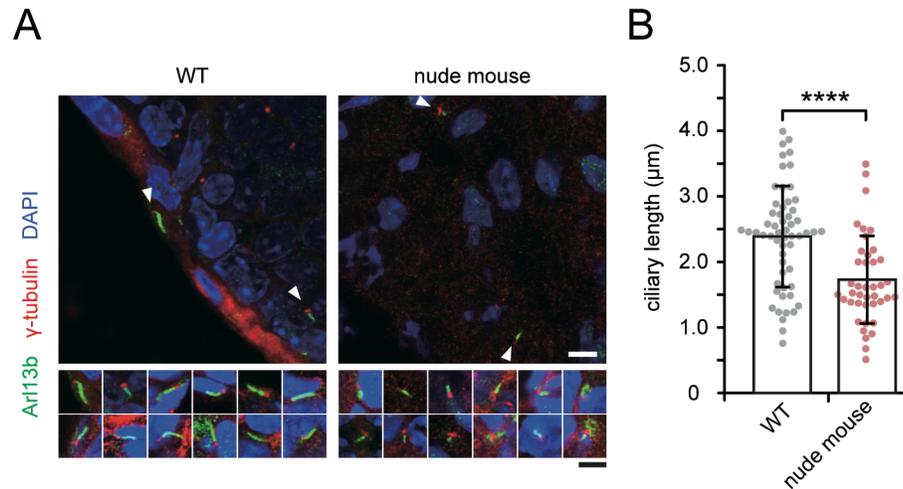


Supplementary Materials:

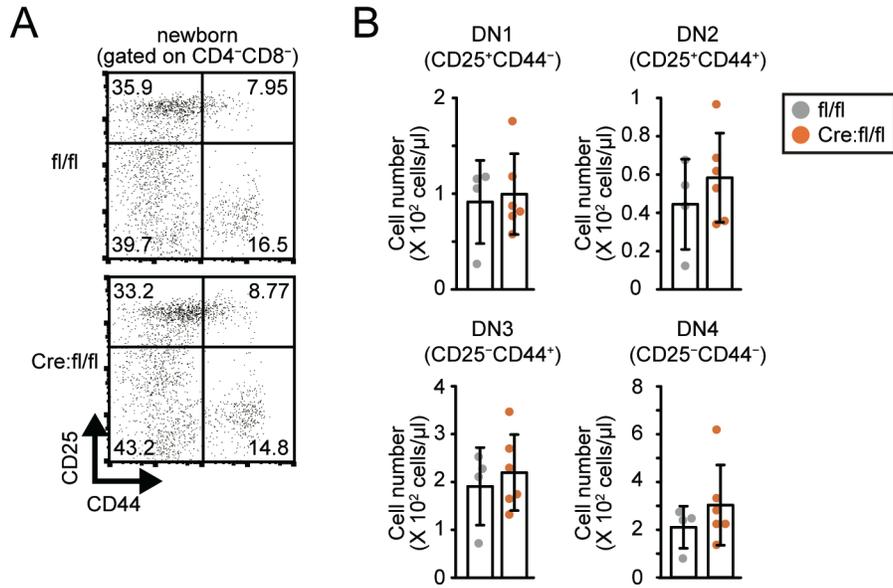
Impact of thymic epithelial primary cilia on T cell development

Osamu Kutomi, Shigenori Nonaka, Katsuto Hozumi and Sen Takeda



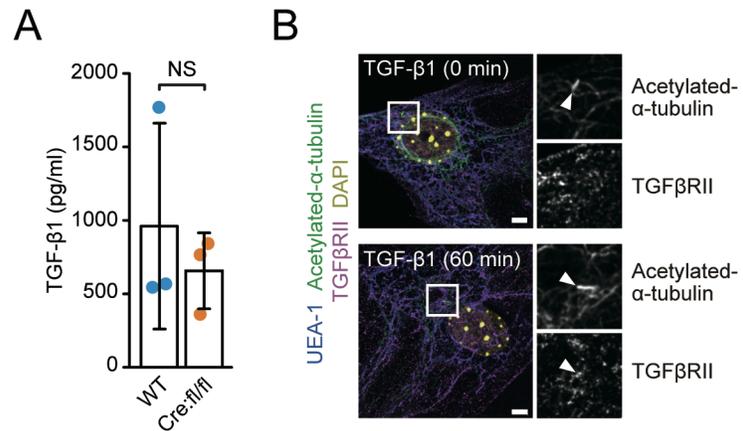
Supplementary Fig. S1. Comparison of primary ciliary length between thymic tissue from newborn WT and thymic primordium in newborn nude mice

(A) Primary cilia indicated by white arrowheads on tissues visualized by immunofluorescence with anti-Arl13b and anti- γ -tubulin antibodies. Bottom panels show typical primary cilia in WT thymic tissue and thymic primordium of nude mice. Primary ciliary length determined by measuring segment path detected by immunofluorescence with anti-Arl13b antibody from ciliary base to tip. Scale bar: 5 μ m. (B) Measured plot and bar (mean \pm SD) showing primary ciliary length distribution in WT thymic tissue and thymic primordium of nude mice (n = 57 and 41 for WT and nude mice, respectively). **** p < 0.0001 (unpaired Student's t -test). WT: wild type



Supplementary Fig. S2. Cell sorting with anti-CD25 and CD44 antibodies gated on newborn mice DN thymocytes

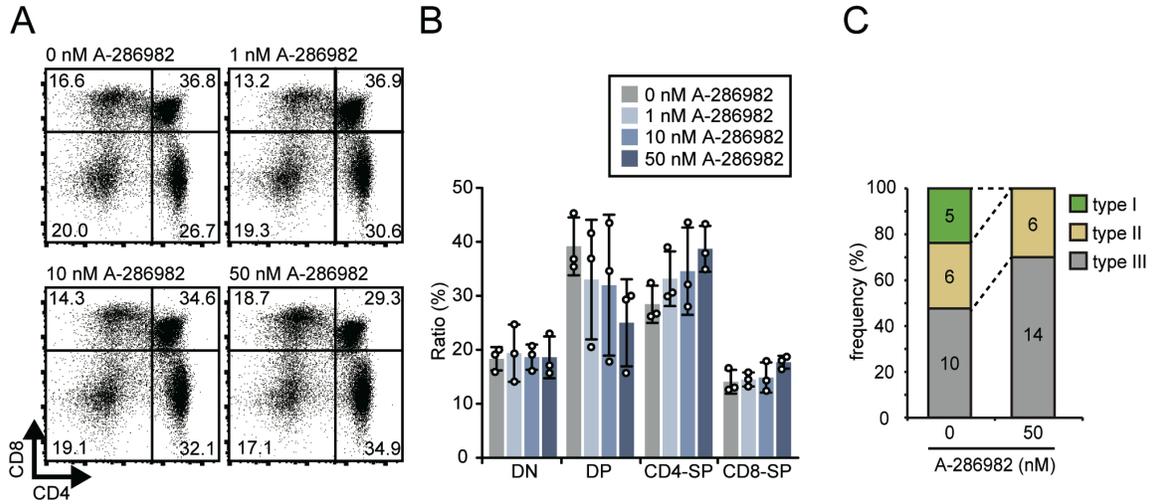
(A) Values in panels represent average ratio of each subset in each quadrant. (B) Graphical representation of cell counts for (mean ± SD) subsets DN1 (CD25⁻CD44⁺CD4⁻CD8⁻), DN2 (CD25⁺CD44⁺CD4⁻CD8⁻), DN3 (CD25⁻CD44⁻CD4⁻CD8⁻), and DN4 (CD25⁺CD44⁻CD4⁻CD8⁻) thymocytes.



Supplementary Fig. S3. Relationship between primary cilia and TGF-β signaling (related to Fig. 4)

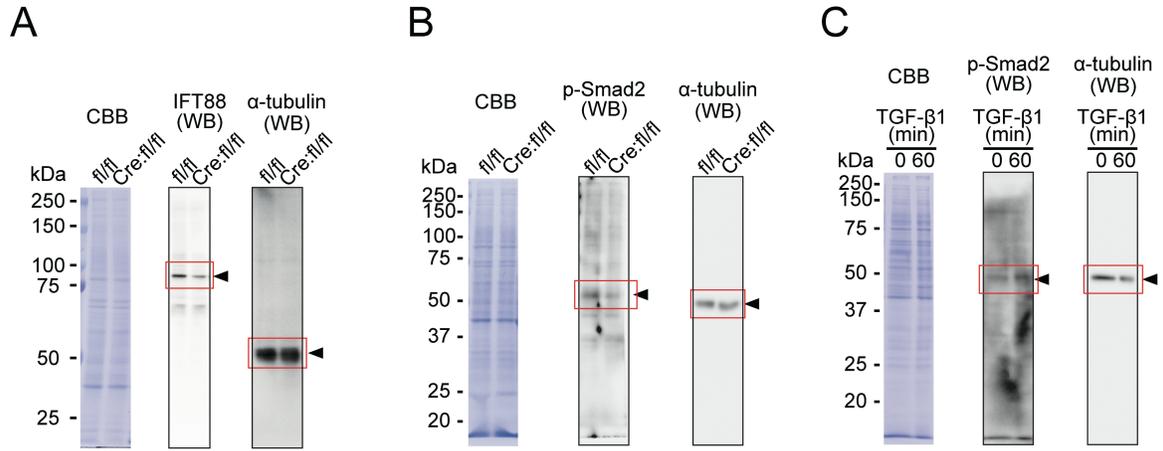
(A) Measured plot and bar (mean ± SD) show distribution of endogenous TGF-μ1 quantity in WT thymus (n = 3) and old *Ifi88* TEC null mice (n = 3). NS: not significant.

(B) Localization of TGF-βRII on cultured mTECs treated with 2 ng/ml TGF-β1 for 0 min and 60 min. Arrowheads indicate ciliary base. Scale bars: 5 μm. WT: wild type; mTEC: medulla thymic epithelial cell; TGF-β: transforming growth factor β.



Supplementary Fig. S4. Effect of inhibition of interaction between LFA-1 and ICAM-1 on T cell development

Fetal thymi cultured for 8 d in presence of inhibitor A-286982 that inhibits interaction between LFA-1 and ICAM-1. Thymocytes analyzed by flow cytometer. **(A)** Flow cytometers showing dot plots of CD4 and CD8 expression in thymocytes. Values in dot plots indicate percentages of thymocyte subsets within indicated area. **(B)** Measured plot and bar (mean \pm SD) show distribution ratio of DN (CD4⁻CD8⁻), DP (CD4⁺CD8⁺), CD4-SP (CD4⁺CD8⁻), and CD8-SP (CD4⁻CD8⁺) in thymocytes. **(C)** Distribution of each TS subtype in the fetal thymi treated with 0 nM (n = 21) and 50 nM (n = 20) A-286982. Each number within bars represents individual TS pattern frequency.



Supplementary Fig. S5. Full-length western blots.

Western blots featured in Fig. 2A (**A**), Fig. 4A (**B**) and Fig. 4B (**C**).

Table S1. Antibodies list used in this study.

Antibodies	Source	Cat#	Dilution	Application
rabbit polyclonal anti-Arl13b	Proteintech	17711-1-AP	1:500	immunofluorescence
rabbit polyclonal anti-IFT88	Proteintech	13967-1-AP	1:100 1:1000	immunofluorescence western blotting
mouse monoclonal anti- α -tubulin (clone: DM1A)	Sigma	T9026	1:1000 or 1:5000	western blotting
rabbit monoclonal phospho-Smad2 (138D4)	Cell Signaling Technology	3108	1:2000 or 1:5000	western blotting
mouse monoclonal anti-acetylated- α -tubulin (clone: 6-11B-1)	Sigma	T6793	1:1000	immunofluorescence
mouse monoclonal anti- γ -tubulin (clone: GTU-88)	Sigma	T6557	1:300	immunofluorescence
rabbit polyclonal anti-keratin 14	BioLegend	905303	1:1000	immunofluorescence
rat monoclonal anti-keratin 8 (clone: TROMA-I)	Developmental Studies Hybridoma Bank		1:222 or 1:200	immunofluorescence
armenian hamster monoclonal anti-ICAM-1 (clone: 3E2B)	abcam	ab1711181	1:500	immunofluorescence
rabbit monoclonal anti-CD3 (clone: SP7)	abcam	ab16669	1:200	immunofluorescence
rat monoclonal anti-CD8 (clone: YTS169.4)	abcam	ab22378	1:200	immunofluorescence
rabbit polyclonal anti-TGF- β receptor II	abcam	ab186838	1:200	immunofluorescence
Ulex Europaeus Agglutinin I (UEA I), Biotinylated	Vector Laboratories	B-1065	1:200	immunofluorescence
Alexa Fluor 488 goat anti-rabbit	Invitrogen	A11008	1:1000	immunofluorescence
Alexa Fluor 488 goat anti-mouse	Invitrogen	A11029	1:1000	immunofluorescence
Alexa Fluor 488 donkey anti-rat	Invitrogen	A21208	1:1000	immunofluorescence
Alexa Fluor 568 goat anti-rabbit	Invitrogen	A11036	1:1000	immunofluorescence
Alexa Fluor 568 goat anti-mouse	Invitrogen	A11004	1:1000	immunofluorescence
Alexa Fluor 568 goat anti-armenian hamster	abcam	ab175716	1:1000	immunofluorescence
Alexa Fluor 594 Streptavidin	BioLenged	405240	1:200	immunofluorescence
Alexa Fluor 647 goat anti-mouse	Invitrogen	A21235	1:1000	immunofluorescence
HRP goat anti-rabbit IgG (H+L)	Cell Signaling Technology	#7074S	1:3000 or 1:5000	western blotting
HRP goat anti-mouse IgG (H+L)	Cell Signaling Technology	#7076	1:3000 or 1:5000	western blotting
FITC anti-CD4 (clone: RM4-5)	BioLegend	100510		flow cytometry
APC anti-CD8 (clone: 53-6.7)	BioLegend	100712		flow cytometry
PE anti-CD25 (clone: PC61.5)	BioLegend	102007		flow cytometry
	Tonbo Biosciences	50-0251		flow cytometry
PerCP-Cy5.5 anti-CD44 (clone: IM7)	Tonbo Biosciences	65-0441		flow cytometry