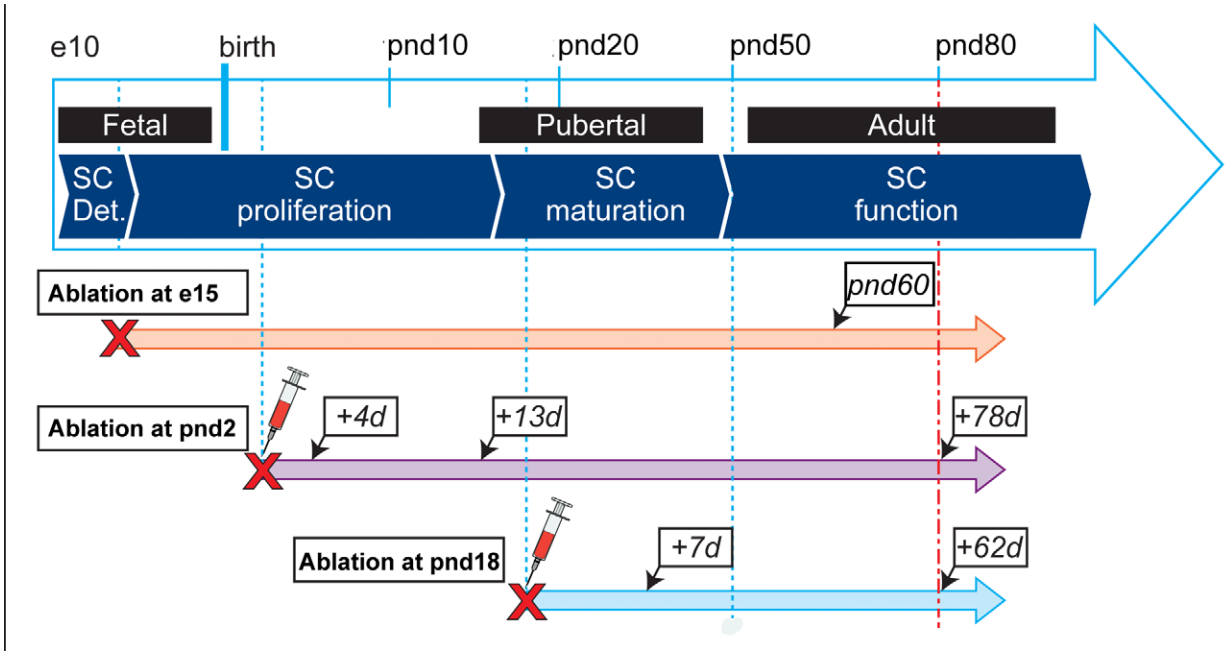


**Figure S1: Overview of experiment**

Schematic diagram indicating ablation time points (e15, post natal day 2 (pnd2) and pnd18) at key periods of testicular development and endpoints of tissue collection.

d:days, pnd: day postnatal, SC det. : SC Determination

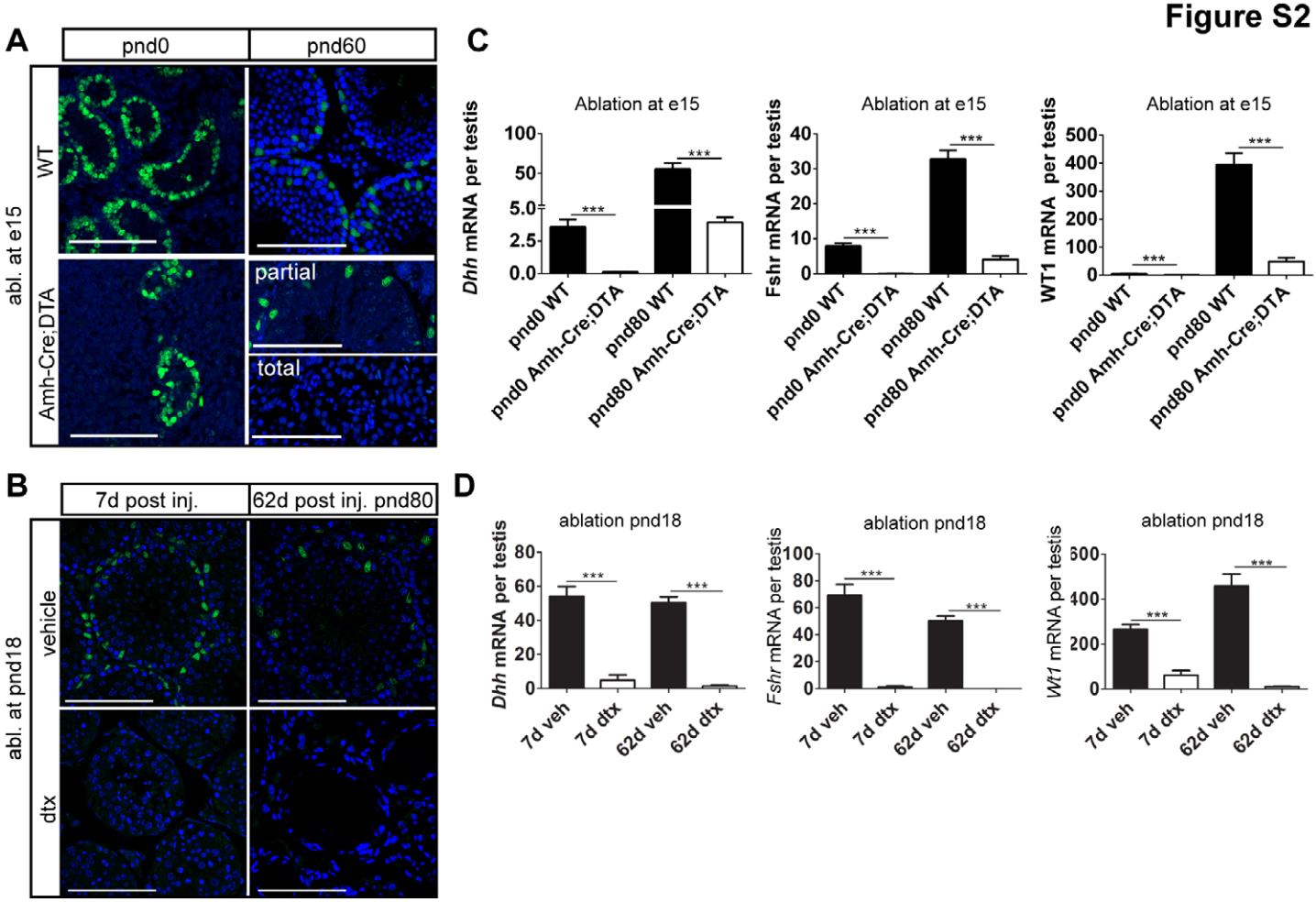


**Figure S2: Ontogeny of SC ablation**

(A) Immunolocalization of SOX9 protein (green) at pnd0 and pnd60 in Amh-Cre;DTA testes confirmed retention of SCs in a proportion of testes examined and complete absence of SCs in other testes examined. SOX9 immunolocalization following DTX-mediated SC ablation at (B) pnd18, when examined 7d later, and absent in adulthood (pnd80) (scale bar: 100 μm). SC specific markers: *Dhh*, *Fshr* and *Wt1* were significantly reduced at all ages following SC ablation. (one-way ANOVA, n=4-9, ***P<0.001).

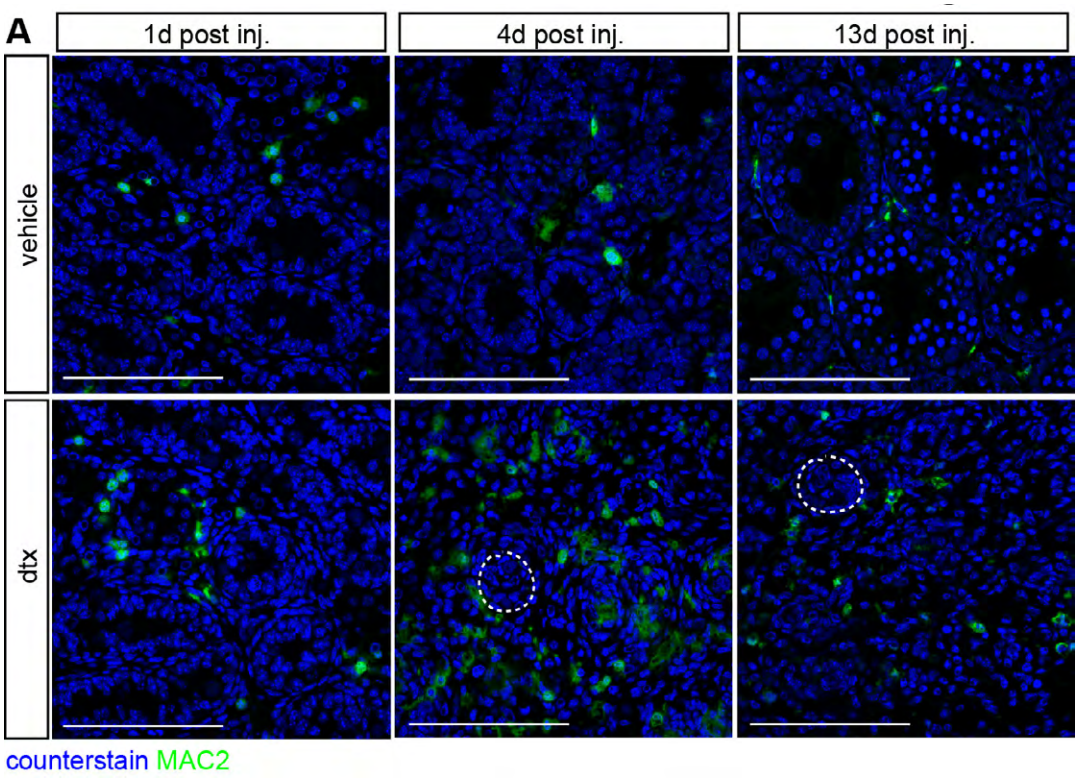


**Figure S3: Macrophages recruitment**

(A) Immunolocalization of MAC2 (green) following DTX-mediated SC ablation at pnd2, showed an apparent peak in macrophages staining 4d post ablation when compared to vehicle and 13d after ablation (scale bar: 100 μm).

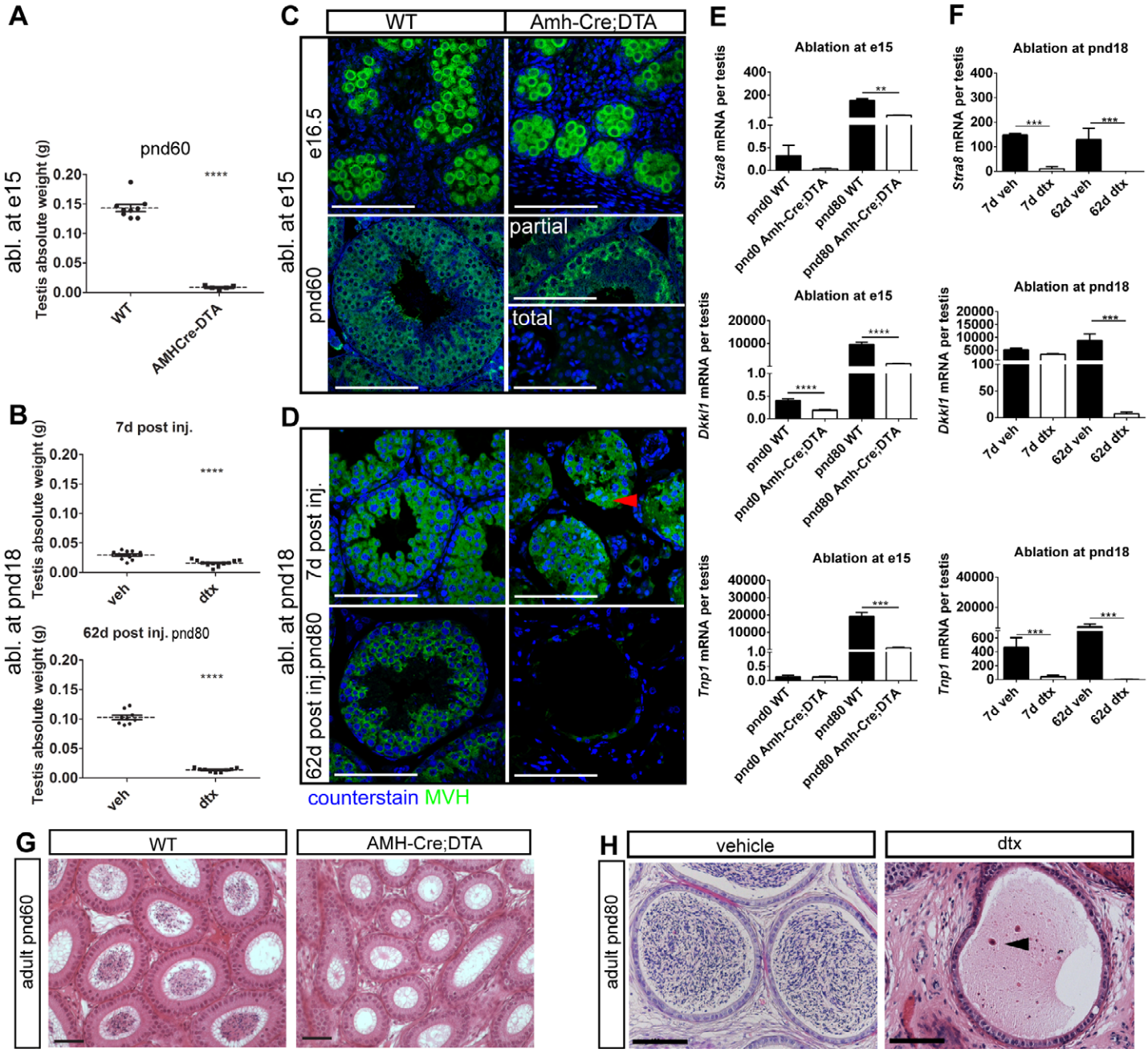


**Figure S4: Ontogeny of germ cell loss**

Testis weight was significantly reduced at all ages following SC ablation (A) e15 and (B) pnd18 (t-test, n=9-13, ****P<0.0001). Immunolocalization of MVH (DDX4) protein (green) identified GC loss following SC ablation. (C) In Amh-Cre;DTA mice, retention of germ cells mirrored presence of SCs. (D) GC loss occurred over an extended period (red arrowhead), but all GC were absent 30d post SC ablation (scale bar: 100 µm). (E, F) Consistent with GC loss, expression of the GC specific markers *Stra8* (spermatogonia), *Dkkl1* (spermatocytes) and *Tnp1* (spermatids) were all significantly reduced following SC ablation at e15 and pnd18 (one-way ANOVA, n=4-9, **P<0.01, ***P<0.001). (G,H) Consistent with GC loss, spermatozoa were absent and cellular debris remained (arrowhead) in cauda epididymides of all SC ablated animals when examined in adulthood (scale bar: 100 µm).

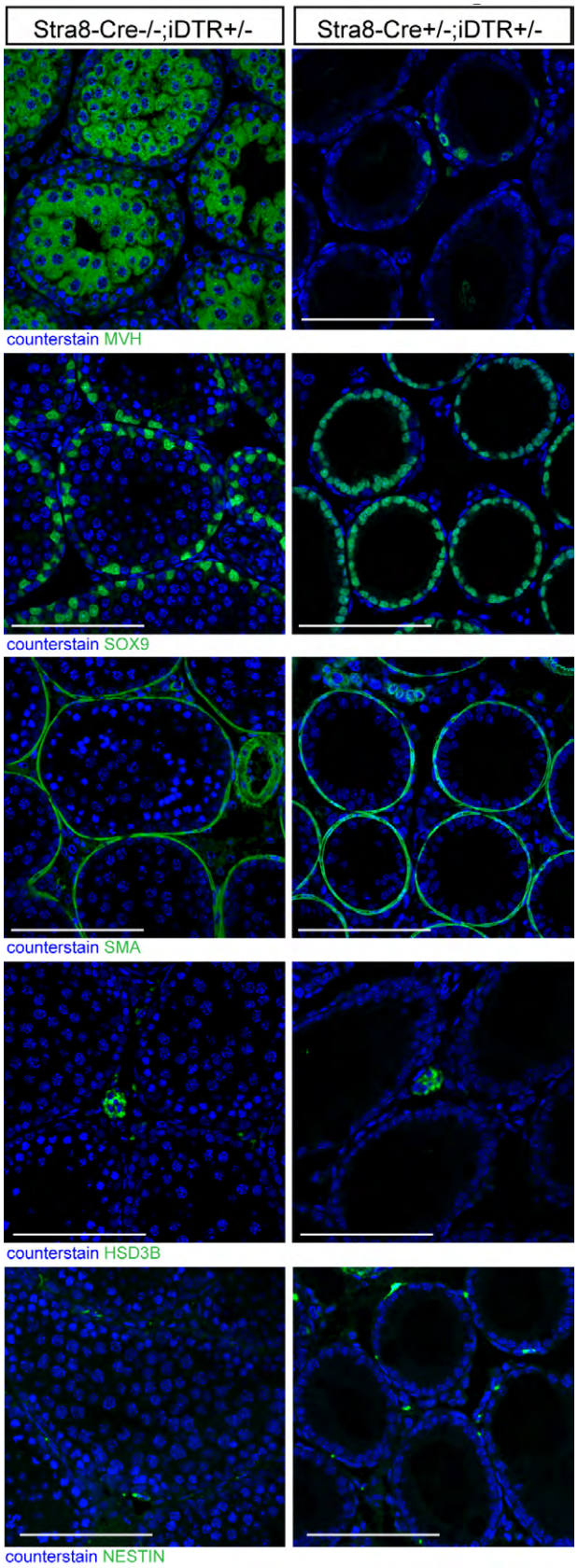


**Figure S5**: **GC-specific cell ablation does not impact testicular architecture**

Stra8-Cre$^{+/-}$;iDTR$^{+/-}$ mice were generated by directing DTR expression to GCs from pnd3. Injection of DTX at pnd10 induced specific GC ablation. Examination of testis histology at pnd25 confirmed near-total ablation of GC (MVH immunolocalization), however, somatic cell testicular architecture remained intact [SC (Sox9), PTMC (SMA), LC (HSD3B) and ALC progenitor cells (NESTIN)] (scale bar: 100 μm).

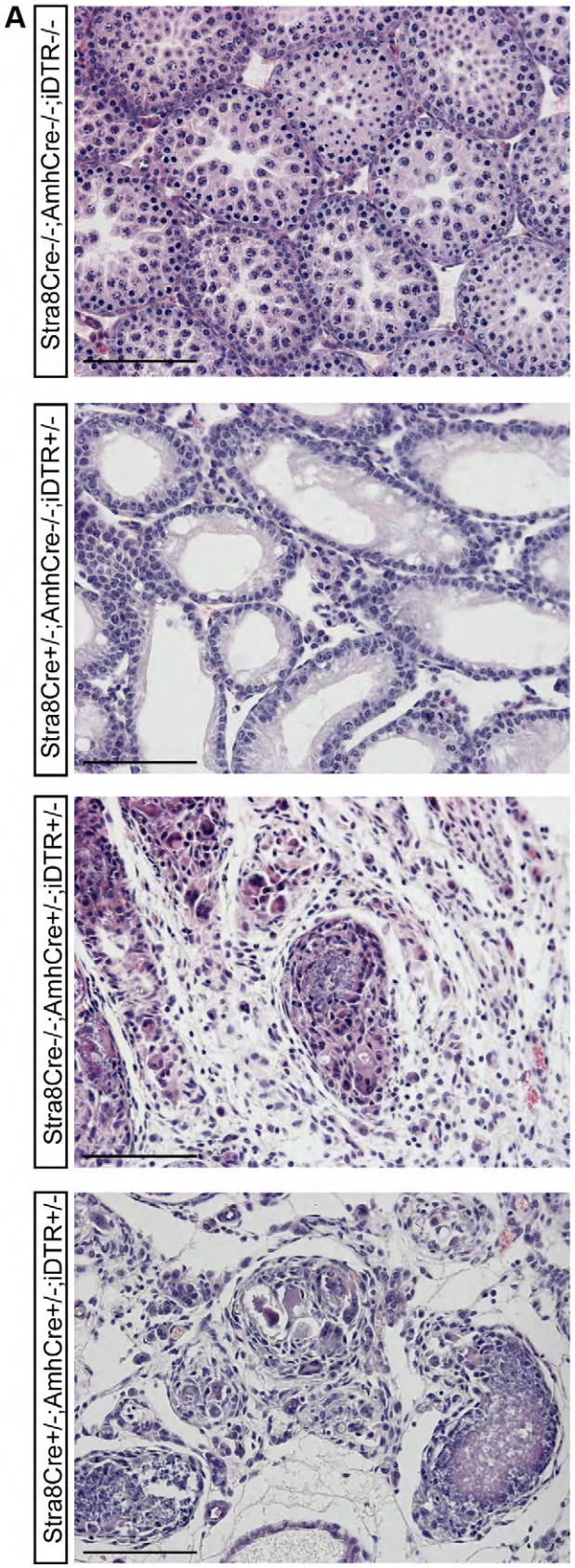


**Figure S6: Testicular histology of the Amh-Cre$^{+/-}$;Stra8-cre$^{+/-}$;iDTR$^{+/-}$ mice**

Testicular histology was examined by hematoxylin /eosin after injection of DTX at pnd10. Amh-Cre$^{-/-}$;Stra8-cre$^{+/-}$;iDTR$^{+/-}$ mice displayed Sertoli cells only tubules confirming ablation of germ cells. Amh-Cre$^{+/-}$;Stra8-cre$^{+/-}$;iDTR$^{+/-}$ mice show an identical testicular histology to Amh-Cre$^{+/-}$;iDTR$^{+/-}$ mice following treatment with DTX (scale bar: 100 µm).

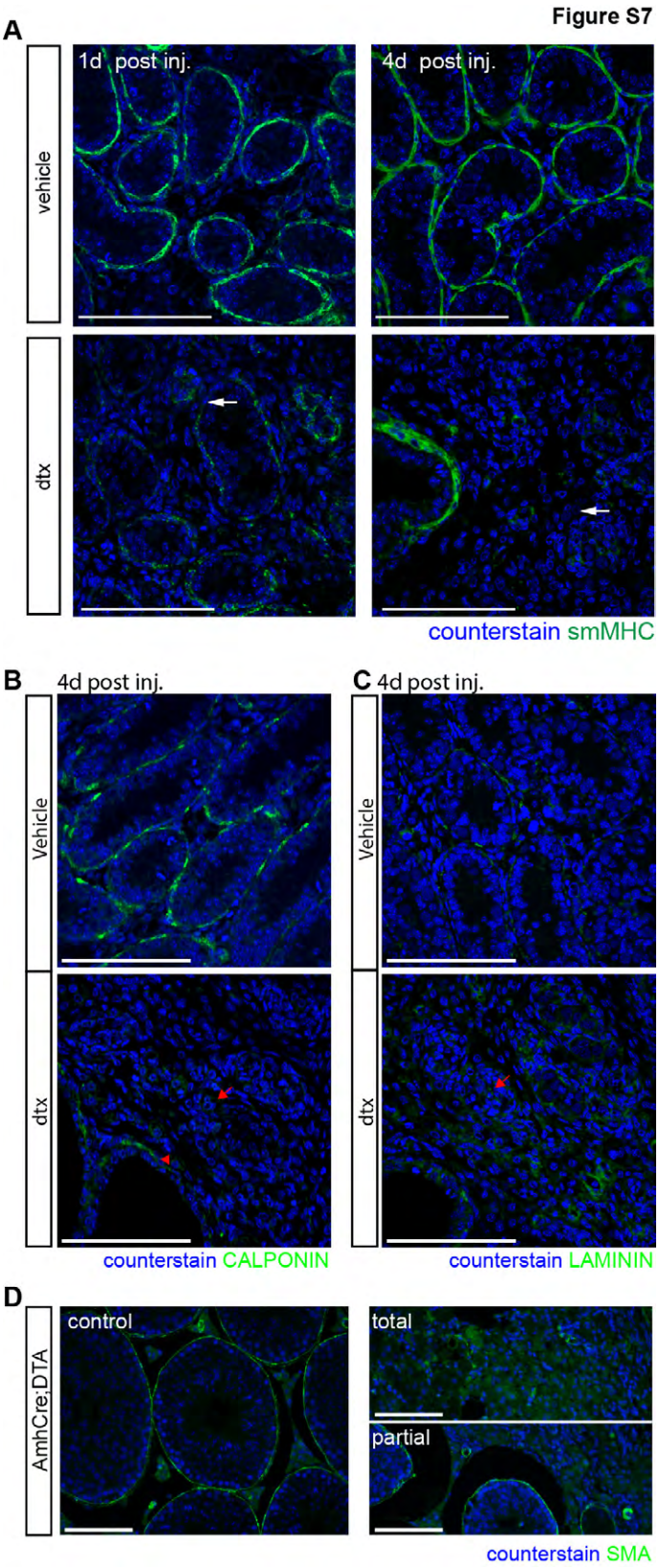


**Figure S7 Disruption to PTMC function following SC ablation at e15 and pnd2**

(A) Immunolocalization of the smooth muscle marker smMHC (green) 1d and 4d after SC ablation at pnd2 reveals disruption to the PTMC specificity and architecture. smMHC expression was retained around the rete testis. (B) Immunolocalization of the functional marker of PTM contractility CALPONIN (green), 4d after SC ablation at pnd2 reveals disruption to the PTMC architecture, but retention of CALPONIN expression around the rete testis. (C) Immunolocalization of LAMININ (green) reveals a similar pattern of expression, consistent with complete disruption of the BM (arrowheads) (scale bar: 100 μm). (D) In Amh-Cre;DTA mice, total ablation reveals disruption to the PTMC architecture whereas the partial ablation retained expression of SMA (scale bar: 100 μm).

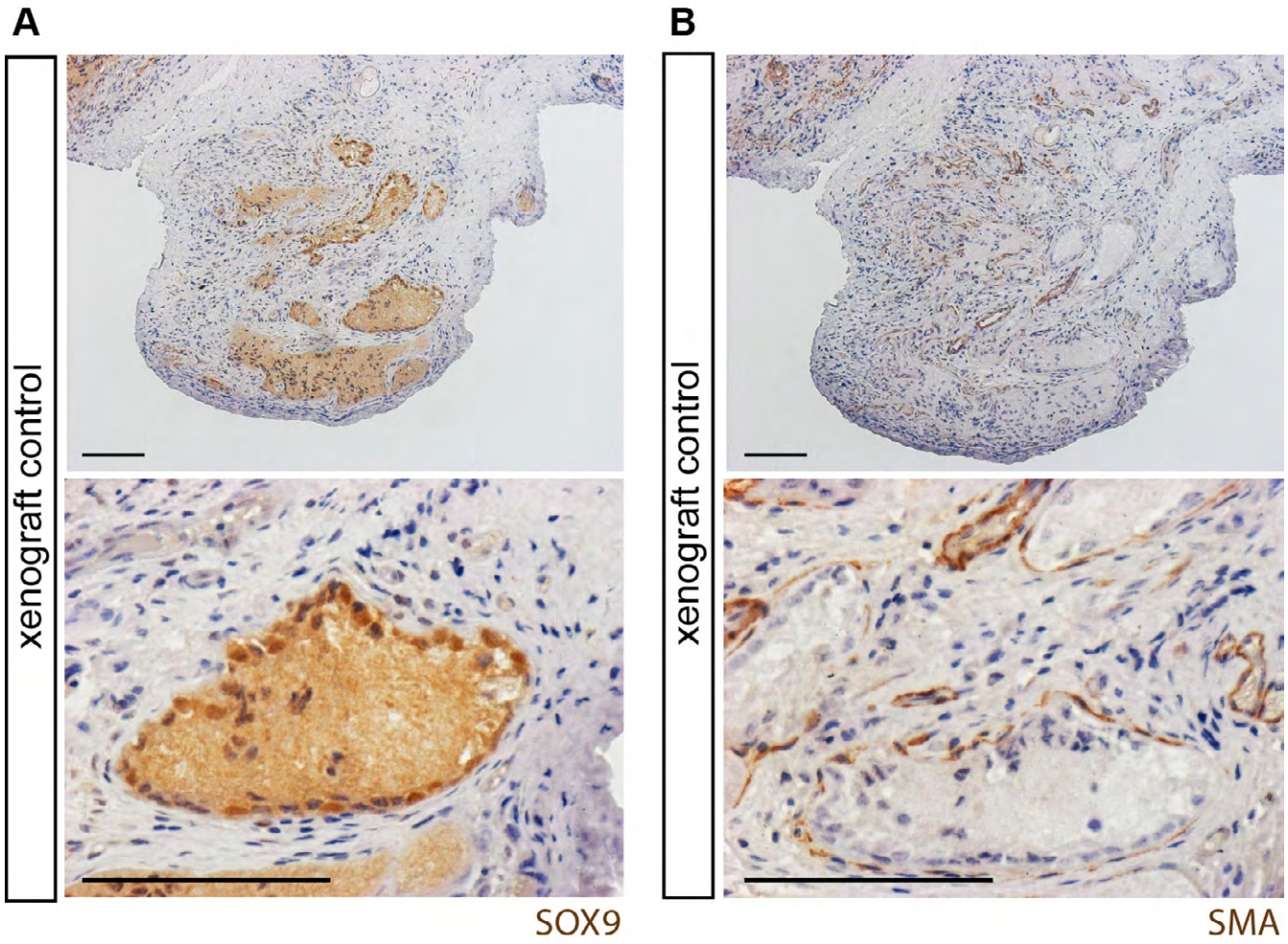


**Figure S8: Tubular architecture reforms after testicular dissociation**

Pnd8 testes dissociated to a single cell suspension and xenografted under the skin of nude mice reform seminiferous tubules. (A) Immunolocalization of the SC marker SOX9 reveals specific localization of SC positive cells into the reformed tubules. (B) Immunolocalization of the smooth muscle marker SMA reveals that SMA positive PTMCs are only found in proximity to SCs. SMA also defines blood vessels present in the graft (scale bar: 100 µm).

**Table S1. Leydig cell counts as percentage of controls based on morphology or on 3bHSD staining.**

| Endpoints | Treatment | Based on 3bHSD | s.e.m. | *n* | Based on morphology | s.e.m. | *n* |
|---|---|---|---|---|---|---|---|
| **2d+4** | veh | **100** | 27.56 | 3 | **100** | 8.008 | 4 |
| | dtx | **80.13** | 10.86 | 3 | **79.66** | 9.434 | 4 |
| **2d+13** | veh | **100** | 37.43 | 3 | **100** | 10.77 | 4 |
| | dtx | **13.16** | 2.75 | 3 | **19.26** | 3.477 | 4 |
| **2d+78** | veh | **100** | 10.57 | 3 | **100** | 5.439 | 12 |
| | dtx | **11.96** | 3.254 | 3 | **8.023** | 3.04 | 4 |

**Table S2. Primers used for genotyping and qRT-PCR**

| Gene symbol | Primers 5'-3' |
|---|---|
| HBEGF (DTR) mutant | CAT CAA GGA AAC CCT GGA CTA CTG |
| HBEGF (DTR) common | AAA GTC GCT CTG AGT TGT TAT |
| HBEGF (DTR) wild type | GGA GCG GGA GAA ATG GAT ATG |
| Stra8-Cre | GTG CAA GCT GAA CAA CAG GA |
| | AGG GAC ACA GCA TTG GAG TC |
| Amh-Cre | CAC ATC AGG CCC AGC TCT AT |
| | GTG TAC AGG ATC GGC TCT GC |
| *Fshr* | GGC CAG GTC AAC ATA CCG CTT G |
| | TGC CTT GAA ATA GAC TTG TTG CAA ATT G |
| *Wt1* | GCT CCA GCT CAG TGA AAT GGA CAG AA |
| | GGC CAC TCC AGA TAC ACG CCG |
| *Cnn1* | CAA GCT GGC CCA GAA ATA CGA CC |
| | TCT TCA CAG AAC CCG GCT GCA G |
| *Myh11* | CTG CAC AAC CTG AGG GAG CGA TAC T |
| | AAT GGC ATA GAT GTG AGG CGG C |
| *Mc2r* | ATT AGT GAC AAA GCC AAG GAG AGG AGC A |
| | GGG TGG TGT TTG CCG TTG ACT TAC |
| *Sult1e1* | TGT TGA AAT GTT CTT GGC AAG GCC |
| | CAT CCT CCT TGC ATT TTT CCA CAT CA |
| *Cyp11a1* | CAC AGA CGC ATC AAG CAG CAA AA |
| | GCA TTG ATG AAC CGC TGG GC |
| *Stra8* | GAA GGT GCAT GGT TCA CCG TGG |
| | GCT CGA TGG CGG GCC TGT G |

| | |
|---|---|
| *Sog1* | GAG CCA GAA CGG AAC CCG GA |
| | GAC ATC GGG CTG GGT CCT CC |
| *Tp1* | GGC GAT GAT GCA AGT CGC AA |
| | CCA CTC TGA TAG GAT CTT TGG CTT TTG G |

**Table S3. Details of antibodies and detection methods used**

| Primary antibody (AbI) name | References | Dilution AbI | Secondary antibody (AbII) conjugated | Dilution AbII | Detection system |
|---|---|---|---|---|---|
| CL.CASPASE 3 | Cell Signaling (NEB) #9661 | 1/100 | Biotin | 1/500 | DAB |
| SOX9 | Millipore Ab5535 | 1/5000 | Peroxidase | 1/200 | IF |
| 3 β-HSD | Santa Cruz Biotechnology sc-30820 | 1/750 | Peroxidase | 1/200 | DAB/IF |
| SMA | Sigma-Aldrich Ab2547 | 1/5000 | Peroxidase | 1/200 | IF |
| MVH | Abcam Ab13840 | 1/400 | Peroxidase | 1/200 | IF |
| CALPONIN | Abcam Ab46794 | 1/1000 | Peroxidase | 1/200 | IF |
| LAMININ | Abcam Ab11575 | 1/1500 | Peroxidase | 1/200 | IF |
| GFP | Molecular Probes | 1/200 | Peroxidase | 1/200 | IF |
| NESTIN | Abcam Ab6142 | 1/1000 | Peroxidase | 1/200 | IF |

DAB: diaminobenzidine, IF: immunofluorescence