SUPPLEMENT

SUPPLEMENTAL METHODS

Immunoelectron Microscopy –Immunoelectron microscopy on ultrathin cryosections was carried out according to the Tokuyasu method (32) with modifications described in (33).

Production of recombinant model proteins - For expression of model proteins containing putative integrin binding motifs the pPET vector (EMD Biosciences) was modified with a 3' double Strep-tag II followed by a thrombin cleavage site, and 5' of the multiple cloning site a foldon domain spanning residues Gly457 to Leu483 of bacteriophage T4 fibritin was introduced (43). Partially overlapping oligonucleotides encoding for the putative integrin binding motifs GEKGER and GTSGER as well as for the integrin binding motif GFPGER and the non-binding motif GFPGDR flanked by $(GPP)_5$ linkers were filled by PCR and introduced into the vector utilizing the 3'Nhe I and 5' BamHI restriction sites. Upon transformation with the recombinant plasmid, *Escherichia coli* cells (Bl21; EMD Biosciences) were induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside and grown for 16 h at 37 °C. The cells were harvested (15 min, 5000 x *g*, 4 °C) and resuspended in phosphate-buffered saline, pH 7.4. The bacteria were sonicated, followed by removal of insoluble cell debris by centrifugation (30 min, 20000 x *g*, 4 °C). The supernatant was applied to a Strep-Tactin sepharose column (IBA) and eluted with binding buffer containing 1 mM desthiobiotin (Sigma). Following removal of desthiobiotin by dialysis, thrombin cleavage was performed overnight at room temperature (5 mM $CaCl_2$, 1 unit/mg thrombin; Sigma-Aldrich), and the cleaved double Strep-tag II was removed by passing the solution again over a Strep-Tactin sepharose column.

CD-spectroscopy – CD spectra of $(GPP)_5GEKGER(GPP)_5$, $(GPP)_5GTSGER(GPP)_5$, $(GPP)_5GFPGER(GPP)_5$ and $(GPP)_5GFPGDR(GPP)_5$ were acquired on a Jasco J-715 spectrometer (Jasco). The far-ultraviolet spectra (190-250 nm) were measured with a 1 mm path-length quarz cell and normalized for concentration and path length to obtain the specific ellipticity after substration of the buffer contributions. The thermal stability was determined by monitoring the change in the specific ellipticity at the wavelength 225 nm as a function of temperature in the range of 15-80°C with a slope of 30°C per hour.

Protein isolation from tissues and western blot analysis – Total Protein was extracted from the skin auf wild-type and intergrin α_2-deficient mice and subsequently used for immunoprecipitation and western blot analysis according to the method described in (4).

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Distribution of collagen XXIII, integrin $\alpha_2\beta_1$, and co-localization of the two proteins in murine tissues. Immunohistochemisty was performed on cryosections of wild type (C57Bl6) embryonic (E18.5) (*A*) as well as adult (P60) mouse skin (*B*) and tongue (*C*). Co-staining was accomplished using sequential incubation with polyclonal anti-collagen XXIII antibody (red) and mixture of monoclonal antibodies against the α_2 integrin subunit (green). Note that the two proteins co-localize (yellow) on the surface of the basal keratinocytes and in hair follicles. Bar = 40 μm.

Fig. S2. CD spectroscopic analysis and adhesion assays with model fusion proteins containing putative integrin binding motifs derived from the amino acid sequence of collagen XXIII. *A*, CD spectra of model proteins containing the putative integrin binding motifs GEKGER and GTSGER or the same proteins containing the known integrin binding motif GFPGER as positive control, and the mutated form GEFGDR as negative control, for integrin binding. CD spectra were recorded with a protein concentration of 200 µg/ml in 20 mM sodium phosphate buffer (pH 7.4) supplemented with 150 mM sodium chloride over the wavelength range 190-250 nm at 15°C. *B*, Thermal transitions of the model proteins monitored by CD spectroscopy. Specific ellipticities at 225 nm of the model proteins were recorded as a function of temperature with a temperature slope of 30°C/h. *C*, Cell attachment of HaCat keratinocytes to the model proteins or the collagen XXIII ectodomain. Serial dilutions of model proteins containing putative integrin binding motifs or the collagen XXIII ectodomain (0.3 – 20 µg/ml) were immobilized on microtiter plates. HaCaT keratinocytes were allowed to attach to the substratum for 30 minutes in the absence or presence of Mg^{2+}/Mn^{2+}, followed by staining with crystal violet. Relative adhesion is presented as ΔE, measured extinction minus non-specific cell attachment to BSA.

Fig. S3. Expression level and distribution of collagen XXIII in the skin of integrin α_2-deficient mice. *A,* Detection of collagen XXIII in immunoprecipitations with subsequent Western blot analysis of tissue extracts form the skin auf wild-type and α_2-deficient mice. *B*, Immunofluorescence analysis of the distribution of collagen XXIII (red) and the basement membrane marker laminin 332 (green) in the skin of newborn (P1) wild-type and α_2-deficient mice. Bar = 40µm.

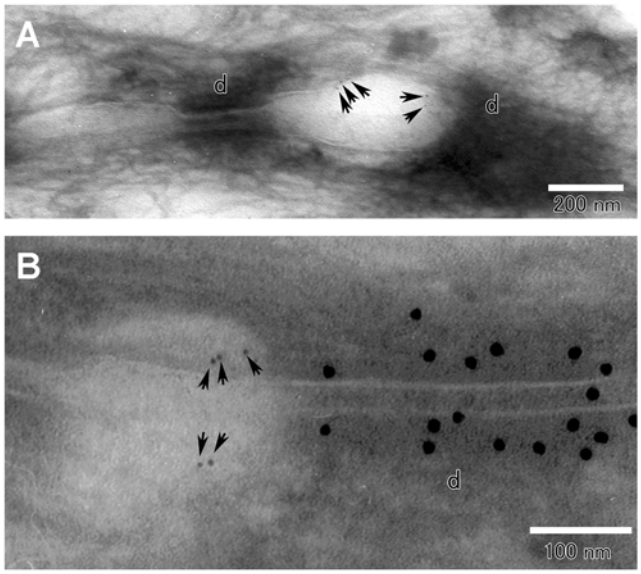

Figure S1

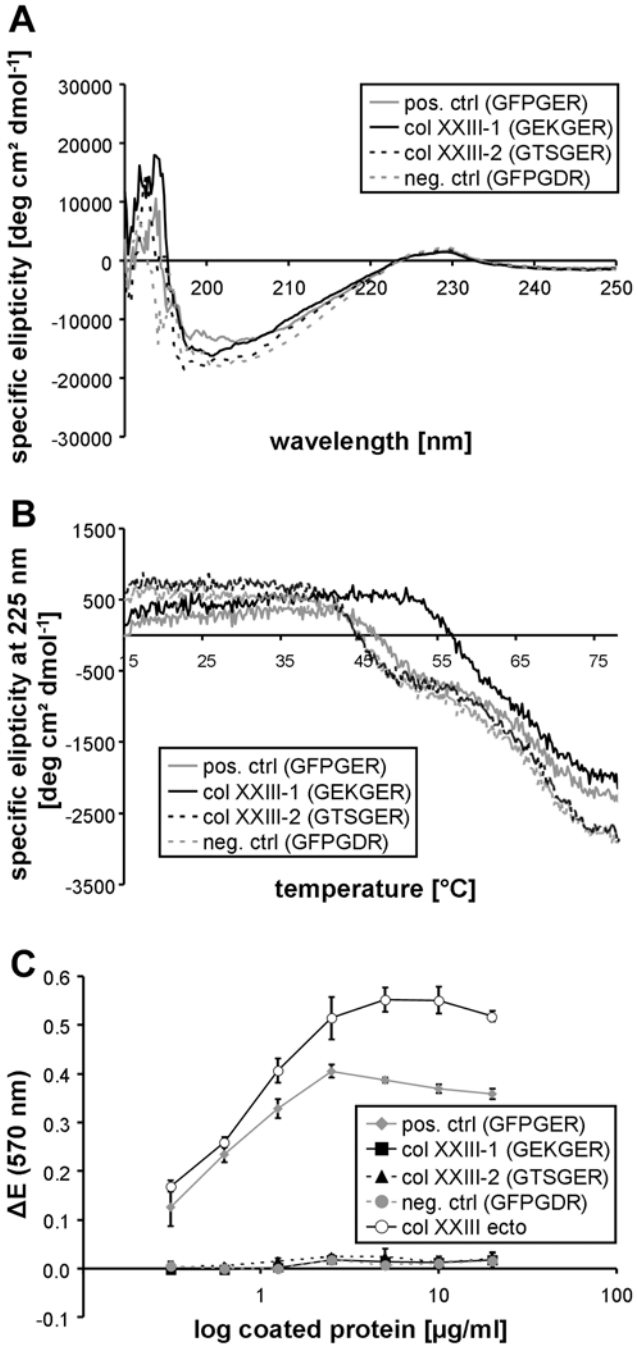
A
specific elipticity [deg cm² dmol⁻¹]
30000
20000
10000
0
-10000
-20000
-30000
200
210
220
230
240
250
wavelength [nm]
pos. ctrl (GFPGER)
col XXIII-1 (GEKGER)
col XXIII-2 (GTSGER)
neg. ctrl (GFPGDR)
B
specific elipticity at 225 nm [deg cm² dmol⁻¹]
1500
500
-500
-1500
-2500
-3500
25
35
55
65
75
temperature [°C]
pos. ctrl (GFPGER)
col XXIII-1 (GEKGER)
col XXIII-2 (GTSGER)
neg. ctrl (GFPGDR)
C
ΔE (570 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
1
10
100
log coated protein [µg/ml]
pos. ctrl (GFPGER)
col XXIII-1 (GEKGER)
col XXIII-2 (GTSGER)
neg. ctrl (GFPGDR)
col XXIII ecto

Figure S2

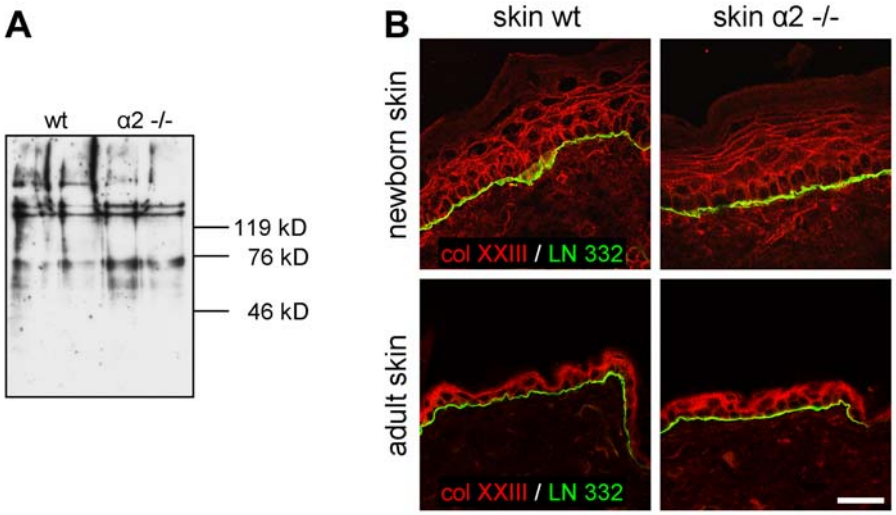
A
wt
α2 -/-
119 kD
76 kD
46 kD
B
skin wt
skin α2 -/-
newborn skin
adult skin
col XXIII / LN 332
col XXIII / LN 332

Figure S3