

1 **Generation of knockout rabbits with X-linked severe combined**  
2 **immunodeficiency (X-SCID) using CRISPR/Cas9**

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4 Shohei Azuma<sup>1</sup>, Mitsuru Kuwamura<sup>5</sup>, Naofumi Takai<sup>6</sup>, Yasuyuki Osada<sup>6</sup>, Yayoi  
5 Kunihiro<sup>4</sup>, Tomoji Mashimo<sup>4, 7\*</sup>, Kohji Nishida<sup>1, 8</sup>

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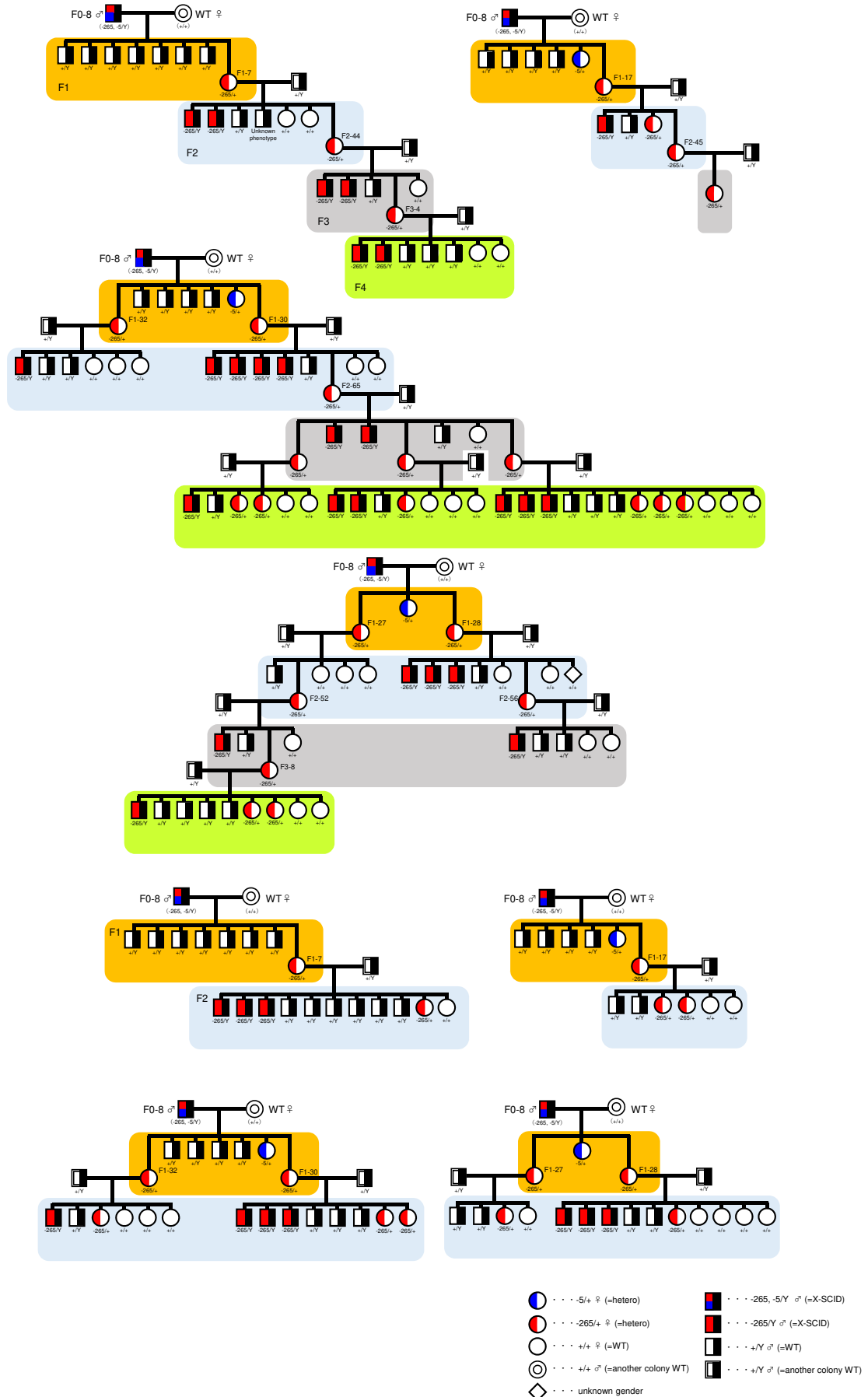
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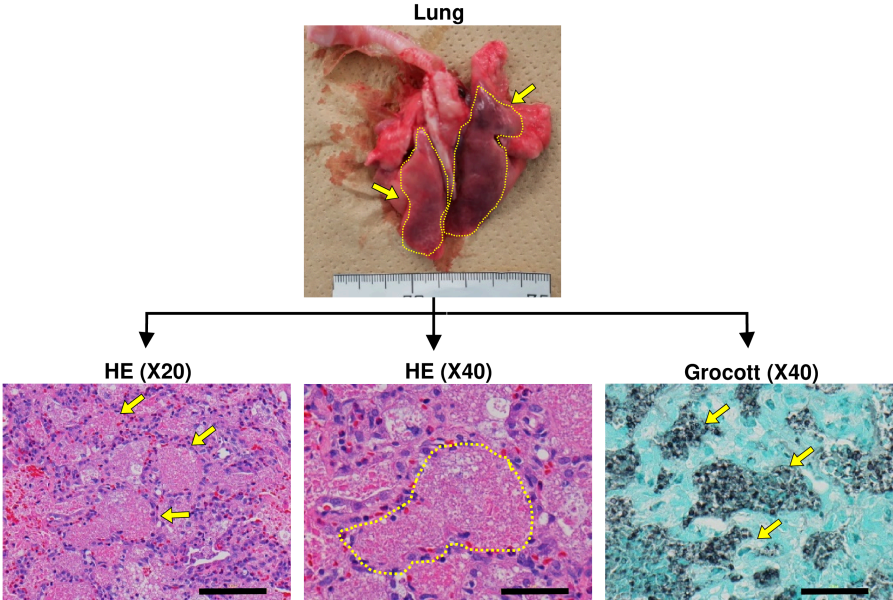
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# Supplementary Figure 1.

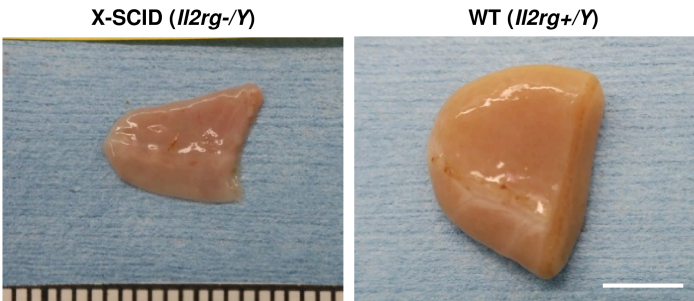


# Supplementary Figure 2.

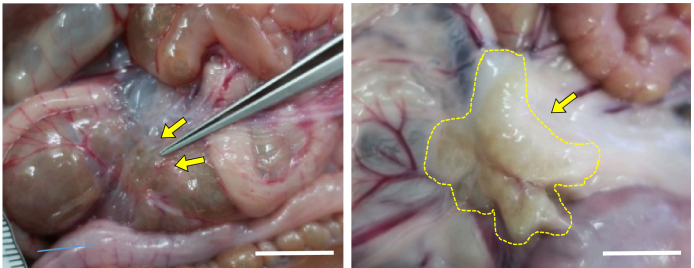


# Supplementary Figure 3.

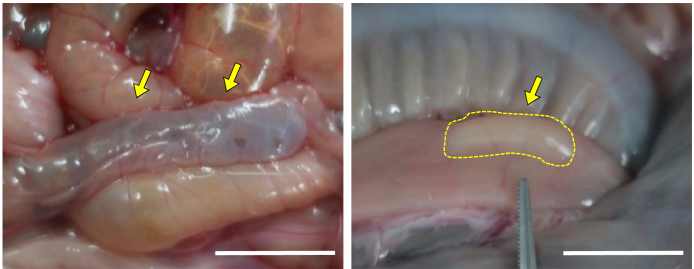
a. appendix



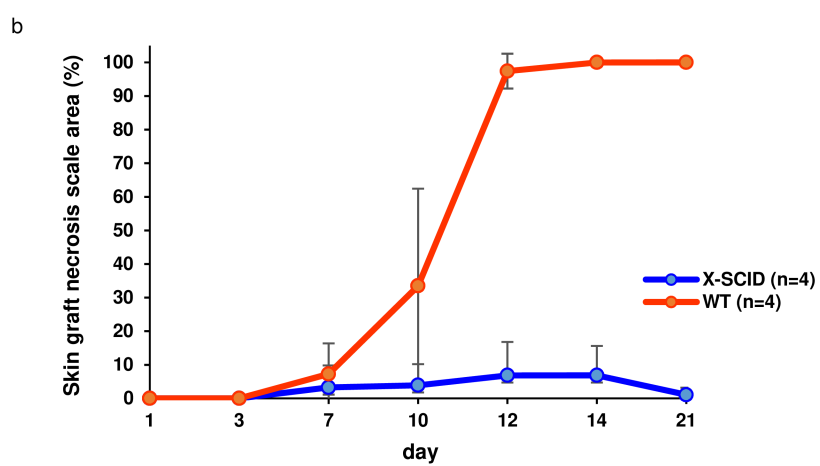
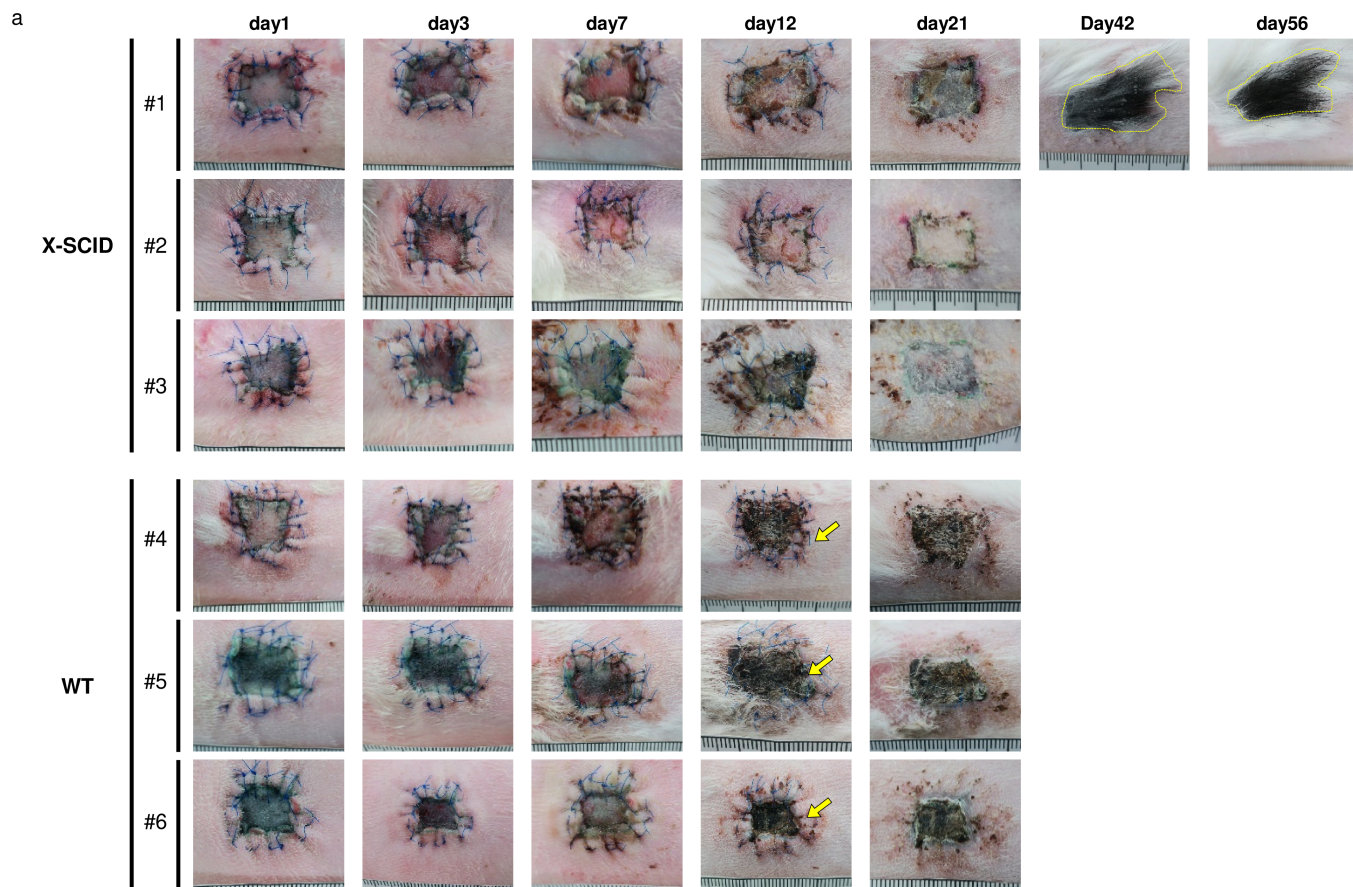
b. Mesenteric lymph node



c. Peyer's patches



# Supplementary Figure 4.



# Supplementary Table 1.

parent			pups generation	<i>Ii2rg</i> genotype				total
mutant	×	WT		male ♂		female ♀		
				X-SCID (%)	WT (%)	Hetero (%)	WT (%)	
♂ (-265, -5)	×	♀ (+/+)	F1	0 (0)	15 (50)	15 (50)	0 (0)	30
♀ (-265/+)	×	♂ (+/+)	F2	21 (26.3)	23 (28.8)	14 (17.5)	22 (27.5)	80
♀ (-265/+)	×	♂ (+/+)	F3	6 (27.3)	5 (22.7)	6 (27.3)	5 (22.7)	22
♀ (-265/+)	×	♂ (+/+)	F4	8 (19.0)	13 (31.0)	9 (21.4)	12 (28.6)	42

## Supplementary Table 2.

Spleen weight (g)	
X-SCID ( <i>Il2rg</i> <sup>-Y</sup> ) n=4	1.2 ± 0.09
WT ( <i>Il2rg</i> <sup>+Y</sup> ) n=5	1.0 ± 0.09

## Supplementary Table 3.

Gene	Sequence	GeneBank accesstion No.	
<i>Ii2rg</i> (gDNA)	Forward	AGGATCCTCACACCCAGTGA	XM-008272760
	Reverse	TAAGCTTCCTCGTGGTCTGC	
	Forward	CCTGGGTCCCTCAGTGTTT	XM-008272760
	Reverse	TGTCTCCATGGGTTAGGTGC *	
<i>Ii2rg</i> (mRNA)	Forward	GAAGAGGAAGCGCCATGTTG	XM-008272760
	Reverse	TTAAGCTTCCTCGTGGTCTGC	
<i>GAPDH</i>	Forward	GGAGAAAGCTGCTAA	L23961
	Reverse	ACGACCTGGTCCCTCGGTGTA	

\* The primer was designed based on our sequencing results of Japanese White rabbit.



28 **Supplementary Figure and Table Legends**

29

30 **Supplementary Figure 1. Pedigrees generated from sequence analyses of**  
31 **the F0 founder, F1, F2, F3, and F4 progeny.**

32

33 **Supplementary Figure 2. X-SCID rabbit abnormality lung.**

34 Macroscopic and Histological analysis of the lung abnormality area (yellow circled  
35 area) of X-SCID rabbit (25 weeks old, male) stained with H&E and Grocott. HE  
36 (X20) scale bar is 100µm, HE (X40) and Grocott (X40) scale bar is 50µm.

37

38 **Supplementary Figure 3. Phenotype during organogenesis in X-SCID**  
39 **rabbits.**

40 **(a)** Histological analysis of the appendix of X-SCID and WT rabbits (25 weeks  
41 old, male). Scale bar, 5 mm. **(b)** Histological analysis of the mesenteric lymph  
42 node (yellow circled area) of X-SCID and WT rabbits (25 weeks old, male).  
43 Scale bar, 10 mm. **(c)** Histological analysis of the Peyer's patches (yellow  
44 circled area) of X-SCID and WT rabbits (25 weeks old, male). Scale bar, 10  
45 mm.

46

47 **Supplementary Figure 4. Allogeneic skin transplantation.**

48 **(a)** Morphological analysis of temporal changes in the skin graft. The area  
49 demarcated by the yellow circled area shows black hair, characteristic of Dutch  
50 rabbits. The yellow arrow indicates darkening of the transplanted skin. **(b)**  
51 Temporal changes in the area of skin graft necrosis.

52

53 **Supplementary Table 1. Number of offspring from F1 to F4 generations in**  
54 **the *Il2rg* genotype.**

55

56 **Supplementary Table 2. Weight of spleen.**

57

58 **Supplementary Table 3. Primer sequences.**

59

## Supplementary Gel Images for

60 **Generation of knockout rabbits with X-linked severe combined**  
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86

Area of interest are highlighted by a white square.  
Labels correspond to those in the main paper.

Figure 1a

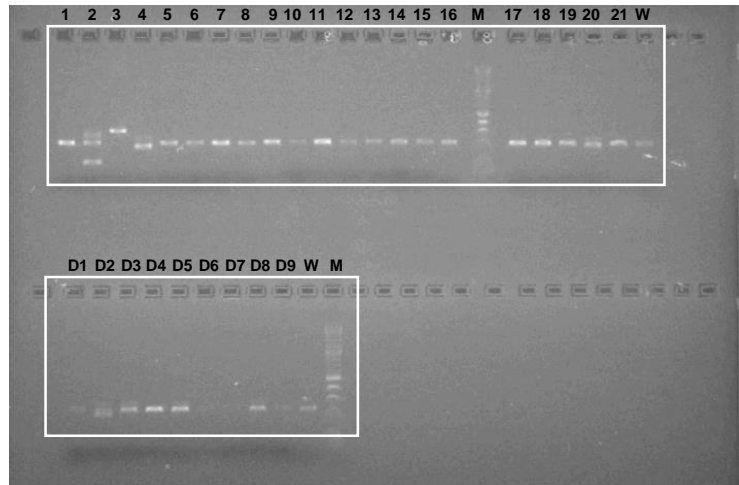


Figure 3c

