SUPPLEMENTARY APPENDIX

Prenatal Correction of X-linked Hypohidrotic Ectodermal Dysplasia

TABLE OF CONTENTS

METHODS 2

SUPPLEMENTARY FIGURES 9

Figure S1. *EDA* mutation Y304C grossly affects solubility of the protein. 9

Figure S2. Phenotypic reversion of EDA-deficient mice depends on the neonatal Fc receptor. 10

Figure S3. Detection of EDAR agonists in milk. 11

Figure S4. Efficacy of EDAR agonists to correct tail hair and sweat glands in EDA-deficient mice in the presence or absence of neonatal Fc receptor (FcRn). 12

Figure S5. Determination of the half-life of Fc-EDA in FcRn-deficient adult mice. 13

Figure S6. Efficient unstimulated sweating and salivation of the twins treated *in utero.* 14

SUPPLEMENTARY TABLES 15

Table S1. Phenotypic correction of individual EDA-deficient mice following treatment with EDAR agonists. 15

Table S2. EDA serum concentrations after a 2-hour intravenous infusion administration of Fc-EDA to pregnant monkeys. 19

Table S3. Adverse events observed in the three human case studies. 20

Table S4. Plasmids used for the experiments. 21

REFERENCES 21

INVESTIGATORS

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METHODS

Animal models

Homozygous *Tabby* mice derived from white-bellied agouti B6CBAa $A^{w\text{-}J}/A\text{-}Eda^{Ta}$/J mice (000314; Jackson Laboratory), in some cases crossed with B6.129-$Fcgrt^{tm1Dcr}$/DcrJ mice (003982; Jackson Laboratory), were housed in individually ventilated cages under standard conditions with a light/dark cycle of 12 hours and free access to standard chow and tap water. *Tabby/Tabby Fcgrt*$^{-/-}$ females were crossed with *Tabby/Y Fcgrt*$^{-/-}$ or *Tabby/Y Fcgrt*$^{+/-}$ males, all selected for agouti coat color. Drug administration to the animals was performed as detailed below. All experimental procedures were conducted in accordance with the German regulations and legal requirements or according to the guidelines and under the authorization of the Swiss Federal Food Safety and Veterinary Office.

Toxicity studies on 26 cynomolgus monkeys were conducted by MPI Research Inc. (Mattawan, USA) in accordance with the United States Food and Drug Administration (FDA) Good Laboratory Practice regulations. The experimental procedures are described below.

Recombinant proteins, cell lines and antibodies

Fc-EDA, a recombinant fusion protein consisting of the receptor-binding domain of EDA (aa 238-391) and the Fc domain of human immunoglobulin G1, was provided as a sterile drug product (EDI200, produced according to Good Manufacturing Practice regulations) with a concentration of 5 mg/mL in 20 mM sodium phosphate, 300 mM sodium chloride, pH 7.2, and 0.02% Polysorbate 20 (w/v) by Edimer Pharmaceuticals, Inc. (Cambridge, USA) and stored frozen at -70°C until further use.

Flag-ACRP-EDA produced by transient transfection of HEK 293T cells was purified on anti-Flag M2-agarose beads (Sigma) as described previously.[1] Endotoxin levels were below

1 unit/µg. The recombinant fusion protein hEDAR-mFc containing the extracellular domain of human EDAR (aa 1-183) and the Fc part of mouse IgG1 was constructed according to standard molecular biology procedures, produced in CHO cells and affinity-purified on immobilized mAbEDAR1. Anti-EDAR agonist antibody number 1 (mAbEDAR1) and hEDAR-hFc were generated as described.[2] Anti-EDA monoclonal antibody Renzo-2 is commercially available (Enzo Life Sciences).

Plasmids coding for Flag-EDA (wild-type and Y304C) were transiently transfected in 293T cells that were then washed and cultured for 6 days in serum-free OptiMEM medium. Cells and supernatants were harvested and analyzed by SDS-PAGE and Western blot with anti-EDA antibody Renzo-2 essentially as described.[3] Sequences of proteins used are detailed in Table S2.

Genotyping

Murine genomic DNA from ear biopsies was analyzed by PCR with the following oligonucleotides: wild-type forward 5'-GGGATGCCACTGCCCTG-3'; mutant forward 5'-GGAATTCCCAGTGAAGGGC; common reverse 5'-CGAGCCTGAGATTGTCAAGTGTATT-3' (378 bp for mutant *Fcgrt* allele and 248 bp for wild-type allele) using a 30-cycle 94°C (7 s) / 63°C (20 s) / 72°C (20 s) program.

Experimental procedures on animals

Intra-amniotic administration of ACRP-EDA or Fc-EDA to mice at different time points of gestation was performed as described previously.[4] In some cases, Fc-EDA or mAbEDAR1 were injected intraperitoneally at doses of 10 mg/kg into dams within 24 hours after delivery and on the two days thereafter (at P1, P2 and P3). Fc-EDA, ACRP-EDA or mAbEDAR1 were also administered intraperitoneally to newborn pups on the day of birth at the doses indicated. The

treated mice were investigated postnatally for various morphological features (tail and coat hair, presence of sweat ducts at the foot pads, shape of the molars). Starch-iodine sweat tests were performed as described previously.[4] For half-life determination, 200 µg of Fc-EDA or mAbEDAR1 were injected into the tail veins of adult mice. Serum was obtained from single drops of blood collected from cheek veins at the time points indicated.

Milk was taken from mice at days 2, 3 or 4 of lactation by gentle liquid aspiration from nipples with the plastic tubing (catheter) of a butterfly needle attached to a collection tube, itself connected to a cell culture vacuum pump (set at low suction power to enable sensitive aspiration). Lactating mice were treated by intraperitoneal injection of two units of oxytocin (03251-500U, Sigma-Aldrich) two minutes before milking.

Fc-EDA was also administered intravenously to cynomolgus monkeys. A group of three male and three female monkeys and a second group of five males and five females were treated by twice weekly infusion of Fc-EDA at dose levels of 30 or 100 mg/kg/dose, respectively, for three weeks. An additional group of five animals of each sex which received the vehicle, 20 mM sodium phosphate, 300 mM sodium chloride, pH 7.2, and 0.02% Polysorbate 20 (w/v), served as control. The dose volume for all groups was 20 mL/kg/dose (10 mL/kg/hour). Following the treatment period, some animals were maintained for a 15-day recovery period. Observations regarding morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Clinical observations took place twice weekly during the treatment period and weekly during recovery. Body weights were measured and recorded weekly. Ophthalmoscopic examinations were conducted before drug delivery and prior to each scheduled necropsy. Electrocardiography was done on day 15 and prior to post-recovery necropsy. Blood samples for determination of the serum concentrations of EDA were collected from all animals

on days 1 and 19 and prior to the post-recovery necropsy. The toxicokinetic parameters were determined for the drug from concentration-time data in the test species. Blood samples for evaluations of immunogenicity and blood and urine samples for further evaluations were obtained before drug delivery and prior to the necropsies. At the necropsies, examinations were performed, organ weights were recorded, and tissues were examined microscopically.

To evaluate the potential placental transfer of the drug, five pregnant monkeys received Fc-EDA intravenously at doses of 10, 30, and 100 mg/kg body weight and were sacrificed one to 12 hours later. Observations of the animals included clinical signs, body weights, heart rate, respiration rate, and body temperature. A toxicokinetic assessment was made.

Protein quantification by ELISA

Fc-EDA in 100 µl of 133-fold diluted sera (and 2-fold dilutions thereof) was quantified using ELISA plates coated with 100 µl of hEDAR-mFc (1 µg/ml) per well and revealed with a peroxidase-coupled anti-human IgG antibody; mAbEDAR1 was quantified similarly on hEDAR-hFc-coated plates and revealed with a peroxidase-coupled anti-mouse IgG antibody. Details of the ELISA procedure have been published.[5] EC50 was determined using the "log(agonist) vs. normalized response – variable slope" function of Prism software.

Prenatal treatment of human patients

In three cases of male human fetuses with the prenatal diagnosis of XLHED (two monochorionic twins and a single fetus), written informed consent for prenatal intra-amniotic administration of Fc-EDA as an individual "Heilversuch" (trial to cure) was given by the parents and approval of the clinical ethics committee of the University Hospital Erlangen was obtained. At week 26 of gestation, some amniotic fluid was removed and Fc-EDA (EDI200, provided by Edimer

Pharmaceuticals Inc.; same batch as used in the clinical trial NCT01775462 on affected neonates; doses of 70 to 75 mg in a total volume of 14 to 15 mL sterile solution) was injected under ultrasound guidance into the amniotic cavity of each fetus. At gestational week 31, a 20-mL sample of amniotic fluid was withdrawn and a second injection of 140 mg of Fc-EDA in a volume of 28 mL was performed in two cases.

Fc-EDA concentrations in the serum of pregnant women, in amniotic fluid samples and in the serum of treated patients were determined by MPI Research Inc. (Mattawan, USA).

Investigation of untreated affected siblings and other control subjects

The older brother of the treated twins who also carries the *EDA* mutation Y304C was investigated in parallel to his siblings. He had suffered several hyperthermic episodes in early infancy and was enrolled in the natural history study ECP-015 (ClinicalTrials.gov NCT02099552). The older brother of the third treated patient carries the *EDA* mutation V309GfsX8. He had been referred to the University Hospital Erlangen because of his characteristic facial appearance and repeated occurrence of unexplained fever soon after birth and was also investigated in parallel to his sibling. Three more boys and one male adult with the *EDA* mutation Y304C who had been examined in previous studies[6-8] or seen at the University Hospital Erlangen served as additional controls. Samples of healthy control subjects age-matched to the treated infants were investigated by confocal laser-scanning microscopy (n = 6) and quantification of pilocarpine-induced sweating (n = 6) at the University Hospital Erlangen. Unstimulated saliva flow was determined in the treated twins, their older brother and two healthy 2-year-old infants. The adult provided written informed consent to participate in the study; in the case of minors, parental consent and if possible assent of the child were obtained.

Confocal laser-scanning microscopy

Reflectance confocal laser-scanning microscopy of skin areas of 36 mm² to determine sweat pore densities was conducted with a VivaScope 1500 (Caliber Imaging & Diagnostics, New York, USA) according to the manufacturer's instructions. The dermal ridges of the infants treated *in utero* were investigated in comparison with older siblings and healthy control infants.

Quantification of pilocarpine-induced sweating

Sweat was collected by a standardized procedure from an area of 57 mm² of the right or left forearm 30 min after stimulation with a pilocarpine gel disk using the Wescor 3700 device (Wescor, Logan, USA). Maximum volume that could be collected in this device was 93 µL. The volume was determined immediately after sampling.

Quantification of saliva flow

Whole saliva was collected with the Quantisal oral fluid collection device (Immunalysis, Pomona, USA) between 10:00 and 13:00 hours, after the subjects had refrained from eating and drinking for a period of 1 hour. All infants were instructed to swallow once before the test started and told to refrain from swallowing or talking during saliva collection for a total of 120 seconds. The pre-weighted fluid collector was positioned under the tongue, and the head of the subject was kept down to allow gravity to help with fluid collection. In none of the tests the volume limit indicator turned blue during the procedure. After the collection period, the weight increase of the collector was determined on a special accuracy weighing machine (Sartorius Lab Instruments, Göttingen, Germany), and the saliva flow rate was calculated in mL/min. Oral fluid collection was repeated three times at different days or with intervals of at least 40 minutes.

Dental imaging

In the treated twins, magnetic resonance imaging (MRI) of the head was conducted at the age of two months. Dental radiography was done when the infants were 6 to 12 months old (age corrected for preterm birth).

Data availability

The datasets generated and/or analyzed during the current study are either included in this article (and its Supplementary appendix) or available from a data depository (DOI: 10.5281/zenodo.1164154), or will be provided by the corresponding author on reasonable request.

SUPPLEMENTARY FIGURES

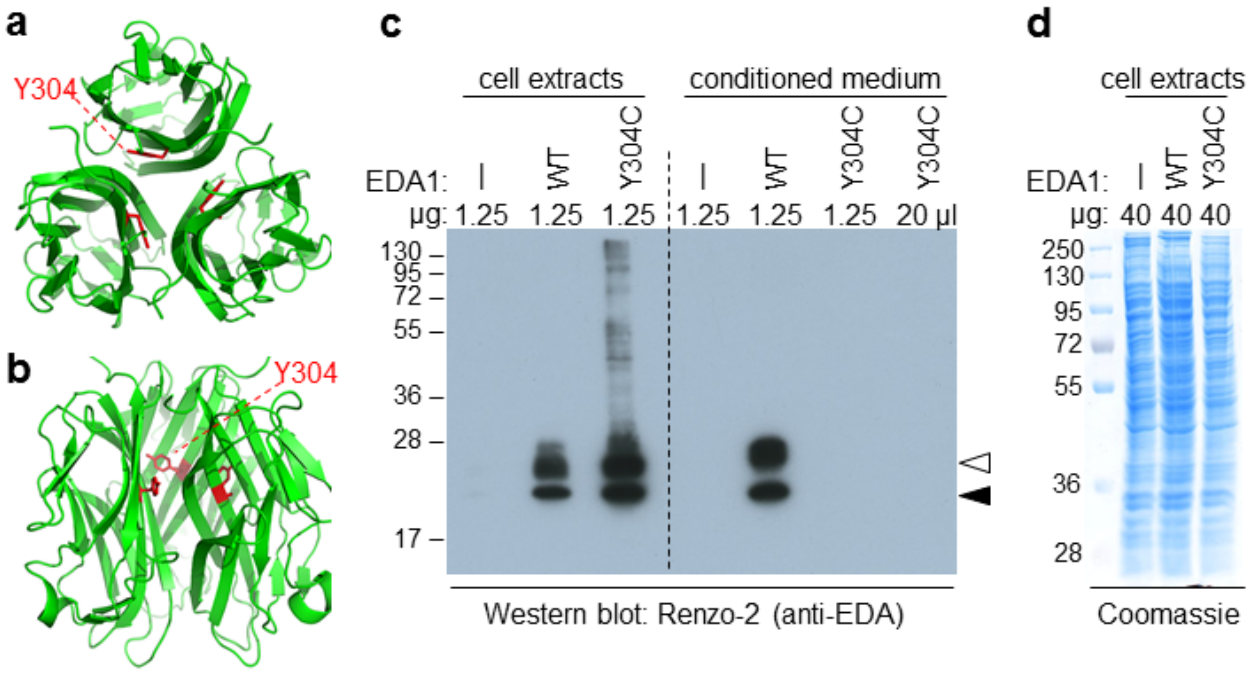

Figure S1. *EDA* mutation Y304C grossly affects solubility of the protein.
a, Position of tyrosine 304 (Y304) in the crystal structure of the C-terminal TNF-homology domain of EDA (pdb accession number 1RJ7) in a top view of the homotrimeric protein. Y304 is situated at the interface of adjacent protomers in the crystal structure.
b, same as panel a, but viewed from the side. In this representation, the receptor-expressing cell would be at the bottom of the picture.
c, Flag-EDA1-WT (wild-type) and Flag-EDA1-Y304C were expressed as recombinant proteins in 293T cells and detected in cell extracts and conditioned culture medium by Western blot using the anti-EDA monoclonal antibody Renzo-2. Although Flag-EDA1-Y304C is expressed, none was found to be secreted. The black and white arrowheads point at the non- and mono-N-glycosylated forms of Flag-EDA1, respectively.[9]
d, Coomassie blue staining of cell extracts used in panel c to document comparable loading.
The experiment in panels c and d was performed twice with similar results.

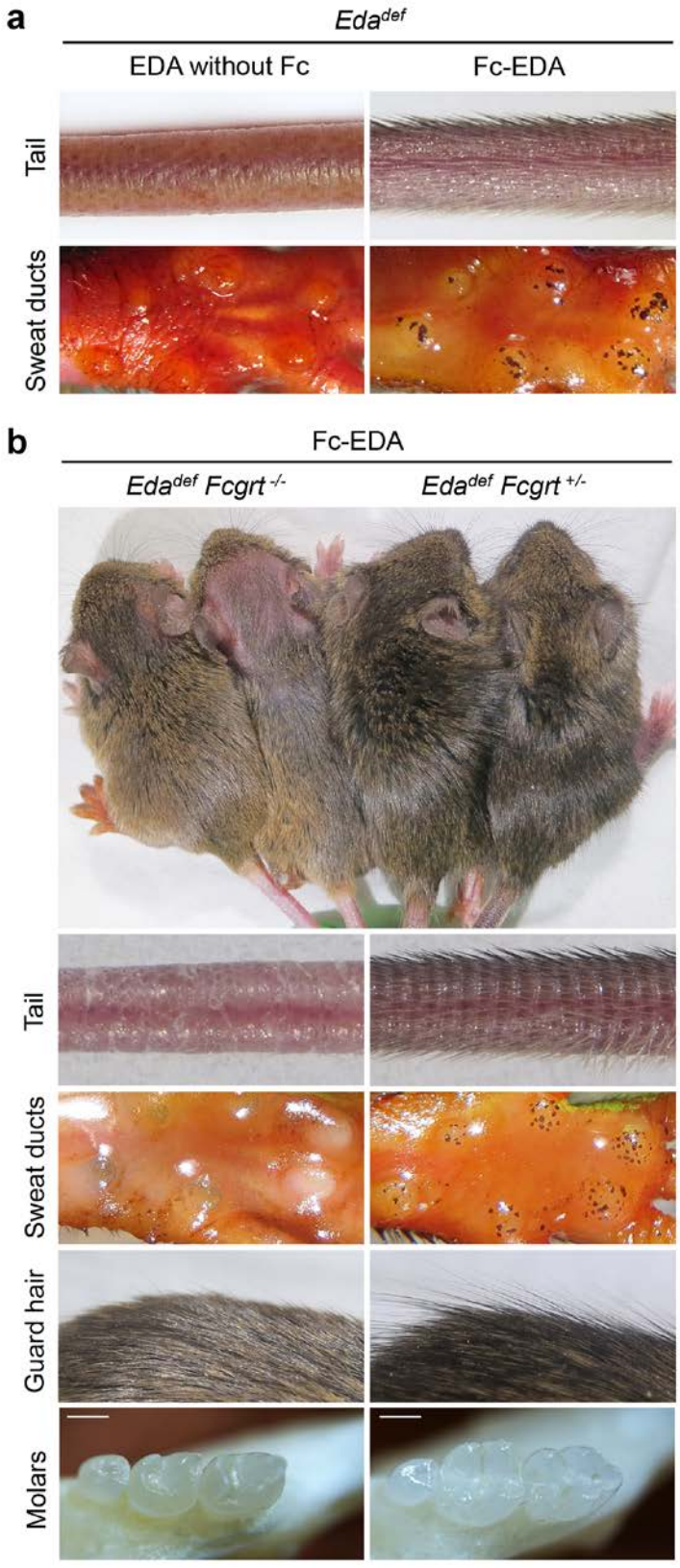

Figure S2. Phenotypic reversion of EDA-deficient mice depends on the neonatal Fc receptor.

a, Representative pictures of tails and paws of EDA-deficient mice treated *in utero* (E14.5) by intra-amniotic administration of ACRP-EDA (EDA without Fc) or Fc-EDA. Sweat gland function was visualized by starch-iodine staining (dark dots with a characteristic pattern).

b, Intra-amniotic administration of Fc-EDA to EDA-deficient mouse fetuses, some of which were devoid of the neonatal Fc receptor, resulted in phenotypic reversion (darker coat with plenty of guard hair, a hairy tail, functional sweat glands, larger and more complex molars) only of those animals expressing the neonatal Fc receptor. Scale bar: 500 µm.

Photographs of 232 further mice used in this study are shown in *Extended Figure S1* accessible under the DOI: 10.5281/zenodo.1164154.

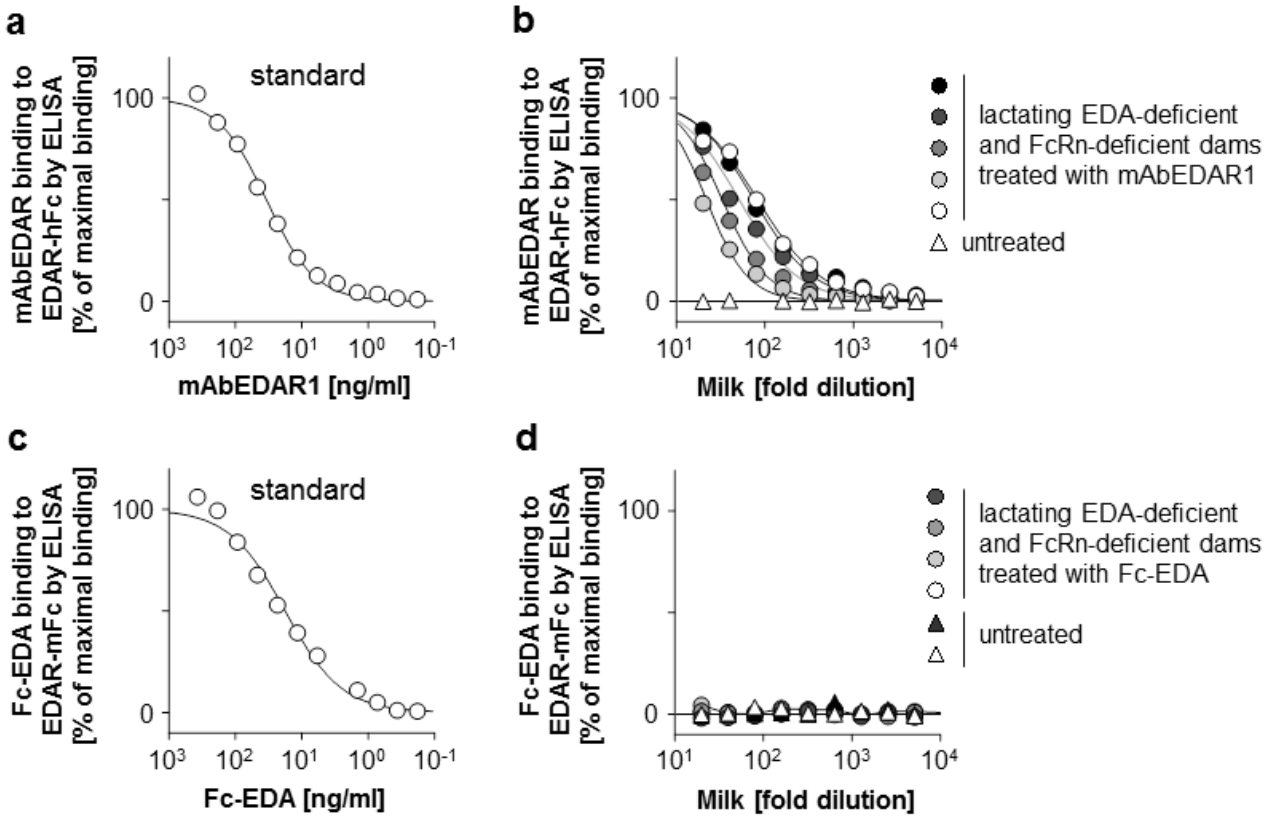

Figure S3. Detection of EDAR agonists in milk after intraperitoneal administration.
EDAR agonists (10 mg/kg) were administrated intraperitoneally to dams after delivery (at days 1, 2 or 3 of lactation) and milk was collected 24 h later.
a, Standard curve of mAbEDAR1 binding to hEDAR-hFc by ELISA.
b, Detection of mAbEDAR1 in milk of neonatal Fc receptor (FcRn)-deficient dams by its ability to bind to hEDAR-hFc by ELISA.
c, Standard curve of Fc-EDA binding to hEDAR-mFc by ELISA.
d, Detection of Fc-EDA in milk of FcRn-deficient dams by its ability to bind to hEDAR-mFc by ELISA.

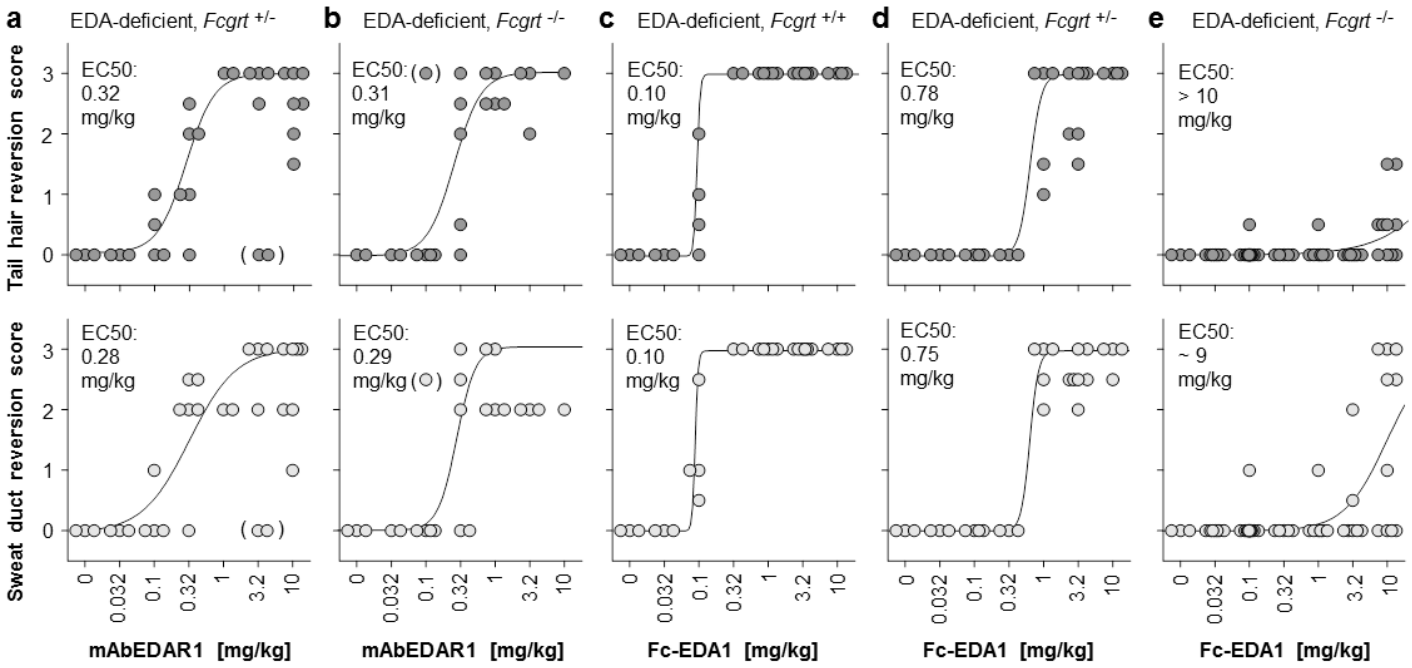

Figure S4. Efficacy of EDAR agonists to correct tail hair and sweat glands in EDA-deficient mice in the presence or absence of neonatal Fc receptor (FcRn).
Mice of the indicated genotypes were treated at birth with the indicated doses of mAbEDAR1 or Fc-EDA. Tail hair and sweating ability were scored three weeks later. *Fcgrt* is the gene encoding FcRn. After exclusion of the results indicated in brackets, the average reversion score for each treatment dose was used to determine EC50. The curve was manually superimposed on graphs.
a, mAbEDAR1 in pups heterozygous for FcRn.
b, mAbEDAR1 in FcRn-deficient pups.
c, Fc-EDA in pups wild-type for FcRn.
d, Fc-EDA in pups heterozygous for FcRn.
e, Fc-EDA in FcRn-deficient pups.

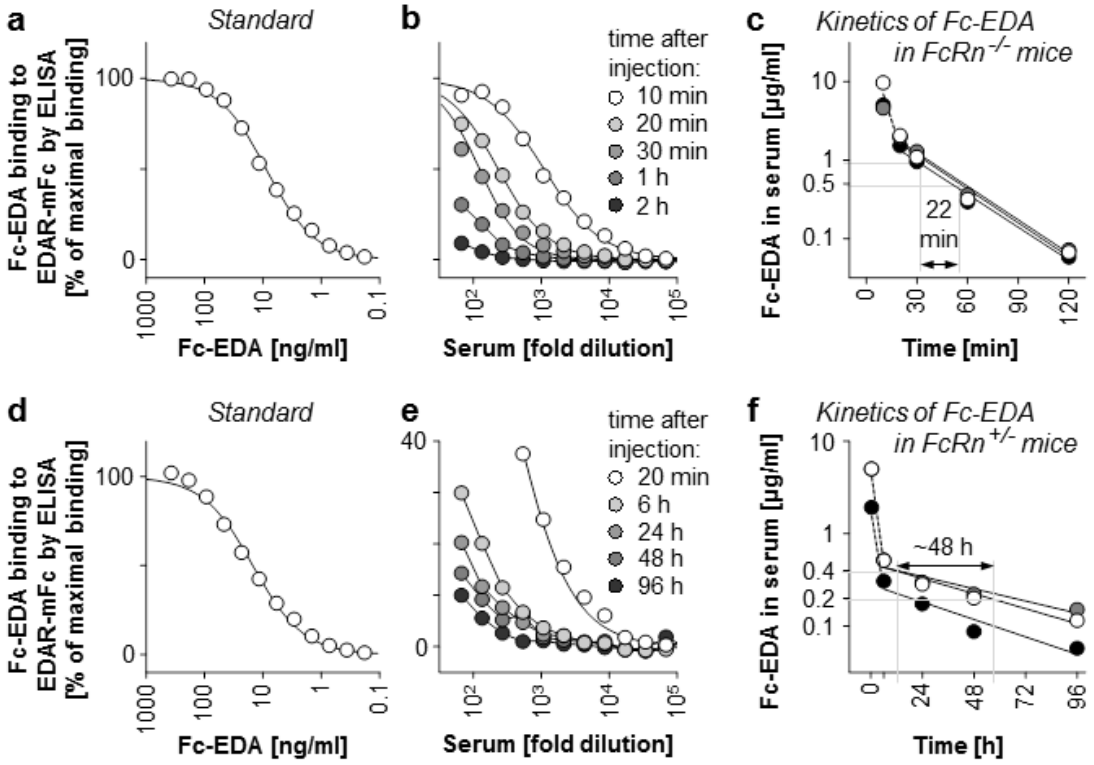

Figure S5. Determination of the half-life of Fc-EDA in FcRn-deficient adult mice.
FcRn-deficient or heterozygous mice were treated by intravenous injection of 200 µg of Fc-EDA. Small blood volumes were collected at the indicated time points for serum preparation and Fc-EDA was detected by ELISA as described in the legend to Fig. S2.
a, Detection of Fc-EDA at the indicated concentrations. The figure shows average values ± SEM of three normalized standard curves.
b, Detection of Fc-EDA in serum samples at the indicated time points after intravenous administration to mice lacking both EDA and FcRn. Data shown are from one mouse out of three with similar results.
c, Fc-EDA concentration in sera was determined assuming that the concentrations at EC50 of curves in panel b are equal to the concentrations of pure Fc-EDA at EC50 in panel a. Results from three mice are shown (white, grey and black circles). The half-life of Fc-EDA was estimated as indicated. For time points where EC50 could not be determined (1 h and 2 h), Fc-EDA concentrations were measured by direct comparison with the standard curve of panel a. The dotted line indicates the distribution phase and the solid line the elimination phase, from which the half-life of Fc-EDA was estimated.
d, same as panel a, but showing the average ± SEM of two standard curves.
e, same as panel b, but in an EDA-deficient mouse heterozygous for FcRn.
f, same as panel c, but in EDA-deficient mice heterozygous for FcRn.

a

b

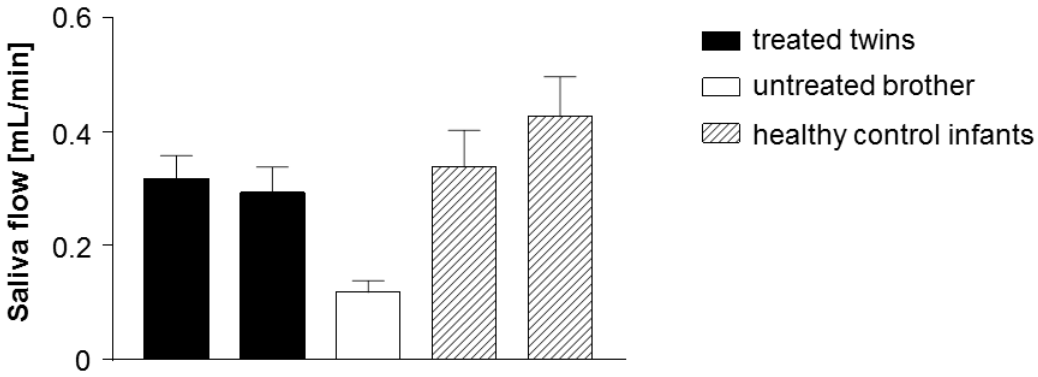

Figure S6. Efficient unstimulated sweating and salivation of the twins treated *in utero*.
Male twins with the *EDA* mutation Y304C who had both received prenatal Fc-EDA treatment were investigated repeatedly after birth.
a, Wet baby car seat of one of the treated twins in the summer documenting unstimulated sweating (spillage of urine or other liquids could be excluded).
b, Assessment of salivation at the age of 21 months (together with the untreated affected brother). Saliva collected over a period of 120 seconds was quantified gravimetrically. All samples were obtained between 10:00 and 13:00 hours, after the subjects had refrained from eating and drinking for a period of 1 hour. Each column shows the average of three independent measurements, error bars indicate the standard deviation.

SUPPLEMENTARY TABLES

Table S1. Phenotypic correction of individual EDA-deficient (*Tabby*) mice following treatment with EDAR agonists.

Drug	Dose	Administration		Genotype of dam		Genotype of the pups		Teeth	Guard hair	Ear hair	Tail hair	Sweat ducts	Remarks
		Time	Route	*Eda*	*Fcgrt*	*Eda*	*Fcgrt*						
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	2	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	3	3	3	3	3	
Fc-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	0	0	0	0	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	0	0	0	0	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	1	0	0	0	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	0	0	0	0	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	0	0	0	0	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	0	0	0	0	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	n.d.	0	0	0	n.d.	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	2	2	3	1	*
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	1	1	0	0	*
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	0	0	0	0	0	
ACRP-EDA	35 µg	E14.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	n.d.	0	0	0	n.d.	

ACRP-EDA	4 mg/kg	P1	i.p.	*Tabby*	+/+	*Tabby*	+/+	n.d.	n.d.	n.d.	2.5	3	
ACRP-EDA	4 mg/kg	P1	i.p.	*Tabby*	+/+	*Tabby*	+/+	n.d.	n.d.	n.d.	3	3	
ACRP-EDA	4 mg/kg	P1	i.p.	*Tabby*	+/+	*Tabby*	+/+	n.d.	n.d.	n.d.	2.5	2	
ACRP-EDA	1 mg/kg	P1	i.p.	*Tabby*	+/+	*Tabby*	+/+	n.d.	n.d.	n.d.	1	3	
ACRP-EDA	1 mg/kg	P1	i.p.	*Tabby*	+/+	*Tabby*	+/+	n.d.	n.d.	n.d.	2	3	
ACRP-EDA	1 mg/kg	P1	i.p.	*Tabby*	+/+	*Tabby*	+/+	n.d.	n.d.	n.d.	3	2	
ACRP-EDA	1 mg/kg	P1	i.p.	*Tabby*	+/+	*Tabby*	+/+	n.d.	n.d.	n.d.	3	3	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	+/+	*Tabby*	+/+	2	3	3	3	3	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	1	3	0	0	0.5	*
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	1	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	n.d.	0	0	0	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	+/-	1.5	3	3	3	3	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	+/-	2	3	3	3	1.5	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	+/-	2	3	3	2	1.5	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	+/-	2	3	3	1.5	0	
Fc-EDA	58 µg	E13.5	i.a.	*Tabby*	-/-	*Tabby*	+/-	1.5	3	3	1.5	1	
Fc-EDA	58 µg	E12.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E12.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	1	0	0	0	
Fc-EDA	58 µg	E12.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E12.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E12.5	i.a.	*Tabby*	-/-	*Tabby*	-/-	0	0	0	0	0	
Fc-EDA	58 µg	E12.5	i.a.	*Tabby*	-/-	*Tabby*	+/-	2	3	3	1	0.5	
Fc-EDA	58 µg	E12.5	i.a.	*Tabby*	-/-	*Tabby*	+/-	0.5	3	3	0.5	0	

mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	1	0.5	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	1	1.5	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0.5	0	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	3	3	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	3	3	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	3	3	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	3	3	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	3	3	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	1	3	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	3	3	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	3	2.5	
mAbEDAR1	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	3	2.5	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	-/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	1	2	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	0	0	
Fc-EDA	10 mg/kg	P1-P3	i.p., dam	*Tabby*	-/-	*Tabby*	+/-	n.d.	n.d.	n.d.	1	2	

E preceding a number indicates an embryonic day, and P denotes a postnatal day. Treatment after birth does not rescue the development of teeth, guard hair, or ear hair; therefore, these scores were not determined for mice that were treated after birth.

Teeth score (excluding the third molar): 0 = narrow molars, shallow cusps, no or rudimentary anterior cusp on molar number 1 (M1); 1 = wider molars, more defined cusps, one small anterior cusp on M1; 2 = wide molars, well-defined cusps, stubby anterior cusp on M1; 3 = like 2, but elongated anterior cusp on M1 (making ~1/3 of the length). Guard hair score (evaluated on a photography of the back of the mouse): 0 = no guard hair; 1 = between one and five guard hairs visible; 2 = sparse guard hair; 3 = numerous guard hairs all over the picture. Ear hair (skin area at the rear side of the ears evaluated): 0 = no hair, naked skin; 1 = very few hairs; 2 = sparse hair; 3 = dense hair, skin covered. Tail hair: 0 = none; 1 = sparse and only on one side of the tail; 2 = hair on both sides of the tail, usually dense on one side and sparse on the other; 3 = dense hair on both sides of the tail. Sweat ducts (evaluated on a photography of the paw showing all six foot pads after starch-iodine staining): 0 = no sweat spot; 1 = few spots on at least one foot pad; 2 = several spots on three or more foot pads; 3 = numerous sweat spots on at least five foot pads.

i.a., intra-amniotic; i.p., intraperitoneal; n.d., not determined; *, partial reversion observed in these animals might have been due to accidental damage of the foetus or a yolk sac vessel during the injection procedure.

Table S2. EDA serum concentrations after intravenous infusion administration of Fc-EDA to pregnant monkeys.

Dose (mg/kg)	Sample	Time (hr)	Day	N	Mean (ng/mL)	SD (ng/mL)	CV%
10	Maternal	0	102	1	0.00	NA	NA
		1	102	1	15300	NA	NA
		12	102	1	5690	NA	NA
10	Fetal	12	102	1	0.00	NA	NA
30	Maternal	0	102	1	0.00	NA	NA
		1	102	1	146000	NA	NA
		12	102	1	15700	NA	NA
30	Fetal	12	102	1	0.00	NA	NA
100	Maternal	0	120	3	0.00	NA	NA
		1	120	3	1260000	262000	20.8
		6	120	2	330000	NA	NA
		12	120	1	106000	NA	NA
100	Fetal	1	120	1	469	NA	NA
		6	120	1	977	NA	NA
		12	120	1	1100	NA	NA

NA, not applicable. Standard deviation (SD) and coefficient of variation (CV) are not reported, when mean concentration equals zero or n<3.

Table S3. Adverse events observed in the three human case studies.

Subject(s) affected	Event	Grade	Treatment	Outcome	Causality assessment (WHO-UMC system)
mother of patients 1 and 2 (twins)	premature contractions with cervical dilation	severe	tocolytic treatment (nifedipine, partusistene, atosiban) for one day, then caesarean section in a hospital close to subject's home	resolved	**possibly related** to prenatal treatment
patients 1 and 2	preterm birth	severe	standard treatment on a neonatal unit close to patients' home for 6 weeks	without sequelae	**possibly related** to prenatal treatment
patient 2 (15 months old)	allergic reaction to egg proteins	severe	medication (prednisolone suppository, Fenistil drops), hospitalization for one night	resolved	**unlikely to be related** to prenatal treatment
mother of patients 1 and 2	atopic dermatitis (since infancy)	mild	topical treatment as needed	unresolved	**not related** to prenatal treatment
patient 2 (8 months old)	atopic dermatitis	moderate	topical treatment as needed	unresolved	**unlikely to be related** to prenatal treatment
patient 1 (8 months old)	atopic dermatitis	mild	topical treatment as needed	unresolved	**unlikely to be related** to prenatal treatment
patient 1 (19 months old)	increased serum urea level	mild	none	resolved	**unlikely to be related** to prenatal treatment
patient 2 (19 months old)	increased serum urea level	mild	none	resolved	**unlikely to be related** to prenatal treatment
patient 2 (19 months old)	thrombocytosis	mild	none	resolved	**unlikely to be related** to prenatal treatment
mother of patients 1 and 2	thrombocytosis (recently)	mild	none	resolved	**unlikely to be related** to prenatal treatment
mother of patients 1 and 2	vitamin D deficiency	mild	vitamin D supplementation as needed	unknown	**not related** to prenatal treatment
mother of patient 3	elective caesarean section	NA	standard obstetric treatment in a hospital close to subject's home	resolved	**not related** to prenatal treatment
patient 3 (4 months old)	Urticaria pigmentosa	moderate	cetirizine drops for two weeks, since then only rarely administered as needed	nearly resolved	**unlikely to be related** to prenatal treatment
patient 3 (10 months old)	Hand-Foot-Mouth disease	moderate	symptomatic treatment	resolved	**not related** to prenatal treatment

Table S4. Plasmids used for the experiments.

Plasmid	Designation (gene product)	Protein encoded	Vector
ps015	Empty vector	None	PCR3
ps869	ACRP-EDA	Signal-Flag-GPGQVQLH-mACRP30 (aa 18-111)-LQ-mEDA1 (aa 245-391)	PCR3
ps930	hEDAR-hFc	hEDAR (aa 1-183)-VD-hFc (aa 224-449 of UniProt entry P0DOX5)	PCR3
ps1938	Fc-EDA1 (EDI200)	Signal-hFc (aa 224-449)-hEDA1 (aa 238-391)	PCR3
ps2270	hEDAR-mFc	hEDAR (aa 1-183)-mFc (aa 237-463 of UniProt entry U5LP42)	pEF1
ps3423	Flag-EDA	Signal-Flag-GPGQVQLQVD-mEDA1 (aa 245-391)	PCR3
ps3754	Flag-EDA Y304C	Signal-Flag-GPGQVQLQVD-mEDA1 (aa 245-391) Y304C	PCR3

Flag = DYKDDDDK HA signal=MAIIYLILLFTAVRG mEDA1 and hEDA1 are 100% identical in amino acids 245-391

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