Supporting Information

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SI Methods

Construction of the *Tgfb1* targeting vector. A TGF-β1 probe corresponding to the exon 1 was generated by PCR using pB2–3 mouse genomic clone (1) as template and used to screen a mouse 129SvEv genomic library (gift of A. Joyner). Positive clones were isolated, amplified, and analyzed by restriction digestion and Southern blotting. Several fragments of the 5′ region containing exon 1 were subcloned into pBluescript SK and partially sequenced. A point mutation (TCC) in the triplet that codes the cysteine 33 (TGC) was generated using a TGF-β1 genomic DNA clone and the megaprimer mutagenesis protocol (2). The mutation results in a cysteine-to-serine change. The PCR product was digested with PstI and SacI, and the PstI–SacI fragment was used to replace the corresponding WT fragment in the genomic DNA clone containing TGF-β1 exon 1 (supporting information (*SI*) Fig. S1*A*).

To generate the targeting vector, a 1.8-kb HindIII-BamHI genomic fragment 5′ from exon 1 was cloned into pBluescript. The BamHI fragment carrying the mutation in exon 1 was subsequently added to this construct to generate the 5′arm (4.3 kb). Next, a 4-kb genomic BamHI–BgII fragment containing exon 2, used as the 3′ arm, was subcloned into the BamHI site of the vector pKS10xPNT, 5′ to the *neo*^{r} gene. Finally, the 5′ arm was excised from pBluescript using the vector's SalI sites and cloned into the SalI site 3′ to the *neo*^{r} gene (Fig. S1*A*) in the 5′ arm-pKS10xPNT construct.

Generation of *Tgfb1*$^{C33S/C33S}$ Mice. ES cells (129/SvEv) were electroporated with the linearized vector, cultured, and selection with G418 and gancylovir performed (3). Resistant ES cell clones were screened for homologous recombination by Southern analysis, using DNA fragments, P1 and P2, as probes (Fig. S1*A*). Seven ES cell lines with normal karyotypes and the correct homologous replacement of the wild-type *Tgfb1* exon 1 were isolated. The presence of the mutated allele was confirmed by sequencing of the exon 1 PCR amplification product. Three ES clones were used to generate chimeric mice using C57BL/6J blastocysts. F_0 chimeric males from two different *Tgfb1*$^{+/C33S}$ ES cell lines were crossed with C57BL/6J or 129 females and two lines (K and J) were maintained by brother–sister matings. The F_1 offspring were genotyped both by Southern analysis and allele-specific PCR, and *Tgfb1*$^{+/C33S}$ mice were crossed to obtain homozygous mutants. The phenotypes of these two lines were identical in all assays described in this paper.

The neo^r cassette, inserted in intron 1, was deleted by crossing *Tgfb1*$^{+/C33S}$ mice with mice that express Cre recombinase in all tissues (3). Animals from *Tgfb1*$^{+/C33S}$ × *Tgfb1*$^{+/C33S}$ crosses were genotyped by PCR using primers from intron 1, surrounding the integrated lox site, or primers flanking the mutated sequence in exon 1 and the restriction enzyme BfuAI that cuts only the *Tgfb1*$^{+/+}$ allele (Fig. S1*B*).

All animal protocols were done with the approval of the NYU Langone Medical Center Animal Use and Care Committee.

1. Kulkarni AB, *et al.* (1993) Transforming growth factor ß1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci USA* 90:770–774.
2. Picard V, Ersdal-Badju E, Lu A, Bock SC (1994) A rapid and efficient one-tube PCR-based mutagenesis technique using Pfu DNA polymerase. *Nucleic Acids Res* 22:2587–2591.
3. Joyner AL (1995) *Gene Targeting, A Practical Approach* (Oxford Univ Press, London).

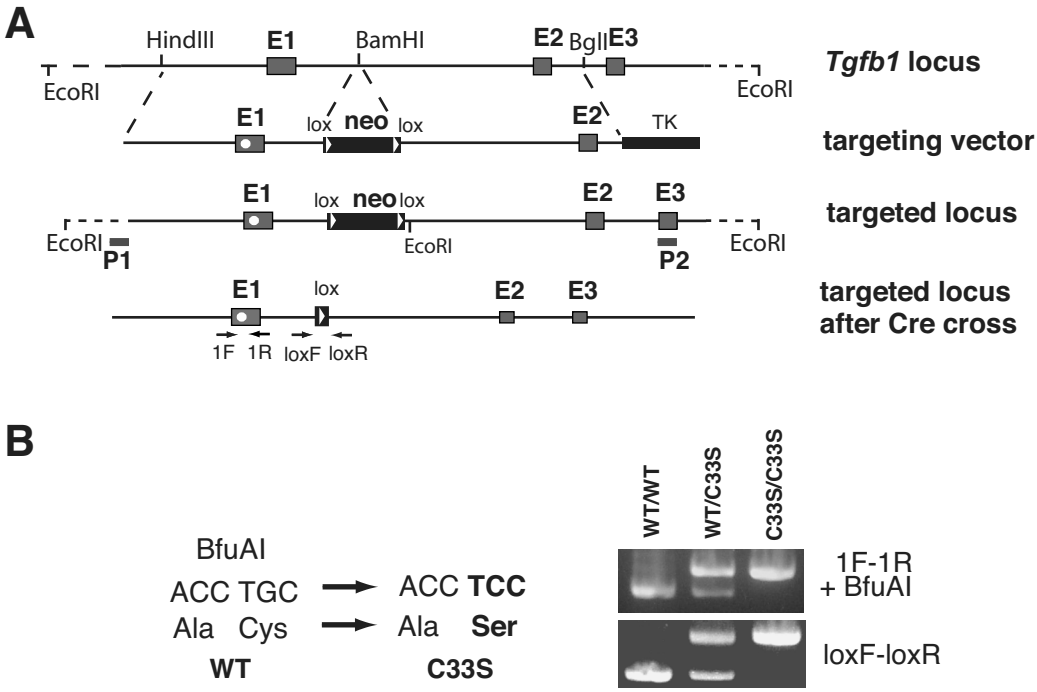

Fig. S1. Creation of *Tgfb1*$^{C33S/C33S}$ mice. (*A*) *Tgfb1* gene targeting strategy to introduce the *Tgfb1*^{C33S} mutation. The C33S mutation (red dot) was introduced into exon 1 (E1) within a 4.3-kb HindIII–BamHI genomic DNA fragment that was used as the 5′ region of homology in the targeting vector. A 4.0-kb BamHI–BglI fragment containing exon 2 (E2) was used as the 3′ region of homology. The targeting vector contained a neo resistance gene expression cassette (neo) required for positive selection and a TK gene cassette for negative selection. Diagnostic restriction digestion by EcoRI and Southern hybridization with the probes P1 and P2 were used for screening of ES clones for homologous recombination with the targeting vector (data not shown). A 20-kb EcoRI fragment encompassing both P1 and P2 corresponded to the *Tgfb1*$^{+}$ allele; the *Tgfb1*^{C33S}-neo allele gave a hybridization signal corresponding to a 14.2-kb fragment with the probe P1 and a 7.2-kb fragment with P2. (*B*) PCR analysis of *Tgfb1*$^{+/+}$, *Tgfb1*$^{+/C33S}$, and *Tgfb1*$^{C33S/C33S}$ mice using either the loss of the BfuA1 restriction site (Upper) or the loss of the lox fragment (Lower) to distinguish different genotypes.

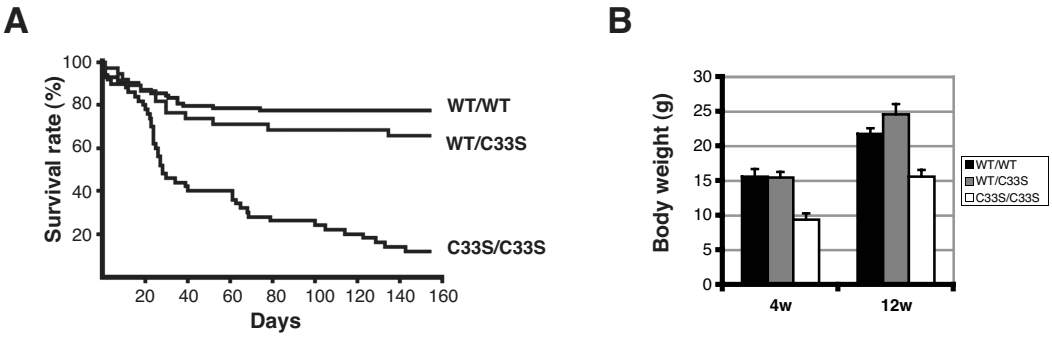

Fig. S2. Survival curves and weights for $Tgfb1^{+/+}$ and $Tgfb1^{C33S/C33S}$ mice. (*A*) Survival curves. The percent survival of $Tgfb1^{+/+}$, $Tgfb1^{+/C33S}$, and $Tgfb1^{C33S/C33S}$ mice is plotted as a function of time: $Tgfb1^{+/+}$ ($n = 84$), $Tgfb1^{+/C33S}$ ($n = 38$), and $Tgfb1^{C33S/C33S}$ mice ($n = 50$). (*B*) Body weights of $Tgfb1^{+/+}$ and $Tgfb1^{C33S/C33S}$ mice at 4 and 12 weeks after birth. At 4 weeks, six mice, three males and three females in each group, were weighed. At 12 weeks, eight mice, three females and five males in each group, were weighed. Animals at each of the two time points and within each group were not from the same litters. Values are presented as SEM.

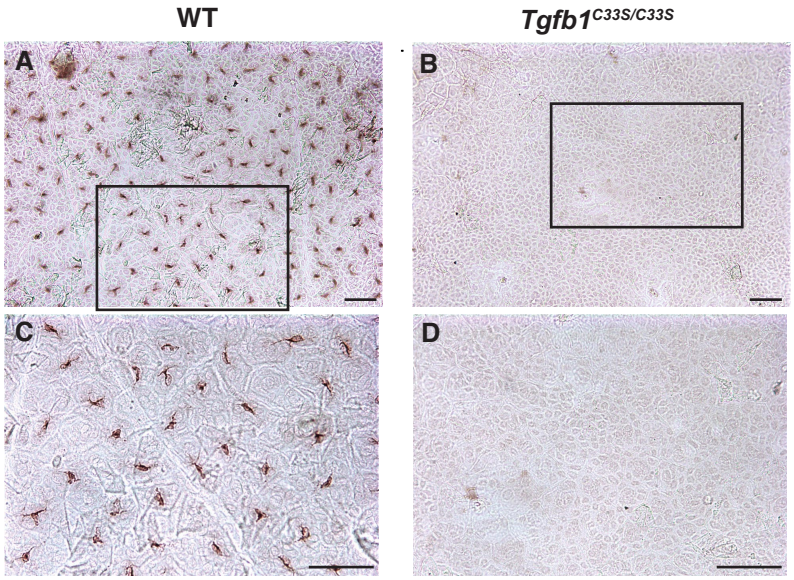

Fig. S3. Langerhans cells in the epidermis of *Tgfb1*$^{+/+}$ and *Tgfb1*$^{C33S/C33S}$ mice. Epidermal sheets were prepared from *Tgfb1*$^{+/+}$ and mutant mice at 21 weeks of age. The skin samples were stained for LC using anti-I-A/I-E antibodies. Numerous LC are present in *Tgfb1*$^{+/+}$ skin, but positively stained cells are absent in *Tgfb1*$^{C33S/C33S}$ skin. (*A*) *Tgfb1*$^{+/+}$ skin. (*B*) *Tgfb1*$^{C33S/C33S}$ skin. *C* and *D* are enlargements of the boxes shown in *A* and *B*, respectively. Bar = 50 μM.

Table S1. Genotypes of *Tgf-β1*$^{C33S/C33S}$ litters

	J Line				K Line		
Genotype	Genotype	*N*	%	% exp	*N*	%	% exp
+/+	+/+	86	29.5	25	115	29.5	25
+/C33S	+/C33S	142	48.6	50	190	48.7	50
C33S/C33S	C33S/C33S	64	21.9	25	85	21.8	25

Mice from the J and K lines were genotyped at weaning by PCR as described in *Methods*.