Analysis of integration site distributions and clonal abundances for gene therapy correction of cystinosis

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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 both mouse and human subjects and assess potential clonal expansions. The list of human oncogenes used throughout this analysis was compiled from the literature (link) and 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. The human oncogene list comprises 7.46% of all human genes (NCBI RefSeq) whereas the mouse oncogene list comprises 2.01% of all mouse genes. The frequency of integration sites in human subjects near oncogenes was not significantly different than a published trial using a comparable vector to correct Wiskott-Aldrich syndrome (WAS) from which no adverse events have been reported². The frequency of integration sites in bone marrow donor mice 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 ³. The integration position profile for human subjects was very similar to that found in the WAS trial. The mouse bone marrow transplant trials yielded varying degrees of persistence in five of the nine trials where detection was limited due to both sequencing depth and vector copy number. No significant enrichment of integration events near oncogenes was identified between donor and recipient mice. The code base for this analysis is available on-line (link).

Human and mouse samples studied

Integration sites were detected in 70 samples from both human and mouse subjects (Tables 1 & S1) while no integration sites were detected in 10 of the provided samples (Table S2).

Table 1. Overview of data collection.

| Organism | Number of samples | Number of reads | Number of inferred cells | Number of integration sites |
|----------|-------------------|-----------------|--------------------------|-----------------------------|
| human | 30 | 19,834,249 | 133,607 | 92,924 |
| mouse | 40 | 20,865,780 | 53,928 | 4,769 |

Subject reports

Subject specific reports for all subjects are available via a protected web archive (link).

user: cherqui

pass: geneTherapy@!#

UCSC browser exploration

UCSC browser sessions pre-loaded with the integration sites identified in this analysis are available via these links: (human subjects), (mouse subjects). Integration sites are shown as blue (positive orientation integration) and red (reverse orientation integration) tick marks. For each integration site, a second track provides the maximum clonal abundance. Entering gene names into the search bar will direct the browser to specific genes.

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.

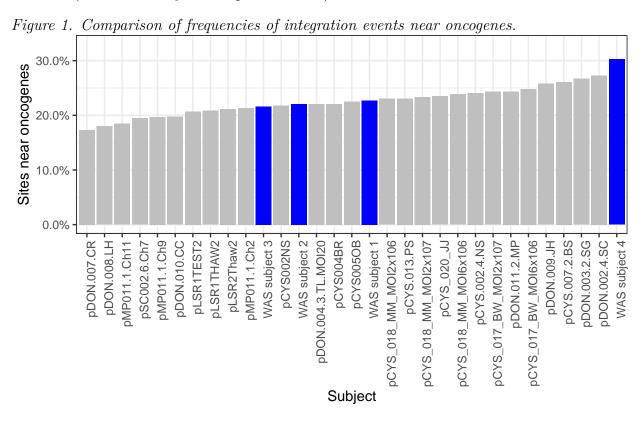
Relative clonal Abundance is determined per sample and is the percentage of identified cells attributed to a given clone. Integration sites and the clones harboring them are sampled from a larger population. It would be rare for all integration sites in a sample to be represented in the sequence data.

For this analysis, four technical replicates of each delivered sample were prepared, sequenced and analyzed with the INSPIIRED integration site analysis pipeline $(v1.2)^5$.

Comparisons to previous trials

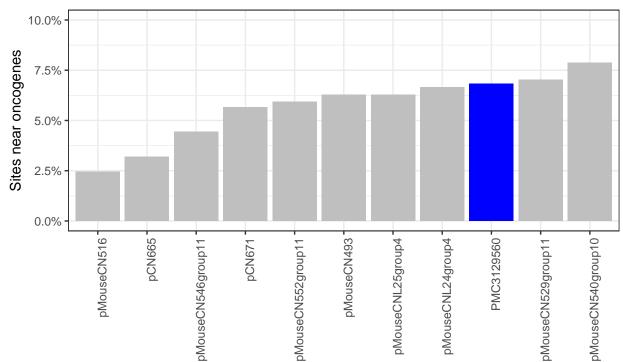
Integration events near oncogenes in human subjects

In order to determine if the experimental vector has a higher propensity of integrating near suspected oncogene in humans than previously employed vectors, the frequency of integration near oncogenes was compared to a previously published human trial¹ which used a comparable lentiviral vector to correct Wiskott-Aldrich syndrome (WAS) where no adverse events have been reported to date. The frequency of integration near oncogenes in four WAS pre-transplant patient samples was compared to the frequency of integration in the experimental day 14 samples (Figure 1 [CYS: gray, WAS: blue]). The frequencies of integration near oncogenes of samples in this study and the previous WAS study were not significantly different (Mann-Whitney U-test p-value: 0.67).



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 trial³ which used a comparable lentiviral vector to correct β -thalassemia. The frequency of integration events near oncogenes in bone marrow donor mice was generally less than the mean frequency of integration events near oncogenes in the published trial (Figure 2 [CYS: gray, β -thalassemia trial: blue]).



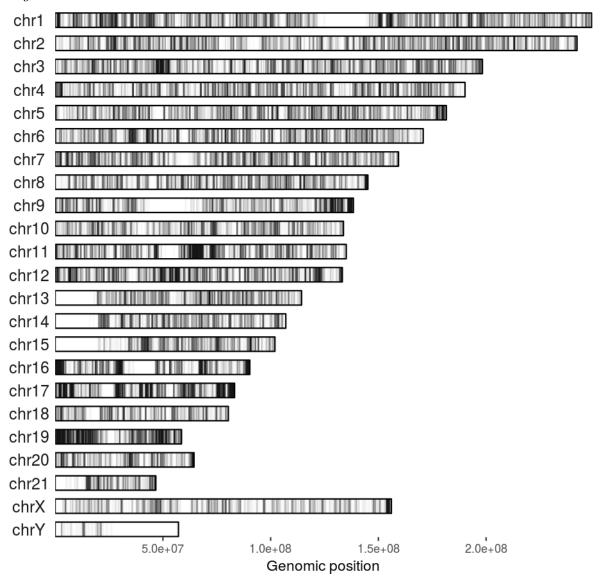
Subject

Figure 2. Comparison of frequencies of integration events near oncogenes.

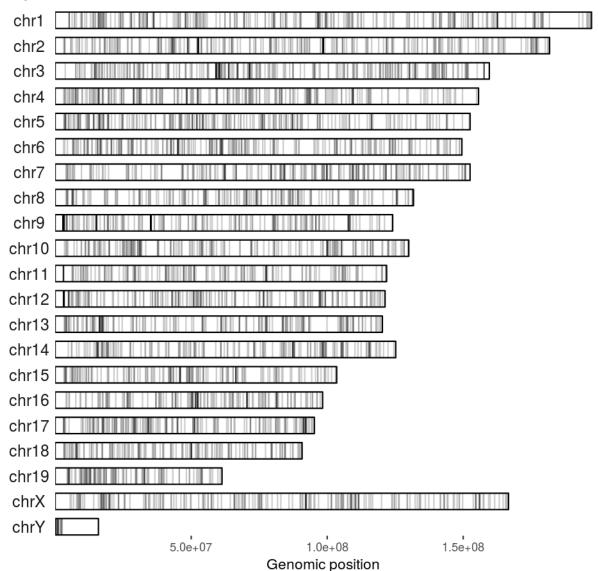
Mapping of integration site positions

Heat maps of identified integration sites are shown below in Figure 3a (human subjects) & 3b (mouse subjects). The integration position profile from human subjects is very similar to the profile from the previous WAS study (Figure S2).









Relative abundances of human subject samples

The sample relative abundance plots below (Figure 4) show the most abundant 25 clones in each human sample as colored bars while less abundant clones were relegated to a single low abundance bar shown in gray. Subject DON.002.4.SC showed an expanded clone (30% relative abundance) with an integration event down stream of the non-coding RNA gene LOC105374704. Subject DON.007.CR showed an expanded clone (15% relative abundance) with three integration events within ZNF337. These two expanded clones are not of immediate concern given that such expanded clones appeared in the WAS trial used for comparison (Figure S1) and both samples contained relatively few inferred cells which inflate relative abundance values.

Figure 4.

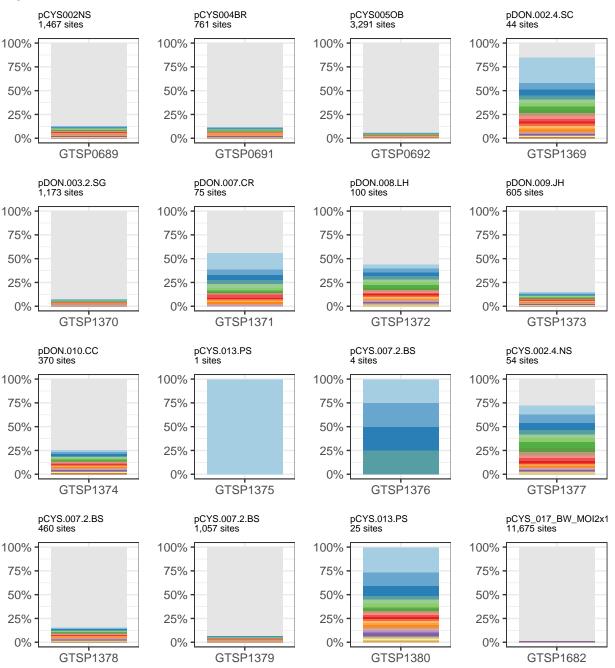
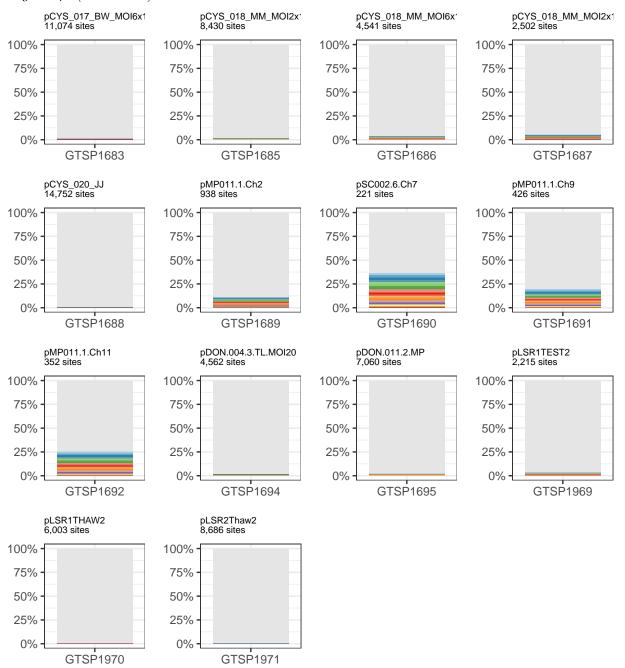


Figure 4. (continued)



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 |
|--------------------------|----------|------------|----------------------|--------------------------------|--------------------|-----------|--------------|
| pDON.002.4.SC | human | D14 | PB_CD34 | chr5 + 30409434 | 27.13% | 35 | LOC105374704 |
| pCYS.013.PS | human | D14 | PBCD34-mock | chr12 + 82136325 | 100.00% | 1 | LOC101928449 |
| pCYS.007.2.BS | human | D14 | PBCD34-mock | chr 1-167962238 | 25.00% | 1 | DCAF6 |
| pCYS.007.2.BS | human | D14 | PBCD34-mock | chr 1-218477558 | 25.00% | 1 | TGFB2 |
| pCYS.007.2.BS | human | D14 | PBCD34-mock | chr6+11842138 | 25.00% | 1 | ADTRP |
| pCYS.007.2.BS | human | D14 | PBCD34-mock | chr8+133682373 | 25.00% | 1 | LOC105375773 |
| pCYS.013.PS | human | D14 | PBCD34-MOI40 | chr8+67244348 | 26.53% | 13 | ARFGEF1 |
| pMouseCNL23group4control | mouse | M6 | Mouse_BM | chr 3-59165408 | 33.33% | 2 | Igsf10 |
| pMouseCNL24group4 | mouse | M6 | Mouse_BM | chr18-79750998 | 27.25% | 785 | Setbp1 |
| pMouseCNL24group4 | mouse | M6 | Mouse_BM | chr2+141127890 | 32.45% | 935 | Macrod2 |
| pMouseCNL25group4 | mouse | M6 | Mouse_BM | chr2+52586806 | 21.70% | 1057 | Stam2 |
| pMouseCNL25group4 | mouse | M6 | Mouse_BM | chr3-59165408 | 22.22% | 1082 | Igsf10 |
| pMouseCNL25group4 | mouse | M6 | Mouse_BM | chr3+71430357 | 20.97% | 1021 | Gm6634 |
| pMouseCNL25group4 | mouse | M6 | Mouse BM | chr9+15188460 | 22.77% | 1109 | Cep295 |
| pMouseCNL38group1A | mouse | M6 | Mouse BM | chr14-102130900 | 50.00% | 1 | Lmo7 |
| pMouseCNL38group1A | mouse | M6 | Mouse BM | chr7-115316991 | 50.00% | 1 | Olfr486 |
| pMouseCN493 | mouse | M6 | Mouse BM | chr14+15556797 | 22.83% | 989 | Slc4a7 |
| pMouseCN493 | mouse | M6 | Mouse BM | chr6-10697710 | 22.25% | 964 | AA545190 |
| pMouseCN493 | mouse | M6 | Mouse BM | chrX-111592392 | 22.30% | 966 | Klhl4 |
| pMouseCN493 | mouse | M6 | Mouse BM | chrX-155983905 | 23.50% | 1018 | Bclaf3 |
| pMouseCNL53 | mouse | M6 | Mouse BM | chr19+18123881 | 100.00% | 2 | Pcsk5 |
| pMouseCNL58 | mouse | M6 | Mouse BM | chr2+141127890 | 100.00% | 3 | Macrod2 |
| pMouseCNL59 | mouse | M6 | Mouse BM | chr2+52586806 | 25.11% | 407 | Stam2 |
| pMouseCNL59 | mouse | M6 | Mouse BM | chr3-59165408 | 22.52% | 365 | Igsf10 |
| pMouseCNL59 | mouse | M6 | Mouse BM | chr9+15188460 | 24.18% | 392 | Cep295 |
| pMouseCN545group11 | mouse | M6 | Mouse BM | chr18-15129653 | 36.11% | 1006 | Kctd1 |
| pMouseCN545group11 | mouse | M6 | Mouse BM | chr5-12432693 | 32.88% | 916 | Sema3d |
| pMouseCNL70group12 | mouse | M6 | Mouse BM | chr11-77602820 | 22.05% | 284 | Myo18a |
| pCNL80 | mouse | M6 | Mouse_BM | chr3+123970190 | 34.43% | 42 | Tram1l1 |
| pCNL82 | mouse | M6 | Mouse_BM | chr10-40246905 | 50.00% | 1 | Slc22a16 |
| pCNL82 | mouse | M6 | Mouse BM | chr10+94767778 | 50.00% | 1 | Cradd |
| pCN552 | mouse | M6 | Bone marrow CD11b | chr3+130245919 | 21.62% | 16 | Col25a1 |
| pCNL59 | mouse | M6 | Unsorted bone marrow | chr2+52586806 | 43.27% | 45 | Stam2 |
| pCNL59 | mouse | M6 | Unsorted bone marrow | chr3-59165408 | 24.04% | 25 | Igsf10 |
| pCNL59 | mouse | M6 | Unsorted bone marrow | chr9+15188460 | 21.15% | 22 | Cep295 |
| pCNL59 | mouse | M6 | Bone marrow CD11b | chr2+52586806 | 30.38% | 24 | Stam2 |
| pCNL59 | mouse | M6 | Bone marrow CD11b | chr3-59165408 | 26.58% | 21 | Igsf10 |
| pCNL59 | mouse | M6 | Bone marrow CD11b | chr9+15188460 | 30.38% | 24 | Cep295 |
| pCNL59 pCNL59 | mouse | M6 | B Cells | chr2+52586806 | 35.00% | 7 | Stam2 |
| pCNL59 | mouse | M6 | B Cells | chr3+71430357 | 30.00% | 6 | Gm6634 |
| pCNL59 pCNL59 | mouse | M6 | B Cells | chr9+15188460 | 30.00% | 6 | Cep295 |
| pCN760 | | M6 | Bone Marrow | chr9+15188460 chr9+45858945 | 29.41% | 5 | Sik3 |
| pCN806 | mouse | | | | 29.41% | 2 | Inhba |
| pon806 | mouse | M6 | Bone Marrow | chr13-16444618 | 22.22% | 2 | шира |

Mouse transplant trials

The positions of identified integration sites from cell transplant trials with nine pairs of mice are shown in Figure 5a (donor mice) and Figure 5b (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 6 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 tilda (\sim) 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. The clonal abundances of clones found in both donor and recipient mice is shown in Table S3. 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 5B & S3).



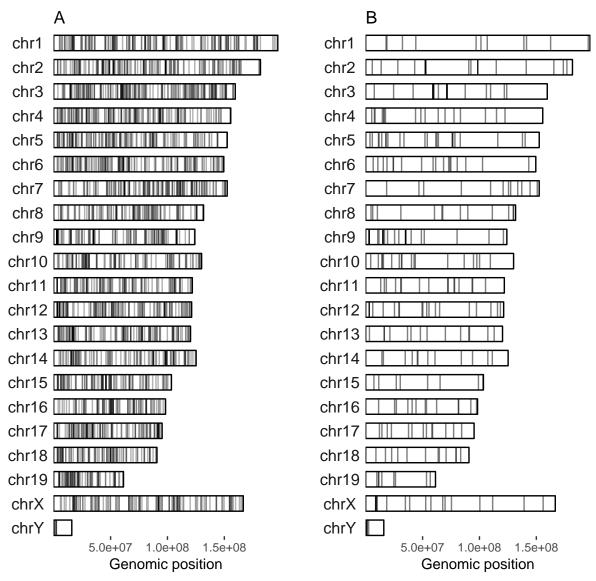
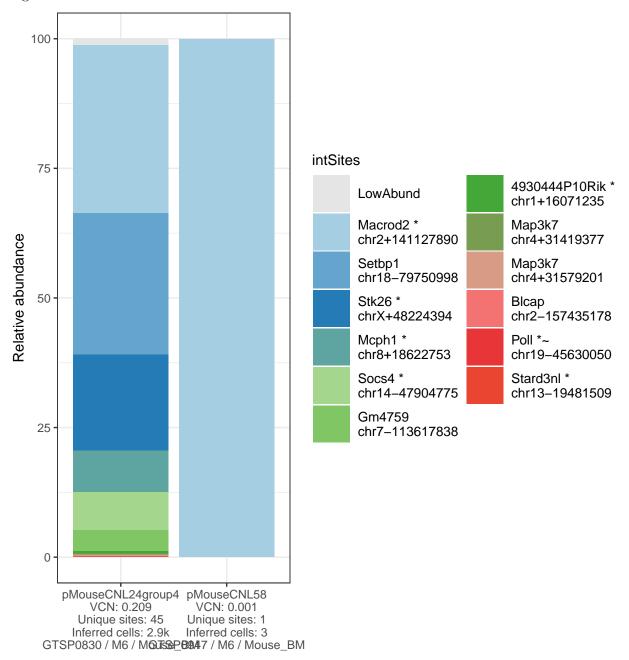


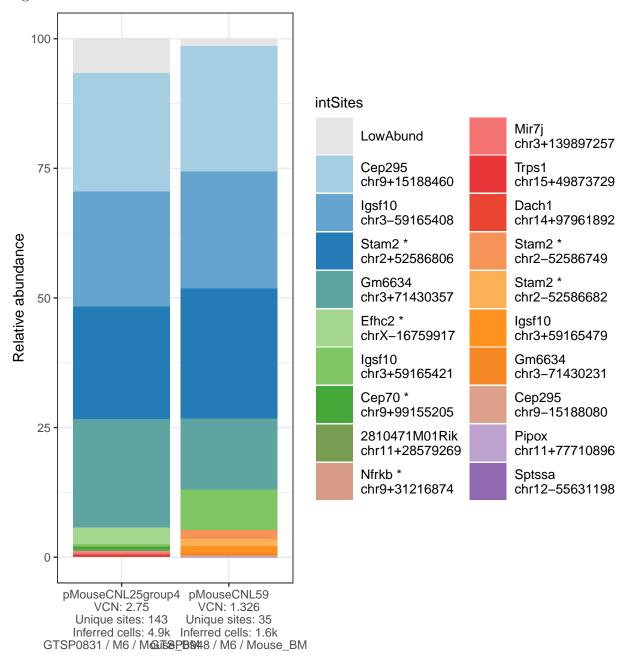
Figure 6a.



Fisher's exact p-value: 1.000

| | Not near onco | Near onco |
|-------------------|---------------|-----------|
| pMouseCNL24group4 | 42 | 3 |
| pMouseCNL58 | 1 | 0 |

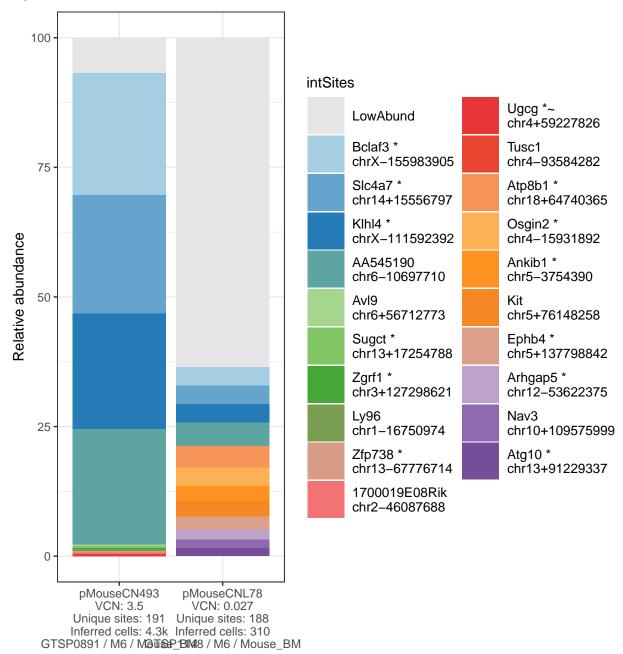
Figure 6b.



Fisher's exact p-value: 0.208

| | Not near onco | Near onco |
|-------------------|---------------|-----------|
| pMouseCNL25group4 | 134 | 9 |
| pMouseCNL59 | 35 | 0 |

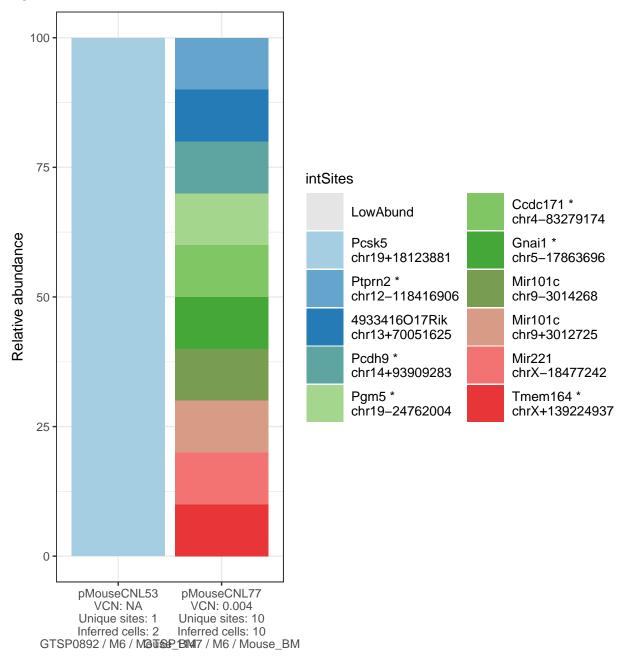
Figure 6c.



Fisher's exact p-value: 0.492

| | Not near onco | Near onco |
|-------------|---------------|-----------|
| pMouseCN493 | 179 | 12 |
| pMouseCNL78 | 180 | 8 |

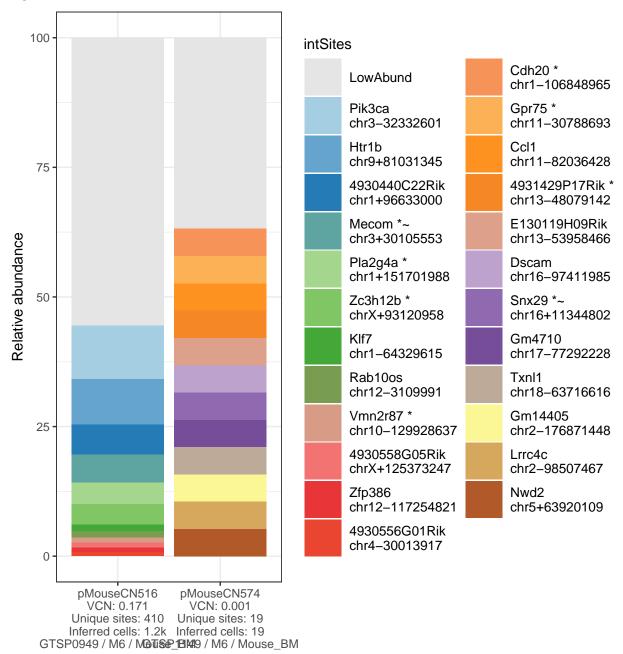
Figure 6d.



Fisher's exact p-value: 1.000

| | Not near onco | Near onco |
|-------------|---------------|-----------|
| pMouseCNL53 | 1 | 0 |
| pMouseCNL77 | 10 | 0 |

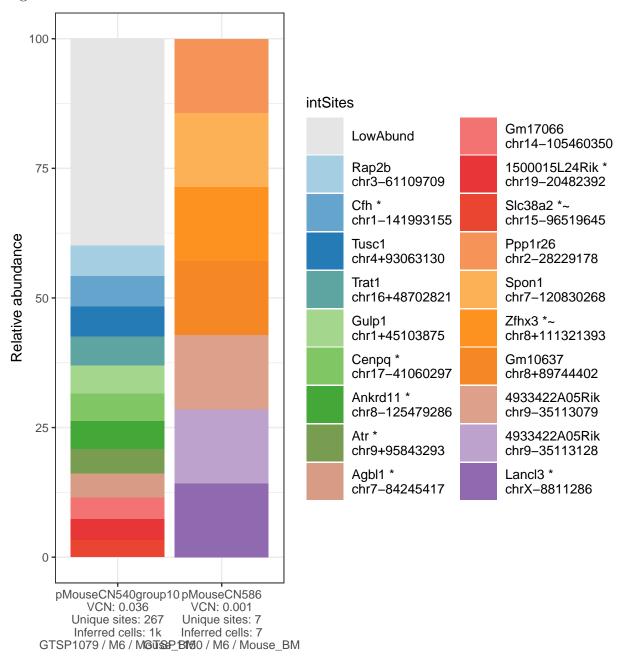
Figure 6e.



Fisher's exact p-value: 0.396

| | Not near onco | Near onco |
|-------------|---------------|-----------|
| pMouseCN516 | 400 | 10 |
| pMouseCN574 | 18 | 1 |

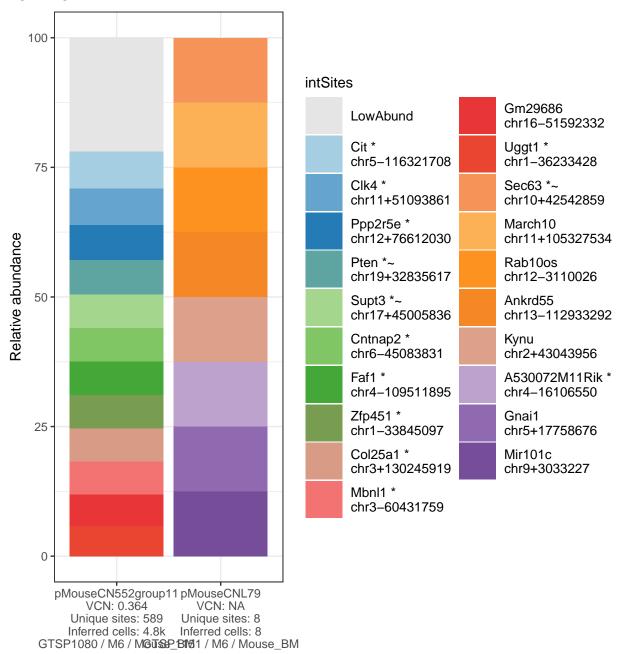
Figure 6f.



Fisher's exact p-value: 0.447

| | Not near onco | Near onco |
|--------------------|---------------|-----------|
| pMouseCN540group10 | 246 | 21 |
| pMouseCN586 | 6 | 1 |

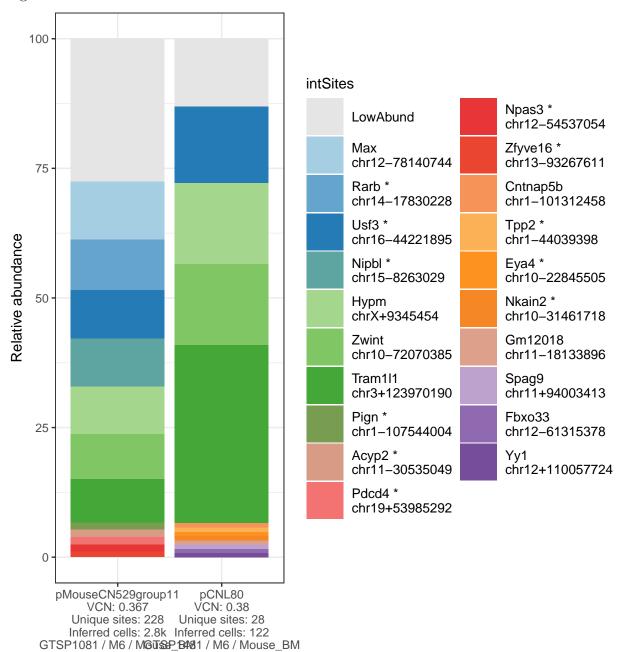
Figure 6g.



Fisher's exact p-value: 0.394

| | Not near onco | Near onco |
|--------------------|---------------|-----------|
| pMouseCN552group11 | 554 | 35 |
| pMouseCNL79 | 7 | 1 |

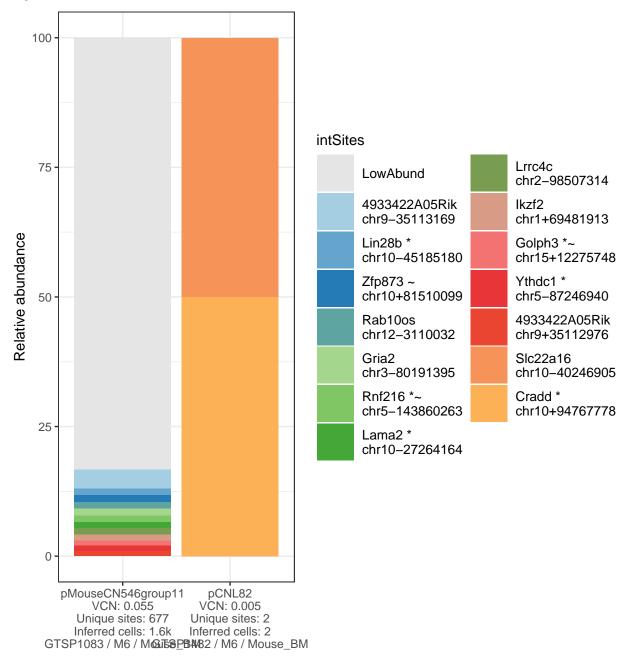
Figure 6h.



Fisher's exact p-value: 1.000

| | Not near onco | Near onco |
|--------------------|---------------|-----------|
| pMouseCN529group11 | 212 | 16 |
| pCNL80 | 26 | 2 |

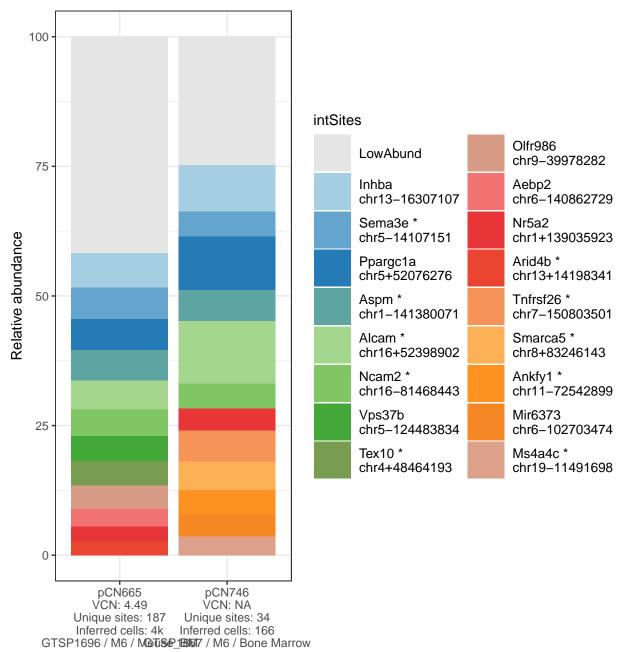
Figure 6i.



Fisher's exact p-value: 1.000

| | Not near onco | Near onco |
|--------------------|---------------|-----------|
| pMouseCN546group11 | 647 | 30 |
| pCNL82 | 2 | 0 |

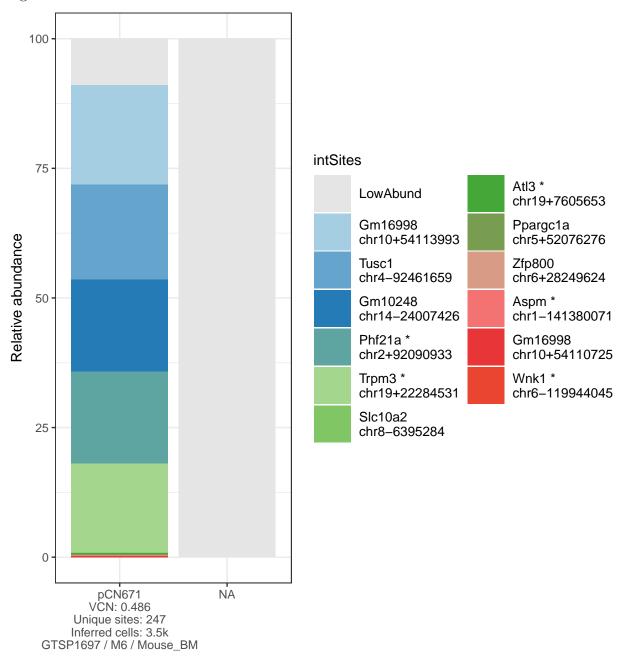
Figure 6j.



Fisher's exact p-value: 0.594

| | Not near onco | Near onco |
|--------|---------------|-----------|
| pCN665 | 181 | 6 |
| pCN746 | 34 | 0 |

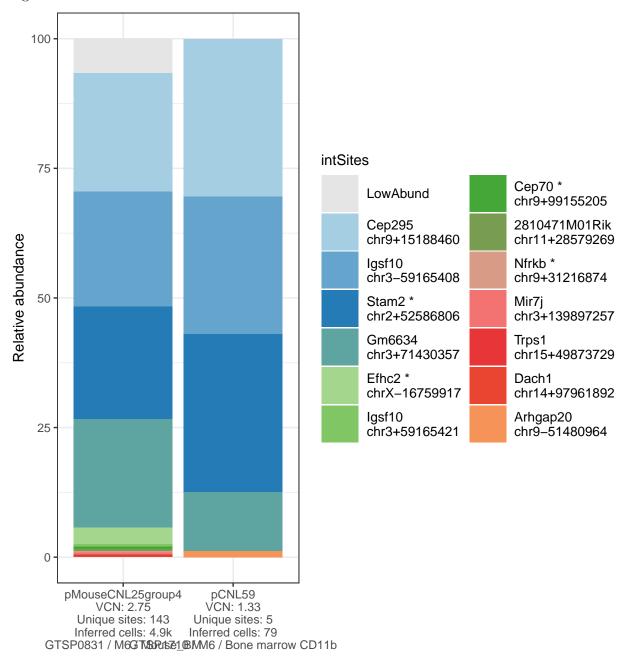
Figure 6k.



Fisher's exact p-value: 1.000

| | Not near onco | Near onco |
|--------|---------------|-----------|
| pCN671 | 233 | 14 |
| NA | 0 | 0 |

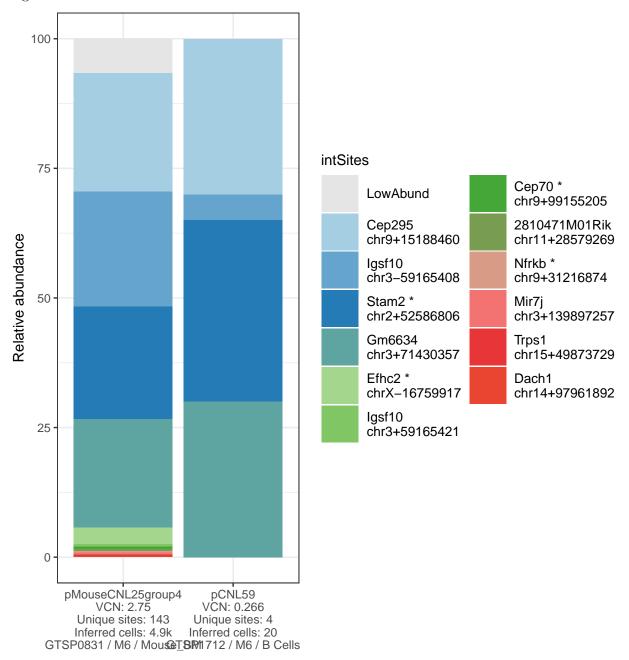
Figure 61.



Fisher's exact p-value: 1.000

| | Not near onco | Near onco |
|-------------------|---------------|-----------|
| pMouseCNL25group4 | 134 | 9 |
| pCNL59 | 5 | 0 |

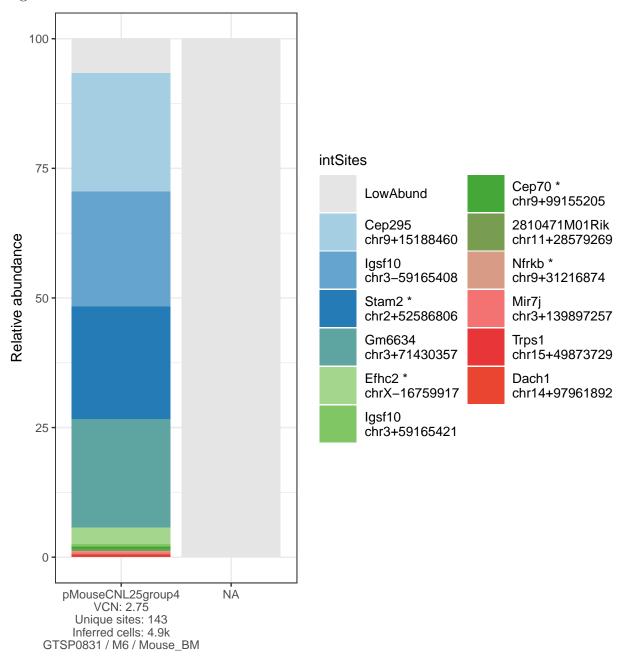
Figure 6m.



Fisher's exact p-value: 1.000

| | Not near onco | Near onco |
|-------------------|---------------|-----------|
| pMouseCNL25group4 | 134 | 9 |
| pCNL59 | 4 | 0 |

Figure 6n.



Fisher's exact p-value: 1.000

| | Not near onco | Near onco |
|-------------------|---------------|-----------|
| pMouseCNL25group4 | 134 | 9 |
| NA | 0 | 0 |

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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 |
|----------|----------|----------------------|----------------|-------|------------|-----------------------|--------------------|
| human | GTSP0689 | pCYS002NS | PBCD34-MOI20 | 0.940 | D14 | 3,146 | 1,467 |
| human | GTSP0691 | pCYS004BR | PBCD34-MOI20 | 0.930 | D14 | 1,091 | 761 |
| human | GTSP0692 | pCYS005OB | PB_CD34 | 0.970 | D14 | 8,698 | 3,291 |
| human | GTSP1369 | pDON.002.4.SC | PB_CD34 | 4.520 | D14 | 129 | 44 |
| human | GTSP1370 | pDON.003.2.SG | PB_CD34 | 2.440 | D14 | 1,489 | 1,173 |
| human | GTSP1371 | pDON.007.CR | PB_CD34 | 1.270 | D14 | 114 | 75 |
| human | GTSP1372 | pDON.008.LH | PB_CD34 | 2.530 | D14 | 134 | 100 |
| human | GTSP1373 | pDON.009.JH | PB_CD34 | 5.280 | D14 | 793 | 605 |
| human | GTSP1374 | pDON.010.CC | PB_CD34 | 3.290 | D14 | 531 | 370 |
| human | GTSP1375 | pCYS.013.PS | PBCD34-mock | NA | D14 | 1 | 1 |
| human | GTSP1376 | pCYS.007.2.BS | PBCD34-mock | 0.002 | D14 | 4 | 4 |
| human | GTSP1377 | pCYS.002.4.NS | PB_CD34 | 2.400 | D14 | 105 | 54 |
| human | GTSP1378 | pCYS.007.2.BS | PBCD34-MOI20 | 3.640 | D14 | 568 | 460 |
| human | GTSP1379 | pCYS.007.2.BS | PBCD34-MOI40 | 6.100 | D14 | 1,225 | 1,057 |
| human | GTSP1380 | pCYS.013.PS | PBCD34-MOI40 | 1.000 | D14 | 49 | 25 |
| human | GTSP1682 | pCYS_017_BW_MOI2x107 | PB_CD34 | 1.660 | D14 | 16,712 | 11,675 |
| human | GTSP1683 | pCYS_017_BW_MOI6x106 | PB_CD34 | 1.415 | D14 | 16,717 | 11,074 |
| human | GTSP1685 | pCYS_018_MM_MOI2x107 | PB_CD34 | 0.958 | D14 | 11,700 | 8,430 |
| human | GTSP1686 | pCYS_018_MM_MOI6x106 | PB_CD34 | 0.530 | D14 | 7,126 | 4,541 |
| human | GTSP1687 | pCYS_018_MM_MOI2x106 | PB_CD34 | 0.331 | D14 | 3,589 | 2,502 |
| human | GTSP1688 | $pCYS_020_JJ$ | PB_CD34 | 2.590 | D14 | 21,755 | 14,752 |
| human | GTSP1689 | pMP011.1.Ch2 | PB_CD34 | 1.607 | D14 | 1,517 | 938 |
| human | GTSP1690 | pSC002.6.Ch7 | PB_CD34 | 2.350 | D14 | 427 | 221 |
| human | GTSP1691 | pMP011.1.Ch9 | PB_CD34 | 2.420 | D14 | 726 | 426 |
| human | GTSP1692 | pMP011.1.Ch11 | PB_CD34 | 1.074 | D14 | 626 | 352 |
| human | GTSP1694 | pDON.004.3.TL.MOI20 | PB_CD34 | 3.250 | D14 | 5,270 | 4,562 |
| human | GTSP1695 | pDON.011.2.MP | PB_CD34 | 3.950 | D14 | 11,241 | 7,060 |
| human | GTSP1969 | pLSR1TEST2 | BM CD34+ Cells | 0.695 | D0 | 2,584 | 2,215 |
| human | GTSP1970 | pLSR1THAW2 | BM CD34+ Cells | 0.709 | D0 | 6,344 | 6,003 |
| human | GTSP1971 | pLSR2Thaw2 | BM CD34+ Cells | 1.020 | D0 | 9,196 | 8,686 |

 $Table \ S1 \ (continued).$

| Organism | GTSP | Subject | Cell type | VCN | Time point | Number inferred cells | Number of intSites |
|----------|----------|--------------------------|----------------------|-------|------------|-----------------------|--------------------|
| mouse | GTSP0829 | pMouseCNL23group4control | Mouse_BM | NA | M6 | 6 | 5 |
| mouse | GTSP0830 | pMouseCNL24group4 | Mouse_BM | 0.209 | M6 | 2,881 | 45 |
| mouse | GTSP0831 | pMouseCNL25group4 | Mouse_BM | 2.750 | M6 | 4,870 | 143 |
| mouse | GTSP0832 | pMouseCNL38group1A | Mouse_BM | 0.001 | M6 | 2 | 2 |
| mouse | GTSP0833 | pMouseCulture | Mouse_Sca1pos | 2.760 | D14 | 89 | 28 |
| mouse | GTSP0891 | pMouseCN493 | Mouse_BM | 3.500 | M6 | 4,332 | 191 |
| mouse | GTSP0892 | pMouseCNL53 | Mouse_BM | NA | M6 | 2 | 1 |
| mouse | GTSP0947 | pMouseCNL58 | Mouse_BM | 0.001 | M6 | 3 | 1 |
| mouse | GTSP0948 | pMouseCNL59 | $Mouse_BM$ | 1.326 | M6 | 1,621 | 35 |
| mouse | GTSP0949 | pMouseCN516 | Mouse_BM | 0.171 | M6 | 1,174 | 410 |
| mouse | GTSP0950 | pMouseCN518 | $Mouse_BM$ | 2.300 | M6 | 3,379 | 101 |
| mouse | GTSP1079 | pMouseCN540group10 | Mouse_BM | 0.036 | M6 | 1,009 | 267 |
| mouse | GTSP1080 | pMouseCN552group11 | $Mouse_BM$ | 0.364 | M6 | 4,849 | 589 |
| mouse | GTSP1081 | pMouseCN529group11 | Mouse_BM | 0.367 | M6 | 2,786 | 228 |
| mouse | GTSP1082 | pMouseCN545group11 | Mouse_BM | 1.339 | M6 | 2,786 | 35 |
| mouse | GTSP1083 | pMouseCN546group11 | Mouse_BM | 0.055 | M6 | 1,644 | 677 |
| mouse | GTSP1084 | pMouseCNL70group12 | Mouse_BM | 0.610 | M6 | 1,288 | 92 |
| mouse | GTSP1146 | pMouseCN557 | Mouse_BM | 2.600 | M6 | 10,839 | 745 |
| mouse | GTSP1147 | pMouseCNL77 | $Mouse_BM$ | 0.004 | M6 | 10 | 10 |
| mouse | GTSP1148 | pMouseCNL78 | Mouse_BM | 0.027 | M6 | 310 | 188 |
| mouse | GTSP1149 | pMouseCN574 | $Mouse_BM$ | 0.001 | M6 | 19 | 19 |
| mouse | GTSP1150 | pMouseCN586 | Mouse_BM | 0.001 | M6 | 7 | 7 |
| mouse | GTSP1151 | pMouseCNL79 | $Mouse_BM$ | NA | M6 | 8 | 8 |
| mouse | GTSP1481 | pCNL80 | Mouse_BM | 0.380 | M6 | 122 | 28 |
| mouse | GTSP1482 | pCNL82 | $Mouse_BM$ | 0.005 | M6 | 2 | 2 |
| mouse | GTSP1696 | pCN665 | Mouse_BM | 4.490 | M6 | 4,012 | 187 |
| mouse | GTSP1697 | pCN671 | $Mouse_BM$ | 0.486 | M6 | 3,453 | 247 |
| mouse | GTSP1705 | pCN552 | Bone marrow CD11b | 0.101 | M6 | 74 | 15 |
| mouse | GTSP1706 | pCN552 | T-Cells | 0.514 | M6 | 192 | 49 |
| mouse | GTSP1707 | pCN552 | B Cells | 4.230 | M6 | 922 | 161 |
| mouse | GTSP1708 | pCN552 | Unsorted bone marrow | NA | M6 | 494 | 103 |
| mouse | GTSP1709 | pCNL59 | Unsorted bone marrow | NA | M6 | 104 | 6 |
| mouse | GTSP1710 | pCNL59 | Bone marrow CD11b | 1.330 | M6 | 79 | 5 |
| mouse | GTSP1712 | pCNL59 | B Cells | 0.266 | M6 | 20 | 4 |
| mouse | GTSP1867 | pCN746 | Bone Marrow | NA | M6 | 166 | 34 |
| mouse | GTSP1972 | pCN752 | Bone Marrow | 0.004 | M6 | 238 | 71 |
| mouse | GTSP1973 | pCN755 | Bone Marrow | NA | M6 | 28 | 27 |
| mouse | GTSP1974 | pCN760 | Bone Marrow | 0.001 | M6 | 17 | 10 |
| mouse | GTSP1980 | pCN804 | Bone Marrow | 0.003 | M6 | 82 | 28 |
| mouse | GTSP1981 | pCN806 | Bone Marrow | 0.001 | M6 | 9 | 8 |

Analyzed samples in which no integration sites were identified

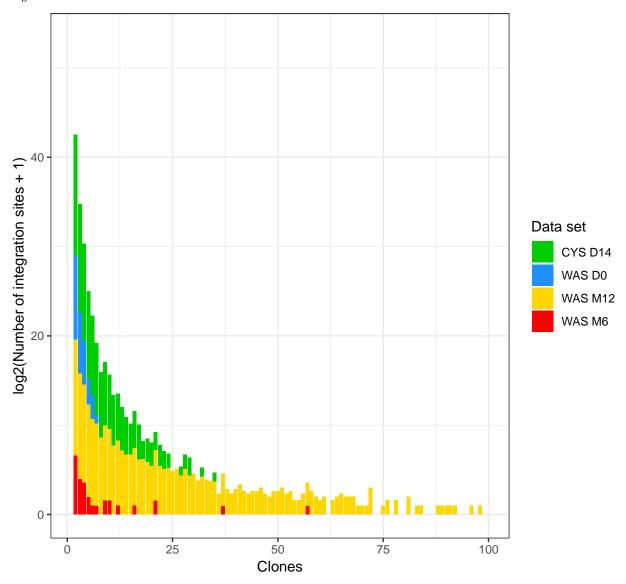
Table S2.

| GTSP | Cell type | Subject | Time point | Comment |
|----------|------------------|----------------------|------------|---|
| GTSP0688 | PBCD34-mock | pCYS002NS | D14 | Mock transduced control; Stage: Mock |
| GTSP0690 | PBCD34-mock | pCYS004BR | D14 | Mock transduced control; Stage: Mock |
| GTSP0828 | $Mouse_BM$ | pMouseCNL1group1 | M6 | pCCL-CTNS; MOI 10; Stage: Primary |
| GTSP0834 | $Mouse_Sca1pos$ | pMouseCultureControl | D14 | Control, DNA was extracted from mouse Sca1+ cells and cultured for 2 weeks (Mock) |
| GTSP1684 | PB_CD34 | pCYS_017_BW_MOCK | D14 | Mock transduced control; Stage: Mock |
| GTSP1693 | PB_CD34 | pDON.004.3.TL.MOCK | D14 | Mock transduced control; Stage: Mock |
| GTSP1711 | T-Cells | pCNL59 | M6 | Mouse Thymus, T cells |
| GTSP1868 | Bone Marrow | pCN748 | M6 | pCCL-CTNS transduced, Secondary graft, Primary graft mouse: CN671, Primary graft |
| GTSP1975 | DNA | pCN752P1 | M6 | Pathology sample 1 from CN752 (found in thorax) |
| GTSP1976 | DNA | pCN752P2 | M6 | Pathology sample 2 from CN752 (thymus) |

Comparison of the number of integration sites and inferred cells

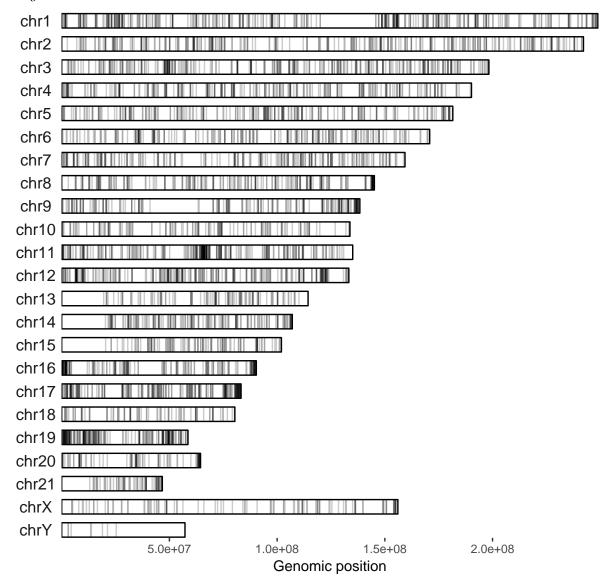
Kolmogorov–Smirnov tests show no significant differences between the distributions of estimated abundances between trials. The plot below was truncated at 100 inferred cells for clarity which eliminated 16 data points from the 'WAS m12' distribution.

Figure S1.



Integration positions of the lentiviral the raputic vector from a previous WAS correction ${\rm trial}^1$





| ${f Persistence}$ | of | clones | in | mouse | \mathbf{BM} | transplant | trials |
|-------------------|----|--------|----|-------|---------------|------------|--------|
|-------------------|----|--------|----|-------|---------------|------------|--------|

Table S3.

| Donor | Recipient | Position | Donor cells | Recipient cells |
|--------------------|-------------|----------------------------------|-------------|-----------------|
| pMouseCNL24group4 | pMouseCNL58 | chr2+141127890 | 935 | 3 |
| pMouseCNL25 group4 | pMouseCNL59 | chr12-55631198 | 3 | 1 |
| pMouseCNL25group4 | pMouseCNL59 | chr2-52586682 | 1 | 22 |
| pMouseCNL25group4 | pMouseCNL59 | chr2-52586749 | 7 | 27 |
| pMouseCNL25group4 | pMouseCNL59 | chr2 + 52586806 | 1,057 | 407 |
| pMouseCNL25group4 | pMouseCNL59 | chr3-59165408 | 1,082 | 365 |
| pMouseCNL25group4 | pMouseCNL59 | chr3-71430231 | 2 | 9 |
| pMouseCNL25group4 | pMouseCNL59 | chr3 + 59165404 | 1 | 1 |
| pMouseCNL25group4 | pMouseCNL59 | chr3+59165421 | 27 | 127 |
| pMouseCNL25group4 | pMouseCNL59 | chr3 + 59165479 | 6 | 22 |
| pMouseCNL25group4 | pMouseCNL59 | chr3 + 71430357 | 1,021 | 222 |
| pMouseCNL25group4 | pMouseCNL59 | chr9+15188460 | 1,109 | 392 |
| pMouseCN493 | pMouseCNL78 | chr14 + 15556797 | 989 | 11 |
| pMouseCN493 | pMouseCNL78 | chr6-10697710 | 964 | 14 |
| pMouseCN493 | pMouseCNL78 | chr X-111592392 | 966 | 11 |
| pMouseCN493 | pMouseCNL78 | chr X-155983905 | 1,018 | 11 |
| pMouseCN540group10 | pMouseCN586 | chr9-35113079 | 1 | 1 |
| pMouseCN552group11 | pMouseCNL79 | chr12-3110026 | 10 | 1 |
| pMouseCN529group11 | pCNL80 | chr 10-72070385 | 242 | 19 |
| pMouseCN529group11 | pCNL80 | chr16-44221895 | 263 | 18 |
| pMouseCN529group11 | pCNL80 | chr3+123970190 | 233 | 42 |
| pMouseCN529group11 | pCNL80 | chrX + 9345454 | 255 | 19 |
| pCN665 | pCN746 | chr1-141380071 | 235 | 10 |
| pCN665 | pCN746 | chr1+139035923 | 115 | 7 |
| pCN665 | pCN746 | chr11-72542899 | 6 | 8 |
| pCN665 | pCN746 | chr13-16307093 | 1 | 2 |
| pCN665 | pCN746 | chr13-16307107 | 265 | 15 |
| pCN665 | pCN746 | chr13+90648207 | 3 | 5 |
| pCN665 | pCN746 | chr16-81468443 | 208 | 8 |
| pCN665 | pCN746 | chr16+52398902 | 224 | 20 |
| pCN665 | pCN746 | chr19-11491698 | 7 | 6 |
| pCN665 | pCN746 | chr2 + 5793957 | 4 | 3 |
| pCN665 | pCN746 | chr4+48464193 | 189 | 4 |
| pCN665 | pCN746 | chr5-14107151 | 245 | 8 |
| pCN665 | pCN746 | chr5+52076276 | 240 | 17 |
| pCN665 | pCN746 | chr6-102703474 | 4 | 7 |
| pCN665 | pCN746 | chr7-150803501 | 105 | 10 |
| pCN665 | pCN746 | chr7+46794970 | 5 | 2 |
| pCN665 | pCN746 | chr8+83246143 | 6 | 9 |
| pCN665 | pCN746 | chr9-39978282 | 181 | 6 |
| pCN665 | pCN746 | chrUn_random+46156 | 4 | 4 |
| pMouseCNL25group4 | pCNL59 | $\frac{-}{\text{chr2}+52586806}$ | 1,057 | 24 |
| pMouseCNL25group4 | pCNL59 | chr3-59165408 | 1,082 | 21 |
| pMouseCNL25group4 | pCNL59 | chr3+71430357 | 1,021 | 9 |
| pMouseCNL25group4 | pCNL59 | chr9+15188460 | 1,109 | 24 |
| pMouseCNL25group4 | pCNL59 | chr2+52586806 | 1,057 | 7 |
| pMouseCNL25group4 | pCNL59 | chr3 35 9165408 | 1,082 | 1 |

Sequencing depth

Identified integration site are shown as colored squares that are positioned by the number of reads leading to their identification.

Figure S3.

