1st CUNEO CITY IMMUNOTHERAPY CONFERENCE (CCITC) - IMMUNOTHERAPY IN HEMATOLOGICAL MALIGNANCIES 2018

Cuneo, 17-19 maggio 2018

DICHIARAZIONE

Relatore: Giulia Casorati

Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

- Posizione di dipendente in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Consulenza ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Partecipazione ad Advisory Board **(Vaxxilon AG, Reinach, Switzerland)**
- Titolarietà di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**

CD1 restricted T cells

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Milano

1st CUNEO CITY IMMUNOTHERAPY CONFERENCE (CCITC) –
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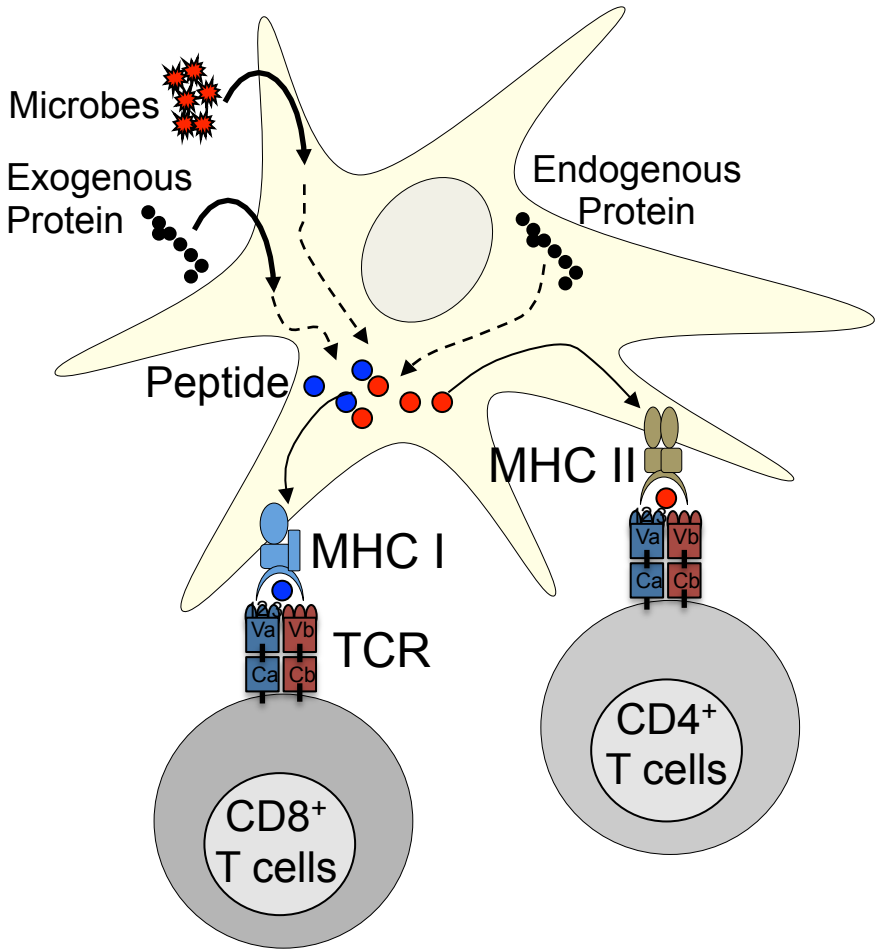
Cuneo, May 17-19, 2018

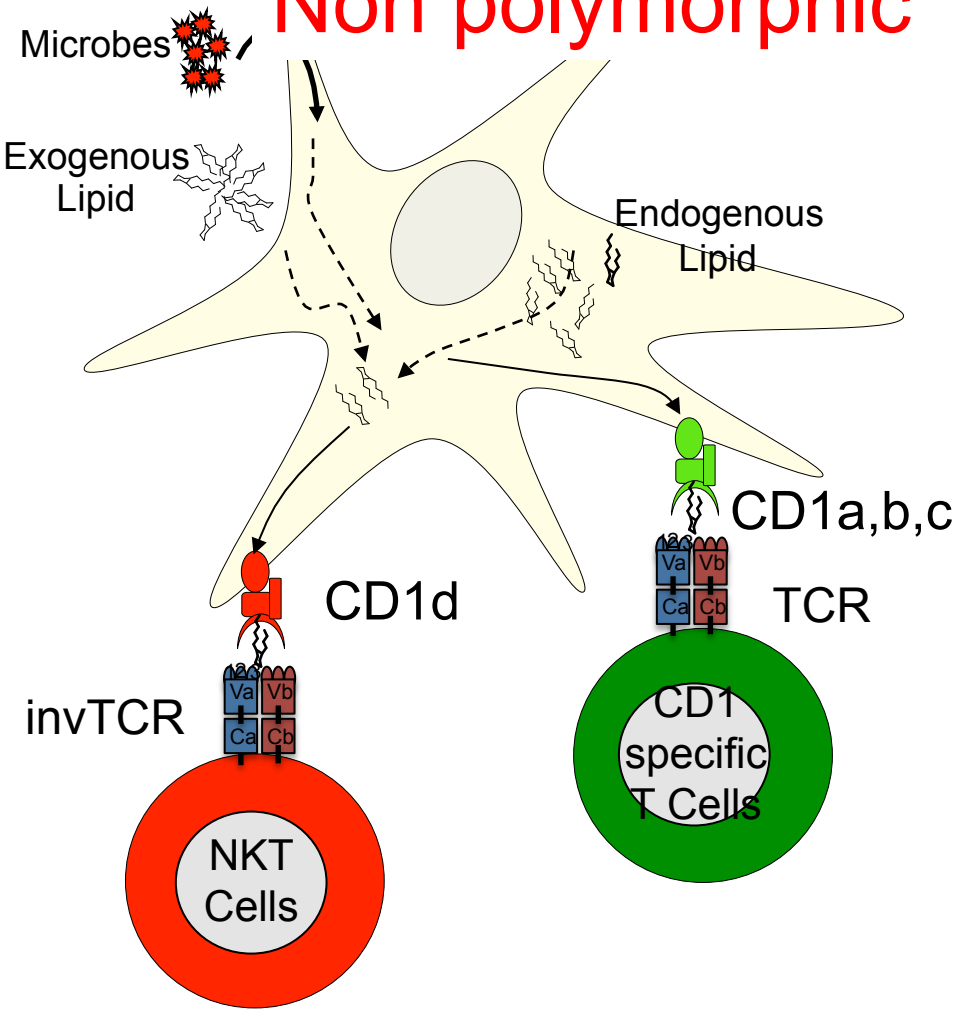
OSPEDALE
SAN RAFFAELE

MHC-restricted T cells
Specific for peptide antigens



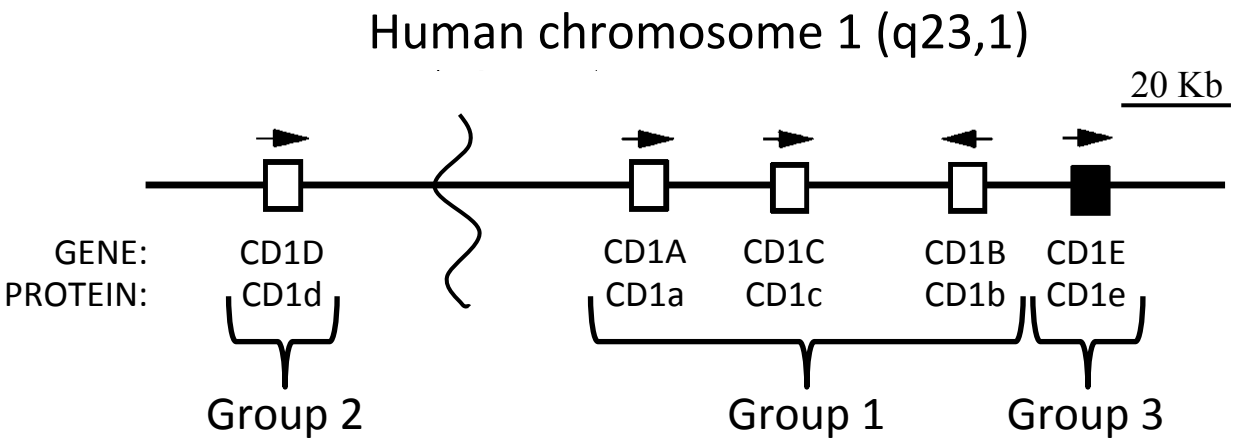
CD1-restricted T cells
Specific for lipid antigens

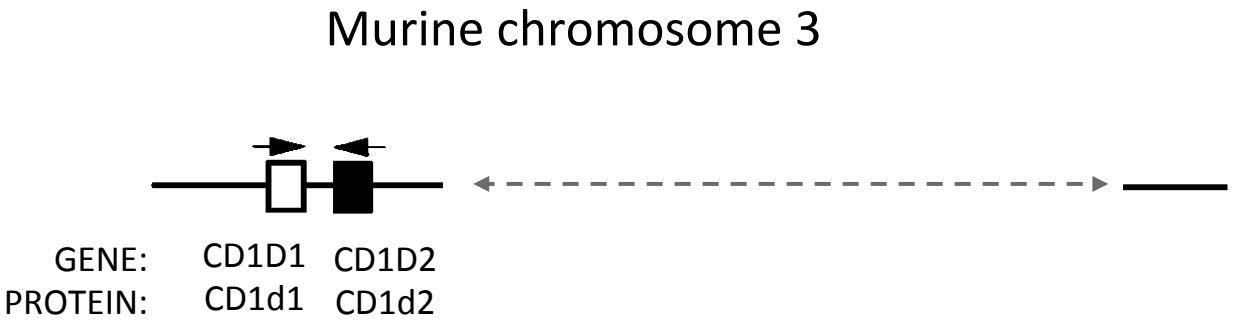
Non polymorphic



OSPEDALE SAN RAFFAELE

CD1 locus

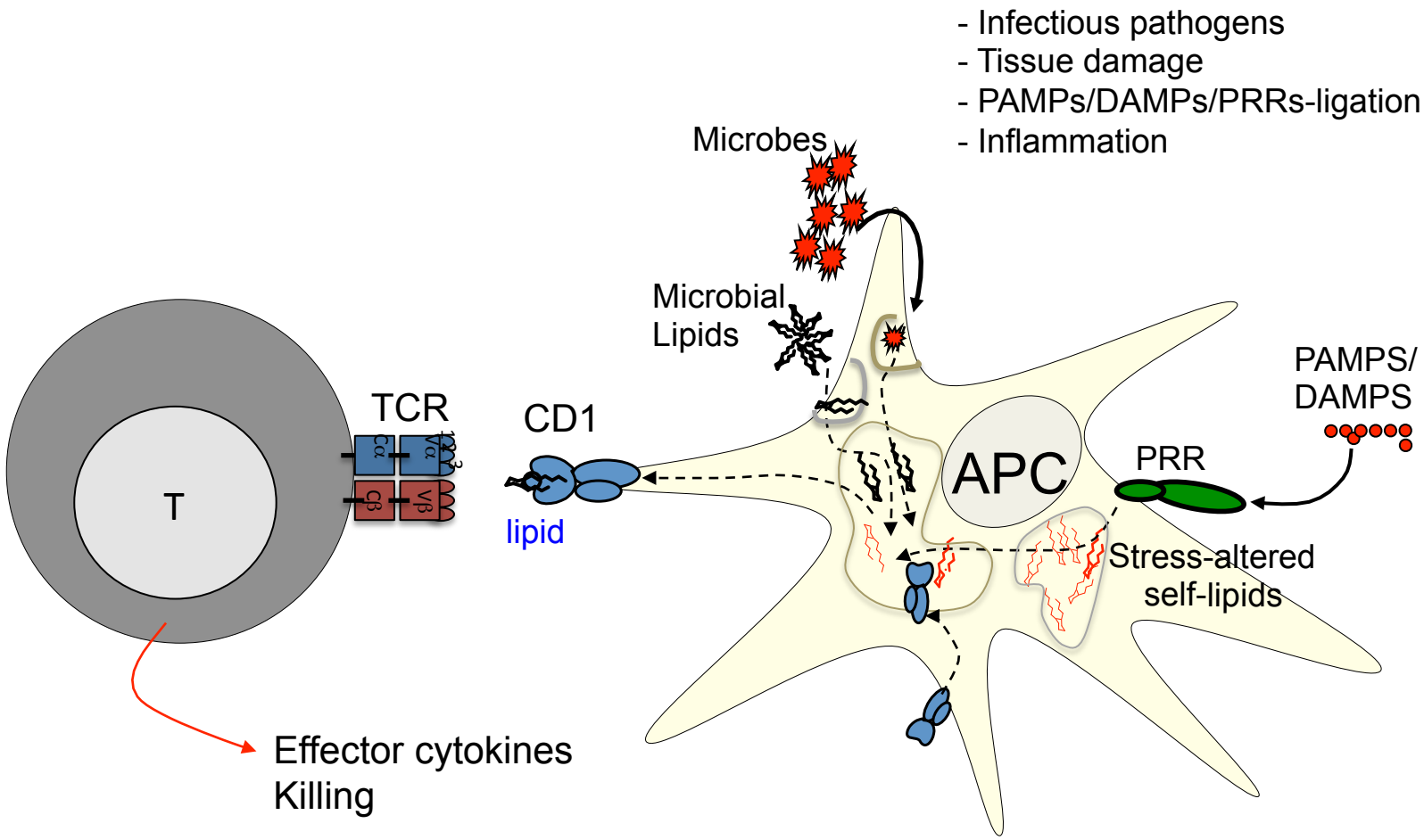




OSPEDALE SAN RAFFAELE

Porcelli et al. 1999

CD1-restricted T cells recognize microbial and stress-altered self-antigens (*autoreactivity*)



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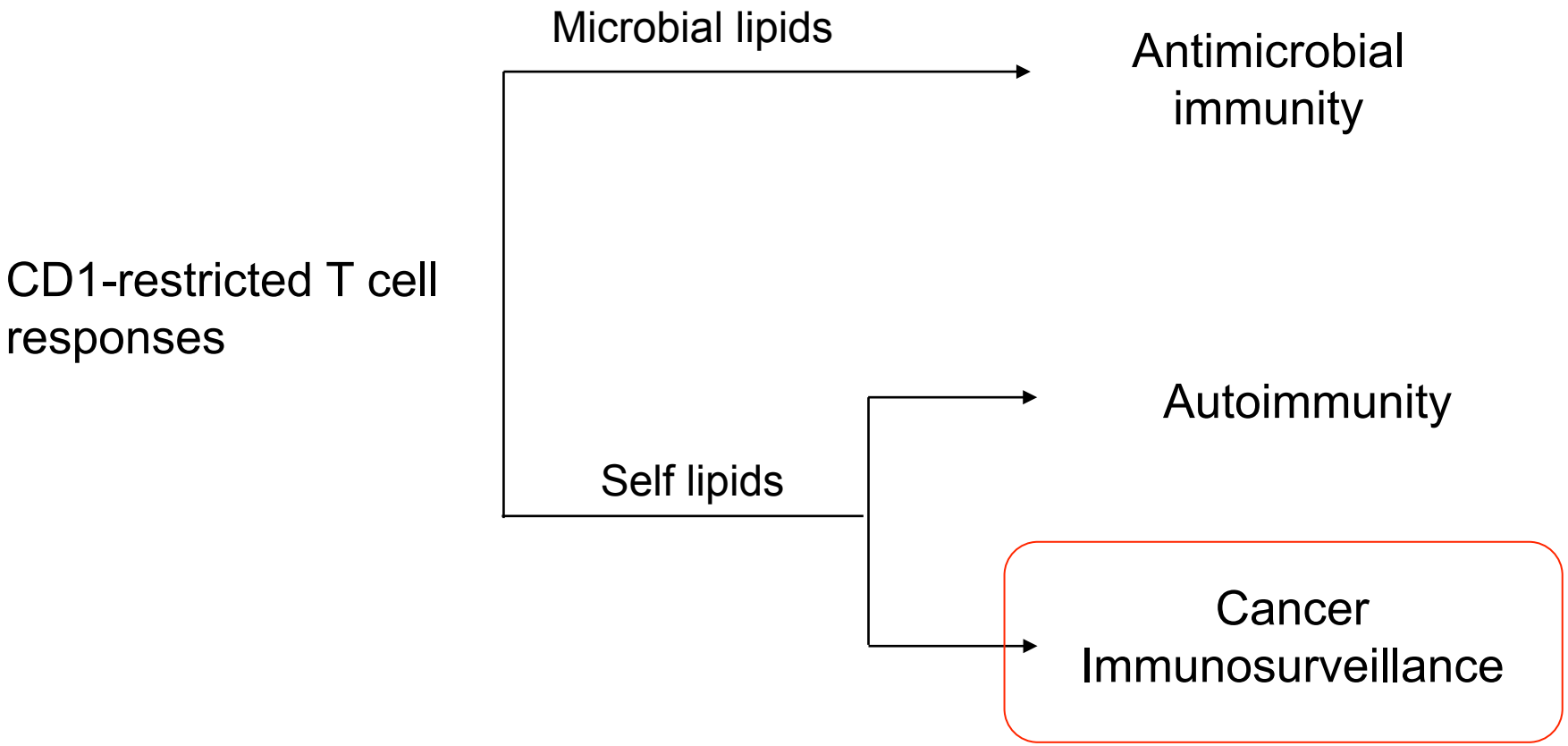
Pathophysiological relevance of lipid-specific T cell response for human health



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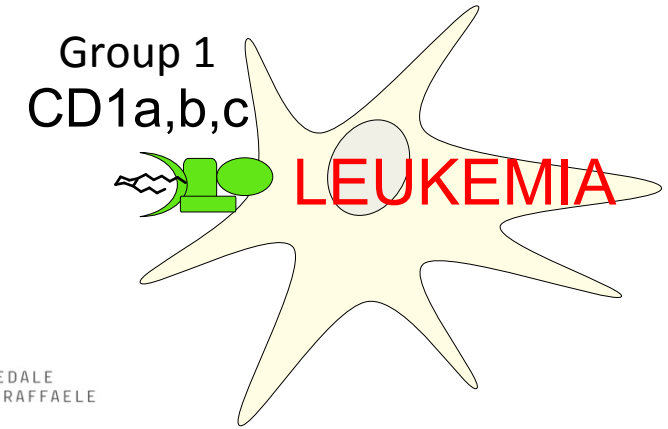
CD1 expression pattern

Group I CD1a, b, c	Group II CD1d
•Cortical immature tymocytes	•Cortical tymocytes
•mDCs (CD1a, b, c)	•mDCs
•Langherans cells (CD1a, c)	------
•Mono/Macrophages (CD1a, b, c variable)	•Mono/Macrophages
•B cells (CD1c on a subset)	•B cells (most)



•Keratinocytes

•Hepatocytes

•Vascular smooth cells (gut, liver)

•Schwann cells

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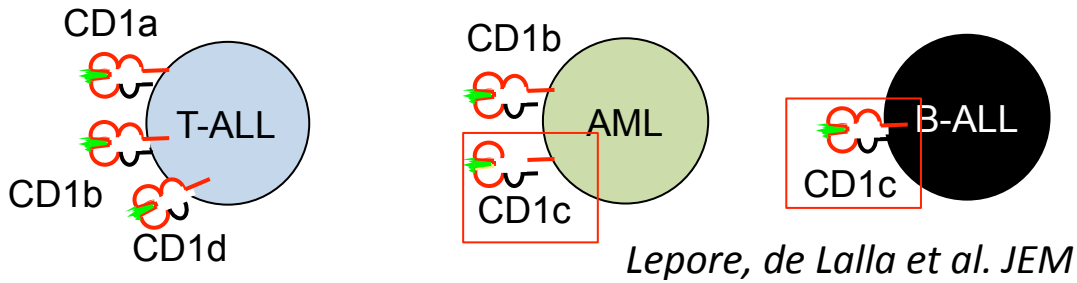
CD1 molecules are frequently expressed by primary acute leukemia blasts

Malignancy	Number	Patients with CD1-expressing blasts			
		CD1a	CD1b	CD1c	CD1d
		%	%	%	%
Pediatric patients					
AML	9	0	45	45	0
B-ALL	23	4	22	26	4
T-ALL	8	75	75	12	50
Adult patients					
AML	33	3	54	51	24
B-ALL	7	0	0	71	29

Data refer to circulating blasts. Similar CD1 expression frequencies were observed in bone marrow biopsy samples.



Lepore, de Lalla et al. JEM 2014

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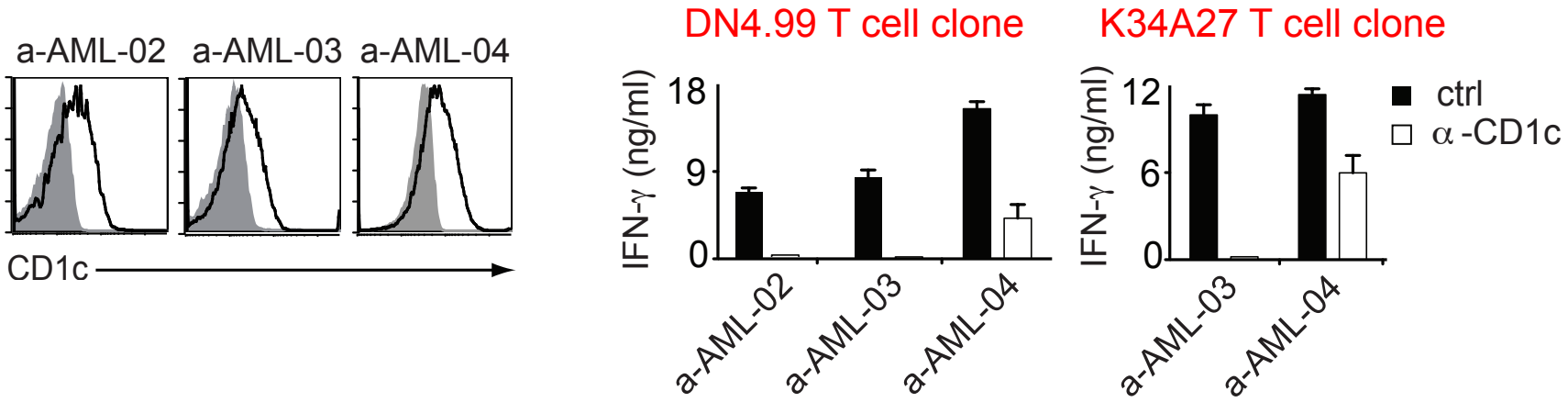
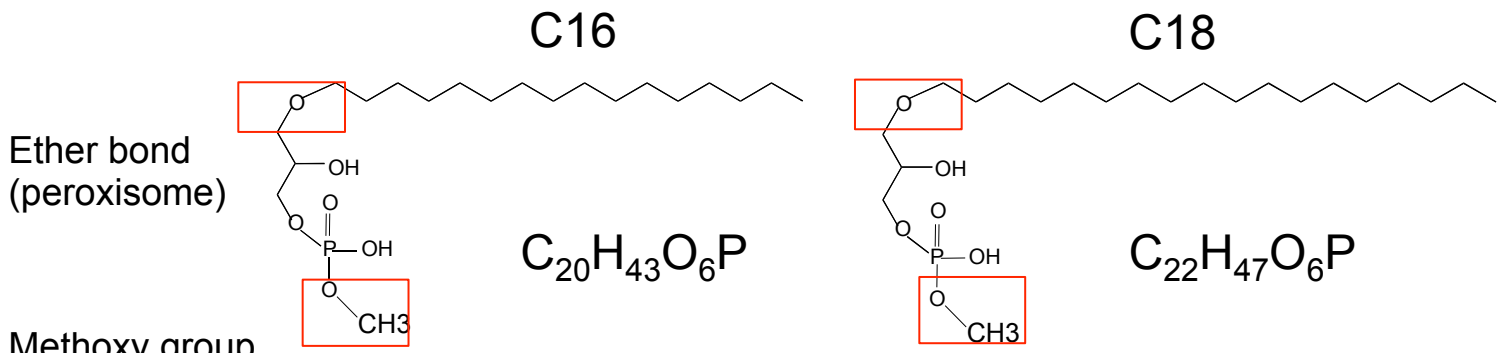
CD1c self-reactive T cell clones recognize acute leukemia expressing CD1c



CD1c self-reactive T cell clones recognize the leukemia-derived methyl-lyso-phosphatidic acid Ag (mLPA)



Lepore, de Lalla et al. 2014

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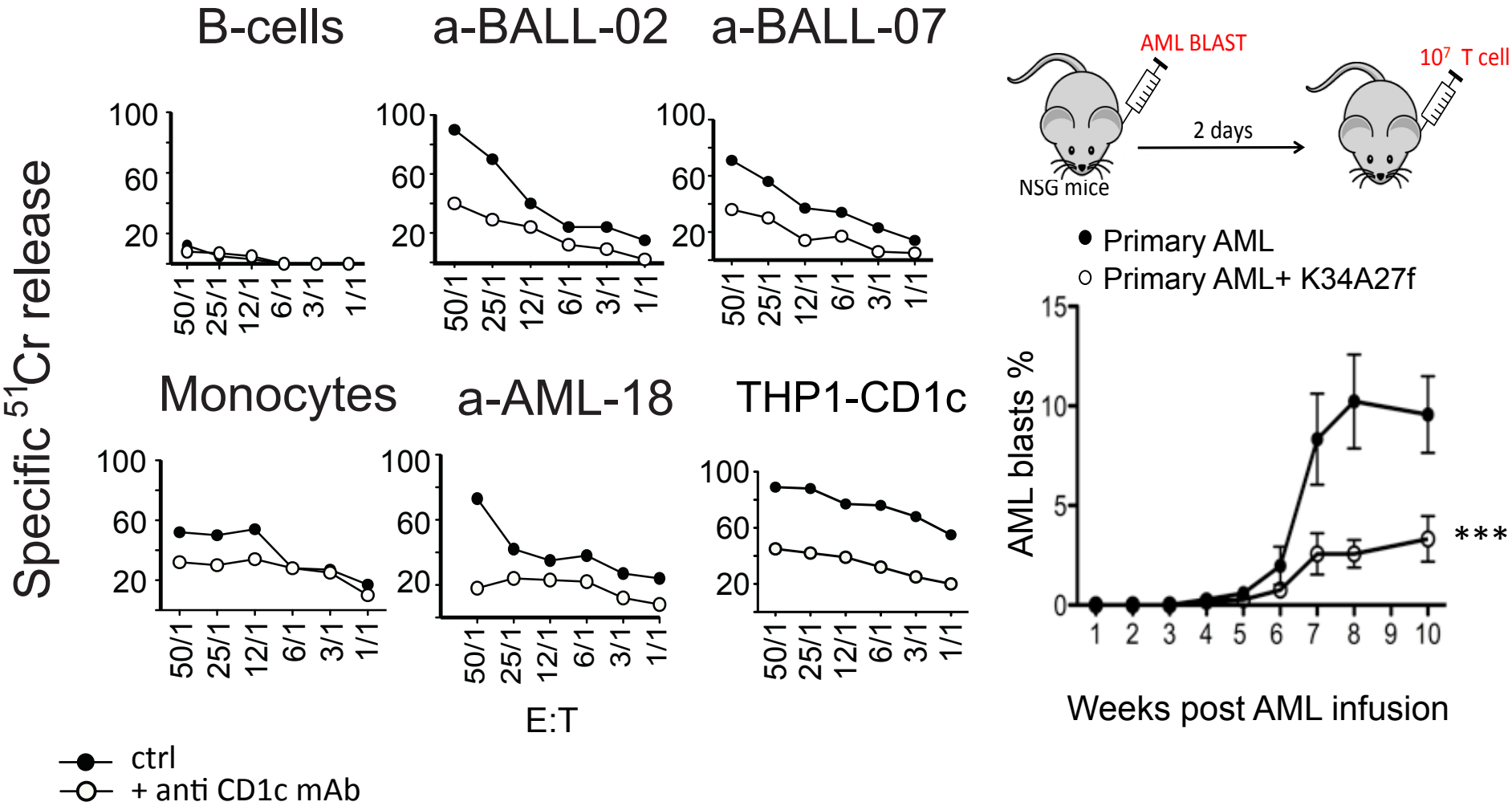
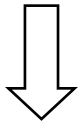
mLPA-specific T cells kill malignant cells *in vitro* *and* impair leukemia progression *in vivo*



Lepore, de Lalla et al. 2014

T cell adoptive immunotherapy of acute leukemia (AML, BCP-ALL, T-ALL)

- Acute leukemia are treated with chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT)
- The major unmet clinical need is post-transplant recurrence of residual leukemia
- Allogeneic T cell transfer can control leukemia recurrence, but can causes detrimental GVHD



- Need strategies to target T cell responses against malignant cells

OSPEDALE
SAN RAFFAELE

Advantages of leukemia targeting by CD1 self-reactive T cells

1. Group 1 CD1 are expressed only by hematopoietic cells =

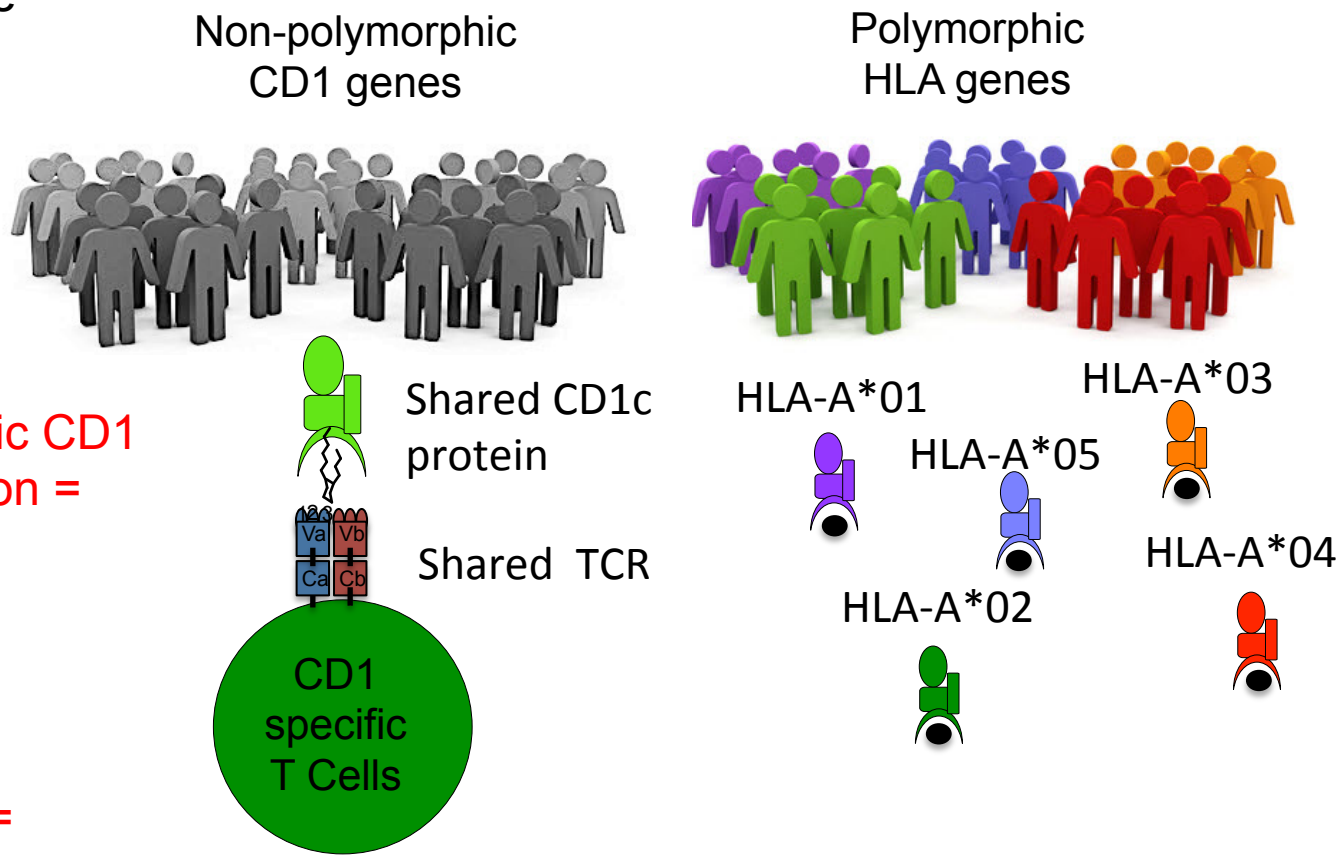
No GVHD upon adoptive transfer

2. Targeting monomorphic CD1 overcomes HLA-restriction =

Universal effector T cells

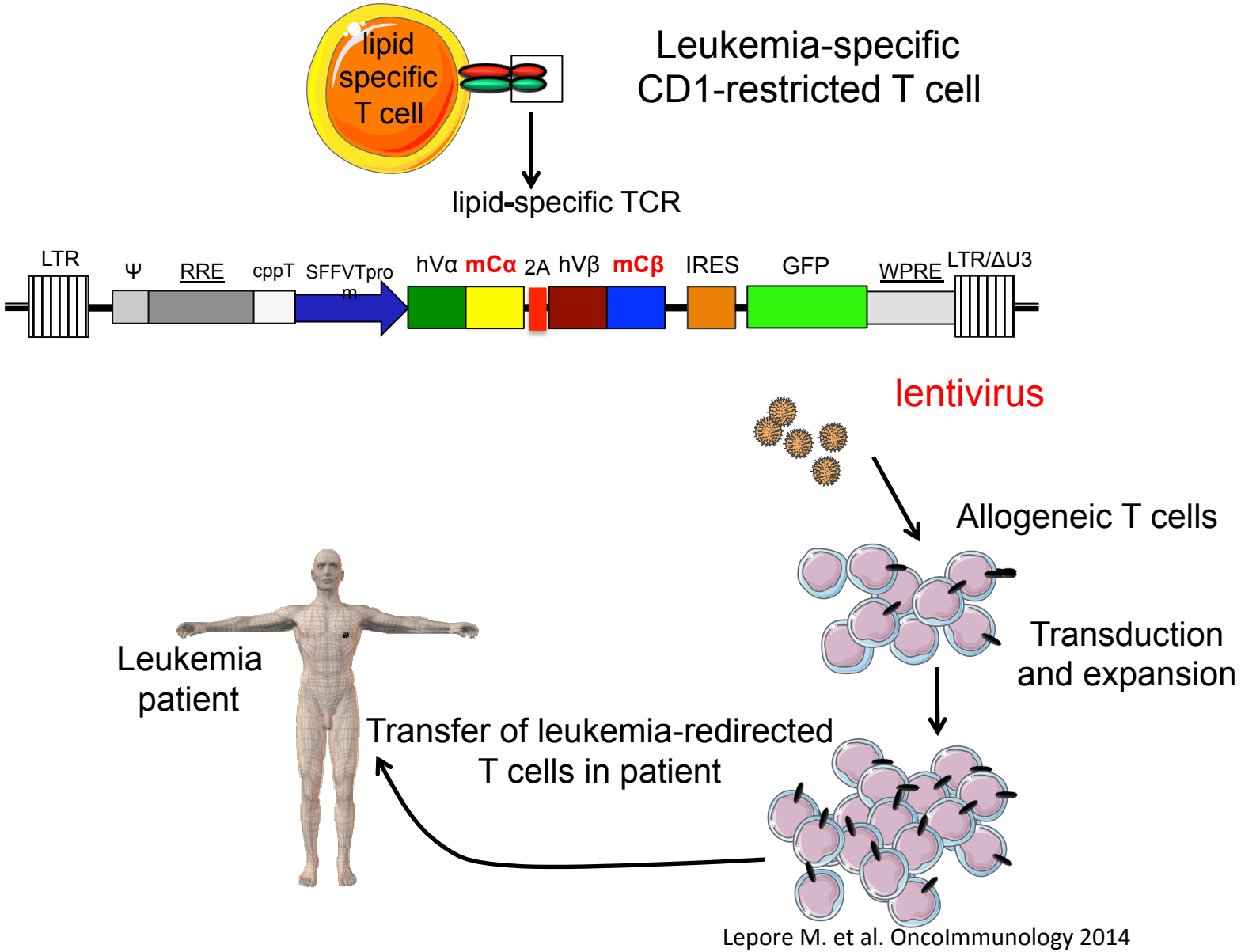
3. Lipids are immutable =

Minimal selection of Ag-loss tumor variants



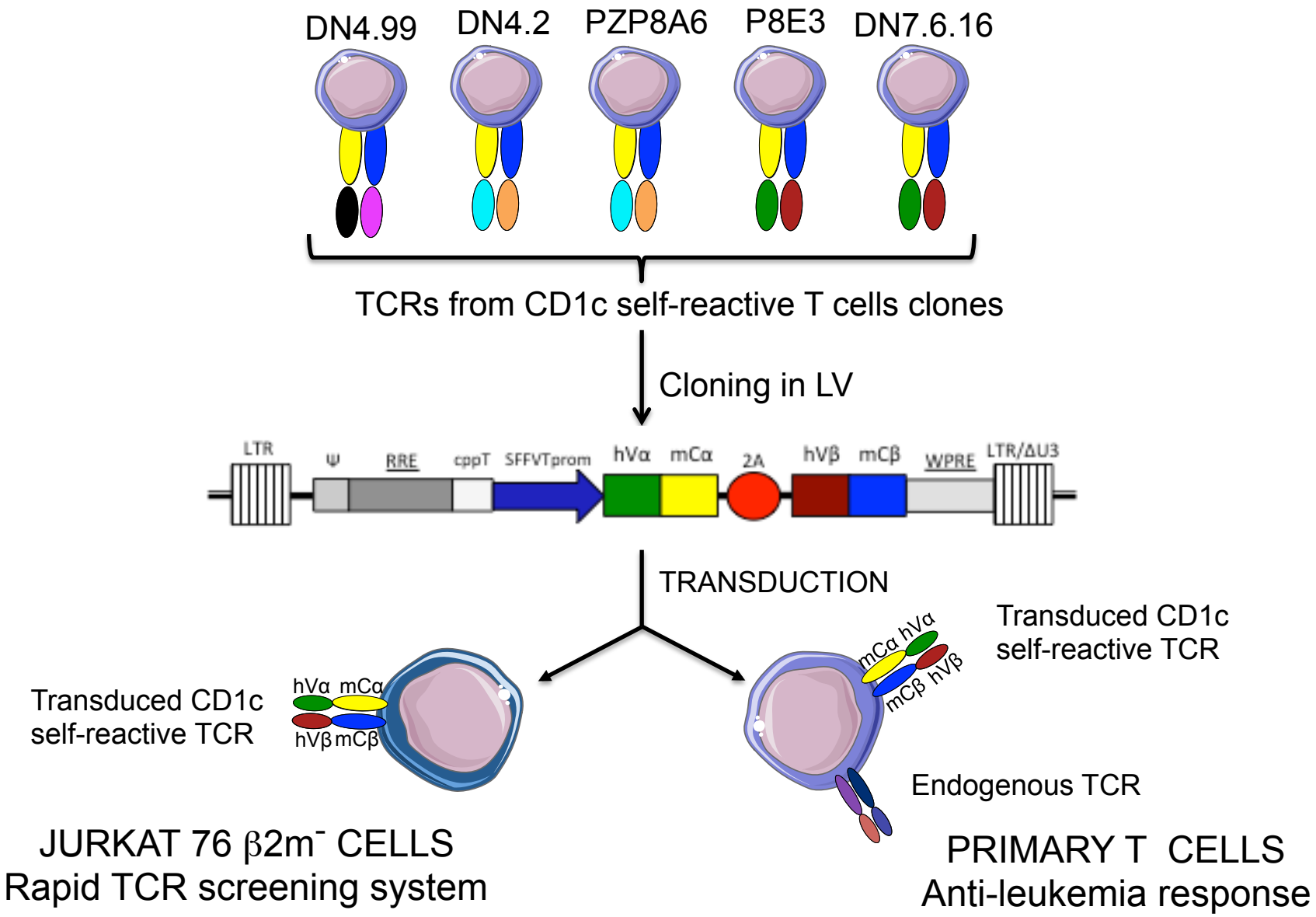
Consonni et al Cytokine & Growth Factor Reviews 36 (2017) 117–123

Retargeting allogeneic T cells against relapsed CD1c^{+} leukemia by transduction with a selected mLPA-specific TCR

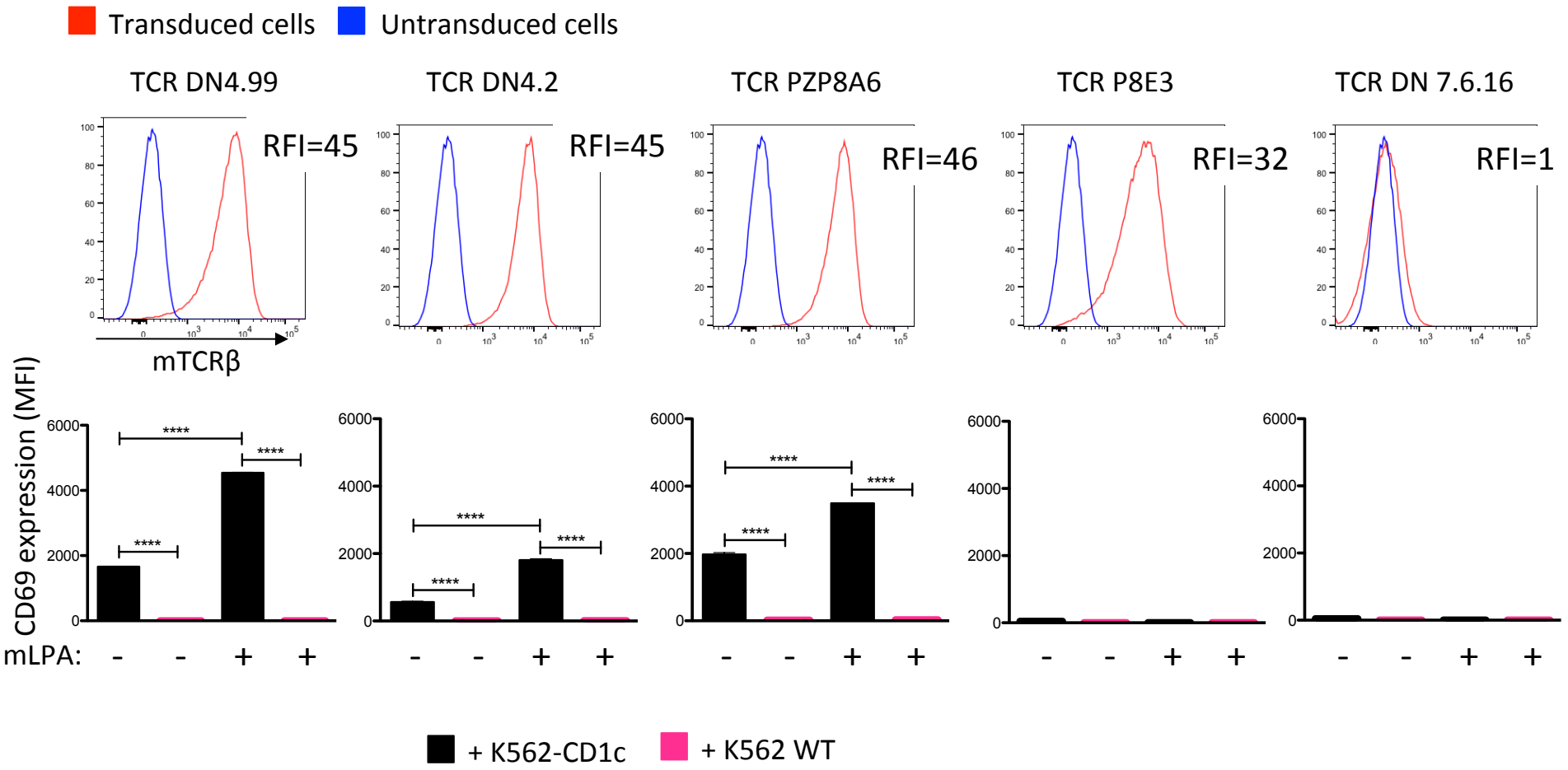


Lepore M. et al. OncoImmunology 2014

2. Identification of the lead mLPA-specific TCR



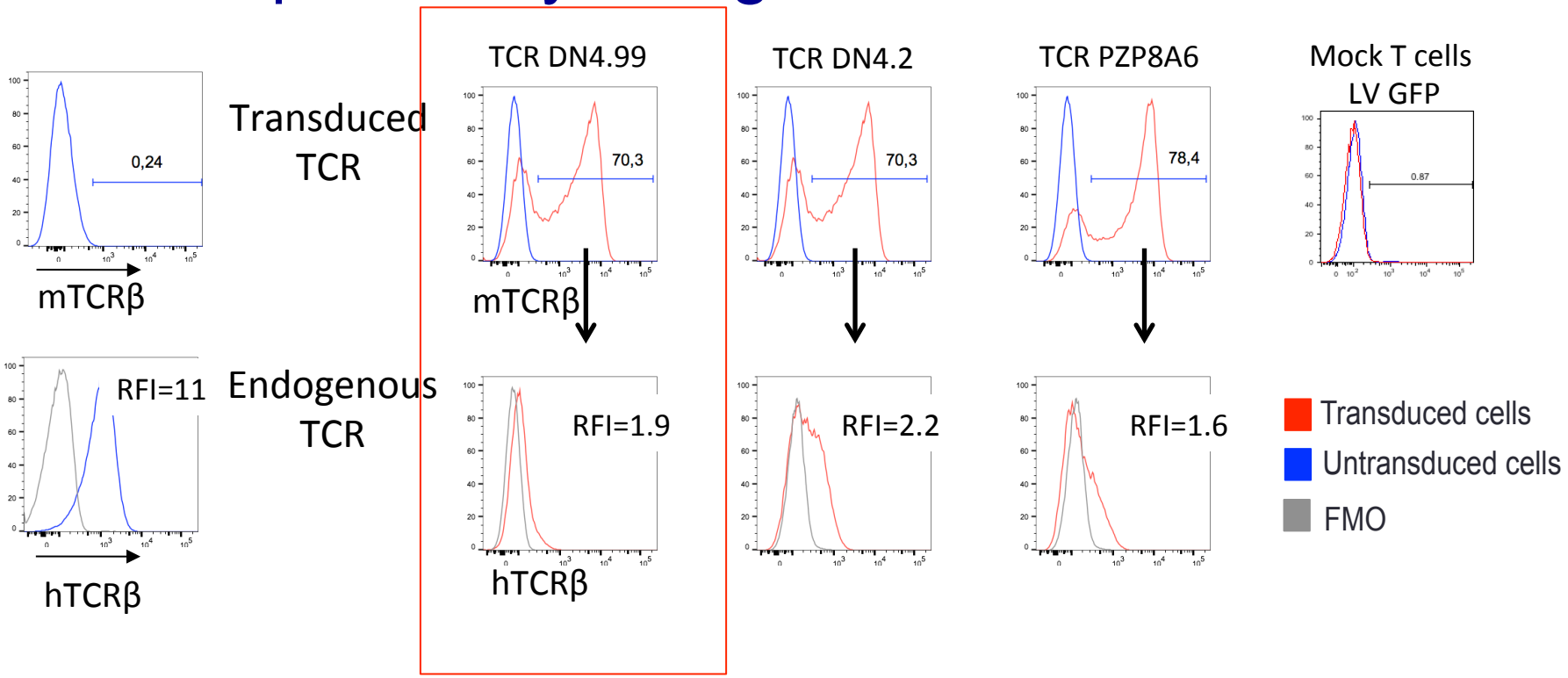
Recognition of K562 cells by Jurkat 76 β2m^{-} cells transduced with mPLA-specific TCRs

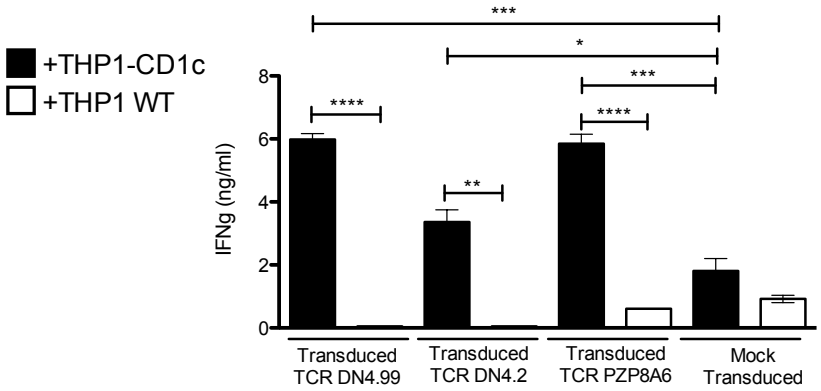


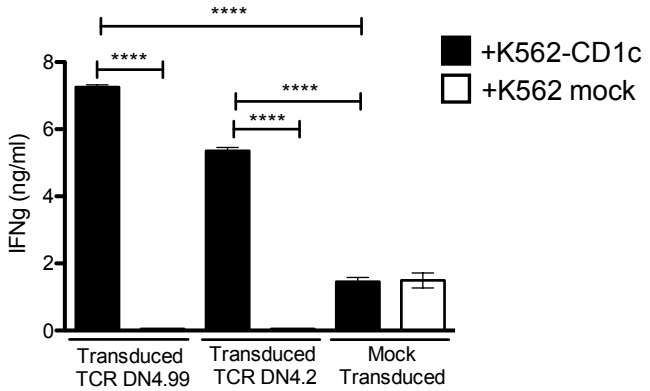
OSPEDALE SAN RAFFAELE

Consonni M. unpublished

Primary T cells transduced with mPLA-specific TCRs specifically recognize leukemia cell lines







Consonni M. unpublished

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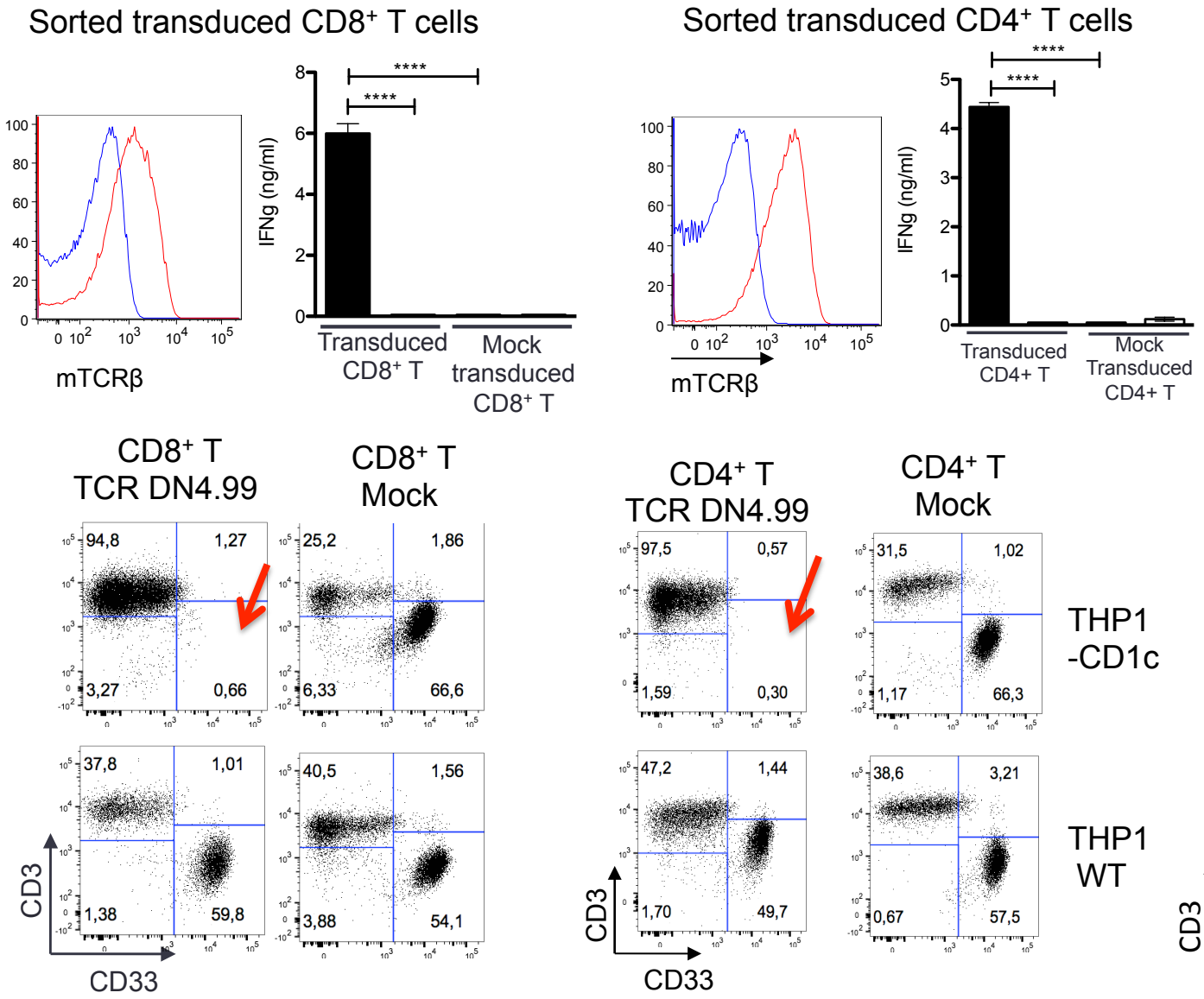
CD4⁺ & CD8⁺ T cells transduced with lead mPLA-specific TCR kill CD1c⁺ leukemia cell lines



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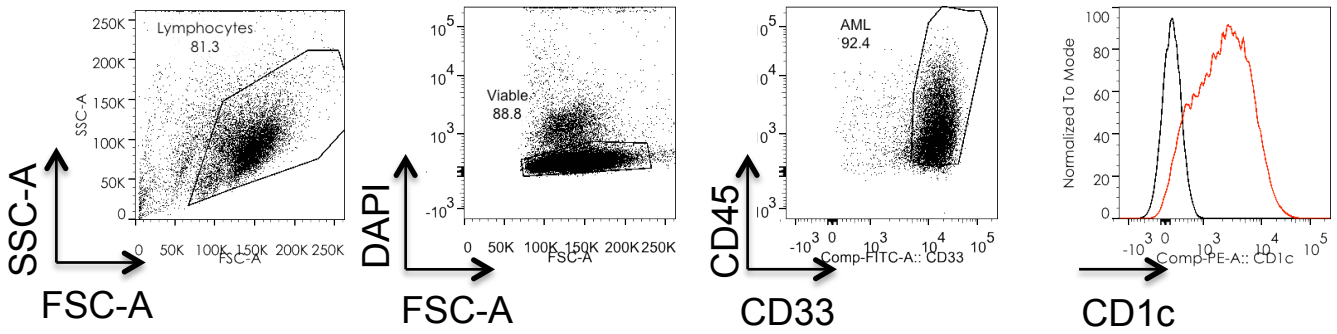
Consonni M. unpublished

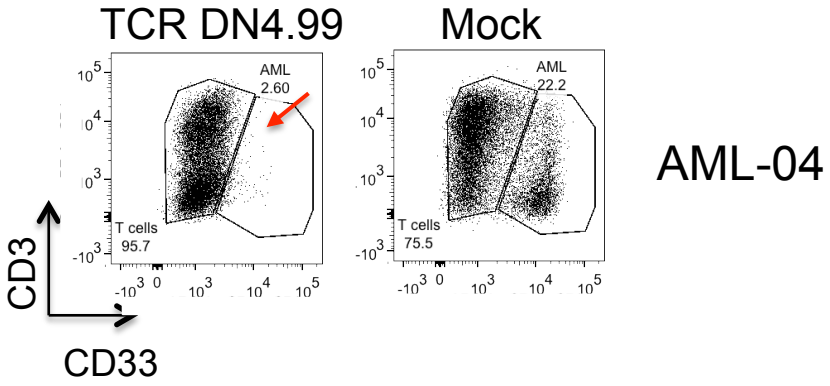
Primary T cells transduced with lead mLPA-specific TCR kill primary CD1c^{+} leukemia blasts

CD1c expression on primary circulating AML blasts

AML-04 ex-vivo



killing of CD1c^{+} AML blasts (CD33^{+}) by TCR transduced primary T cells

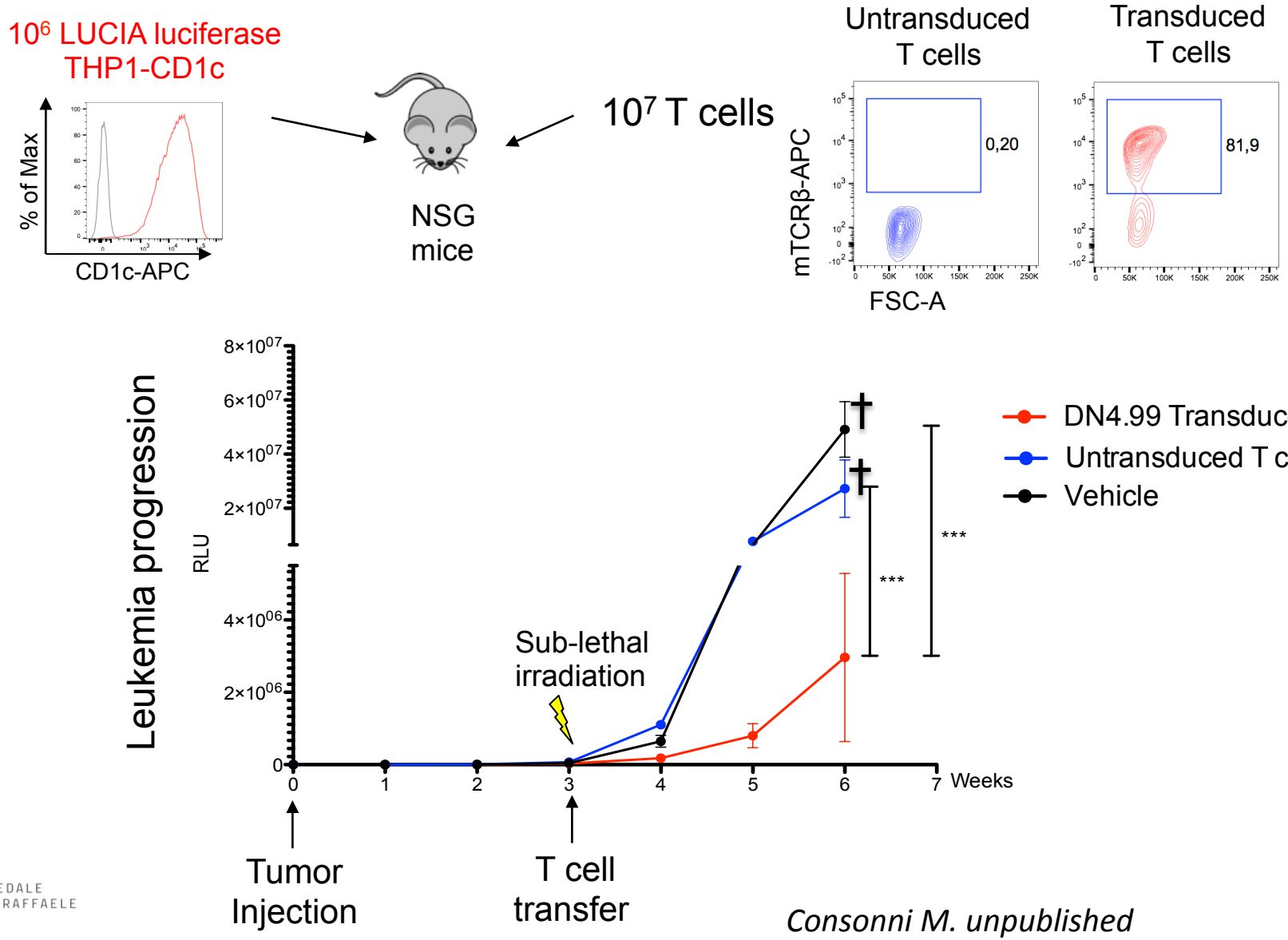


AML-04

72 h co-culture at 10:1 effector/target ratio

De Lalla C. Consonni M. unpublished

Primary T cells transduced with lead mLPA-specific TCR impair CD1c^{+} leukemia cell progression in vivo

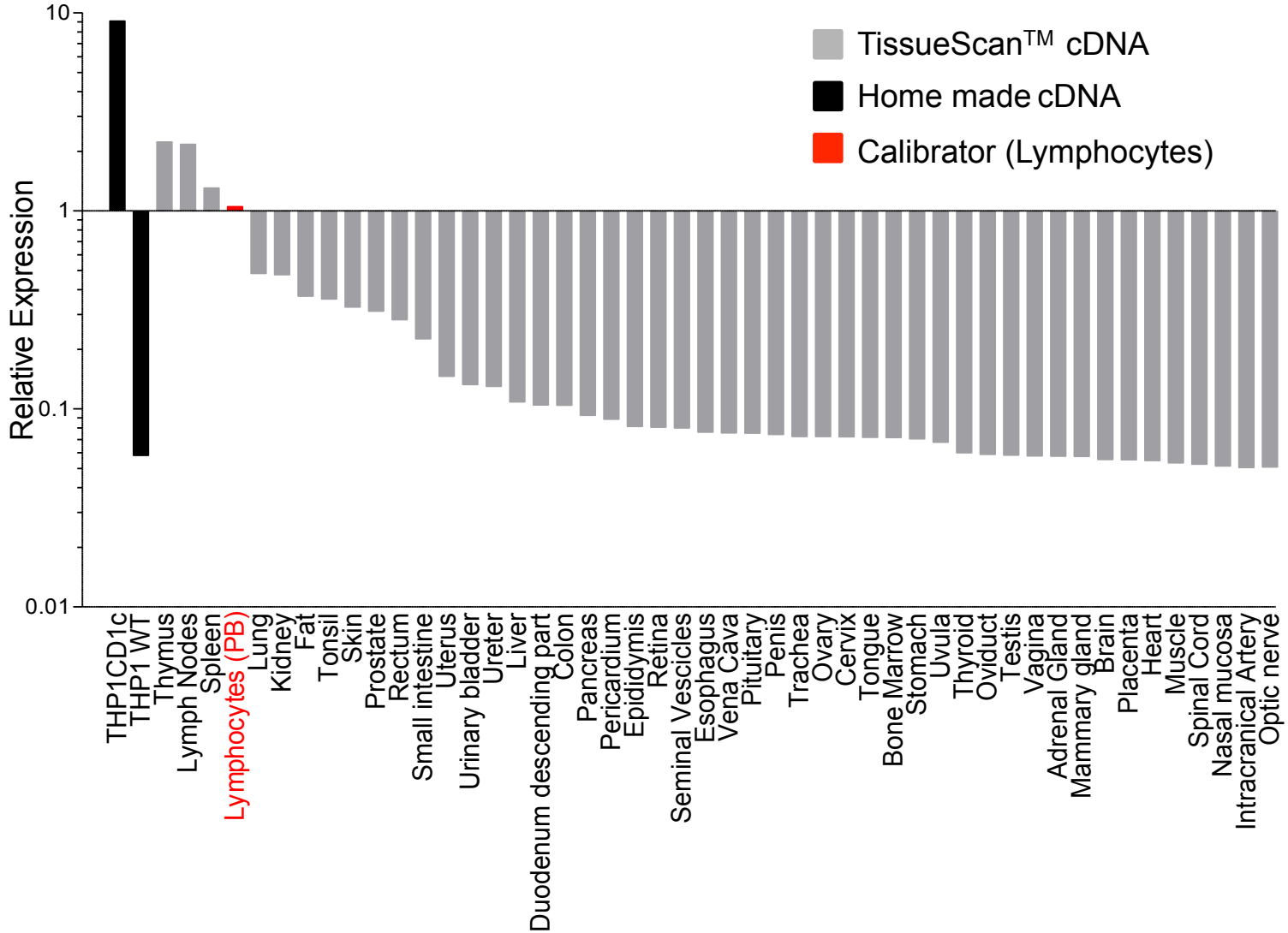


Consonni M. unpublished

Safety issues

1. On-target off-tumor recognition

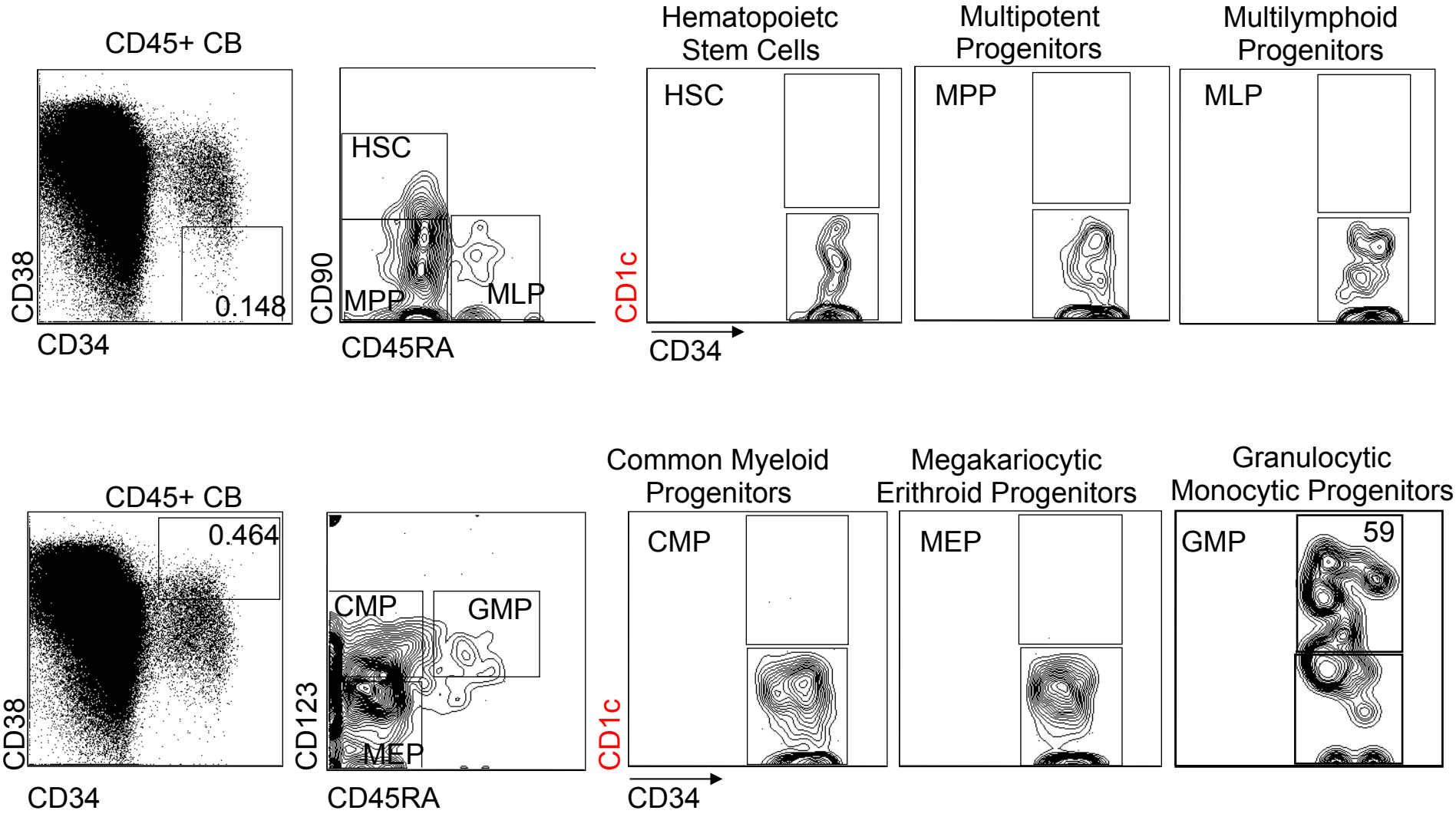
Absent CD1c expression in non-hematopoietic tissues of healthy individual



Lepore, de Lalla et al. JEM 2014

Absence of CD1c expression in normal hematopoietic precursors

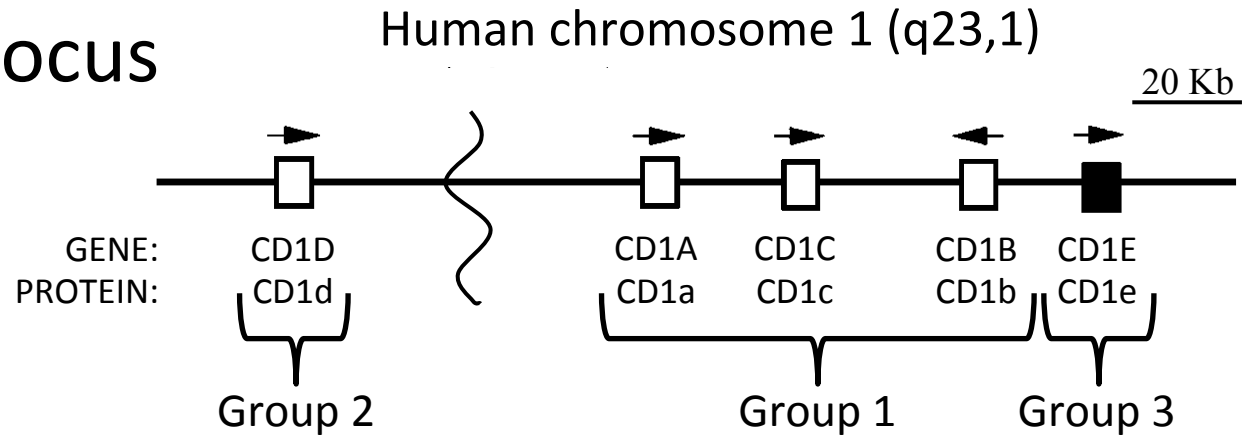
Cord Blood cells from healthy donors

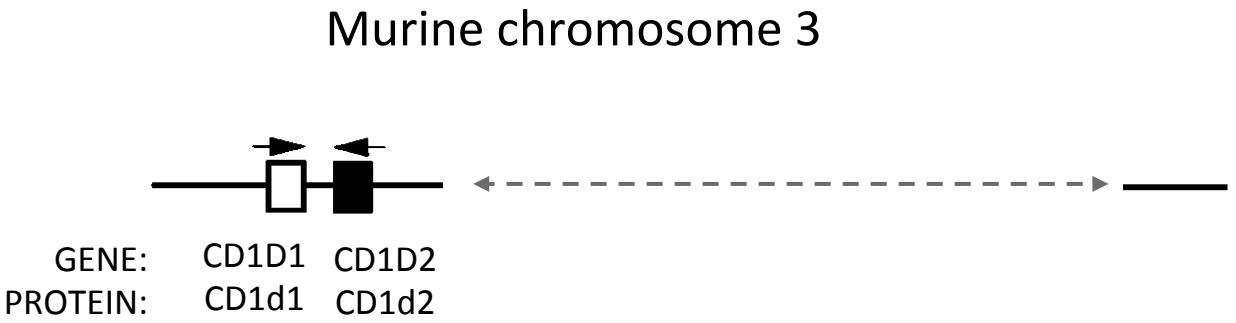


Lepore, de Lalla et al. JEM 2014

Assessing efficacy and safety of ACT with T cells engineered with mLPA-specific TCRs in syngeneic animal models

CD1 locus

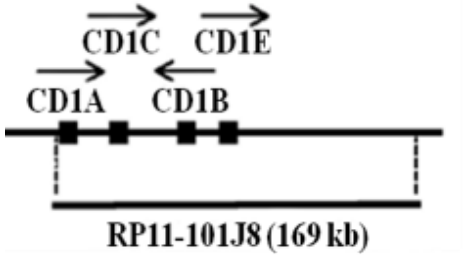




Porcelli et al. 1999

OSPEDALE SAN RAFFAELE

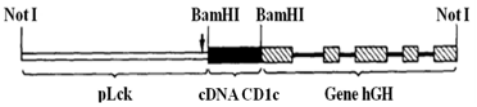
Humanized CD1c transgenic mice



BAC-CD1 Tg mice

x

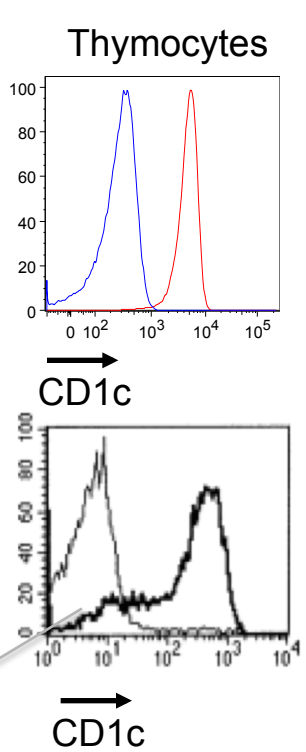
pLck-CD1c Tg mice

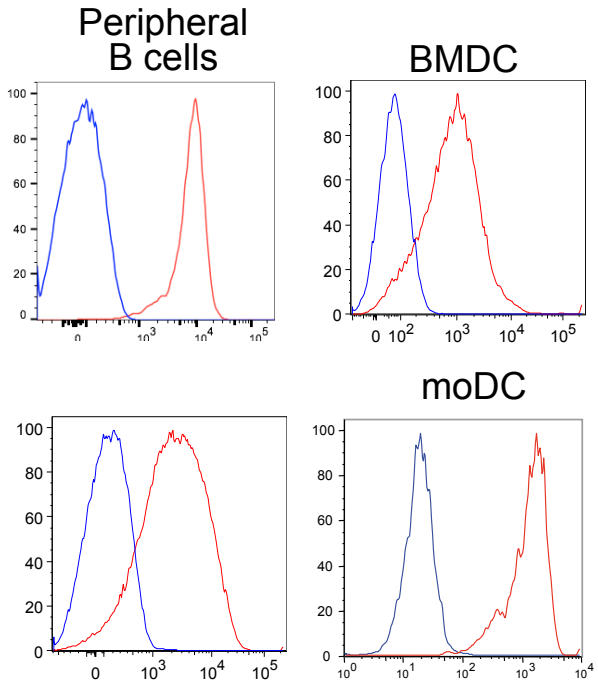


BAC x pLck-CD1c Tg mice

BAC x pLck CD1c Tg mice

HUMAN





Tg mice

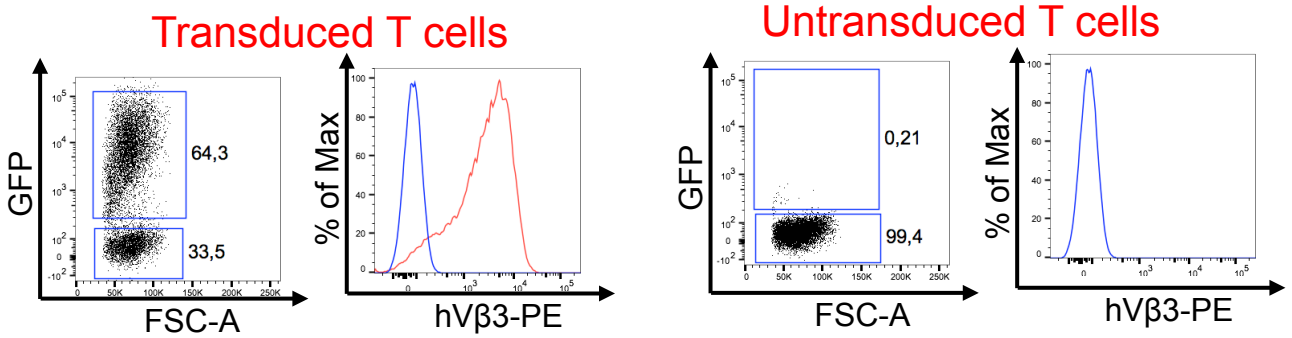
WT mice

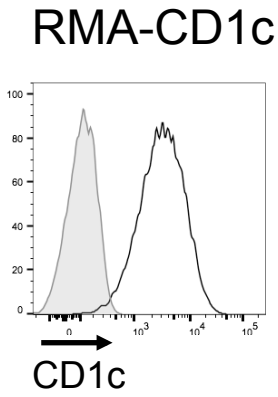
(Exley M et al, 2000)

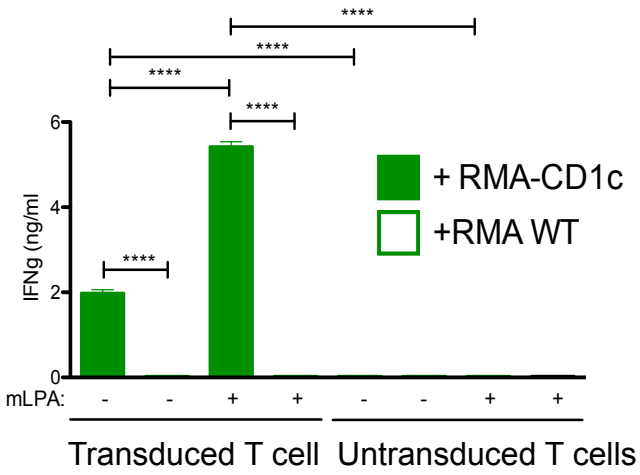
Consonni M. unpublished

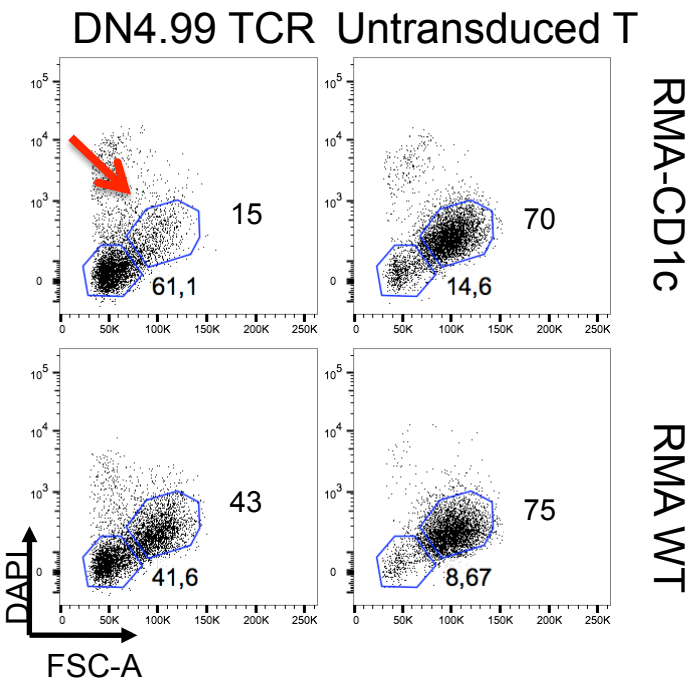
OSPEDALE SAN RAFFAELE

Mouse T cells engineered with lead human mLPA-specific TCR recognize and kill mouse CD1c^{+} lymphoma cells









OSPEDALE SAN RAFFAELE

Consonni M. unpublished

Conclusions

1. Polyclonal T cells are efficiently retargeted against CD1c-expressing leukemia in vitro and in vivo (NSG mice) by mLPA-specific TCR transfer

2. We have identified a lead mLPA specific TCR for further clinical development

3. CD1 humanized mice allow assessing efficacy and safety of ACT with T cells engineered with mLPA-specific TCRs

Ongoing experiments

1. Assessing efficacy of ACT with engineered T cells against primary AML-ALL xenografts in NSG mice

2. Investigating leukemia control and safety by mLPA-specific T cells in CD1 humanized mice

OSPEDALE
SAN RAFFAELE

Acknowledgments

Experimental immunology unit

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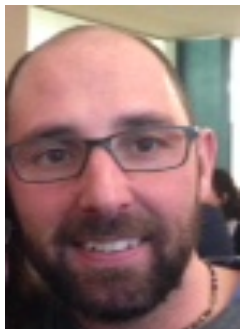

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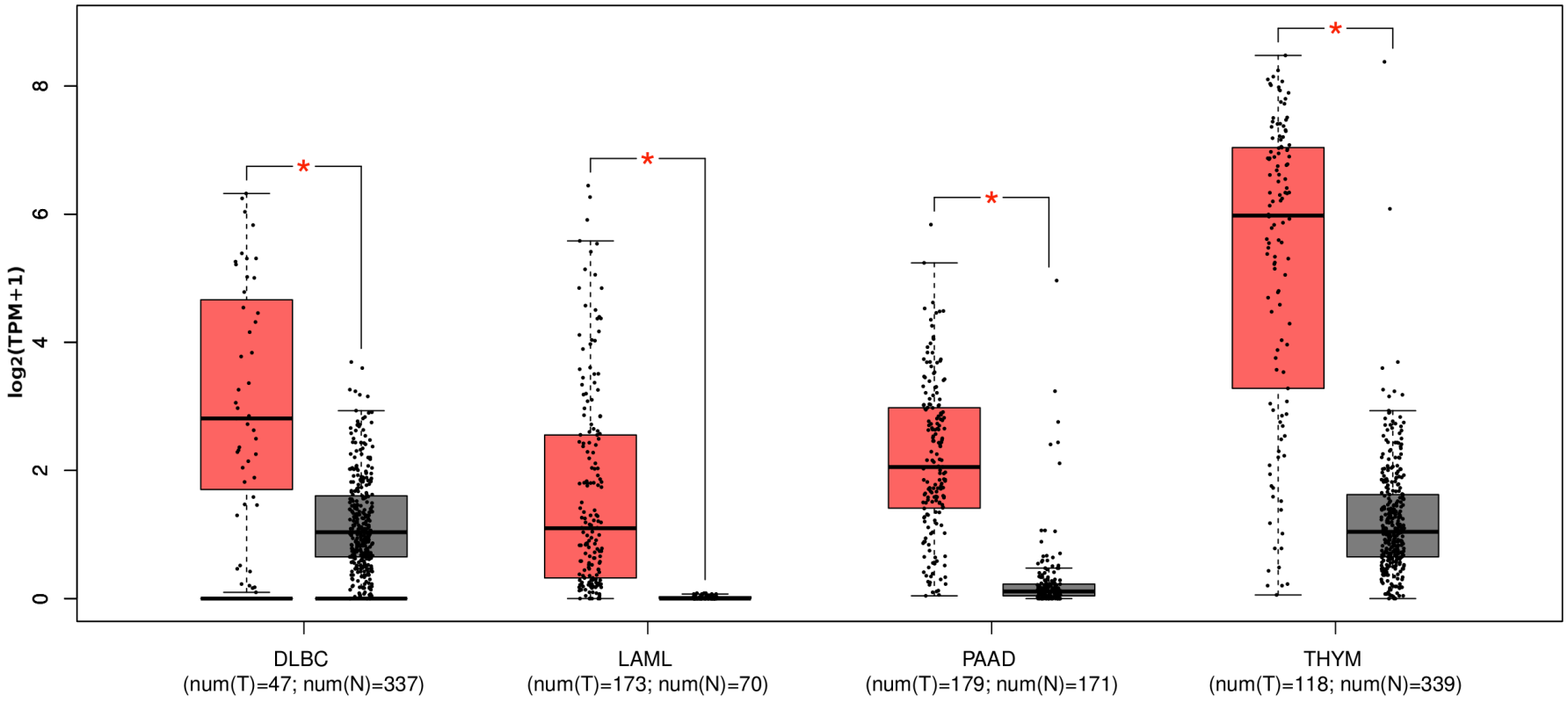
Franco Locatelli

Cohort	Disease Name	Cases	Normal
ACC	Adrenocortical carcinoma	77	128
BLCA	Bladder urothelial carcinoma	404	28
BRCA	Breast invasive carcinoma	1085	291
CESC	Cervical and endocervical cancers	306	13
CHOL	Cholangiocarcinoma	36	9
COAD	Colon adenocarcinoma	275	349
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	47	337
ESCA	Esophageal carcinoma	182	286
GBM	Glioblastoma multiforme	163	207
HNSC	Head and Neck squamous cell carcinoma	519	44
KICH	Kidney Chromophobe	66	53
KIRC	Kidney renal clear cell carcinoma	523	100
KIRP	Kidney renal papillary cell carcinoma	286	60
LAML	Acute Myeloid Leukemia	173	70
LGG	Brain Lower Grade Glioma	518	207
LIHC	Liver hepatocellular carcinoma	369	160
LUAD	Lung adenocarcinoma	483	347
LUSC	Lung squamous cell carcinoma	486	338
OV	Ovarian serous cystadenocarcinoma	426	88
PAAD	Pancreatic adenocarcinoma	179	171
PCPG	Pheochromocytoma and Paraganglioma	182	3
PRAD	Prostate adenocarcinoma	492	152
READ	Rectum adenocarcinoma	92	318
SARC	Sarcoma	262	2
SKCM	Skin Cutaneous Melanoma	461	558
STAD	Stomach adenocarcinoma	408	211
TGCT	Testicular Germ Cell Tumors	137	165
THCA	Thyroid carcinoma	512	337
THYM	Thymoma	118	339
UCEC	Uterine Corpus Endometrial Carcinoma	174	91
UCS	Uterine Carcinosarcoma	57	78

CD1c expression in human cancers vs normal tissues



Lymphoid Neoplasm Diffuse Large B-cell Lymphoma

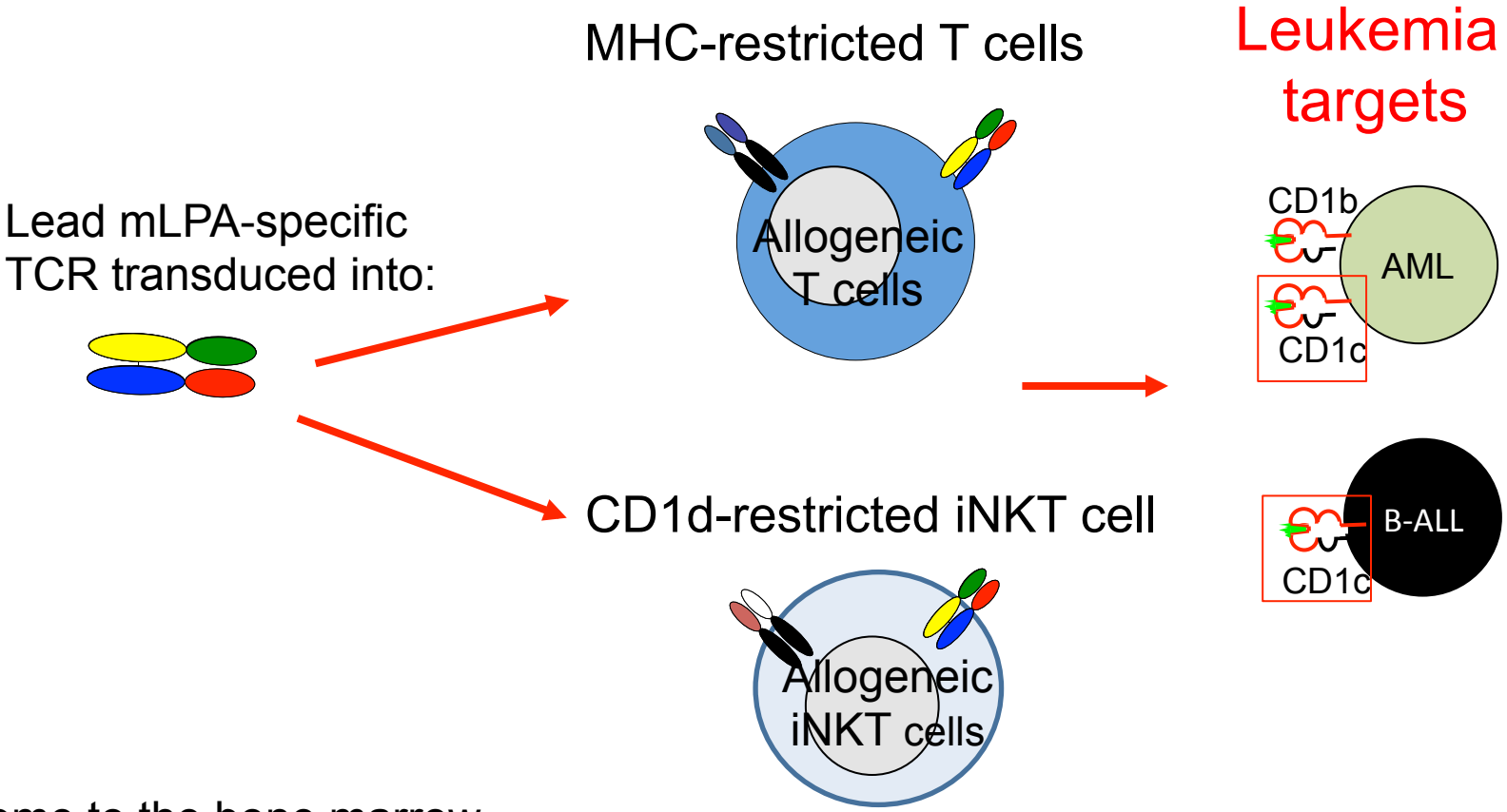
Acute Myeloid Leukemia

Pancreatic adenocarcinoma

Thymoma

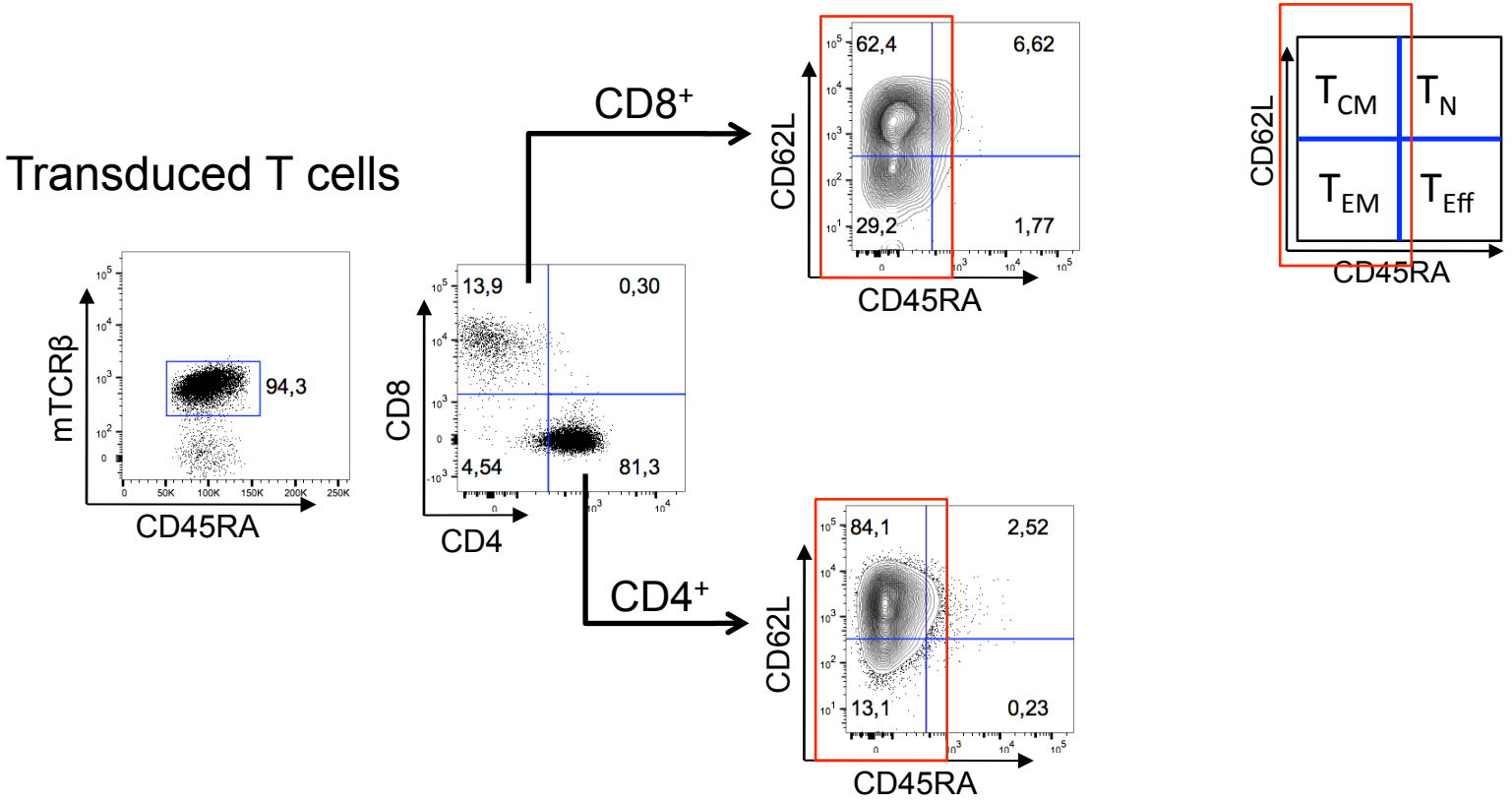
Targeting any CD1c^{+} acute leukemia with T or iNKT cells engineered with mLPA-specific TCR

Selection of the optimal recipient effector T cell subset

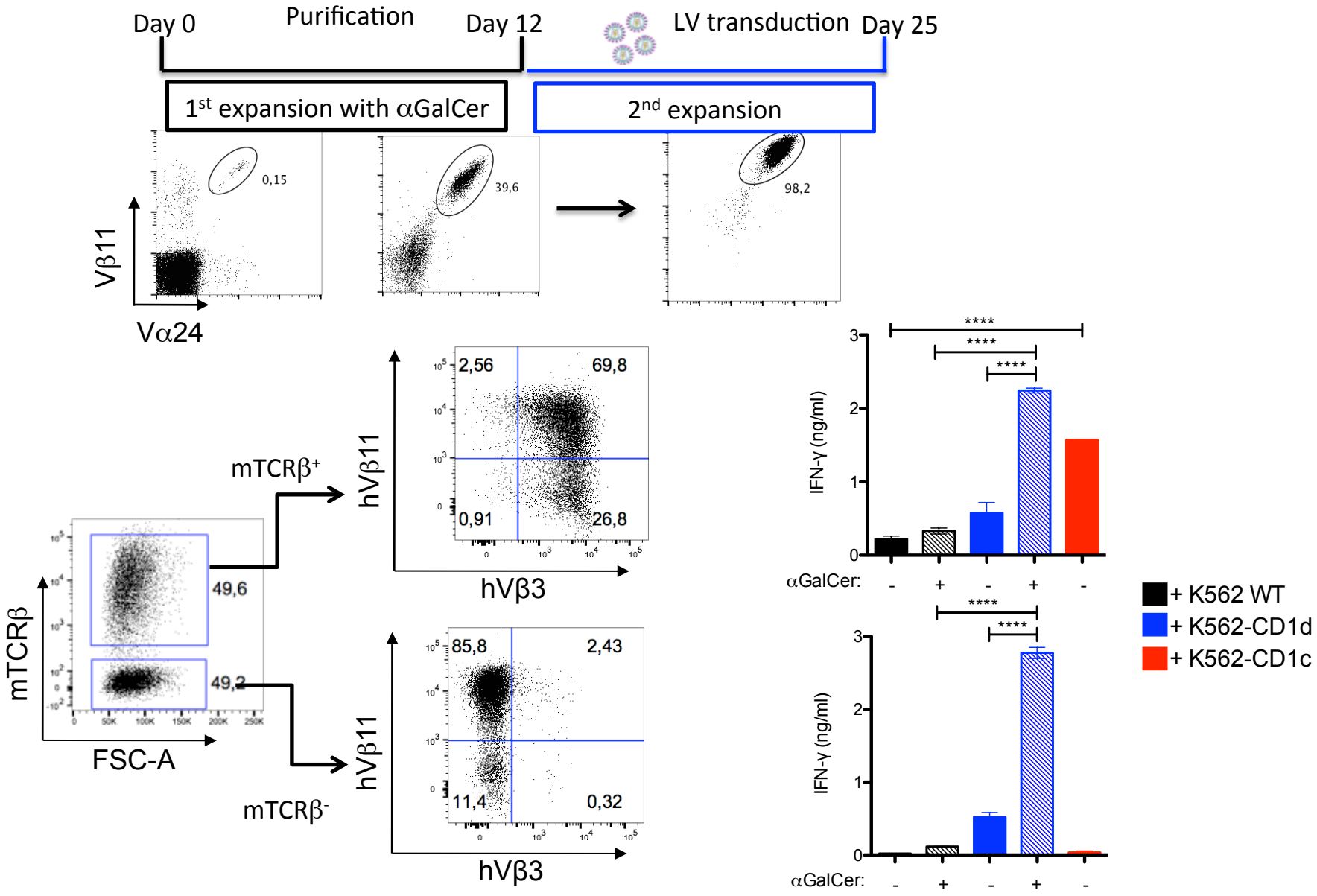


- Home to the bone marrow
- Promote GVL while preventing GVHD *(de Lalla 2011; Chaidos 2012; Rubio 2012; Morris 2005; Pillai 2007)*
- Reprogram cancer-opposing functions in the tumor microenvironment

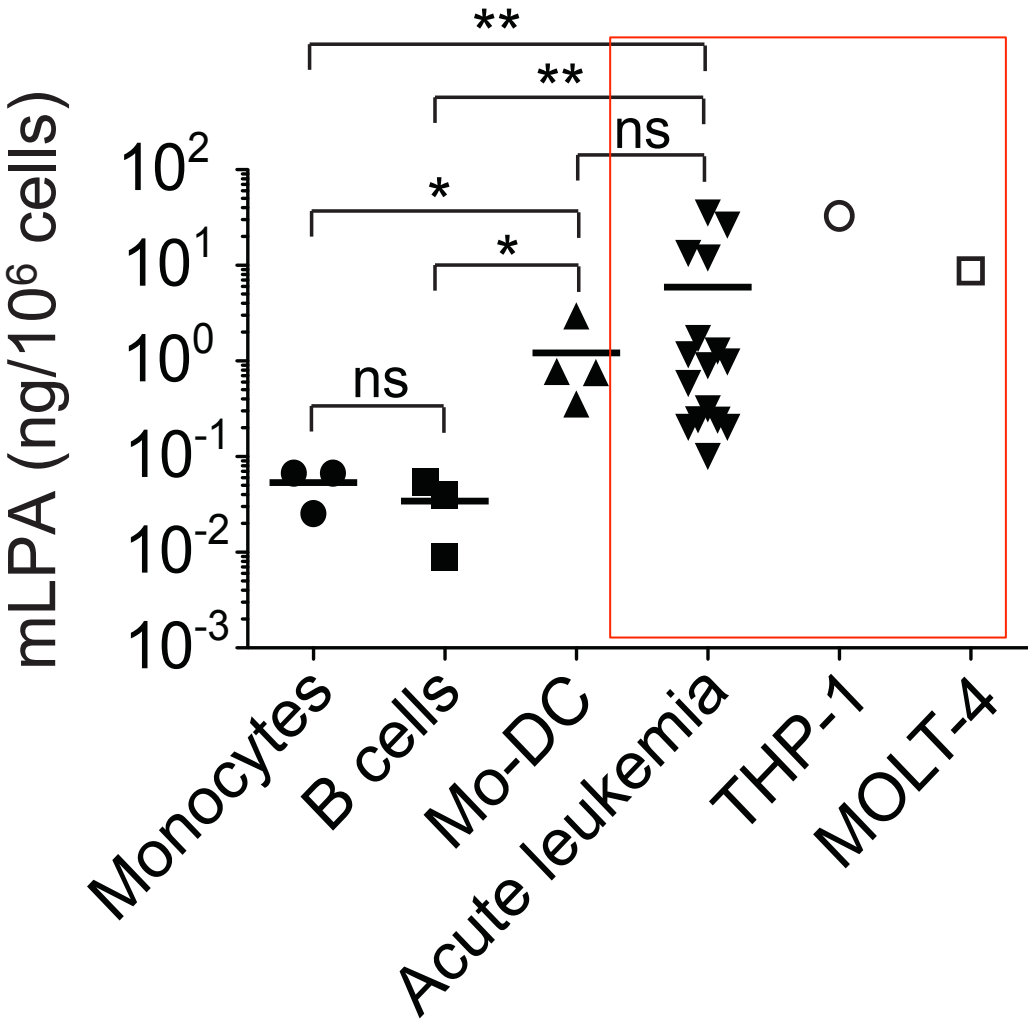
TCR transduction of in vitro expanded $CD4^+$ and $CD8^+$ $T_{CM/EM}$ cells (anti-CD3/CD28 beads + IL-7/IL-15)



Efficient iNKT cell transduction with the lead mLPA-specific TCR DN4.99



mLPA is enriched in leukemia and in Mo-DCs



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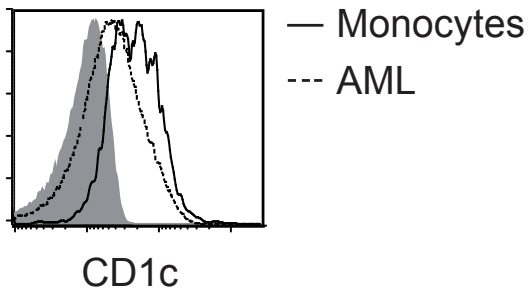
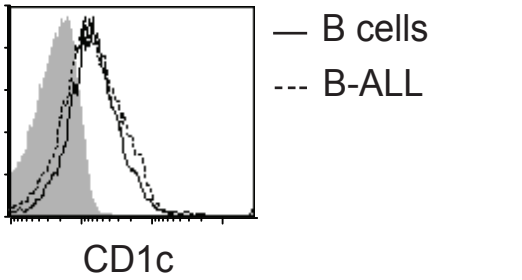
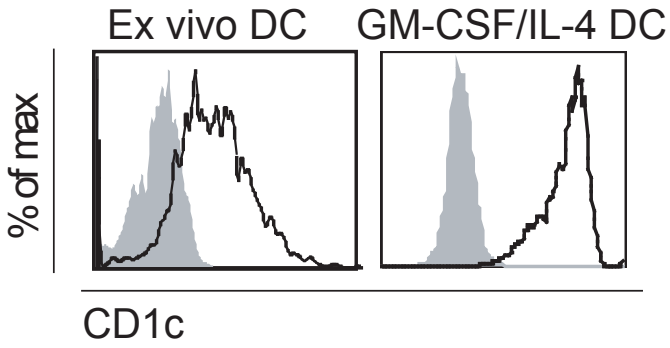
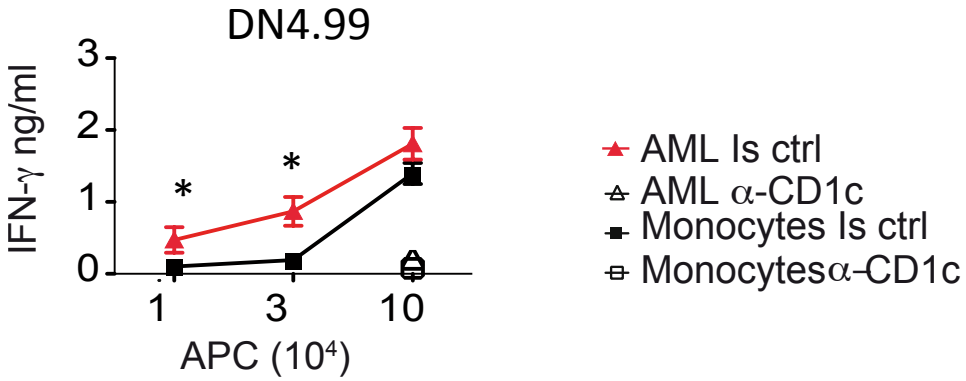
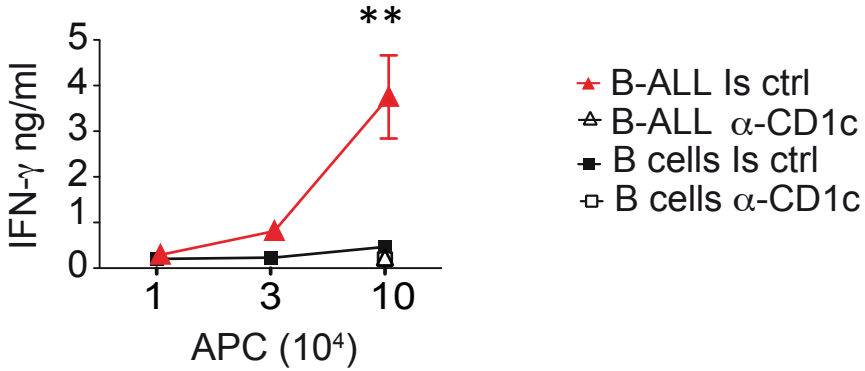
Differential recognition of normal and malignant cells by mLPA-specific T cells

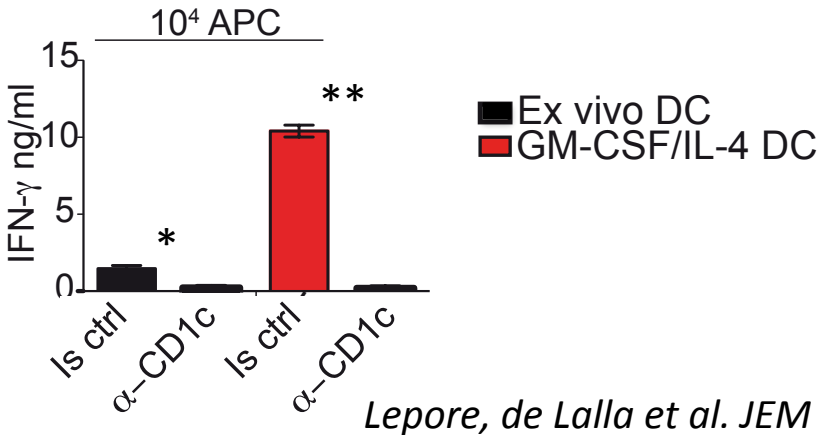












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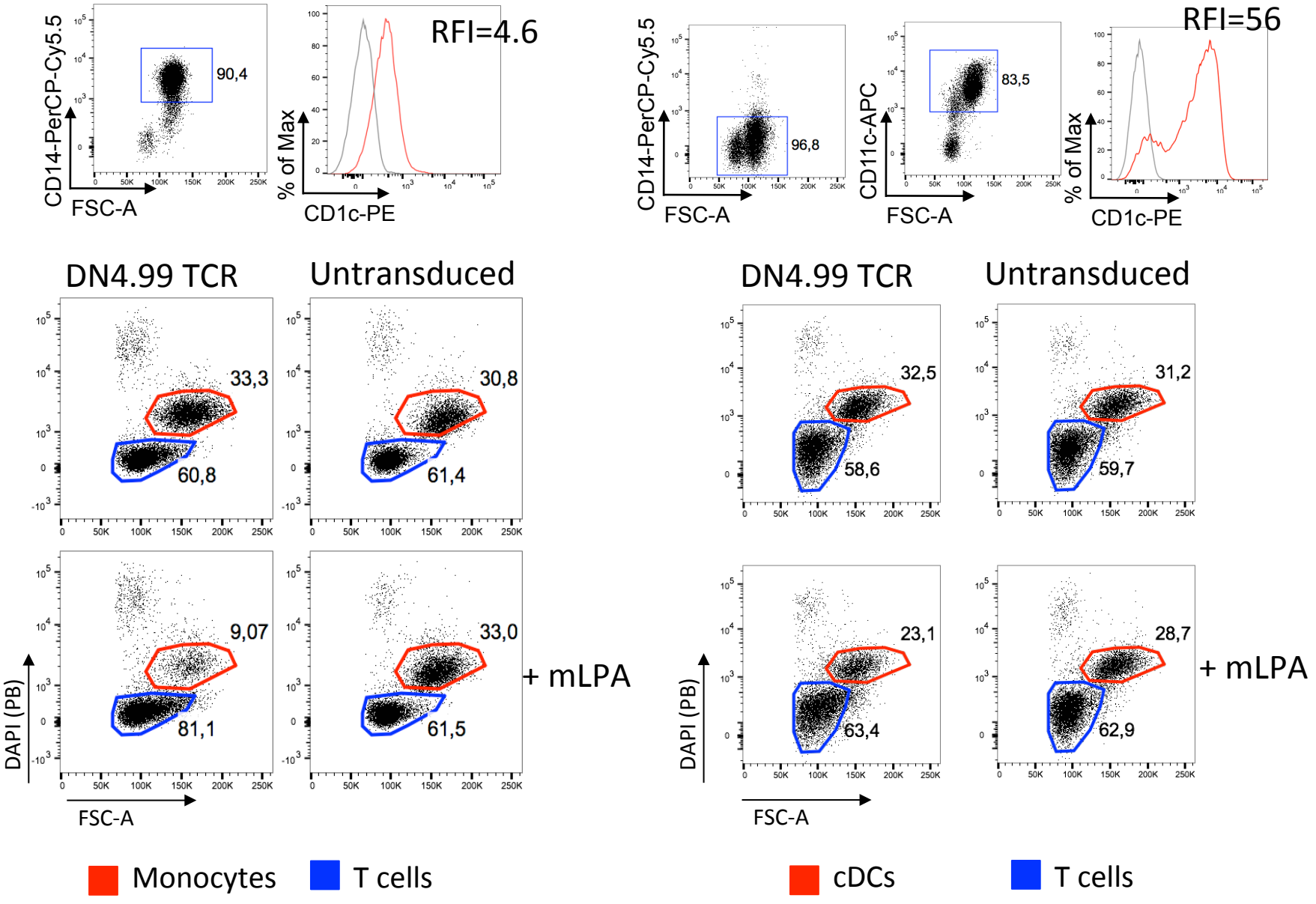
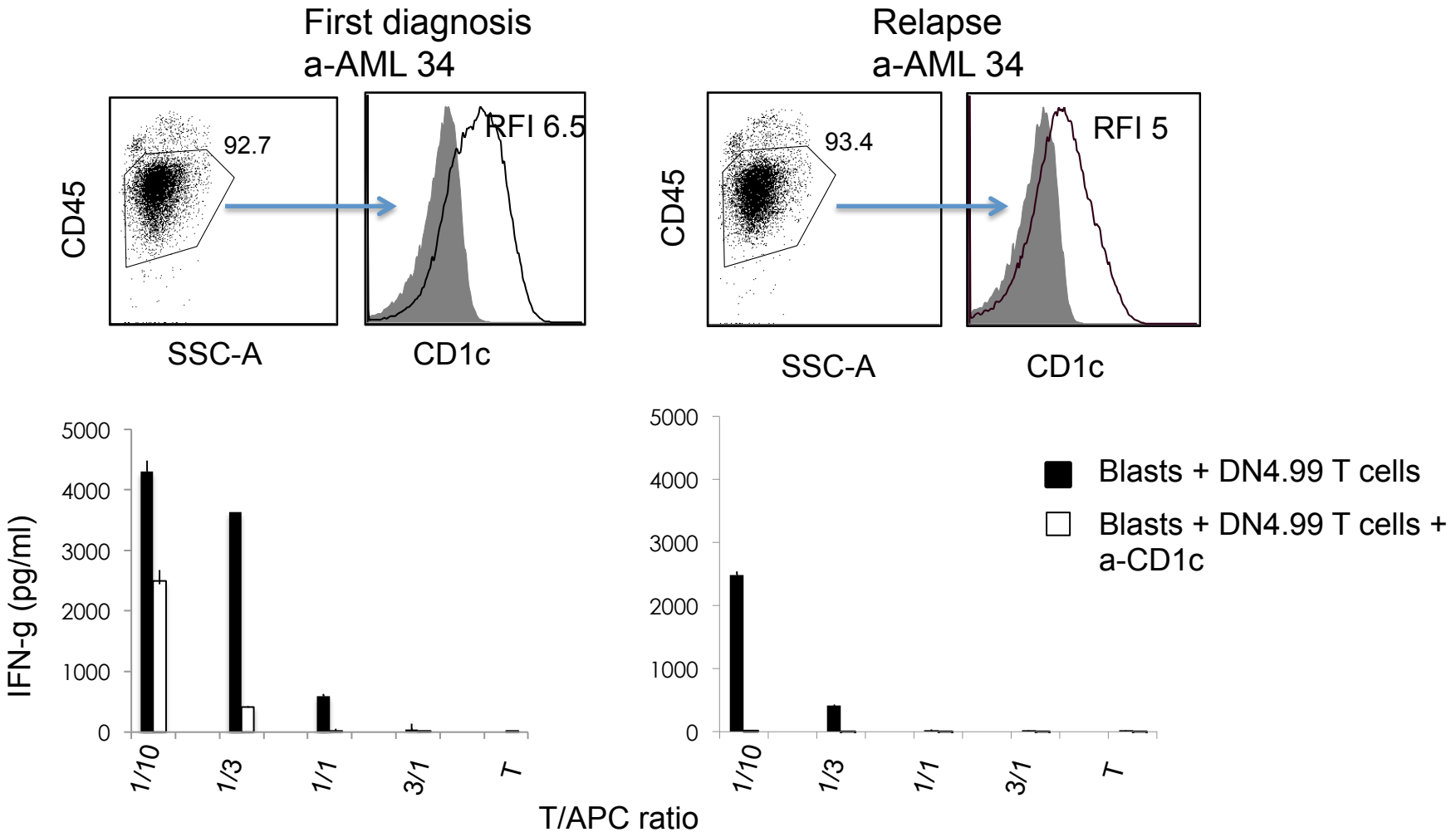
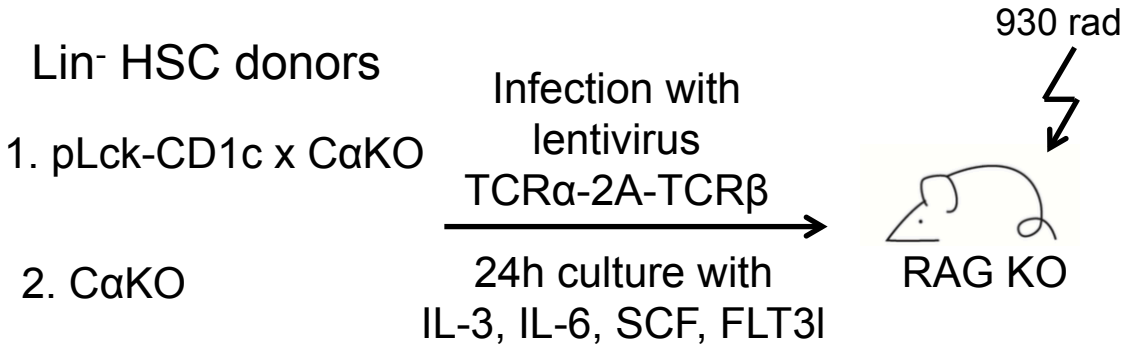
T cells transduced with DN4.99 TCR do not kill monocytes and DC



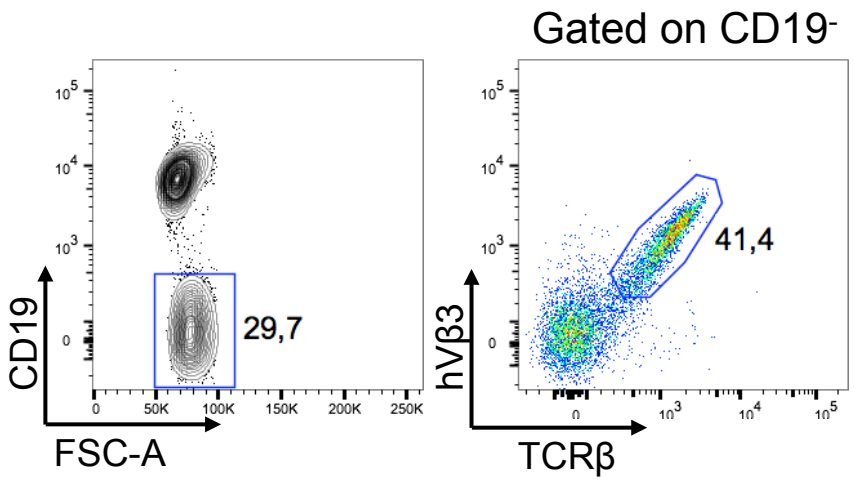
self-reactive CD1c restricted T cells recognize also relapsed leukemia blasts



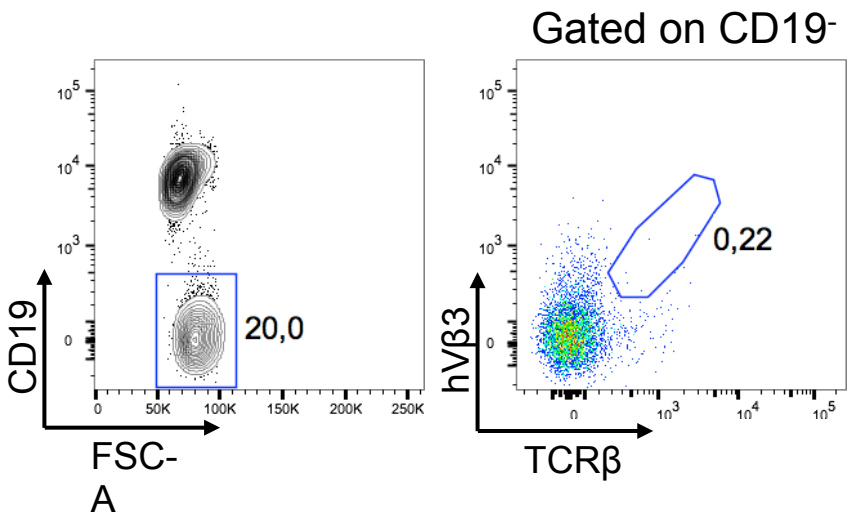
Generation of retrogenic mice expressing mLPA specific TCR and CD1c molecules



1.pLck-CD1c x Cα KO mice
EXPERIMENTAL GROUP



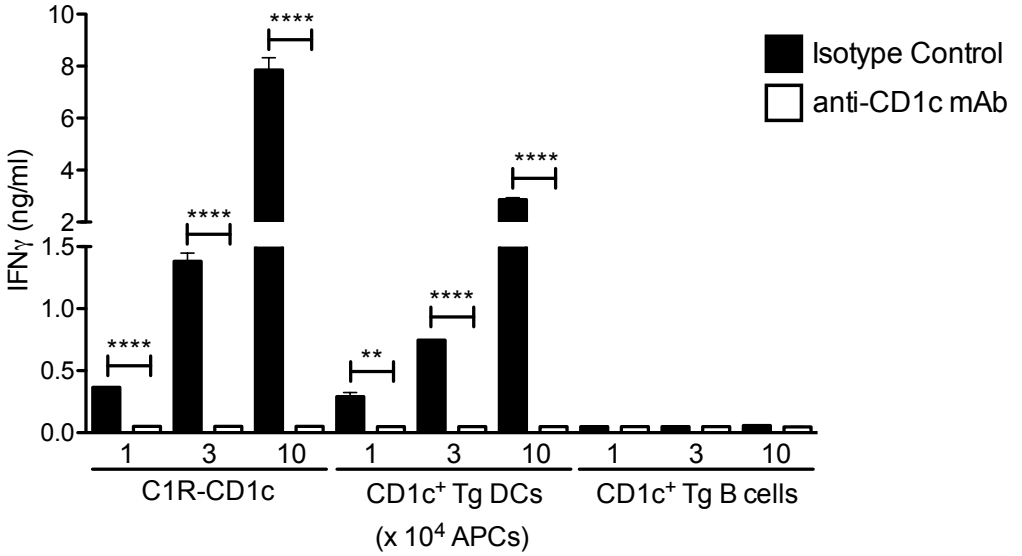
2. Cα KO mice
CONTROL GROUP

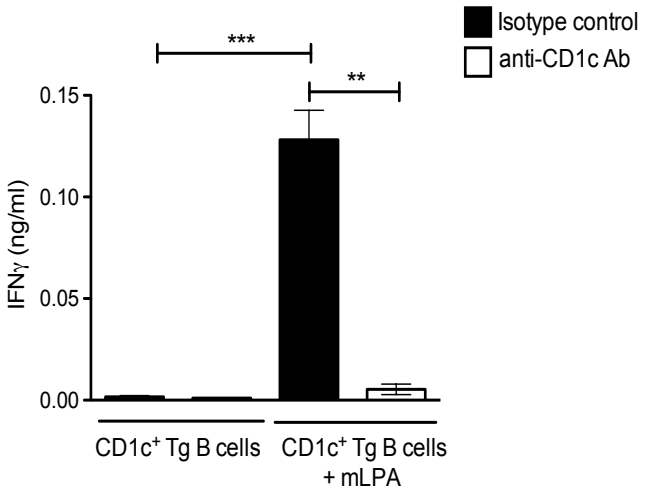


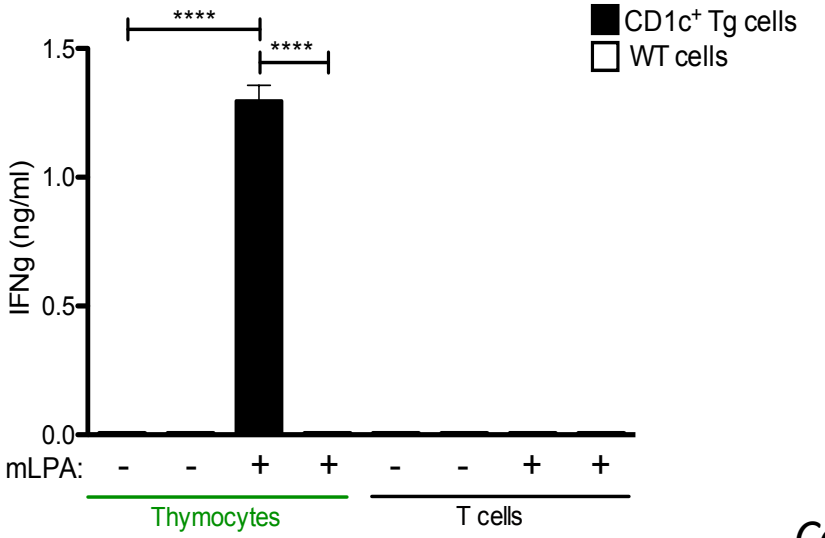
OSPEDALE SAN RAFFAELE

Consonni M. unpublished

Mouse Tg DCs, but not B cells, stimulate CD1c self-reactive human T cell clones

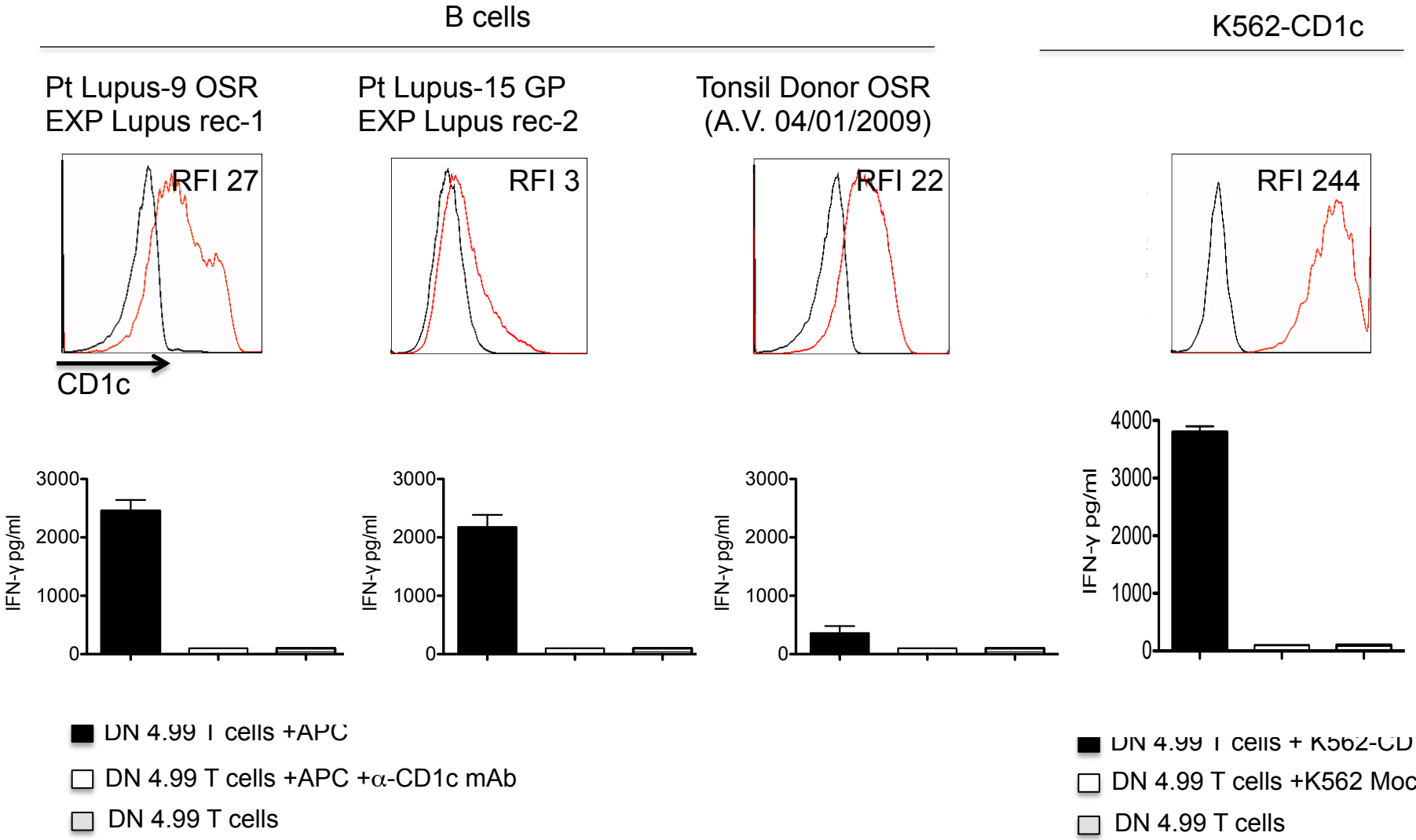






Consonni M. unpublished

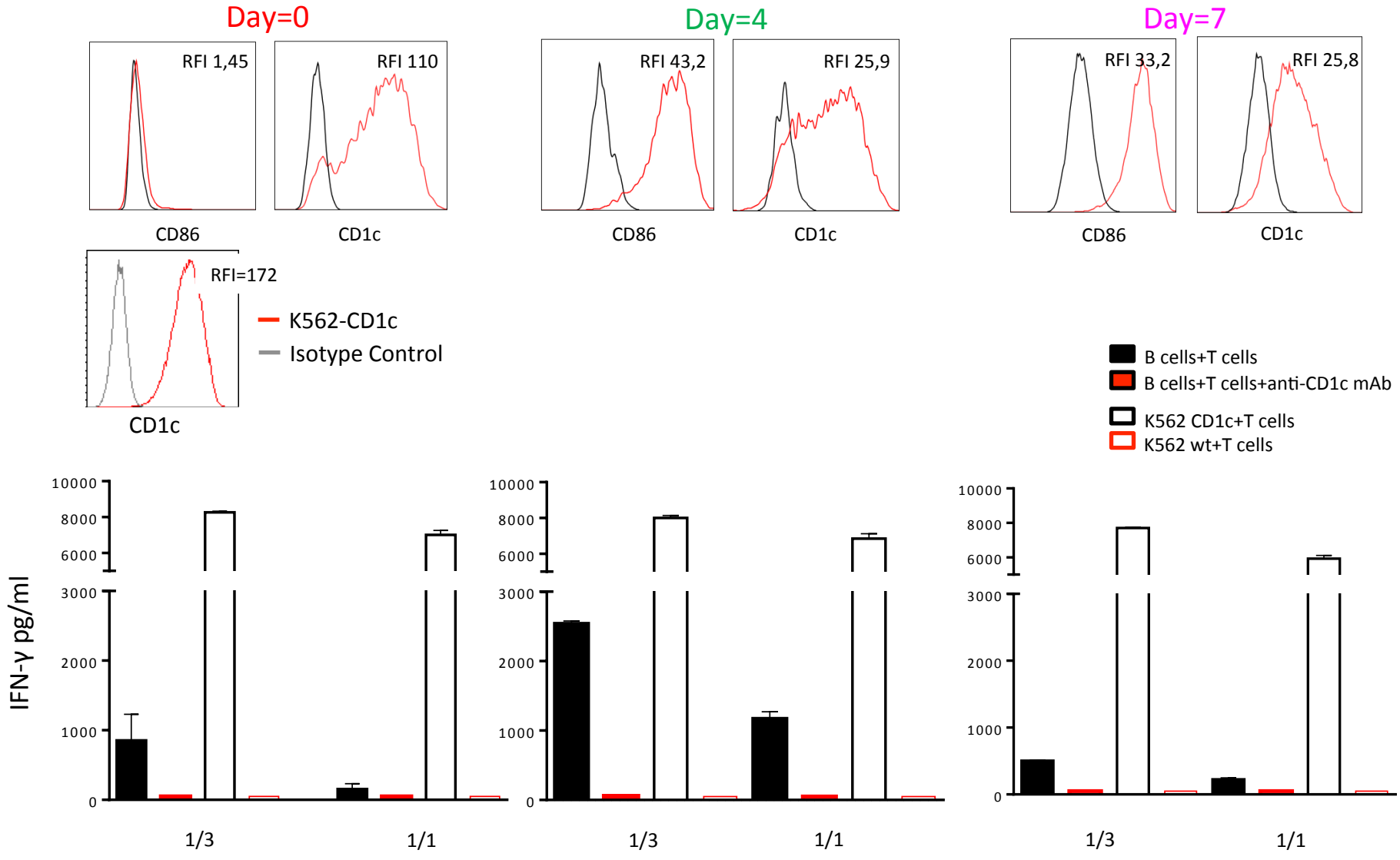
B cells from Lupus patients are recognized by mLPA specific T cells



T cells 20.000/well / 200.000 APC/well
α-CD1c mAb (20 μg/ml M241)
48 hrs co-culture 37^C

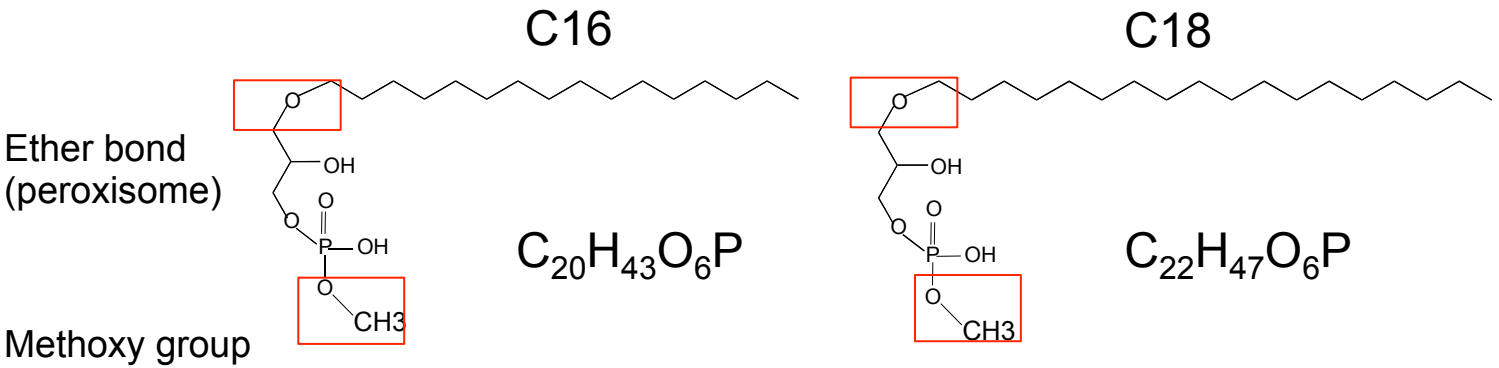
B cells were purified with α-CD19 beads

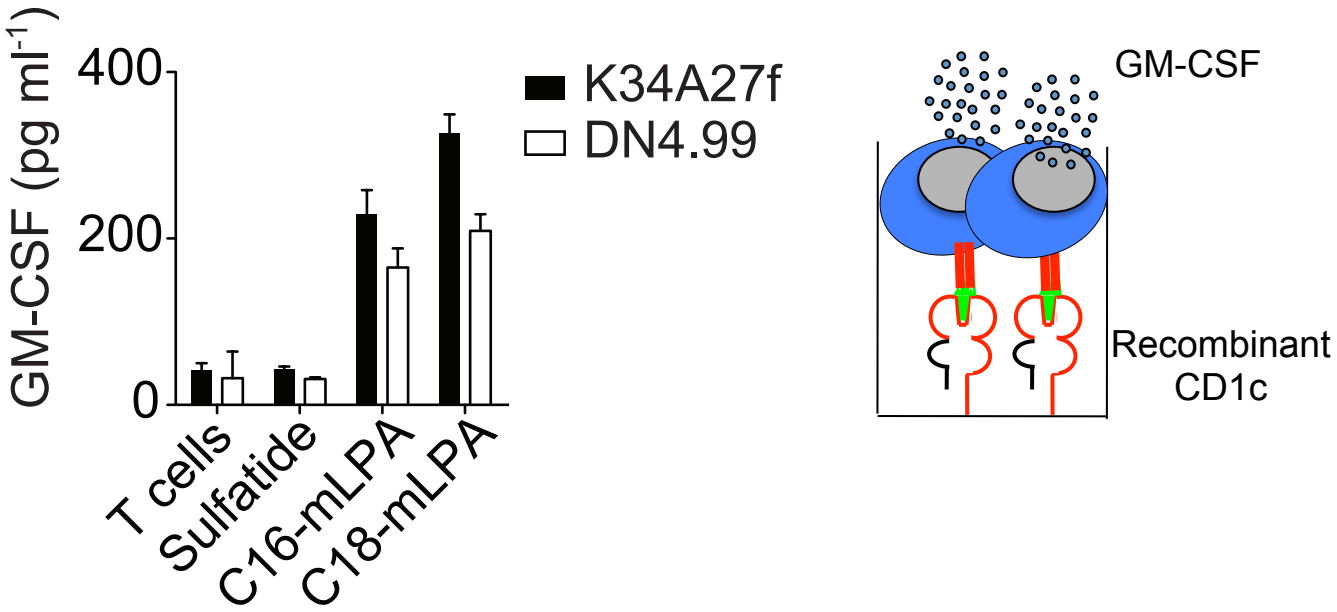
Normal B cells activated in vitro by CD40L + IL-21 stimulate mLPA specific T cell clones



20.000 T cell DN 4.99

Methyl lysophosphatidic acid (mLPA) stimulates CD1c self-reactive T cells





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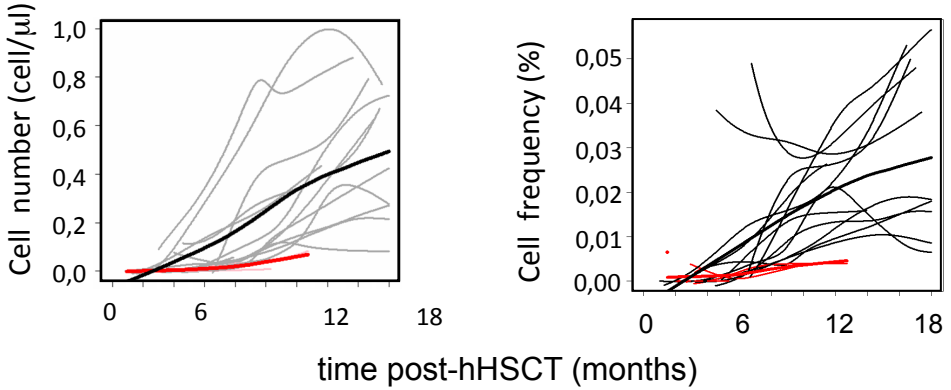
iNKT cells fail to reconstitute post-hHSCT in relapsing patients



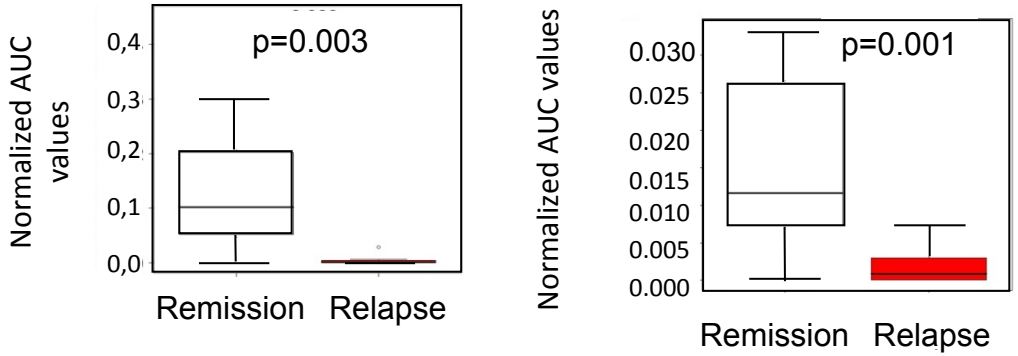
Longitudinal means:

remission

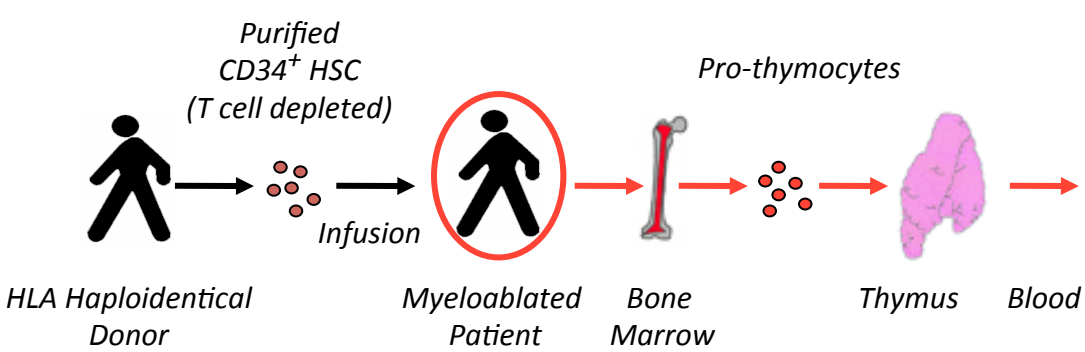
average >100 iNKT cells/10^6 T cells

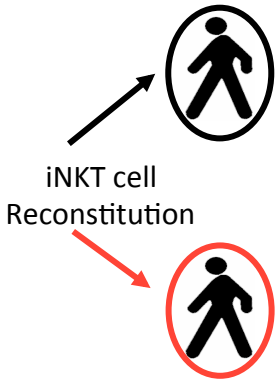
relapse

average <20 iNKT cells/10^6 T cells



de Lalla 2011

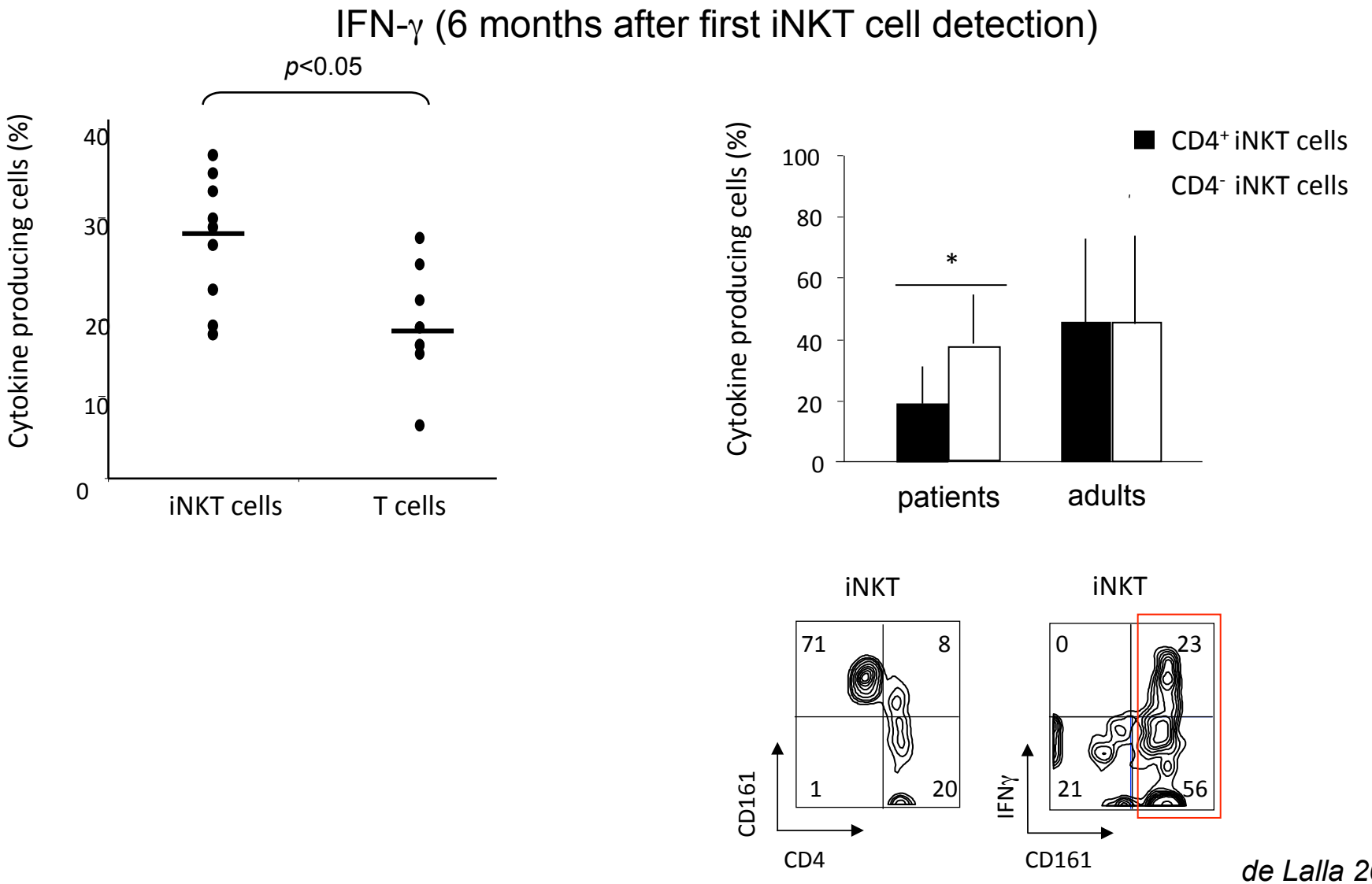




REMISSION
iNKT cell reconstitution
(iNKT cells >100/10^6 T cells)

RELAPSE
No iNKT cell reconstitution
(iNKT cells <20 /10^6 T cells)

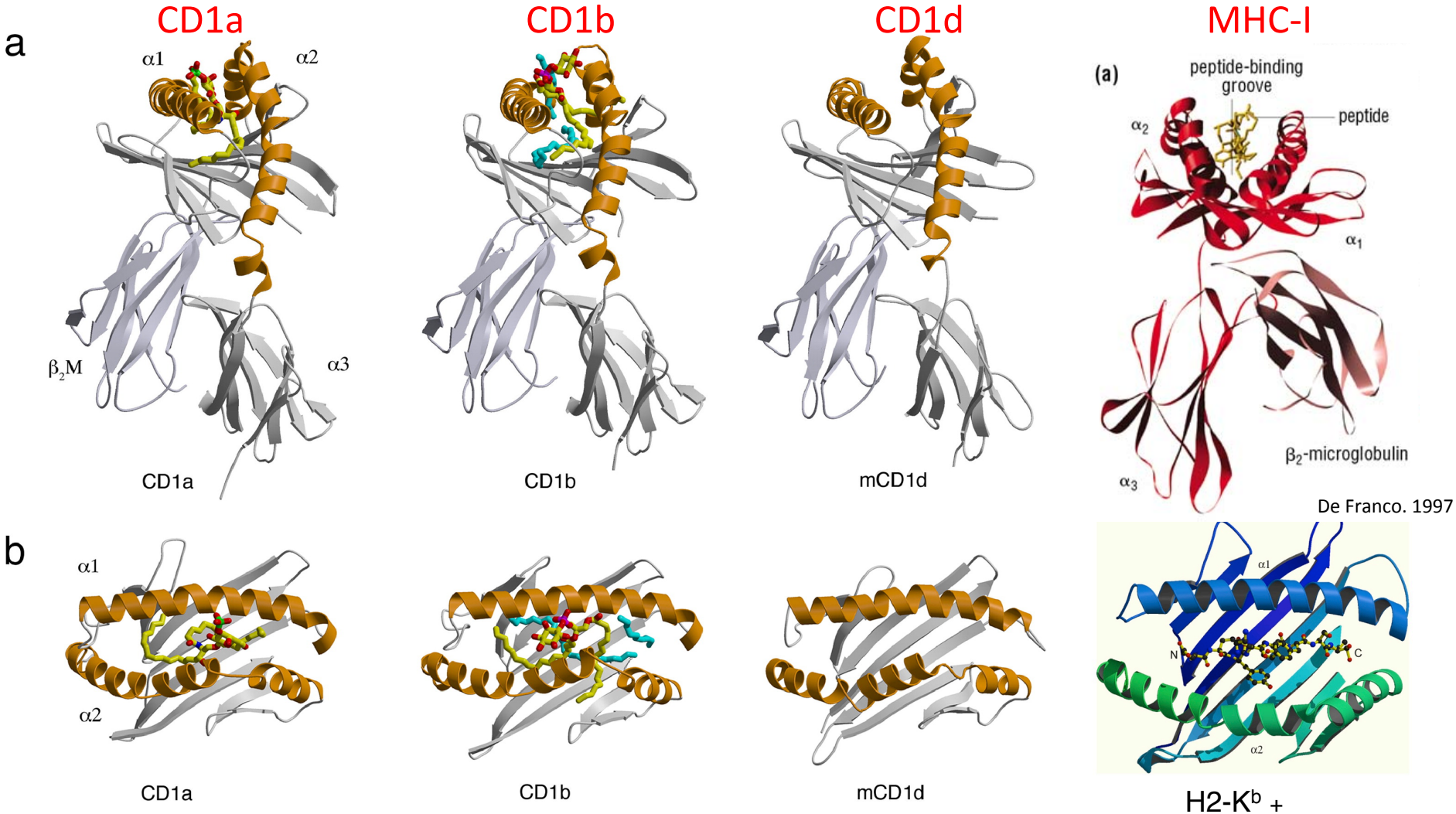
iNKT cells rapidly acquire anti-tumor effector competence post-hHSCT



de Lalla 2011

CD1 molecules display MHC-I like structures

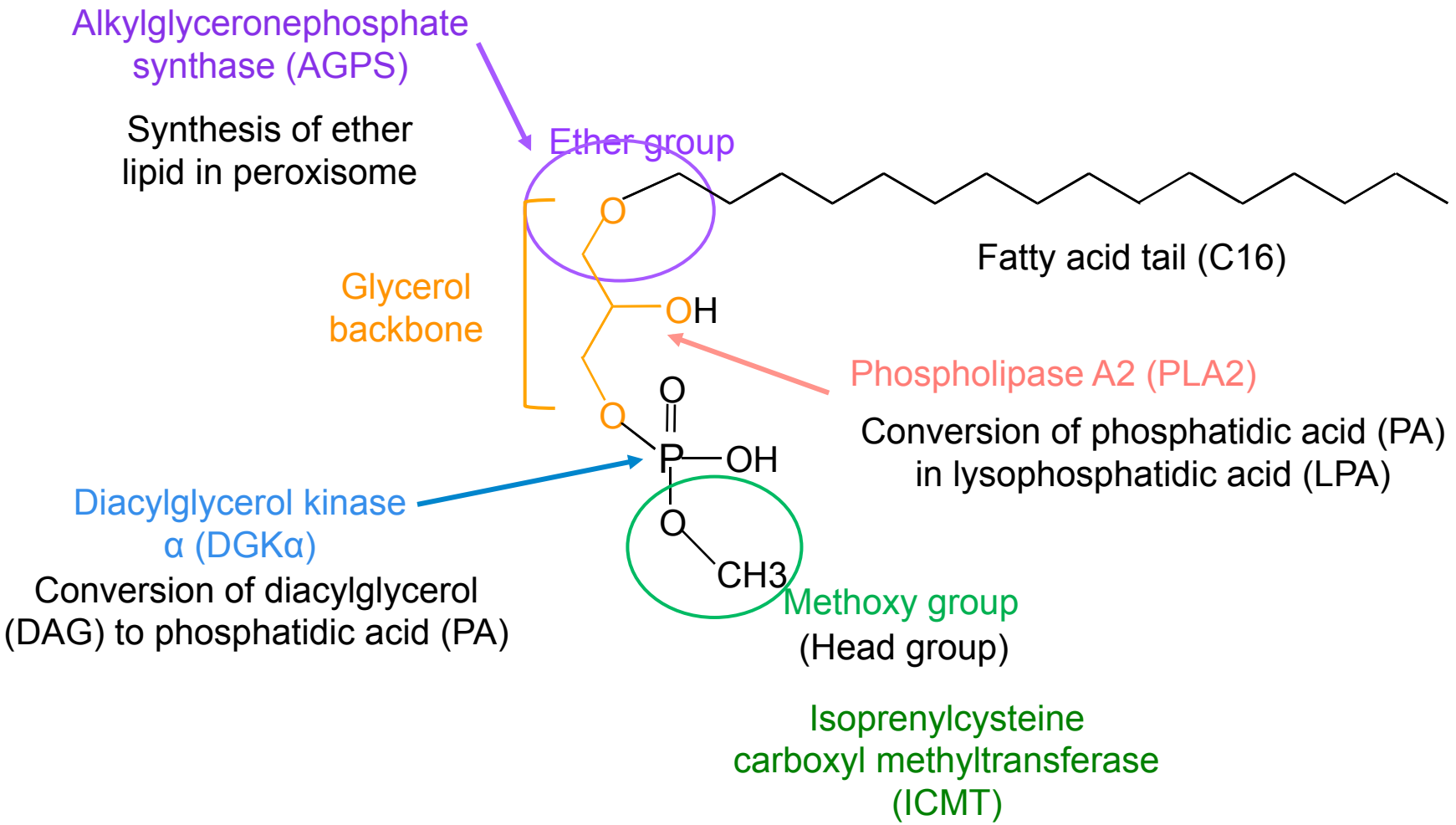
The CD1 groove is significantly deeper and lined almost entirely with non-polar or hydrophobic amino acid side chains



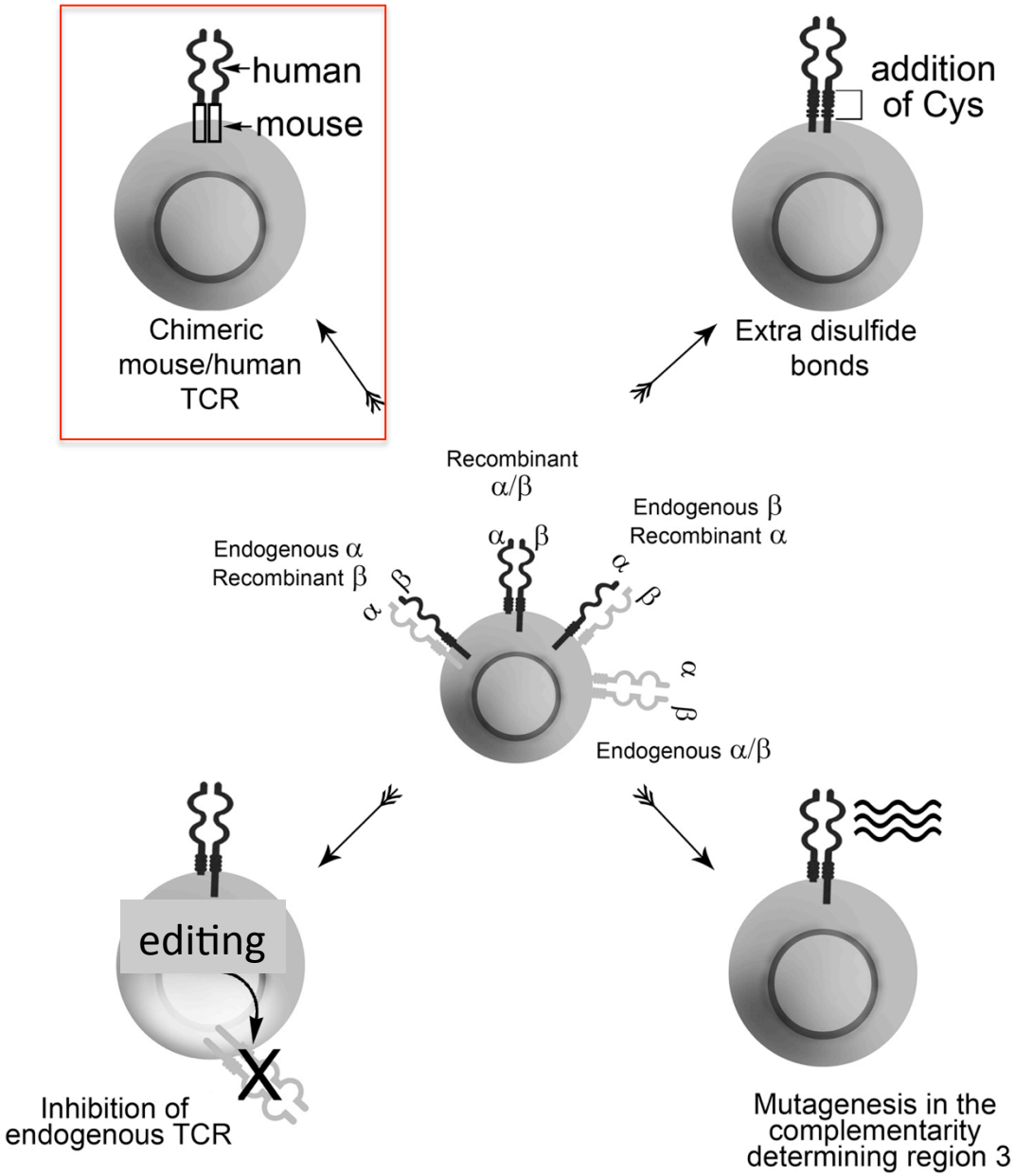
Brigl & Brenner Annu Rev Immunol 2004

Unravel possible pathways of mLPA synthesis
(inhibition of target enzymes by shRNA or CRISPR/Cas9)

with Massimo Degano (Biocrystallography Unit)
Andrea Graziani (Lipid Signaling in cancer and metabolism Unit)
Annapaola Andolfo (Protein Microsiquencing Facility)



Strategies to overcome mispairing with endogenous TCRαβ chains

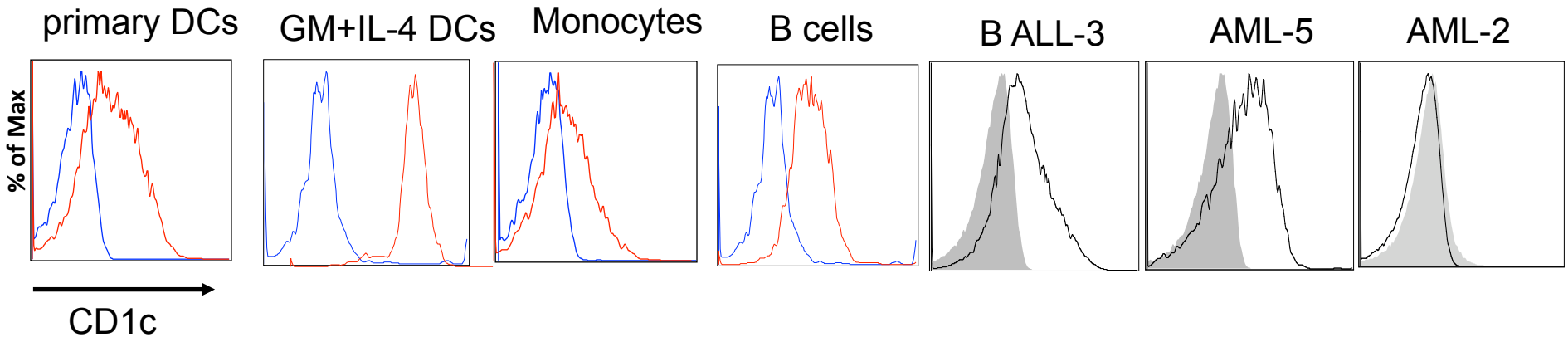


Define the epigenetic and transcriptional control of CD1 genes in normal APCs vs ALL

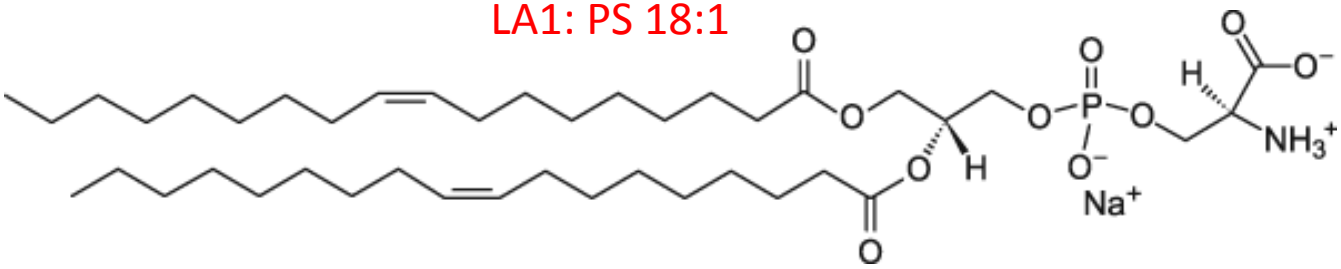
(by ATAC and RNAseq, with G. Natoli Humanitas Inst; OSR Translational Genomics and Bioinformatics)

Malignancy	Number	Patients with CD1-expressing blasts			
		CD1a	CD1b	CD1c	CD1d
		%	%	%	%
Pediatric patients					
AML	9	0	45	45	0
B-ALL	23	4	22	26	4
T-ALL	8	75	75	12	50
Adult patients					
AML	33	3	54	51	24
B-ALL	7	0	0	71	29

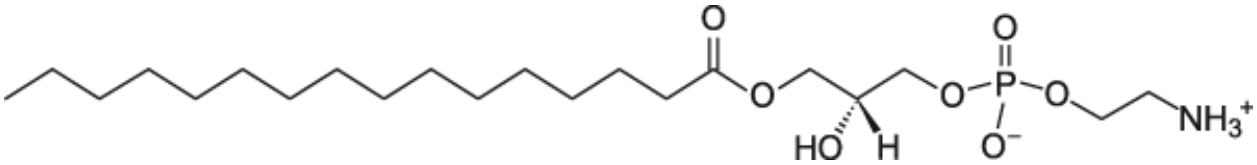
Data refer to circulating blasts. Similar CD1 expression frequencies were observed in bone marrow biopsy samples.



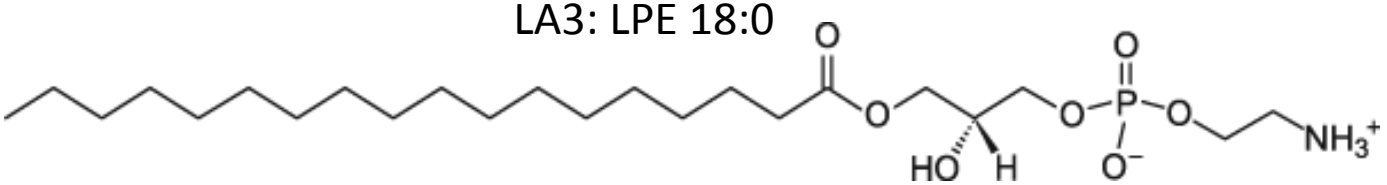
LA1: PS 18:1



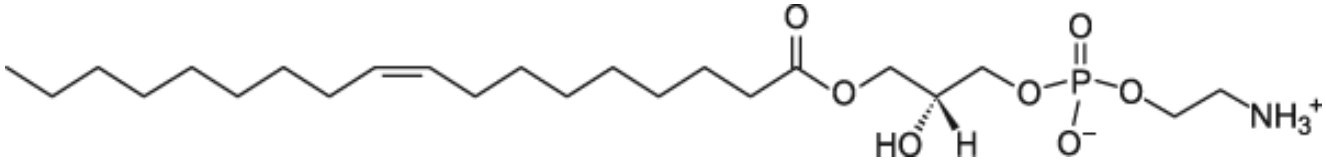
LA2: LPE 16:0



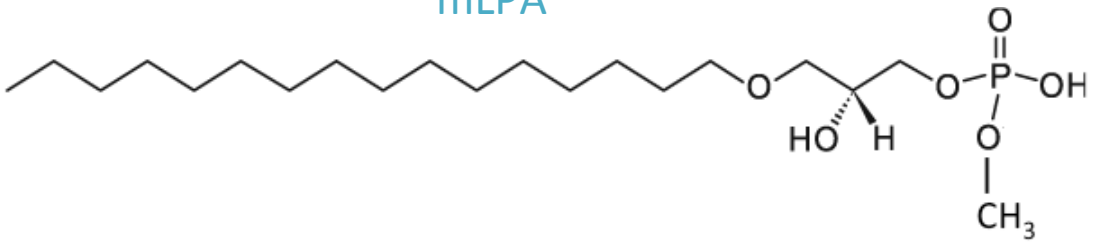
LA3: LPE 18:0



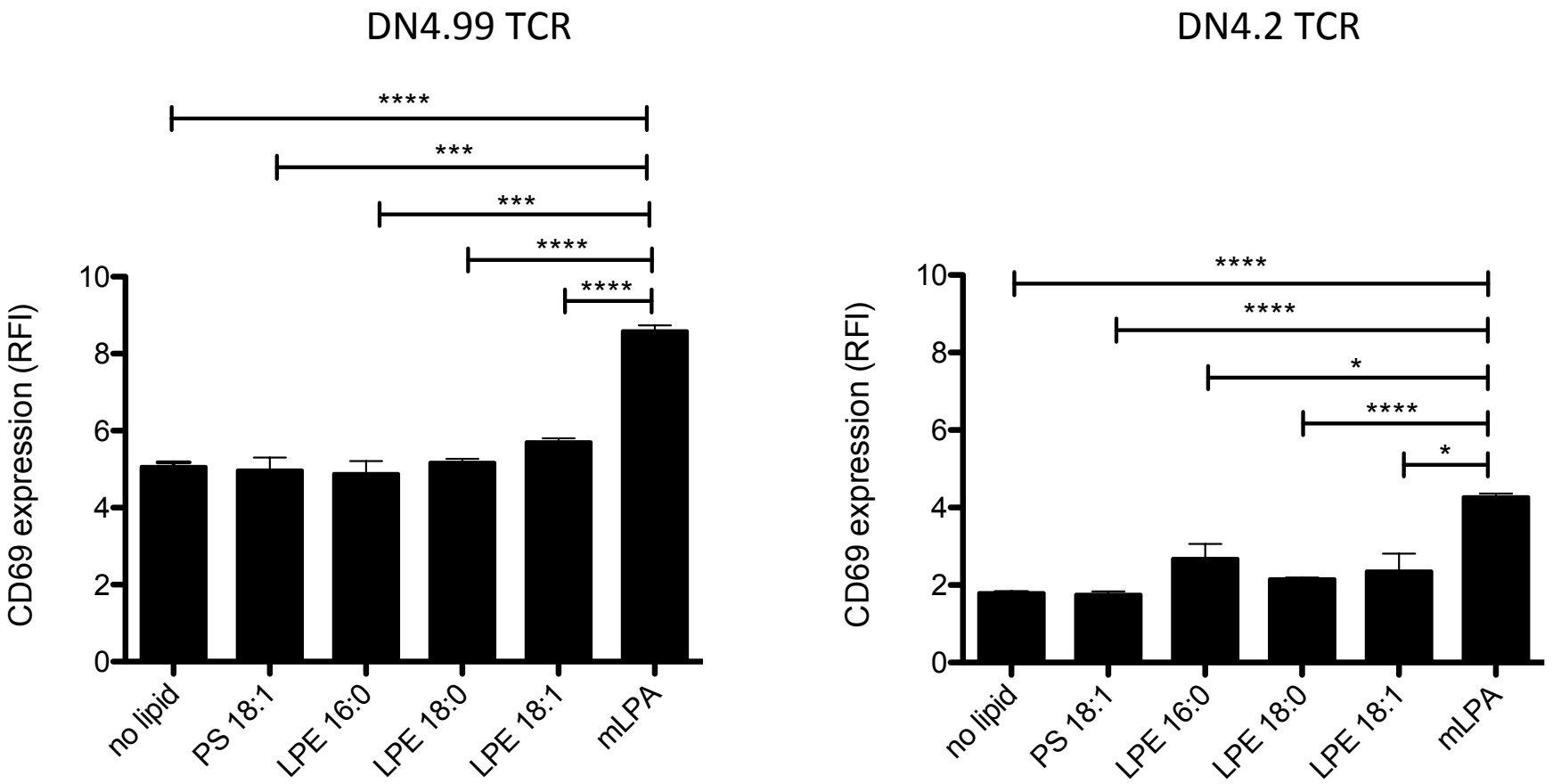
LA4: LPE 18:1



mLPA

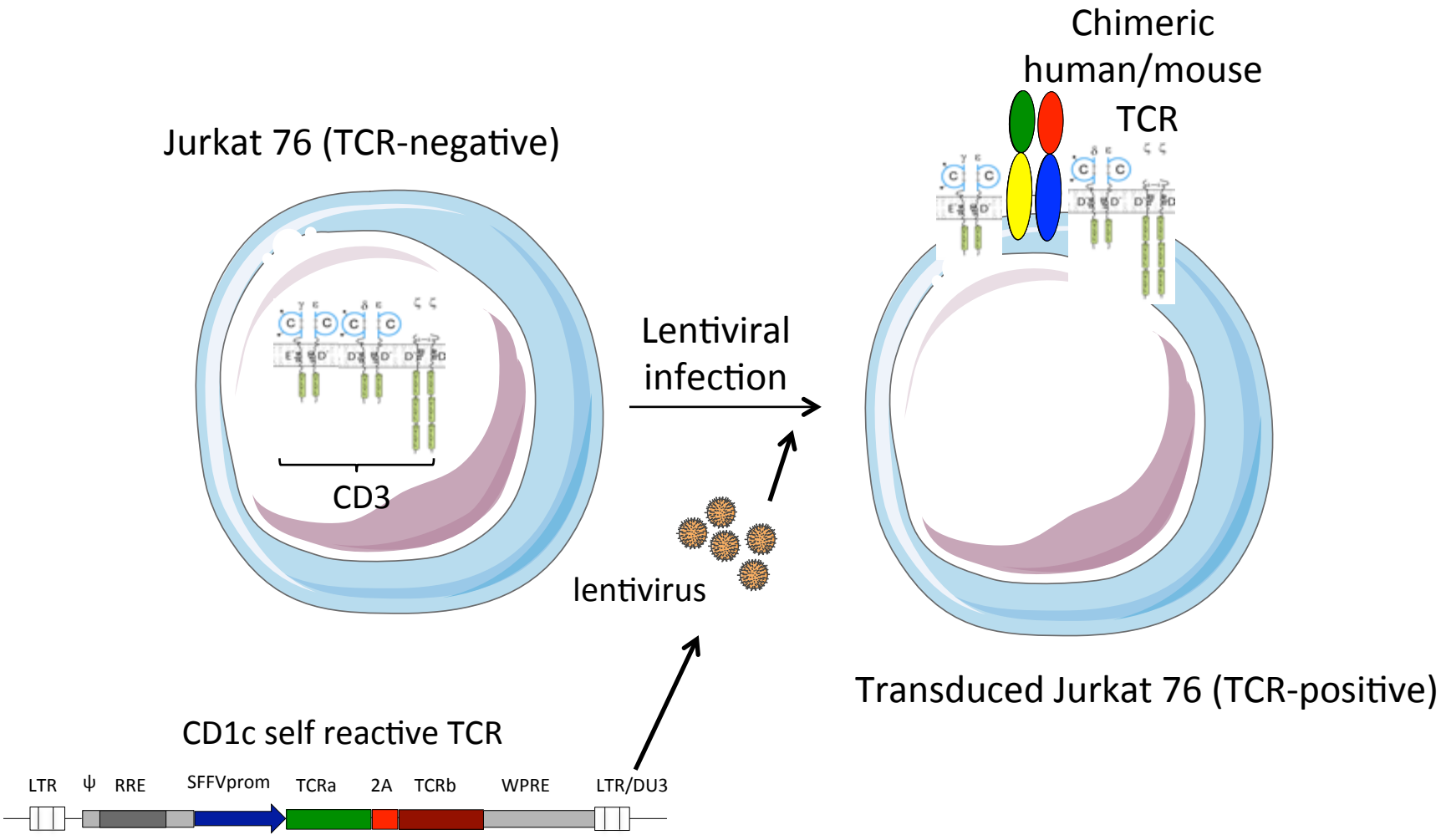


CD69 expression on Jurkat 76 cells transduced with TCRs DN4.99 and DN4.2 T cell clones



ON co-culture with K562-CD1c cells loaded with 4µg/ml of indicated lipids

Transduction of Jurkat 76 cell line with the CD1c-self reactive TCRs



CD1 present both exogenous (bacterial) and endogenous (self) lipid antigens

Exogenous (Bacterial)

Endogenous (self)

Source	Antigen	CD1 isoform	Refs
Mycobacterium tuberculosis and other mycobacteria	Mycolic acids	CD1b	3
	Glucose monomycolate	CD1b	132
	Sulpholipid (diacylated sulphoglycolipid)	CD1b	8
	Phosphatidylinositol mannosides	CD1b, CD1d	5,133
	Mannosylated lipoarabinomannan	CD1b	5
	Mannosyl-β1-phosphomycoketides	CD1c	6,134
	Didehydroxymycobactin	CD1a	124
Sphingomonas spp.	α-Glucuronosylceramide	CD1d	9,10
Borrelia burgdorferi	α-Galactosyldiacylglycerol	CD1d	11
Leishmania donovani	Lipophosphoglycan	CD1d	12
Mammalian (self)	Phosphatidylinositol	CD1d	18
	Phosphatidylglycerol	CD1d	18
	Phosphatidylethanolamine	CD1d	18
	GM1	CD1b	20,21
	GD3	CD1d	22
	Sulphatide	CD1a, CD1b, CD1c	19
	Isoglobotrihexosylceramide	CD1d	10,23
Synthetic or marine sponge	α-Galactosylceramide	CD1d	125

Barral DC, Brenner MB. Nat Rev Immunol. 2007