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Supplemental Information

Beta-Catenin Causes Adrenal Hyperplasia by Blocking Zonal Transdifferentiation

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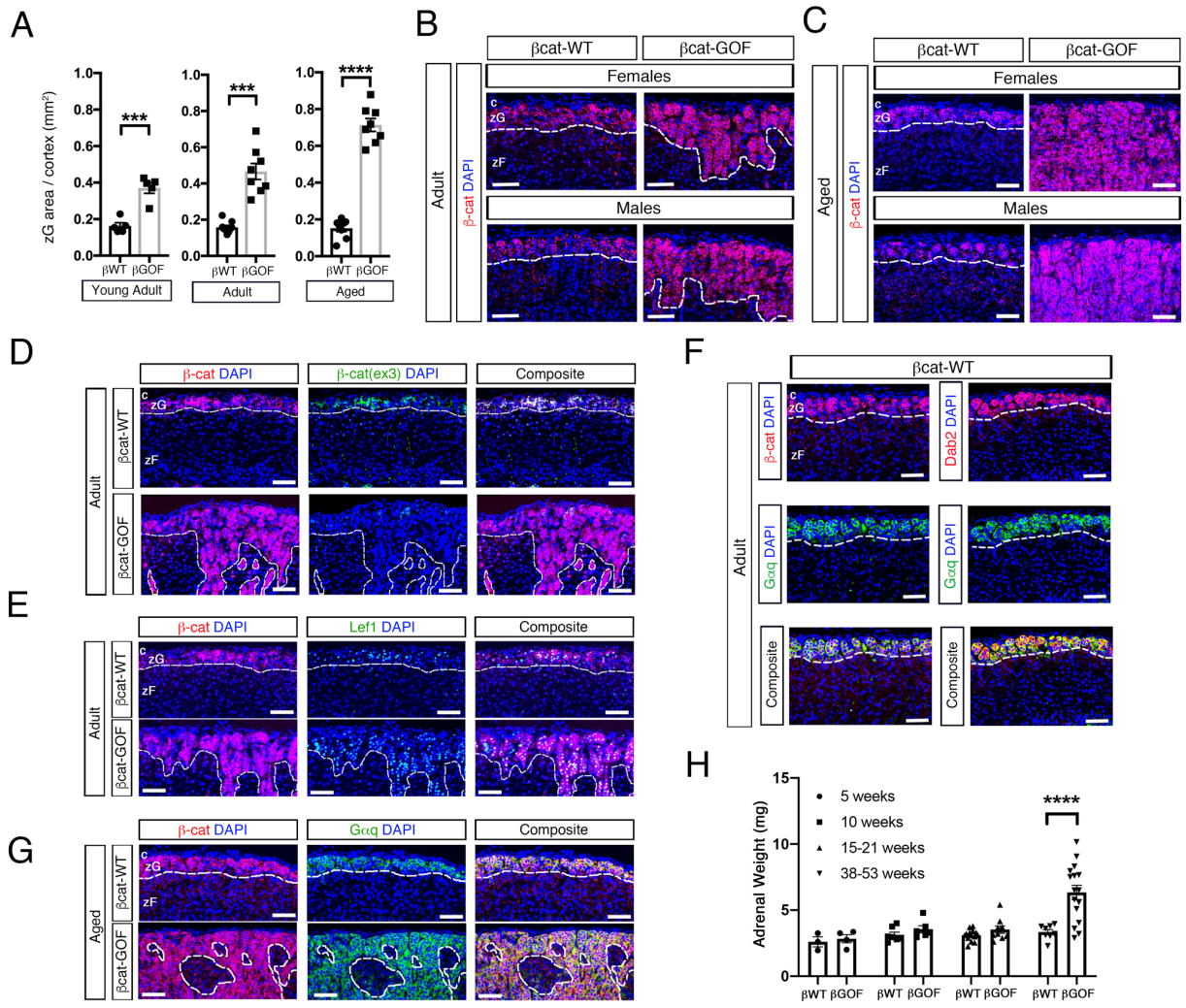
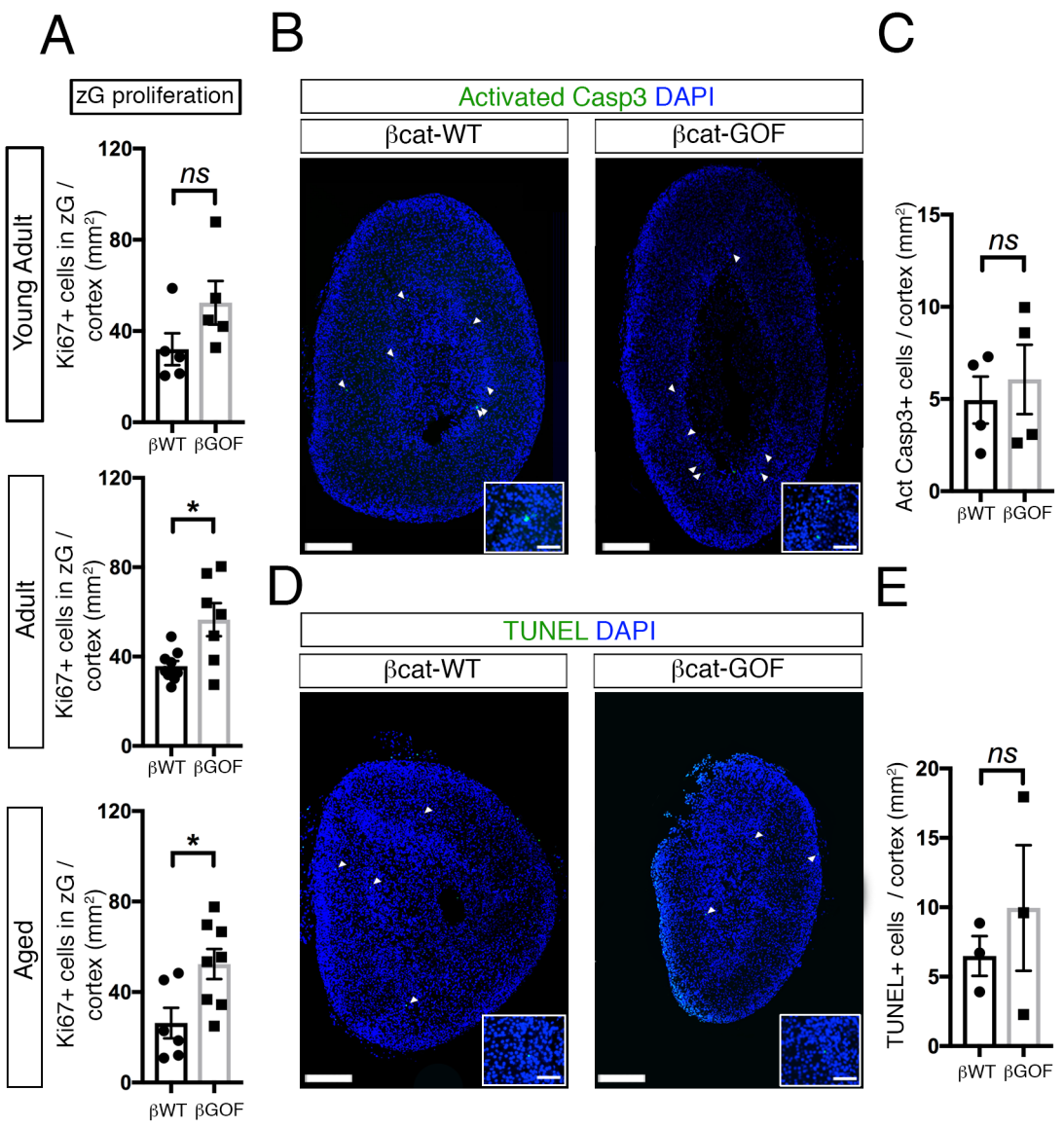
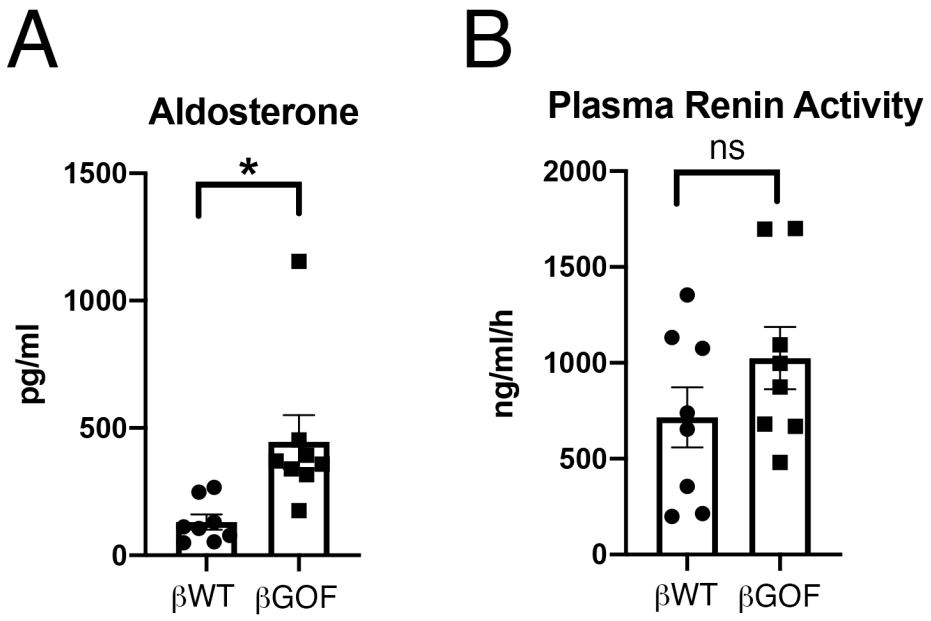


Supplemental Figure 1. Stabilization of β-catenin in zG cells results in zG expansion, Related to Figure 1. (**A**) Quantification of the β-catenin-positive region, normalized by cortical area, (from left to right: *n* = 5, 5, 5, 8, 7 and 8 mice). (**B**) Representative cortical sections from adult female (*n* = 15 for each genotype) and male (*n* = 3 for each genotype) mice stained for β-catenin (β-cat, red). (**C**) Representative cortical sections from aged female (*n* = 5 for each genotype) and male (*n* = 3 for each genotype) mice stained for β-catenin (β-cat, red). (**D**) Representative adrenal sections from adult mice co-stained for an antibody recognizing all forms of β-catenin (β-cat, red) and a β-catenin antibody recognizing exon 3 (green) (*n* = 3 for each genotype). (**E**) Representative adrenal sections from adult mice co-stained for all forms of β-catenin (β-cat, red) and Lef1 antibody (green) (*n* = 3 for each genotype). (**F**) Representative cortical sections co-stained for Gαq (green) and β-catenin (red) (left panels) or Dab2 (red), (right panels, *n* = 3 mice each group). (**G**) Representative adrenal sections from aged mice co-stained for all forms of β-catenin (β-cat, red) and Gαq (green) (*n* = 5 for each genotype). (**H**) Average weight of adrenal glands from mice at indicated ages (from left to right, *n* = 3, 4, 7, 6, 14, 9, 8 and 16 mice). All sections are counter-stained with nuclear DAPI (blue). The dotted lines define the border between positive and negative regions for β-catenin or Dab2, as indicated. c, capsule. zG, zona Glomerulosa. zF, zona Fasciculata. Med, medulla. Scale bars: 50 μm. ***p < 0.001; ****p < 0.0001. Data are represented as mean ± SEM.



Supplemental Figure 2. Analysis of cellular proliferation and apoptosis following β-catenin stabilization, Related to Figure 2. (**A**) Quantification of the Ki67-positive cells in the zG, normalized per cortical area (from left to right, top to bottom: n = 5, 5, 9, 7, 6 and 8 mice. The samples used here are the same as in Figure 2A). (**B**) Representative adrenal sections were stained for activated Caspase 3-positive (green), (n = 4 mice for each genotype). (**C**) Quantification of activated Caspase 3-positive cells from (C) per cortical area, (n = 4 mice for each genotype). (**D**) Representative adrenal sections were stained for TUNEL (green), (n = 3 mice for each genotype). (**E**) Quantification of TUNEL-positive cells from (E) per cortical area, (n = 3 mice for each genotype). All sections are counter-stained with nuclear DAPI (blue) Scale bar: 200 µm. Scale bar in magnified insets: 50 µm. *ns,* not significant. *ns,* not significant. *p < 0.05. Data are represented as mean ± SEM.



Supplemental Figure 3. Stabilization of β-catenin in zG cells results in mild hyperaldosteronism, Related to Figure 1. Quantification of (**A**) plasma aldosterone and (**B**) plasma renin activity in aged mice (n = 8 mice for each genotype). *ns,* not significant. *p < 0.05. Data are represented as mean ± SEM.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
TaqMan™ probe _ mouse *Axin2*	Thermo Fisher Scientific	Cat# Mm00443610_m1
TaqMan™ probe _ mouse *Lef1*	Thermo Fisher Scientific	Cat# Mm00550265_m1
TaqMan™ probe _ mouse *Cyp11b2*	Thermo Fisher Scientific	Cat# Mm01204955_g1
TaqMan™ probe _ mouse *Pde2a*	Thermo Fisher Scientific	Cat# Mm01136644_m1
TaqMan™ probe _ mouse *Actb*	Thermo Fisher Scientific	Cat# Mm026119580_g1
Primer: ChIP-qPCR, Pde-A Forward: CTCTCAGTTCCAAGGCAC	This paper	N/A
Primer: ChIP-qPCR, Pde-A Reverse: CTCCCACCTAACTCCACC	This paper	N/A
Primer: ChIP-qPCR, Pde-B Forward: CTTCCCCTCCCAGTGATATT	This paper	N/A
Primer: ChIP-qPCR, Pde-B Reverse: AACACATCACTAGTTGCTGG	This paper	N/A
Primer: ChIP-qPCR, Axin2 Forward: CTCTAGGAGGTGGACTGG	This paper	N/A
Primer: ChIP-qPCR, Axin2 Reverse: CCCAAAGCAGAAACCAGTT	This paper	N/A
Primer: ChIP-qPCR, Ped-X Forward: CCCAGGATAGTGTAGTGAGA	This paper	N/A
Primer: ChIP-qPCR, Ped-X Reverse: CTTGGAACTTCTCACAGTGG	This paper	N/A

Supplemental Table 1. List of primer sequences, including source and identifiers, used in the manuscript for quantitative PCR. This table is related to all main figures including experiment of quantitative PCR.