HCV RNA Activates APCs via TLR7/TLR8 While Virus Selectively Stimulates Macrophages Without Inducing Antiviral Responses

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Supplementary information

Supplementary Figure Legends

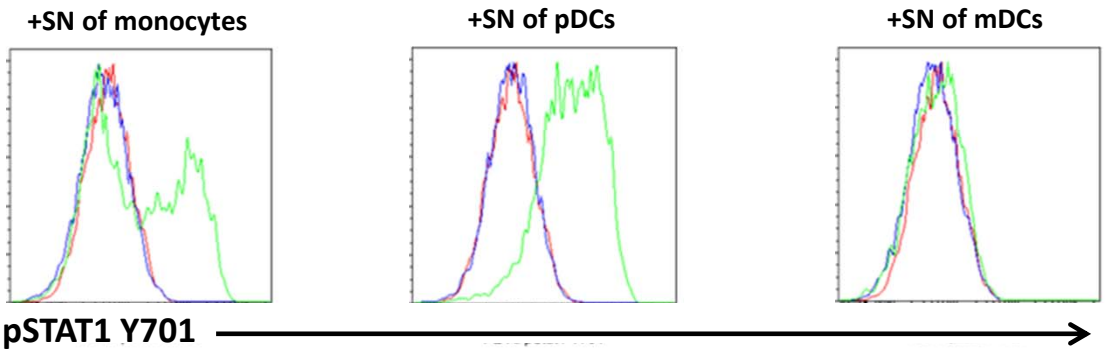
Supplementary Figure S1: STAT1 phosphorylation reveals type I IFN activity in supernatants from HCV ssRNA-exposed monocytes and pDCs but not mDCs. Monocytes, mDCs, and pDCs were isolated from 3 healthy donors by negative selection using magnetic beads. Cells were stimulated with the newly HCV ssRNA sequence HCVL1 (7.5 µg/ml). Cell-free supernatants (SN) were harvested at 24 h post-stimulation. PBMCs (10^6/ml) were incubated for 15 min. with SN from monocytes, mDCs, or pDCs that were exposed to medium only (blue), DOTAP alone (Red), or HCV ssRNA-DOTAP complex (Green). Cells were then fixed and stained with anti-phosphorylated STAT1 (Y701) Ab according to the manufacturer's Phosflow protocol (BD Biosciences). Cells were analyzed by flow cytometry for the intracellular expression of phosphorylated STAT1.

Supplementary Figure S2: Macrophages but not monocytes produce TNF-α in response HCV particles. (a) PBMCs (10^5/200µl) from 3 different donors were incubated with HCV particles (virus/cell ratio of 100), DOTAP alone or HCV RNA-DOTAP complex for 24 h. **(b)** Macrophages or monocytes (10^5/200µl) were stimulated with HCV particles at a virus/cell ratio of 20 for 24h. TNF-α production was measured by ELISA. Results were generated with cells from twenty different healthy donors. Macrophages **(c)** and monocytes **(d)** were stimulated with HCV particles, medium, CL097 (1µg/ml), polyI:C (30µg/ml) or LPS (1ng/ml) for 24h. Macrophages **(e)** and monocytes **(f)** were stimulated with Sendai virus (200 hemagglutinating units/ml) or CL097 for 24h. Supernatants were harvest at 24 h post-stimulation and TNF-α production was measured by ELISA (n=3).

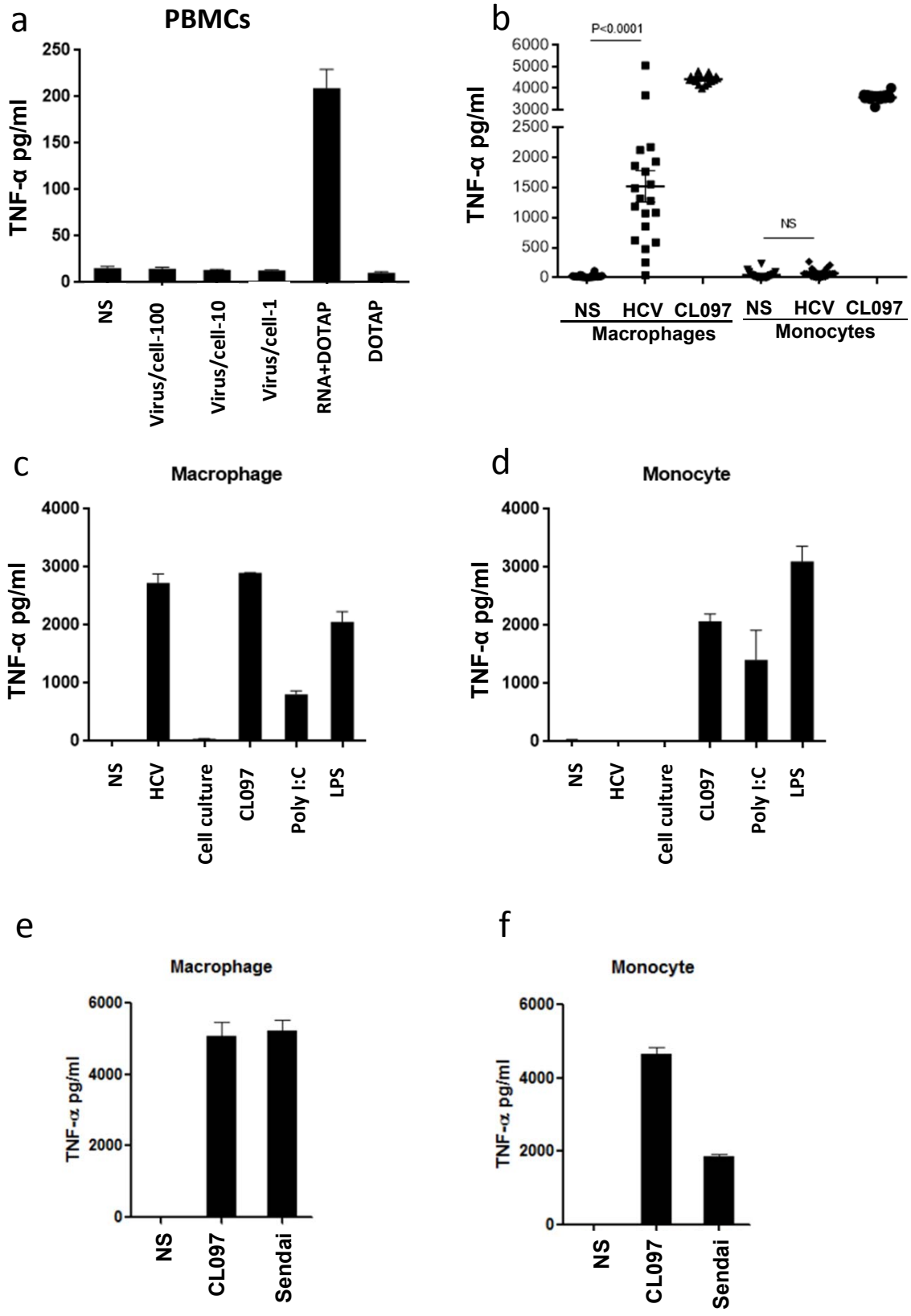
Supplementary Figure S3: Both monocyte-derived macrophages and DCs express DC-SIGN but only macrophages are stimulated by HCV particles. Monocytes were cultured in the presence

of GM-CSF or GM-CSF/IL-4 for 6 days to generate macrophages and MDDCs, respectively. (a-b) DC-SIGN expression on macrophages and DCs was measured by flow cytometry. Shown are representative results generated with cells from one donor (a) and statistical analysis of DC-SIGN MFI expression on cells from 3 different donors (b, c) Macrophages and MDDCs (10^5/200µl) were stimulated with HCV particles at a virus/cell ratio of 20, or with CL097 (1µg/ml) or Sendai virus (200 hemagglutinating units/ml) for 24h. TNF-α production was measured by ELISA. Results represent mean ± SD of values generated with cells from three different donors.

Supplementary Table S1: Novel GU-rich sequences in the HCV genome. GU-rich ssRNA sequences were analyzed within HCV genome based on the sequence of JFH-1 strain. Nine sequences (HCVL1 to HCVL9) that contain around 65% G/U or more were selected.



Zhang et al., Supplemental Figure S1



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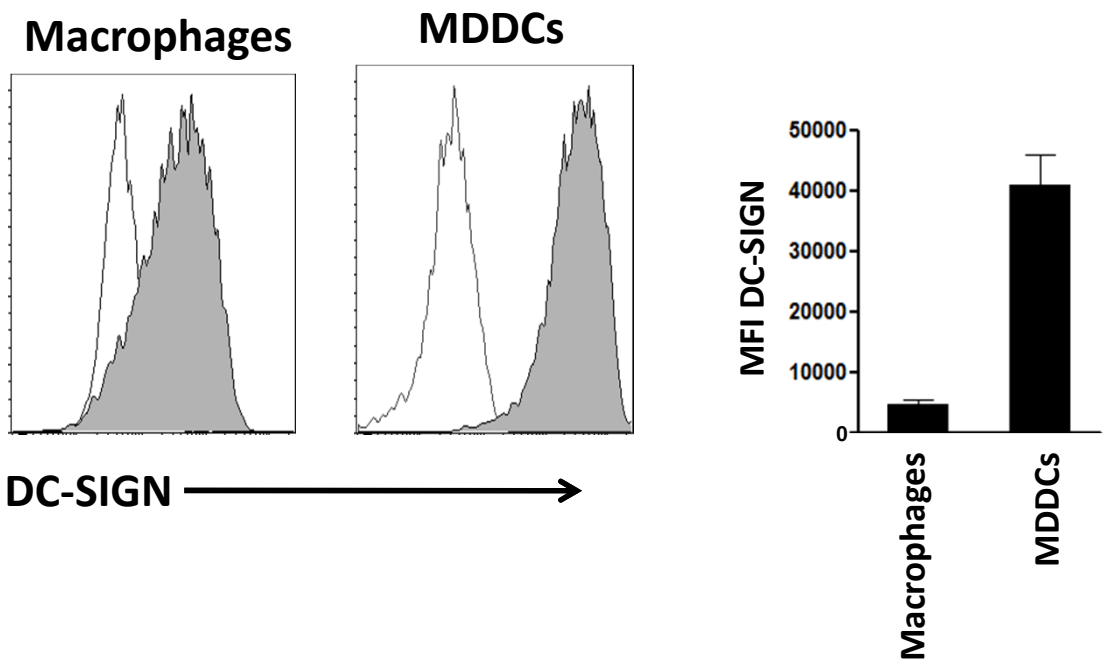
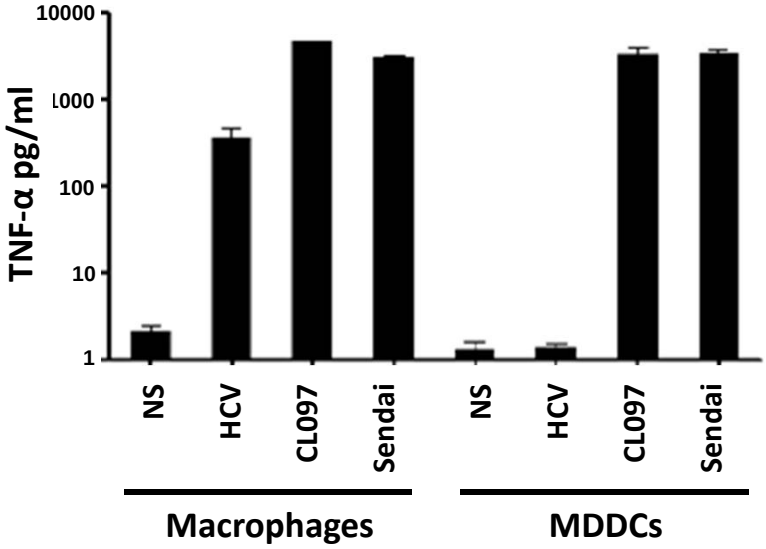
a



b



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Supplemental Table S1

ID	RNA sequences	Regions
HCVL1	GUAGUGUUGGGUCGCGAAAGGCCUUGUGGU	5'UTR
HCVL2	GUCAAGUUCCCGGGUGGCGGUCAGAUCGUUGGUGGAGUUUACUUGUUG	Core
HCVL3	UGUGCGGGUCUGUCUUUCUUGUUGGUCAACUGUUUACCUU	E1
HCVL4	UUGUGGACGUGCAGUACUUGUACGGGGUAGGGU	E2
HCVL5	GUCUUGUGUCCUUCCUCGUGUUCUUCUGCUUUGCGUGGUAUCUGAAGG GUAGGUGGGU	p7
HCVL6	GUCUUGUGUCCUUCCUCGUGUUCUUCUGCUUUGCGUGGU	p7
HCVL7	GUGGCCGCGUCGUGUGGCGGCGUUGUUCUUGUCGGGUU	NS2
HCVL8	GUUGUCGUCGUGUCGACCGA	NS3
HCVL9	GUGUGUGGCGACGACUUAGUCGUUAUCUGUGAAAGUG	NS5B