

Gel Images

FoxO1-zDHH4 axis increases CD36 S-acylation driving metabolic dysfunction in the type 2 diabetic heart

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⁵Division of Experimental Cardiology, Department of Cardiology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands.

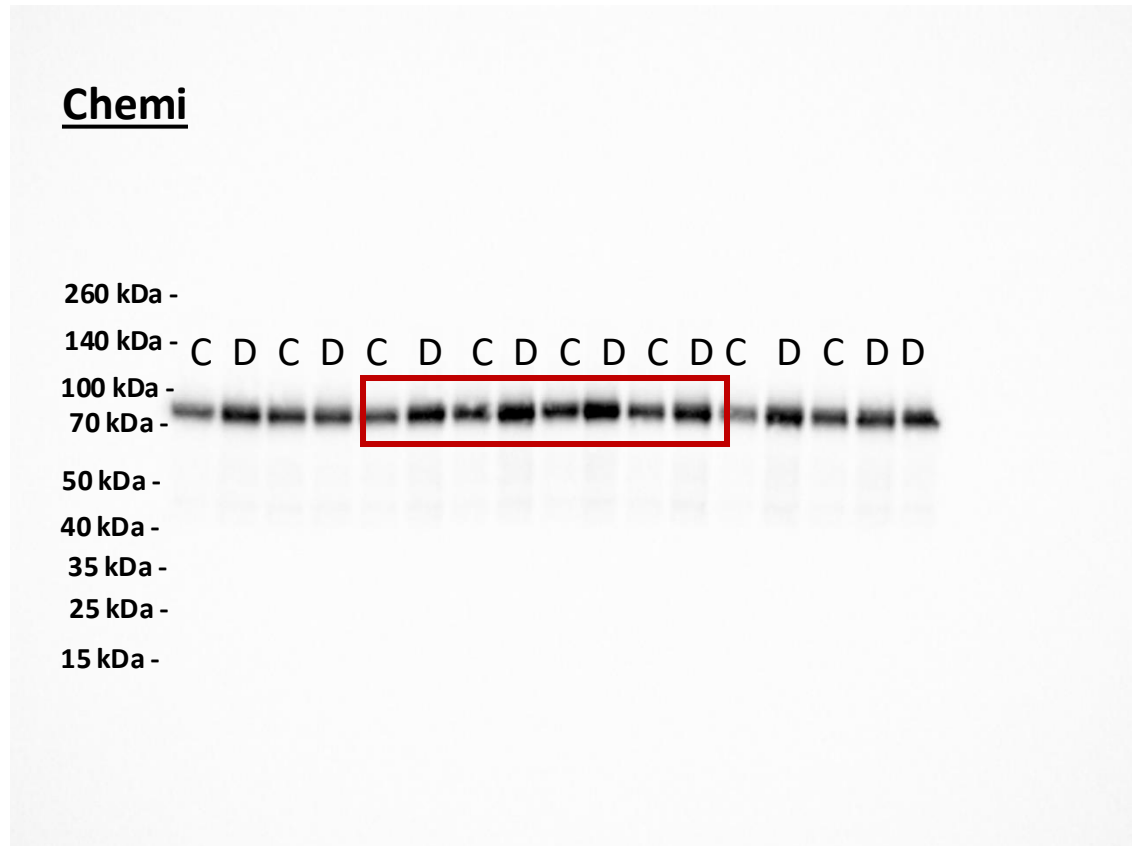
⁶Turku PET Centre, University of Turku and Turku University Hospital, Turku, Finland.

⁷Department of Genetics and Cell Biology, Faculty of Health, Medicine and Life Sciences, Maastricht University, 6200 MD Maastricht, The Netherlands.

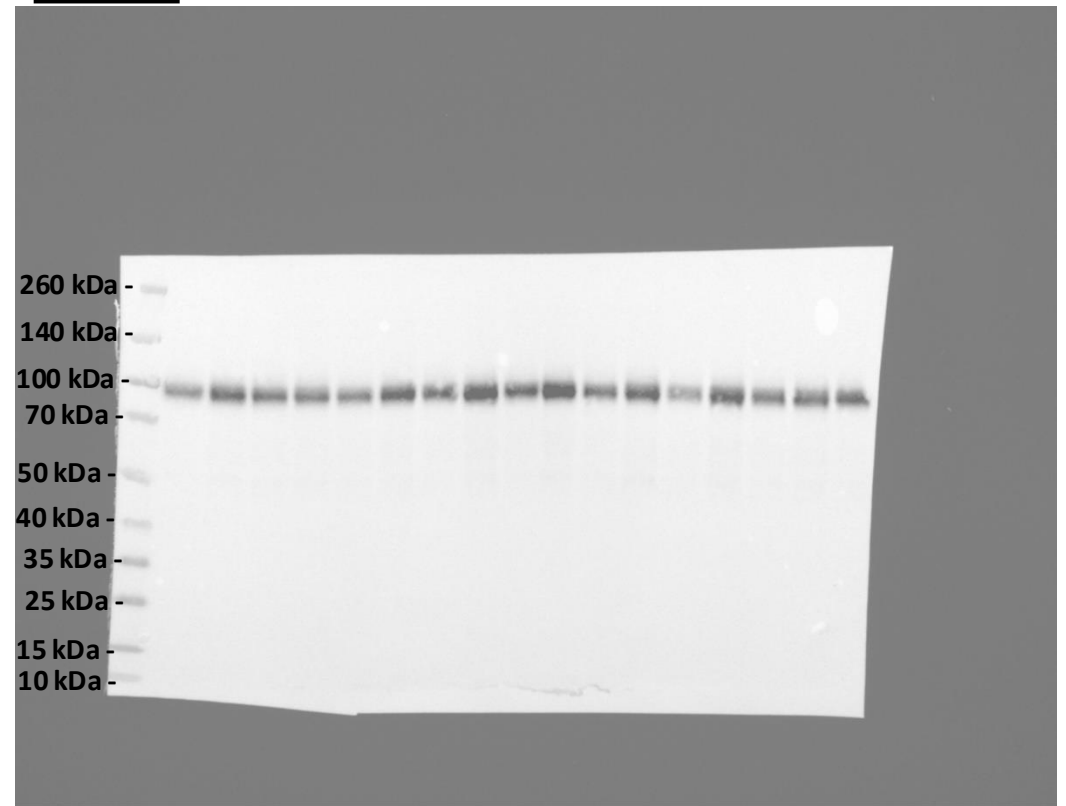
⁸William Harvey Research Institute, Barts and the London Faculty of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom.

⁹School of Cardiovascular and Metabolic Health, University of Glasgow, United Kingdom.

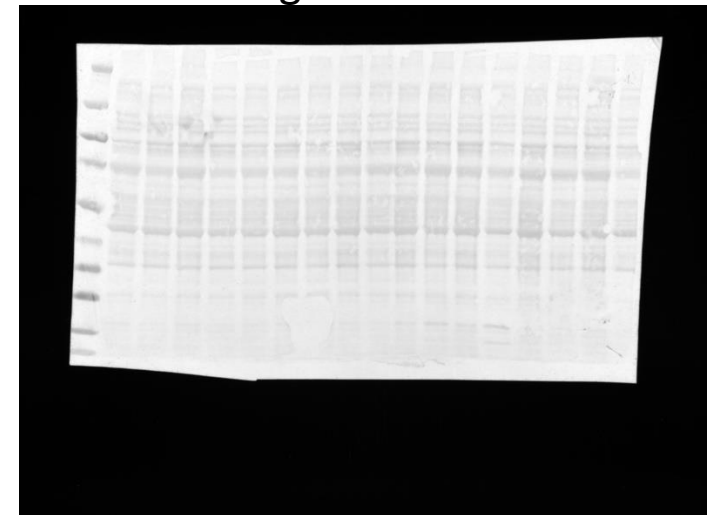
Figure 1 D: Total CD36



Marker



Ponceau image of membrane

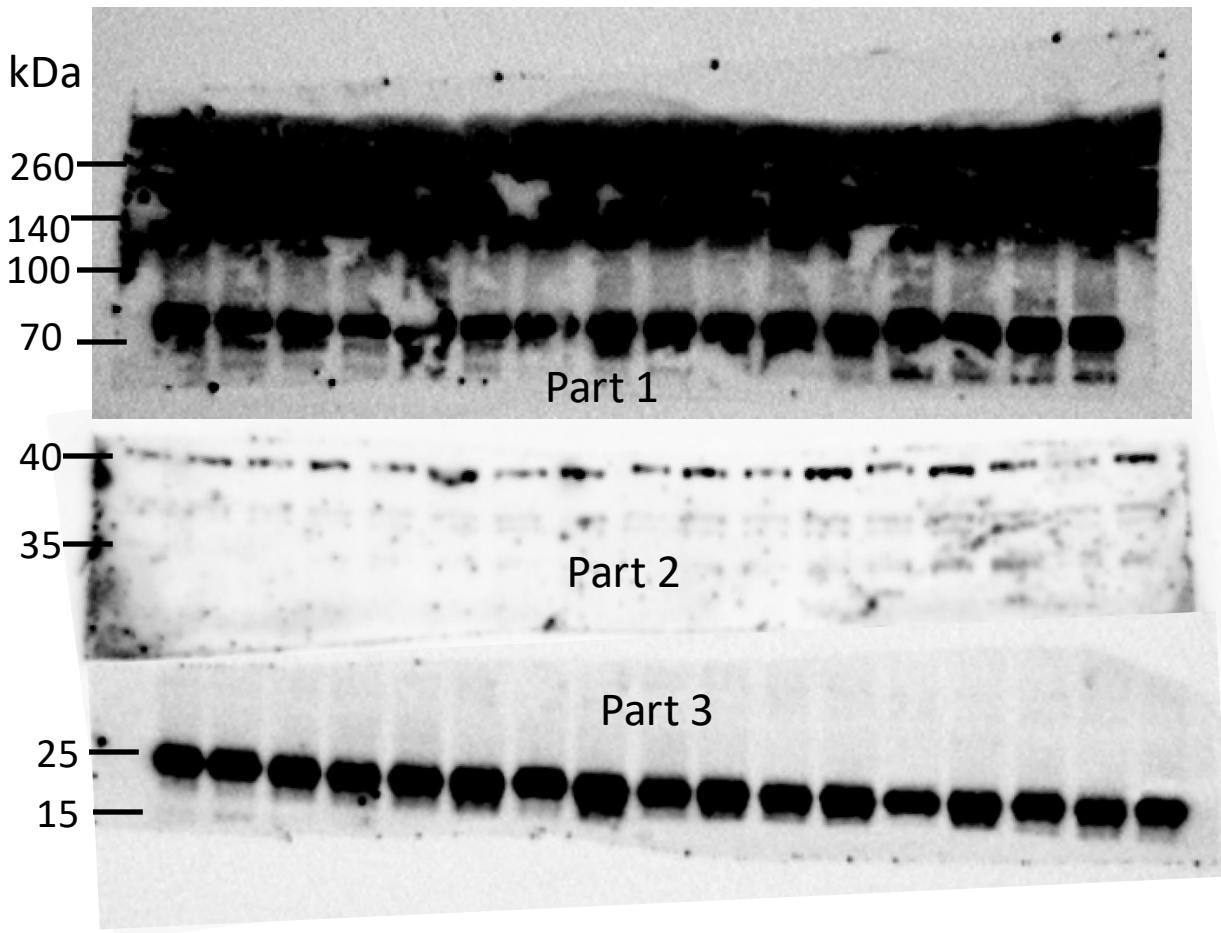


Uncropped gel and membrane – only one band for CD36 at 88kDa

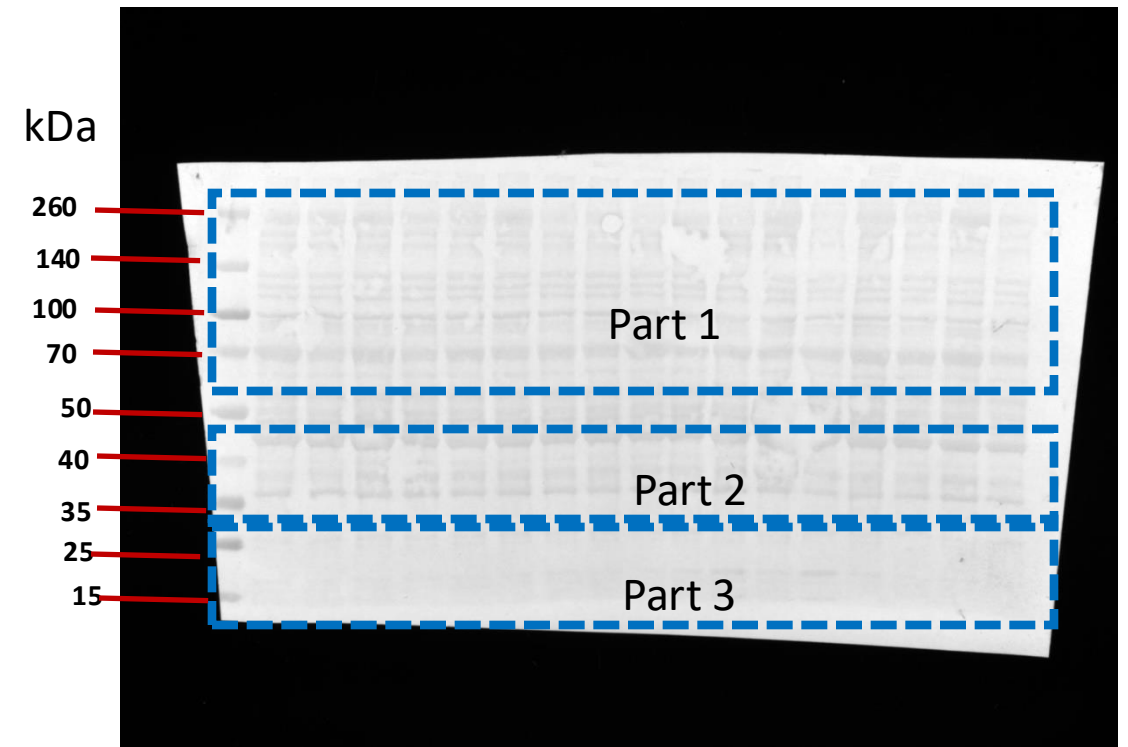
Figure 3:

zDHHHC4 (44 kDa), APT1 (25 kDa) and zDHHHC5 (78kDa) all cut from the same membrane – same Ponceau housekeeper

Chemi overexposed to show outline of membranes



Markers

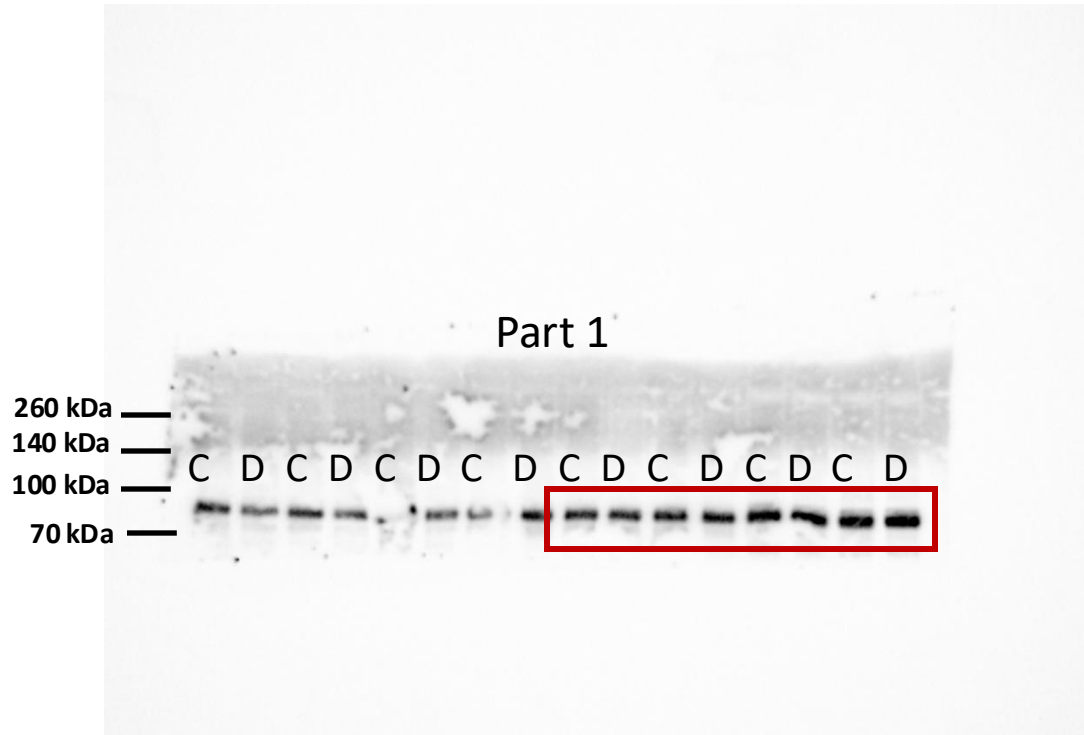


APT1, zDHHHC5 and zDHHHC4 all taken from the same membrane, which was cut to blot for the multiple targets

Figure 3: zDHHC5 (MW 78 kDa)

zDHHC4 (44 kDa), APT1 (25 kDa) and zDHHC5 (78kDa) all cut from the same membrane – same Ponceau housekeeper

Chemi



Markers

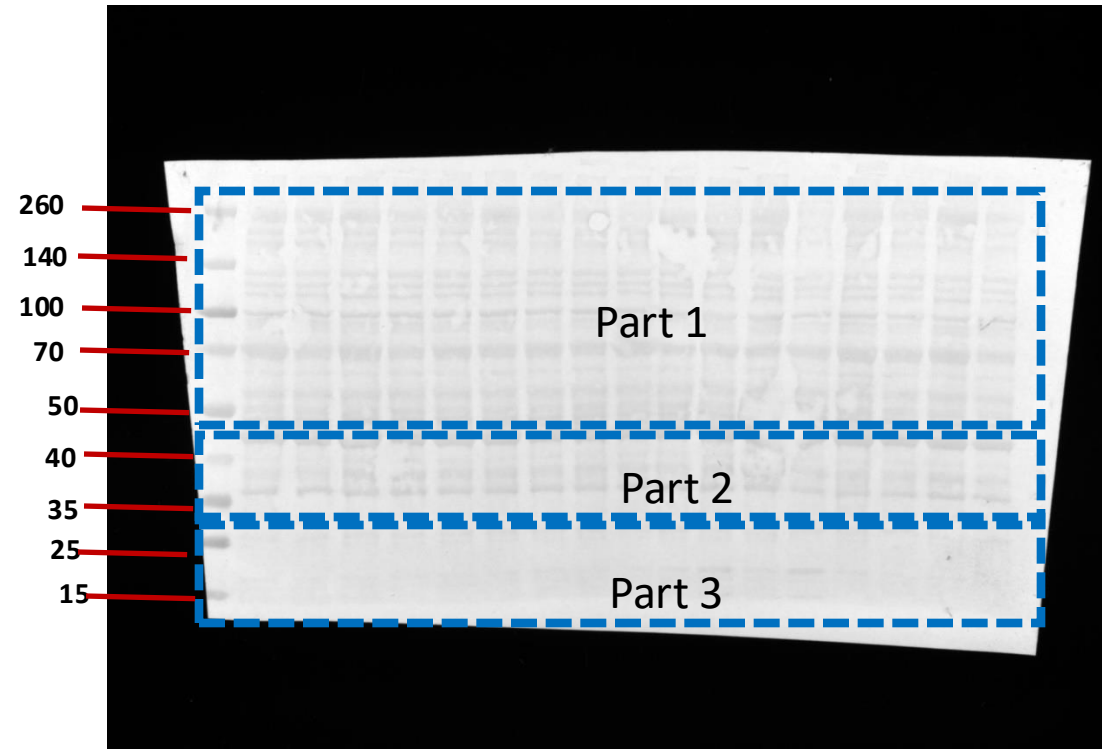
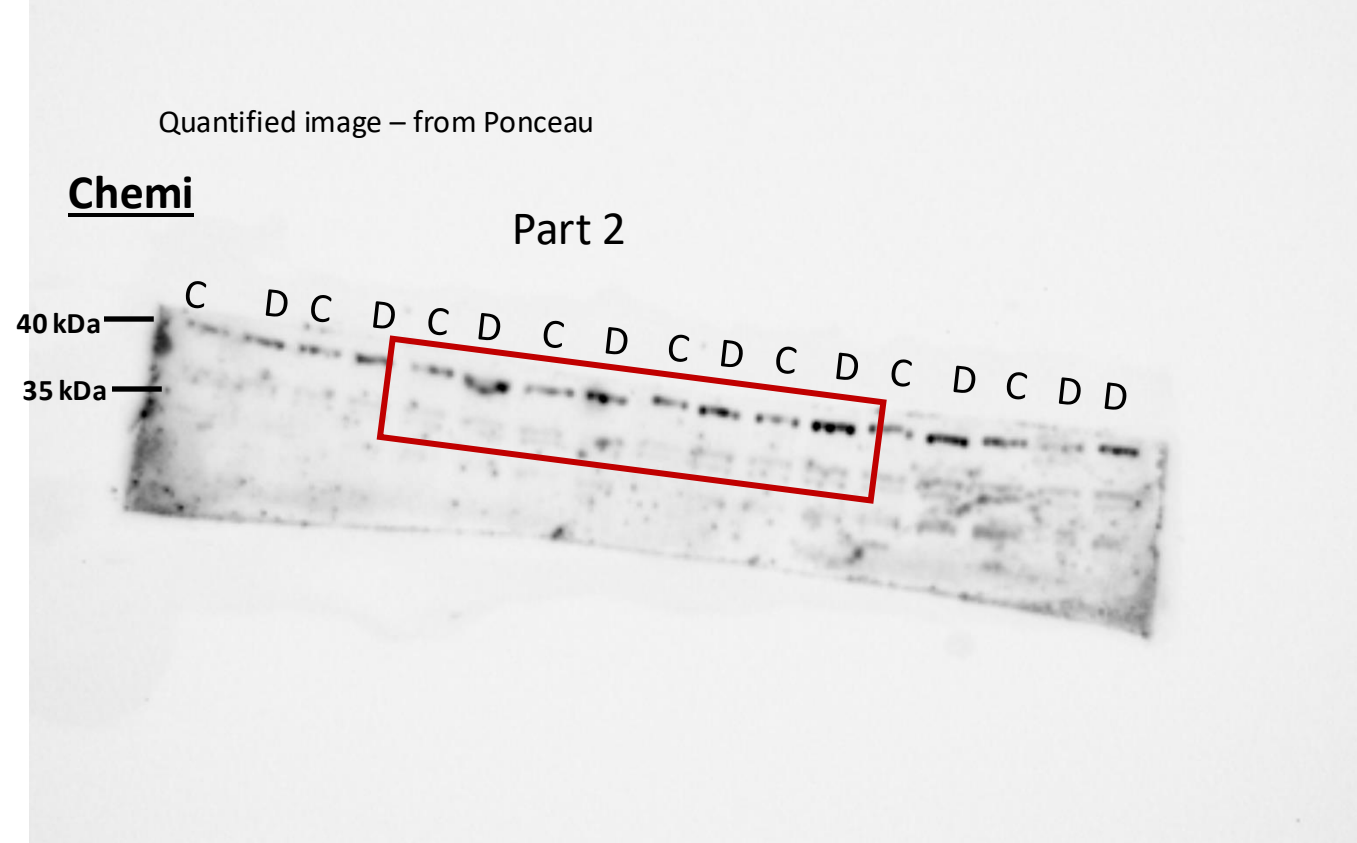


Figure 3: zDHHC4 (MW: 40 kDa)

zDHHC4 (44 kDa), APT1 (25 kDa) and zDHHC5 (78kDa) all cut from the same membrane – same Ponceau housekeeper



Markers

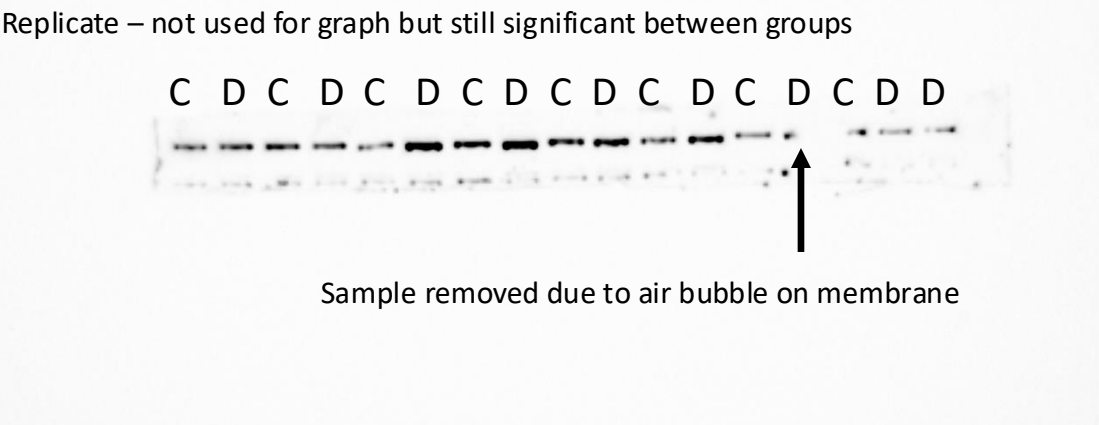
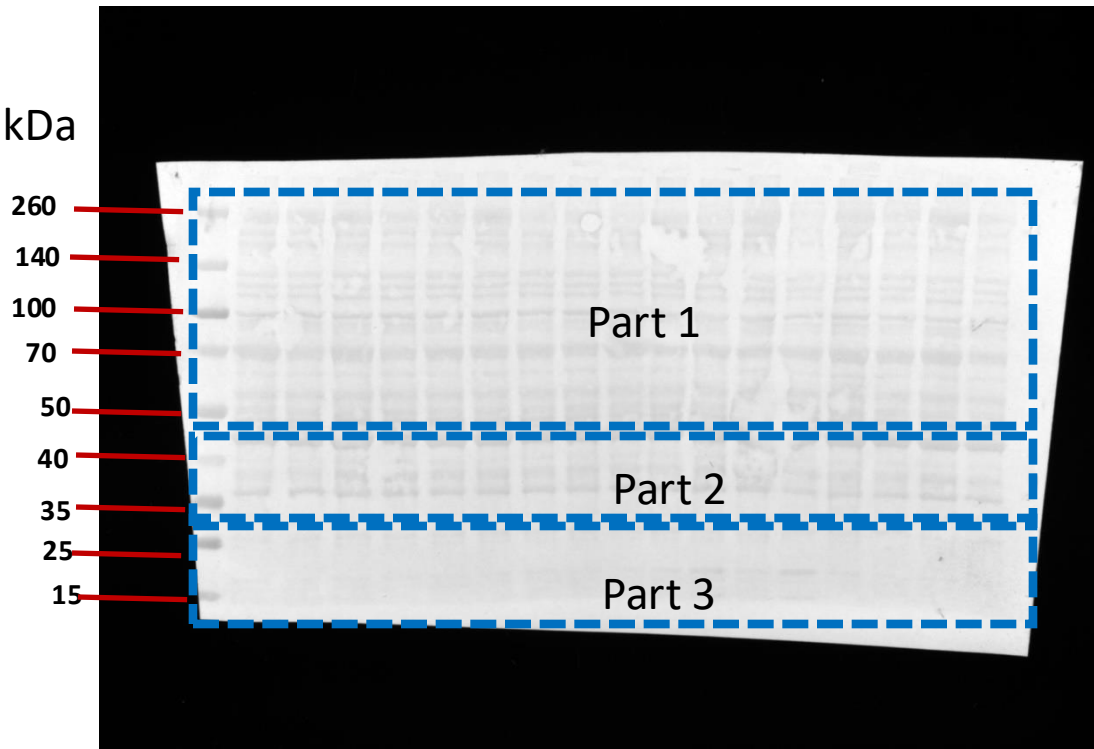
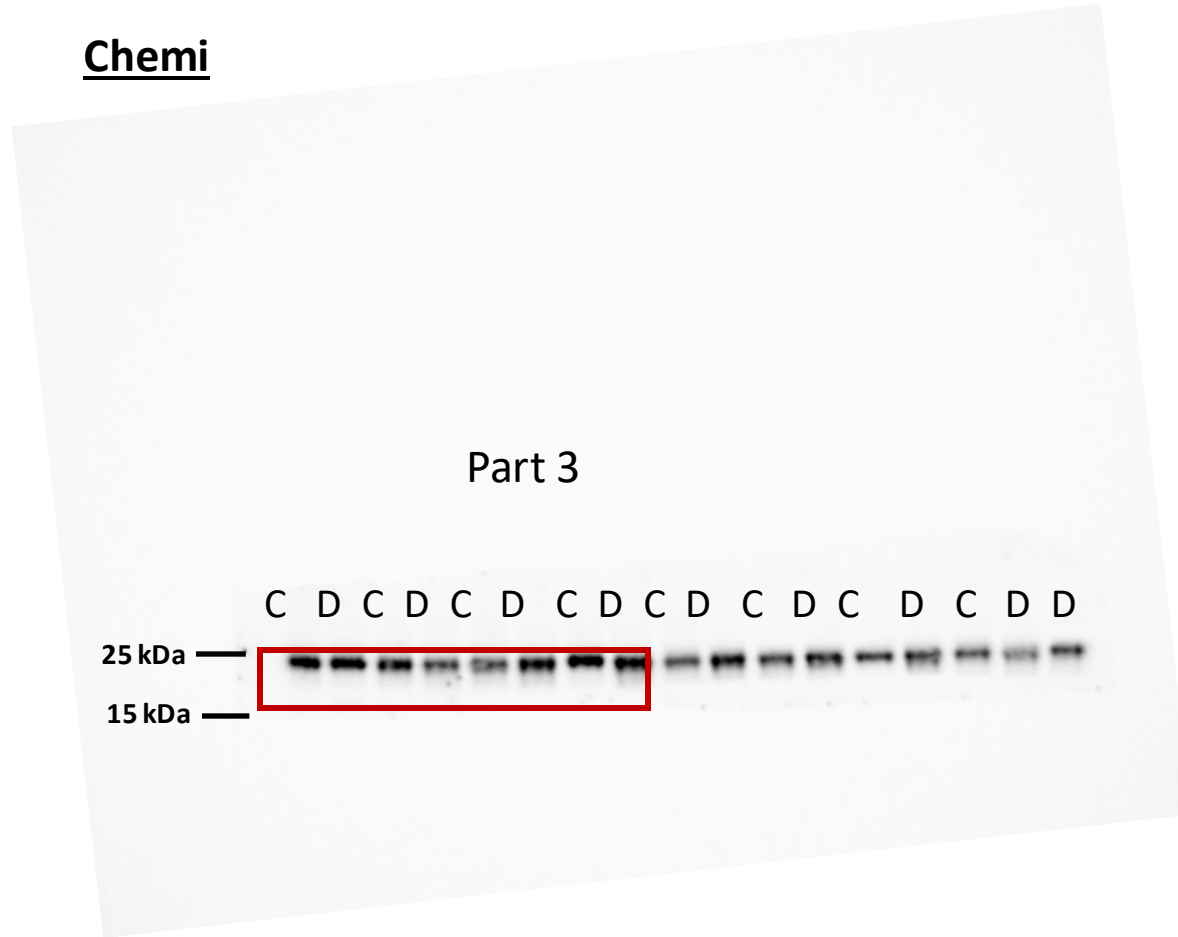


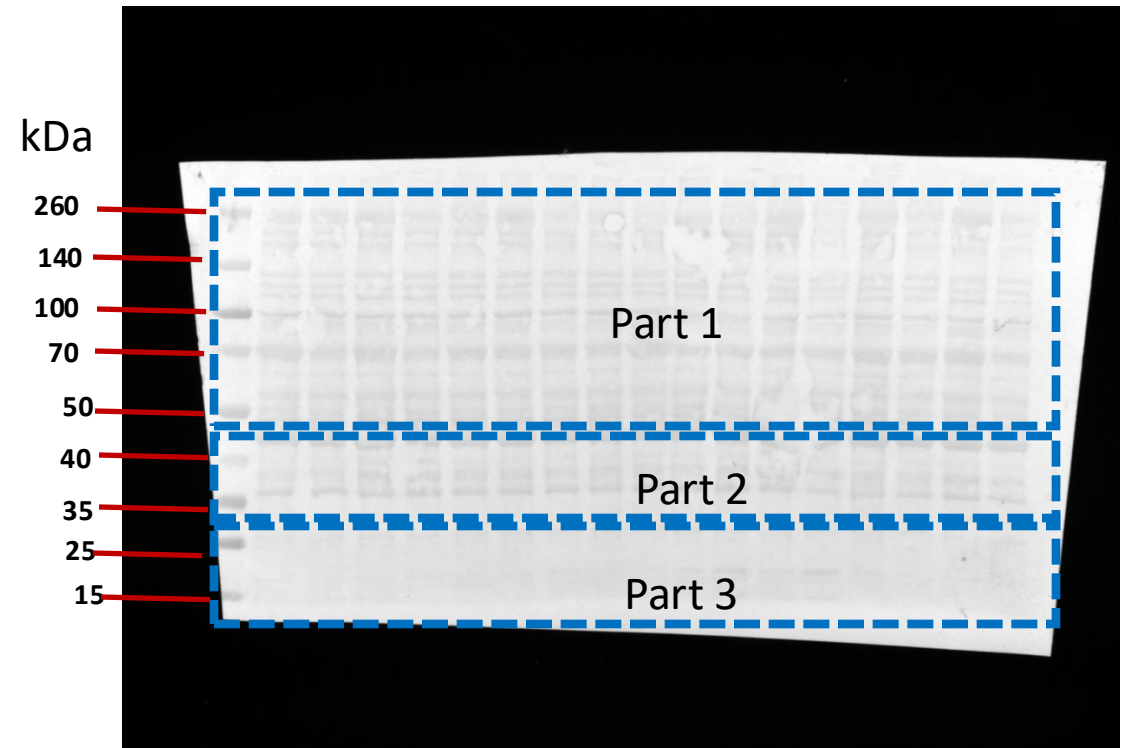
Figure 3: APT1 (MW 25 kDa)

zDHHC4 (44 kDa), APT1 (25 kDa) and zDHHC5 (78kDa) all cut from the same membrane – same Ponceau housekeeper

Chemi



Markers



APT1, zDHHC5 and zDHHC4 all taken from the same membrane, which was cut to blot for the multiple targets

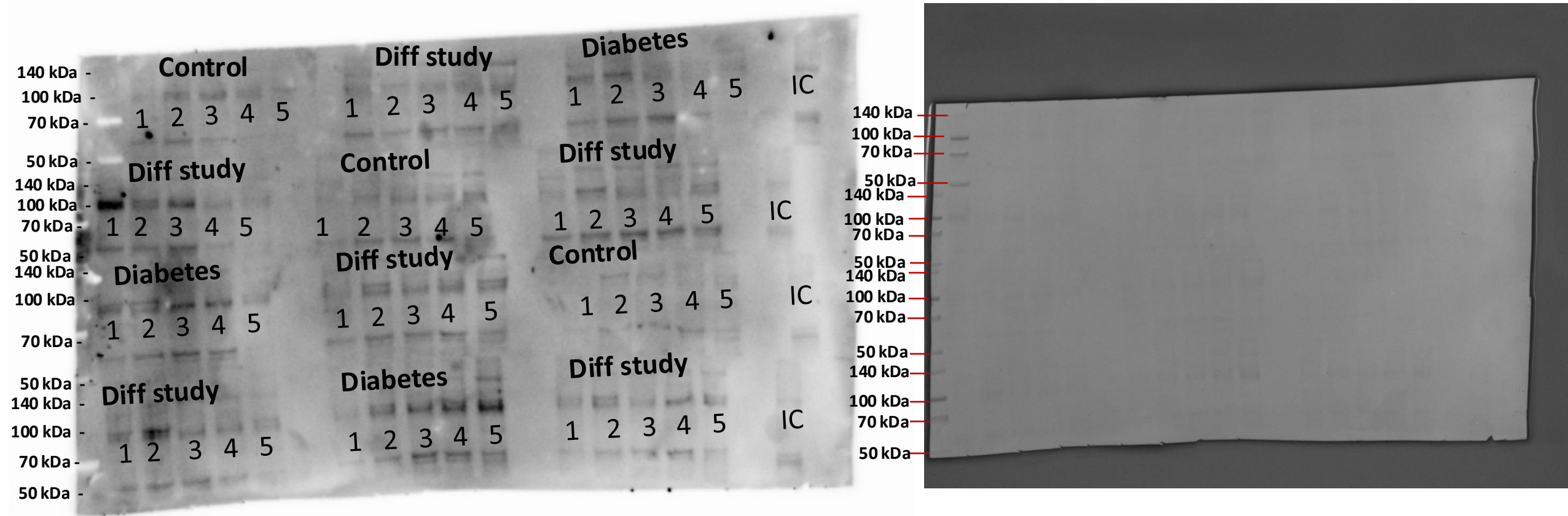
Figure 1 E and F: Sarcolemmal CD36 membrane 1 of 2

4 gels transferred on to one membrane – due to the large number of fractions generated per heart.

Gels cut just above the 140 Mw band and below the 50 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Chemi

Marker



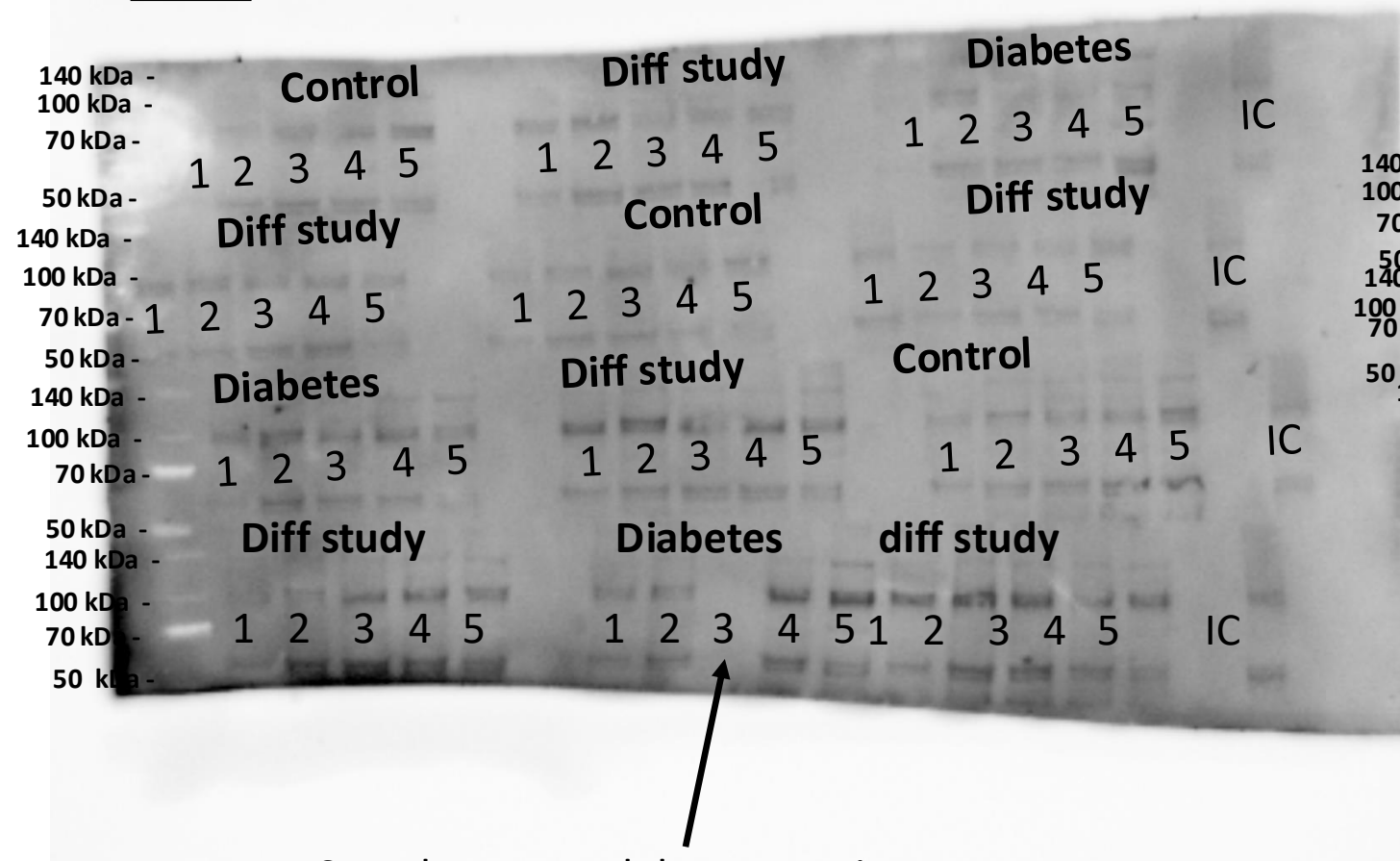
“Diff Study” = samples analysed for a different study

Figure 1 E and F: Sarcolemmal CD36 membrane 2 of 2

4 gels transferred on to one membrane – due to the large number of fractions generated per heart.

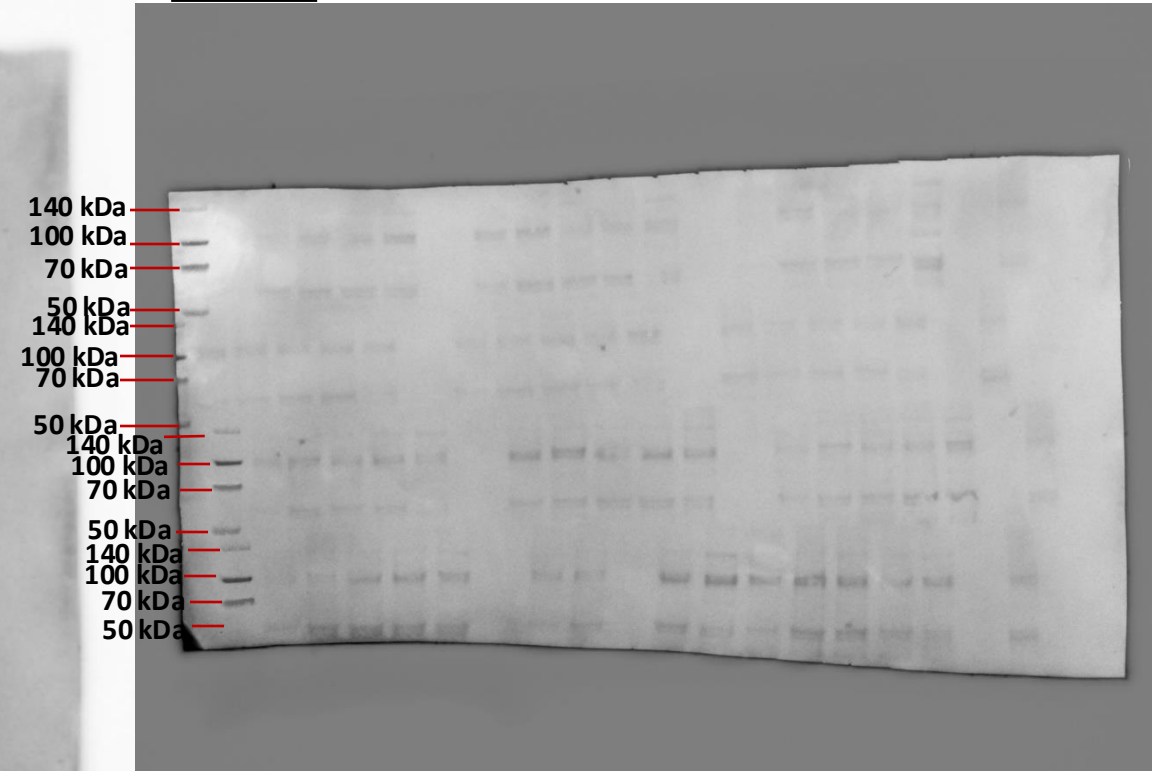
Gels cut just above the 140 Mw band and below the 50 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Chemi



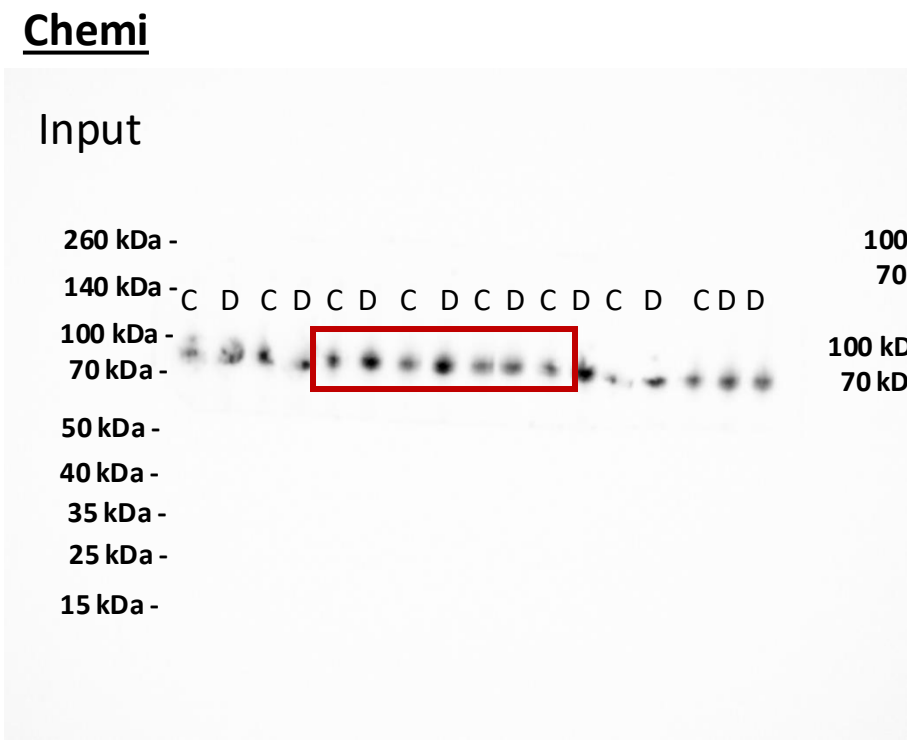
Sample removed due extraction error as no protein present in P3

Marker

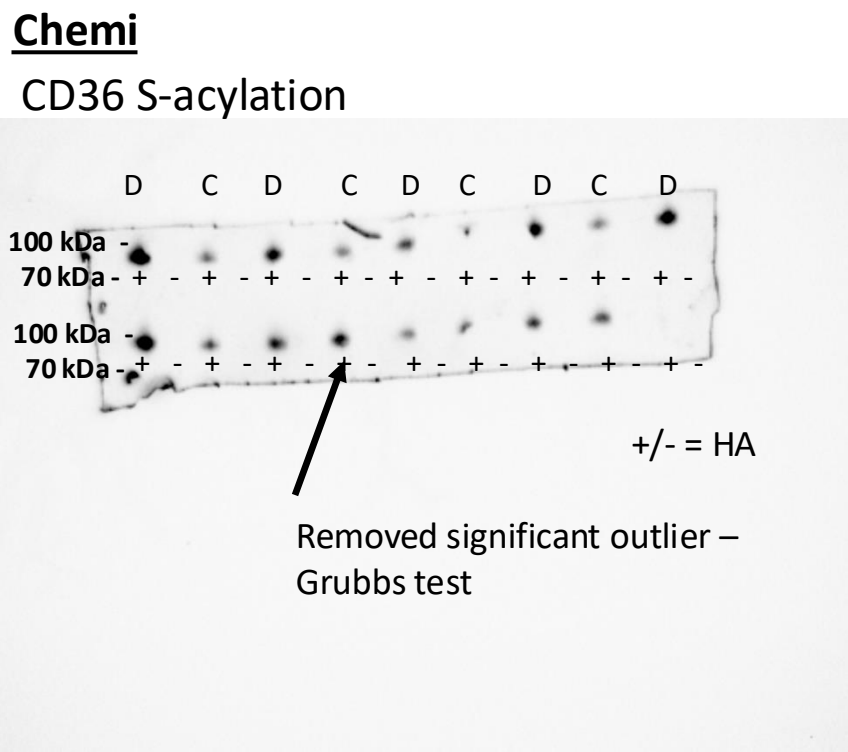
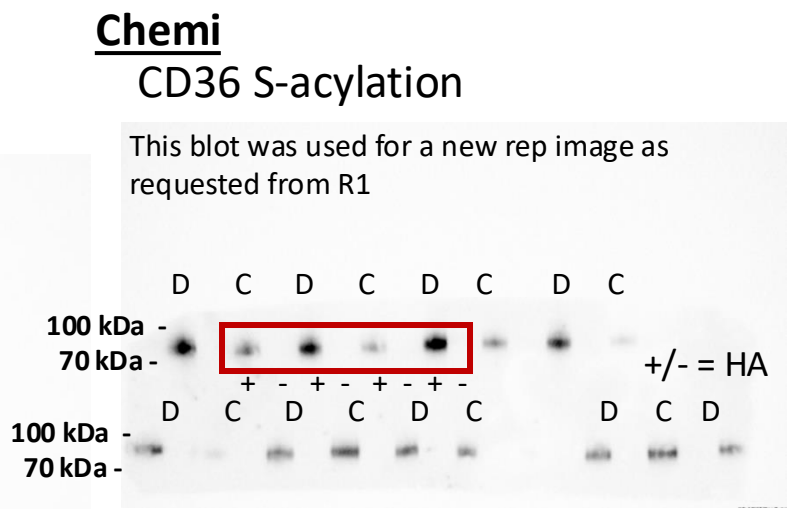


“Diff Study” = samples analysed for a different study
“IC” = internal control

Figure 2 A: CD36 S-acylation Rat



Uncropped gel and membrane

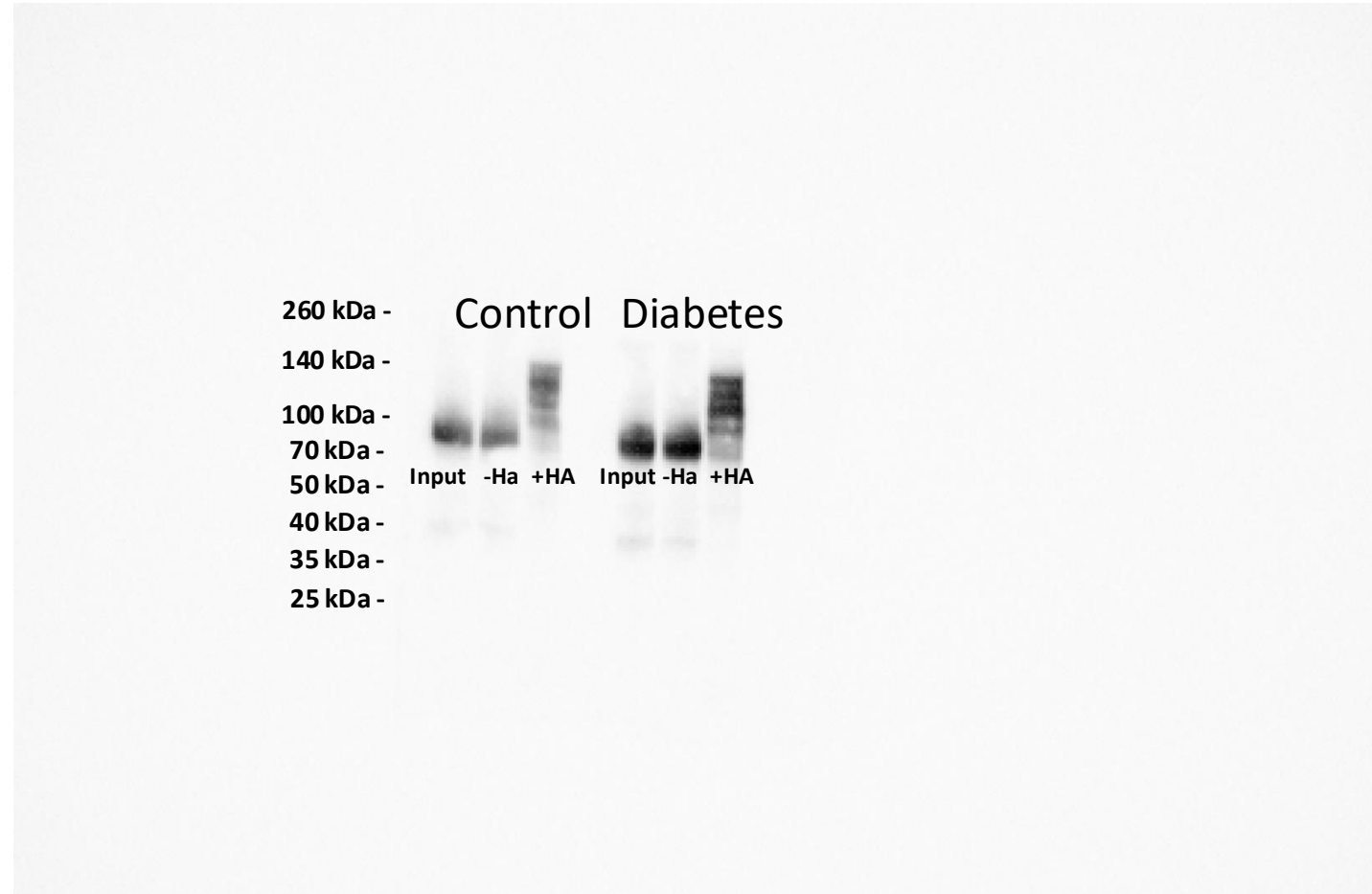


2 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

Gels cut at the 140 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Figure 2C: Acyl PEG CD36

Chemi

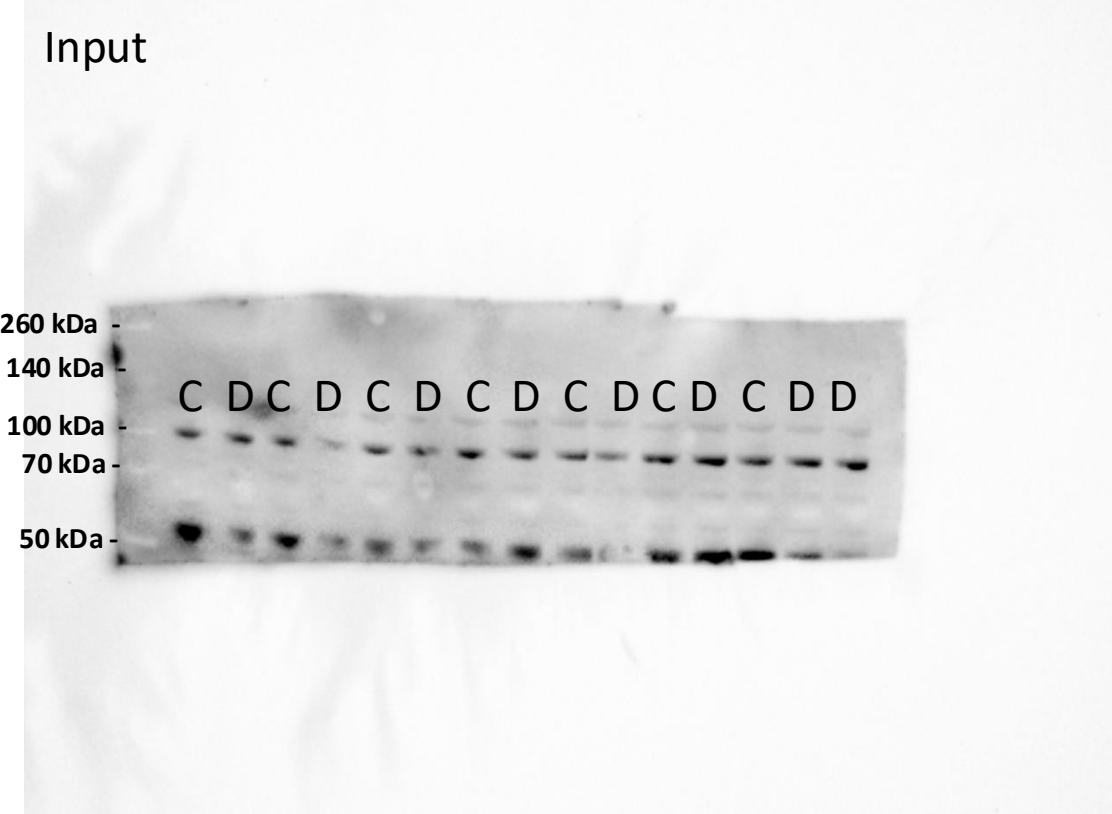


Uncropped gel and membrane

Figure 2 D: CD36 S-acylation Pig

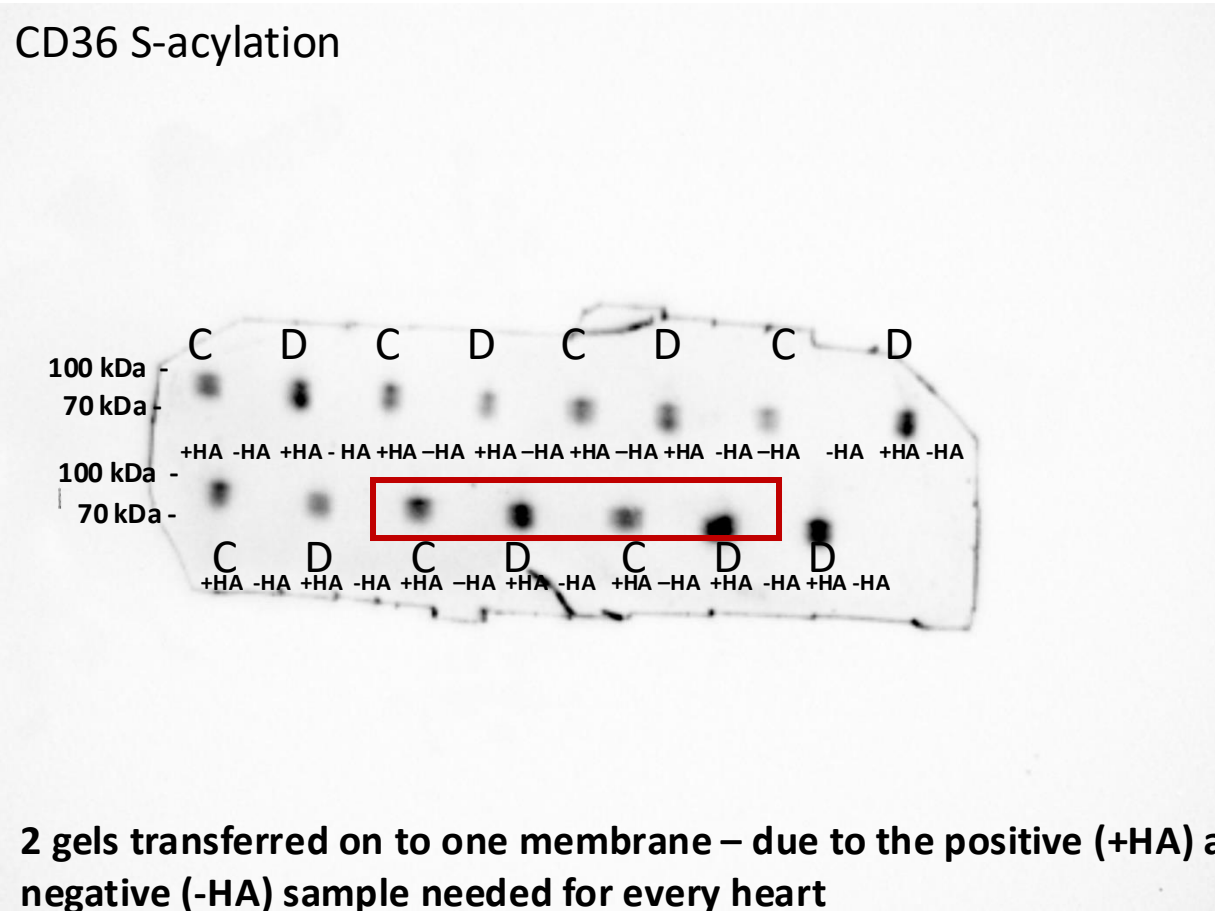
Chemi

Input



Chemi

CD36 S-acylation

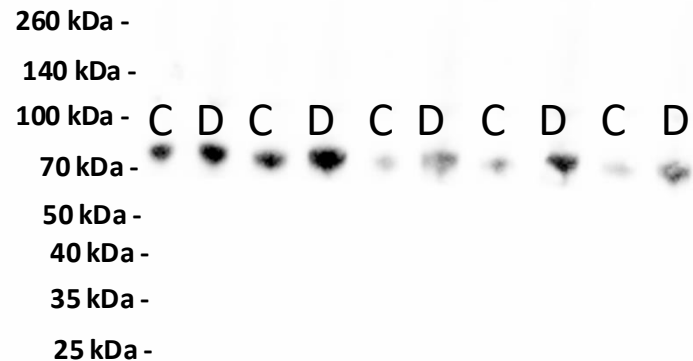


2 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

Gels cut just above the 100 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Figure 2 E: CD36 S-acylation db/db

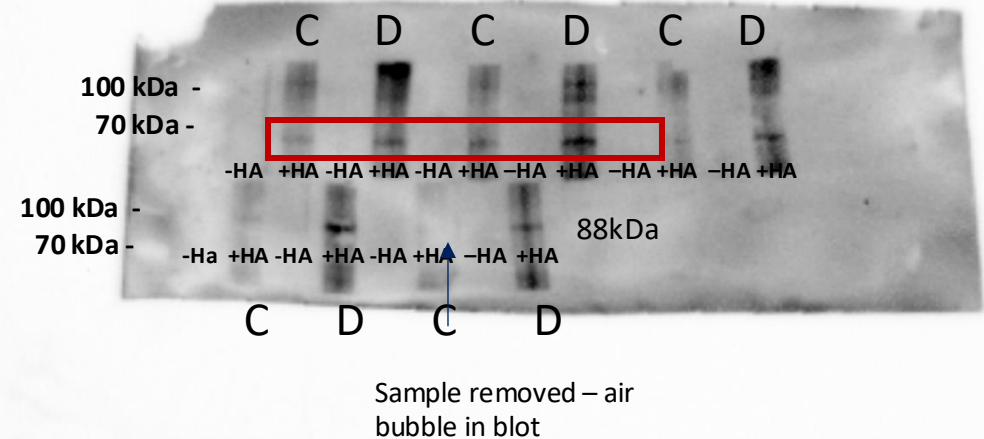
Chemi Input



Uncropped gel and membrane

Chemi

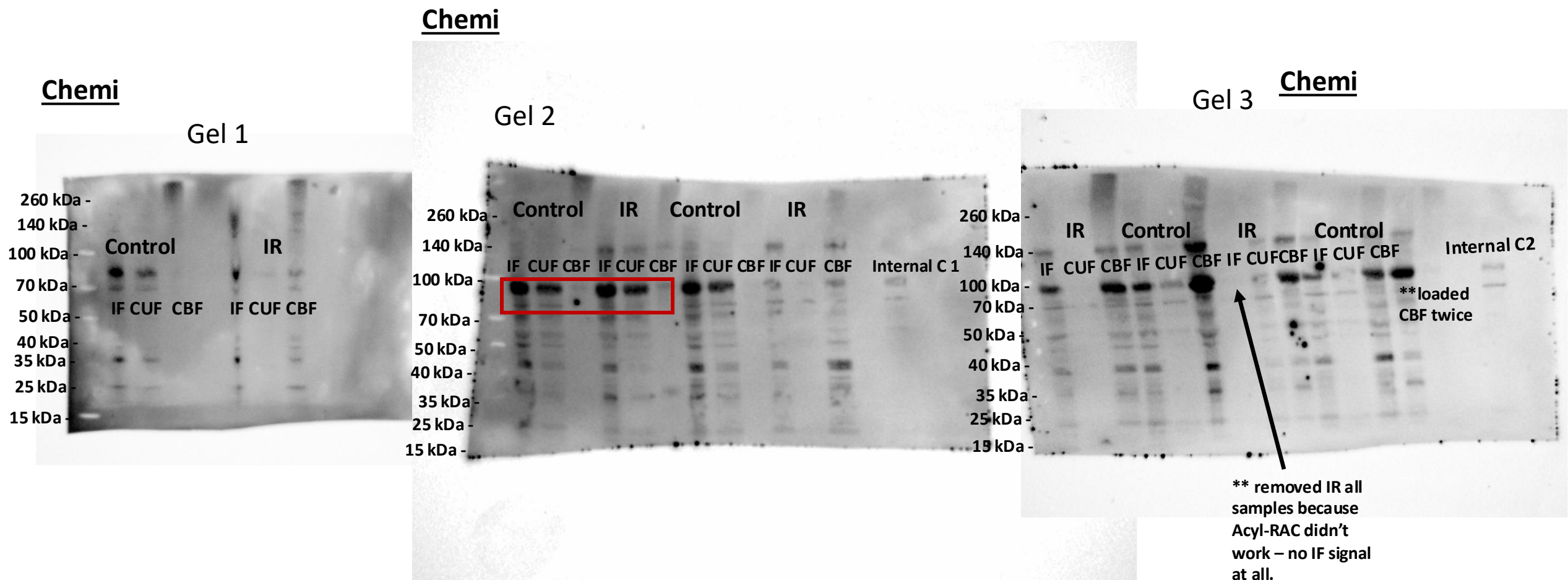
CD36 S-acylation



2 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

Gels cut at the 140 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

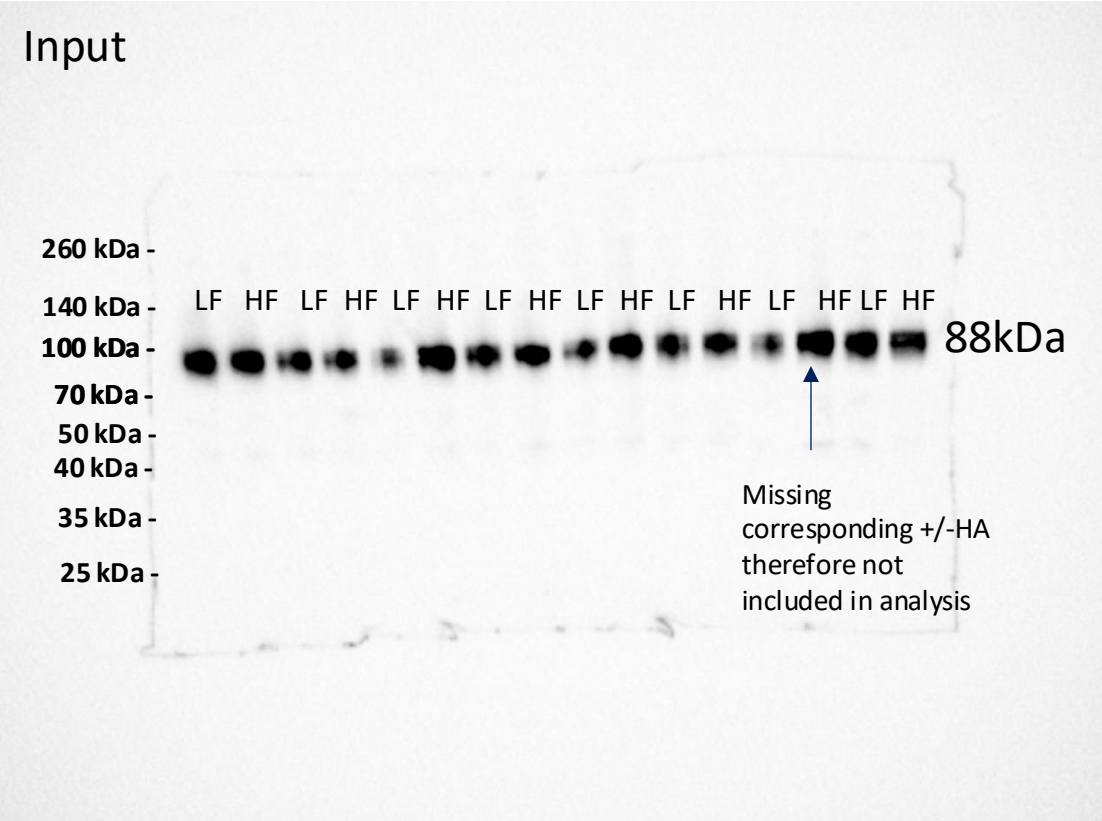
Figure 2F: CD36 S-acylation hiPSC-CM



Uncropped gel and membrane

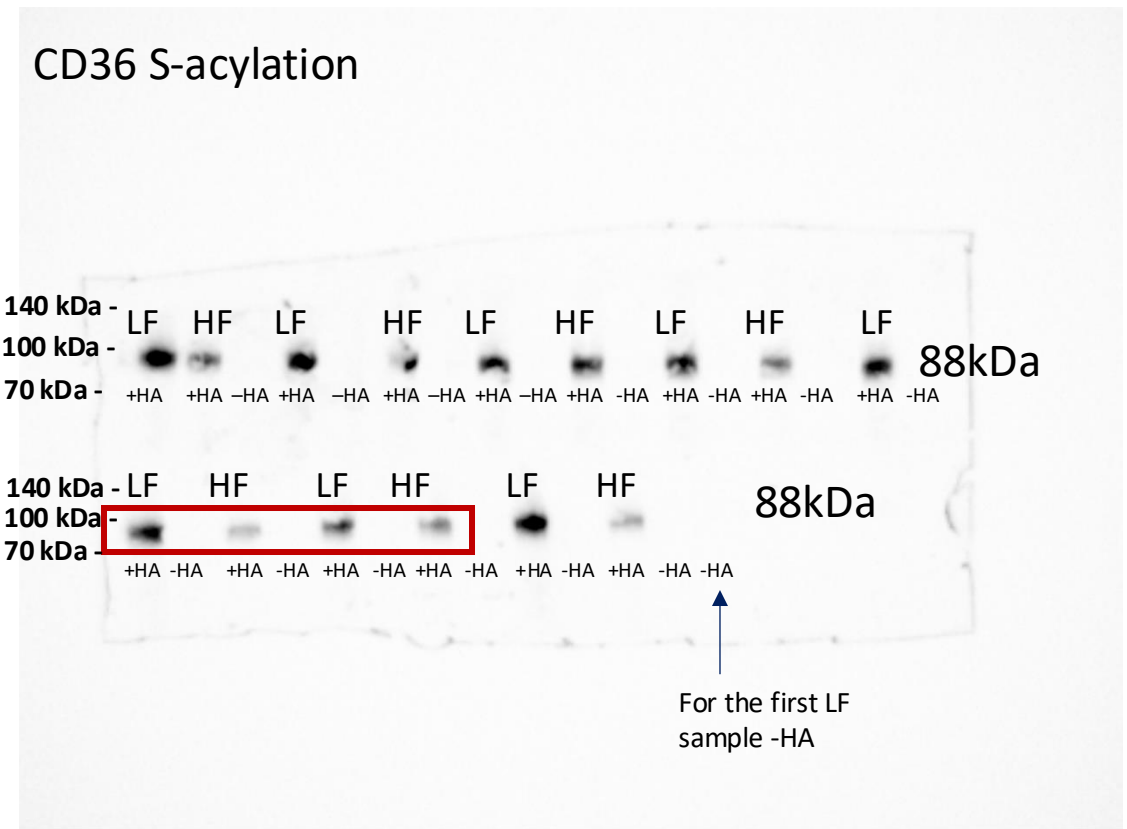
Figure 3 A-B: Low Fat High Fat

Chemi



Uncropped gel and membrane

Chemi



2 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

Gels cut just at the 140 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Figure 5 D-E: hiPSC-CM CD36 S-acylation

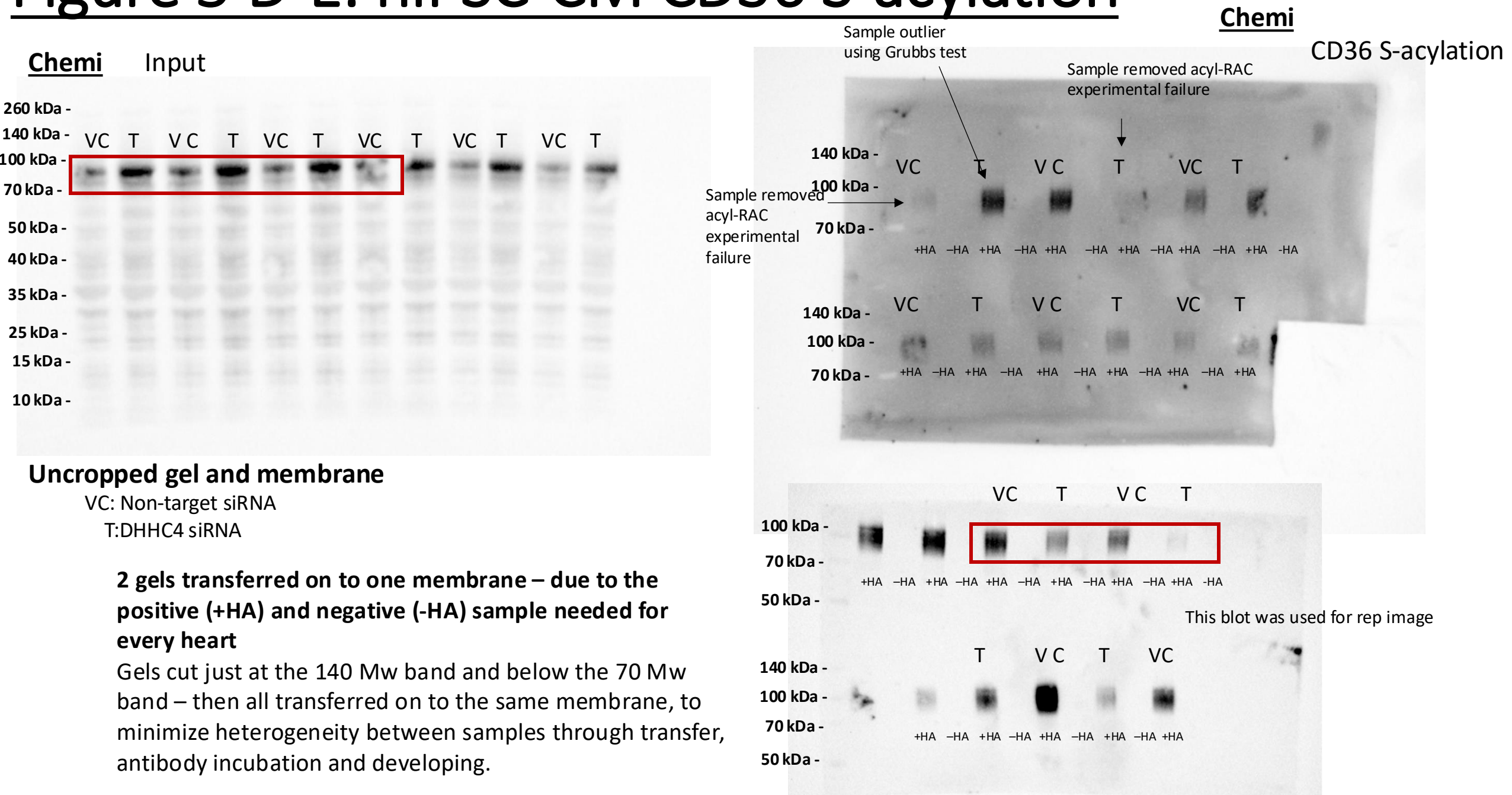
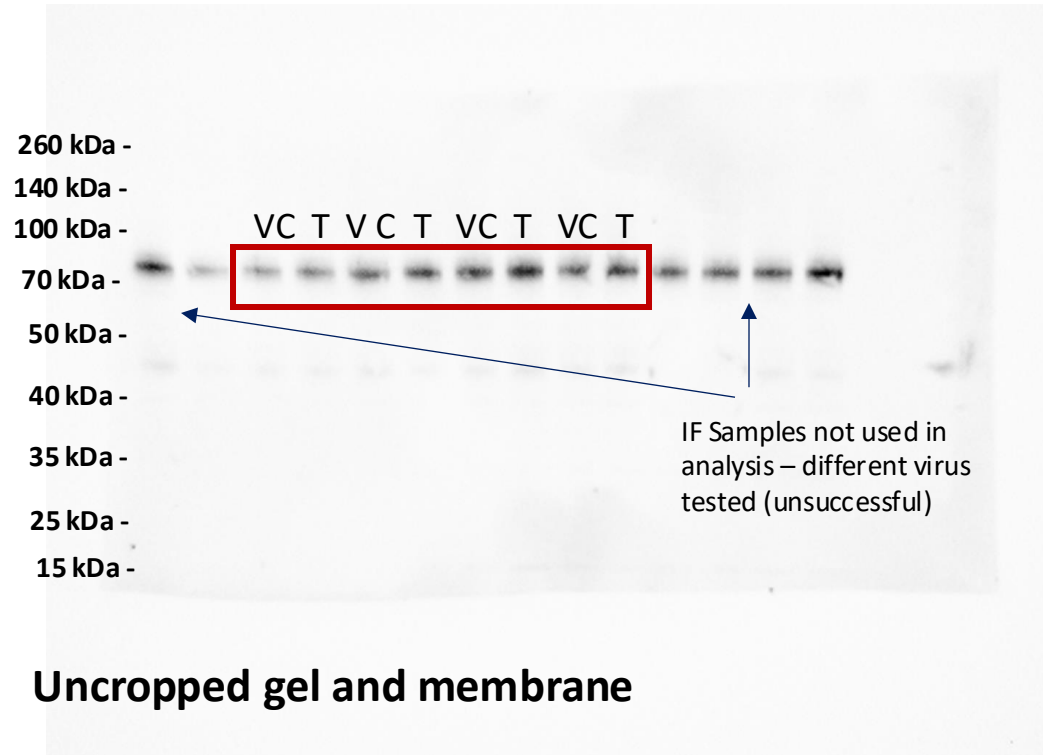


Figure 5 F-G: NRMV CD36 S-acylation

Chemi Input



VC: Scramble ShRNA
T: DHH4 ShRNA

Chemi CD36 S-acylation

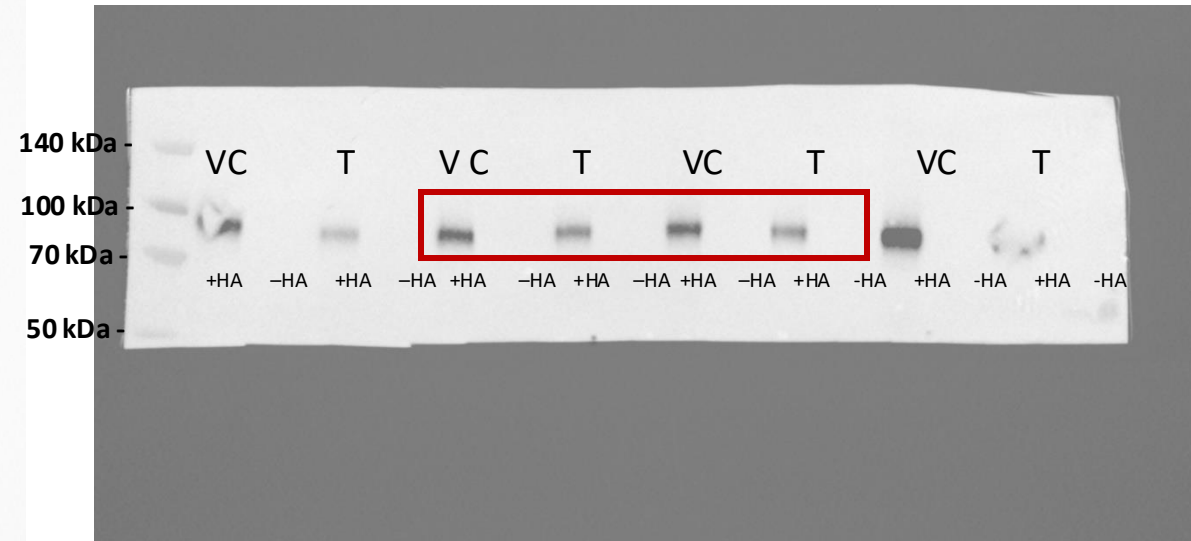


Figure 5 I, J, L : FoxO1 KO and AS1842856 CD36 S-acylation input

Chemi Input

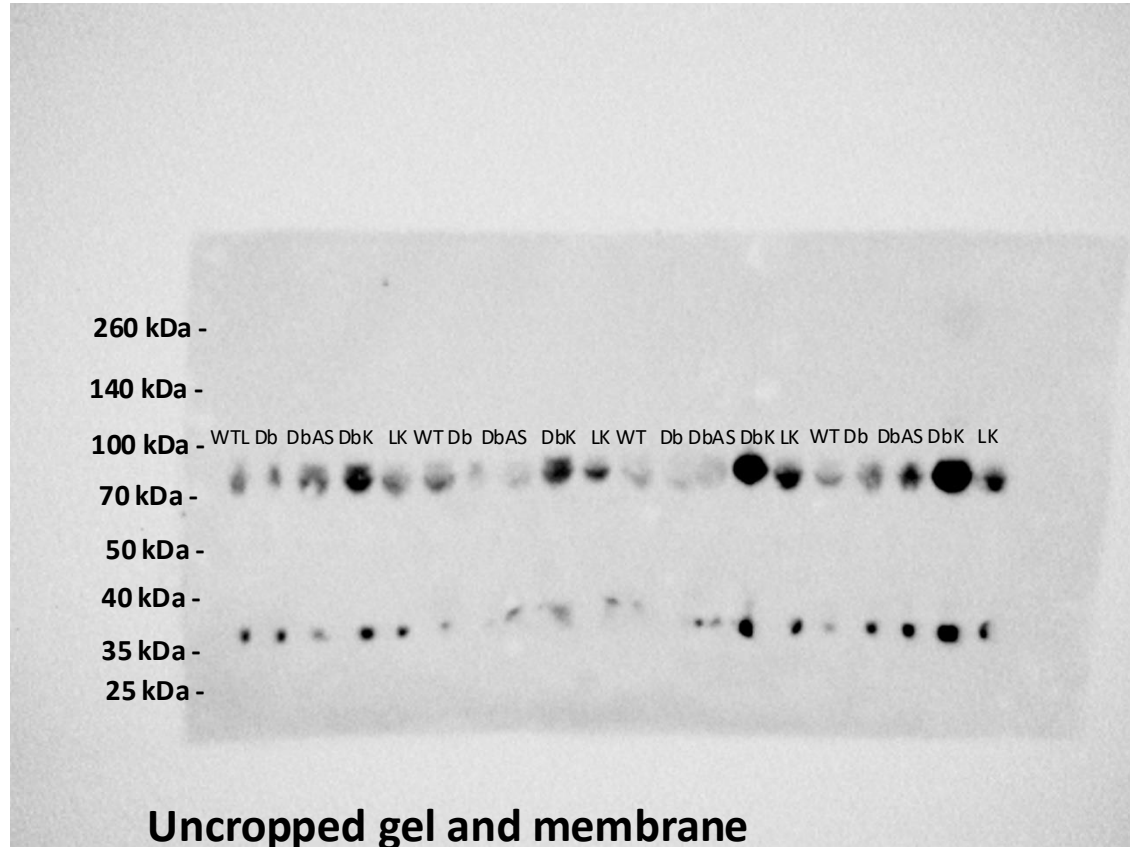
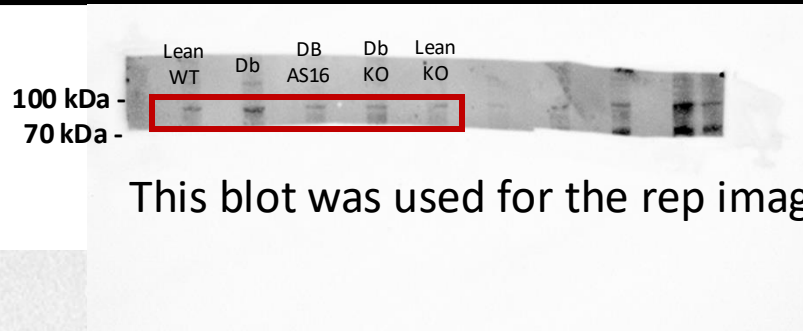


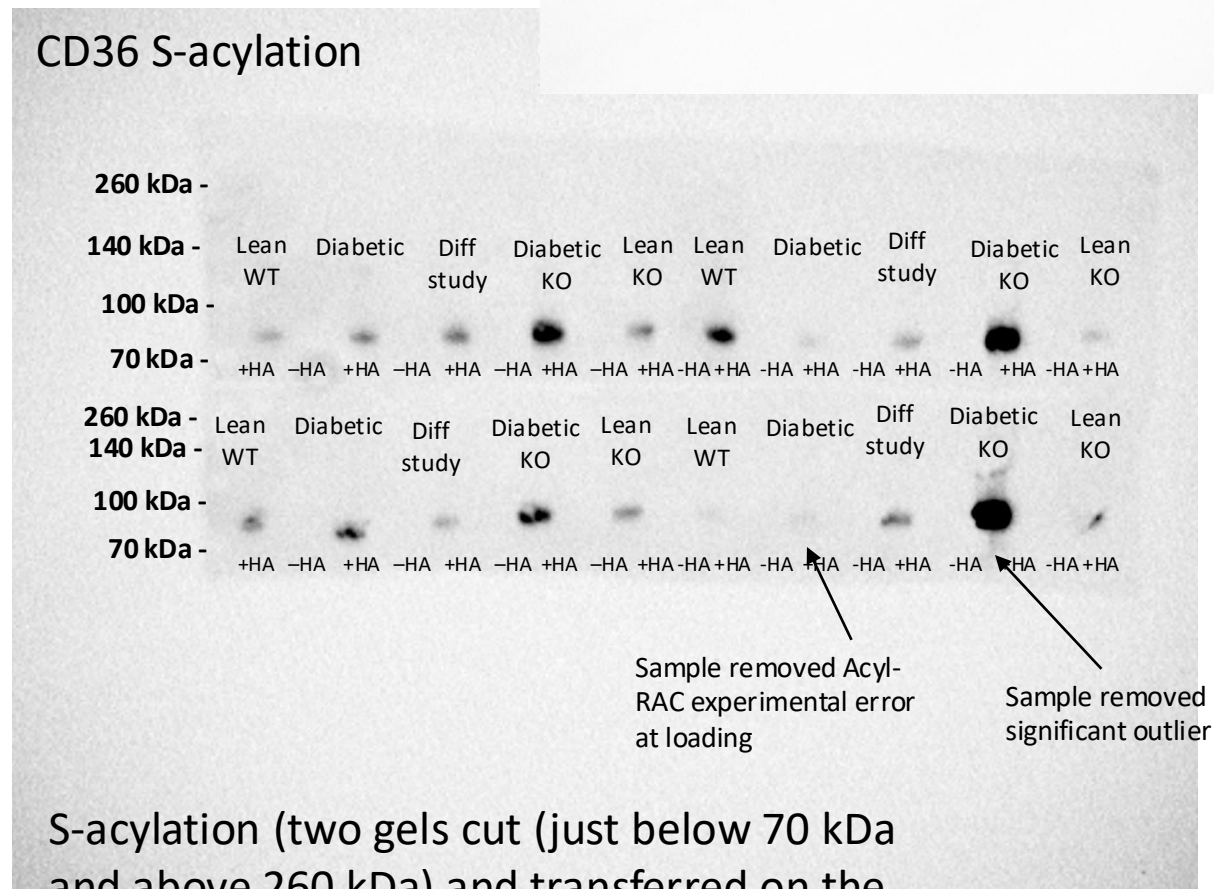
Figure 5 I, J : FoxO1 KO CD36 S-acylation



Chemi

This blot was used for the rep image

Marker



S-acylation (two gels cut (just below 70 kDa and above 260 kDa) and transferred on the same membrane). The membrane was cut after imaging

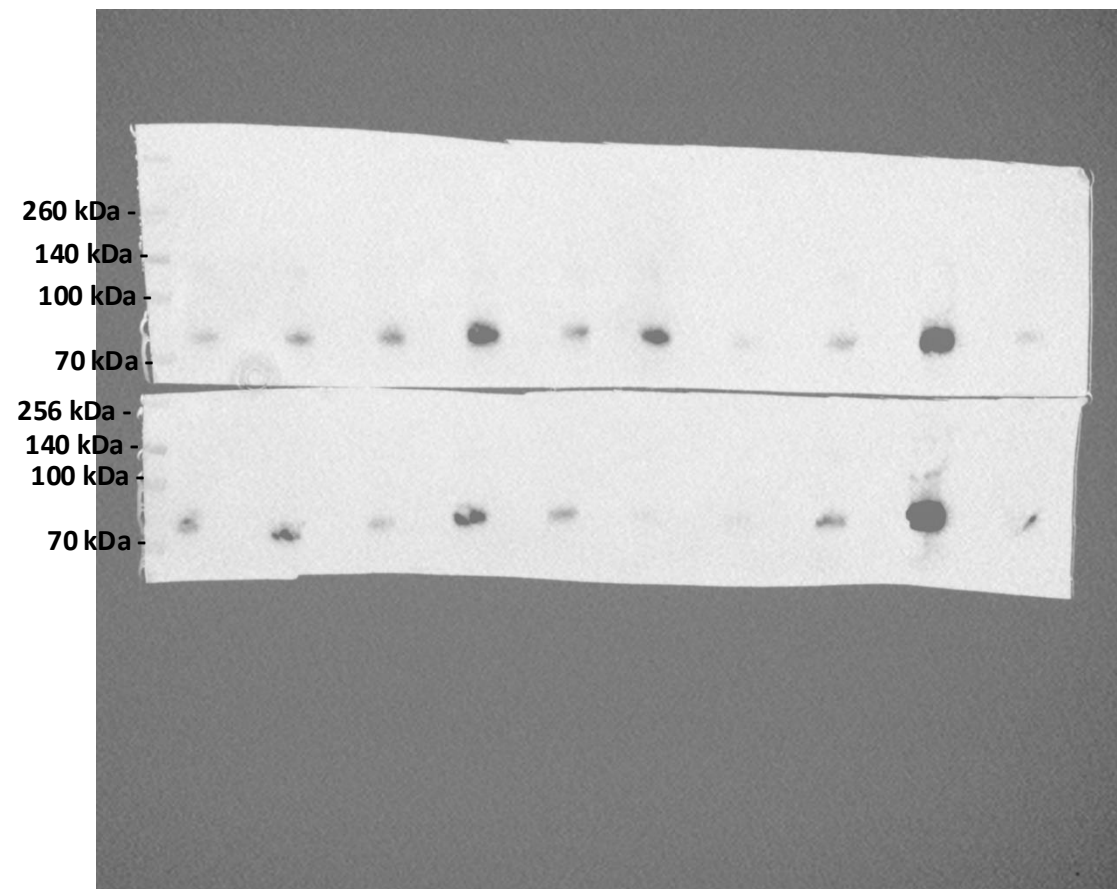
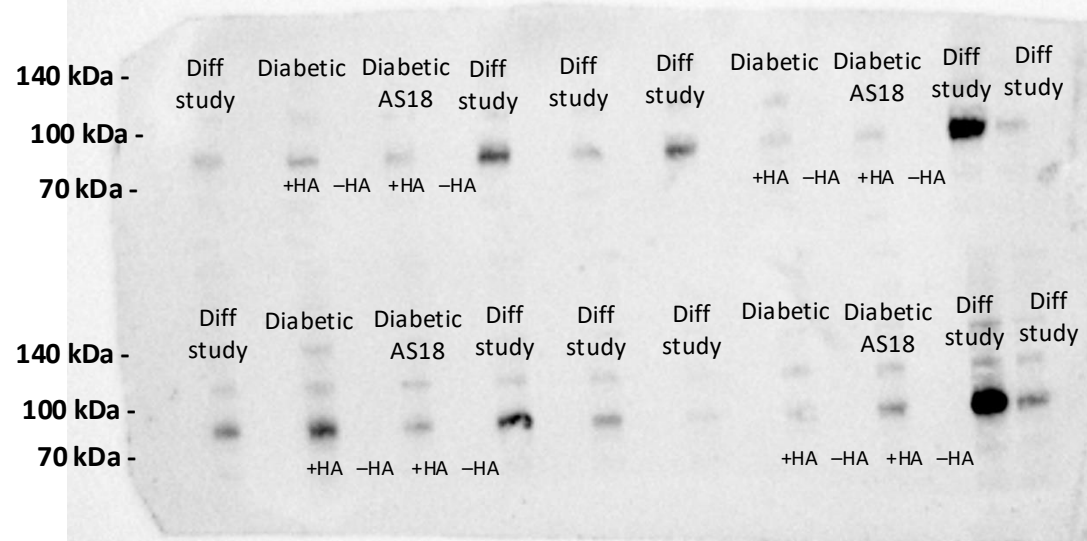


Figure 5 J, L: AS1842856 CD36 S-acylation

Chemi



CD36 S-acylation



S-acylation (two gels cut (just below 70 kDa and above 140 kDa) and transferred on the same membrane).

Marker

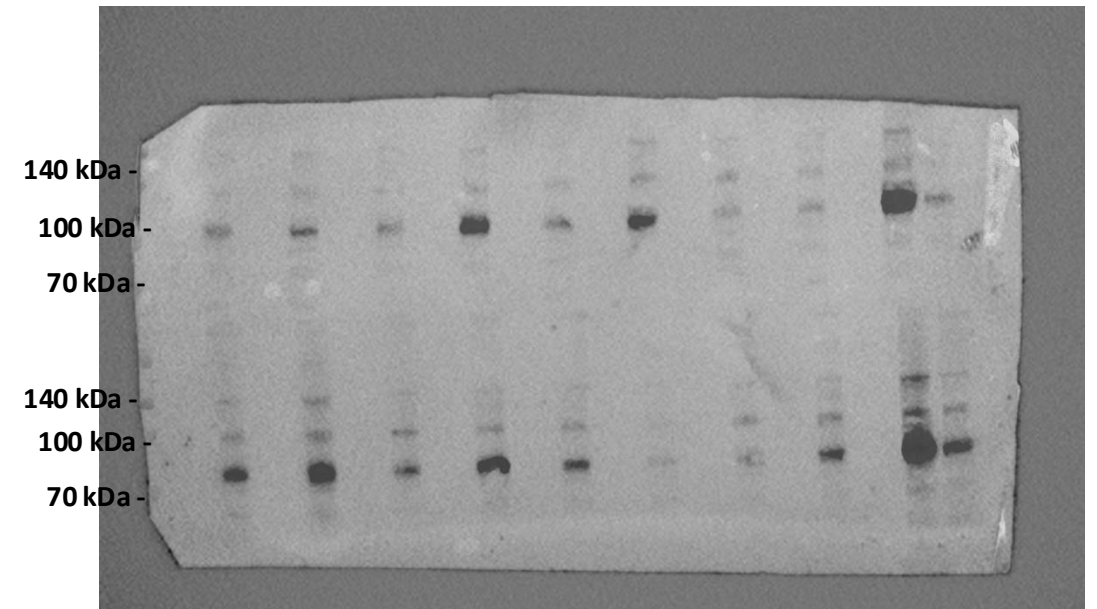
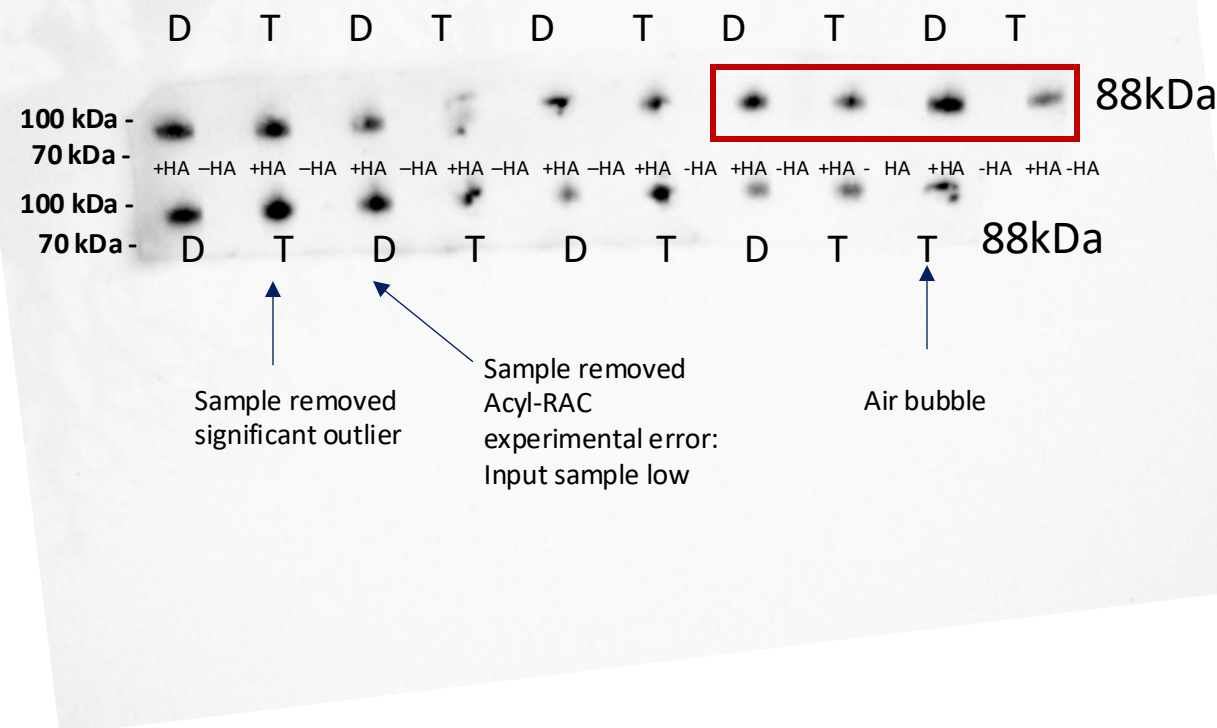
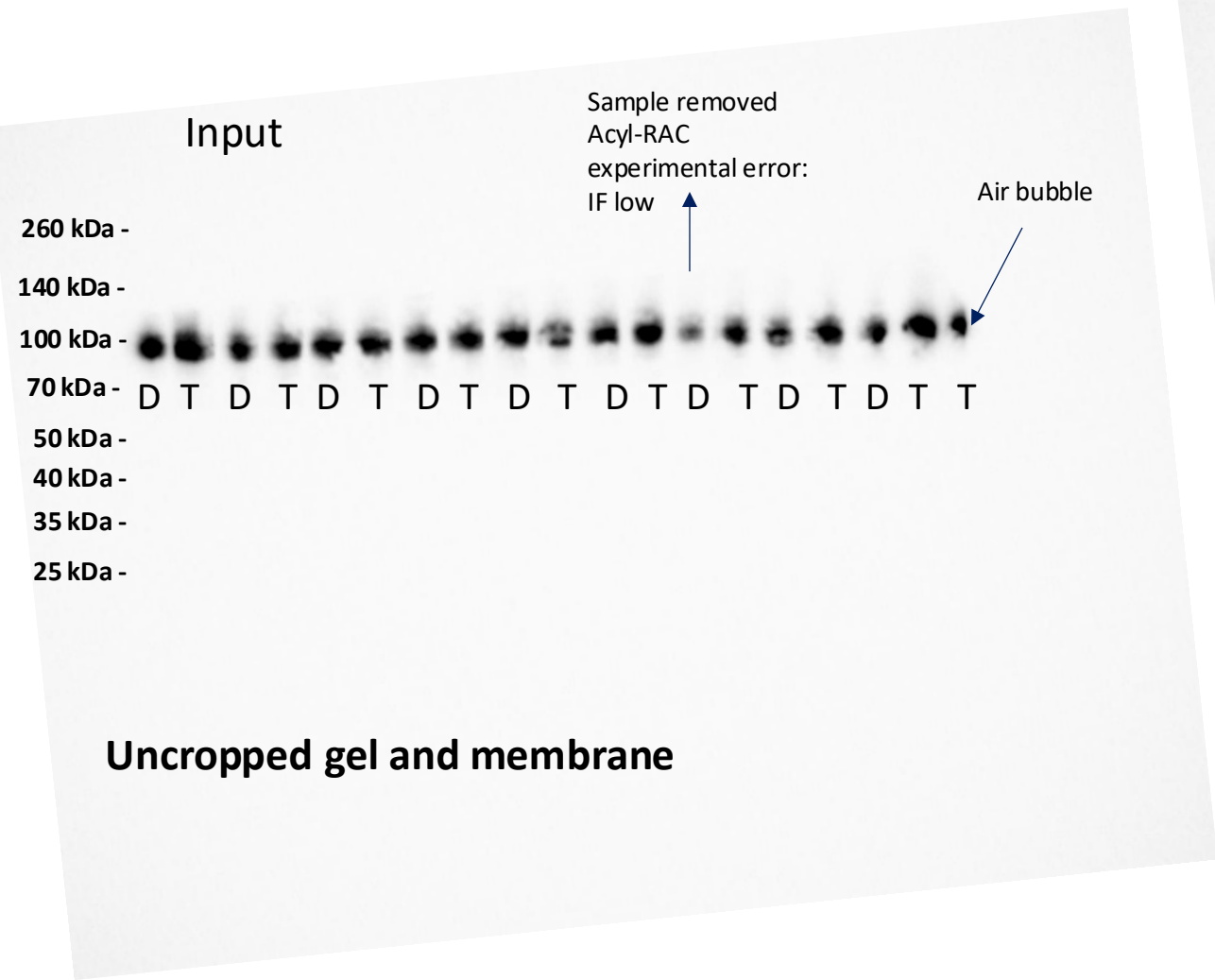


Figure 6B-C: CMA CD36 S-acylation



2 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

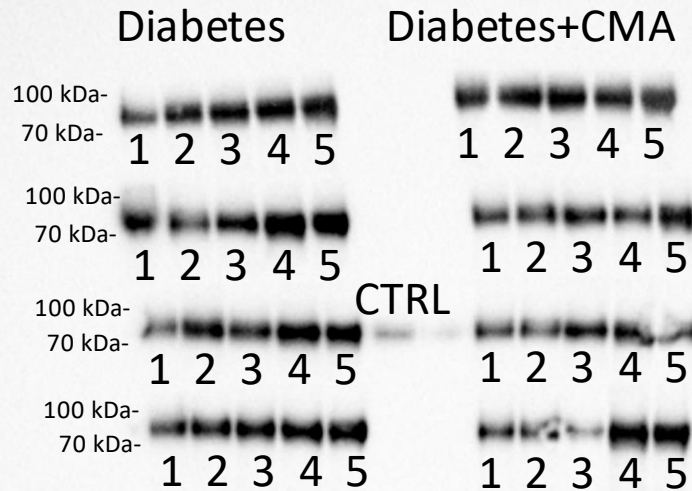
Gels cut just above the 100 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Figure 6D: CMA Subcellular fractionation CD36 membrane 1/3

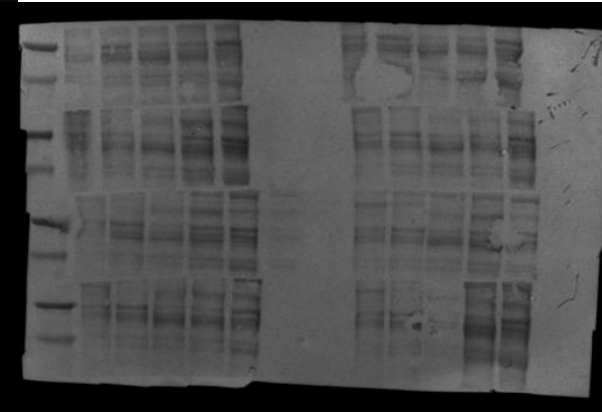
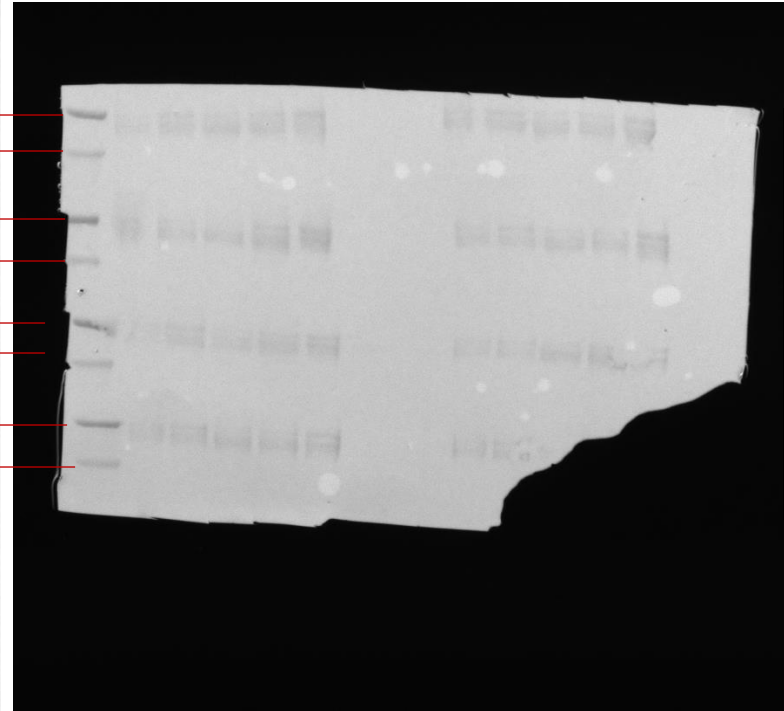
Chemi

Marker

Ponceau



100 kDa
70 kDa
100 kDa
70 kDa
100 kDa
70 kDa
140 kDa
100 kDa



Membrane tore after
chemiluminescence

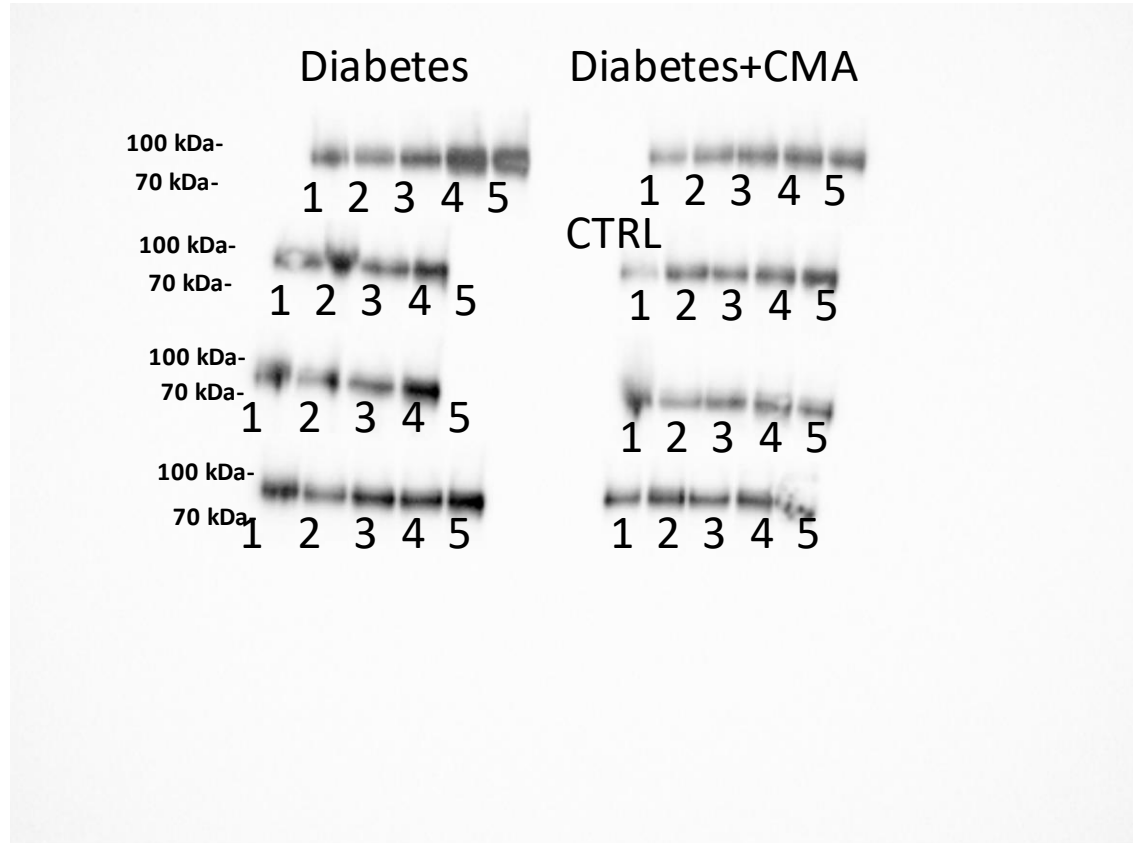
4 gels transferred on to one membrane – due to the large number of fractions generated per heart.

Gels cut just above the 100 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

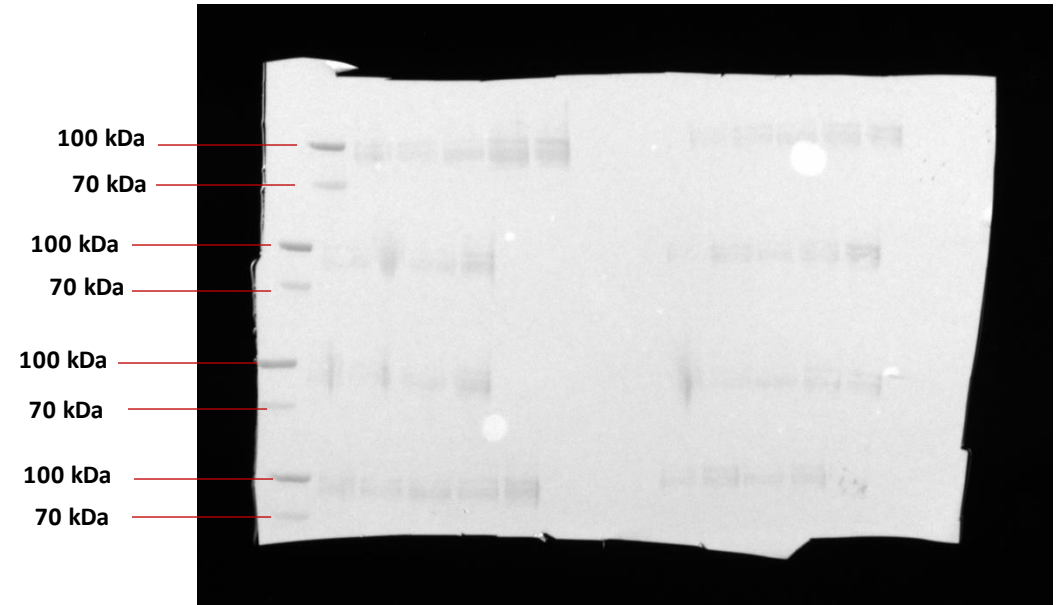
Figure 6D: CMA Subcellular fractionation CD36

membrane 2/3

Chemi



Markers



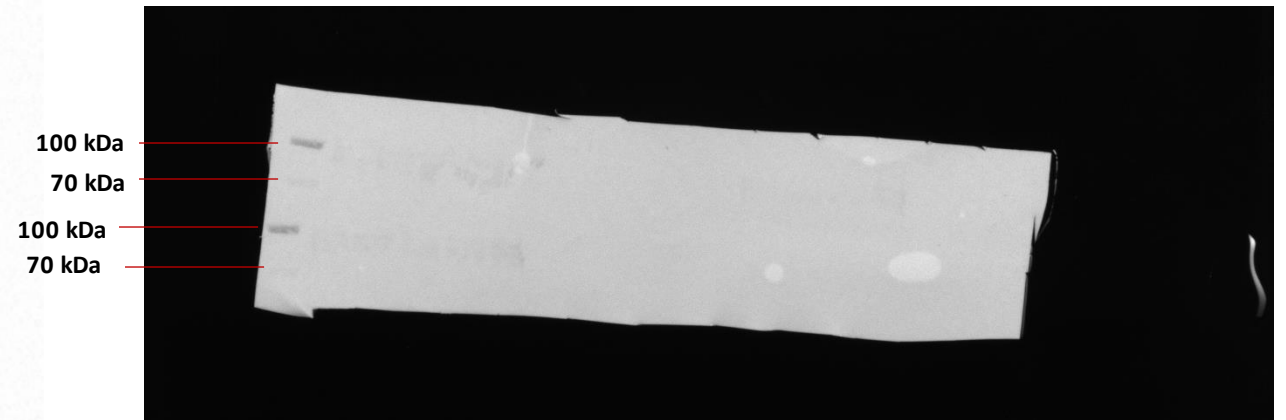
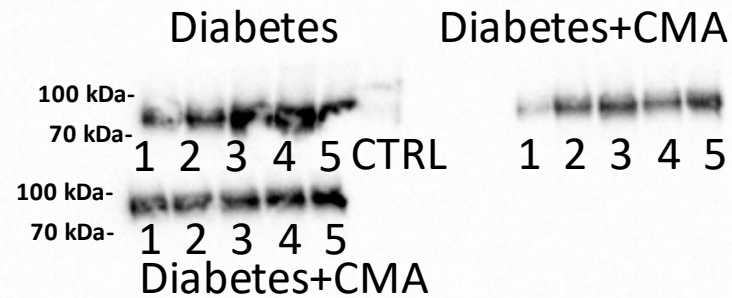
4 gels transferred on to one membrane – due to the large number of fractions generated per heart.

Gels cut just above the 100 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Figure 6D: CMA Subcellular fractionation CD36 membrane 3/3

Markers

Chemi



4 gels transferred on to one membrane – due to the large number of fractions generated per heart.

Gels cut just above the 100 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Chemi Input

260 kDa -
140 kDa -
100 kDa -
70 kDa -
50 kDa -
40 kDa -
35 kDa -
25 kDa -
15 kDa -

C T C T C T C T C T C T C T C T T 88kDa

Sample removed
Crushed lane

Sample removed
Acyl-RAC failure

Chemi CD36 S-acylation

140 kDa -
100 kDa -
70 kDa -

C T C T C T C T C T

-HA +HA -HA +HA -HA +HA -HA +HA -HA +HA -HA +HA -HA +HA -HA +HA

88kDa

140 kDa -
100 kDa -
70 kDa -

C T C T C T C T T

-HA +HA -HA +HA -HA +HA -HA +HA -HA +HA -HA +HA -HA +HA -HA +HA

88kDa

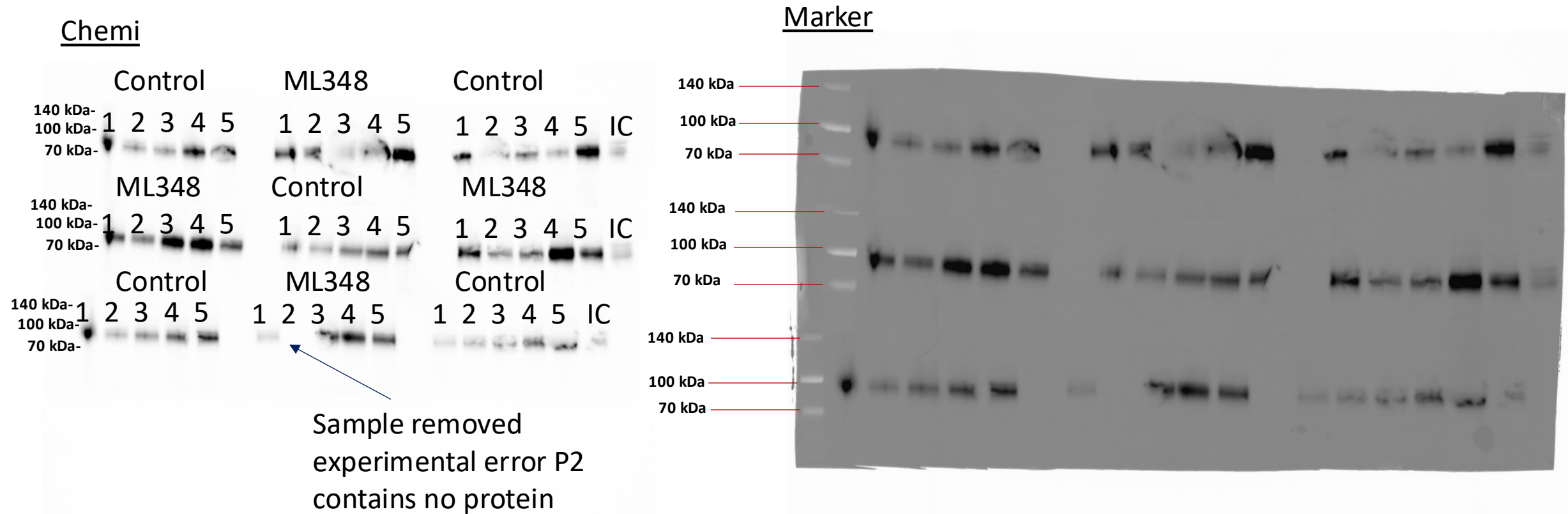
Sample removed
significant outlier

Sample removed Acyl
RAC failure due to IF
removal

Sample removed due
to IF removal (crush
lane)

Gels cut just above the 140 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

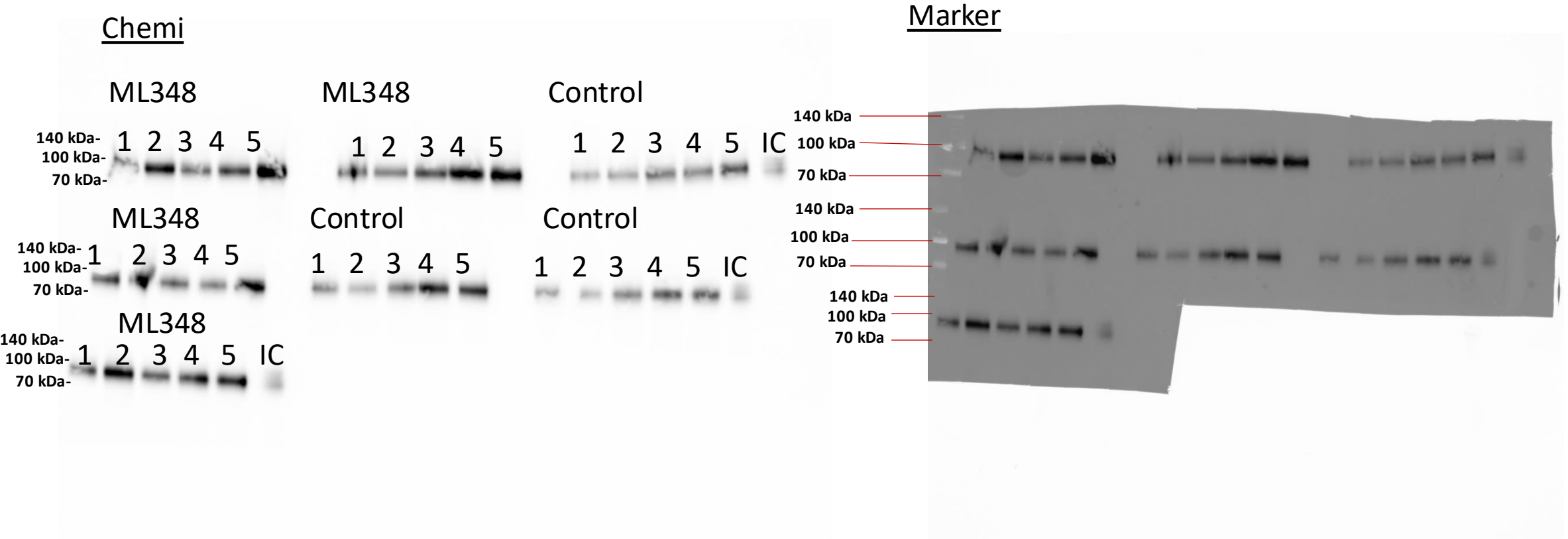
Figure 7D: ML348 Subcellular fractionation CD36 membrane 1/2



3 gels transferred on to one membrane – due to the large number of fractions generated per heart.

Gels cut just above the 140 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Figure 7D: ML348 Subcellular fractionation CD36 membrane 2/2

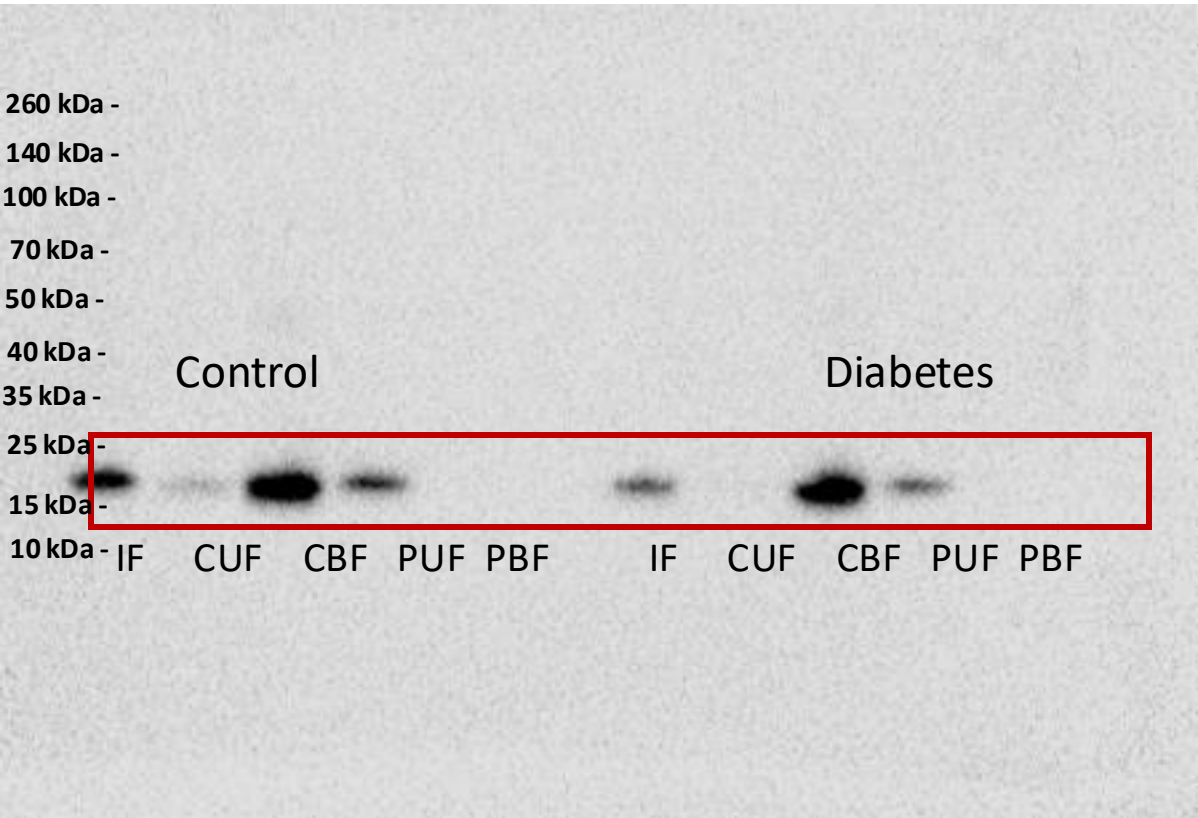


3 gels transferred on to one membrane – due to the large number of fractions generated per heart.

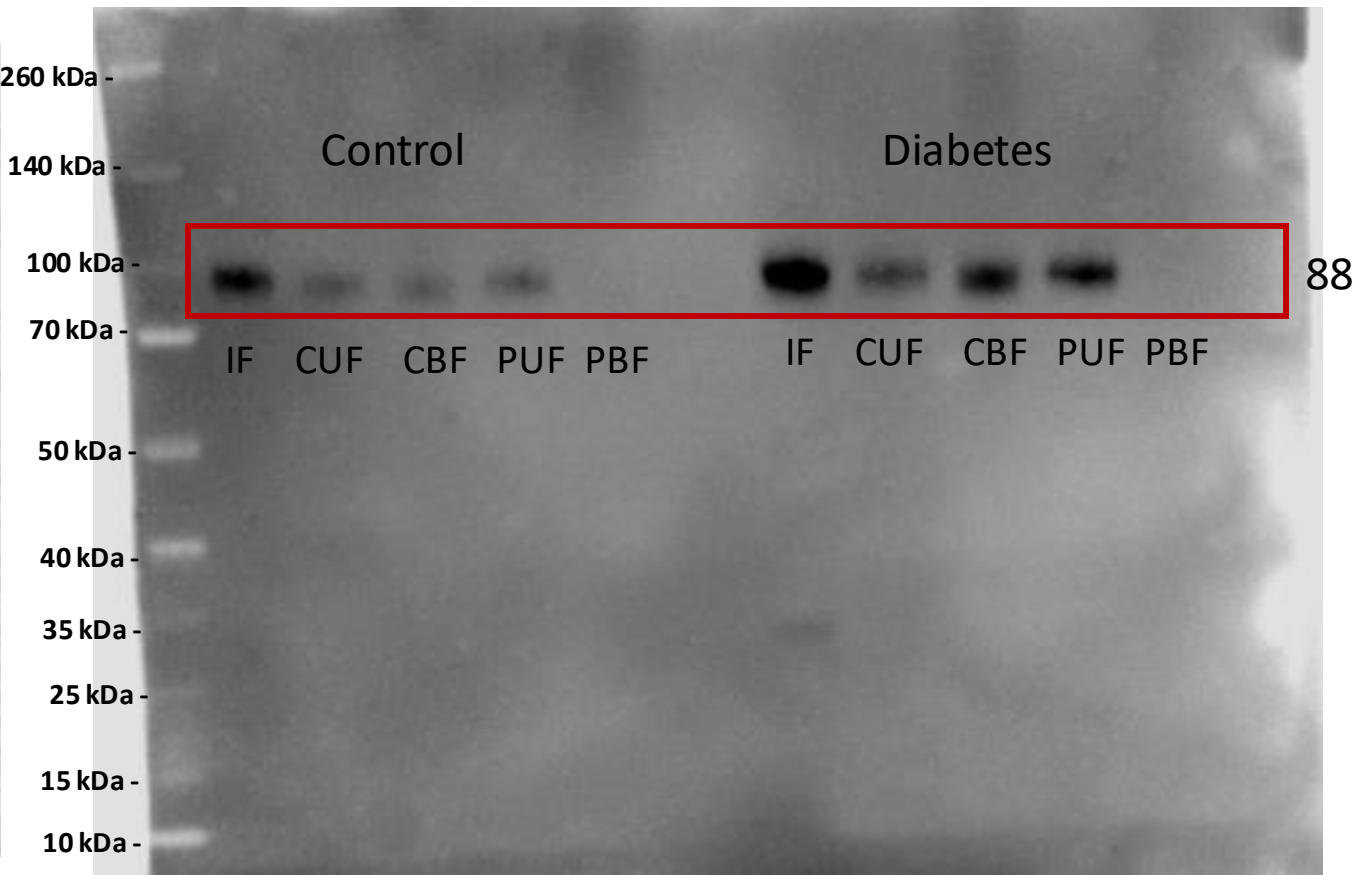
Gels cut just above the 140 Mw band and below the 70 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Supplemental Figure 2 A (CAV3 and CD36 validation - not quantified)

Chemi – CAV3



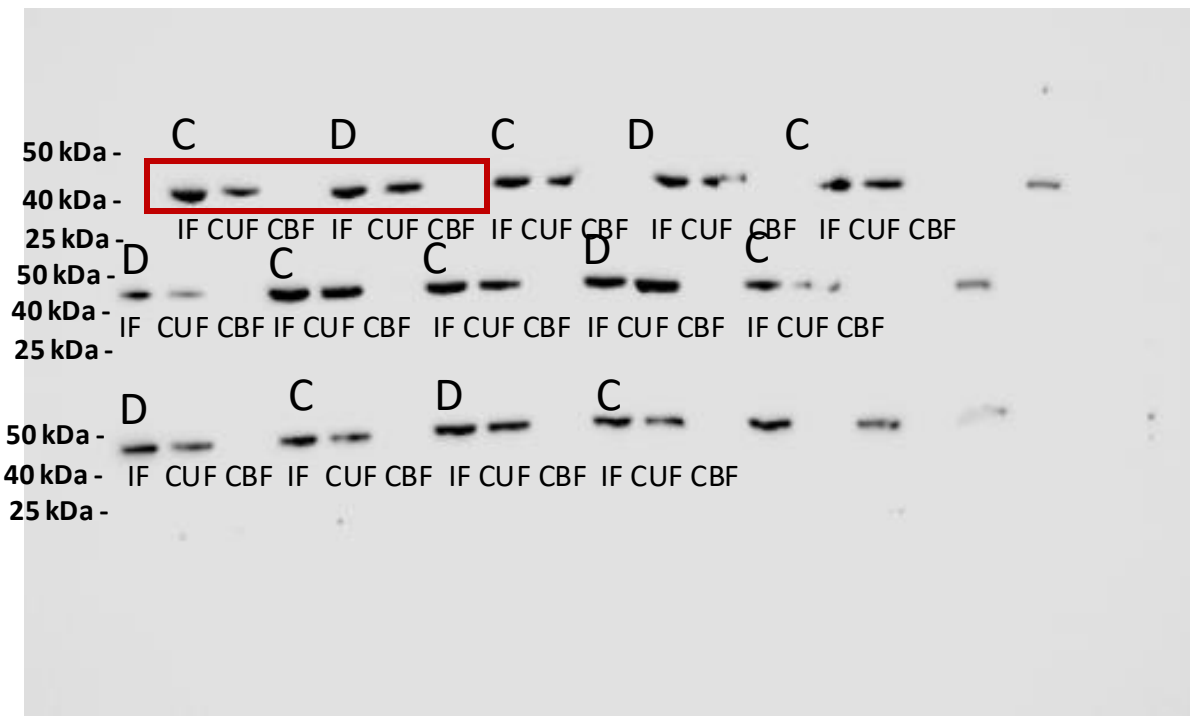
Chemi – CD36



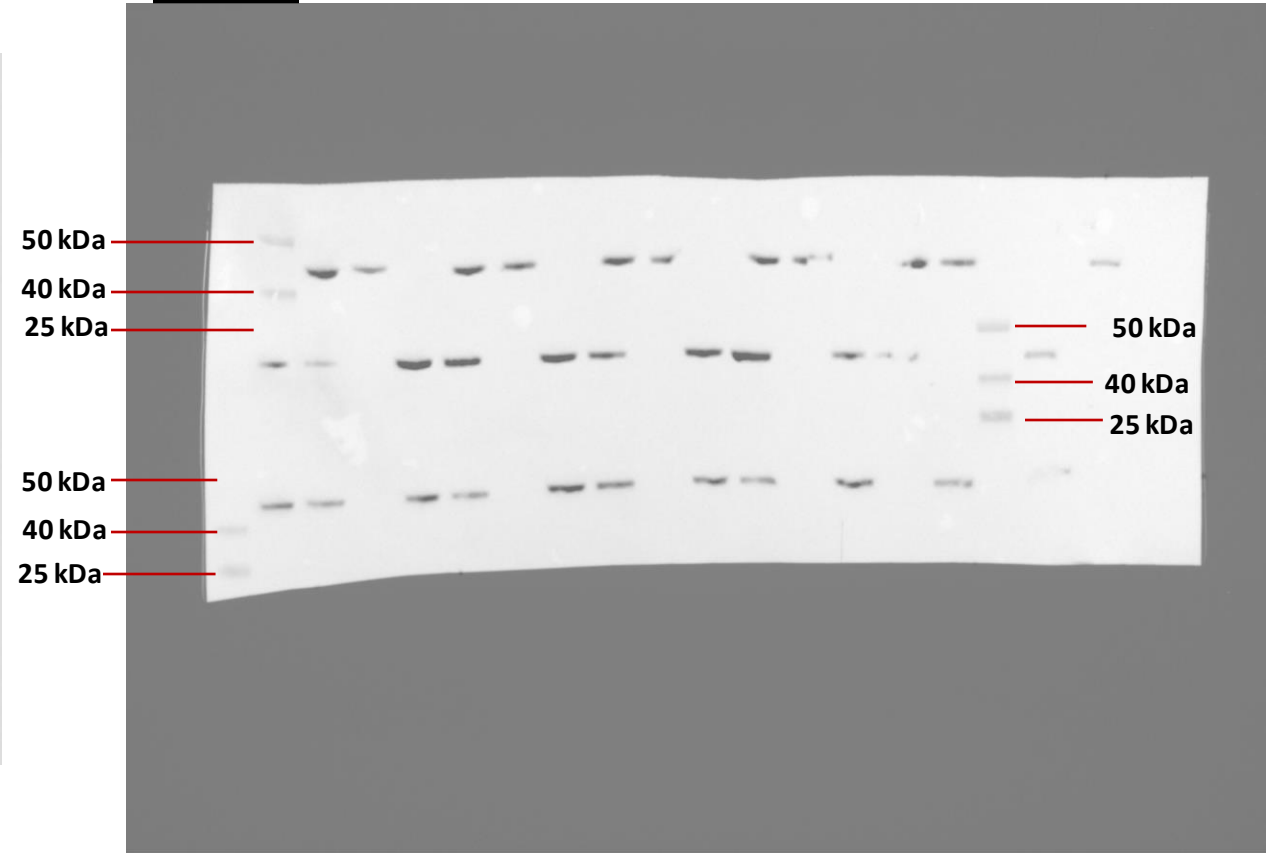
Uncropped gel and membrane – only one band for CD36

Supplemental Figure 2 B (not quantified) FABPpm

Chemi



Marker

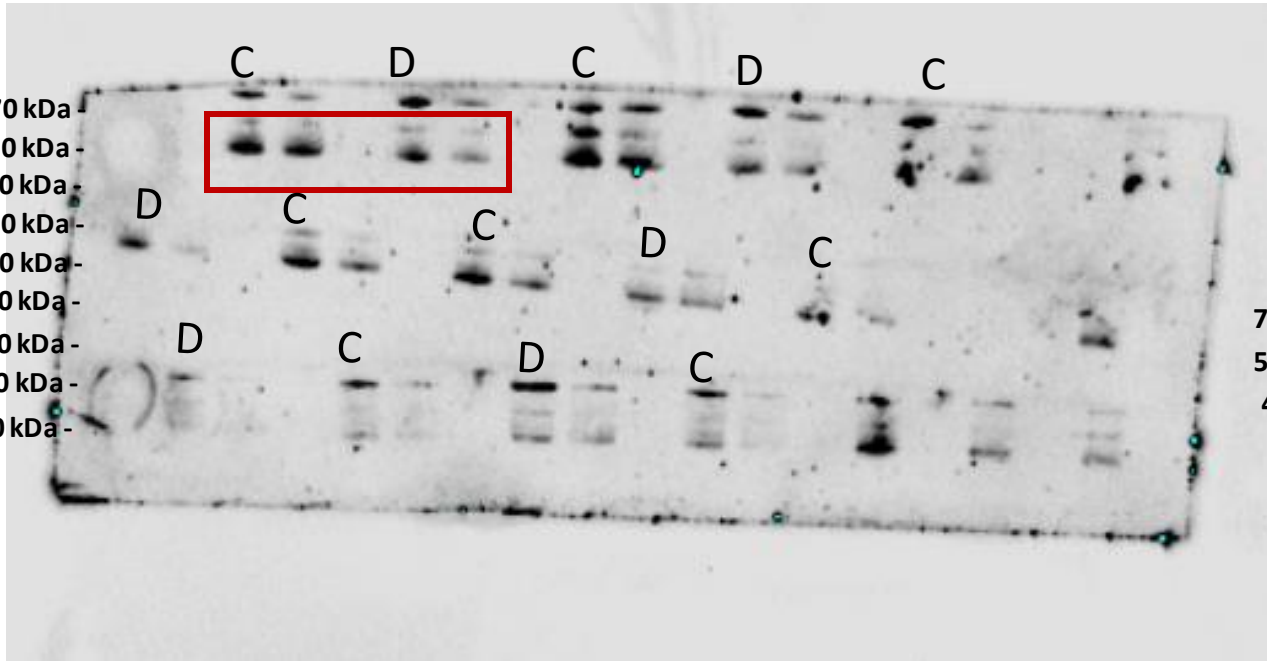


3 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

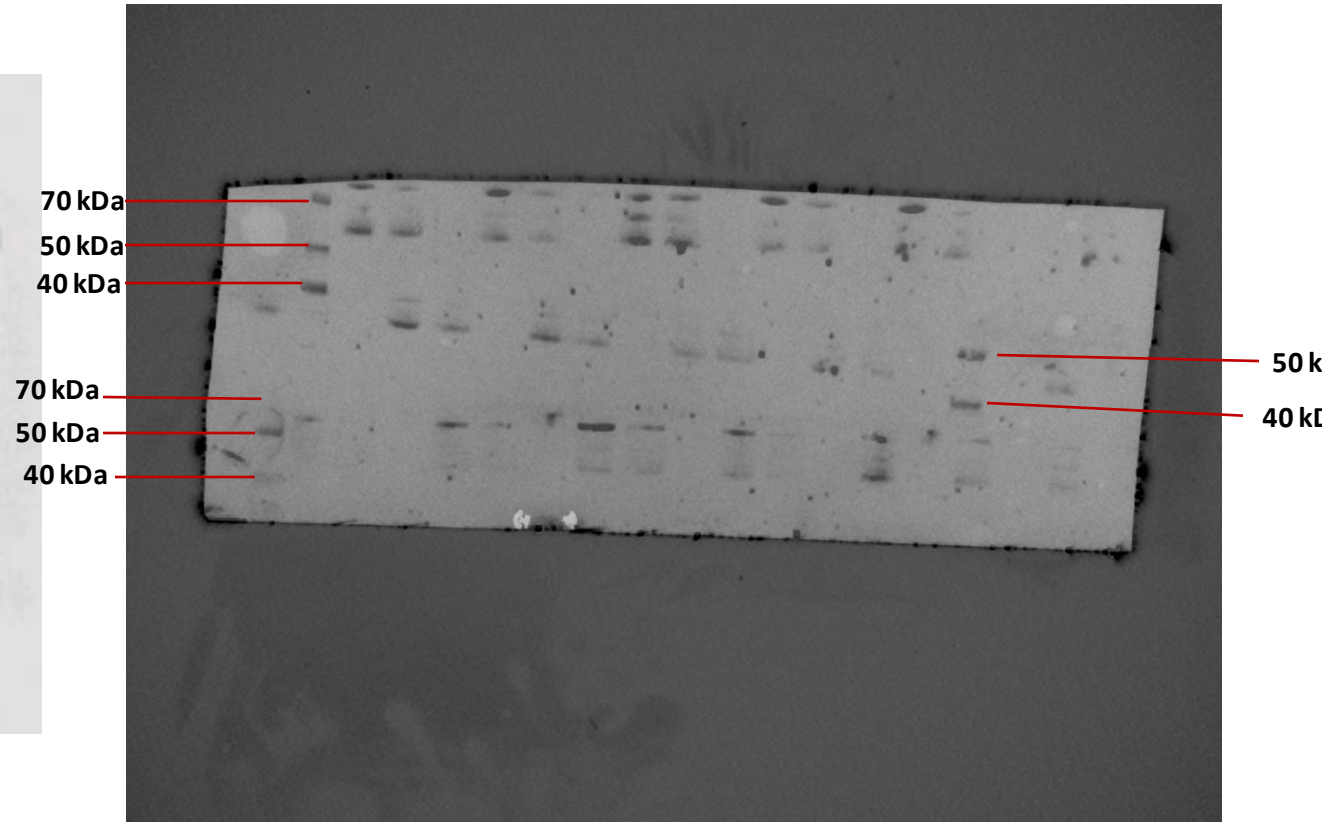
Gels cut above the 50 kDa and below the 25 kDa then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Supplemental Figure 2 B (not quantified) GLUT1

Chemi



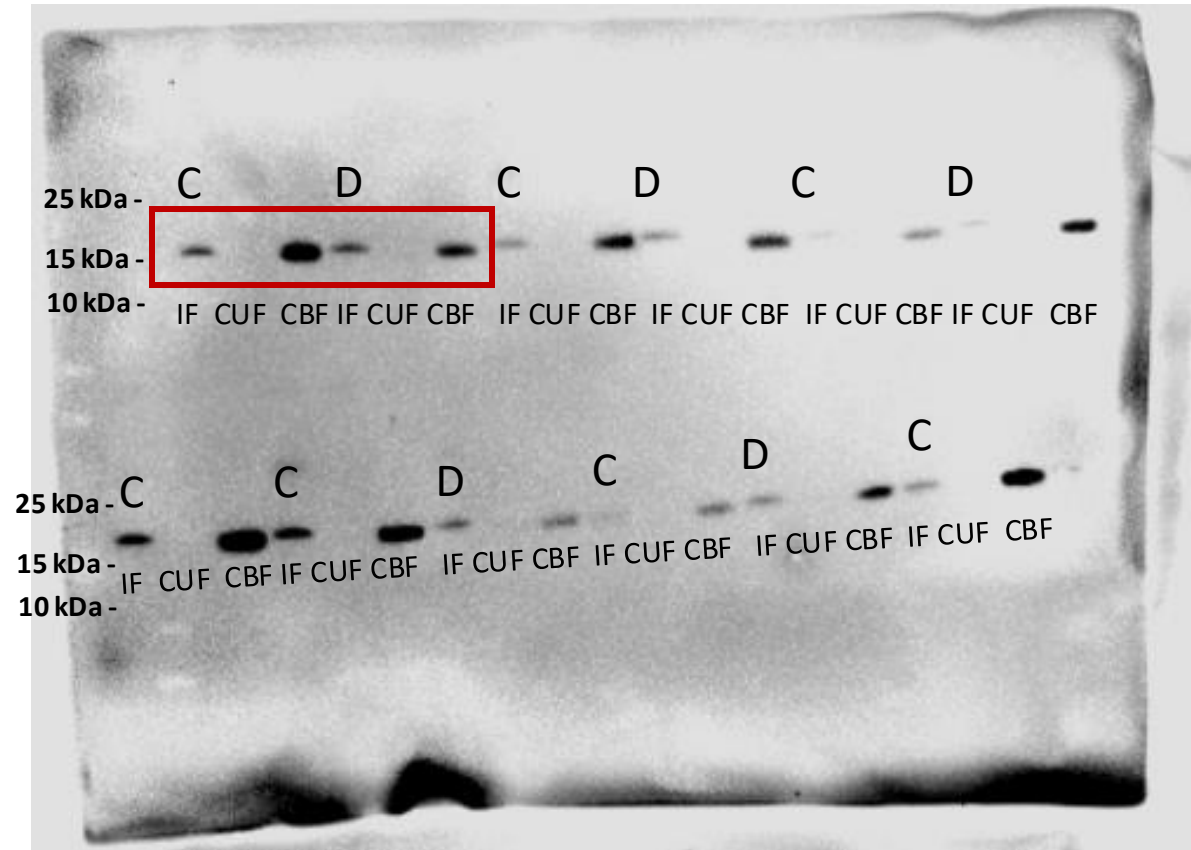
Marker



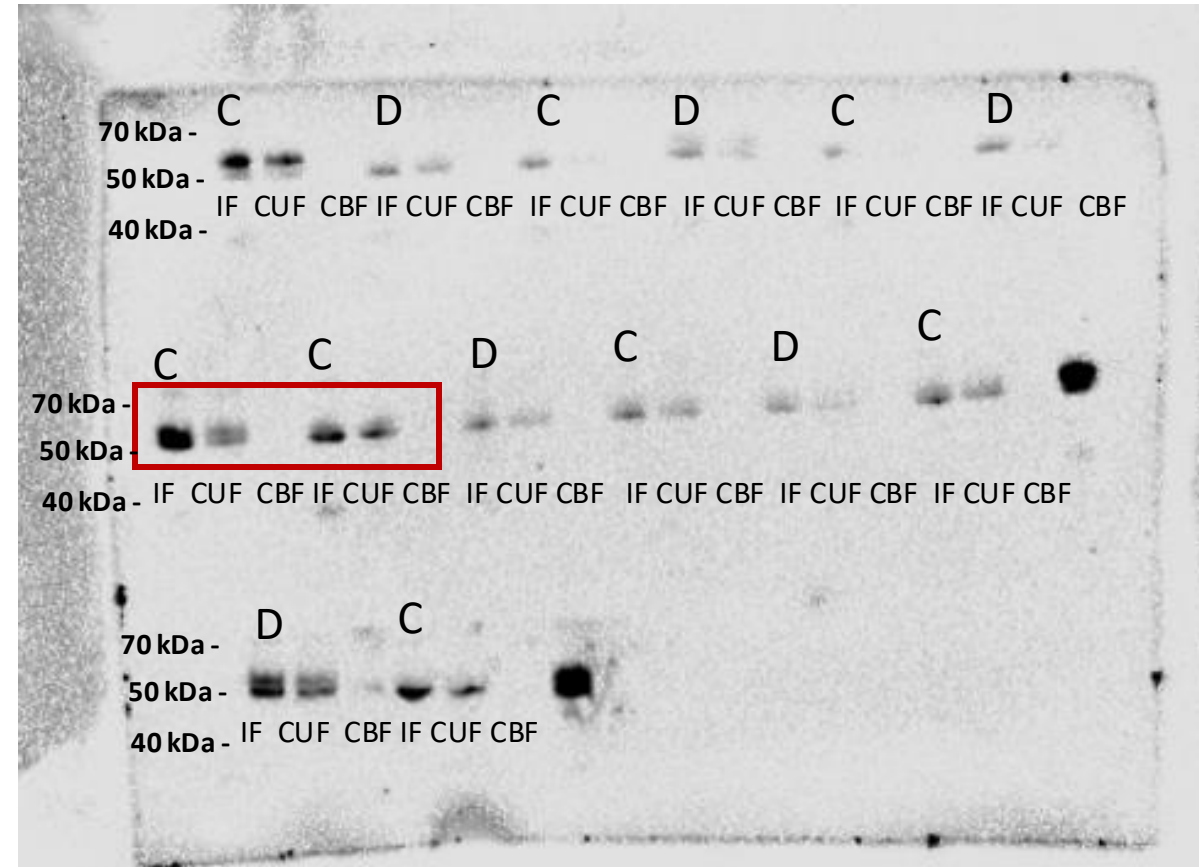
3 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart. Gels were cut above the 70 kDa and below the 40 kDa, then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Supplemental Figure 2 B (Not quantified)

Chemi CAV3



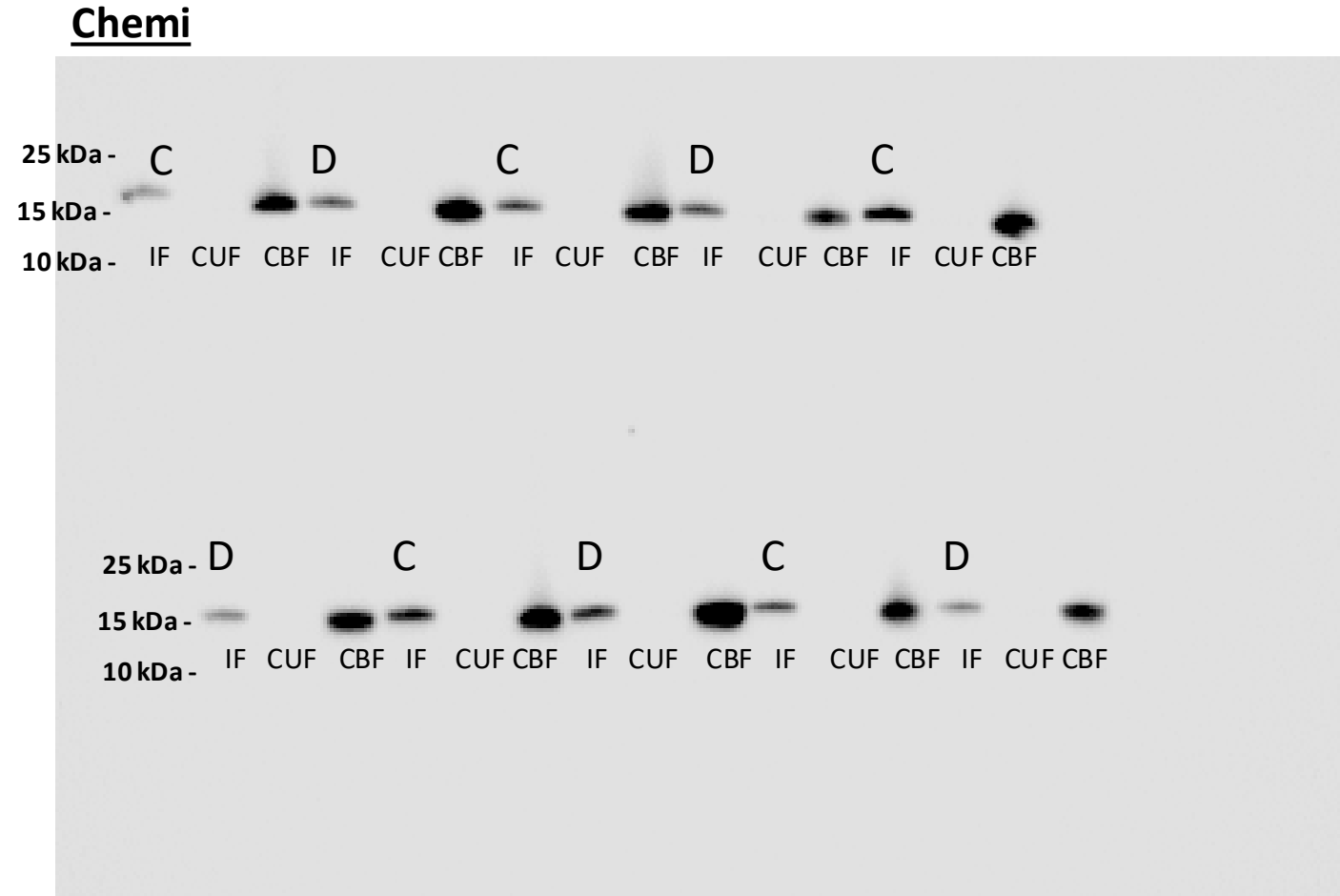
Chemi GLUT4



2-3 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

Gels cut just above the 25 Mw band (for CAV3) and above the 70Mw band (for GLUT4) – then gels transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

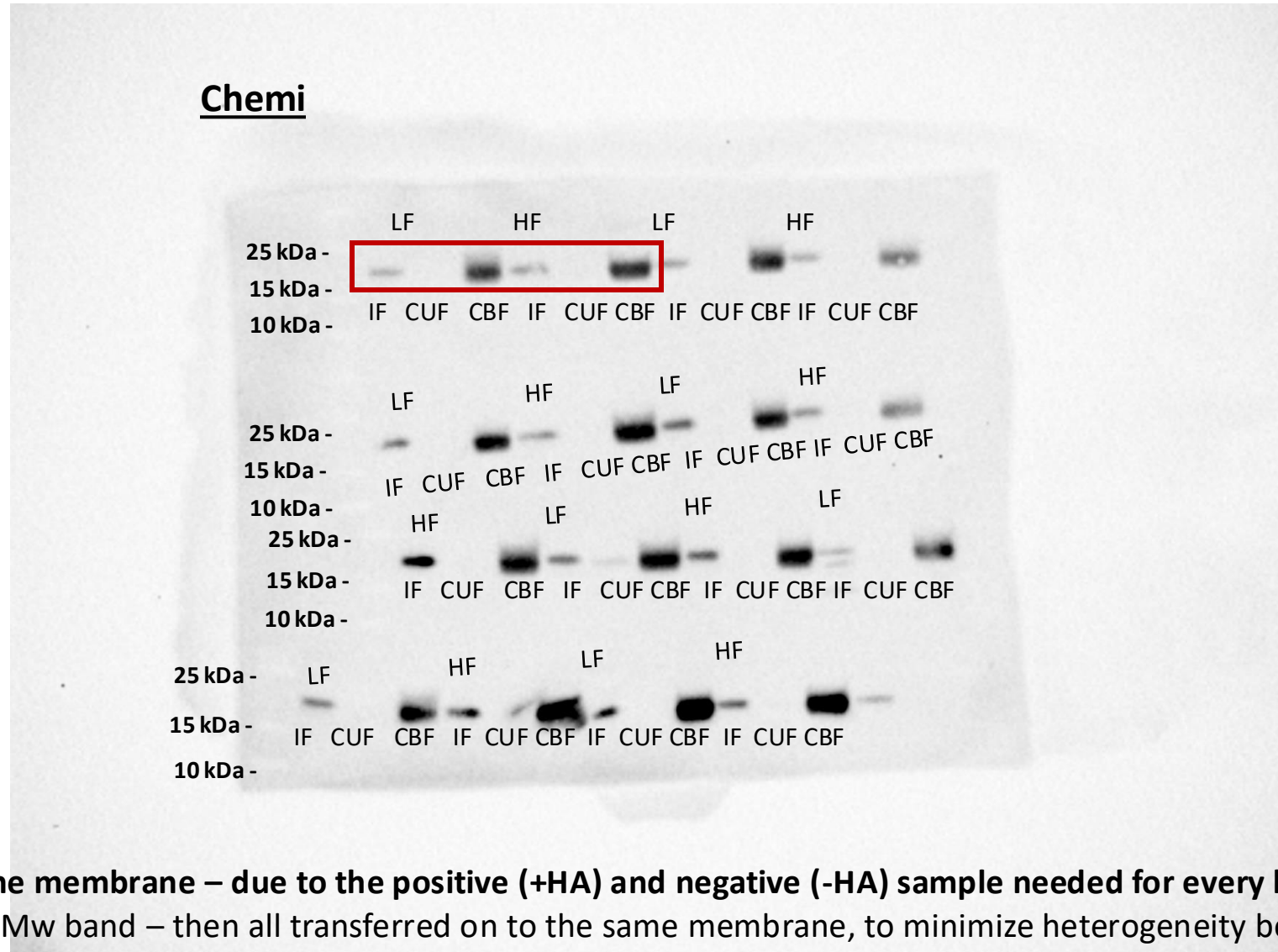
Supplemental Figure 2 C - CAV3



2 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

Gels cut just above the 25 Mw band and below the 10 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

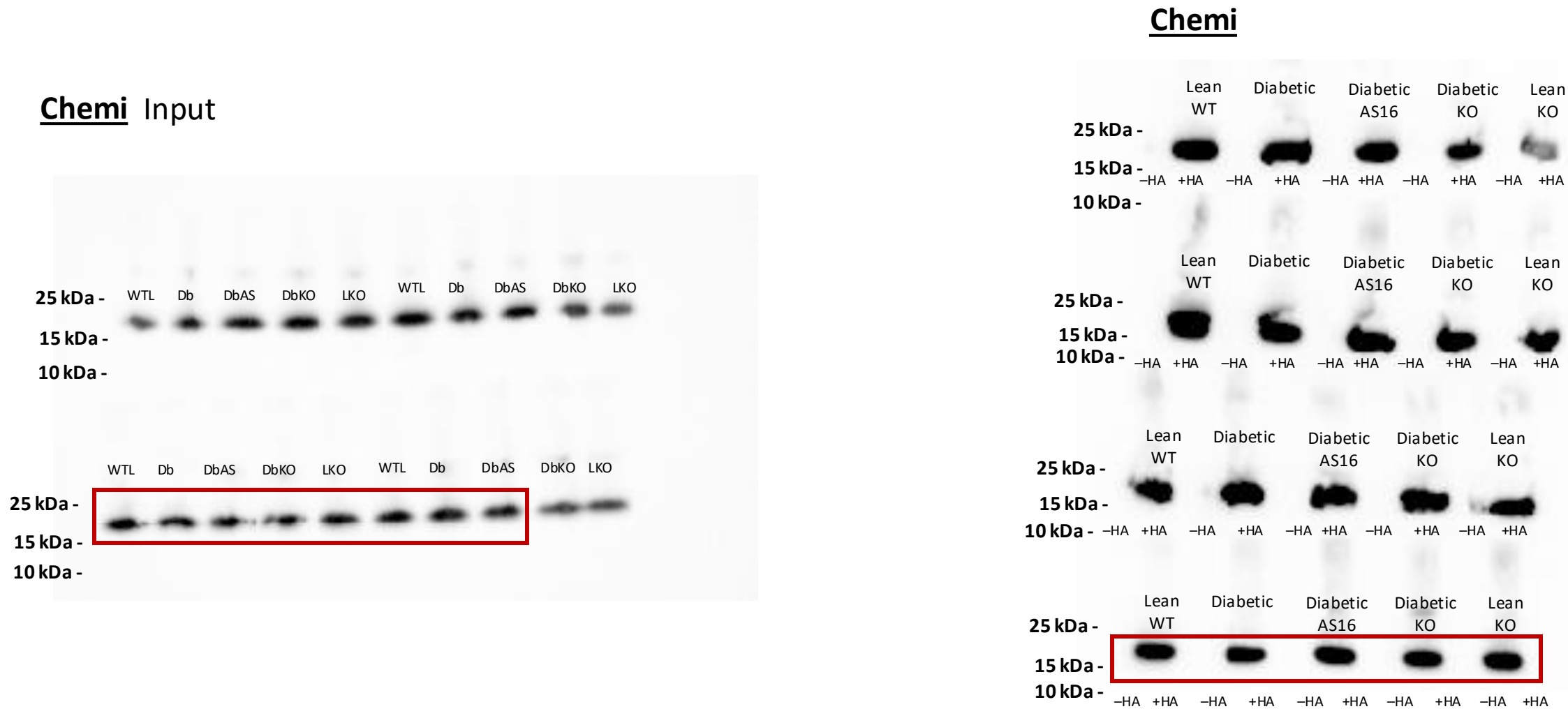
Supplemental Figure 3 A-B: CAV3 S-acylation



4 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

Gels cut just above the 25 Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Supplemental Figure 4A-B : CAV3 S-acylation



Input (two gels cut (just below 10 kDa and above 25 kDa) and transferred on the same membrane) from the same CD36 gels

Four gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart. Gels cut just above the 25 Mw band and below the 10Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Chemi Input

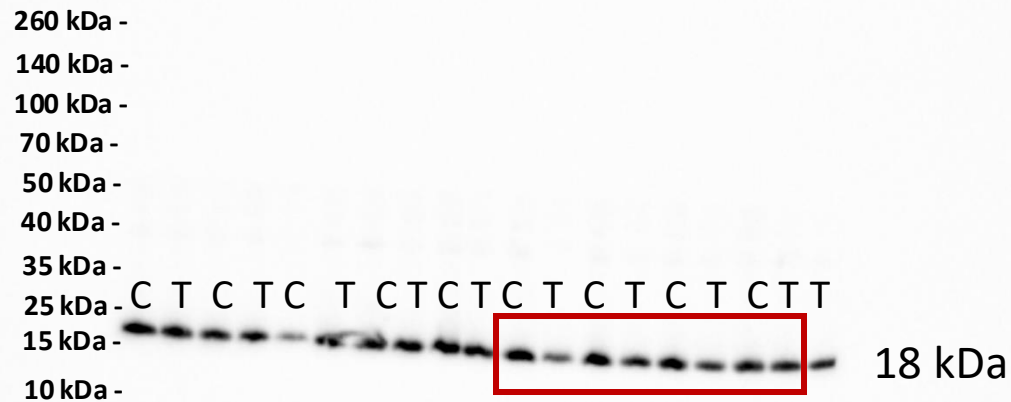


Western blot analysis of HA-tagged proteins. The blot shows bands at 25 kDa, 15 kDa, and 10 kDa. A red box highlights the 15 kDa band across all lanes, indicating consistent protein expression levels.

Gels cut just above the 25 Mw band and below the 10Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.

Supplemental Figure 6A-B : CAV3 S-acylation

Chemi Input



Uncropped gel and membrane

Chemi



2 gels transferred on to one membrane – due to the positive (+HA) and negative (-HA) sample needed for every heart

Gels cut just above the 25 Mw band and below the 10Mw band – then all transferred on to the same membrane, to minimize heterogeneity between samples through transfer, antibody incubation and developing.