

## Supplementary Figures for

Farnesoid X Receptor activation by bile acids suppresses lipid peroxidation and ferroptosis

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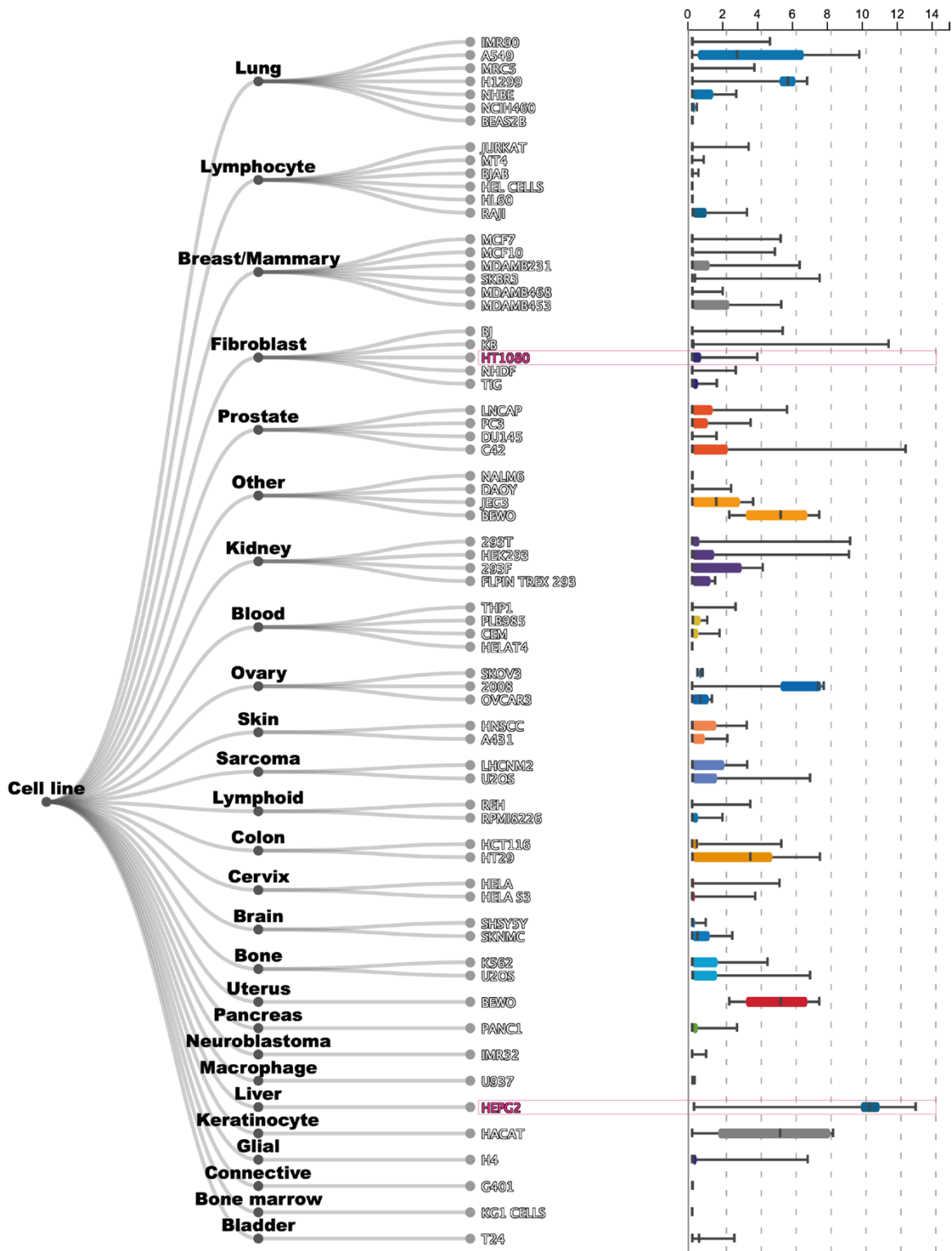
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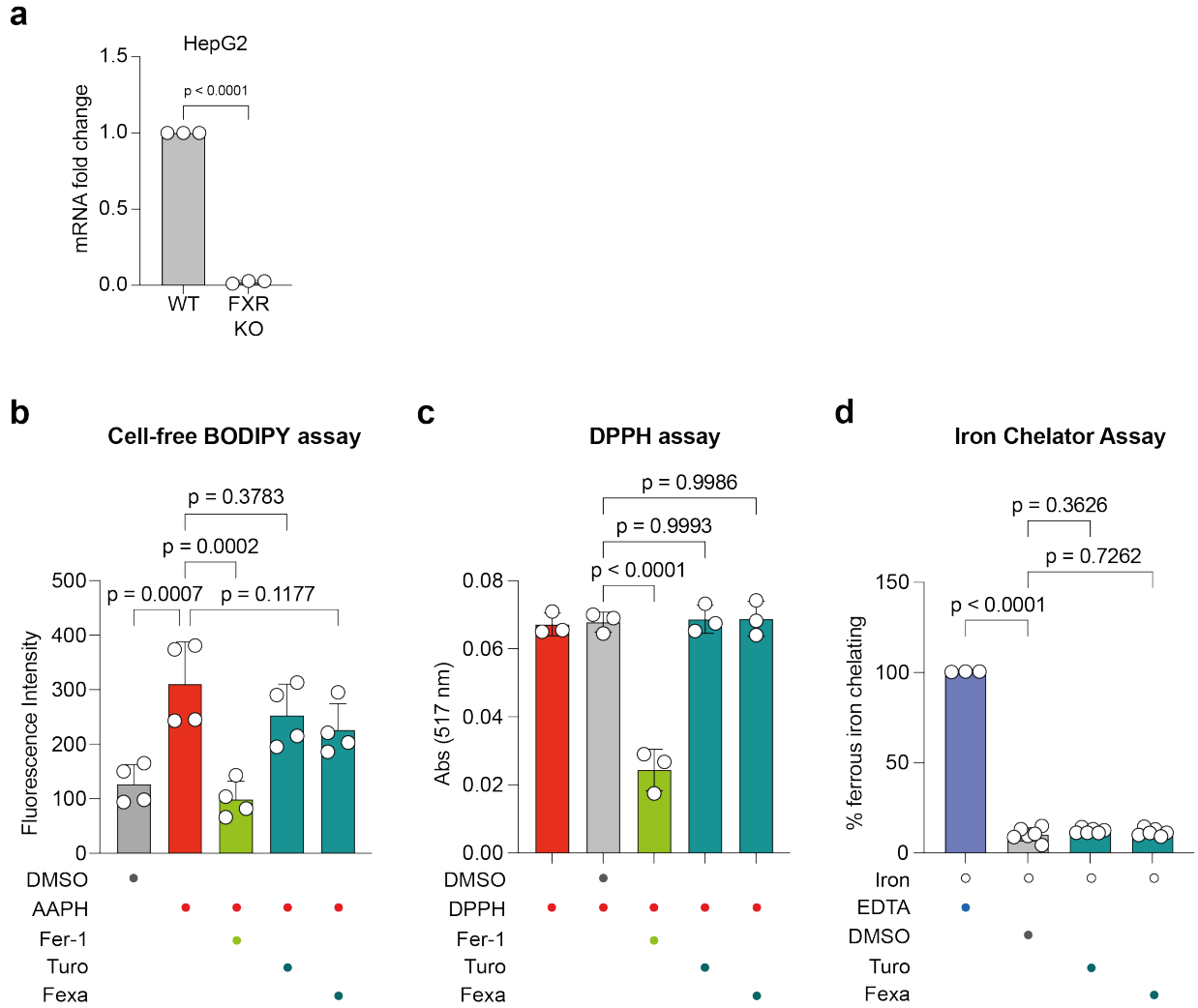
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# Supplementary Figure 1



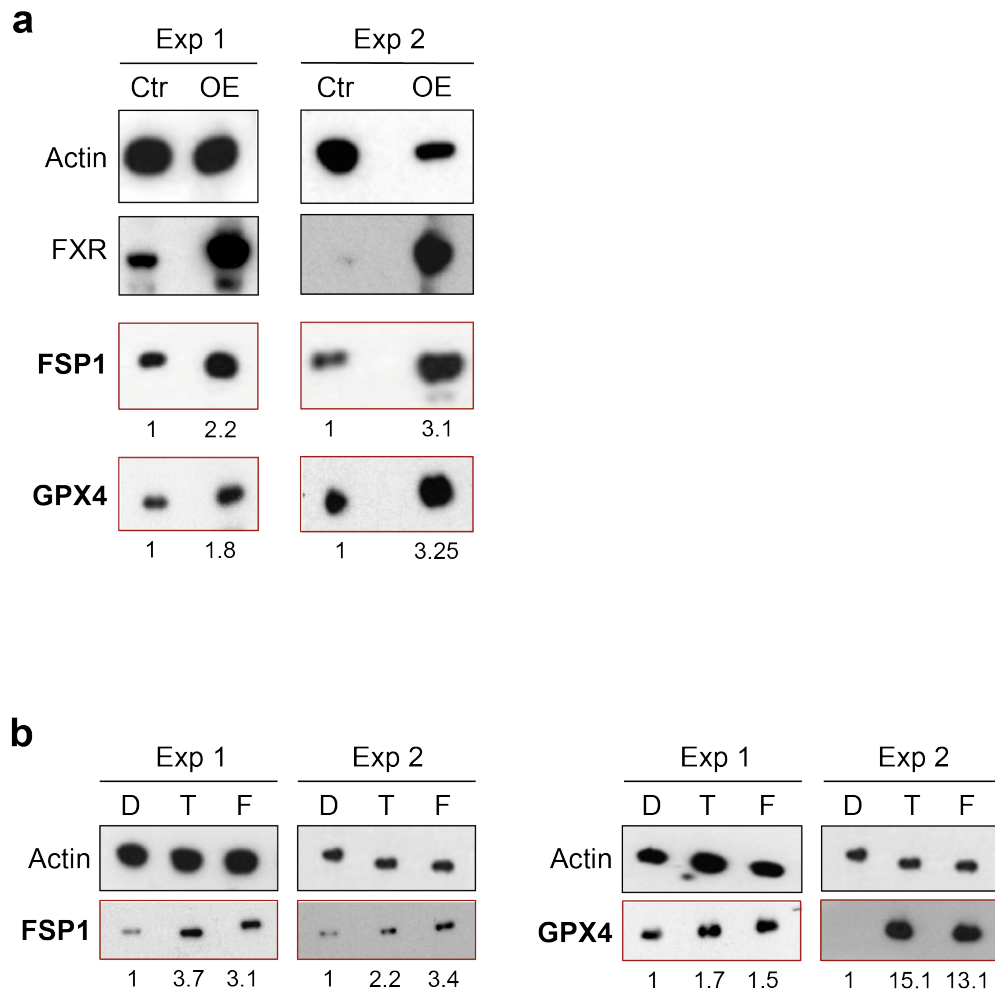
**Supplementary Figure 1.** Expression levels of FXR (NR1H4) across several cell lines extracted from ARCH<sup>4</sup> database (Lachmann, A., Torre, D., Keenan, A.B., Jagodnik, K.M., Lee, H.J., Wang, L., et al., 2018. Massive mining of publicly available RNA-seq data from human and mouse. Nat Commun 9(1):1366.)

## Supplementary Figure 2



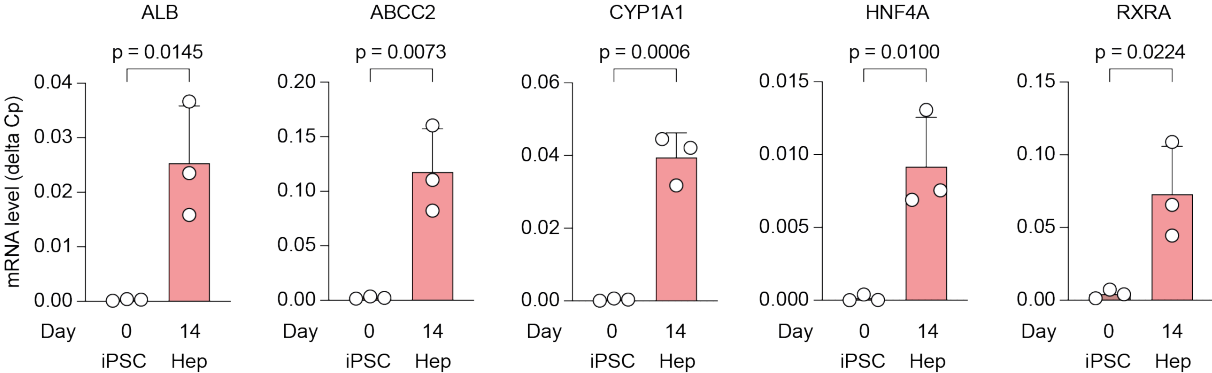
**Supplementary Figure 2.** **a** mRNA expression of FXR in wildtype (WT) and FXR knockout (KO) cells. Data are mean  $\pm$  SD of  $n = 3$  biological replicates; unpaired two-tailed  $t$ -test. **b** Turofexorate and Fexaramine have no antioxidant effects in a cell-free oxidizable BODIPY-C11 assay treated with 7.5 mM free-radical-producing 2,2'-azobis(2-methyl-propanimidamide) dihydrochloride (AAPH). In contrast, we observed significant decrease in oxidative fluorescence by Fer-1. Data are mean  $\pm$  SD of  $n = 4$  biological replicates; one-way ANOVA with Dunnett's test. **c** DPPH assay showed no antioxidant activity of Turofexorate and Fexaramine. All compounds were tested at a final concentration of 50  $\mu$ M. DPPH was used at 0.05 mM. Data are mean  $\pm$  SD of  $n = 3$  biological replicates, one-way ANOVA with Tukey's test. **d** 12  $\mu$ M Turofexorate or Fexaramine are not able to chelate iron (Fe(II)) in a Ferrozine-based assay. 100  $\mu$ M EDTA were used as a positive control. Data are mean  $\pm$  SD of  $n = 3$  (EDTA) or 6 (all other treatments) biological replicates; one-way ANOVA with Dunnett's test.

### Supplementary Figure 3



**Supplementary Figure 3. a** Western Blots of HT-1080 cells overexpressing FXR show an upregulation of target genes FSP1 and GPX4. **b** Western Blots of HepG2 cells treated with 12  $\mu$ M Turofexorate or Fexaramine for 2h show an upregulation of target genes FSP1 and GPX4. Bands were quantified using ImageJ and signals were normalized to  $\beta$ -Actin signals. Data from 2 biological replicates are shown. Antibodies are listed in Supplementary Tables 1 and 2.

**Supplementary Figure 4**



**Supplementary Figure 4.** Canonical differentiation markers of hepatocytes are upregulated on mRNA level after 14 days of differentiation compared to iPSCs at day 0. RP2 was used as a housekeeper gene for normalization. Data plotted are mean  $\pm$  SD of  $n = 3$  biological replicates; unpaired two-tailed t test.

## Supplementary Figure 5

Figure 2a

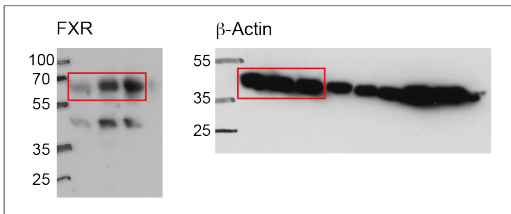
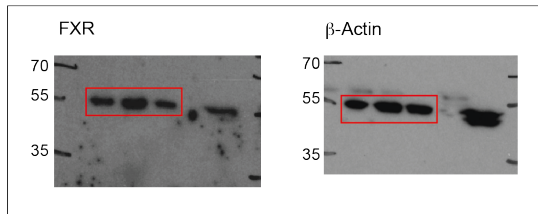
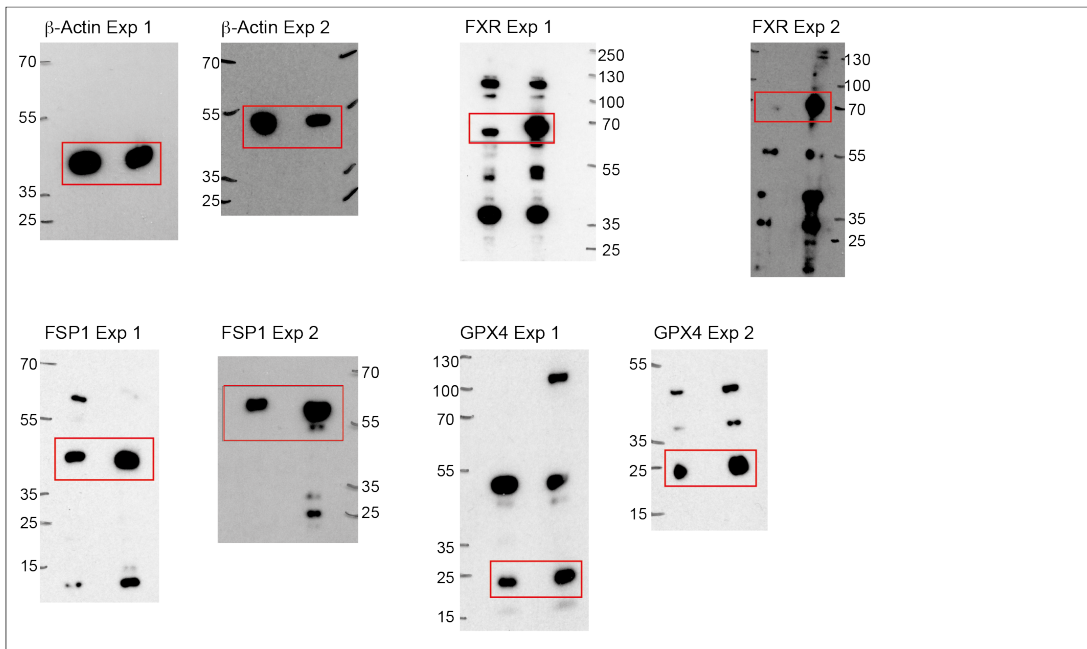


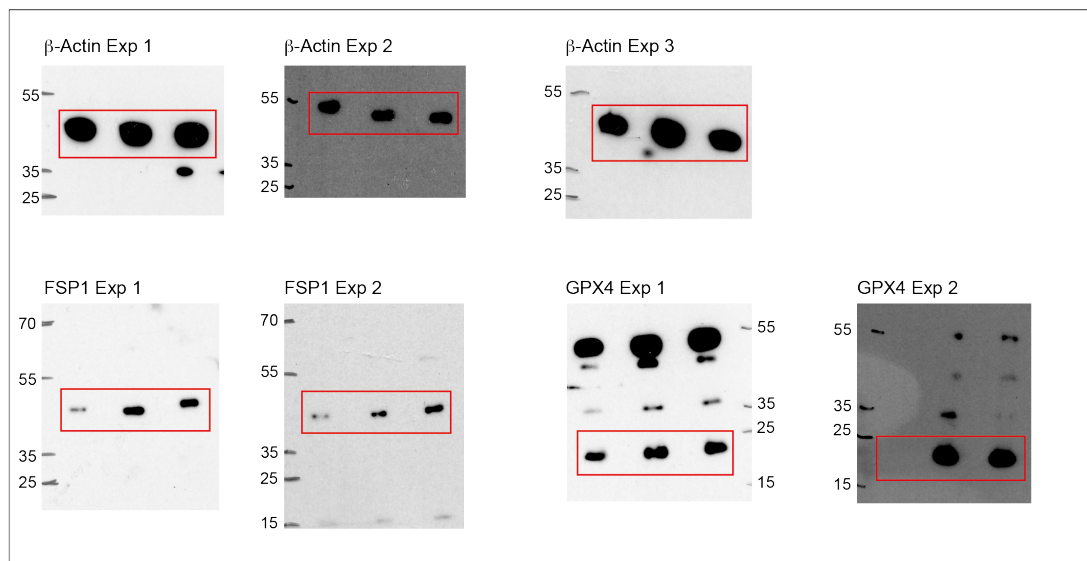
Figure 3b



Supplementary Figure 3a

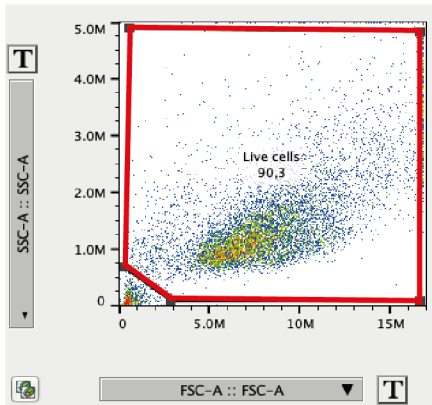


Supplementary Figure 3b

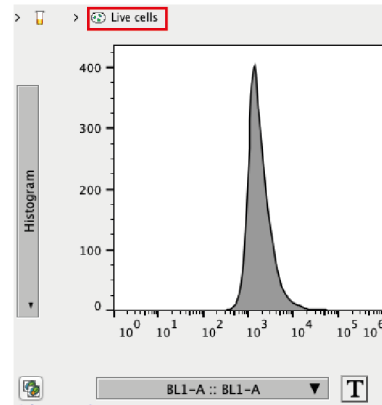


Supplementary Figure 5. Full scans of the Western Blots in Fig. 2a, 3b and Supplementary Fig. 3a, 3b

## Supplementary Figure 6



1. Exclude dead cells from scatter plot



2. Plot histogram of live cells in BL1-A channel

	Sample Name	Subset Name	Count	Median : BL1-A
	Specimen Flexa 1.fcs	HT1080	9010	2588
	Specimen Turo 2.fcs	HT1080	3822	4957
	Specimen Fer-1 1.fcs	HT1080	9062	1441
	Specimen RSL3 2.fcs	HT1080	5374	16428
	Specimen DMSO.fcs	HT1080	9098	1585

3. Extract median intensities of BL1-A channel

**Supplementary Figure 6.** Flow cytometry gating strategy and analysis for Fig. 1f, 4a, 4c, and 4d.

## Supplementary Tables

Supplementary Table 1:

<b>Primary Antibodies</b>	<b>Dilution</b>	<b>Species</b>	<b>Supplier</b>
b-Actin (C4)	1:500	mouse	sc-47778, Santa Cruz Biotechnology
NR1H4 (FXR)	1:500	rabbit	ab228949, Abcam
FSP1 (AMID)	1:1000	rabbit	PA5-103183, Thermo Fisher Scientific
GPX4	1:1000	rabbit	ab125066, Abcam

Supplementary Table 1:

<b>Secondary Antibodies</b>	<b>Dilution</b>	<b>Species</b>	<b>Supplier</b>
HRP-conjugated anti-mouse IgG	1:7500	donkey	715-035-150, Jackson ImmunoResearch Laboratories, Biozol
HRP-conjugated anti-rabbit IgG	1:7500	donkey	711-035-152, Jackson ImmunoResearch Laboratories, Biozol