Supplementary information

Supplementary Materials and Methods

Animals

The generation of *Adam17*$^{flox/flox}$*Krt14-Cre*, *Egfr*$^{flox/flox}$*Krt14-Cre* and *Adam10*$^{flox/flox}$*Krt14-Cre* mice has been described previously (Franzke *et al.*, 2012; Weber *et al.*, 2011). All mice were of mixed genetic background (129Sv, C57BL/6), and all comparisons were between littermates. The mice were maintained in the Biomedical Research Center of the University Medical Center in Freiburg, and all experiments were performed according to the guidelines of the German Animal Welfare association and approved by the Regierungspräsidium Freiburg.

Immunoblotting

For western blot analysis the tissues were lysed in 50 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate supplemented with 2 mM EDTA, 5 mM 1,10-ortho-phenanthroline (Sigma-Aldrich) and protease inhibitor cocktail set III (Calbiochem). The epidermis was detached from the dermis by heat separation as described previously (Lichti and Yuspa, 1988). Tissues were homogenized in lysis buffer on ice with a T18 basic Ultra Turrax (Ika, Germany). Total protein content was determined using the BCA^{TM} protein assay kit (Invitrogen). We have extensively validated the S3 (Val1744) antibody on cells after ligand activation or with constitutive overexpression of Notch1 mutants by comparing Notch S3 cleavage in the presence and absence of γ-secretase inhibitor (van Tetering *et al.*, 2009). Such samples were taken along to verify the molecular weight of cleavage products on western blots with those from epidermal lysates (not shown). 30µg of protein was separated and transferred onto PVDF membranes. After membranes were blocked with 5% skimmed milk in PBS/0.05% Tween-20, protein detection on membranes was performed with subsequent primary antibodies: rabbit anti-Notch1 (Santa Cruz Biotechnology), rabbit anti-Cleaved Notch1 (Val1744) (Cell Signaling) and rabbit anti-Lamin A (C-terminal) (SIGMA). Visualization was performed with secondary rabbit IgG-HRP linked antibodies (Cell Signaling) and Amersham ECL prime western blotting detection reagent on Hyperfilm as described by the manufacturer (GE Healthcare).

Serology

Serum TSLP levels were determined according the manufacturers recommendations using the mouse TSLP Quantikine ELISA kit (R & D systems, Germany).

Quantitative RT-PCR analysis

Total RNA from mouse skin or dispase-separated epidermal splits (Franzke *et al.*, 2012) was extracted using RNeasy (Qiagen). 1µg of total RNA was reverse transcribed using a First Strand cDNA Synthesis kit (Fermentas). Relative quantification of gene expression was performed by real-time quantitative PCR using iQ SYBR-Green Supermix on the CFX96^{TM} C1000^{TM} Thermal Cycler (Bio-Rad, Germany) following the manufacturer's protocols. The used primer sequences for *Tslp* (Murthy *et al.*, 2012) and *Gapdh* (Franzke *et al.*, 2012) were described previously. Relative expression was normalized for levels of GAPDH. The generation of the correct amplification products was confirmed using agarose gel electrophoresis.

Statistics

The data are presented as means ± SEM. Data of two groups were analyzed for significance using the unpaired Student´s t test and differences are considered to be statistically significant at $p < 0.05$.

References

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van Tetering G, van Diest P, Verlaan I, van der Wall E, Kopan R, Vooijs M (2009) Metalloprotease ADAM10 is required for Notch1 site 2 cleavage. *The Journal of biological chemistry* 284:31018-27.

Weber S, Niessen MT, Prox J, Lullmann-Rauch R, Schmitz A, Schwanbeck R*, et al.* (2011) The disintegrin/metalloproteinase Adam10 is essential for epidermal integrity and Notch-mediated signaling. *Development* 138:495-505.

Supplementary Figures

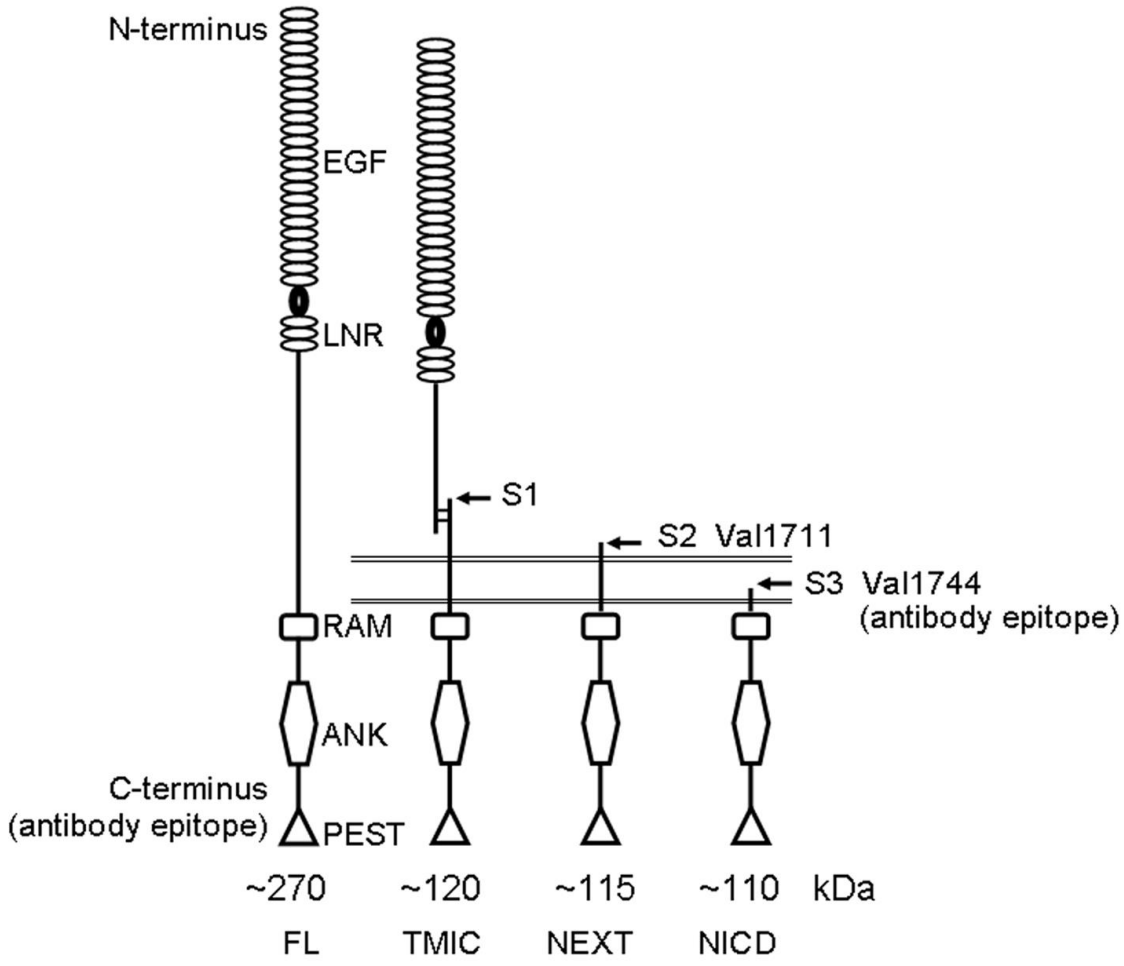

Figure S1. Schematic presentation of Notch1 receptor processing. The full length (FL) Notch1 receptor forms after S1 cleavage a non-covalently bound heterodimer composed of the membrane embedded C-terminal Transmembrane/Intracellular fragment (TMIC) and an ectodomain with extracellular EGF-like repeats for ligand binding and three Lin12-Notch Repeats (LNR) for S2 cleavage site protection. Upon ligand binding S2 site is cleaved at Val1711 resulting in Notch Extracellular Truncation (NEXT) protein, which subsequently is cleaved at Val1744 (S3), releasing the Notch Intracellular Domain (NICD). RAM, Rbp-jκ association module; ANK, ankyrin repeat; PEST, proline (P), glutamate (E), serine (S) and threonine (T) rich domain. The approximated molecular weights of the Notch1 fragments are indicated in kDa.