Circulating and exosome packaged single-stranded Hepatitis C RNA induce monocyte differentiation via TLR7/8 to polarized macrophages and fibrocytes (128/130 characters)

Running Title: Fibrocytes and polarized macrophages induced by TLR7/8

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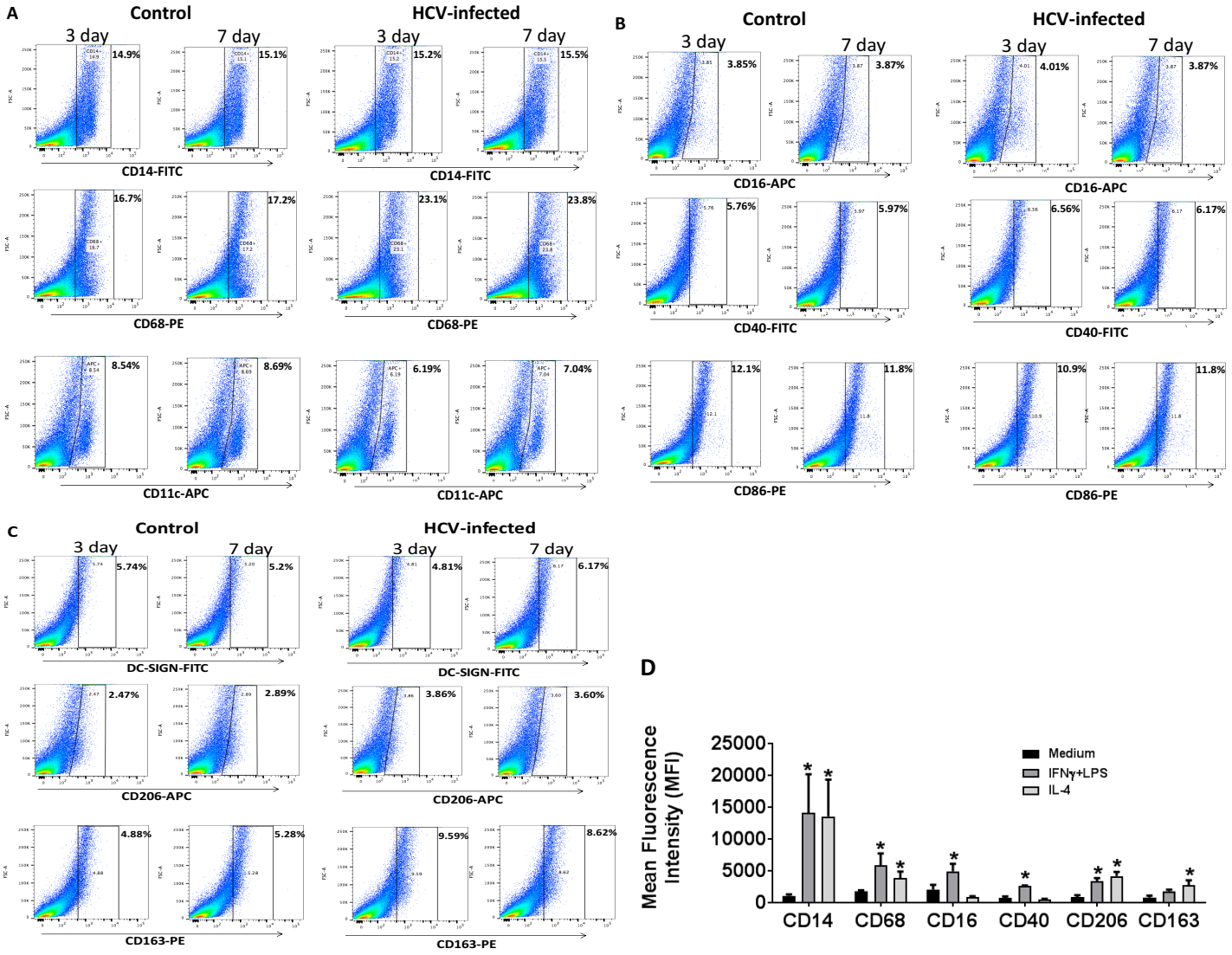
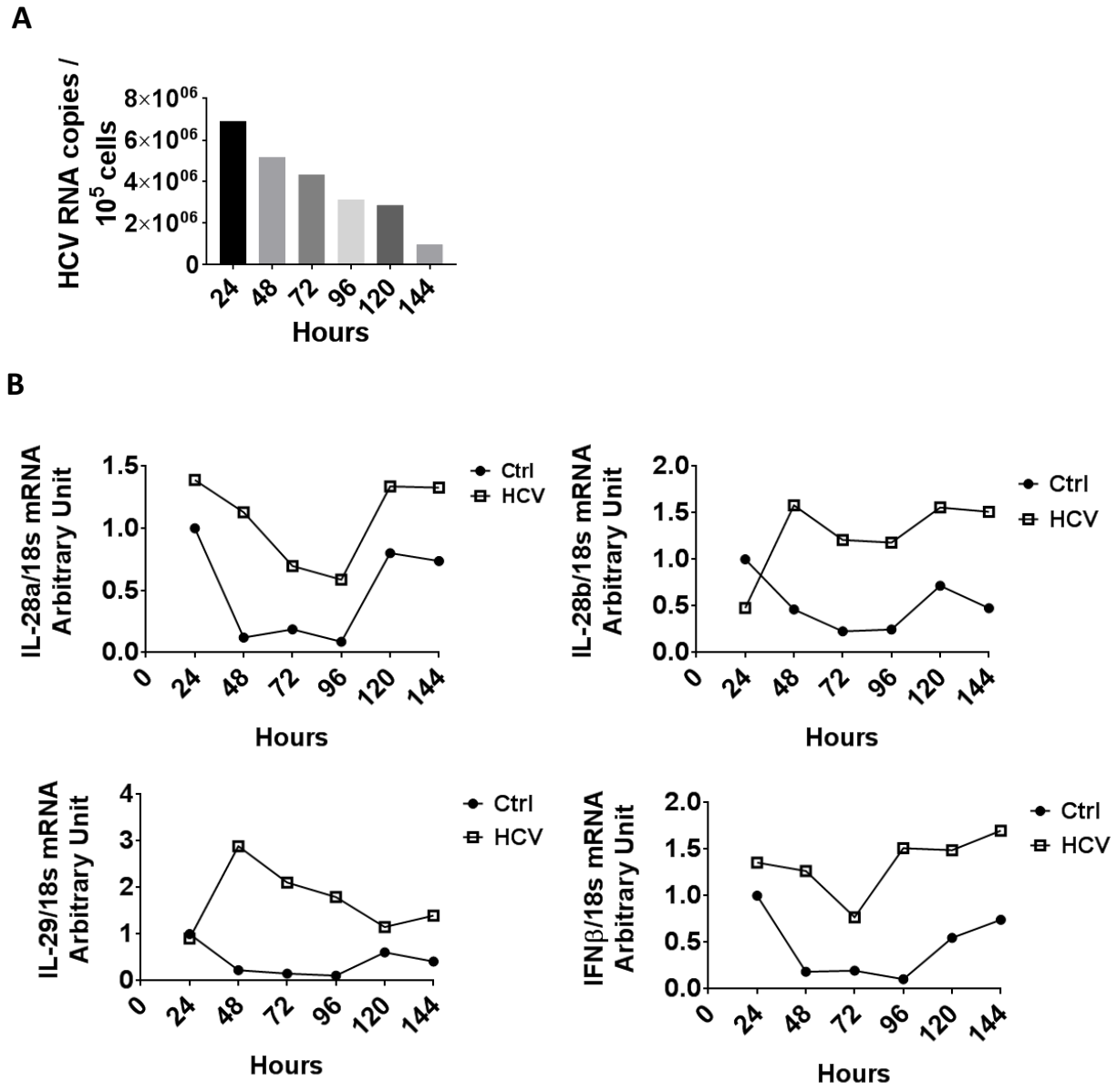


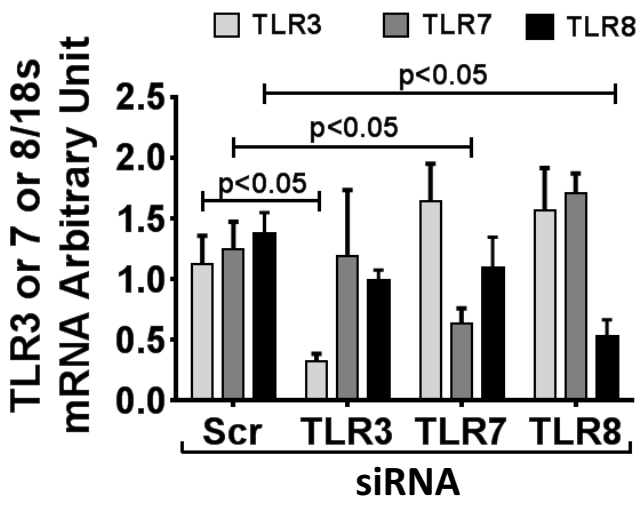
Supplementary Fig. 1. HCV-infected hepatocytes induce monocyte differentiation into macrophages. Monocytes were isolated from healthy donors and cultured with primary hepatocytes alone or infected with HCV for 3 or 7 days. The cells were harvested and immunophenotyped using fluorescently labelled antibodies. Dot plots showing the expression of (A) CD14, CD68, CD11c, (B) CD16, CD40 and CD86 and (C) DC-SIGN, CD206, CD163 has been shown. The data is representative of 2 independent experiments. (D) Monocytes were cultured in the presence of macrophage colony–stimulating factor (M-CSF) (50 ng/mL) and then treated with interferon (IFN)γ (20 ng/mL) lipopolysaccharide (LPS) (100 ng/mL) or IL4 (20 ng/mL) for 18 hours to generate M1 and M2 MΦs, respectively. The cells were harvested and the phenotypic characteristics were evaluated by flow cytometry. The bar graphs represent the levels of CD14, CD68, M1 markers (CD16, CD40) and M2 markers (CD206, CD163). Results are expressed as means ± SEM, n= 3. *$p<0.05$ as compared to respective medium control.

Supplementary Fig. 1



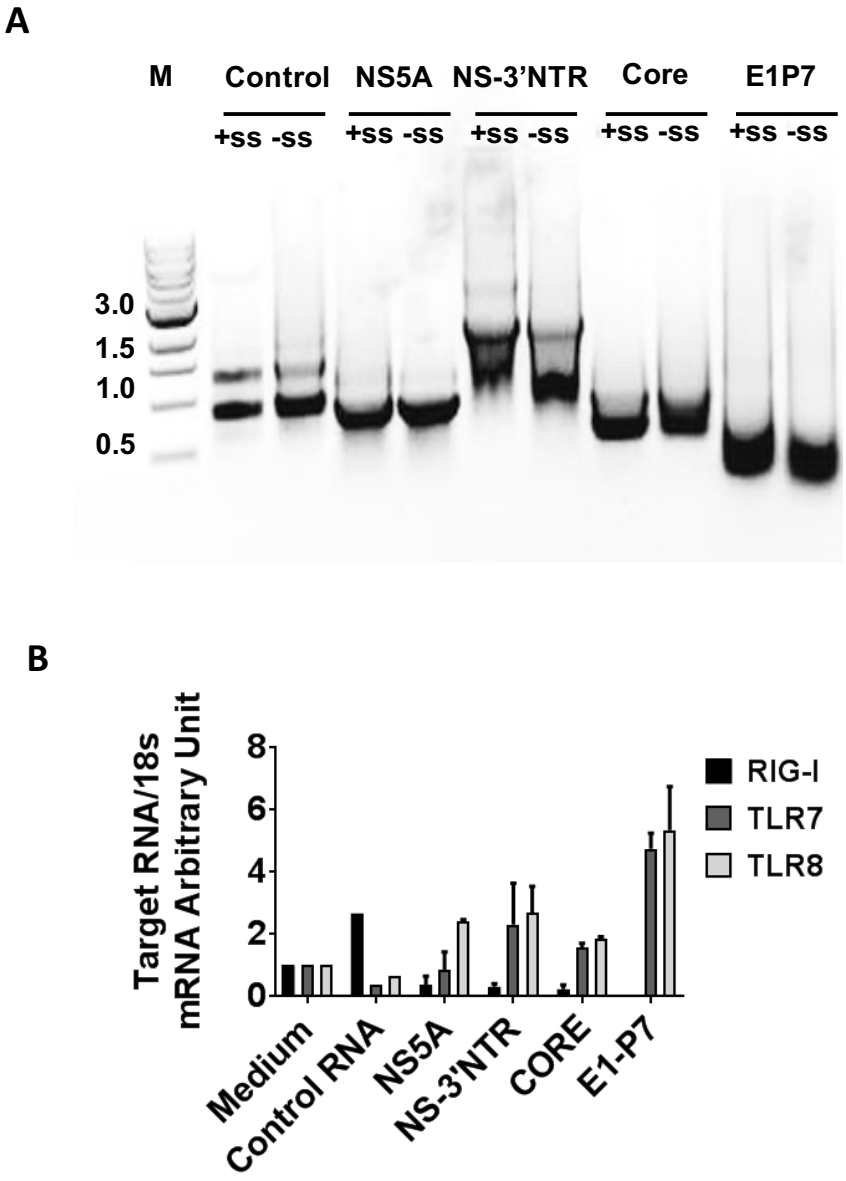
Supplementary Fig 2. HCV infection levels and IFN secretion pattern in PHH. PHH were infected with concentrated JFH-1 (HCV) virions or were left uninfected. (A) Cells were harvested at indicated times and the levels of HCV RNA was determined by quantitative PCR. (B) IL-28a, IL-28b, IL-29 and IFNβ RNA levels were also determined by quantitative PCR. The data is representative of 2 independent experiments.

Supplementary Fig. 2



Supplementary Fig 3. Expression levels of TLRs after knockdown in primary human monocytes. Monocytes were transfected with scrambled or TLR3, TLR7 or TLR8 siRNA for 48 hours and mRNA expression of TLR3, TLR7 and TLR8 was measured. The data is representative of 2-3 independent experiments.

Supplementary Fig. 3



Supplementary Fig. 4. In vitro synthesis of HCV ssRNA and transfection in monocytes. (A) Gel electrophoresis of in vitro synthesized positive (+) and negative (-) HCV ssRNA derived from indicated HCV genome region. M, 1 kb DNA ladder (New England Biolabs Inc, Ipswich, MA). The data is representative of 3 independent experiments. (B) Healthy monocytes were transfected with 5μg/ml of different regions of HCV ssRNA or control RNA and cultured for 7 days. Cells were harvested and total RNA was isolated, cDNA transcribed and PCR for RIG-I, TLR7 and TLR8 was performed. The data is represented as Mean±SEM (n=3).

Supplementary Fig. 4