

Supplementary Material

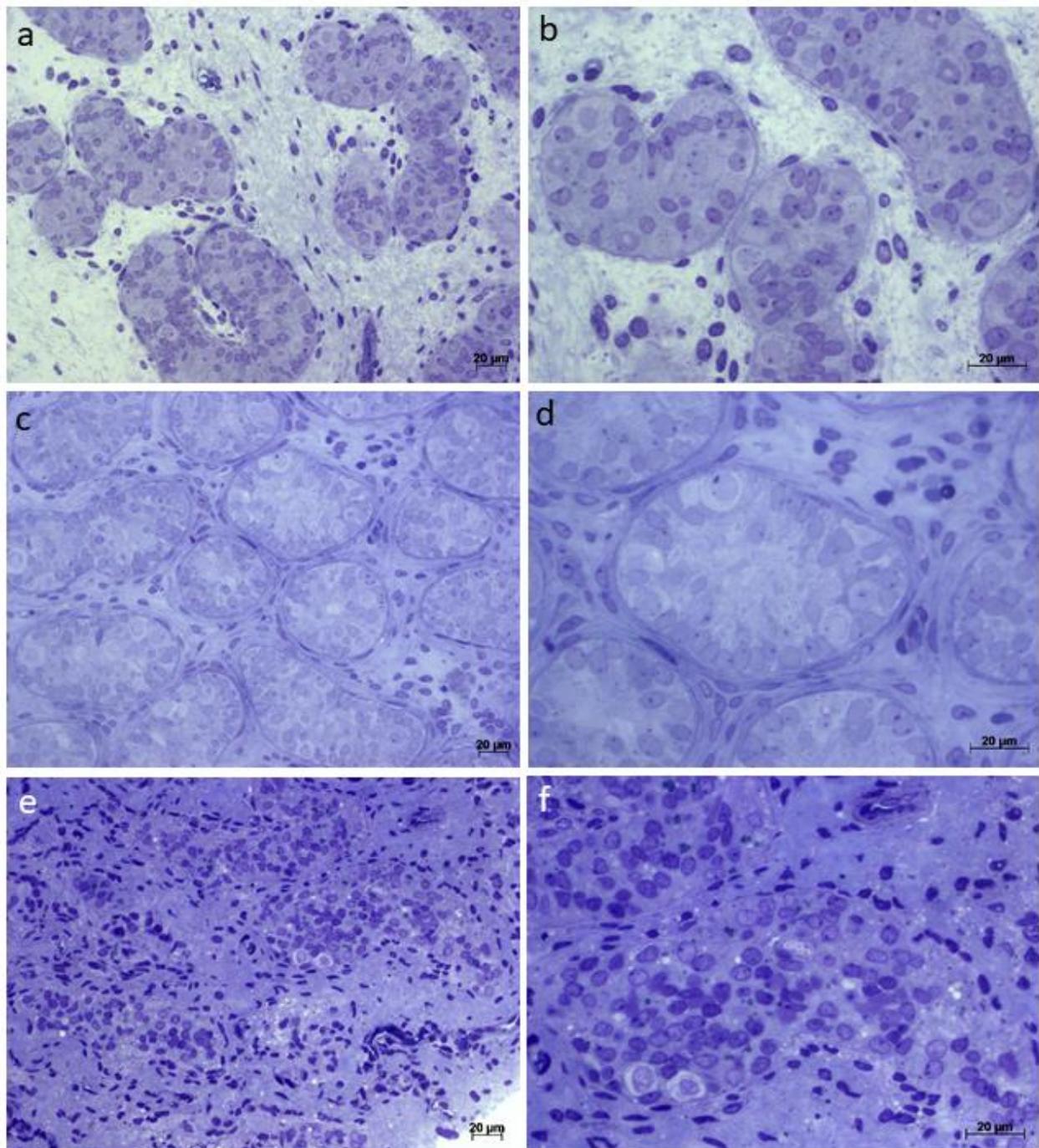


Figure S1 Histology of the tissues used for organ culture in the native state, shown on semi-thin sections stained with toluidine blue and pyronine. (a, b) 7-year-old β -thalassemia major patient; (c, d) 12-year-old XIAP deficiency patient; (e, f) 9-year-old sickle cell disease patient.

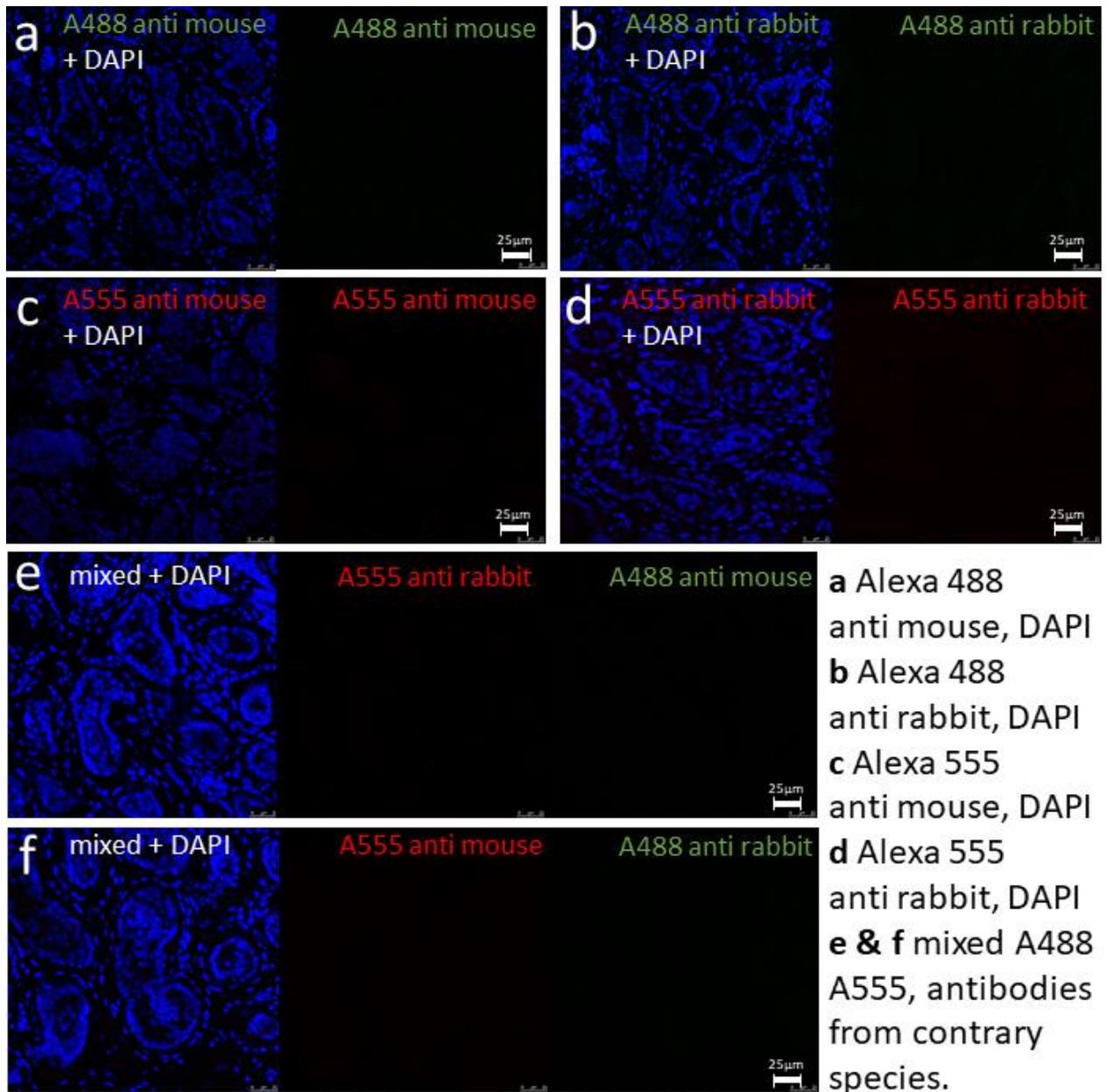


Figure S2 Negative controls, prepubertal tissue.

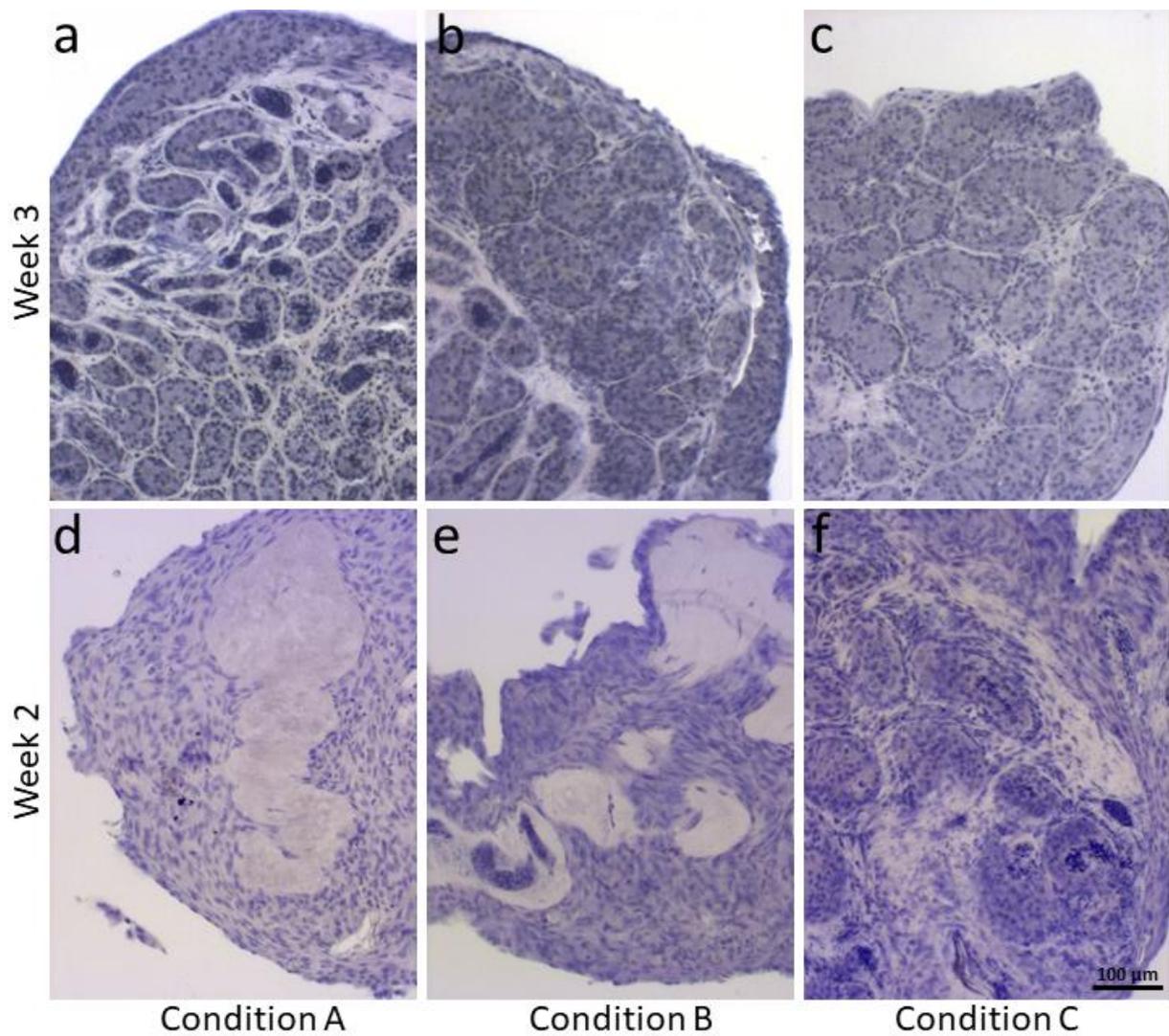


Figure S3 Mayer's Hematoxylin staining of the tissue cultured under all 3 conditions. (a-c) 12-year-old with XIAP deficiency, week 3. (d-f) 9-year-old with sickle cell disease, insufficient tissue preservation with many fibrous areas, week 2.

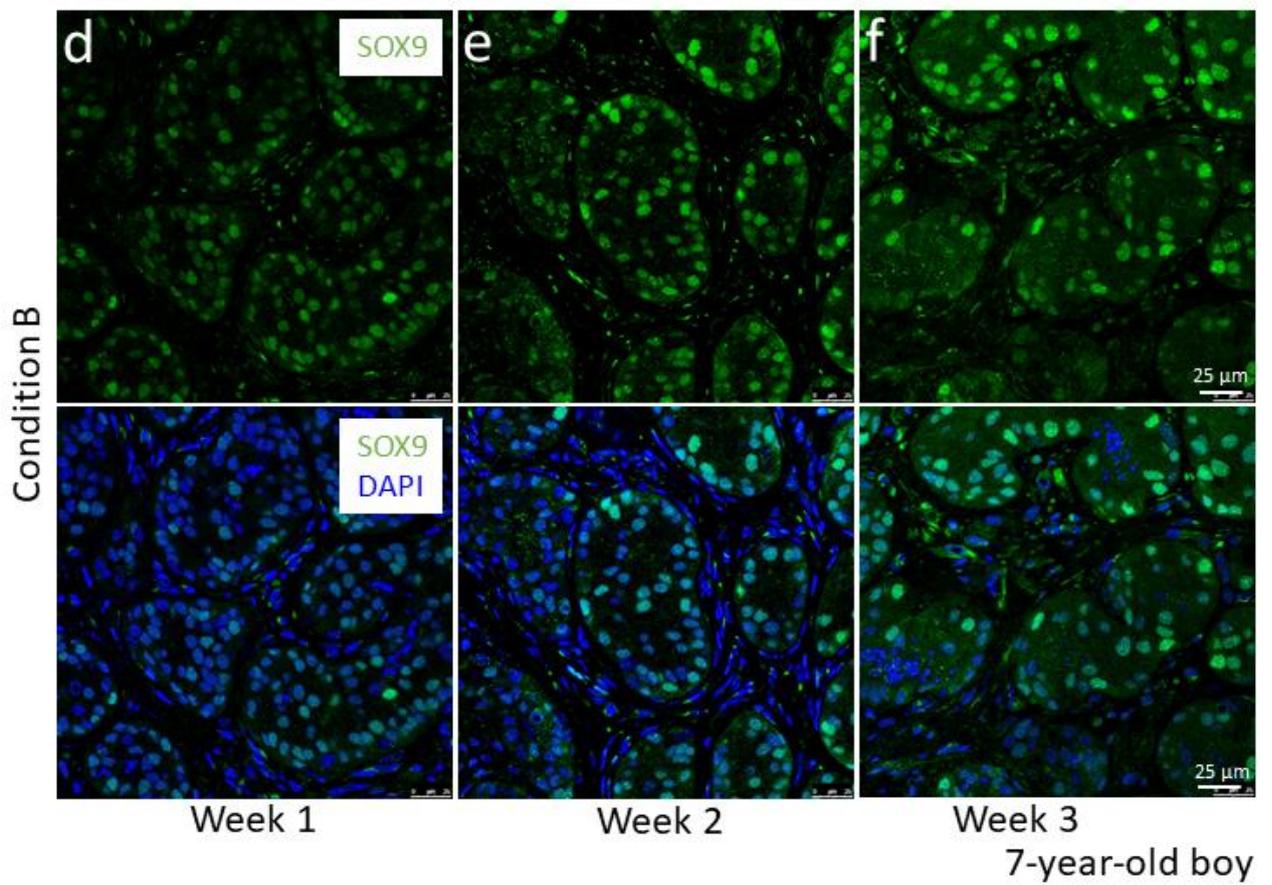
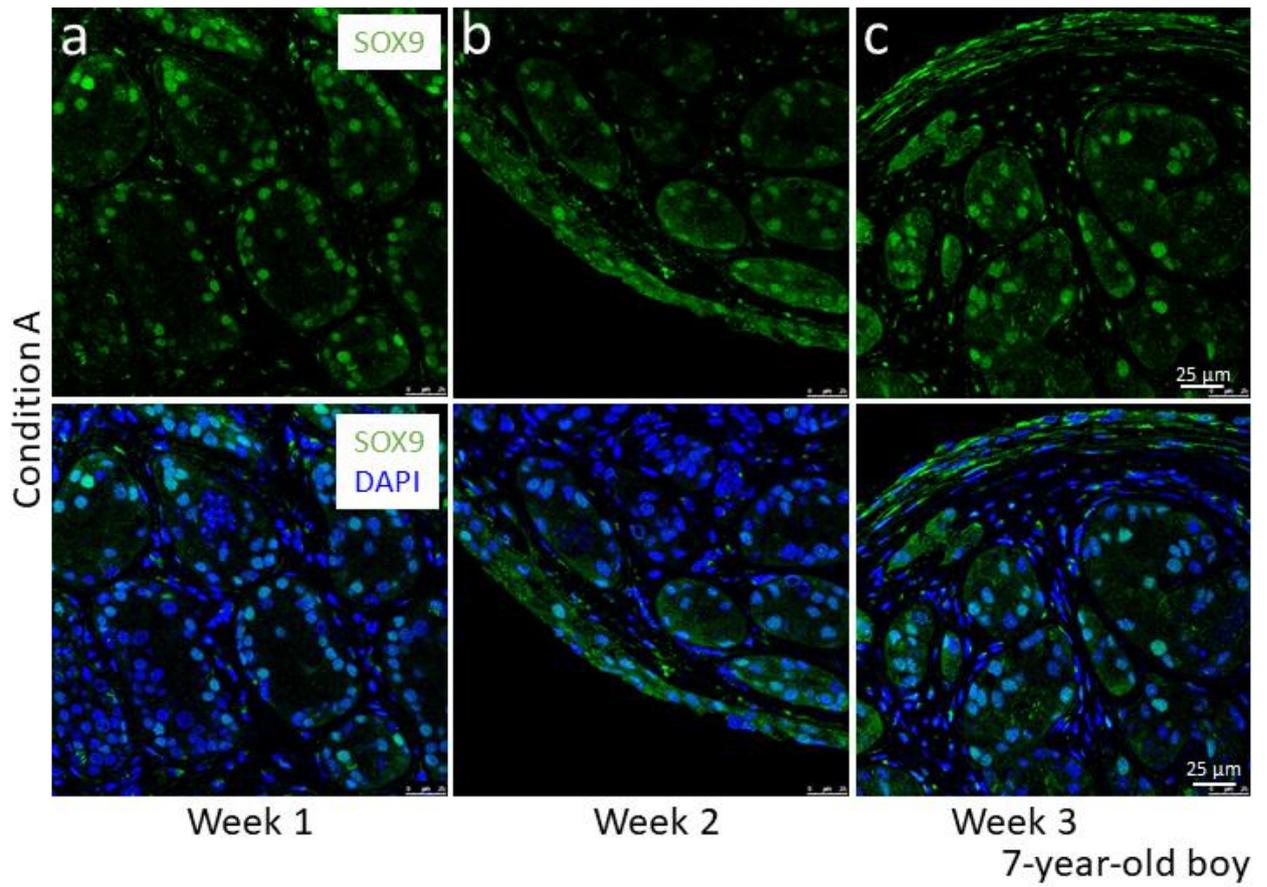


Figure S4 (1/2) SOX9 expression in Sertoli cell nuclei.

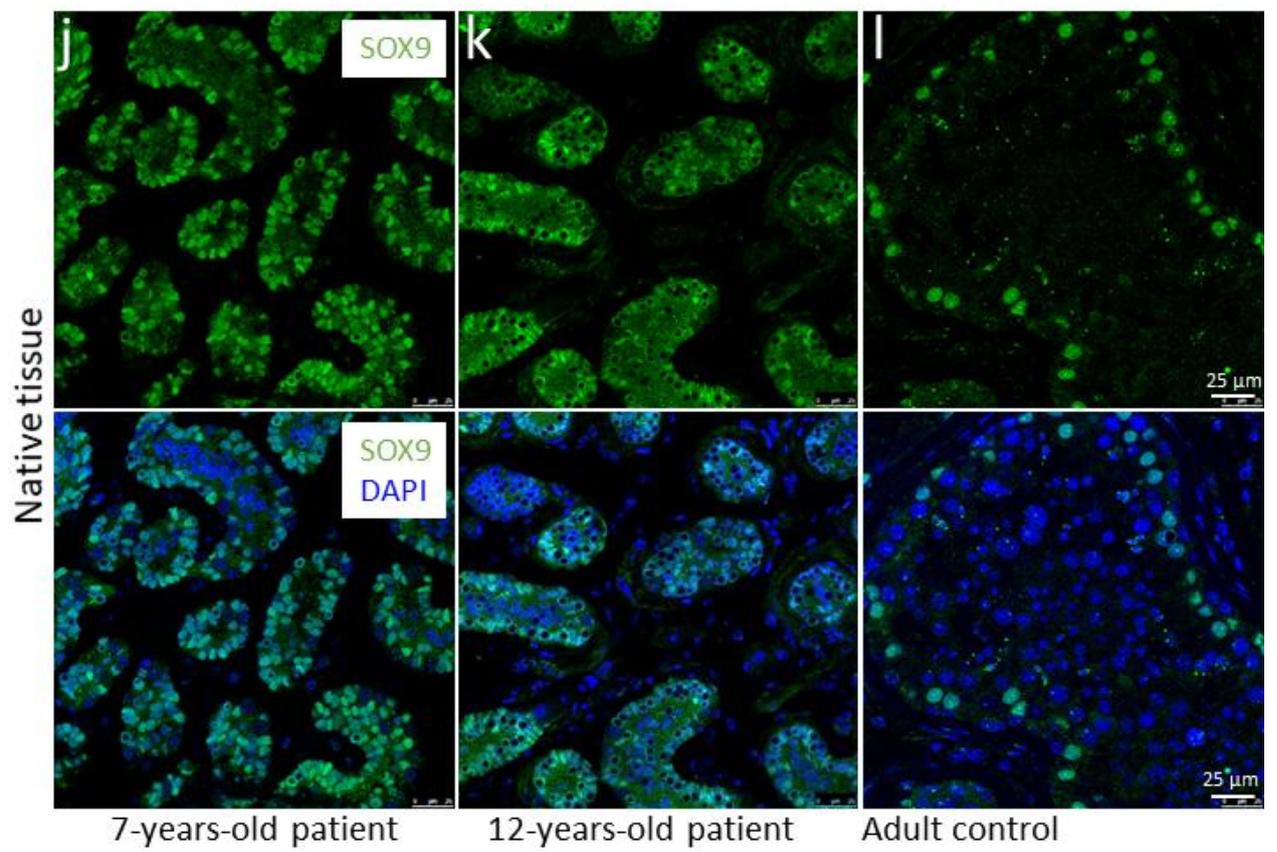
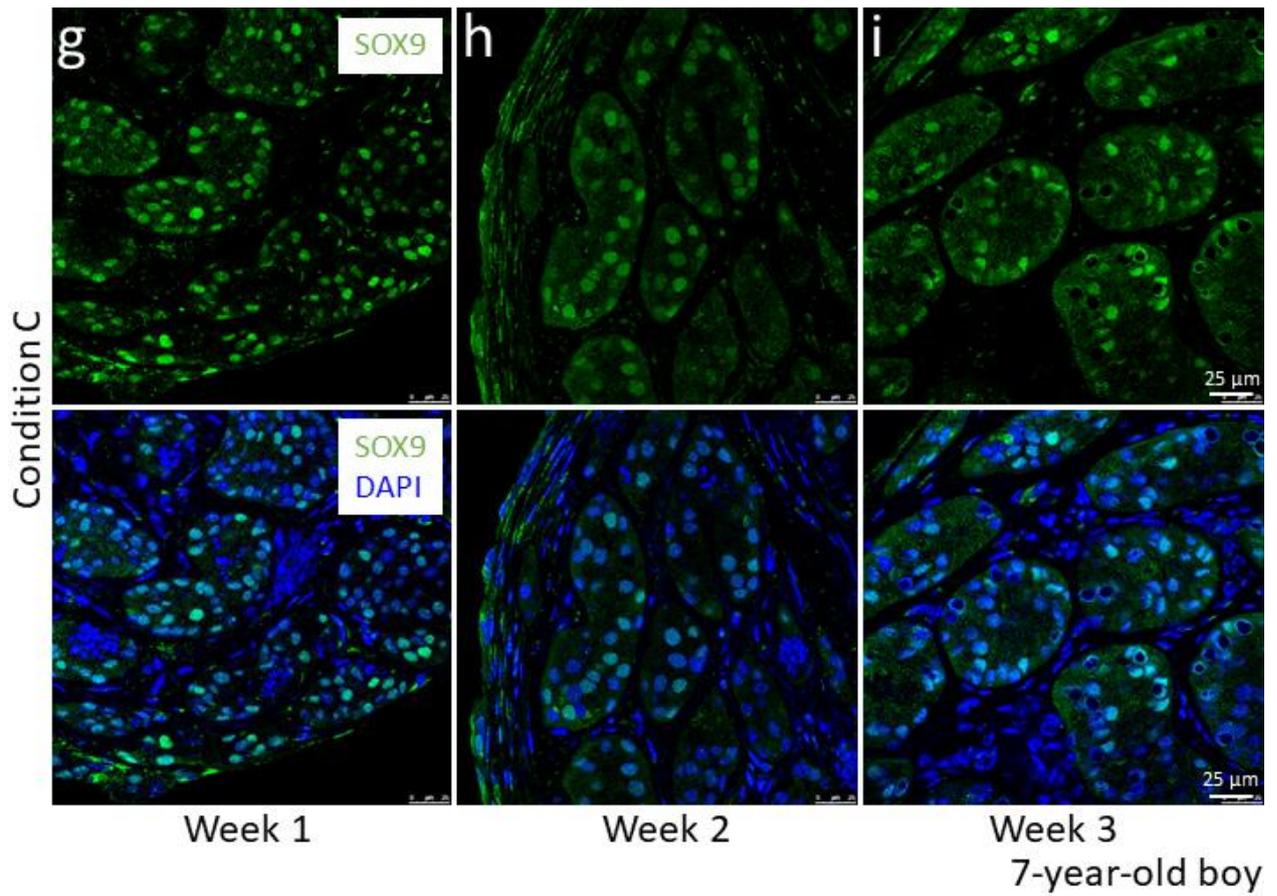


Figure S4 (2/2) SOX9 expression in Sertoli cell nuclei.

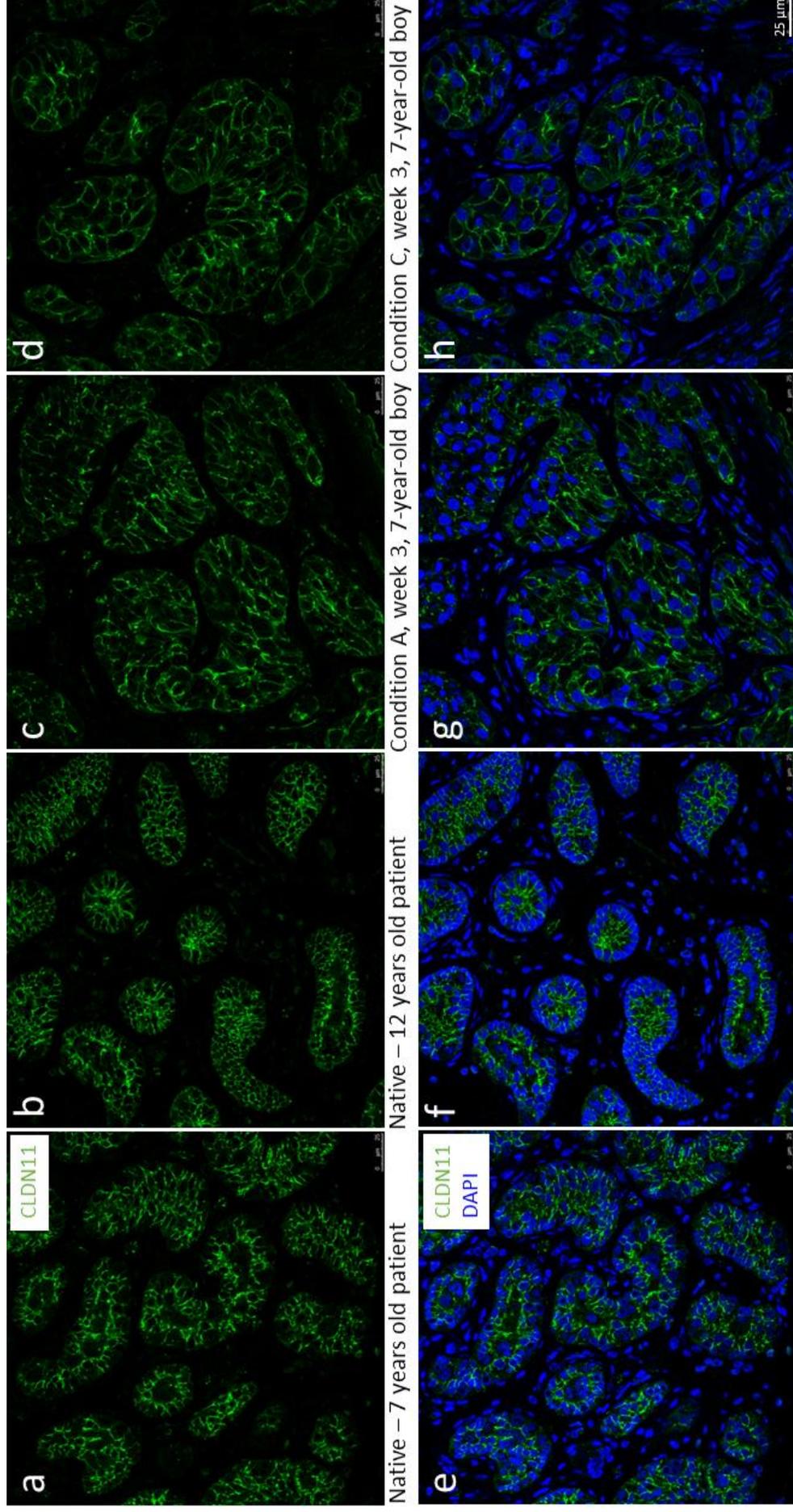


Figure S5 Expression of the BTB component CLDN11 in Sertoli cells in the native prepubertal tissue and after 3 weeks of culture. For reasons of conciseness, Condition B, which showed a similar result, is not presented here.

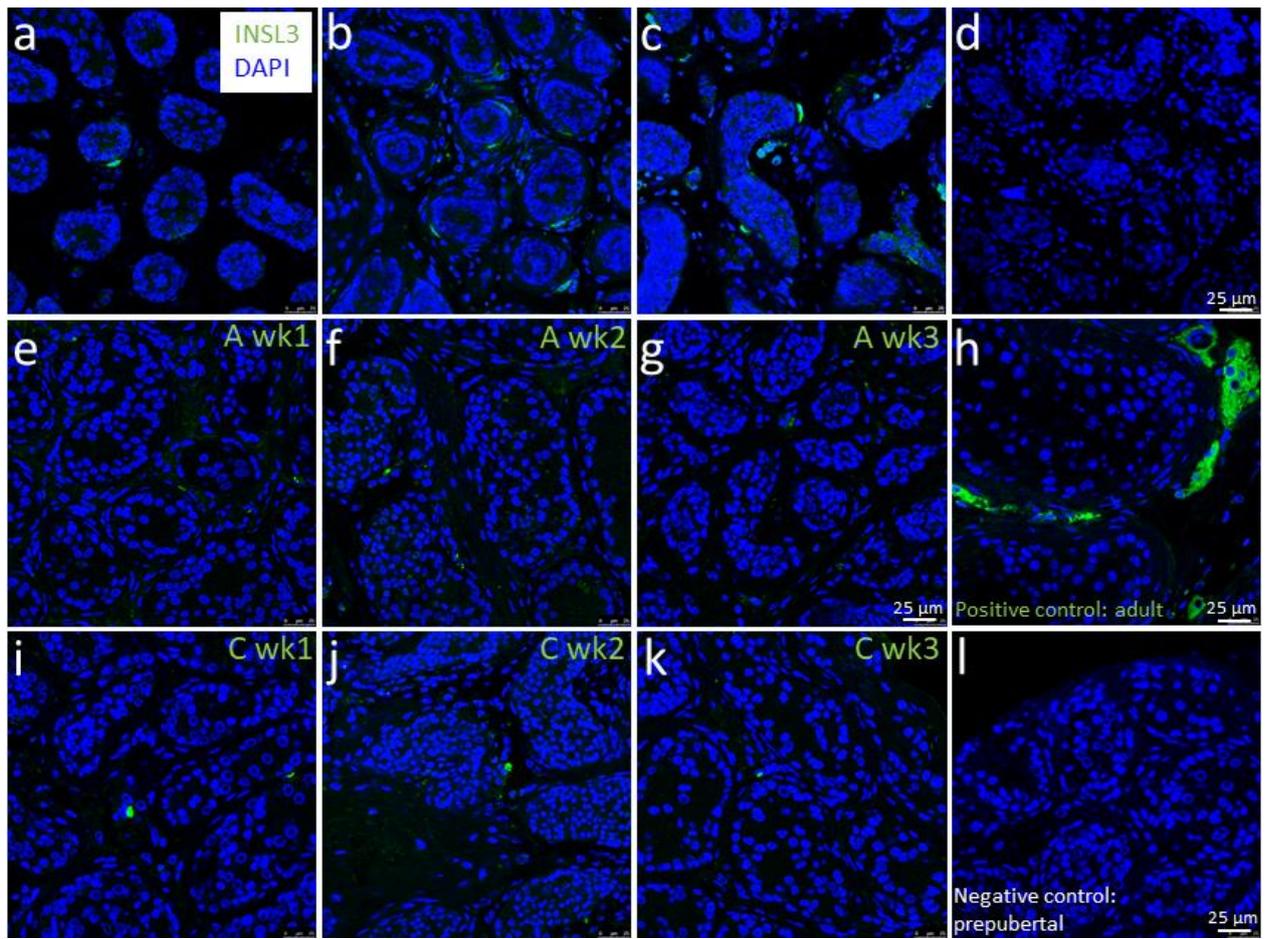
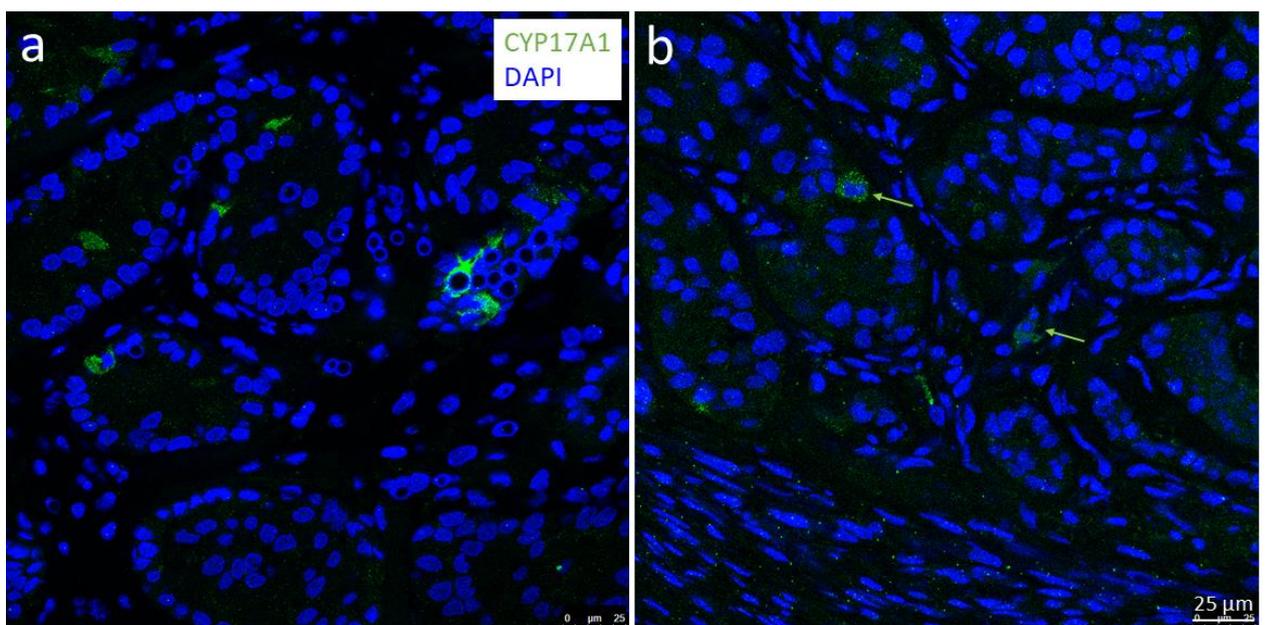


Figure S6 INSL3 expression in the native and cultured prepubertal tissue and controls. (a-c) Native tissue of the 12-year-old patient, and (d) of the 7-year-old patient. (e, f, g, i, j, k) Cultured tissue of the 12-year-old patient. (h, l) Controls.



Condition C, week 3, 12-year-old patient Condition C, week 3, 7-year-old patient
Figure S7 CYP17A1 expression in the cultured prepubertal tissue.

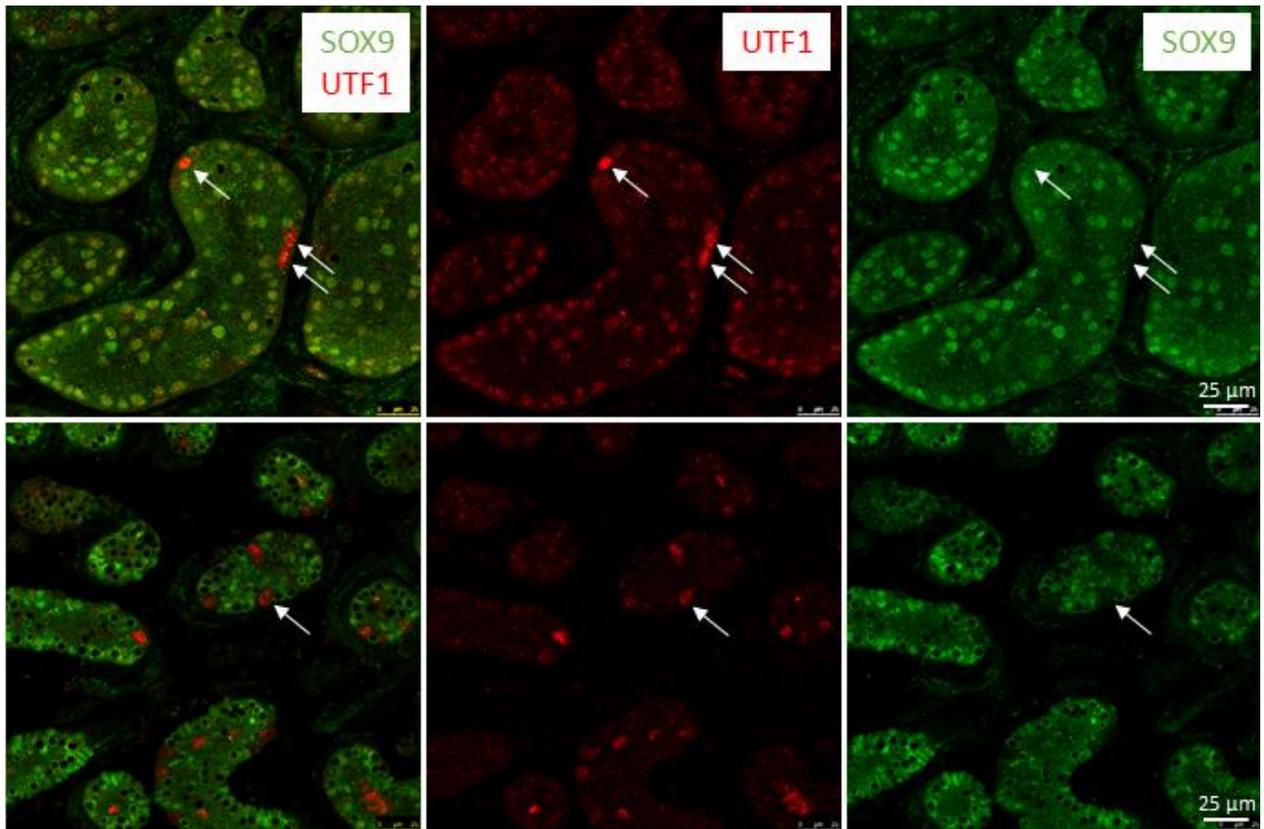


Figure S8 Weak UTF1 expression in SOX9+ Sertoli cells in cultured tissue (upper row) vs. uncultured tissue (bottom row). UTF1+ spermatogonia marked by arrows.

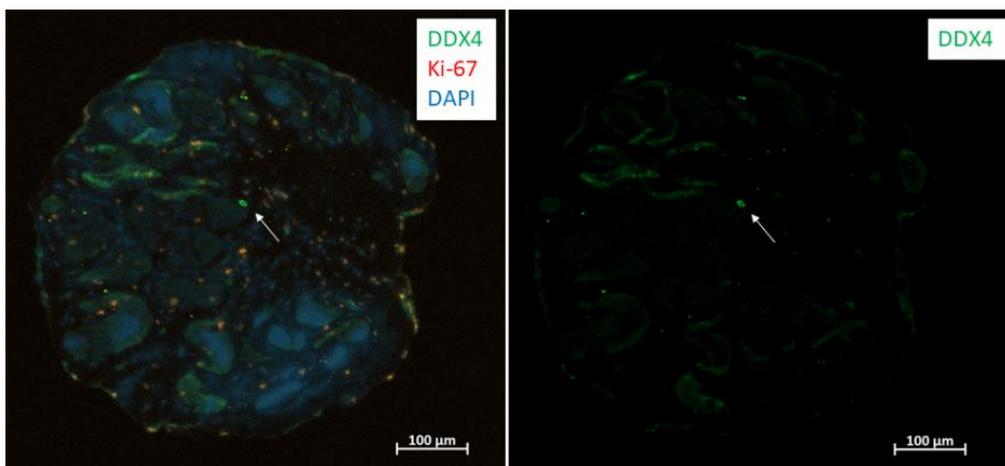


Figure S9 DDX4/Ki-67 staining of cultured tissue from 9-year-old sickle cell disease patient, condition C, week 1.

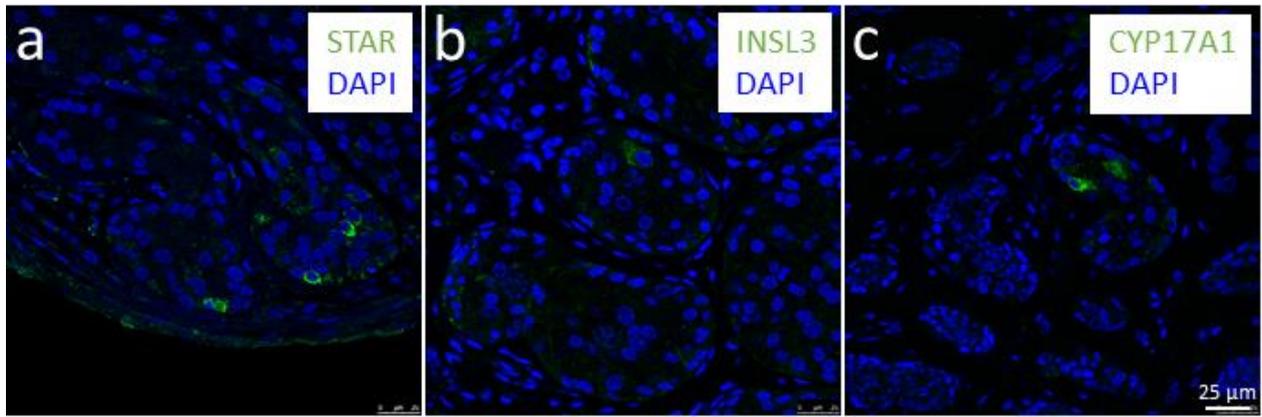


Figure S10 Atypical staining of tubular cells from cultured tissue expressing (a) STAR, (b) INSL3, (c) CYP17A1.

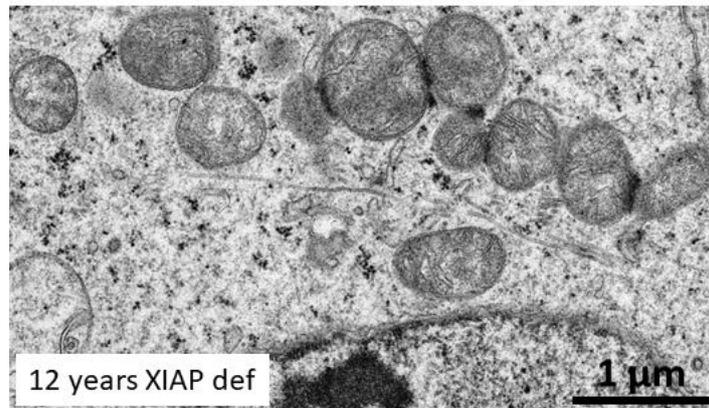
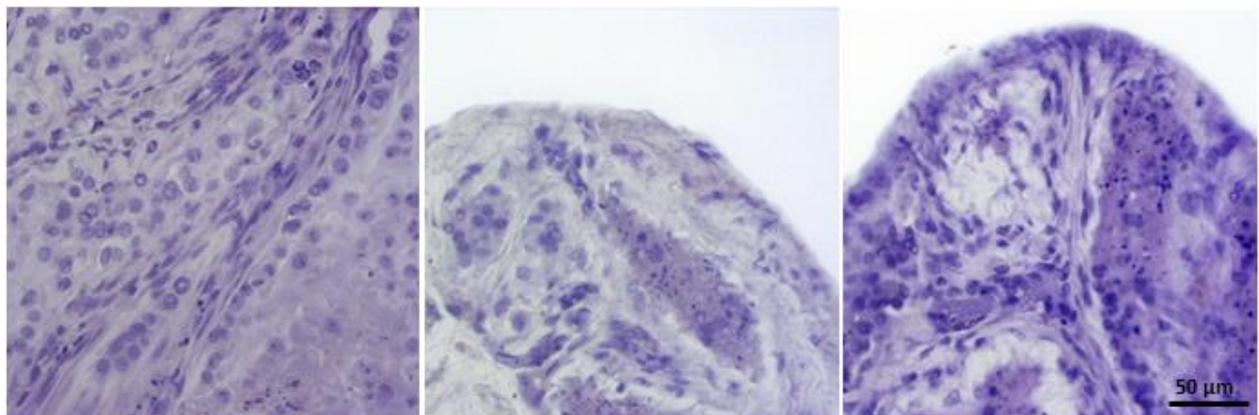


Figure S11 Larger round mitochondria in undifferentiated SPG of the 12-year-old XIAP deficiency patient.



StemFlex™ Medium StemPro®-34 SFM Nutristem®hPSC XF

Figure S12 Adult human testicular tissue cultivated for 2 weeks in different media.

Supplementary table S1: Primary antibody information

Antigen	Company	Order number	Species	Clonality	Dilution
BOULE	Santa Cruz Biotechnology	sc-166660	mouse	monoclonal	1:100
CLDN11	Invitrogen	36-4500	rabbit	polyclonal	1:50
CYP17A1	Santa Cruz Biotechnology	sc-374244	mouse	monoclonal	1:500
DDX4	Abcam	ab13840	rabbit	polyclonal	1:500
INSL3	Abcam	ab227974	rabbit	polyclonal	1:100
LAMA1	Abcam	ab210954	mouse	monoclonal	1:20
MKI67	Agilent Technologies	M7240	mouse	monoclonal	1:50
PIWIL4	Sigma-Aldrich	HPA036588	rabbit	polyclonal	1:100
SMA	Sigma-Aldrich	A2547	mouse	monoclonal	1:500
SOX9	EMD Millipore	AB5535	rabbit	polyclonal	1:100
STAR	Sigma-Aldrich	HPA023644	rabbit	polyclonal	1:100
UTF1	EMD Millipore	MAB4337	mouse	monoclonal	1:50

Supplementary table S2: Secondary antibody information

Secondary antibody name	Company	Order number	Dilution
Alexa Fluor 488 goat anti-mouse IgG	Invitrogen	A11001	1:200
Alexa Fluor 488 goat anti-rabbit IgG	Invitrogen	A11034	1:200
Alexa Fluor 555 goat anti-mouse IgG	Life technologies	A21422	1:80
Alexa Fluor 555 goat anti-rabbit IgG	Life technologies	A21429	1:80

Supplementary table 3: Organ/organotypic culture using human prepubertal testis tissues (2017-2022)

	De Michele et al. 2017	De Michele et al. 2018	Medrano et al. 2018	Portela et al. 2019	Kurek et al. 2021	Wang et al. 2022	Aden et al. 2023
N (experiment)	3	5	4	9	16	4	3 (2)
Donor tissue	2-12 y/o	2-12 y/o	7-14 y/o	6-14 y/o	1.6-13.4 y/o	0.5-1.4 y/o	7-12 y/o
Experiment length	139d	139d	70d	35d	14d	60d	21d
Germ cell markers	MAGEA4 ¹	MAGEA4 ¹ , SYCP3 ¹ , BOULE ¹ , ACE ¹ , PRM2 ¹ , TNP1 ¹	PLZF ² , FGFR3 ² , UTF1 ^{1,2} , SALL4 ² , c-KIT ¹ , DDX4 ¹ , DAZL ² , SYCP3 ^{1,2} , ACR ²	MAGEA4 ¹	DDX4 ¹ , MAGEA4 ¹ , POU5F1 ¹	ACR ¹ , BOULE ¹ , CREM ¹ , DDX4 ¹ , GAGE ¹ , MAGEA ¹ , PRM1 ¹	BOULE ¹ , DDX4 ¹ , PIWIL4 ¹
SPG count after culture	↓	↓	↓	↓	↓	↓	↓
Most advanced germ cell type during culture	not examined	SYCP3+ SPCs, BOULE+ SPCs/ rSPT, rare ACE+ eSPT	SYCP3+ cells	SPG	not examined	BOULE+ SPCs	SPG
SPG proliferation markers	MAGEA4/Ki-67 ¹	MAGEA4/Ki-67 ¹	not examined	MAGEA4/PCNA ¹	only Ki-67 ¹	Morphology, Ki-67 ¹	DDX4/Ki-67 ¹ , PIWIL4/Ki-67 ¹
Proliferative SPG during culture	proliferating SPG↓, ratio* ↑ (maintained until d64)	proliferating SPG↓, ratio* ↑ (maintained until d139)	not examined	↓	Ki-67↑	Ki-67↑	proliferation maintained
Markers used for SC detection	AMH ¹ , AR ¹ , GDNF ¹	AMH ¹ , AR ¹ , GDNF ¹ , SOX9 ¹	AMH ¹ , AR ¹ , SOX9 ^{1,2} , ZO1 ¹	AMH ¹ , AR ¹	-	AMH ¹ , AR ¹ , SOX9 ¹ , ZO1 ¹	ultrastructure ³ , SOX9 ¹ , CLDN11 ¹
SCs during culture	AMH ↓, GDNF, AR constant, AMH secretion↓	AMH↓ (ns), SOX9, AR, GDNF constant, AMH secretion↓	SOX9↓, AMH↓, SOX9/AR↓, ZO1 unorganized, AMH secretion↓	AMH↓, AR not expressed	not examined	SOX9↑, AMH↓, AR induced	SOX9, CLDN11 maintained
LC markers	3β-HSD ¹	STAR ¹	STAR ²	-	-	CYP17A1 ¹ , STAR ¹	Morphology, ultrastructure ³ , CYP17A1 ¹ , INSL3 ¹ , STAR ¹
LCs during culture	no significant changes, testosterone secretion	STAR↑, testosterone secretion	STAR↑ in one condition after 14 days, testosterone secretion	histologically not examined, testosterone secretion	not examined	STAR induced, CYP17A1 maintained	CYP17A1↑, STAR present, INSL3 partially present, steroid hormones secreted
PTC markers	-	-	SMA ²	-	LAMA1 ¹ , LAMA5 ¹ ; type IV collagen ¹ ; fibronectin ¹	SMA ¹	SMA ¹ , LAMA1 ¹
PTCs during culture	not examined	not examined	SMA↑	not examined	LAMA1↓ and weak seminiferous LAMA 5 expression in some samples	induction of SMA in peritubular walls	SMA↑
Supernatant analysis	testosterone, AMH, LDH	testosterone, AMH	testosterone, AMH	testosterone	no	no	progesterone, 17-OHP, androstenedione, testosterone, estradiol via LCMS/MS
	1 IHC	2 PCR	3 TEM		*ratio = proliferating SPG/total number of SPG		

Abbreviations: 17-OHP, 17α-hydroxyprogesterone; 3β-HSD, 3β-hydroxysteroid dehydrogenase; ACE, angiotensin I converting enzyme; ACR, acrosin; AMH, anti-muellerian hormone; AR, androgen receptor; BOULE (BOLL), boule-like RNA binding protein; CLDN11, claudin 11; c-KIT (KIT), KIT proto-oncogene, receptor tyrosine kinase; CREM, CAMP-responsive element modulator; CYP17A1, cytochrome P450 family 17 subfamily A member 1; DAZL, deleted in azoospermia-like; DDX4, DEAD-box helicase 4; FGFR3, fibroblast growth factor receptor 3; GAGE, G antigen; GDNF, glial cell derived neurotrophic factor; IHC, immunohistochemistry; INSL3, insulin like 3; Ki-67, marker of proliferation Ki-67; LAMA1, laminin subunit alpha-1; LAMA5, laminin subunit alpha-5; MAGEA, melanoma-associated antigen A (family); MAGEA4, melanoma-associated antigen 4; PCNA, proliferating cell nuclear antigen; PCR, polymerase chain reaction; PIWIL4, piwi like RNA-mediated gene silencing 4; PLZF (ZBTB16), zinc finger and BTB domain-containing protein 16; POU5F1, POU class 5 homeobox 1; PRM1, protamine 1; PRM2, protamine 2; SALL4, spalt like transcription factor 4; SMA (ACTA2), actin alpha 2, smooth muscle; SOX9, SRY-box transcription factor 9; SPCs, spermatocytes; SPG, spermatogonia; eSPTs, elongated spermatids; rSPTs, round spermatids; STAR, steroidogenic acute regulatory protein; SYCP3, synaptonemal complex protein 3; TEM, transmission electron microscopy; TNP1, transition protein 1; UTF1, undifferentiated embryonic cell transcription factor 1; ZO1 (TJP1), tight junction protein 1