SUPPORTING INFORMATION

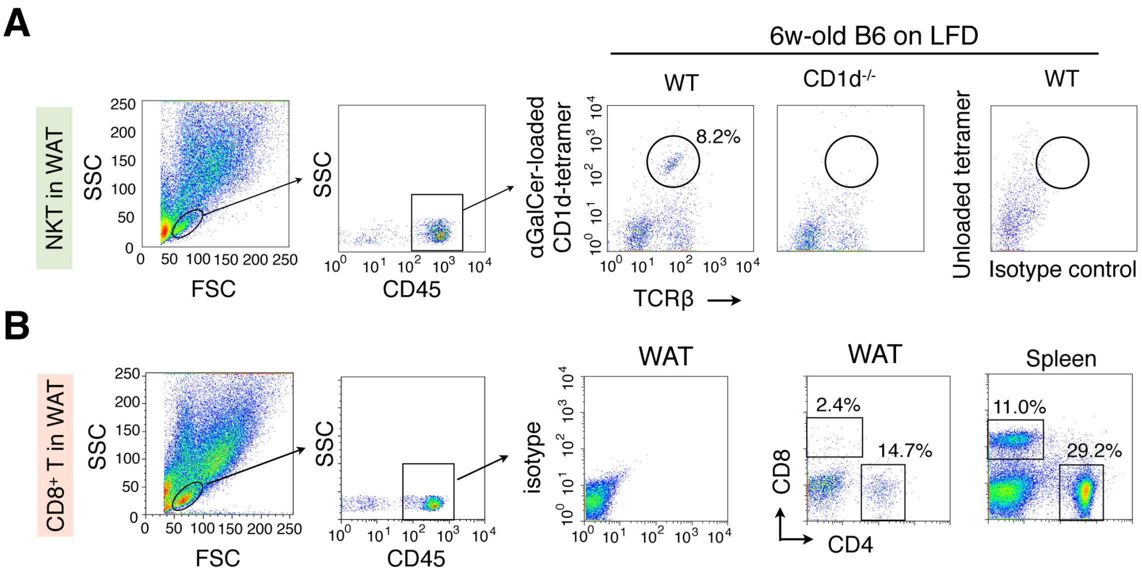
Activation of NKT Cells Promote M2 Macrophage Polarization in Adipose Tissue and Improves Systemic Glucose Tolerance via the IL-4/STAT6 signaling axis in Obesity

Yewei Ji[1], Shengyi Sun[2], Aimin Xu[3], Prerna Bhargava[4], Liu Yang[2], Karen S. L. Lam[3], Bin Gao[5], Chih-Hao Lee[4], Sander Kersten[1,6], and Ling Qi[1,2,]*

This supplementary information contains 7 figures and 1 table.

SUPPLEMENTARY FIGURES:

Fig. S1. (**A-B**) The diagrams illustrate our methods to gate NKT (**A**) and CD8$^+$ T cells (**B**) in various tissue of 6w-old B6 mice on LFD. Proper controls are included in each cell types. (**C**) Fatty acid compositions of diets used in this study provided by the suppliers.



C

Dietary fatty acid composition (mg/g diet)	**13% LFD (Teklad 2914)**	**60% HFD (Bio-Serv, F3282)**
Myristic (C14:0)	-	5
Palmitic (C16:0)	5	85
Palmitoleic (C16:1n7)	-	10
Stearic (C18:0)	1	48
Oleic (C18:1n9)	7	148
Linoleic (C18:2n6)	20	37
Linolenic (C18:3n3)	1	4
Calorie of fat	**13%**	**59%**
Calorie of protein	**20%**	**15%**
Calorie of CHO	**67%**	**26%**

Fig. S2. Diagram of germline loci of human *TCR* genes. Sizes of exons and introns not shown to scale. The α chain gene cluster contains ~50 variable (V) segments, ~70 joining (J) segments, and a single constant (C) segment. The β chain gene cluster consists of 75 V segments and two sets of D, J and C segments. Human type 1 NKT cells express invariant TCR containing Vα24 segment. Positions of three oligo sets used in **Fig. 2** are indicated. The distance between the forward (F) and reverse (R) primers of each primer set are indicated next to the R primer with the size of Q-PCR products shown in parentheses. Sequences for each primer is listed in **Table S1**.

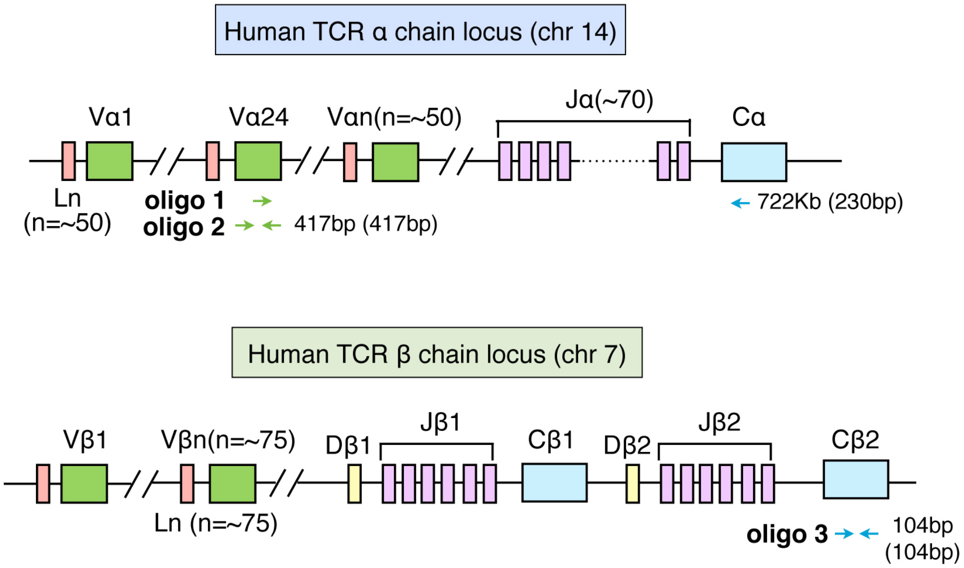


Fig. S3. GTT (**A**) of WT and CD1d$^{-/-}$ mice on 8-week HFD. n=8-9 mice each, 2 repeats. (**B-D**) Body and epididymal fat weights of mice under LFD or HFD with vehicle (veh) or αGalCer injection. Values represent mean ± s.e.m. *, *P*<0.05, **, *P*<0.01, ***, *P*<0.005.

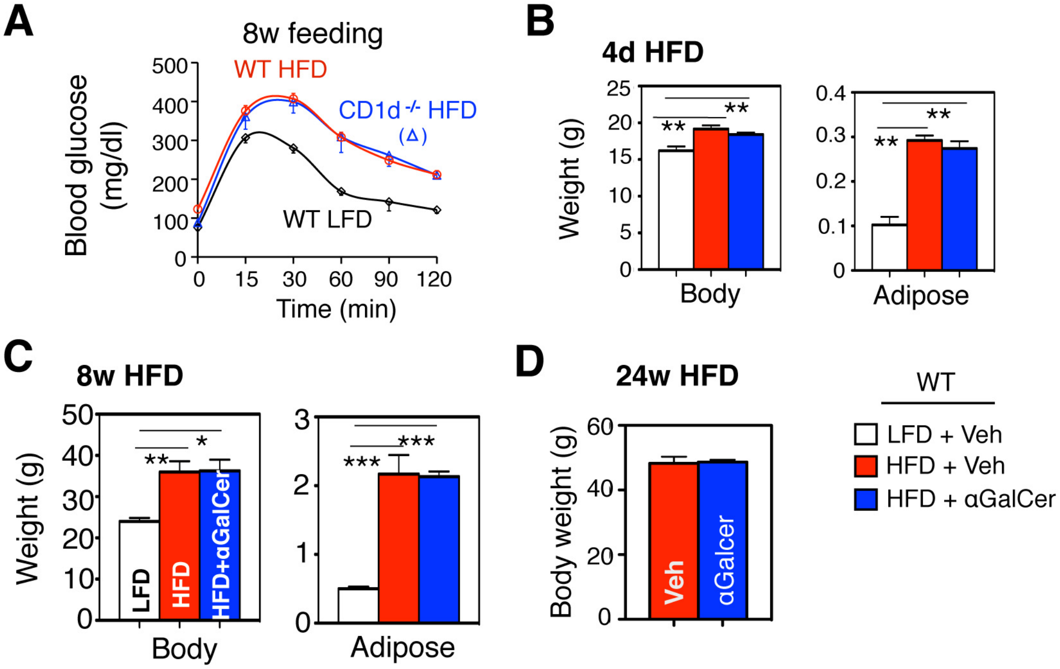


Fig. S4. (**A-B**) Quantitation of cell numbers per g adipose tissue of total CD45$^+$ lymphocytes, NKT cells, F4/80$^+$ CD11b$^+$ macrophages and CD8$^+$ T cells of mice that have been on LFD or 4d HFD (**A**) or 8w HFD (**B**) with vehicle or αGalCer challenge. N=4 mice each. (**C**) Flow analysis of NKT cells in adipose tissue of 14w-old mice that have been on HFD for 24w injected with αGalCer or vehicle (veh). Number refers to the percentage of NKT cells in total CD45$^+$ lymphocytes in SVC of adipose tissue. N=3-4 mice per cohort. Values represent mean ± s.e.m.*, $P<0.05$, **, $P<0.01$, and ***, $P<0.005$.

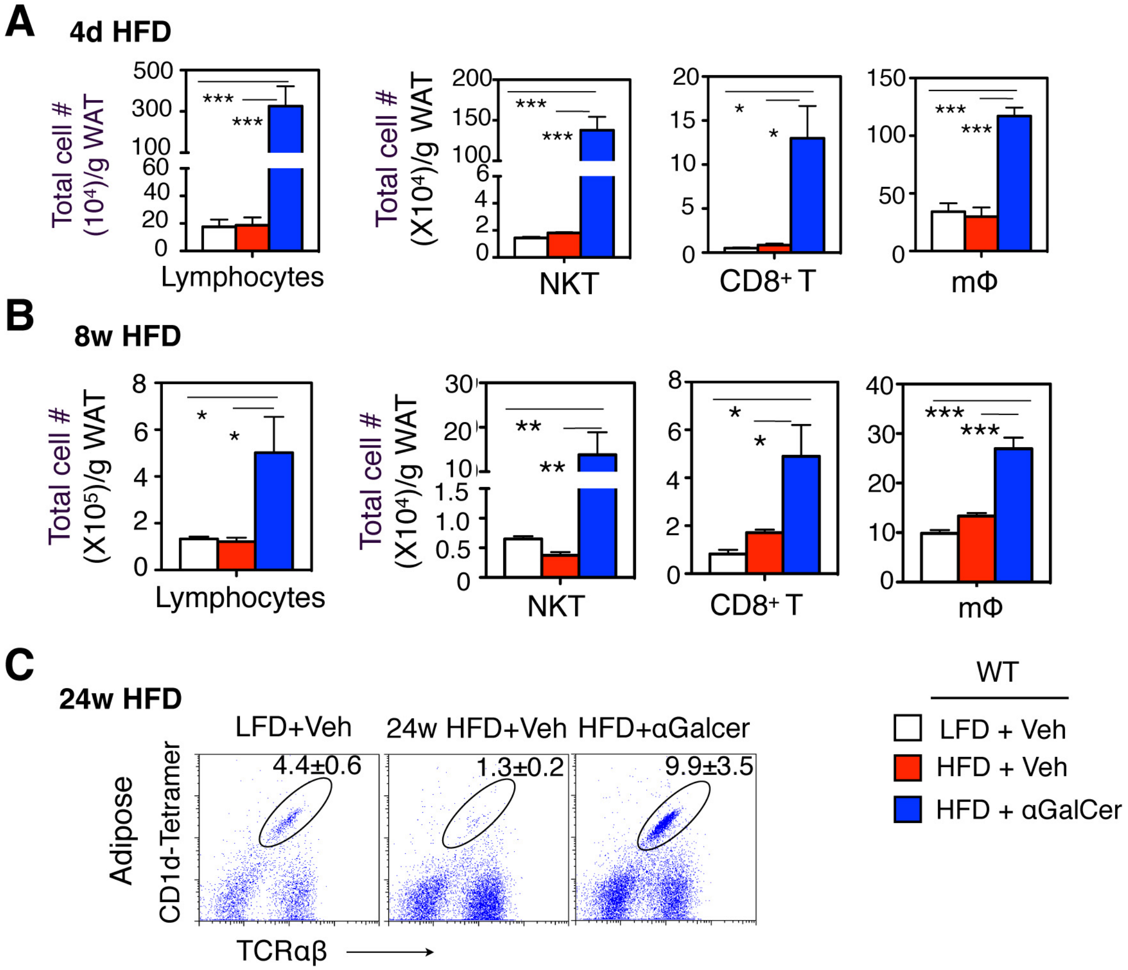


Fig. S5. (**A**) Q-PCR analysis of adipose tissue of 30w-old mice that have been on HFD for 24w were injected with αGalCer or vehicle (veh). N=3-4 mice per cohort. (**B**) Western blot analysis of Arg1 expression in the liver of various cohorts under 4d (upper) or 8w (lower) HFD feeding. n=4-5 mice each, 2 repeats. HSP90, a loading control.

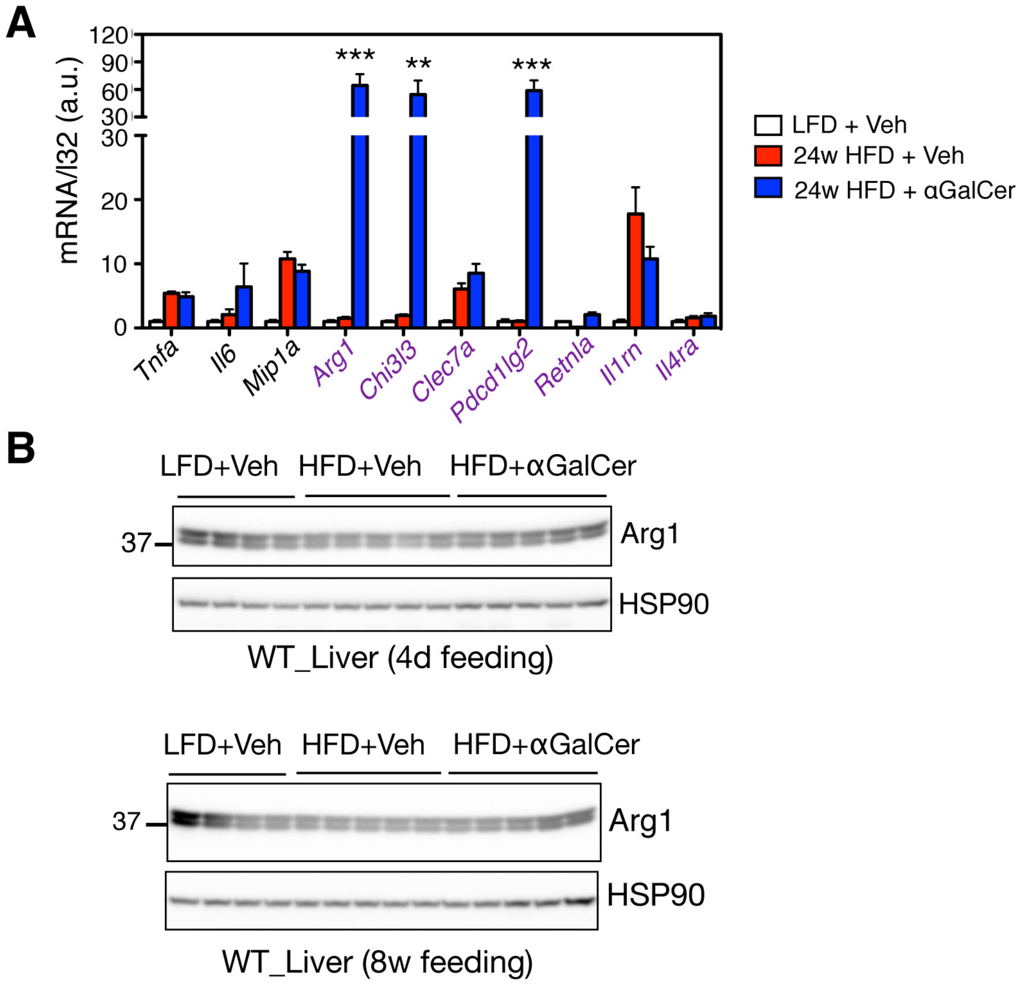


Fig. S6. (**A**) Hierarchical clustering of microarray data of individual mice depicted as dendogram showing clear separation of WT mice injected with αGalCer from the other groups. (**B**) Normalized enrichment score (NES) showing the most significant upregulated pathways identified by GSEA (all with false discovery rate q-value < 0.0003) in WT mice injected with αGalCer. "T_H1/T_H2 differentiation" and "IL-4 pathway" highlighted in red. (**C**) Diagram of the canonical IL-4 pathway illustrating the fold induction of each key component by αGalCer in WAT. Color scale shown at the bottom. Note that STAT6 is activated by phosphorylation, not at the transcriptional level.

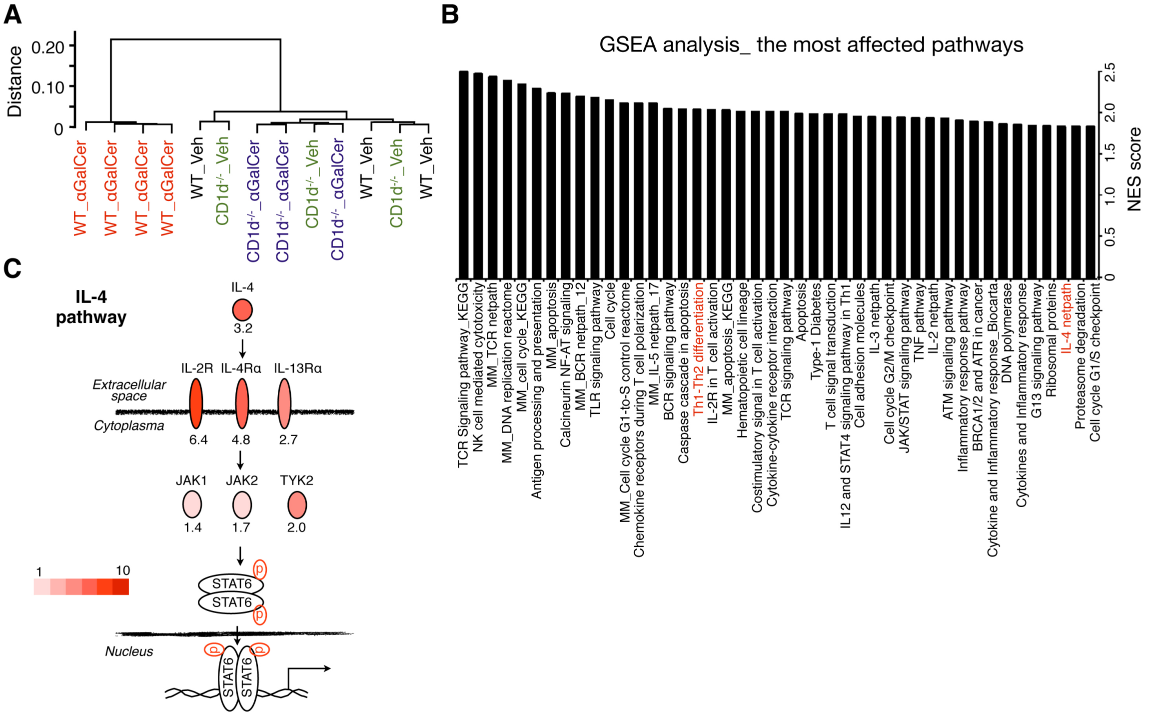


Fig. S7. (**A**) H&E section of adipose tissue of 4d HFD mice with veh or αGalCer injection (n=4-5 mice). (**B**) Total number of lymphocytes in adipose tissue of WT or IL-4$^{-/-}$ mice on 4d HFD with or without αGalCer challenge. N=5-6 mice each, 2 repeats. (**C**) Q-PCR analysis of M1 (black) and M2 genes (purple) in WAT of WT, PPARγ$^{-/-}$ (lower), PPARδ$^{-/-}$ (upper) mice following 4 day HFD feeding with or without αGalCer injection. Data normalized to "WT+vehicle" whose value was set at 1. Values represent mean ± s.e.m. **, *P*<0.01, and ***, *P*<0.005.

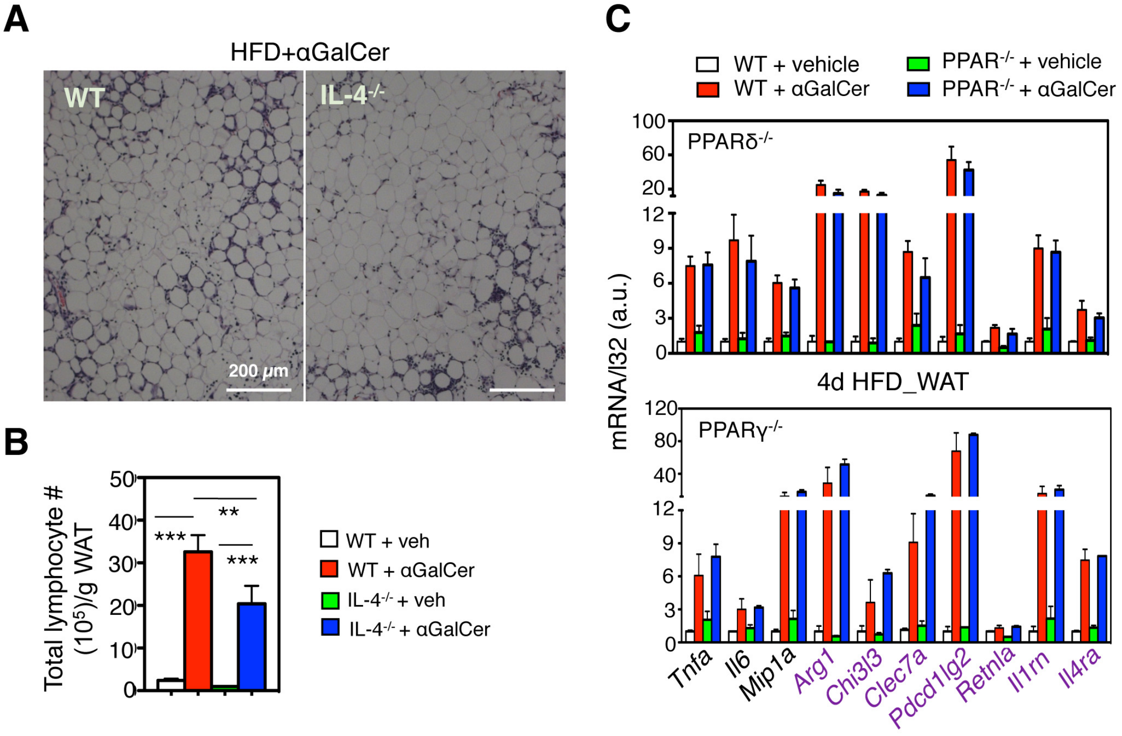


Table S1. Q- and RT-PCR primers used in this study.

Inflammatory gene analysis (mouse)		
Target genes	**Sequence F**	**Sequence R**
TNF-α (*Tnfa*)	TCAGCCGATTTGCTATCTCATA	AGTACTTGGGCAGATTGACCTC
Interleukin 4 (*Il4*)	CATGGGAAAACTCCATGCTT	TGGACTCATTCATGGTGCAG
Interleukin 6 (*Il6*)	AGACAAAGCCAGAGTCCTTCAG	TGCCGAGTAGATCTCAAAGTGA
MIP-1α (*Mip1a*)	TTCTCTGTACCATGACACTCTGC	CGTGGAATCTTCCGGCTGTAG
Arginase 1 (*Arg1*)	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
chitinase 3-like 3 (*Chi3l3*)	GGCTCAAGGACAACAATTTAGG	ACTGTGGAAAAACCGTTGAACT
C-type lectin domain family 7,member a (*Clec7a*)	TCATTGAAAGCCAAACATCG	CCTGGGGAGCTGTATTTCTG
Programmed cell death 1 ligand 2 *(Pdc1lg2)*	ACGTGGCCACTTCATGTTTT	TCTTGAGGGTTTCCCATCAG
Resistin like alpha (*Retnla*)	TATGAACAGATGGGCCTCCT	AGCTGGGTTCTCCACCTCTT
Interleukin 1 receptor antagonist (*Il1rn*)	TTGTGCCAAGTCTGGAGATG	TTCTCAGAGCGGATGAAGGT
Interleukin receptor 4 alpha (*Il4ra*)	GAAGCCAGGAGTCAACCAAG	ATACAGCGCACCACACTGAC
NKT TCR analysis (Human)		
Oligo 1: *TRAV10* (T cell receptor alpha variable 10 or Vα24) – *TRAC* (TCR constant)	GATATACAGCAACTCTGGATGCA	GGCAGACAGACTTGTCACTGGAT
Oligo 2: *TRAV10* (T cell receptor alpha variable 10 or Vα24)	AAGCATCTGACGACCTTCTTG	AACAGGACCTCTCCCAGTATC
Oligo 3: *TRBC2* (T cell receptor beta constant 2)	CAGCGAGCCCTACTCAAATTAG	GACCTGTGGAAGAGAGAACATT
18S	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA