

Supplemental data

**Desmoglein 3-specific CD4⁺ T cells induce pemphigus vulgaris and
interface dermatitis in mice**

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1 GTCTCCCGGG **AV8S13**
 TGC ACT CAAG GACCAAGTGT CATTTCTTCC ATGAAC ATG CAT CCT 55
XmaI ← **M H P**

56 GTC ACC TGC TCA GTT CTT GTG CTC CTC CTA ATG CTC AGA AGG AGC AAT 103
 V T C S V L V L L L M L R R S N
← **Leader** →

104 GGA GAC TCA GTG ACC CAG ACA GAA GGC CTG GTC ACT CTC ACC GAG GGG 151
 G D S V T Q T E G L V T L T E G
← **FR1** →

152 TTG CCT GTG ATG CTG AAC TGC ACC TAT CAG ACT GCT TAC TCA ACT TTC 199
 L P V M L N C T Y Q T A Y S T F
← **CDR1** →

200 CTT TTC TGG TAT GTG CAA CAT CTC AAT GAA GCC CCT AAA CTA CTC CTG 247
 L F W Y V Q H L N E A P K L L L
← **FR2** →

248 AAG AGC TCC ACA GAC AAC AAG AGG ACC GAG CAC CAA GGG TTC CAC GCC 295
 K S S T D N K R T E H Q G F H A
← **CDR2** →

296 ACT CTC CAT AAG AGC AGC AGC TCC TTC CAT CTG CAG AAG TCC TCA GCG 343
 T L H K S S S S F H L Q K S S A
← **FR3** →

344 CAG CTG TCA GAC TCT GCC CTG TAC TAC **AV8S13** **J21 exon**
 Q L S D S A L Y Y C A L I R ACC AAT 391
← **CDR3** →

392 ACA GGC AAA TTA ACC TTT GGG GAT GGG ACC GTG CTC ACA GTG AAG CCA A 440
 T G K L T F G D G T V L T V K P
←

..... **J21 intron**
 441 GTAAGTTACC CTCTTCTTAG CCTGGGCCTT TGGGGGGAAA TCAATCAAAT CATTAGTTTG 500
 501 AAAAAATACTT TAATCTTATG CATCTGCTTG GGCTCTTTAT TAATGTTCTT TCCTCGGGCC 560
 561 AAAAAAAAAA AAAAAAAAAA AAGCTGTTTC TCTTCCCTGC TTTAAAATGA GGTAGGAGTG 620
 621 GAGGTGGAGG GAGAGTAATG ATATAAACAA TATTTAAGCC AATGAGGACA TTAGGAGGGA 680
 681 GCTCTGGGGA ATCCAGTACC CCACCACTTT TGACTAGACT TCCTGGCAGG CCGCGGC 738
SacII

Supplemental Figures 1 - 6 The nucleotide and amino acid sequences of the TCR α - and β -chains from clones 140#27 (Supplemental Figures 1 and 2), 162#24 (Supplemental Figures 3 and 4), and 164#2 (Supplemental Figures 5 and 6). Dotted arrows indicate the beginning or end of each rearranged gene. The restriction enzyme sites used for subcloning are underlined. The segmentation of the amino acid sequences was determined using the IMTG web site (available at <http://imgt.cines.fr/>). Shaded and hatched nucleotides were mutated to avoid digestion by the restriction enzyme noted above the nucleotide.

Supplemental Figure 1

Dsg3H TCR α -chain from T cell clone, 140#27

1 GTCTCGAGT TCTCTTTTAA CTAATGCCCA GAGCCAAAGA AAGTCCCTCC AA ACT ATG 58
 XhoI M

59 AAC AAG TGG GTT TTC TGC TGG GTA ACC CTT TGT CTC CTT ACT GTA GAG 106
 N K W V F C W V T L C L L T V E
 —————Leader—————

107 ACC ACA CAT GGT GAT GGT GGC ATC ATT ACT CAG ACA CCC AAA TTC CTG 154
 T T H G D G G I I T Q T P K F L

155 ATT GGT CAG GAA GGG CAA AAA CTG ACC TTG AAA TGT CAA CAG AAT TTC 202
 I G Q E G Q K L T L K C Q Q N F
 ————FR1—————

203 AAT CAT GAT ACA ATG TAC TGG TAC CGA CAG GAT TCA GGG AAA GGA TTG 250
 N H D T M Y W Y R Q D S G K G L
 —CDR1—————KpnI—————FR2—————

251 AGA CTG ATC TAC TAT TCA ATA ACT GAA AAC GAT CTT CAA AAA GGC GAT 298
 R L I Y Y S I T E N D L Q K G D
 —————CDR2—————

299 CTA TCT GAA GGC TAT GAT GCG TCT CGA GAG AAG AAG TCA TCT TTT TCT 346
 L S E G Y D A S R E K K S S F S
 —————FR3—————

347 CTC ACT GTG ACA TCT GCC CAG AAG AAC GAG ATG GCC GTT TTT CTC TGT 394
 L T V T S A Q K N E M A V F L C

395 *BV6S1*←……………*Jβ1.3 exon*
 GCC AGC AGT ATA AGC ACC CGT TCT GGA AAT ACG CTC TAT TTT GGA GAA 442
 A S S I S T R S G N T L Y F G E
 ←—————CDR3—————

443 GGA AGC CGG CTC ATT GTT GTA GGTAAAGTTAG GGCCAAATGG CTGGGTACTG GGTG 497
 G S R L I V V *Jβ1.3 intron*

498 AAGTTTCTCT CTGAGGTGCC TAGGTTGCAG TGTACTTCTA TCAGAAAGAA CTAAACTCCT 557
 558 CTGCTCATGC ATGCACGAAG GAATGAATGC CCACAGGTGG CTATGCGAAA CTCGGCATCG 617
 618 TTTAATGCAG GCAAGCTCAA CTGGGAGTAA GCCTCATGTA CTGTTACAG TCCTACGCCG 677
 678 CGGGC SacII 682

Supplemental Figure 2
Dsg3H TCR β-chain from 140#27

1 GCCC GG GGA GAGATAACTC AAAGCTTCAG AGAAGAC ATG ACC GGC TTC CTG AAG 55
XmaI M T G F L K

56 GCC TTG CTG TTG GTT CTG TGC CTG CGG CCA GAA TGG GTA AAG AGT CAA 103
A L L L V L C L R P E W V K S Q
Leader

104 CAG AAG ACT GGT GGC CAG CAA GTT AAA CAA AGC TCT CCA TCG CTG ACT 151
Q K T G G Q Q V K Q S S P S L T
FR1

152 GTT CAA GAG GGA GGG ATA TTG ATC CTG AAT TGT GAT TAC GAG AAT GAT 199
V Q E G G I L I L N C D Y E N D

200 ATG TTT GAC TAT TTT GCC TGG TAC AAA AAA TAC CCT GAC AAC AGC CCC 247
M F D Y F A W Y K K Y P D N S P
CDR1 FR2

248 ACA CTC CTG ATA TCC GTA CGC TCA AAT GTG GAT AAG AGG GAA GAC GGA 295
T L L I S V R S N V D K R E D G
CDR2

296 AGA TTC ACA GTT TTC TTG AAC AAA AGC GGC AAA CAC TTC TCA CTG CAC 343
R F T V F L N K S G K H F S L H
FR3

344 ATC ACA GCC TCC CAG CCT GAA GAC ACA GCA GTG TAC CTC TGC GCA GCA 391
I T A S Q P E D T A V Y L C A A

392 GGG ATG GAC TAT GCA AAC AAG ATG ATC TTT GGC TTG GGA ACC ATT TTG 439
G M D Y A N K M I F G L G T I L
CDR3

440 AGA GTC AGA CCT GTTAAGTACC AAACACATTC TGTTCCTCCA CCATCCCAAC CTGAG 496
R V R P
J39 intron

497 CTTCTCTGTA CACTGAAGAG ACTAACTTAG AAGTGCTTGG GTTCTAAGAA ACAAACAAG 556

557 CTCCAGGTGC AGCGCAGACA GATCACTGTG TGGCTCACTT AAGGATCTAC ACGCAGTGCC 616

617 TTGGGGAGCA AGGCAAACAC CAAATTCACCT GGTGTGGCCC ACAGCTTGGG CCGCGGC 673
SacII

Supplemental Figure 3

Dsg3M TCR α-chain from T cell clone, 162#24

1 GCTCGAGTTCAG TTCTGAG ^{BV8S1} ATG GGC TCC AGA CTC TTC TTT GTG GTT TTG ATT 50
XhoI M G S R L F F V V L I
← Leader →

51 CTC CTG TGT GCA AAA CAC ATG GAG GCT GCA GTC ACC CAA AGT CCA AGA 98
L L C A K H M E A A V T Q S P R

99 AGC AAG GTG GCA GTA ACA GGA GGA AAG GTG ACA TTG AGC TGT CAC CAG 146
S K V A V T G G K V T L S C H Q
← FR1 →

147 ACT AAT AAC CAT GAC TAT ATG TAC TGG TAT CGG CAG GAC ACG GGG CAT 194
T N N H D Y M Y W Y R Q D T G H
→ ← CDR1 → ← FR2 →

195 GGG CTG AGG CTG ATC CAT TAC TCA TAT GTC GCT GAC AGC ACG GAG AAA 242
G L R L I H Y S Y V A D S T E K
← CDR2 →

243 GGA GAT ATC CCT GAT GGG TAC AAG GCC TCC AGA CCA AGC CAA GAG AAT 290
G D I P D G Y K A S R P S Q E N
← FR3 →

291 TTC TCT CTC ATT CTG GAG TTG GCT TCC CTT TCT CAG ACA GCT GTA TAT 338
F S L I L E L A S L S Q T A V Y

^{BV8S1} TTC TGT GCC AGC CAA GGA CTG GGG GGG GGC GAT GAA CAG TAC TTC GGT 386
F C A S Q G L G G G D E Q Y F G
← CDR3 → ^{Jβ2.7 exon}

387 CCC GGC ACC AGG CTC ACG GTT TTA GGTAAAGATTC ACATCTCTCG CTCCACCCA 440
P G T R L T V L
← Jβ2.7 intron →

441 AATTCCTGGG TCCCTGGAGT AGTTTGGAGT AGACCAGGGT TAGGGAATCT CCGGGAGGGG 500
501 AATCGATAGT AGGAGACAGC TCTCAACTTC ACCCTTTCTG GGGGAGCGGG ATGAAAAGGG 560
561 ATTGGGTGCC AAACGTTGTT CGCTCTCTCG CAAGCCAGGA CAAGGTAGGT TTCTTTCTCT 620
621 TTTCTCTGAG GTATGCATGC ACTGGGTGGG CCGCGGC 657
SacII

Supplemental Figure 4
Dsg3M TCR β-chain from 162#24

1 GCCCGGGGCTT GCATGGCAAG AGATTGCAAG TCTGCATTGA TCCCAGGCAG GAAGAATG 58
 XmaI

59 ATG AAG ACA TCC CTT CAC ACT GTA TTC CTA TTC TTG TGG CTG TGG ATG 106
 M K T S L H T V F L F L W L W M
 ← Leader →

107 GAC TGG GAG AGC CAT GGA GAG AAG GTC GAG CAA CAC GAG TCT ACA CTG 154
 D W E S H G E K V E Q H E S T L

155 AGT GTT CGA GAG GGA GAC AGC GCT GTC ATC AAC TGC ACT TAC ACA GAT 202
 S V R E G D S A V I N C T Y T D
 ← FR1 →

203 ACT GCT TCA TCA TAC TTC CCT TGG TAC AAG CAA GAA GCT GGA AAG GGT 250
 T A S S Y F P W Y K Q E A G K G
 ← CDR1 → ← FR2 →

251 CTC CAC TTT GTG ATA GAC ATT CGT TCA AAT GTG GAC AGA AAA CAG AGC 298
 L H F V I D I R S N V D R K Q S
 ← CDR2 →

299 CAA AGA CTT ATA GTT TTG TTG GAT AAG AAA GCC AAA CGA TTC TCC CTG 346
 Q R L I V L L D K K A K R F S L
 ← FR3 →

347 CAC ATC ACA GCC ACA CAG CCT GAA GAT TCA GCC ATC TAC TTC TGT GCA 394
 H I T A T Q P E D S A I Y F C A

AV15S1
 395 GCAAGC CCC TCG AAC AGT GGA GGC AGC AAT TAC AAA CTG ACA TTT GGG 442
 A S P S N S G G S N Y K L T F G
 ← CDR3 →

443 AAA GGA ACT CTC TTA ACT GTG ACT CCA AATAAGTATT ACAGGAGCGG CAAAGGG 496
 K G T L L T V T P

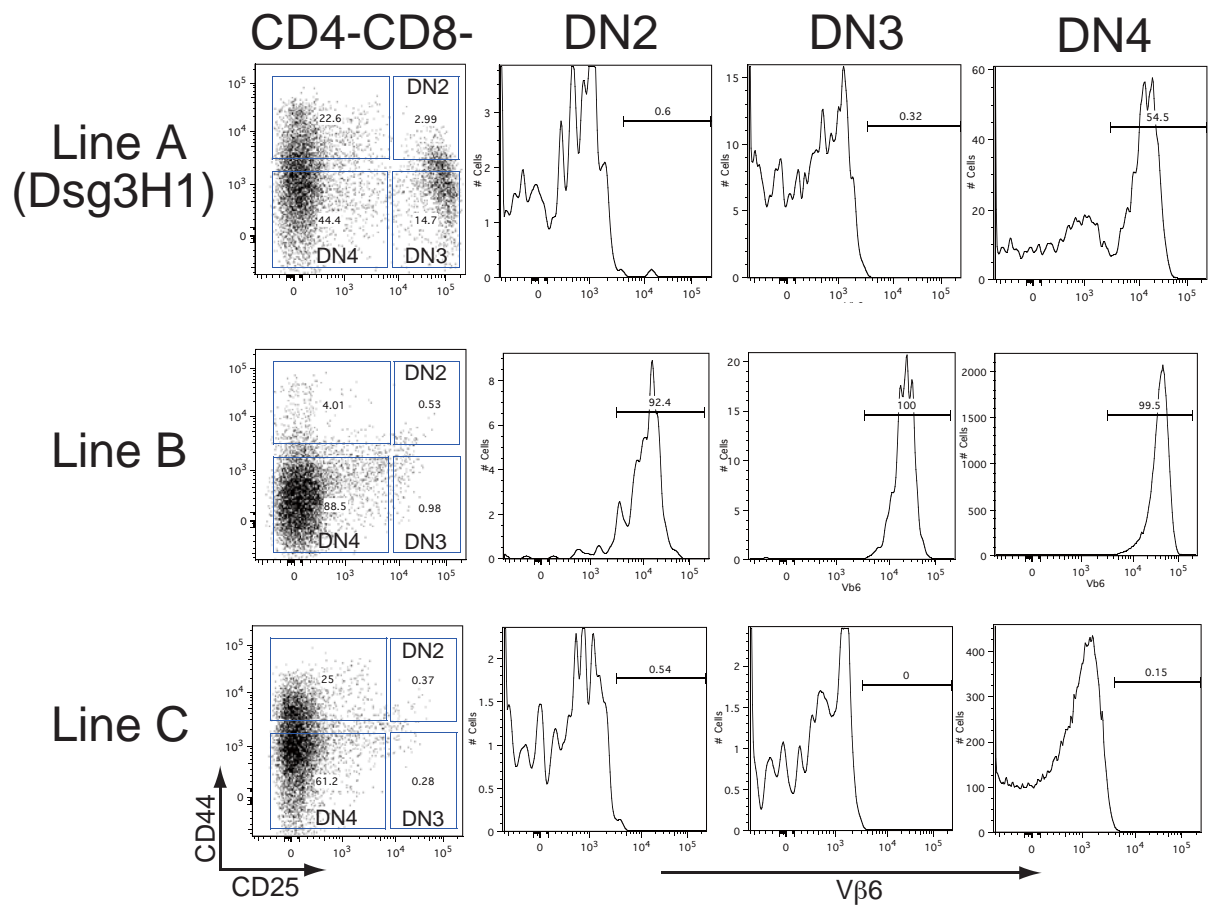
497 ATACTTCTCA TTCCAAGAAC CGCCCCTCTA AAGCACCCCC GCTTACCTTC TAAAGCTGAG 556
 557 CGGATTTATT ACAGAACGGT GGTCCCCATC GCCACTGTAC AAAGACTAAA TTGCCTCTAG 616
 617 CCAACGATAT CCAATCATT CATCTTAAAT GAAATCTTTG CTACAGGGAG GCACATTGAA 676
 677 TACAGGATCC AGCCGCGGC 695
 SacII

Supplemental Figure 5
 Dsg3L TCR α -chain from T cell clone, 164#2

1	<u>GTCTCGAG</u> TT TCTCTTTTAA ^{BV6S1} CTAATGCCCA GAGCCAAAGA AAGTCCCTCC AAAC T M	58
	<u>XhoI</u> ←	
59	AAC AAG TGG GTT TTC TGC TGG GTA ACC CTT TGT CTC CTT ACT GTA GAG	106
	<u>Leader</u> →	
107	ACC ACA CAT GGT GAT GGT GGC ATC ATT ACT CAG ACA CCC AAA TTC CTG	154
	←	
155	ATT GGT CAG GAA GGG CAA AAA CTG ACC TTG AAA TGT CAA CAG AAT TTC	202
	<u>FR1</u> →	
203	AAT CAT GAT ACA ATG TAC TGG TAT CGA CAG GAT TCA GGG AAA GGA TTG	250
	<u>CDR1</u> → <u>FR2</u> ←	
251	AGA CTG ATC TAC TAT TCA ATA ACT GAA AAC GAT CTT CAA AAA GGC GAT	298
	<u>CDR2</u> →	
299	CTA TCT GAA GGC TAT GAT GCG TCC CGA GAG AAG AAG TCA TCT TTT TCT	346
	<u>FR3</u> →	
347	CTC ACT GTG ACA TCT GCC CAG AAG AAC GAG ATG GCC GTT TTT CTC TGT	394
	←	
395	^{BV6S1} GCC AGC AGT ATG AGG GGT GGT GCA GAA ACC CTG TAT TTT GGC TCA GGA	442
	←	
443	ACC AGA CTG ACT GTT CTC GGT ^{Jβ2.3 exon} TAAGTTGG GAGCTAGTAA TGAAGGGGAG GGAGCATT	498
	<u>Jβ2.3 intron</u> →	
499	TCCCAGGCTA AGGATAGCCA GAGCCAGTTT TTGTCCTGTA CCAAGAGGCT GTGAGTCAAA	558
559	ACACCTTGTA CTTTGGTGGC GGCACCCGAC TATCGGTGCT AGGTAAGCTG GGGTATAGTT	618
619	TTTGTGTTGG GTTCTGGGGC TGTGAACCAA GACACCCAGT ACTTTGGGCC AGGCACTCGG	678
679	CTCCTCGCCG CGGC	
	<u>SacII</u>	

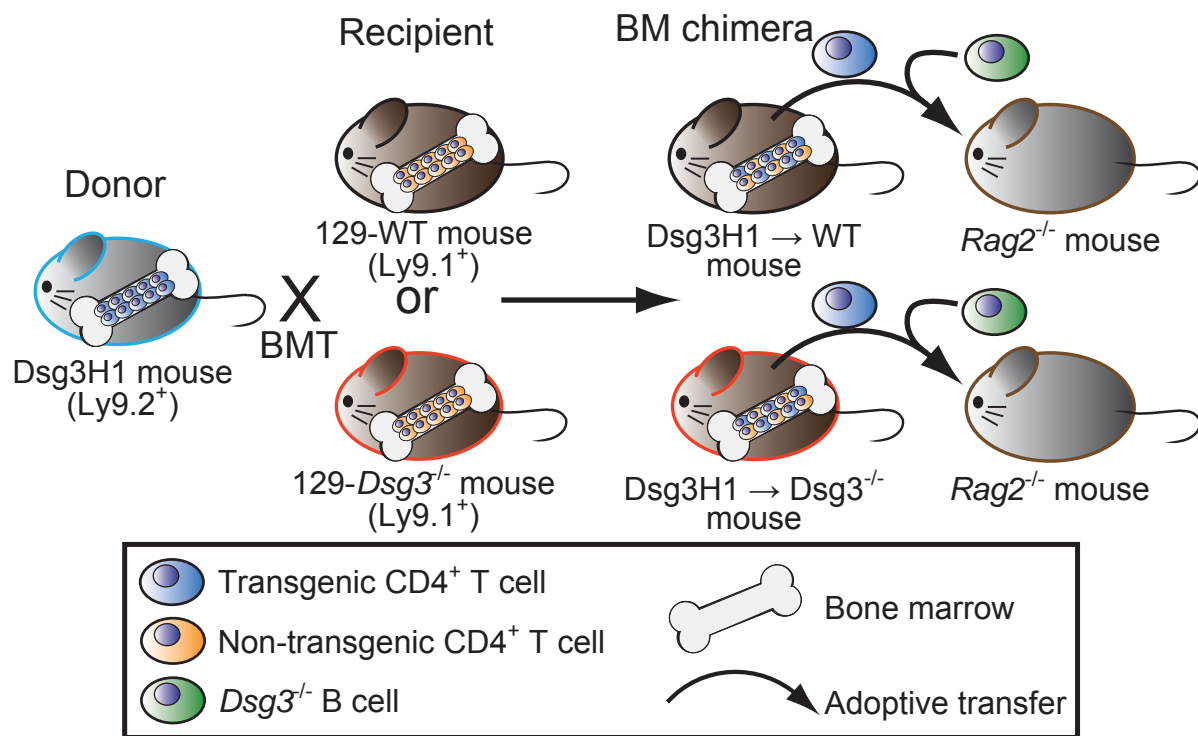
Supplemental Figure 6

Dsg3L TCR β-chain from 164#2



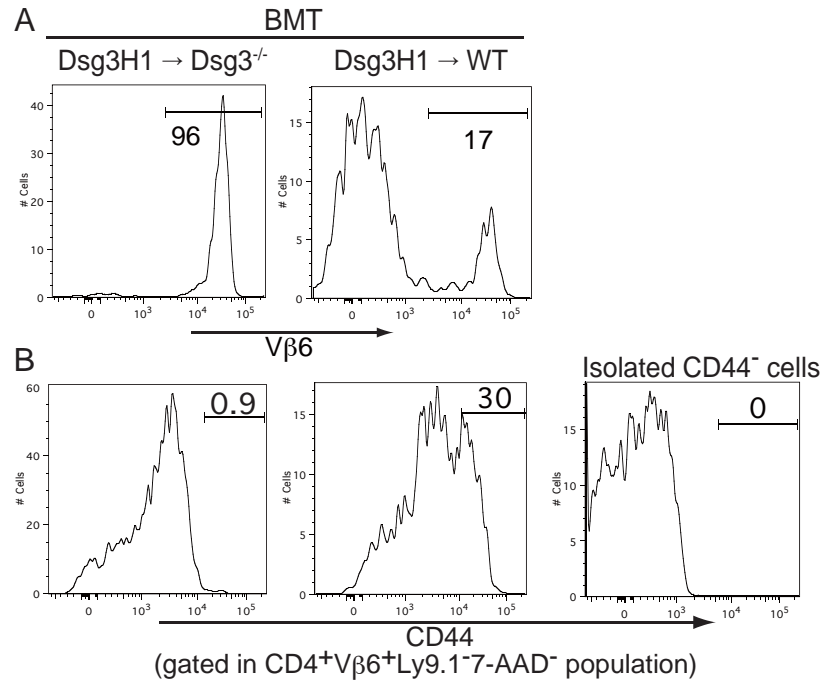
Supplemental Figure 7 Time points of TCR β -chain expression in lines A to C of the Dsg3H TCR transgenic mouse. Thymocytes were stained with anti-CD4, anti-CD8, anti-CD25, anti-CD44, and anti-V β 6 antibodies. The CD4⁻CD8⁻ double negative (DN) population was gated and analyzed using CD44 and CD25 expression to classify them as DN2 to DN4 (left column). In each population of DN2-4, V β 6 expression was evaluated. Transgenes for the Dsg3H TCR α - and β -chains were detected by genotyping in all lines.

Supplemental Figure 7



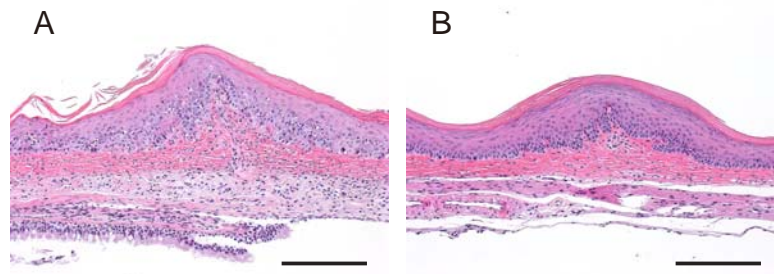
Supplemental Figure 8 Outline of the bone marrow transfer experiments. BM cells were isolated from Dsg3H1 mice (Ly9.2) and transferred into sublethally irradiated 129-Dsg3^{-/-} or 129-WT mice (Ly9.1). Two months later, naïve CD4⁺Ly9.1⁻ cells were isolated from the recipient mice by depleting CD8⁺, CD11b⁺, B220⁺, Gr-1⁺, Ly-9.1⁺, DX5⁺, and CD44⁺ cells from splenocytes and LN cells, using magnetic beads, and transferred with Dsg3^{-/-} B cells into Rag2^{-/-} mice.

Supplemental Figure 8



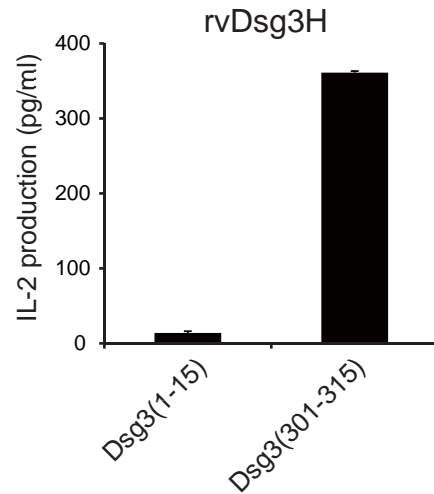
Supplemental Figure 9 Transgenic Dsg3H1 T cells undergo Dsg3-dependent T cell selection. BM cells from Dsg3H1 mice were transferred into sublethally irradiated 129-WT ($n = 5$) or 129-Dsg3^{-/-} mice ($n = 3$). (A) Eight weeks later, peripheral blood mononuclear cells (PBMCs) from recipients of BM cells from Dsg3H1 mice were stained with anti-CD4, anti-Ly9.1, and anti-Vβ6 antibodies. The numbers in the histogram indicate the proportion of Vβ6⁺ cells in CD4⁺Ly9.1⁻ lymphocytes. (B) CD44 expression by Dsg3H1 → Dsg3^{-/-} CD4⁺ T cells and Dsg3H1 → WT CD4⁺ T cells was analyzed using flow cytometry after gating on the CD4⁺Vβ6⁺Ly9.1⁻7-AAD⁻ population (left and middle panels). CD44^{hi} memory T cells were depleted from Dsg3H1 → Dsg3^{-/-} CD4⁺ T cells by the MACS cell separation system before adoptive transfer (right panel).

Supplemental Figure 9



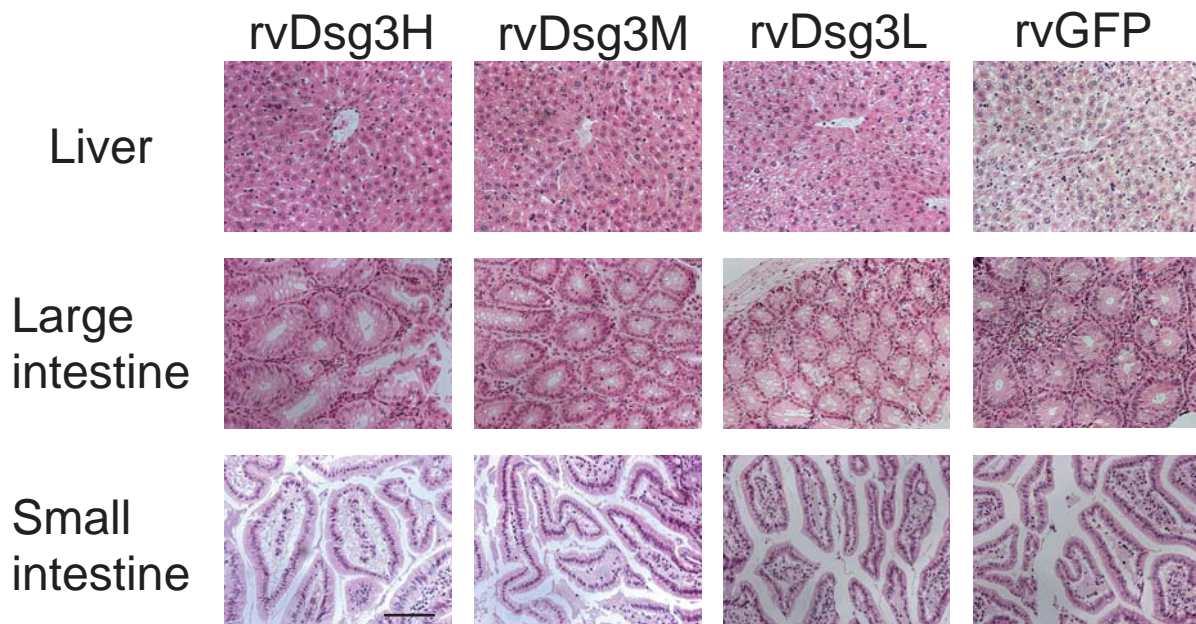
Supplemental Figure 10 Tolerized Dsg3H1 T cells induced interface dermatitis. The palates of *Rag2*^{-/-} mice given CD4⁺Vβ6⁺ cells from Dsg3H1 mice (A) or CD4⁺ T cells from C57BL/6 mice (B) in combination with *Dsg3*^{-/-} B cells were stained with H&E. Bars indicate 200 μm.

Supplemental Figure 10



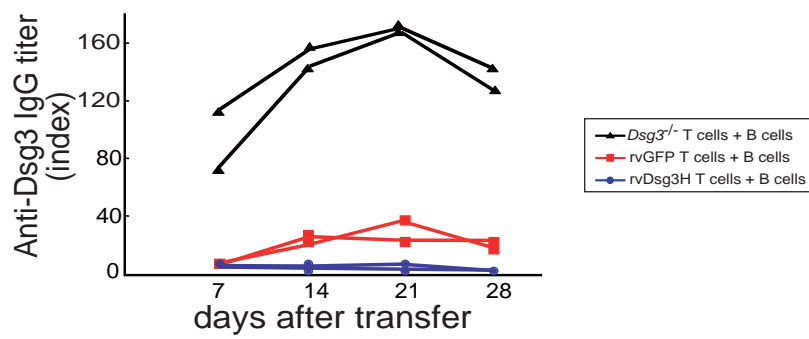
Supplemental Figure 11 The reactivity of retrovirally transduced Dsg3-specific T cells. After retrovirally transducing Dsg3H TCR into CD4⁺ T cells, rvDsg3H T cells were cultured with irradiated splenocytes and a Dsg3 peptide, Dsg3(1–15) or Dsg3(301-315), for 18 h. The supernatant was evaluated for IL-2 production from T cells by ELISA.

Supplemental Figure 11



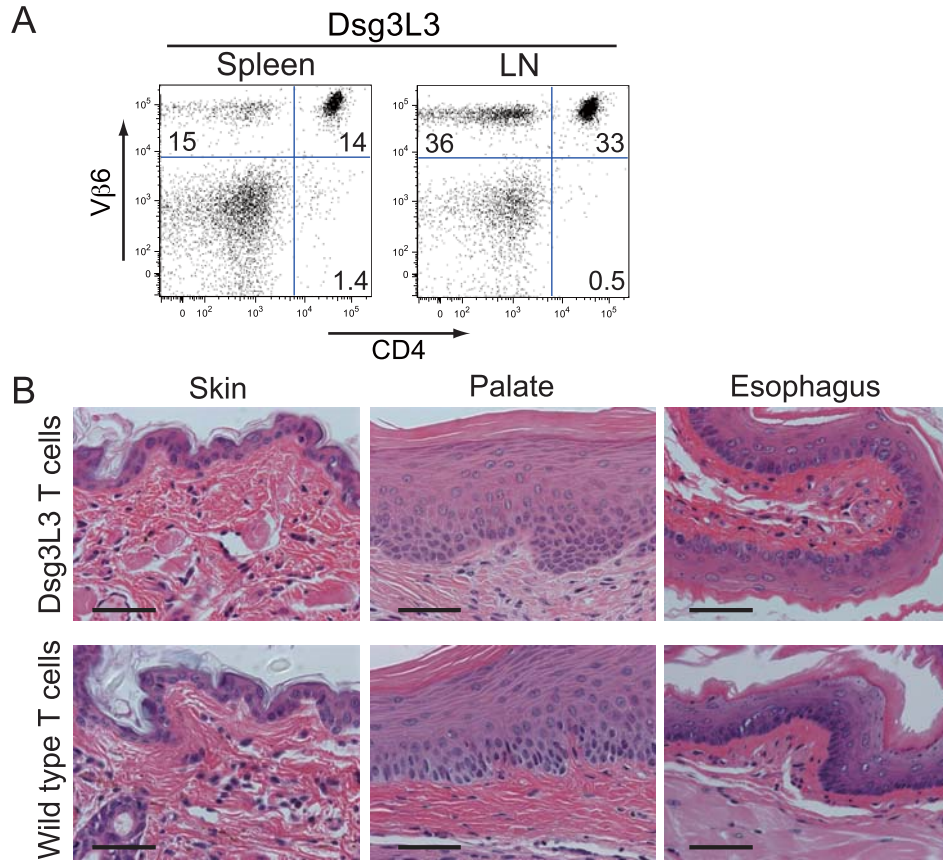
Supplemental Figure 12 Dsg3-specific CD4⁺ T cells do not induce interface dermatitis in non-Dsg3-expressing tissues. Retrovirally transduced rvDsg3H, rvDsg3M, rvDsg3L, and rvGFP T cells were adoptively transferred into *Rag2*^{-/-} mice. Four weeks later, the liver and small and large intestines were evaluated histologically using H&E staining. Bar indicates 100 μ m.

Supplemental Figure 12



Supplemental Figure 13 Retrovirally transduced Dsg3-specific T cells do not induce anti-Dsg3 IgG production. *Dsg3*^{-/-} CD4⁺ T cells (black), rvGFP T cells (red), and rvDsg3H T cells (blue) were adoptively transferred with *Dsg3*^{-/-} B cells into *Rag2*^{-/-} mice, from which serum samples were collected at the time points shown at the bottom and the anti-Dsg3 IgG titer was evaluated using ELISA.

Supplemental Figure 13



Supplemental Figure 14 Generation of the Dsg3-specific TCR transgenic mouse, Dsg3L3 mouse, and inability of Dsg3L3 T cells to induce interface dermatitis. (A) A single-cell suspension from the spleen and LN was stained with anti-CD4 and anti-TCRV β 6 antibodies. (B) CD4⁺V β 6⁺ cells were isolated from Dsg3L3 mice and adoptively transferred with *Dsg3*^{-/-} B cells into *Rag2*^{-/-} mice. The skin, palate, and esophagus were collected 4 weeks after transfer and stained with H&E. The histopathology of each tissue from recipient mice transferred with wild-type CD4⁺ T cells is shown as a control. Similar results were obtained from two separate experiments. Bars indicate 50 μ m.

Supplemental Figure 14

Supplemental Table 1

Features on Dsg3-specific TCRs

Name		Gene segment		Pathogenicity of parental T cell clone for PV phenotype
T cell clone	TCR	TCR α	TCR β	
140#27	Dsg3H	AV8S13-J21	BV6S1-XDX-J β 1.3	+
162#24	Dsg3M	AV20S1-J39	BV8S1-XDX-J β 2.7	-
164#2	Dsg3L	AV15S1-J45	BV6S1-XDX-J β 2.3	+