Supporting Information

Wang et al. 10.1073/pnas.0902253106

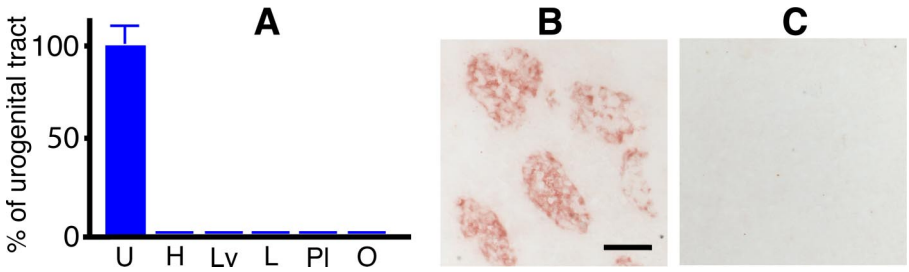

Fig. S1. MIS production in male embryos. (*A*) The levels of MIS mRNA in various parts of E12.5 male embryos. The bars are mean ± standard error of the mean of 5 embryos. The values are expressed as percentage of those in the urogenital tract (U), after normalization to the level of GAPDH mRNA. The embryos were divided into the head (H), liver (Lv), limbs (L), placenta (Pl), and the remainder [other (O)]. (*B* and *C*) Transverse section through the testes (*B*) and brain (*C*) stained with anti-MIS. (Scale bar, 50 μm.)

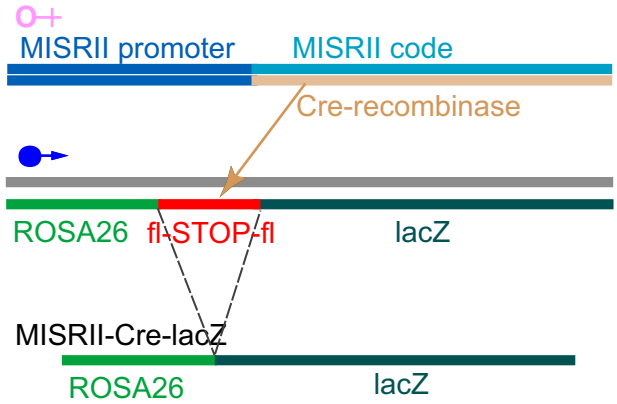

Fig. S2. Genetics of the MISRII-Cre-lacZ (*Misrii*$^{+/-}$, ROSA-lacZ^{+ve}) mice. Dams in which the coding region of *Misrii* allele had been replaced by Cre-recombinase [Smith DE, Johanson CE, Keep RF (2004) Peptide and peptide analog transport systems at the blood-CSF barrier. *Adv Drug Deliv Rev* 56:1765–1791] were mated with ROSA26 Cre reporter studs [Jamin SP, Arango NA, Mishina Y, Hanks MC, Behringer RR (2002) Requirement of Bmpr1a for Mullerian duct regression during male sexual development. *Nat Genet* 32:408–410]. Cells that produce MISRII in the resulting MISRII-Cre-lacZ offspring also produce Cre-recombinase, which deletes the floxed stop sequence in the reporter gene. These cells will therefore produce lacZ under the control of the ROSA26 promoter.

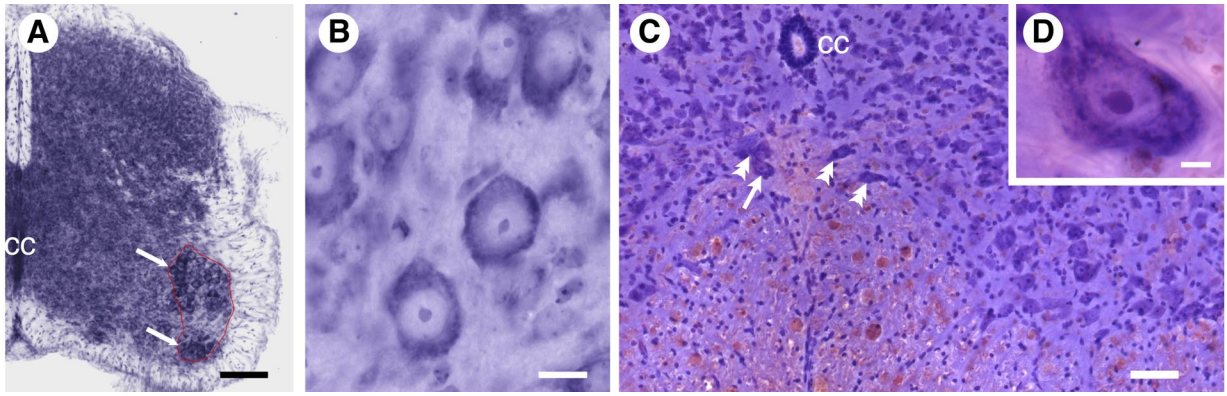

Fig. S3. Spinal motor neurons. The location of the spinal motor neurons is illustrated in transverse sections of the lumbar spinal cord of neonatal (*A* and *B*) and adult (*C* and *D*) mice. The lateral motor column, which innervates the limb musculature, is indicated by arrows and delineated by a red-dotted line in *A*, with the motor neurons illustrated in higher magnification in *B*. The limb innervating motor neurons are at the ventral-lateral extreme of the gray matter. The testosterone-sensitive spinal bulbocavernosus motor neurons are indicated by arrowheads in *C*, with the motor neuron indicated by the arrow shown at higher magnification in *D*. The bulbocavernosus motor neurons innervate the muscles associated with penile function (bulbocavernosus and levator ani), as well as contributing to the innervation of the anal sphincter. (Scale bars: *A*, 100 μm; *B*, 50 μm; *C*, 200 μm; *D*, 20 μm.)

Table S1. Oligonucleotide primer sequences

Primer	Sequence	Product size
MISRII-F	5′-CTTCCCACATAGCTCCCTTGTCT-3′	230-bp wild-type allele
MISRII-R	5′-GAACCTCCAGGAGTGCCACAG-3′	
Cre-F	5′-GTTGATGCCGGTGAACGTGCAAA-3′	322-bp MISRII-Cre allele
Cre-R	5′-ATCAGCTACACCAGAGACGGAAA-3′	
ROSA-F	5′-GTTGCAGTGCACGGCAGATACACTTGCTGA-3′	389-bp LacZ allele
ROSA-R	5′-GCCACTGGTGTGGGCCATAATTCAATTCGC-3′	
MIS-F	5′-ggaacacaagcagagcttcc-3′	243-bp wild-type allele
MIS-R	5′-gagacagagtccatcacgtacc-3′	
Neo-F	5′-TGCTCCTGCCGAGAAAGTATCCATCATGGC-3′	380-bp MIS null allele
Neo-R	5′-CGCCAAGCTCTTCAGCAATATCACGGGTAG-3′	

The forward and reverse primers used for RT-PCR detection of the listed genes are indicated by ″-F″ and ″-R,″ respectively.