Supplementary figures

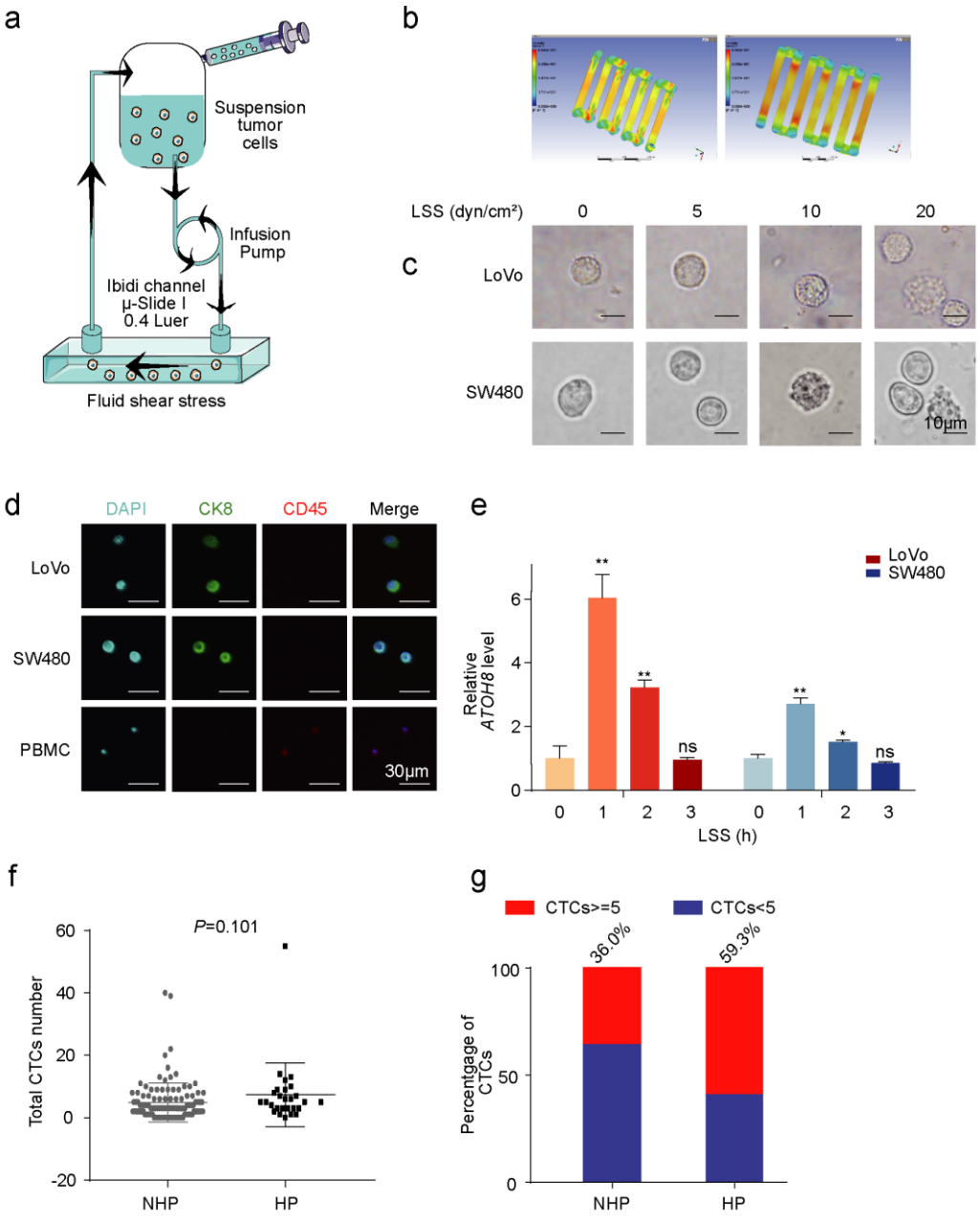

Figure S1. Construction of mimic circulating tumour cells and the effects of LSS on CTCs. a Schematic diagram of laminar shear stress (LSS) loading platform *in vitro*. **b** Flow velocity map simulated by ANSYS software, to verify that this LSS loading system consisting of seven IBIDI channels in series can generate a relatively stable and uniform LSS. **c** The suspended LoVo and SW480 cells can maintain normal

cell morphology after 30 min of shear stress stimulation in the physiological range of 0-20 dyn/cm^2. **d** Representative immunofluorescence images of DAPI, CD45, and CK8 expression in suspended LoVo and SW480 cells and peripheral blood mononuclear cells (PBMCs). **e** Quantitative polymerase chain reaction (qPCR) analysis of ATOH8 expression in suspended LoVo and SW480 cells treated with longer time gradient (10 dyn/cm^2; 0, 1, 2, 4h) LSS. **f** In colorectal cancer patients with or without hypertension, the total number of CTCs detected. **g** The percentage of colorectal cancer patients with CTCs ≥ 5 were higher in hypertension group. *$P < 0.05$ and **$P < 0.01$.

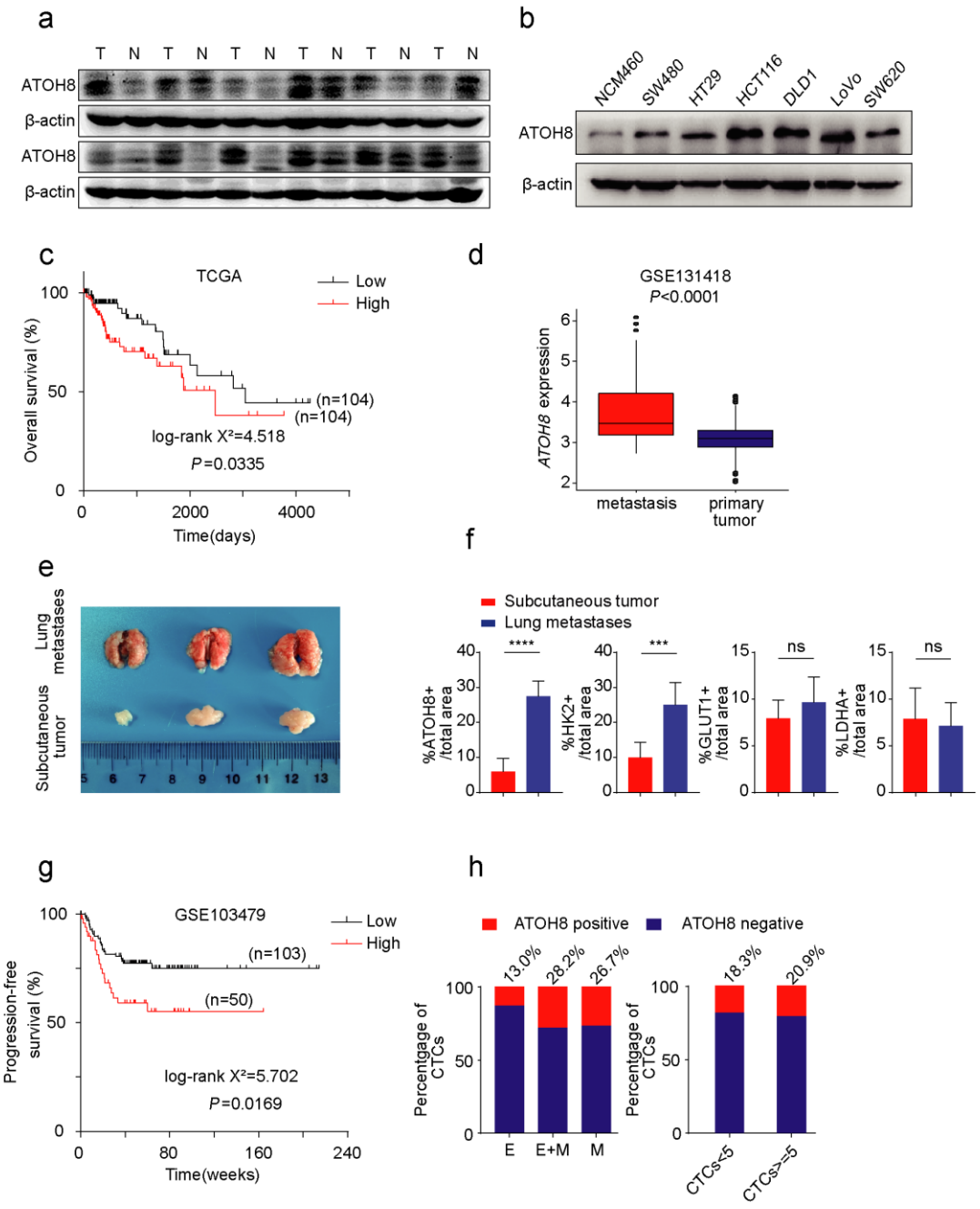

Figure S2. Shear stress responsive molecule ATOH8 is associated with poor prognosis in colorectal cancer patients. a Western blot (WB) analysis showed the ATOH8 expression from 12 colorectal cancer patients with tumour tissues and matched adjacent normal tissues. **b** WB results showing baseline protein levels of ATOH8 in NCM460 and six colon cancer cell lines. **c** Kaplan-Meier analysis of overall survival according to the expression of the ATOH8 in 208 colorectal cancer

patients from TCGA. **d** The expression of *ATOH8* in colorectal cancer metastases was significantly higher than that in primary tumours in GSE131418. **e** Gross view of lung metastasis and subcutaneous tumour from nude mice. **f** ATOH8, HK2, GLUT1 and LDHA protein expression quantified via immunohistochemically staining intensity based on Fig 1i. **g** Kaplan-Meier analysis of progression-free survival according to the expression of the ATOH8 in 153 surgically treated patients with stage II-III colorectal cancer from GSE103479. **h** The proportion of ATOH8-positive CTCs from 141 colorectal cancer patients were detected and quantified in different groups.

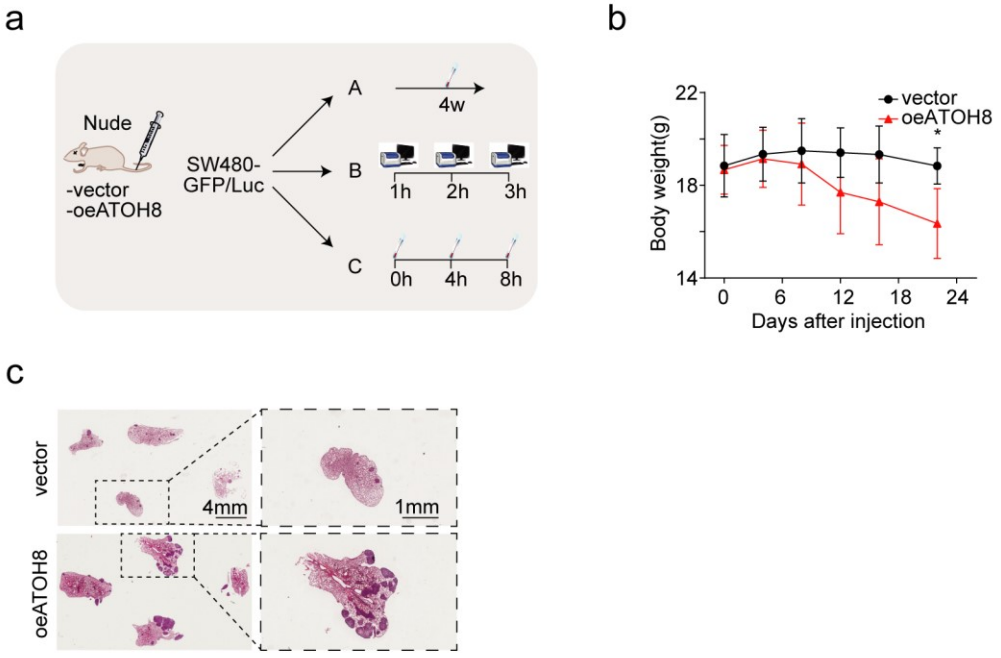

Figure S3. Overexpression of ATOH8 facilitates colorectal tumour cells to form metastases. **a** Experimental scheme detailing observation times and detection indicators of *in vivo* experiment. **b** Vector or ATOH8-overexpressing SW480 cells with GFP labelling were injected intravenously into nude mouse, and the mice body weight was tested within 3 weeks. **c** Representative images of haematoxylin and eosin staining of metastatic nodules in the lungs from vector or ATOH8-overexpressing group. *$P < 0.05$.

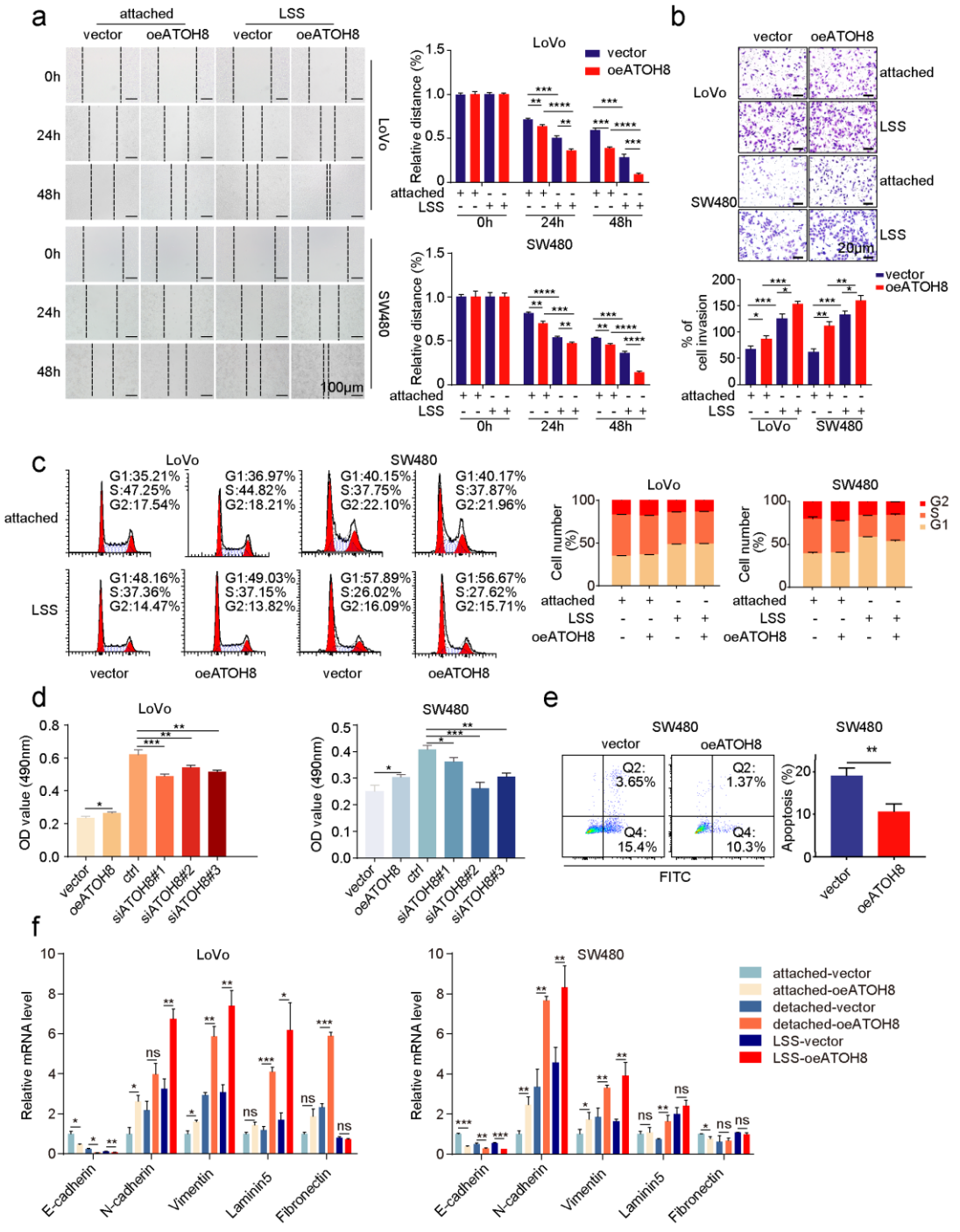

Figure S4. ATOH8 promotes the invasion, metastasis and anoikis resistance of colorectal cancer cells. a Wound healing assay was performed to compare cell migration ability between vector or ATOH8-overexpressing LoVo and SW480 cells with or without LSS (10 dyn/cm^2, 30min) stimulation. The representative plots (Left)

and quantification (Right) results were presented. **b** Matrigel invasion assay was performed to compare cell invasion ability between vector or ATOH8-overexpressing LoVo and SW480 cells with or without LSS (10 dyn/cm^2, 30min) stimulation. The representative plots (Upper) and quantification (Down) results were presented. **c** Flow cytometry analysis of cell cycle phase distribution of vector or ATOH8-overexpressing LoVo and SW480 cells with or without LSS (10 dyn/cm^2, 30min) stimulation. The representative plots (Left) and quantification (Right) results were presented. **d** MTT assays were conducted to measure the anoikis rate of suspended LoVo and SW480 cells after overexpressing or silencing ATOH8. **e** Apoptosis rates of suspended SW480 cells with or without ATOH8 overexpression were measured using flow cytometry with double staining of Annexin V and PI. Left, representative scatter plots of PI vs. Annexin V, while percentage of apoptotic cells was shown in Right. **f** qPCR analysis of the expression level of anoikis markers, including *E-acdherin*, *N-cadherin*, *Vimentin*, *Laminin5* and *Fibronecctin* in vector or ATOH8-overexpressing LoVo and SW480 cells with attached, detached or LSS (10 dyn/cm^2, 30min) stimulation. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ and $****P < 0.0001$.

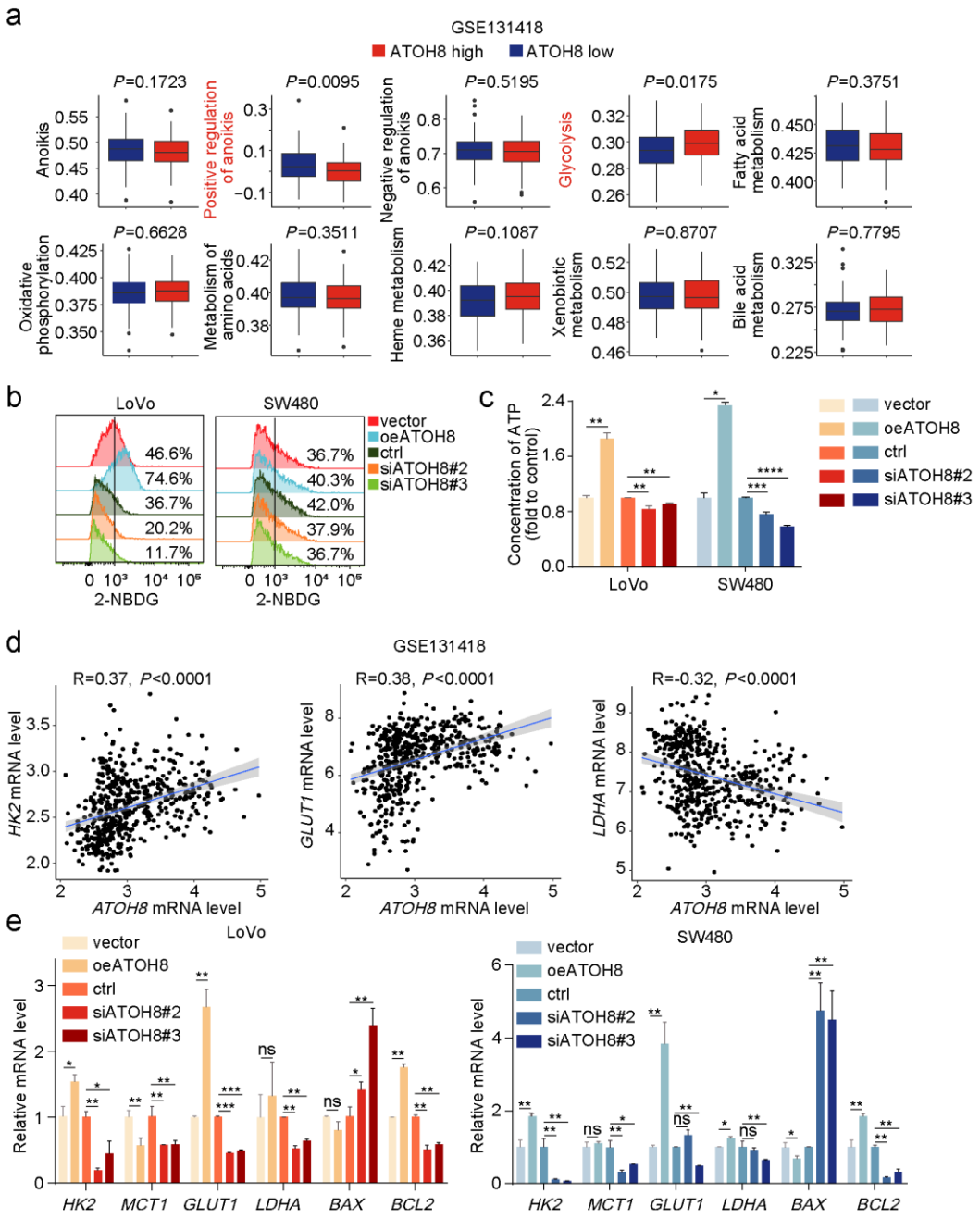

Figure S5. ATOH8 is associated with glycolysis in colorectal cancer. a Single sample gene set enrichment analysis (ssGSEA) of gene-containing signature in $ATOH8_{high}$ and $ATOH8_{low}$ group in the colorectal cancer metastasis cohort from GSE131418 and the results of anoikis-related and key metabolic pathways were presented. **b** 2-NBDG uptake assays showing that ATOH8 promoted glucose absorption in suspended LoVo and SW480 cells. **c** Overexpression of ATOH8

promoted ATP production in suspended LoVo and SW480 cells, while opposite effects were observed when silencing ATOH8. **d** Pearson correlation analysis of the expression of *ATOH8* and the glycolytic enzymes *HK2, LDHA* and *GLUT1* in colorectal cancer tissues from GSE131418. **e** qPCR analysis of the expression level of glycolytic enzymes *HK2*, *LDHA*, *GLUT1* and *MCT1* and apoptotic markers *BAX*, *BCL2* in LoVo and SW480 cells after overexpressing or silencing ATOH8. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

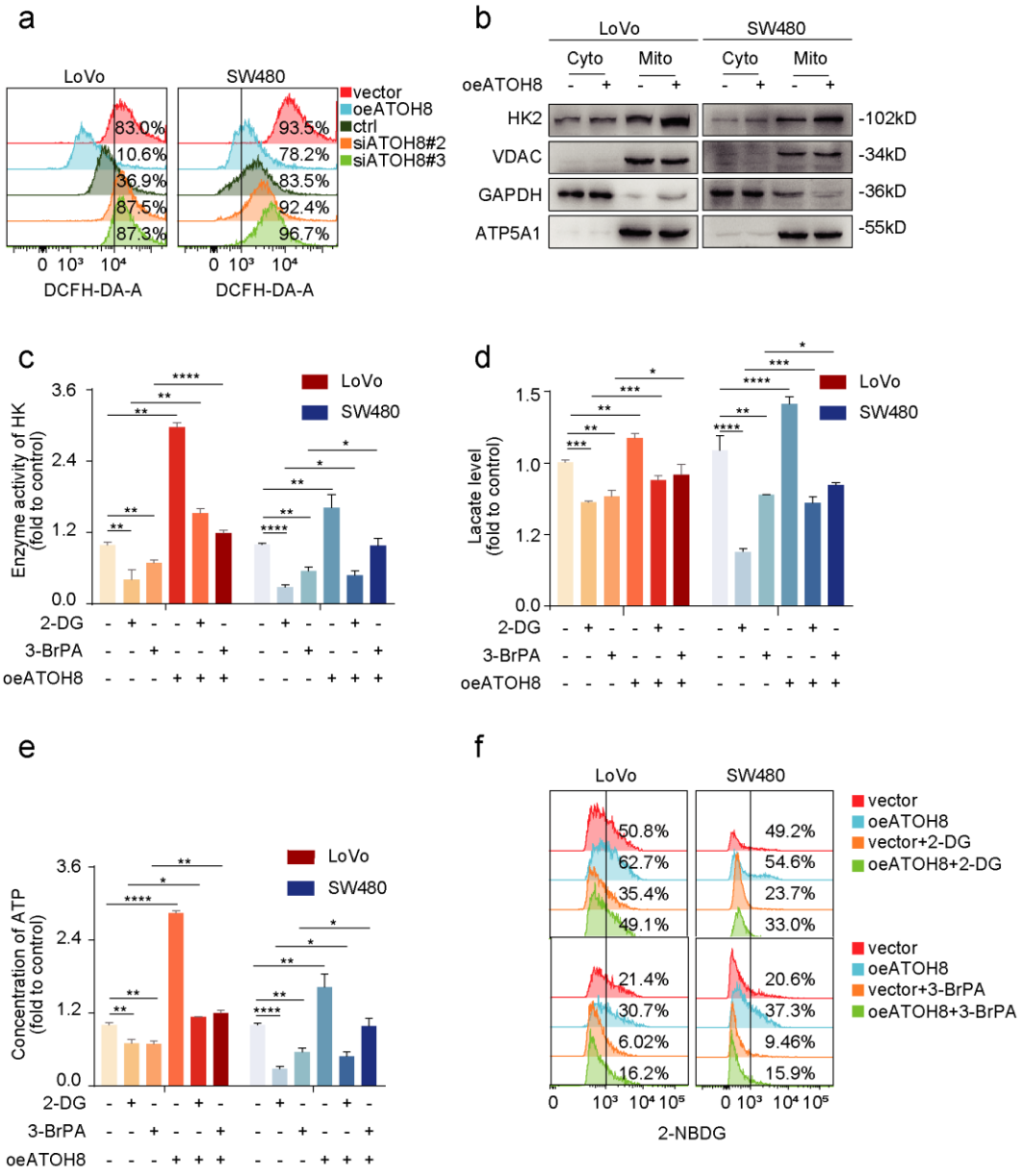

Figure S6. ATOH8 inhibits intravascular death of circulating colorectal tumour cells by targeting HK2. a ROS assay kit was used to measure ROS accumulation in LoVo and SW480 cells with ATOH8 overexpression or silencing. **b** WB results showing that ATOH8 promote the distribution of HK2 in the cytoplasm and especially mitochondria. **c-f** HK2 enzyme activity (**c**), lactate production (**d**), ATP (**e**), glucose absorption (**f**) in LoVo and SW480 cells after overexpressing ATOH8 and treating with or without 1 mM 2-Deoxy-D-glucose (2-DG) or 2 nM 3-bromopyruvate (3-BrPA)

for 24 h. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

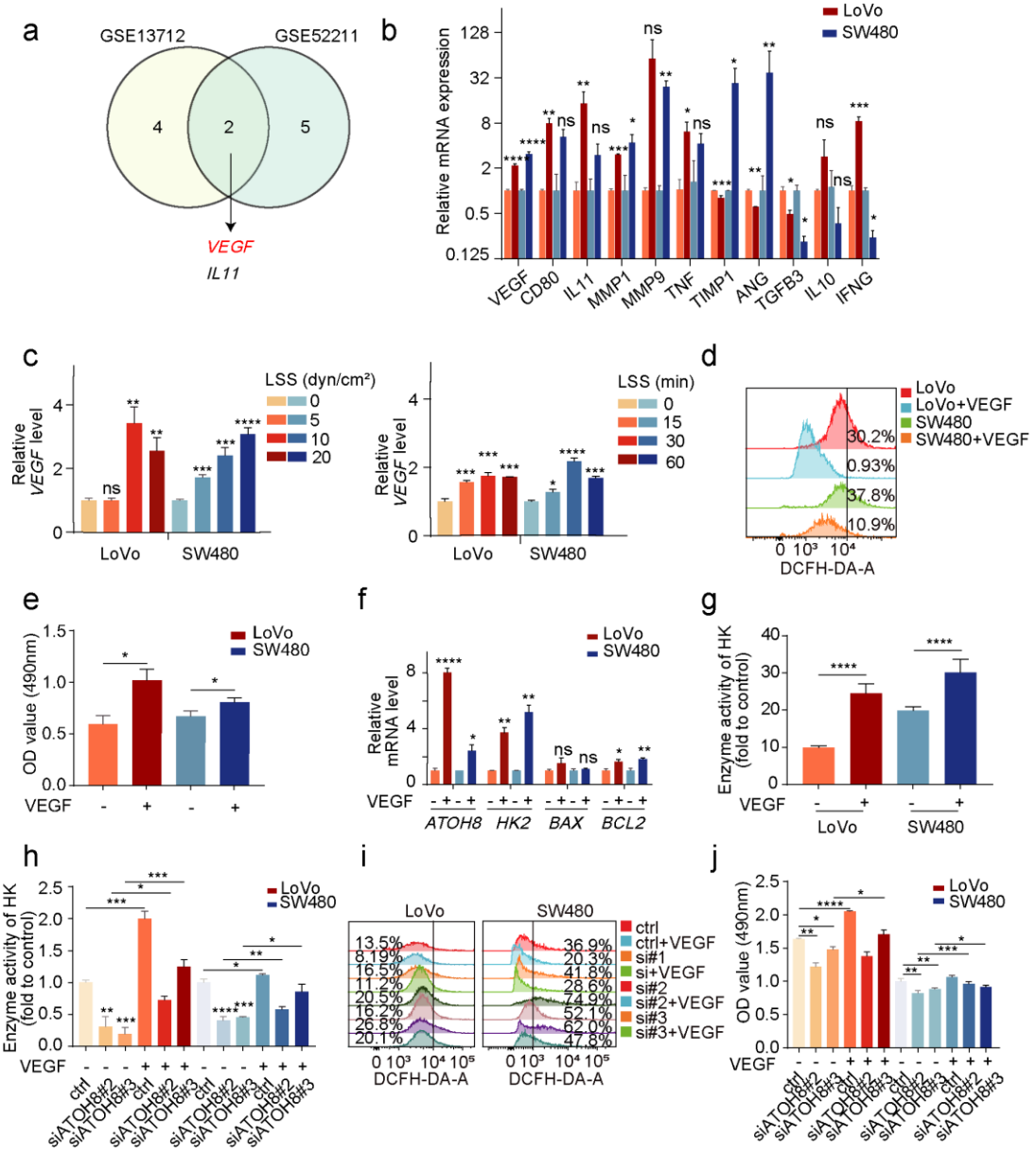

Figure S7. VEGF is responsible for ATOH8 upregulation in colorectal tumour cells in suspension and under LSS. a Venn diagram showing the number of differentially expressing cytokines and cytokine receptors in endothelial cells undergoing LSS in GSE13712 and GSE52211. **b** The qPCR analysis of 11 cytokines or cytokine receptors expression in suspended LoVo and SW480 cells treated with LSS (10dyn/cm^2, 30min). **c** The qPCR analysis of *VEGF* expression in suspended LoVo and SW480 cells treated with size gradient (0,5,10,20 dyn/cm^2; 30min; Left)

and time gradient (10 dyn/cm^2; 0,15, 30, 60min; Right) LSS. **d, e** ROS accumulation (**d**) and MTT assay (**e**) analysis in suspended LoVo and SW480 cells treated with 10ng/mL VEGF for 24h. **f, g** The qPCR analysis of expression level of *ATOH8*, *HK2*, *BAX* and *BCL2* (**f**) and HK2 enzyme activity (**g**) in suspended LoVo and SW480 cells treated with 10ng/mL VEGF for 24h. **h-j** Suspended LoVo and SW480 cells transfected with ctrl or si-ATOH8 were seeded in low attachment 6-well plate and treated with 10ng/mL VEGF for 24h. HK2 enzyme activity (**h**), ROS accumulation (**i**) and cell vitality (**j**) were measured. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

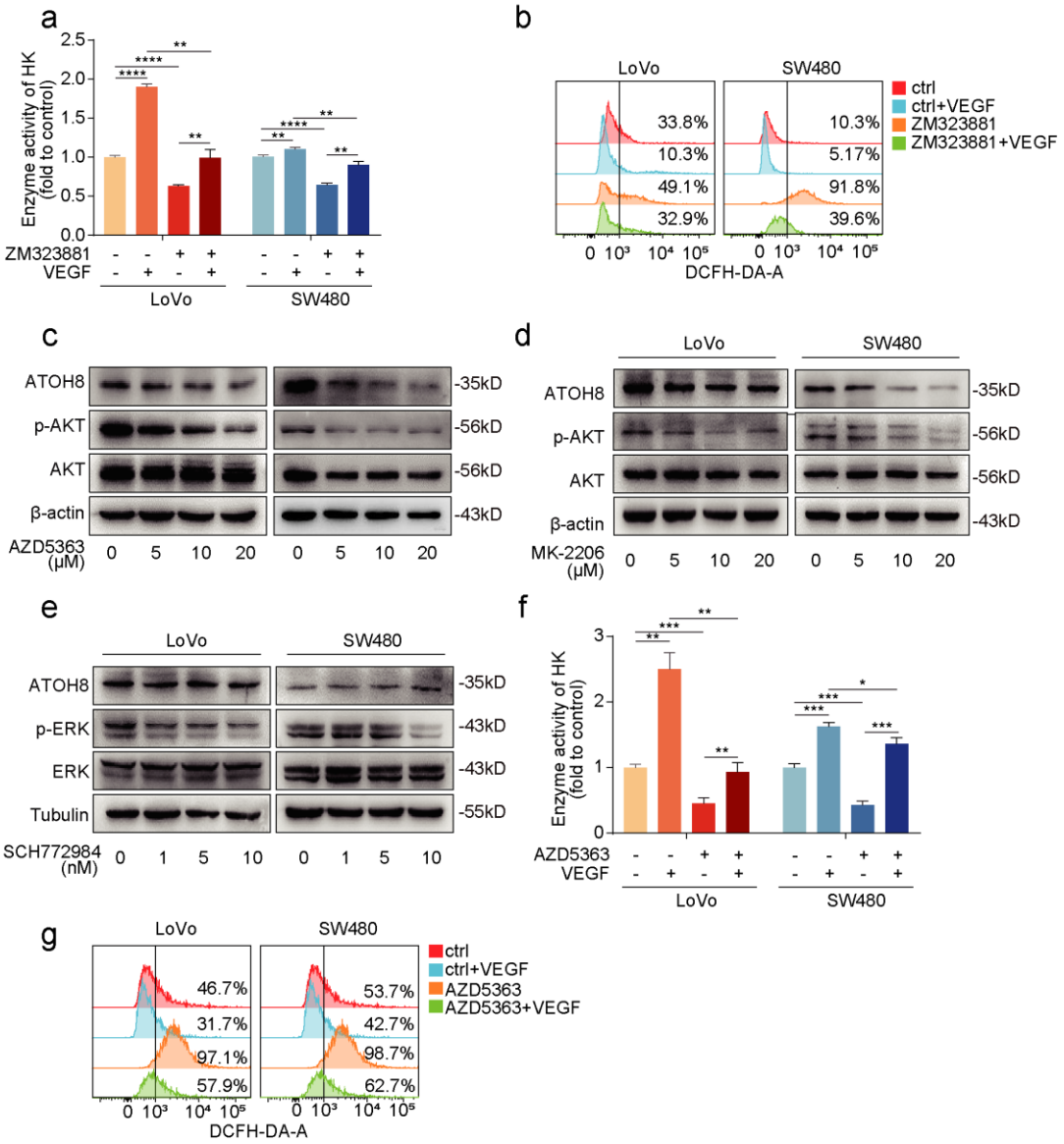

Figure S8. VEGF-VEGFR2-AKT signalling axis activates ATOH8 and its downstream glycolysis pathway. a, b Suspended LoVo and SW480 cells treated with 10ng/mL VEGF for 24h and with or without 10 μM VEGFR2 inhibitor (ZM323881), HK2 enzyme activity (**a**) and ROS accumulation (**b**) were analysed. **c, d** LoVo and SW480 cells was treated with AKT inhibitors AZD5363 (0, 5, 10, 20 μM) (**c**) or MK-2206 (0, 5, 10, 20 μM) (**d**), and the relative change in ATOH8, AKT, p-AKT expression was analysed by WB. **e** LoVo and SW480 cells was treated with ERK inhibitors SCH772984 (0, 1, 5, 10 nM), and the relative change in ATOH8, ERK,

p-ERK expression was analysed by WB. **f, g** Suspended LoVo and SW480 cells treated with 10ng/mL VEGF for 24h and with or without 10 μM AKT inhibitor (AZD5363), HK2 enzyme activity (**f**) and ROS accumulation (**g**) were analysed. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ and $****P < 0.0001$.