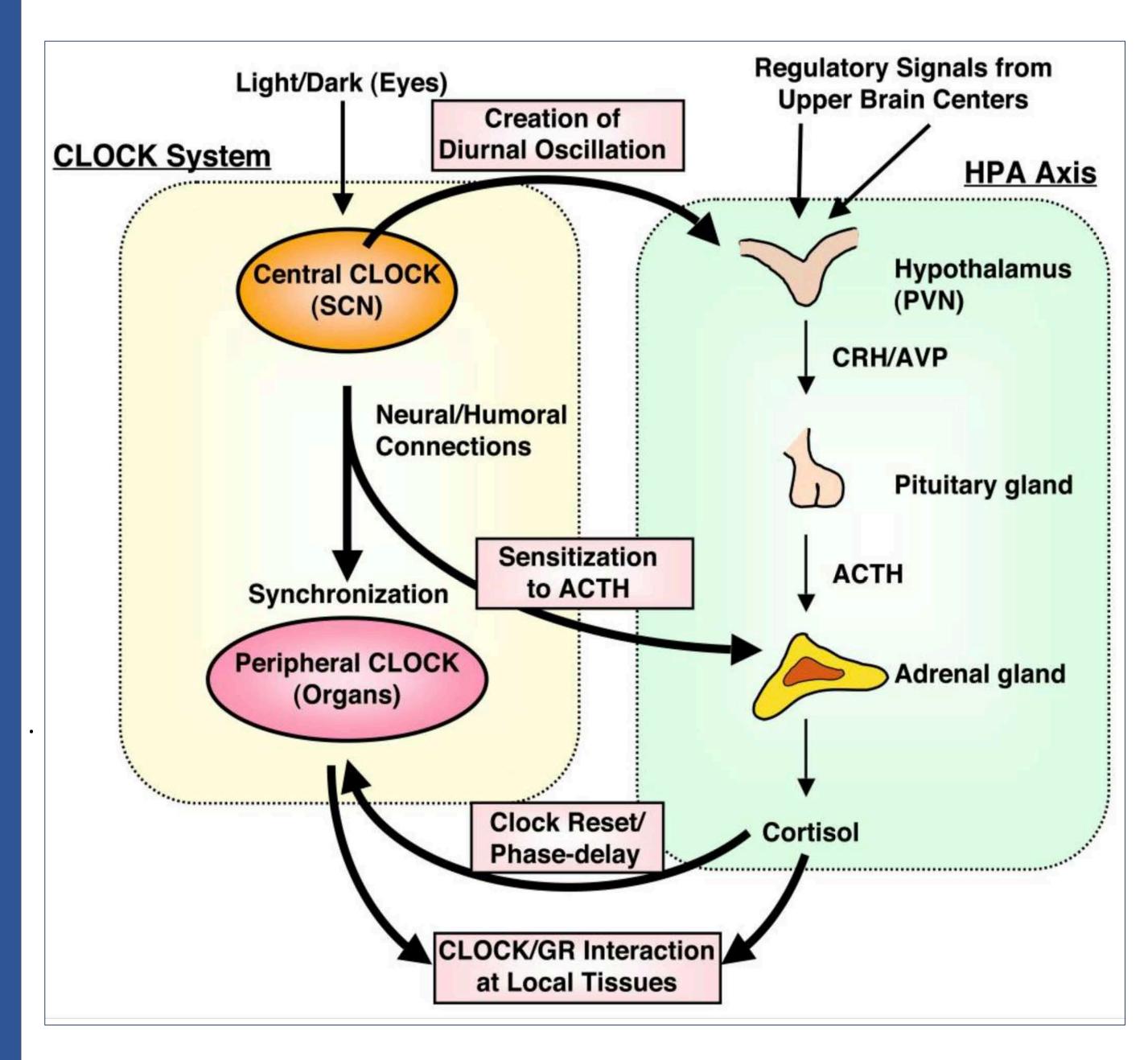
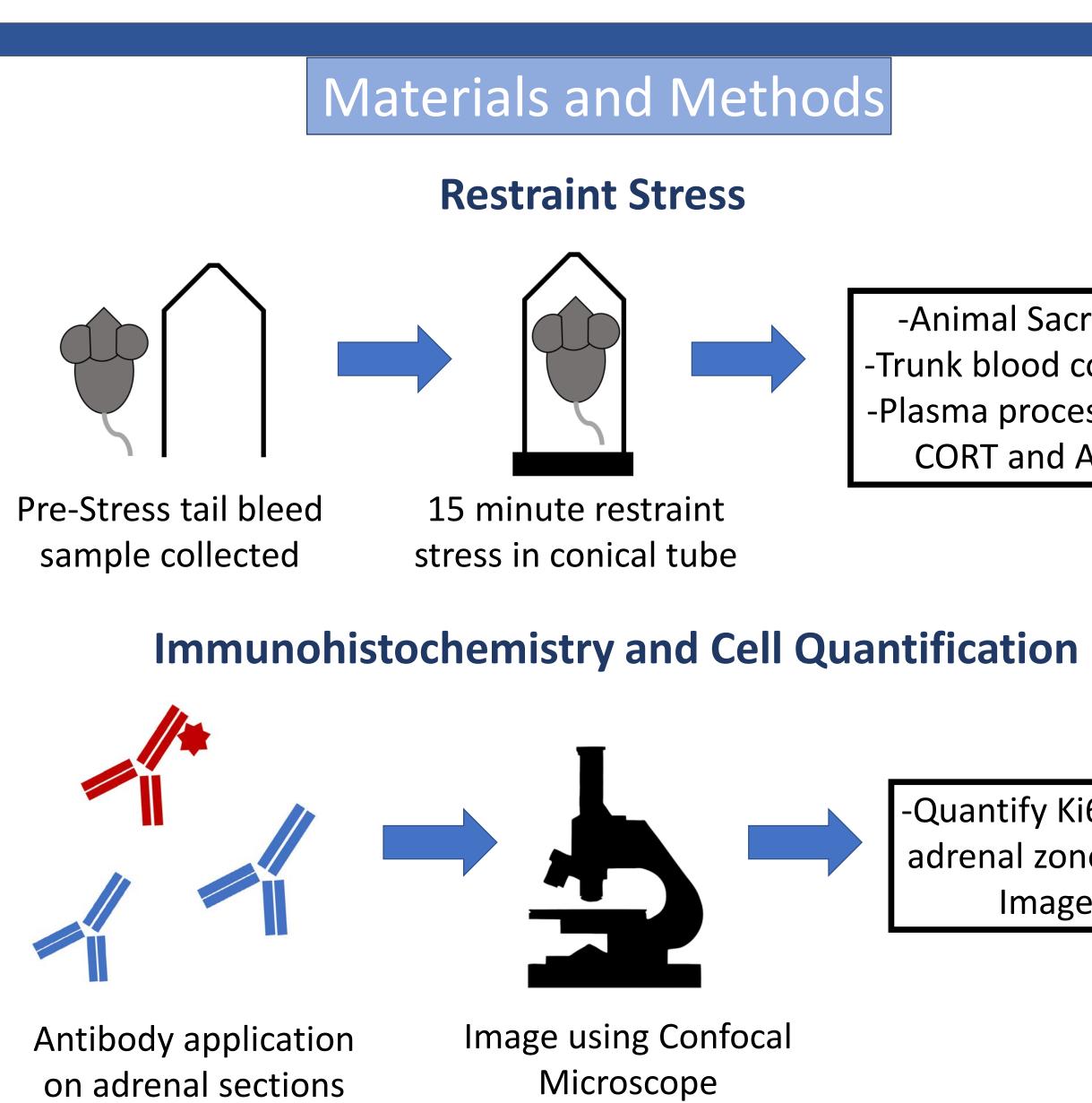
Understanding the Role of the Adrenal Clock in the Stress Response

The HPA Axis, Clock Genes, and Stress¹

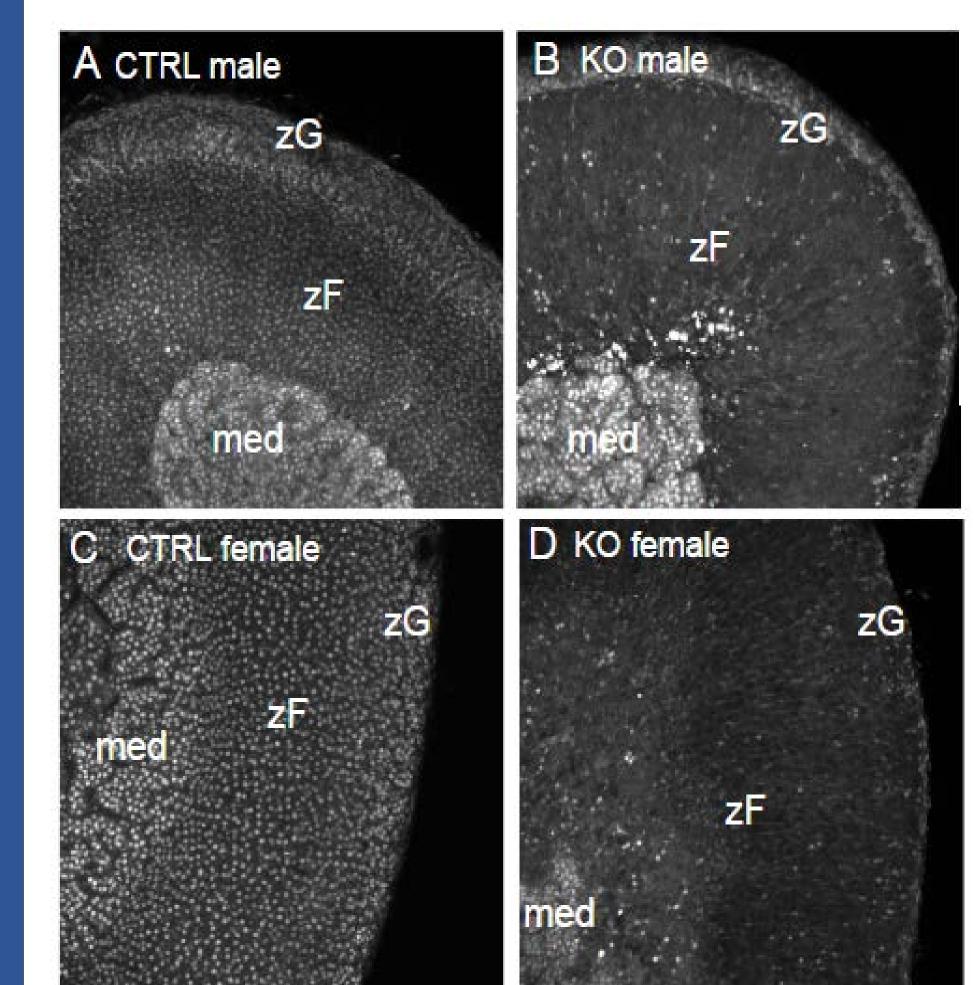


The primary goal of this research is to better understand the purpose of the adrenal clock in gating adrenal responsiveness to ACTH under conditions of acute stress.

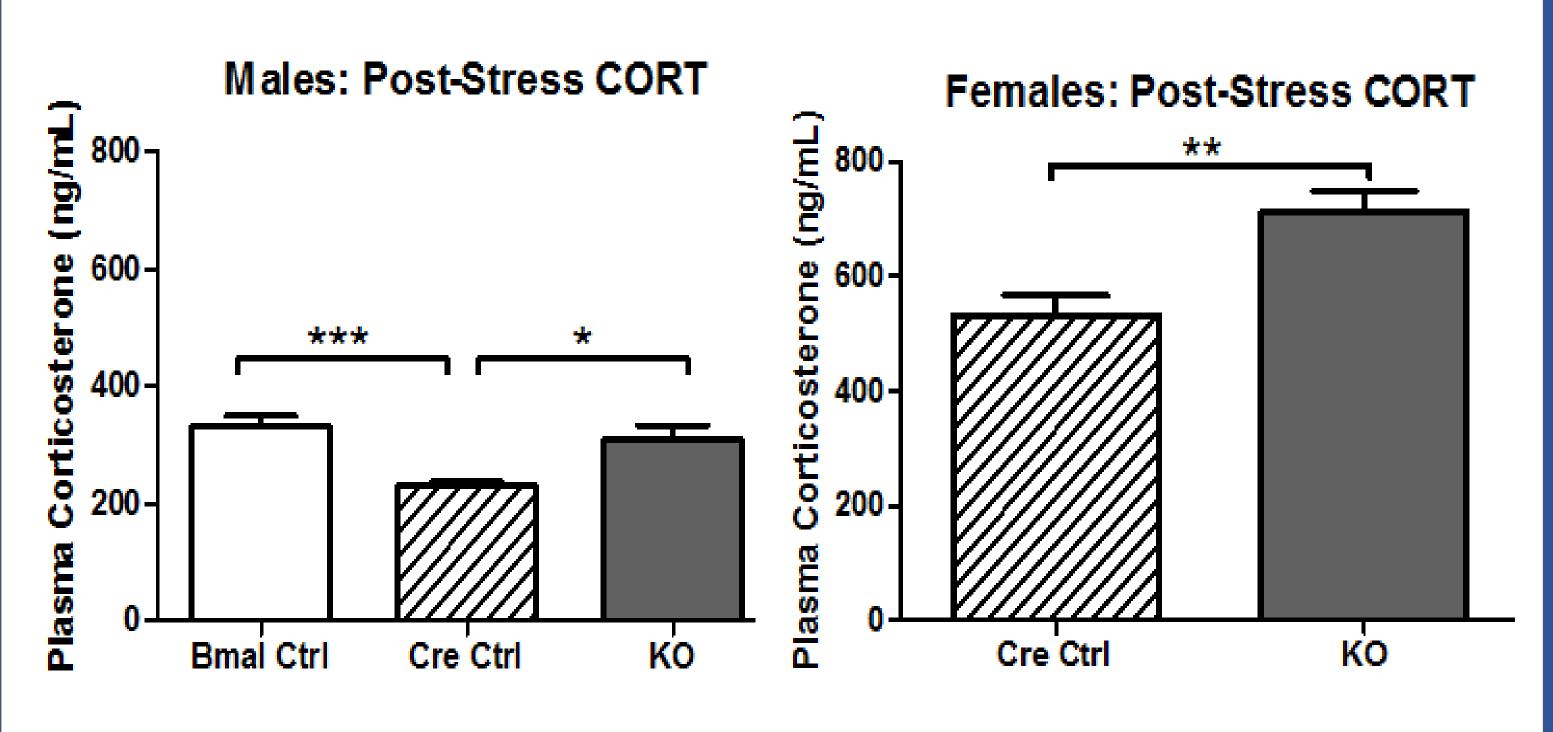


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Generating an Adrenal Clock KO Model



Adrenal Clock KO Animals Have a Heightened Glucocorticoid Response to Acute Stress



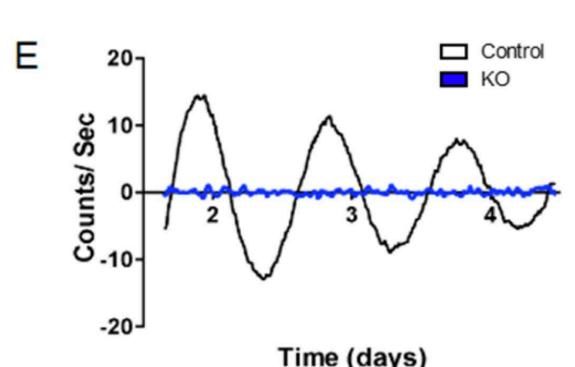
Animals were restrained for 15 minutes using a conical tube. Blood samples preand post-stress were taken and analyzed using a radioimmunassay (RIA) for plasma corticosterone and ACTH concentrations. Cre Controls (SCC^{cre/+}::Bmal^{+/+}) in addition to Bmal1 Controls (SCC^{+/+}::Bmal^{fl/fl}) were used to account for presence of only one functional p450scc gene in the KO animal due to the Cre-Recombinase insertion. Contrary to previous studies^{2,3}, both male and female *Bmal1* KO animals exhibited a *higher* plasma corticosterone response to stress relative to Cre Control animals, suggesting the adrenal clock is necessary for proper response to stress.

1.) Buhr, E.D., and Takahashi, J.S. (2013). Molecular components of the mammalian circadian clock. Handb Exp Pharmacol 3–27 2.) Son, G.H., Chung, S., Choe, H.K., Kim, H.-D., Baik, S.-M., Lee, H., Lee, H.-W., Choi, S., Sun, W., Kim, H., et al. (2008). Adrenal peripheral clock controls the autonomous circadian rhythm of glucocorticoid by causing rhythmic steroid production. Proc Natl Acad Sci U S A 105, 20970–20975 3.) Dumbell, R., Leliavski, A., Matveeva, O., Blaum, C., Tsang, A.H., and Oster, H. (2016). Dissociation of Molecular and Endocrine Circadian Rhythms in Male Mice Lacking Bmal1 in the Adrenal Cortex. Endocrinology 157, 4222–4233.



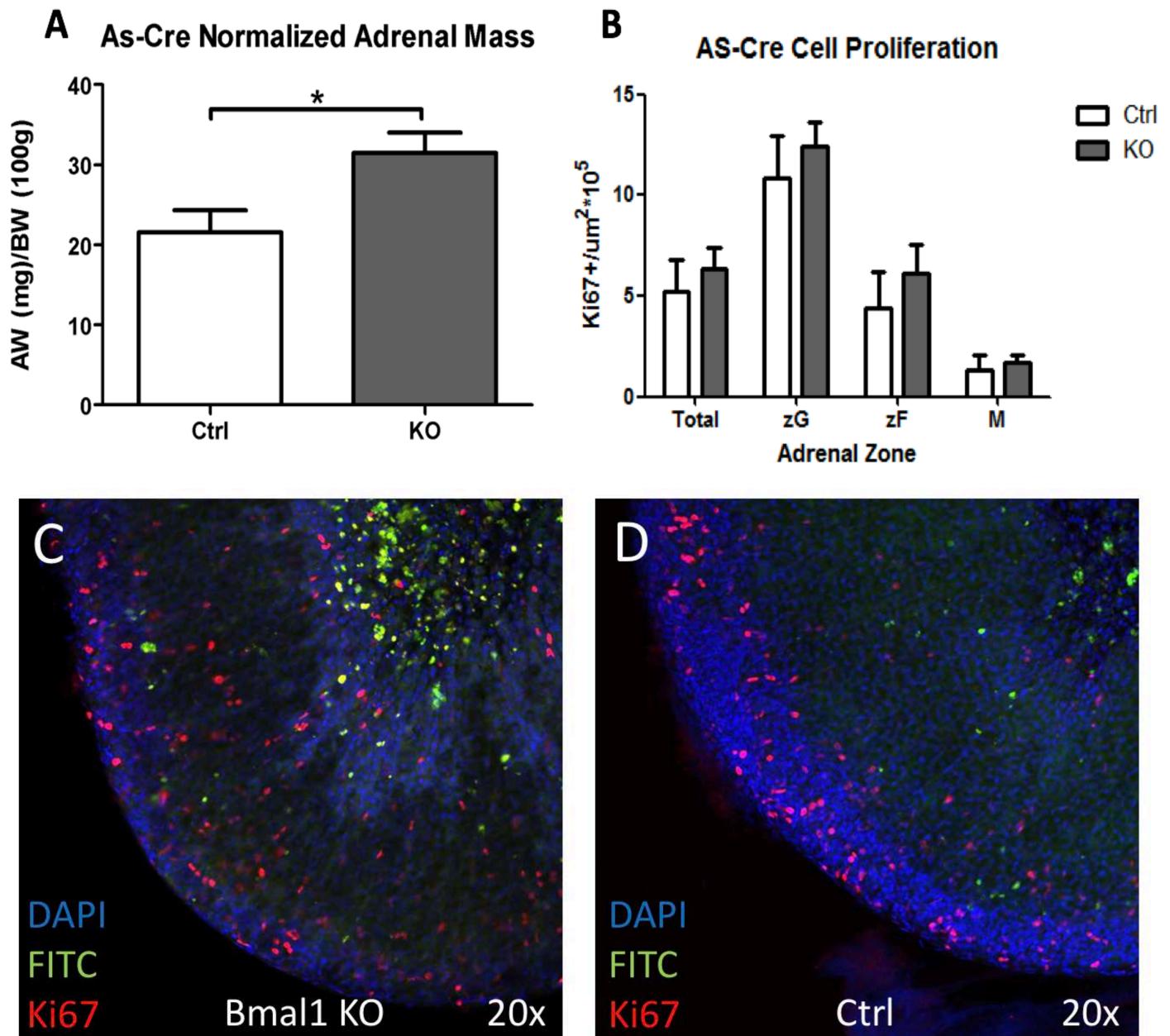
-Animal Sacrificed -Trunk blood collected -Plasma processed for CORT and ACTH

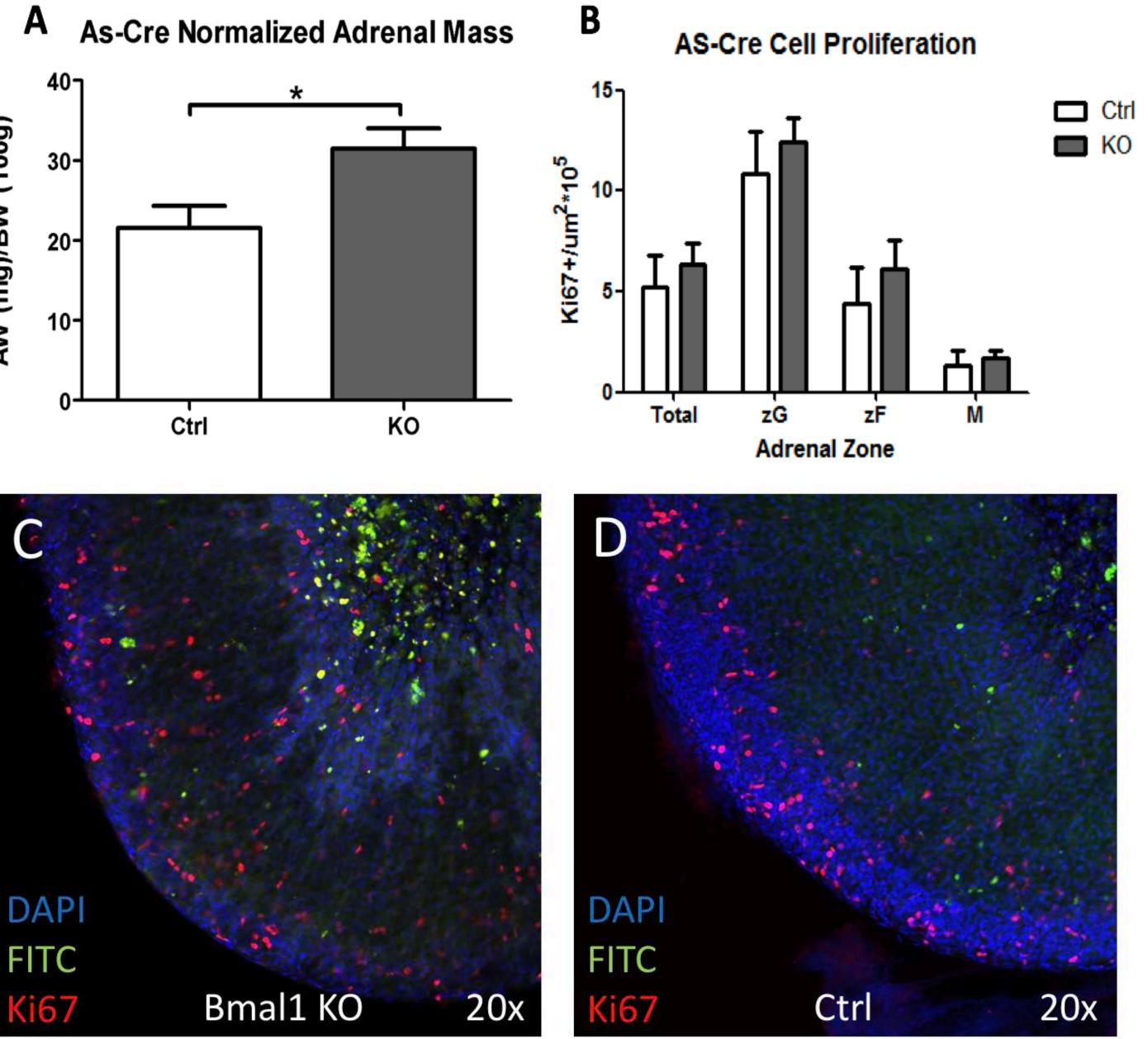
-Quantify Ki67+ per adrenal zone using ImageJ



Using a Cre-LoxP construct, an adrenal clock knockout animal was generated through the ablation of *Bmal1. Bmal1* KO animals lose expression of BMAL1 in the adrenal cortex. Furthermore, PER2Luc rhythmicity is lost in KO mice, confirming the loss of the adrenal clock.

Animals with and Ablated Adrenal Clock Exhibit an Increase in Adrenal Mass





Cell proliferation was analyzed to examine a possible connection with the increase in adrenal mass in clock knockout animals. Immunohistochemistry was used to label Ki67+ cells in 100um adrenal sections (5C-D). Ki67+ cells were quantified and corrected for zone area using ImageJ (5B). Overall, there was no significant difference in cell proliferation between control and knockout mice, thus there is no correlation between proliferation and the increased adrenal mass. These data suggest an alternative mechanism other than increase in cell number is responsible for the increase in plasma corticosterone responses in *Bmal1* KO animals.

Conclusions and Future Directions

- Contrary to previous findings, we have shown that adrenal clock knockout animals produce an *increased* response to acute stress
- The increase in adrenal mass seen in adrenal clock knockout animals is not due to an increase in cell proliferation
- Adrenal sensitivity with new control consideration warrants further investigation
- Continue to explore responsiveness to stress; vary stress intensity Examine alternative mechanisms for increase in adrenal mass and increase adrenal responsiveness to acute stress in *Bmal1* KO animals
- In vitro adrenal sensitivity experiments
- Examine glucocorticoid response at different time points (AM vs PM)

References and Acknowledgements

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