SUPPLEMENTARY FIGURES

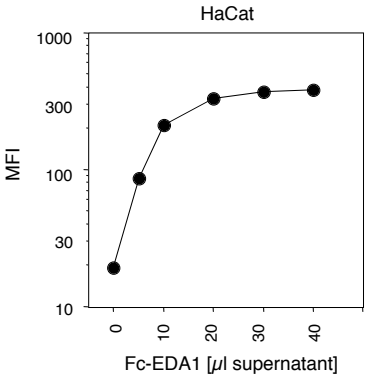


Fig. S1. **Dose dependence of Fc-EDA1 staining of HaCat cells**.

HaCat cells were stained with Fc-EDA1-E245 is the presence of heparin, followed by PE-coupled anti-human IgG and analyzed by FACS. Staining was performed in a final volume of 50 µl with the indicated volumes of 293T cell supernatant (containing about 5 µg/ml of Fc-EDA1-E245 in Opti-MEM medium). MFI: mean fluorescence intensity.

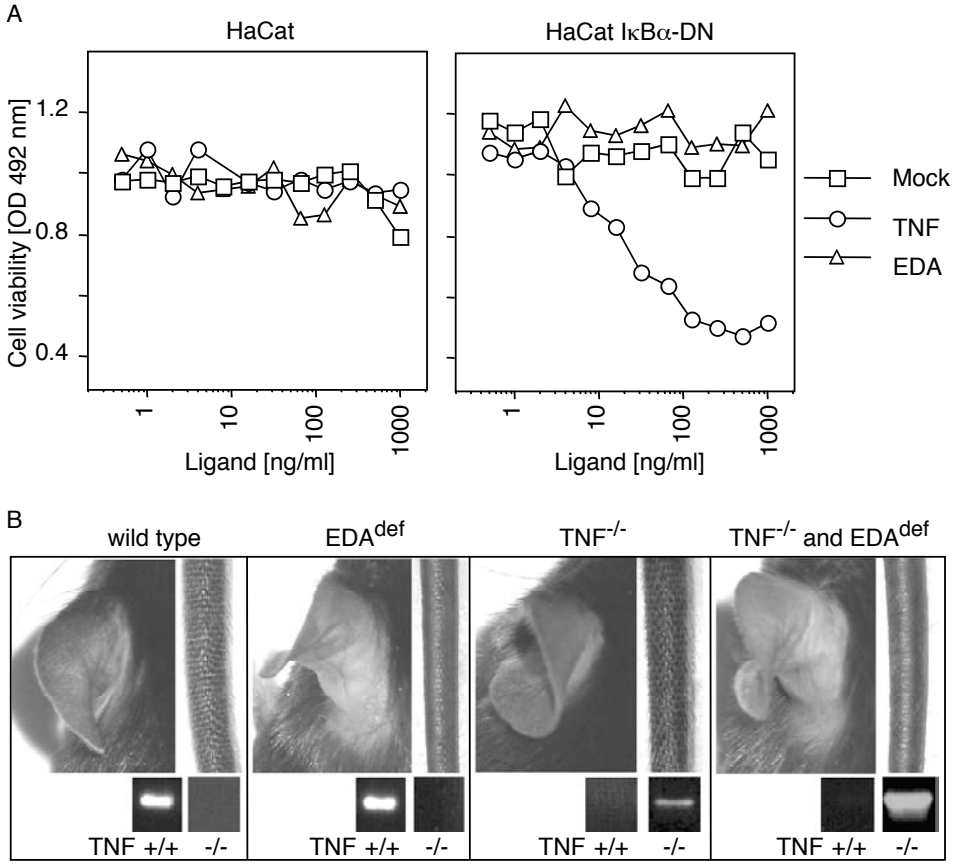


Fig. S2. **Endogenous EDAR does not signal cell death. TNF-deficiency does not rescue the *Tabby* phenotype**.

Panel A: HaCat cell populations stably transduced with a dominant-negative form of Iκ-Bα or a control vector were treated for 16 h with the indicated dose of Fc-TNF or Fc-EDA1-E245, after which time cell viability was quantified with the PMS/MTS assay.

Panel B: *Tabby* mice were crossed with $TNF^{-/-}$ mice. The F2 generation was genotyped for the presence of TNF, and screened morphologically for signs of ectodermal dysplasia such as lack of tail hair and lack of hair behind the ears.

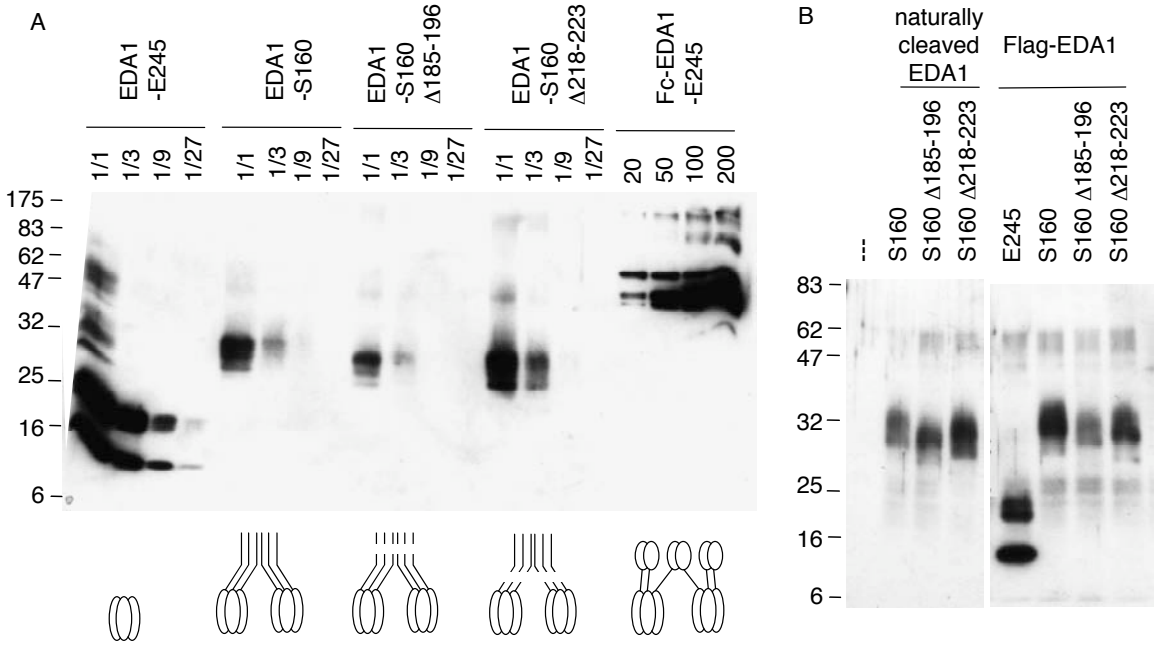


Fig. S3. **PreScission cleavage, Western blot analysis and quantification of several EDA1 proteins**.

Panel A: The indicated proteins were produced as Fc-PreScission-EDA1 in supernatants of transfected 293T cells. They were captured on protein A-Sepharose, and eluted by cleavage with Prescission protease. The same amount of EDA1 that served to stimulate HaCat cells in Fig.3A was immunoprecipitated with EDAR-Fc/Protein A-Sepharose and analyzed by Western blotting at various dilutions (1/1, 1/3, 1/9 and 1/27) with polyclonal anti-EDA AL166 anti-serum. Known amounts (20, 50, 100 and 200 ng) of purified, unprocessed Fc-EDA1-E245 were loaded for quantification purpose. Note that EDA migrates as a doublet of glycosylated and non-glycosylated proteins (1). Cartoons indicate the putative structural arrangement of the native corresponding proteins

Panel B: Supernatants of 293T cells transfected with the indicated full length or Flag-tagged EDA1 proteins. The same proteins served to stimulate HaCat cells in Fig. 3B. Proteins were immunoprecipitated with EDAR-Fc, and the immunoprecipitates were analyzed by Western blotting with polyclonal anti-EDA AL166 anti-serum.

1. Schneider, P., Street, S. L., Gaide, O., Hertig, S., Tardivel, A., Tschopp, J., Runkel, L., Alevizopoulos, K., Ferguson, B. M., and Zonana, J. (2001) *J Biol Chem* **276**, 18819-18827

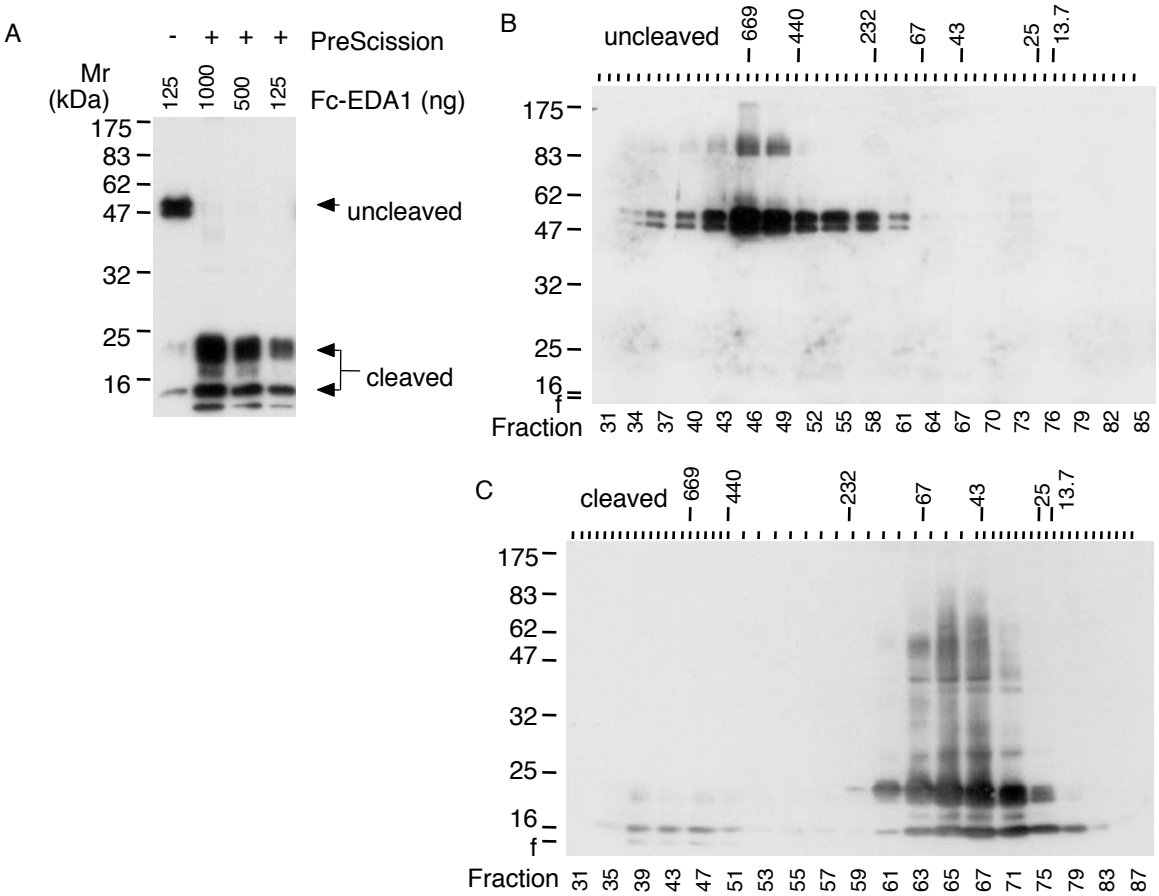


Fig. S4. **Cleavage of Fc-EDA1 with PreScission protease**.
Panel A: Fc-PreScission-EDA1-E245, with a protease site engineered between the Fc and EDA1 moieties, was digested or not with PreScission protease and analyzed by Western blotting with rabbit anti-EDA AL166 anti-serum. The same proteins served to stimulate HaCat cells in Fig. 3B.
Panel B: size exclusion chromatography of uncleaved Fc-PreScission-EDA1-E245 on Superdex-200. Marks at the top indicate the fractions. Fractions selected for Western blotting with anti-EDA1 AL166 anti-serum are numbered at the bottom. The size (in kDa) and elution position of standard proteins for the chromatography and for the SDS-PAGE are indicated at the top and on the left of the blot, respectively.
Panel C: as in B, but with Fc-PreScission-EDA1-E245 processed with PreScission protease.
Samples analyzed in A and C originated from different digestions. f: migration front.

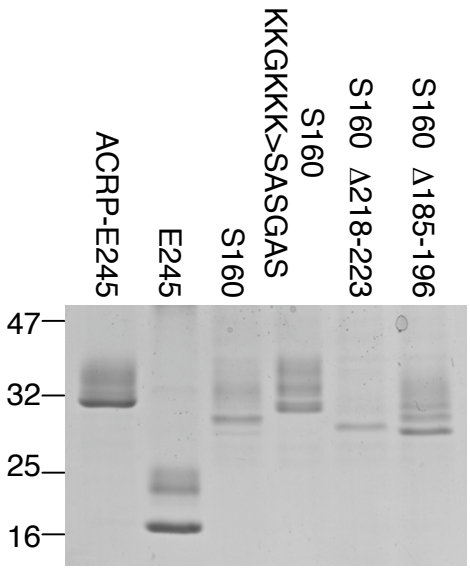


Fig. S5. **Coomassie blue staining of Flag-EDA1 proteins**.
Flag-tagged proteins were purified form conditioned supernatant of transiently transfected 293T cells, and analyzed by SDS-PAGE and Coomassie blue staining. Note the reduction in molecular weight in EDA1 proteins with in-frame deletions in the collagen domain. The following amounts of Flag-EDA1 were loaded on the gel: ACRP-E245 (4 $\mu$g), E245 (3.5 $\mu$g), S160 (2.5 $\mu$g), S160 KKGKKK>SASGAS (3.5 $\mu$g), S160 Δ218-223 (2.5 $\mu$g), S160 Δ185-196 (3.5 $\mu$g). 5 $\mu$g of total protein was loaded in each lane, of which 1 to 2.5 $\mu$g was contaminating bovine serum albumin (not shown).

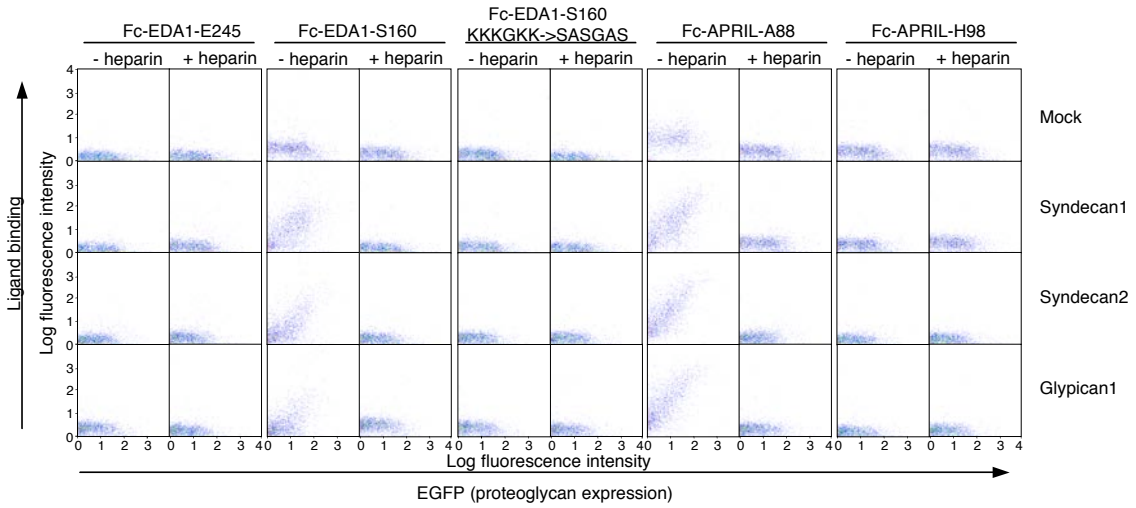


Fig. S6. **EDA with an intact proteoglycan binding site interacts with proteoglycans**.
Jurkat cells were electroporated with proteoglycan expression constructs together with an EGFP tracer plasmid using the transfection solution V and the electoporation program O-17 (Amaxa Biosystems). After electroporation, cells were cultured for 16 h, and then stained with the indicated Fc-EDA constructs containing or not an intact proteoglycan-binding sequence, in the presence or absence of heparin, and analyzed by FACS. APRIL constructs with (A88) or without (H98) proteoglycan-binding sequence were included as controls. APRIL A88 and EDA-S160 bound cells in a heparin-inhibitable manner, whereas forms of EDA without the proteoglycan-binding region did not.

SUPPLEMENTARY TABLE

| Plasmid | Designation | Protein encoded | Figure |
|---|---|---|---|
| ps515 | EGFP | Enhanced green fluorescent protein | 8C |
| ps1752 | EDA1 full | hEDA1(aa 1-391) | 3B,S3B |
| ps1753 | EDA1 full Δ185-196 | hEDA1 Δ185-196 (aa 1-391) | 3B,S3B |
| ps1754 | EDA1 full Δ218-223 | hEDA1 Δ218-223 (aa 1-391) | 3B,S3B |
| ps548 | EDA1-E245 (short) | Signal-Flag-Linker 1-VD-mEDA1 (aa 245-391) | 3B,4,5,7,8B,S3B,S5,8D |
| ps1001 | EDA1-S160 (long) | Signal-Flag-Linker 1-hEDA1 (aa 160-391) | 3B,4,5,7,8B,S3B,S5,8D |
| ps1002 | EDA1-S160 Δ185-196 | Signal-Flag-Linker 1-hEDA1 Δ185-196 (aa 160-391) | 3B,4,8B,S3B,S5 |
| ps1177 | EDA1-S160 Δ218-223 | Signal-Flag-Linker 1-hEDA1 Δ218-223 (aa 160-391) | 3B,4,8B,S3B,S5 |
| ps2266 | EDA1-S160 KKKGKK ->SASGAS | Signal-Flag-Linker 1-hEDA1 KKKGKK>SASGAS (aa 160-391) | 4,5,7,S5,8D |
| ps2343 | EDA1-S160 Δ181-234 | Signal-Flag-Linker 1-hEDA1 Δ181-234 (aa 160-391) | 8D |
| ps2344 | EDA1-S160 Δ181-234 KKKGKK->SASGAS | Signal-Flag-Linker 1-hEDA1 Δ181-234 KKKGKK>SASGAS (aa 160-391) | 8D |
| ps869 | ACRP-EDA1-E245 | Signal-Flag-Linker 1 (D>H)-mACRP30 (aa 18-111)-LQ-mEDA1 (aa 245-391) | 4,5,7,8B,S5 |
| ps167 | FasL | Signal-Flag-Linker 1-hFasL (aa 139-281) | 5 |
| ps959 | EDA1-S160-242 :FasL | Signal-Flag-Linker 1-hEDA1 (aa 160-242)-MHVD-hFasL (aa 139-281) | 5 |
| ps579 | ACRP-FasL | Signal-Flag-Linker 1 (D>H)-mACRP30 (aa 18-111)-LQ-hFasL (aa 139-281) | 5 |
| ps1661 | Fc-PS-EDA1-E245 | Signal-LD-hFc (aa 245-470)-PreSci linker-EDA1 (aa 245-391) | 2A,2B,3A,3C,8C,S1,S2A,S3A,S4,S6 |
| ps1888 | Fc-PS-EDA1-S160 | Signal-LD-hFc (aa 245-470)-PreSci linker-EDA1 (aa 160-391) | 3A,8C, S3A,S6 |
| ps1707 | Fc-PS-EDA1 Δ185-196 | Signal-LD-hFc (aa 245-470)-PreSci linker-EDA1 Δ185-196 (aa 160-391) | 3A, S3A |
| ps1708 | Fc-PS-EDA1 Δ218-223 | Signal-LD-hFc (aa 245-470)-PreSci linker-EDA1 Δ218-223 (aa 160-391) | 3A, S3A |
| ps1943 | Fc-PS-EDA1 KKKGKK->SASGAS | Signal-LD-hFc (aa 245-470)-PreSci linker-EDA1 KKKGKK>SASGAS (aa160-391) | 8C,S6 |
| ps2274 | Fc-PS-EDA1 Δ181-234 | Signal-LD-hFc (aa 245-470)-PreSci linker-EDA1 Δ181-234 (aa 160-391) | 8C,S6 |
| ps2287 | Fc-PS-EDA1 Δ181-234 KKKGKK->SASGAS | Signal-LD-hFc (aa 245-470)-PreSci linker-EDA1 Δ181-234 KKKGKK>SASGAS (aa 160-391) | 8C,S6 |
| ps1938 | Fc-EDA1-A238 | HA signal-hFc (aa 245-470)-hEDA1 (aa 238-391). | 6 |
| ps1236 | Fc-EDA1-E245 | Signal-LD-hFc (aa 245-470)-Linker 2-GSLQVD-mEDA1 (aa 245-391) | 3C |
| ps1181 | Fc-TNF | Signal-LD-hFc (aa 245-470)-Linker 2-GSLQ-hTNF (aa 85-233) | 2B,3B,S2A |
| ps1252 | sol. EDAR | hEDAR (aa 1-183)-Linker 2-GGCC-hCOMP (aa 32-80)-EF-Flag | 2A |
| ps1282 | sol. BCMA | Ig signal-PRGS-hBCMA (aa 2-54)-Linker 2-GGCC-hCOMP (aa 32-80)-EF-Flag | 2A |
| ps1431 | EDAR-GPI | hEDAR (aa 1-183)-VD-hTRAILR3 (aa 157-259) | 8C |
| ps1432 | XEDAR-GPI | Ig signal-DVT-hXEDAR (aa 1-134)-VD-hTRAILR3 (aa 157-259) | 8C |
| ps930 | EDAR-Fc | hEDAR (aa 1-183)-VD-hFc (aa 245-470) | S3, S5 |
| ps1130 | EDAR-PS-Fc | hEDAR (aa 1-183)-VD-PreSci-hFc (aa 245-470) | 6 |
| ps2199 | EDAR:Fas | hEDAR (aa 1-183)-VD-hFas (aa 169-335) | 4,7A |
| ps1889 | IκBα-DN | Flag-EFR-IκBα (aa 1-317) S32G | S2A |
| ps1155 | Fc-APRIL-A88 | Signal-LD-hFc (aa 245-470)-linker 2-SLQ-hAPRIL (aa 88-233, from 2nd methionine) | 6S |
| ps1307 | Fc-APRIL-H98 | Signal-LD-hFc (aa 245-470)-linker 2-PreSci-GSLQ-hAPRIL (aa 98-233, from 2nd methionine) | 6S |
| ps1880 | Syndecan-1 | hSyndecan-1 (aa 1-308)-EFGSPGVD-VSV tag | 6S |
| ps1666 | Syndecan-2 | hSyndecan-2 (aa 1-201)-VD-VSV tag | 6S |
| ps1812 | Glypican-1 | Signal-VSV tag-Linker 1-VD-hGlypican-1 (aa24-558) | 6S |

Table S1. **List of plasmids used in the study**.

Amino acids are indicated with the one letter code or, for large stretches of sequence, by a written description. Signal (signal peptide of haemaglutinin: MAIIYLILLFTAVRG⬇); Flag (DYKDDDDK); VSV tag (YTDIEMNRLGK); Linker 1 (GPGQVQLQ); Linker 2 (RSPQPQPKPQPKPEPEG); PreSci (PreScission site: LEVLFQ⬇GP); PreSci linker (RSPQPQPKPQPKPEPEG-LEVLFQ⬇GP-GSLQVD: the sequence in the middle corresponds to the PreScission site); mEDA1 (SwissProt accession number O54693. Note that the protein sequence of mouse and human EDA1 is identical for aa 245-391); hEDA1 (SwissProt accession number Q92838); hFc (IgG1 encoded by GenBank accession number BC018747 or aa105-330 of SwissProt accession number P01857); hTNF (SwissProt accession number P01375); hEDAR (SwissProt accession number Q9UNE0); hTRAILR3 (SwissProt accession number O14798); hCOMP (cartilage oligomeric matrix protein; SwissProt accession number P49747); Ig signal (Signal peptide of mouse Ig, heavy chain: MNFGFSLIFLVLVLKGVQC⬇EVKLV); hBCMA

(SwissProt accession number Q02223), hFas (SwissProt accession number P25445), hIκBα (SwissProt accession number P25963), hAPRIL (SwissProt accession number O75888), hFasL (SwissProt accession number P48023), hSyndecan-1 (SwissProt accession number P18827), hSyndecan-2 (SwissProt accession number P34741), and hGlypican-1 (SwissProt accession number P35052). The "⬇" indicate predicted proteolytic cleavage sites by signal peptidase or PreScission proteases.