

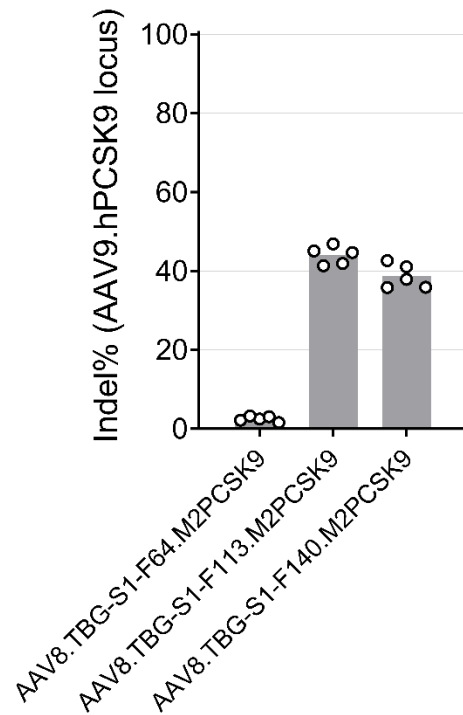
**YMTHE, Volume 29**

## **Supplemental Information**

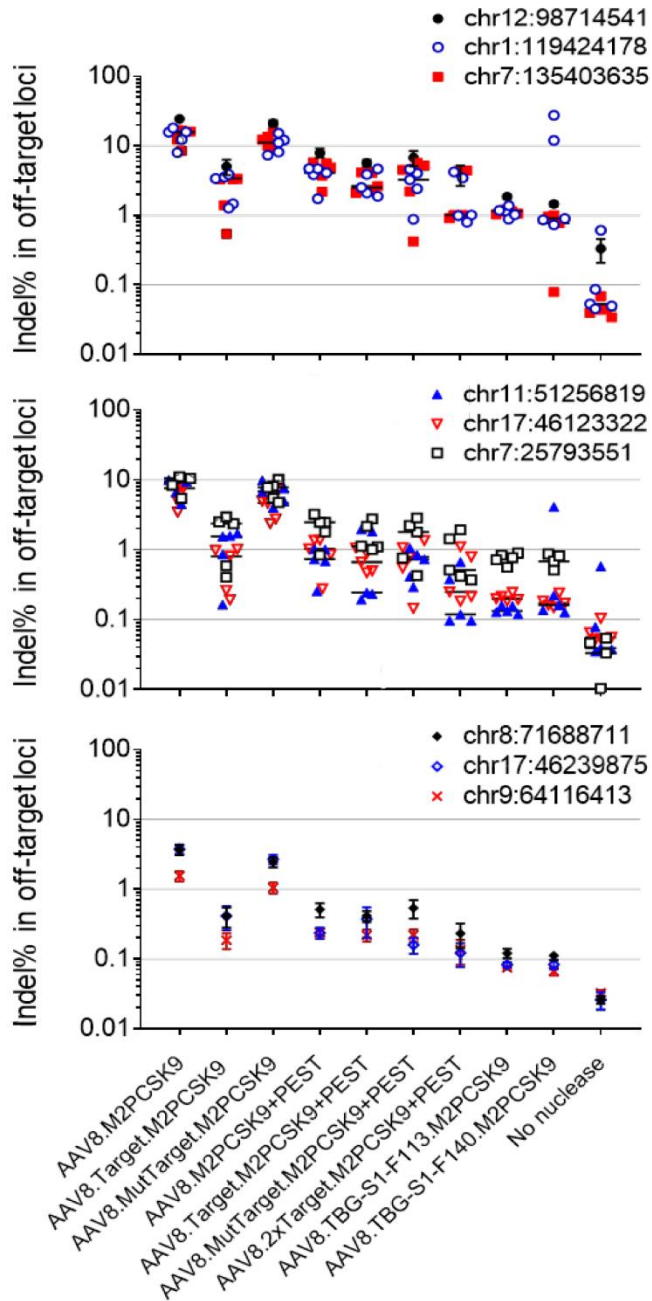
### **Increasing the Specificity of AAV-Based Gene Editing through Self-Targeting and Short-Promoter Strategies**

**Camilo Breton, Thomas Furmanak, Alexa N. Avitto, Melanie K. Smith, Caitlin Latshaw, Hanying Yan, Jenny A. Greig, and James M. Wilson**

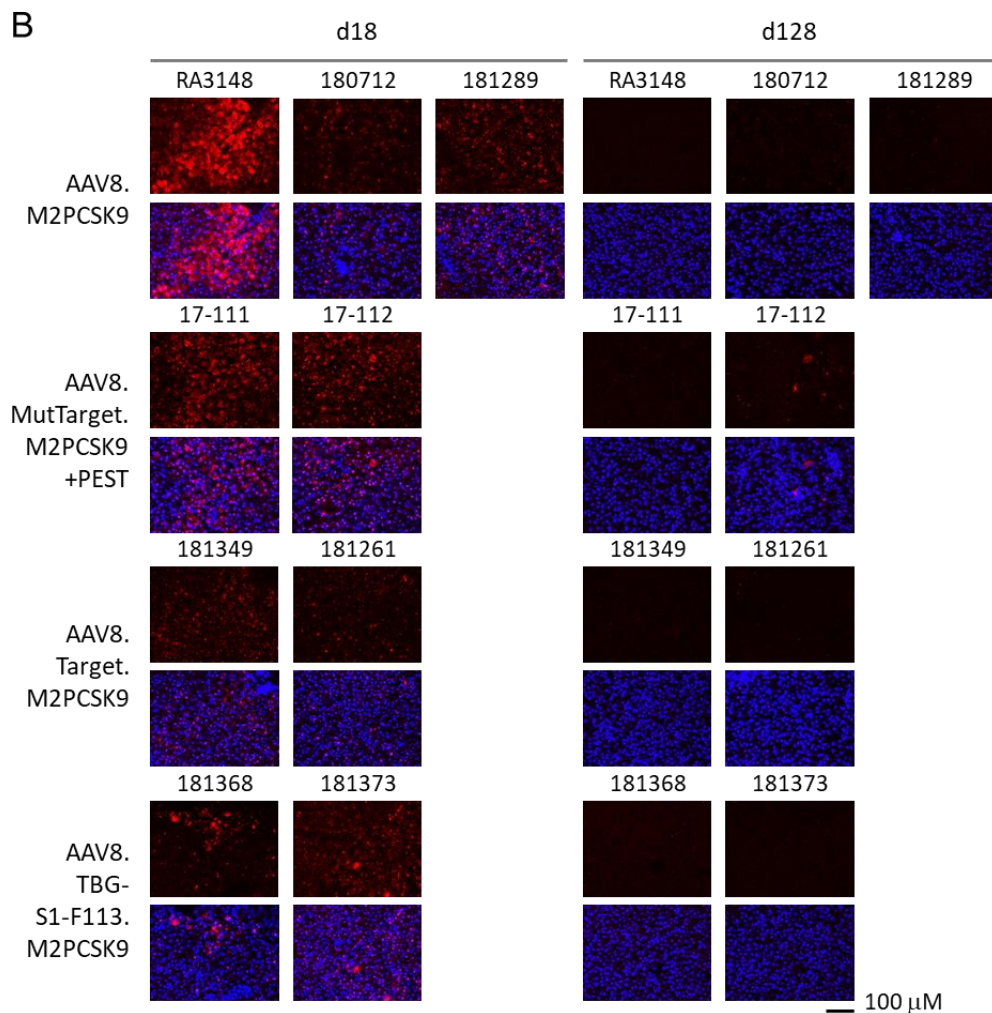
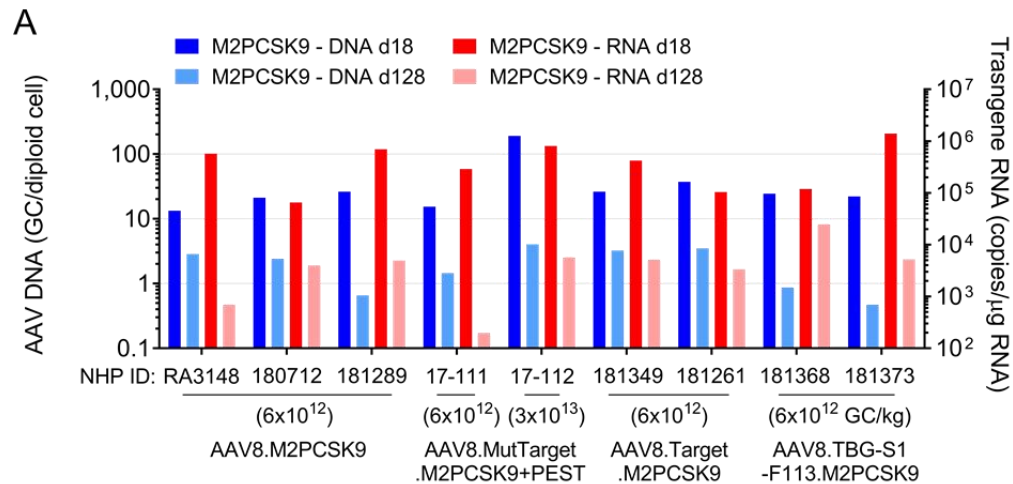
## Supplemental Figures



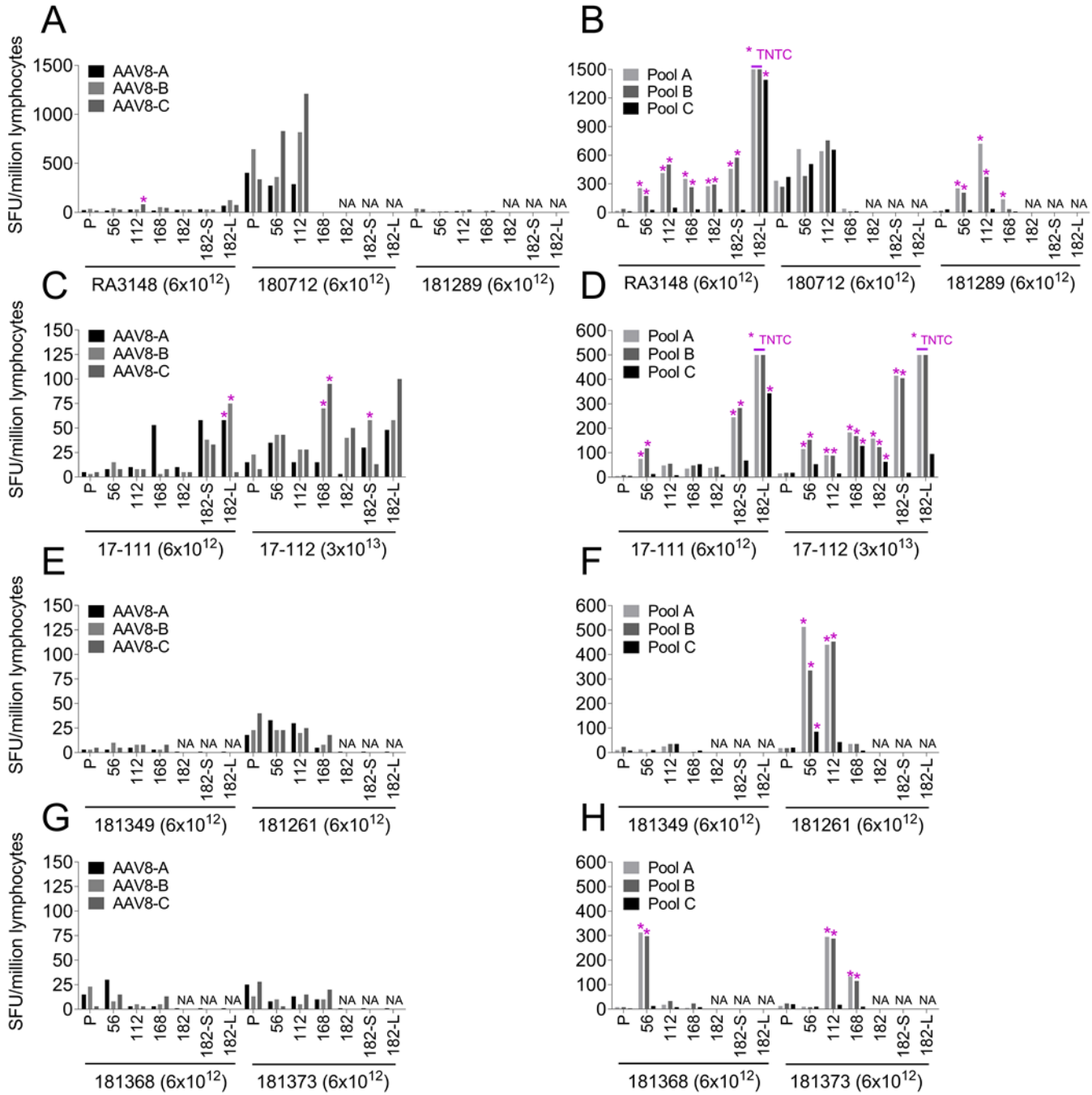
**Figure S1. M2PCSK9 on-target editing in mice treated with shortened-promoter AAV vectors.** *Rag1* knockout mice were treated with AAV9.hPCSK9 and shortened-promoter AAV vectors expressing M2PCSK9. At nine weeks post AAV9.hPCSK9, livers were collected and Indel% in the target region present in AAV9.hPCSK9 was calculated.



**Figure S2. Editing level in selected off-targets.** ITR-Seq-identified off-targets were ranked according to the number of reads per off-target loci and a subset of nine high-rank off-targets was selected for further NGS analysis. Indel% per mouse is plotted. Off-targets are plotted in groups of three off-targets each to facilitate the visualization.



**Figure S3. Liver transduction and transgene RNA expression in NHP. A.** AAV genome copies per diploid cell and M2PCSK9 RNA per microgram of total RNA calculated by qPCR from day 18 or day 128 liver DNA of AAV-treated NHP **B.** In situ hybridization using specific probes to detect M2PCSK9 RNA (red color) along with DAPI nuclei staining (blue) in liver biopsies samples taken at the indicated time points. Scale bar displayed on the bottom right.



**Figure S4. T-cell responses to AAV8- and M2PCSK9-derived peptide pools.** Number of spot-forming unit (SFU) per million lymphocytes as quantified in the IFN- $\gamma$  ELISpot using three different pools for AAV8 capsid (AAV8-A, AAV8-B, and AAV8-C) or peptide pools derived from M2PCSK9 sequence (Pool A, Pool B, and Pool C). NHP were treated with AAV8.M2PCSK9 (Panels A and B), AAV8.MutTarget.M2PCSK9+PEST (Panels C and D), AAV8.Target.M2PCSK9 (Panels E and F) or AAV8.TBG-S1-F113.M2PCSK9 (Panels G and H). For the AAV8.MutTarget.M2PCSK9+PEST group, we replaced the Pool C with a peptide pool derived from the PEST amino acid sequence. TNTC, too numerous to count. \* indicates a positive T cell activation, defined as >55 SFU per million cells and threefold higher than the negative (medium only) control (P). NA indicates that samples are not available as the study was ongoing. NHP identification number and AAV dose (in GC/kg) are displayed below the timepoints.

## Supplemental Tables

**Table S1. Titers of the produced AAV vectors**

| <b>Vector name</b>          | <b>GC/mL</b> | <b>Yield</b> | <b>Cells</b> |
|-----------------------------|--------------|--------------|--------------|
| AAV8.M2PCSK9                | 9.89E+13     | 2.7E+14      | 3E+9         |
| AAV8.Target.M2PCSK9         | 7.98E+13     | 4.11E+14     | 6E+9         |
| AAV8.MutTarget.M2PCSK9      | 4.89E+13     | 5.74E+13     | 1E+9         |
| AAV8.M2PCSK9+PEST           | 5.66E+13     | 6.93E+13     | 1E+9         |
| AAV8.Target.M2PCSK9+PEST    | 4.66E+13     | 5.01E+13     | 1E+9         |
| AAV8.MutTarget.M2PCSK9+PEST | 1.07E+14     | 5.53E+14     | 6E+9         |
| AAV8.2XTarget.M2PCSK9+PEST  | 8.60E+13     | 1.01E+14     | 1E+9         |
| AAV8.TBG-S1-F113.M2PCSK9    | 1.62E+14     | 8.72E+14     | 6E+9         |
| AAV8.TBG-S1-F140.M2PCSK9    | 1.74E+14     | 9.82E+14     | 6E+9         |

**Table S2. M2PCSK9 off-targets selected for validation.** Bold letters indicate a mismatch to the intended target sequence (5'-TGGACCTCTTTGCCCCAGGGGA-3'), dashes indicate a gap in the off-target sequence and inserted bases are indicated by an underline in the off-target sequence alignment. Ranking was obtained from the ITR-Seq result tables, sorted by number of reads per off-target in descending order.

| Off-target     | Sequence alignment                                | Target sequence match | Ranking in AAV.M2PCSK9 (week 9) ITR-Seq results |         |         |         |         |
|----------------|---|-----------------------|---|---------|---------|---------|---------|
|                |   |                       | Mouse A   | Mouse B | Mouse C | Mouse D | Mouse E |
| chr12:98714541 | TG <b>T</b> CCCTCT <b>G</b> TCCCCAGGGCA           | 17/22                 | 1   | 1       | 1       | 1       | 1       |
| chr1:119424178 | TGGCCCTCT <b>G</b> TCCCCAGGGAA                    | 19/22                 | 10  | 8       | 7       | 9       | 5       |
| chr7:135403635 | <b>G</b> GGACCTCTTT <b>C</b> CTCCAGGGAGA          | 19/22                 | 4   | 3       | 3       | 3       | 3       |
| chr7:25793551  | TGGACCTCT <b>G</b> T <u>AC</u> CCCCAGTGTA         | 18/22                 | 5   | 4       | 4       | 4       | 5       |
| chr11:51256819 | TGG-CCTCTCT--CACAGGAGGA                           | 17/22                 | 7   | 5       | 5       | 6       | 7       |
| chr17:46123322 | TG <b>T</b> CCCTTT <b>G</b> TCCCCAGGGTA           | 16/22                 | 11  | 9       | 11      | 11      | 10      |
| chr8:71688711  | TGGTCCTT <b>G</b> GCTCGCCAGGGGA                   | 15/22                 | 3   | 2       | 2       | 2       | 2       |
| chr17:46239875 | TGGAC <b>C</b> CT <b>G</b> TT <b>A</b> CCAGAGGTCA | 14/22                 | 6   | 6       | 6       | 7       | 8       |
| chr9:64116413  | <b>C</b> ATACCTCT <b>G</b> T <u>AC</u> CCCCAGGGGA | 17/22                 | 16  | 18      | 8       | 14      | 11      |

**Table S3. Primer sequences used to generate the plasmids for AAV production.** Sequences corresponding to the target or mutant target sequences are in bold.

| Name          | Sequence (5' – 3')   |
|---------------|--|
| C-Target-F    | TTGCTTTCTGAGAGACTGCAGT <b>GGACCTCTTTGCCCCAGGGG</b> AAAGTTGG<br>TCGTGAGGCAC                             |
| C-Target-R    | TCGCCCTTGCTCACCATGGCGGCCGCTGGACACCTGTGGAGAG  |
| C-MutTarget-F | TTGCTTTCTGAGAGACTGCAGT <b>GGCCCTTTTTATTCCAGGG</b> AAAGTTGG<br>TCGTGAGGCAC                              |
| PEST-F        | AGAAGAAGAAGTCGTCCCCCAAGCTTAGCCATGGCTTCCCGCCGGAGGTG<br>GAGGAGCAGGATGATGGCACGCTGCCCATGTCTTGTGCCCAGGAGAGC |
| PEST-R        | CTAGAAGGCACAGTCGAGGCTTACTACACATTGATCCTAGCAGAAGCAC<br>GGCTGCAGGGTGACGGTCCATCCCGCTCTCCTGGGCACAAGACATGGG  |
| PEST-Target-F | CGGCCTCTCCGCGTCTTCGAAGCTTAGCCATGGCTTCCCGCCG  |
| PEST-Target-R | TAGAAGGCACAGTCGAGGCAGATCTCCCTGGGGCAAAGAGGTCCATTAC<br>TACACATTGATCCTAGCAGAAGCACAGG                      |
| TBG-S1-F140-F | TACTTATCTACTTAAGCCTCTTGGCCTTGGTTTTGTACATCAG  |
| TBG-S1-F113-F | TACTTATCTACTTAAGCTTTGAAAATACCATCCCAGGGTTAATGCTG  |
| TBG-S1-F64-F  | TACTTATCTACTTAAGGAGTGCTCTAGTTTTGCAATACAGGACATG   |
| TBG-S1-R      | CCGCTACACTGCGGCCGCTGTCTCTCAGAAAGC  |



**Table S4. Primers used to amplify the region of interest for indel% calculation.**

| <b>Name</b> | <b>Sequence (5' – 3')</b>                    | <b>Region of interest</b>                                    |
|-------------|--|--|
| Target_5F   | TGCTTTGTTTCCTCTTGGCCTTGG                     | 5' target sequence in AAV vectors                            |
| Target_5R   | TCCTGGCAAAGATGGAACCGTC                       |  |
| Target_3F   | GACTCCAAGACCCGCAAGACCACTTC                   | 3' target sequence in AAV vectors                            |
| Target_3R   | TTATTAGGAAAGGACAGTGGGAGTGGCACC               |  |
| PCS_MMF     | TGCCACCTACCTCCTCACCTTTC                      | On-target region in rhesus PCSK9 gene                        |
| PCS_MMR     | GGCTGTTAGCATCACGGTGG                         |  |
| AAV_PCS_F   | CCTCAGCTCCCGAGGTCATCACAGTTG                  | On-target region in AAV9.hPCSK9 vector                       |
| AAV_PCS_R   | TGACATCTTTGGCAGAGAAGTGGATCAGTC               |  |
| Pos_GSP1    | GGATCTCGACGCTCTCCCTCCAGGACCAGCCGGTGAC        | AMP-Seq analysis of rhesus PCSK9 gene (positive primers set) |
| Pos_GSP2    | CCTCTCTATGGGCAGTCGGTGATGACCCTGGGGACTTTGGGG   |  |
| Neg_GSP1    | GGATCTCGACGCTCTCCCTGCAGCAGCCTGCGATGTC        | AMP-Seq analysis of rhesus PCSK9 gene (negative primers set) |
| Neg_GSP2    | CCTCTCTATGGGCAGTCGGTGATCGATGTCCCACTCCGTGACAC |  |