

Supplementary Information

LIPID DROPLET DEGRADATION BY AUTOPHAGY CONNECTS MITOCHONDRIA METABOLISM TO PROX1-DRIVEN EXPRESSION OF LYMPHATIC GENES AND LYMPHANGIOGENESIS

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Supplementary Fig. 1

Supplementary Fig. 2

Supplementary Fig. 3

Supplementary Fig. 4

Supplementary Fig. 5

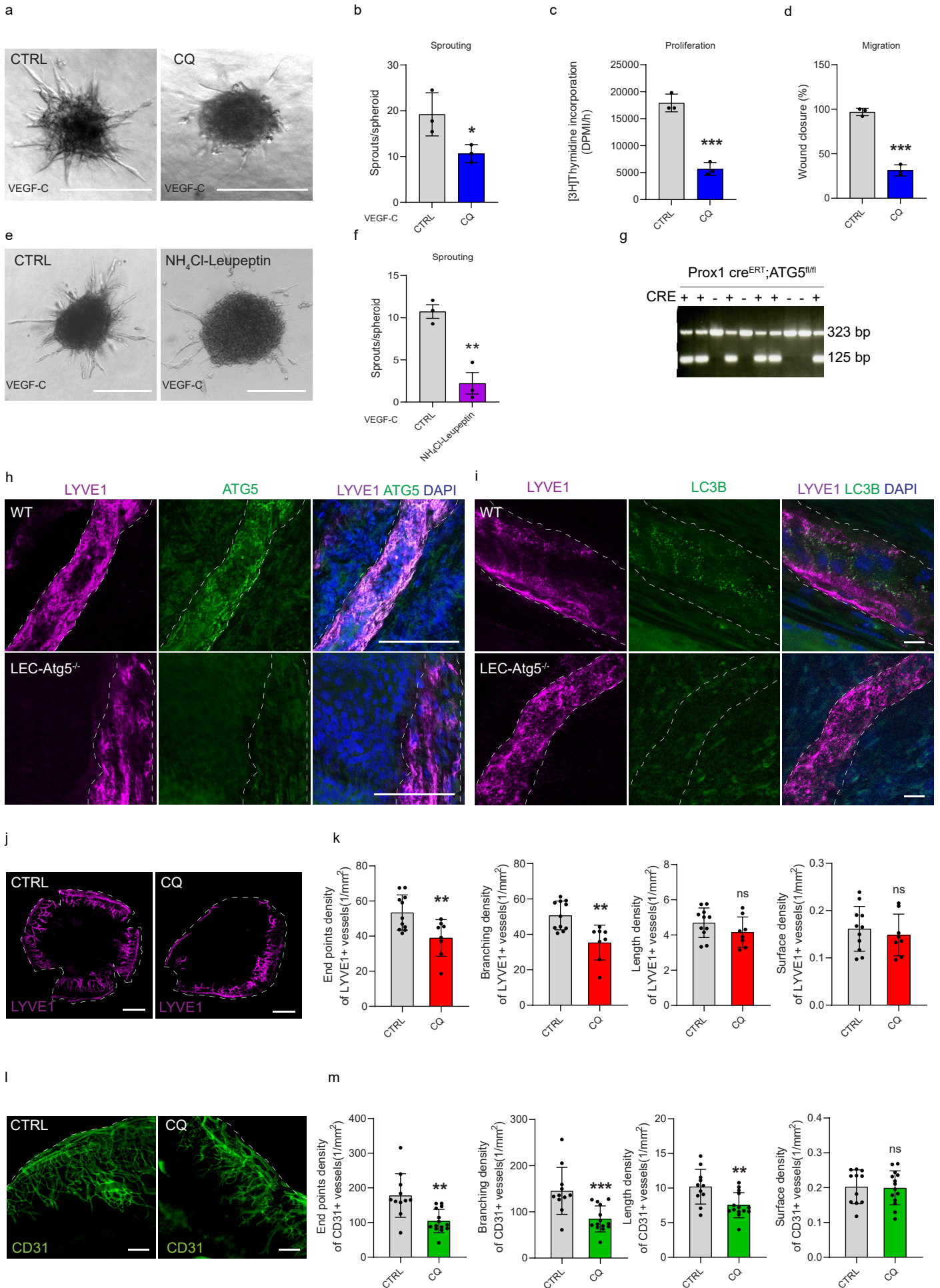
Supplementary Fig. 6

Supplementary Fig. 7

Supplementary Table 1: Primer sequences

Supplementary Table 2: Antibodies, Reagents and other Resources

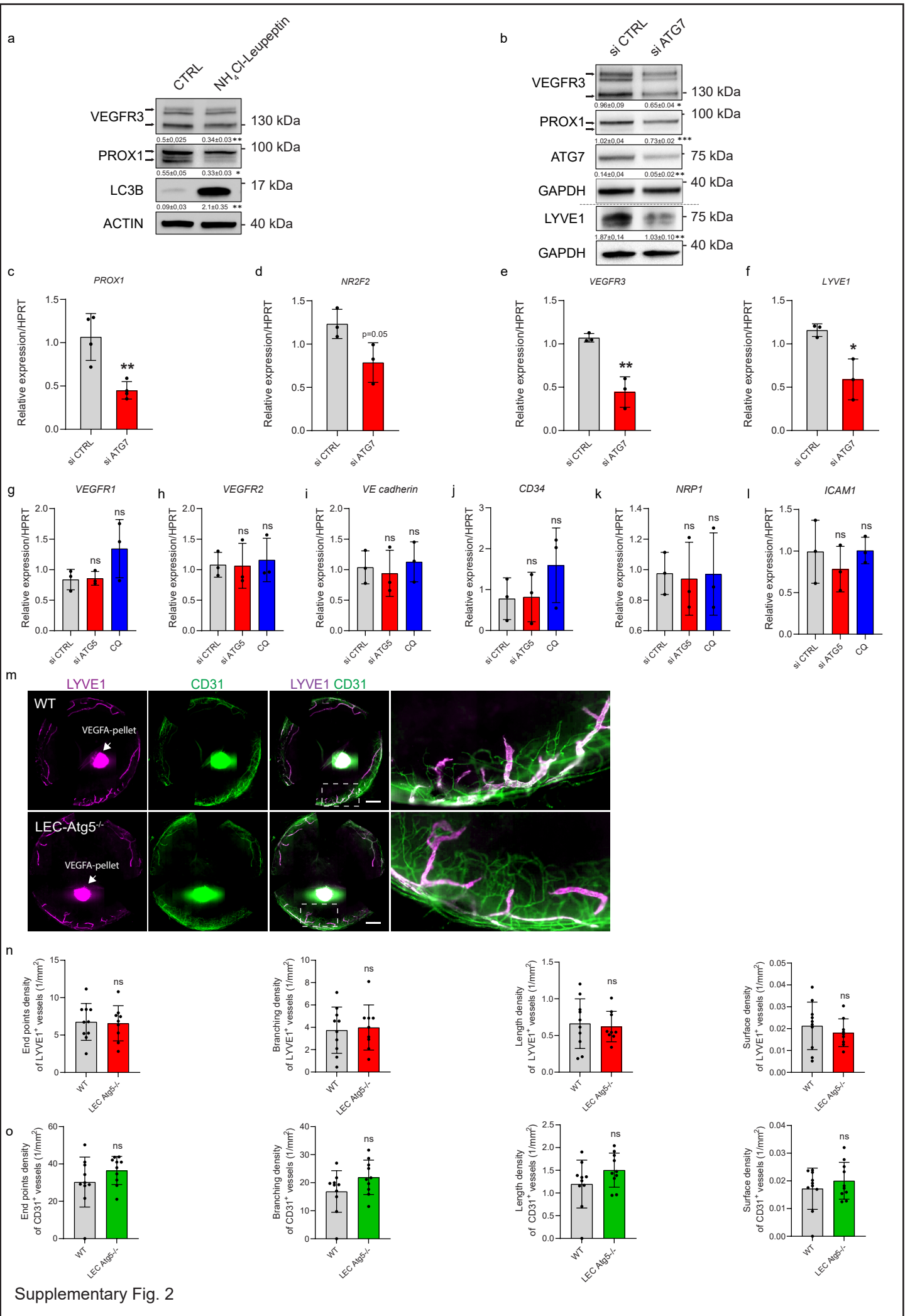
Gating strategies Extended data 3i



Supplementary Fig. 1

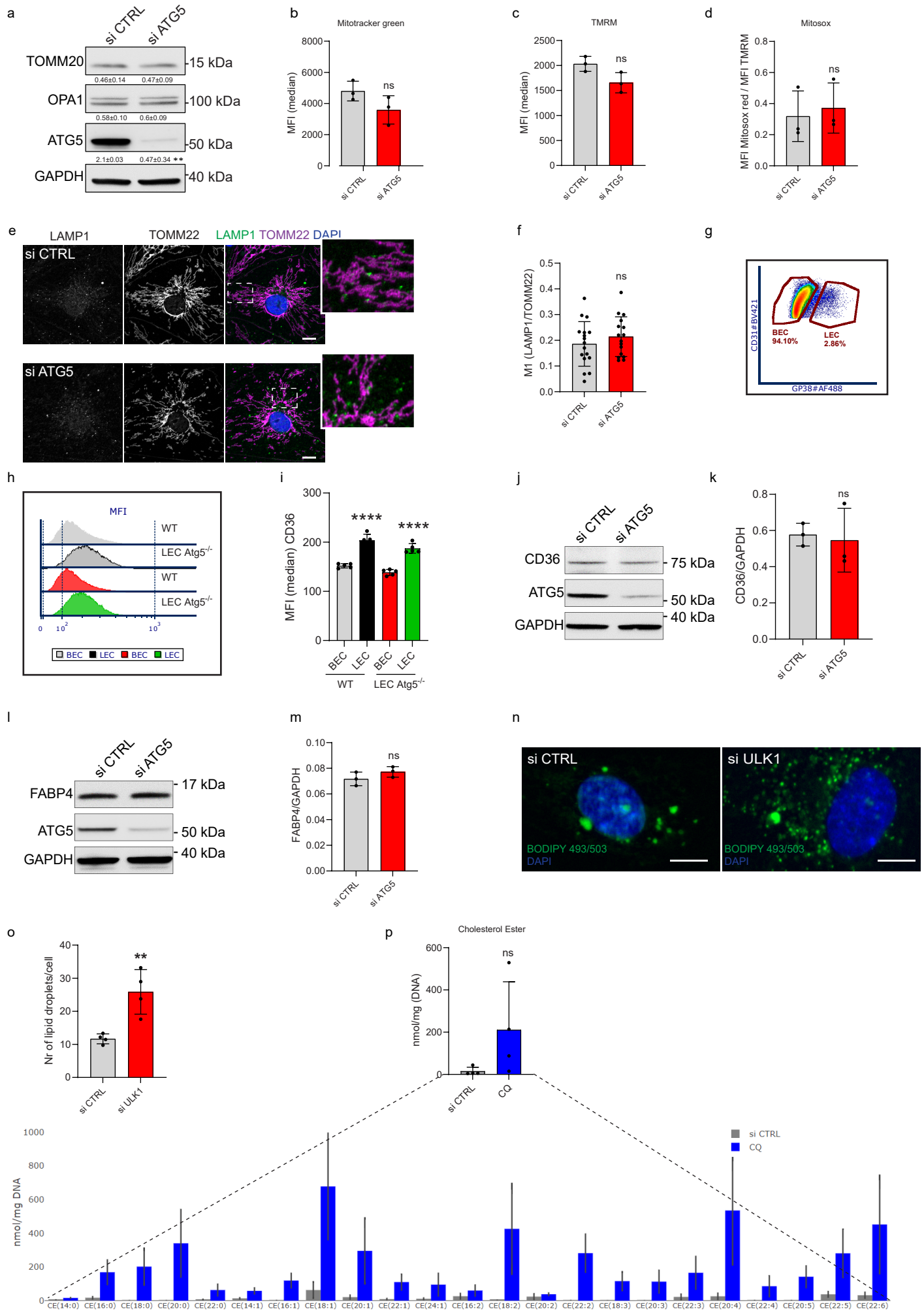
Supplementary Fig.1 | Effects of chloroquine and NH₄Cl-Leupeptin on LEC function and confirmation of LEC-ATG5^{-/-} mouse model.

a,b) Representative images (a) and quantification (b) of CTRL and CQ-(25 μ M, 48h) treated LEC spheroids upon stimulation with VEGF-C (100 ng/mL, 48h). Scale bar represents 100 μ m. Mean \pm SD, N=3 independent donors analyzed using unpaired Student's t test (two-tailed), *p = 0.04. Mean represents mean per independent experiment, with a minimum of 10 spheroids analyzed per condition per independent experiment. **c)** [³H]Thymidine incorporation assay into DNA of CTRL and CQ-treated LEC upon stimulation with VEGF-C. Mean \pm SD, N=3 biological replicates analyzed using unpaired Student's t test (two-tailed), ***p = 0.0005. **d)** Quantification of scratch wound healing for CTRL and CQ-treated LEC monolayers in the presence of 500 μ g/ml Mitomycin C. Mean \pm SD, N=3 biological replicates analyzed using unpaired Student's t test (two-tailed), ***p = 0.0001. **e,f)** Representative images (e) and quantification (f) of CTRL and NH₄Cl-Leupeptin (20mM and 100 μ M, 48h) treated LEC spheroids upon stimulation with VEGF-C. Mean \pm SD, N=3 independent donors analyzed using unpaired Student's t test (two-tailed), **p = 0.005. Mean represents mean per independent experiment, with a minimum of 9 spheroids analyzed per condition per independent experiment. **g)** Genotyping for the presence of Atg5 floxed (323bp) and excision band upon tamoxifen administration (125bp) in Prox1 creERT; ATG5^{fl/fl} mice upon tamoxifen administration. Genotyping was performed prior to every in vivo experiment to confirm knockout of Atg5. **h)** Representative super resolution AiryScan images of LYVE1+ lymphatic vessels (dashed lines) from corneal sections dissected from wild type (WT) and LEC-Atg5 knock out mice (LEC-Atg5^{-/-}) stained for ATG5 and ATG5 from corneas of WT and LEC-Atg5^{-/-} mice. Note the absence of ATG5 immunoreactive signal in the LYVE1+ lymphatic vessel. Three corneas per condition were stained with similar results. Nuclei are stained with DAPI. Scale bar represents 100 μ m. **i)** Representative super resolution AiryScan images of LYVE1+ lymphatic vessels (dashed lines) from corneal sections dissected from wild type (WT) and LEC-Atg5^{-/-} mice stained for LC3B showing absence of autophagosomal bound LC3B in the lymphatic vessels of LEC-Atg5^{-/-} mice. Three corneas per condition were stained with similar results. Nuclei are stained with DAPI. Scale bar represents 10 μ m. **j)** Representative immunofluorescent images of whole corneal mounts (dashed lines) of vehicle treated or CQ-(50 mg/kg daily) treated wild type (WT) mice, stained for LYVE1, 8 days post corneal cauterization. Scale bar represents 1 mm. **k)** Quantification for the number of end points, number of branch points, average of cumulative length and surface density for LYVE1+ lymphatic vessels. Mean \pm SD, N=11 corneas for CTRL and N=8 corneas for CQ analyzed using unpaired Student's t test (two-tailed), **p < 0.01. **l)** Representative immunofluorescent images of corneal sections dissected from vehicle treated or CQ-treated mice stained for CD31, 8 days post corneal cauterization. Scale bar represents 100 μ m. **m)** Quantification for the number of end points, number of branch points, average of cumulative length and surface density for CD31+ blood vessels. Mean \pm SD, N=11 corneas for CTRL and N=14 corneas for CQ analyzed using unpaired Student's t test (two-tailed), **p < 0.01 and ***p < 0.001.



Supplementary Fig.2 | Autophagy deficiency does not impair general transcription of endothelial markers and responses to VEGF-A in vivo.

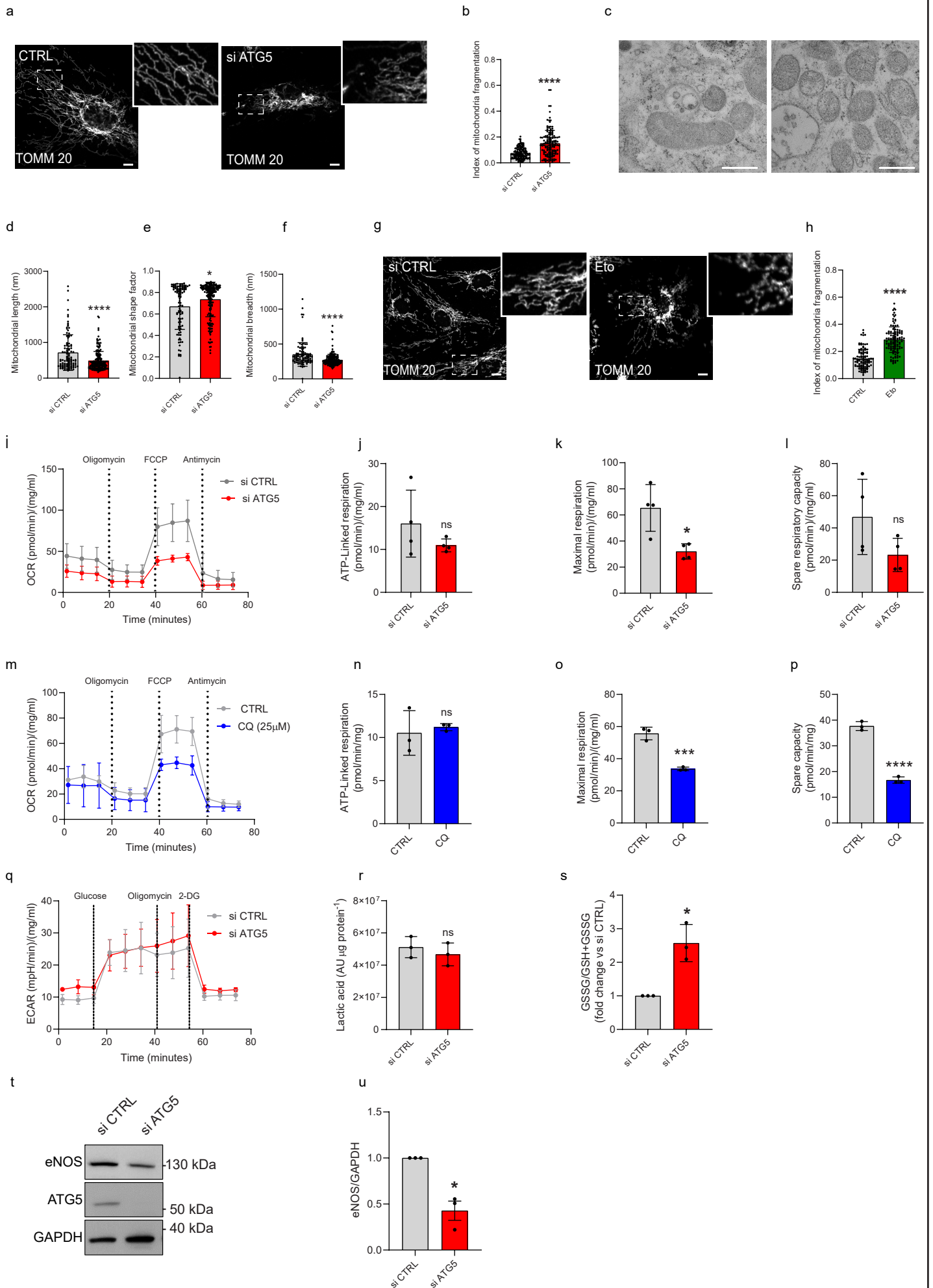
a) Representative blots for the indicated proteins in CTRL and NH₄CL-Leupeptin-treated (20mM and 100μM, 48h) LEC. Densitometric quantification is indicated beneath the blots Mean ± SD, N=3 biological replicates analyzed using unpaired Student's t test, *p < 0.05 and **p < 0.01. **b)** Representative blots for the indicated proteins in si CTRL and si ATG7 LEC. Densitometric quantification is indicated beneath the blots. Mean ± SD, N≥3 biological replicates analyzed using unpaired Student's t test, *p < 0.05, **p < 0.01 and ***p<0.001. **c-f)** RT-qPCR analysis of si CTRL and si ATG7 LEC. mRNA expression of PROX1, VEGFR3 and LYVE1 (relative to HPRT). Mean ± SD, N=3 (d,e,f) and N=4 (c) biological replicates analyzed by unpaired Student's t test (two-tailed), *p < 0.05, **p < 0.01. **g-l)** RT-qPCR analysis of si CTRL and si ATG5 LEC or CQ-(25μM, 48h) treated LEC. mRNA expression of VEGFR1, VEGFR2, VE cadherin, CD34, NRP1 and ICAM1 (relative to HPRT). Mean ± SD, N=3 biological replicates analyzed by one-way ANOVA with Tukey's test for multiple comparisons. p=not significant. **m)** Representative immunofluorescent images from whole corneal mounts of wild type (WT) and LEC-Atg5 knock out mice (LEC-Atg5^{-/-}) stained for LYVE1 and CD31, 5 days post pocket assay with VEGF-A (300ng/pellet) stimulation. Zoomed boxed areas show outgrowth of lymphatic vessel (magenta) and blood vessels (green). Scale bar represents 1 mm. **n)** Quantification for the number of end points, number of branch points, average of cumulative length and surface density for LYVE1+ vessels. Mean ± SD. N=10 corneas for WT and N=9 corneas for LEC-Atg5^{-/-} analyzed using unpaired Student's t test (two-tailed), p=not significant. **o)** Quantification for the number of end points, number of branch points, average of cumulative length and surface density for CD31+ vessels. Mean ± SD. N=10 corneas for WT and N=9 corneas for LEC-Atg5^{-/-} analyzed using unpaired Student's t test (two-tailed), p=not significant.



Supplementary Fig. 3

Supplementary Fig.3 | Effect of genetic or pharmacological inhibition of autophagy on mitochondrial protein turnover, mitochondrial ROS, mitochondrial membrane potential and lipid droplets.

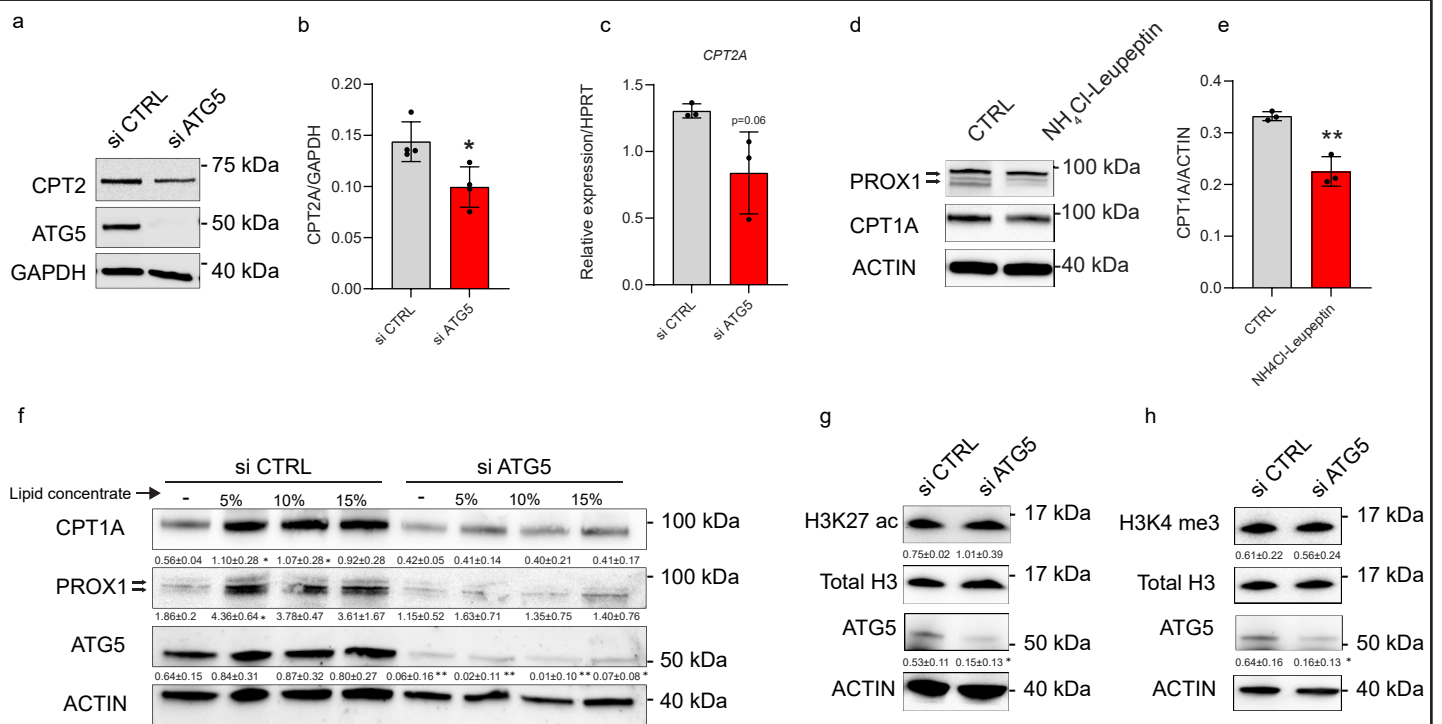
a) Representative blots for the indicated proteins in si CTRL, si ATG5 LEC. Densitometric quantification is indicated beneath the blots. Mean \pm SD, N=3 biological replicates analyzed using unpaired Student's t test, $**p < 0.01$. **b-d)** Flow cytometry analysis for mitochondrial mass using MitoTracker green (b), mitochondrial membrane potential using TMRM (c) and mitochondrial ROS using MitoSOX (d) in si CTRL and si ATG5 LEC. Mean \pm SD, N=3 analyzed using unpaired Student's t test (two-tailed). $p =$ not significant. **e)** Representative super resolution AiryScan images of si CTRL and si ATG5 immunostaining of TOMM22 and LAMP1. Nuclei are stained with DAPI. Scale bars represent 10 μm . **f)** Co-localization of LAMP1 and TOMM22 in si CTRL and si ATG5 LEC (Manders M1 coefficient). Mean \pm SD, N=2 biological replicates with a minimum of 16 cells per condition in total analyzed using unpaired Student's t test (two-tailed), $p =$ not significant. **g)** Flow cytometry histograms showing distinct populations of lymphatic (LEC, gated as double CD31+GP38+ double positive) and blood endothelial cells (BEC, gated as CD31+ positive), isolated from lung tissues of WT and LEC-Atg5^{-/-} mice. **h,i)** median fluorescence intensity (MFI) of surface expression of CD36 in BEC and LEC from WT and LEC-Atg5^{-/-} mice. Mean \pm SD, N=5 mice analyzed by one-way ANOVA, with Tukey's test for multiple comparisons, $****p < 0.0001$ to corresponding BEC. **j-m)** Representative blots for the indicated proteins (j,l) and densitometric quantification (k,m) in si CTRL, si ATG5 LEC. Mean \pm SD, N=3 biological replicates analyzed using unpaired Student's t test (two-tailed), $p =$ not significant. **n)** Representative images of BODIPY 493/503 staining of lipid droplets in si CTRL and si ULK1 LEC. Scale bars represent 10 μm . **o)** Quantification of lipid droplet number per cell. Mean \pm SD, N=4 biological replicates analyzed using unpaired Student's t test (two-tailed), $**p < 0.006$. Mean represents mean per independent experiment, with a minimum of 29 cells analyzed per condition per independent experiment. **p)** Cholesterol ester (CE) bar plots calculated as an average of the lipid subspecies analyzed across si CTRL and CQ-treated LEC (25 μM , 48h). Mean \pm SD, N=4 analyzed by unpaired Student's t test (two-tailed), $p = 0.14$.



Supplementary Fig. 4

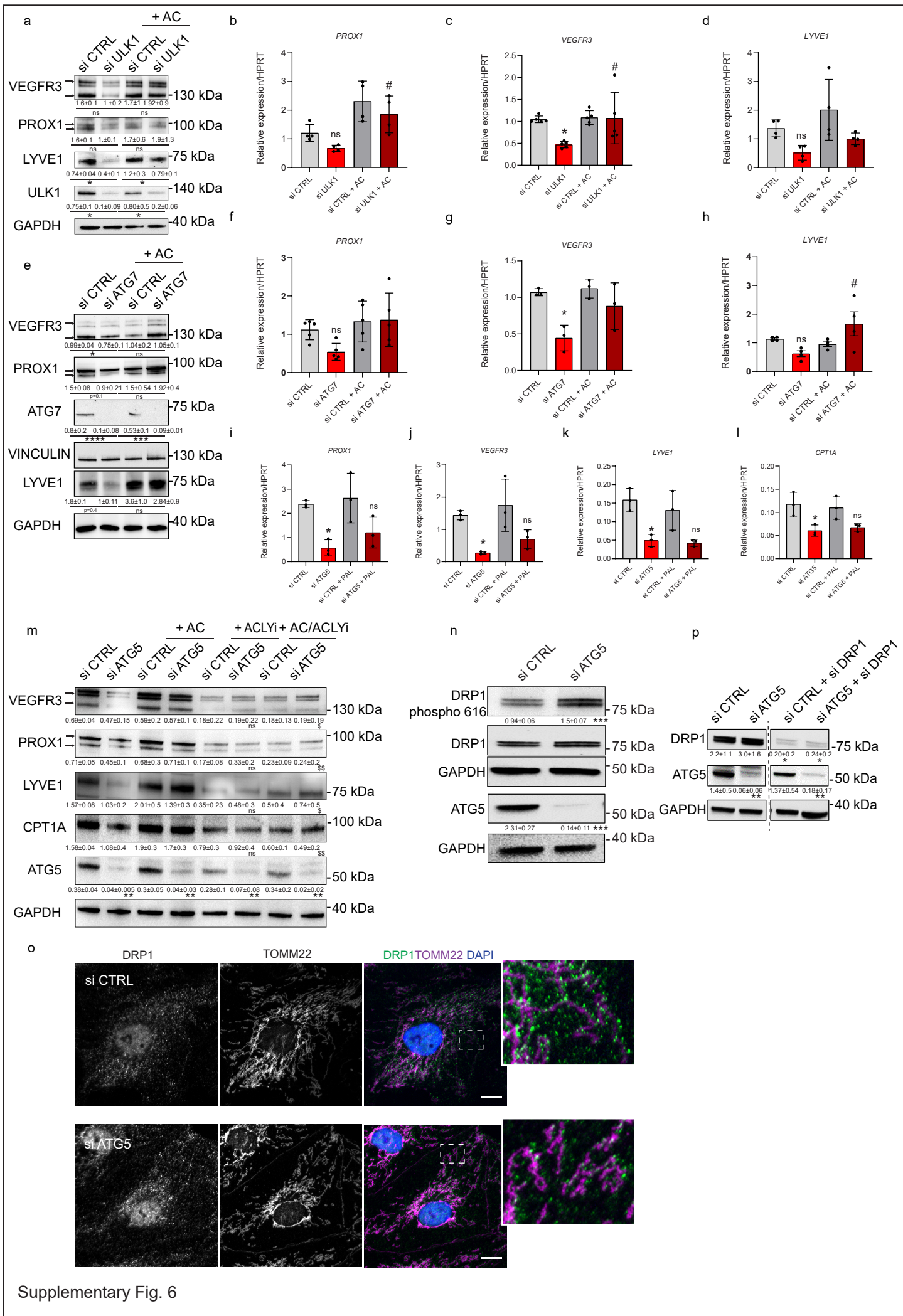
Supplementary Fig.4 | Assessment of mitochondrial respiration parameters in LEC upon ATG5 inhibition or CQ treatment and etomoxir effects on mitochondrial morphology.

a,b) Representative confocal immunofluorescent images (a) of the mitochondrial protein TOMM20 from si CTRL and si ATG5 LEC and quantification (b) of the mitochondrial fragmentation index (number of mitochondria/ total mitochondrial area). Zoomed boxed areas show the mitochondria shape. Mean \pm SD, N=5 biological replicates with 144 cells for si CTRL and 153 cells for si ATG5 cells in total analyzed using Mann-Whitney test (two-tailed), **** $p < 0.0001$. Scale bars represent 10 μ m. **c-f)** Representative electron microscopy images (c) of si CTRL and si ATG5 LEC (48h) and quantification of mitochondria morphometric parameters; mitochondrial length (d), shape (e) and breadth (f). Mean \pm SD, N=104 (si CTRL) and N=189 (si ATG5) mitochondria from two biological replicates analyzed using Mann-Whitney test (two-tailed), * $p < 0.05$ and **** $p < 0.0001$. Scale bars represent 500 nm. **g,h)** Representative immunofluorescent images (g) of the mitochondrial protein TOMM20 in si CTRL LEC and after treatment with etomoxir (Eto, 100 μ M, 48h,) and quantification (h) of mitochondrial fragmentation index (number of mitochondria/ total mitochondrial area). Mean \pm SD, N=3 biological replicates with a minimum of 104 cells per condition in total analyzed using Mann-Whitney test (two-tailed), **** $p < 0.0001$. **i)** Oxygen consumption rate (OCR) of si CTRL or si ATG5 LEC determined at baseline, after oligomycin, FCCP and antimycin treatment, analyzed via the Seahorse XFp Extracellular flux analyzer. **j)** ATP-linked respiration, **k)** Maximal respiration and **l)** Spare respiratory capacity calculated from OCR in i. Mean \pm SD, N=4 biological replicates analyzed using unpaired Student's t test (two-tailed), * $p = 0.01$. **m)** OCR of CTRL or CQ-treated LEC (25 μ M, 48h) determined at baseline, after oligomycin, FCCP and antimycin treatment, analyzed via the Seahorse XFp Extracellular flux analyzer. **n)** ATP-linked respiration, **o)** Maximal respiration and **p)** Spare respiratory capacity calculated from OCR in m. Mean \pm SD, N=3 biological replicates analyzed using unpaired Student's t test (two-tailed), *** $p < 0.001$ and **** $p < 0.0001$. **q)** Extracellular acidification rate (ECAR) of si CTRL or si ATG5 LEC determined at baseline, after glucose, oligomycin and 2-deoxy-D-glucose (2-DG) treatment, analyzed via the Seahorse XFp Extracellular flux analyzer. **r)** Intracellular lactic acid levels. Mean \pm SEM, N=3 biological replicates analyzed using unpaired Student's t test (two-tailed), $p = 0.47$. **s)** Oxidized glutathione calculated by the ratio of oxidized to total glutathione levels. Mean \pm SEM, N=3 biological replicates analyzed using one sample t test (two-tailed), * $p = 0.04$. **t,u)** Representative blots (t) and densitometric quantification (u) of the indicated proteins in si CTRL and si ATG5 LEC. Mean \pm SD, N=3 biological replicates analyzed using one sample t test (two-tailed), * $p = 0.03$.



Supplementary Fig.5 | Expression of CPT1A and PROX1 is regulated by fatty acids and autophagy.

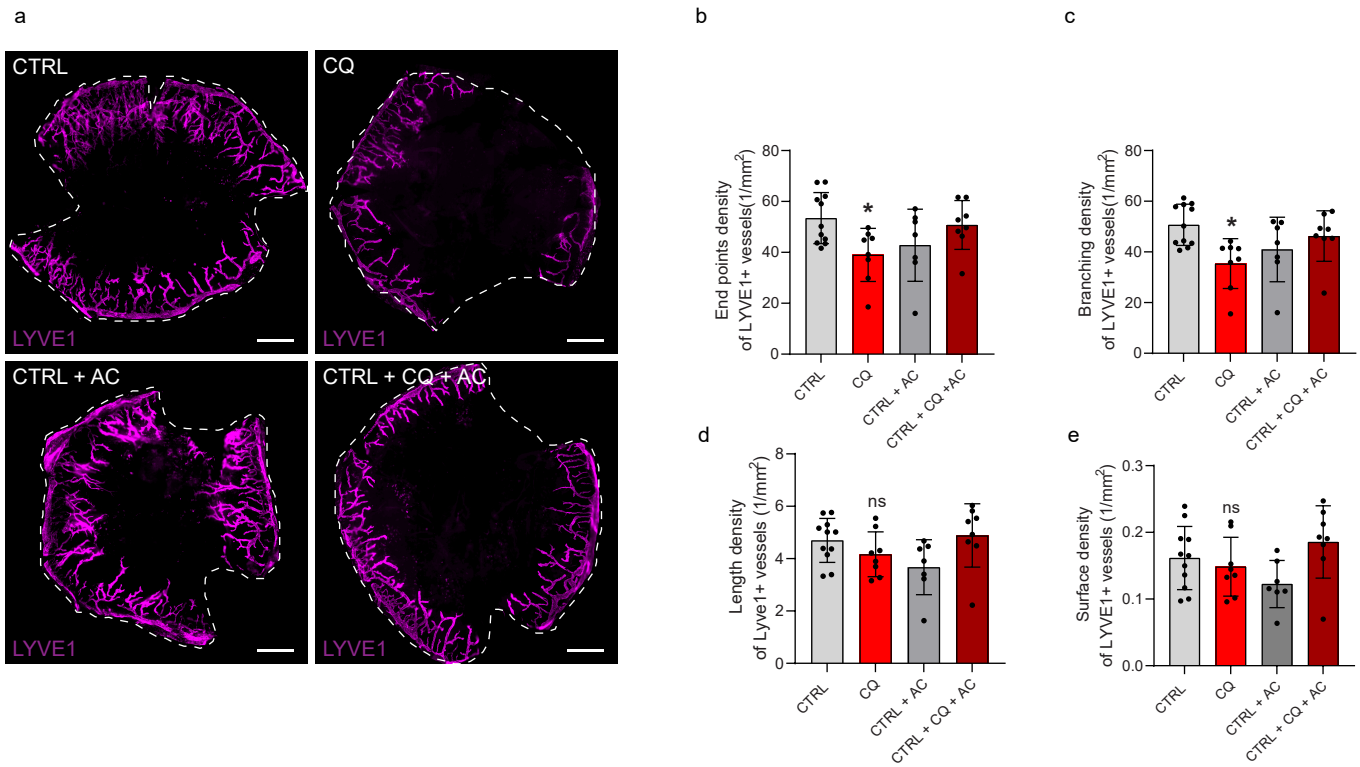
a,b) Representative blots (a) and densitometric quantification (b) for CPT2 levels of si CTRL or si ATG5 LEC. Mean \pm SD, N=3 biological replicates analyzed using unpaired Student's t test (two-tailed), *p = 0.02. **c)** RT-qPCR analysis of si CTRL and si ATG5 LEC. mRNA expression of CPT2 (relative to HPRT). Mean \pm SD, N=3 biological replicates analyzed by unpaired Student's t test (two-tailed). **d,e)** Representative blots (d) for indicated proteins and densitometric analysis (e) for CPT1A in CTRL and NH₄Cl-Leupeptin (20mM and 100 μ M, 48h) treated LEC. Mean \pm SD, N=3 biological replicates analyzed using unpaired Student's t test (two-tailed), **p < 0.004. **f)** Representative blots for indicated proteins in si CTRL and si ATG5 LEC upon stimulation with 5%, 10%, 15% lipid concentrate added to 10% dialyzed and lipid depleted serum for 48h. Densitometric quantification is indicated beneath the blots. Mean \pm SD, N \geq 3 biological replicates analyzed using one way ANOVA with Tukey's test for multiple comparisons, *p < 0.05, **p<0.01, ***p<0.001 vs si CTRL. **g,h)** Representative blots for indicated proteins in si CTRL or siATG5 LEC. Densitometric quantification is indicated beneath the blots (H3K27 ac and H3K4 me3 levels relative to total H3 and ATG5 levels relative to ACTIN). Mean \pm SD, N=3 biological replicates analyzed using unpaired Student's t test (two-tailed), *p < 0.05.



Supplementary Fig. 6

Supplementary Fig.6 | Rescue of PROX-1 driven lymphatic markers by acetate in autophagy compromised LEC.

a,e) Representative blots for indicated proteins in si CTRL, si ULK1 or si ATG7 LEC treated with sodium acetate (AC, 20mM, 48h) or vehicle. Densitometric quantification is indicated beneath the blots. Mean \pm SD, N=3 biological replicates analyzed by one-way ANOVA, with Tukey's test for multiple comparisons, * $p < 0.05$, *** $p < 0.001$ and **** $p < 0.0001$. **b-d)** and **f-h)** RT-qPCR analysis in si CTRL, si ULK1 or si ATG7 LEC treated with AC or vehicle. mRNA expression of PROX1, VEGFR3 and LYVE1 genes (relative to HPRT). Mean \pm SD N=3 (g), N=4 (b,d,h), N=5 (c,f) biological replicates analyzed by one-way ANOVA, with Tukey's test for multiple comparisons, * $p < 0.05$ vs si CTRL and # $p < 0.05$ vs si ULK1 or si ATG7. **i-l)** RT-qPCR analysis of si CTRL and si ATG5 LEC supplemented with palmitate (PAL, 500nM, 48h) or BSA. mRNA expression of PROX1, VEGFR3 and LYVE1 and CPT1A genes (relative to HPRT). Mean \pm SD, N=3 biological replicates analyzed by one-way ANOVA, with Tukey's test for multiple comparisons, * $p < 0.05$ vs si CTRL and $p = ns$ vs siATG5. **m)** Representative blots for indicated proteins in si CTRL and si ATG5 LEC upon ACLY inhibitor, AC alone or in combination (10 μ M and 20 mM respectively, 48h). Densitometric quantifications indicated beneath the blots. Mean \pm SD, N \geq 3 biological replicates analyzed by two-way ANOVA, with Tukey's test for multiple comparisons, ** $p < 0.01$ vs si CTRL, \$ $p < 0.05$, \$\$ $p < 0.01$ vs si ATG5 + AC. and ns vs si ATG5. **n)** Representative blots for indicated proteins in si CTRL and si ATG5 LEC. Densitometric quantification indicated beneath the blot. Mean \pm SD, N=3 biological replicates analyzed by Student's t test, ** $p < 0.01$. **o)** Representative super resolution AiryScan images of si CTRL and si ATG5 LEC stained with TOMM22 and DRP1. Nuclei are stained with DAPI. Scale bars represent 10 μ m. **p)** Representative blots for indicated proteins in si CTRL, si ATG5, si CTRL + si DRP1 and si ATG5 + si DRP1 LEC. Densitometric quantification indicated beneath the blot. Mean \pm SD, N \geq 3 biological replicates analyzed by one-way ANOVA, with Tukey's test for multiple comparisons, * $p < 0.05$ and ** $p < 0.01$ vs si CTRL.



Supplementary Fig.7 | Acetate partly rescues injury-driven lymphangiogenesis in chloroquine treated mice.

a) Representative immunofluorescent images of whole corneal mounts (dashed lines) of vehicle control mice (CTRL) and mice daily treated with chloroquine (CQ, 50 mg/kg i.p daily), sodium acetate (AC, 400 μ l 0.5 M i.p daily) or in combination stained for LYVE1, 8 days post corneal cauterization. Scale bar represents 1 mm. **b-e)** Quantification of the number of end points, number of branch points, average of cumulative length and surface density for LYVE1+ lymphatic vessels. Mean \pm SD, N=11 corneas for CTRL, N=8 corneas for CQ, N=7 corneas for CTRL + AC and N=8 corneas for CQ + AC analyzed using one-Way ANOVA corrected for multiple comparisons using Tukey's test, * $p < 0.05$ vs CTRL.

Supplementary Table 1: Primer sequences

| Gene | Forward primer | Reverse primer |
|--------------------------|--------------------------|----------------------------|
| Human <i>VEGFR3</i> | AAGATGTTTGCCCAGCGTAG | GCACTGTGGCATGAGGTCT |
| Human <i>LYVE1</i> | TGAAGGGGTAGGTGTGATGG | ATGACACCTGGATGGAAAGC |
| Human <i>PROX1</i> | TCACCTTATTCGGGAAGTGC | GAGCTGGGATAACGGGTATAAA |
| Human <i>NR2F2</i> | CCATAGTCCTGTTACCTCAGA | AATCTCGTCGGCTGGTTG |
| Human <i>CPT1A</i> | CAATCGGACTCTGGAACCG | CCGCTGACCACGTTCTTC |
| Human <i>CPT2A</i> | CCACCATGCACTACCAGGA | TGGTGTCTTCAAGTTTGGGAAT |
| Human <i>HPRT</i> | GACCAGTCAACAGGGGACAT | GTGTCAATTATATCTCCACAATCAAG |
| Human <i>ATG5</i> | CAACTTGTTTCACGCTATATCAGG | CACTTTGTCAGTTACCAACGTCA |
| Human <i>VEGFR1</i> | GCATACCTCACTGTTCAAGGA | GCCACACAGGTGCATGTT |
| Human <i>VEGFR2</i> | GCTCAAGACAGGAAGACC | GGTGCCACACGCTCT |
| Human <i>VE cadherin</i> | GCAGTCCAACGGACCAGAA | CATGAGCCTCTGCATCTTCC |
| Human <i>CD34</i> | GCGCTTTGCTTGCTGAGT | GGGTAGCAGTACCGTTGTTGT |
| Human <i>NRP1</i> | CACATTTCAACAAGAAGATTGTGC | CATCAATTTTAATTTCTGGGTTCTTT |
| Human <i>ICAM1</i> | CCTTCCTCACCGTGTACTGG | AGCGTAGGGTAAGGTTCTTGC |

Supplementary Table 2: Antibodies, Reagents and other Resources

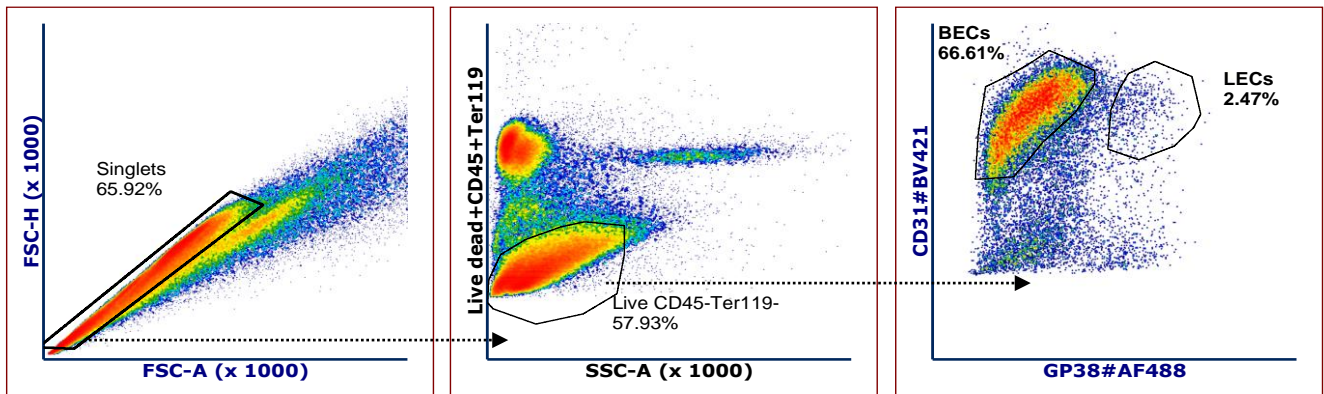
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|--------------------------------------|---------------------------|--|
| Antibodies | | |
| Anti Goat IgG, HRP-linked | Thermo Fisher Scientific | PA1-28823 RRID:AB_10986856 |
| anti-CD31 | BD Biosciences | 563356 |
| anti-CD36 | Biolegend | 102605 RRID:AB_389348 |
| anti-CD45 | BD Biosciences | 557659 |
| Anti-Goat IgG, Alexa Fluor 488 | Thermo Fisher Scientific | A21222 RRID: AB_10373853 |
| anti-GP38 | Biolegend | 156207 RRID:AB_2814079 |
| Anti-Mouse IgG, Alexa Fluor 488 | Thermo Fisher Scientific | A11001 RRID:AB_2534069 |
| Anti-Mouse IgG, Alexa Fluor 647 | Thermo Fisher Scientific | A21235 RRID:AB_2535804 |
| Anti-Mouse IgG, HRP-linked | Cell Signaling Technology | 7076V RRID: AB_330924 |
| Anti-Rabbit IgG, Alexa Fluor 488 | Thermo Fisher Scientific | A21206 RRID:AB_2535792 |
| Anti-Rabbit IgG, Alexa Fluor 647 | Thermo Fisher Scientific | A21222 RRID:AB_2535812244 |
| Anti-Rabbit IgG, HRP-linked | Cell Signaling Technology | 7074V RRID:AB_2099233 |
| Anti-Rat IgG, Alexa Fluor 546 | Thermo Fisher Scientific | A11081 RRID:AB_141738 |
| anti-ter119 | BD Biosciences | 560509 |
| Goat anti-LYVE1 | R&D systems | AF2089 RRID: AB_355144 |
| Goat anti-LYVE1 | R&D systems | AF2125 RRID: AB_2297188 |
| Goat anti-Mouse IgG, Alexa Fluor 647 | Thermo Fisher Scientific | A21235 RRID: AB_2535804 |
| Hamster anti-PDPN | Biolegend | 127401 RRID: AB_1089186 |
| Mouse anti-ATG5 | Biolegend | 847410 RRID:AB_2814563 |
| Mouse anti-CD36 | Abcam | ab17044 RRID: AB_443600 |
| Mouse anti-DRP1 | BD Biosciences | 611113 RRID:AB_398424 |
| Mouse anti-DRP1 | BD Biosciences | 61113 |
| Mouse anti-LAMP1 | Abcam | ab25630 |

| | | |
|---|---------------------------|--|
| Mouse anti-NR2F2 | Abcam | ab41859 |
| Mouse anti-OPA1 | BD Biosciences | 612607 RRID: AB_399889 |
| Mouse anti-TOMM20 | BD Biosciences | BD 612278 RRID: AB_399595 |
| Mouse anti-ULK1 | Abcam | ab56344 |
| Rabbit anti- Phospho-DRP1 (Ser616) | Cell Signaling Technology | 3455S RRID: AB_2085352 |
| Rabbit anti-acetyl histone H3 (lysine 9) | Cell Signaling Technology | 9671 RRID: AB_331532 |
| Rabbit anti-ATG5 | Cell Signaling Technology | 12994S RRID: AB_2630393 |
| Rabbit anti-ATG7 | Cell Signaling Technology | 8558S RRID: AB_10831194 |
| Rabbit anti-CPT1 | Cell Signaling Technology | D3B3 RRID: AB_2797857 |
| Rabbit anti-CPT2 | Abcam | ab181114 |
| Rabbit anti-ENOS | BD Biosciences | 610297 |
| Rabbit anti-FABP4 | Cell Signaling Technology | 2120S RRID: AB_2102466 |
| Rabbit anti-GAPDH | Cell Signaling Technology | 2118S RRID: AB_561053 |
| Rabbit anti-H3K27 ac | Cell Signaling Technology | 8173T RRID: AB_10949503 |
| Rabbit anti-H3K4me3 | Abcam | ab8580 |
| Rabbit anti-LC3 | Cell Signaling Technology | 3868S RRID: AB_2137707 |
| Rabbit anti-p62 | Millipore | p0067 RRID: AB_1841064 |
| Rabbit anti-pan-acetyl-histone H3 | Active Motif | 39139 RRID: AB_2687871 |
| Rabbit anti-PROX1 | Proteintech | 11067-2 RRID: AB_2268804 |
| Rabbit anti-TOMM22 | My Biosource | MBS7605092 |
| Rabbit anti-VEGFR3 | Abcam | ab154079 |
| Rabbit anti-Vinculin | Cell Signaling Technology | #4650 RRID: AB_10559207 |
| Rabbit anti-β-actin | Sigma-Aldrich | A5441 RRID: AB_476744 |
| Rat anti-CD102 | BD Biosciences | 553326 |
| Rat anti-CD31 | Pharmigen | 553370 RRID: AB_394816 |
| Rat anti-VEGFR3 | Thermo Fisher Scientific | 14-5988-82 RRID: AB_467795 |
| Chemicals, Peptides, and Recombinant Proteins | | |
| [6- ³ H]thymidine | Perkin Elmer | NET355L005MC |
| 2-deoxy-D-glucose | Sigma Aldrich | D-6134 |
| Antimycin A | Sigma-Aldrich | A8674 |
| BMS-303141 (ATP citrate lyase inhibitor) | Sigma-Aldrich | SML0784 |
| Chloroquine | Sigma-Aldrich | C6628 |
| Etomoxir | Sigma-Aldrich | E1905 |
| Glucose | Sigma-Aldrich | 8769 |
| Glutamine | Sigma-Aldrich | G7513 |
| L-Carnitine | Sigma-Aldrich | 8400920025 |
| Methylcellulose | Sigma-Aldrich | M6385 |
| Mitomycin C | Sigma-Aldrich | M4287 |
| Oligomycin | Sigma-Aldrich | 75351 |
| Palmitic acid N-hydroxysuccinimide ester | Sigma-Aldrich | 14464-31-4 |
| Palmitic acid- ¹³ C ₁₆ | Sigma-Aldrich | 605573 |
| Phenylhydrazone (FCCP) | Sanbio | 1528-10 |
| Propidium iodide | Sigma-Aldrich | P4170 |
| Recombinant Mouse VEGF | R&D systems | 493-MV-025 |

| | | |
|--|--------------------------|---|
| Saponin | Sigma-Aldrich | S-4521 |
| Sodium acetate | Sigma-Aldrich | S2889 |
| Sodium Pyruvate | Thermo Fisher Scientific | S8636 |
| Tamoxifen | Sigma-Aldrich | T5648 |
| Trichloroacetic acid | Sigma-Aldrich | T6399 |
| VEGF-C human recombinant | Sigma-Aldrich | SRP3184 |
| Critical Commercial Assays | | |
| ORA qPCR Green L mix | HighQu | QPD0105 |
| Reverse transcription kit | Qiagen | 205313 |
| RNeasy Plus mini kit | Qiagen | 74136 |
| Deposited Data | | |
| Raw and analyzed data | This paper | |
| Experimental Models: Cell Lines | | |
| Human dermal lymphatic endothelial cells | Promo Cell | C-12217 |
| Experimental Models: Organisms/Strains | | |
| Mouse: Atg5 ^{fl/fl} | Kuma et al. 2004 | N/A |
| Mouse: Prox1-cre ^{ERT2} | Bazigou et al. 2011 | N/A |
| Oligonucleotides | | |
| ON-TARGETplus Non-targeting Pool | Dharmacon | D-001810-10-20 |
| SMARTpool: ON-TARGETplus ATG5 siRNA | Dharmacon | L-004374-00-0020 |
| SMARTpool: ON-TARGETplus ATG7 siRNA | Dharmacon | L-020112-00-0050 |
| SMARTpool: ON-TARGETplus DRP1 siRNA | Dharmacon | L-012092-00-0005 |
| SMARTpool: ON-TARGETplus ULK1 siRNA | Dharmacon | L-005049-00-0005 |
| Recombinant DNA | | |
| pBABEpuro GFP-LC3 plasmid | Addgene | #22405 |
| mCherry-hLC3B-pcDNA3.1 | Addgene | #40827 |
| Software and Algorithms | | |
| Fiji (Image J) | Open Source | https://fiji.sc |
| FlowJo 8.8.6 software | FlowJo, LLC | https://www.flowjo.com |
| Image Lab Software | Licor | https://www.licor.com/bio/image-studio/?gclid=EAIaIQobChMlrP3L7_uS6wIVGed3Ch1uCw9yEAAYASAAEgJimPD_BwE |
| Prism v9 | Graphpad | http://www.graphpad.com |
| Seahorse Wave Desktop Software | Agilent | https://www.agilent.com/?gclid=EAIaIQobChMIo5rHq-qS6wIVTe7tCh0YYw7gEAAAYASAAEgJd1vD_BwE&gclsrc=aw.ds |
| FCS express V7 | De Novo Software). | https://denovosoftware.com |
| MATLAB 8.3 (R2014a) software | Mathworks | https://www.mathworks.com/products/matlab.html |
| Leica MetaMorph AF2.1 morphometric software | Leica | https://www.moleculardevices.com/products/cellular-imaging-systems/acquisition-and-analysis-software/metamorph-microscopy |
| Other | | |
| 2x Antibiotic-Antimycotic | Sigma Aldrich | A5955 |
| 4% Paraformaldehyde | Thermo Fisher Scientific | J19943K2 |
| 4',6-Diamidino-2-Phenylindole, Dihydrochloride | Thermo Fisher Scientific | D1306 |

| | | |
|---|---------------------------|-------------|
| BODIPY 493/503 | Thermo Fisher Scientific | D3922 |
| BODIPY™ FL C ₁₆ | Thermo Fisher Scientific | D3821 |
| Bovine serum albumin | Sigma-Aldrich | A6003-25G |
| CD31 magnetic beads | Miltenyi Biotec | 130-097-418 |
| CellVue™ Jade | Thermo Fisher Scientific | 88-0876-16 |
| CellVue™ NIR780 | Thermo Fisher Scientific | 88-0875-16 |
| Charcoal stripped serum | Thermo Fischer Scientific | A3382101 |
| Chemically Defined Lipid Concentrate | Thermo Fisher Scientific | 11905031 |
| Collagen gel (rat tail) | Merck | 08-115 |
| collagenase II | Thermo Fisher Scientific | 17101015 |
| collagenase IV | Thermo Fisher Scientific | 17104019 |
| DNase | Sigma Aldrich | D4527-10KU |
| ECGMV2 | Promo Cell | C-22211 |
| ECGMV2 Supplement Mix | Promo Cell | C-39226 |
| FACS buffer | BD Biosciences | 554656 |
| FC block | Biologend | 101319 |
| Fixable Viability Dye eFluor™ 780 | Thermo Fisher Scientific | 65-0865-14 |
| Gelatin from bovine skin | Sigma-Aldrich | G9391 |
| Gentle Macs C Tubes | Miltenyi Biotec | 130-093-237 |
| Goat serum | Cell Signaling Technology | #5425 |
| Hepes | Gibco | 12509079 |
| Ketamine hydrochloride | Dechra | |
| KnockOut™ DMEM | Thermo Fisher Scientific | 10829018 |
| Lipofectamine 2000 | Invitrogen | 16688 |
| Magnetic beads CELLection | Invitrogen | 11533D |
| MitoSOX™ Red Mitochondrial Superoxide Indicator | Thermo Fischer Scientific | M36008 |
| MitoTracker Green | Thermo Fischer Scientific | M7514 |
| MitoTracker Deep Red | Thermo Fisher Scientific | M22426 |
| Nile Red | Thermo Fisher Scientific | N1142 |
| Non-Essential Amino Acids Solution | Sigma Aldrich | M7145 |
| penicillin/streptomycin | Sigma Aldrich | P4333 |
| Prolong® Gold Antifade Reagent | Thermo Fisher Scientific | P36934 |
| Seahorse XF Assay Medium | Agilent | 103335-100 |
| Seahorse Xp culture plates | Agilent | 103022-100 |
| Sodium pyruvate | Sigma Aldrich | S8636 |
| Supplement Mix 2 | Promo Cell | C-39216 |
| Supplement Mix MV2 | Promo Cell | C-39226 |
| TMRM | Thermo Fischer Scientific | T-668 |
| Unicaïne | Thea | 048720 |
| Xylazine | VMD | |

Gating strategies Supplementary Fig. 3i



Endothelial cells were isolated from mice lungs as described in the method section. Single cells were gated based on FSC-A and FSC-H (first panel). Cells were further gated based on CD45, Ter119 and Live dead triple negative populations (middle panel). Lymphatic endothelial cells were further identified as CD31 and GP38 double positive cells (last panel).