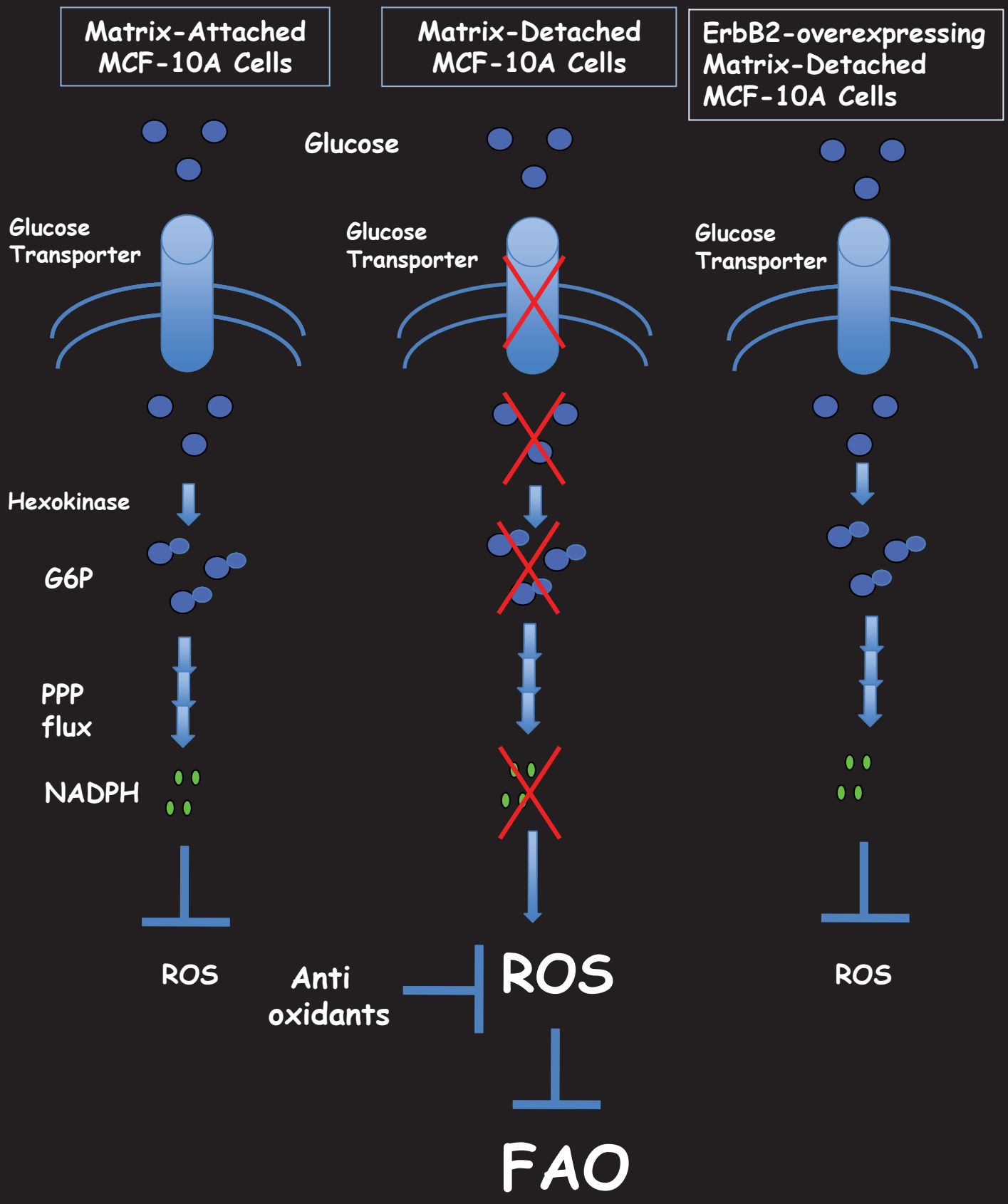
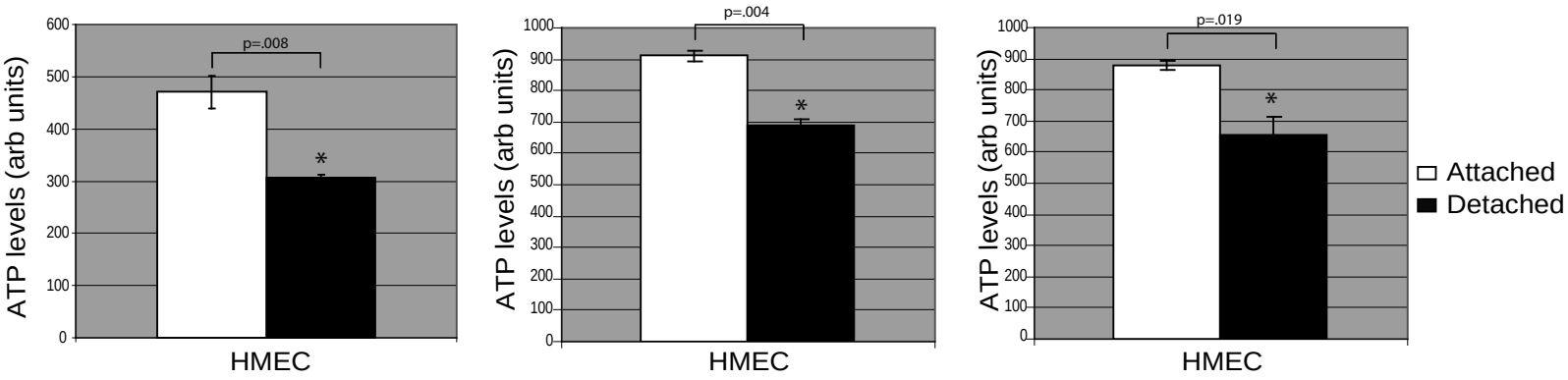
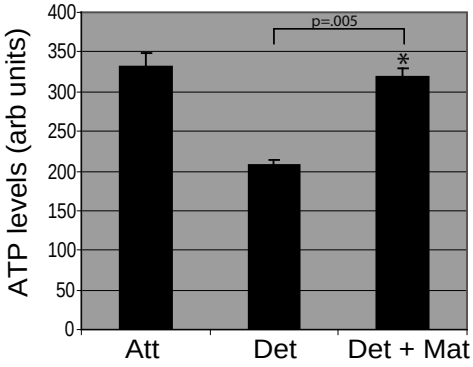


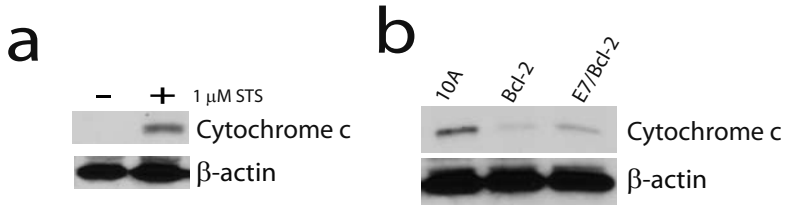
Supplementary Figure 1. Schematic representation of ROS regulation by ECM. ECM-detachment suppresses glucose uptake, preventing production of the antioxidant NADPH through the pentose phosphate pathway. This leads to an increase in ROS, which suppresses fatty acid oxidation (FAO), and reduces ATP. ErbB2 rescues glucose uptake in matrix-detached cells leading to production of NADPH and suppression of ROS. Inhibitors of G6PDH prevent the production of NADPH and lead to an increase in ROS.



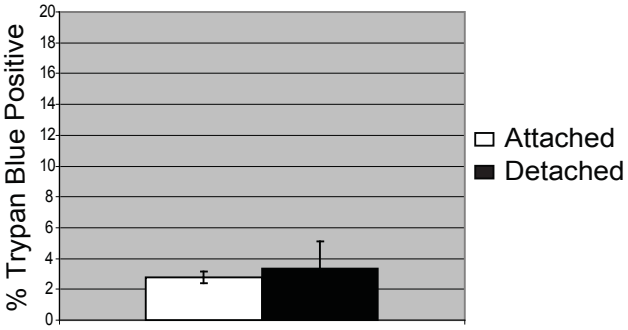
Supplementary Figure 2. Primary HMECs exhibit ATP deficiencies in detached cells. Primary human mammary epithelial cells (HMEC) were plated in either normal (attached) or poly-HEMA coated (detached) plates and after 24 hours ATP was measured using ATPlite assay. Error bars represent standard deviation (n=3). Shown are 3 independent replicates of this experiment. A * represents a statistically significant change calculated using a 2 tailed t test.



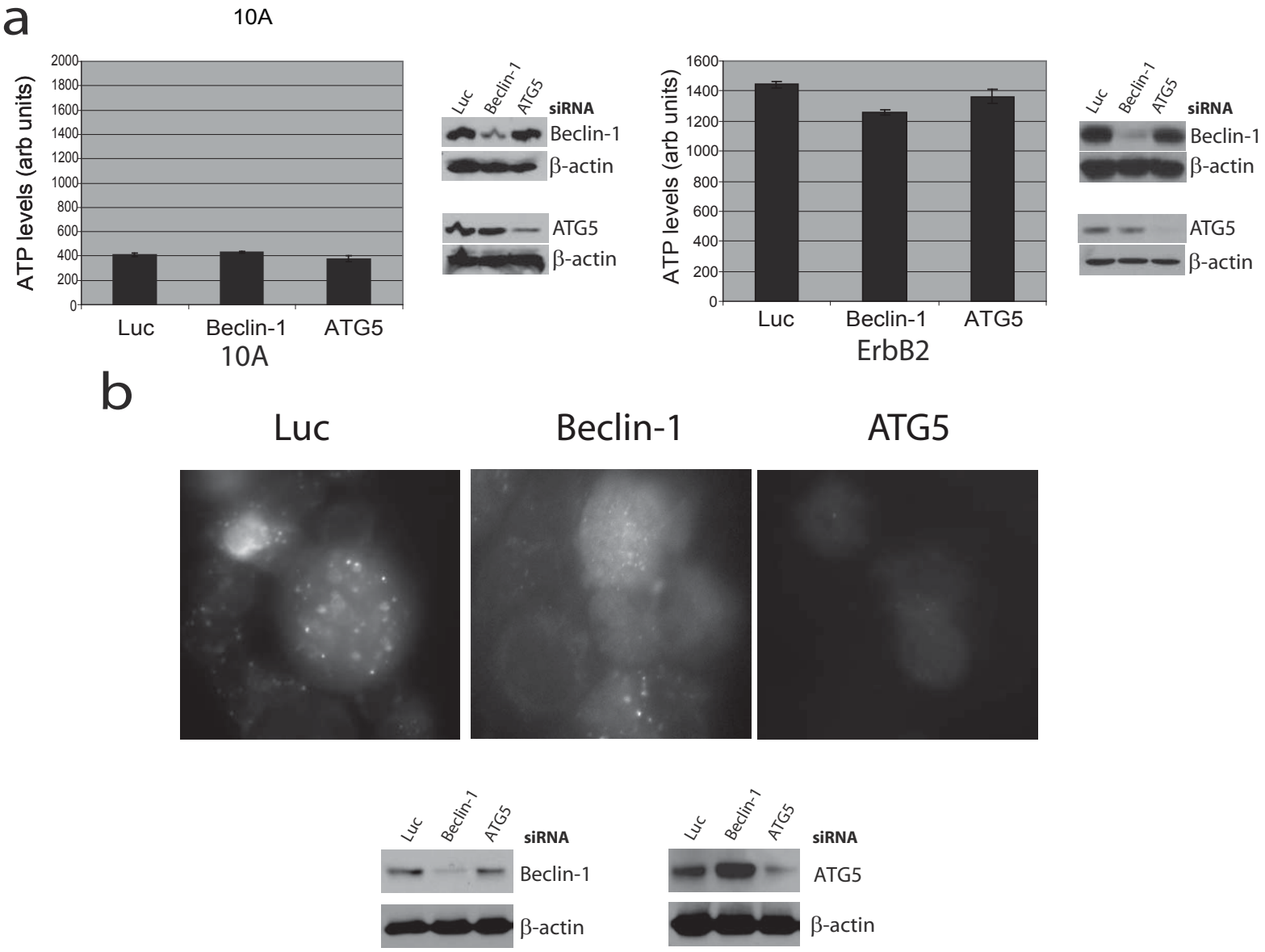
Supplementary Figure 3. Matrigel rescues low ATP levels in detached cells. MCF-10A cells were plated in either normal (attached) or poly-HEMA coated (detached) plates. 1% Matrigel was added where indicated to detached cells. After 24 hours, ATP was measured using ATPlite assay. Error bars represent standard deviation (n=3).



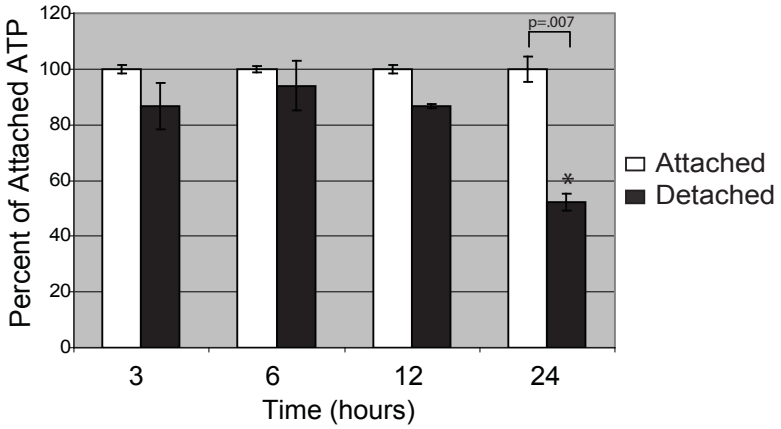
Supplementary Figure 4. Cytochrome c release in detached MCF-10A cells. (a) MCF-10A cells were plated on poly-HEMA coated (detached) plates and after 24 hours, cytochrome c release was measured by immunoblotting in the presence or absence of staurosporine (STS). (b) The indicated cells were plated on poly-HEMA coated (detached) plates and after 48 hours, cytochrome c release was measured by immunoblotting. β-actin was used as a loading control. These results demonstrate that cytochrome c is not released after 24 hours of detachment (STS is a positive control for cytochrome c release). In addition, Bcl-2 effectively inhibits cytochrome c release in Bcl-2-MCF-10A and E7/Bcl-2-MCF-10A cells.



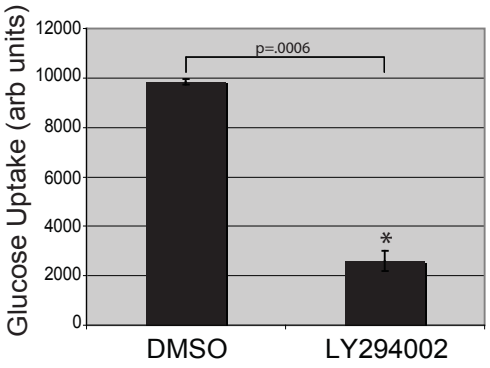
Supplementary Figure 5. ECM detachment does not induce trypan blue positivity at 24 hours. MCF-10A cells were plated on either normal (attached) or poly-HEMA coated (detached) plates and after 24 hours the number of trypan blue positive cells was counted. These results provide evidence that 24 hours of ECM detachment does not induce cell death. Error bars represent standard deviation (n=3).



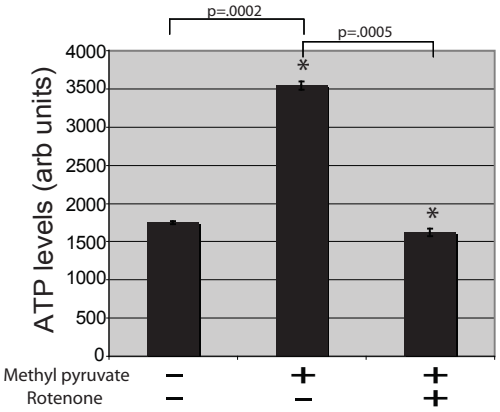
Supplementary Figure 6. Autophagy inhibition does not affect ATP levels in detached cells. (a) Parental MCF-10A or ErbB2-MCF-10A cells were transfected with siRNA SMARTpools targeting either luciferase (luc), Beclin-1, or ATG5. Lysates were generated and knockdown of Beclin-1 or ATG5 was confirmed by immunoblotting. ATP levels were then measured using ATP determination kit. β-actin was used as a loading control. (b) MCF-10A cells expressing GFP-LC3 were transfected with siRNA SMARTpools targeting either luciferase (luc), Beclin-1, or ATG5 and then were plated on poly-HEMA coated plates in Hanks Buffered Salt Solution (HBSS, to induce autophagy). Knockdown was confirmed by immunoblotting and GFP puncta were visualized as an indicator of autophagy. Error bars represent standard deviation (n=3).



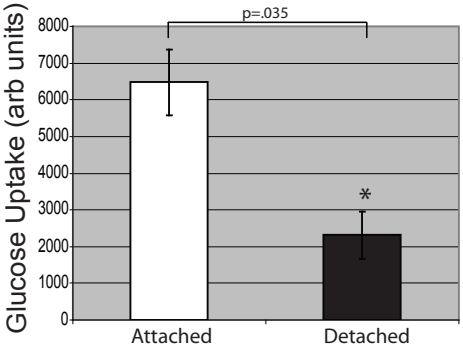
Supplementary Figure 7. Time course of ATP reduction after matrix detachment. MCF-10A cells were plated on normal (attached) or poly-HEMA coated (detached) plates and ATP levels were measured using ATPlite assay at the indicated timepoints. Results are graphed as the percentage of ATP levels in attached cells. A * represents a statistically significant change calculated using a 2 tailed t test. Error bars represent standard deviation (n=3).

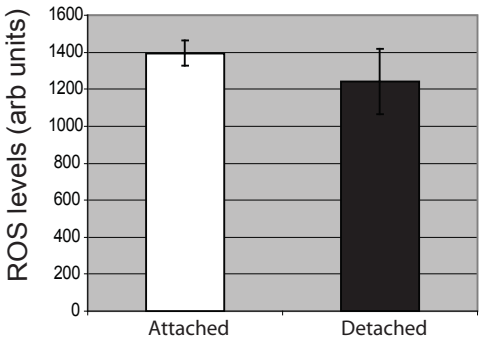


Supplementary Figure 8. Inhibition of the PI(3)K pathway blocks the ability of ErbB2 to rescue glucose uptake. ErbB2-MCF10A cells were plated on poly-HEMA coated plates and treated with 50 μM LY294002. After 24 hours, glucose uptake was measured as described previously. A * represents a statistically significant change calculated using a 2 tailed t test. Error bars represent standard deviation (n=3).

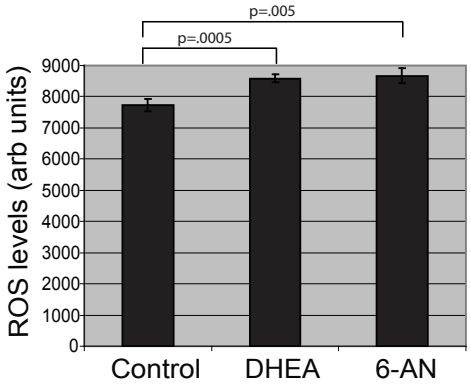


Supplementary Figure 9. Methyl pyruvate rescue is dependent on oxidative phosphorylation. MCF-10A cells were plated on poly-HEMA coated plates and treated with 1 mM methyl pyruvate in the presence or absence of 5 μM rotenone treatment. ATP levels were measured using the ATP determination kit as described in the methods section. A * represents a statistically significant change calculated using a 2 tailed t test. Error bars represent standard deviation (n=3).

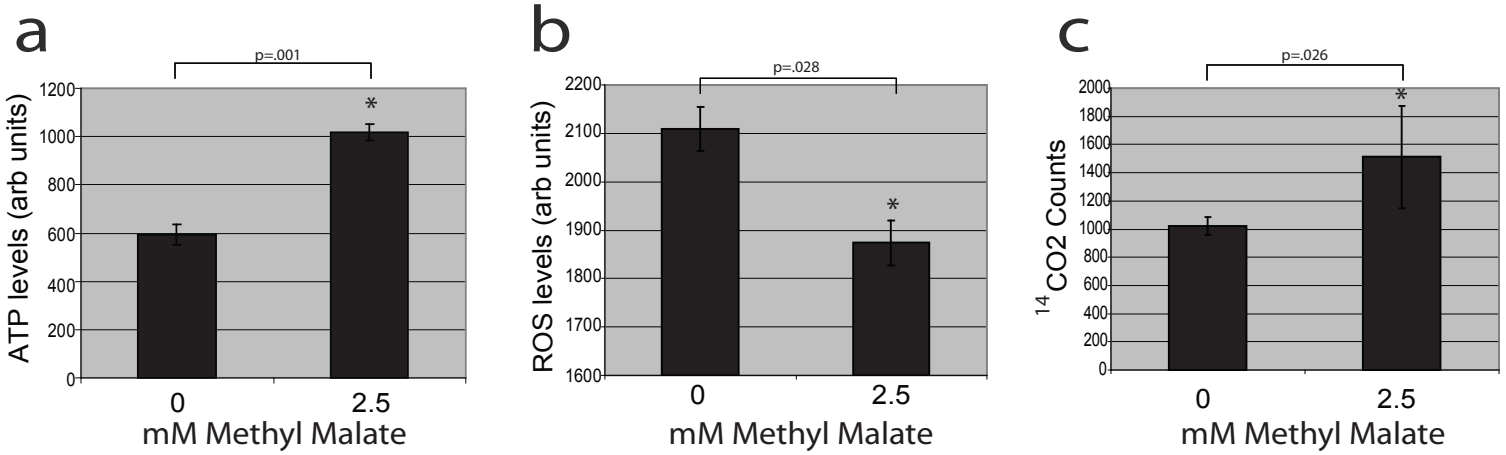




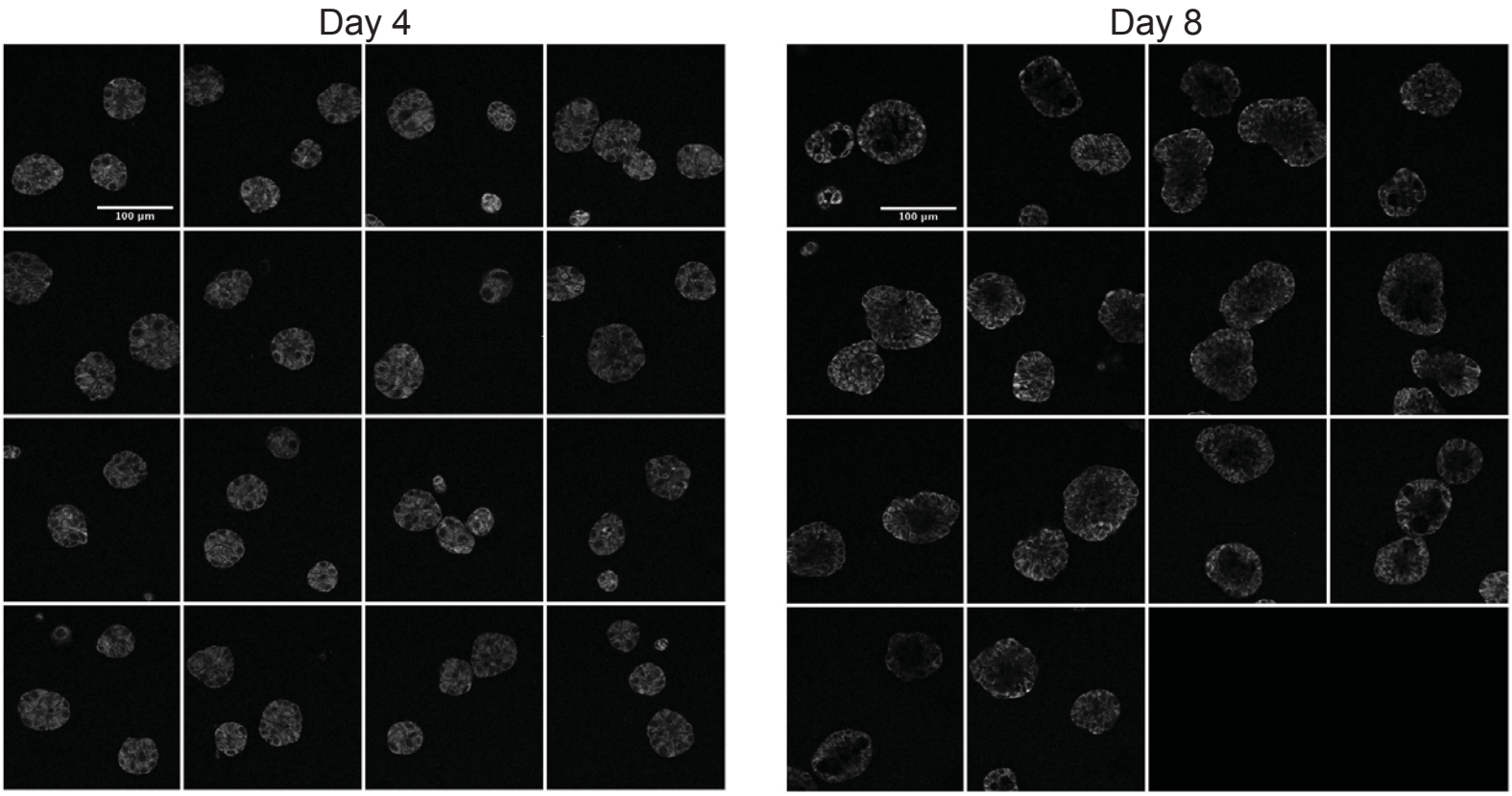
Supplementary Figure 10. Detachment-induced loss in glucose uptake precedes the induction of ROS. MCF-10A cells were plated on either normal (attached) or poly-HEMA coated (detached) plates. After 3 hours, glucose uptake and ROS were measured (using DCF-DA) as described in the methods section. A * represents a statistically significant change calculated using a 2 tailed t test. Error bars represent standard deviation (n=3).



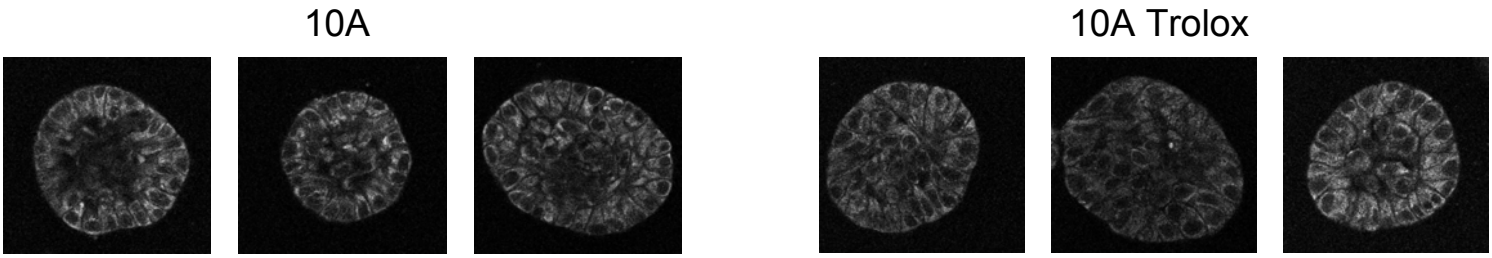
Supplementary Figure 11. PPP inhibition leads to an increase in ROS levels. MCF-10A cells expressing ErbB2 were plated in 96 well poly-HEMA coated plates and treated with either control, 150 μM DHEA, or 150 μM 6-AN. After 24 hours, 10 μM DCF-DA was added and ROS levels were measured on a fluorometer. Error bars represent standard deviation. A * represents a statistically significant change calculated using a 2 tailed t test. Error bars represent standard deviation (n=3).



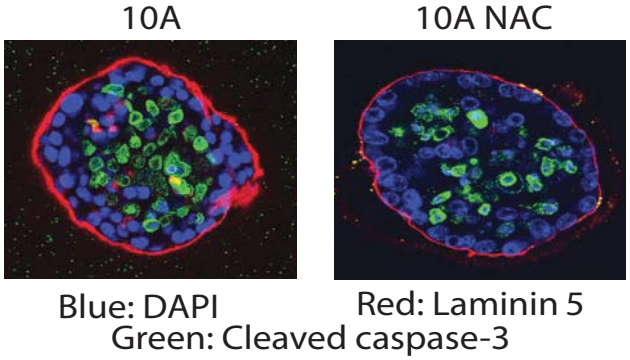
Supplementary Figure 12. Methyl malate treatment elevates ATP levels in detached cells. MCF-10A cells were plated on poly-HEMA coated plates and treated with the indicated dose of methyl malate. After 24 hours, ATP (a), ROS (b, DCF-DA), or fatty acid oxidation (c) were measured as described in methods section. A * represents a statistically significant change calculated using a 2 tailed t test. Error bars represent standard deviation (n=3).



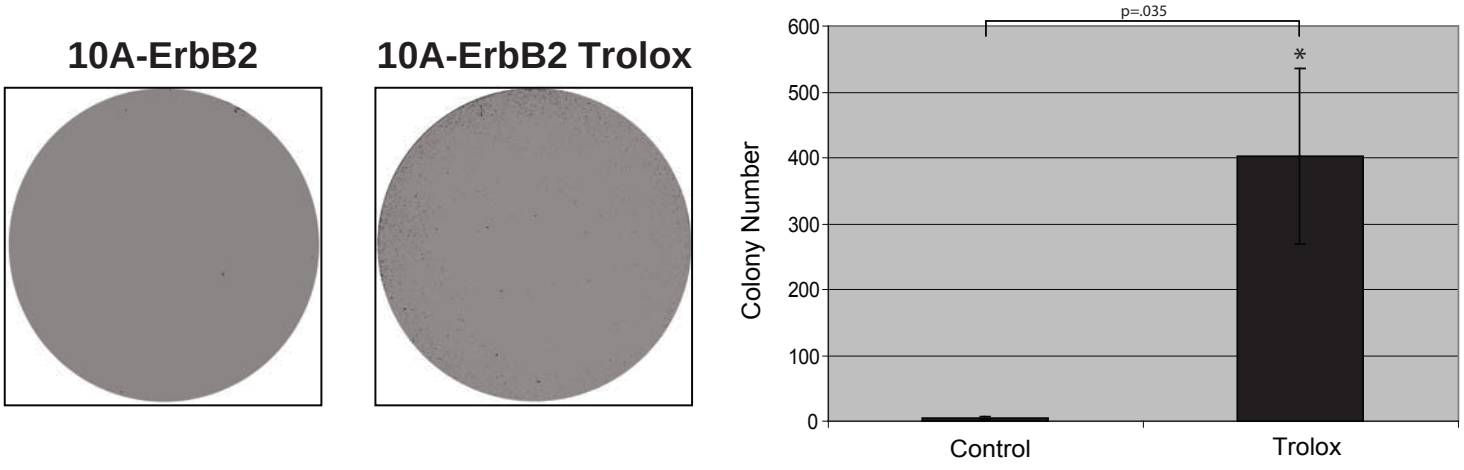
Supplementary Figure 13. Analysis of NAD(P)H fluorescence of MCF-10A structures. Multiple equatorial cross sections of MCF-10A structures were imaged after excitation at 730nm on d4 (left) and d8 (right) after seeding in Matrigel. Most of the cells in the structures from d4 showed relatively homogenous intensity distributions, whereas the majority of structures from d8 showed lower intensities in the inner cells within the structures. The fact that most, but not all, structures on Day 8 show a contrast in fluorescence intensity between the inner and outer cells reflects the asynchronous development among the acinar structures.



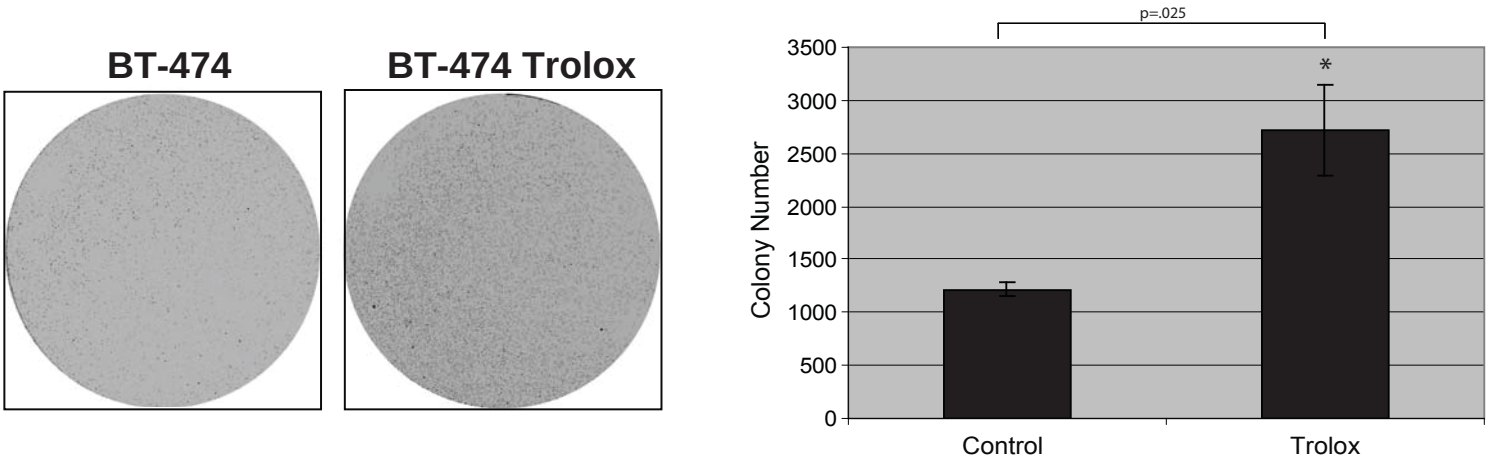
Supplementary Figure 14. Effects of Trolox Treatment of NAD(P)H fluoresence in MCF-10A acini. Representative equatorial cross-sections showing NAD(P)H fluorescence of Day 8 control (left) and Trolox-treated (right, 50 μM) acinar structures. The Trolox-treated structures show substantially less dichotomy between the inner and outer cells than control structures.



Supplementary Figure 15. Antioxidant treatment does not affect caspase activation in MCF-10A acini. MCF-10A cells were cultured in Matrigel to form acini in the presence or absence of 1 mM NAC and after 12 days, acini were fixed and stained for laminin 5, cleaved caspase-3, and with DAPI. Acini were imaged using confocal microscopy and representative pictures were taken of both treated and untreated acini.



Supplementary Figure 16. Antioxidant treatment leads to enhanced colony formation in ErbB2-MCF-10A cells. Cells were plated in soft agar and treated with 50 μM Trolox where indicated. Photos were taken after staining with INT-violet after 30 days. Colony number was then determined using ImageJ. A * represents a statistically significant change calculated using a 2 tailed t test. Error bars represent standard deviation (n=3).



Supplementary Figure 17. Antioxidant treatment leads to enhanced soft agar colony formation in BT-474 cells. BT-474 cells were plated in soft agar and treated with 50 μM Trolox where indicated. Photos were taken after staining with INT-violet after 28 days. Colony number and average colony size were then determined using ImageJ. A * represents a statistically significant change calculated using a 2 tailed t test. Error bars represent standard deviation (n=3).