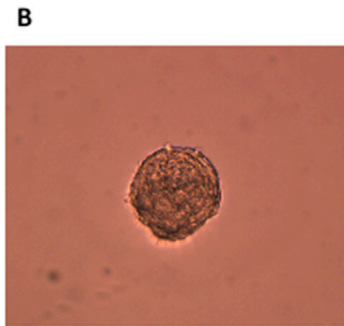


Serotonin transporter antagonists target tumor-initiating cells in a transgenic mouse model of breast cancer

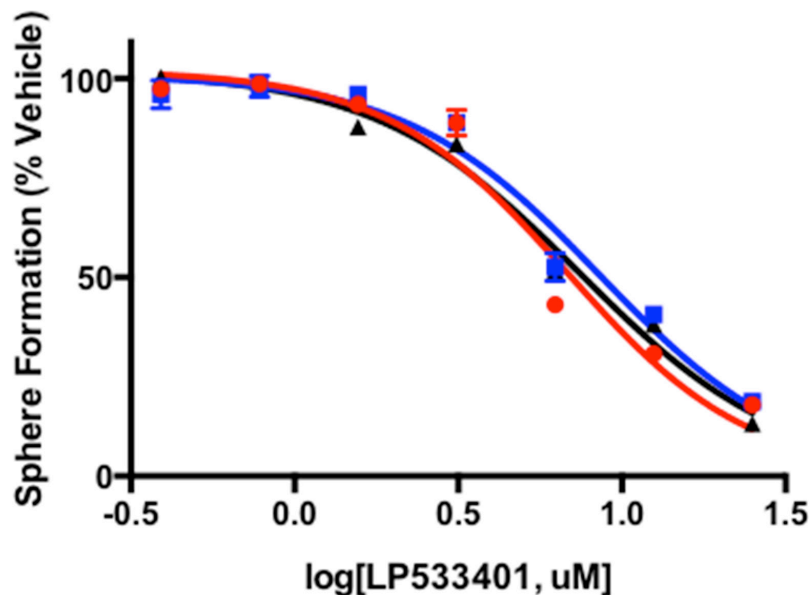
Supplementary Materials

A

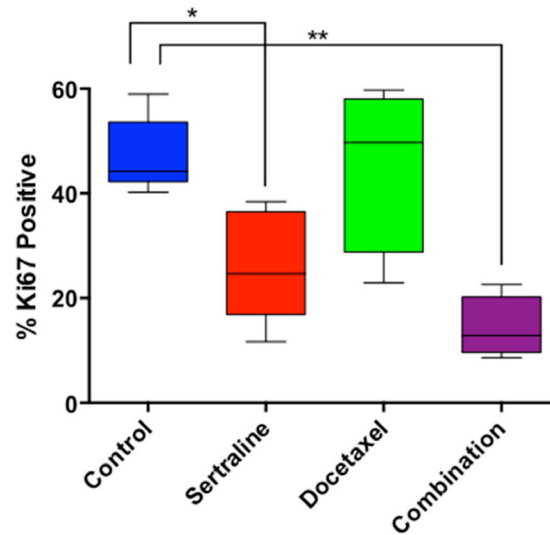
	Number of Wells with Single Cells	Number of Wells with a Single Sphere	Frequency of Wells with Spheres (%)
15 96-well plates	601	11	1.8
16 96-well plates	336	3	0.9
16 96-well plates	447	15	3.2
			Mean = 2.0



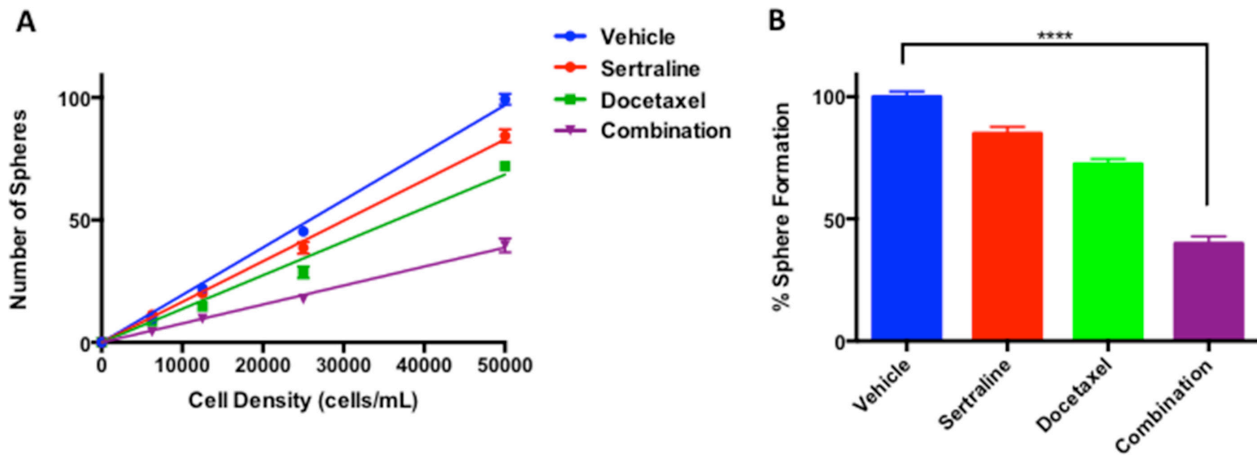
Supplementary Figure S1: Single mouse mammary tumorsphere-derived cells form spheres. (A) Single mouse mammary tumor cells from 3 independent mouse mammary tumors were seeded into the wells of 15 or 16 96-well plates and the number of wells with single cells identified a day later; wells seeded with tumorspheres were enumerated a week after the single cells were seeded. (B) Image of a well comprising a single tumorsphere.



Supplementary Figure S2: An inhibitor of TPH1 reduces sphere formation in a dose-dependent fashion. Dilutions of LP533401 were assayed in triplicate replicates for its capacity to affect sphere formation by tumorsphere-derived cells isolated from 3 independent tumors.



Supplementary Figure S3: Sertraline and docetaxel synergize to reduce the fraction of Ki67-positive cells. *P*-values were calculated using one-way ANOVA; (*) < 0.05; (**) < 0.01. *P* = 0.0009.



Supplementary Figure S4: Limiting dilution tumorsphere-forming assays reveal that tumors harvested from mice administered sertraline and docetaxel comprised fewer sphere initiating cells relative to the other treatment groups. (A and B) Frequency of tumorsphere forming cells in residual tumors remaining after treating tumor-bearing mice with the vehicle, sertraline, docetaxel, or the combination of sertraline and docetaxel. *P*-values were calculated using one-way ANOVA; (****) < 0.00001.

Supplementary Table S1: Selective antagonists of 4 different 5-HT receptors reduced the frequency of tumorsphere-forming cells

Selective Antagonist	5-HT Receptor Target	IC ₅₀ Prestoblue Reduction	IC ₅₀ Sphere Formation
SB 2242489	1B	2.8 μM	0.6 μM
SB 216641	1B	3.4 μM	3.3 μM
N -Desmethylozapine	2C	7.0 μM	9.0 μM
SB 699551	5A	0.3 μM	0.5 μM
MS 245	6	5.1 μM	6.1 μM

The IC₅₀ of selective 5-HT receptor antagonists was determined in sphere forming assays in triplicate at each concentration of the inhibitor.