Supplementary Figure Legends

Fig. S1. Gene enrichment analysis between MSCs with inflammatory stimulated and unstimulated. RNA of MSCs with inflammatory stimulated and unstimulated was collected for RNA-sequencing analysis. Volcano plot of changed genes when MSCs stimulated by TNF-α (10 ng/mL) plus IFN-γ (10 ng/mL) for 24 h compared with unstimulated MSCs was shown.

Fig. S2. Identification of the phenotypes of WT-MSCs and OPN-/--MSCs. (a) OPN expression was determined in WT-MSCs and $OPN^{-/-}$-MSCs by quantitative real-time PCR and immunoblotting analysis. Full-length blots are presented in Additional file 1: Fig. S2a. **(b)** Markers of WT-MSCs and $OPN^{-/-}$-MSCs were analyzed by flow cytometry. **(c)** WT-MSCs and $OPN^{-/-}$-MSCs were cultured in osteogenic differentiation medium, adipogenic differentiation medium and chondroblast differentiation medium, and staining was performed to indicate the effects of OPN on MSC differentiation. The expression of sox9, col2 and aggrecan, markers of chondroblast differentiation of MSCs, was also determined by quantitative real-time PCR. The results are representative of three to six independent experiments. Values are shown as the mean ± SEM and statistical significance is indicated as *P < 0.05, **P < 0.01 and ***P<0.001.

Fig. S3. iOPN promoted proliferation and inhibited IFN-γ plus TNF-α-induced apoptosis in MSCs. (a-g) MSCs were stimulated with or without TNF-α (10 ng/mL) plus IFN-γ (10 ng/mL) for 24 h. Recombinational sOPN or neutralizing antibody of sOPN was added into the medium of MSCs for 24 h before inflammation stimulation. **(e)** iOPN overexpression in MSCs was determined by immunoblotting analysis and quantitative real-

time PCR. Full-length blots are presented in Additional file 1: Fig. S3e. **(a, c, f)** The proliferation of MSCs was analyzed by a CCK8 kit. **(b, d, g)** The percentage of apoptotic MSCs after the indicated treatments was analyzed by flow cytometry. The results are representative of three to six independent experiments. Values are shown as the mean ± SEM and statistical significance is indicated as *P < 0.05, **P < 0.01 and ***P<0.001. ns = no significance.

Fig. S4. iOPN overexpression in MSCs suppressed immune responses more effectively *in vivo*. (a-c) Mice were intravenously injected with ConA (20 mg/kg), and untreated, Vector-MSCs and iOPN-MSCs were transfused immediately (n = 5 per group). Eight hours later, livers and spleens were sampled. **(a)** Percentages of $CD4^+$ T cells in livers and spleens were determined by flow cytometry. The absolute numbers and percentages of $CD4^+$ T cells in livers and spleens were counted. **(b)** BrdU was intraperitoneally injected into naïve mice, ConA mice, Vector-MSC-treated mice or iOPN-MSC-treated mice. Eight hours later, MNCs were isolated from livers and spleens. The percentages of $CD4^+BrdU^+$ T cells in the livers and spleens were determined by flow cytometry. **(c)** The levels of CD25 and CD69 on $CD4^+$ T cells in livers and spleens were analyzed by flow cytometry. **(d-e)** Mice were fed DSS, untreated, Vector-MSCs and iOPN-MSCs were transfused every two days (n = 5 per group). **(d)** Percentages of $CD4^+$ T cells in the colon and MLNs were determined by flow cytometry. The absolute numbers and percentages of $CD4^+$ T cells in the colon and MLNs were counted. **(e)** Percentages and absolute numbers of $LY6G^+$ cells and $F4/80^+$ cells in colons were determined by flow cytometry. The results are representative of three

to six independent experiments. Values are shown as the mean ± SEM and statistical significance is indicated as *P < 0.05, **P < 0.01 and ***P<0.001.

Fig. S5. Analysis of gene correlation between OPN and iNOS in MSCs. RNA of MSCs with inflammatory stimulated (TNF-α (10 ng/mL) plus IFN-γ (10 ng/mL) for 24 h) and unstimulated was collected for RNA-sequencing analysis. Heatmap of the correlation between gene expression (rows and columns) was shown.

Fig. S6. OPN was not regulated by T-bet or hnRNP-A/B in MSCs under inflammation. **(a)** MSCs were treated with TNF-α (10 ng/mL) plus IFN-γ (10 ng/mL) for 24 h. The mRNA was isolated from the cells and opn, T-bet, hnRNP-A/B levels were measured by quantitative real-time PCR. **(b-c)** siRNA targeting T-bet or hnRNP-A/B was transfected into MSCs. Then, MSCs were treated with TNF-α (10 ng/mL) plus IFN-γ (10 ng/mL) for 24 h. Total mRNA was collected, and the levels of opn, T-bet and hnRNP-A/B expression were measured by quantitative real-time PCR. The results are representative of three to six independent experiments. Values are shown as the mean ± SEM and statistical significance is indicated as *P < 0.05, **P < 0.01 and ***P<0.001. ns = no significance.

Figure S1

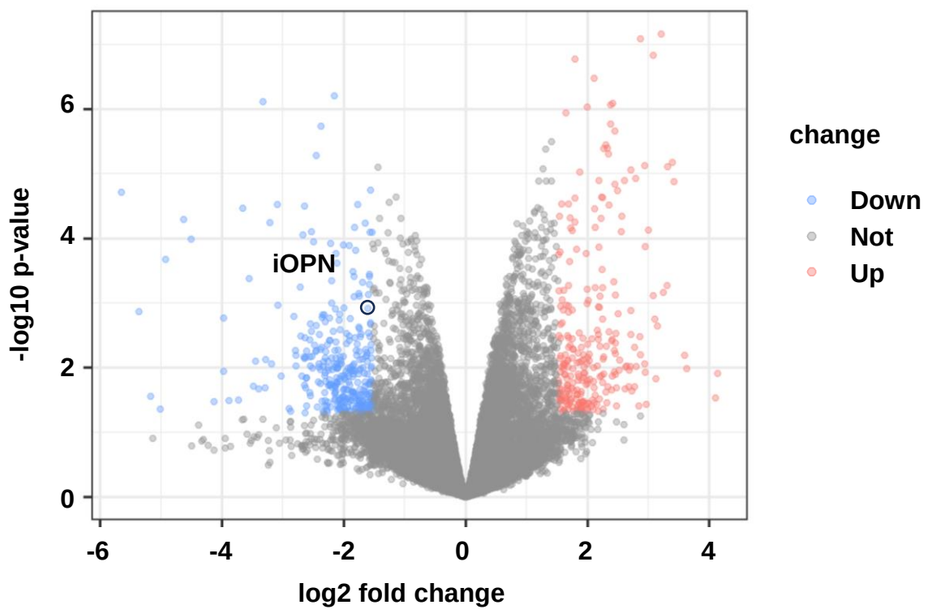

-log10 p-value
6
4
2
0
iOPN
-6
-4
-2
0
2
4
log2 fold change
change
Down
Not
Up

Figure S2

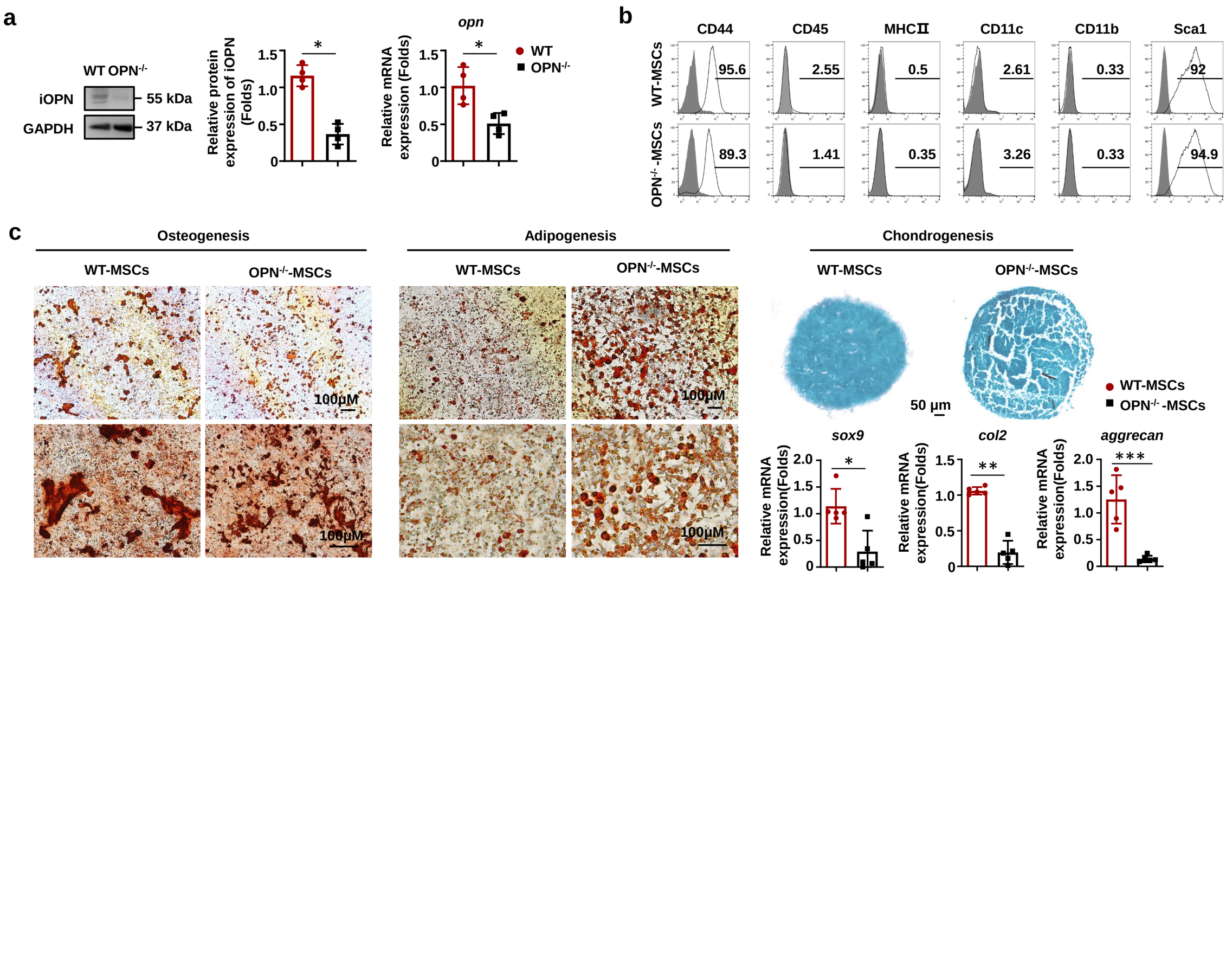

a
WT OPN-/-
iOPN
GAPDH
55 kDa
37 kDa
Relative protein expression of iOPN (Folds)
1.5
1.0
0.5
0
*
opn
Relative mRNA expression (Folds)
WT
OPN-/-
b
CD44
CD45
MHCⅡ
CD11c
CD11b
Sca1
WT-MSCs
OPN-/- -MSCs
95.6
2.55
0.5
2.61
0.33
92
89.3
1.41
0.35
3.26
0.33
94.9
c
Osteogenesis
Adipogenesis
Chondrogenesis
WT-MSCs
OPN-/-MSCs
100μM
50 μm
WT-MSCs
OPN-/- -MSCs
sox9
col2
aggrecan
Relative mRNA expression(Folds)
2.0
1.5
1.0
0.5
0
*
**
***

Figure S3

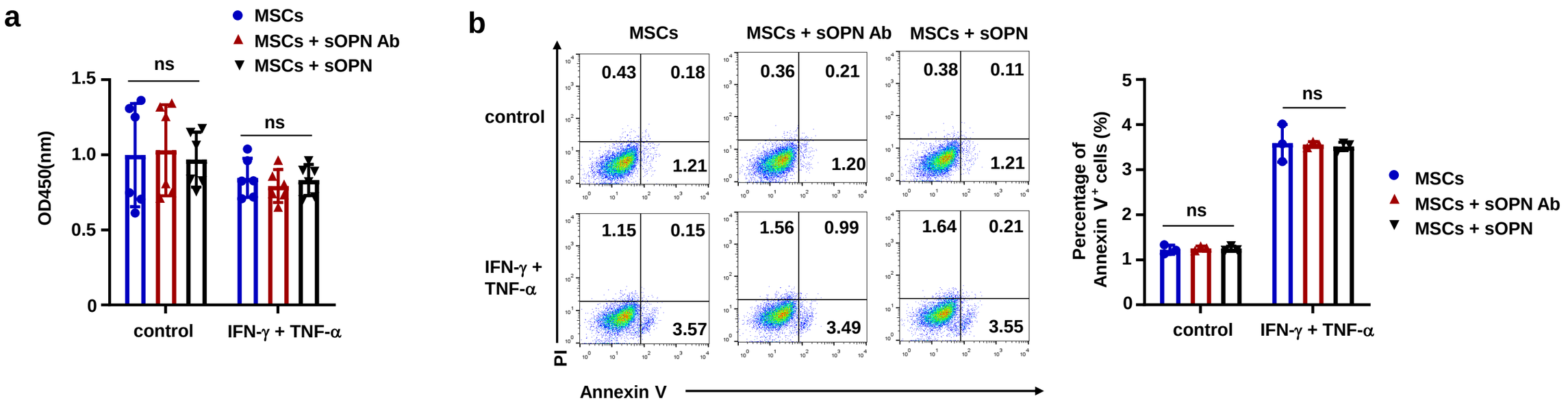

a
MSCs
MSCs + sOPN Ab
MSCs + sOPN
OD450(nm)
ns
ns
control
IFN-γ + TNF-α
b
MSCs
MSCs + sOPN Ab
MSCs + sOPN
control
IFN-γ + TNF-α
0.43
0.18
1.21
0.36
0.21
1.20
0.38
0.11
1.21
1.15
0.15
3.57
1.56
0.99
3.49
1.64
0.21
3.55
PI
Annexin V
Percentage of Annexin V+ cells (%)
ns
ns
MSCs
MSCs + sOPN Ab
MSCs + sOPN
c
WT-MSCs
OPN-/--MSCs
OPN-/--MSCs + sOPN
OD450(nm)
**
ns
*
ns
control
IFN-γ + TNF-α
d
WT
OPN-/-
OPN-/- + sOPN
control
IFN-γ + TNF-α
1.09
0.06
0.31
0.65
0.18
1.5
0.36
0.17
1.53
5.12
0.27
2.54
1.32
0.18
6.72
1.03
0.37
6.91
PI
Annexin V
Percentage of Annexin V+ cells (%)
*
ns
WT-MSCs
OPN-/--MSCs
OPN-/--MSCs + sOPN
control
IFN-γ + TNF-α
e
Vector-MSCs
iOPN-MSCs
iOPN
55 kDa
GAPDH
37 kDa
Relative protein expression of iOPN (Folds)
*
Vector
iOPN
opn
Relative mRNA expression (Folds)
*
Vector
iOPN
f
Vector-MSCs
iOPN-MSCs
OD450(nm)
***
***
control
IFN-γ + TNF-α
g
Vector
iOPN
control
IFN-γ + TNF-α
0.7
0.99
9.89
1.10
0.75
9.58
2.45
2.80
49.1
2.23
2.47
38.5
PI
Annexin V
Vector-MSCs
iOPN-MSCs
Percentage of Annexin V+ cells (%)
*
control
IFN-γ + TNF-α

Figure S4

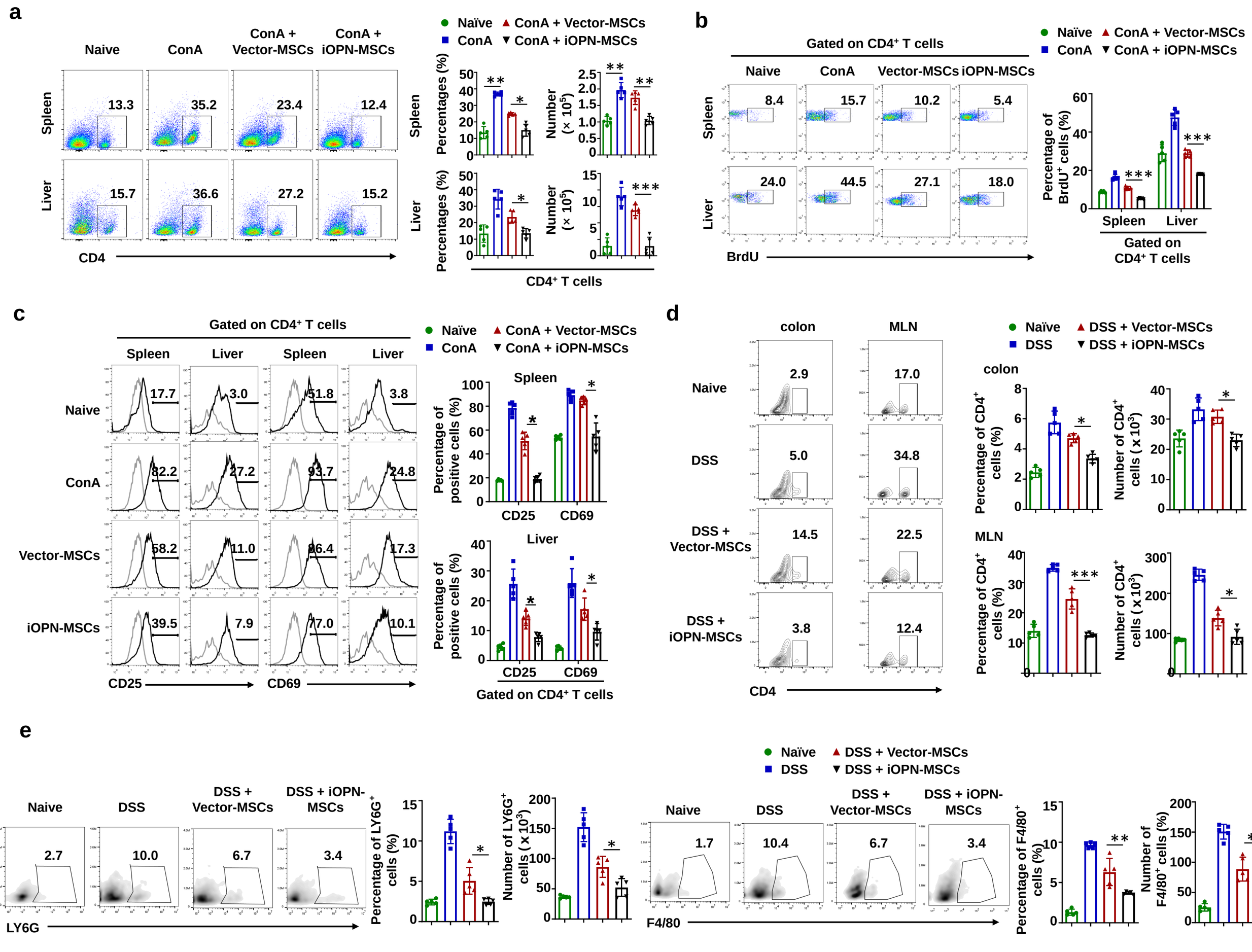

a
Naive
ConA
ConA + Vector-MSCs
ConA + iOPN-MSCs
Spleen
Liver
13.3
35.2
23.4
12.4
15.7
36.6
27.2
15.2
CD4
Percentages (%)
Number (× 10^5)
CD4+ T cells
b
Gated on CD4+ T cells
Vector-MSCs
iOPN-MSCs
8.4
15.7
10.2
5.4
24.0
44.5
27.1
18.0
BrdU
Percentage of BrdU+ cells (%)
Spleen
Liver
c
Gated on CD4+ T cells
17.7
3.0
51.8
3.8
82.2
27.2
93.7
24.8
58.2
11.0
86.4
17.3
39.5
7.9
77.0
10.1
CD25
CD69
Percentage of positive cells (%)
Gated on CD4+ T cells
d
colon
MLN
2.9
17.0
5.0
34.8
14.5
22.5
3.8
12.4
CD4
Percentage of CD4+ cells (%)
Number of CD4+ cells (x 10^3)
e
2.7
10.0
6.7
3.4
LY6G
Percentage of LY6G+ cells (%)
Number of LY6G+ cells (x 10^3)
1.7
10.4
6.7
3.4
F4/80
Percentage of F4/80+ cells (%)
Number of F4/80+ cells (%)

Figure S5

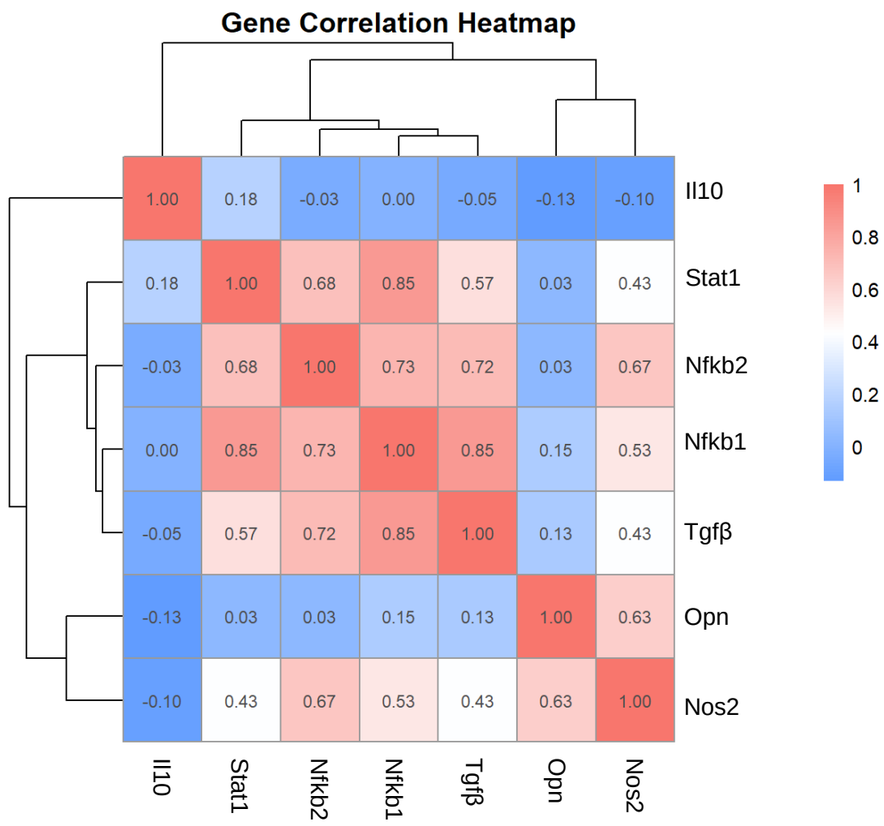
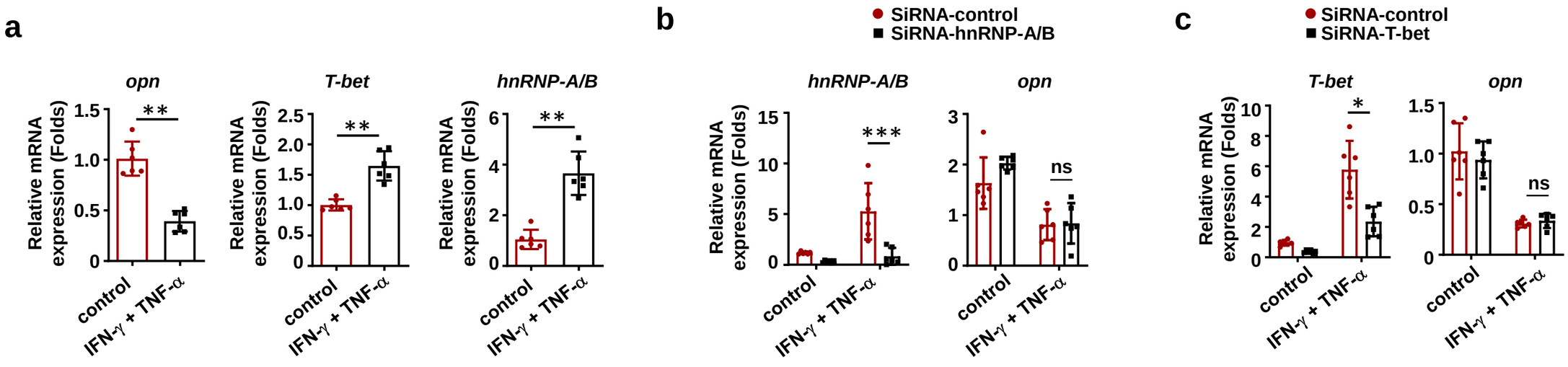

Gene Correlation Heatmap
1.00 0.18 -0.03 0.00 -0.05 -0.13 -0.10
0.18 1.00 0.68 0.85 0.57 0.03 0.43
-0.03 0.68 1.00 0.73 0.72 0.03 0.67
0.00 0.85 0.73 1.00 0.85 0.15 0.53
-0.05 0.57 0.72 0.85 1.00 0.13 0.43
-0.13 0.03 0.03 0.15 0.13 1.00 0.63
-0.10 0.43 0.67 0.53 0.43 0.63 1.00
Il10
Stat1
Nfkb2
Nfkb1
Tgfβ
Opn
Nos2
Il10
Stat1
Nfkb2
Nfkb1
Tgfβ
Opn
Nos2
1
0.8
0.6
0.4
0.2
0

Figure S6


a
opn
T-bet
hnRNP-A/B
Relative mRNA expression (Folds)
**
control
IFN-γ + TNF-α
b
SiRNA-control
SiRNA-hnRNP-A/B
hnRNP-A/B
opn
***
ns
c
SiRNA-control
SiRNA-T-bet
T-bet
opn
*
ns