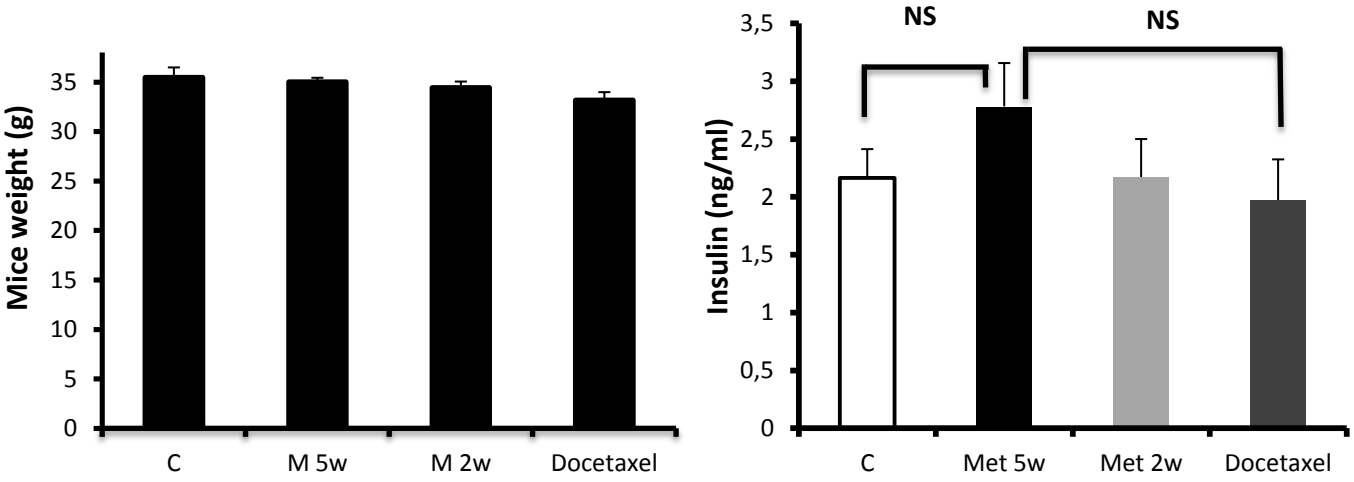

FIG. S1: Metformin does not affect mouse weight an insulinemia

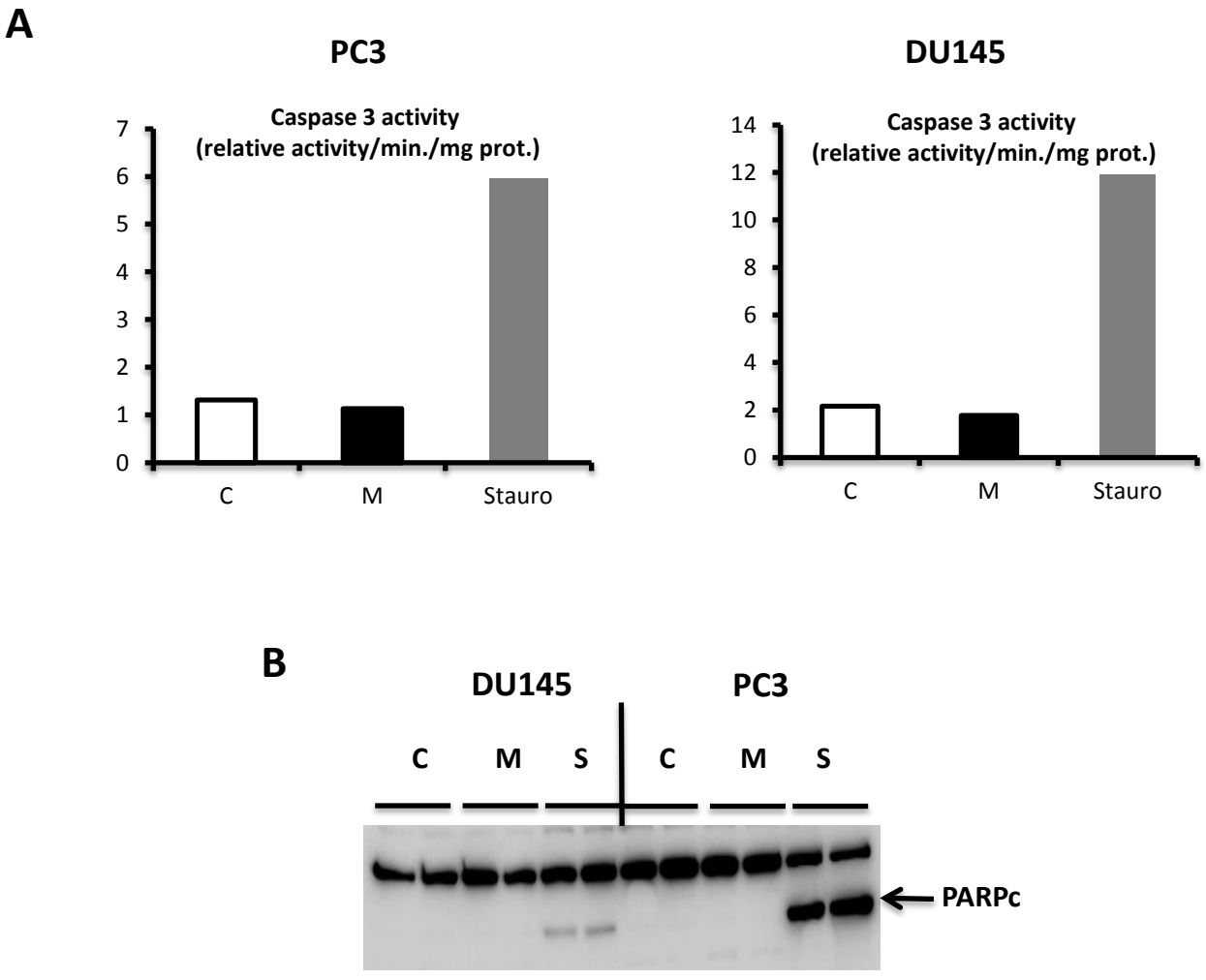

FIG. S2: Metformin does not induce apoptosis in prostate cancer cells.

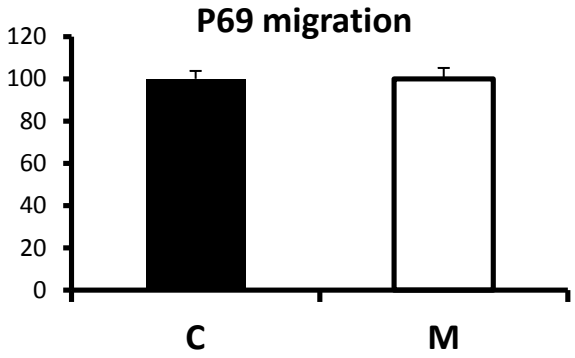

Figure S3: Metformin does not affect P69 cell migration

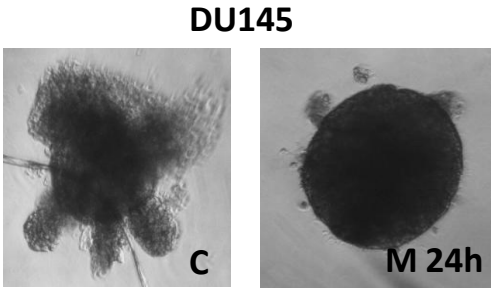

Figure S4: Metformin blocks cell migration out of the spheroid

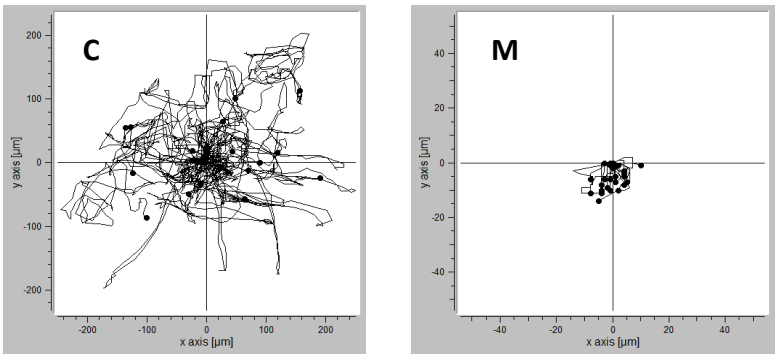

Figure S5: Metformin inhibits cell motility

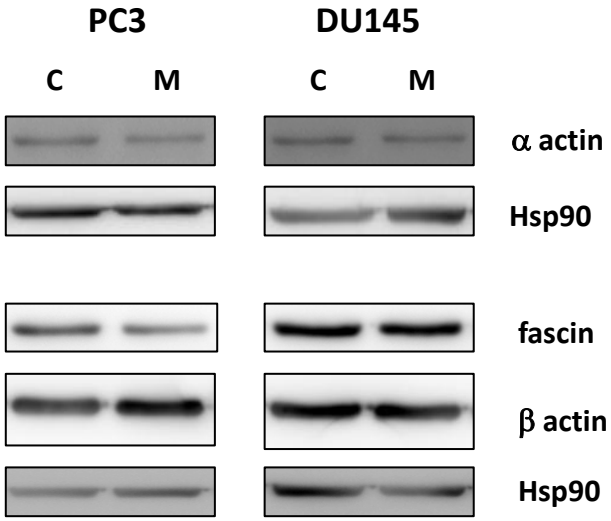

Figure S6: Metformin effects on actin and fascin levels

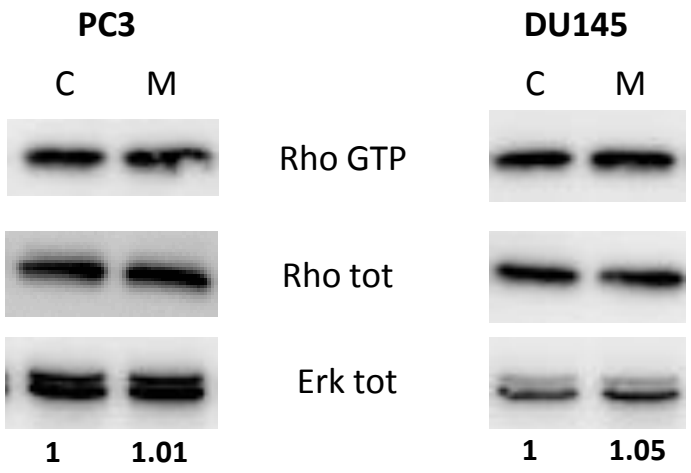

Figure S7: Metformin does not affect Rho GTP levels

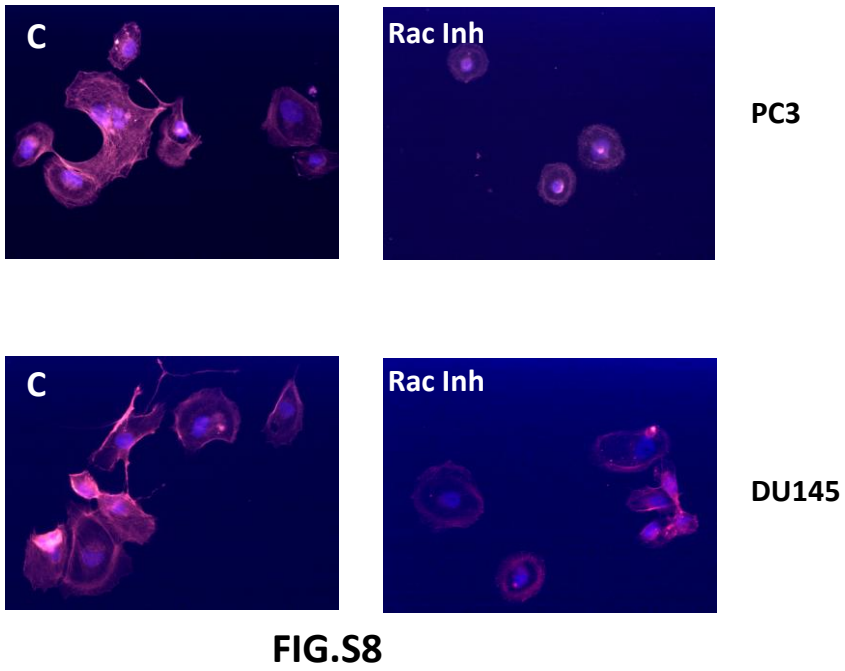

Figure S8: A rac1 inhibitor affects cytoskeletal organisation

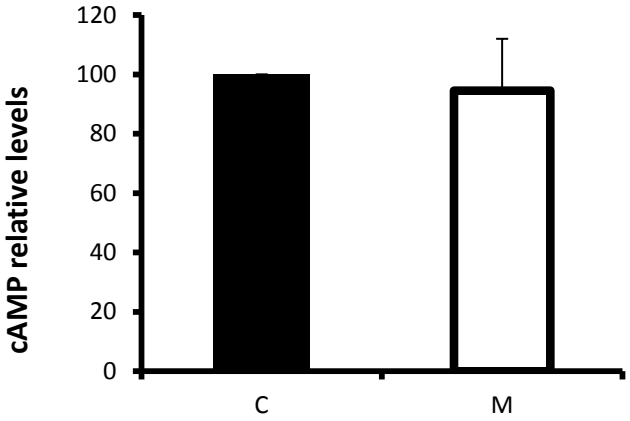

Figure S9: Metformin does not affect cAMP levels in PC3 cells

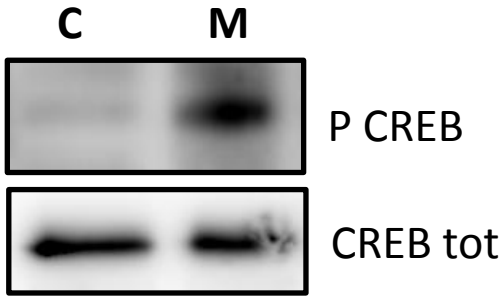

Figure S10: Metformin increases P CREB in DU145 cells

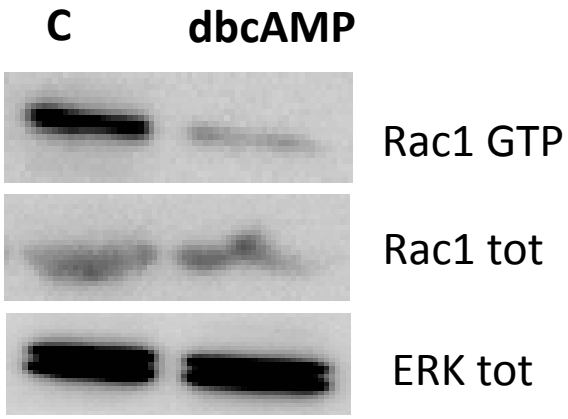

FIG.S11: dbcAMP decreases Rac1GTP levels

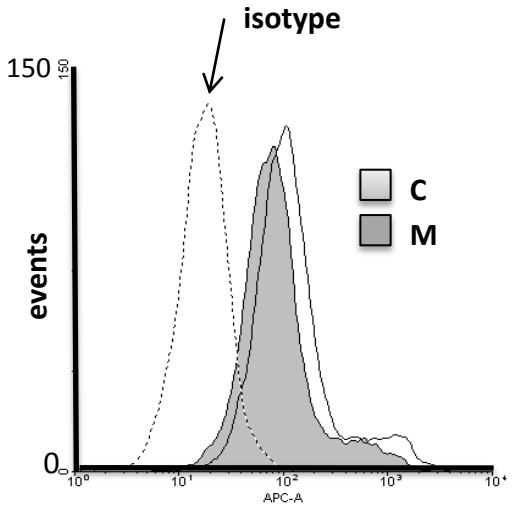

FIG. S12: Metformin decreases CXCR4 at the cell surface

Figure legends

Figure S1: Average mice body weight and insulin concentration at the end of the treatment with metformin (5 weeks or 2 weeks) or Docetaxel (2weeks).

Figure S2: PC3 and DU145 were serum starved overnight and treated for 4 hours with 5mM metformin or 0.5µM Staurosporine. (A) Caspase 3 activity. (B) Western blot analysis of PARP in cells treated as described above.

Figure S3: P69 cells (normal epithelial prostate cells) were seeded in Boyden chamber and metformin (5mM) was added during the migration for 4 hours. The graph represents the average number of cells which migrates across the Boyden chamber.

Figure S4: Pictures of DU145 cells spheroid treated with 5mM metformin for 24h (M).

Figure S5: Recording of PC3 cell tracks using Chemotaxis software, cells were analysed during a period of 24h in presence or not of 5mM metformin (M).

Figure S6: PC3 and DU145 were serum starved overnight and treated for 4 hours with 5mM metformin and an immunoblot was performed using antibodies against the mentioned proteins.

Figure S7: Immunoblot of Rho after a pull-down assay, the assay was performed with DU145 and PC3 prostate cancer cells treated with 5mM metformin for four hours as described in material and methods.

Figure S8: Immunofluorescence performed with Texas red Phalloidin in DU145 and PC3 treated or not with the Rac1 Inhibitor (50µM) during 4h as described in Material and Methods.

Figure S9: cAMP relative concentration in PC3 cells treated or not with 5mM metformin for 4h

Figure S10: Immunoblot of Phospho CREB Ser133 and Total CREB in DU145 cells treated with 5mM metformin.

Figure S11: Immunoblot of Rac1 after a pull-down assay performed in DU145 cells treated with the analog of cAMP (dbcAMP) for four hours as described in material and methods.

Figure S12: FACS analysis after CXCR4-APC staining in DU145.

Supplemental Material and methods:

Insulinemia: Blood samples were withdrawn from the tail vein at the end of the experiment and insulinemia was determined using a mouse ultrasensitive assay ELISA kit (ALPCO, Salem, NH, USA)

Caspase 3 activity: Cells were incubated with 5mM Metformin for 4h or with 2µM staurosporine after overnight starvation in DMEM 0.5% BSA medium. Caspase 3 activity was fluorimetrically measured in presence or not of Ac-DEVD-CHO (caspase 3 inhibitor) (Calbiochem, Merck, Darmstadt, Germany). Enzyme activities were expressed in relative intensity per minute and per milligram of protein.

Western blot: Antibodies againt Fascin were from Millipore; α and β actin from Sigma-Aldrich.