Supplementary Data

Supplementary Figure S1

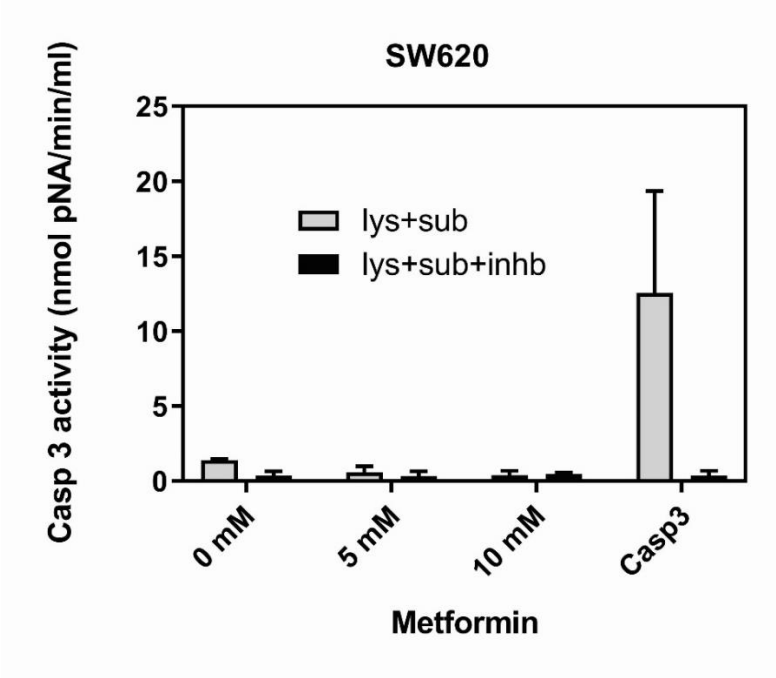

Figure S1: Metformin did not induce caspase 3 activity in SW620 cells. For the caspase 3 activity assay, lysates were prepared from 10^7 untreated cells (0 mM metformin), and cells treated with 5 mM and 10 mM metformin for 48 hrs. Recombinant caspase 3 (a component of the kit) served as a positive control for the caspase 3 reaction (Casp3). Ten µl of lysates were applied in one reaction, and each was added with caspase 3 substrate (Ac-DEVD-pNA) and caspase 3 substrate + caspase 3 inhibitor (Ac-DEVD-CHO). Caspase 3 activity is presented in nmol pNA/min/ml (Caspase 3 Assay Kit, Sigma #CASP-3-C). Data show mean values ±SD from three independent experiments.
Abbreviations: lys, lysate; sub, substrate; inhb, inhibitor.

Supplementary Figure S2

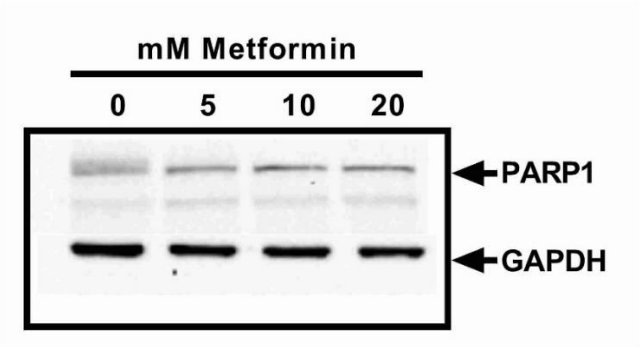

Figure S2: Metformin did not induce PARP1 cleavage in SW620. Cells were treated with 0, 5, 10, and 20 mM metformin for 48 hours. Protein lysates were prepared as mentioned in Materials and Methods. Thirty µg proteins were loaded on 10% SDS PAGE and separated proteins were transferred onto nitrocellulose membranes. Antibody detections were performed using anti PARP1 antibody (#9542, CST) in (1:1000) dilution in blocking buffer. Loading controls for this SW620 blot was GAPDH (anti-GAPDH antibody, #G9545, Sigma). The data shown are representative of three independent experiments.