Supplemental information

Adeno-associated-virus-mediated gene delivery to ovaries restores fertility in congenital infertile mice

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Figure S1 Infection of ovaries by AAV transduction. Related to Figure 1. (A) Expression levels of MCHERRY in ovaries after virus infection (n = 3 ovaries). AAV9 was used to normalize the relative values. Statistics with t-test. (B) Immunostaining of granulosa (AMH) and theca (HSD3B) cells 1 week after virus injection. Arrows indicate cells expressing MCHERRY and AMH. (C, D) Quantification of MCHERRY+ area in AMH+ (C) and HSD3B+ (D) cells (n = 4 ovaries). Statistics with t-test. Scale bar, 50-µm (B). Counterstain, Hoechst 33342 (B). Asterisk indicates statistical significance (p < 0.05). Data are represented as mean ± SEM.
Figure S2 Infection of R26R-Eyfp ovaries by AAV9-Cre transduction. Related to Figure 1. (A) Immunostaining of granulosa (AMH) and theca (HSD3B) cells at indicated time points after virus injection. (B) Quantification of EYFP$^+$ area in AMH$^+$ and HSD3B$^+$ cells (n = 7 follicles). Statistics with t-test. Arrows indicates cells expressing EYFP. Scale bar, 50-µm (A). Counterstain, Hoechst 33342 (A). Data are represented as mean ± SEM.
Figure S3 Analysis of gene expression after virus injection. Related to Figures 2, 3, and 5. (A) Real-time PCR analysis of mCherry expression in WT ovaries 12 days after virus injection (n = 3 ovaries). Statistics with t-test. (B) Western blot analysis of MCHERRY expression in WT ovaries 12 days after virus injection (n = 3 ovaries). Statistics with t-test. (C) Real-time PCR analysis of Kitl expression in WT ovaries 12 days after virus injection (n = 3 ovaries). Statistics with t-test. (D) Western blot analysis of KITL expression in WT ovaries 12 days after virus injection (n = 3 ovaries). Statistics with t-test. (E) Real-time PCR analysis of Kitl expression in WT and KitlSl−/KitlSl− ovaries 12 days after virus infection (n = 3 ovaries). Statistics with t-test. Asterisk indicates statistical significance (p < 0.05). Data are represented as mean ± SEM.
Figure S4 Apoptosis and inflammation after AAV transduction. Related to Figure 3. (A) TUNEL staining of ovaries. Note the lack of oocyte apoptosis. (B, C) Immunostaining of ovary and spleen with anti-CD4 (B) or CD8 (C) antibodies. Bar = 50 µm. Counterstain, Hoechst 33342.
Supplemental Figure S5

Figure S5 PCR analysis of transgene integration using AAV-specific primers. Related to Figure 2. Controls represent viral DNA in amounts equivalent to the indicated copies of viral DNA per diploid genome.
Supplemental Figure S6

Figure S6 Comparison of Kitl sequences between WT and KitlSl-t/KitlSl-t mutant mice. Related to Figure 4.
DNA was extracted from tail sample.
Table S1. List of PCR primers. Related to STAR Methods.

| Integration of AAV9 | Forward | Reverse |
|--------------------|---------|---------|
| TCGTCCTTTCCCTTGGCTGCT | AGGGAGTGGGCAACTCCATCACTA |

| RT-PCR | Forward | Reverse |
|--------|---------|---------|
| Kitl   | TAAACCAAGGAGATTCGCTG | CACCTCTTTGAATTTTCTCTC |
| Hprt   | GCTGGTGAAAAGGACCTCT | CACAGGAAGGAAACACCTGC |

| qPCR | Forward | Reverse |
|------|---------|---------|
| Kitl | GATCTGCGGGAATTCGTA | ACTAGGCAACATCCATCC |
| mCherry | CCCTATGCAGAAGAGACATG | TCAGCCTCCAGGCTCTGGATCT |

| COBRA | Forward | Reverse |
|-------|---------|---------|
| H19   | GGAATATTTTGTGTTTGGAGGG | TTAAACCCCAAACCTCTACTTTTATAAC |
| Igf2r | TTAGTGGGGGTATTTTATTTGTATG | AAATATCCTAAAAATACAAACTACA |

| Mutation screening | Forward | Reverse |
|--------------------|---------|---------|
| 2nd exon | TGCATTTCACCACATATGG | GTCAGCAGACAGGGA |
| 7th exon | ATGGAATCCTTTGGGAGC | GTAAGAATGATTGTTTCTTC |

Bisulphite genomic DNA methylation analysis by Ion PGM sequencing

| H19 bi Forward | GATTTATAAGGGTTATGAGGGTGG |
| H19 bi Reverse | TTAAACCCCAAACCTCTACTTTTATAAC |
| Igf2r bi Forward | TTAGTGGGGGTATTTTATTTGTATG |
| Igf2r bi Reverse | AAATATCCTAAAAATACAAACTACA |
| H19 bi F Tag5 | CCATCTCATCCTCCGTTGCTTCGACTCAGAAGGCAACGATT |
| H19 bi F Tag6 | CCATCTCATCCTCCGTTGCTTCGACTCAGAAGGCAACGATT |
| trP1 H19 bi R | CCTCTCTATGGCAGTCGGGTATCAAACCCTACTTTTATAACTAT |
| Igf2r bi F Tag 1 | CCATCTCATCCTCCGTTGCTTCGACTCAGAAGGCAACGATT |
| Igf2r bi F Tag 2 | CCATCTCATCCTCCGTTGCTTCGACTCAGAAGGCAACGATT |
| trP1 Igf2r bi R | CCTCTCTATGGCAGTCGGGTATCAAACCCTACTTTTATAACTAT |