

Figure S1: Specificity of GFP Antibody in Paraffin Sections and Cre Expression is Restricted to Fetal Adrenal Descendant Cells. Tomato and EGFP can be detected endogenously in cryosections, however for the majority of our studies, paraffin sections were used. In paraffin sections, a GFP antibody can be utilized to detect EGFP expression by immunofluorescence (IF) and thereby identify cells with active recombination by Cre. IF was carried out on paraffin sections of adrenal glands from adult mice. A. No primary antibody. B. Anti-GFP shows green cytoplasm in cortical cells indicative of Cre-recombination. Cre recombination is specific to cells derived from fetal adrenocortical cells (expressing *FAdE-Ad4bp-Cre*), including Nr5a1-expressing cells (white nuclei). C. Co-staining with anti-GFP (green), anti-Nr5a1 (white), and anti-Th (red) shows cre recombination in Nr5a1-expressing cells but not in the Th expressing medulla. Panel D is magnified from the box in Panel C. Scale bars = 50 μ m.

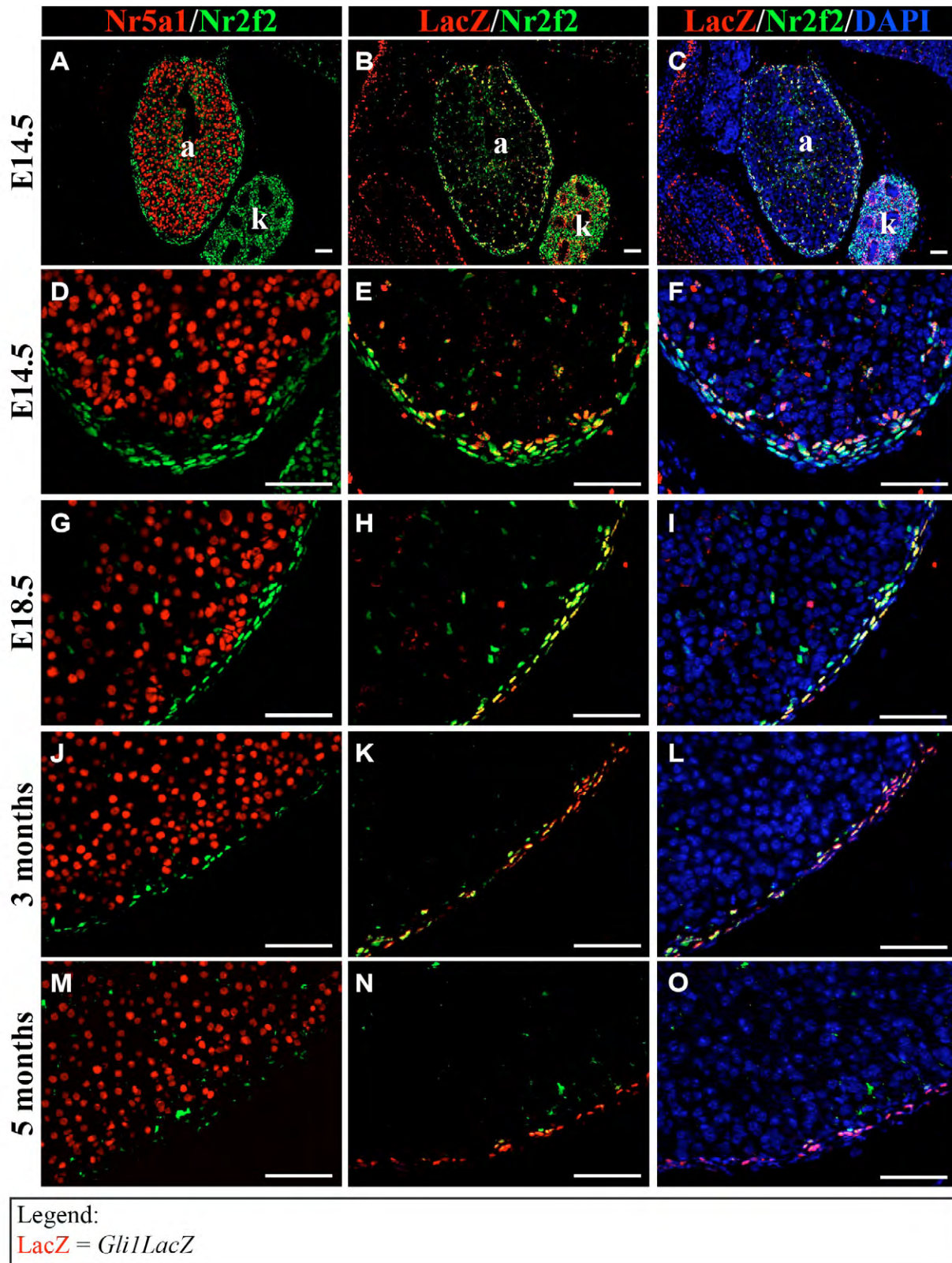
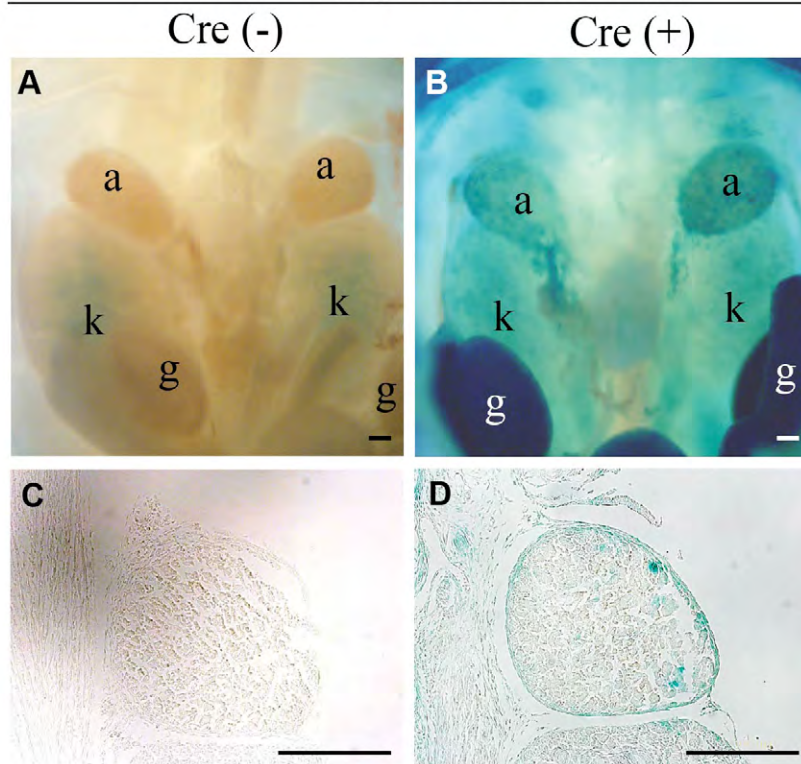
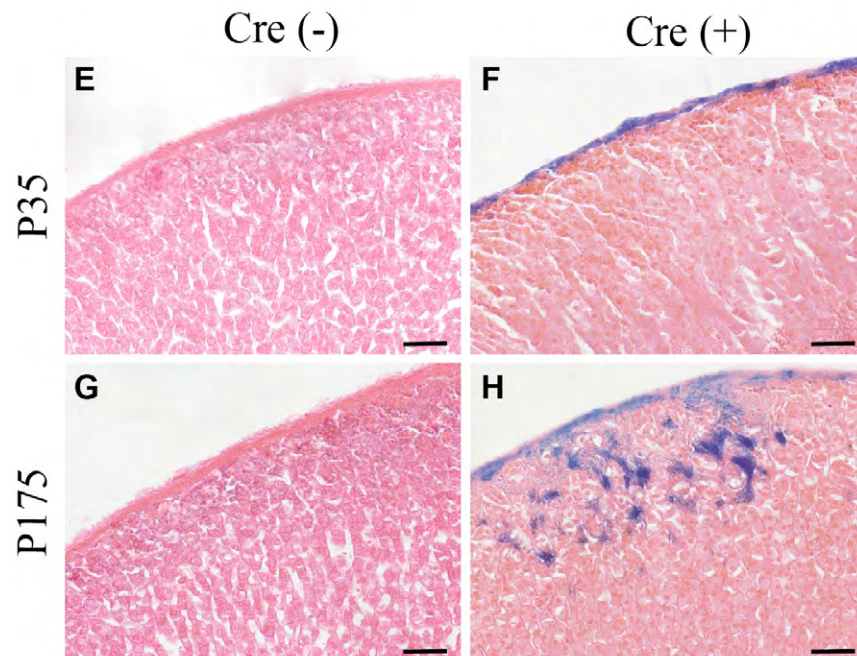


Figure S2: Adrenal Capsule Contains Nr2f2- and Gli1-Expressing Cells. *Gli1-LacZ* mice were used to characterize the cellular milieu of the adrenal capsule by immunofluorescence in paraffin sections. After formation of the adrenal capsule, Nr2f2 (green nuclei) is expressed from E14.5 (panels A-F), through E18.5 (panels G-I), and into adulthood (3 months; panels J-L) where upon cessation of organogenesis, Nr2f2 levels decrease (5 months; panels M-O). Panels B, E, H, K, and N show that β -galactosidase expression (LacZ, indicative of *Gli1* expression; red nuclei) can also be detected throughout the adrenal capsule at all ages evaluated. As shown in panels A, D, G, J, and M, Nr5a1 expression (red nucleus) is restricted to the adrenal cortex. Panels D-E are high power images from A-C, respectively. Yellow indicates overlap of green and red nuclei. Nuclei are shown by DAPI in panels C, F, I, L, and O. a = adrenal, k = kidney. Scale bars = 50 μ m.

Pulse at E14.5; Harvest at E16.5



Pulse from P21 to P35



Legend:
 $LacZ = Gli1Cre-ER^{T2}:R26R^{LacZ}$

Figure S3: *Gli1*-Expressing Cells of the Adrenal Capsule Give Rise to Adrenocortical Cells. In panels A-D, embryos were harvested at E16.5 from pregnant *Gli1Cre-ER^{T2}:R26R^{LacZ}* (Ahn and Joyner, 2004) mice that were administered tamoxifen at E14.5. In Panels A and C, adrenals from animals lacking Cre expression have no β-galactosidase (βgal) activity (blue cytoplasm) as detected by whole mount LacZ staining. In contrast, panels B and D show that animals with Cre expression reveal βgal activity throughout the adrenal capsule and some cells with βgal activity in the adrenal cortex. Panels C and D are sagittal sections from embryos. In panels E-H, postnatal *Gli1Cre-ER^{T2}:R26R^{LacZ}* mice administered tamoxifen from P21 through P35, display βgal activity predominantly in the adrenal capsule of Cre expressing mice at P35 (F) and in cells of the adrenal cortex at P175 (H) when compared to animals lacking Cre expression (E, G). Scale bars = 100 μm.

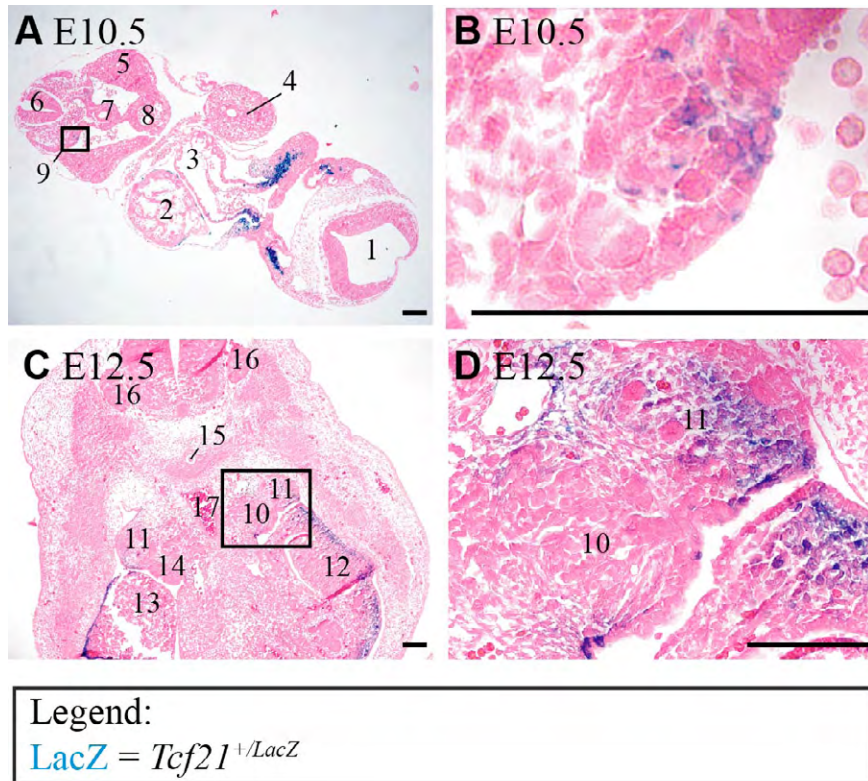


Figure S4: Characterization of *Tcf21* Promoter Activity in the Embryonic Adrenal Gland. Embryos harvested from *Tcf21*^{lacZ/+} mice were evaluated by whole mount LacZ staining. Transverse sections of E10.5 (panels A and B) and E12.5 (Panels C and D) embryos reveal β gal activity (blue cytoplasm) in the developing adrenogonadal primordia and coalescing adrenal primordia, respectively. Panels B and D are enlargements of boxes in Panels A and C. Key: (1) caudal region of fourth ventricle; (2) common ventricular chamber of heart (3) bulbus cordis region of heart; (4) caudal extremity of notochord; (5) hindlimb bud; (6) neural tube and lumen; (7) hindgut; (8) umbilical artery; (9) urogenital ridge; (10) adrenal primordial; (11) mesonephric tubules; (12) stomach; (13) liver; (14) rostral extremity of gonadal ridge; (15) descending aorta; (16) dorsal root ganglion; (17) aorta. Scale bars = 100 μ m.

Table S1. Antibodies used for immunohistochemistry

A. Primary antibodies				
Antibody	Species	Company	Catalog code	Working Dilution
Nr2f2 (CoupTFII)	Mouse	R & D Systems (Minneapolis, MN)	pp-H7147-00	1:200
GFP	Chicken	Abcam (Cambridge, MA)	ab13970	1:2000
GFP	Rabbit	Invitrogen (Carlsbad, CA)	A11122	1:500
LacZ	Chicken	Abcam	ab9361	1:1000
LacZ	Rabbit	Millipore (Billerica, MA)	AB1211	1:1000
Pdgfra	Rabbit	Cell Signaling Technology (Danvers, MA)	3164S	1:500
Nr5a1	Rabbit	Proteintech Group (Chicago, IL)	Custom	1:1000
Th	Mouse	Millipore (Billerica, MA)	MAB318	1:200
Acta1	Mouse	Sigma (St Louis, MO)	A2547 (Clone 1A4)	1:200
Desmin	Rabbit	ThermoScientific/Pierce (Rockford, IL)	PA5-16705	1:200
B. Secondary antibodies from Jackson ImmunoResearch (West Grove, PA, USA) used at 1:500 dilution				
Host	Reactivity	Fluorophore	Catalog code	
Donkey	Chicken	AlexaFluor 647	703-605-155	
Donkey	Chicken	Dylight 488	703-485-155	
Donkey	Goat	AffiniPure 488	705-545-147	
Donkey	Goat	AffiniPure Cy3	705-165-147	
Donkey	Goat	AlexaFluor 647	705-605-147	
Donkey	Mouse	Dylight 649	715-495-150	
Donkey	Rabbit	Dylight 649	711-495-152	
Goat	Chicken	Dylight 549	103-505-155	
Goat	Mouse	Dylight 488	115-485-146	
Goat	Mouse	Dylight 549	115-505-146	
Goat	Rabbit	Dylight 549	111-505-144	
Rabbit	Goat	Dylight 488	305-485-045	
Rabbit	Goat	Dylight 549	305-505-045	