

Analysis of integration site distributions and clonal abundances for gene therapy correction of cystinosis (Groups 24-28)

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Summary of results

The goal of this analysis is to investigate the integration profile of a gene therapy vector for the correction of cystinosis in mouse subjects and assess potential clonal expansions. The list of mouse oncogenes was compiled from the retroviral tagged cancer gene database (RTCGD)¹ using an inclusion threshold of three or more incidents where the mouse oncogene list comprises 2.5% of all mouse genes. The frequency of integration near oncogenes was generally less than that of mice in a previously published β -thalassemia mouse trial from which no adverse events have been reported ². The code base for this analysis is available online ([link](#)).

Twenty-eight mice were tested by serial transfer of vector-treated bone marrow. In both donor and recipient samples, there was no overall increase in integration sites near annotated murine cancer-associated genes, thus providing no evidence for genotoxicity. Some cells expanded more than others, but analysis of cells showing the most pronounced expansion showed no enrichment in integration sites in or near cancer-associated genes. In a number of cases, a single cell appears to have mainly populated a test mice. These clones, for the most part, appear to multi-integrated cells which appear as multiple nearly equal size bars within the relative abundance plots within this report.

Mouse samples studied

Integration sites were detected in 48 samples from mouse subjects (Tables 1 & S1).

Table 1. Overview of data collection.

Organism	Number of samples	Number of reads	Number of inferred cells	Number of integration sites
mouse	48	24,335,872	79,167	4,048

Subject reports

Subject specific reports for all subjects are available via an online archive ([link](#)).

UCSC browser exploration

UCSC browser sessions pre-loaded with the integration sites identified in this analysis are available via this ([link](#)). Integration sites are shown as blue (positive orientation integration) and red (reverse orientation integration) tick marks. For each integration site, the intensity of the color reflects the maximum abundance observed.

Description of analysis techniques

We investigate effects of integration on cell growth using the following criteria: Integration Frequency is the frequency at which unique integration sites are observed in or near a given gene. Clonal Abundance is determined by quantifying the number of sites of linker ligation associated with each unique integration site. This samples the number of DNA chains at the start of the experiment allowing clonal expansion to be quantified⁴.

Relative clonal Abundance is determined per sample and is the percentage of identified cells attributed to a given clone. Integration sites and the clones harboring them are sampled from a larger population. It would be rare for all integration sites in a sample to be represented in the sequence data.

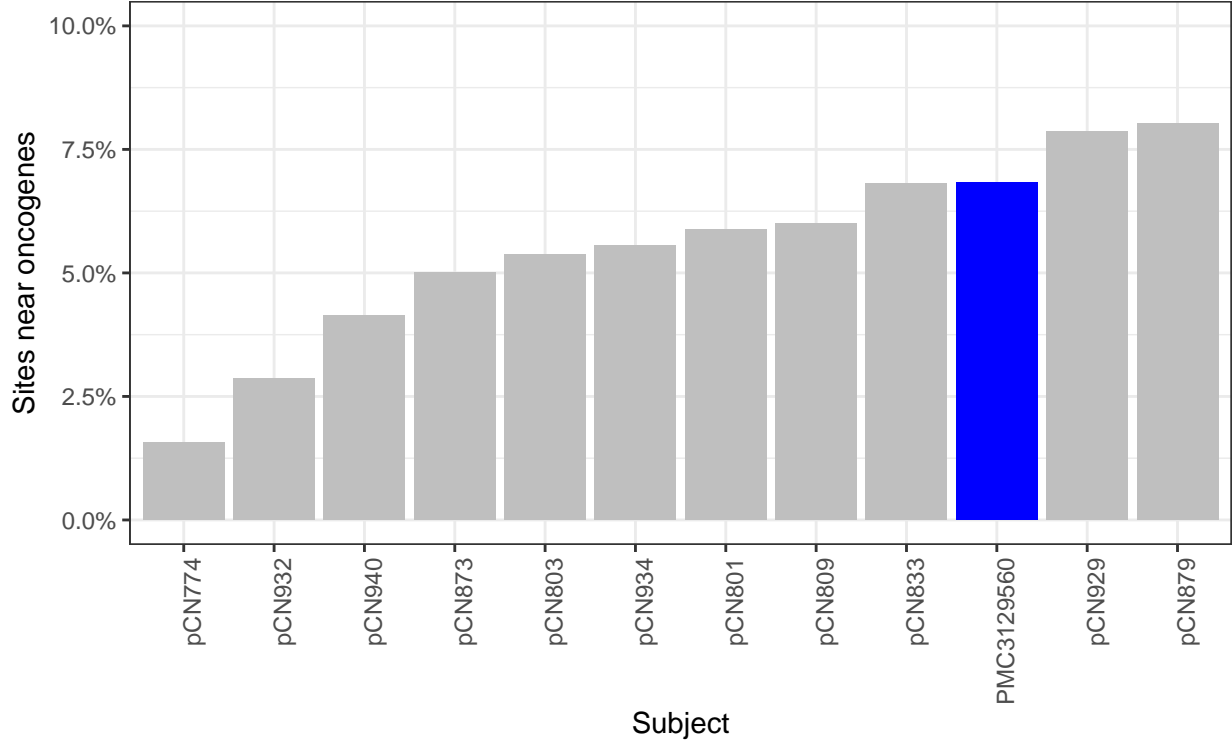
For this analysis, four technical replicates of each delivered sample were prepared, sequenced and analyzed with the INSPIRED integration site analysis pipeline (v1.2)⁴.

Comparisons to previous trials

Integration events near oncogenes in mouse subjects

In order to determine if the experimental vector has a higher propensity of integrating near suspected oncogene in mice than previously employed vectors, the frequency of integration near oncogenes was compared to a previously published mouse trial² which used a comparable lentiviral vector to correct β -thalassemia. The frequency of integration events near onco genes in *bone marrow donor mice* was less than the mean frequency of integration events near oncogenes in the published trial (Figure 1 [experimental subjects: gray, previous β -thalassemia trial: blue]).

Figure 1. Comparison of frequencies of integration events near oncogenes.



Relative abundances of mouse subject samples

The sample relative abundance plots below (Figure 2) show the most abundant 25 clones in each sample as colored bars while less abundant clones were relegated to a single low abundance bar shown in gray.

Figure 2.

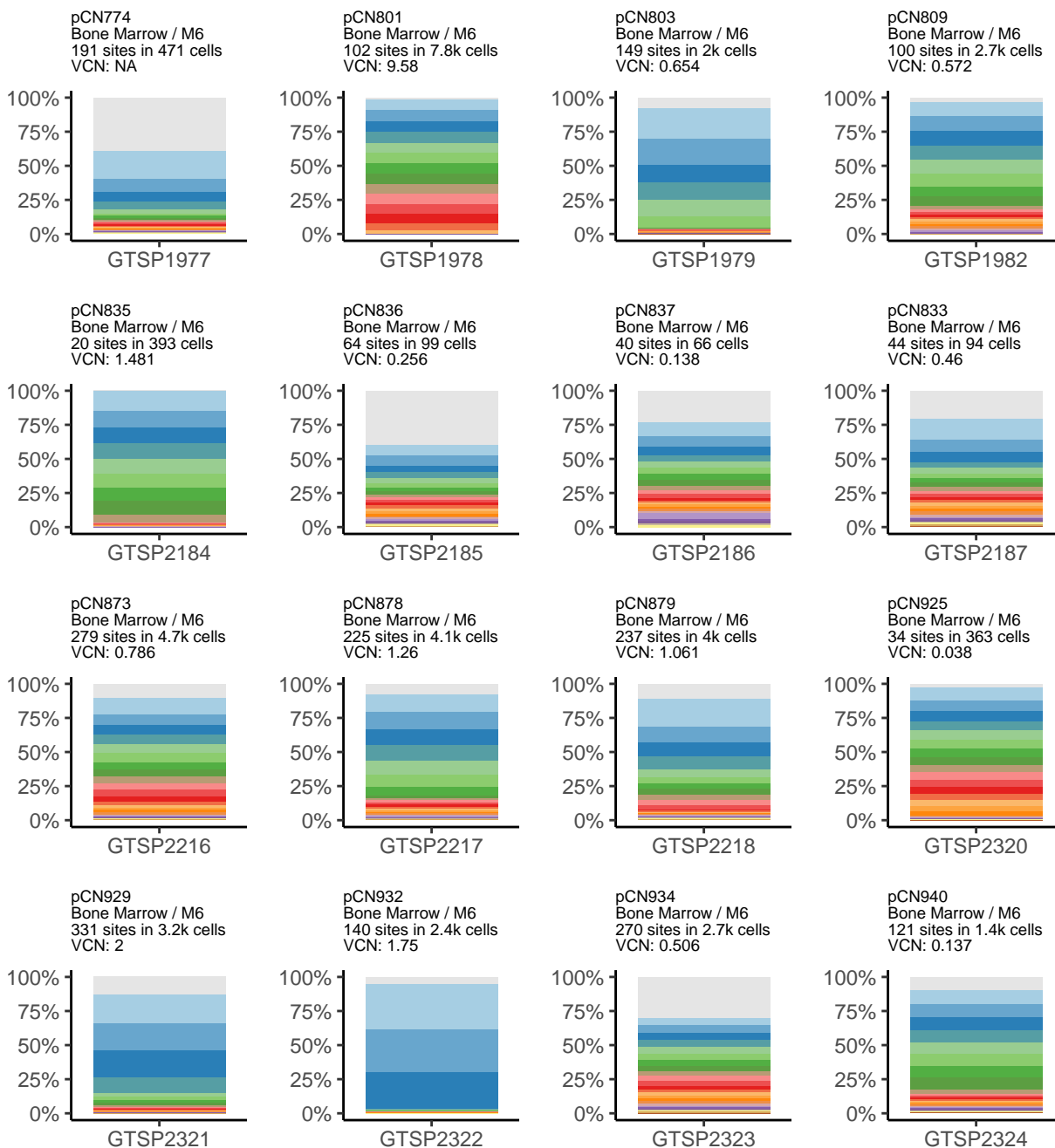


Figure 2 (continued).

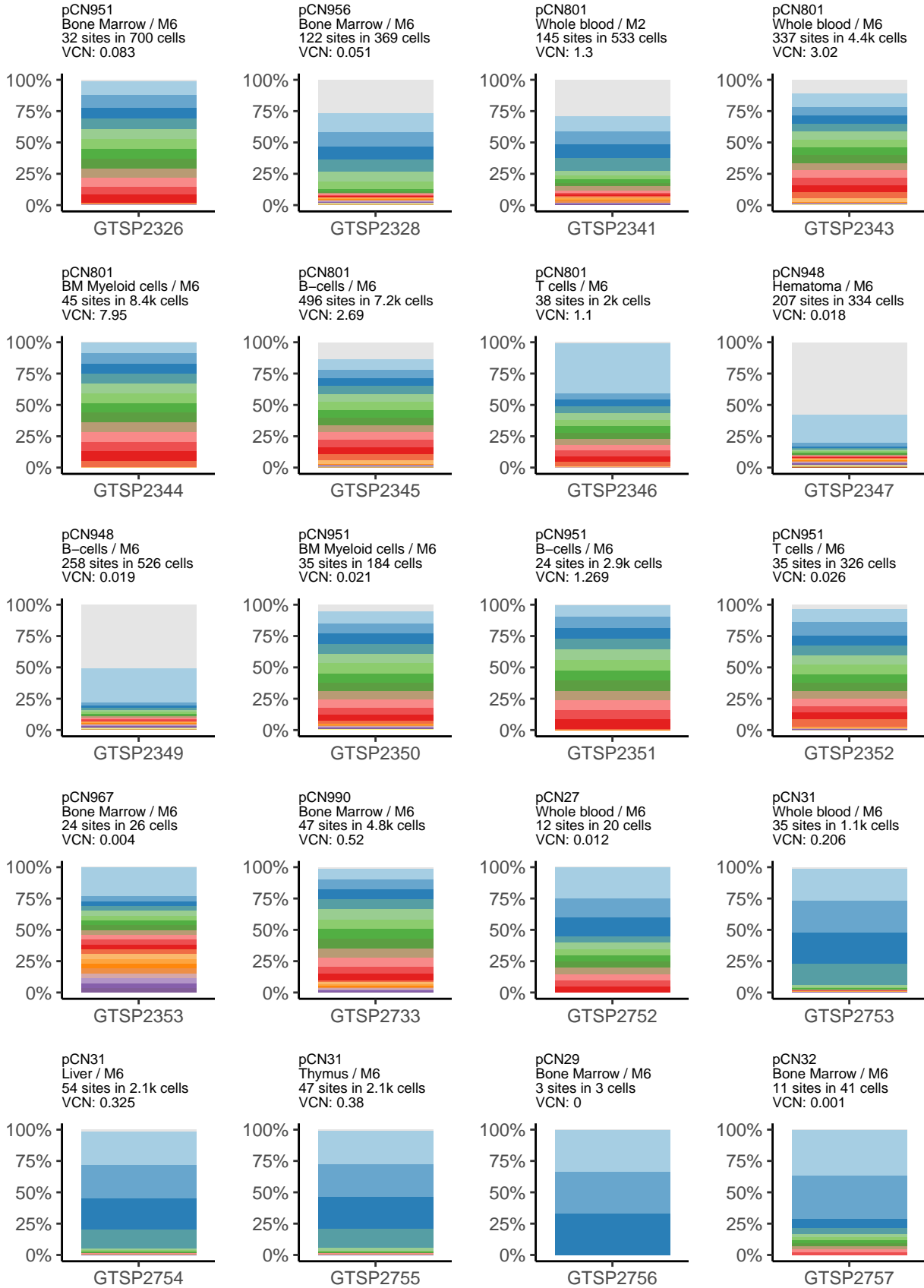
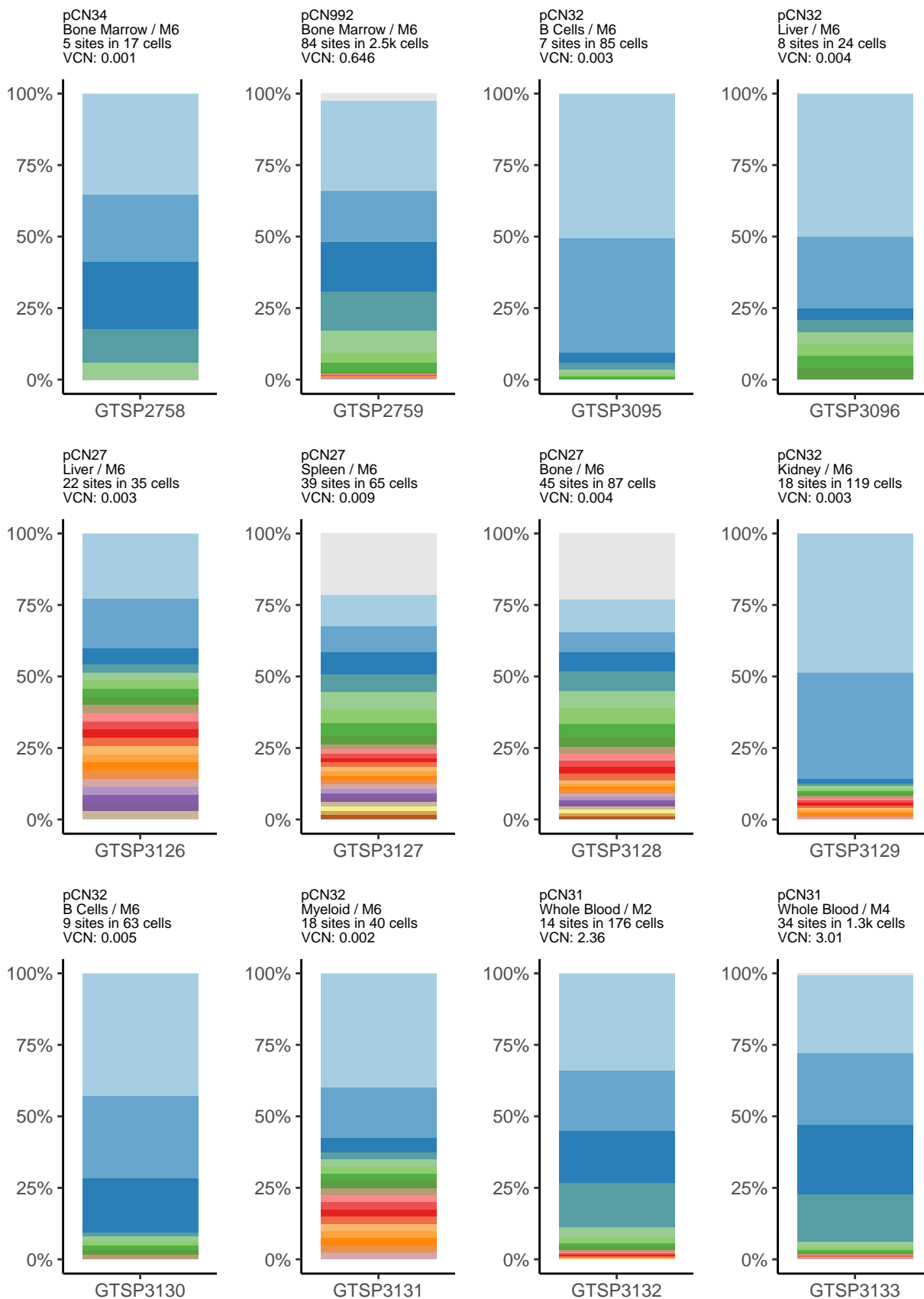


Figure 2 (continued).



Expanded clones

Table 2 below lists clones with relative clonal abundances $\geq 20\%$. The estimated number of cells harboring each integration (Abundance) is shown for context.

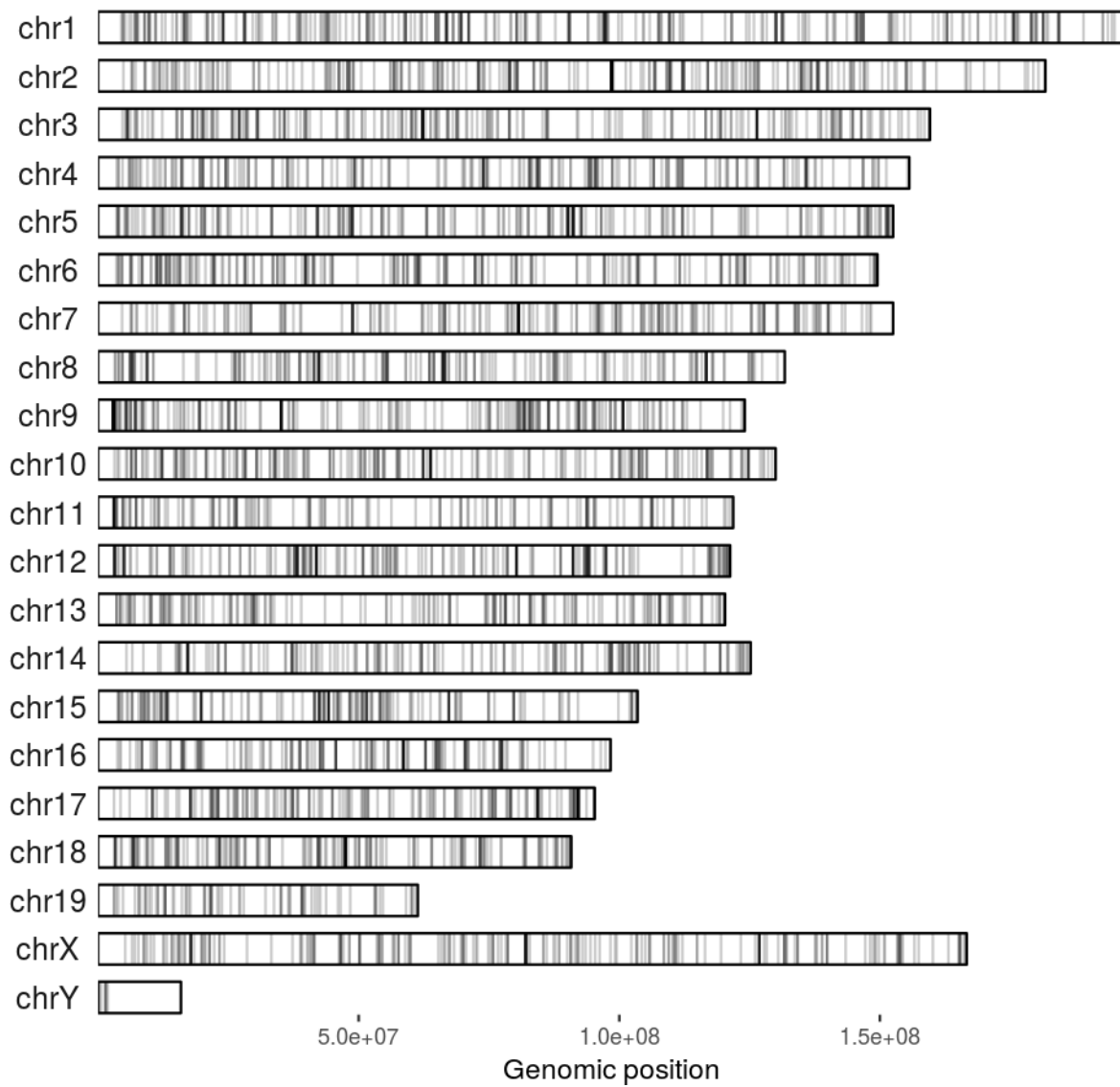
Table 2.

Subject	Organism	Time point	Cell type	Position	Relative abundance	Abundance	Nearest gene
pCN27	mouse	M6	Whole blood	chr4-106608629	25.00%	5	Ssbp3
pCN27	mouse	M6	Liver	chr14+119353687	22.86%	8	Dnajc3
pCN29	mouse	M6	Bone Marrow	chr12-38193729	33.33%	1	Agmo
pCN29	mouse	M6	Bone Marrow	chr18+26909782	33.33%	1	Celf4
pCN29	mouse	M6	Bone Marrow	chr9-76663996	33.33%	1	Tinag
pCN31	mouse	M6	Whole blood	chr12-38193729	25.31%	283	Agmo
pCN31	mouse	M6	Whole blood	chr9+100734703	25.04%	280	Stag1
pCN31	mouse	M6	Whole blood	chrX-126866229	25.76%	288	Diaph2
pCN31	mouse	M6	Liver	chr12-38193729	26.90%	560	Agmo
pCN31	mouse	M6	Liver	chr9+100734703	24.78%	516	Stag1
pCN31	mouse	M6	Liver	chrX-126866229	26.66%	555	Diaph2
pCN31	mouse	M6	Thymus	chr12-38193729	26.38%	554	Agmo
pCN31	mouse	M6	Thymus	chr9+100734703	26.33%	553	Stag1
pCN31	mouse	M6	Thymus	chrX-126866229	25.19%	529	Diaph2
pCN31	mouse	M2	Whole Blood	chr9+100734703	21.02%	37	Stag1
pCN31	mouse	M2	Whole Blood	chrX-126866229	34.09%	60	Diaph2
pCN31	mouse	M4	Whole Blood	chr12-38193729	25.34%	340	Agmo
pCN31	mouse	M4	Whole Blood	chr9+100734703	24.07%	323	Stag1
pCN31	mouse	M4	Whole Blood	chrX-126866229	27.05%	363	Diaph2
pCN32	mouse	M6	Bone Marrow	chr12+80277915	34.15%	14	Rdh11
pCN32	mouse	M6	Bone Marrow	chr8-66234933	36.59%	15	Sgo2b
pCN32	mouse	M6	B Cells	chr12+80277915	50.59%	43	Rdh11
pCN32	mouse	M6	B Cells	chr8-66234933	40.00%	34	Sgo2b
pCN32	mouse	M6	Liver	chr12+80277915	50.00%	12	Rdh11
pCN32	mouse	M6	Liver	chr8-66234933	25.00%	6	Sgo2b
pCN32	mouse	M6	Kidney	chr12+80277915	36.97%	44	Rdh11
pCN32	mouse	M6	Kidney	chr8-66234933	48.74%	58	Sgo2b
pCN32	mouse	M6	B Cells	chr12+80277915	28.57%	18	Rdh11
pCN32	mouse	M6	B Cells	chr8-66234933	42.86%	27	Sgo2b
pCN32	mouse	M6	Myeloid	chr8-66234933	40.00%	16	Sgo2b
pCN34	mouse	M6	Bone Marrow	chr2+135509372	23.53%	4	Plcb4
pCN34	mouse	M6	Bone Marrow	chr6+22170407	35.29%	6	Cped1
pCN34	mouse	M6	Bone Marrow	chr6+29837095	23.53%	4	Ahcyl2
pCN774	mouse	M6	Bone Marrow	chr1-21011655	21.02%	99	Tram2
pCN801	mouse	M6	T cells	chr5-90109547	39.80%	780	Adamts3
pCN803	mouse	M6	Bone Marrow	chr3+60155698	22.64%	461	Mbnl1
pCN879	mouse	M6	Bone Marrow	chr15+44227316	20.85%	829	Nudcd1
pCN929	mouse	M6	Bone Marrow	chr9+100734703	20.67%	669	Stag1
pCN929	mouse	M6	Bone Marrow	chrX-126866229	20.02%	648	Diaph2
pCN932	mouse	M6	Bone Marrow	chr12+80277915	33.13%	807	Rdh11
pCN932	mouse	M6	Bone Marrow	chr8-66234933	31.53%	768	Sgo2b
pCN932	mouse	M6	Bone Marrow	chr8+9405148	26.56%	647	Fam155a
pCN948	mouse	M6	Hematoma	chr1-21011655	22.75%	76	Tram2
pCN948	mouse	M6	B-cells	chr1-21011655	27.19%	143	Tram2
pCN992	mouse	M6	Bone Marrow	chr15+44227316	31.39%	799	Nudcd1

Mapping of integration site positions

Integration events were observed across all mouse subject chromosomes (Figure 3).

Figure 3.



Mouse transplant trials

The positions of identified integration sites from cell transplant trials with nine pairs of mice are shown in Figure 4a (donor mice) and Figure 4b (recipient mice). The gRxCluster software package did not identify clusters of integration sites between donor and recipient mice with a false discovery rate of $\leq 10\%$. The relative clonal abundances of samples from the transplant trials are shown in Figure 5 where donor mice are shown on the left and recipient mice are shown on the right. Integration sites are denoted by both nearest gene and genomic coordinate and annotated with an asterisk (*) if located within transcription units and with a tilde (~) if the integration site is within 50 KB of an oncogene. Below each abundance plot is a Fisher's exact test for the enrichment of oncogenes. None of the tests returned a significant result. Instances where no integration sites were identified in the recipient mouse are listed as 'NA'. The clonal abundances of clones found in both donor and recipient mice is shown in Table S2. The identification of relatively few persistent clones is likely due to sequencing experiments sampling only a subset of existing integration sites and a number of samples with low vector copy numbers (Figures 4B & S3).

Figure 4.

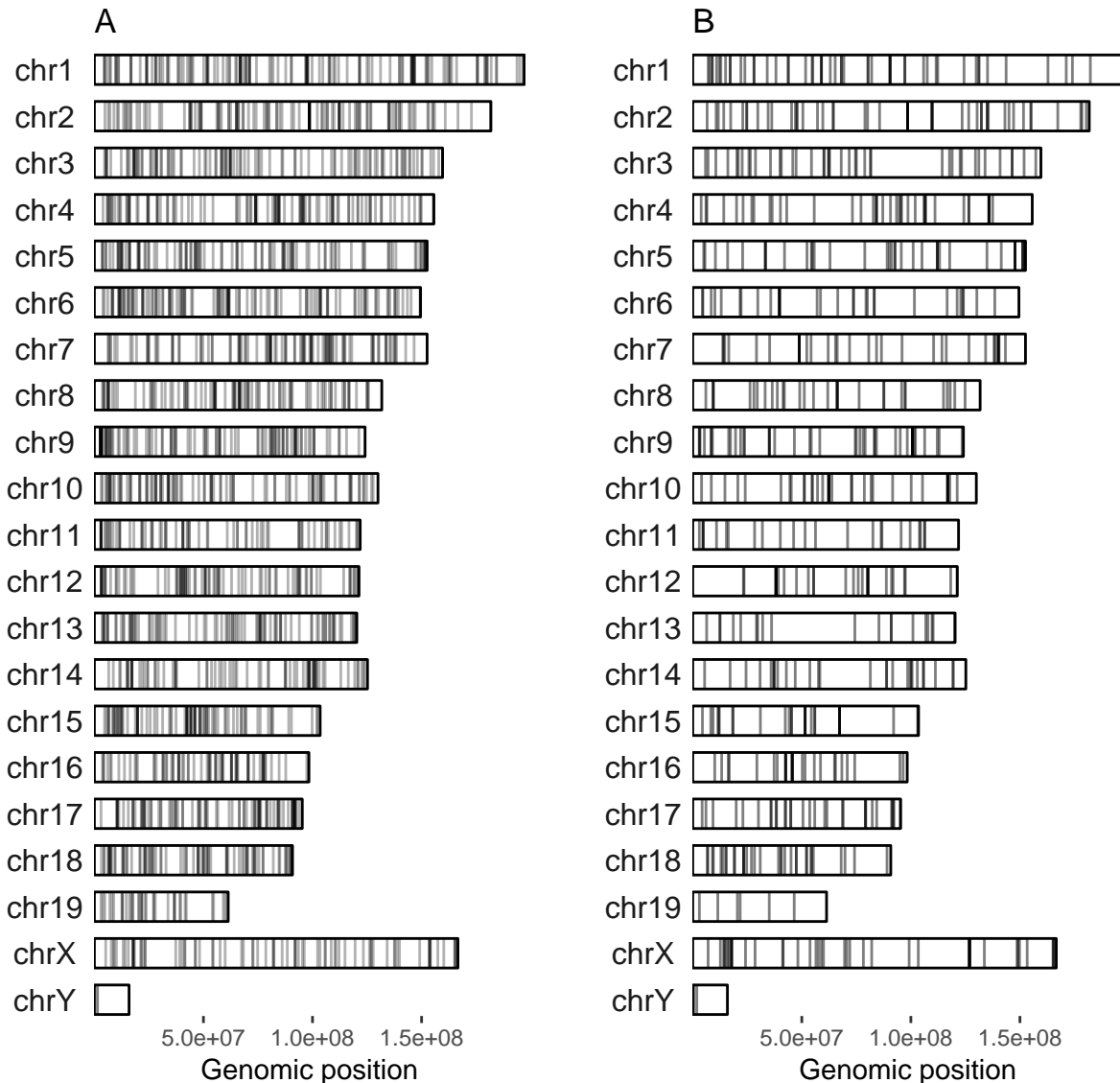
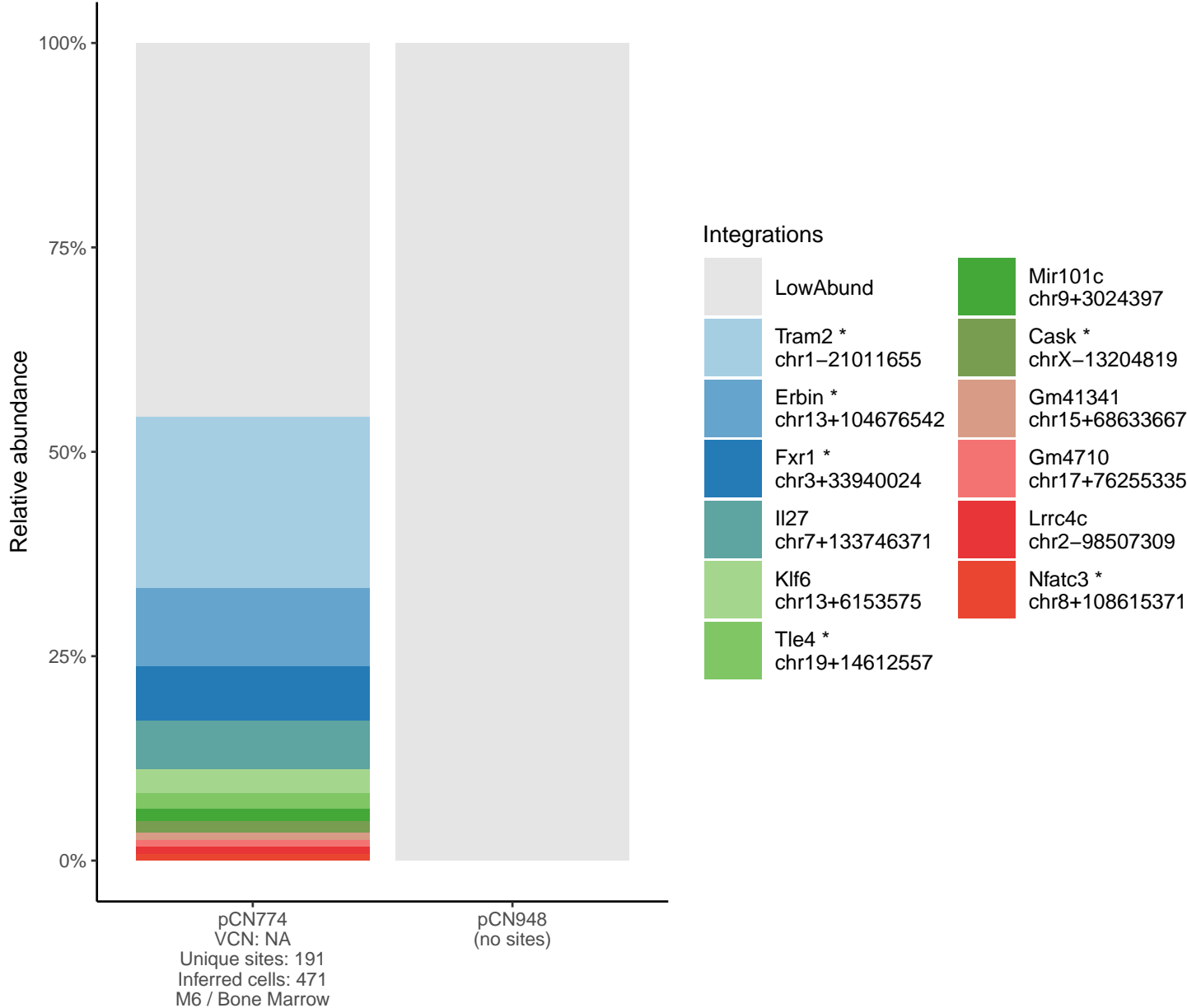


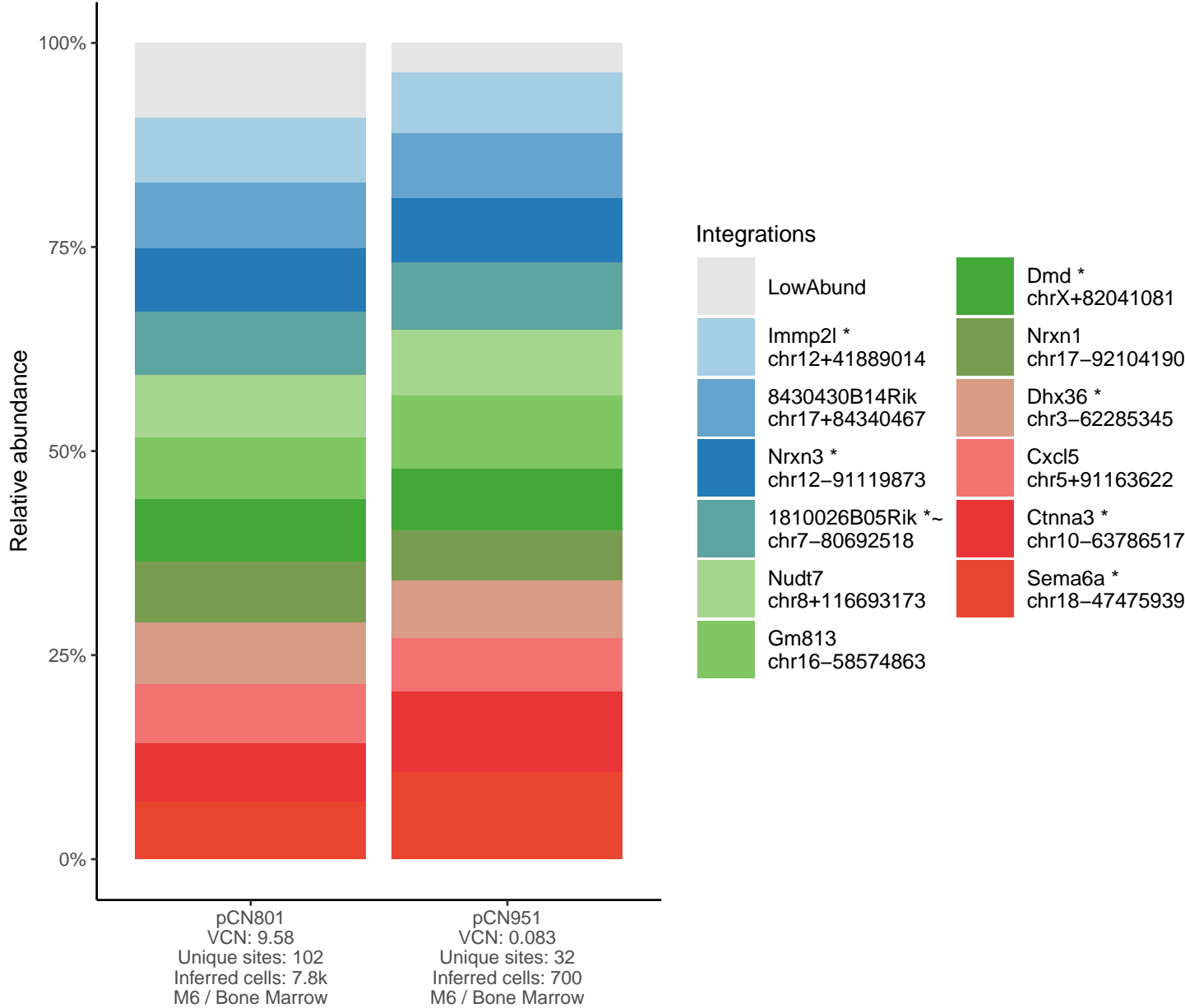
Figure 5a.



Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN774	188	3
NA	0	0

Figure 5b.

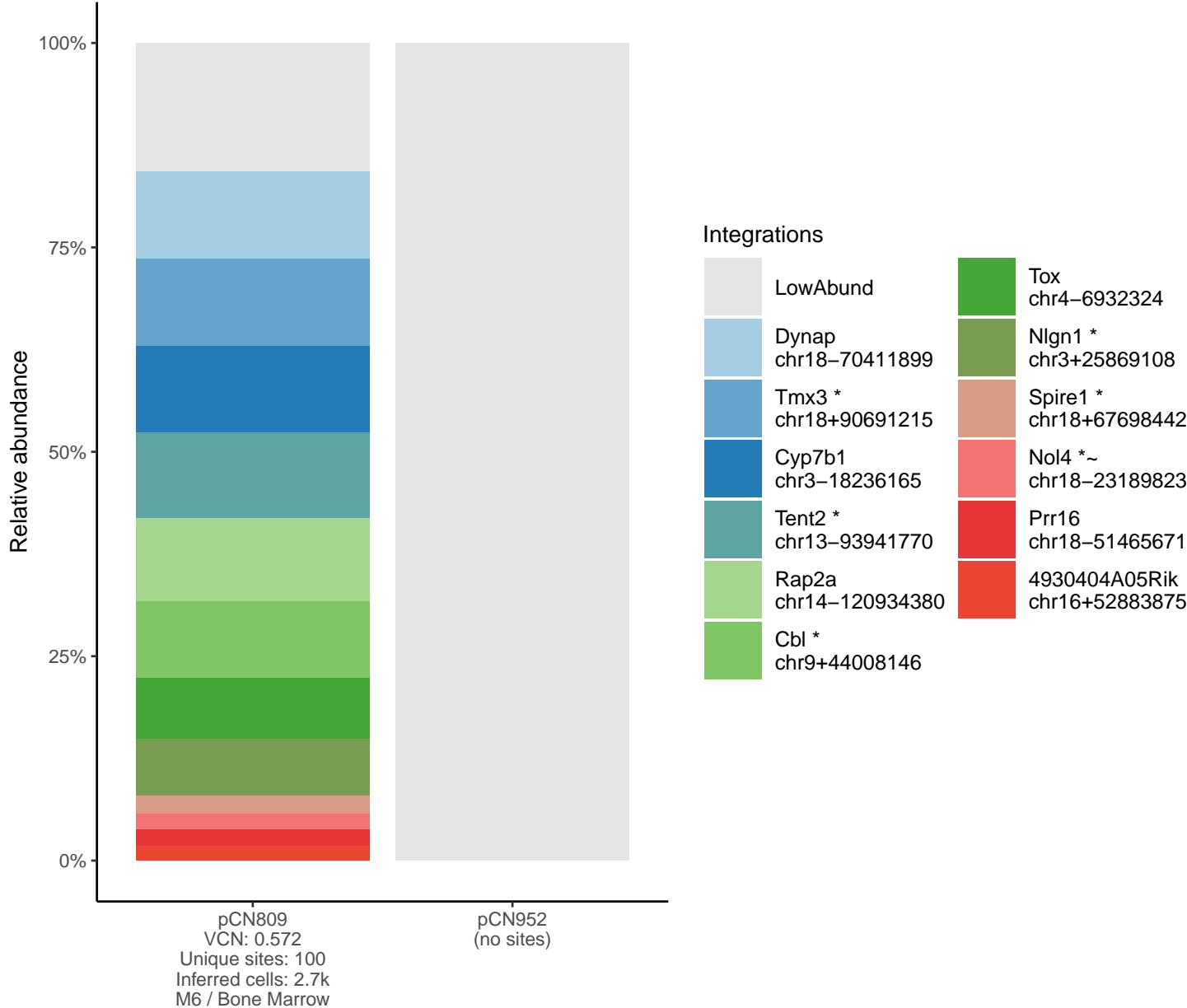


Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN801	96	6
pCN951	31	1

• Integration at chr7-80692518, near 1810026B05Rik, is 6 KB upstream of oncogene Chd2.

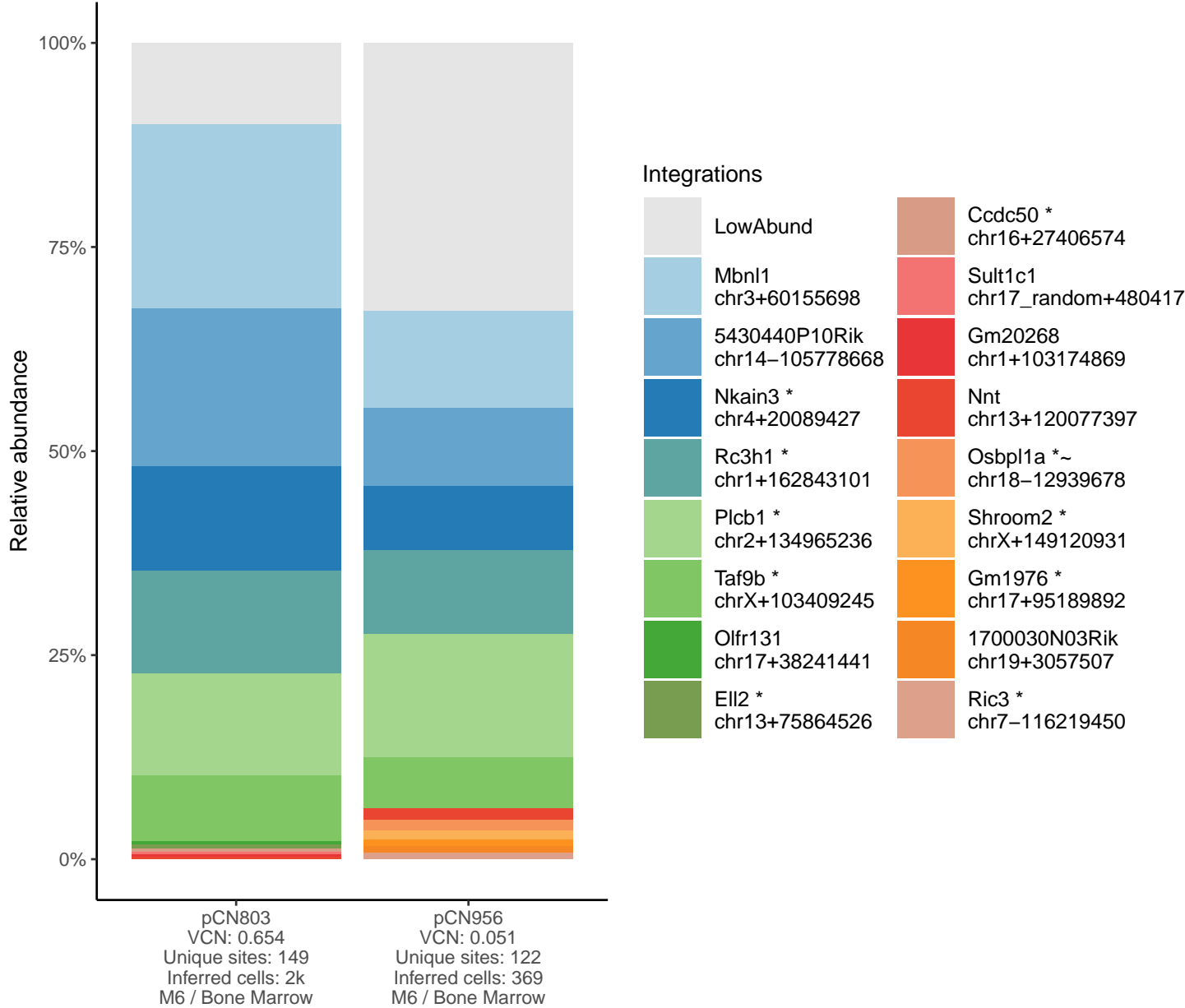
Figure 5c.



Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN809	94	6
NA	0	0

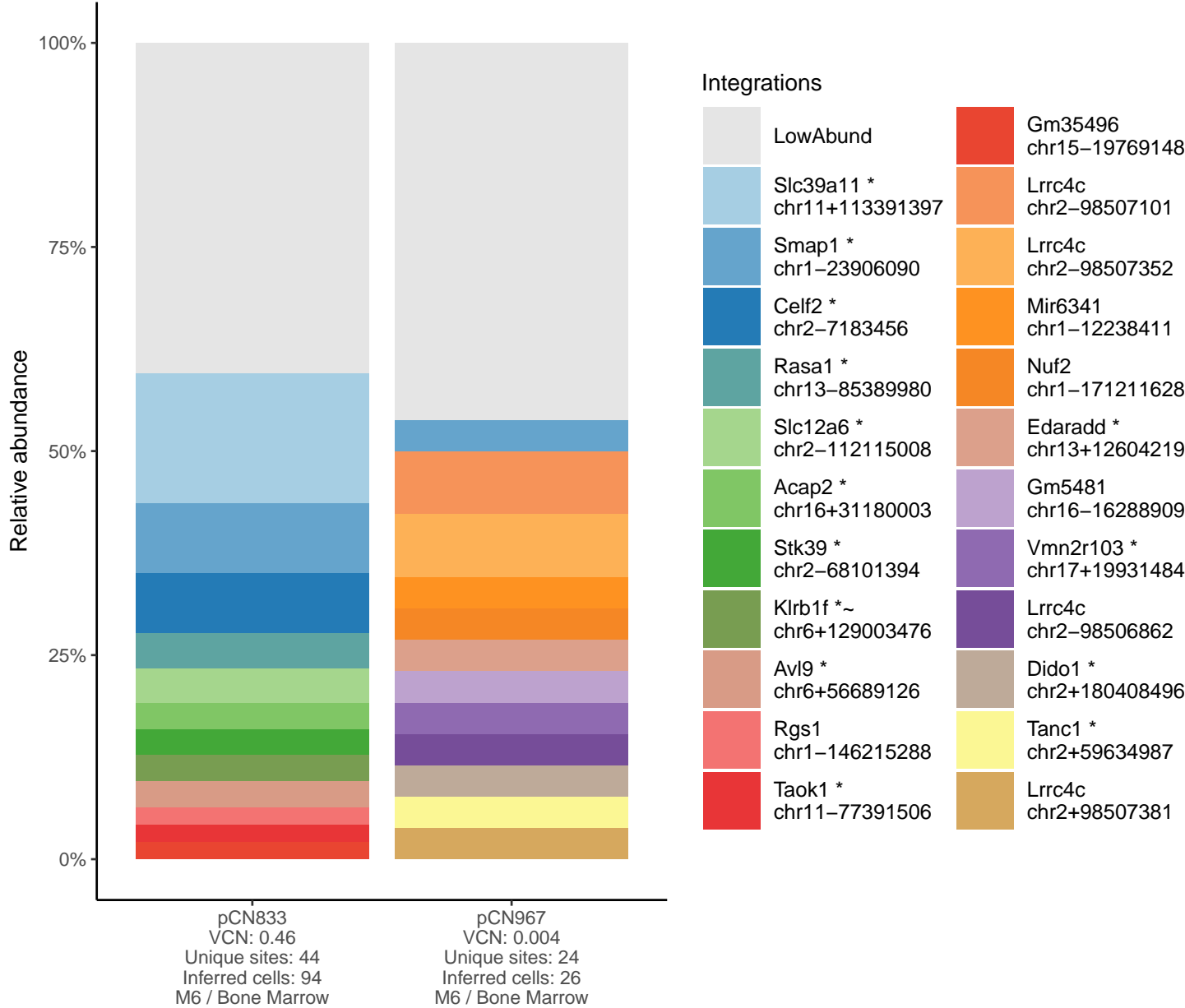
Figure 5d.



Fisher's exact p-value: 0.355

	Not near onco	Near onco
pCN803	141	8
pCN956	119	3

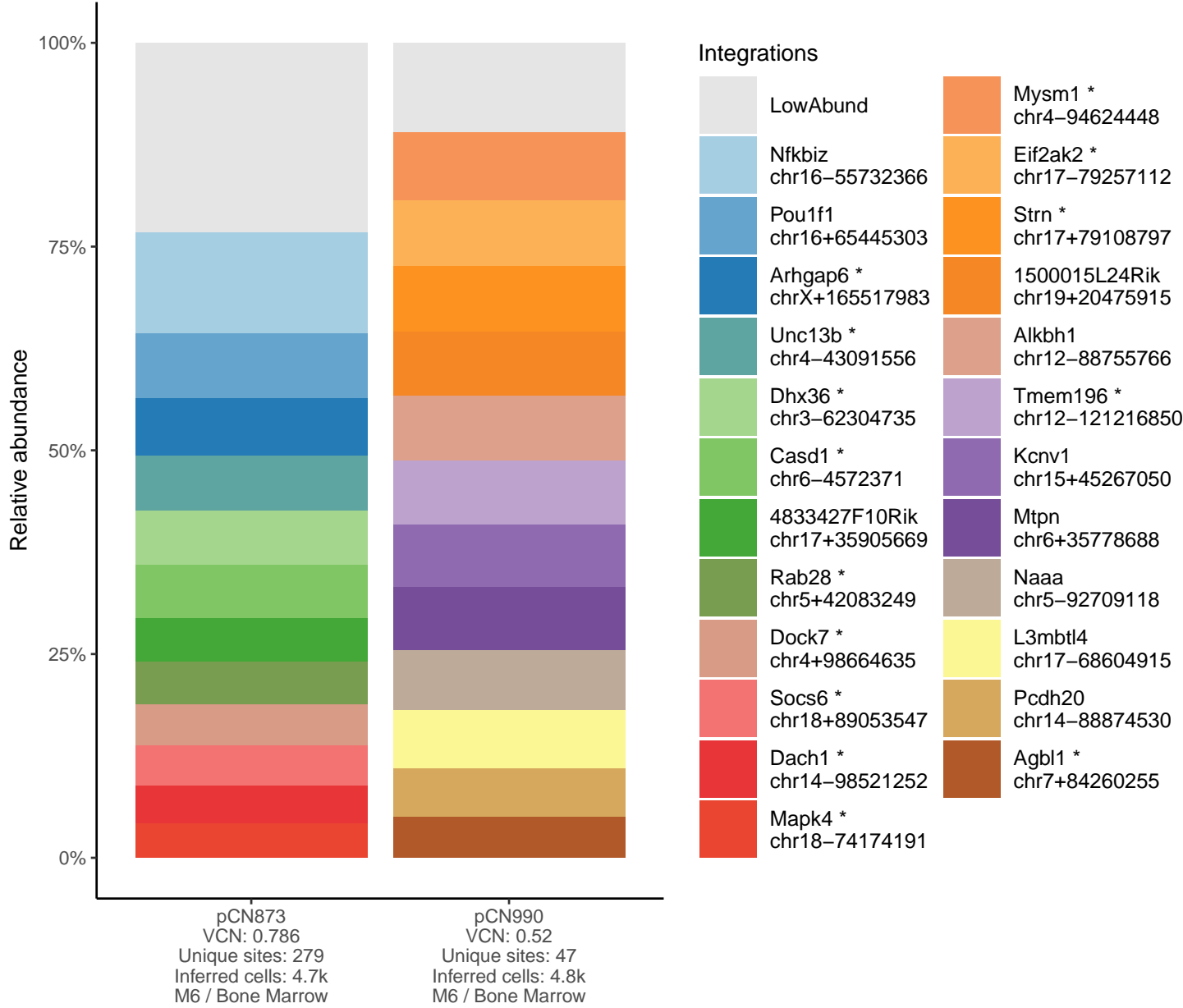
Figure 5e.



Fisher's exact p-value: 0.547

	Not near onco	Near onco
pCN833	41	3
pCN967	24	0

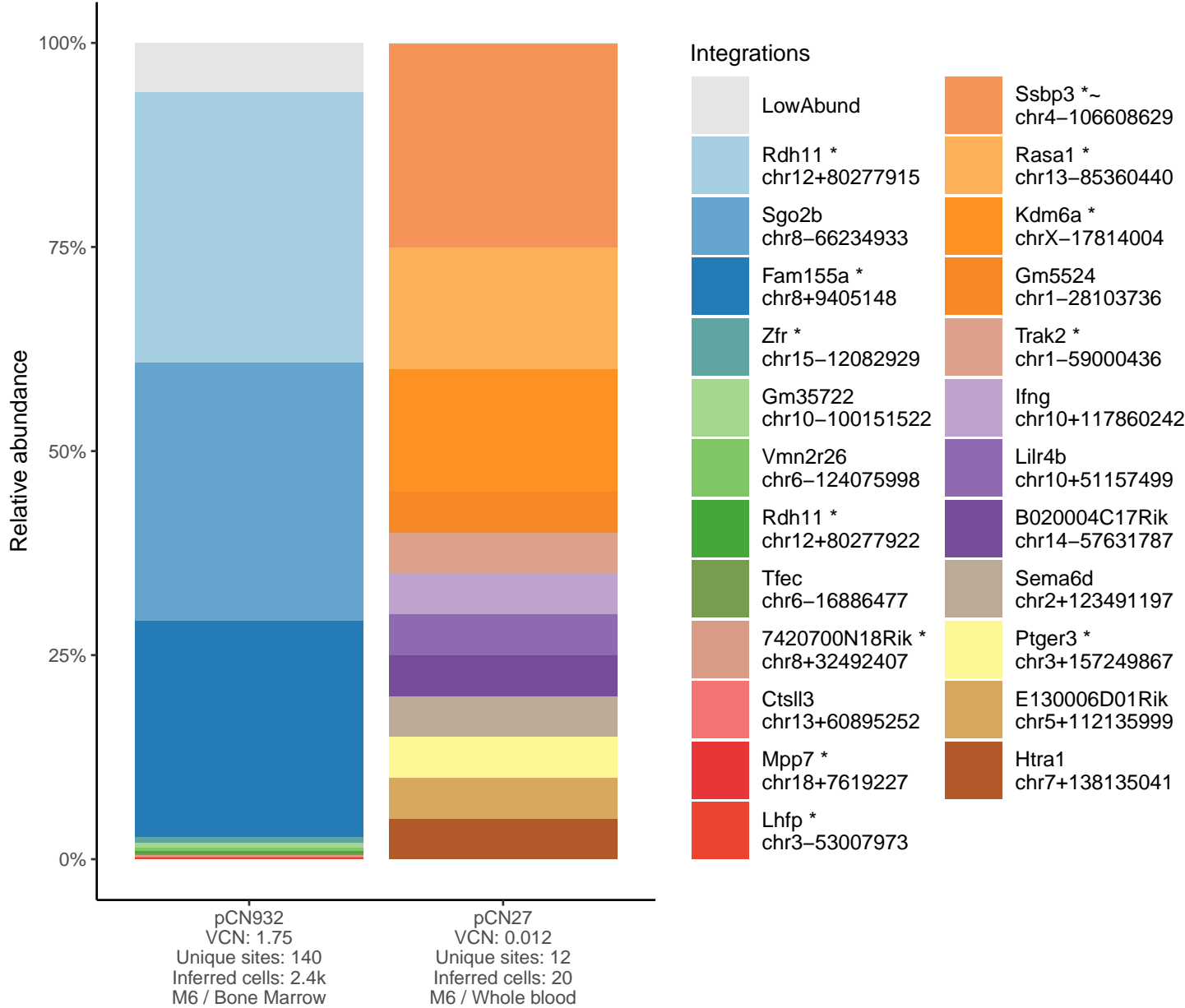
Figure 5f.



Fisher's exact p-value: 0.705

	Not near onco	Near onco
pCN873	265	14
pCN990	46	1

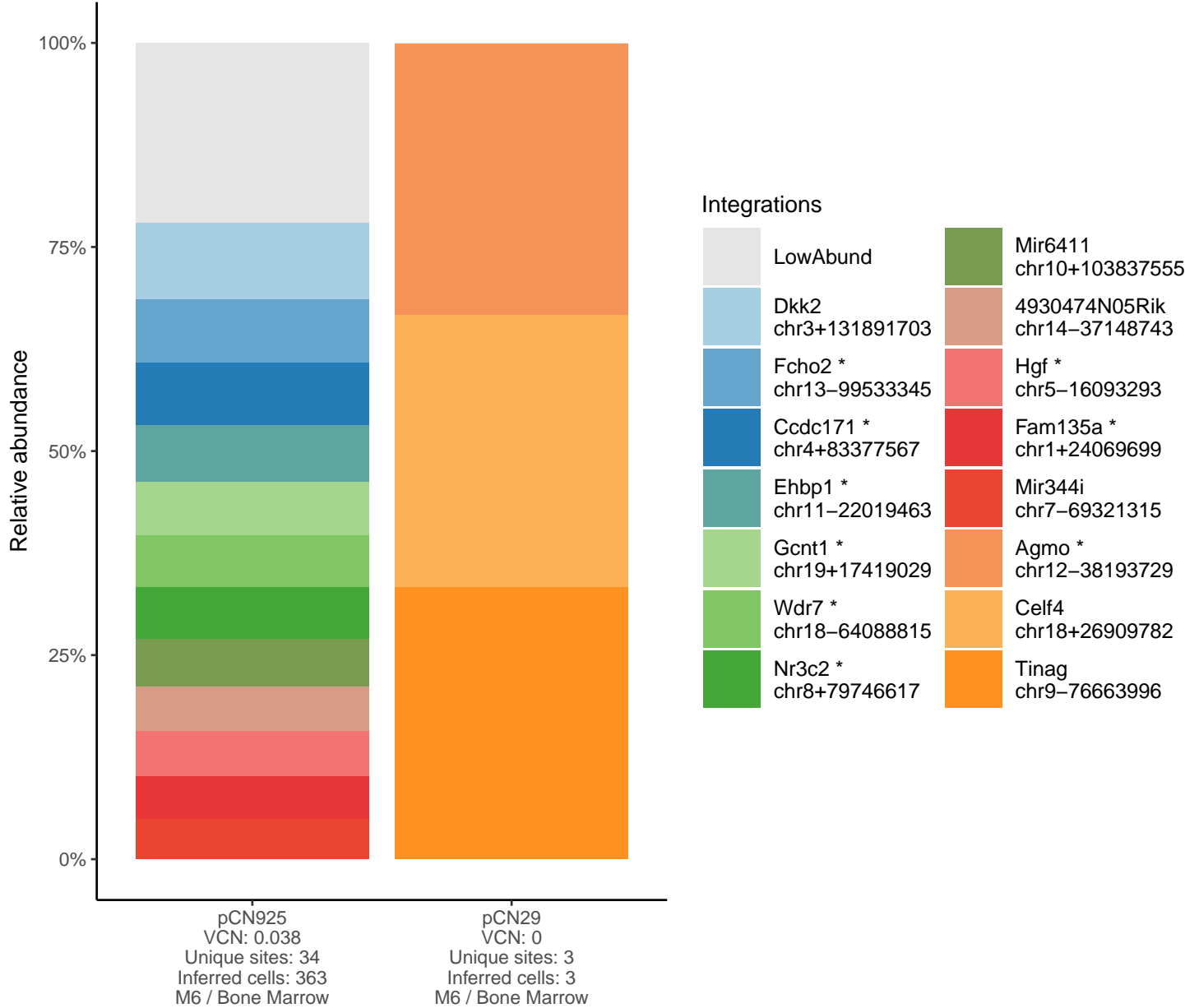
Figure 5g.



Fisher's exact p-value: 0.341

	Not near onco	Near onco
pCN932	136	4
pCN27	11	1

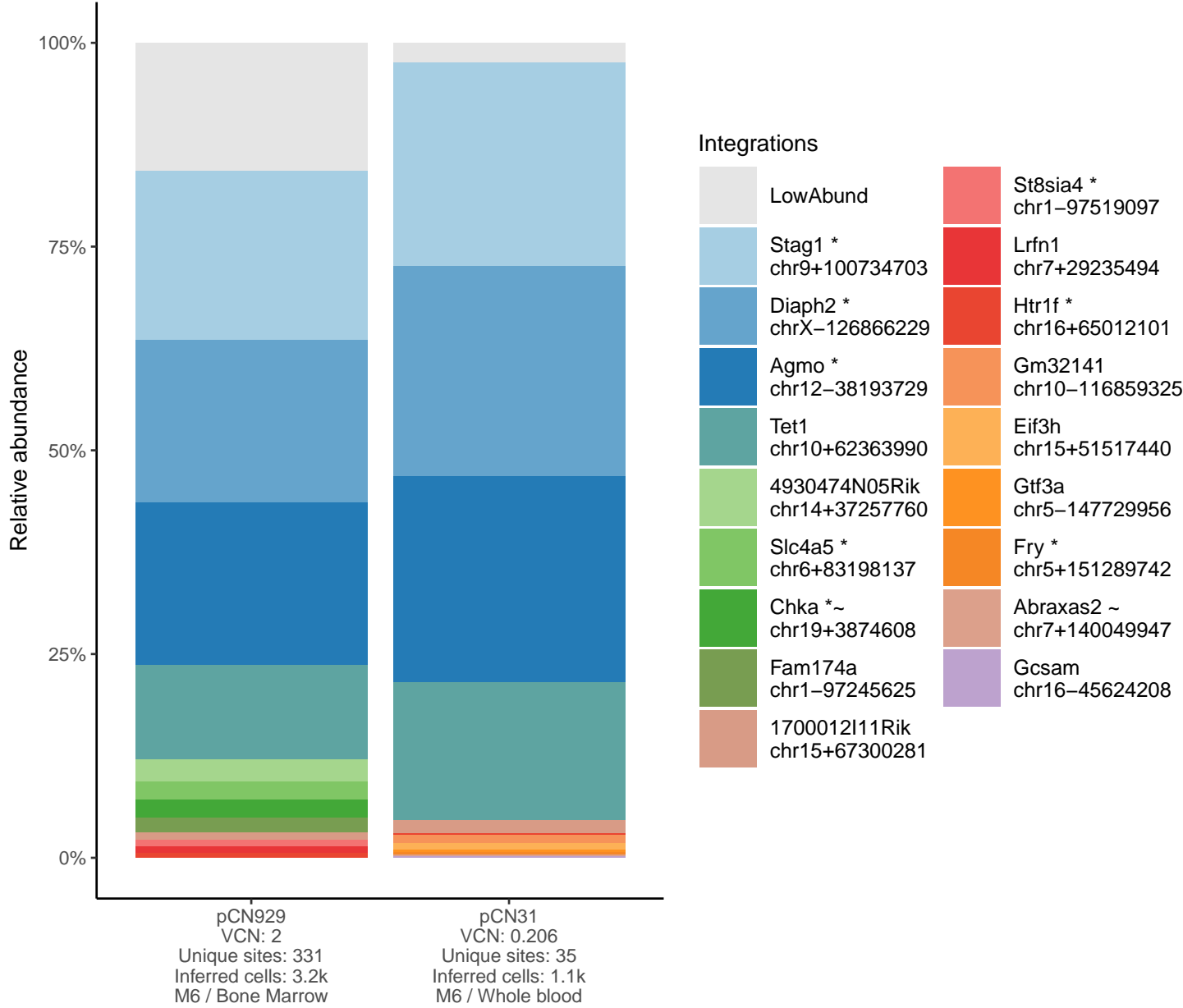
Figure 5h.



Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN925	34	0
pCN29	3	0

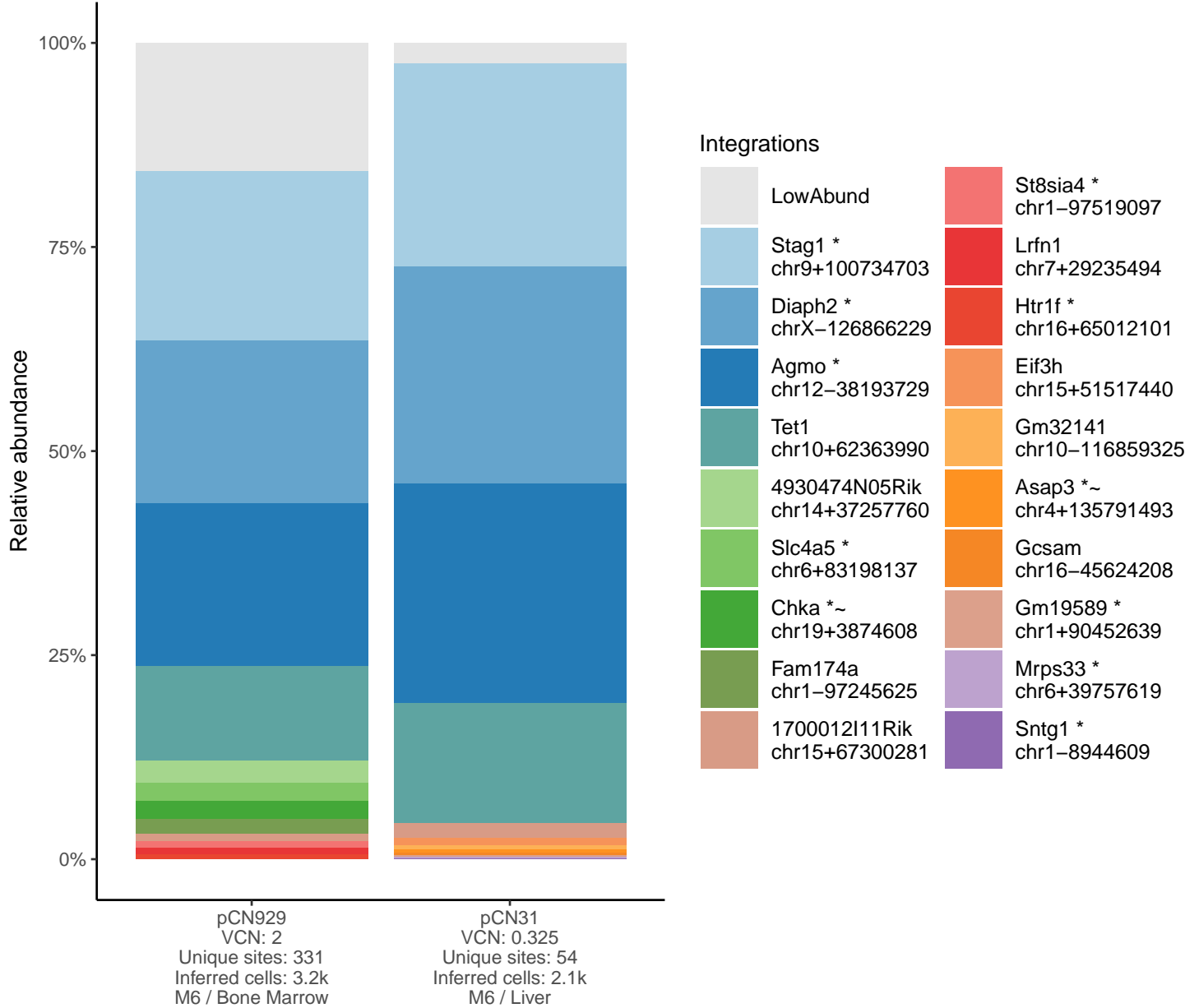
Figure 5i.



Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN929	305	26
pCN31	33	2

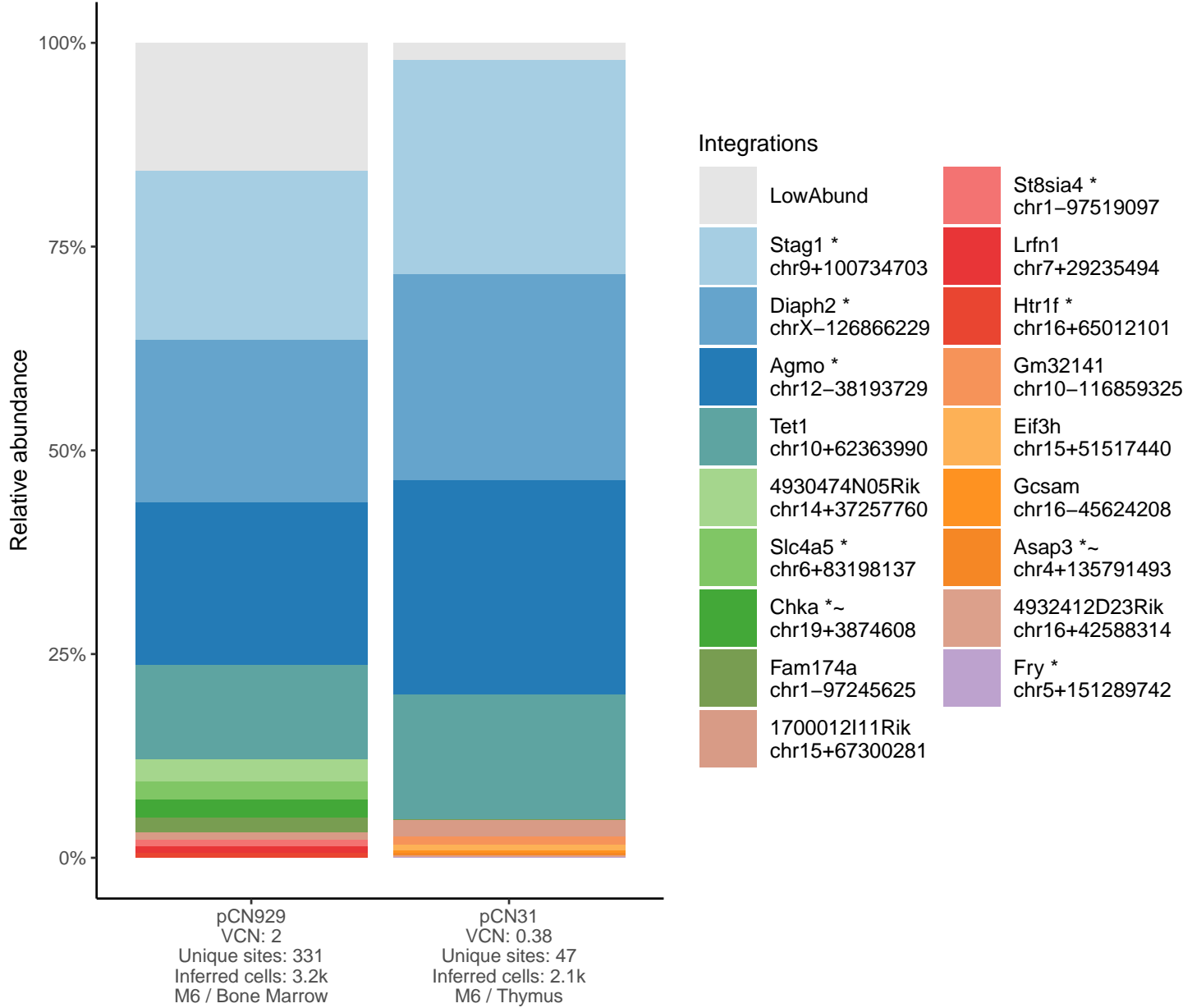
Figure 5j.



Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN929	305	26
pCN31	50	4

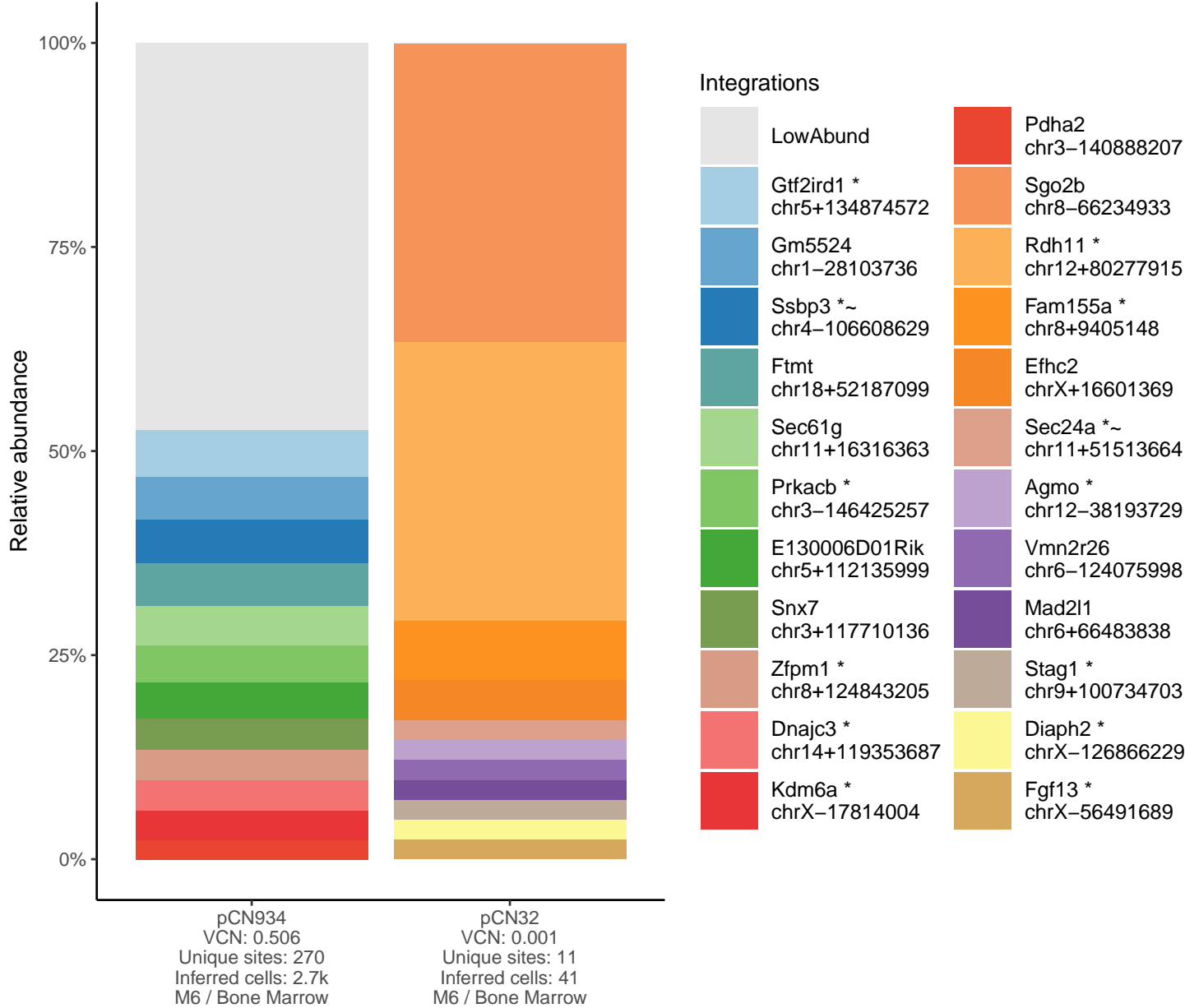
Figure 5k.



Fisher's exact p-value: 0.778

	Not near onco	Near onco
pCN929	305	26
pCN31	43	4

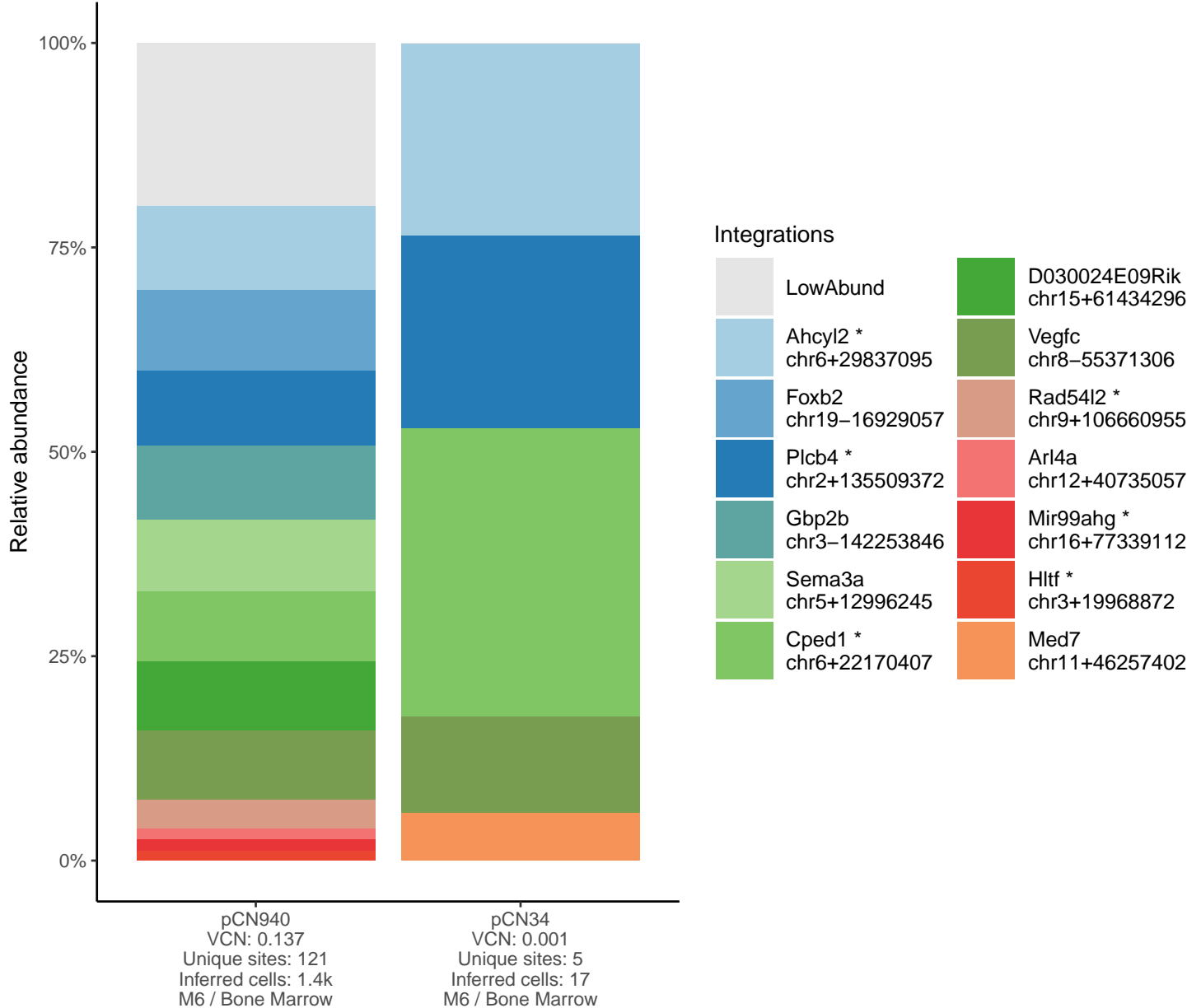
Figure 5l.



Fisher's exact p-value: 0.482

	Not near onco	Near onco
pCN934	255	15
pCN32	10	1

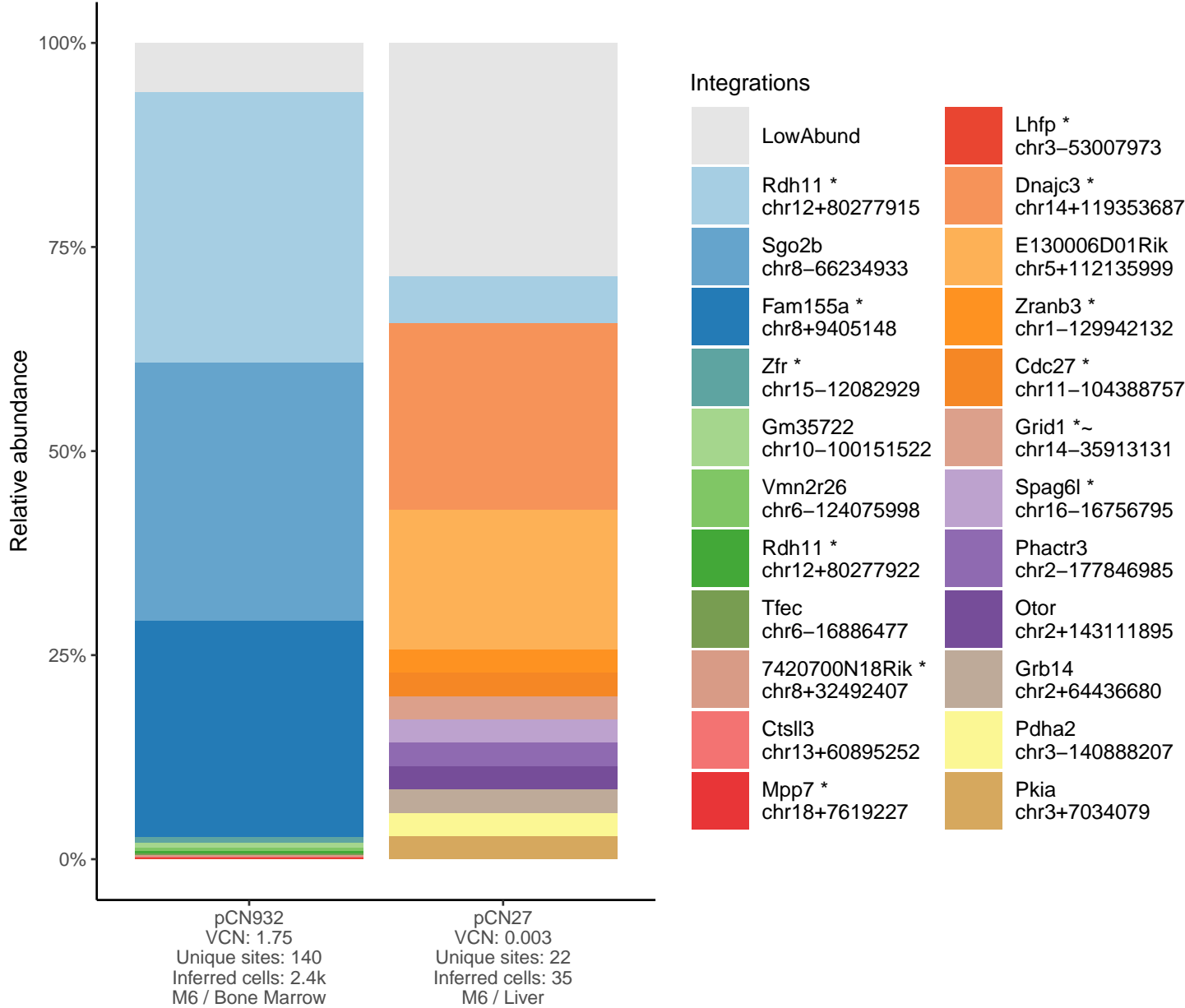
Figure 5m.



Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN940	116	5
pCN34	5	0

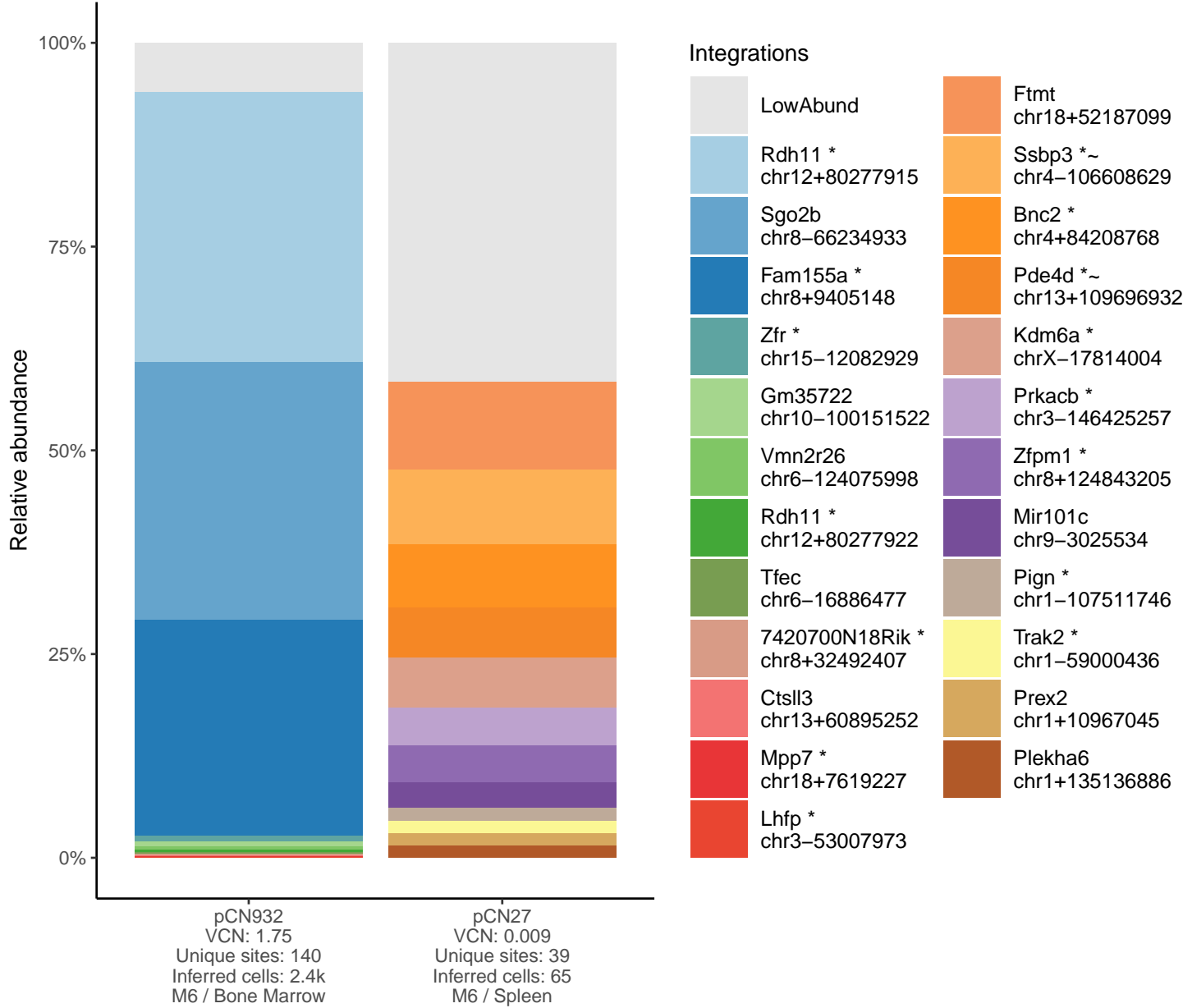
Figure 5n.



Fisher's exact p-value: 0.523

	Not near onco	Near onco
pCN932	136	4
pCN27	21	1

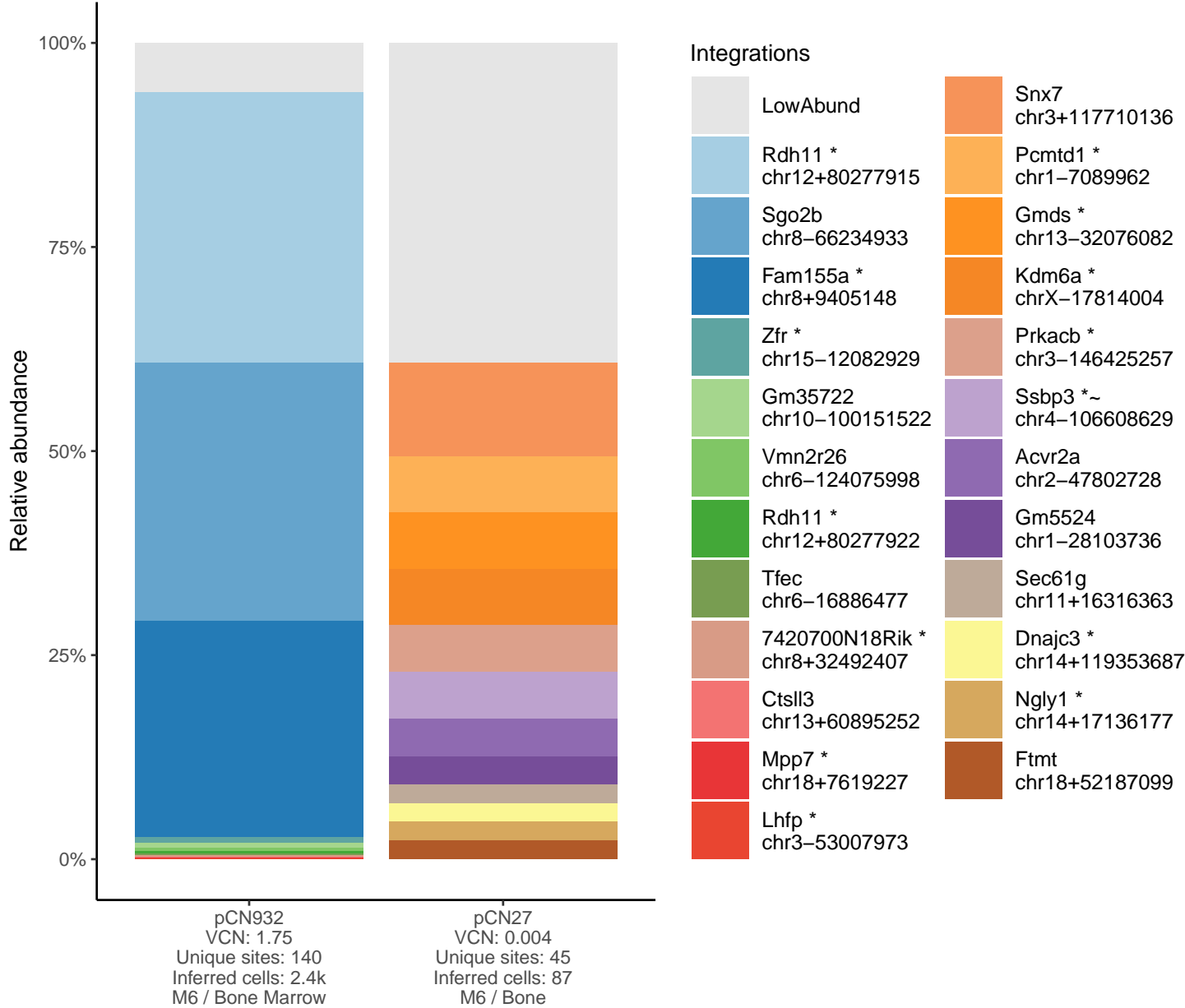
Figure 5o.



Fisher's exact p-value: 0.176

	Not near onco	Near onco
pCN932	136	4
pCN27	36	3

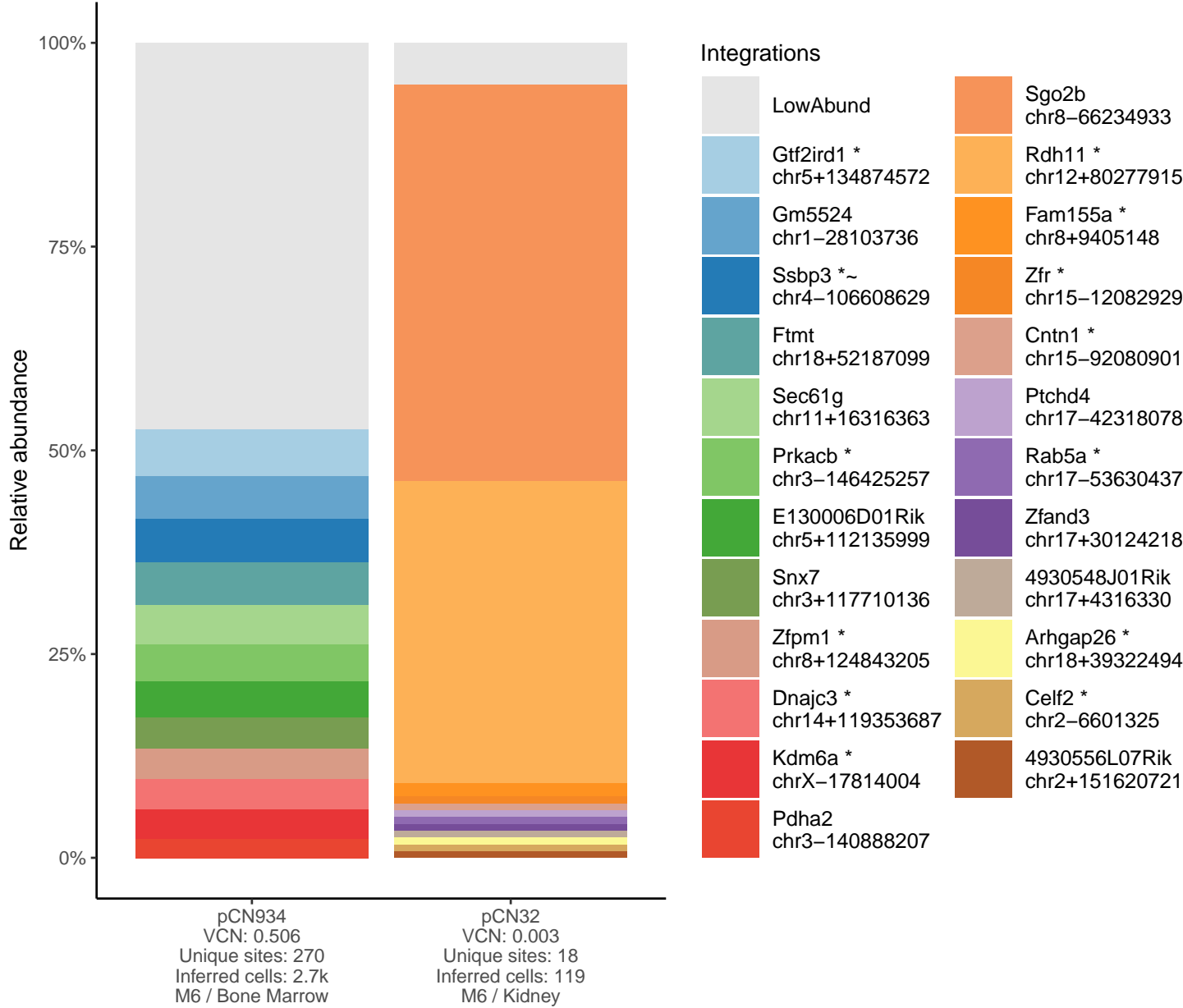
Figure 5p.



Fisher's exact p-value: 0.634

	Not near onco	Near onco
pCN932	136	4
pCN27	43	2

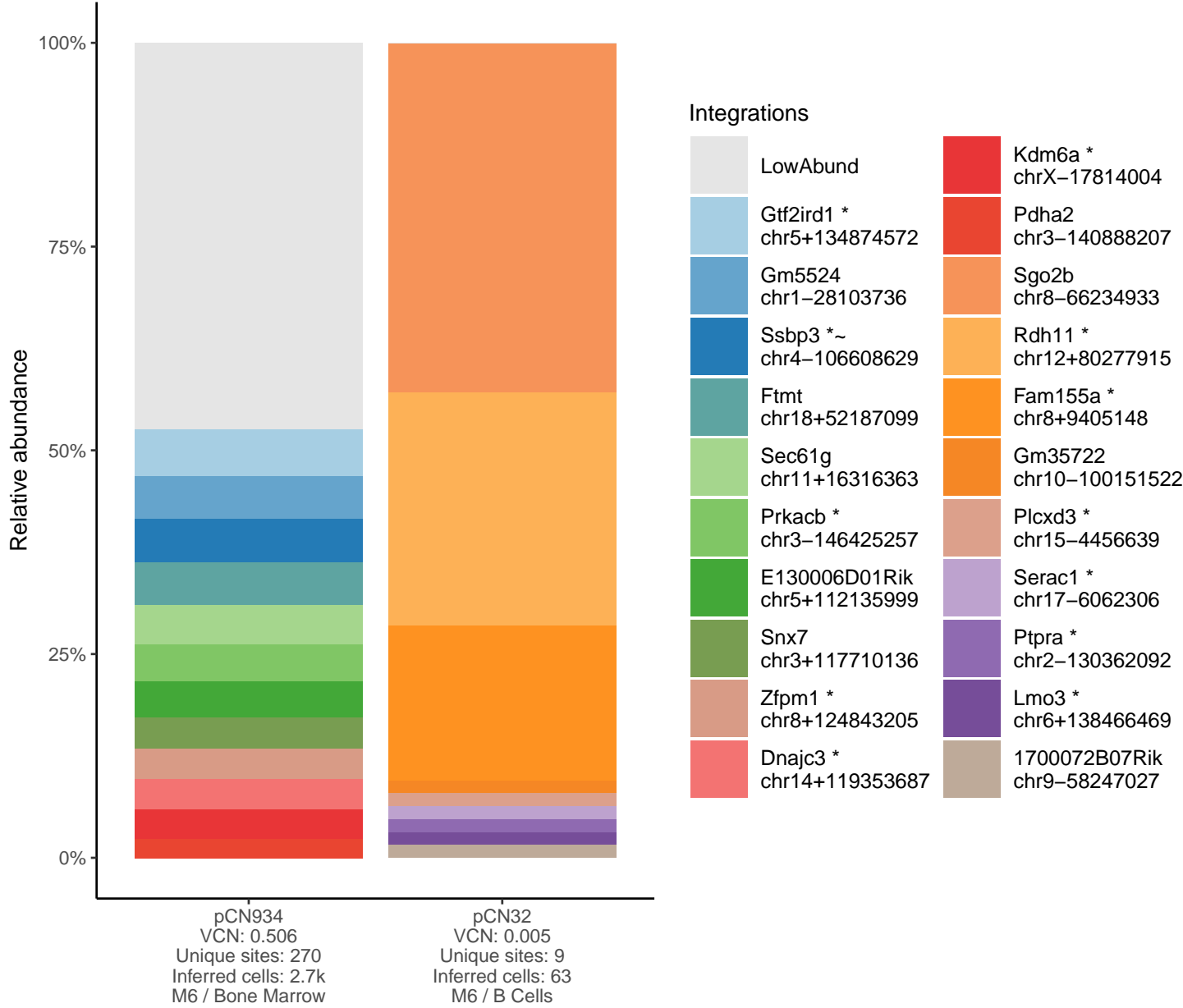
Figure 5q.



Fisher's exact p-value: 0.609

	Not near onco	Near onco
pCN934	255	15
pCN32	18	0

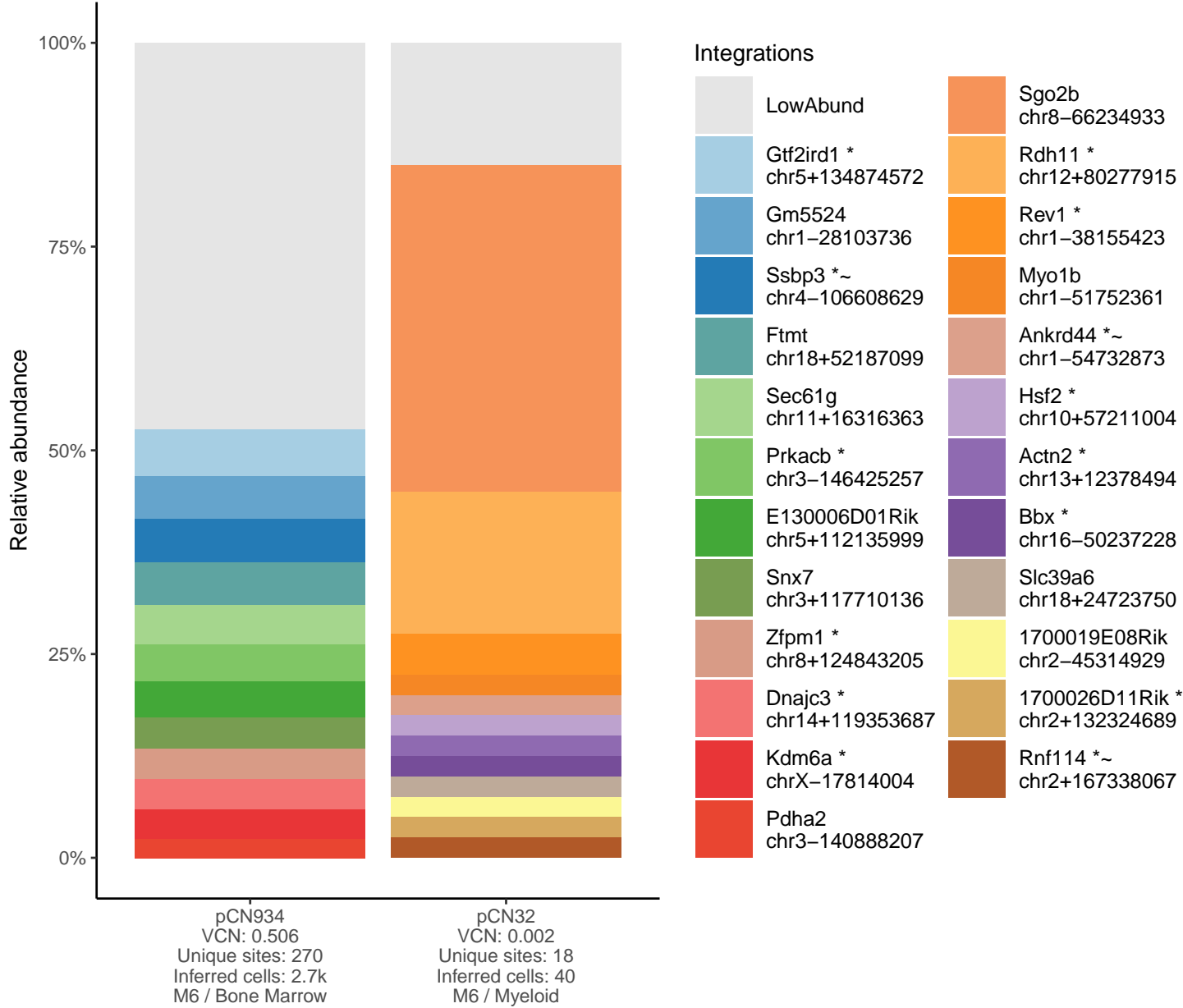
Figure 5r.



Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN934	255	15
pCN32	9	0

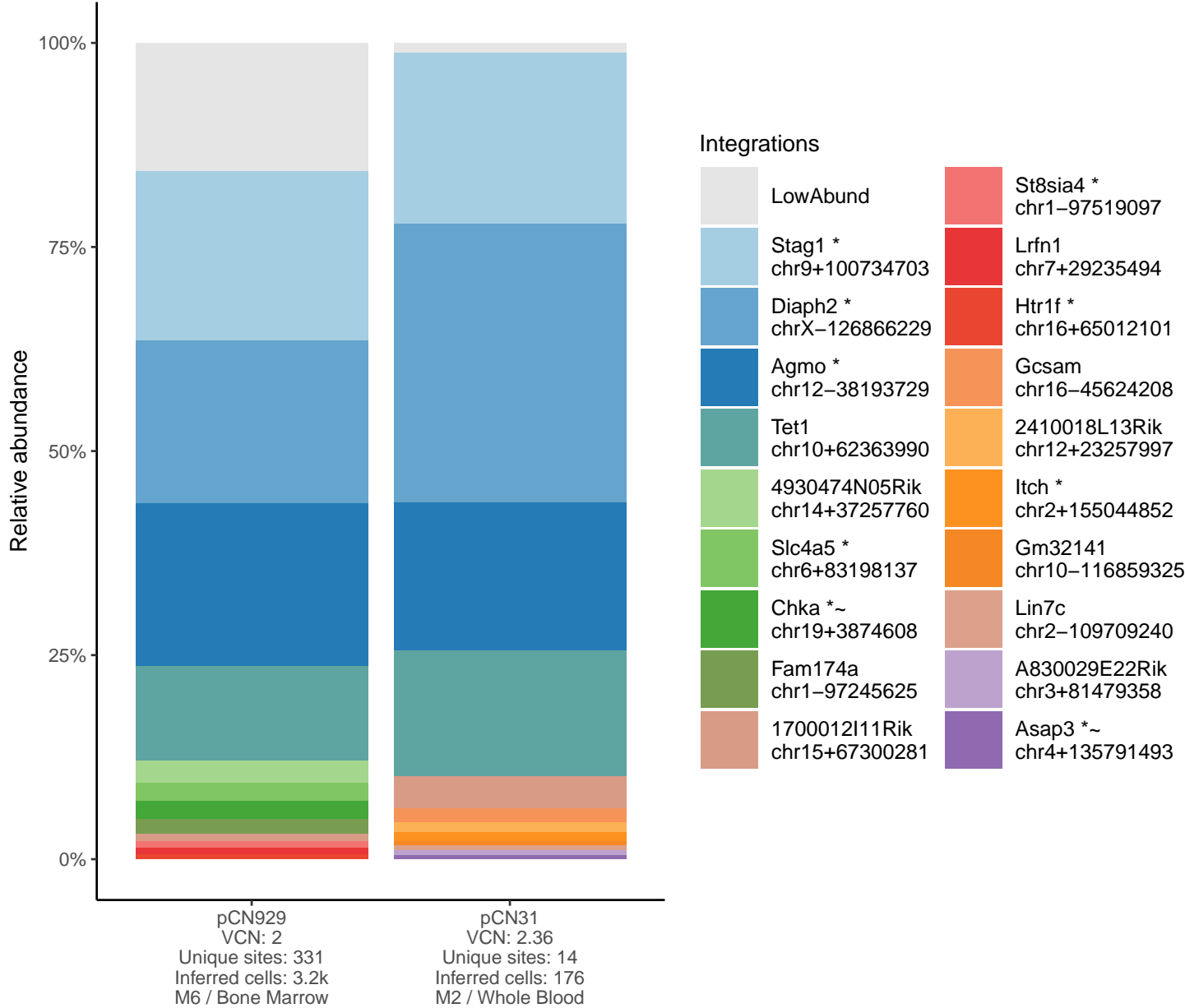
Figure 5s.



Fisher's exact p-value: 0.023

	Not near onco	Near onco
pCN934	255	15
pCN32	14	4

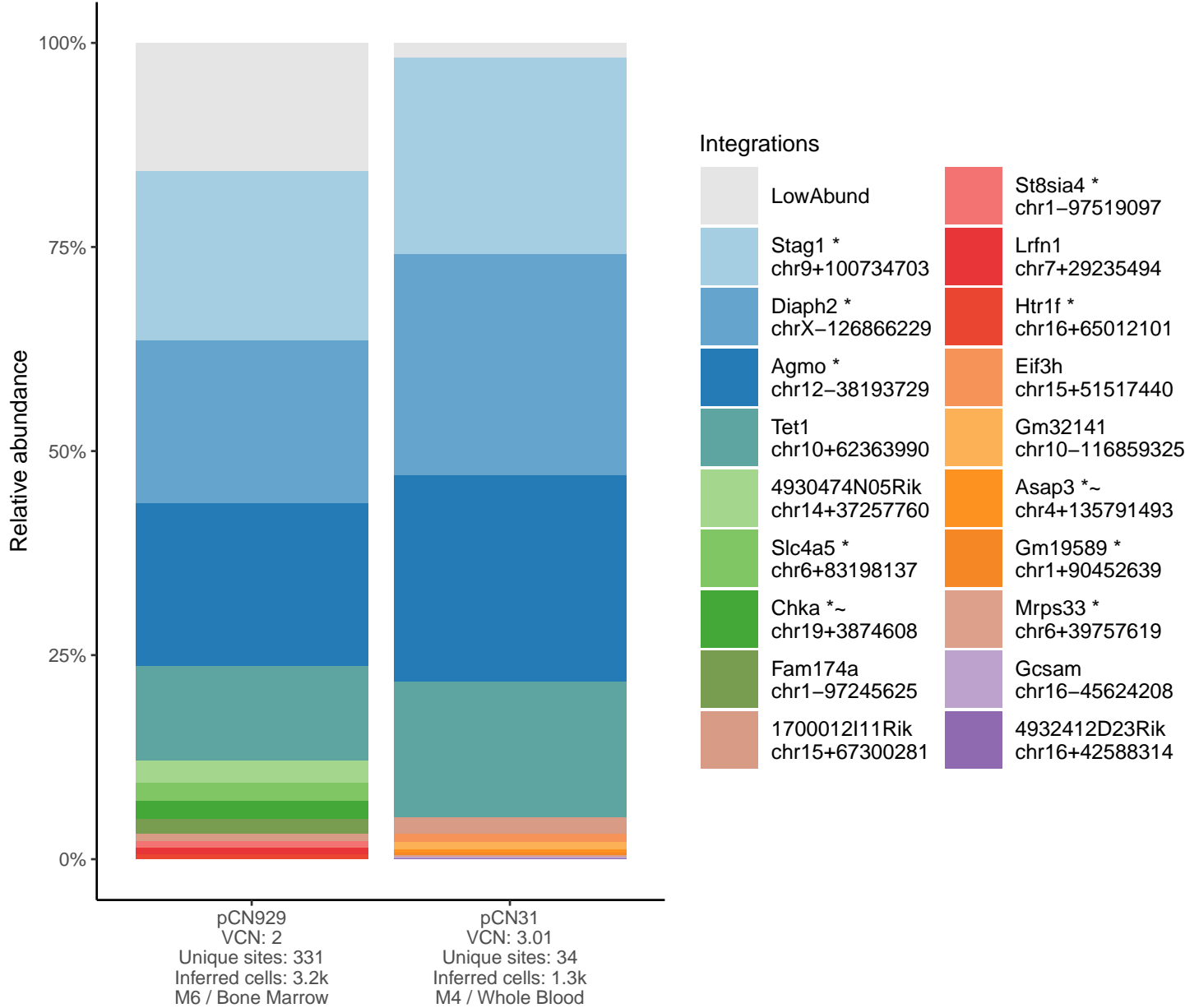
Figure 5t.



Fisher's exact p-value: 1.000

	Not near onco	Near onco
pCN929	305	26
pCN31	13	1

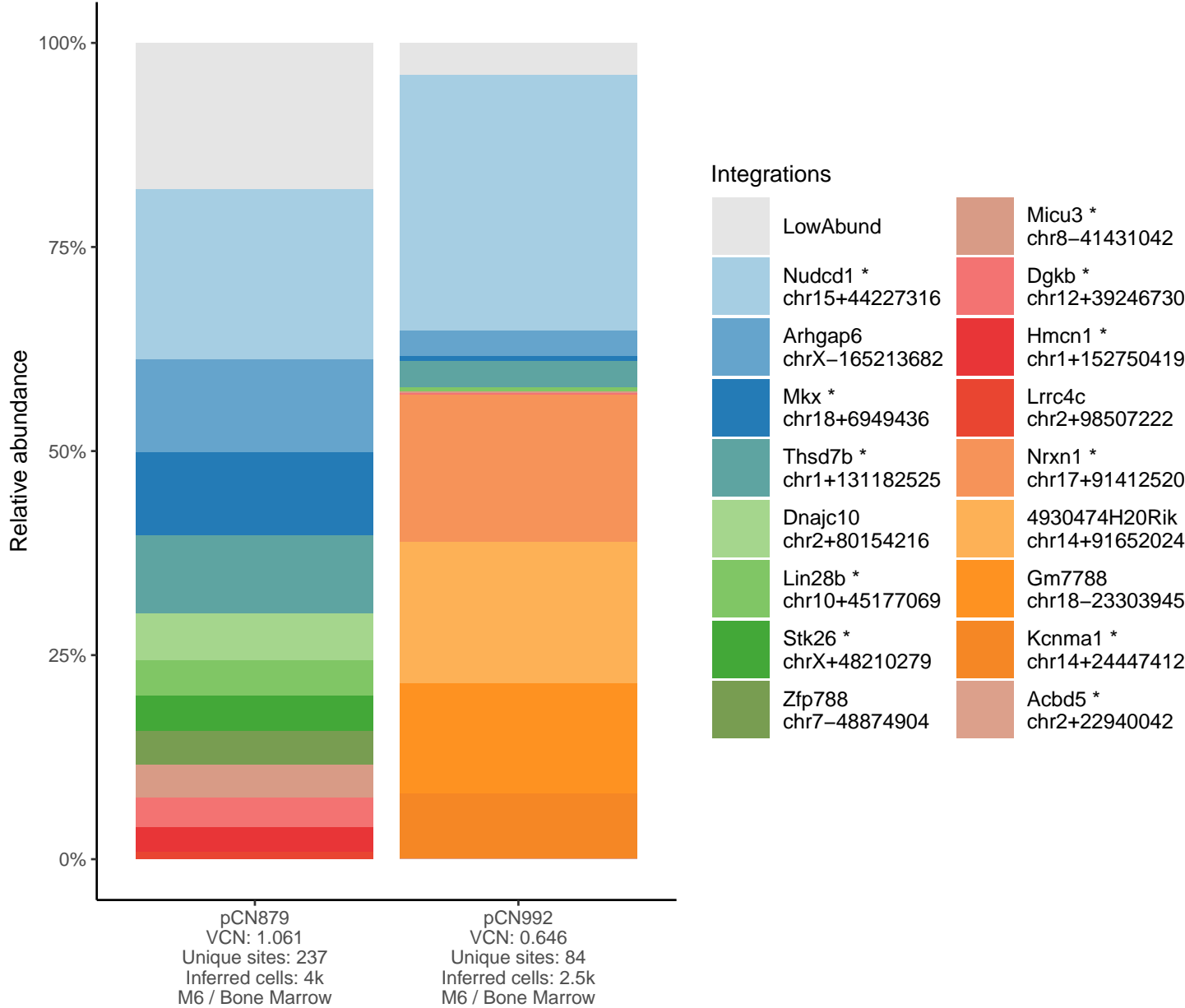
Figure 5u.



Fisher's exact p-value: 0.743

	Not near onco	Near onco
pCN929	305	26
pCN31	31	3

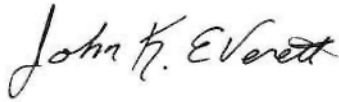
Figure 5v.



Fisher's exact p-value: 0.213

	Not near onco	Near onco
pCN879	218	19
pCN992	81	3

Analyst



John K. Everett, Ph.D.

Laboratory director



Frederic D. Bushman, Ph.D.

References

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Supplementary tables and figures

Numbers of inferred cells and integration sites identified in provided samples

Table S1.

Organism	GTSP	Subject	Cell type	VCN	Time point	Number inferred cells	Number of intSites
mouse	GTSP1977	pCN774	Bone Marrow	NA	M6	471	191
mouse	GTSP1978	pCN801	Bone Marrow	9.580	M6	7,842	102
mouse	GTSP1979	pCN803	Bone Marrow	0.654	M6	2,036	149
mouse	GTSP1982	pCN809	Bone Marrow	0.572	M6	2,675	100
mouse	GTSP2184	pCN835	Bone Marrow	1.481	M6	393	20
mouse	GTSP2185	pCN836	Bone Marrow	0.256	M6	99	64
mouse	GTSP2186	pCN837	Bone Marrow	0.138	M6	66	40
mouse	GTSP2187	pCN833	Bone Marrow	0.460	M6	94	44
mouse	GTSP2216	pCN873	Bone Marrow	0.786	M6	4,681	279
mouse	GTSP2217	pCN878	Bone Marrow	1.260	M6	4,125	225
mouse	GTSP2218	pCN879	Bone Marrow	1.061	M6	3,976	237
mouse	GTSP2320	pCN925	Bone Marrow	0.038	M6	363	34
mouse	GTSP2321	pCN929	Bone Marrow	2.000	M6	3,237	331
mouse	GTSP2322	pCN932	Bone Marrow	1.750	M6	2,436	140
mouse	GTSP2323	pCN934	Bone Marrow	0.506	M6	2,720	270
mouse	GTSP2324	pCN940	Bone Marrow	0.137	M6	1,431	121
mouse	GTSP2326	pCN951	Bone Marrow	0.083	M6	700	32
mouse	GTSP2328	pCN956	Bone Marrow	0.051	M6	369	122
mouse	GTSP2341	pCN801	Whole blood	1.300	M2	533	145
mouse	GTSP2343	pCN801	Whole blood	3.020	M6	4,356	337
mouse	GTSP2344	pCN801	BM Myeloid cells	7.950	M6	8,411	45
mouse	GTSP2345	pCN801	B-cells	2.690	M6	7,174	496
mouse	GTSP2346	pCN801	T cells	1.100	M6	1,960	38
mouse	GTSP2347	pCN948	Hematoma	0.018	M6	334	207
mouse	GTSP2349	pCN948	B-cells	0.019	M6	526	258
mouse	GTSP2350	pCN951	BM Myeloid cells	0.021	M6	184	35
mouse	GTSP2351	pCN951	B-cells	1.269	M6	2,869	24
mouse	GTSP2352	pCN951	T cells	0.026	M6	326	35
mouse	GTSP2353	pCN967	Bone Marrow	0.004	M6	26	24
mouse	GTSP2733	pCN990	Bone Marrow	0.520	M6	4,792	47
mouse	GTSP2752	pCN27	Whole blood	0.012	M6	20	12
mouse	GTSP2753	pCN31	Whole blood	0.206	M6	1,118	35
mouse	GTSP2754	pCN31	Liver	0.325	M6	2,082	54
mouse	GTSP2755	pCN31	Thymus	0.380	M6	2,100	47
mouse	GTSP2756	pCN29	Bone Marrow	0.000	M6	3	3
mouse	GTSP2757	pCN32	Bone Marrow	0.001	M6	41	11
mouse	GTSP2758	pCN34	Bone Marrow	0.001	M6	17	5
mouse	GTSP2759	pCN992	Bone Marrow	0.646	M6	2,545	84
mouse	GTSP3095	pCN32	B Cells	0.003	M6	85	7
mouse	GTSP3096	pCN32	Liver	0.004	M6	24	8
mouse	GTSP3126	pCN27	Liver	0.003	M6	35	22
mouse	GTSP3127	pCN27	Spleen	0.009	M6	65	39
mouse	GTSP3128	pCN27	Bone	0.004	M6	87	45
mouse	GTSP3129	pCN32	Kidney	0.003	M6	119	18
mouse	GTSP3130	pCN32	B Cells	0.005	M6	63	9
mouse	GTSP3131	pCN32	Myeloid	0.002	M6	40	18
mouse	GTSP3132	pCN31	Whole Blood	2.360	M2	176	14
mouse	GTSP3133	pCN31	Whole Blood	3.010	M4	1,342	34

Persistence of clones in mouse BM transplant trials

Table S2.

Donor	Recipient	Position	Donor cells	Recipient cells
pCN801	pCN951	chr10-63786517	568	69
pCN801	pCN951	chr12-91119873	615	55
pCN801	pCN951	chr12+41889014	629	52
pCN801	pCN951	chr16-58574863	596	63
pCN801	pCN951	chr17-92104190	589	43
pCN801	pCN951	chr17+84340467	626	56
pCN801	pCN951	chr18-47475939	552	75
pCN801	pCN951	chr18-6677426	5	4
pCN801	pCN951	chr3-62285345	585	49
pCN801	pCN951	chr5-90109547	195	2
pCN801	pCN951	chr5+91163622	569	46
pCN801	pCN951	chr7-80692518	604	58
pCN801	pCN951	chr8+116693173	599	56
pCN801	pCN951	chrX+82041081	596	53
pCN803	pCN956	chr1+162843101	258	38
pCN803	pCN956	chr1+43686997	1	1
pCN803	pCN956	chr10-20694821	2	1
pCN803	pCN956	chr10+54685831	1	1
pCN803	pCN956	chr10+72683496	1	2
pCN803	pCN956	chr12+75878946	4	1
pCN803	pCN956	chr13+120077397	6	5
pCN803	pCN956	chr14-105778668	392	35
pCN803	pCN956	chr17+38241441	10	2
pCN803	pCN956	chr17+55932173	1	1
pCN803	pCN956	chr17+95189892	2	3
pCN803	pCN956	chr18-42192342	1	1
pCN803	pCN956	chr19+3057507	2	3
pCN803	pCN956	chr2+126512656	6	2
pCN803	pCN956	chr2+134965236	254	56
pCN803	pCN956	chr3-25751852	1	1