SUPPLEMENTARY FIGURE LEGENDS

Suppl. Fig. 1. Characterization of ovarian cancer cell lines and xenografts with different metabolic features.

A: Increased cell death of OC316 compared to IGROV-1 cells following glucose deprivation (left) or treatment with 2DG (right). Cells were plated in P6 wells at 1.5 x 10^5 cells/well. Following 72 h incubation, cells were stained with Annexin V Alexa Fluor 488 conjugate and PI and the level of cell death was measured by flow cytometry. Columns show mean ± SD values of n = 3 different experiments. *, $P <$ 0.05 normalized to control bars.

B: High resolution quantitative ^{1}H MRS analyses at 16.4 T of OC316 and IGROV-1 cells *in vitro*. Main differences are shown by the red circles. Alanine, creatine (Cr), phosphocreatine (PCr), glutamate and glutamine pool (Glx), Choline containing metabolites (tCho), *myo*-inositol (Ins), lactate (Lac), taurine (Tau), glutathione (GS).

C: Left side: coronal multislice T2-weighted spin echo images acquired from the OC316 and IGROV1 s.c. implants at 20 dpi (TR/TE = 3000/70, 4 transients, 15 slices, FOV = 20 x 20 mm^2, matrix 256 x 128, thickness = 0.6 mm). Areas of haemorrhage and necrosis are identified as hypointense and hyperintense regions, respectively. Right side: ADC maps calculated from coronal multislice DWI (TR/TE = 2500/50 ms, 2 transients, 11 slices, FOV = 20 x 20 mm^2, matrix 128 x 64, thickness = 1 mm, b-values =123, 491, 1105 s/mm^2) and the H & E staining in the corresponding slice.

D: Typical ^{1}H MR localised spectra collected from vital areas of IGROV1 and OC316 tumour by using a PRESS sequence (TR/TE = 4000/23 and 136 ms, ROIs

ranging from 8 to 40 μL, averages ranging from 256 to 512). Average lactate content is also given.

E: Expression of genes involved in glycolysis (*GAPDH*, *HKII* and *LDH-A*) was analized by quantitative PCR analysis in ovarian cancer xenografts. β2-microglobulin transcript was used as housekeeping gene. The expression levels in IGROV-1 cells were then set at 1 and relative expression levels were calculated. Mean ± SD values are shown (n = 4 samples/group). *, $P < 0.05$.

Suppl. Fig. 2. HIF-1α levels are increased in tumors following anti-VEGF therapy.

Detection of HIF-1α protein by Western blot analysis in lysates of IGROV-1 and OC316 tumors treated or not with the anti-VEGF Ab A4.6.1. Results of 3 representative samples per group are shown. Tubulin was used as a loading control. Columns report the mean values ± SD of HIF-1α/tubulin ratios in all samples analyzed. Samples were normalized to the ratio measured in the weakest one of the series, which was set at 1. Signal intensity was measured using a Biorad XRS chemioluminescence detection system. *, $P < 0.05$ compared to control tumor values.

Suppl. Fig. 3. Morphologic effects of anti-VEGF therapy on oesophageal cancer xenografts with different glycolytic phenotypes.

A: Measurement of glucose consumption and lactate production *in vitro*. Cells were plated in P6 wells at 1.5 x 10^5 cells/well, incubated for 24 h under normoxic

conditions and metabolic parameters were quantified by an automatic analyzer. Mean ± SD of three experiments is shown. *, $P < 0.05$ compared to OE19 values.

B: Increased cell death of Kyse-30 compared to OE19 cells following glucose deprivation. Cells were plated in P6 wells at 1.5 x 10^5 cells/well. Following 72 h incubation, cells were stained with Annexin V Alexa Fluor 488 conjugate and PI and the level of cell death was measured by flow cytometry. Columns show mean ± SD values of $n = 3$ different experiments. *, Annexin V^+ cells under glucose starvation versus standard medium; $P < 0.05$.

C: Histological analysis shows large necrotic areas in Kyse-30 tumors after 3 injections of A4.6.1 (100 µg/dose, every 2 days). A4.6.1 administration started when tumors reached a volume of 120 ± 22 mm^3. Representative images (original magnification: x25) are shown. The continuous line marks the boards of necrotic tissue. Columns indicate quantitative analysis of necrotic areas in 6 samples of each group. *, $P < 0.05$ compared to control values.

Suppl. Fig. 4: ADC distribution and spectral profiles of IGROV-1 xenografts treated with anti-VEGF mAb.

A: ADC distribution in A4.6.1-treated IGROV-1 xenografts showed a significant reduction in the median value compared with controls ($P < 0.05$).

B: Top panel, T2-weighted images of IGROV-1 xenografts acquired 48 h after the second dose of anti-VEGF mAb (A4.6.1, 100 µg/dose). Bottom panel: ^1H MR localised spectra (TR/TE=4000/136 ms VOI ranging between 15 and 37 µl) acquired in treated and in control tumors.

Suppl. Fig. 5. Impaired AMPK activation in OC316 cells *in vitro* under stress conditions or treatment with AICAR or metformin.

A: Western blot analysis of p-AMPK and p-ACC levels in OC316 and IGROV-1 cells grown in vitro under standard conditions (normoxia / 2 g/l glucose), glucose starvation, hypoxia (0.5% pO2) or a combination of the two. Levels of total AMPK and ACC are also shown; α-tubulin has been used as loading control. One representative experiment of five performed is shown.

B: Western blot analysis of p-AMPK levels following *in vitro* culture in the presence of the indicated concentrations of AICAR. Levels of total AMPK and ACC are also shown; α-tubulin has been used as loading control. One representative experiment of three performed is shown.

C: Western blot analysis of p-AMPK levels following *in vitro* culture in the presence of the indicated concentrations of metformin. Levels of total AMPK and ACC are also shown; α-tubulin has been used as loading control. One representative experiment of three performed is shown.

Suppl. Fig. 6. Analysis of LBK1 status in OC316 cells.

A: Western blot analysis of cell lysates shows expression of LKB1 both in IGROV-1 and OC316 cells, but not in HeLa cells, which were used as negative control (30). β-Actin was used as loading control.

B: Immunofluorescence analysis of LKB1 expression in IGROV-1 and OC316 cells cultivated under hypoxic conditions (20 h, 0.5% pO2) and glucose starvation. Magnification, x400.

C: Electropherogram of LKB1 sequence analysis in OC316 cells. The 958 G>A substitution found in exon 8 is boxed.

Suppl. Fig. 7. Proliferation of AMPKα2 silenced IGROV-1 cells is similar to that of control cells.

Proliferation of AMPKα2 versus shRNA-transduced IGROV-1 cells was measured by the Vialight ATP assay. CPS, counts per second. *Columns*, mean values of five replicates; *bars*, SD.

Suppl. Fig. 8. Assessment of HIF1α activity *in vitro* following AMPKα2 silencing in IGROV-1 cells.

Attenuation of AMPKα2 by shRNA lentiviral vectors increases HIF1α activity in IGROV-1 cells. Twenty-four hours after lipofection of cells with an HRE-luciferase plasmid and a β-galactosidase-encoding plasmid, HIF1α activity was measured by a luciferase reporter assay and normalized to β-galactosidase activity. The bars represent mean value + SD of three independent experiments.

Suppl. Fig. 9. Akt activation in IGROV-1 and OC316 cells.

Western blot analysis shows increased expression of p-Akt^{ser473} and p-Akt^{thr308} in IGROV-1 compared to OC316 cells; total Akt levels (Akt 1/2) are also shown. α-tubulin was used as loading control.