

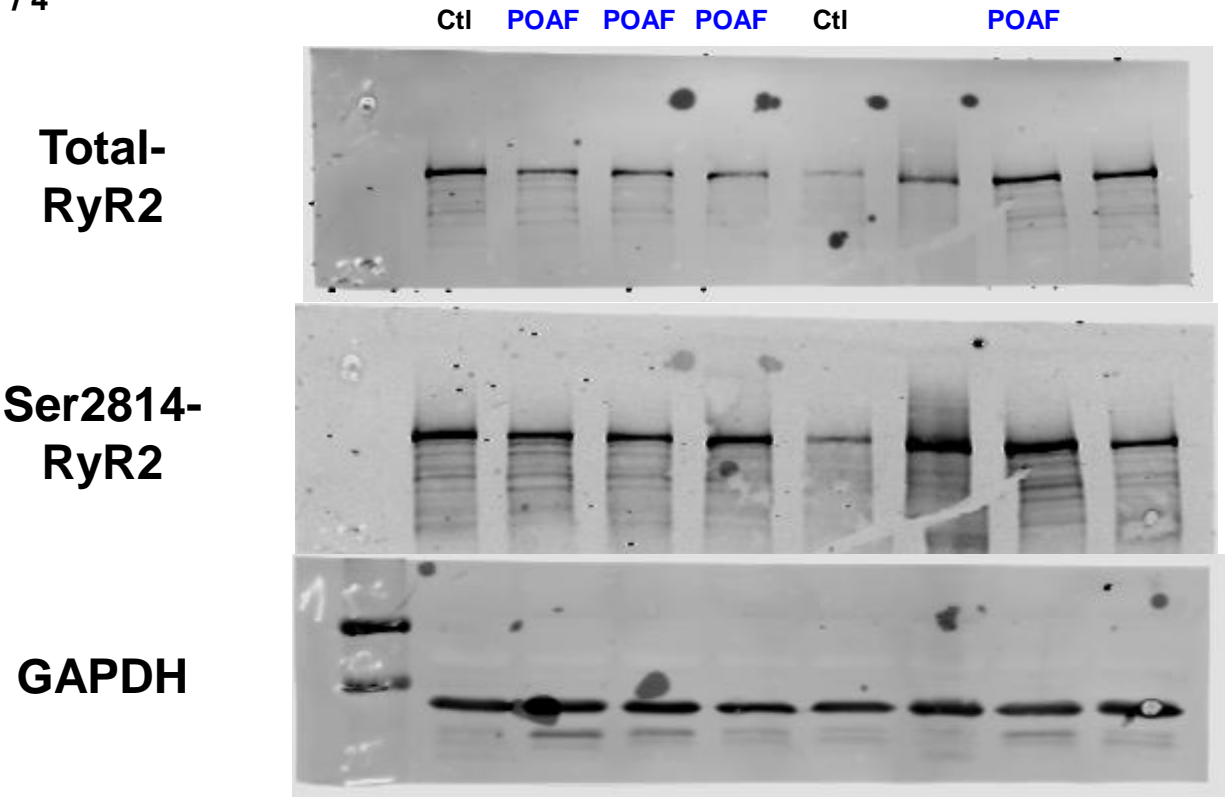
Western Blot Overview

Cardiomyocyte NLRP3-inflammasome and Calcium/Calmodulin-dependent Kinase II Signaling Create a Cellular Substrate for Post-operative Atrial Fibrillation

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Marcel Gorka; Stephan Künzel, MD; Ali El-Armouche, MD;
Hermann Reichenspurner, MD, PhD; Markus Kamler, MD;
Viacheslav Nikolaev, PhD; Ursula Ravens, MD; Na Li, PhD;
Stanley Nattel, MD; Xander H.T. Wehrens, MD, PhD;
Dobromir Dobrev, MD

Note: Given the limited availability of human atrial tissue samples, most gels were physically cut to enable incubation with different antibodies against proteins with distinct molecular weights. These fragments are shown in the subsequent slides. Unless otherwise indicated, samples are loaded in pairs: two Ctl samples followed by two POAF samples, etc. with molecular weight markers in the first and/or last lanes. Only samples that were used in the representative blots and/or quantitative analysis are marked.

Gel 1 / 4



Gel 2 / 4

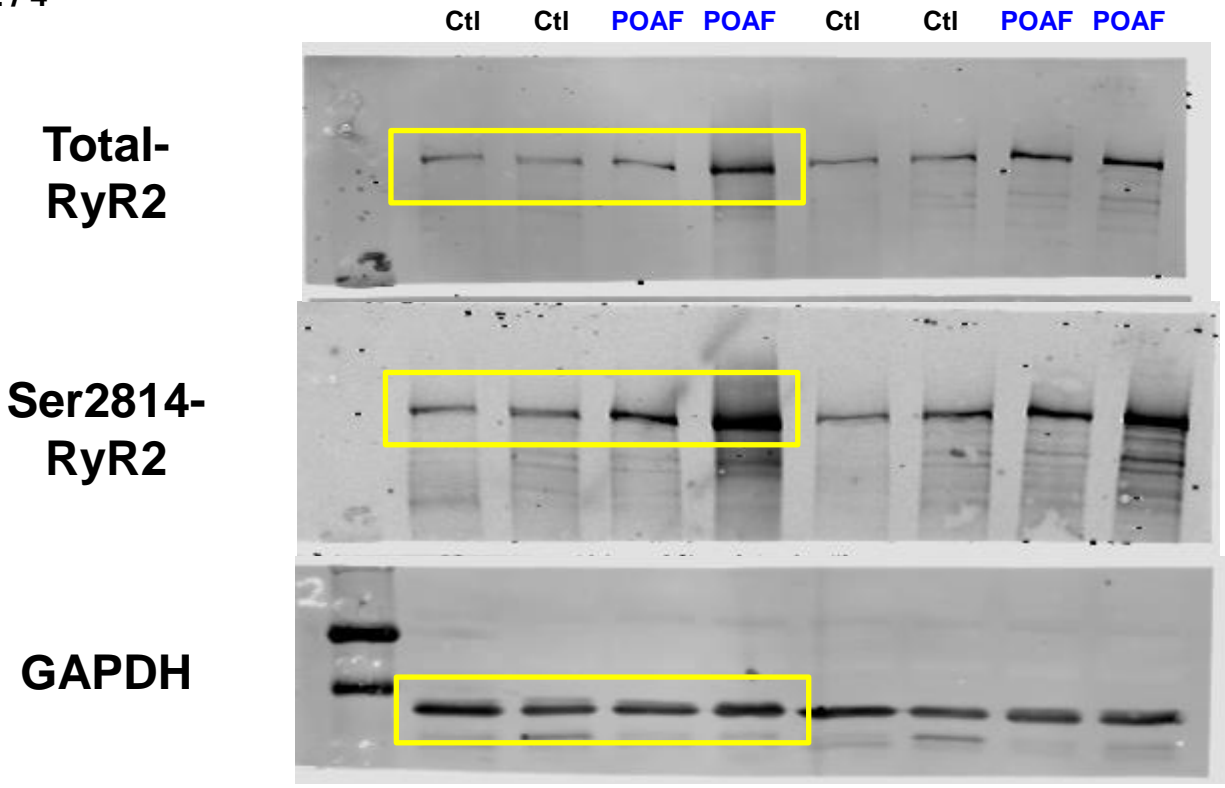
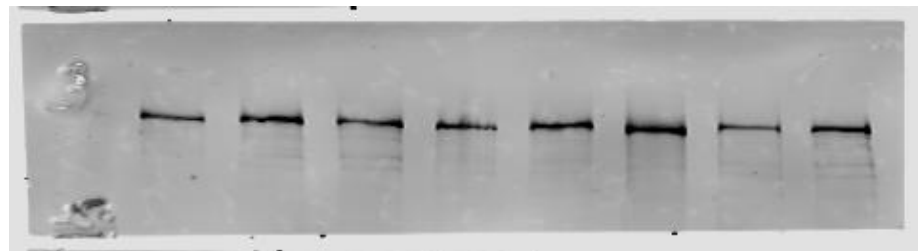


Figure 4B – Human atrial whole-tissue lysates

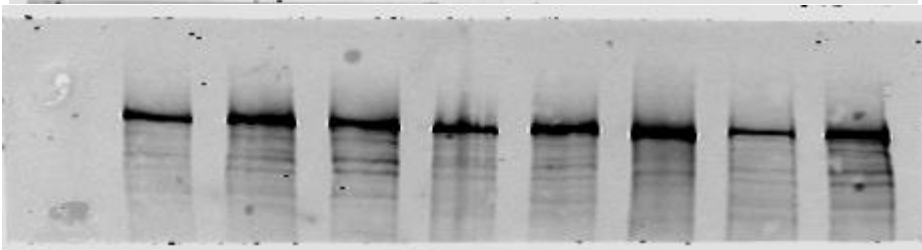
Gel 3 / 4

Ctl Ctl POAF POAF Ctl Ctl POAF POAF

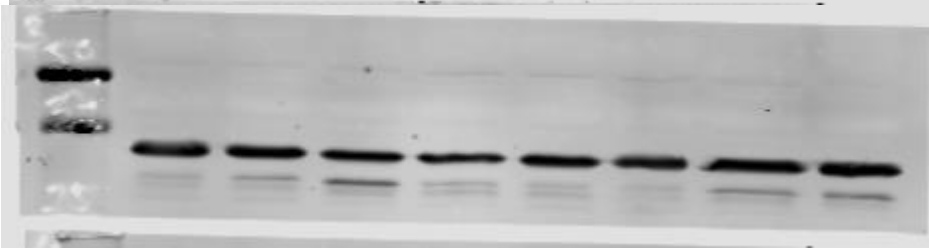
Total-RyR2



Ser2814-RyR2



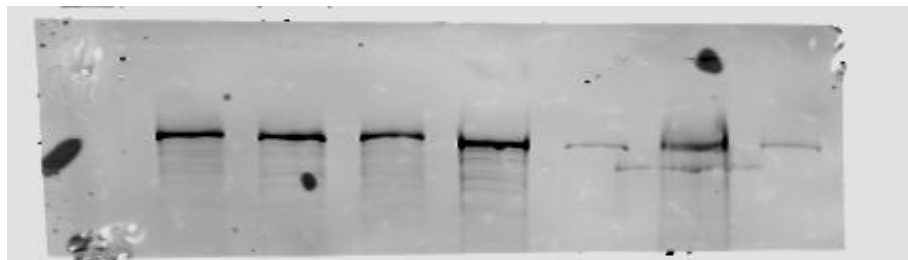
GAPDH



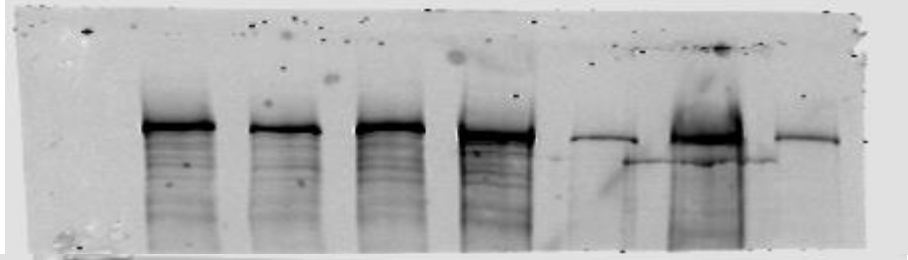
Gel 4 / 4

Ctl

Total-RyR2



Ser2814-RyR2



GAPDH

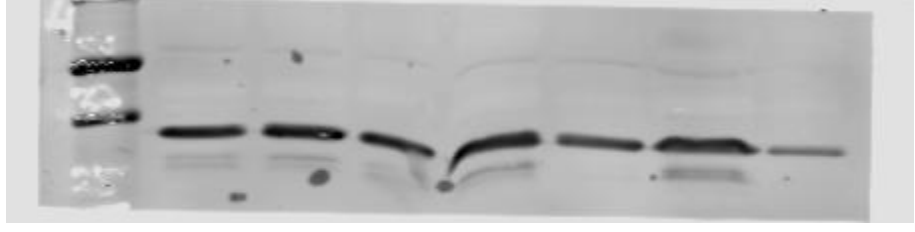
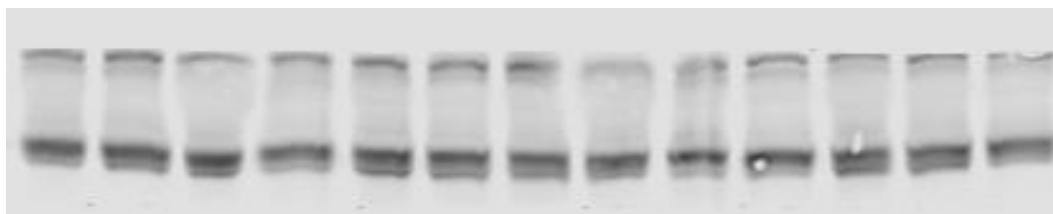


Figure 4B – Human atrial whole-tissue lysates

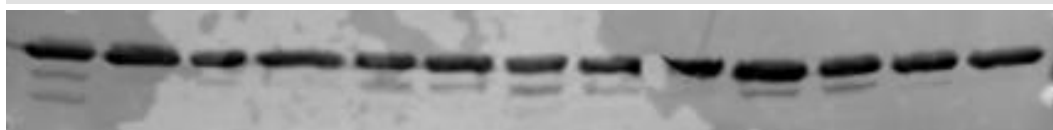
Gel 1 / 3

Ctl Ctl POAF POAF POAF POAF Ctl Ctl POAF POAF Ctl

Total-CaMKII δ



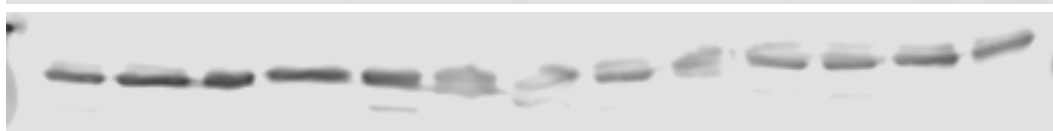
GAPDH



Thr287-CaMKII δ



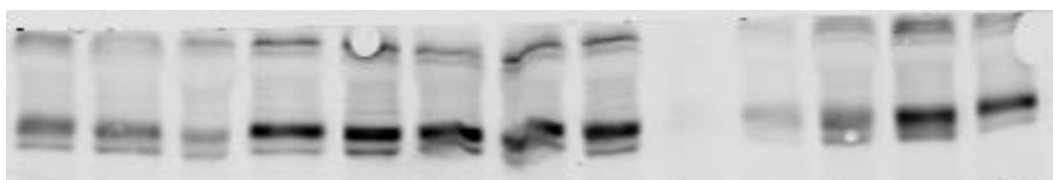
GAPDH



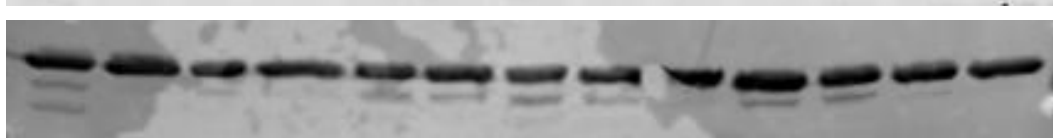
Gel 2 / 3

Ctl Ctl POAF POAF Ctl Ctl POAF

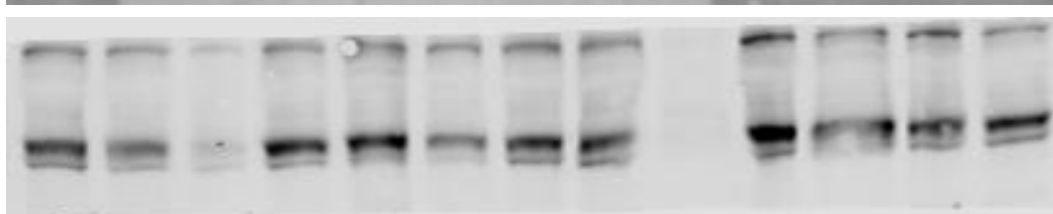
Total-CaMKII δ



GAPDH



Thr287-CaMKII δ



GAPDH

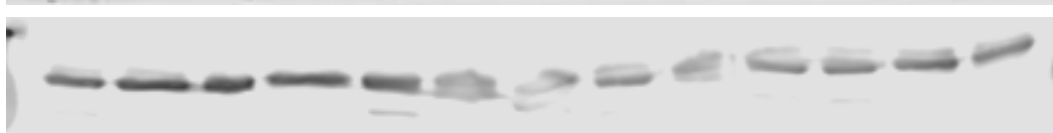
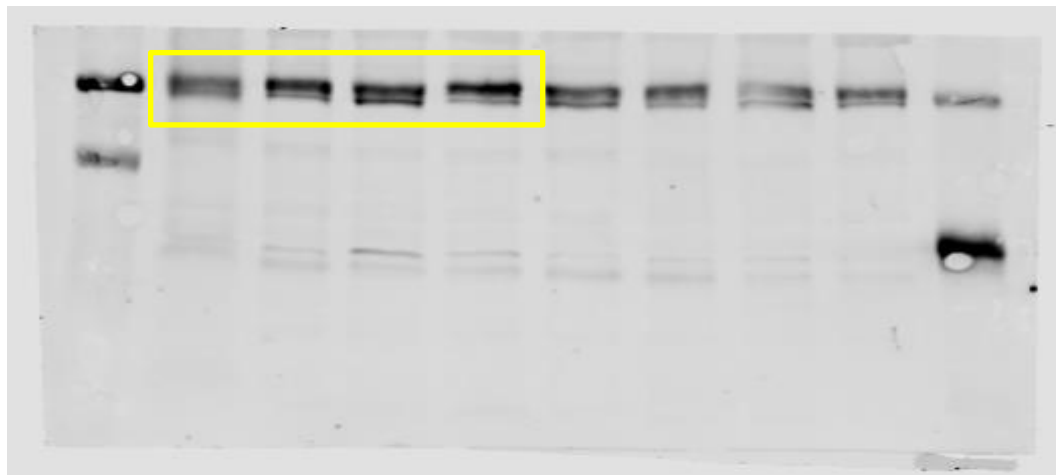


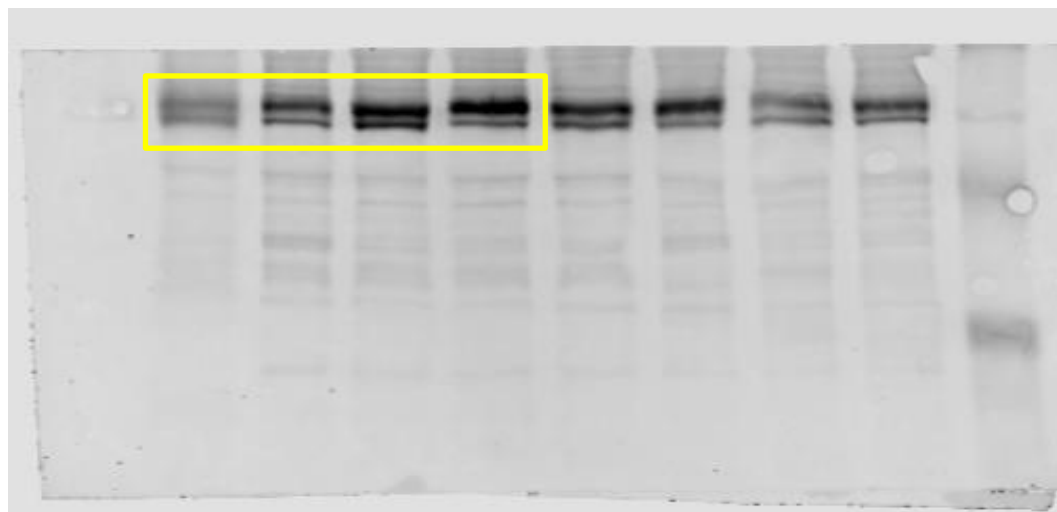
Figure 4B + Online Figure X – Human atrial whole-tissue

Gel 3 / 3

Ctl Ctl POAF POAF



Total-CaMKII δ



Thr287-CaMKII δ

GAPDH

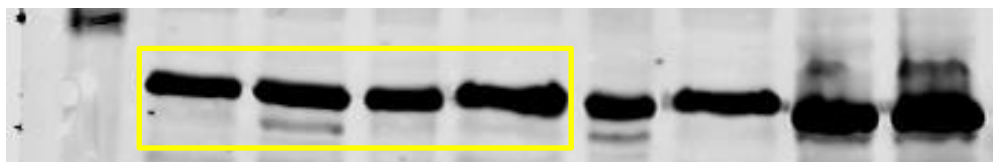
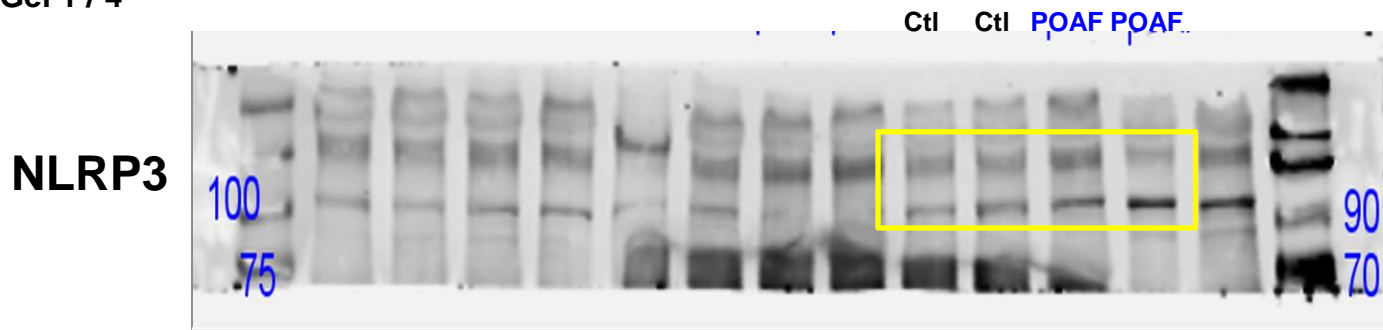


Figure 4B + Online Figure X – Human atrial whole-tissue

Gel 1 / 4



Gel 2 / 4

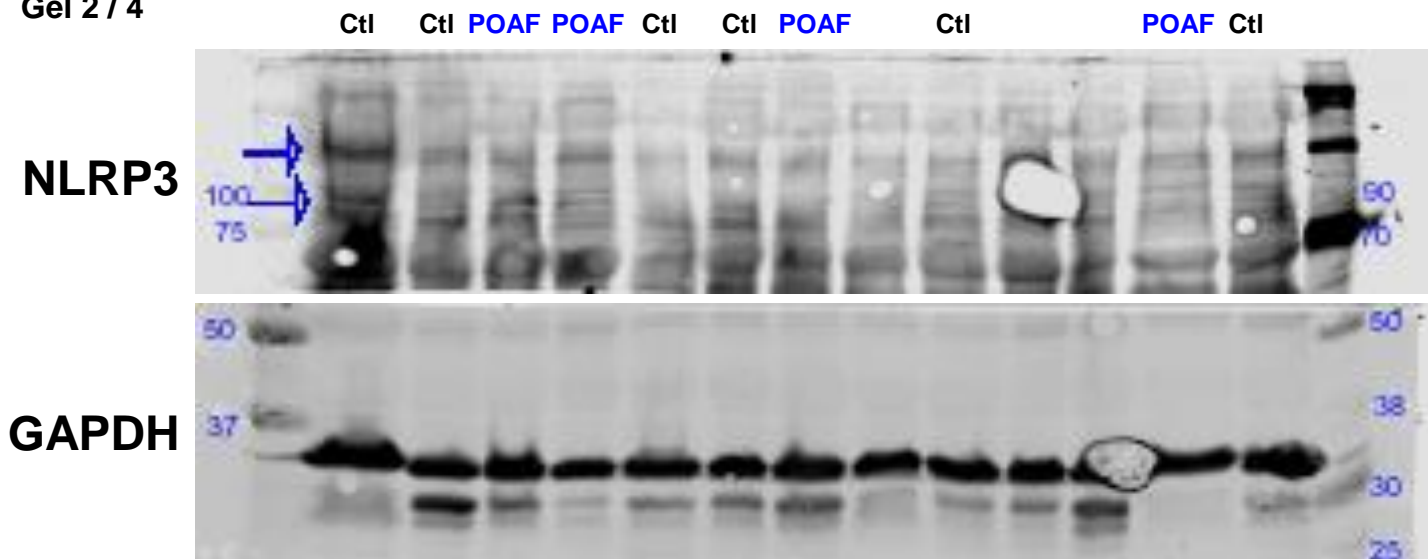
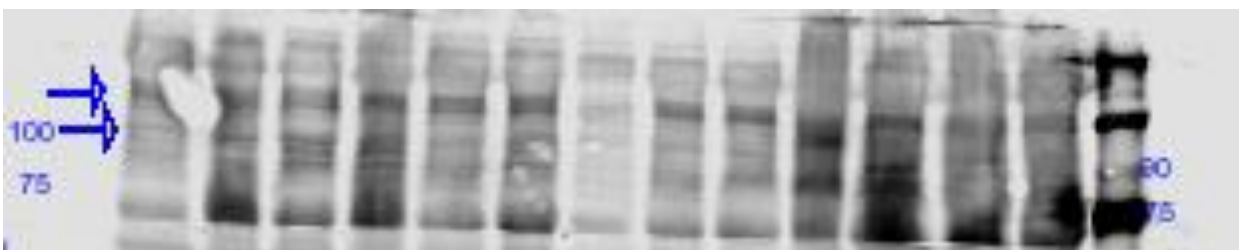


Figure 5A – Human atrial whole-tissue lysates

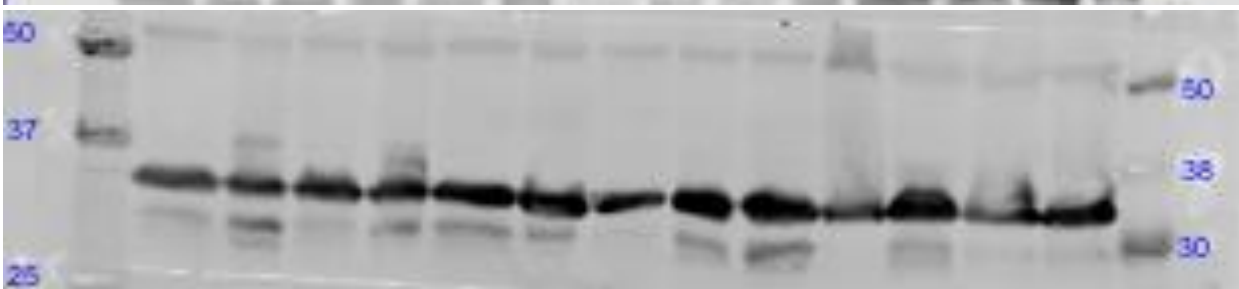
Gel 3 / 4

Ctl POAF POAF POAF Ctl Ctl POAF Ctl Ctl POAF POAF POAF

NLRP3



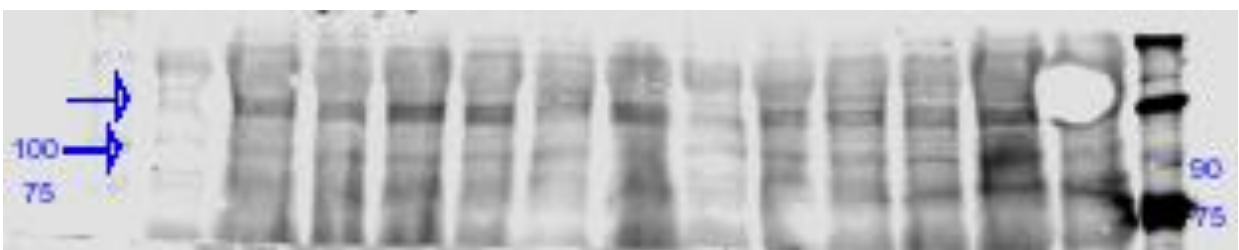
GAPDH



Gel 4 / 4

Ctl POAF POAF Ctl Ctl POAF POAF Ctl Ctl POAF POAF

NLRP3



GAPDH

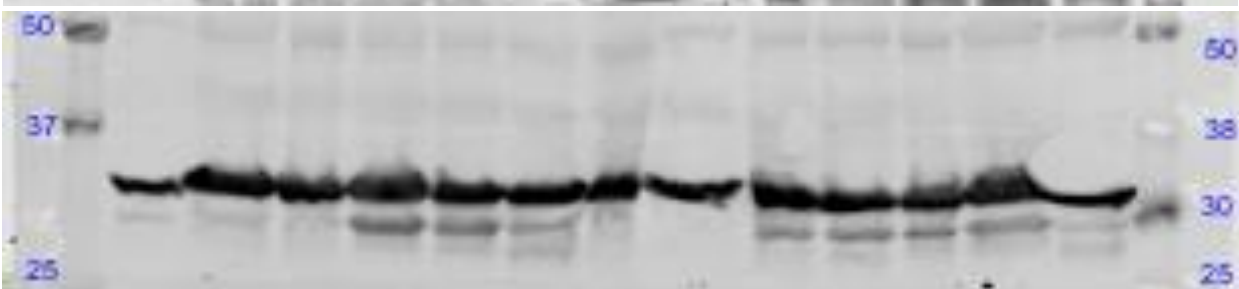
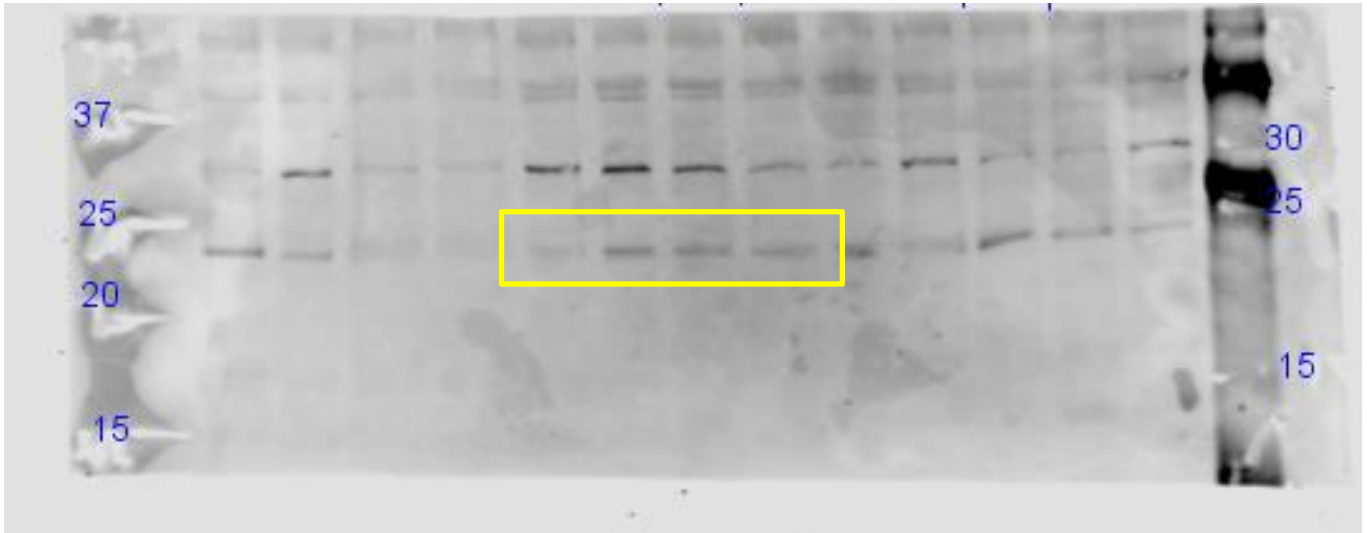


Figure 5A – Human atrial whole-tissue lysates

ASC

Gel 1 / 2

Ctl POAF Ctl Ctl POAF POAF Ctl Ctl POAF POAF Ctl



GAPDH

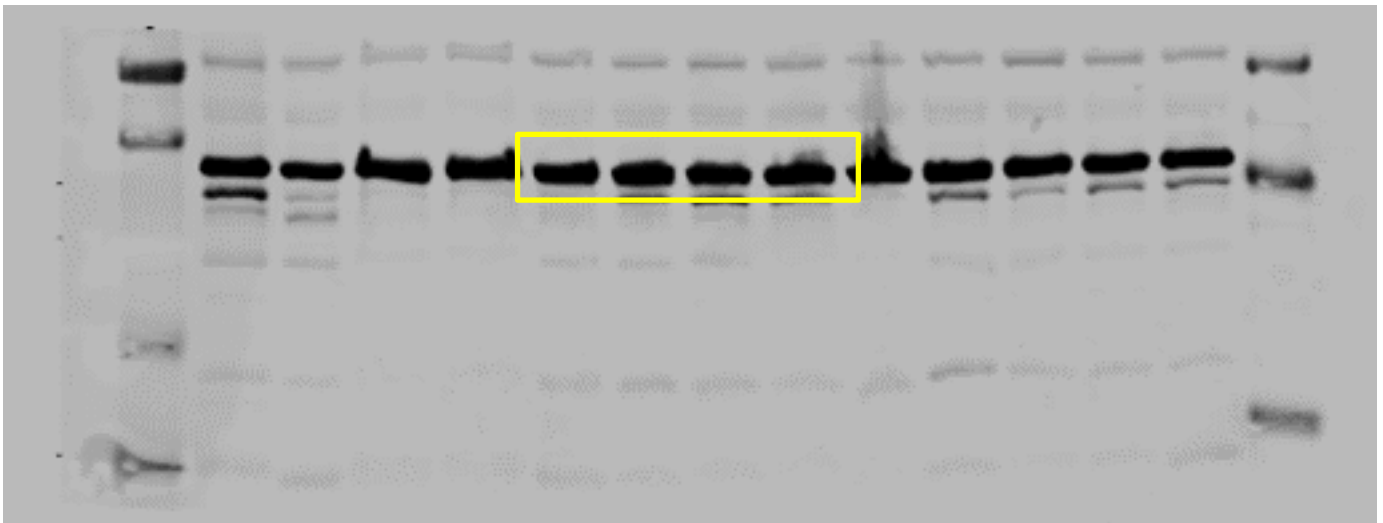
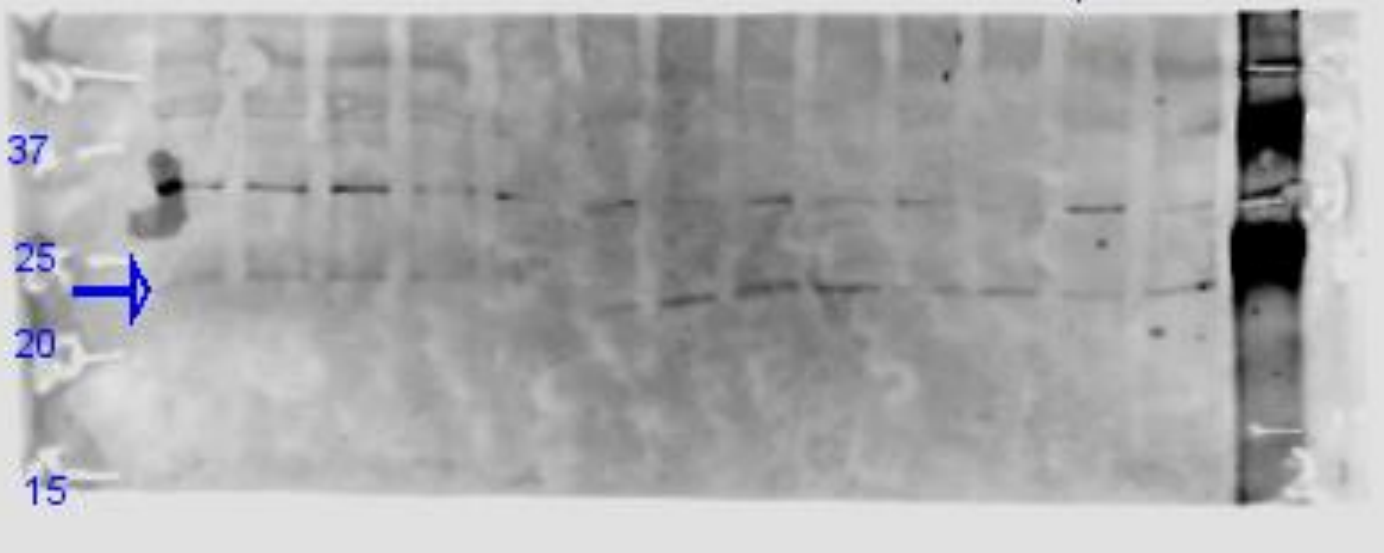


Figure 5A – Human atrial whole-tissue lysates

ASC

Gel 2 / 2

Ctl Ctl POAF POAF POAF POAF Ctl POAF POAF



GAPDH

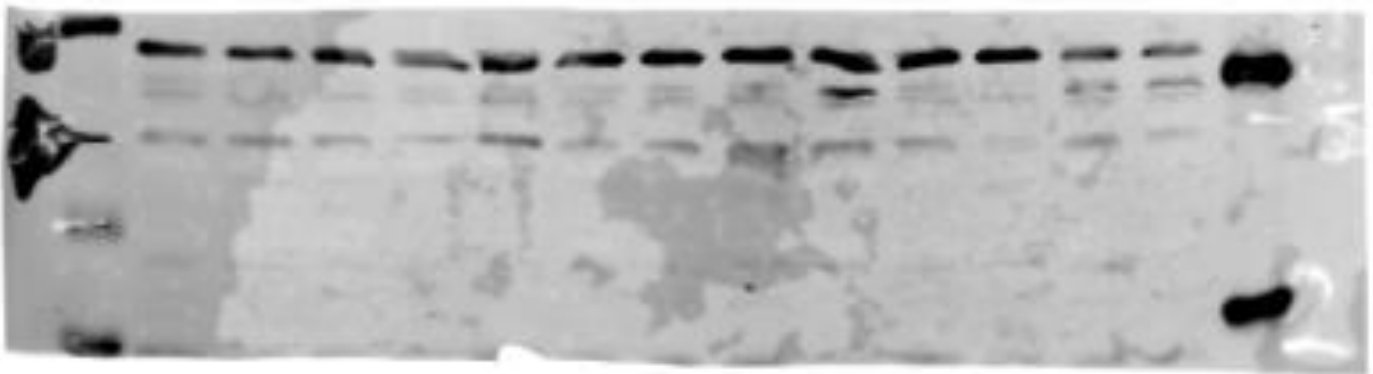


Figure 5A – Human atrial whole-tissue lysates

Pro-Caspase-1

POAF POAF Ctl Ctl

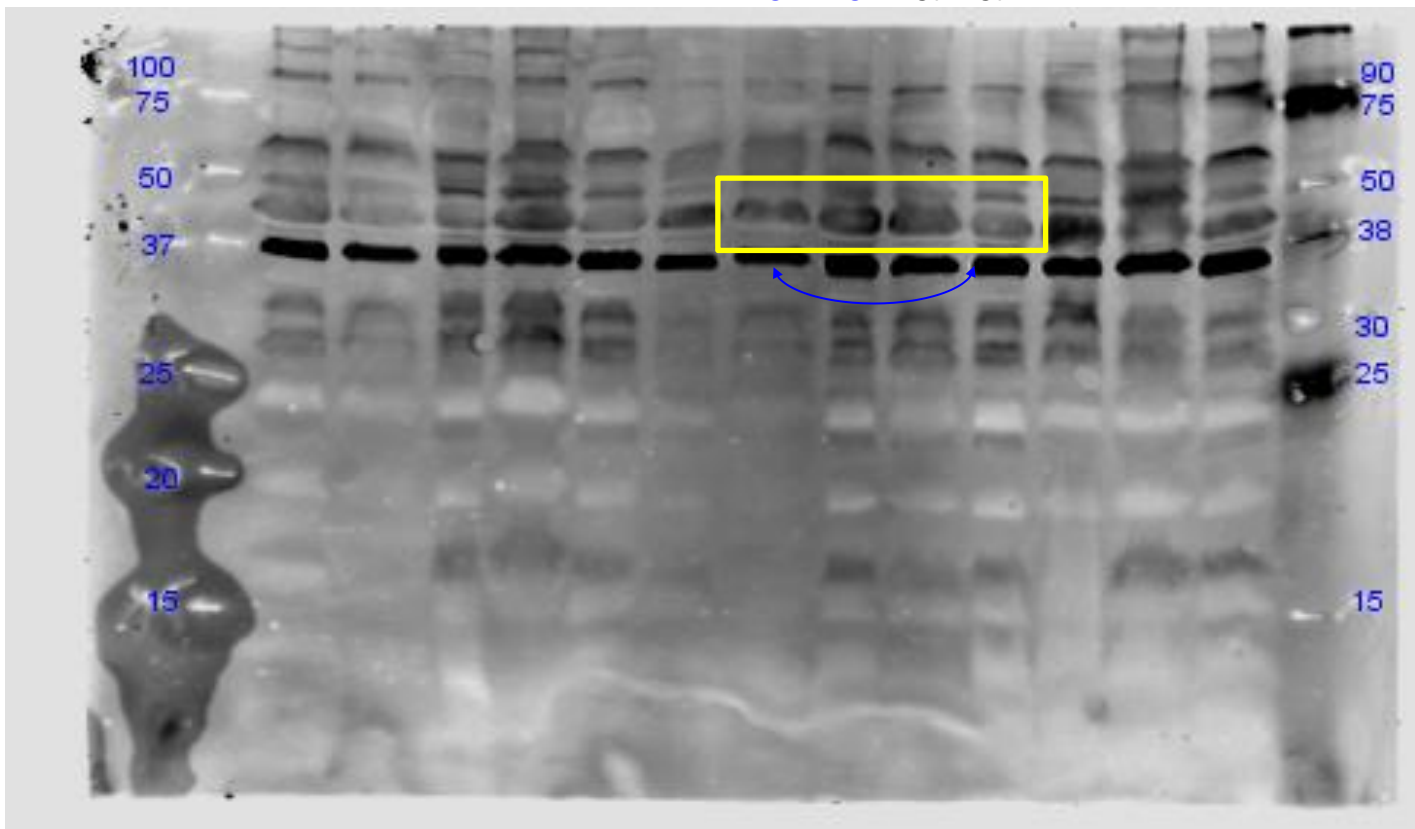
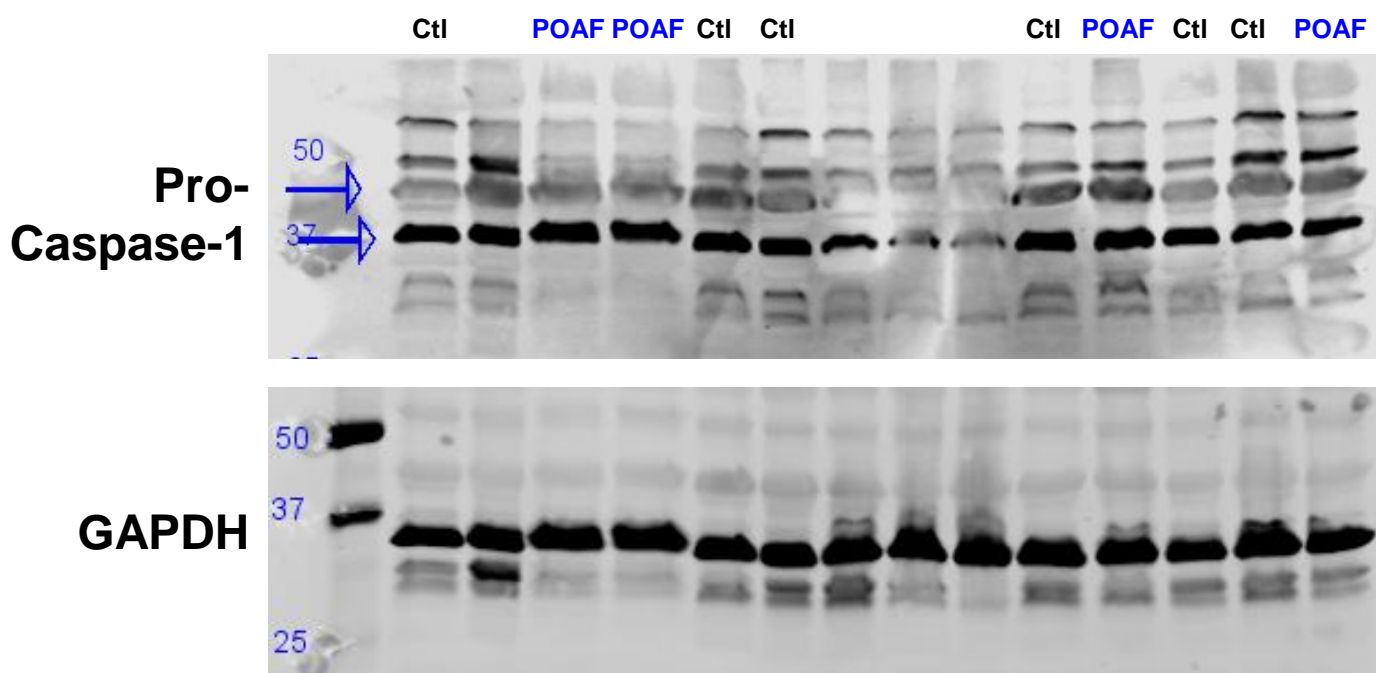


Figure 5A – Human atrial whole-tissue lysates

Gel 2 / 3



Gel 3 / 3

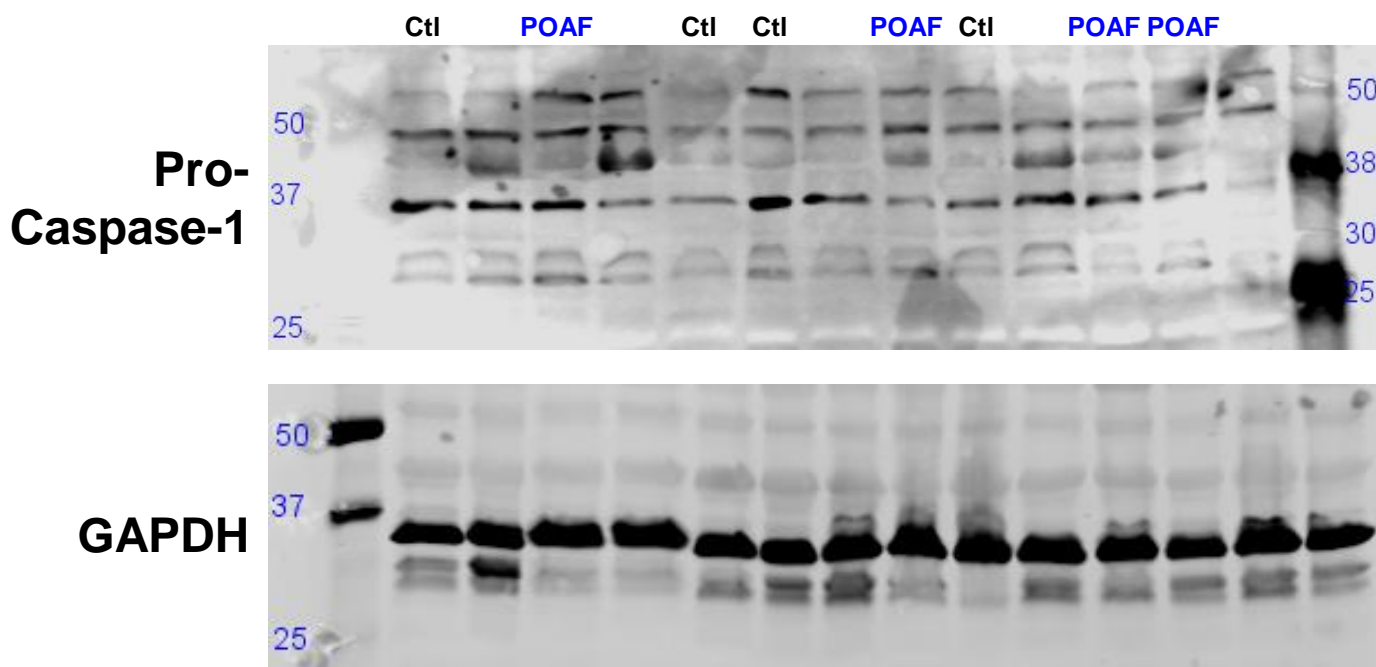
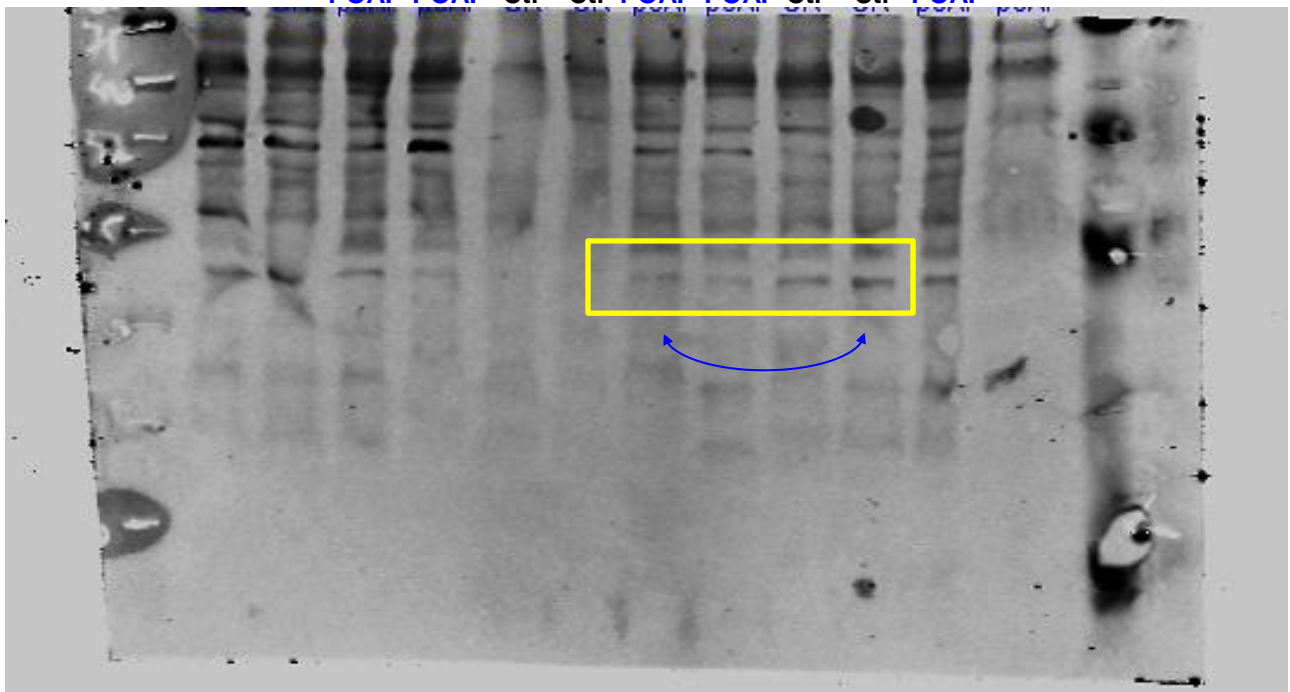


Figure 5A – Human atrial whole-tissue lysates

Caspase-1-p20

POAF POAF Ctl Ctl POAF POAF Ctl Ctl POAF POAF



GAPDH

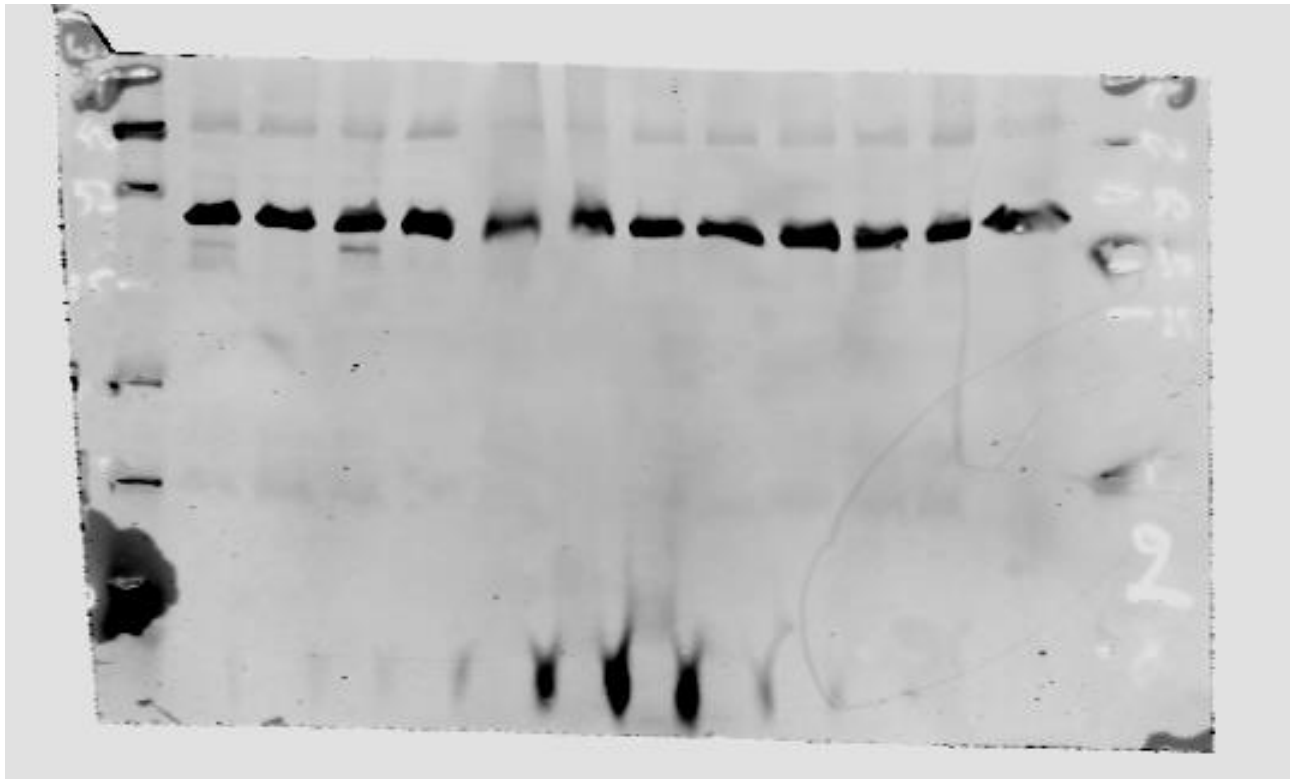
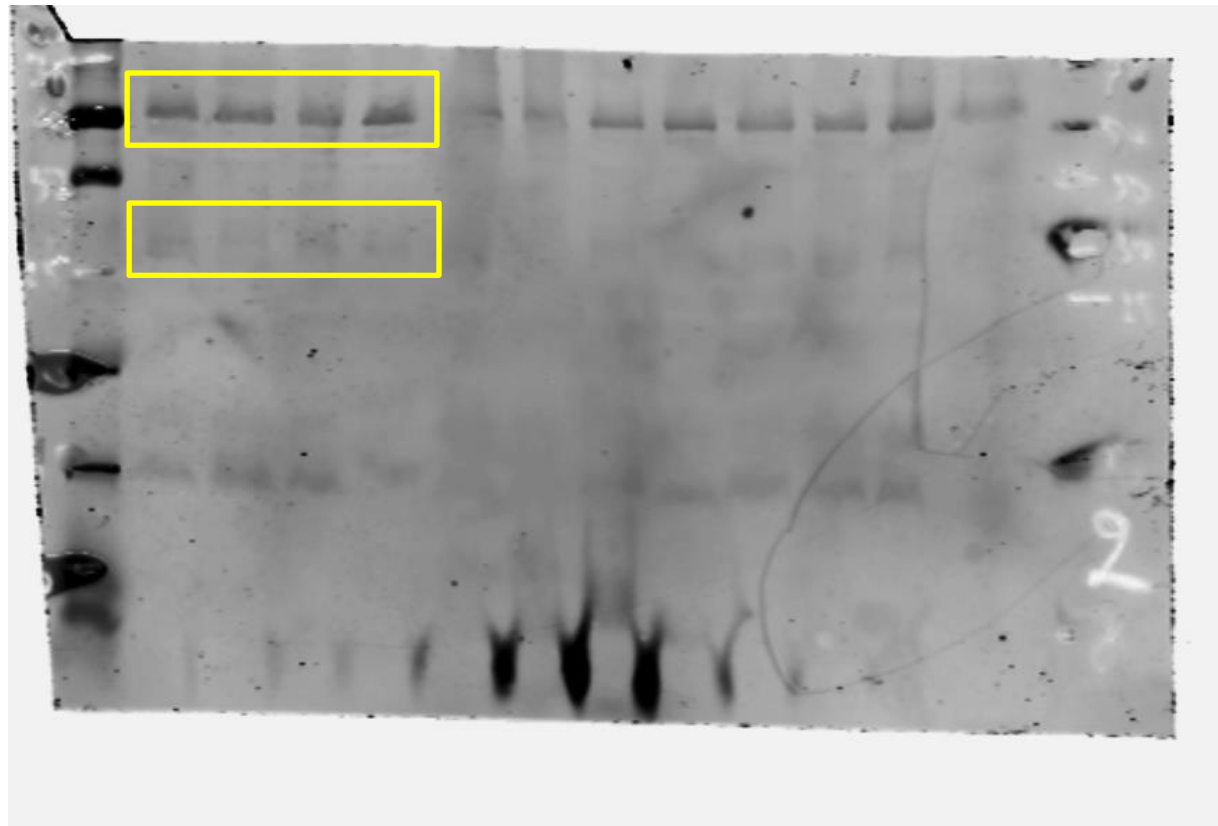


Figure 5A – Human atrial whole-tissue lysates

Gasdermin-D (full-length + N-terminal fragment)

Ctl Ctl POAF POAF Ctl Ctl POAF POAF Ctl POAF POAF



GAPDH

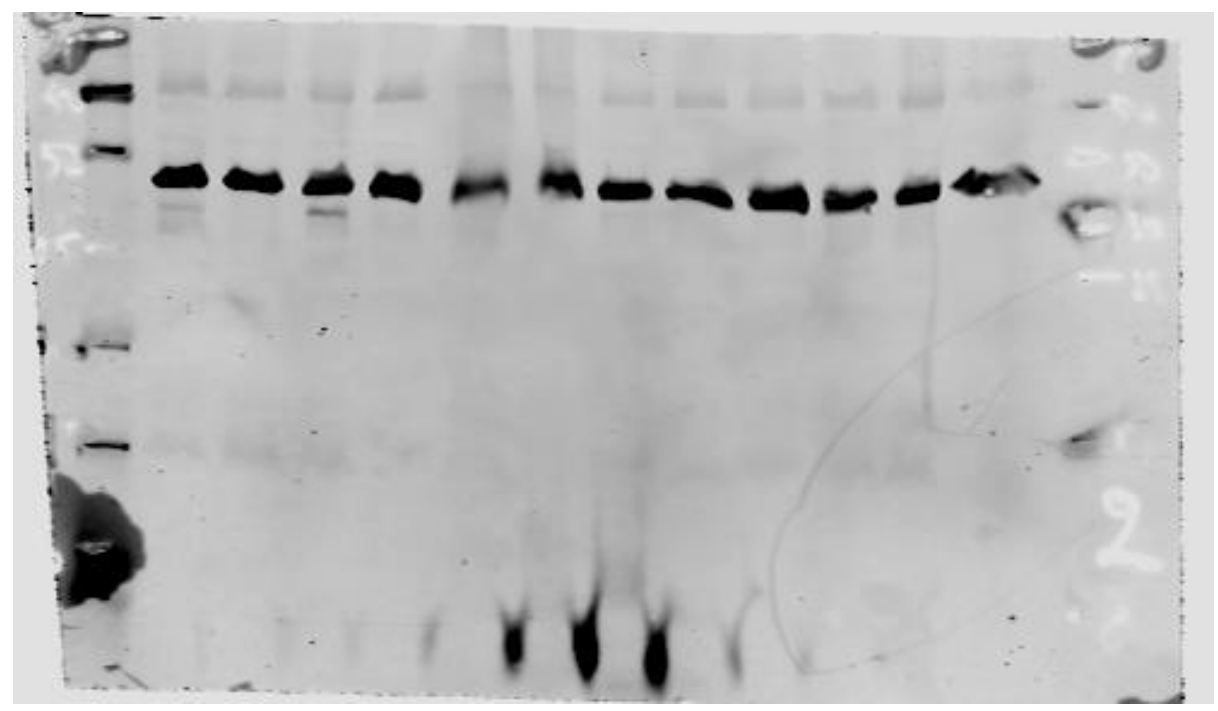
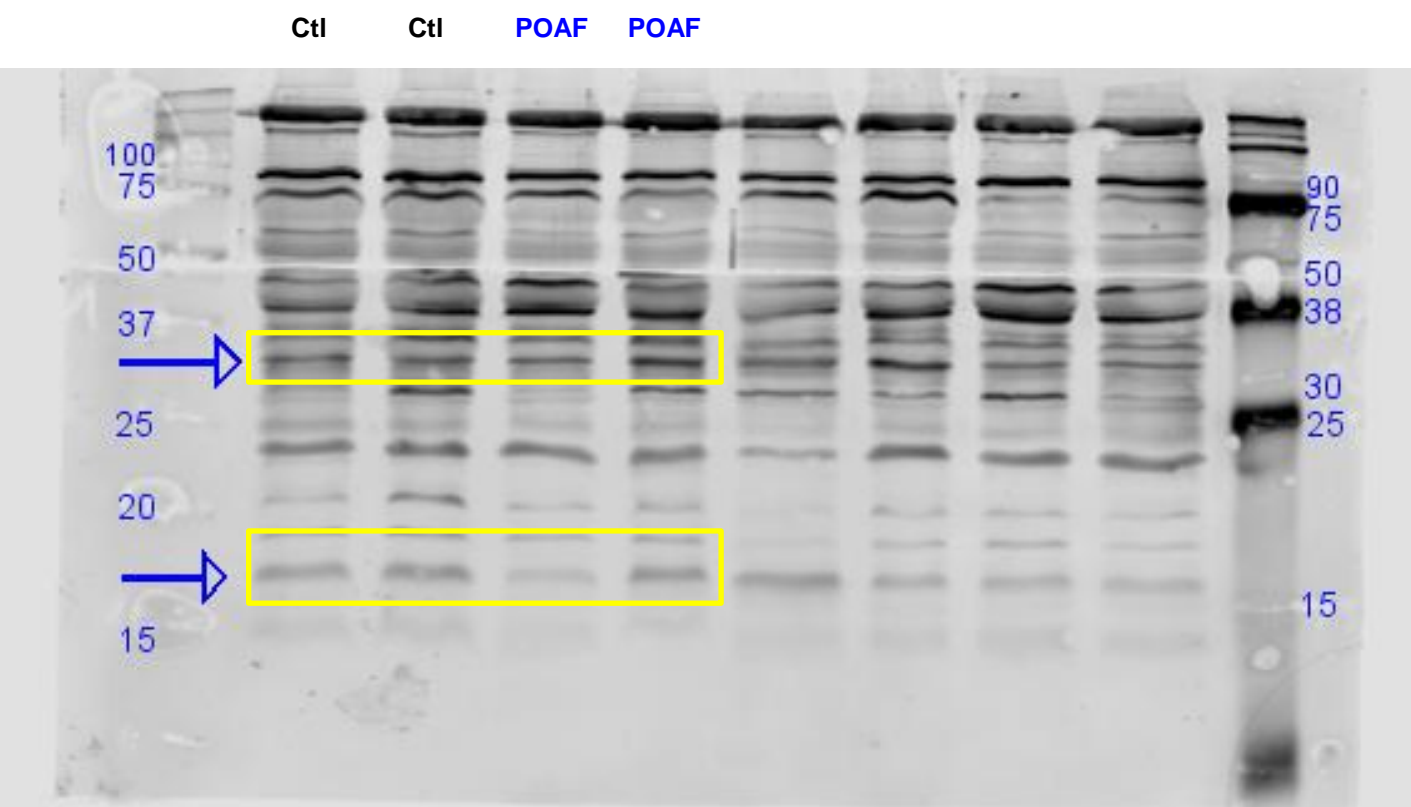


Figure 5A – Human atrial whole-tissue lysates

(Pro-)Interleukin-1 β



GAPDH

Ctl Ctl POAF POAF

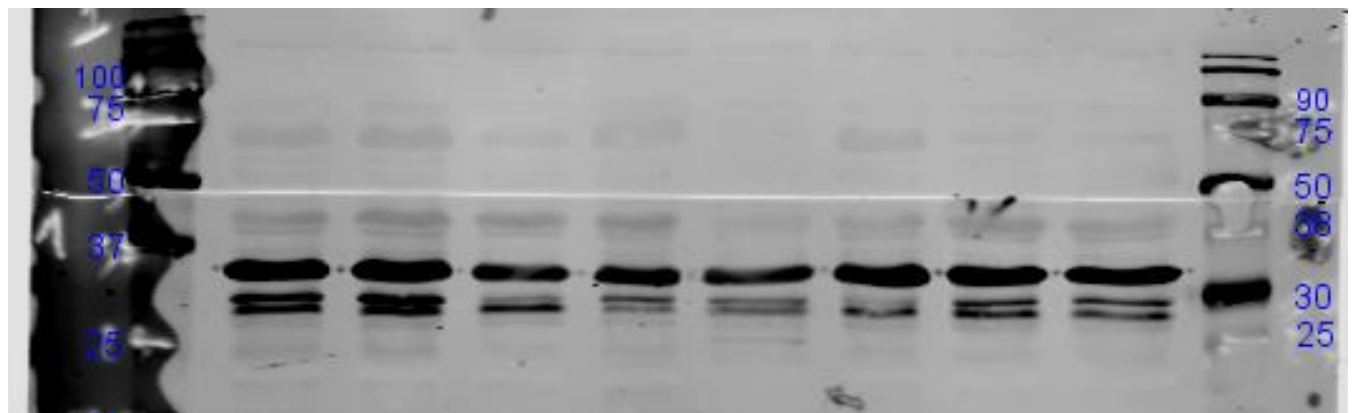
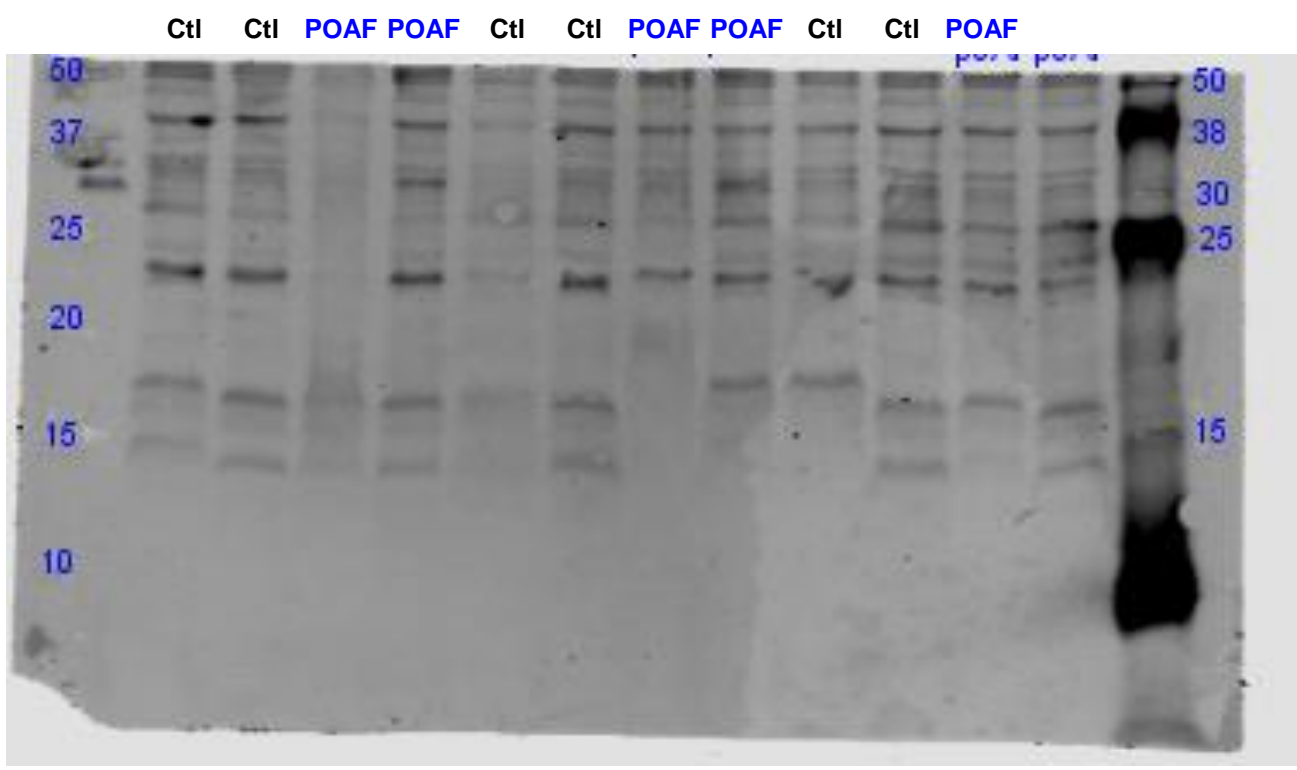


Figure 5A – Human atrial whole-tissue lysates

(Pro-)Interleukin-1 β



GAPDH

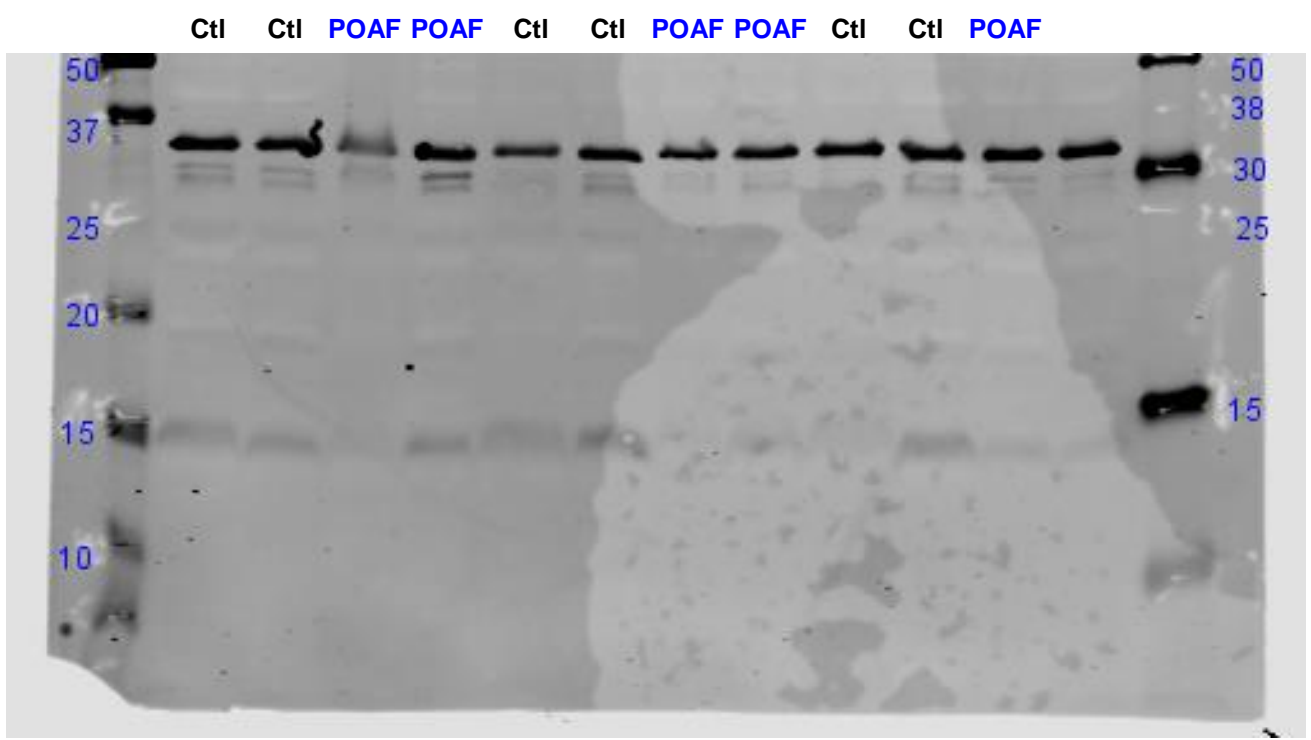
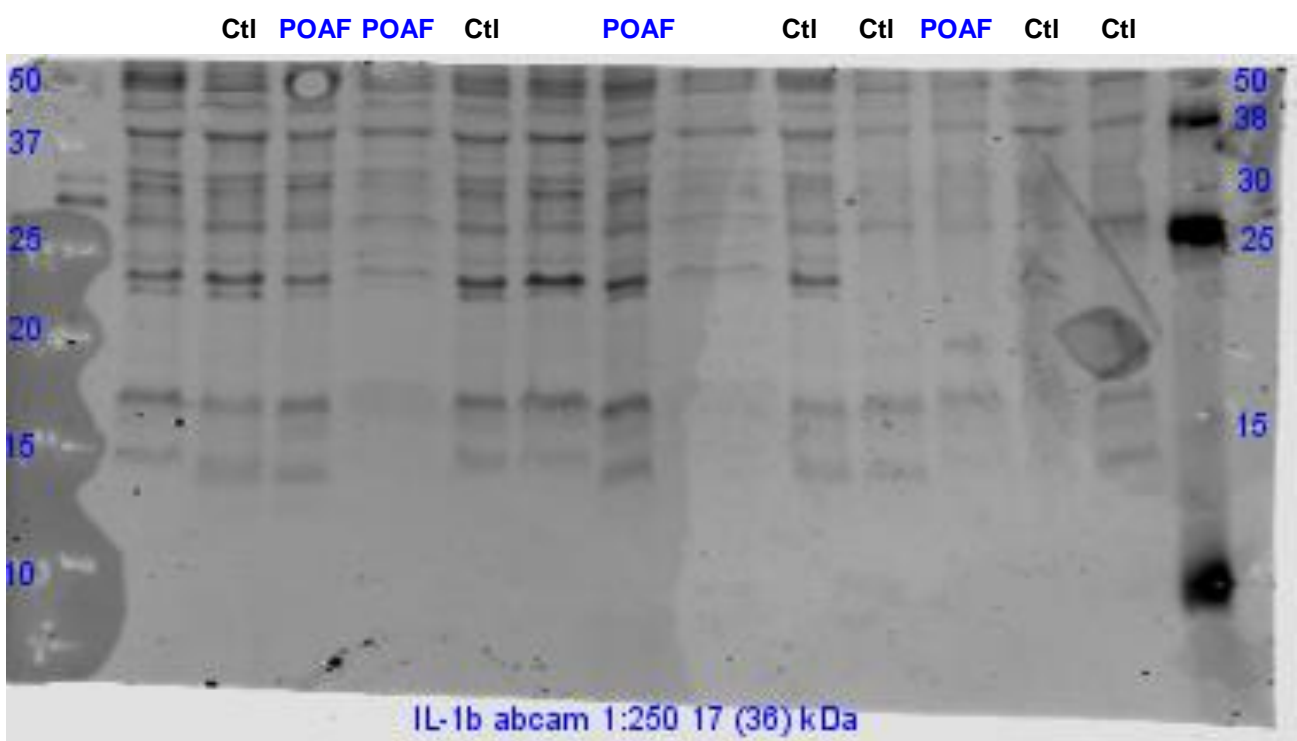


Figure 5A – Human atrial whole-tissue lysates

(Pro-)Interleukin-1 β



GAPDH

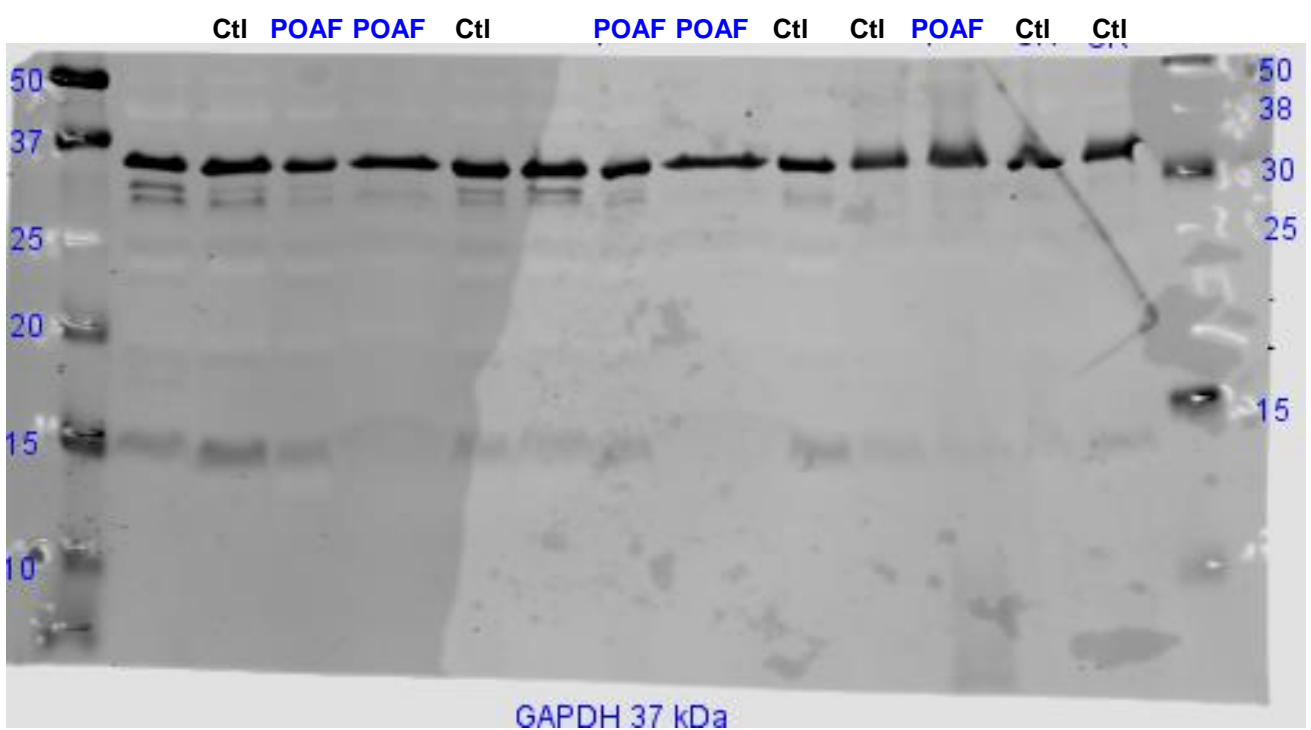


Figure 5A – Human atrial whole-tissue lysates

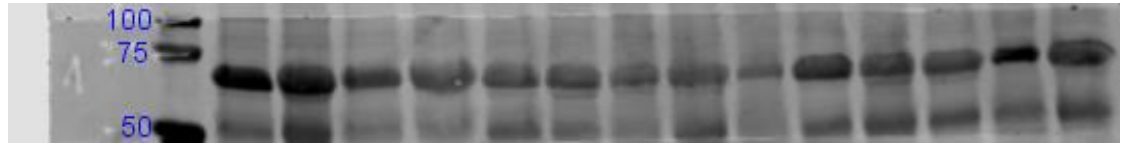
Gel 1 / 3

Ctl Ctl POAF POAF Ctl Ctl POAF POAF Ctl POAF Ctl Ctl POAF

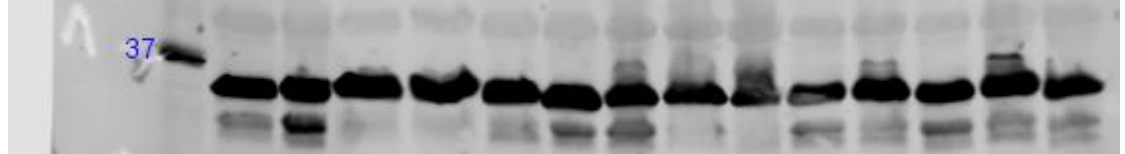
Total-NFκB



Ser536-NFκB



GAPDH



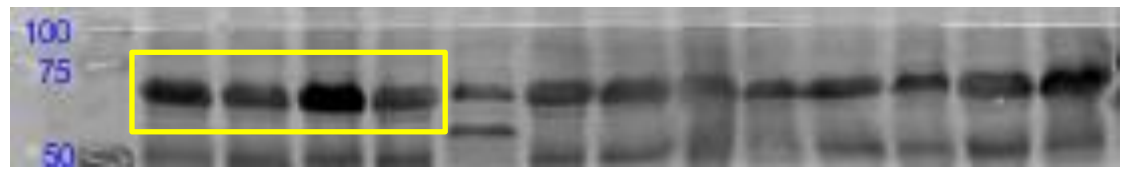
Gel 2 / 3

Ctl Ctl POAF POAF Ctl Ctl POAF POAF Ctl Ctl POAF

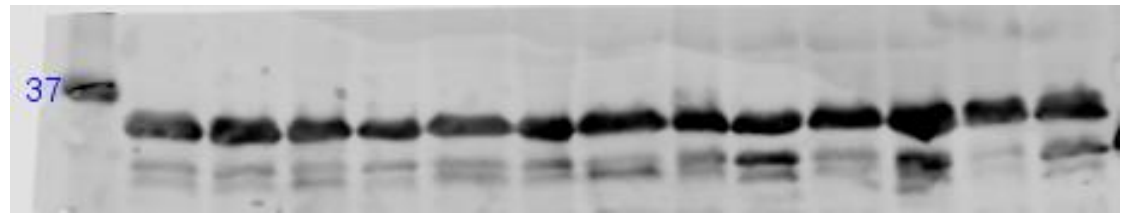
Total-NFκB



Ser536-NFκB



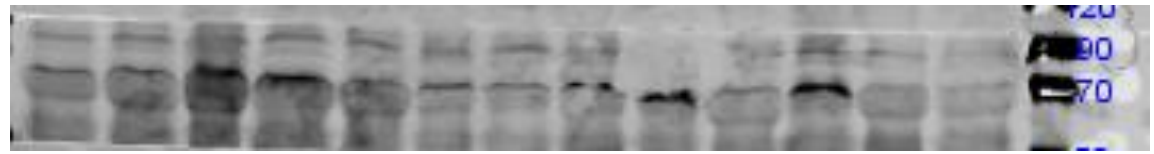
GAPDH



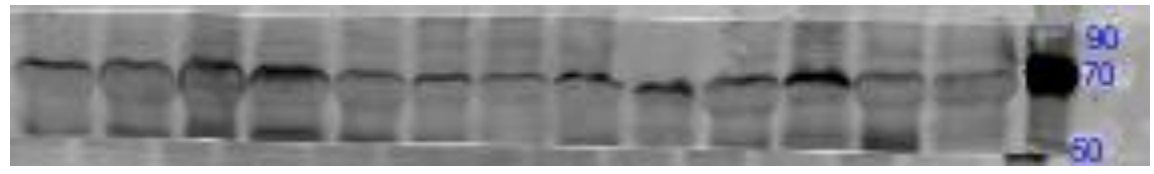
Gel 3 / 3

Ctl Ctl POAF POAF Ctl Ctl Ctl Ctl Ctl Ctl Ctl

Total-NFκB



Ser536-NFκB



GAPDH

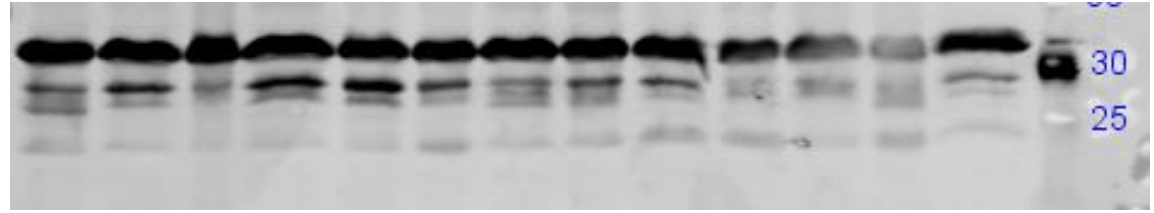
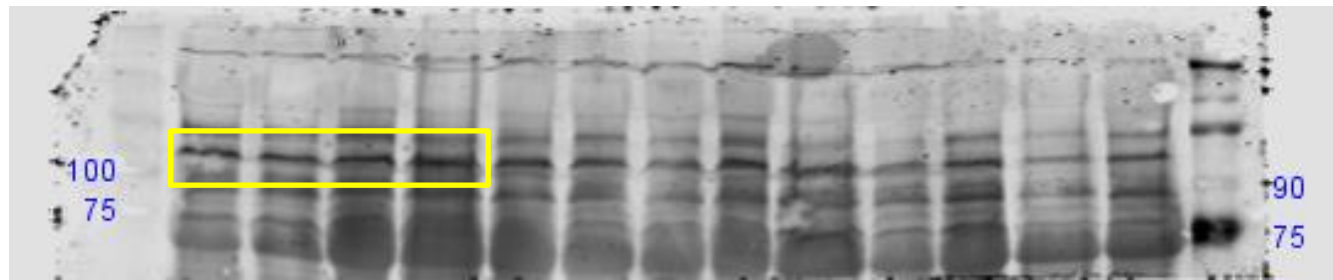


Figure 5B – Human atrial whole-tissue lysates

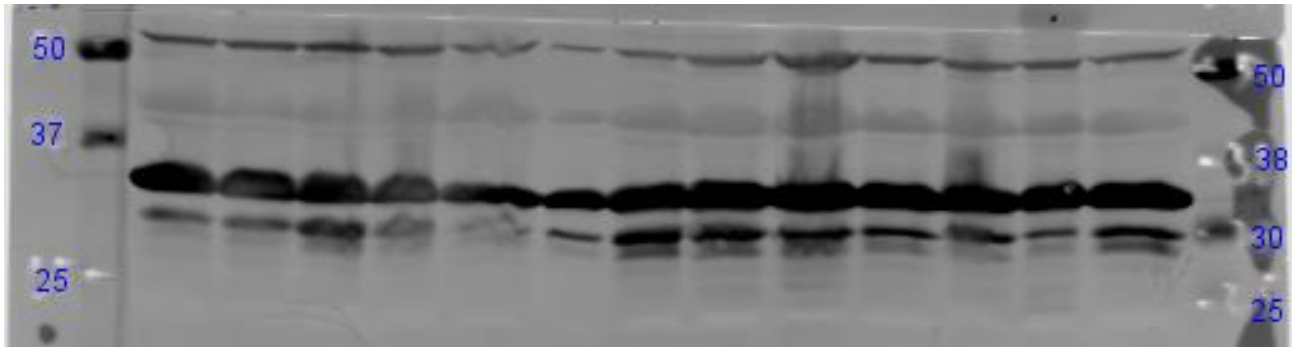
Gel 1 / 2

TLR4

Ctl Ctl POAF POAF Ctl POAF POAF Ctl Ctl POAF POAF Ctl



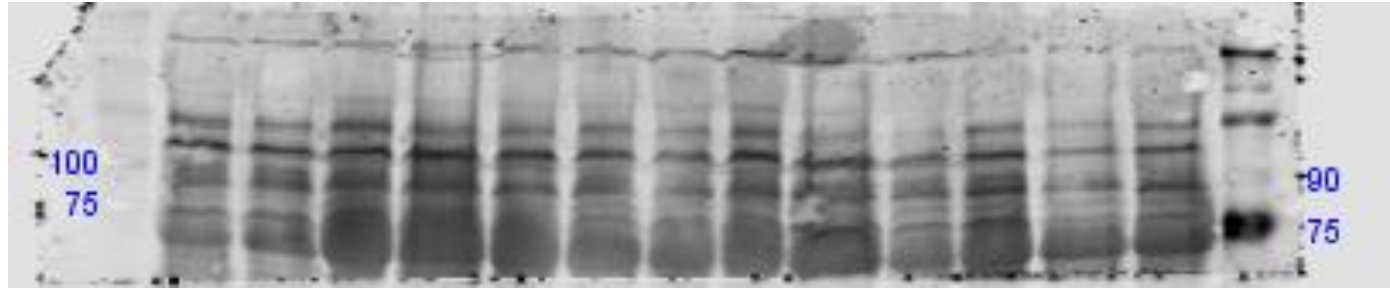
GAPDH



Gel 2 / 2

TLR4

Ctl POAF POAF Ctl Ctl Ctl POAF Ctl Ctl POAF POAF Ctl



GAPDH

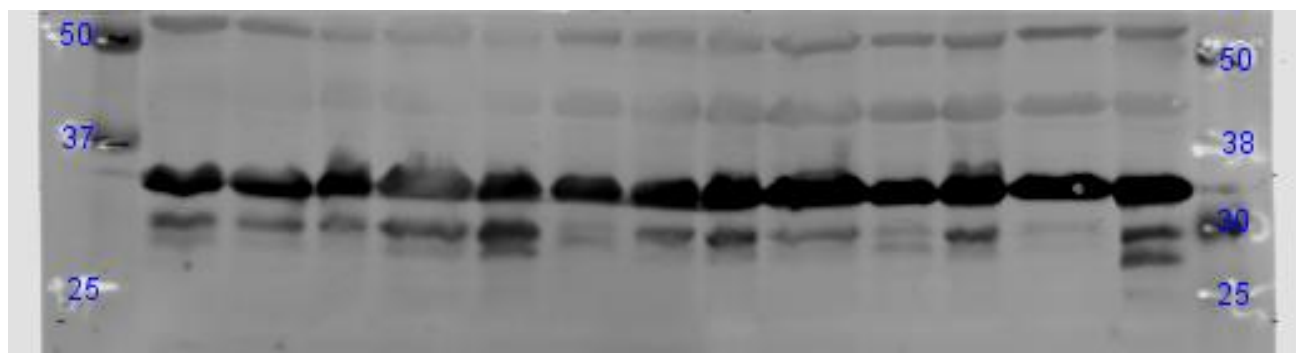
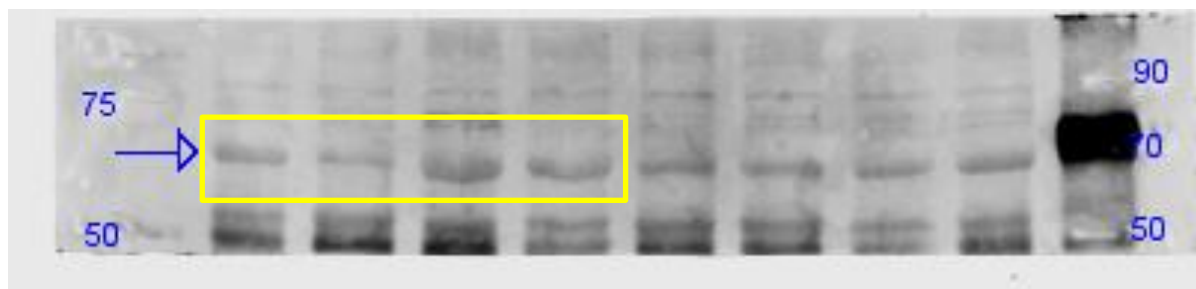


Figure 5B – Human atrial whole-tissue lysates

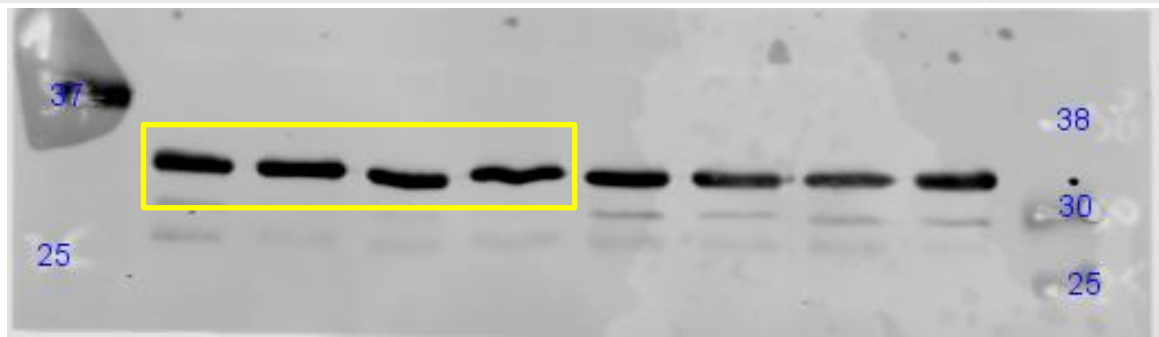
Gel 1 / 4

Ctl Ctl POAF POAF Ctl Ctl POAF POAF

P2X7R



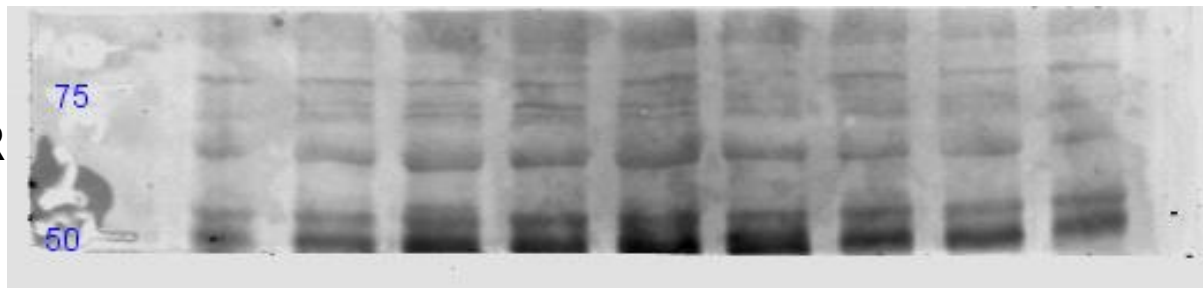
GAPDH



Gel 2 / 4

Ctl Ctl POAF POAF Ctl Ctl POAF POAF Ctl

P2X7R



GAPDH

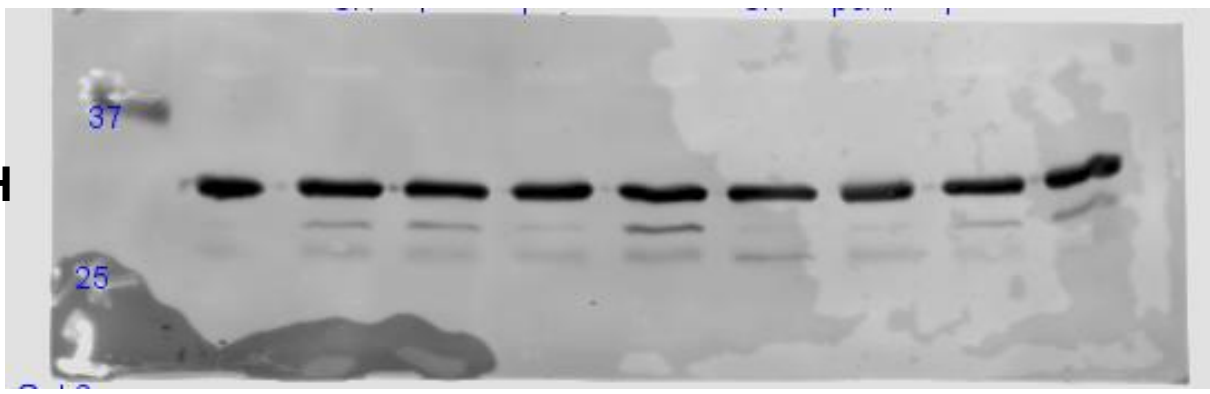
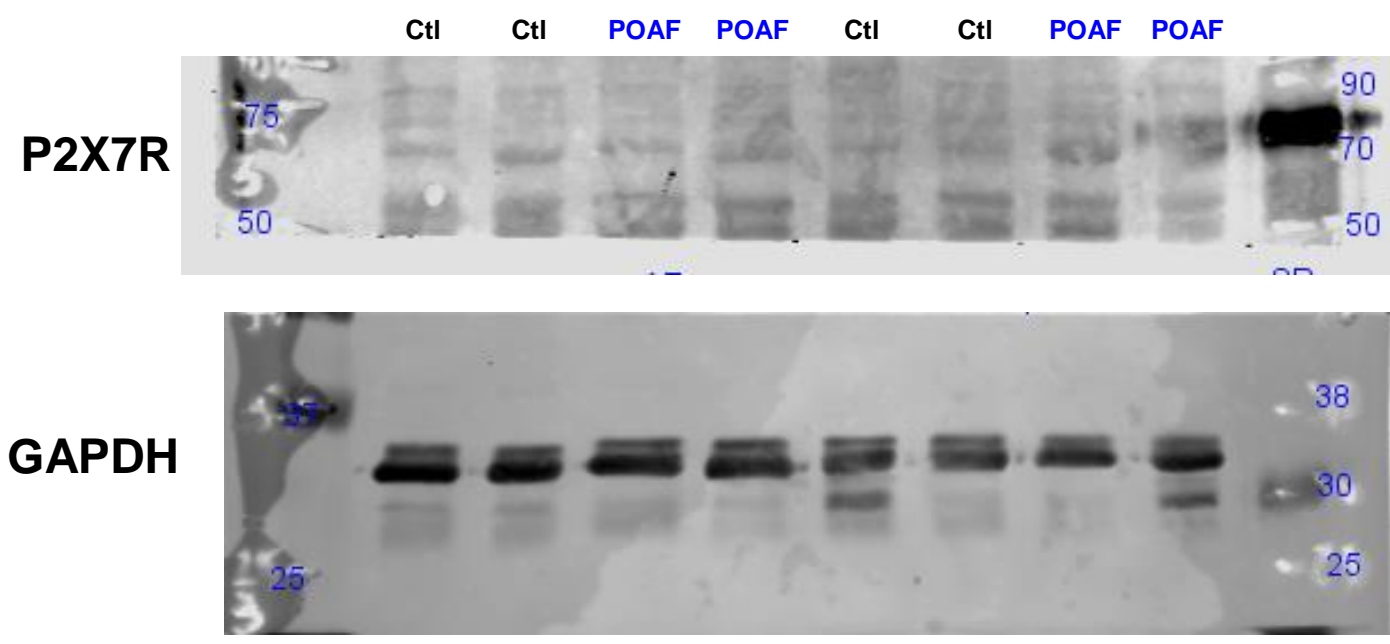


Figure 5B – Human atrial whole-tissue lysates

Gel 3 / 4



Gel 4 / 4

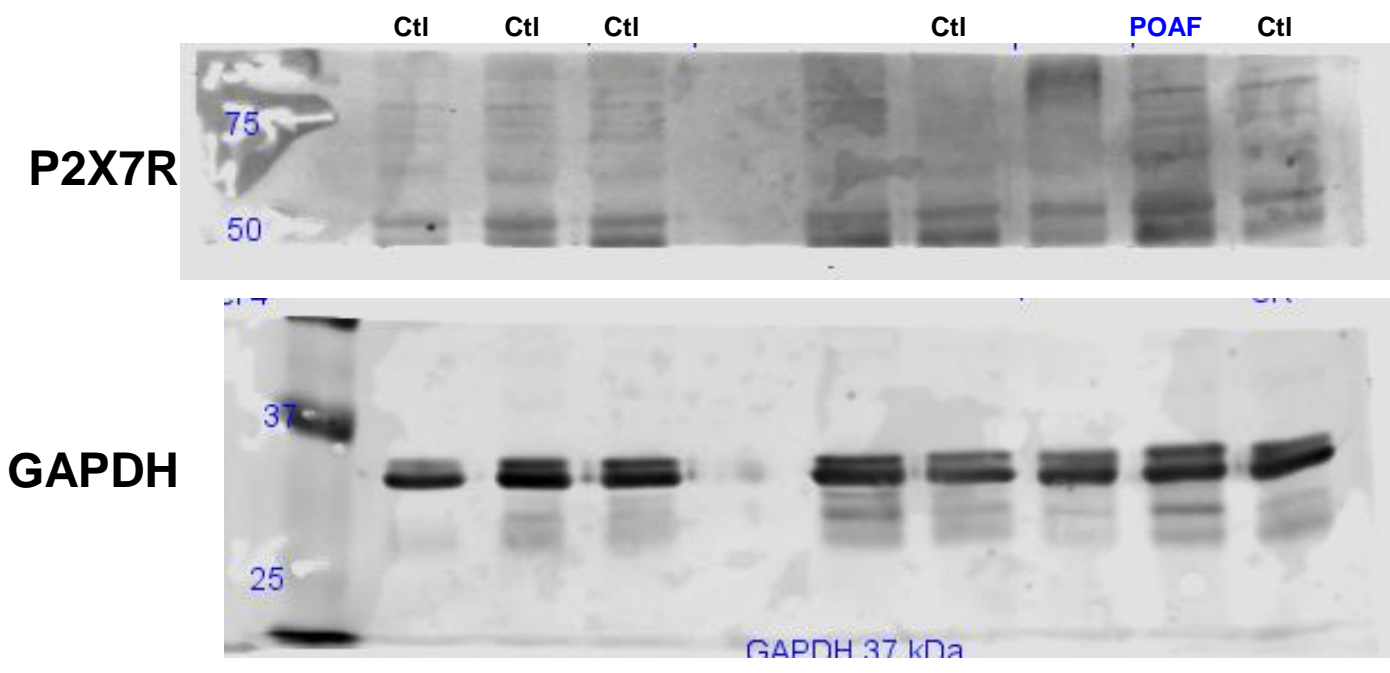
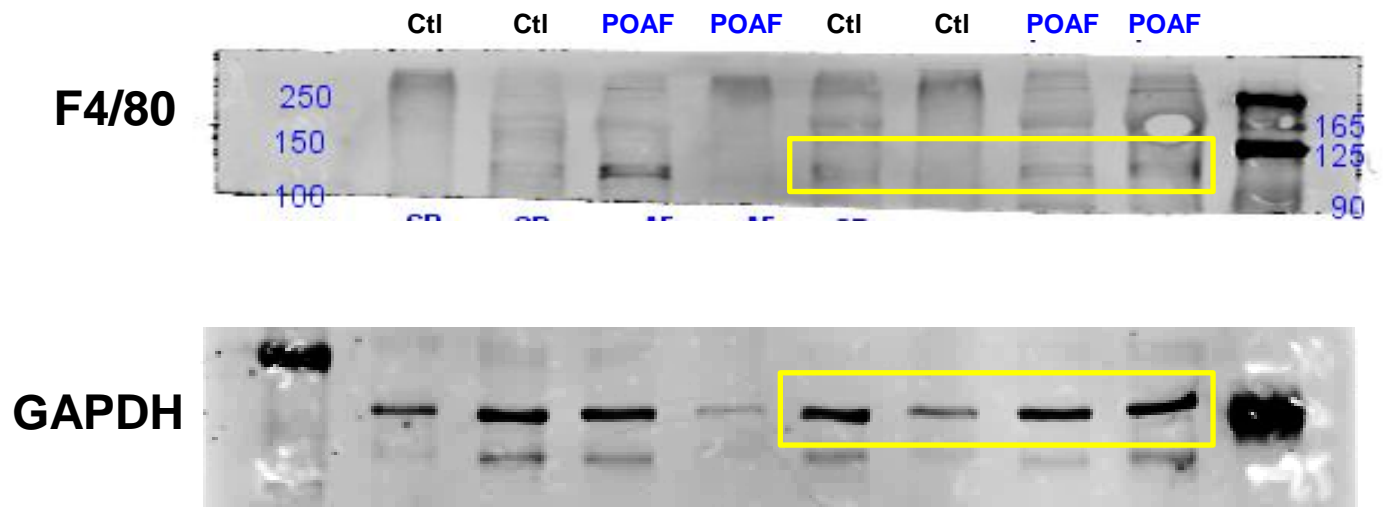


Figure 5B – Human atrial whole-tissue lysates

Gel 1 / 2



Gel 2 / 2

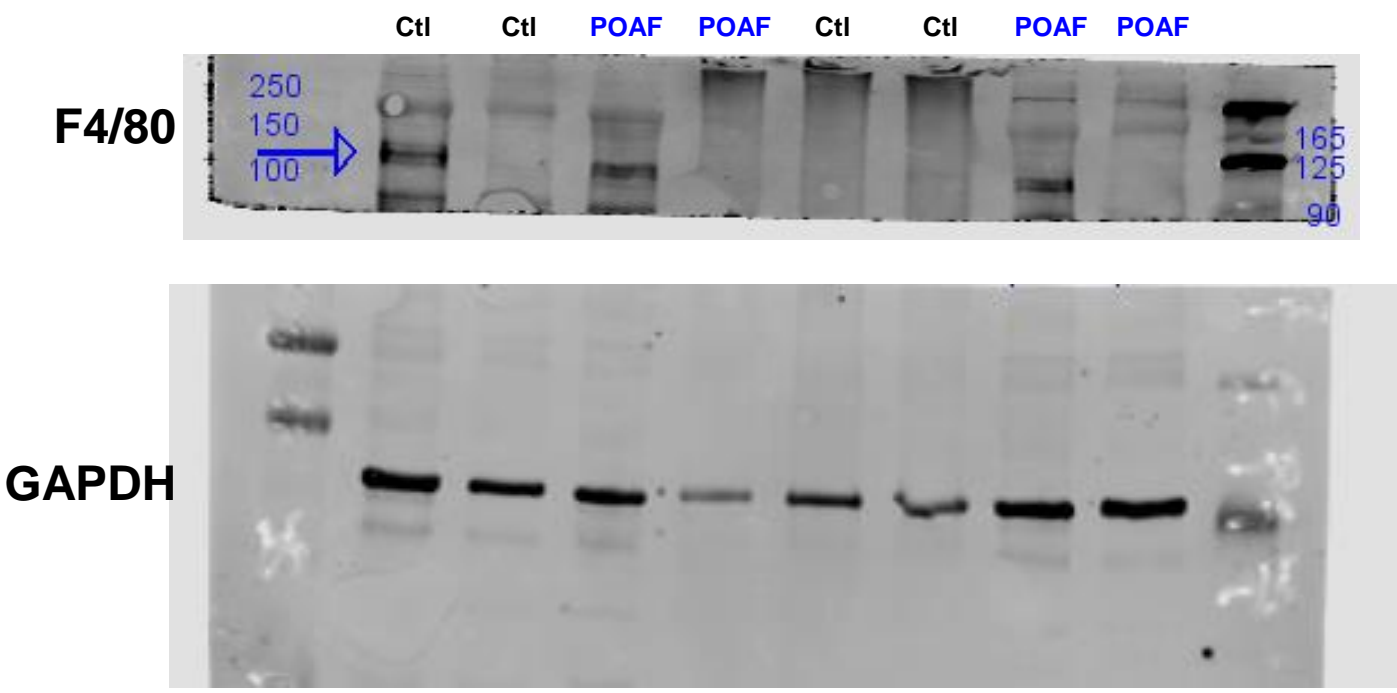
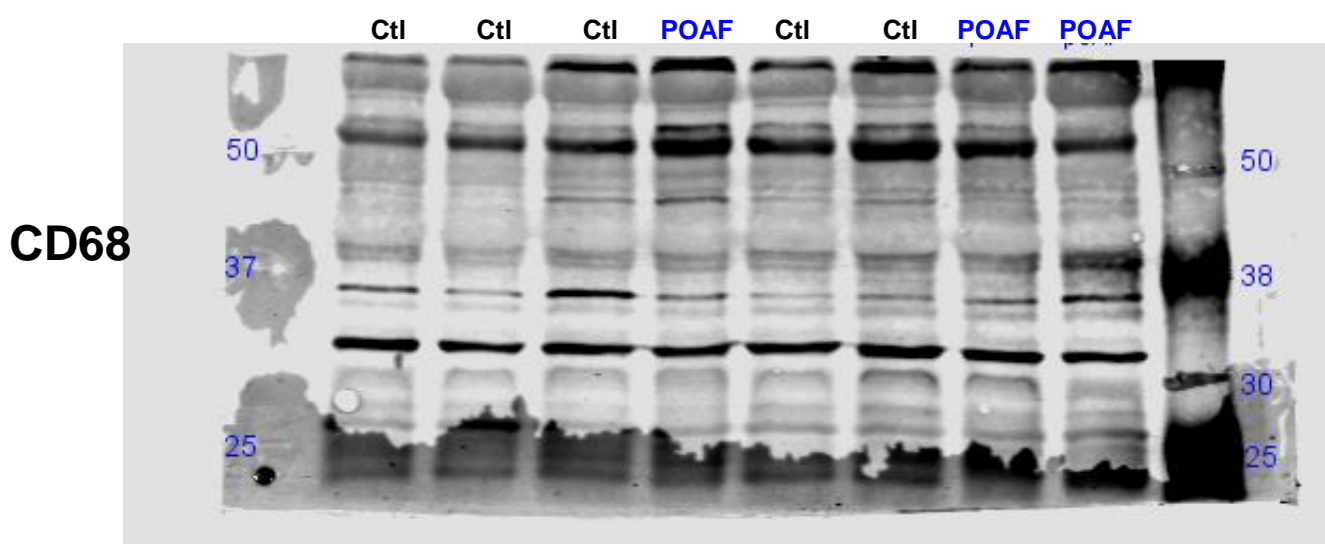
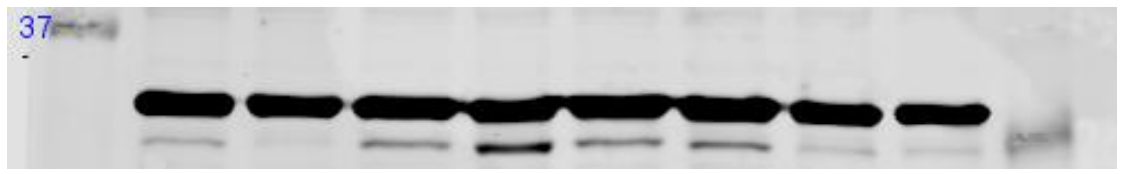


Figure 5C – Human atrial whole-tissue lysates

Gel 1 / 4

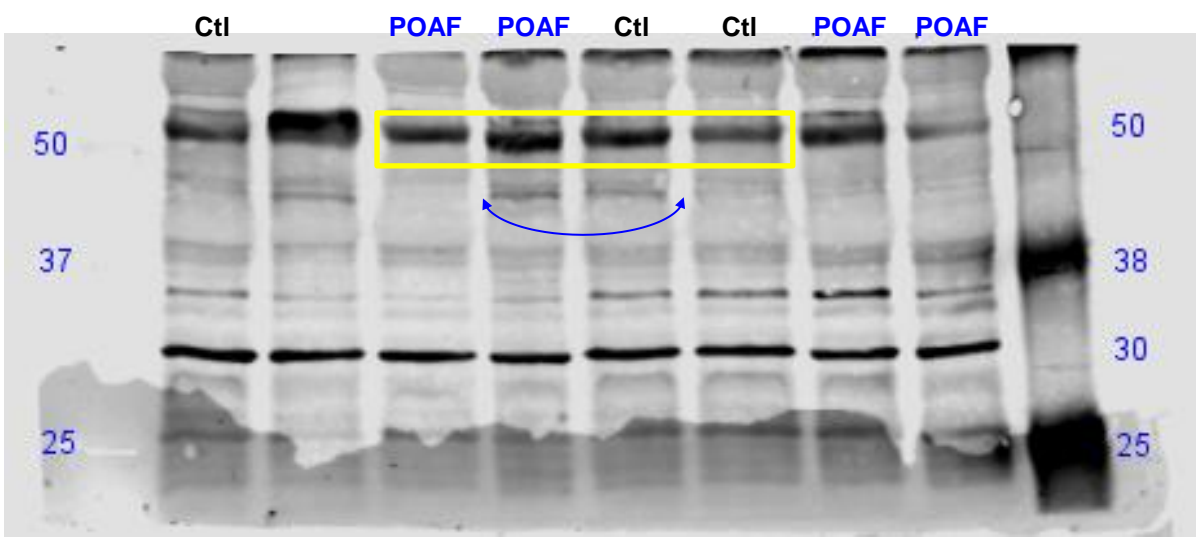


GAPDH



Gel 2 / 4

CD68



GAPDH

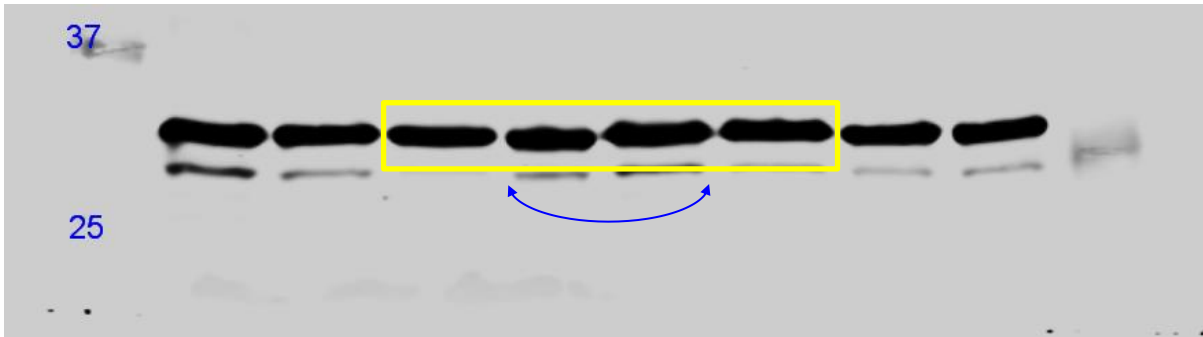
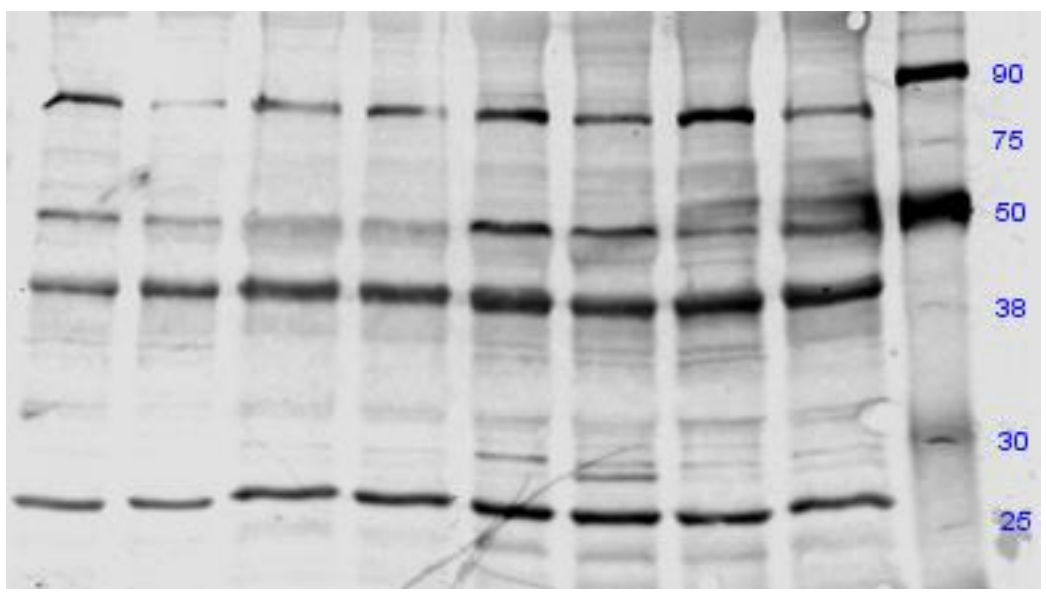


Figure 5C – Human atrial whole-tissue lysates

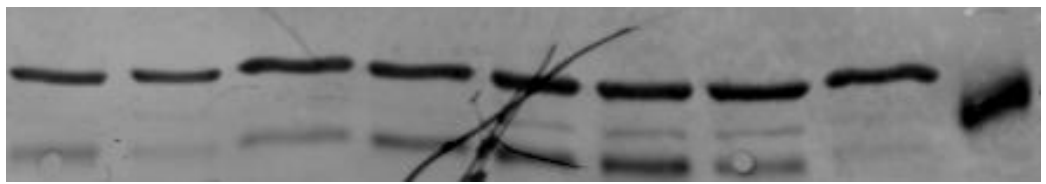
Gel 3 / 4

Ctl Ctl POAF POAF Ctl Ctl POAF POAF

CD68



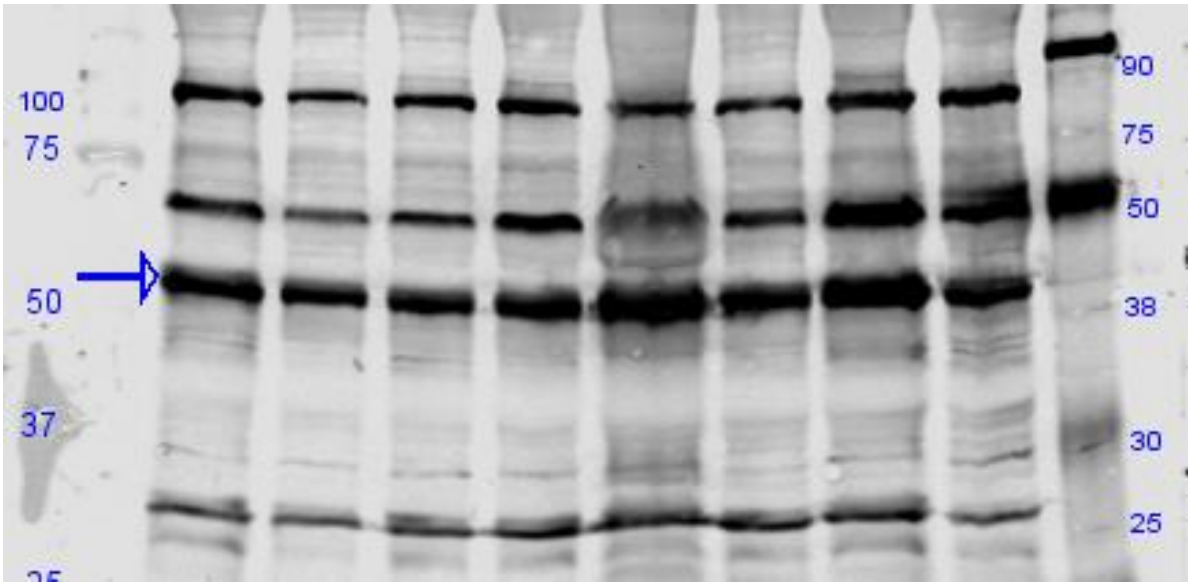
GAPDH



Gel 4 / 4

Ctl Ctl POAF Ctl Ctl POAF POAF

CD68



GAPDH

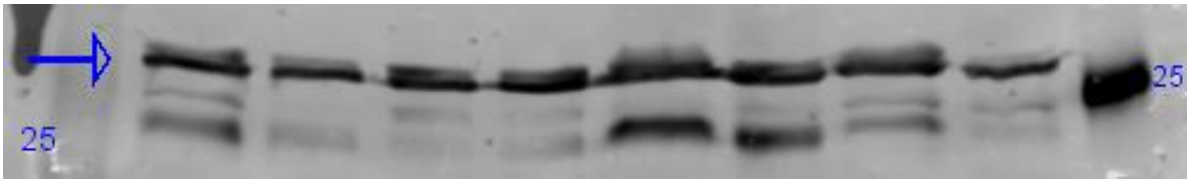
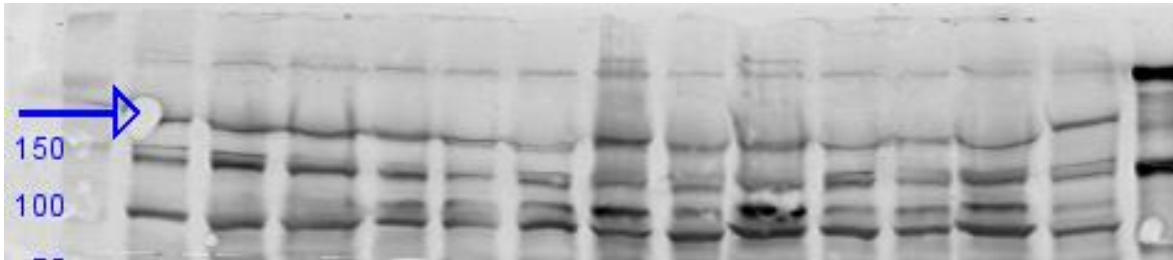


Figure 5C – Human atrial whole-tissue lysates

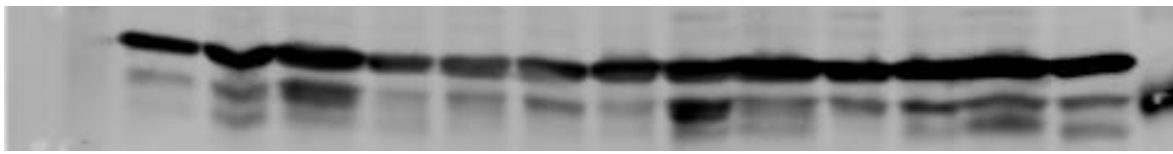
Gel 1 / 3

Ctl POAF POAF Ctl Ctl POAF POAF Ctl Ctl POAF POAF Ctl

CD206



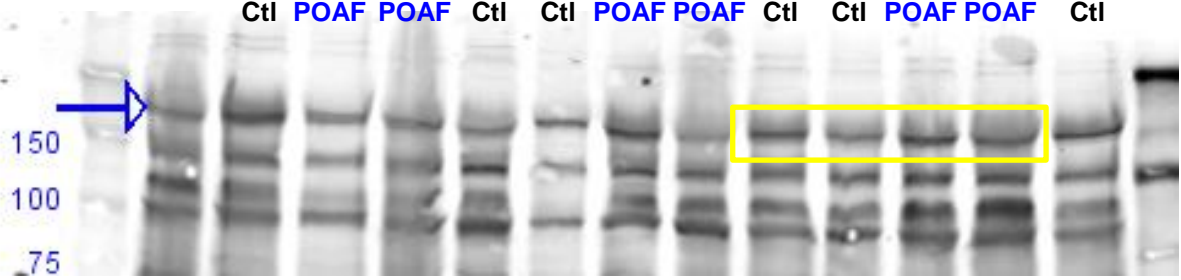
GAPDH



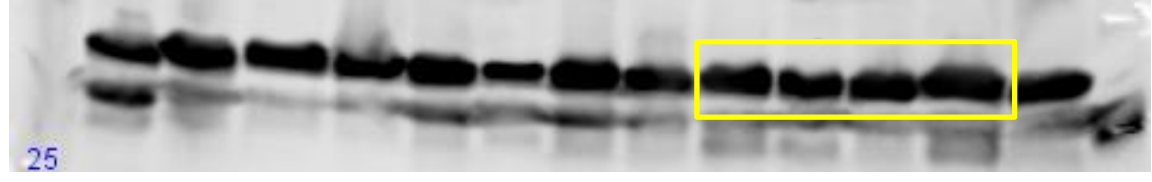
Gel 2 / 3

Ctl POAF POAF Ctl Ctl POAF POAF Ctl Ctl POAF POAF Ctl

CD206



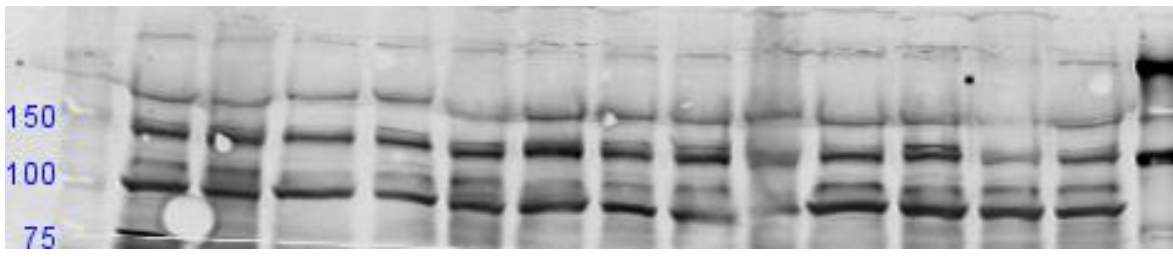
GAPDH



Gel 3 / 3

Ctl Ctl POAF POAF Ctl Ctl POAF Ctl POAF Ctl POAF

CD206



GAPDH

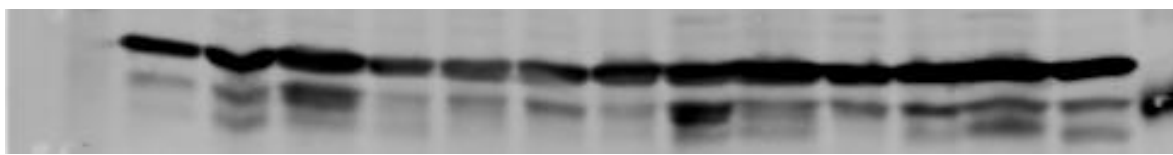
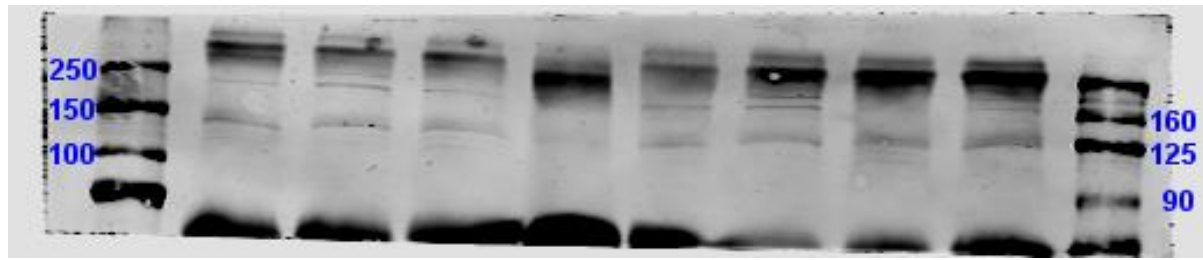


Figure 5C – Human atrial whole-tissue lysates

NLRP3

Ctl Ctl POAF POAF Ctl Ctl POAF POAF



Ponceau

Ctl Ctl POAF POAF Ctl Ctl POAF POAF

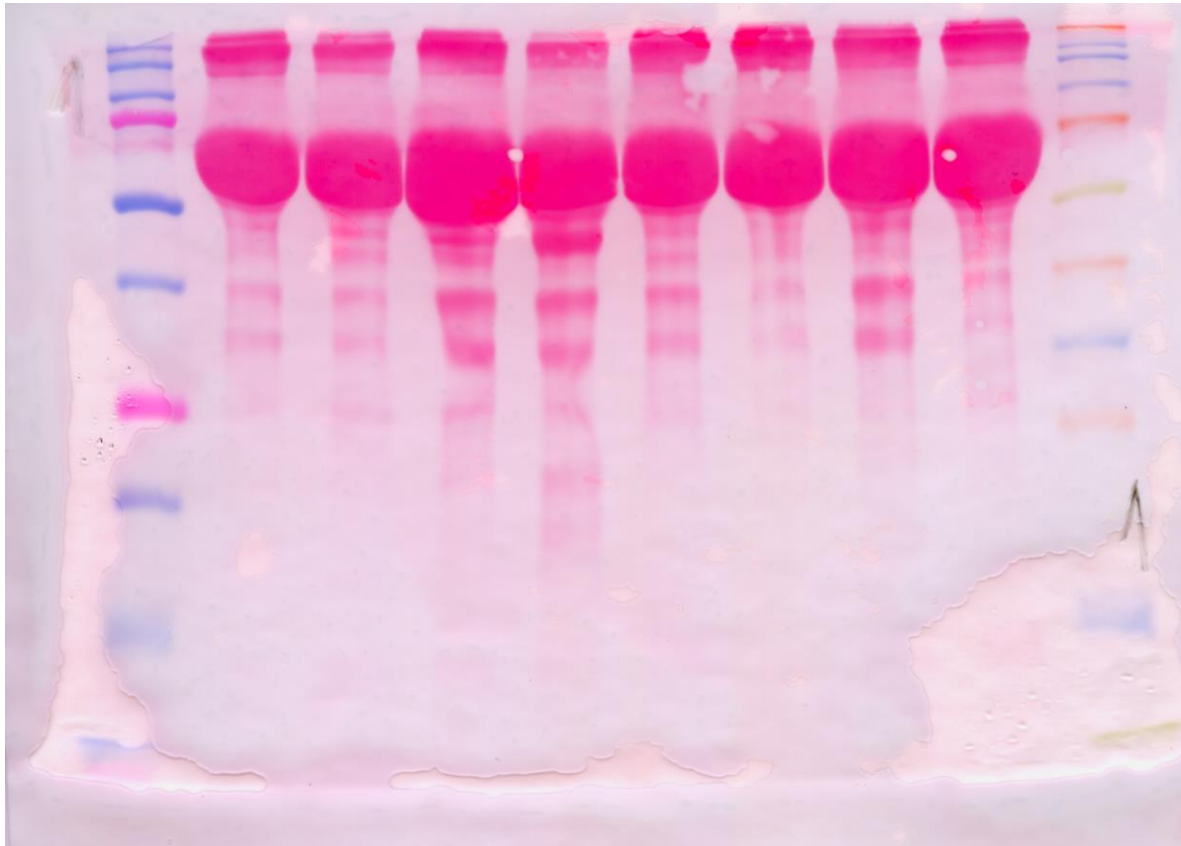
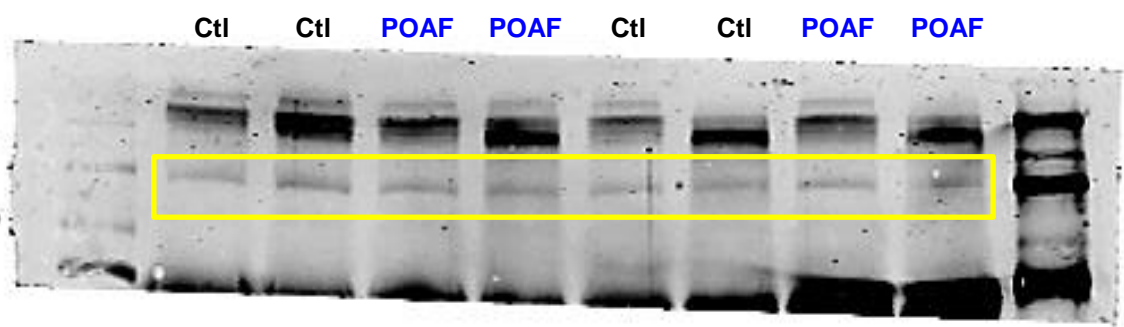


Figure 6A – Human atrial cardiomyocytes

NLRP3



Ponceau

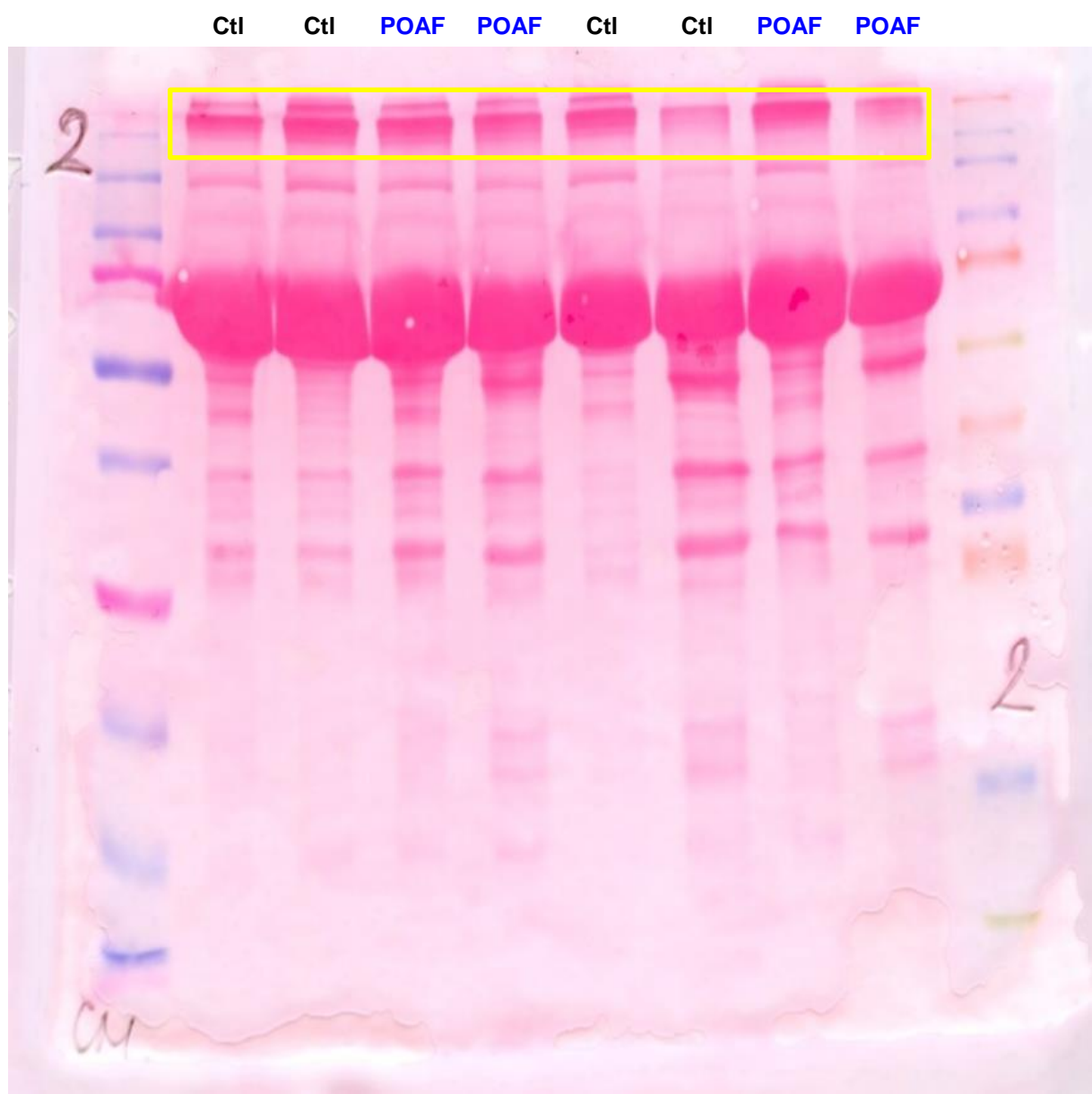
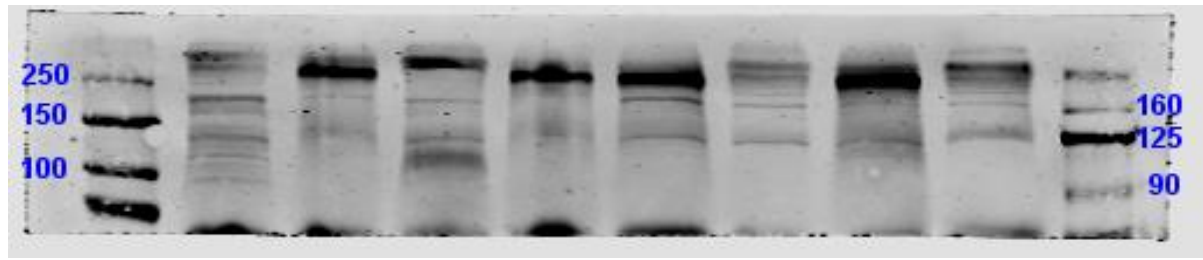


Figure 6A – Human atrial cardiomyocytes

NLRP3

Ctl Ctl POAF POAF Ctl Ctl POAF POAF



Ponceau

Ctl Ctl POAF POAF Ctl Ctl POAF POAF

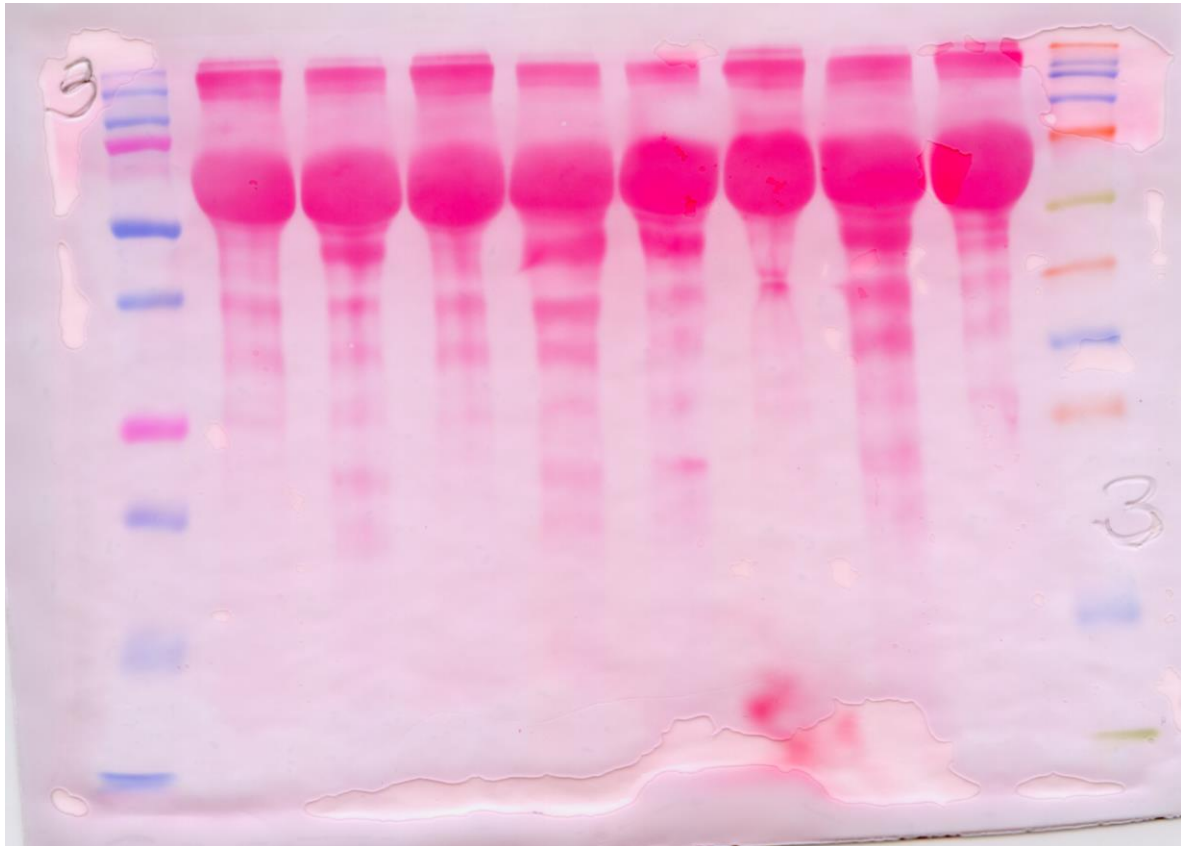
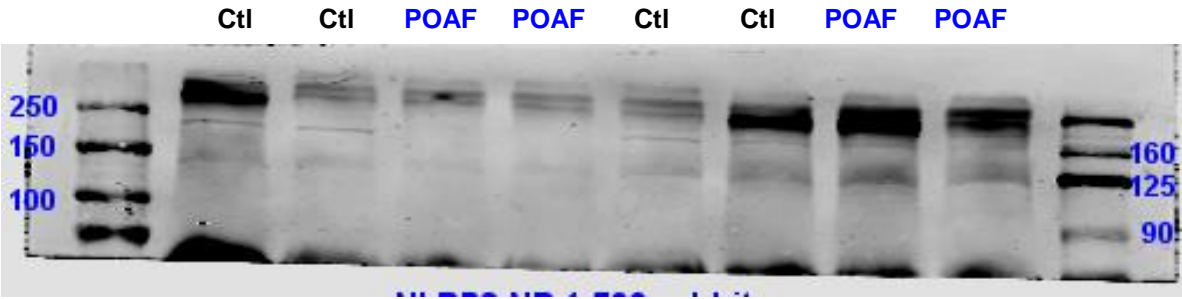


Figure 6A – Human atrial cardiomyocytes

NLRP3



Ponceau

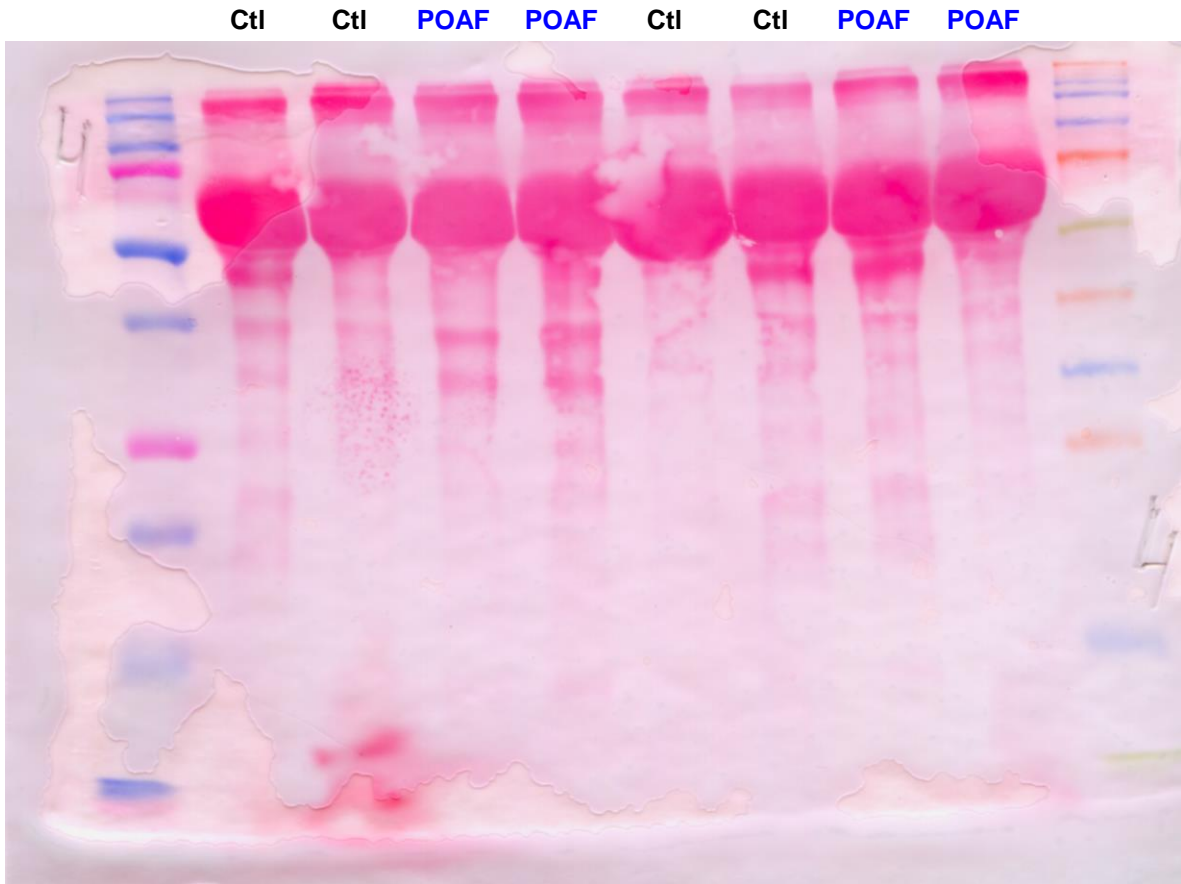
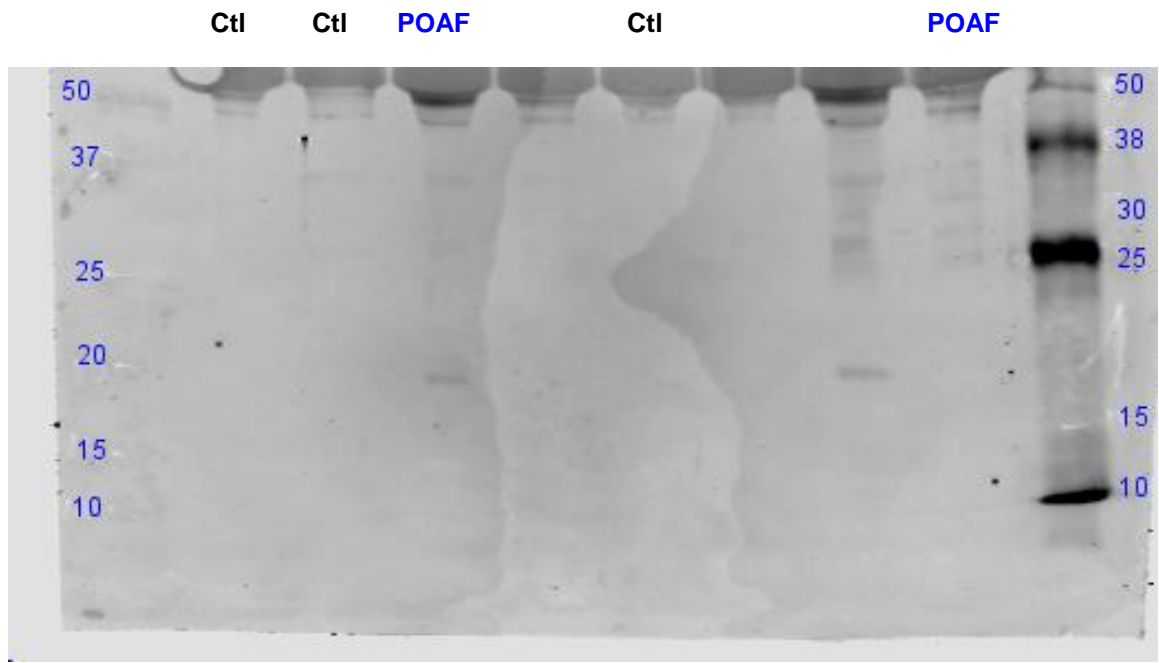


Figure 6A – Human atrial cardiomyocytes

ASC



Ponceau

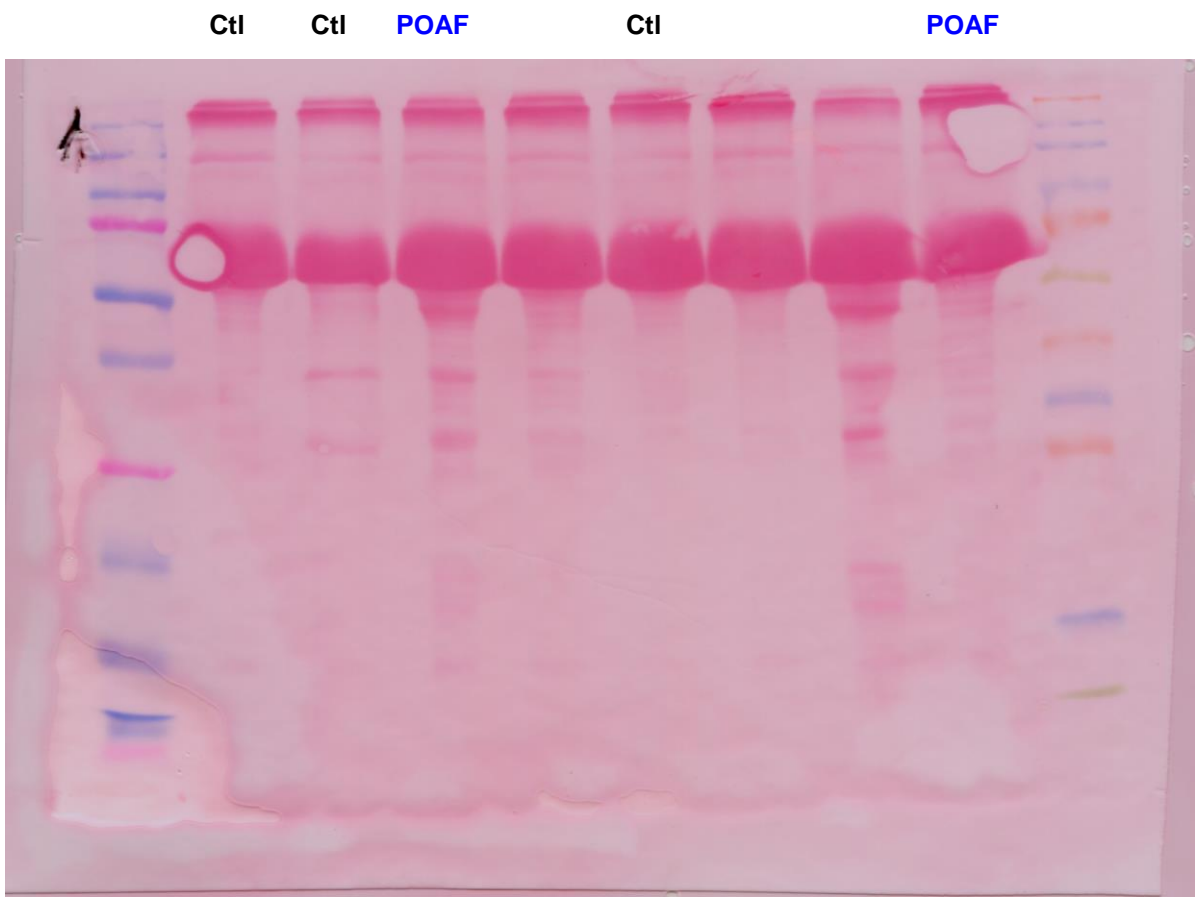
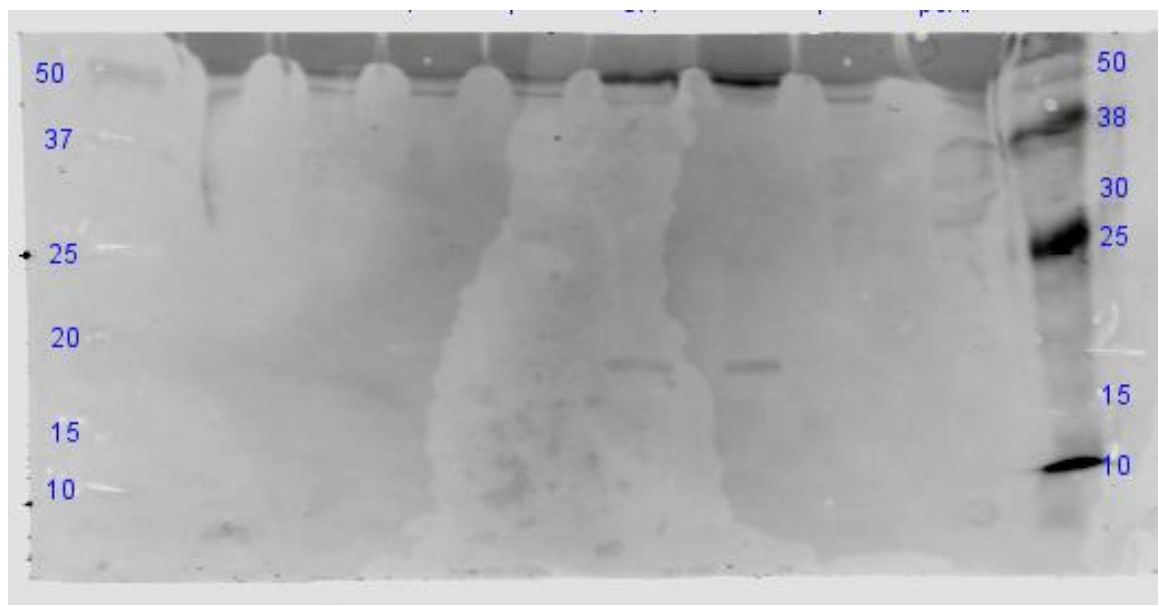


Figure 6B – Human atrial cardiomyocytes

ASC

Ctl POAF Ctl Ctl POAF POAF



Ponceau

Ctl POAF Ctl Ctl POAF POAF

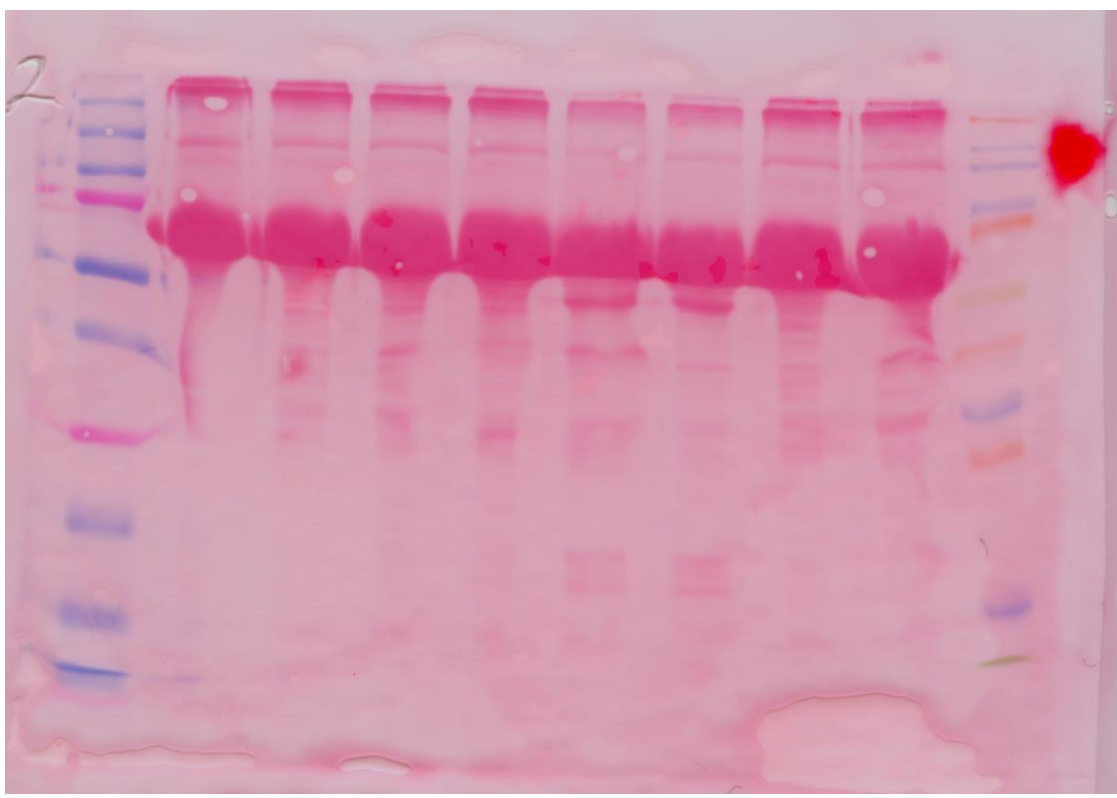
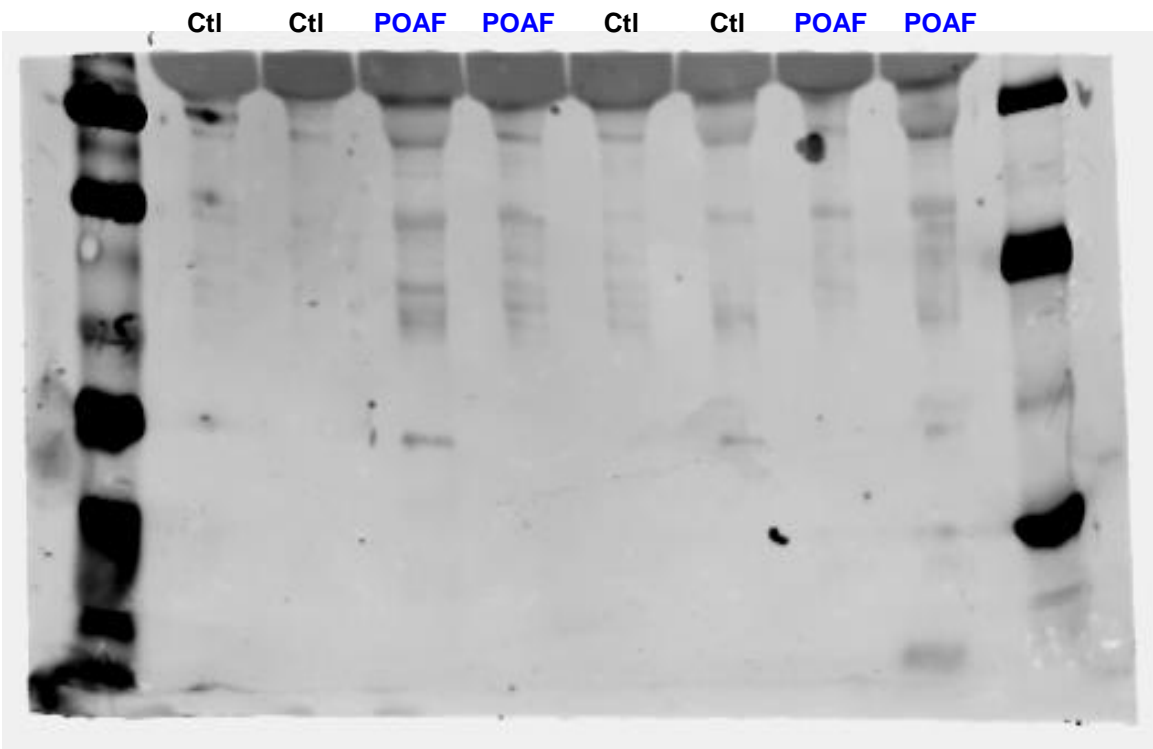


Figure 6B – Human atrial cardiomyocytes

ASC



Ponceau

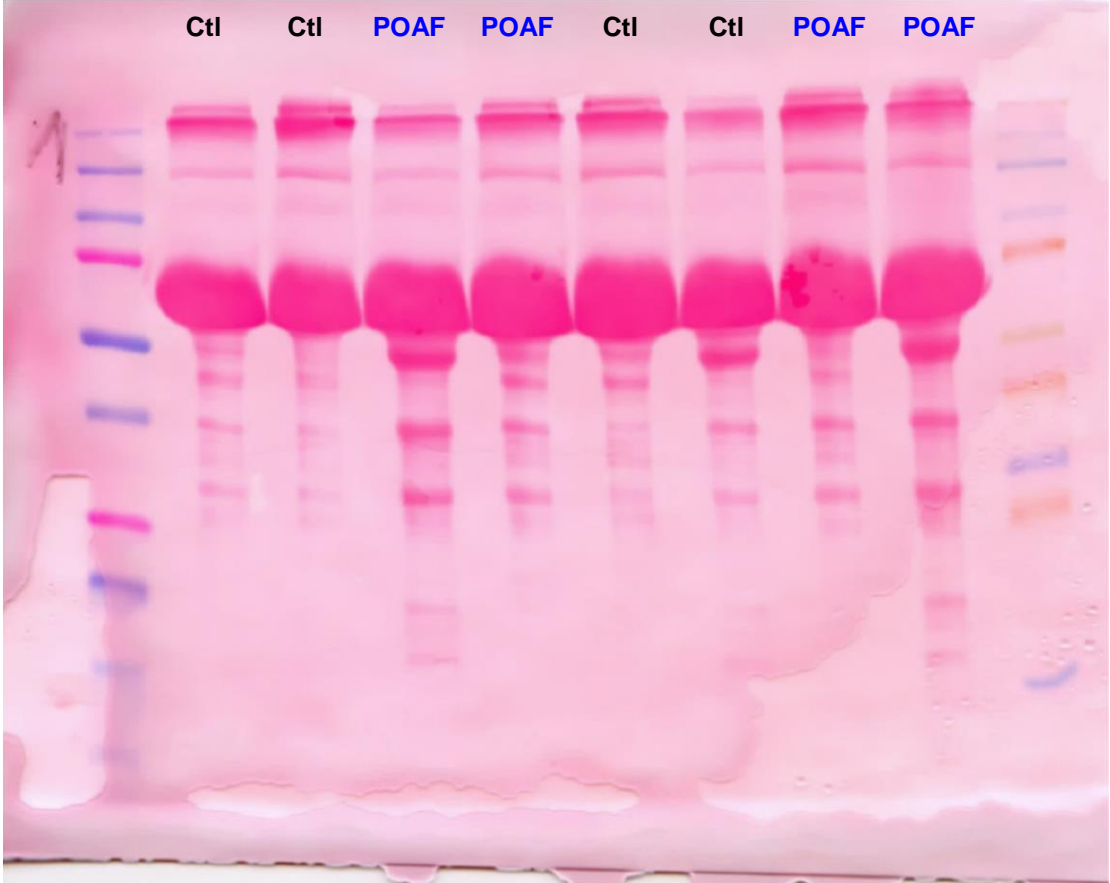
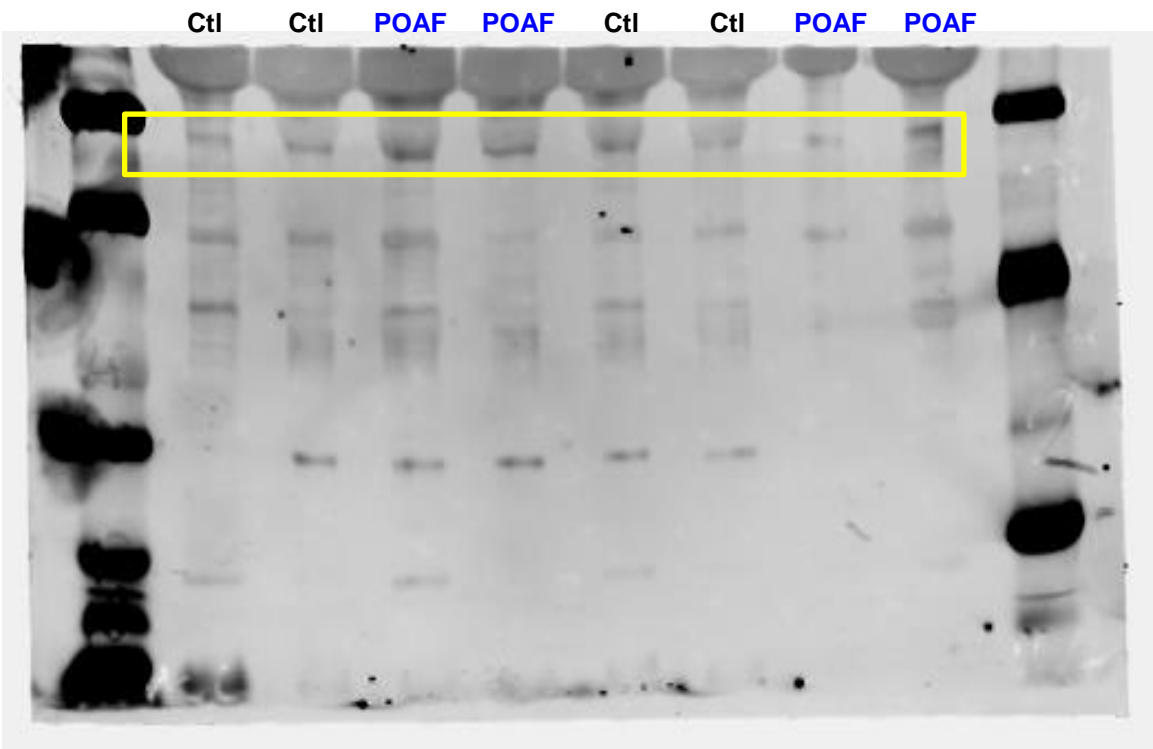


Figure 6B – Human atrial cardiomyocytes

ASC



Ponceau

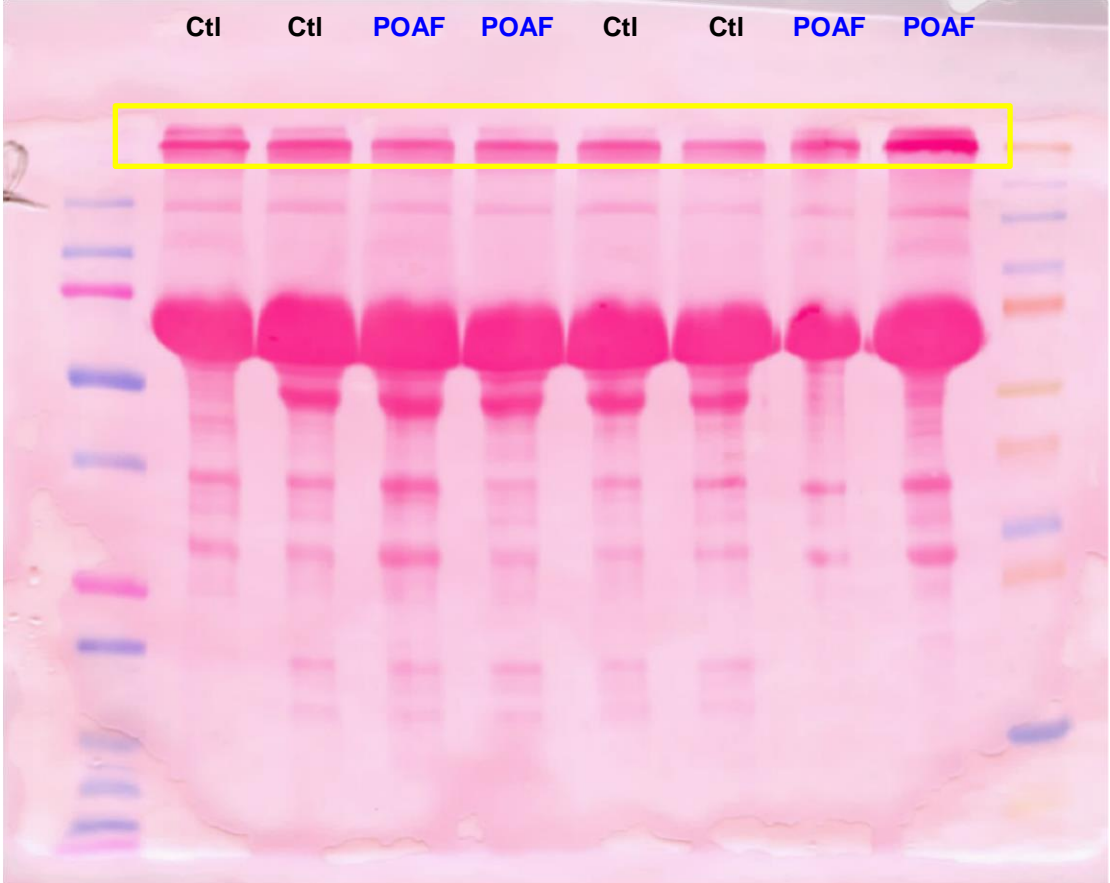
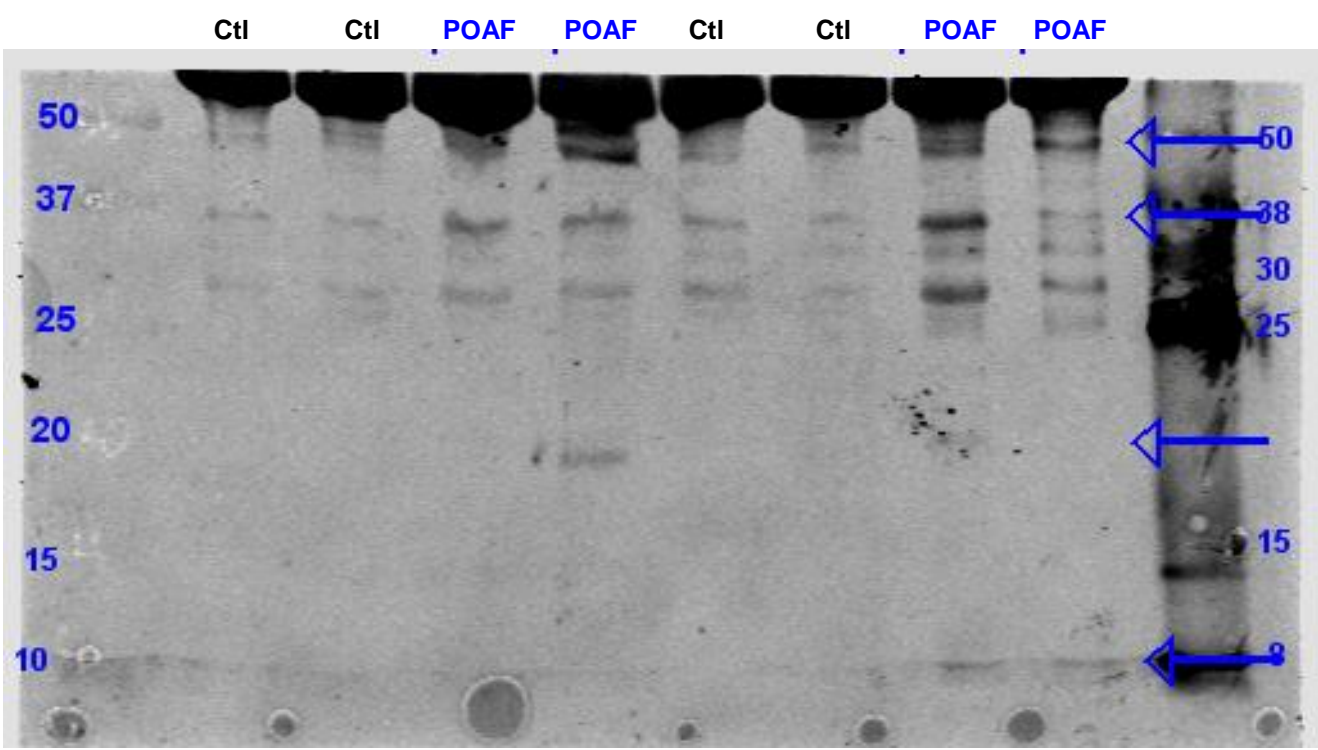


Figure 6B – Human atrial cardiomyocytes

(Pro-)Caspase-1



Ponceau

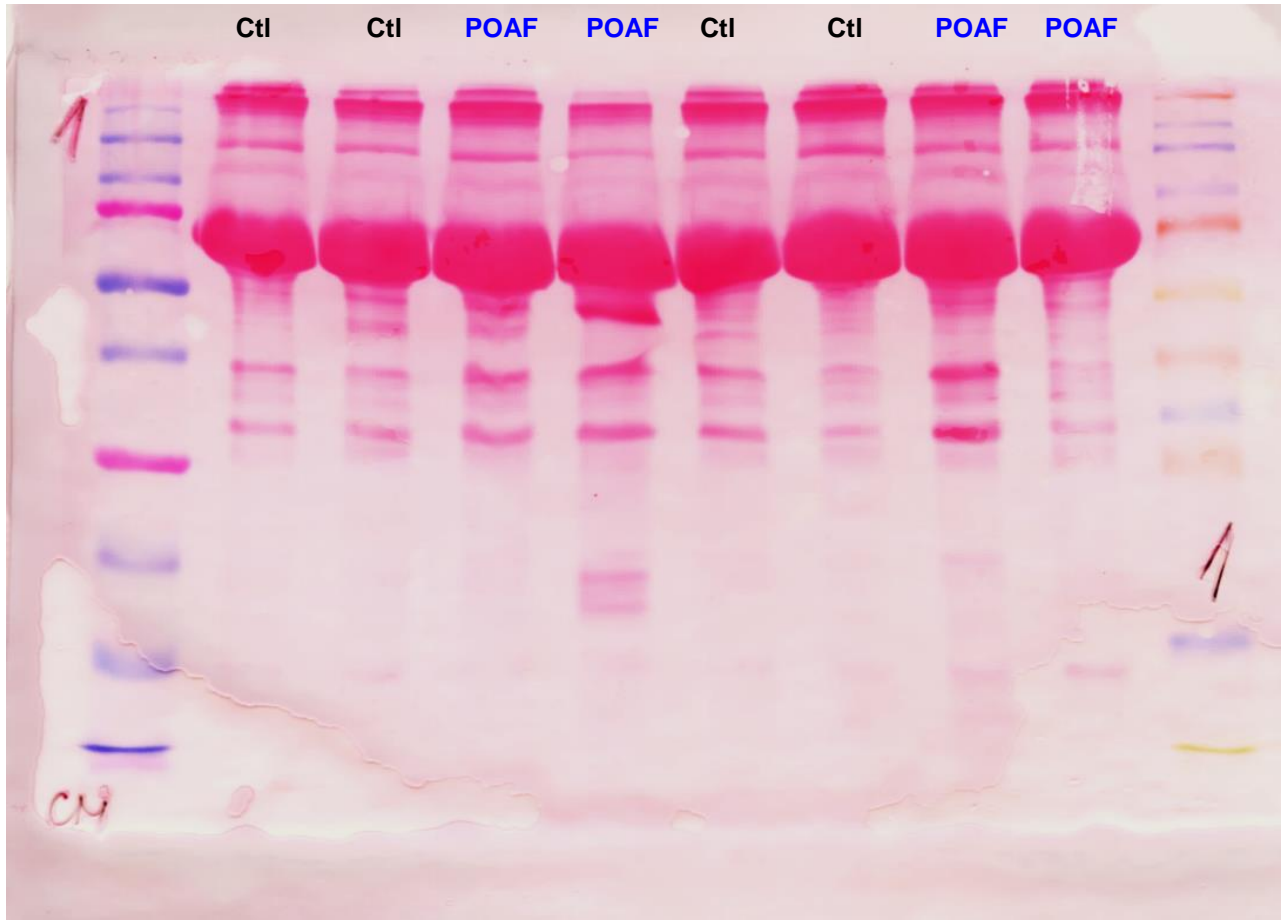
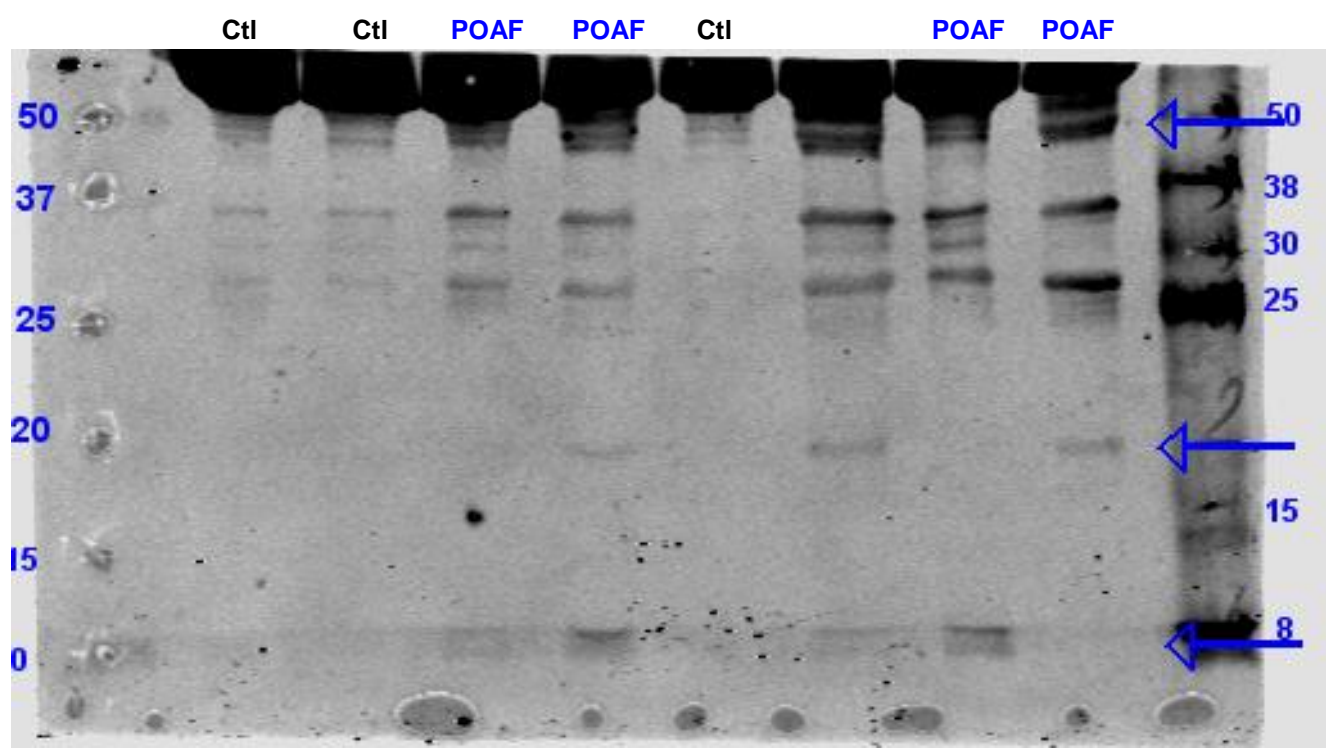


Figure 6C – Human atrial cardiomyocytes

(Pro-)Caspase-1



Ponceau

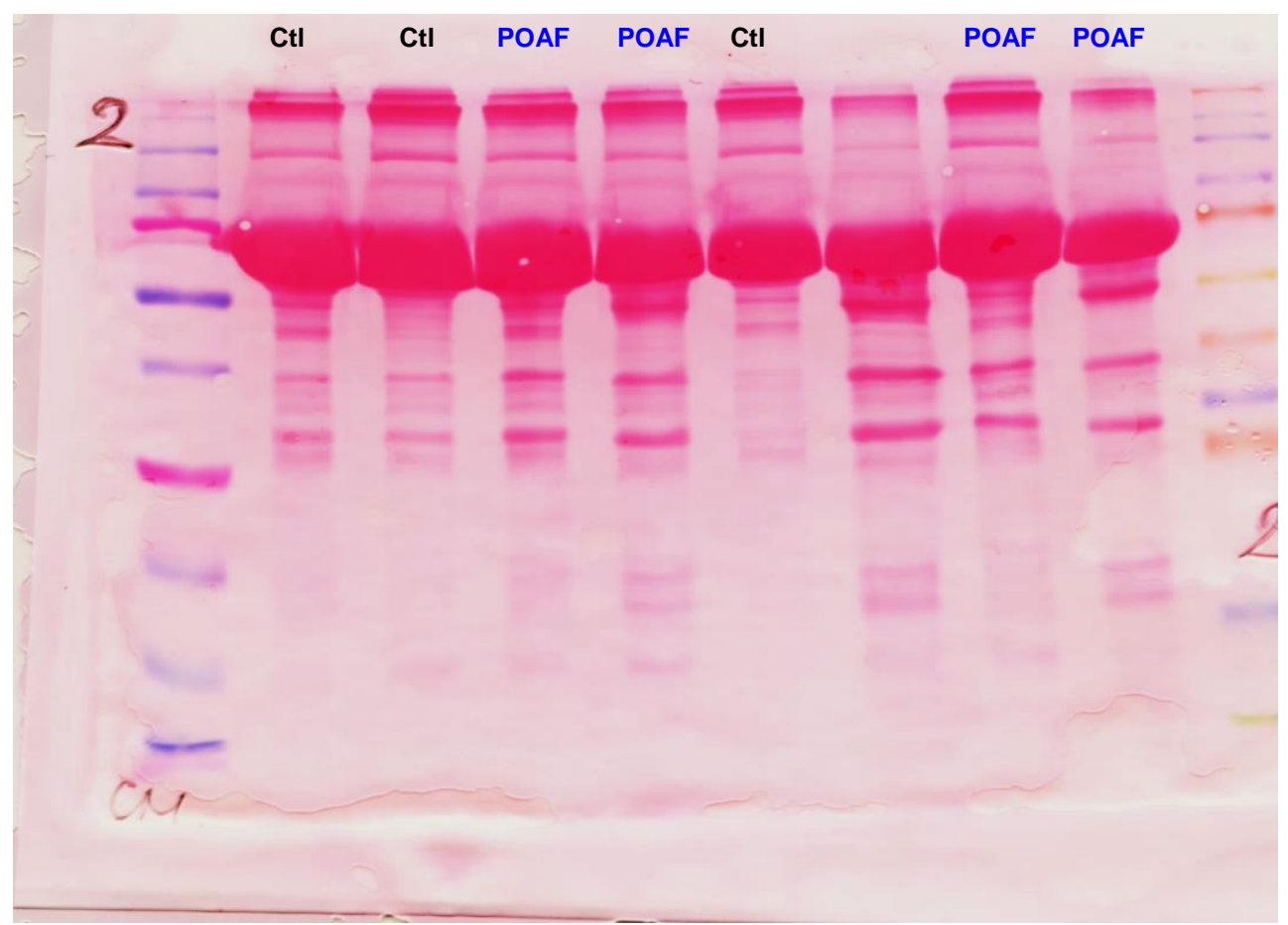
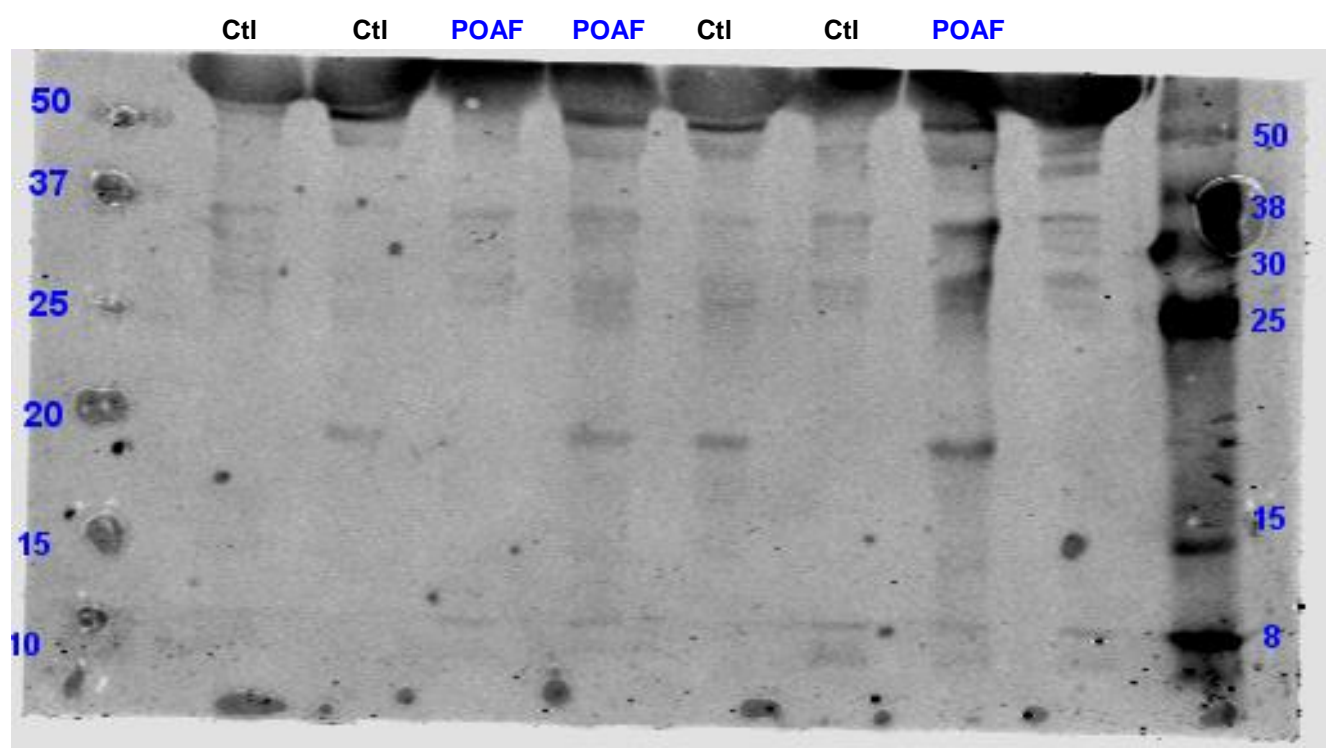


Figure 6C – Human atrial cardiomyocytes

(Pro-)Caspase-1



Ponceau

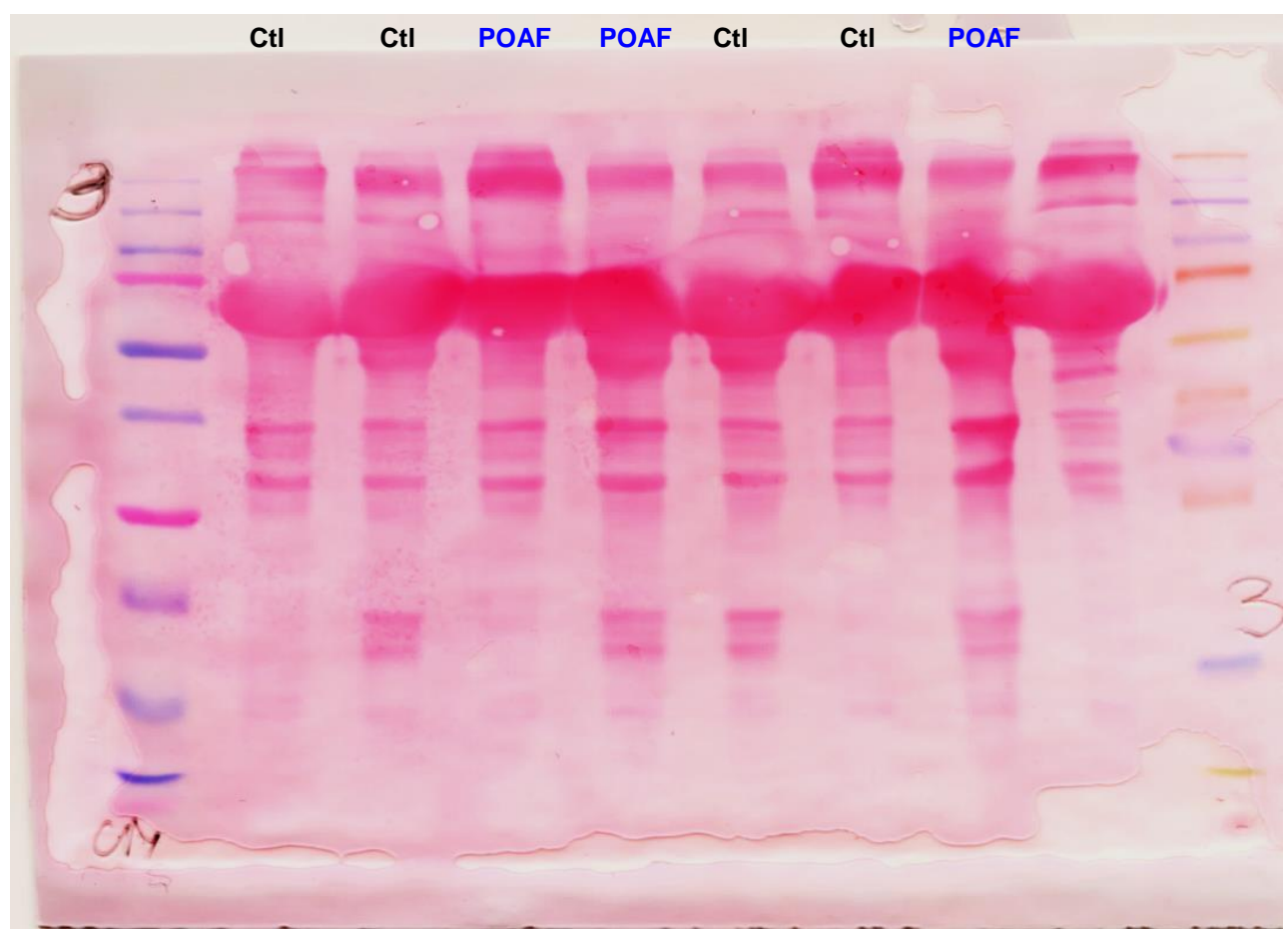
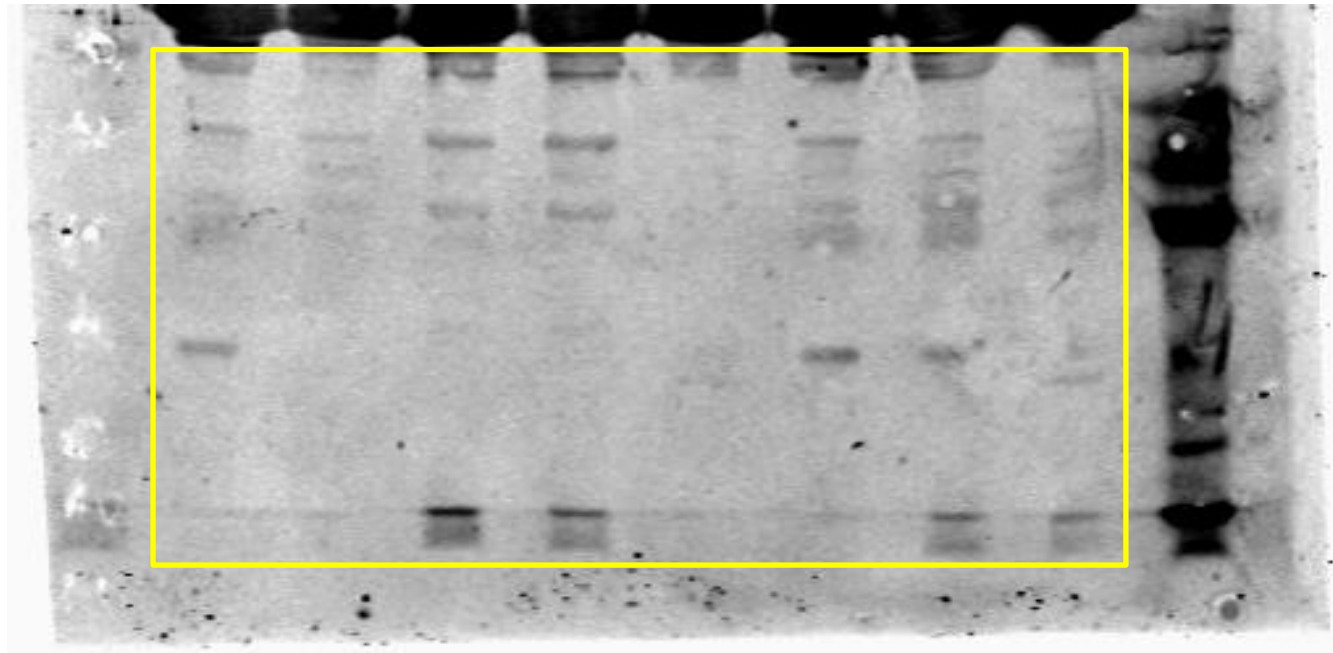


Figure 6C – Human atrial cardiomyocytes

(Pro-)Caspase-1

Ctl Ctl POAF POAF Ctl Ctl POAF POAF



Ponceau

Ctl Ctl POAF POAF Ctl Ctl POAF POAF

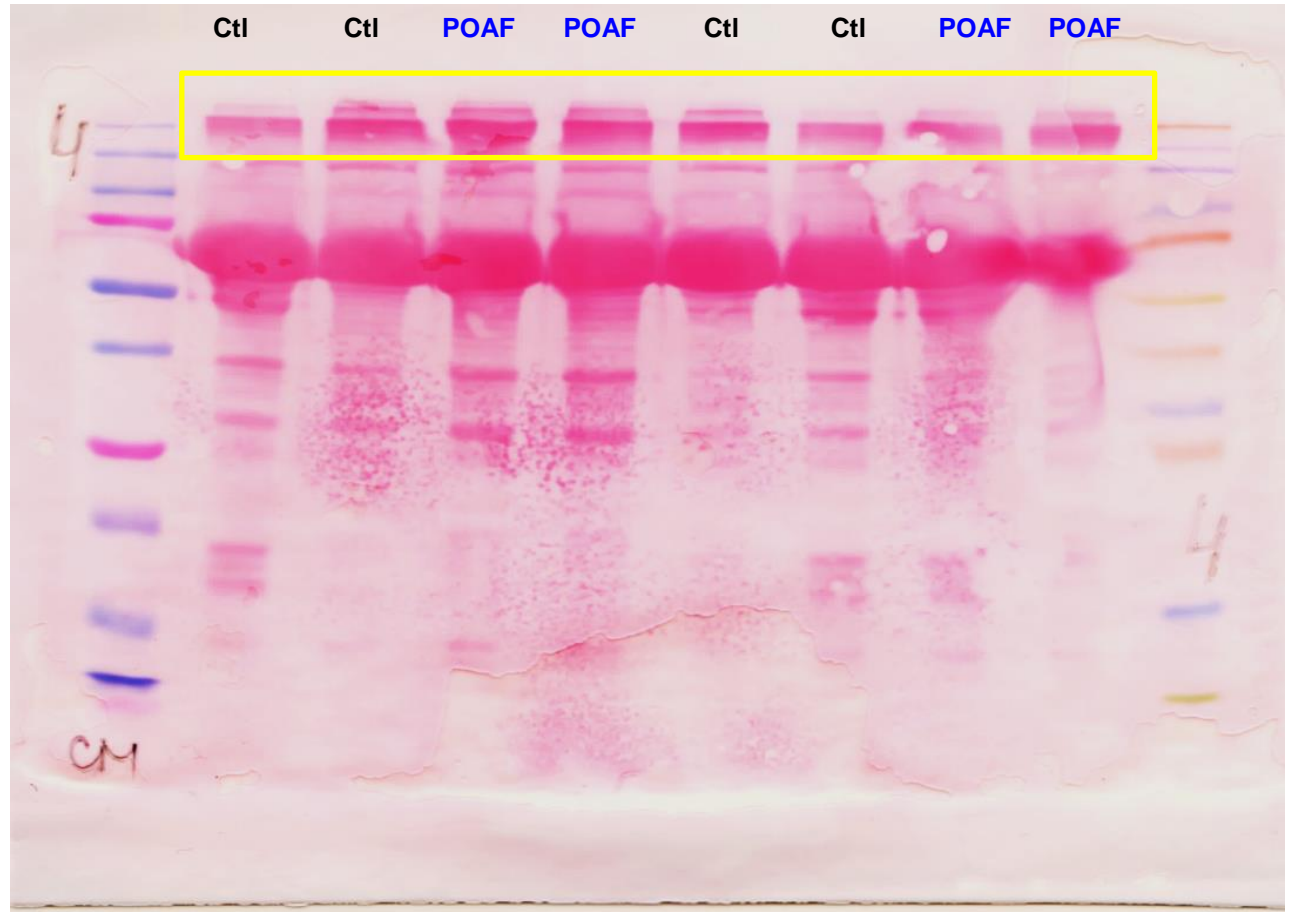
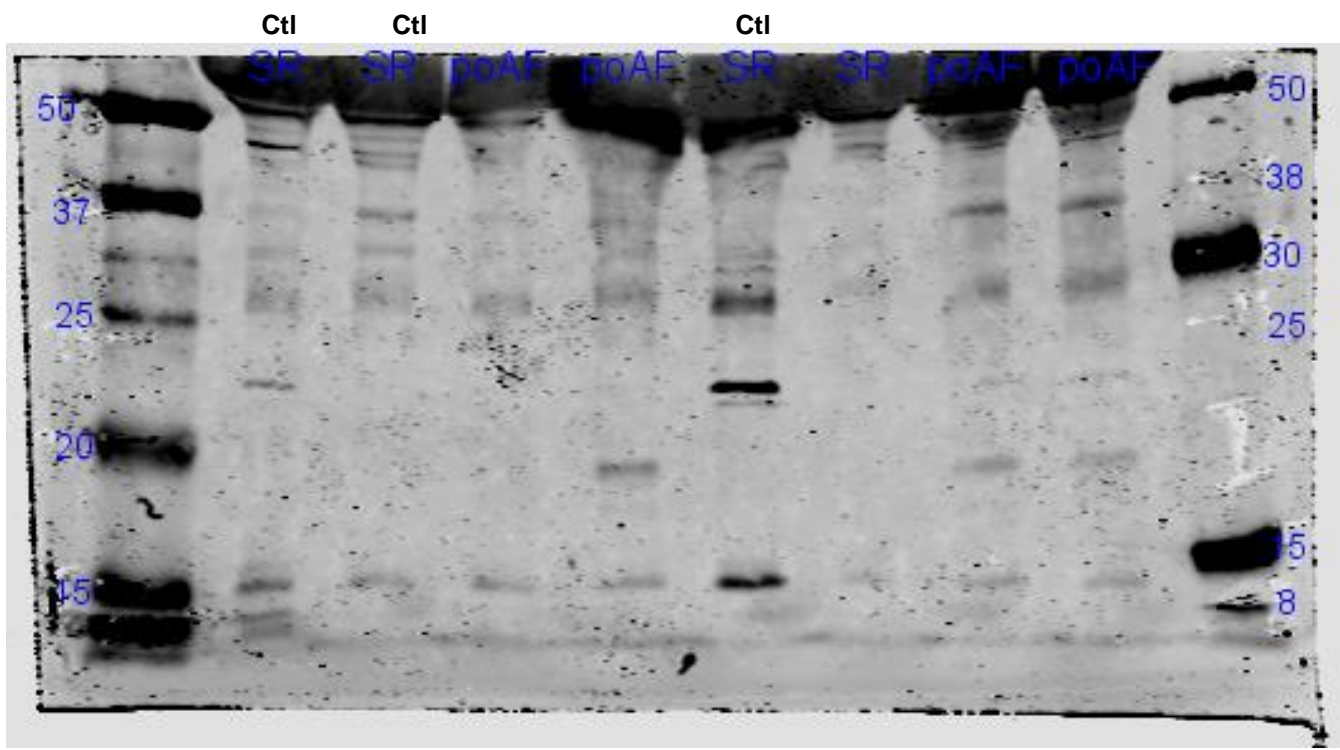


Figure 6C – Human atrial cardiomyocytes

(Pro-)Interleukin-1 β



Ponceau

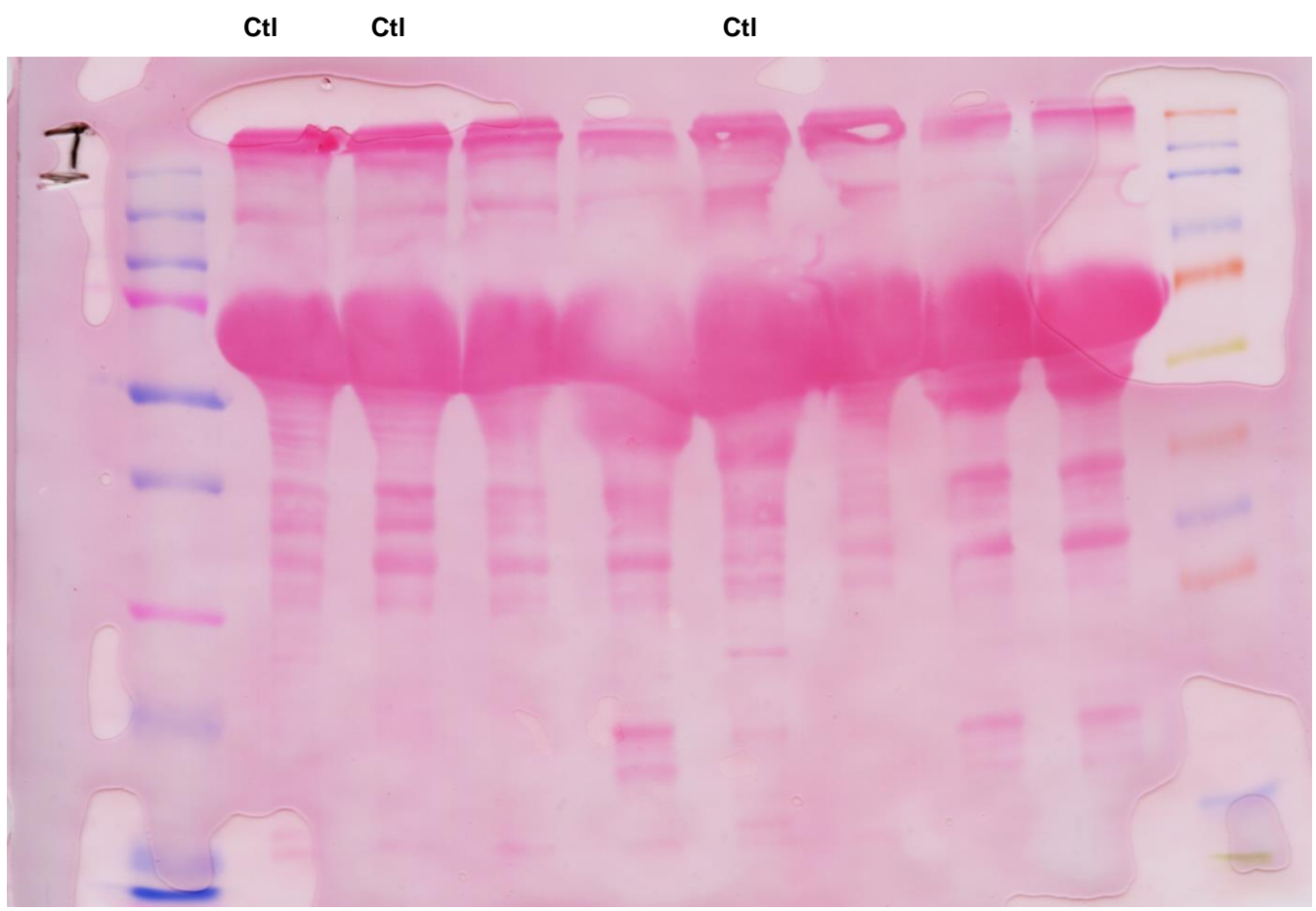
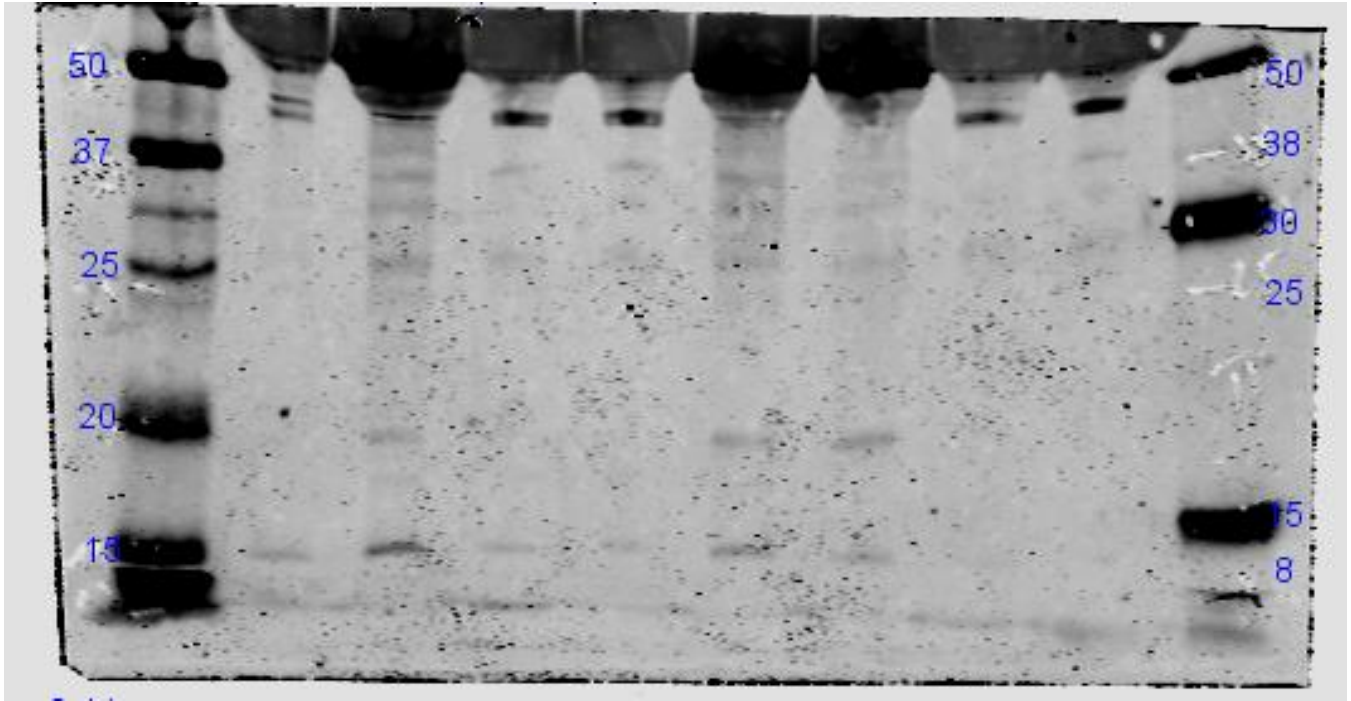


Figure 6D – Human atrial cardiomyocytes

(Pro-)Interleukin-1 β

Ctl Ctl POAF Ctl Ctl POAF POAF



Ponceau

Ctl Ctl POAF Ctl Ctl POAF POAF

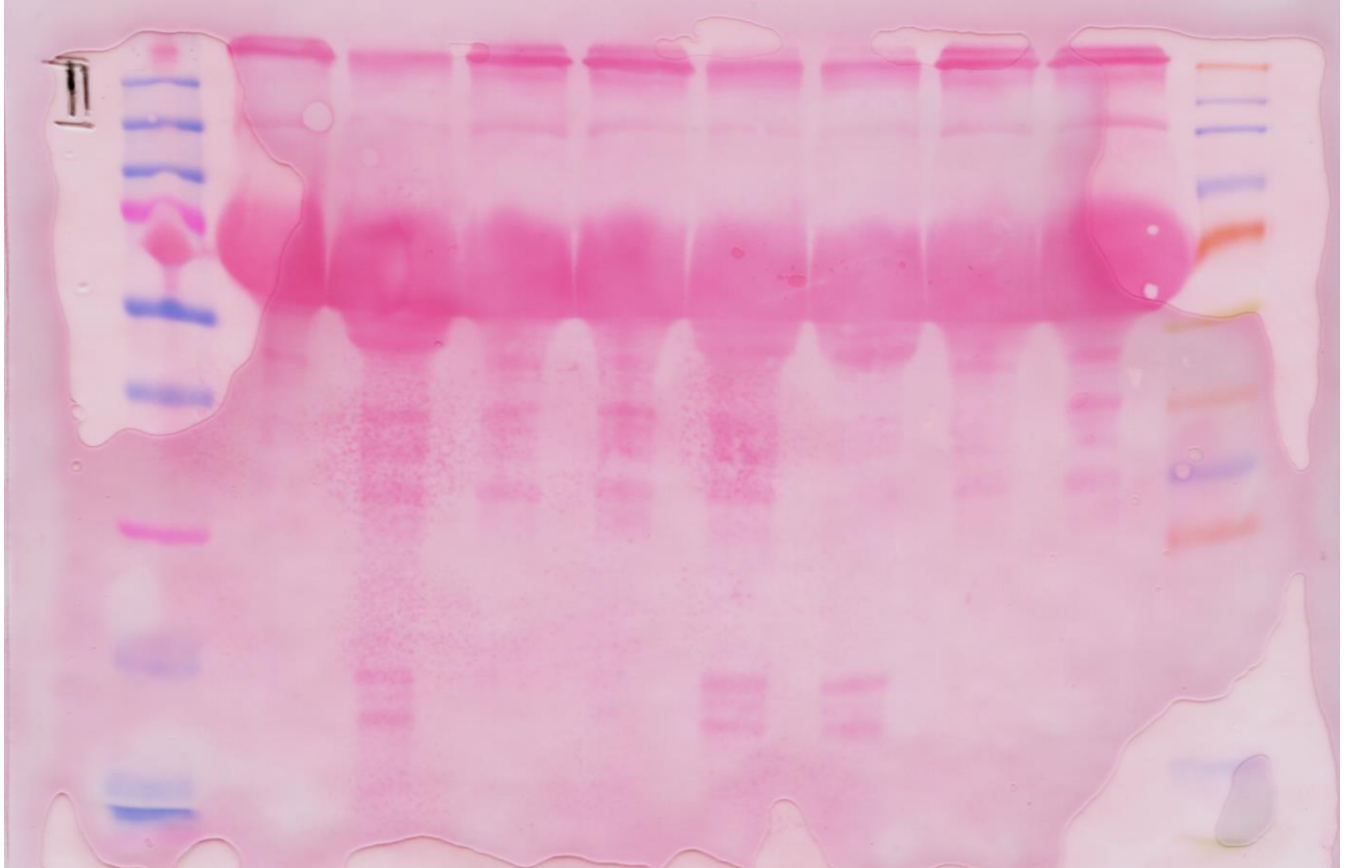
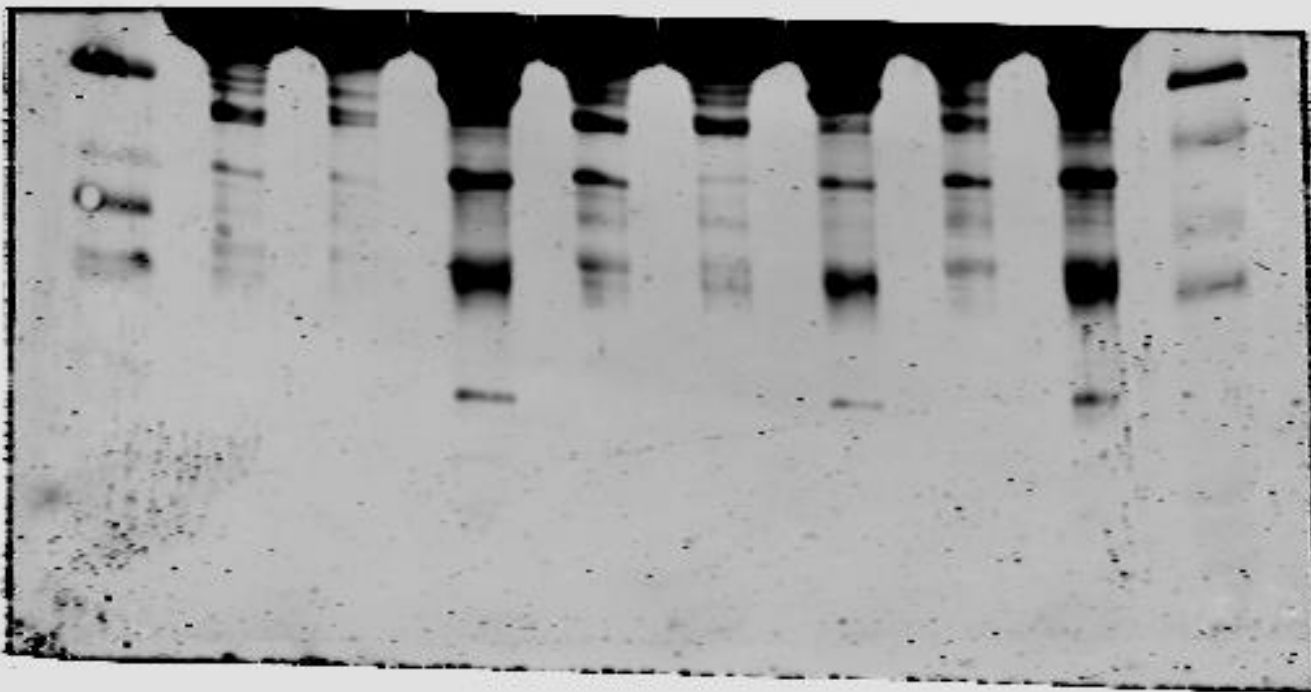


Figure 6D – Human atrial cardiomyocytes

(Pro-)Interleukin-1 β

Ctl Ctl POAF POAF Ctl Ctl POAF POAF



Ponceau

Ctl Ctl POAF POAF Ctl Ctl POAF POAF

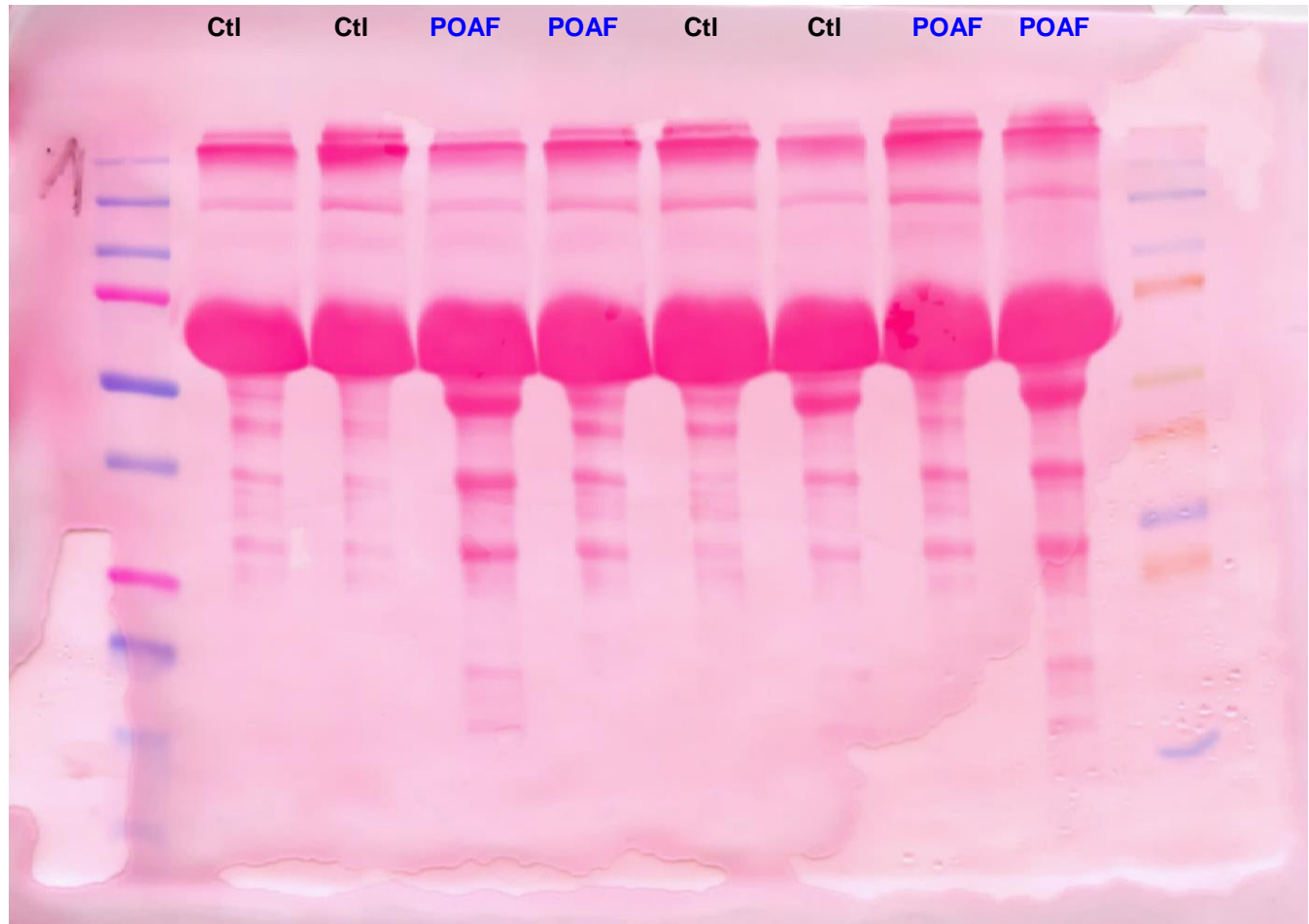
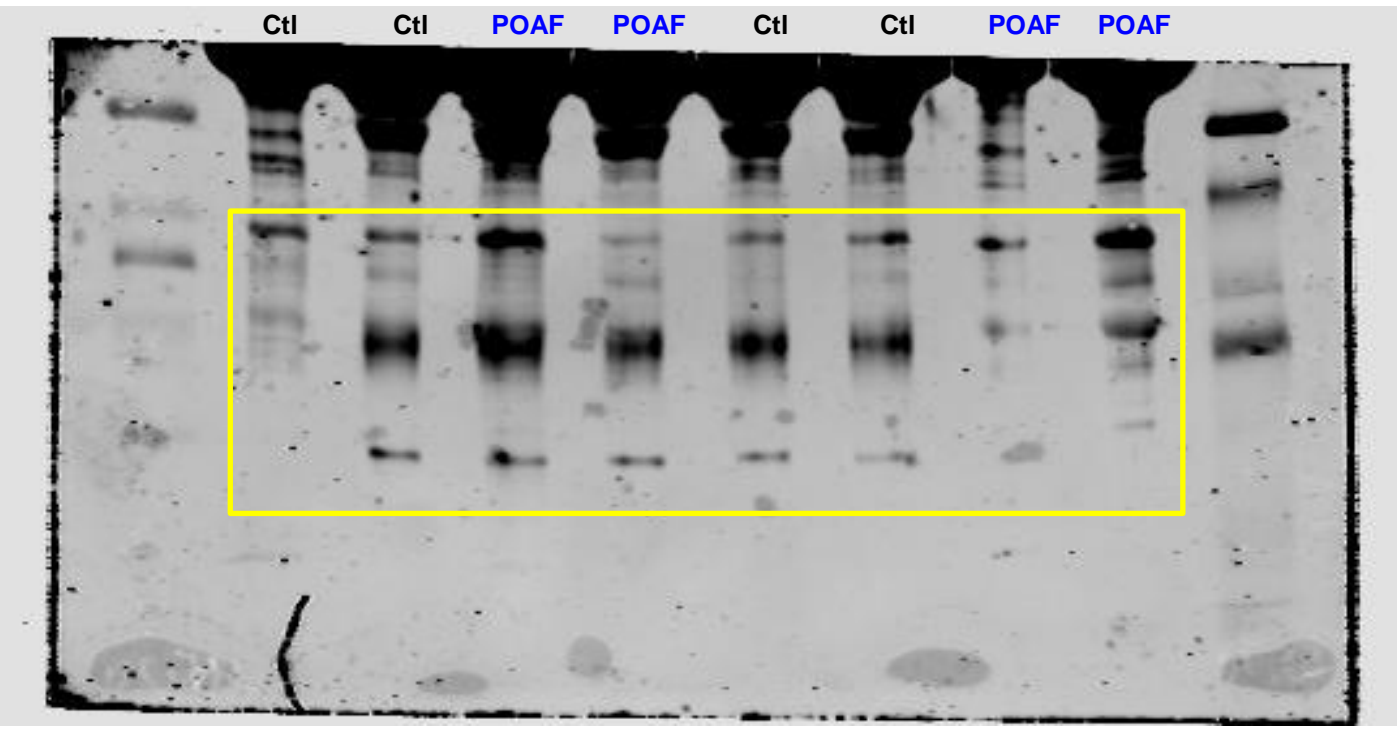


Figure 6D – Human atrial cardiomyocytes

(Pro-)Interleukin-1 β



Ponceau

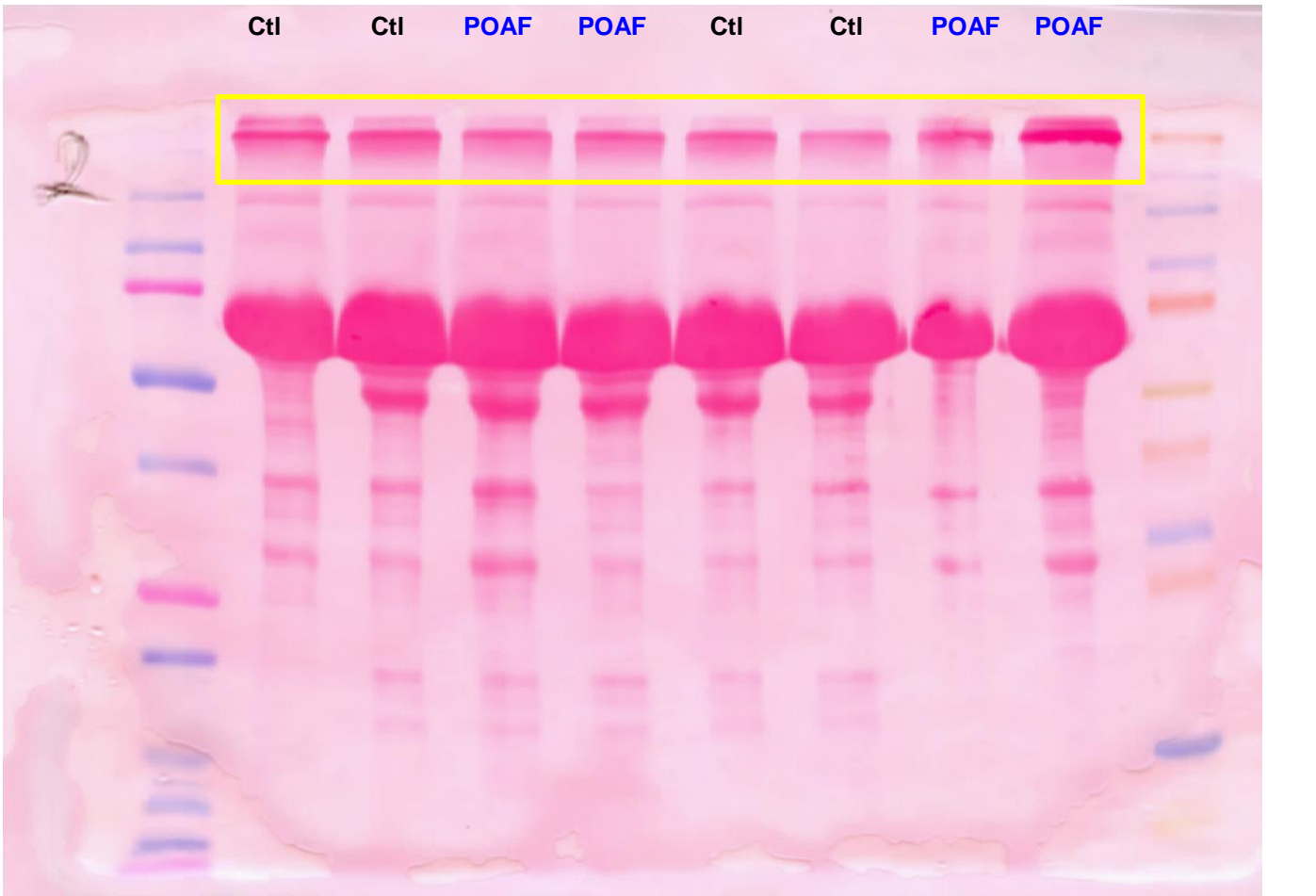
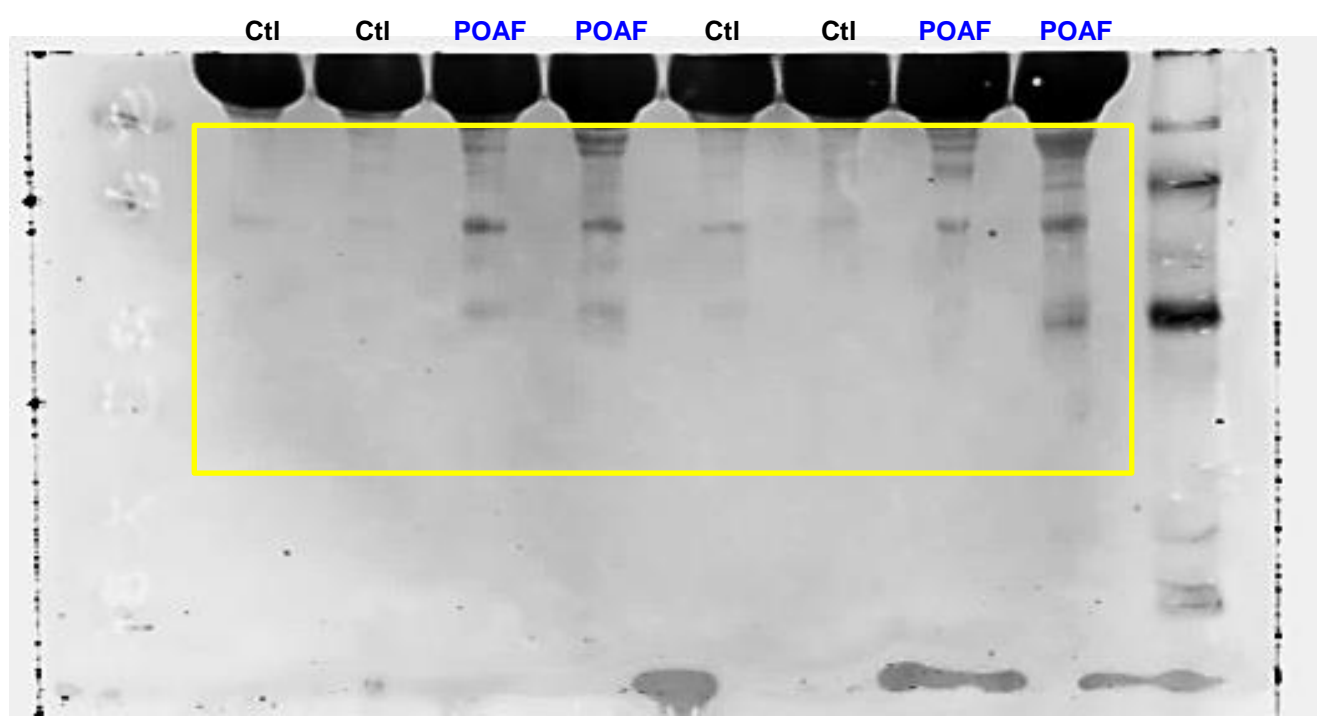


Figure 6D – Human atrial cardiomyocytes

Gasdermin-D



Ponceau

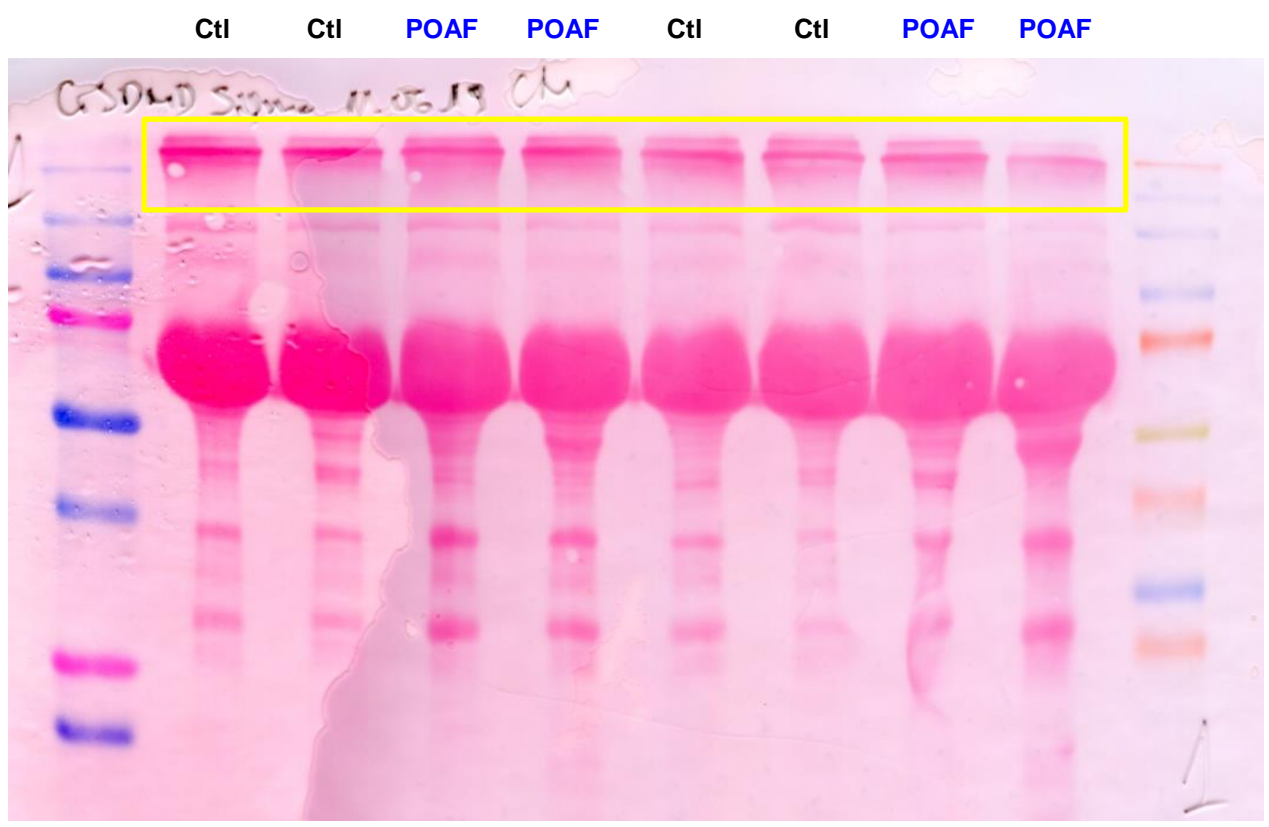
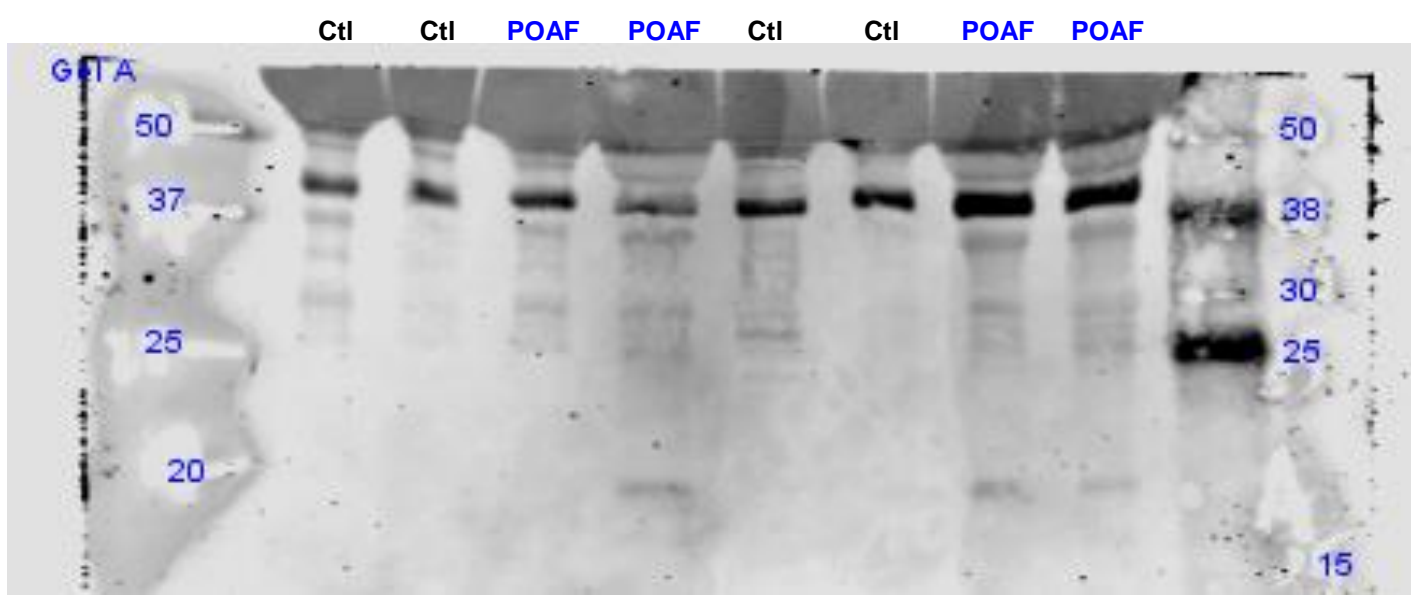


Figure 6E – Human atrial cardiomyocytes

Gasdermin-D



Ponceau

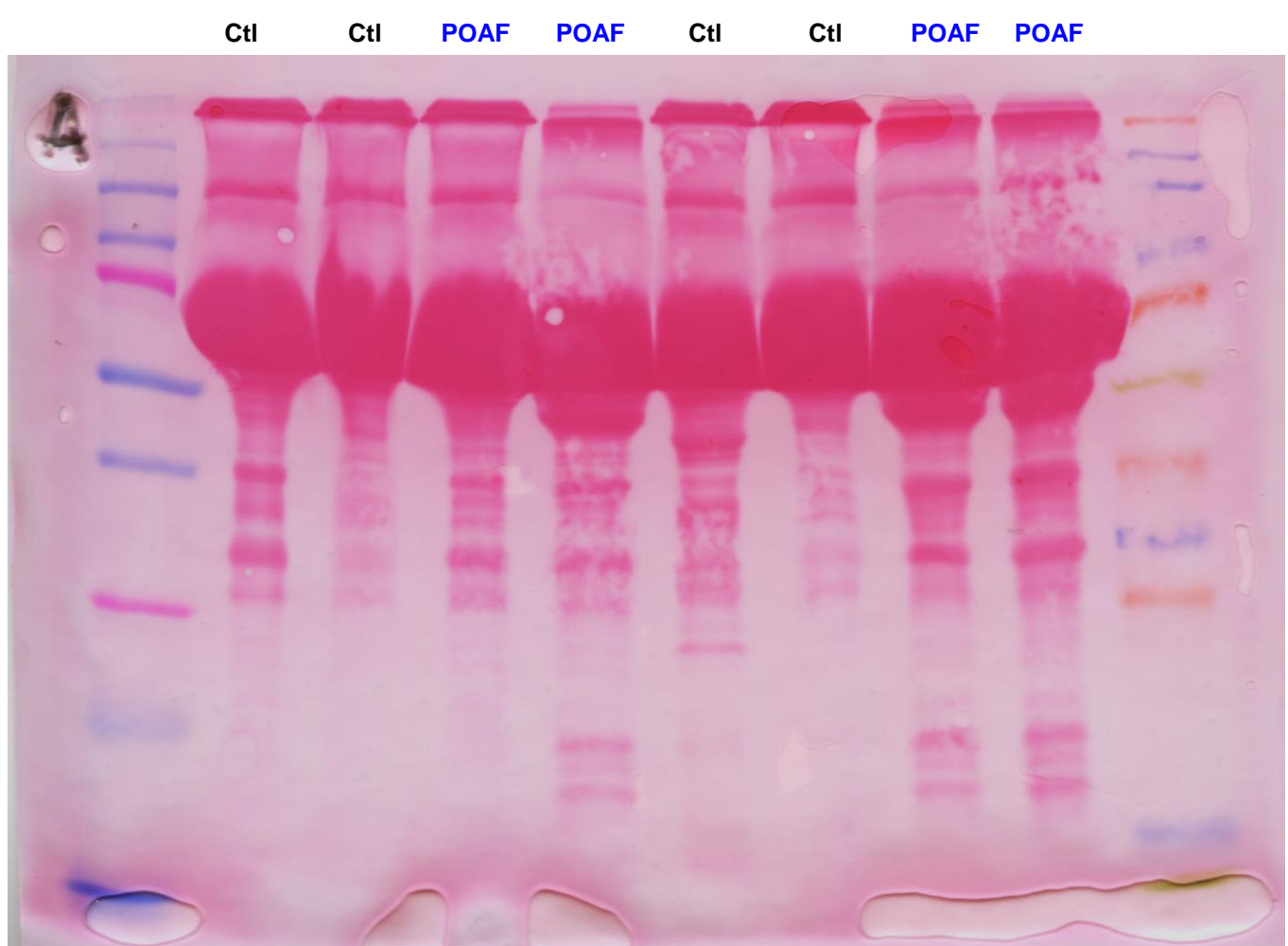
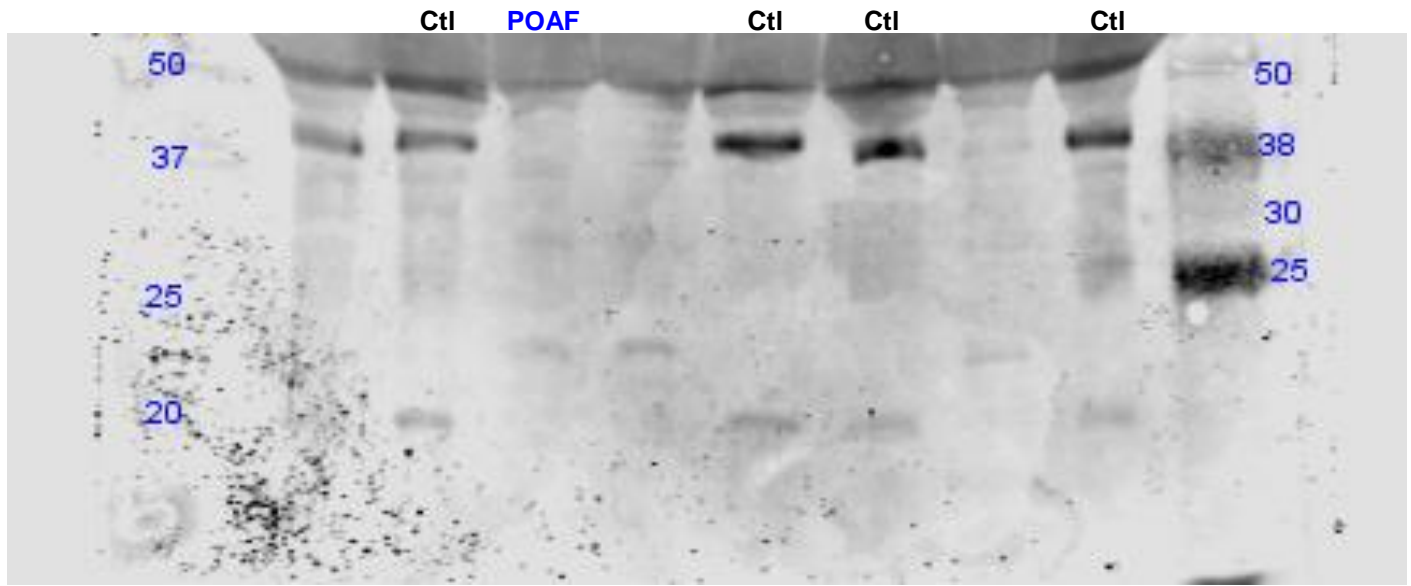


Figure 6E – Human atrial cardiomyocytes

Gasdermin-D



Ponceau

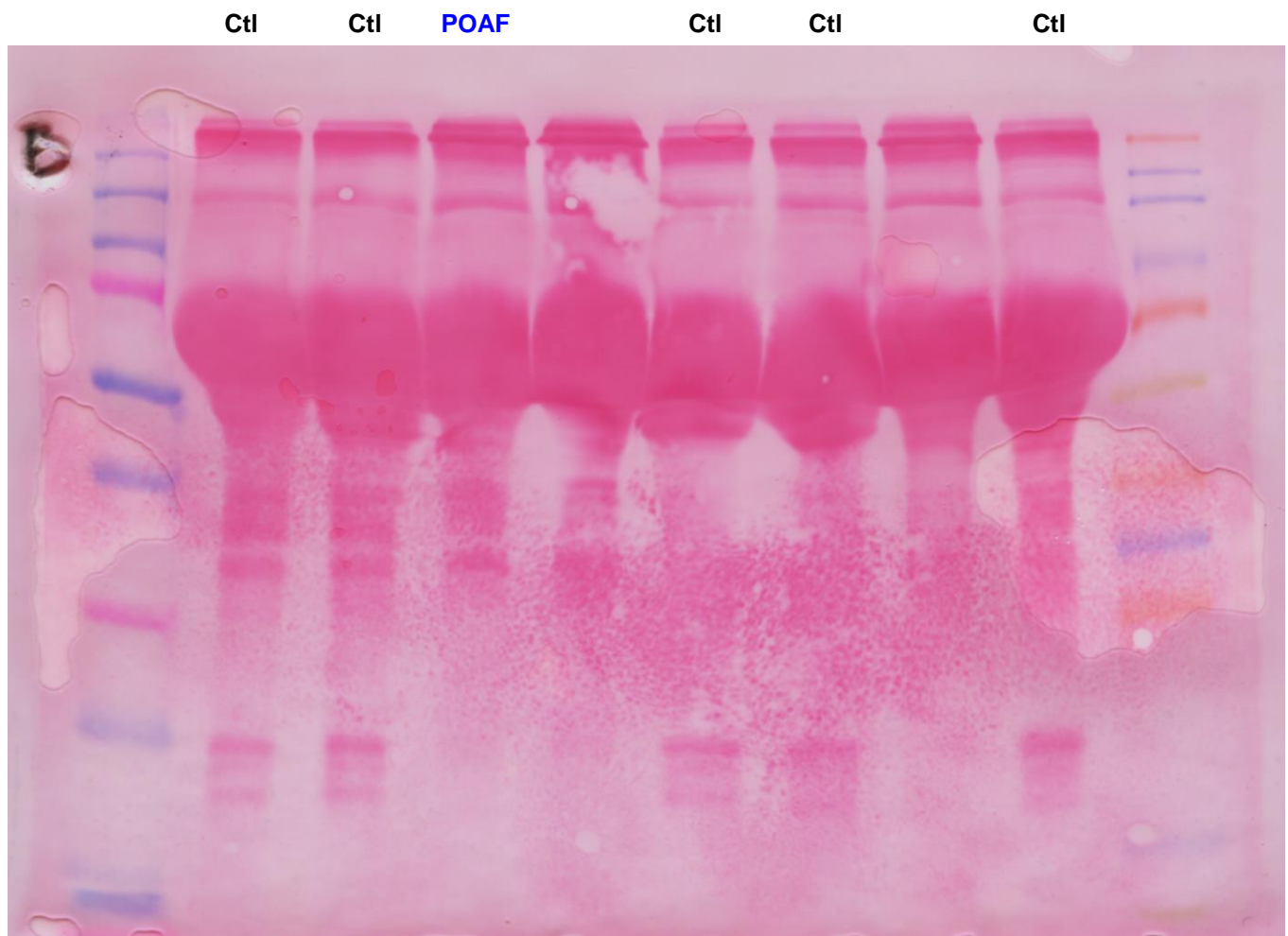
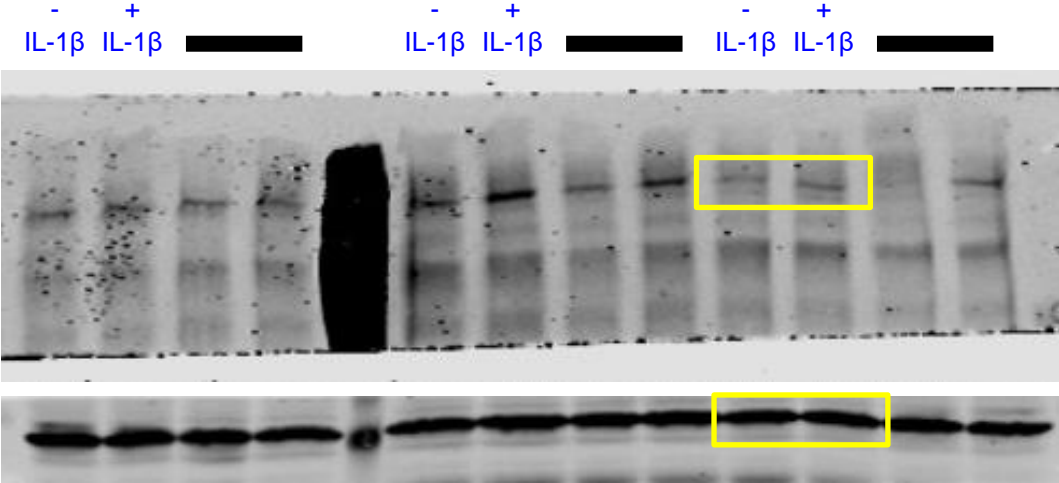


Figure 6E – Human atrial cardiomyocytes

Ser2808-RyR2 (-IL-1 β vs. IL-1 β)

Gel 1 / 4



Gel 2 / 4

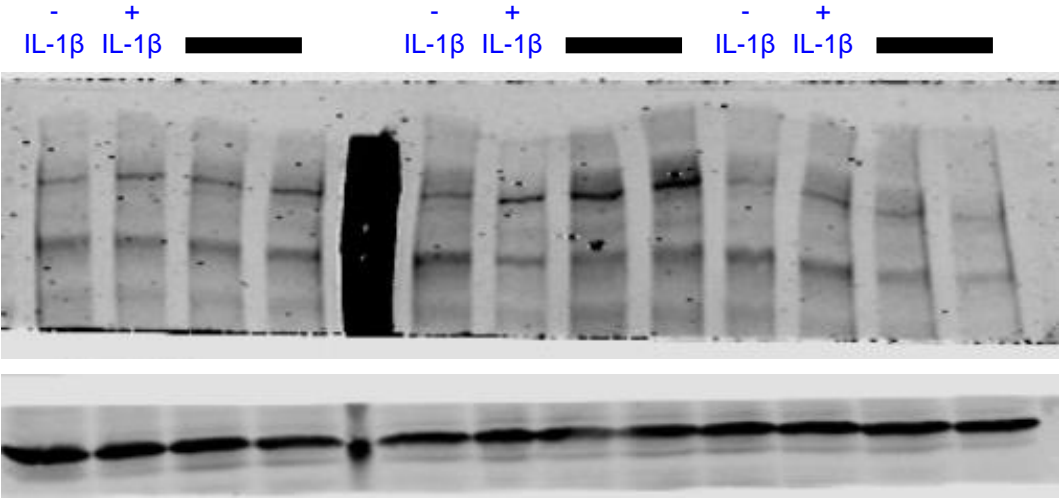


Figure 8B – HL1 cells

Excluded from this analysis

Ser2808-RyR2 (-IL-1 β vs. IL-1 β)

Gel 3 / 4

IL-1 β - IL-1 β + IL-1 β - IL-1 β + IL-1 β

Ser2808
-RyR2



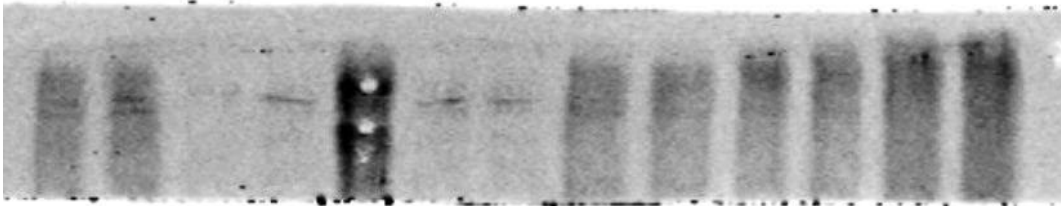
GAPDH



Gel 4 / 4

IL-1 β - IL-1 β + IL-1 β - IL-1 β + IL-1 β - IL-1 β + IL-1 β

Ser2808
-RyR2



GAPDH



Figure 8B – HL1 cells

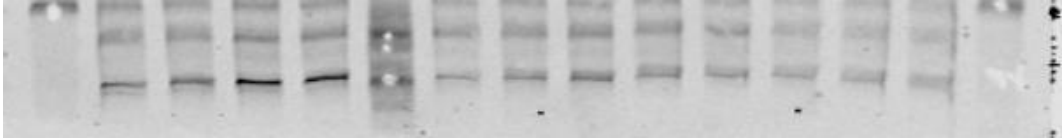
Excluded from this analysis

Ser2808-RyR2 (KN-92 vs. KN-93)

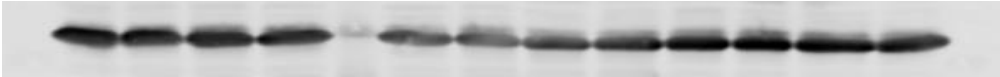
Gel 1 / 5

KN-93 + IL-1 β KN-92 + IL-1 β KN-93 + IL-1 β KN-92 + IL-1 β KN-93 + IL-1 β KN-92 + IL-1 β

Ser2808-RyR2



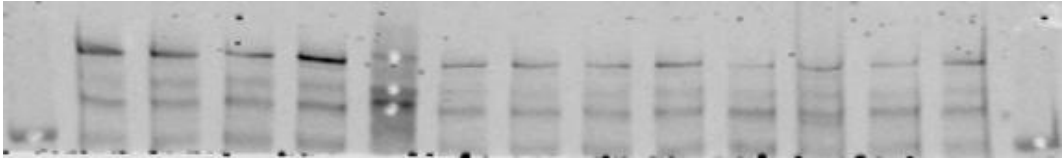
GAPDH



Gel 2 / 5

KN-93 + IL-1 β KN-92 + IL-1 β KN-93 + IL-1 β KN-92 + IL-1 β KN-93 + IL-1 β KN-92 + IL-1 β

Ser2808-RyR2



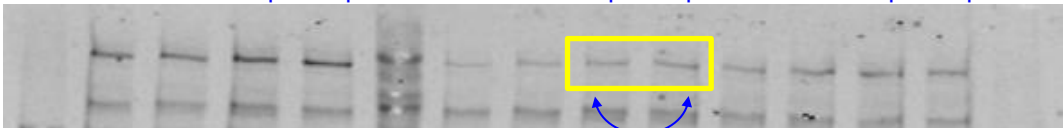
GAPDH



Gel 3 / 5

KN-93 + IL-1 β KN-92 + IL-1 β KN-93 + IL-1 β KN-92 + IL-1 β KN-93 + IL-1 β KN-92 + IL-1 β

Ser2808-RyR2



GAPDH

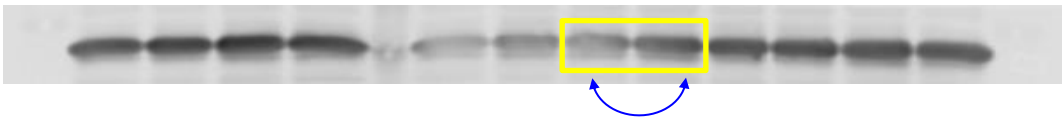


Figure 8B – HL1 cells

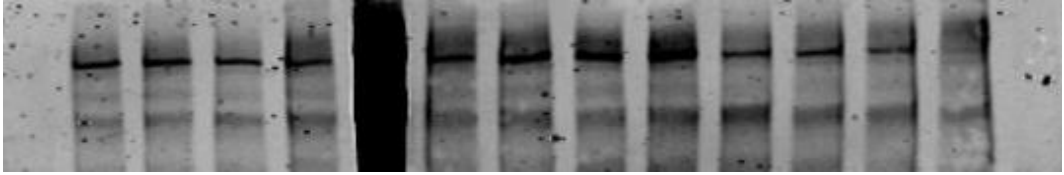
Excluded from this analysis

Ser2808-RyR2 (KN-92 vs. KN-93)

Gel 4 / 5

KN- 93 +
KN- 92 +
IL-1 β IL-1 β

Ser2808
-RyR2



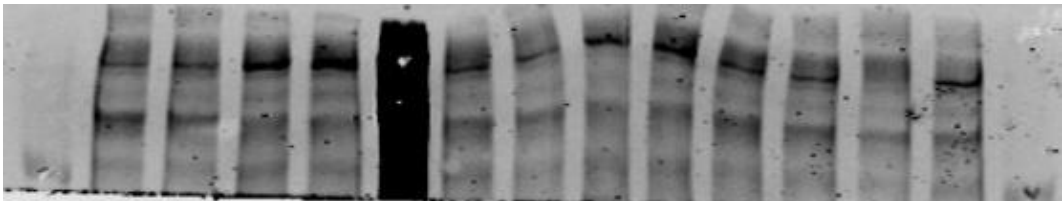
GAPDH



Gel 5 / 5

KN- 93 + KN- 92 + KN- 93 + KN- 92 + KN- 93 + KN- 92 +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Ser2808
-RyR2



GAPDH



Figure 8B – HL1 cells

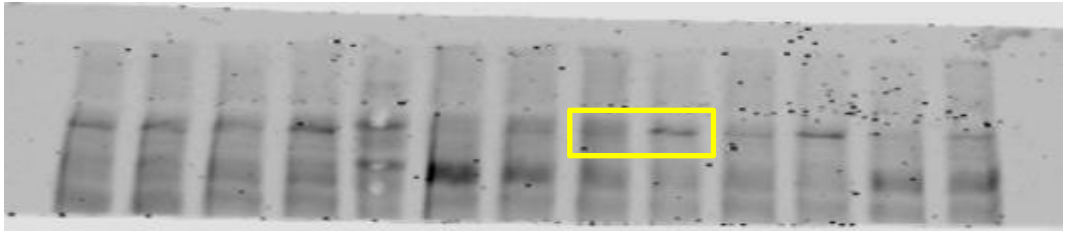
Excluded from this analysis

Ser2814-RyR2 (-IL-1 β vs. + IL-1 β)

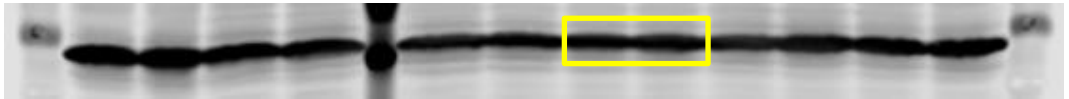
Gel 1 / 3

- + - + - + - + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Ser2814
-RyR2



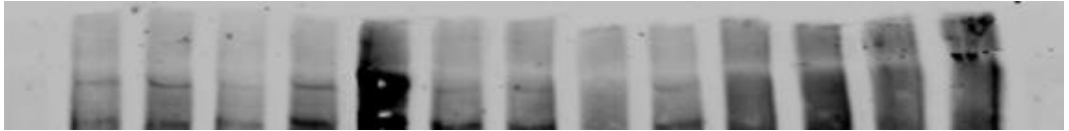
GAPDH



Gel 2 / 3

- + - + - + - + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Ser2814
-RyR2



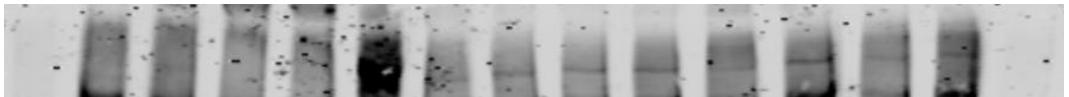
GAPDH



Gel 3 / 3

- + - + - + - + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Ser2814
-RyR2



GAPDH



Figure 8B – HL1 cells

Excluded from this analysis

Ser2814-RyR2 (KN-92 vs. KN-93)

Gel 1 / 4

KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β

Ser2814-RyR2

GAPDH

Gel 2 / 4

KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β

Ser2814-RyR2

GAPDH

Gel 3 / 4

KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β

Ser2814-RyR2

GAPDH

Gel 4 / 4

KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β

Ser2814-RyR2

GAPDH

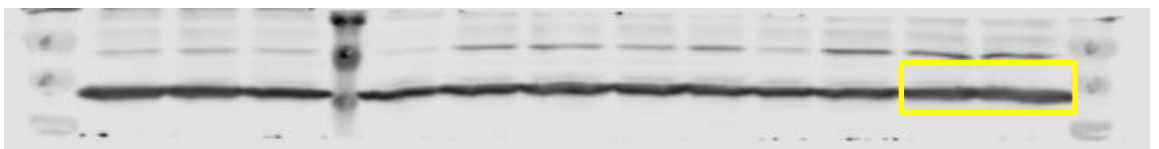
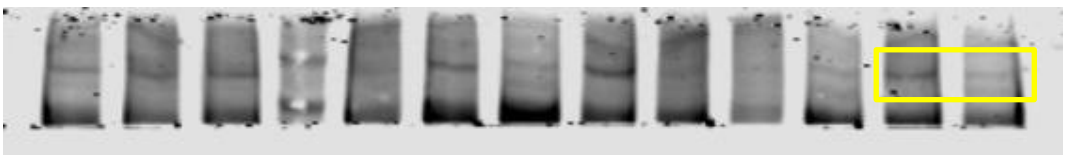
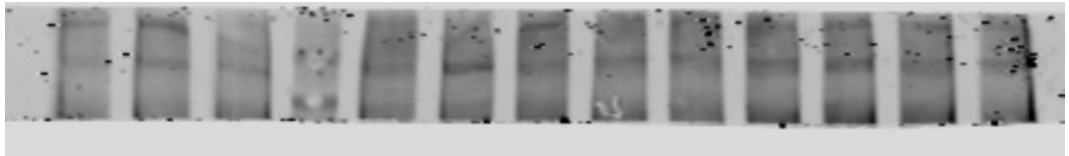
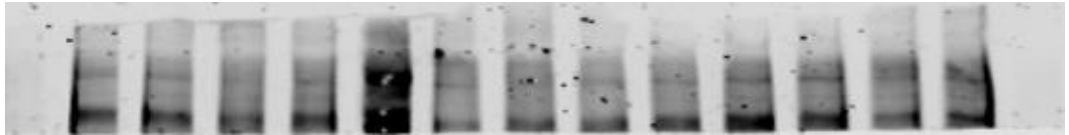
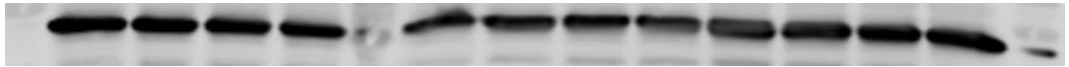
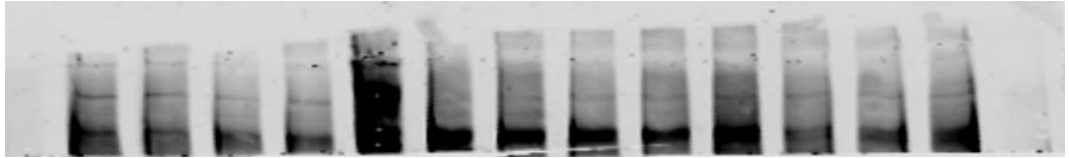


Figure 8B – HL1 cells

Excluded from this analysis

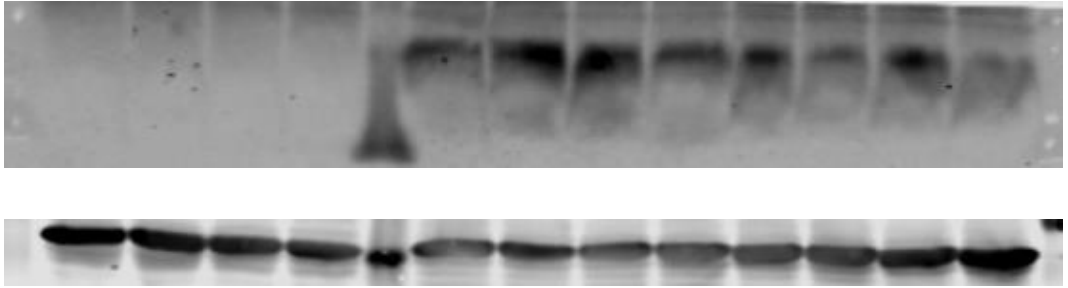
Ser16-PLB (-IL-1 β vs. + IL-1 β)

Gel 1 / 5

- + - +
IL-1 β IL-1 β IL-1 β IL-1 β

Ser16
-PLB

GAPDH

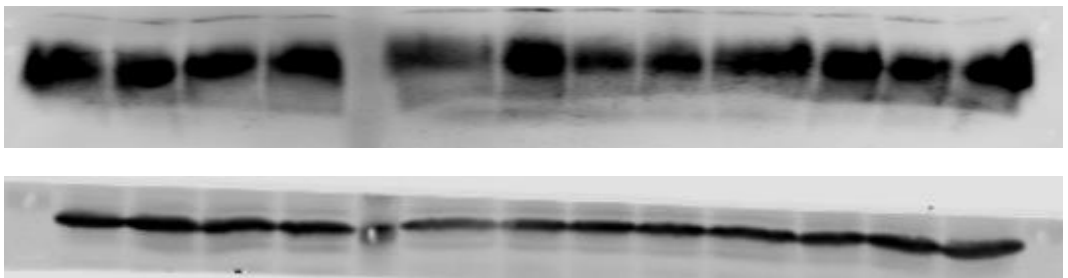


Gel 2 / 5

- + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Ser16
-PLB

GAPDH



Gel 3 / 5

- + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Ser16
-PLB

GAPDH

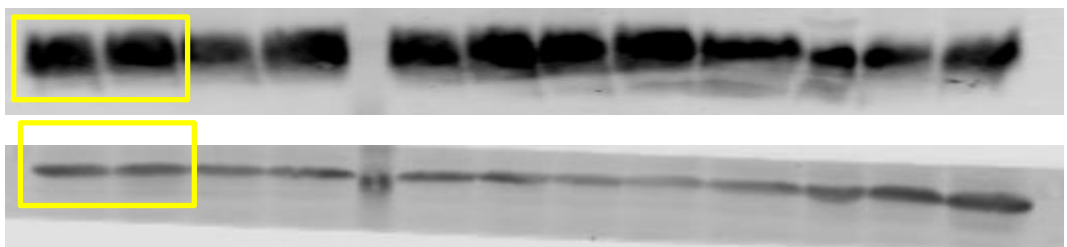


Figure 8B – HL1 cells

Excluded from this analysis

Ser16-PLB (-IL-1β vs. + IL-1β)

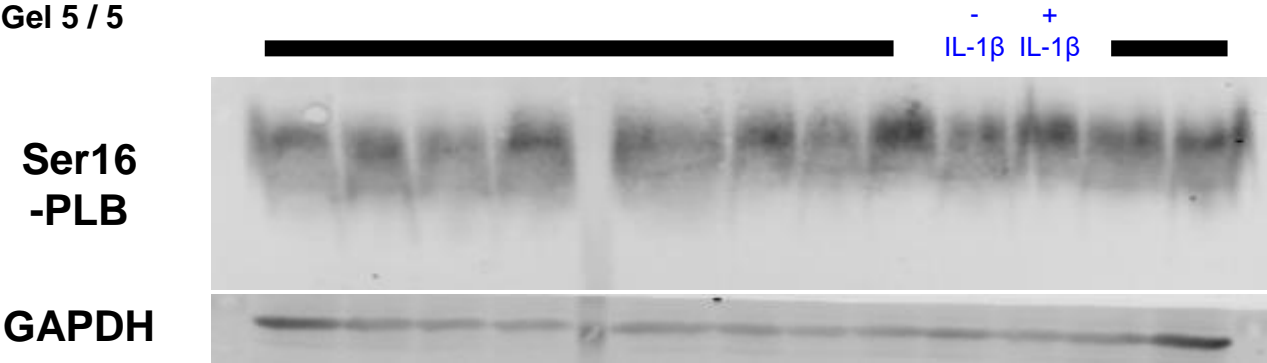
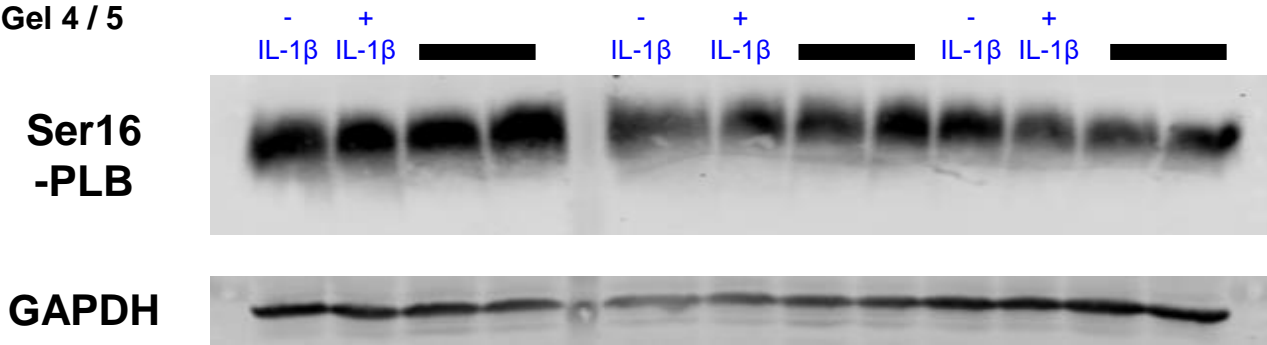


Figure 8B – HL1 cells

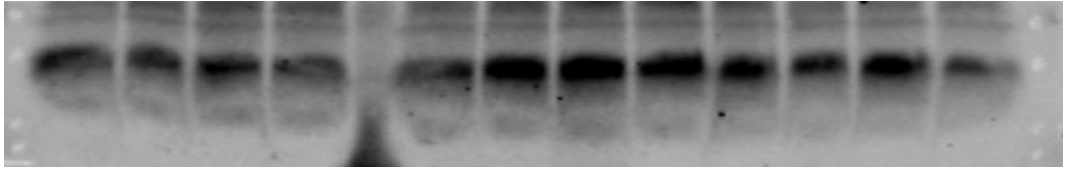
Excluded from this analysis

Ser16-PLB (KN-92 vs. KN-93)

Gel 1 / 4

KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β

Ser16-PLB



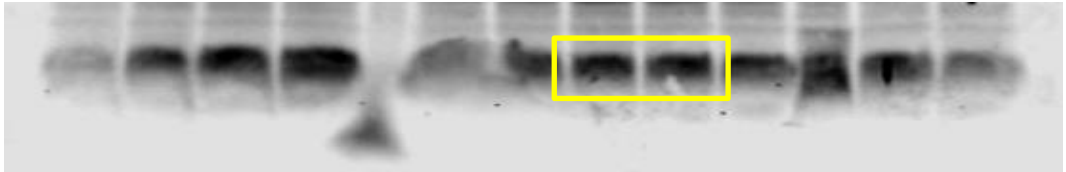
GAPDH



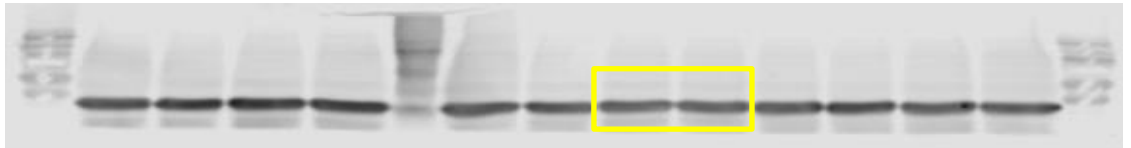
Gel 2 / 4

KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β

Ser16-PLB



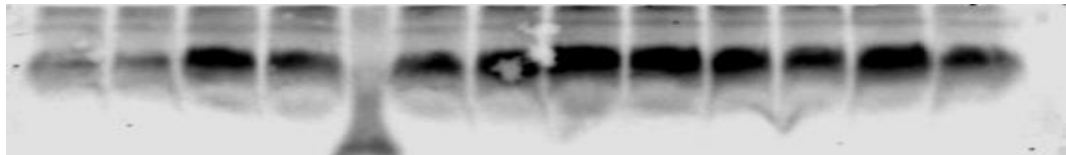
GAPDH



Gel 3 / 4

KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β

Ser16-PLB



GAPDH



Gel 4 / 4

KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β

Ser16-PLB



GAPDH



Figure 8B – HL1 cells

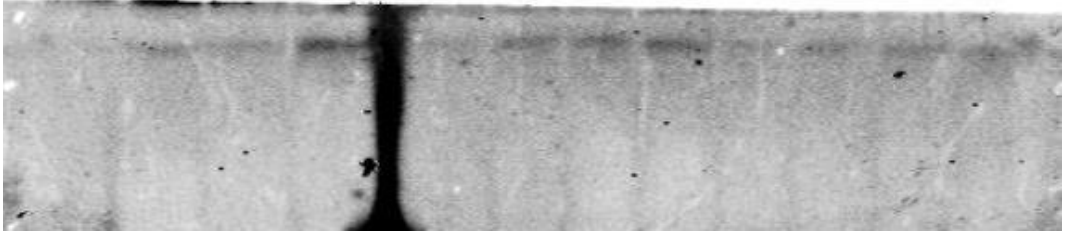
Excluded from this analysis

Thr17-PLB (-IL-1 β vs. + IL-1 β)

Gel 1 / 5

- + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Thr17
-PLB



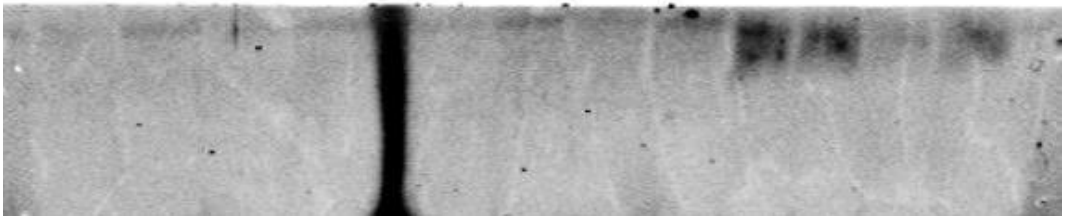
GAPDH



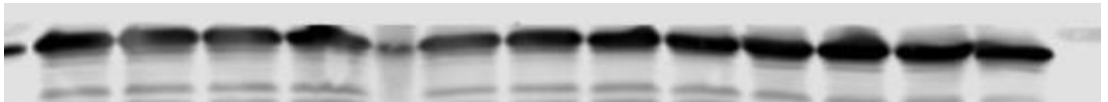
Gel 2 / 5

- + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Thr17
-PLB



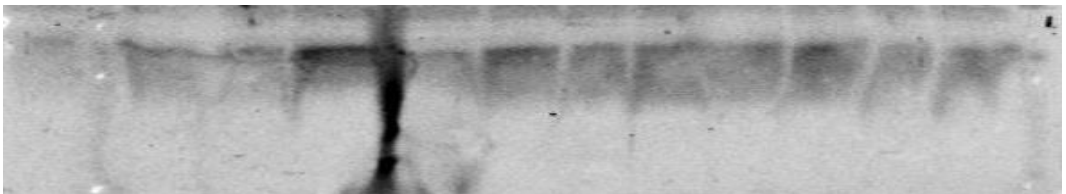
GAPDH



Gel 3 / 5

- + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Thr17
-PLB



GAPDH



Figure 8B – HL1 cells

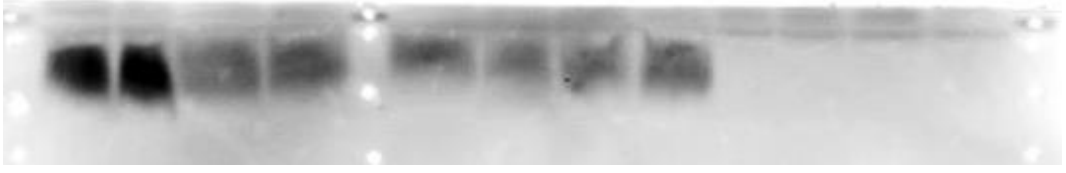
Excluded from this analysis

Thr17-PLB (-IL-1 β vs. + IL-1 β)

Gel 4 / 5

- + - + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Thr17
-PLB



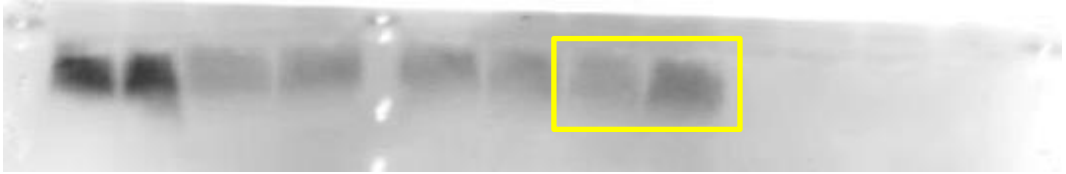
GAPDH



Gel 5 / 5

IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

Thr17
-PLB



GAPDH



Figure 8B – HL1 cells

Excluded from this analysis

Thr17-PLB (KN-92 vs. KN-93)

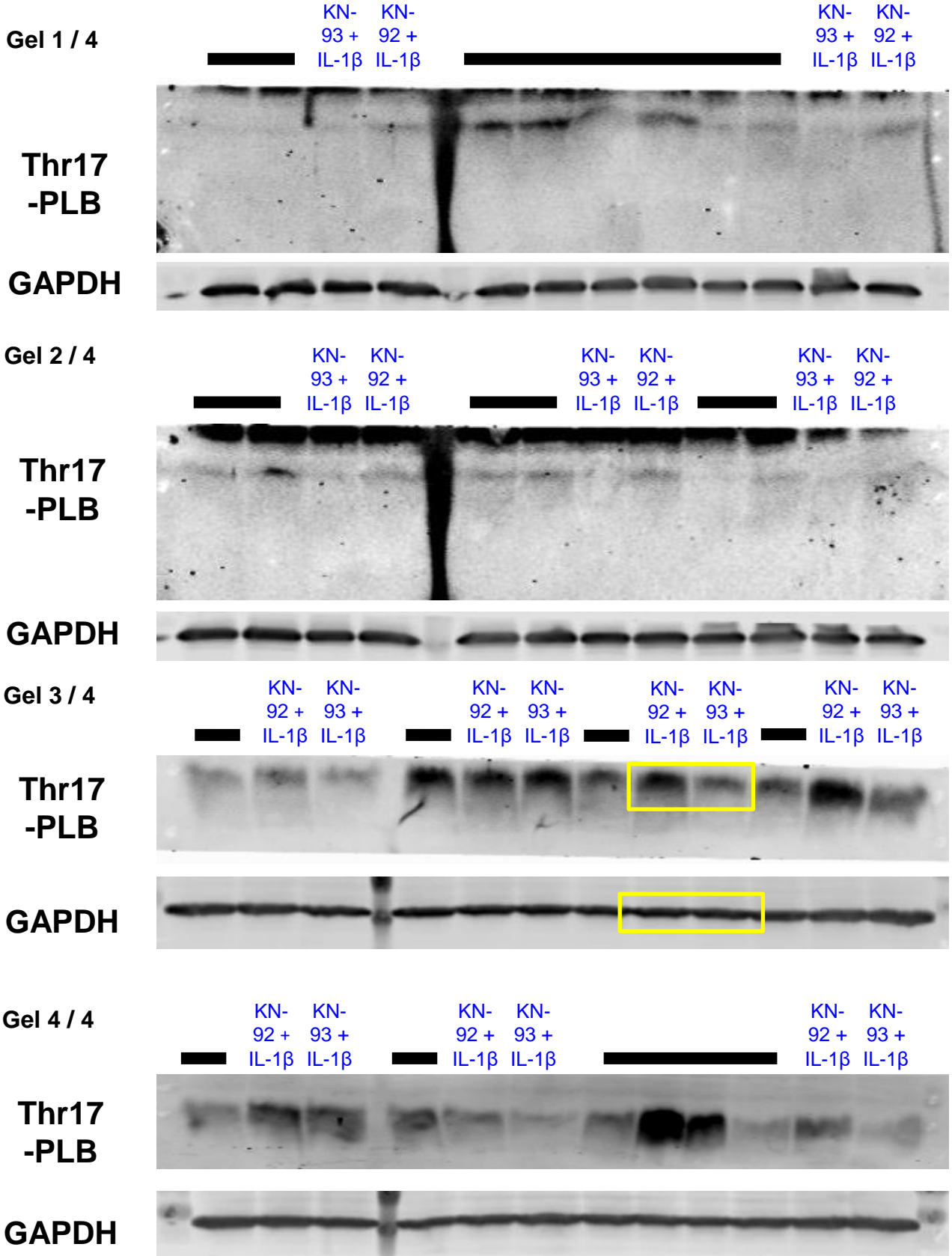





Figure 8B – HL1 cells

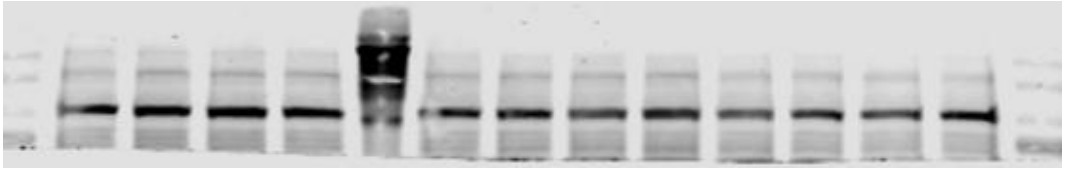
Excluded from this analysis

NLRP3 (-IL-1 β vs. + IL-1 β)

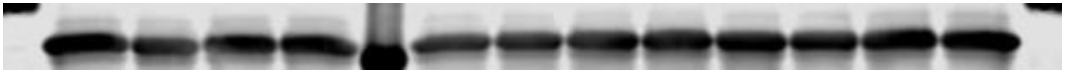
Gel 1 / 5

IL-1 β ⁻ IL-1 β ⁺  IL-1 β ⁻ IL-1 β ⁺  IL-1 β ⁻ IL-1 β ⁺ 




NLRP3



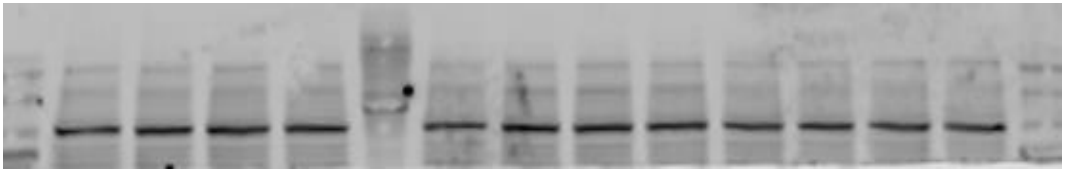
GAPDH



Gel 2 / 5

IL-1 β ⁻ IL-1 β ⁺  IL-1 β ⁻ IL-1 β ⁺  IL-1 β ⁻ IL-1 β ⁺ 




NLRP3



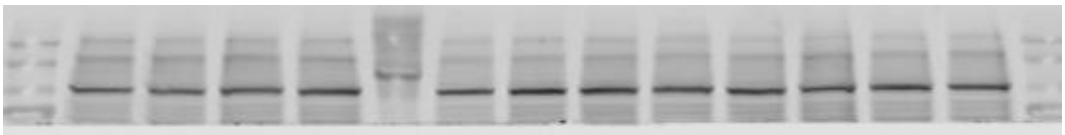
GAPDH



Gel 3 / 5

IL-1 β ⁻ IL-1 β ⁺  IL-1 β ⁻ IL-1 β ⁺  IL-1 β ⁻ IL-1 β ⁺ 

NLRP3



GAPDH

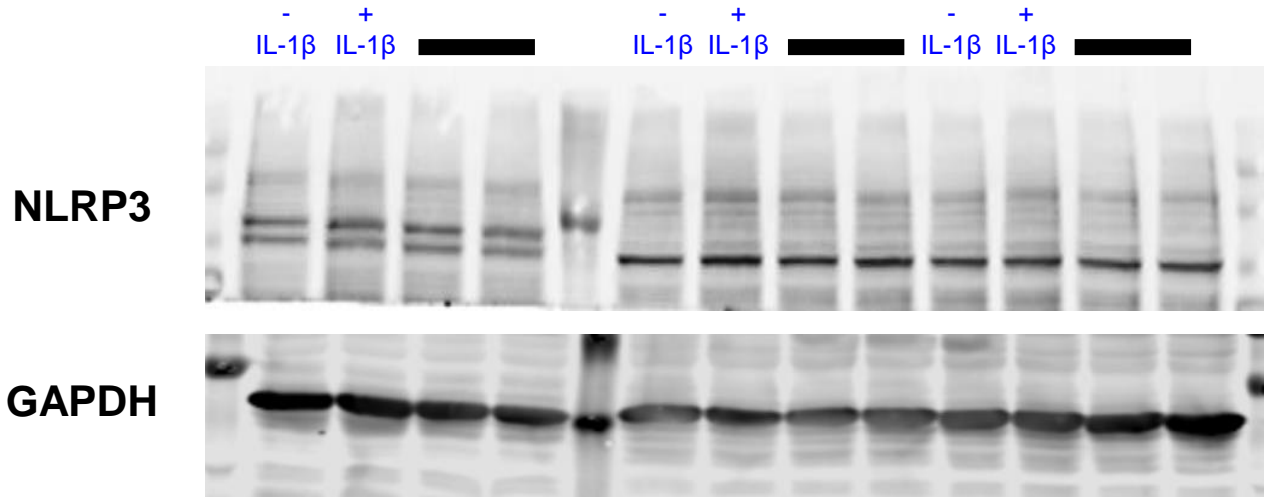


Figure 8E – HL1 cells

 Excluded from this analysis

NLRP3 (-IL-1 β vs. + IL-1 β)

Gel 4 / 5



Gel 5 / 5

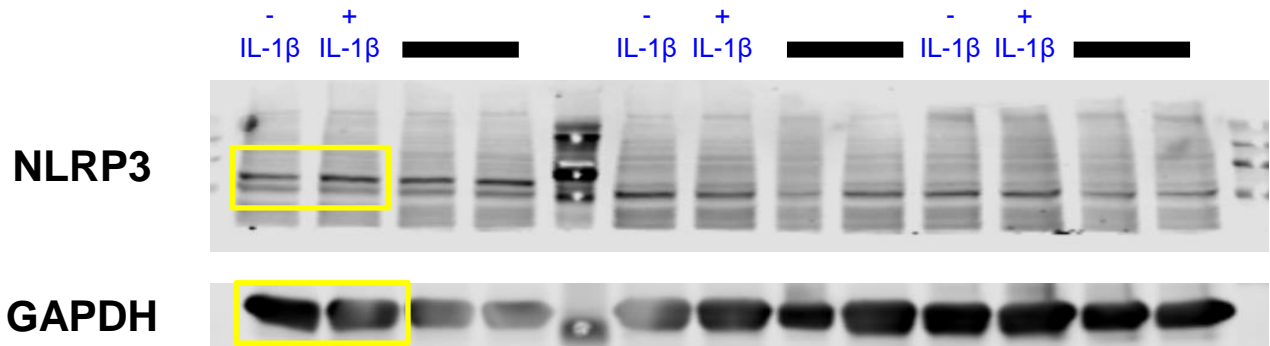


Figure 8E – HL1 cells

Excluded from this analysis

Pro-Caspase-1 & Caspase-1-p20 (-IL-1 β vs. + IL-1 β)

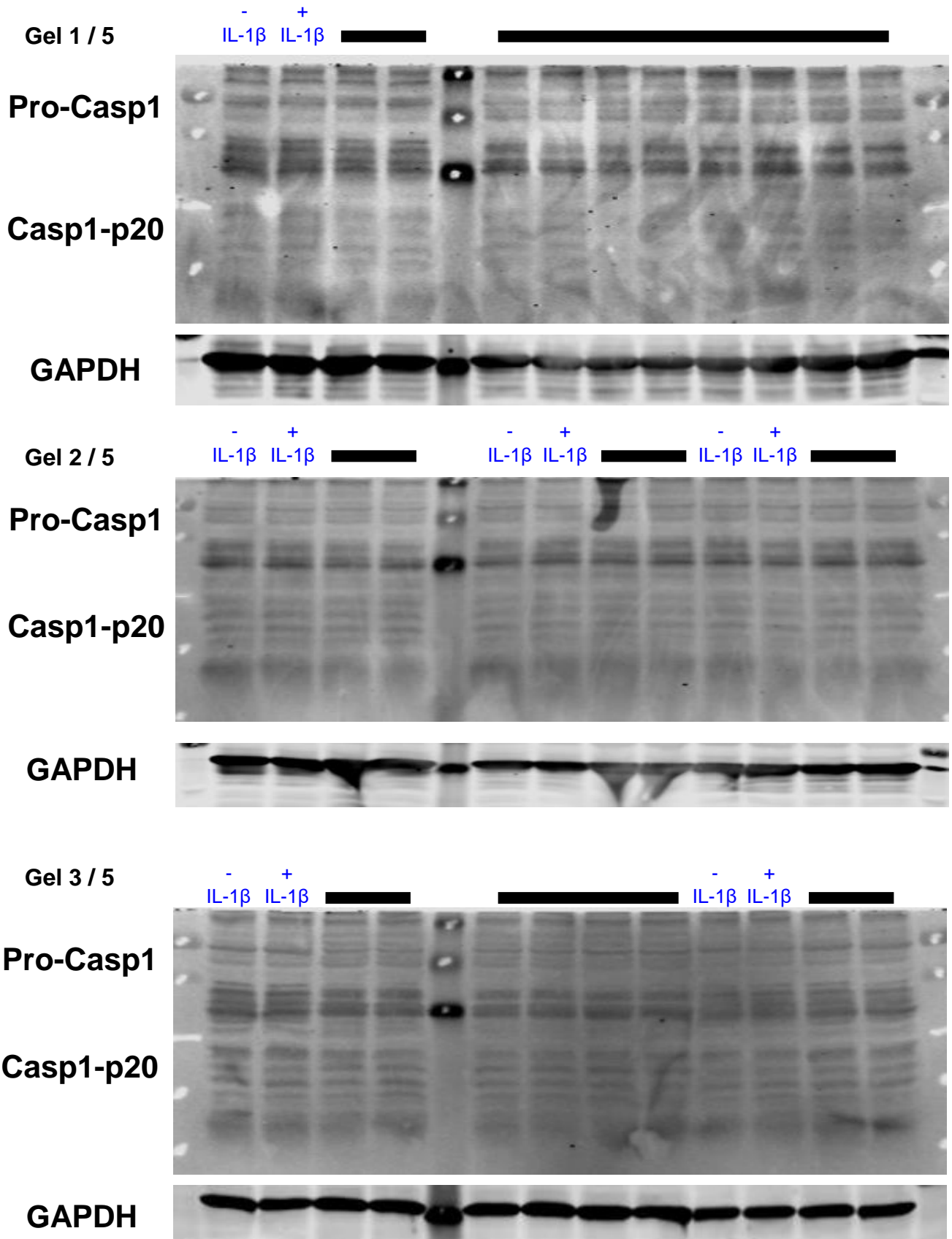


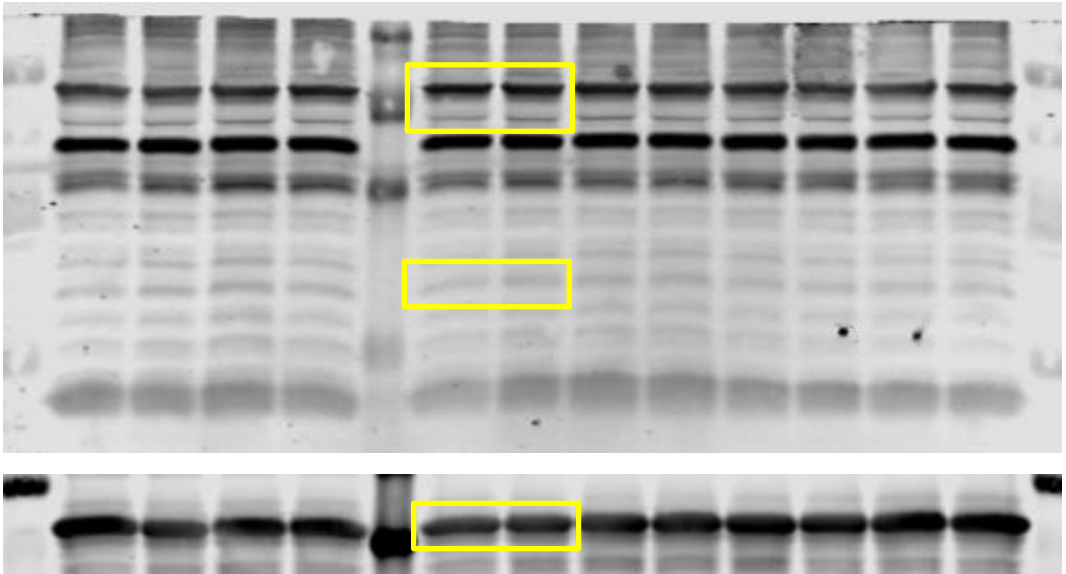
Figure 8E – HL1 cells

■ Excluded from this analysis

Pro-Caspase-1 & Caspase-1-p20 (-IL-1 β vs. + IL-1 β)

Gel 4 / 5

- + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β



Gel 5 / 5

- + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

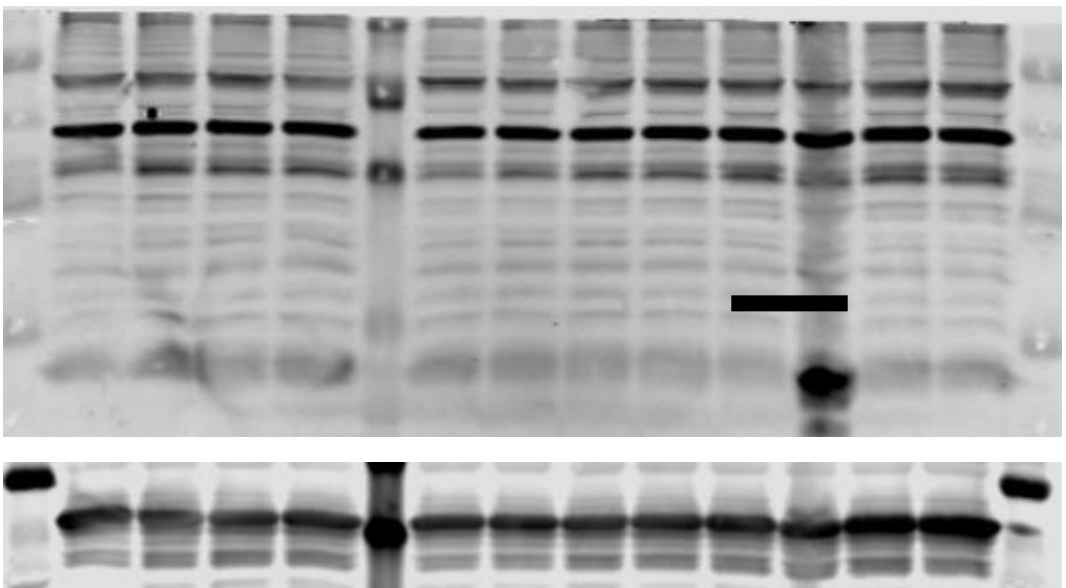


Figure 8E – HL1 cells

Excluded from this analysis

Pro-Caspase-1 & Caspase-1-p20 (KN-92 vs. + KN-93)

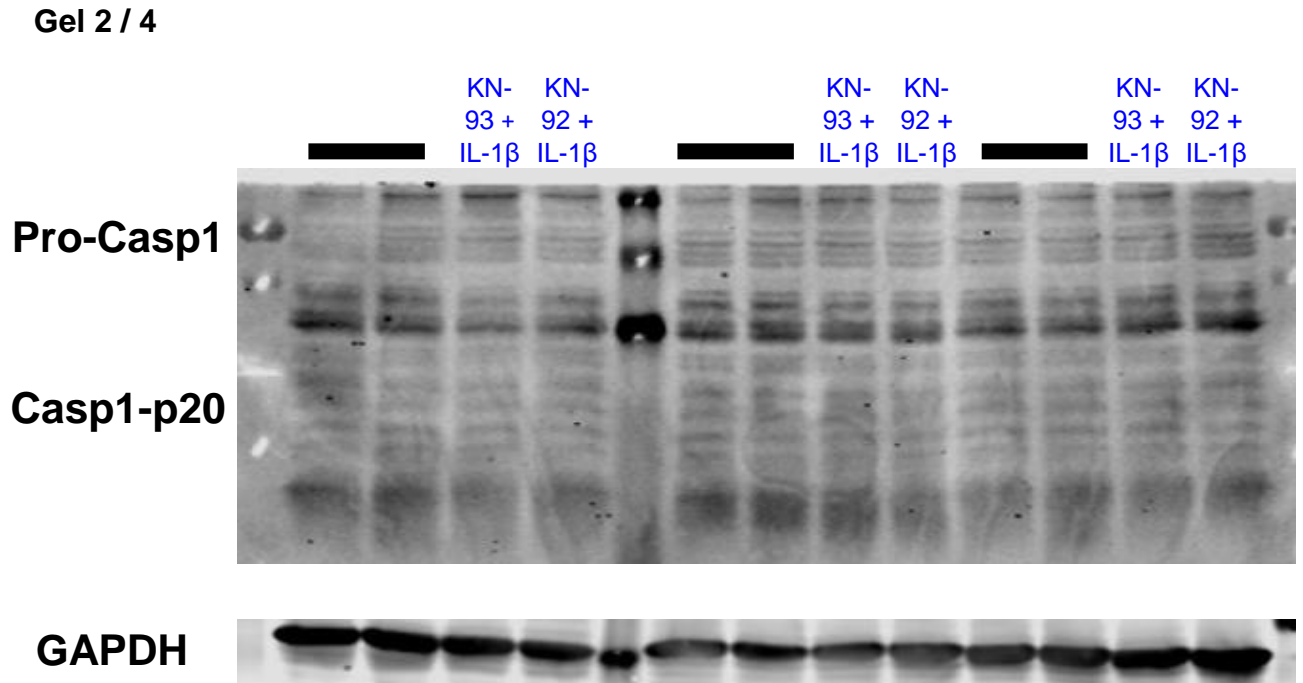
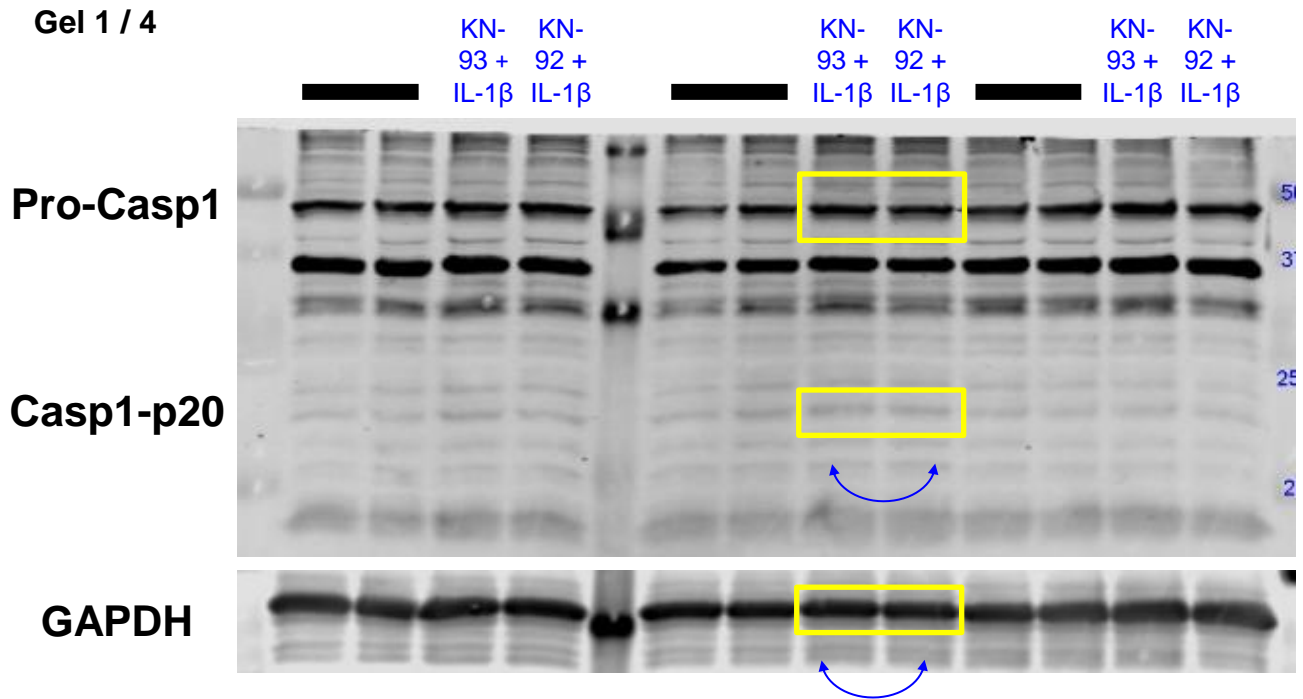


Figure 8E – HL1 cells

Excluded from this analysis

Pro-Caspase-1 & Caspase-1-p20 (KN-92 vs. + KN-93)

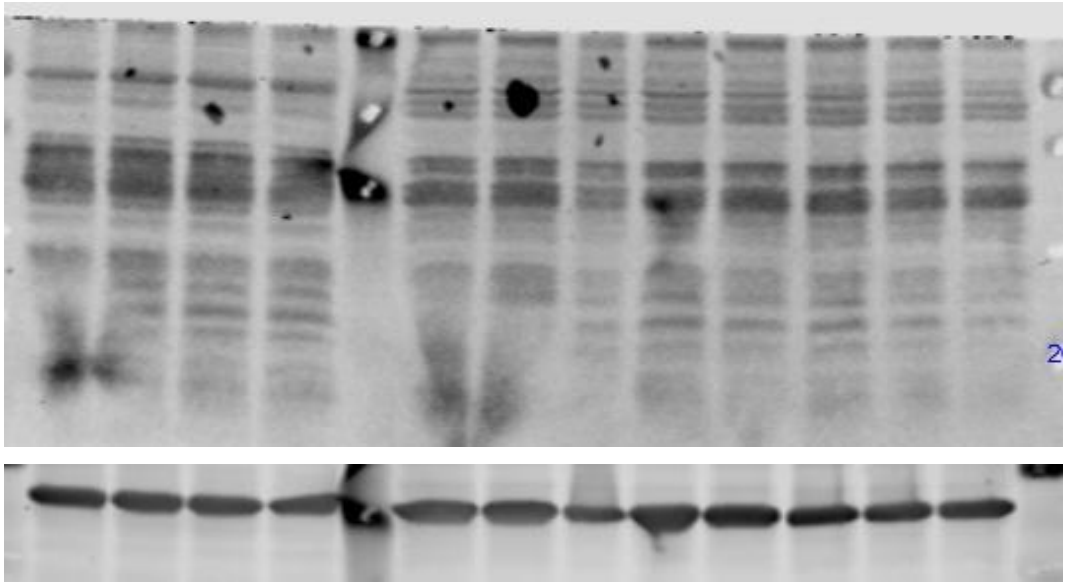
Gel 3 / 4

KN- 93 +
KN- 92 +
IL-1 β IL-1 β

Pro-Casp1

Casp1-p20

GAPDH



Gel 4 / 4

KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β KN- 92 + IL-1 β KN- 93 + IL-1 β

Pro-Casp1

Casp1-p20

GAPDH

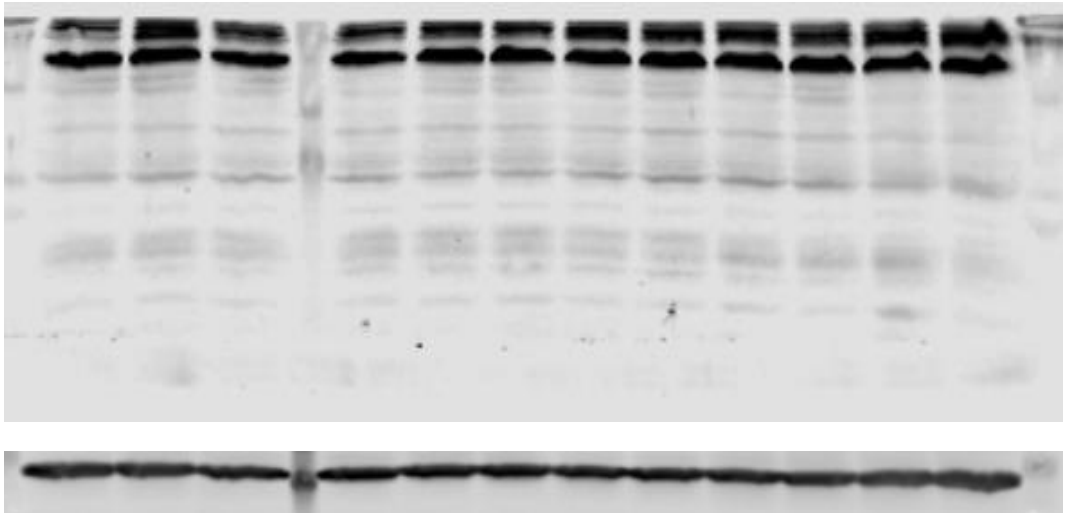
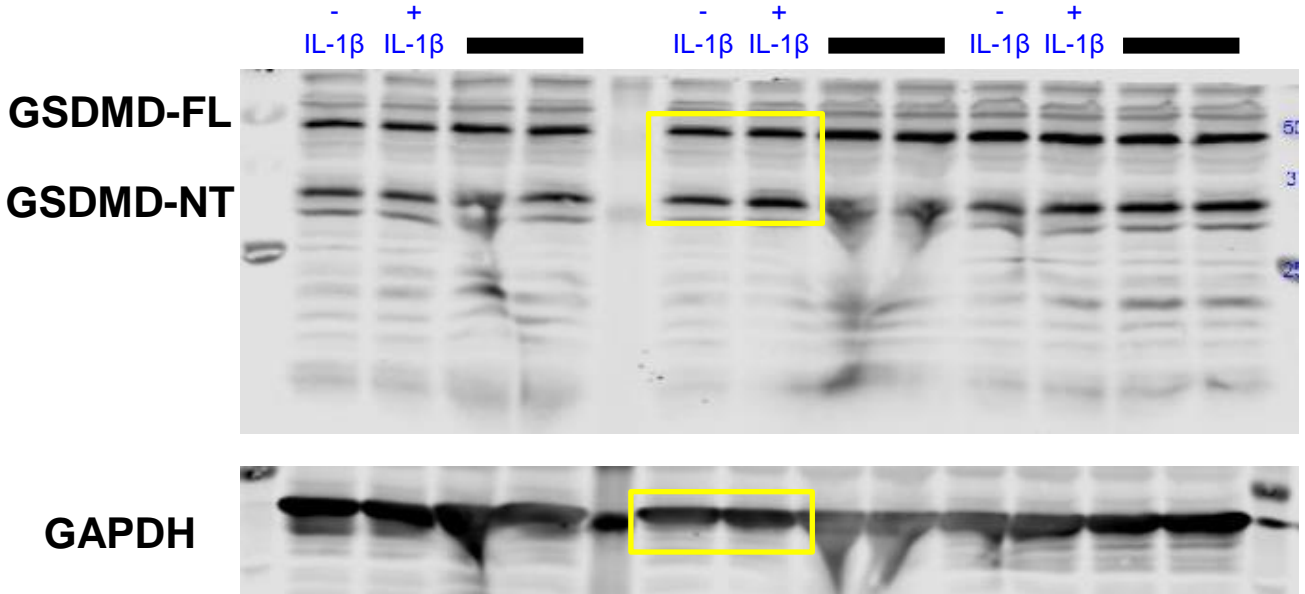


Figure 8E – HL1 cells

Excluded from this analysis

Gasdermin-D (GSDMD) FL + NT (-IL-1 β vs. + IL-1 β)

Gel 1 / 4



Gel 2 / 4

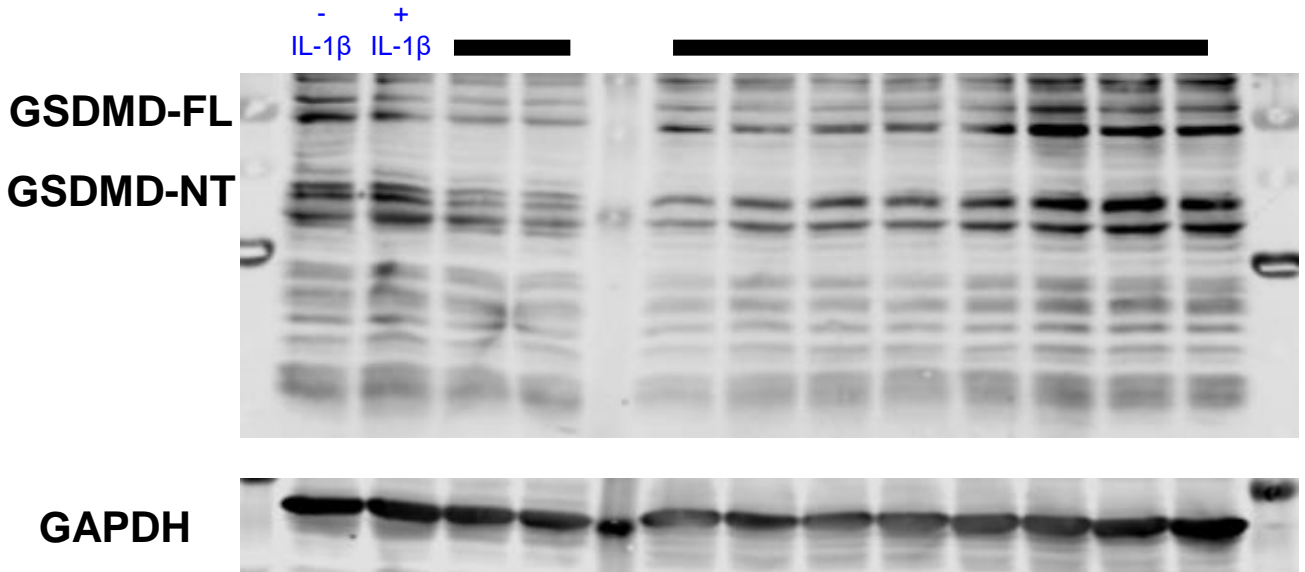


Figure 8E – HL1 cells

Excluded from this analysis

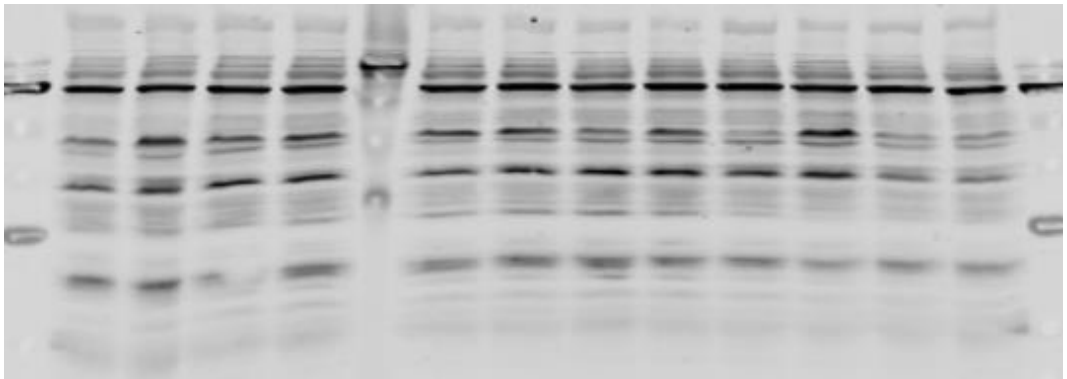
Gasdermin-D (GSDMD) FL + NT (-IL-1 β vs. + IL-1 β)

Gel 3 / 4

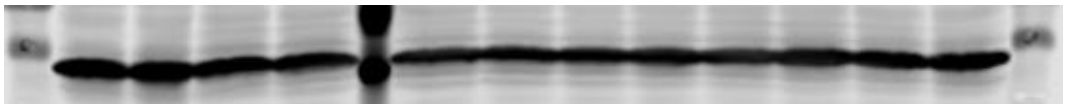
- + - + - + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

GSDMD-FL

GSDMD-NT



GAPDH

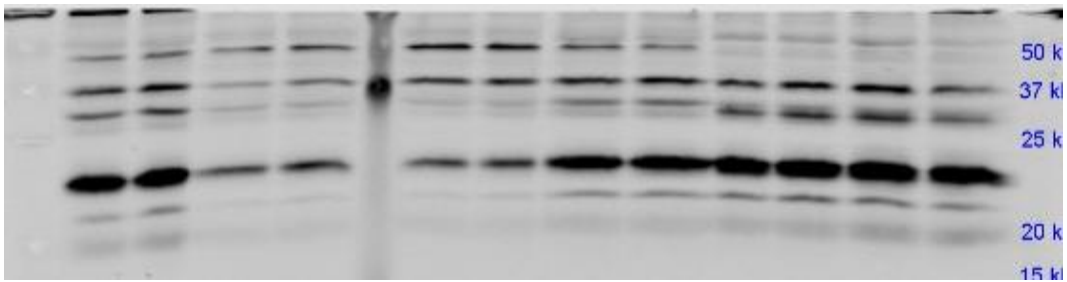


Gel 4 / 4

- + - + - + - + - +
IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β IL-1 β

GSDMD-FL

GSDMD-NT



GAPDH



Figure 8E – HL1 cells

Excluded from this analysis

Gasdermin-D (GSDMD) FL + NT (KN-92 vs. KN-93)

Gel 1 / 5

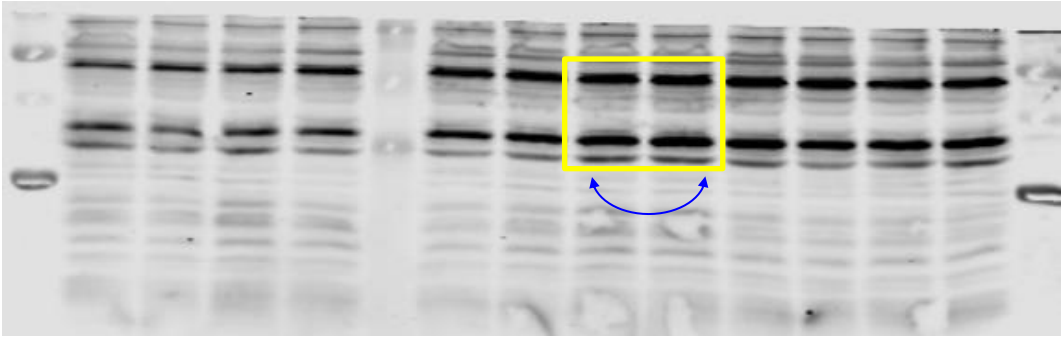
KN- 93 +
KN- 92 +
IL-1 β IL-1 β

KN- 93 +
KN- 92 +
IL-1 β IL-1 β

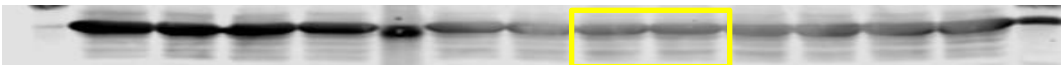
KN- 93 +
KN- 92 +
IL-1 β IL-1 β



GSDMD-FL
GSDMD-NT



GAPDH



Gel 2 / 5

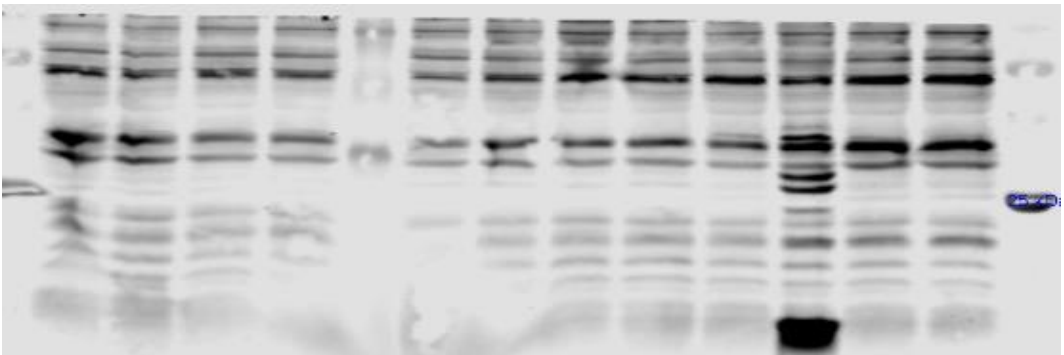
KN- 93 +
KN- 92 +
IL-1 β IL-1 β

KN- 93 +
KN- 92 +
IL-1 β IL-1 β

KN- 93 +
KN- 92 +
IL-1 β IL-1 β



GSDMD-FL
GSDMD-NT



GAPDH



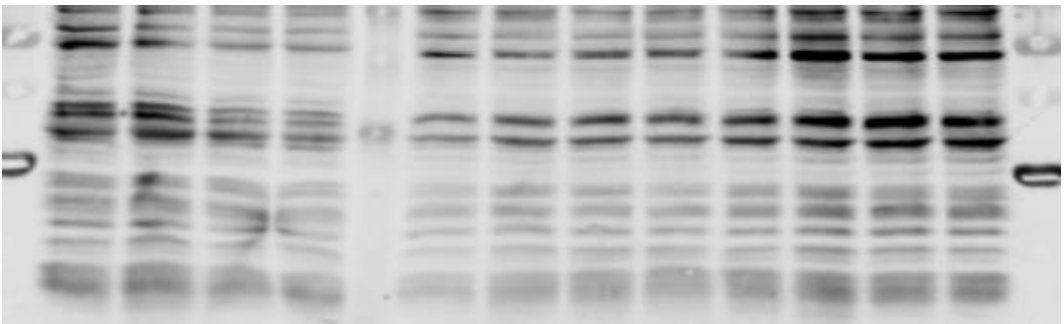
Gel 3 / 5

KN- 93 +
KN- 92 +
IL-1 β IL-1 β

KN- 93 +
KN- 92 +
IL-1 β IL-1 β



GSDMD-FL
GSDMD-NT



GAPDH



Figure 8E – HL1 cells

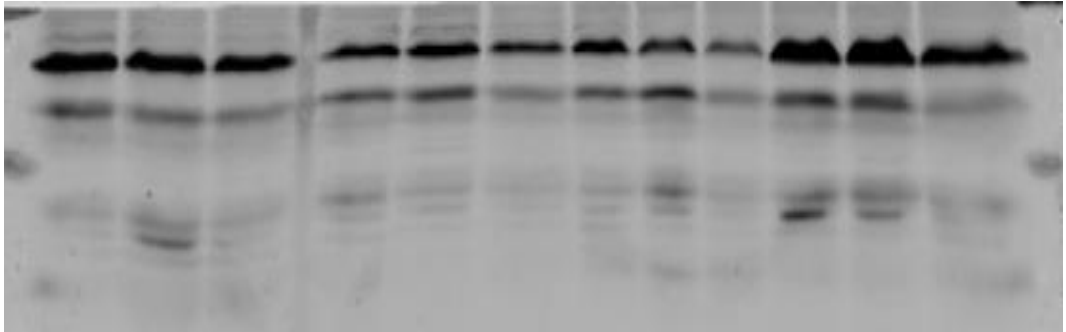
 Excluded from this analysis

Gasdermin-D (GSDMD) FL + NT (KN-92 vs. KN-93)

Gel 4 / 5

■ KN-92 + IL-1 β KN-93 + IL-1 β ■ KN-92 + IL-1 β KN-93 + IL-1 β ■

GSDMD-FL
GSDMD-NT



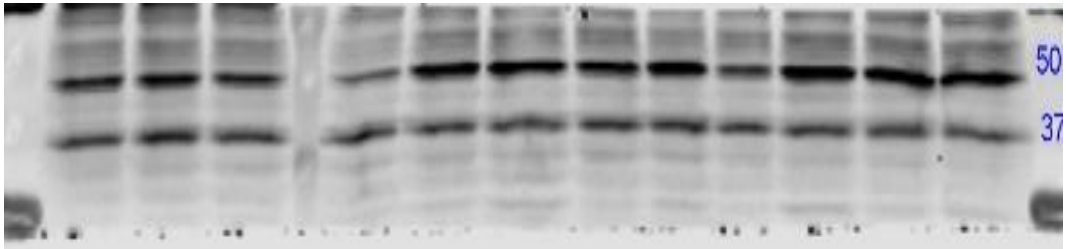
GAPDH



Gel 5 / 5

■ KN-92 + IL-1 β KN-93 + IL-1 β ■ KN-92 + IL-1 β KN-93 + IL-1 β ■ KN-92 + IL-1 β KN-93 + IL-1 β ■

GSDMD-FL
GSDMD-NT



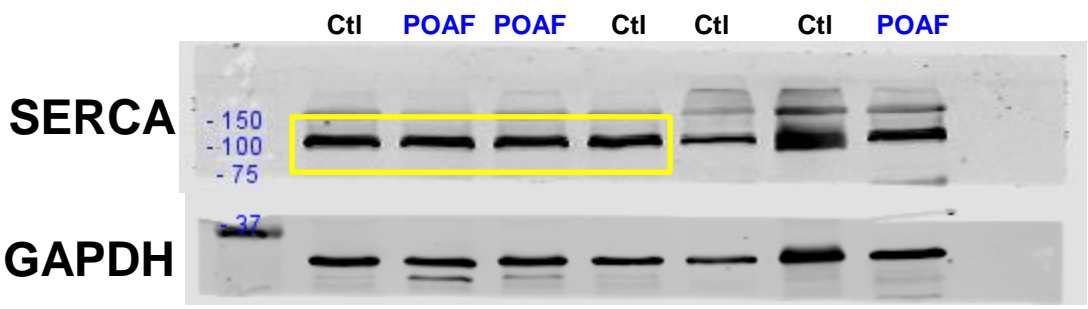
GAPDH



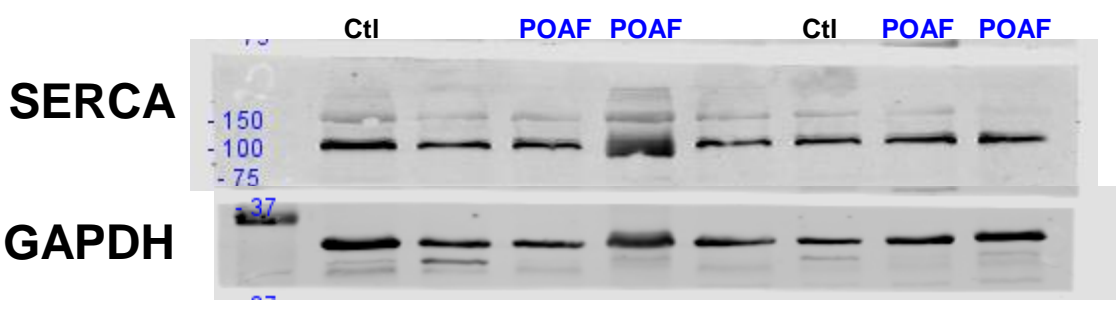
Figure 8E – HL1 cells

■ Excluded from this analysis

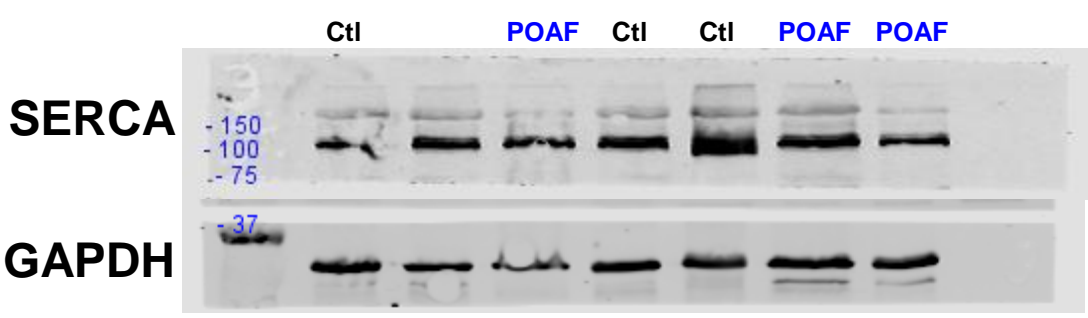
Gel 1 / 4



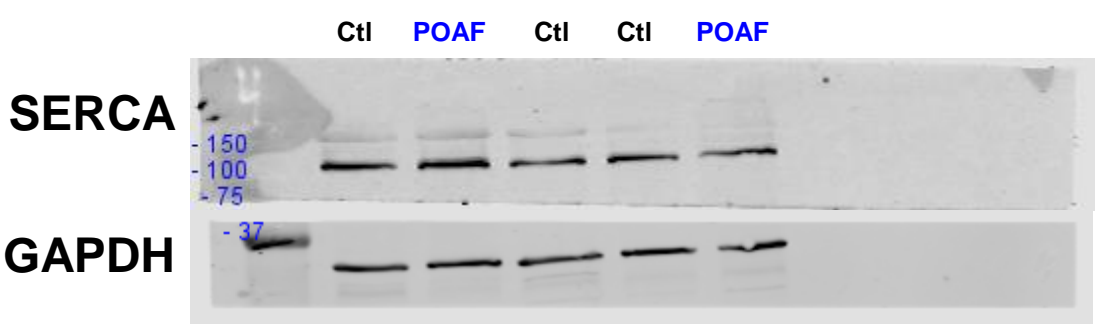
Gel 2 / 4



Gel 3 / 4

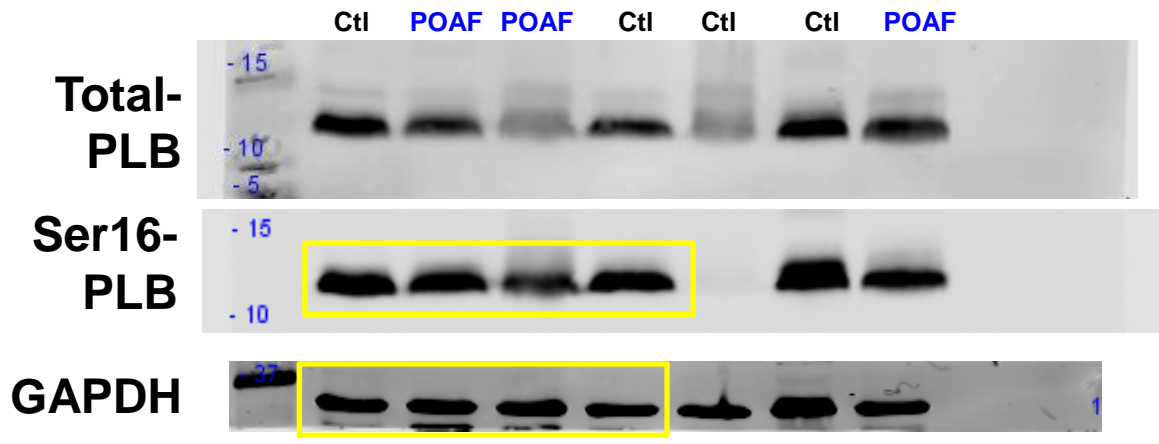


Gel 4 / 4

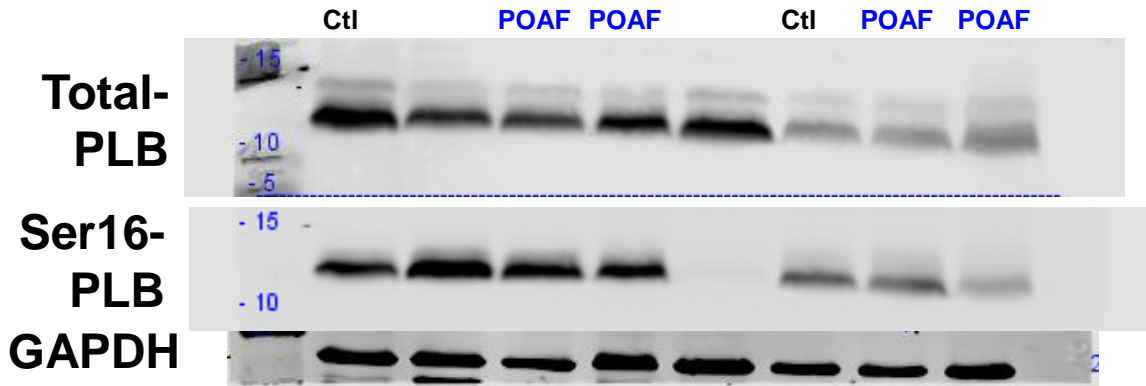


Online Figure VIIA

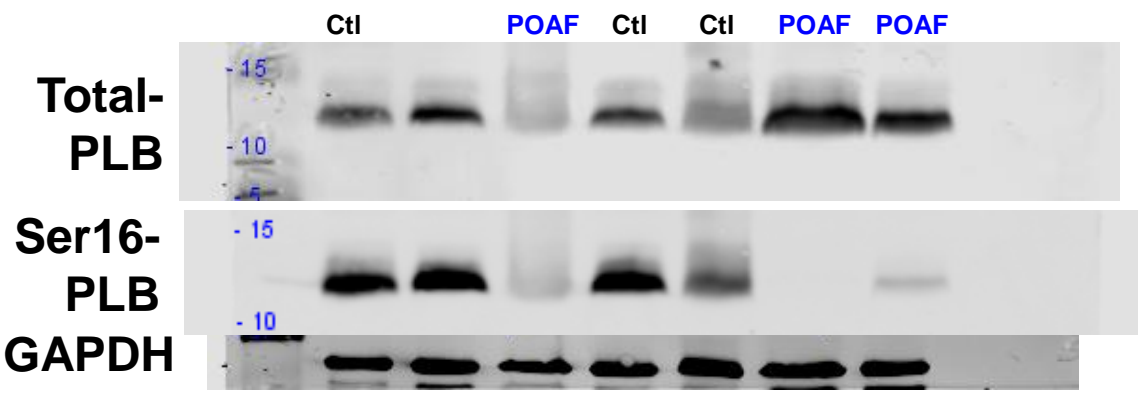
Gel 1 / 4



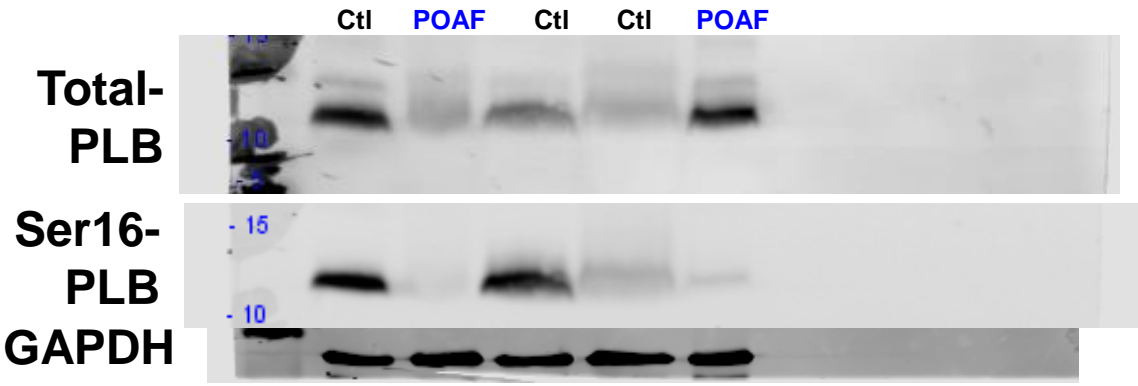
Gel 2 / 4



Gel 3 / 4

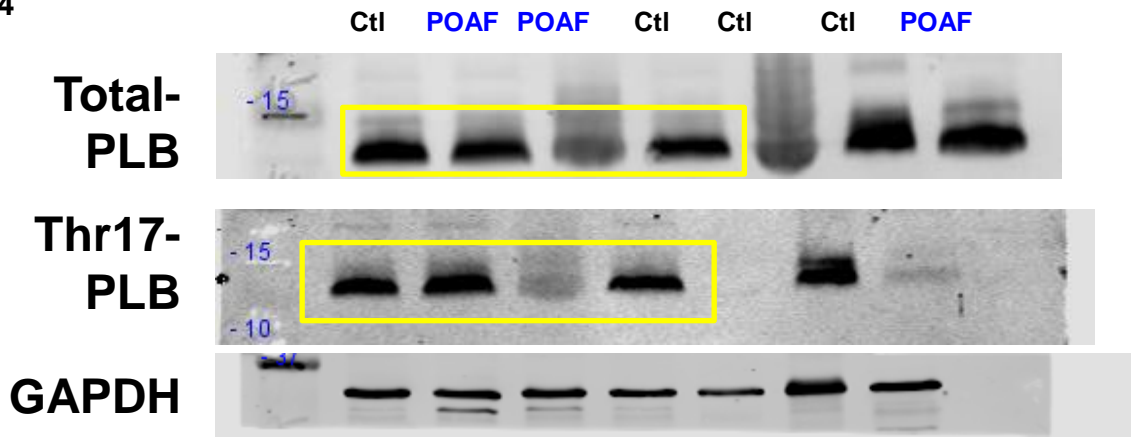


Gel 4 / 4

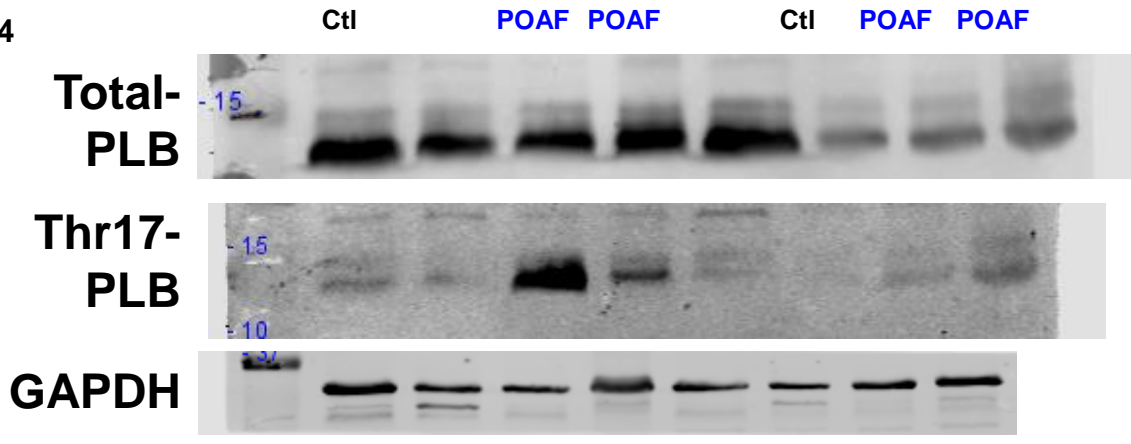


Online Figure VIIA

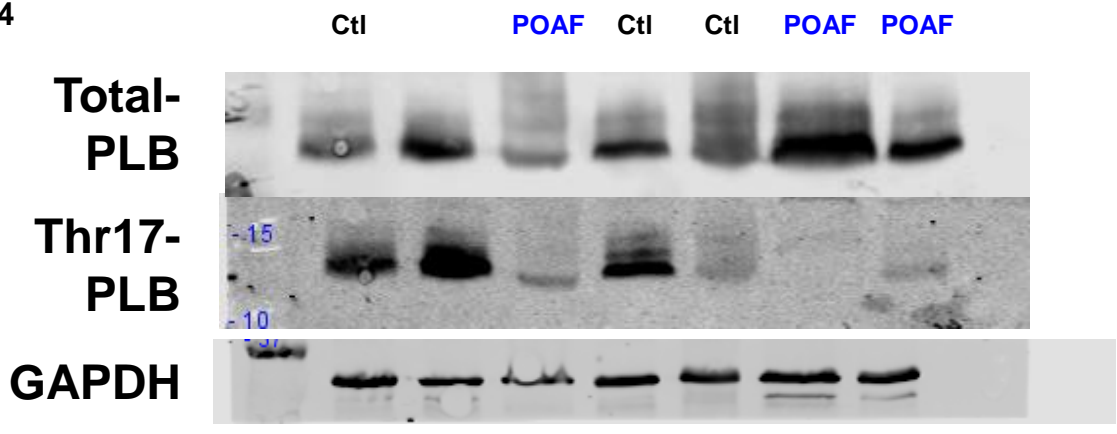
Gel 1 / 4



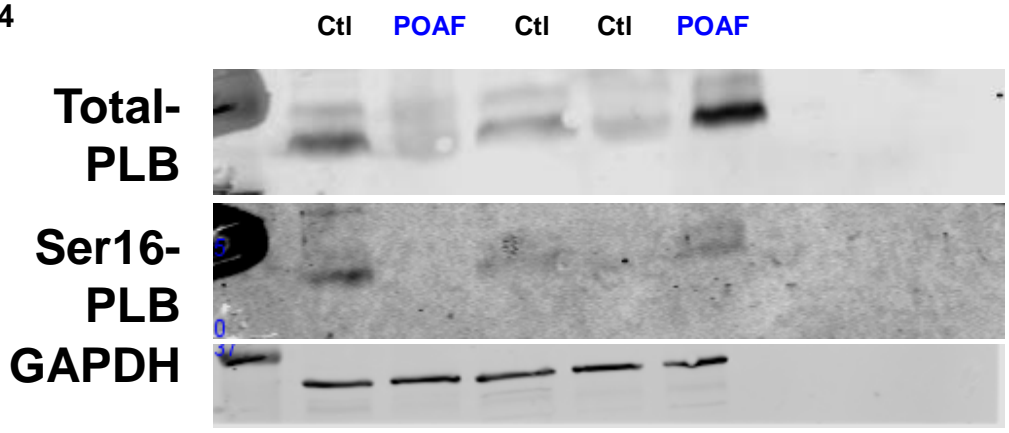
Gel 2 / 4



Gel 3 / 4

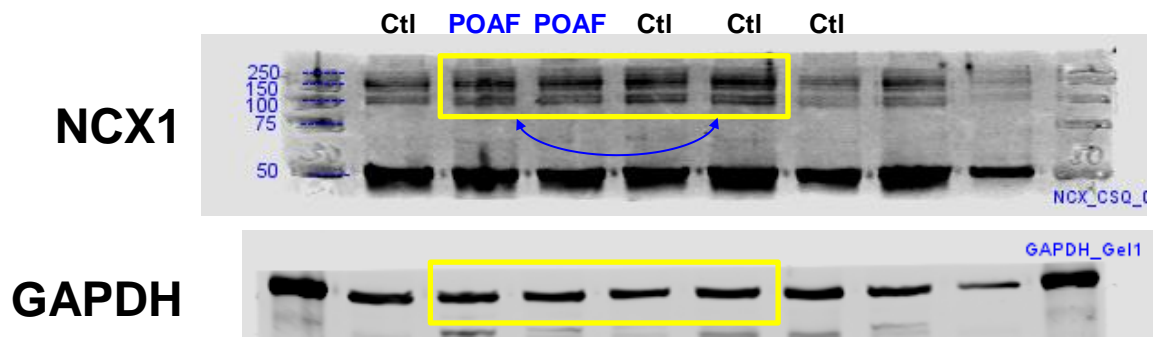


Gel 4 / 4

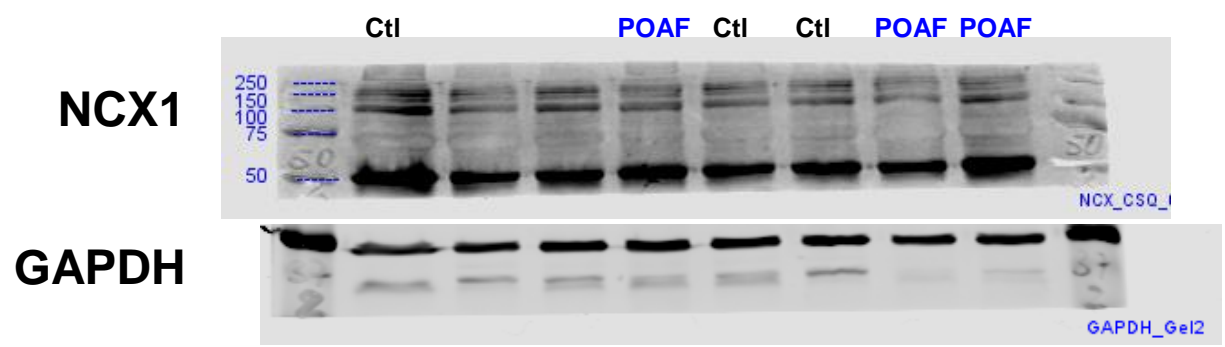


Online Figure VIIA

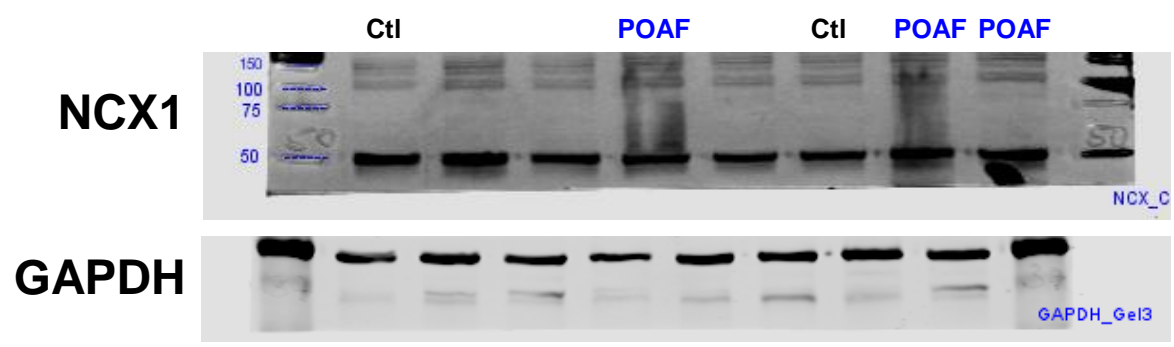
Gel 1 / 3



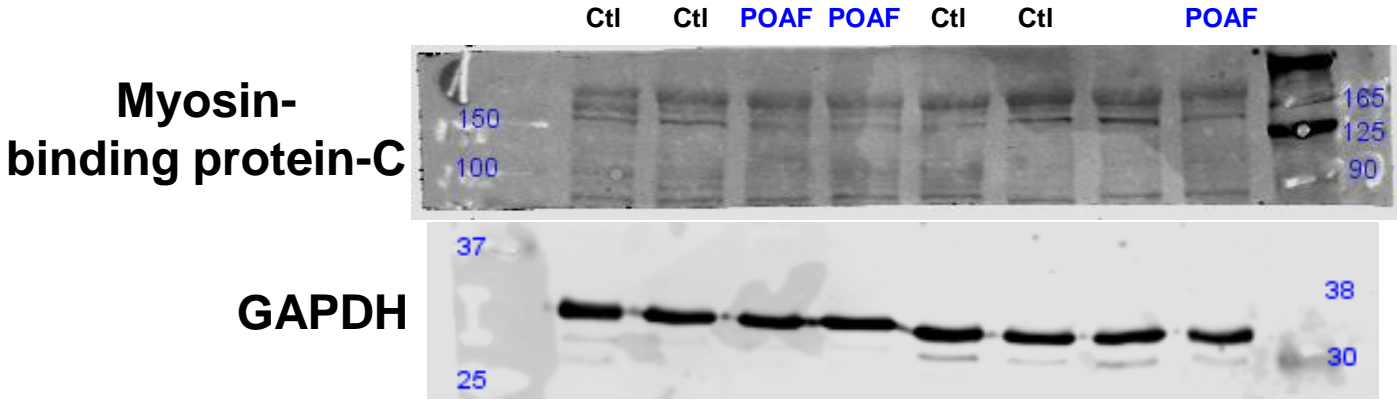
Gel 2 / 3



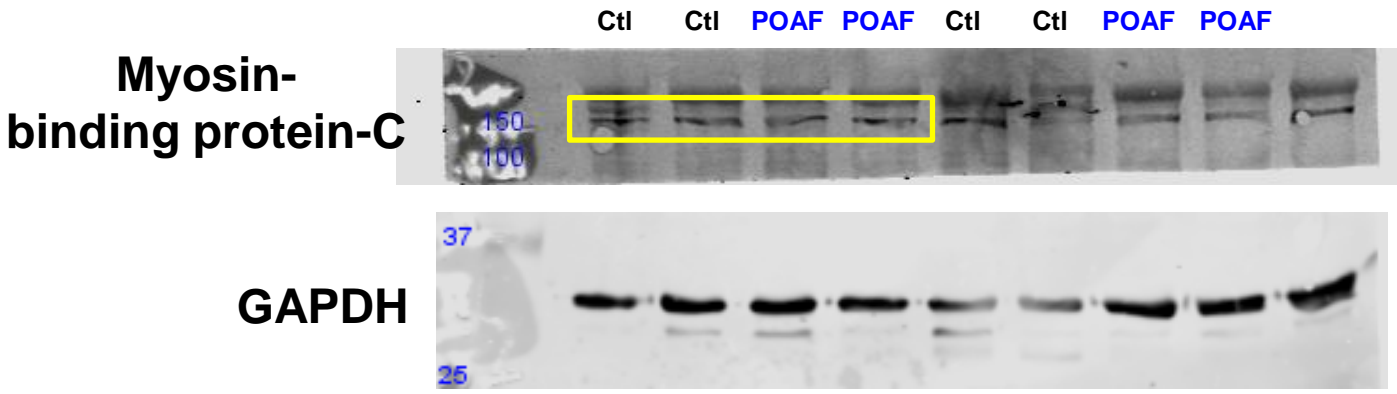
Gel 3 / 3



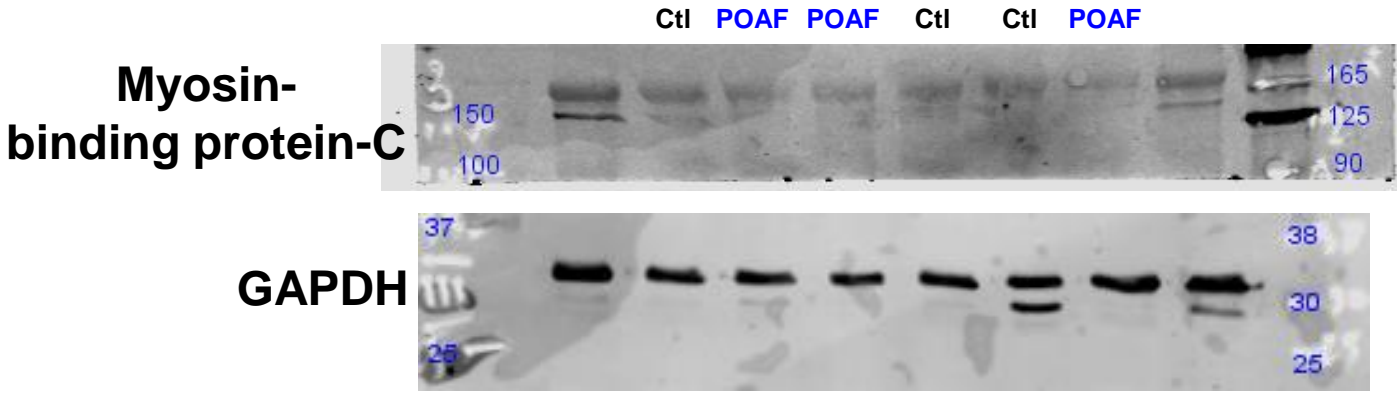
Gel 1 / 3



Gel 2 / 3

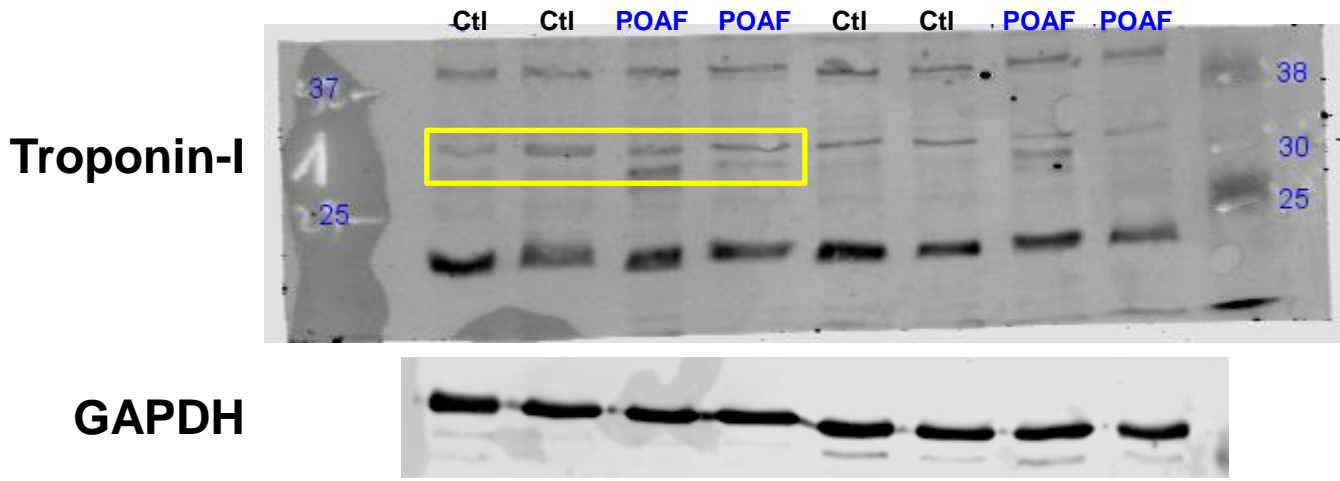


Gel 3 / 3

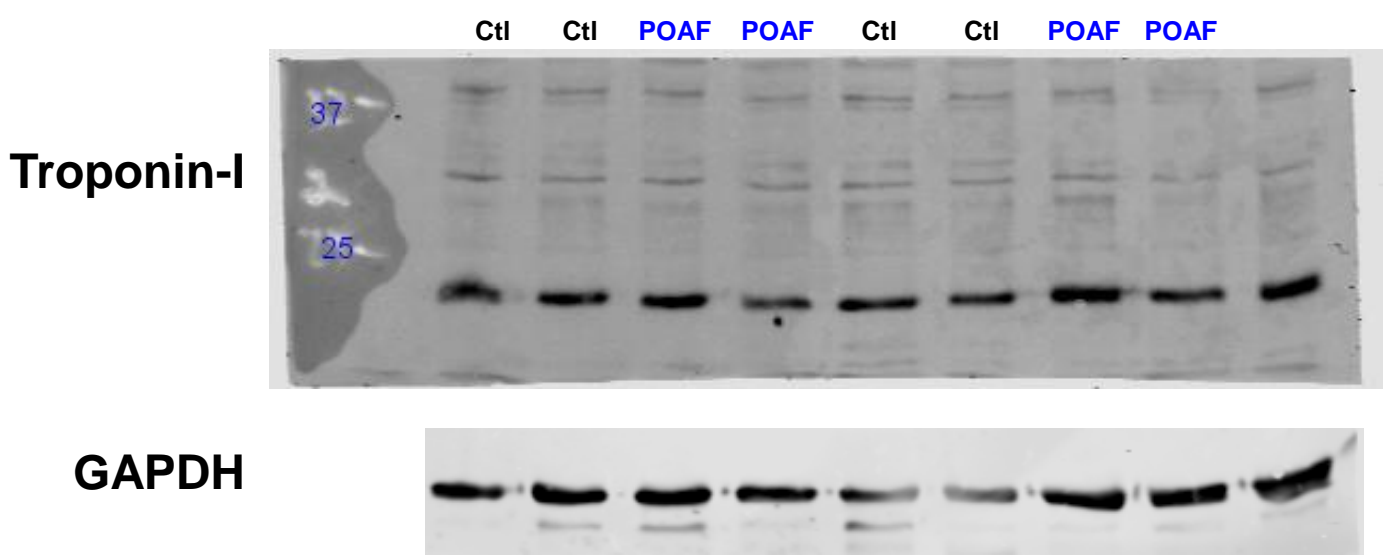


Online Figure VIID – Human atrial whole-tissue lysates

Gel 1 / 2

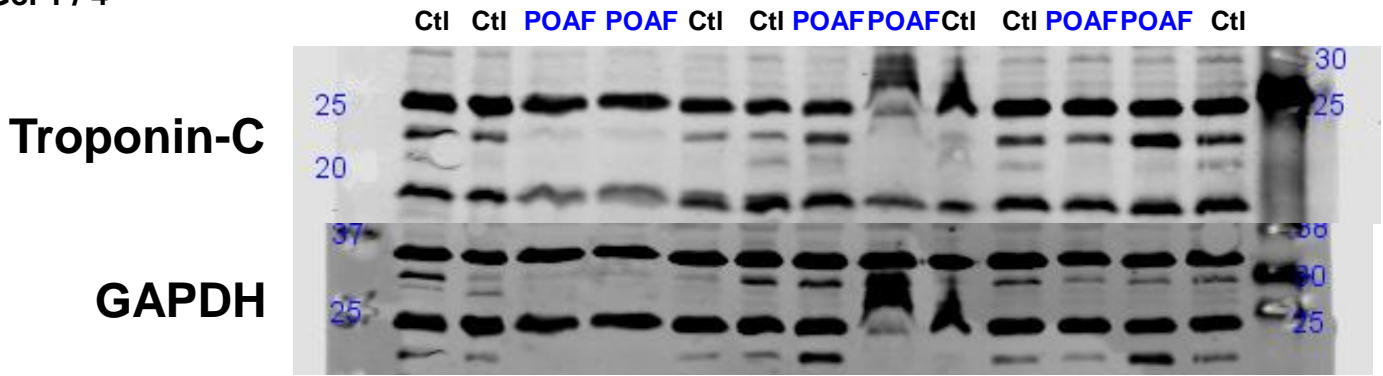


Gel 2 / 2

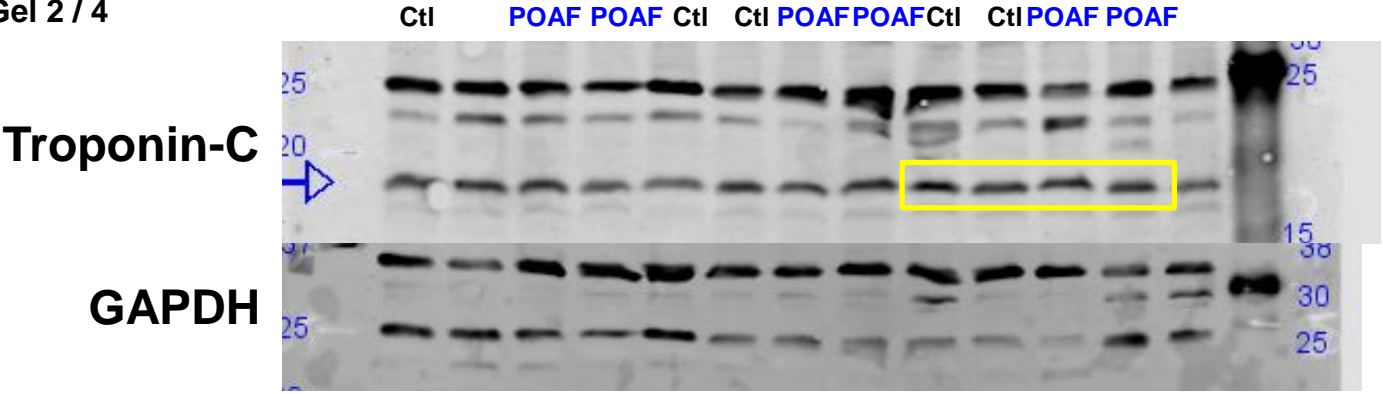


Online Figure VIID – Human atrial whole-tissue lysates

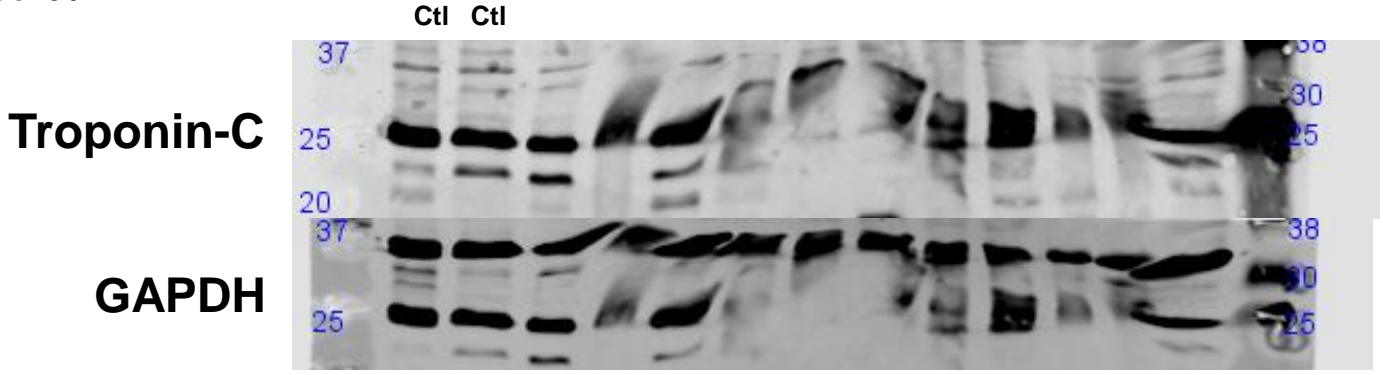
Gel 1 / 4



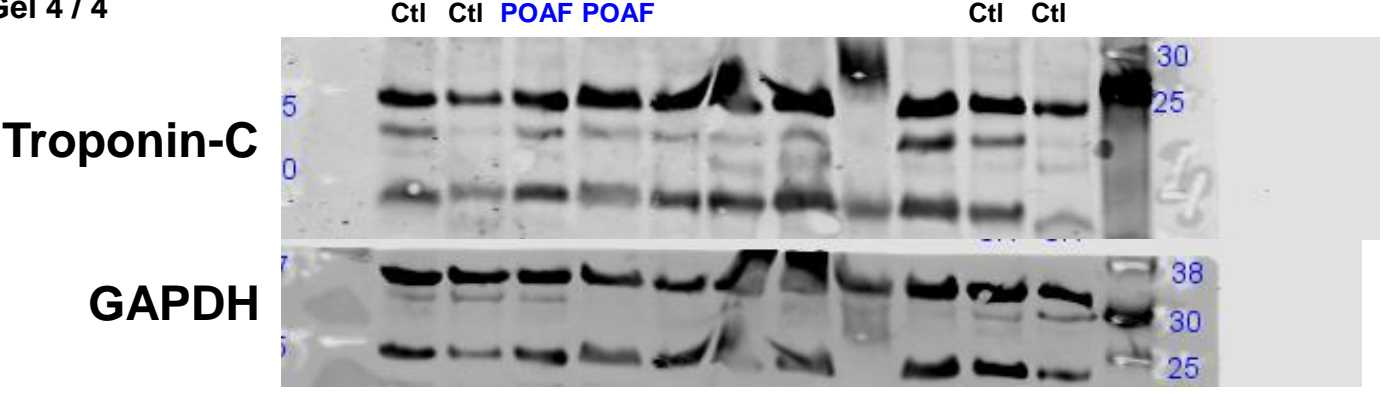
Gel 2 / 4



Gel 3 / 4

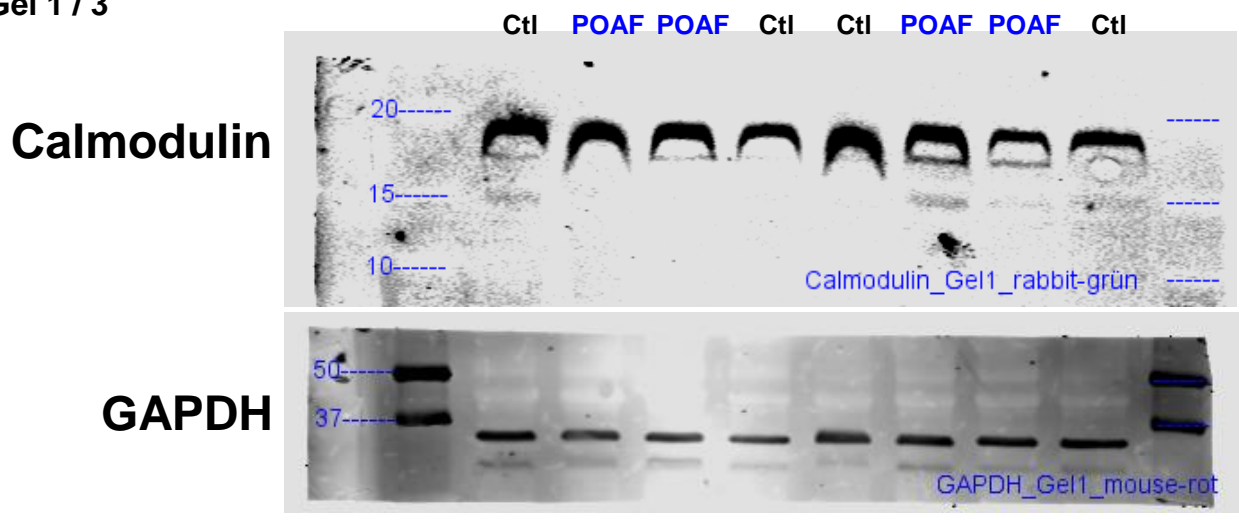


Gel 4 / 4

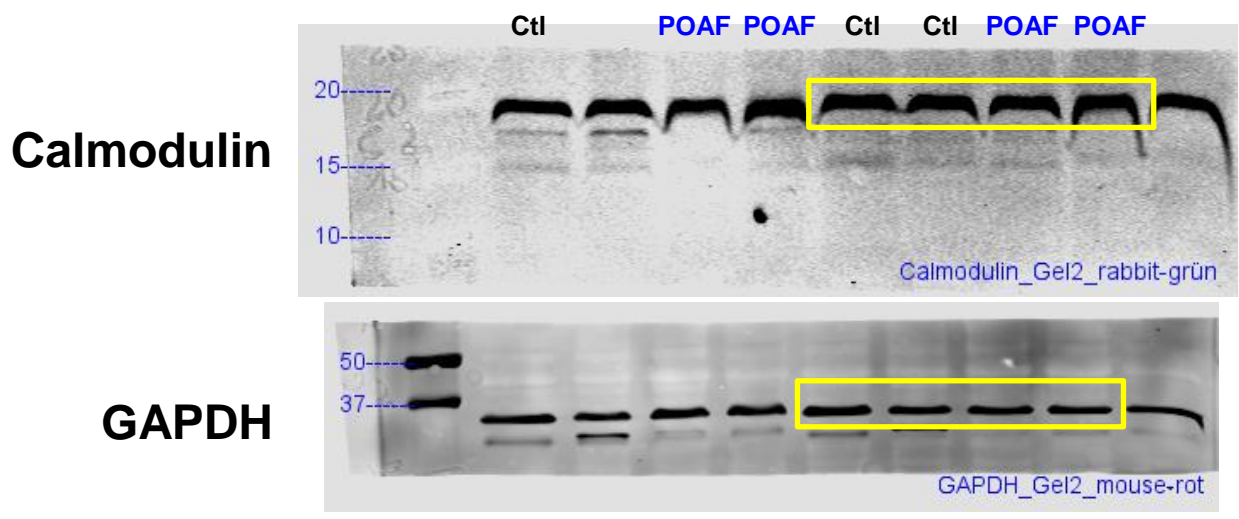


Online Figure VIID – Human atrial whole-tissue lysates

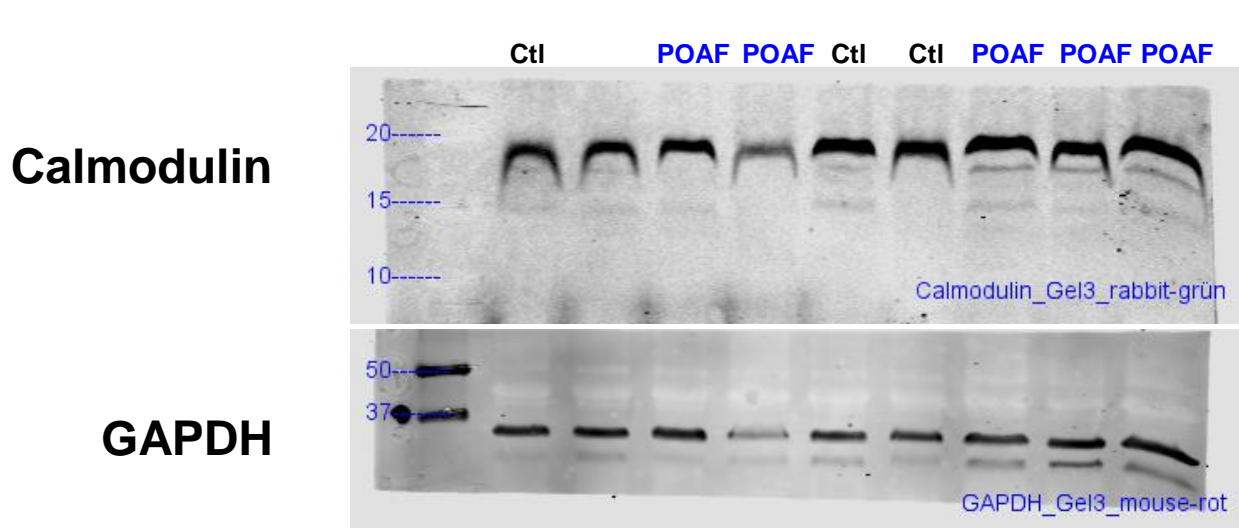
Gel 1 / 3



Gel 2 / 3

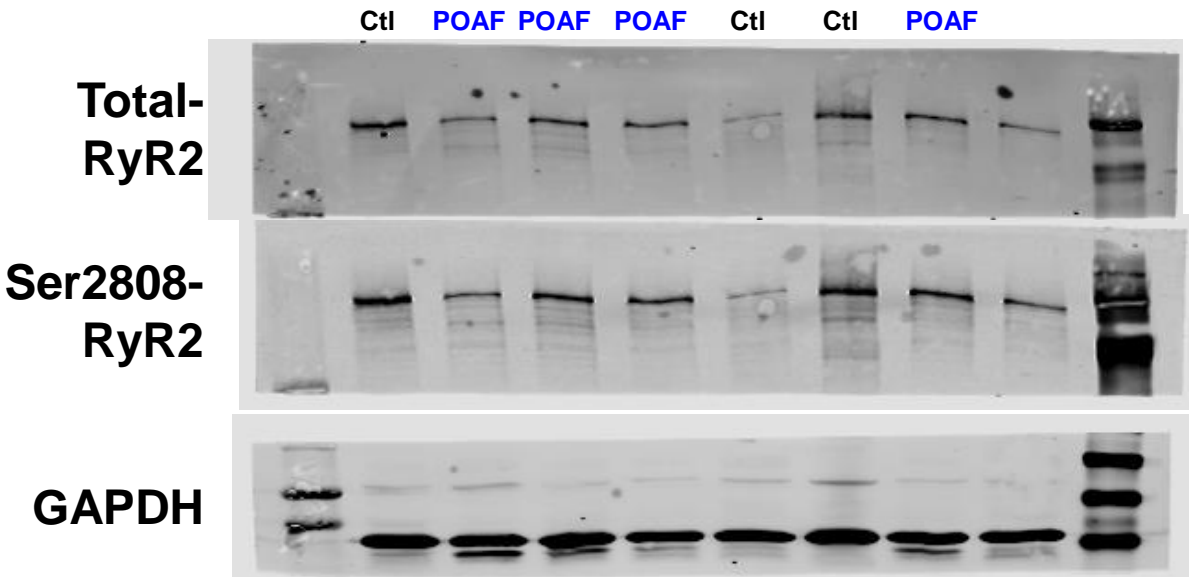


Gel 3 / 3

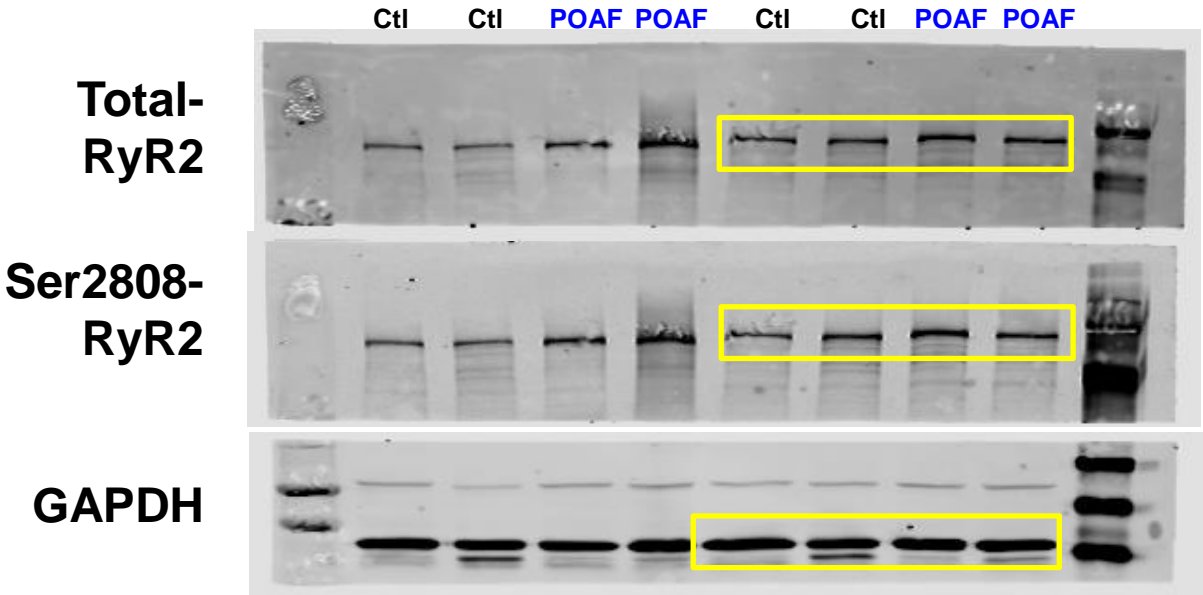


Online Figure VIID – Human atrial whole-tissue lysates

Gel 1 / 4

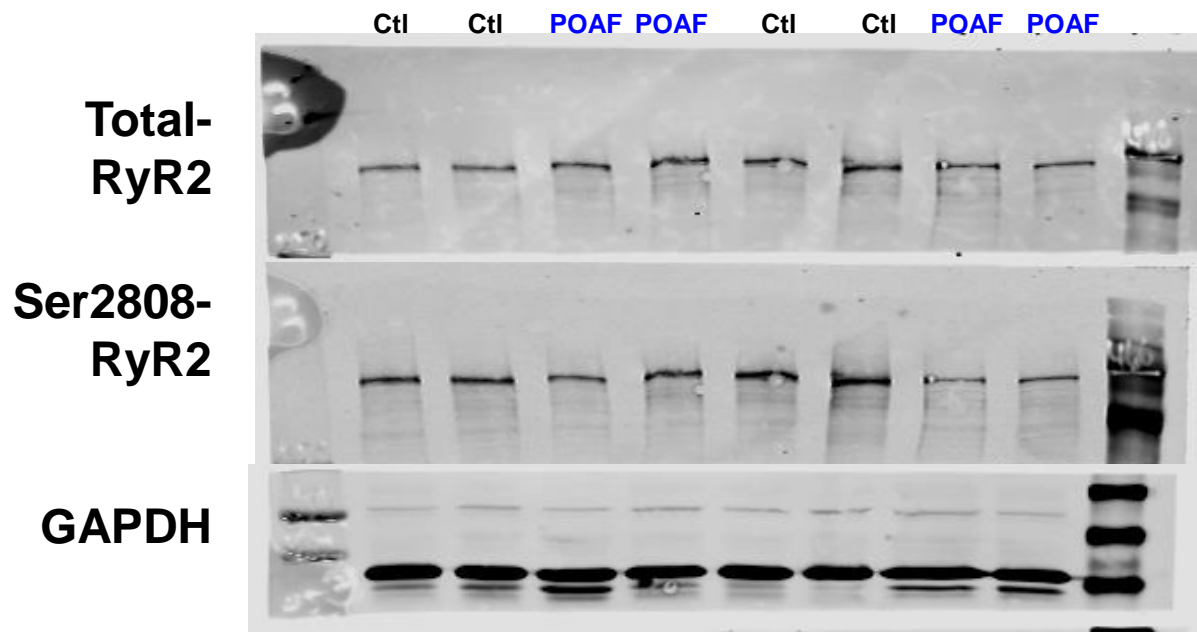


Gel 2 / 4

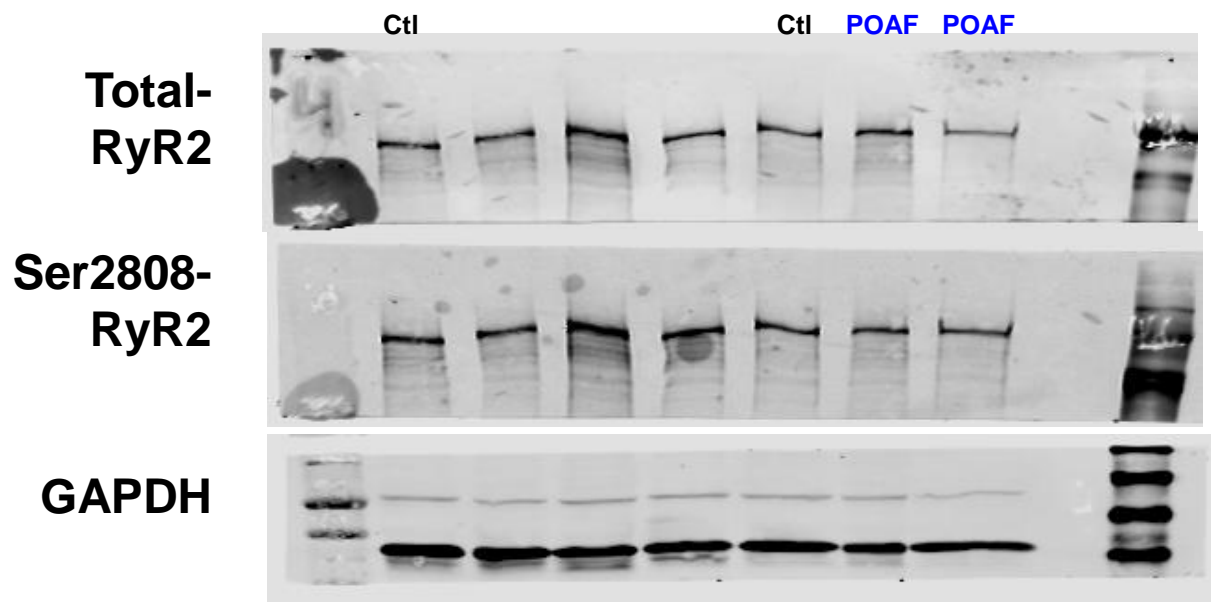


Online Figure IXA – Human atrial whole-tissue lysates

Gel 3 / 4

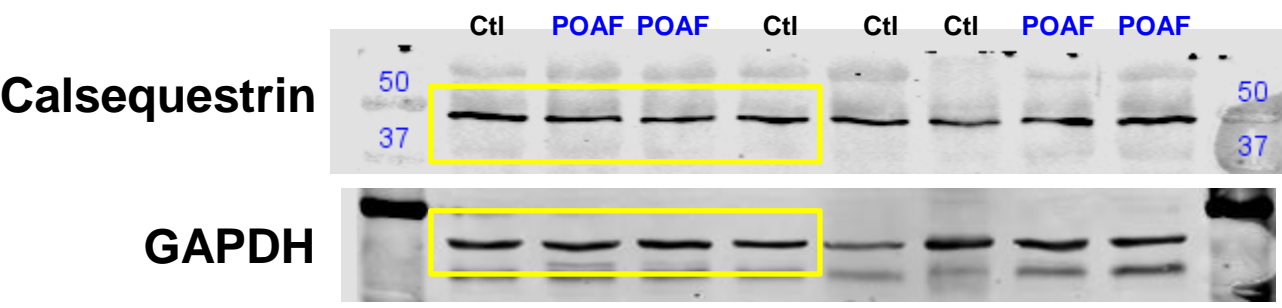


Gel 4 / 4

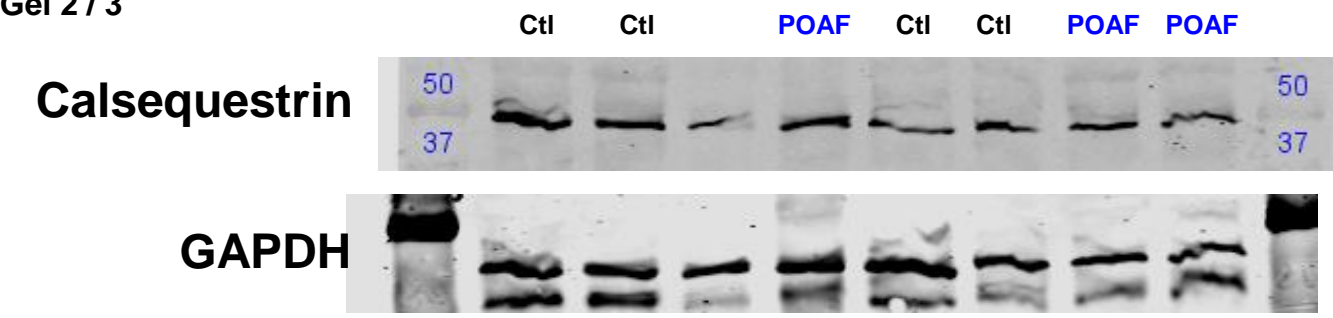


Online Figure IXA – Human atrial whole-tissue lysates

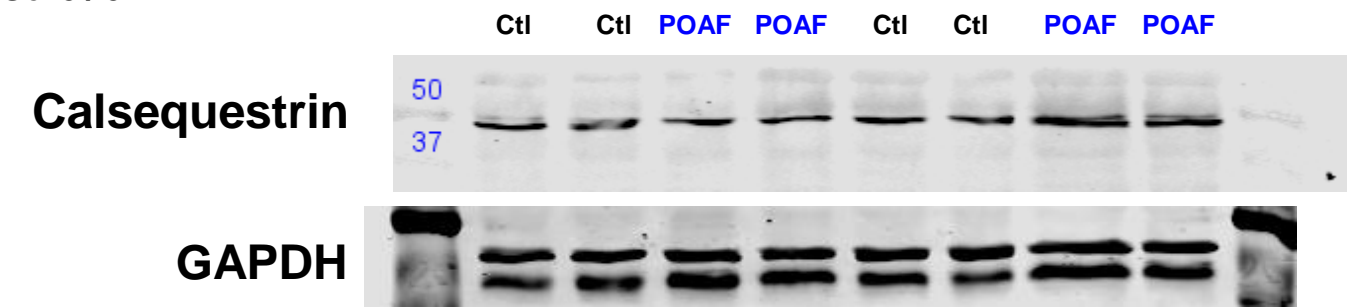
Gel 1 / 3



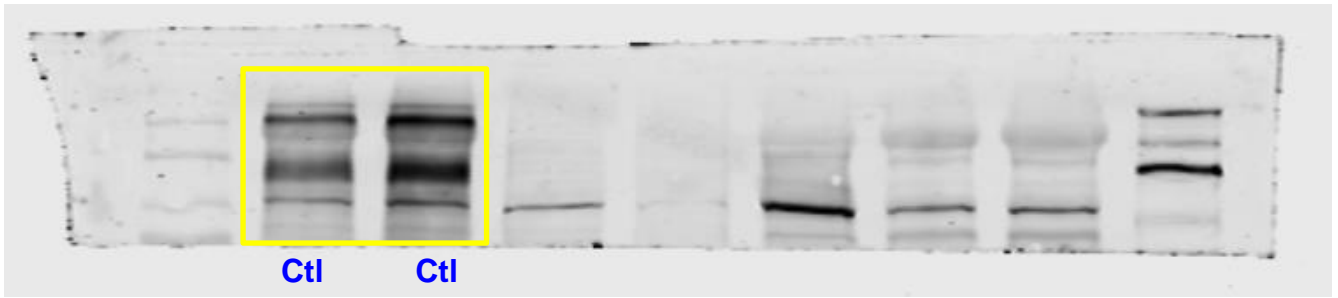
Gel 2 / 3



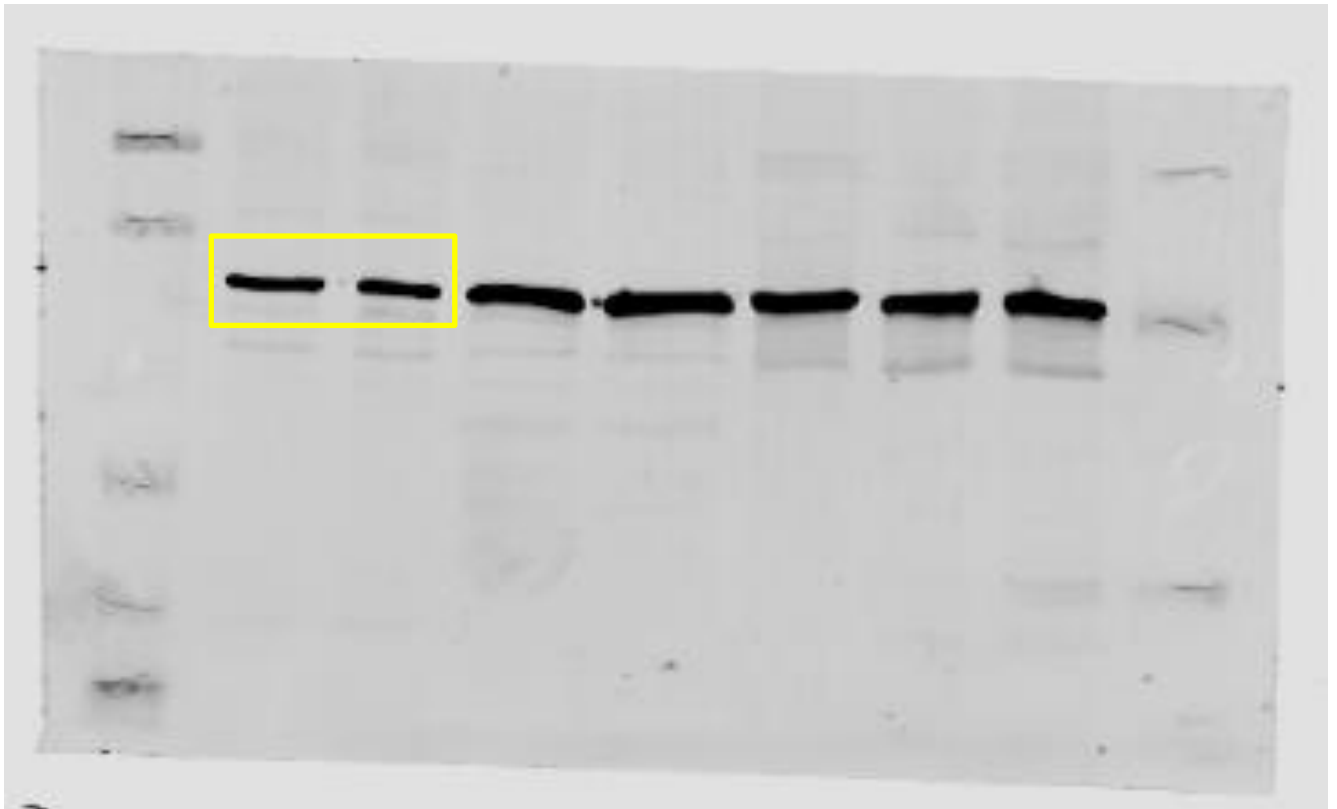
Gel 3 / 3



NLRP3



GAPDH



NLRP3

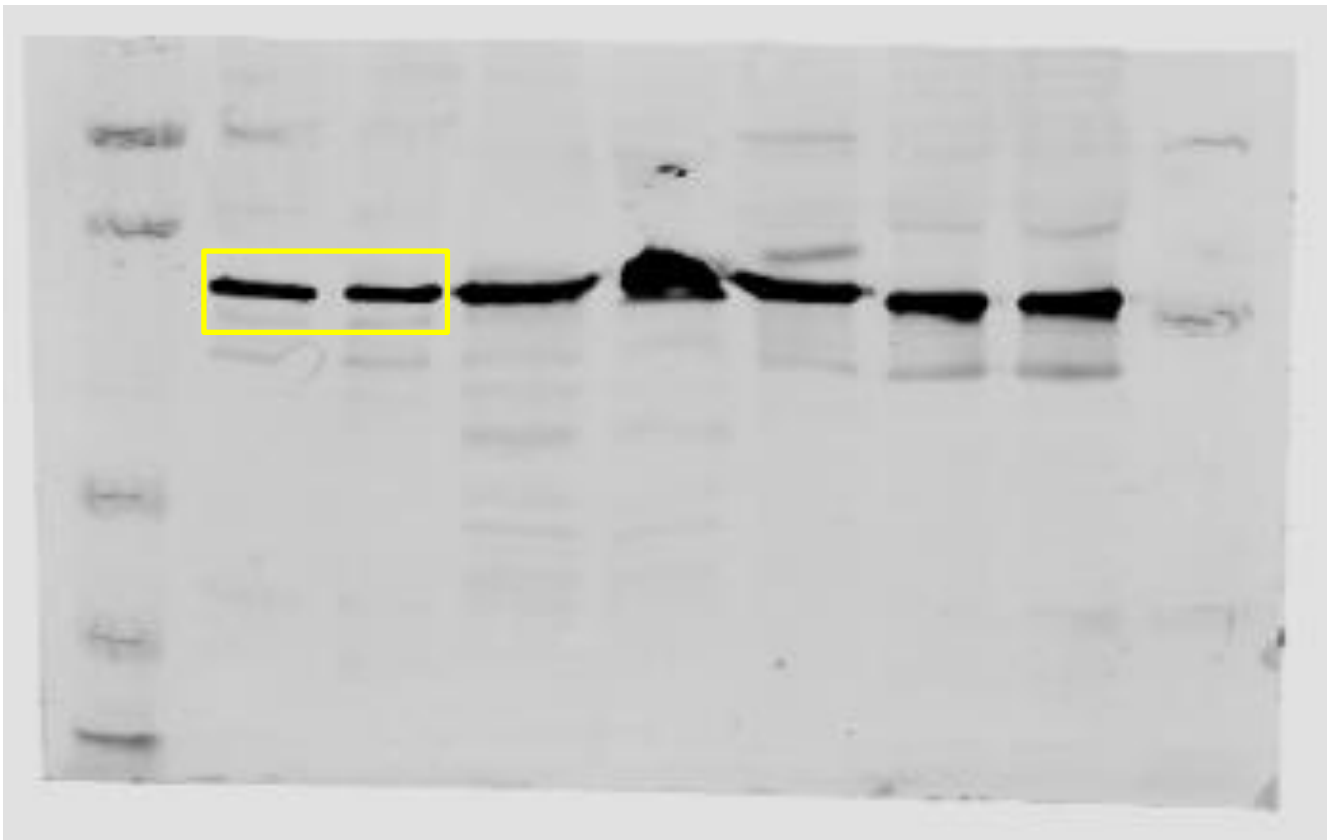


Ctl

Ctl

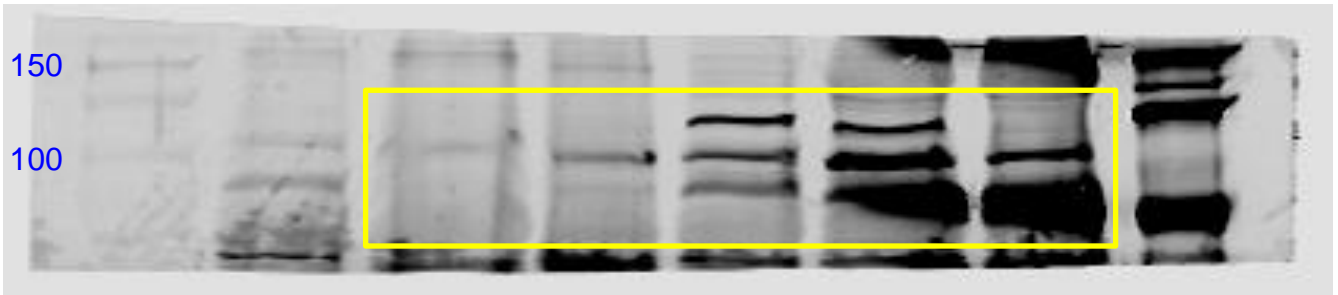
Blocking peptide

GAPDH

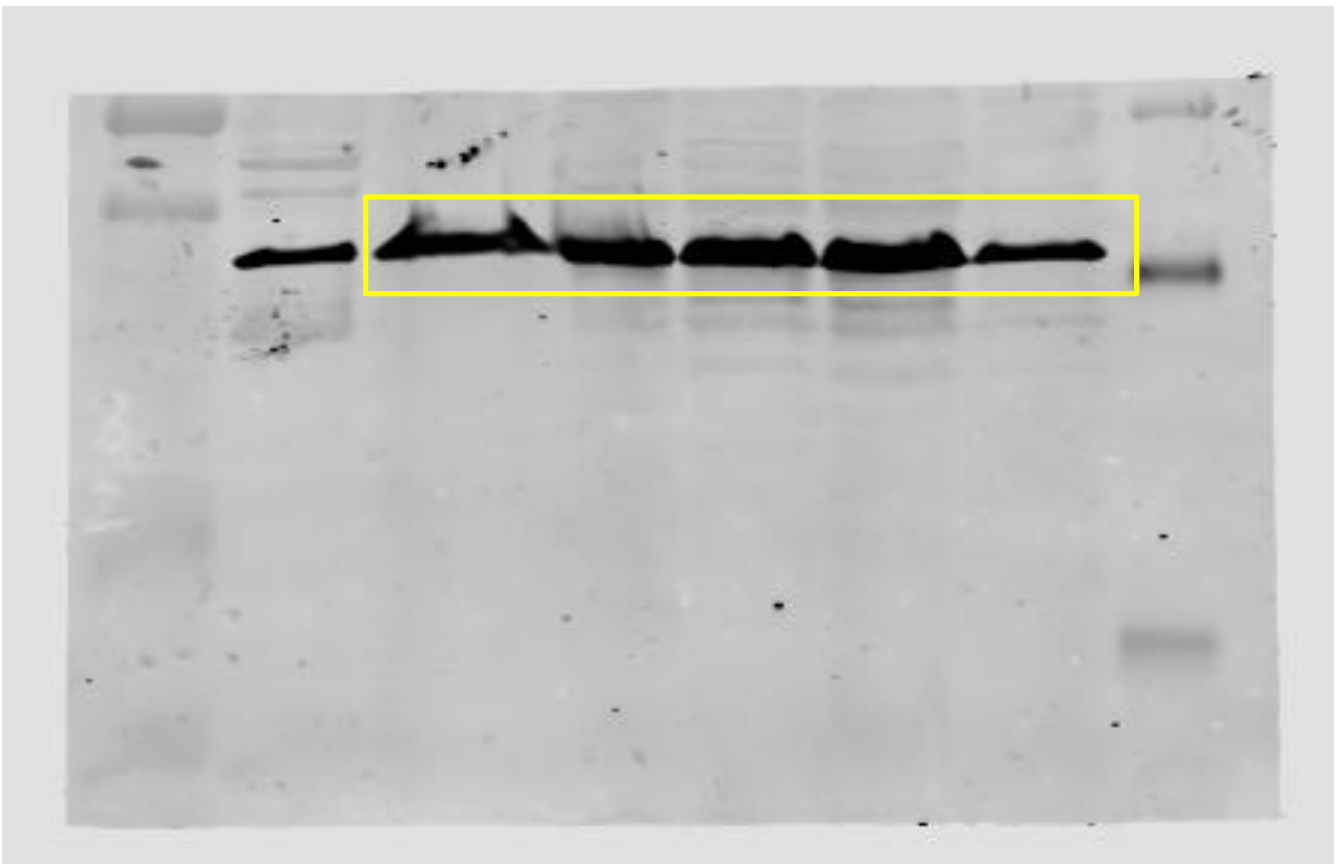


NLRP3

ATR: $\bar{}$ $\bar{}$ $\bar{}$ $\bar{}$ $\bar{}$ $\bar{}$
CF CF CF CM CM CF

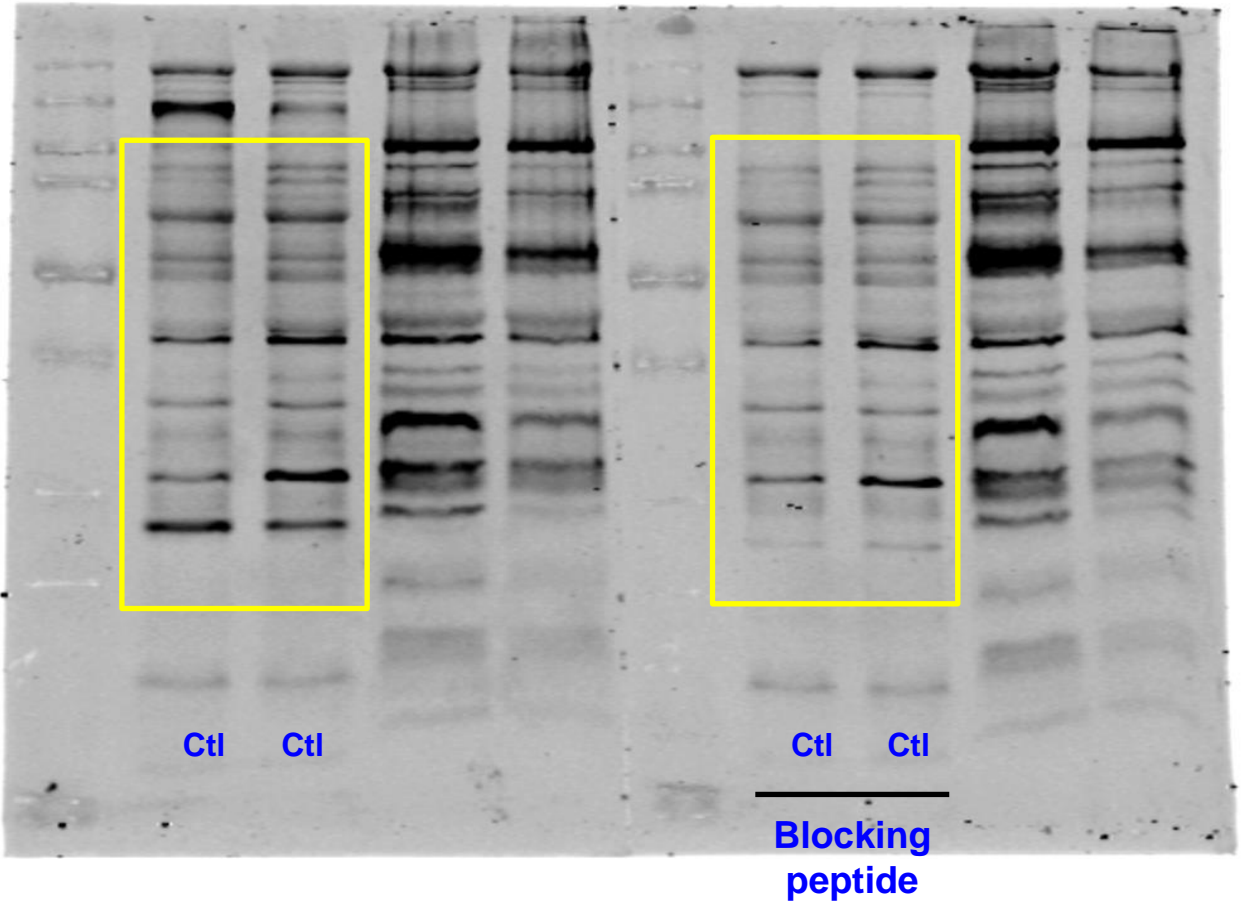


GAPDH

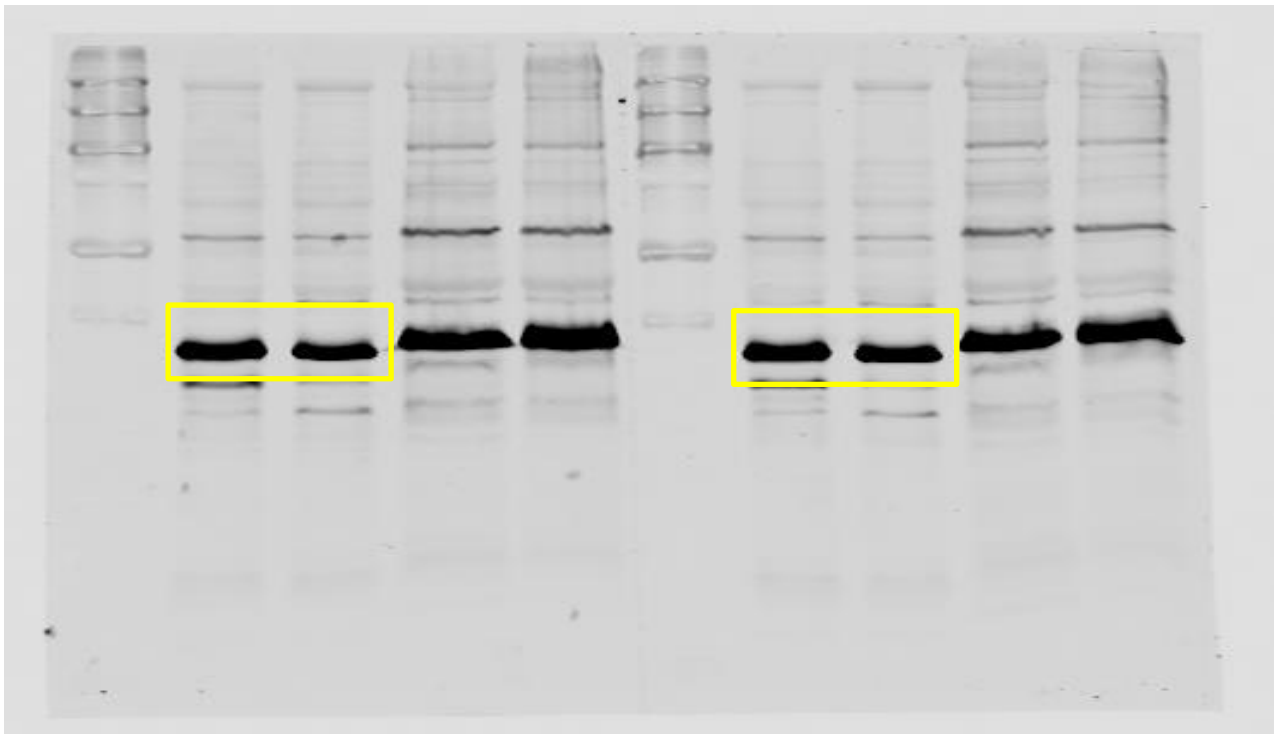


Online Figure XIA – Canine atrial fibroblasts (CF) and atrial cardiomyocytes (CM)

ASC

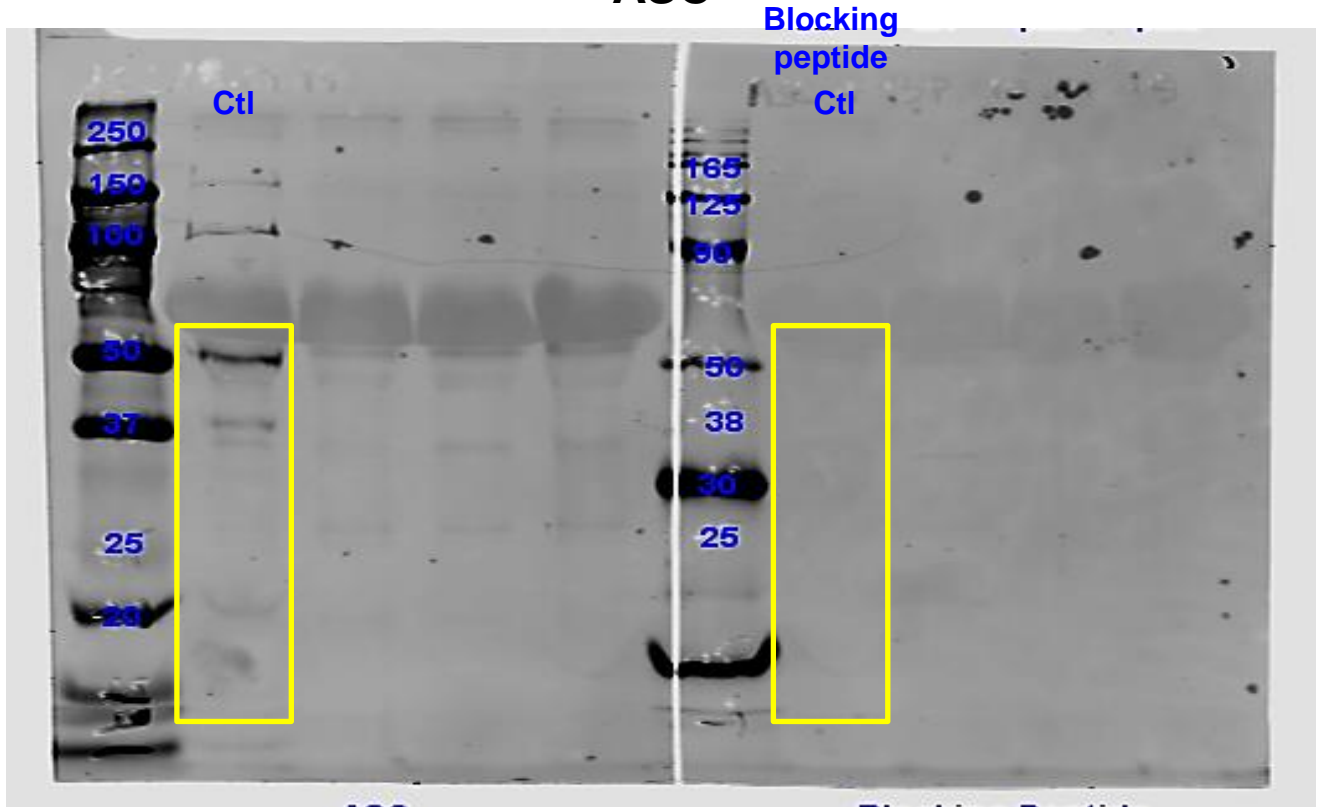


GAPDH

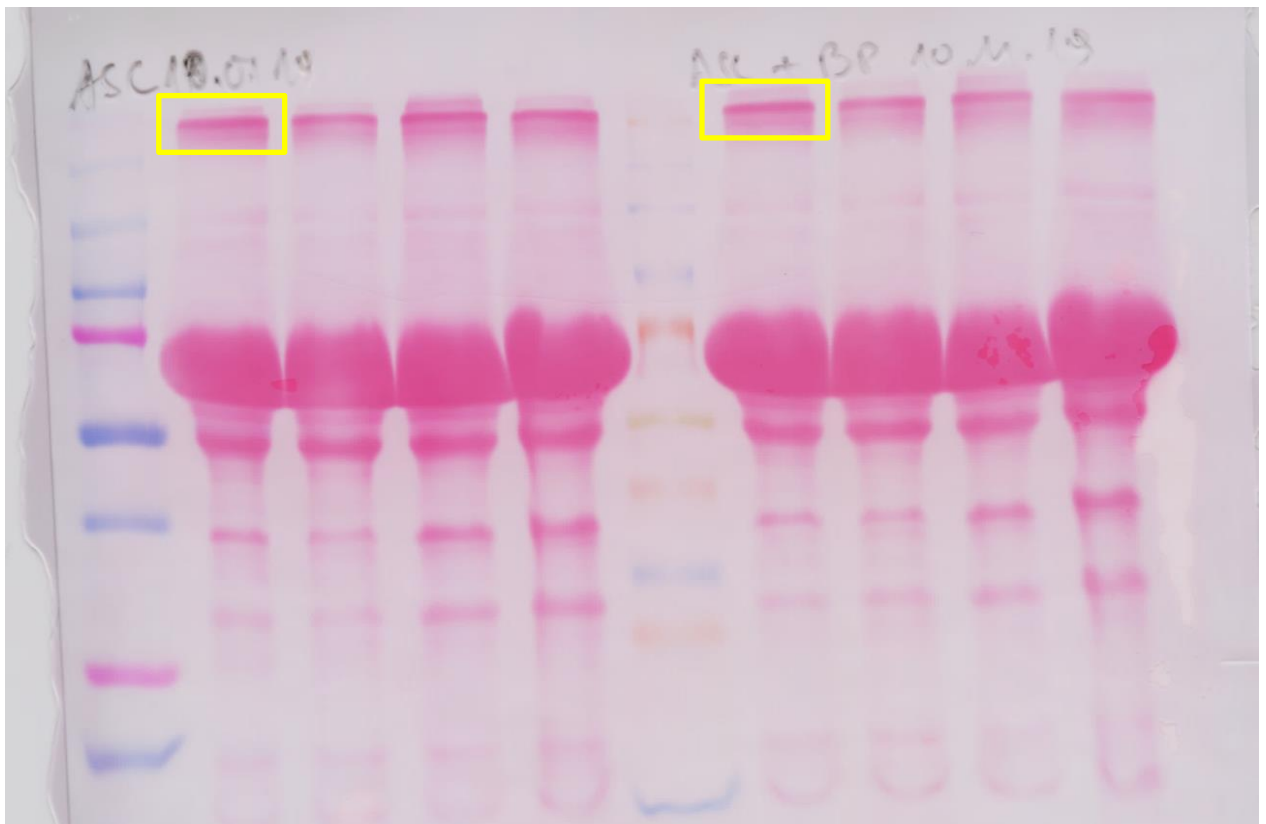


Online Figure XIB – Human atrial whole-tissue lysates

ASC



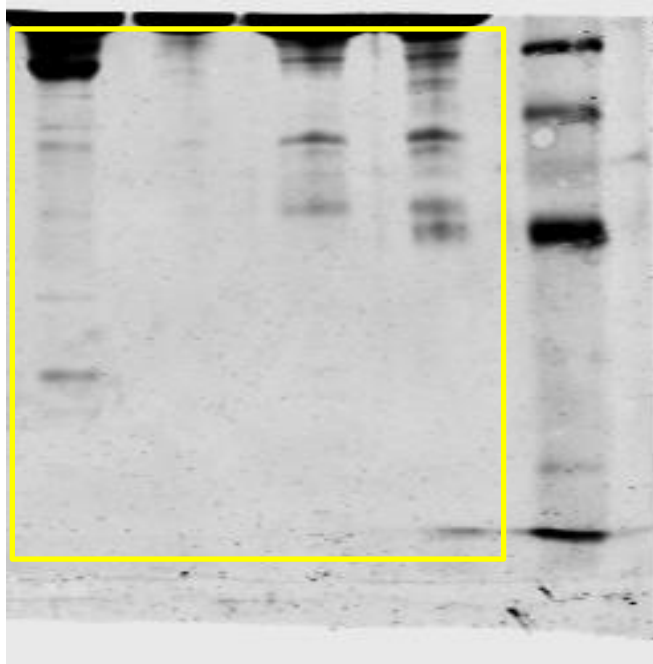
Ponceau



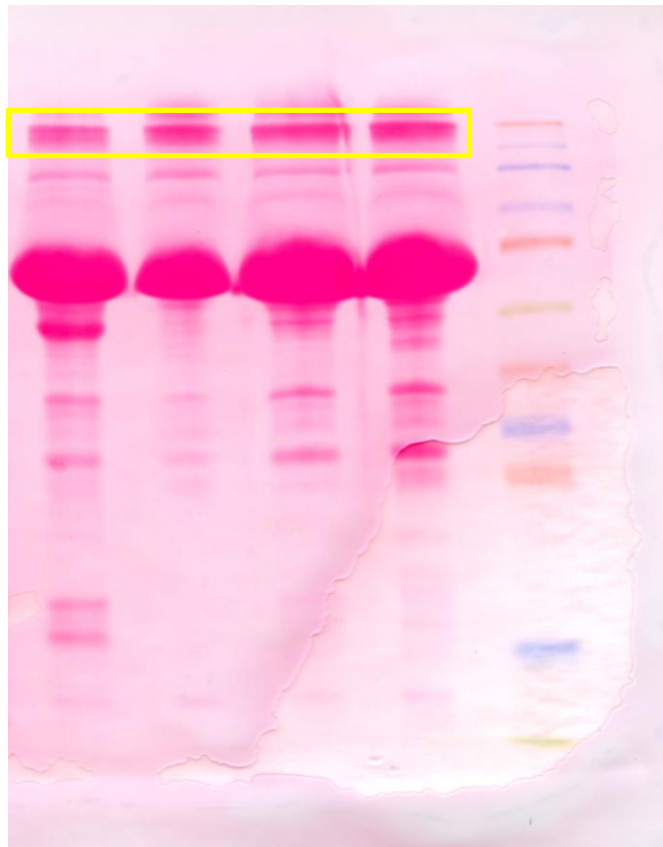
Online Figure XIC – Human atrial cardiomyocytes

(Pro-)Caspase-1

Ctl Ctl POAF POAF

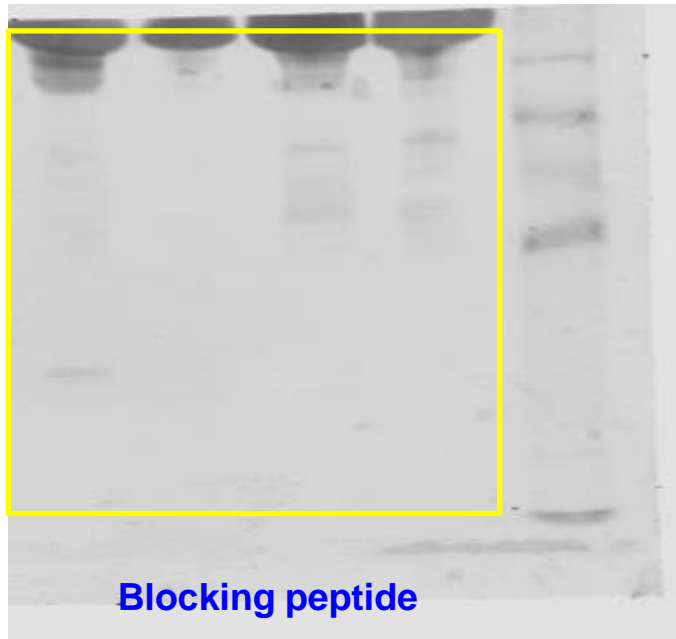


Ponceau

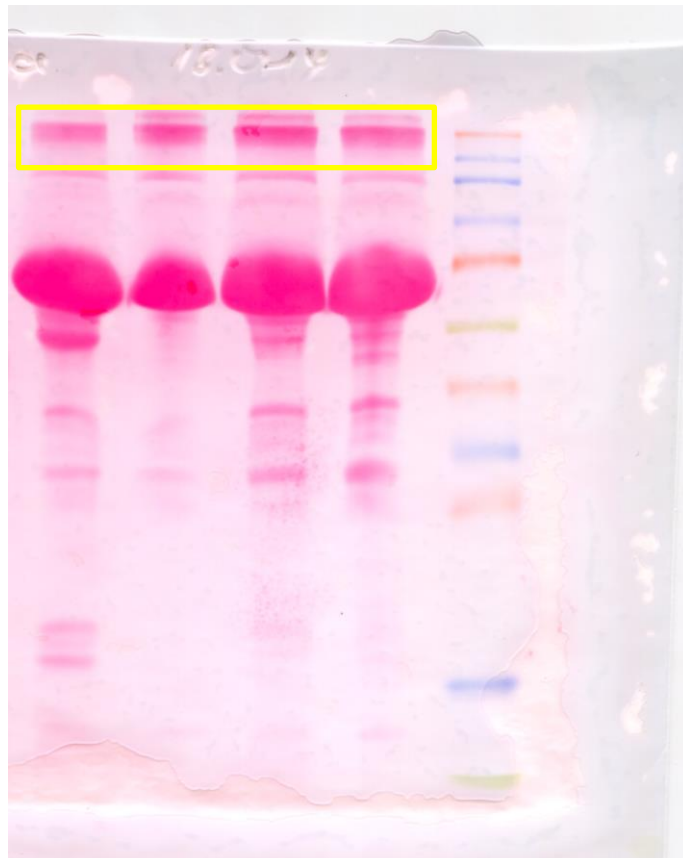


(Pro-)Caspase-1

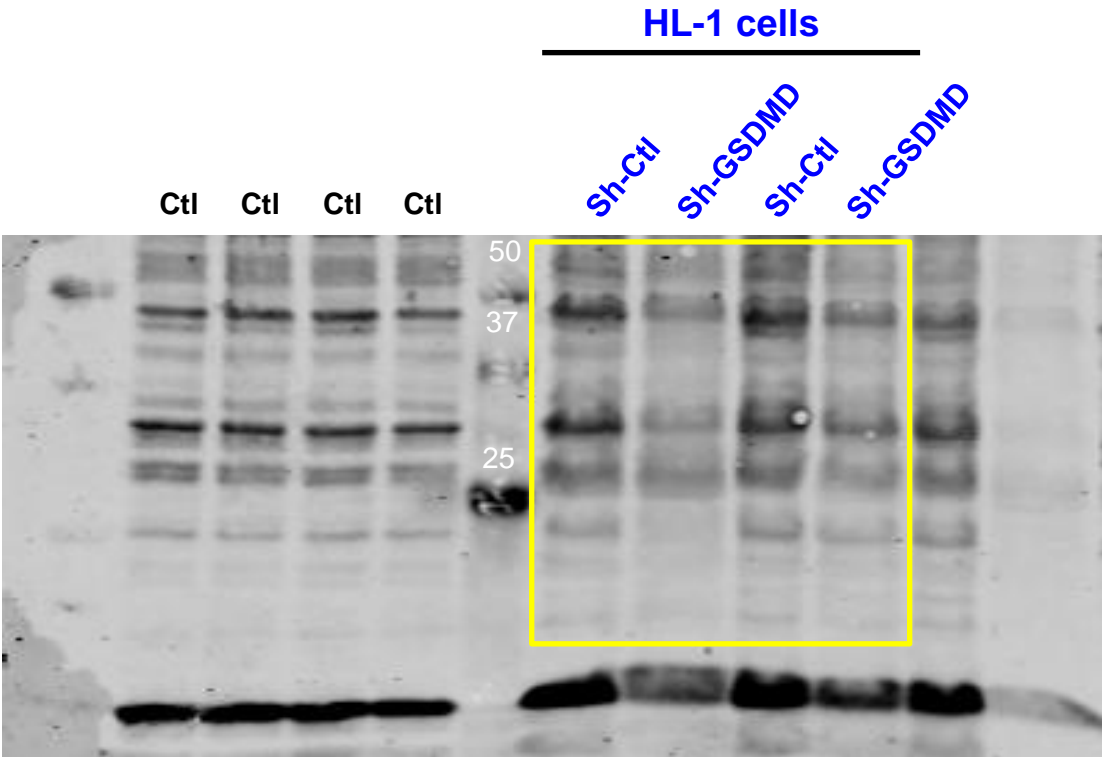
Ctl Ctl POAF POAF



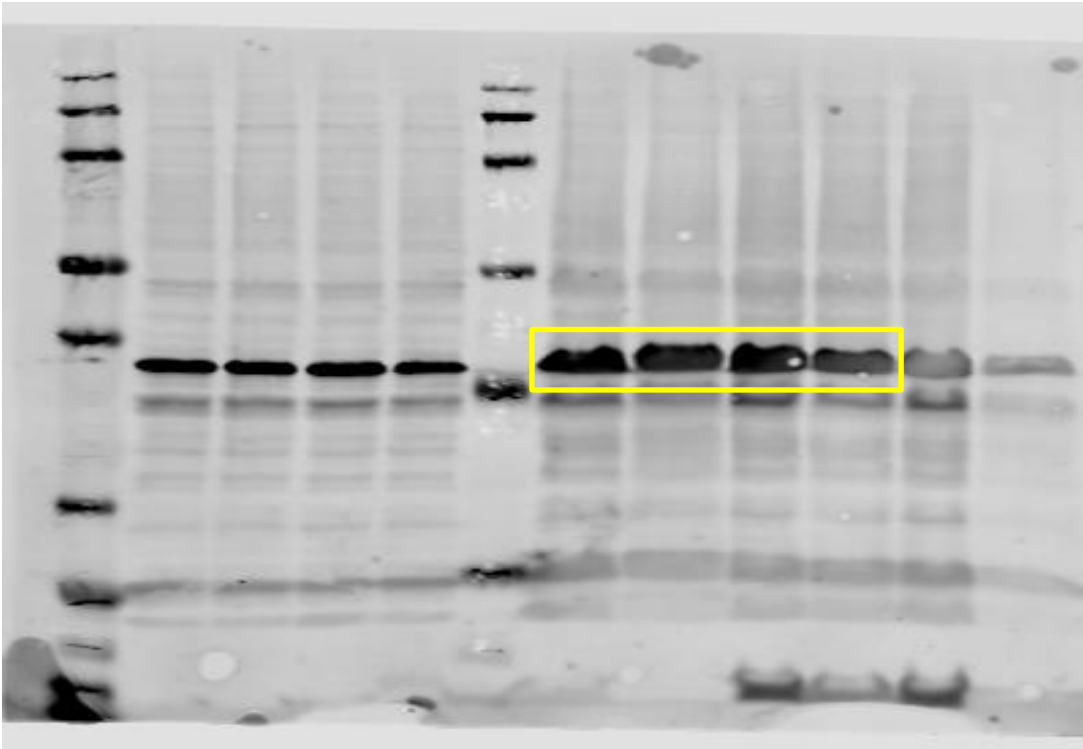
Ponceau



Gasdermin-D



GAPDH

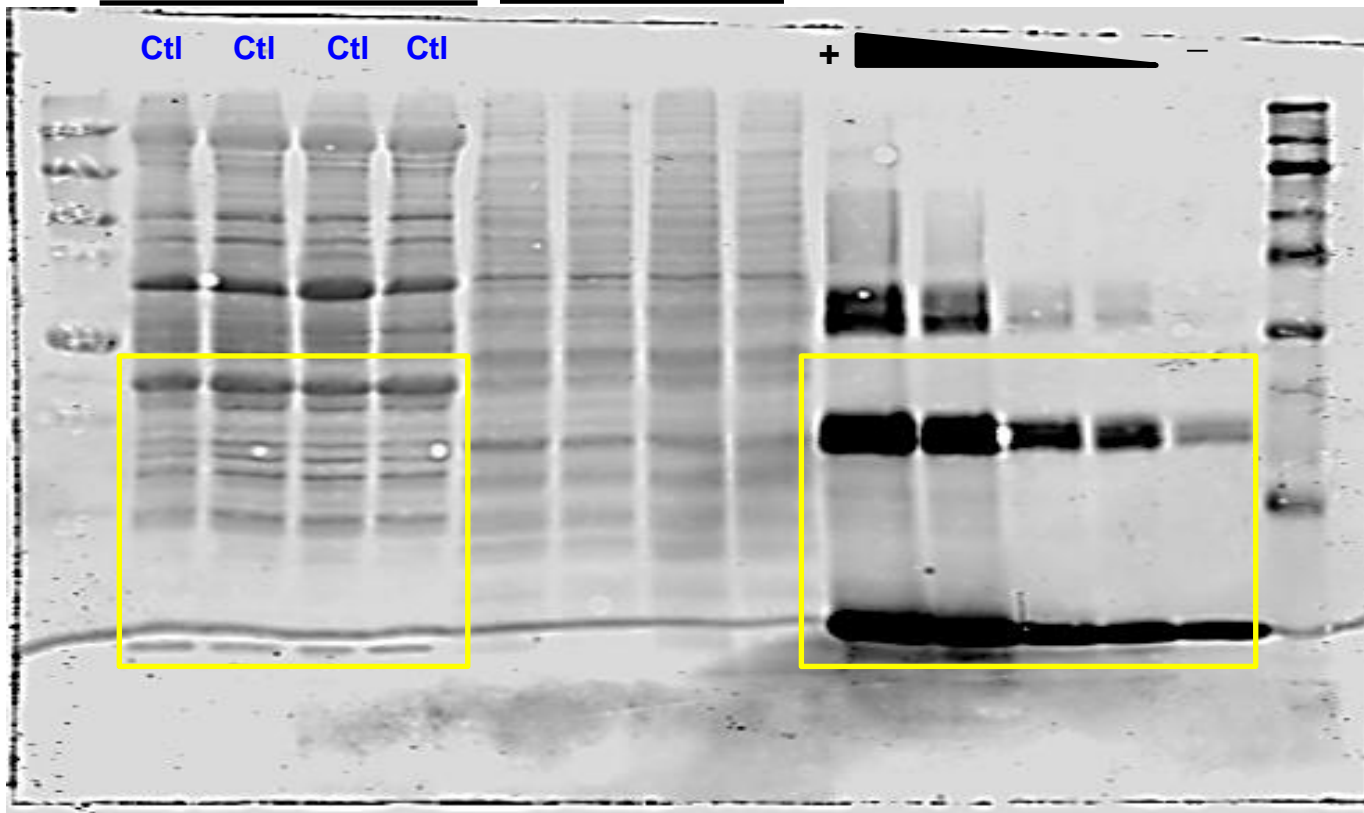


(Pro-)Interleukin-1 β

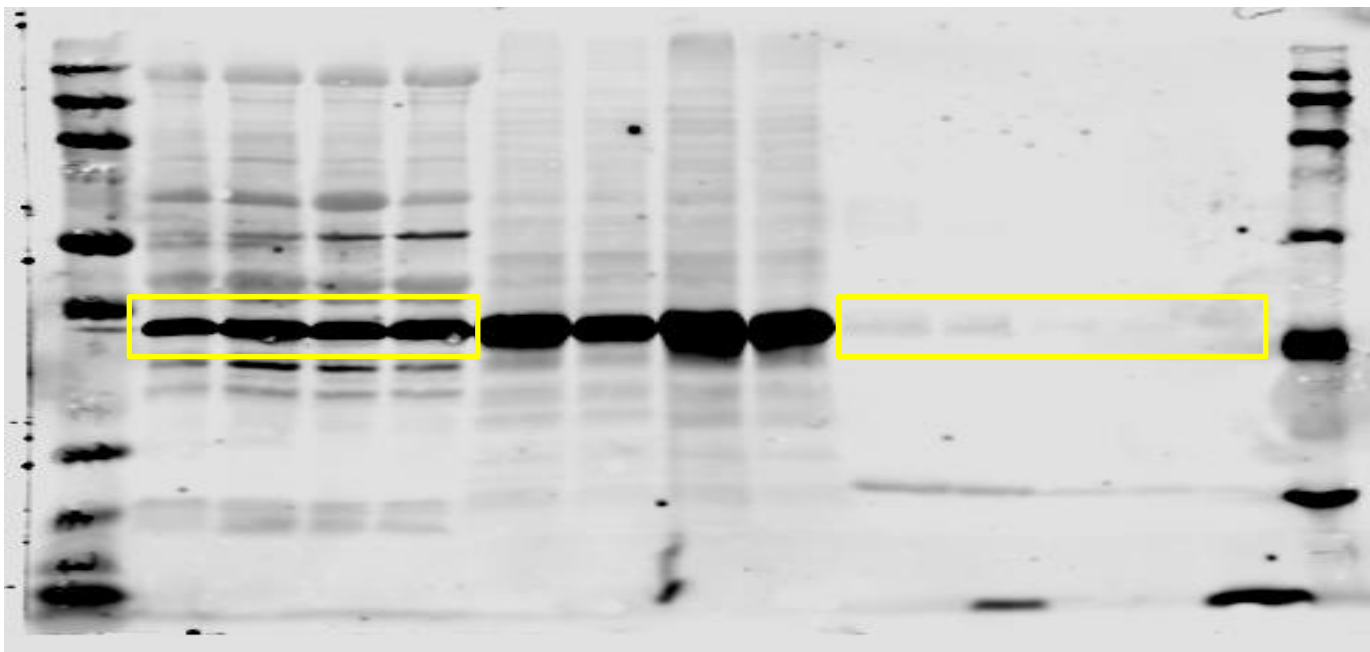
Human atrial whole-tissue lysates

HL-1 cells

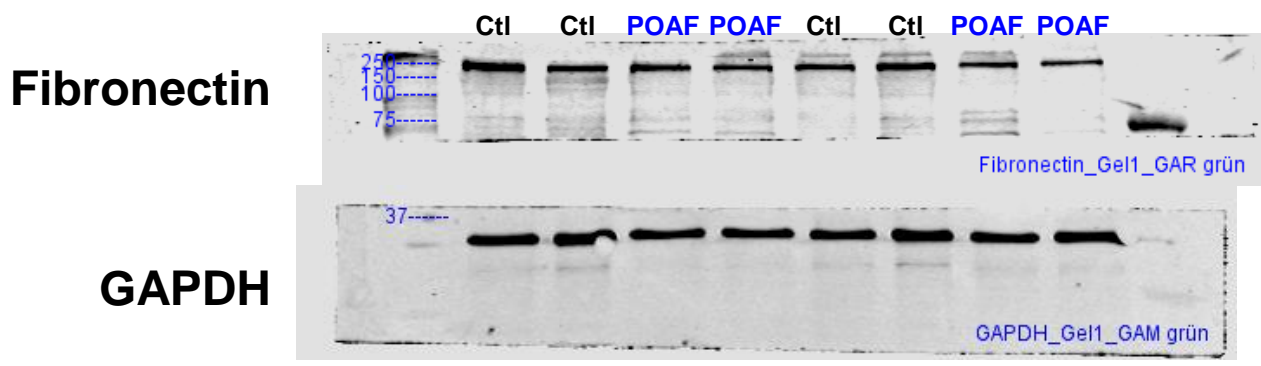
Recombinant IL-1 β



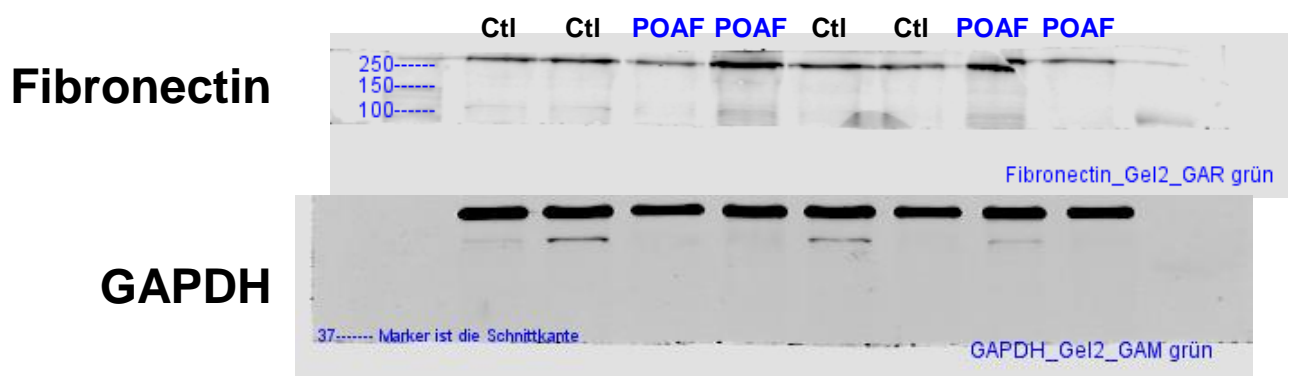
GAPDH



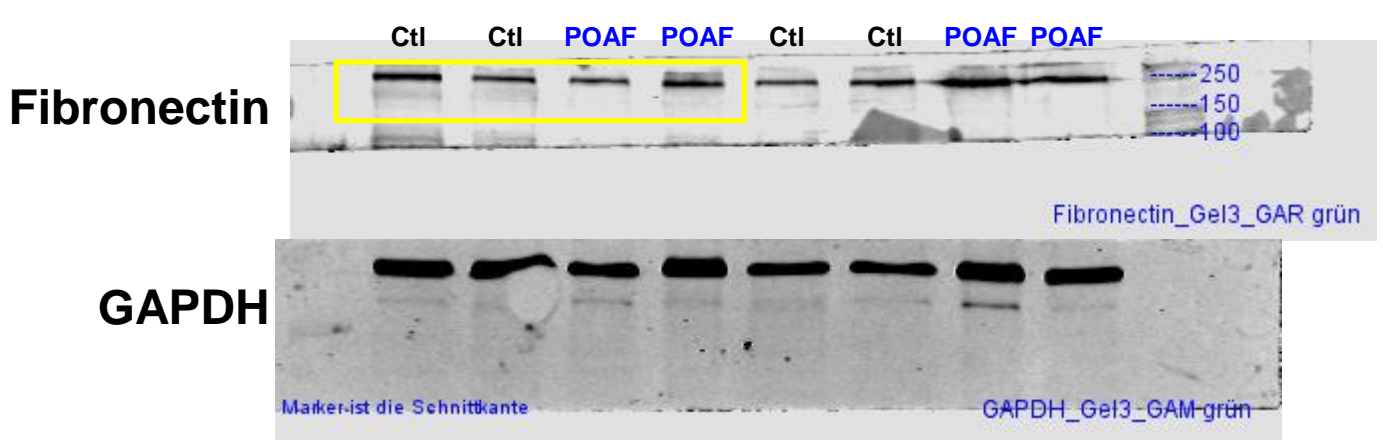
Gel 1 / 3



Gel 2 / 3



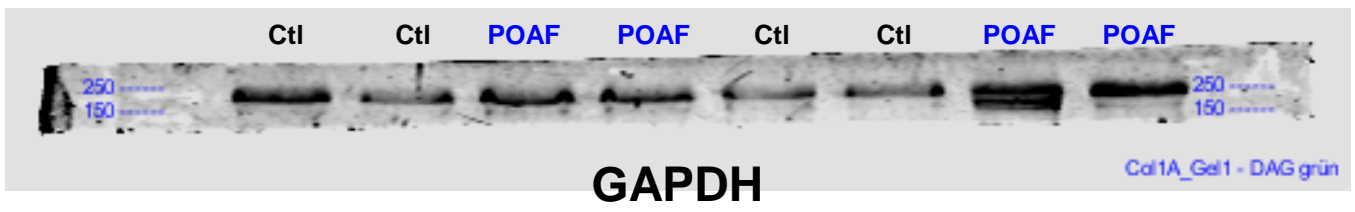
Gel 3 / 3



Online Figure XIII – Human atrial whole-tissue lysates

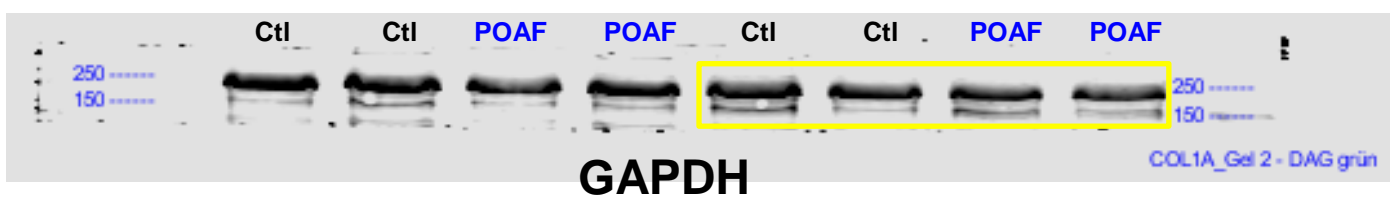
Gel 1 / 3

Collagen-1a



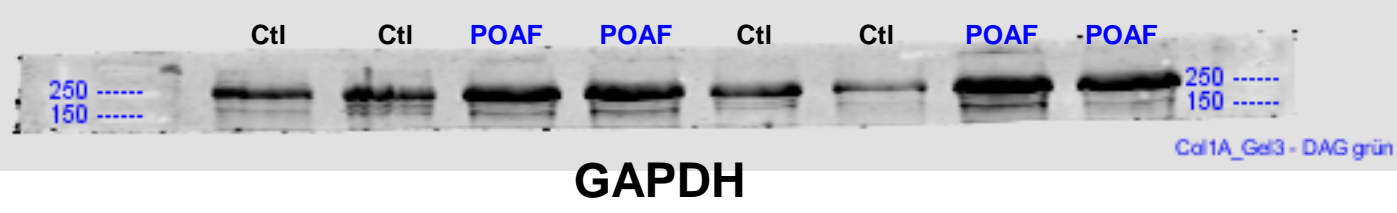
Gel 2 / 3

Collagen-1a

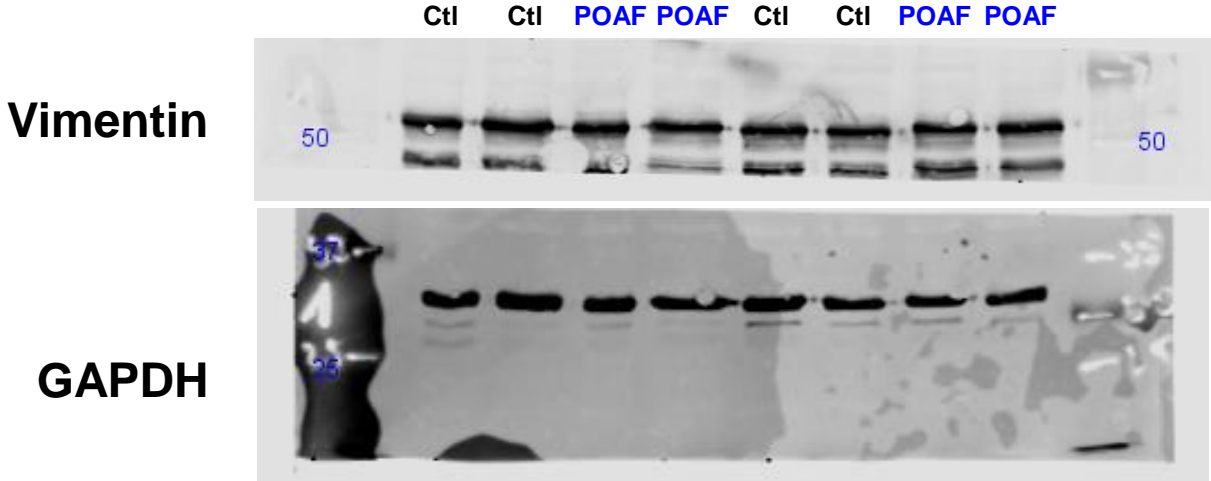


Gel 3 / 3

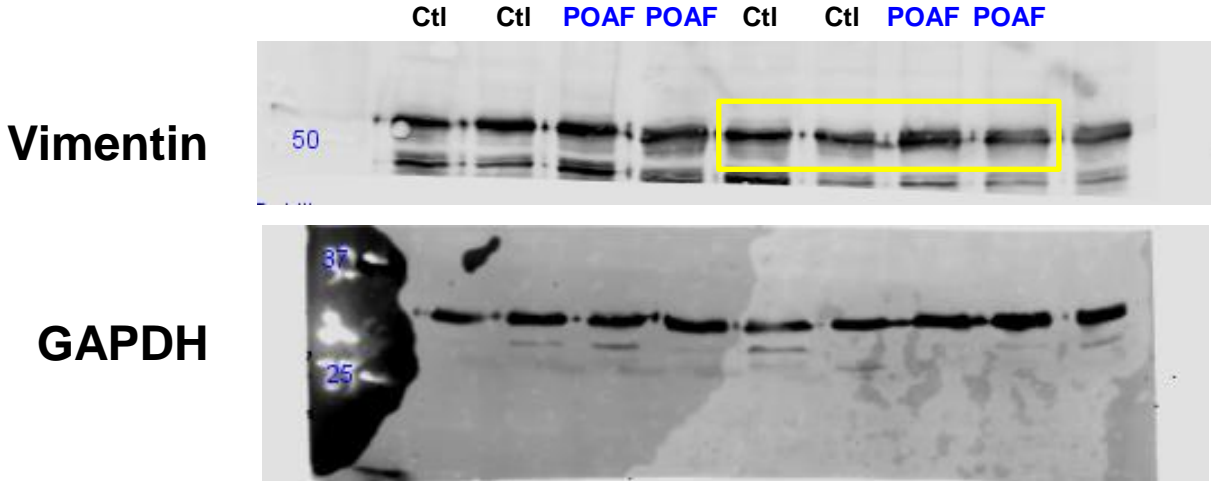
Collagen-1a



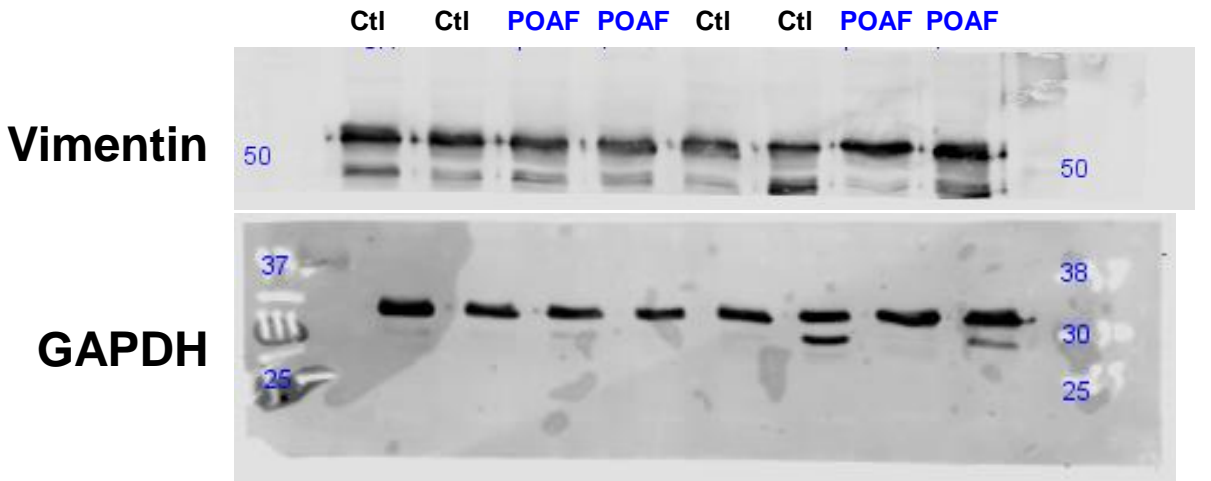
Gel 1 / 3



Gel 2 / 3

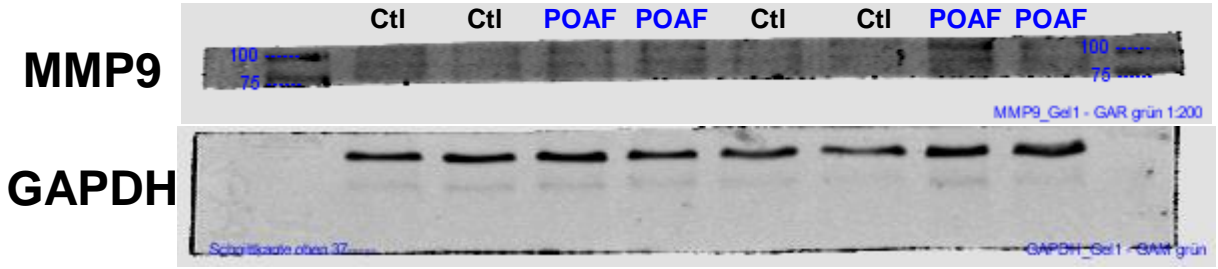


Gel 3 / 3

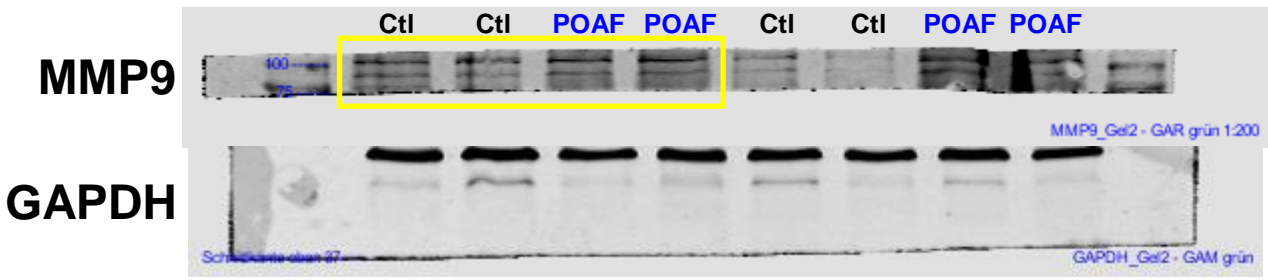


Online Figure XIII – Human atrial whole-tissue lysates

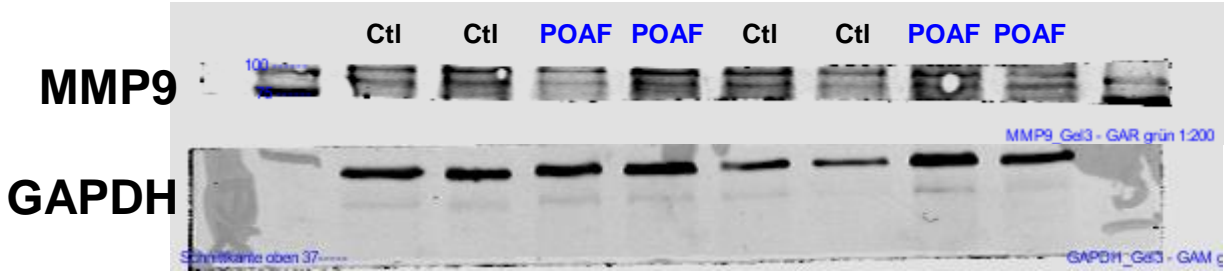
Gel 1 / 3



Gel 2 / 3



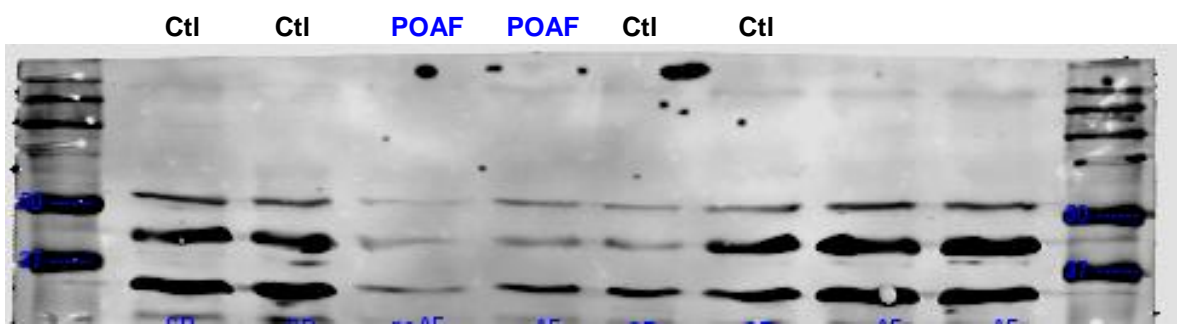
Gel 3 / 3



Online Figure XIII – Human atrial whole-tissue lysates

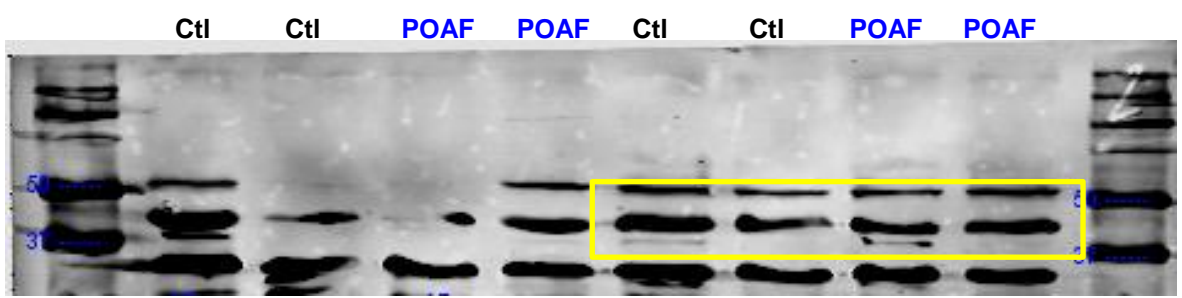
Gel 1 / 2

α -SMA



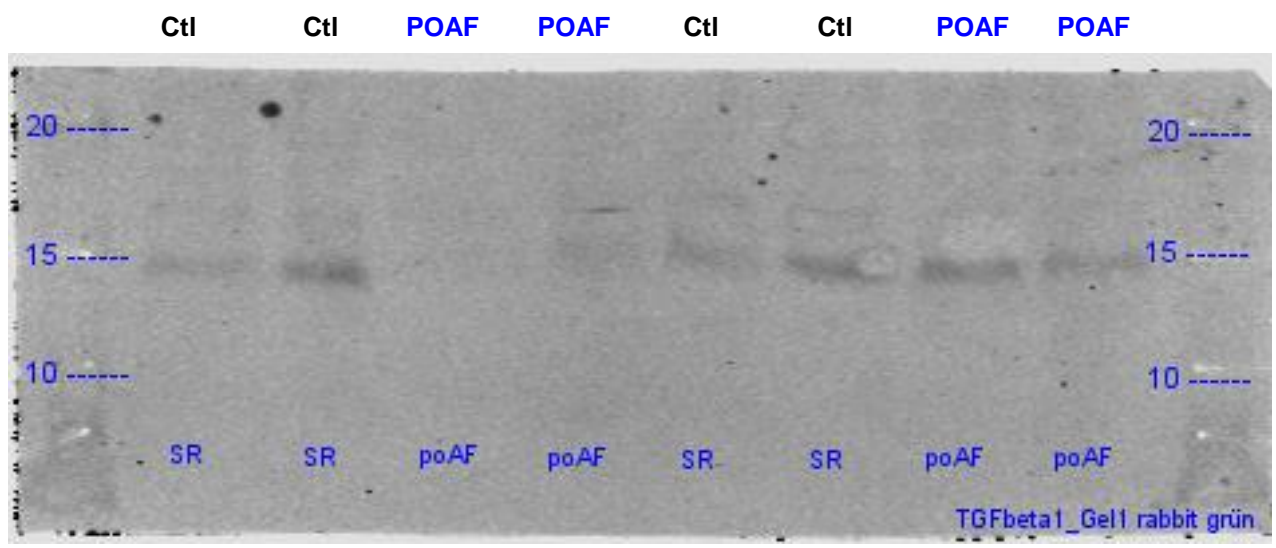
Gel 2 / 2

α -SMA

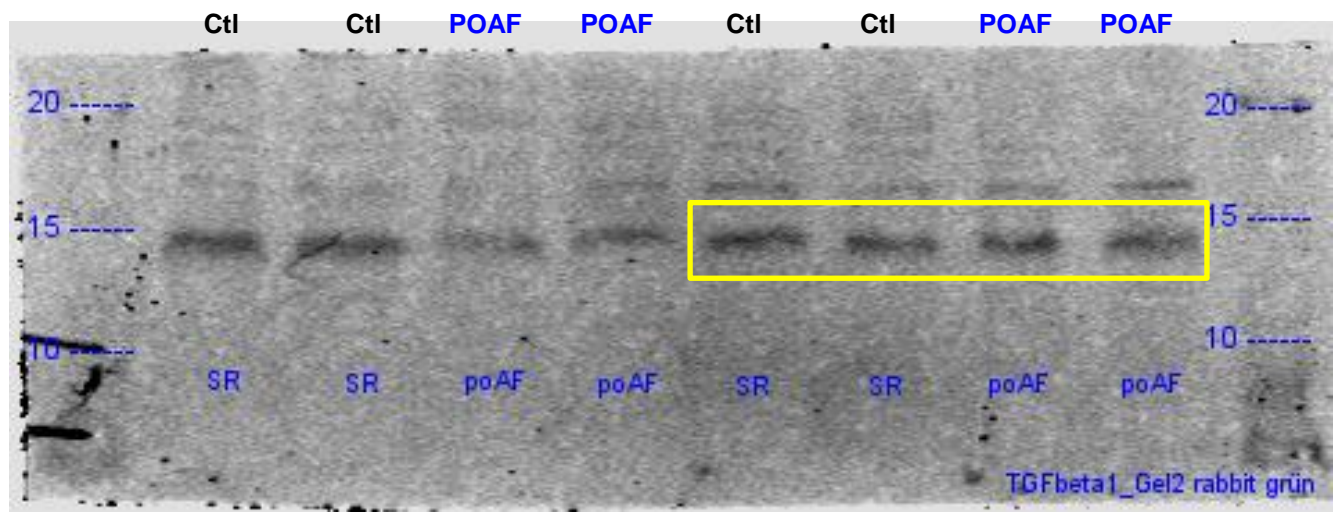


Online Figure XIII – Human atrial whole-tissue lysates

TGF-β1

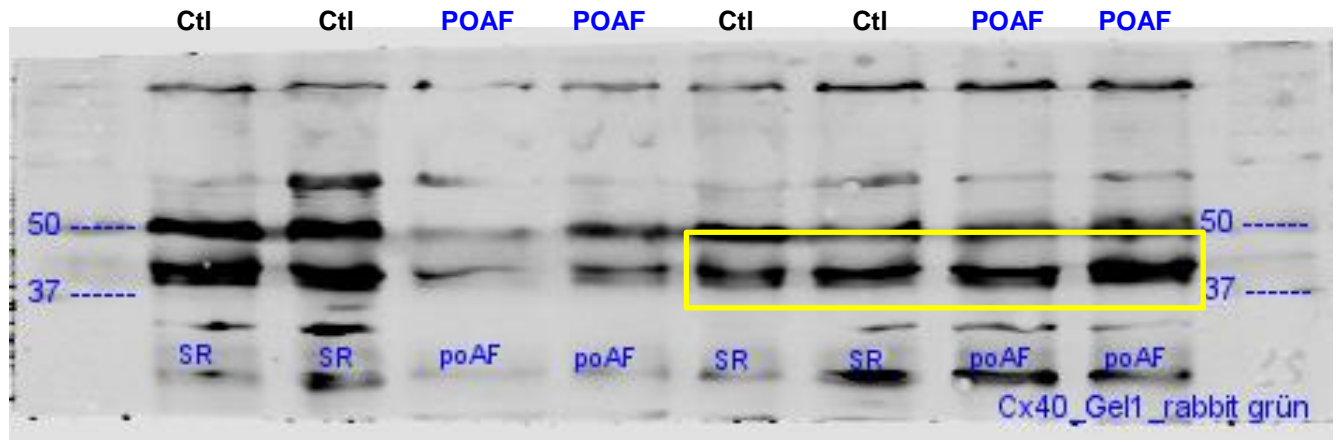


TGF-β1



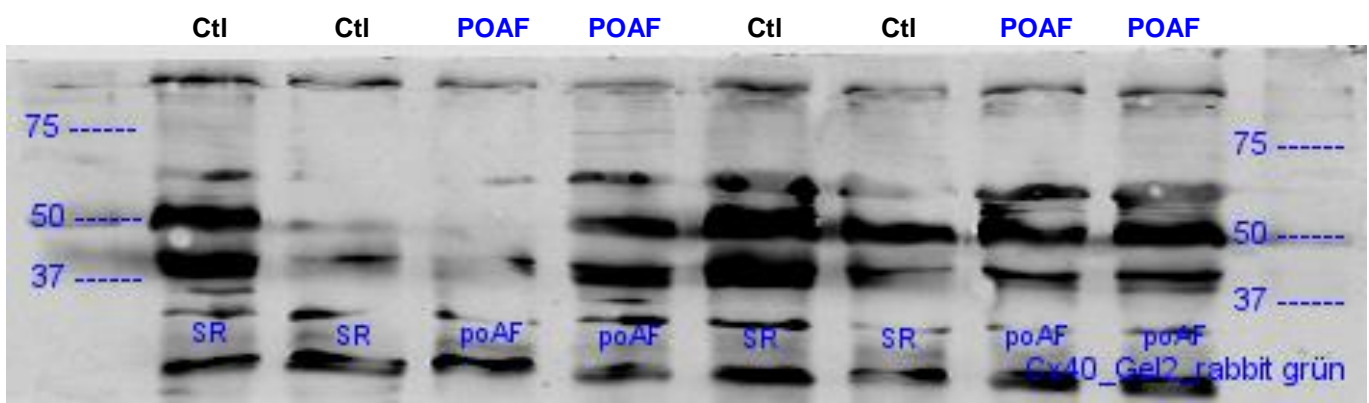
Gel 1 / 2

Total-Connexin-40



Gel 2 / 2

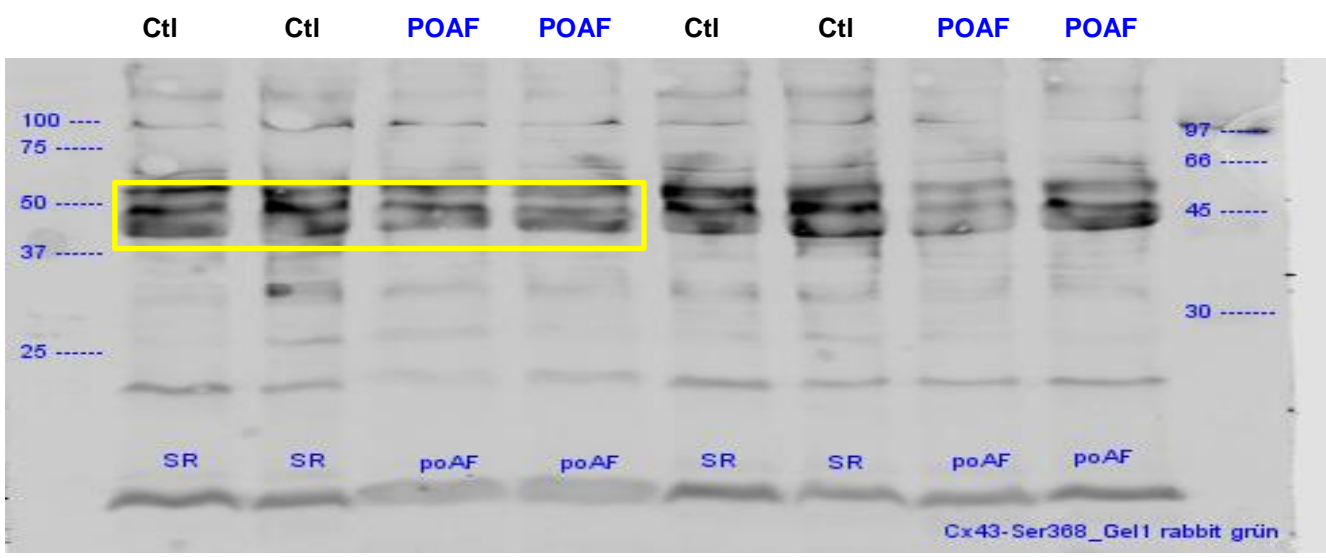
Total-Connexin-40



Online Figure XIV – Human atrial whole-tissue lysates

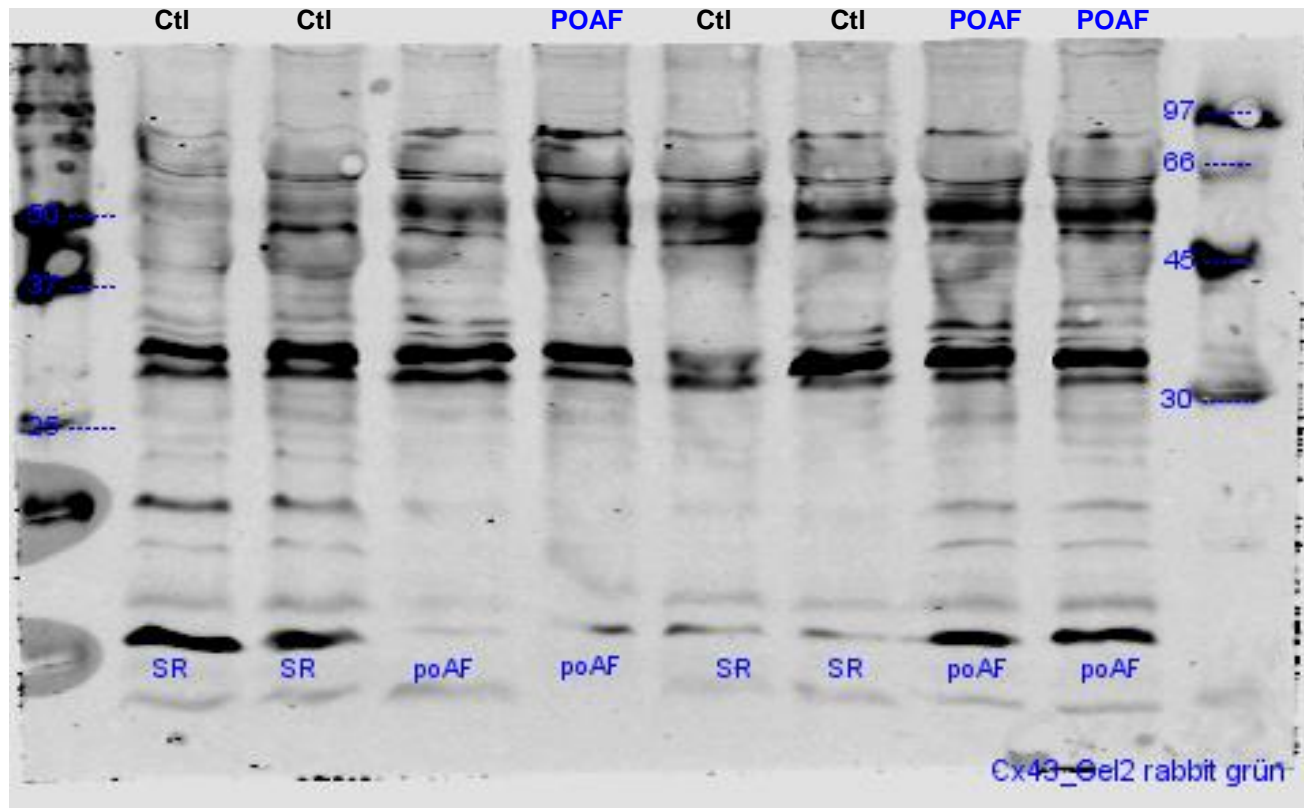
Gel 1 / 2

Connexin-43



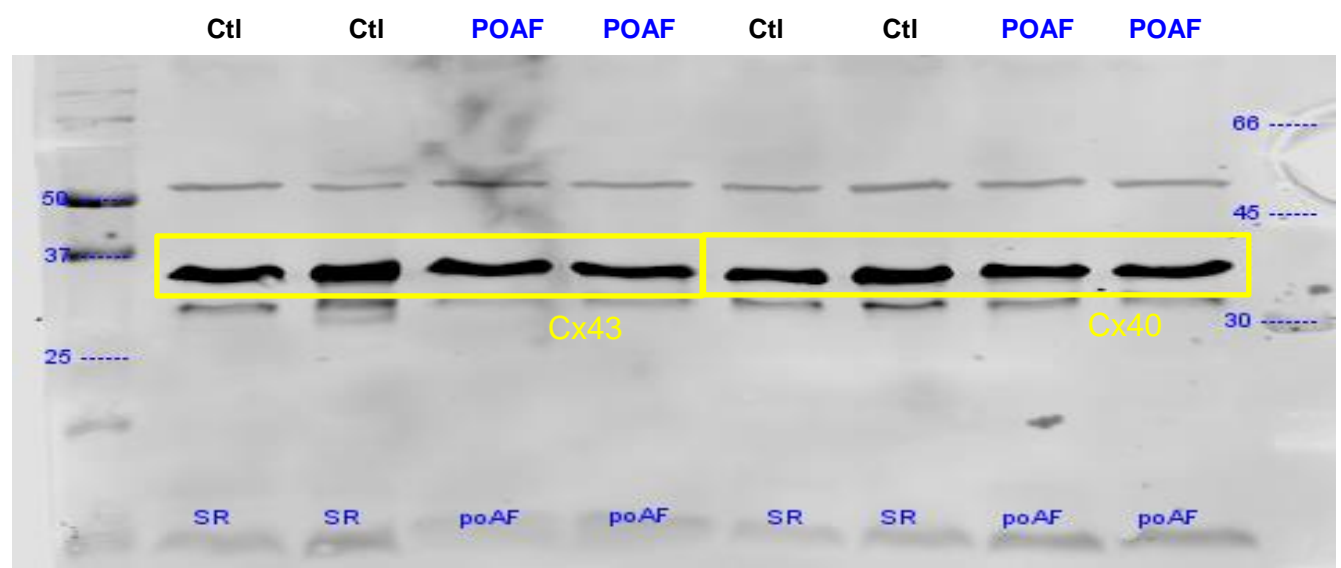
Gel 2 / 2

Connexin-43



Online Figure XIV – Human atrial whole-tissue lysates

GAPDH



GAPDH

