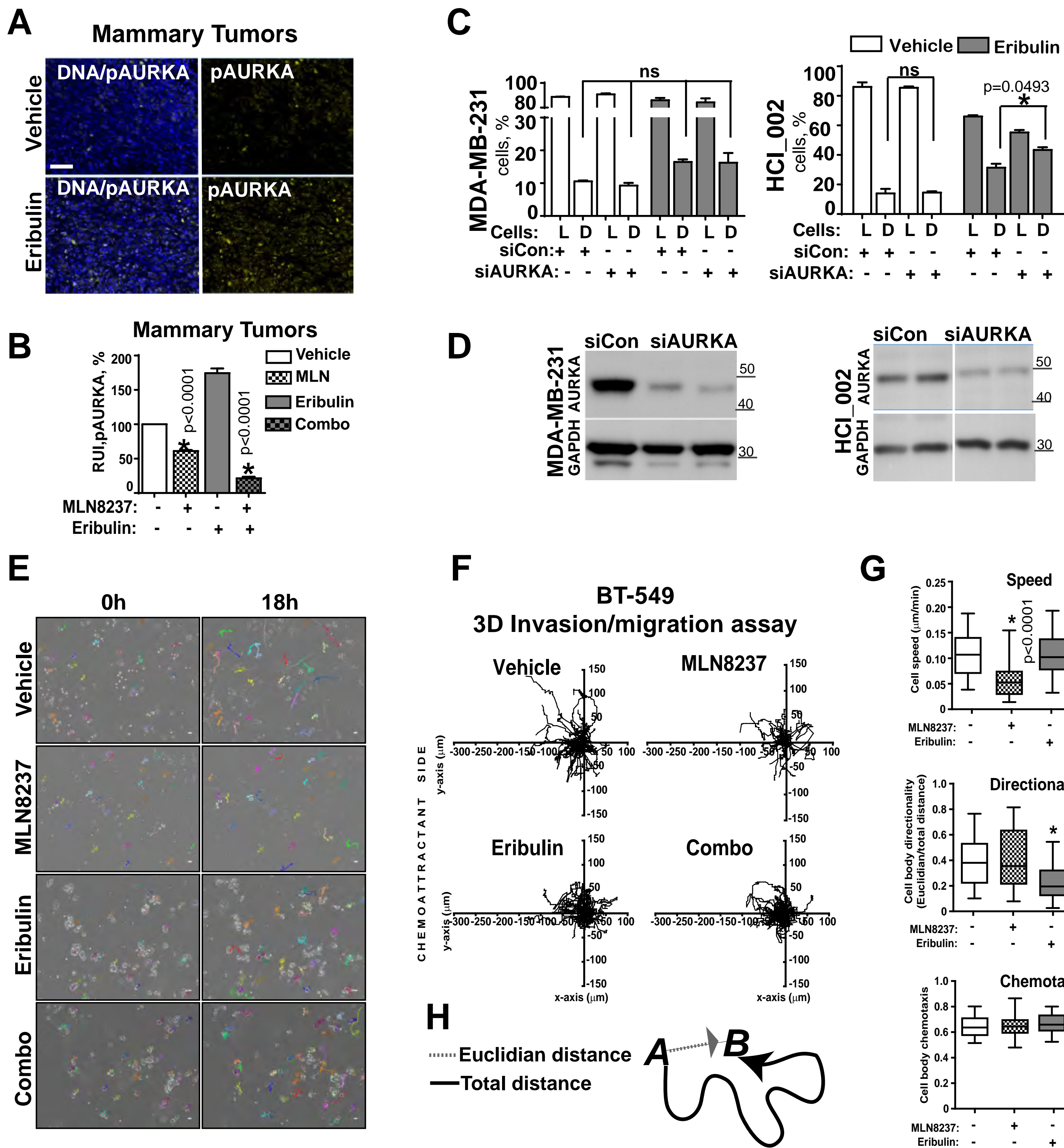
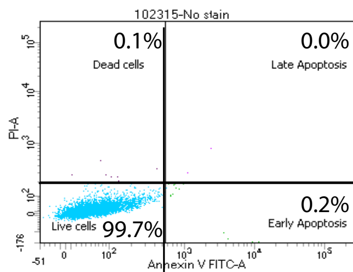
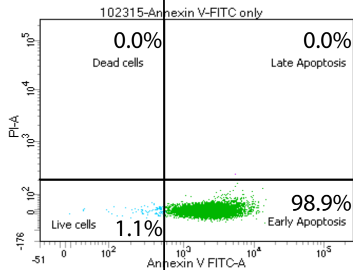


A. Fluorescent-immunohistochemistry (F-IHC) analysis of pAURKA-T288/yellow, DNA/blue in MDA-MB-231LN xenografts treated with eribulin or vehicle, 2 weeks; scale bar-50µm. **B.** Quantification of F-IHC staining as in (A), plus other treatment groups, n=4, one-way ANOVA, vehicle vs. treatments. **C**. Viability assay:Trypan blue cell staining to quantify live/dead cells 48h post treatment with siRNAs and drugs. Graph is % of live (L) or dead (D) cells +/-SEM, n=100 cells; one-way ANOVA, *p as indicated; control vs. AURKA siRNAs. **D.** Western blot analysis of AURKA expression in TNBC cell lines treated with siRNAs (pools) against AURKA (siAURKA) or non-targeting control (siCon) with anti-AURKA and -GAPDH antibodies. **E.** Representative bright field time-lapse microscopy images of BT-549 cells pre-treated as indicated or vehicle, embedded in the Matrigel, 0h and 18h, in the presence of drugs(individual tracks color coded, scale bar -10um). **F.** Individual cell tracking plots (as in E) were made using Ibidi Chemotaxis/Migration software.
G. Quantification of speed, directionality, chemotaxis of moving cells as in (E) were obtained by tracking individual cell movements using MTrackJ plugin for ImageJ software. Data presented as box and whisker plots of maximum and minimum values from three independent experiments, n=30-50 per/treatment group, + S.E.M, one-way ANOVA vehicle vs. treatments; *p as indicated, ns-non significant. **H.** Schematic representation of quantified Directionality as euclidian over total distances as in (G).

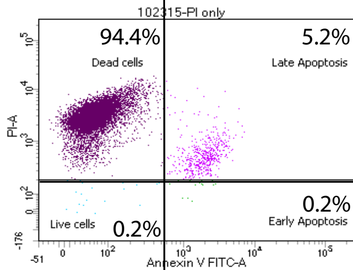
Supplementary Figure 1, Kozyreva



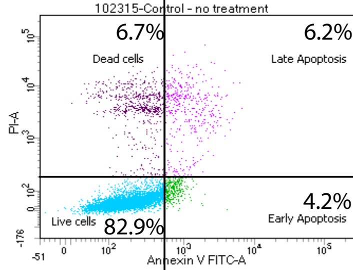
Control for live cells.
Attached cells not treated, stained with Hoechst 33342
Live cells are AnnexinV and PI negative, DNA positive



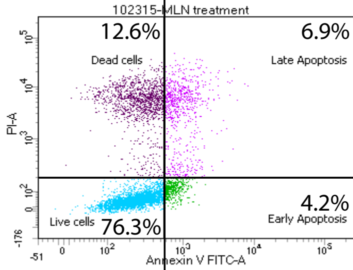
Control for AnnexinV positive cells
*short term (10min) UV exposure of attached cells
Early Apoptosis=AnnexinV positive



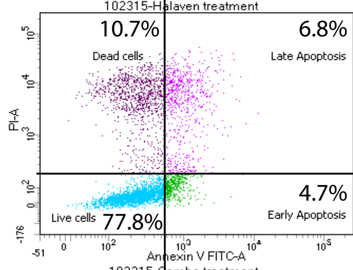
Control for dead (PI positive) cells.
*Cell were boiled for 1 min.
Dead cells=PI positive
Late Apoptosis=PI and AnnexinV positive



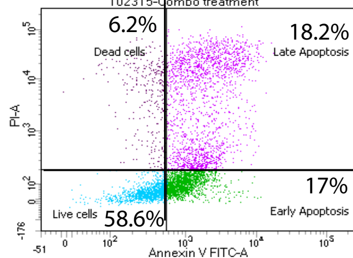
Vehicle:



MLN8237:



Eribulin:



Combo:

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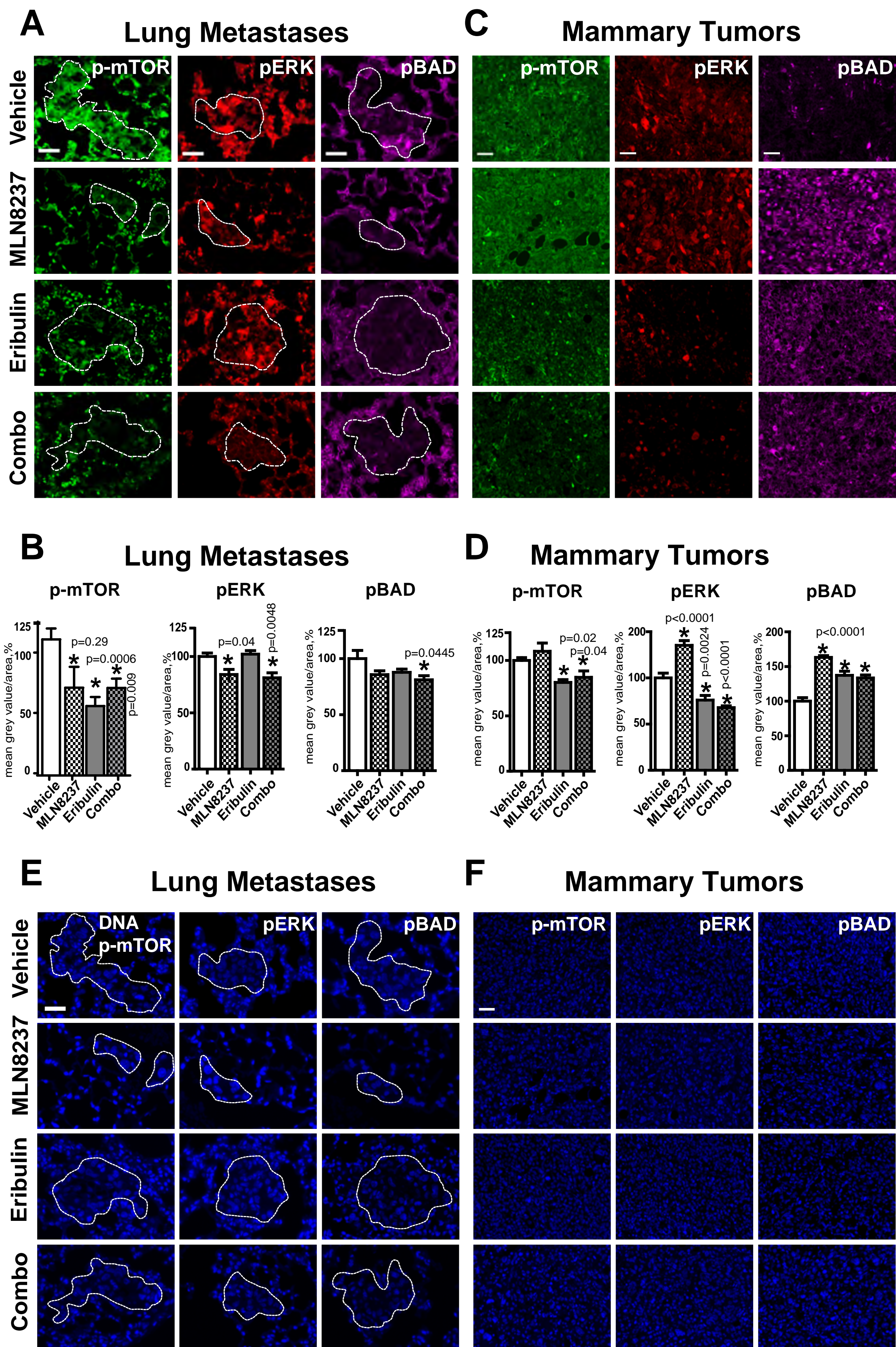
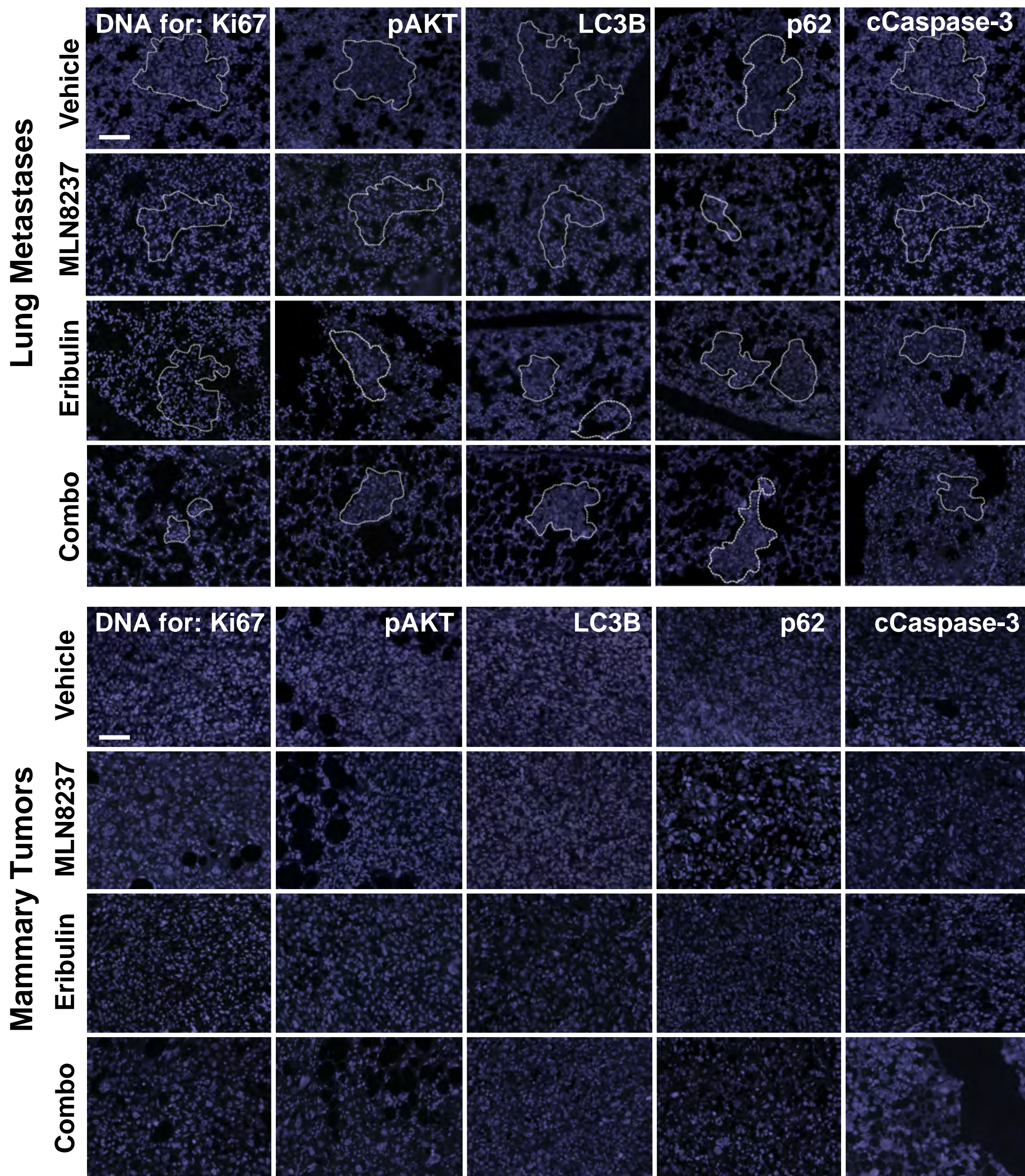
Representative FACS analysis data as plots of annexin-V/PI/Hoechst33342 distribution in MDA-MB-231 cells treated with drugs attached for 48h. Percentage of annexin-V/PI positive cells (Fig.2D) was calculated based on # of events counted (10,000), which was referenced as 100%.

Supplementary Figure 2, Kozyreva



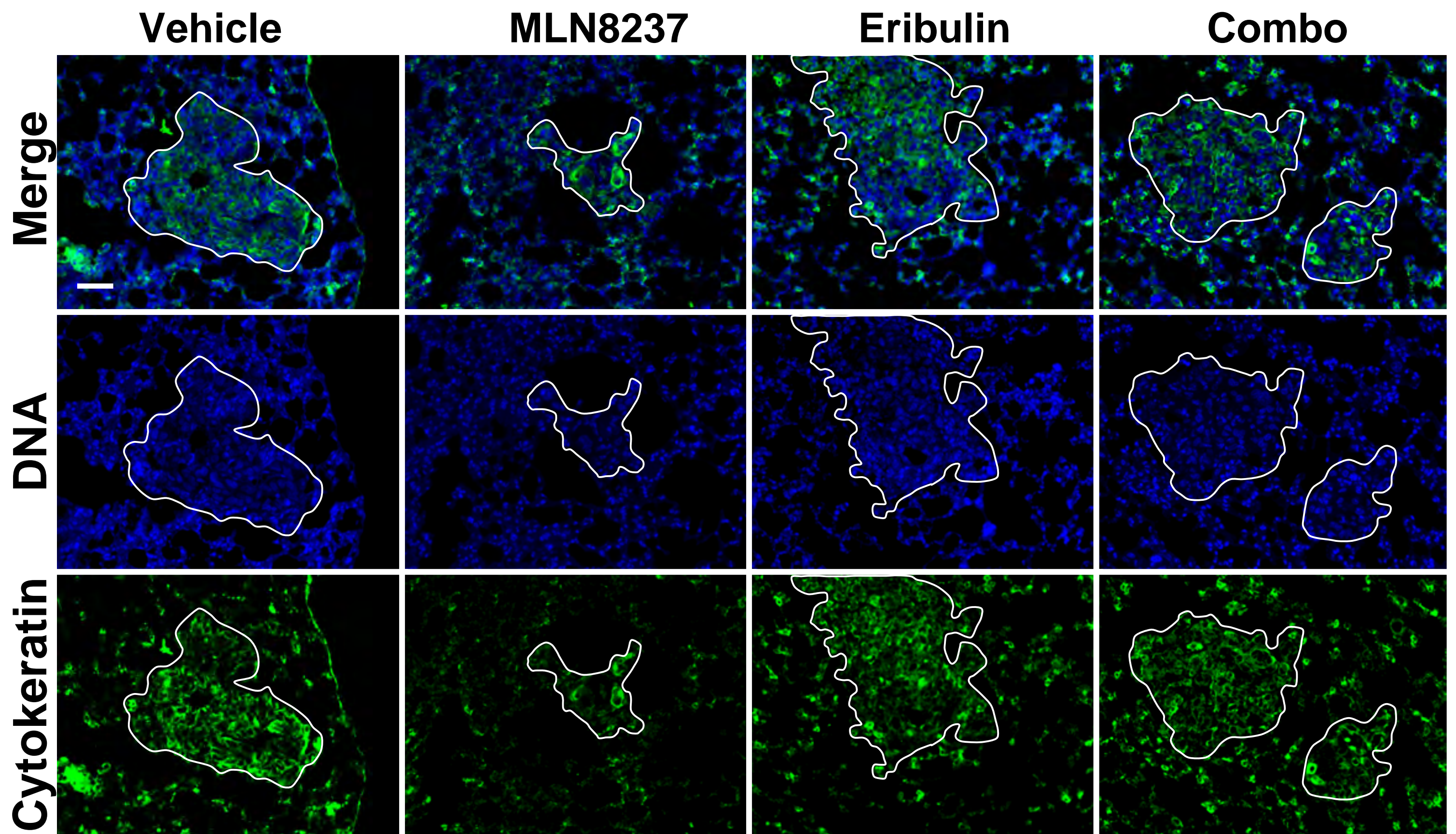
A, C. F-IHC analysis of tumors and lung metastases at the end point of treatment with vehicle, MLN8237, eribulin or combination using indicated phospho-specific antibodies. Scale bar is 50 um for tumors, 30 um for lung metastasis. B, D. Quantification of F-IHC as in A, C from three independent experiments n=3, based on 3D projections or whole section. One-Way ANOVA, *p-values as indicated in the figure compared to vehicle. E,F DNA staining for F-IHC panels in A,C.

Supplementary Figure 3, Kozyreva



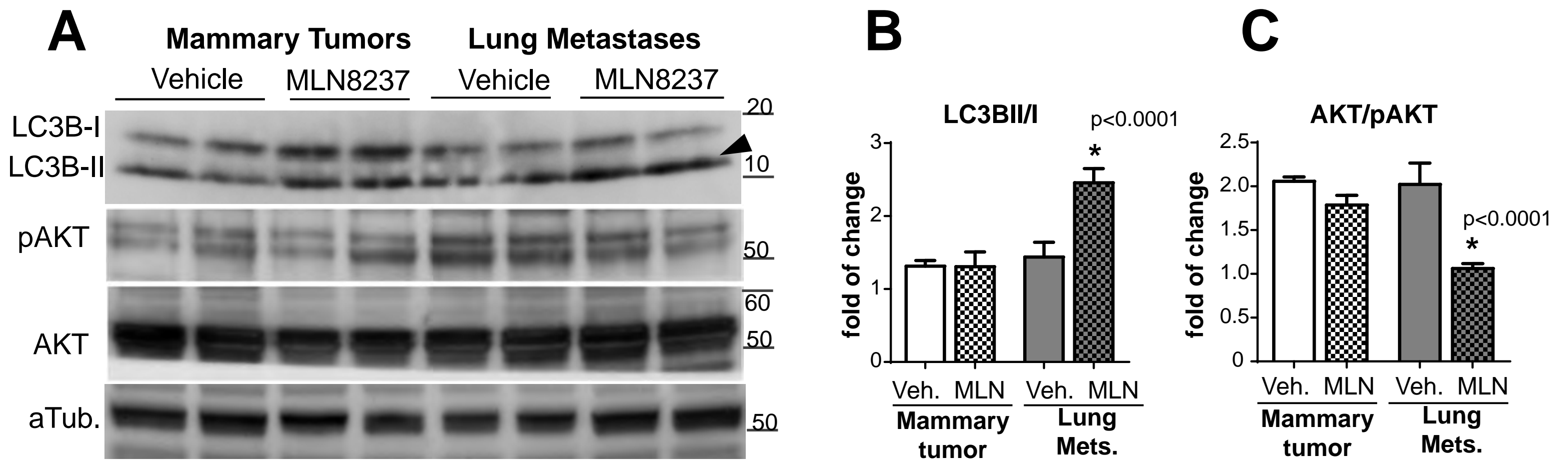
Representative images of F-IHC, DNA (DAPI) staining of pulmonary tissue (metastases defined by the white outline) and mammary tumors.Treatments groups as indicated, scale bar is 100μm.

Supplementary Figure 4, Kozyreva



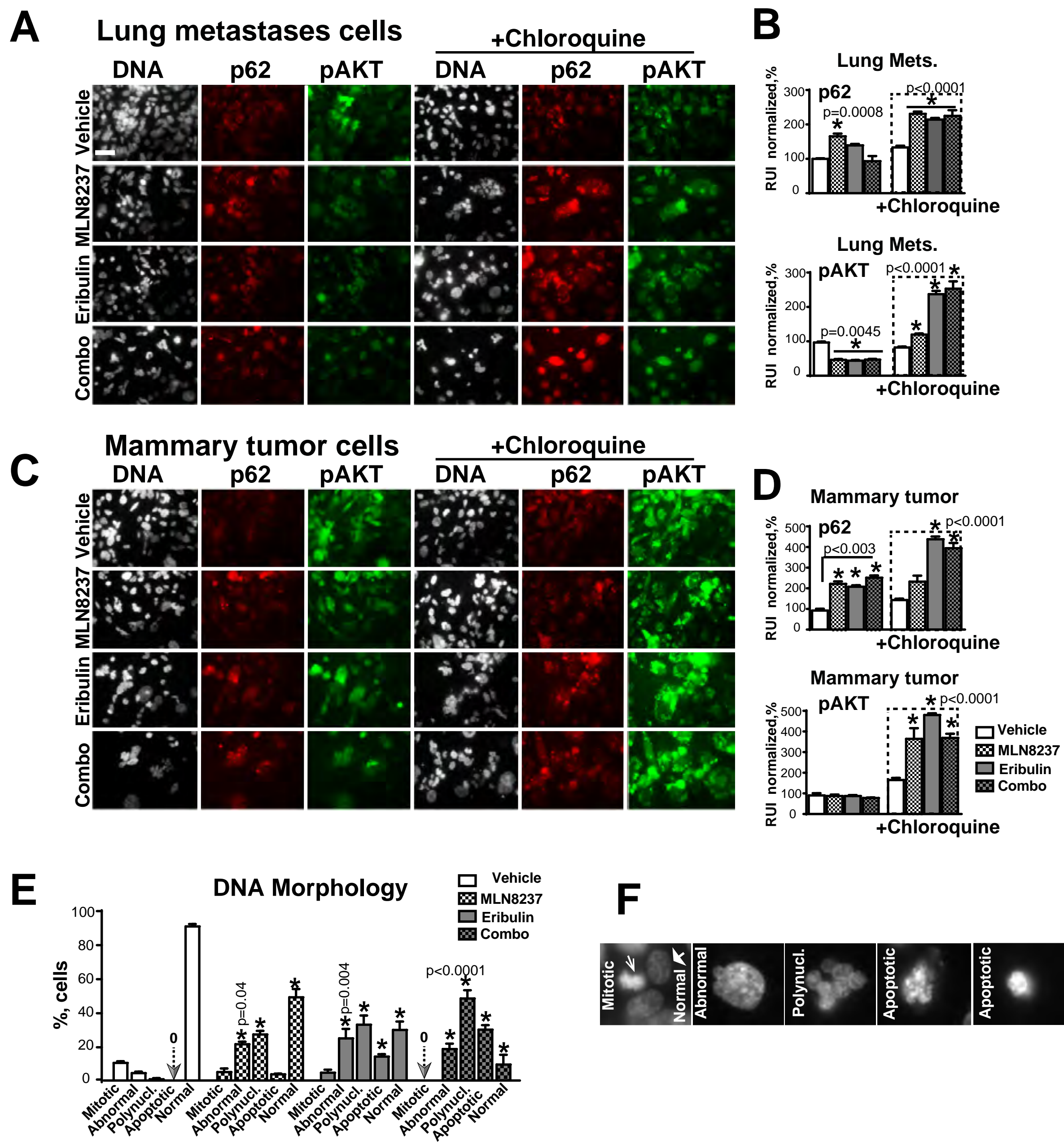
Fluorescent IHC analysis of metastases using pan-cytokeratin antibodies (Dako, green) to detect human tumor cells in lungs (mouse), DNA-DAPI, blue. Treatments as indicated in the figure. Scale bar is 100μm.

Supplementary Figure 5, Kozyreva



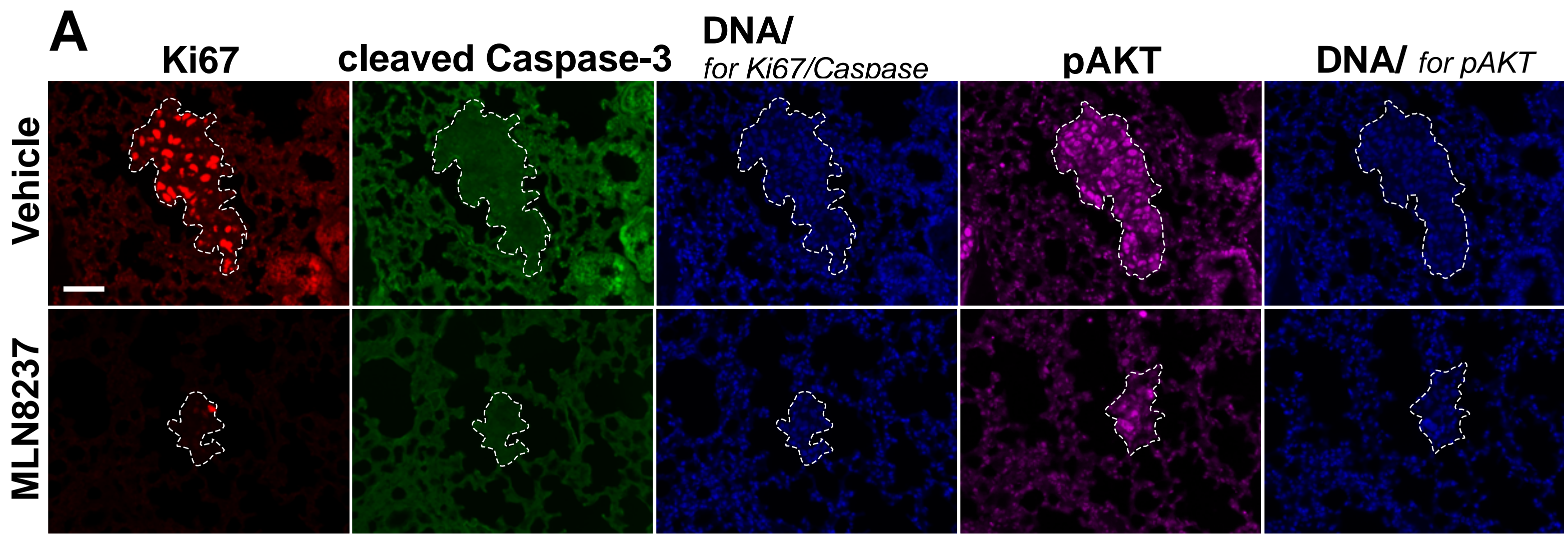
A. WB analysis of LC3BI/II, pAKT, AKT, alpha-tubulin expression with respective antibodies in tumor cells isolated (not cultured in vitro) from freshly collected mammary tumors and lungs of MDA-MB-231LN xenograft tumor bearing mice treated with MLN8237 or vehicle. **B-C** Quantification of digital images as in (A) using GeneTool software (Syngene), mean grey value of corresponding band/area (as fold of change/vehicle) +S.E.M, n=3, one-way ANOVA, p is non-significant (ns, tumors), *p<0.0001 (metastases).

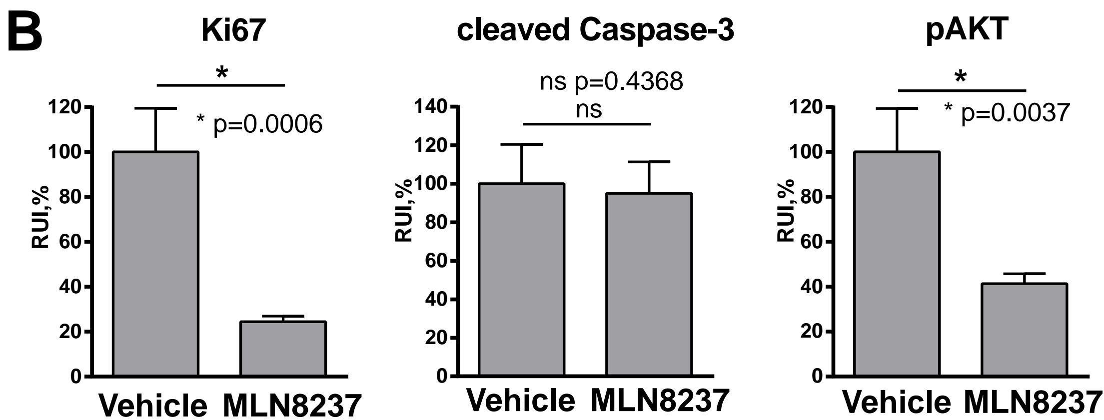
Supplementary Figure 6, Kozyreva



MLN8237/eribulin combination induces cytotoxic autophagy, apoptosis.
A, C Representative IF images of MDA-MB-231 cells isolated from lung metastases (A) or mammary tumors (C), treated with drugs/or vehicle with and without chloroquine; stained with pAKT/green, p62/red and DNA/white; scale bar-20um. **B-D** Quantification of images as in (A, C) using ImageJ software, n=3, 100 cells/treatment, one-way ANOVA: vehicle vs. treatments. **E** Quantification of nuclear morphology, cells as in (A); arrows with zero indicate absence of cells with such morphology. **F** Representative images of mitotic, abnormal, polynucleated, apoptotic and normal nuclei. Apoptotic cells were also confirmed by staining with anti-cleaved caspase-3 antibodies; white arrows indicate mitotic cells and normal size/shape nucleus; n=3, 100 cells/treatment, one-way ANOVA: vehicle vs. treatments, all *p are <0.001 unless otherwise indicated.

Supplementary Figure 7, Kozyreva

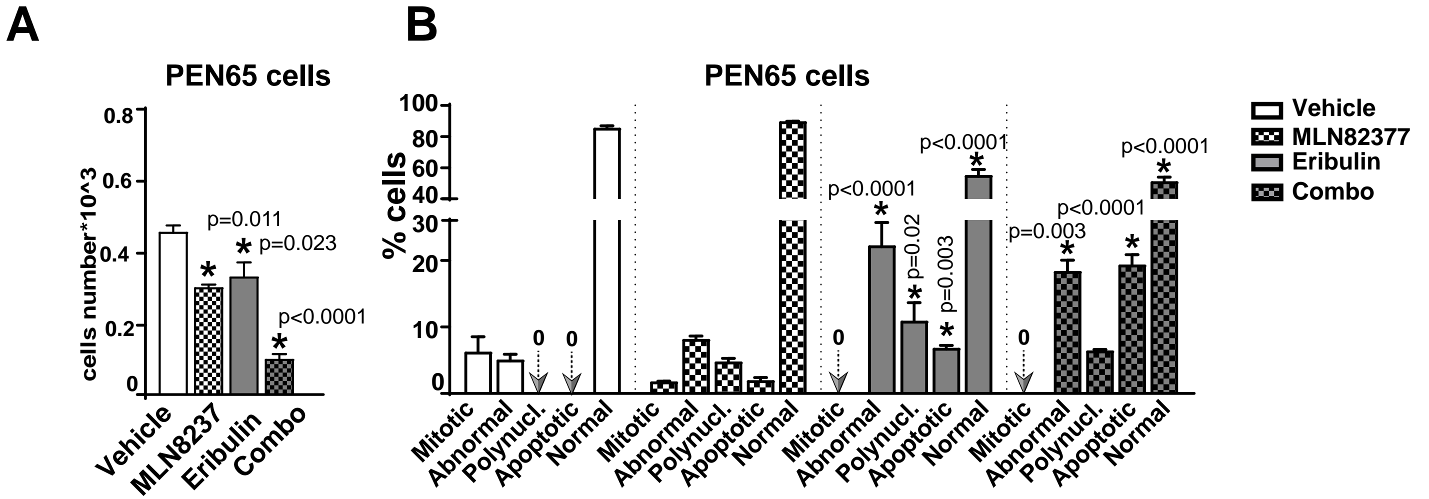




A. Representative images of F-IHC staining of pulmonary tissue (metastases defined by the white outline) from vehicle and MLN8237 treated mice with anti-Ki67 (red), -cleaved Caspase-3 (green), -phosphoAKT1/2 (purple) antibodies & DAPI (DNA-blue).scale bar is 50μm.

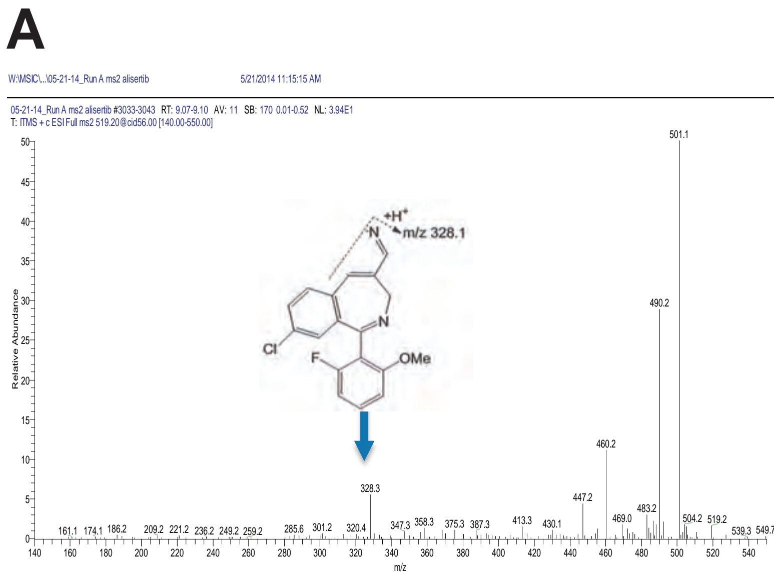
B. Quantification of multiple metastases from 5-6 animals in 3 independent experiments, was used for calculation of fluorescent intensity (mean grey value, presented as relative units of intensity RUI) of corresponding area +S.E.M, normalized to the vehicle; unpaired t-test comparison of vehicle/MLN8237, p as indicated in the figure.

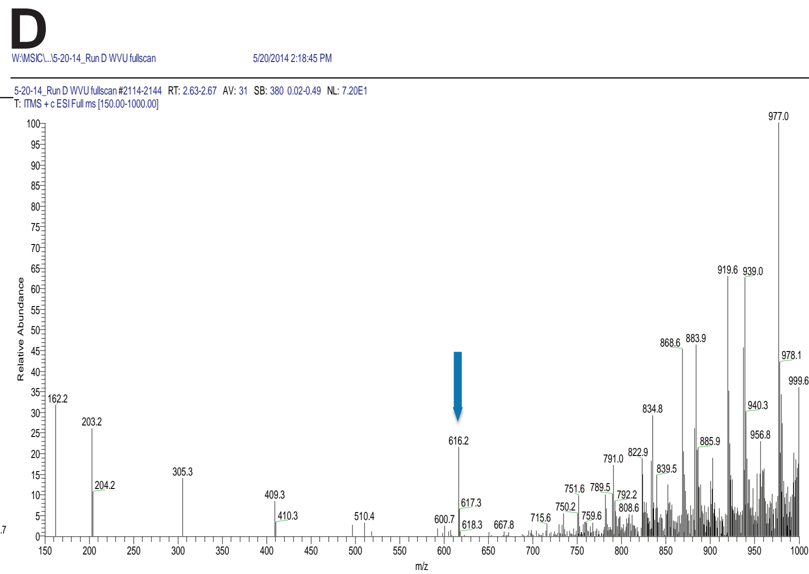
Supplementary Figure 8, Kozyreva

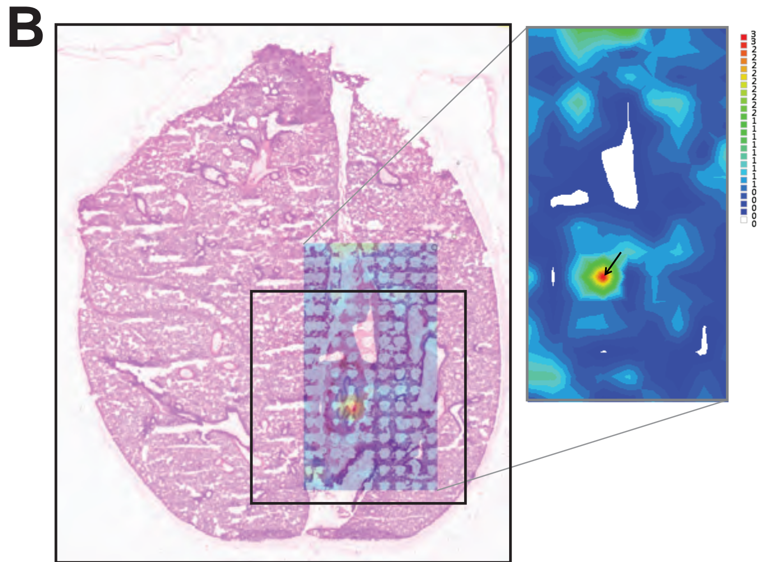


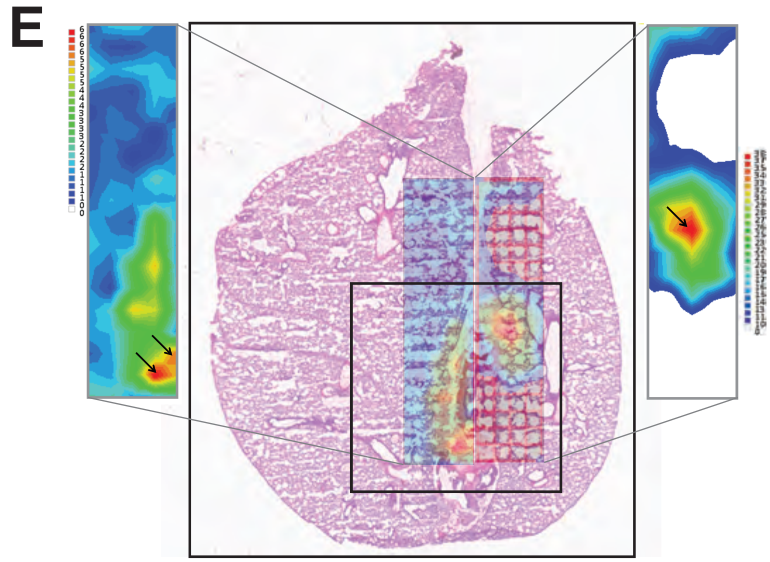
A. Cell growth analysis after 48h of treatment with 100nM of MLN8237 , 3nM of eribulin, combination or vehicle. Tumor cells PEN65 derived from PDX, 100 cells per treatment, n=3. one-way ANOVA, p values as indicated compared to vehicle. **B** Analysis of the nuclear morphology and cleaved caspase-3 staining for apoptotic cells. One-way ANOVA, p values as indicated in the figure.

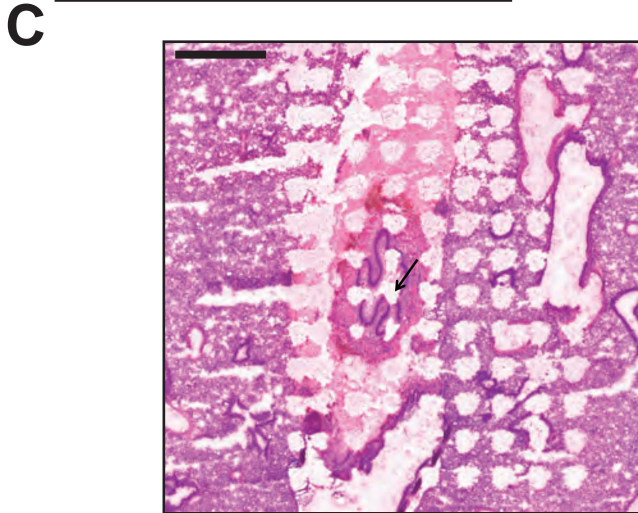
Supplementary Figure 9, Kozyreva

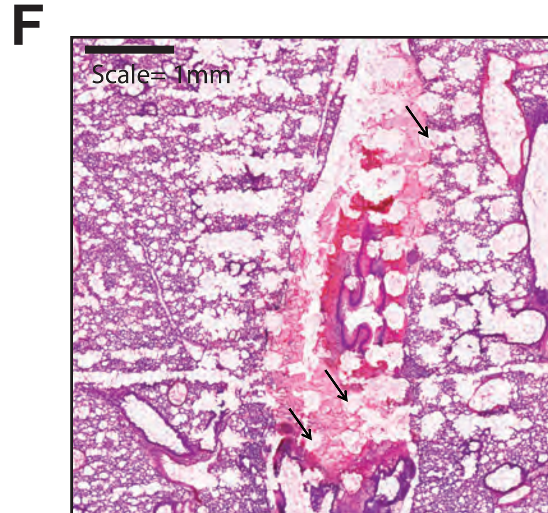












A. LAESI-MS MS/MS fragmentation of MLN8237 (m/z 519.1) in dosed animal tumor tissue. Arrow denotes confirmed fragment ion m/z 328.2 . **B.** Ion map [M+H]+ of MLN8237 MS/MS fragmentation ion m/z 328.2 ± 1.0 with H&E overlay. Arrow denotes where drug was detected. **C.** Post LAESI-MS analysis slide was H&E stained and scanned with VS-120 microscope. **D.** LAESI-MS full scan spectra from dosed animal tissue. Arrow denotes [M+H]+ of heme m/z 616.2. **E**. Ion map [M+H]+ of heme m/z 616.2 ± 1.0 with H&E stain overlay. **F.** H&E stain post-analysis and scanned with VS-120 microscope. Arrows denote the approximate location of heme.

Supplementary Figure 10, Kozyreva