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ARTICLE

Chronic viral infections persistently alter marrow stroma and impair hematopoietic stem cell fitness

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Chae Dong Hoon
Journal meeting

Introduction

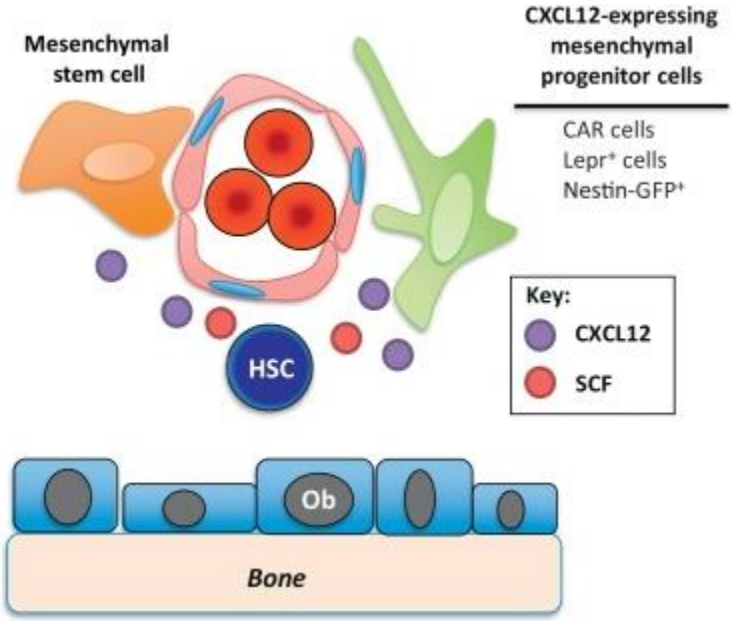
- **CARc(CXCL12-abundant reticular cell)**

- **Major component of BM stroma**

- make up the largest fraction of mesenchymal stroma and comprise a variety of multifunctional and heterogeneous adipogenic and osteogenic progenitor cells

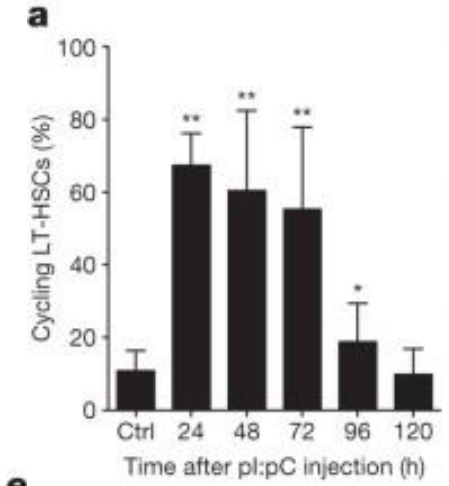
- **Production of ECM and abundant secretion of key factors**, CXCL12 , SCF and etc…

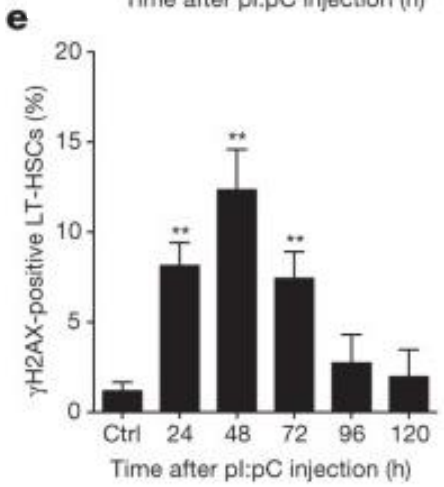
->govern hematopoietic development and contribute to the maintenance of HSCs and progenitors



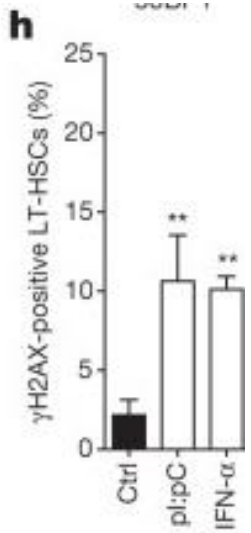
Previous study

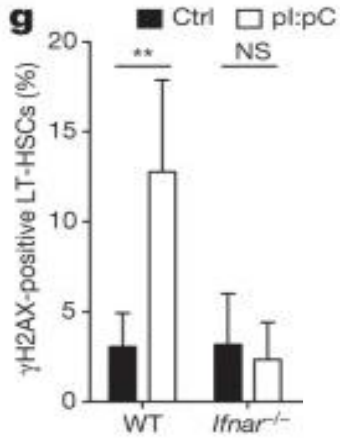
(Interferon family effect on in HSC)





*γH2AX: DNA double-strand break marker





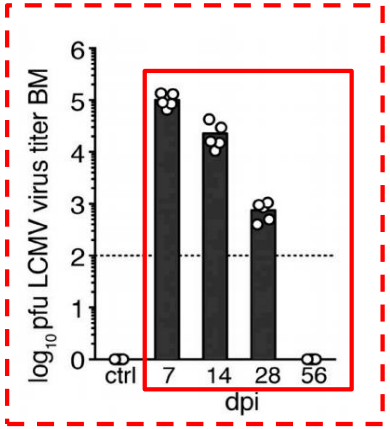
✓ pI:pC-activated LT-HSC show increased proliferation and DNA damage

✓ *IFNAR* KO LT-HSC avoid DNA damage

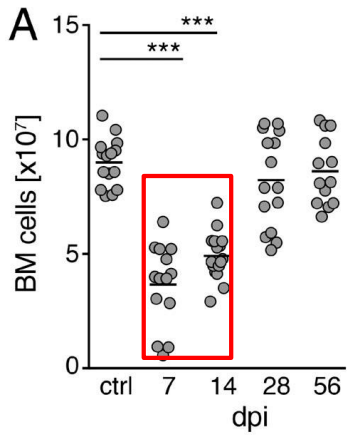
->IFNα-induced proliferation of LT-HSC induces DNA damage and defective HSC function

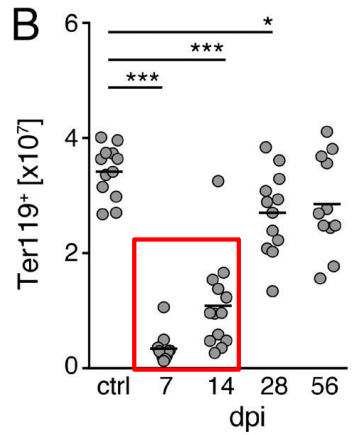
Walter et al., 2015

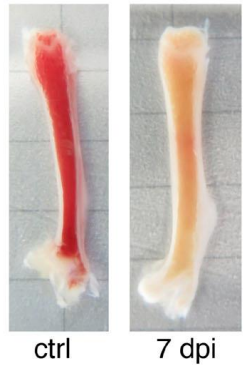
LCMV infection causes transient BM hypoplasia and prolonged impairment of HSC function



Viral infection titer





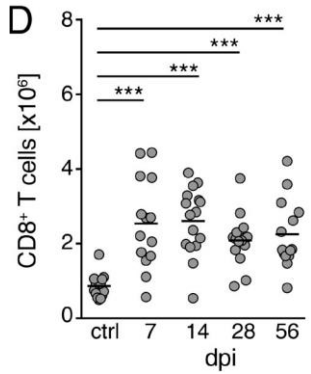


*LCMV-Lymphocytic choriomeningitis virus

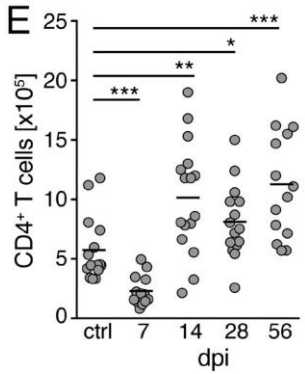
*dpi – day per infection

✓ Total number of BM cells and erythrocytes are dropped and recovered to normal state

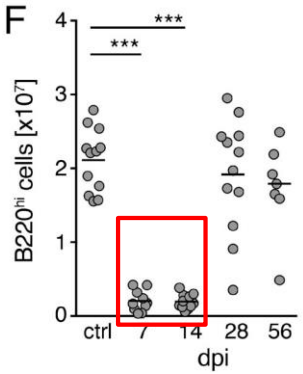
CD8+ T cell



CD4+ T cell



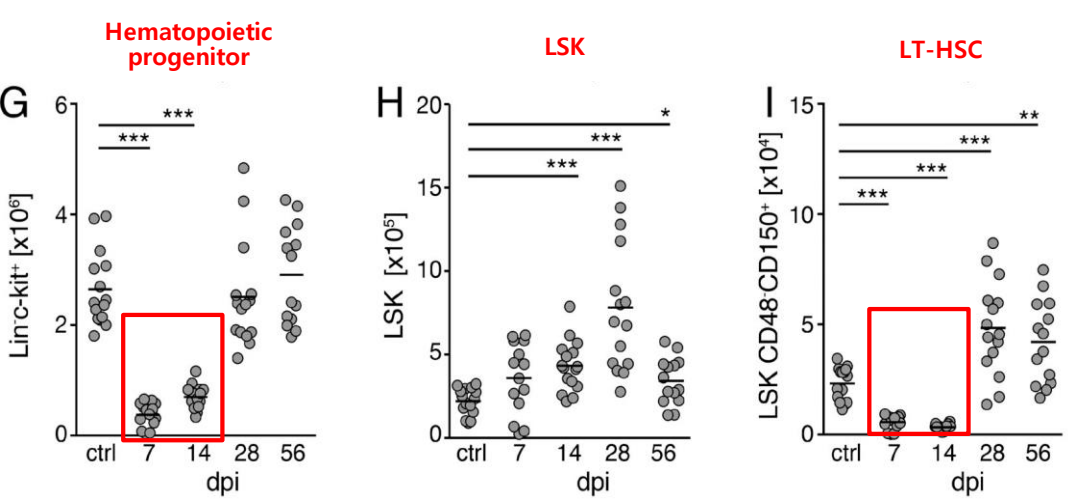
B220+ B cell

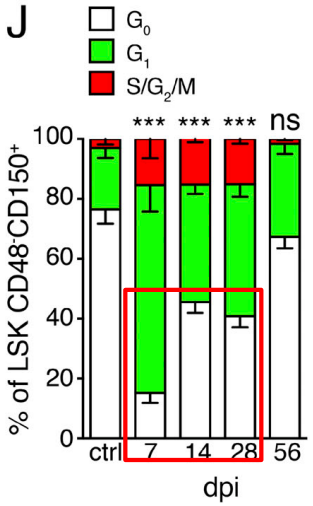


✓ CD8+ T cells are increased after LCMV infection detected

✓ CD4+ T cells and B220+ B cells are reduced during initial infection phases and rose to abnormally high levels

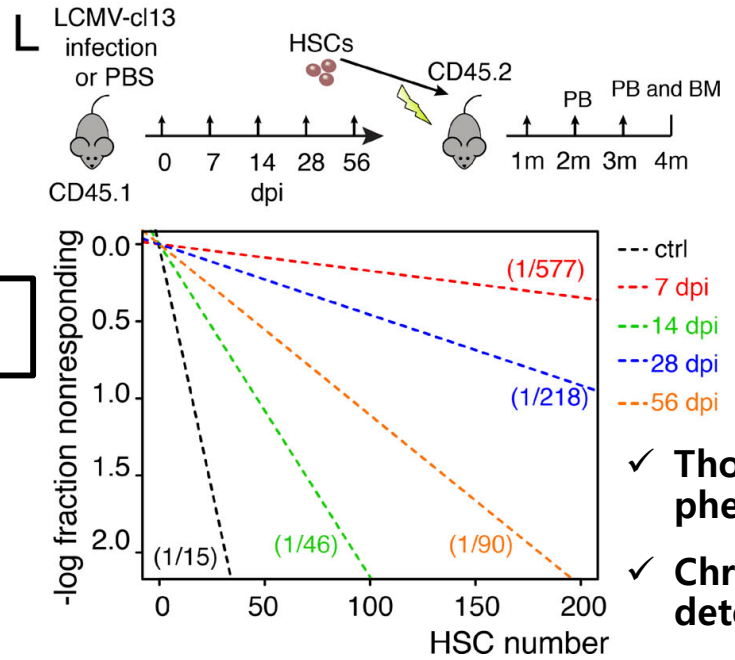
LCMV infection causes transient BM hypoplasia and prolonged impairment of HSC function





- ✓ Hematopoietic progenitor and LT-HSC rapidly dropped and recover to normal levels 2 week later
- ✓ G_0 state HSCs are declined until 28 dpi
- ✓ HSC numbers and hematopoietic parameters were restored at 28 dpi

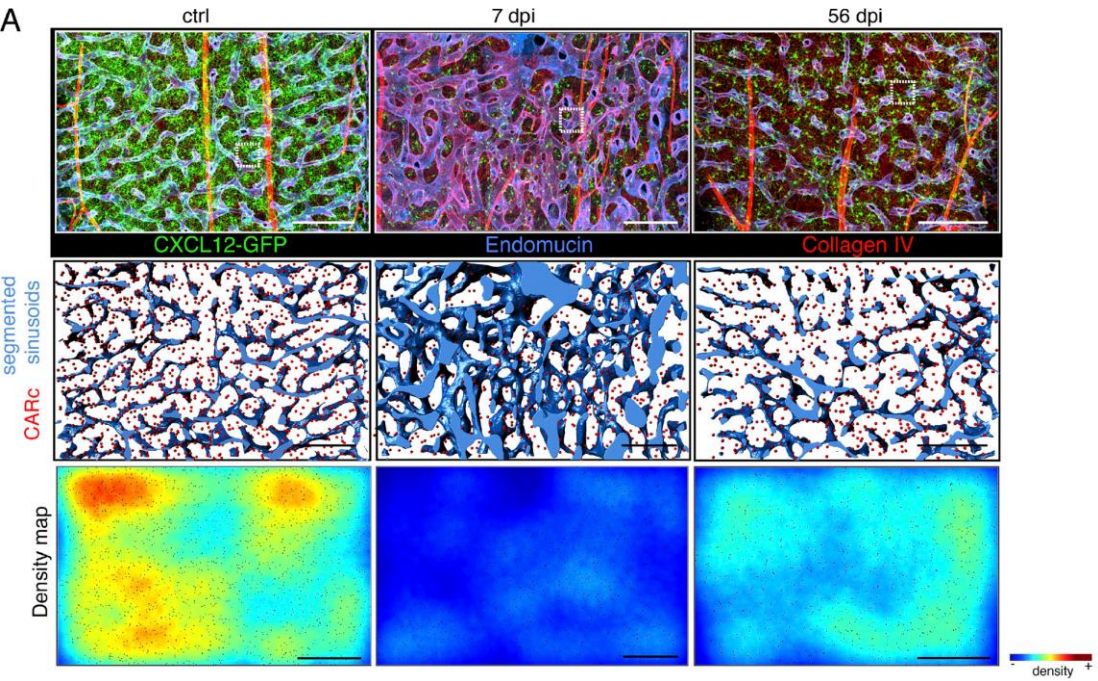
Limited dilution transplantation



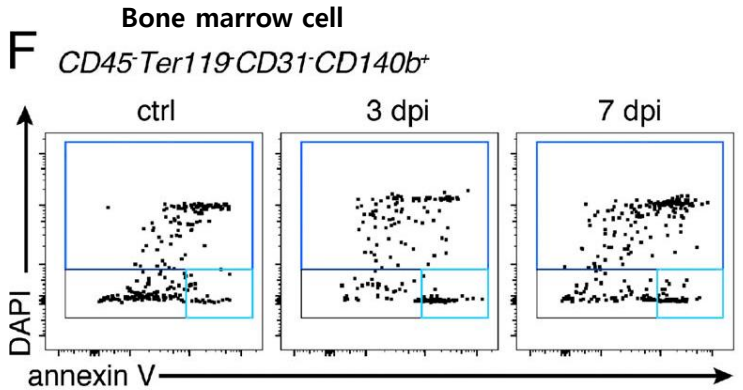
- ✓ Though engraftment of CD45.1 HSC to CD45.2 mouse, check remaining LT-HSC phenotype frequency
- ✓ Chronic viral infection effect to HSPC function even when the virus is no longer detected.

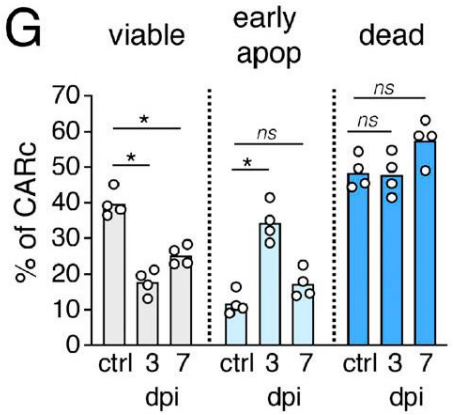
Chronic LCMV infection induces transient remodeling of BM vascular and ECM networks

3D quantitative microscopy(3D QM)

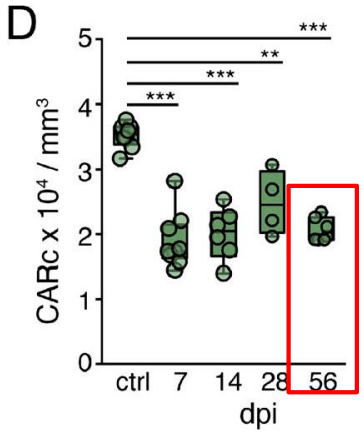


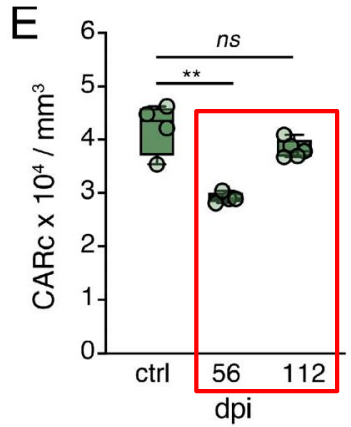
- ✓ **Under infected condition, CARcs were decreased and ECM network was destroyed**
- ✓ **Expansion of the vessel network and contraction of the extravascular space was observed**





- ✓ **Significant increases in the fraction of apoptotic CARcs could be detected as early as 3 dpi ~ 7 dpi**



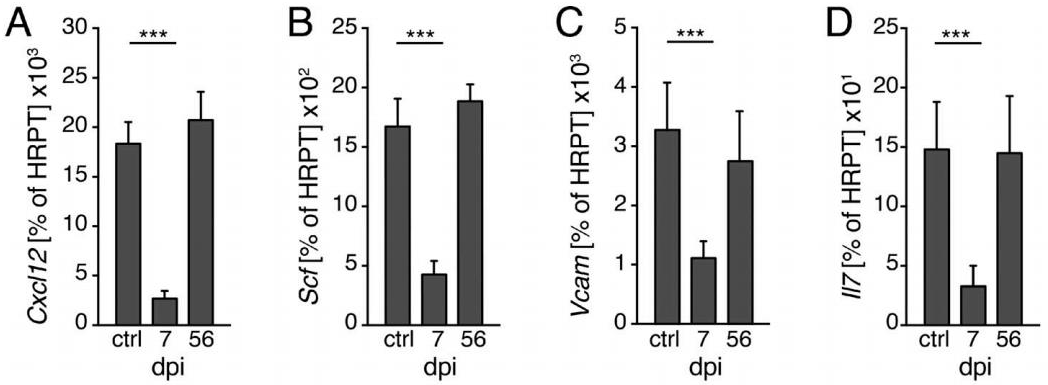


- ✓ **Total number of CARcs wasn't recover in 56dpi**
- ✓ **Long-term damage was reversible**

->CARc densities were almost restored by 4 month after infection

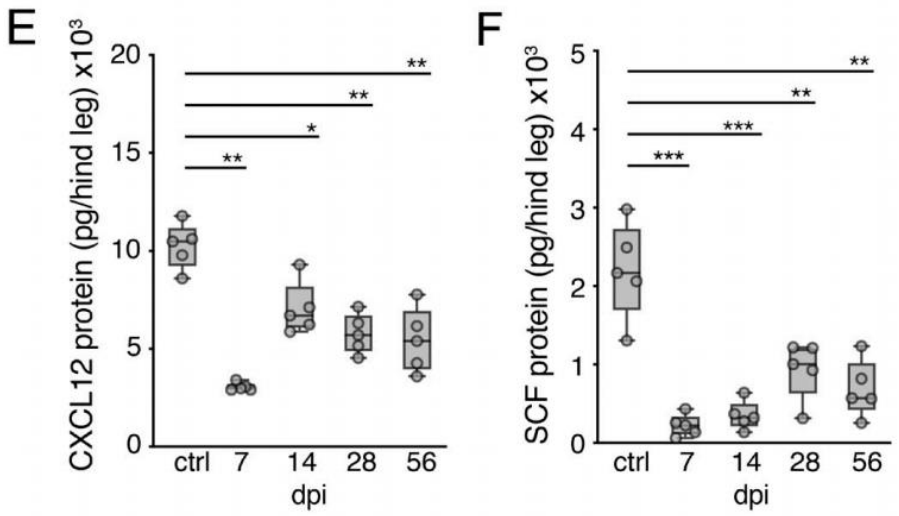
Durable functional impairment of CARc after chronic LCMV-cl13 infection

Real-time PCR

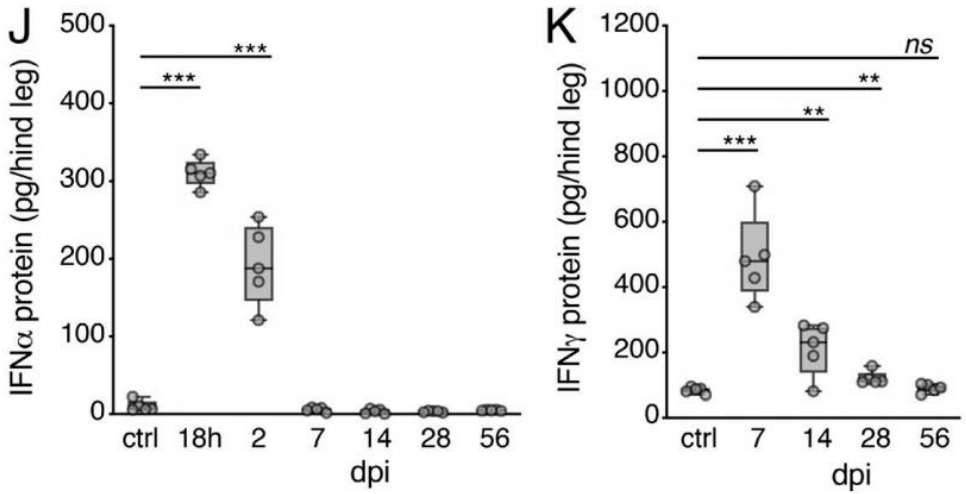


✓ **CARc functions were decreased at 7 dpi and recovered at 56 dpi**

ELISA



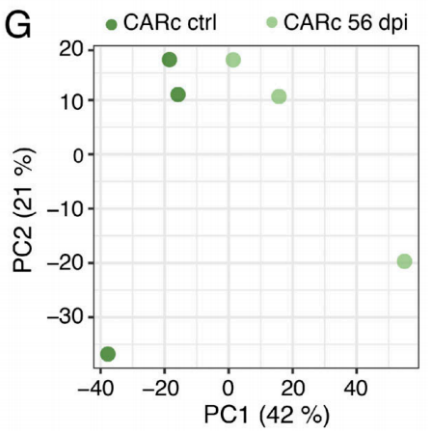
✓ **CARc functions were decreased at 7 dpi and maintained up to 56 dpi**

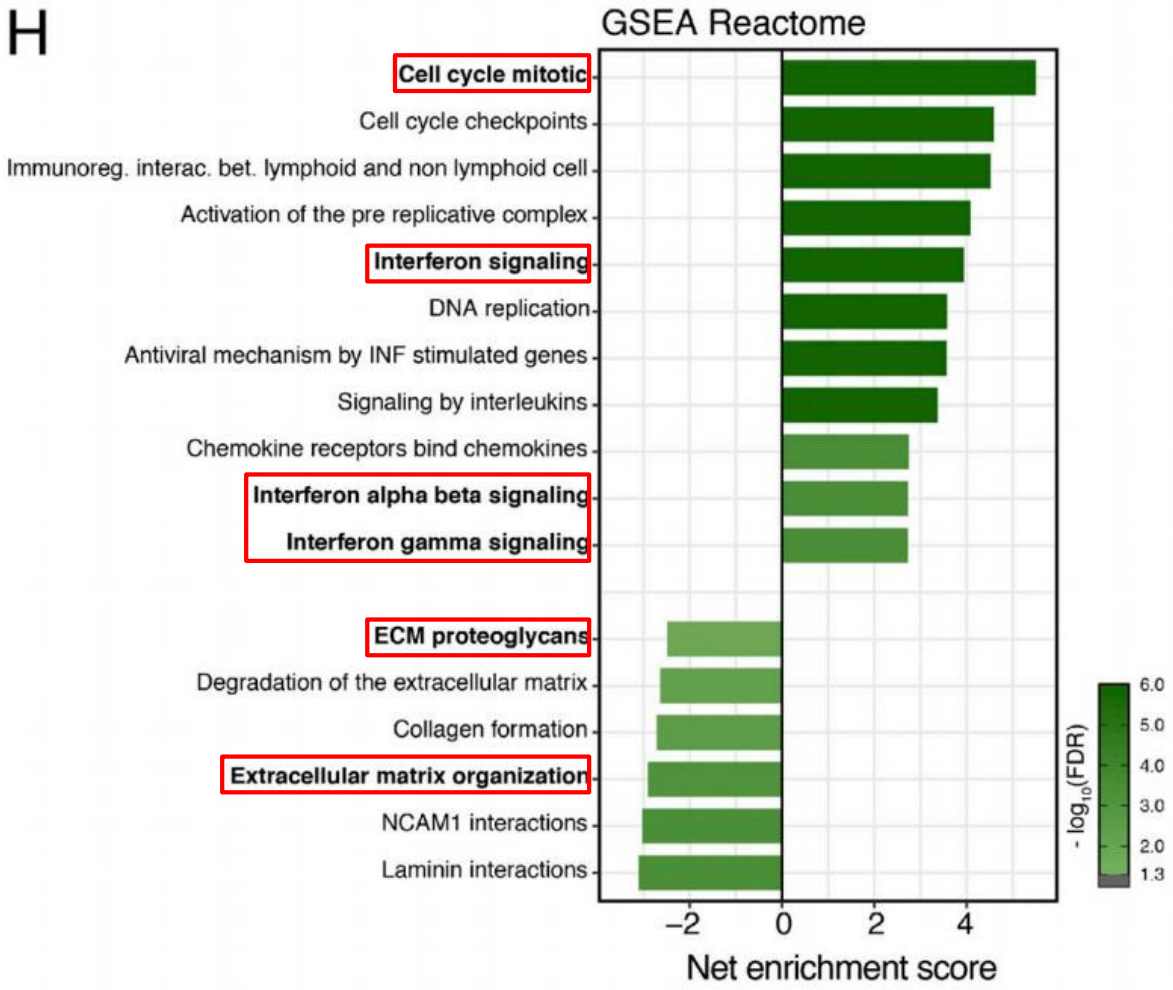


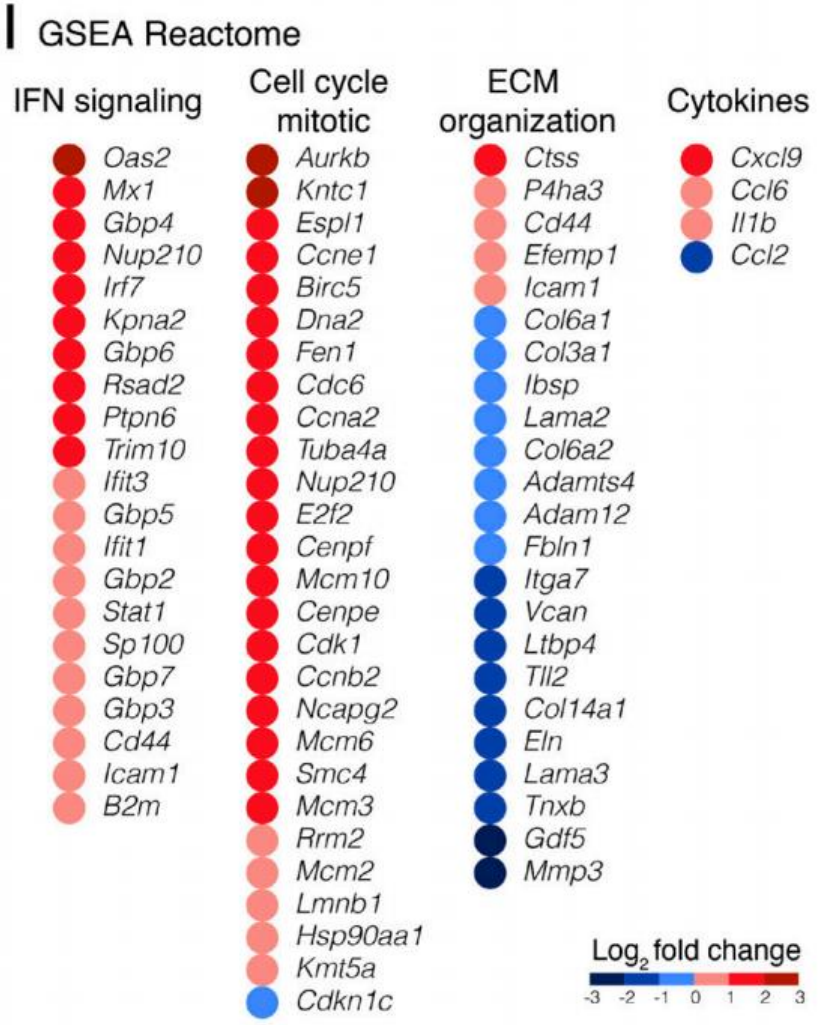
✓ **Secretion of IFN-α/IFN-γ was increased at early stage of virus infection in BM lysate and serum**

Durable functional impairment of CARc after chronic LCMV-cl13 infection

Gene set enrichment analysis (GSEA)

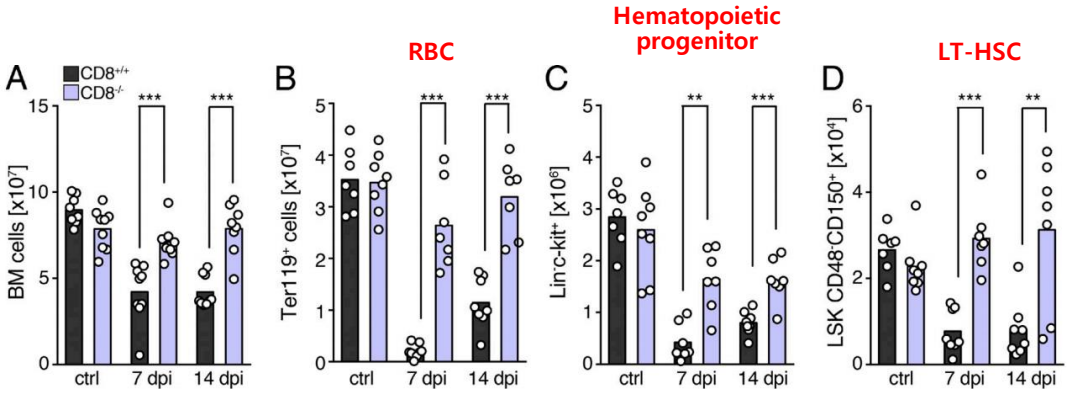




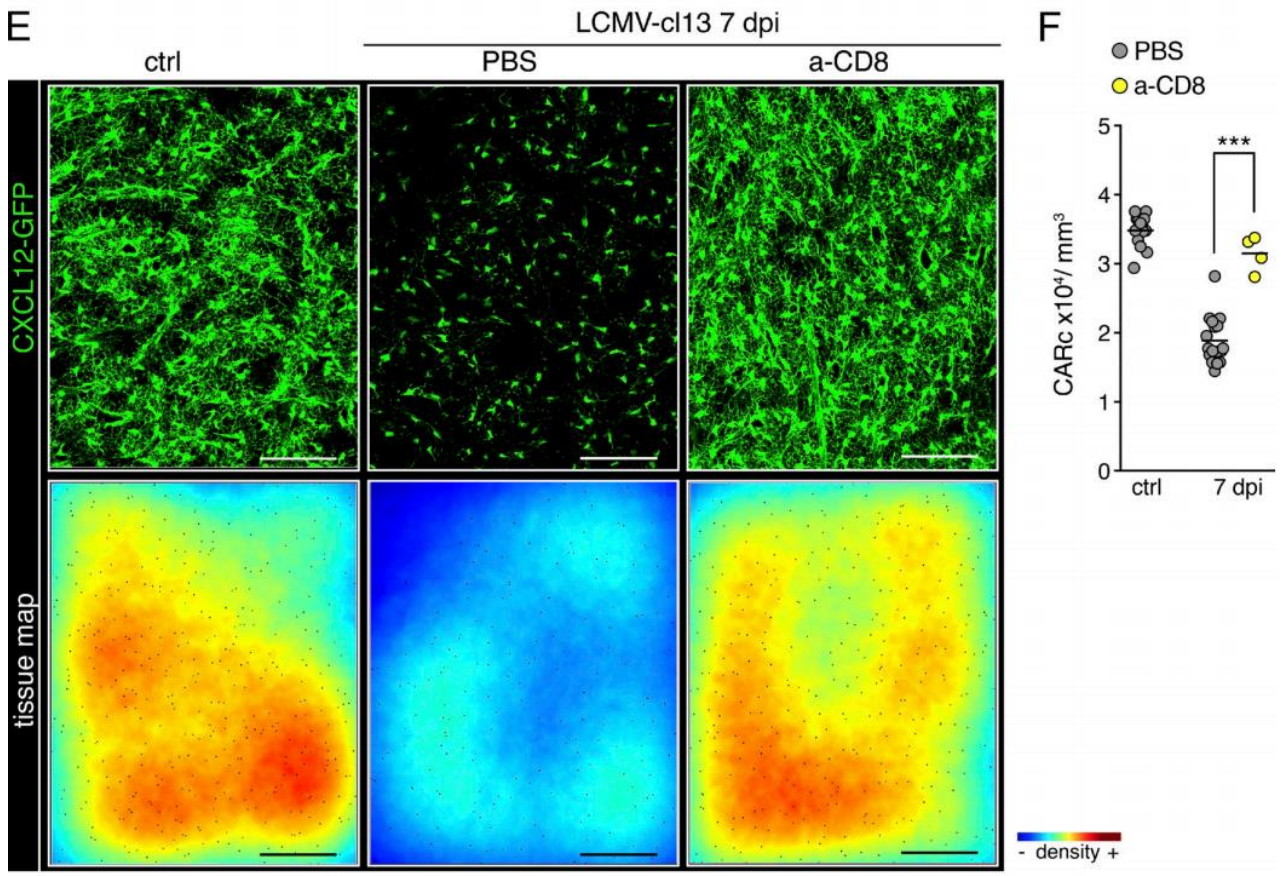


✓ **RNA seq data of control/LCMV 56 dpi were showed that increase in cell proliferation and interferon signaling and decrease in ECM matrix organization**

CD8 T lymphocytes mediate infection-induced hematopoietic effects and destruction of BM CARc

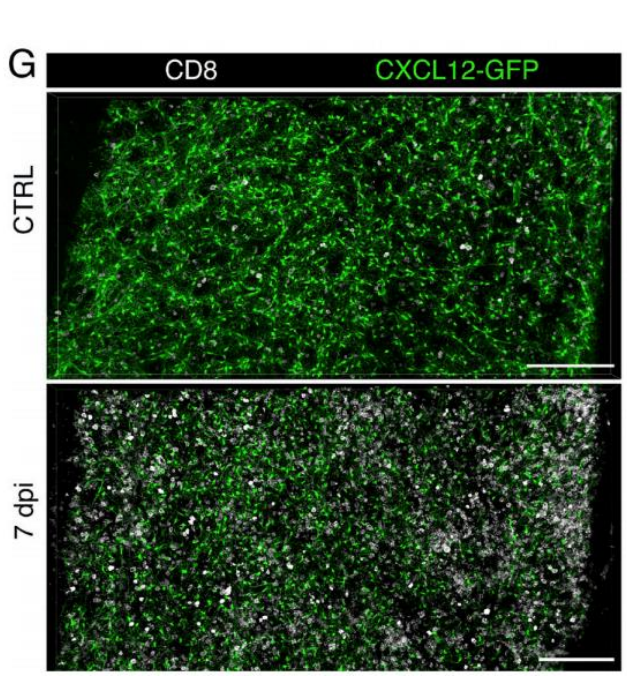


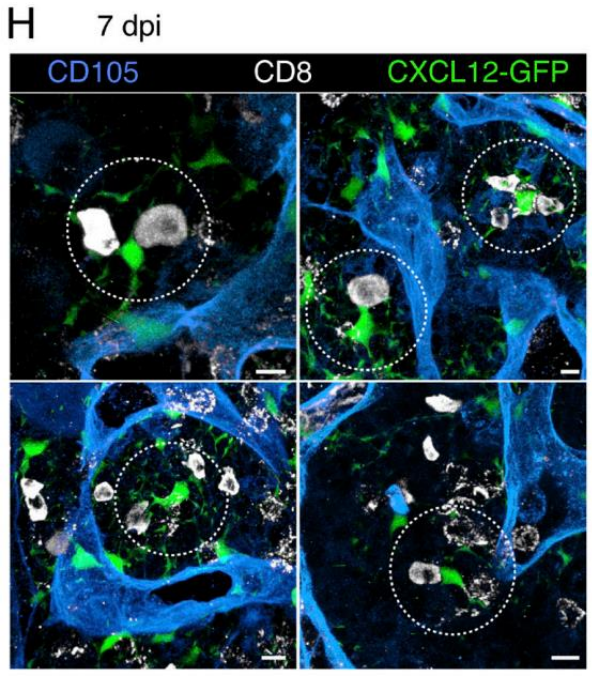
✓ **Blockade of CD8+ T cell was affected to depletion of BM cell and RBC in early stage**



✓ **Absence of CD8 T cells largely prevented destruction of CARc networks triggered by viral challenge**

CD8 T lymphocytes mediate infection-induced hematopoietic effects and destruction of BM CARc





✓ CARcs were mostly found in direct spatial association with CD8 T cells during early phase of infection

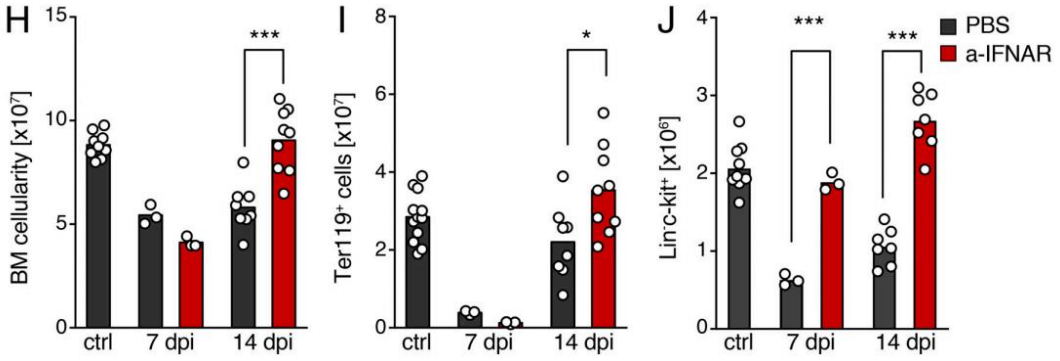
->CARcs are directly exposed to the cytotoxic action and cytokine secretion of CD8 lymphocytes

Type I IFN signaling drives BM accumulation of activated, antigen-specific CD8 T cells

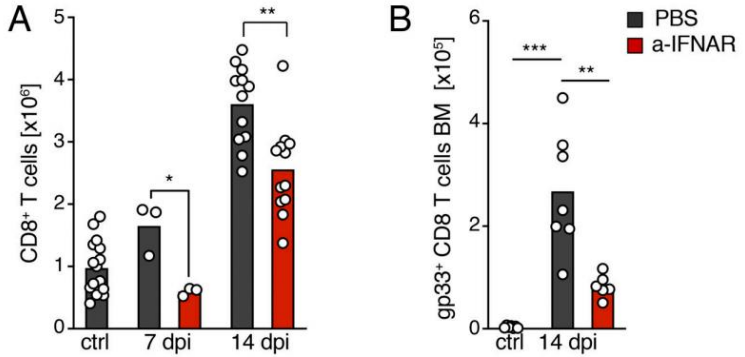
*gp33+ : viral gp33 peptide recognition

RBC

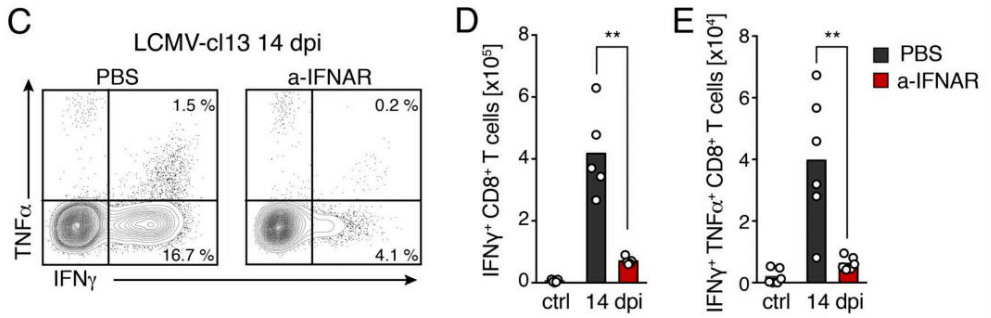
Hematopoietic progenitor



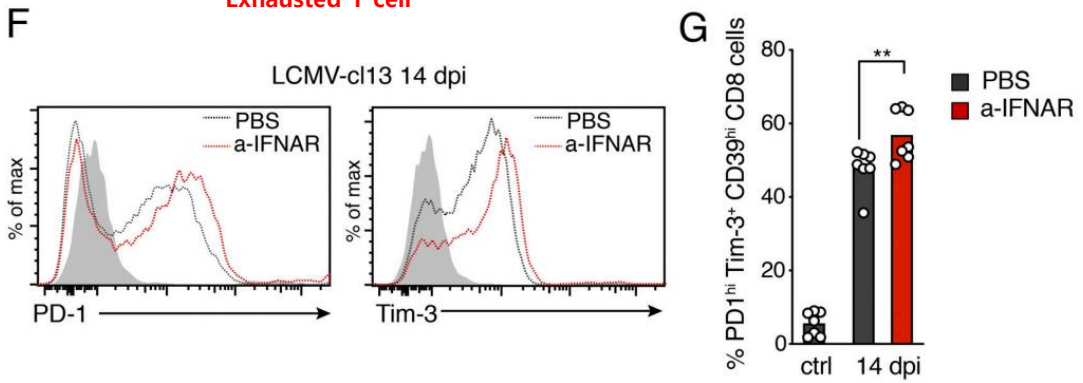
Virus-specific CD8+ T cell



Virus-specific and cytokine producing T cell



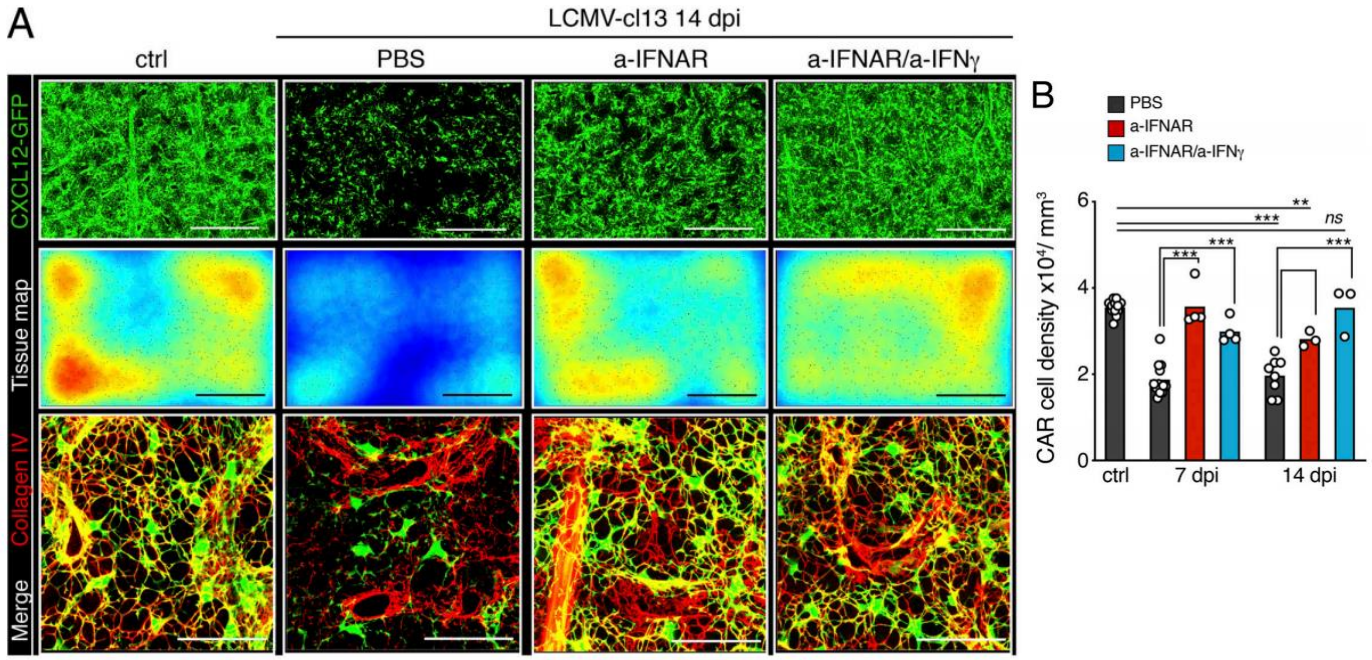
Exhausted T cell



- ✓ Blockade of IFNAR was affected to depletion of BM cell and RBC in early stage
- ✓ Blockade of IFNAR was affected to activation of CD8+/ virus specific T cell

- ✓ Blockade of IFNAR led to a very pronounced reduction of gp33+ antigen-specific and cytokine-producing activated CD8 T cells
- ✓ Blockade of IFNAR increased expression of prototypical markers of cellular exhaustion upon infection

Type I and II IFN signaling mediates structural and functional damage to BM CARc networks



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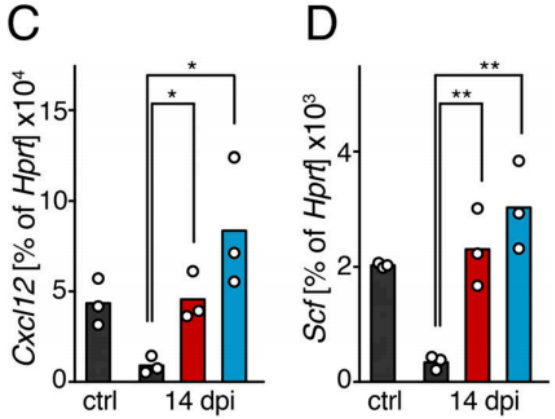
Real-time PCR



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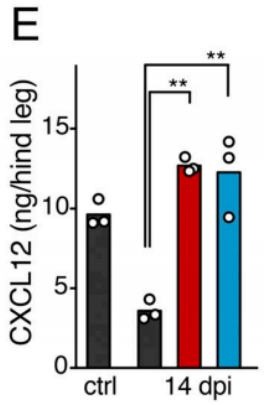
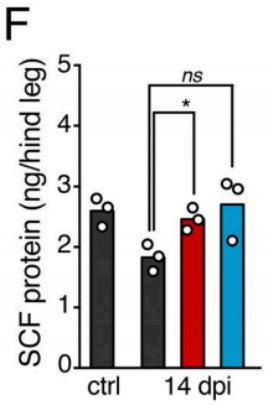
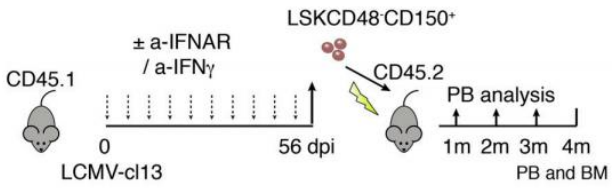
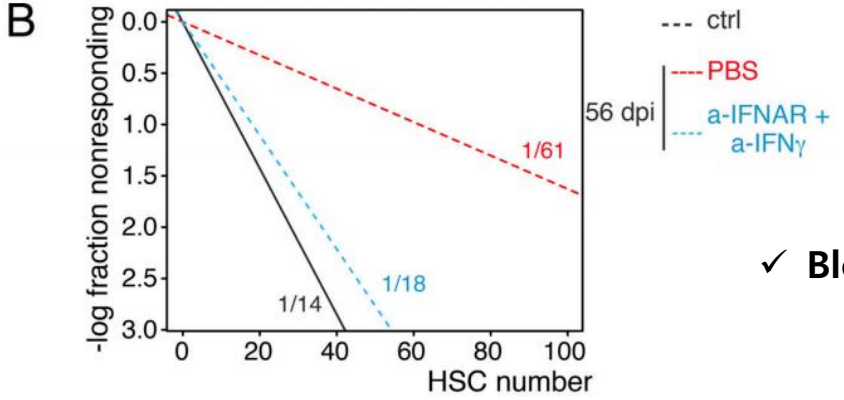




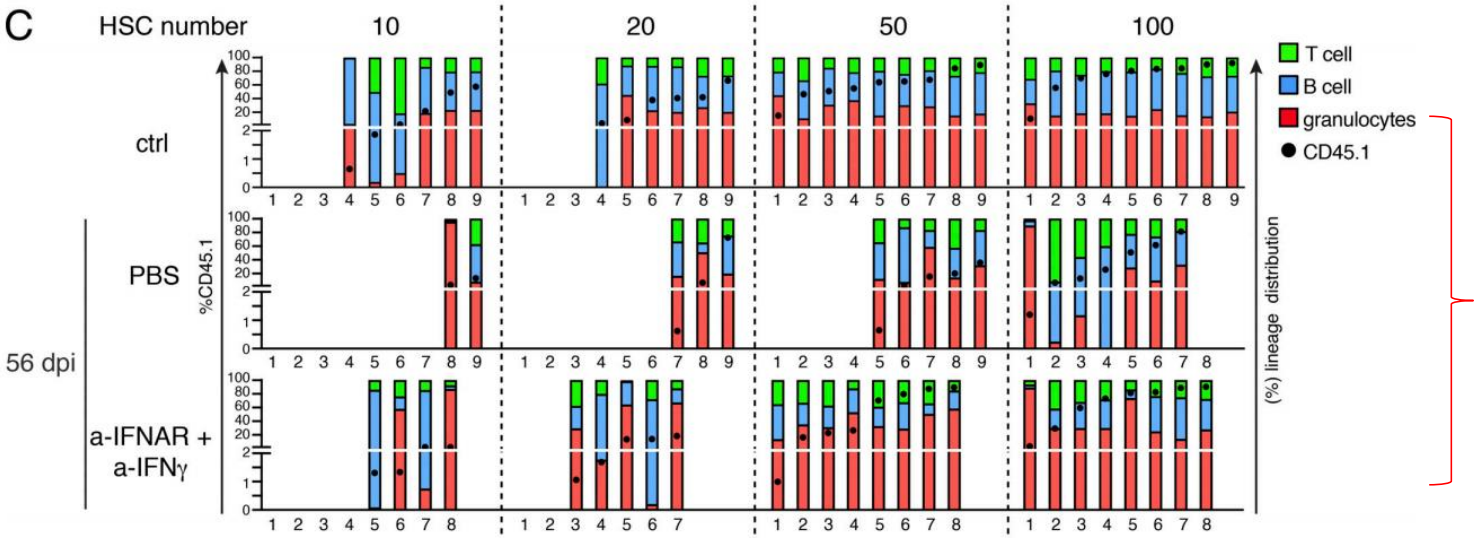
- ✓ Blockade of IFNAR was affected to depletion of BM cell and RBC in early stage
- ✓ Blockade of IFNAR was affected to activation of CD8+/ virus specific T cell

Combined blockage of type I and II IFN signaling prevents persistent decline in HSC functionality



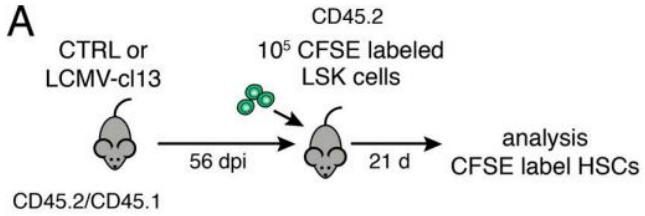


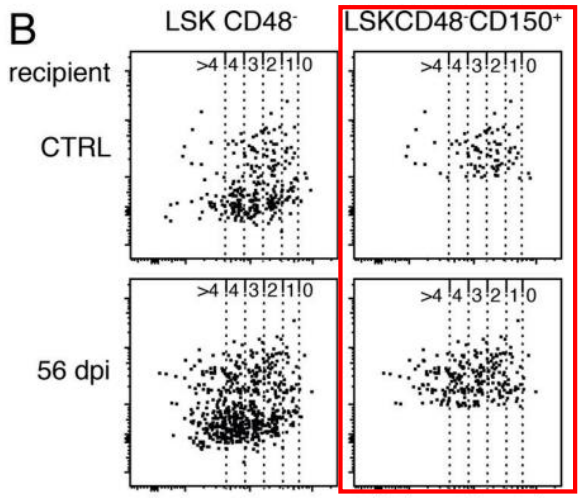
✓ **Blockade of IFNAR recover LT-HSC frequency**

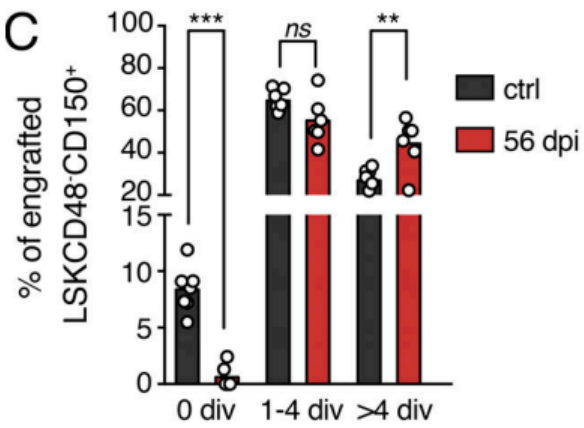


✓ **Blockade of IFNAR was showed similar HSC fraction**

LCMV infection results in persistent functional impairment of the BM microenvironment to maintain HSC quiescence

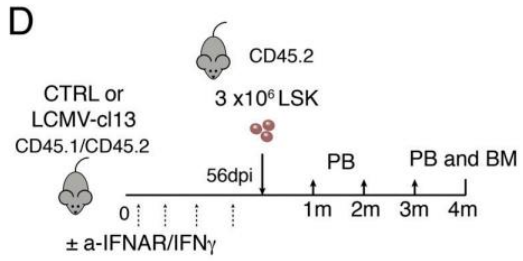


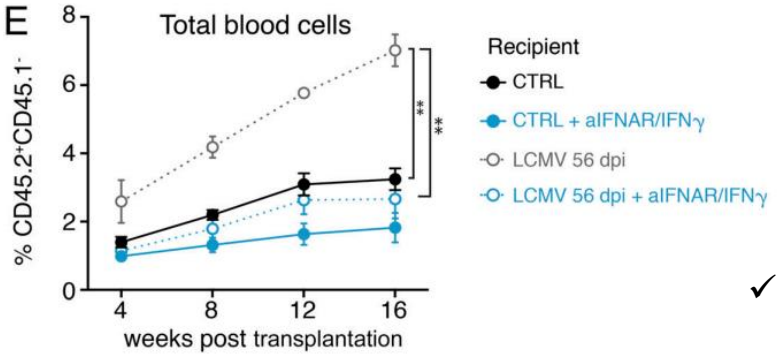




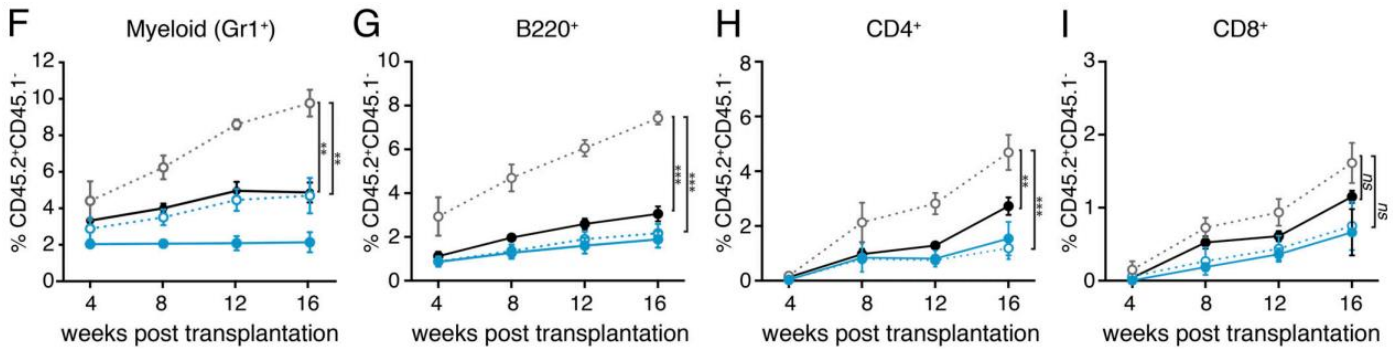
✓ **Chronic stress state in BM stroma lead HSC expansion**

LCMV infection results in persistent functional impairment of the BM microenvironment to maintain HSC quiescence



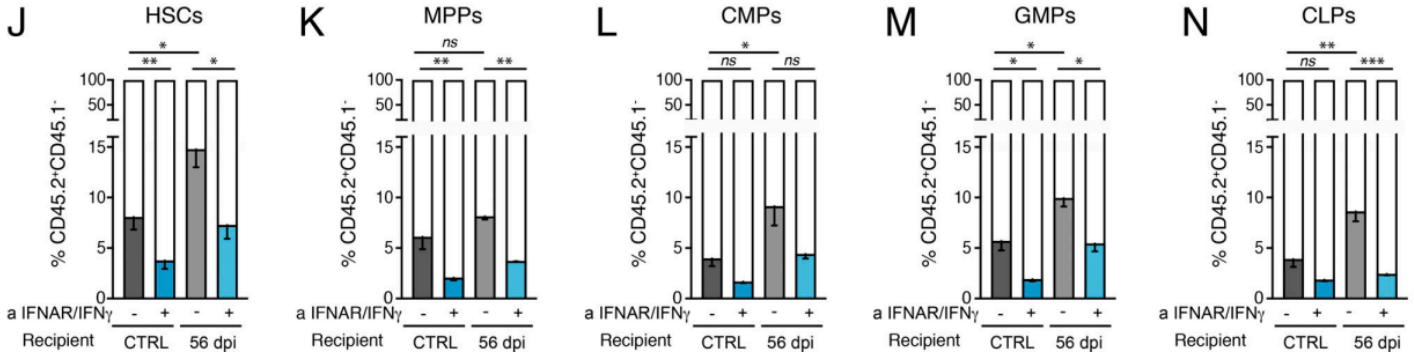


✓ IFN blockade reduce competitive HSC engraftment



✓ Virus-infected BM stroma maintain functions that support HSC and progenitor

4-month BM



Summary

- Chronic infection results in a strong impairment of HSC functionality and competitive fitness.
- Injury to CARc networks and loss of HSCs are immune mediated and triggered by BM-resident virus-activated CD8 T cells
- Immunopathological alterations are mediated by type I and type II IFN signaling.
- While a damaged CARc network suffices to support basal HSC and progenitor function, it may fall short in maintaining full HSC fitness required during stress.