Supplementary Information (SI) for Nanoscale.

SUPPLEMENTARY DATA

Identification of DYRK1b as a novel regulator of small extracellular vesicle release using a high throughput nanoscale flow cytometry screening platform.

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SUPPLEMENTARY FIGURE 1

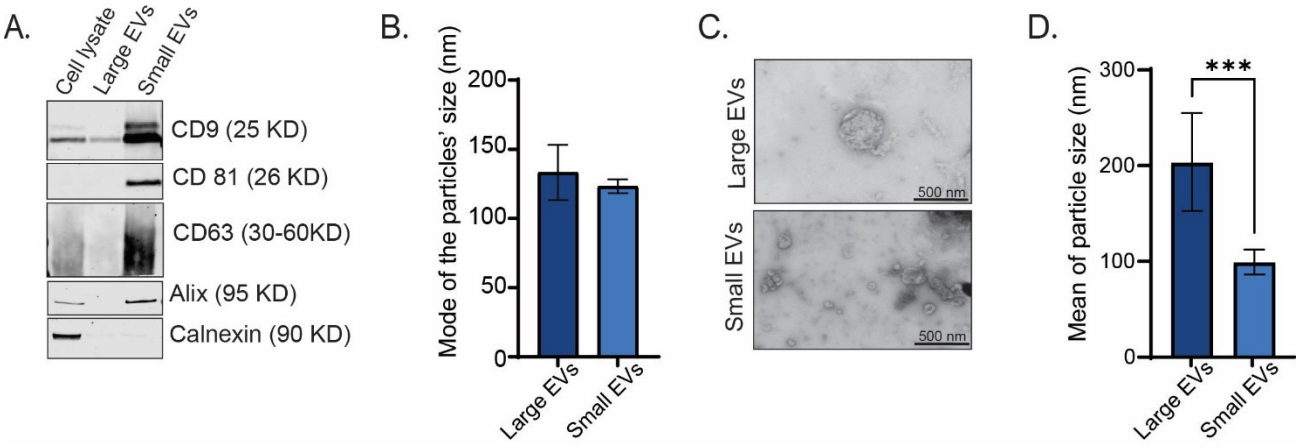

Figure-S1. EV characterization. (**A**) Representative western blot of EV-markers CD9, CD63, CD81, Alix, and EV contaminant marker calnexin from cell lysates, large EVs, and small EVs.(**B**) EVs isolated in (A) were diluted 1:200 in PBS and run on NanoSight LM10 for nanoparticle tracking analysis. The mode of the particle size ±SEM in each sample is represented by the bar graph. (**C**) EVs isolated in (A) were fixed by dilution 1:1 in 4% paraformaldehyde and adsorbed onto formvar/carbon-coated copper 200 mesh grids and were imaged using the scanning transmission mode of a Helios NanoLab 650, fitted with a STEM detector. Scale bar = 500nm. (**D**) Ten images per grid per sample obtained from (C) were analyzed via ImageJ to measure the particle size. The mean of particle size ±SEM in each sample is presented by the bar graph. Mann-Whitney test. ***$p < 0.001$.

SUPPLEMENTARY FIGURE 2

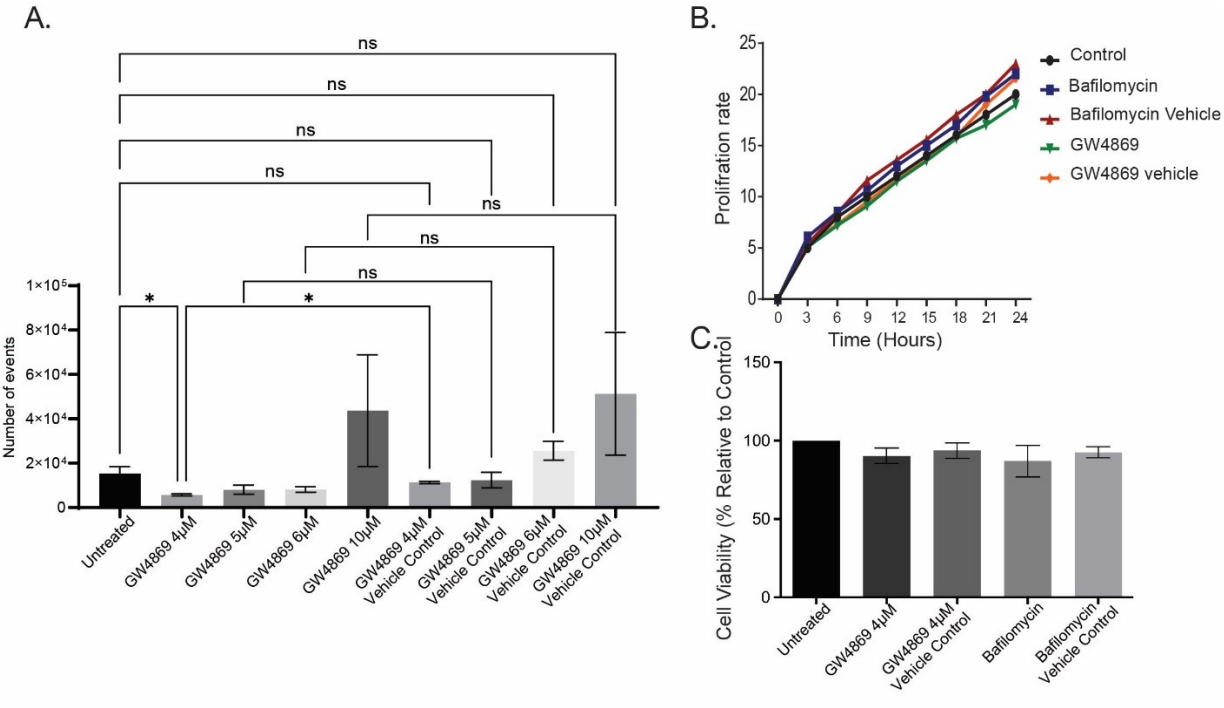

Figure-S2. Bafilomycin and GW4869 optimization (**A**) MDA-MB-231 cells seeded into a 96-well plate (15*10^3/well) were treated with different concentrations of GW4869 for 24 h; 4 µM, 5 µM, 6 µM, and 10 µM. 4 µM GW4869 was the only concentration that significantly reduced the number of EVs. Kruskal-Wallis test with Uncorrected Dunn's Test. ±SEM, n=2-3, ns: not significant, *$p < 0.05$. (**B**) Proliferation rates for MDA-MB-231 cells seeded in a 96-well plate (15*10^3/well), treated with 99.2 nM bafilomycin or 4 µM GW4869 for 24 h, and imaged by an IncuCyte every three hours. ±SEM, n=3. (**C**) Cell viability at end-point of the proliferation assay, (B), quantified using 0.01% thiazolyl blue tetrazolium bromide. The absorbance was measured at 570 nm to determine viability. Compared to vehicle treated control, difference in cell viability per condition was not statistically significant. GW4869 and bafilomycin were dissolved in DMSO; as different volumes for each compound were used, GW4869 and Bafilomycin Vehicle Control (DMSO alone) was matched to the volume of GW4869 and bafilomycin, respectively. ±SEM, n=3.

SUPPLEMENTARY FIGURE 3

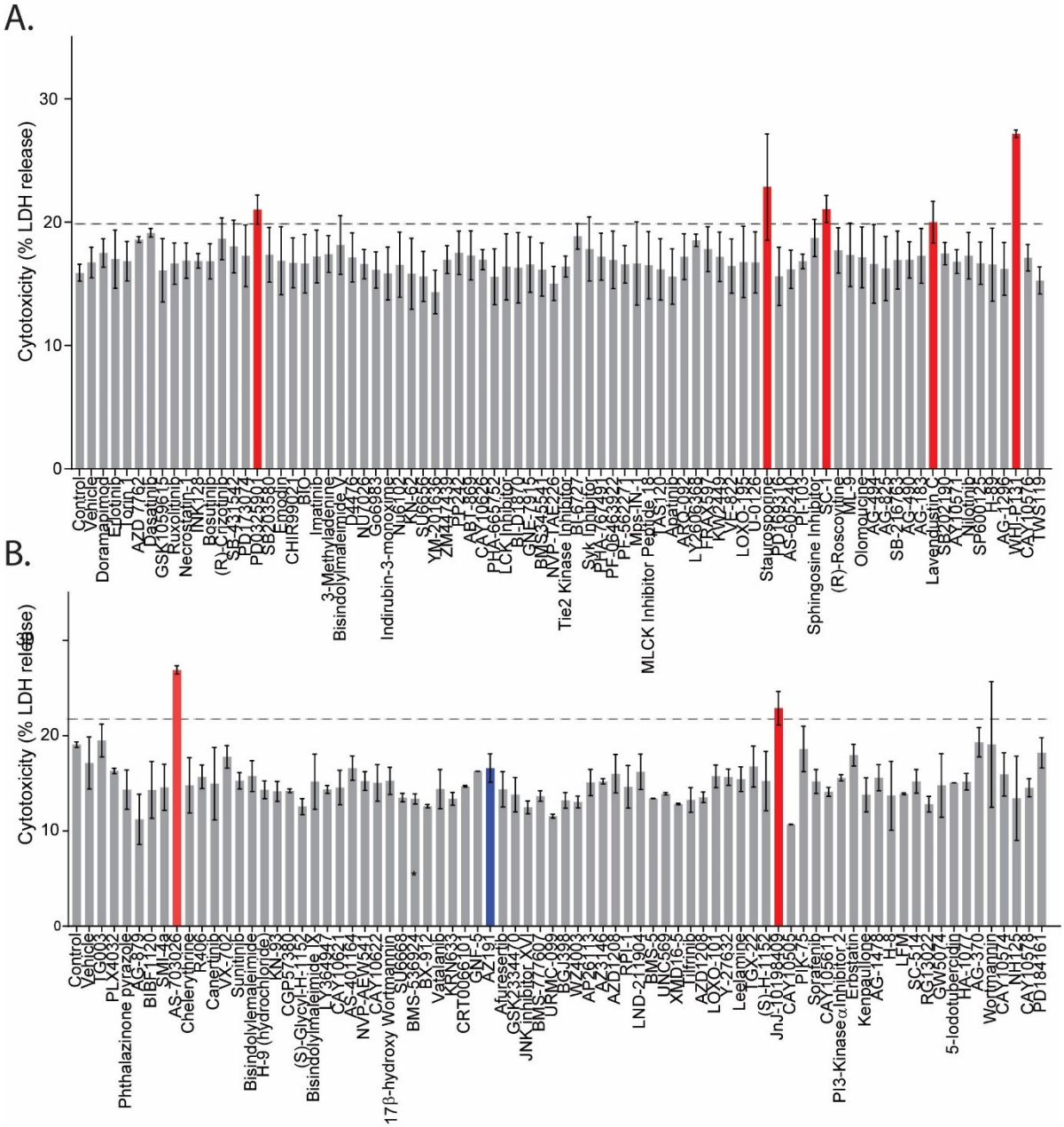

Figure-S3. High-throughput screening of 156 kinase inhibitors on cytotoxicity. MDA-MB-231 cells seeded into 96-well plates ($15*10^3$/well) were treated with 500 nM of each compound for 24 h. Conditioned media was collected and cytotoxicity measured by LDH concentration. LDH absorbance was measured at 490 nm. The dotted line on top and bottom graphs marks $\geq$ 10% increase of cytotoxicity relative to Mean+S.E.M of the vehicle control. The red bars indicate the 7 compounds that were eliminated from the Volcano Plot because of increased cytotoxicity. The lead compound, AZ191, is marked with blue. ±SEM, n=2.

SUPPLEMENTARY FIGURE 4

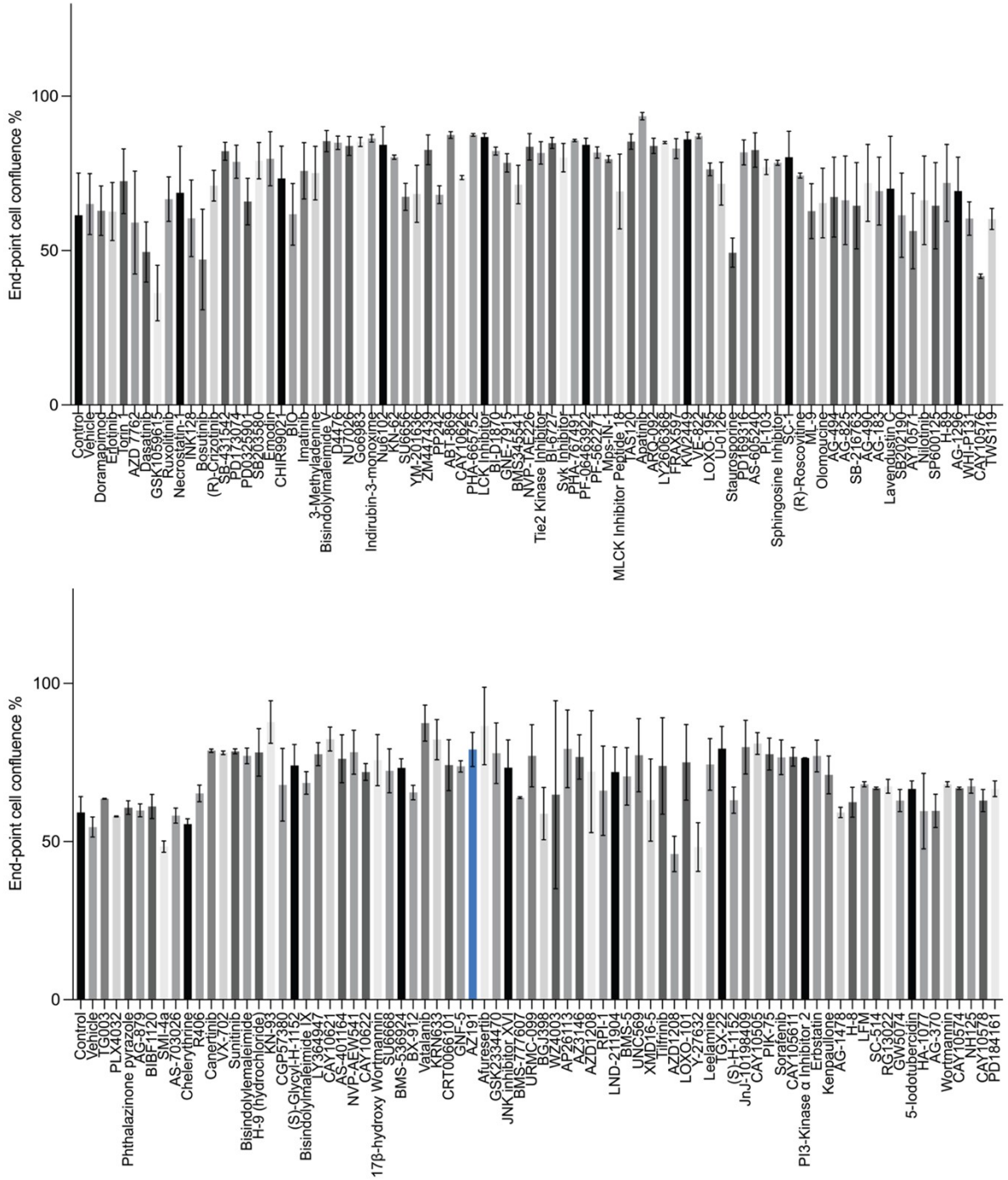

Figure-S4. High-throughput screening of 156 kinase inhibitors on end-point cell confluence. MDA-MB-231 cells seeded into 96-well plates ($15*10^3$/well) were treated with 500 nM of each compound for 24 h. The plate was imaged at 24 h post treatment on the IncuCyte Zoom HD72CLR (Essen BioScience, USA), 9 phase contrast images per well using 20X magnification. The confluence of cells was analyzed via IncuCyte Zoom 2018A software according to the manufacturer's instructions for a label-free proliferation assay. The lead compound, AZ191, is marked with blue. ±SEM, n=2.

Table S1. Antibody product information and concentrations used in the study.

Antibody	Catalogue number	Company	Concentration
CD63	Ab59479	Abcam	1:1000
CD9	CBL162	EMD Millipore	1:1000
Alix	Ab88743	Abcam	1:5000
Calnexin	Ab22595	Abcam	1:5000
Beta-actin	0000120485	Sigma	1:2000
Vinculin	Ab91459	Abcam	1:5000
DYRK1b	Sc-390417	Santa Cruz Biotechnologies	1:50
DYRK1a	Sc-100376	Santa Cruz Biotechnologies	1:50
Secondary anti mouse IgG IRdye® 680RD	926-68070	LI-COR	1:10000