

**Supplemental Figure 1:** Confocal images of 7-day growth OVCAR-8 cell lines in 3D Matrigel ECM

Red fluorescence is generated from expression of DsRed2 proteins and blue fluorescence is from Hoechst 33342 staining of DNA. a) OVCAR-8 cells grown in 21%  $O_2$ , b) in 3%  $O_2$ , c) and in gradient  $O_2$ .



Supplemental Figure 2: Brightfield images of 14-day growth conditions



0.25

0.00

-0.25

-10

10

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log2 Fold Change

- log10 p-value

MCF-7 cell cultures, including outlier replicate.

(a) MDS plot depicting the Euclidean distance between Log2 fold changes of genes between MCF-7 samples, outlier replicate samples are circled. (b) Volcano plot of transcriptomes for MCF-7 21% O2 vs. 2D comparison; genes with a log2 fold change greater than I±0.6l and a False Discovery Rate (FDR)  $\leq$  0.05 are shown in green, genes not meeting the criteria are grey. (c) Volcano plots of OVCAR-8 3% vs. 21% O2, (d) 3% vs. gradient, and (e) gradient vs. 21% O2. (f) Top 10 up-regulated (red) and down-regulated (blue) Gene Ontology (GO) Biological Processes for MCF-7 21% O2 vs. 2D comparison, (g) 3% vs. 21%O2, and (h) 3% O2 vs. gradient.