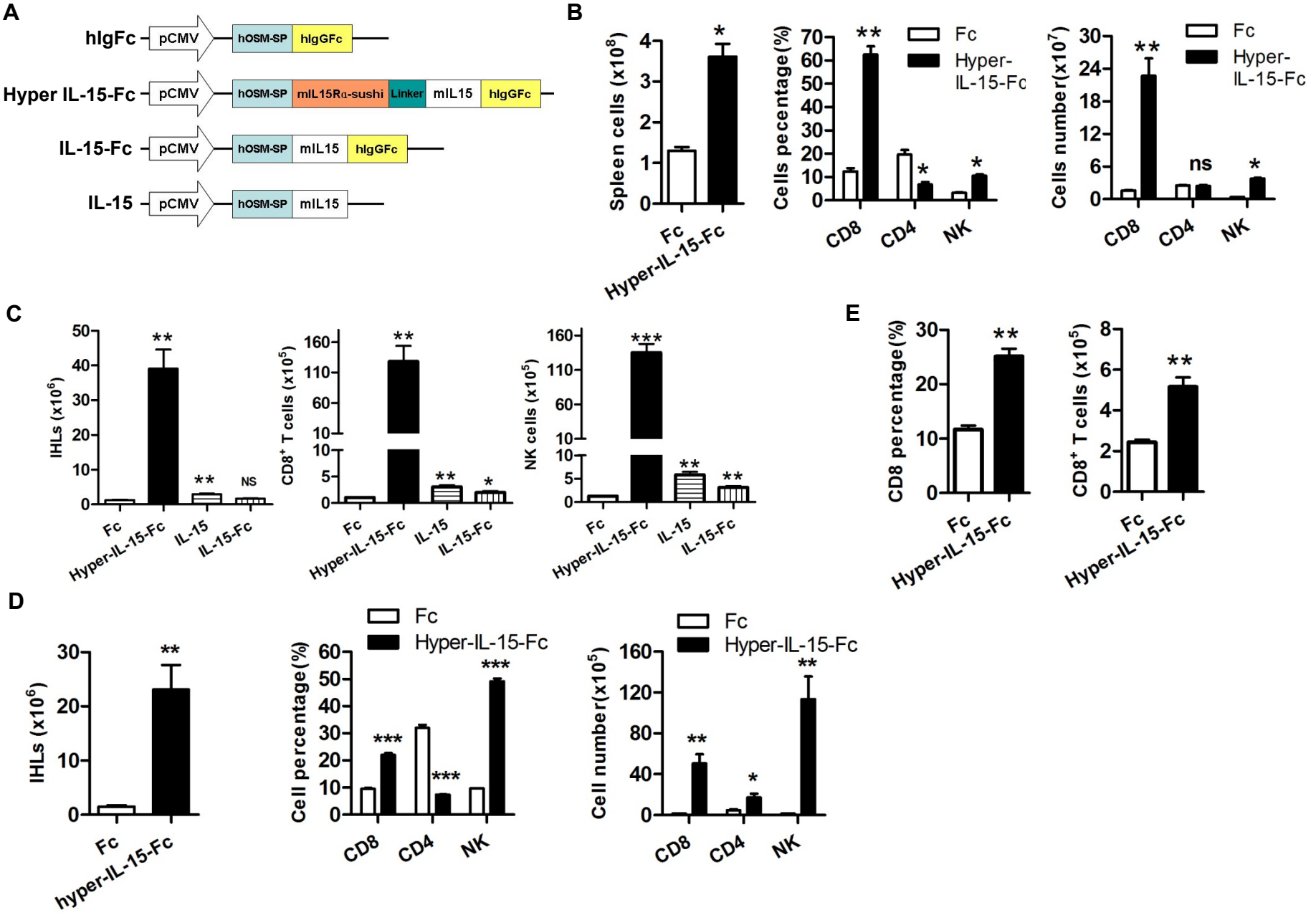
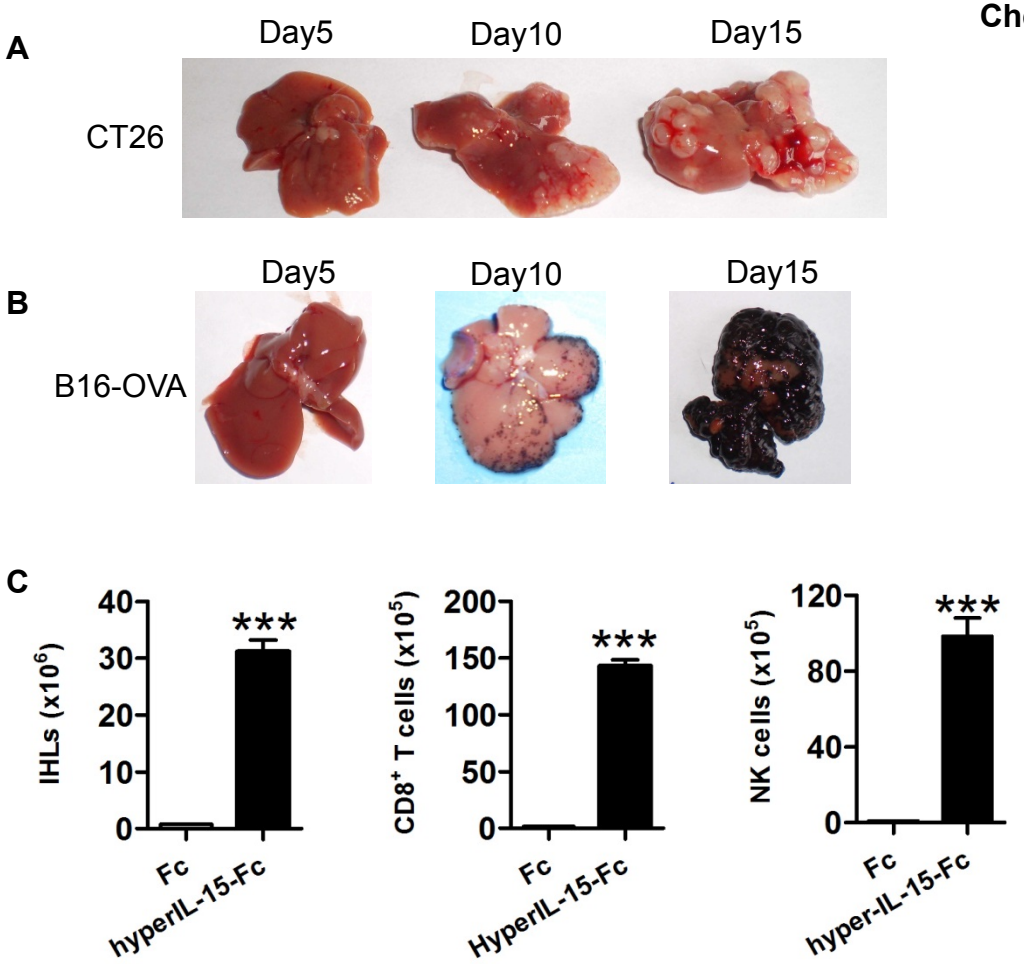
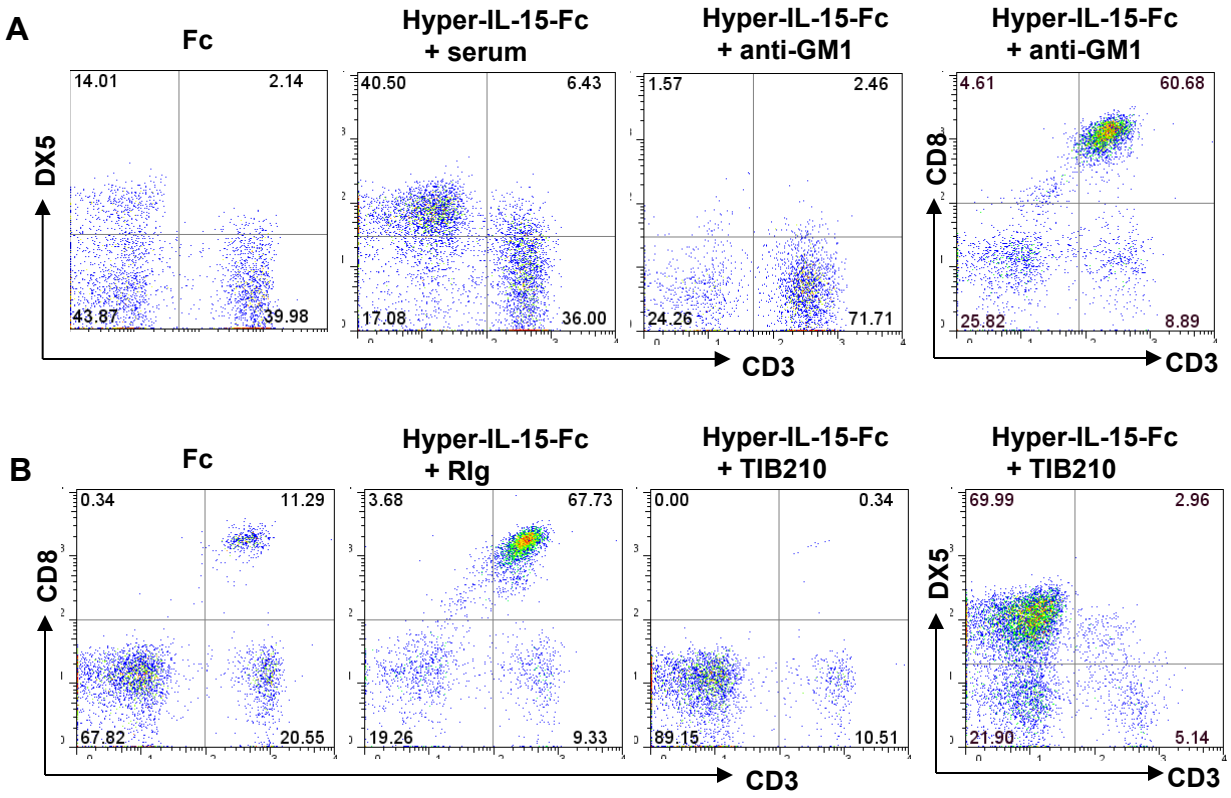

A
hIgFc
pCMV
hOSM-SP
hIgGFc
Hyper IL-15-Fc
mIL15Rα-sushi
Linker
mIL15
IL-15-Fc
IL-15
B
Spleen cells (x10^8)
Fc
Hyper-IL-15-Fc
Cells pecentage(%)
CD8
CD4
NK
Cells number(x10^7)
ns
C
IHLs (x10^6)
NS
IL-15
IL-15-Fc
CD8+ T cells (x10^5)
NK cells (x10^5)
D
hyper-IL-15-Fc
Cell percentage(%)
Cell number(x10^5)
E
CD8 percentage(%)
CD8+ T cells (x10^5)

**Supplementary Fig.1 Hyper-IL-15 is much more efficient than IL-15 alone to stimulate CD8$^+$ T cells and NK cells *in vivo*.**

(A) Schematic representation of hIgGFc (Fc), hyper-IL-15, IL-15-Fc and IL-15 constructs. The signal peptide of human oncostatin M (OSM) was used for all the proteins. For hyper-IL-15-Fc construction, the cDNA encoding mouse IL-15Ra-sushi domain (amino acids 1-78) and mouse IL-15 mature sequence were linked by a 20-amino acid linker and then fused with Fc.21 All the fragments were sub-cloned into the pTT3 plasmid. (B) C57BL/6 mice were hydrodynamically injected with 10μg pTT3-hyper-IL-15-Fc plasmid or pTT3-hIgGFc (pTT3-Fc) as control at day 0. At day 4, the mice were sacrificed and splenic cells were isolated for cytofluorimetric analysis. Numbers of total splenic cells (left), percentages (middle) and numbers (right) of CD8+ T cells, CD4+ T cells, NK cells in spleen in each group were showed. Graphs represent the mean ± SEM of 3 mice each group. Three experiments with similar results were performed. (C) C57BL/6 mice were hydrodynamically injected with pTT3-Fc, pTT3-hyper-IL15-Fc, pTT3-IL15, pTT3-IL15-Fc plasmids at day 0. The mice were sacrificed at day 4 and intrahepatic lymphocytes (IHLs) were isolated for flow cytometry analysis. Numbers of total IHLs (left), numbers of CD8$^+$ T cells (CD3$^+$CD8$^+$) (middle) and NK cells (CD3$^-$NK1.1$^+$) (right) in liver in each group were showed. Graphs represent the mean ± SEM of 3-5 mice per each group. (D) C57BL/6 mice were tail vein injected with 10μg hyper-IL-15-Fc protein or Fc protein as control at day 0. The mice were sacrificed at day 4 and intrahepatic lymphocytes (IHLs) were isolated for flow cytometry analysis. Numbers of total IHLs (left), percentages (middle) and numbers (right) of CD8+ T cells (CD3+CD8+), CD4+ T cells (CD3+CD4+), NK cells (CD3-NK1.1+) in liver in each group were showed. Graphs represent the mean ± SEM of 3 mice each group. (E) C57BL/6 mice were hydrodynamically injected with pTT3-Fc or pTT3-hyper- IL15-Fc plasmids at day 0. The mice were sacrificed at day 40 and intrahepatic lymphocytes (IHLs) were isolated for flow cytometry analysis. Percentage of CD8$^+$ T cells (left), numbers of CD8$^+$ T cells (right) in IHLs in each group were showed. Graphs represent the mean ± SEM of 3 mice in each group.




**Supplementary Fig.2 Intraportal injection of CT26 and B16-OVA tumor cells leads to disseminated metastatic tumors in the liver.** (A-B) 1x105 CT26 colon carcinoma cells or 3x105 B16-OVA melanoma cells were injected into the liver of BALB/c mice or C57BL/6 mice through portal vein. Mice were sacrificed at each time point and representative photograph of livers with metastatic CT26 colon carcinoma (A) or B16-OVA melanoma (B) were shown. (C) $3x10^5$ B16-OVA melanoma cells were intraportally injected into the liver of C57BL/6 mice at day 0 and hydrodynamically injected with pTT3- hyper-IL-15-Fc or pTT3-Fc as control at day 10. The mice were sacrificed at day14 and intrahepatic lymphocytes (IHLs) were isolated for cytofluorimetric analysis. Numbers of total IHLs (left), numbers of $CD8^+$ T cells ($CD3^+CD8^+$) (middle) and NK cells ($CD3^-NK1.1^+$)(right) in liver in each group were showed. Graphs represent the mean ± SEM of 3 mice in each group.




**Supplementary Fig.3 Specific epletion of CD8$^+$ T cells and NK cells *in vivo*.** 1x10$^5$ CT26 colon carcinoma cells were injected into the liver of BALB/c mice through portal vein at day0. And at day9, mice were left untreated or intraperitoneally injected with 200μg of NK cells depletion antibody (anti-asialo GM-1) or CD8$^+$ T cells depletion antibody (TIB210), control rabbit serum or RatIgG (RIg) as control. And at day 10 they were hydrodynamically injected with pTT3-Fc or pTT3-hyper-IL-15-Fc plasmid. Four days later, PBMCs were harvested for cytofluorimetric analysis. Representative flow cytometric results showed the specific depletion of NK cells **(A)** and CD8$^+$ T cells **(B)**.