Supplemental Materials for

CD8$^+$ T cells in beige adipogenesis and energy homeostasis

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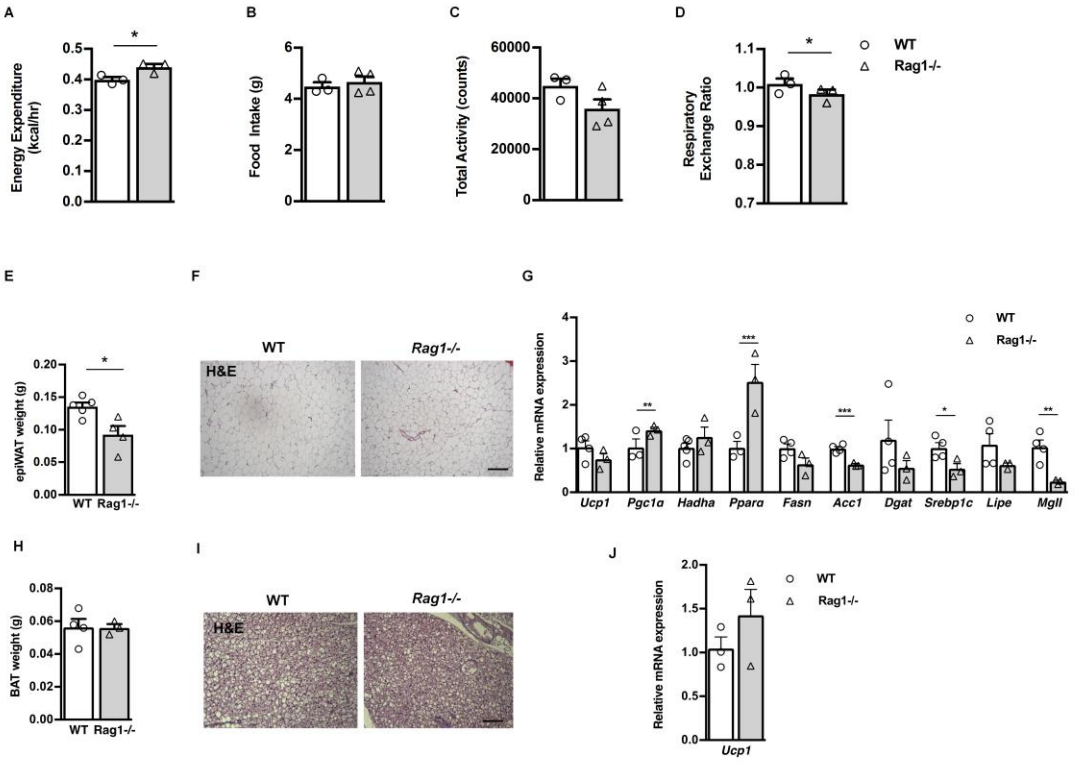
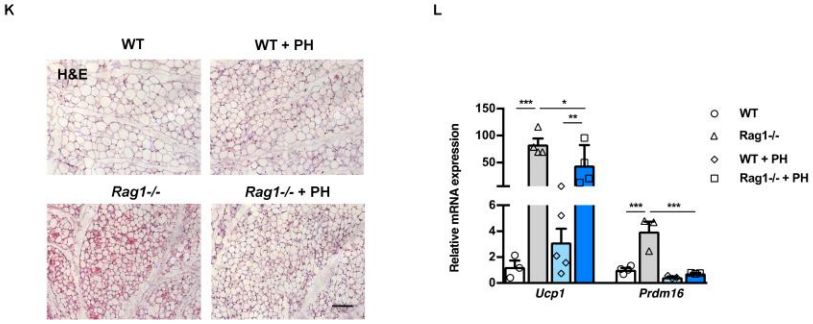
SUPPLEMENTAL FIGURES

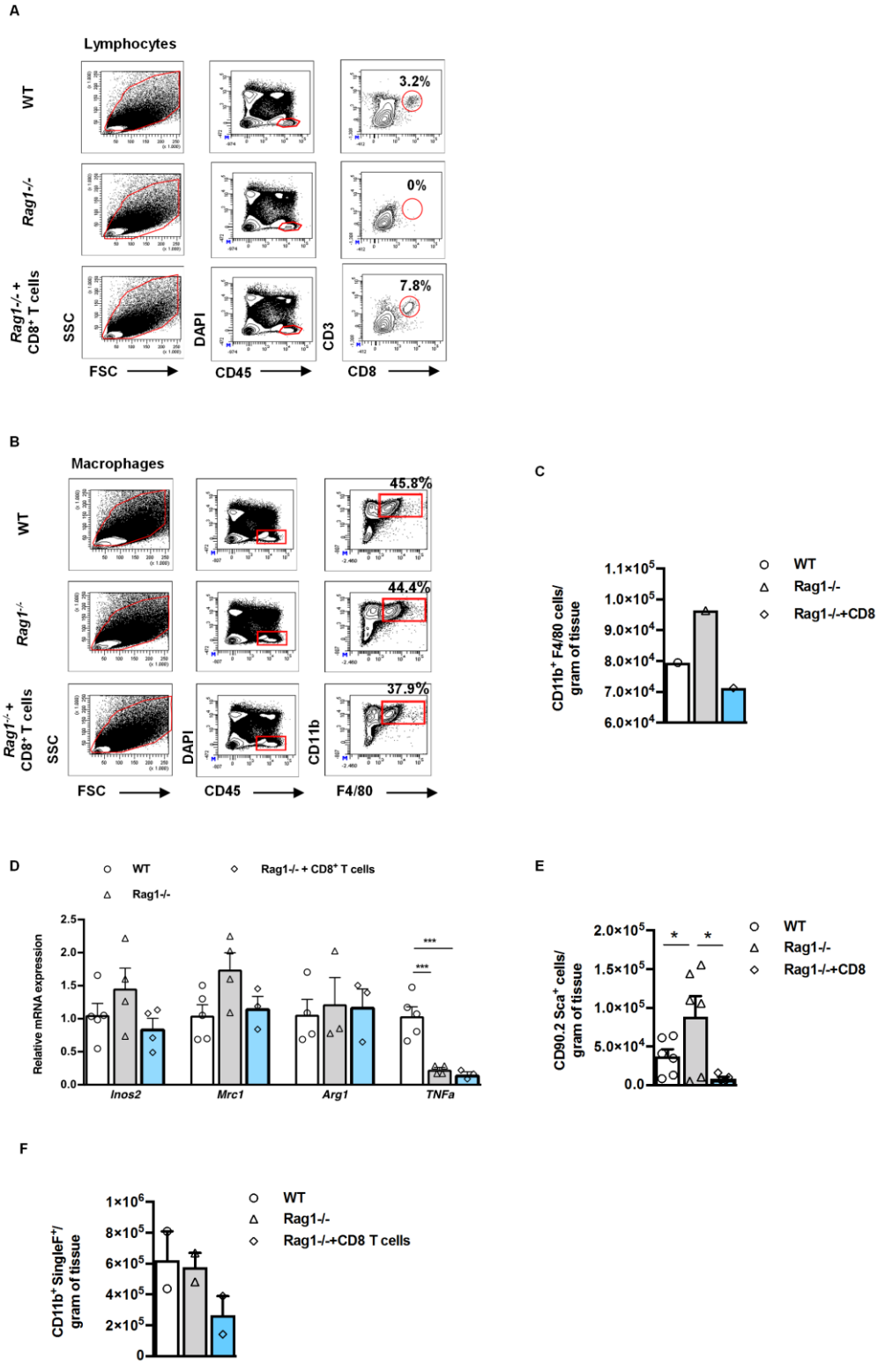




Supplemental Figure 1. Lymphocyte deficiency promotes more efficient lipid utilization in epiWAT.

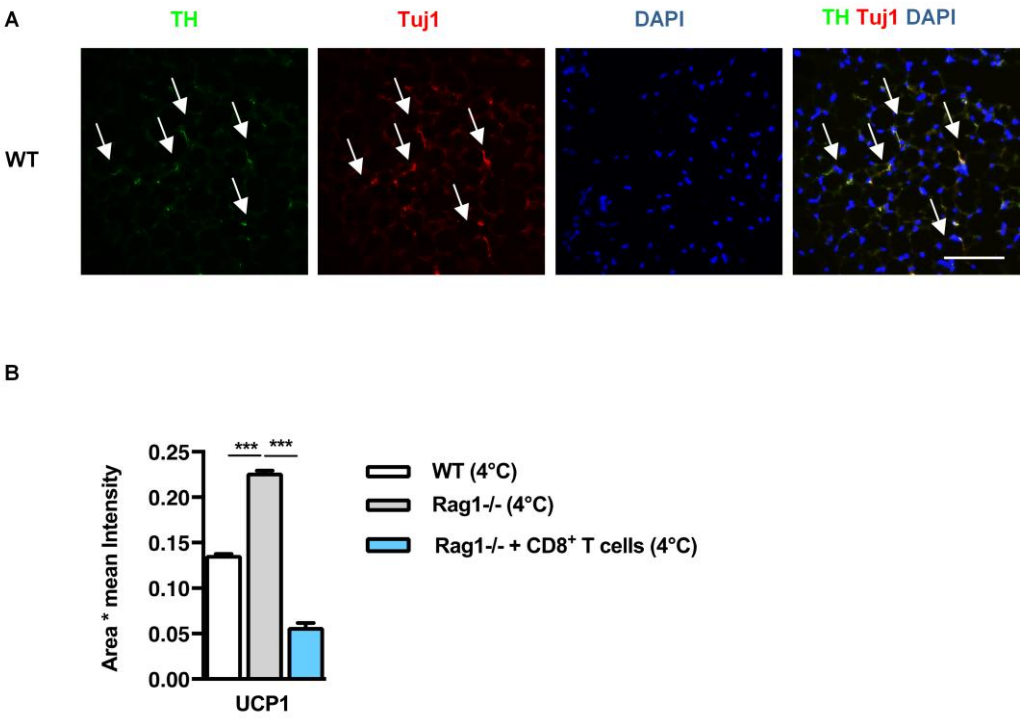
(A-D) Assessment of metabolic behavior using indirect calorimetry, including energy expenditure adjusted to body weight (A) food intake (B), total activity (C) and respiratory exchange ratio (RER) (D). n = 4 per group. Data shown are derived from one experiment

(E) Characterization of the absolute epiWAT weight of age and weight-matched WT and *Rag1*$^{-/-}$ mice. n=5 (F) Representative images of H&E staining. Scale bar: 100μm. (G) Gene expression analysis of *Ucp1* and lipid metabolism related genes in the above groups. Data are presented as mean expression normalized to actin ± S.E.M. n = 4 per group. P values, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, Student's t-test. (H) Characterization of the BAT weight in age and weight-mathced WT and *Rag1*$^{-/-}$ mice. n=5 (I) Representative images of H&E staining in the above groups. Scale bar:100 μm. (J) Gene expression analysis for *Ucp1* in the above groups. n = 4 per group. Data are mean expression normalized to actin ± S.E.M. Data shown was derived from one out of two independent experiments. (K) Representative images of H&E staining in the scWAT of WT, *Rag1*$^{-/-}$ and WT and *Rag1*$^{-/-}$ mice administered PH (8mg/kg) pre-diluted in PBS, via the drinking water. Control groups have received PBS containing drinking water for 5 days. Scale bar: 100μm. (L) Gene expression analysis of thermogenic markers in the above groups. Data shown are derived from one experiment. Data are presented as mean expression normalized to actin ± S.E.M. n = 4 per group. ***p* < 0.01, ****p* < 0.001, 2-way Anova with Bonferroni's post test.


A
Lymphocytes
WT
Rag1-/-
Rag1-/- + CD8+ T cells
3.2%
0%
7.8%
SSC
FSC
DAPI
CD45
CD3
CD8
B
Macrophages
WT
Rag1-/-
Rag1-/- + CD8+ T cells
45.8%
44.4%
37.9%
SSC
FSC
DAPI
CD45
CD11b
F4/80
C
CD11b+ F4/80 cells/ gram of tissue
1.1×10^5
1.0×10^5
9.0×10^4
8.0×10^4
7.0×10^4
6.0×10^4
WT
Rag1-/-
Rag1-/-+CD8
D
WT
Rag1-/-
Rag1-/- + CD8+ T cells
Relative mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
***
***
Inos2
Mrc1
Arg1
TNFa
E
CD90.2 Sca+ cells/ gram of tissue
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0.0
*
*
WT
Rag1-/-
Rag1-/-+CD8
F
CD11b+SingleF+/ gram of tissue
1×10^6
8×10^5
6×10^5
4×10^5
2×10^5
0
WT
Rag1-/-
Rag1-/-+CD8 T cells

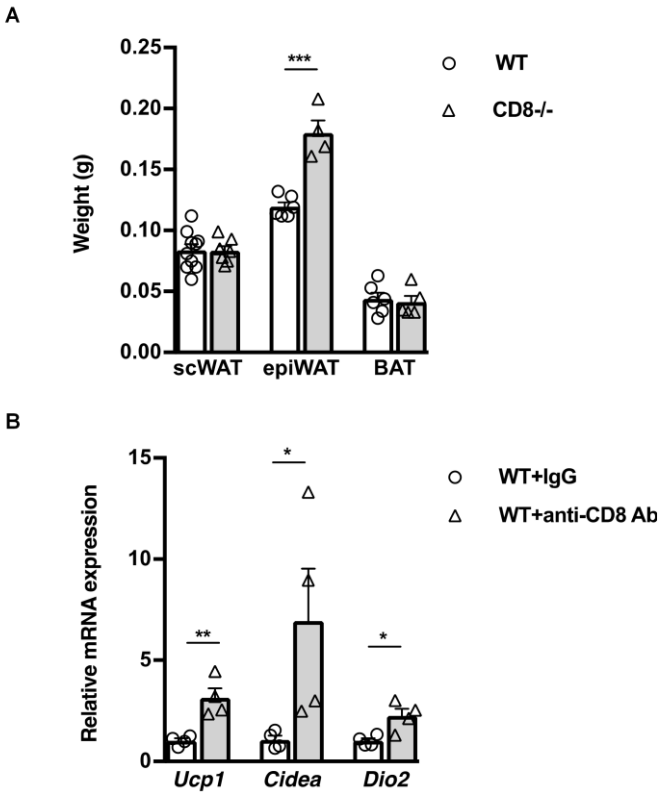
Supplemental Figure 2. Increased homing of $CD8^+$ T cells in the scWAT of *Rag1*$^{-/-}$ following adoptive transfer

(A)Representative gating strategy to identify mouse $CD8^+$ T cells in WT and *Rag1*$^{-/-}$ mice treated either with PBS or adoptively transferred with $CD8^+$ T cells ($5x10^6$), once/week for 2 weeks. Percentages of $CD3\varepsilon^+$ $CD8^+$ cells gated on Viable $CD45^+$ $DAPI^-$ cells with a low side scatter profile are depicted. Data shown are representative of two independent experiments. Flow cytometry was performed after pooling n = 5 mice per group (B) Gating strategy to identify murine $CD11b^+F480^+$ macrophages in WT mice or *Rag1*$^{-/-}$ mice treated with PBS or adoptively transferred with $CD8^+$ T cells ($5x10^6$), once/week for 2 weeks. Percentages of $CD11b^+F480^+$ cells gated on Viable $CD45^+$ $DAPI^-$ cells with a low side scatter profile are depicted. Data shown are one experiment. Flow cytometry was performed after pooling n=5 mice per group. (C) The absolute number of resident macrophages per gram of tissue in the above treatments. Data shown are one experiment. Flow cytometry was performed after pooling n = 5 mice per group. (D) Gene expression analysis for M1 (*inos2*, *TNFα*) and M2 (*Arg1*, *Mrc1*) markers in the above groups. n=4 per group. Data are mean expression normalized to actin ± S.E.M. Data shown are derived from one experiment. ***$p < 0.001$, Student's t-test. (E) The absolute numbers of total lineage (Lin)-negative $CD90.2^+$ $Sca\text{-}1^+$ ILCs per gram of tissue in the scWAT of WT, *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$ mice reconstituted with $CD8^+$ T cells. n=5 mice per group. Data shown are representative of two independent experiments and were analyzed by Student's t-test. Values are means ± S.E.M. *$p < 0.05$. (F) The absolute number of $CD11b^+$ Siglec F^+ gated on the viable $CD45^+DAPI^-$ cells per gram of tissue in the scWAT of WT, *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$ mice reconstituted with $CD8^+$ T cells. n = 5 per group. Data shown are derived from one experiment. Flow cytometry was performed after pooling n ≥ 5 mice per group.



Supplemental Figure 3. **Reconstitution of *Rag1*$^{-/-}$ mice with CD8^{+} T cells compromised significantly their response to cold.**

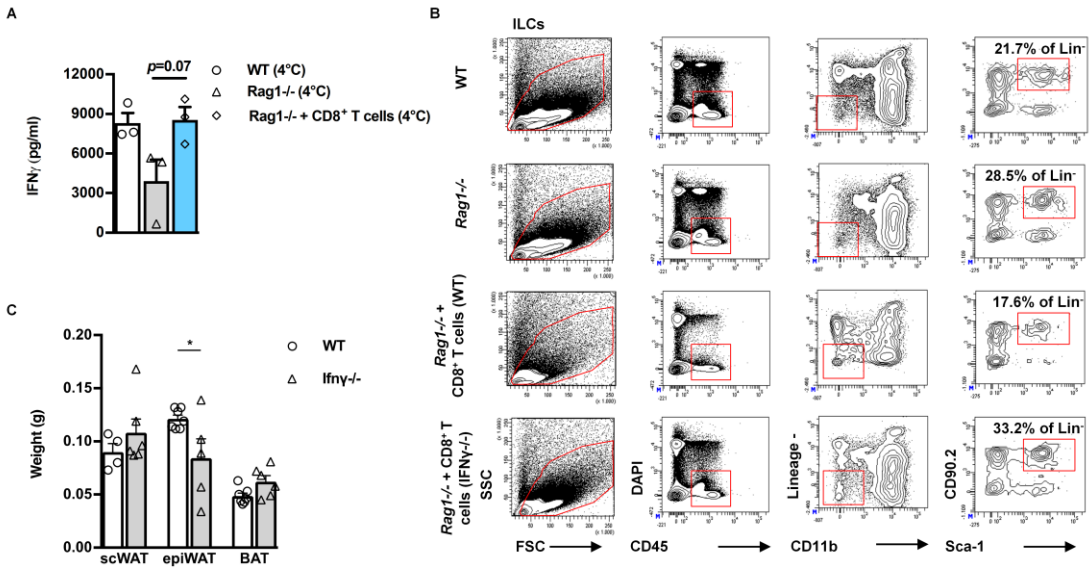
(**A**)Representative images of double IF staining for TH and Tuj1 of WT scWAT at baseline conditions. n=3 per group. Scale bar: 100μm. (**B**) Quantitation of mean intensity of UCP1 protein expression following cold exposure in the scWAT of WT, *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$ mice reconstituted with CD8^{+} T cells (5x10^{6}), once/week for 2 weeks. n = 3 per group. Data are means ± SD intensity of 15 patches for every image. ***p < 0.001. Data shown are derived from one out of two separate experiments.



Supplemental Figure 4. Impact of CD8$^+$ T cell depletion in the development of beige adipogenesis

(A) Characterization of the absolute scWAT, epiWAT and BAT weight of WT and *IFNγ*$^{-/-}$ mice. n=5. Data are presented as means ± S.E.M. ***p < 0.001.

(B) Relative expression of beige genes (*Ucp1, Cidea, Dio2*) of age and weight-matched WT either received anti-CD8 antibody or IgG. n=4. Data are mean expression normalized to actin ± S.E.M. *p < 0.05, **p < 0.01. t-test.



Supplemental Figure 5. IFNγ deficiency is associated with increase in total ILCs in the scWAT.

(A)ScWAT tissue levels of IFNγ (pg/ml) in WT mice or *Rag1*$^{-/-}$ following adoptive transfer of 5x10^{6} CD8^{+} T cells, or PBS injection (control), once/week for 2 weeks. n = 3 per group. Data are presented as means ± S.E.M. *p = 0,07*, 1-way Anova with Bonferroni's post test. (B) Gating strategy for the identification of lineage (Lin)-negative CD90.2^{+} Sca-1^{+} ILCs of WT or *Ifnγ*$^{-/-}$ scWAT, pre-gated on live CD45^{+} DAPI^{-} cells. Percentage of cells stained positive for CD90.2^{+} Sca-1^{+} is depicted on the flow cytometry plots. Data shown are from one single experiment. Flow cytometry was performed after pooling n ≥ 4 mice per group. (C) Characterization of the absolute scWAT, epiWAT and BAT weight of WT and *IFNγ*$^{-/-}$ mice. n=6. Data are presented as means ± S.E.M. *$p < 0.05$, t-test.

Supplemental Table 1: Primers used for real-time PCR

Gene	***Forward***	***Reverse***
Actin	5′-CCCAGGCATTGCTGACAGG- 3′	5′-TGGAAGGTGGACAGTGAGGC-3′
Pgc1α	5′-TCACCCTCTGGCCTGACAAATCTT- 3′	5′-TTTGATGGGCTACCCACAGTGTCT- 3′
Lipe	5'-AAGGACTTGAGCAACTCAGA-3'	5'-TTGACTATGGCTGACGTGTA-3'
Pparα	5′-AAGAACCTGAGGAAGCCGTTCTGT- 3′	5′-GCAGCCACAAACAGGGAAATGTCA-3′
MgII	5′-GACGGACAGTACCTCTTTTG- 3′	5′-AGAAAAGTAGGTTGGCCTCT-3′
Hadha	5′-AGCAAGTGTTCAAAGGGCTGAACG- 3′	5′-TGTGCTTTACACCGAGGTCCTCAA- 3′
Ucp1	5'-TCTTCTCAGCCGGAGTTTCAGCTT-3'	5'-ACCTTGGATCTGAAGGCGGACTTT-3'
Cidea	5'-ATCACAACTGGCCTGGTTACG-3'	5'-TACTACCCGGTGTCCATTTCT-3'
Prdm16	5'-CAGCACGGTGAAGCCATTC-3'	5'-GCGTGCATCCGCTTGTG-3'
Dio2	5'-CAGTGTGGTGCACGTCTCCAATC-3'	5'-TGAACCAAAGTTGACCACCAG-3'
Fgf21	5'-TACACAGATGACGACCAAGA-3'	5'-GGCTTCAGACTGGTACACAT-3'
AdR1α	5'-GGGTCCTTCTTCCCGAATTT-3'	5'-GCTGGAGCATGGGTATATGATAG-3'
AdR3β	5'-TGAAACAGCAGACAGGGACA-3'	5'-TCTTGACACTCCCTCAGCAC-3'
Fasn	5'-CTCCGTGGACCTTATCACTA-3'	5'-CTGGGAGAGGTTGTAGTCAG-3'
Acc1	5'-TAACAGAATCGACACTGGCTGGCT-3'	5'-ATGCTGTTCCTCAGGCTCACATCT-3'
Dgat	5'-TCATGGGTGTCTGTGGGTTA-3'	5'-CAGAGTGAAACCAGCCAACA-3'
Srepb1c	5'-TGGCTTGGTGATGCTATGTT-3'	5'-TAAGGGGTTGGGAGTAGAGG-3'
Cyclophilin	5'- CATCCTAAAGCATACAGGTCCTG-3'	5'-TCCATGGCTTCCACAATGTT-3'
iNOS	5'- CAGAGGACCCAGAGACAAGC-3'	5'-CCTGGCCAGATGGGCCTCTA-3'
Arg1	5'- AGACCACAGTCTGGCAGTTG-3'	5'-CCACCCAAATGACACATAGG-3'