The Role of BAP1 in the Enteric Nervous System

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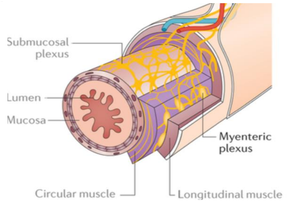
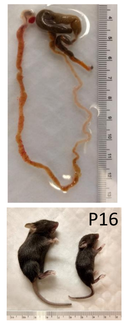
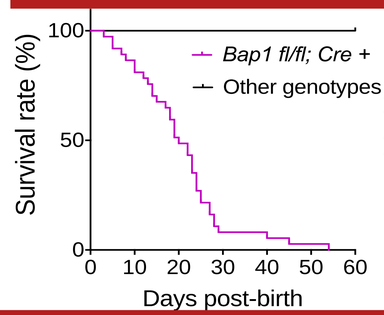
Introduction

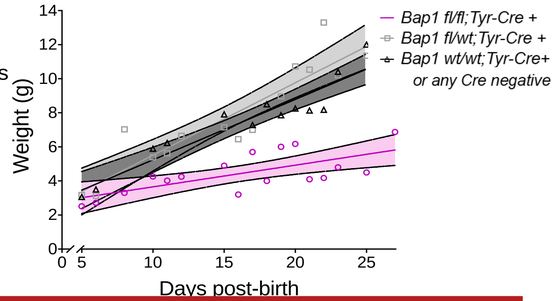
The enteric nervous system (ENS) is an extensive network of neural crest-derived neurons and glia that control bowel motility and other vital aspects of bowel function such as absorption and secretion. To perform these functions, the ENS utilizes more than 20 neuron types and relies on an extraordinary level of neurotransmitter diversity (1). When the ENS does not develop or function properly, symptoms include constipation, distension, and abdominal pain, which impair quality of life and cause life-threatening complications. Recently, we began investigating the role of BAP1 in ENS development and function using a mouse strain with a conditional deletion of *Bap1* induced by Cre-recombinase under the control of the tyrosinase promotor. Tyrosinase is expressed in the majority of migrating neural crest cells. Previously, BAP1 has been linked to decreased expression of genes involved in neural crest migration (2). BAP1 is an epigenetic modifier involved in regulating gene expression through chromatin remodeling and deubiquitylation (3-6). Loss of BAP1 causes mice to die at 3-4 weeks of age with megacolon. Currently, we hypothesize that Bap1 is involved in regulating genes important for neuronal development and function.



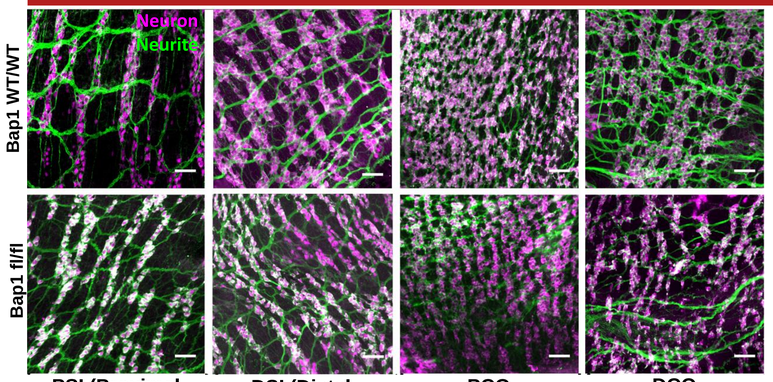


TyrCre+; Bap1 fl/fl mice die at 3-4 weeks of age and exhibit failure to thrive



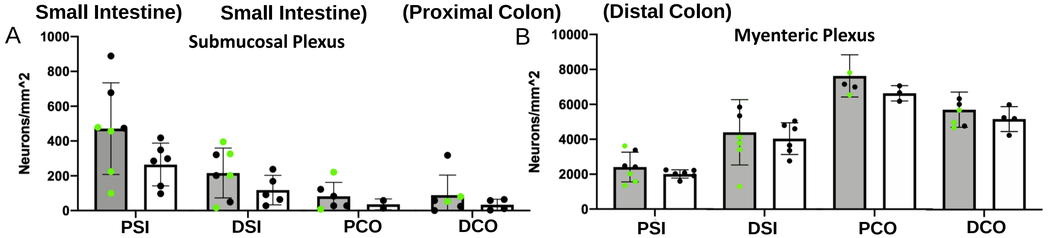


Wnt1-Cre+; Bap1 fl/fl mice have no obvious ENS defects at birth



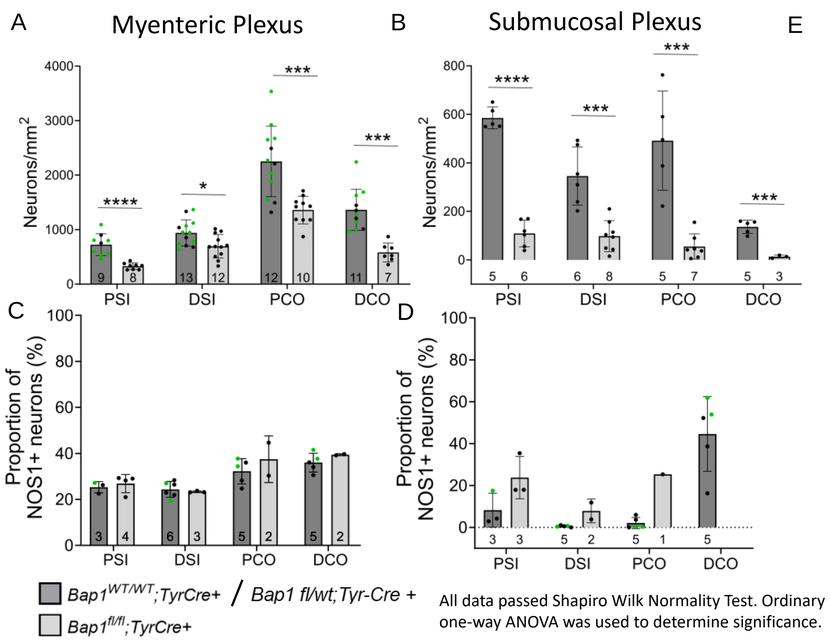
Loss of BAP1 in ~96% of neural crest cells demonstrates complete migration of the enteric neural crest and normal ENS density at birth in both the submucosal (A) and myenteric (B) plexuses.

Bap1$^{WT/WT}$;TyrCre+
Bap1 fl/wt;Tyr-Cre +
Bap1$^{fl/fl}$;TyrCre+

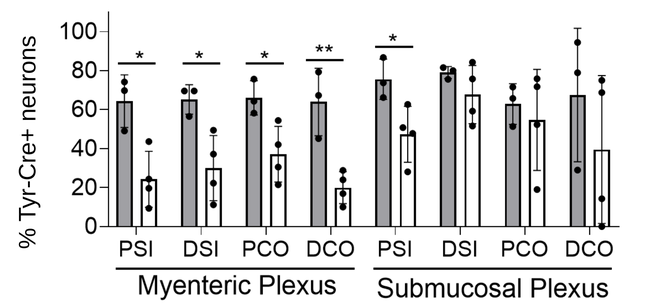


All data passed Shapiro Wilk Normality Test. Ordinary one-way ANOVA was used to determine significance. All data non-significant.

Tyr-Cre+; Bap1 fl/fl mice experience a disproportionate loss of Tyr+ neurons by P15

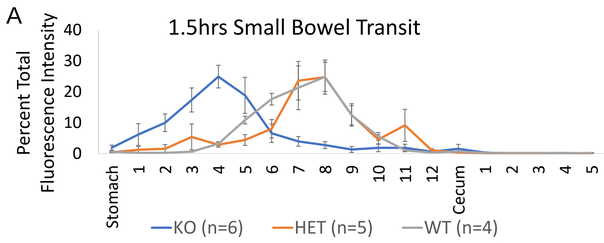


All data passed Shapiro Wilk Normality Test. Ordinary one-way ANOVA was used to determine significance.

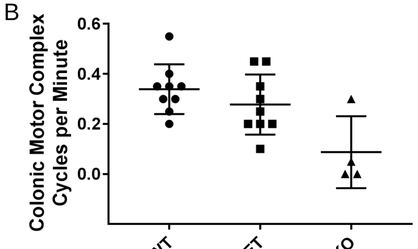


Loss of BAP1 in the tyrosinase lineage results in decreased neuron density in the myenteric (A) and submucosal (B) plexuses at P15. The proportion of NOS1+ neurons to total neurons remains the same (C and D), suggestng NOS1+ neurons are also lost. There is a significant decrease in the percent of ENS neurons that are *Tyr-Cre+* in *Tyr-Cre+; Bap1 fl/fl* mice (E), indicating loss of *Tyr-Cre+* neurons is responsible for the decrease in total neuron density.

in vivo and in vitro assays show slower motility in small and large bowel of TyrCre+; Bap1 fl/fl mice at P15



(A) *in vivo* FITC Dextran small bowel transit assay shows decreased small bowel motility in *TyrCre+; Bap1 fl/fl* ("KO") mice.



(B) *In vitro* observation of colon motility via organ bath shows fewer cycles of colonic motor complexes (CMCs)/minute in *TyrCre+; Bap1 fl/fl* ("KO") mice than in WT's or Het's. (KO= 0.0875±0.1436 cpm, Het= 0.2770±0.1201 cpm, WT= 0.3388±0.0993 cpm (stdev)). *p=0.0283, Pairwise Kruskal-Wallis Test

Future Directions and Discussion

We determined *Tyr-Cre+; Bap1 fl/fl* mice contain a fully migrated and fully colonized ENS at birth. *Tyr-Cre+* neurons are present at birth and functional (data not shown) but lost over time. At P15, an age where a large proportion of the *Tyr-Cre+* ENS is lost, slower motility is seen in the proximal small bowel and colon. To determine the cause of the neuronal loss, we aim to characterize expression abnormalities in neuronal populations by collecting single cell-RNA Seq data from P5 *TyrCre+; Bap1 fl/fl* and WT mice.

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Acknowledgements

Thank you to Dr. Robert Heuckeroth, Professor of Pediatrics at PSOM for his mentor- and sponsorship, Sabine Schneider for her mentorship. Thank you to the CURF office for awarding me the Jumpstart for Juniors grant allowing me to do this work.