Supplementary material

Humanization of a therapeutic claudin-1-specific antibody to prevent and cure HCV infection without escape

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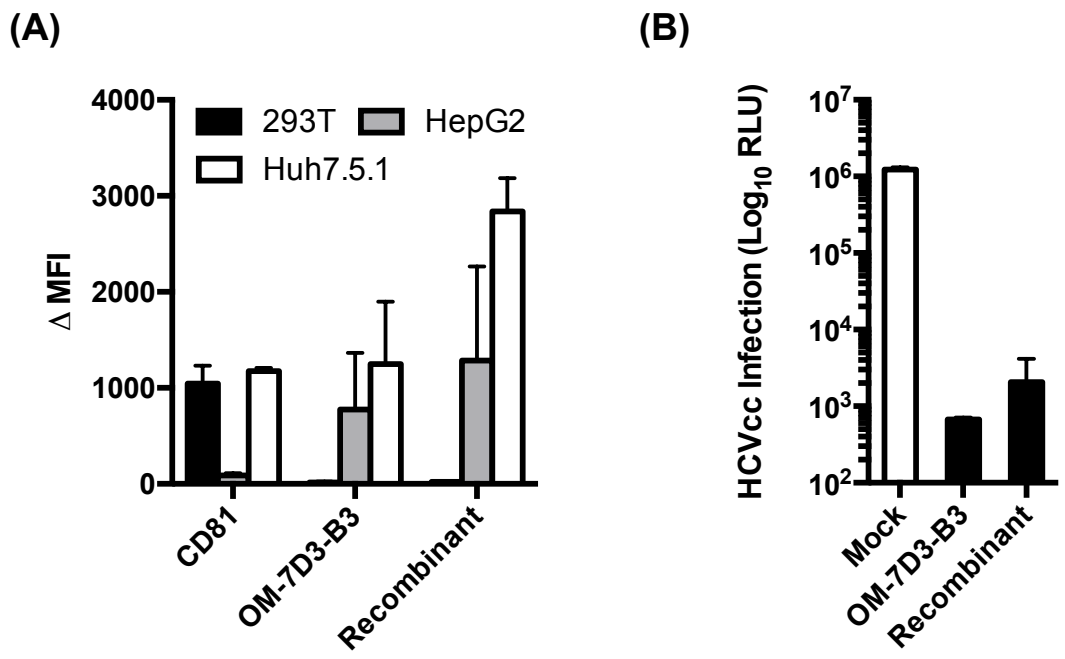
Materials and Methods

Generation of recombinant IgG2b antibody

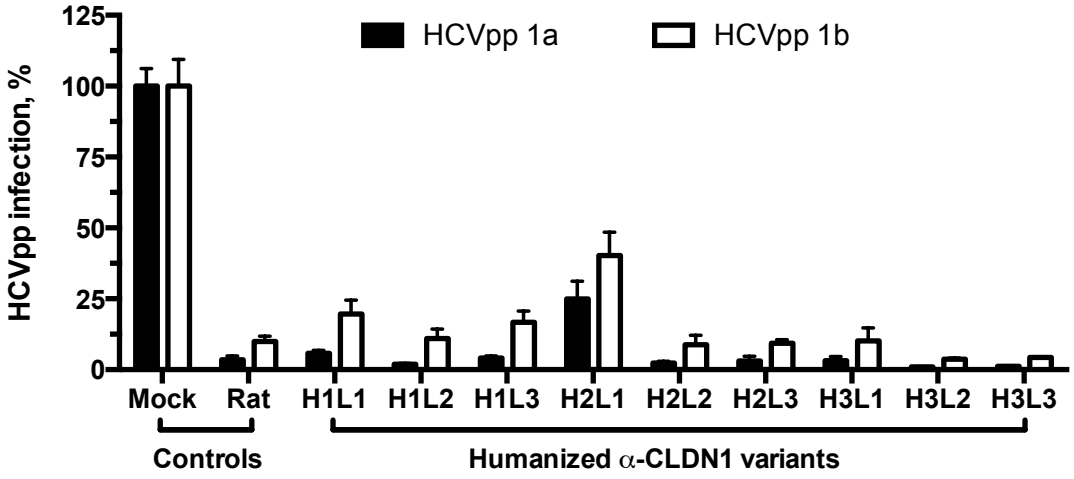
A recombinant full-length IgG2b (parent isotype) antibody was produced by co-transfecting plasmids containing the appropriate heavy and light chain variants into CHO cells. Supernatants were harvested and antibodies purified using the MAbTrap Kit (GE Healthcare). The antibody was buffer-changed into PBS prior to evaluation of its binding and inhibitory properties.

Cytotoxicity assessment

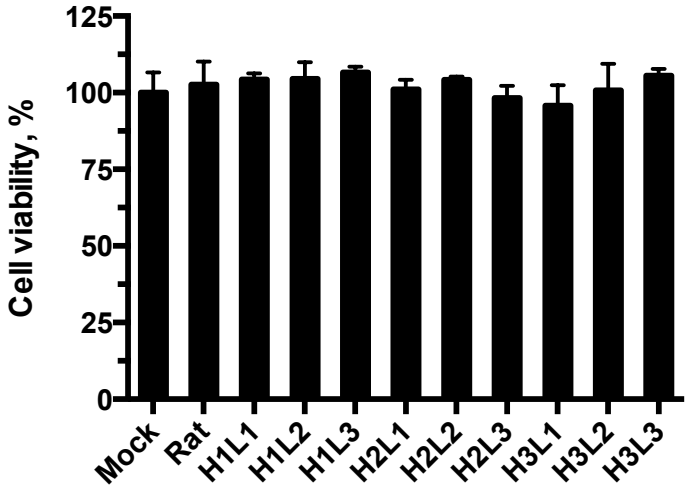
Potential cytotoxicity was evaluated using the Presto Blue assay (Sigma), according to manufacturer's instructions, following treatment of Huh7.5.1 cells with humanized antibodies. Briefly, Huh7.5.1 cells were treated with humanized antibody (20 µg/mL) at 37°C for 72 h. Presto Blue reagent was then added to the cells and the plate incubated at 37°C to allow the viable cells to take up the dye. Cell viability was evaluated by measuring absorbance at 570 nm.



Supplementary Figure 1. Activity of recombinant IgG2b anti-CLDN1 antibody. **(A)** Flow cytometry analysis of the binding of parental rat OM-7D3-B3 and recombinant rat IgG2b mAbs (20 μg/mL) to cell lines. Both antibodies bind to Huh7.5.1 and HepG2 cells expressing human CLDN1 but not to 293T cells lacking CLDN1 expression. Binding is expressed as delta median fluorescence intensity (ΔMFI). **(B)** The recombinant rat IgG2b antibody efficiently inhibits HCVcc infection. Huh7.5.1 cells were incubated with parental rat OM-7D3-B3 or recombinant rat IgG2b mAbs (25 μg/mL) at 37°C for 1 h prior to infection with HCVcc. Infectivity was assessed after 72 h by measuring luciferase activity and is expressed as log relative light units (RLU).



Supplementary Figure 2. Humanized anti-CLDN1 mAbs inhibit entry of HCVpp. PHH were treated with humanized antibody (20 μg/mL) for 1 h at 37°C prior to infection with HCVpp bearing envelope glycoproteins of strains H77 (genotype 1a) or HCV-J (genotype 1b). Infectivity was measured by luciferase activity after 72 h and is expressed as RLU.



Supplementary Figure 3. Cytotoxicity evaluation of humanized antibodies. Following treatment of Huh7.5.1 cells with humanized antibodies (20 μg/mL) for 72 h at 37°C, Presto Blue reagent was added to the cells. Cells were then incubated at 37°C and viability evaluated by measuring absorbance at 570 nm (one experiment performed in triplicate). Cell viability is expressed as a percentage compared to mock-treated cells.

Supplementary Table 1. IC_{50} and IC_{90} of H3L3 against major HCV genotypes in a panel of PHH.

HCV genotype	PHH donor	IC_{50} (μg/mL)	IC_{90} (μg/mL)
1a (H77)	283	8.6	28.9
	297	3.3	23.8
	S2310	3.7	16.7
1b (HCV-J)	216	6.1	143.6
	223	1.1	149.5
	228	1.5	90.0
	235	5.9	200.0
	283	9.9	164.0
	295	2.7	65.1
	297	13.6	65.4
	S2310	11.8	370.6
	S1443	8.6	310.2
2a (JFH-1)	283	0.2	1.3
2b (J8)	283	0.3	1.3
3a (UKN3A1.28)	283	2.0	24.8
	297	1.1	31.9
	S2310	0.7	46.2
3a (NIH S52)	216	1.3	7.3
	223	2.4	15.4
	228	3.3	100.3
	235	2.0	24.8
	283	4.0	22.8
	295	1.3	58.0
	297	2.3	16.8
	S1437	0.1	12.6
	S2310	0.1	12.1
	S1443	3.2	85.2
3a (gt3SXB1)	233	16.7	62.4
	283	4.2	67.8
	288	5.3	103.4
4 (UKN4.21.16)	283	4.9	114.9
5 (UKN5.14.4)	283	5.3	100.1
6 (UKN6.5.340)	283	8.4	290.8