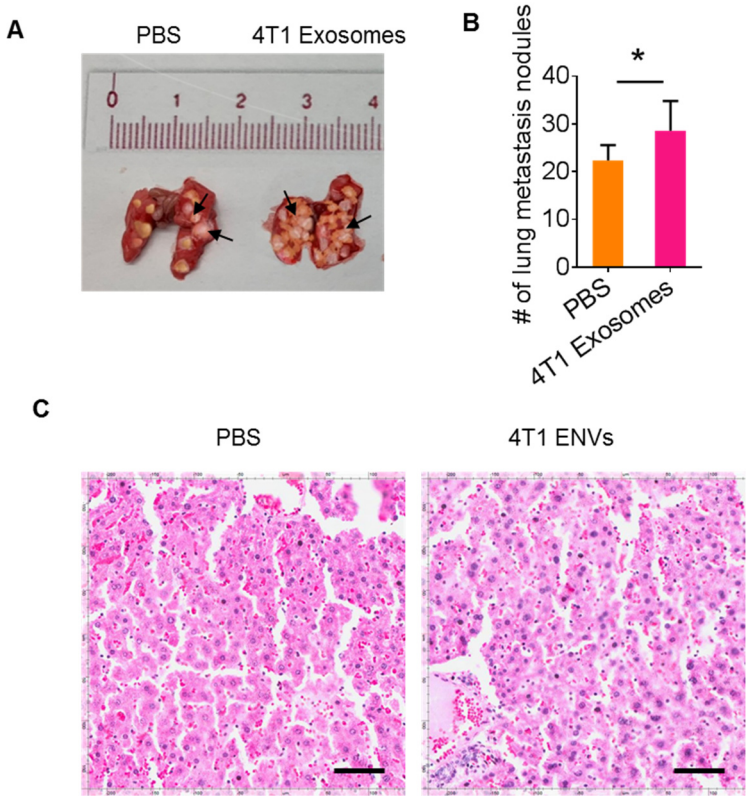
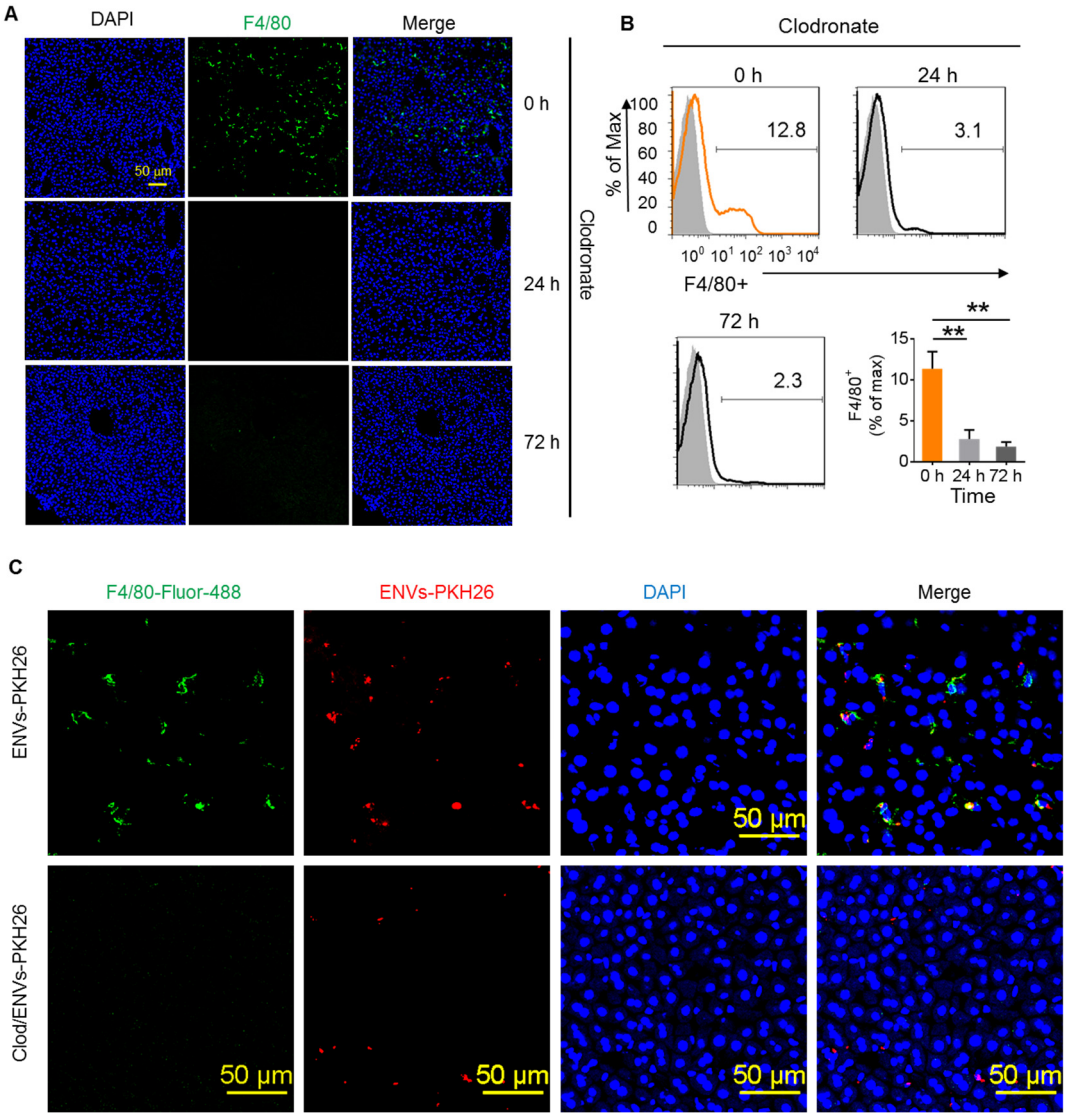
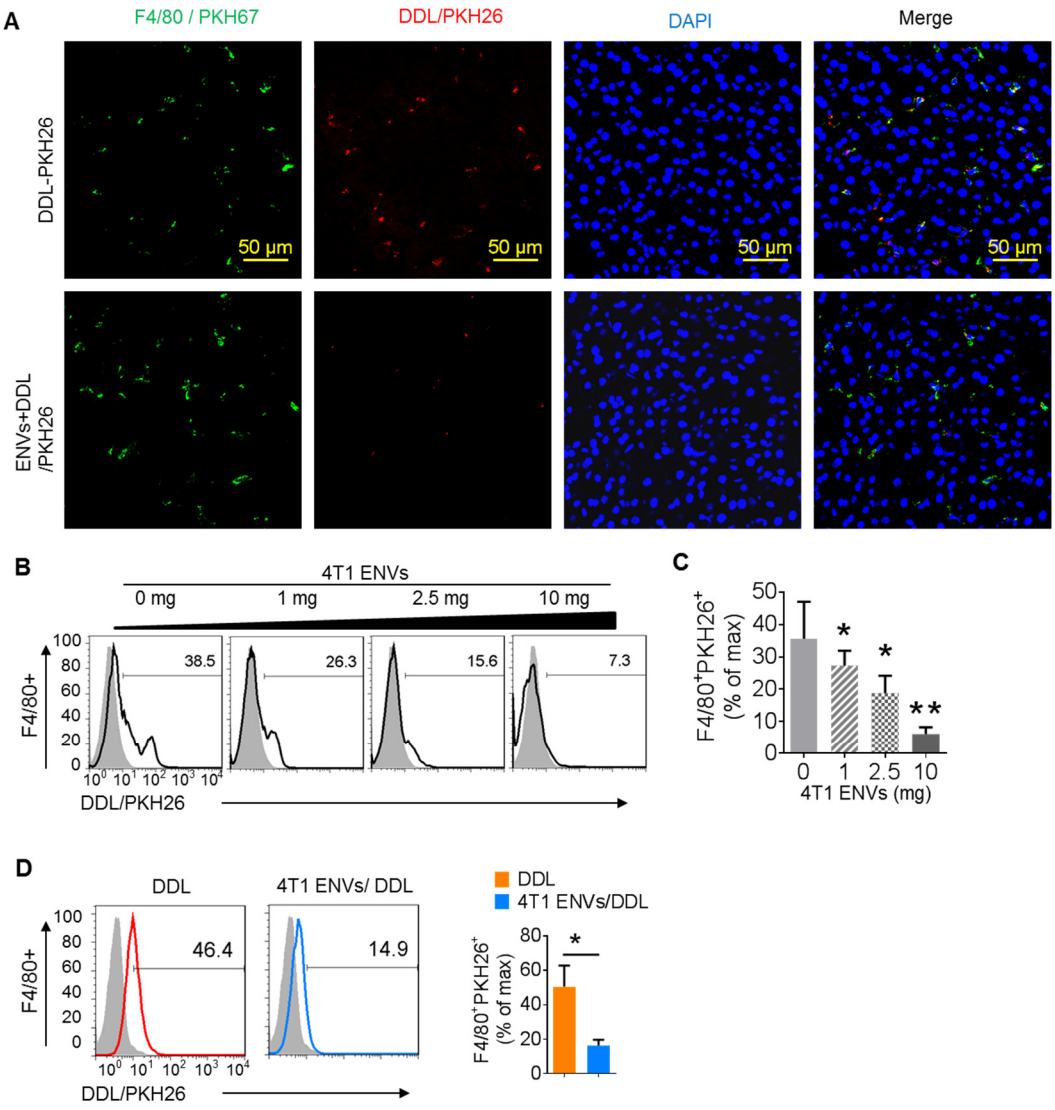
Supplementary Figures



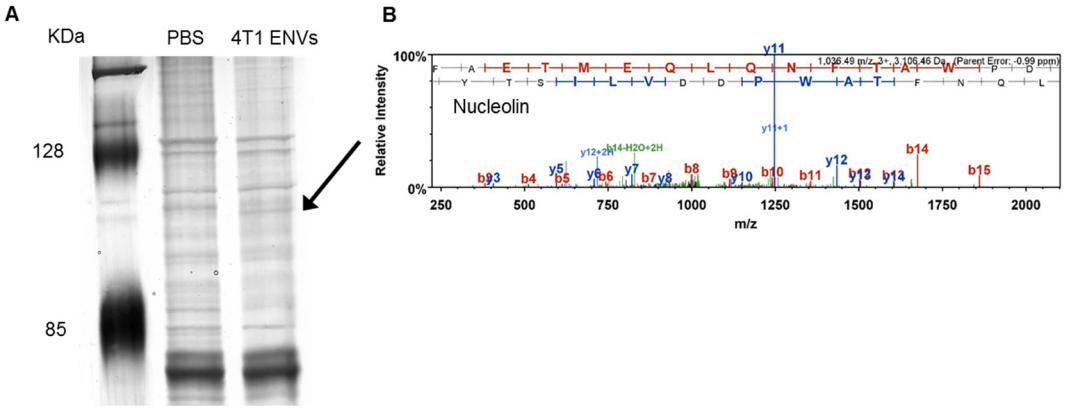
Supplementary Fig. 1 4T1-derived exosomes enhance tumor metastasis. (A) Representative lung (metastatic nodules indicated by arrows) from 4T1 tumor bearing BALB/c mice (n=5) at 21 d objected to breast pad injection of 4T1 tumor cells along with 4T1-derived exosomes (50 pmol per mouse, n=5) or PBS via intravenous (i.v.) injection. (**B**) Quantification of metastasis nodule number (> 1 μM). (**C**) Representative H&E-stained sections of formalin-fixed, paraffin-embedded liver (400x magnification) from tumor bearing BALB/c mice at 28 d objected to breast pad injection of 4T1 tumor cells. Scale bars, 200 μM. Paired t-test. *$P < 0.05$. Each data point was measured in triplicate (error bars, s.e.m.).



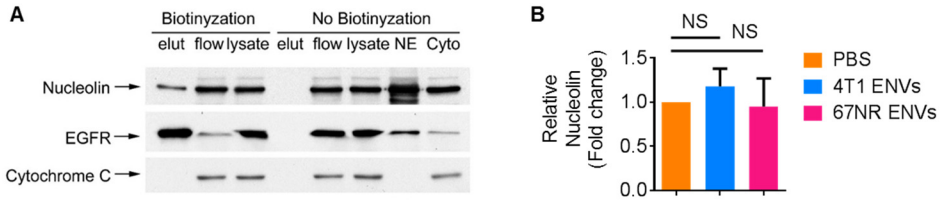
Supplementary Figure 2 Depletion of macrophages in liver by clodronate. (**A**) BALB/c mice given i.v. injection of clodronate (110 mg/kg). Liver kupffer cells (F4/80+, green) of mice visualized with confocal microscopy, assessed 24 h and 72 h after administration of clodronate. Data are representative of three independent experiments. Scale bars, 50 μM. (**B**) Frequency of F4/80+ cells in liver leukocytes from clodronate treated mice assessed by flow cytometry. Numbers above bracketed lines indicate percent F4/80$^+$ cells; gray, isotype-matched control antibody. Quantification of percent F4/80$^+$ cells (bottom right). Paired t-test. $^{**}P < 0.01$. Each data point was measured in triplicate (error bars, s.e.m.). (**C**) PKH26-labeled (red) 4T1 ENVs located in liver kupffer cells (F4/80+, green) from BALB/c mice treated with clodronate for 24 h following i.v. injection of ENVs, visualized with confocal microscopy, assessed 1 d after administration of clodronate.



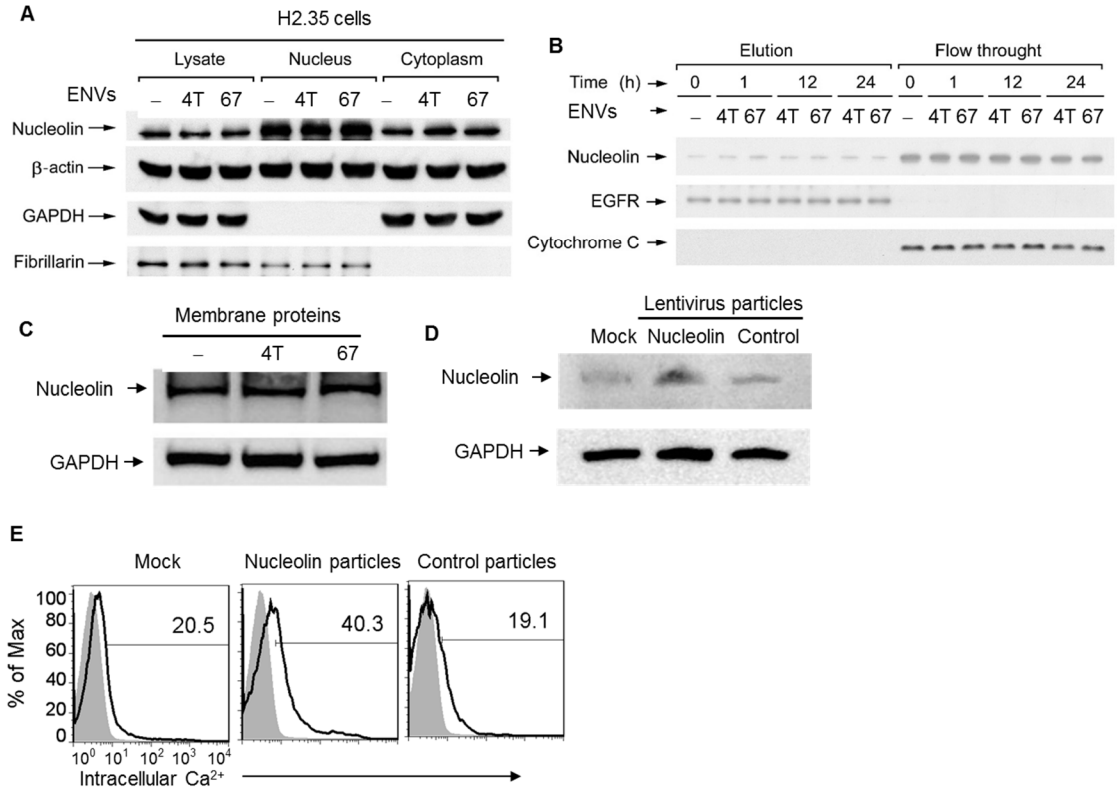
Supplementary Figure 3 4T1 ENVs inhibit macrophage uptaking DDL on dose-dependence. (**A**) PKH26-labeled (red) DDL located in liver kupffer cells (F4/80+, green) from BALB/c mice treated with clodronate for 24 h following i.v. injection of ENVs, visualized with confocal microscopy, assessed 1 d after administration of DDL. (**B**) Pre-treated with PKH26 labeling 4T1 ENVs 24 h prior to treat with DOTAP/DOPE liposome (DDL) for 24 h, frequency of $PKH26^+/F4/80^+$ cells in liver leukocytes from DDL/PKH26 treatment mice assessed by flow cytometry. Numbers above bracketed lines indicate percent $PKH26^+/F4/80^+$ cells; gray, non-staining particles. (**C**) Quantification of results at left. (**D**) Kupffer cells isolated from BALB/c mice pretreated with PKH26 labeling 4T1 ENVs 24 h prior to treat with DOTAP/DOPE liposome (DDL) for 24 h, frequency of $PKH26^+/F4/80^+$ cells in liver leukocytes from DDL/PKH26 treatment mice assessed by flow cytometry. $^*P < 0.05$ and $^{**}P < 0.01$ (two-tailed t-test). Data are representative of three independent experiments (error bars, s.e.m.).



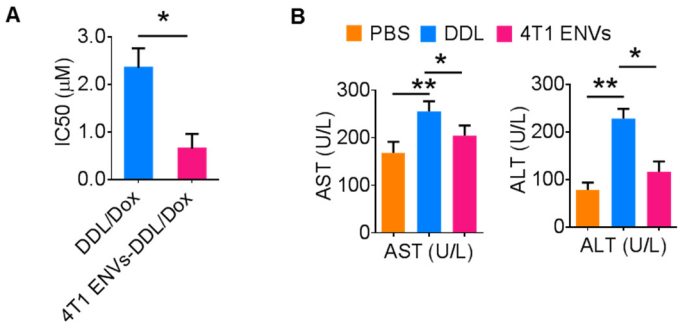
Supplementary Figure 4 4T1 ENVs bind to nucleolin of cell membrane. (**A**) RAW264.7 cells membrane proteins extracted and incubated with 4T1 ENVs at 37°C for 2 h. ENVs complex was isolated by centrifugation at 100,000g and then separated by 10% SDS-PAGE followed coomassie staining. (**B**) Representative spectrum of nucleolin from MALDI-TOF mass spectrometry analysis of the differential band shown in (**A**).



Supplementary Figure 5 Identification of cell surface nucleolin expression. (**A**) RAW264.7 cells were treated or not treated with Sulfo-NHS-SS-Biotin and processed according to the kit protocol. Labeled proteins are isolated with immobilized NeutrAviding Gel. Meanwhile, whole cell lysate, cytoplasmic protein, and nuclear protein were extracted with lysate buffer, buffer B, and buffer C, respectively. All extracts, elution fractions, and flow-through were alalyzed by Western blot for cell surface protein marker EGFR, cytoplasmic protein cytochrome C as negative control, and nucleolin. (**B**) Nucleolin expression in RAW264.7 cells treated with 4T1 ENVs or 67NR ENVs using qPCR. NS, no statistical significance, paired t-test. Data are representative of three independent experiments (error bars, s.e.m.).



Supplementary Figure 6 Effects of 4T1 ENVs on H2.35 cell surface nucleolin. (**A**) Hepatocytes H2.35 cells treated with 0.5g/kg ENVs from 4T1 (4T) and 67NR cells (67). Nucleolin expression in whole cell lysate, nucleus and cytoplasm was estimated by western blot. (**B**) Analysis of cell surface nucleolin on H2.35 cells in time course by Western blot. (**C**) Analysis of whole cell membrane nucleolin on RAW264.7 cells by Western blot. (**D**) Overexpression of nucleolin using CRISPR/dCas9 lentiviral activation particles. Analysis of nucleolin on cell surface from RAW264.7 cells by Western blot. (**E**) Analysis of intracellular Ca^{2+} using FACS. Numbers above bracketed lines indicate percent calcium^{+} cells; gray, negative control. Data are representative of three independent experiments (error bars, s.e.m.).



Supplementary Figure 7 4T1 ENVs have no effect on AST and ALT. (**A**) BALB/c mice (n=5 mice per group) objected with 4T1 ENVs at 0.5g/kg (body weight), DDL at 50 mM/kg (body weight) or PBS as control by i.v. injection every other day for a total of 3 times. The 50% inhibitory concentration (IC_{50}) has been used here to represent the concentration of a drug that is required for 50% inhibition of tumor size. (**B**) Analysis of AST and ALT in serum of mice using Piccolo Xpress chemistry analyzer. *P < 0.05; *P < 0.05 (two-tailed t-test).