# Metformin prevents NGF-dependent proliferative and pro-angiogenic effects in epithelial ovarian cancer cells and endothelial cells

| A2780 | | B | N | M | M+N | GW | Ab | GW+N | Ab+N |
|---|---|---|---|---|---|---|---|---|---|
| G0/G1 | Mean | 75.9 | 68.1 | 82.9 | 78.3[c] | 77.8[d] | 76.2[d] | 76.8[d] | 76.9[d] |
| | SEM | 1.4 | 3.5 | 2.2 | 3.5 | 2.0 | 1.6 | 1.7 | 1.4 |
| S | Mean | 11.8 | 16.7 | 5.5[a] | 9.8[c] | 11.4 | 12.3 | 12.0 | 13.5 |
| | SEM | 1.7 | 2.8 | 1.5 | 2.5 | 2.1 | 2.1 | 2.2 | 1.6 |
| G2/M | Mean | 12.3 | 15.2[a] | 11.6 | 11.9 | 10.8[d] | 11.5[c] | 11.2[c] | 9.6[d] |
| | SEM | 0.8 | 0.8 | 1.3 | 1.5 | 0.4 | 0.8 | 0.8 | 0.6 |

HOSE

| G0/G1 | Mean | 82.1 | 79.1[a] | 86.1[a] | 86.1[d] | 82.1[c] | 82.3[c] | 82.6[c] | 84.9[c] |
|---|---|---|---|---|---|---|---|---|---|
| | SEM | 1.0 | 0.5 | 1.2 | 1.2 | 1.0 | 1.1 | 1.0 | 1.0 |
| S | Mean | 8.6 | 8.1 | 5.2[b] | 5.2[d] | 9.0 | 8.7 | 8.7 | 8.0 |
| | SEM | 0.6 | 0.1 | 0.4 | 0.3 | 0.7 | 0.7 | 0.7 | 0.7 |
| G2/M | Mean | 9.3 | 12.8[a] | 8.7 | 8.7[c] | 8.9[c] | 9.0 | 8.7[c] | 7.1[c] |
| | SEM | 0.9 | 0.5 | 0.9 | 0.9 | 0.7 | 1.1 | 0.5 | 0.4 |

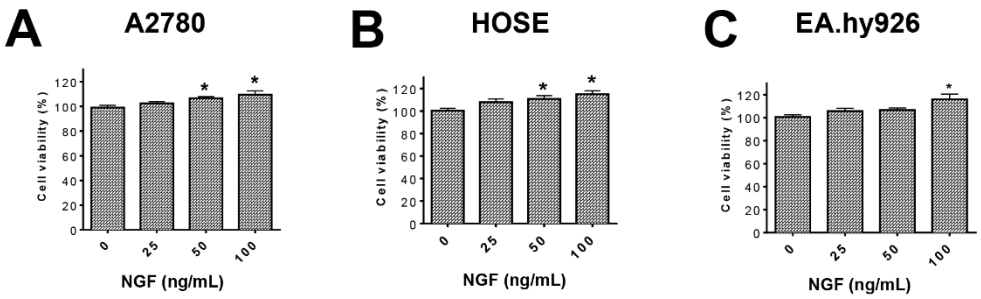
EA.hy926

| G0/G1 | Mean | 80.6 | 77.4 | 84.2 | 83.8[c] | 83.7[c] | 83.7[c] | 87.0[c] | 86.8[d] |
|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | SEM | 1.6 | 1.2 | 2.7 | 2.4 | 2.4 | 2.0 | 1.2 | 1.3 |
| S | Mean | 11.6 | 13.6 | 10.6 | 9.9 | 10.1 | 9.8 | 8.1[c] | 7.9[c] |
| | SEM | 1.3 | 1.2 | 1.8 | 1.6 | 1.6 | 1.2 | 0.2 | 0.5 |
| G2/M | Mean | 7.8 | 9.0[a] | 5.2[a] | 6.3 | 6.2[d] | 6.5[c] | 4.9[d] | 5.3[d] |
| | SEM | 0.6 | 0.3 | 0.9 | 1.1 | 1.0 | 1.0 | 1.0 | 1.0 |

## Table S1. Cell cycle stages of A2780, HOSE and EA.hy926 cells.

Cells were treated with metformin 10 mM (M) for 48 hours or NGF 100 ng/mL (N), TRKA inhibitor GW441756 (GW, 20 nM) or a NGF-neutralizing antibody (Ab, 5 µg/mL) for 6 hours. The percentages of cells in different stages of the cell cycle were measured (N=4). Statistically significant changes are indicated as a= $p<0.05$ and b= $p<0.01$ respect to basal condition (B); c=$p<0.05$ and d= $p<0.01$ respect to NGF group.




## Figure S1: NGF increases viability of A2780, HOSE and EA.hy926 cells.

Cells were stimulated with NGF (25, 50 and 100 ng/mL) for 48 hours and then cell viability was evaluated in A2780 (A), HOSE (B) and EA.hy926 (C) cells (N=3, triplicate). Statistically significant changes are indicated as *= $p<0.05$, **= $P<0.01$ and ***= $P<0.001$. Statistical analysis: Kruskal Wallis test.

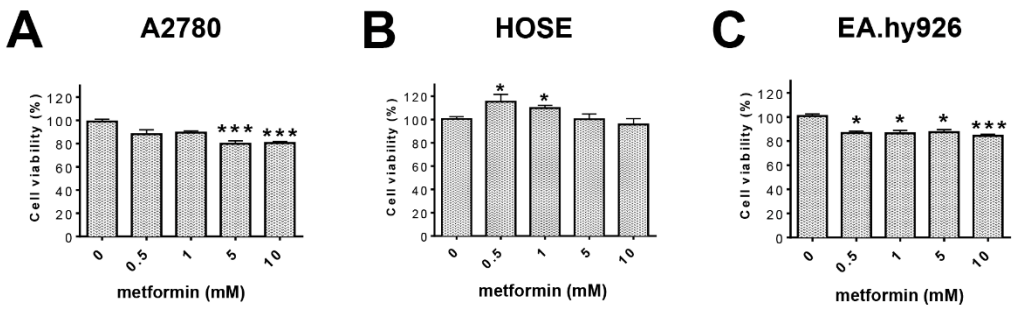



Figure S2: Metformin decreases viability of A2780 and EA.hy926 cells.

Cells were stimulated with NGF (25, 50 and 100 ng/mL) for 48 hours and then viability was evaluated in A2780 (A), HOSE (B) and EA.hy926 (C) cells (N=3, triplicate). B: basal; M0.5: metformin 0.5 mM; M5: metformin 5 mM; N: NGF 100 ng/mL. Statistically significant changes are indicated as *= $p<0.05$, **= $P<0.01$ and ***= $P<0.001$. Statistical analysis: Kruskal Wallis test.

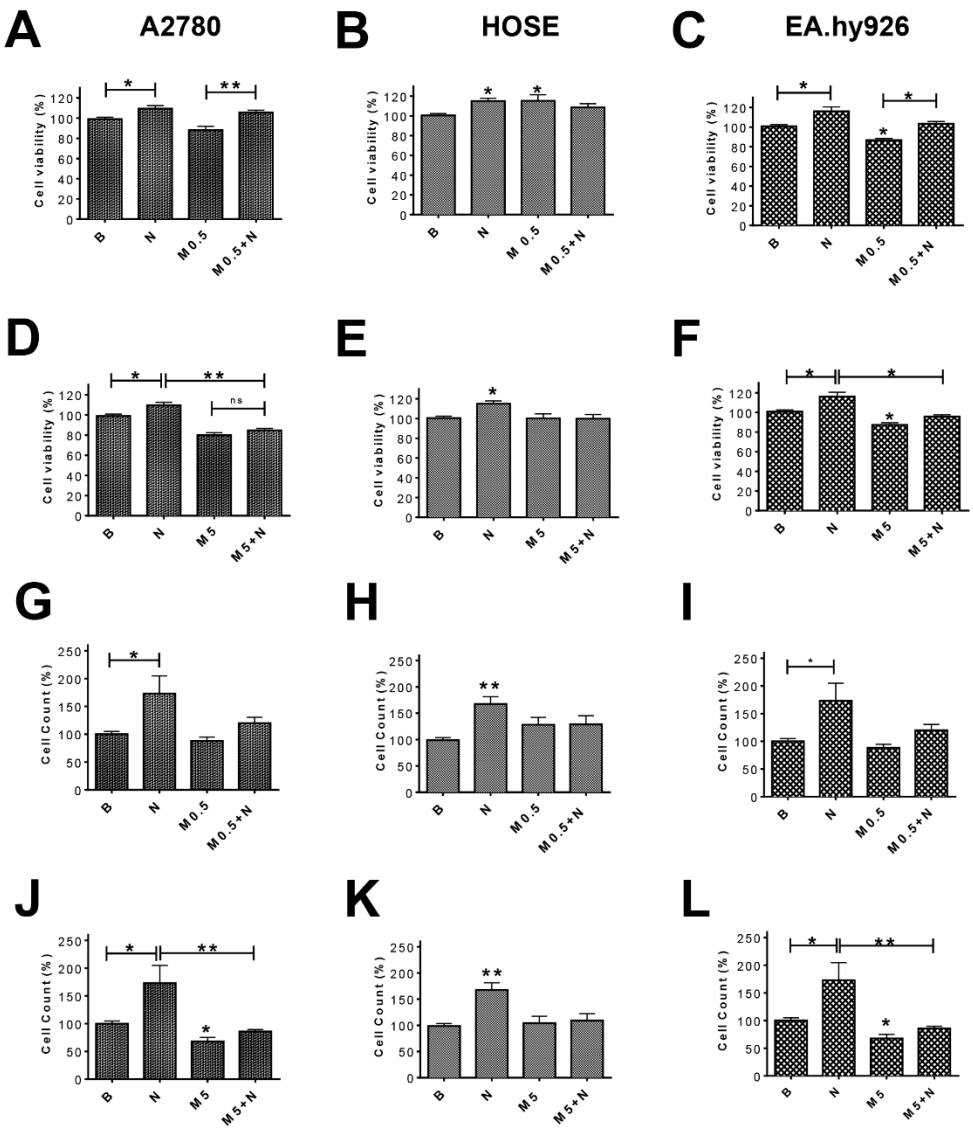
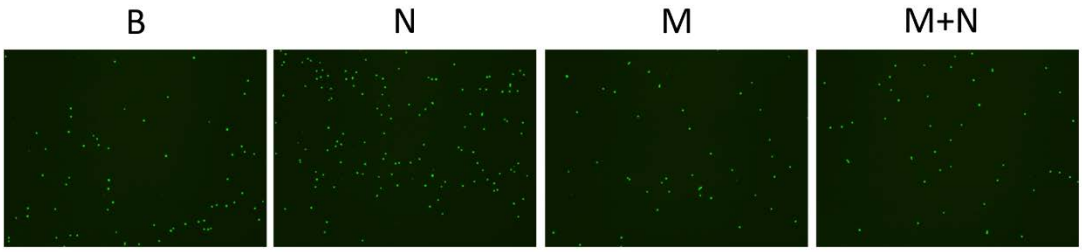


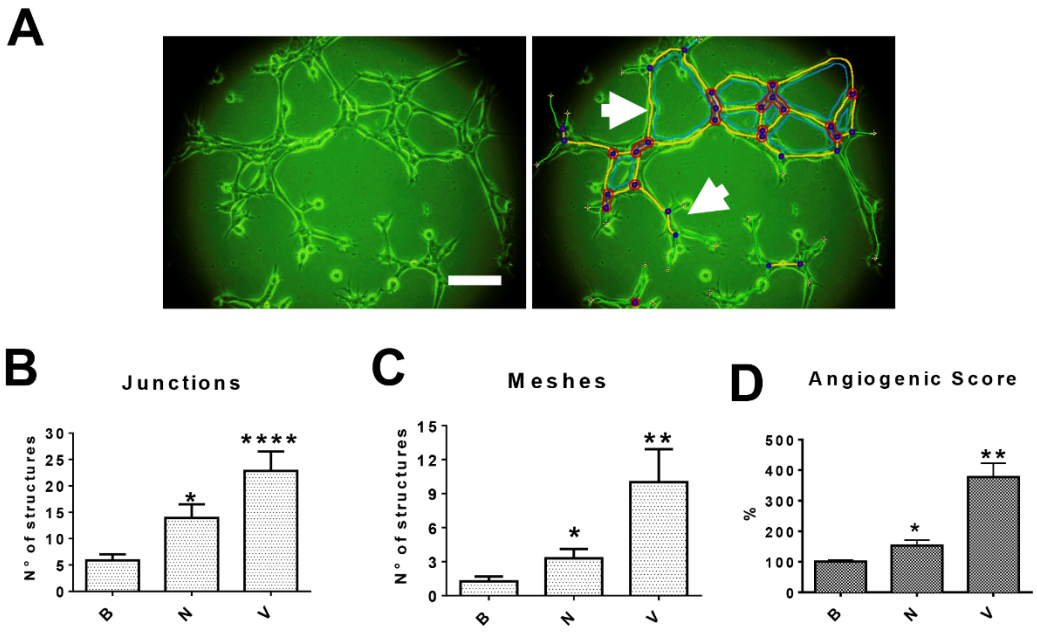

Figure S3: Metformin treatment precludes NGF-enhanced viability and numbers of A2780, HOSE and EA.hy926 cells.

A2780 cells (A,D,G,J), HOSE cells (B,E,H,K) and EA.hy926 cells (C,F,I,L) were treated with metformin (0.5 mM and 5 mM) and NGF (100 ng/mL) for 48 hours. (N=3, triplicate). B: basal; M0.5: metformin 0.5 mM; M5: metformin 5 mM; N: NGF 100 ng/mL. Statistically significant differences are indicated as *= $p<0.05$ **= $p<0.01$ and ***= $p<0.001$. Statistical analysis: Kruskal Wallis test.




## Figure S4. Metformin treatment precludes NGF-enhanced A2780 cell numbers

Representative images of A2780 cells treated with metformina 10 mM (M) and NGF 100 ng/mL (N) for 48 hours are shown. Cells were counted after Acridine Orange & Propidium Iodide staining. B: basal condition. Green dots indicate live cells in each condition.




## Figure S5: NGF and VEGF enhanced the angiogenic score of EA.hy926 cells.

Cells seeded in fresh medium were stimulated with NGF (100 ng/mL) or the positive control VEGF (20 ng/mL) and then incubated on matrigel-covered plates for 8 hours. A: Representative images after analysis by Image J Angiogenesis Analyzer, which highlight cell rows as blue dots and where light blue circles correspond to junctions (B), polygonal structures or meshes (C) respectively. D: Angiogenic score of EA.hy926 cells. (8 images per group; N=3 for VEGF group and N=4 for rest of groups). Statistically significant

differences are indicated as *= $p<0.05$; **= $p<0.01$ ****= $p<0.0001$, according to Kruskal Wallis test. Magnification bar = 50 μm.