

**Type 2 diabetes progression differently affects endothelial function and vascular contractility in the aorta and the pulmonary artery**

Bernardete F. Melo<sup>1,\*</sup>, Jesus Prieto-Lloret<sup>1,\*</sup>, Marlene D. Cabral<sup>1</sup>, Fatima O. Martins<sup>1</sup>, Inês B. Martins<sup>1</sup>, Joana F. Sacramento<sup>1</sup>, Pedro Ruivo<sup>2</sup>, Tânia Carvalho<sup>2</sup> and Silvia V. Conde<sup>1</sup>

## Supplementary Data

**Table S1 – Composition of Standard diet**

<b>Composition</b>	<b>%</b>
<b>Proximate Analysis</b>	
Moisture	10.00
Crude Oil	4.16
Crude Protein	21.86
Crude Fibre	4.33
Ash	7.89
Nitrogen Free Extract	51.24
<b>Amino Acids</b>	
Arginine	1.39
Histidine	0.54
Isoleucine	0.96
Leucine	1.81
Lysine	1.30
Phenylalanine	1.20
Tyrosine	0.85
Alanine	0.27
Aspartic Acid	1.34
Glutamic Acid	4.30
Proline	1.53
Serine	0.98
<b>Fatty acids</b>	
Lauric	0.05
Myristic	0.17
Palmitic	0.37
Stearic	0.10
Myristoleic	0.01
Palmitoleic	0.09
Oleic	1.00
Linoleic	1.25
Linolenic	0.17
Arachidonic	0.12
<b>Fiber and Carbohydrates</b>	
Total Dietary Fibre	15.70
Pectin	1.47
Hemicellulose	9.33
Cellulose	4.04
Lignin	1.50
Starch	33.61
Sugar	5.84
<b>Energy</b>	
Gross Energy	15.10 MJ/kg
Digestible Energy	12.27 MJ/kg

Metabolizable energy	11.24 MJ/kg
Atwater Fuel Energy (AFE)	13.79 MJ/kg
AFE from Oil	11.35
AFE from Protein	26.51
AFE from Carbohydrate	62.13
<b>Minerals</b>	
Calcium	1.24
Phosphorus	0.80
Potassium	0.78
Magnesium	0.28
Sodium	0.24
Chloride	0.36
Fluorine	8.53 mg/kg
Iron	161.01 mg/kg
Zinc	46.90 mg/kg
Manganese	101.71 mg/kg
<b>Vitamins</b>	
Vitamin A	42522.66 IU/kg
Vitamin D-3 (added)	4369.41 IU/kg
Vitamin E	171.70 IU/kg
Vitamin K	40.72 mg/kg
Thiamin	49.77 mg/kg
Riboflavin	37.74 mg/kg

**Table S2 – Composition of 60% lipid-rich diet**

<b>Composition</b>	<b>%</b>
<b>Protein</b>	<b>23.1</b>
Arginine	0.90
Histidine	0.67
Isoleucine	1.24
Leucine	2.24
Lysine	1.88
Phenylalanine	1.24
Tyrosine	1.31
Threonine	1.0
Valine	1.47
Aspartic Acid	1.66
Glutamic Acid	5.28
Proline	3.04
Serine	1.43
<b>Fat</b>	<b>34.9</b>
Cholesterol	301 ppm
Linoleic Acid	4.70
Linolenic Acid	0.39
Arachidonic Acid	0.06
Omega-3 Fatty Acids	0.39
Total Saturated Fatty A	13.68
Total Monounsaturated Fatty Acids	14.00
Polyunsaturated Fatty Acids	5.15
<b>Fiber (max)</b>	<b>6.5</b>
<b>Carbohydrates</b>	<b>25.9</b>
<b>Energy</b>	<b>5.10 kcal/g</b>
<b>From:</b>	<b>%</b>
Protein	18.1
Fat	61.6
<b>Minerals</b>	
Calcium	0.79
Phosphorus	0.59
Potassium	0.77
Magnesium	0.07
Sodium	0.15
Chloride	0.25
Fluorine	1.2 ppm
Iron	65 ppm
Zinc	46 ppm

Manganese	76 ppm
<b>Vitamins</b>	
Vitamin A	5.2 IU/g
Vitamin D-3 (added)	1.3 IU/g
Vitamin E	67.2 IU/kg
Vitamin K	0.65 ppm
Thiamin	6.2 ppm
Riboflavin	8.7 ppm
Niacin	39 ppm
Pantothenic Acid	21 ppm

# Supplementary Data - Representative Western Blot images

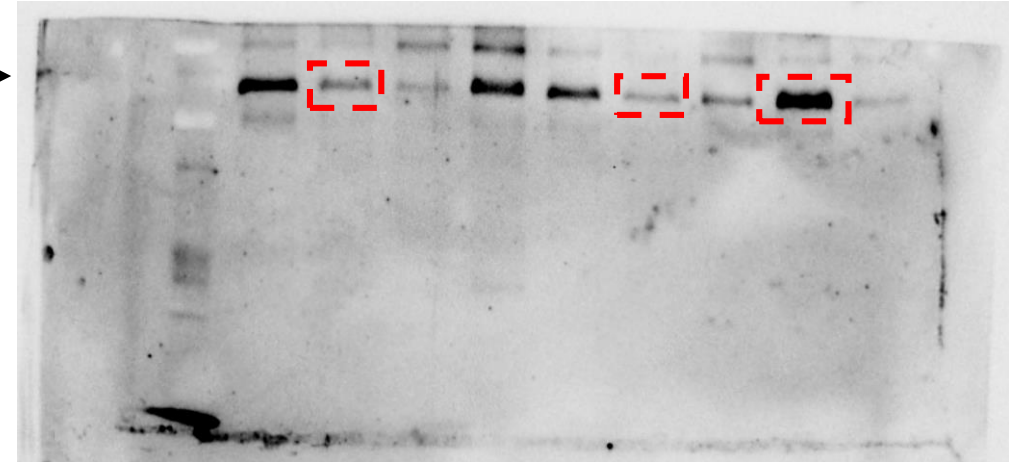
## Figure 3 b

PGF<sub>2α</sub> receptor

Aorta



PGF<sub>2α</sub>  
receptor  
60KDa

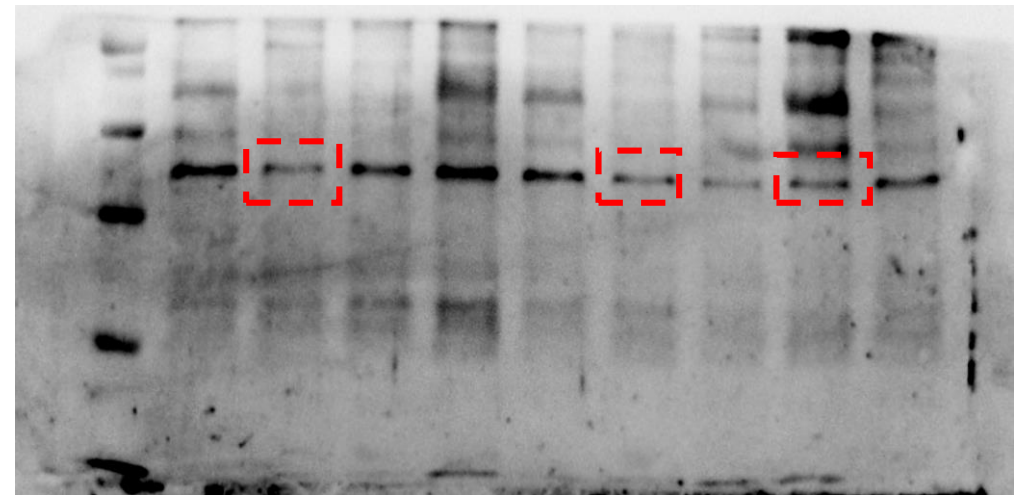


CTL

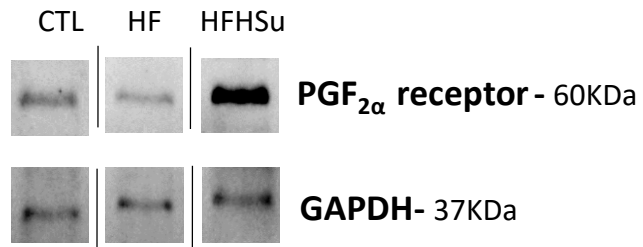
HF

HF HFSU

GAPDH  
37KDa



Representative bands  
cropped for the  
manuscript



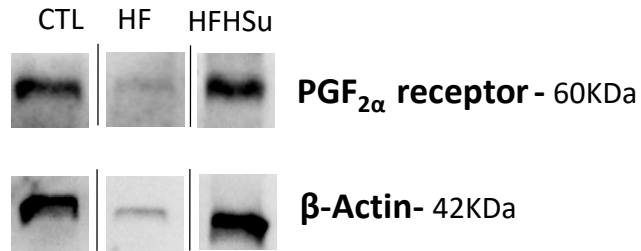
**Figure 3 b**

**PGF<sub>2α</sub> receptor**

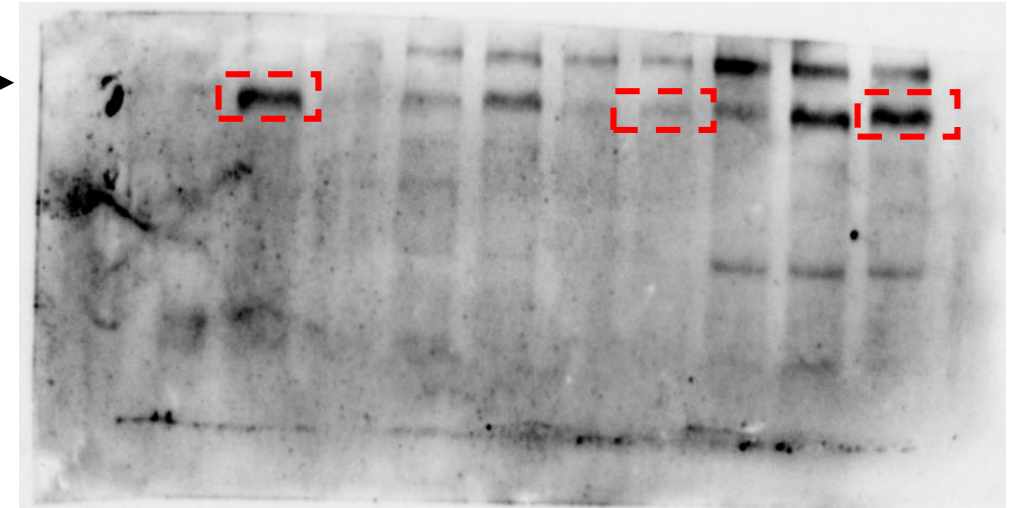
**Pulmonary Artery**



Representative bands  
cropped for the  
manuscript



**PGF<sub>2α</sub>  
receptor**  
60KDa

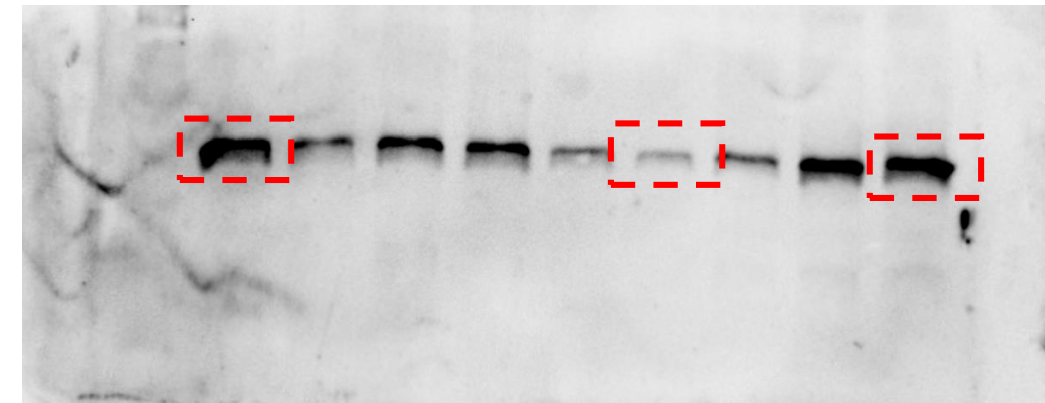


CTL

HF

HF HFSU

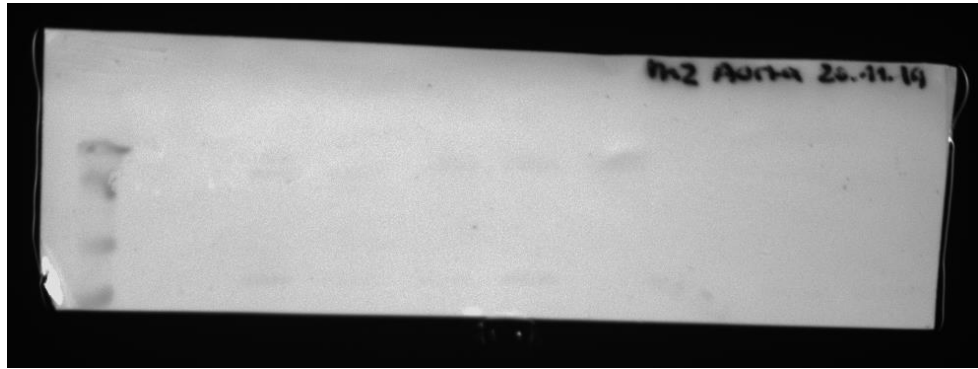
**β-Actin**  
42KDa



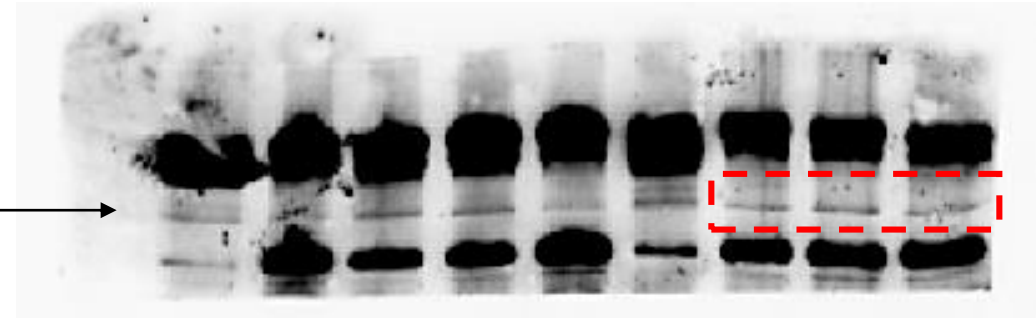
**Figure 4 b**

**iNOS**

**Aorta**



**iNOS**  
130KDa



CTL

HF

HFHSu

CTL

HF

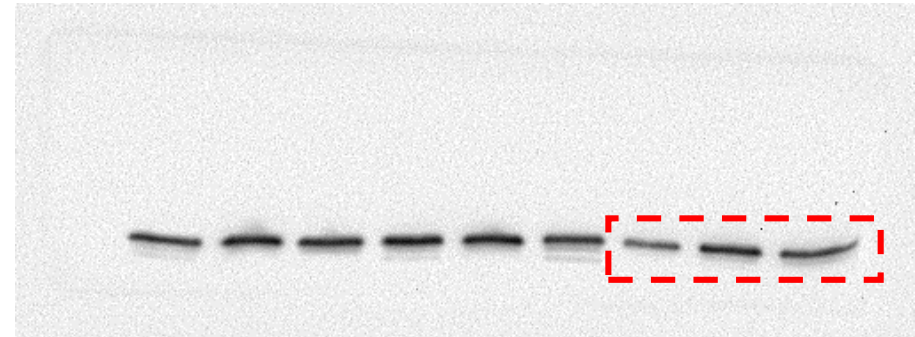
HFHSu

CTL

HF

HFHSu

**Calnexin**  
90KDa





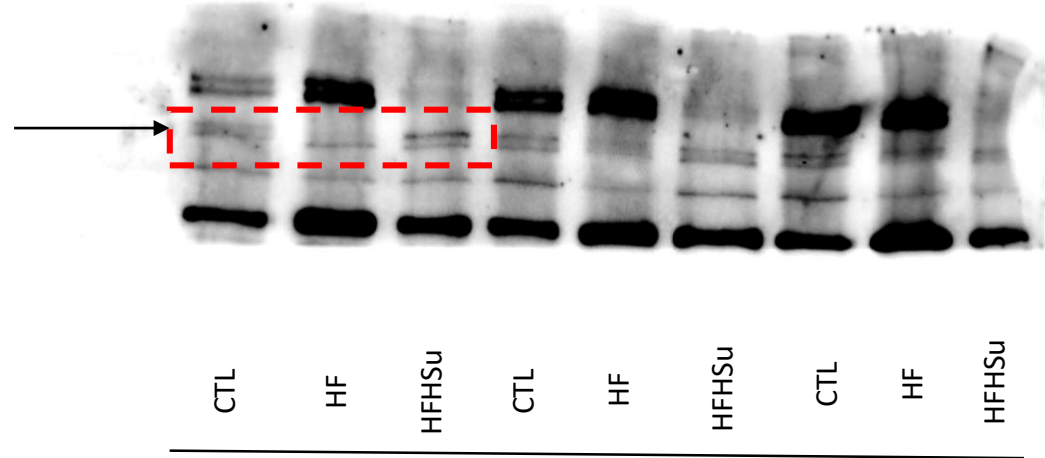
**Figure 4 b**

**iNOS**

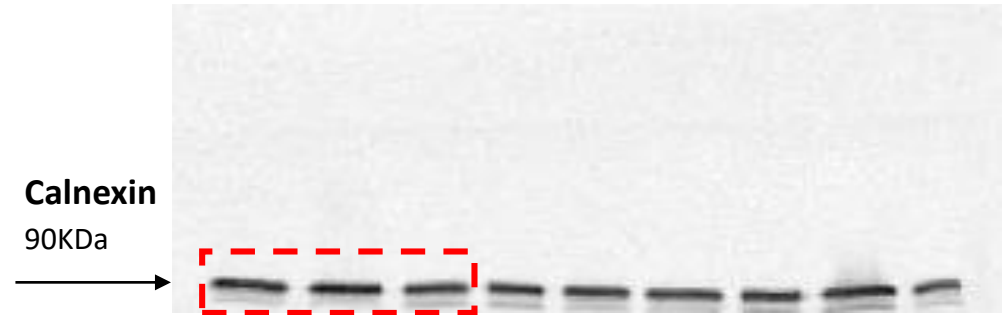
**Pulmonary artery**



**iNOS**  
130KDa



**Calnexin**  
90KDa



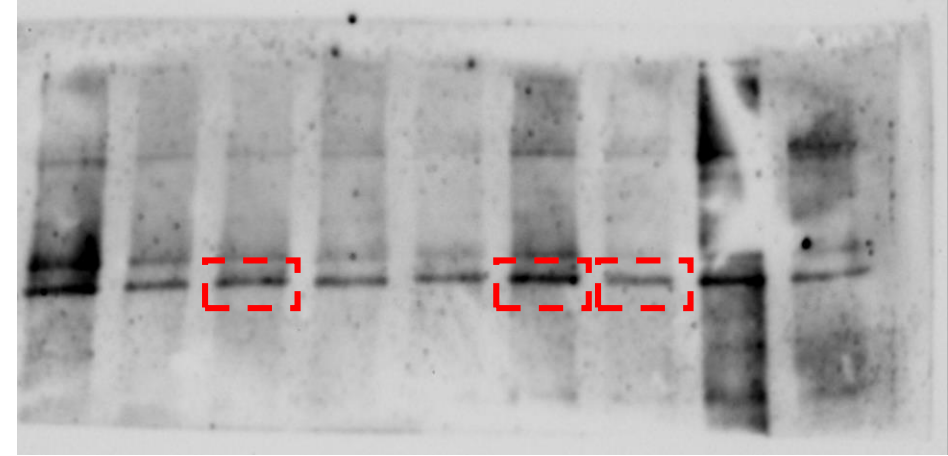
**Figure 4 c**

**eNOS**

**Aorta**

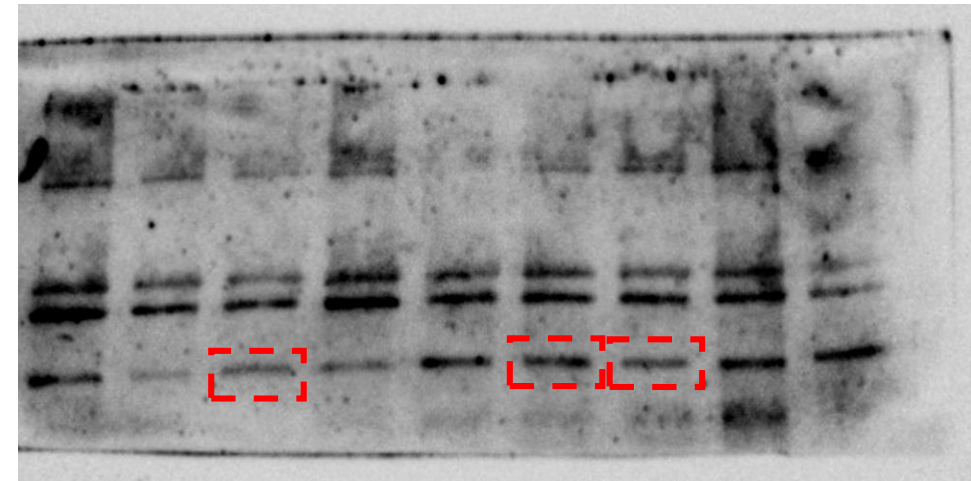


eNOS  
140KDa  
→

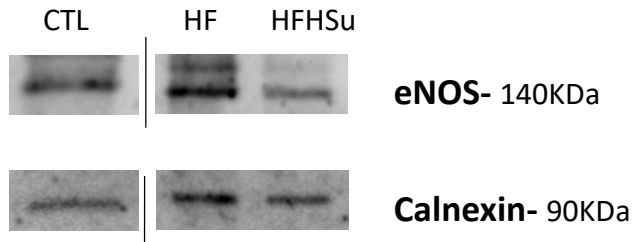


CTL      HF      HF HFSU

Calnexin  
90KDa  
→



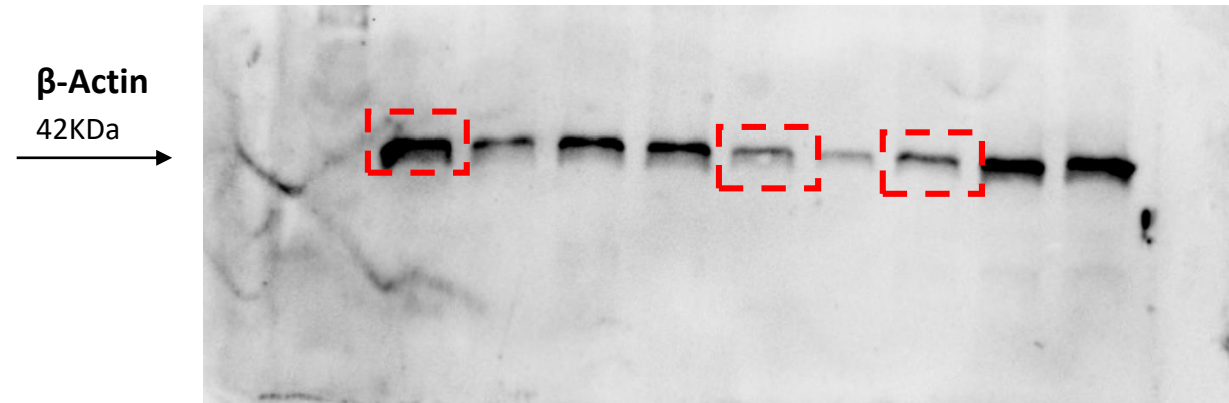
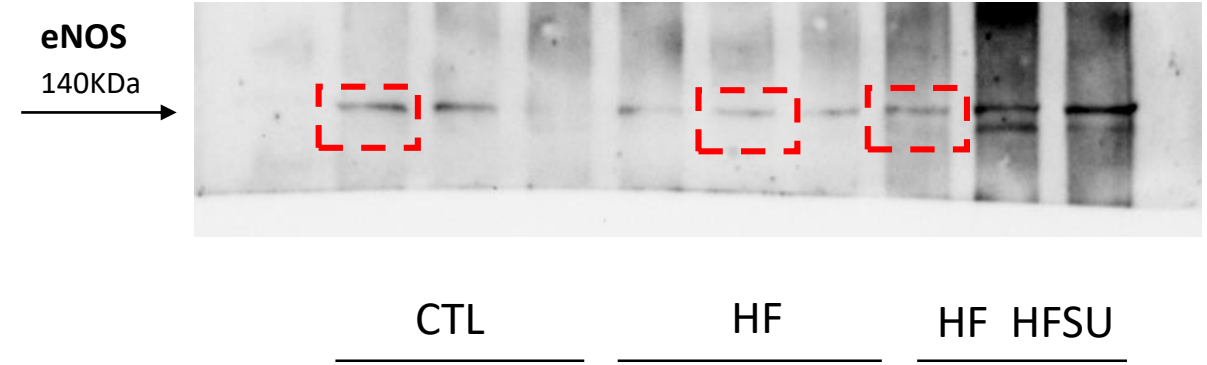
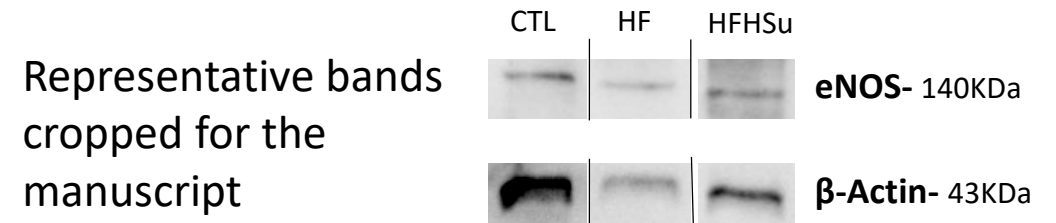
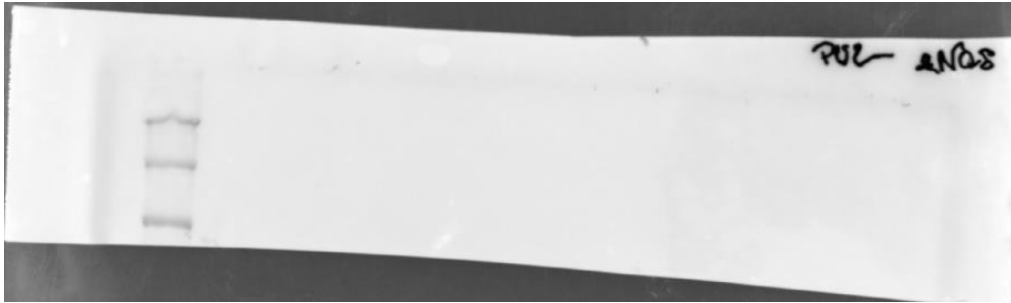
Representative bands  
cropped for the  
manuscript



**Figure 4 c**

**eNOS**

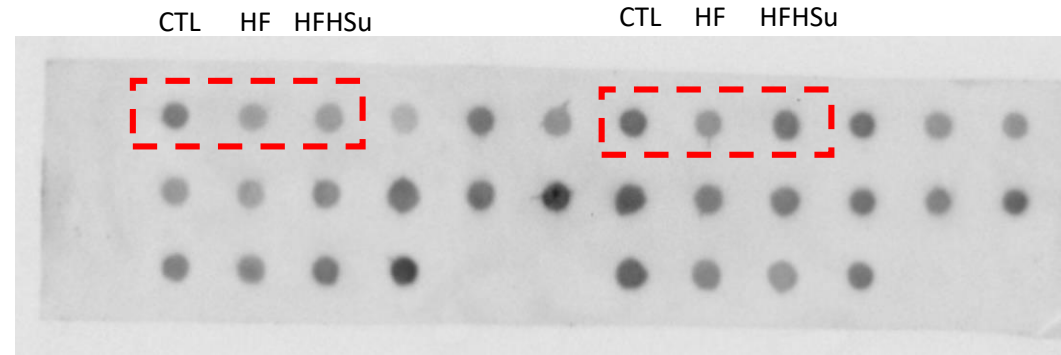
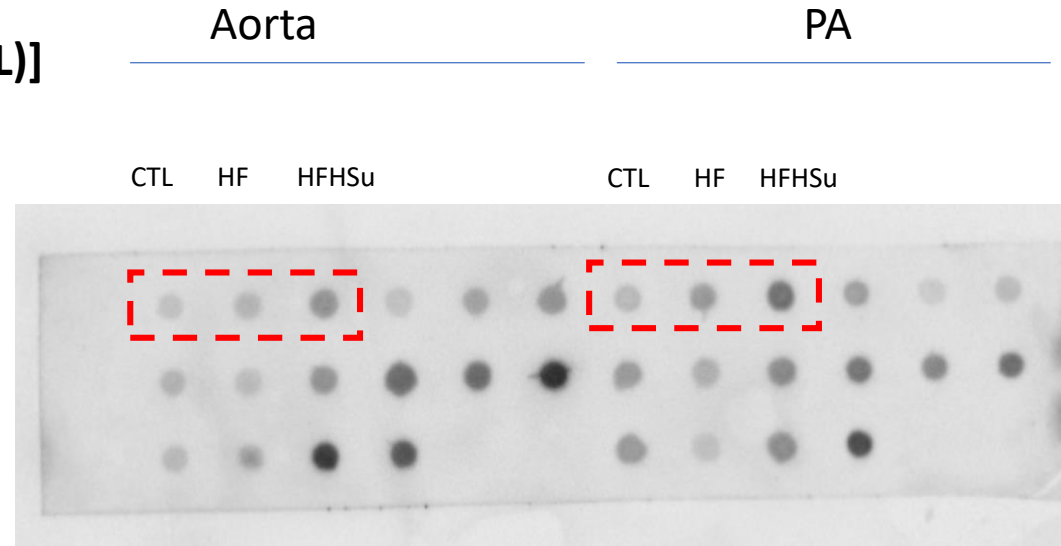
**Pulmonary Artery**



**Figure 5 a**

**AGEs [N<sup>ε</sup>-(carboxyethyl) lysine (CEL)]**

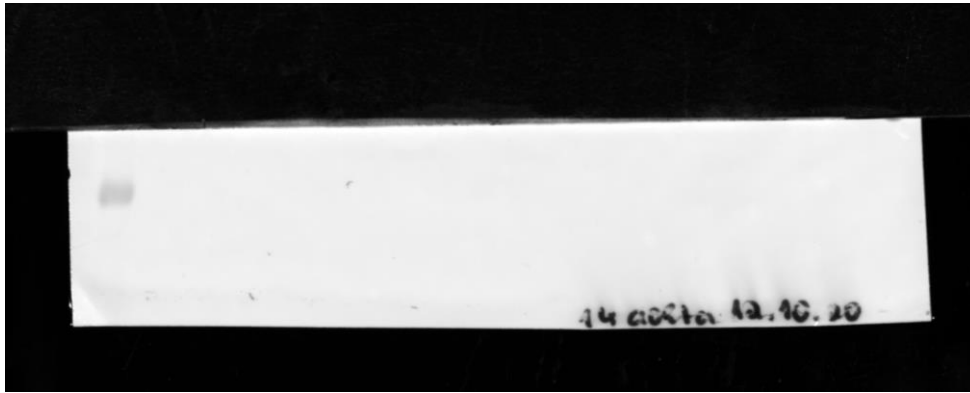
**Aorta and Pulmonary artery**



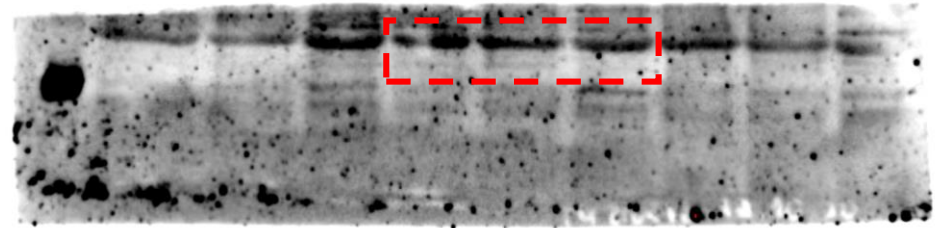
**Figure 5 b**

**AGEs receptor**

**Aorta**

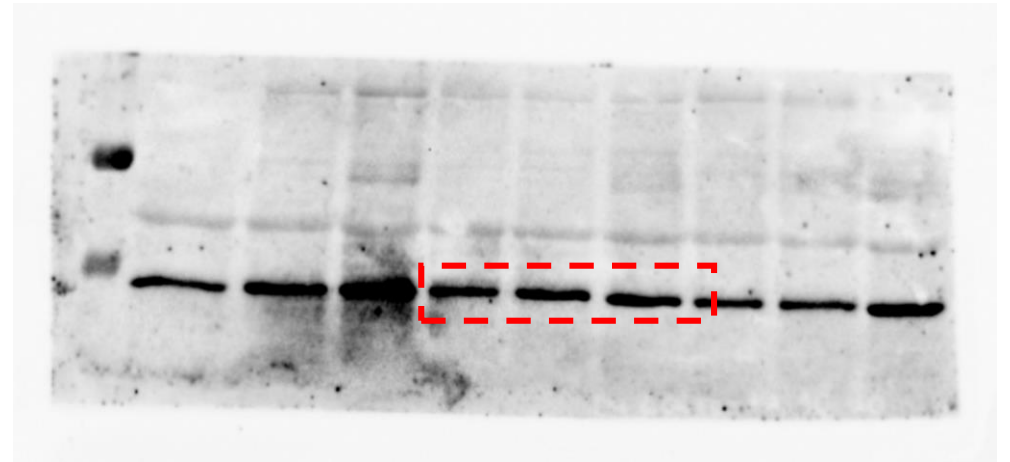


**AGEs  
receptor**  
42KDa



CTL HF HFHSu CTL HF HFHSu CTL HF HFHSu

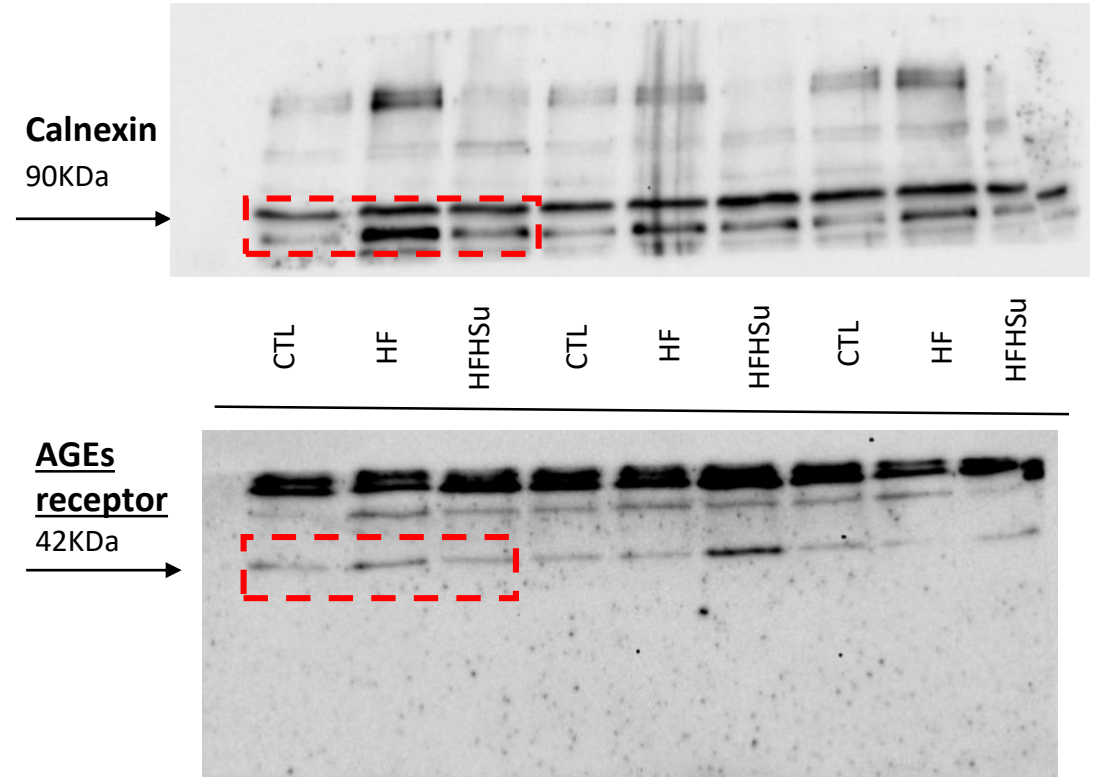
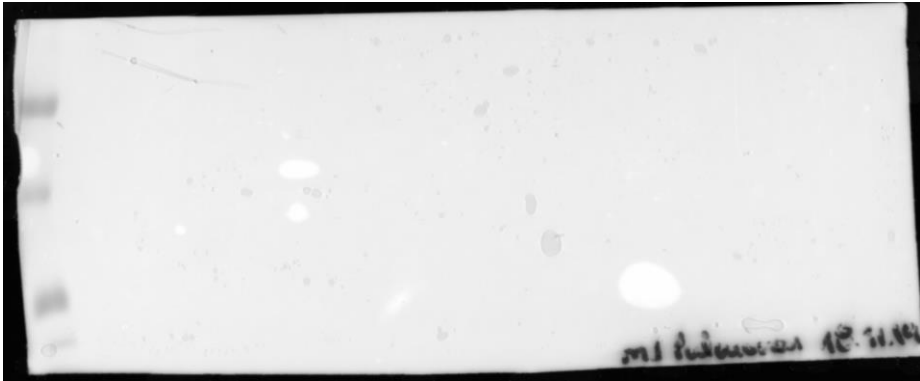
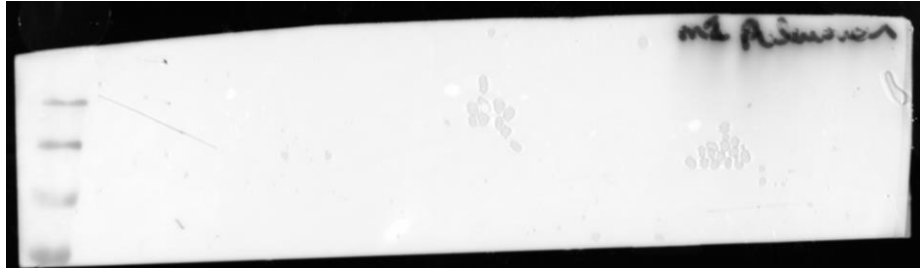
**GAPDH**  
37kDa



**Figure 5 b**

**AGEs receptor**

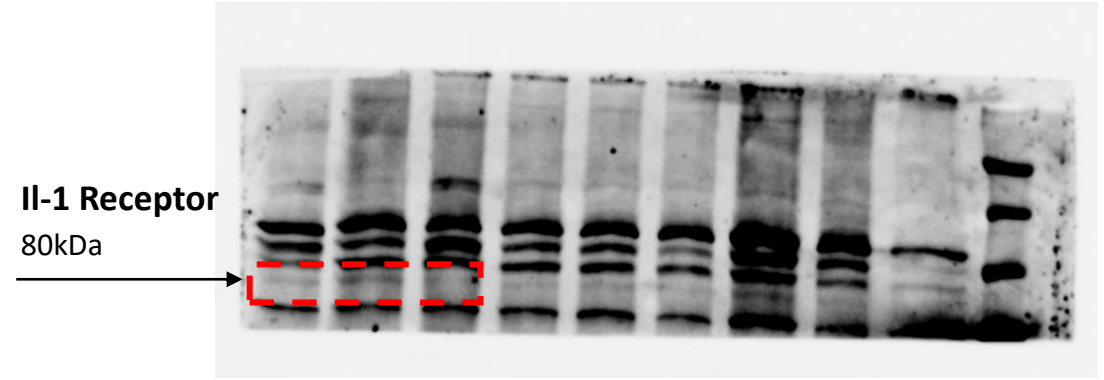
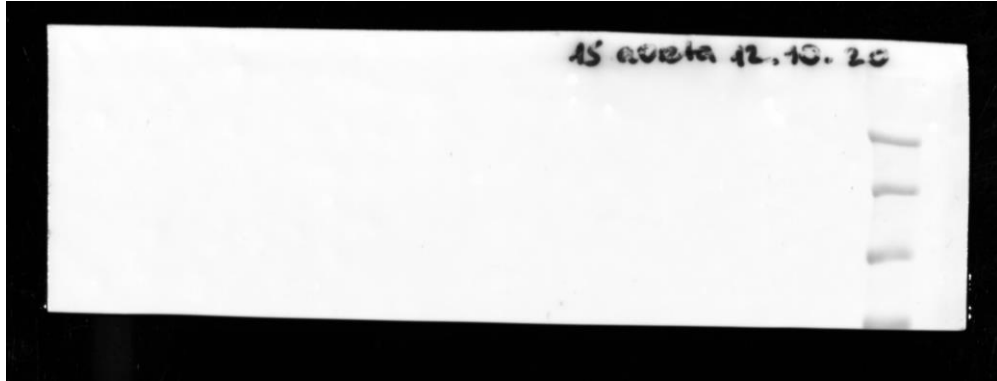
**Pulmonary Artery**



**Figure 6 a**

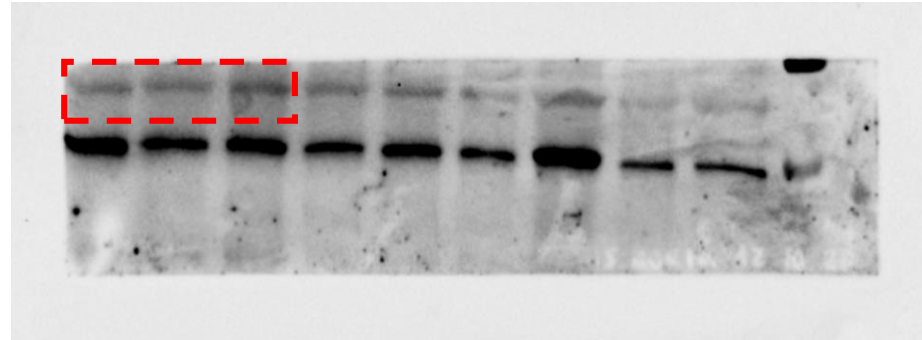
**IL-1 receptor**

**Aorta**

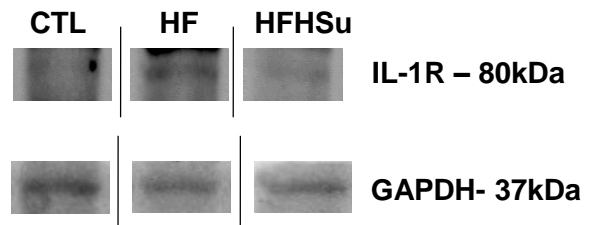


HFHSu HF CTL HFHSu HF CTL HFHSu HF CTL

**GAPDH**  
37kDa



Representative bands  
cropped for the  
manuscript





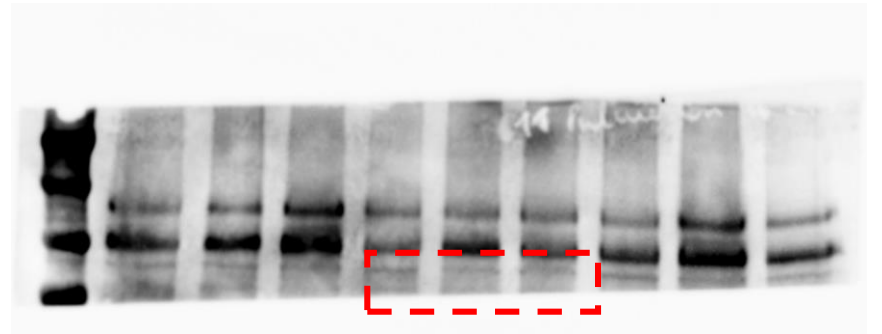
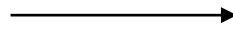
**Figure 6 a**

**IL-1 receptor**

**Pulmonary artery**

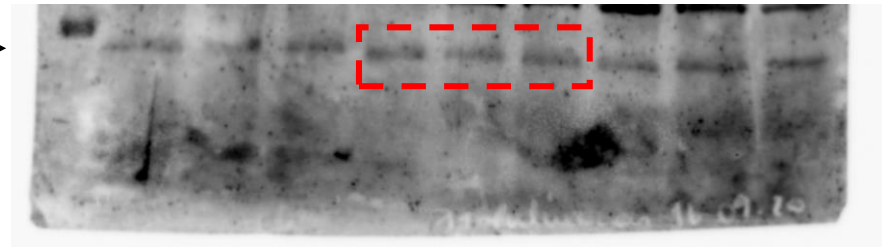


**IL-1 Receptor**  
80kDa



CTL HF HFHSu CTL HF HFHSu CTL HF HFHSu

**GAPDH**  
37kDa

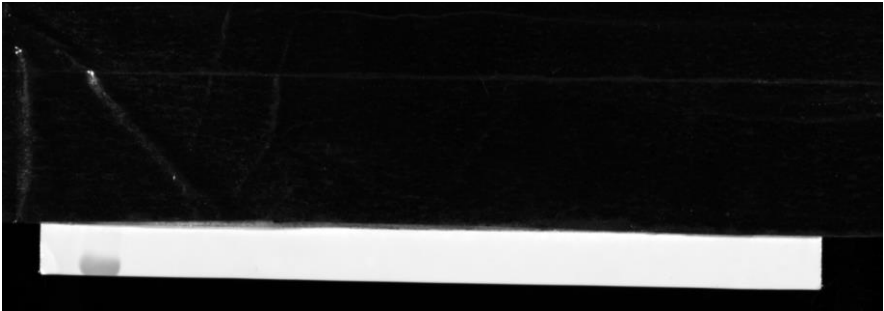
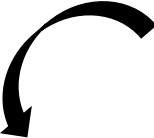




**Figure 6 b**

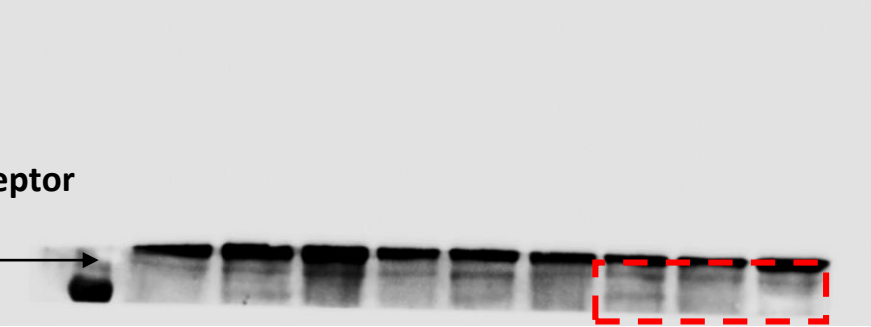
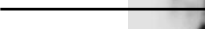
**IL-6 receptor**

**Aorta**



**IL-6 Receptor**

80kDa



CTL

HF

HFHSu

CTL

HF

HFHSu

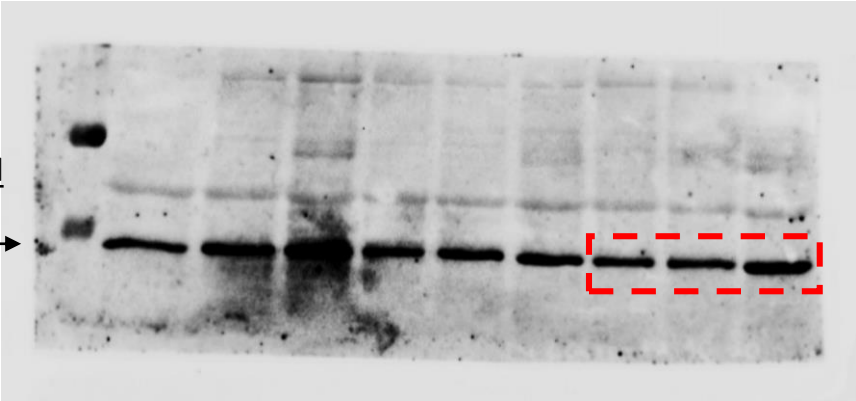
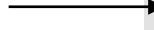
CTL

HF

HFHSu

**GAPDH**

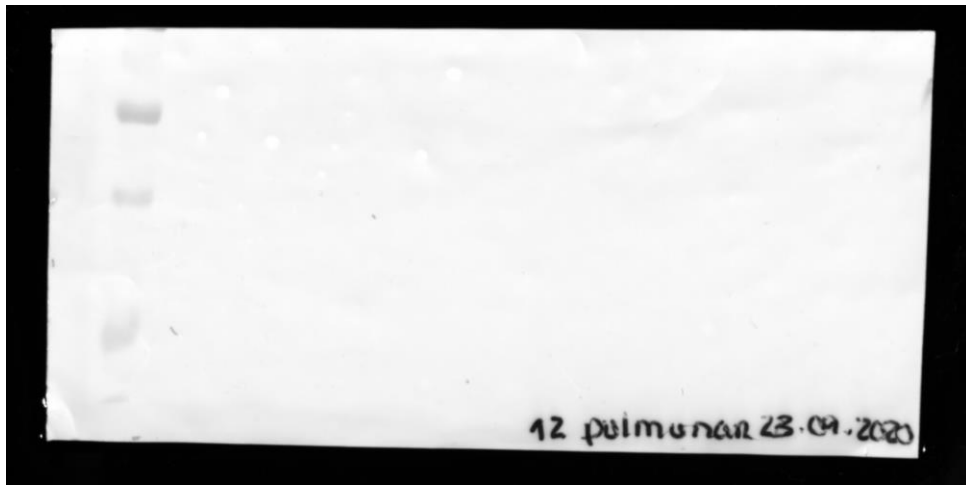
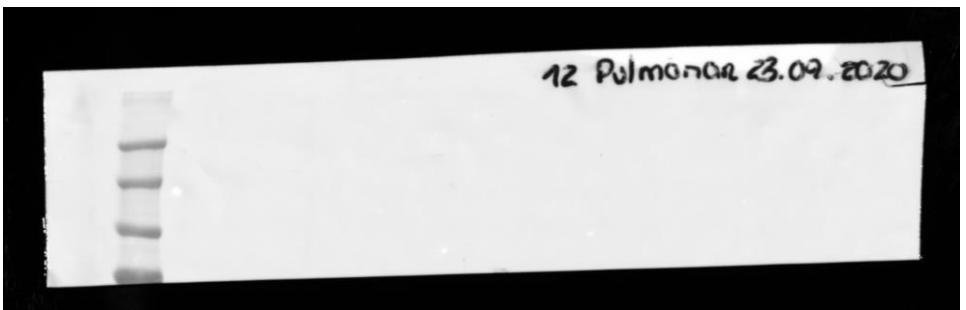
37kDa



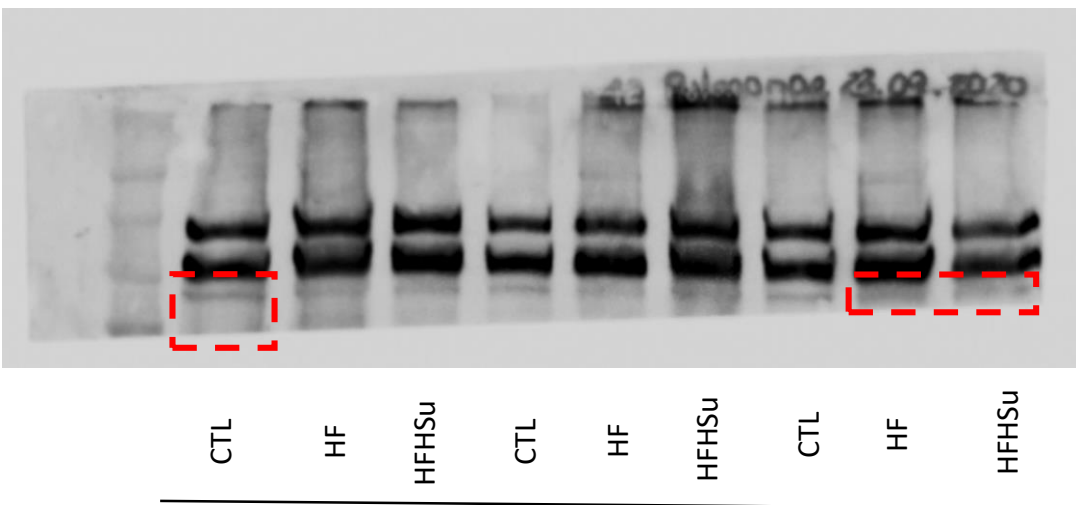
**Figure 6 b**

**IL-6 receptor**

**Pulmonary artery**



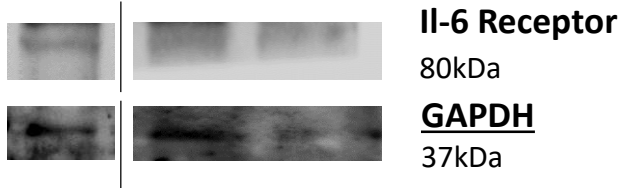
**IL-6 Receptor**  
80kDa  
→



**GAPDH**  
37kDa  
→



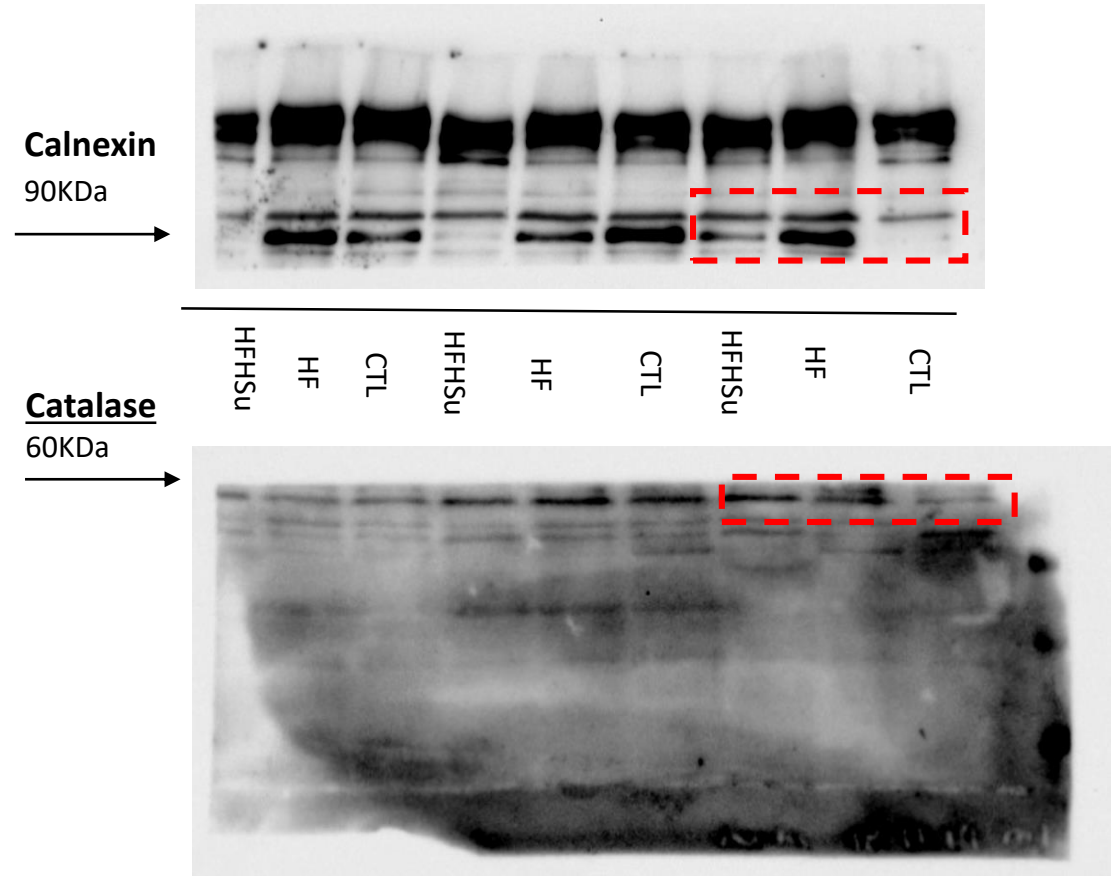
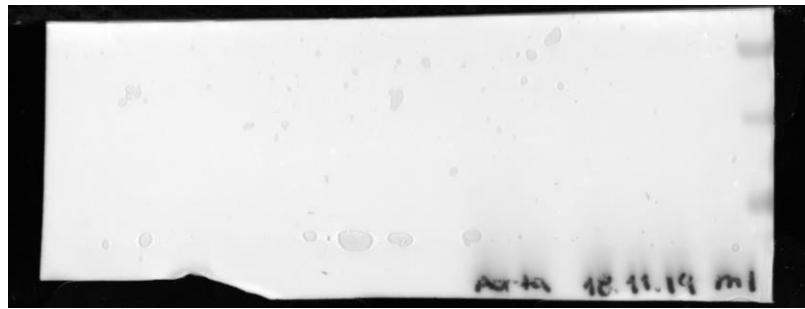
Representative bands  
cropped for the  
manuscript



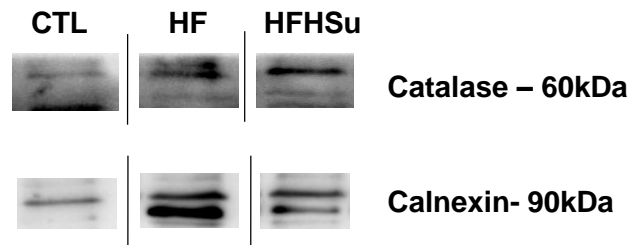
# Figure 7

## Catalase

### Aorta



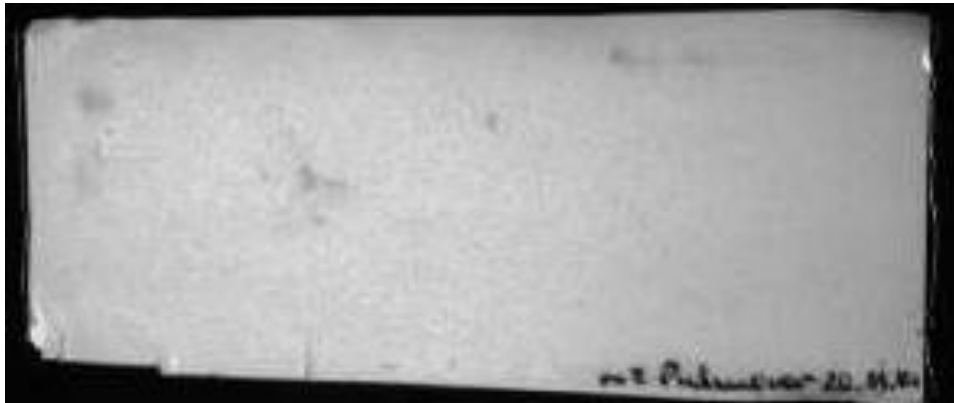
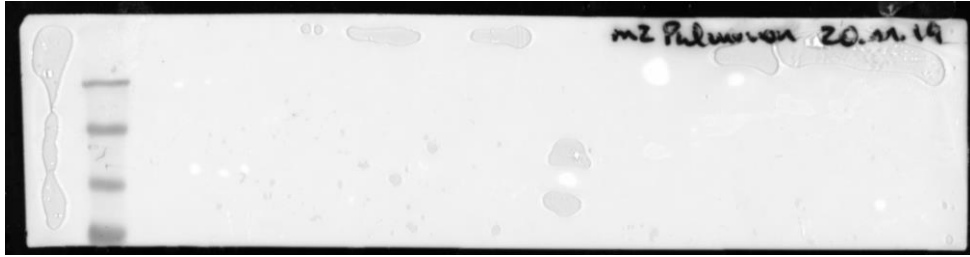
Representative bands  
cropped for the  
manuscript



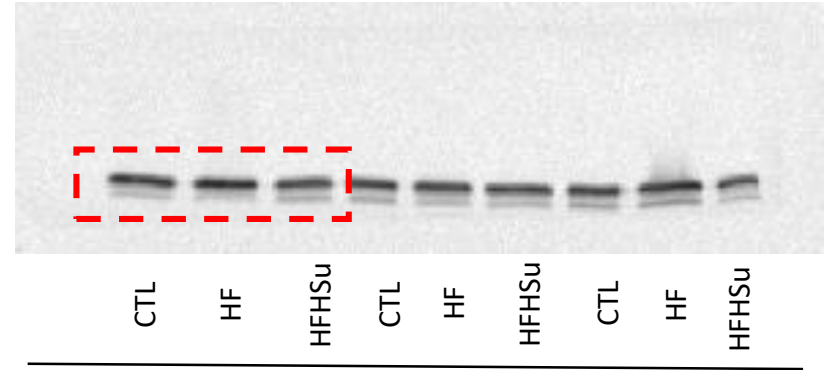
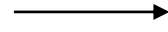
# Figure 7

## Catalase

### Pulmonary Artery



**Calnexin**  
90KDa



**Catalase**  
60KDa

