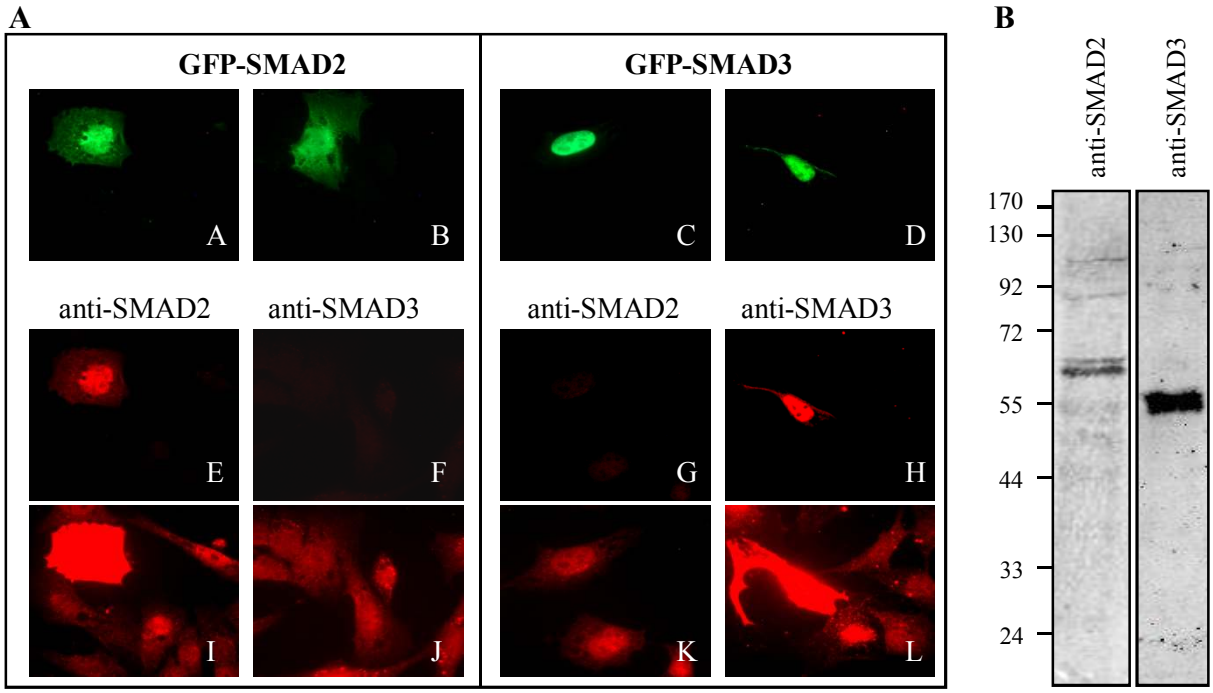
Itman_Figure S1



S1A: Validation of anti-Smad2 and anti-Smad3 antibodies by immunofluorescence.
Immunofluorescence with anti-SMAD2 and anti-SMAD3 antibodies using the TM-4 Sertoli cell line expressing GFP-SMAD2 and GFP-SMAD3 fusion proteins. The detection of GFP-tagged SMAD2 by the anti-SMAD2 antibody (A, E), and GFP-tagged SMAD3 by the anti-SMAD3 antibody (D, H) confirms these antibodies will detect their respective SMAD proteins. Absence of SMAD2 detection with the anti-SMAD3 antibody (B, F), and lack of SMAD3 detection with the anti-SMAD2 antibody (C, G) confirms there is no reactivity of antibodies with the other, closely related, SMAD protein. Lower panels I, J, K, L are higher exposure images of E, F, G, H (respectively) showing detection of overexpressed GFP-SMAD proteins in addition to endogenous Smad expression.

S1B: Validation of anti-SMAD2 and anti-SMAD3 antibodies by Western blot analysis.
Western blot on lysate prepared from an enriched preparation of Sertoli cells isolated from 6 dpp mouse testes. The anti-SMAD2 antibody detects a 58-60 kDa band, the expected size for SMAD2. The anti-SMAD3 antibody detects a 55 kDa band, the expected size for SMAD3.

TM-4 cells (a Sertoli cell derived line Mather *et al* 1990 *Endocrinology* 127:3206-3214) were grown on glass coverslips without laminin, in DMEM-F12 supplemented with penicillin/streptomycin (Life Technologies, Inc), D-glucose (Life Technologies, Inc) and 7.5% fetal calf serum (Invitrogen). TM-4 cells were fixed in the same manner as described for primary cells. Transfection was carried out using 1µg of DNA per well of a 24 well plate using Lipofectamine 2000 (Invitrogen) at a ratio of 1:2, according to manufacturer's specifications. GFP-tagged human SMAD2 (99% amino acid identity with murine SMAD2) and GFP-tagged human SMAD3 (100% amino acid identity with murine SMAD3) were a kind gift from Dr. Caroline Hill, UK. Immunofluorescence on transfected TM-4 cells was carried out in an identical manner to that described below for primary cells, except streptavidin-Texas Red (Chemicon) was used to detect bound primary antibody, and to co-localize with GFP.

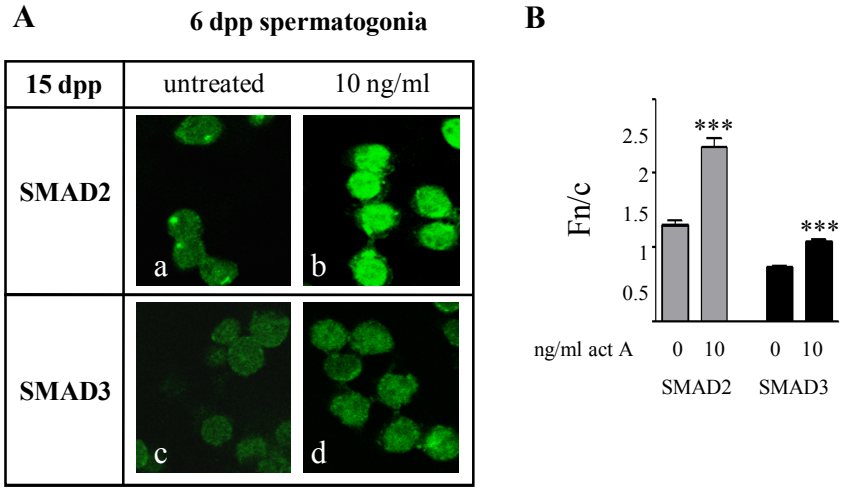
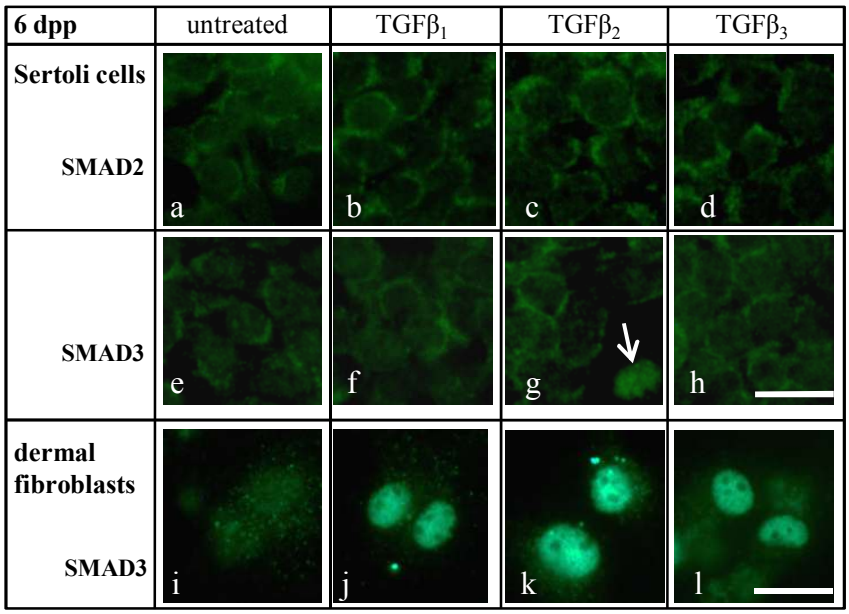


Figure S2A: SMAD2 and SMAD3 accumulate in the nuclei of spermatogonia following exposure to activin A.
A: Detection of SMAD2 (a,b) and SMAD3 (c,d) proteins by immunofluorescence in spermatogonia isolated from 6 dpp mouse testes. Cells were cultured for 24 hours in serum-free media then either left untreated or stimulated with activin A at 10 ng/ml for 45 minutes. In contrast to that observed in immature Sertoli cells, in spermatogonia, 10 ng/ml activin A induces nuclear accumulation of both SMAD2 and SMAD3 (representative images from three independent experiments).
B: Quantitation of SMAD2 and SMAD3 nuclear accumulation in spermatogonia following activin exposure.
Changes in subcellular localization of SMAD2 and SMAD3 in spermatogonia following activin A treatment (from Figure S2A) was measured by analysis of confocal images, with an increase in the ratio of nuclear to cytoplasmic fluorescence (Fn/c) indicating accumulation of protein in the nucleus. These data confirm that significant accumulation of SMAD2 and SMAD3 is induced following exposure to 10 ng/ml activin. Statistical significance was determined using one-way ANOVA and Tukey's *post hoc* test ($P<0.05$). Different letters signify significant differences between samples and error bars indicate SEM. ***$P<0.001$.



S3: 6 dpp Sertoli cells do not appear responsive to TGFβ$_1$, TGFβ$_1$ or TGFβ$_3$ *in vitro*.
Detection of SMAD2 and SMAD3 by immunofluorescence in 6 dpp mouse Sertoli cells (Fig S3 a-h) following TGFβ stimulation. Cells were cultured as described in Materials and Methods and either left untreated or stimulated with TGFβ$_1$, TGFβ$_1$ or TGFβ$_3$ at 1 ng/ml (above) or 5 ng/ml (data not shown) for 45 minutes and then fixed. SMAD2 and SMAD3 localization was then determined by immunofluorescence as described in Materials and Methods. In contrast to that observed following activin A treatment, no nuclear accumulation of SMAD2 or SMAD3 was observed in cells treated with TGFβ$_1$ (b,f) or TGFβ$_3$ (d,h) whereas TGFβ$_2$ induced nuclear accumulation of SMAD3 in less than 0.5% of cells (white arrow; c,g). Bioactivity of each of the three TGFβs was confirmed by treatment of dermal fibroblasts (prepared as per Verbruggen and Salomon 1980, *Archives of Dermatological Research* 269:111-126) followed by immunofluorescence to detect SMAD3 localization (i-l). Nuclear accumulation of SMAD3 was observed following treatment of dermal fibroblasts with 5 ng/ml of TGFβ$_1$, TGFβ$_2$ and TGFβ$_3$. We are grateful to Dr. Sarah Robertson for the gift of TGFβ$_{1, 2, 3}$ proteins. Scale bar = 25 μm.