Establishment of a protocol to generate Kupffer cells from induced pluripotent stem cells

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1. Background

- Kupffer cells (KCs) play a vital role in hepatic tissue homeostasis by engulfing harmful particles and sensing danger.
- Human KCs are characterized by expression of folate receptor beta (FOLRb), V-set and immunoglobulin domain containing 4 (VSIG4) and CD163, and have increased phagocytic and efferocytic activity.
- In vitro models that accurately resemble human KCs are scarce.
- KCs originate from erythro-myeloid progenitors (EMPs) in the yolk-sac, instead of hematopoietic stem cells (HSCs).
- Induced pluripotent stem cells (iPSCs) offer a promising tool to generate KC-like cells since iPSC differentiation towards macrophages follows the yolk-sac route.

Aim

Development of an iPSC-based differentiation protocol to generate cells with a KC-like phenotype and KC-like functional properties.

2. Experimental set-up

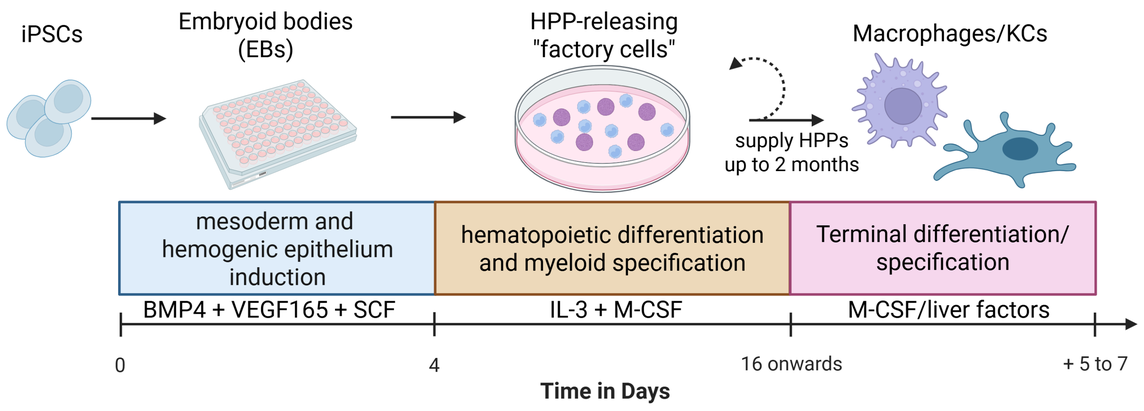


Figure 1. Schematic overview of the differentiation method to generate iPSC-derived macrophages (iMacs) and KCs.

3. iPSC-derived macrophages express common macrophage markers

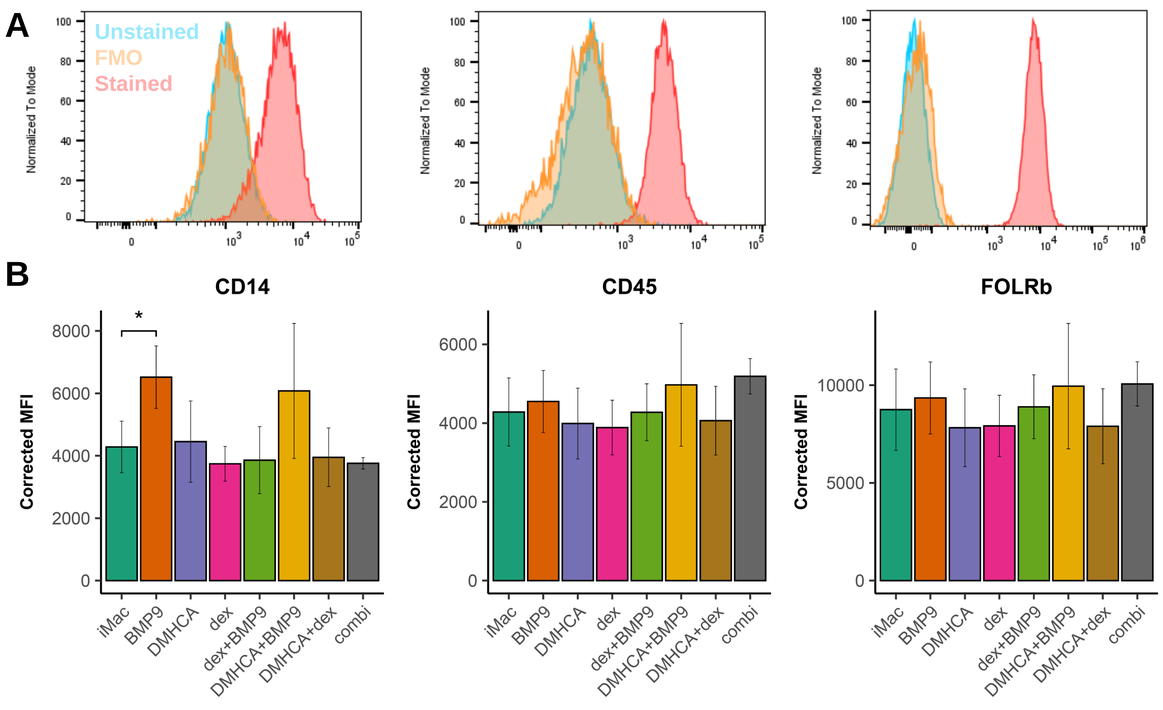


Figure 2. Qualitative expression of CD14, CD45 and FOLRb on iMacs (A). Abundance of CD14, CD45 and FOLRb on iMacs treated with different liver relevant stimuli (B). Mean±SD (n=5).

4. Dexamethasone alone and in combination boosts KC marker expression

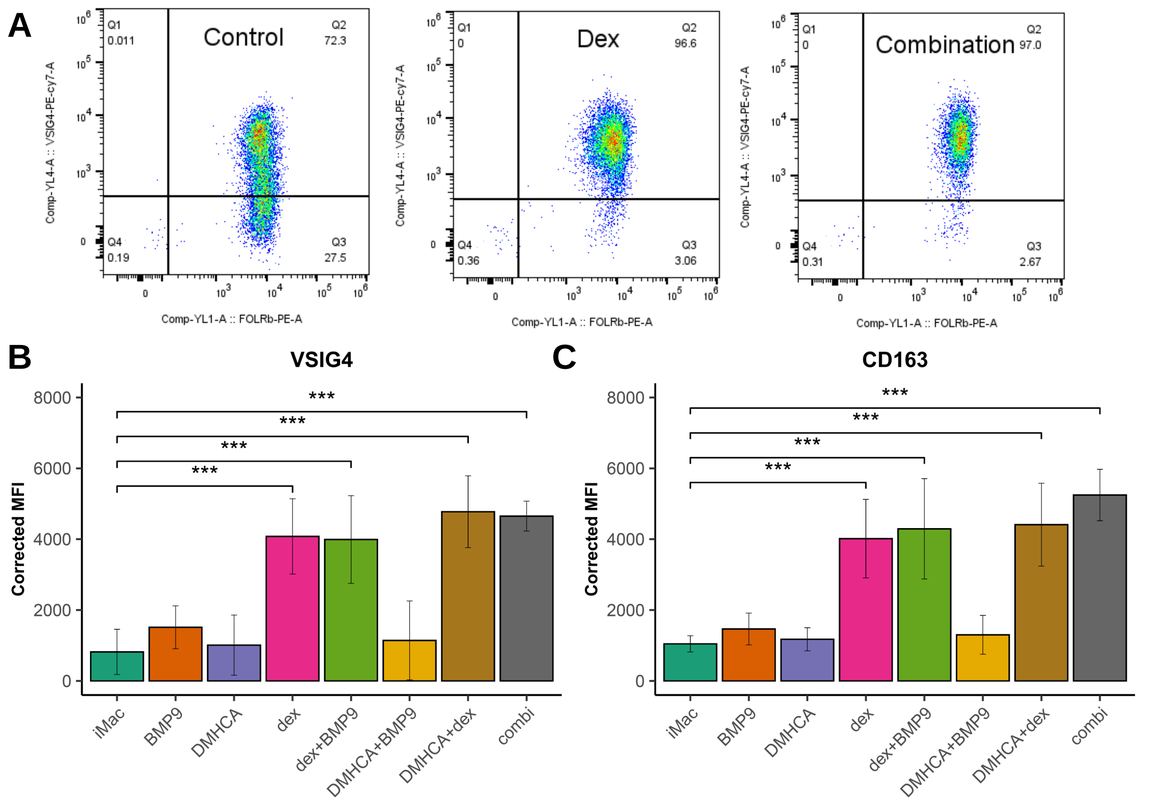


Figure 3. Expression of FOLRb and VSIG4 in control, dexamethasone and combination treated iMacs (A). Abundance of VSIG4 (B) and CD163 (D) on iMacs treated with different liver relevant stimuli. Mean±SD (n=5).

5. Liver relevant stimuli enhance phagocytic activity

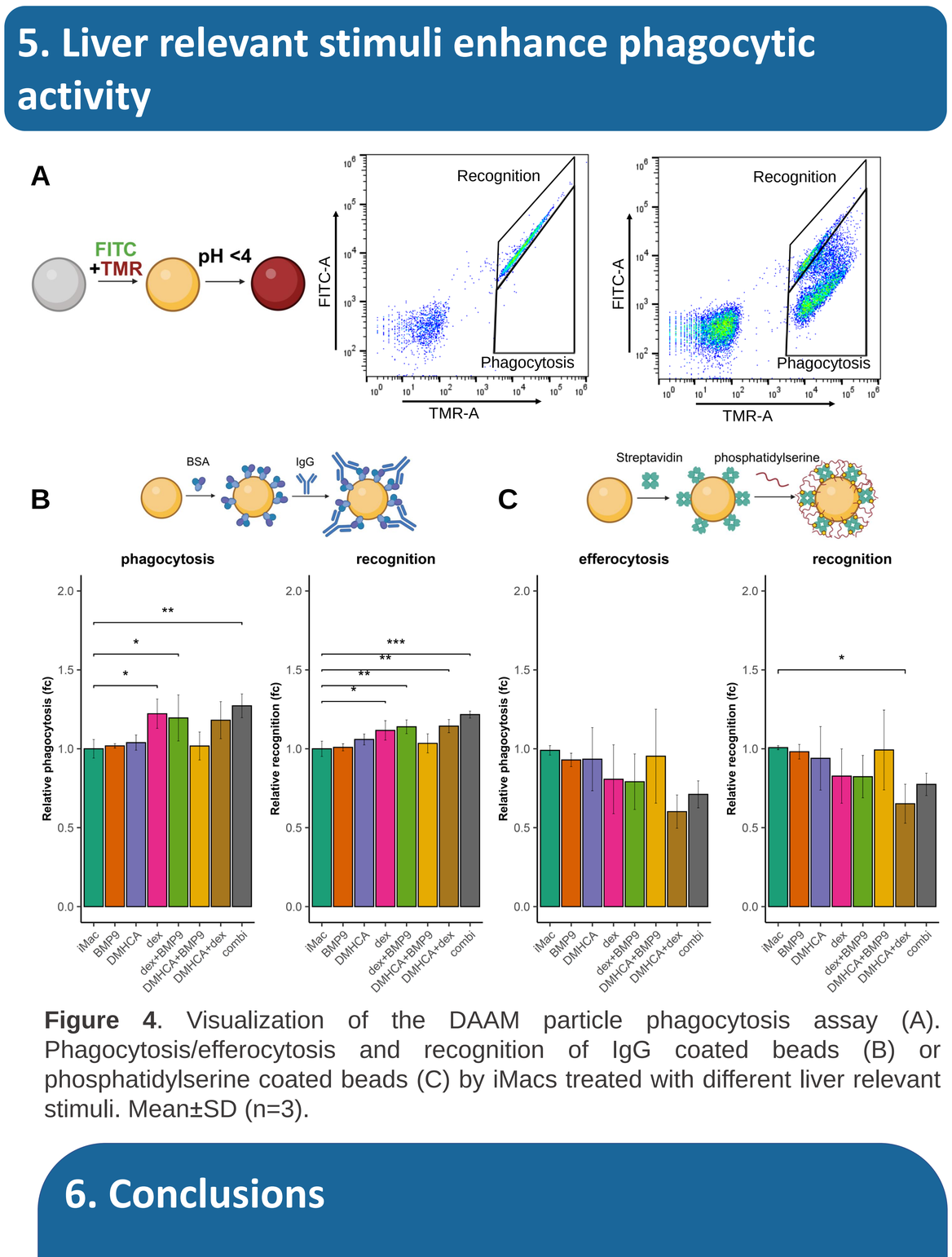


Figure 4. Visualization of the DAAM particle phagocytosis assay (A). Phagocytosis/efferocytosis and recognition of IgG coated beads (B) or phosphatidylserine coated beads (C) by iMacs treated with different liver relevant stimuli. Mean±SD (n=3).

6. Conclusions

- **The current model expresses the desired macrophage and KC markers.**
- **Liver relevant stimuli enhance phagocytosis.**
- **iPSCs offer a promising tool to generate KC-like cells.**

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