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Adult Adrenal Cortex undergoes Rapid Tissue Renewal in a Sex-Specific Manner

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SUMMARY

Evolution has resulted in profound differences between males and females that extend to non-reproductive organs and are reflected in the susceptibility and progression of diseases. However, the cellular and molecular basis for these differences remain largely unknown. Here we report that adrenal gland tissue renewal is highly active and sexually dimorphic with female mice showing a 3-fold higher turnover than males. Moreover, while in males, homeostasis relies on the proliferation of cells within the steroidogenic zone, females employ an additional stem/progenitor compartment situated in the adrenal capsule. Using lineage tracing, sex reversal models, gonadectomy and dihydrotestosterone treatments, we further show that sex-specific stem cell activity is driven by male hormones that repress recruitment of *Gli1*⁺ stem cells from the capsule and cell proliferation. Taken together our findings provide a molecular and cellular basis for adrenal sex dimorphism that may contribute to the increased incidence of adrenal diseases in females.

INTRODUCTION

Tissue renewal is an essential process that ensures continued organ function in the aging organism. Dramatic progress towards an understanding of tissue homeostasis in many tissues has been made. By contrast, little is known about the extent to which the adrenal cortex renews, despite the fact that it represents one of the main sites of steroid synthesis in the mammalian organism.

Adrenals are composed of a neural crest derived medulla and the adrenal cortex that originates from the intermediate mesoderm. The development of the adrenal cortex commences with an adrenal primordium around E9.5 (embryonic day) in mice (for review see (Bandiera et al., 2015)). SHH, released from this primordium, induces recruitment of surrounding *Gli1*⁺ mesenchymal cells to the steroidogenic lineage (King et al., 2009; Lerario et al., 2017). Once formed, the adult cortex adopts its zonated organization with the zona glomerulosa (zG), zona fasciculata (zF) and zona reticularis (zR) producing mineralocorticoids, glucocorticoids and androgens, respectively. Mice lack the zR, but instead have an X-zone that represents a developmental remnant disappearing at puberty in males and during first pregnancy in females. Recent studies have demonstrated that zonation depends on the antagonistic action of β -catenin and PKA signaling (Drelon et al., 2016). RSPO3, a signalling molecule released from the mesenchymal adrenal capsule, activates β -catenin in sub-capsular steroidogenic cells thus conferring the zG fate (Vidal et al., 2016). Steroidogenic cells situated closer to the medullary position do not receive R-spondin/WNT signals and thus adopt the zF fate (Figure 1A).

Several reports suggested continuous turnover of the adrenal cortex with steroidogenic cells being born in the outer cortex, followed by centripetal migration and stereotypic apoptosis when they reach the cortico-medullary boundary (Chang et al., 2013; Finco et al., 2018; Freedman et al., 2013; Penny et al., 2017; Vidal et al., 2016). However, the vast majority of studies have been restricted to males and differences/similarities in adult tissue turnover between the two sexes remain unknown. This is surprising given the well-established sexual dimorphism in the adrenal gland. Indeed, female adrenals in mice are larger, which has been attributed to an increased zF resulting from differential growth after puberty (Bielohuby et al., 2007). Sexual dimorphism has also been observed on the phenotypic level with several genetic models showing a sex-dependent phenotype (Berthon et al., 2014; Dumontet et al., 2018; Levasseur et al., 2017) and transcriptomic studies revealing sex-specific differences (El

Wakil et al., 2013; Jopek et al., 2017). Most importantly, adrenal pathologies including Cushing syndrome (Steffensen et al., 2010), Addison's disease (Ten et al., 2001) and certain adrenal cortical cancers are up to 6 times more frequent in women than in men (Audenet et al., 2013; Ayala-Ramirez et al., 2013; Scollo et al., 2016). Despite these striking differences, the cellular basis of these sex bias remains largely unknown.

Here, we addressed the cellular dynamics of adrenal tissue homeostasis in both sexes. We show that the adult adrenal cortex is subject to rapid and sex-specific turnover that results in a complete replacement of the steroidogenic tissue every 3 months in females. We further show that while renewal in male adrenals is primarily driven through proliferation of cells situated in the zG, female adrenals also recruit mesenchymal cells from the capsule. Both, proliferation and stem cell recruitment are suppressed by male hormones, thus providing a molecular explanation for the sex-specific activity.

RESULTS

Adrenal cortex renewal is sex-dependent

To address whether adrenal sex differences may be reflected in tissue renewal, we first used BrdU labelling (2 days pulse) (Figure 1B) in fully matured (12 weeks of age) mice and quantified proliferation rates in different adrenal compartments. BrdU labelled steroidogenic cells were predominantly observed in the zG and the outer zone of the zF, whereas deeper zones were devoid of proliferation (FigureS1A), consistent with a previous report (Chang et al., 2013). Overall, females showed a 6.3-fold ($p=0.007$) higher proliferation rate in steroidogenic cells, when compared to males (Figure 1B, Figure S1A). Differences were particularly striking in the outer zF, where we observed a 15-fold higher number of proliferating cells in females ($p<0.0001$; Figure 1B). The much higher proliferation rate in females was surprising, as organ size is maintained throughout adult life in both sexes. We therefore speculated that the high level of proliferation in females maybe counter-balanced by increased apoptosis. Indeed, quantification revealed a 2.0-fold ($p\text{-value}=0.038$) higher number of Caspase 3 positive cells at the juxtamedullary boundary (Figure1C, FigureS1B), thus ensuring maintenance of tissue size.

To gain insights into the cellular dynamics of adrenal cortex homeostasis, we next performed lineage-tracing experiments using tamoxifen-inducible Cre lines to activate GFP expression (*mTmG*) (Muzumdar et al., 2007) in different cellular compartments. *Axin2* and *Wnt4* are two

β -catenin (CTNNB1) downstream targets and tamoxifen activation with *Axin2-CreERT2* (Wang et al., 2015) or *Wnt4-CreERT2* driver lines (Kobayashi et al., 2008) resulted in GFP-labelling of zG cells in both sexes two days after induction (Figure 1D; Figure S1C, D). Over time the number of GFP-labelled cells increased and in females the entire cortex was replaced by 3 months after induction (Figure 1D). Quantification of GFP-labelled cells using the *Wnt4-CreERT2* line revealed that renewal was considerably slower in males (Figure 1E, Figure S1E) and only 41,8% and 74,6% of cells were replaced after 3 and 6 months, respectively, despite the fact that males exhibit a significantly thinner cortex when compared to females (Bielohuby et al., 2007).

Female-specific recruitment of capsular stem cells

A higher index of proliferation in females was not limited to steroidogenic cells, but was also detected in NR2F2⁺ capsular cells (4-fold, p-value=0.0016, Figure 2A; Figure S2A). Since capsular cells are essential for the initial formation of the adult adrenal cortex (King et al., 2009), we wondered whether this population may also contribute to sex-specific adrenal cell renewal. Lineage-tracing experiments using the *Gli1-CreERT2* strain (Ahn and Joyner, 2004) in 3-weeks-old animals (1-month chase) revealed recruitment of capsular cells to the steroidogenic lineage in both sexes (Figure S2B, C). By 6 weeks after birth, however, this contribution had almost entirely ceased in males (Figure S2B) and only occasional GFP⁺ steroidogenic cells were detected in adults even after long-term labelling (Figure 2B). In contrast, induction in adult females (12 weeks) resulted in recruitment of GFP-labelled steroidogenic (zG and zF) cells as early as 1 week after induction (Figure 2B, Figure S2D). After 1 month, the number of capsule-derived steroidogenic cells was 16.1-fold higher in females than in males (Figure 2C, Figure S2B, p-value=0,013). Upon reaching the zF, a proportion of GFP⁺ cells further proliferated (Figure 2D; Figure S2E). Taken together these data show that while cell turnover in adult males relies on progenitors located within the zG, in females, capsular stem cells continuously proliferate and contribute significantly to tissue renewal. GFP-label was maintained in the capsule over the entire period analyzed (6 months) indicating continuous self-renewal of this compartment (Figure S2F).

Adrenal cortex homeostasis depends on gonadal rather than chromosomal sex.

To assess whether the observed sexual dimorphism is sex-chromosome dependent, we employed *Sfl-Cre; Sox9^{fl/fl}* XY (Lavery et al., 2012) and *Tg(Wt1:Sox9)* XX (Vidal et al., 2001) mice, two genetic models that present sex reversal phenotypes and develop as XY females and XX males, respectively (Figure 3A and Figure 3B). Morphological and histological analysis revealed that *Tg(Wt1-Sox9)* XX males developed adrenals that lacked the X-zone and resembled wildtype male organs in both weight and histology (Figure S3A and Figure S3B). Analogously, XY females (*Sfl-Cre; Sox9^{fl/fl}* XY) had significantly larger adrenals than male controls and displayed the X-zone, thus resembling control females (Figure S3C and Figure S3D). Quantification of BrdU incorporation in the capsular compartment revealed a significant increase in proliferation in XY females (Figure 3A; p-value=0.0024) and a decrease in XX males (Figure 3B; p-value=0.0006), when compared to control males and females, respectively. Strikingly, lineage-tracing analysis of *Gli1*⁺ cells on a *Tg(Wt1-Sox9)* transgenic background (induction at 12 weeks, 1 month chase) showed that GFP⁺ cells in XX males remained restricted to the capsule, a pattern resembling XY males rather than XX females (Figure 3C, D; p-value=0.0051).

The above sex reversal analysis suggested that sex-specific proliferation and recruitment does not depend on sex chromosomes, but is rather controlled by hormones. To confirm this hypothesis, we gonadectomized 10-weeks old *Gli1-CreERT2;mTmG* males and females, induced GFP expression by tamoxifen treatments 2 weeks later and analyzed adrenal glands after 4 weeks chase (Figure 4A). Consistent with a previous report (Hershkovitz et al., 2007), bilateral orchiectomy induced a secondary X-zone (20 α HSD⁺) at the juxta-medullary boundary (Figure S4A). Proliferation and cell recruitment of capsular cells was unaffected in adrenals in females that had undergone ovariectomy (Figure 4A-C) suggesting that ovarian hormones, such as estrogens and progesterone, do not significantly influence adrenal cell renewal. By contrast, orchiectomized males showed a 3.5-fold increase of the proliferative index when compared to sham males (Figure 4A; p-value<0.0001). Similarly, recruitment of capsular cells to the steroidogenic lineage was 3 times higher after testis removal than in sham males (Figure 4B, C; p-value=0.0012) suggesting that the reduction of androgens permitted activation of this stem cell compartment.

Capsular stem cell activity is inhibited by androgen treatment

To further assess the contribution of androgens to sex-specific differences in adrenal homeostasis, we performed ovariectomy of *Gli1-CreERT2;mTmG* females at 10 weeks of age and treated them for 6 consecutive weeks with dihydrotestosterone (DHT), the primary

androgen produced by testes (Figure 4D). As reported previously (HersHKovitz et al., 2007), DHT administration induced X-zone regression (Figure S4B), demonstrating successful treatment. BrdU labeling revealed a significant reduction of capsular cell proliferation 3.2 fold compared to controls (Figure 4D; p-value<0.0001). Importantly, the recruitment of *Gli1*⁺ capsular cells was almost entirely suppressed (81-fold decrease, Figure 4E and Figure S4C; p-value=0,0002). Moreover, DHT treatment of pregnant females (E14.5 onwards) also strongly inhibited capsular cell recruitment during adrenal development (26.7-fold; p-value<0.0001; Figure S4 D-F). We conclude that androgens negatively impact proliferation and capsular cell recruitment.

Discussion

Studying tissue homeostasis is an important objective when considering aging, responses to tissue damage and its links to cancer. Previous studies have suggested that the adrenal cortex is undergoing active cell replacement, but the degree of tissue turnover and a potential influence of sex on this process remained poorly characterized. Here we have performed an in-depth analysis of tissue dynamics in the adult adrenal cortex and demonstrate that this tissue is subject to an unexpectedly high level of cell renewal that leads to a complete replacement of the steroidogenic compartment every few months. The directed proliferation in the outer cortex, centripetal migration of cells and stereotypic apoptosis at the cortico-medullary boundary suggest an organ-intrinsic requirement for this rapid turnover. As steroid hormone synthesis generates reactive oxygen substrates (ROS) as byproducts (Prasad et al., 2014; Zangar et al., 2004), we can speculate that the active tissue renewal has been adopted to avoid an accumulation of damage in steroid producing cells. Future experiments are needed to address this hypothesis.

Even more surprising than the rapid turnover of the adrenal cortex are the dramatic differences observed in stem cell dynamics between males and females. A recent paper by Dumontet et al. (2018) reported sex differences in the migration of adrenal steroidogenic cells in the postnatal period, but their experimental setup (constitutive Cre-line) did not allow them to analyze tissue turnover in adults. Our study now demonstrates that a striking sex-difference in tissue renewal persists throughout life. Although we did not perform lineage tracing beyond 6 months, by extrapolating from our data we estimate that the male adrenal cortex appears to be replaced every 9 months, which is in stark contrast to the rapid turnover of 3 months in females. In addition to the differences in proliferation, our study demonstrates that females,

but not males, continuously recruit capsular stem cells to the steroidogenic cell lineage. Why females require this high level of tissue turnover and the need for continuous recruitment of capsular cells is presently unknown, but we can assume a physiological reason for this dimorphism. In this respect, it will be interesting to determine whether the recruited cells are distinct in terms of transcriptional signature and/or hormone production.

Apart from organs involved in the reproduction (including the mammary gland), stem cell activity is usually not considered to be sex-specific. However, a recent study in *Drosophila* reported differences in gut stem cell proliferation that appears to be under control of the chromosomal sex determination system (Hudry et al., 2016). By contrast, the here reported stem cell dimorphism involves hormonal control. The fact that orchietomy rather than ovariectomy influenced proliferation and stem cell recruitment, suggested that androgens rather than female hormones regulate adrenal cortex turnover. In line with this hypothesis, hormone treatment with dihydrotestosterone suppressed proliferation and capsular stem cell recruitment in ovariectomised females. Testosterone has been shown previously to impact proliferation of human mesenchymal stem and endothelial progenitor cells at least *in vitro*, although this study described a pro-proliferative action (Corotchi et al., 2016). That androgens can influence adrenal gene expression and the phenotype in a mouse model for Cushing syndrome has been recently reported (Dumontet et al., 2018) and it will now be important to determine how transcriptional changes translate into altered stem cell activity.

It is tempting to speculate that the dramatic differences in adrenal stem cell activity of opposite sex are linked to the much higher prevalence of adrenal diseases in women. Indeed, the significantly higher proliferation rate observed in females is expected to cause an accumulation of mutations and thus increase the risk of developing cancer. Future research will need to focus on how these differences translate into sex-specific diseases, which in the long run may pave the way to the development of sex-specific treatment options.

As sexual dimorphism on the histological, molecular and phenotypic level is not restricted to the adrenal gland (Karp et al., 2017), it is likely that sex-specific stem cell activity may also occur in other organ systems. Surprisingly, research in many fields, but in particular in endocrinology, is often limited to one sex (often male) to avoid the recurring physiological changes due to changes of hormones in the menstrual cycle (Beery and Zucker, 2011; Yoon et al., 2014). Our data highlight the profound differences sex hormones have on tissues and underline the importance of analyzing phenotypes and basic molecular processes in both sexes.

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Declaration of Interests

The authors declare no competing interests.

Author contributions

Conceptualization, A.G., M-C.C. and A.S. Investigation, A.G., B.D. and B.K. Surgical procedures, F.J.M. and A.S. Writing - Original Draft, A.G. and A.S. Writing – Review and Editing, all authors.

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Figure Legends

Figure 1. Adrenal cortex renewal is sex-dependent. **A)** Schematic drawing of the adrenal cortex with *Gli1*⁺ and *Axin2*⁺/*Wnt4*⁺ stem/progenitor cells residing within the capsule and zG, respectively. **B)** BrdU analysis (treated for 2 days) in 12 weeks old animals demonstrated increased proliferation rates in the female zG (5-fold; p=0.0038), outer zF (15-fold; p<0.0001) and the overall adrenal cortex (6.3-fold; p=0.007), when compared to males. **C)** Similarly, apoptosis (active Caspase3⁺ cells) in female adrenals was also higher than in males (2-fold; p<0.05). **D)** Cell lineage-analysis (*Axin2-CreERT2*) confirmed the more rapid turnover in females. Scale bar 100 μm. **E)** Quantification using *Wnt4-CreERT2* mice shows complete replacement of the female, but not the male adrenal cortex in 3 months. Lineage analysis with the *Axin2-CreERT2* line gave similar results (not shown). See also Figure S1.

Figure 2. Female-specific recruitment of capsular stem cells. **A)** Capsular (NR2F2⁺) cells show a higher proliferation rate in females than in males (3.7-fold; p=0.0016; 12 weeks old animals treated for 1 week with BrdU). **B)** Lineage tracing revealed continuous recruitment of capsular cells to the adrenal cortex in females. Inset: *Gli1*⁺ cells give rise to both zF and zG (DAB2⁺) cells. Scale bar 100 μm. **C)** The number of cortical cells derived from the capsule is significantly higher in females (zG: 18.1-fold, p-value=0.0003; zF: 15.9-fold, p-value=0.0044; total cortex: 16.1-fold, p-value=0.0026). **D)** Once in the outer zF, proliferation of recruited female capsular cells is increased in females (11.5-fold; p=0.013; For all statistical analysis: two-tailed t-test). See also Figure S2

Figure 3. Adrenal cortex homeostasis depends on gonadal rather than chromosomal sex. **(A)** Proliferation of capsular cells in a genetic model for male to female sex reversal (*SflCre*⁺/*Sox9flox/flox* XY) was increased when compared to XY males (3.1-fold, p-value=0.0024), but remained lower than in XX females (1.8-fold, p-value=0.0003). and **B)** Proliferation of capsular cells was decreased in sex reversed XX males (*Tg(Wt1:Sox9)* XX) when compared to XX females (4.7-fold; p-value=0.0006). **C)** *Gli1-CreERT2* lineage tracing demonstrates absence of capsular cell recruitment in adult XX males. Scale bar 100 μm. **D)** Quantification of the lineage tracing experiment. XX males show a decreased recruitment compared to XX females (55-fold; p-value=0.0051; For all statistical analysis: two-tailed t-test). See also Figure S3.

Figure 4. Capsular stem cell activity is inhibited by dihydrotestosterone (DHT). **A)** Orchiectomized males exhibit increased proliferation of capsular cells (3.5-fold, p-value<0.0001), albeit slightly lower than control females (1.4fold; p-value=0.0041). **B, C)** Recruitment of capsular cells is significantly increased in orchiectomized males (3-fold; p-value=0.0012), but unaffected in oophorectomized females. Scale bar 50 μ m. **D)** DHT-treatment of adult ovariectomised females results in a dramatic decrease of cortical proliferation (3.2-fold; p-value<0.0001) and **(E)** the number of GFP⁺ cells derived from the capsule (81-fold; p-value=0,0002). See also Figure S4.

STAR Methods

Lead contact details:

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Andreas Schedl (schedl@unice.fr)

Experimental models

In vivo animal studies

All animal work was conducted according to national and international guidelines and approved by the local ethic committee (CIEPAL: PEA-NCE/335) and the French ministry for agriculture. *Axin2-CreERT2* (Wang et al., 2015), *Wnt4-CreERT2* (Kobayashi et al., 2008), *Gli1-CreERT2* (Ahn and Joyner, 2004), *Wt1:Sox9* (Vidal et al., 2001), *Sfl-Cre;Sox9flox* (Lavery et al., 2012) and *mTmG* (Muzumdar et al., 2007) lines were previously reported. If not otherwise stated, all lines were maintained and bred on mixed genetic background. Male and female mice were equally used and littermates were preferentially chosen for experimental group. Unless specifically stated, all experiments were conducted at 12 weeks of age.

Method details

Proliferation quantification

For proliferation quantification in the adrenal capsule and cortex, 1mg/ml of BrdU (Sigma) was dissolved in autoclaved water with 5% sugar and given as drinking water to 12weeks of age C57Bl/6J (Charles River, Europe) mice. The solution was changed every other day and mice were sacrificed after 48hours (cortex) or 7 days of treatment (capsule). Tissues were processed as described for Immunofluorescence analysis. Every 5th section was stained for NR2F2 and BrdU (capsule) or DAB2 and BrdU (cortex).

Cell lineage tracing

Axin2-CreERT, *Wnt4-CreERT2*, and *Gli1-CreERT2* were activated by a single intraperitoneal injection on two consecutive days of Tamoxifen (Sigma). 100 μ l/20g of mouse weight from a solution of 20mg/ml of Tamoxifen in corn oil was administrated per mouse. For adult experiments, animals were induced at 12 weeks of age (unless stated otherwise). For experiments during embryogenesis, the CRE recombinase was activated at E15.5 by intraperitoneally injecting 100 μ l/20g of mouse weight (pregnant female) from a solution of 20mg/ml of Tamoxifen (made up in corn oil).

Hormonal treatment

Beginning two weeks after surgery, oophorectomized females were injected twice daily subcutaneously with 5 α -androstane-17 β -ol-3-one (75 μ g in 5% ethanol and corn oil) or oil only for a period of 6 weeks. For developmental studies, pregnant females were injected twice daily subcutaneously with 5 α -androstane-17 β -ol-3-one (75 μ g in 5% ethanol and corn oil) from E14.5 onwards.

Immunofluorescence analysis

For immunofluorescence analysis of paraffin-embedded samples, adrenal tissues were fixed overnight at 4°C with 4% paraformaldehyde, progressively dehydrated and paraffin embedded. Sections of 5 μ m were cut and unmasked with PT Link (Dako Agilent Pathology Solutions) at pH6. Sections were blocked for 1h with 10% Normal Donkey Serum (Jackson Immunoresearch), 3% BSA (Sigma) and 0,1% Tween-20 (Sigma). Primary antibodies were applied overnight at 4 degrees diluted in 3% NDS, 3% BSA, 0,1% Tween-20 as follows: anti-NR2F2, anti-DAB2, anti-3 β -HSD, anti-AR and anti- α 20-HSD at 1:200; anti-BrdU (NB500-169) at 1:100; anti-BrdU (3D4) at 1:250; anti-GFP at 1:500. The next day, sections were incubated at room temperature with secondary antibodies diluted 1:400 in PBS-0.1%Tween-20 and mounted in antifade mounting medium with DAPI (Vectashield). For immunofluorescence analysis of thick sections, freshly dissected adrenals were fixed for 1hour in 4% paraformaldehyde at 4°C, embedded in low melting point agarose and cut with a vibratome at a thickness of 50 μ m per section. Sections were mounted in antifade mounting medium with DAPI.

Quantification and statistical analysis

Proliferation analysis

Counting results were processed using Graph Pad Prism 7.00 for Windows (GraphPad Software). Statistical significance was determined with a two-tailed unpaired Students t-test. For the quantification of the proliferation in the capsule, 5 sections of whole adrenals were counted per animal (n=5 males, n=6 females). The number of double positive cells for NR2F2 and BrdU was normalized on the total number of NR2F2 positive cells. In the cortex, 3 sections were counted per animal (n=4 males and females). The number of double positive cells for BrdU and DAB2 was normalized on the total number of DAB2 positive cells for the *zona glomerulosa*. For the

zona fasciculata, the number of BrdU positive cells was normalized on the total number of cells. The quantification of the cell turnover was used by measuring the distance from the capsule to the innermost GFP positive cell normalized on the total thickness of the cortex (excluding the X-zone) in 3 regions of 3 nonconsecutive sections per animal ($n \geq 3$). For proliferation analysis of the recruited capsular cells, the number of BrdU/GFP double positive cells in the cortex was normalized on the total number of GFP positive cells in the cortex ($n=4$ for males and females). For the sex reverse models, both adrenals were weighed for each animal. Mean adrenal weights were normalized on body weight ($n \geq 5$). Gonadectomized animals were compared to age matched animals undergoing the surgical procedure without removal of the gonads. DHT-treated females after oophorectomy were compared to oil-treated females after oophorectomy. For all graphs, error bars represent the standard error of mean and asterisks represent the p-values as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****).

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER	RRID
Antibodies			
Anti-CouptfII (NR2F2)	R&D systems	PP-H7147-00	AB_1964214
Anti-BrdU	Novus Bio	NB500-169	AB_10002608
Anti-DAB2 (E11)	Santa Cruz	sc-136964	AB_2245763
Anti-BrdU (3D4)	Pharmingen™	555627	AB_10015222
Anti-GFP	Abcam	ab13970	AB_300798
Anti-3 β -HSD (P-18)	Santa Cruz	sc-30820	AB_2279878
Anti- α 20-HSD	from Yacob Weinstein	n/a	n/a
Anti-AR	Santa Cruz	sc-816	AB_1563391
Chemicals, Peptides, and Recombinant Proteins			
BrdU	Sigma	B9285	n/a
Tamoxifen	Sigma	T5648	n/a
5 α -androstane-17 β -ol-3-one	Sigma	A8380	n/a
Anti-fade mounting medium with DAPI	Vectashield	H-1200	n/a
Experimental Models: Organisms/Strains			
Mouse: C57Bl/6J	Charles River	n/a	IMSR_JAX:000664
Mouse: mTmG	(Muzumdar et al., 2007)	n/a	IMSR_JAX:007576
Mouse: Axin2-CreERT2	(Wang et al., 2015)	n/a	IMSR_JAX:018867
Mouse: Wnt4-CreERT2	(Kobayashi et al., 2008)	n/a	IMSR_JAX:032489
Mouse: Gli1-CreERT2	(Ahn and Joyner, 2004)	n/a	IMSR_JAX:007913
Mouse: Wt1:Sox9	(Vidal et al., 2001)	n/a	n/a
Mouse: Sox9flox	(Lavery et al., 2011)	n/a	IMSR_JAX:013106
Mouse: Sf1-Cre (high)	(Bingham et al., 2006)	n/a	n/a
Software and Algorithms			
Graph Pad Prism 7.00 for Windows	GraphPad Software	n/a	n/a

Figure 2. Female-specific recruitment of capsular stem cells.

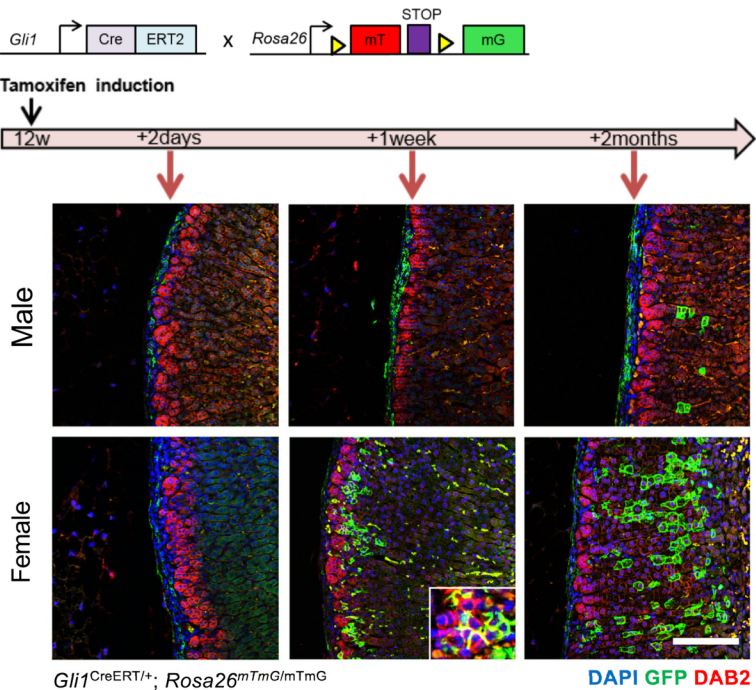
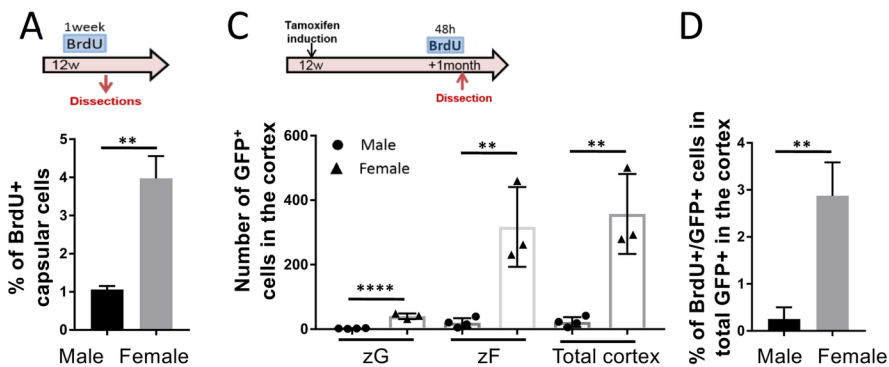


Figure 3. Adrenal cortex homeostasis depends on gonadal rather than chromosomal sex.

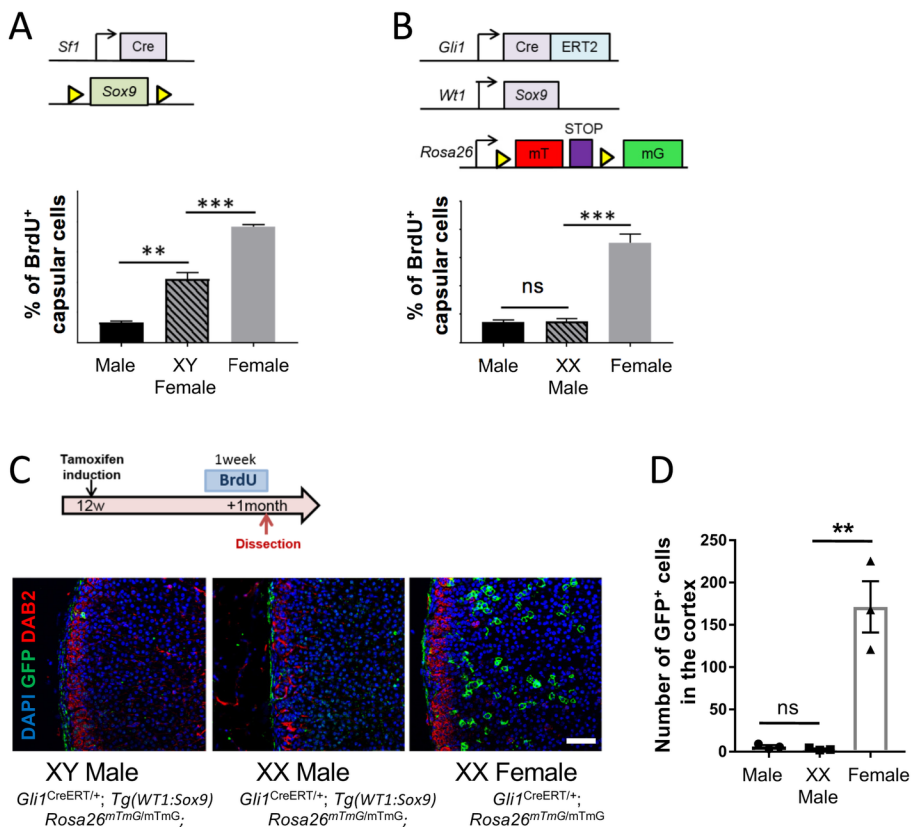
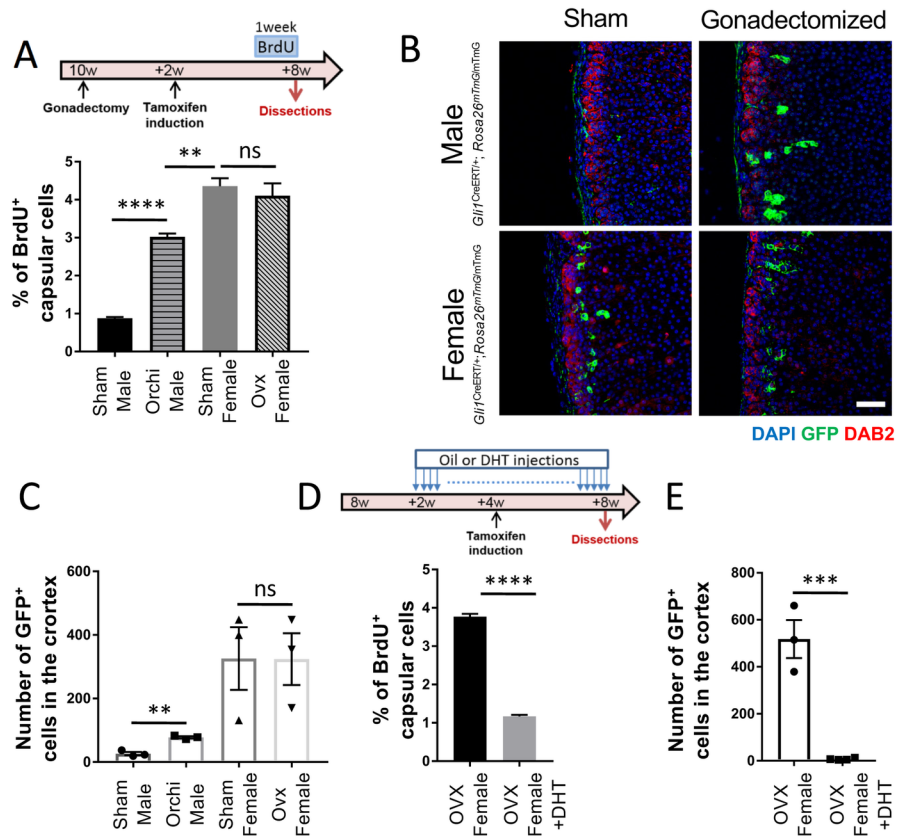
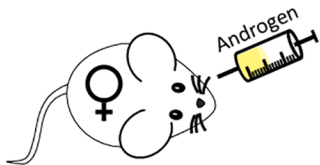
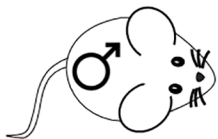


Figure 4. Capsular stem cell activity is inhibited by dihydrotestosterone



**ADRENAL
CORTEX**



Adrenal
Capsule



Zona
Glomerulosa



Zona
Fasciculata

