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Identification and Characterization of an Alternate, AAVR Independent, AAV Entry Mechanism Using a Genome-Wide CRISPR/Cas9 Knock-Out Screen

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Recently KIAA0319L (AAVR) was identified as a requirement for entry of many AAV serotypes. Here, a large set of human and simian AAVs and putative evolutionary intermediate capsids were interrogated for AAVR usage. We identified a distinct AAV capsid lineage that can bind and transduce cells in the absence of AAVR, and are unable to be rescued by AAVR re-introduction into non-permissive cells. Cell binding and viral overlay assays demonstrate that these serotypes cannot bind the AAVR protein. Importantly, AAVrh32.33 is able to transduce *Aavr* KO and WT mice to a similar level, demonstrating that these AAVs use an alternate entry mechanism *in vivo*. Further investigation revealed several serotypes that are able to undergo entry, although to a decreased extent, in the absence of AAVR both *in vitro* and *in vivo*. We have determined that these partially AAVR-dependent AAV serotypes are able to transduce an AAVR KO mouse, although with delayed onset of gene expression, and with an altered tissue expression pattern. To identify alternate entry pathway receptors, we have carried out a genome-wide CRISPR/Cas9-based knock-out screen and have identified several important cellular factors involved in this alternate AAV entry pathway. Knock-out of the identified entry factors prevents entry of AAVR-independent serotypes up to 100-fold. Additionally, some identified factors can be co-immunoprecipitated by purified AAV4 and AAVrh32.33 capsids. Our results demonstrate that AAVR usage is highly conserved amongst primate AAVs aside from the AAVrh32.33 lineage, yet a non-AAVR pathway may be available to other serotypes, suggesting a multi-modal entry pathway used by some AAV capsids. We have also further defined the molecular details that dictate AAV entry of diverse AAV serotypes. By elucidating serotype specific entry pathway differences we aim to further inform the development of AAV as a gene therapy vector.