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Supplemental information

Matrix-driven changes in metabolism support cytoskeletal activity to promote cell migration

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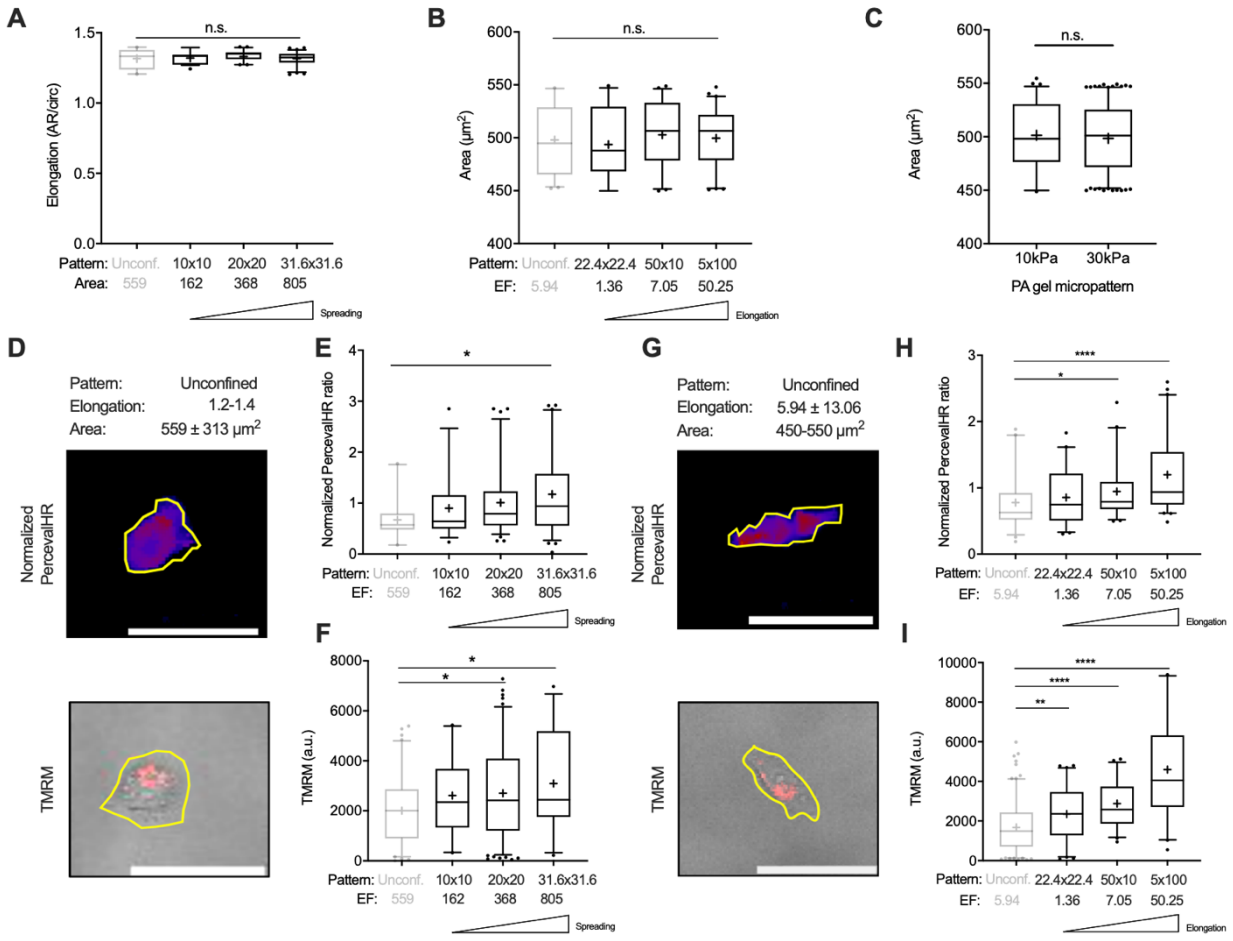
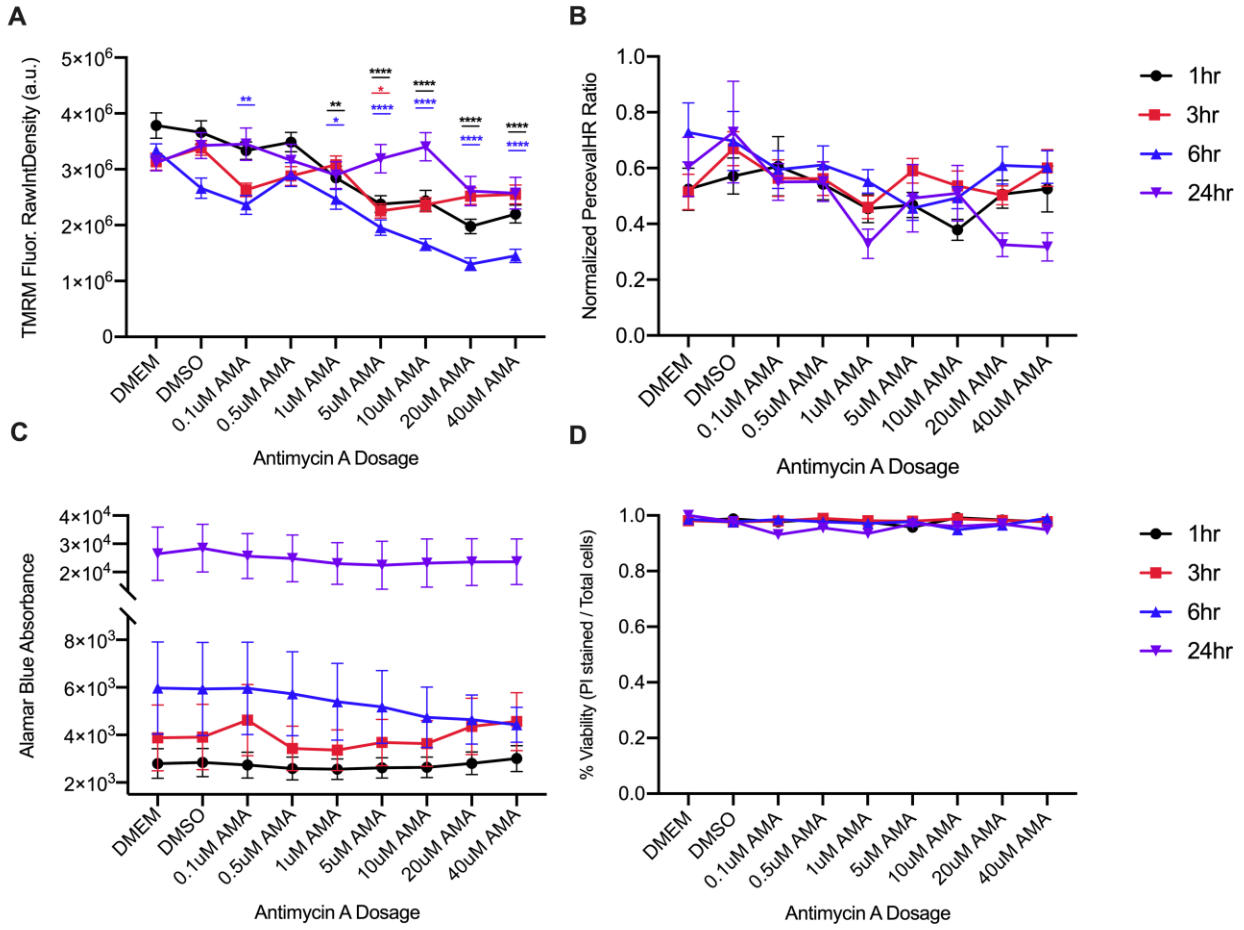
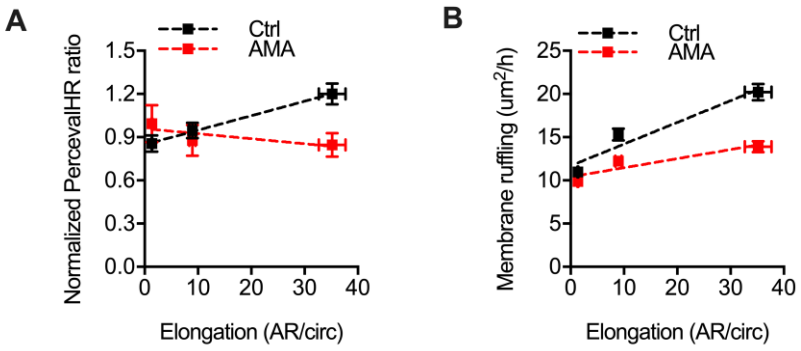
Supplemental figures.



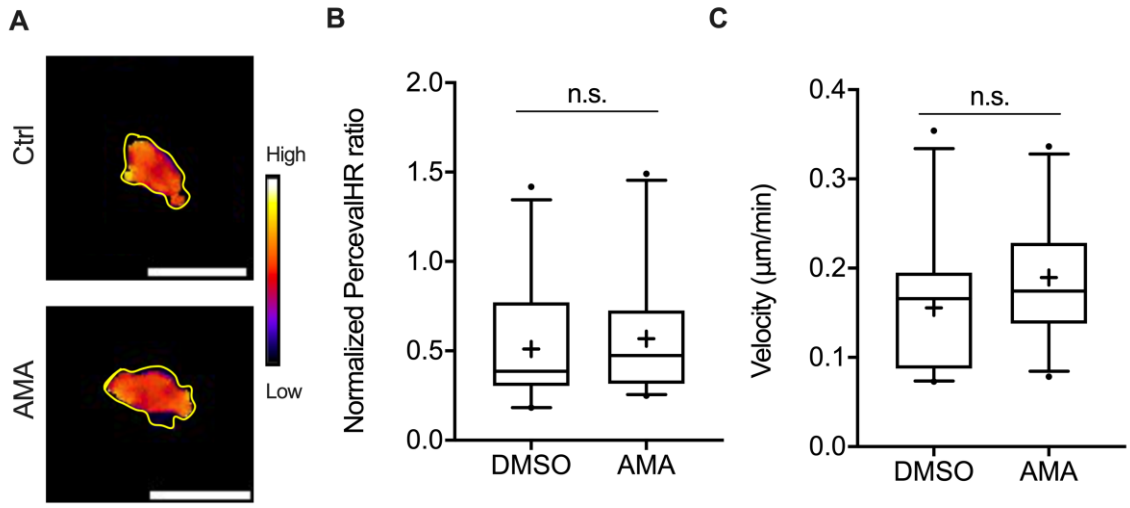
Supplemental Figure 1. Controlling for cell area/elongation and cell confinement across micropattern shapes. Maintaining (**A**) cell elongation factor across micropattern shape with varying cell area for quantification of MDA-MB-231 PercevalHR signal (n = 20-76 cells) and (**B**) cell area across micropattern shape with varying cell elongation (n = 50-64 cells). (**C**) Maintaining cell area across all micropattern shapes of varying gel stiffness (n > 100 cells). (**D**) Normalized PercevalHR ratio and TMRM fluorescence intensity for MDA-MB-231 cells unconfined on collagen micropatterns. Quantification of (**E**) normalized PercevalHR ratio (n = 36-76 cells) and (**F**) mitochondrial membrane potential via TMRM (n = 30-155 cells) for cells with increasing spreading. (**G**) Normalized PercevalHR ratio and TMRM fluorescence intensity for MDA-MB-231 cells unconfined on collagen micropatterns. (**H**) Quantification of normalized PercevalHR (n = 55-64 cells) and (**I**) TMRM for cells with increasing elongation (n = 40-62 cells). Data shown as box-and-whisker plots denote mean (+), medians and 25th/75th and 5th/95th percentiles, gray as unconfined and black as patterns; yellow lines show cell outlines. n.s.= non-significant, *P < 0.05, **P < 0.01, ****P < 0.0001 with one-way ANOVA. Scale bar = 50 μm.



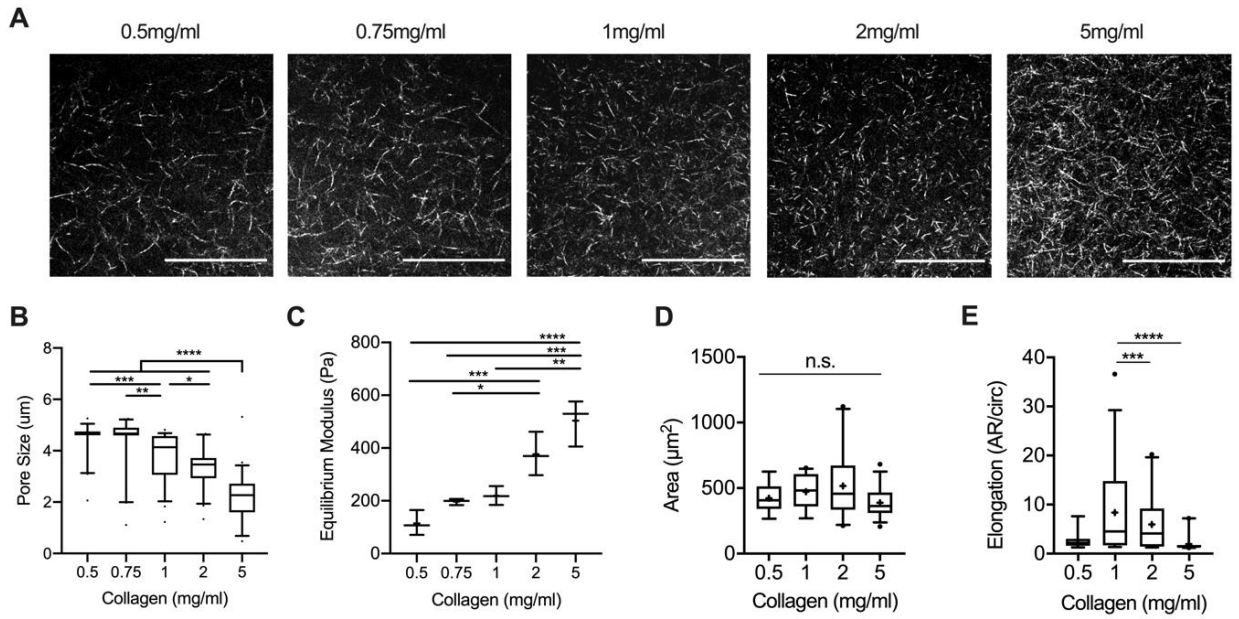
Supplemental Figure 2. OXPHOS inhibition across AMA concentrations. Quantification of (**A**) mitochondria membrane potential via TMRM integrated fluorescence intensity, (**B**) normalized PercevalHR ratio, (**C**) metabolic activity via AlamarBlue fluorescent intensity, and (**D**) cell viability (propidium iodide (PI) / Hochest) for 1, 3, 6, and 24 h of treatment with 0.1–40 μM AMA (n > 30 cells). Data shown as mean ± s.e.m., *P < 0.05, **P < 0.01, ****P < 0.0001 with one-way ANOVA.



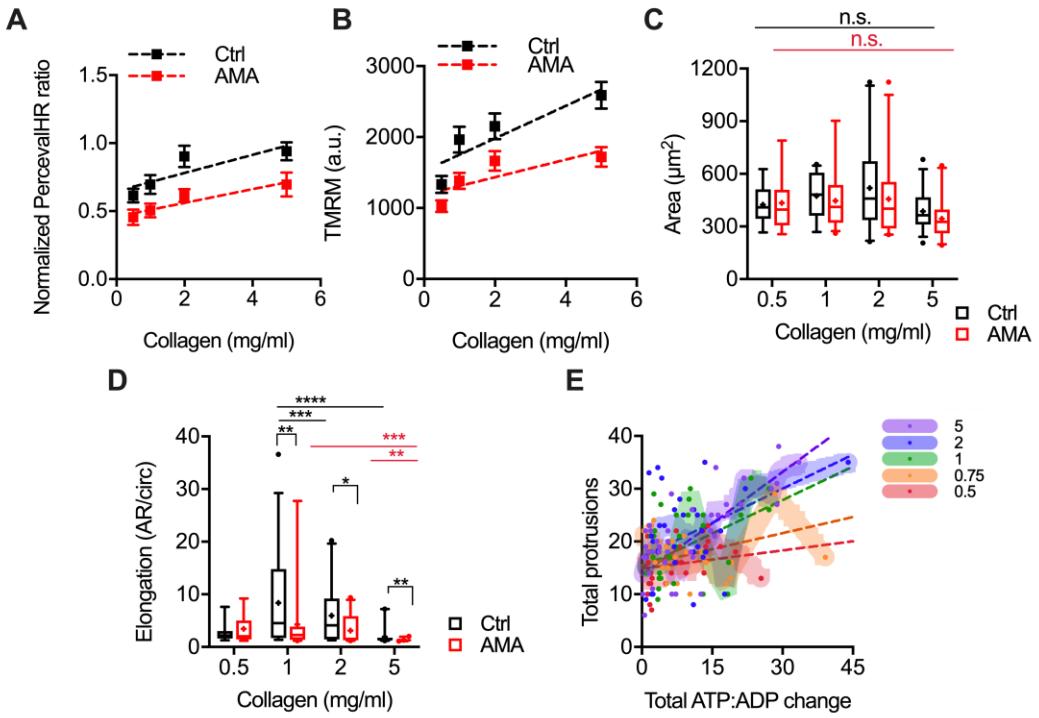
Supplemental Figure 3. OXPHOS impacts intracellular ATP:ADP ratio and membrane ruffling during 2D elongation. (**A**) Normalized PercevalHR ratio (n = 55-64 cells) and (**B**) membrane ruffling (*n* = 30-35 cells) following AMA treatment on collagen micropatterns with increasing elongation. Data shown as mean ± s.e.m.; dashed lines show linear regression.



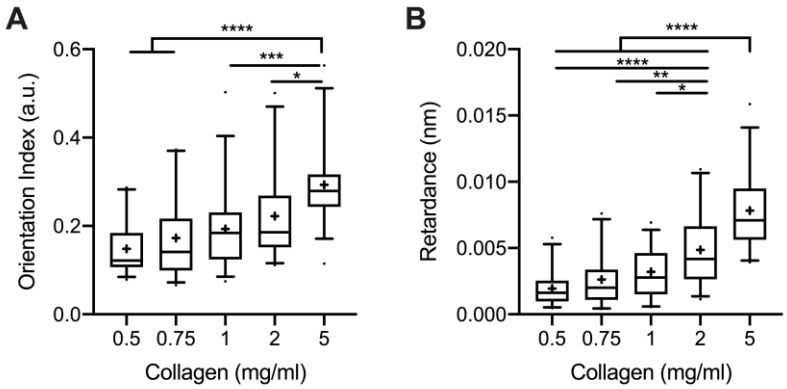
Supplemental Figure 4. OXPHOS inhibition does not influence 2D migration. (**A**) Normalized PercevalHR ratio of MDA-MB-231 cells following AMA treatment on 2D glass surface. Quantification of (**B**) Normalized PercevalHR ratio and (**C**) stepwise velocity after AMA treatment in for 2D migration ($n > 20$ cells). Data shown as box-and-whisker plots denote mean (+), medians and 25th/75th and 5th/95th percentiles; yellow lines show cell outlines; n.s.= not significant with two tailed t-test. Scale bar = 50 µm.



Supplemental Figure 5. MDA-MB-231 cell morphology as a function of 3D collagen density with mechanical characterization. (**A**) Confocal reflectance images of collagen matrices with varying density. Quantification of (**B**) pore size and (**C**) equilibrium modulus of collagen gels with varying density (n = 3 gels). Quantification of (**D**) cell area and (**E**) cell elongation factor (*n* = 16-33 cells) in matrices with increasing collagen density. Data shown as box-and-whisker plots denote mean (+), medians and 25th/75th and 5th/95th percentiles; multiple comparisons with ANOVA, n.s. = not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Scale bar = 50 μm.



Supplemental Figure 6. OXPHOS inhibition impacts morphology and cellular energetics which correlates with protrusions. Linear correlation of collagen density (0.5, 1, 2, and 5mg/ml) with (**A**) normalized PercevalHR ratio (n = 32-61 cells) and (**B**) TMRM fluorescent signal (n = 42-93 cells) following AMA treatment. Quantification of (**C**) cell area and (**D**) cell elongation factor (n = 13-33 cells) in matrices of increasing collagen densities. (**E**) Total number of new protrusions and total ATP:ADP change of single MDA-MB-231 cell in collagen matrices (*n* = 30-40 cells). Data shown as box-and-whisker plots denote mean (+), medians and 25th/75th and 5th/95th percentiles, or mean ± s.e.m.; two-tailed t-test when comparing between ctrl and AMA, multiple comparisons with ANOVA when comparing across densities; lines show LOWESS smoothing and dashed lines show linear regression. n.s. = not significant, **P < 0.01, ***P < 0.001, ****P < 0.0001.



Supplemental Figure 7. Matrix remodeling and cell contractility increases with collagen density. Quantification of (**A**) collagen fiber orientation within 10μm to cell protrusions and (**B**) cell contractility for MDA-MB-231 cells in matrices of increasing collagen density. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 with one-way ANOVA.