Retroviral and Lentiviral Vectors for Long-Term Gene Correction:
Clinical Challenges in Vector and Trial Design

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Mouse Models for Predicting Insertional Mutagenesis

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Mouse models

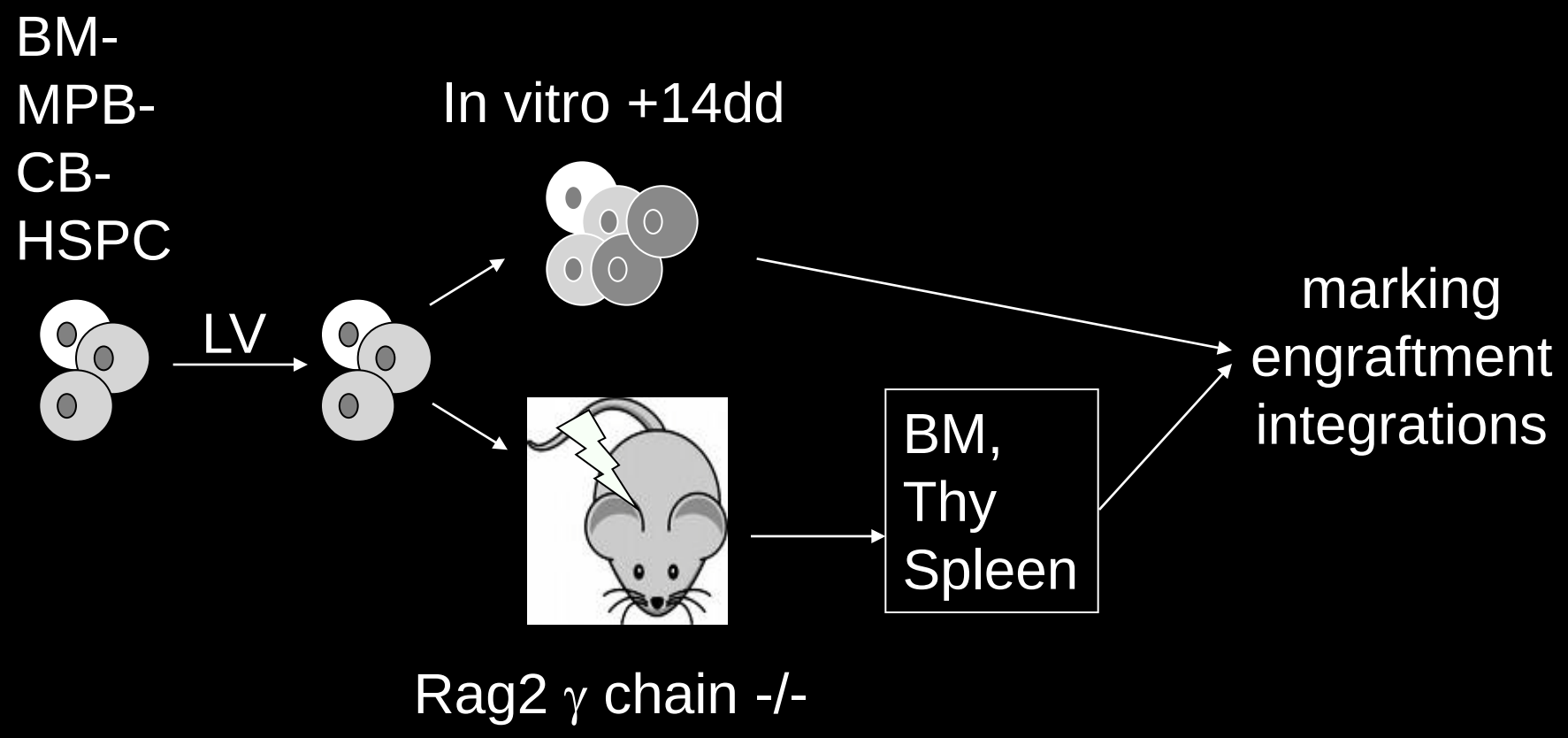
- *May provide sensitive readouts to test insertional mutagenesis in vivo and to study integration site selection*

  - *Tumor-prone transgenic mice (Cdkn2a$^{-/-}$)*
    - genetic background sensitized to activation of oncogenes
    - single-hit at synergizing locus sufficient to accelerate oncogenesis

  - *Human/mouse hematochimeras*
    - Immunodeficient models allow engraftment proliferation and differentiation of human hematopoietic cells
    - Model to study vector integrations in human cells in vivo

Immunodeficient mouse models to study integration profiles in human cells

In collaboration with Alessandra Biffi



Integrations from 12 Rag2$^{-/-}$γchain$^{-/-}$ mice

CD34 Source	*Sample*	*N Replicates*	*N Reads*	*N Ints*
BM	LC (*in vitro*)	1	1897	126
	BM	6	8451	293
	Spleen	3	3505	77
	Thymus	3	7332	57
MPB	LC (*in vitro*)	2	14437	641
	BM	2	436	222
	Spleen	2	531	68
	Thymus	2	1447	54
	LC GFP (*in vitro*)	1	344	267
GFP CB	LC (*in vitro*)	2	490	361
	Blood	5	427	12
	BM	4	1831	73
	Spleen	5	933	42
	Thymus	5	1846	32
Totals	3 MPB *in vitro* samples (1 GFP); 2 MBP mice;1 BM *in vitro* sample; 6 BM mice; 2 CB *in vitro* GFP samples; 5 CB GFP mice	**43**	**43907**	**2325**

CIS in LV integration datasets

N Hits	***Genes (Interval Kb)***
4	CARD8 (23), NSD1 (90), QRICH1 (31), SAPS2 (47), USP48 (48)
5	GPATCH8 (53)
6	FCHSD2 (272)
7	NPLOC4 (76), SMARCC1 (95), NF1 (140)
8	PACS1 (110), HLA (542)
9	FBXL11 (107)

- 85% of our CIS overlap with CIS found in ALD clinical trial

First LV HSC gene therapy trial

RESEARCH ARTICLE

Science AAAS

Nov. 2009

Hematopoietic Stem Cell Gene Therapy with a Lentiviral Vector in X-Linked Adrenoleukodystrophy

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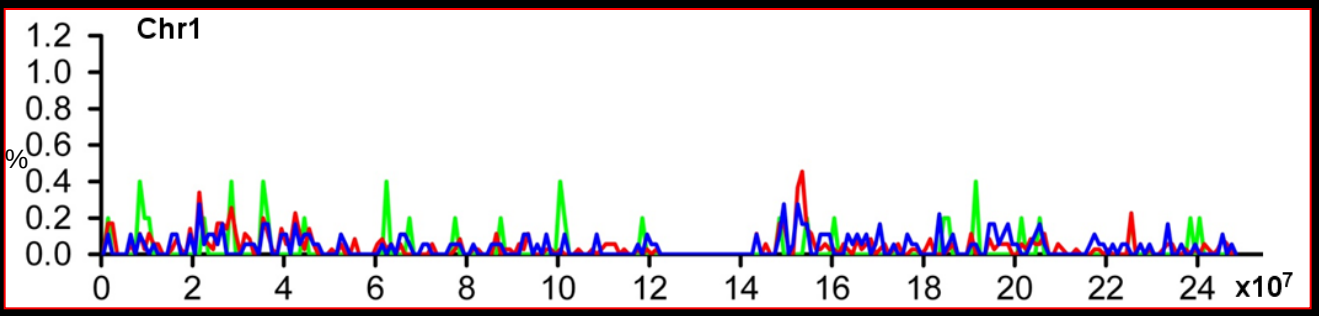
- ALD (Adrenoleukodystrophy)
- HIV-derived self-inactivating (SIN) LV, expressing ABCD1 transgene
- 2 patients treated
- Good safety and efficacy, 2 years follow-up

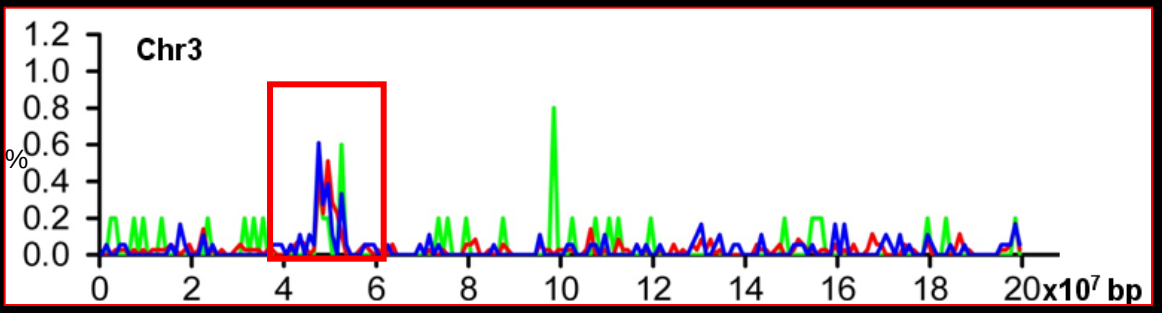
- Detailed integration studies show the appearance of many CIS

Are CIS in ALD a prelude to malignancy?

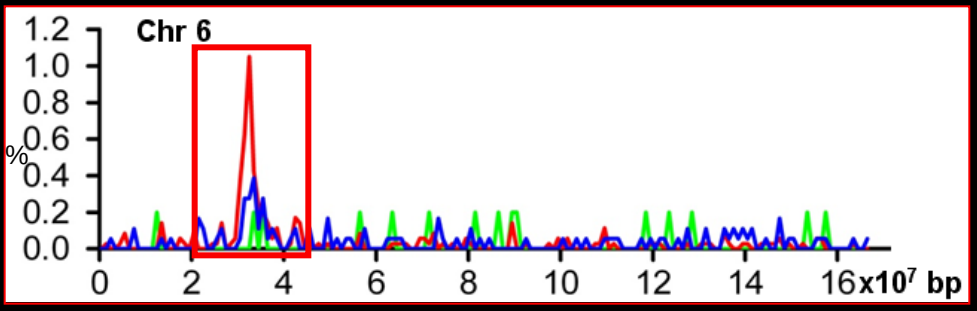
Chromosomal distribution of LV integrations

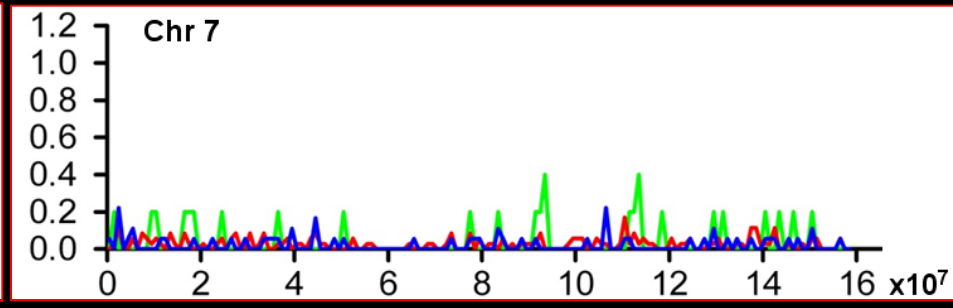


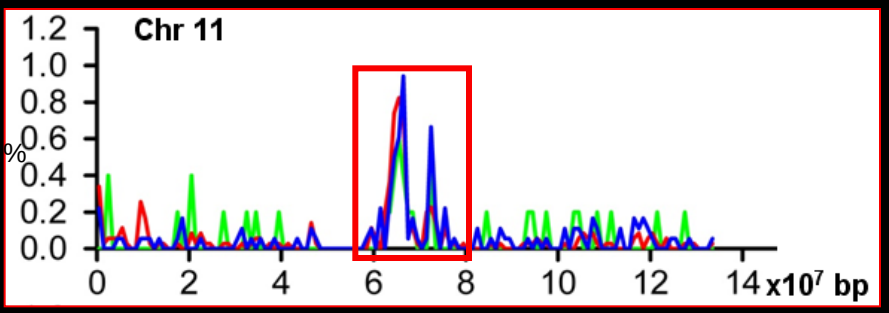
LV.ALD
LV.ARSA
LV.GFP

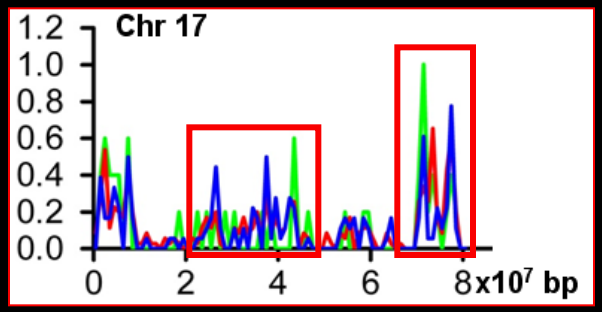


14% of LV integrations map in 2.5% of the genome

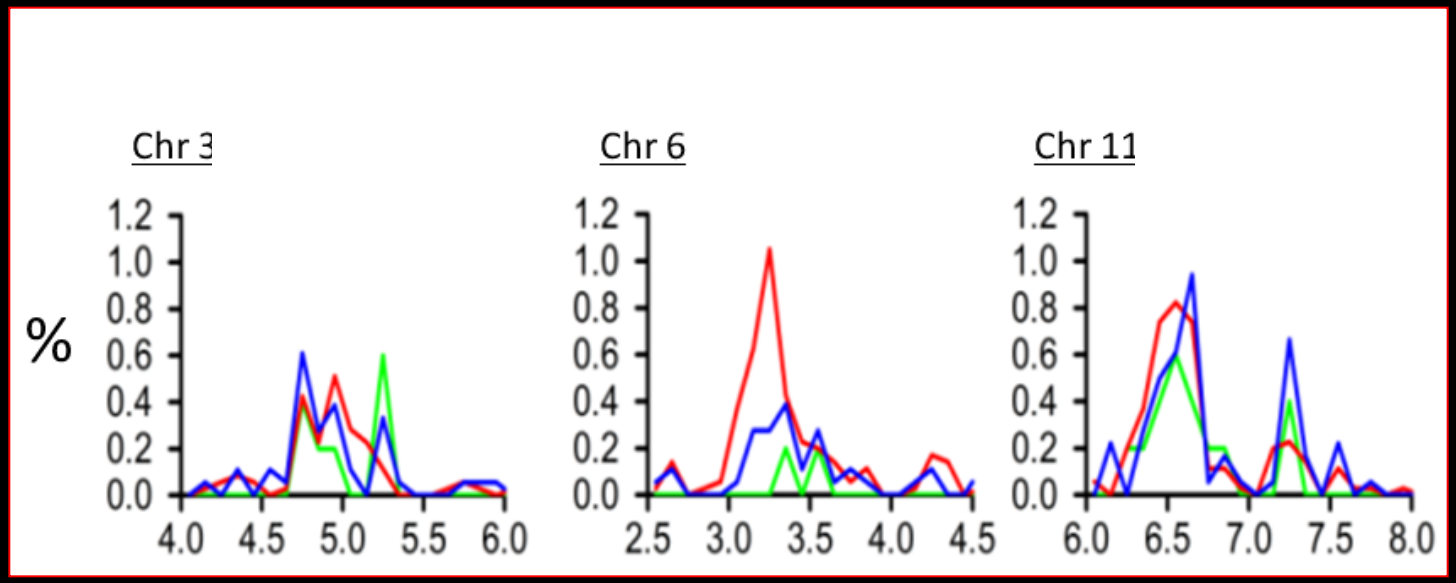


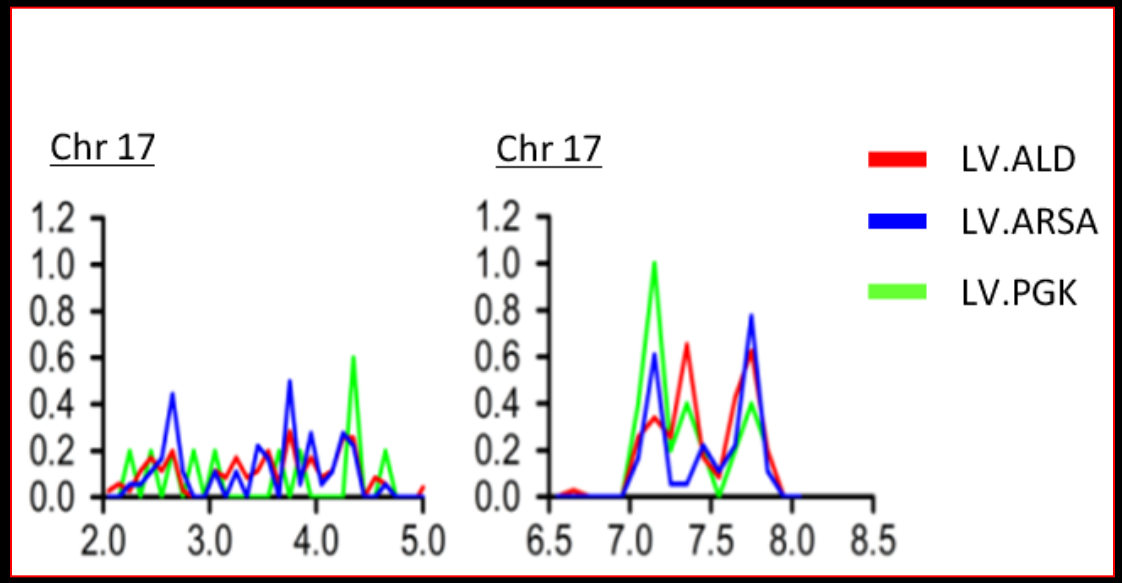






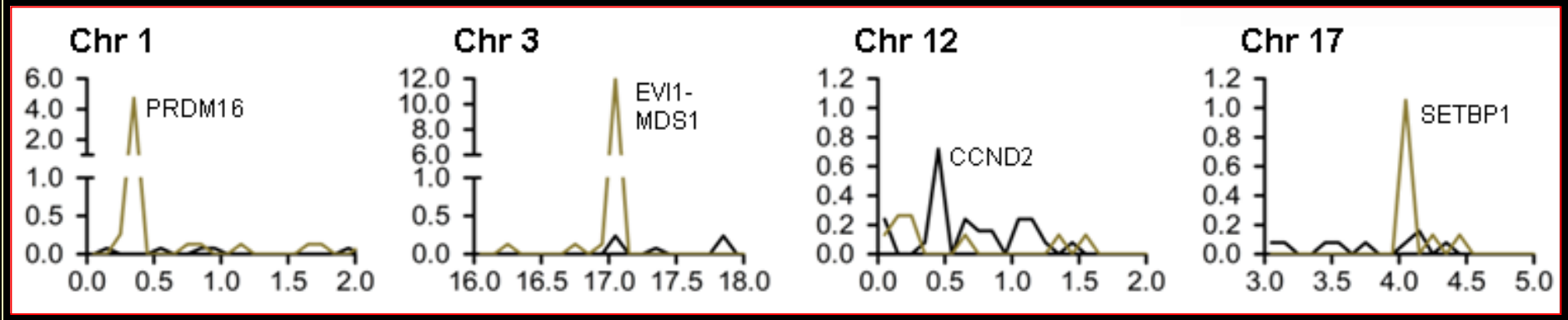
LV CISs are clustered in genomic macro-areas





Genotoxic CISs are narrow and isolated

GENOTOXIC gRV CISs from the X-SCID and X-CGD gene therapy clinical trials



RV-SCID

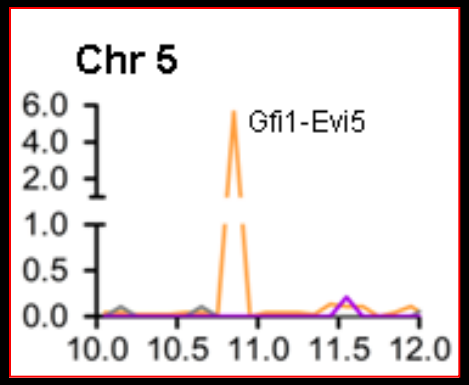
RV-CGD

GENOTOXIC CISs from insertional mutagenesis studies in mice

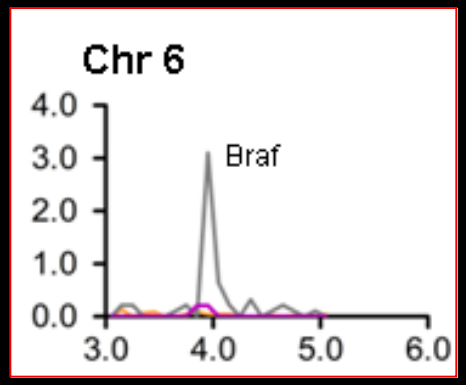
MLV-RB Pathway

SB-p19$^{-/-}$

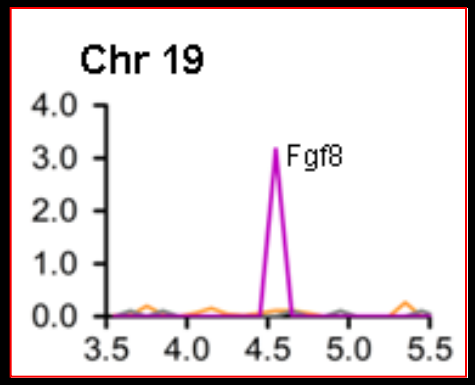
MMTV-CH3



gRV: Mo-MLV



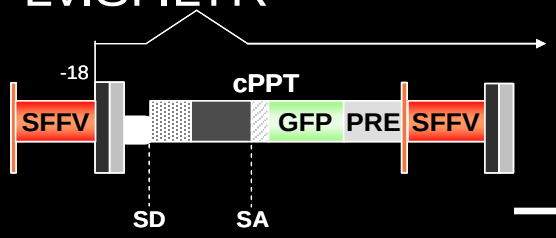
transposon: SB



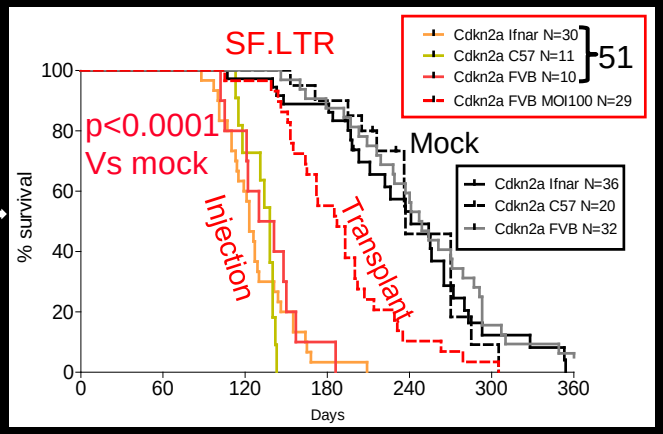
gRV: MMTV

LV genotoxic CIS

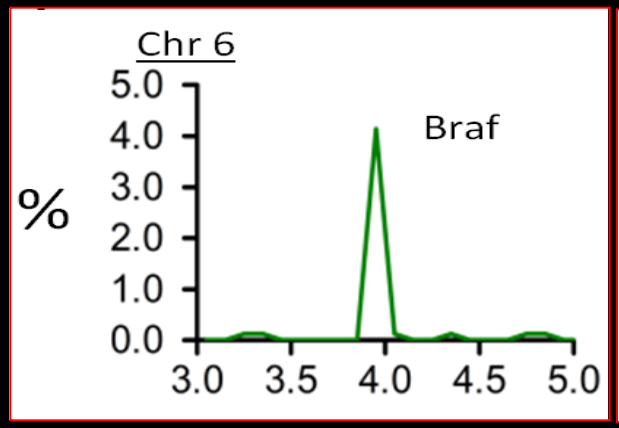
LV.SF.LTR



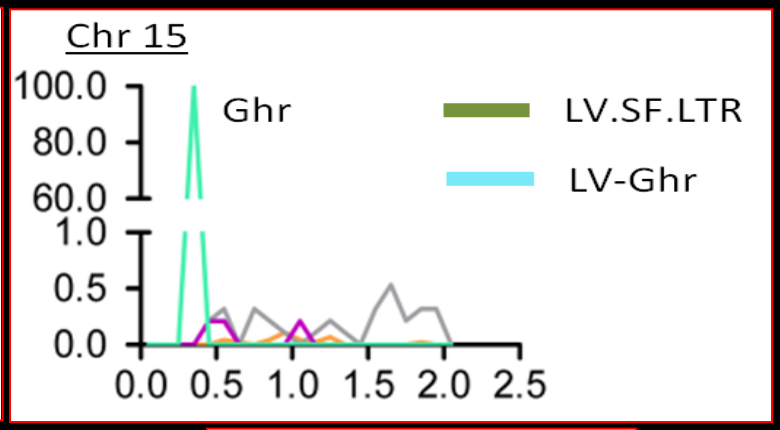
Cdkn2a deficient



- Myeloid F4/80+ tumors
- 1455 integrations
- CIS, Braf predominant

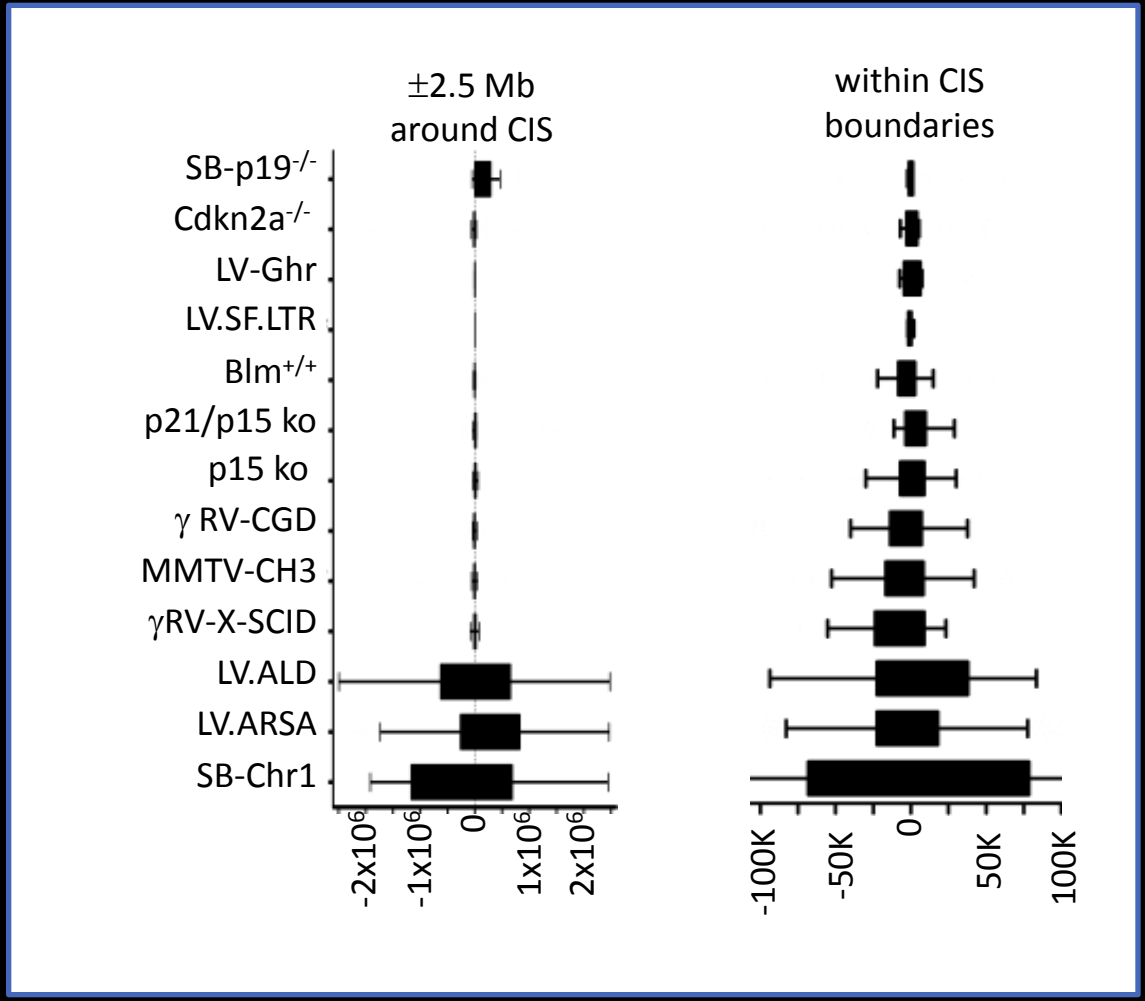


LTR.LV
in tumor-prone mouse



LVs in
IL-3-dependent cell line

Distribution of integrations around CIS



- LV integrations at CIS are more widespread than genotoxic CIS integrations

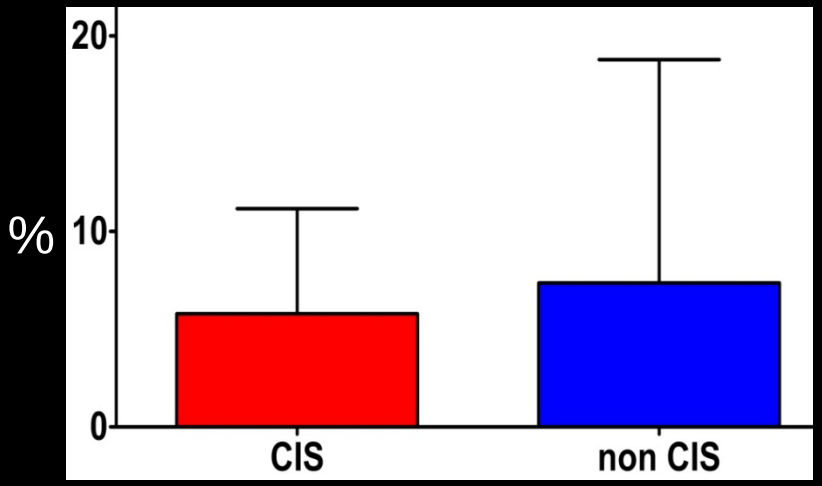
Integrations in 5 Mbp around CIS
- box: 50% of integrations
- whiskers: 90% of integrations

Fisher's exact test comparison

Fisher on ±25 Kb Bin	ALD	MLD	SB chr 1	CGD	X-SCID	LV CIS Ghr	SB p19-/-	p15ko	p15/p21ko	Cdkn2a	Blm+/+	C3H-MMTV	NIH-Swiss
ALD	1	0.75	0.03	2.2E-16	2.9E-07	2.5E-12	2.4E-08	2.2E-16	2.2E-16	1.9E-15	2.2E-16	2.2E-16	1.3E-05
MLD		1	0.023	2.2E-16	4.2E-06	7.7E-11	8.6E-07	1.5E-15	1.1E-12	1.2E-10	1.1E-13	2.2E-16	7.7E-05
SB chr 1			1	2.8E-15	2.8E-07	1.5E-11	1.2E-07	1.4E-11	2.1E-10	4.3E-09	6.4E-12	2.2E-16	2.7E-06
CGD				1	0.20	0.10	0.12	0.18	0.23	0.02	1.00	0.16	0.16
X-SCID					1	0.02	1.00	0.70	0.83	0.83	0.23	0.03	1.00
LV-CIS Ghr						1	0.02	0.02	0.03	0.004	0.13	0.36	0.02
SB p19-/-							1	0.60	0.69	1.00	0.18	0.01	1.00
p15ko								1	1.00	0.37	0.29	0.01	0.64
p15/p21ko									1	0.43	0.26	0.01	0.64
Cdkn2a										1	0.07	0.0007	1.00
Blm+/+											1	0.33	0.20
C3H-MMTV												1	0.02
NIH-Swiss													1

Significant $p<0.0005$ (Bonferroni correction for 91 comparisons)

Cells harboring LV CIS integrations are not over-represented in transplanted mice



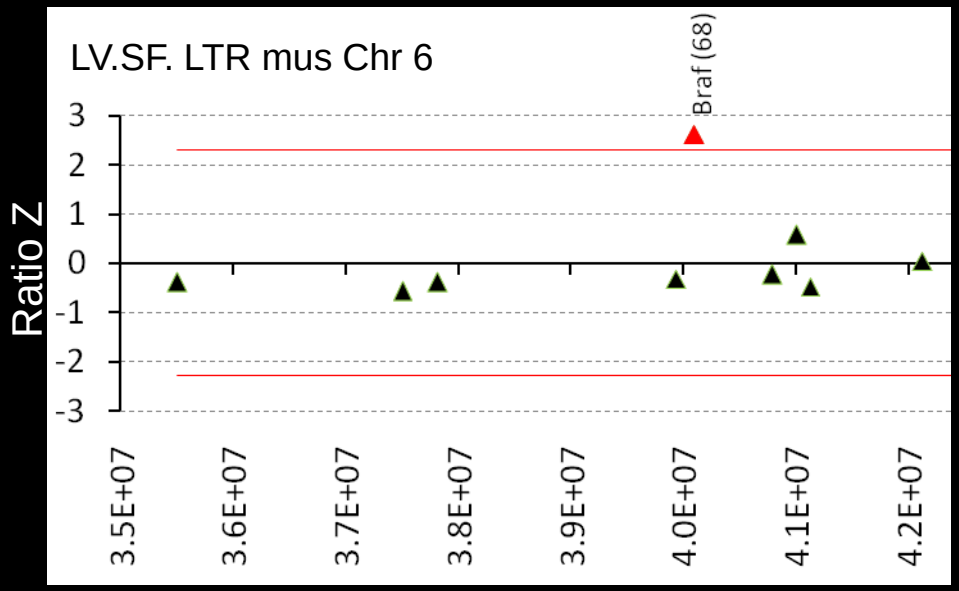
- no difference in representation of cells harboring CIS integrations and cells harboring non-CIS integrations

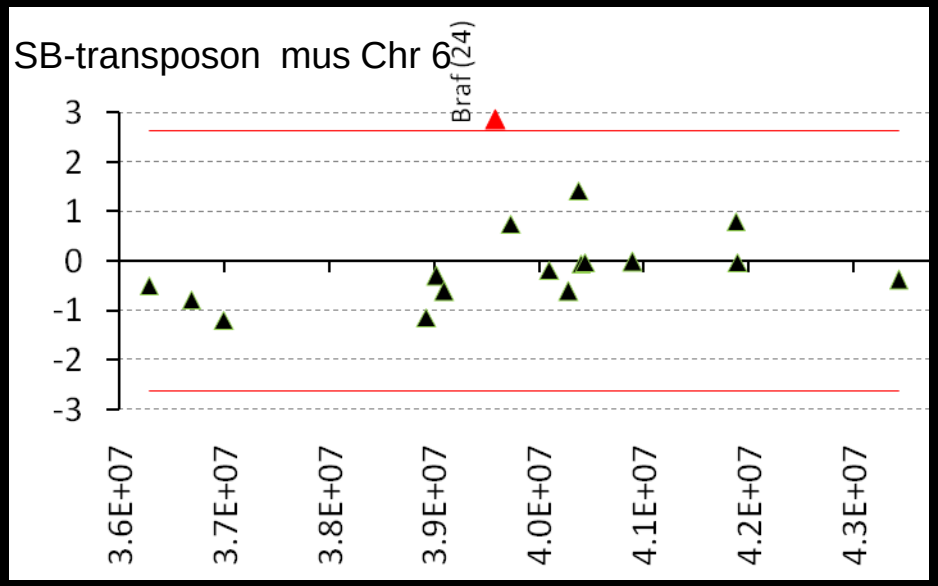
condition	*BM*		*MPB*		*MPB GFP*
	vitro	*vivo*	*vitro*	*vivo*	*vitro*
% CIS	6.4	7.5	8.6	6.4	5.2

- No enrichment of CIS from in vitro to in vivo conditions
- no clonal dominance is associated with LV CIS

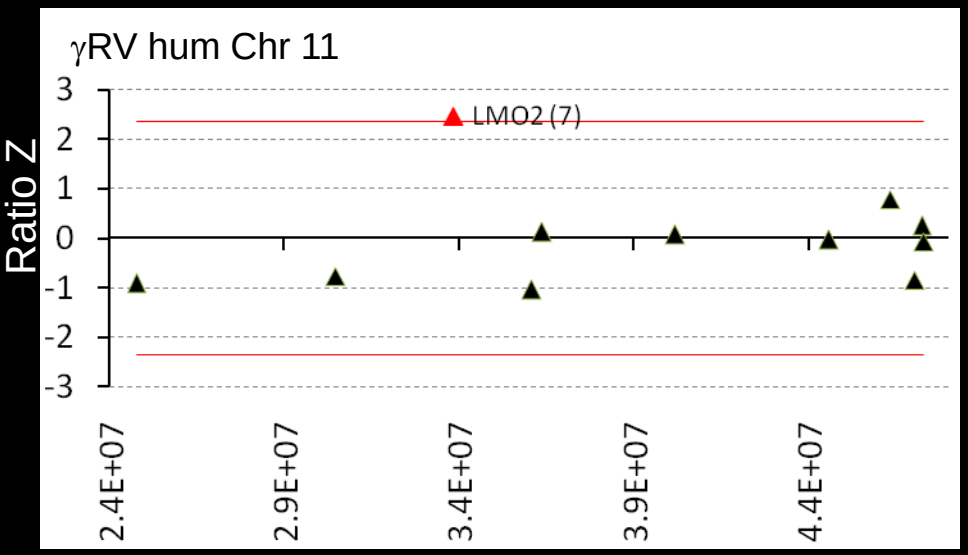
Statistical validation of CIS by the Grubbs' test for outliers

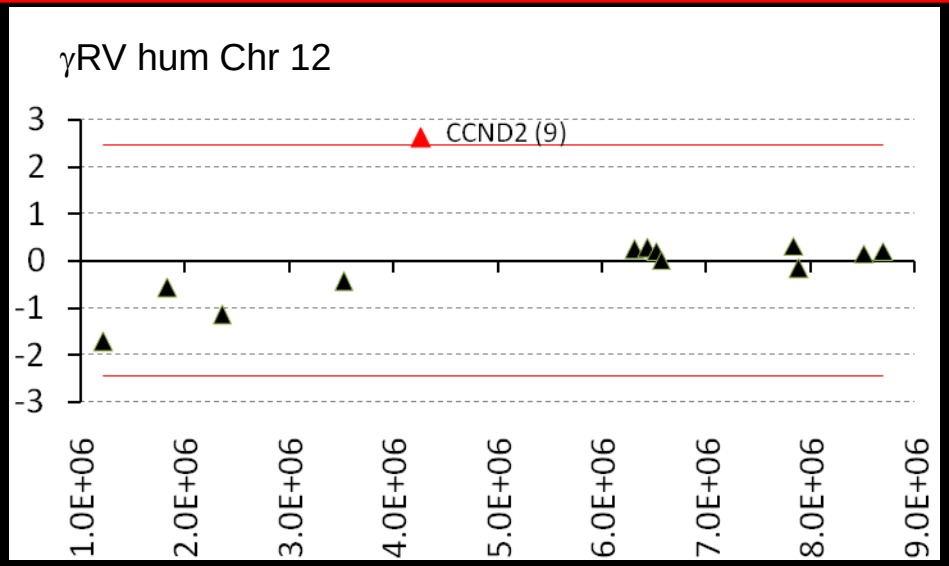
- *postulate:*
  - A genotoxic CIS is a genomic-region/gene targeted at a frequency so high that can be identified as significant outlier with respect to all other targeted-regions/genes contained in the same interval.

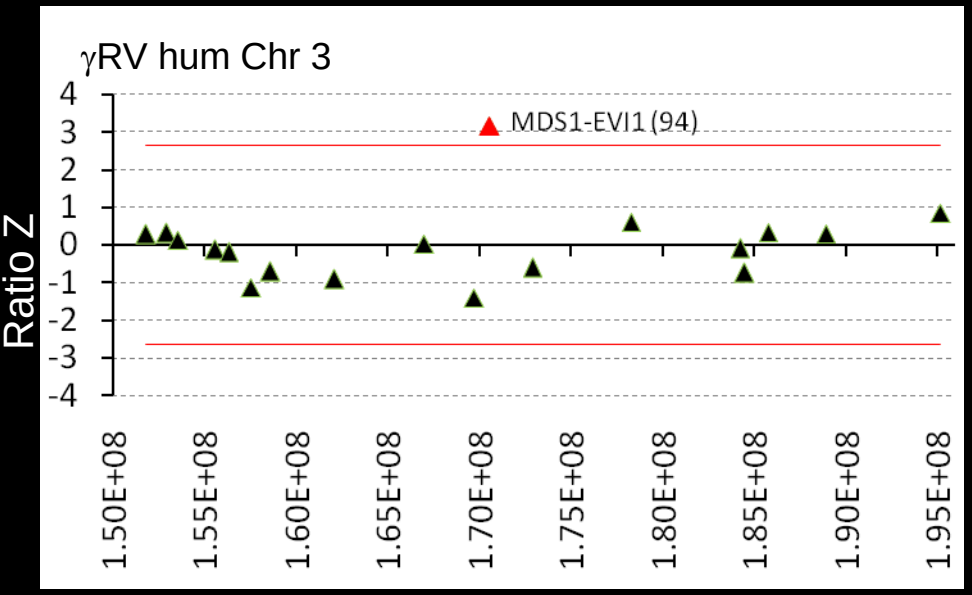


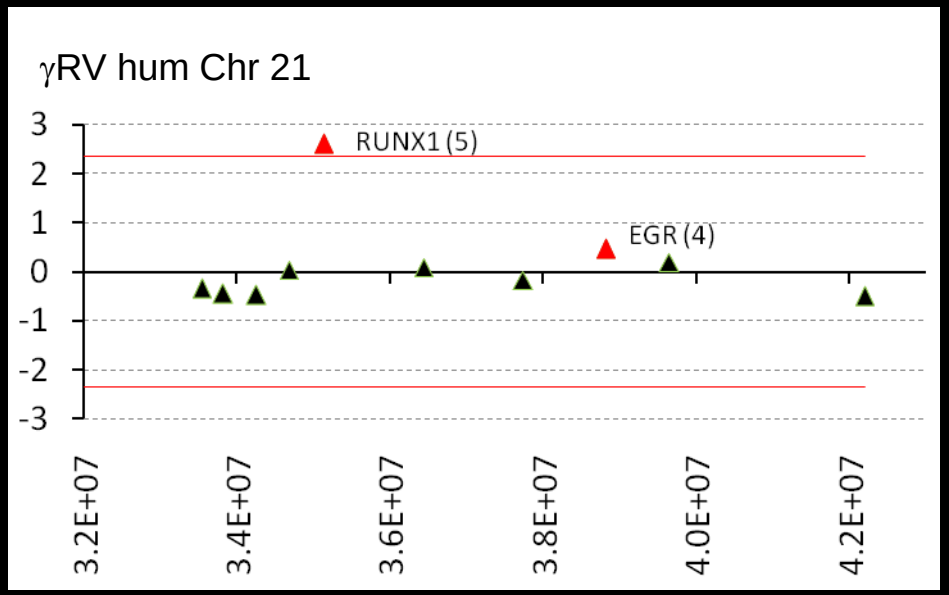


Grubbs' test results on CIS form X-SCID and CGD clinical trials





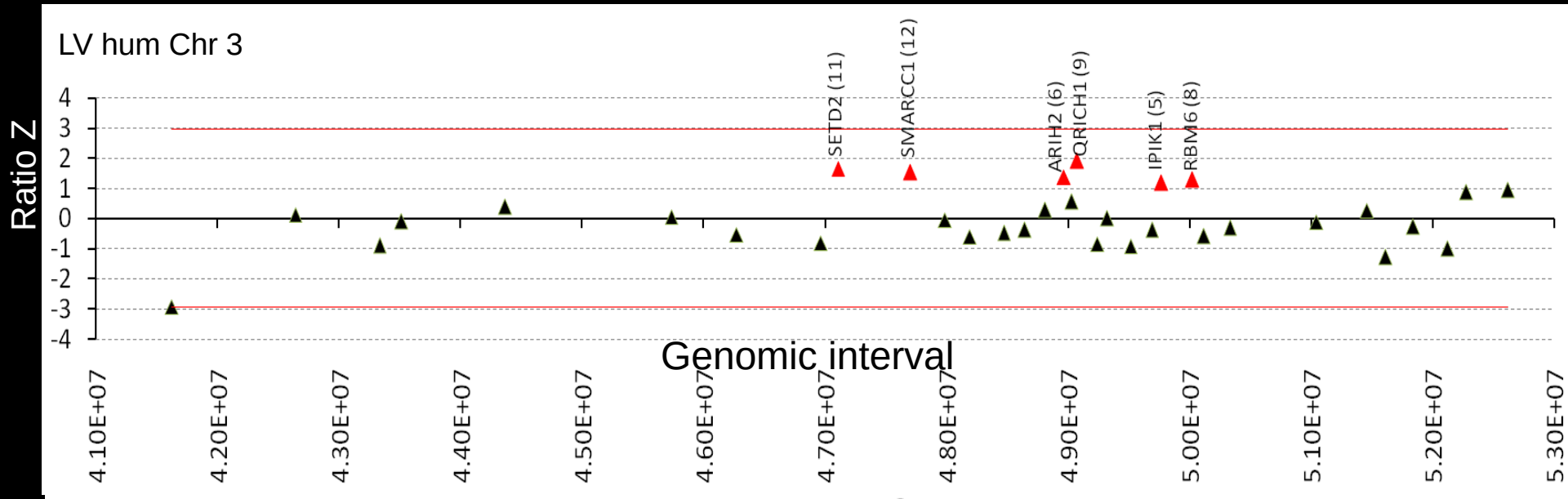


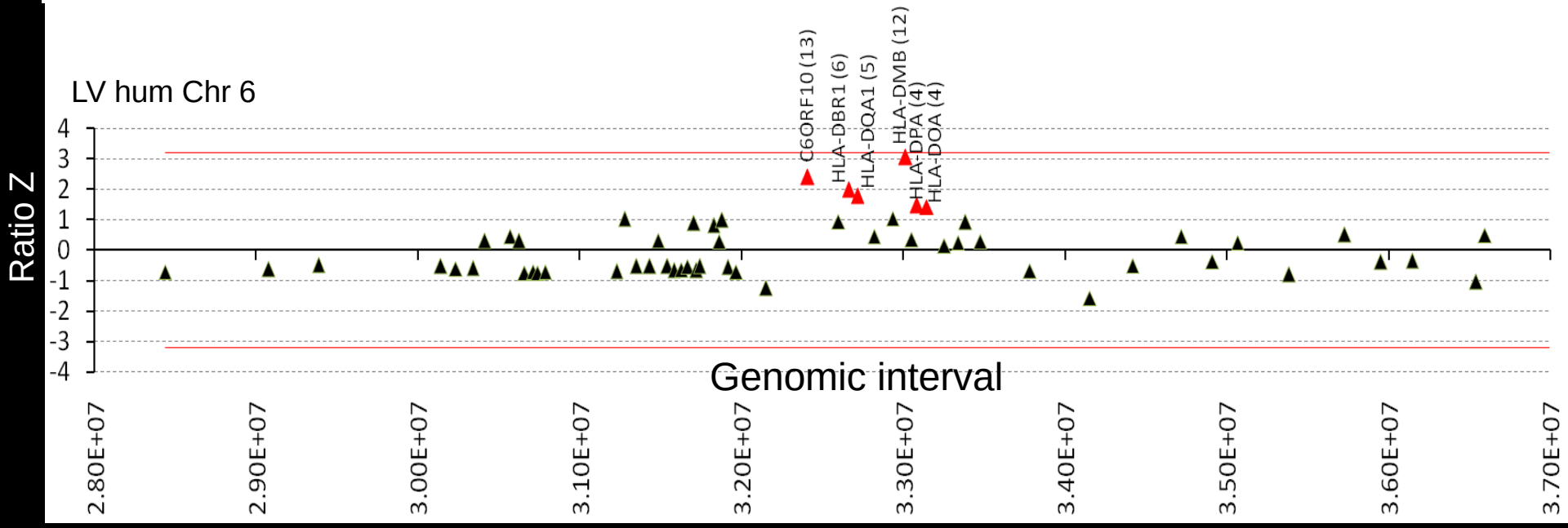
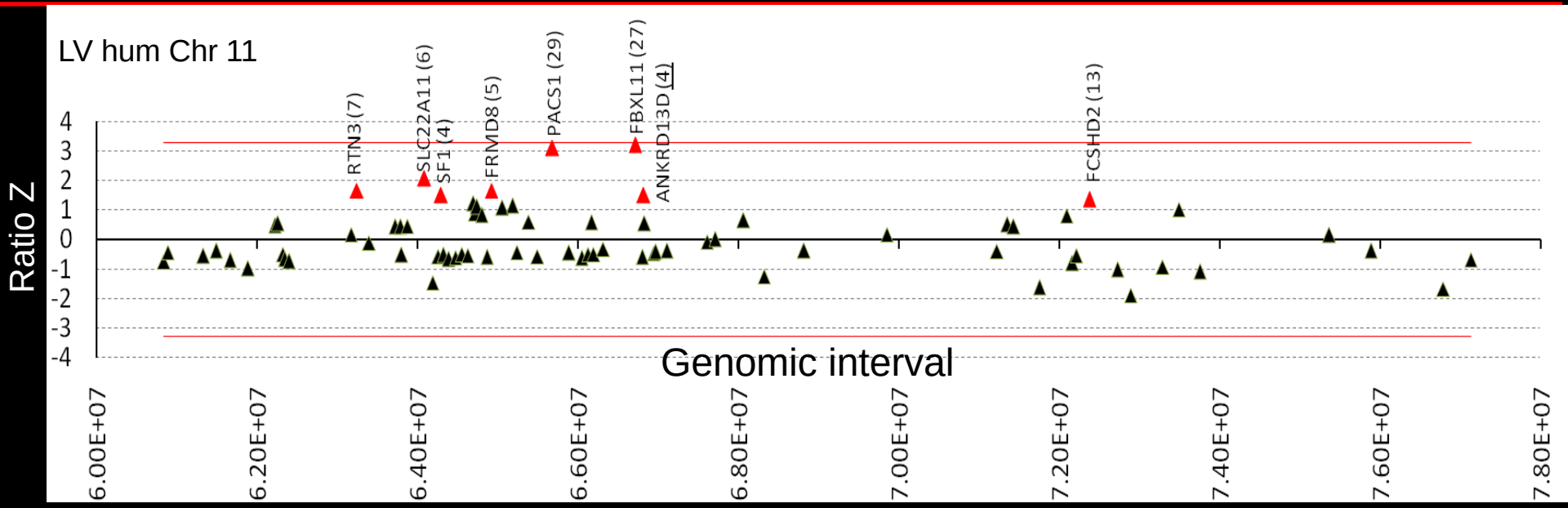
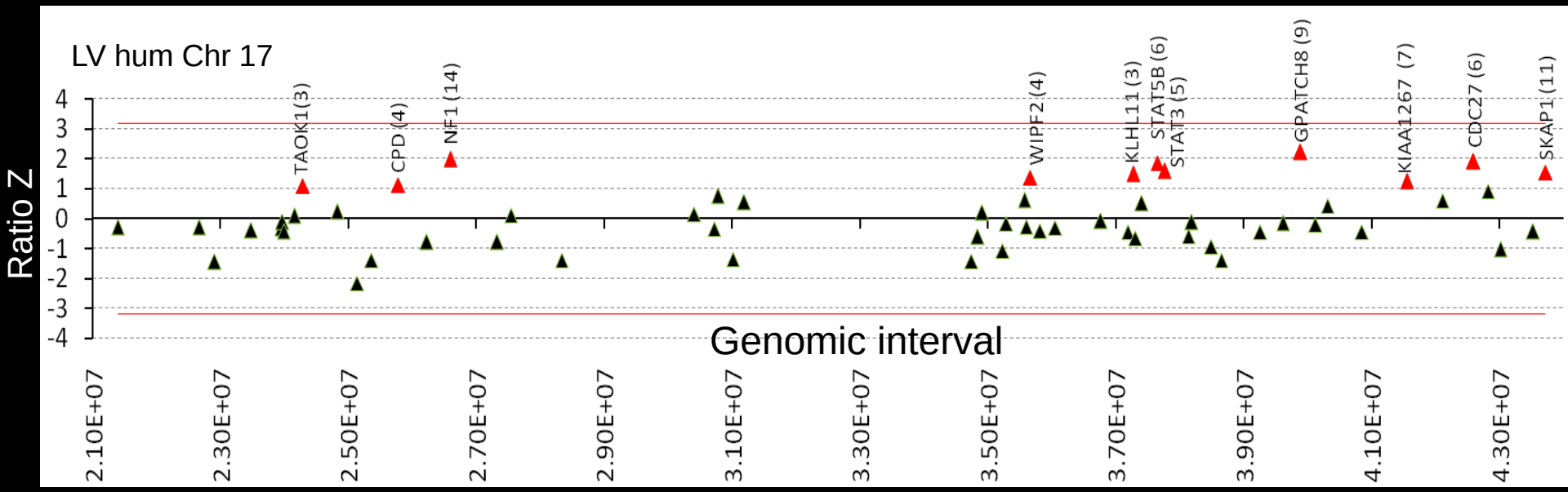


Genomic interval

Genomic interval

Grubbs' test results on CIS form ALD clinical trial and hematochimeras

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