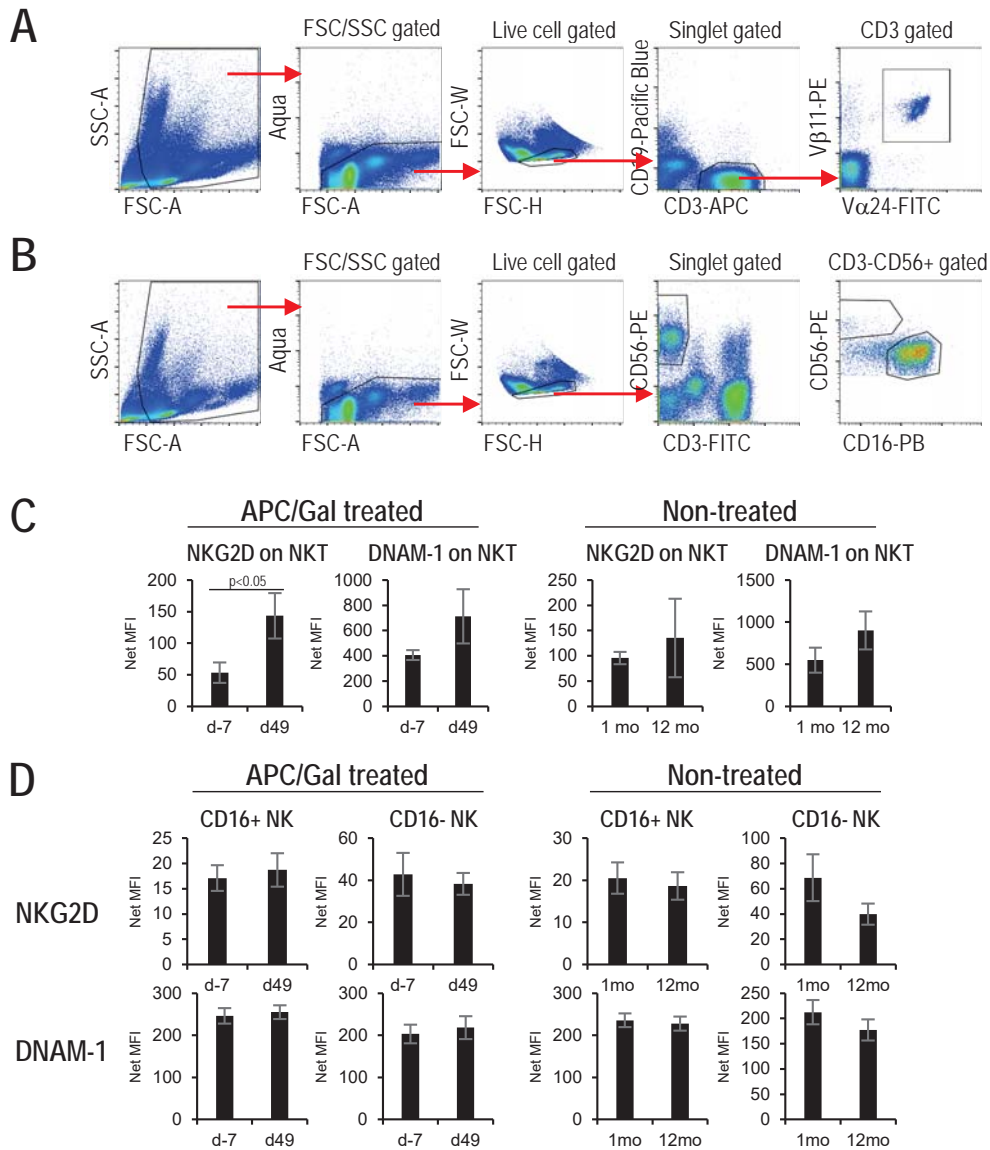


New Supplemental figure 1



Supplemental Figure 1 Gating strategies for iNKT cells and NK cells, and expression of stimulatory and co-receptor molecules of iNKT and NK cells before and after APC/Gal injection

(A) PBMCs were stained with FITC-Vα24, PE-Vβ11, APC-CD3, and Pacific Blue-CD19. Dead cells were labeled with Aqua (LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit). (B) PBMCs were stained with FITC-CD3, PE-CD56, Pacific Blue-CD16, and PE-Cy7-NKG2D, -DNAM-1, or isotype control. Dead cells were labeled with aqua. After gating out dead cells, NK cells were CD3-CD56+ cells, which were subsequently divided into two subsets by CD16 expression. (C) Alteration of stimulatory and co-receptor expression of iNKT cells. NKG2D and DNAM-1 expression is shown as geometric mean of the fluorescence intensity minus the isotype value. Results for ten patients in the treated group and five patients in the non-treated group are summarized, analyzed by Student's t-test. (D) Alteration of the stimulatory and co-receptor expression of NK cells. NKG2D and DNAM-1 expression is shown as geometric mean of the fluorescence intensity minus the isotype values. Results for 26 patients in the treated group and 23 in the non-treated group are summarized. No significant differences were observed in all groups.

Supplemental Table I. Surface phenotypes of injected APC/Gal

Case No.	Percentage of Cells \pm SD			
	HLA-DR+	CD11c+	CD86+	CD3+
002	60.4 \pm 17.4	38.6 \pm 14.9	65.9 \pm 28.5	82.6 \pm 12.0
004	84.4 \pm 7.6	45.5 \pm 8.0	64.7 \pm 27.4	58.8 \pm 10.3
006	86.8 \pm 2.8	24.9 \pm 7.5	67.9 \pm 18.7	90.6 \pm 4.7
009	77.7 \pm 10.7	32 \pm 12.1	59.7 \pm 17.5	77.0 \pm 6.5
013	87.9 \pm 6.4	56.5 \pm 11.4	63.0 \pm 28.0	73.3 \pm 12.6
014	92.2 \pm 6.4	50 \pm 9.5	80.5 \pm 18.6	53.9 \pm 2.8
016	72.9 \pm 18.3	33.7 \pm 12.9	54.0 \pm 29.8	65.5 \pm 22.2
018	73.8 \pm 7.2	53.9 \pm 8.2	51.8 \pm 18.5	71.8 \pm 14.4
020	88.2 \pm 5.7	50.3 \pm 9.6	74.0 \pm 22.3	79.7 \pm 9.6
022	93.0 \pm 4.4	69.4 \pm 14.9	79.4 \pm 7.5	88.5 \pm 1.9
024	87.9 \pm 6.9	52.2 \pm 11.3	79.6 \pm 12.9	52.6 \pm 9.5
026	83.4 \pm 7.8	55.3 \pm 10.5	67.7 \pm 20.2	82.8 \pm 12.3
030	84.6	32.1	57.4	70.1
034	90.1 \pm 7.6	47 \pm 5.8	78.9 \pm 18.2	76.7 \pm 8.0
035	80.3 \pm 15.9	43.8 \pm 16.4	76.6 \pm 13.5	42.6 \pm 16.9
038	82.4 \pm 7.1	69.6 \pm 9.4	68.1 \pm 20.9	50.0 \pm 6.4
039	81.2 \pm 9.1	47.7 \pm 10.6	74.3 \pm 24.9	70.7 \pm 8.3
040	89.7 \pm 5.8	58.6 \pm 11.0	78.5 \pm 15.3	74.5 \pm 31.6
041	94.4 \pm 2.5	52.7 \pm 11.5	87.0 \pm 7.3	35.5 \pm 4.7
043	77.5 \pm 7.8	41.0 \pm 9.3	67.9 \pm 12.5	82.7 \pm 9.1
045	90.1 \pm 4.1	58.1 \pm 13.6	83.7 \pm 8.0	86.2 \pm 4.8
046	79.5 \pm 9.5	63.8 \pm 10.4	50.5 \pm 24.2	52.8 \pm 19.9
047	90.0 \pm 7.2	34.9 \pm 6.1	69.9 \pm 20.2	71.1 \pm 4.8
049	85.2 \pm 15.2	50.0 \pm 16.6	75.1 \pm 16.2	58.2 \pm 18.3
051	92.8 \pm 7.1	53.0 \pm 12.2	80.1 \pm 20.1	88.5 \pm 4.4
054	84.0 \pm 15.9	38.4 \pm 11.2	71.9 \pm 22.3	43.9 \pm 15.2
055	87.0 \pm 6.0	54.5 \pm 10.0	76.7 \pm 6.8	57.7 \pm 22.8
Average \pm SEM	84.3 \pm 1.1	48.7 \pm 1.4	70.8 \pm 1.9	68.0 \pm 1.9

Supplemental Table II. Surface phenotypes of injected APC/Gal

Case No.	Percentage of Cells \pm SD					
	HLA-DR ⁺ /CD3 ⁺	HLA-DR ⁺ /CD3 ⁻	CD11c ⁺ /CD3 ⁺	CD11c ⁺ /CD3 ⁻	CD86 ⁺ /CD3 ⁺	CD86 ⁺ /CD3 ⁻
002	66.3 \pm 19.9	38.0 \pm 13.5	34.8 \pm 24.6	63.4 \pm 17.4	n.d.	n.d.
004	78.9 \pm 7.6	89.9 \pm 5.2	26.6 \pm 5.8	74.5 \pm 4.2	n.d.	n.d.
006	87.5 \pm 3.4	78.3 \pm 19.8	20.6 \pm 8.4	69.1 \pm 10.7	71.8	31.4
009	79.0 \pm 13.5	73.2 \pm 6.4	27.3 \pm 15.0	62.7 \pm 22.6	67.7 \pm 21.3	33.6 \pm 12.1
013	87.8 \pm 9.1	83.5 \pm 10.8	44.9 \pm 19.0	83.2 \pm 6.3	68.0 \pm 28.4	42.5 \pm 24.4
014	88.4 \pm 10.7	97.6 \pm 0.6	27.7 \pm 10.0	74.4 \pm 7.4	78.8 \pm 21.8	78.9 \pm 15.4
016	70.1 \pm 22.1	82.5 \pm 13.1	20.2 \pm 11.0	74.1 \pm 8.3	56.0 \pm 32.1	48.8 \pm 32.3
018	74.0 \pm 12.8	64.3 \pm 21.6	39.0 \pm 18.9	78.2 \pm 9.5	59.0 \pm 21.5	15.5 \pm 5.4
020	87.0 \pm 8.5	92.8 \pm 1.1	45.5 \pm 18.4	73.0 \pm 7.6	72.7 \pm 23.1	73.3 \pm 20.8
022	94.7 \pm 4.0	81.7 \pm 7.9	68.4 \pm 14.3	74.4 \pm 16.3	82.1 \pm 6.8	61.5 \pm 13.3
024	81.1 \pm 12.1	95.1 \pm 2.3	30.4 \pm 17.5	73.9 \pm 11.9	75.2 \pm 15.4	83.4 \pm 10.8
026	82.2 \pm 10.3	85.8 \pm 3.5	49.8 \pm 12.4	80.5 \pm 8.0	69.9 \pm 18.8	49.3 \pm 23.1
030	86.0	81.6	17.6	68.5	67.4	27.6
034	89.0 \pm 9.0	92.9 \pm 4.8	37.6 \pm 7.7	76.2 \pm 8.8	77.3 \pm 20.5	74.4 \pm 16.8
035	70.0 \pm 30.9	89.7 \pm 8.1	28.9 \pm 18.5	55.5 \pm 13.9	65.3 \pm 28.9	85.1 \pm 11.1
038	79.0 \pm 10.2	86.8 \pm 5.5	53.8 \pm 17.8	85.1 \pm 2.7	65.3 \pm 23.1	68.7 \pm 19.9
039	78.5 \pm 14.9	86.4 \pm 6.6	37.1 \pm 12.1	72.0 \pm 14.1	72.1 \pm 28.1	73.6 \pm 21.3
040	89.2 \pm 10.2	71.7 \pm 20.1	51.7 \pm 17.4	77.1 \pm 11.3	81.4 \pm 15.5	44.9 \pm 21.3
041	88.7 \pm 5.9	98.0 \pm 1.0	19.4 \pm 10.8	70.6 \pm 10.9	81.9 \pm 12.0	89.7 \pm 4.3
043	78.1 \pm 8.2	74.6 \pm 17.9	35.1 \pm 12.2	77.2 \pm 12.7	69.8 \pm 11.0	53.4 \pm 26.3
045	89.7 \pm 4.5	93.8 \pm 2.6	55.2 \pm 14.1	72.4 \pm 9.8	84.1 \pm 7.7	78.8 \pm 10.6
046	74.6 \pm 17.1	81.4 \pm 2.2	42.4 \pm 24.4	81.5 \pm 5.4	54.8 \pm 27.1	40.5 \pm 15.4
047	87.9 \pm 10.0	96.0 \pm 1.8	16.5 \pm 3.3	81.0 \pm 5.5	73.6 \pm 21.4	57.6 \pm 23.5
049	74.6 \pm 29.5	94.0 \pm 8.5	34.7 \pm 19.8	70.9 \pm 16.6	66.7 \pm 30.0	78.1 \pm 7.9
051	92.7 \pm 7.7	94.5 \pm 3.8	48.9 \pm 14.2	82.7 \pm 7.9	80.6 \pm 20.9	69.1 \pm 17.0
054	72.6 \pm 23.3	97.3 \pm 1.4	24.2 \pm 4.9	54.0 \pm 27.4	60.9 \pm 22.9	80.0 \pm 21.5
055	79.6 \pm 10.3	95.3 \pm 4.3	34.5 \pm 5.9	76.3 \pm 9.4	67.2 \pm 14.2	84.9 \pm 6.9
Average \pm SEM	81.7 \pm 1.4	85.1 \pm 1.5	36.4 \pm 1.8	73.5 \pm 1.3	70.8 \pm 6.8	62.3 \pm 8.4

n.d., not determined

Supplemental Table III. NKT and NK cell responses of non-treated patients at 12-month

Case No.	Base line NKT (%)	NKT (12m) [#]	Base line NK (%)	NK (12m) [#]	ELISPOT (12m) [§]	NK GzmB (12m)
052	N.D.	0.09	N.D.	14	97	138.5
032	0.081	0.14	2.9	3.67	86.0	1.0
048	0.000345	0.000211	2.4	2.66	72.0	2.3
057	0.01	0.013	8.4	5.44	42.0	1128.5
044	0.015	0.016	8.5	5.96	25.5	2.7
012	0.00589	0.00212	6.7	6.01	23.0	6.8
019	0.00482	0.00321	9.1	15.4	16.5	1.4
010	0.011	0.00412	8.3	10.3	12.5	0.5
042	0.018	0.022	14.5	16.4	10.0	0.2
015	0.00596	0.00455	9.2	13.1	8.0	2.9
031	0.00815	0.024	9.7	11.6	7.5	0.0
029	0.00407	0.00643	17.1	22.1	7.0	0.0
001	N.D.	0.00584	n.d.	16.1	6.5	6.4
007	0.01	0.00258	15.9	11.7	3.5	1.5
005	0.009	0.00951	4.8	8.54	3.0	1.0
008	0.013	0.012	7.1	4.54	2.5	0.9
011	0.00456	0.00332	15.8	16	2.0	1.8
023	0.00149	0.00679	31.2	11.3	1.5	2.8
033	0.11	0.00543	4.7	4.36	1.5	0.0
053	0.00756	0.00519	8.9	7.15	1.5	457.6
027	0.000949	0.027	9.6	8.31	1.0	0.0
036	0.00257	0.00396	9.9	7.49	1.0	0.7
017	0.00209	0.00397	18.0	24.3	0.5	0.8
058	0.00142	0.00421	8.4	10.9	0.0	152.7
021	0.00912	n.d.	5.9	n.d.	n.d.	n.d.
025	0.00403	n.d.	5.1	n.d.	n.d.	n.d.
028	0.011	n.d.	11.9	n.d.	n.d.	n.d.
037	0.0062	n.d.	6.6	n.d.	n.d.	n.d.
056	0.01	n.d.	48.9	n.d.	n.d.	n.d.

[#] Fold increase of 12-month to base line in NKT cell or NK cell number during treatment.

[§] ELISPOT fold increase of 12-month to base line in IFN- γ -producing cell number. n.d., not determine.

The line in the table shows the boundary of 2.0 times increase rate of ELISPOT on 12-month.