## Derivation of xenospheres.

Tumor explants were collected in DMEM medium supplemented with 2 mM glutamine (Sigma) and penicillin-streptomycin (1:100, EuroClone), kept on ice and processed within few hours to preserve cell viability. Tumor samples were first mechanically dissociated with scalpels, and then enzymatically digested for 2h at 37°C with 1 mg/ml of Type-I Collagenase (Life Technologies-Invitrogen) resuspended in Leibovitz’s L-15 medium (Life Technologies-GIBCO). Digested material was washed in PBS and filtered through a 70 um cell strainer (BD Falcon) to eliminate cell debris. Cleared cells were subsequently pelleted, resuspended in DMEM/F-12 serum-free medium, loaded over histopaque-1077 (Sigma) with a volume ratio of 2,5:1 and centrifuged for 20’ at 13.000 rpm. Viable cells at the interface between Histopaque-1077 and medium were recovered, while pelleted red-blood and dead cells were discarded. Cell were then washed twice in PBS, resuspended in stem-cell complete medium, plated into ultra-low attachment plastics (Sigma-Corning) and incubated at 37°C, with 5% O2 and CO2. Xenospheres were passaged once a week by enzymatic dissociation (5mM EDTA; Trypsin 8mM), followed by re-plating of both single cells and residual small aggregates in complete fresh stem-cell medium, supplemented with a 30% of the recovered conditioned medium.

**Stem-cell complete medium**: DMEM/F-12 (Life Technologies-GIBCO) supplemented with 2 mM glutamine (Sigma), penicillin-streptomycin (1:100, EuroClone), N-2 supplements, 0.4% BSA (Sigma), 4 ug/ml heparin (Sigma), CD Lipid Concetrate (1:100, Life Technologies-GIBCO), human recombinant epidermal growth factor (EGF, 20 ng/ml; Peprotech), and basic fibroblast growth factor 2 (bFGF, 10 ng/ml; Peprotech).