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Supplemental Information

Codon-Optimization of Wild-Type Adeno-Associated Virus Capsid Sequences Enhances DNA Family Shuffling while Conserving Functionality

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Supplementary Figure S1. Schematic representation of localized codon-optimization (LCO).

The upper panel represents schematic of wtAAV donors and sample alignment of fragment of *cap* sequence from AAV2, AAV5 and AAV6 showing DNA and amino acid sequences. Vertical lines indicate positions with same residues, while * represents mismatches at the DNA level. DNA residues modified using LCO algorithm are shown in purple in the lower panel.

Supplementary Figure S2. Schematic illustration of AAV library selection protocol.

At each round of selection cells were infected with the library at various MOIs. QPCR was used to identify dilution (shown as tubes 1, 2 and 3, where tube 1 has the highest MOI) with the lowest MOI that led to detectable library amplification, when compared to cells infected with the library at same MOI but without wtAd5 co-infection.

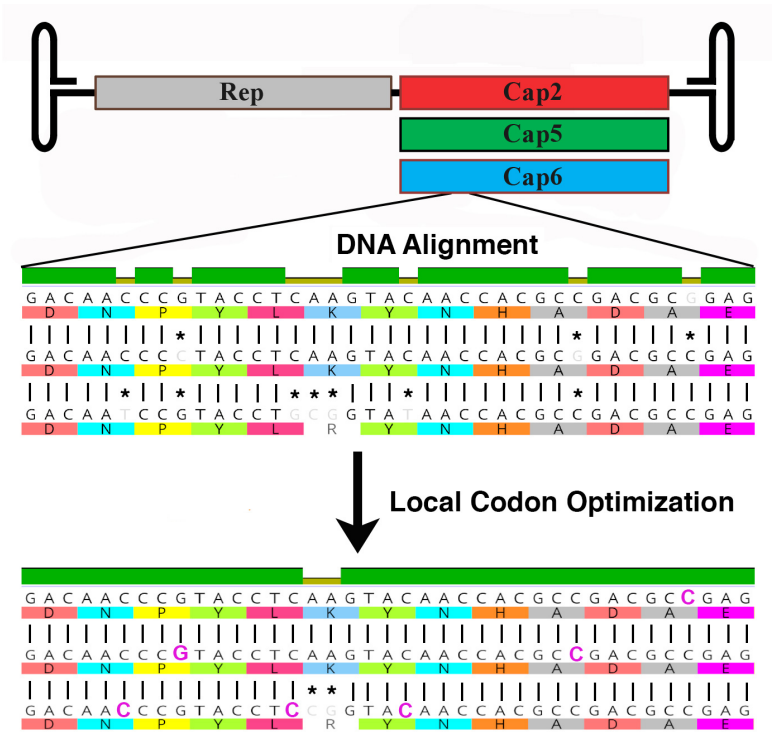
Supplementary Figure S3. Sequences of AAV capsids used in the study optimized using canonical codon-optimization (hcoAAV).

Supplementary Figure S4. Aligment of AAP coding regions from wild type and LCO AAV1 and AAV2. Alignemnt performed using Clustal Omega tool. Asterisk "*****" indicates positions with conserved residue. Colon "**:**" indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. Period "**.**" indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix. Color coding reflects physicochemical properties: **Red** – small (small + hydrophobic (inc. aromatic –Y); **Blue** – acidic; **Magenta** – Basic –H; **Green** – Hydroxyl + sulfhydryl + amine + G.

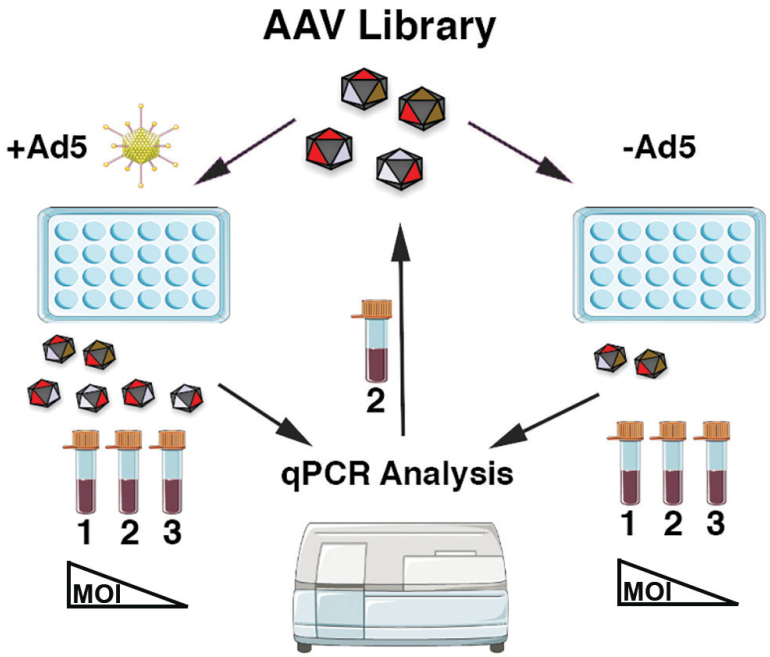
Supplementary Figure S5. Western blot analysis of VP and Rep expression from wtAAV2, hcoAAV2 and lcoAAV2. Levels of capsid proteins (VP1, VP2 and VP3) as well as replicase (Rep78, Rep68, Rep52, and Rep40) in 293T packaging cells transfected with AAV-LSP-GFP, pAd5 and: pAAV2 packaging plasmid encoding wild-type AAV2 Rep and Cap (first lane: **wtAAV2**), plasmid encoding wild-type Rep2 and cap2 optimized using conventional codon-optimization method (second lane: **hcoAAV2**), and plasmid encoding wild-type Rep2 and cap2 optimized using localized codon-optimization method (lane three: **lcoAAV2**), without providing AAP2 *in trans*, or with AAP2 expressed *in trans* (lane five, six, seven: **+AAP2**). 293T transfected with pAd5 and AAV-LSP-GFP only served as negative control (lane eight: **Negative control**)

Supplementary Figure S6. Sequences of lcoAAVs used in the study.

Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3

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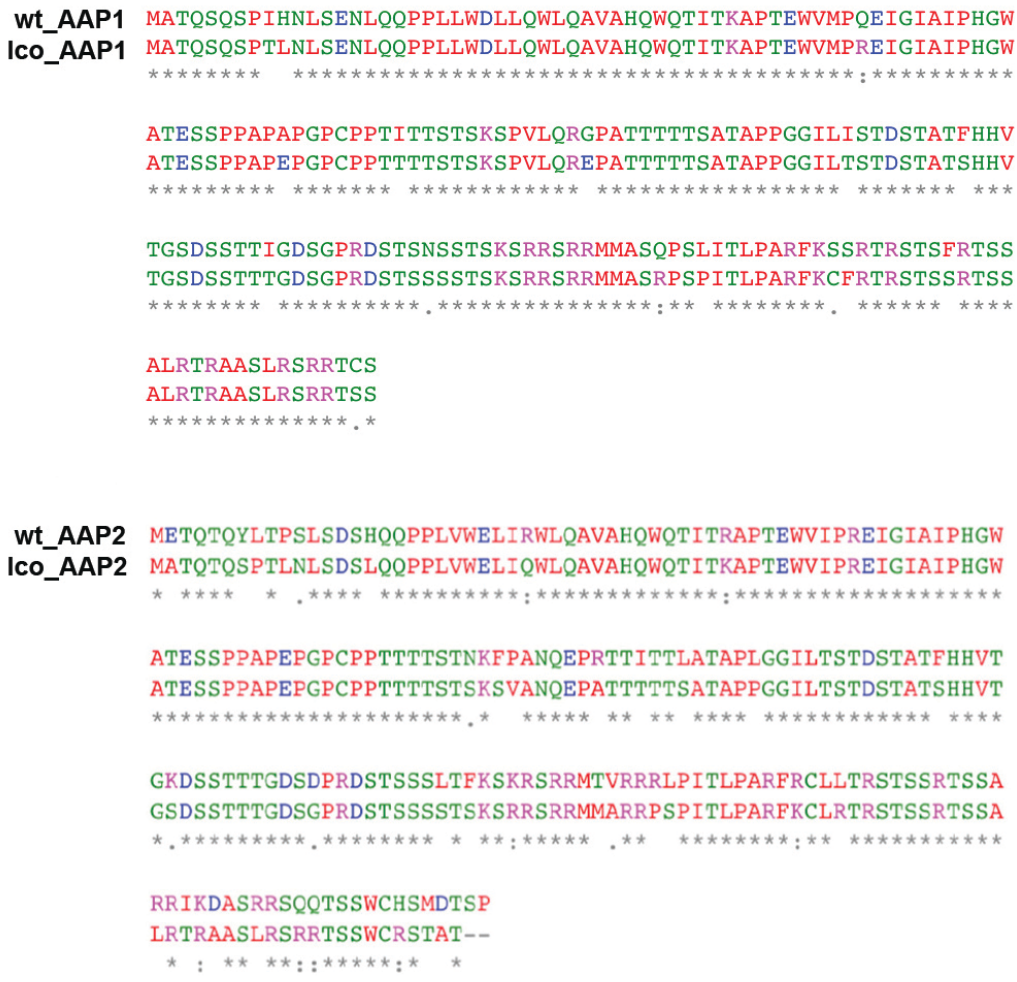
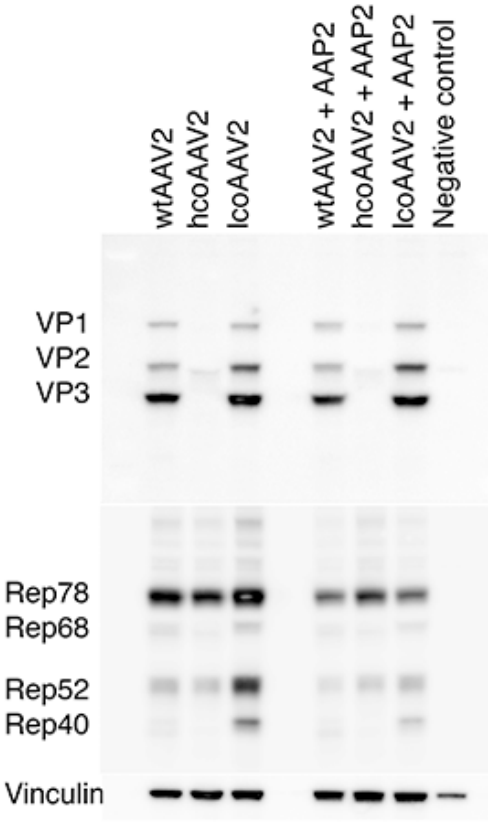
Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6

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