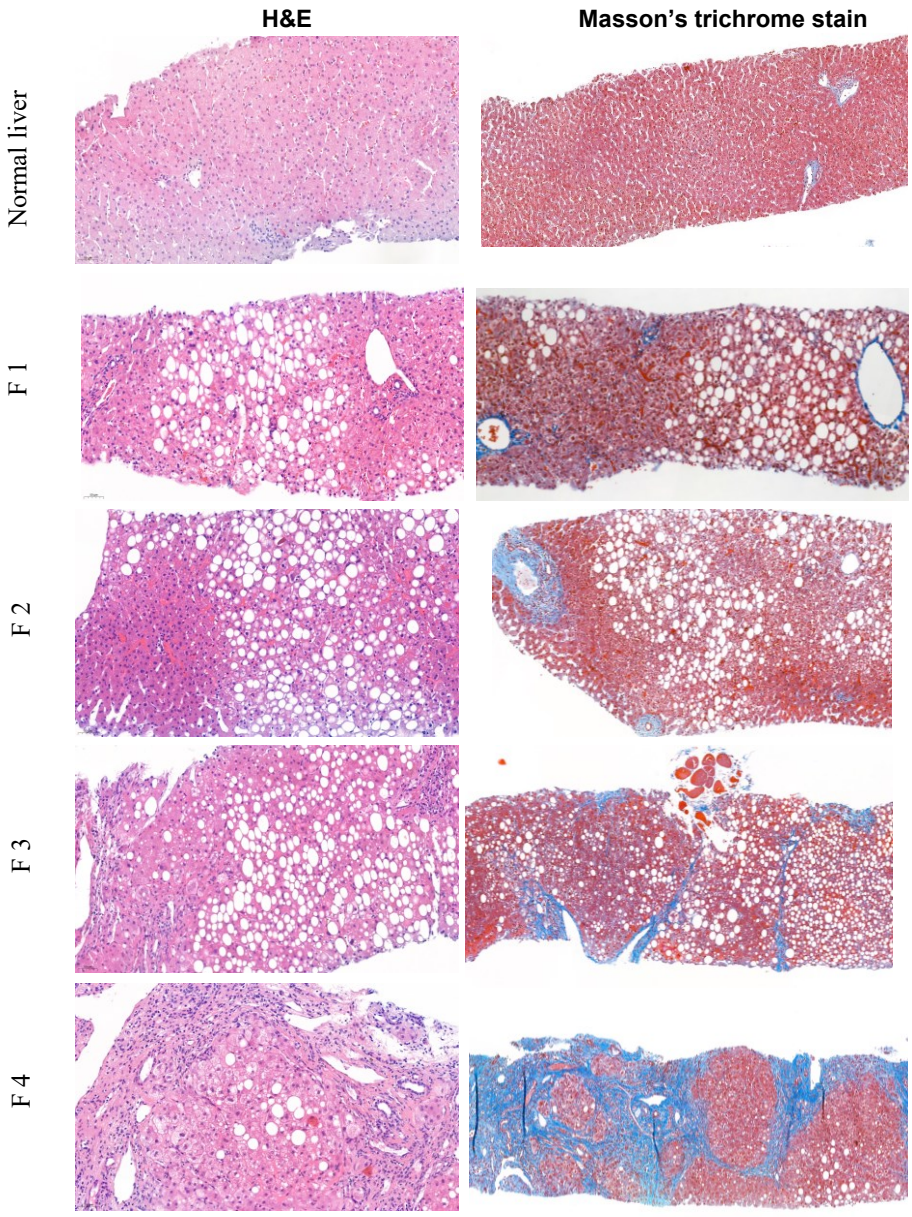
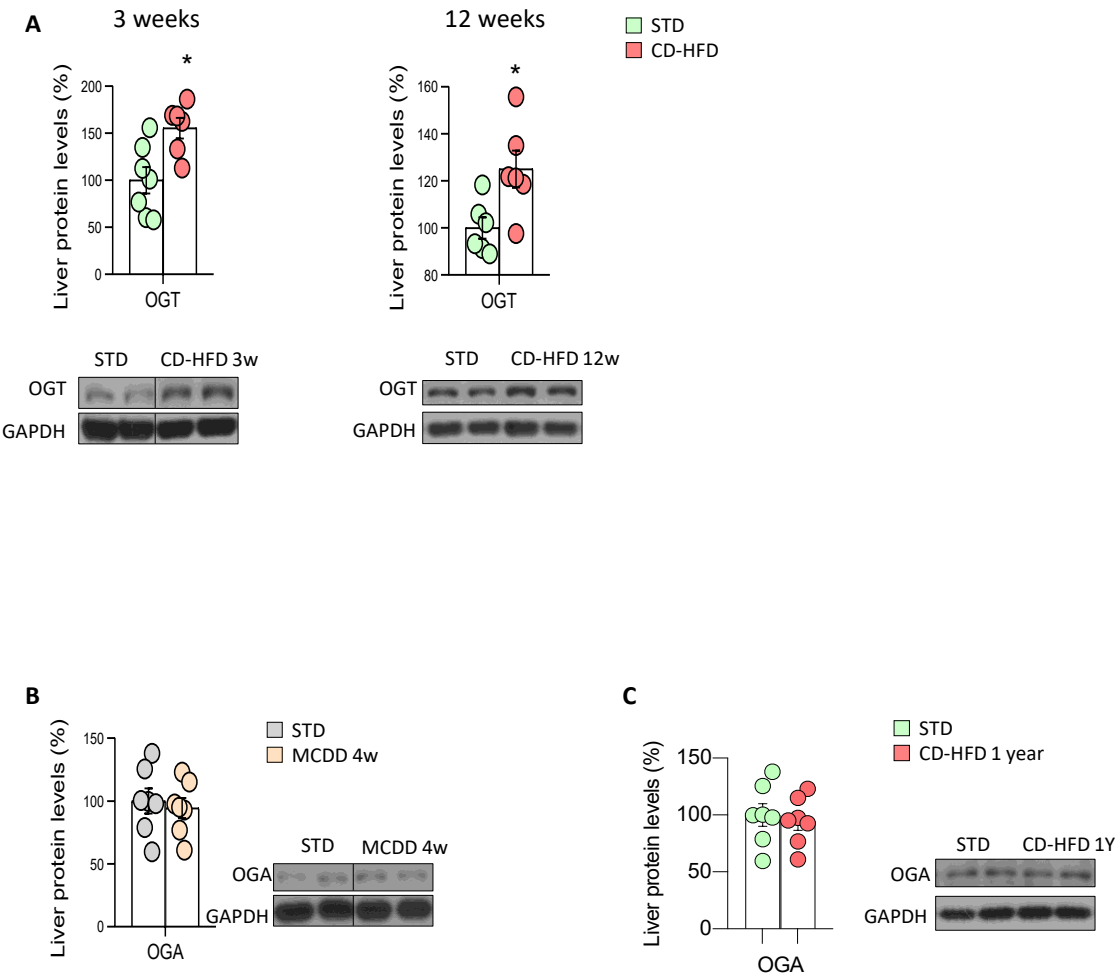
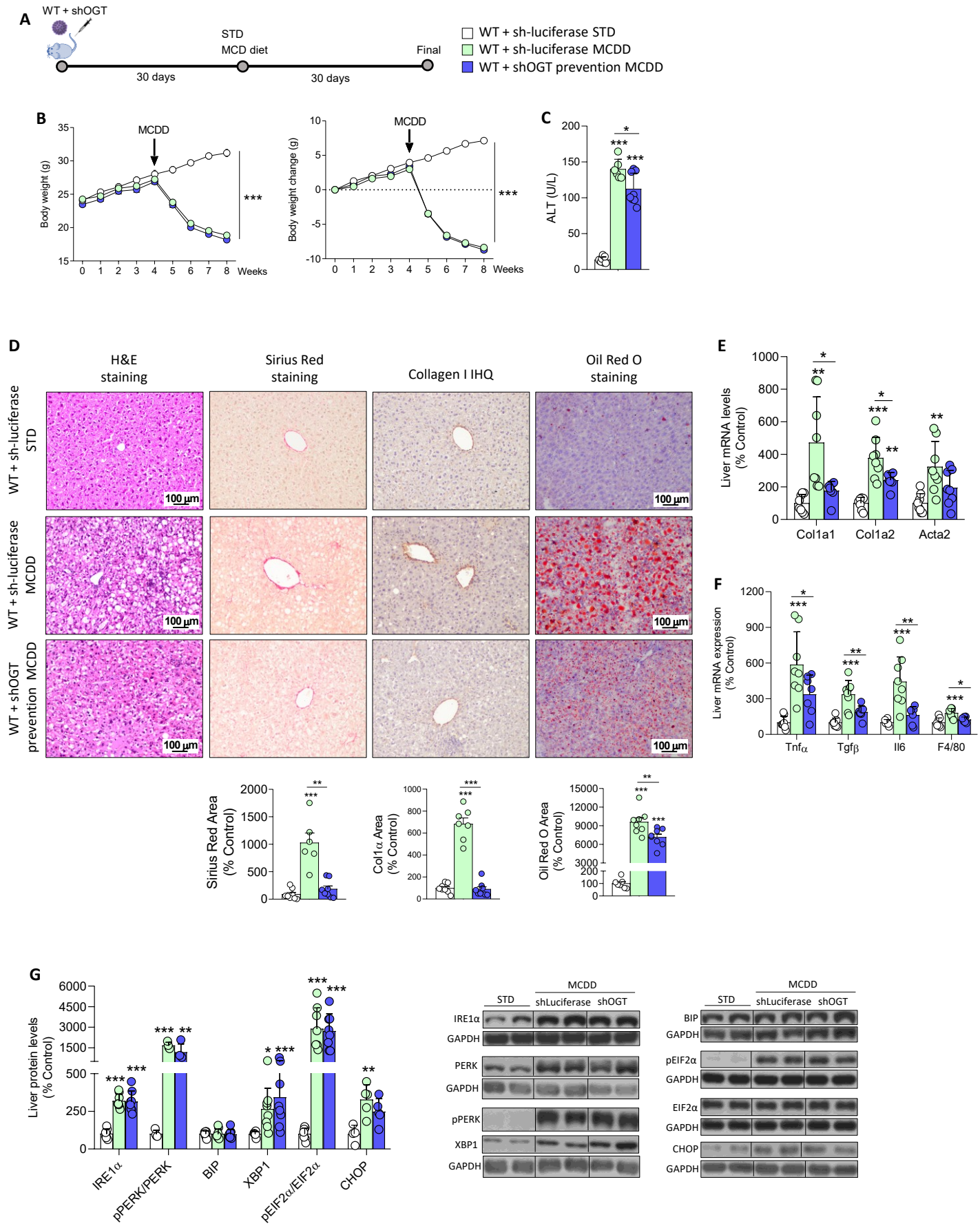
**Supplementary Figure 1**



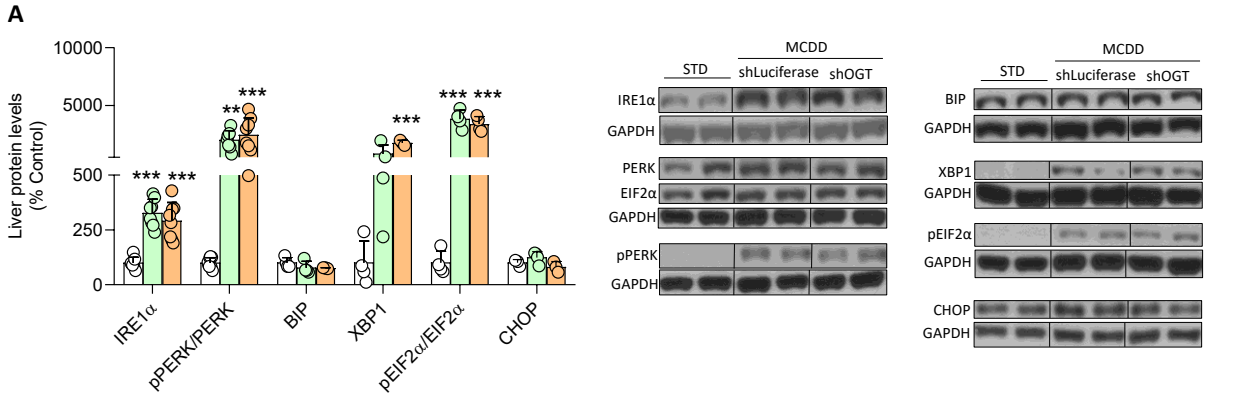
**Supplementary Figure 2**

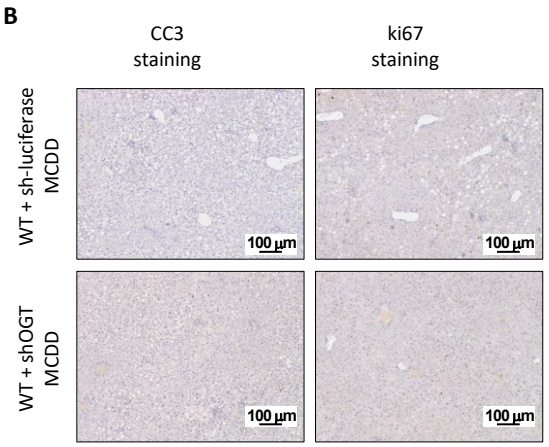


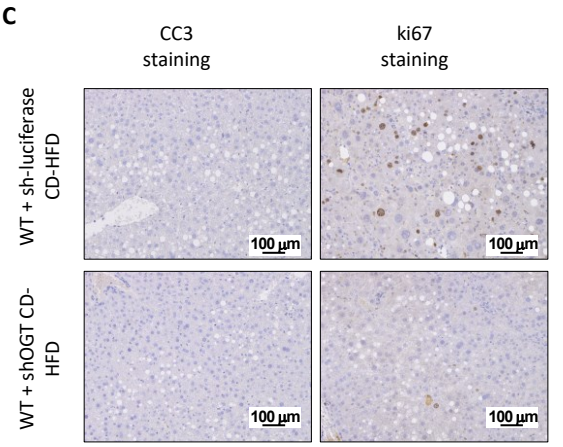
**Supplementary Figure 3**



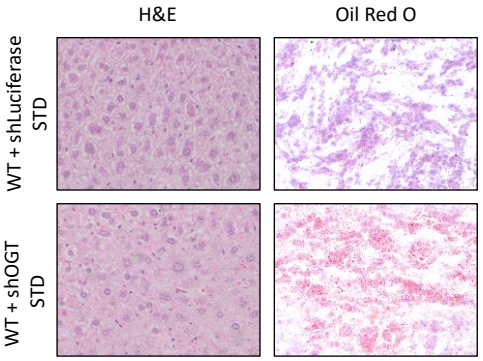
**Supplementary Figure 4**

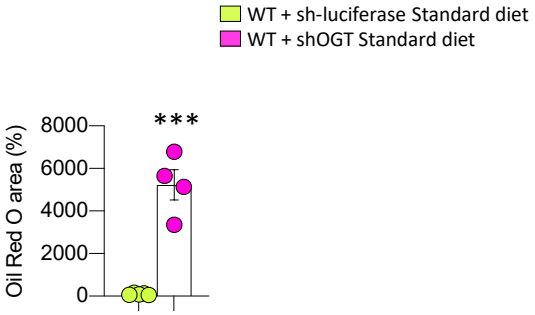


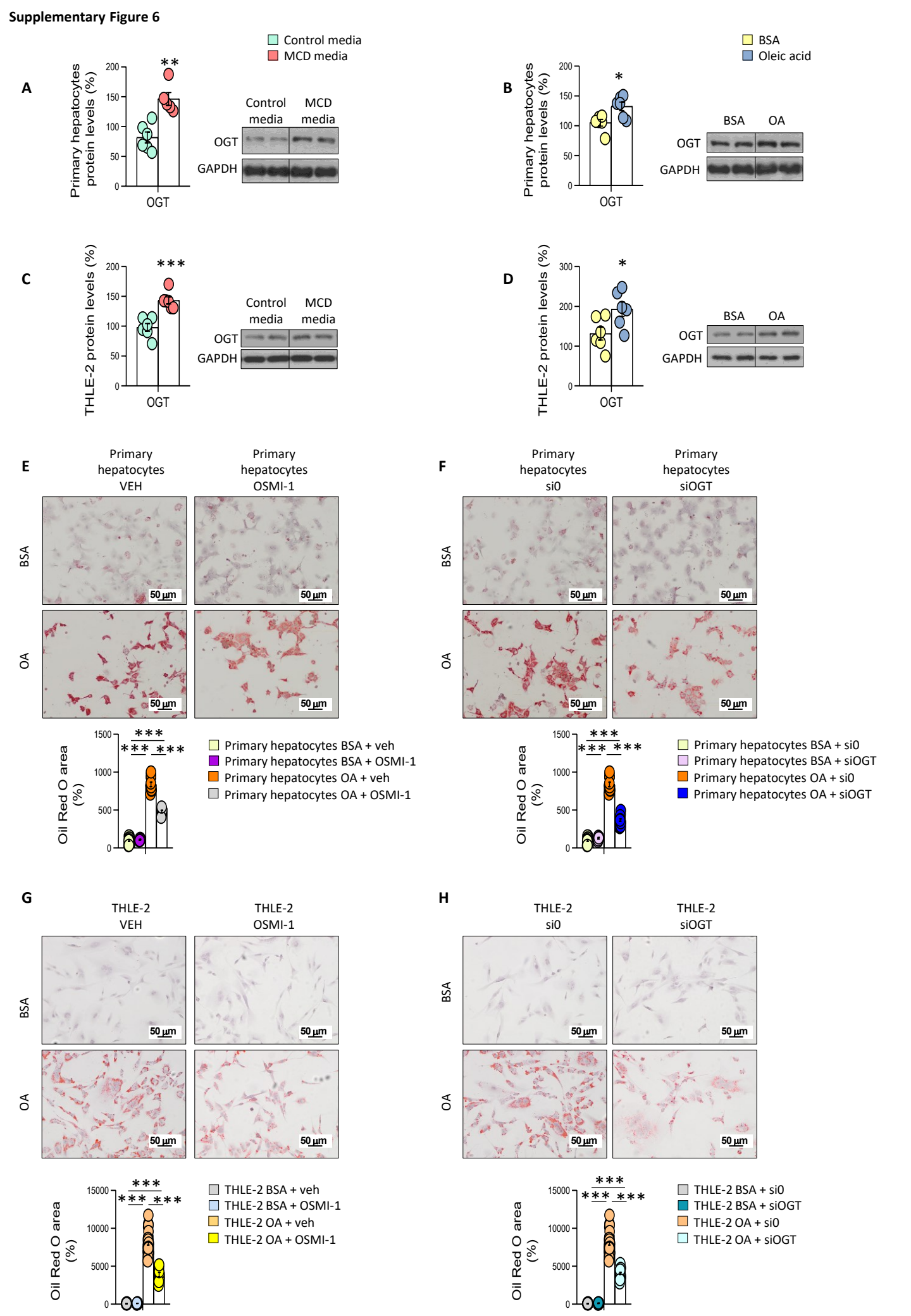



**Supplementary Figure 5**

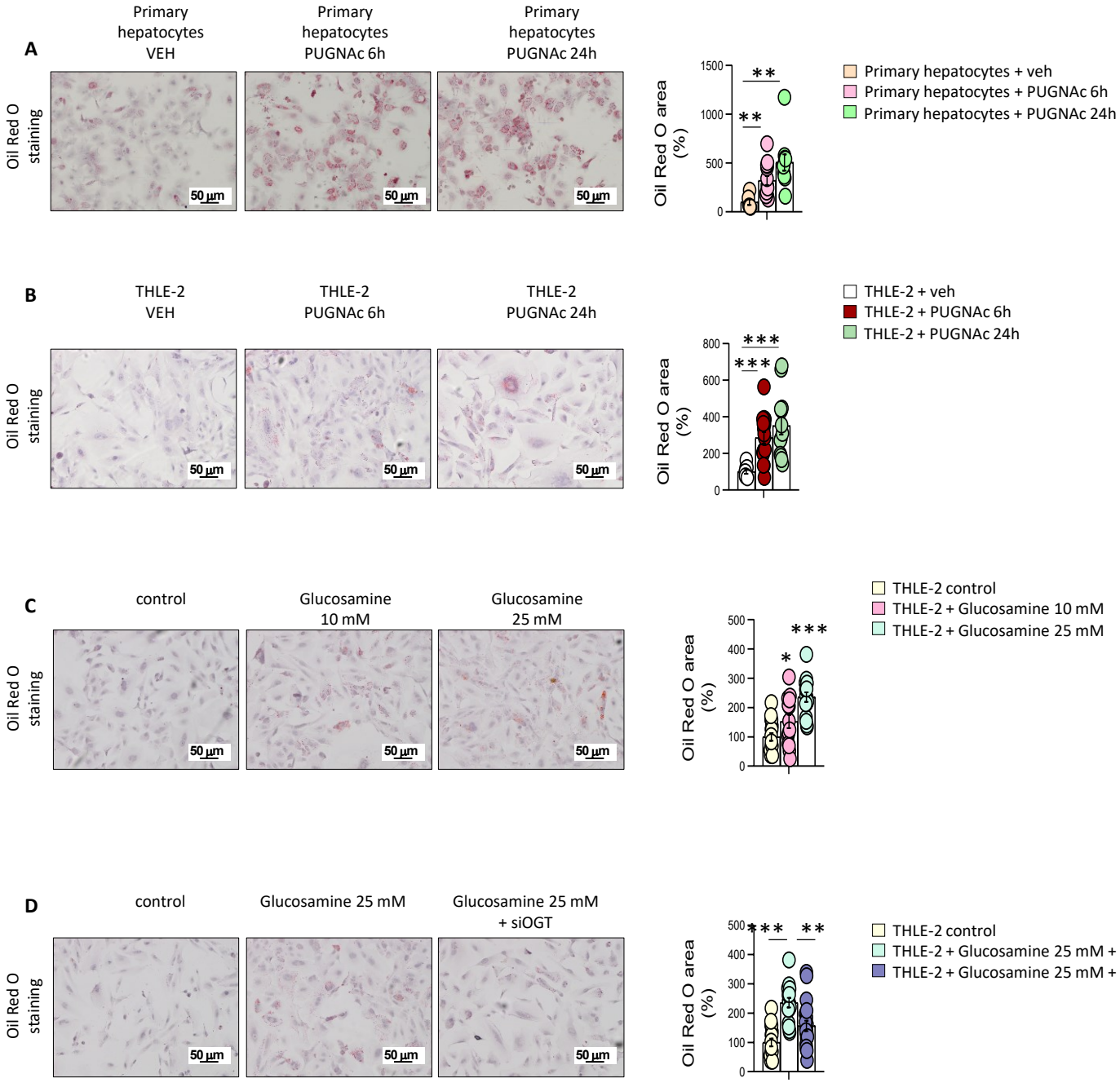


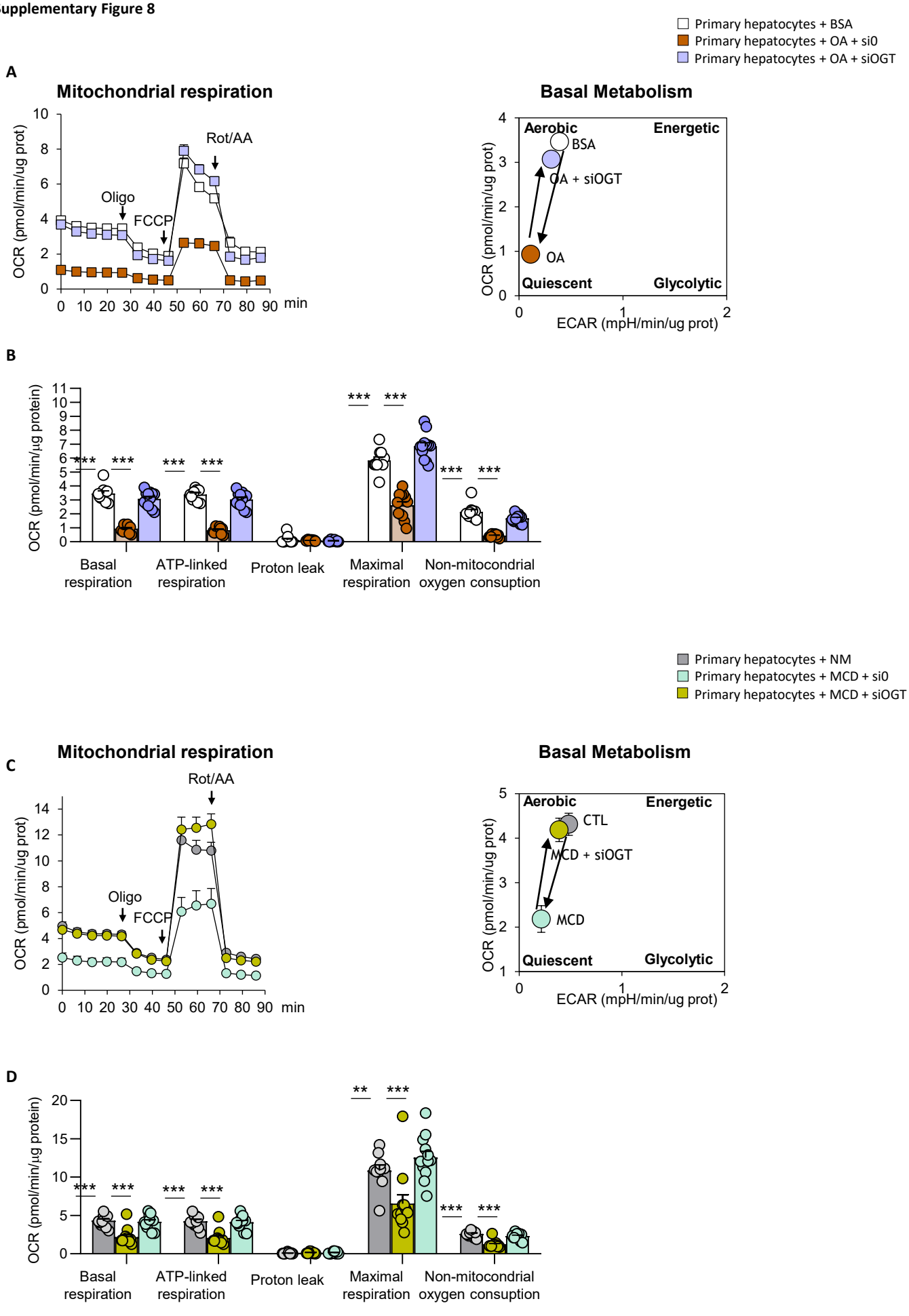
**Supplementary Figure 6**



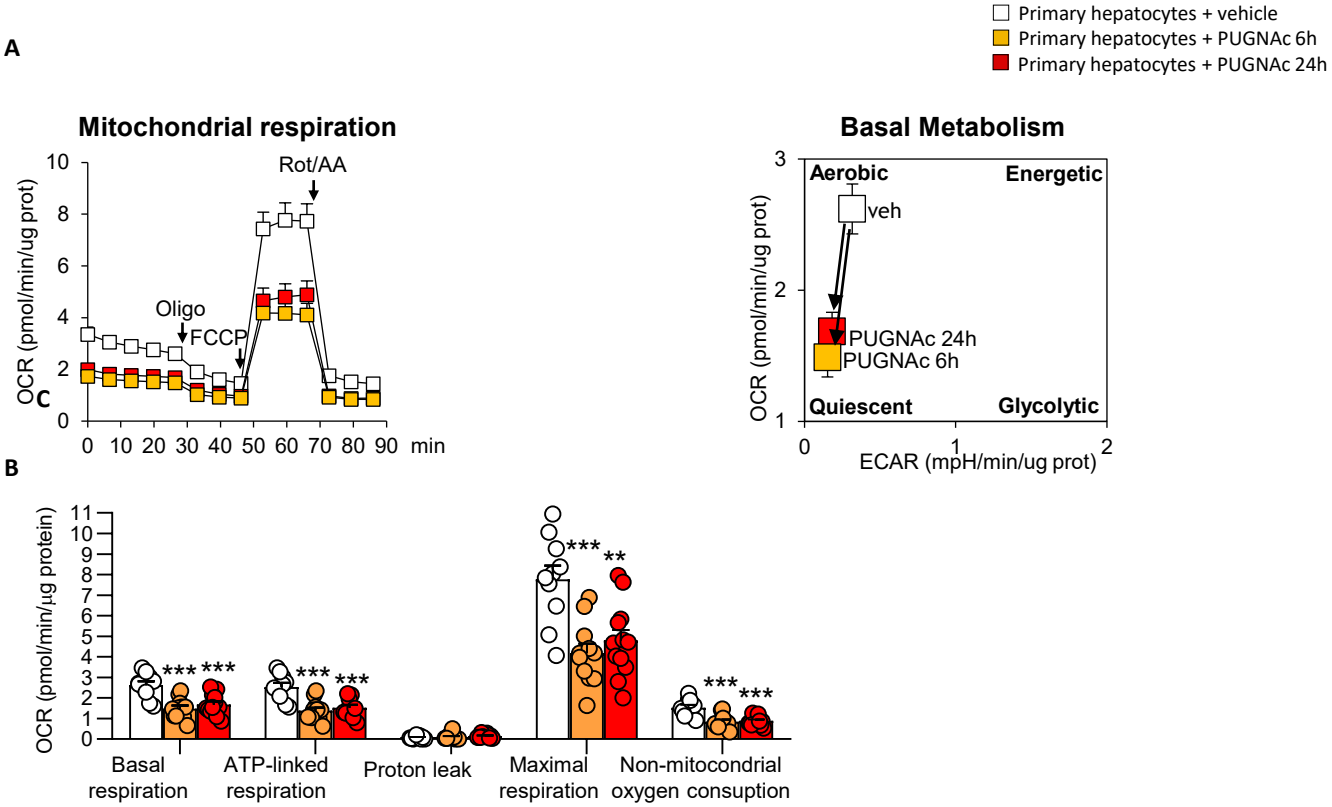
**Supplementary Figure 7**



**Supplementary Figure 8**



**Supplementary Figure 9**



**Supplementary Figure 10**

Fig 1A

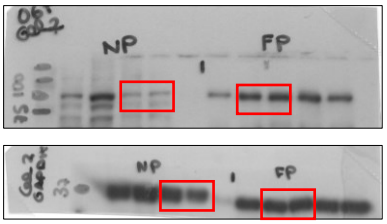



Fig 2C

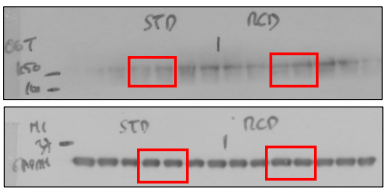



Fig 2F

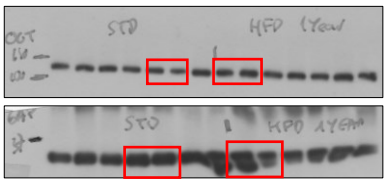



Fig 3A

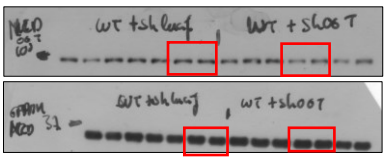



Fig 3G

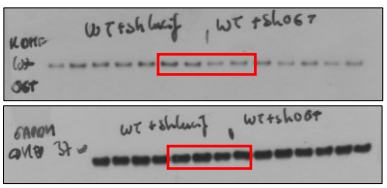



Fig 4A

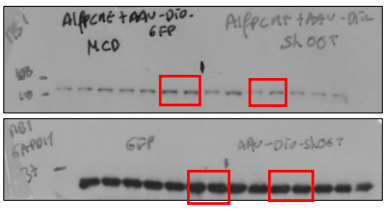



Fig 5A

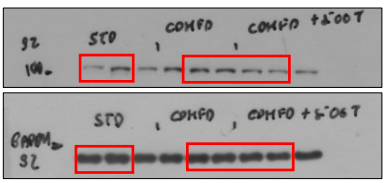



Fig 7C

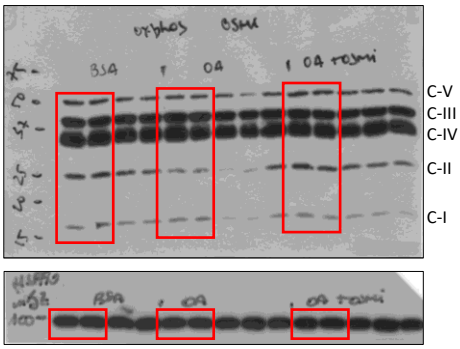






Fig S2

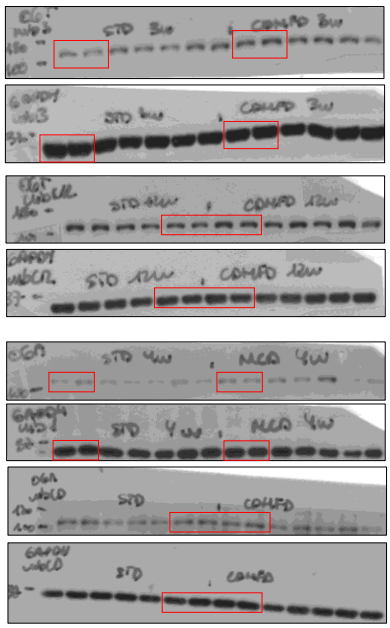
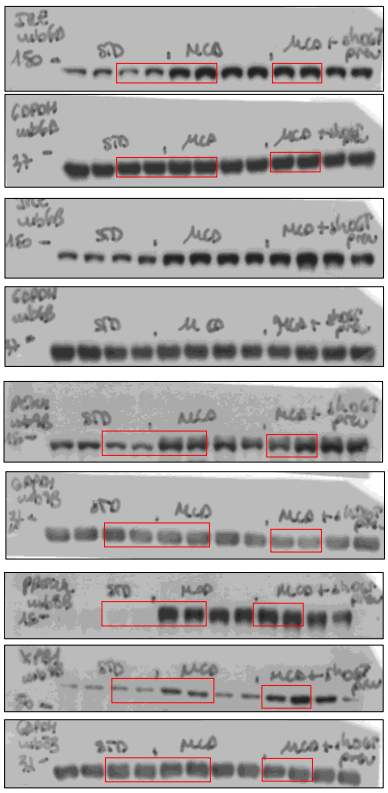



Fig S3G



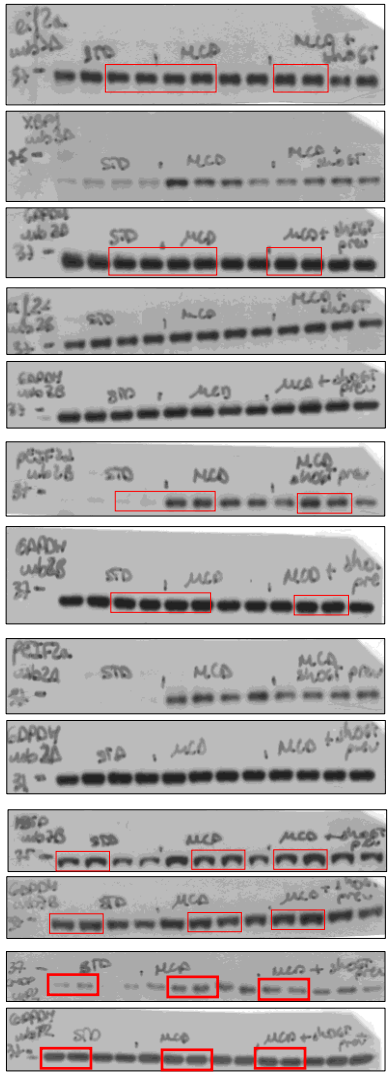
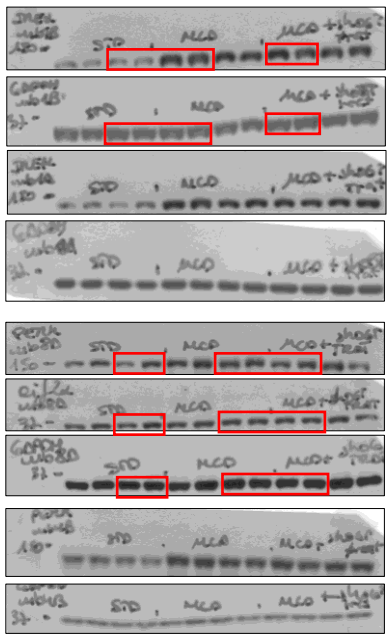




Fig S4A




Uncropped blots of each figure. Red squares indicate the selected bands of each gel shown in each figure.

**Supplementary Figure 10 (cont)**

Fig S4A

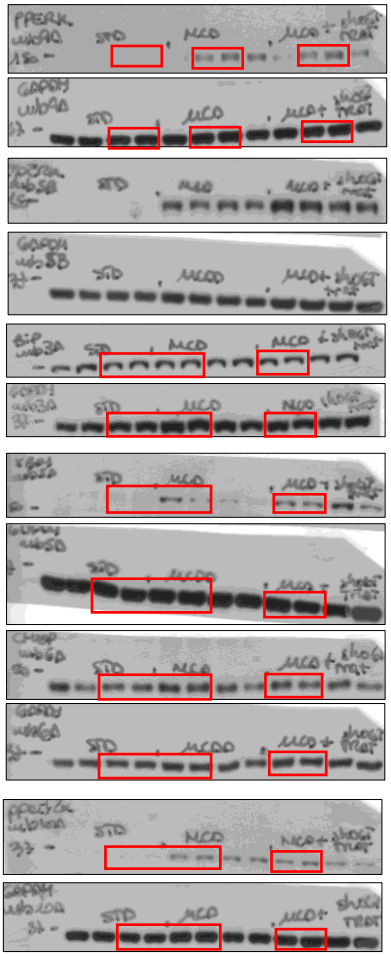
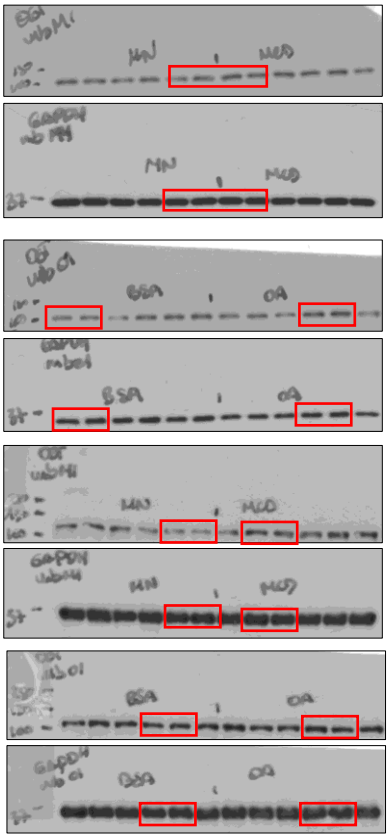


Fig S6



Uncropped blots of each figure. Red squares indicate the selected bands of each gel shown in each figure.

**Supplementary figures**

**Supplementary Figure 1. Hematoxylin and eosin staining and Masson's trichrome staining in healthy (normal liver) and patients with NASH and fibrosis.** Representative microphotographs are shown of hematoxylin and eosin staining (H&E; upper panel) and Masson's trichrome staining in healthy (normal liver) and patients at different stages of fibrosis.

**Supplementary Figure 2. Protein levels of OGT and OGA in *in vivo* preclinical models of NASH.** A) OGT protein levels in the liver of mice fed a choline-deficient high-fat diet (CD-HFD) for 3 and 12 weeks (n=6-7). OGA protein levels in the liver of mice fed a: B) standard diet (STD) and methionine-and-choline-deficient diet (MCDD) or STD and choline-deficient high-fat diet (CD-HFD) for one year (n=7).

**Supplementary Figure 3. Early OGT inhibition ameliorates MCD-induced hepatic fibrosis.** A) OGT inhibition in wild-type (WT) mice fed a MCD diet compared to mice fed a standard diet (STD) and analysed by: B) Body weight of mice; C) ALT; D) hematoxylin & eosin, Sirius red, collagen 1 and oil red O staining. E, F) Expression of fibrosis (E) and inflammation (F) markers; G) protein levels of endoplasmic reticulum markers in mice injected with sh-luciferase or shOGT and fed a MCD diet (n=3-8). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, using a one-way ANOVA followed by a Bonferroni Multiple Comparison Test.

**Supplementary Figure 4. OGT downregulation in *in vivo* models of liver fibrosis does not affect endoplasmic reticulum stress, apoptosis or proliferation.** A) Protein levels of IRE1α, pPERK/PERK, BIP, XBP1, pEIF2α/EIF2α and CHOP in the liver (n=3-8); B-C) Representative microphotographs are shown of cleaved caspase 3 (CC3) and ki67 staining of liver sections of WT: B) mice fed with MCD diet, and C) mice fed with CD-HFD injected with shLuciferase or shOGT. *p<0,05, ∗∗p <0.01, ***p<0.001, using a one-way ANOVA followed by a Bonferroni Multiple Comparison Test.

**Supplementary Figure 5. OGT inhibition in mice fed a standard diet induces hepatic lipid accumulation.** OGT inhibition in WT mice fed a standard diet injected with sh-luciferase or shOGT (n=4). ***p<0.001, using a two-tail Student's *t*-test.

**Supplementary Figure 6. OGT is increased in *in vitro* models of hepatocyte injury.** A-D) OGT protein levels in primary hepatocytes (upper panel) and human THLE-2 cells (lower panel) treated with methionine and choline-deficient media (MCD) and BSA or oleic acid (OA) (n=5-6). Oil red O staining of primary mouse hepatocytes challenged with BSA or OA, and then treated with E) vehicle or OSMI-1, or F) empty siRNA or siRNA against OGT. Oil red O staining of human THLE-2 cells challenged with BSA or OA, and then treated with G) vehicle or OSMI-1, or H) empty siRNA or siRNA against OGT (n=6-29). ∗p <0.05, ∗∗p <0.01, ***p<0.001, using a Student's t test (A) (B) (C) (D), or one-way ANOVA followed by a Bonferroni Multiple Comparison Test (E) (F) (G) (H).

**Supplementary Figure 7. *O*-GlcNAcylation mediates lipid accumulation in hepatocytes.** Oil Red O in A) primary hepatocytes from STD-fed mice, and B) THLE-2 cells treated with vehicle or PUGNAc for 6 and 24 hours (n=6-16). Oil Red O in THLE-2 cells treated with C) Glucosamine 10 and 25 mM; and D) glucosamine 25 mM, and empty siRNA or siRNA against OGT (n=8-16). ∗p <0.05, ∗∗p <0.01, ***p<0.001, using a one-way ANOVA followed by a Bonferroni Multiple Comparison Test.

**Supplementary Figure 8. Inhibition of *O*-GlcNAcylation increases mitochondrial activity in *in vitro* models.** A) Oxygen consumption rate (OCR) in primary hepatoctyes treated with BSA or oleic acid (OA) and then with empty si-RNA or si-RNA-OGT. Arrows indicate the timepoint at which mitochondrial respiration modulators (oligomycin [Oligo], phenylhydrazone [FCCP], or rotenone/antimycin A [Rot/AA]) were added to the assay. Right, graph depicting the effect of OA and si-RNA-OGT on aerobic or quiescent metabolic states, based on quantification of glycolysis and oxygen consumption rate during basal metabolism. B) Parameters of mitochondrial function (n=10-12). C) Oxygen consumption rate (OCR) in primary hepatoctyes incubated with normal medium (NM) or MCD media, and then with empty siRNA or siOGT. Arrows indicate the timepoint at which mitochondrial respiration modulators were added to the assay. Right, graph depicting the effect of MCD and siOGT on aerobic or quiescent metabolic states, based on quantification of glycolysis and oxygen consumption rate during basal metabolism. D) Parameters of mitochondrial function (n=9-12). ∗∗p <0.01 and ***p<0.001, using a one-way ANOVA followed by a Bonferroni multiple comparison test.

**Supplementary Figure 9. Activation of *O*-GlcNAcylation reduces mitochondrial activity in *in vitro* models.** A) Oxygen consumption rate (OCR) in primary hepatoctyes treated with vehicle or PUGNAc. Arrows indicate the timepoint at which mitochondrial respiration modulators (oligomycin [Oligo], phenylhydrazone [FCCP], or rotenone/antimycin A [Rot/AA]) were added to the assay. Right, graph depicting the effect of OA and si-RNA-OGT on aerobic or quiescent metabolic states, based on quantification of glycolysis and oxygen consumption rate during basal metabolism. B) Parameters of mitochondrial function (n=10-12). ∗∗p <0.01 and ***p<0.001, using a one-way ANOVA followed by a Bonferroni multiple comparison test.

**Supplementary Figure 10.** Uncropped blots of each figure. Red squares indicate the selected bands of each gel shown in each figure.

## Supplementary Tables

**Table S1. Anthropometric, biochemical and clinical characteristics of patients with NASH used for *O*-GlcNAc immunostaining analysis.** BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase.

| Variable | Normal liver (n=3) | NASH with fibrosis (n=13) |
|---|---|---|
| **Weight (kg)** | 122,7 ± 8,6 | 107,2 ± 3,9 |
| **BMI** | 42,4 ± 1,8 | 36,6 ± 1,0 |
| **Glucose (mg/dl)** | 90,7 ± 2,9 | 118,8 ± 10,6 |
| **Urea (mg/dl)** | 34 ± 3 | 32,7 ± 2,6 |
| **Cr (mg/dl)** | 0,8 ± 0,2 | 0,7 ± 0,05 |
| **ALT (U/l)** | 37,7 ± 12,5 | 61,6 ± 7,9 |
| **AST (U/l)** | 27,7 ± 7,5 | 47,5 ± 7,0 |
| **GGT (U/l)** | 22,3 ± 9,1 | 121,4 ± 39,2 |
| **Fatty acids (U/l)** | 68,3 ± 15,5 | 88,4 ± 9,9 |
| **Bilirubin (mg/dl)** | 0,6 ± 0,1 | 0,75 ± 0,1 |
| **Triglycerides (mg/dl)** | 208,5 ± 93,5 | 226,6 ± 74,2 |
| **Total cholesterol (mg/dl)** | 178,3 ± 10,9 | 171,2 ± 7,3 |
| **LDL (mg/dl)** | 88 ± 17 | 100,9 ± 9,6 |
| **HDL (mg/dl)** | 57 ± 11 | 40,2 ± 3,6 |
| **Albumin (g/dl)** | 4,4 ± 0,1 | 4,5 ± 0,03 |
| **Ferritin (ng/ml)** | 236,7 ± 148,2 | 178,8 ± 36 |
| **Hemoglobin (g/dl)** | 14,9 ± 1,3 | 14,7 ± 0,5 |
| **Platelets x$10^3$/ul** | 263,7 ± 54,7 | 217,7 ± 21,3 |
| **AP (%)** | 86 ± 4,2 | 89,6 ± 3,8 |
| **Steatosis (0-3)** | 0,0 ± 0,0 | 1,7 ± 0,1 |
| **Lobular inflammation (0-3)** | 0,0 ± 0,0 | 1 ± 0,1 |
| **Ballooning (0-2)** | 0,3 ± 0,3 | 1,3 ± 0,1 |
| **Fibrosis (0-4)** | 0,0 ± 0,0 | 2,3 ± 0,3 |

| | | |
|---|---|---|
| **NAS Score (0-8)** | 0,3 ± 0,3 | 4 ± 0,2 |

**Table S2. Anthropometric, biochemical and clinical characteristics of patients with NASH used for *O*-GlcNAc western blot analysis.**

| Variable | Normal liver (n=9) | NASH with fibrosis (n=9) |
|---|---|---|
| **Weight (kg)** | 122,1 ± 7,3 | 117,9 ± 10,7 |
| **BMI** | 40,7 ± 1,9 | 38,3 ± 1,6 |
| **Glucose (mg/dl)** | 110,6 ± 17,3 | 112 ± 10,2 |
| **ALT (U/l)** | 25,6 ± 3,6 | 28,8 ± 8,3 |
| **AST (U/l)** | 18,7 ± 1,9 | 21,3 ± 5,2 |
| **GGT (U/l)** | 25,5 ± 3,9 | 36,7 ± 16,1 |
| **Bilirubin (mg/dl)** | 0,67 ± 0,12 | 0,61 ± 0,27 |
| **Triglycerides (mg/dl)** | 108,2 ± 16,9 | 159 ± 93,5 |
| **Total cholesterol (mg/dl)** | 184 ± 9,9 | 220,6 ± 28,2 |
| **LDL (mg/dl)** | 109,2 ± 7,9 | 131,4 ± 23,1 |
| **HDL (mg/dl)** | 52,5 ± 5,7 | 48,4 ± 9,4 |
| **Fibrosis (0-4)** | 0,0 ± 0,0 | 2 ± 0,1 |
| **NAS Score (0-8)** | 1 ± 0,2 | 4 ± 0,5 |

BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase.

**Table S3. Primers and probes used for gene amplification.**

| Name | Primer sequence 5′→ 3′ |
|---|---|
| Mus musculus Collagen 1α1 | FW cctaatgctgccttttctgc<br>RV atgtcccagcaggatttgag |
| Mus musculus Collagen 1α2 | FW ccgtgcttctcagaacatca<br>RV cttgccccattcatttgtct |
| Mus musculus F4/80 | FW tgcatctagcaatggacagc<br>RV gccttctggatccatttgaa |
| Mus musculus HPRT | FW aagcttgctggtgaaaagga<br>RV ttgcgctcatcttaggcttt |
| Mus musculus IL6 | FW agttgccttcttgggactga<br>RV tccacgatttcccagagaac |
| Mus musculus α-Smooth Muscle Actin | FW ctgacagaggcaccactgaa<br>RV catctccagagtccagcaca |
| Mus musculus TGFβ1 | FW ttgcttcagctccacagaga<br>RV tggttgtagagggcaaggac |
| Mus musculus TNFα | FW agcccccagtctgtatcctt<br>RV ctccctttgcagaactcagg |
| Mus musculus OGT | FW caccgttcagtattctgtgccgcc<br>RV tagggcaattctcctgtgcg |

**Table S4. Antibodies used for western blot.**

| Protein target | Manufacturer (catalog number) | Species reactivity | Dilution |
|---|---|---|---|
| *O*-GlcNAc transferase (OGT) | Cell Signaling (D1D8Q) | Rabbit monoclonal | 1:1000 |
| Glyceraldehyde 3- phosphate dehydrogenase (GAPDH) | Merck (CB1001) | Mouse monoclonal | 1:5000 |
| O-GlcNAcase (OGA) | Abcam (ab124807) | Rabbit monoclonal | 1:1000 |
| OXPHOS | Abcam (ab110413) | Mouse cocktail | 1:1000 |
| Heat shock protein 90 (HSP90) | Santa Cruz Biotechnology (Sc-13119) | Mouse monoclonal | 1:5000 |
| Protein Kinase RNA-Like ER Kinase (PERK) | Cell Signaling Technology (3192S) | Rabbit monoclonal | 1:1000 |
| Protein Kinase RNA-Like ER Kinase Thr980 (phospho-PERK) | Cell Signaling Technology (3179S) | Rabbit monoclonal | 1:1000 |
| Inositol requiring enzyme 1 alpha (IRE1α) | Abcam (ab37073) | Rabbit monoclonal | 1:1000 |
| Binding immunoglobulin protein (BIP) | Cell Signaling Technology (3183S) | Rabbit monoclonal | 1:1000 |
| X-Box Binding Protein 1 (XBP1) | Abcam (ab220783) | Rabbit monoclonal | 1:1000 |
| Eukaryotic translation initiation factor 2 alpha (EIF2α) | Santa Cruz Biotechnology (Sc-11386) | Rabbit polyclonal | 1:1000 |
| Eukaryotic translation initiation factor 2 alpha Ser52 (phospho-EIF2α) | Santa Cruz Biotechnology (Sc-101670) | Rabbit polyclonal | 1:1000 |

| | | | |
|---|---|---|---|
| C/EBP Homologous Protein (CHOP) | Santa Cruz Biotechnology (Sc-793) | Rabbit polyclonal | 1:1000 |