**Supplementary Materials**

**Supplementary Figure legends**

**Supplementary Figure S1. *KAP1* transcript level is positively associated with some of mitochondrial complex genes expression in breast cancer patients. (A)** *KAP1 gene expression is positively correlated with message abundance of some of mitochondrial complex genes.* Breast tumor dataset from the Netherlands Cancer Institute was used. The 50 highest and lowest *KAP1*-expression samples were selected for the GENE-E analyses. OXPHOS genes were selected based on Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Genes with most significant co-expression pattern with *KAP1* among these samples were selected for heatmap. **(B - G)** *Correlation between KAP1 message abundance and abundance of selected message encoding mitochondrial respiratory complexes***. (B, C)** the complex I genes: *NDUFS8* **(B)** and *NDUFA3* **(C);** **(D)** the complex III gene: *UQCRCI*; **(E, F)** the complex IV genes: *COX6B1* **(E)** and *COX6C* **(F)**; **(G)** the complex V gene: *ATP5D*. \*: *p*<0.05; \*\*: *p*<0.01; \*\*\*: *p*<0.001.

**Supplementary Figure S2. *KAP1* overexpression is associated with breast cancer poor prognosis. (A)** *Elevated* *KAP1 transcript abundance in breast tumors compared to normal-like samples*. A histogram revealed the correlation of KAP1 message abundance in different types of breast cancer. The 103 best classified samples from the breast tumor dataset of the Netherlands Cancer Institute were used for the analysis. \*: *p*<0.05; \*\*: *p*<0.01; \*\*\*: *p*<0.001**.** Normal-like: n=17;Luminal A: n=24; Luminal B: n=19; Basal: n=20; HER2: n=23. **(B)** *KAP1 transcript abundance is positively correlated with breast tumor grades*. A histogram revealed an positive correlation of KAP1 message abundance and the corresponding tumor grade using a breast tumor dataset from the Netherlands Cancer Institute. Grade 1: n=158, Grade 2: n=358, Grade 3: n=276. \*\*: *p*<0.01; \*\*\*: *p*<0.001. **(C)** *KAP1 expression level is negatively correlated with relapse free survival in breast cancer patients.* 295breast tumor samples of the Netherlands Cancer Institute were used for the Kaplan-Meier analysis.

**Supplementary Figure S3. p38MAPK, but not AMPK or ATM, activates metabolic stress-induced KAP1 phosphorylation at Ser473**. **(A)** *Glutamine depletion induced AMPK-independent pS473-KAP1.* MDA-MB-231 cells, in the absence or presence of compound C, an AMPK inhibitor, were subjected to glutamine deprivation (or not) for 2- and 6-h prior to cell harvesting (*upper panel*). *AMPKα1/α2* double knockout and wild-type (wt) MEFs were glutamine depleted for the indicated time periods (*lower panel*). **(B)** *ATM signaling is not responsible for the glucose starvation-induced pS473-KAP1.* MDA-MB-231 cells were treated with vehicle or the ATM inhibitor, Ku55933 (10 µM) in the presence or absence of glucose (25 mM) for 6-h prior to cell harvesting. Non-contiguous lanes in the same membrane were separated by a dashed line. **(C)** *Knockdown of CHK2 and PKCδ does not reduce glutamine deprivation-elevated pS473-KAP1.* MDA-MB-231 cells were transfected with siCtrl, siCHK2 or siPKCδ for 24-h prior to subjecting to glutamine starvation for an additional 6-h. **(D)** *Glucose or glutamine depletion induces ROS.* Flow cytometry was used to detect oxidization of DCFDA to DCF in MDA-MB-231 cells subjected to glucose and glutamine starvation for 6-h (*left panel*). Oxidation-insensitive fluorescent probe (DCF) was used as a control (*right panel*). \*\*\*: *p*<0.005. **(E)** *ROS mediates pS473-KAP1 induction upon metabolic stress.* MDA-MB-231 cells, in the absence and presence of NAC (5 mM), were cultured in glucose- or glutamine-depleted medium for the indicated periods prior to cell harvesting. **(F)** *Arginine depletion induces p38 activation.* Knockdown of p38β dampened pS473-KAP1 induction in response to arginine depletion(*left panel*)*.* MDA-MB-231 cells were subjected to arginine deprivation for the indicated time periods prior to cell harvesting (*right panel*). **(G - H)** *p38 signaling is critical for pS473-KAP1 induction*. **(G)** MDA-MB-231 cells were, in the presence of increasing concentrations of SB203580, a p38 inhibitor, subjected to glucose depletion for 6-h. **(H)** MDA-MB-231 cells were, in the presence of vehicle, SB203580 (10 μM), or NAC (5 mM), subjected to H2O2-treatment (100 μM) for 1-h. **(I - J)** *NAC reduces glucose deprivation-induced apoptosis*. **(I)** MDA-MB-231 cells were subjected to glucose starvation for 24-h in the presence or absence of NAC (10 mM) prior to analyses. Annexin V staining followed by flow cytometric analysis quantified Apoptosis. One representative histogram is shown from n=3. **(J)** MDA-MB-231 cells were subjected to glucose starvation for 48-h in the presence or absence of NAC (1 mM) prior to clonogenic assays. Survival fraction was calculated by designating control cells in complete medium as 1. \*\*\*: *p*<0.001. **(A - C, E - H)** Equal amounts of cell lysates were subjected to Western analyses with the indicated antibodies. One representative Western image is shown from n=3.

**Supplementary Figure S4. Time-dependent alterations of Mitochondrial dynamics upon glucose depletion. (A)** *KAP1 Ser473-phosphorylation does not affect ATP (left panel) and mitochondrial DNA (mtDNA) amount (right panel)*. The relative ATP amount was calculated, after normalization with cell numbers, by designating the value in wt-KAP1-expressing cells in complete medium as 1. Mitochondrial DNA (mtDNA) abundance was assessed by quantitative PCR using primer pairs targeting the mitochondrial D loop. Relative mtDNA amount was calculated, after normalization with nuclear DNA *Lamin B*, by designating the value in MDA-MB-231/shKAP1 cells as 1. Mean±SD from n=3 is shown. **(B)** MDA-MB-231 cells expressing COX4-DsRed-labeled mitochondria were imaged at indicated time points.Images were obtained by 40X objective. The enlarged view of respective boxed area is shown in below.

**Supplementary Figure S5. S473A-KAP1 promotes mitochondrial network and sensitizes MDA-MB-231/shKAP1 cells to glucose starvation.** **(A)** *S473A-KAP1 favors mitochondrial fusion under glucose depletion (8-h)*. Mitochondrial morphology was scored (Figure 2C) by the following criteria: fragmented: more than 70% of mitochondria are small and round (<3 µm); intermediated: mixture of globular and shorter tubulated mitochondria (3 to 5 µm); and tubulated: more than 70% of mitochondria are filamentous (>5 µm); scale bar: 10 µm. One representative image is shown**. (B, C)** *Mitochondrial surface area is increased in S473A-KAP1-expressing cells.*MDA-MB-231 cells with different KAP1-re-expression were subjected to glucose depletion for 8-h (or not). **(B)** One representative SEM image from each treatment group is shown. Scale bar: 50μm. **(C)** Total mitochondrial surface area in a cell was calculated by Image-Pro software. (n=30). **(D)** *Mdivi-1 sensitizes MDA-MB-231 cells to prolonged glucose depletion*. MDA-MB-231 cells were incubated with the indicated concentrations of glucose, in the presence of vehicle or Mdivi-1 (10 µM), for 72-h prior to cell harvesting. Cell viability was measured by ACP assay. Mean±SD from n=3 is shown; \*: *p*<0.05. **(E, F)** *The early response to arginine depletion is mitochondrial fusion*. MDA-MB-231 cells were subjected to arginine depletion for 8-h (or not). An anti-TOM20 antibody was used for staining mitochondria. **(E)** One representative image is shown. Scale bar: 10 µm. **(F)** Mitochondrial morphology was scored by the following criteria: fragmented: more than 70% of mitochondria are small and round (<3 µm); intermediate: mixture of globular and shorter tubulated mitochondria (3 to 5 µm); and tubulated: more than 70% of mitochondria are filamentous (>5 µm). 80 images were analyzed to quantitate the percentage of respective cells with different mitochondrial morphology**.**

**Supplementary Movie legends**

**Movie 1. Time-lapse imaging of MDA-MB-231 cells in complete medium.** Cells expressing COX4-DsRed-labeled mitochondria were imaged in a 20-min interval for 20-h.Images were obtained by 40X objective**.**

**Movie 2. Time-lapse imaging of MDA-MB-231 cells in glucose-depleted medium.** Cells expressing COX4-DsRed-labeled mitochondria were imaged in a 20-min interval for 20-h.Images were obtained by 40X objective**.**

**Movie 3. Time-lapse imaging of MDA-MB-231/shKAP1/S473A-KAP1 cells in glucose-depleted medium.** Equal numbersofMDA-MB-231/shKAP1/S473A-KAP1 cells harboring Su9-GFP-labeled mitochondria and MDA-MB-231/shKAP1/S473D-KAP1 with COX4-DsRed-labeled mitochondria were co-cultured in glucose-depleted medium and imaged in a 20-min interval for 20-h.Images of MDA-MB-231/shKAP1/S473A-KAP1 cells harboring Su9-GFP-labeled mitochondria, obtained by 40X objective, are shown**.**

**Movie 4. Time-lapse imaging of MDA-MB-231/shKAP1/S473D-KAP1 cells in glucose-depleted medium.** Equal numbersofMDA-MB-231/shKAP1/S473A-KAP1 cells harboring Su9-GFP-labeled mitochondria and MDA-MB-231/shKAP1/S473D-KAP1 with COX4-DsRed-labeled mitochondria were co-cultured in glucose-depleted medium and imaged in a 20-min interval for 20-h.Images of MDA-MB-231/shKAP1/S473D-KAP1 with COX4-DsRed-labeled mitochondria, obtained by 40X objective, are shown**.**