Supplementary Materials for

Immunological mechanisms of the antitumor effects of supplemental oxygenation

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Supplementary Materials

Supplementary Materials and Methods

Adoptive NK cell Transfer. For adoptive NK cell transfer experiments and *in vitro* analyses, splenocytes from naïve mice were isolated and cultured with high dose (6000 U/ml) IL-2 for 6 days. To exclude other lymphocytes, only adherent cells were retained and replenished with IL-2, every 2 days. Activated NK cells were isolated, resuspended in HBSS, and infused (10×10^6) into mice with 4-day established B16 lung tumors. Mice were placed in either 60% or 21% oxygen, and the number of tumor nodules per lung was assessed after 21 days.

LPS-induced lung injury. LPS was intra-tracheally injected at a dose of 4 mg/kg body weight in a total volume of 50 µl per mouse under isoflurane anesthesia. After IT injection of LPS into unconscious but spontaneously breathing mice, animals were held in an upright position for 15 s and then briefly shaken in all directions to ensure homogenous fluid dispersion in the lung. Experimental mice were injected i.p. daily for 48 h with 150 mg/kg N-acetylcysteine in HBSS. After 48 h, mice were sacrificed, their lungs were lavaged with 500 µl PBS, and cells were incubated for 15 min at 37°C with 10 µm dichlorofluoresceine diacetate (DDF-DA, Molecular Probes) as a reactive oxygen species detection reagent. Flow cytometry was used to detect the mean fluorescent intensity of 2' 7'-dichlorofluoresceine in LPS control and NAC-treated mice.

Supplementary Figures

Fig. S1. The tumor-regressing effects of respiratory hyperoxia are lost in cγ/*Rag-2*$^{-/-}$ mice.
Comparison of cγ/*Rag-2*$^{-/-}$ and wild type mice breathing 21% and 60% oxygen are provided in
(**A**), showing an image of each lung and direct enumeration of the number of MCA205 lung
tumors (n = 5 mice/group, P = 0.0001), (**B**) calculations comparing the total tumor-bearing
lung weight (n = 5 mice/group, P = 0.0002), (**C**) representative image of H/E-stained lung
sections used to quantify percent tumor saturation versus normal tissue (shown are the areas of
tumor tissue outlined in pink on lung sections from tumor-bearing mice breathing 21% or 60%
oxygen), and (**D**) the calculation of the ratio between area occupied by tumor versus normal
lung tissue on lung sections (n = 5 mice/group, P = 0.004).
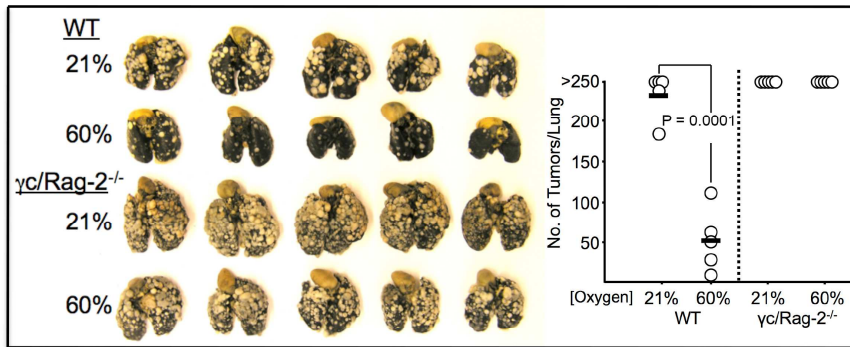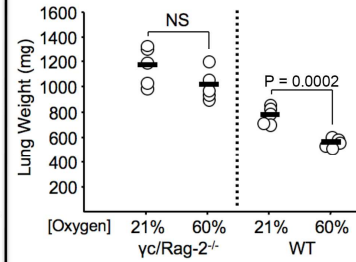
Fig. S2. ROS scavenger does not prevent the antitumor effects of respiratory hyperoxia.

A. N-acetylcysteine inhibits the production of reactive oxygen species in an LPS-induced lung injury model, where ROS have been implicated in tissue damage (26-29). Flow cytometric assays demonstrate that NAC (150 m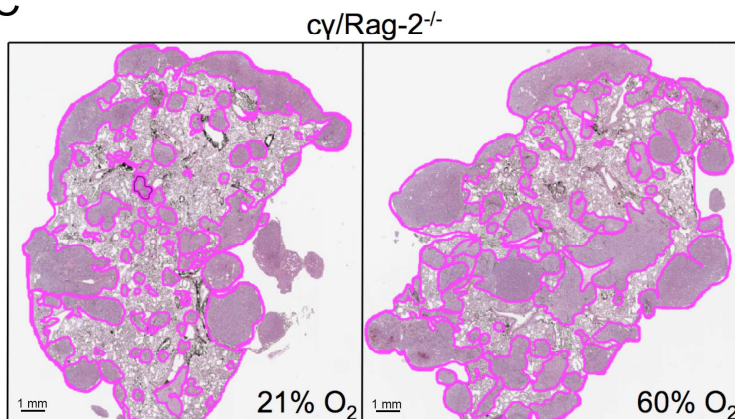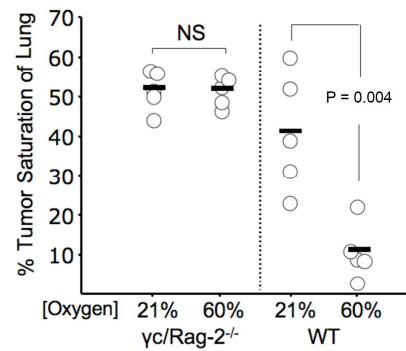g/kg) reduces the mean fluorescent intensity of DCF, a reactive oxygen species indicator (ROS Detection Reagents, Molecular Probes). The average mean fluorescent intensity of DCF was 452 in LPS control mice versus 174 in LPS mice treated with NAC (n = 4 mice/group, P = 0.001). Dotted line represents unstained control.
B. N-acetylcysteine was unable to interfere with the tumor-regressing effects of 60% oxygen in mice with established tumors treated daily with the same dose (150 mg/kg, i.p.) of the broad ROS scavenger, starting immediately before respiratory hyperoxia (n = 5 mice/group, P = 0.0001.

S2
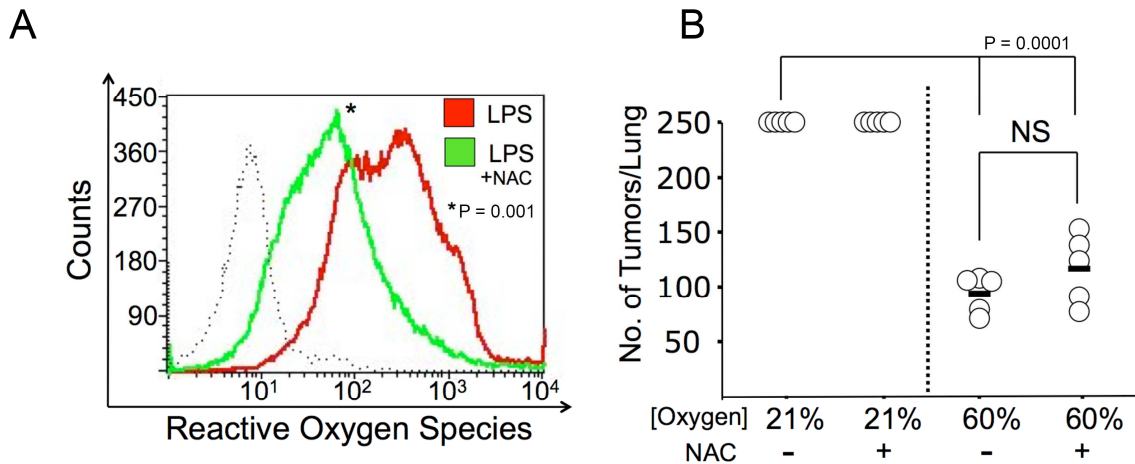
Fig. S3. Respiratory hyperoxia reverses hypoxia-adenosinergic inhibition of NK cells.

A, B. Expression of **(A)** NKG2D and **(B)** CD69 on NK cells is reduced by hypoxia and A2 adenosine receptor activation in in vitro experiments. Interleukin-2-activated NK cells were cultured in vitro in 1% or 21% oxygen or with and without 10 μm CGS (A2AR agonist) or NECA (A2AR/A2BR agonist) for 48 h. The expression levels were determined by the mean fluorescent intensity of NKG2D and CD69 as measured by flow cytometry (n = 4/group). [**A.** Average MFI for NKG2D: Control = 139; NECA =118 (P = 0.006), 1% O_2 = 76 (P = 4.3×10^{-6}). **B.** Average MFI for CD69: Control = 201; CGS = 150 (P = 0.015), 1% O_2 = 153 (P = 6.1×10^{-5})].

C. After 24 and 48 h incubation of activated NK cells with IL-2, levels of IFN-γ were measured by ELISA (R&D Systems) (n = 4/group, $P = 1.9 \times 10^{-7}$ for CGS and $P = 2.5 \times 10^{-7}$ for 1% O_2).

D. Adoptive transfer of activated NK cells in combination with respiratory hyperoxia enables stronger regression of 4-day established B16 pulmonary tumors [n = 5 mice/group; averages represented as horizontal bars, P = 0.0002 (21% O_2+NK vs. 60% O_2+NK) and P = 0.001 (60% O_2 vs. 60% O_2+NK)]. For adoptive NK cell transfer experiments and in vitro analysis, splenocytes from naïve mice were isolated and cultured with high dose (6000 U/ml) IL-2 for 6 days. To exclude other lymphocytes, only adherent cells were retained and replenished with IL-2 every 2 days. Activated NK cells were isolated, resuspended in HBSS, and infused (10×10^6) into mice with 4-day established B16 lung tumors. Mice were placed in either 60% or 21% oxygen, and the number of tumor nodules per lung was assessed after 21 days.

S3
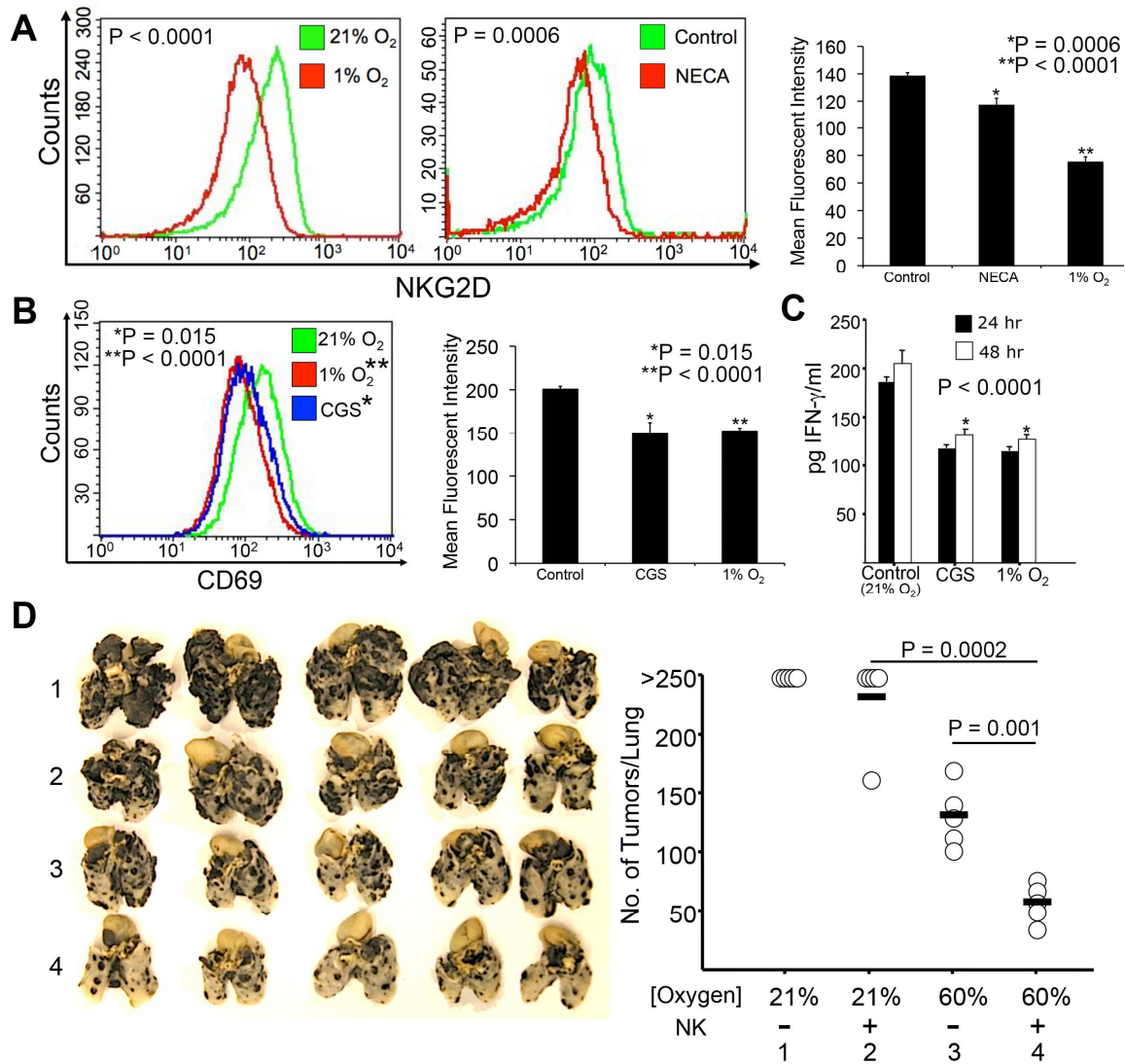
Fig. S4. Respiratory hyperoxia does not further improve the activity of tumor-reactive A2AR$^{-/-}$ T cells. Adoptively transferred WT or A2AR$^{-/-}$ TDLN T cells (5×10^6) were transferred into cγ/Rag-2$^{-/-}$ mice with 11-day established MCA205 lung tumors, and the mice were placed in either 21% or 60% oxygen. To overcome inherent difficulties in quantitation of the number of lung tumor foci in control cγ/Rag-2$^{-/-}$ mice, where tumors grow rapidly, tumor burden was assessed using dry lung weight on day 21 [(n = 5 mice/group, averages represented as horizontal bars, P = 0.001(WT 21% → 60%)].
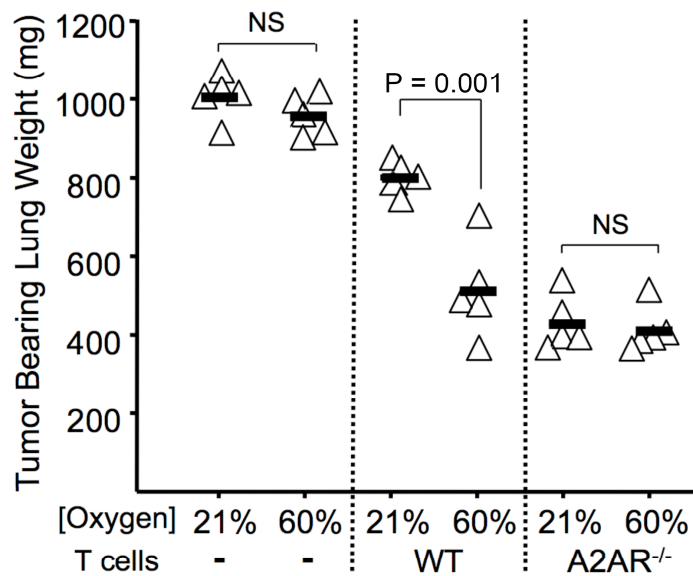
Fig. S5. CD8 and CD4 T cells avoid hypoxic TME.

A. Fluorescence micrographs (scale bar = 100 µm) of the preferential localization of CD8 and CD4 T cells (red) in normoxic TME (blue, DAPI) compared to hypoxic TME (green, Hypoxyprobe) of intra-dermal MCA205 tumors.

B. Comparison of the number of CD8 or CD4 T cells in normoxic versus hypoxic TME (n = 3 mice; *P = 0.001 comparing CD4 T cells from normoxic and hypoxic areas, **P = 0.0002 comparing CD8 T cells from normoxic and hypoxic areas).

C. Fluorescence micrograph (scale bar = 1 mm) of CD8 T cells avoiding hypoxic areas of an intradermal tumor section using a Zeiss LSM 710 confocal microscope, stitched using Zeiss Zen Blue Imaging software.

S5 A

Intradermal Tumors

CD4

CD8

B

**P = 0.0002
*P = 0.001

6
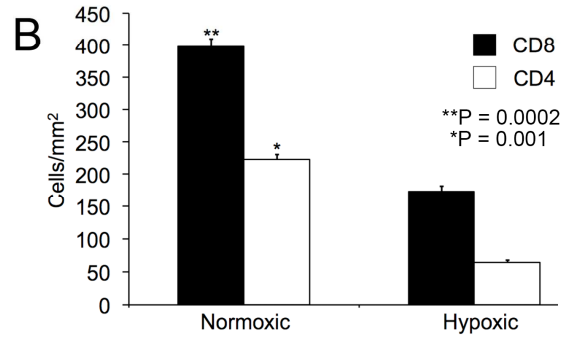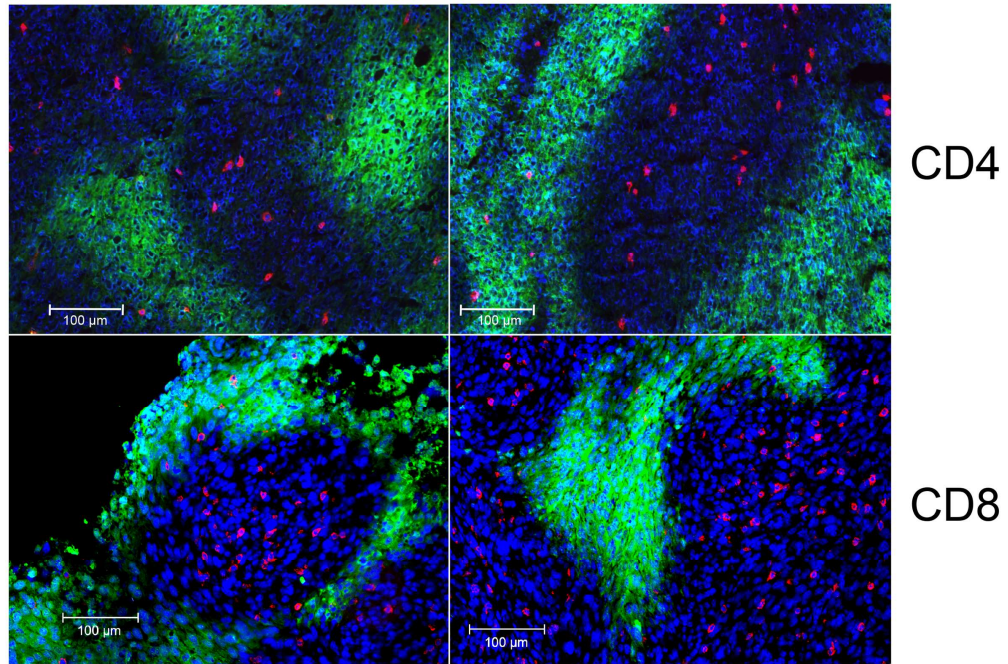
S5 C   Intradermal Tumor
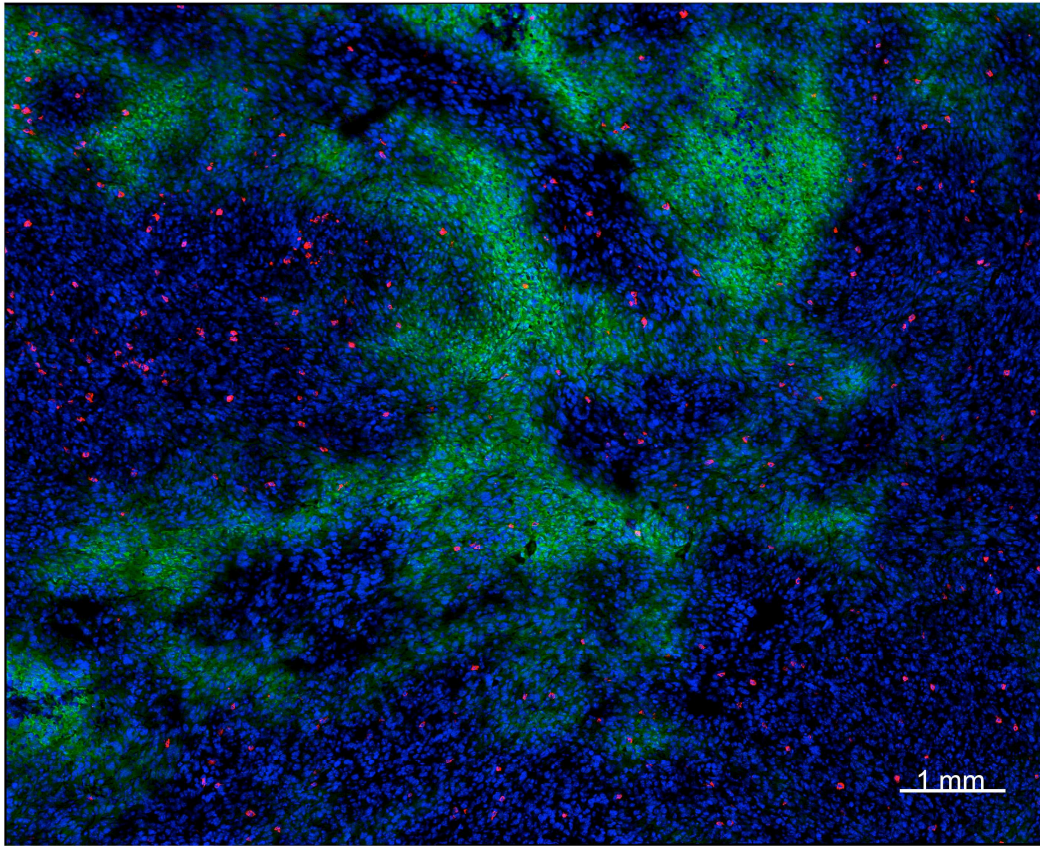
Fig. S6. T$_{regs}$ with higher expression of CTLA-4 are more hypoxic. CTLA-4^{High} T regs from the spleen of tumor-bearing mice were also Hypoxyprobe^{High}, reflecting in vivo exposure to deeper levels of hypoxia. Hyperoxic breathing decreased the number of CTLA-4^{High} T regs compared to mice breathing 21% oxygen (P = 0.0001, n = 4 mice/group).
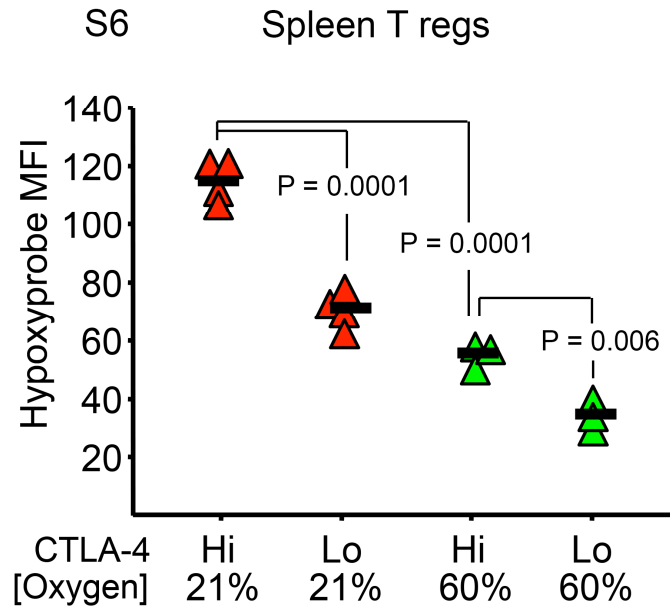
Fig. S7. CD8 T cells from TDLN are enriched after culture activation for adoptive transfer. In a typical preparation, adoptively-transferred cells consist of approximately 70-80% CD8$^+$ and 15-20% CD4$^+$ cells. Shown in red are the percentages of CD8 (75.8) and CD4 (11.6) T cells after culture activation.
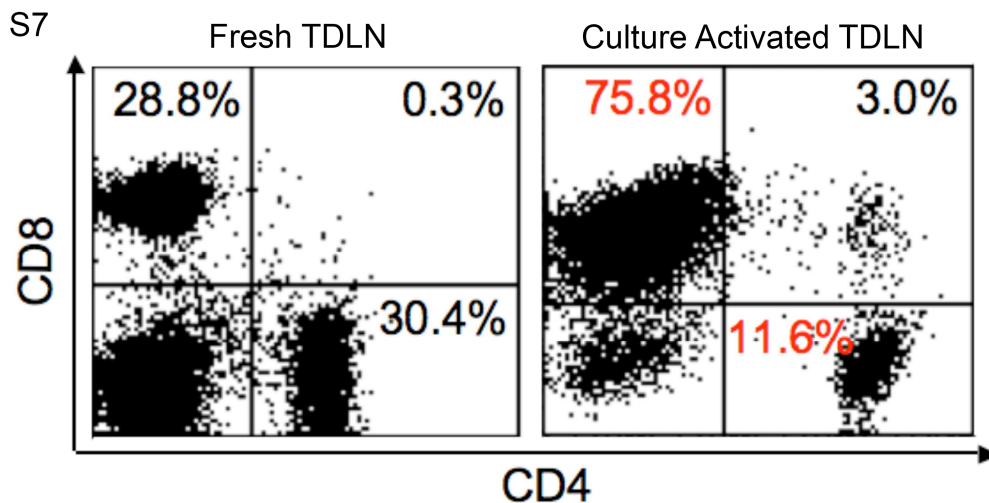
Fig. S8. Breathing 60% oxygen increased IFN-γ production by CD8 T cells in the lung TME. After 4 days of respiratory hyperoxia, adoptively transferred CD8$^+$ (Thy 1.1$^+$) and endogenously developed CD8$^+$ (Thy 1.2$^+$) demonstrate increased IFN-γ production. The average number of CD8 T cells (Thy 1.1$^+$ and Thy 1.2$^+$) expressing IFN-γ in mice treated with respiratory hyperoxia was 42% compared to 32% in mice breathing ambient oxygen (n = 3 mice/group, P = 0.002).
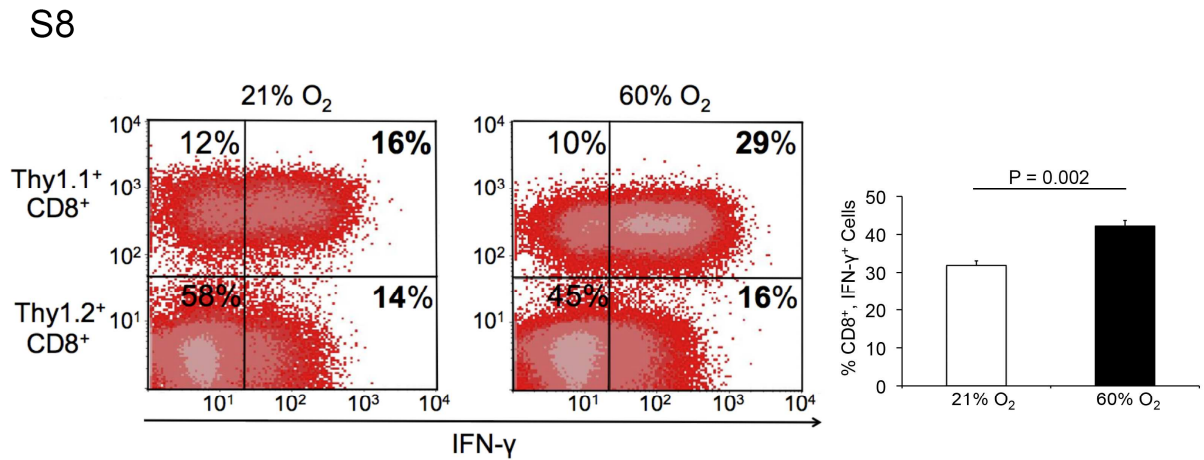
Table S1. Immunostimulating cytokines/chemokines increased by respiratory hyperoxia.
The fold-increase in the RNA levels and the exact P-value for each gene is listed.

Cytokine/Chemokine	Fold-Increase	P value
IL-12a	35.19703181	0.001361776
IL-2	2.05436743	0.052619196
CXCL9	8.57769879	0.029648982
CXCL10	2.478411813	0.012345832
CXCL11	2.2332708	0.037631946
CCL3	6.51554072	0.007171583
CCL9	13.47993625	0.002393171
CCL20	26.64894485	0.015096529
CCL26	17.74878418	0.023460324
CCR3	4.0747839	0.045264066

Table S2. Full list of primer sets in RT-PCR arrays.

Taylor Schreiber
Primer Designs for Chemokine and Cytokine RT-PCR (mouse)

CCL4
>NM_013652 NM_013652 Mus musculus chemokine (C-C motif) ligand 4 (Ccl4),
mRNA. 3/2007
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CAGCAGTCTTTGCTCCAAGCCAGCTGTGGTATTCCTGACCAAAAGAGGCAGACAGATCTG
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	Sequence	Tm
Forward	TCTGCCCTCTCTCTCCTCTTGCT	62
Reverse	TTGGTGCTGAGAACCCTGGA	60

CCL17
>NM_011332 NM_011332 Mus musculus chemokine (C-C motif) ligand 17 (Ccl17),
mRNA. 12/2006
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AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

	Sequence	Tm
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Reverse	TCGGCCTACATTGGTGGCT	60

IL4
>NM_021283 NM_021283 Mus musculus interleukin 4 (Il4), mRNA. 3/2007
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AATGT

	Sequence	Tm
	Sequence	Tm

| Forward | TTGAACGAGGTCACAGGAGAAGG | 61 |
| Reverse | GAGGACGTTTGGCACATCCA | 60 |

IL13
>NM_008355 NM_008355 Mus musculus interleukin 13 (Il13), mRNA. 1/2007
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GCCGCCCCAGGGCCGGTGCCAAGATCTGTGTCTCTCCCTCTGACCCTTAAGGAGCTTATT
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GACAGAT

	Sequence	Tm
Forward	CGCTGGCGGGTTCTGTG	61
Reverse	ATGGCATTGCAATTGGAGATGT	60

CCL8
>NM_021443 NM_021443 Mus musculus chemokine (C-C motif) ligand 8 (Ccl8), mRNA. 11/2006
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	Sequence	Tm
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Reverse	GCACCCGAAGGGGGATCTT	62

CCL9
>NM_011338 NM_011338 Mus musculus chemokine (C-C motif) ligand 9 (Ccl9), mRNA. 3/2007
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Reverse	CGCTGGGAAACCGAGTGG	61

CCR1L1

>NM_007718 NM_007718 Mus musculus chemokine (C-C motif) receptor 1-like 1
(Ccr1l1), mRNA. 11/2006

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TGGACGCCAACAAAGGCC
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	Sequence	Tm
Forward	CACTTGCCTACACCCACTGCTG	62
Reverse	CCGGAACCGTTTACCCACA	60

CXCL4

>NM_019932 NM_019932 Mus musculus chemokine (C-X-C motif) ligand 4 (Cxcl4), mRNA. 11/2006

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AATTGTGTTACAAGACTCCTGGAATCTTGTCTACTTTTAATGTAACTGCAATCTTCCGAT
GTTTATATTATCCTTCAAGATTTAAATAAATGCATTGAACCAAAG
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	Sequence	Tm
Forward	GCTGCTTCTGGGCCTGTTG	61
Reverse	TTCTTCGGGACCAGCGCT	60

CCR3

>NM_009914 NM_009914 Mus musculus chemokine (C-C motif) receptor 3 (Ccr3), mRNA. 1/2007

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GGTTATCTCTGTTTCATTAGCAGTGAAACTTAAGTTACTACTGGACTCATAAAGGACTTA
GCAAAATTCACCAGAGACAAGTAGAATGGCATTCAACACAGATGAAATCAAGACTGTGGT
TGAAAGCTTTGAGACCACACCCTATGAATATGAGTGGGCACCACCCTGTGAAAAAGTCAG
AATCAAAGAGCTGGGGTCATGGCTCCTGCCTCCACTGTACTCCCTGGTGTTCATCATCGG
CCTCCTGGGCAACATAATGGTTGTGTTGATCCTCATAAAGTACAGGAAGCTACAAATTAT
GGCTAATATCTACCTGTTCAACTTGGCAATTTCTGACCTGCTCTTTCTCTTCACTGTCCC
ATTCTGGATTCACTATGTTCTGTGGAATGAGTGGGGTTTTGGCCACTACATGTGCAAAAT
GCTGTCTGGGTTTTATTACCTGGCCTTGTACAGCGAGATCTTTTTCATCATTCTGCTGAC
AATTGACAGATACCTGGCTATCGTCCATGCTGTGTTTGCCCTTCGAGCCCGAACTGTGAC
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ATTTATCTTCCATGAGTCTCAAGACAGCTTTGGAGAGTTTTCCTGCAGTCCTCGCTATCC
AGAGGGTGAAGAAGACAGCTGGAAACGTTTCCATGCTCTAAGAATGAATATCTTTGGTCT
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GATTGCCTACACCCACTGCTGTATTAATCCAGTAATCTACGCCTTTGTTGGTGAGAGGTT
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TCCGTTTCTTCCTGGTGAGAAAATGGAAGAACAAGCTCTGTCTCCCCATCAACTGGGGA
GCAAGAAATCTCTGTGGTGTTTTTAGTTGGGCAGAGAAAATTGTCACTTATTCCATGGACT
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CTCCATGCAATGCTGATGCTCTCAAAGACCTGTAAACACACATAATAACTGCAGAATATC
TTCATCTACCCCAAGATCATTAGTAGAGGACATGTGCTGGCCAGTTGACAATTCATCAAA
CATGACATGAAAAAGACAGCTTTTTTTTTTTATTCTCCAAAGAGTTATACATGTTCTGAT
TCACTTAAATGTTAAATAATCCATTCACAAGTAGGAAGGTTTAAAAAATATTTAAACTAC
TTTTGCCACTTTTTGACATCAGTGAAATGTTTCACAAATGCATTTATTTTATTTCCTAA
CGTAAGTAGTTCTTTTTCTTCTTGATGAATGGCTTTCTTTTTCCTCTTTTTAAAAAAATT
ATTATTTGAATATAGACTGGATGTTATATCTTGTTTAATTTTTTCTTAAAAGTCAAATGA
AATCATATTTCCTATTAGTGATTGAGAGCATCTTGGAAGGAACACACTCTCCTCATAATG
CCAGTTTTATGTTGAAAAACAGGCTGGGGCCATCTGTATTGAGAAGTCCAGGTGTGGTGC
TGTCGTTAATTCTTCCTAGACTTGACATCATAAGTACCTGGAGGGTATAGTGACACATGC
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GATGAAGTAAGACTGGAAACCCCTTTCTACCGGCCCTCACATACCAAATAGAGGATGTAC
AAGAAAGTATATGATATCACACAGAAAGAAGTTGGCACTATGCAAATAACCCATGAATG
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ATCTATATGTTCATTATTCTGGCACACAGACCCTAGAAATCTCAGGAGGTGCTCTCTGGA
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AGAATTATGTTGTAACTGTGATGCGTTCAGAGACTCAACAATTGAAAATGAGGATG
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	Sequence	Tm
Forward	CAGAATCAAAGAGCTGGGGTCATG	63
Reverse	GAGGCCGATGATGAACACCA	60

CCR4

>NM_009916 NM_009916 Mus musculus chemokine (C-C motif) receptor 4 (Ccr4),
mRNA. 11/2006

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TTTCAGAAGAGCAAGGCAGCTCAACTGTTCTCATTGGCTTCTCCTGCTGGTACCCGGAGC
GCGACGATTCCAAAGATGAATGCCACAGAGGTCACAGACACCACCCAGGATGAAACTGTG
TACAATAGTTATTACTTCTACGAAAGCATGCCAAAGCCTTGCACCAAGGAAGGTATCAAG
GCATTTGGGGAGGTCTTCCTGCCTCCTCTCTACTCCTTGGTCTTCTTGTTGGGTCTGTTT
GGAAATTCTGTTGTGGTTCTGGTCCTGTTCAAATACAAGAGGCTCAAGTCCATGACGGAC
GTGTACCTGCTGAACCTGGCCATCTCGGATTTGCTGTTCGTCCTGTCCCTCCCATTCTGG
GGCTACTACGCCGCCGACCAGTGGGTTTTTGGACTAGGTCTGTGCAAGATCGTTTCATGG
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TACCTGGCCATCGTGCACGCGGTATTCTCCTTGAAGGCAAGGACCCTGACCTATGGGGTC
ATCACCAGCCTGATCACGTGGTCAGTGGCTGTGTTTGCCTCCCTCCCAGGCCTCTTGTTC
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GGCATCATGCTGTTTTGGTATTCCATGATCATTAGGACTCTGCAACACTGCAAGAATGAG
AAGAAGAACAGAGCAGTGCGCATGATCTTCGGCGTGGTGGTCCTCTTCCTCGGCTTCTGG
ACGCCGTACAACGTGGTGCTTTTCCTGGAGACGCTGGTGGAGCTTGAAGTCCTTCAGGAC
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CACTGCTGCCTTAACCCCGTCATTTACTTCTTTCTCGGGGAGAAATTCCGCAAGTACATC
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CAGGTCTACTCGGCTGACATGTCCAGCTCCTCTTACACGCAGTCCACTGTGGATCATGAC
TTCCGTGACGCTTTGTAAGGTGTGAGTGGGGGTAACATGGCGTTAACAAGCTCCACACAC
CCAGCACCTGCTCGCCTTGTTTCAGTCAGGGTGCCCTGAACAGGGCTCTGAGGAAGAAAA
CAAGTAAAACCAAGACCATGGCAAGATGGCTTCTCACCCTGCAGGTGGCTCCCAAGAGGT
TCAGAGCCCTGCTGGGTGGAGGAAATCACCCCTTCATGACAATGAGCCCTTGAGTGGATC
TCTAGTTTGGTTGAACTACCTAGAATTCTTGGACATGCTGTATTCCATAAAGCCAGATGT
CTGGAGAAAAAAAAAAAAAAAAAAAAAAAAAAA
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	Sequence	Tm
Forward	CGACGGCATTGCTTCATAGACTG	63
Reverse	CAGTTGAGCTGCCTTGCTCTTC	60

MCSF
>NM_007778 NM_007778 Mus musculus colony stimulating factor 1 (macrophage)
(Csf1), mRNA. 3/2007
GACAGAGCGACGGGGAAGAGAGCTAGCGGGGACGACCAGGCGGCCCGCTTGGGGGAAGGG
AGTCGGCGGCTCAGTGGGCCTCTGGGGTGTAGTATGTGTCAGTGCCTGTGAGTGTGTTTG
TGTGTGTGTATGTCTGTGTGTGTCTGGCGGAGAGCCAGGGTGATTTCCCATAAACCACAT
GCCCCGCCAGCCCGCCCCGCTTAAAAGGCTGTGCCGAGGGCTGGCCAGCGAAGCTCGGCCA
GGGGAAAGTGAAAGTTTGCCTCGGTGCTCTCGGTGTCGCTGCGGCTCTCTGCATCCCAGG
ACAGCGGCGTGGCCCTCGACCGGGGCGCGGGCTCTTCAGCCACTAGCGAGCAAGGGAGCG
AGCGAACCAGGGCGGCCAACACGCCGTGCCGGGACCCAGCTGCCCGTATGACCGCGCGGG
GCGCCGCGGGGCGCTGCCCTTCTTCGACATGGCTGGGCTCCCGGCTGCTGCTGGTCTGTC
TCCTCATGAGCAGGAGTATTGCCAAGGAGGTGTCAGAACACTGTAGCCACATGATTGGGA
ATGGACACCTGAAGGTCCTGCAGCAGTTGATCGACAGTCAAATGGAGACTTCATGCCAGA
TTGCCTTTGAATTTGTAGACCAGGAACAGCTGGATGATCCTGTTTGCTACCTAAAGAAGG
CCTTTTTTCTGGTACAAGACATAATAGATGAGACCATGCGCTTTAAAGACAACACCCCCA
ATGCTAACGCCACCGAGAGGCTCCAGGAACTCTCCAATAACCTGAACAGCTGCTTCACCA
AGGACTATGAGGAGCAGAACAAGGCCTGTGTCCGAACTTTCCATGAGACTCCTCTCCAGC
TGCTGGAGAAGATCAAGAACTTCTTTAATGAAACAAAGAATCTCCTTGAAAAGGACTGGA
ACATTTTTACCAAGAACTGCAACAACAGCTTTGCTAAGTGCTCTAGCCGAGATGTGGTGA
CCAAGCCTGATTGCAACTGCCTGTACCCTAAAGCCACCCCTAGCAGTGACCCGGCCTCTG
CCTCCCCTCACCAGCCCCCCGCCCCCTCCATGGCCCCTCTGGCTGGCTTGGCTTGGGATG
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CCACGCCTGTAGGGGGCAGCATCCAGGCAGAGACTGACAGACCCAGGGCCCTCTCAGCAT
CTCCATTCCCTAAATCAACAGAGGACCAAAAGCCAGTGGATATAACAGACAGGCCGTTGA
CAGAGGTGAACCCTATGAGACCCATTGGCCAGACACAGAATAATACTCCTGAGAAGACTG
ATGGTACATCCACGCTGCGTGAAGACCACCAGGAGCCAGGCTCTCCCCATATTGCGACAC
CGAATCCCCAACGAGTCAGCAACTCAGCCACCCCCGTTGCTCAGTTACTGCTTCCCAAAA
GCCACTCTTGGGGCATTGTGCTGCCCCTTGGGGAGCTTGAGGGCAAGAGAAGTACCAGGG
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TGGCCCGTTTTAATTCCATTCCTTTGACTGACACAGGCCATGTGGAGCAGCATGAGGGAT
CCTCTGACCCCCAGATCCCTGAGTCTGTCTTCCACCTGCTGGTGCCGGGCATCATCCTAG
TCTTGCTGACTGTTGGGGGCCTCCTGTTCTACAAGTGGAAGTGGAGGAGCCATCGAAACC
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ACAGACAGGTGGAACTGCCAGTATAGAAAGGATTCTATGCTGGGCACACAGGACTATCTC
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CCCCCACATACTGCCACTATTTATTGTGGGCCCTGGAGGCTCCCTGCATTGGAGGAAGGG
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CCGCCTGGGCTACCAGTCAGAGCTGGTCTTTGGGCTGTGTTCCTTGCCCAGGTTTCTGCA
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TCTGAAACCTGGAGCTAAGCAGGTTGTCCTGTCACAAGCTCTGGGGACTGAGCTCCATGC
TCCAACCCCACCCTCTTCTGACCTTTGTTCTCCAGACCTGACCCAGGTAGGCAAGGGTAC
CCTCCCAGTCTCACCTACCATACTGTGCCATCTCTAGCCAAGCAAGCCAGGTTTAGAGAA
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TTCCCAGGCCAGGCTTGTCTGTGGTGACCTGGGCATGGGTGACAGGGCTCTCATTTGCCC
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GAAAATCCAACCCATTGCAAGAAGCTCTTTGTGGGTGCCTGGTTACATCGGAGCAGGGGA
GCCTCAAATCCACCTTTGGAGCCGCCCCTGTGTGCATTAGGAACCCTTCTCTCCTCTGAG
AAAGCTCAGAGGGAGCACTGCCTCACAAACTGTGAGACTGCGTTTTTTATACTTGGAAGT
GGTGAATTATTTTATATAAGGTCATTTAAATATCTATTTAAAAAATAGGAAGCTGCTTTT
ATATTTAATAATAAAAGAAGTGCACAAGC
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	Sequence	Tm
Forward	AGCCTGATATTCAAAGGGTGGG	60
Reverse	CCGTCTTTTGGGCTGCGTG	63

GCSF

>NM_009971 NM_009971 Mus musculus colony stimulating factor 3 (granulocyte)
(Csf3), mRNA. 11/2006

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GCTGTGGCAAAGTGCACTATGGTCAGGACGAGAGGCCGTTCCCCTGGTCACTGTCAGCGC
TCTGCCACCATCCCTGCCTCTGCCCCGAAGCTTCCTGCTTAAGTCCCTGGAGCAAGTGAG
GAAGATCCAGGCCAGCGGCTCGGTGCTGCTGGAGCAGTTGTGTGCCACCTACAAGCTGTG
TCACCCCGAGGAGCTGGTGTTGCTGGGCCACTCTCTGGGGATCCCGAAGGCTTCCCTGAG
TGGCTGCTCTAGCCAGGCCCTGCAGCAGACACAGTGCCTAAGCCAGCTCCACAGTGGGCT
CTGCCTCTACCAAGGTCTCCTGCAGGCTCTATCGGGTATTTCCCTGCCCTGGCCCCCAC
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AAACCTAGGGGTGGCCCCTACTGTGCAGCCCACACAGAGCGCCATGCCAGCCTTCACTTC
TGCCTTCCAGCGCCGGGCAGGAGGTGTCCTGGCCATTTCGTACCTGCAGGGCTTCCTGGA
GACGGCTCGCCTTGCTCTGCACCACTTGGCCTAGACCTGAGCAGAAAGCCCTTTCCAGAT
AGTTTATTTATCTCTATTTAATATTTATGCATATTTAAGCCTACTATTTAAAGACAAAGA
CGAGAAAATGGAGCTCTAAGCTTCTAGATCATTCTCTCCACTTCCGAGTTTTGTTCTCCT
GCTTAGAGCAGAGAGAGAAGGCTCTTGTGTCCTCCTGTGGAGGCCAGGGAAGGAGATGGG
TAAATACCAAGTATTGATTCCTGCTGCTGCTCCAGGCACCCAGTTCTGTGGCAGTACCCC
CAAAAAATCAGTGAGCCCTGCCGTGCTGAGGCACCATCTCAGGGGGGCCCAGGCAGCATC
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ACTGGGTTCTTATATCCCTTGCTCTGGTCAACCAGGTTGCAGGGTTTCCTGTCCTCACAG
GAACGAAGTCCCTAAAGAAACAGTGGCAGCCAGGTTTAGCCCCGGAATTGACTGGATTCC
TTTTTTAGGGCCCTGCTGGCCTGGAAGTTGGAGTGGGGGGCAGAGGAGGCAGGAGGAAGC
CTGGGGGGGGGTTGGCATGGAGGGAGGCCTTCCCATCCACCCTCACCCTCCACCCCACC
TGTCACTATAGCCAAGCTTGCGGATAATAAAGTGTGGTGTTCC
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	Sequence	Tm
Forward	GCCAACTTTGCCACCACCA	61
Reverse	CTGCACAGTAGGGGCCACC	60

GMCSF

>NM_009969 NM_009969 Mus musculus colony stimulating factor 2 (granulocyte-
macrophage) (Csf2), mRNA. 2/2007

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GTTCTTGGAAGGGCTTATTAATGAAAACCCCCCAAGCCTGACAACCTGGGGGAAGGCTCA
CTGGCCCCATGTATAGCTGATAAGGGCCAGGAGATTCCACAACTCAGGTAGTTCCCCCGC
CCCCCTGGAGTTCTGTGGTCACCATTAATCATTTCCTCTAACTGTGTATATAAGAGCTCT
TTTGCAGTGAGCCCAGTACTCAGAGAGAAAGGCTAAGGTCCTGAGGAGGATGTGGCTGCA
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GAATTTACTTTTCCTGGGCATTGTGGTCTACAGCCTCTCAGCACCCACCCGCTCACCCAT
CACTGTCACCCGGCCTTGGAAGCATGTAGAGGCCATCAAAGAAGCCCTGAACCTCCTGGA
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GAAGCTAACATGTGTGCAGACCCGCCTGAAGATATTCGAGCAGGGTCTACGGGGCAATTT
CACCAAACTCAAGGGCGCCTTGAACATGACAGCCAGCTACTACCAGACATACTGCCCCCC
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AGGAACTTGGAATTTCTGCCTTAAAGGGACCAAGAGATGTGGCACAGCCACAGTTGGAAG
GAAGTATAGCCCTCTGAAAACGCTGACTCAGCTTGGACAGCGGAAGACAAACGAGAGATA
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	Sequence	Tm
Forward	CACCCACCCGCTCACCC	62
Reverse	TTGATGGCCTCTACATGCTTCC	60

IL12 alpha
>NM_008351 NM_008351 Mus musculus interleukin 12a (Il12a), mRNA. 2/2007

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CCTCGGCATCCAGCAGCTCCTCTCAGTGCCGGTCCAGCATGTGTCAATCACGCTACCTCC
TCTTTTTGGCCACCCTTGCCCTCCTAAACCACCTCAGTTTGGCCAGGGTCATTCCAGTCT
CTGGACCTGCCAGGTGTCTTAGCCAGTCCCGAAACCTGCTGAAGACCACAGATGACATGG
TGAAGACGGCCAGAGAAAAACTGAAACATTATTCCTGCACTGCTGAAGACATCGATCATG
AAGACATCACACGGGACCAAACCAGCACATTGAAGACCTGTTTACCACTGGAACTACACA
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CCCCACAGAAGACGTCTTTGATGATGACCCTGTGCCTTGGTAGCATCTATGAGGACTTGA
AGATGTACCAGACAGAGTTCCAGGCCATCAACGCAGCACTTCAGAATCACAACCATCAGC
AGATCATTCTAGACAAGGGCATGCTGGTGGCCATCGATGAGCTGATGCAGTCTCTGAATC
ATAATGGCGAGACTCTGCGCCAGAAACCTCCTGTGGGAGAAGCAGACCCTTACAGAGTGA
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ACACAGATAGGAAACAAAGAAAGATTCATAAGAGTCAGGTGGTCTTGGCCTGGTGGGCCT
TAAGCTCCTTCAGGAATCTGTTCTCCCATCACATCTCATCTCCCCAAAGGTGGCACAGCT
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CAAGAACTGATAAGCTATTGTTTTTGTGCCAAAGTGTTTATGAAAACACTCAGTCACCCC
TTATTTAAAAATATTTATTGCTATATTTTATACTCATGAAAGTACATGAGCCTATTTATA
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	Sequence	Tm
Forward	CCCATCACATCTCATCTCCCCA	63
Reverse	AAGCGATGGAGGGGACCAT	60

IL12 beta
>NM_008352 NM_008352 Mus musculus interleukin 12b (Il12b), mRNA. 3/2007

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CAGGGGACAGGCTACCCCTGAACCAGGCAGCGTGGGAGTGGATGTGTCCTCAGAAGCTA
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AACCTCACCTGTGACACGCCTGAAGAAGATGACATCACCTGGACCTCAGACCAGAGACAT
GGAGTCATAGGCTCTGGAAAGACCCTGACCATCACTGTCAAAGAGTTTCTAGATGCTGGC
CAGTACACCTGCCACAAAGGAGGCGAGACTCTGAGCCACTCACATCTGCTGCTCCACAAG
AAGGAAAATGGAATTTGGTCCACTGAAATTTTAAAAAATTTCAAAAACAAGACTTTCCTG
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GAACTGGCGTTGGAAGCACGGCAGCAGAATAAATATGAGAACTACAGCACCAGCTTCTTC
ATCAGGGACATCATCAAACCAGACCCGCCCAAGAACTTGCAGATGAAGCCTTTGAAGAAC
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AAGACATTAAGGAAGAAAAATTTAAACTCAGGATGGAAGAGTCCCCCAAAAGCTGTCTTC
TGCTTGGTTGGCTTTTTCCAGTTTTCCTAAGTTCATCATGACACCTTTGCTGATTTCTAC
ATGTAAATGTTAAATGCCCGCAGAGCCAGGGAGCTAATGTATGCATAGATATTCTAGCAT
TCCACTTGGCCTTATGCTGTTGAAATATTTAAGTAATTTATGTATTTATTAATTTATTTC
TGCATTTCACATTTGTATACCAAGATGTATTGAATATTTCATGTGCTTGTGGCCTGATCC
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AAGCTGTAAAGTTGACTCTGAAGAGTTTAGCACTAGTTTCAACACCAAGAAAGACTTTTT
AGAAGTGATATTGATAAGAAACCAGGGCCTTCTTTAGAAGGGTACCTAAATTTAAAAGAA
TTTTGAAAGGCTGGGTATCGGTGGTATATGCTTTTAATTCCAGCACTCAGGAGACCAAGG
CAGGCAGATCTCTGTGAGTTTGAGGACAGCCTGGTGTACAGAGGGAGTTCCAGCACAGCC
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	Sequence	Tm
Forward	TCCAGATTCAGACTCCAGGGG	60
Reverse	GACACATCCCACTCCCACGC	62

IL1 alpha

>NM_010554 NM_010554 Mus musculus interleukin 1 alpha (Il1a), mRNA. 2/2007
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GGCTCACTTCATGAGACTTGCACAGATCAGTTTGTATCTCTGAGAACCTCTGAAACGTCA
AAGATGTCCAACTTCACCTTCAAGGAGAGCCGGGTGACAGTATCAGCAACGTCAAGCAAC
GGGAAGATTCTGAAGAAGAGACGGCTGAGTTTCAGTGAGACCTTCACTGAAGATGACCTG
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CAGAGTGATTTGAGATACAAACTGATGAAGCTCGTCAGGCAGAAGTTTGTCATGAATGAT
TCCCTCAACCAAACTATATATCAGGATGTGGACAAACACTATCTCAGCACCACTTGGTTA
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GAGACCGACCTCATTTTCTTCTGGAAAAGTATCAACTCTAAGAACTACTTACATCAGCT
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TACCCTATAGTTTGCTCAGTACTAATCATTTTAATTACTATTCTGCATGGCATTCTTAGG
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AAGTTATTTTATCTATGTATTTATAAATATATTTATGATAATTATATTATTTATGGAACA
TCCTTAAATCCTCTGAGCTTGACGGCACCCTCGCAGCAGGGTTTTCTAGGTGGTCAGTTA
GATGTAGTCTCCTCTAGAGCTCCATGCTACAGACTTTTACACTTTTTCCACAGCCACGAA
GCTCTCCGTACATTCCTGCACTTGGGAGCCCTTTCATCATGATCTTAATCTGTGCTGTTT
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ACTTTGTGCATCTAAAATGATAATTGAGTCAGTCTTTCTCCCTCCCGTCCTTAAAGCTGT
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AAGAGCTTAACTGTGACAACCACATCACTGATACCTGAAGTTTCTTTTCTAGAATGTAAT
CAGTGTTTCCCCTGGATTCCAATTTTTTTTTCAAACCACAGTGTCATGTAACTATCAACA
ATAACAATCAACTCATTATTATTAATCATAATTAAATAAAACAGGTTTGAGCTG
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	Sequence	Tm
Forward	GCACGGGGACTGCCCTCTAT	62
Reverse	TAGACTCCCGAAATAAGGCTGCTT	60

IL1 beta

>NM_008361 NM_008361 Mus musculus interleukin 1 beta (Il1b), mRNA. 2/2007

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AGAATGACCTGTTCTTTGAAGTTGACGGACCCCAAAAGATGAAGGGCTGCTTCCAAACCT
TTGACCTGGGCTGTCCTGATGAGAGCATCCAGCTTCAAATCTCGCAGCAGCACATCAACA
AGAGCTTCAGGCAGGCAGTATCACTCATTGTGGCTGTGGAGAAGCTGTGGCAGCTACCTG
TGTCTTTCCCGTGGACCTTCCAGGATGAGGACATGAGCACCTTCTTTTCCTTCATCTTTG
AAGAAGAGCCCATCCTCTGTGACTCATGGGATGATGATGATAACCTGCTGGTGTGTGACG
TTCCCATTAGACAACTGCACTACAGGCTCCGAGATGAACAACAAAAAGCCTCGTGCTGT
CGGACCCATATGAGCTGAAAGCTCTCCACCTCAATGGACAGAATATCAACCAACAAGTGA
TATTCTCCATGAGCTTTGTACAAGGAGAACCAAGCAACGACAAAATACCTGTGGCCTTGG
GCCTCAAAGGAAAGAATCTATACCTGTCCTGTGTAATGAAAGACGGCACACCCACCCTGC
AGCTGGAGAGTGTGGATCCCAAGCAATACCCAAAGAAGAAGATGGAAAAACGGTTTGTCT
TCAACAAGATAGAAGTCAAGAGCAAAGTGGAGTTTGAGTCTGCAGAGTTCCCCAACTGGT
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ACATAATTGACTTCACCATGGAATCCGTGTCTTCCTAAAGTATGGGCTGGACTGTTTCTA
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CTATTTATTTATGTATTTATTGATTGGTTGATCTATTTAAGTTGATTCAAGGGGACATTA
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CCGCACTGAGGTCTTTCATTGAAGCTGAGAATAAATAGGTTCCTATAATATGGATGAGAC
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CAAACAAA
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	Sequence	Tm
Forward	CTCGTGCTGTCGGACCCATA	61
Reverse	TTGGTTGATATTCTGTCCATTGAGG	60

IL1f9

>NM_153511 NM_153511 Mus musculus interleukin 1 family, member 9 (Il1f9), mRNA. 11/2006

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ACCCATTTTCTACACACATCTCAGGAAGAGAAACTCCTGACTTTGGGGAGGTTTTTGACTT
TGGACCAGCAGGTGTGGATCTTTCGTAATCAGGCCCTTGTGACAGTTCCACGAAGCCACA
GAGTAACCCCAGTCAGCGTGACTATCCTCCCATGCAAGTACCCAGAGTCTCTTGAACAGG
ACAAAGGGATTGCCATTTATTTGGGAATTCAGAATCCAGATAAATGCCTGTTTTGTAAGG
AAGTTAATGGACACCCTACTTTGCTGCTAAAGGAAGAGAAGATTTTGGATTTGTACCACC
ACCCTGAGCCAATGAAGCCATTCCTGTTTTACCACACCCGGACAGGTGGAACATCCACCT
TTGAATCAGTGGCTTTCCCTGGCCACTATATTGCCTCCTCCAAGACTGGCAACCCCATCT
TCCTCACATCAAAAAAGGGAGAATATTACAACATTAACTTCAATTTAGATATAAAGTCTT
AAACTCAGCATGGAAGTGGAGGGTTGGTTAGAACTCTT
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	Sequence	Tm
Forward	TGGGGAGGTTTTTGACTTGGA	60
Reverse	GGAACTGTCACAAGGGCCTGA	61

IL1rn

>NM_031167 NM_031167 Mus musculus interleukin 1 receptor antagonist (Il1rn),
transcript variant 1, mRNA. 12/2006
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CAAGTCTCTCTGGAGTGAGACGTTGGAAGGCAGTGGAAGACCTTGTGTCCTGTTTAGCTC
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CTTCAGAATCTGGGATACTAACCAGAAGACCTTTTACCTGAGAAACAACCAGCTCATTGC
TGGGTACTTACAAGGACCAAATATCAAACTAGAAGAAAAGATAGACATGGTGCCTATTGA
CCTTCATAGTGTGTTCTTGGGCATCCACGGGGGCAAGCTGTGCCTGTCTTGTGCCAAGTC
TGGAGATGATATCAAGCTCCAGCTGGAGGAAGTTAACATCACTGATCTGAGCAAGAACAA
AGAAGAAGACAAGCGCTTTACCTTCATCCGCTCTGAGAAAGGCCCCACCACCAGCTTTGA
GTCAGCTGCCTGTCCAGGATGGTTCCTCTGCACAACACTAGAGGCTGACCGTCCTGTGAG
CCTCACCAACACACCGGAAGAGCCCCTTATAGTCACGAAGTTCTACTTCCAGGAAGACCA
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GCCTGAGGAACAACTTTTGCAGGGTGTATGTACAGTAGAAGGAGACAGAAGAGTTCTGAT
GATAGATCTCTGCCTCAGTCTGTTGGCTGGCCTAATCCCCATGATGATTCCAGAATAATC
TTGCAAATTGGATCATGGCAGGTGCTTGTTCAAAGCCCTTTCTTGTTGCCTCTGCCATCT
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CCTGCCACAAACACACACTTTTTTTGTTTTTGTTTTTTCCATTGTTCTGCACTTCCACAG
TCCAGACCAATCAAGTCACTTGACAATATGCCCCAAGTGACTCCCTTACCCTGTTTTATA
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CAGCTCAGGGGGGCACTTTTAAGGATCTAGAAAACAGAAATTAAGGATCTCATAGTTATT
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TGAGAGGAGCTGGGGATTAGATGCTCCTGCATTTGTGAAATGGTTATAAGCATAGAAAAA
TAGGTGGTAAGCTTTCCTTCTTTCCTTATTTTGTGTGATGCCTTAAGCTGAAAAGTTAAA
AATTGATGGATTGTAGCATTCCCATAATCTCCCCCTTCTTTTTTTTTCCTTTGGAAATGT
CCAATAGTCTATATTCCTCTGTCCCGCCCAAACACCATCTTCACTCCAAGCCTACCACAG
ATGCCTGAAGAAGTTCCTCACTATCTGCAAATGTGGCTCTCAGGCCCTTCCTGATGTGAT
GAATGAATCTACTAATCATTTCTTGACCATTCATTTTATCACTTCTAACCTTGAAACATG
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GCGTGCGCACGCACGTGCATGCACATGCTATGTATTGGGTCCCTCCAAGGATGAACCCTC
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CTCATCCCTGTGACTTTGGCTTTATTTTACAATAAAACACTGAAAATGTCCACTTTGTTA
GTTGTGAACATGAGCCCAGGCCTAAGGTGCTGGGAAACAGAAAGGGGCGGGAGATTTTTC
TTTATTCTATGGCTAGAAAATAGTTACCTCCTCTCTGAAAGTCTTCTTCCTCATTTCTGG
GTAACAGAATATCAAACACCTTGCTTATAAGTTATAAAGTAGTGTTGTCCACCATGAACC
CACCAAGTAAAAACAACCCAAATACCTATCATGGATGAATAATCATGCAAGTATCAGATC
TGCACTCAATGCCACACAATGACAAAGATAGCAAATGAGCCACAGACGGCTCCACCCAAC
CCAATAGATGAACACTTGGTTCAAAATCACTAAAGCTCAAATACTCCCAGGTCAAACACC
AGGTAACAAGTTAATACTCAACAAAGGGGGAAAACAAATGTTCCACTGAATCCTGTGACC
CTGTGGGCGTGGTTCACCTCCTGTGTTGTTTGCCATGTGTGCTCAGGATGAGCTGATTAA
AGCTCTTCTCAGGGGTTCAGTTTTCCCATCTCTGCTTGTAAATAAAAGCTTATGC

	Sequence	Tm
Forward	TCACTCCAAGCCTACCACAGATG	60
Reverse	AGGGCCTGAGAGCCACATTTG	62

IL2

>NM_008366 NM_008366 Mus musculus interleukin 2 (Il2), mRNA. 1/2007
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AGCTCGCATCCTGTGTCACATTGACACTTGTGCTCCTTGTCAACAGCGCACCCACTTCAA
GCTCCACTTCAAGCTCTACAGCGGAAGCACAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC
AGCAGCACCTGGAGCAGCTGTTGATGGACCTACAGGAGCTCCTGAGCAGGATGGAGAATT
ACAGGAACCTGAAACTCCCCAGGATGCTCACCTTCAAATTTTACTTGCCCAAGCAGGCCA
CAGAATTGAAAGATCTTCAGTGCCTAGAAGATGAACTTGGACCTCTGCGGCATGTTCTGG
ATTTGACTCAAAGCAAAAGCTTTCAATTGGAAGATGCTGAGAATTTCATCAGCAATATCA
GAGTAACTGTTGTAAAACTAAAGGGCTCTGACAACACATTTGAGTGCCAATTCGATGATG

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AGTCAGCAACTGTGGTGGACTTTCTGAGGAGATGGATAGCCTTCTGTCAAAGCATCATCT
CAACAAGCCCTCAATAACTATGTACCTCCTGCTTACAACACATAAGGCTCTCTATTTATT
TAAATATTTAACTTTAATTTATTTTTGGATGTATTGTTTACTATCTTTTGTAACTACTAG
TCTTCAGATGATAAATATGGATCTTTAAAGATTCTTTTTGTAAGCCCCAAGGGCTCAAAA
ATGTTTTAAACTATTTATCTGAAATTATTTATTATATTGAATTGTTAAATATCATGTGTA
GGTAGACTCATTAATAAAAGTATTTAGATGATTCAAATATAAATAAGCTCAGATGTCTGT
CATTTTTAGGACAGCACAAAGTAAGCGCTAAAATAACTTCTCAGTTATTCCTGTGAACTC
TATGTTAATCAGTGTTTTCAAGAAATAAAGCTCTCCTCT
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	Sequence	Tm
Forward	GCCCAAGCAGGCCACAGA	62
Reverse	TGCCGCAGAGGTCCAAGTT	60

Ifn alpha 1

>NM_010502 NM_010502 Mus musculus interferon alpha 1 (Ifna1), mRNA. 11/2006
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ATGGCTAGGCTCTGTGCTTTCCTGATGGTCCTGGCGGTGCTGAGCTACTGGCCAACCTGC
TCTCTAGGATGTGACCTTCCTCAGACTCATAACCTCAGGAACAAGAGAGCCTTGACACTC
CTGGTACAAATGAGGAGACTCTCCCCTCTCTCCTGCCTGAAGGACAGGAAGGACTTTGGA
TTCCCGCAGGAGAAGGTGGATGCCCAGCAGATCAAGAAGGCTCAAGCCATCCCTGTCCTG
AGTGAGCTGACCCAGCAGATCCTGAACATCTTCACATCAAAGGACTCATCTGCTGCATGG
AATACAACCCTCCTAGACTCATTCTGCAATGACCTCCACCAGCAGCTCAATGACCTGCAA
GGCTGTCTGATGCAGCAGGTGGGGGTGCAGGAATTTCCCCTGACCCAGGAAGATGCCCTG
CTGGCTGTGAGGAAATACTTCCACAGGATCACTGTGTACCTGAGAGAGAAGAAACACAGC
CCCTGTGCCTGGGAGGTGGTCAGAGCAGAAGTCTGGAGAGCCCTGTCTTCCTCTGCCAAT
GTGCTGGGAAGACTGAGAGAAGAGAAATGA
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	Sequence	Tm
Forward	CCCGCAGGAGAAGGTGGAT	61
Reverse	ACTCAGGACAGGGATGGCTTG	60

Ifn gamma

>NM_008337 NM_008337 Mus musculus interferon gamma (Ifng), mRNA. 3/2007
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TTGAGACAGAAGTTCTGGGCTTCTCCTCCTGCGGCCTAGCTCTGAGACAATGAACGCTAC
ACACTGCATCTTGGCTTTGCAGCTCTTCCTCATGGCTGTTTCTGGCTGTTACTGCCACGG
CACAGTCATTGAAAGCCTAGAAAGTCTGAATAACTATTTTAACTCAAGTGGCATAGATGT
GGAAGAAAAGAGTCTCTTCTTGGATATCTGGAGGAACTGGCAAAAGGATGGTGACATGAA
AATCCTGCAGAGCCAGATTATCTCTTTCTACCTCAGACTCTTTGAAGTCTTGAAAGACAA
TCAGGCCATCAGCAACAACATAAGCGTCATTGAATCACACCTGATTACTACCTTCTTCAG
CAACAGCAAGGCGAAAAAGGATGCATTCATGAGTATTGCCAAGTTTGAGGTCAACAACCC
ACAGGTCCAGCGCCAAGCATTCAATGAGCTCATCCGAGTGGTCCACCAGCTGTTGCCGGA
ATCCAGCCTCAGGAAGCGGAAAAGGAGTCGCTGCTGATTCGGGGTGGGGAAGAGATTGTC
CCAATAAGAATAATTCTGCCAGCACTATTTGAATTTTTAAATCTAAACCTATTTATTAAT
ATTTAAAACTATTTATATGGAGAATCTATTTTAGATGCATCAACCAAAGAAGTATTTATA
GTAACAACTTATATGTGATAAGAGTGAATTCCTATTAATATATGTGTTATTTATAATTTC
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TTGTAGCTTGTACCTTTACTTCACTGACCAATAAGAAACATTCAGAGCTGCAGTGACCCC
GGGAGGTGCTGCTGATGGGAGGAGATGTCTACACTCCGGGCCAGCGCTTTAACAGCAGGC
CAGACAGCACTCGAATGAGTCAGGTAGTAACAGGCTGTCCCTGAAAGAAAGCAGTGTCTC
AAGAGACTTGACACCTGGTGCTTCCCTATACAGCTGAAAACTGTGACTACACCCGAATGA
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TTGCAACC
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	Sequence	Tm
Forward	GCATCTTGGCTTTGCAGCTCTTC	63
Reverse	ATGACTGTGCCGTGGCAGTAA	60

Cmklr1

>NM_008153 NM_008153 Mus musculus chemokine-like receptor 1 (Cmklr1), mRNA.
11/2006

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GAGGGAGGCTCTTAGGATGTTGTGCTCCGCGGGGCTCAGACGAAATCTTCTGTGAATGGA
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GTTCTCAAACCCTGAAGTCGCAGGAGCCGGAGGGGGATATTGGAGAGAAGGTATTTCCAG
TCACGCGCAGTAACAGACCAGCCAAGGACCAGGACTGGAGTTCTGTTCTACAACGGTGAA
CAGTGAAAGGTCTCCAAAGAGATGGAGTACGACGCTTACAACGACTCCGGCATCTATGAT
GATGAGTACTCTGATGGCTTTGGCTACTTTGTGGACTTGGAGGAGGCGAGTCCGTGGGAG
GCCAAGGTGGCCCCGGTCTTCCTGGTGGTGATCTACAGCTTGGTGTGCTTCCTCGGTCTC
CTAGGCAACGGCCTGGTGATTGTCATCGCCACCTTCAAGATGAAGAAGACCGTGAACACT
GTGTGGTTTGTCAACCTGGCTGTGGCCGACTTCCTGTTCAACATCTTTTTGCCGATGCAC
ATCACCTACGCGGCCATGGACTACCACTGGGTGTTCGGGAAGGCCATGTGCAAGATCAGC
AACTTCTTGCTCAGCCACAACATGTACACCAGCGTCTTCCTGCTGACTGTCATCAGCTTT
GACCGCTGCATCTCCGTGCTGCTCCCCGTCTGGTCCCAGAACCACCGCAGCATCCGCCTG
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GTCTTCCGGGACACCGCCAACATTCATGGGAAGATAACCTGCTTCAACAACTTCAGCTTG
GCCGCGCCTGAGTCCTCCCCACATCCCGCCCACTCGCAAGTAGTTTCCACAGGGTACAGC
AGACACGTGGCGGTCACTGTCACCCGCTTCCTTTGCGGCTTCCTGATCCCCGTCTTCATC
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GTCATCTGGGCAGCTGACCAGCTTTCCCGAGTCCTCCTTGAATGTATCAACAGCCCCGTG
GGCAGCCCAGGATCAGGCTTTCCTCCCCTGGCAGGAAAATGGCTCACTCACCCCCCCCCC
CCCACACACACTGTTCATCAGTAGCAAAAGACCCGAGCCCTGCTCCGTCTGGCTTGAC
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TATTTCACATGCACCTTCTCATTTATTCTTCACAGAAACCTCATATGTATGAGGACAAGC
AGAGACTCAGAGAGGTTAAGCAACTGGCCCGAGGGGACCCAGTTAGATGTTGGTAGAGAC
TGGATTCGAATCGCAGTGGGCTTGGCTCGAAGGAAGATGAAGAGTCTGGGGAAACTAATA
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	Sequence	Tm
Forward	AAGAGGCCAGAGCGCGAGT	61
Reverse	CTCCAATATCCCCCTCCGG	60

CXCL1

>NM_008176 NM_008176 Mus musculus chemokine (C-X-C motif) ligand 1 (Cxcl1),
mRNA. 1/2007

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CCTGGCCACAGGGGCGCCTATCGCCAATGAGCTGCGCTGTCAGTGCCTGCAGACCATGGC
TGGGATTCACCTCAAGAACATCCAGAGCTTGAAGGTGTTGCCCTCAGGGCCCCACTGCAC
CCAAACCGAAGTCATAGCCACACTCAAGAATGGTCGCGAGGCTTGCCTTGACCCTGAAGC
TCCCTTGGTTCAGAAAATTGTCCAAAAGATGCTAAAAGGTGTCCCCAAGTAACGGAGAAA
GAAGACAGACTGCTCTGATGGCACCGTCTGGTGAACGCTGGCTTCTGACAACACTATACA
ATTTCTTTTGAGGGTCCTATTTATTTATGTATTTATTTATTCCACAAAGTGTGTGGTTTT
TATTTTACATTAATATTTAACAGTGTGGATACATTTCATCGATGGTAGTTCAGTTCTGCT
TGTTCAGTTTAAAGATGGTAGGCTTAAAATATTTCATTAAAACTAATATTTATTGGGAGA
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ATAGAGTTTAGTATTATGTTTTGTATGTATTAGGGTGAGGACATGTGTGGGAGGCTGTGT
TTGTATGTCTTGAAAAGAATGTCAGTTATTTATTGAAAGTCGTCTTTCATATTGTATGGT
CAACACGCACGTGTTGACGCTTCCCTTGGACATTTTGTGTCTAGTTGGTAGCCCATAATG
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CAAAGGACTGTTACAAATGAAGTAAAAATAAAAGTTTTATTAAG
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	Sequence	Tm
Forward	CCCACTGCACCCAAACCG	62
Reverse	GGCAAGCCTCGCGACC	60

CXCL2

>NM_009140 NM_009140 Mus musculus chemokine (C-X-C motif) ligand 2 (Cxcl2),
mRNA. 1/2007
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ACAACATTTTTGATGCTGGATTTCAATGTAATGTTGTGAGTAACCCTTGGACATTTTATG
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GTT
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	Sequence	Tm
Forward	AAAGATACTGAACAAAGGCAAGGCTAA	61
Reverse	GAGGGACAGCAGCCCAGG	60

CXCL5

>NM_009141 NM_009141 Mus musculus chemokine (C-X-C motif) ligand 5 (Cxcl5),
mRNA. 11/2006
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TGTGTTTGCTTAACCGTAACTCCAAAAATTAATCCCAAATTGATCGCTAATTTGGAGGTG
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	Sequence	Tm
Forward	GGCATTTCTGTTGCTGTTCACG	62
Reverse	TGAGGAAGGGGCAGCTTCA	60

CXCL12

>NM_001012477 NM_001012477 Mus musculus chemokine (C-X-C motif) ligand 12
(Cxcl12), transcript variant 3, mRNA. 2/2007
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TCCGGGCAGGTGCTCAAACCTGACGGTAAACTCTTCCCTCTTCGGGGGCAGACCCCATAC
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	Sequence	Tm
Forward	CCTTCAGATTGTTGCACGGC	60
Reverse	TCTCCAGGTACTCTTGGATCCACTTT	61

IL8 R beta

>NM_009909 NM_009909 Mus musculus interleukin 8 receptor, beta (Il8rb),
mRNA. 3/2007

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Forward	GCGATGACATTGACAAGGCC	60
Reverse	GGGTTAAGGCAGCTGTGGAGG	62

IL18

>NM_008360 NM_008360 Mus musculus interleukin 18 (Il18), mRNA. 3/2007
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TGGAGTTTGAATCTTCACTGTATGAAGGACACTTTCTTGCTTGCCAAAAGGAAGATGATG
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TCACTAACTTACATCAAAGTTAGGTGGGGAGGGTTTGTGTTCCAGAAAGATGATTAGCAC
ACATGCGCCTTGTGATGACCTCGCCTGTATTTCCATAACAGAATACCCGAGGCTGCATGA
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Forward	ACCTCCAGCATCAGGACAAAGAAA	62
Reverse	CTGGGCCAAGAGGAAGTGATTT	60

IL-25 (IL-17e)

>NM_080729 NM_080729 Mus musculus interleukin 25 (Il25), mRNA. 11/2006
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GAAGCCATACTCCTGGCTCCTTTCCCCTGAATCCCCCAACTCCTGGCACAGGCACTTTCT
CCACCTCTCCCCCTTTGCCTTTTGTTGTGTTTGTTTGTGCATGCCAACTCTGCGTGCAGC
CAGGTGTAATTGCCTTGAAGGATGGTTCTGAGGTGAAAGCTGTTATCGAAAGTGAAGAGA
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Forward	CCACTTTACCACAACCAGACGG	60
Reverse	GGGTACCTTCCTCGCCATGG	62

IL-27

>NM_145636 NM_145636 Mus musculus interleukin 27 (Il27), mRNA. 2/2007
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ACCCAGGGGACCTGGACCAGCTCAGAGAGGGAGCAGCTGTGGGCCATGAGGCTGGATCTC
CGGGACCTGCACAGGCACCTCCGCTTTCAGGTGCTGGCTGCAGGATTCAAATGTTCAAAG
GAGGAGGAGGACAAGGAGGAAGAGGAAGAGGAGGAAGAAGAAGAAAAGAAGCTGCCCCTA
GGGGCTCTGGGTGGCCCCAATCAGGTGTCATCCCAAGTGTCCTGGCCCCAGCTGCTCTAT
ACCTACCAGCTCCTTCACTCCCTGGAGCTTGTCCTGTCTCGGGCTGTTCGGGACCTGCTG
CTGCTGTCCCTGCCCAGGCGCCCAGGCTCAGCCTGGGATTCCTAA
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	Sequence	Tm
Forward	ATGAGGCTGGATCTCCGGGA	63
Reverse	GAATCCTGCAGCCAGCACC	60

IL-5

>NM_010558 NM_010558 Mus musculus interleukin 5 (Il5), mRNA. 2/2007
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CGCTCTTCCTTTGCTGAAGGCCAGCGCTGAAGACTTCAGAGTCATGAGAAGGATGCTTCT
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CTTTCAGGGGCTAGACATACTGAAGAATCAAACTGTCCGTGGGGGTACTGTGGAAATGCT
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CATATGCTGTTTCCATTTATATTTATGTCCTGTAGTCAGTTAAACCTATCTATGTCCATA
TATGCAAAGTGTTTAACCTTTTTGTATACGCATAAAAGAAATTCCTGTAGCGCAGGCTGG
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GTTGCATCAGGGTCTCAAGTATTCCAGGCTGACTTTGAACTCAGTGTGTAGCCAAGGGTG
ACCCTGAACTCTTGGTCCAGATGGACGCAGGAGGATCACATACCCAACCTTAGCATCCTT
TCTCCTAGCCCCTTTAGATAGATGATACTTAATGACTCTCTTGCTGAGGGATGCCACACC
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	Sequence	Tm
Forward	ACCCAAGGCTTTGTGCATGTT	60
Reverse	GCAACGAAGAGGATGAGGGG	61

Stat6

>NM_009284 NM_009284 Mus musculus signal transducer and activator of
transcription 6 (Stat6), mRNA. 2/2007
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Reverse	CAGTTCGTCCCGAGTGAGGAT	60

IL-22
>NM_016971 NM_016971 Mus musculus interleukin 22 (Il22), mRNA. 2/2007
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	Sequence	Tm
Forward	CTGCCCGTCAACACCCG	61
Reverse	ACGATGTACGGCTGCTGGAA	60

TGF-beta

>NM_011577 NM_011577 Mus musculus transforming growth factor, beta 1
(Tgfb1), mRNA. 2/2007

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AGCCCCGCCCCGCCCCGCCCCTCCCGGCAGGCCCGGCCCCGCCCCGCCCCGCC
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Forward	CGCGGGACTCTCCACCTG	61
Reverse	GATGCGCTTCCGTTTCACC	60

Tbx21 (TBET)
>NM_019507 NM_019507 Mus musculus T-box 21 (Tbx21), mRNA. 3/2007
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CTGTCCAGTCAGTAACTTTCAACTGTTGGTCTGACACCTGTGTTAATCTCTGACCTGAAA
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Forward	TGGCGGTACCAGAGCGG	60
Reverse	AAGCGGTTCCCTGGCATG	61

Gata-3
>NM_008091 NM_008091 Mus musculus GATA binding protein 3 (Gata3), mRNA. 2/2007
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CGAGGACATGGAGGTGACTGCGGACCAGCCGCGCTGGGTGAGCCACCATCACCCCGCGGT
CCTCAACGGTCAGCACCCAGACACGCACCACCCGGGCCTCGGCCATTCGTACATGGAAGC
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CGTCCCGTCCTACTACGGAAACTCCGTCAGGGCTACGGTGCAGAGGTATCCTCCGACCCA

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	Sequence	Tm
Forward	TGAACTGCGGGGCAACCTC	63
Reverse	TGCAAAGGTAGTGCCCGGTA	60

CXCL9

>NM_008599 NM_008599 Mus musculus chemokine (C-X-C motif) ligand 9 (Cxcl9),
mRNA. 1/2007

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	Sequence	Tm
Forward	AGCCTAGAGCCCCTGCACAC	60
Reverse	TCTCCACTGTAGAACCCCAAAACC	61

CXCL10

>NM_021274 NM_021274 Mus musculus chemokine (C-X-C motif) ligand 10
(Cxcl10), mRNA. 1/2007

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	Sequence	Tm
Forward	CTGAAAGGTGACCAGCCGTG	60
Reverse	GGCTTGACCATCATCCTGCA	61

CXCL11

>NM_019494 NM_019494 Mus musculus chemokine (C-X-C motif) ligand 11
(Cxcl11), mRNA. 2/2007
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	Sequence	Tm
Forward	ATAAACGACAAAGGTGCCTGGAC	60
Reverse	CTATTGCCTGCATTATGAGGCG	61

CCL6

>NM_009139 NM_009139 Mus musculus chemokine (C-C motif) ligand 6 (Ccl6),
mRNA. 3/2007
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	Sequence	Tm
Forward	TGCCGACCCCAGCGA	60
Reverse	TTGGGCCTTGCTTCAGGG	61

CXCR3

>NM_009910 NM_009910 Mus musculus chemokine (C-X-C motif) receptor 3
(Cxcr3), mRNA. 1/2007

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Forward	ATGCTGCTGTCCAGTGGGTTTT	62
Reverse	GAACAAGGCGCCTGCCA	60

TNF-alpha

>NM_013693 NM_013693 Mus musculus tumor necrosis factor (Tnf), mRNA. 3/2007

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TAP1
>NM_013683 NM_013683 Mus musculus transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (Tap1), mRNA. 2/2007

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Reverse	GGGCACTGGTGGCATCATC	61

TIRAP

>NM_054096 NM_054096 Mus musculus toll-interleukin 1 receptor (TIR) domain-
containing adaptor protein (Tirap), mRNA. 12/2006
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Reverse	AGTGCAGGCCTCCAAACTAACC	60
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RIP1 (Ripk1)
>NM_009068 NM_009068 Mus musculus receptor (TNFRSF)-interacting serine-
threonine kinase 1 (Ripk1), mRNA. 12/2006
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Reverse	TATCTGGGTCTTTAGCACGTGCA	61

NFkB1

>NM_008689 NM_008689 Mus musculus nuclear factor of kappa light chain gene enhancer in B-cells 1, p105 (Nfkb1), mRNA. 2/2007
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Forward	CGAAATCCAACGCAGGGGT	62
Reverse	GCACAATCTTTAGGGCCATTTTTAA	60

TRAM1

>NM_028173 NM_028173 Mus musculus translocating chain-associating membrane
protein 1 (Tram1), mRNA. 11/2006
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Forward	GGTGGAGGGAACATTCTGCC	60
Reverse	GGTCACGGCCGGTTTCCT	62

TICAM-2

>NM_173394 NM_173394 Mus musculus toll-like receptor adaptor molecule 2
(Ticam2), mRNA. 11/2006

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Reverse	AATGGGTCGAAGTTCTATGAGCG	60

Calreticulin

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Reverse	CCGTGAACTGTACCACCAGTGTC	60

Erp57
>NM_007952 NM_007952 Mus musculus protein disulfide isomerase associated 3
(Pdia3), mRNA. 11/2006

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Reverse	CCGCCGAGCCCGTGT	61

Tapasin

>NM_001025313 NM_001025313 Mus musculus TAP binding protein (Tapbp),
transcript variant 1, mRNA. 11/2006
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MyD88

>NM_010851 NM_010851 Mus musculus myeloid differentiation primary response gene 88 (Myd88), mRNA. 2/2007
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TRIF (Ticam-1)

>NM_174989 NM_174989 Mus musculus toll-like receptor adaptor molecule 1 (Ticam1), mRNA. 2/2007

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Delta-4

>NM_019454 NM_019454 Mus musculus delta-like 4 (Drosophila) (Dll4), mRNA.
1/2007

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Jagged-1

>NM_013822 NM_013822 Mus musculus jagged 1 (Jag1), mRNA. 1/2007
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48

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Syk

>NM_011518 NM_011518 Mus musculus spleen tyrosine kinase (Syk), mRNA. 2/2007
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Forward	GTGGAACTGAGGCTTCGCAAT	60
Reverse	GGACAGGCGCCGGAGCT	63

Card-9

>BC065797 BC065797 Mus musculus caspase recruitment domain family, member 9,
mRNA (cDNA clone IMAGE:573639), complete cds. 12/2006
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Forward	AGAAGGGGATCGCGGGA	60
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ICAM-1

>NM_010493 NM_010493 Mus musculus intercellular adhesion molecule (Icam1),
mRNA. 2/2007
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Forward	CGAGATCGGGGAGGACAGC	62
Reverse	ACGACTGCACGGTGCCAC	60

VCAM

>NM_011693 NM_011693 Mus musculus vascular cell adhesion molecule 1 (Vcam1),
mRNA. 2/2007
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Forward	CGCCCGAACTCCTTGCA	60
Reverse	TCCCGATGGCAGGTATTACCA	61

LFA-1

>NM_008400 NM_008400 Mus musculus integrin alpha L (Itgal), mRNA. 3/2007
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53

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Forward	GGACACACGGCCTACGCAGA	63
Reverse	GCAAGACCTGGTACCCAAAATGT	60

VLA-4
>NM_010576 NM_010576 Mus musculus integrin alpha 4 (Itga4), mRNA. 2/2007
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	Sequence	Tm
Forward	TCCGGGAAGCGGTGATG	60
Reverse	AGTGCATTCTCCGGGTCCAG	61

VEGF-A
>NM_001025250 NM_001025250 Mus musculus vascular endothelial growth factor A
(Vegfa), transcript variant 1, mRNA. 2/2007
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GGTCTGCGCCGCAGGAGACAAACCGATCGGAGCTGGGAGAAGTGCTAGCTCGGGCCTGGA
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CTCCTTACCCCACCTCCTGGGTACAGCCCAGGAGGACCTTGTGTGATCAGACCATTGAAA
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	Sequence	Tm
Forward	CCGCAGACGTGTAAATGTTCCT	60
Reverse	ACTCAAGCTGCCTCGCCTTG	62

VEGF-B

>NM_011697 NM_011697 Mus musculus vascular endothelial growth factor B
(Vegfb), mRNA. 11/2006

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TTAACCACCCTGGTCAAGTGAGCATCTTCTGGCTGGCTGTCTCCCCTCACTATGAAAACC
CCAAACTTCTACCAATAACGGGATTTGGGTTCTGTTATGATAACTGTGACACACACAC
ACTCACACTCTGATAAAAGAGATGGAAGACACTAAC
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	Sequence	Tm

| Forward | CCCGGCTGCTTTTATGGC | 60 |
| Reverse | AGGAGGTTCGCCTGTGCTCC | 63 |

VEGF-C

>NM_009506 NM_009506 Mus musculus vascular endothelial growth factor C
(Vegfc), mRNA. 12/2006
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ACCTCAGCCTCACGCCCCAGCCTGCGCCAGCCAACGGACCGGCCTCCCTGCTCCCGGTCC
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GATCCCCAGTCCGCGCGAGGCGCCCGCCACCGTCGCCGCCTTCGAGTCGGGACTGGGCTT
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GGAGTTTGGAGCAGCCACAAACACCTTCTTTAAACCTCCATGTGTGTCCGTCTACAGATG
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	Sequence	Tm
Forward	GGCCCCAAACCAGTCACAA	60
Reverse	TAGACATGCACCGGCAGGAAG	62

B7-1

>NM_009855 NM_009855 Mus musculus CD80 antigen (Cd80), mRNA. 2/2007
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TGGATGCCATCCAGGCTTCTTTTTCTACATCTCTGTTTCTCGATTTTTGTGAGCCTAGGA
GGTGCCTAAGCTCCATTGGCTCTAGATTCCTGGCTTTCCCCATCATGTTCTCCAAAGCAT
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59

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Forward	TTCAGAAGAAATGAGGCAAGCAGA	61
Reverse	CTAATGCTTCTTCAGGCCCGA	60

B7-2

>NM_019388 NM_019388 Mus musculus CD86 antigen (Cd86), mRNA. 1/2007
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Reverse	ACTCCGTTTCCAGAACACACACA	60

IL-10

>NM_010548 NM_010548 Mus musculus interleukin 10 (Il10), mRNA. 2/2007
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Forward	GGATGCGGCTGAGGCG	61
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MEK1

>NM_008927 NM_008927 Mus musculus mitogen activated protein kinase kinase 1
(Map2k1), mRNA. 12/2006
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Reverse	GGCGACATGTAGGATCTCGTGC	63

TLR1
>NM_030682 NM_030682 Mus musculus toll-like receptor 1 (Tlr1), mRNA. 11/2006
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Reverse	TTCAAATGAGCAATCAGCTGCA	60

TLR2

>NM_011905 NM_011905 Mus musculus toll-like receptor 2 (Tlr2), mRNA. 2/2007
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TLR3

>NM_126166 NM_126166 Mus musculus toll-like receptor 3 (Tlr3), mRNA. 2/2007
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Reverse	GAGCGAGGGGACAGACGC	60

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>NM_021297 NM_021297 Mus musculus toll-like receptor 4 (Tlr4), mRNA. 3/2007
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Forward	TCACCTGGGGCTCTGCTATGG	63
Reverse	TTCTGCCCGGTAAGGTCCA	60

OX40
>NM_011659 NM_011659 Mus musculus tumor necrosis factor receptor
superfamily, member 4 (Tnfrsf4), mRNA. 1/2007
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CACGTTCACATACCTTCTTGCCTGTCCGCCTACTCTTCTTGCCCCACCTCCATAGTTCTT

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	Sequence	Tm
Forward	TTGCCCTCTCCACCCACC	60
Reverse	GGCCTAGGAGAACAGCAAATGC	61

OX40L
>NM_009452 NM_009452 Mus musculus tumor necrosis factor (ligand)
superfamily, member 4 (Tnfsf4), mRNA. 1/2007
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Forward	CCCCGTGGAAAGCAGGAC	60
Reverse	GGCCAGGAAAAGAGTGCACC	61

TL1A (Tnfsf15)

>NM_177371 NM_177371 Mus musculus tumor necrosis factor (ligand)
superfamily, member 15 (Tnfsf15), mRNA. 11/2006
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GAAAAAAAAAAAAAAAAA

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Forward	CGACCAAACAAGCCAGACTCC	61
Reverse	CGGGCAGGCTCAGGGTAG	60

CXCR4

>NM_009911 NM_009911 Mus musculus chemokine (C-X-C motif) receptor 4
(Cxcr4), mRNA. 2/2007
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TTCCGGGATGAAAACGTCCATTTCAATAGGATCTTCCTGCCCACCATCTACTTCATCATC
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Forward	TCCTCTATGCCTTCCTCGGG	60
Reverse	CATGGAGTTGAGTGCATGCTGG	62

CXCL13

>NM_018866 NM_018866 Mus musculus chemokine (C-X-C motif) ligand 13
(Cxcl13), mRNA. 12/2006
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Forward	CATCATGAGGTGGTGCAAAGC	60
Reverse	AAGTAGGACTGTTTCCCCCAGGA	61

CD91

>NM_008512 NM_008512 Mus musculus low density lipoprotein receptor-related
protein 1 (Lrp1), mRNA. 2/2007
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	Sequence	Tm

| Forward | ATCTCAAAGGGCTGGCGGT | 61 |
| Reverse | GCTTCATCAGAGCCGTCGG | 60 |

APRIL (Tnfsf13)

>NM_023517 NM_023517 Mus musculus tumor necrosis factor (ligand)
superfamily, member 13 (Tnfsf13), mRNA. 1/2007
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Forward	GCATCCAGAACAGCAGCACC	60
Reverse	TTTTCCCTAGCAGAGACCATAGTCTTTC	61

DR3 (Tnfrsf25)

>NM_033042 NM_033042 Mus musculus tumor necrosis factor receptor
superfamily, member 25 (Tnfrsf25), mRNA. 11/2006
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Forward	CACACCCTCCTGGCACCTC	60
Reverse	TGTTGCCTACCAACTGGACAGTG	61

RANKL

>NM_011613 NM_011613 Mus musculus tumor necrosis factor (ligand)
superfamily, member 11 (Tnfsf11), mRNA. 2/2007

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Genomic DNA Control from Beta Actin Intron Sequence

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Tnfsf8 (CD30L)

>NM_009403 NM_009403 Mus musculus tumor necrosis factor (ligand)
superfamily, member 8 (Tnfsf8), mRNA. 11/2006
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	Sequence	Tm
Forward	GAAGAGTTTCTCCATCCGGCA	60
Reverse	TGCATCCTGGATCATGTGACTTG	62