Supplementary Text and Figures for Luo Y., et al.

METABOLIC REGULATOR BKLOTHO INTERACTS WITH FIBROBLAST GROWTH FACTOR RECEPTOR 4 (FGFR4) TO INDUCE APOPTOSIS AND INHIBIT TUMOR CELL PROLIFERATION

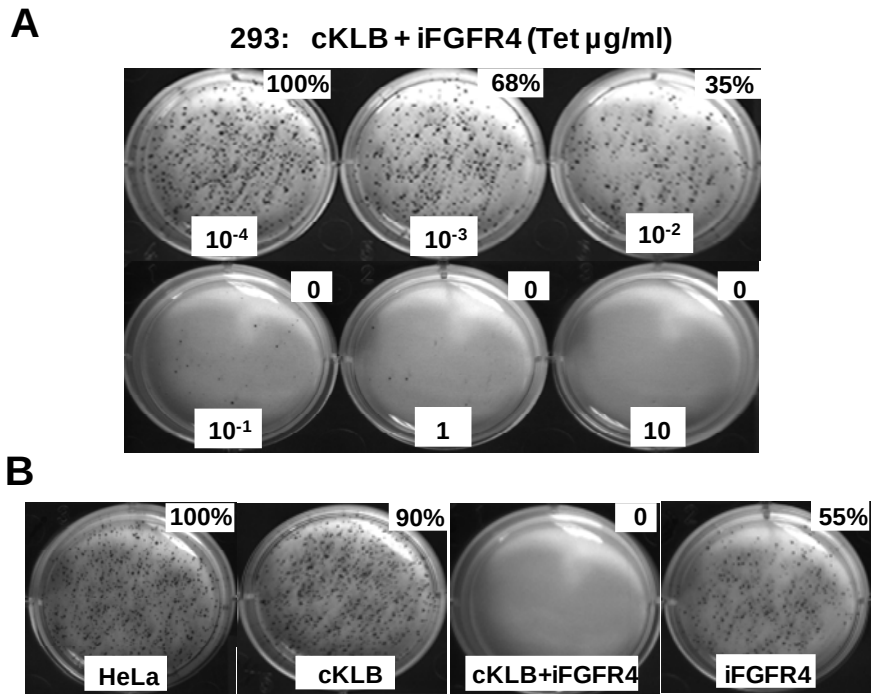

Fig. S1. Suppression of anchorage-independent growth by the KLB-FGFR4 partnership. A. Population growth and clonal expansion of 293 cells expressing KLB in which FGFR4 was induced at the indicated concentrations of Tet was analyzed in soft agarose. Percentages indicate number of colonies relative to plates containing 10^{-4} µg/ml Tet. B. HeLa cell cultures induced at 1 µg/ml Tet. One ml of a 1:1 mixture of 1.4% low melting point agarose (Invitrogen, Carlsbad, CA) and DMEM containing 20% FBS was de-aerated and added to each well of 6-well plates at 37°C and allowed to solidify at room temperature. A mixture of 1 ml 1.4% agarose and 3 ml DMEM medium containing 4 x 10^4 cells with 12% FBS and the indicated concentrations of Tet was prepared at 37° C. An aliquot of 1.5 ml of the mixture was slowly added to the solidified bottom layer. Cultures were incubated with addition of 0.5 ml fresh DMEM containing 10% FBS and the indicated treatments every 4 days. All cultures contained 1 µg/ml of FGF19. After about two weeks, resultant colonies were stained with 100 µg per ml of MTT for 1 hr at 37°C, photographed and counted for quantization.

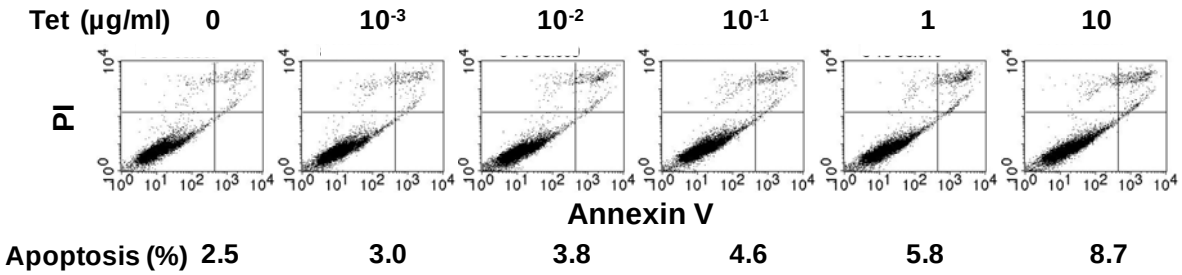

Fig. S2. Basal levels of apoptotic cell death in 293 cells induced to express FGFR4 alone in absence of KLB. FGFR4 was induced by the indicated concentrations of Tet. Apoptosis was monitored as described in text Fig. 3.

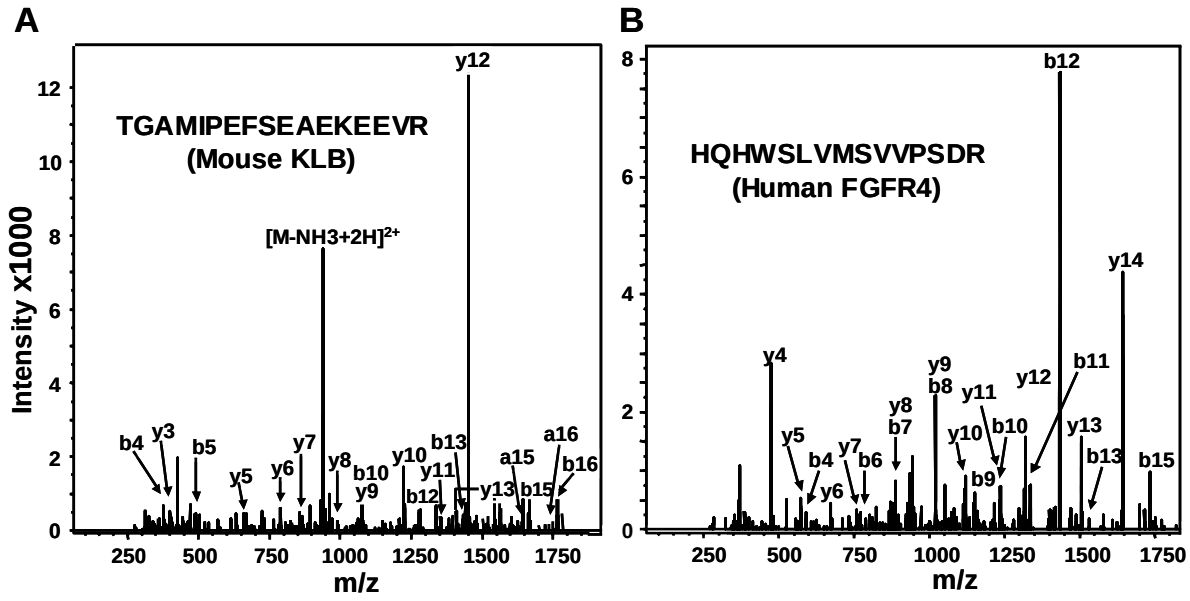

Fig. S3. Direct analysis of interaction of KLB and FGFR4 by co-immunoprecipitation and MS. Bands from SDS-PAGE gel corresponding to the predicted molecular mass of mouse KLB (A) and human FGFR4 (B) were excised and identified by nanoLC-MS/MS. 293 cells stably expressing KLB were induced to express FGFR4 by 300 ng/ml of Tet overnight and exposed to serum-free medium containing Tet for 6 hrs. Cells after exposure to 1 µg/ml FGF19 for 10 min in fresh serum-free medium were extracted in modified cold RIPA buffer. Supernatant from cell lysate was immunoprecipitated by anti-FGFR4 antibody that specifically recognizes the COOH-terminus of FGFR4 (Santa Cruz Biotechnology, Santa Cruz, CA) and was non-covalently immobilized on Protein A/G beads. The precipitates were resolved on 10% SDS-PAGE. Bands from SDS-PAGE gel corresponding to the predicted molecular mass of mouse KLB and human FGFR4 were directly excised without staining, and processed for in-gel tryptic digest. The peptides were then extracted, desalted by ZipTip C18, and identified by nanoLC-MS/MS as described previously (Luo et al., 2009). IgG or beads alone were used as negative controls (not shown). Non-specific interactions of recombinant FGFR4 or KLB with the beads was negligible.

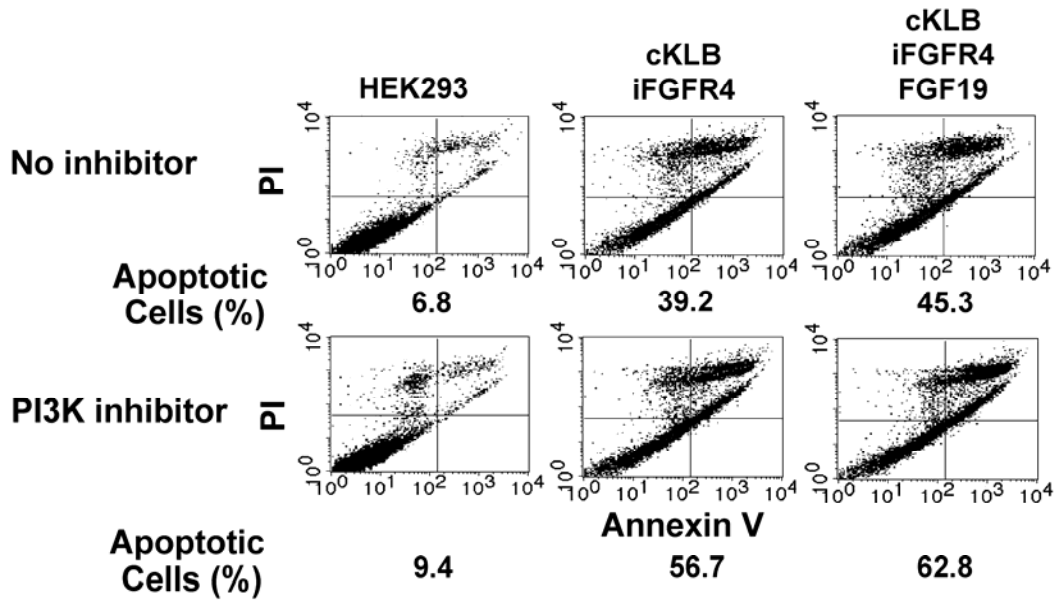

Fig. S4. A PI3K inhibitor increases apoptotic cell death in addition to the KLB-FGFR4 partnership. 2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002) (Calbiochem, CA) was added during induction by 1 μg/ml Tet. Apoptosis was monitored as described in text Fig. 3. The indicated data is representative of three reproductions. The similar percent increase in apoptosis in control cells and those expressing KLB and FGFR4 due to the inhibitor indicates the increase in apoptosis may be in addition to and independent of that caused by the KLB-FGFR4 partnership.

Supplementary Reference

Luo Y, Yang C, Jin C, Xie R, Wang F, McKeehan WL (2009) Novel phosphotyrosine targets of FGFR2IIIb signaling. *Cell Signal* **21:** 1370-8.