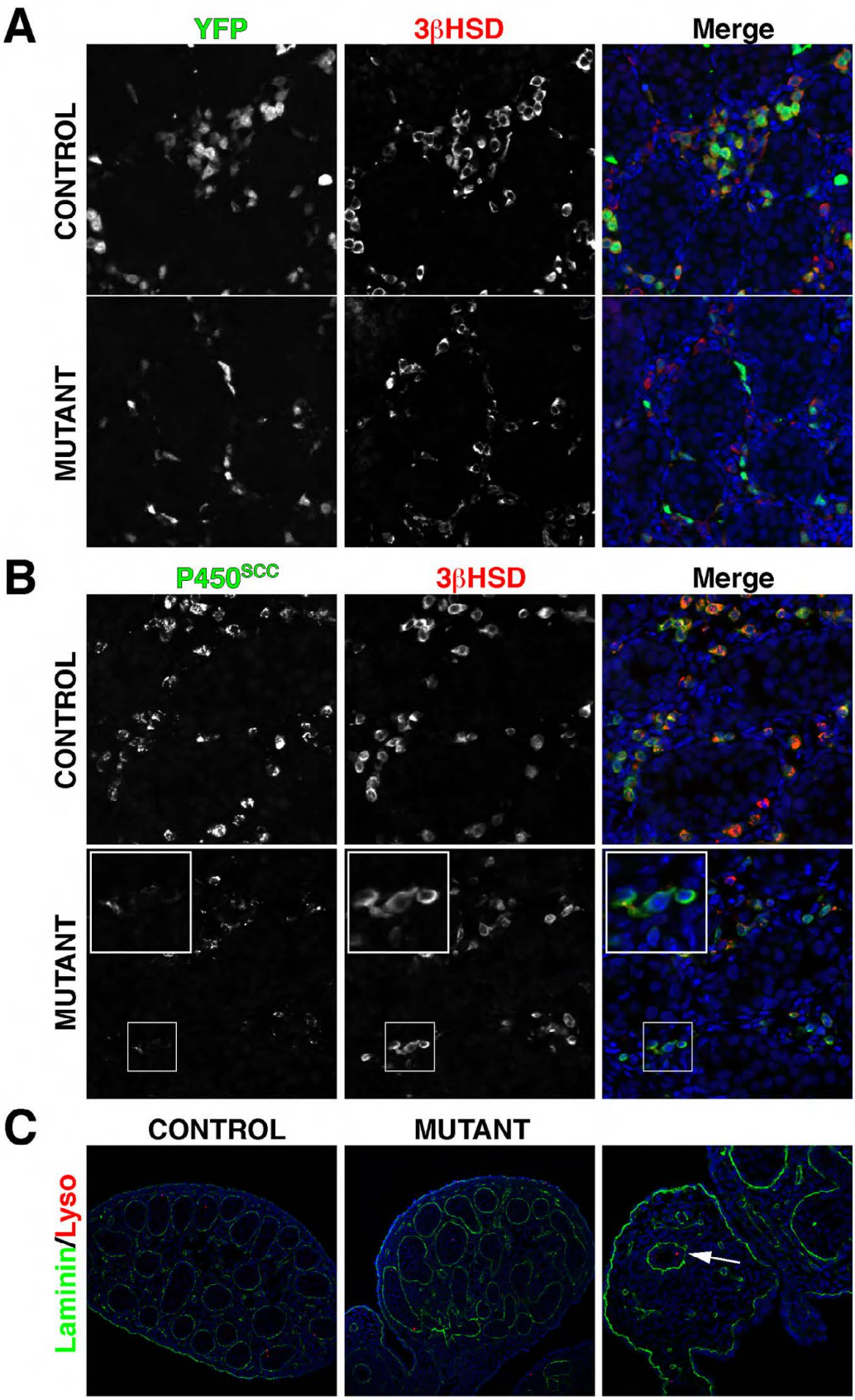

Fig. S1. 3βHSD expression and cell death analysis in E14.5 foetal *Cyp11a1*-Cre;*Sf1*$^{Fl/Fl}$ testes. (**A**) Protein expression of YFP (green) and 3βHSD (red) as detected by co-immunofluorescence in control and mutant E14.5 testes. Interstitial YFP-positive cells in controls are always positive for 3βHSD. These cells exhibit YFP expression throughout the nuclear and cytoplasmic compartments with strong non-uniform endoplasmic reticulum-localized 3βHSD that is devoid from the nucleus. Mutant testes continue to express 3βHSD in a large number of cells. (**B**) Protein expression of P450^{SCC} (green) and 3βHSD (red) in control and mutant E14.5 testes. Control interstitial P450^{SCC} cells co-express 3βHSD whereas mutant Leydig cells exhibit a notable loss of P450^{SCC} but continue to express high levels of 3βHSD (inset). (**C**) Apoptosis, detected by Lysotracker (red) is not altered in mutant foetal testes. Laminin (green) identifies the testis cords (left two panels) and control mesonephric ducts (far right panel) that are known to have cells undergoing apoptosis at this developmental time point (arrow).

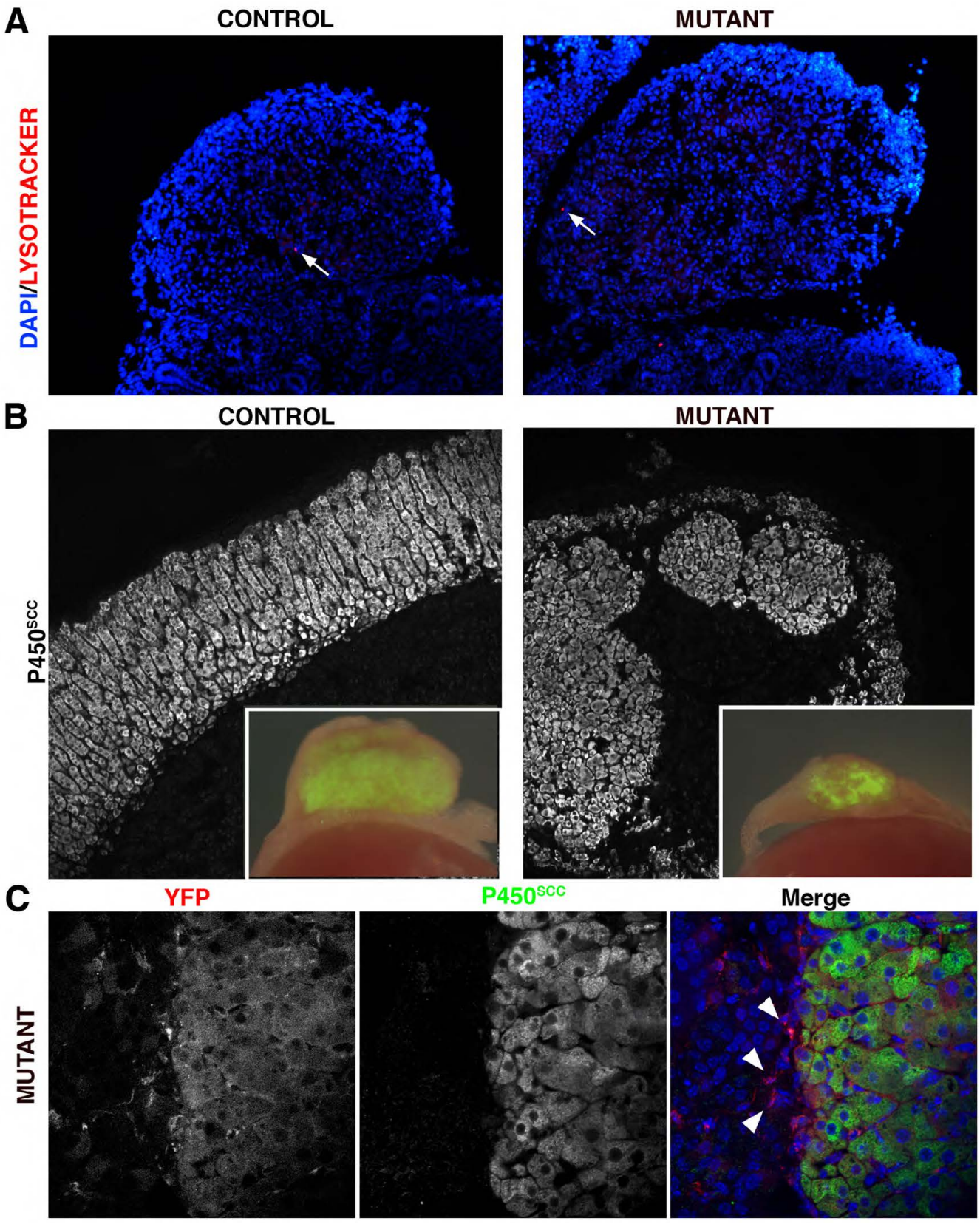

Fig. S2. In-depth analysis of adrenal defects in *Cyp11a1*-Cre;*Sf1*$^{Fl/Fl}$ mice. (**A**) Apoptosis, detected by Lysotracker (red), is not altered in mutant adrenal glands compared with controls (arrows). (**B**) Most adult mutant adrenals appeared morphologically normal, and were indistinguishable from controls. However, a small number were hypoplastic and showed disorganization within the cortex, demonstrated both by immunofluorescence of P450^{SCC} and by whole-mount analysis of YFP expression in freshly dissected glands (inset). (**C**) Double-fluorescence immunohistochemistry for YFP (red) and P450^{SCC} (green) in adult adrenal glands. In female mutants, but never in controls, a population of YFP-positive, P450^{SCC}-negative cells was present at the cortex-medulla boundary (arrowheads). This cell population exhibited a small, flat morphology.

Table S1. qRT-PCR primer sequences

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
Cyp11a1	AAAGACCGAATCGTCCTAAACC	CTTGATGCGTCTGTGTAAGACT
Cyp17a1	GATCTAAGAAGCGCTCAGGCA	GGGCACTGCATCACGATAAA
Hsd3b1	CAGGCCTCCAATAGGTTCTG	GTTGTCATCCACACTGCTGC
Sox9	AGTACCCGCATCTGCACAAC	TACTTGTAATCGGGGTGGTCT
Lhcgr	TCAGGAATTTGCCGAAGAAAGAACAG	GAAGTCATAATCGTAATCCCAGCCACTG
Insl3	TGGTCCTTGCTTACTGCGATCT	CCTGGCTATGTCATTGCAACA
Hmgcs1	TTCAAAGGAAGTGACCCAGG	GGTCTGATCCCCTTTGGTG
Rps2	CTGACTCCCGACCTCTGGAAA	GAGCCTGGGTCCTCTGAACA

Table S2. Antibodies used in immunofluorescence studies

Antibody	Supplier	Method	Working dilution
3β-HSD (rabbit)	Generous gift, Prof. Ian Mason (University of Edinburgh MRC Centre for Reproductive Health, UK)	IF-TSA	1/2000
AR (rabbit)	Millipore, 06-680	IF-TSA	1/250
c-Kit (rabbit)	Cell Signaling, 3074	IF	1/200
GFP (rabbit)	Invitrogen, A6455	IF	1/500
		IF-TSA	1/2000
Ki67 (rat)	Dako, M7249	IF	1/25
		IF-TSA	1/200
Laminin (rabbit)	Sigma, L9393	IF	1/400
LIFR (rabbit)	Santa Cruz, sc-659	IF	1/100
$P450^{SCC}$ (rabbit)	Millipore, AB1244	IF	1/200
		IF-TSA	1/1000
p-S6 (rabbit)	Cell Signaling, 4857	IF-TSA	1/500
PECAM (rat)	BD Pharmingen, 553370	IF	1/50
SF-1 (rabbit)	Generous gift, Prof. Ken Morohashi (Kyushu University, Japan)	IF	1/200
		IF-TSA	1/2000
SMA (mouse)	Generous gift, Dr David Robertson (Breakthrough Toby Robins Breast Cancer Centre, London, UK)	IF	1/4000

IF, immunofluorescence performed using a fluorescently labelled secondary antibody;
F-TSA, immunofluorescence performed using the TSA system.