

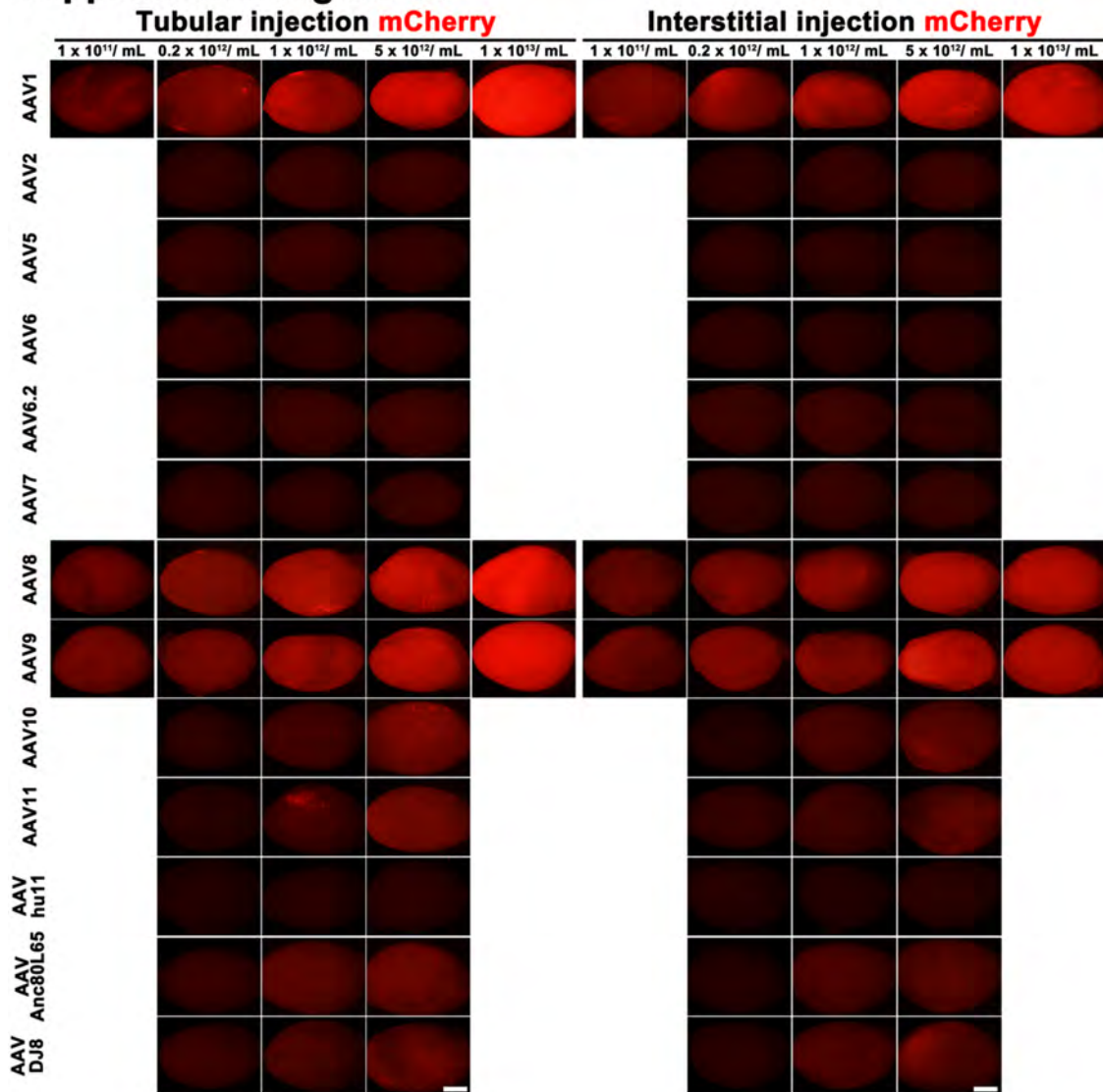
Stem Cell Reports, Volume 10

Supplemental Information

***In Vivo* Genetic Manipulation of Spermatogonial Stem Cells and Their
Microenvironment by Adeno-Associated Viruses**

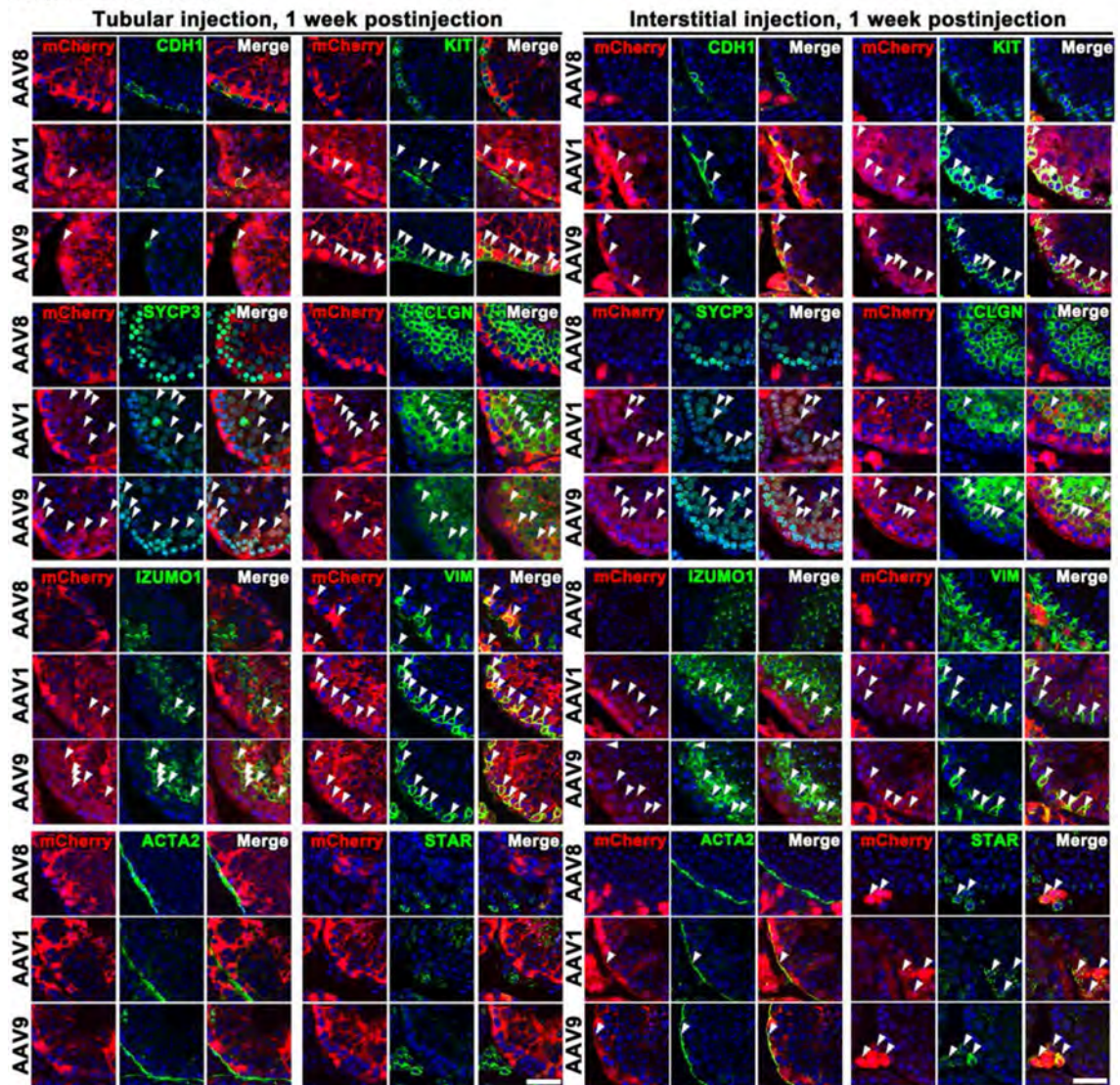
Satoshi Watanabe, Mito Kanatsu-Shinohara, Narumi Ogonuki, Shogo Matoba, Atsuo Ogura, and Takashi Shinohara

Supplemental Figure S1



Supplementary Figure S1, related to Figure 1. Macroscopic appearance of wild-type testes 1 week after AAV infection. Bar, 1 mm.

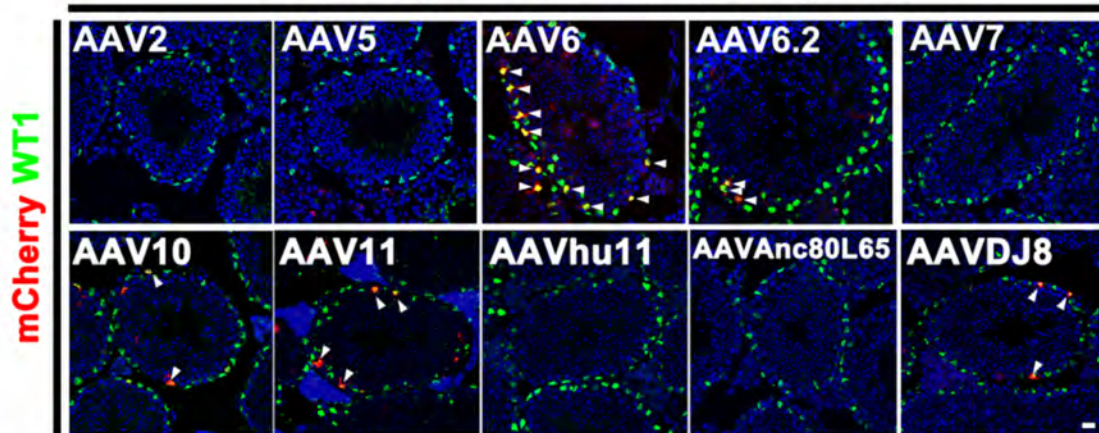
Supplemental figure S2



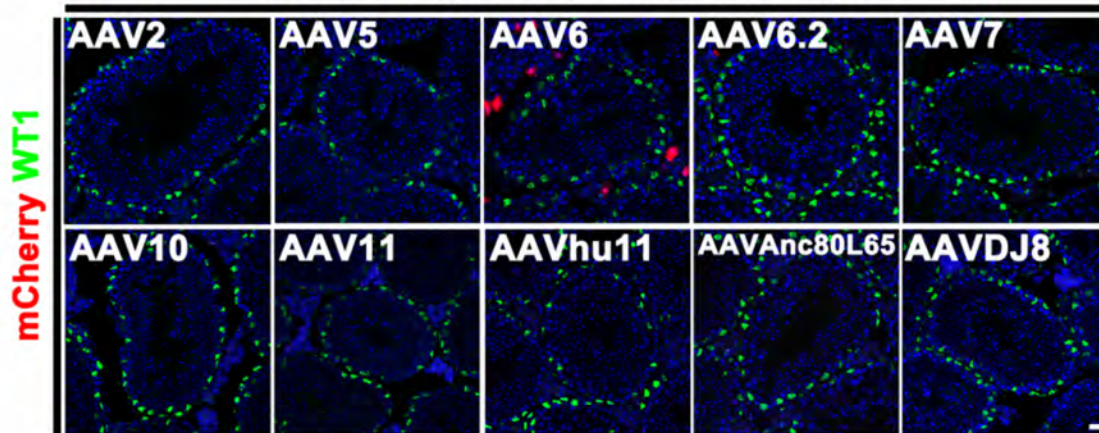
Supplementary Figure S2, related to Figure 1. Immunostaining of wild-type testes 1 week after AAV8, 1, and 9 infection. Bar, 40 μ m. Counterstain, Hoechst 33342.

Supplemental figure S3

Tubular injection, 1 week postinjection



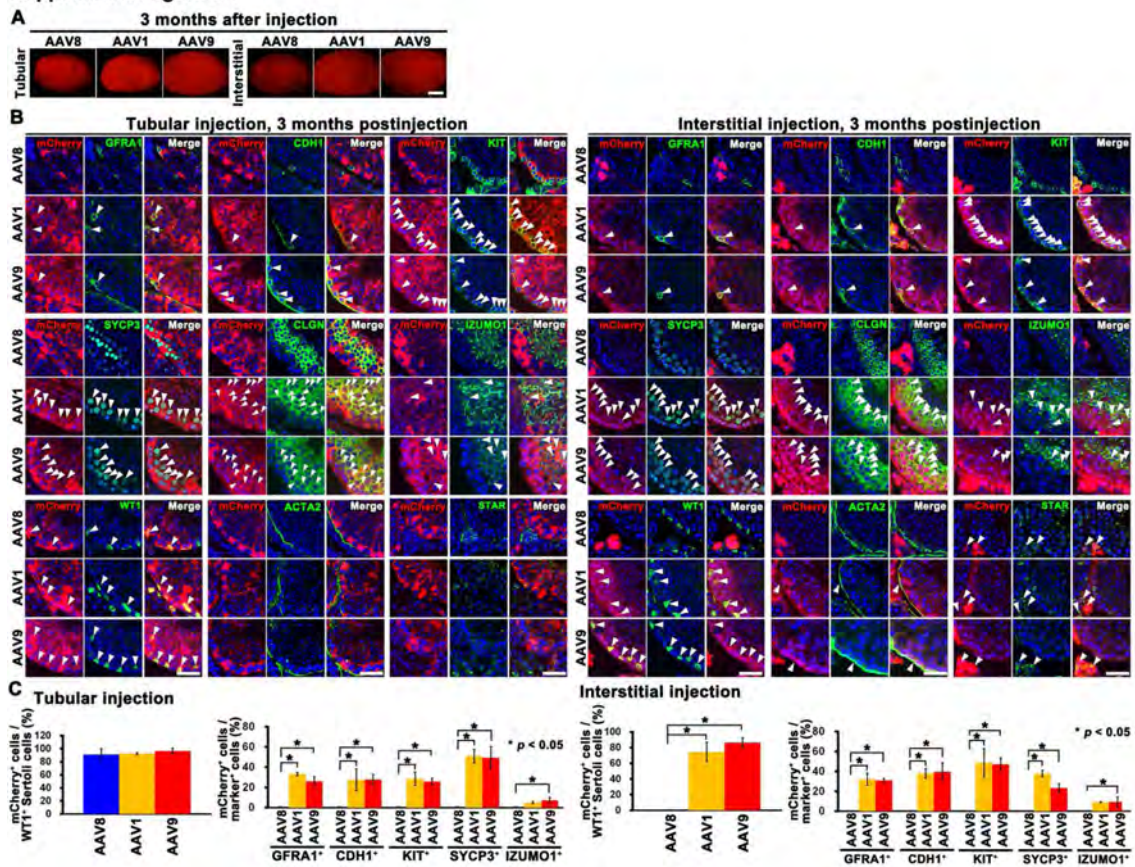
Interstitial injection, 1 week postinjection



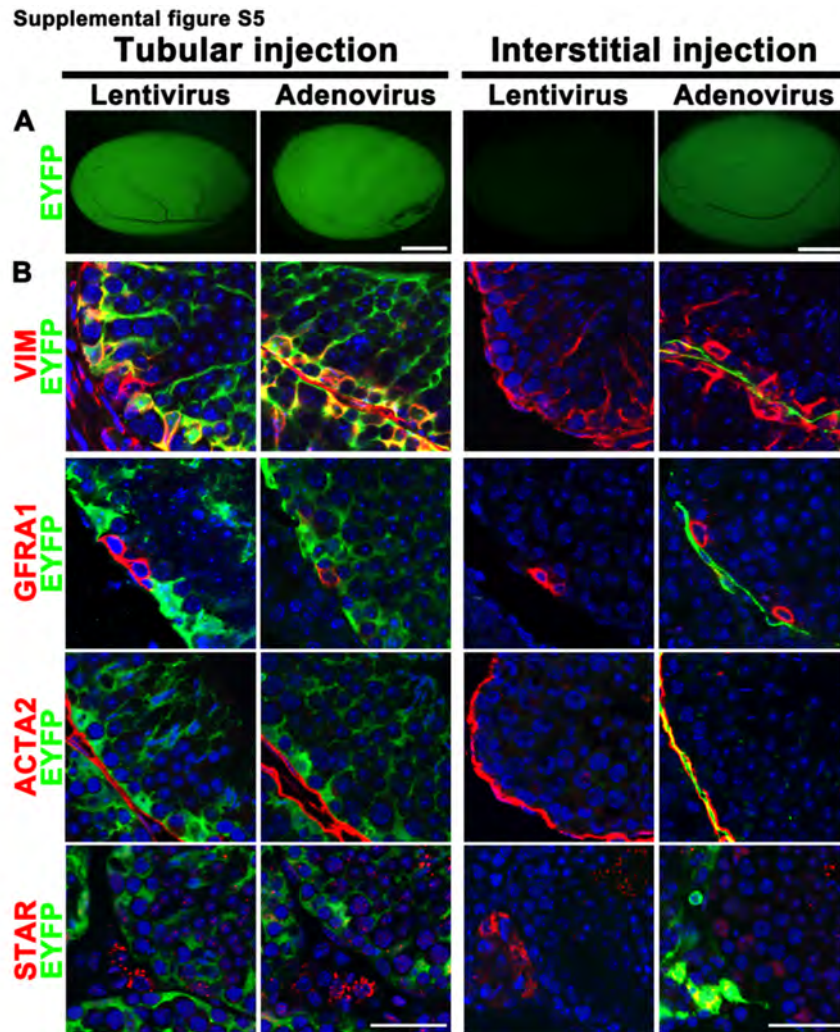
Supplementary Figure S3, related to Figure 1. Immunostaining of Sertoli cells in wild-type testes 1 week AAV2, 5, 6, 6.2, 7, 10, 11, hu11, Anc80L65, and DJ8 infection.

Bar, 40 μ m. Counterstain, Hoechst 33342.

Supplemental figure S4

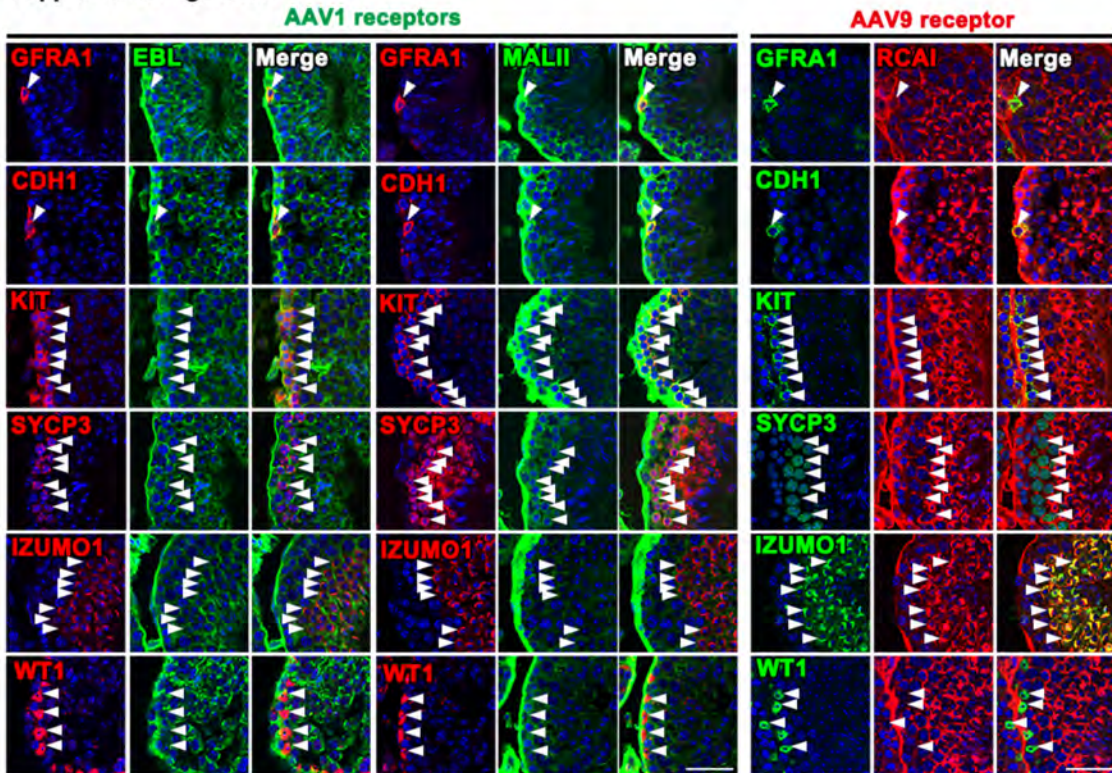


Supplementary Figure S4, related to Figure 1. Macroscopic appearance and immunostaining of wild-type testes 3 months after AAV8, 1, and 9 infection. (A) Macroscopic appearance ($1 \times 10^{12}/\text{ml}$). (B) Immunostaining. (C) Quantification of immunostaining. Three tubules from 3 different testes were counted for each type. Bar, 1 mm (A), 40 μm (B). Counterstain, Hoechst 33342 (B).



Supplementary Figure S5, related to Figure 1. Transduction of *R26R-Eyfp* testes by adeno- or lentiviral vectors. (A) Macroscopic appearance of *R26R-Eyfp* mouse testes 1 week after microinjection with AxCANCre and CSII-EF1-Cre. (B) Immunostaining. Bar, 1 mm (A), 40 μ m (B). Counterstain, Hoechst 33342 (B).

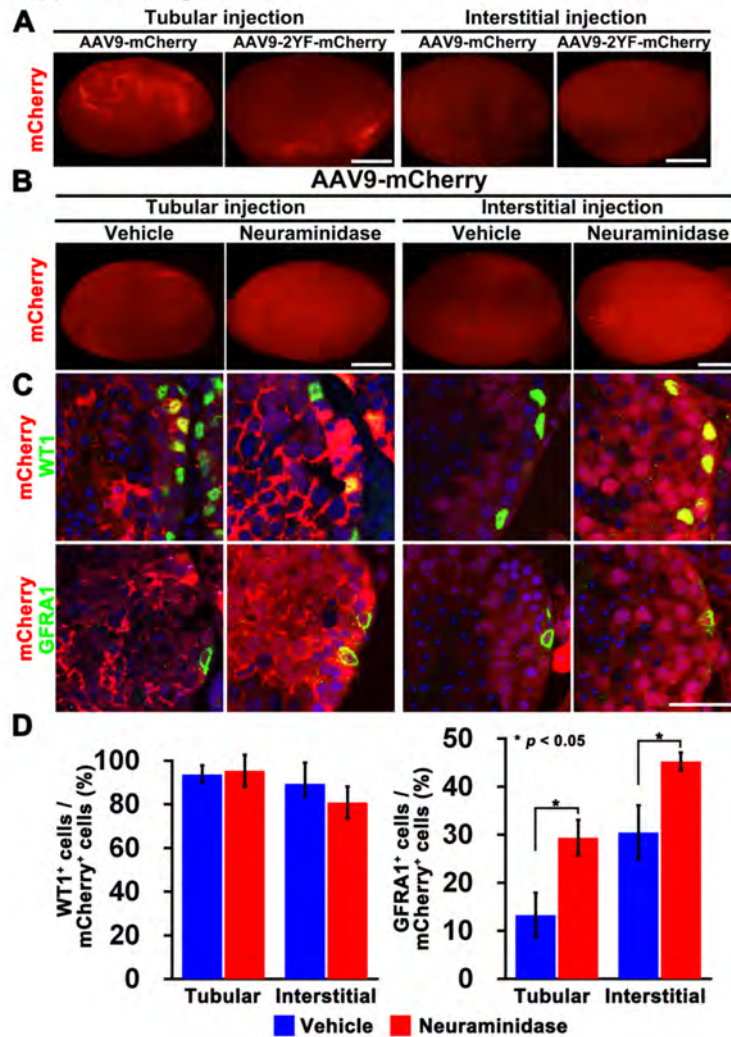
Supplemental figure S6



Supplementary Figure S6, related to Figure 1. Immunostaining and lectin staining.

Wild-type testes were stained with lectins that react with AAV1 (EBL, MALII) or AAV9 (RCA1) receptors along with immunostaining using germ cell makers. Bar, 40 μ m. Counterstain, Hoechst 33342.

Supplemental figure S7



Supplementary Figure S7, related to Figure 4. Improvement of AAV9 transduction by neuraminidase treatment. (A) Macroscopic appearance of wild-type testes 1 week after microinjection with AAV9-2YF-mCherry. (B) Macroscopic appearance of wild-type testes 7 days after microinjection with AAV9-mCherry and neuraminidase. (C) Immunostaining. (D) Quantification of immunostaining. Three tubules from 3 different testes were counted for tubular and interstitial injection. Bar, 1 mm (A, B), 40 μ m (C). Counterstain, Hoechst 33342 (C).

Supplementary Table S1, Antibodies and lectins

Name	Catalog number	Origin
Rabbit anti-VIM	5741S	Cell Signaling, Danvers, MA
Mouse anti-ACTA2	A2547	Sigma-Aldrich, St. Louis, MO
Rabbit anti-mouse WT1	sc-192	Santa Cruz, Dallas, TX
Goat anti-rat GFRA1	AF560	R & D systems, Minneapolis, MN
Rat anti-GFP	04404-26	Nacalai, Tokyo, Japan
Rabbit anti-GFP	MBL598	MBL, Nagoya, Japan
Rabbit anti-SYCP3	-	Gift from Dr. S. Chuma (Kyoto University)
Rat anti-KIT	14-1171	eBioscience, San Diego, CA
Rabbit anti-CLDN3	34-1700	Thermo Fisher Scientific, Waltham, MA
Mouse anti-CLDN5	35-2500	Thermo Fisher Scientific, Waltham, MA
Rabbit anti-CLDN11	Ab53041	Thermo Fisher Scientific, Waltham, MA
Rat anti-IZUMO1	KS64-125	Gift from Dr. M. Okabe (Osaka University)
Rat anti-CDH1 (ECCD2)	-	Gift from Dr. M. Takeichi (RIKEN)
Rabbit anti-CLGN	Ab171971	Abcam, Cambridge, UK
Rabbit anti-STAR	sc-25806	Santa Cruz, Dallas, TX
Rat anti-EPCAM (CD326)	118201	Biologend, San Diego, CA
Rat anti-CD4	12-0041	eBioscience, San Diego, CA
Rat anti-CD8	Ab22378	Abcam, Cambridge, UK
Alexa fluor 488 anti-rabbit IgG	A21206	Thermo Fisher Scientific, Waltham, MA
Alexa fluor 488 anti-mouse IgG	A21202	Thermo Fisher Scientific, Waltham, MA
Alexa fluor 488 anti-goat IgG	A11055	Thermo Fisher Scientific, Waltham, MA
Alexa fluor 488 anti-rat IgG	A21208	Thermo Fisher Scientific, Waltham, MA
Alexa fluor 555 anti-rabbit IgG	A31572	Thermo Fisher Scientific, Waltham, MA
Alexa fluor 555 anti-mouse IgG	A31570	Thermo Fisher Scientific, Waltham, MA
Alexa fluor 555 anti-goat IgG	A21432	Thermo Fisher Scientific, Waltham, MA, USA
Alexa fluor 568 anti-rat IgG	A11077	Thermo Fisher Scientific, Waltham, MA
Alexa fluor 647 anti-rat IgG	712-605-153	Jackson ImmunoResearch Laboratories, West Grove, PA
Rhodamine-labeled peanut agglutinin	RL-1072	Vector Laboratories, Burlingame, CA
Biotinylated elderberry bark lectin	B-1305	Vector Laboratories, Burlingame, CA
Biotinylated maackia amurensis lectin II	B-1265	Vector Laboratories, Burlingame, CA
Rhodamine ricinus communis agglutinin I	RL-1082	Vector Laboratories, Burlingame, CA

Supplementary Table S2, Primer sequences

Name	Forward	Reverse
<i>Fgf2</i>	CATAGCAAGGTACCGGTTGG	CTCTACTGCAAGAACGGCG
<i>Gdnf</i>	CTTCGAGAAGCCTCTTACCG	GCCACTTGGAGTTAATGTCC
<i>Kitl</i>	TGGTGGCATCTGACACTAGT	TGCCATGGCTGTCCATTGTA
<i>Hprt</i>	AAAGCGGTCTGAGGAGGAAC	CGTTTCTGAGCCATTGCTGA
<i>CAG-promoter</i>	CCTGGCATTATGCCCAGTACATG	GCTCACCTCGACCATGGTAATAG
<i>Cre</i>	GACGATGCAACGAGTGATGA	AGCATTGCTGTCACTTGGTC
<i>R26R-Eyfp</i>	GGAGTGTTGCAATACCTTTCTGGG	AGCTCCTCGCCCTTGCTCACCCAT
<i>AAV-5'end</i>	AGGGAGTGGCCAACTCCATCACTA	GGCGTTACTATGGGAACATACGTC
<i>AAV-Cre-3'end</i>	CAATACCGGAGATCATGCAAGCTG	AGGGAGTGGCCAACTCCATCACTA
<i>AAV-Kitl-3'end</i>	GCCGGCTCTCATTTCGCTTGTAAT	AGGGAGTGGCCAACTCCATCACTA