

1 **Synergy of entry inhibitors with direct-acting antivirals uncovers**
2 **novel combinations for prevention and treatment of hepatitis C**

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8
9 **SUPPLEMENTARY TABLES**

10 **Supplementary Table 1. Synergy of entry inhibitors and direct-acting antivirals on**

11 **inhibition of HCV infection.** Huh7.5.1 cells were pre-incubated with serial concentrations of

12 DAAs (protease inhibitors telaprevir, boceprevir, simeprevir or danoprevir; NS5A inhibitor

13 daclatasvir; or polymerase inhibitors mericitabine or sofosbuvir) and 0.01 µg/ml receptor-

14 specific (anti-CD81, anti-SR-BI or anti-CLDN1) or respective isotype control mAbs or 0.1 µM

15 PKIs (erlotinib or dasatinib) for 1h at 37°C. The mix was removed and Huh7.5.1 cells were

16 incubated for 4h at 37°C with HCVcc Luc-Jc1 in the presence of both compounds. HCVcc

17 infection was analyzed by luciferase reporter gene expression[1]. The Combination Index

18 (CI) at (A) IC₅₀, (B) IC₇₅ and (C) IC₉₀ was calculated as described[2]. A CI less than 0.9,

19 between 0.9 and 1.1, and more than 1.1 indicates synergy, additivity, and antagonism,

20 respectively. Means [95% credible intervals] from three independent experiments performed

21 in triplicate are shown for the CI.

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A.

Compound 1	IC ₅₀ (μM or nM^1)	Compound 2	IC ₅₀ (μM or nM^1) for combination	CI
telaprevir	0.15 \pm 0.06	anti-CD81	0.004 \pm 0.001	0.53 [0.46-0.61]
		anti-SR-BI	0.015 \pm 0.001	0.11 [0.03-0.19]
		anti-CLDN1	0.006 \pm 0.003	0.11 [0.03-0.18]
		erlotinib	0.001 \pm 0.002	0.22 [0.14-0.30]
		dasatinib	0.02 \pm 0.03	0.39 [0.30-0.46]
boceprevir	0.14 \pm 0.02	anti-CD81	0.0004 \pm 0.0001	0.50 [0.42-0.58]
		anti-SR-BI	0.02 \pm 0.002	0.16 [0.08-0.24]
		anti-CLDN1	0.0005 \pm 0.001	0.06 [-0.01-0.14]
		erlotinib	0.008 \pm 0.004	0.27 [0.19-0.35]
		dasatinib	0.001 \pm 0.0005	0.26 [0.18-0.34]
simeprevir	0.013 \pm 0.001	anti-CD81	0.0007 \pm 0.0001	0.56 [0.48-0.64]
		anti-SR-BI	0.006 \pm 0.0007	0.47 [0.39-0.55]
		anti-CLDN1	0.0005 \pm 0.0002	0.1 [0.04-0.20]
		erlotinib	0.005 \pm 0.002	0.59 [0.50-0.66]
		dasatinib	0.0025 \pm 0.002	0.44 [0.36-0.52]
danoprevir	0.006 \pm 0.003	anti-CD81	0.0006 \pm 0.0004	0.6 [0.53-0.68]
		anti-SR-BI	0.0007 \pm 0.001	0.13 [0.05-0.21]
		anti-CLDN1	0.0003 \pm 0.0004	0.11 [0.03-0.19]
		erlotinib	0.001 \pm 0.0005	0.37 [0.29-0.45]
		dasatinib	0.0016 \pm 0.0007	0.52 [0.44-0.60]
daclatasvir	0.012 \pm 0.003	anti-CD81	N.A.	N.A.
		anti-SR-BI	0.0002 \pm 0.0004	0.03 [-0.04-0.12]
		anti-CLDN1	0.0006 \pm 0.0002	0.11 [0.03-0.19]
		erlotinib	0.0009 \pm 0.0004	0.28 [0.20-0.36]
		dasatinib	0.002 \pm 0.0008	0.42 [0.34-0.49]
mericitabine	0.12 \pm 0.03	anti-CD81	0.00015 \pm 0.0001	0.50 [0.43-0.59]
		anti-SR-BI	0.019 \pm 0.007	0.17 [0.09-0.25]
		anti-CLDN1	0.0028 \pm 0.00035	0.08 [0.01-0.17]
		erlotinib	0.003 \pm 0.006	0.23 [0.16-0.32]
		dasatinib	0.035 \pm 0.01	0.54 [0.46-0.62]
sofosbuvir	0.021 \pm 0.002	anti-CD81	0.0006 \pm 0.0004	0.53 [0.45-0.61]
		anti-SR-BI	0.003 \pm 0.0007	0.15 [0.07-0.23]
		anti-CLDN1	0.0012 \pm 0.0003	0.12 [0.04-0.20]
		erlotinib	0.0001 \pm 0.00002	0.20 [0.13-0.29]
		dasatinib	0.00029 \pm 0.0001	0.26 [0.18-0.34]

37 ¹ Telaprevir, boceprevir, danoprevir, simeprevir, mericitabine, sofosbuvir: μM ; daclatasvir: nM

38 IC₅₀ of individual entry inhibitors: anti-CD81, 0.02 \pm 0.01 $\mu\text{g/ml}$; anti-SR-BI, 1.3 \pm 0.4 $\mu\text{g/ml}$; anti-CLDN1,

39 0.18 \pm 0.03 $\mu\text{g/ml}$; erlotinib, 0.5 \pm 0.06 μM ; dasatinib, 0.4 \pm 0.3 μM . N.A.: not applicable.

B.

Compound 1	IC ₇₅ (μM or nM^1)	Compound 2	IC ₇₅ (μM or nM^1) for combination	CI
telaprevir	0.5 \pm 0.15	anti-CD81	0.1 \pm 0.09	0.35 [0.27-0.43]
		anti-SR-BI	0.2 \pm 0.05	0.4 [0.32-0.48]
		anti-CLDN1	0.2 \pm 0.02	0.42 [0.34-0.50]
		erlotinib	0.2 \pm 0.05	0.4 [0.32-0.48]
		dasatinib	0.25 \pm 0.05	0.52 [0.44-0.60]
boceprevir	0.4 \pm 0.04	anti-CD81	0.15 \pm 0.04	0.52 [0.44-0.60]
		anti-SR-BI	0.2 \pm 0.02	0.51 [0.43-0.59]
		anti-CLDN1	0.06 \pm 0.03	0.17 [0.1-0.25]
		erlotinib	0.15 \pm 0.09	0.4 [0.32-0.48]
		dasatinib	0.15 \pm 0.04	0.4 [0.32-0.48]
simeprevir	0.04 \pm 0.01	anti-CD81	0.02 \pm 0.002	0.65 [0.57-0.72]
		anti-SR-BI	0.03 \pm 0.01	0.74 [0.67-0.82]
		anti-CLDN1	0.015 \pm 0.003	0.39 [0.31-0.47]
		erlotinib	0.03 \pm 0.005	0.76 [0.69-0.84]
		dasatinib	0.02 \pm 0.002	0.52 [0.44-0.60]
danoprevir	0.045 \pm 0.003	anti-CD81	0.02 \pm 0.006	0.6 [0.52-0.68]
		anti-SR-BI	0.004 \pm 0.002	0.1 [0.02-0.18]
		anti-CLDN1	0.0018 \pm 0.0003	0.07 [-0.01-0.15]
		erlotinib	0.02 \pm 0.005	0.47 [0.39-0.55]
		dasatinib	0.01 \pm 0.009	0.25 [0.18-0.33]
daclatasvir	0.045 \pm 0.01	anti-CD81	0.00025 \pm 0.0002	0.17 [0.01-0.24]
		anti-SR-BI	0.001 \pm 0.0005	0.04 [-0.04-0.12]
		anti-CLDN1	0.02 \pm 0.01	0.47 [0.39-0.55]
		erlotinib	0.006 \pm 0.004	0.17 [0.09-0.24]
		dasatinib	0.02 \pm 0.006	0.47 [0.39-0.55]
mericitabine	0.45 \pm 0.05	anti-CD81	0.03 \pm 0.01	0.22 [0.15-0.30]
		anti-SR-BI	0.2 \pm 0.05	0.45 [0.38-0.53]
		anti-CLDN1	0.15 \pm 0.05	0.35 [0.28-0.43]
		erlotinib	0.2 \pm 0.04	0.47 [0.39-0.55]
		dasatinib	0.4 \pm 0.05	0.90 [0.82-0.98]
sofosbuvir	0.07 \pm 0.003	anti-CD81	0.025 \pm 0.005	0.51 [0.43-0.59]
		anti-SR-BI	0.05 \pm 0.03	0.70 [0.63-0.78]
		anti-CLDN1	0.03 \pm 0.005	0.45 [0.37-0.53]
		erlotinib	0.002 \pm 0.001	0.07 [-0.01-0.15]
		dasatinib	0.025 \pm 0.005	0.38 [0.30-0.46]

41 ¹ Telaprevir, boceprevir, danoprevir, simeprevir, mericitabine, sofosbuvir: μM ; daclatasvir: nM
42 IC₇₅ of individual entry inhibitors: anti-CD81, 0.065 \pm 0.02 $\mu\text{g}/\text{ml}$; anti-SR-BI, 10 \pm 0.3 $\mu\text{g}/\text{ml}$; anti-CLDN1,
43 0.5 \pm 0.06 $\mu\text{g}/\text{ml}$; erlotinib, 3.8 \pm 0.2 μM ; dasatinib, 4 \pm 0.8 μM .

C.

Compound 1	IC ₉₀ (μM or nM^1)	Compound 2	IC ₉₀ (μM or nM^1) for combination	CI
telaprevir	1 \pm 0.2	anti-CD81	0.45 \pm 0.05	0.48 [0.38-0.59]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	0.6 \pm 0.09	0.61 [0.50-0.71]
		erlotinib	0.5 \pm 0.1	0.5 [0.40-0.61]
		dasatinib	0.6 \pm 0.1	0.61 [0.50-0.71]
boceprevir	0.7 \pm 0.04	anti-CD81	0.4 \pm 0.1	0.6 [0.49-0.70]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	0.35 \pm 0.05	0.51 [0.41-0.62]
		erlotinib	0.5 \pm 0.13	0.71 [0.61-0.82]
		dasatinib	0.4 \pm 0.05	0.58 [0.48-0.68]
simeprevir	0.08 \pm 0.02	anti-CD81	0.05 \pm 0.01	0.65 [0.54-0.75]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	0.05 \pm 0.01	0.64 [0.53-0.74]
		erlotinib	0.05 \pm 0.017	0.63 [0.52-0.73]
		dasatinib	0.05 \pm 0.03	0.64 [0.53-0.74]
danoprevir	0.2 \pm 0.02	anti-CD81	0.08 \pm 0.02	0.43 [0.33-0.54]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	0.006 \pm 0.001	0.06 [-0.05-0.17]
		erlotinib	0.06 \pm 0.01	0.32 [0.21-0.42]
		dasatinib	0.04 \pm 0.03	0.22 [0.12-0.33]
daclatasvir	0.09 \pm 0.005	anti-CD81	0.02 \pm 0.01	0.27 [0.17-0.38]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	0.08 \pm 0.01	0.89 [0.78-0.99]
		erlotinib	0.03 \pm 0.01	0.35 [0.24-0.45]
		dasatinib	0.06 \pm 0.01	0.67 [0.57-0.78]
mericitabine	1.5 \pm 0.2	anti-CD81	0.2 \pm 0.1	0.18 [0.08-0.29]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	0.4 \pm 0.1	0.28 [0.19-0.40]
		erlotinib	0.7 \pm 0.15	0.47 [0.37-0.58]
		dasatinib	1 \pm 0.1	0.68 [0.57-0.78]
sofosbuvir	0.25 \pm 0.02	anti-CD81	0.1 \pm 0.08	0.43 [0.33-0.54]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	0.1 \pm 0.05	0.41 [0.31-0.52]
		erlotinib	0.1 \pm 0.03	0.41 [0.31-0.52]
		dasatinib	0.15 \pm 0.06	0.61 [0.50-0.71]

46 ¹ Telaprevir, boceprevir, danoprevir, simeprevir, mericitabine, sofosbuvir: μM ; daclatasvir: nM
47 IC₉₀ of individual entry inhibitors: anti-CD81, 0.3 \pm 0.2 $\mu\text{g/ml}$; anti-CLDN1, 0.8 \pm 0.05 $\mu\text{g/ml}$; erlotinib,
48 10 \pm 0.6 μM ; dasatinib, 10 \pm 0.7 μM . N.A.: not applicable.

49 **Supplementary Table 2. Synergy of host-targeting agents on the inhibition of HCV**
50 **infection.** Huh7.5.1 cells were pre-incubated with (A) serial dilutions of receptor-specific
51 (anti-CD81, anti-SR-BI or anti-CLDN1) mAbs or respective isotype control mAbs and 0.1 μ M
52 PKIs (erlotinib or dasatinib); or with (B) serial concentrations of alisporivir and 0.01 μ g/ml of
53 receptor-specific (anti-CD81, anti-SR-BI or anti-CLDN1) mAbs or 0.1 μ M PKIs (erlotinib or
54 dasatinib) for 1h at 37°C. HCVcc infection was carried out and analyzed and the CI at (A)
55 IC_{50} , (B) IC_{75} and (C) IC_{90} was calculated as described in supplementary table 1. Means
56 [95% credible intervals] from three independent experiments performed in triplicate are
57 shown for the CI. IC_{50} of individual entry inhibitors: anti-CD81, 0.02 ± 0.01 μ g/ml; anti-SR-BI,
58 1.3 ± 0.4 μ g/ml; anti-CLDN1, 0.18 ± 0.03 μ g/ml; erlotinib, 0.5 ± 0.06 μ M; dasatinib, 0.4 ± 0.3 μ M.

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60 **A.**

Compound 1	IC_{50} (μ g/ml or μ M)	Compound 2	IC_{50} (μ g/ml) for combination	CI
anti-CD81	0.02 \pm 0.01	erlotinib	0.0025 \pm 0.007	0.33 [0.25-0.41]
		dasatinib	0.0029 \pm 0.002	0.44 [0.36-0.52]
		anti-SR-BI	0.0006 \pm 0.0003	0.4 [0.32-0.48]
anti-SR-BI	1.3 \pm 0.4	erlotinib	0.002 \pm 0.001	0.21 [0.13-0.29]
		dasatinib	0.015 \pm 0.007	0.26 [0.18-0.34]
anti-CLDN1	0.18 \pm 0.03	erlotinib	0.005 \pm 0.03	0.24 [0.16-0.32]
		dasatinib	0.005 \pm 0.003	0.28 [0.20-0.36]
		anti-SR-BI	0.014 \pm 0.005	0.09 [0.01-0.17]
		anti-CD81	0.086 \pm 0.002	0.85 [0.77-0.93]
erlotinib	0.5 \pm 0.06	dasatinib	0.027 \pm 0.005	0.3 [0.22-0.38]

Compound 1	IC ₅₀ (μM)	Compound 2	IC ₅₀ (μM) for combination	CI
alisporivir	2.2±0.5	anti-CD81	0.00045±0.0004	0.50 [0.42-0.57]
		anti-SR-BI	0.31±0.02	0.15 [0.07-0.23]
		anti-CLDN1	0.0007±0.0006	0.06 [-0.02-0.14]
		erlotinib	0.0007±0.006	0.20 [-0.01-0.28]
		dasatinib	0.048±0.01	0.28 [0.21-0.36]

78 IC₅₀ of individual entry inhibitors: erlotinib, 0.5±0.06 μM; dasatinib, 0.4±0.3 μM.

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81 **B.**

Compound 1	IC ₇₅ (μg/ml or μM)	Compound 2	IC ₇₅ (μg/ml) for combination	CI
anti-CD81	0.065±0.02	erlotinib	0.04±0.009	0.64 [0.56-0.71]
		dasatinib	0.03±0.01	0.49 [0.41-0.57]
		anti-SR-BI	0.004±0.002	0.06 [-0.02-0.14]
anti-SR-BI	10±0.3	erlotinib	0.06±0.01	0.04 [-0.04-0.12]
		dasatinib	0.4±0.09	0.08 [-0.01-0.16]
anti-CLDN1	0.5±0.06	erlotinib	0.15±0.1	0.32 [0.24-0.40]
		dasatinib	0.025±0.015	0.09 [0.01-0.17]
		anti-SR-BI	0.2±0.06	0.4 [0.32-0.48]
erlotinib	3.8±0.2	dasatinib	1.8±0.3	0.78 [0.7-0.86]

Compound 1	IC ₇₅ (μM)	Compound 2	IC ₇₅ (μM) for combination	CI
alisporivir	10±2	anti-CD81	1.5±0.5	0.3 [0.23-0.38]
		anti-SR-BI	7±1	0.69 [0.62-0.77]
		anti-CLDN1	1.8±0.4	0.20 [0.12-0.27]
		erlotinib	1.5±0.9	0.19 [0.11-0.27]
		dasatinib	4±1	0.43 [0.35-0.51]

94 IC₇₅ of individual entry inhibitors: erlotinib, 3.8±0.2 μM; dasatinib, 4±0.8 μM.

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Compound 1	IC ₉₀ (µg/ml or µM)	Compound 2	IC ₉₀ (µg/ml) for combination	CI	99
anti-CD81	0.3±0.2	erlotinib	0.2±0.09	0.68 [0.57-0.78]	
		dasatinib	0.08±0.04	0.27 [0.18-0.38]	100
		anti-SR-BI	N.A.	N.A.	
anti-SR-BI	N.A.	erlotinib	N.A.	N.A.	101
		dasatinib	N.A.	N.A.	
anti-CLDN1	0.8±0.05	erlotinib	0.5±0.09	0.64 [0.53-0.74]	
		dasatinib	0.08±0.02	0.13 [0.02-0.23]	102
		anti-CD81	0.75±0.05	0.95 [0.85-1.06]	
		anti-SRBI	N.A.	N.A.	103
erlotinib	10±0.6	dasatinib	7.7±1.0	0.78 [0.88-0.68]	104
					105

106 IC₉₀ of individual entry inhibitors: anti-CD81, 0.3±0.2 µg/ml; anti-CLDN1, 0.8±0.05 µg/ml; erlotinib,
 107 10±0.6 µM; dasatinib, 10±0.7 µM. N.A.: not applicable.

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112 **Supplementary Table 3. Synergy of entry inhibitors and IFN- α 2a or IFN- α 2b on**
 113 **inhibition of HCV infection.** Huh7.5.1 cells were pre-incubated with serial concentrations of
 114 IFN- α 2a or IFN- α 2b and 0.01 μ g/ml of receptor-specific (anti-CD81, anti-SR-BI or anti-
 115 CLDN1) or respective isotype control mAbs for 1h at 37°C. Means \pm SD from at least three
 116 independent experiments performed in triplicate are shown. HCVcc infection was carried out
 117 and analyzed and the CI at (A) IC₅₀, (B) IC₇₅ and (C) IC₉₀ was calculated as described in
 118 supplementary table 1. Means [95% credible intervals] from three independent experiments
 119 performed in triplicate are shown for the CI.

120 **A.**

Compound 1	IC ₅₀ (UI/ml)	Compound 2	IC ₅₀ (UI/ml) for combination	CI
IFN- α 2a	0.3 \pm 0.16	anti-CD81	0.032 \pm 0.001	0.60 [0.53-0.69]
		anti-SR-BI	0.08 \pm 0.04	0.27 [0.19-0.36]
		anti-CLDN1	0.02 \pm 0.01	0.13 [0.06-0.22]
IFN- α 2b	0.2 \pm 0.05	anti-CD81	0.035 \pm 0.01	0.67 [0.59-0.75]
		anti-SR-BI	0.07 \pm 0.04	0.36 [0.29-0.45]
		anti-CLDN1	0.02 \pm 0.05	0.17 [0.09-0.25]

130 IC₅₀ of individual receptor-specific mAbs: anti-CD81, 0.02 \pm 0.01 μ g/ml; anti-SR-BI, 1.3 \pm 0.4 μ g/ml; anti-
 131 CLDN1, 0.18 \pm 0.03 μ g/ml.

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136 **B.**

Compound 1	IC ₇₅ (UI/ml)	Compound 2	IC ₇₅ (UI/ml) for combination	CI
IFN-α2a	1±0.7	anti-CD81	0.5±0.09	0.65 [0.56-0.72]
		anti-SR-BI	0.6±0.05	0.6 [0.52-0.67]
		anti-CLDN1	0.3±0.1	0.32 [0.24-0.40]
IFN-α2b	0.7±0.07	anti-CD81	0.3±0.1	0.58 [0.50-0.66]
		anti-SR-BI	0.4±0.05	0.57 [0.49-0.65]
		anti-CLDN1	0.3±0.05	0.44 [0.36-0.52]

146 IC₇₅ of individual receptor-specific mAbs: anti-CD81, 0.065±0.02 µg/ml; anti-SR-BI, 10±0.3 µg/ml; anti-
 147 CLDN1, 0.5±0.06 µg/ml.

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149 **C.**

Compound 1	IC ₉₀ (UI/ml)	Compound 2	IC ₉₀ (UI/ml) for combination	CI
IFN-α2a	5±09	anti-CD81	2±0.17	0.43 [0.33-0.54]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	0.7±0.18	0.16 [0.06-0.27]
IFN-α2b	2±0.2	anti-CD81	1±0.26	0.53 [0.43-0.64]
		anti-SR-BI	N.A.	N.A.
		anti-CLDN1	1±0.43	0.51 [0.41-0.62]

159 IC₉₀ of individual receptor-specific mAbs: anti-CD81, 0.3±0.2 µg/ml; anti-CLDN1, 0.8±0.05 µg/ml. N.A.:
 160 not applicable.

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166 **SUPPLEMENTARY FIGURE LEGEND**

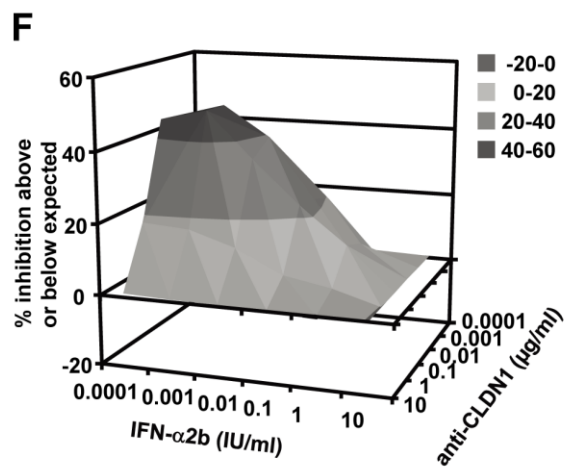
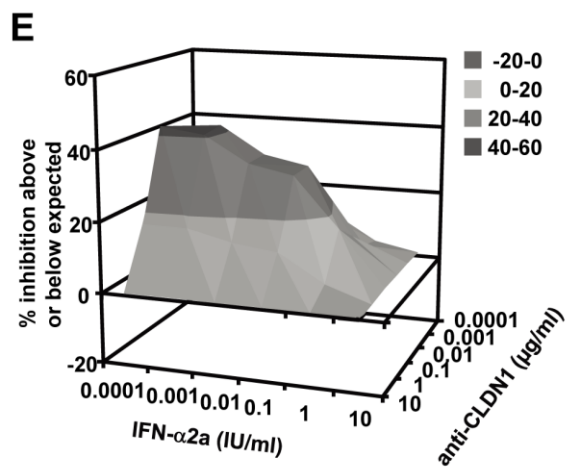
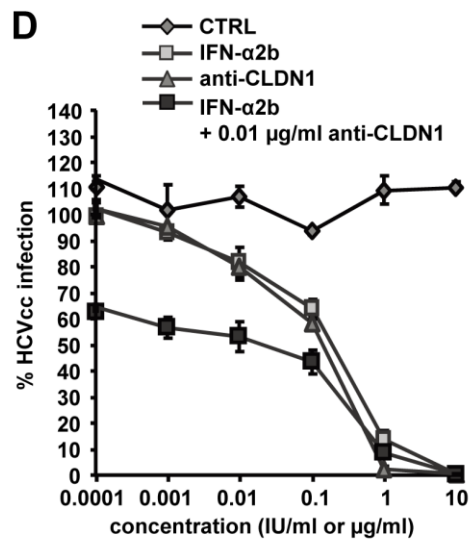
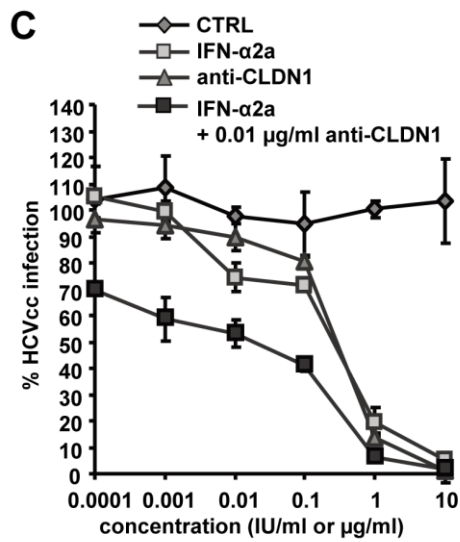
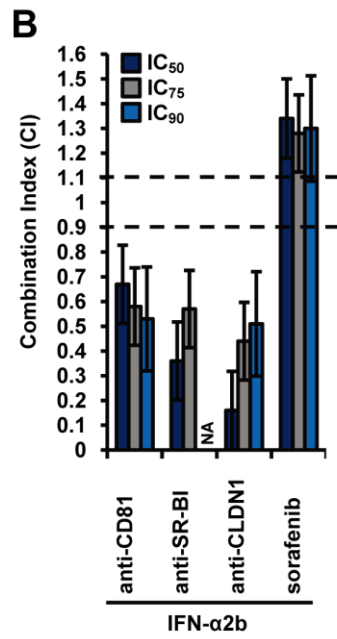
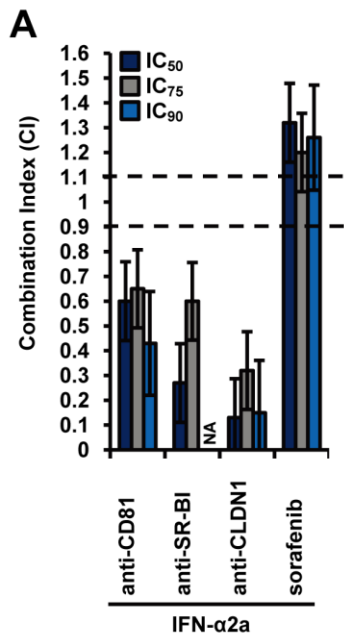
167 **Supplementary Figure 1. Antiviral activity of IFN- α and entry inhibitors in combination.**

168 Huh7.5.1 cells were pre-incubated for 1h with serial concentrations of (A) IFN- α 2a or (B) IFN-
169 α 2b and 0.01 μ g/ml of receptor-specific (anti-CD81, anti-SR-BI, anti-CLDN1) mAbs before
170 incubation with HCVcc Luc-Jc1 in the presence of both compounds. HCVcc infection was
171 analyzed by luciferase activity as described[1] (A-B) The CIs at IC₅₀, IC₇₅ and IC₉₀ are
172 indicated in Supporting Table 3. Dotted lines at combination values of 0.9 and 1.1 indicate
173 the boundaries of additivity. Means \pm 95% credible intervals from at least three independent
174 experiments performed in triplicate are shown. (C-D) Combination of IFN- α 2a or IFN- α 2b
175 with anti-CLDN1 mAb. (E-F) Synergy was confirmed using the method of Prichard and
176 Shipman[3].

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Supplementary Figure 1

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SUPPLEMENTARY REFERENCES

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