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Generation and Validation of Mice Carrying a Conditional Allele of the Epidermal Growth Factor Receptor

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Summary:

The epidermal growth factor receptor (EGFR) is important for normal homeostasis in a variety of tissues and, when abnormally expressed or mutated, contributes to the development of many diseases. However, in vivo functional studies are hindered by the lack of adult mice lacking EGFR because of the pre- and postnatal lethality of EGFR deficient mice. We generated a conditional allele of *Egfr* (*Egfr*^{tm1Dwt}) by flanking exon 3 with *loxP* sites in order to investigate tissue-specific functions of this widely expressed receptor tyrosine kinase. The activity of the *Egfr*^{tm1Dwt} allele is indistinguishable from wildtype *Egfr*. Conversely, the Egfr$^{\Delta}$ allele, generated by Cre-mediated deletion of exon 3 using the germline *EIIa-Cre* transgenic line, functions as a null allele. Egfr$^{\Delta/\Delta}$ embryos that have complete ablation of EGFR activity and die at mid-gestation with placental defects identical to those reported for mice homozygous for the *Egfr*^{tm1Mag} null allele. We also inactivated the *Egfr*^{tm1Dwt} allele tissue-specifically in the skin epithelium using the *K14-Cre* transgenic line. These mice were viable but exhibited wavy coat hair remarkably similar to mice homozygous for the *Egfr*^{wa2} hypomorphic allele or heterozygous for the *Egfr*^{Wa5} antimorphic allele. These results suggest that the hairless phenotype of *Egfr* nullizygous mice is not solely due to absence of EGFR in the epithelium, but that EGFR activity is required also in skin stromal cells for normal hair morphogenesis. This new mouse model should have wide utility to inactivate *Egfr* conditionally for functional analysis of EGFR in adult tissues and disease states.

Keywords

EGFR; receptor tyrosine kinase; gene targeting; Cre/loxP; conditional allele

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The epidermal growth factor receptor (EGFR) is the prototypical member of the ERBB family of tyrosine kinase receptors that also includes ERBB2, ERBB3, and ERBB4. *Egfr* is widely expressed and is essential for normal placental development and function of many organ systems. Many abnormal phenotypes associated with loss of EGFR are dependent on genetic background. On some backgrounds the placenta does not develop normally and has a variably reduced spongiotrophoblast layer and a disorganization of the labyrinth layer (Dackor *et al.,* 2007; Strunk *et al.,* 2004), while on other backgrounds EGFR deficient mice survive until term and have abnormalities of the skin and gastrointestinal tract, immature lungs, and cell death in the cortex and olfactory bulb (Miettinen *et al.,* 1995; Sibilia and Wagner, 1995; Threadgill *et al.,* 1995). *Egfr* null mice also have abnormal craniofacial development including defects in palate closure, elongated snouts, and underdeveloped lower jaws (Miettinen *et al.,* 1999).

To study the role of EGFR in adult tissues and disease models, the $Egfr^{wa2}$ hypomorphic allele is frequently used. The $Egfr^{wa2}$ allele is a spontaneously arising point mutation causing a Val743Gly substitution in the ATP-binding site of the tyrosine kinase domain (Fowler *et al.,* 1995; Luetteke *et al.,* 1994). The receptor produced from the $Egfr^{wa2}$ allele has an 80–95% reduction in EGFR activity leading to lactational defects (Fowler *et al.,* 1995) and an increased frequency of cardiac hypertrophy and death caused by enlarged aortic valves on some genetic backgrounds (Chen *et al.,* 2000). The only overt abnormal phenotype observed on all backgrounds is wavy coat hair, which results from loss of EGFR signaling during the anagen phase of the hair cycle (Mak and Chan, 2003).

A conditional humanized allele, $Egfr^{KI}$, has also been created, containing a human *EGFR* cDNA that replaces the mouse *Egfr* allele (Sibilia *et al.,* 2003). Mice homozygous for $Egfr^{KI}$ can live up to 6 months, but display a variety of organ defects making this allele unsuitable for studying the role of EGFR in specific tissues through conditional ablation. More recently, another conditional allele was reported lacking the abnormal phenotypes (Natarajan *et al.,* 2007). This allele appears to function normally before deletion. However, exon 1 was flanked by *loxP* sites, which may lead to altered gene regulation on some backgrounds or under some experimental conditions.

To overcome the real or potential deficiencies with existing *Egfr* alleles, we generated a new conditional allele of *Egfr* by flanking exon 3 with *loxP* sites (Fig. 1a). Deletion of exon 3 introduces a frameshift resulting in two stop codons in exon 4 and early termination of translation (Reiter *et al.,* 2001). In addition, exon 3 encodes residues 57–117, which is part of the L1 subdomain of the extracellular domain that is essential for ligand-binding (Garrett *et al.,* 2002), ensuring an inactive *Egfr* allele upon Cre-mediated recombination even if residual altered protein is produced. After electroporating TL1 embryonic stem (ES) cells from 129S6/SvEvTAC (129S6) mice, 13 targeted ES cell clones were identified out of 300 screened ES cell clones (4.3% targeting frequency). The targeted allele was identified as a 6.9 kbp *Pfl* F1 fragment by Southern blot analysis as opposed to the wildtype allele that produced a 9.4 kbp fragment. One of the clones was found to have lost the third *loxP* site as determined by PCR analysis, indicating that in this clone homologous recombination occurred in the region between the second and the third *loxP* sites. A second clone contained

a correctly targeted allele but the other allele differed from the predicted wildtype allele (data not shown). The remaining 11 ES cell clones were correctly targeted.

On the basis of the colony morphology and karyotype analysis, two clones were chosen to remove the *Neo* cassette by recombination between the first and the second *loxP* sites using transient Cre expression. This generated the conditional $Egfr^{tm1Dwt}$ allele (also called $Egfr^{f)}$ that only has the two *loxP* sites franking exon 3 remaining in the *Egfr* locus. To verify that the remaining *loxP* sites do not perturb *Egfr* expression, RT-PCR analysis was performed to show that $Egfr^{f}$ transcripts are indistinguishable from those of the wildtype allele (Fig. 1b). ES cells containing $Egfr^{f}$ were used to produce chimeras by injection into C57BL/6J (B6) blastocysts. Ten male chimeras were obtained, five of which had high coat color chimerism (85–100%). These were mated with wildtype B6 and 129S6 females, which produced agouti pups containing the $Egfr^{f}$ allele as determined by PCR.

The activity of the $Egfr^{f}$ allele was tested by crossing $Egfr^{f/+}$ mice with mice carrying the $Egfr^{tm1Mag}$ null allele. Progeny with $Egfr^{f/tm1Mag}$ and $Egfr^{+/tmIMag}$ genotypes were born in expected Mendelian ratios (Fig. 2a). $Egf/^{tm1Mag}$ mice have normal coat hair (Fig. 2b), body weights (Fig. 2c), and fertility (data not shown). Statistically, there was no significant difference between genotypes. These results demonstrate that the $Egfr^{f}$ allele produces EGFR with wildtype activity in vivo.

After Cre-mediated excision of exon 3, the $Egfr^{\Delta}$ allele is produced and is predicted to act like a null allele. To test this prediction, mice carrying the $Egfr^{\Delta}$ allele were produced by crossing $Egfr^{f/f}$ mice with mice carrying the *EIIa-Cre* transgene (Fig. 3a); the *EIIa* promoter expresses *Cre* during preimplantation development (Lakso *et al.,* 1996). At mid-gestation, placentas from $Egfr^{\Delta/\Delta}$, *EIIa-Cre* embryos were grossly smaller in size than those of their wildtype littermates (Fig. 3b,c). Histological analysis showed that the spongiotrophoblast and labyrinth layers were affected similarly to that reported for mice homozygous for the $Egfr^{tmlMag}$ null allele (Dackor *et al.,* 2007; Strunk *et al.,* 2004; Threadgill *et al.,* 1995). In the spongiotrophoblast layer, the numbers of trophoblast and glycogen cells were moderately to severely reduced, with few spongiotrophoblast cells remaining in some $Egfr^{\Delta/\Delta}$, *EIIa-Cre* placentas. The labyrinth layer in the mutant placentas had a disorganized architecture, which was also reduced in size. Western blot analysis for EGFR revealed total loss of EGFR in placentas from $Egfr^{\Delta/\Delta}$, *EIIa-Cre* embryos (Fig. 3d). Taken together, these results demonstrate that the $Egfr^{\Delta}$ allele functions identically to an *Egfr* null allele.

Since most uses of the $Egfr^{f}$ allele will be for tissue or temporal ablation of EGFR activity, $Egfr^{f/f}$ mice were crossed with *K14-Cre* transgenic mice that express Cre in the developing skin (Dassule *et al.,* 2000). $Egfr^{f/f}$, *Kl4-Cre* mice were obtained at Mendelian ratios and were viable and fertile (data not shown). However, unlike their wildtype littermates that had normal, straight coat hair, $Egfr^{f/f}$, *Kl4-Cre* mice displayed wavy coat hair similar to the phenotype of $Egfr^{wa2/wa2}$ mice (Fig. 4a; Keeler; 1935); as with *EIIa-Cre,* the $Egfr^{\Delta}$ allele was efficiently generated in the skin of $Egfr^{f/f}$, *K14-Cre* mice (Fig. 4b). The phenotype of *Egfr* nullizygous mice is absence of coat hair due to severely disrupted hair follicle morphogenesis, suggesting that deletion of *Egfr* in the epithelium alone is insufficient to recapitulate the null phenotype. Rather, these results suggest that disruption of hair

morphogenesis seen in *Egfr* nullizygous mice is caused by the combined loss of EGFR in the epithelium and stroma of the skin. This result indicates that the $Egfr^{f}$ allele undergoes tissue-specific Cre-mediated excision.

The development and validation of the conditional $Egfr^{tm1Dwt}$ allele reported here will be a valuable resource for investigating the role of EGFR in vivo, particularly at time points after mice constitutionally lacking EGFR die. The *Egfr* allele can be inactivated spatially and temporally, overcoming the limitations caused by embryonic pre- or early postnatal lethality of mice lacking EGFR. Since conditional alleles have been generated for all four *Erbb* genes (Crone *et al.,* 2002; Long *et al.,* 2003; Qu *et al.,* 2006), it is now possible to contemplate generation of combinatorial tissue-specific knockouts to elucidate the role for specific ERBB combinations.

METHODS

Generation of the $Egfr^{tm1Dwt}$ Conditional Null Allele

BAC clone 158K10 containing the *Egfr* genomic locus was digested with *Eco* R1 to isolate DNA fragments of 6.0 kbp and 12.8 kbp surrounding exon 3 (Reiter *et al.,* 2001). The 6.0 kbp *Eco* R1 fragment was further digested with *Nhe* 1 to produce a 4.3 kbp *Eco R1-Nhe* 1 fragment for the 5′ homology arm that was inserted upstream of a *loxP-Neo-loxP* cassette to generate 5′-*loxP-Neo-loxP.* The remaining 1.7 kbp *Nhe 1-Eco* R1 fragment was ligated with a 7.5 kbp *Eco R1-Xma* 1 fragment isolated from the 12.8 kbp *Eco* R1 fragment after digestion with *Xma* 1. After subcloning the 92 kbp *Nhe* 1-*Xma* 1 fragment, a *loxP* site was inserted into the *Spe* I site within intron 3 before cloning the fragment downstream of the *5′-loxP-Neo-loxP* cassette to form the targeting vector (Fig. 1a).

The targeting vector was electroporated into TL1 ES cells (derived from 129S6/SvEvTAC mice and provided by Dr. Brigid Hogan, Duke University) as previously described (Threadgill *et al.,* 1995). After selection with G418, resistant ES cell clones were digested with *Pfl* F1 and subjected to agarose gel electrophoresis before placing the gel in denaturation solution (0.4M NaOH, 3M NaCl). The restriction fragments were then Southern blotted by capillary action to a nylon membrane (Nytran Supercharge, Schleicher & Schuell, Keene, NH). After UV cross-linking, a 1.3 kbp ^{32}P-labeled probe corresponding to a region upstream of the 5′ homology arm was hybridized to the membrane. The membrane was washed and exposed to X-ray film to reveal bands of 6.9 kbp *Pfl* F1 for a correctly targeted allele and 9.4 kbp for the wildtype allele. Additional confirmation of putatively targeted clones was performed using Southern blots with *Eco* N1 and *Nde* 1 digested ES cell DNA. A correctly targeted allele produced bands of 10.9 kbp and 11.2 kbp, respectively, while the wildtype allele produced bands of 8.8 kbp and 91 kbp, respectively.

Two correctly targeted ES cell clones were used by the University of North Carolina Animal Models Core for injection into C57BL/6J blastocysts and re-implantation into surrogate dams. After weaning, male chimeras were tested for germline transmission by mating to C57BL/6J females. Tail DNA samples from agouti offspring were genotyped for the $Egfr^{tm1Dwt}$ allele (also called $Egfr^{f}$) by PCR. Conditions were 35 cycles (30 s at 94°C, 1 min at 60°C and 1 min at 72°C) with Taq DNA polymerase (Qiagen,

Valencia, CA). The primers were lox3s 5′-CTTTGGA GAACCTGCAGATC-3′ and lox3as 5′-CTGCTACTGGCT CAAGTTTC-3′. A 375 bp PCR product is generated from the *Egfr*^{f} allele and a 320 bp PCR product from the wildtype allele. The Cre-reduced Egfr$^{\Delta}$ allele was detected by PCR using 40 cycles (30 s at 94°C, 20 s at 60°C and 20 s at 72°C) with primers Delta-3 5′-CTCAGCCAGAT GATGTTGAC-3′ and Delta-4 5′-CCTCGTCTGTGGAA GAACTA-3′. A 129 bp PCR fragment is amplified from the Egfr$^{\Delta}$ allele. The *Egfr* null allele, *Egfr*^{tm1Mag} (Threadgill *et al.,* 1995), was detected by amplifying DNA for 40 cycles (30 s at 94°C, 30 s at 55°C and 1 min at 72°C). The primers were EGFR common 5′-GCCCTGCCTTTCCCACCATA-3′ and EGFR knockout 5′-AACGTCGTGACTGGGAAAAC-3′. A 450 bp PCR product is detected from the *Egfr*^{tm1Mag} allele.

RT-PCR

Total RNA were extracted from targeted and wildtype ES cell clones using TRizol reagent (Invitrogen) according to the manufacturer's instructions. One microgram of RNA was used as template for synthesis of cDNA using random primers (Gibco-BRL, Carlsbad, CA) and SuperScript II RT (Gibco-BRL) in a total reaction volume of 20 μl. The cDNA was used as template for PCR amplification of exons 2–4 using 35 cycles (30 s at 93°C, 1 min at 58°C and 1 min at 72°C) with primers RNA2 5′-TGCCAAGGCACAAG-TAACAG-3′ and RNA4 5′-GCTCGGATGGCTCTGTAAGT-3′. The predicted wildtype band is 465 bp.

Mouse Crosses

Mice carrying the *Egfr*^{f} allele were crossed with *Egfr*$^{+/tm1Mag}$ mice to generate *Egfr*$^{f/tm1Mag}$ and *Egfr*$^{+/tm1Mag}$ F1 mice that were used as experimental and control groups, respectively. Body weights were measured and compared by gender. To evaluate whether *Egfr*$^{\Delta/\Delta}$ functions as a null allele, *Egfr*$^{f/f}$ mice were crossed with *EIIa-Cre* mice to generate *Egfr*$^{\Delta/+}$, *EIIa-Cre* progeny, which were then crossed with *Egfr*$^{f/f}$ mice to generate *Egfr*$^{\Delta/+}$*, EIIa-Cre, and Egfr*$^{\Delta/\Delta}$*, EIIa-Cre* mice. To determine whether the *Egfr*^{f} allele can function as a tissue-specific conditional allele, *Egfr*$^{f/f}$ mice were crossed to *K14-Cre* mice to obtain *Egfr*$^{f/+}$*, K14-Cre* progeny, which were then crossed to *Egfr*$^{f/f}$ mice to generate *Egfr*$^{f/f}$, *K14-Cre,* and *Egfr*$^{f/f}$, *K14-Cre* offspring that were used as experimental and control groups, respectively. Coat phenotypes were examined by visual inspection and histology.

Egfr$^{f/+}$ and *Egfr*$^{f/f}$ mice were on a 129,B6 mixed background, while *Egfr*$^{+/tm1Mag}$ were on a 129S1/SvImJ background (Threadgill *et al.,* 1995). *Ella-Cre* mice, on a B6,FVB mixed background, were a gift from Dr. Mark Majesky (UNC) and *K14-Cre* mice, on a FVB background, were obtained from The Jackson Laboratory. Genotyping for the *Ella-* and *K14-Cre* transgenes from genomic DNA was done by PCR using 38 cycles (30 s at 94°C, 1 min at 56°C and 1 min at 72°C) with primers CRE-1 5′-GTGAT GAGGTTCGCAAGAAC-3′ and CRE-2 5′-AGCATTGCTGT CACTTGGTC-3′. A 278 bp PCR fragment is generated from the *Cre* transgenes.

Histological Analysis

Placentas from 12.5 days post coitus (dpc) embryos were isolated and fixed in 10% neutral buffered formalin solution (NBF) for 24 h. After fixation, the samples were dehydrated

using a graded alcohol series, 70% (1 h), 85% (1 h), 95% (1 h), 100% (1 h), and 100% (1 h), followed by two washes in xylene for 1 h for each. Finally, the samples were infiltrated with paraffin at 60°C twice for 1 h each before embedding and cutting 7-μm sections. Sections were stained with hematoxylin and eosin and after mounting, examined under light microscopy as previously (Strunk *et al.,* 2004; Threadgill *et al.,* 1995).

Western Blot Analysis

Embryos and yolk sacs isolated at 10.5 dpc from crosses containing *Egfr*$^{+/\Delta}$, *EIIa-Cre,* and *Egfr*$^{f/f}$ were homogenized in 10 ml tissue lysis buffer/g of tissue. The lysis buffer contained 20 mM HEPES, pH 7.4, 150 mM MaCl, 2 mM ethylenediamine tetraaceticacid (EDTA), pH 8.0, 2 mM ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA), pH 8.0, 1% Triton X-100, and 10% glycerol adjusted to final pH 7.4 before adding protease inhibitors [1 mM phenyl sulfonyl fluoride (PMSF) in isopropanol, 10 μg/ml leupeptin and 10 μg/ml aprotinin] and phosphatase inhibitors [1 mM sodium orthovanadate (Na3VO4) and 1 mM NaF].

Twenty-five micrograms of total protein from each homogenized lysate were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), before transferring to a polyvinylidene fluoride (PVDF) membrane. The membrane was placed in blocking solution containing 5% milk in TBST (150 mM NaCl, 10 mM Tris-HCl, pH 8.0, 0.1% Tween-20), and then probed with a sheep anti-EGFR polyclonal IgG primary antibody (Upstate, Billerica, MA) in blocking solution (1:500). The secondary antibody was rabbit anti-sheep polyclonal IgG conjugated with horseradish peroxidase (HRP) (1:10,000). To visualize antibody binding, the membrane was incubated with enhanced chemiluminescent (ECL) substrate (Pierce, Rockford, IL) for 1–5 min and exposed to X-ray film. The amount of β-tubulin in each sample was used as an internal control. β-tubulin was detected using a mouse primary anti-β-tubulin polyclonal IgG antibody (1:1,000), with a secondary goat anti-mouse polyclonal IgG antibody conjugated with HRP (1:15,000).

Statistical Analysis

An unpaired *t*-test was used for all comparisons.

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LITERATURE CITED

Chen B, Bronson RT, Klaman LD, Hampton TG, Wang JF, Green PJ, Magnuson T, Douglas PS, Morgan JP, Neel BG. 2000. Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. Nat Genet 24:296–299. [PubMed: 10700187]

Crone SA, Zhao YY, Fan L, Gu Y Minamisawa S, Liu Y, Peterson KL, Chen J, Kahn R, Condorelli G, Ross J, Chien KR, Lee KF. 2002. ErbB2 is essential in the prevention of dilated cardiomyopathy. Nat Med 8:459–465. [PubMed: 11984589]

Dackor J, Strunk KE, Wehmeyer MM, Threadgill DW 2007. Altered trophoblast proliferation is insufficient to account for placental dysfunction in Egfr null embryos. Placenta 28:1211–1218. [PubMed: 17822758]

Dassule HR, Lewis P, Bei M, Maas R, McMahon AP 2000. Sonic hedgehog regulates growth and morphogenesis of the tooth. Development 127:4775–4785. [PubMed: 11044393]

Fowler KJ, Walker F, Alexander W, Hibbs ML, Nice EC, Bohmer RM, Mann GB, Thumwood C, Maglitto R, Danks JA, Chetty R, Burgess AW, Dunn AR. 1995. A mutation in the epidermal growth factor receptor in waved-2 mice has a profound effect on receptor biochemistry that results in impaired lactation. Proc Natl Acad Sci USA 92:1465–1469. [PubMed: 7533293]

Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Zhu HJ, Walker F, Frenkel MJ, Hoyne PA, Jorissen RN, Nice EC, Burgess AW, Ward CW. 2002. Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha. Cell 110:763–773. [PubMed: 12297049]

Keeler CE. 1935. A second rexoid coat character in the house mouse. J Hered 26:189–191.

Lakso M, Pichel JG, Gorman JR, Sauer B, Okamoto Y, Lee E, Alt FW, Westphal H. 1996. Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. Proc Natl Acad Sci USA 93:5860–5865. [PubMed: 8650183]

Long W, Wagner KU, Lloyd KC, Binart N, Shillingford JM, Hennighausen L, Jones FE. 2003. Impaired differentiation and lactational failure of Erbb4-deficient mammary glands identify ERBB4 as an obligate mediator of STAT5. Development 130:5257–5268. [PubMed: 12954715]

Luetteke NC, Phillips HK, Qiu TH, Copeland NG, Earp HS, Jenkins NA, Lee DC. 1994. The mouse waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase. Genes Dev 8:399–413. [PubMed: 8125255]

Mak KK, Chan SY 2003. Epidermal growth factor as a biologic switch in hair growth cycle. J Biol Chem 278:26120–26126. [PubMed: 12714603]

Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, Werb Z, Derynck R. 1995. Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. Nature 376:337–341. [PubMed: 7630400]

Miettinen PJ, Chin JR, Shum L, Slavkin HC, Shuler CF, Derynck R, Werb Z. 1999. Epidermal growth factor receptor function is necessary for normal craniofacial development and palate closure. Nat Genet 22:69–73. [PubMed: 10319864]

Natarajan A, Wagner B, Sibilia M. 2007. The EGF receptor is required for efficient liver regeneration. Proc Natl Acad Sci USA 104:17081–17086. [PubMed: 17940036]

Qu S, Rinehart C, Wu HH, Wang SE, Carter B, Xin H, Kotlikoff M, Arteaga CL. 2006. Gene targeting of ErbB3 using a Cre-mediated unidirectional DNA inversion strategy. Genesis 44:477–486. [PubMed: 16991114]

Reiter JL, Threadgill DW, Eley GD, Strunk KE, Danielsen AJ, Sinclair CS, Pearsall RS, Green PJ, Yee D, Lampland AL, Balasubramaniam S, Crossley TD, Magnuson TR, James CD, Maihle NJ. 2001. Comparative genomic sequence analysis and isolation of human and mouse alternative EGFR transcripts encoding truncated receptor isoforms. Genomics 71:1–20. [PubMed: 11161793]

Sibilia M, Wagner B, Hoebertz A, Elliott C, Marino S, Jochum W, Wagner EF. 2003. Mice humanised for the EGF receptor display hypomorphic phenotypes in skin, bone and heart. Development 130:4515–4525. [PubMed: 12925580]

Sibilia M, Wagner EE 1995. Strain-dependent epithelial defects in mice lacking the EGE receptor. Science 269:234–238. [PubMed: 7618085]

Strunk KE, Amann V, Threadgill DW 2004. Phenotypic variation resulting from a deficiency of epidermal growth factor receptor in mice is caused by extensive genetic heterogeneity that can be genetically and molecularly partitioned. Genetics 167:1821–1832. [PubMed: 15342520]

Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, LaMantia C, Mourton T, Herrup K, Harris RC, Barnard JA, Yuspa SH, Coffey RJ, Magnuson T. 1995. Targeted disruption of mouse EGE receptor: Effect of genetic background on mutant phenotype. Science 269:230–234. [PubMed: 7618084]

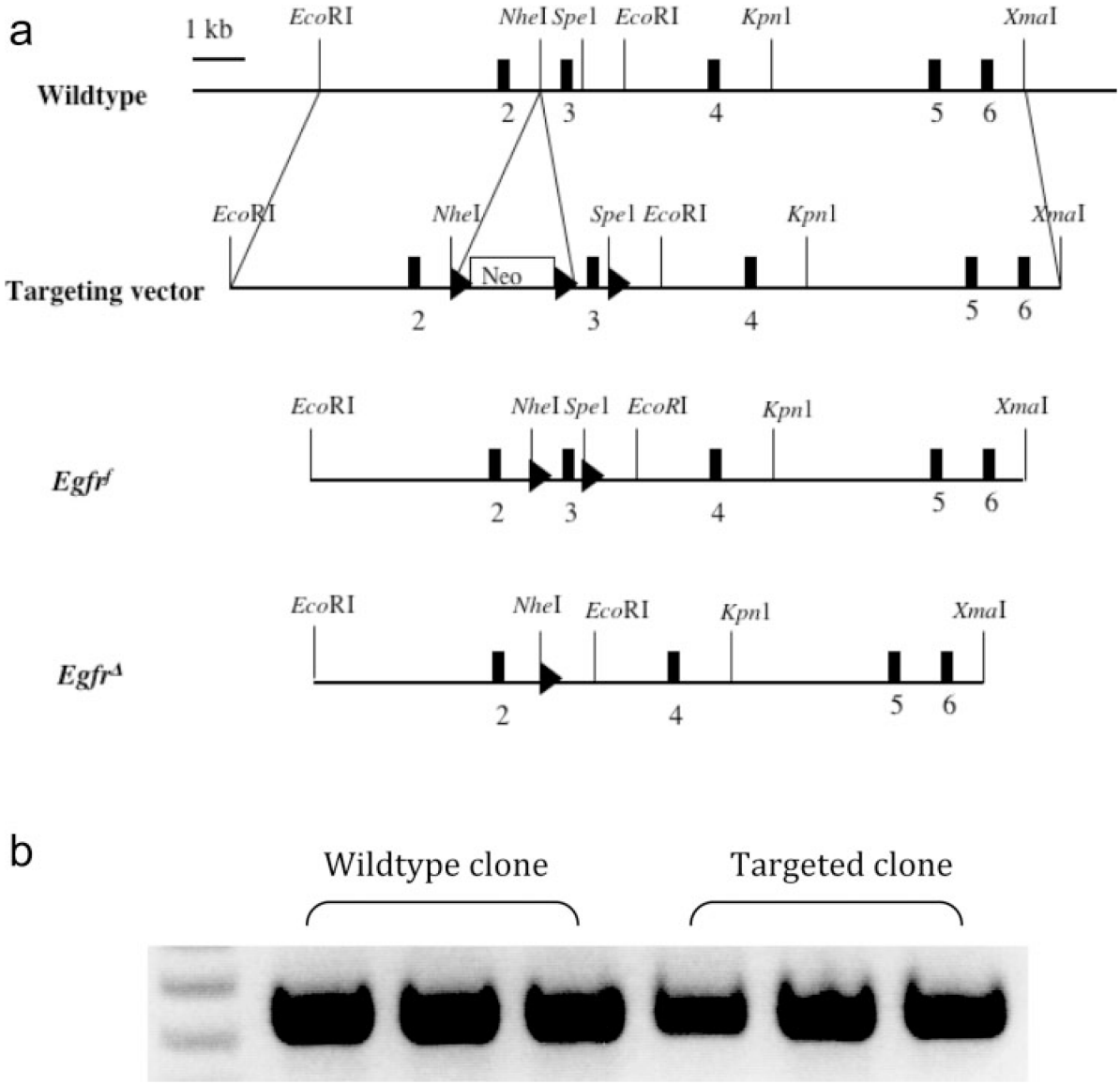

FIG. 1.

Structure of wildtype and engineered *Egfr* alleles. (**a**) Targeting approach to flank exon 3 with *loxP* sites and generation of the $Egfr^{f}$ allele by Cre-mediated deletion of the *Neo* cassette. The $Egfr^{\Delta}$ allele is generated by Cre-mediated deletion of exon 3 from the $Egfr^{f}$ allele. (**b**) RT-PCR analysis of RNA corresponding to exons 2–4 in $Egfr^{f/+}$ ES cells.

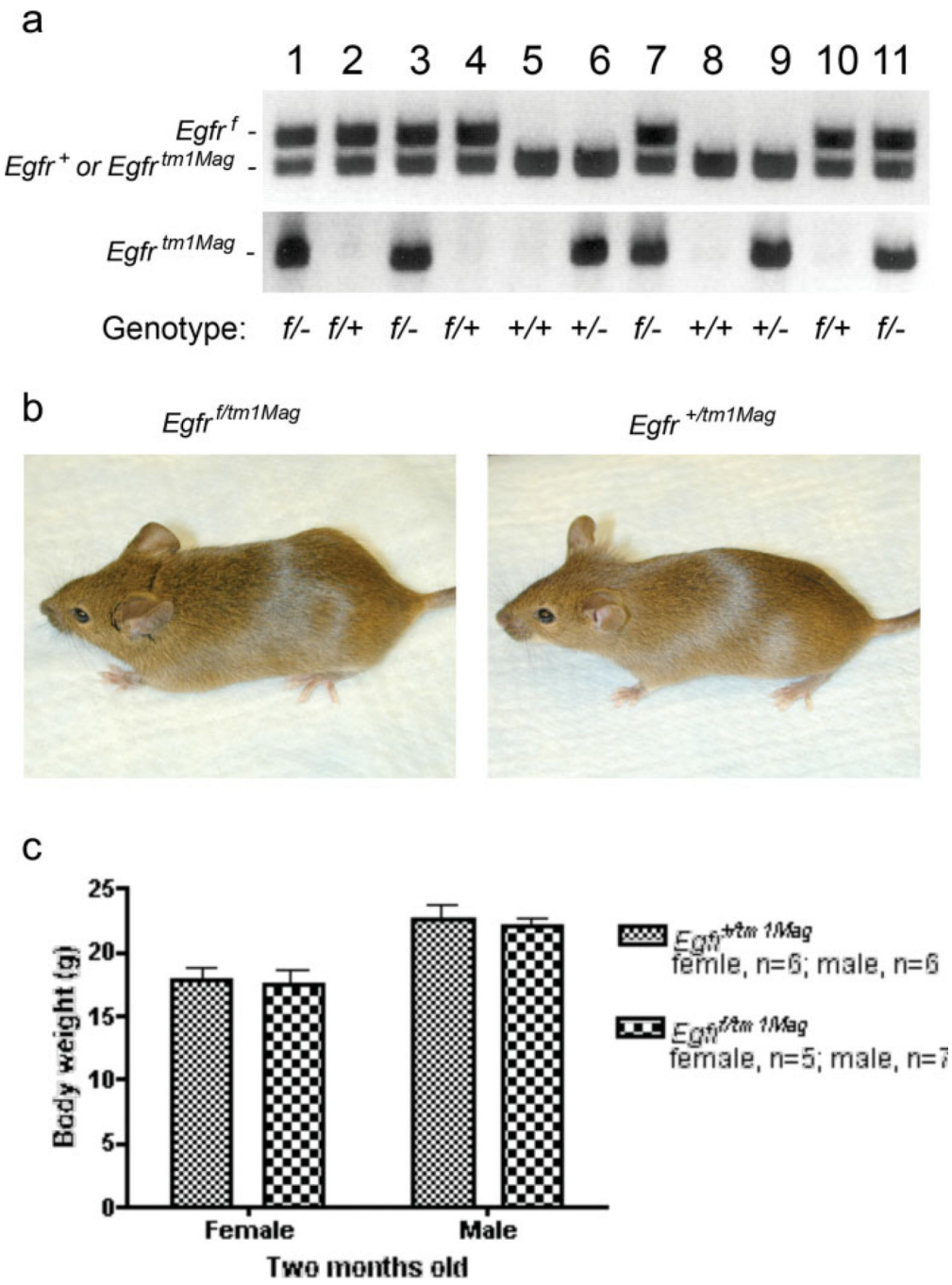

FIG. 2.

Evaluation of *Egfr*^{f} function. (**a**) *Egfr*$^{f/tm1Mag}$ mouse (left) that is indistinguishable from an *Egfr*$^{+/tm1Mag}$ mouse (right). (**b**) Genotypes of progeny from a cross between *Egfr*$^{+/tm1Mag}$ and *Egfr*$^{f/+}$ mice. (**c**) Comparison of body weights between *Egfr*$^{f/tm1Mag}$ and *Egfr*$^{+/tm1Mag}$ mice.

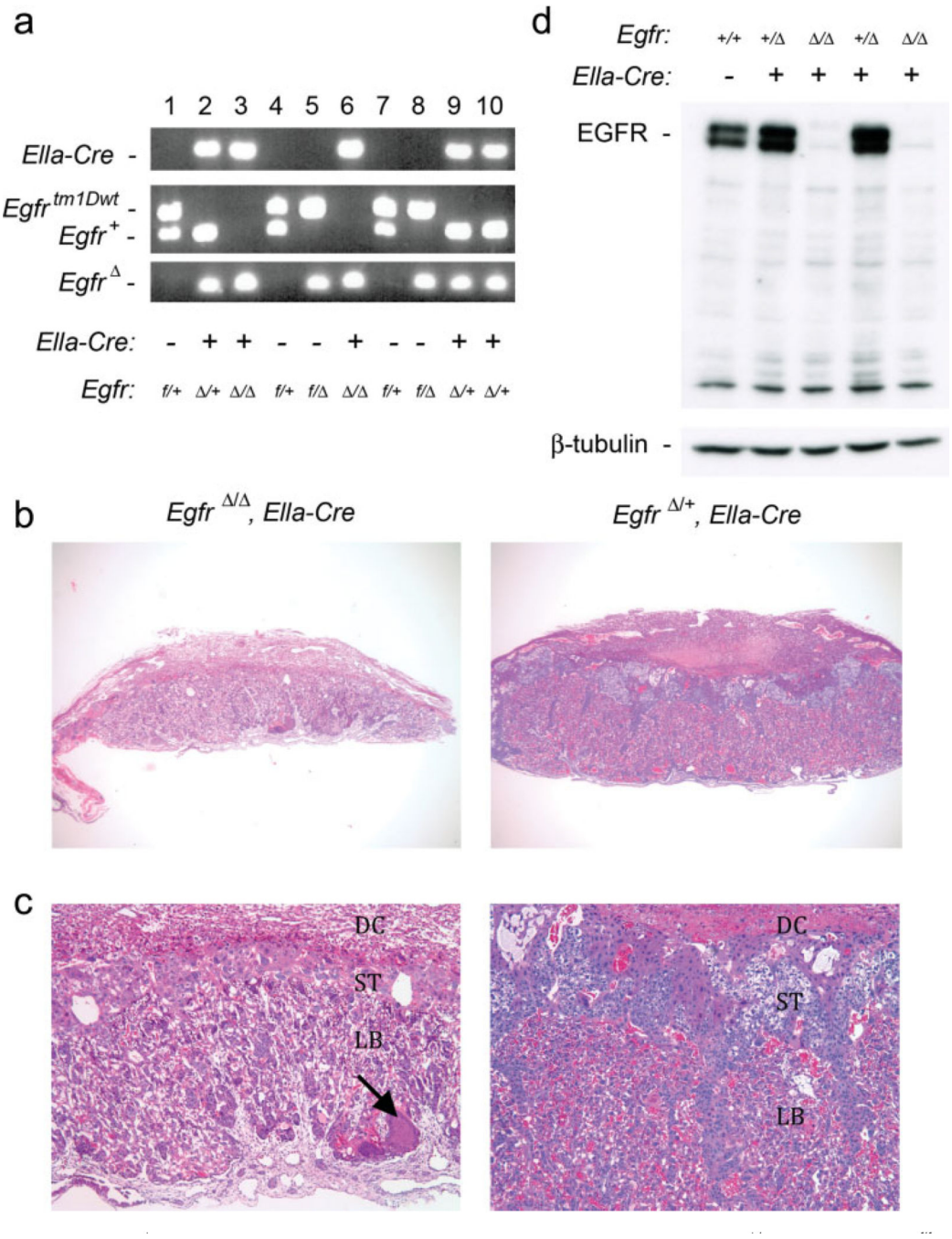

FIG. 3.

Analysis of Egfr$^{\Delta}$ allele activity. (**a**) Genotypes of 12.5 dpc embryos from crosses between Egfr$^{\Delta/+}$, *Ella-Cre,* and *Egfr*$^{f/f}$ mice. (**b**) H&E staining of Egfr$^{\Delta/\Delta}$, *Ella-Cre* placenta (left, ×30) with smaller size than wildtype placenta (right, ×30). (**c**) H&E staining of Egfr$^{\Delta/\Delta}$, *Ella-Cre* placenta (left, ×100) showing reduced spongiotrophoblast layer and disorganized labyrinth layer with amorphous material (arrow) compared to wildtype placenta (right, ×100). (**d**) Western blot analysis showing loss of EGFR in Egfr$^{\Delta/\Delta}$, *Ella-Cre* embryos at 10.5 dpc. DC, decidua; ST, spongiotrophoblast layer; LB, labyrinth layer.

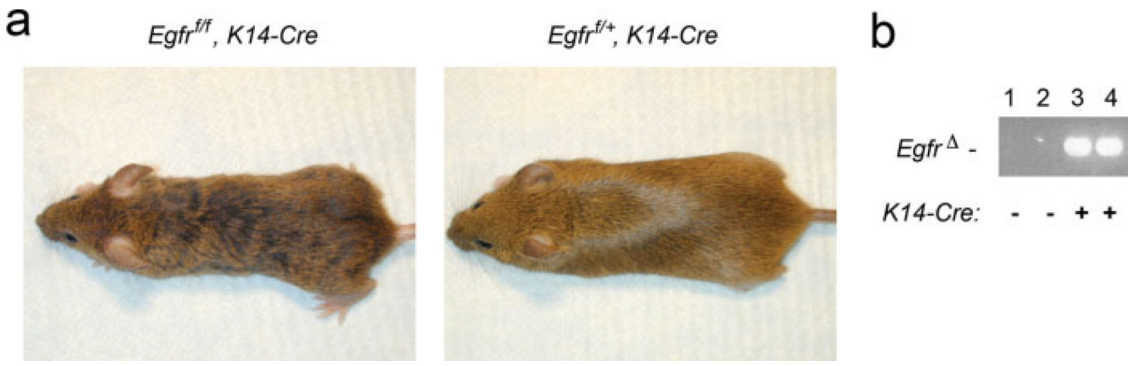

FIG. 4.

Tissue-specific deletion of *Egfr.* (**a**) *Egfr$^{f/f}$, K14-Cre* mouse (left) at 3 months of age displaying a wavy coat compared with the straight coat of wildtype mouse (right) at the same age. (**b**) PCR products using primers specific for Egfr$^{\Delta}$ allele showing generation in skin from *K14-Cre* mice.