**Supplementary Fig. S1**

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**Supplementary Fig. S1. The effect of cyclophosphamide on liposome delivery is dose dependent, requires a predose rather than a co-injection, and is not mediated by changes in blood clearance.** (**A**) BT474-M3 tumor bearing mice (n=3-4/group with bilateral tumors) were predosed with either PBS (blue squares) or with cyclophosphamide (IP) at 40 mg/kg (light red diamonds), 80 mg/kg (red diamonds) or 170 mg/kg (dark red diamonds) 4 days prior to HER2-tPLD (3 mg/kg). 24 h post-liposome injection, tumors were collected and processed for quantification of doxorubicin by HPLC. (**B**) BT474-M3 tumor bearing mice (n=4-5/group) received either HER2-tPLD alone (3 mg/kg; blue squares) or were co-injected with cyclophosphamide (170 mg/kg, IP) and HER2-tPLD (3 mg/kg) (orange diamonds). 24 h post-liposome injection, tumors were collected and processed for quantification of doxorubicin by HPLC. BT474-M3 (**C**) or SUM190 (**D**) tumor bearing mice (n=5/group) were predosed with either PBS (no C), or with cyclophosphamide (170 mg/kg, IP) for 2, 4, or 5 days as indicated prior to PLD (3 mg/kg). 24 h post-liposome injection, tumors were collected and processed for quantification of doxorubicin by HPLC. (**E**) BT474-M3 tumor bearing mice (n=5/group) received either HER2-tPLD alone (3 mg/kg; blue squares) or received cyclophosphamide (170 mg/kg, IP) 4 days prior to HER2-tPLD alone (3 mg/kg; green diamonds). An additional group of mice was co-injected with HER2-tPLD (3 mg/kg) and cyclophosphamide (170 mg/kg, IP; orange diamonds). Blood samples were collected at 5 min, 30 min, 2 h, 6 h and 24 h post liposome injection, and they were processed for quantification of doxorubicin by HPLC.

**Supplementary Fig.S2**

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**Supplementary Fig. S2. Cyclophosphamide induces DNA-damage and tumor cell apoptosis.** Mice (n=5/group) were dosed with cyclophosphamide (170 mg/kg, IP) and tumors were collected 2-5 days post-injection. Tumor sections were stained for γ-H2AX, cleaved caspase 3, cleaved PARP and cytokeratin. Slides were scanned and images were analyzed to quantify the percent of γ-H2AX positive cells (**A**), the percent of cleaved caspase 3 positive cells (**B**), the percent of cleaved PARP positive tumor cells (**C**).

**Supplementary Fig. S3**

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**Supplementary Fig. S3. Effects of cyclophosphamide, ifosfamide, paclitaxel and eribulin on HER2-tPLD delivery, stromal cell density, total cell density, and interstitial space area.** (**A**) SUM190 tumor-bearing mice (n=4/group) were predosed with either PBS (no agent, blue squares), paclitaxel (P, green triangles) or Cremophor (empty green triangles) prior to injection of HER2-tPLD (3 mg/kg). 24 h post HER2-tPLD injection, mice were sacrificed and tumors were collected and analyzed by HPLC to quantify the doxorubicin content. (**B**) BT474-M3 tumor-bearing mice (n=2-4/group with bilateral tumors) were predosed with either PBS (no agent, blue squares) or a single dose of paclitaxel given either 2 weeks (P, 2 weeks; green triangles) or 3 weeks (P, 3 weeks; dark green triangles) prior to injection of HER2-tPLD (3 mg/kg). 24 h post HER2-tPLD injection, mice were sacrificed and tumors were collected and analyzed by HPLC to quantify the doxorubicin content. (**C**) MDA-MB-361 tumor-bearing mice (n=3-6/group) were predosed with either PBS (no agent, blue squares), cyclophosphamide (C, dark blue diamonds) or eribulin (E). 24 h post-liposome injection, tumors were collected, processed for FFPE, and tumor sections were stained with an anti-cytokeratin antibody and counterstained with Hoechst. Slides were scanned and the images were analyzed to quantify the stromal cells density (# stromal cells/tumor area in mm2). (**D**, **E**) BT474-M3 tumor bearing mice (n=4/group with bilateral tumors) were predosed with either PBS (no agent, blue squares), cyclophosphamide (C, dark blue diamonds), ifosfamide (I, magenta diamonds), paclitaxel (P, green triangles) or eribulin (E, orange circles) prior to injection of HER2-tPLD (3 mg/kg). 24 h post-liposome injection, tumors were collected, processed for FFPE, and tumor sections were stained with an anti-cytokeratin antibody and counterstained with Hoechst. Slides were scanned and the images were analyzed to quantify the total (tumor cells + stromal cells) cell density (# total cells/tumor area in mm2) (**D**) and changes in interstitial space area (**E**). Black circles in panels **C**, **D** and **E** indicate control (CTL) tumors that received PBS only (no chemotherapeutic agent and no HER2-tPLD).

**Supplementary Fig. S4**

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**Supplementary Fig. S4. Pretreatment of tumors with cyclophosphamide significantly enhances nuclear delivery of doxorubicin following HER2-tPLD** **injection.** BT474-M3 tumor bearing mice (n=5/group) were dosed with PBS or with cyclophosphamide (170 mg/kg, IP) 4 days before injection of fluorescently labelled HER2-tPLD (3 mg/kg) (DiI5-HER2-tPLD). Tumors were collected 24 h post-liposome injection, prepared for cryosections, counterstained with Hoechst, and scanned to visualize the microdistribution of DiI5-liposomes and doxorubicin relative to the vasculature. (**A**) The DiI5-liposome MFI is plotted against the % i.d./g tissue of matched tumors (linear regression fit, R2=0.8164); HER2-tPLD, light blue; HER2-tPLD predosed with cyclophosphamide (HER2-tPLD + C), dark blue. (**B**) Changes in distribution of doxorubicin positive (top panels) and negative nuclei (bottom panels) distance away from the nearest blood vessel in a representative HER2-tPLD (left), and HER2-tPLD + C tumor (right).

**Supplementary Fig. S5**

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**Supplementary Fig. S5. Pretreatment of tumors with cyclophosphamide enhances the delivery of HER2-tPLD in multiple tumor models.** Micewere inoculated with SUM190 (n=4-5/group), NCI-N87 (n=4-5/group with bilateral tumors) or MDA-MB-361 (n=3-6/group) tumor cells. When tumor volume reached 200-300 mm3, mice were treated with PBS or predosed with cyclophosphamide (170 mg/kg, IP) 4-7 days prior to injection of HER2-tPLD (3 mg/kg). 24 h post-liposome injection, tumors were collected and processed for quantification of doxorubicin by HPLC (**A, i-iii**). (**B, i-iii**) Tumor sections were stained for cytokeratin and counterstained with Hoechst. Slides were imaged and analyzed to quantify changes in tumor cell density. (**C, i-iii**) Frozen tumor sections were counterstained with Hoechst, imaged, and analyzed to quantify the percent of doxorubicin positive nuclei. CTL in panels **B** and **C** indicates control tumors that received PBS only (no cyclophosphamide and no HER2-tPLD). (**D**) Cells (BT474-M3, NCI-N87, SUM190 and MDA-MB-361) were plated in a 96-well plate at 5000 cells/well (n=3). Cells were treated with 4-hydroxy-cyclophosphamide (~ 1 uM) for 72 hrs and viability was evaluated by PrestoBlue Assay. The data is presented as % viability relative to untreated.

**Supplementary Fig. S6**

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**Supplementary Fig. S6. The combination of a cyclophosphamide predose with HER2-tPLD results in synergistic anti-tumor activity.** Mice (n=10/group) were inoculated with BT474-M3 tumor cells, followed by treatment with PBS (CTL), HER2-tPLD, cyclophosphamide (C) or a combination of the two agents (C given 4 days prior to HER2-tPLD, HER2-tPLC + C). (**A**) A Bliss independence analysis was performed on the groups at day 55. Statistical analysis was performed by z-test. The data is presented as mean ± SEM.