SUPPLEMENTAL DATA

Supplement 1

HPLC method for quantification of Ald: 10 µl of liposome sample was lyophized and reconstituted in 10 µl of 30% acetonitrile and 90µl of 18mM amylamine (10-fold dilution). A 40µl sample was chromatographed on a Zorbax300SB-C18 column (4.6 mm ID x150mm, 5 µm) using the following mobile phase gradient: A, composed of 18mM amylamine pH 7.0 (adjusted with acetic acid); and, B, composed of acetonitrile (ACN). The elution gradient was 3% B for 4 minutes, ramp to 100% B in 2 minutes, hold at 100% B for 5 minutes, and ramp down to 3% B in 2 minutes. A calibration stock Ald solution was prepared at 2 mg/ml in 33mM NaCl and further diluted to 500 µg/mL with 18 mM amylamine. Alendronate was detected by charged aerosol detector (CAD, ESA Corona Ultra). The nebulizer gas was compressed nitrogen with flow-rate of 1.68 L/min and pressure 35.1 psi.

Supplement 2

HPLC method for quantification of Dox: Calibration standards of doxorubicin (Fluka) were prepared at various concentrations ranging from 10–100 µg/mL in 25% (v/v) acetonitrile (ACN)/water. For doxorubicin concentration determination, 10 µL of liposome sample was lyophilized and reconstituted with 10 µL 30% (v/v) ACN. To the reconstituted samples, 90 µL of 18 mM amylamine was added. A 40 µL aliquot of this solution was mixed with 120 µL of 30% (v/v) ACN (40-fold dilution). A 40µl sample was chromatographed on a Zorbax 300SB-C18 column (4.6 mm ID x150mm, 5 µm) using the following mobile phase gradient: water with 0.1% (v/v) trifluoroacetic acid (TFA)/acetonitrile with 0.1% (v/v) TFA (A/B). The elution gradient was 25% B for 3 minutes, ramp to 100% B in 8 minutes, hold at 100% B for 4 minutes, and ramp down to 25% B in 3 minutes. Doxorubicin was detected using a diode array detector (DAD) at 234 nm. The flow rate was 1 mL/min.

Supplement 3

Liposome processing for cryoTEM: A liposomal sample was diluted 1:10 in the dextrose-histidine buffer and 5 µL was applied to a 200-mesh copper grid coated with lacey carbon (SPI supplies). Samples were blotted and plunged into liquid ethane, using the Leica automated EM-GP plunger in a controlled environment at 25 °C and 100% relative humidity. Samples were transferred to a Gatan 626 cryo-holder under liquid nitrogen. Cryo-transmission

electron microscopy was performed using a Tecnai T12 transmission electron microscope operating at 120 kV, and images were obtained with a TVIPS F224 CCD digital camera.

Supplement 4:

Stability of PLAD in tissue culture medium was tested for 72 h at 37^0C as a control experiment for the in vitro cytotoxicity assays. No significant leakage of Dox was detected. Results were comparable to those with PLD (Figure S1). This experiment indicates that leakage of Dox from PLAD is not the reason for greater cytotoxicity.

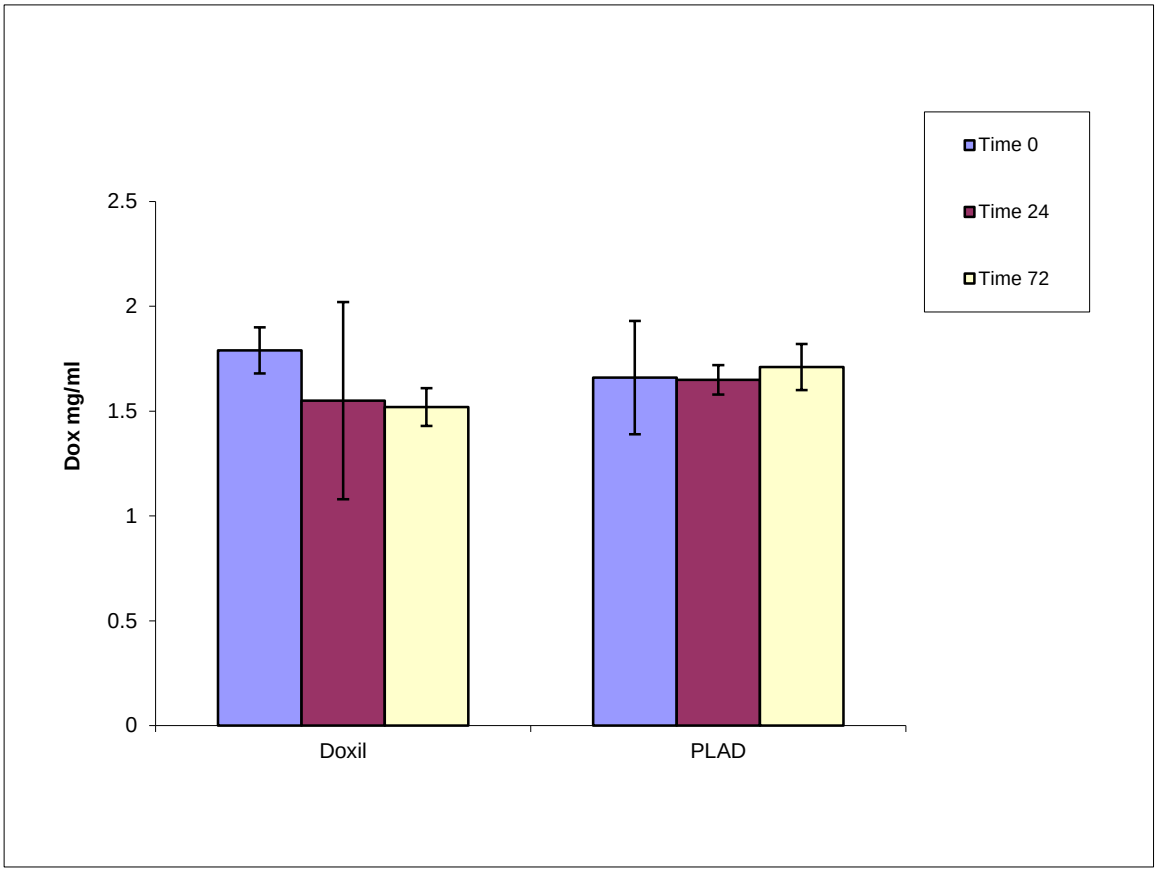

Figure S1. Stability of PLAD incubated at 37 ^{o}C for 72 hours in cell culture medium with 10% bovine serum (free drug removed by Dowex cation-exchange resin beads). There was no significant leakage of Dox from either Doxil (PLD) or PLAD.

Confocal microscopy of human KB ca cells treated with PLAD or PLD for 3 hr confirmed equally weak intracellular uptake of Dox from both PLD and PLAD (Fig S2), as expected from non-targeted stealth/pegylated liposomes and confirming non leakage of drug.

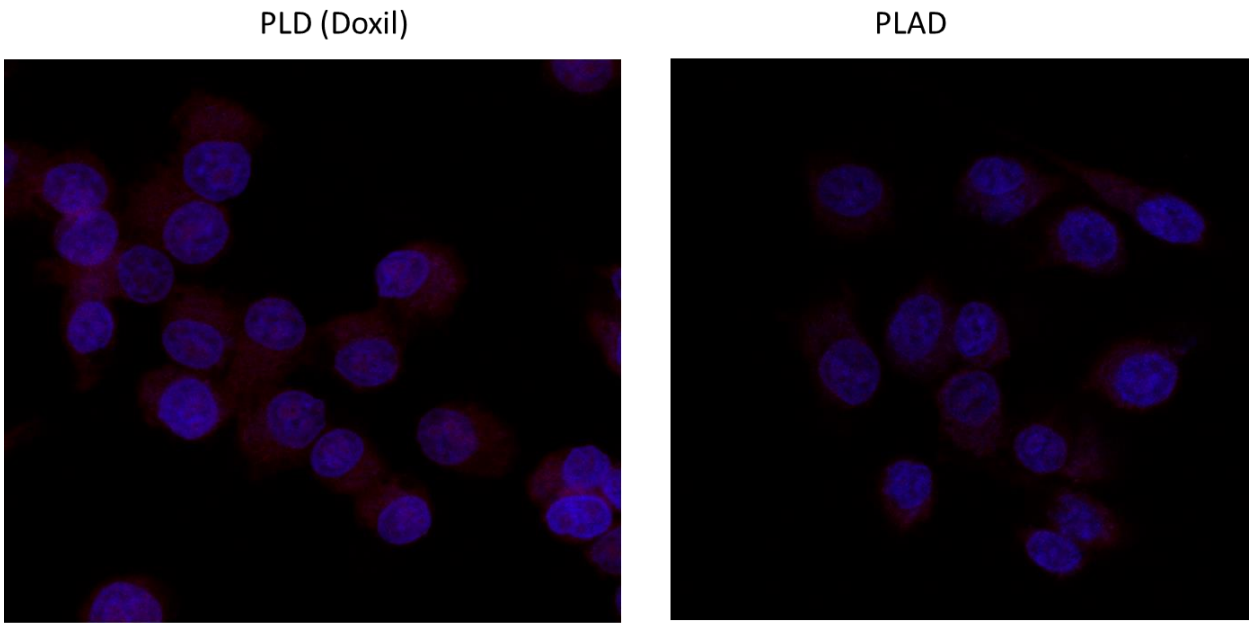

Fig S2: Confocal imaging of KB cells: incubated for 3 hr with PLAD or PLD liposomes (5µg/ml based on Dox content). Cells were stained with DAPI, and observed using a Zeiss Model 410 confocal microscope (488_{ex}, 520_{em}). Weak cytoplasmic fluorescence of Dox was observed and was similar in PLAD and PLD treated cells. In few cells, Dox fluorescence appears to spread to nucleoli.

Supplement 5:

Individual tumor growth curves of mice inoculated with M109R and 4T1 tumors and treated with PLD and PLAD, corresponding to experiments in Figures 10A and 10B are presented in Figure S3.

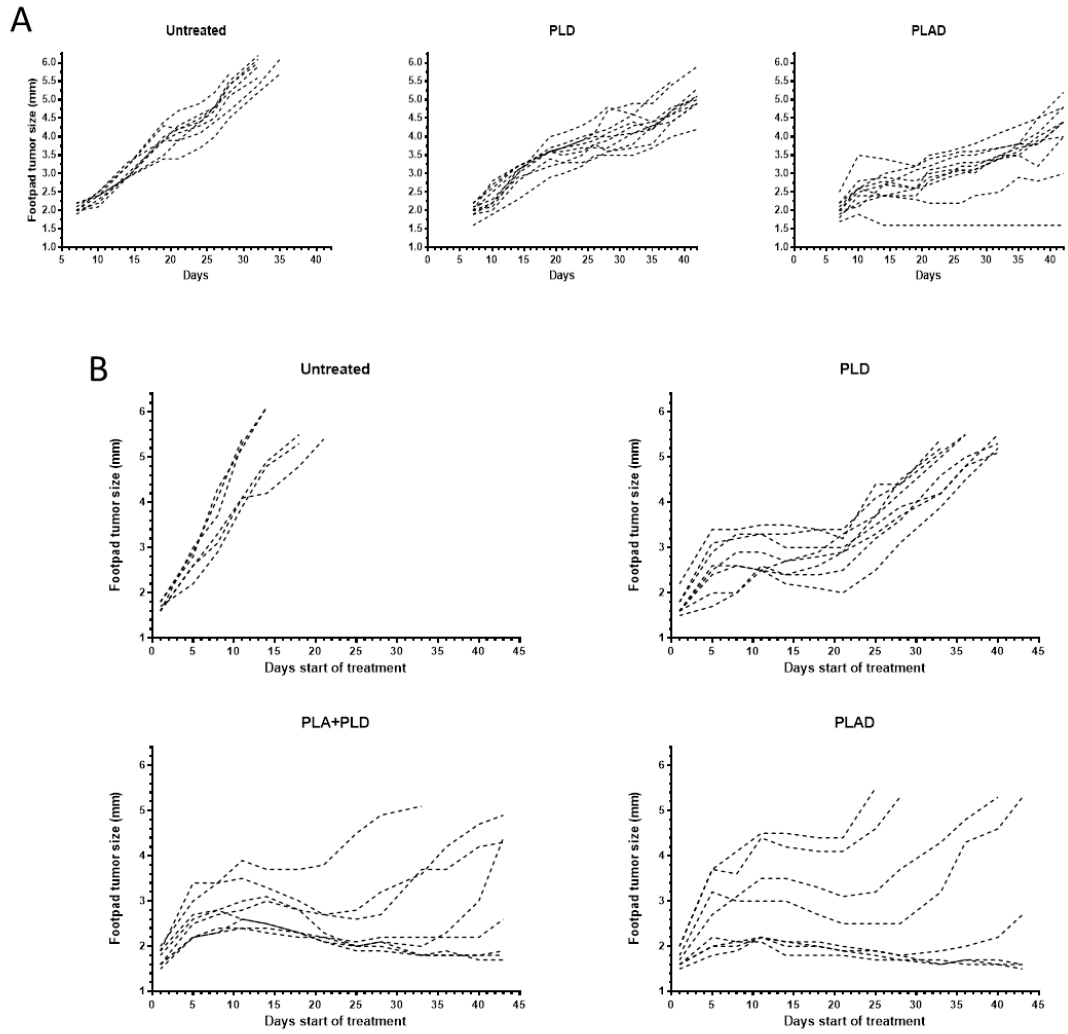

Figure S3. Individual mouse tumor growth curves (see Figures 10A and 10B). A- M109R tumor; B- 4T1 tumor.