Supplementary Materials for

A universal system to select gene-modified hepatocytes in vivo

Sean Nygaard, Adi Barzel, Annelise Haft, Angela Major, Milton Finegold, Mark A. Kay, Markus Grompe*

*Corresponding author. Email: grompem@ohsu.edu

Published 8 June 2016, Sci. Transl. Med. 8, 342ra79 (2016)
DOI: 10.1126/scitranslmed.aad8166

The PDF file includes:

Fig. S1. Integration site analysis after selection in gene-targeted mouse liver DNA.
Table S1. shRNA sequences tested in lentiviral vectors.
Data values in tabular format
Figure S1. Integration site analysis after selection in gene-targeted mouse liver DNA. (A) Experimental time line. Two cohorts of P1 Fah<sup>−/−</sup> mice (n = 3 each) were administered 2 × 10<sup>11</sup> vector genomes of gene-ride rAAV8. Fah selection was initiated in one cohort (mice 1-3) at 5 weeks of age by withdrawing NTBC for three weeks. The second cohort (mice 4-6) remained on NTBC for the duration of the experiment. All mice were euthanized at 8 weeks of age and genomic DNA was purified from whole liver and used as template for PCR. (B) PCR primer pairs flanking the predicted 5-prime (primers 1 and 2) or 3-prime (primers 3 and 4) albumin integration sites of the selectable gene-ride AAV were designed to generate 1.7 kb or 2.5 kb amplicons respectively. (C and D) 5’ PCR (C) and 3’ PCR (D). Gel bands were excised and sequenced. No mismatches to the predicted integration sites were found.
**SUPPLEMENTARY TABLE**

**Table S1. shRNA sequences tested in lentiviral vectors.** Sequences of shRNAs (5’→3’) against targets in the tyrosine degradation pathway. The protective *Hpd* shRNA sequence is highlighted in bold italic font.

| Tat shRNAs: | CCGGCCCATCAAGATTTGAAGATTACTCGAGTAATCTTCAATCTTGATGCGTTTTTG |
|-------------|------------------------------------------------------------|
|             | CCGGCCCTCTGACATGCTCAATAAACATCGAGTTTTATTTGGACATGTCAGAGGGTTTTTG |
|             | CCGGCCATGTACCTTATGTTGGGAACTCGACTCCACCATAAGGTACATGGTTTTTG |
|             | CCGGCTACAATCTATTGCCTGAGAAGTCGAGTTCTCAGATGAGAGGTGTTTTTG |

| Hgd shRNAs: | CCGGCCGTGCATAGCTTCCTTGAGATCTCGAGATCTCAAGGAAGCTATGCACGTTTTTG |
|-------------|------------------------------------------------------------|
|             | CCGGCCGAGATTTCTTGATGCCTCGTTTCGAGAAGCTCAAGAAATCTCGTTTTTG |
|             | CCGGCTTCTCATTACACCAGAGTTTCGAGTAGAAGATGCTCGTTTTTG |
|             | CCGGCTACGGAAACTACACCTATCGAGTATGCCATGCTGTTTTTG |

| Hpd shRNAs: | CCGGGTGATCTCCAAGCTGGCTGAAGATCTCGAGTTTGAGCTGAGATGACTTTTTG |
|-------------|------------------------------------------------------------|
|             | CCGGGCTGATCTCCAGCAGTGGCTGAAGATCTCGAGTTTGAGCTGAGATGACTTTTTG |
|             | CCGGTCAGGGAATATGCTGAGATGCTCGAGATGCTCGAGAGTTTTTG |

**Protective sequence** ➔ **CCGGGCCTCAGAATGGTACCTGAGTTTGAGCTGAGATGACTTTTTG**

| Protect. sequence | CCGGTCAGGAATATGCTGAGATGCTCGAGATGCTCGAGAGTTTTTG |
|------------------|-------------------------------------------------|
## Data values in tabular format

### Data for Table 1

| Mouse ID | ALT (U/L) | AST (U/L) | ALK PHOS (U/L) | TOTAL BILIRUBIN (mg/dL) | ALBUMIN (g/dL) | TOTAL PROTEIN (g/dL) |
|----------|-----------|-----------|----------------|-------------------------|----------------|---------------------|
| Saline-L | 15        | 65        | 83             | 0.2                     | 2.7            | 4.9                 |
| Saline-R | 18        | 38        | 106            | 0.2                     | 3.2            | 6                   |
| Saline-B | 19        | 102       | 143            | 0.2                     | 2.9            | 5.4                 |
| CEHPOBA-L| 14        | 33        | 122            | 0.1                     | 3.1            | 5.6                 |
| CEHPOBA-R| 17        | 36        | 157            | 0.2                     | 2.9            | 5.4                 |
| CEHPOBA-B| 15        | 49        | 63             | 0.1                     | 2.8            | 5.3                 |

### Data for Figure 1C

#### Control Group

| Age (weeks) | Mouse ID, Weight (grams) |
|-------------|--------------------------|
|             | A | B | C |
| 4.3         | 14.8 | 15.8 | 11.1 |
| 4.7         | 17.1 | 16 | 11.8 |
| 5.1         | 17.8 | 16.9 | 17.2 |
| 5.7         | 15.5 | 15.8 | 12.4 |
| 6.0         | 13.2 | 15.6 | 15.1 |
| 6.3         | 12.2 | 13.6 | 16.2 |
| 6.7         | 16.5 | 16.8 | 16.5 |
| 7.1         | 17.3 | 16 | 15.6 |
| 7.7         | 18.4 | 15.6 | 13.8 |
| 8.0         | 17.4 | 16.1 | 13.5 |
| 8.3         | 15.1 | 14.7 | 13 |
| 8.7         | 14.7 | 13 | 12.7 |
| 9.1         | 19.2 | 17.3 | 17.1 |
| 9.7         | 22.3 | 20.4 | 20 |
| 10.1        | 18.5 | 16.8 | 14.4 |
| 10.7        | 20.1 | 20.1 | 21.1 |
| 11.1        | 23.8 | 22.4 | 21.8 |
| 11.7        | 21.6 | 18.9 | 19.2 |
| 12.1        | 21.9 | 20.2 | 16.2 |
| 12.7        | 16 | 20.3 | 21.4 |
| 13.1        | 24.8 | 19.3 | 21.9 |

#### TAT Group

| Age (weeks) | Mouse ID, Weight (grams) |
|-------------|--------------------------|
|             | A | B | C | D |
| 4.3         | 17.9 | 18.5 | 17.3 | 19 |
| 4.7         | 17.5 | 18.6 | 16.6 | 18.8 |
| 5.1         | 18.1 | 18.4 | 16.3 | 18.8 |
| 5.7         | 18 | 19.4 | 16.2 | 19.4 |
| 6.0         | 17.6 | 18.6 | 15.4 | 17.9 |
| 6.3         | 16.5 | 18 | 14.9 | 17.3 |
| 6.7         | 15.3 | 16.5 | 13.8 | 15.8 |
| 7.1         | 14.7 | 17.6 | 13 | 14.7 |
| 7.7         | 20.6 | 21.6 | 19.8 | 21.7 |
### HPD Group

| Age (weeks) | Mouse ID, Weight (grams) |  |  |  |
|-------------|--------------------------|---|---|---|
| 3.3         | 15.8 16.3 14.5           | B | C |   |
| 3.7         | 16.3 17 15.3             | B | C |   |
| 4.0         | 17.5 18.2 17.1           | B | C |   |
| 4.3         | 18.1 19 17.7             | B | C |   |
| 4.9         | 15.9 16.2 15.2           | B | C |   |
| 5.4         | 15.8 15.5 15.2           | B | C |   |
| 5.7         | 15.9 15 13.4             | B | C |   |
| 6.0         | 16 14.6 12.9             | B | C |   |
| 6.4         | 16.4 15.1 13.1           | B | C |   |
| 6.9         | 17.3 17.5 14.9           | B | C |   |
| 7.4         | 18.3 18.8 16             | B | C |   |
| 8.0         | 20.8 21.8 19             | B | C |   |
| 8.4         | 22.7 24 22.2             | B | C |   |
| 9.0         | 23.2 23.4 22.2           | B | C |   |
| 9.4         | 23.2 23.5 22.4           | B | C |   |
| 10.0        | 23.8 24 22.6             | B | C |   |
| 10.4        | 23.4 24 23.3             | B | C |   |

### HGD Group

| Age (weeks) | Mouse ID, Weight (grams) |  |  |  |  |
|-------------|--------------------------|---|---|---|---|
| 3.3         | 14 13.9 15.3 15.1 16.1   | B | C | D | E |
| 3.7         | 13.4 13.5 15.5 14.6 16    | B | C | D | E |
| 4.0         | 12.6 12.8 13.9 13.2 14.4  | B | C | D | E |
| 4.3         | 12 12.3 12.3 12.9 13.4    | B | C | D | E |
| 4.9         | 10.7 10.7 10.5 10.8 13.8  | B | C | D | E |
| 5.4         | 12.8 14 10.1 9.9 15.3     | B | C | D | E |
| 5.7         | 16 15.8 12.7 13 16.9      | B | C | D | E |
| 6.0         | 17.5 18.1 15.3 16.2 19.3  | B | C | D | E |
| 6.4         | 14.5 15.3 12.4 15.5       | B | C | D | E |
| 6.9         | 14.1                       | B | C | D | E |
| 7.4         | 17.5                       | B | C | D | E |
| 8.0         | 18.4                       | B | C | D | E |
| 8.4         | 17.3                       | B | C | D | E |
| 9.0         | 17.7                       | B | C | D | E |
| 9.4         | 16.9                       | B | C | D | E |
| 10.0        | 15.9                       | B | C | D | E |
| 10.4        | 14.2                       | B | C | D | E |
### Data for Figure 2D

| Age (weeks) | Control Mice Mouse ID, Plasma hFIX (ng/ml) | HPD shRNA Mouse ID, Plasma hFIX (ng/ml) |
|-------------|------------------------------------------|----------------------------------------|
|             | A  | B  | C  | D  | E  | A  | B  | C  | D  |     |
| 4.1         | 93.0 | 103.0 | 165.0 | 55.0 | 184.0 | 20.3 | 12.7 | 14.2 | 28.6 |     |
| 5.7         | 252.0 | 295.0 | 252.0 | 92.0 | 365.0 | 44.0 | 23.5 | 28.6 | 56.0 |     |
| 7.7         | 31.7 | 16.9 | 21.0 | 73.0 | 22.3 | 19.0 | 11.8 | 16.1 | 35.8 |     |
| 9.9         | 19.8 | 25.8 | 28.6 | 9.5 | 52.4 | 48.2 | 7.2 | 27.2 | 48.7 |     |
| 12.1        | 14.8 | 22.7 | 26.4 | 26.8 |     | 95.2 | 21.4 | 124.5 | 158.0 |     |
| 14.0        | 8.0 | 14.0 | 19.0 | 19.0 |     | 360.0 | 111.0 | 404.0 | 158.0 |     |
| 15.1        | 17.0 | 19.0 | 24.0 | 22.0 |     | 551.0 | 241.0 | 455.0 | 784.0 |     |

### Data for Figure 3C

| Age (weeks) | Gene-rider rAAV Mouse ID, Plasma hFIX (ng/ml) | Selectable gene-rider rAAV Mouse ID, Plasma hFIX (ng/ml) |
|-------------|-----------------------------------------------|---------------------------------------------------|
|             | A  | B  | C  | D  | A  | B  | C  | D  | A  | B  | C  | D  |
| 4.5         | 18.0 | 48.0 | 67.4 | 39.6 | 121 | 98 | 144 | 175 |     |     |     |     |
| 11.0        | 11.4 | 12.7 | 24.9 | 9.5  | 2133 | 1472 | 1918 | 2386 |     |     |     |     |
| 14.9        | 4.8  | 15.6 | 37.5 | 6.9  | 38058 | 50713 | 47645 | 47054 |     |     |     |     |
| 18.2        | 19.5 | 24.8 | 31.9 | 20.4 | 14700 | 26733 | 15195 | 10948 |     |     |     |     |

### Data for Figure 3E

| Age (weeks) | Saline-treated Mouse ID, Plasma hFIX (ng/ml) |
|-------------|---------------------------------------------|
|             | A  | B  | C  |
| 5.6         | 125 | 219 | 298 |
| 8.9         | 396 | 514 | 1115 |
| 11.0        | 402 | 702 | 1109 |
| 14.3        | 487 | 791 | 1115 |

### Data for Figure 5B

| Age | Saline-treated Mouse ID, Plasma hFIX (ng/ml) |
|-----|---------------------------------------------|
|     | A  | B  | C  | D  |
|     | 125 | 219 | 298 |     |
| 8.9 | 396 | 514 | 1115 |     |
| 11.0| 402 | 702 | 1109 |     |
| 14.3| 487 | 791 | 1115 |     |
CEHPOBA-treated

| Age (weeks) | Mouse ID, Plasma hFIX (ng/ml) |
|-------------|------------------------------|
|             | A   | B   | C   |
| 4.1         | 38.0| 57.0| 41.0|
| 5.3         | 90.0| 193.0| 73.0|
| 6.3         | 72.0| 189.0| 78.0|
| 7.0         | 131.0| 398.0| 208.0|
| 8.0         | 398.0| 1155.0| 779.0|
| 9.1         | 95.0| 1323.0| 363.0|
| 10.1        | 117.0| 1526.0| 361.0|
| 11.1        | 111.0| 1361.0| 412.0|
| 12.0        | 94.2| 1460.0| 396.3|

Data for Figure 6B

| Saline-treated | CEHPOBA-treated | Fah-/-Control vector | Fah-/-shHpd vector |
|----------------|-----------------|----------------------|-------------------|
| 0.00026110     | 0.0003643       | 5.67112E-06          | 0.057719          |
| 0.00011528     | 0.3413005       | 2.40924E-05          | 0.592205          |
| 0.00011284     | 0.0087441       | 1.48618E-05          | 0.223240          |