Supporting Information

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SI Materials and Methods

Ovalbumin Sensitization and Challenge. Mice were sensitized on days 0, 7, and 14 by i.p. injection of 50 μg ovalbumin (OVA) (Sigma-Aldrich) emulsified in 1 mg aluminum potassium sulfate in a total volume of 200 μL as previously described (1). Control groups received an equal volume of aluminum potassium sulfate without OVA. Subsequently, mice anesthetized by isoflurane inhalation were challenged with 100 μg OVA in 50 μL saline, or saline alone as control by intranasal aerosol, at days 21, 22, and 23 following the first i.p. injection (Fig. 1*A*). A linear regression model was fit to the data to test whether drug and genotype had a significant effect on response (Fig. 1*B*). A second linear regression model was fit to the data (Fig. 2*C*) to test whether the genetic background was significantly associated with AHR when comparing OVA-treated NIH to OVA-treated Tgfbm2/3 mice.

Measurement of Airway Responsiveness to Acetylcholine. Twenty-four hours after the last intranasal challenge, mice were anesthetized with ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight). A tracheostomy was performed and a 20-gauge tubing adaptor was used to cannulate the trachea. Mice were then attached to a rodent ventilator and pulmonary mechanics analyzer (FlexiVent; SIRAQ) and ventilated at a tidal volume of 9 mL/kg, a frequency of 150 breaths/min and 2 cm H_2O positive end-expiratory pressure. Mice were paralyzed with pancuronium (0.1 mg/kg i.p.), given increasing doses of acetylcholine (0.1, 0.3, 1, and 3 μg/mL) administered through the tail vein to generate a dose–response curve. Measurements of airway mechanics were made continuously using the linear single compartment model.

Assessment of Pulmonary Inflammation and Mucous Production. Lungs were lavaged five times with 0.8 mL PBS. After centrifugation of total bronchoalveolar lavage (BAL) ($200 \times g$, 5 min), the supernatant was removed for cytokine analysis. After red blood cell lysis, the cell pellet was resuspended in saline, and total cells were counted using a hemocytometer. Cytospin preparations were stained with HEMA 3 stain set (Fisher Scientific), and BAL fluid cell differential percentages were determined based on light microscopic evaluation of >300 cells per slide.

OVA-Specific IgE Assay. Sera were obtained from blood collected by cardiac puncture from antigen- or vehicle-treated mice after airway responsiveness measurement. OVA-specific IgE levels were measured by ELISA using microplates coated with OVA. Diluted serum samples were added to each well, and the bound IgE was detected with biotinylated antimouse IgE (Pharmingen; R35-118). Color development was achieved using streptavidin-conjugated horseradish peroxidase (Pharmingen) followed by addition of HRP substrate (TMB; BD Biosciences Pharmingen). OD readings of samples at 450 nm were obtained and the results were expressed as OD value.

RNA Extraction and TaqMan Analysis. Lungs were lysed with a benchtop homogenizer AHS 200 (VWR) in TRIzol and RNA was isolated according to manufacturer protocol. cDNA was synthesized starting from 1 μg of RNA using MMLV reverse transcriptase and buffer from Promega with oligo-dT primer (Invitrogen) according to manufacturer protocol.

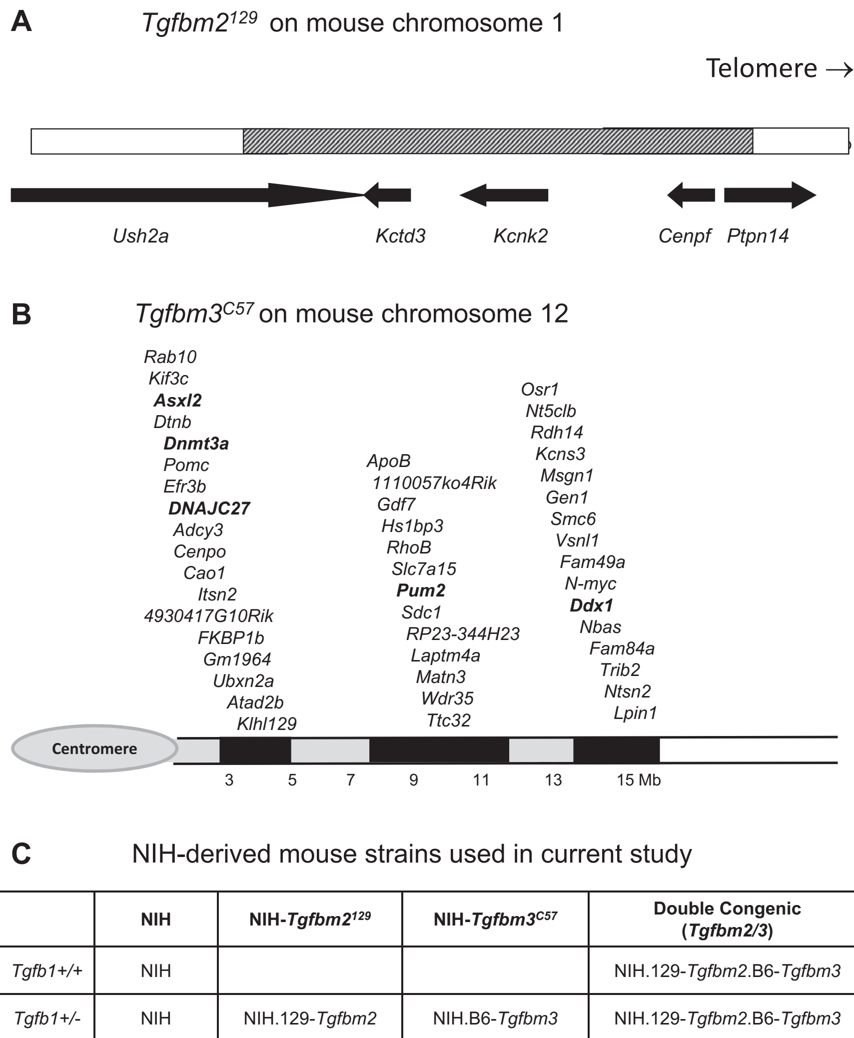
TaqMan analysis was performed on an ABI7900 using TaqMan gene expression assays from Applied Biosystems. Data were normalized to endogenous GAPDH gene expression and displayed as fold induction compared with saline-treated NIH mice. A list of TaqMan probes can be provided upon request.

MicroArray Analysis. RNA was extracted as described above and quality standard was assessed using the Agilent 2100 bioanalyzer. RNA concentration was established by NanoDrop measurement. Each microarray used RNA from three independent mouse lungs of the same mouse strain and treatment group, pooled in a 1:1:1 ratio. Microarray gene expression analysis was performed on GeneChip Mouse Gene 1.0 ST arrays from Affymetrix, using two independent pools of RNA (six mice total) for each mouse strain and treatment group.

Western Blotting Analysis. Western blot analysis on proteins from lung tissue was performed using NuPAGE Novex PAGE (Invitrogen) according to manufacturer protocol. Proteins were detected using primary antibody against phospho-Smad2 (Cell Signaling; 3101) and Smad2/3 (BD Transduction Laboratories; 610842). As internal loading control all membranes were probed with monoclonal anti–β-actin unconjugated antibody (Sigma-Aldrich; A5441). Antigen–antibody reactions were detected by enhanced chemiluminescence (ECL) (Amersham Pharmacia) and exposed to autoradiographic film.

Immunohistochemistry/Immunofluorescence. Immunofluorescence was carried out on 5-μm tissue cryofrozen sections embedded in optimal cutting temperature (OCT) compound (Tissue-Tek). The specimen was blocked in 10% (vol/vol) normal goat serum (Invitrogen) and primary antibody (α-SMA-Cy3; Sigma) was added at a 1:100 dilution in blocking solution, and staining was performed according to the antibody manufacturer's instructions. Sections were then washed three times in PBS and mounted with DAPI (ProLong Gold antifade; Invitrogen). Immunohistochemistry was carried out on 5 μm 4% PFA-fixed, paraffin-embedded tissue sections using anti–P-Smad2 (Cell Signaling) antibody. Quantification used 10 fields of view per sample. National Institutes of Health ImageJ software was used to determine positive cell counts (P-Smad staining) and α-SMA thickness.

1. Kudo M, et al. (2012) IL-17A produced by αβ T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. *Nat Med* 18:547–554.



	NIH	NIH-*Tgfbm2*^{129}	NIH-*Tgfbm3*^{C57}	Double Congenic (*Tgfbm2/3*)
Tgfb1+/+	NIH			NIH.129-*Tgfbm2*.B6-*Tgfbm3*
Tgfb1+/-	NIH	NIH.129-*Tgfbm2*	NIH.B6-*Tgfbm3*	NIH.129-*Tgfbm2*.B6-*Tgfbm3*

Fig. S1. (*A*) Murine *Tgfbm2* locus, indicating the -Mb 129 interval around *Tgfbm2* (hatched box) spanning from exons 57–60 of *Ush2a* to exons 2–6 of *Ptpn14*. (*B*) The murine Tgfbm3 locus, indicating position of the 14-Mb C57 region spanning from the centromere to the Lpin1 gene within Tgfbm3. Black box represents unique, gene-encoding DNA blocks of C57 origin; gray regions represent large stretches of repetitive DNA of C57 origin. White blocks in *A* and *B* are NIH genomic DNA, i.e., the rest of the genome. (*C*) Table indicates the genetically distinct mouse lines used in the study. Throughout the main text, NIH.129-*Tgfbm2*.C57-*Tgfbm3* mice are denoted as *Tgfbm2/3*.

Fig. S2. (*A* and *B*) Respiratory resistance was measured in response to escalating doses of Ach in *Tgfb1*$^{+/-}$ mice sensitized and challenged with OVA (O) or saline (S) in two experiments independent of those in Fig. 2. Respiratory curves of OVA-stimulated lungs are displayed for NIH versus *Tgfbm2/3* mice. Data are shown as mean ± SEM from each group (n = 10).

Fig. S3. Quantification of percentage of bronchiolar circumference invested in smooth muscle in parental NIH and *Tgfbm2/3* lungs treated with either OVA or saline. NIH ImageJ was used to determine α-SMA circumference. Measurement used five fields of view per sample (n = 5–10) and data are shown as mean ± SEM.

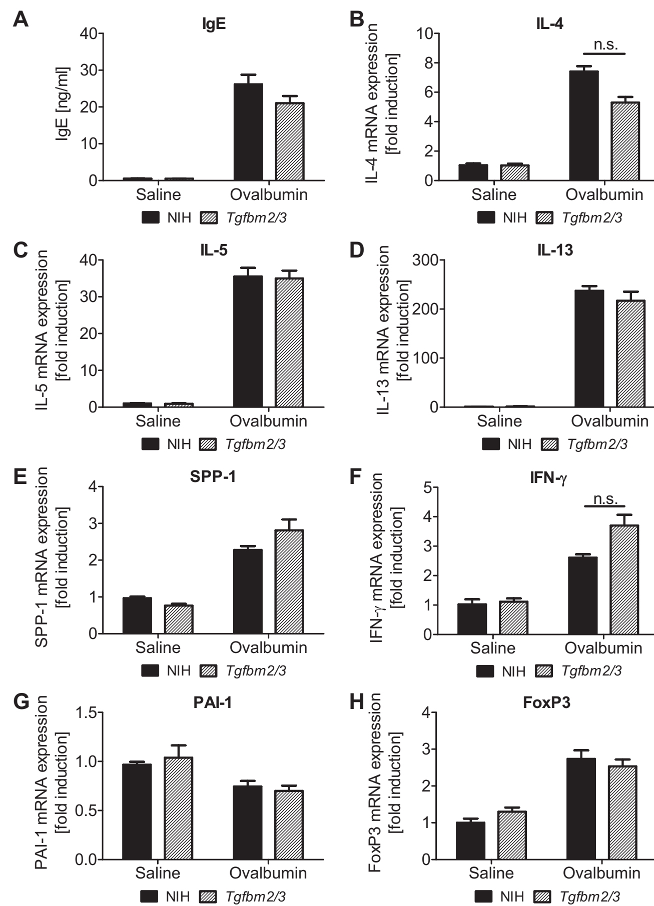


Fig. S4. (*A*) IgE levels measured by ELISA using microplates coated with OVA comparing parental NIH and *Tgfbm2/3* lungs after either saline or OVA challenge. (*B*) IL-4, (*C*) IL-5, (*D*) IL-13, (*E*) SPP1, (*F*) IFN-γ, (*G*) PAI-1, and (*H*) FoxP3 mRNA expression analysis of parental NIH and *Tgfbm2/3* lungs after either saline or OVA challenge. Results are represented compared with *Gapdh* Ct value. Data are shown as mean ± SEM from each group ($n = 8$). *P* values were determined using a Student *t* test (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$).

Other Supporting Information Files

Dataset S1 (PDF)
Dataset S2 (PDF)
Dataset S3 (PDF)
Dataset S4 (PDF)