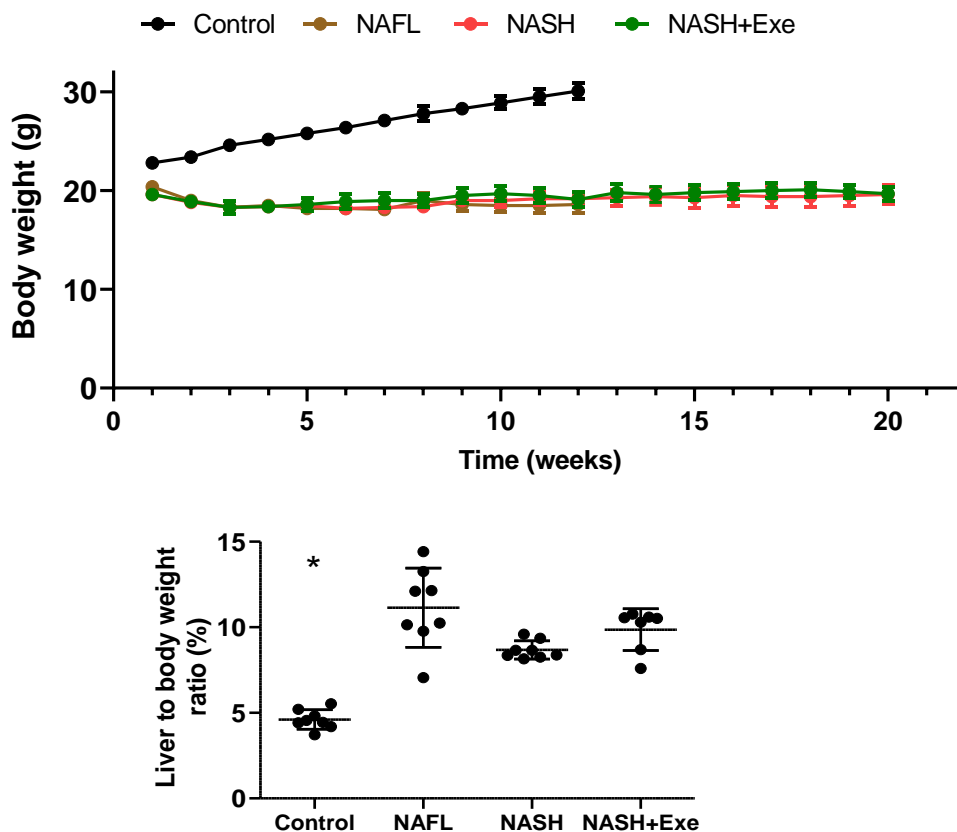


## Exercise attenuates the transition from fatty liver to steatohepatitis and reduces tumor formation in mice

Maria Guarino, Pavitra Kumar, Andrea Felser, Luigi M. Terracciano, Sergi Guixé-Muntet, Bostjan Humar, Michelangelo Foti, Jean-Marc Nuoffer, Marie V. St-Pierre and Jean-François Dufour

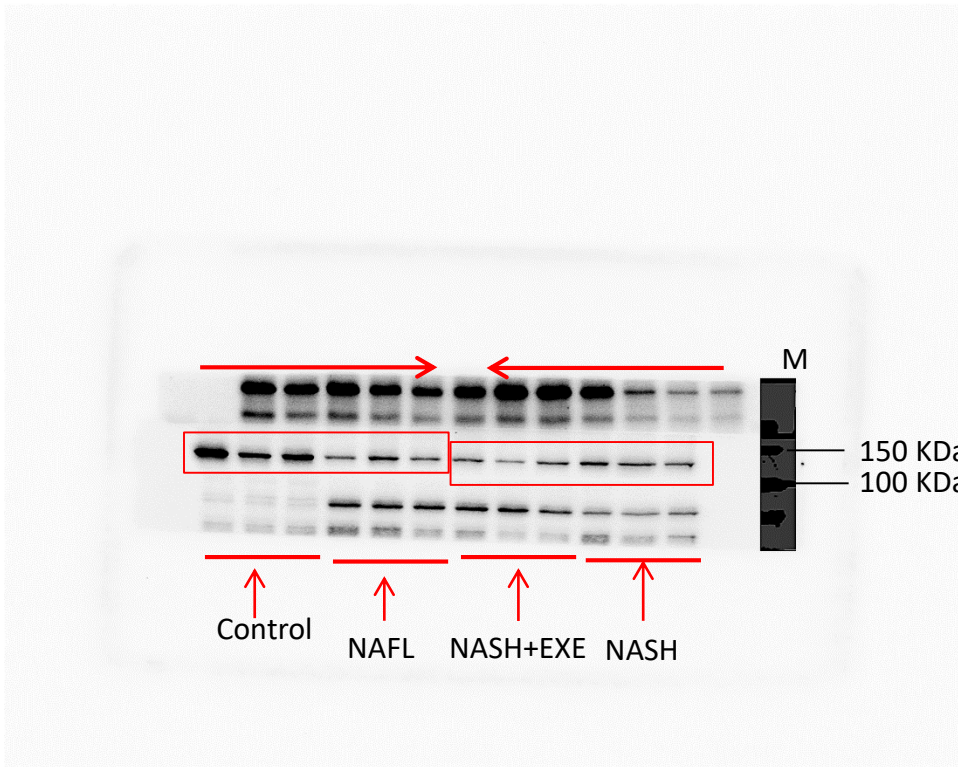
Figure S1



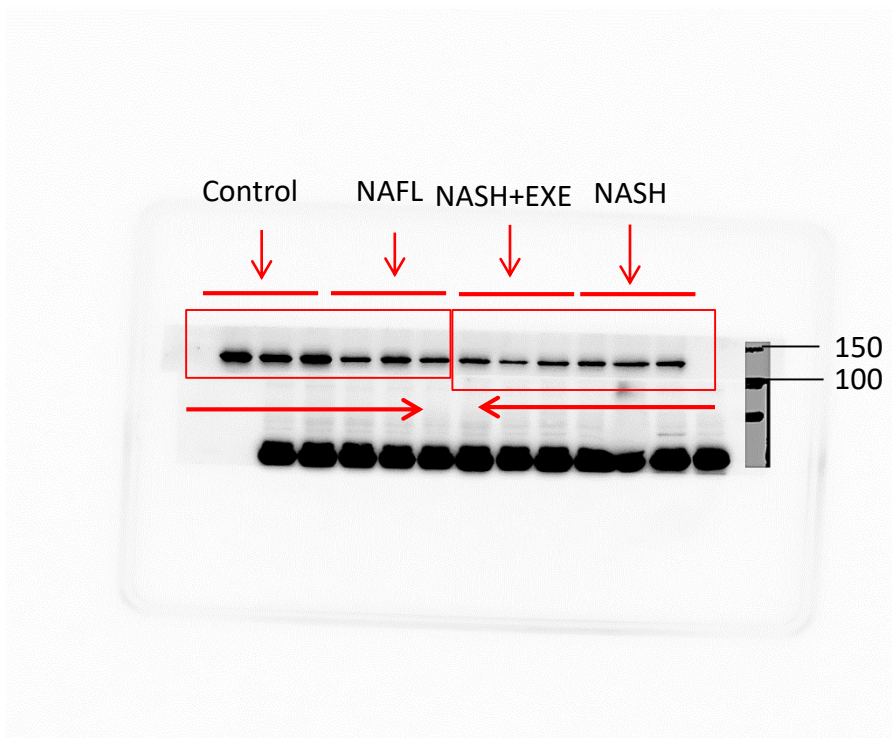
**Figure S1:** Comparison of body weight and of liver to body weight ratios. C57Bl/6N mice were fed a standard or choline-deficient high-fat (CD-HFD) diet. Mice were randomized to one of four groups: (1) control (n = 11) fed a standard diet for 12 weeks; (2) non-alcoholic fatty liver (NAFL) group (n = 11) fed a CD-HFD diet for 12 weeks; (3) non-alcoholic steatohepatitis (NASH) sedentary group (n = 11) fed a CD-HFD for 20 weeks; (4) NASH + exercise (EXE) group (n = 11) fed a CD-HFD for 20 weeks but with treadmill running at 12.5 m/min imposed from weeks 12 to 20. Body weights were recorded weekly after 7 days of habituation to the diet (upper panel). Tissues were harvested after 12 weeks (control, NAFL) or 20 weeks (NASH sedentary, NASH+ EXE) (lower panel). The liver/body weight ratio was increased in all groups fed the CD-HFD. ( $p < 0.05$ , One way ANOVA).

## Figure S2. Unabridged western blot images

### Figure 5D



P-ATPCL  
(predicted MW  
125 KDa)



ATPCL (predicted  
MW 125 KDa)

Figure 5D continued

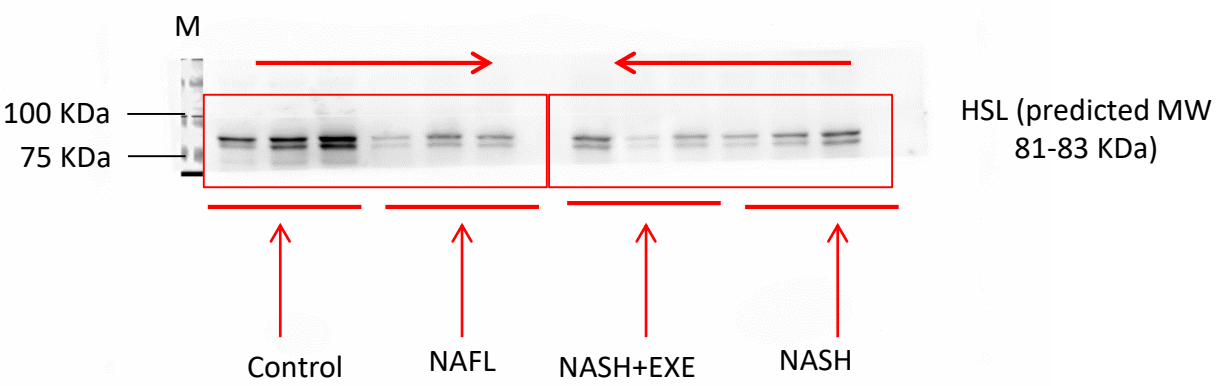
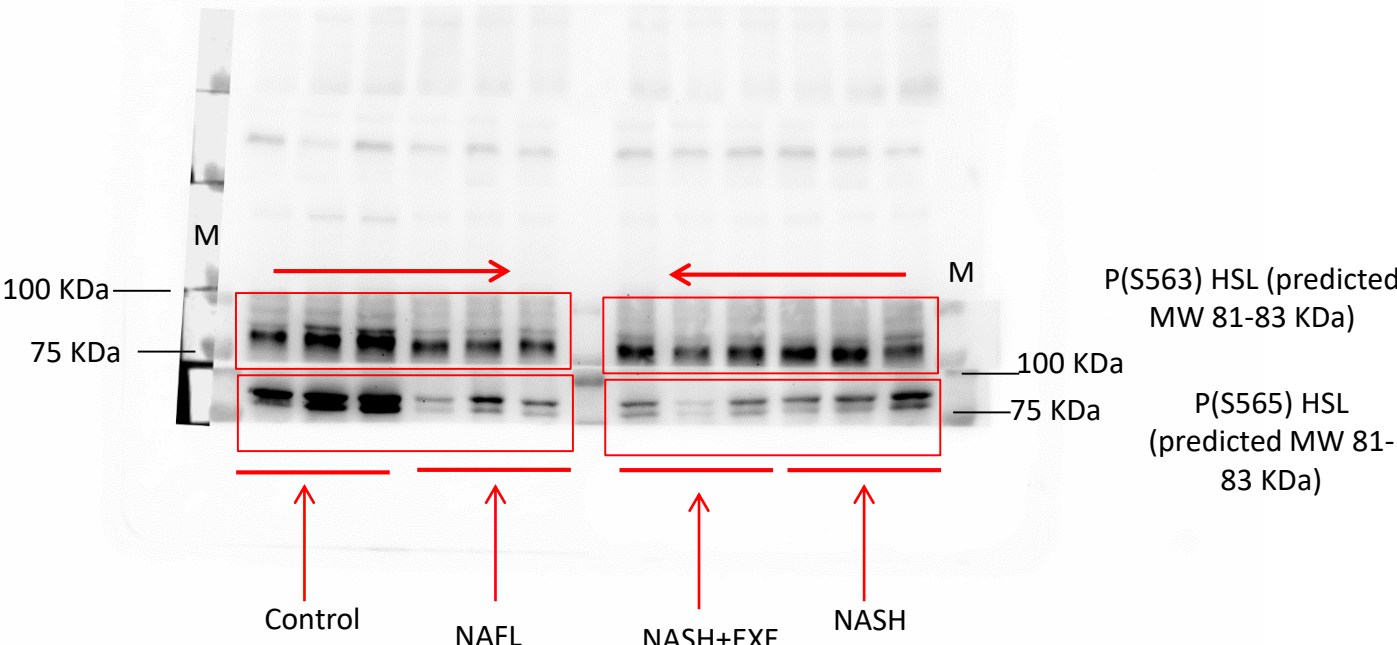


Figure 5D continued

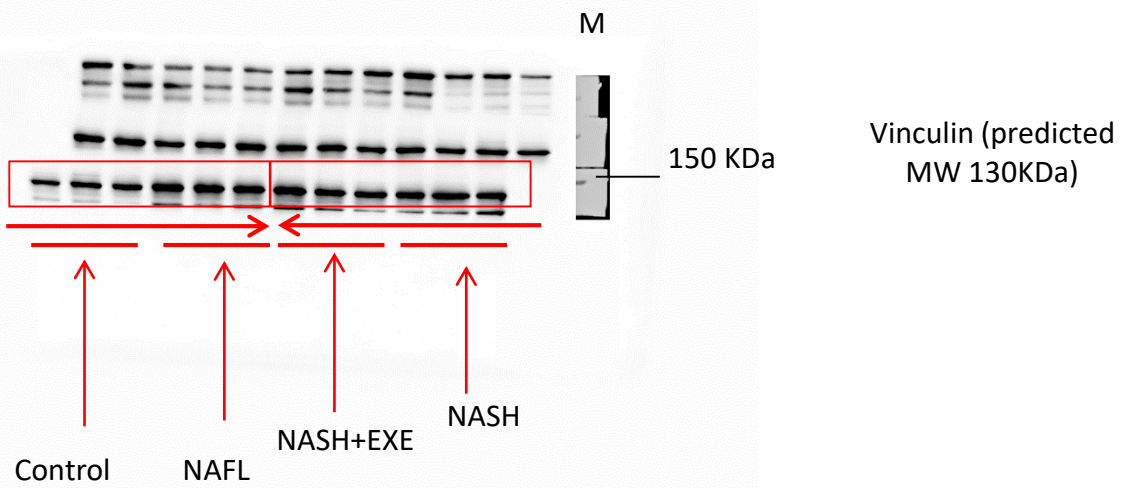
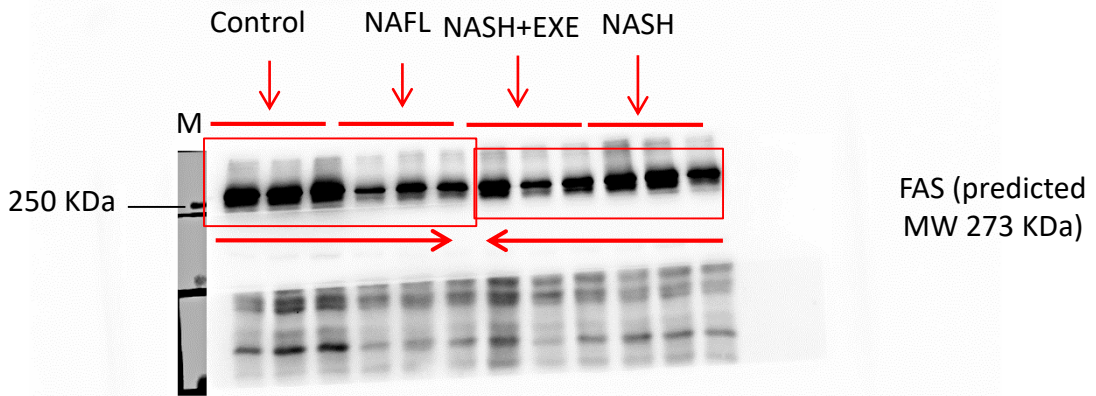


Figure 5D continued

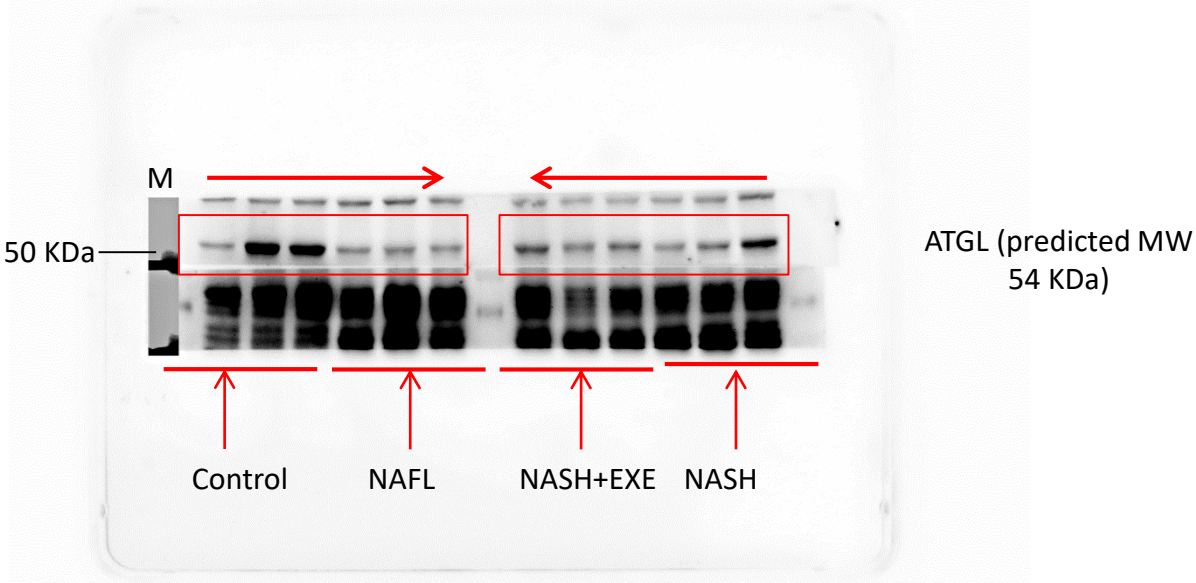
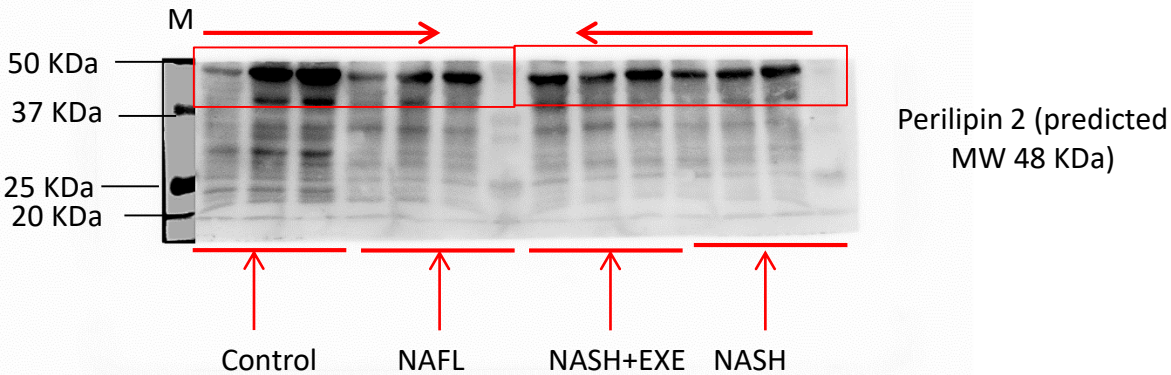
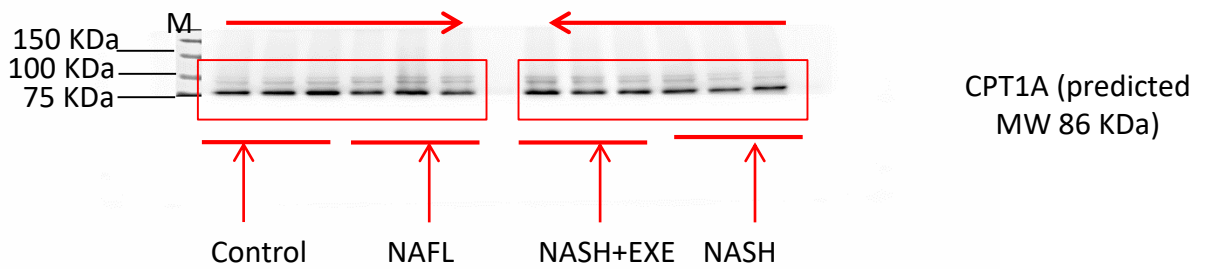
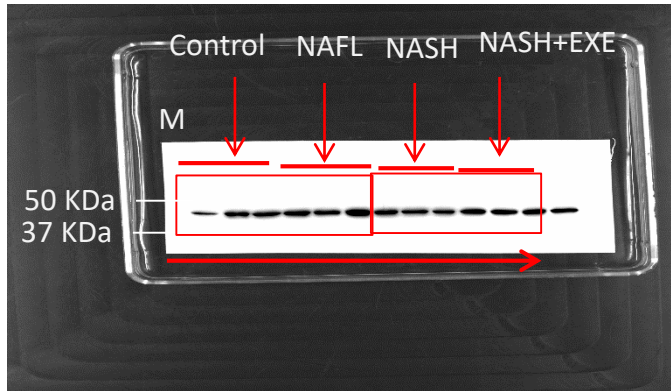


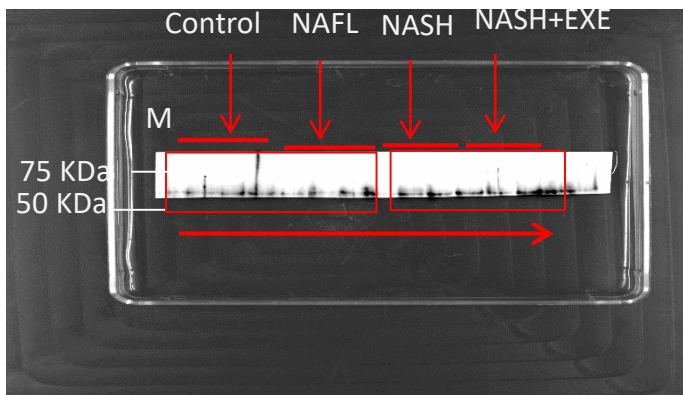
Figure 5D continued



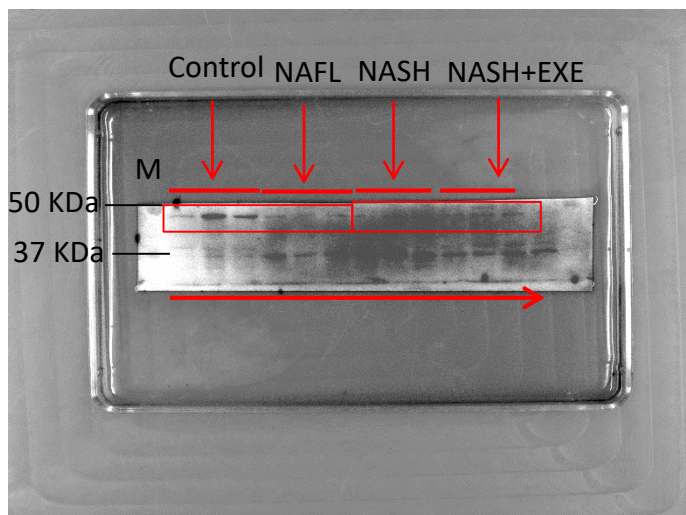
# Figure 5D continued



MCAD (predicted  
MW 47 KDa)



DGAT1 (predicted  
MW 60 KDa)



DGAT2 (predicted  
MW ~47 KDa)

**Figure 7**

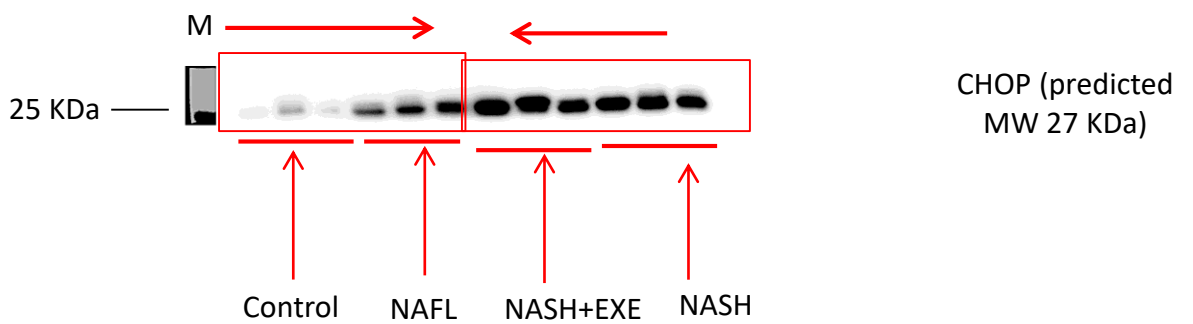
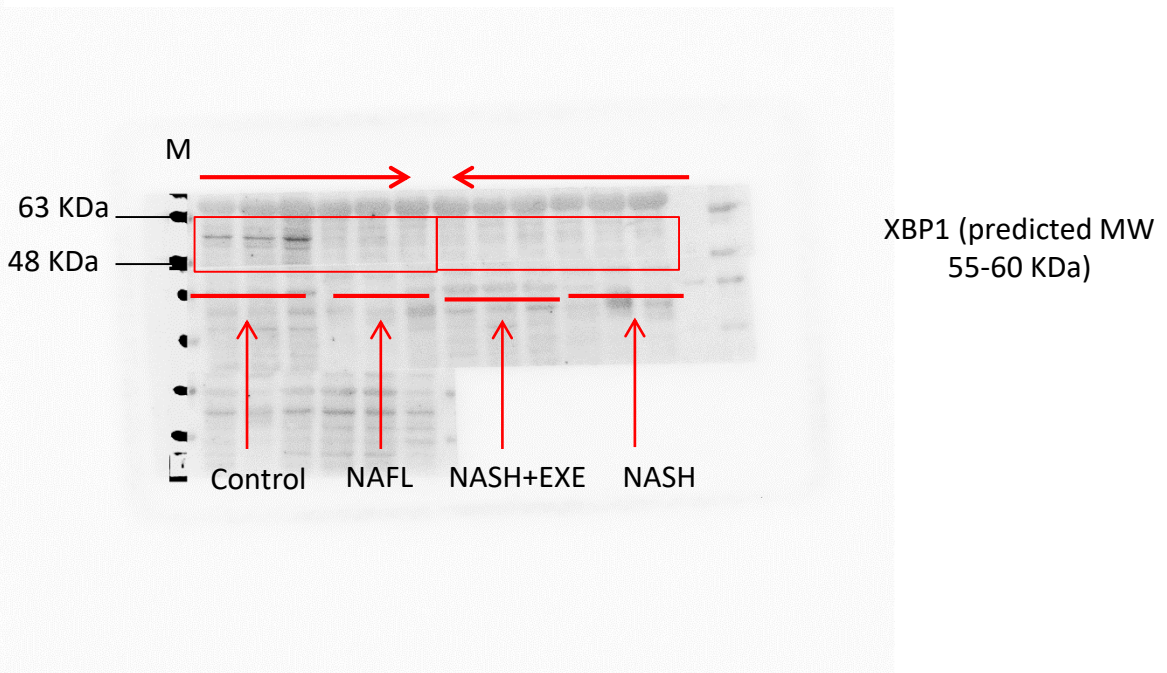
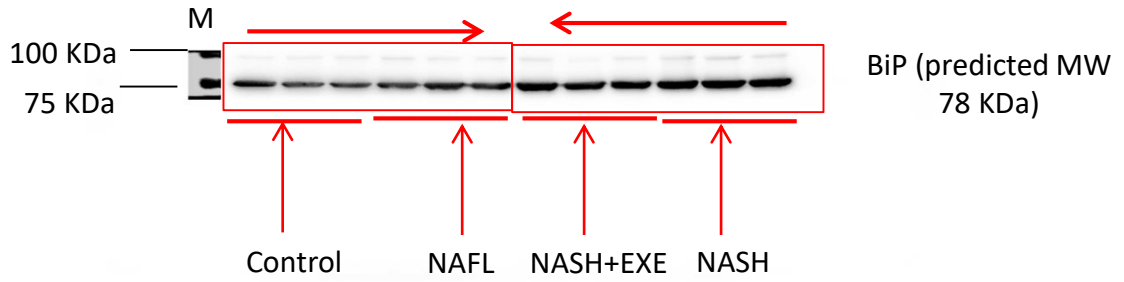
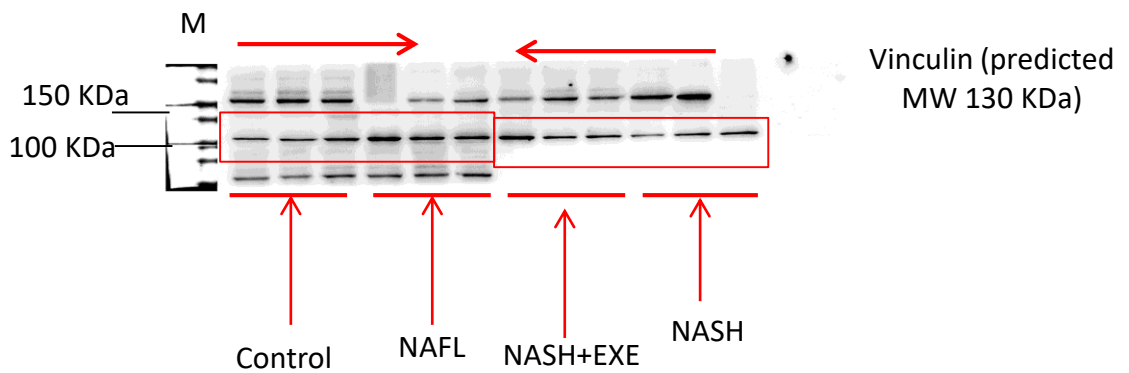
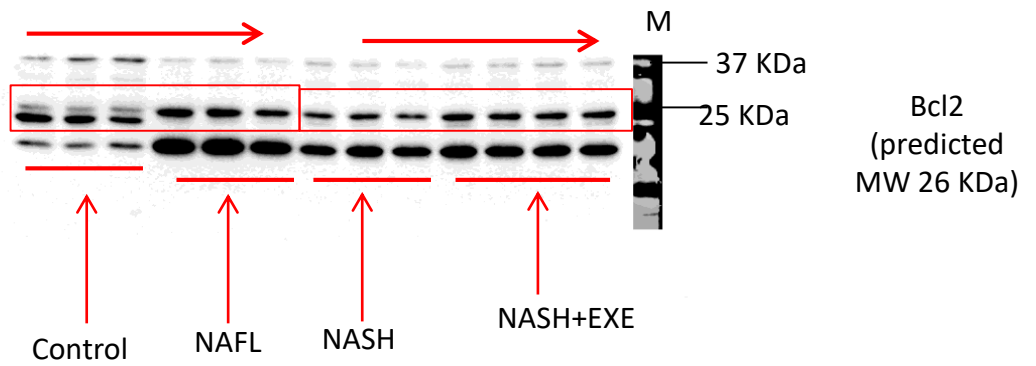
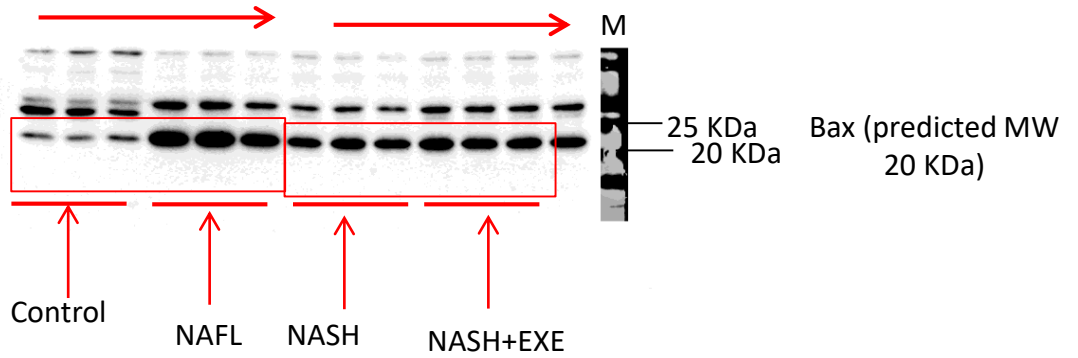




Figure 7 continued



**Figure 8**

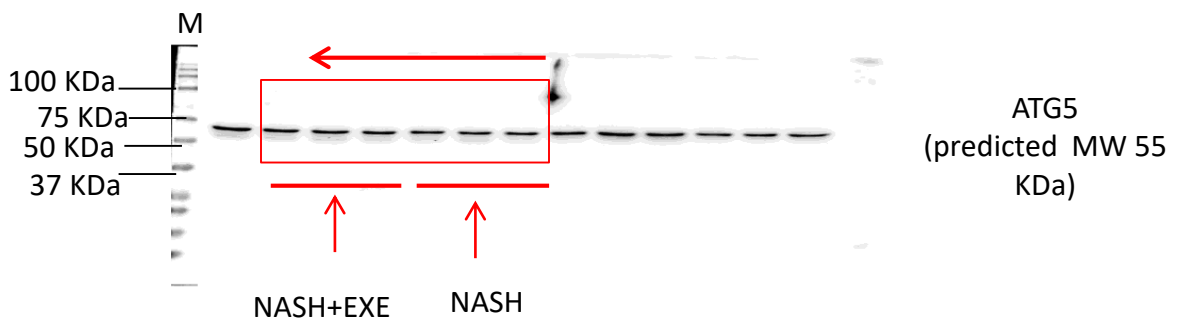
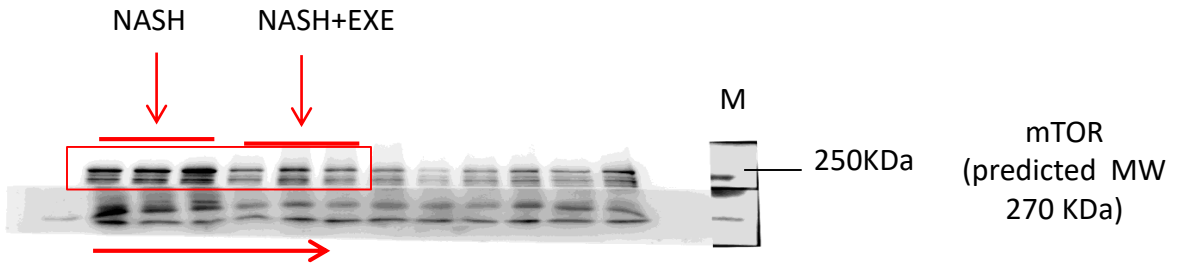
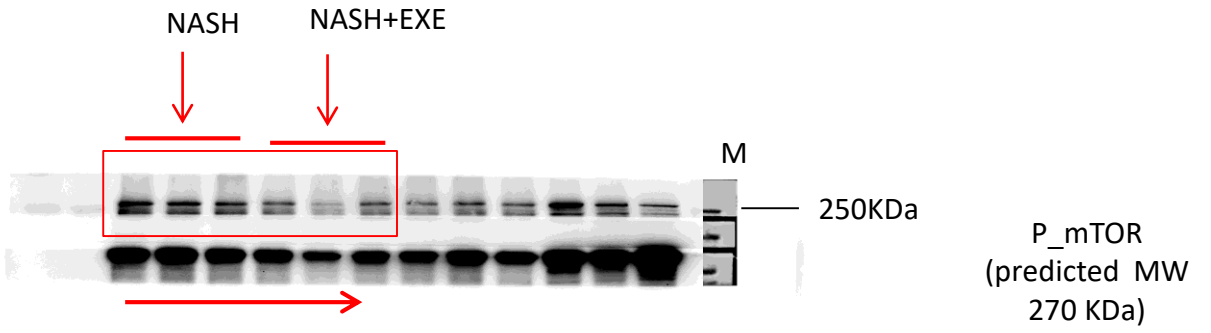


Figure 8 continued

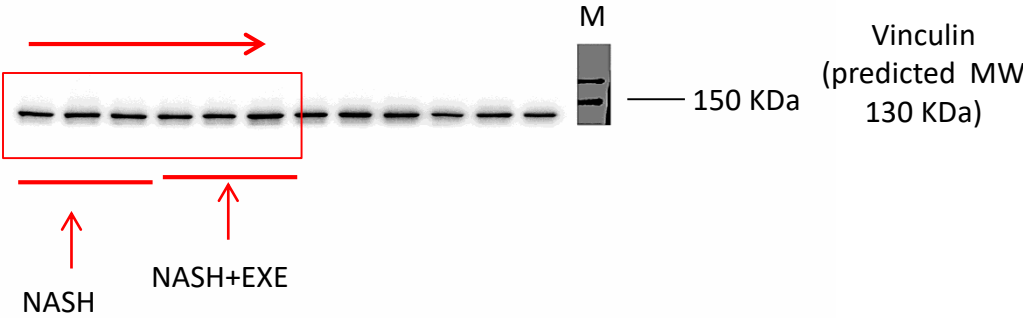
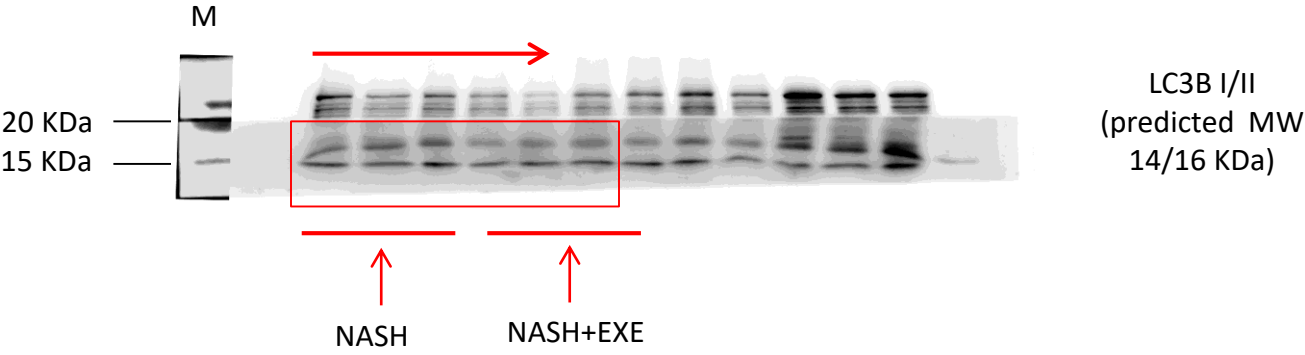
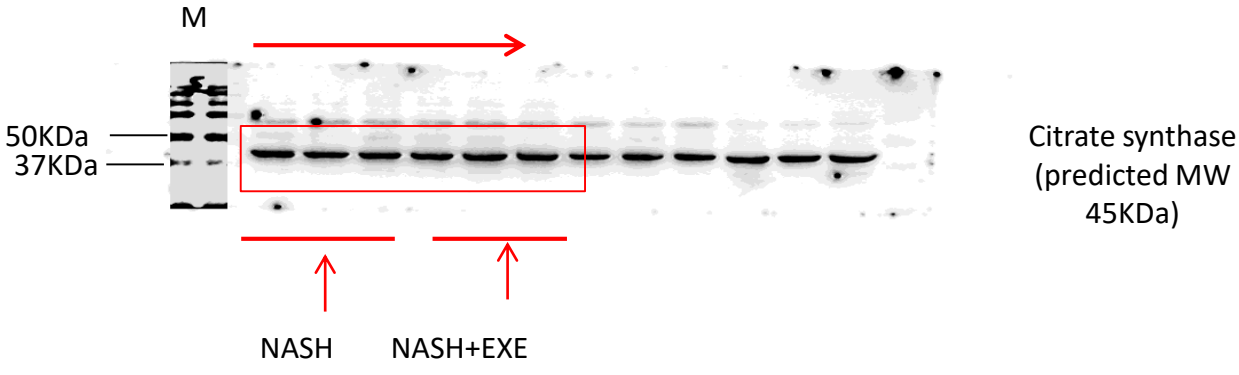
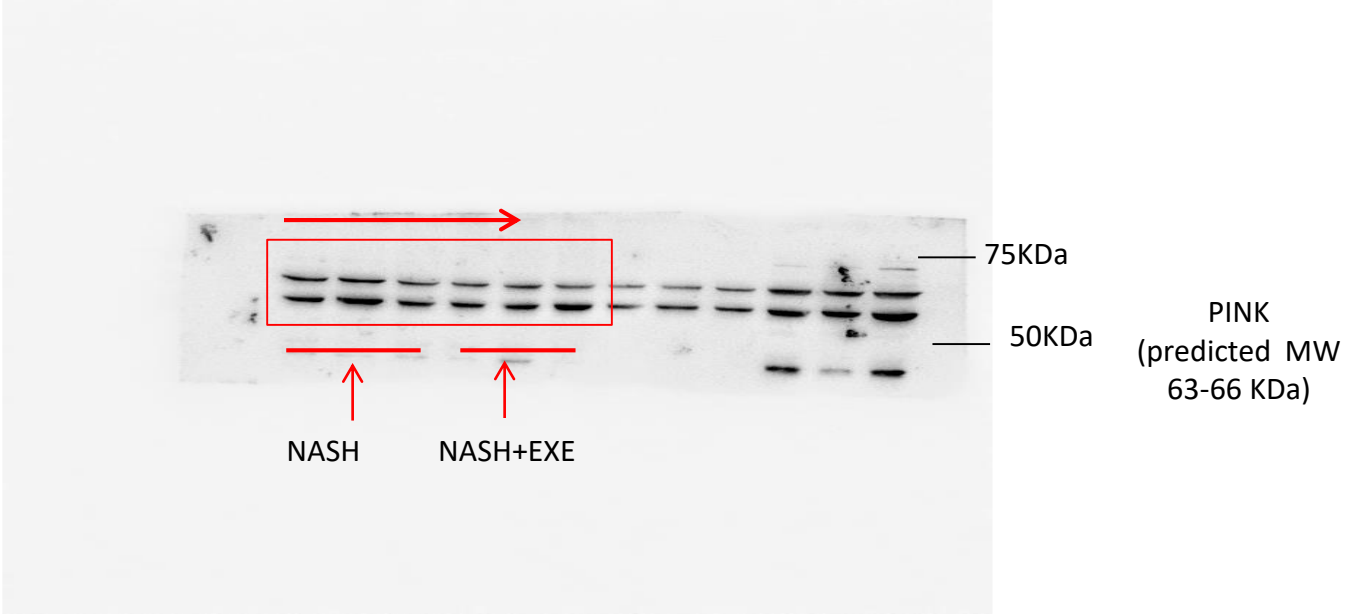
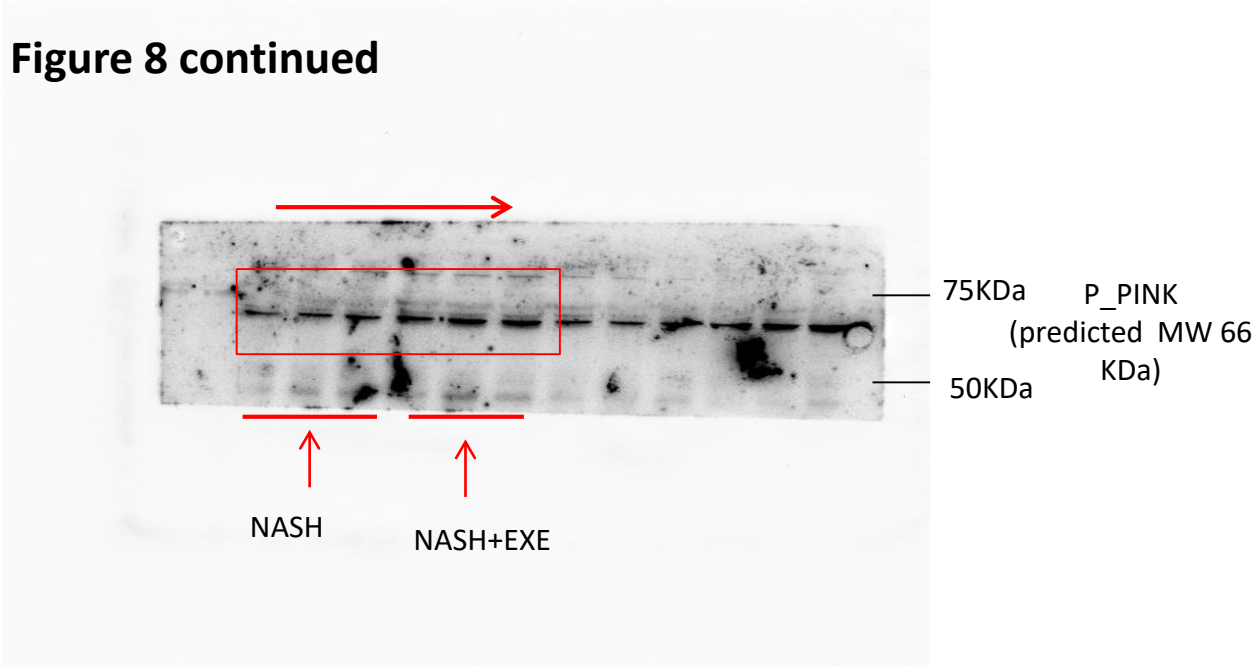


Figure 8 continued



**Figure 9C**

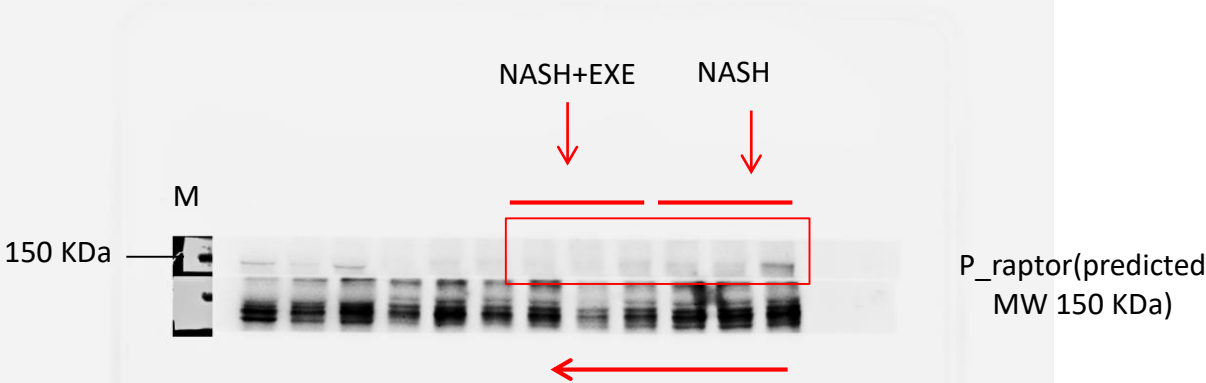
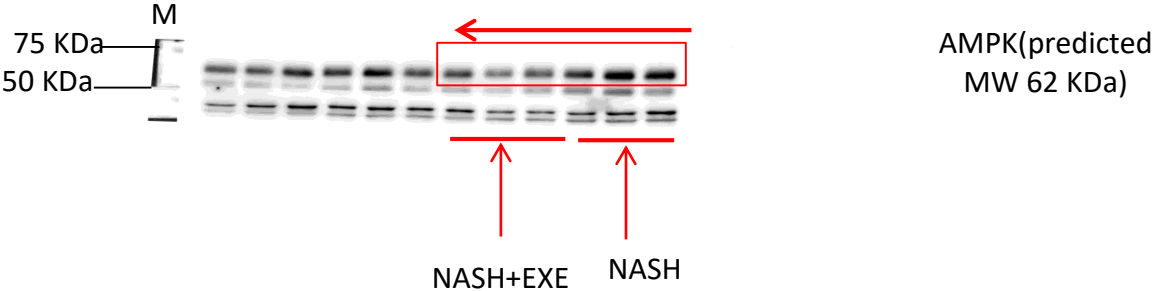
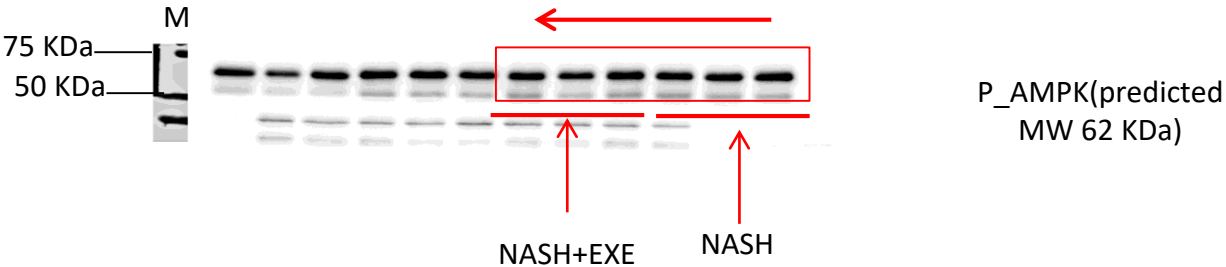


Figure 9C continued

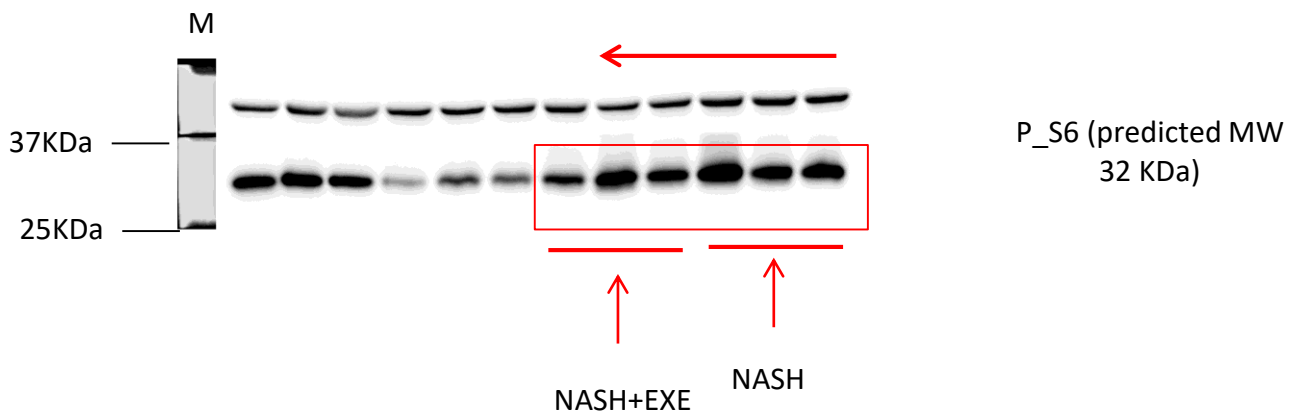
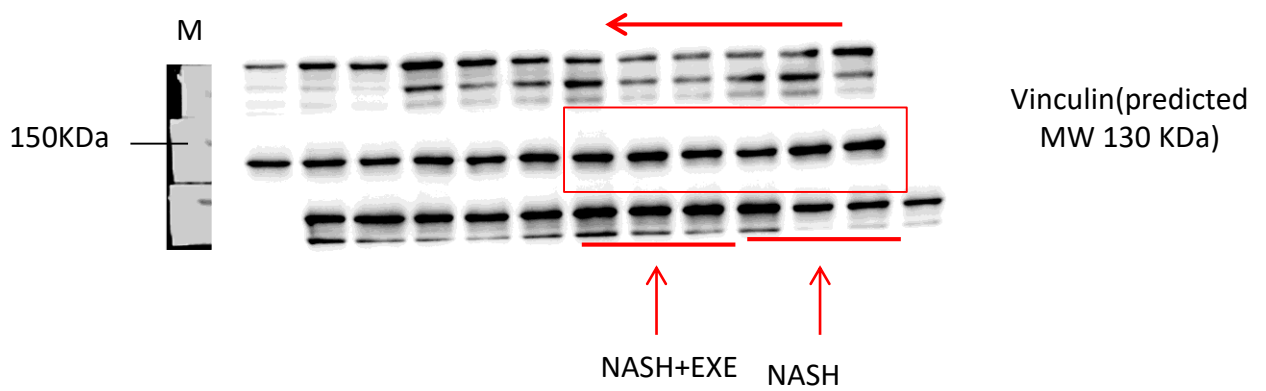
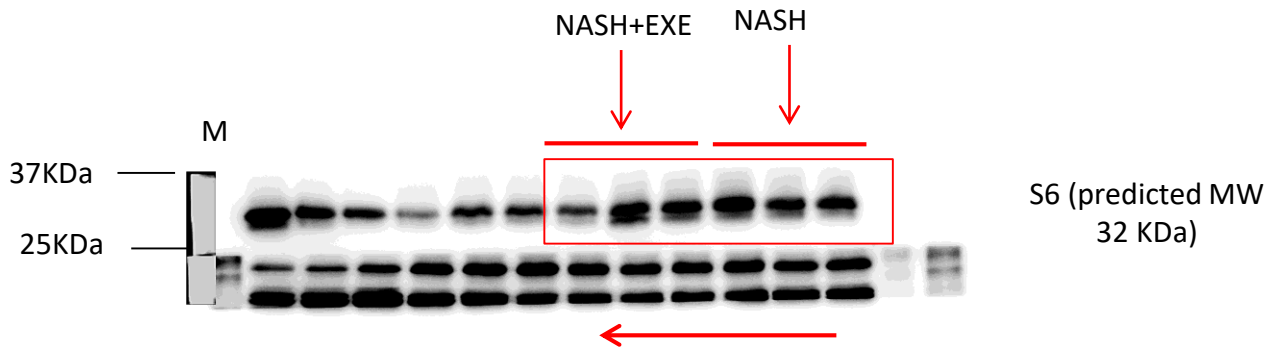


Figure 9C continued



**Figure 10 D**

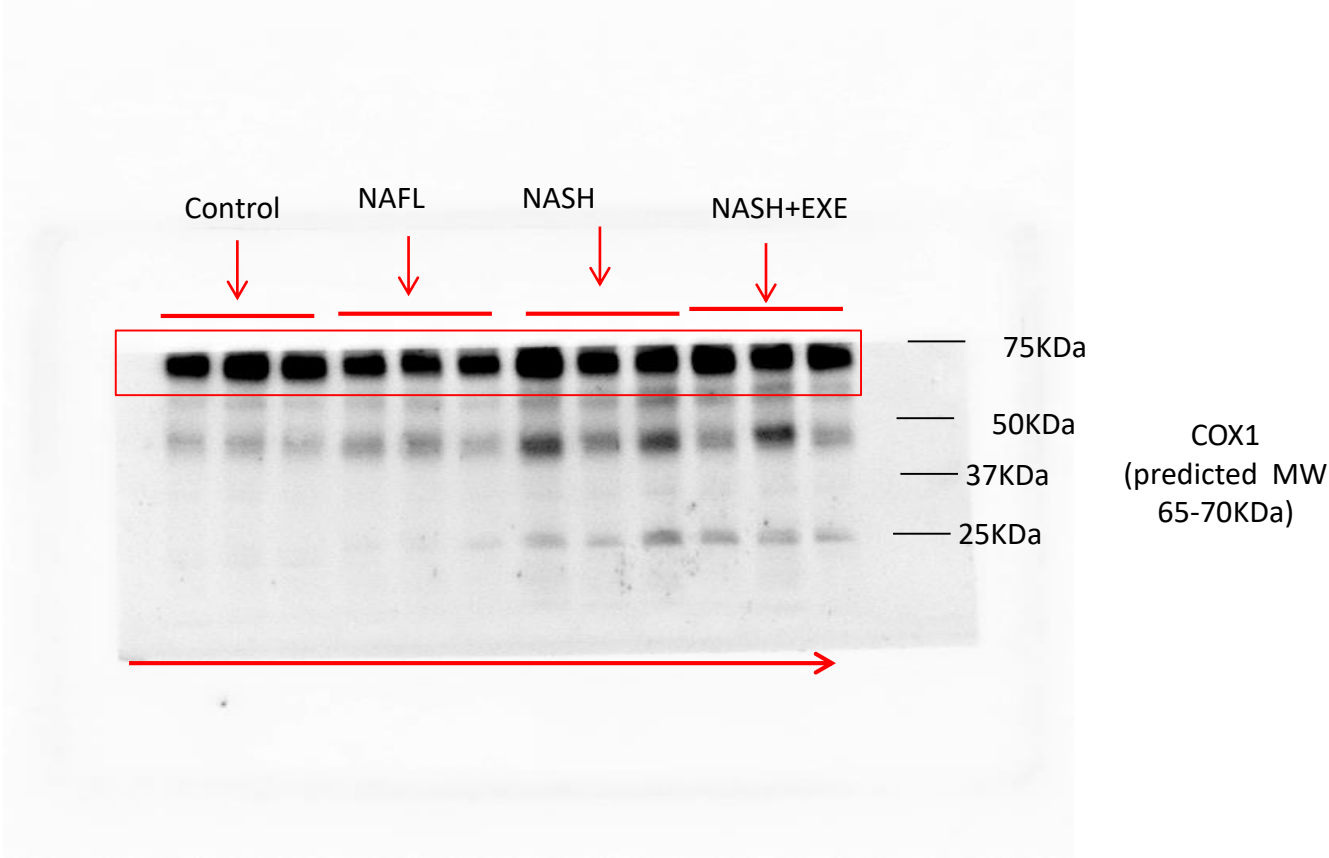
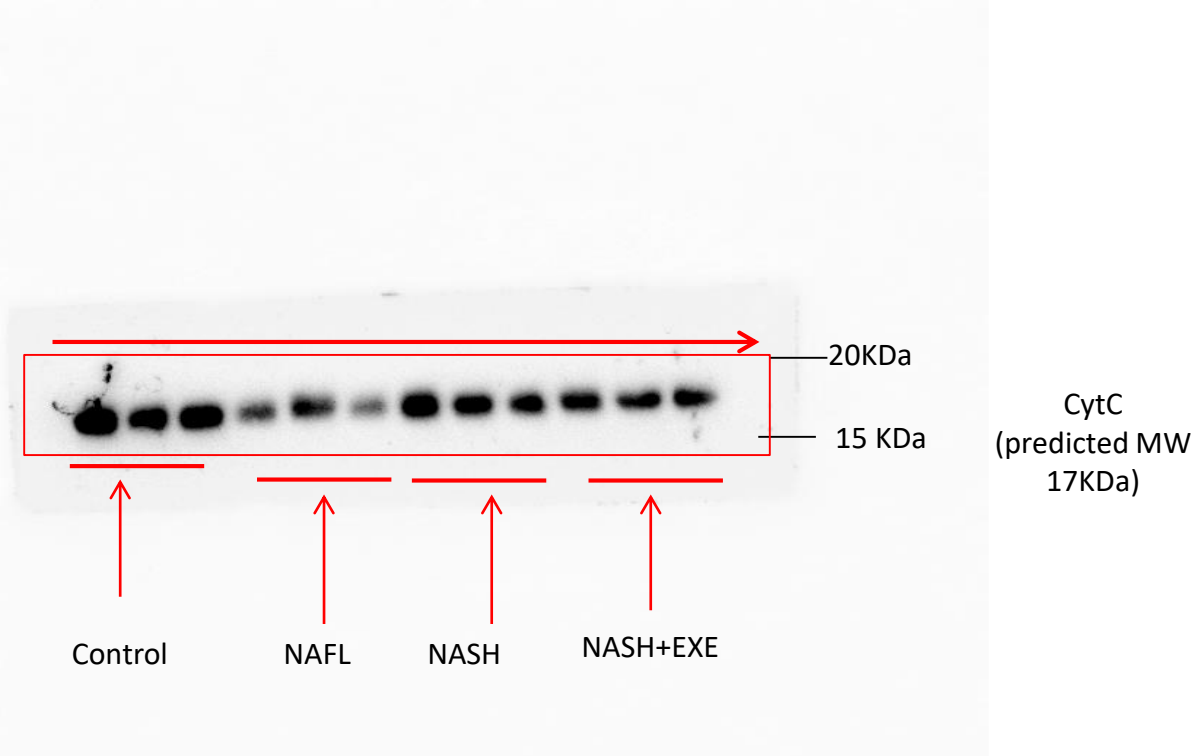
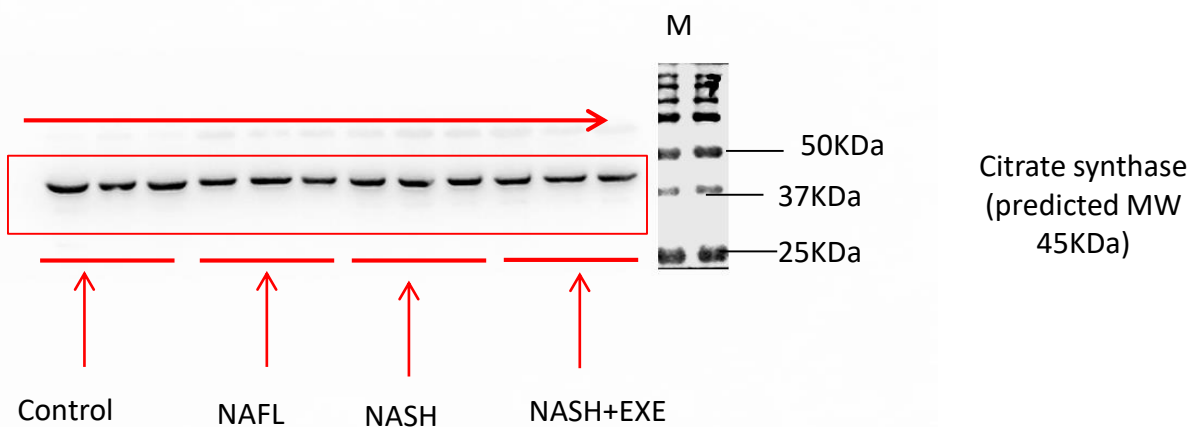
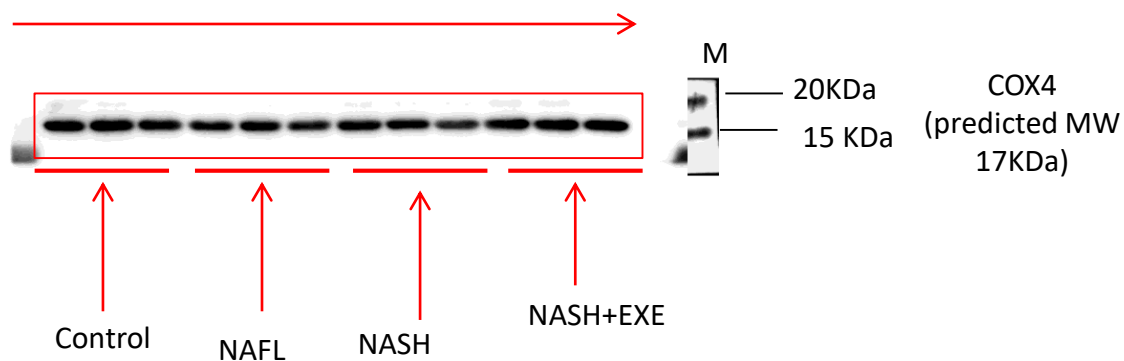
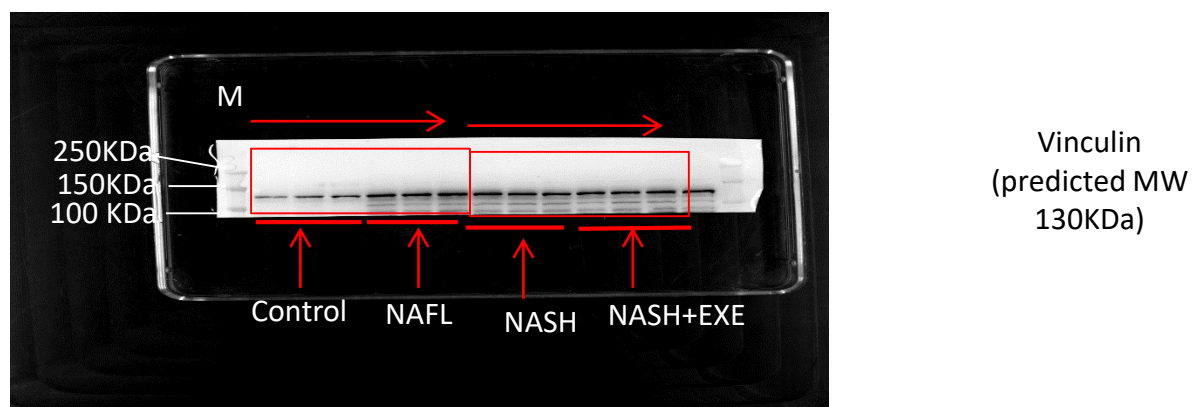
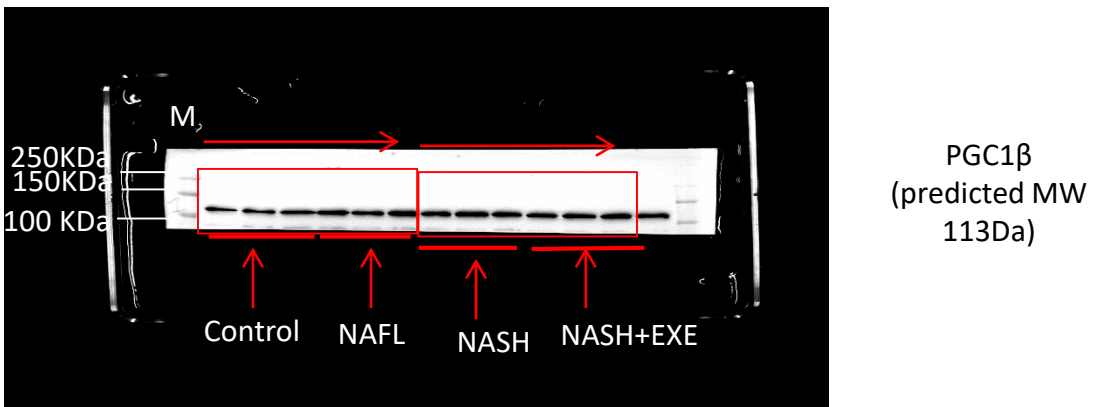
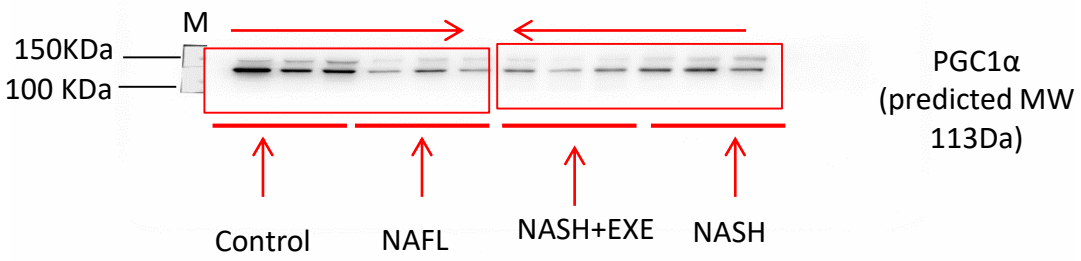




Figure 10 D continued



**Figure 10 E**



**Figure 11B**

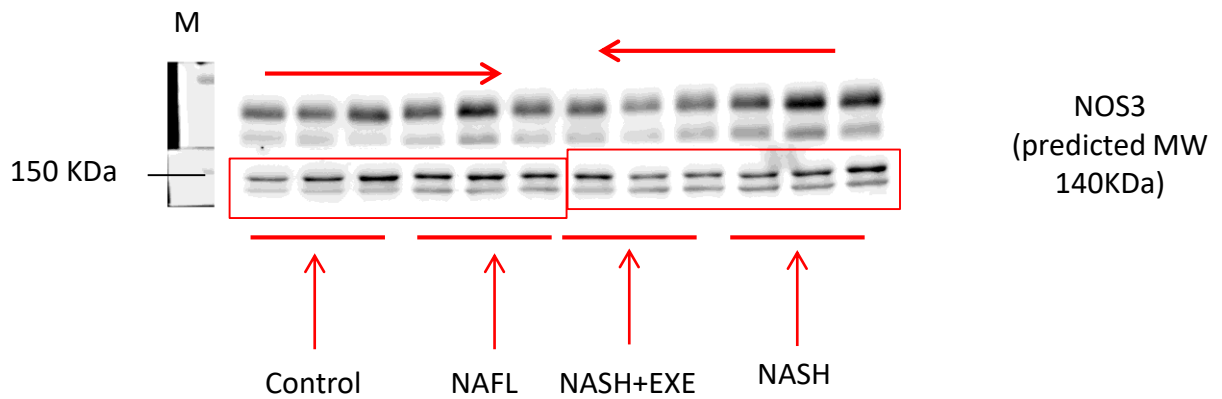
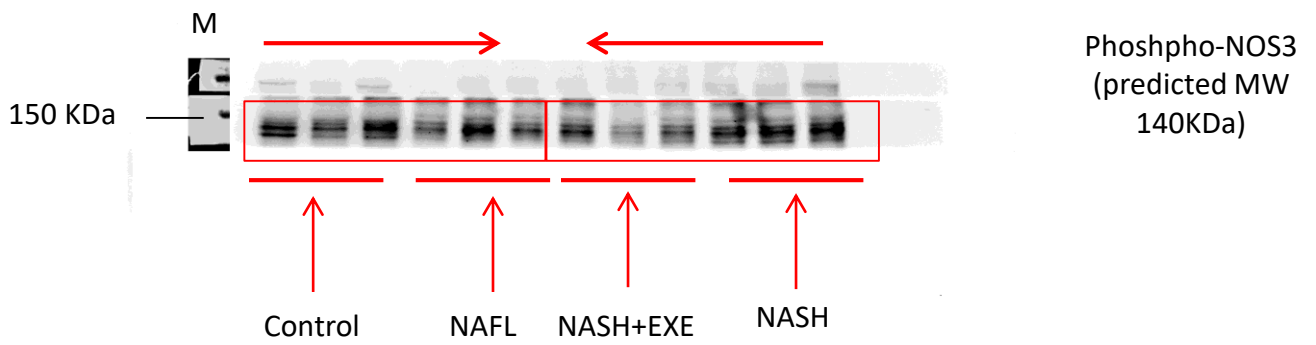
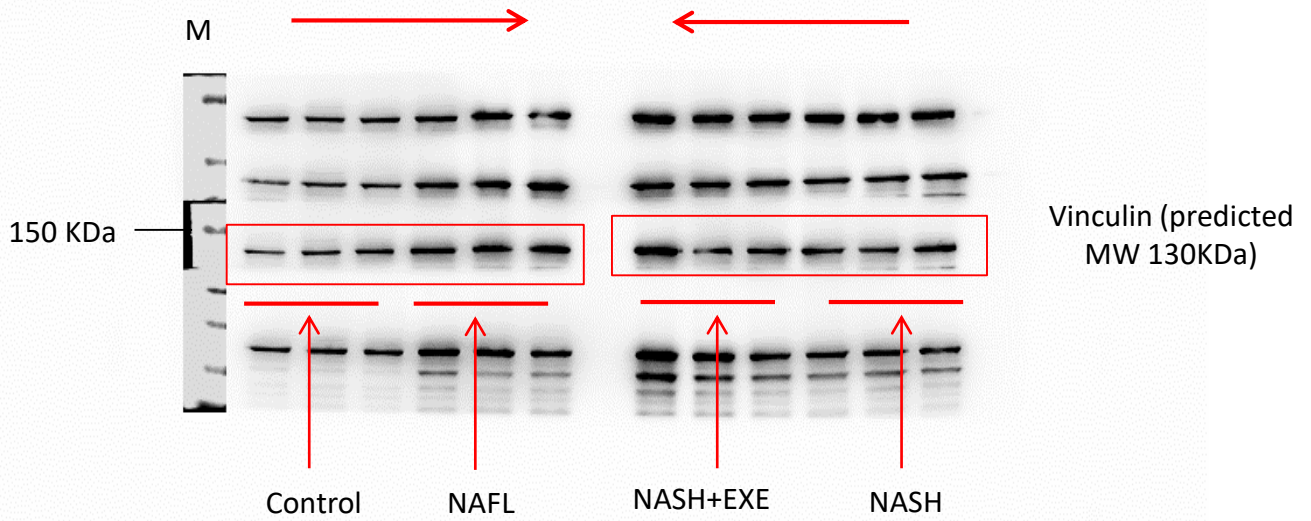
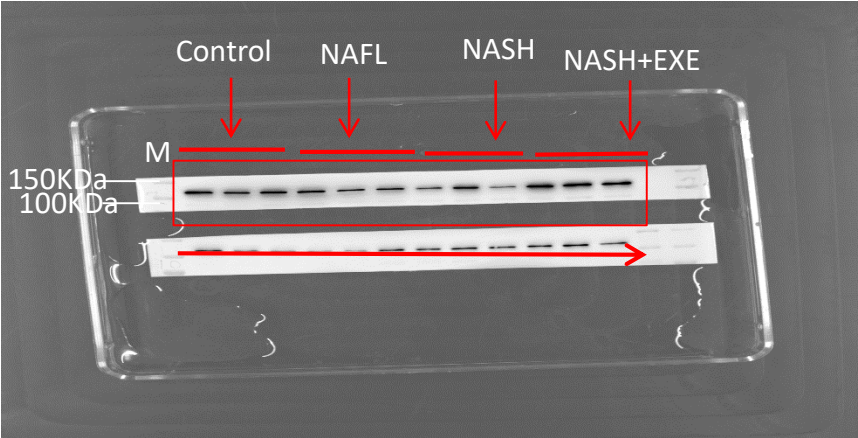
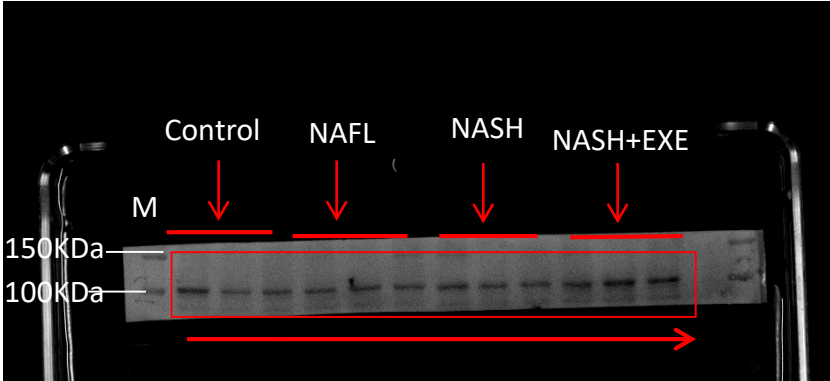


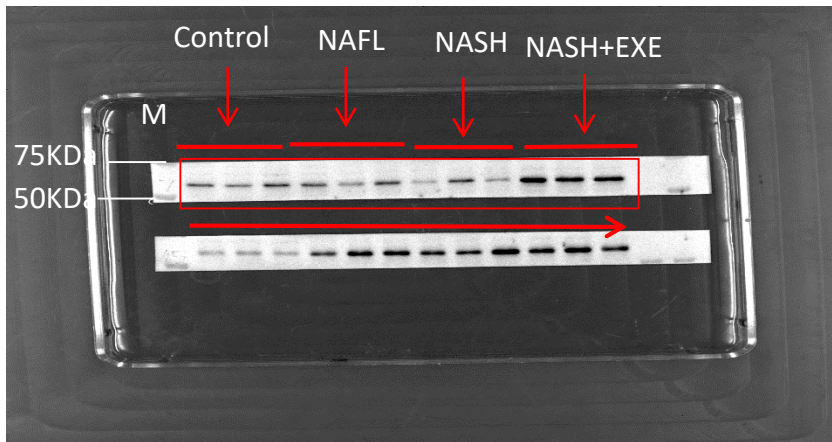
Figure 11B continued



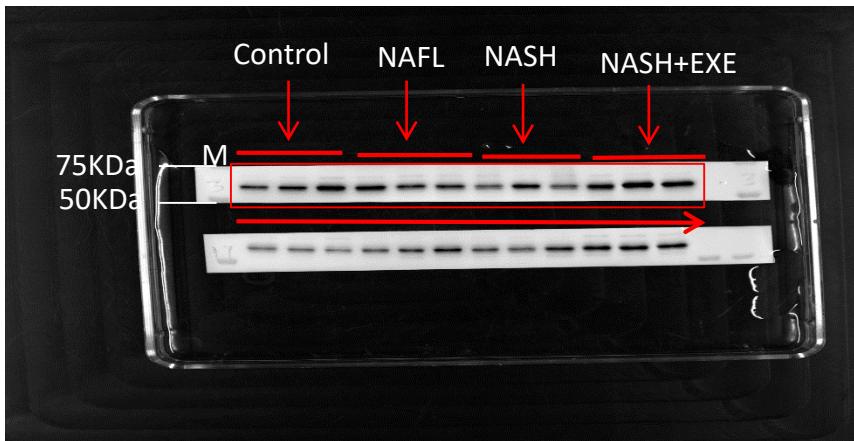
**Figure 12**



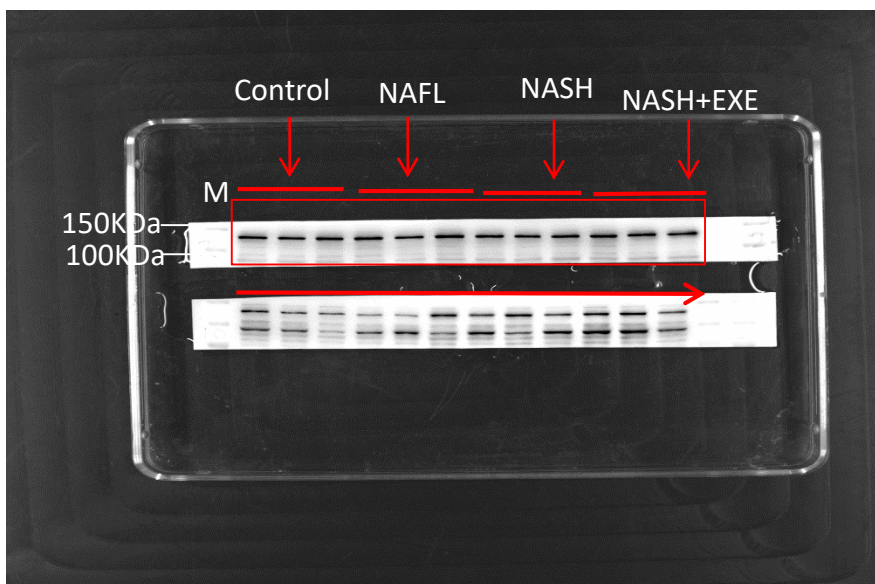
**Figure 12 continued**



Phospho Akt  
(predicted MW  
60Da)



Akt  
(predicted MW  
60Da)



Vinculin  
(predicted MW  
130KDa)

**Table S1**

Composition of standard and choline-deficient high-fat diets		
Ingredient	Standard diet (E15668-04) %	CD-HFD (E15673-94) %
Corn starch, pre-gelatinated	40.00	10.00
Maltodextrin	10.00	6.00
Sucrose	18.29	22.00
Cellulose, microcrystalline	5.00	4.95
<b>Amino acid mixture</b>	<b>14.53</b>	<b>14.37</b>
Vitamin premix AIN w/o choline	1.00	1.00
Mineral & trace element premix	3.50	3.50
Choline Cl (50%)	0.25	-
Sodium bicarbonate	0.43	0.43
Cholesterol	-	2.10
Coconut oil, hydrogenated	-	-
Corn oil	5.00	-
Pork lard	-	34.60
Soybean oil	2.00	1.00
<b>Composition of the amino acid mixture</b>		
L-Lysine HCl	0.91	0.91
L-Methionine	0.40	0.17
L-Cystine	0.30	0.37
L-Threonine	0.46	0.46
<b>Energy contribution of diet components</b>		
Energy (Atwater) MJ/Kg	16.60	22.50
Kcal% Protein	12.00	9.00
Kcal% Fat	16.00	60.00
Kcal% Carbohydrates	72.00	31.00

The diets and their compositions were provided by Ssniff Spezialdiäten GmbH, Germany.

<b>Table S2</b>			
<b>S.No</b>	<b>Reagent</b>	<b>Source</b>	<b>Identifier</b>
1	Rabbit polyclonal anti-phospho ATP citrate lyase	Cell Signaling Technology	Cat #4331
2	Rabbit polyclonal anti-ATP citrate lyase	Cell Signaling Technology	Cat #4332
3	Rabbit monoclonal anti-fatty acid synthase	Cell Signaling Technology	Cat #3180
4	Rabbit polyclonal anti-Phospho (S563)-HSL	Cell Signaling Technology	Cat #4139
5	Rabbit polyclonal anti-Phospho (S565)-HSL	Cell Signaling Technology	Cat #4137
6	Rabbit polyclonal anti-HSL	Cell Signaling Technology	Cat #4107
7	Rabbit polyclonal anti-perilipin 2	Proteintech	Cat #15294-1-AP
8	Rabbit monoclonal anti-ATGL	Cell Signaling Technology	Cat #2439
9	Rabbit polyclonal anti-PPAR $\alpha$	Santa Cruz Biotechnology	Cat #sc-9000
10	Rabbit polyclonal anti-CPT1A	Proteintech	Cat #15184-1-AP
11	Mouse monoclonal anti-MCAD	Abcam	Cat #ab110296
12	Rabbit polyclonal anti-DGAT1	Abnova	Cat #PAB8674
13	Goat polyclonal anti-DGAT2	Novus Biologicals	Cat #NB100-57851
14	Mouse monoclonal anti-vinculin	Merck Milipore	Cat #CP74
15	Rabbit polyclonal anti-(GRP78) BiP	Thermo Fisher	Cat #PA5-34941
16	Rabbit polyclonal anti-XBP1s	Cell Signaling Technology	Cat #83418
17	Mouse monoclonal anti-CHOP	Cell Signaling Technology	Cat #2895
18	Rabbit polyclonal anti-Bax	Cell Signaling Technology	Cat #2772
19	Rabbit polyclonal anti-Bcl2	Cell Signaling Technology	Cat #2876
20	Rabbit polyclonal anti-phospho mTOR	Cell Signaling Technology	Cat #2971



<b>Table S2 continued</b>			
<b>S.No</b>	<b>Reagent</b>	<b>Source</b>	<b>Identifier</b>
21	Rabbit monoclonal anti-mTOR	Cell Signaling Technology	Cat #2983
22	Rabbit monoclonal anti-ATG5	Cell Signaling Technology	Cat #12994
23	Rabbit polyclonal anti-LC3B	Abcam	Cat #ab48394
24	Rabbit polyclonal anti-phospho PINK	Affinity Biosciences	Cat #AF7081
25	Rabbit polyclonal anti-PINK	Cayman Chemical	Cat #10006283
26	Rabbit monoclonal anti-citrate synthase	Cell Signaling Technology	Cat #14309
27	Rabbit polyclonal anti-phospho AMPK	Cell Signaling Technology	Cat #2531
28	Rabbit polyclonal anti-AMPK	Cell Signaling Technology	Cat #2532
29	Rabbit polyclonal anti-phospho raptor	Cell Signaling Technology	Cat #2083
30	Rabbit monoclonal anti-raptor	Cell Signaling Technology	Cat #2280
31	Rabbit polyclonal anti-phospho S6	Cell Signaling Technology	Cat #2215
32	Rabbit monoclonal anti-S6	Cell Signaling Technology	Cat #2217
33	Rabbit polyclonal anti-cytochrome C	Cell Signaling Technology	Cat #4272
34	Rabbit polyclonal anti-(MTCO1)COX1	Novus Biologicals	Cat #NBP2-29949
35	Rabbit polyclonal anti-COXIV	Proteintech	Cat #11242-1-AP
36	Mouse monoclonal anti-PGC1 $\alpha$	Merck Milipore	Cat #ST1202
37	Rabbit monoclonal anti-PGC1 $\beta$	Abcam	Cat #ab176328
38	Rabbit polyclonal anti-phospho NOS3	Cell Signaling Technology	Cat #9571
39	Mouse monoclonal anti-NOS3	BD biosciences	Cat #610297
40	Rabbit polyclonal anti-phospho (S473) AKT	Cell Signaling Technology	Cat #9271
41	Rabbit polyclonal anti-AKT	Cell Signaling Technology	Cat #9272

<b>Table S3</b>					
<b>S.No</b>	<b>Assay ID</b>	<b>Dye</b>	<b>Gene Symbol</b>	<b>Gene Name</b>	<b>Identifier</b>
1	Mm99999056_m 1	FAM	Ccl2	Chemokine ligand 2 MCP-1	Thermo Fisher Scientific
2	Mm01135198_m 1	FAM	Cd36	CD36 antigen	Thermo Fisher Scientific
3	Mm00439560_m 1	FAM	IGF1	Insulin-like growth factor 1	Thermo Fisher Scientific
4	Mm00439564_m 1	FAM	IGF2	Insulin-like growth factor 2	Thermo Fisher Scientific
5	Mm00449517_m 1	FAM	FATP2	Fatty acid transporter, member 2	Thermo Fisher Scientific
6	Mm00447768_m 1	FAM	FATP5	Fatty acid transporter, member 5	Thermo Fisher Scientific
7	Mm01178820_m 1	FAM	Tgfb1	Transforming growth factor, beta 1	Thermo Fisher Scientific
8	Mm00437762 PL	VIC	B2m	B-microglobulin Primer Limited	Thermo Fisher Scientific
9	Mm00443258_m 1	FAM	TNF	Tumor necrosis factor	Thermo Fisher Scientific
10	Mm00446190_m 1	FAM	Il6	Interleukin 6	Thermo Fisher Scientific
11	Mm00840165_g1	FAM	FGF21	Fibroblast growth factor 21	Thermo Fisher Scientific