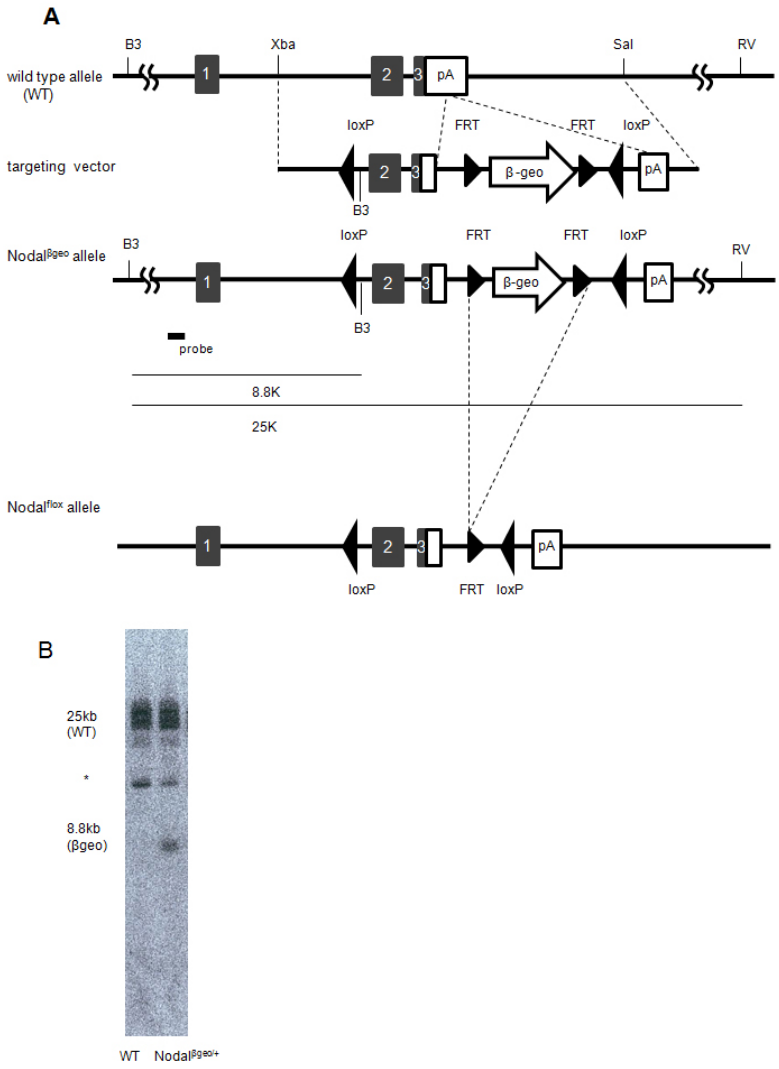

Fig. S1. Generation of floxed alleles for *Nodal*. (**A**) Targeting strategy. The genomic organization of the *Nodal* gene is shown at the top of the panel. Homologous recombination would insert a *loxP* site into the intron 1 and a [*FRT*-β*geo*-*FRT*-*loxP*] cassette into the 3′-untranslated region, generating the *Nodal*$^{\beta geo}$ allele. B3, *Ban*III; RV, *Eco*RV; Sal, *Sal*I; Xba, *Xba*I. Southern blot analysis of *Eco*RV- and *Ban*III-digested DNA with the 5′-external probe (probe) detects an 8.8-kb fragment and a 25-kb fragment in the wild-type *Nodal* allele and the *Nodal*$^{\beta geo}$ allele, respectively. (**B**) Southern blot analysis of ES clones. Genomic DNA was digested with *Eco*RV and *Ban*III and subjected to hybridization with the 5′ external probe indicated in A. An 8.8-kb fragment was detected in correctly targeted ES clones. The 15-kb fragment shown by the asterisk was not derived from the *Nodal* gene.

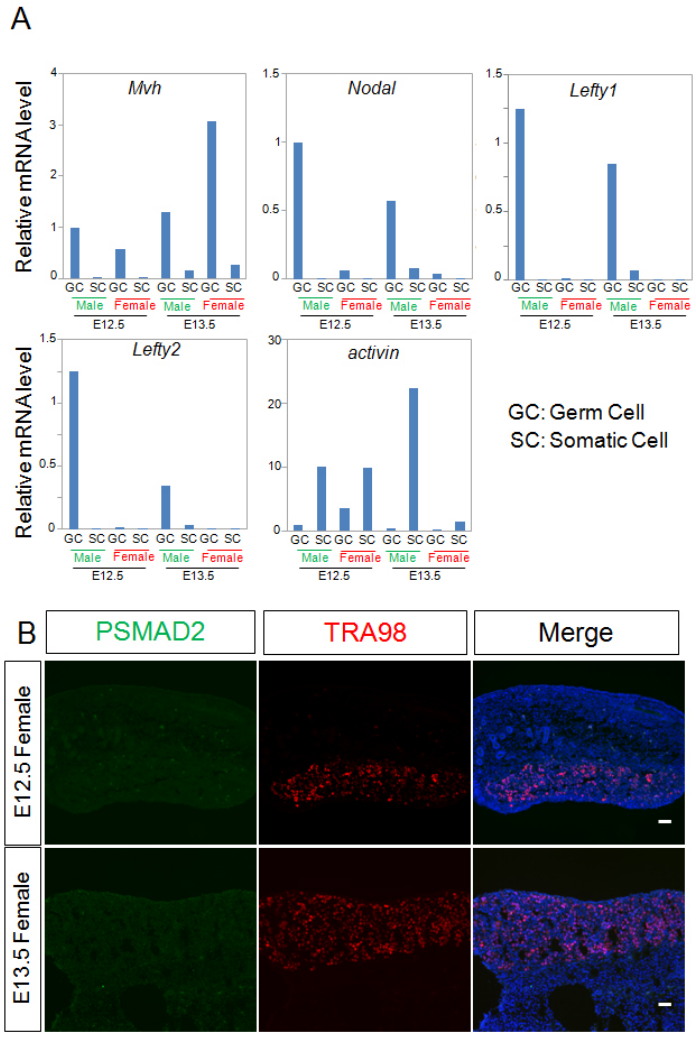

Fig. S2. Nodal/activin signaling is activated in male germ cells but not in fetal ovaries. (**A**) Germ cells and somatic cells were separated immunomagnetically from E12.5-13.5 gonads and RT-qPCR was performed to analyze the expression levels of *Mvh*, *Nodal*, *Lefty1/2* and *Inhba*. (**B**) Immunostaining for pSMAD2 (green) in ovaries at E12.5. TRA98 (red) was used as a marker of germ cells. DAPI (blue) was used to detect nuclei. pSMAD2 was not observed in either germ cells or somatic cells. Scale bar: 50 μm.

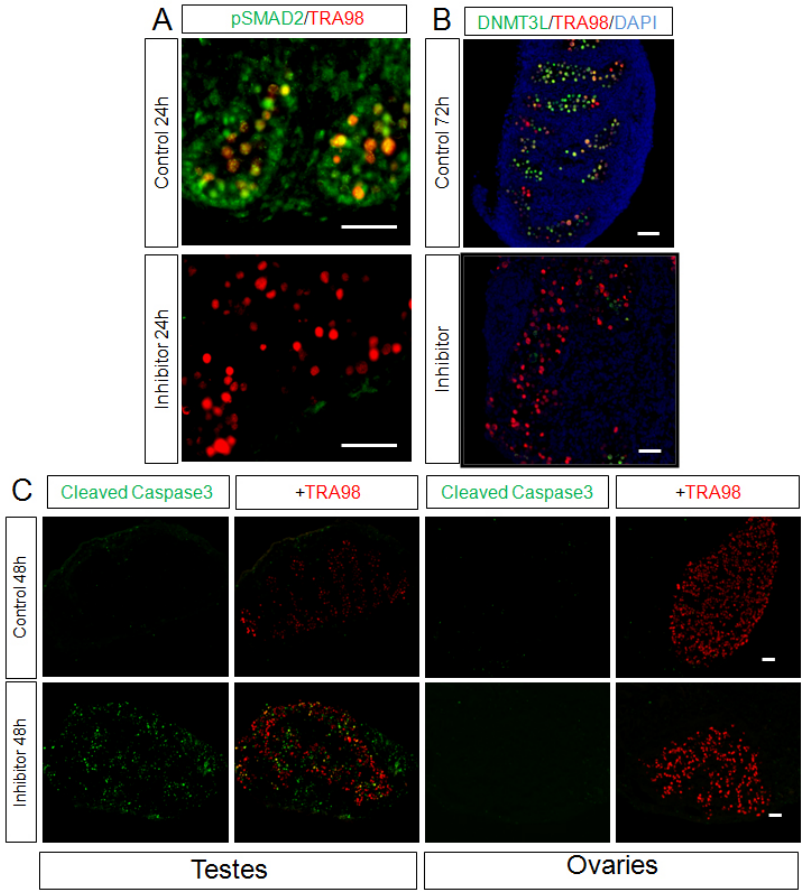

Fig. S3. Treatment with SB431542 suppresses the expression levels of pSMAD2 and DNMT3L and induces apoptotic cell death in the fetal testis. (**A**,**B**) Testes from mice at E12.5 were cultured with the TGFβ receptor inhibitor SB431542 (40 μM) or DMSO (control vehicle) for 24 (A) or 72 (B) hours. Results of immunostaining for pSMAD2 (green in A) and DNMT3L (green in B) together with TRA98 (red), indicate that SB431542 suppressed the expressions of both pSMAD2 and DNMT3L. (**C**) Testes or ovaries from mice at E12.5 were dissected and cultured with SB431542 (40 μM) or DMSO for 24 hours. Gonads were then immunostained with anti-cleaved caspase 3 (green) and TRA98 (red) antibodies. Many male germ cells and somatic cells entered apoptosis (C, left panel). However, no apoptotic cells were observed in inhibitor-treated ovaries (C, right panel). Scale bars: 50 μm.

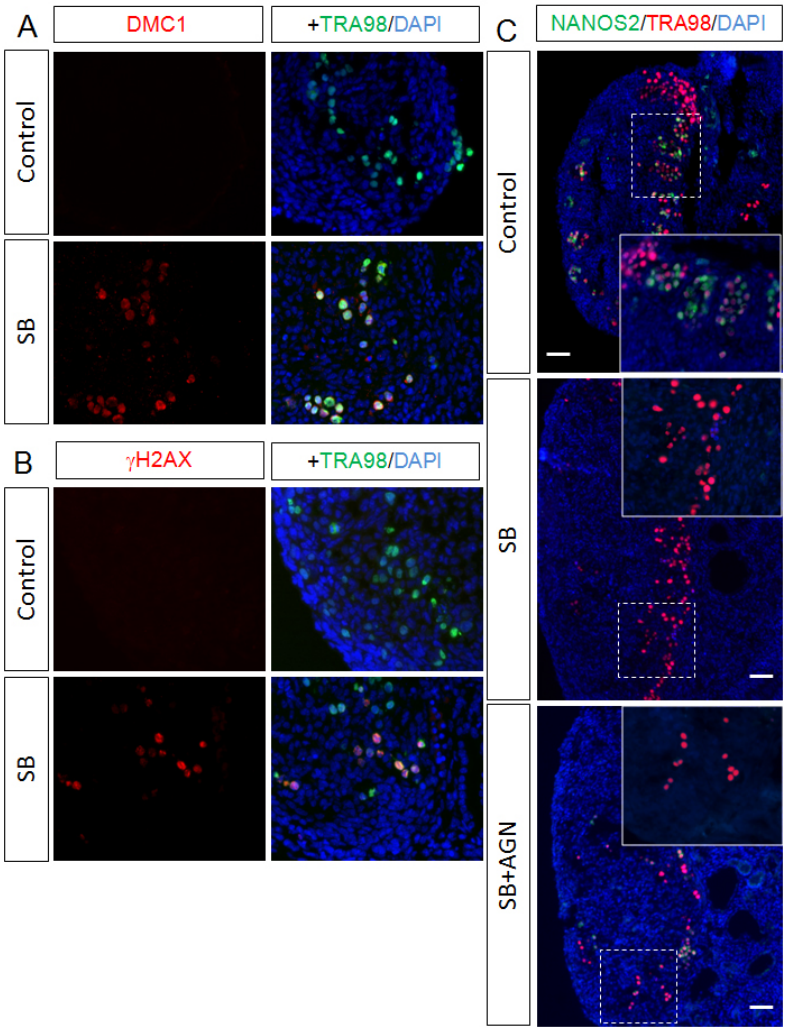

Fig. S4. SB431542 promotes meiosis and inhibits NANOS2 expression independently of RA signaling. (**A**,**B**) Testes from mice at E11.5 were cultured with the TGFβ receptor inhibitor SB431542 (40 μM) or DMSO (control vehicle) for 72 hours. Immunostaining with DMC1 (A) and γH2AX (B) indicated that treatment of testes with SB431542 promoted meiosis. (**C**) Immunostaining with NANOS2 protein in testes treated with SB431542 alone or together with RA receptor antagonist AGN 193109. Testes from mice at E12.5 were cultured with DMSO (control vehicle; upper panel), the TGFβ receptor inhibitor SB431542 (40 μM; middle panel) or the RA receptor antagonist AGN 193109 (5 μM; lower panel) for 48 hours. After treatment, the expression of NANOS2 was investigated by immunostaining (*n*=2). Scale bars: 50 μm.

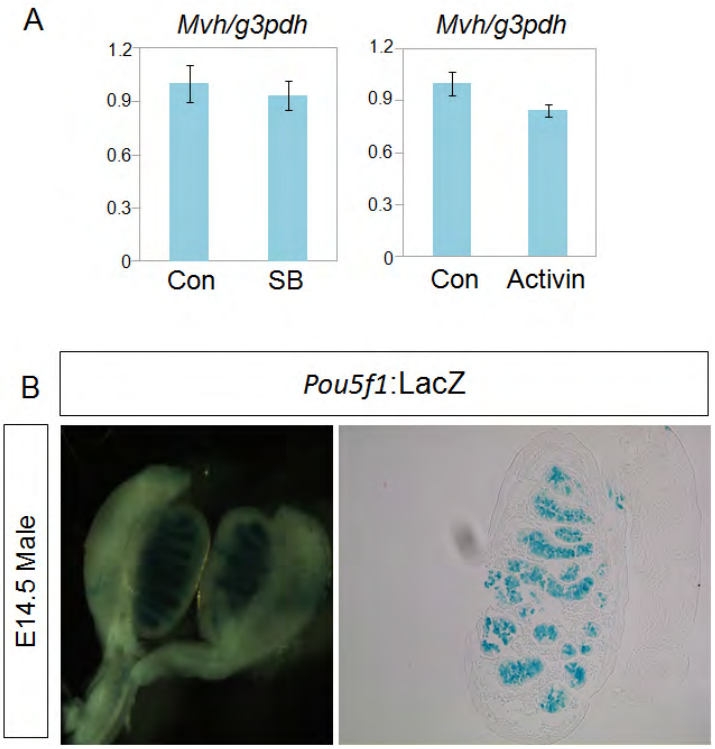

Fig. S5. Nodal/activin signaling directly acts on male germ cells. (**A**) Male germ cells were isolated from E12.5 testes and cultured with SB431542 or activin-A for 24 hours; RT-qPCR was used to assay the expression levels of *Mvh*. (**B**) X-gal staining of *Pou5f1-CreERT2*$^{lacZ/+}$ testes at E14.5. Tamoxifen was injected at E10.5 and E11.5 (*n*=3; only one sample is shown). Bars in graphs represent mean ± s.e.m.

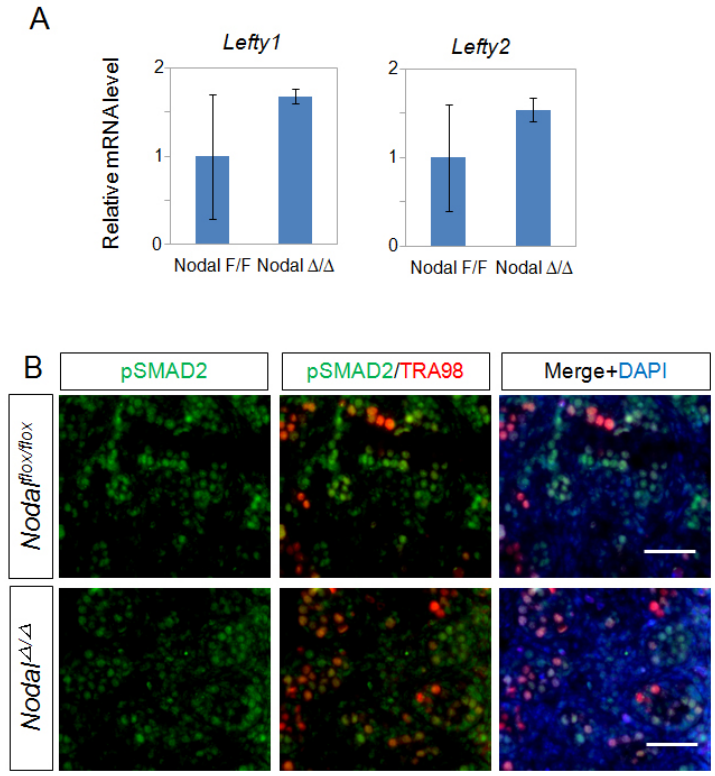

Fig. S6. Expression of Lefty1/2 and pSMAD2 persists in the testes of *Nodal*$^{\Delta/\Delta}$ mice. (**A**) Expression of *Lefty1/2* in E14.5 testes from *Nodal*$^{flox/flox}$ and *Nodal*$^{\Delta/\Delta}$ mice was analyzed using RT-qPCR. (**B**) Immunostaining with anti-TRA98 (red) and anti-pSMAD2 (green) antibodies in E13.5 testes from *Nodal*$^{flox/flox}$ and *Nodal*$^{\Delta/\Delta}$ mice. Bars in graphs represent mean ± s.e.m. Scale bars: 50 μm.

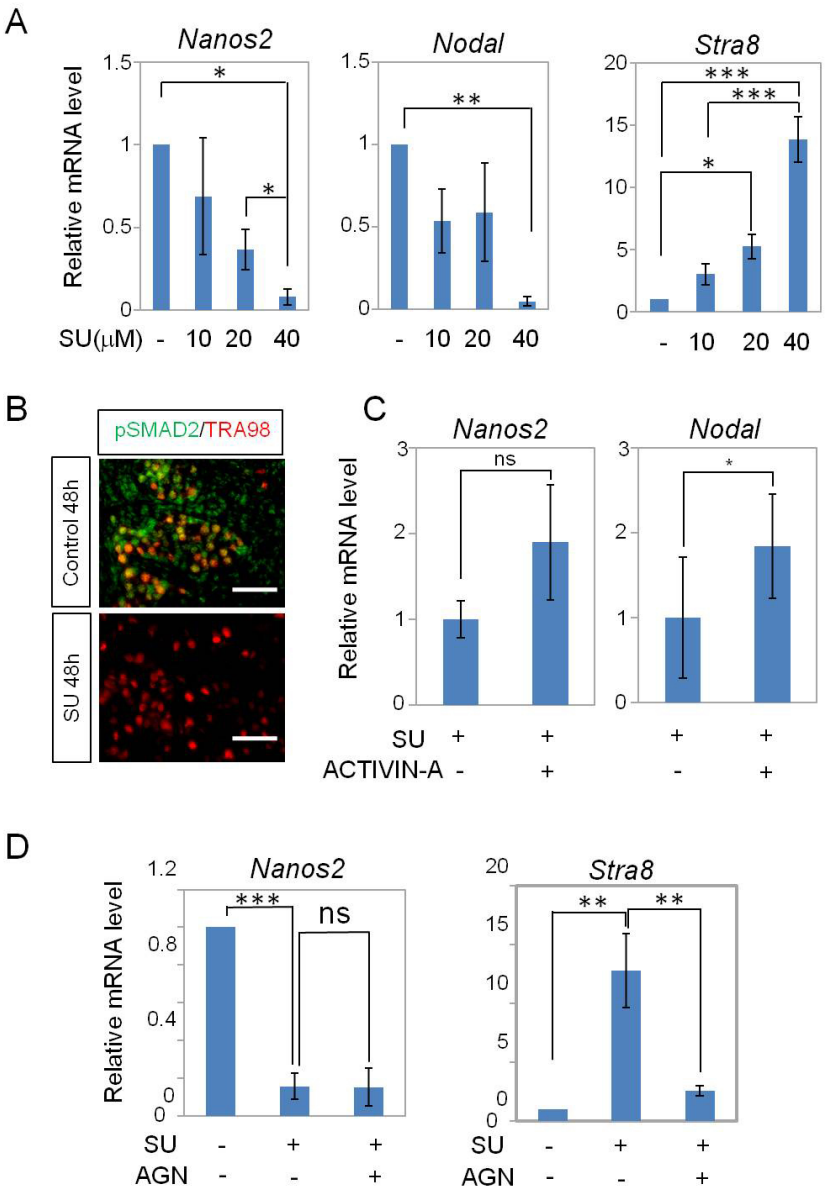

Fig. S7. Nodal signaling acts downstream of FGF signaling. (**A**) E11.5 testes were cultured with the FGF receptor inhibitor SU5402 (10, 20 or 40 μM) or DMSO (control vehicle) for 48 hours and the expression levels of *Lefty1/2*, *Nodal* and *Nanos2* were examined using real-time RT-qPCR (n=3, using *Mvh* as a normalization control). (**B**) E11.5 testes were treated with DMSO or SU5402 (40 μM) for 24 hours and the localization of pSMAD2 was examined using immunostaining. (**C**) E11.5 testes were cultured for 48 hours with SU5402 alone (40 μM) or together with activin-A (100 ng/ml), as indicated. Expression levels of *Nodal* and *Nanos2* were assayed using RT-qPCR (n=3). (**D**) Testes at E11.5 were cultured with DMSO or SU5402 (40 μM; middle panel) or SU5402 together with the RA receptor antagonist AGN 193109 (5 μM) for 48 hours. The mRNA levels of *Nanos2* and *Stra8* were then examined using RT-qPCR. Bars in the graphs represent the mean±s.e.m. *P<0.05, **P<0.01, ***P<0.001. ns, not significant. Scale bar: 50 μm.

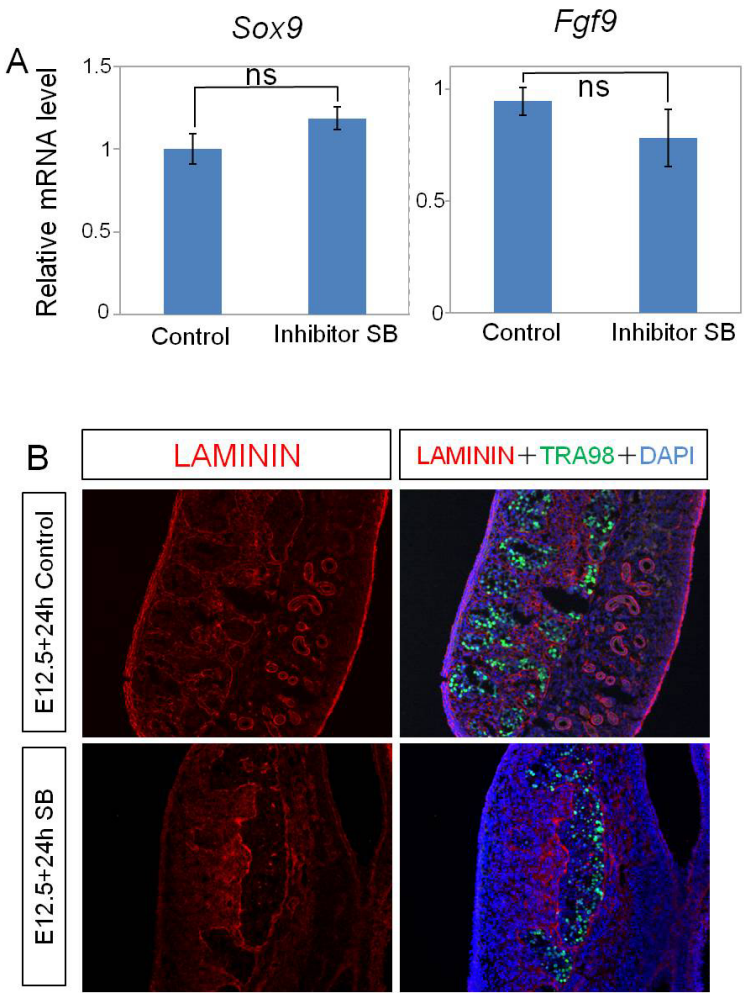

Fig. S8. SB431542 treatment disrupts testicular cords but does not affect *Ffg9* and *Sox9* expression. (**A**,**B**) E12.5 testes were cultured with the TGFβ receptor inhibitor SB431542 (40 μM) or DMSO (control vehicle) for 48 (A) or 24 (B) hours. (A) The expression levels of *Sox9* and *Fgf9* were investigated using RT-qPCR (*n*=3). Bars on the graphs represent the mean±s.e.m. (B) Immunostaining with anti-laminin in the testes with or without SB431542 treatment. ns, not significant.