

**1 Supplementary Fig. 1: Expression and distribution of SREBP isoforms and SCAP in TM**

2 (A) Expression profile of SREBP isoforms and SCAP in primary human trabecular meshwork (HTM) cells by  
 3 reverse-transcription polymerase chain reaction (RT-PCR) showing that SREBP1 transcript 2, SREBP2 transcript 1,  
 4 and SCAP were expressed in HTM. (B) Protein expression of proform SREBP1 (Pro-SREBP1) and SREBP2 (Pro-  
 5 SREBP2), and nuclear form SREBP1 (N-SREBP1) and SREBP2 (N-SREBP2), and SCAP in primary HTM cells.  
 6 GAPDH was used as the loading control. (C) Protein expression of Pro-SREBP1 and Pro-SREBP2, N-SREBP1 and  
 7 N-SREBP2, and SCAP in the primary porcine trabecular meshwork (PTM) cells. GAPDH was used as the loading  
 8 control. (D) Immunofluorescence (IF) shows the cytosolic distribution of SREBP1 (green staining), SREBP2 (green  
 9 staining) and SCAP (green staining) in primary HTM cells. The nucleus was stained with DAPI in blue. Images  
 10 were captured in z-stack in a confocal microscope, and stacks were orthogonally projected. Scale bar 20 microns.  
 11 (E) Tissue distribution of SREBP1 (green staining) and SREBP2 (green staining) and SCAP (green staining) in the  
 12 aqueous humor (AH) outflow pathway of a normal human eye specimen by IF. The nucleus was stained with DAPI  
 13 in blue. Images were captured in z-stack in a confocal microscope, and stacks were orthogonally projected. Scale bar  
 14 50 microns. (F) The negative control for SREBPs and SCAP distribution in AH outflow pathway (in the presence of  
 15 secondary antibody alone) did not show any significant staining (green panel).

**16 Supplementary Fig. 2: Dose-dependent effects of fatostatin on SREBPs activation in PTM cells**

17 (A) and (B) Dose-dependent treatment of fatostatin on PTM cells to test the optimal concentration of fatostatin on  
 18 TM cells. Immunoblotting shows 20  $\mu$ M fatostatin for 24 h treatment significantly decreased both N-SREBP1 and  
 19 N-SREBP2 expression. GAPDH was used as a loading control. (C) Examination of the effects of 20  $\mu$ M fatostatin  
 20 on primary PTM cell viability. Cell viability assay in serum-starved PTM cells treated with 20  $\mu$ M fatostatin for 24  
 21 h was performed using fluorometric live/dead staining of FDA-PI staining. The first row represents the control  
 22 DMSO treatment, and the second row represents 20  $\mu$ M fatostatin treatment of serum starved PTM cells. The first,  
 23 second, and the third columns are bright field image of PTM cells 24 h after treatment, FDA, and PI staining  
 24 respectively. The green channel shows viable cells that take up FDA and emit green fluorescence, and the red  
 25 channel shows dead cells, which take up PI and give out red fluorescence. The graphical representation denotes the  
 26 mean percentage ratio of FDA/PI, including the viable cells (green bar) and the non-viable cells (red bar) in both  
 27 control and 20  $\mu$ M fatostatin treated cells. No significant cell death was observed after 20  $\mu$ M fatostatin for 24 h  
 28 treatment. Scale bar 100 micron. Values represent the mean  $\pm$  SEM, where n = 4 (biological replicates). \*p < 0.05  
 29 was considered statistically significant.

**30 Supplementary Fig. 3: 6 h mechanical stretch induces SREBPs activation**

31 (A) and (B) Localization of SREBPs in HTM cells subjected to cyclic mechanical stretch was checked using  
 32 immunofluorescence (IF). After 6 h of mechanical stress, HTM cells shows a strong nuclear localization of both  
 33 SREBP1 and SREBP2 (second-row third column). Phalloidin was used to stain the distribution of filamentous actin  
 34 (F-actin) fiber in the cells. After 6 h of mechanical stress, there was increased F-actin distribution inside the HTM  
 35 cells (second-row fourth column). Quantification of immunofluorescence images using ImageJ-based fluorescence  
 36 intensity measurements shows a significant increase in both nuclear SREBP1 and nuclear SREBP2's mean  
 37 fluorescence intensity in 6 h stretched HTM cells (right panel).

**38 Supplementary Fig. 4: HTM cell changes after Ad5-N-SREBPs treatment**

39 HTM cells were treated with either AdMT or Ad5-N-SREBPs for 24 h followed by 48 h serum starvation. (A)  
 40 Compared to AdMT, Ad5-N-SREBP1a and Ad5-N-SREBP1c treatment on HTM cells significantly increased N-  
 41 SREBP1 mRNA expression, and Ad5-N-SREBP2 significantly increased N-SREBP2 mRNA expression in HTM  
 42 cells. 18S were used as internal controls for qPCR analysis. (B) Compared to AdMT, Ad5-N-SREBP1a and Ad5-N-  
 43 SREBP1c significantly increased N-SREBP1 protein expression, and Ad5-N-SREBP2 significantly increased N-  
 44 SREBP2 protein expression. The results were based on semi-quantitative immunoblotting with subsequent  
 45 densitometric analysis.  $\beta$ -actin was used as a loading control. (C) Bright-field cell culture images were captured by  
 46 Nikon TS100 Inverted Phase Contrast Microscope. Scale bar 25 micron. After treatment, Ad5-N-SREBPs treated  
 47 HTM cells displayed increased lamellipodia and filopodial extension formation (indicated by black arrows),

48 compared to AdMT treatment. **(D)** and **(E)** Immunofluorescence (IF) shows the distribution of SREBP1, SREBP2,  
 49 filamentous actin (F-actin) fibers, and paxillin in HTM cells under AdMT and Ad5-N-SREBPs treatments. **(D)** Ad5-  
 50 N-SREBP1a (second-row third column), and Ad5-N-SREBP1c (third-row third column) induced strong staining of  
 51 SREBP1 in the nucleus in HTM cells compared to AdMT (first-row third column). Similarly, **(E)** Ad5-N-SREBP2  
 52 (second-row third column) induced strong staining of SREBP2 in the nucleus in HTM cells compared to AdMT  
 53 (first-row third column). Compared to AdMT (first-row fifth column), **(D)** Ad5-N-SREBP1a (second-row fifth  
 54 column) and Ad5-N-SREBP1c (third-row fifth column), and **(E)** Ad5-N-SREBP2 (second-row fifth column) caused  
 55 the increased distribution of F-actin fibers stained by phalloidin (purple/grayscale) in HTM cells and induced  
 56 increased lamellipodia and filopodia formation (indicated by yellow arrows). **(D)** Ad5-N-SREBP1a (second-row  
 57 fourth column), Ad5-N-SREBP1c (third-row fourth column), and **(E)** Ad5-N-SREBP2 (second-row fourth column)  
 58 also induced more distribution of paxillin (green/grayscale) at the edges of F-actin fibers (indicated by white arrows)  
 59 in HTM cells compared to the AdMT (first-row fourth column). The nucleus was stained with DAPI in blue.  
 60 Images were captured in z-stack in a confocal microscope, and stacks were orthogonally projected. Scale bar 20  
 61 micron. Values represent the mean  $\pm$  SEM, where  $n = 4$  (biological replicates). \* $p < 0.05$  was considered statistically  
 62 significant.

63 **Supplementary Fig. 5: Molecular inactivation of SREBPs by knocking down SCAP in old and adult SCAP<sup>ff</sup>**  
 64 **mice lowers IOP in vivo**

65 **(A)** In old SCAP<sup>ff</sup> mice, starting from 10 days after saline and viral injection, IOP was consistently and significantly  
 66 decreased in Ad5.CMV.iCre-eGFP injection group compared to Ad5.CMV.eGFP injection group, and sustained  
 67 until 50 days post-injection. Compared to the untouched and saline injection group, IOP was also lower in  
 68 Ad5.CMV.iCre-eGFP injection group, but not consistently statistically significant. **(B)** In old SCAP<sup>ff</sup> mice, IOP  
 69 changes in each group were analyzed and compared to their baseline normal IOP (before injection/time point 0  
 70 days), the graphical representation shows that IOP was significantly decreased in Ad5.CMV.iCre-eGFP injection  
 71 group starting from 10 days after injection and sustained until 50 days post-injection. There were no significant  
 72 changes in IOP levels in other control groups. **(C)** In old SCAP<sup>ff</sup> mice, IOP percentage change (Delta IOP)  
 73 compared to their baseline normal IOP in each group was calculated, and graphical representation shows that IOP in  
 74 Ad5.CMV.iCre-eGFP injection group was decreased as much as 42.72 % of baseline normal (before injection/time  
 75 point 0 days) with an average decrease of 27.61 %. The IOP in other control groups showed less than 17 % changes  
 76 of baseline normal IOP. **(D)** In adult SCAP<sup>ff</sup> mice, start from 10 days after saline and viral injection, IOP was  
 77 consistently and significantly decreased in Ad5.CMV.iCre-eGFP injection group compared to Ad5.CMV.eGFP  
 78 injection group, and sustained until 50 days post-injection. Compared to the untouched, saline injection group, IOP  
 79 was also lower in Ad5.CMV.iCre-eGFP injection group, but not consistently statistically significant. **(E)** In adult  
 80 SCAP<sup>ff</sup> mice, IOP was significantly decreased in Ad5.CMV.iCre-eGFP injection group starting from 10 days after  
 81 injection and sustained until 50 days post-injection. There were no significant changes in IOP levels in other control  
 82 groups. **(F)** In adult SCAP<sup>ff</sup> mice, IOP in Ad5.CMV.iCre-eGFP injection group decreased as much as 43.14 % of  
 83 baseline normal (before injection/time point 0 days) with an average decrease of 28.10 %. The IOP in other control  
 84 groups showed less than 17 % changes of baseline normal IOP. Values represent the mean  $\pm$  SEM, where  $n = 4-5$   
 85 (biological replicates). \* $p < 0.05$  was considered statistically significant.

86 **Supplementary Fig. 6: Lipidomic volcano analysis and top 80 lipid features heatmap**

87 **(A)** In the volcano plot, the lipids were presented as log 2-fold changes (FC) against the  $-\log_{10}(p)$  of the differential  
 88 expression between the control (CTL) and fatostatin treatment (FATO). Lipidomic volcano analysis revealed 79  
 89 significantly changed lipids above the threshold ( $|FC| > 1$  and  $p < 0.05$ ) in fatostatin treatment, compared to the  
 90 control. Blue color dots indicate significantly decreased lipids, red color dots indicate significantly increased lipids,  
 91 gray color dots are non-significantly changed lipids. **(B)** Heatmap illustrating the lipid profiles of the control (CTL)  
 92 and fatostatin (FATO) treated HTM cells. Top 80 lipid features were ranked using the t test, distance was measured  
 93 using the Pearson correlation, and clustering was determined using the Ward algorithm.

94 **Supplementary Fig. 7: ECM changes due to response to SREBPs activation**

95 Immunofluorescence (IF) shows the FN and COL1A expression and distribution in HTM cells after fatostatin  
96 combined with AdMT or Ad5-N-SREBPs treatments. Increased FN and COL1A distribution in HTM cells were  
97 observed in fatostatin combined with Ad5-N-SREBP1a (second-row third column and sixth column), Ad5-N-  
98 SREBP1c (third-row third column and sixth column) and Ad5-N-SREBP2 (fourth-row third column and sixth  
99 column) treatments, compared to fatostatin combined with AdMT treatment (first-row third column and sixth  
100 column). The nucleus was stained with DAPI in blue. Images were captured in z-stack in a confocal microscope,  
101 and stacks were orthogonally projected. Scale bar 20 micron. White box in the images indicate the chosen cells and  
102 magnifications of these cells are included in Figure 7F and 7G.