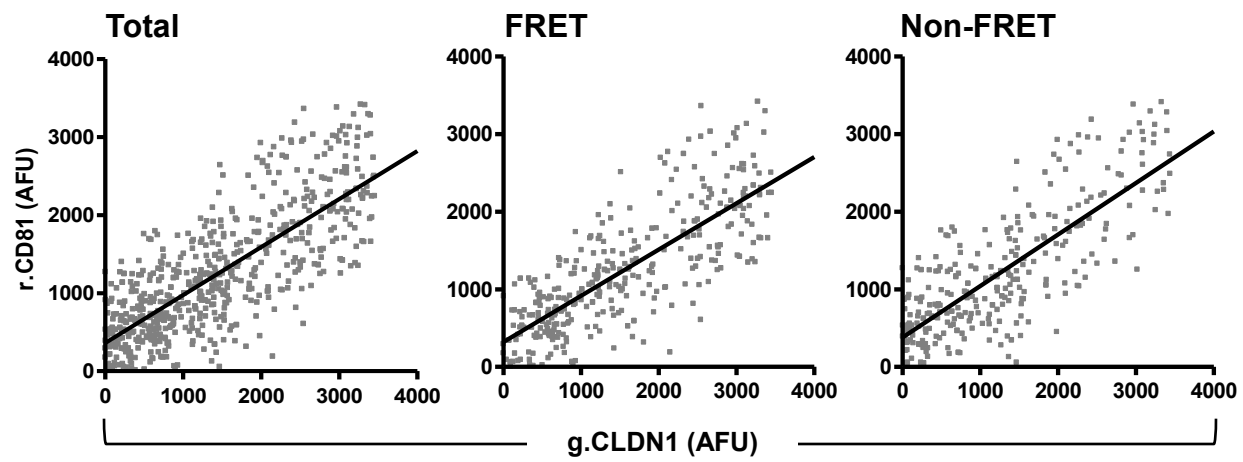**Supplementary figure legends.**
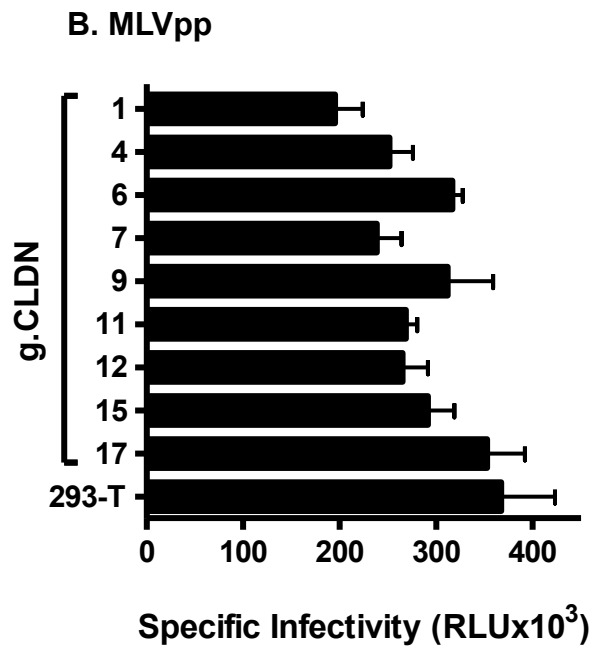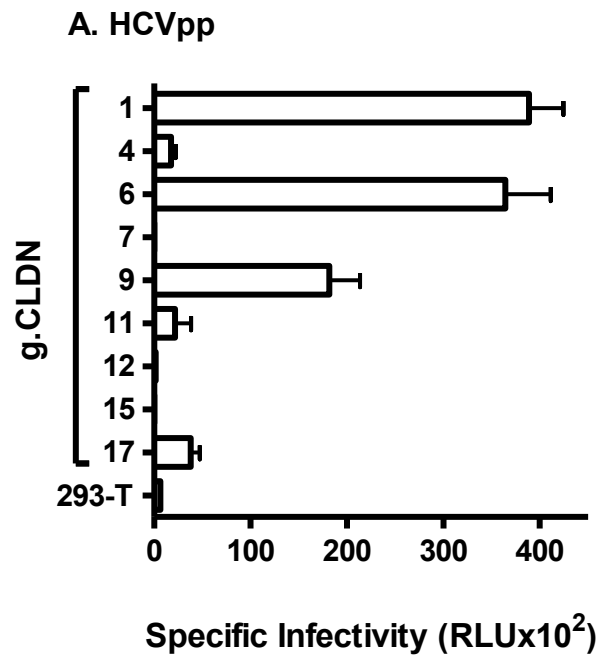
Fig.S1. Effect of FRET on CLDN1-CD81 FIR. To determine if FRET between AcGFP (g) and DsRED (r) tagged g.CLDN1 and r.CD81 affects FIR, pixels where FRET occurred were identified and plotted on a separate scatter plot to those pixels without FRET and the mean FIR determined. FRET occurred between g.CLDN1 and r.CD81 at a frequency of 52%. Median FIR values of 0.59 ± 0.03 (r^2 0.48) and 0.55 ± 0.03 (r^2 0.52) were noted in the presence or absence of FRET, respectively, demonstrating that FRET has a minimal effect on the inferred protein stoichiometry.
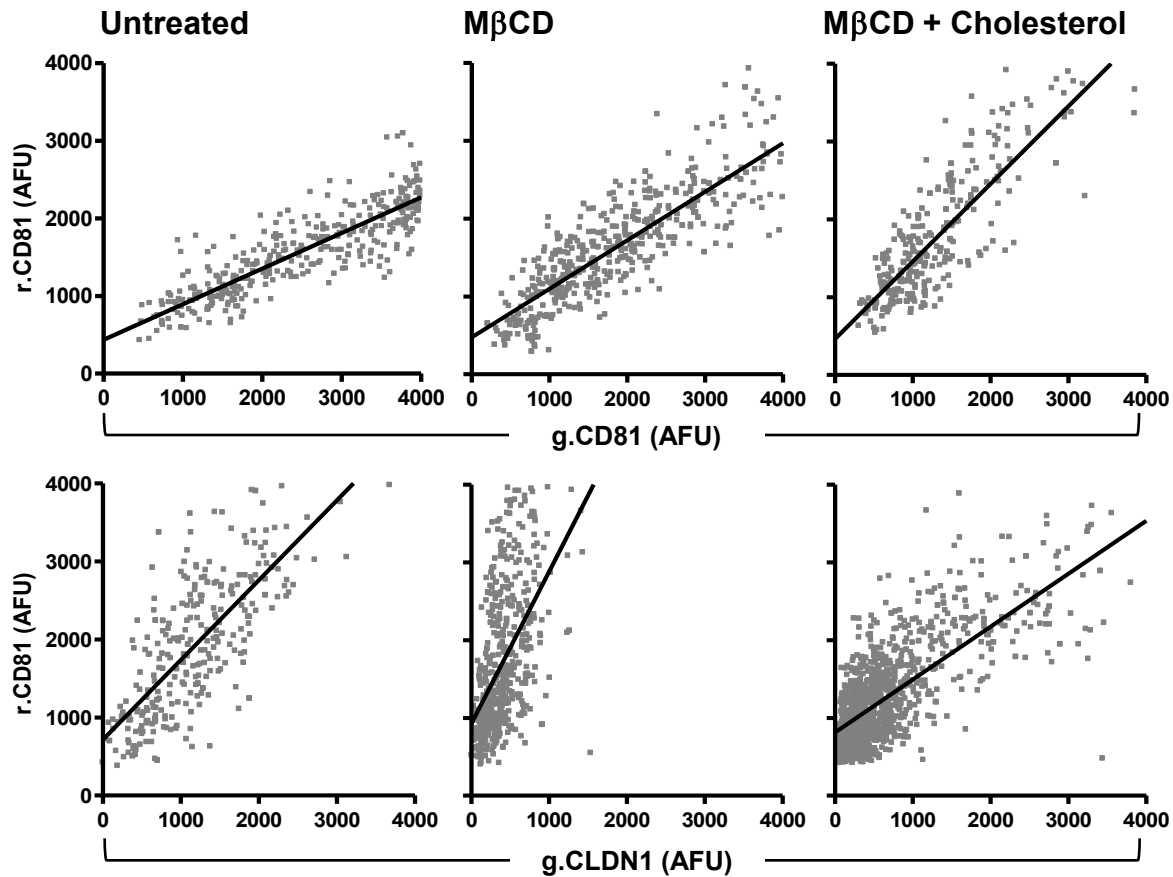
Fig.S2. CLDN viral receptor activity. The panel of AcGFP (g) tagged CLDN proteins were expressed in 293T cells and infected with either HCVpp, MLVpp or Env-pp. Data is represented as specific infectivity where the mean luciferase levels (relative light units [RLU]) for triplicate wells infected with HCVpp (**A**) or MLVpp (**B**) are shown, where the mean Env⁻pp value was subtracted. Data from a single representative experiment is shown.

Fig.S3. The role of cholesterol in CD81-CD81 and CLDN1-CD81 association(s). 293T cells were transfected to express AcGFP (g) and DsRED (r) fluorescent tagged g.CD81-r.CD81 or g.CLDN1-r.CD81 and treated with the cholesterol depleting agent methyl-β-cyclodextrin (MβCD) for 1h at 37°C prior to measuring viral co-receptor association(s). CD81-CD81 and CLDN1-CD81 FIR and FRET analysis of ten individual cells is summarised and representative scatter plots shown.

Fig.S4. Effect of CLDN1 and CLDN7 EC1 mutations on HCV entry. 293T cells were transfected to express AcGFP tagged wild type and CLDN1 and CLDN7 EC1 mutations and DsRED-CD81 and infected with either HCVpp, MLVpp or Env-pp. Data is represented as specific infectivity where the mean luciferase levels (relative light units [RLU]) for triplicate wells infected with HCVpp (**A**) or MLVpp (**B**) are shown, where the mean Env⁻pp value was subtracted. Data from a single representative experiment is shown.

Total FRET Non-FRET

r.CD81 (AFU)

g.CLDN1 (AFU)

A. HCVpp

g.CLDN

1
4
6
7
9
11
12
15
17
293-T

0   100   200   300   400

Specific Infectivity (RLUx10^2)

B. MLVpp

g.CLDN

1
4
6
7
9
11
12
15
17
293-T

0   100   200   300   400

Specific Infectivity (RLUx10^3)

Supp Figure 2 Harris *et al*

	Cholesterol (μM/mg protein)	CD81-CD81 association			CLDN1-CD81 association		
		r² (IQR)	FIR (IQR)	%FRET	r²	FIR (IQR)	%FRET
Untreated	3.0 ± 0.04	0.7 (0.58 - 0.81)	0.8 (0.68 - 0.91)	63 ± 5	0.4 (0.27 - 0.42)	0.8 (0.71 - 1.02)	50 ± 2
MβCD	1.5 ± 0.02	0.6 (0.42 - 0.71)	0.6 (0.53 - 0.70)	55 ± 2	0.3 (0.22 - 0.50)	1.3 (1.00 - 2.00)	35 ± 10
MβCD + Chol	2.1 ± 0.09	0.8 (0.63 - 0.84)	1.0 (0.81 - 1.17)	72 ± 2	0.4 (0.37 - 0.49)	0.7 (0.56 - 0.72)	50 ± 5

A. HCVpp

B. MLVpp

X-axis (A): Specific Infectivity (RLUx10^2) — 0, 100, 200, 300

X-axis (B): Specific Infectivity (RLUx10^4) — 0, 250, 500, 750, 1000

Y-axis categories (both panels, top to bottom): 293-T, CLDN7 M32I/K48E, CLDN7 K48E, CLDN7 M32I, CLDN7, CLDN1 I32M/E48K, CLDN1 E48K, CLDN1 I32M, CLDN1