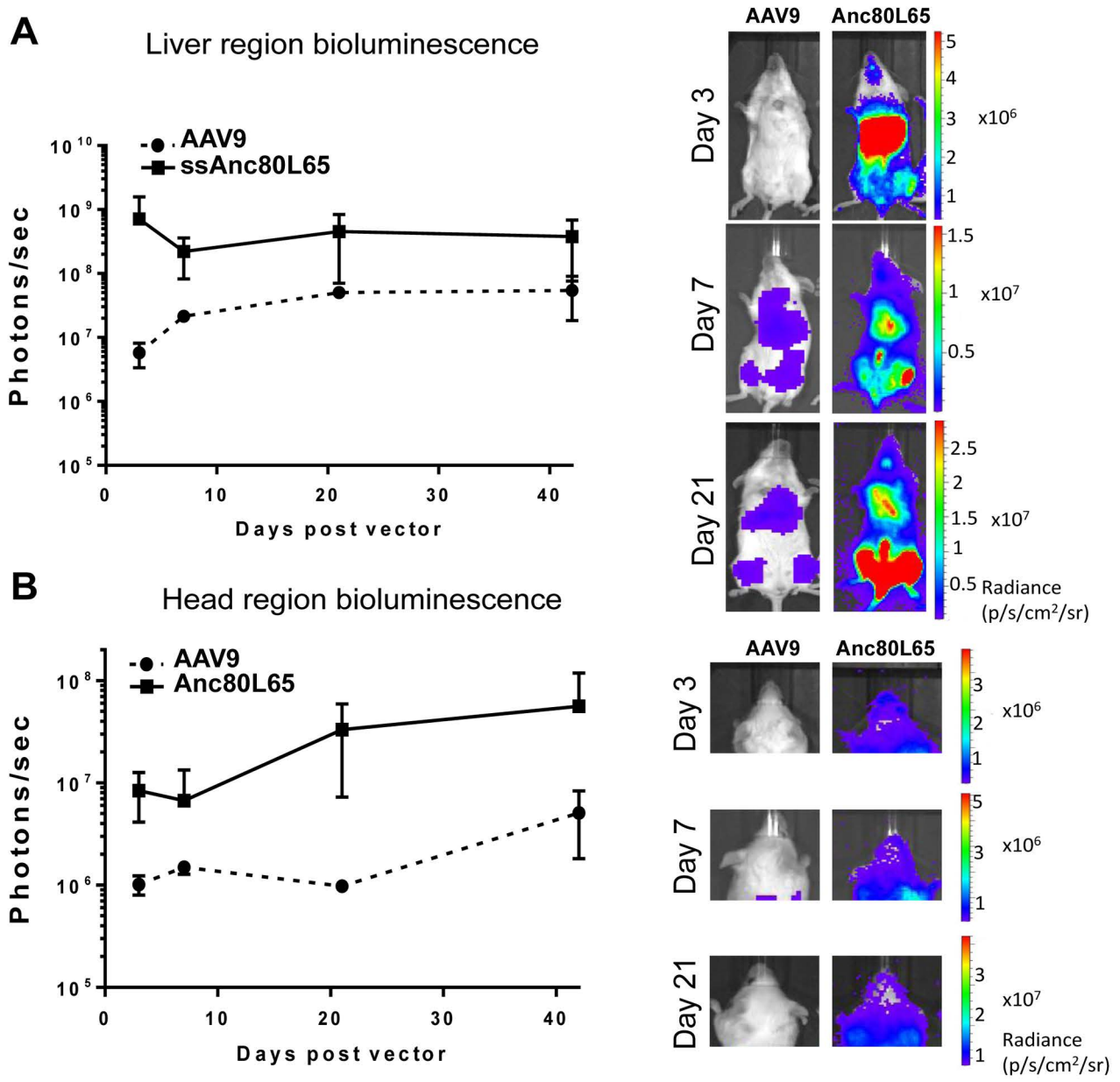
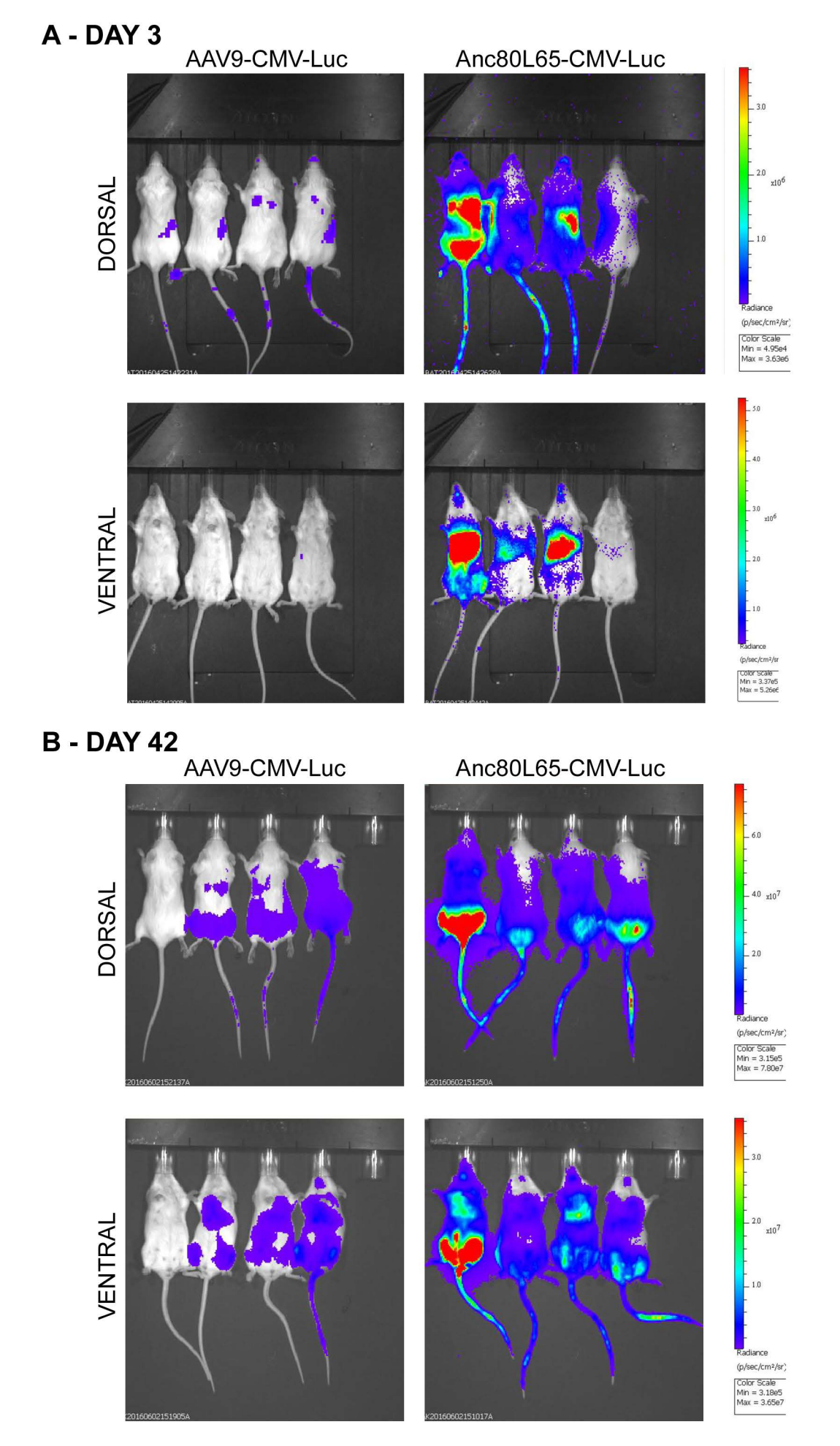
OMTM, Volume *10*

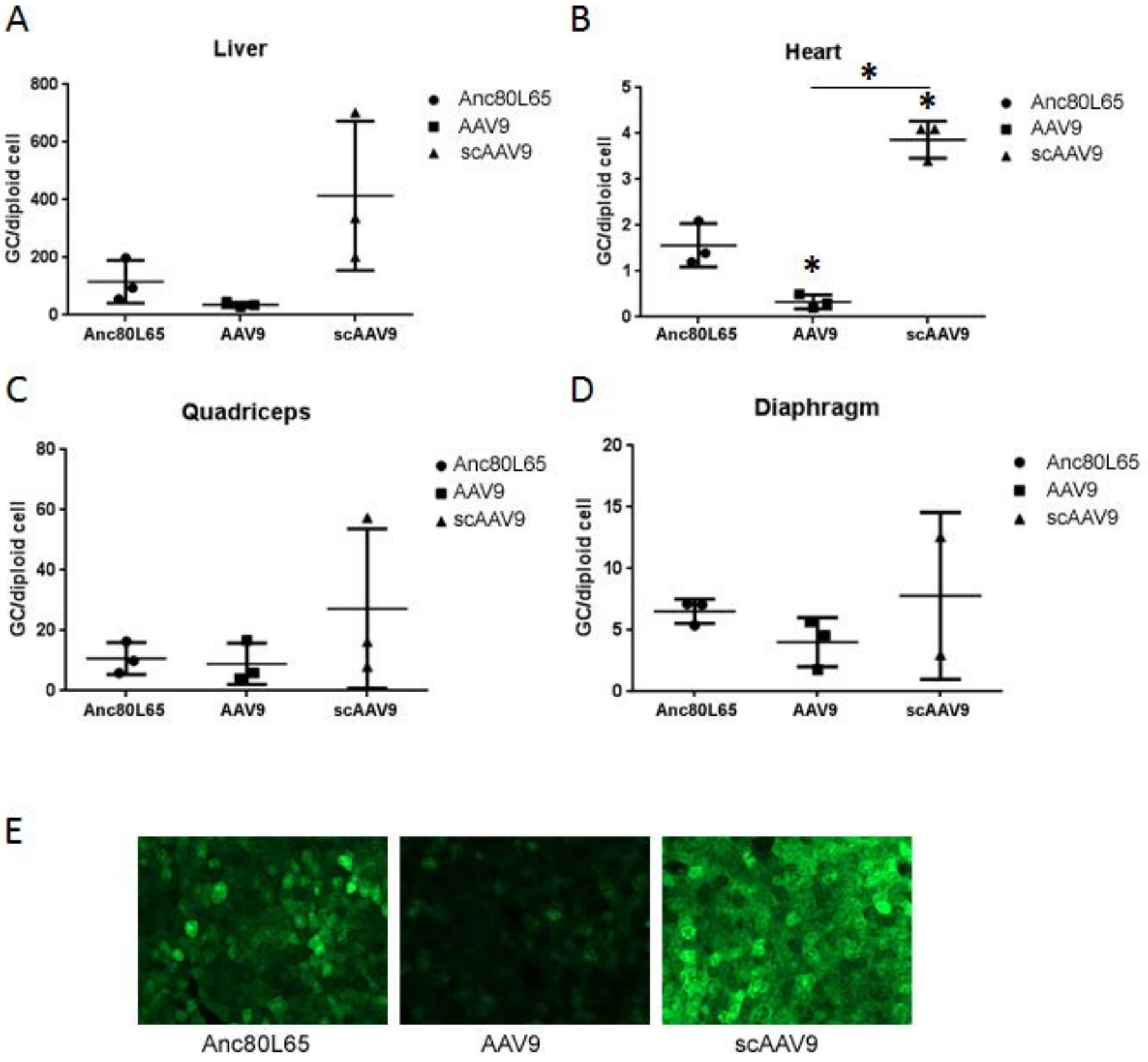
Supplemental Information

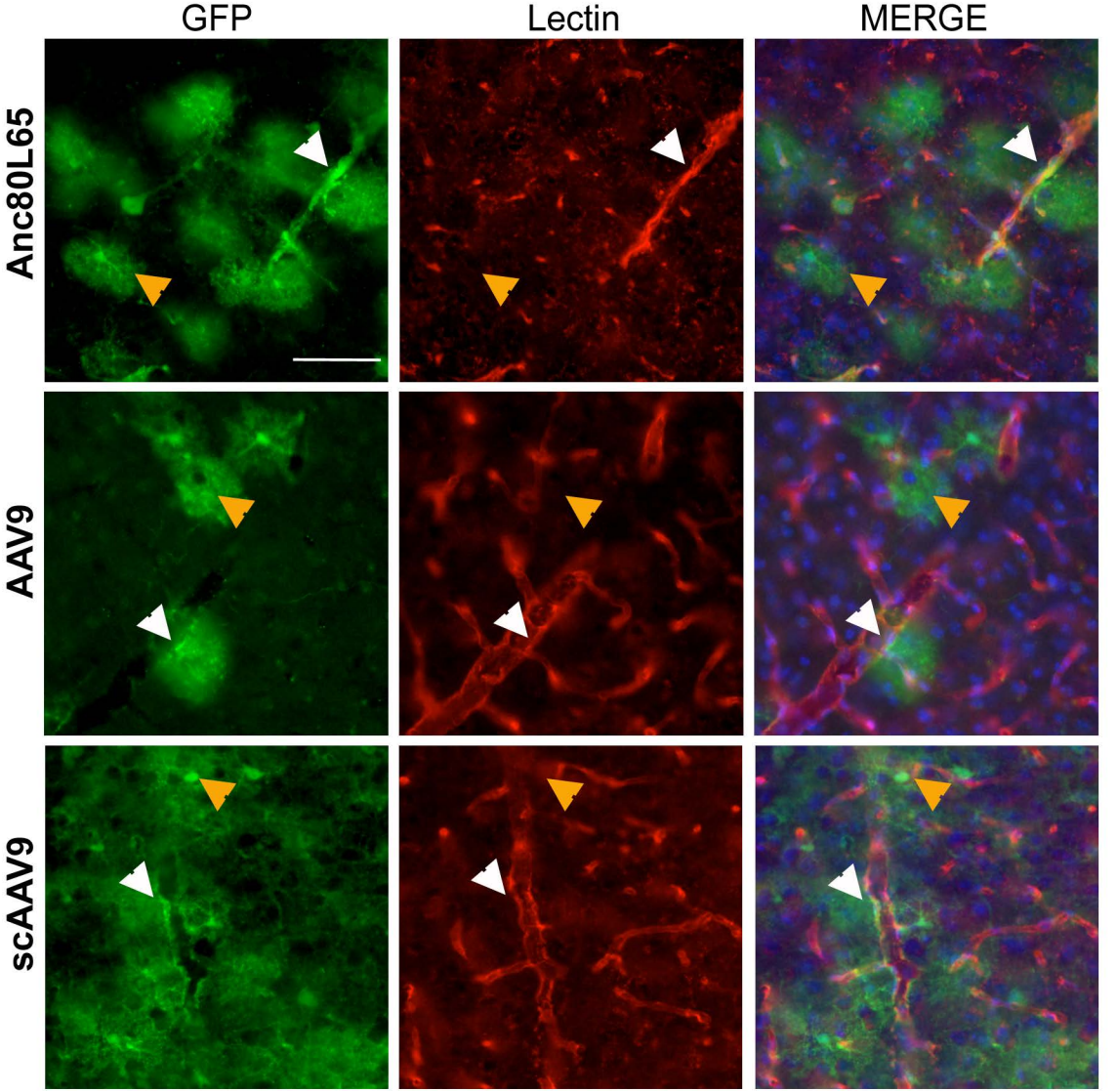
Efficient Gene Transfer to the Central Nervous System by Single-Stranded Anc80L65

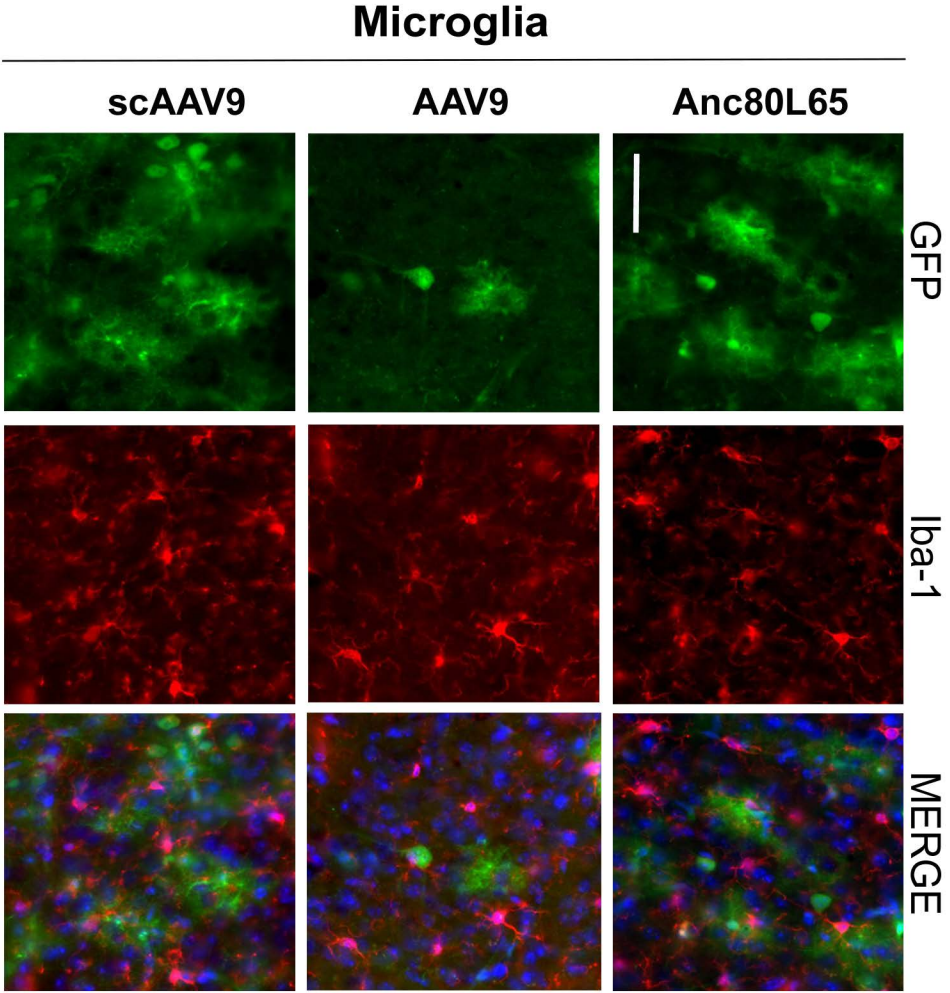
Eloise Hudry, Eva Andres-Mateos, Eli P. Lerner, Adrienn Volak, Olivia Cohen, Bradley T. Hyman, Casey A. Maguire, and Luk H. Vandenberghe


A
Liver region bioluminescence
AAV9
ssAnc80L65
Photons/sec
Days post vector
B
Head region bioluminescence
AAV9
Anc80L65
Photons/sec
Days post vector
AAV9
Anc80L65
Day 3
Day 7
Day 21
x10^6
x10^7
Radiance (p/s/cm²/sr)


A - DAY 3
AAV9-CMV-Luc
Anc80L65-CMV-Luc
DORSAL
VENTRAL
3.0
2.0
x10^6
1.0
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 4.95e4
Max = 3.63e6
5.0
4.0
3.0
x10^6
2.0
1.0
Radiance
(p/sec/cm²/sr
Color Scale
Min = 3.37e5
Max = 5.26e6
B - DAY 42
AAV9-CMV-Luc
Anc80L65-CMV-Luc
DORSAL
VENTRAL
6.0
4.0
x10^7
2.0
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 3.15e5
Max = 7.80e7
3.0
2.0
x10^7
1.0
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 3.18e5
Max = 3.65e7


A
Liver
GC/diploid cell
800
600
400
200
0
Anc80L65
AAV9
scAAV9
B
Heart
GC/diploid cell
5
4
3
2
1
0
*
*
*
Anc80L65
AAV9
scAAV9
C
Quadriceps
GC/diploid cell
80
60
40
20
0
Anc80L65
AAV9
scAAV9
D
Diaphragm
GC/diploid cell
20
15
10
5
0
Anc80L65
AAV9
scAAV9
E
Anc80L65
AAV9
scAAV9


GFP
Lectin
MERGE
Anc80L65
AAV9
scAAV9

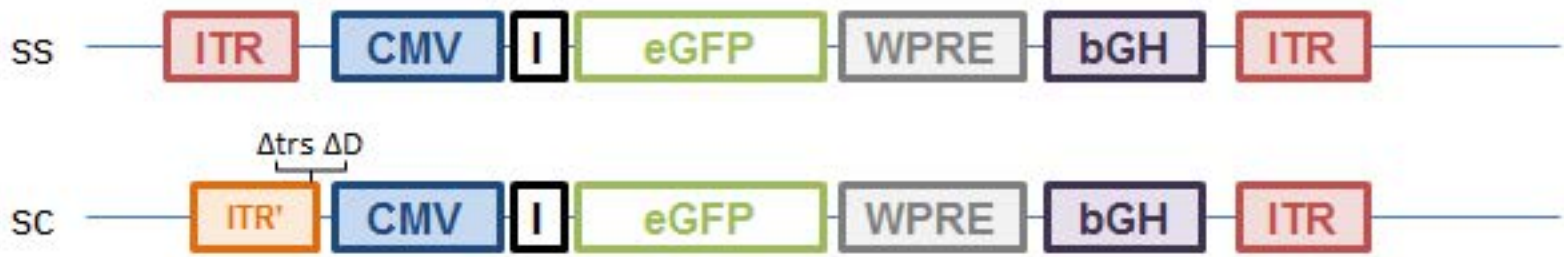

Microglia
scAAV9
AAV9
Anc80L65
GFP
Iba-1
MERGE

A

Bioluminescence

B

Systemic and intracerebral injection



C

VECTOR	DOSE	ROUTE OF ADMINISTRATION
BIOLUMINESCENCE		
AAV2/9.CMV.EGFP.T2A.LUCIFERASE.SVPA	2E12 gc/kg	Intravenous
AAV2/Anc80.CMV.EGFP.T2A.LUCIFERASE.SVPA	2E12 gc/kg	Intravenous
SYSTEMIC INJECTION		
AAV2/9.CMV.EGFP.WPRE.bGH	4E13 gc/kg	Intravenous
AAV2/Anc80.CMV.EGFP.WPRE.bGH	4E13 gc/kg	Intravenous
SC AAV2/9.CMV.EGFP.WPRE.bGH	4E13 gc/kg	Intravenous
INTRACEREBRAL INJECTION		
AAV2/9.CMV.EGFP.WPRE.bGH	1.5E10 gc/mouse	Intra-striatal
AAV2/Anc80.CMV.EGFP.WPRE.bGH	1.5E10 gc/mouse	Intra-striatal
AAV2/9.CMV.EGFP.WPRE.bGH	4.5E10 gc/mouse	Intra-ventricular
AAV2/Anc80.CMV.EGFP.WPRE.bGH	4.5E10 gc/mouse	Intra-ventricular

SUPPLEMENTAL FIGURES LEGENDS

Supplementary Figure 1. Kinetics and localization of bioluminescence signal after intravenous injection of BALB/c with Anc80L65-CMV-FLuc or AAV9-CMV-FLuc.
Mice were injected with $2x10^{12}$ gc/kg of vector and imaged at days 3, 7, 21, and 42 post injection. (A) Bioluminescence signal from liver region. A region of interest (ROI) was drawn on each image and photons/sec calculated. Left: graph depicting kinetics of expression. Right: representative images of mice injected with AAV9 or Anc80 at days 3, 7, and 21 post injection. (B) Bioluminescence signal from head region. ROI was drawn on each image and photons/sec calculated. Left: graph depicting kinetics of expression. Right: representative images of mice injected with AAV9 or Anc80L65 at days 3, 7, and 21 post injection. n=5 mice/group.

Supplementary Figure 2. Whole-body bioluminescence images at days 3 and 42 post intravenous injection of Anc80L65-CMV-FLuc or AAV9-CMV-FLuc
Representative bioluminescence images of BALB/c mice injected systemically with $2x10^{12}$ gc/kg of AAV9-CMV-FLuc or Anc80-CMV-FLuc, at 3 and 42 days after injection in the lateral tail vein.

Supplementary Figure 3. Quantification of AAV genome-copy numbers in peripheral tissues after systemic injection with Anc80L65-CMV-eGFP, AAV9-CMV-eGFP and scAAV9-CMV-eGFP
Genomic DNA qPCR analysis was performed to measure the gc/diploid cell in liver (A), heart (B), quadriceps (C) and diaphragm (D), n=3 mice per group; two-way ANOVA followed by Tukey's multiple comparison test; *$p<0.05$. (E) Representative images of eGFP fluorescence signal detected across the liver after intravenous injection of the same dose of Anc80L65, AAV9 and scAAV9 in wild-type mice ($4x10^{13}$gc/kg).

Supplementary Figure 4. Anc80L65, AAV9 and scAAV9 transduction of intraparenchymal and vessel-associated astrocytes
Double immunostaining with Lycopersicon Esculentum lectin ("Lectin") and eGFP shows that both astrocytic endfeet and parenchymal astrocytes were equivalently transduced by Anc80L65, AAV9 or scAAV9 after systemic infusion. Scale bar: 50µm

Supplementary Figure 5. Absence of microglial transduction after intravenous delivery of Anc80L65, AAV9 and scAAV9 harboring a self-complementary genome
Double immunostaining with Iba-1 and eGFP was performed in order to determine if Anc80L65, AAV9 or AAV9 containing a self-complementary genome (scAAV9) transduced microglial cells. As shown on those representative images, no colocalization between the two markers was detected. Scale bar: 50µm

Supplementary Figure 6. Vector and experimental design
(A and B) Schematic representation of the expression cassette used to produce the viral vectors. (A) For the bioluminescence experiments a multicistronic vector with a T2A peptide signal was used. A 133 base pairs chimeric intron (I) and the viral SV40 polyA signal (SVPA) were introduced in the cassette. (B) For the systemic and intracerebral injection, the self-complementary (sc) vector 5' ITR contained a deletion of the D-sequence and the terminal resolution site mutation (trs). The transgene design (CMV.eGFP.WPRE.bGH, 2266 base pairs) was identical for the self-complementary and single stranded vectors, and included a 133 bp chimeric intron (I) and the bovine growth hormone polyA signal (bGH). (C) Table including the vector, dose and route of administration used in all the experiments.