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Supplemental Information

Directed Evolution of AAV Serotype 5 for Increased Hepatocyte Transduction and Retained Low Humoral Seroreactivity

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Supplemental Figures

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>MV1

MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEP
VNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQAKKRVLE
PFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAQPASSLGADT
MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSG
SVDRSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQ
DSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTER
SSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSLAPSQNLFKLANPLVDQYLYRFVSTNNTGGV
QFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPN
GMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATN
NQSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMM
LIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDF
APDSTGEYRSTRPIGTRYLTRPL

>MV18

MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEP
VNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQAKKRVLE
PFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAQPASSLGADT
MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSG
SVDRSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQ
DSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTER
SSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGV
QFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPN
GMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATN
NQSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMM
LIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDF
APDSTGEYRTTRPIGTRYLTRPL

>MV20

MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEP
VNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQAKKRVLE
PFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAQPASSLGADT
MSAGGGGSLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSG
SVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTV
QDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTE
RSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGG
VQFNKNLAGRYANTYKNWFPGPIGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPN
GMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATN
NQSSTTTPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMM
LIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDF
APDSTGEYRSTRPIGTRYLTRPL

>MV50
```

MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEP
VNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQAKKRVLE
PFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAQPASSLGADT
MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSG
SVDRSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQ
DSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTER
SSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGV
QFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPN
GMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATN
NQSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMM
LIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDF
APDGTGEYRSTRPIGTRYLTRPL

>MV53

MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEP
VNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQAKKRVLE
PFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLRIPAQPASSLGADT
MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSG
SVDRSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQ
DSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTER
SSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGV
QFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPN
GMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATN
NQSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMM
LIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDF
APDSTGEYRTTRPIGTRYLTRPL

Figure S1: Amino Acid Sequence for Best Performing AAV5 Variants

Amino acid sequences for best performing variants. The mutations that differ from AAV5 are highlighted in red.

```
AAV5     1 MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDR
MV1      1 MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDR
MV18     1 MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDR
MV20     1 MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDR
MV50     1 MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDR
MV53     1 MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDR

AAV5    61 GEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQA
MV1     61 GEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQA
MV18    61 GEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQA
MV20    61 GEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQA
MV50    61 GEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQA
MV53    61 GEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQA

AAV5   121 KKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQI
MV1    121 KKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQI
MV18   121 KKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQI
MV20   121 KKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQI
MV50   121 KKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQI
MV53   121 KKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLRI

AAV5   181 PAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLP
MV1    181 PAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLP
MV18   181 PAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLP
MV20   181 PAQPASSLGADTMSAGGGGSLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLP
MV50   181 PAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLP
MV53   181 PAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLP

AAV5   241 SYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPR
MV1    241 SYNNHQYREIKSGSVDRSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPR
MV18   241 SYNNHQYREIKSGSVDRSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPR
MV20   241 SYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPR
MV50   241 SYNNHQYREIKSGSVDRSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPR
MV53   241 SYNNHQYREIKSGSVDRSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPR

AAV5   301 SLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV
MV1    301 SLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV
MV18   301 SLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV
MV20   301 SLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV
MV50   301 SLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV
MV53   301 SLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV

AAV5   361 FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPS
MV1    361 FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSLAPS
MV18   361 FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPS
MV20   361 FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPS
MV50   361 FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPS
MV53   361 FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPS

AAV5   421 QNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSG
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MV1   421 QNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSG
MV18  421 QNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSG
MV20  421 QNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPIGRTQGWNLGSG
MV50  421 QNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSG
MV53  421 QNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSG


AAV5  481 VNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTA
MV1   481 VNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTA
MV18  481 VNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTA
MV20  481 VNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTA
MV50  481 VNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTA
MV53  481 VNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTA


AAV5  541 TYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERD
MV1   541 TYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERD
MV18  541 TYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERD
MV20  541 TYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTTPATGTYNLQEIVPGSVWMERD
MV50  541 TYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERD
MV53  541 TYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERD


AAV5  601 VYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFIT
MV1   601 VYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFIT
MV18  601 VYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFIT
MV20  601 VYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFIT
MV50  601 VYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFIT
MV53  601 VYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFIT


AAV5  661 QYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYL
MV1   661 QYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYL
MV18  661 QYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYL
MV20  661 QYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYL
MV50  661 QYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDGTGEYRTTRPIGTRYL
MV53  661 QYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYL


AAV5  721 TRPL
MV1   721 TRPL
MV18  721 TRPL
MV20  721 TRPL
MV50  721 TRPL
MV53  721 TRPL
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Figure S2: Amino Acid Sequence Alignment of AAV5 and MV Mutants

Alignment of the amino acid sequences for each of the MV mutants and AAV5. The mutations that differ from AAV5 are highlighted in black with white font.

```
AAV1            MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLD
AAV6            MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLD
AAV2            MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD
AAV3            MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGYKYLGPGNGLD
AAV7            MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFNGLD
AAV8            MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLD
AAV9            MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
AAV4            -MTDGYLPDWLEDNLSEGVREWWALQPGAPKPKANQQHQDNARGLVLPGYKYLGPGNGLD
MV              MSFVDHPPDWLEEVG-EGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLD
                    .: *****:   **:*::  *:.* * **. ::::*: ********:**** ****

AAV1            KGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQ
AAV6            KGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQ
AAV2            KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
AAV3            KGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLQEDTSFGGNLGRAVFQ
AAV7            KGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQ
AAV8            KGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQ
AAV9            KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
AAV4            KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQQRLQGDTSFGGNLGRAVFQ
MV              RGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQ
                :***** ** .* *** :*:.**.:******:*********::*  *********:****

AAV1            AKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQE-PDSSSGIGKTGQQPAKKRLNFGQTGDS
AAV6            AKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQE-PDSSSGIGKTGQQPAKKRLNFGQTGDS
AAV2            AKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVE-PDSSSGTGKAGQQPARKRLNFGQTGDA
AAV3            AKKRILEPLGLVEEAAKTAPGKKRPVDQSPQE-PDSSSGVGKSGKQPARKRLNFGQTGDS
AAV7            AKKRVLEPLGLVEEGAKTAPAKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDS
AAV8            AKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDS
AAV9            AKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQE-PDSSAGIGKSGAQPAKKRLNFGQTGDT
AAV4            AKKRVLEPLGLVEQAGETAPGKKRPLIESPQQ-PDSSTGIGKKGKQPAKKKLVFEDETGA
MV              AKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKR----------KKARTEEDSKPSTSSDAE
                ****:***:****:  :***  **     * .             :. ..     .

AAV1            ESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRV
AAV6            ESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRV
AAV2            DSVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRV
AAV3            ESVPDPQPLGEPPAAPTSLGSNTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWLGDRV
AAV7            ESVPDPQPLGEPPAAPSSVGSGTVAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRV
AAV8            ESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRV
AAV9            ESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRV
AAV4            GDGPP-----EGSTSGAMSDDSEMRAAAGGAAVEGGQGADGVGNASGDWHCDSTWSEGHV
MV              AGPSGSQQLRIPAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRV
                 . .        . . :  .   : :..*..  :..:******.:**:***** *  .:*

AAV1            ITTSTRTWALPTYNNHLYK---QIS-SASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPR
AAV6            ITTSTRTWALPTYNNHLYK---QIS-SASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPR
AAV2            ITTSTRTWALPTYNNHLYK---QIS-SQSG-ASNDNHYFGYSTPWGYFDFNRFHCHFSPR
AAV3            ITTSTRTWALPTYNNHLYK---QIS-SQSG-ASNDNHYFGYSTPWGYFDFNRFHCHFSPR
AAV7            ITTSTRTWALPTYNNHLYK---QISSETAG-STNDNTYFGYSTPWGYFDFNRFHCHFSPR
AAV8            ITTSTRTWALPTYNNHLYK---QISNGTSGGATNDNTYFGYSTPWGYFDFNRFHCHFSPR
AAV9            ITTSTRTWALPTYNNHLYK---QISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPR
AAV4            TTTSTRTWVLPTYNNHLYKRLG--------ESLQSNTYNGFSTPWGYFDFNRFHCHFSPR
MV              VTKSTRTWVLPSYNNHQYR----EIKSGSVDRSNANAYFGYSTPWGYFDFNRFHSHWSPR
                 *.*****.**:**** *:              :  * * *:*************.*:***

AAV1            DWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTVQVFSDSEYQLPYVL
AAV6            DWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTVQVFSDSEYQLPYVL
AAV2            DWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVL
AAV3            DWQRLINNNWGFRPKKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVL
AAV7            DWQRLINNNWGFRPKKLRFKLFNIQVKEVTTNDGVTTIANNLTSTIQVFSDSEYQLPYVL
AAV8            DWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVL
AAV9            DWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVL
AAV4            DWQRLINNNWGMRPKAMRVKIFNIQVKEVTTSNGETTVANNLTSTVQIFADSSYELPYVM
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MV              DWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVV
                ******** **:**: : .*:********* .:. .*:*******:*:*:*..*:****:

AAV1            GSAHQGCLPPFPADVFMIPQYGYLTLNNG---SQAVGRSSFYCLEYFPSQMLRTGNNFTF
AAV6            GSAHQGCLPPFPADVFMIPQYGYLTLNNG---SQAVGRSSFYCLEYFPSQMLRTGNNFTF
AAV2            GSAHQGCLPPFPADVFMVPQYGYLTLNNG---SQAVGRSSFYCLEYFPSQMLRTGNNFTF
AAV3            GSAHQGCLPPFPADVFMVPQYGYLTLNNG---SQAVGRSSFYCLEYFPSQMLRTGNNFQF
AAV7            GSAHQGCLPPFPADVFMIPQYGYLTLNNG---SQSVGRSSFYCLEYFPSQMLRTGNNFEF
AAV8            GSAHQGCLPPFPADVFMIPQYGYLTLNNG---SQAVGRSSFYCLEYFPSQMLRTGNNFQF
AAV9            GSAHEGCLPPFPADVFMIPQYGYLTLNDG---SQAVGRSSFYCLEYFPSQMLRTGNNFQF
AAV4            DAGQEGSLPPFPNDVFMVPQYGYCGLVTGNTSQQQTDRNAFYCLEYFPSQMLRTGNNFEI
MV              GNGTEGCLPAFPPQVFTLPQYGYATLNRDN-TENPTERSSFFCLEYFPSKMLRTGNNFEF
                . . :*.**.** :** :*****  *  .   .: . *.:*:*******:******** :

AAV1            SYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGSPAGM
AAV6            SYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGSPAGM
AAV2            SYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN-TPSGTTTQSRLQFSQAGASDI
AAV3            SYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAGPQSM
AAV7            SYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLARTQSNPGGTAGNRELQFYQGGPSTM
AAV8            TYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTTGG-TANTQTLGFSQGGPNTM
AAV9            SYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSG--QNQQTLKFSVAGPSNM
AAV4            TYSFEKVPFHSMYAHSQSLDRLMNPLIDQYLWGLQSTTTGTTLNAGTATTNFTKLRPTNF
MV              TYNFEEVPFHSSLAPSQNLFKLANPLVDQYLYRFVSTN-------NTGGVQFNKNLAGRY
                :* **.*****  * **.* :* ***:****: :  *              *    .

AAV1            SVQPKNWLPGPCYRQQRVSKTKTD-----NNNSNFTWTGASKYNLNGRESIINPGTAMAS
AAV6            SVQPKNWLPGPCYRQQRVSKTKTD-----NNNSNFTWTGASKYNLNGRESIINPGTAMAS
AAV2            RDQSRNWLPGPCYRQQRVSKTSAD-----NNNSEYSWTGATKYHLNGRDSLVN--PAMAS
AAV3            SLQARNWLPGPCYRQQRLSKTAND-----NNNSNFPWTAASKYHLNGRDSLVNPGPAMAS
AAV7            AEQAKNWLPGPCFRQQRVSKTLDQ-----NNNSNFAWTGATKYHLNGRNSLVNPGVAMAT
AAV8            ANQAKNWLPGPCYRQQRVSTTTGQ-----NNNSNFAWTAGTKYHLNGRNSLANPGIAMAT
AAV9            AVQGRNYIPGPSYRQQRVSTTVTQ-----NNNSEFAWPGASSWALNGRNSLMNPGPAMAS
AAV4            SNFKKNWLPGPSIKQQGFSKTANQNYKIPATGSDSLIKYETHSTLDGRWSALTPGPPMAT
MV              ANTYKNWFPGPMGRTQGWNLGSGVN-----RASVSAFATTNRMELEGASYQVPPQPNGMT
                    :*::***  : *  .             *       .   *:*            :

AAV1            HKDDEDKFFPMSGVMIFGKESAGA---SNTALDNVMITDEEEIKATNPVATERFGTVAVN
AAV6            HKDDKDKFFPMSGVMIFGKESAGA---SNTALDNVMITDEEEIKATNPVATERFGTVAVN
AAV2            HKDDEEKFFPQSGVLIFGKQGSEK---TNVNIEKVMITDEEEIGTTNPVATEQYGSVSTN
AAV3            HKDDEEKFFPMHGNLIFGKEGTTA---SNAELDNVMITDEEEIRTTNPVATEQYGTVANN
AAV7            HKDDEDRFFPSSGVLIFG-KTGAT---NKTTLENVLMTNEEEIRPTNPVATEEYGIVSSN
AAV8            HKDDEERFFPSNGILIFGKQNAAR---DNADYSDVMLTSEEEIKTTNPVATEEYGIVADN
AAV9            HKEGEDRFFPLSGSLIFGKQGTGR---DNVDADKVMITNEEEIKTTNPVATESYGQVATN
AAV4            AGPADSKFS-NSQLIFAGPKQNGN---TATVPGTLIFTSEEELAATNATDTDMWGNLPGG
MV              NNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATN
                     ..       :: . :              :::*.*.*  ..* .  :  * :. .

AAV1            FQSSSTDPATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKNP
AAV6            LQSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHP
AAV2            LQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHP
AAV3            LQSSNTAPTTRTVNDQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHP
AAV7            LQAANTAAQTQVVNNQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHP
AAV8            LQQQNTAPQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHP
AAV9            HQSAQAQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHP
AAV4            DQSNSNLPTVDRLTALGAVPGMVWQNRDIYYQGPIWAKIPHTDGHFHPSPLIGGFGLKHP
MV              NQSSTTTPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHP
                 *     .          :** ** :**:* *********.*..:***** :****:*:*

AAV1            PPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEVQYTS
AAV6            PPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEVQYTS
AAV2            PPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTS
AAV3            PPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTS
AAV7            PPQILIKNTPVPANPPEVFTPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTS
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AAV8            PPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTS
AAV9            PPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTS
AAV4            PPQIFIKNTPVPANPATTFSSTPVNSFITQYSTGQVSVQIDWEIQKERSKRWNPEVQFTS
MV              PPMMLIKNTPVPGN-ITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTN
                ** ::*******.:    *.   . ***********:*:::**::**.*******:*:*.

AAV1            NYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL
AAV6            NYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL
AAV2            NYNKSVNRGLTVDTNGVYSEPRPIGTRYLTRNL
AAV3            NYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
AAV7            NFEKQTGVDFAVDSQGVYSEPRPIGTRYLTRNL
AAV8            NYYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL
AAV9            NYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL
AAV4            NYGQQNSLLWAPDAAGKYTEPRAIGTRYLTHHL
MV              NYNDPQFVDFAPDGTGEYRSTRPIGTRYLTRPL
                *: .      : :  * * ..*.*******: *
```
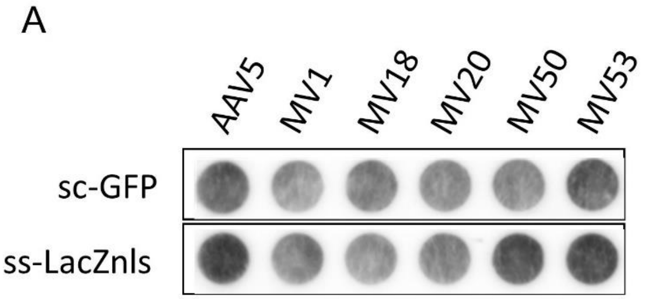

Figure S3: Alignment of All Mutations found in MV Mutants with Other AAV Serotypes

Alignment of AAV serotypes 1 through 9 against MV which contains all of the mutations found in the MV mutants pooled into one sequence. Mutations are highlighted in red.



B

AAV5 and MV Mutant Viral Titer

Virus	sc-GFP (vg/ml)	ss-LacZ (vg/ml)
AAV5	2.10E+12	3.78E+12
MV1	1.35E+12	1.46E+12
MV18	1.50E+12	1.96E+12
MV20	1.40E+12	2.36E+12
MV50	1.45E+12	3.54E+12
MV53	2.30E+12	3.76E+12

Figure S4: Production Yield of AAV5 and MV Mutants

(A) DNA dotblot for the yield of each mutant and AAV5 for two versions of the virus, sc-GFP and ss-LacZnls. (B) Production titers for each capsid and each transgene version.

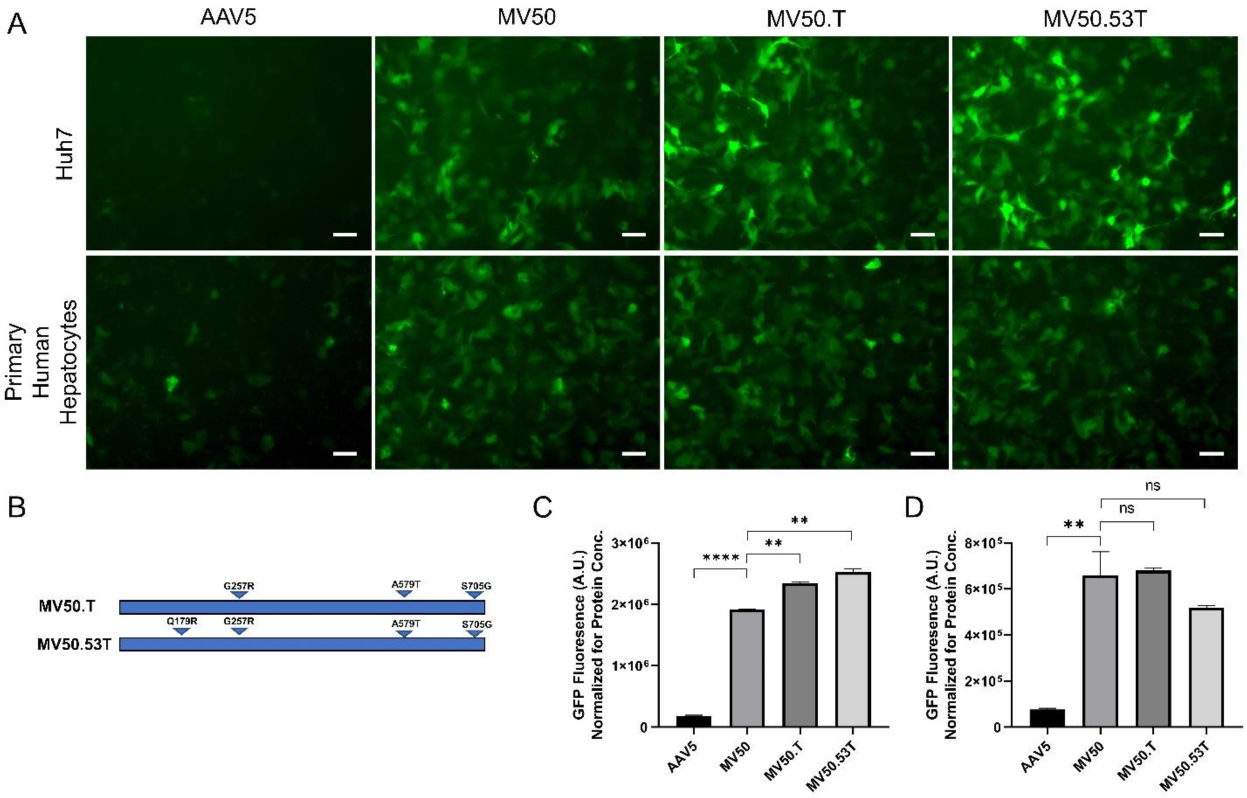


Figure S5. Combination of Mutations from multiple AAV5 Variants

(A) Fluorescent imaging of Huh7 cells and primary human hepatocytes(TRL HUM4020) transduced with self-complementary GFP vectors with AAV5, MV50, MV50.T, and MV50.53T. Huh7 cells were transduced at a MOI of 1e5 vg/cell and human hepatocytes were transduced at a MOI of 5e5vg/cell. Images were taken 96 hours after infection. Expression of GFP (green) indicates transduced cells. 10x magnification. Scale Bar: 100um. (B) Depiction of the combined mutations that are in MV50.T and MV50.53T. Location of amino acid mutations in the AAV5 VP1 amino acid sequence are indicated by triangles. The exact mutation is indicated above the triangle. (C) Comparison of normalized GFP expression in Huh7 cells between AAV5, MV50, MV50.T, and MV50.53T. Data are shown as mean values ± SD. **p<0.01; ****p<0.0001. (D) Comparison of normalized GFP expression in primary human hepatocytes between AAV5, MV50, MV50.T, and MV50.53T. Data are shown as mean values ± SD. **p<0.01.

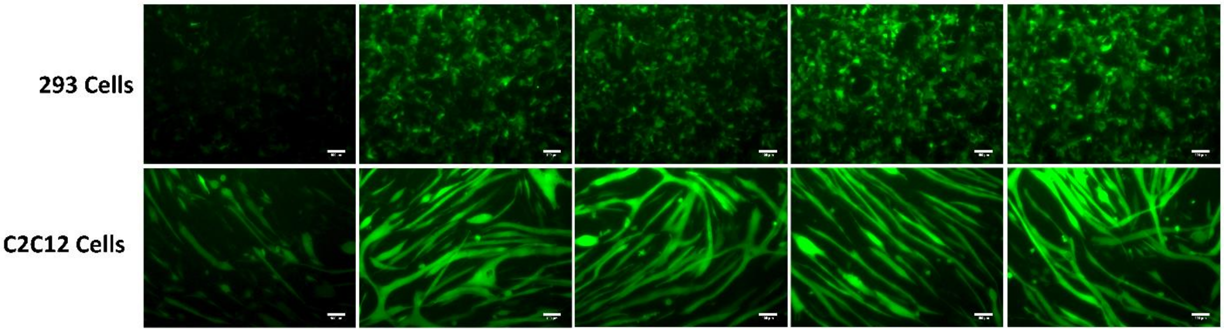


Figure S6. Transduction Efficiency of MV Mutants Compared to AAV5 in 293 and Differentiated C2C12 Cells

(A) Fluorescent imaging of 293 cells and differentiated C2C12 cells transduced with self-complementary GFP vectors packaged by AAV5, MV1, MV18, MV50, and MV53. Both cell lines were transduced at a MOI of 4e5 vg/cell. Images were taken 96 hours after infection. Expression of GFP (green) indicates transduced cells. 10x magnification. Scale Bar: 100um.

Table S1: Replacement of AAV5 Loop1/VRI Amino Acid Sequence with Other AAV Serotype Loop1

AAV Serotype	Loop1 Sequence	Packages DNA?	Infectious on 293?	Infectious in Mice?
1	ISSASTGASNDNHY	No	No	No
2	ISSQSGASNDNHY	No	No	No
4	LGESLQSNTYN	No	No	No
7	ISSETAGSTNDNTY	Yes	No	No
8	ISNGTSGGATNDNTY	No	No	No
9	ISNGTSGGSTNDNTY	No	No	No
Avian	IQGPGGTDPNNKFF	Yes	No	No

Replacement of AAV5 Loop1/VRI amino acid sequence with other AAV serotypes Loop1/VRI sequence. The sequence IKSGSVDGSNANAY was replaced with the sequence indicated in the table using PCR-based cloning. Modified AAV5 capsids were then used to package DNA and tested for their infectivity in 293 cells and mice.

Supplemental Methods

Transduction of 293 Cells by AAV Vectors

Variant AAV5 vectors were used to packaged sc-GFP reporter genes for comparison to wt-AAV5. 293 cells in 12 well plates were infected with AAV5 and mutant self-complementary(sc)-GFP encoding viruses at a MOI of 400,000 viral genomes(vg) per cell and co-infected with 5000 vg/cell of wt-ad5. Ninety-six hours after infection, cells were taken for fluorescent imaging.

Transduction of Differentiated C2C12 Cells by AAV Vectors

Variant AAV5 vectors were used to packaged sc-GFP reporter genes for comparison to wt-AAV5. C2C12 myoblasts were seeded in 12 well plates were cultured with DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (p/s). Upon reaching 70% confluency, culture medium was changed to differentiated medium (DMEM with 2% horse serum and 1% p/s). C2C12 cells were differentiated for five days prior to infection with AAV5 and mutant self-complementary(sc)-GFP encoding viruses at a MOI of 400,000 viral genomes(vg) per cell and co-infected with 5000 vg/cell of wt-ad5. Ninety-six hours after infection, cells were taken for fluorescent imaging. Differentiation medium was changed every two days.