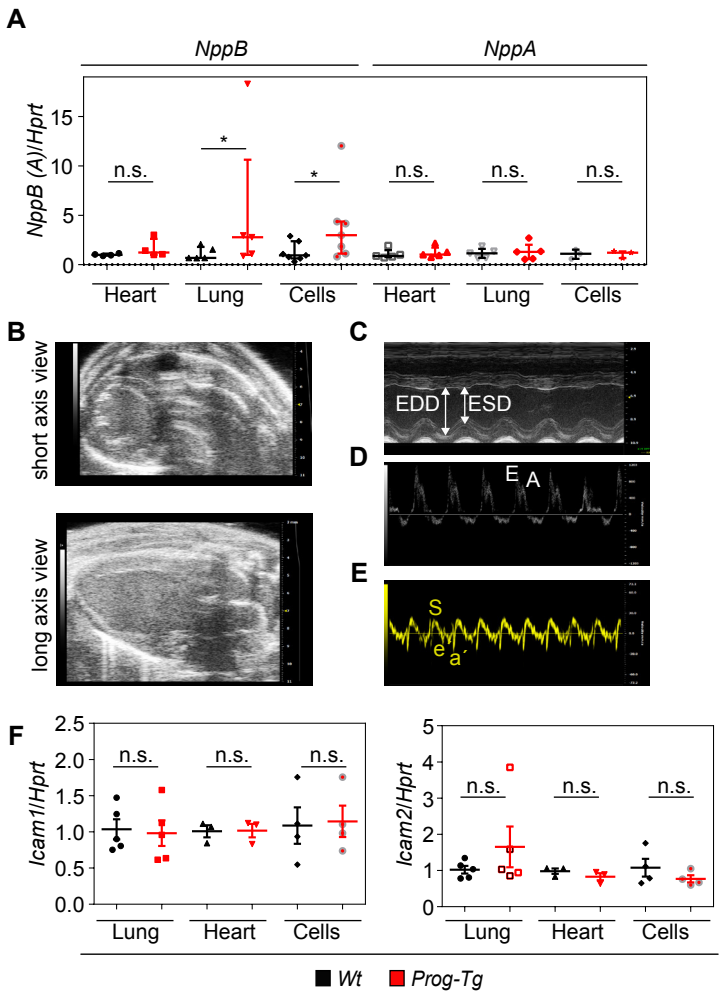
**SUPPLEMENTAL DATA, Osmanagic-Myers et al.**

1. Supplemental Figures and Figure Legends

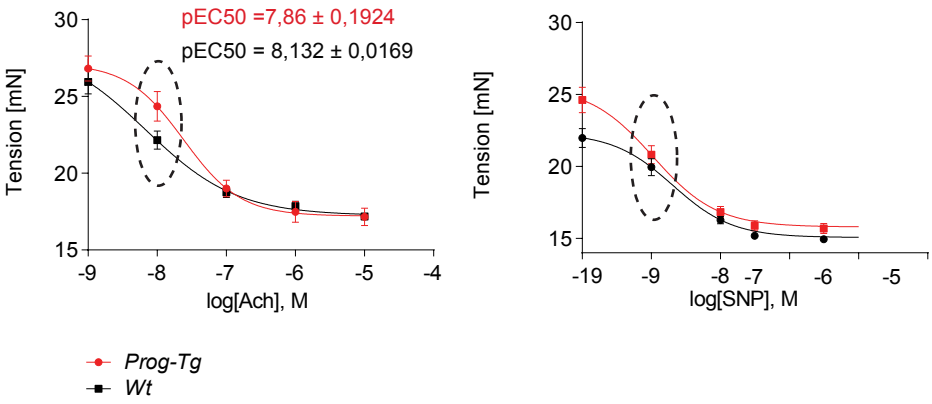
2. Supplemental Tables



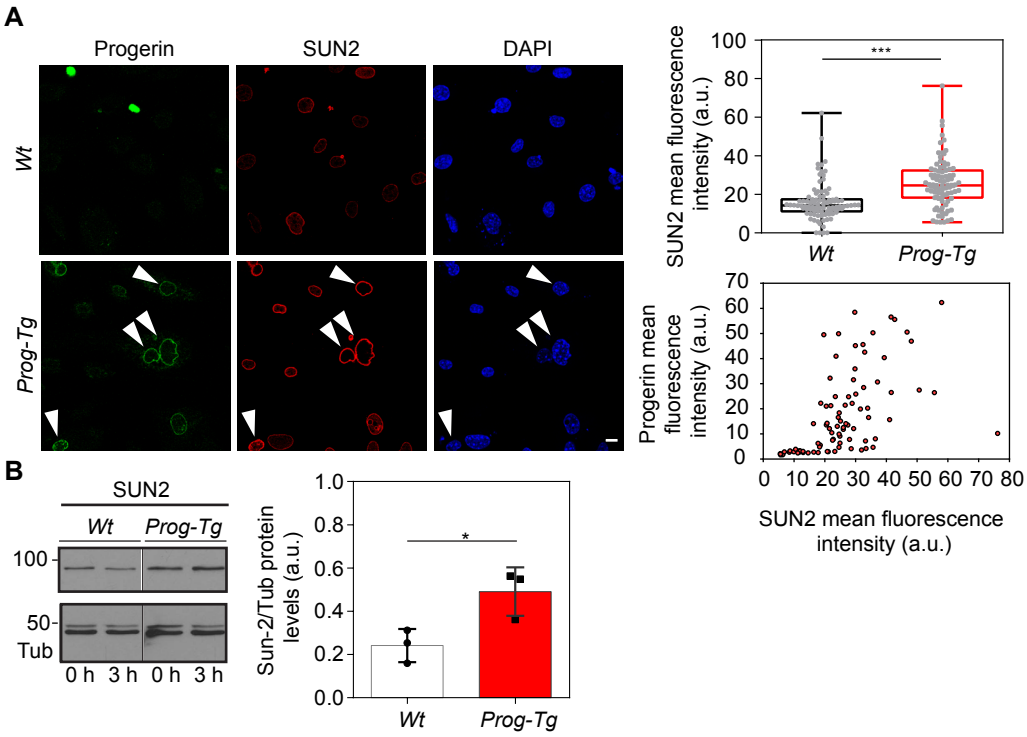

**Supplemental Figure 1. Endothelium-specific expression of human lamin A and progerin in *Prog-Tg* mice. (A)** Immunofluorescence microscopy of a mixture of cell types obtained from lung tissue using antibodies to human lamin A (Jol) and to endothelial marker VE-cadherin. Arrowheads, VE-cadherin marked endothelial-specific cell-cell junctions. Encircled cells, non-endothelial cells (absence of VE-cadherin cell-cell junctions). Note that only cells positively stained for VE-cadherin junction marker were detected positive for human lamin A. Scale bar, 10 µm.
**(B)** Immunofluorescence image of larger area from coronary artery (compared to Figure 1C) showing surrounding cardiac tissue from *Prog-Tg* animals stained with progerin antibody, VE-cadherin antibody and DNA dye Hoechst. Progerin expression is confined to intimal layer (arrowheads) and absent in surrounding area. Hoechst and green autofluorescence signals are used to identify internal- and external elastic lamina (IEL) to mark boundaries of the medial layer (media, double arrow). Note, that the VE-cadherin marked intimal layer signal merges with the IEL autofluorescence signal. Scale bar, 10 µm. (A and B, representative of n=3 independent experiments).



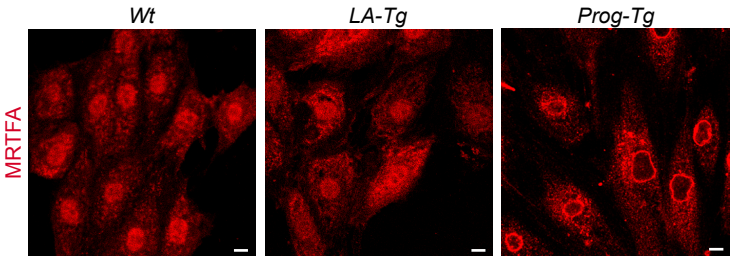

**Supplemental Figure 2. Analysis of hypertrophy and inflammatory markers and representative echocardiography measurements in the heart. (A)** Expression of *NppB* and *NppA* in heart and lung tissue of *Prog-Tg* animals at ~25 weeks (n=5 littermate pairs) and in *Prog-Tg* ECs (n=7) normalized to hypoxanthine phosphoribosyltransferase 1 (*Hprt*) and compared to those from *Wt* littermates (fold change) and *Wt* ECs, respectively. n.s. not significant, *p<0.05. Wilcoxon signed rank test (littermate tissues) and Mann-Whitney U test (cells). Median (middle line) and whiskers, 25th and 75th percentile. **(B)** Representative echocardiography 2D mode with a short and long axis view of the left ventricle allowing to obtain a M Mode tracing of the left ventricle in **(C)** in order to evaluate the left ventricle EF, diameters and wall thickness. **(D)** Representative mitral Doppler flow profile for measurement of IVRT and the combined parameter E/e'. **(E)** Representative tissue Doppler tracing of the septum at the mitral annulus level. (B-E) representative of n=5 littermate pairs. **(F)** Gene expression of *Icam1* and *Icam2* in lung, heart and isolated lung endothelial cell cultures (n= 5 and 3 littermate pairs, n=4 independent experiments for cells). All mice were at the age of ~25 weeks. Values normalized to *Hprt* were compared to those from *Wt* littermate or *Wt* cells (fold change). n.s. not significant. Paired (littermate tissues) and unpaired (cells) Student's t-test. Mean and error bars indicate SEM.




**Supplemental Figure 3. Vascular reactivity in isolated aortic segments of *Prog-Tg* mice.** Aortic segments were isolated from mice and were mounted onto a Multi Wire Myograph System (DMT 620 M) for determination of endothelium-dependent relaxation and endothelium-independent relaxation by cumulatively increasing concentrations of acetylcholine (Ach, $10^{-9}$ to $10^{-5}$ mol/l) and sodium nitroprusside (SNP, $10^{-10}$ to $10^{-6}$ mol/l), respectively. Tension in (mN) and log (concentration) in (mol/l) is shown (n=4 animals per genotype; age>30 weeks). Note that in *Prog-Tg* versus *Wt* aortic segments a tendentiously prolonged lag phase and reduced pEC50 values (p=0.2) were observed only for endothelium-dependent relaxation. Error bars denote mean ± SEM.




**Supplemental Figure 4. Accumulation of SUN2 in *Prog-Tg* endothelial cells. (A)** Representative image of endothelial cells from *Wt* and *Prog-Tg* mice immunostained with SUN2 antibody (representative of n=3 independent experiments). Scale bar, 10 µm. Box plots show the distribution of SUN2 mean fluorescence intensities in *Prog-Tg* and *Wt* endothelial cells (n=100 cells per genotype). *** p<0.001, Mann-Whitney U test. Median (middle line) with boxes encompassing 25th to 75th percentile and whiskers, minimum to maximum values Lower panel, mean progerin fluorescence intensities plotted over mean SUN2 fluorescence intensities in *Prog-Tg* cells (n=87 cells). Arbitrary units (a.u.) of fluorescence are shown. (B) Quantitative immunoblot analysis of lysates (0 h, untreated) from *Wt* and *Prog-Tg* endothelial cells (*Wt* and *Prog-Tg* samples from different parts of the same gel; n=3), * p<0.05, unpaired Student's t-test. Mean and error bars indicate SEM. 3h, flow exposed samples.




**Supplemental Figure 5. MRTFA localization is unaltered in *LA-Tg* endothelial cells.** Endothelial cells isolated from *Wt*, *LA-Tg* and *Prog-Tg* mice were processed for immunofluorescence microscopy using MRTFA antibody (representative of n=3 independent experiments). Scale bars, 10 µm.

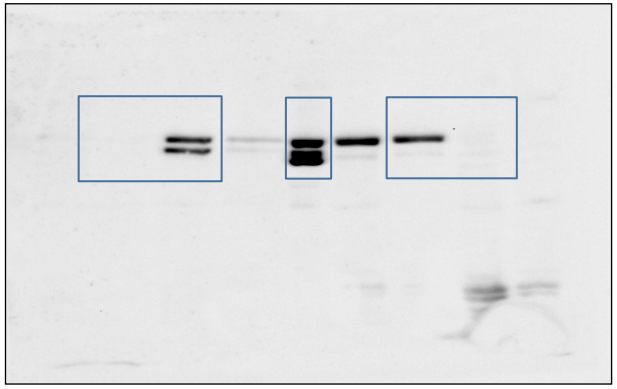
**Supplemental Table 1. Hemodynamic and echocardiographic parameters**.

| **Hemodynamic parameters** | **LM_*Prog-Tg*** | ***Prog-Tg*** | **LM_*LA-Tg*** | ***LA-Tg*** |
|---|---|---|---|---|
| Heart rate [beats/min] | 410 ± 24 | 441 ± 20 | 445 ± 39 | 420 ± 21 |
| LVEDP [mmHg] | 2.12 ± 0.20 | 3.99 ± 0.35** | 2.52 ± 0.36 | 2.60 ± 0.36 |
| LVSP [mmHg] | 85.2 ± 2.3 | 99.8 ± 4.9* | 84.2 ± 4.4 | 84.75 ± 4.18 |
| LV +dP/dt [mmHg/s] | 5368 ± 348 | 6009 ±514‡ | 5857 ± 618 | 4871 ±417 |
| LV -dP/dt [mmHg/s] | -5465 ± 428 | -5177 ± 605 | -5355 ± 650 | -4573 ± 384 |
| WS_dias_posterior [mm Hg] | 5.8 ± 0.5 | 7.7 ± 0.4* | 6.7 ± 0.95 | 6.3 ± 1.41 |
| WS_diastolic_septum [mm Hg] | 5.1 ± 0.6 | 7.5 ± 0.5* | 5.8 ± 0.6 | 7.16 ± 1.32 |
| WS_systolic_posterior [mm Hg] | 92.5 ± 9.8 | 96.8 ± 15.4 | 113 ± 7.7 | 108 ± 10.8 |
| WS_systolic_septum [mm Hg] | 77.8 ± 6.4 | 91.5 ± 7.5 | 106.5 ± 8.9 | 102 ± 10.6 |
| | | | | |
| **Echocardiographic parameters** | **LM_*Prog-Tg*** | ***Prog-Tg*** | **LM_*LA-Tg*** | ***LA-Tg*** |
| | | | | |
| Ejection fraction [%] | 62.8 ± 2 | 65.1 ± 2 | 60.6± 1.28 | 61.2± 4.01 |
| EDD/BW [mm/g] | 0.149 ± 0.033 | 0.155 ± 0.031 | 0.138 ± 0.013 | 0.142 ± 0.023 |
| ESD/BW [mm/g] | 0.094 ± 0.018 | 0.101 ± 0.026 | 0.089 ± 0.02 | 0.086 ± 0.03 |
| EDSW/BW [mm/mg] | 0.298 ± 0.020 | 0.397 ± 0.030* | 0.247 ± 0.024 | 0.254 ± 0.048 |
| EDPW/BW [mm/mg] | 0.276 ± 0.025 | 0.363 ± 0.037* | 0.258 ± 0.019 | 0.296 ± 0.036 |
| ESSW/BW [mm/g] | 0.051 ± 0.013 | 0.05 ± 0.008 | 0.038 ± 0.01 | 0.04 ± 0.01 |
| ESPW/BW [mm/g] | 0.036 ± 0.009 | 0.053 ± 0.009 | 0.037 ± 0.01 | 0.038 ± 0.01 |
| E/e´ ratio | 3.03 ± 0.10 | 2.92 ± 0.16 | 2.67 ± 0.08 | 2.77 ±0.07 |
| IVRT [ms] | 19.45 ± 0.5 | 17.7 ± 0.56* | 18.33 ± 0.71 | 19.05 ± 0.7 |
| MGTA [mmHg] | 4.2 ± 0.21 | 4.7 ± 0.28 | 4.4 ± 0.26 | 4.5 ± 0.27 |
| | | | | |

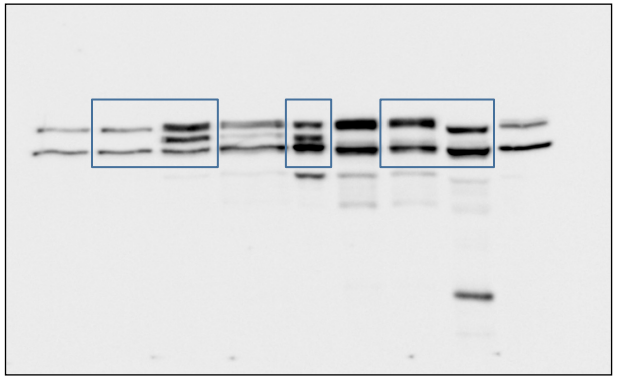
**Supplemental Table 1. Hemodynamic and echocardiographic parameters**. *Prog-Tg* and *LA-Tg* and corresponding littermates were analyzed (age >27 weeks; n=11 Wt, n=5 *LA-Tg*, n=6 *Prog-Tg*;). LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; LV +dP/dt, maximal value of the first derivative of LV pressure; LV -dP/dt, minimal value of the first derivative of LV pressure; WS, wall stress; EDD, end-diastolic diameter; ESD, end-systolic diameter; EDSW, end-diastolic septal wall width; EDPW, end-diastolic posterior wall width; ESSW, end-systolic septal wall width, ESPW, end-systolic posterior wall width; E/e´, Ratio of E to e'; IVRT, isovolumic relaxation time; MGTA, mean gradient of transverse aorta. ‡ trend $p<0.08$,*$p<0.05$ and **$p<0.01$ Tg vs LM,. Unpaired Student´s *t*-test. Mean and error bars indicate SEM.

**Supplemental Table 2. Primers used for quantitative real-time PCR analysis**

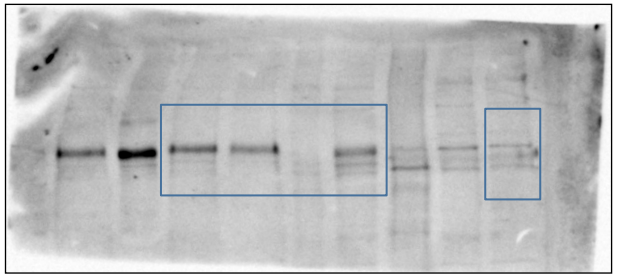
| Gene | Genbank accession number | Primer Sequences |
|---|---|---|
| ***Cdh5*** | NC_000074.6 | Forward: 5'-CTGCTCACGGACAAGATCAGC-3'<br>Reverse: 5'-CTCTTTTGGCGATGGTGGGC-3' |
| ***Hprt*** | NM_013556.2 | Forward: 5'-GCAGTCCCAGCGTCGTGATTA-3'<br>Reverse: 5'-TGATGGCCTCCCATCTCCTTCA-3' |
| ***Nos3*** | NM_008713.4 | Forward: 5'-GCATGGGCAACTTGAAGAGTG-3'<br>Reverse: 5'-GCTGCCCACTTCCCAATTCT-3' |
| ***Col1a1*** | NM_007742.3 | Forward: 5'-CTGACGCATGGCCAAGAAGA-3'<br>Reverse: 5'-ATACCTCGGGTTTCCACGTC-3' |
| ***Col3a1*** | NC_000067.6 | Forward: 5'-TCCGGGAATAACGTCAGTC-3'<br>Reverse: 5'-GGAAGCCCATTTGCACCAGG-3' |
| ***Col4a5*** | NM_001163155.1 | Forward: 5'-CCCAAGTGCACCAGCATAAC-3'<br>Reverse: 5'-AGAAGAACACCCATGGCAGG-3' |
| ***Actb*** | NM_007393.3 | Forward: 5'-ACAGCTTCTTTGCAGCTCCT-3'<br>Reverse: 5'-TTGTCGACGACCAGCGCA-3' |
| ***Nppb*** | NM_008726.5 | Forward: 5'-GGGCACAAGATAGACCGGAT-3'<br>Reverse: 5'-GCCAGGAGGTCTTCCTACAA-3' |
| ***Icam1*** | NC_000075.6 | Forward: 5'-CAGATGCCGACCCAGGAGAG-3'<br>Reverse: 5'-CCGCTAGCTCCAAAACGCAG-3' |
| ***Icam2*** | NC_000077.6 | Forward: 5'-GCTCACCGGCACAGAGGAGA-3'<br>Reverse: 5'-TATGGGCTTCAGGGGCACAG-3' |
| ***Acta2*** | NP_031418.1 | Forward: 5'-GTACCACCATGTACCCAGGC-3'<br>Reverse: 5'-GAAGGTAGACAGCGAAGCCA-3' |
| ***Nos3 (promoter, ChIP)*** | NP_032739.3 | Forward: 5'-CCCTCTAGCAGACAACCCAC-3'<br>Reverse: 5'-CTCTCAGATGCTGGCCTTCG-3' |
| ***Nos3 (gene body, ChIP)*** | NP_032739.3 | Forward: 5'-AAGTGGGCAGCATCACCTAC-3'<br>Reverse: 5'-GGGACCAGGCCTAGAAACAC-3' |



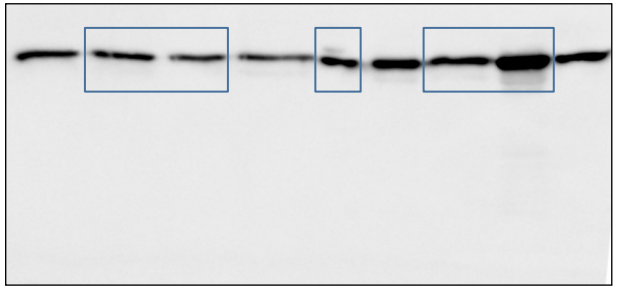
human lamin A



h+m lamin A



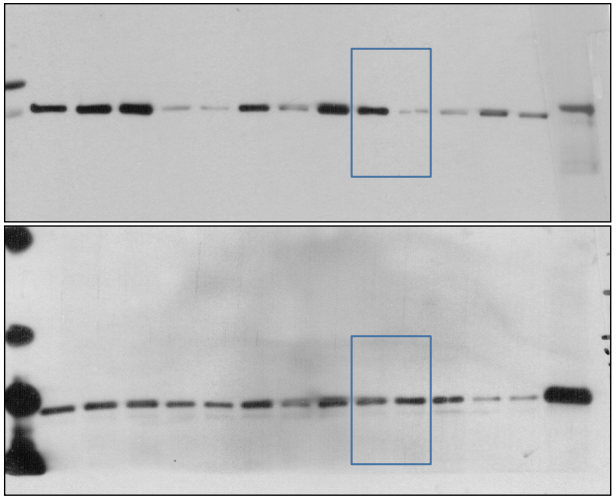
VE-cadherin



tubulin

* lower part of laminA (E1) blot separated and used for tubulin and also emerin (see Figure 5C)

Full unedited gels for Figure 1A.



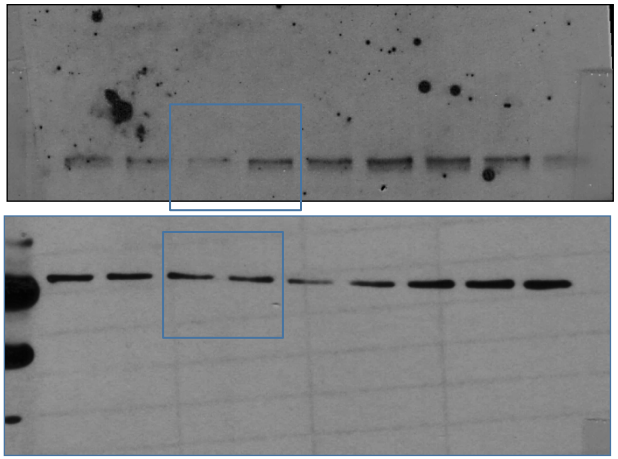
**upper gel part**

eNOS

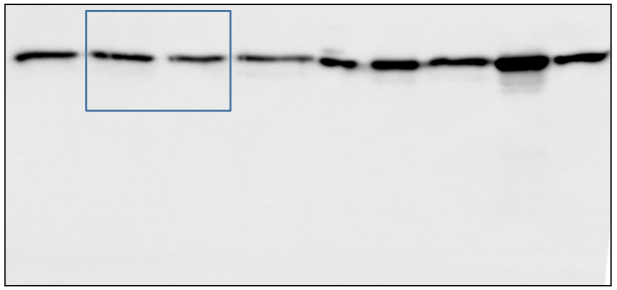
**lower gel part**

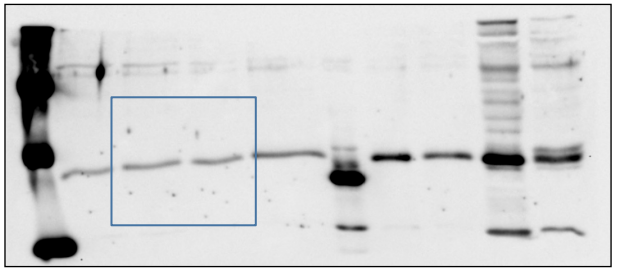
tubulin

Full unedited gels for Figure  4B.

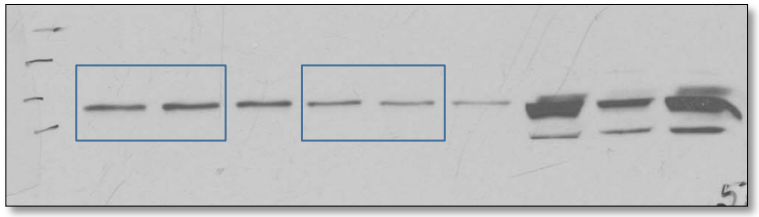



Full unedited gels for Figure 6B.



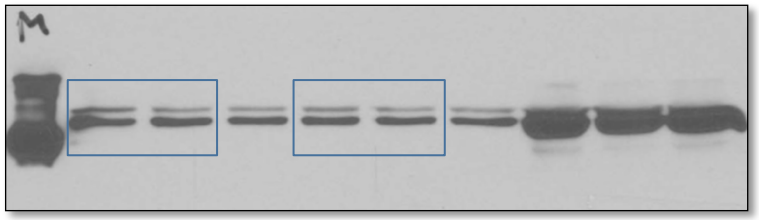




Full unedited gels for Figure 6C.



**upper gel part**

Sun2




**lower gel part**

tubulin

Full unedited gels for Supplemental Figure 4.