

A paracrine activin A–mDia2 axis promotes squamous carcinogenesis via fibroblast reprogramming

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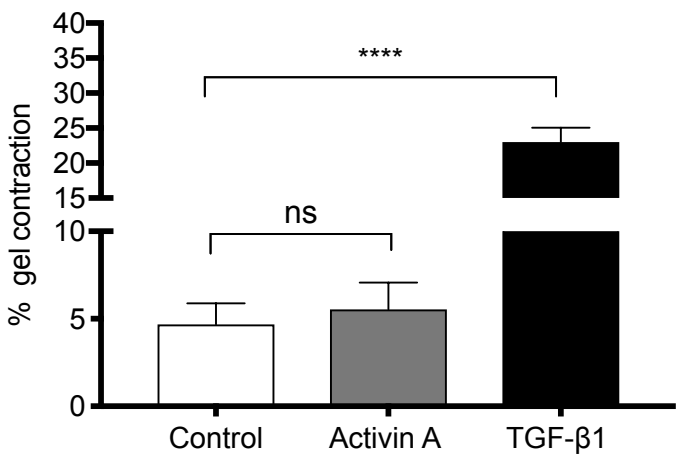
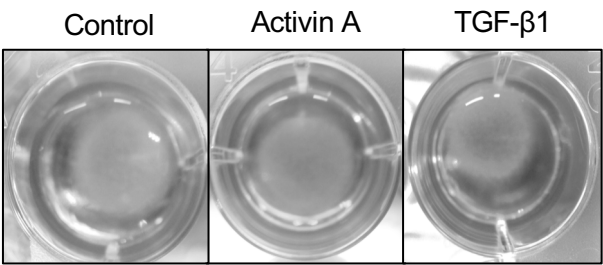
Appendix: Supplementary Information

Appendix Figure S1–S3

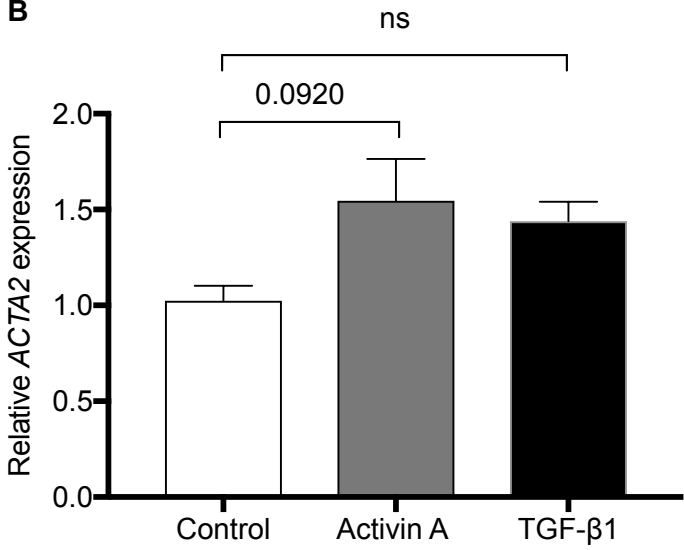
Appendix Table S1–S5

Appendix Fig S1.

A



B



Appendix Fig S1 Activin A does not promote myofibroblast differentiation

A Representative images of collagen gels with embedded primary human dermal fibroblasts and quantification of the extent of gel contraction upon treatment with activin A (20 ng/ml) or TGF- β 1 (1 ng/ml). N=3.

B qRT-PCR analysis for *ACTA2* relative to *RPL27* using RNA from fibroblasts treated with activin A or TGF- β 1 for 6 h. N=3.

Bar graphs show mean $\pm$ SEM. ns $p > 0.05$, **** $p < 0.0001$, one-way ANOVA with Bonferroni post-hoc test.

A

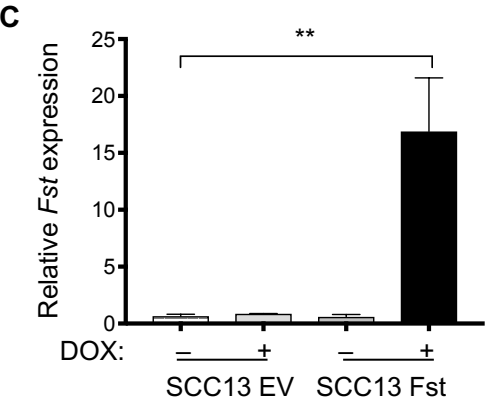
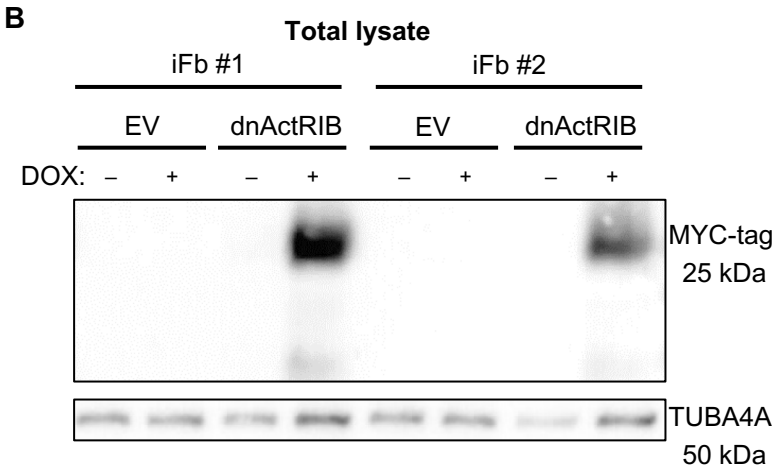
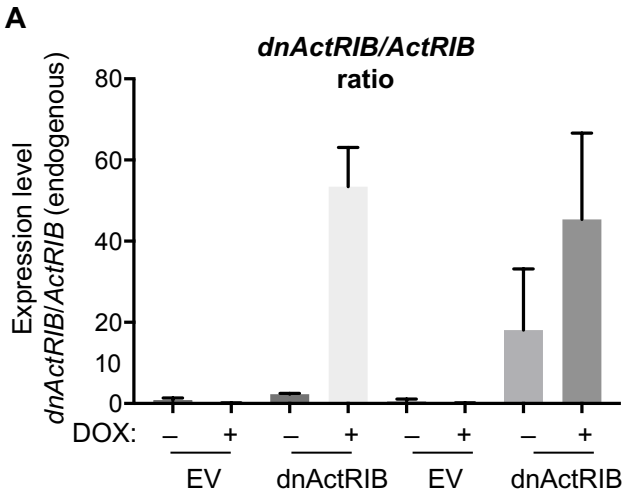
IPA Upstream Regulator Analysis: **TP53**

Comparison	Activation Z-score	–Log10 (p-value)
Act/WT vs WT/WT	-2.98	11.08
Act/HPV8 vs WT/HPV8	-2.52	10.63
Act/HPV8 vs WT/WT	-3.78	10.72

Appendix Fig S2 Negative correlation between *INHBA* and p53 target gene expression in FACS-isolated fibroblasts from Act vs. wt mice

A Ingenuity pathway analysis of differentially expressed genes in FACS-isolated CD45⁻CD140a⁺ fibroblasts from activin A-overexpressing compared to control mice, showing enrichment score of p53 target genes. Negative Z-scores predict that activin A reduces the expression of p53-target genes.

Appendix Fig. S3.



Appendix Figure S3 Expression of a dominant-negative type I activin receptor (dnActRIB) mutant or of follistatin in immortalized fibroblasts from PDGFR α -eGFP mice or in SCC13 cells, respectively.

- A Ratio of dnActRIB vs. endogenous ActRIB mRNA in transduced fibroblasts from PDGFR α -eGFP mice (each normalized to *Rps29*) with and without DOX treatment (N=3).
- B Western blot analysis of total lysate from fibroblasts transduced with EV or dnActRIB lentiviruses with an antibody detecting the Myc epitope in the dnActRIB-Myc fusion protein.
- C qRT-PCR for *Fst* relative to *RPL27* using RNA from SCC13 cells transduced with a lentiviral vector expressing murine *Fst* in a DOX-inducible manner or EV. N=3. Bar graphs show mean and SEM. ** $p < 0.01$. one-way ANOVA with Bonferroni post-hoc test.

Appendix Table S1: Primer sequences for amplification of fragments required for cloning

Gene Name	Forward primer (5'-3')	Reverse primer (5'-3')
<i>INHBA</i> *	CAC CAT GCC CTT GCT TTG	CTA TGA GCA CCC ACA CTC CTC C
<i>FST</i>	CAC CAT GTA CCC ATA CGA TGT TCC AGA TTA CGC TGT CTG CGC CAG GCA CC	TTA CCA CTC TAG AAT GGA AGA GAT AGG AAA GCT GTA GT
<i>dnActRI</i> †	CAC CAT GGC GGA GTC GGC CGG AGC CTC	TTA CAG GTC TTC TTC AGA GAT CAG TTT CTG TTC

(an in-frame *HA-tag or †MYC-tag sequence was fused in-frame at the 3' end of the coding sequence)

Appendix Table S2: shRNA sequences

shRNA mDia2	Sequence (5'-3')
sh-mDia2 #1 (TRCN0000108778)	CCGGGCAGAGAAAGAGCGACTTGAAGTCGAGTTCAAGTCG CTCTTTCTCTGCTTTTGTG
sh-mDia2 #2 (TRCN0000108779)	CCGGCGACCCAAGTTGCATTTAAATCTCGAGATTTAAATGC AACTTGGGTCGTTTTTG
sh-mDia2 #3 (TRCN0000108777)	CCGGCCCTGACTTCACATACAGAAACTCGAGTTTCTGTATG TGAAGTCAGGGTTTTTG
sh-mDia2-human (TRCN0000150903)	GCTCAGTGCTATTCTCTTTAA

Appendix Table S3: Primer sequences for qRT-PCR

	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Acta2</i>	TCC CTG GAG AAG AGC TAC GA	CTT CTG CAT CCT GTC AGC AA
<i>ACTA2</i>	GTG ACG AAG CAC AGA GCA AA	GAG TCA TTT TCT CCC GGT TG
<i>Inhba</i>	GGA GAA CGG GTA TGT GGA GA	ACA GGT CAC TGC CTT CCT TG
<i>INHBA</i>	CCT CGG AGA TCA TCA CGT TT	CCC TTT AAG CCC ACT TCC TC
<i>INHBB</i>	AGC TTC GCC GAG ACA GAT G	CGT AGG GCA GGA GTT TCA GG

<i>INHA</i>	CCA GCT GTG AGG ACA AGT CA	CTA GCA GGG GCT CAG AGC TA
<i>mDia1</i>	GGA TCC TGC TGT TCC CAA TA	CCC ACT TTT TAA TCC GTC CA
<i>mDia2</i>	TTT GCA TAG ATC AGGCCA AAC	GCT TGT AAC TCT GCT TGA AGC TC
<i>DIAPH3</i>	GCG GGA AAA GGA CTT CAG TA	TTG CTG GTC AAA GAC ACT CG
<i>mDia3</i>	AGC AAT TGA CCC GAA ACA AC	TCC ACA ATT GGT GAA AAT CG
<i>Fst</i>	AGG GAA AGT GTA TCA CAA AGT	GAG TTG CAA GAT CCA GAA TG
<i>FST</i>	CGT GAA TGA CAA CAC ACT CTT C	TTT TTC CCA GGT CCA CAG TC
<i>TGFB1</i>	QT00000728 (Forward + Reverse) (QuantiTect, Qiagen)	
<i>Postn</i>	AAG ACT GCT TCA GGG AGA CAC A	TCA GTG TGG TGG CTC TTA CA
<i>POSTN</i>	CAG CCT TTC ATT CCT TCC AT	CAA ATG TCT GTG CCC TTC AA
<i>Spp1</i>	CCA GCA GCT CAC ACT GAA GA	CCA AAC AGG CAA AAG CAA AT
<i>Mmp13</i>	ATC CTG GCC ACC TTC TTC TT	TTT CTC GGA GCC TGT CAA CT
<i>Fn1</i>	TGT GAC AAC TGC CGT AGA CC	GAC CAA CTG TCA CCA TTG AGG
<i>FN1</i>	GCA GGT ACA GTC CCA GAT CA	AGC GGA CCT ACC TAG GCA AT

<i>Trp53</i>	TTT TGA AGG CCC AAG TGA AG	TGA GGG GAG GAG AGT ACG TG
<i>Il6</i>	CCG GAG AGG AGA CTT CAC AG	TTC TGC AAG TGC ATC ATC GT
<i>Serpine1</i>	CGC CTC CTC ATC CTG CCT AAG	CTG TGC CGC TCT CGT TTA CC
<i>SERPINE1</i>	CAT CAT GGG CAC AGA GAC AG	GGT GGA GAG AGC CAG ATT CA
<i>Fmn1</i>	ATA AGG ATG GAG GGG TTT GG	CTT GGA GTC TGC CTG GAG TC
<i>Fmn2</i>	AAC CCA CAC ATT CGA TGG AT	CTG GAC ACC ATT TGA GCA TTT
<i>Alk4</i>	GGG TGG GGA CCA AAC GAT AC	GAG GGC ATA GAT GTC GGC AC
<i>dnAlk4- myc</i>	TCA TCG TCT TCC TGG TCA TCA A	ATC AGT TTC TGT TCC CCA CCA C
<i>Rbp-Jκ (Csl)</i>	ACG CCA GTT CAC AAC AGT G	GTC TGC CCG TAA TGG ATG TA
<i>RPL27</i>	TCA CCT AAT GCC CAC AAG GTA	CCA CTT GTT CTT GCC TGT CTT
<i>Rps29</i>	GGT CAC CAG CAG CTC TAC TG	GTC CAA CTT AAT GAA GCC TAT GTC C

**Appendix Table S4: List of antibodies and dyes used for fluorescence/
immunofluorescence staining**

Name (Prod. No.)	Company	Dilution
Alexa Fluor® 488 AffiniPure donkey anti-mouse IgG (H+L) (#715-545-150)	Jackson ImmunoResearch	1:200
Alexa Fluor® 594 AffiniPure donkey anti-rabbit IgG (H+L) (#711-587-003)	Jackson ImmunoResearch	1:200
Alexa Fluor™ 555 phalloidin (#A34055)	Thermo Fisher Scientific	1:200
Chicken anti- β -catenin (C7207)	Sigma-Aldrich	1:1000
Cy™2 AffiniPure donkey anti-goat IgG (H+L) (#705-225-147)	Jackson ImmunoResearch	1:200
Cy™3 AffiniPure donkey anti-rabbit IgG (H+L) (#711-165-152)	Jackson ImmunoResearch	1:200
Cy™3 AffiniPure donkey anti-chicken IgG (H+L) (#703-165-155)	Jackson ImmunoResearch	1:200
Hoechst 33342 (B2261)	Sigma-Aldrich	1:1000
Mouse anti-E-cadherin (#610181)	BD Pharmingen	1:100
Mouse anti-SMAD2/3 (C-8) (sc-133098)	Santa Cruz	1:500

Mouse anti- α -SMA (A2547)	Sigma-Aldrich	1:800
Mouse anti- α -SMA FITC (F3777)	Sigma-Aldrich	1:1000
Mouse anti-p53 (sc-126)	Santa Cruz	1:500
Rabbit anti-DIAPH3 (H-42) (sc-135261)	Santa Cruz	1:1000
Rabbit anti-keratin 14 (#PRB-155P)	Biolegend	1:5000
Rabbit anti-LYVE1 (#11034)	Angiobio	1:500
Rat anti-MECA32 (#553849)	BD Pharmingen	1:500
Goat anti-PDGFR- α (#AF1062)	R&D Systems	1:500
Mouse anti-collagen IV (#10710)	Progen	1:500

Appendix Table S5: Primer sequences for ChIP

Gene Name	Forward primer (5'-3')	Reverse primer (5'-3')
<i>mDia2</i> (Intron 1)	CTGGAGGAGGATGTGTCCAT	AGCCAAGGCACAAAGGACTA