

## Supplementary Material

### Supplementary tables

#### Suppl. Table S1: (A) Primer sequences used for quantitative real-time PCR (qPCR) and (B) antibody for immunofluorescence

List of forward and reverse primer sequences and antibody used for gene expression analysis in human and murine samples.

#### A

| Gene                               | Sequence |                                       |
|------------------------------------|----------|---------------------------------------|
| Human                              |          |                                       |
| <i>HO-1</i>                        | Forward  | 5'-CCA GGC AGA GAA TGC TGA GTT C-3'   |
|                                    | Reverse  | 5'- AAG ACT GGG CTC TCC TTG TTG C-3'  |
| <i>Beta-2-microglobulin (b2mg)</i> | Forward  | 5'-TGA CTT TGT CAC AGC CAA AGA TA-3'  |
|                                    | Reverse  | 5'-AAT CCA AAT GCG GCA TCT TC-3'      |
| <i>Ferritin</i>                    | Forward  | 5'-TAC GAG CGT CTC CTG AAG ATG C-3'   |
|                                    | Reverse  | 5'-GGT TCA GCT TTT TCT CCA GGG C-3'   |
| <i>Nrf2</i>                        | Forward  | 5'-CAC ATC CAG TCA GAA ACC AGT GG-3'  |
|                                    | Reverse  | 5'-GGA ATG TCT GCG CCA AAA GCT G-3'   |
| <i>ASGPR1</i>                      | Forward  | 5'-GAA GCA GTT CGT GTC TGA CCT G-3'   |
|                                    | Reverse  | 5'-AGC GAG AGA ACC AGT AGC AGC T-3'   |
| <i>ASGPR2</i>                      | Forward  | 5'-AGA ACG CAC ACC TGG TGG TCA T-3'   |
|                                    | Reverse  | 5'-TTC CAA GAG CCA TCA CTG TCC G-3'   |
| <i>SCARF1</i>                      | Forward  | 5'-AGC TAC CGT GTC CAG GAT GAA G-3'   |
|                                    | Reverse  | 5'-GGC TCG ATG AAG CTG TGG TTG A-3'   |
| <i>TLR4</i>                        | Forward  | 5'-CCC TGA GGC ATT TAG GCA GCT A-3'   |
|                                    | Reverse  | 5'-AGG TAG AGA GGT GGC TTA GGC T-3'   |
| <i>Bmp6</i>                        | Forward  | 5'-GTC GTA ATC GCT CTA CCC AGT CC-3'  |
|                                    | Reverse  | 5'-CTG GGT AAT AAG GCA CTG GCA TG -3' |

**B**

| <b>Antigen</b>    | <b>Host species</b>                | <b>Dilution</b> | <b>Stock concentration (mg/mL)</b> | <b>Company</b>           | <b>Cat. No.</b> |
|-------------------|------------------------------------|-----------------|------------------------------------|--------------------------|-----------------|
| <b>CD206</b>      | rabbit                             | 1:500           | 0.5                                | HistoSure                | HS-488003       |
| <b>GAPDH</b>      | rabbit                             | 1:3000          |                                    | CST                      | 2118S           |
| <b>Annexin V</b>  | rabbit                             | 1:200           | 2.0                                | Proteintech              | 11060-1-AP      |
| <b>Rabbit IgG</b> | Donkey Alexa Fluor® 647-conjugated | 1:500           |                                    | jacksonimmuno.com        | 711-605-152     |
| <b>Hemoglobin</b> | rabbit                             | 1:250           | 0.5                                | Thermo Fisher Scientific | PA5-145321      |

**Suppl. Table S2: Clinical characteristics of heavy-drinking patients included in hepatic Stabilin-1 expression analysis**

Summary of demographic and laboratory parameters of patients whose liver tissue was used for Stabilin-1 mRNA quantification.

| <b>Patient characteristics (N=47)</b> |                            |             |           |
|---------------------------------------|----------------------------|-------------|-----------|
|                                       | <b>Normal range</b>        | <b>Mean</b> | <b>SD</b> |
| <b>General information</b>            |                            |             |           |
| Sex (male)                            |                            | 66%         |           |
| Age (years)                           |                            | 52.7        | 13.0      |
| BMI (kg/m <sup>2</sup> )              | 18-25                      | 25.5        | 5.1       |
| Alcohol consumption (g/day)           | <40 (m), <20 (f)           | 187         | 124       |
| <b>Routine laboratory</b>             |                            |             |           |
| AST (U/L)                             | <50                        | 132         | 145       |
| ALT (U/L)                             | <50                        | 71          | 66        |
| GGT (U/L)                             | <60                        | 509         | 726       |
| AP (U/L)                              | 40-130 (m), 35-105 (f)     | 113         | 65        |
| Bilirubin total (mg/dL)               | <1.3                       | 1.7         | 2.3       |
| INR                                   | 0.95-1.10                  | 1.04        | 0.39      |
| Hemoglobin (g/dL)                     | 13.5-17.5 (m), 12-16 (f)   | 13.6        | 2.0       |
| Hematocrit (%)                        | 40-53 (m), 36-48 (f)       | 38.4        | 5.4       |
| MCV (fL)                              | 80-96                      | 95.3        | 7.1       |
| Erythrocytes (/pL)                    | 4.5-5.9 (m), 4.1-5.1 (f)   | 4.1         | 0.7       |
| Leukocytes (/nL)                      | 3.7-10.0 (m), 3.5-10.0 (f) | 8.3         | 3.4       |
| Platelets (/nL)                       | 150-360                    | 184.2       | 74.0      |
| Ferritin (ng/mL)                      | 30-400 (m), 13-150 (f)     | 818         | 779       |
| CRP (mg/L)                            | <0.5                       | 12.7        | 31.5      |
| Albumin (g/dL)                        | 3.8-5.9                    | 4.1         | 0.7       |
| Transferrin saturation (%)            | 16-45                      | 43.0        | 25.1      |
| Serum iron (µg/dL)                    | 59-158 (m), 37-145 (f)     | 125.2       | 68.8      |
| Triglycerides (mg/dL)                 | <150                       | 162.1       | 145.3     |
| LDH (U/L)                             | <250                       | 254         | 82        |
| CD163 (ng/mL)                         | <800                       | 1210        | 813       |
| <b>Ultrasound</b>                     |                            |             |           |
| Liver size (cm)                       | <16                        | 16.4        | 4.4       |
| Hepatic steatosis (0-3)               | 0                          | 1.9         | 0.8       |
| Spleen size (cm)                      | <11.5                      | 10.1        | 1.9       |
| Ascites (1 or 0)                      | 0                          | 13%         |           |
| Signs of cirrhosis (1 or 0)           | 0                          | 18%         |           |
| <b>Transient elastography</b>         |                            |             |           |
| Liver stiffness (kPa)                 | <6                         | 21.5        | 26.7      |
| CAP (dB/m)                            | <240                       | 300         | 48        |

**Suppl. Table S3: Calculation of hemin equivalents corresponding to lysed red blood cell (RBC) percentages**

Approximate conversion of lysed RBC percentages into micromolar hemin concentrations used in *in vitro* stimulation assays

**Calculation of hemin equivalents from lysed RBCs**

The hemoglobin concentration in whole blood was assumed to be 130 g/L (within the normal range of 110–150 g/L in females). Accordingly, 250  $\mu$ L whole blood contains 32,500  $\mu$ g hemoglobin, which is assumed to be fully retained within the packed RBC fraction after centrifugation (200  $\mu$ L).

Thus, 10  $\mu$ L packed RBCs contain  $32,500 \mu\text{g} \times (10 / 200) = 1,625 \mu\text{g Hb}$ .

1% (v/v) RBC = 10  $\mu$ L RBCs in a total volume of 1 mL

Hb molecular weight: 67,000 g/mol

Mass (g) = Concentration (M)  $\times$  Volume (L)  $\times$  Molecular weight (g/mol)

Hb concentration of 1% RBCs ( $\mu$ M) =

$1,625 (\mu\text{g}) / 0.001 (\text{L}) / 67,000 (\mu\text{g}/\mu\text{mol}) = 24.25 \mu\text{M}$

Globin accounts for ~96%, heme for ~4%

1,625  $\mu$ g Hb contains:

$1,625 \times 4\% = 65 \mu\text{g hemin}$

Hemin molecular weight: 616.5 g/mol

Hemin concentration of 1% RBCs ( $\mu$ M) =

$65 (\mu\text{g}) / 0.001 (\text{L}) / 616.5 (\mu\text{g}/\mu\text{mol}) = 105.44 \mu\text{M}$

**Conclusion: 1% RBC corresponds to 24.25  $\mu$ M Hb and 105.44  $\mu$ M hemin**

**Suppl. Table S4: Mouse characteristics**

| <b>Parameter</b>                                           | <b>Controls</b> |           | <b>Chronic EtOH model</b> |           | <b>PHZ model</b> |           |
|------------------------------------------------------------|-----------------|-----------|---------------------------|-----------|------------------|-----------|
|                                                            | <b>Mean</b>     | <b>SD</b> | <b>Mean</b>               | <b>SD</b> | <b>Mean</b>      | <b>SD</b> |
| <b>Leukocytes (<math>\times 10^3/\mu\text{L}</math>)</b>   | 43              | 20        | 26                        | 14        | 24               | 4         |
| <b>Erythrocytes (<math>\times 10^6/\mu\text{L}</math>)</b> | 9.2             | 0.9       | 9.7                       | 0.6       | 3.9              | 0.3       |
| <b>Hemoglobin (g/dL)</b>                                   | 15.4            | 1.1       | 16.4                      | 1.4       | 7.8              | 0.4       |
| <b>Hematocrit (%)</b>                                      | 45.1            | 3.5       | 47.4                      | 3.7       | 19.8             | 1.5       |
| <b>LDH (U/L)</b>                                           | 424.3           | 363.1     | 1045.0                    | 791.1     | 4433.0           | 671.8     |
| <b>AST (U/L)</b>                                           | 193.0           | 133.4     | 583.8                     | 459.9     | 237.5            | 17.7      |
| <b>ALT (U/L)</b>                                           | 38.8            | 13.4      | 127.2                     | 67.8      | 36.5             | 9.2       |
| <b>Serum iron (<math>\mu\text{mol/L}</math>)</b>           | 37.0            | 28.0      | 20.8                      | 6.0       | 26.9             | 5.9       |
| <b>Albumin (g/L)</b>                                       | 24.5            | 12.7      | 23.7                      | 6.1       | 23.7             | 1.6       |

**Suppl. Table S5: Clinical characteristics of heavy-drinking patients included in LSECs immunofluorescence analyses**

Demographic, clinical, and laboratory features of patients whose liver cryosections were used for immunofluorescent staining of RBC-derived hemoglobin and CD206-positive LSECs.

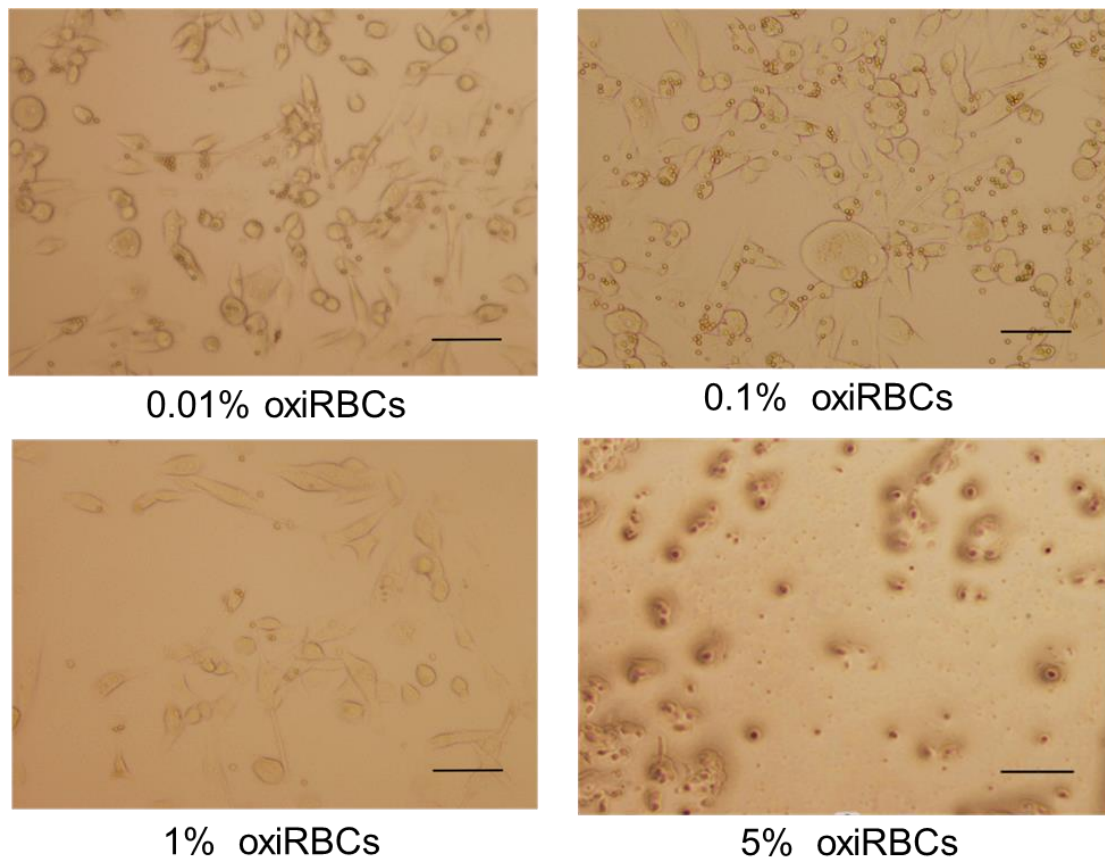
| Parameter                 | Units             | Category     | Heavy drinkers<br>without hemolysis |      |      | Heavy drinkers<br>with hemolysis |      |      |
|---------------------------|-------------------|--------------|-------------------------------------|------|------|----------------------------------|------|------|
|                           |                   |              | P1                                  | P2   | P3   | P4                               | P5   | P6   |
| <b>Sex</b>                | male: 1           | General      | 0                                   | 1    | 1    | 0                                | 0    | 1    |
| <b>Age</b>                | years             | General      | 50                                  | 42.2 | 41   | 70.8                             | 44.4 | 58.6 |
| <b>BMI</b>                | Kg/m <sup>2</sup> | Morphometric | 26.3                                | 26.1 | 27.8 | 20.4                             | 23.7 | 21.3 |
| <b>AST</b>                | U/L               | Laboratory   | 33                                  | 31   | 43   | 177                              | 285  | 175  |
| <b>ALT</b>                | U/L               | Laboratory   | 32                                  | 39   | 71   | 95                               | 66   | 49   |
| <b>GGT</b>                | U/L               | Laboratory   | 51                                  | 78   | 291  | 841                              | 3533 | 822  |
| <b>AP</b>                 | U/L               | Laboratory   | 58                                  | 71   | 85   | 135                              | 371  | 127  |
| <b>Bilirubin total</b>    | mg/dL             | Laboratory   | 0.4                                 | 0.3  | 0.2  | 2                                | 8.3  | 2.5  |
| <b>Hemoglobin</b>         | g/dL              | Laboratory   | 15.1                                | 15.5 | 16.7 | 9.6                              | 12.7 | 13.4 |
| <b>MCV</b>                | fL                | Laboratory   | 87                                  | 86   | 88   | 114                              | 112  | 105  |
| <b>Erythrocytes</b>       | /pL               | Laboratory   | 4.9                                 | 5.2  | 5.0  | 2.4                              | 3.3  | 3.6  |
| <b>Ferritin</b>           | ng/mL             | Laboratory   | 132                                 | 96   | 147  | 2420                             | 1887 | 1573 |
| <b>CRP</b>                | mg/L              | Laboratory   | 0.5                                 | 0.5  | 3.8  | 1                                | 67.7 | 61.8 |
| <b>Liver size</b>         | cm                | Ultrasound   | 18.4                                | 16.8 | 17   | 15.5                             | 40   | 20   |
| <b>Hepatic steatosis</b>  | 0-3               | Ultrasound   | 2                                   | 0    | 0    | 2                                | 2    | 3    |
| <b>Spleen size</b>        | cm                | Ultrasound   | 8.3                                 |      | 11   | 8.6                              | 9.5  | 13.9 |
| <b>Ascites</b>            | 1 or 0            | Ultrasound   | 0                                   | 0    | 0    | 0                                |      | 1    |
| <b>Signs of cirrhosis</b> | 1 or 0            | Ultrasound   | 0                                   | 0    | 0    | 0                                |      | 1    |
| <b>Liver stiffness</b>    | kPa               | Ultrasound   | 2.5                                 | 6.5  | 4.3  | 12.2                             | 70.6 | 73.5 |
| <b>Kleiner score</b>      | 0-4               | Histology    | 0                                   | 2    | 1    | 1                                | 4    | 4    |

**Suppl. Table S6: Clinical characteristics of heavy-drinking patients included in hemoglobin immunofluorescence analyses**

Demographic, clinical, and laboratory features of patients whose liver cryosections were used for immunofluorescent staining of autofluorescence and hemoglobin antibody.

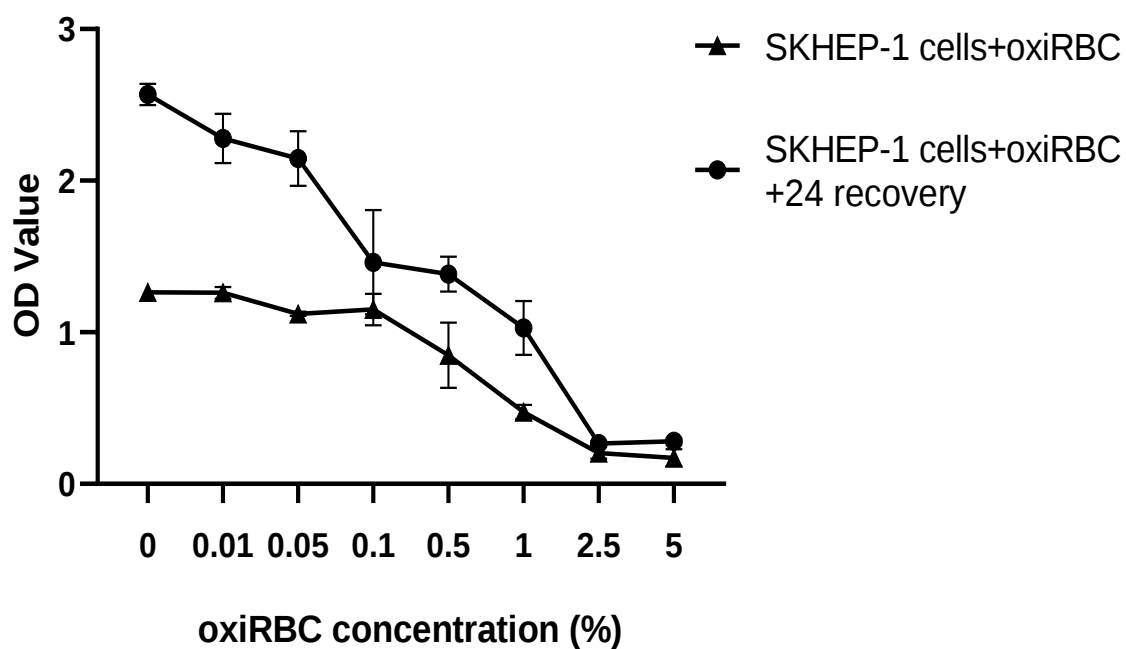
| Parameter                 | Units             | Category     | Heavy drinkers<br>without hemolysis |      | Heavy drinkers<br>with hemolysis |       |
|---------------------------|-------------------|--------------|-------------------------------------|------|----------------------------------|-------|
|                           |                   |              | P7                                  | P8   | P9                               | P10   |
| <b>Sex</b>                | male: 1           | General      | 0                                   | 1    | 1                                | 0     |
| <b>Age</b>                | years             | General      | 50                                  | 42.2 | 49                               | 59    |
| <b>BMI</b>                | Kg/m <sup>2</sup> | Morphometric | 26.3                                | 26.1 | 23.7                             | 18.1  |
| <b>AST</b>                | U/L               | Laboratory   | 33                                  | 31   | 271                              | 104   |
| <b>ALT</b>                | U/L               | Laboratory   | 32                                  | 39   | 149                              | 88    |
| <b>GGT</b>                | U/L               | Laboratory   | 51                                  | 78   | 391                              | 241   |
| <b>AP</b>                 | U/L               | Laboratory   | 58                                  | 71   | 56                               | 195   |
| <b>Bilirubin total</b>    | mg/dL             | Laboratory   | 0.4                                 | 0.3  | 0.8                              | 9.2   |
| <b>Hemoglobin</b>         | g/dL              | Laboratory   | 15.1                                | 15.5 | 13.7                             | 12.1  |
| <b>MCV</b>                | fL                | Laboratory   | 87                                  | 86   | 112                              | 99    |
| <b>Erythrocytes</b>       | /pL               | Laboratory   | 4.9                                 | 5.2  | 3.5                              | 3.4   |
| <b>Ferritin</b>           | ng/mL             | Laboratory   | 132                                 | 96   | 1126                             | 1110  |
| <b>CRP</b>                | mg/L              | Laboratory   | 0.5                                 | 0.5  | 2.27                             | 56.86 |
| <b>Liver size</b>         | cm                | Ultrasound   | 18.4                                | 16.8 | 17.8                             | 12    |
| <b>Hepatic steatosis</b>  | 0-3               | Ultrasound   | 2                                   | 0    | 3                                |       |
| <b>Spleen size</b>        | cm                | Ultrasound   | 8.3                                 |      |                                  | 11    |
| <b>Ascites</b>            | 1 or 0            | Ultrasound   | 0                                   | 0    | 0                                | 1     |
| <b>Signs of cirrhosis</b> | 1 or 0            | Ultrasound   | 0                                   | 0    | 1                                | 1     |
| <b>Liver stiffness</b>    | kPa               | Ultrasound   | 2.5                                 | 6.5  | 28.4                             | 59    |
| <b>Kleiner score</b>      | 0-4               | Histology    | 0                                   | 2    | 4                                | 3     |

## Supplementary figures



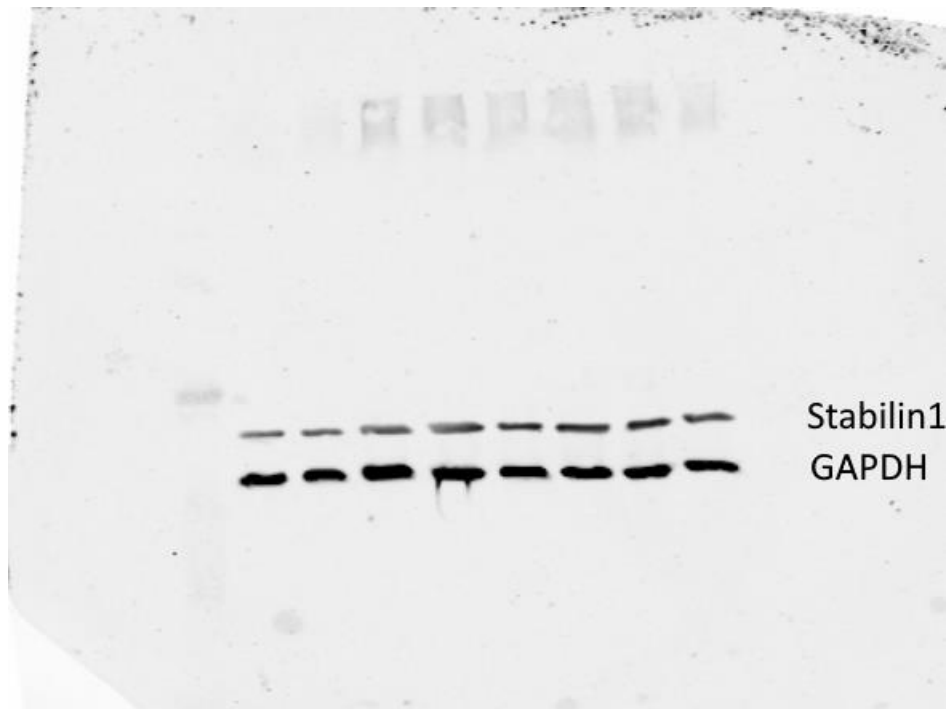
### **Supplementary Figure S1: Cytotoxic effects of oxidized RBCs on SK-HEP1 cells.**

SK-HEP1 cells were exposed to increasing concentrations of oxiRBCs for 24 hours. Cell density was visibly reduced at concentrations exceeding 0.5%, consistent with cytotoxic effects. Scale bar: 100 μm.



**Supplementary Figure S2: Independent replicate confirming oxiRBC-induced toxicity in SK-HEP1 cells.**

An independent biological replicate under identical conditions confirmed the concentration-dependent cytotoxicity of oxiRBCs, as shown in Supplementary Figure 1.



**Supplementary Figure S3: Original stabilin-1 Western blot for Figure 4B.**