Supplemental Information

Efficient gene transfer to kidney mesenchymal cells using a synthetic adeno-associated viral vector

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Inventory of Supplemental Information

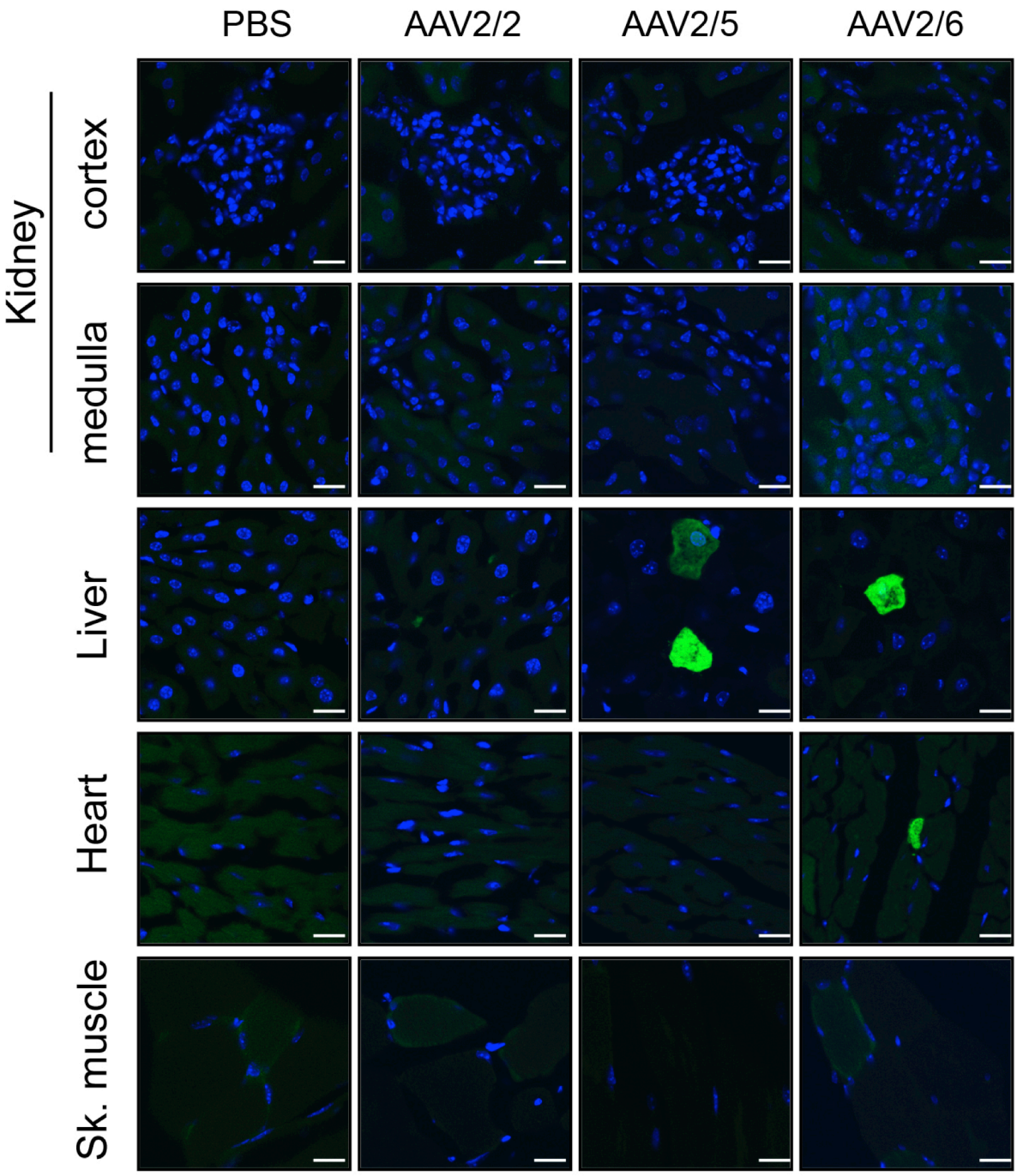


Figure S1. Transduction of various organs by AAV pseudotypes 2, 5 and 6. 1 x 10^{11} GC/mouse of the indicated AAV pseudotypes were injected intravenously, and transduction efficiencies were assessed 3 weeks later. None of the AAVs transduced kidney, with low rates in other tissues. N = 3 mice tested for each AAV. Scale bar 50 μm.

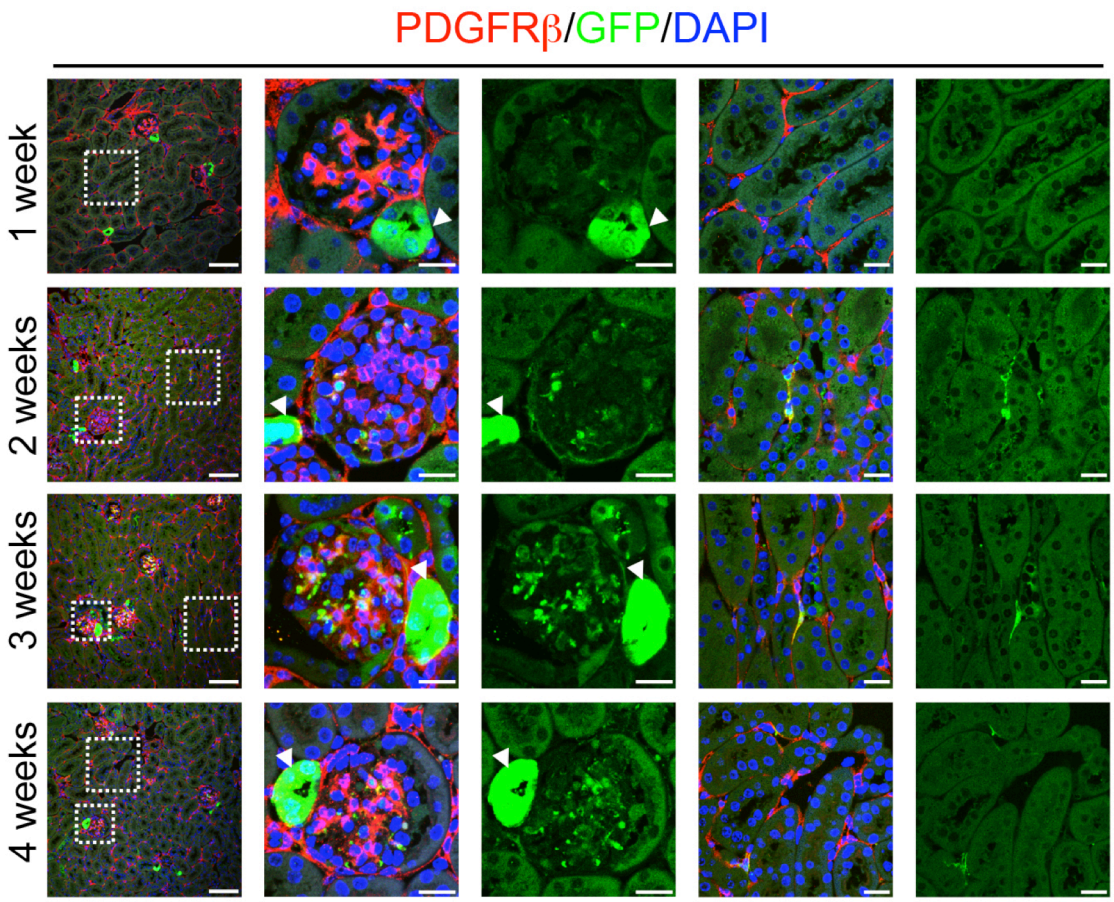


Figure S2. Time course for GFP expression after administration of AAV2/Anc80. A dose of 1 x 1011 GC/mouse of Anc80 (CASI.eGFP.WPRE) was administered intravenously, and kidney transduction assessed weekly up to four weeks. Strong eGFP expression adjacent to the juxtaglomerular apparatus could be seen by one week, whereas mesangial and interstitial expression could be detected at two weeks, and was maximal by three weeks. Left column scale bar 100 μm, all other scale bars 20 μm.

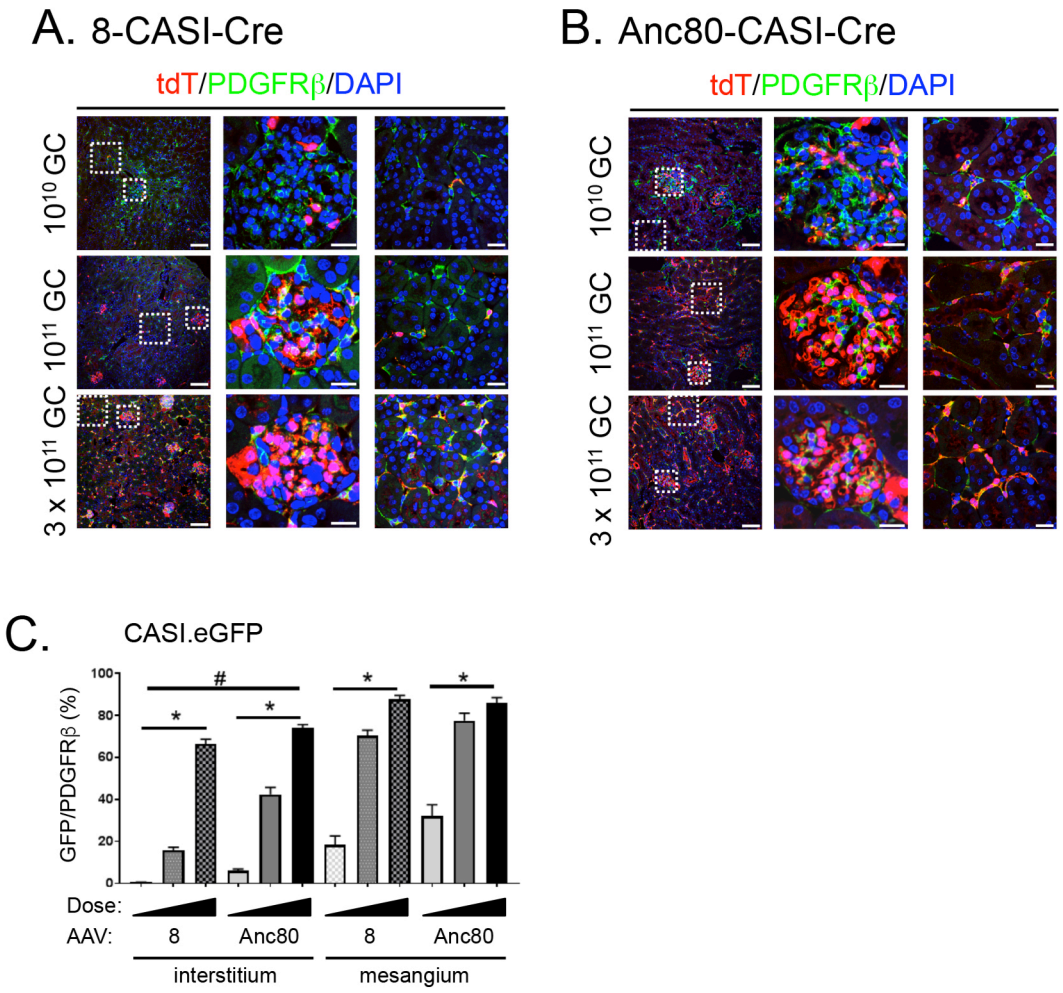


Figure S3. Comparison of recombination efficiencies between AAV2/8-CASI-Cre and AAV2/Anc80-CASI-Cre. A,B. Varying doses of both AAVs were injected intravenously into R26tdTomato reporter mice, and tdTomato expression was assessed three weeks later. Both AAVs transduced mesangium and interstitium, but AAV2/Anc80-CASI-Cre had higher transduction efficiencies at every dose compared to AAV2/8-CASI-Cre. Green stain is PDGFRβ. Left column scale bar 100 μm, all other scale bars 20 μm. **C.** Quantitation of transduction efficiencies between these AAVs at each dose. N = 3 per group.

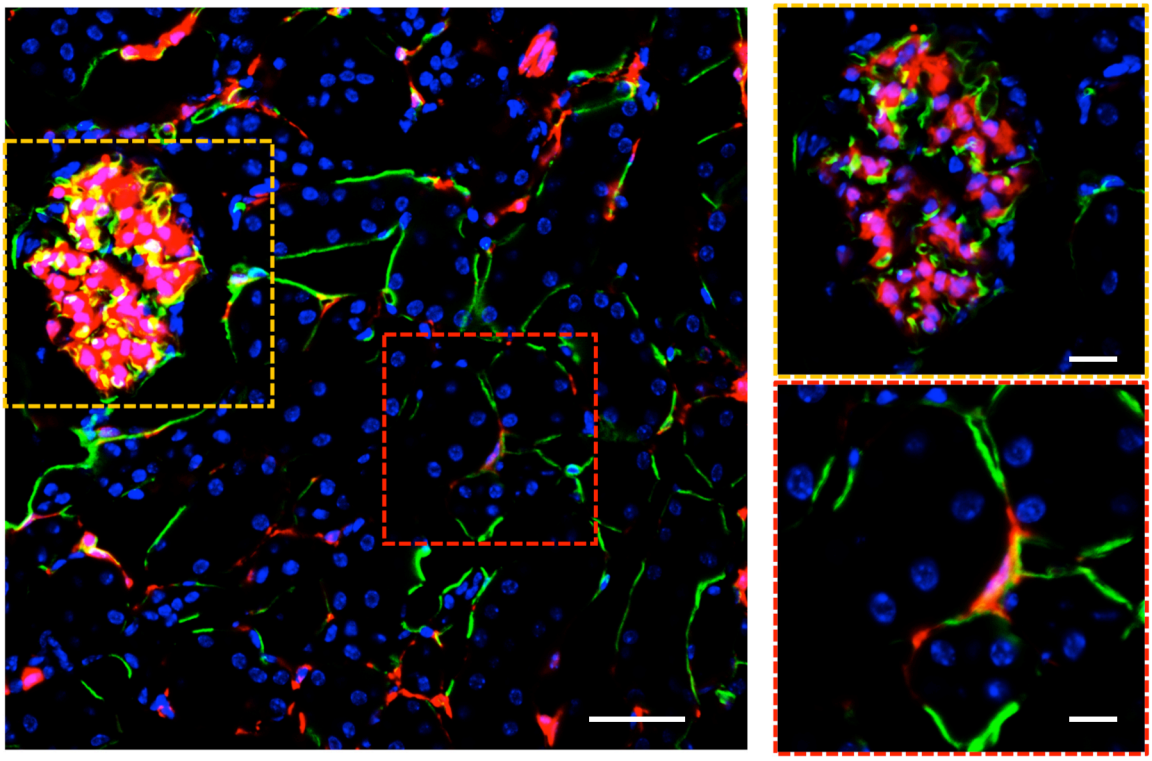
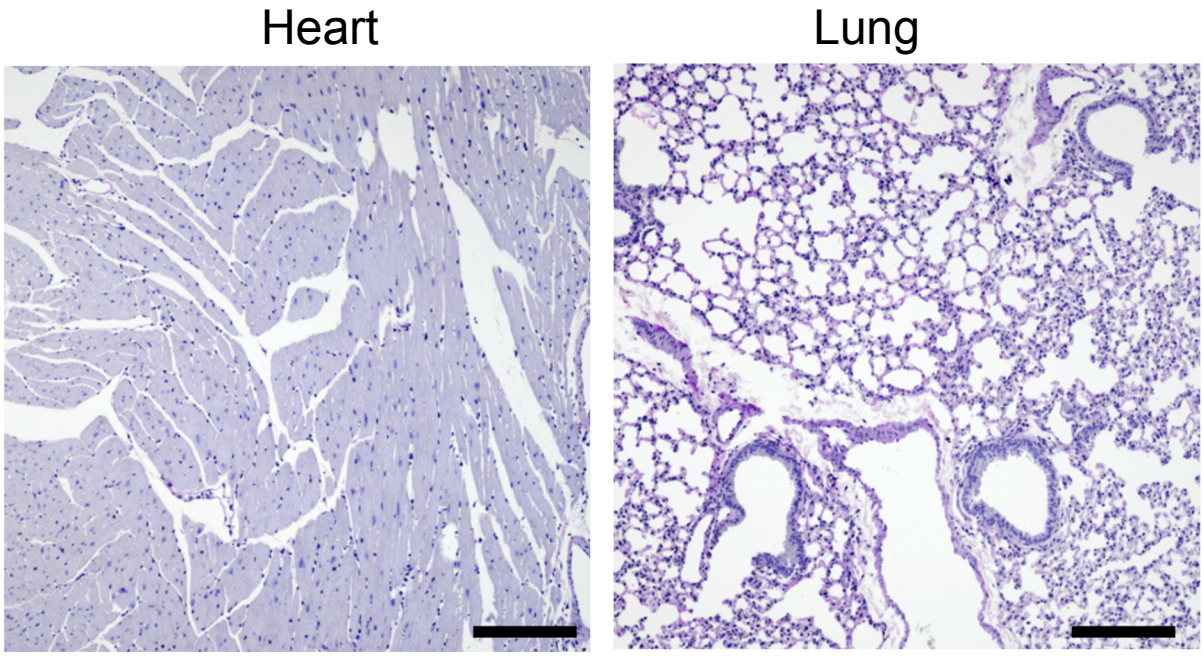


Figure S4. Anc80-Cre does not transduce endothelial cells. Anc80-Cre was injected into R26tdTomato reporter mice Costaining of tdTomato and the endothelial marker endomucin revealed no detectable transduction of endothelium by AAV. At high power, some tdTomato+ pericytes could clearly be seen directly adjacent endomucin+ endothelial cells (Lower right). Similarly, in the glomerulus, high power views clearly show a lack of overlap between tdTomato+ mesangium and endomucin+ glomerular endothelial cells (Upper right). Left scale bar 40 μm, right scale bars 20 μm.



Supplementary Figure 5. No toxicity of heart or lung in Anc80-injected mice. AAV8-CASI-Cre was injected into tdTomato reporter mice at a dose of 3 x 10^{11} GC per mouse (n = 3). Organs were harvested 8 weeks later. There was no evidence of heart or lung inflammation or fibrosis on histology. Scale bar, 100 μM.

Supplementary Table 1:

Antibody	application	host species	cat #	Manufacturer	Dilution
aSMA	WB (tissue)	rabbit	ab5694	Abcam	1:1000
aSMA-FITC	IF-F or P	mouse	F3777	Sigma	1:1000
aSMA-Cy3	IF-F or P	mouse	C6198	Sigma	1:1000
collagen1	IF-F	goat	1310-01	Southern Biotech	1:40
fibronectin	WB	rabbit	ab23750	Abcam	1:3000
PDGFRb	IF-F or P	rabbit	ab32570	Abcam	1:400
β-catenin	WB	rabbit	ab32572	Abcam	1:3000
β-catenin	IF-F	rabbit	ab16051	Abcam	1:200
tdTomato	IF-P	goat	AB8181	Acris	1:100
SLC12A1	IF-P	rabbit	ab171747	Abcam	1:200
GFP	IF-P	chicken	GFP-1020	AVES	1:500
NOS1	IF-P	rabbit	sc-648	Santa Cruz	1:200
AQP2	IF-P	rabbit	ab15116	Abcam	1:200

Antibodies used

Supplementary Table 2:

Primer pairs used for qt-RT-PCR in mouse:

Gene	Sequence
GAPDH	Fw 5´-AGGTCGGTGTGAACGGATTTG -3´
	Rv 5`-TGTAGACCATGTAGTTGAGGTCA -3´
Gli1	Fw5´- ATCACCTGTTGGGGATGCTGGAT-3´
	Rv5´- CGTGAATAGGACTTCCGACAG -3´
Gli2	Fw5´- ACGCATGATTCGGACCTCTC -3´
	Rv5´- AGCCTCAGTCTTGACCTTGC-3´
Ptch1	Fw5´- GCTGGAGGAGAACAAGCAAC-3´
	Rv5´- GAGCAAACATGTGCTCCAGA -3´
Col1α1	Fw5´- TGACTGGAAGAGCGGAGAGT-3´
	Rv5´- GTTCGGGCTGATGTACCAGT -3´
Col3α1	Fw5´- CTGTAACATGGAAACTGGGGAAA
	Rv5´- CCATAGCTGAACTGAAAACCACC
Fibronectin	Fw5´- ATCTGGACCCCTCCTGATAGT -3´
	Rv5´- GCCCAGTGATTTCAGCAAAGG-3´
Axin2	Fw5´- GAGTAGCGCCGTGTTAGTGACT -3´
	Rv5´- CCAGGAAAGTCCGGAAGAGGTATG-3´
Lef1	Fw5´- TGTTTATCCCATCACGGGTGG-3´
	Rv5´- CATGGAAGTGTCGCCTGACAG-3´
α-SMA	Fw5´- GTCCCAGACATCAGGGAGTAA -3´
	Rv5´- TCGGATACTTCAGCGTCAGGA-3´

Supplementary Methods

AAV transduction of human kidney organoids

Human kidney organoids were generated as described by Morizane et al.[1] Briefly, BJFF6, a human iPSC line reprogrammed by Sendai virus from human foreskin fibroblasts (Washington University Genome Engineering and iPSC Core), cells were cultured in basic differentiation medium consisting of Advanced RPMI 1640 medium (Life Technologies) and 1X L-GlutaMAX(Life Technologies) supplemented with 8 uM CHIR99021(Tocris Bioscience) and 5ng/mL Noggin(PeproTech) for 4 days, followed by 3 days of Activin(10ng/mL, R&D Systems) and another 2 days of FGF9(10 ng/mL R&D Systems) treatment. On day 9 of differentiation, cells were dissociated with Accutase (Innovative Cell Technology) and resuspended in the basic differentiation medium containing 3 uM CHIR99021 and 10ng/mL FGF9, and seeded in 96 well round bottom ultra-low-attachment plate with $1X10^5$ cells per well. After 2 days, the medium was changed to basic differentiation medium supplemented with 10 ng/mL FGF9 and cultured for another 3 days. At day 14, the kidney organoids were cultured in the basic differentiation medium without any additional factor for 7-20 days. For AAV transduction in kidney organoids, $5x10^{10}$ GC Anc80 viruses were added to the differentiation medium at day 11. After 2 days, the viruses were withdrawn with new differentiation medium. At day 21, kidney organoids were harvested for experiments.

Reference

1. Morizane, R, Bonventre, JV: Generation of nephron progenitor cells and kidney organoids from human pluripotent stem cells. *Nat Protoc,* 12**:** 195-207, 2017.