Supplementary Material

The tumor suppressor 5A2, a synthetic miR-7-5p mimic, targets oncogenic and metabolic pathways, as revealed by transcriptome-wide analysis

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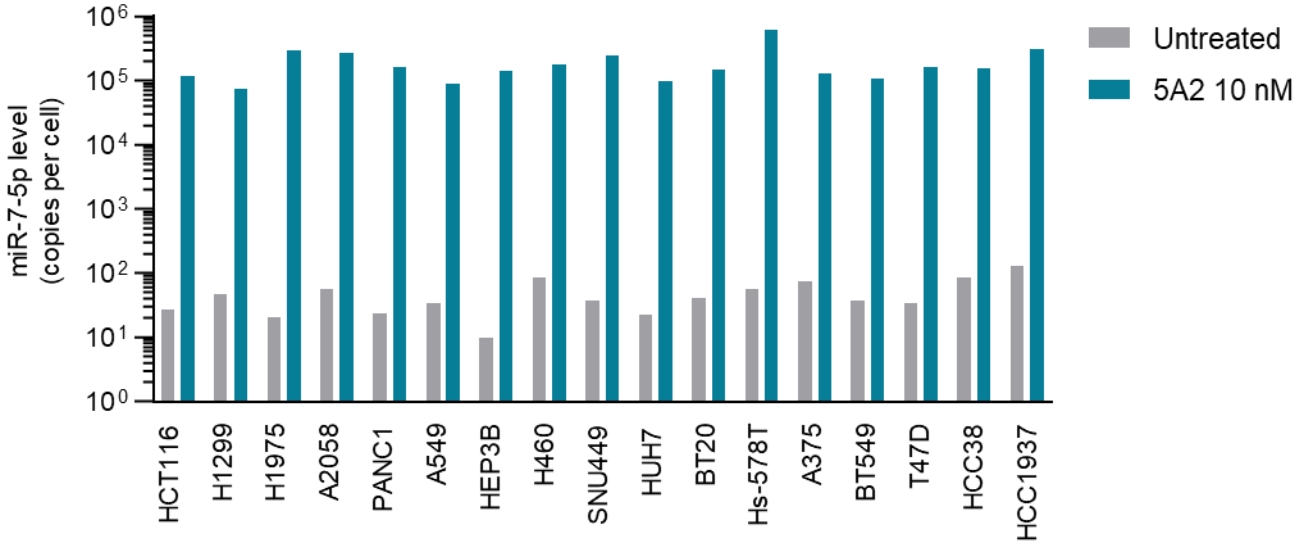
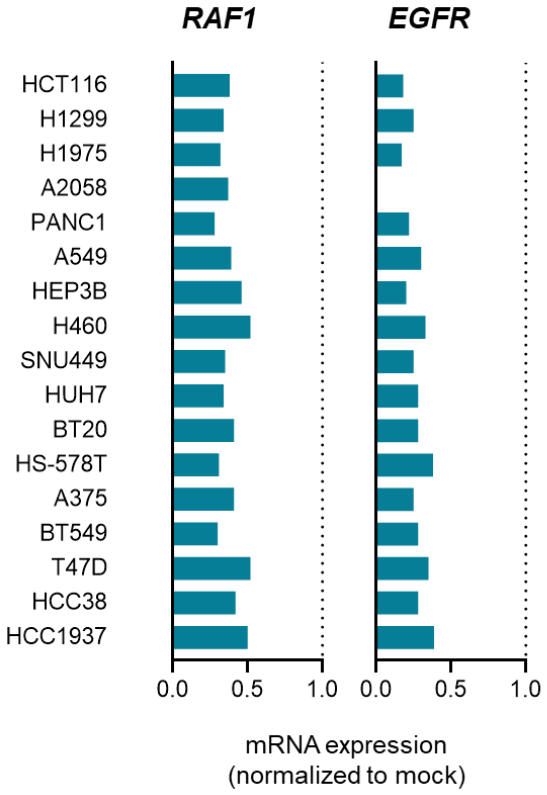
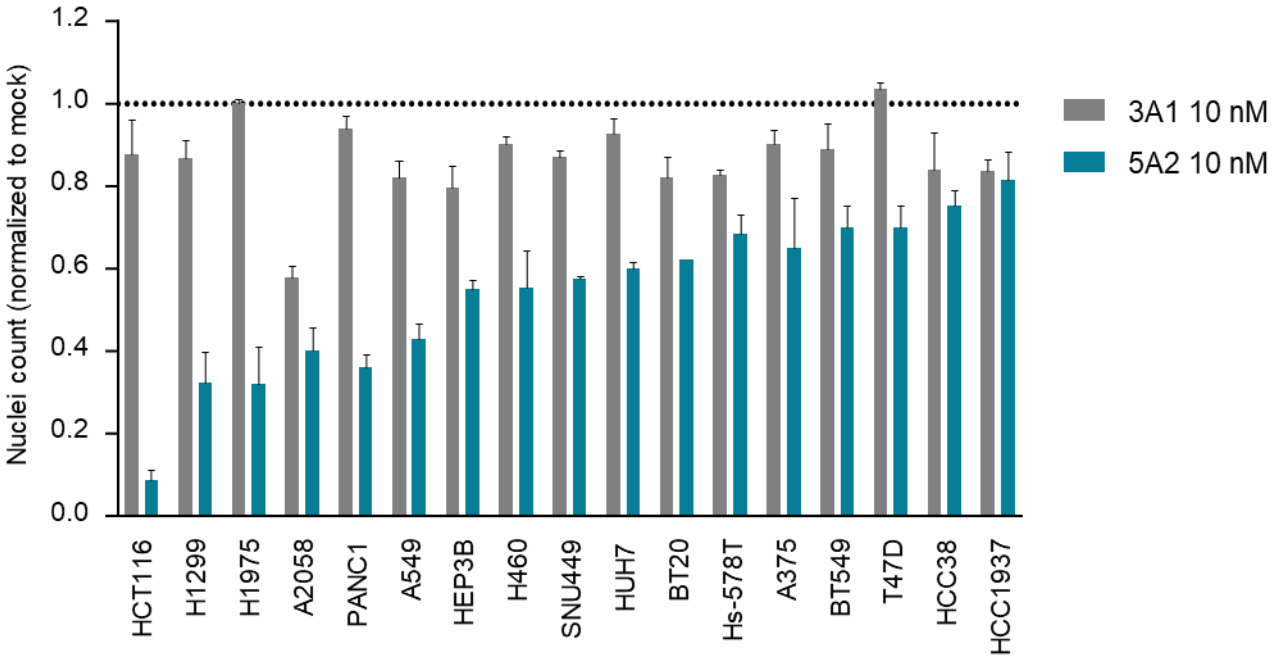


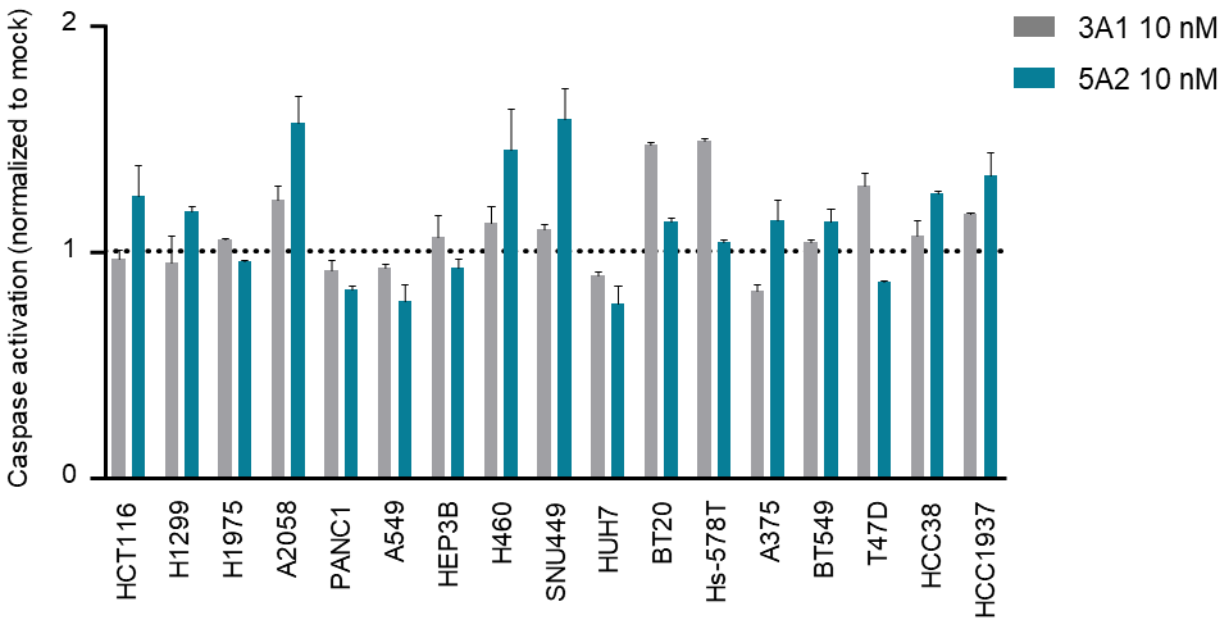
Supplementary figure 1: Effect of 5A2 transfection on 5A2 levels in a range of human cancer cell lines. Cells were transfected with 10 nM 5A2. Non-transfected cells were also included to define endogenous miR-7-5p levels. RNA was harvested after 24 h and 2-tailed q-RTPCR to detect 5A2/miR-7-5p was performed. The y-axis is labeled 'miR-7-5p' but it should be assumed that this also includes 5A2 in the transfected conditions given that the 5A2 guide strand sequence is identical to miR-7-5p. Bars show the mean of triplicate reactions from one biological replicate.



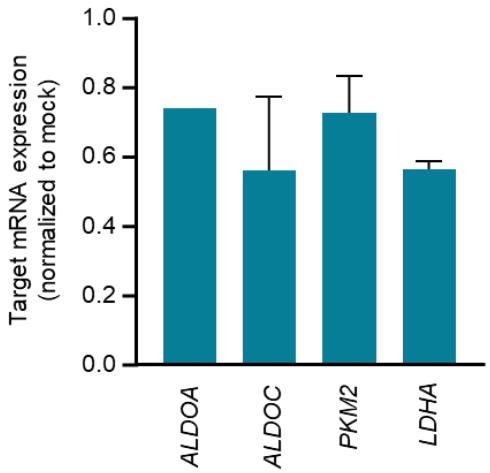
Supplementary Figure 2: Effect of 5A2 transfection on *RAF1* and *EGFR* mRNA expression in a range of human cancer cell lines. Cells were transfected with 10 nM 5A2. Non-transfected cells (mock) were also included to define baseline readout. RNA was harvested after 24 h and qRT-PCR was used to detect *RAF1* and *EGFR* mRNA levels. Values were normalized to mock (dashed line) and show the mean of triplicate reactions from one biological replicate.



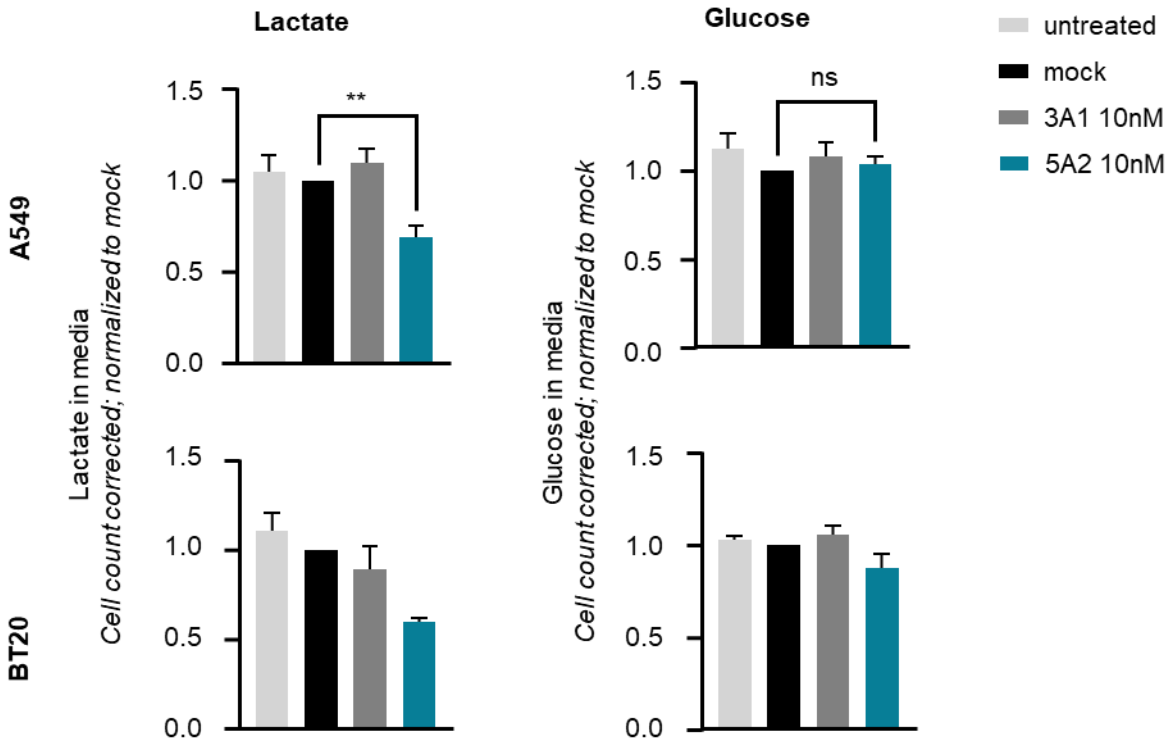
<u>Supplementary figure 3</u>: Effect of 5A2 and 3A1 on cell proliferation in a panel of human cancer cell lines. Cells were transfected with either 10 nM 5A2 or negative control miRNA 3A1. Non-transfected cells (mock) were also included to define baseline readout. Nuclei count was determined 96 h after transfection by staining with Hoeschst-33342 and imaging. Values were normalized to mock (dashed line). Values are the mean of at least three independent replicates and error bars show standard error of the mean.



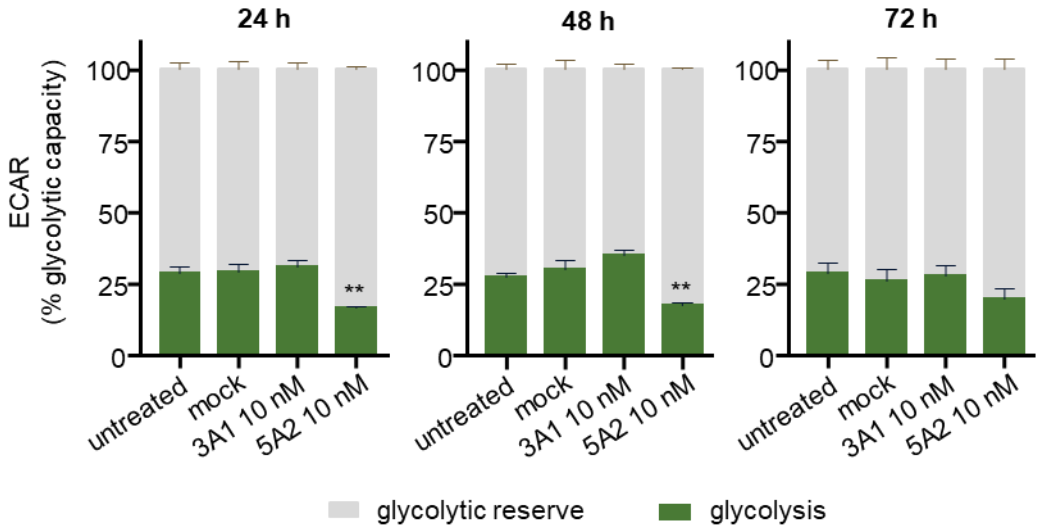
<u>Supplementary figure 4</u>: Effect of 5A2 and 3A1 on caspase 3/7 activation in a panel of human cancer cell lines. Cells were transfected with either 10 nM 5A2 or negative control miRNA 3A1. Non-transfected cells (mock) were also included to define baseline readout. Caspase activation was measured 72 h after transfection. Values were normalized to mock (dashed line). Values are the mean of at least three independent replicates and error bars show standard error of the mean.



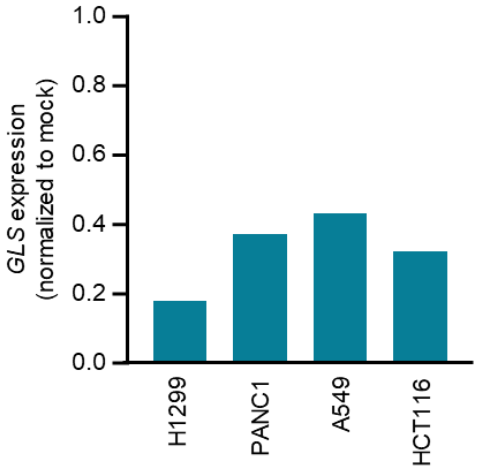
Supplementary figure 5: Effect of 5A2 on selected glycolysis target mRNA expression in A549 cells. A549 cells were transfected with 10 nM 5A2. Non-transfected cells (mock) were also included to define baseline readout. RNA was harvested after 24 h and qRT-PCR was used to detect *ALDOA, ALDOC, PKM2, and LDHA* mRNA levels. Values were normalized to mock and show the mean of triplicate reactions from two biological replicates (except *ALDOA,* which shows one biological replicate). Error bars show the standard deviation of the mean.



<u>Supplementary figure 6:</u> Effect of 5A2 and negative controls on glucose and lactate levels. Cells transfected with 10 nM 5A2 or negative control miRNA 3A1 were seeded 24h before the assay. Non-transfected (mock) and untreated cells were included to define baseline readout. Media was aspirated 24 h later (after 72 h (A549) or 96 h (BT20) transfection) to measure glucose and lactate levels. Cells were then fixed and imaged to count cells. Glucose and lactate levels were corrected for cell proliferation differences using this cell count. Values were normalized to mock-transfected cells. A549 data are the mean of three independent replicates and error bars show standard error of the mean (***p* < 0.01). BT20 data are the mean of two independent replicates and error bars show the standard deviation.



<u>Supplementary figure 7:</u> Effect of 5A2 and negative controls on glycolysis. A549 cells were transfected with 5A2 or negative control miRNA 3A1. Non-transfected cells (mock) and untreated cells were included to define a baseline readout. Cells were seeded 24h before the assay. The extracellular acidification rate (ECAR), an indicator of glycolysis, was measured using the Seahorse FX96 extracellular Flux analyzer. Shown is the mean of three independent replicates and error bars show the standard deviation of the mean (**$p < 0.01$).



<u>Supplementary figure 8</u>: Effect of 5A2 on *GLS* target mRNA expression in cancer cell lines. H1299, PANC1, A549, and HCT116 cells were transfected with 10 nM 5A2. Non-transfected cells (mock) were also included to define baseline readout. RNA was harvested after 24 h. qRT-PCR was used to detect *GLS* mRNA level. Values were normalized to mock and show the mean of triplicate reactions.