

Supplementary Materials for

IGFBP7 Binds to the IGF-1 Receptor and Blocks Its Activation by Insulin-Like Growth Factors

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The PDF file includes:

- Fig. S1. IGFBP7 does not interfere with activation of the IGF1R or INSR pathways when added simultaneously with their cognate ligands.
- Fig. S2. Pretreatment with IGFBP7 or its ectopic expression in cancer cell lines inhibits IGF-1–mediated activation of IGF1R.
- Fig. S3. Plasma membrane localization of IGFBP7 in treated or transfected cell lines.
- Fig. S4. Full-length but not the truncated form of IGFBP7 induces apoptosis in the absence of IGF-1.

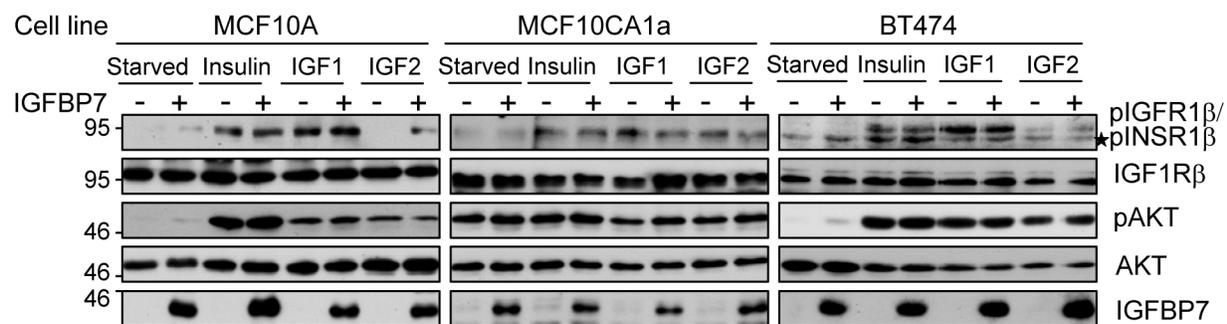


Fig. S1. IGFBP7 does not interfere with activation of the IGF1R or INSR pathways when added simultaneously with their cognate ligands. Serum-starved MCF10A, MCF10CA1a and BT474 cell lines were induced for 15 min with insulin (10 μ g/ml), IGF-1 or IGF-2 (50 ng/ml of each) in the absence or presence of IGFBP7 (20 μ g/ml). Cell extracts were analyzed by immunoblotting using the indicated antibodies. IGFBP7 was detected using c-myc-tag antibodies. The star indicates a cross-reacting protein in BT474 cells. Representative results from three independent experiments are shown.

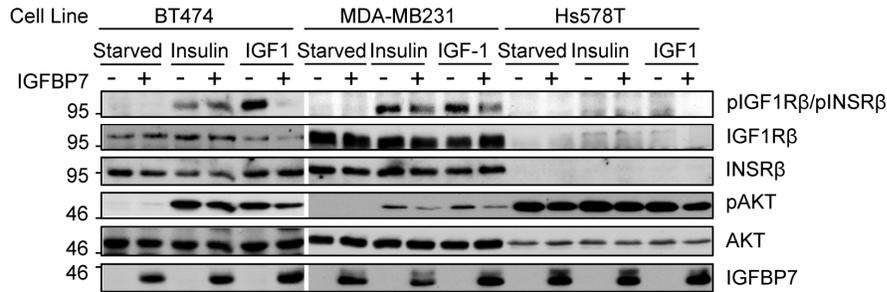
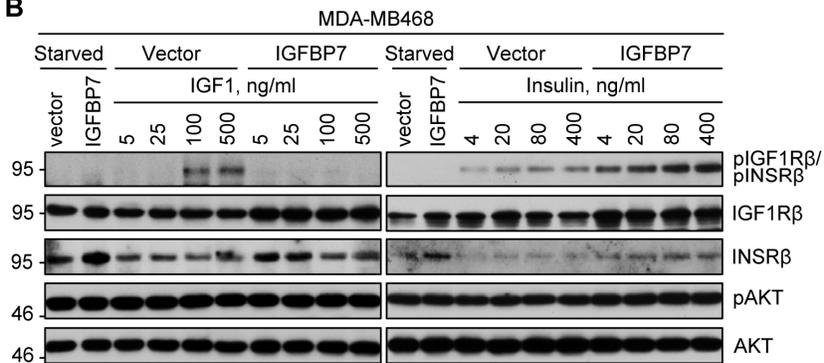
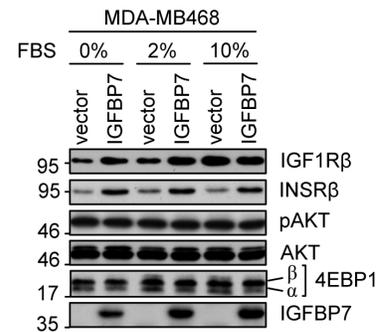
A**B****C**

Fig. S2. Pretreatment with IGFBP7 or its ectopic expression in cancer cell lines inhibits IGF-1–mediated activation of IGF1R. **(A)** Indicated cell lines were serum-starved for 18 hours, pretreated with IGFBP7 (10 μ g/ml) for the last 4 hours and then left untreated (Starved) or induced with IGF-1 (50 ng/ml) or insulin (10 μ g/ml) for 15 min. Representative immunoblotting results from two independent experiments are shown. **(B)** MDA-MB468 cells stably expressing vector alone or c-myc-tagged IGFBP7 were serum-starved for 4 hours and then treated with the indicated concentrations of IGF-1 or insulin for 5 min. Cell extracts were examined by immunoblotting using corresponding antibodies. IGFBP7 was detected using c-myc-tag antibodies. **(C)** Vector alone or IGFBP7-expressing MDA-MB468 cells were grown in the absence (0%) or in presence of 2% or 10% FBS for 24 hours, and then examined by immunoblotting using the corresponding antibodies. Results shown in (B-C) are representative of three independent experiments.

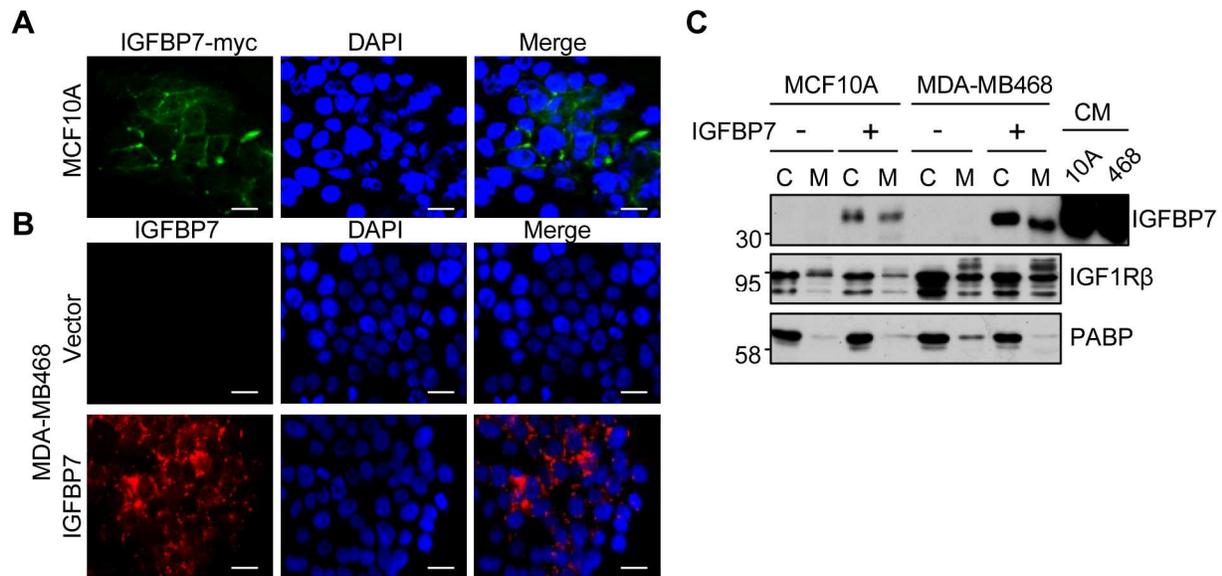


Fig. S3. Plasma membrane localization of IGFBP7 in treated or transfected cell lines. **(A, B)** Immunofluorescence microscopy showing plasma membrane localization of IGFBP7 in MCF10A cells treated with IGFBP7 (10 $\mu\text{g/ml}$) in serum-free medium for 1 hour (A) or in MDA-MB468 cells ectopically expressing IGFBP7 or vector alone (B). IGFBP7 was detected using c-myc (A) or IGFBP7 (B) antibodies followed by the corresponding Alexa Fluor-labeled secondary antibodies. DAPI was used to visualize nuclei. Scale bars, 50 μm . $n = 2$ independently repeated experiments. **(C)** Immunoblotting showing distribution of IGFBP7 and IGF1R between cytosolic (C) and membrane (M) fractions derived from MCF10A cells treated with IGFBP7 (10 $\mu\text{g/ml}$) or from MDA-MB468 cells ectopically expressing IGFBP7 or vector alone. Cells were treated for 18 hours in the medium containing 2% horse serum or FBS, respectively. To remove weakly or non-specifically bound IGFBP7 from the cell surface, cells were briefly washed with 0.01%-NP-40 lysis buffer prior to lysis. The poly(A)-binding protein (PABP) was used as a marker for cytosolic fraction. “CM” indicates IGFBP7 found in the conditioned medium from MCF10A or MDA-MB468 cells; 1/10,000th aliquot was taken for the analysis, compared to 1/100th of those from the cytosolic and membrane fractions. IGFBP7 was detected using c-myc-tag antibodies. $n = 2$ experiments.

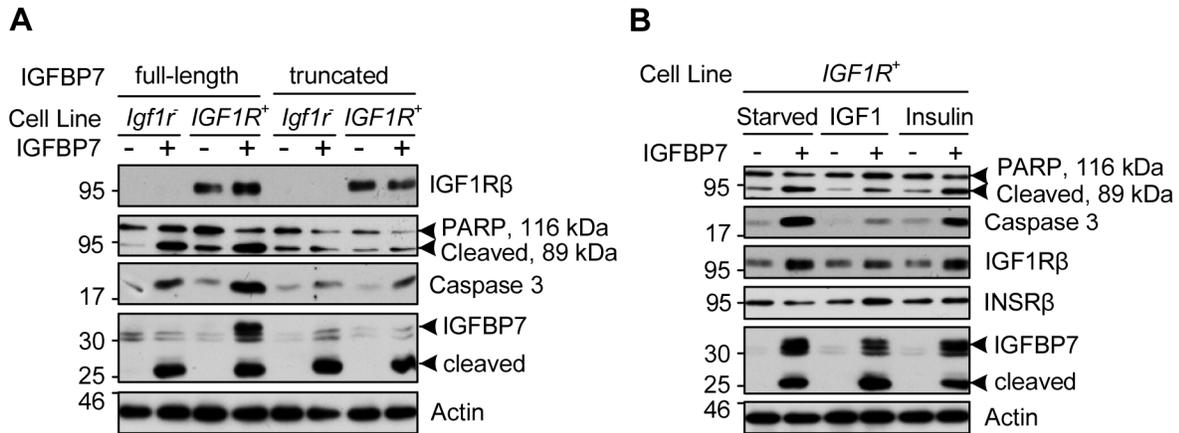


Fig. S4. Full-length but not the truncated form of IGFBP7 induces apoptosis in the absence of IGF-1. **(A)** MEFs were treated with the recombinant full-length or truncated IGFBP7 proteins (20 μ g/ml of each) in the medium containing 2% FBS for 3 days. Floating and adherent cells were collected and examined by immunoblotting using corresponding antibodies. IGFBP7 was detected using c-myc-tag antibodies. Note that full-length IGFBP7 appears to be more stable in *IGFBP7*⁺ cells, whereas it was completely cleaved in *Igf1r*⁻ cells. This form is referred to as “cleaved”, in contrast to the recombinant “truncated” form. Representative results from three independent experiments are shown. **(B)** Serum-starved *IGFBP7*⁺ cells were treated with full-length IGFBP7 as in (A) in the absence (Starved) or presence of IGF-1 (50 ng/ml) or insulin (10 μ g/ml) and analyzed by immunoblotting. Representative results from two independent experiments are shown.