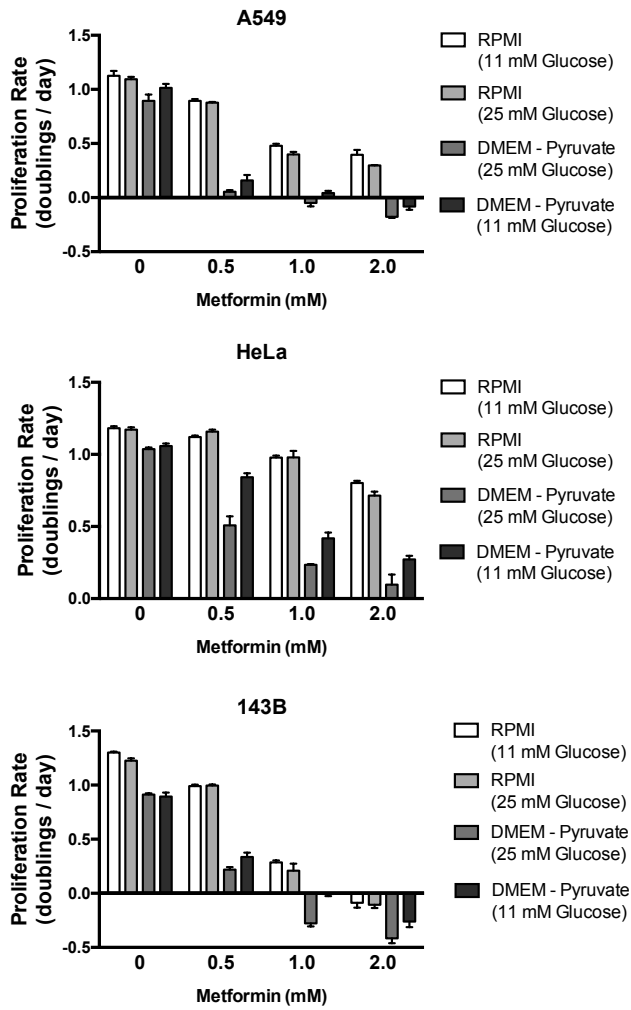


**Figure S1**

**A**



**B**

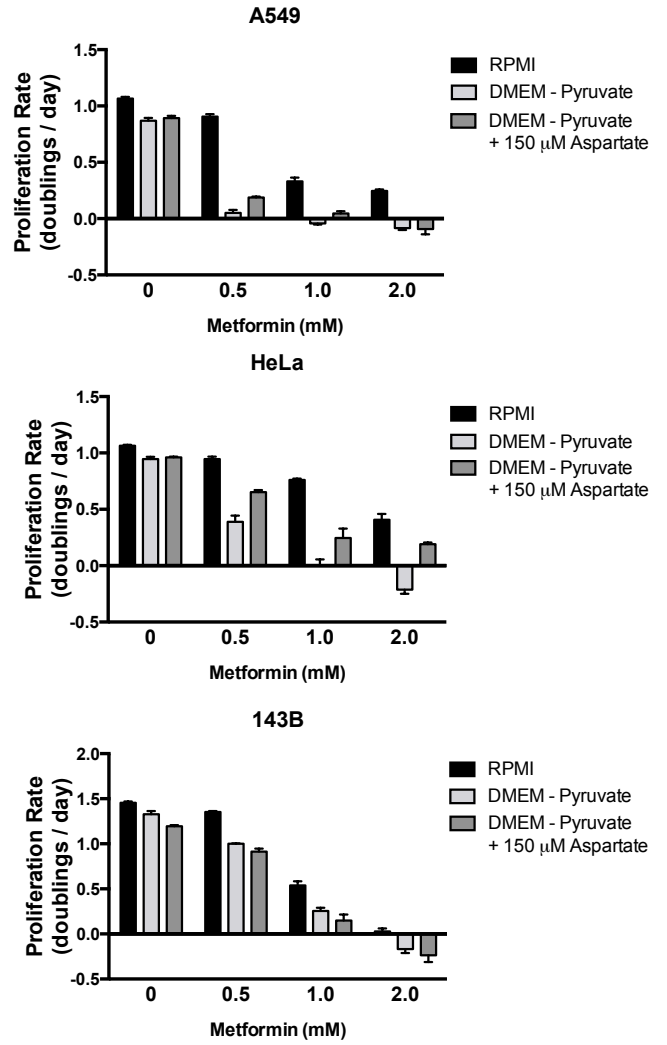
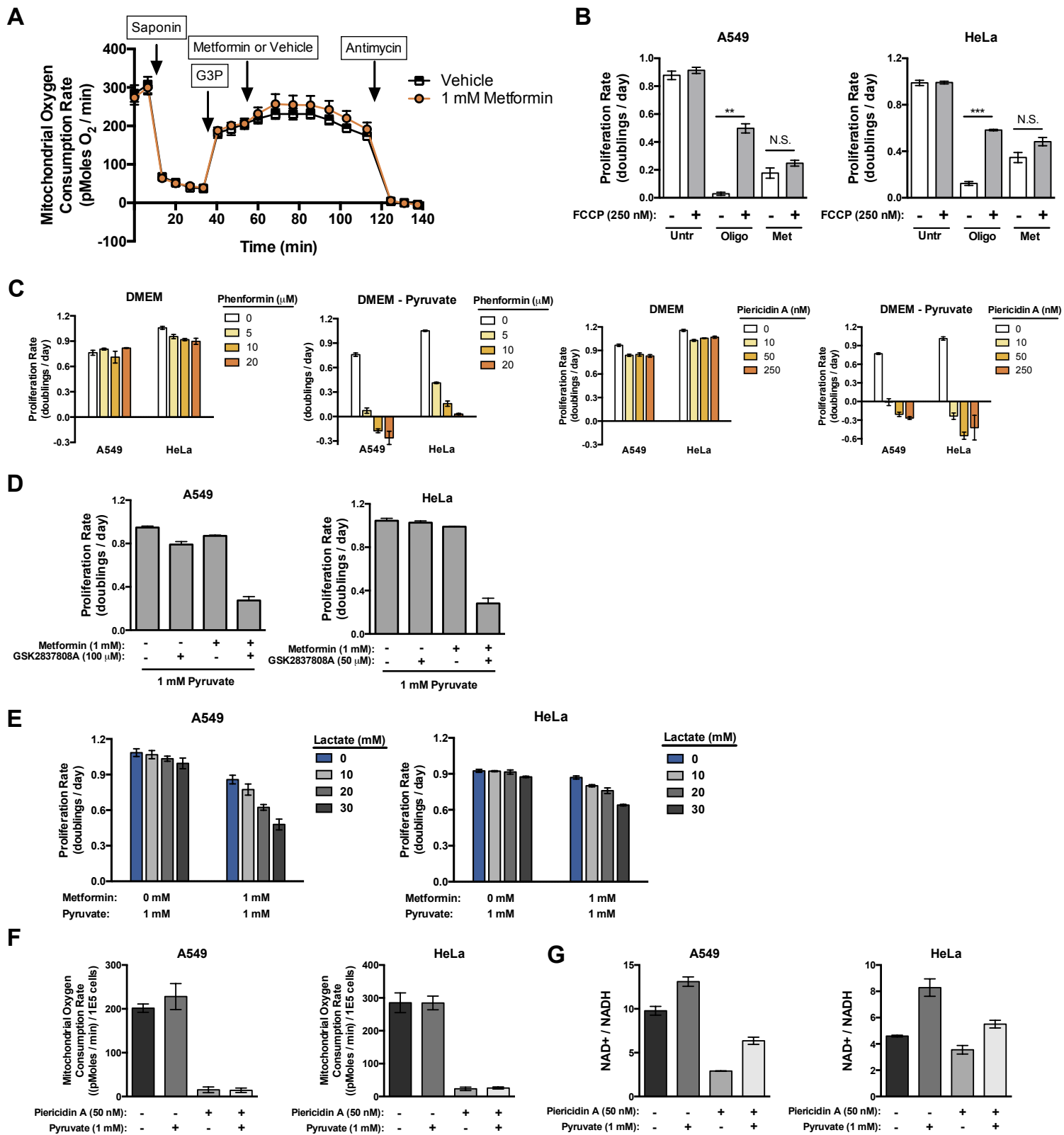
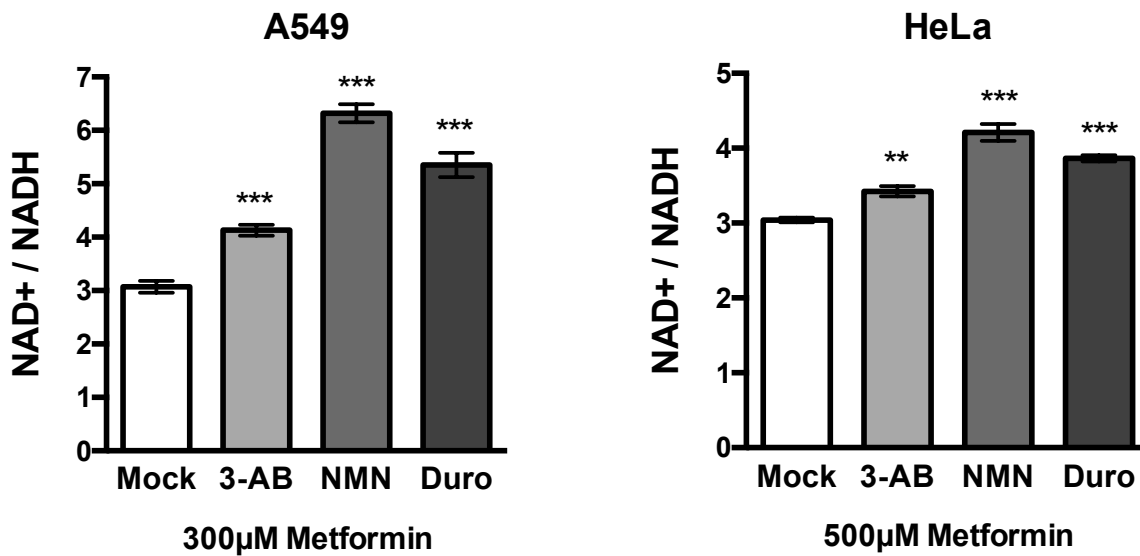


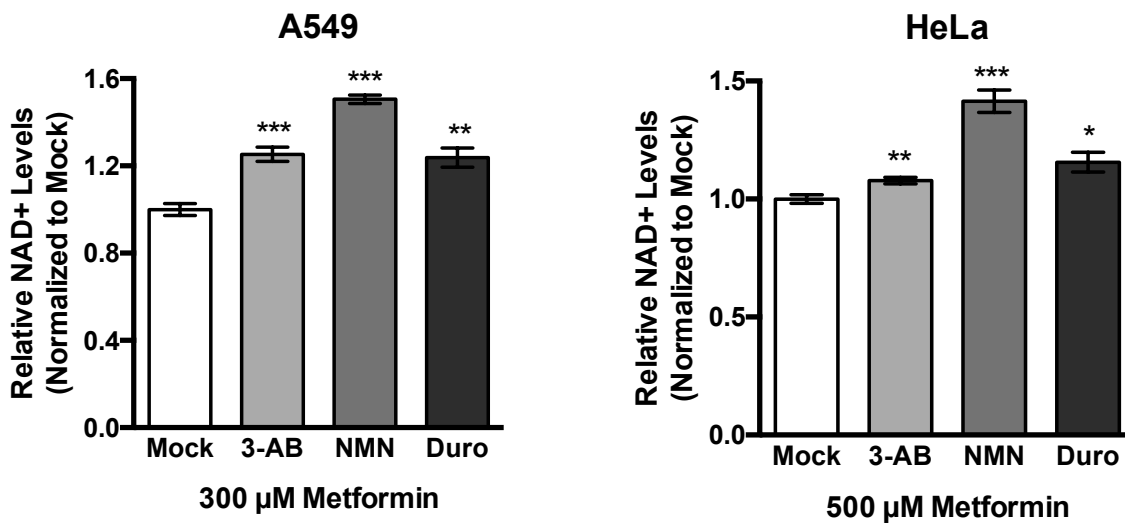
Figure S2



A



B



C

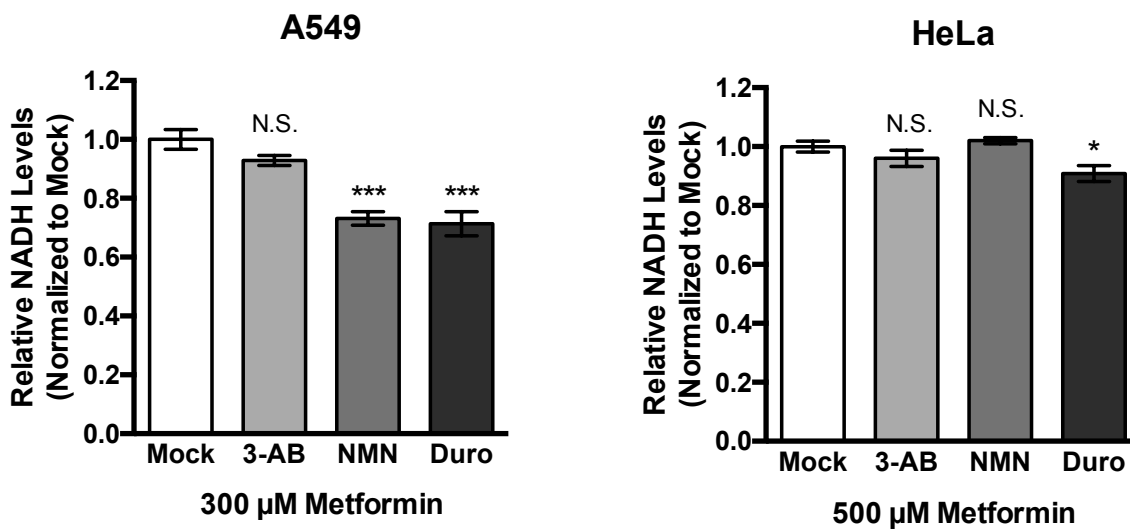
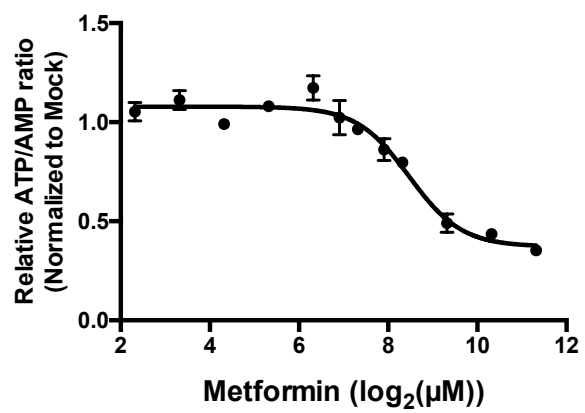
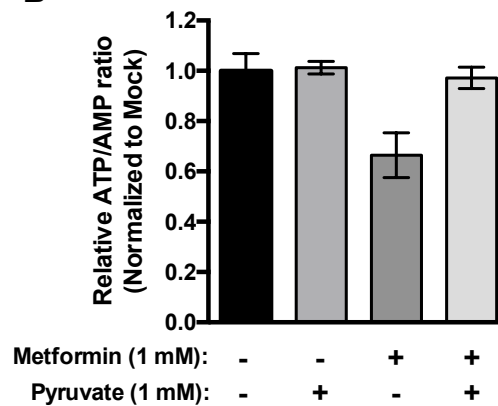


Figure S4

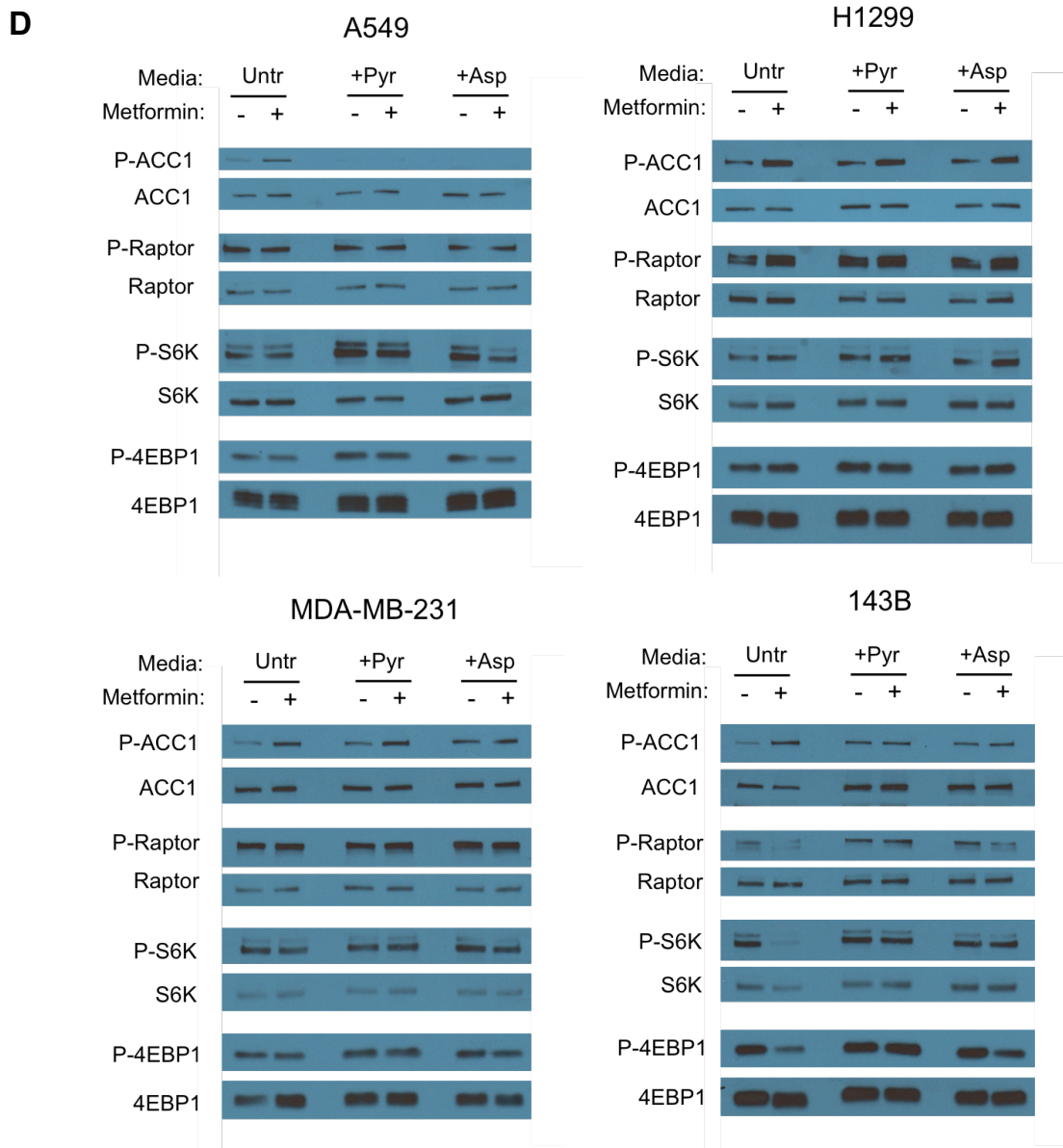
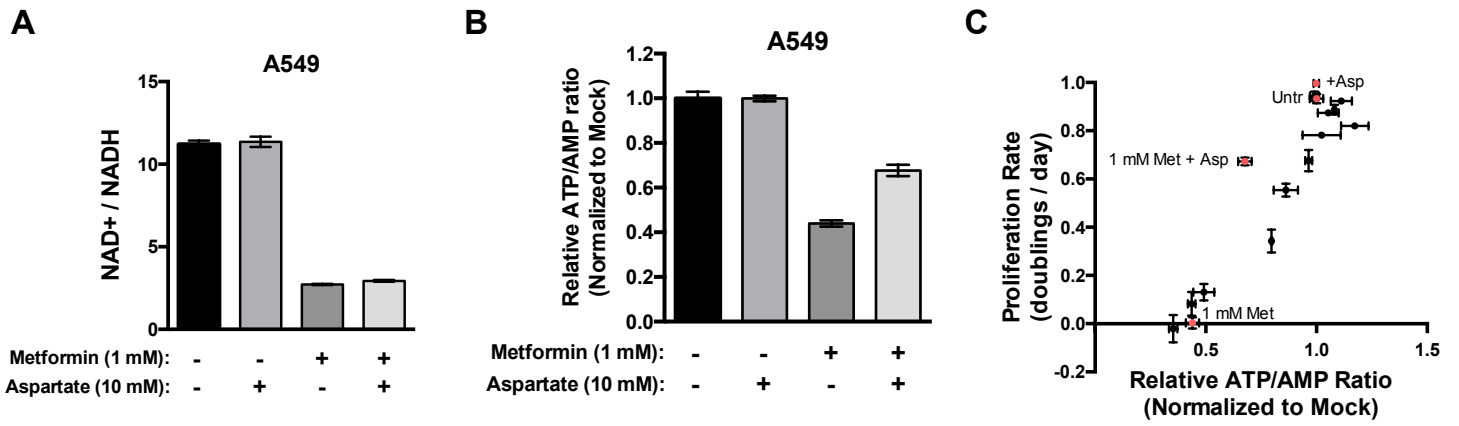
A



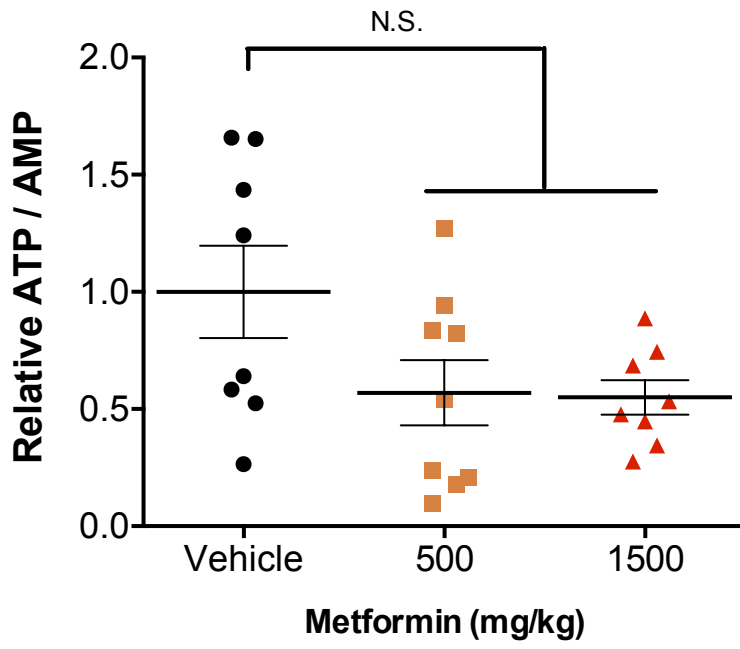
B



**Figure S5**



A



## Supplementary Figure Legends

### **Figure S1. The antiproliferative effect of metformin is influenced by media composition, related to Figure 1.**

Proliferation rates for A549, HeLa, and 143B cells in media treated with the indicated concentrations of metformin. Cells were cultured in DMEM without pyruvate or RPMI 1640 with the indicated concentration of glucose (A) or with addition of RPMI levels of aspartate (150 $\mu$ M) (B). Values denote mean  $\pm$  SEM.  $n=3$ .

### **Figure S2. Conversion of pyruvate to lactate is required to restore proliferation upon complex I inhibition, related to Figure 2.**

(A) Mitochondrial oxygen consumption of A549 cells following permeabilization by saponin, addition of glycerol-3-phosphate (G3P), addition of either metformin or vehicle and addition of antimycin as indicated. (B) Proliferation rates for A549 and HeLa cells treated with oligomycin or metformin in the presence or absence of FCCP. (C) Proliferation rates for A549 and HeLa cells in DMEM or DMEM without pyruvate treated with the indicated doses of the complex I inhibitors phenformin or piericidin A. (D) Proliferation rates for A549 and HeLa cells cultured in DMEM with pyruvate treated as indicated with metformin and the LDH inhibitor GSK2837808A. (E) Proliferation rates for A549 and HeLa cells with and without metformin in the presence of the indicated amounts of pyruvate and lactate in the media. (F) Mitochondrial oxygen consumption rates for A549 and HeLa cells treated with the indicated concentrations of piericidin A and pyruvate. (G) Intracellular NAD<sup>+</sup>/NADH ratios of A549 and HeLa cells treated with the indicated concentrations of piericidin A and pyruvate. Values denote mean  $\pm$  SEM.  $n=5$  (A,F),  $n=3$  (B-E, G). \* $p < 0.05$ , \*\* $p < 0.01$ .

### **Figure S3. Treatment with compounds that alter NAD<sup>+</sup>/NADH metabolism result in altered NAD<sup>+</sup>/NADH ratio, related to Figure 3.**

(A) Intracellular NAD<sup>+</sup>/NADH ratios of A549 and HeLa cells treated with 3-aminobenzamide (3-AB), nicotinamide mononucleotide (NMN), and duroquinone (duro). Normalized intracellular levels of NAD<sup>+</sup> (B) and NADH (C) in A549 and HeLa cells treated with 3-AB, NMN, and duro. Values denote mean  $\pm$  SEM.  $n=3$ . \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

### **Figure S4. ATP/AMP ratio is affected by metformin treatment, related to Figure 4.**

(A) Intracellular ATP/AMP ratios were measured after treatment with metformin at the indicated doses in A549 cells. (B) Intracellular ATP/AMP ratios were measured in A549 cells with the indicated doses of metformin and pyruvate. Values denote mean  $\pm$  SEM.  $n=3$ .

**Figure S5. Aspartate suppression of the anti-proliferative effects of metformin is downstream of changes in cellular NAD<sup>+</sup>/NADH, and does not correlate with AMPK or mTORC1 signaling, related to Figure 5.**

Intracellular NAD<sup>+</sup>/NADH ratio (A) and ATP/AMP ratio (B) in A549 cells treated with the indicated doses of metformin and aspartate. (C) Proliferation rate and relative ATP/AMP ratios of A549 cells were plotted independent of treatment condition, cells treated with the indicated concentration of metformin and aspartate are highlighted in red. (D) Western blot for P-ACC, P-Raptor, P-S6K, P-4EBP1 from cell lysates from A549, H1299, MDA-MB231, and 143B cells treated with metformin in the presence or absence of either pyruvate or exogenous aspartate. Values denote mean  $\pm$  SEM.  $n=3$  (A-C).

**Figure S6. Intratumoral ATP/AMP ratios following metformin treatment, related to Figure 6.**

Relative intratumoral ATP/AMP ratio was measured following 10 days of vehicle or metformin treatment at the indicated dose with tissue harvested 2 hours after the last dose. N.S. indicates  $p > 0.05$ .