**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure S1**

A-B: Changes in RNA Pol II pausing after AKTi treatment. Comparison of the genome-wide log2 Pausing Index between AKTi and DMSO treated cells (i.e., HCT116 (A), MCF7 (B)). Pearson correlation coefficient is shown on the bottom right corner of each plot. Only genes with a Pausing Index higher than 0.1 (log2) in at least one of the samples were included in the plot, yielding a total of 12652 genes.

**Supplementary Figure S2**

A-B: Immunofluorescence images for both HCT116 and MCF7 cells, treated either with DMSO or Akti-1/2. Cells were stained for DAPI, MCM2, H3K9me2, FDFT1 (A), and MCM2, H3K9me2, cholesterol, and lipid rafts (B). Merged images represent overlay of respective stains. Size bar = 10μm.

**Supplementary Figure S3**

A-E: Effect of AKTi microvesicles on cell vitality (i.e., MTS assay). Different tumor cell lines (e.g., HCT116 (A), MCF7 (B), MDA-MB-231 (C), PC9 (D), and A375 (E)) were exposed to microvesicles derived from Akti-1/2, MK-2206, or DMSO treated cells of the same type for 1 hour, and a subsequent MTS assay was performed in triplicate for each cell line and treatment. Bars depict the log2 fold-change in cell survival between AKTi-treated cells (i.e., Akti-1/2 or MK-2206) and DMSO-treated cells after 120h. Error bars show the standard error of the mean (SEM). Asterisks on top of bars designate statistically significant increases (i.e., p < 0.05, t-test).

F-J: Effect of AKTi microvesicles on cell colony counts after exposure to stress conditions. Barplots depict the log2 fold change (i.e., Akti-1/2 - DMSO, grey bar; MK-2206 - DMSO, open bar) in the number of colonies formed after exposure to microvesicles derived from either AKTi or DMSO treated cells of the same type for 1 hour. Exposed cells were placed under three different stress conditions (e.g., 1% serum, 4% oxygen, standard chemotherapy agent) for 72h. Experiments were done in triplicate for each different cells line (e.g., HCT116 (F), MCF7 (G), MDA-MB-231 (H), PC9 (I), and A375 (J)), treatment, and stress condition. Error bars show the standard error of the mean (SEM). Asterisks on top of bars designate statistically significant increases (i.e., p < 0.05, one-sided t-test) in colony counts after exposing cells to microvesicles derived from AKTi-treated cells, compared to microvesicles derived from DMSO-treated cells.

**Supplementary Figure S4**

A-E: Effect of microvesicles on growth rate using MK-2206, a different AKT1 allosteric inhibitor. Different tumor cell lines (e.g., HCT116 (A), MCF7 (B), MDA-MB-231 (C), PC9 (D), and A375 (E)) were exposed to microvesicles derived from either MK-2206 or DMSO treated cells of the same type for 1 hour. Experiments were done in triplicate for each cell line and treatment. Error bars show the standard error of the mean (SEM). P-values shown at the top-left corner on each panel correspond to the model comparing the two slopes of the linear models fitted for each condition (i.e., DMSO or Akti-1/2 microvesicles). Non-significant p-values in all five cell lines support the finding that there is no evidence of any significant difference in cell growth based on the differential microvesicle treatment.

F-J: MK-2206 microvesicles bioactivity *in vitro*. Barplots depict the log2 fold change (MK-2206 - DMSO) in the total number of cells exposed to microvesicles derived from either MK-2206 or DMSO treated cells of the same type for 1 hour. Cells were then placed under three different stress conditions (e.g. 1% serum, 4% oxygen, standard chemotherapy agent) for 72h. Experiments were done in triplicates for 5 different cells lines (e.g., HCT116 (F), MCF7 (G), MDA-MB-231 (H), PC9 (I), and A375 (J)), treatment, and stress condition. Error bars show the standard error of the mean (SEM). Asterisks on top of bars designate statistically significant increases (i.e., p < 0.05, one-sided t-test) in cell count after exposing them to microvesicles derived from MK-2206 treated cells, compared to microvesicles derived from DMSO treated cells.

**Supplementary Figure S5**

Akti-1/2 microvesicles *in vitro* cross-cell line bioactivity assay. Five cancer cell lines (e.g., HCT116, MCF7, MDA-MB-231, PC9, and A375) and one human fibroblast cell line (e.g., AG11726), represented in the panel rows, were exposed to microvesicles secreted by each one of the five cancer cell lines (i.e., panel columns). Barplots depict the log2 fold change (Akti1/2 - DMSO) in the total number of cells exposed to microvesicles derived from either Akti-1/2 or DMSO treated cells for 1 hour. Cells were then placed under three different stress conditions (e.g. 1% serum, 4% oxygen, standard chemotherapy agent) for 72h (grey bar). Experiments were done in triplicates for each cell line, treatment, and stress condition. Error bars show the standard error of the mean (SEM). Asterisks on top of bars designate statistically significant increases (i.e., p < 0.05, one-sided t-test) in cell count after exposing them to microvesicles derived from Akti-1/2 treated cells, compared to microvesicles derived from DMSO treated cells.

**Supplementary Figure S6**

A-D: Effect of microvesicle treatment *in vitro* on tumor cell proliferation *in vivo*. HCT116 (A), MDA-MB-231 (B), PC9 (C), and A375 (D) cancer cell lines were exposed to isogenic microvesicles derived from either Akti-1/2 or DMSO treated cells for 1-hour and injected into NU/NU mice (n = 1 / condition / cell line). At sacrifice, MKI67 protein staining was performed on resulting tumors to assess cell proliferation. Three 40x high-power fields were analyzed for each slide. Bars depict the average percentage of MKI67+ cells across of the three high-power fields. Error bars show the standard error of the mean (SEM) for the three observed proportions. Asterisks on top of bars designate statistically significant variation (i.e., p < 0.05, two-sided Fisher test) in the number of MKI67+ cells.

**Supplementary Figure S7**

A-B: Effect of AKT1 inhibition *in vitro* on tumor cell proliferation *in vivo*. HCT116 (A) and MCF7 (B) cancer cell lines were treated either with DMSO or Akti-1/2 for 72h and then injected into NU/NU mice (n = 1 / condition / cell line). At sacrifice, MKI67 protein staining was performed on resulting tumors to assess cell proliferation. Three 40x high-power fields were analyzed for each slide. Bars depict the average percentage of MKI67+ cells across of the three high-power fields. Error bars show the standard error of the mean (SEM) for the three observed proportions. Asterisks on top of bars designate statistically significant variation (i.e., p < 0.05, two-sided Fisher test) in the number of MKI67+ cells.

**SUPPLEMENTARY DATASET LEGENDS**

**Supplementary Datasets S1A-H**

Lists of analytes (i.e., mRNA transcripts, proteins, metabolites, small RNAs) with concordant behavior (i.e., log2 fold-change either positive or negative in both HCT116 and MCF7 cell lines).

- 1A: GRO-Seq

- 1B: RNA-Seq

- 1C: Proteomics

- 1D: Metabolomics

- 1E: Secreted proteins

- 1F: Microvesicle small RNA-Seq

- 1G: Cell small RNA-Seq

**Supplementary Datasets S2A-E**

Summary of GSEA results for GRO-Seq, RNA-Seq and protein datasets. Absent datasets (GRO-Seq up/down, RNA up) contain no significant (FWER <5%) gene sets. Up-regulated gene sets show increased levels in Akti-1/2 treated cells compared to DMSO treated cells. Down-regulated gene sets show decreased levels in Akti-1/2 treated cells compared to DMSO treated cells. Only canonical gene sets were included in these analyses (i.e., KEGG, REACTOME, BIOCARTA, PID, GO).

- 2A: List of significantly down-regulated gene sets in the RNA-Seq (FWER < 5%).

- 2B: List of significantly down-regulated gene sets in the protein dataset (FWER < 5%).

- 2C: Genes (rows) by gene sets (columns) matrix of the GSEA protein down results. Genes are the union of the leading edge of all the significantly downregulated gene sets at the protein level. Each column corresponds to one of the significant gene sets. Value of 0 means that a gene does not belong to the leading edge of that gene set, while a value of 1 means that the gene is included in the leading edge of that gene set.

- 2D: List of significantly upregulated gene sets in the protein dataset (FWER < 5%).

- 2E: Genes (rows) by gene sets (columns) matrix of the GSEA protein up results. Genes are the union of the leading edge of all the significantly upregulated gene sets at the protein level. Each column corresponds to one of the significant gene sets. Value of 0 means that a gene does not belong to the leading edge of that gene set, while a value of 1 means that the gene is included in the leading edge of that gene set.