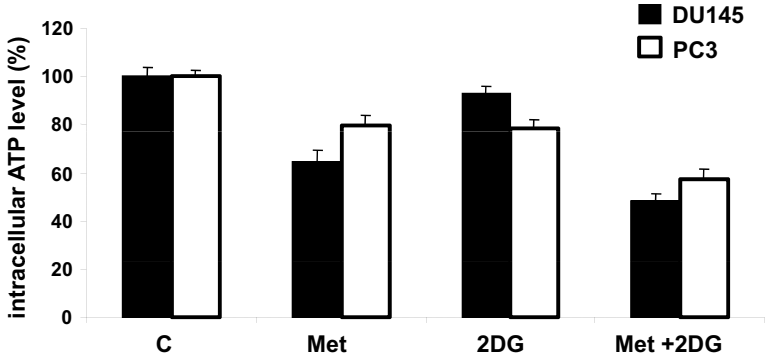
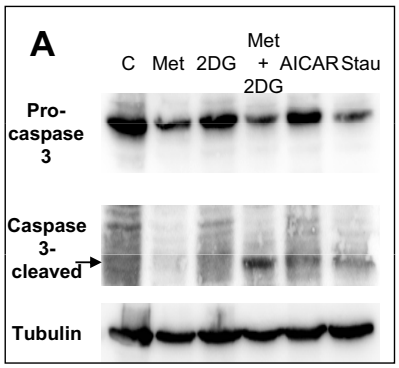
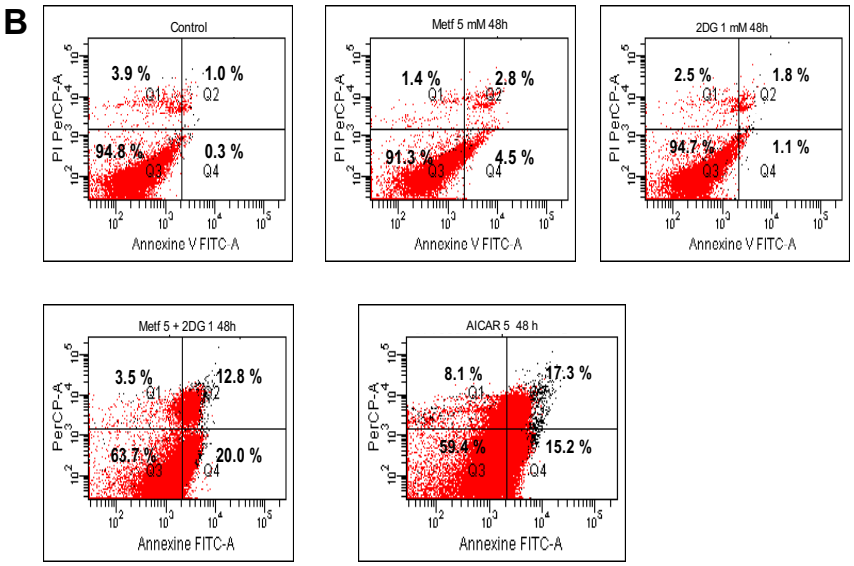
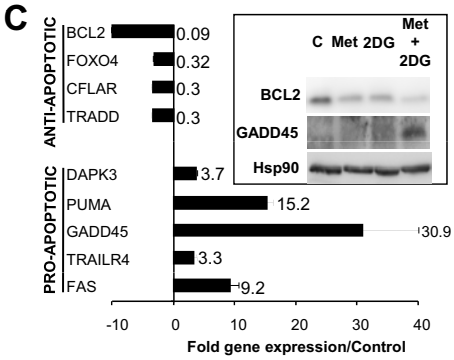



## Supplementary S1

Intracellular concentration of ATP in DU145 and PC3 cells treated for 24 hours with the indicated agents (Metformin 5mM and 2DG 1mM). Results are expressed as percent of control (100%).



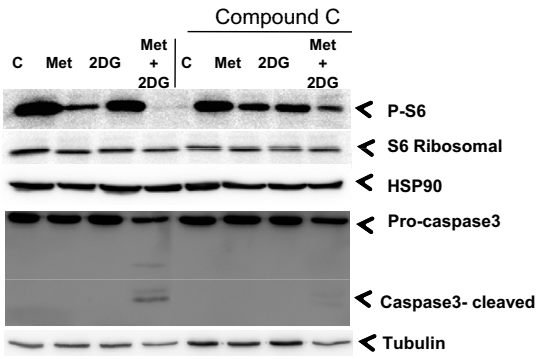




## Supplementary S2

**A:** Immunoblot of caspase 3 in LNCaP cells treated with metformin 5mM (Met); 2DG 1mM, the combination of the two drugs, AICAR (positive control), 1mM and Staurosporine (100µM) for 48 h.

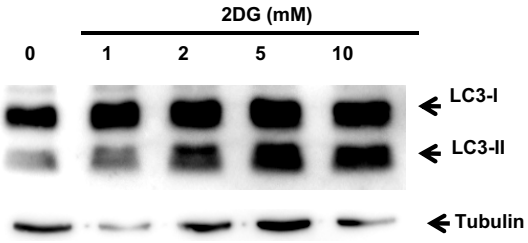
**B:** Flow cytometry analysis of LNCaP cells treated with the indicated agents and labelled with Annexin V. The percentage of cells positively labelled with Annexin V is indicated for each condition. Metformin 5mM, 2DG 1mM, the combination and AICAR 5mM.

**C:** The expression of 88 pro- and anti-apoptotic genes was analysed by RT-PCR in LNCaP by comparing untreated cells and cells treated with the combination of metformin and 2DG. The variation of the significantly modified genes ($p<0.05$) is represented. (Inset). Imunoblotting of BCL2 and GADD45 in LNCaP cells treated for 24 hours with the indicated agents.




## Supplementary S3: AMPK mediates metformin/2DG induced apoptosis

**The inhibition of AMPK was monitored by the analysis of PS6 ribosomal** Inhibition of AMPK by compound C (20μM) reversed the inhibition of PS6 ribosomal by the combination of metformin and 2DG and prevented caspase 3 cleavage induced by the drugs.




## Supplementary S4

Immunoblot of LC3 in LNCaP cells treated with increasing concentration of 2DG for 48 h.

# Materials and methods for supplementary data

**AnnexinV/PI staining assay**

Apoptosis was assessed by measuring membrane redistribution of phosphatidylserine using an annexin V-FITC apoptosis detection kit (Roche Diagnostics, Mannheim, Germany). According to the protocol kit, cells were collected, washed twice with PBS and resuspended in 500 µl of staining solution containing FITC-conjugated annexin V antibody and propidium iodide (PI). After incubation on ice for 30 min, cells were analyzed by flow cytometry. Basal apoptosis and necrosis were identically determined on untreated cells.

**Inhibition of AMPK with compound C**

LNCaP cells were treated with 20µM of compound C (Calbiochem, Merck, Darmstadt, Germany) for 48 hours. The indicated agents were then added to the culture media: Metformin 5mM, 2DG 1mM and the combination of the two drugs.

***Quantitative PCR analysis of the apoptosis genes.***

Total RNA (1µg) was reverse transcribed using random priming and Superscript II reverse transcriptase (Invitrogen). Real-time PCR was performed in an ABI PRISM 5700 Sequence Detector System using a dedicated “apoptosis plate” (Applied Biosystems, Courtaboeuf, France) containing the major genes regulated upon apoptosis {Ref: 20}.The relative expression level of target genes was normalized for RNA concentrations with four different house-keeping genes (GAPDH, β-actin, HPRT and ubiquitin). mRNA values are expressed as arbitrary units.