Supporting Information

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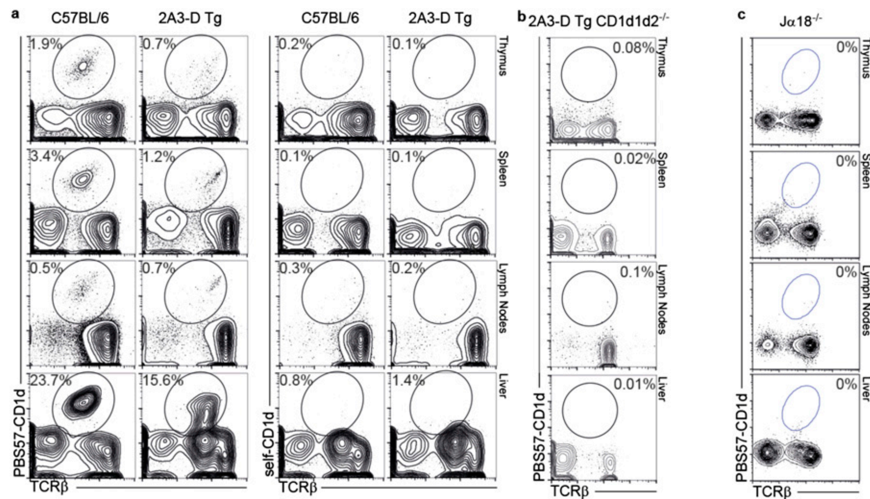

Fig S1. Invariant NK T cells (iNKT) cells in 2A3-D Tg mice depends on CD1d for thymic development. (*A*) Purified lymphocytes from the thymus, spleen, liver, and peripheral lymph nodes (pLN) of C57BL/6 or 2A3-D Tg mice were stained for indicated markers to assess reactivity of iNKT cells to self-CD1d and PBS57-CD1d (data representative of $n = 3$). (*B*) Purified lymphocytes from the thymus, spleen, liver, and pLN of 2A3-D Tg CD1d1d2$^{-/-}$ mice were stained for indicated markers to confirm that iNKT cell positive selection is still restricted to CD1d (data representative of $n = 2$). (*C*) Purified lymphocytes from the thymus, spleen, liver, and pLN of Jα18$^{-/-}$ mice were stained for indicated markers to confirm the specificity of iNKT cell staining (data representative of $n = 2$).

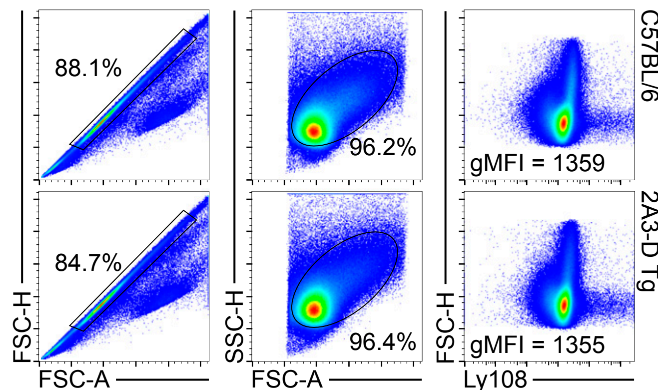

Fig S2. Ly108 expression levels are similar on C57BL6 and 2A3-D Tg thymocytes. Purified lymphocytes from the thymus of C57BL/6 or 2A3-D Tg mice were stained for indicated markers to assess Ly108 expression level. The gating strategy to obtain total thymocytes is depicted and Ly108 gMFI (geometric mean flouorescent intensity) is indicated as a representative value of $n = 3$.

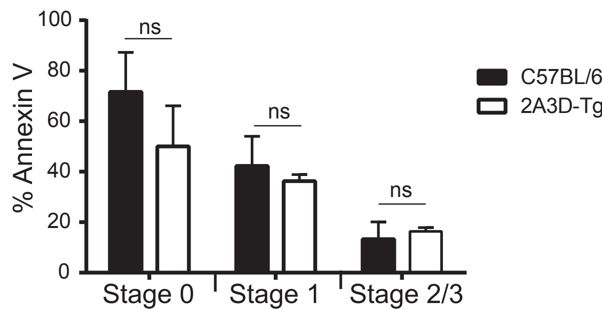

Fig S3. 2A3-D Tg NKT cells do not express more Annexin V compared with wild-type control. Total thymocytes were stained for CD24 and CD44 to define the different developmental stages. Here, the stages are defined as follows: stage 0 ($CD24^{high}CD44^{low}$), stage 1 ($CD24^{low}CD44^{low}$), stage 2/3 ($CD24^{low}CD44^{high}$). Total thymocytes were stained for Annexin V and 7-ADD to assess apoptosis and necrosis respectively (data representative of $n = 2$). ns, not significant.

A

Vα chain 1 2 3 4 5 6 7 8 9 10 11 12 13 14 16 17 18 19 H_2O

Spleen

pLN

B

C57BL/6

Jα18 Germline

Vα14 TA GAT AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT

TGT GTG GTG GGC G

Sequence	nb of sequences
TGT GTG GTG GGC GAT AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	2339
TGT GTG GTG GGA GAT AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	874
TGT GTG GTG GGT GAT AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	634
TGT GTG GTG GGG GAT AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	178
TGT GTG GTG GGC GAC AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	67

C V V G D R G S A L G R L H F

90 91 92 93 94 95 96 97 98 99 100 101 102 103 104

2A3-D

Jα18 Germline

Vα14 TA GAT AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT

TGT GTG GTG GGC G

Sequence	nb of sequences
TGT GTG GTG GGC GCG AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	3431
TGT GTG GTG GGC GCA AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	695
TGT GTG GTG GGC GCT AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	316
TGT GTG GTG GGC GCG CGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	294
TGT GTG GTG GGC GCC AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	182
TGT GTG GTG GGC GCG AGG GGT TCA GCC TTA GGG AGG CTG CAT TTT	85
TGT GTG GTG GGC GCG CGG GGT TCA GCC TTA GGG AGG CTG CAT TTT	58
TGT GTG GTG GGC GCA CGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	47
TGT GTG GTG GGC GCT CGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	40
TGT GTG GTG GGC GCC CGA GGT TCA GCC TTA GGG AGG CTG CAT TTT	20

C V V G A R G S A L G R L H F

90 91 92 93 94 95 96 97 98 99 100 101 102 103 104

C

Liver (22552 sequences)

Lymph Nodes (31727 sequences)

% Usage

TRAJ

D

94

CVVGVRGSALGRLHF

CVVGHRGSALGRLHF

CVVGIRGSALGRLHF

CVVGSRGSALGRLHF

CVVGERGSALGRLHF

CVVGNRGSALGRLHF

CVVGARGSALGRLHF

CVVGARGSALGRLHF

% Usage

CDR3 size (aa)

Fig S4. Complementary data for Fig. 5. (*A*) PBS57-CD1d tetramer-positive T-cell receptor (TCR)-β^{+} cells were sorted from the spleen and peripheral lymph nodes of 2A3-D Tg mice. mRNA was extracted and transformed into cDNA. cDNAs were amplified by PCR with Vα-specific forward primers and a Cα-specific reverse primer. (*B*) Sequences of the Vα14-Jα18 rearrangements found in iNKT cells from the spleen of C57BL/6 and 2A3-D Tg mice. For each observed rearrangement, the number of sequences, contribution of the Vα14 and Jα18 chain and existence of p-addition (blue) or n-addiction is depicted (data representative of $n = 2$). (*C*) PCR analysis was performed to evaluate the use frequency of TCR-α joining (TRAJ) genes encoding productive, in-frame rearrangements with the TRAV11 family in iNKT cells from the liver or the pLN or C57BL/6 or 2A-D Tg mice. (*D*) Amino acid composition and size of the CDR3 of the α-chain in C57BL/6 or 2A3-D Tg in iNKT cells from the liver and pLN.

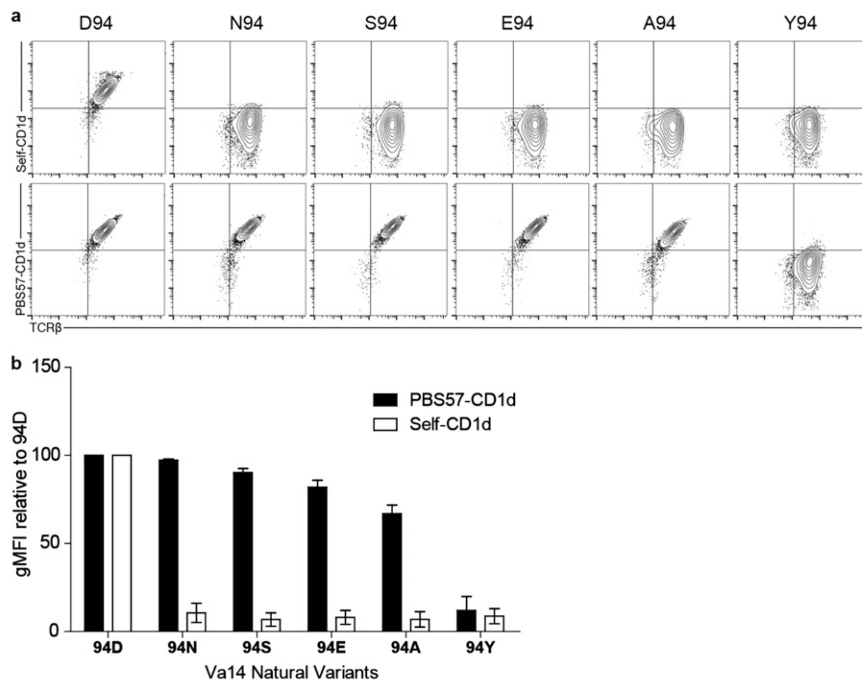

Fig S5. Specific loss of self-reactivity in Vα14 natural variants paired with 2A3-D Tg Vβ. (*A*) 5KC Hybridoma expressing 2A3-D Tg Vβ were transduced with indicated Vα14 natural variants and tested for reactivity with self-CD1d and PBS57-CD1d tetramers (data representative of $n = 4$). (*B*) For each 5KC hybridoma expressing a Vα14 natural variant, the gMFI of the PBS57-CD1d or self-CD1d tetramer staining was evaluated for a narrow slice of TCR expression. Relative percentage of this gMFI compared with the gMFI of the D94 variant with PBS57-CD1d and self-CD1d is shown ($n = 3$).

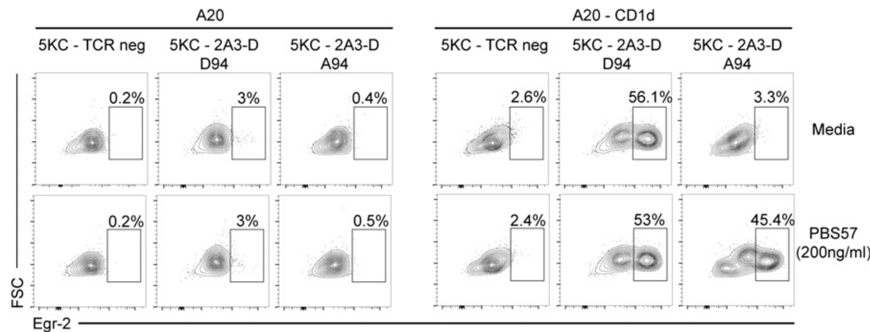

Fig S6. Hybridomas expressing the D94A Vα14 natural variant paired with 2A3-D Tg Vβ do not induce Egr-2 upon autoreactive response to CD1d-transfected A20 cells. The TCR^{-} 5KC hybridoma was transduced with the 2A3-D β-chain paired either with the D94 wild-type Vα14 iNKT chain or the A94 variant α-chain. Hybridoma were stimulated for 2 h with A20 lymphoma cells transfected or not with mouse CD1d in the presence or not of 200 ng/mL of the antigen PBS57. Following stimulation the levels of Egr-2 were measured by intracellular staining. The percentage of $Egr\text{-}2^{+}$ cells in each condition is shown. Results are representative of two independent experiments.