Supplementary Figure Legends

Supplementary figure 1. Survival curve of BCSCs/Progenitors from sphere forming capacity assay of cells derived from SUM159PT, MCF-7, and T47D. Monolayers were irradiated with 0, 2, 4, 6, and 8 Gy and plated into mammosphere media into 96-well ultra-low adhesion plates to allow spheres formation. After 21 days, spheres were counted for each dose point. To

determine the surviving fractions, counts were normalized using the plating efficiency of the non-irradiated corresponding control. We used the surviving fraction for each dose point to calculate the theoretical survival curve used in Figure 1e and Supplementary figure 3b.

Supplementary figure 2. Low proteasome cells partially overlap with ALDH1-positive cells and drive tumor growth. (a), T47D-dsRed-cODC, MCF-7-dsRed-cODC and MDA-MB-231-dsRed-cODC cells were stained for ALDH1 activity using Aldefluor assay (Stemcell Technologies Inc.). 3.2%, 4.41% and 4.01% of total population were respectively positive for ALDH1, 19.6%, 14.2% and 10.8% of cells with low proteasome activity (dsRed-cODC-positive cells) were ALDH1-positive. **(b)** Percentages of ALDH1-positive in total population, dsRed-cODC-negative and dsRed-cODC-positive are shown for T47D, MCF-7 and MDA-MB-231 dsRed-cODC expressing cells.

Supplementary Figure 3. Radiation induced *de novo* generation of ALDH1 positive cells. (a) T47D cells were stained for ALDH1 activity using the Aldefluor assay. ALDH1-negative cells were sorted and plated as monolayers. The following day, cells were irradiated with 0, 4, or 8 Gy. The presence of ALDH1-positive cells was analyzed 5 days after irradiation. Representative dot blots are shown. **(b)** ZsG-cODC-negative cells from T47D, MCF-7 and SUM159PT were sorted and plated as mammospheres. The following day, cells were treated with 0, 2, 4, 6, 8 or 12 Gy (at a dose rate of 2.789 Gy/min). Five days after treatment,

MCF-7, T47D, and SUM159PT cells were seeded at low density to assess sphere forming capacity. Solid lines represent the means and S.Ds. (n=3), dashed lines represent the hypothetical sphere forming capacity. Secondary sphere forming capacity was assessed 15 days after.

Supplementary Figure 4. Heat map of significantly modified stem cells related genes (86 analyzed). Details of Figure 3.

Supplementary Figure 5. SUM159PT-ZsG-cODC-negative cells were sorted from monolayer cultures, plated as monolayers and irradiated with 0, 4, and 8 Gy. 5 days after irradiation, expression levels of ZsG-cODC, Oct4 (rabbit anti-Oct4, anti-rabbit APC-Alexa 750-conjugated), Sox2 (mouse anti-Sox2, anti-mouse APC-conjugated), Nanog (rabbit anti-Nanog), Klf4 (mouse anti-Klf4) and c-Myc (mouse anti-c-Myc), and DNA content (Propidium Iodide) were analyzed by flow cytometry. **(a)** Representative histograms of DNA content of irradiated cells; (b) bar graphs of DNA content of irradiated cells treated with γ-secretase inhibitor; **(c)** dot blots of DNA content vs. Sox2/Oct4 expression, respectively. **(d)** The means and S.E.M.s of radiation-induced polyploid cells for replicates of **(c)** are shown. *$p<0.05$.

Supplementary Figure 6. (a) Protein expression levels of ZsGreen-cODC, Oct4, Sox2, and Nanog, and DNA content were analyzed by flow cytometry in MCF-7 and T47D. **(b)** Number of polyploid Oct4-, Sox2-, and Nanog-positive cells after

irradiation are shown in MCF-7 and T47D. **(d)** Distribution of BCSCs in the total population, in non-polyploid population, in polyploid Oct4/Sox2/Nanog positive, for MCF-7 and T47D cells.

Supplementary figure 7. Polyploidy induces *de novo* generation of CSCs. Non-tumorigenic cells were treated with Noscapine to induced polyploidy, and the presence of iBCSCs was analyzed 5 days later. Dose response analysis of noscapine-induced polyploidy in SUM159PT, MCF-7, and T47D. **(a)** Representative histograms of DNA contain and **(b)** means of polyploid cells are shown. ZsGreen-cODC-negative cells from MCF-7, T47D and SUM159PT were treated with noscapine 0, 10, 25 or 50μM. **(c)** The presence of iBCSCs was analyzed after 5 day by FACS.