

Combined MSC/CD and α -GalCer therapy in colon cancer

Supplementary Table 1. Change in WBCs after MSC/CD with 5-FU and α -GalCer treatment

	Normal Mice	PBS	α -GalCer	MSC/CD	MSC/CD+ α -GalCer
Lymphocytes	58.93 \pm 5.71	23.87 \pm 20.75*	30.27 \pm 6.73**	36.97 \pm 2.80**	53.17 \pm 8.54
Neutrophils	27.63 \pm 2.34	79.10 \pm 42.03	47.73 \pm 3.59**	52.50 \pm 0.75***	20.70 \pm 1.04**
Eosinophils	0.10 \pm 0.10	0.07 \pm 0.06	0.07 \pm 0.12	0.10 \pm 0.10	0.13 \pm 0.23
Basophils	5.40 \pm 2.69	11.37 \pm 16.74	4.83 \pm 5.12	1.40 \pm 0.75	4.20 \pm 2.79
Monocytes	2.77 \pm 1.08	3.70 \pm 3.27	13.37 \pm 3.76**	6.07 \pm 0.40**	15.53 \pm 3.04**

Blood was collected on Day 12. WBC analysis was performed using ADVIA 2021i. Results are expressed as Mean \pm S.D. Statistical analysis was performed by t-test based on the normal mouse group. *P*-value: * <0.05, ** <0.01, *** <0.001.

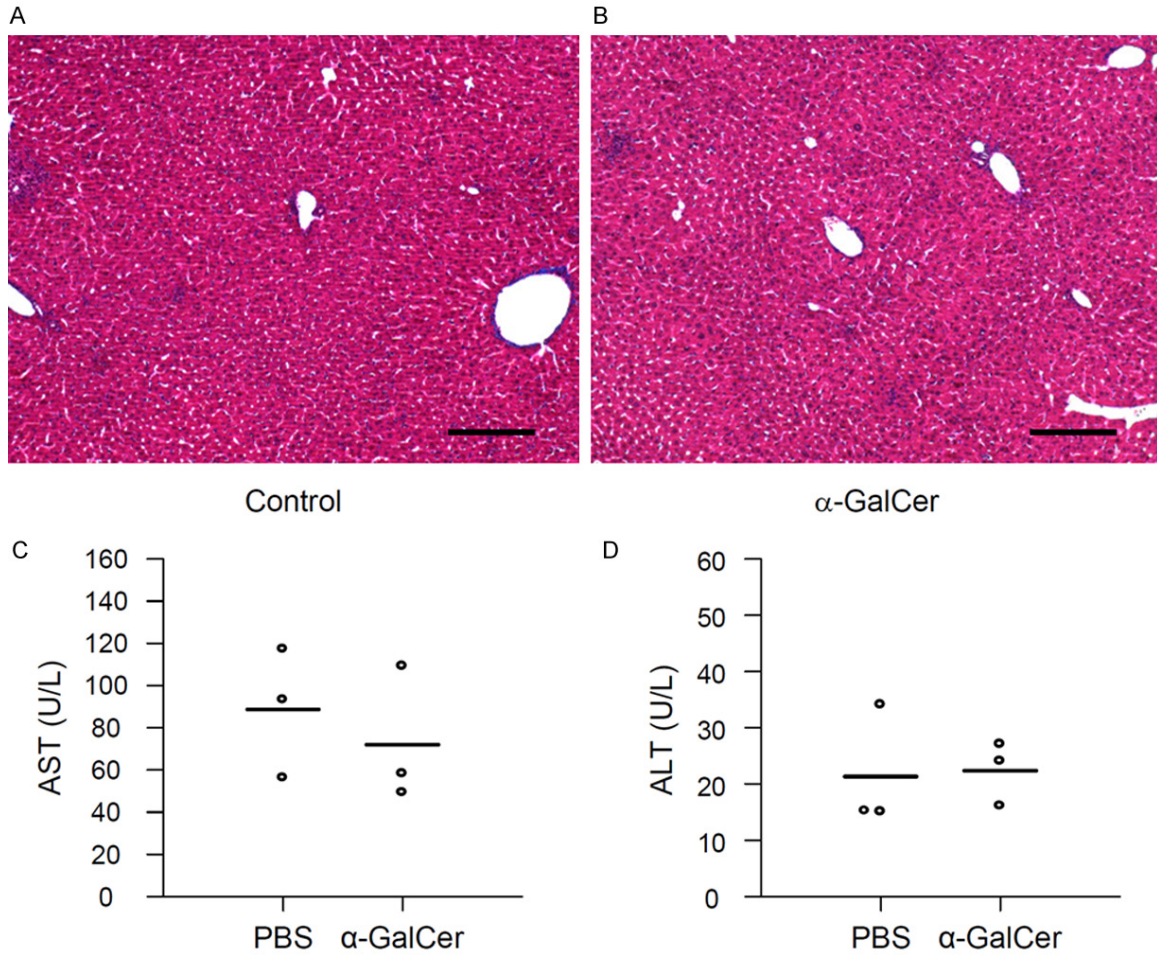
Supplementary Table 2. Immune cell markers

Marker	Cell type
CD3 ⁺ CD4 ⁺	T _H cells
CD3 ⁺ CD8 ⁺	T _C cells
CD3 ⁺ NK1.1 ⁺	NK cells
CD3 ⁺ NK1.1 ⁺	NKT cells
CD3 ⁺ CD4 ⁺ CD25 ⁺	Tregs
CD11b ⁺ GR-1 ⁺	MDSCs
CD3 ⁺ CD19 ⁺	B cells
CD3 ⁺ CD11c ⁺ CD64 ⁺	Macrophages
CD3 ⁺ CD11c ⁺ MHCII ⁺	Dendritic cells

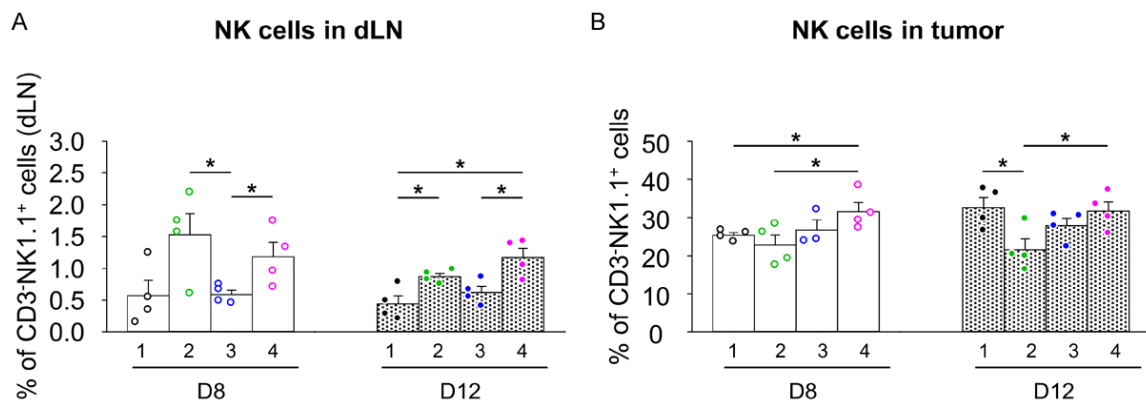
Supplementary Table 3. Antibodies used in experiments

	Clone	Conjugate/ Excitation	Tested dilution (μ g/test)	Supplier	Cat No.
anti-CD3	17A2	FITC	0.25	invitrogen	11-0032-82
anti-CD4	GK1.5	PE-Cyanine7	0.25	invitrogen	25-0041-82
anti-CD8a	53-6.7	APC-eFluor 780	0.5	invitrogen	47-0081-82
anti-CD19	eBio1D3(1D3)	PE-Cyanine7	0.25	invitrogen	25-0193-82
anti-CD25	PC61.5	APC	0.125	invitrogen	17-0251-82
anti-NK1.1	PK136	PE	0.25	invitrogen	12-5941-82
anti-Ly-6G/Ly-6C	RB6-8C5	PerCPCyanine5.5	0.06	invitrogen	45-5931-80
anti-CD11b	M1/70	AlexaFluor 700	0.25	invitrogen	56-0112-82
anti-CD11c	N418	APC-eFluor 780	0.5	invitrogen	47-0114-82
anti-CD64	X54-5/7.1	PE	0.5	invitrogen	12-0641-82
anti-MHCII (I-A/I-E)	M5/114.15.2	APC	0.03	invitrogen	17-5321-82
anti-CD16/CD32	93	-	0.5	invitrogen	14-0161-86
SYTOX AADvanced Dead cell stain kit		488 nm excitation	-	invitrogen	S10349
Live/Dead Fixable Red Dead cell stain kit	-	488 nm excitation	-	invitrogen	L34972

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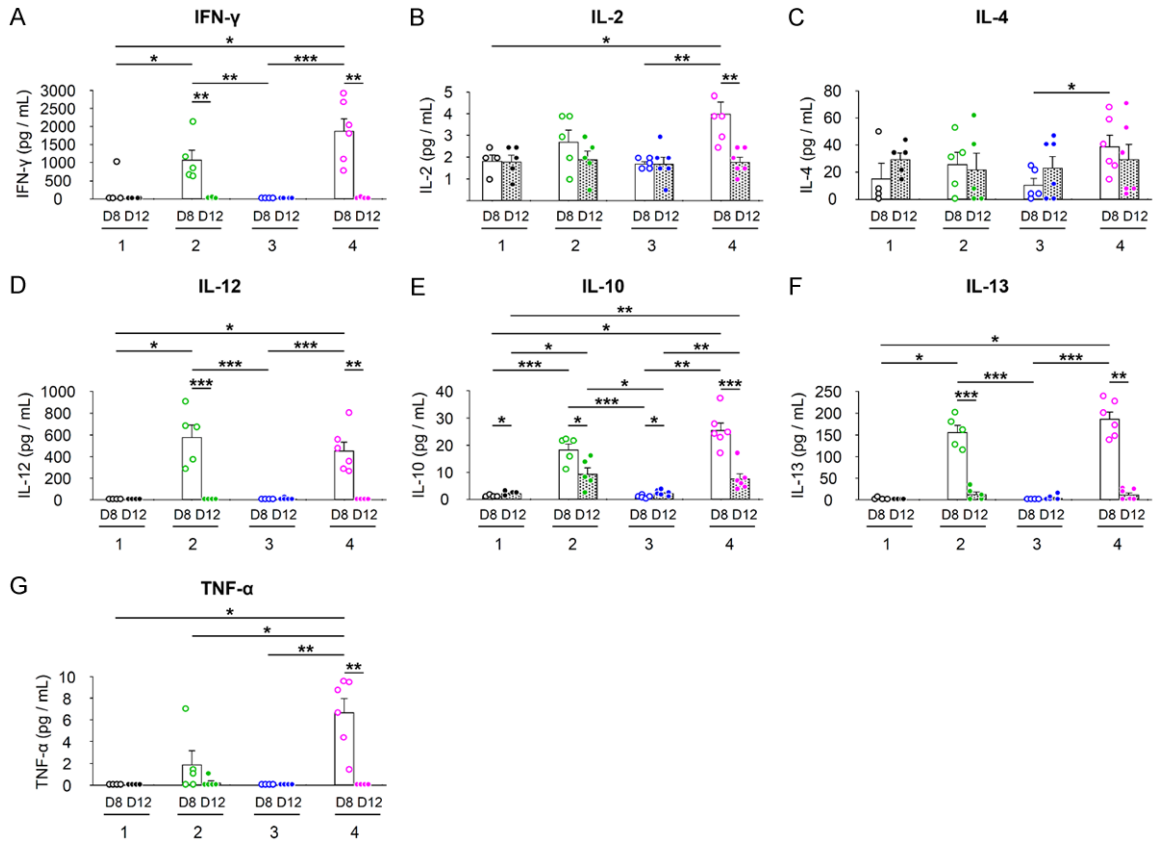


Supplementary Figure 1. Hepatotoxicity following intratumoral injection of α -GalCer. Toxicity assessment after α -GalCer administration: CT26 (2×10^6 cells, 20% Matrigel, $n = 3$) was inoculated into the left flank of BALB/c mice. Following tumor formation, 120 μ g/kg α -GalCer was injected, and analysis was performed 3 days later. A, B. Livers were removed and subjected to Masson's trichrome staining. C, D. AST and ALT levels were measured in serum using a Cobas 8000 c702 instrument from Roche (Tokyo, Japan).



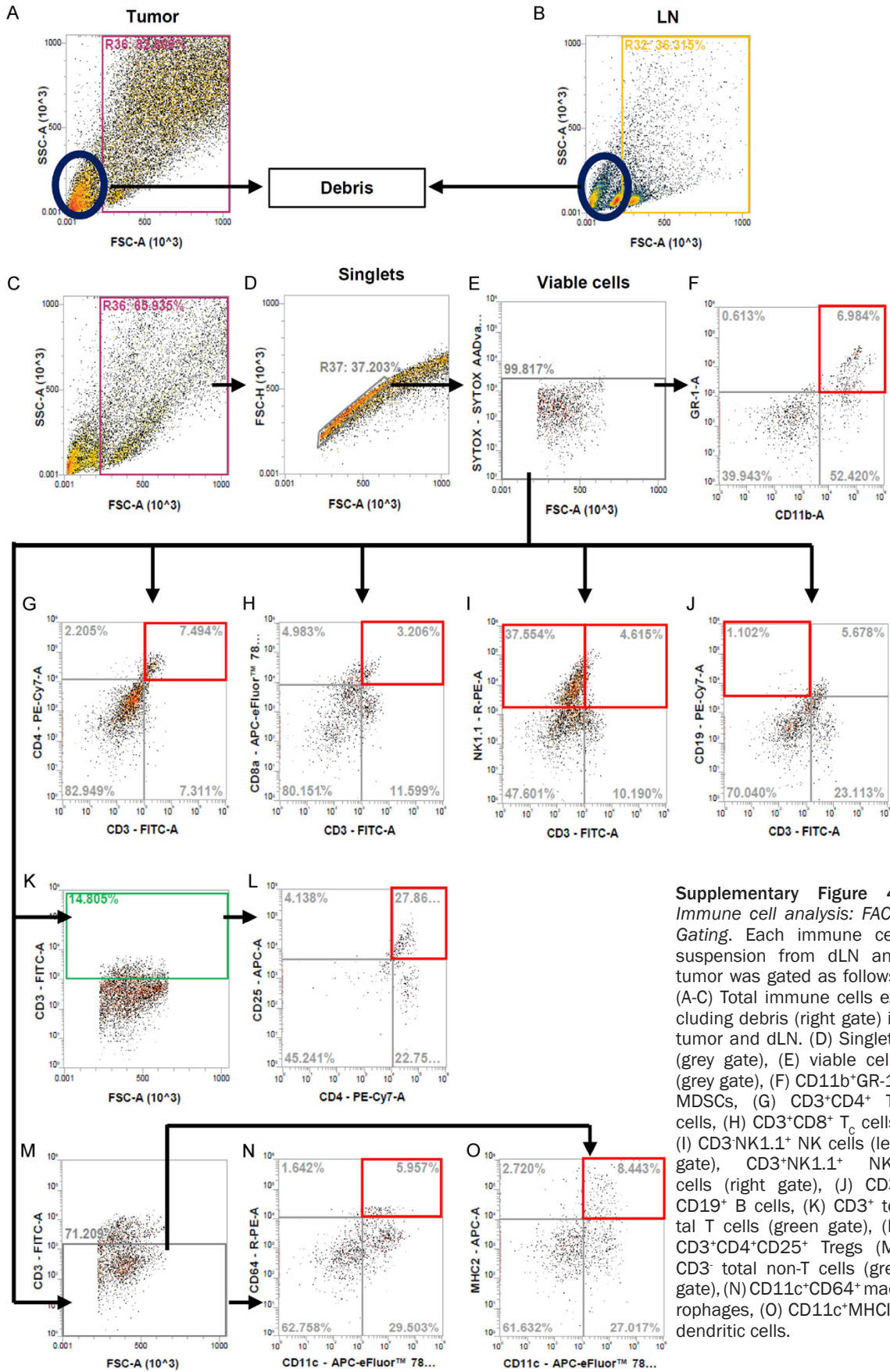
Supplementary Figure 2. Effect on NK cells after combined treatment with MSC/CD containing 5-Fc and α -GalCer in CT26 colorectal cancer model. Experiments were conducted according to Figure 3A. NK cells in each tissue were analyzed by FACS by gating for CD3⁺NK1.1⁺. (A) NK cell ratio in dLN, (B) in tumors. $n = 3-4$. * $P < 0.05$.

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Supplementary Figure 3. Cytokine levels in serum after combined treatment of MSC/CD with 5-FU and α -GalCer in CT26 colon cancer model. Experiments were conducted according to **Figure 3A**; blood was collected, serum was separated, and cytokine levels were measured. (A) IFN- γ , (B) IL-2, (C) IL-4, (D) IL-12, (E) IL-10, (F) IL-13, (G) TNF- α . * P <0.05, ** P <0.01, *** P <0.001.

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Supplementary Figure 4. Immune cell analysis: FACS Gating. Each immune cell suspension from dLN and tumor was gated as follows. (A-C) Total immune cells excluding debris (right gate) in tumor and dLN. (D) Singlets (grey gate), (E) viable cells (grey gate), (F) CD11b⁺GR-1⁺ MDSCs, (G) CD3⁺CD4⁺ T_H cells, (H) CD3⁺CD8⁺ T_C cells, (I) CD3⁺NK1.1⁺ NK cells (left gate), CD3⁺NK1.1⁺ NKT cells (right gate), (J) CD3⁺CD19⁺ B cells, (K) CD3⁺ total T cells (green gate), (L) CD3⁺CD4⁺CD25⁺ Tregs (M) CD3⁺ total non-T cells (grey gate), (N) CD11c⁺CD64⁺ macrophages, (O) CD11c⁺MHCII⁺ dendritic cells.