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Supplemental Information

The Bile Acid Nuclear Receptor FXRα Is a Critical Regulator of Mouse Germ Cell Fate

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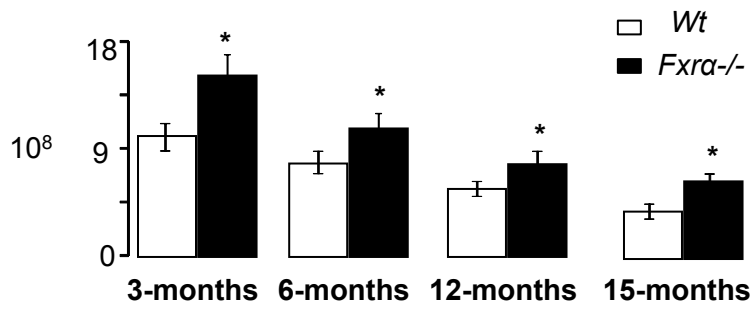
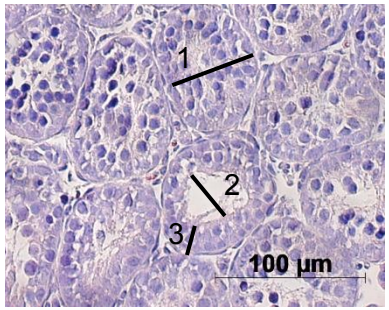
A



B



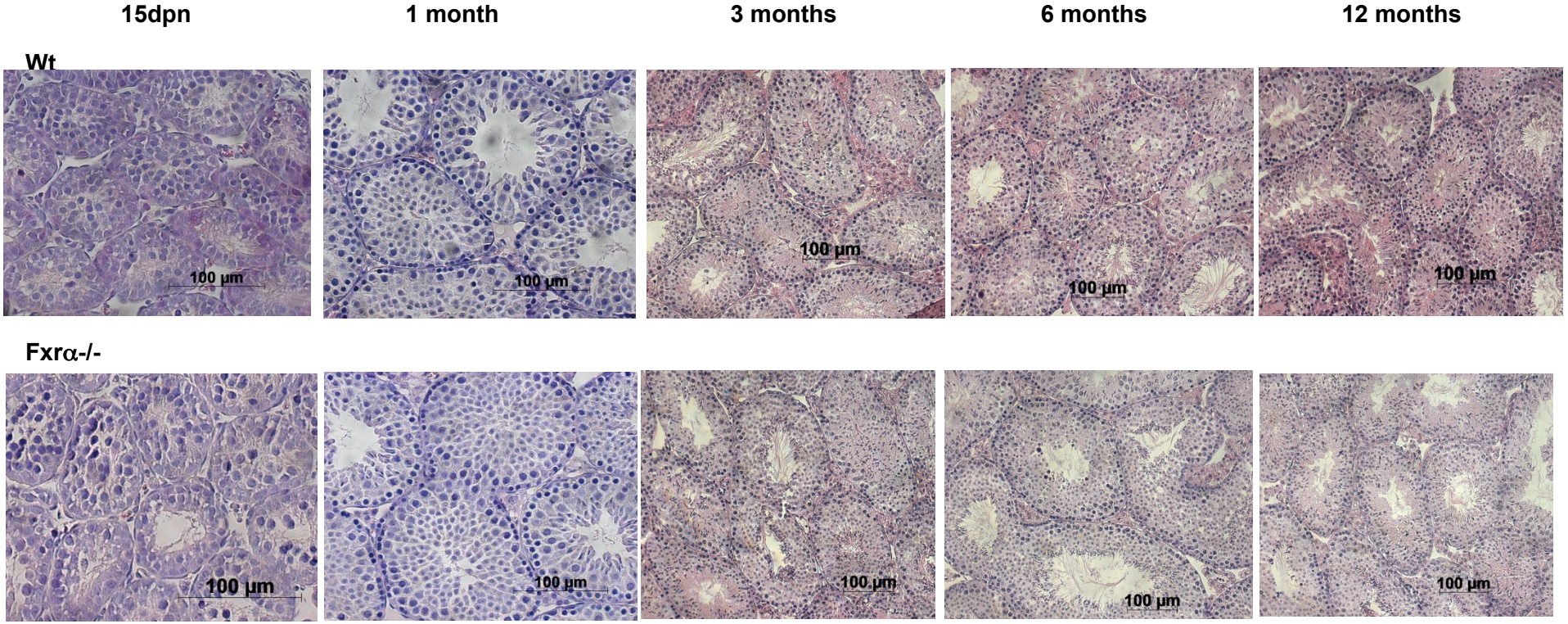
1: illustrate the diameter of the seminiferous tubule

2: illustrate the diameter of the lumen

3: illustrate the height of the epithelium

C

HE from 1 to 12 months



Suppl. 1

Figure S 1. Illustrates the sperm production and testicular histology, Related to Figure 1

A/ Relative number of sperm count in the epididymis head normalized to body weight in wild-type and *Fxrα*-/- at 3, 6, 12 or 15-month-old.

B/ A Representative image of a 10dpn testis; it is illustrate here the way how were quantified the diameter of the seminiferous tubule and the height of the epithelium.

C/ Representative images of a testis stained with eosine hematoxyline from wild-type and *Fxrα*-/- at 15dpn, 1, 3, 6, 12 or 15-month-old.

In all panel for each group, n =10 males from 3 to 4 independent litters; * denotes significance; $p<0.05$.

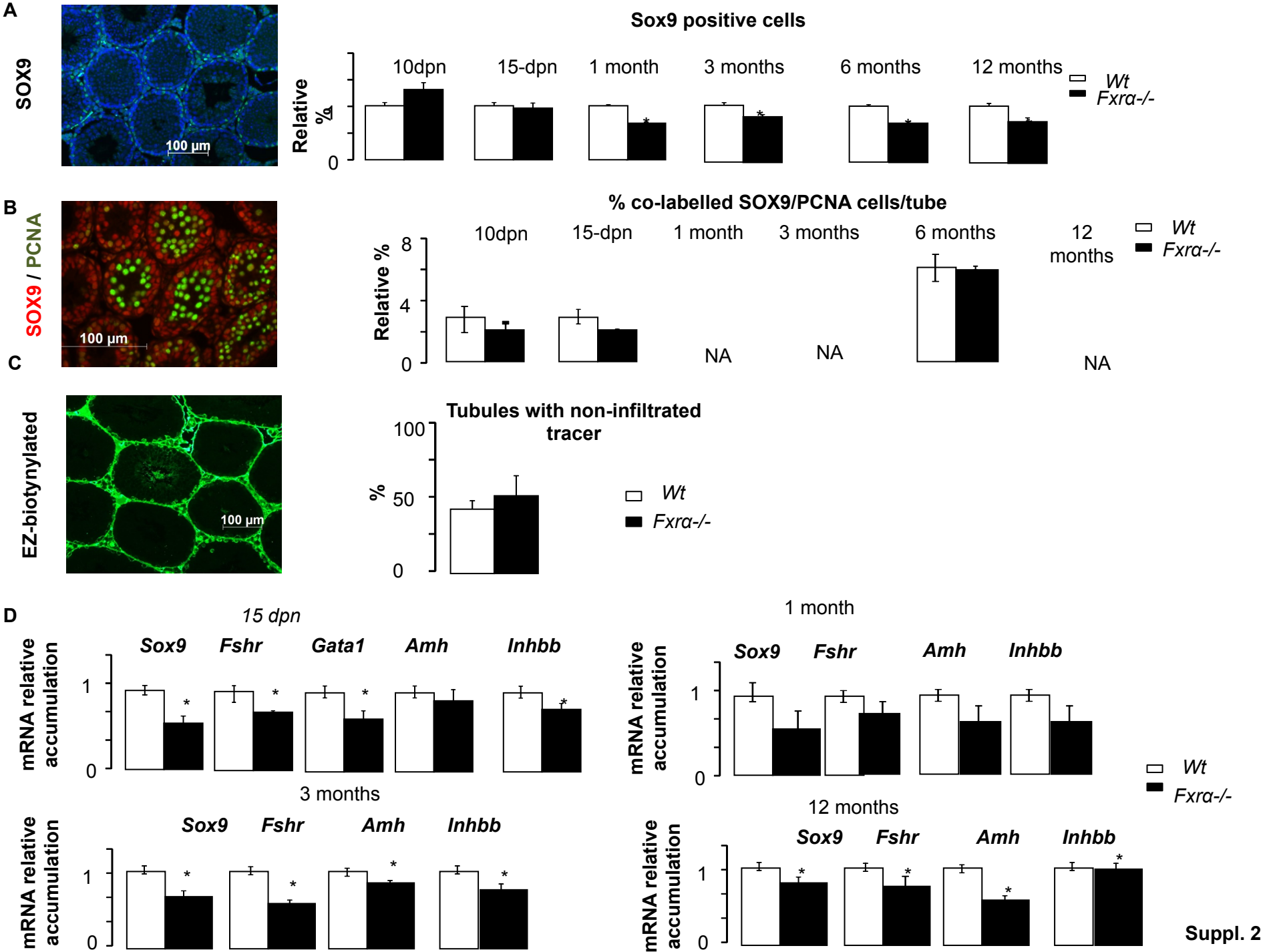
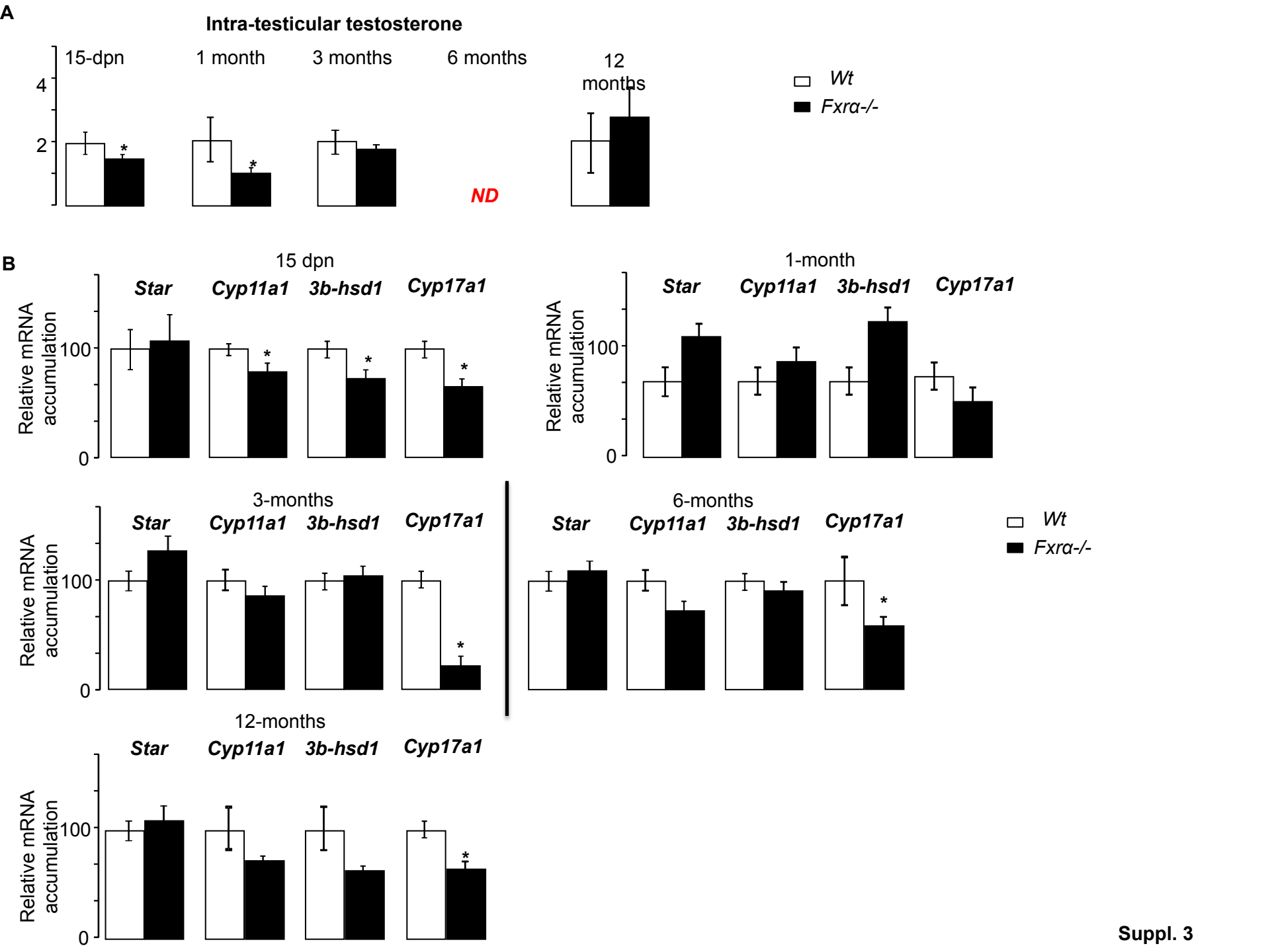

A
SOX9
100 µm
Sox9 positive cells
Relative %
0
10dpn
15-dpn
1 month
3 months
6 months
12 months
Wt
Fxrα-/-
B
SOX9 / PCNA
100 µm
% co-labelled SOX9/PCNA cells/tube
Relative %
8
4
0
10dpn
15-dpn
1 month
3 months
6 months
12 months
NA
NA
NA
Wt
Fxrα-/-
C
EZ-biotynylated
100 µm
Tubules with non-infiltrated tracer
%
100
50
0
Wt
Fxrα-/-
D
15 dpn
mRNA relative accumulation
Sox9
Fshr
Gata1
Amh
Inhbb
1
0
1 month
3 months
12 months
Wt
Fxrα-/-
Suppl. 2

Figure S 2. Impact of Fxrα deficiency on Sertoli cell homeostasis, Related to figure 2.

A/ Representative micrograph of the SOX9 staining in testis of Fxrα-/- male at 15 dpn. Quantification of the number of SOX9 positive cells is indicated as the number of positive cells per 100 seminiferous tubule in wild-type and Fxrα$^{-/-}$ mice at 10dpn, 15dpn, 1 months, 6 months and 12 months. **B/** Representative micrograph of the testis of 15-day-old *Fxrα-/-* male. Stained for PCNA and, SOX9. The original magnification was x200. Quantification of the percentage of cells co-labeled for PCNA and SOX9 per seminiferous tubule in wild-type and Fxrα$^{-/-}$ mice at 10dpn, 15dpn, 1 months, 6 months and 6 months. **C/** Representative micrograph of the efficiency of the BTB in testis of Fxrα$^{-/-}$ mice at 15 dpn visualized by staining of a biotynylated tracer. Quantification of the percentage of seminiferous tubules with tracer infiltration. **D/** Testicular mRNA accumulation of *Sox9*, *Fshr, Inhbb* and *Amh* normalized to β–actin mRNA levels in the whole testes of wild-type and Fxrα-/- mice at 15 days old, 1 month, 3 months and 12 months.

In all panels for each group, n =6 males from 4 independent litters; * denotes significance; $p<0.05$.


A
Intra-testicular testosterone
15-dpn
1 month
3 months
6 months
12 months
ND
Wt
Fxrα-/-
B
15 dpn
1-month
3-months
6-months
12-months
Star
Cyp11a1
3b-hsd1
Cyp17a1
Relative mRNA accumulation
100
0

Suppl. 3

Figure S 3. Impact of Fxrα deficiency on Leydig cell homeostasis, Related to figure 2.

A/ Intra-testicular testosterone concentrations in wild-type and Fxrα-/- mice at 15 dpn, 1 month, 3 months, 6 months and 12 months. **B/** Testicular mRNA accumulation of *Star*, *Cyp11a*1,*3β-Hsd1* and *Cyp17a1* normalized to *β–actin* mRNA levels in the whole testes of wild-type and Fxrα$^{-/-}$ mice at 15 dpn, 1 month, 3 months, 6 months and 12 months.

In all panels for each group, n =6 to 10 males from 4 to 5 independent litters; * denotes significance; $p<0.05$.

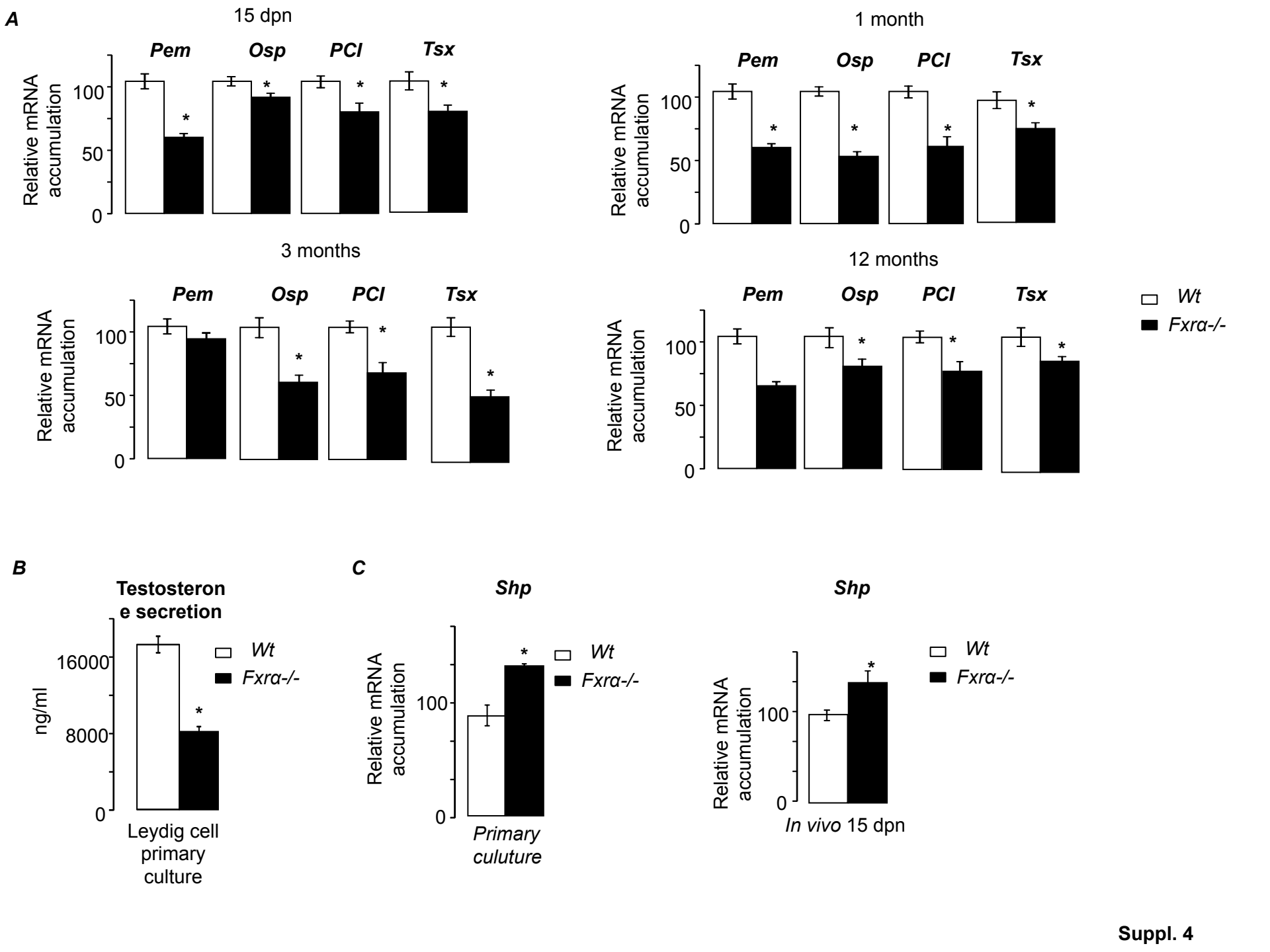

A
15 dpn
Pem
Osp
PCl
Tsx
Relative mRNA accumulation
100
50
0
*
1 month
3 months
12 months
Wt
Fxrα-/-
B
Testosteron e secretion
ng/ml
16000
8000
0
Leydig cell primary culture
C
Shp
Relative mRNA accumulation
Primary culuture
In vivo 15 dpn

Figure S 4. Impact of Fxrα deficiency on expression of androgeno-dependent genes, Related to figure 2.

A/ Testicular mRNA accumulation of *Pem*, *Osp*, *Pci* and *Tsx*normalized to *β–actin* mRNA levels in the whole testes of wild-type and Fxrα$^{-/-}$ mice at 15 dpn, 1 month, 3 months and 12 months. **B/** Testosterone secretion in Leydig cells (primary culture) from adult *wild-type* and *Fxrα*$^{-/-}$ mice. **C**/ mRNA accumulation of *Shp* normalized to *β–actin* mRNA levels evaluated in Leydig cells (primary culture) from 3 month-old *wild-type* and *Fxrα*$^{-/-}$ mice or in in the whole testes of wild-type and Fxrα$^{-/-}$ mice at 3 months.

In panels B and C, results were obtained from one experiment of primary culture from 20 Wt or 20 Fxrα-/- males.

In panel A & C, for in vivo experiments each group, n =6 males from 4 independent litters; * denotes significance; $p<0.05$.

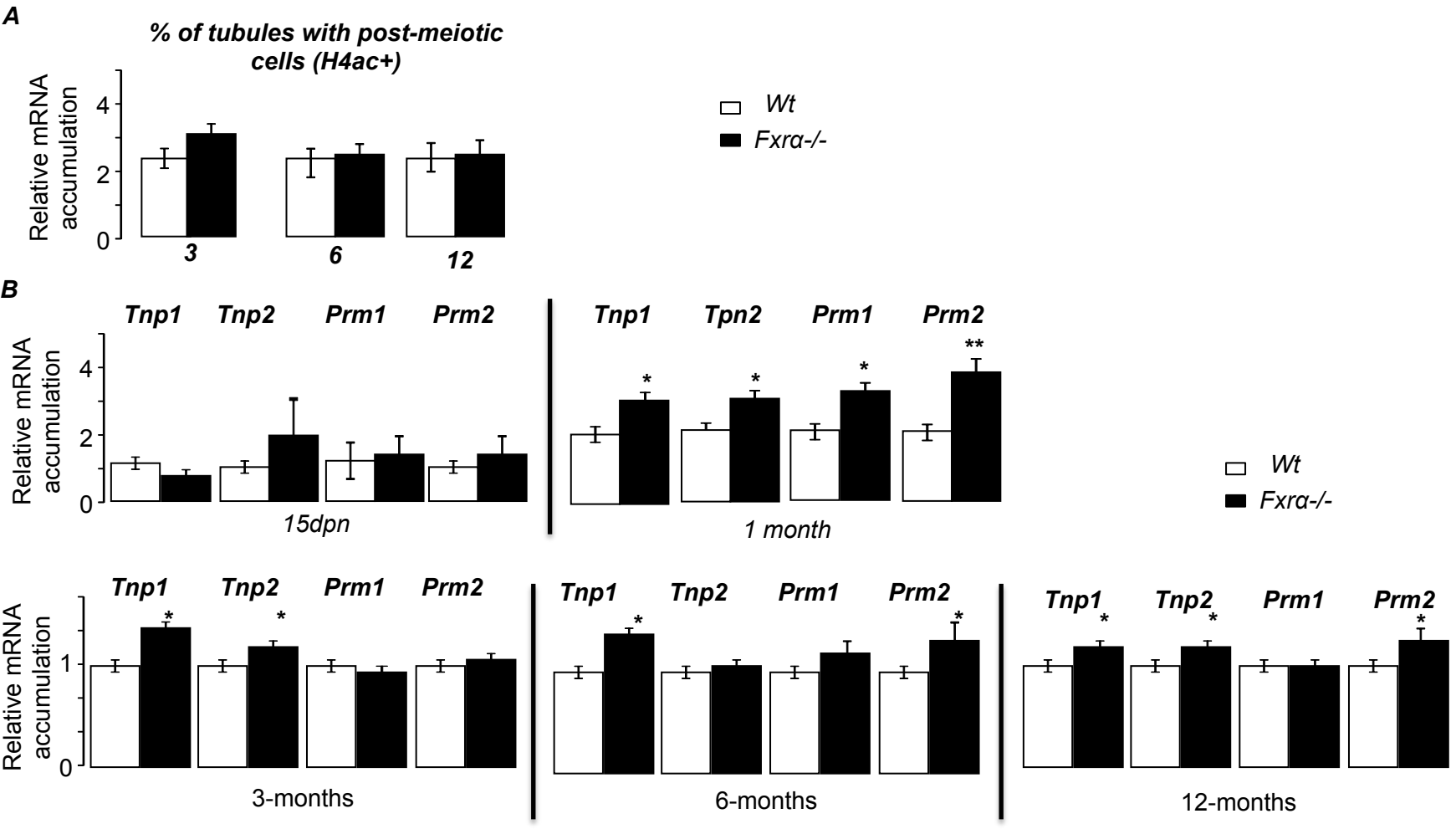
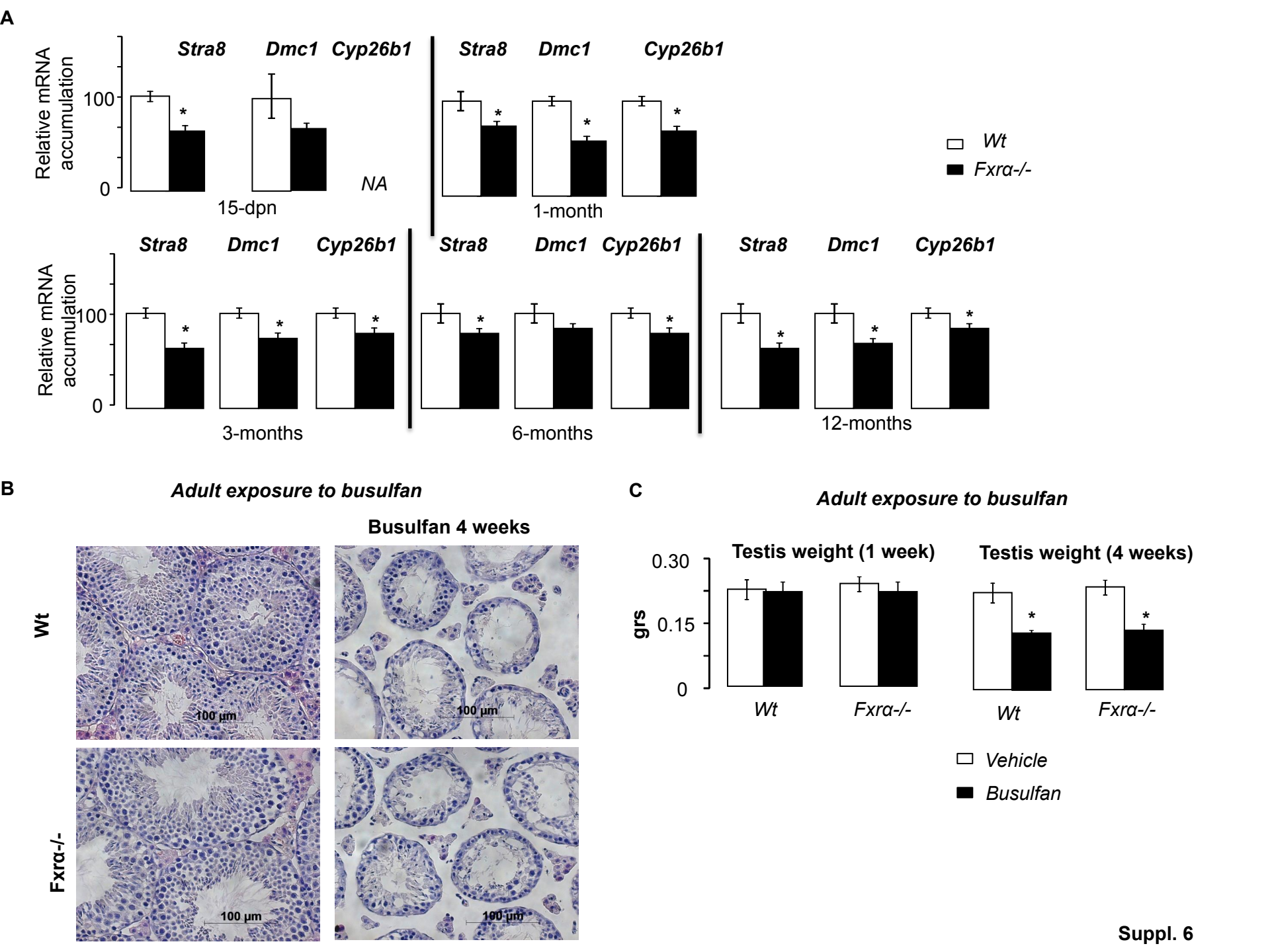

A
% of tubules with post-meiotic cells (H4ac+)
Relative mRNA accumulation
0
2
4
3
6
12
Wt
Fxrα-/-
B
Tnp1
Tnp2
Prm1
Prm2
Relative mRNA accumulation
15dpn
Tnp1
Tpn2
Prm1
Prm2
1 month
Wt
Fxrα-/-
Tnp1
Tnp2
Prm1
Prm2
Relative mRNA accumulation
0
1
3-months
Tnp1
Tnp2
Prm1
Prm2
6-months
Tnp1
Tnp2
Prm1
Prm2
12-months

Figure S 5. Fxrα deficiency improves germ cell differentiation, Related to figure 2.

A/ Quantification of the percentage of seminiferous tubules showing elongated H4ac positive germ cells in *wild-type* and *Fxrα*$^{-/-}$ mice from 3, 6 and 12 months of age.

B/ Testicular mRNA accumulation of *Tpn1, Tpn2, Prm1* and *Prm2* normalized to *β–actin* mRNA levels *wild-type* and *Fxrα*$^{-/-}$ mice at 15dpn, 1 month, 3 months and 12 months. .

In all panel for each group, n =5 to 10 males from 3 to 4 independent litters; * denotes significance; $p<0.05$.

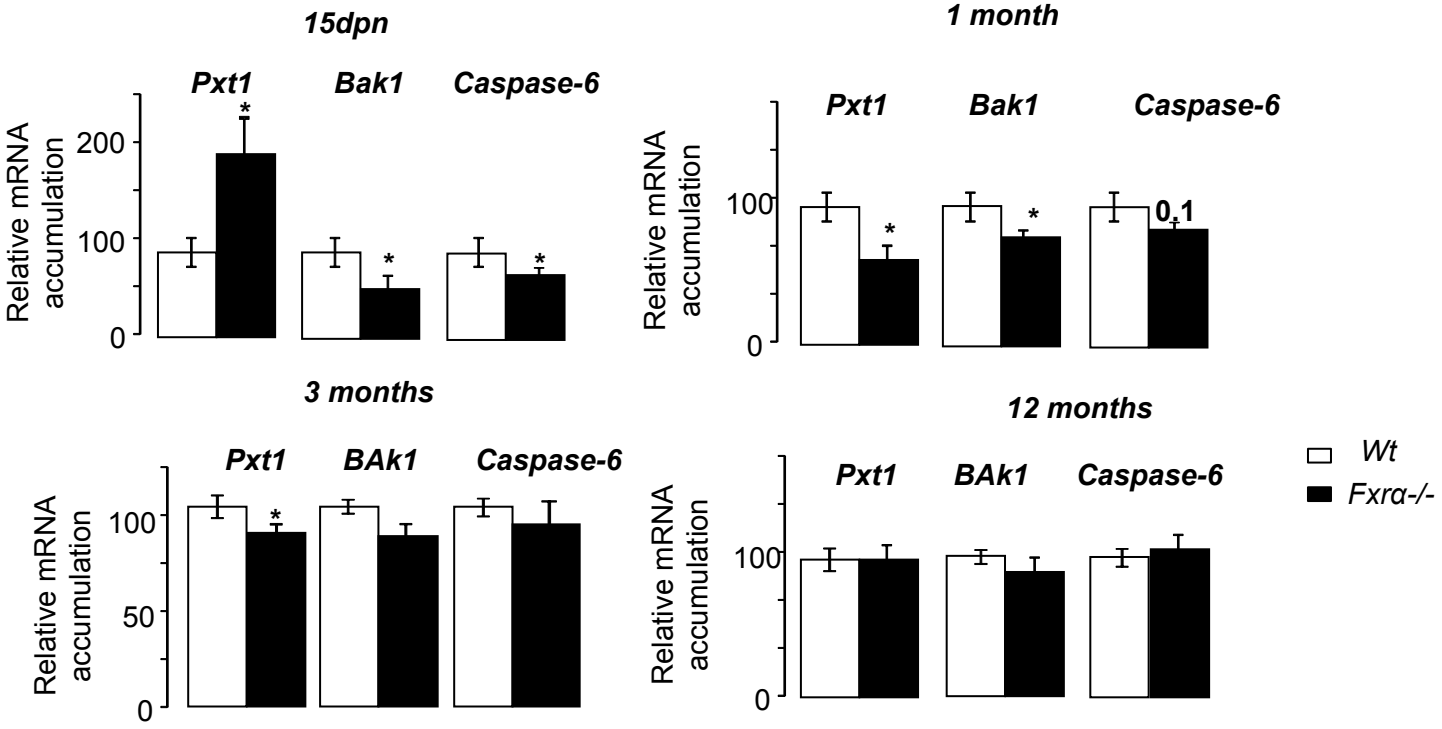

A
Relative mRNA accumulation
100
0
Stra8
Dmc1
Cyp26b1
*
NA
15-dpn
1-month
3-months
6-months
12-months
Wt
Fxrα-/-
B
Adult exposure to busulfan
Busulfan 4 weeks
Wt
Fxrα-/-
100 µm
C
Adult exposure to busulfan
Testis weight (1 week)
Testis weight (4 weeks)
grs
0.30
0.15
0
Vehicle
Busulfan

Suppl. 6

Figure S 6. Fxrα deficiency alters the expression of meiotic genes, Related to figure 3.

A/ Testicular mRNA accumulation of *Stra8*, *Dmc1 and Cyp26b1* normalized to *β–actin* mRNA levels in *wild-type* and *Fxrα*$^{-/-}$ mice at 15dpn, 1 month, 3 months, 6 months and 12 months. n=5-10 per group from 4 to 5 independent litters; * denotes significance; $p<0.05$. **B/** Representative micrographs of the testis H&E stained from 3 months old *wild-type* and *Fxrα-/-* mice treated with busulfan. **C/** Testis weights in *wild-type* and *Fxrα*-/- mice from 1 week and 4 weeks after busulfan exposure. In all panel n=5 to 6- per group from 3 different litters; * denotes significance; $p<0.05$.

A


15dpn
Pxt1
Bak1
Caspase-6
Relative mRNA accumulation
200
100
0
1 month
100
0
0.1
3 months
BAk1
50
12 months
Wt
Fxrα-/-

B

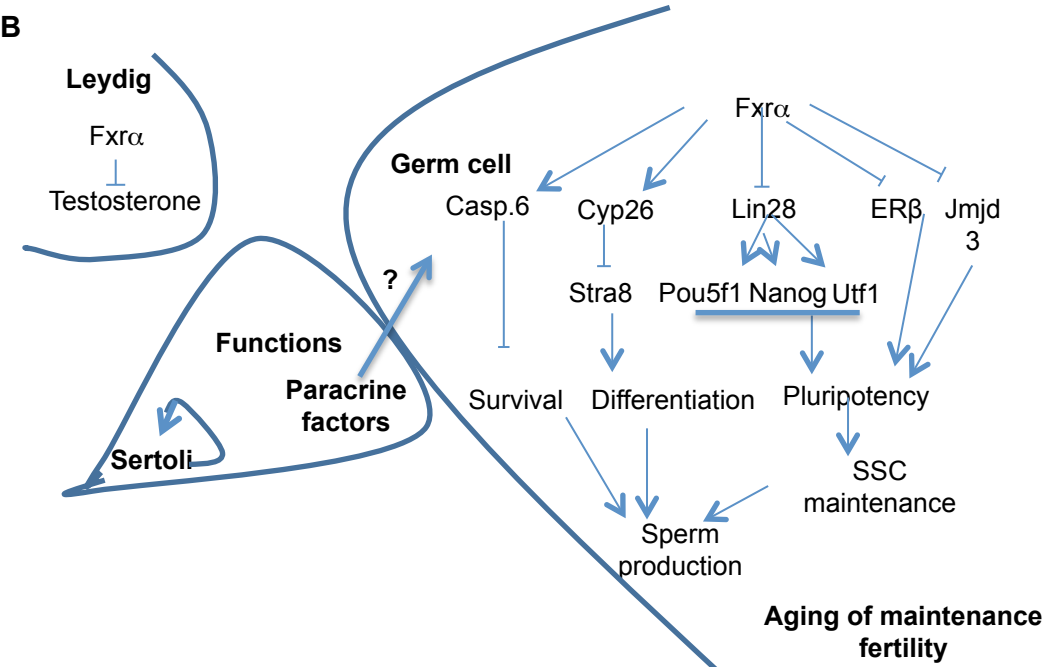

Leydig
Fxrα
Testosterone
Germ cell
Casp.6
Cyp26
Fxrα
Lin28
ERβ
Jmjd 3
Stra8
Pou5f1 Nanog Utf1
?
Functions
Paracrine factors
Sertoli
Survival
Differentiation
Pluripotency
SSC maintenance
Sperm production
Aging of maintenance fertility

Figure S 7. Fxrα deficiency alters the expression of genes involved in apoptosis, Related to figure 5

A/ Testicular mRNA accumulation of *Pxt1*, *Bak1 and Caspase-6* normalized to *β–actin* mRNA levels in the whole testes of wild-type and Fxrα$^{-/-}$ mice at 15 dpn, 1 month, 3 months, 6 months and 12 months. For each group, n =5 to 10 males from 3 to 4 independent litters; * denotes significance; $p<0.05$.

B/ Schematic model representing the putative roles of FXRα on testicular physiology**.**