

Fig S1. dAKAP1 expression is inversely related to mesenchymal markers in multiple analyses. (A) Pearson's r values quantifying the correlation of dAKAP1 mRNA and 36 mesenchymal markers from

gene array analysis data of 1.037 CCLE cell lines (29). Cell lines were analyzed separately according to cancer etiology (n=22). Median and interquartile ranges (red bars) are indicated. (B) Heat map displaying r values of correlation of dAKAP1 mRNA and mesenchymal genes. Rows (mesenchymal genes) are organized with hierarchical clustering; columns (cancer cell line etiology) are organized by mean r value. Intensity scale indicates r values ranging from high (yellow) to low (teal). (C) Pearson's r values quantifying the correlation of mitochondrial proteins and 29 mesenchymal markers from gene array analysis data of 77 tissue samples (32). Note that 7 of the 36 mesenchymal markers and several mitochondrial proteins were not quantified in the published dataset. (D) Heat map displaying r values of correlation mitochondrial and mesenchymal proteins. Rows (mesenchymal proteins) are organized with hierarchical clustering; columns (mitochondrial proteins) are organized by mean r value. Intensity scale indicates r values ranging from high (vellow) to low (teal). (E) Bar graphs of iBAQ intensity of mitochondrial proteins (x-axis) in breast cancer cell lines (y-axis)(34). Cell lines are classified as in figure 3. A value of zero is indicative of a protein that was not quantified in the dataset. (F) Heat map of dAKAP1 mRNA intensity and mean mesenchymal marker mRNA across 57 CCLE breast cancer cell lines used in this dataset organized by ascending dAKAP1 mRNA expression as determined by RNAseq (29). (G) Scatter plot of dAKAP1 mRNA against mean mesenchymal marker mRNA by RNAseq (29). Error bars represent s.e.m. (H) Heat map displaying r values quantifying the correlation of AKAPs mRNA and a panel of 84 metastasis-related genes from gene array analysis data of the breast cancer CCLE cell lines (n=59) (29). Rows (AKAPs) are annotated; columns (metastasis-related genes) are organized by molecular function. Some genes are repeated when functions span multiple classifications. Intensity scale indicates r values ranging from high (yellow) to low (teal). (I) RII overlay in the four breast cancer cell lines used in this study shows AKAPs' abundance in whole cell lysate (top) and loading control Eif2α (bottom).

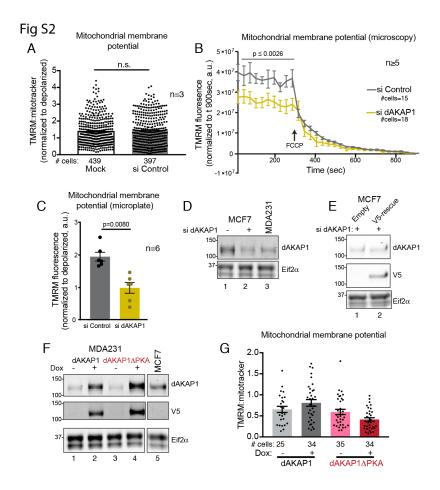


Fig S2. Classification of breast cancer cell lines (mRNA analysis) and mitochondrial membrane potential with siRNA. (A) Quantification of amalgamated data (n=3 independent experiments). Number of cells used in analyses are listed below each column. Mitochondrial membrane potentials were calculated by comparing the ratio of TMRM to Mitotracker Green FM fluorescence with untreated (mock) and control siRNA (si Control) treated cells. Error bars represent s.e.m. Statistical significance was determined by two-tailed Student's t-test. (B) Images were acquired from "dAKAP1-high" MCF7 cells treated with control (dark grey) or dAKAP1-targeted (yellow) siRNA and loaded with $\Delta\Psi_{m-}$ dependent fluorophore TMRM. After 300 seconds (5 mins), cells were completely depolarized with FCCP. TMRM fluorescence intensity was measured from individual cells over 900 seconds (15 mins). Data was collected over n≥5 independent experiments. Error bars represent s.e.m. Statistical significance was determined by two-tailed Student's t-test for each time point. (C) MCF7 cells were seeded onto a 96well microplate and treated with control (dark grey) or dAKAP1-targeted (yellow) siRNA and loaded with $\Delta\Psi_{\rm m}$ -dependent fluorophore TMRM. Fluorescent signal intensity was read with and without FCCP depolarization. Data shown was normalized to post-FCCP treated intensity values. Data was collected over n=6 independent experiments; individual data points are shown. Error bars represent s.e.m. Statistical significance was determined by two-tailed Student's t-test. (D) Comparison of dAKAP1 knockdown in MCF7 cells to "dAKAP1-low" cell line MDA231. Immunoblot detection of (top) dAKAP1 and (bottom) Eif2α loading control. Analysis of dAKAP1 expression in MCF7 cells treated with (lane 1) si Control or (lane 2) siRNA against dAKAP1 and compared (lane 3) to wild-type MDA231 cells. (E) Immunoblot of (top) dAKAP1, (mid) V5, and (bottom) Eif2α in MCF7 cells transfected with (lane 1) siRNA against dAKAP1 or (lane 2) siRNA against dAKAP1 and V5-tagged murine dAKAP1 construct. Note that the dAKAP1 antibody used only recognizes the human protein, thus V5 expression confirms

rescue with the murine ortholog. (**F**) Comparison of induced dAKAP1 expression in MDA231 cells to "dAKAP1-high" cell line MCF7. Immunoblot detection of (top) dAKAP1, (mid) V5, and (bottom) Eif2 α loading control. dAKAP1 expression was compared between un-induced MDA231 cells (lanes 1 and 3), those induced with doxycycline (lanes 2 and 4), and wild-type MCF7 cells (lane 5). Note that MCF7 lane 5 was run on the same blot as lanes 1-4, but spliced to exclude repeated data. (**G**) Quantification of mitochondrial membrane potentials calculated by comparing the ratio of TMRM to Mitotracker Green FM fluorescence with either un-induced or doxycycline-induced dAKAP1 expression in MDA231 cells. Number of cells used in analyses are listed below each column. Error bars represent s.e.m.

Table S1. Detailed description of patient breast tumor samples used in this study.

- Movie S1. Time course video of mitochondrial dynamics in MCF7 cells treated with si Control. Two color images captured every 2 minutes for 30 minutes. Mitochondrial photoactivation occurs after the first acquisition (2 minutes).
- Movie S2. Time course video of mitochondrial dynamics in MCF7 cells treated with si AKAP1. Two color images captured every 2 minutes for 30 minutes. Mitochondrial photoactivation occurs after the first acquisition (2 minutes).
- Movie S3. Time course video of mitochondrial dynamics in MCF7 cells treated with si AKAP1 with non-targeted dAKAP1-SNAP rescue. Two color images captured every 2 minutes for 30 minutes. Mitochondrial photoactivation occurs after the first acquisition (2 minutes).
- Movie S4. Time course video of un-induced control MDA231 cells migrating into scratch wound. Images captured every 10 minutes for 18 hours.
- Movie S5. Time course video of doxycycline-induced MDA231 cells expressing dAKAP1 migrating into scratch wound. Images captured every 10 minutes for 18 hours.
- Movie S6. Time course video of doxycycline-induced MDA231 cells expressing dAKAP1DPKA migrating into scratch wound. Images captured every 10 minutes for 18 hours.