

Dominant-negative *IKZF1* mutations cause a T, B, and myeloid cell combined immunodeficiency

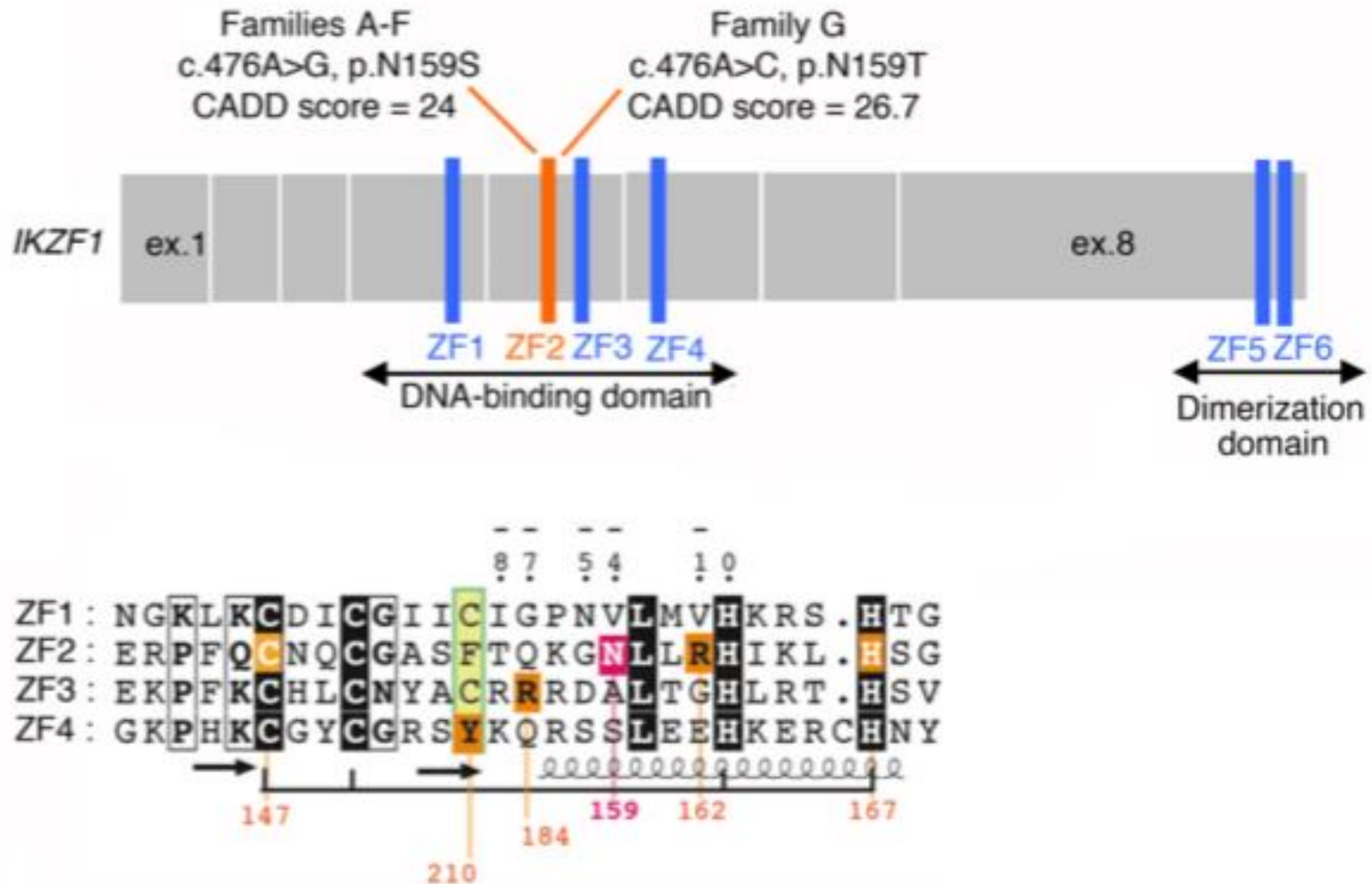
Aug. 2. 2018 Journal club

別役 / 真部

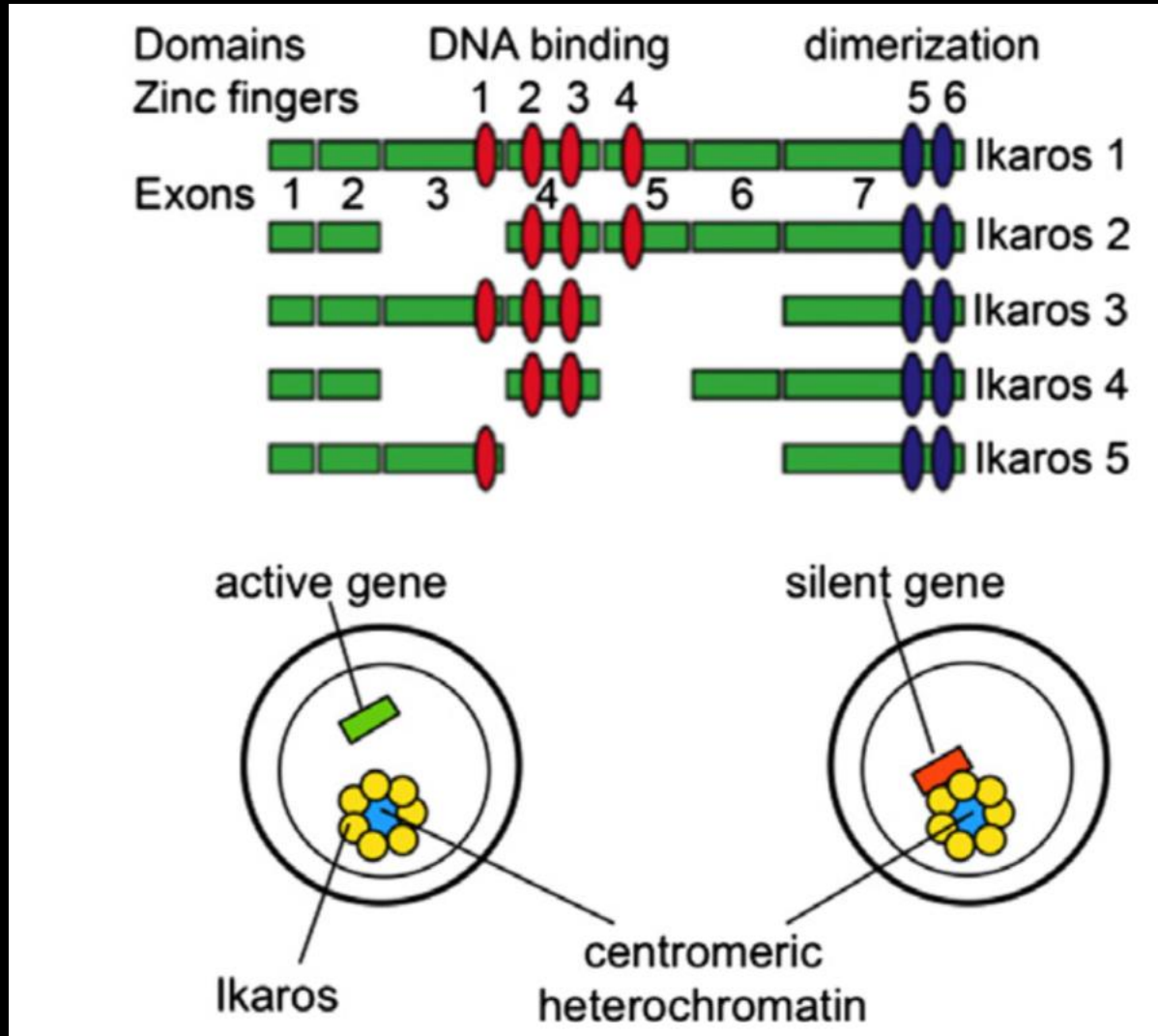
Ikarosの作用

- リンパ球分化の促進
- 細胞増殖の抑制
- Th2応答
- 濾胞B細胞活性抑制
- 骨髄系細胞の分化

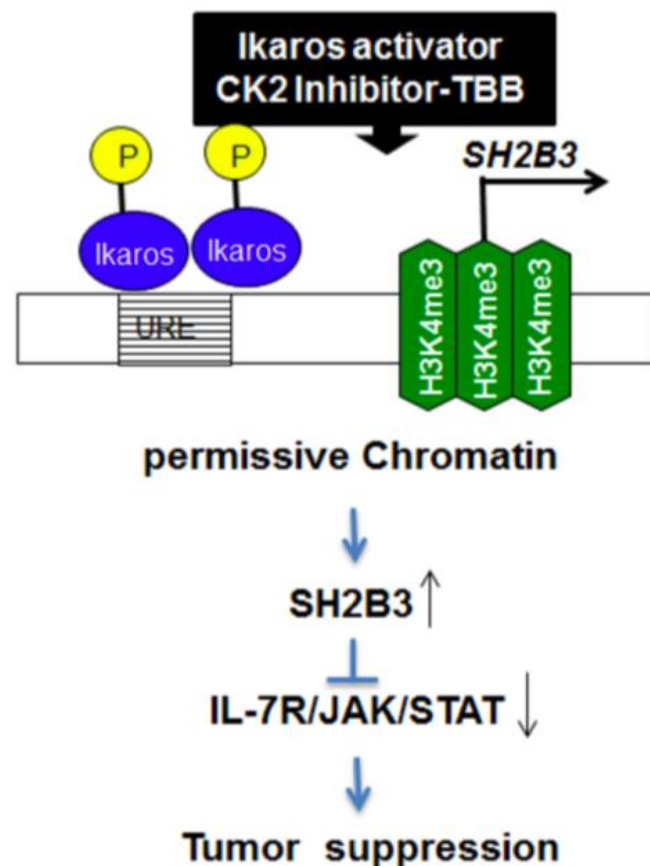
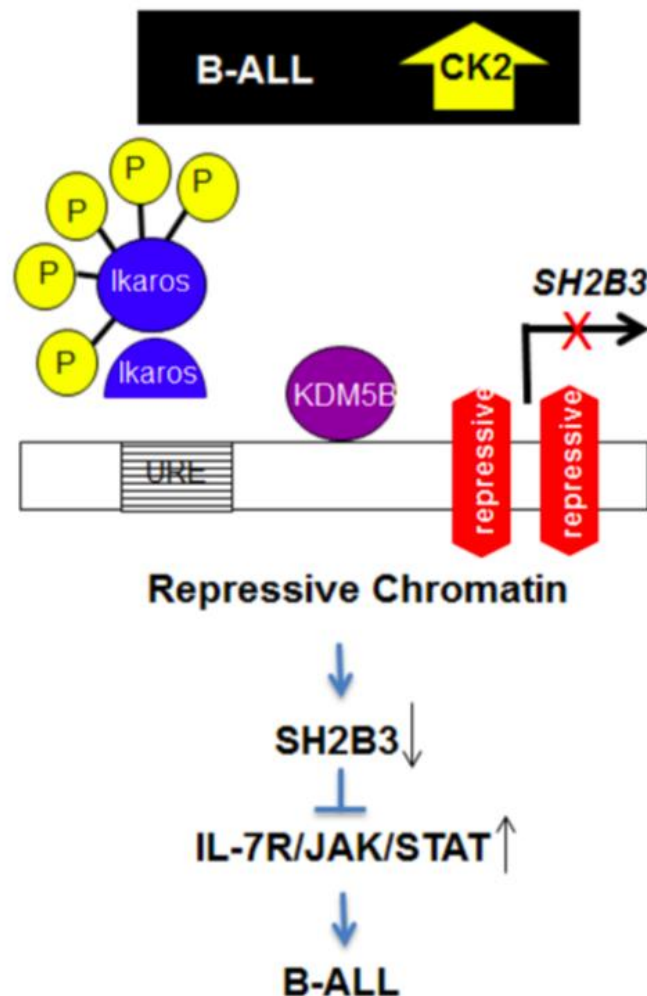
IKAROSはZinc finger motifを6つ持ち、DimerとしてDNAに結合する転写因子である



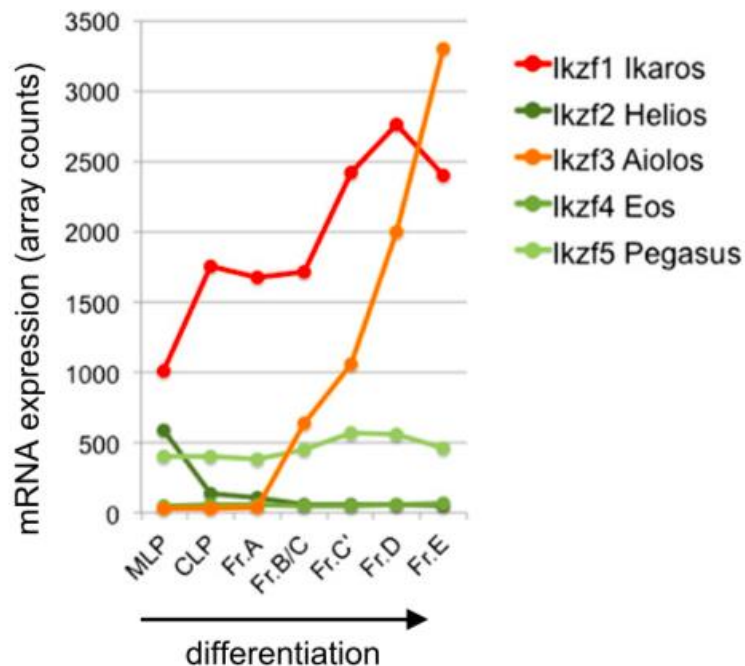
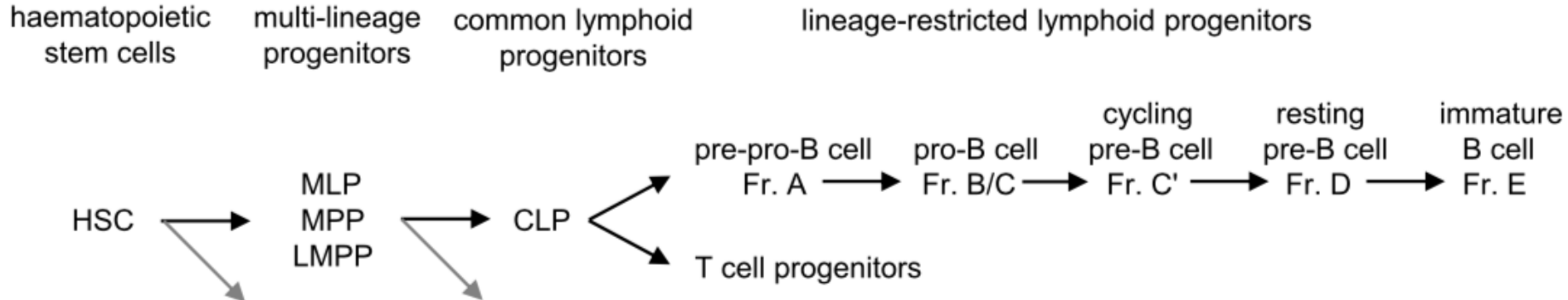
Ikarosは5つ以上のスプライシングアイソフォームを持つ



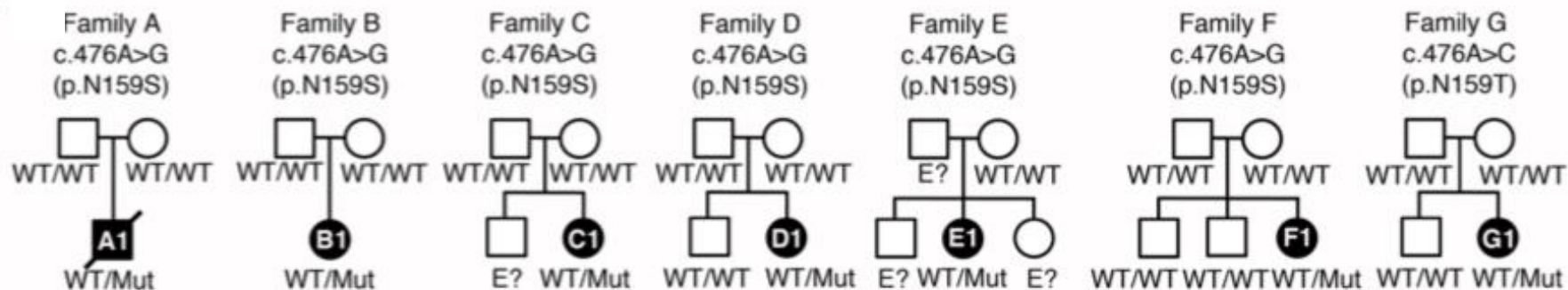
Ikarosのリン酸化がB-ALLに関与する



Ikaros familyはB cell分化の過程で転写が変化する



Dominant-negative変異(DN)は孤発的に生じる



DN-IKZF1は易感染性を呈する

Patient	Mutation	Origin	Age at first symptoms	Pneumocystis pneumonia (age)	Infections			Live vaccines, complications	Other manifestations	Treatment (prior to HSCT)	Outcome (age [yr])
					Viral	Bacterial	Others				
A1	p.N159S	European descent (Europe)	2 mo	Yes (17 mo)	Severe RSV bronchiolitis	<i>Klebsiella</i> sp. lung infection	Cryptosporidial cholangitis	No		Azithromycin, cotrimoxazole, nitazoxanide, fluconazole, IVIG	HSCT (2), died (2)
B1	p.N159S	Asian (Japan)	2 mo	Yes (2, 3, and 17 yr)	No	Lung abscess, recurrent sinopulmonary infections, bronchiectasis, pulmonary <i>Mycobacterium avium</i> complex infection	Pulmonary aspergillosis	BCG and MMR, uneventful	No	Cotrimoxazole, IVIG	Alive (17)
C1	p.N159S	European descent (US)	10 mo	Yes (10 mo)	Influenza type A	Recurrent otitis media and pneumonia	No	No	No	Cotrimoxazole, SCIG	Alive (4)
D1	p.N159S	European descent (US)	1.5 yr	Yes (2 yr)	Severe RSV bronchiolitis, adenoviral pneumonia requiring cidofovir	Recurrent otitis media and pneumonia	No	No	Recurrent oral ulcers	Cotrimoxazole, IVIG	HSCT (3), alive and symptom-free (8)
E1	p.N159S	European descent (US)	1 yr	Yes (1 yr)	Recurrent HSV (oral, genital), uneventful chickenpox	<i>Streptococcus pneumoniae</i> meningitis, <i>Pseudomonas</i> spp. pneumonia, skin abscesses	Recurrent oral candidiasis, <i>Candida parapsilosis</i> fungemia	No	DVT/PE, pancreatitis	Clindamycin, cotrimoxazole, aciclovir, fluconazole, SCIG, G-CSF	Alive (28)
F1	p.N159S	Asian (Japan)	9 mo	Yes (20 mo)	No	Severe pneumonia, otitis media, chronic sinusitis	Recurrent oral candidiasis	BCG and oral polio, uneventful	T-ALL	Cotrimoxazole, G-CSF, IVIG	HSCT (14), alive and symptom-free (20)
G1	p.N159T	European descent (Europe)	6 mo	Yes (6 mo, 5 yr)	Molluscum contagiosum and warts, uneventful chickenpox	No	Localized onychomycosis	BCG, uneventful	Recurrent oral ulcers	Cotrimoxazole, IVIG	Alive and symptom-free (12)

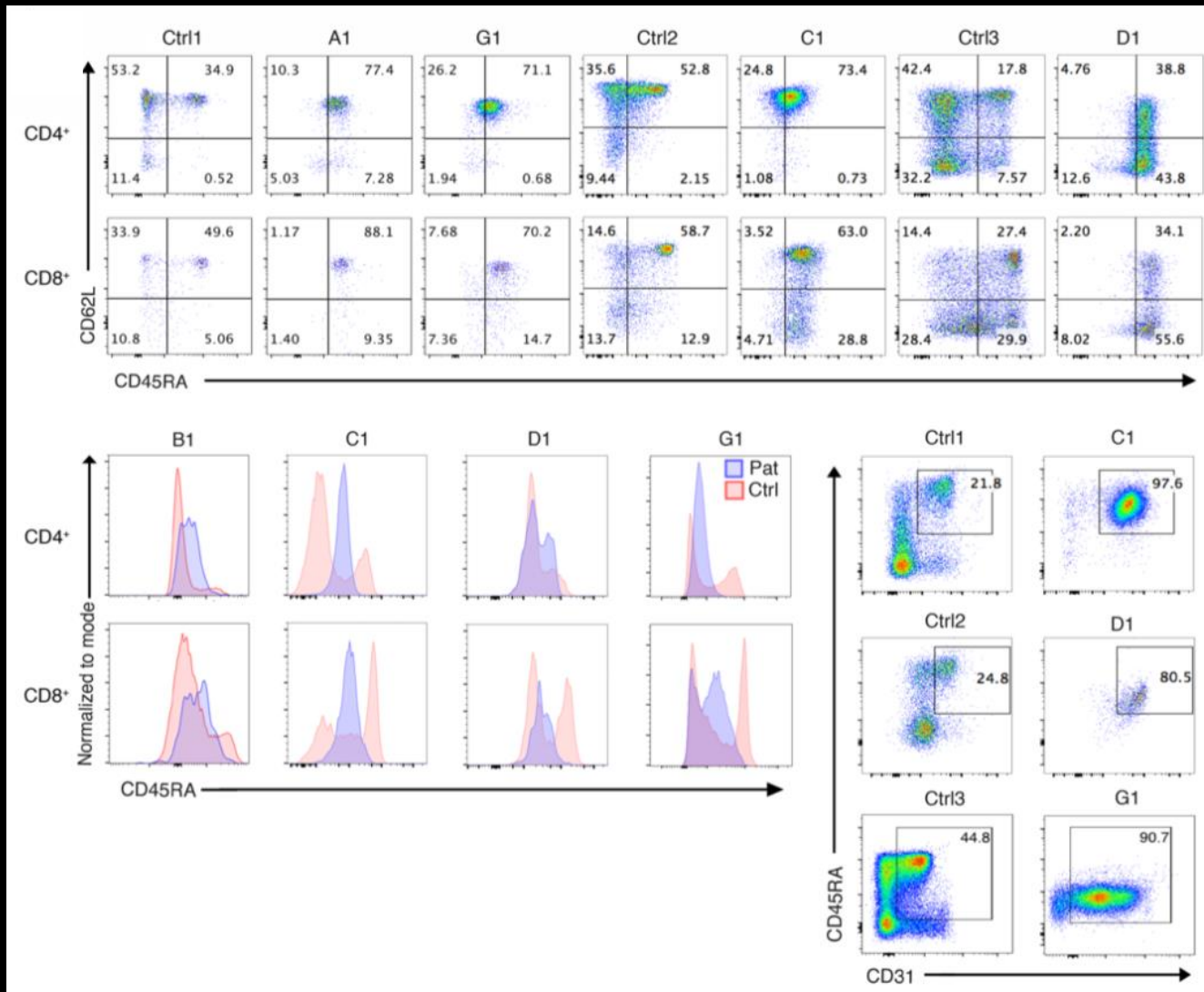
IVIG, intravenous immunoglobulin; SCIG, subcutaneous immunoglobulin; G-CSF, granulocyte-CSF; DVT, deep venous thrombosis; PE, pulmonary embolism.

DN-IKZF1は多種の血球減少させる

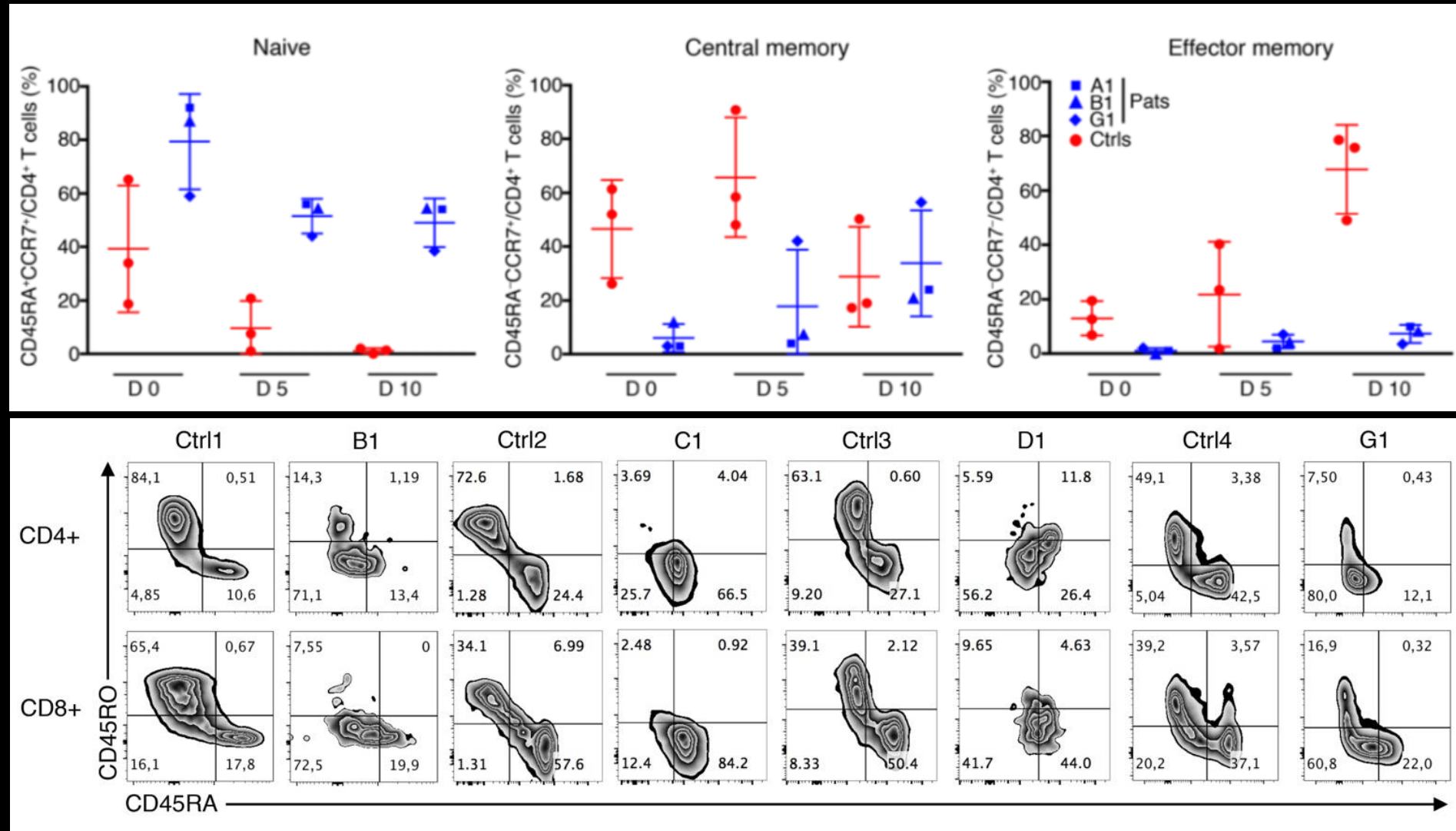
Patients	A1 (16 mo)	B1 (17 yr)	C1 (3 yr)	D1 (16 mo)	E1 (26 yr)	F1 (9 mo/4 yr)	G1 (7 yr/13 yr)
Leukocytes (cells/mm ³)	4,600	7,700	7,820	11,500	1,030	6,100/2,600	6,876
Hemoglobin, mean corpuscular volume (g/dl)	12.6-78	11.2-91	12.7-93.2	12.4-87.6	10.0-86.1	10.5-80	12.3-77
Neutrophils (cells/mm ³)	1,900	704	720	670-5,080	200/900	5,650/494	1,300/2,700
Eosinophils (cells/mm ³)	0	0	0	0	0	ND	0
Platelets (cells/mm ³)	<i>619,000</i>	240,000	299,000	386,000	155,000	180,000	270,000
Monocytes (cells/mm ³)	260	360	640	280	40/80	150/170	376
Lymphocytes (cells/mm ³)	2,300	6,550	6,530	6,120	640/810	260/2,054	5,200
T cells							
CD3 ⁺ (cells/mm ³)	2,208	<i>5,700</i>	6,354	<i>5,072</i>	604	192/1,970	<i>4628</i>
CD4 ⁺ (cells/mm ³)	989	<i>2,587</i>	3,232	<i>3,383</i>	284	140/430	<i>2,823</i>
CD8 ⁺ (cells/mm ³)	874	<i>2,692</i>	2,579	1,218	209	63/1,355	1,040
CD4/CD8 ratio	1.1	0.96	1.25	2.8	1.36	2.2/0.3	2.7
TCR-γδ/CD3 ⁺ (%)	6.9	2.4	0.9	6.2	3.8	0.2	2.12
HLA-DR ⁺ /CD3 ⁺ (%)	8.2	12.4	34	3.4	21	12.9/ND	16.3
CD3 ⁺ /CD4 ⁺ CD8 ⁺ (DNT) (%)	ND	6.5	ND	ND	ND	ND	4.3
CCR7 ⁺ (or CD62L ⁺) CD45RA ⁺ /CD4 ⁺ (naïve) (%)	92	<i>814</i>	93	98	9.5	ND	97
CD31 ⁺ CD45RA ⁺ /CD4 ⁺ (recent naïve thymic emigrant) (%)	90	<i>97.9</i>	98	>90	2.1	ND	91
CD45RO ⁺ /CD4 ⁺ (memory) (%)	8	17.9	ND	2	ND	ND	3
CCR7 ⁺ (or CD62L ⁺) CD45RA ⁺ /CD8 ⁺ (naïve) (%)	85	<i>92.3</i>	79	55.6	12	ND	55
CCR7 ⁺ (or CD62L ⁺) CD45RA ⁺ /CD8 ⁺ (central memory) (%)	1	ND	1	2.2	18	ND	5
CCR7 ⁺ (or CD62L ⁺) CD45RA ⁺ /CD8 ⁺ (effector memory) (%)	3	ND	1	8.2	38	ND	19
CCR7 ⁺ (or CD62L ⁺) CD45RA ⁺ /CD8 ⁺ (exhausted effector memory, EMRA) (%)	11	ND	19	34.1	32	ND	21
FoxP3 ⁺ CD25 ⁺ /CD4 ⁺ (regulatory) (%)	ND	0.5	0.5	1.3	ND	ND	2.6
Vα2 ⁺ CD161 ⁺ /CD3 ⁺ (MAIT) (%)	ND	ND	ND	ND	ND	ND	2.1
Vα24 ⁺ Vβ11 ⁺ CD161 ⁺ /CD3 ⁺ (iNKT) (%)	ND	0	0	0.001	ND	ND	0.071
T cell proliferation (cpm/10 ³)							
PHA (6.25 mg/ml)	>50	>50	>50	>50	>50	>50	74
OKT3 (50 ng/ml)	<30	<30	<30	ND	ND	ND	11
Tetanus toxoid/candidin	0/0	<10/<10	<10/<10	<10/<10	<10/<10	ND	2.5/0.5
NK cells							
CD16 ⁺ CD56 ⁺ (cells/mm ³)	230	314	163	146	30	ND/53	208
CD16 ⁺ CD56 ⁺ (%)	1	4.8	2.5	3	4.7	ND/2.6	4
B cells							
CD19 ⁺ (cells/mm ³)	0	32	7	0	7	15/26	468/74
CD19 ⁺ (%)	0	0.5	0.1	0	1.1	6/1.3	9/3.1
CD21 ⁺ CD27 ⁺ /CD19 ⁺ (memory) (%)	ND	0.7	0	ND	0.3	ND	2/0.1
DCs							
pDCs (% total PBMCs)	ND	0.02	ND	ND	ND	ND	0.07
mDC (% total PBMCs)	ND	0.09	ND	ND	ND	ND	0.017
Immunoglobulin levels (mg/dl)							
IgG	35	18	1,240 ^a	65	1,786 ^a	21	329
IgM	0	13	<4	<3	19	9	0
IgA	0	5	<6	<5	<5	<5	0
IgE (U/ml)	ND	30	<5	<1	1.4	0	5

^aBold indicates below-normal values, and italics above-normal values, according to age-matched data (44, 45). ^bOn immunoglobulin replacement therapy. ND, not determined.

DN-IKZF1では幼若なnaïve T cell
(CD45RA⁺CD62⁺CD31⁺)が多く見られる

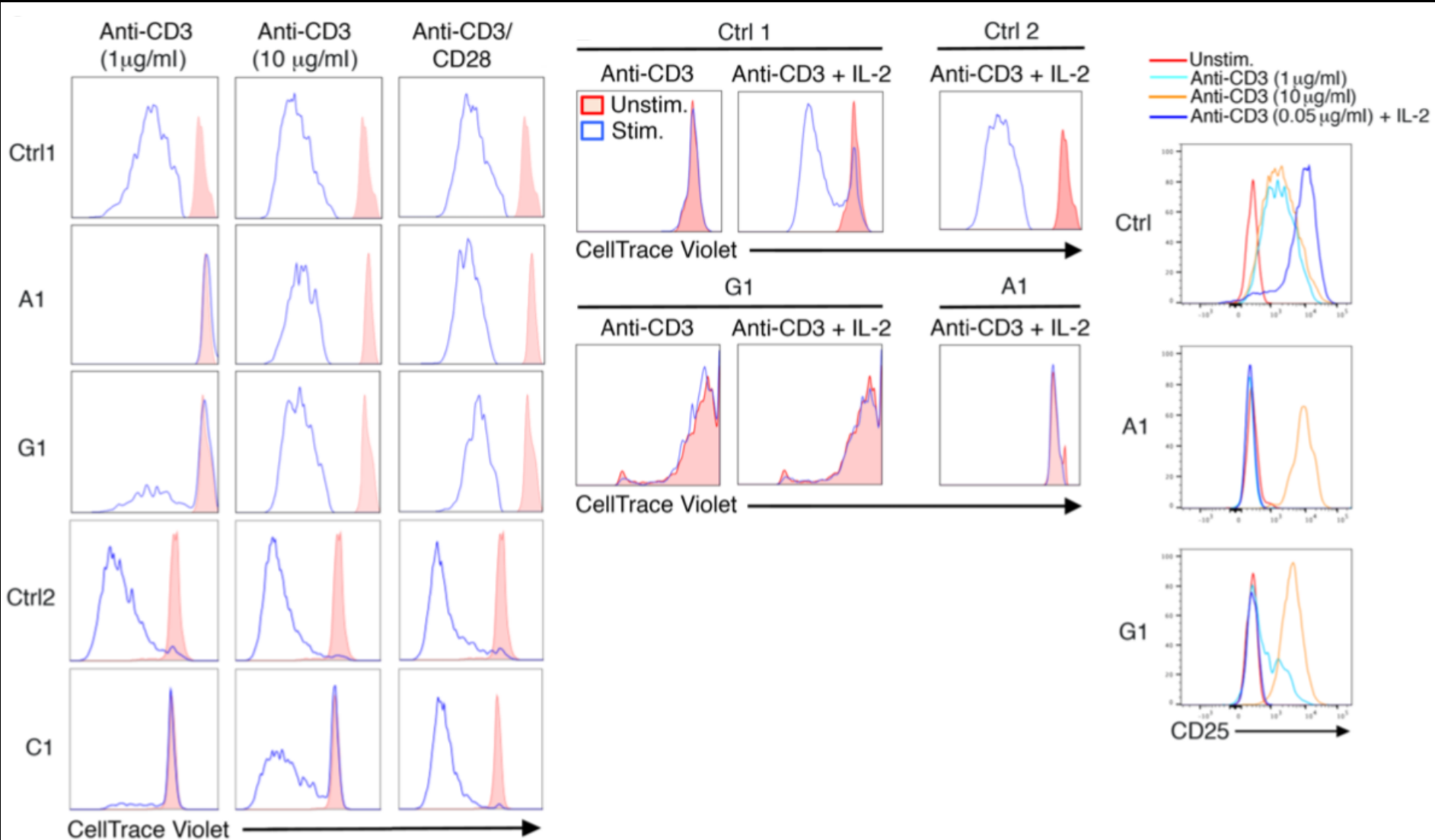


DN-IKZF1はMemory cell が減少し、Naïve Tが増加する

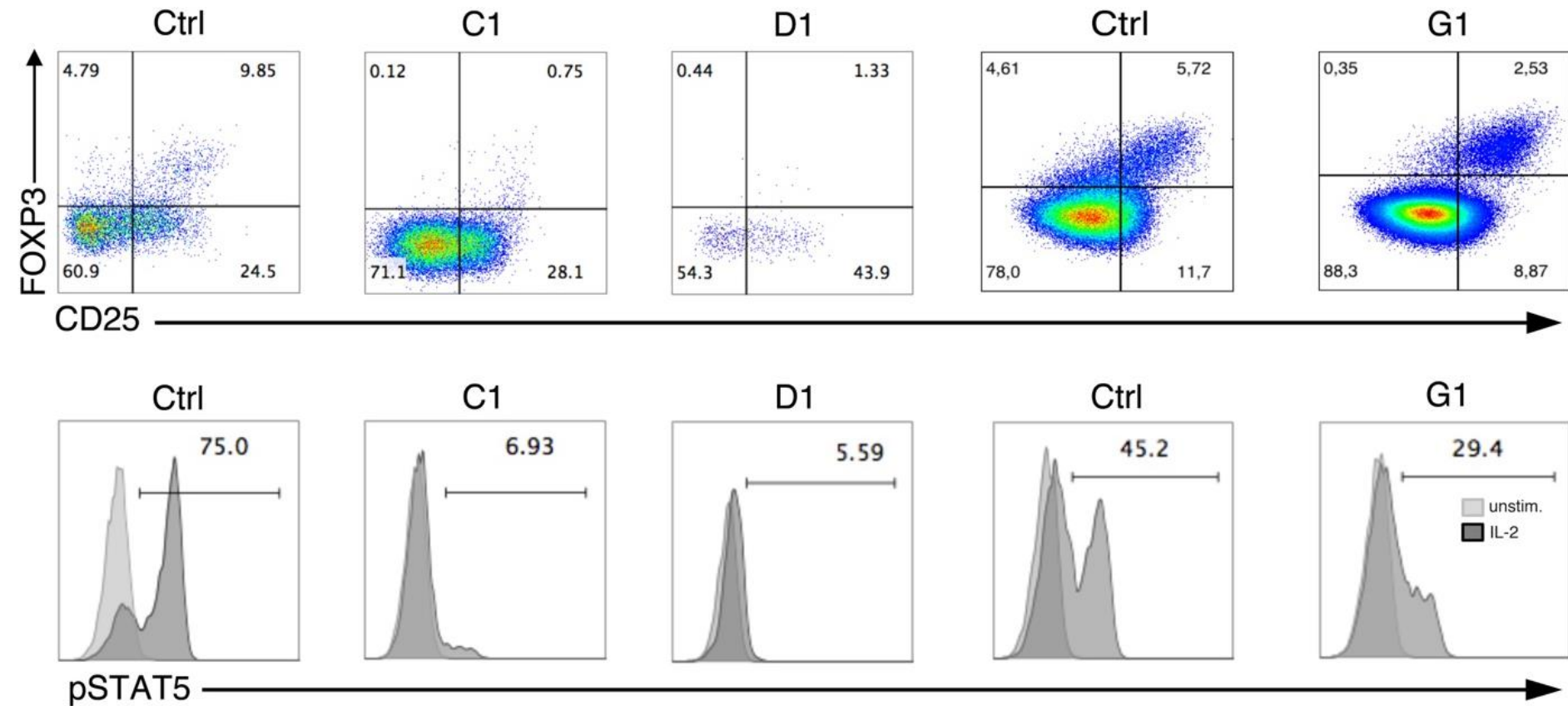


Naïve T cell: CD45RA⁺CD45RO⁻
Memory cell: CD45RA⁻CD45RO⁺

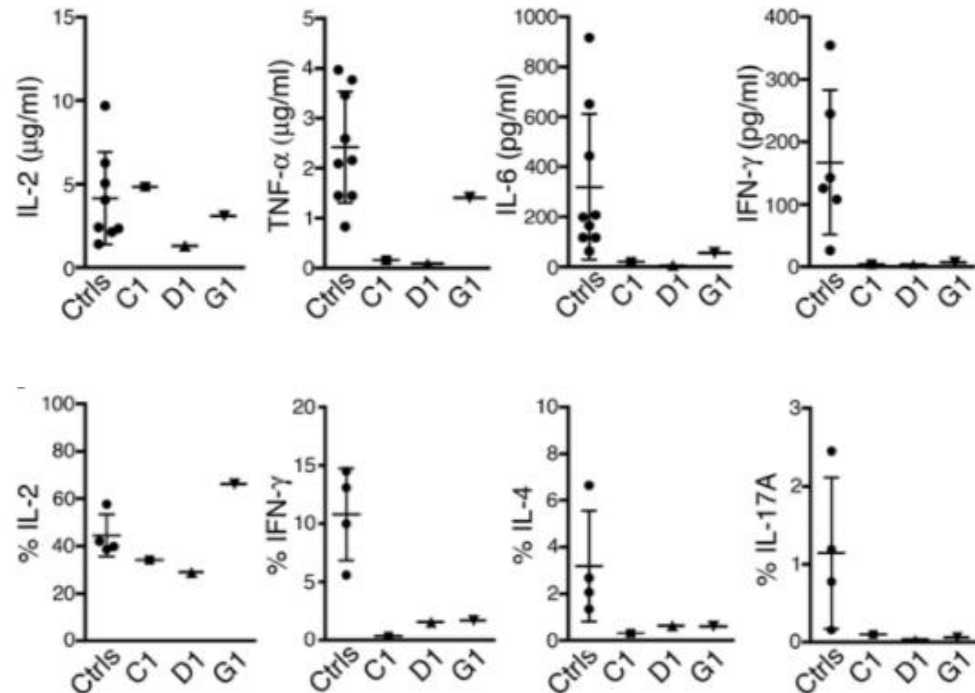
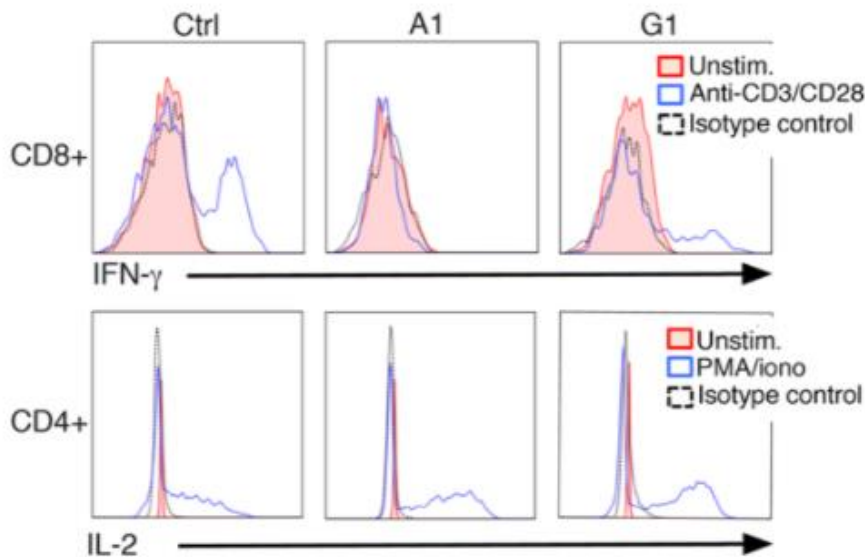
DN-IKZFではCD3 stimulationに対して鈍感になる



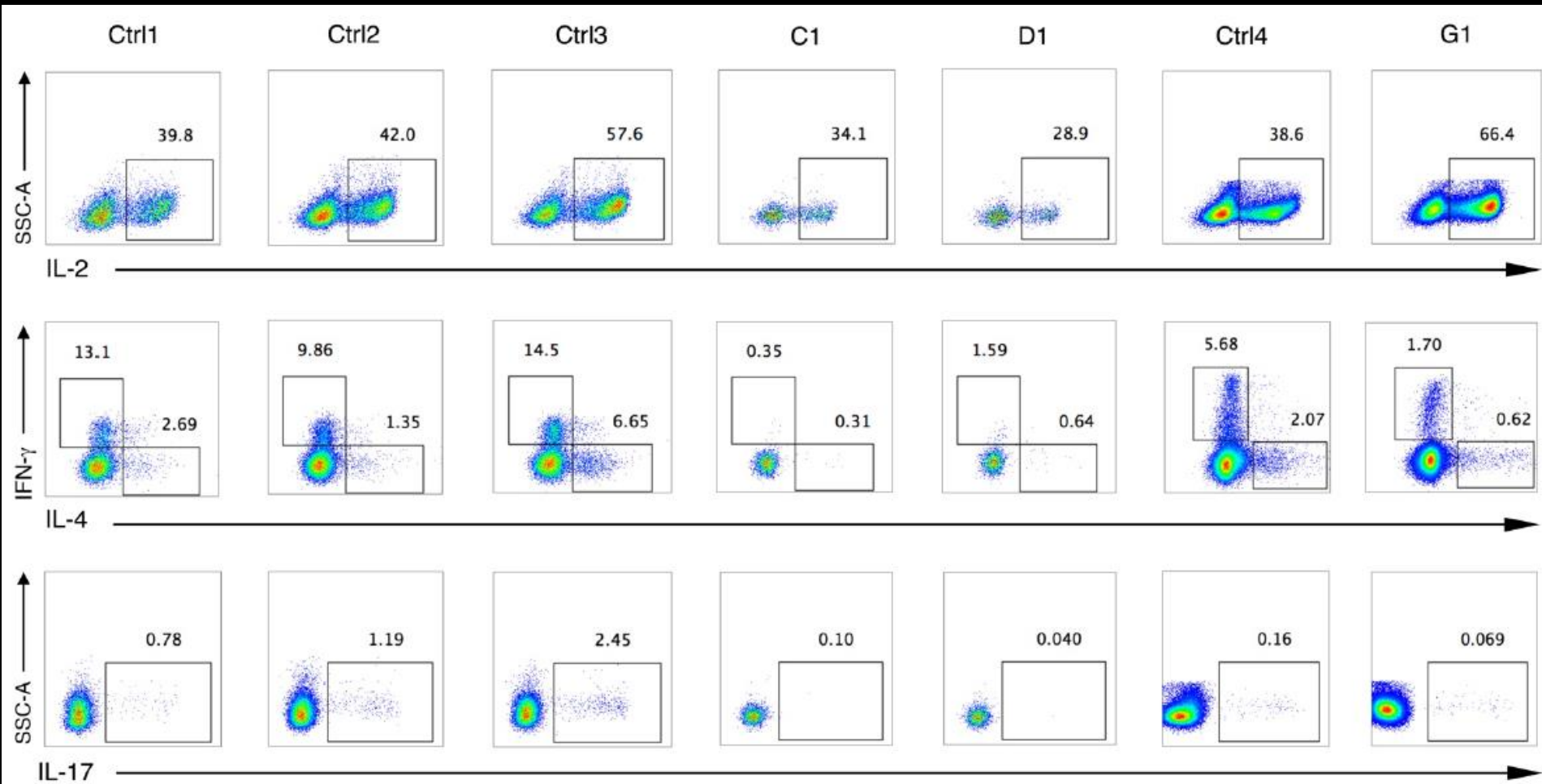
DN-IKZF1ではTreg(CD4⁺CD25⁺FoxP3⁺)内でのSTAT5のリン酸化が抑制される



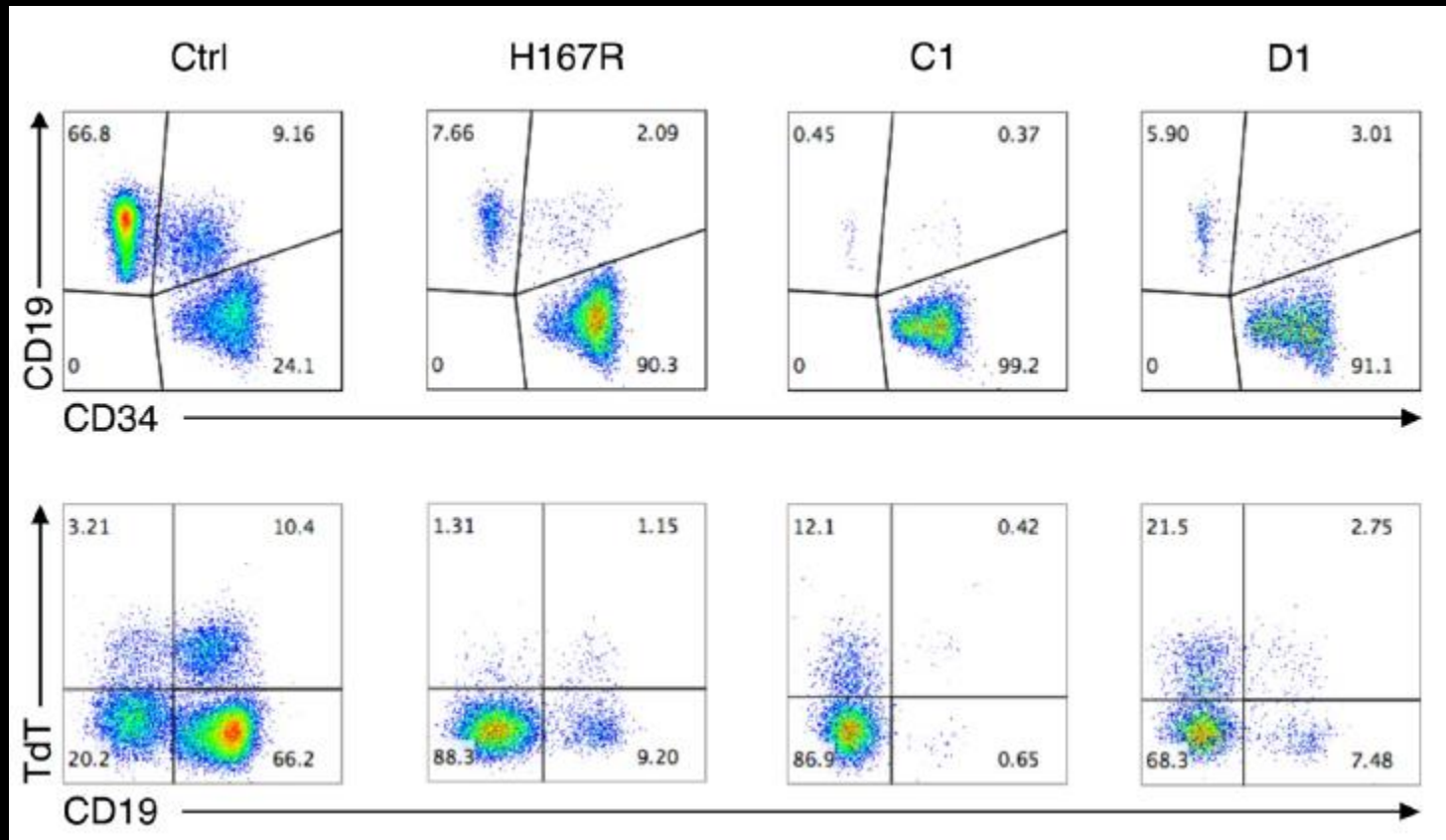
DN-IKZF1ではサイトカイン産生が低下する



DN-IKZF1ではIL-2産生は変わらないが Th1/Th2/Th17の分化が阻害される



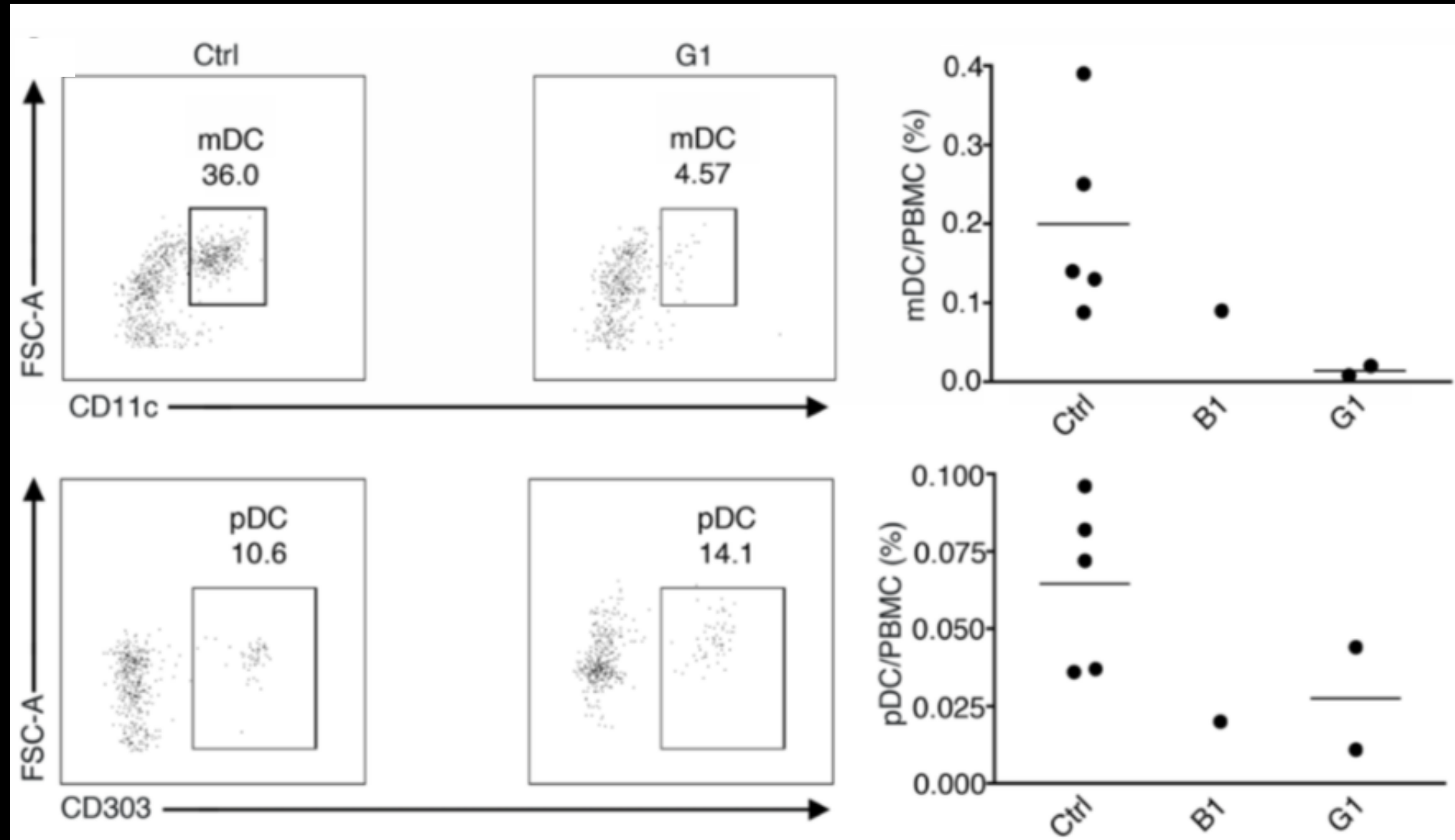
DN-IKZF1ではPrecursorが増加する



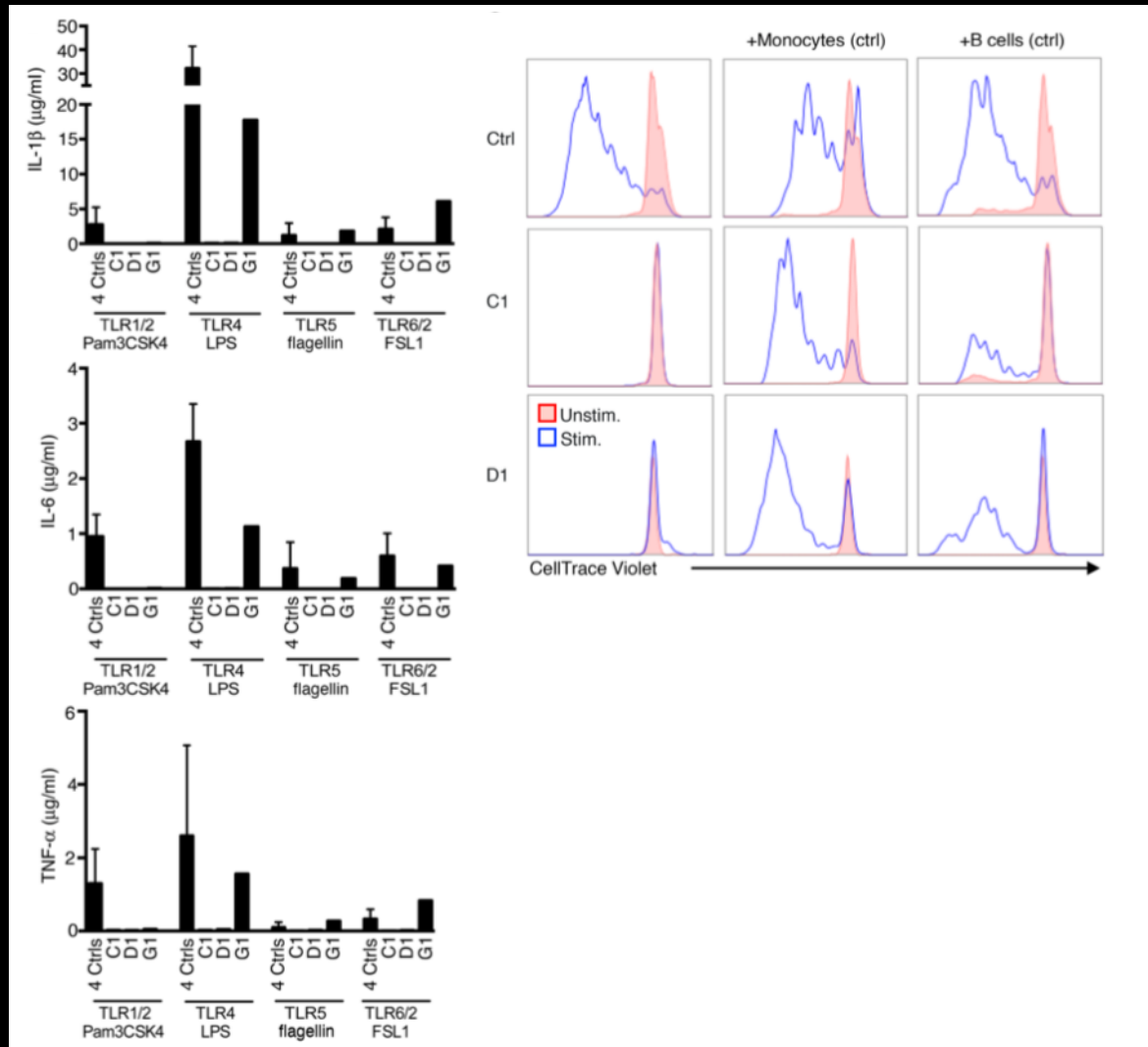
Pro-B cell: CD34⁺CD19⁺

Precursor of pro-B cell: CD34⁺TdT⁺CD19⁻

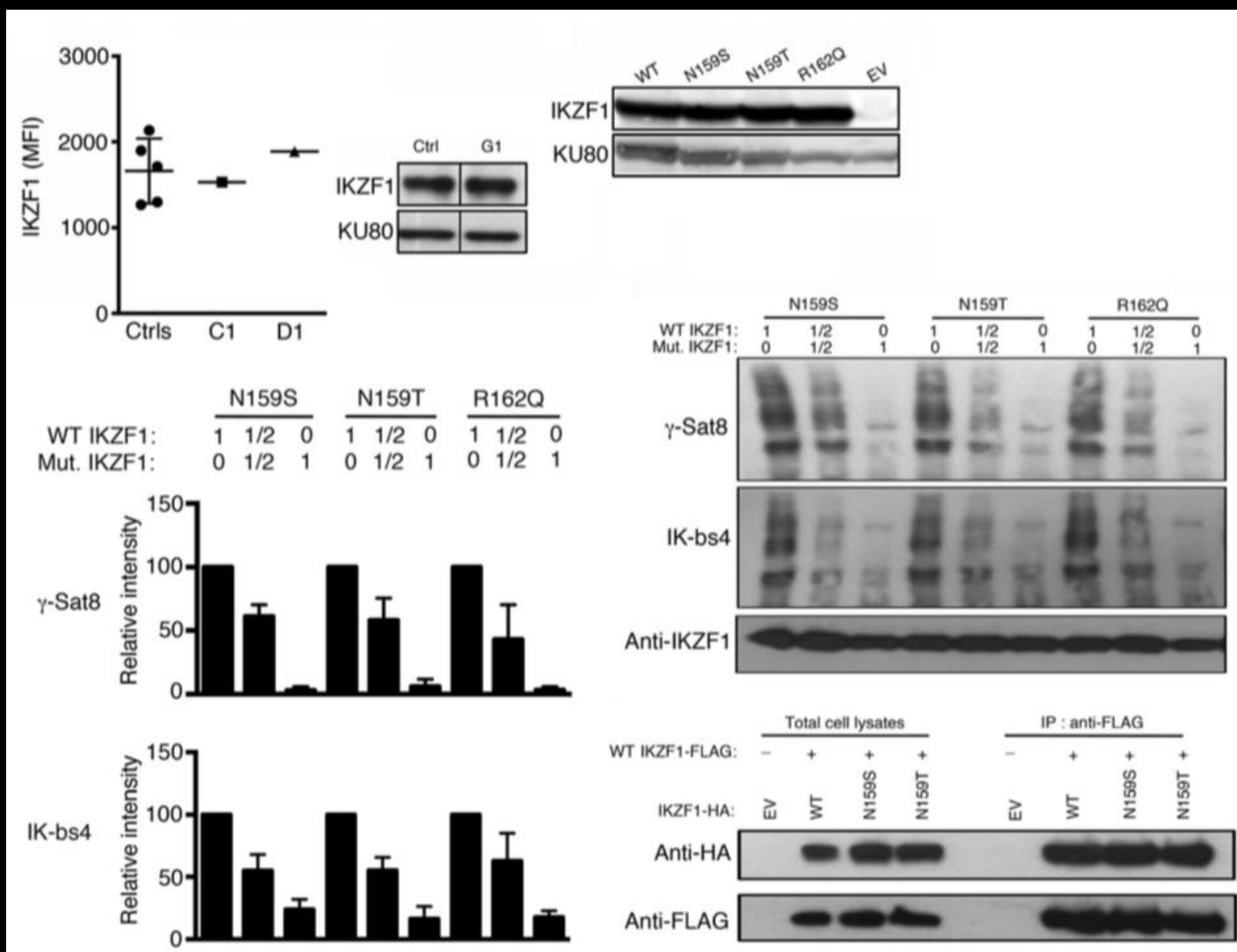
DN-IKZF1はDCの減少を誘導する



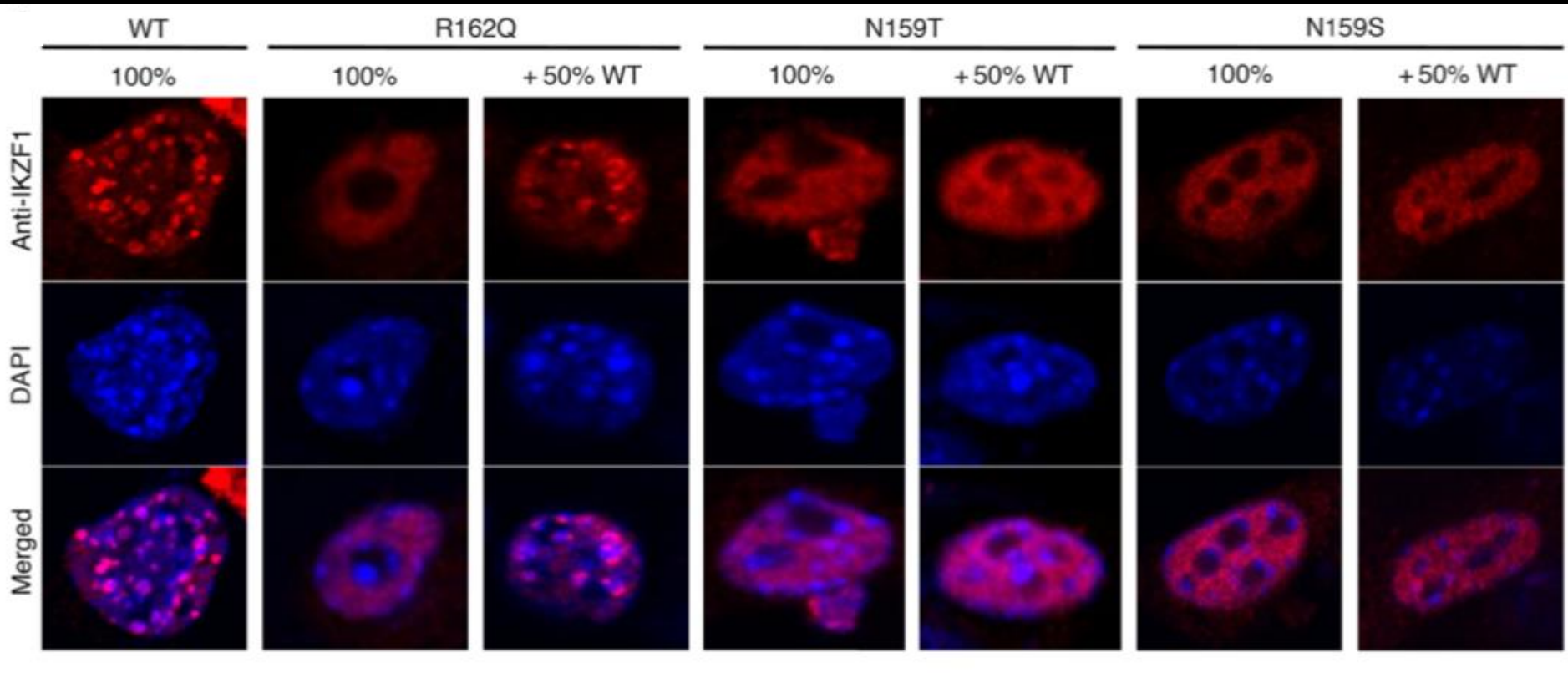
DN-IKZF1はTNF family cytokine産生を減少させ、また野生型Monocyte/B cellが入るとT cellの数が増加する



DN-IKZF1ではDNA結合能が低下する



DN-IKZF1ではIKAROSの核移行性が低下する



複合免疫不全はT細胞の機能不全に由来する

- Naïve Th0-like phenotype が増加する
- Th polarizationするサイトカイン産生が減少し、末梢血T細胞増殖が抑制される
- 他の複合免疫不全と異なり、memory Tが減少する→IKZF1はmemory cellの分化に必要である

IKZF1変異は造血能を低下させる

- T cell
- B cell
- Myeloid (RBC, Eosinophil, Neutrophil)
- Megakaryocyte異型

Summary

- 本研究では、臨床的にも生物学的にもIKZF1変異のヒトでのLoss-of-functionを証明した
- IKZF1は造血やT cell memory の獲得に必須である