SUPPLEMENTAL INFORMATION

Figure S1. Lineage differentiation was not involved in the reversion of MSC immunosuppression by IFNα.

(A) MSCs were treated with IFNγ and TNFα or IFNγ, TNFα and IFNα for 24 hours. According to the manufacturer's instructions, total RNA was collected and the expression of osteoblast marker genes or adipocyte marker genes was quantitated by real-time PCR. Values are means ± SD of a representative result of two independent experiments. (**B**) MSCs with treated by different cytokines for 72 hours. Calcium deposits were stained by Alizarin Red S (osteogenesis, upper panel). MSCs were stained with Oil Red O to reveal lipid droplets (adipogenesis, lower panel). (bar = 100 μm)

Figure S2. IFNα does not inhibit iNOS expression in bone marrow-derived macrophages.

Bone marrow-derived macrophages were stimulated by various combinations of TNFα (10 ng/ml), IFNγ (10 ng/ml) or IFNα (2500 U/ml) for 24 hours. The expression of iNOS and pTyr701-Stat1 were examined by western blotting analysis. Values are means ± SD of a representative result of two independent experiments.

Figure S3. IFNα does not inhibit the production of other cytokines or cytokines by

MSCs.

MSCs were stimulated by IFNγ and TNFα with or without IFNα for 24 hours. Supernatants were collected and analyzed by Bio-Plex protein array system for cytokines and chemokines, or by Griess assay for nitrate concentration. IFNγ/TNFα: 10 ng/ml; IFNα 2500 U/ml. Values are means ± SD of four wells from a representative of two independent experiments.

Figure S4. IFNα does not inhibit the expression of IFNγ receptors.

(A) Relative expression levels of IFNγ receptor 1 (IFNGR1), IFNGR2, H2-D1, and H2-K1 from microarray data were compared with or without 24-hour treatment of cytokines. **(B)** Surface expression levels of IFNGR1 and IFNGR2 were determined by flow cytometry analysis at 24 hours. Isotype controls were shown in filled grey histograms.

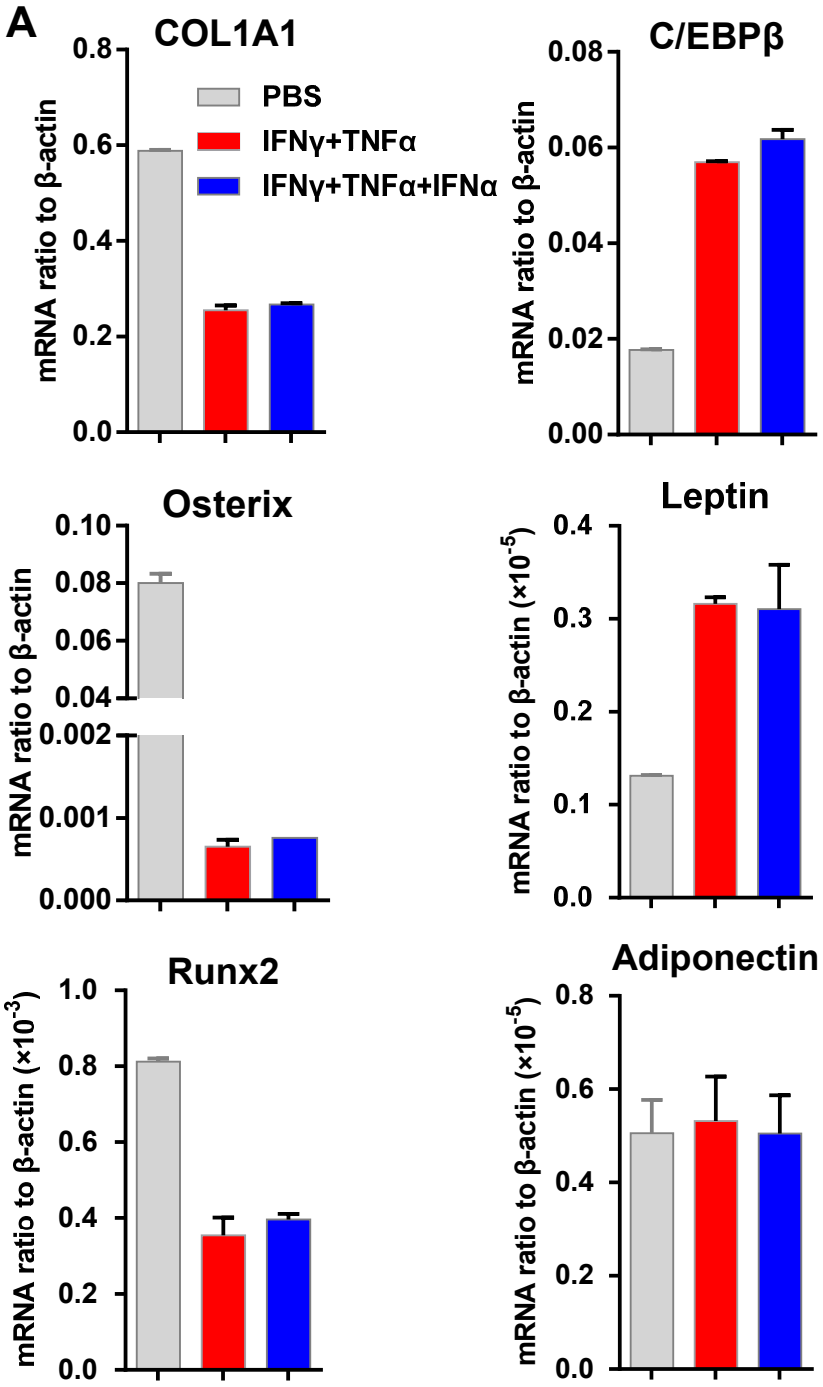
Figure S5. NF-κB signaling pathway is not involved in IFNα-induced iNOS inhibition.

(A) MSCs were stimulated with combinations of IFNγ and TNFα or IFNγ, TNFα and IFNα for indicated time. Total protein was collected and the expression of pSer32-IκBα, IκBα and pSer536-p65 were examined by western blotting analysis. **(B)** MSCs were treated for indicated time. Nucleic proteins were extracted. The distribution of p65 in nucleus was determined by western blotting analysis. Lamin B was reference for nucleic

proteins. **(C)** MSCs were stimulated as previously for 24 hours. Total proteins were collected. p65 was precipitated by sequence-specific oligonucleotide agarose beads. The precipitants were determined by western blotting analysis. Total proteins were inputs. Experiments were repeated twice.

Figure S6. IFNα inhibits NO production by L-MSCs.

MSCs and lymphoma-derived MSCs (L-MSCs) were cultured in the presence of TNFα and IFNγ with or without IFNα for 12 hours. Supernatants were collected and nitrate concentration was determined by a modified Griess reagent. Values are mean ± SD of 3 replicates.


A
COL1A1
PBS
IFNγ+TNFα
IFNγ+TNFα+IFNα
mRNA ratio to β-actin
0.0
0.2
0.4
0.6
0.8
C/EBPβ
mRNA ratio to β-actin
0.00
0.02
0.04
0.06
0.08
Osterix
mRNA ratio to β-actin
0.000
0.001
0.002
0.04
0.06
0.08
0.10
Leptin
mRNA ratio to β-actin (×10-5)
0.0
0.1
0.2
0.3
0.4
Runx2
mRNA ratio to β-actin (×10-3)
0.0
0.2
0.4
0.6
0.8
1.0
Adiponectin
mRNA ratio to β-actin (×10-5)
0.0
0.2
0.4
0.6
0.8

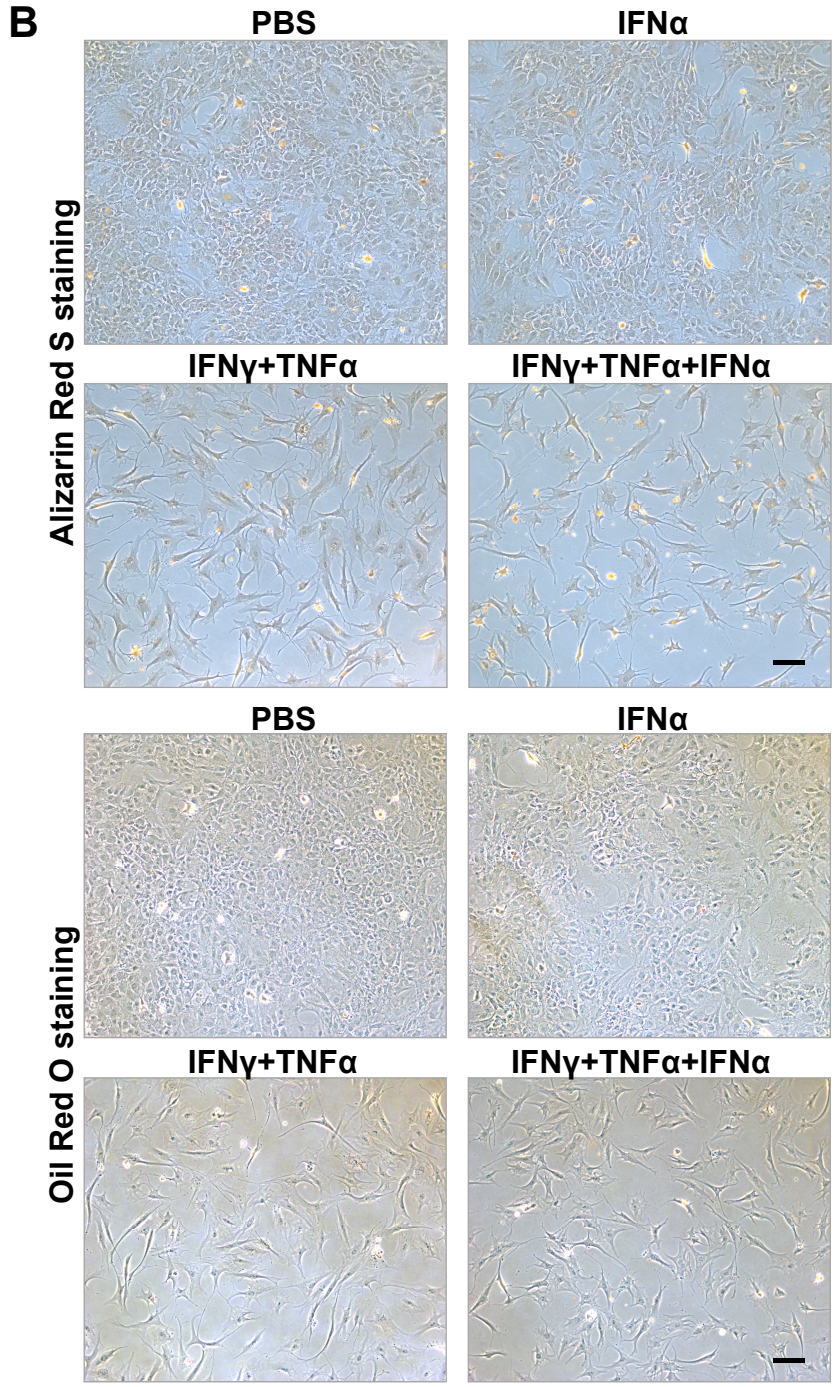
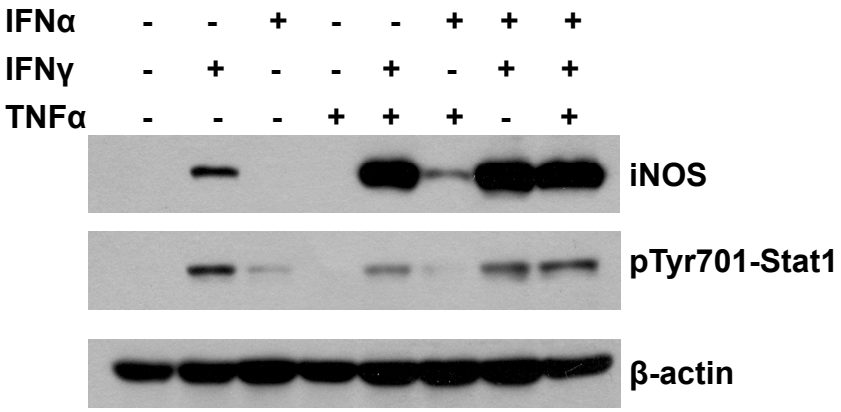

B
PBS
IFNα
IFNγ+TNFα
IFNγ+TNFα+IFNα
Alizarin Red S staining
PBS
IFNα
IFNγ+TNFα
IFNγ+TNFα+IFNα
Oil Red O staining

Fig. S1



Macrophages derived from bone marrow

Fig. S2

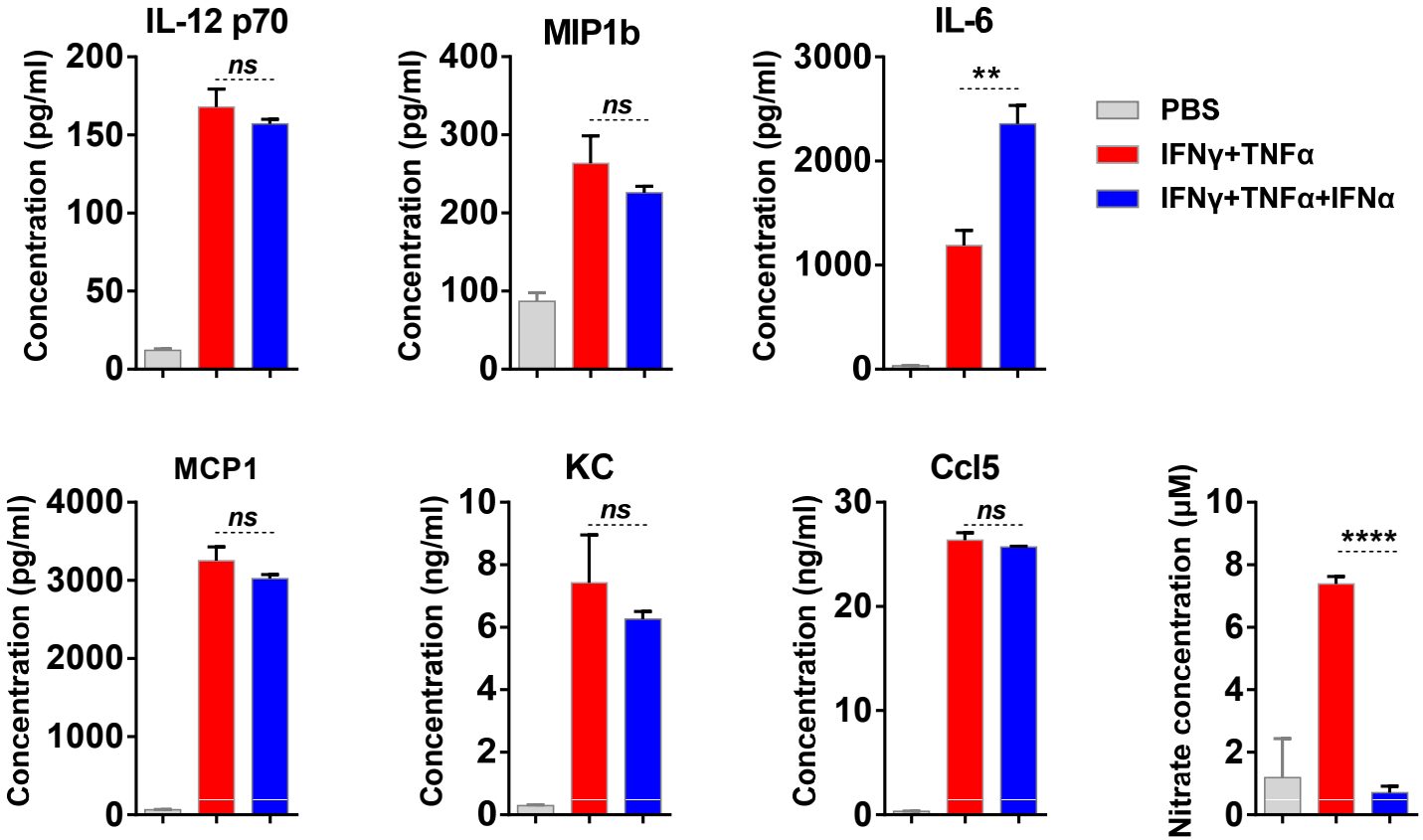
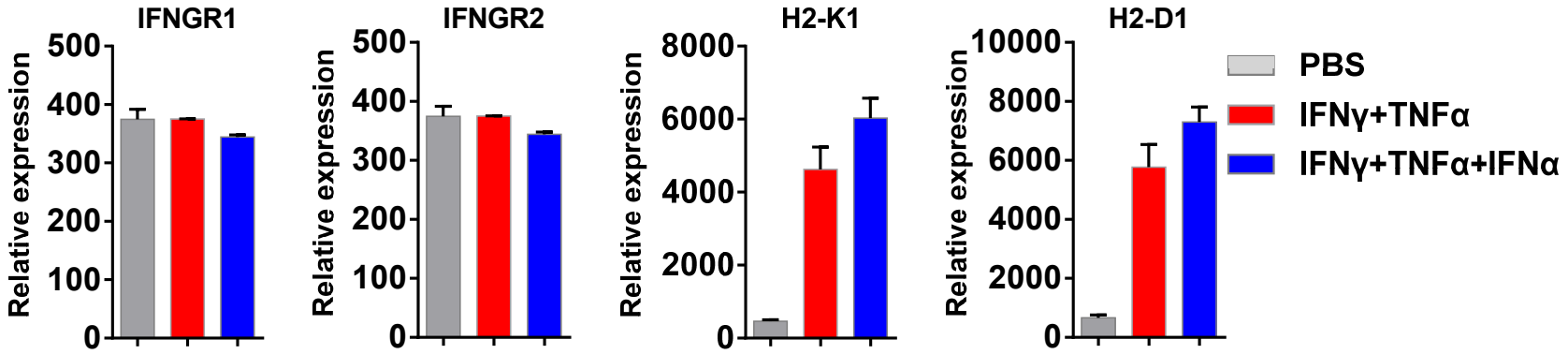

IL-12 p70
Concentration (pg/ml)
200
150
100
50
0
ns
MIP1b
Concentration (pg/ml)
400
300
200
100
0
ns
IL-6
Concentration (pg/ml)
3000
2000
1000
0
**
PBS
IFNγ+TNFα
IFNγ+TNFα+IFNα
MCP1
Concentration (pg/ml)
4000
3000
2000
1000
0
ns
KC
Concentration (ng/ml)
10
8
6
4
2
0
ns
Ccl5
Concentration (ng/ml)
30
20
10
0
ns
Nitrate concentration (μM)
10
8
6
4
2
0
****

Fig. S3

A


IFNGR1
IFNGR2
H2-K1
H2-D1
Relative expression
500
400
300
200
100
0
8000
6000
4000
2000
10000
PBS
IFNγ+TNFα
IFNγ+TNFα+IFNα

B

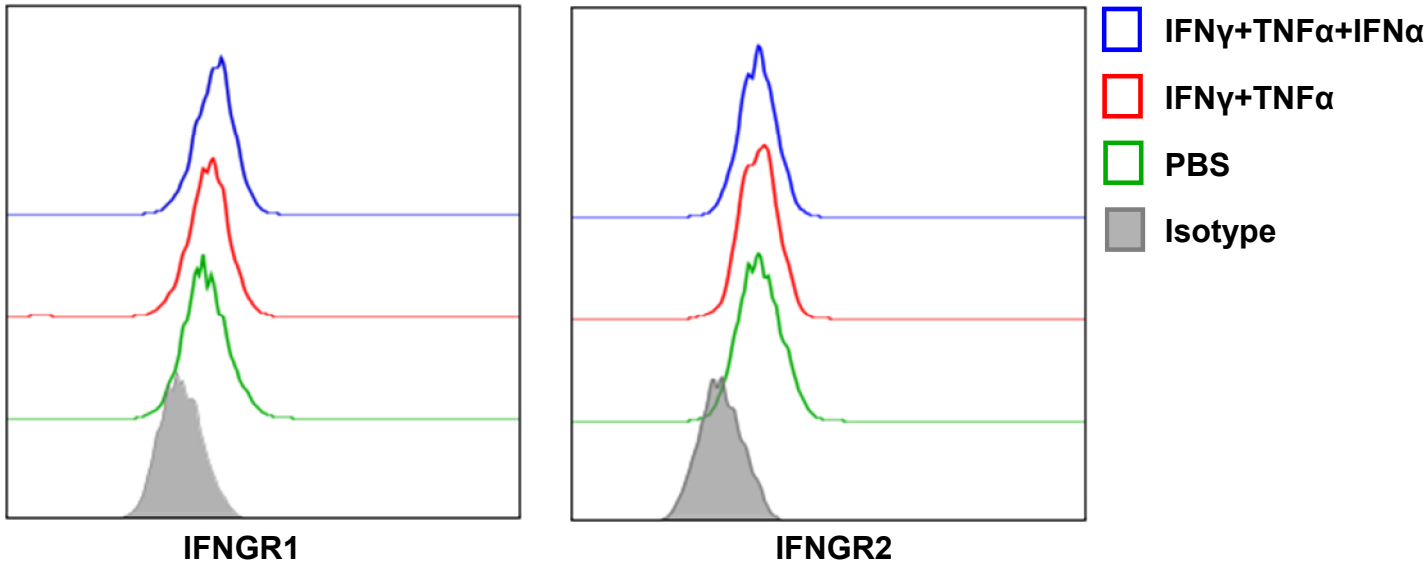
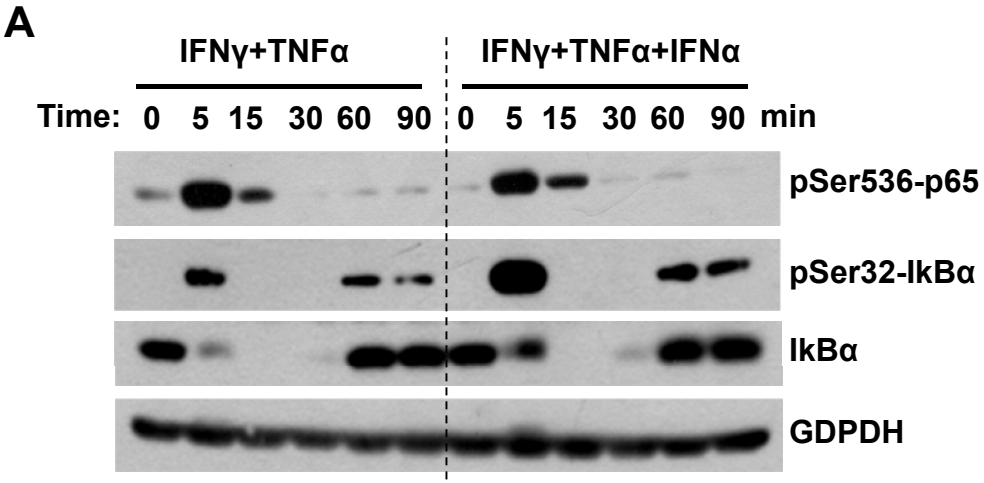
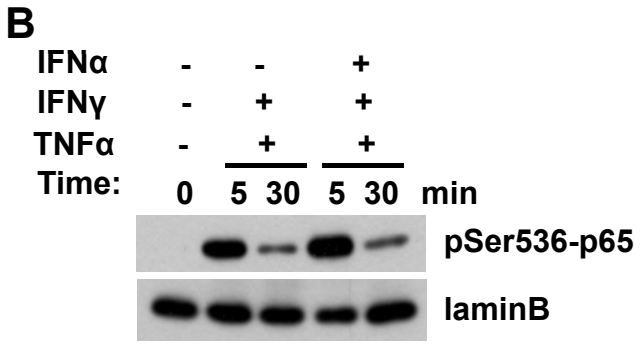

IFNγ+TNFα+IFNα
IFNγ+TNFα
PBS
Isotype
IFNGR1
IFNGR2

Fig. S4


A
IFNγ+TNFα
IFNγ+TNFα+IFNα
Time: 0 5 15 30 60 90 0 5 15 30 60 90 min
pSer536-p65
pSer32-IkBα
IkBα
GDPDH


B
IFNα - - +
IFNγ - + +
TNFα - + +
Time: 0 5 30 5 30 min
pSer536-p65
laminB

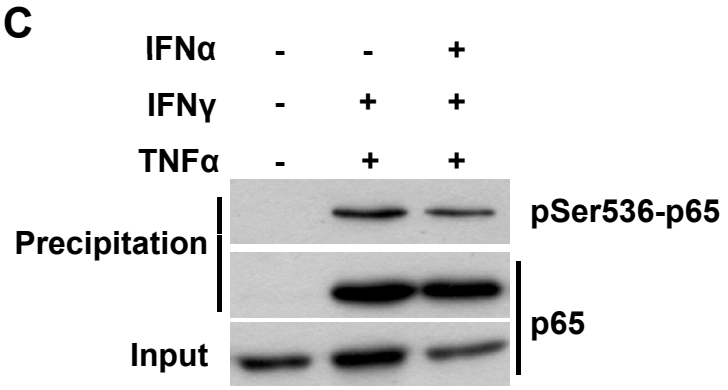

C
IFNα - - +
IFNγ - + +
TNFα - + +
Precipitation
Input
pSer536-p65
p65

Fig. S5

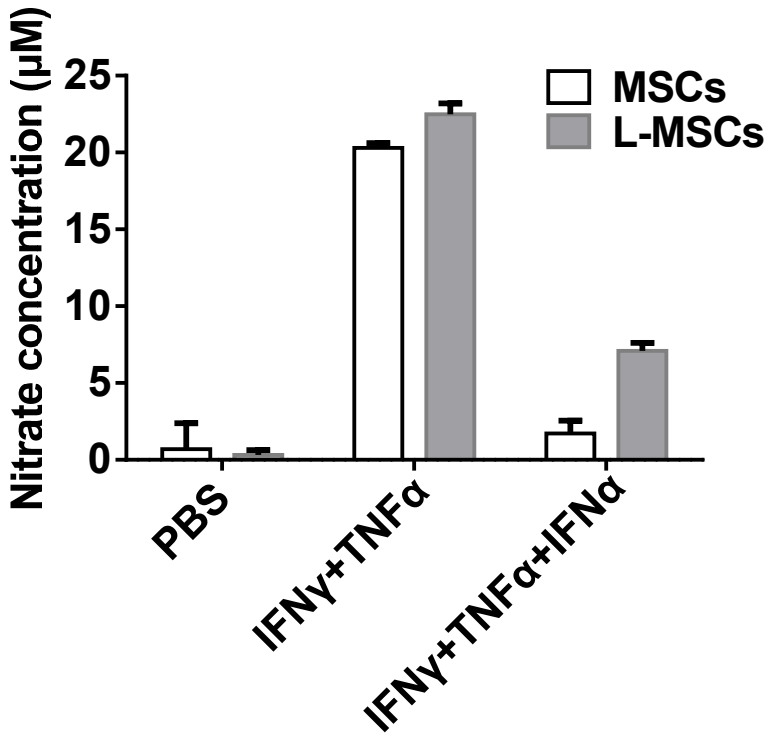

Nitrate concentration (μM)
25
20
15
10
5
0
MSCs
L-MSCs
PBS
IFNγ+TNFα
IFNγ+TNFα+IFNα

Fig. S6