Supplemental figure legends

Supplemental Figure 1. Validation of antibody specificity for H-98 IGFBP3 antibody employed for immunofluorescence and immunohistochemistry in this study
Confocal imaging of PrSCs treated for 24 h with 1 ng/ml bFGF or TGFβ1 as indicated and stained with H-98 anti-IGFBP3 antibody (Santa Cruz, red) that was pre-incubated with 200 ng/ml rhIGFBP3 (block) or PBS (mock) for 2 h with end-over-end rotation prior to immunofluorescent staining. Nuclei were counterstained with DAPI. Images are representative of two independent experiments using PrSCs isolated from two different donors. Original magnification x40.

Supplemental Figure 2. Basal expression levels of IGFBPs in PrSCs
qPCR of basal expression level of the indicated IGFBP isoform in PrSCs maintained under routine culture conditions. Relative expression level of IGF1 is shown for comparison. Values represent mean expression (±SEM) relative to IGFBP7 mRNA levels (set as 1.0) after normalization against the housekeeping gene HMBS. (IGFBP, insulin-like growth factor binding protein; IGF1, insulin-like growth factor 1; HMBS, hydroxymethylbilane synthase).

Supplemental Figure 3. Isoform specificity of IGFBP3 shRNA
PrSCs were incubated with scrambled (scr) control or IGFBP3 shRNA-expressing lentivirus at MOI 4 for 96 h and subsequently stimulated for 72 h with 1 ng/ml bFGF before qPCR of the indicated genes. Values represent relative gene expression (± SEM) compared to scr control after normalization against the housekeeping gene HMBS using PrSCs isolated from three independent donors. Statistical significance is indicated (ns, not significant where \( P > 0.05 \); ***, \( P < 0.001 \)). (IGFBP, insulin-like growth factor binding protein; HMBS, hydroxymethylbilane synthase).

Supplemental Figure 4. rhIGFBP3 potentiates TGFβ1-induced changes in phosphorylation levels of key signaling transducers of differentiation
Western blotting with the indicated antibodies of PrSCs treated for 8 h with rhIGFBP3 (300 ng/ml), TGFβ1 (1 ng/ml) alone or in combination as indicated. Total ERK1/2 is shown as loading control.
Images are representative of four independent experiments using PrSCs from different donors.

(SMAD2, SMAD family member 2; AKT, v-akt murine thymoma viral oncogene homolog 1; JNK, c-Jun N-terminal kinase; ERK1/2; extracellular regulated kinase 1/2).

Supplemental Figure 5. **IGF1 enhances proliferation of PrSCs**

PrSCs were incubated with 100 ng/ml IGF1, LongR3-IGF1 or vehicle equivalent in the presence of the indicated concentration of rhIGFBP3 for 72 h as stated before analysis of cell proliferation via BrdU incorporation ELISA. Values represent mean absorbance at 450 nm (±SEM) from quadruplicate wells relative to mock treated control using PrSCs isolated from three independent donors.

Supplemental Figure 6. **Validation of antibody specificity in immunohistochemistry of prostate tissue sections**

Immunohistochemistry of prostate tissue sections stained in parallel with anti-rabbit IGFBP3 antibody (left) or control rabbit immunoglobulin (right). Images are representative of three independent experiments using tissue sections from four different patients. Original magnification 100x.
Supplemental Fig. 1
Supplemental Fig. 3
A

Mean BrdU incorporation (absorbance 450 nm)

rhIGFBP3 (ng/ml)

mock
IGF1

B

Mean BrdU incorporation (absorbance 450 nm)

rhIGFBP3 (ng/ml)

mock
LongR3-IGF1

Supplemental Fig. 4
### Supplemental Table 1 Primer sequences

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene ID</th>
<th>Primer sequence</th>
<th>Gene symbol</th>
<th>Gene ID</th>
<th>Primer sequence</th>
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</thead>
</table>
| ACTA2 (SMA)  | 72      | F: AGAAGAGCTATGAGCTGCCA  
R: GCTGTGATCTCCTCTCTCAT | IGFBP3       | 3486     | F: CAAGCGGGGAGACAGAATATG  
R: TTATCCACACACCCAGAGAA |
| CNN1         | 1264    | F: GGTGAACGTTGGAGTGAAATG  
R: GGTCCAGAGGCTGGTCTGT | IGFBP4       | 3487     | F: AAATTCGAGACGGAGACAC  
R: AGCTTCACCCCCGTCTTC |
| HMBS         | 3145    | F: CCAGGACATCTTGGATCTGG  
R: ATGGTAGCCTGCATGGTCTC | IGFBP5       | 3488     | F: GTACCTGCCCAATTGTGACC  
R: AGGTGTGCGACTGAAAGTCC |
| IGF1         | 3479    | F: GGAGGCTGGAGATGTATTGC  
R: GATGTTGCTTTGGGCAACCT | IGFBP6       | 3489     | F: AAGGAGAGTAAACCCCAAGCA  
R: TTTGAGCCCCCTCGGTAGAC |
| IGF1R        | 3480    | F: CAAGTCCTTCGCCTGTC  
R: GAGAGGGCGCCTGATCTTG | IGFBP7       | 3490     | F: TATGAGTGCCATGACATCCAA  
R: CCATGACTACTTTTAACCATGCAG |
| IGFBP1       | 3484    | F: CTGCCAAAAGCTGCAAAGAAGA  
R: TATCTGCAGTGGGTCTCTC | NOX4         | 50507    | F: TGGCAAGAGAACAGACCTGA  
R: TGGGTCCACACACAGAAAACA |
| IGFBP2       | 3485    | F: CGGAAGCCCTCAAGTCG  
R: GCCTCTGCTGCTCATTG |             |         | *Primer sequences are given 5' to 3', annealing temperature for all primers in qPCR is 56°C, all primers span at least one intron |