Figure S1

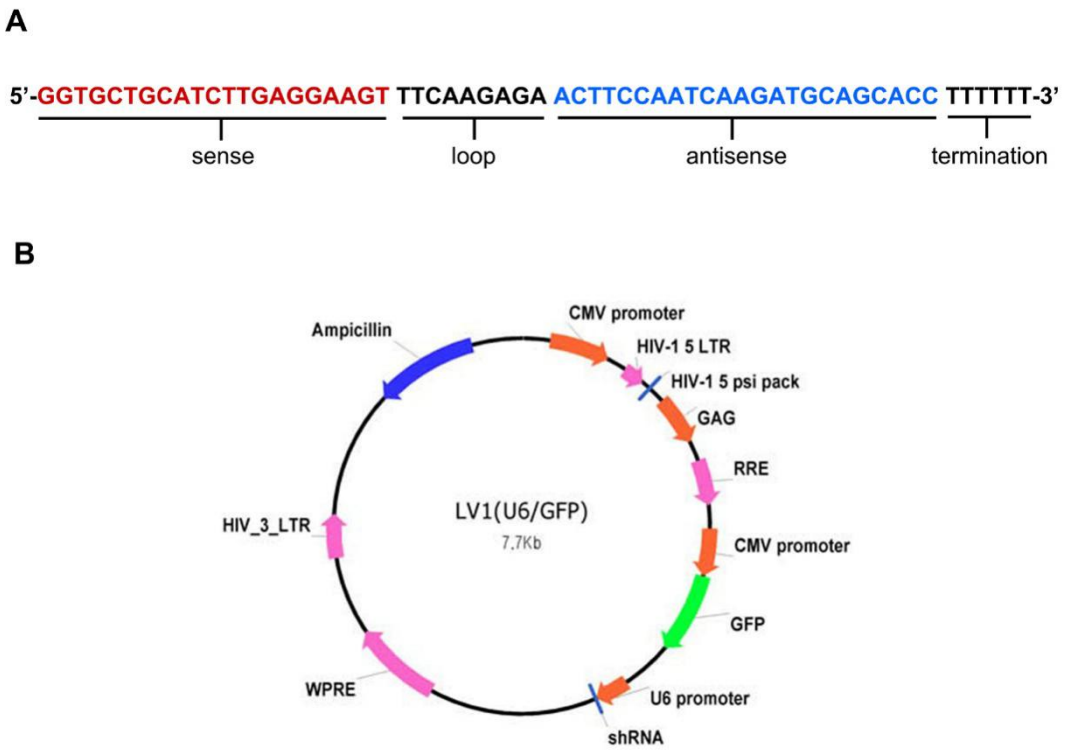

Figure S1. The shRNA construct targeting *Amh* and vector map. (A) The designed shRNA construct contained a unique double-stranded *Amh* sequence that presented as an inverted complementary repeat (5'-GGTGCTGCATCTTGAGGAAGT-3'), a loop sequence (5'-TTCAAGAGA-3') and the RNA Plo-II terminator (5'-TTTTTT-3') . (B) The pU6/GFP plasmid used for cloning GFP and shRNA against turtle *Amh*.

Figure S2

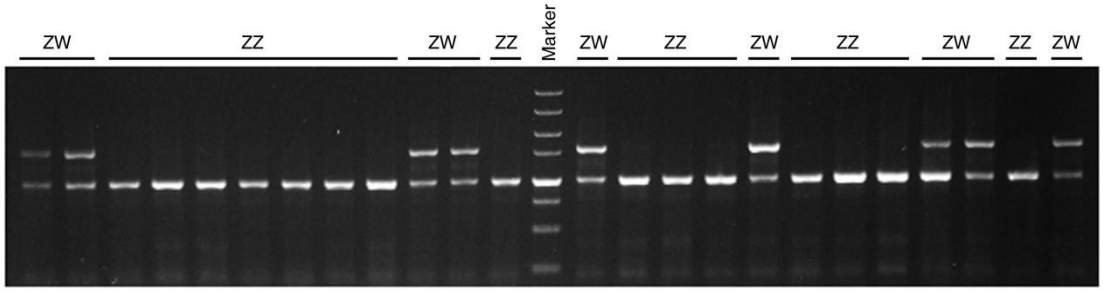

Figure S2. Sex-diagnostic amplification in *Pelodiscus sinensis.* Lower bands represent Z-linked amplified fragments, and higher bands represent W-linked sex-diagnostic fragments. One- and two-band indicated genetic male (ZZ) and female (ZW), respectively.

Figure S3

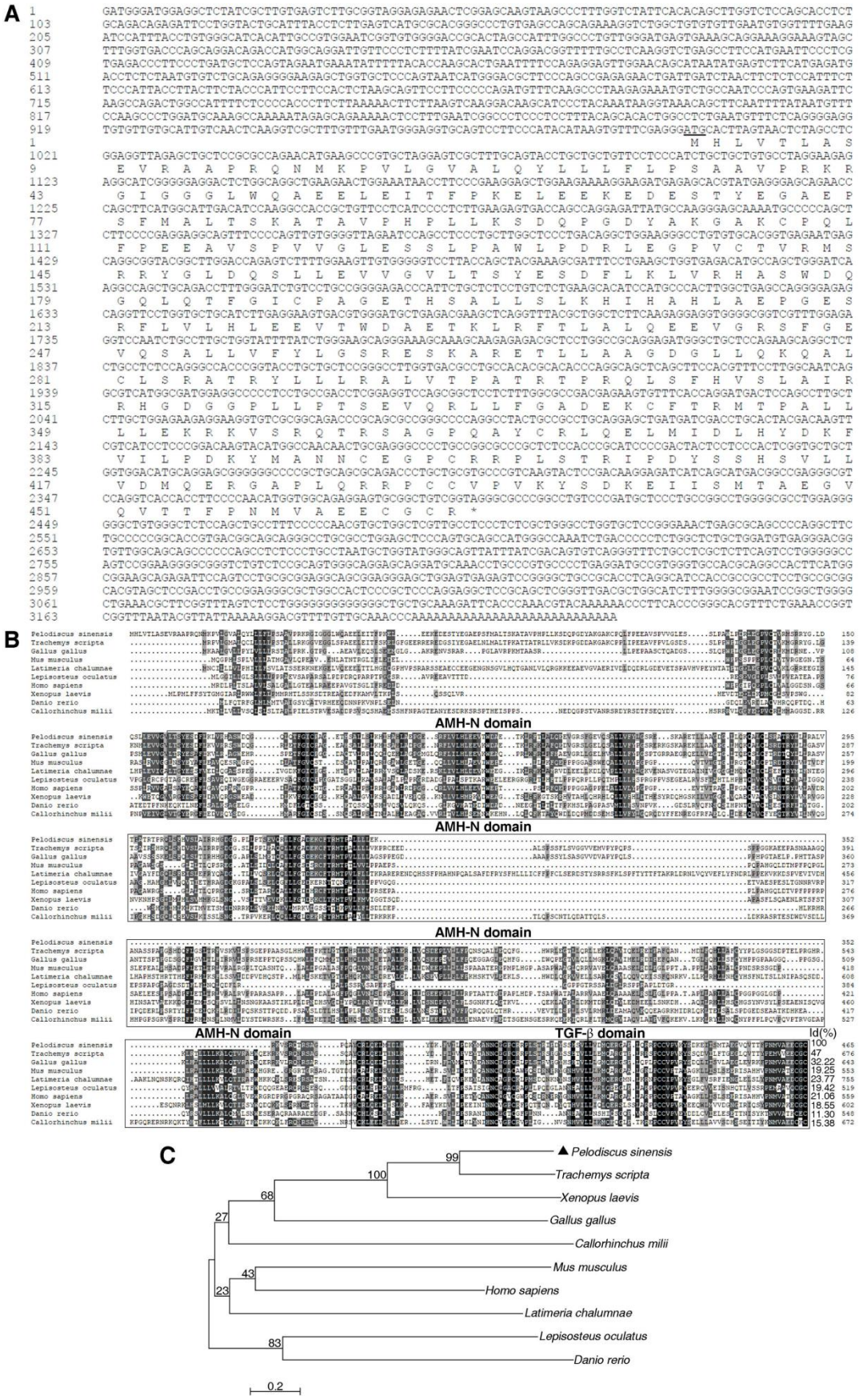
A
B
AMH-N domain
AMH-N domain
AMH-N domain
AMH-N domain
TGF-β domain
Id(%)
C
Pelodiscus sinensis
Trachemys scripta
Xenopus laevis
Gallus gallus
Callorhinchus milii
Mus musculus
Homo sapiens
Latimeria chalumnae
Lepisosteus oculatus
Danio rerio
99
100
68
27
43
23
83
0.2

Figure S3. Sequence and phylogenetic analyses of *P. sinensis Amh*. (A) The complete cDNA sequence of *P. sinensis Amh* and deduced amino acid sequence. The start codon ATG was underlined, and the stop codon was indicated by an asterisk. (B) Alignment of amino acid sequence of *P. sinensis* AMH with those from other typical species. The two characteristic functional domains of the TGF-β superfamily, AMH-N and TGF-β domain, were marked. (C) AMH phylogenetic tree from *P. sinensis* and other typical species based on Neighbor-Joining (N-J) method. Numbers at branches were confidence values based on 1000 bootstraps. Each branch length scale in terms of genetic distance was indicated above the tree.

Figure S4

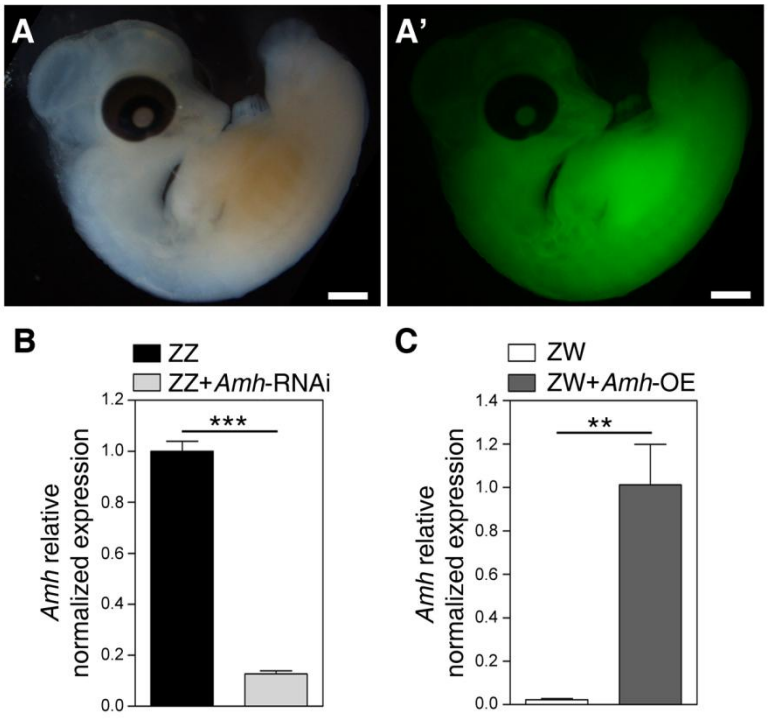

Figure S4. Establishment of *Amh*-Knockdown and -overexpressing turtle model using lentivirus vectors. (A, A') The whole embryos of stage 15 infected with scrambled lentiviral vector (LV-NC) at stage 14 showed widespread GFP expression. Bright (A) and epifluorescence (A') images. Scale bars are 1 mm. (B, C) qRT-PCR of *Amh* showed >5-fold downregulation in ZZ gonads with LV-*Amh*-shRNA treatment (B) and >50-fold upregulation in ZW gonads with LV-*Amh*-OE treatment (C), respectively. Data are shown as means ± S.D. N≥3. **, $P < 0.01$; ***, $P < 0.001$.