Filomicelles deliver a chemo-differentiation combination of Paclitaxel and Retinoic Acid that durably represses carcinomas in liver to prolong survival

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Supplementary Figure 1: RA arrests the rapid proliferation of mouse liver carcinoma EC4 cells

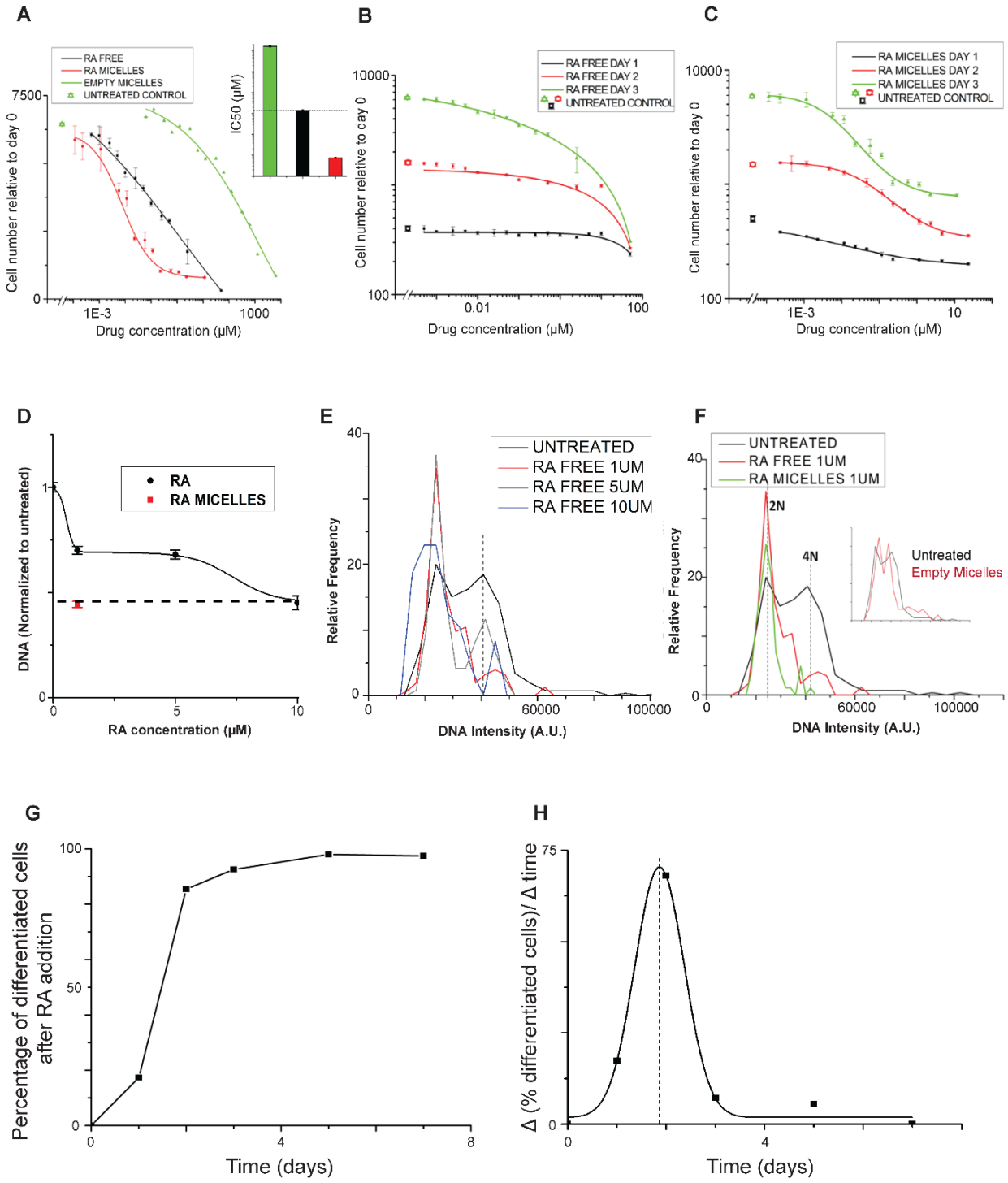

Figure S1: A) Comparison of free and encapsulated RA (bar graph IC50 inset). RA loaded filomicelles (red curve) are 200 times more potent than free RA (black curve). **B)** and **C)** Kinetics of cell numbers treated with free and encapsulated RA. Encapsulated RA is more successful at stopping cell growth at physiologically relevant concentrations (~1 μM). **D)** RA decreases average DNA content per cell. Average intensity decreases with increasing concentrations of RA and RA loaded onto filomicelles (red point) were more effective than that administered in free (black points). **E)** and **F)** DNA histograms after treatment with free RA show a suppression of the replicating peak (labeled 4N). Filomicelles with loaded RA suppressed the proliferating fraction even more. **G** and **H)** Estimation of fraction of EC4 cells differentiated by RA into a quiescent, non-proliferative state, follows a hill curve. Most cells get differentiated 1-2 days (2-4 cell cycles) after incubation with the drug, consistent with necessitation of secondary transcription factors to enforce the decisions.

Supplementary Figure 2: RA drives the differentiation of EC4 cells

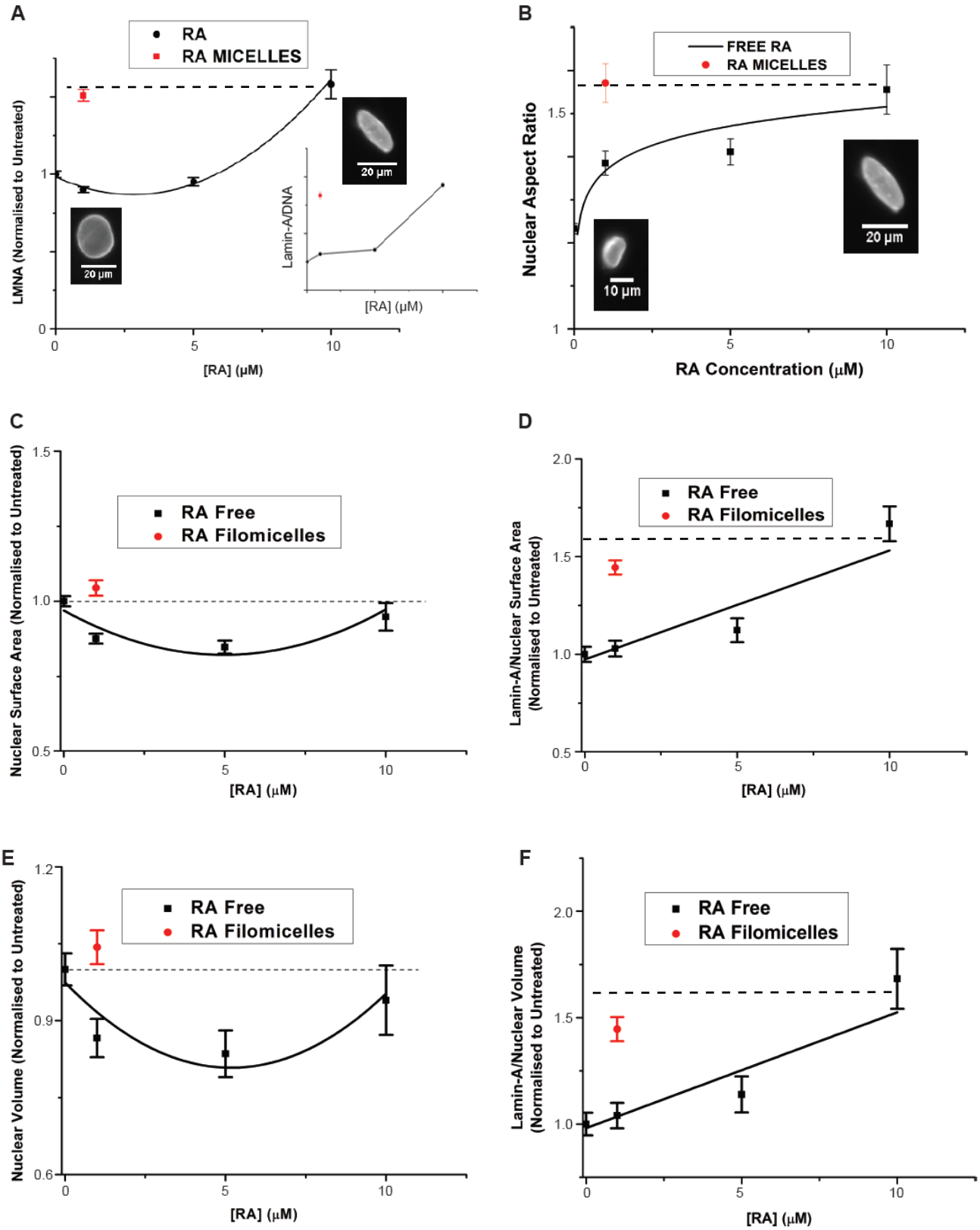

Figure S2: RA induces differentiation of cells **A)** RA affects levels of Lamin-A (a marker of differentiation), with RA in nanocarriers being an order of magnitude more effective than free drug. Treatment with RA increases levels of Lamin-A (normalized to DNA), hinting at differentiation. **B)** Nuclear aspect ratio increases hyperbolically with RA concentrations. As with Lamin-A levels, encapsulated RA is an order of magnitude more effective than free RA. **C)** and **E)** RA has a parabolic effect on nuclear surface area and volume. As with previous plots, encapsulated RA is an order of magnitude more effective than free RA. **D)** and **F)** Levels of Lamin-A normalized to either nuclear surface area or volume increase linearly with RA concentration, indicating that changes in protein level are not solely due to change in nuclear shape (morphology).

Supplementary Figure 3: RA-TAX treatment leads to larger cells with increased DNA content

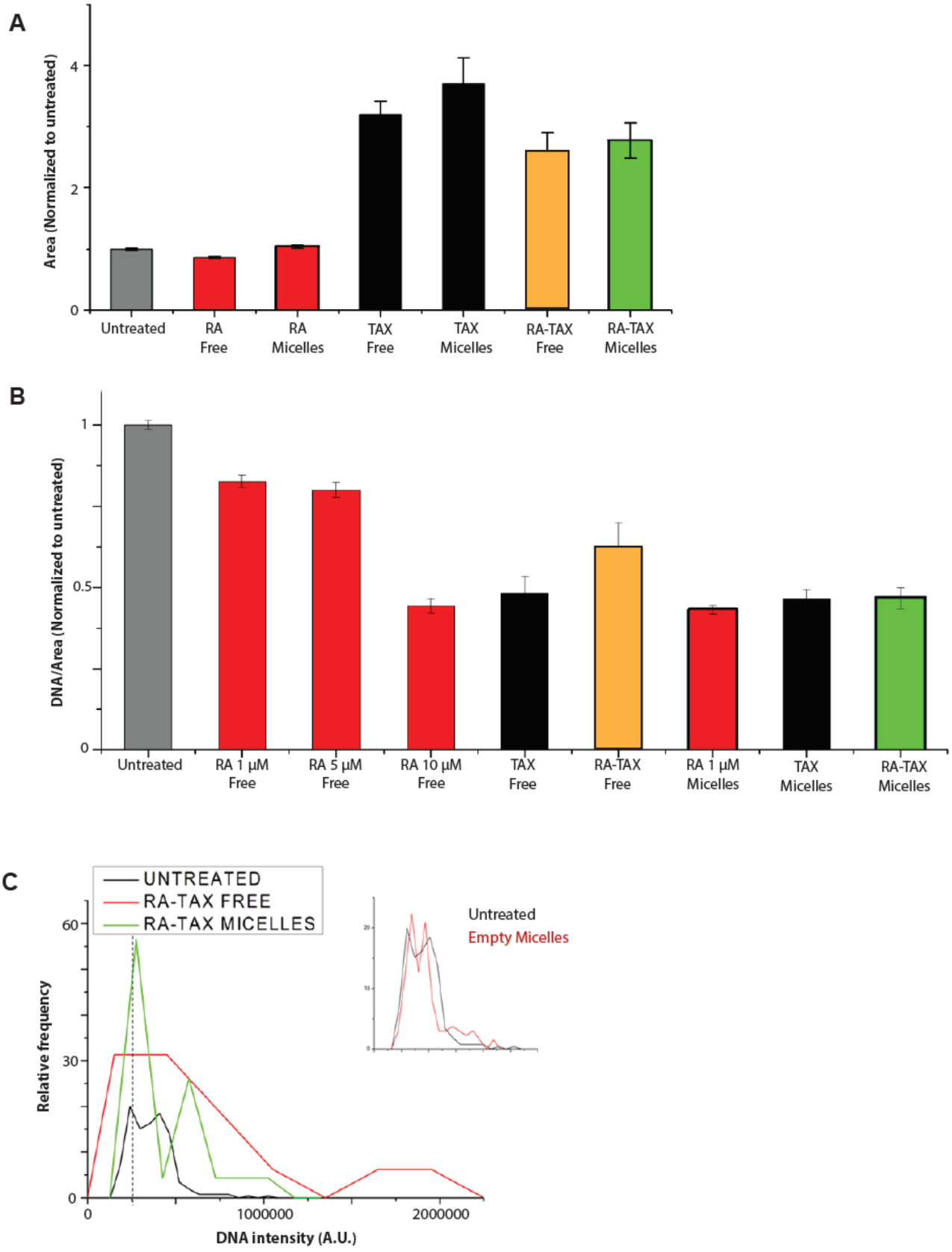

Figure S3: A) Treatments involving TAX causes about a 3-fold increase in nuclear area compared to untreated nuclei. While the presence of RA decreases the area slightly as compared to nuclei treated exclusively with TAX, the effects of TAX are still evident in images and DNA distribution. **B)** All drug treatments decrease levels of DNA (normalized to area), although through different mechanisms. **C)** DNA histogram of treated and untreated cells show polyploidy in surviving cells as well as a reduction in the replicating fraction. Empty filomicelles do not alter the distribution (inset).

Supplementary Figure 4: RA-TAX treatment shrinks subcutaneous tumors, prolonging survival

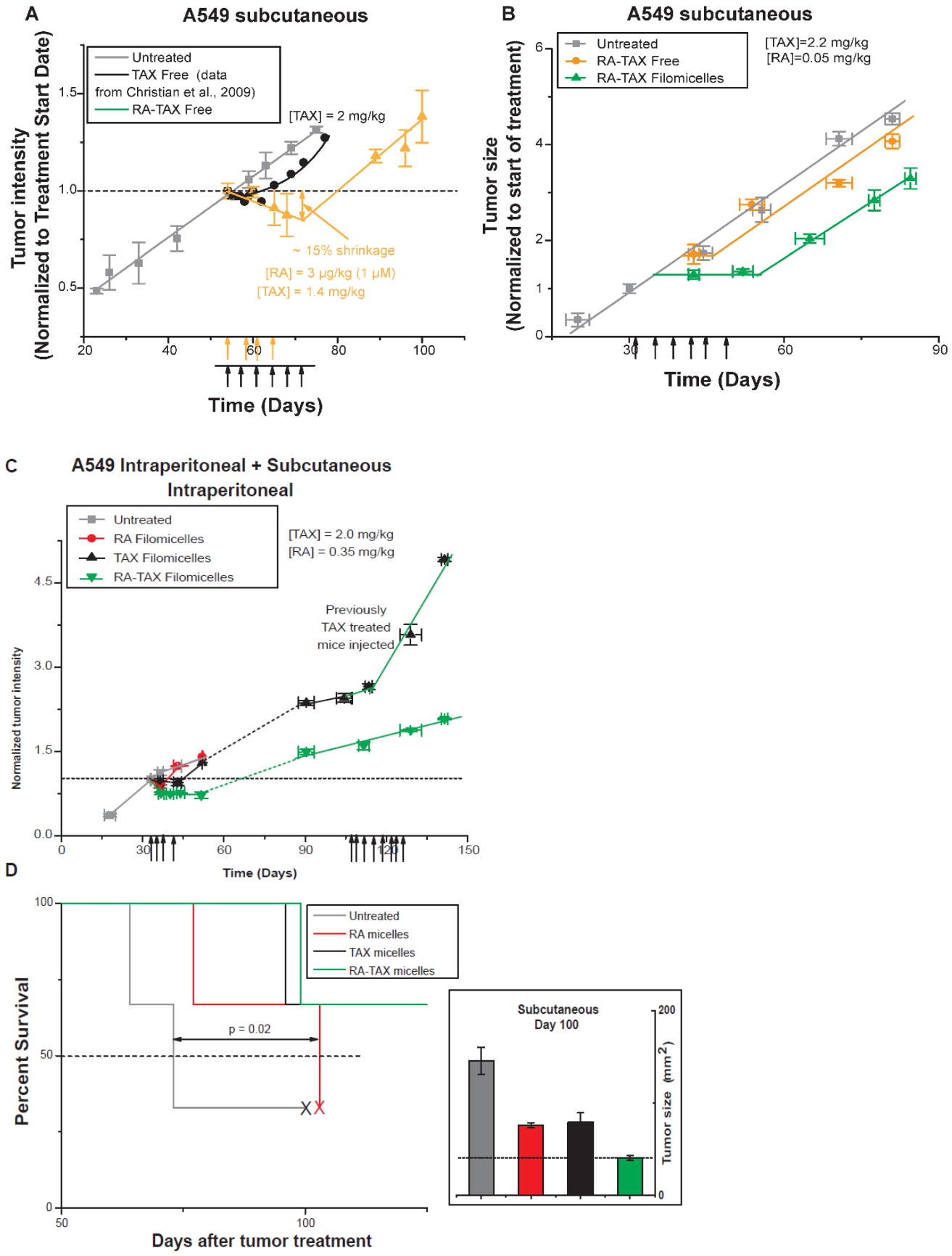

Figure S4: A) Free RA-TAX injections in NSG with A549 xenografts led to a 15% shrinkage in tumor size after 4 injections over the course of 11 days. **B)** Lower dosage of RA failed to shrink tumors in A549 subcutaneous tumors, although it did retard its growth. **C)** Mice bearing A549 intraperitoneal tumors were injected 4 times with single drugs or combination loaded into filomicelles. RA-TAX combination produced the most tumor shrinkage, 15%. D**)** Durable tumor shrinkage was evident in prolonged survival of RA-TAX treated mice. TAX and RA treated mice also displayed extended survival. Inset: Subcutaneous tumor sizes measured by calipers 100 days after tumor treatment.

Supplementary Figure 5: RA-TAX treatment shrinks tumors in vivo and is more effective than single drugs

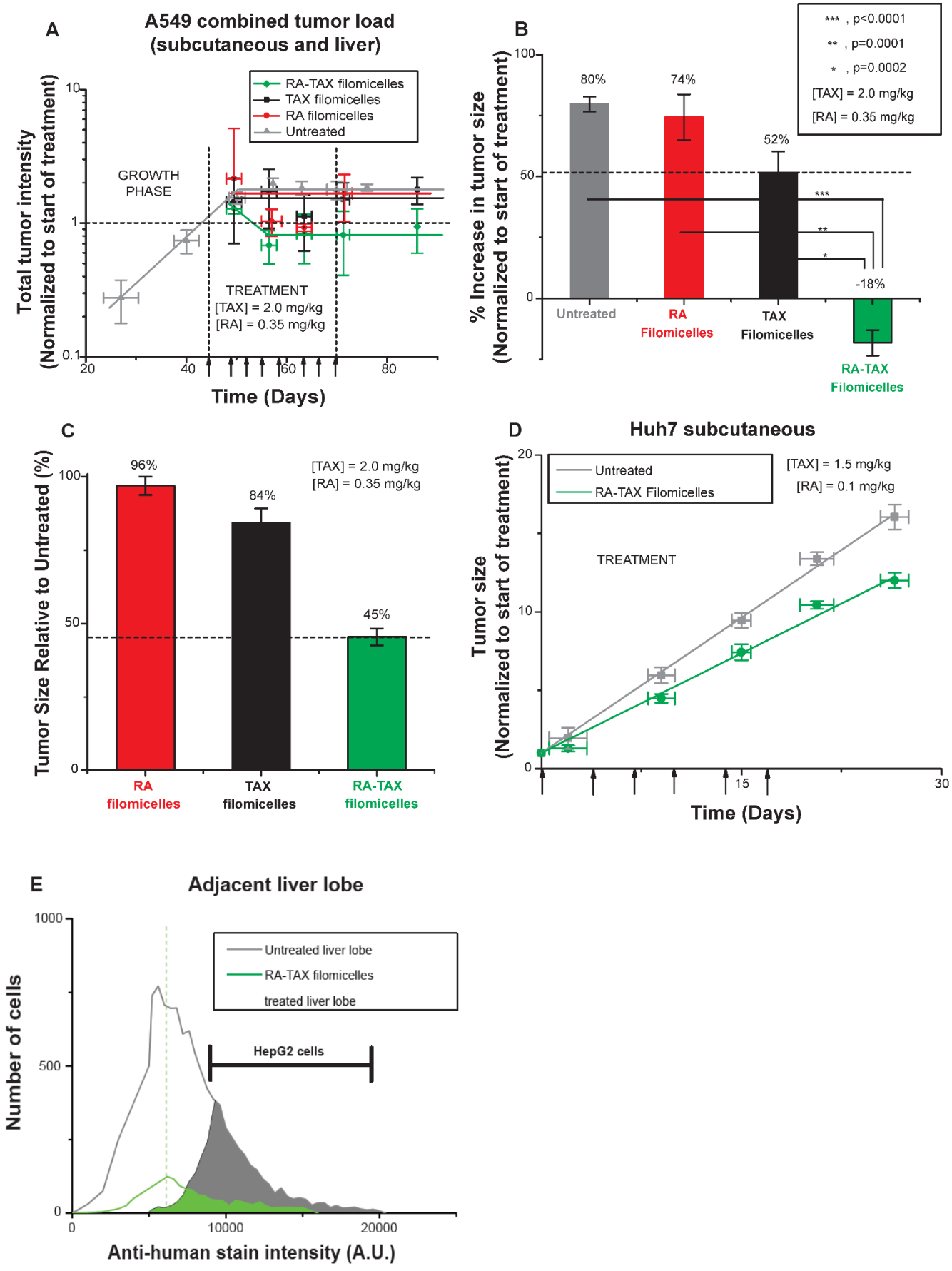

Figure S5: A) Quantification of total tumor burden in mice, bearing A549 subcutaneous and liver tumors, with time. RA-TAX filomicelles were the only treatment that shrunk tumors at conclusion. **B)** and **C)** Quantification of total tumor size. RA-TAX filomicelles produce the only shrinkage from start of treatment, and final size is less than half of the final untreated tumors. **D)** RA-TAX reduces growth rate of Huh7 subcutaneous tumors, leading to 25% reduction in size. **E)** Histogram of anti-human intensity from adjacent liver lobe. Untreated lobe shows a peak of anti-human staining (black shaded area), indicating migrated cells, that is minimal in treated mouse.

Supplementary Figure 6: RA-TAX treatment does not cause body weight loss in vivo

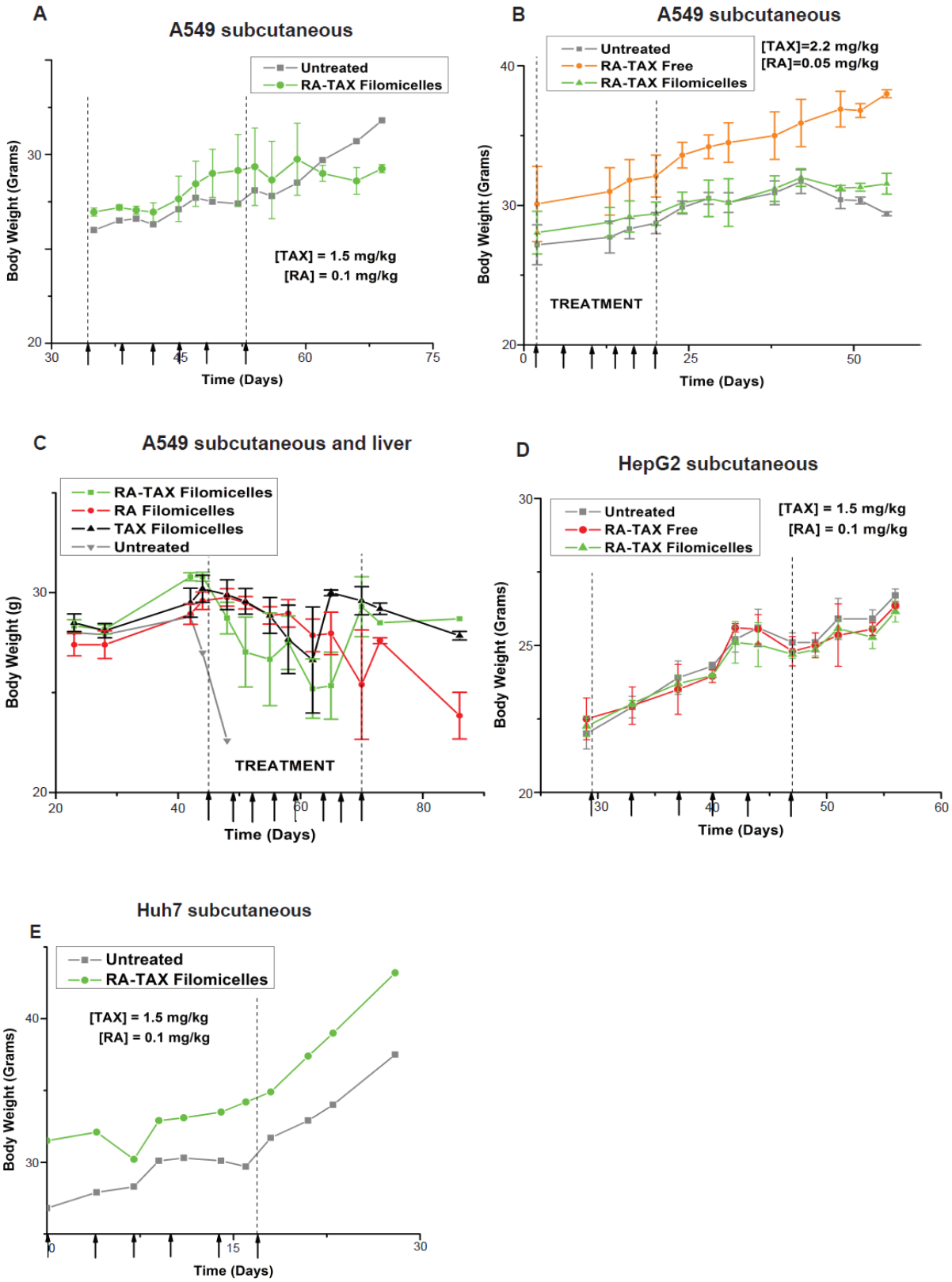

Figure S6: Mouse body weight data for *in vivo* treatments corresponding to figures **A)** S4A **B)** S4B **C)** 3B-D **D)** 4A **E)** S5D

Supplementary Figure 7: RA synergizes better with Paclitaxel than other DNA damaging drugs

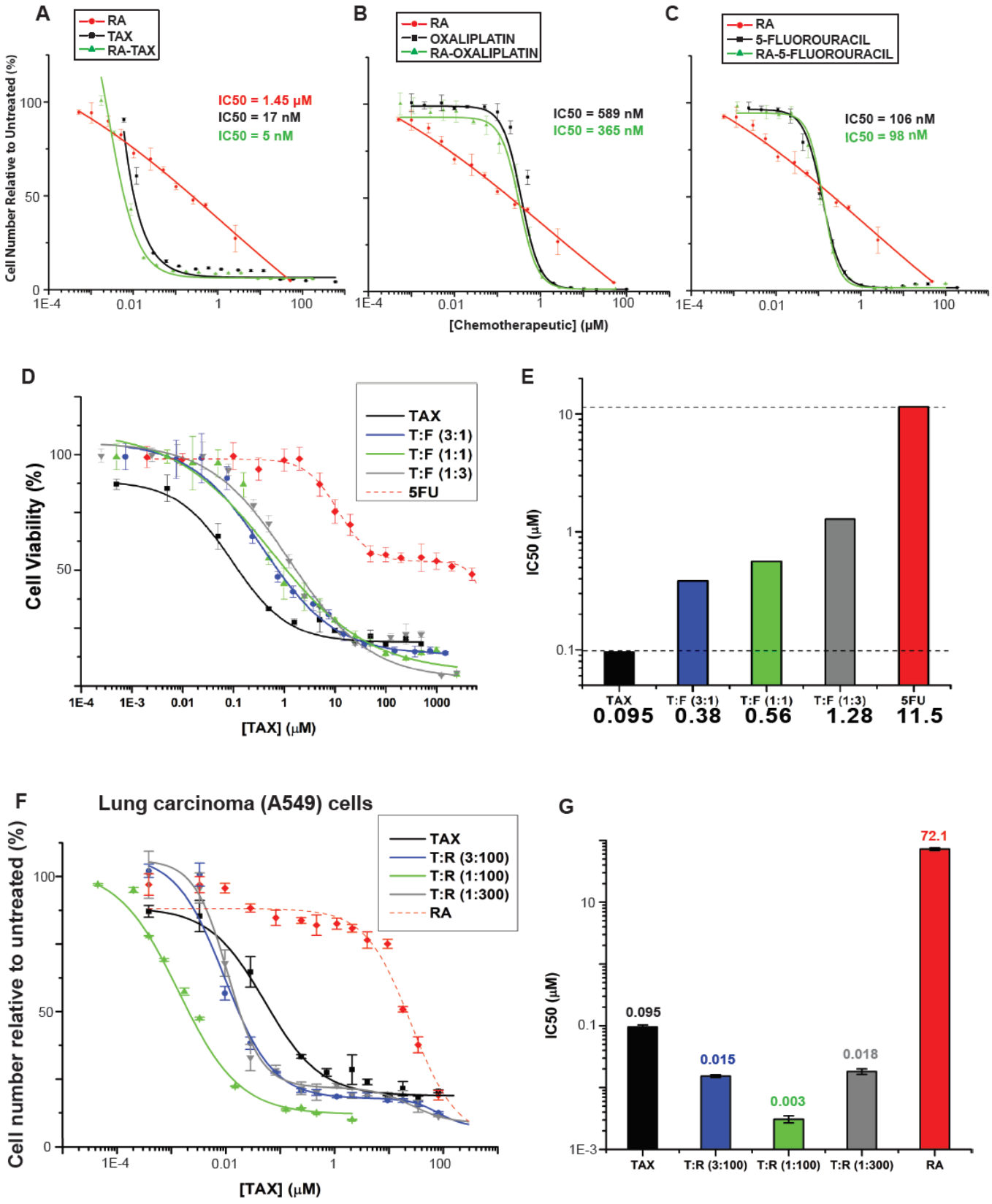

Figure S7: Synergy between different drugs tested with free drug solutions. Combinations of RA with **A)** 5-Fluorouracil, **B)** Oxaliplatin, and **C)** Paclitaxel. While all combination treatments had a lower IC50 on EC4 cells than the involved chemotherapeutic, the change in IC50 was different for each combination. Empty symbol represents untreated control. **D** and **E)** The combination of Paclitaxel with 5-Fluorouracil indicates lack of synergy, with IC50s quantified in **E)**. **F)** Combination of a Paclitaxel (black curve) with RA (red) for a chemo-differentiative treatment is better than that of two chemotherapeutics, with 1:100 molar ratio (green curve) being the most potent. **G)** Bar graph depiction of the IC50 quantifications of the free drug combinations tested in Figure S1D.

Supplementary Figure 8: RA-TAX treatment maximizes cell death and minimizes IC50, with filomicelles further increasing potency

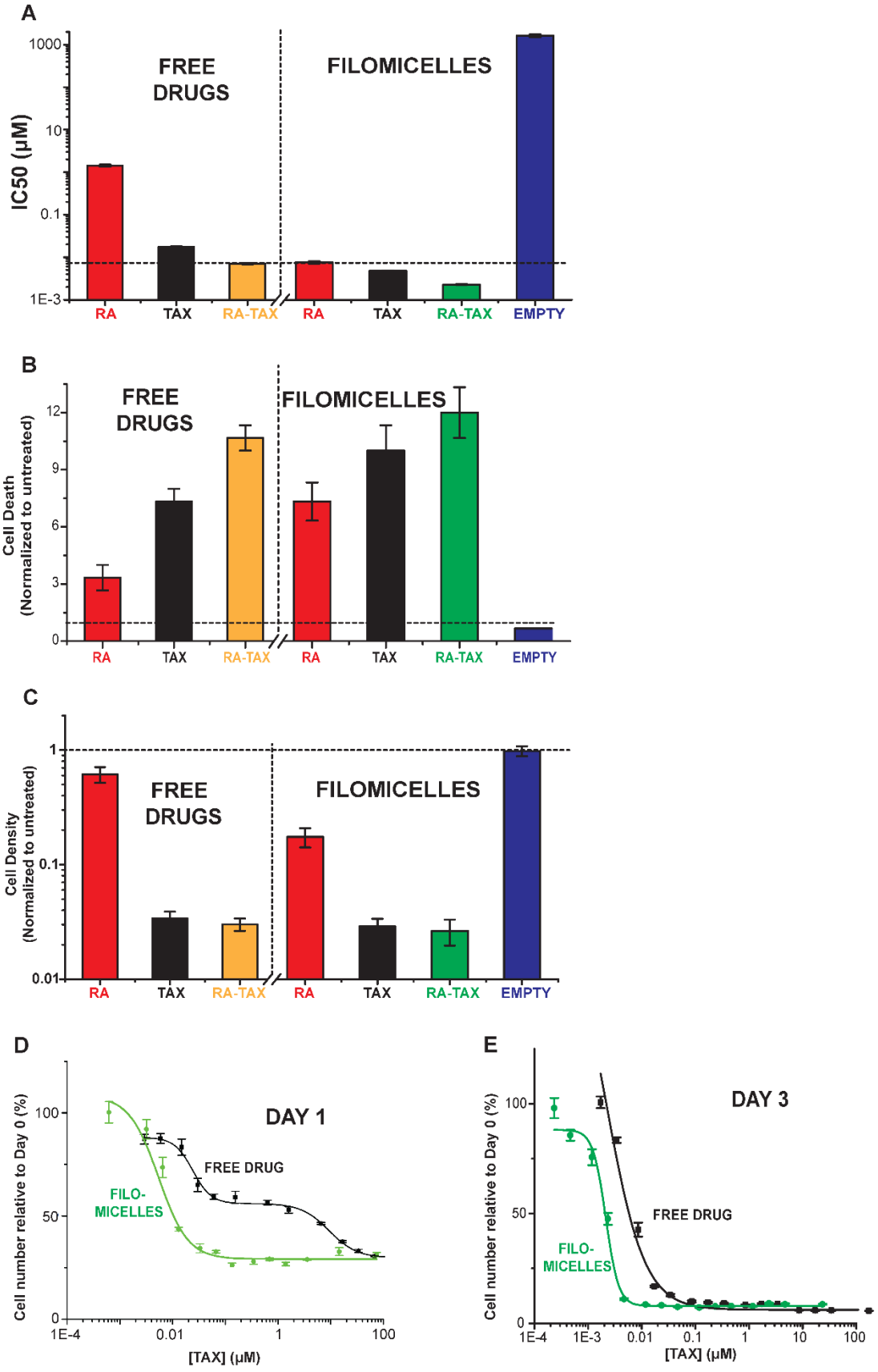

Figure S8: A) Bar graph representation of IC50 of different formulations. Micellar RA-TAX had the lowest IC50 among all tested formulations, almost 3 times lower than that of free drug. **B)** and **C)** Cell density and Cell death measurements done on well plates further highlight the potency of the system. RA-TAX treated cells had the highest cell death (D) and lowest cell density on plate (E). As with previous experiments, filomicelles were more effective than free drug. **D)** and **E)** Kinetic studies reveal an effect as early as a day, with peak death observed after two days. These limitations may be overcome to certain extent by loading them onto filomicelles

Supplementary Figure 9: RA-TAX co-loads into filomicelles with no loss in integration efficiency, while RA-TAX resistant cells proliferate slower.

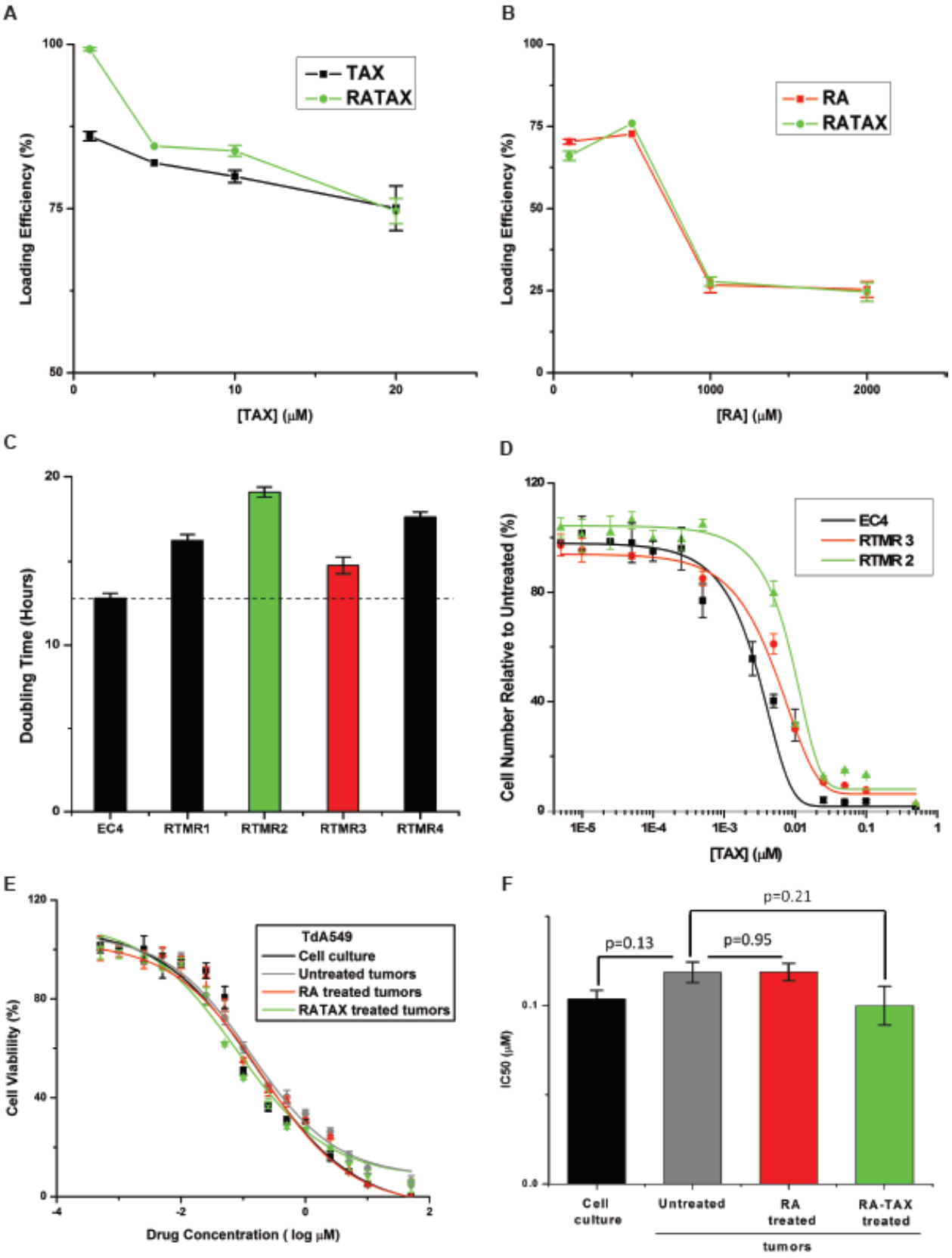

Figure S9: A) and **B)** Quantification of drug loading efficiency with change in added drug concentration reveals a decrease with increasing concentrations. Vitally, there is no loss in integration efficiency between single and dual drug loading for either drug. **C)** Quantification of doubling time of RA-TAX micelle resistant (RTMR) EC4 cells. All RTMR cells proliferate slower than untreated EC4 cells. **D)** Quantification of response of RTMR cells to RA-TAX treatment. RTMR cells have higher IC50 than WT EC4 cells, and hence higher resistance to RA-TAX treatment. **E-F**) TdA549 cells disaggregated from in vivo tumors treated with RA and RA-TAX as well as untreated tumors displayed identical dose responses to TdA549 cells grown in culture. Quantification of IC50s revealed no significant differences between the groups.

Supplementary Figure 10: RA-TAX treatment increases Lamin-A, while decreasing Cyclin-D1 and Ki67

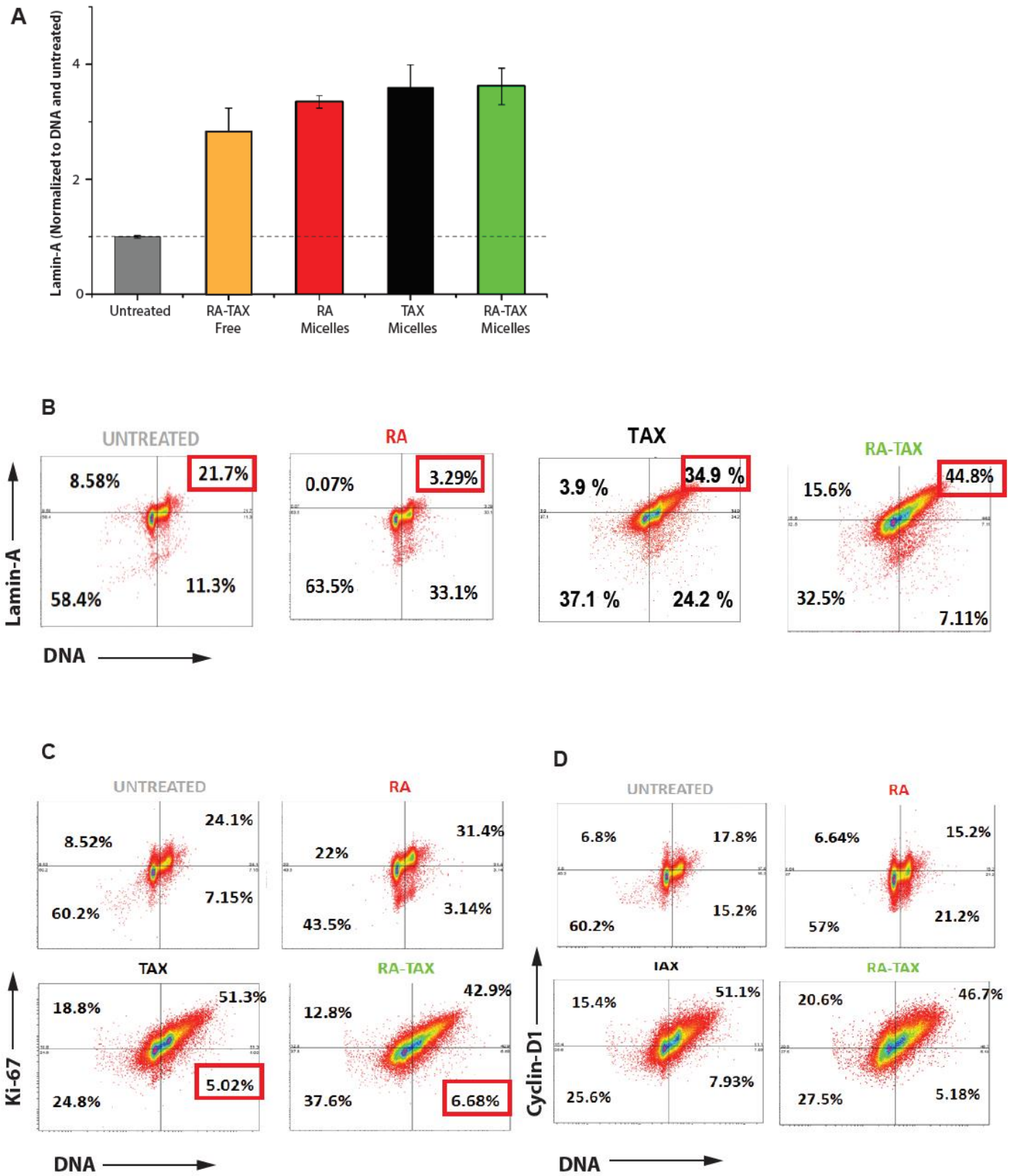

Figure S10: A) As with RA, treatment with TAX or RA-TAX increased Lamin-A levels. Flow cytometry scatter plots for **B)** Lamin A, **C)** Ki67 and **D)** Cyclin D1 on the y-axis and DNA (Hoechst) on the x-axis.

Supplementary Figure 11: Efficacy of RA-TAX treatment can be extended to carcinomas and sarcomas other than the liver

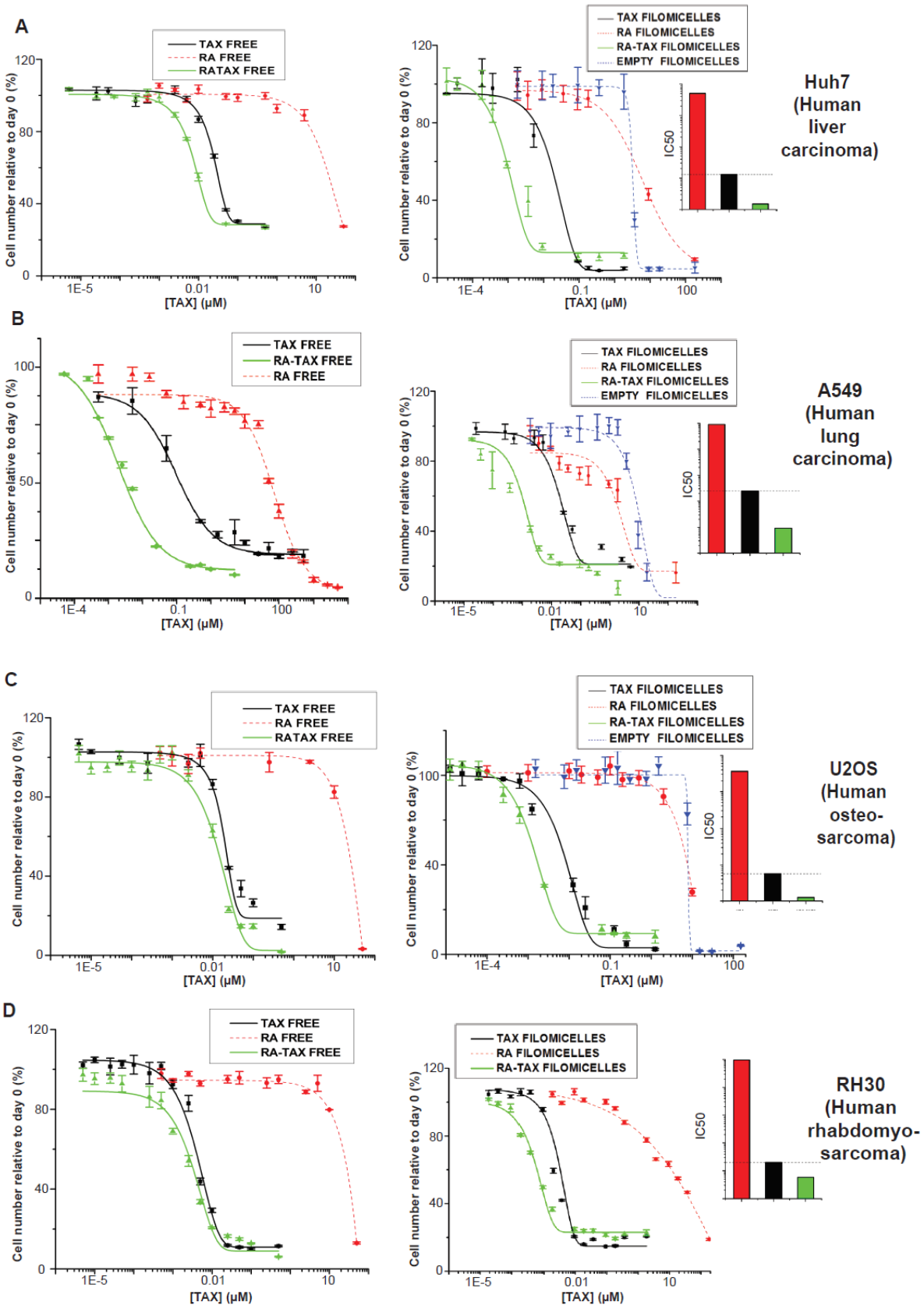

Figure S11: Dose response curves for free and filomicelle drug treatments with **A)** Huh7 (human hepatocellular carcinoma), **B)** A549 (human lung epithelial adenocarcinoma), **C)** U2OS (human osteosarcoma), and **D)** RH30 (human rhabdomyosarcoma) cell lines respectively. The dual drug treatment retains its efficacy despite varying responses to TAX and RA across different cell lines.