Figure S1. Exemplary propidium iodide FACS plots for hypoxic and normoxic conditions and a glucose concentration of 2 mM in the absence of glutamine. SVG cells were exposed to serum-free DMEM with 2 mM glucose, DMSO, 2DG or A-769662 and hypoxia or normoxia. The fraction of propidium iodide (PI) positive cells represent non-viable cells. 2DG, 2-deoxy-d-glucose.

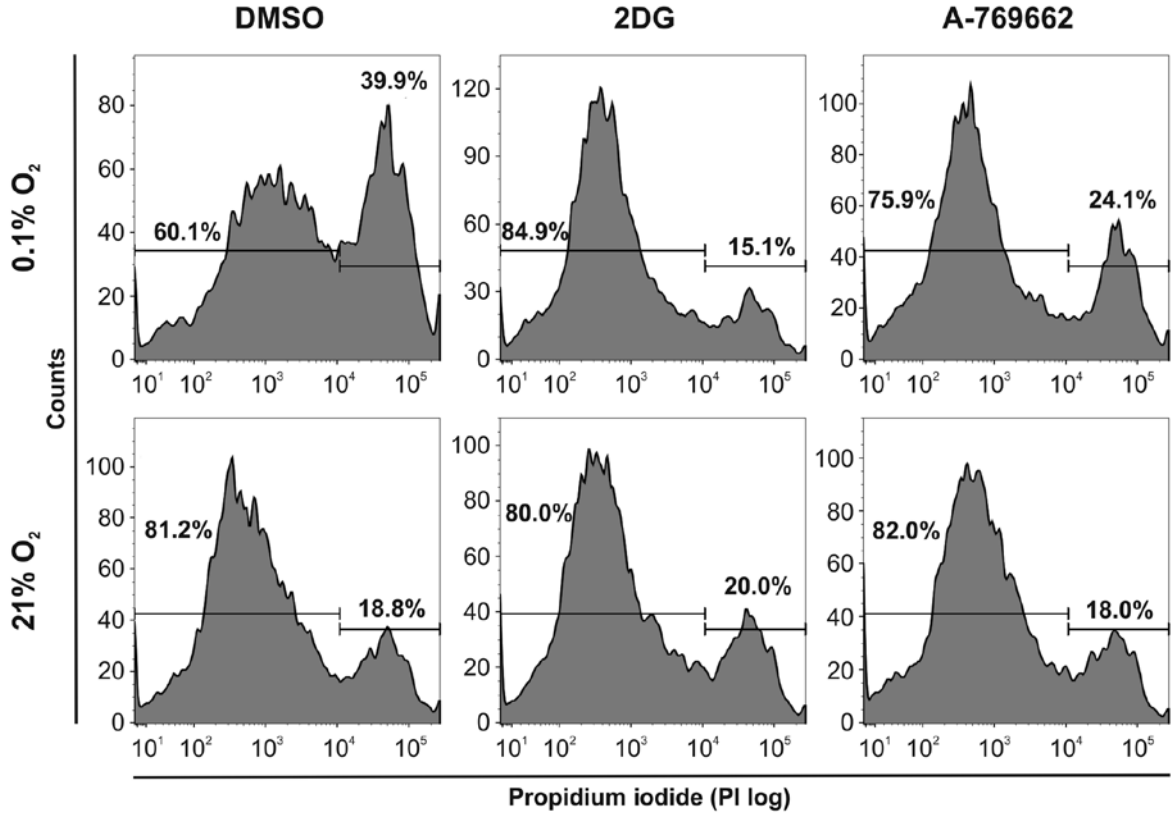

Figure S2. Caspase-3 and PARP cleavage under normoxic and hypoxic conditions. SVG cells were treated with 2DG, A-769662, the vehicle DMSO or staurosporin as a positive control, a glucose concentration of 2 mM and a glutamine concentration of 0 mM for 12 h. Cellular lysates were examined by western blot analysis with antibodies for PARP, cleaved PARP (cPARP), cleaved caspase-3 (cCaspase-3) and actin (A). Intensities of an exemplary blot were normalized to actin expression (B-D). 2DG, 2-deoxy-d-glucose.

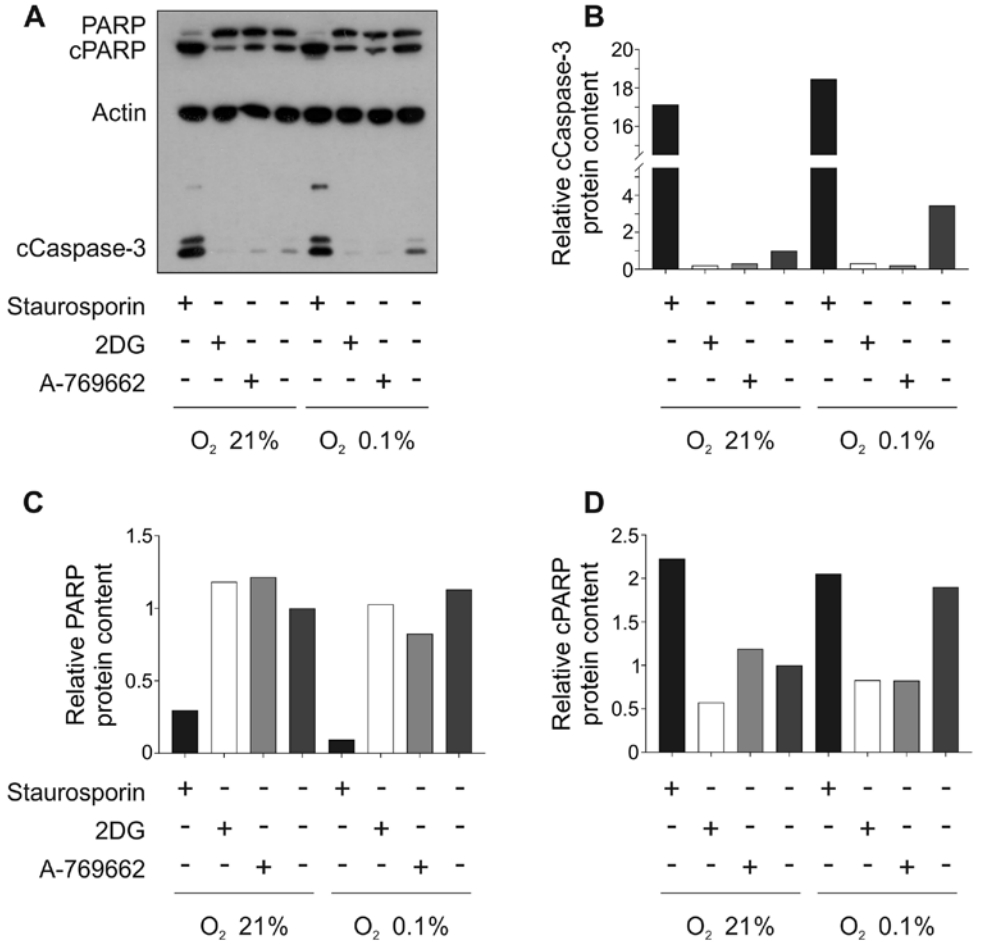

Figure S3. Inhibition of glycolysis and activation of AMPK protects SVG from hypoxia induced cell death at glutamine concentrations of 4 mM. Effects of 2DG and A-769662 on cell viability under hypoxic (0.1% O_2) and normoxic (21% O_2) conditions measured with PI-FACS. SVG cells were exposed to serum-free DMEM containing 4 mM glutamine supplemented with 2, 5 or 25 mM glucose, vehicle DMSO, 2DG or A-769662, incubated under hypoxic or normoxic conditions. PI-positive dead cells are shown in percentage of the total cell count. (A and B) Exposure to 2 mM glucose and hypoxia or normoxia, 24 h treatment. (C and D) Exposure to 5 mM glucose and hypoxia or normoxia, 48 h treatment. (E and F) Exposure to 25 mM glucose and hypoxia or normoxia, 96 h treatment [n=4; n.s., not significant ($P \geq 0.05$); $^{*}P<0.05$, $^{***}P<0.001$, ANOVA with Dunnett's multiple comparisons test]. 2DG, 2-deoxy-d-glucose.

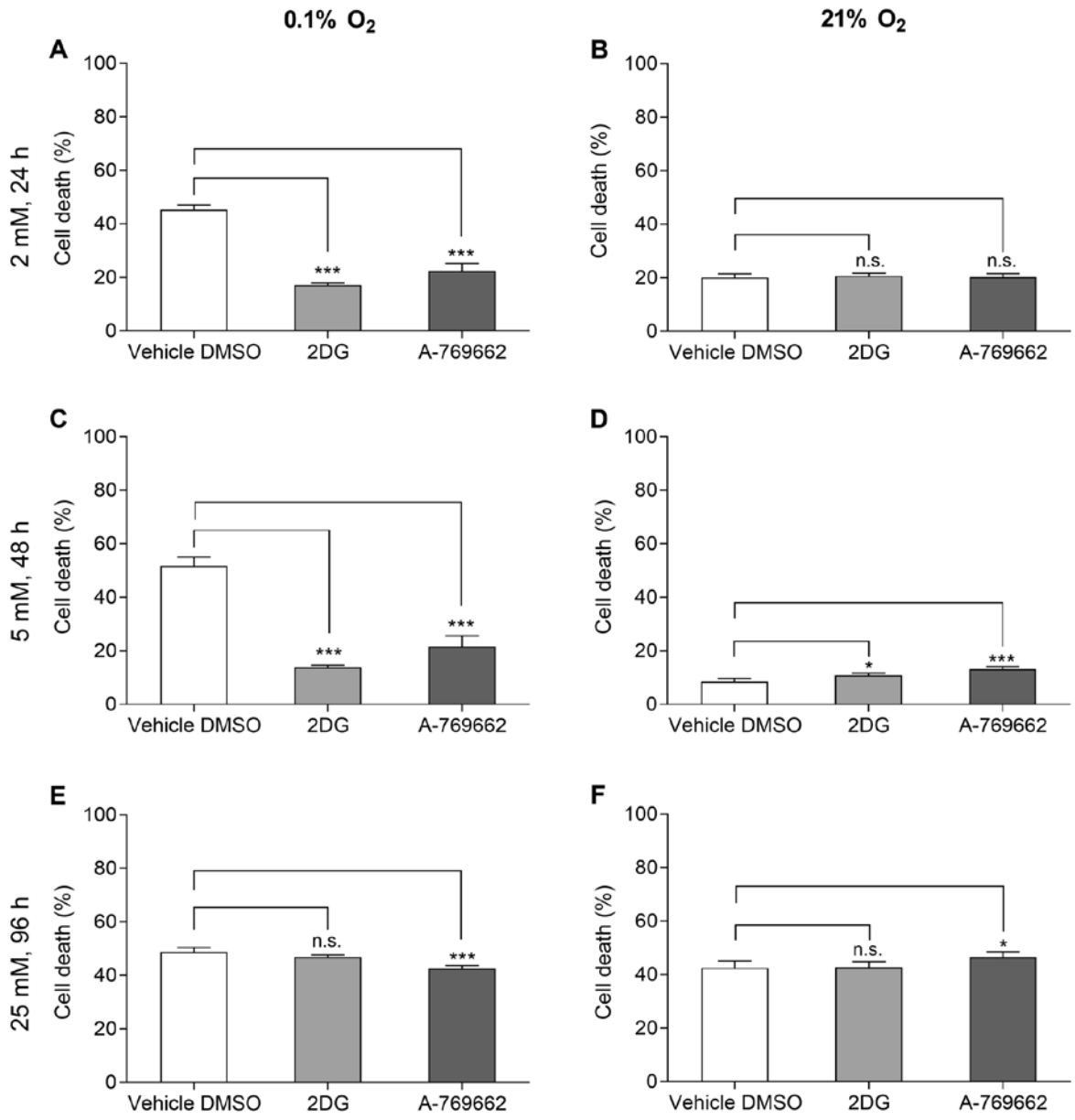

Figure S4. Effect of 2DG and A-769662 on cell viability under hypoxic (0.1% O_2) conditions measured with LDH assay. SVG cells were exposed to serum-free DMEM lacking glutamine supplemented with 2 or 5 mM of glucose, vehicle DMSO, 2DG or A-769662 and hypoxia in the absence of glutamine. Cell death was quantified by LDH release, which was measured with a photometric-based assay. (A) Exposure to 2 mM glucose, 24 h treatment. (B) Exposure to 5 mM glucose, 48 h treatment. Inhibition of glycolysis and activation of AMPK under hypoxic conditions led to reduction in cell death, measured by a decreased LDH release when cells were treated with 2DG or A-769662 (n=4, $^{**}P<0.01$, $^{***}P<0.001$, ANOVA with Dunnett's multiple comparisons test). 2DG, 2-deoxy-d-glucose.