Supplemental Information

Supplemental Materials and Methods

Cell Authentication and Sequencing

Fibroblast and epithelial cell lines used were authenticated by Genetica DNA Laboratories. All epithelial cell lines were a 97%-100% match to the correct cell line in both the ATCC and DSMZ database. L3.6 cells and fibroblasts did not have a match in either database, suggesting there was no cell contamination. CAF1 and CAF2 cell lines (pancreatic tumor derived), which were used in the majority of studies, were sequenced at the KRAS exon 2 locus. Cells were washed with PBS, lysed with lysis buffer containing 25mM NaOH, 0.2mM EDTA, and boiled at 95°C for 30 minutes. Lysate was treated with 40mM Tris-HCl, 1.8mM EDTA, vortexed, and spun at 14,000 RPM for 10 minutes. PCR was performed with extracted DNA (forward primer: 5′-GGCCTGCTGAAAATGACTGA-3’; reverse primer: 5′-GTCCTGCACCAGTAATATGC-3′), and run on a 2.5% agarose gel via electrophoresis. DNA was spliced from gel and DNA extracted from gel with GeneElute™ Gel Extraction Kit (Sigma) according to manufacturer’s protocol. Samples were distributed to the Notre Dame Bioinformatics and Genomics Core for Sanger sequencing with an Applied Biosystems 96-capillary 3730xl DNA Analyzer.

Cell Viability Assays

Cell media was collected and adherent cells were trypsinized. Both cells in the cell media and adherent cells were spun down and counted using the Bio-Rad automated cell counter. Live adherent cells were counted as well as detached dead cells in the media.

TACS® MTT assays were performed according to the manufacture's protocol: https://www.trevigen.com/docs/protocol_4890-XX-K.pdf.

Immunocytochemistry

Immunocytochemistry was performed according to standard procedure in chamber slides: http://www.abcam.com/protocols/immunocytochemistry-immunofluorescence-protocol. Briefly, cells were washed with PBS, treated with 4% paraformaldehyde and 0.5% Triton™ X-100, goat serum blocking buffer, primary antibody, and secondary antibody in blocking buffer. See Supplementary Table S2 for a list of antibodies used.

miRNA-Seq

miRNA-Seq was performed by the Genomics and Bioinformatics Core at the University of Notre Dame. Initial RNA concentration and integrity was assessed using the Qubit 2.0 Fluorometer (Life Technologies Corp., Carlsbad, CA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The sequencing library was constructed using the NEBNext Small RNA Library Prep Set and protocol companion (New England Biolabs, Ipswich, MA). Briefly, starting with 1 mg of total RNA, a 3'-tagging sequence (the 3' Adaptor Oligo) is ligated to the 3' end of the RNA. Small RNA with 3' ends containing either a 2',3'-OH or 2'-O-Me,3'-OH are efficiently tagged in the reaction. Excess 3' Adaptor is removed using a reverse transcription primer. A 5'-tagging sequence (the 5' Adaptor Oligo) is ligated to the 5' end of 5'-monophosphorylated RNA. cDNA was synthesized using reverse transcriptase. The cDNA is then amplified by PCR using a standard forward primer and an indexing reverse primer. The library was then cleaned up using a Zymo DNA Clean & Concentrator-5 (Zymo Research, Irvine, CA). Size selection is performed on the library using the BluePippin (Sage Science, Inc.,

Beverly, MA) and the 3% agarose gel cassette (Sage Science, Inc.). To calculate final library concentration the quantity and average fragment length for each library was measured using the Agilent DNA 7500 Assay (Agilent Technologies, Santa Clara, CA), and Qubit High Sensitivity DNA Assay (Life Technologies Corp.) Libraries were normalized to 2nM in buffer EB (Qiagen, Santa Clarita, CA) and combined in equal molar amounts. Single read sequencing of 51bp was performed using the Illumina MiSeq platform and MiSeq Reagent Kit v2 (50 cycles) (Illumina, Inc.). A total of 11.7 million single reads passing filter were attained.

Processing of miRNA sequences, including adapter trimming, mapping, and statistical analysis was performed in iMir. iMir utilizes Cutadapt for adapter trimming, Bowtie for read mapping, and the DESeq bioconductor package for differential expression analysis of replicated samples. Processed reads were assessed for quality with FastQC.

Study data are deposited in NCBI GEO under accession number (pending).

siRNA Knockdown

120,000 cells were plated per well of a 12-well plate in 893µl of complete DMEM. 150ng of Snail-targeting (QIAGEN; SI00083398; 10nM) or negative control siRNA (QIAGEN; SI03650325; 10nM) was diluted in 100µl DMEM without serum and 6µl HiPerfect Transfection Reagent (QIAGEN). Diluted siRNA was vortexed and incubated at room temperature for 10 minutes, then added directly to the cells. 48 hours later cells were washed with PBS and RNA was collected with TRIzol®. The exact method was used

with a fluorescently tagged control siRNA (Cell Signaling; 6201S) to ensure uptake of siRNA into cells.

Subcutaneous Mouse Model

AsPC1 cells and CAF1 cells were trypsinized, spun down, washed in PBS, and resuspended in PBS with $CaCl_2$ and $MgCl_2$. NOD/SCID male mice (species *Mus musculus*) were shaved and injected subcutaneously with one million AsPC1 cells and 200,000 CAF1 cells in 100µl PBS. Mice were 13-15 weeks old with an average weight of 28g at time of implantation. Two weeks post cell implantation mice were intraperitoneally-injected with 100µl PBS and 200µl 3.75% DMSO in PBS (control; DMSO+PBS), 100mg/kg mouse gemcitabine in 100ul PBS and 3.75% DMSO in 200µl PBS (DMSO+GEM), or 100ul PBS and 3.75% GW4869 in 200µl PBS (GW4869+PBS) twice weekly for two weeks. Mice were randomly allocated into each treatment group (n=6) by randomly assigning 1-2 mice per cage to each group. Investigators were blinded at time of tumor measurements by writing down the size of each tumors next to mouse I.D. prior to knowledge of the assigned treatment group. Tumors were measured with a digital caliper utilizing the formula volume=0.5(length x width^2). Net tumor growth was utilized to generate data, measuring tumor growth from the first day of drug treatment. GW4869 (Sigma) was maintained in DMSO at 8mg/ml and was intraperitoneally injected into mice at 0.3mg/ml in 200ul PBS resulting in 2-2.5µg GW4869/g body weight as previously described [41].

Supplemental Table S1

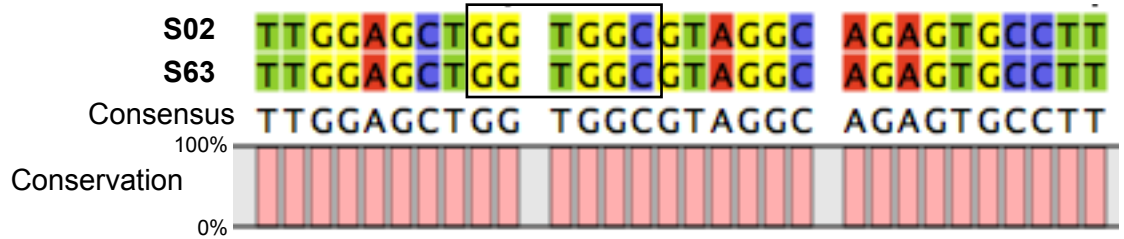
microRNA ID	Fold Change	log2FoldChange
hsa-miR-146a-5p	10.49805228	3.392049782
hsa-miR-4301	10.14629571	3.342881208
hsa-miR-139-5p	9.582667558	3.26042732
hsa-miR-585-3p	4.917201984	2.297837618
hsa-miR-4725-3p	4.633368096	2.212061301
hsa-miR-615-3p	4.58614293	2.197281318
hsa-miR-4787-3p	4.515596324	2.174916521
hsa-miR-4461	0.246877743	-2.018131314
hsa-miR-758-3p	0.246373185	-2.021082853
hsa-miR-27a-5p	0.239878732	-2.059622841
hsa-miR-490-3p	0.229441151	-2.12380393
hsa-miR-199b-5p	0.227409351	-2.136636517
hsa-miR-335-3p	0.224170866	-2.157329305
hsa-miR-494-3p	0.215677006	-2.213055721
hsa-let-7a-3p	0.203542732	-2.296596387
hsa-miR-4448	0.201524561	-2.310972417
hsa-miR-299-3p	0.195997294	-2.351094359
hsa-miR-20a-5p	0.194194457	-2.36442607
hsa-miR-10a-5p	0.180473765	-2.470138966
hsa-miR-145-3p	0.165668569	-2.593628178
hsa-miR-143-5p	0.151737125	-2.720353987
hsa-miR-411-3p	0.140846089	-2.82780859
hsa-miR-21-5p	0.139408994	-2.842604459
hsa-miR-490-5p	0.138845307	-2.848449681
hsa-miR-143-3p	0.119514112	-3.064747122
hsa-miR-424-5p	0.070334991	-3.829613595

Supplemental Table S1. miR-Seq. Three separate patient-derived PDAC CAF cell lines (CAF1-3) were left untreated as a control baseline or were treated with 1μM gemcitabine for 6 days. MicroRNAs that showed a log2 fold change of at least 2 or -2 in all gemcitabine-treated CAFs is shown in the table.

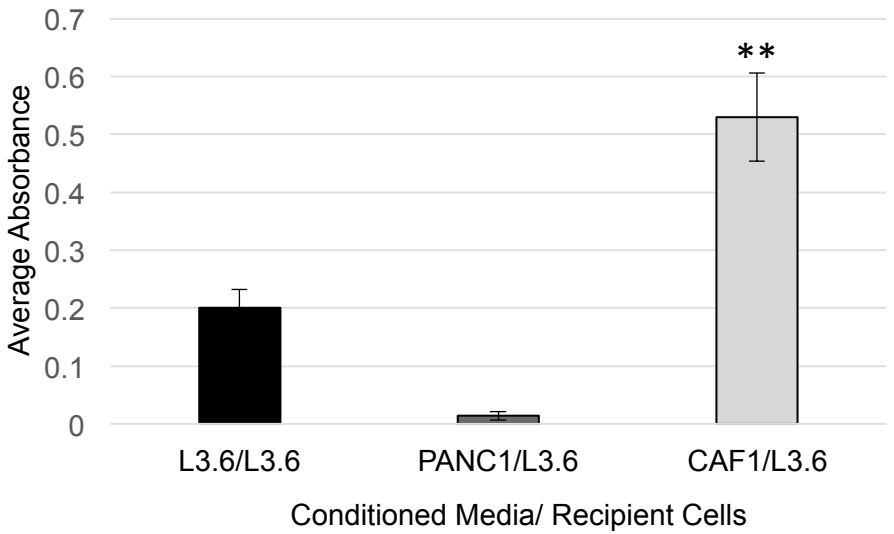
Supplementary Table S2.

Antibody	Company	Catalog Number
Beta Actin	Cell Signaling	4970L
CD81	System Biosciences	ExoAB-CD81A-1
Vimentin	Novus Biologicals	NBP1-40730
α-SMA	Novus Biologicals	NB600-531
Secondary; 488	Life Technologies	A11008
Secondary; 594	Life Technologies	A11020
Secondary HRP-Conjugated	Cell Signaling	7074S

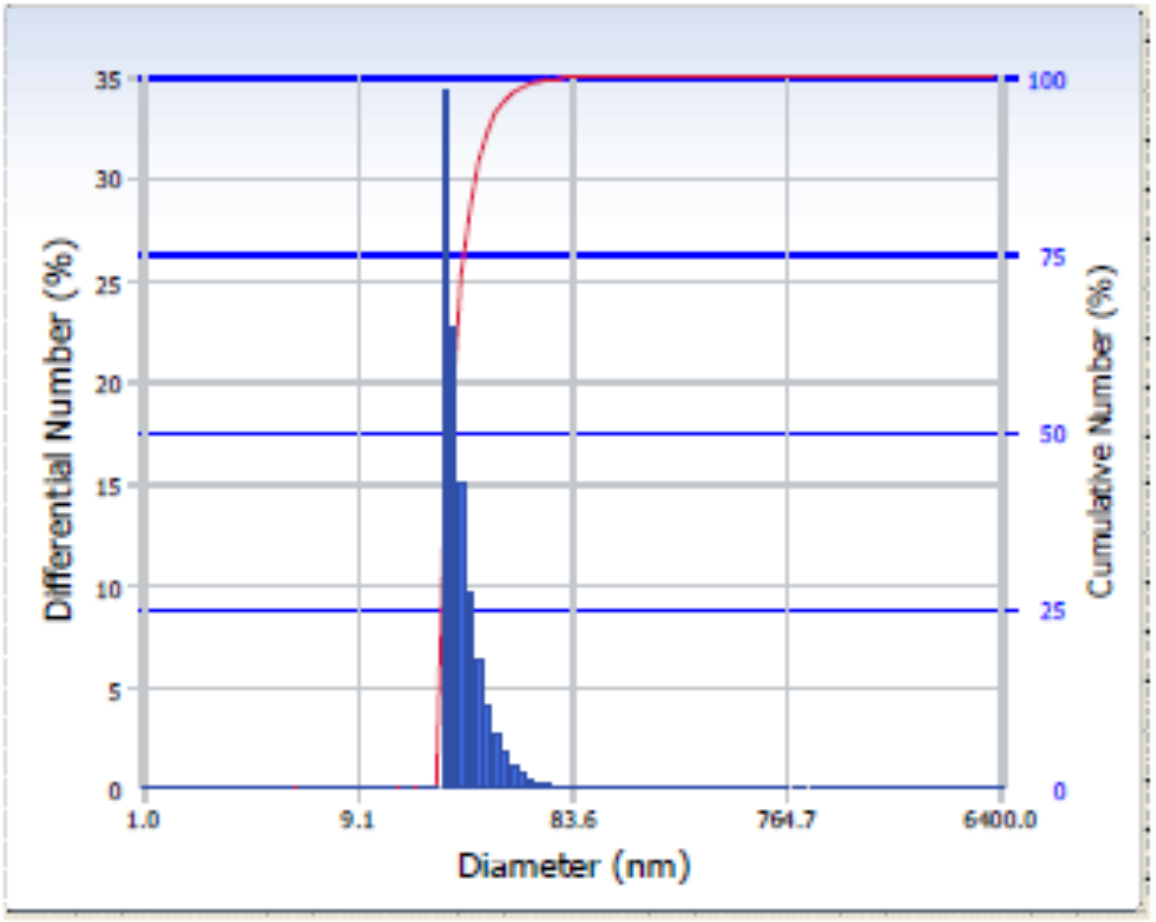
Supplementary Table S2. List of antibodies used during experiments shown in this study.



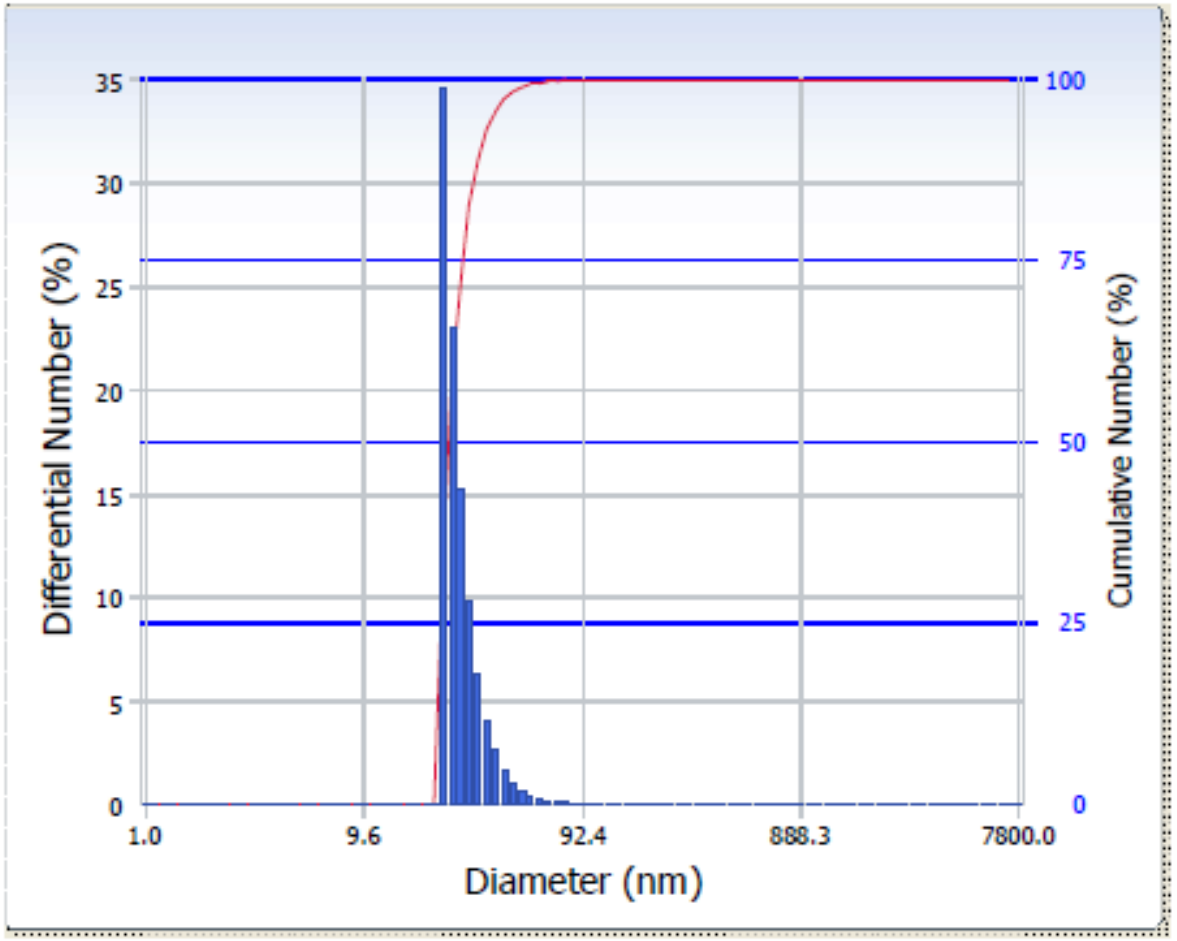
Supplemental Figure S1. Patient derived cancer-associated fibroblasts (CAFs) are KRAS wild type. CAF lines CAF1 and CAF2 were sequenced to determine if KRAS mutations were present in exon 2 at codon 12 and 13 (black outlined area). Both cell lines were identified as KRAS wild type.



Supplemental Figure S2. Proliferation assay (MTT) of L3.6 cells which received cell-conditioned media for 8 days. ***p*-value<0.01



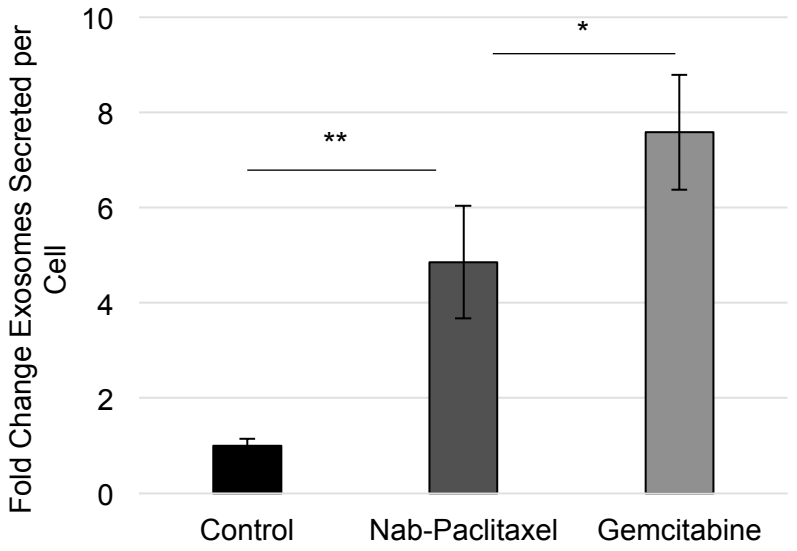
NT-CAF1 Exosome Sample
Particle Size Range (d): 20.9nm-84.8nm



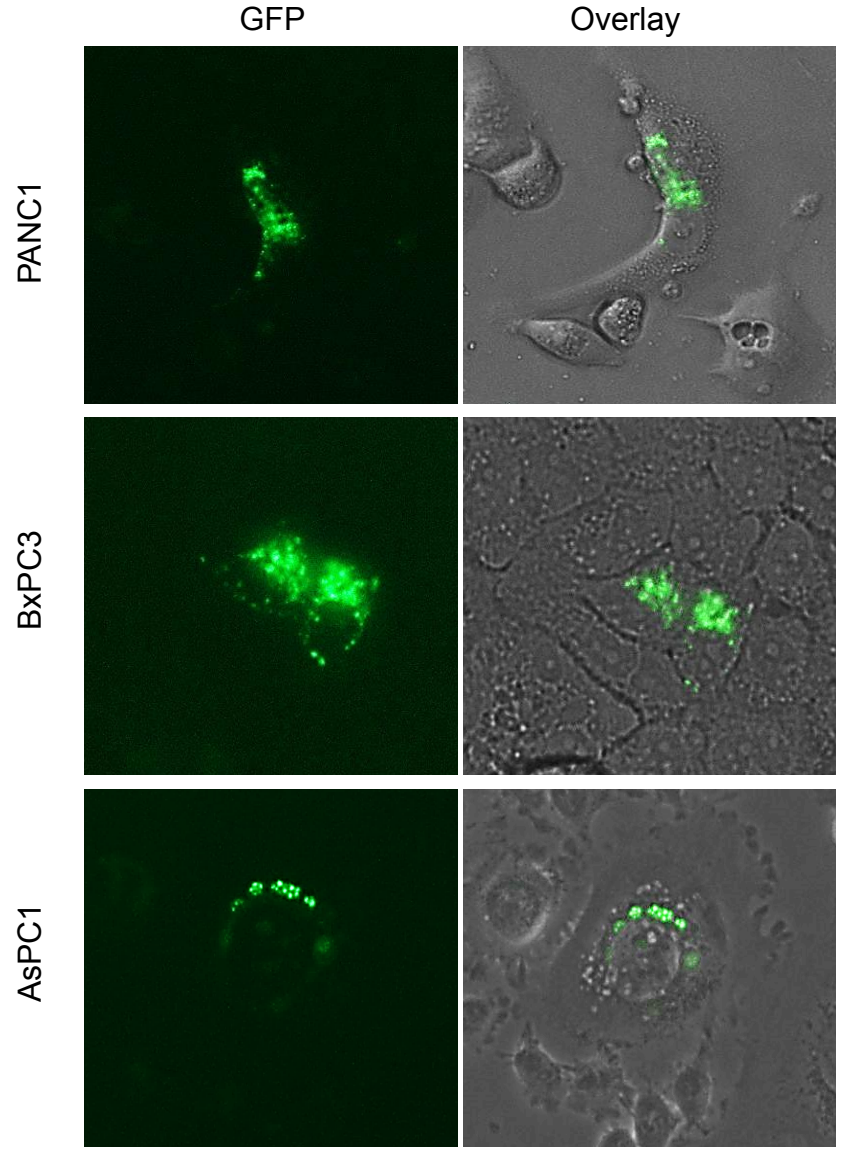
GT-CAF1 Exosome Sample
Particle Size Range (d): 20.4nm-77.0nm

Supplemental Figure S3. CAF1 exosomes were isolated from untreated CAF1s (NT-CAF1) or gem-treated CAF1s (GT-CAF1) and resuspended in PBS. Their size was determined by dynamic light scattering technique using a particle size analyzer.

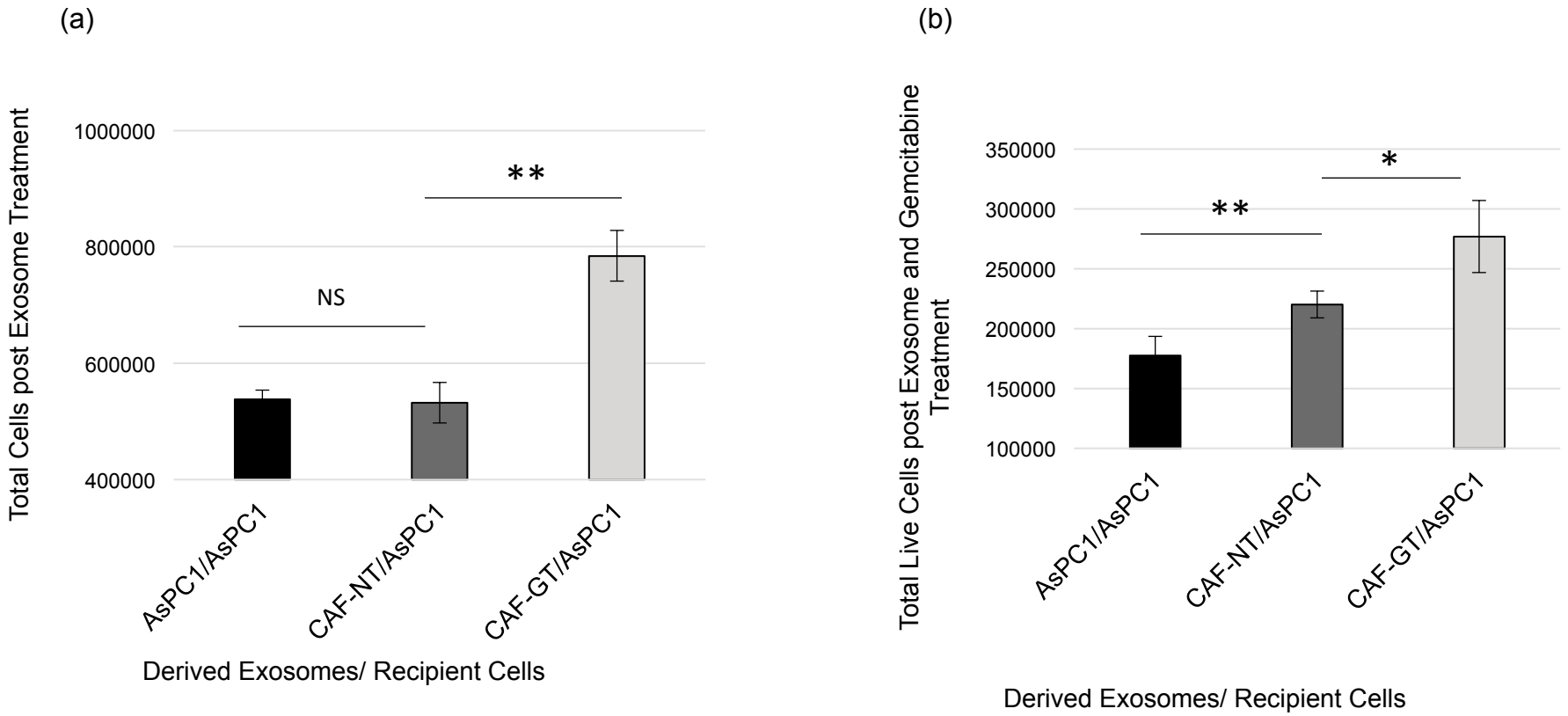
(a)



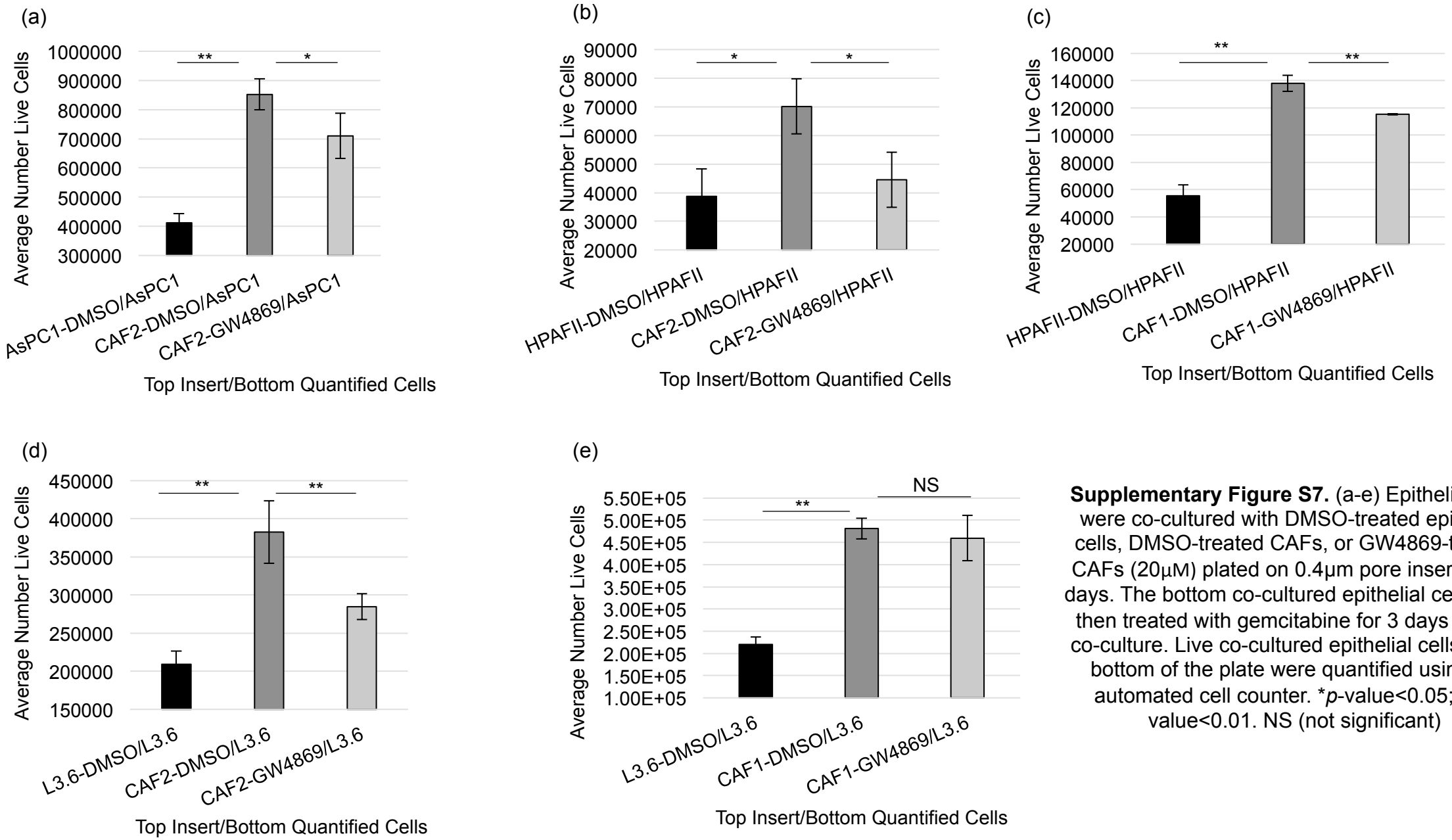
Supplemental Figure S4. (a) CAF1s were plated at 500,000 cells/flask and left untreated (control), treated with 1μM gemcitabine, or 10nM Nab-Paclitaxel for 4 days. Live cells were counted. Exosomes were isolated from conditioned-media and quantified. *p-value<0.05;**p-value<0.01



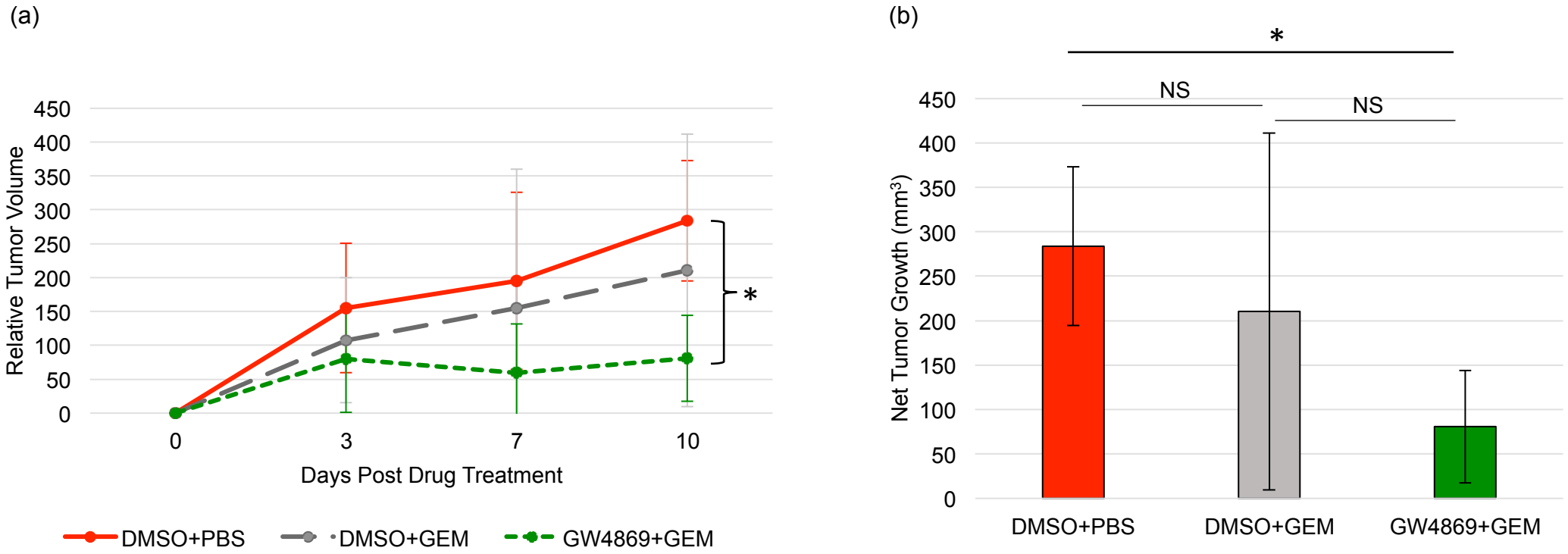
Supplemental Figure S5. Epithelial cells were cultured in CD63-GFP transgenic CAF1 conditioned-media for 2 days. Cells were washed, and GFP-labeled exosomes taken up by epithelial cells are visualized.



Supplemental Figure S6. AsPC1 cells were treated directly with AsPC1 exosomes (AsPC1), untreated CAF1 exosomes (CAF-NT), and GEM-treated CAF1 exosomes (CAF-GT) for 6 days and total cells were counted (a) or were subsequently treated with 1μM gemcitabine for 3 days and live cells were counted (b). **p*-value<0.05;***p*-value<0.01; NS (not significant)



Supplementary Figure S7. (a-e) Epithelial cells were co-cultured with DMSO-treated epithelial cells, DMSO-treated CAFs, or GW4869-treated CAFs (20μM) plated on 0.4μm pore inserts for 3 days. The bottom co-cultured epithelial cells were then treated with gemcitabine for 3 days during co-culture. Live co-cultured epithelial cells at the bottom of the plate were quantified using an automated cell counter. **p*-value<0.05; ***p*-value<0.01. NS (not significant)



Supplementary Figure S8.
NOD/SCID mice were subcutaneously implanted with one million AsPC1 cells and 200,00 CAF1 cells. Two weeks post implantation mice were treated intraperitoneally with DMSO+PBS, DMSO+gemcitabine (GEM), or PBS+GW4869 twice weekly for two weeks. (a) Tumor growth over the course of the ten day post drug treatment. (b) Change in tumor volume from first day of drug treatment to 10 days post initial drug treatment. *p-value<0.05. NS (not significant).