

## **Supplementary data for**

# **Mitochondrial fusion via OPA1 and MFN1 supports liver tumor cell metabolism and growth**

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### **Table of contents**

Supplementary Figure 1

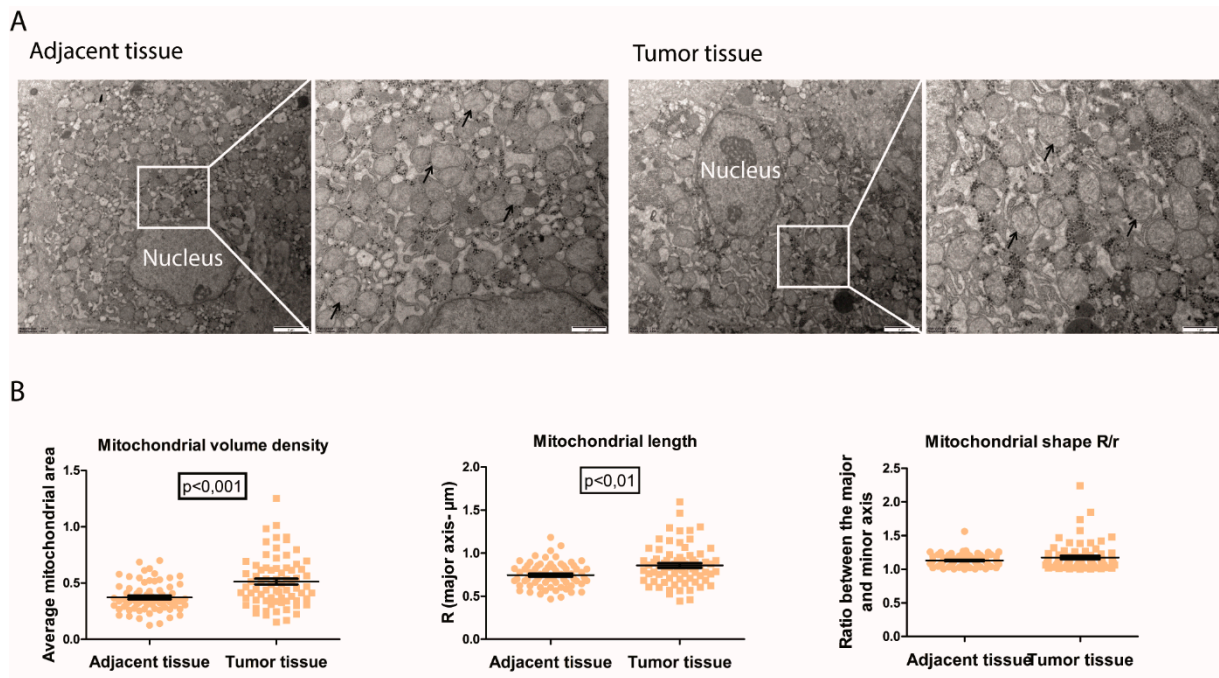
Supplementary Figure 2

Supplementary Figure 3

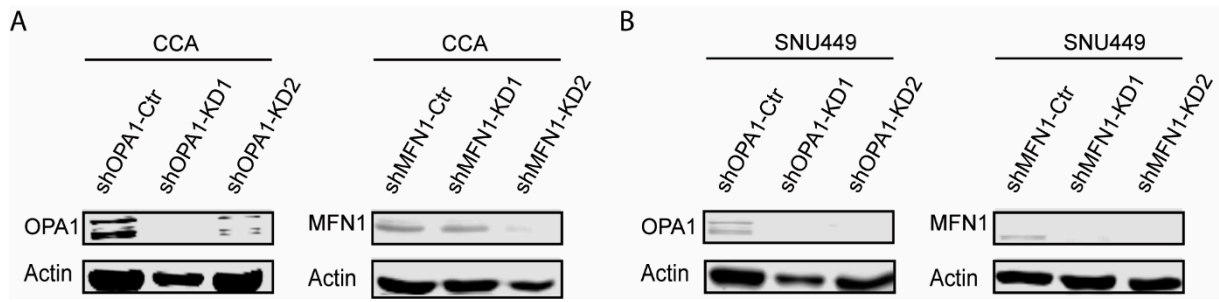
Supplementary Figure 4

Supplementary Figure 5

Supplementary Figure 6



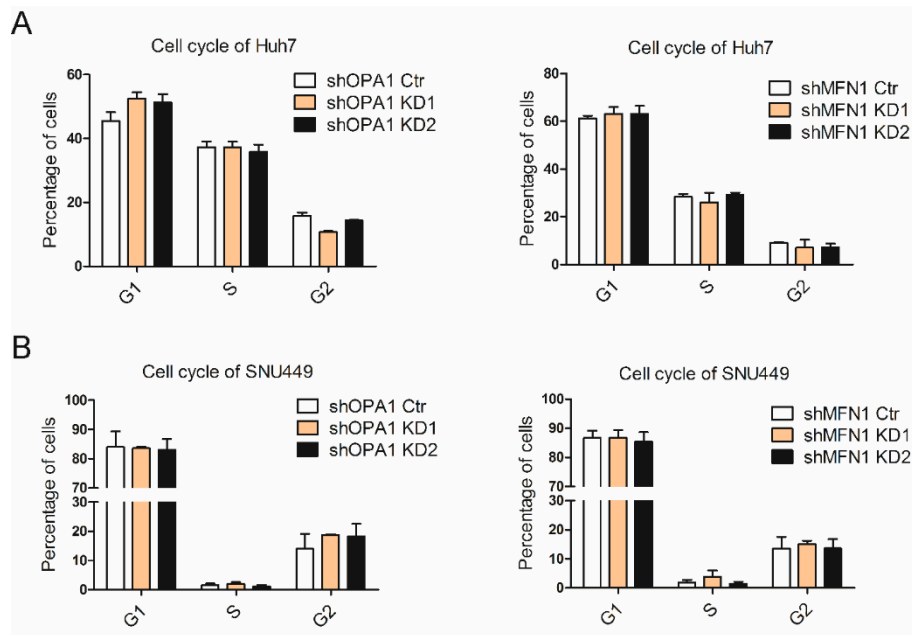
**Supplementary Fig 1** Mitochondrial morphology in a HBV-related HCC patient. **A** Representative electron microscopy images of cells from adjacent tissue and tumor tissue of the HCC patient (scale bar = 2  $\mu\text{m}$ ). The white boxed regions were further magnified in the expanded images (right side) (scale bar = 1  $\mu\text{m}$ ). Mitochondria (arrows) were observed. **B** Images of paired tissue were analysed by ImageJ software (n = 5 images/sample). Mitochondrial volume (mitochondrial area) per cell and mitochondrial length (mitochondrial major axis) were valued. Mitochondrial shape was calculated by the ratio of major axis and minor axis. Histograms are mean  $\pm$  SEM, with *p* values by Mann Whitney test.



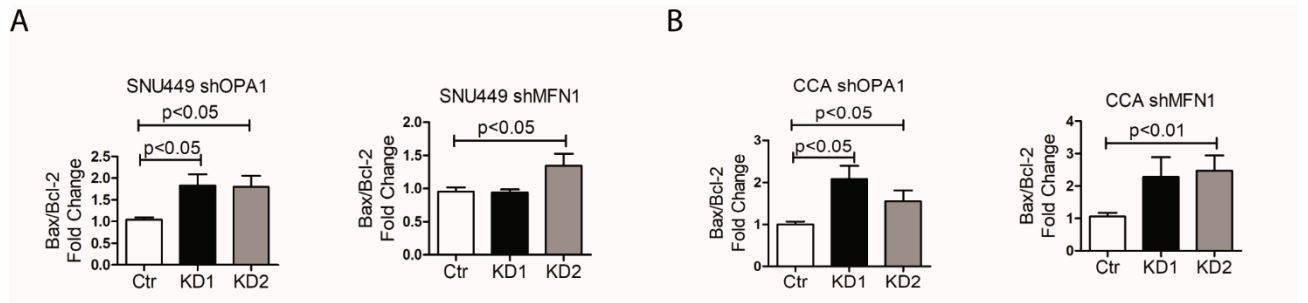
**Supplementary Fig 2** Western blot analysis of mitochondrial fusion genes in liver cancer cells

SNU449 and CCA organoids. **A** OPA1/MFN1 protein level of CCA organoids with OPA1/MFN1

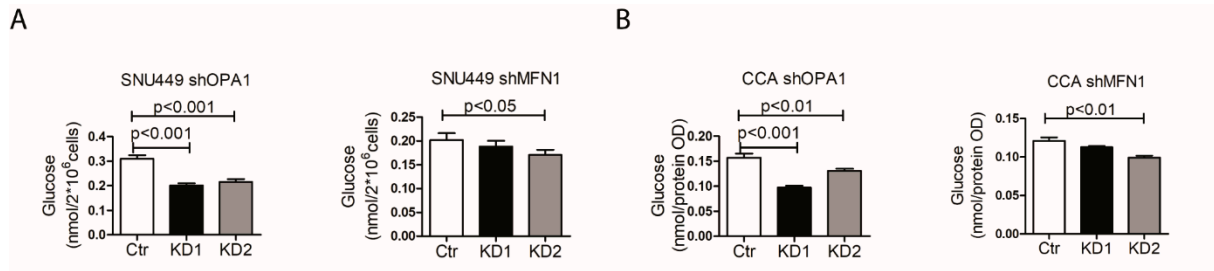
downregulation. **B** OPA1/MFN1 protein level of SNU449 cells with OPA1/MFN1 downregulation.



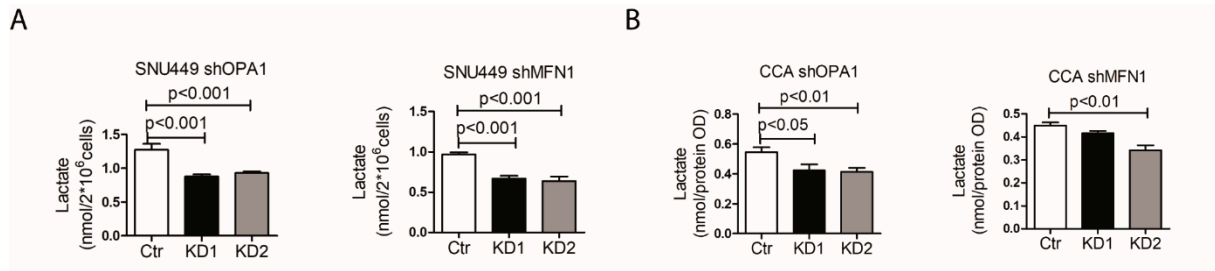
**Supplementary Fig 3** Dysfunction of mitochondrial fusion inhibited tumor cell growth independent of cell cycle arrest. **A** The distribution of Huh7 cells with OPA1/MFN1 downregulation did not show significantly difference with control group (n = 3). **B** The distribution of SNU449 cells with OPA1/MFN1 downregulation did not show significantly difference with control group (n = 3). Histograms are mean  $\pm$  SEM.



**Supplementary Fig 4** Dysfunction of mitochondrial fusion is correlated with increased ratio of Bax/Bcl-2 gene expression in liver cancer cells. **A** Bax/Bcl-2 fold change of SNU449 cells with OPA1/MFN1 downregulation increased compared with control group (n = 6). **B** Bax/Bcl-2 fold change of CCA organoids with OPA1/MFN1 downregulation increased compared with control group (n = 6). Histograms are mean  $\pm$  SEM, with *p* values by Mann Whitney test.



**Supplementary Fig 5** Dysfunction of mitochondrial fusion down-regulated glucose level in liver cancer cells. **A** The glucose product level of SNU449 cells with OPA1/MFN1 downregulation showed decreased glucose level compared with control group (n = 6). **B** The glucose product level of CCA organoids with OPA1/MFN1 downregulation showed decreased glucose level compared with control group (n = 6). Histograms are mean  $\pm$  SEM, with *p* values by Mann Whitney test.



**Supplementary Fig 6** Dysfunction of mitochondrial fusion down-regulated lactate level in liver cancer cells. **A** The lactate product level of SNU449 cells with OPA1/MFN1 downregulation showed decreased lactate level compared with control group (n = 6). **b** The lactate product level of CCA organoids with OPA1/MFN1 downregulation showed decreased lactate level compared with control group (n = 6). Histograms are mean  $\pm$  SEM, with *p* values by Mann Whitney test.