

Supplementary Materials

Complement Factor D Is a Novel Biomarker and Putative Therapeutic Target in Cutaneous Squamous Cell Carcinoma

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Original uncropped Western blots for Figures 1B, 3B, 4B, 5A, 5C

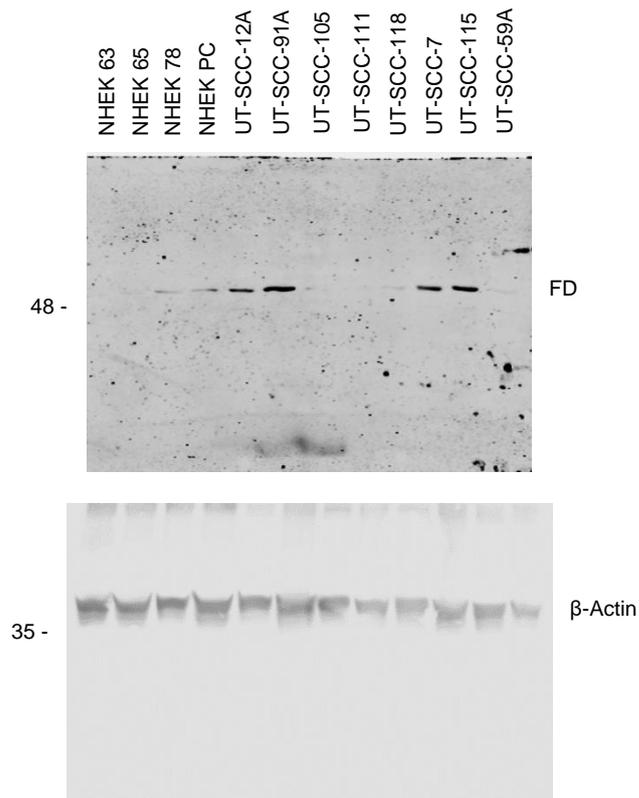


Figure S1. Expression of FD is up-regulated in cSCC tumor cells. B: FD levels in conditioned media of NHEKs and primary and metastatic cSCC cell lines were determined by Western blot analysis under nonreducing conditions. β -Actin levels in the cell lysates were determined as the sample controls. Migration positions of molecular weight markers in kDa are shown on the left.

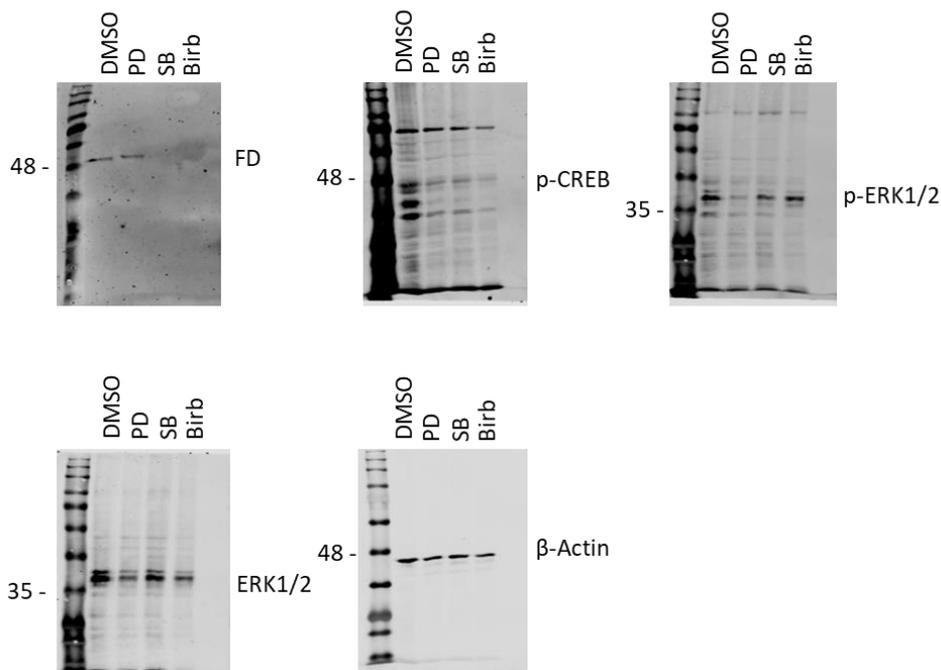


Figure S2. Up-regulation of FD expression in cSCC cells by IFN- γ and IL-1 β , and p38 MAPK signaling pathway. B: cSCC cells (UT-SCC-12A) were treated with MEK1/2 inhibitor (PD98059; 30 μ M) or p38 inhibitor specific for p38 α/β (SB203580; 10 μ M) or the inhibitor of all p38 isoforms α , β , γ and δ (BIRB796; 10 μ M) for 24 hours. The conditioned media were analyzed for FD levels by Western blot analysis. Cell lysates were analyzed for levels of phosphorylated CREB (p-CREB), phosphorylated ERK1/2 (p-ERK1/2) and ERK1/2 to verify the proper effects of SB203580, BIRB, and PD98059, respectively. β -actin was used as a sample and loading control. Migration positions of molecular weight markers in kDa are shown on the left.

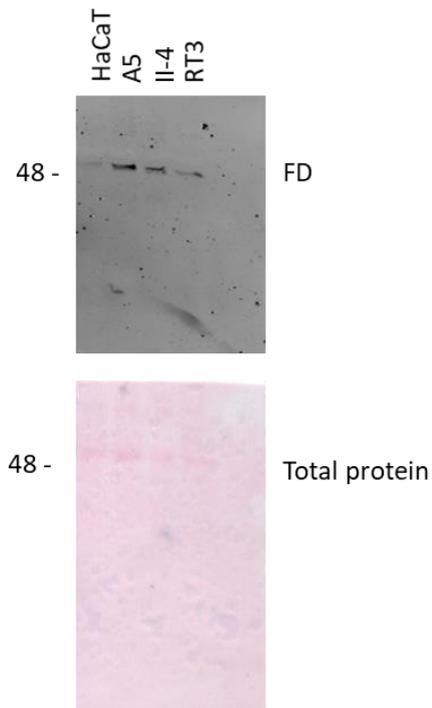


Figure S3. Regulation of FD expression in HaCaT and tumorigenic Ha-ras-Transformed HaCaT cell lines in culture. B: The expression of FD protein in conditioned media of HaCaT and Ha-ras-transformed HaCaT cell lines (A5, II-4, and RT3) was determined by Western blot analysis under nonreducing conditions. Equal total protein loading was controlled by Ponceau (0.2%) staining. Migration positions of molecular weight markers in kDa are shown on the left.

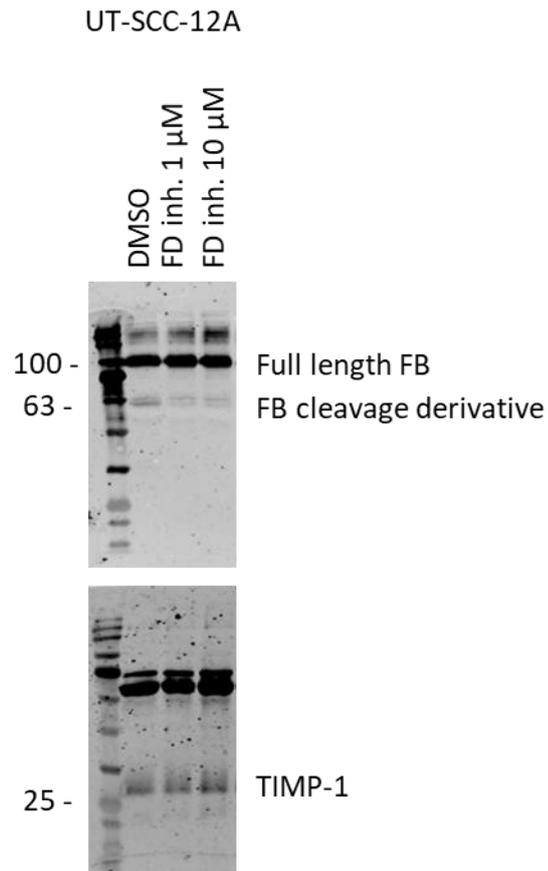


Figure S4. Targeted inhibition of FD suppresses proliferation of cSCC cells through blockade of ERK1/2 activation. A: cSCC cell cultures (UT-SCC-12A) were treated with small-molecule factor D inhibitor (Danicopan; ACH-4471) or DMSO as vehicle control for 24 h. The levels of complement factor B (FB) and its 60 kDa cleavage derivative in conditioned media of cSCC cells were determined by Western blot analysis. TIMP-1 was used as a loading control. Migration positions of molecular weight markers in kDa are shown on the left.

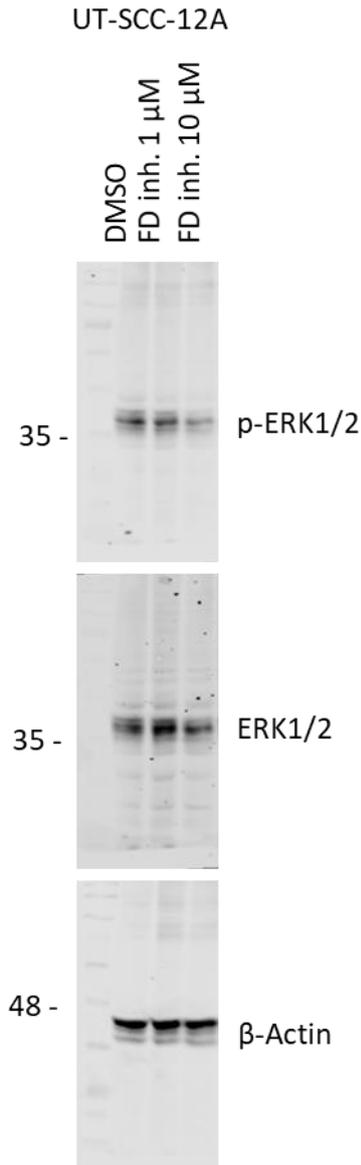


Figure S5. Targeted inhibition of FD suppresses proliferation of cSCC cells through blockade of ERK1/2 activation. C: Levels of phosphorylated ERK1/2 (p-ERK1/2) and ERK1/2 in small-molecule factor D inhibitor (Danicopan; ACH-4471) or DMSO vehicle treated cSCC cell lysates were determined by Western blot analysis 24 h following targeted FD inhibition. β -Actin was used as loading control. Migration positions of molecular weight markers in kDa are shown on the left.