Supporting Information

Meves et al. 10.1073/pnas.1105689108

SI Materials and Methods

Two-Stage Carcinogenesis. Cutaneous two-stage chemical carcinogenesis was performed as described previously (1) using topical applications of 100 nmol (25 μg) 7,12-dimethylbenz[a]anthracene (Sigma-Aldrich) in 100 μL of acetone and twice-weekly applications of 3.4 nmol (2.1 μg) TPA (Sigma-Aldrich) in 100 μL of acetone for 20 wk. Control animals were treated with acetone only. Fisher's exact probability test was used for week-by-week analysis of tumor incidence, and the Mann–Whitney *U* test was used for tumor multiplicity. LRCs were identified using a chase period of 90 d after injection of BrdU (2). All experiments were approved by the government of Upper Bavaria.

Antibodies Used for Immunohistochemistry and Western Blot Analysis. The following antibodies were used for immunohistology: FITC-conjugated mAb against α6 integrin (clone GoH3; BD Biosciences), mouse mAb against K15 (clone LHK15; Millipore), rat mAb against β1 (clone MB1.2; Millipore), rat mAb against BrdU (clone ICR1; Abcam), rabbit pAb against Laminin-332 (a kind gift from M. Aumailley, University of Cologne, Cologne, Germany), and rabbit pAbs against K5 (ab24647) and CSRP1 (ab70010; both from Abcam), K10 (PRB-159P) and Loricrin (PRB-145P; both from Covance), and Src (44-656G; Invitrogen). Rabbit Abs against FAK-pY397 (44-624G; Invitrogen), FAK-pY861 (44-626G; Invitrogen), and total FAK (06-543; Millipore) were used for Western blot analysis.

Primers Used for RT-PCR and FAK siRNA. The following primers were used for RT-PCR (sense and antisense): GAPDH, 5′-TCGTGGATCTGACGTGCCGCCTG-3′ and 5′-CACCACCCTGTTGCTGTAGCCGTAT-3′; K1, 5′-CTGTCTGTTCCCCTAGTGGC-3′ and 5′-GTCCGGGTTGTGGTGTCTAC-3′; K10, 5′-TTTGGTGGCGGTAGCTATGGAG-3′ and 5′-CTCTCGCTGGCTTGAGTTG-3′; CSRP1, 5′-AACAGCTTCCATAAATCCTGCTT-3′ and 5′-CCATACTTCTTGCCGTAACATGA-3′. The following siRNA duplexes were used for mouse FAK siRNA: siRNA #1, 5′-GGGCAUCAUUCAGAAGAUA-[dT][dT]-3′; siRNA #2, 5′-GUACAGCACUCGCGUAUCU-[dT][dT]-3′; siRNA #3, 5′-GAAGUUGGGUUGUUUGGAA-[dT][dT]-3′.

1. Matsumoto T, et al. (2003) Targeted expression of c-Src in epidermal basal cells leads to enhanced skin tumor promotion, malignant progression, and metastasis. *Cancer Res* 63:4819–4828.

2. Ferreira M, Fujiwara H, Morita K, Watt FM (2009) An activating β1 integrin mutation increases the conversion of benign to malignant skin tumors. *Cancer Res* 69: 1334–1342.

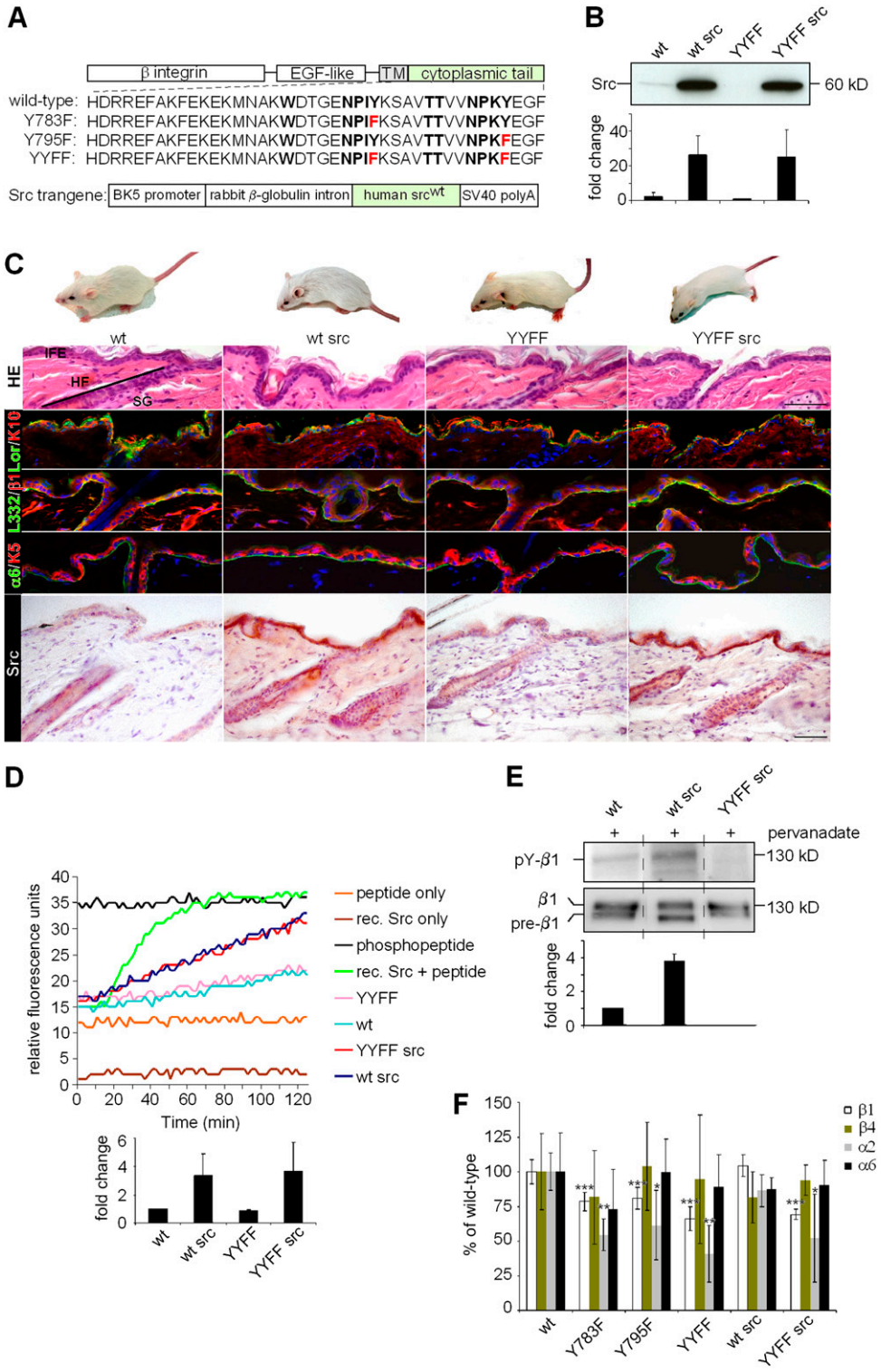

Fig. S1. Analysis of skin and β1 integrin tyrosine phosphorylation. (*A*) Amino acid alignment of the cytoplasmic regions of WT and mutant β1 integrin subunits used in this study. Substituted residues are highlighted in red. The structure of the Src transgene is depicted as well. (*B*) Src levels are ~20-fold increased in primary keratinocytes derived from transgenic animals as determined by densitometry analysis. (*C*) Six-wk-old mutant mice appear phenotypically normal. H&E staining of skin sections details epidermal integrity. The basement membrane is visualized by immunofluorescence staining of laminin332 and α6 integrin. K10 and loricrin are epidermal differentiation markers. Immunostainings show increased Src levels in transgenic animals. IFE, interfollicular epidermis; HF, hair follicle; SG, sebaceous gland. (*D*) Src kinase activity was determined by incubation of immunoprecipitated Src with a chelation-enhanced fluorophore, 8-hydroxy-5-(N,*N*-dimethylsulfonamido)-2-methylquinoline, which increases in fluorescence on phosphorylation. Changes in emission were monitored at 460 nm every 2 min for 2 h at 30 °C using a Synergy HT fluorescence plate reader (Bio-Tek) at an excitation of 360 nm. Synthesized phosphopeptide was used as a positive control (maximum fluorescence), and a nonphosphorylated peptide without the addition of immunoprecipitate served as a negative control (background fluorescence). Peptide substrate incubated with 0.3 μg of recombinant human c-Src (Invitrogen) instead of cell lysate immunoprecipitate was used as an additional positive control. (*E*) Immunoprecipitation of β1 using anti-β1 rabbit serum and subsequent antiphosphotyrosine (4G10) immunoblotting reveal

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an approximate fourfold increase in β1 tyrosine phosphorylation in Src transgenic keratinocytes vs. nontransgenic controls in the presence of pervanadate. Dotted lines indicate the parts of the same gel that were rearranged for presentation purposes. Because of the overall low signal despite maximum exposure time, the image was autocontrasted using Adobe Photoshop in an effort to visualize bands. No significant phosphotyrosine signal is seen in the β1YYFF Src transgenic keratinocytes. (*F*) Integrin subunit profile of freshly isolated keratinocytes. Data are mean ± SD fluorescence intensities minus isotype control of five animals per group. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ vs. WT. β1 and α2 integrin levels were significantly reduced in cells carrying a Y-to-F integrin knock-in vs. WT. β3 integrin was not detected in freshly isolated keratinocytes (not shown). (Scale bars: 60 μM.)

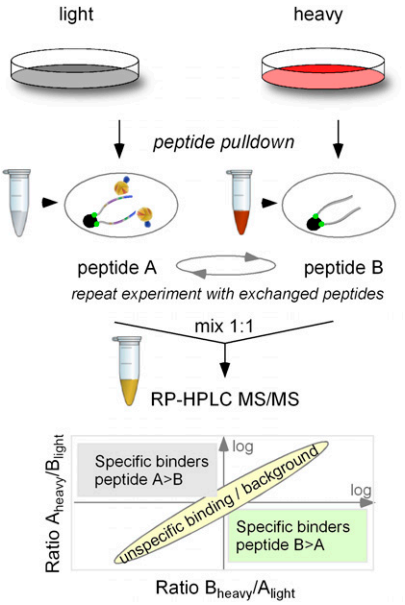

Fig. S2. Schematic of the approach used for SILAC-based peptide pull-downs. Binding of heavy (labeled) or light (unlabeled) protein lysate to a reference or mutated immobilized peptide was tested. Pulled-down protein from corresponding peptide pairs were pooled and analyzed by reverse-phase HPLC MS/MS. Results were visualized by scatterplot. True peptide-binding proteins were identified by their reversed SILAC ratios after a lysate labeling swap.

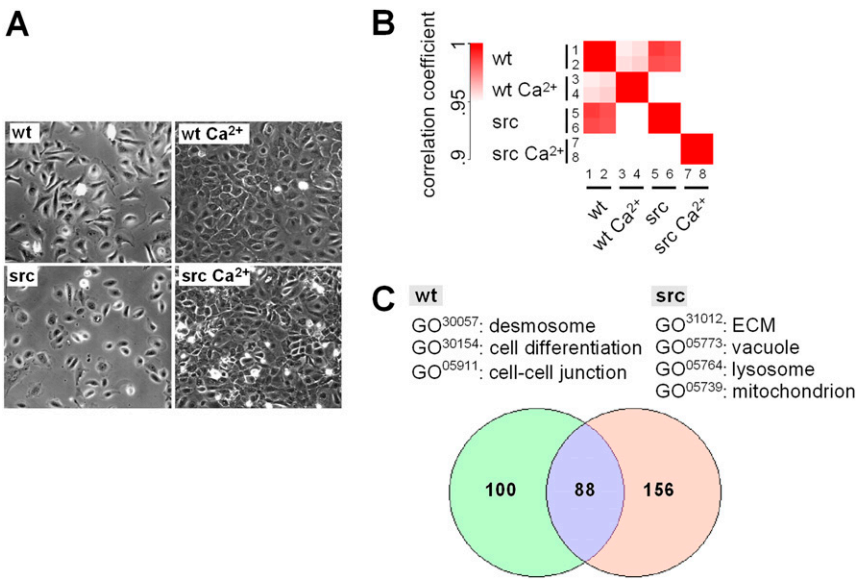

Fig. S3. Differentiation response to Ca^{2+}. (*A*) Phase-contrast images of WT and Src transgenic subconfluent and confluent keratinocytes. Confluent keratinocytes are shown after 8 h with high Ca^{2+} (2 mM). (*B*) Correlation plot of microarrays after RMA normalization. Conditions were run in duplicate. (*C*) Proteins up-regulated after Ca^{2+} at least threefold and assigned to enriched Gene Ontology (GO) terms. Ca^{2+} exposure of WT and Src transgenic keratinocytes led to the up-regulation of proteins associated with different GO terms.

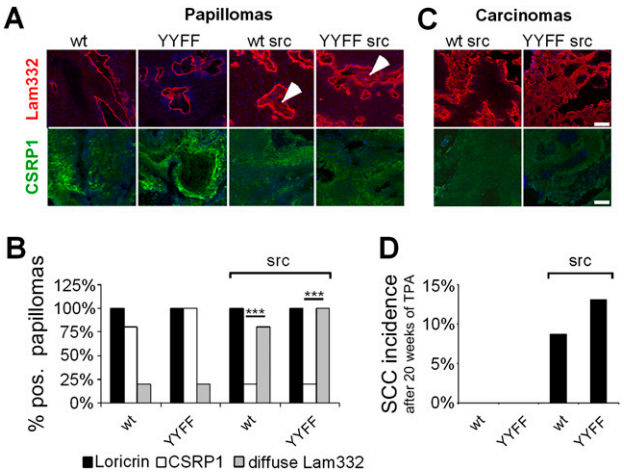

Fig. S4. Papilloma differentiation. (*A* and *C*) Histological analysis of papillomas (*A*) and carcinomas (*C*), using CSRP1 as a differentiation marker. Laminin332 is a component of the basement membrane, and β1 is expressed on basal keratinocytes. Arrows indicate areas of diffuse laminin332 deposition in Src transgenic papillomas. (*B*) Ten papillomas from three different mice per group scored for loricrin expression, CSRP1 expression, and laminin332 deposition. Statistical analysis was performed using Pearson's χ^2 test . $^{***}P < 0.001$ vs. non-Src transgenic papillomas. (*D*) Incidence of squamous cell carcinomas within 20 wk of TPA promotion. (Scale bars: 60 μM.)

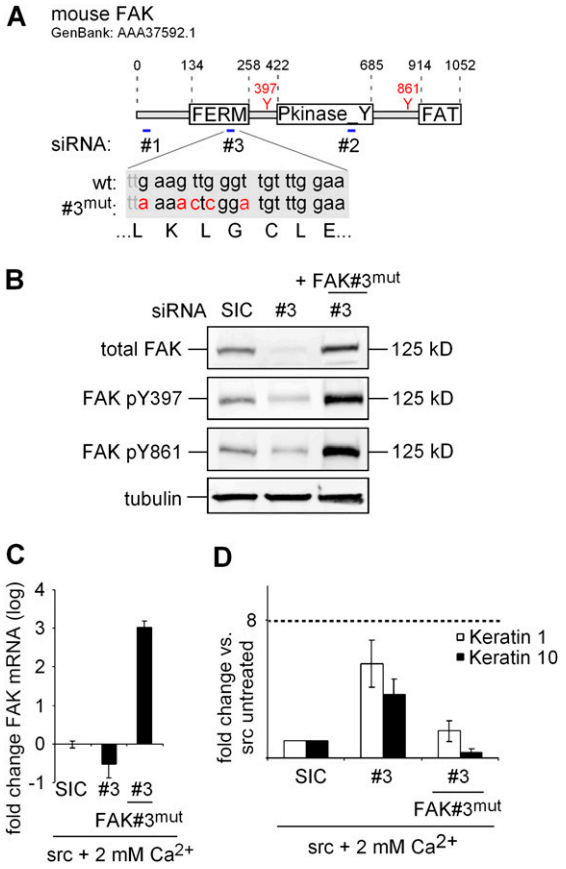

Fig. S5. Rescue of FAK siRNA-mediated knockdown. To confirm the specificity of the siRNA-mediated knockdown, we reexpressed a mouse FAK cDNA (GenBank accession no. AAA37592.1). Five silent nucleotide exchanges were introduced into the 19-bp stretch of siRNA #3 target sequence in FAK (termed FAK#3^{mut}) to prevent binding of siRNA #3 to the rescue mRNA (*A*). The mutated construct pCMV.FAK#3^{mut} is expressed at both the protein (*B*) and mRNA (*C*) levels. Importantly, expression of FAK#3^{mut} reinstituted a differentiation block in Src transgenic cells, as shown by reduced K1 and K10 mRNA in the presence of high Ca^{2+} (*D*). K1 and K10 were used as keratinocyte differentiation markers.