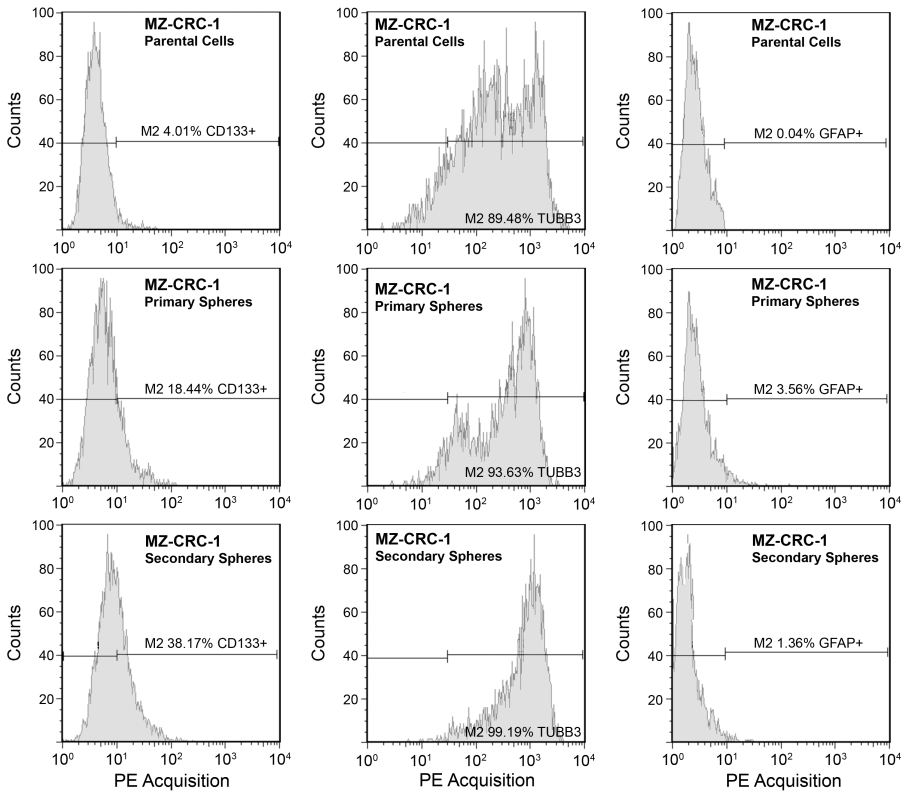
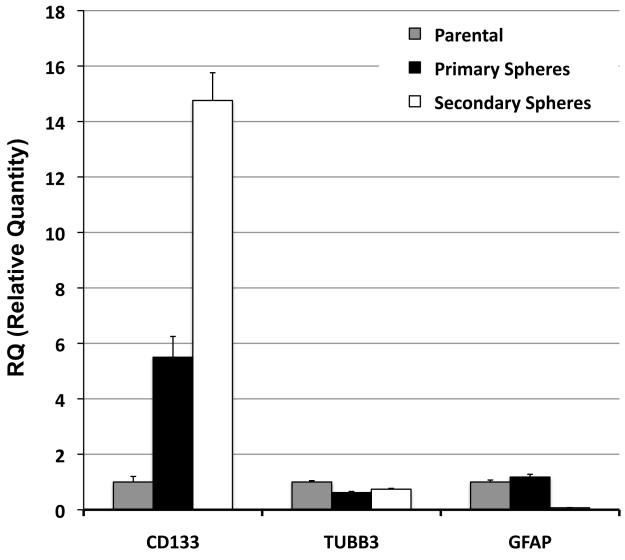
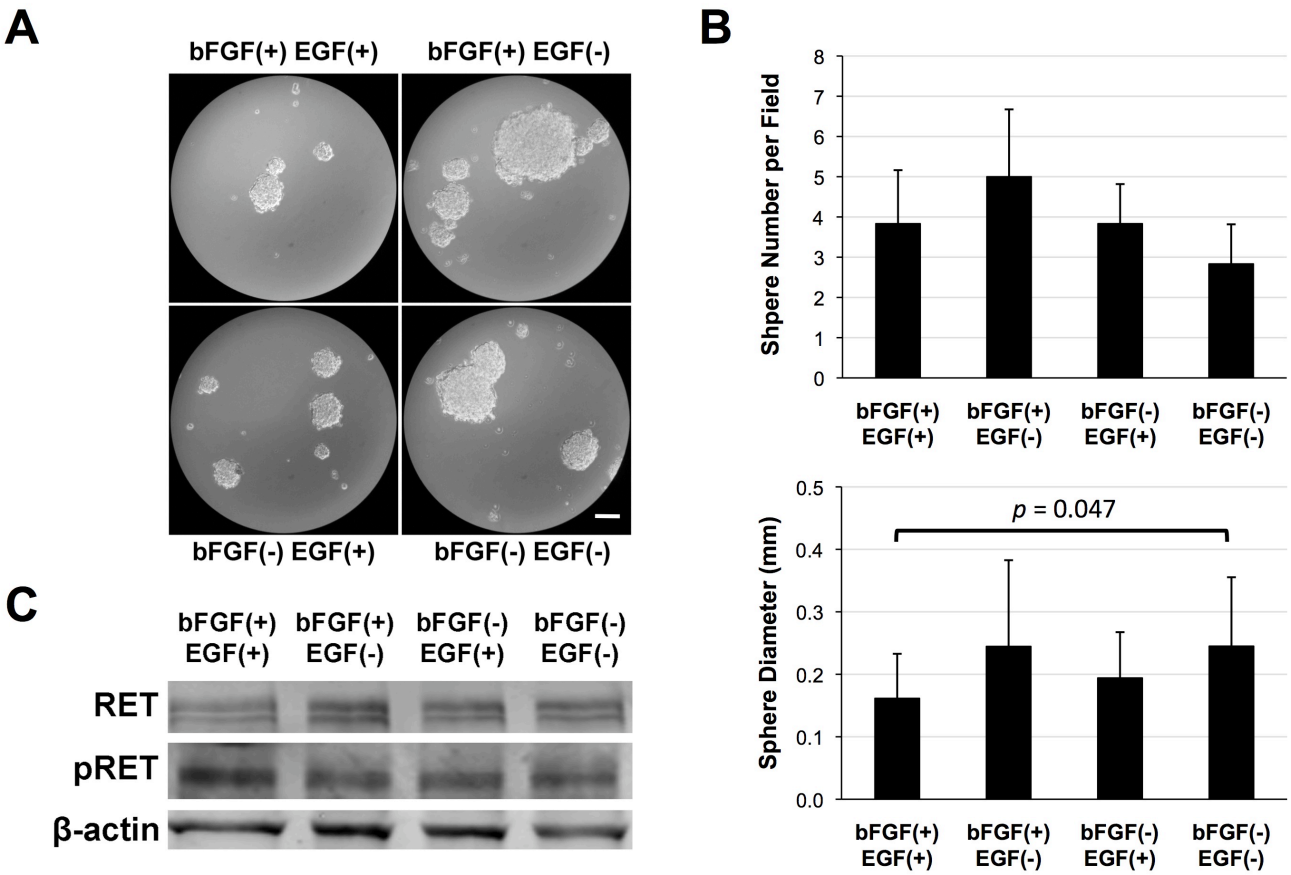
A



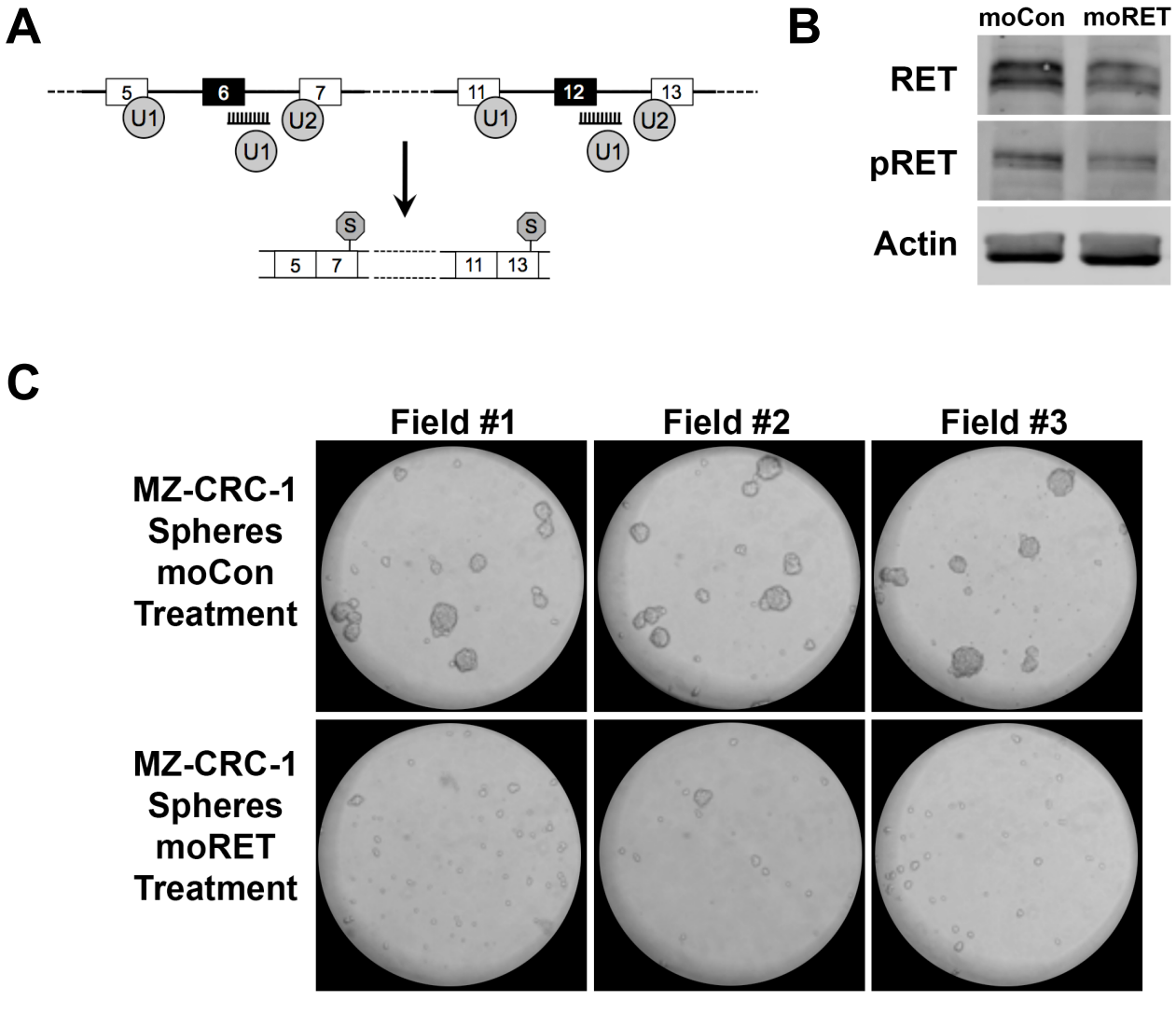
B



Supplemental Figure 1. Expression of stem and progenitor cell markers in MZ-CRC-1 parental cells and tumor spheres. (A) Flow cytometric analysis of CD133, GFAP and TUBB3 expression in MZ-CRC-cells as described in Methods. Representative profiles are shown for the indicated cell populations and antibodies. The quantification of these data appear in Figure 2. (B) Quantitative mRNA analysis was performed on parallel cultures. A specific increase in stem cell marker CD133 is observed at both the protein and RNA levels.



Supplemental Figure 2. MZ-CRC-1 cells formed spheres without the addition of exogenous growth factors. (A) The sphere forming assay was performed using MZ-CRC-1 cells in the indicated culturing conditions (presence or absence of bFGF and/or EGF). Representative micrographs show sphere formation in the indicated culturing conditions 14 days after plating. (Scale bar 0.1mm). (B) Numbers and sizes of the MZ-CRC-1 spheres in the four different groups were analyzed in 10 random fields under 10× magnification on Day 14. Values provided represent the mean ± SD. Data are representative of three independent experiments. (C) Western blot analysis of RET expression in lysates derived from spheres treated as described in A. RET activity was determined as a measure of phosphoRET/totalRET by Odyssey imaging system (Li-Cor Biosciences, Lincoln, NE). No significant difference in RET activation level was detected among the four groups.



Supplemental Figure 3. Inhibition of tumor sphere formation by morpholino oligonucleotide-mediated RET knockdown. (A) RET knockdown was performed using an antisense morpholino oligonucleotide (MO) that targeted the 5' splice sites of RET exons 6 (AGGCAATAGGTATGGGCTCACCTGG) and 12 (TGTGCCTGTGCCTGGCAGGTACCTT) to induce exon skipping (Gene Tools Inc., Philomath, OR). The skipping of exon 6 and 12 is predicted to induce a frameshift resulting premature stop codons 47 and 17 amino acids downstream respectively. Exon 6 encodes the third cadherin-like domain, while exon 12 encodes the beginning of the tyrosine kinase domain. (B) Western analysis of MZ-CRC-1 cells following treatment with either control (moCon) or targeted (moRET) morpholinos. Cells were collected 96 hours after double scrape loading of morpholino (Cheung et al. Brain 132:2277, 2009). Parallel cultures were replated and subjected to neurosphere culture conditions as described in the methods section immediately following a the second scraping. (C) Representitive micrographs of MZ-CRC-1 sphere formation at 10 days following replating. A clear inhibition of MTC tumor sphere size is observed.