Supplementary Data

Nuclear FAK and Runx1 cooperate to regulate IGFBP3, cell cycle progression and tumor growth

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Supplemental Material for the manuscript includes:

A: Supplementary Materials and Methods.

B: Supplementary Table 1 and **Supplementary Table 2** provided as separate Excel spreadsheets. The legends for the Supplementary Tables are replicated below.

> **Supplementary Table 1 |** Supplementary table 1 details nuclear FAK-binding proteins with known associations to Runx1.
>
> **Supplementary Table 2 |** Supplementary table 2 details pathway enrichment analysis of nuclear FAK-binding proteins with known associations to Runx1.

C: Supplementary Figures 1-5 (included in this file).

> **Supplementary Figure 1 |** Supplementary figure 1shows relative levels of angiogenesis related proteins in conditioned medium, that are subject to FAK dependent regulation. Results are presented as a heat map.
>
> **Supplementary Figure 2** | Supplementary figure 2 shows qRT-PCR results from mouse liver cDNA used to validate primer sets for the IGFBP family.
>
> **Supplementary Figure 3** | Supplementary Figure 3 shows transcription factors with predicted binding sites in the promoters of the top 3 angiogenesis related proteins up-regulated upon FAK deletion. Graphs represent the number of predicted binding sites in the human promoter.

Supplementary Figure 4 | Supplementary figure 4 shows a representative western blot of Runx1 siRNA in SCC FAK-wt and -/- cells, and the effect of Runx1 knock down on IGFBP3 expression, as measured by qRT-PCR.

Supplementary Figure 5 | Supplementary figure 5 shows interaction network analysis of physical or predicted direct or indirect binders of Runx1 that interact with FAK in the nucleus of SCC cells.

Supplementary Materials and Methods

Antibodies used were as follows:

Antibody	Species	Supplier
Anti-IGFBP3	Goat	R & D Systems
Anti-Runx1	Rabbit	Abcam
Anti-FAK 4.47	Mouse	Millipore
Anti-FAK 4.47 agarose	Mouse	Millipore
Anti-CD31	Rat	BD Biosciences
Anti-Runx1	Mouse	Sigma
Anti-γ-tubulin	Rabbit	Sigma
Anti-FAK	Rabbit	Cell Signaling Technology
Anti-PARP	Rabbit	Cell Signaling Technology
Anti-GAPDH	Rabbit	Cell Signaling Technology
Anti-Sin3a	Rabbit	Cell Signaling Technology
Anti-mouse IgG–DyLight-conjugated	Mouse	Cell Signaling Technology
Anti-rabbit IgG–DyLight-conjugated	Rabbit	Cell Signaling Technology
Anti-mouse IgG–HRP-conjugated	Mouse	Cell Signaling Technology
Anti-rabbit IgG–HRP-conjugated	Rabbit	Cell Signaling Technology

Primers used to generate nuclear targeting mutants were as follows:

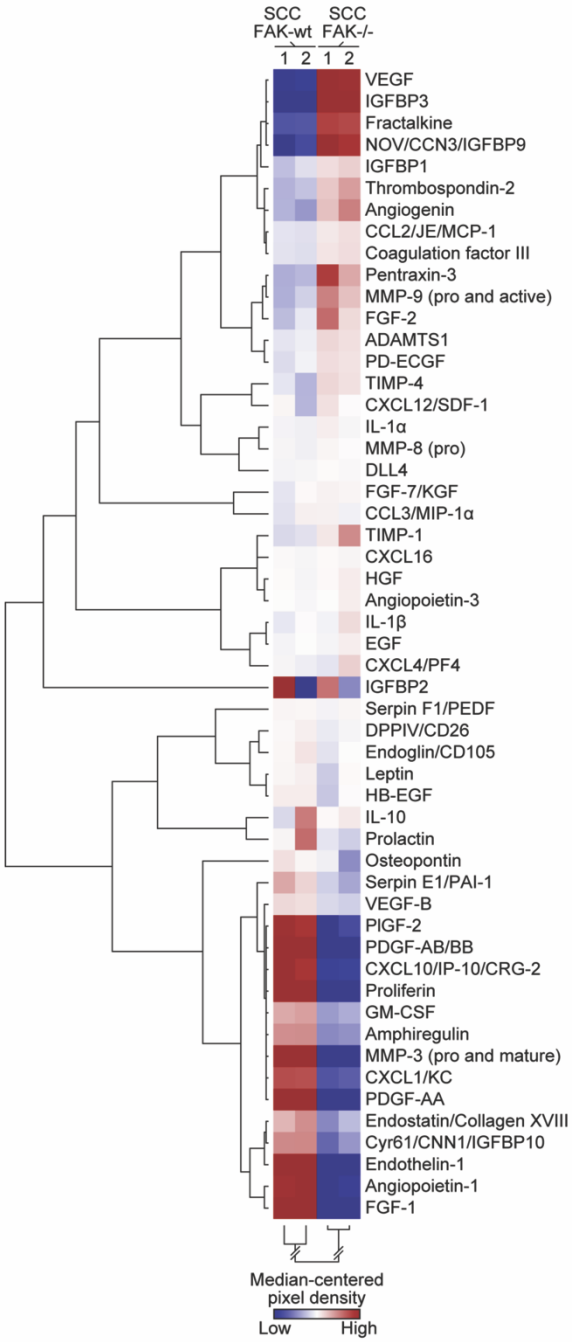
Primer Name	Sequence
FAK-R177A/R178A FWD	CTAGGTTGCCTTGAAATCGCGGCATCCTACGGAGAGATGAG
FAK-R177A/R178A REV	CTCATCTCTCCGTAGGATGCCGCGATTTCAAGGCAAC CTAG
FAK-K190A/K191A FWD	GAGGCAATGCATTAGAGGCGGCATCCAACTATGAAGTGCTAG
FAK-K190A/K191A REV	CTAGCACTTC ATAGTTGGATGCCGCCTCTAATGCATTGCCTC
FAK-K216A/K218A FWD	GAAGAGTTTGCTAGATTCAGTGGCGGCCGCAACACTACGAAAATTAAT
FAK-K216A/K218A REV	GATTAATTTTCGTAGTGTTGCGGCCGCCACTGAATCTAGCAAACTCTTC

IGFBP family q(RT)-PCR primers used were as follows:

Primer Name	Sequence
IGFBP1 FWD	AGCCCAGAGATGACAGAGGA
IGFBP1 REV	GTTGGGCTGCAGCTAATCTC
IGFBP2 FWD	GCGGGTACCTGTGAAAAGAG
IGFBP2 REV	AACACAGCCAGCTCCTTCAT

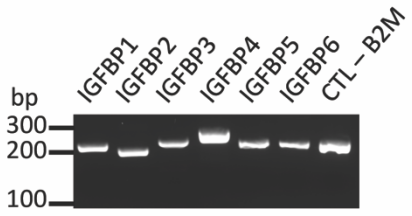
IGFBP3 FWD	AATGCTGGGAGTGTGGAAAG
IGFBP3 REV	TTCTGGGTGTCTGTGCTTTG
IGFBP4 FWD	AGAGCGAACATCCCAACAAC
IGFBP4 REV	ACAGTTTGGAATGGGGATGA
IGFBP5 FWD	TGCACCTGAGATGAGACAGG
IGFBP5 REV	TTGTCCACACACCAGCAGAT
IGFBP6 FWD	CCGTCGGAGG AGACTACAAA
IGFBP6 REV	CTCGGAAGACCTCAGTCTGG
B2M FWD	GGGAAGCCGAACATACTGAA
B2M REV	TGCTTAACTCTGCAGGCGTAT

Supplementary Figure 1 | FAK-dependent regulation of secretion of angiogenesis-related proteins. Relative levels of 53 angiogenesis-related proteins in conditioned media derived from SCC FAK-wt and FAK-/- cells were measured by antibody array. Array spot pixel densities from duplicate spots were quantified, median centered, hierarchically clustered and displayed as a heat map.



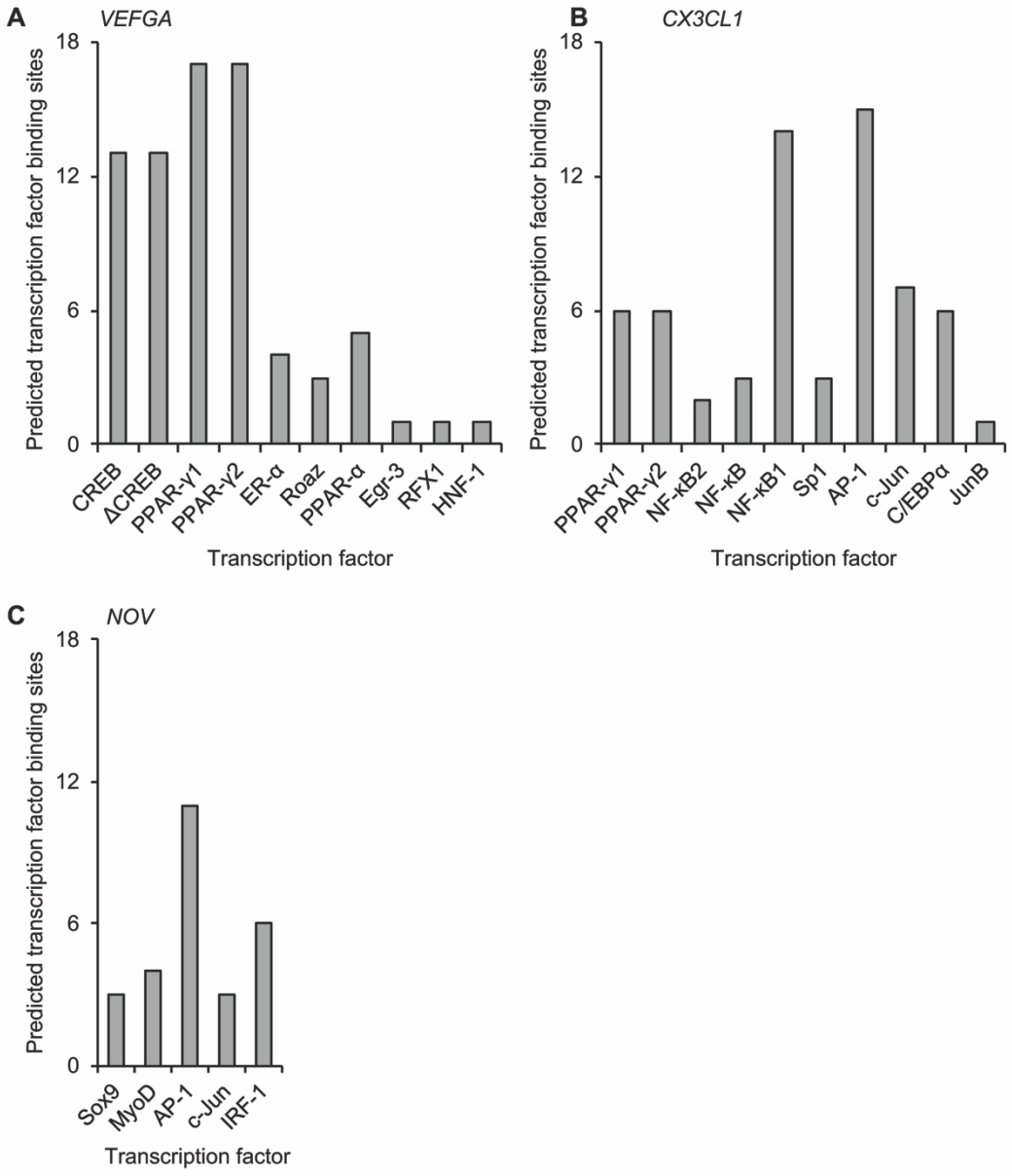
Supplementary Figure 1.

Supplementary Figure 2 | Validation of primer sets for IGFBP family PCR. Representative PCR from mouse liver cDNA using IGFBP1-6 specific primer sets.



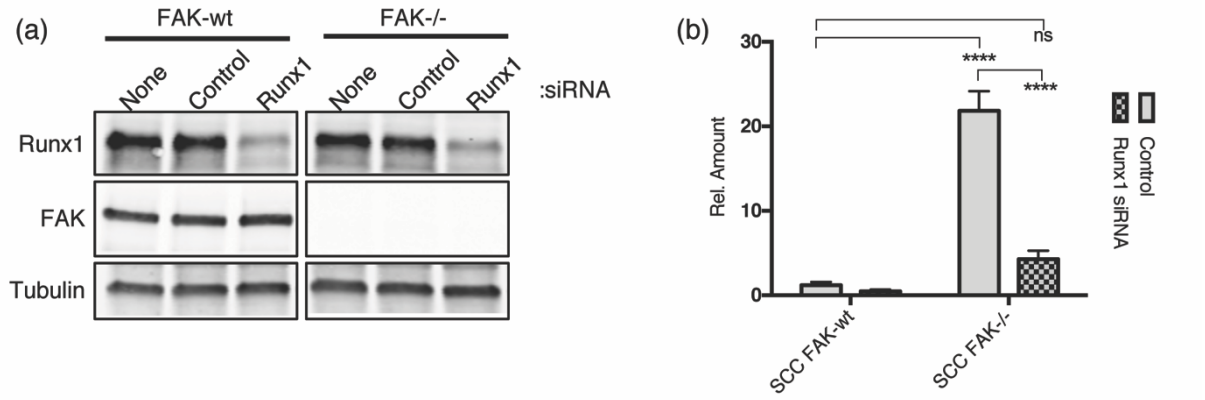
Supplementary Figure 2.

Supplementary Figure 3 | Predicted transcription factor binding sites in the promoters of FAK-downregulated angiogenesis-related genes. (A–C) Transcription factors with predicted binding sites in the *VEGFA* (A), *CX3CL1* (fractalkine) (B), and *NOV* (CCN3/IGFBP9) (C) promoters. The most relevant transcription factors, as predicted by text mining of human data, and the corresponding numbers of predicted binding sites in respective promoters are shown.



Supplementary Figure 3.

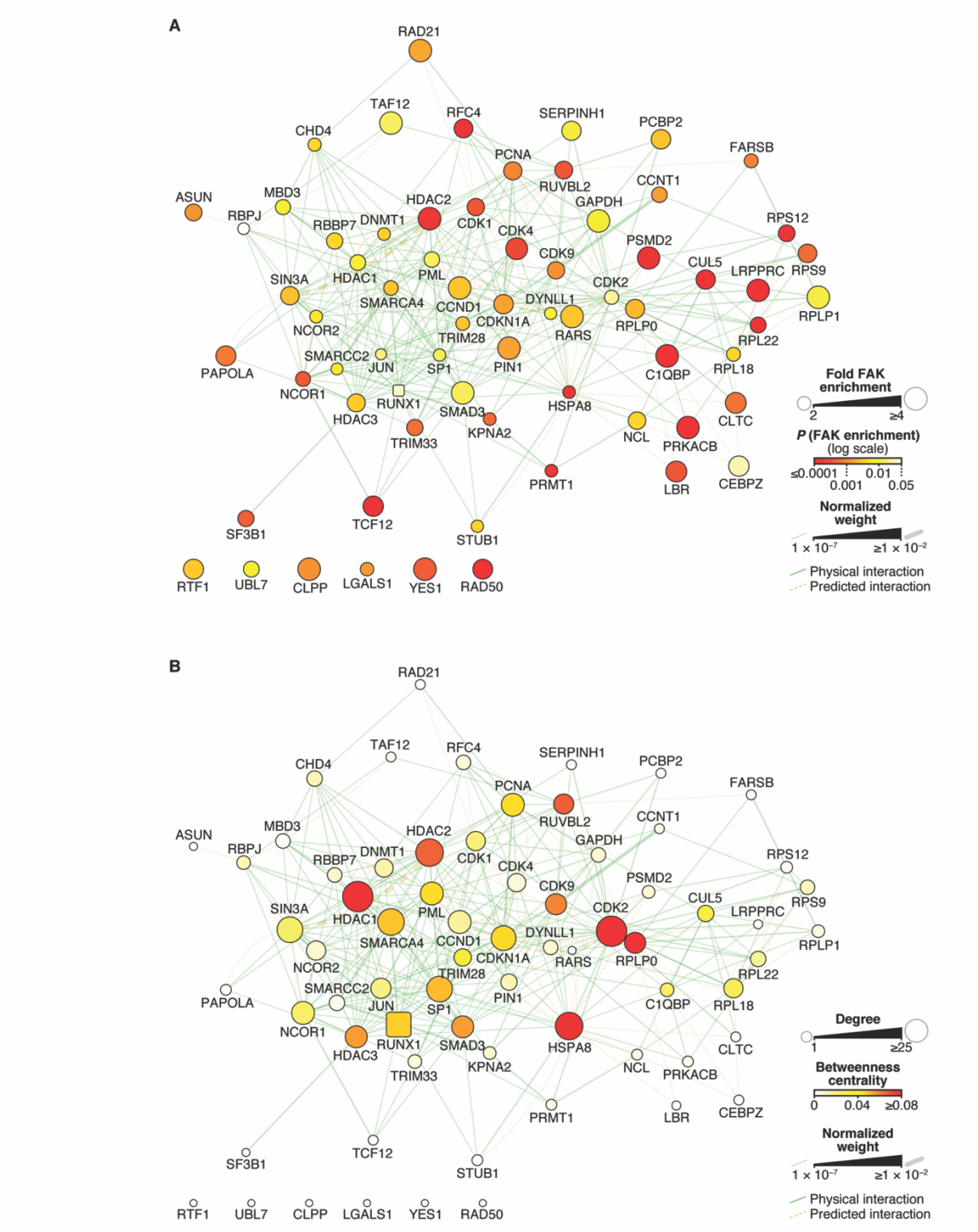
Supplementary Figure 4 | Runx1 is required for IGFBP3 expression in SCC FAK-/- cells. (a) Representative anti-Runx1 western blot from SCC FAK-wt and SCC FAK-/- cells transiently transfected with Runx1 siRNA. None = untransfected control, Control = scrambled siRNA, Runx1 = Runx1 siRNA. (b) (q)RT-PCR analysis of IGFBP3 expression in control and Runx1 depleted SCC FAK-wt and SCC FAK-/- cells. Sidak's corrected 2way ANOVA, ****p < 0.0001. ns = not significant. Data represented as mean +/- s.e.m. n = 3.



Supplementary Figure 4.

Supplementary Figure 5 | Nuclear FAK interactome in the context of Runx1 protein interactions. (A and B) Interaction network analysis of physical or predicted direct or indirect binders of Runx1 that interact with FAK in the nucleus of SCC cells. Runx1 is shown as a square node. Protein node size is proportional to fold enrichment in nuclear FAK immunoprecipitations (A) or number of interaction partners in the network (degree) (B). Node color indicates significance of enrichment in nuclear FAK immunoprecipitations (A) or betweenness centrality (normalized number of shortest paths between proteins; a measure of the control a protein exerts over the interactions of other proteins in the network) (B).

Interaction edge (line) thickness is proportional to network weighting, as determined by GeneMANIA. Proteins are labeled with gene names for clarity (see Supplementary Table 1).



Supplementary Figure 5.