SUPPORTING INFORMATION

Defining the growth factor binding motif in the extracellular matrix protein MAGP-1

Thomas J. Broekelmann. Nicholas K. Bodmer, and Robert P. Mecham*

Date Included:

Figure S1: Amino acid sequence of mouse MAGP-1 showing exon boundaries that defined the protein domains described in this study.

Figure S2. Inhibition of TGFβ-1 binding to a MAGP-1 chip (600 RU) by peptides listed in Figure 2a.

Figure S3. SDS-PAGE and western blot analysis of purified fibrillin-2.

Figure S4. BMP binds fibrillin-2.

Figure S5. TGFβ production by WT and MAGP knockout cells.

SUPPORTING INFORMATION

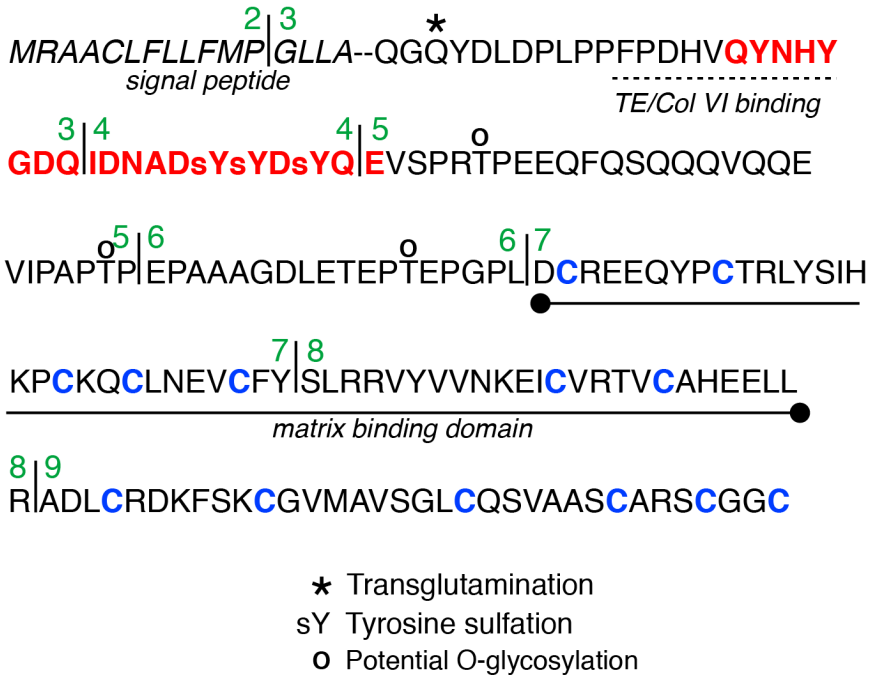

Figure S1: Amino acid sequence of mouse MAGP-1 showing exon boundaries that define the protein domains described in this study. Also shown are the post-translational modification sites that include transglutamination, tyrosine sulfation, and O-glycosylation. The solid underline indicates the matrix binding domain, and the segmented underline indicates the tropoelastin and collagen VI binding region. The growth factor-binding domain (TGFβ and BMP) defined in this study is indicated by red letters.

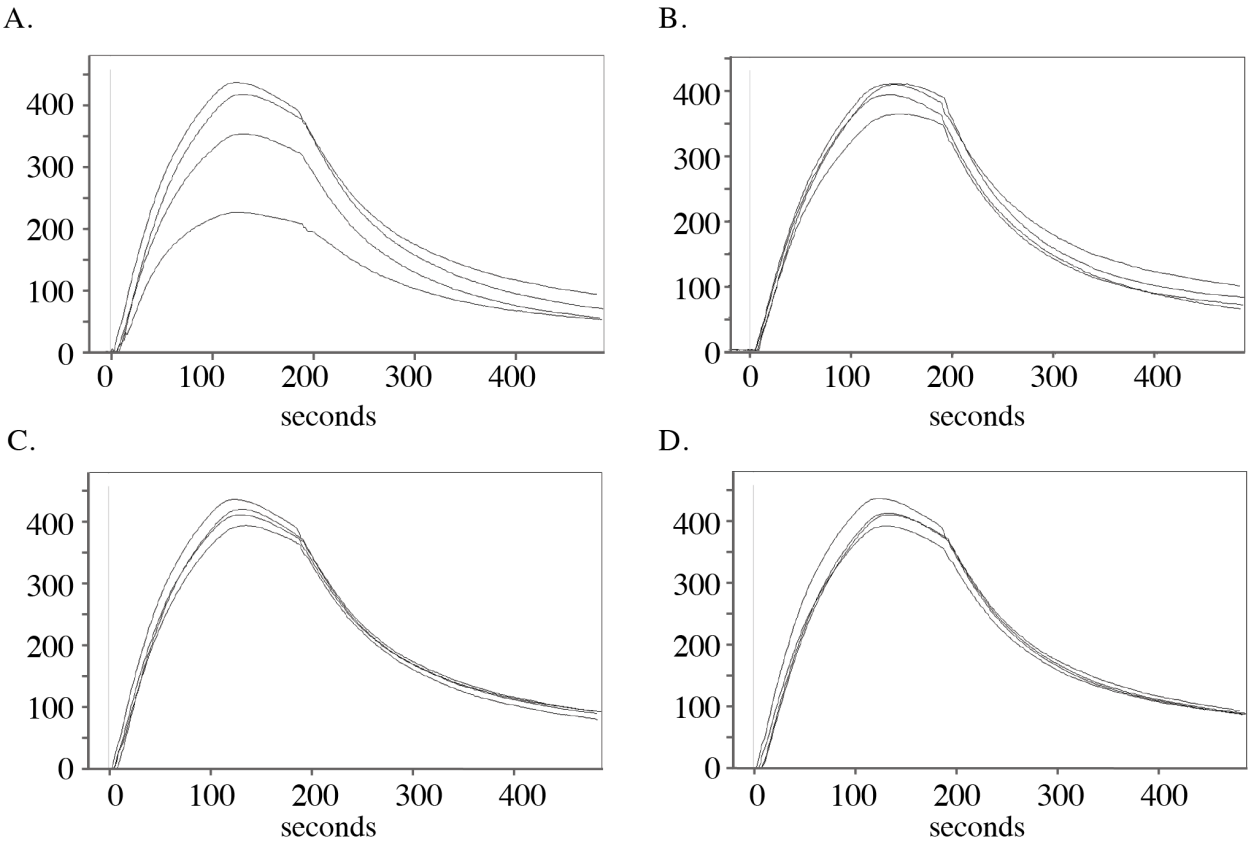

Figure S2. Inhibition of TGFβ-1 binding to a MAGP-1 chip (600 RU) by peptides listed in Figure 3a. A) is a graph of isotherms from the binding of 50nM TGFβ-1, premixed with varying concentrations of peptide p1-35 (120, 60, 30, 15, and 0 μM), showing binding inhibition by the peptide. **B, C, and D)** show that peptides p22-36 (120, 60, 30, 15, and 0 μM), peptide p1-18 (120, 60, 30, 15, and 0 μM), and peptide p26-35 (120, 60, 30, 15, and 0 μM) do not inhibit TGFβ-1 binding to full-length MAGP-1.

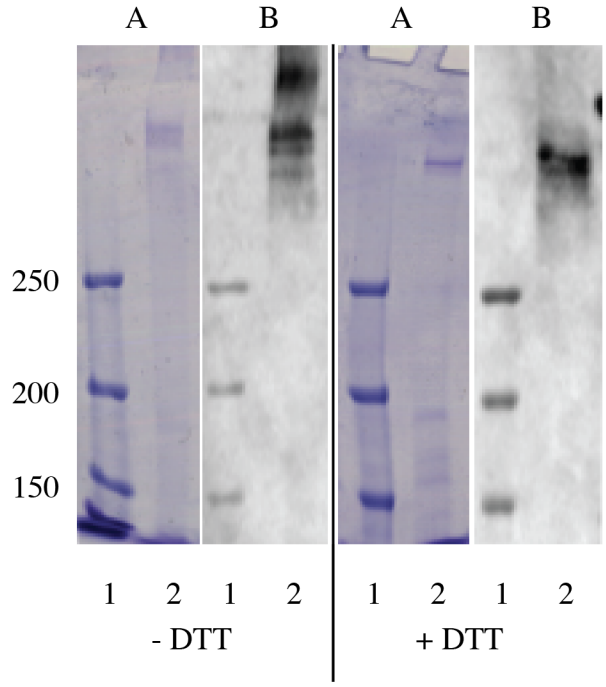

Figure S3. SDS-PAGE and western blot analysis of purified fibrillin-2. A lanes: gels stained with Simply Blue Coomassie G-250, **B lanes:** immunoblots of proteins transferred to nitrocellulose and detected with an antibody to fibrillin-2. Lanes marked 1 are molecular weight standards, lanes marked 2 contain fibrillin-2 either with or without reducing agent (DTT).

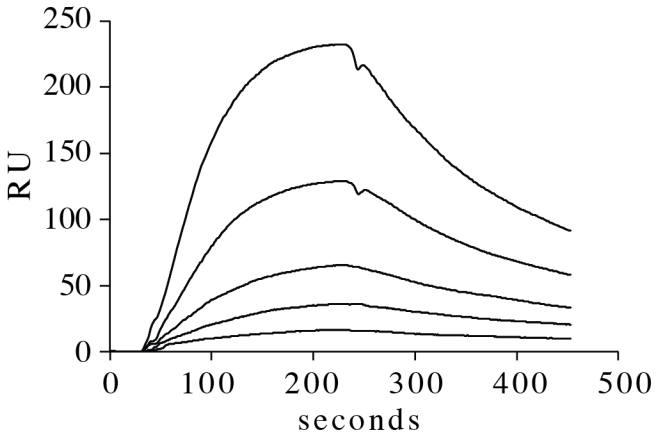

Figure S4. **BMP-2 binds directly to fibrillin-2.** Isotherms showing the interaction of BMP-2 with a fibrillin-2-coated SPR chip. From the top, the BMP-2 concentrations are: 100, 50, 25, 12.5, and 6.25 nM.

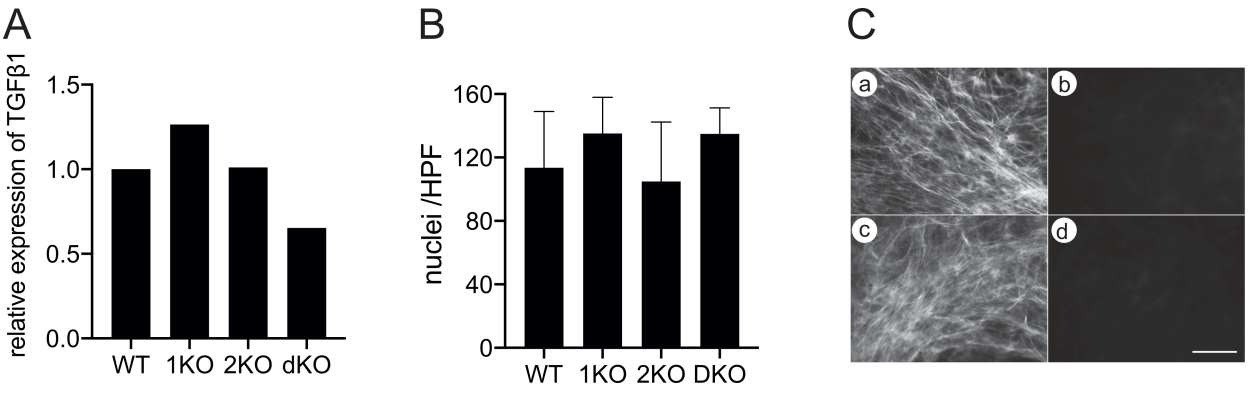

Figure S-5. TGFβ production by WT and MAGP knockout cells. **A)** shows the relative expression of TGFβ-1 mRNA in 14-day post-confluent wild type (WT), MAGP-1 knockout (1KO), MAGP-2 knockout (2KO) and MAGP1-2 double knockout (DKO) fibroblast cultures. Values are normalized to WT levels. **B)** shows the number of nuclei (DAPI stained) averaged from10 high power fields taken from 14-day post-confluent WT, 1KO, 2KO and DKO fibroblast cultures. **C)** shows representative MAGP-1 immunofluorescence microscopy photographs of fibroblasts taken from WT (**Panel A**), MAGP-1KO (**B**), MAGP-2KO (**C**), and MAGP-1;MAGP-2-KO (DKO) (**D**) fibroblasts. The scale bar is 100μM.