

# **AAV8-Mediated Angiotensin-Converting Enzyme 2 Gene Delivery Prevents Experimental Autoimmune Uveitis by Regulating MAPK, NF- $\kappa$ B and STAT3 Pathways**

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## **Supplementary Methods:**

### **Recombinant adeno-associated virus construction and packaging**

AAV vectors containing a secreted form of human *ACE2* gene or enhanced green fluorescent protein (eGFP) under the control of the chicken  $\beta$ -actin (CBA) promoter were constructed as previous studies<sup>1,2</sup>. The secreted ACE2 protein has been proved to be enzymatically activated in the preceding study<sup>3</sup>. These constructs contained expression cassettes flanked by the rAAV8 terminal repeats. Expressions of eGFP and ACE2 were driven by a CBA with a human cytomegalovirus enhancer. Site-directed mutagenesis of surface-exposed tyrosine residues on the capsids to generate AAV8(Y733F) mutation has been described recently<sup>4</sup>. Vector plasmid was packaged in AAV8 (Y733F) by transfection of HEK cells according to previously published

methods<sup>5</sup>. Vector doses were expressed as genome copies.

## References

1. Verma, A. et al. ACE2 and Ang-(1-7) confer protection against development of diabetic retinopathy. *Mol Ther* **20**,28-36 (2012).
2. Li, H. et al. Macrophage migration inhibitory factor in hypothalamic paraventricular nucleus neurons decreases blood pressure in spontaneously hypertensive rats. *Faseb J* **22**,3175-3185 (2008).
3. Huentelman, M.J., Zubcevic J., Katovich M.J. & Raizada M.K. Cloning and characterization of a secreted form of angiotensin-converting enzyme 2. *Regul Pept* **122**,61-67 (2004).
4. Petrs-Silva, H. et al. High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. *Mol Ther* **17**,463-471 (2009).
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## Supplementary Table S1

**Table S1.** Fold changes of IFN- $\gamma$  and IL-17 at the protein level (VS. IRBP group)

group	cytokines	protein fold change
SB+IRBP	IFN- $\gamma$	7.58
	IL-17	22.67
SP+IRBP	IFN- $\gamma$	8.76
	IL-17	12.83
PD+IRBP	IFN- $\gamma$	1.88
	IL-17	11.41
BAY+IRBP	IFN- $\gamma$	29.47
	IL-17	6.39
S3I-201+IRBP	IFN- $\gamma$	70.29
	IL-17	11.34

## Supplementary Table S2

**Table S2. Primers used for Real-Time PCR analysis**

Gene name	Accession number	Sequences
ACE2	NM_001130513.1	Forward: 5'-GATGTCATTCCTAGAAAGTGAAG-3' Reverse: 5'-ACATACAGGAAGATGACAAGTG-3'
IL-6	NM_031168.1	Forward: 5'-AGATAACAAGAAAGACAAAGCCAGAGTC-3' Reverse: 5'-GCATTGGAAATTGGGGTAGGAAG-3'
IL-1 $\beta$	NM_008361.3	Forward: 5'-TTGAAGAAGAGCCCGTCC-3' Reverse: 5'-CTTATGTTCTGTCCATTGAGGT-3'
TNF- $\alpha$	NM_001278601.1	Forward: 5'-AGGCGCCACATCTCCCTCCA-3' Reverse: 5'-CGGTGTGGGTGAGGAGCACG-3'
MCP-1	NM_011333.3	Forward: 5'-AGTTGCCGGCTGGAGCATCC-3' Reverse: 5'-TCTTTGGGACACCTGCTGCTGG-3'
IL-10	NM_010548.2	Forward: 5'-GAAGACCCTCAGGATGCG-3' Reverse: 5'-CCAAGGAGTTGTTTCCGTTA-3'
IFN- $\gamma$	NM_008337.3	Forward: 5'-TCAAGTGGCATAGATGTGGAAGAA-3' Reverse: 5'-TGGCTCTGCAGGATTTTCATG-3'
IL-17	NM_010552.3	Forward: 5'-CTCAACCGTTCCACGTCACCCT-3' Reverse: 5'-CCAGCTTCCCTCCGCATT-3'
iNOS	NM_010927.3	Forward: 5'-GGGCTGTCACGGAGATCA-3' Reverse: 5'-CCATGATGGTCACATTCTGC-3'
Arg-1	NM_007482.3	Forward: 5'-GAACACGGCAGTGGCTTTAAC-3' Reverse: 5'-TGCTTAGCTCTGTCTGCTTTGC-3'
GAPDH	NM_001289726.1	Forward: 5'-GTATGACTCCACTCACGGCAA-3' Reverse: 5'-GGTCTCGCTCCTGGAAGATG-3'