Supplemental Information

Figure S1

Regulation SOCS1 expression in BCG-infected BMM, related to Figure 1

Total RNA was isolated from WT BMM treated or not with 5 μM cytochalasin D, 6 h after incubation with BCG (MOI 5:1) or 1 μg/ ml Pam3 (A).

The accumulation of *SOCS1* and *HPRT* mRNA were measured by real time PCR in triplicate samples for each group and time point. The mean fold induction of *SOCS1* mRNA ± SEM is depicted.

Total RNA was isolated from BMM treated with the indicated concentrations of BAY-11 7082 or vehicle alone (B) 1h before BCG infection. The accumulation of *MCP-1* and HPRT mRNA was measured by real time PCR. The mean fold induction of *MCP-1* mRNA ± SEM is depicted.

*Differences with WT controls are significant ($p<0.05$ Student t test)

Figure S.2

SOCS1 expression LysM-cre SOCS1$^{fl/fl}$ M. tuberculosis-infected BMM, related to Figure 2

The relative concentration of *SOCS1* and *HPRT* mRNA were measured from total RNA isolated from *LysM-cre SOCS1$^{fl/fl}$* and *SOCS1$^{fl/fl}$* BMM at the indicated time points after infection with *M. tuberculosis* by real time PCR. The mean fold induction of SOCS1 mRNA ± SEM is depicted (A).

SOCS1 hinders clearance of BCG by macrophages in an IFN-γ-mediated manner, related to Figure 3

Total RNA was extracted from *SOCS1$^{-/-}$* and WT BMM (B-D) and BMDC (E-G) at the indicated time points after infection with BCG at a MOI 5:1. The accumulation of *IFN-α*, *IFN-β* and *IFN-γ* and *HPRT* mRNA was measured by real time PCR. The mean fold accumulation of the transcripts of triplicate cultures per time point in relation to HPRT ± standard error of the mean is depicted.

IFN-γ-secreting *SOCS1$^{-/-}$* and WT BMDC were measured by ELISPOT assay 24 h after incubation with BCG or with culture medium as a control. The mean number of spots ± SEM from triplicate cultures in each experimental condition is shown (H).

IFN-γ$^{-/-}$ and IFN-γ$^{-/-}$/*SOCS1$^{-/-}$* BMM were infected with BCG at MOI 5:1 (I), and the CFU were determined in lysates from triplicate cultures. The mean CFU per well ± SEM from one of two independent experiments is depicted.

Total RNA was isolated from *IFN-γ R$^{-/-}$* and WT BMM at different time points after infection with BCG at a MOI 5: 1 (J). The accumulation of SOCS1 and HPRT mRNA was measured by real time PCR.

*Differences with control BMM are significant ($p<0.05$ Student t-test).

Figure S.3

SOCS1-dependent expression of IL12Rb1 mRNA by M. tuberculosis infected BMM is IFN-γ-dependent, related to Figure 4.

Total RNA was isolated from IFN-$\gamma^{-/-}$ and IFN-$\gamma^{-/-}$/ SOCS1$^{-/-}$ BMM at the indicated times after infection with *M. tuberculosis.* The accumulation of *IL12Rβ1* and *HPRT* mRNA were measured by real time PCR in triplicate samples for each group and time point. The mean fold induction of *IL12Rβ1* mRNA ± SEM is depicted (A).

M. tuberculosis-infected BMM are not tolerant to IFN-γ (B, C), related to Figure 4

*SOCS*1$^{-/-}$ and WT BMM were treated with 100 U IFN-γ 3 h after infection with *M. tuberculosis*. Total RNA was isolated at the indicated time points after infection. Uninfected controls were harvested 3 h after IFN-γ stimulation. The accumulation of *CXCL10* (B), *iNOS* (C) and *HPRT* mRNA was measured by real time PCR. The mean fold accumulation of *CXCL10* mRNA of triplicate cultures per time point in relation to HPRT ± standard error of the mean is depicted. Differences between IFN-γ-treated and untreated cells are significant ($p<0.05$ Student t test).

Figure S.4

SOCS1 in non-macrophage cells hinders detrimental pulmonary inflammation, related to Figure 7

Total RNA was extracted from lungs of individual *RAG1*$^{-/-}$, *RAG1*$^{-/-}$*/ SOCS1*$^{-/-}$, IFN-γR$^{-/-}$, *IFN-γ* $^{-/-}$, *IFN-γ*$^{-/-}$*/ SOCS1*$^{-/-}$ and C57Bl/6 mice after aerosol infection with *M. tuberculosis*. The mean fold accumulation of *SOCS1* (D, E), *IFN-γ* (B), *iNOS* (A, F), *CXCL9* (G) and *CXCL10* (H) transcripts ± SEM is depicted.

*Differences with mutant uninfected controls are significant ($p<0.05$ Student t test).

Spleens and lung cells were obtained from *LysM-cre SOCS1*$^{fl/fl}$ and *SOCS1* $^{fl/fl}$ mice before or at 6 weeks after aerosol infection with *M. tuberculosis.* The frequency of IFN-γ secreting CD4+ cells in response to PPD stimulation was assessed by FACS analysis as described in the supplementary experimental procedures section. The mean frequency of IFN-γ-secreting CD4+ cells of at least 4 animals per group ± SEM is depicted (C). IFN-γ-secreting cells were not detected in uninfected mice.

Histopathological scoring of hematoxylin-eosin stained paraffin lung sections from *LysM-cre SOCS1*$^{fl/fl}$ and *SOCS1* $^{fl/fl}$ 6 weeks after aerosol infection with *M. tuberculosis*. The mean % area or the relative histopathological score of 8 mice per group ± SEM is depicted (I-J). *Differences with controls are significant ($p<0.05$ Student t test).

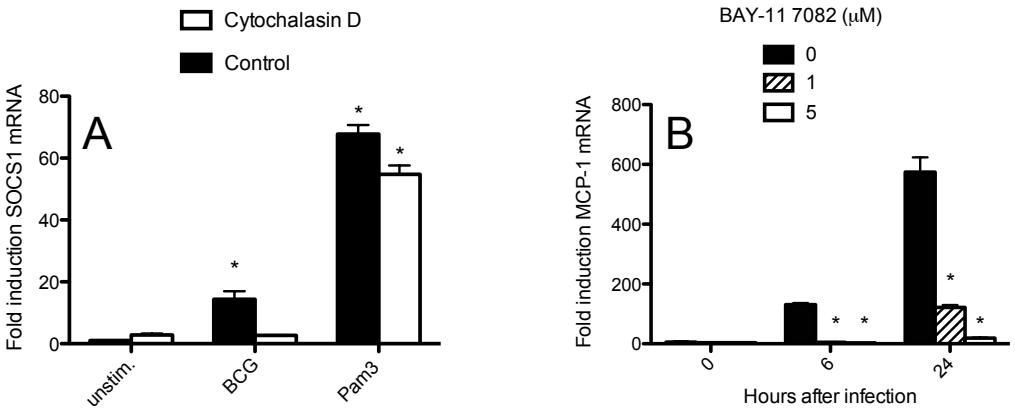
Cytochalasin D
Control
A
Fold induction SOCS1 mRNA
80
60
40
20
0
unstim.
BCG
Pam3
*
*
*
BAY-11 7082 (μM)
0
1
5
B
Fold induction MCP-1 mRNA
800
600
400
200
0
0
6
24
Hours after infection
*
*
*
*

Figure S1

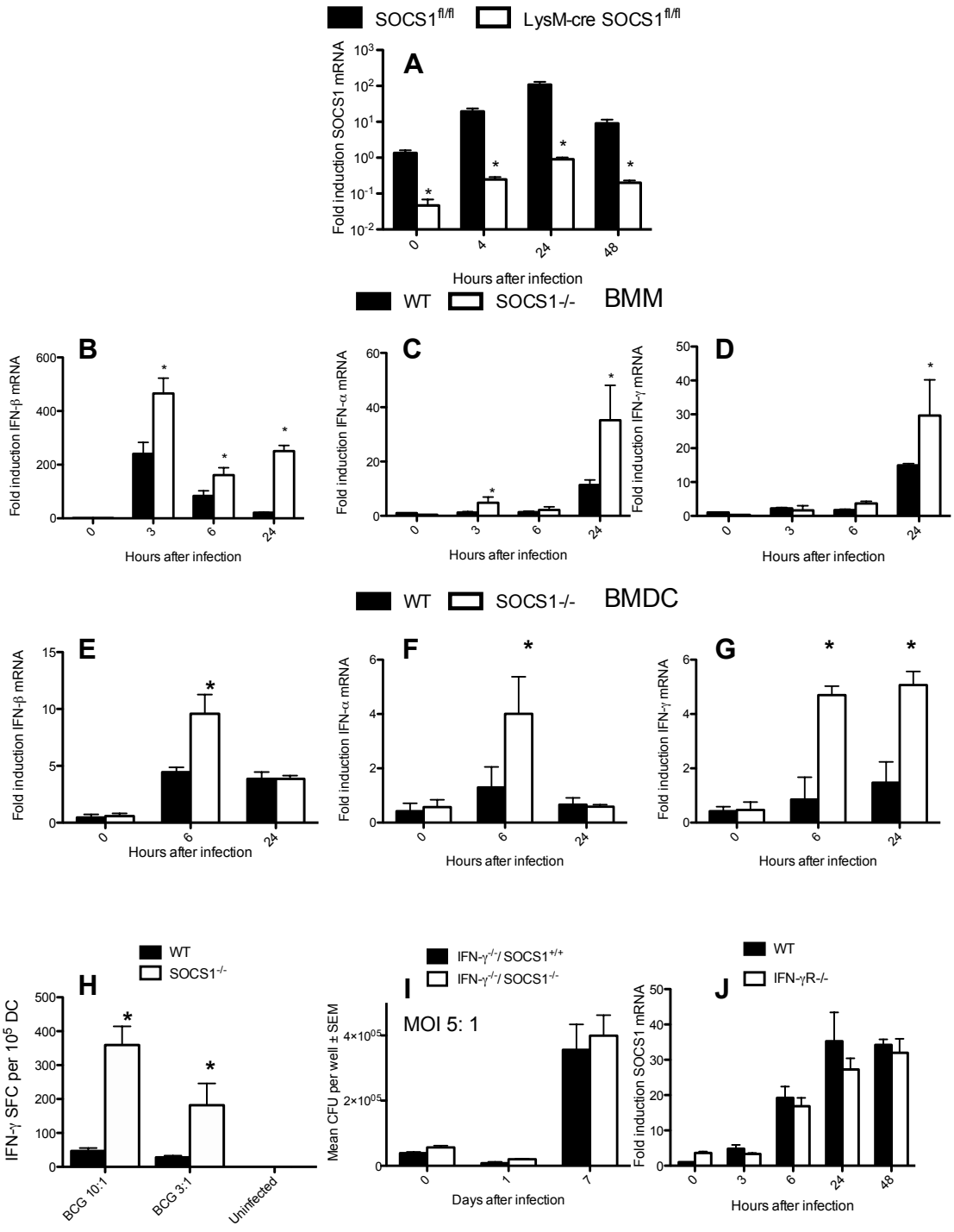
SOCS1fl/fl
LysM-cre SOCS1fl/fl
A
Fold induction SOCS1 mRNA
Hours after infection
WT
SOCS1-/-
BMM
B
Fold induction IFN-β mRNA
C
Fold induction IFN-α mRNA
D
Fold induction IFN-γ mRNA
BMDC
E
F
G
H
WT
SOCS1-/-
IFN-γ SFC per 10^5 DC
BCG 10:1
BCG 3:1
Uninfected
I
IFN-γ-/- / SOCS1+/+
IFN-γ-/- / SOCS1-/-
MOI 5: 1
Mean CFU per well ± SEM
Days after infection
J
IFN-γR-/-

Figure S2

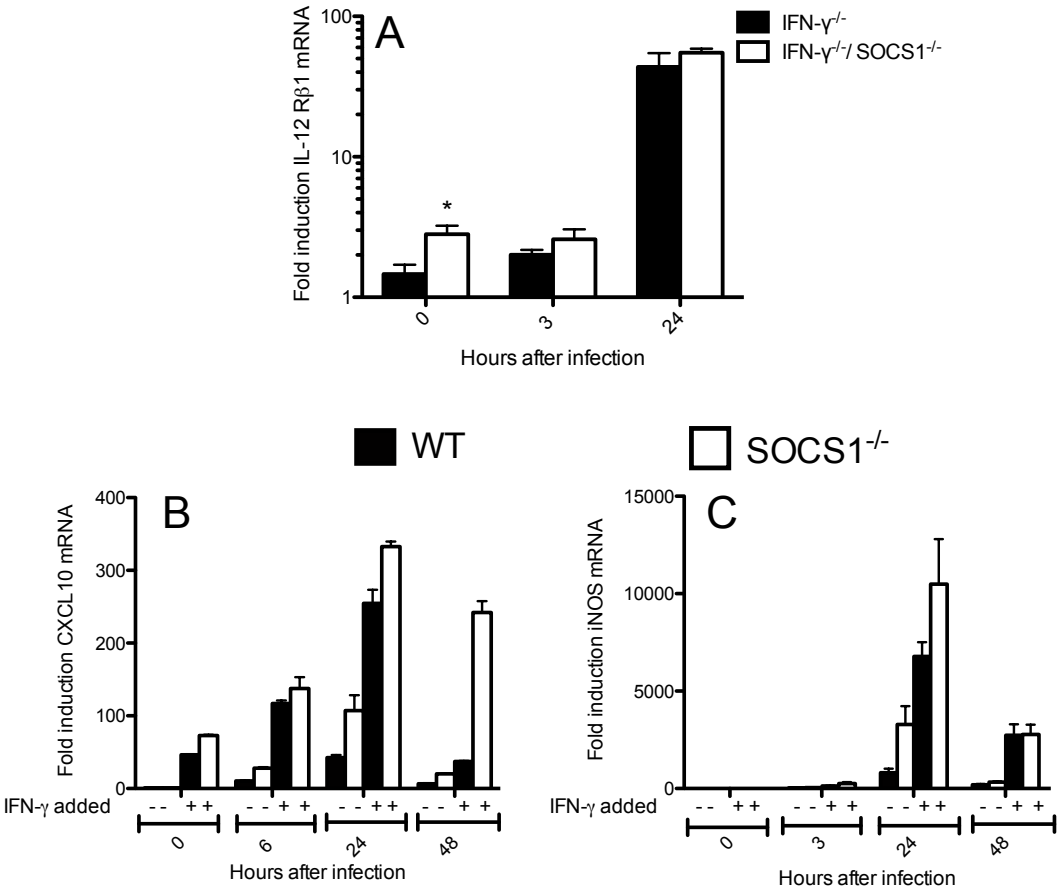
A
IFN-γ-/-
IFN-γ-/- / SOCS1-/-
Fold induction IL-12 Rβ1 mRNA
100
10
1
*
0
3
24
Hours after infection
WT
SOCS1-/-
B
Fold induction CXCL10 mRNA
400
300
200
100
0
IFN-γ added
- - + + - - + + - - + + - - + +
0
6
24
48
Hours after infection
C
Fold induction iNOS mRNA
15000
10000
5000
0
IFN-γ added
- - + + - - + + - - + + - - + +
0
3
24
48
Hours after infection

Figure S3

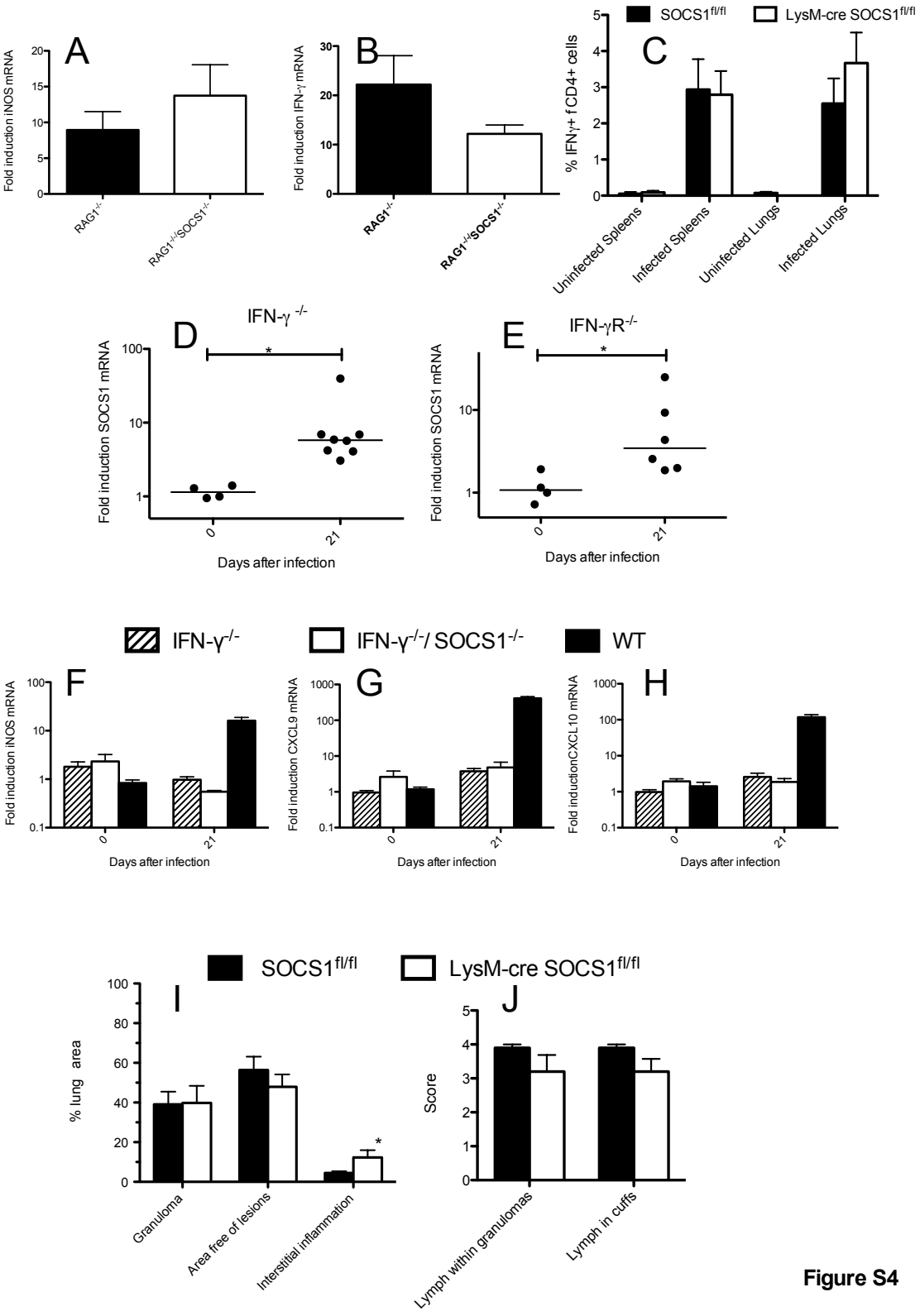
A
Fold induction iNOS mRNA
RAG1-/-
RAG1-/-SOCS1-/-
B
Fold induction IFN-γ mRNA
RAG1-/-
RAG1-/-SOCS1-/-
C
SOCS1fl/fl
LysM-cre SOCS1fl/fl
% IFNγ+ f CD4+ cells
Uninfected Spleens
Infected Spleens
Uninfected Lungs
Infected Lungs
D
IFN-γ -/-
Fold induction SOCS1 mRNA
Days after infection
E
IFN-γR-/-
Fold induction SOCS1 mRNA
Days after infection
IFN-γ-/-
IFN-γ-/-/ SOCS1-/-
WT
F
Fold induction iNOS mRNA
Days after infection
G
Fold induction CXCL9 mRNA
Days after infection
H
Fold inductionCXCL10 mRNA
Days after infection
SOCS1fl/fl
LysM-cre SOCS1fl/fl
I
% lung area
Granuloma
Area free of lesions
Interstitial inflammation
J
Score
Lymph within granulomas
Lymph in cuffs

Figure S4

Real time PCR primer sequences used

Gene	Forward	Reverse
SOCS1	GCT GTG CCG CAG CAT TAA G	CCA GAA GTG GGA GGC ATC TC
SOCS3	TTC CCA TGC CGC TCA CA	CCC ACC CAG CCC CAT AC
IFN-γ	GCT TTG CAG CTC TTC CTC AT	CAC ATC TAT GCC ACT TGA GTT AAA ATA GT
IFN-β	CTG GAG CAG CTG AAT GGA AAG	TCC GTC ATC TCC ATA GGG ATCT
IFN-α4	TCT GAT GCA GCA GGT GGG	AGG GCT CTC CAG AYT TCT GCT CTG
IL-12p40	CGT GCT CAT GGC TGG TGC AAA G	CTT CAT CTG CAA GTT CTT GGG C
IL-12p35	AGT TTG GCC AGG GTC ATT CC	TCT CTG GCC GTC TTC ACC AT
IL-12Rβ1	TGC CTG TGT GTG TTC CAC CT	TCC TTG CAT GGT TAG ACG CC
IL-12Rβ2	GCA TCA GTG TCT GCA GCC AA	GAG ACC TGG TGA GGA GCC AG
IL-1β	TGG TGT GTG ACG TTC CCA TT	CAG CAC GAG GCT TTT TTG TTG
IL-6	ACA AGT CGG AGG CTT AAT TAC ACA T	TTG CCA TTG CAC AAC TCT TTT C
TNF-α	GGC TGC CCC GAC TAC GT	GAC TTT CTC CTG GTA TGA GAT AGC AAA
iNOS	CAG CTG GGC TGT ACA AAC CTT	CAT TGG AAG TGA AGC GTT TCG
CXCL9	CTT TTC CTC TTG GGC ATC AT	GCA TCG TGC ATT CCT TAT CA
CXCL10	GCT GCC GTC ATT TTC TGC	TCT CAC TGG CCC GTC ATC
CXCL1	GGC GCC TAT CGC CAA TG	CTG GAT GTT CTT GAG GTG AAT CC
CXCL2	CCC CCT GGT TCA GAA AAT CA	GGT TCT TCC GTT GAG GGA C
HPRT	CCC AGC GTC GTG ATT AGC	GGA ATA AAC ACT TTT TCC AAA TCC