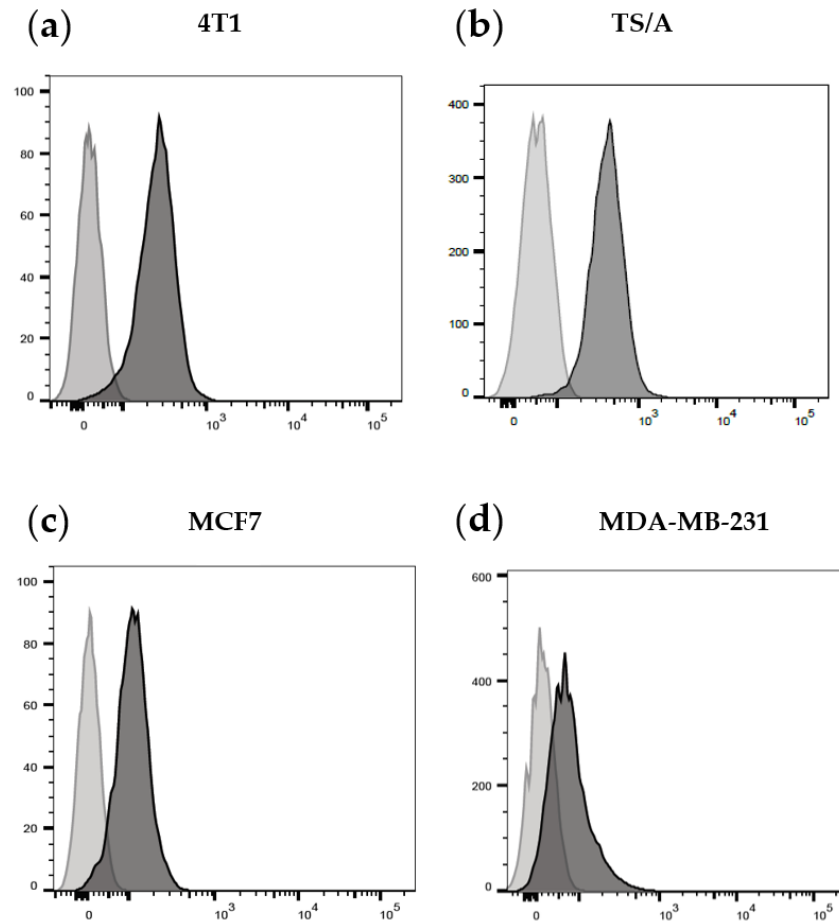
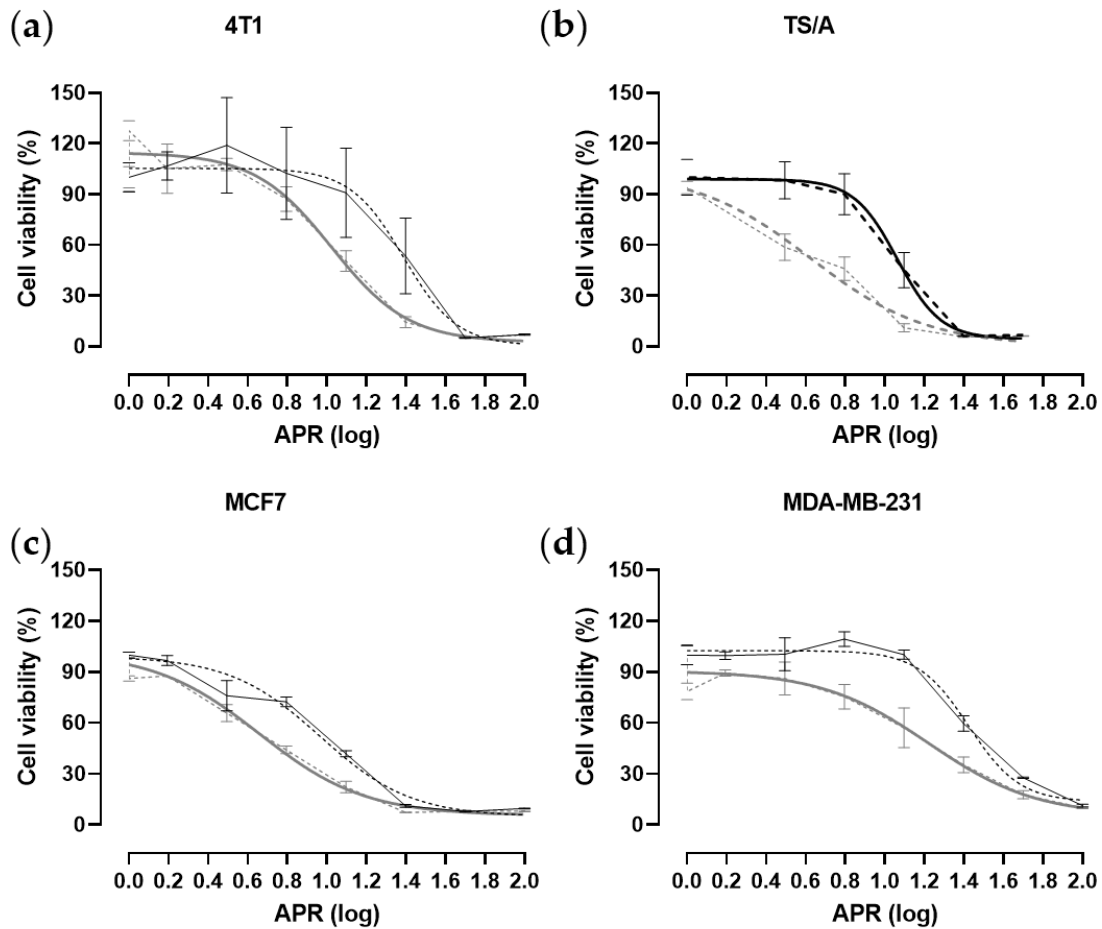


**Supplementary figures: Immunotherapy against the cystine/glutamate antiporter xCT improves the efficacy of APR-246 in preclinical breast cancer models.**

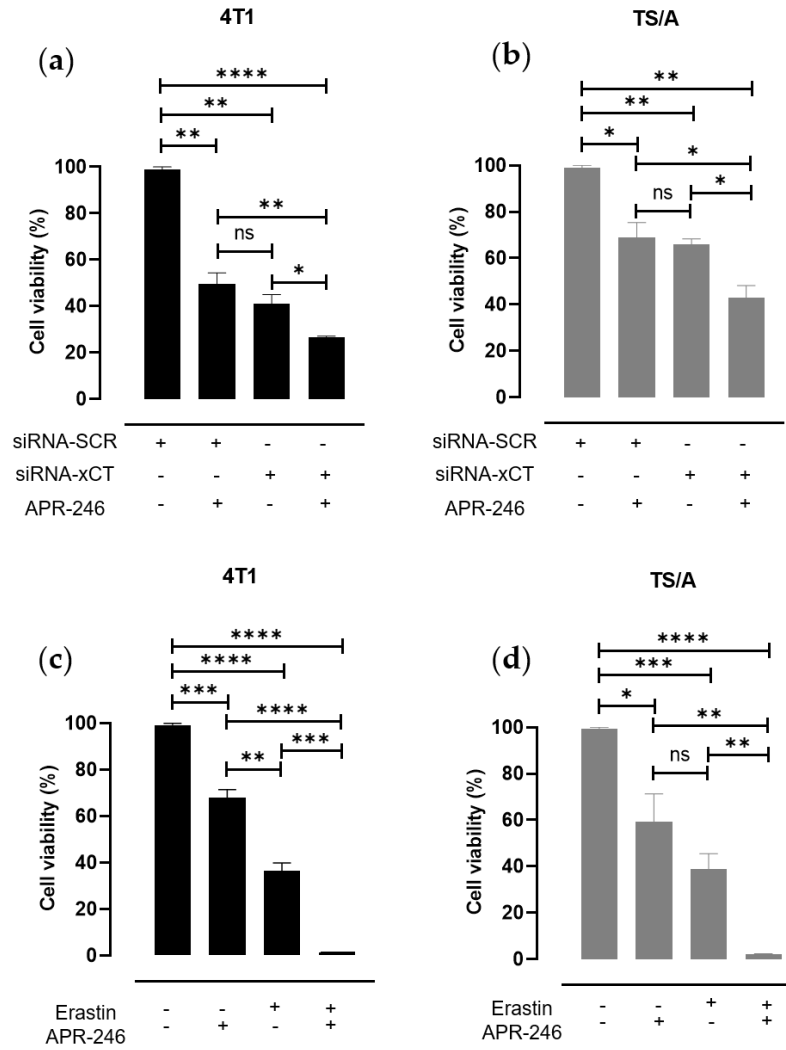
Giuseppina Barutello, Antonino Di Lorenzo, Alessandro Gasparetto, Chiara Galiazzi, Elisabetta Bolli, Laura Conti and Federica Cavallo



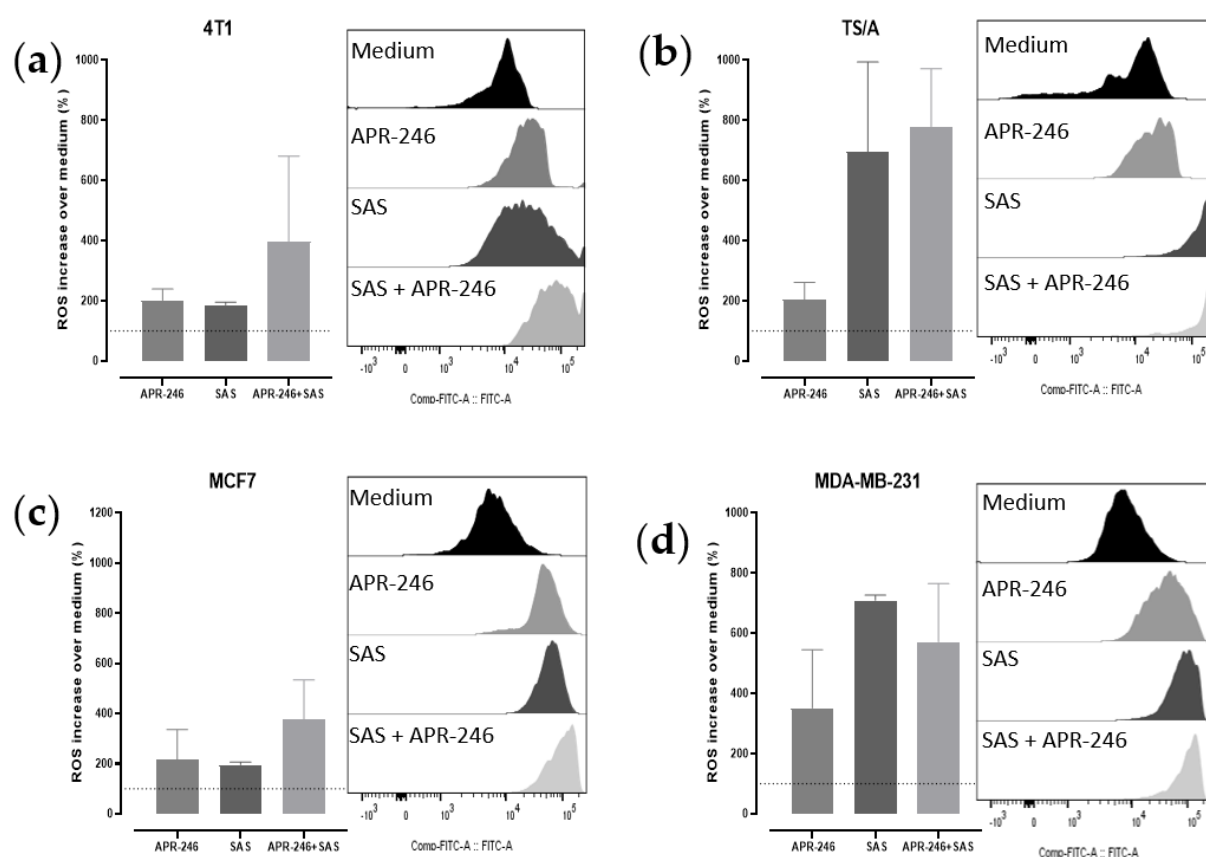
**Figure S1. Assessment of xCT expression in breast cancer cell line panel.** Representative FACS histograms of xCT (dark grey) in comparison to the control antibody control (light grey) staining on (a) 4T1, (b) TS/A, (c) MCF7, and (d) MDA-MB-231 cells.



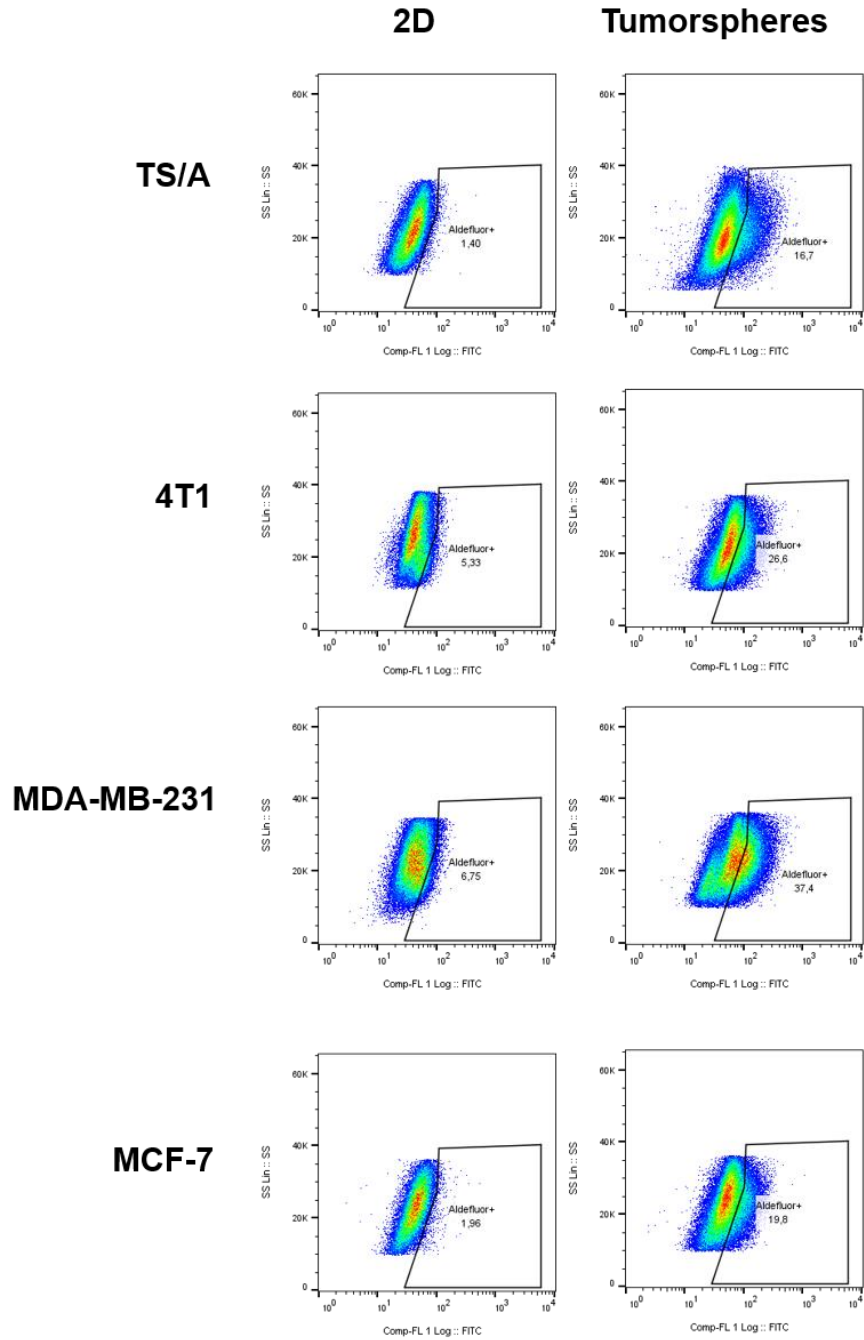
**Figure S2. MTT assays to determine the IC<sub>50</sub> value of APR-246 and its effect on cell viability.** The results are expressed as the percentage of cell viability of cells following 24 hours incubation with APR-246 alone (black lines) or in combination with SAS (grey lines). The graphs show log(inhibitor) vs. response - variable slope (four parameters) non-linear regression of data from three independent experiments, calculated with GrapPad9 software.



**Figure S3. Effect of APR-246 in combination with xCT depleted mouse mammary tumor cells.** Histograms represent the percentage (mean value  $\pm$  SEM) of the cell viability of (a) 4T1 and (b) TS/A previously treated with specific mouse xCT siRNA mix (siRNA-xCT), following 24 hours incubation with 25  $\mu$ M or 12.5  $\mu$ M of APR-246 respectively, and of (c) 4T1 and (d) TS/A following 24 hours incubation with APR-246 alone, or in combination with 0.2  $\mu$ M of Erastin. Results are reported in comparison to cells grown in the presence of medium added with the control siRNA (siRNA-SCR) (a, b) or medium (c, d), considered as control (100%). \* $p = 0.02$ ; \*\* $p \leq 0.009$ ; \*\*\* $p = 0.0008$ ; \*\*\*\* $p < 0.0001$ ; ns = not significant; Student t test.



**Figure S4. Cytofluorimetric analysis of intracellular ROS in treated cells.** FACS analysis of intracellular ROS in (a) 4T1, (b) TS/A, (c) MCF7, and (d) MDA-MB-231 cells treated with APR-246 and/or SAS, reported as percentage of increase of the DCF MFI in treated cells as compared to cells cultured in medium (means  $\pm$  SEM from 3 independent experiments) and as representative FACS histograms.



**Figure S5. Tumorspheres are enriched in cancer stem cells.** FACS analysis of aldehyde dehydrogenase 1 activity, measured using the Aldefluor kit (Stem Cell Technologies), in TS/A, 4T1, MDA-MB-231 and MCF-7 cells cultured in standard 2D conditions or as tumorspheres. Representative density plots are shown. The gates were set based on the corresponding control samples, and the percentage of Aldefluor<sup>+</sup> cells is reported.