SUPPLEMENTAL DATA

Supplemental Figure 1. Recruitment of $CD4^+CD25^+Foxp3^-$ T effector cells in intrahepatic compartment. Percentage of $CD4^+CD25^+Foxp3^-$ cells in HD-PBLs, HCV-PBLs, and HCV-IHLs. Statistical analyses of values between IH- and P-cell populations from HCV patients were performed with the nonparametric Mann-Whitney U-test for paired data, whereas those between cell populations from HCV-patients and HDs were performed with the nonparametric Mann-Whitney U-test for unpaired data. *** $P <$ 0.0005. n.s. = not significant. Each symbol represents the value in PBLs or IHLs of a single individual.

Supplemental Figure 2. Upregulation of PD-1 on Teff cells infiltrating HCV-infected livers. (**A, B**) Percentage (A) and MFI (B) of $PD\text{-}1^+$ cells in $CD4^+CD25^+Foxp3^-$ ($CD4^+$ Teff) from HD-PBLs, HCV-PBLs or HCV-IHLs. (**C, D**) Percentage (C) and MFI (D) of $PD\text{-}1^+$ cells in $CD4^-CD25^+Foxp3^-$ ($CD8^+$ Teff) from HD-PBLs, HCV-PBLs or HCV-IHLs. Statistical analyses of values between IH- and P-cell populations from HCV patients were performed with the nonparametric Mann-Whitney *U*-test for paired data, whereas those between cell populations from HCV-patients and HDs were performed with the nonparametric Mann-Whitney *U*-test for unpaired data. * $P < 0.02$; ** $P < 0.0025$; *** $P <$ 0.0008. n.s. = not significant. Each symbol represents the value in PBLs or IHLs of a single individual.

Supplemental Figure 3. Upregulation of PD-1 on Treg cells infiltrating HCV-infected livers. (**A, B)** Percentage (A) and MFI (B) of $PD\text{-}1^+$ cells in $CD4^+CD25^+Foxp3^+$ (Treg) cells from HD-PBLs, HCV-PBLs, and HCV-IHLs. Statistical analyses of values between

IH- and P-cell populations from HCV-patients were performed with the nonparametric Mann-Whitney *U*-test for paired data, whereas those between cell populations from HCV patients and HDs were performed with the nonparametric Mann-Whitney *U*-test for unpaired data. ** $P < 0.009$; *** $P < 0.0001$. n.s. = not significant. Each symbol represents the value in PBLs or IHLs of a single individual.

Supplemental Figure 4. PD-1 blockade enhances Ki67 expression in Treg cells. Representative experiment of four, in which sorted $CD4^+CD25^+PD\text{-}1^+$ or $CD4^+CD25^+PD\text{-}1^-$ cells were stimulated or not for 6 d with anti-CD3/CD28 and IL-2 (100 U/ml), in the presence or absence of anti-PD-L1, and finally stained with anti-CD4, -CD25, -PD-1, -Foxp3, and –Ki67 mAbs. Contour plot analyses are gated on $CD4^+$ cells and show $CD25^+$ and/or $PD\text{-}1^+$ cells, or on the latter and show $Foxp3^+$ and/or $Ki67^+$ cells. α = anti- .

Supplemental Figure 5. Treg cells upregulate B7.1 at a lesser extent than PD-1 in patients with HCV infection. (A) Flow cytometry analysis of HCV-PBLs or –IHLs stained with mAbs to CD4, CD25, Foxp3, PD-1, and B7.1. Dot plot analyses are gated on $CD4^+CD25^{-/lo}Foxp3^-$ or $CD4^+CD25^{hi}Foxp3^+$ cells and show percentages of $B7.1^+$ and/or $PD\text{-}1^+$ cells. The percentage of cells is indicated in each quadrant. (**B**) Representative analysis of highly-purified $CD4^+CD25^+$ cells from HCV-PBLs that were stimulated or not with anti-CD3/CD28 and IL-2 in the presence or absence of anti-PD-L1. After 6 d, cells were stained with mAbs to CD4, CD25, Foxp3, PD-1, and B7.1. Dot plot analyses are gated on $CD4^+CD25^{hi}Foxp3^+$ cells and show percentages of $B7.1^+$ and/or $PD\text{-}1^+$ cells. The percentage of cells is indicated in each quadrant. α = anti-.

Supplemental Figure 6. **B7.1 blockade enhances IL-2-dependent proliferation of HCV-specific Treg cells at a lesser extent than PD-L1 blockade.** (**A**) One representative

of three experiments, in which CFSE-labeled (#) P-$CD4^+CD25^+$ cells from an HCV patient were stimulated with anti-CD3/CD28 and IL-2, in the presence or absence of 10 μg/ml anti-PD-L1 mAb or CTLA-4/Ig. After 6 d, cells were stained with mAbs to CD4, CD25, and Foxp3. Dot plot analyses are gated on $CD4^+CD25^+$ cells and show cells stained with both CFSE and anti-Foxp3. The percentages of cells are indicated in each quadrant. α = anti-. (**B**) Kinetics of CFSE-labeled $Foxp3^+$ cell percentage in $CD4^+CD25^+$ (Treg) cells upon stimulation with anti-CD3/CD28 and IL-2 in the presence or absence of different concentrations of either anti-PD-L1 (represented by square symbols) or CTLA-4/Ig (represented by circle symbols).

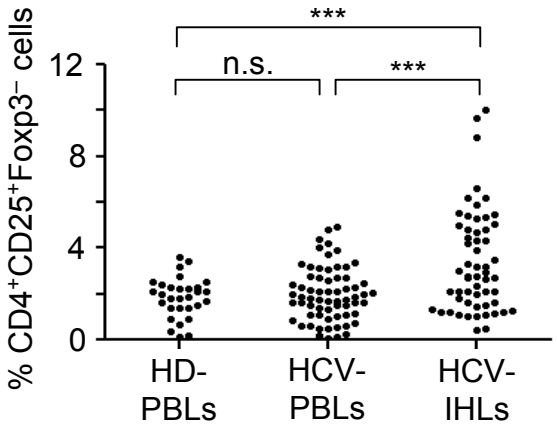
Supplemental Figure 7. PD-1 blockade does not improve suppression function by fresh (non-previously expanded) PD-1^+ Treg cells. (**A**) Flow cytometry analysis of cells expressing PD-1 and/or Foxp3 in highly purified $CD4^+CD25^+$PD-1^- Treg cells, $CD4^+CD25^+$PD-1^+ Treg cells, or $CD4^+CD25^-$ Tresp cells. (**B**) One representative of three experiments in which CD4+$CD25^-$ (Tresp) cells were stained with CFSE (indicated with the # symbol) and stimulated or not with anti-CD3/CD28 in the presence or absence of freshly sorted $CD4^+CD25^+$PD-1^- or $CD4^+CD25^+$PD-1^+ Treg cells, with or without anti-PD-L1. After 6 d, cells were stained with mAbs to CD4, CD25, and Foxp3. Dot plot analyses are gated on $CD4^+CD25^-$ cells and show cells stained with both CFSE and anti-Foxp3. The percentages of cells are indicated in each quadrant. α = anti- .

Supplemental Figure 8. Upregulation of phosphorylated STAT-5 in peripheral Treg cells over-expressing PD-1. Percentage of peripheral pSTAT-5^+ cells in cell populations from HCV-patients indicated under the horizontal axis. Statistical analyses were performed with nonparametric Mann-Whitney *U*-test for paired data. * $P < 0.015$; *** $P < 0.0001$. n.s. = not significant. Each symbol represents the value of cells from a single individual.

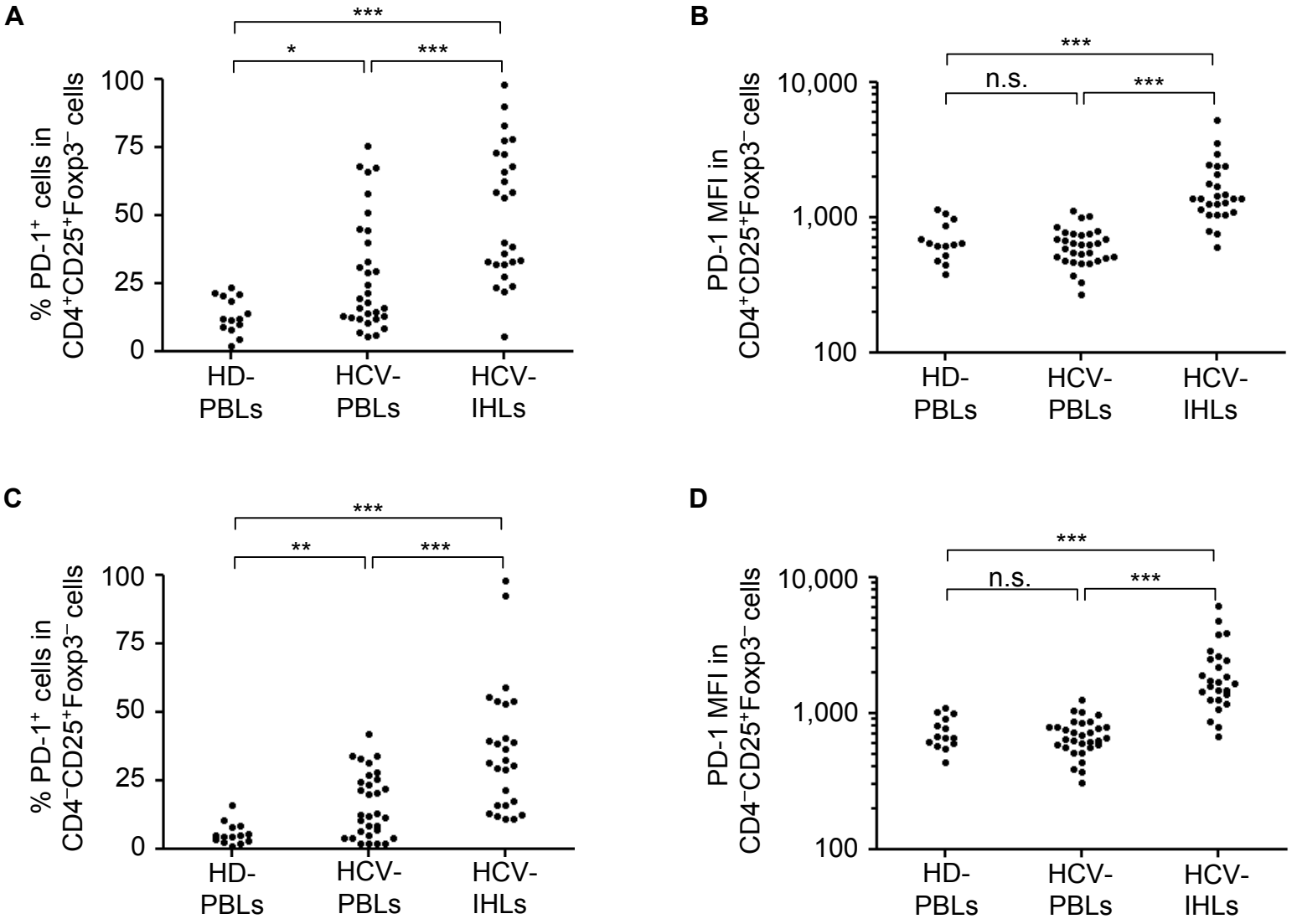
Supplemental Figure 9. Lack of upregulation of phosphorylated STAT-5 in PD-1$^-$ Treg cells by PD-1/PD-L1 blockade *ex vivo*. (**A**) Representative flow cytometry experiment out of six, in which HCV-IHLs were stimulated for 6 h with anti-CD3/CD28 and IL-2 (50 U/ml), in the presence or absence of anti-PD-L1. Cells were then stained with the antibodies to the indicated molecules. Contour plot analyses are gated on $CD4^+CD25^+Foxp3^+PD\text{-}1^-$ cells and show percentages of pSTAT-5$^+$ cells. The counter plot analyses of samples stained with the isotype control of the anti-pSTAT-5 are placed on the top of the pSTAT-5 analyses. The percentages of cells are indicated in each quadrant. Values of pSTAT-5 MFI are shown under the flow cytometry analyses. α = anti-. (**B**) Representative flow cytometry experiment of three, in which $CD4^+CD25^+PD\text{-}1^-$ sorted from PBLs were stimulated or not for 6 h with anti-CD3/CD28 and IL-2 (100 U/ml), in the presence or absence of anti-PD-L1. Cells were then stained with the antibodies to the indicated molecules. Contour plot analyses are gated both on $CD4^+CD25^+PD\text{-}1^-$ cells and show percentage of PD-1$^+$ and/or Foxp3$^+$ cells, and on the latter and show percentages of $PD1^+$pSTAT-5$^+$ or $Foxp3^+$pSTAT-5$^+$ cells. Values of pSTAT-5 MFI are shown under the flow cytometry analyses. α = anti-.

Supplemental Figure 10. Rapid upregulation of pSTAT-5 in $CD4^+CD25^+$ Teff cells following IL-2 contact. Representative kinetics analysis of two, in which highly purified $CD4^+CD25^-$ Tresp cells were stimulated with anti-CD3/CD28 alone for 1h, then cultured with IL-2 (100 U/ml), and tested for the expression of both CD25 and pSTAT-5 after 1, 8, and 24 h. Histogram analyses are gated on both $CD4^+CD25^-$ (Tresp) and $CD4^+CD25^+$ (Teff) cells and show percentage and MFI of p-STAT-5$^+$ cells. Numbers in parenthesis represent the percentage of each population during the kinetics analysis. α = anti-.

Supplemental Figure 11. Foxp3$^+$ Treg cells upregulate pSTAT-5 later, but in a higher extent and in a more sustained fashion than Teff cells. (**A-C**) Representative kinetics analysis of two, in which highly purified CD4$^+$CD25$^+$ cells were stimulated with anti-CD3/CD28 in the presence of 100 U/ml IL-2, and then tested for the expression of CD25, Foxp3, and pSTAT-5 in the times indicated. Histogram analyses are gated on CD4$^+$CD25^{hi}Foxp3$^+$ (A), CD4$^+$CD25^{lo}Foxp3$^+$ (B), and CD4$^+$CD25^{lo}Foxp3$^-$ (C) cells and show percentage and MFI of p-STAT-5$^+$ cells. Numbers in parenthesis represent the percentage of each population during the kinetics analysis. α = anti-.



Supplemental Figure 1 Barnaba



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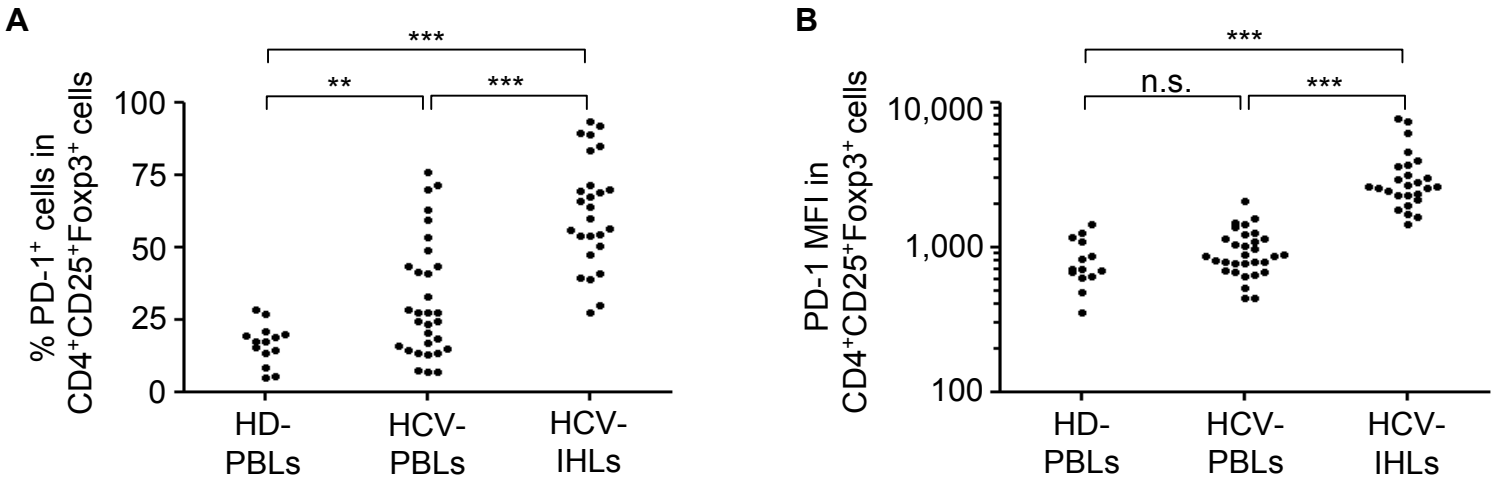
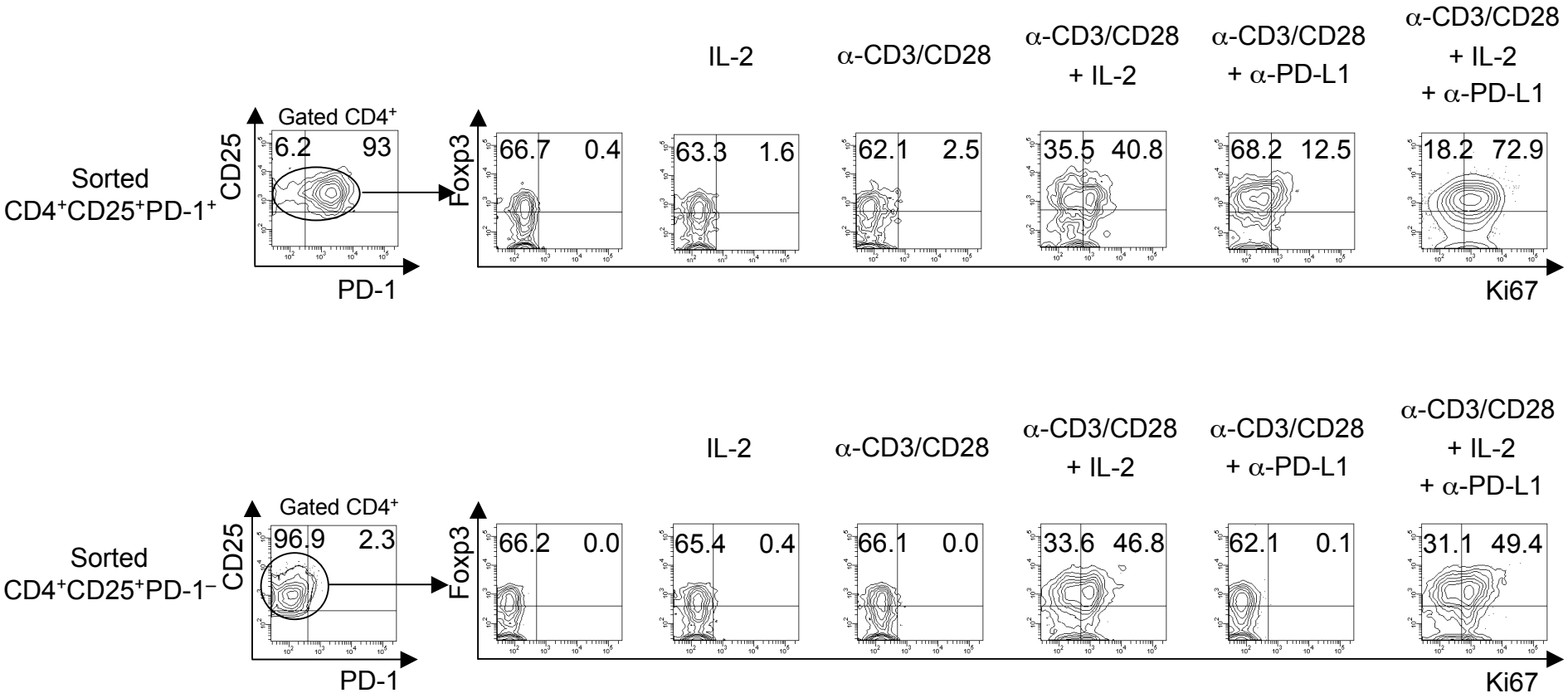


Supplemental Figure 3 Barnaba



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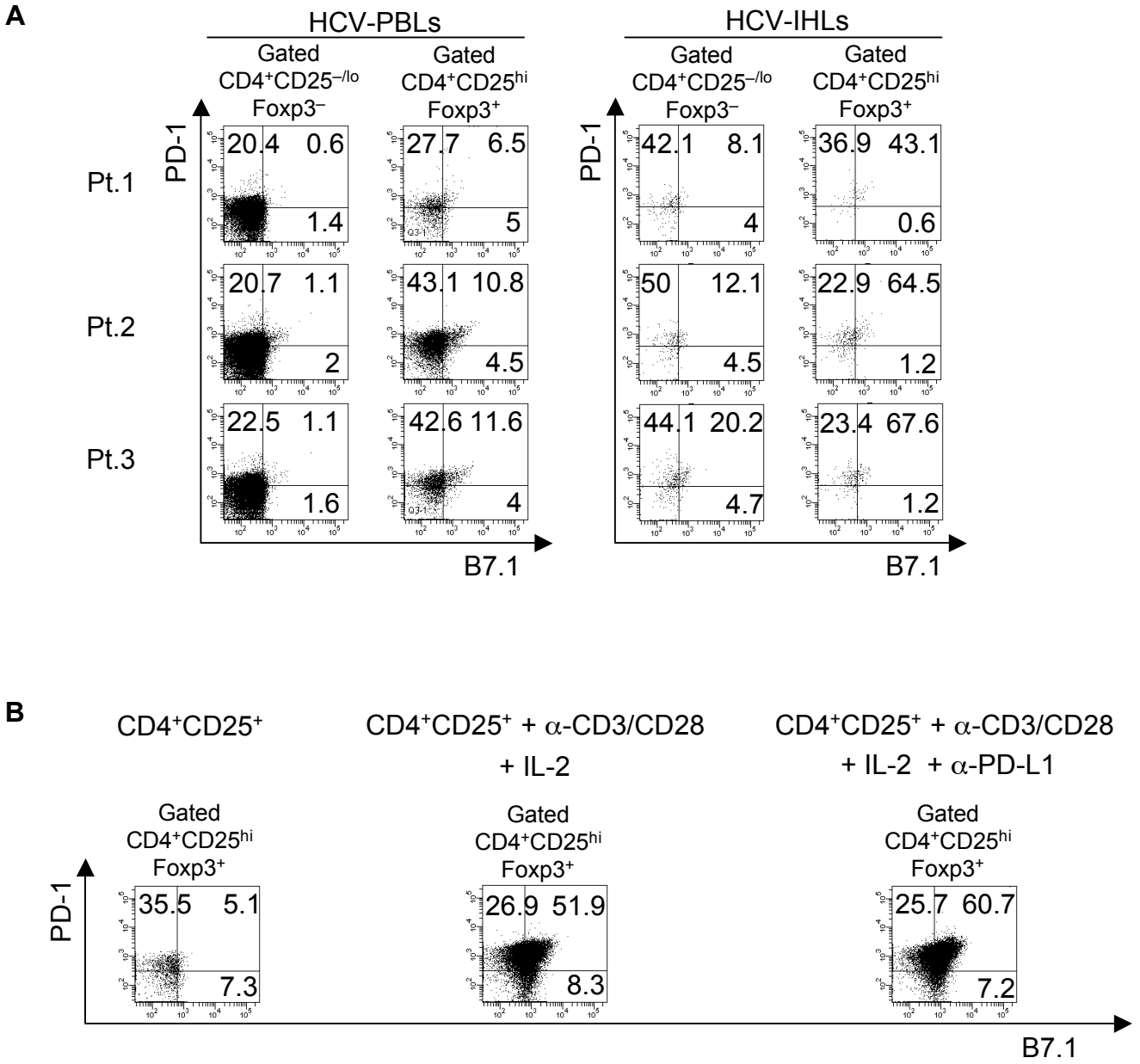
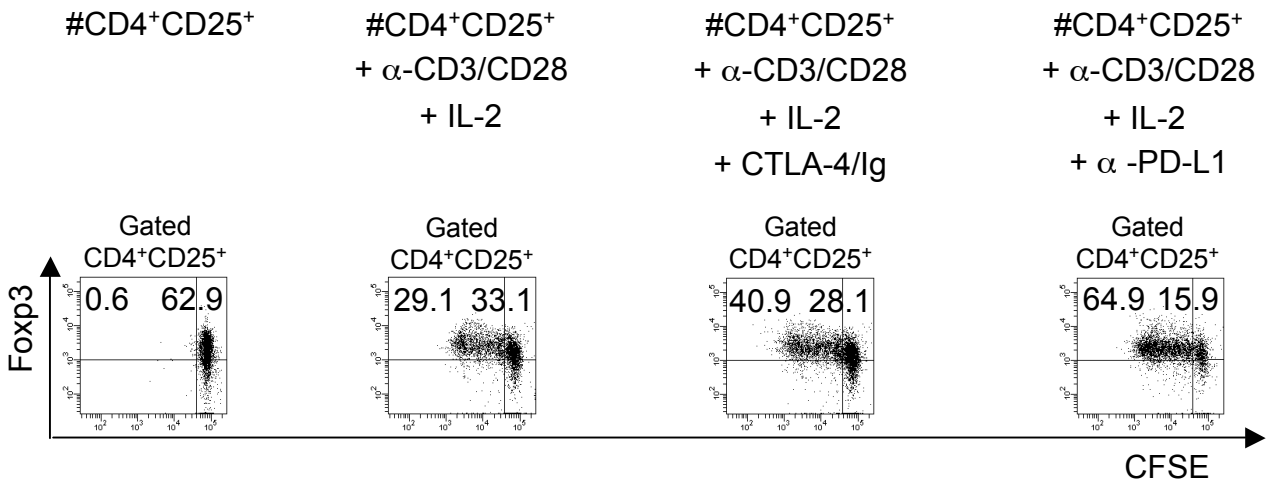


Supplemental Figure 5 Barnaba

A



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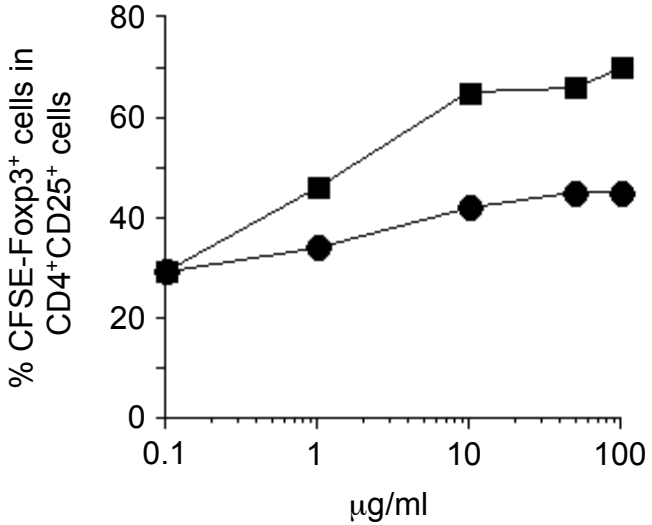
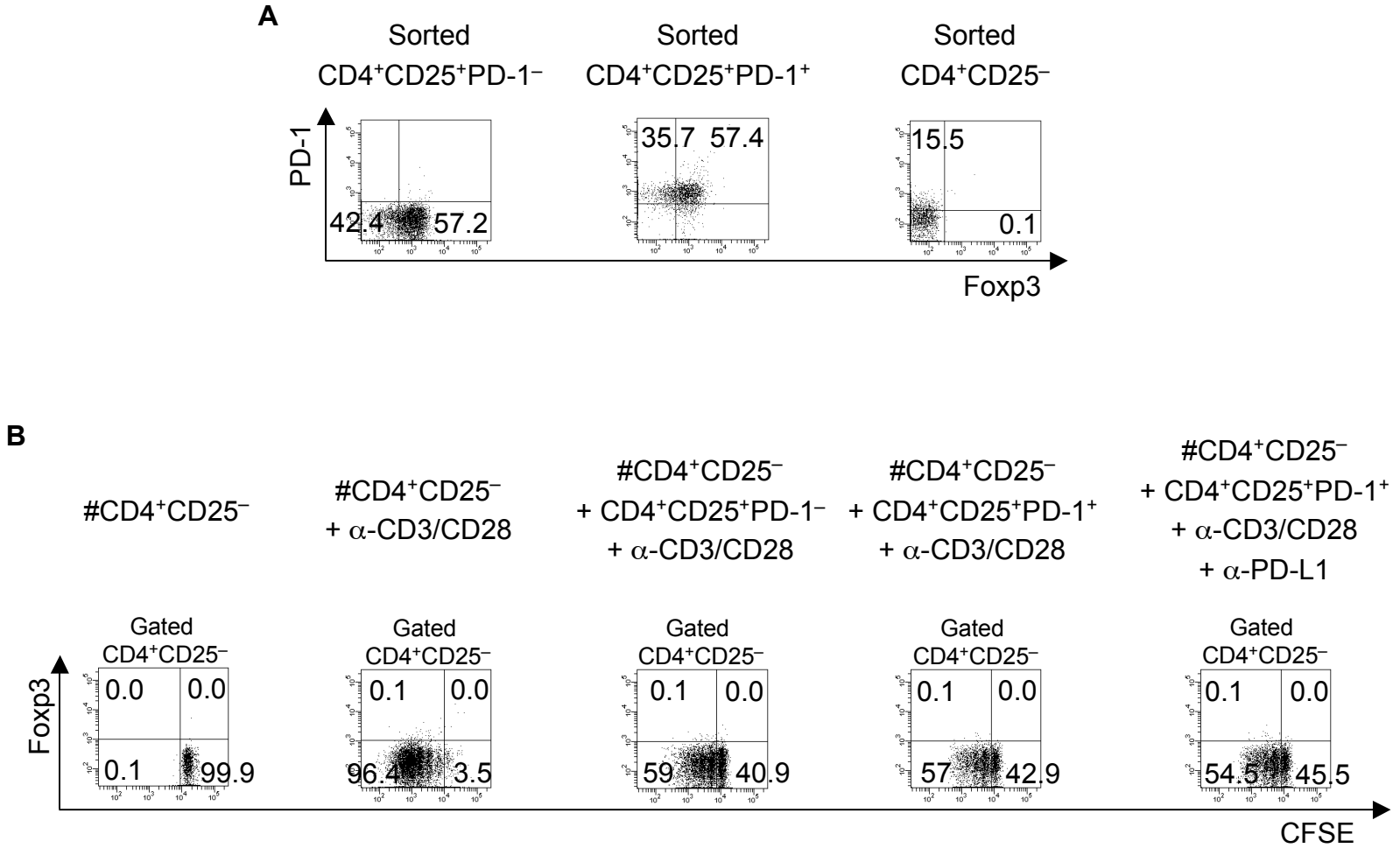
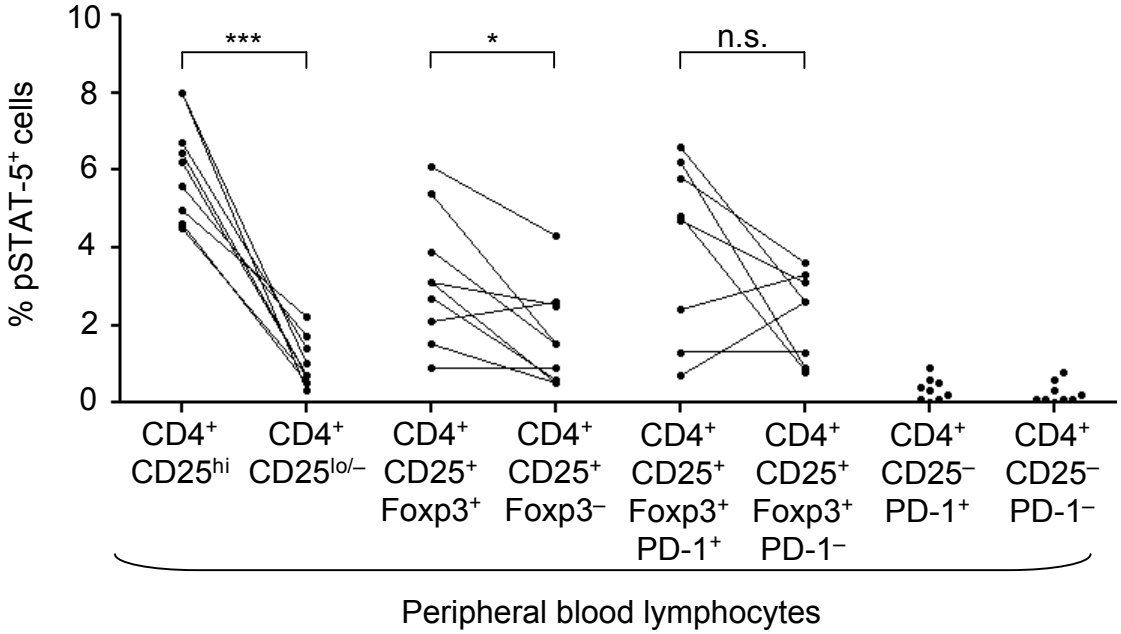


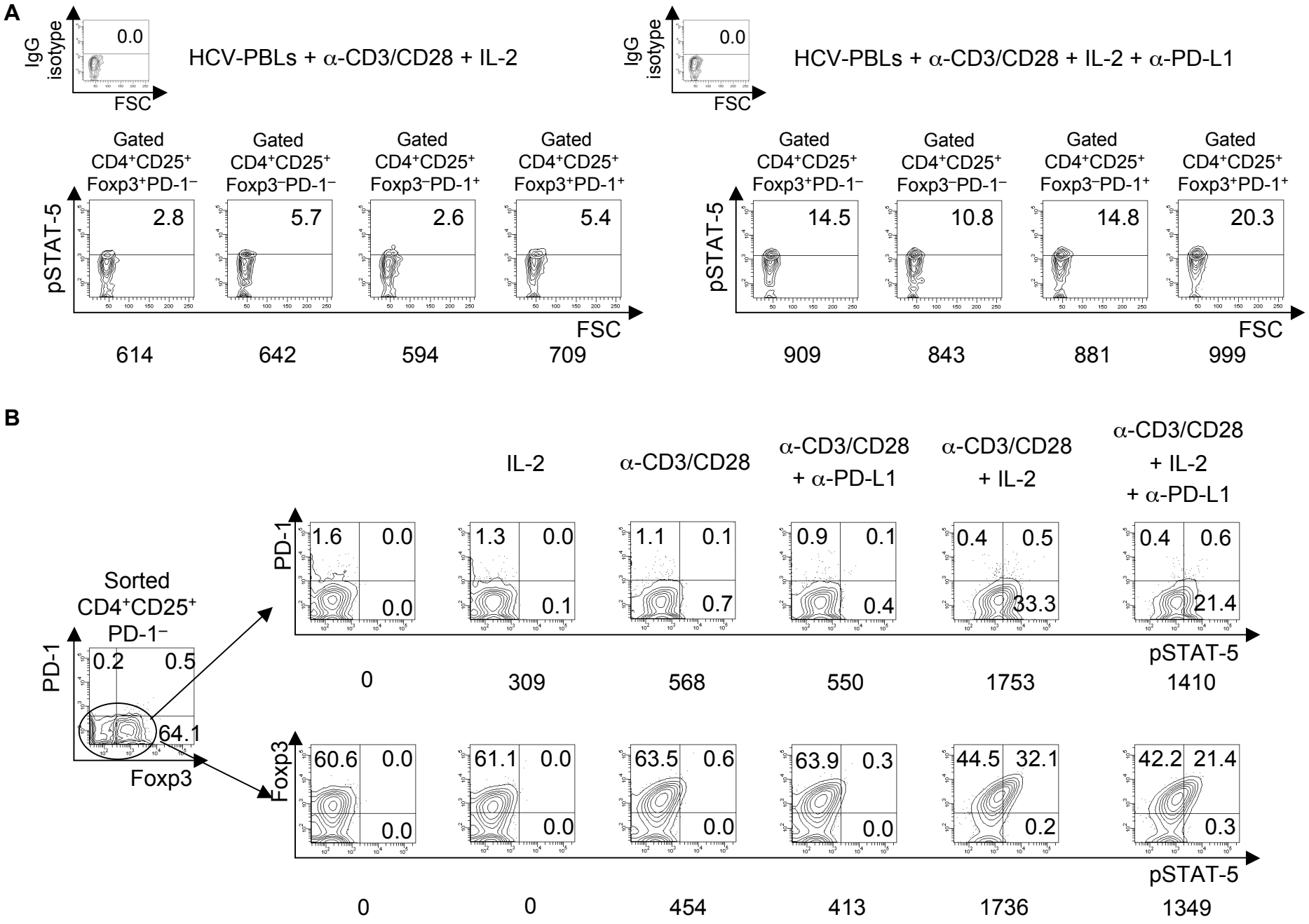
Supplemental Figure 6 Barnaba



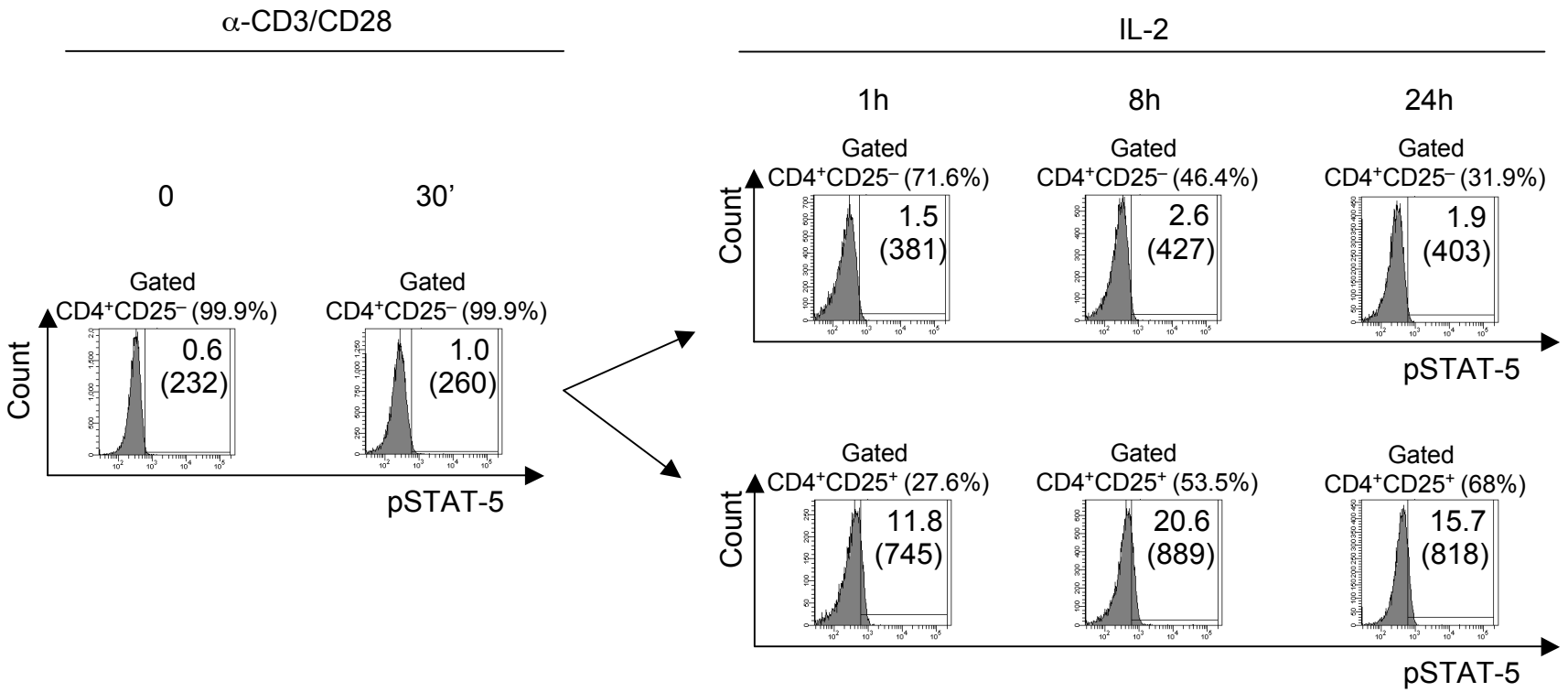
Supplemental Figure 7 Barnaba



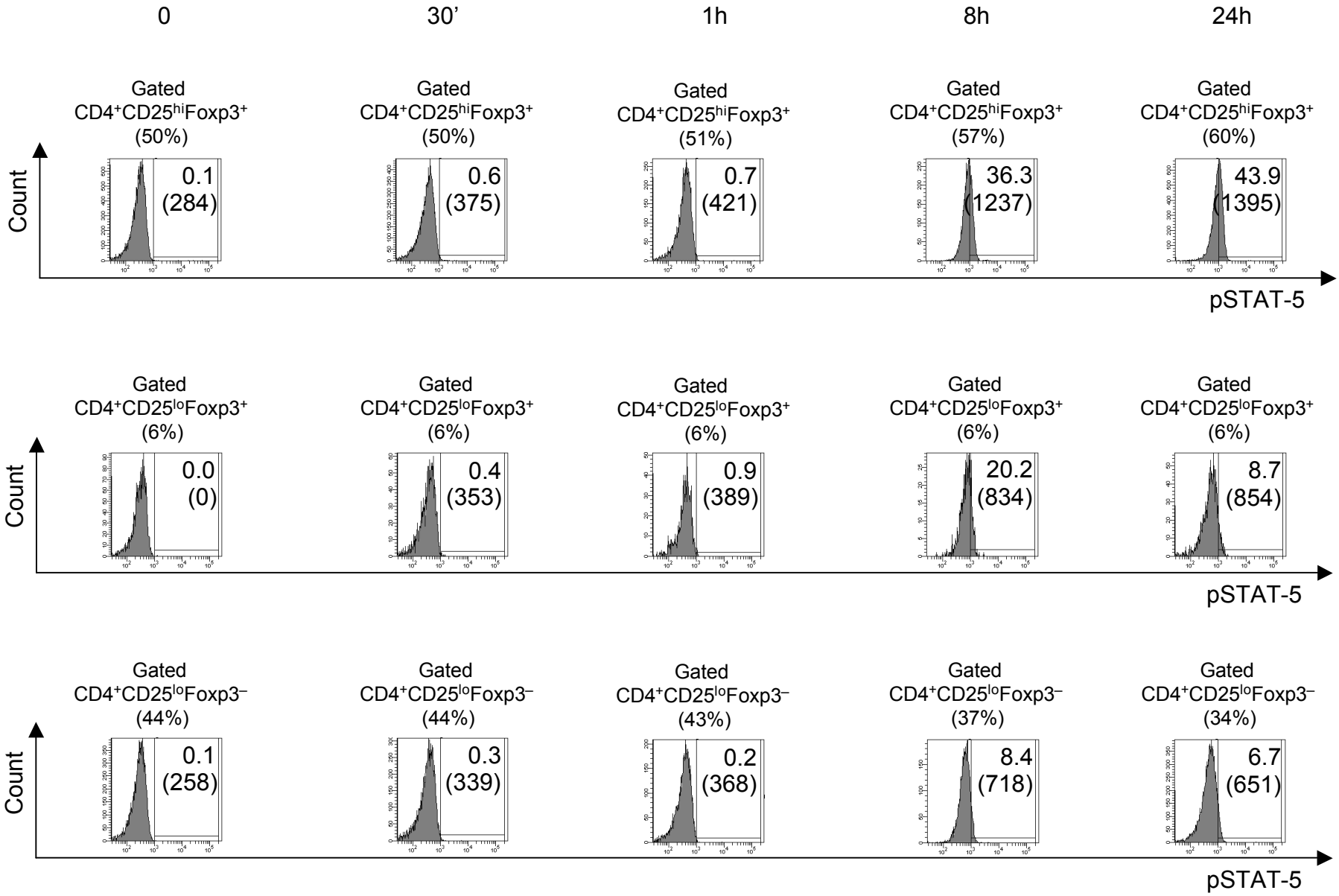
Supplemental Figure 8 Barnaba



Supplemental Figure 9 Barnaba



Supplemental Figure 10 Barnaba



Supplemental Figure 11 Barnaba

Supplemental Table 1
Clinical parameters of patients with chronic HCV infection

Pts	Age (yr)	Sex	Genotype	ALT (IU/ml)	HCV-RNA (IU/ml)	HAI		
						Total score	Grading	Staging
1	29	F	N.D.	173	N.D.	13	10	3
2	52	M	N.D.	118	N.D.	N.D.	N.D.	N.D.
3	24	M	1b	30	1699850	8	6	2
4	42	M	1b	52	135302	5	3	2
5	67	M	3a	420	560070	6	6	0
6	26	M	2a	271	1071990	9	8	1
7	45	F	N.D.	122	N.D.	5	4	1
8	49	M	2a	29	24011	2	2	0
9	47	F	1b	47	1994	7	6	1
10	30	M	4c/4d	122	875130	5	3	2
11	35	F	1b	35	307799	2	2	0
12	28	M	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
13	31	F	3a	122	7000	10	9	1
14	49	M	N.D.	111	neg	3	2	1
15	48	M	1	74	245704	1	0	1
16	42	M	2	191	958777	13	10	3
17	25	M	2a	32	2874630	N.D.	N.D.	N.D.
18	23	F	2	32	1462050	5	5	0
19	53	M	2a/2c	51	N.D.	5	3	2
20	46	M	4	288	113975	3	0	3
21	37	F	2a/2c	16	3128170	3	2	1
22	30	F	3a	625	220786	5	4	1
23	59	F	1b	60	806793	5	3	2
24	62	M	2a/2c	29	7692310	1	0	1
25	49	F	2a/2c	76	350621	2	0	2
26	49	M	1	42	319072	3	2	1
27	N.D.	F	2a/2c	146	10220	10	9	1
28	48	M	2a/2c	53	765254	1	0	1
29	34	M	1b	23	2413410	2	1	1
30	35	F	2a/2c	22	6036620	3	3	0
31	37	M	4	175	1182879	12	9	3
32	18	M	2a/2c	N.D.	737277	5	4	1
33	62	M	2	19	4330810	3	2	1
34	42	M	3a	34	N.D.	5	4	1
35	35	M	4	60	921123	5	5	0
36	59	F	2a	19	3791031	5	4	1
37	37	F	1a	51	390000	7	6	1
38	64	F	5a	71	970994	1	0	1
39	58	M	2a/2c	24	5348630	3	2	1
40	64	F	1b	70	999358	5	5	0
41	49	F	3a	168	53377	13	10	3
42	63	M	2a/2c	36	2674420	3	2	1
43	59	M	4c/4d	152	1589247	4	0	4
44	49	F	1b	26	80059	2	2	0
45	40	F	1a	28	752221	2	2	0
46	62	M	2a	49	141067	5	3	2
47	46	F	1b	43	835000	6	3	3
48	46	M	3a	152	3270000	2	2	0

Pts	Age (yr)	Sex	Genotype	ALT (IU/ml)	HCV-RNA (IU/ml)	HAI		
						Total score	Grading	Staging
49	53	M	1a	N.D.	6000	7	3	4
50	69	F	1b	45	12500000	N.D.	N.D.	N.D.
51	48	F	1b	26	501362	9	6	3
52	52	M	3a	47	5769509	1	1	0
53	33	M	4c/4d	39	115236	1	1	0
54	67	F	N.D.	39	283430	7	3	4
55	59	F	1b	130	34370	8	7	1
56	36	M	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
57	33	M	3a	35	1948880	7	5	2
58	27	F	3a	90	3632267	9	7	2
59	52	M	1b	66	500000	10	6	4
60	43	M	1a	82	665000	10	6	6
61	53	F	2a/2c	21	203926	3	2	1
62	48	M	1a	92	166096	12	9	3
63	60	F	N.D.	20	N.D.	7	6	1
64	65	F	1b	55	1630000	8	5	3
65	58	F	1b	55	7440000	12	8	4

The sum of values of peri-portal necrosis, intralobular necrosis, or portal inflammation (range from 0 to 10, 0 to 4, or 0 to 4, respectively) provides grading. Values of fribosis (from 0 to 4) provides staging. Grading + staging provides total score.
ALT (n.v., 0-40)
Pts, patients; n.v., normal value; F, female; M, male; N.D., not determined