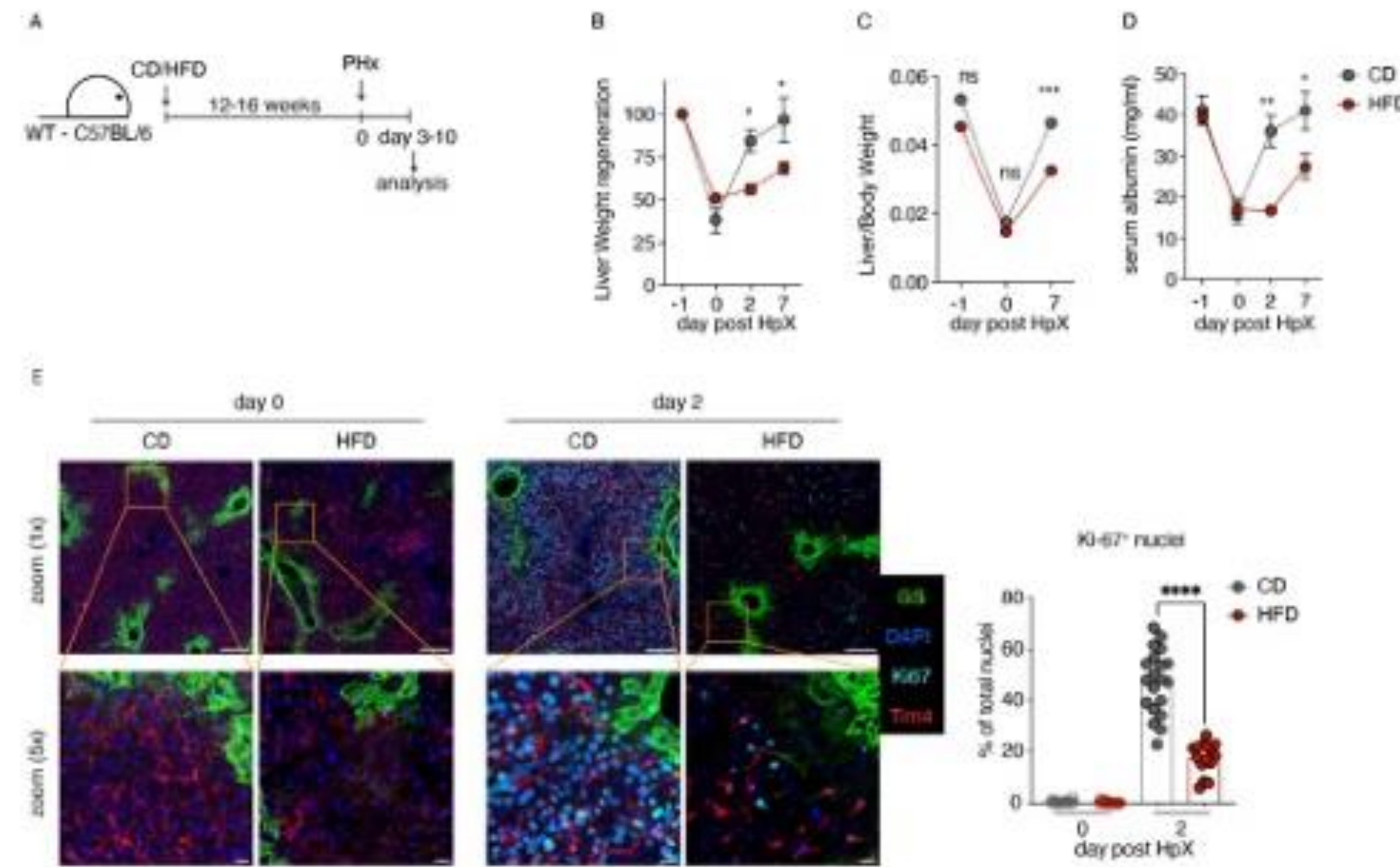


Abstract

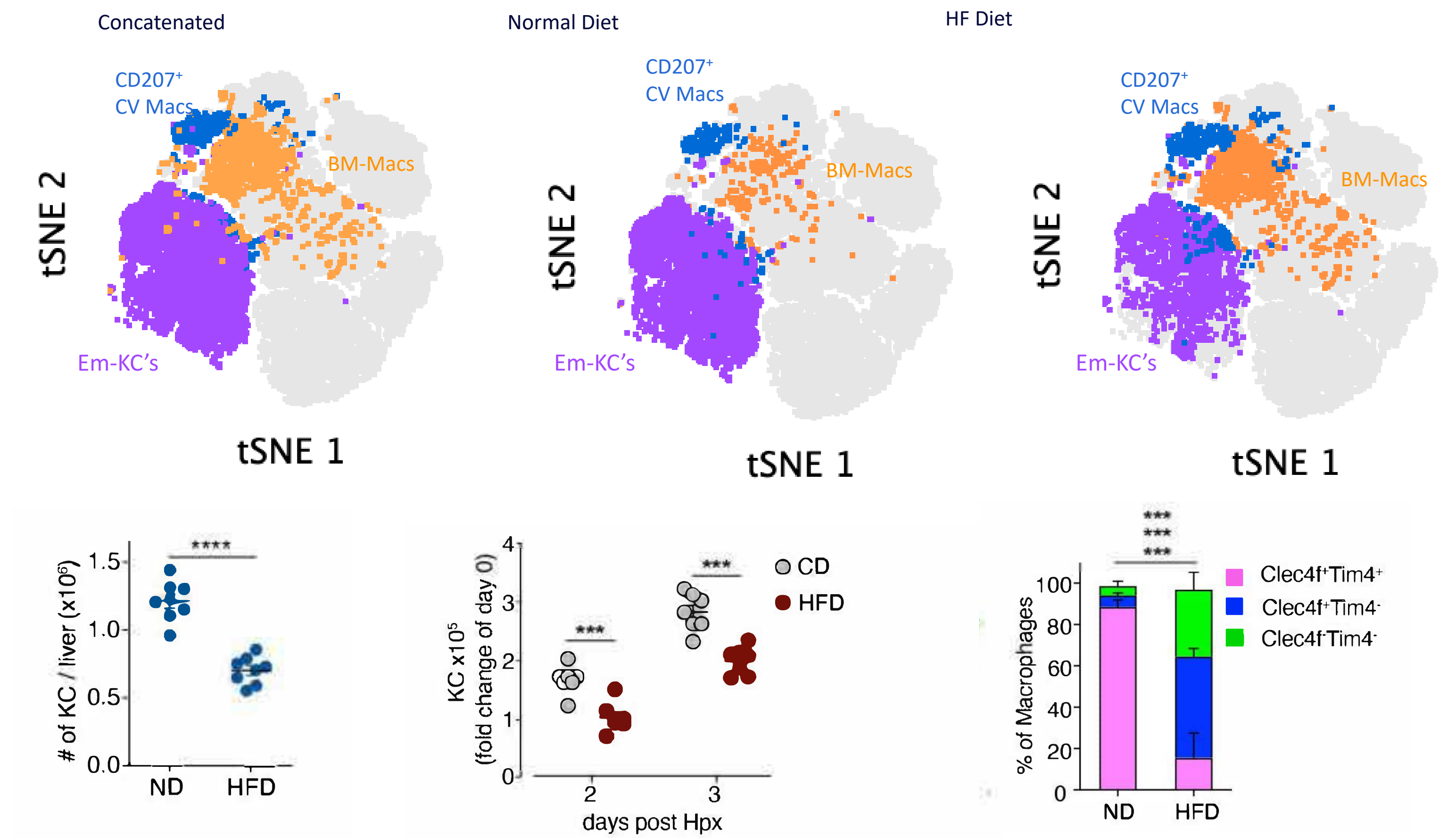
Liver regeneration is essential for restoring hepatic function after surgical resection or transplantation. Obesity and metabolic-driven chronic liver inflammation (metaflammation) are recognized as major inhibitors of liver regrowth. In a murine model of partial hepatectomy (Phx), we identified Kupffer cells (KCs) rather than monocyte-derived macrophages as the key mediators of liver regeneration. Transcriptomic and proteomic analyses of KCs during the early phase of regeneration revealed that in lean mice, resident KCs commit to tissue regeneration by suppressing innate and IFNAR-associated transcriptional programs to meet the metabolic demands for the activation of liver regeneration programs. Conversely, KCs from obese mice exhibited a pronounced interferon-alpha/beta receptor (IFNAR) and innate immunity-associated signature, coupled with impaired clonal expansion and reduced metabolic fitness. Obesity-induced gut dysbiosis and microbial translocation exacerbated hepatic inflammation and inhibited regeneration by inducing type I interferon (IFN-I) signaling in KCs. Systemic inhibition or genetic deletion of IFNAR in KCs restored their proliferative capacity and metabolic fitness, leading to liver regeneration in obese mice comparable to that in lean mice. This study highlights the critical role of KCs in liver regeneration, the deleterious effects of obesity-driven chronic IFN-I signaling, and the potential of targeting this pathway to enhance liver regeneration in obese individuals.

Results (1)

1A Obesity is associated with impaired liver regeneration in mice



1B Altered functions and replenishment of hepatic macrophage populations in obese mice.



Methods

1. Experimental Animals:

- Mouse Strains:** C57BL/6 mice were either bred in-house or purchased. Clec4f-cre-DTR, IFNAR1^{flx/flx} Mafia, and CX3CR1-CreERT2 x confetti mice were used, with some strains obtained from Jackson Laboratory.
- Diet-Induced Obesity:** Mice were fed a high-fat diet (60 kcal% fat) starting at 6-8 weeks of age for 12-15 weeks. Control mice were fed a matched control diet.

2. Partial Hepatectomy:

- Surgical Procedure:** Performed under general anesthesia with isoflurane. A midline incision was made, and partial hepatectomy was conducted by ligating and removing the median and left liver lobes. The abdomen was closed with a two-layer running suture.
- Sham Operation:** Involved a midline incision, liver mobilization, and abdominal cavity rinsing, followed by closure with a two-layer running suture.

3. Macrophage Depletion:

- Clodronate Liposomes:** Mice received intravenous injections of clodronate or PBS liposomes for macrophage depletion.
- Kupffer Cell Depletion:** Clec4f-DTR mice were injected intraperitoneally with diphtheria toxin (DT) at specified time points.

4. Flow Cytometry:

- Antibodies:** Fluorochrome-conjugated or biotinylated monoclonal antibodies (mAbs) specific to various mouse markers (e.g., Ly6C, Ly6G, CD11b, CD45, CD11c, Iba1, F4/80, IAb, CD146, GS, and Live/Dead) were used.
- Staining:** Cells were fixed, permeabilized, and stained with specific antibodies, including anti-Ki67 and Clec4f.
- Analysis:** Multiparameter analysis was performed using an LSRII flow cytometer and FlowJo software.

5. Bulk RNA Sequencing and Gene Set Enrichment Analysis (GSEA):

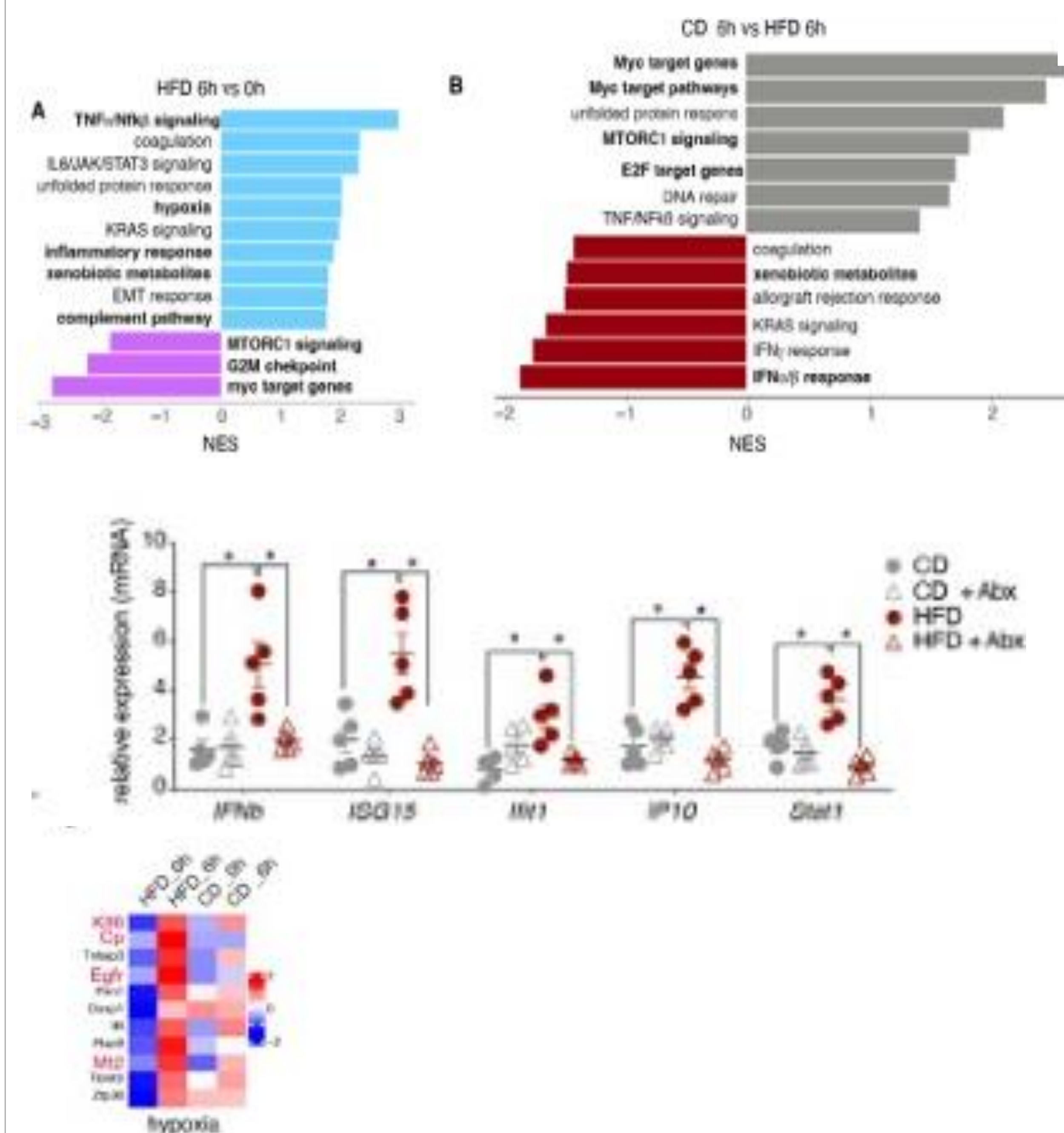
- Cell Sorting:** TIM4⁺ (Kupffer cells) and TIM4⁻ (MoMs) macrophages were sorted using a BD FACSAria Fusion.
- RNA Isolation:** RNA was isolated and purified, followed by library production for 3'-mRNA sequencing.
- Sequencing and Analysis:** Sequencing was done on a HiSeq2500, with alignment to the murine reference genome. Differentially expressed genes were identified and visualized using various bioinformatics tools.

6. Immunofluorescence Microscopy:

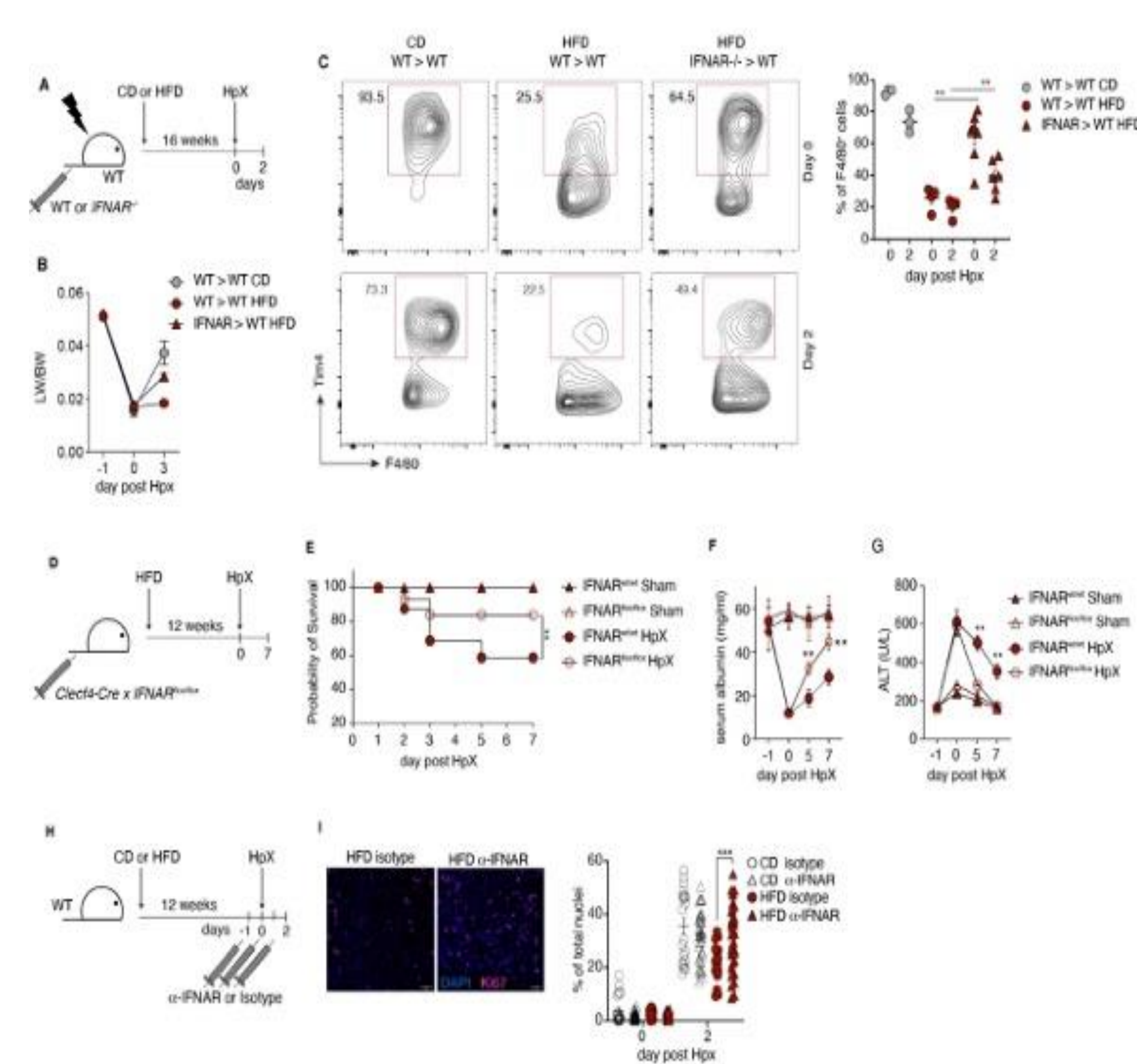
- Sample Preparation:** Liver tissues were fixed, dehydrated, and snap-frozen. Sections were stained with antibodies and imaged using confocal microscopes.

Results (2)

2A Resident Kupffer cells commit to tissue regeneration by shutting down innate and IFNAR-associated transcriptional programs



2B Targeting IFNAR signaling in Kupffer cells restores liver regeneration in obese mice



Conclusions

Findings:

- KCs are crucial for liver regeneration in mice, unlike monocyte-derived macrophages (Mo-MΦ).
- Obesity-induced chronic inflammation compromises KC function.
- Both KCs and Mo-MΦ expand during liver regeneration, but only KCs are indispensable.
- KCs perform phagocytosis and clearance of translocated gut microbiota, foreign particles, and apoptotic cells.
- KCs adapt their metabolic and cellular functions during liver regeneration in mice.
- Obesity alters KC functions, reducing their proliferative activity and protein translation.
- Chronic IFN-I signaling in obesity impairs KC functions and liver regeneration in mice.
- IFNAR signaling suppresses MTORC1 and Myc-associated genes, affecting KC proliferation.
- Inhibition of IFNAR signaling in KCs promotes liver regeneration in obese mice.
- Obesity-driven chronic IFN-I signaling may affect tissue regeneration in other organs.

Implications:

- Provides a molecular basis for impaired KC functions in metabolic syndrome.
- Highlights potential therapeutic targets for enhancing tissue-resident macrophage functions and tissue regeneration in obesity.

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