COLLOQUE RECHERCHE
FIRENDO - 7ème ÉDITION -

MODÈLES EXPÉRIMENTAUX : NOUVEAUX HORIZONS POUR LES MALADIES RARES ENDOCRINIENNES

MARDI
5 DÉCEMBRE
2023

FIRENDO
FILIÈRE MALADIES RARES ENDOCRINIENNES

MODÈLES EXPÉRIMENTAUX :
NOUVEAUX HORIZONS POUR
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COLLOQUE RECHERCHE
FIRENDO - 7ème ÉDITION -

FIRENDO
FILIÈRE MALADIES RARES ENDOCRINIENNES

QUEL MODÈLE EXPÉRIMENTAL POUR COMPRENDRE LA PHYSIOPATHOLOGIE DE LA PUBERTÉ PRÉCOCE CENTRALE :

RÔLE ET MÉCANISMES D'ACTION DE MKRN3 ?

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INSTITUT DE BIOLOGIE PARIS SEINE
Comprendre le vivant pour agir

SORBONNE UNIVERSITÉ

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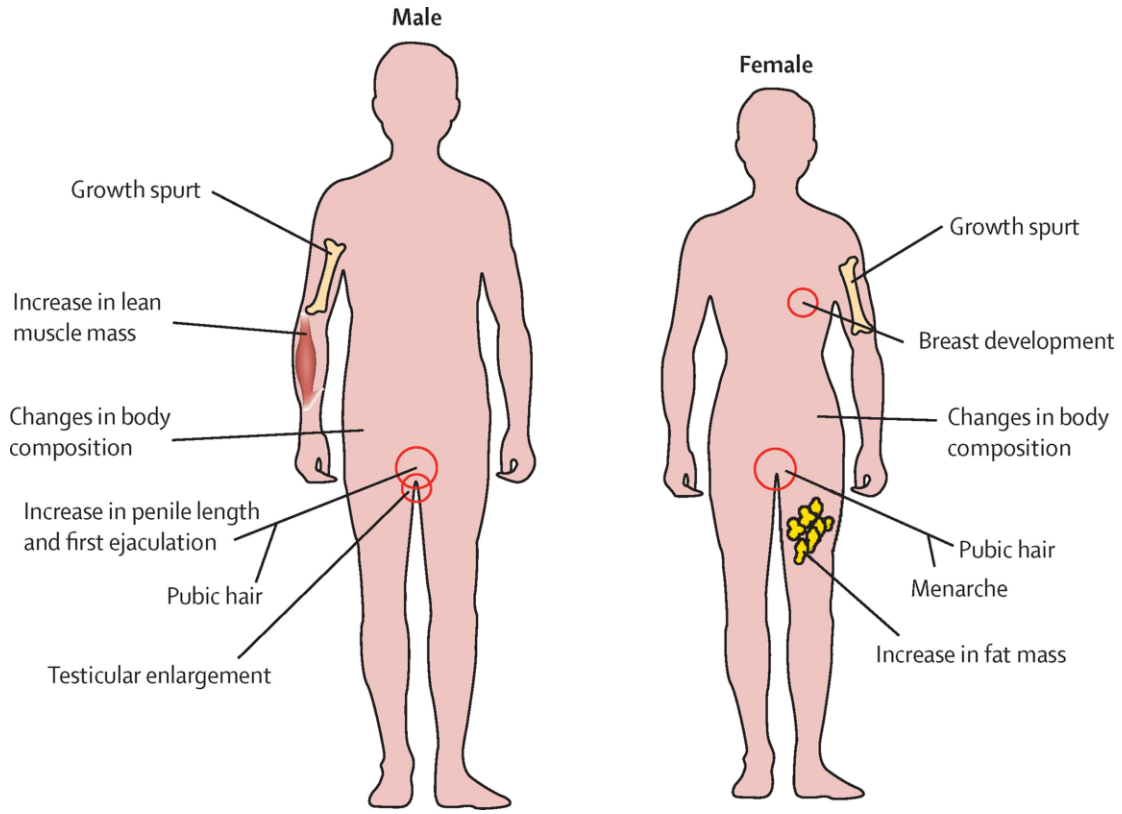
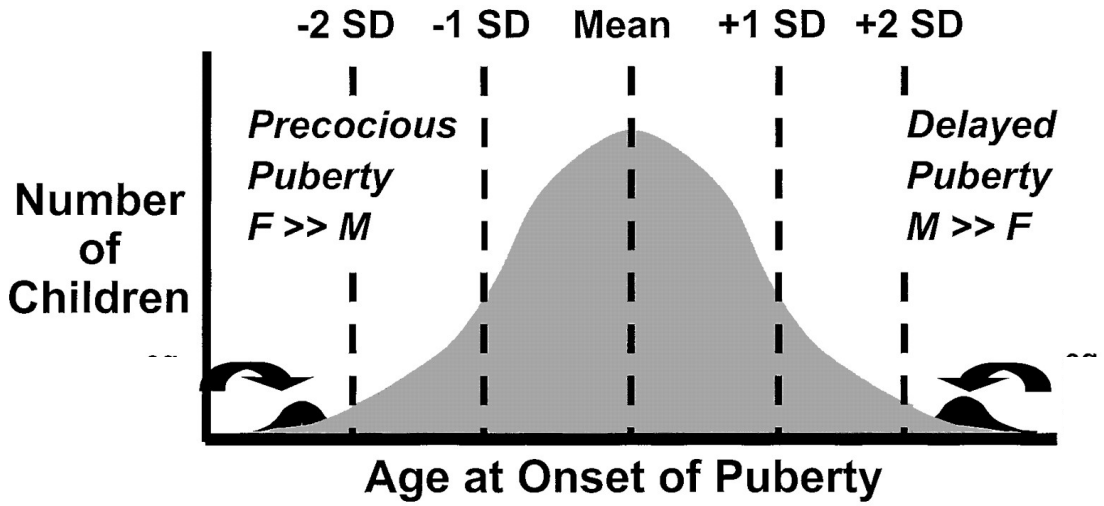
PUBERTY



Physical changes and secondary sexual characteristics that appear during pubertal development.

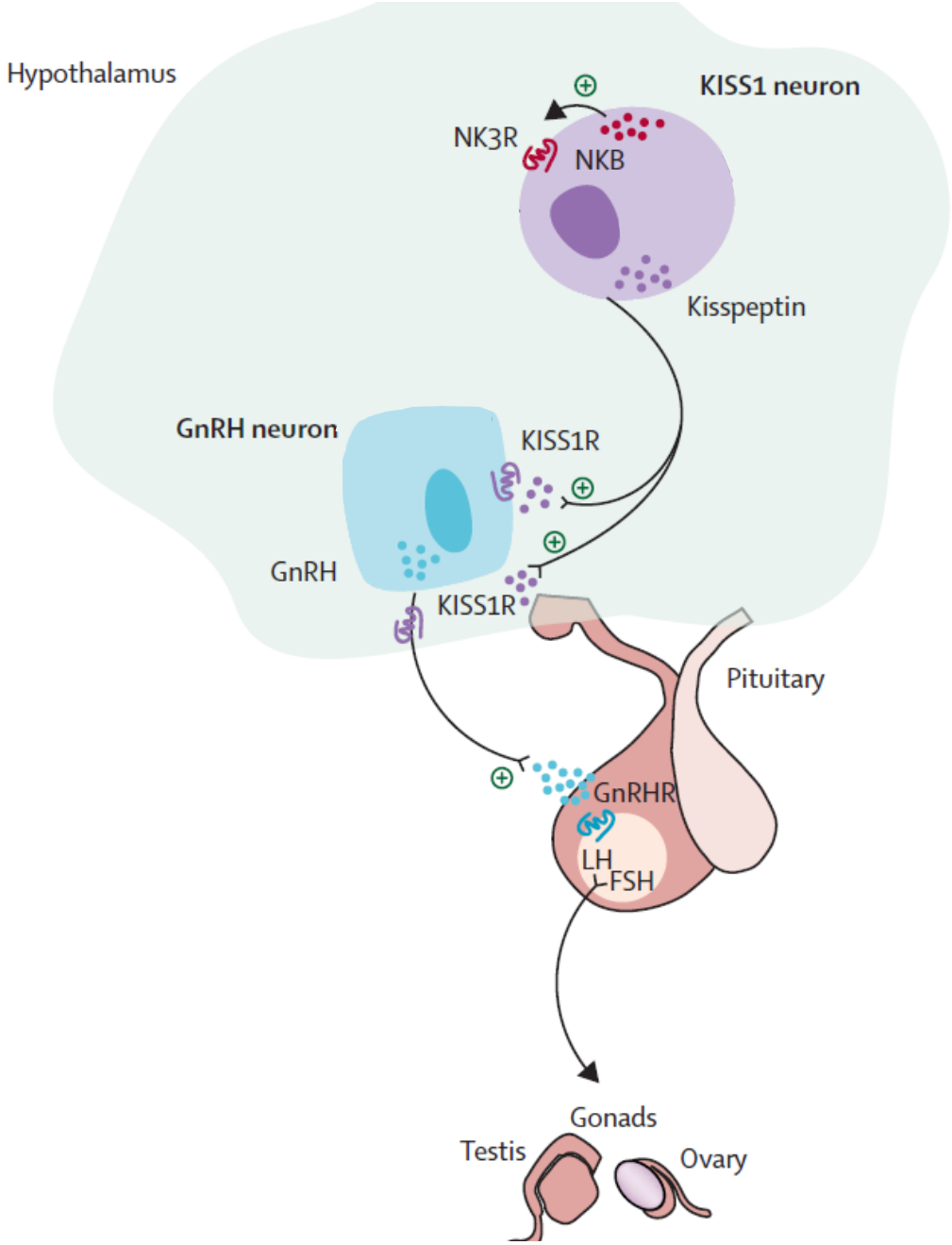
Abreu et Kaiser, 2016



Precocious Puberty	Delayed Puberty
< 8 yo girls	13 yo girls
< 9 yo boys	14 yo boys

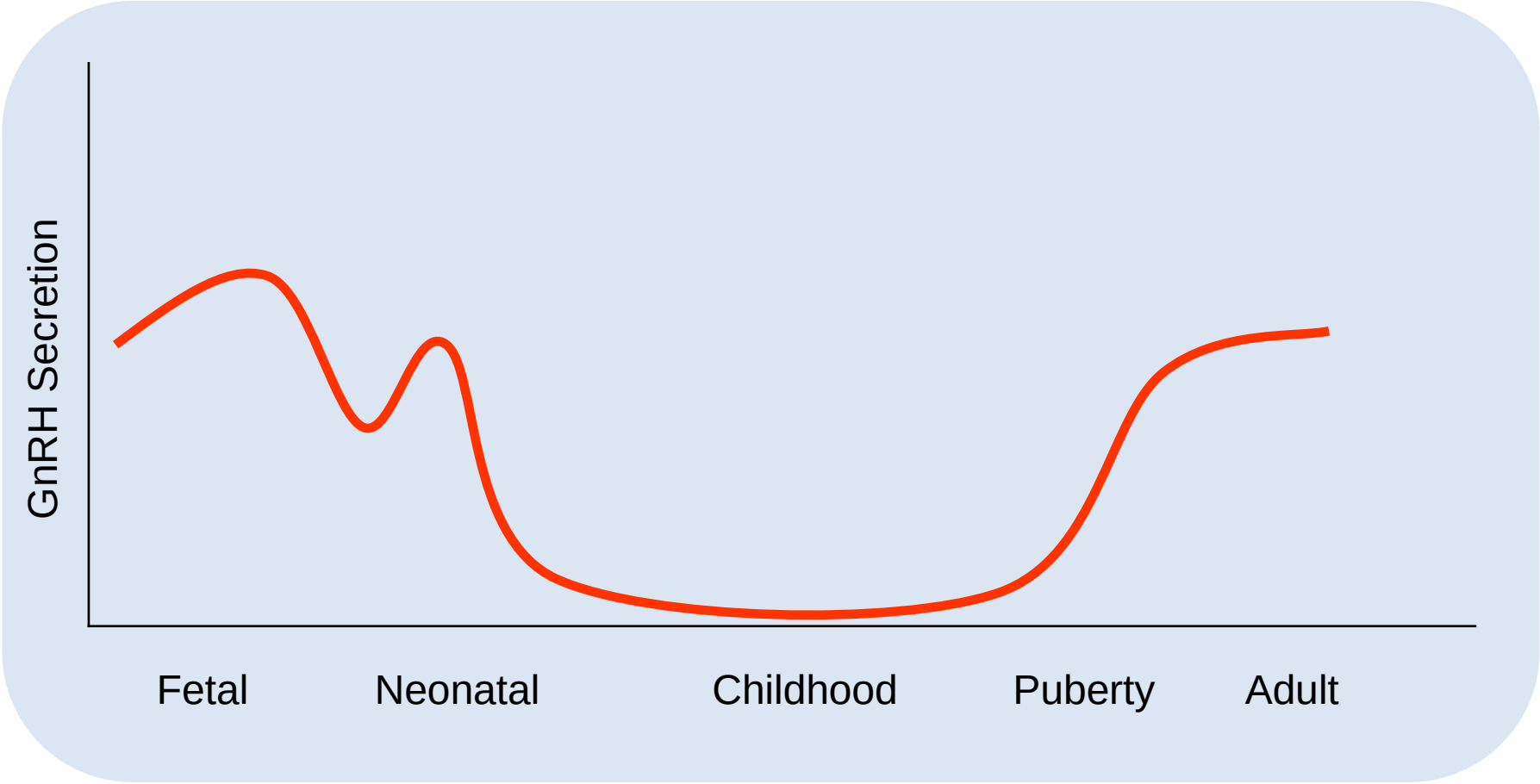
Palmert & Boepple, 2001

HYPOTHALAMIC-PITUITARY-GONADAL AXIS



Abreu et Kaiser, 2016

REGULATION OF GnRH SECRETION



Adapted from Palmert and Boepple., 2001

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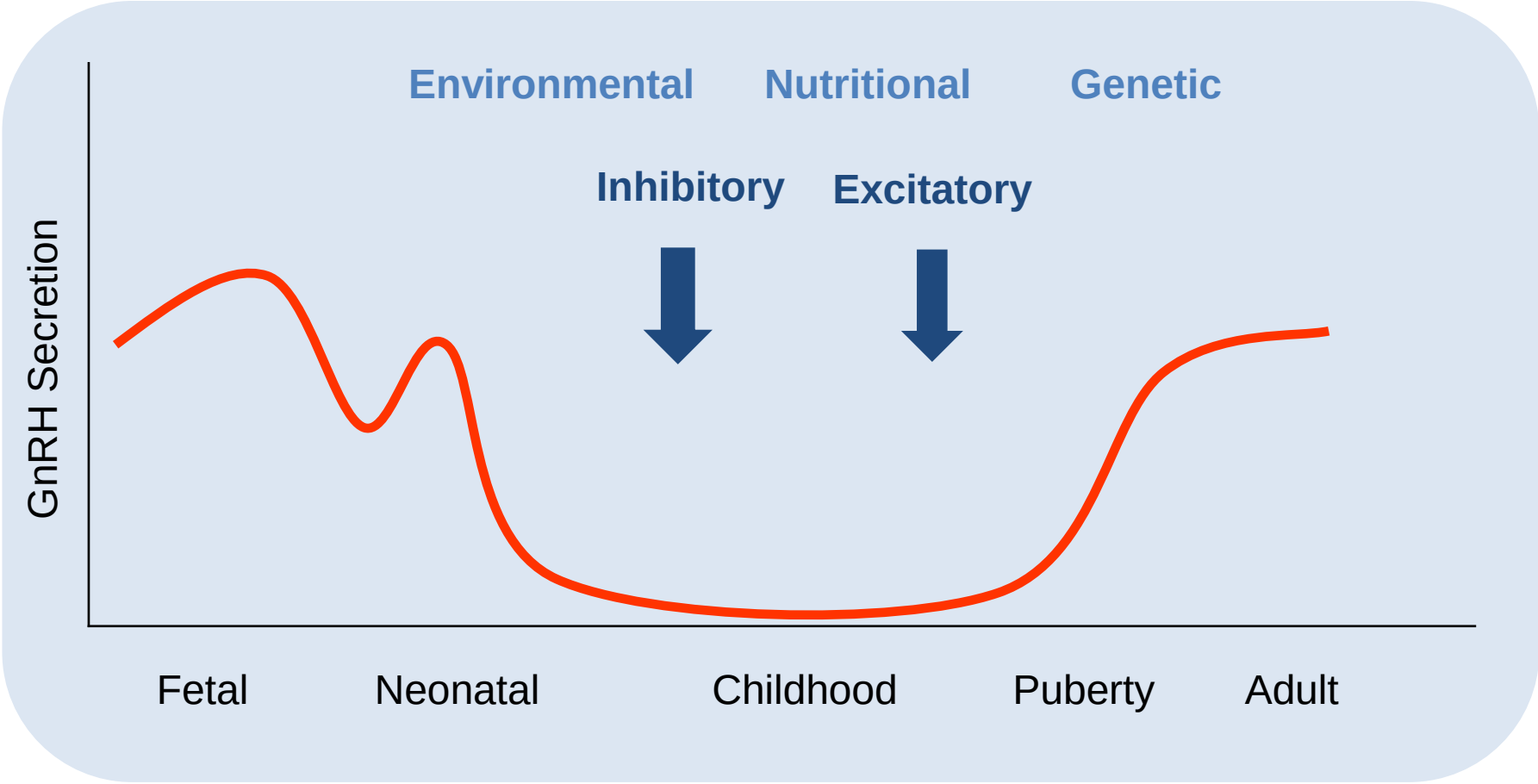

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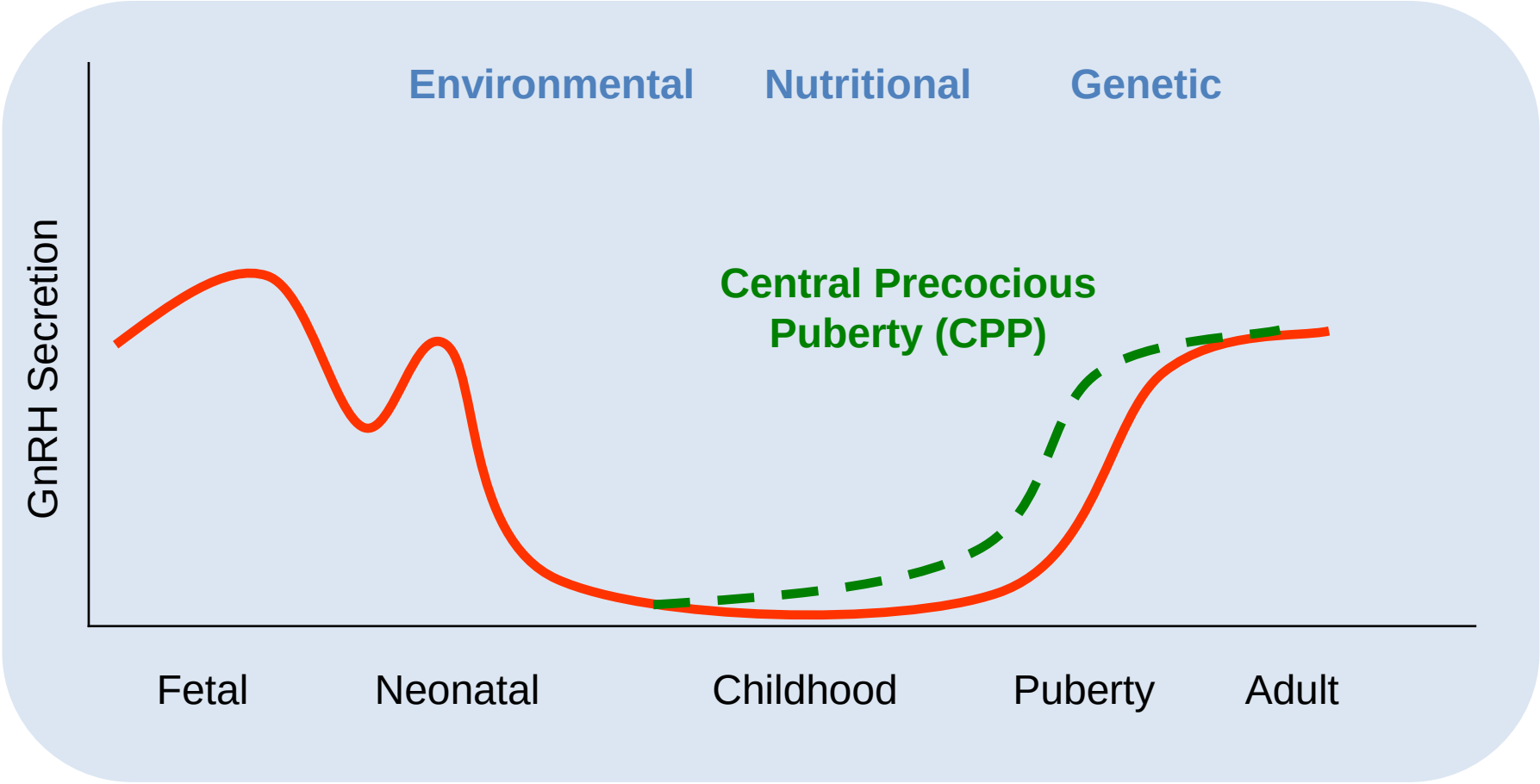

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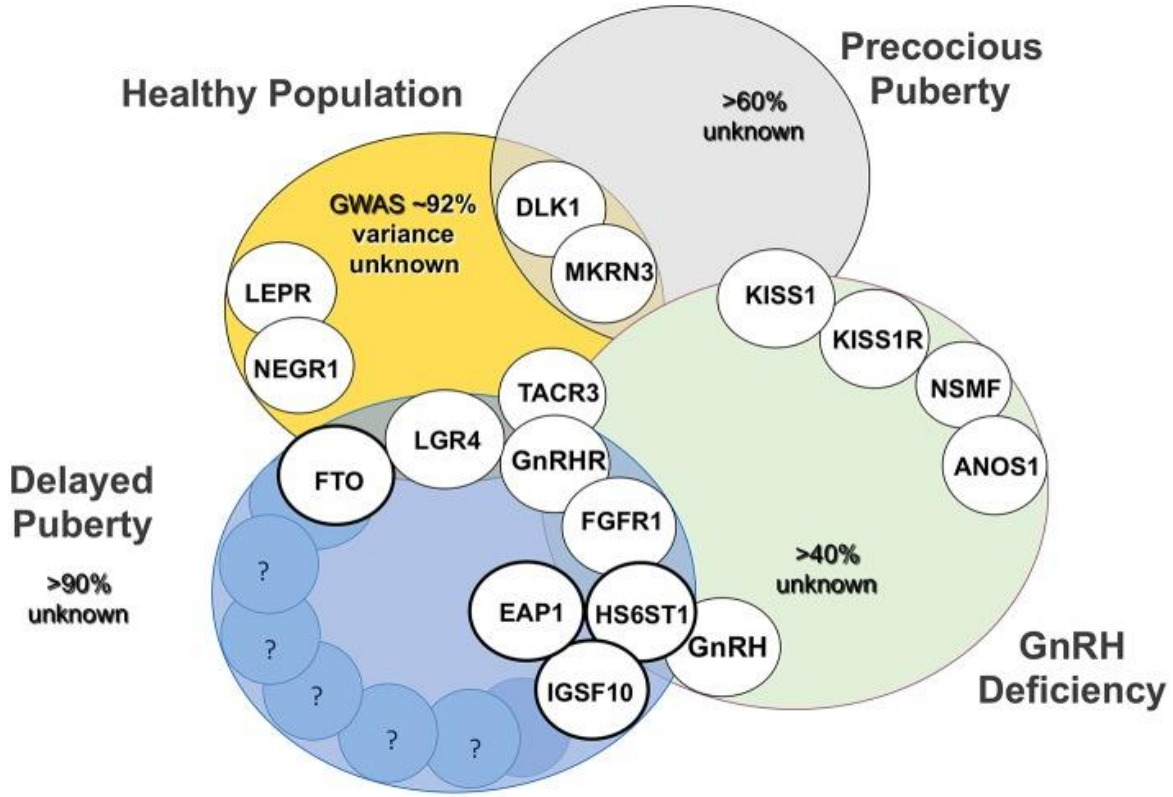
GENETIC REGULATION OF PUBERTY INITIATION



Established genetic basis of common genetic variants of pubertal timing from genome wide association studies (GWAS), conditions of GnRH deficiency (CHH and KS), precocious puberty and delayed puberty and their overlap. Activating and inactivating mutations in KISS1 and KISS1R cause the opposite phenotypes, precocious puberty and CHH, respectively.

Abreu et Kaiser, 2016

Howard, Clin Endocrinol (Oxford) 2021

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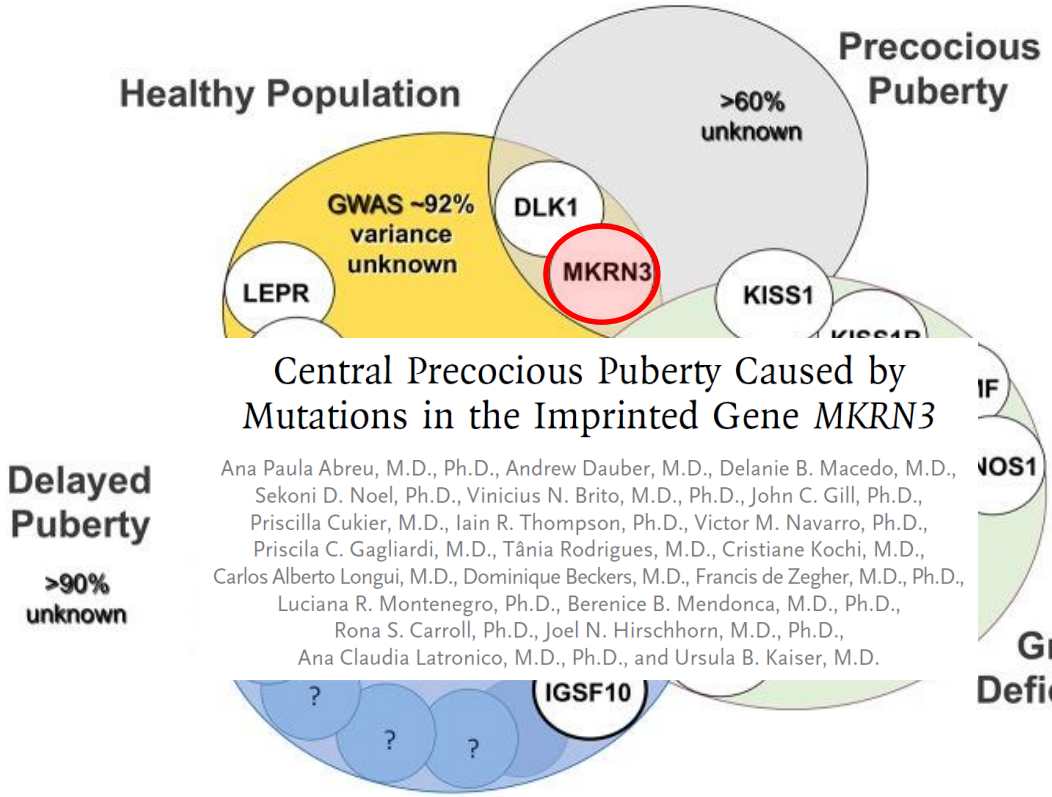

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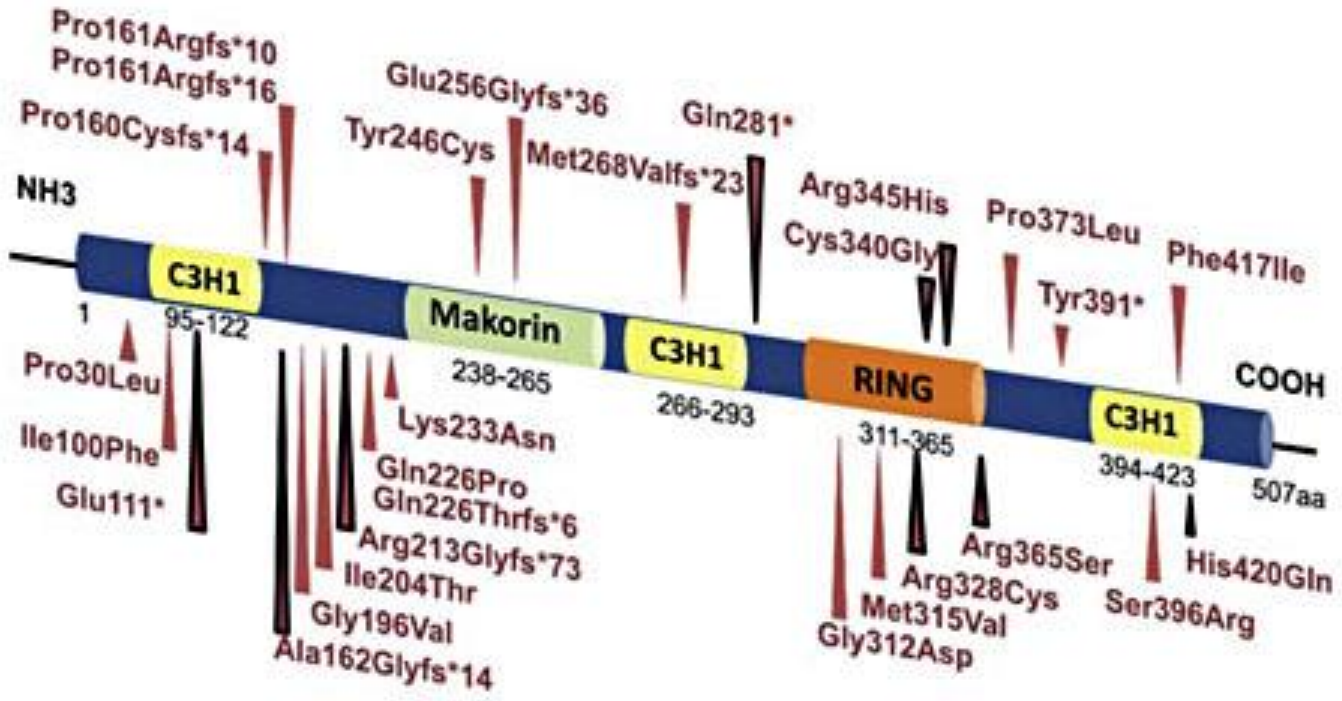
MKRN3 PROTEIN STRUCTURE

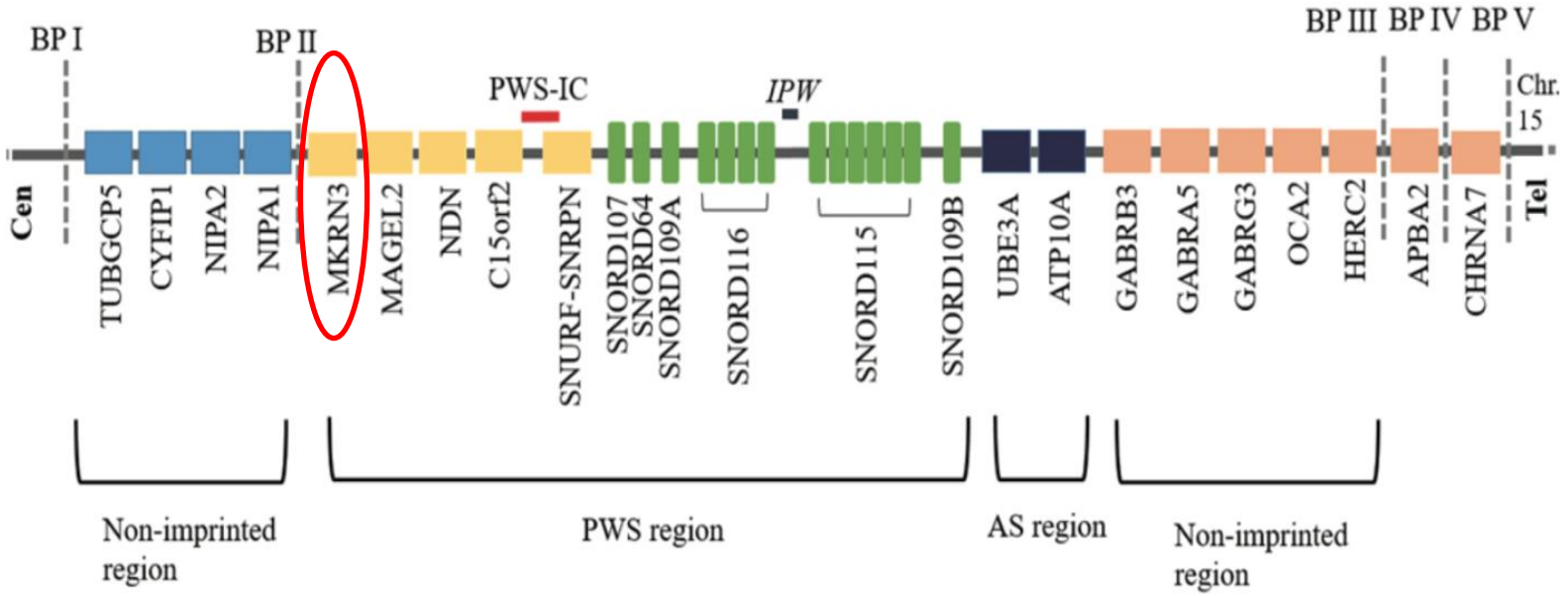


E3 ubiquitin ligase activity

RNA-binding activity

Abreu, Dauber et al., 2013
Bessa et al., 2017

MKRN3 IS LOCATED ON THE CHROMOSOME 15q11-q13 IN THE PWS CRITICAL REGION



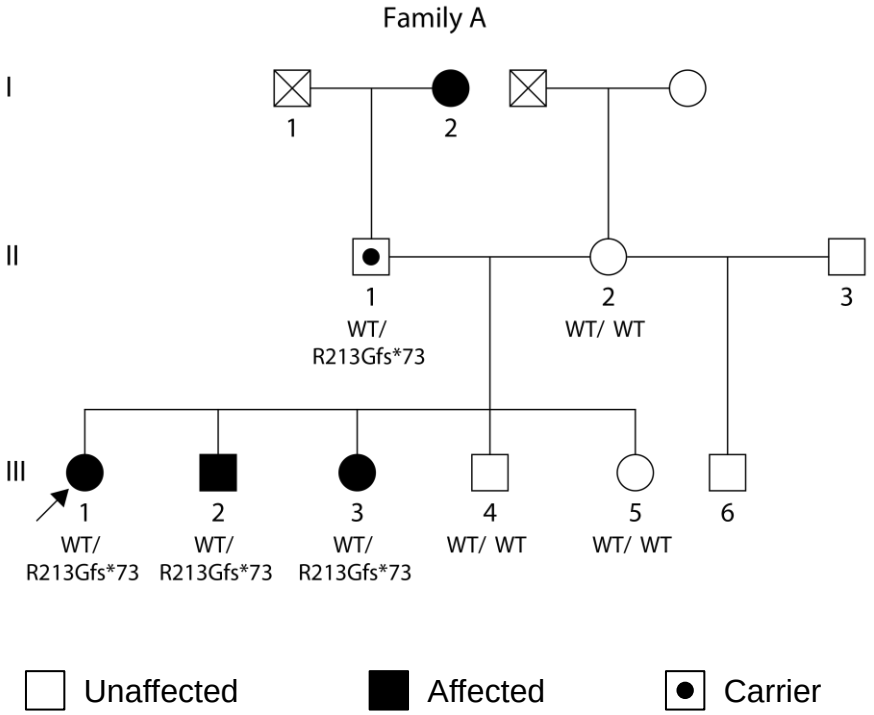
- Intronless gene

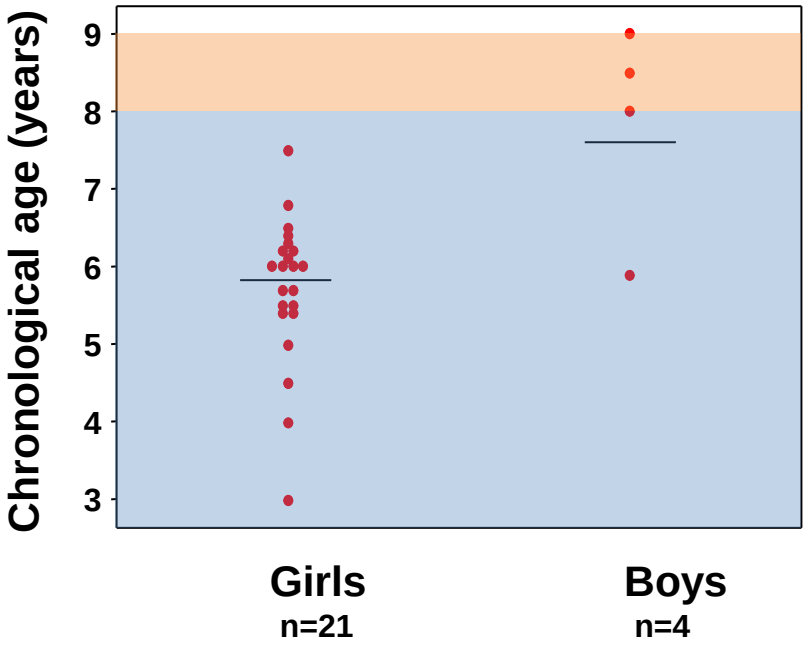
- Maternally imprinted

(expressed only from the paternally inherited allele)

Cheon et al., 2016

LOSS-OF-FUNCTION MUTATIONS IN THE *MKRN3* GENE IN PATIENTS WITH CPP





Precocious Puberty

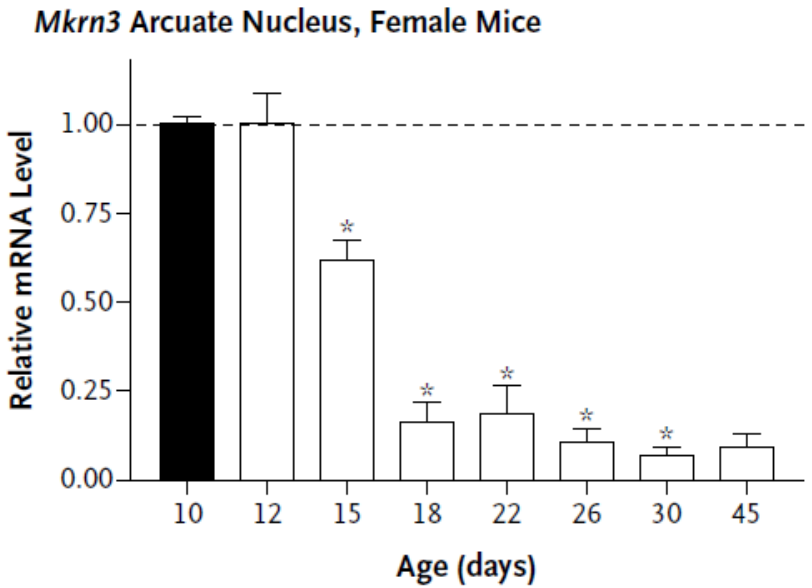
< 8 yo girls
< 9 yo boys

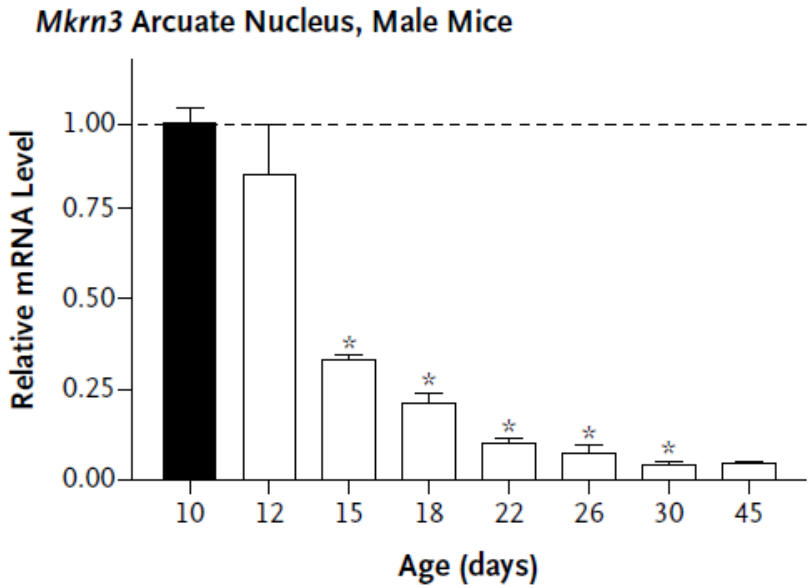
- Maternally imprinted

(expressed only from the paternally inherited allele)

Abreu, Dauber et al., 2013
Macedo, Abreu et al., 2014

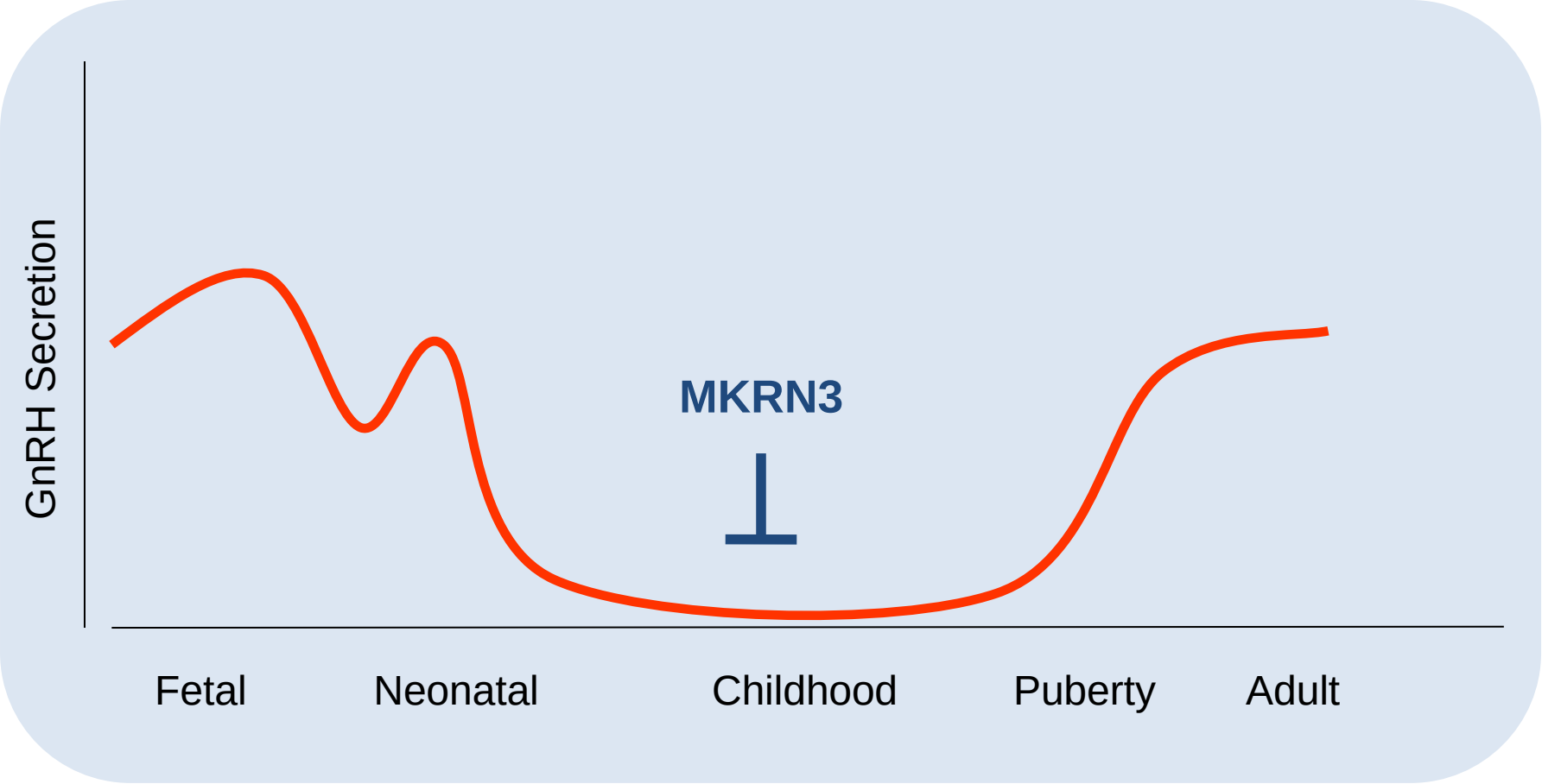
Mkrn3 EXPRESSION IN THE MOUSE HYPOTHALAMUS





Abreu et al., 2020
Abreu, Dauber et al., 2013

MKRN3 ACTS AS AN INHIBITOR OF PUBERTY ONSET



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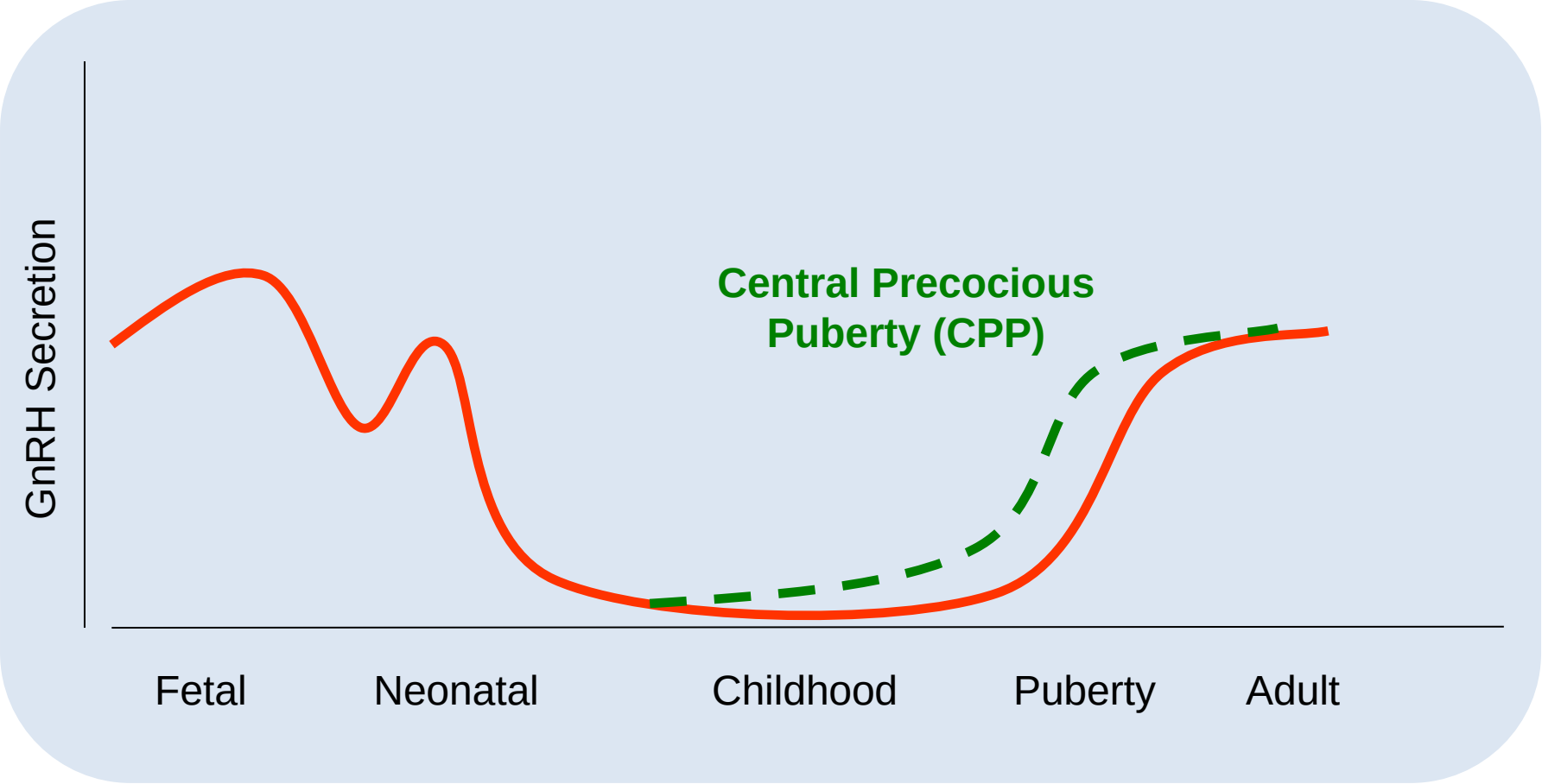

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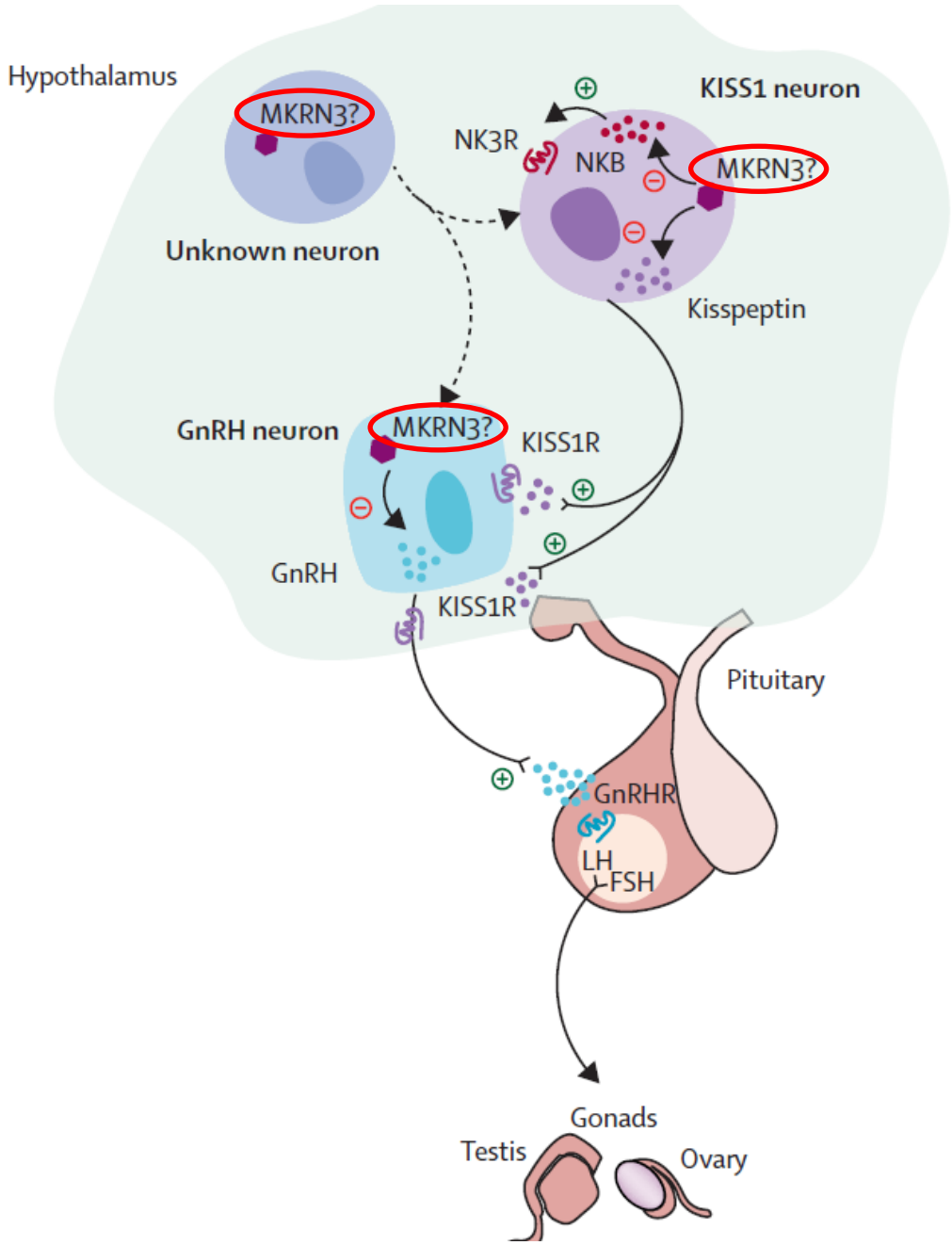
MECHANISMS OF ACTION OF MKRN3 ?

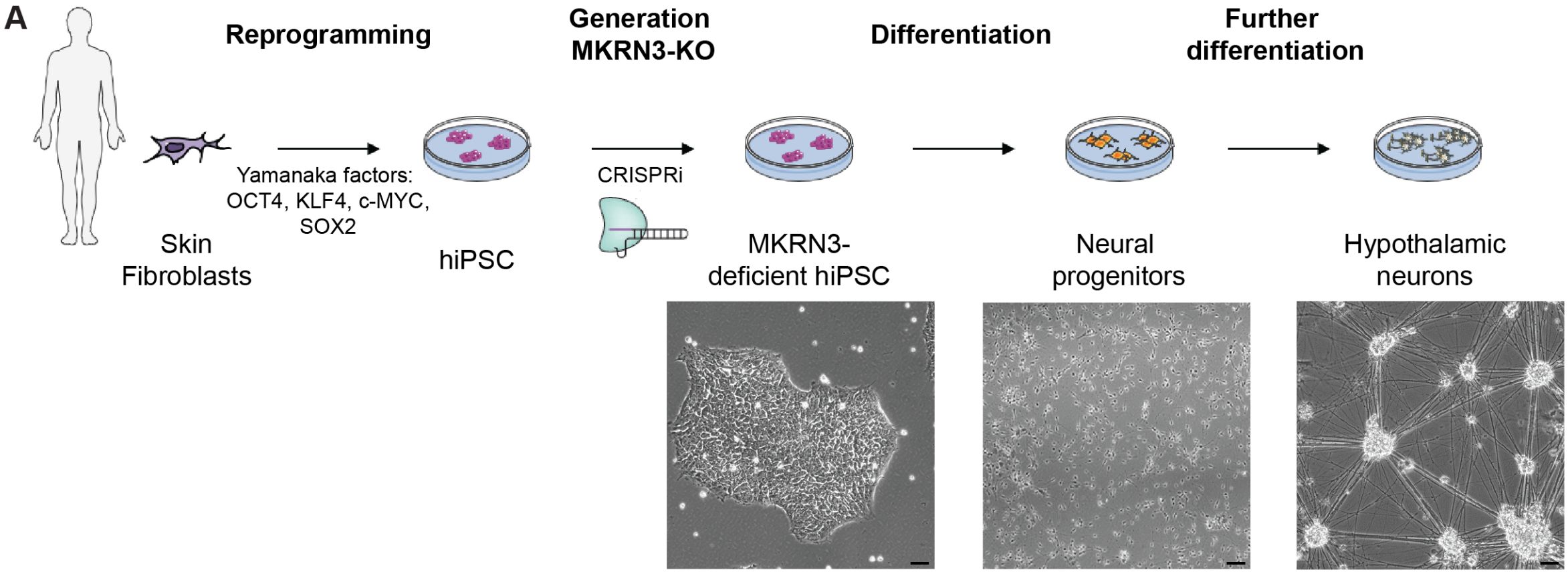


Abreu et Kaiser., 2016

AIM

- **Elucidate the mechanism of action of MKRN3 in puberty initiation within the hypothalamus using both *in vitro and in vivo* approaches.**

HYPOTHALAMIC DIFFERENTIATION OF MKRN3 DEFICIENT AND WT ISOGENIC hiPSCs



Naulé et al., 2023

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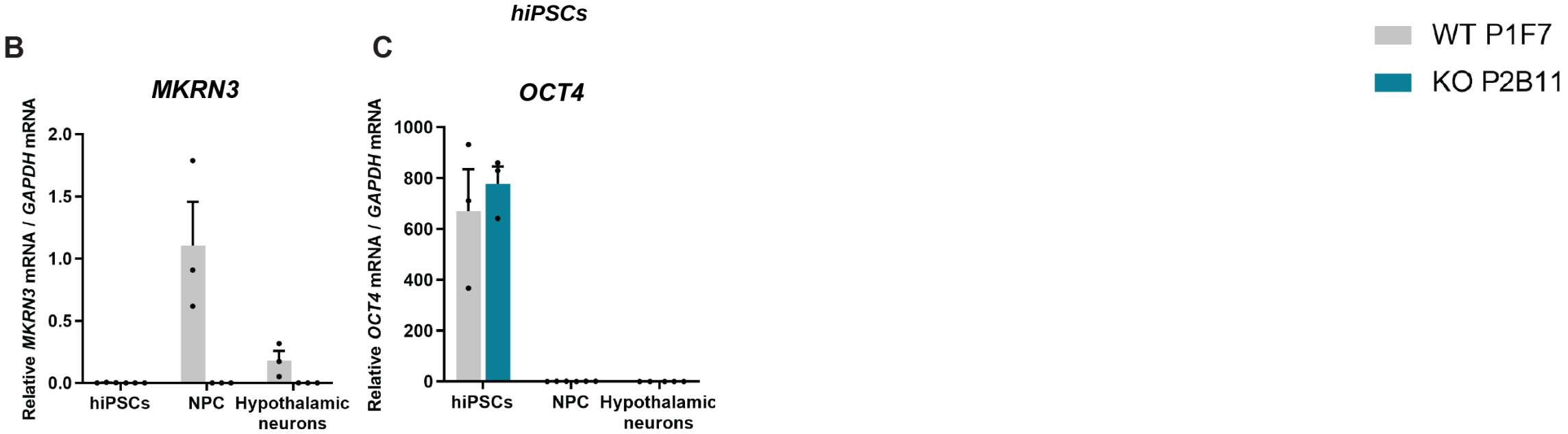

Naulé et al., 2023

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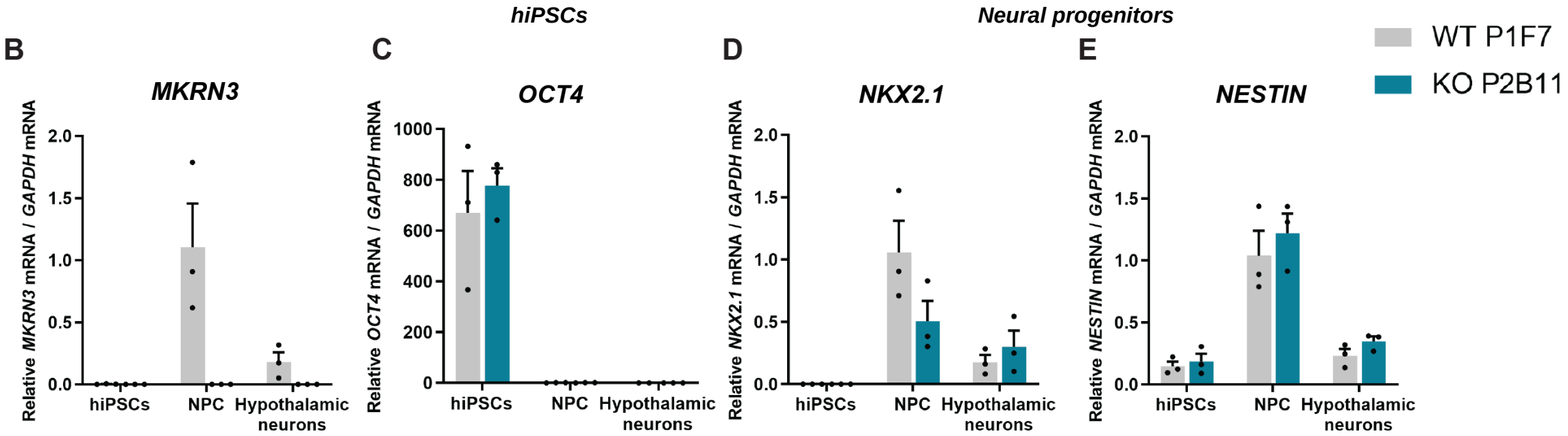

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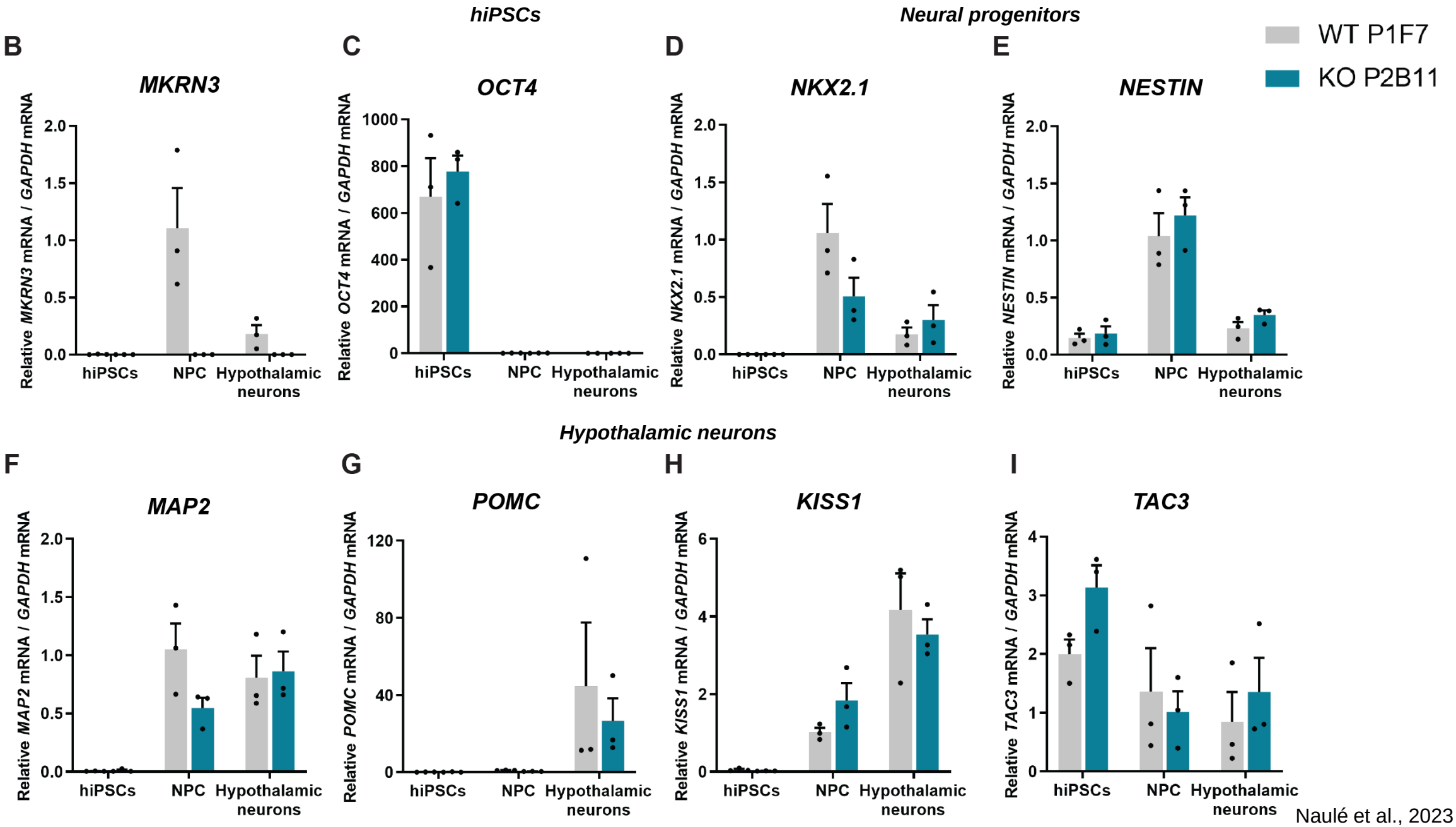
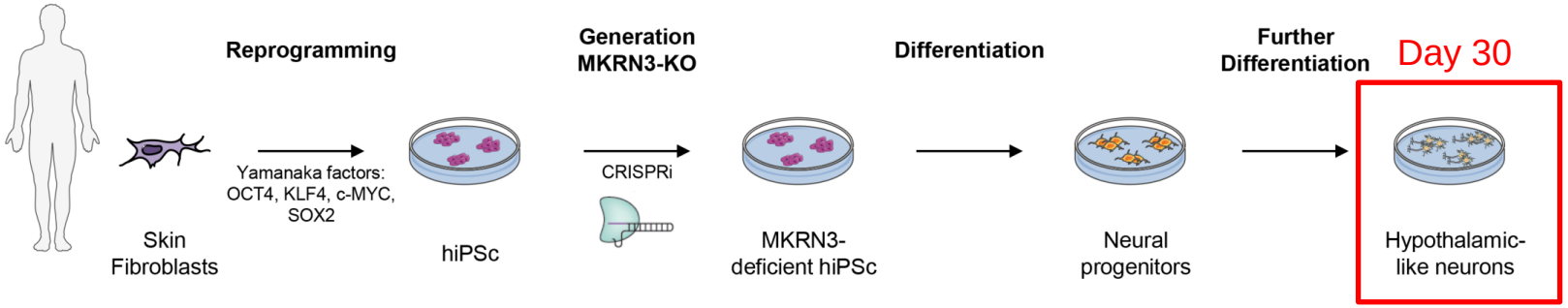


Naulé et al., 2023

TRANSCRIPTOME ANALYSIS BY RNA-SEQUENCING – DIFFERENTIAL EXPRESSION



176 genes upregulated,
228 downregulated
in the MKRN3-deficient compared to WT cells.

Naulé et al., 2023

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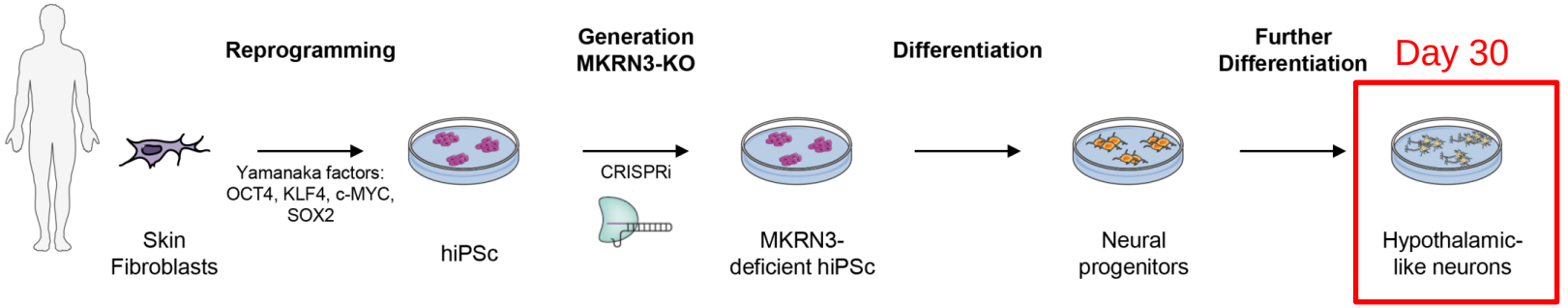

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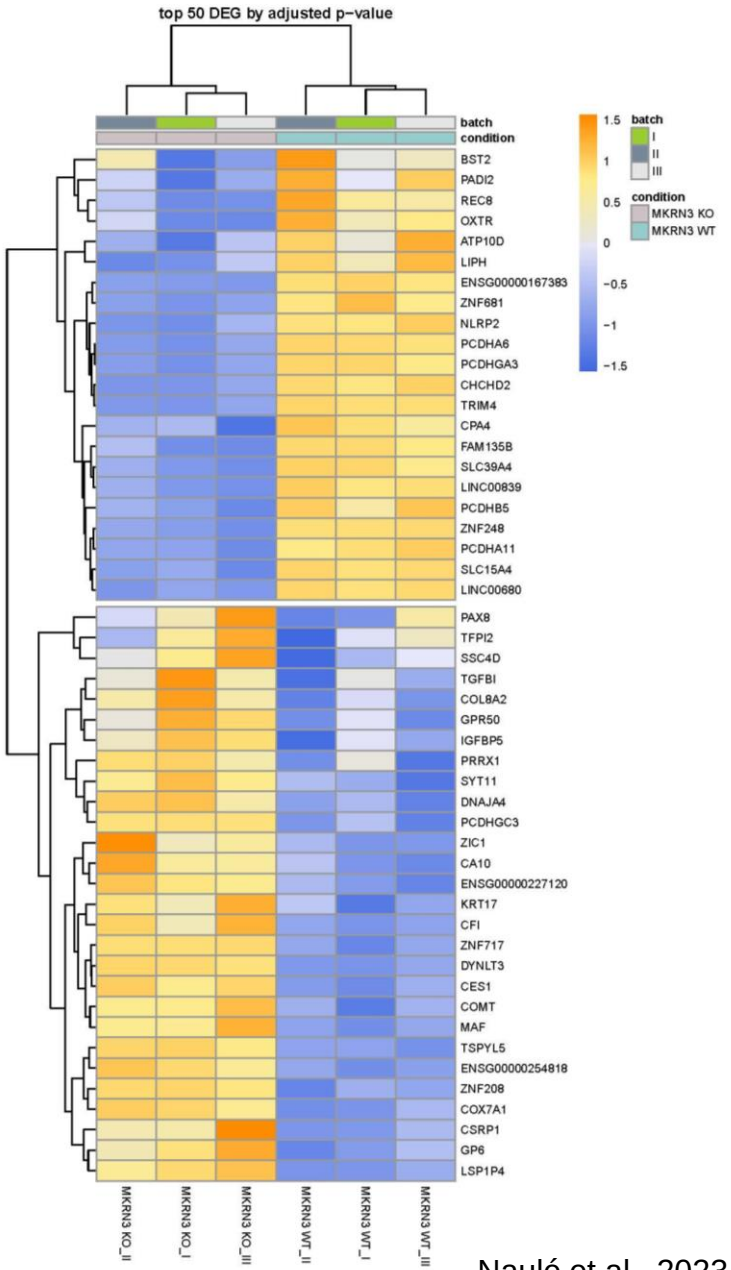


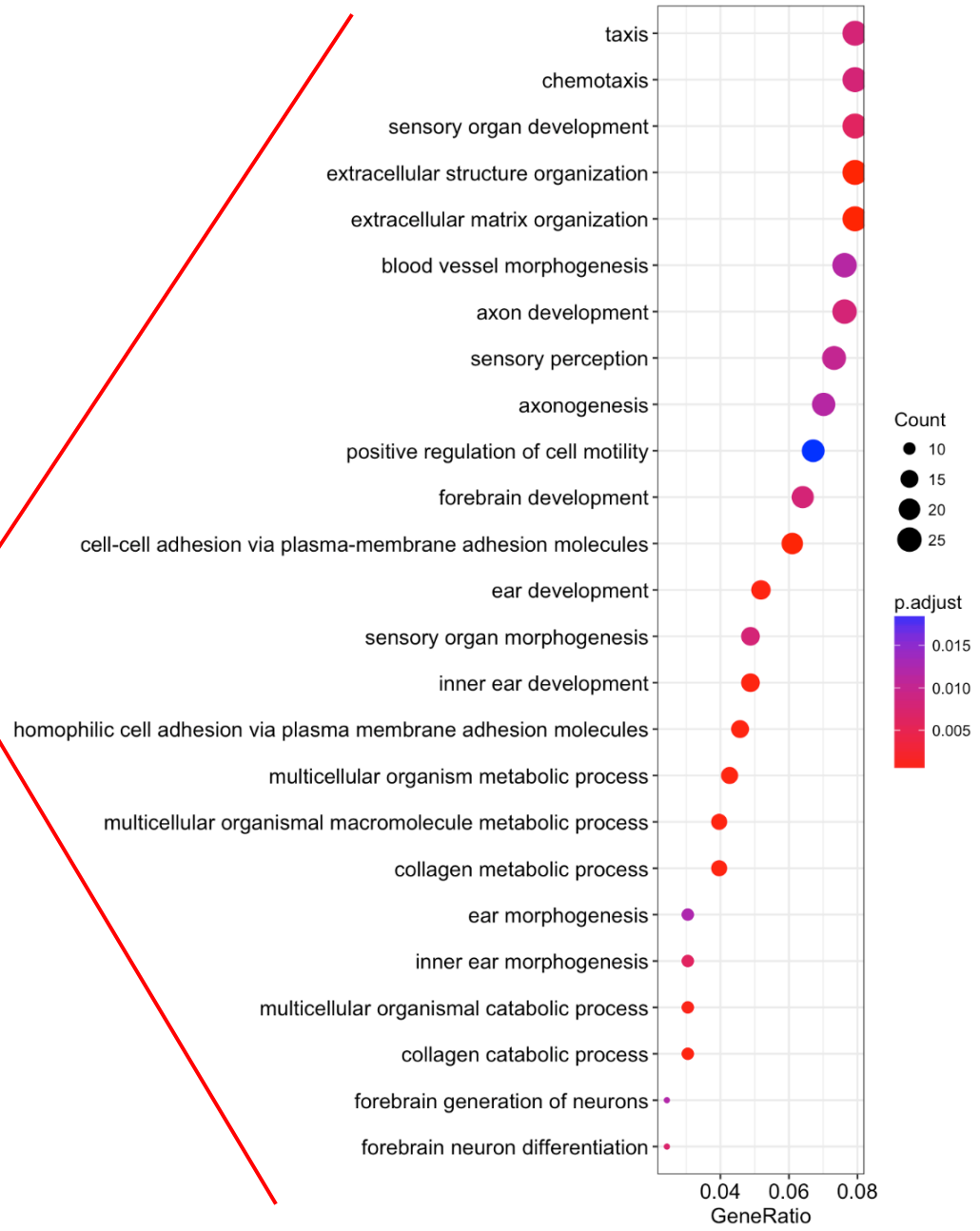
Naulé et al., 2023

53 Gene Ontology (GO) pathways were significantly different between the MKRN3-deficient and WT hypothalamic neurons.

Taxis/Chemotaxis
Extracellular matrix organization

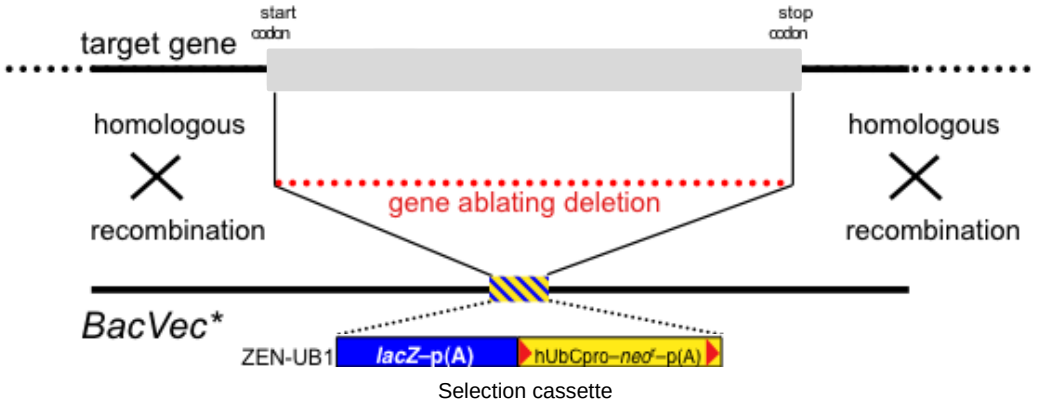
Axon development
Axonogenesis
Axon guidance
Neuron projection guidance
Synapse organization

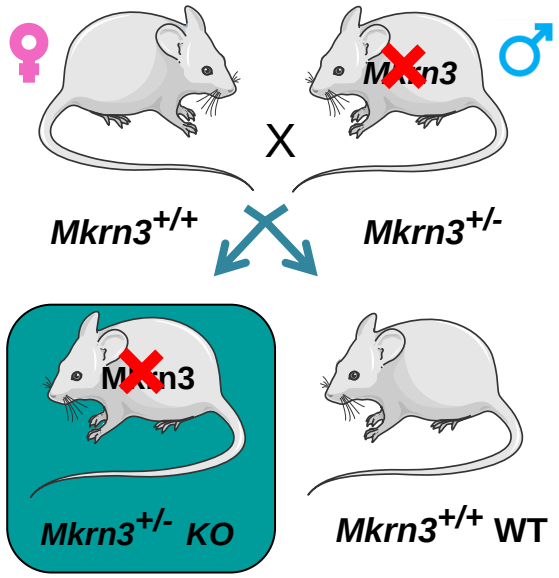
Forebrain development
Forebrain generation of neurons
Forebrain neuron differentiation

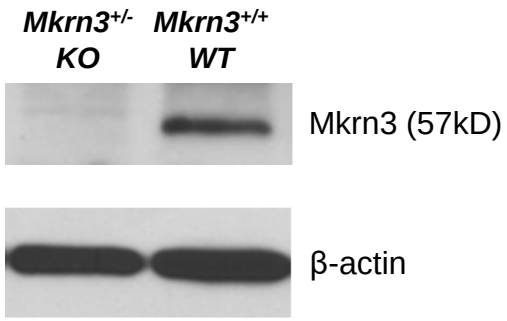


Naulé et al., 2023

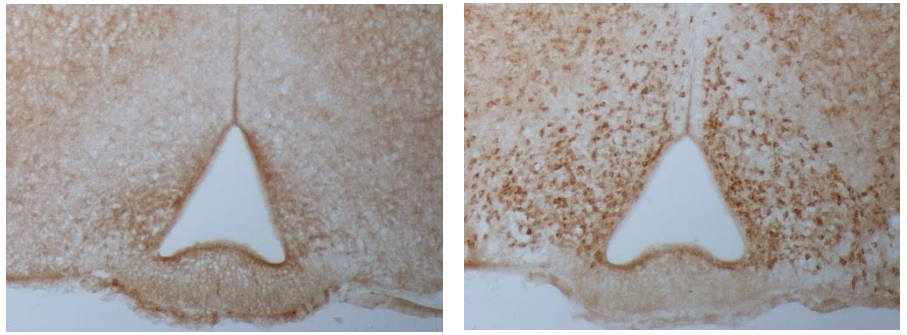
Mkrn3 DEFICIENT MOUSE MODEL







Mkrn3 protein expression in the mediobasal hypothalamus of postnatal day 10 female mice



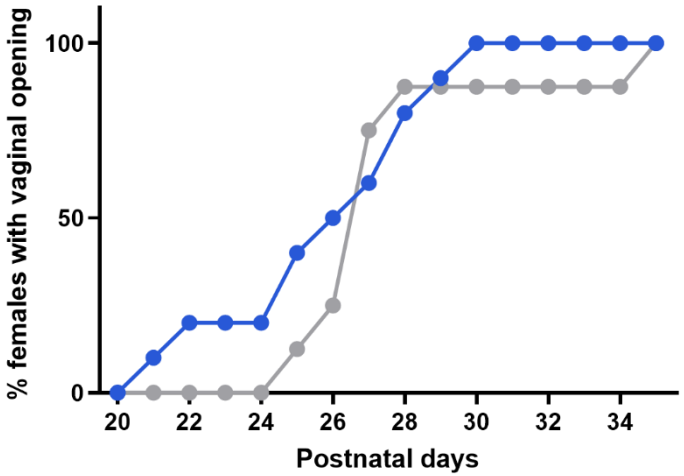
***Mkrn3*$^{+/-}$ KO** ***Mkrn3*$^{+/+}$ WT**

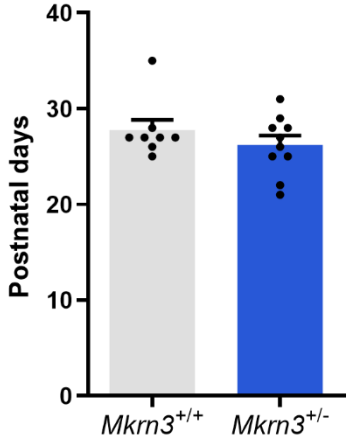
Mkrn3 protein expression in the arcuate nucleus postnatal day 10 mice

Naulé et al., 2023

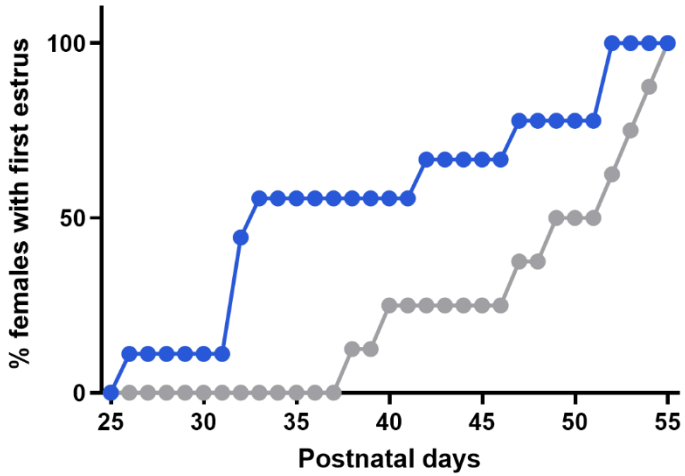
EARLY PUBERTY ONSET IN FEMALE *Mkrn3* KO MICE

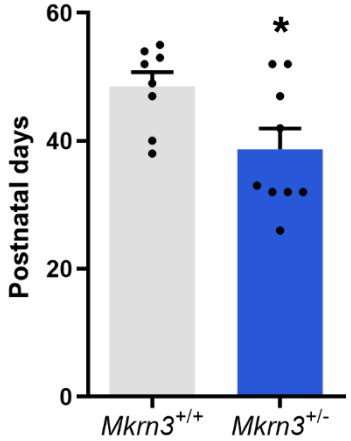
Age of vaginal opening

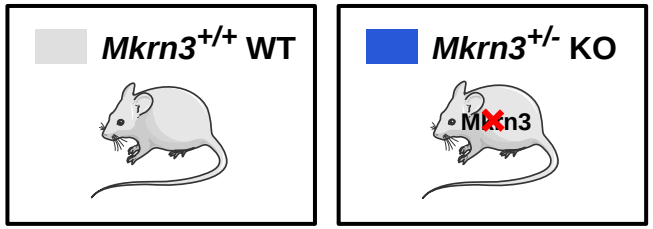




Age of first estrus







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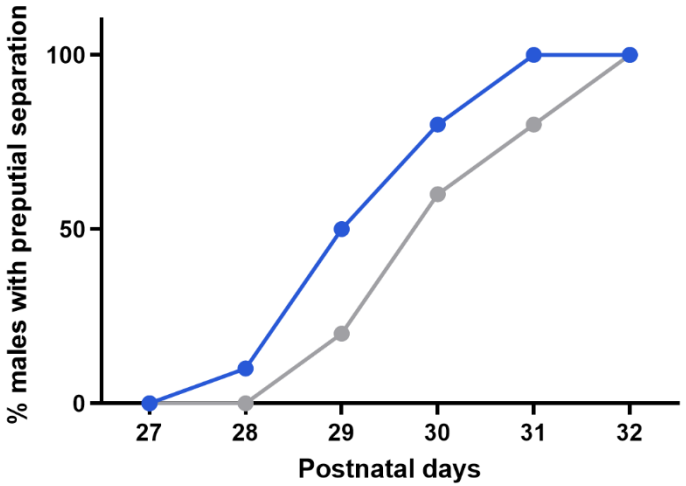
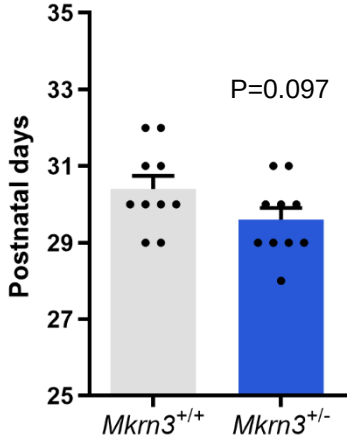
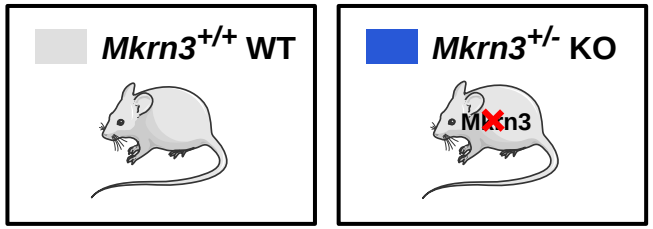
TENDENCY TOWARDS EARLY PUBERTAL ONSET IN MALE *Mkrn3* KO MICE

Age of preputial separation







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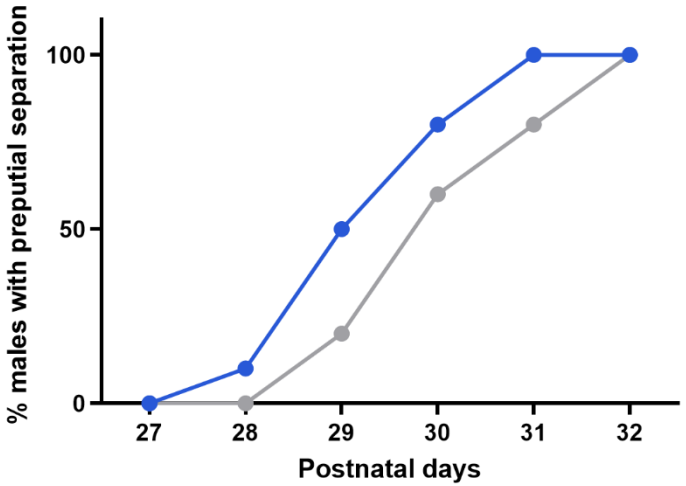
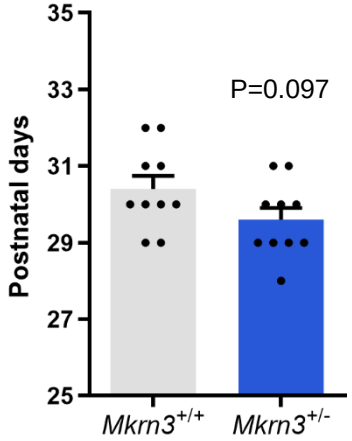
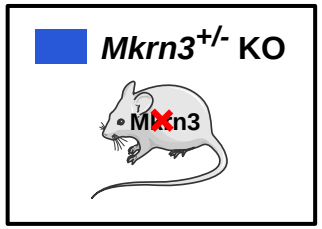




Mkrn3 **deletion is associated with accelerated puberty onset in female mice and a tendency towards early puberty in male mice.**



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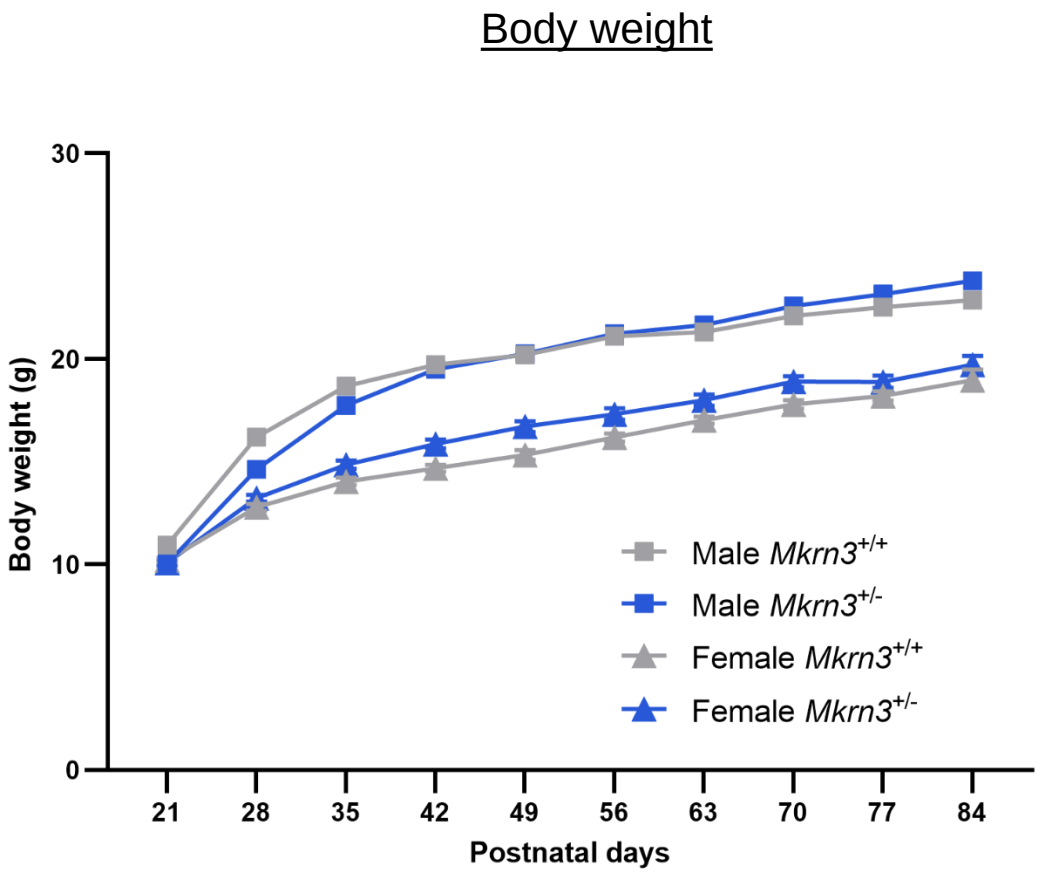
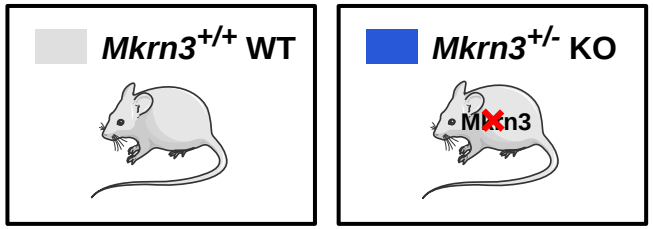
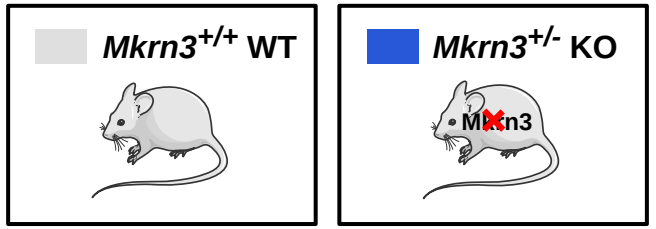
NO DIFFERENCE IN BODY WEIGHT BETWEEN MALE AND FEMALE *Mkrn3* KO MICE COMPARED TO WT



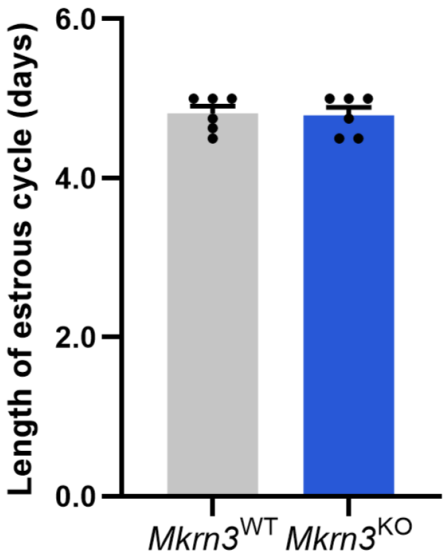


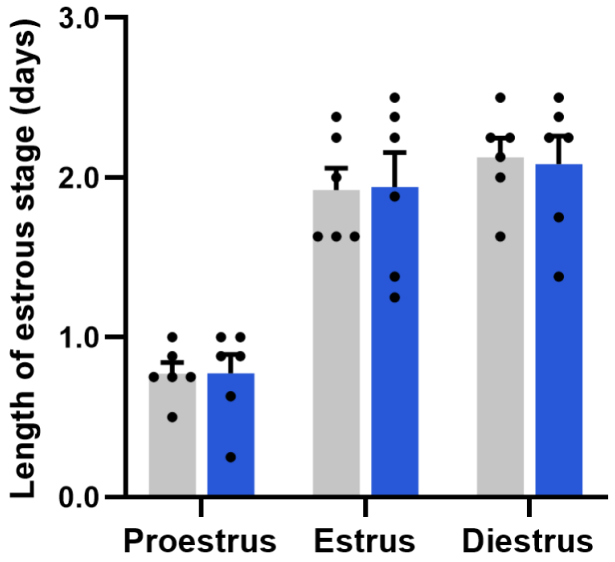
Naulé et al., 2023

NORMAL CYCLICITY AND FERTILITY IN *Mkrn3* KO MICE



Cyclicity



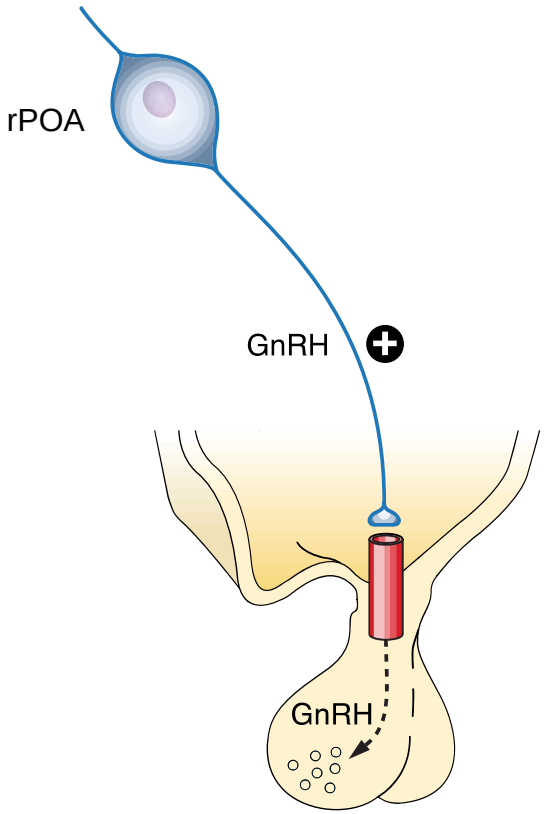


Fertility

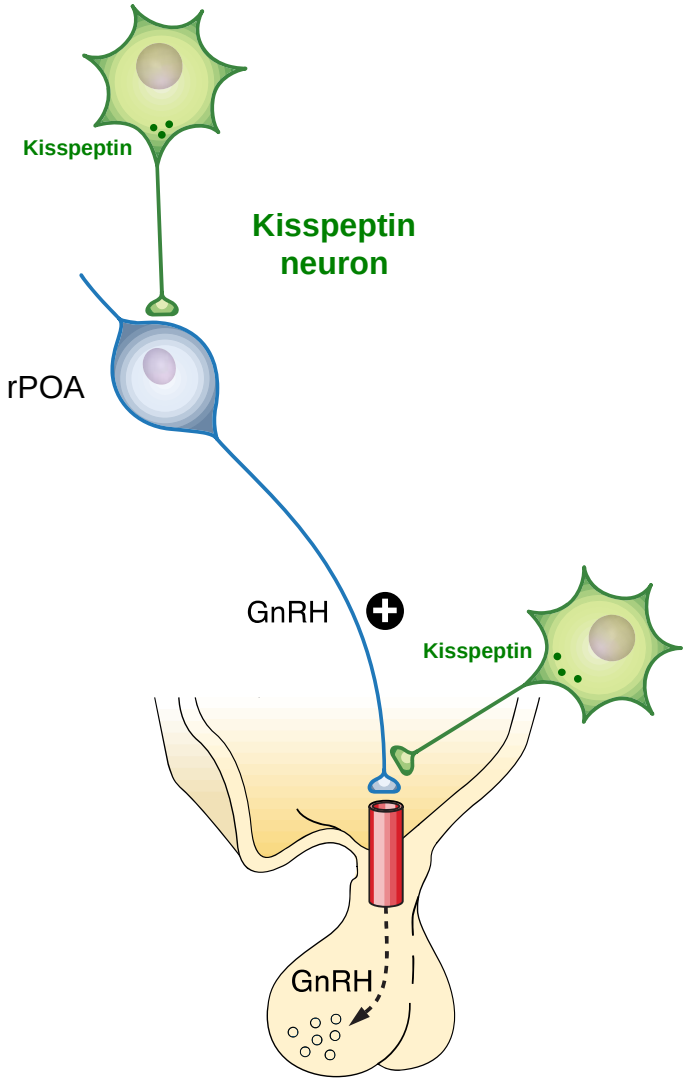
	Female		Male	
	Mkrn3^{WT}	Mkrn3^{KO}	Mkrn3^{WT}	Mkrn3^{KO}
Time to first litter	23.3 ± 0.3	26.3 ± 1.9	23.3 ± 0.3	26.3 ± 3.3
Number of litters	3.7 ± 0.3	4.0 ± 0.6	3.7 ± 0.3	4 ± 0.0
Number of pups	18.3 ± 2.3	20.3 ± 1.5	18.3 ± 2.3	19.7 ± 1.86
Number of pups per litter	5.00 ± 0.3	5.6 ± 0.6	5.00 ± 0.3	5.00 ± 0.5

Naulé et al., 2023

KISSPEPTIN NEURONS: CRITICAL FOR PUBERTY ONSET AND FERTILITY



KISSPEPTIN NEURONS: CRITICAL FOR PUBERTY ONSET AND FERTILITY



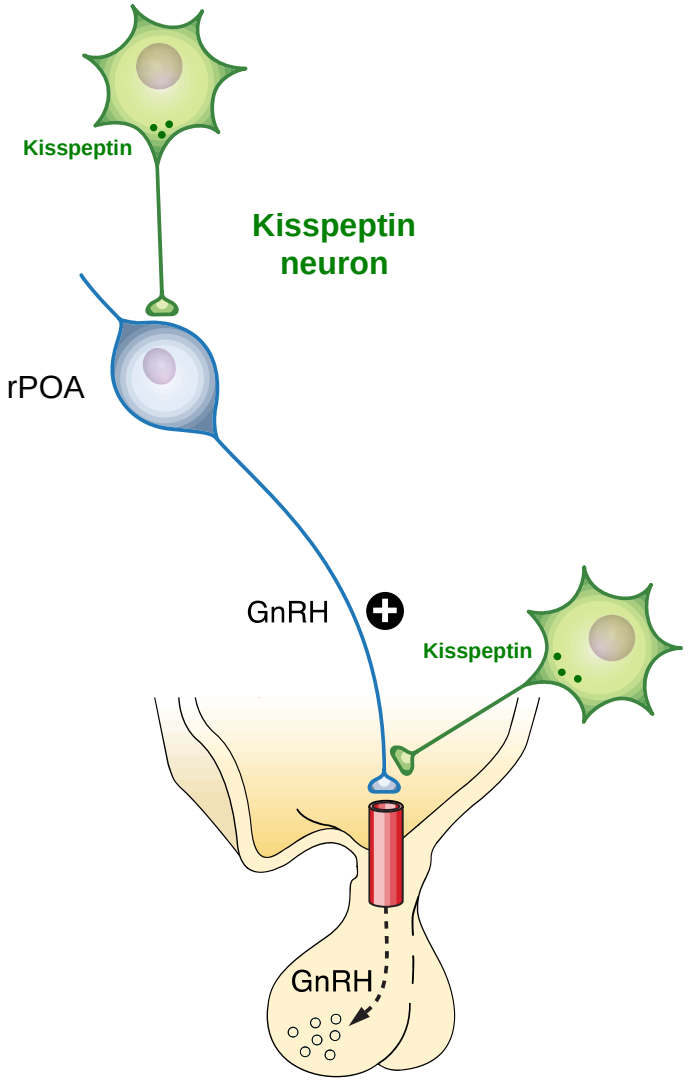
In human

Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54

Nicolas de Roux*†‡, Emmanuelle Genin§, Jean-Claude Carel¶, Fumihiko Matsuda‖, Jean-Louis Chaussain¶, and Edwin Milgrom*

| PNAS | September 16, 2003 |

KISSPEPTIN NEURONS: CRITICAL FOR PUBERTY ONSET AND FERTILITY



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In primates

The role of *KiSS-1* in the regulation of puberty in higher primates

Tony M Plant

Department of Cell Biology and Physiology and Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, 3550 Terrace Street, Rm 828 Scaife Hall, Pittsburgh, Pennsylvania 15261, USA

European Journal of Endocrinology (2006)

In mice

Hypogonadotropic hypogonadism in mice lacking a functional *Kiss1* gene

Xavier d'Anglemont de Tassigny*, Lisa A. Fagg*, John P. C. Dixon†, Kate Day†, Harry G. Leitch*, Alan G. Hendrick†, Dirk Zahn†, Isabelle Franceschini‡, Alain Caraty‡, Mark B. L. Carlton†, Samuel A. J. R. Aparicio§, and William H. Colledge*¶

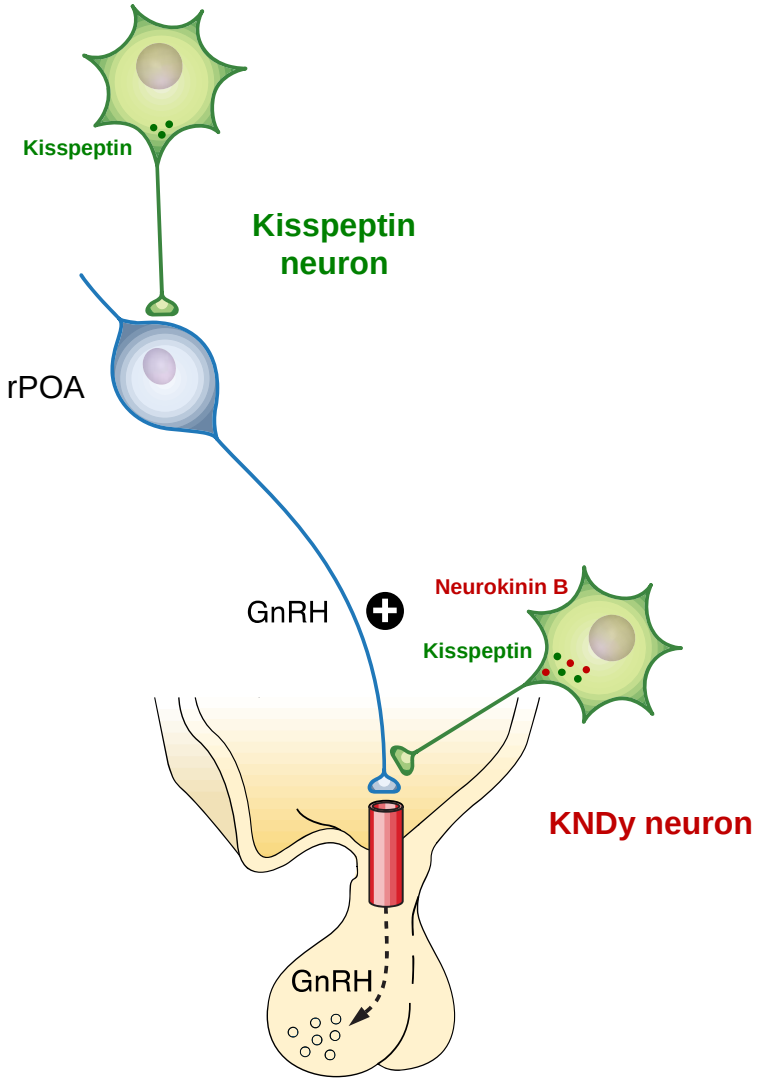
| PNAS | June 19, 2007 | vol. 104 | no. 25

The GPR54 Gene as a Regulator of Puberty

Stephanie B. Seminara, M.D., Sophie Messager, Ph.D.,
Emmanouella E. Chatzidaki, B.Sc., Rosemary R. Thresher, Ph.D.,

N ENGL J MED 349;17OCTOBER 23, 2003

MECHANISMS OF ACTION OF MKRN3 IN PUBERTY INITIATION?

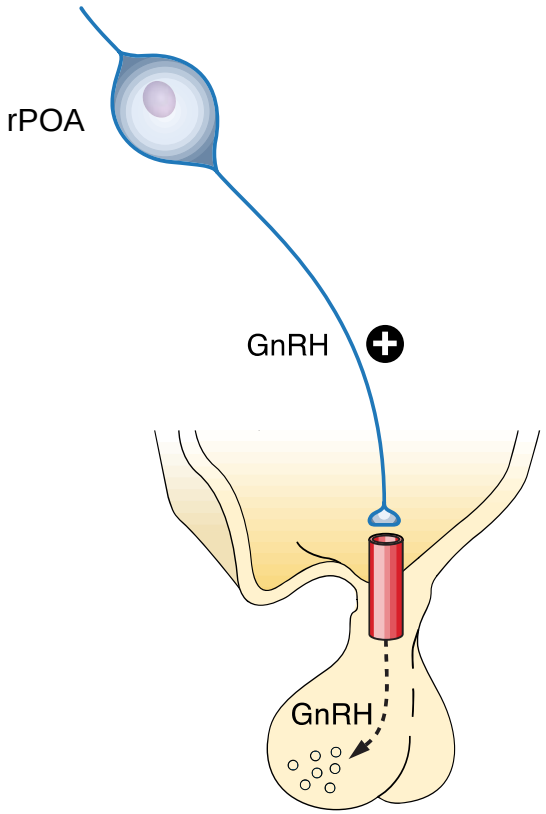


TAC3 and *TACR3* mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction

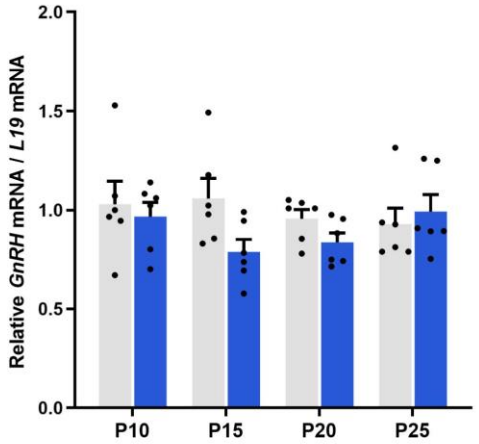
A Kemal Topaloglu[1,7], Frank Reimann[2,7], Metin Guclu[3], Ayse Serap Yalin[4], L Damla Kotan[5], Keith M Porter[6], Ayse Serin[5], Neslihan O Mungan[1], Joshua R Cook[6], Mehmet N Ozbek[1], Sazi Imamoglu[3], N Sema Akalin[4], Bilgin Yuksel[1], Stephen O'Rahilly[6] & Robert K Semple[6]

VOLUME 41 | NUMBER 3 | MARCH 2009 | NATURE GENETICS

MECHANISMS OF ACTION OF MKRN3 IN PUBERTY INITIATION?

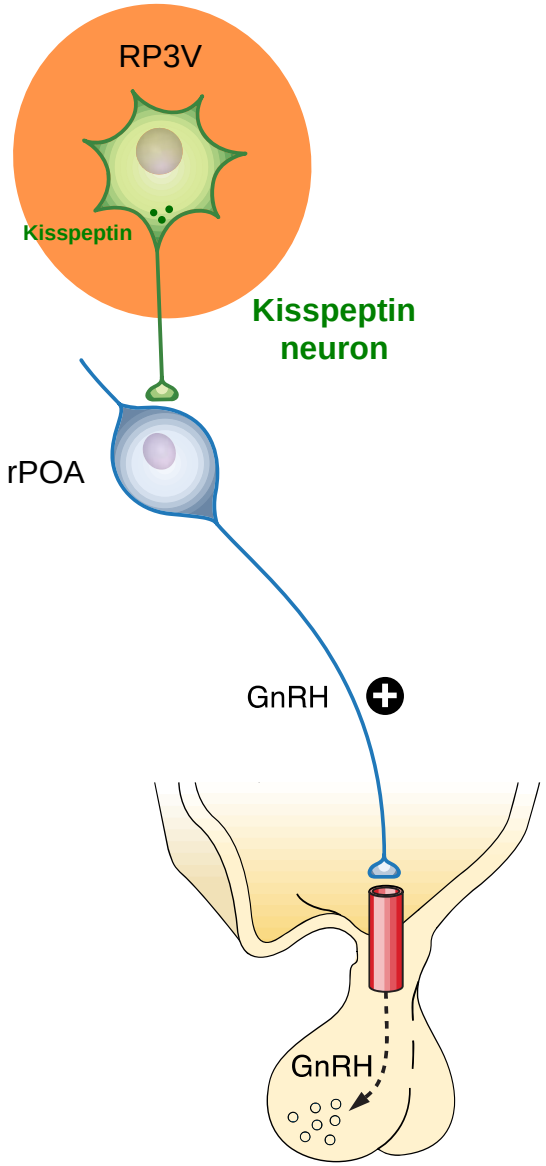


GnRH expression in the preoptic area (rPOA)



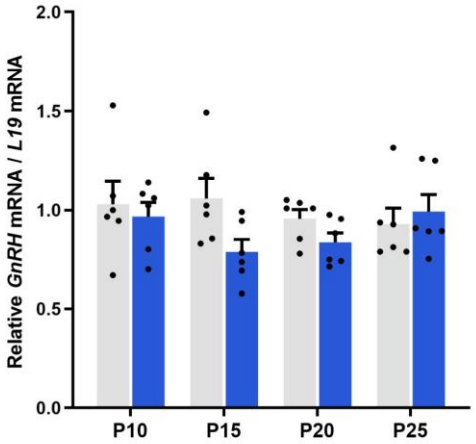
Naulé et al., 2023

MECHANISMS OF ACTION OF MKRN3 IN PUBERTY INITIATION?

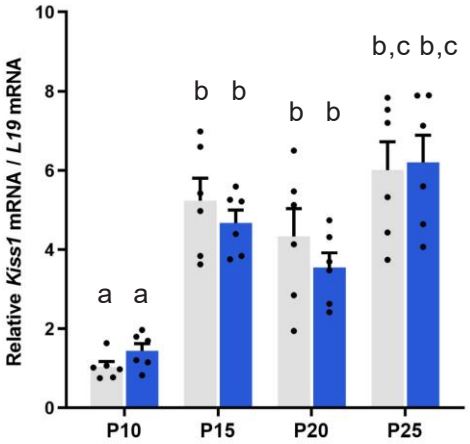


Mkrn3$^{+/+}$ WT *Mkrn3*$^{+/-}$ KO

GnRH expression in the preoptic area (rPOA)



Kiss1 expression in the preoptic area (POA)



Naulé et al., 2023

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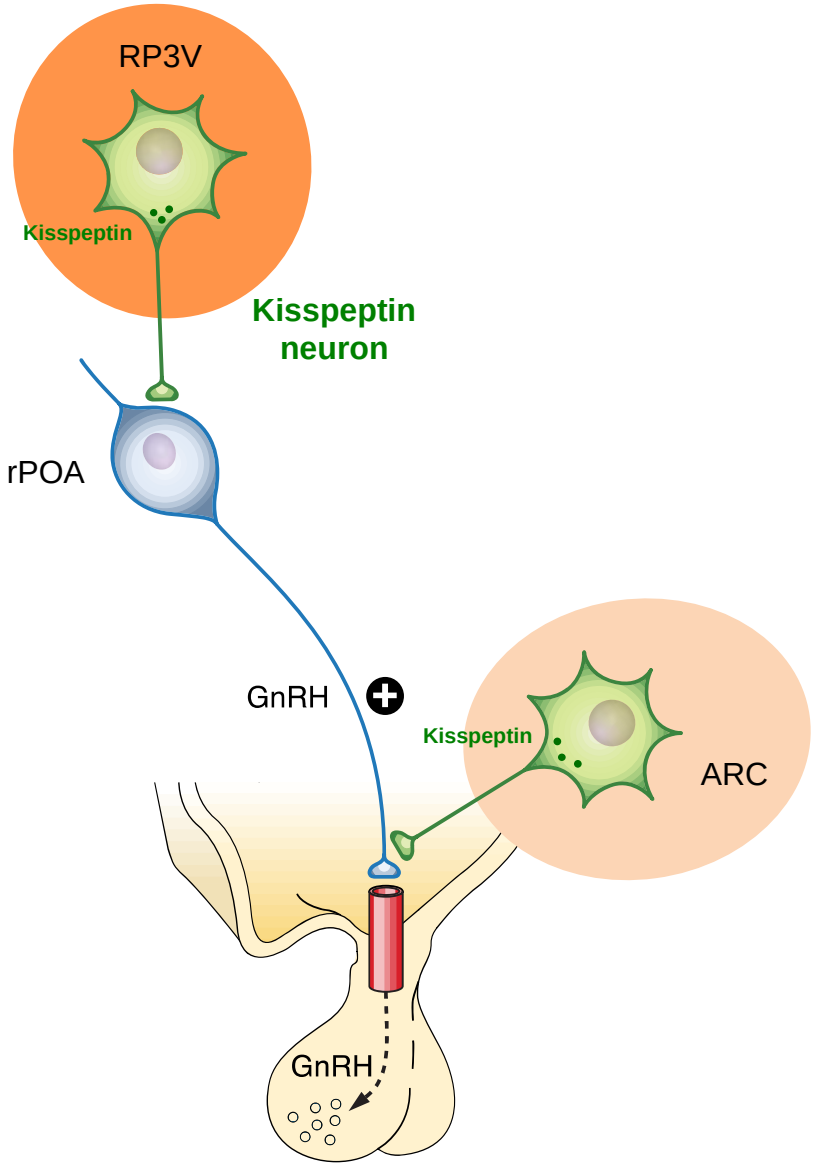

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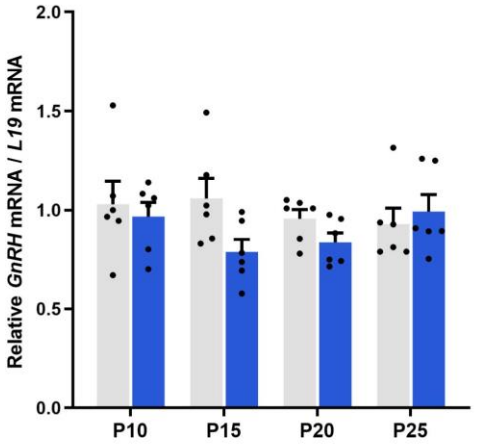

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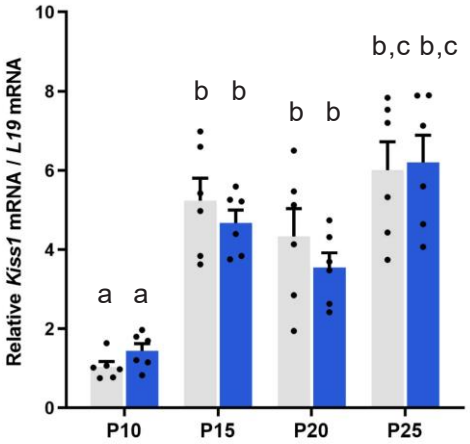
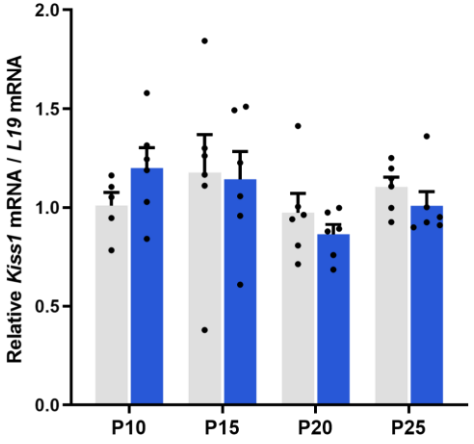
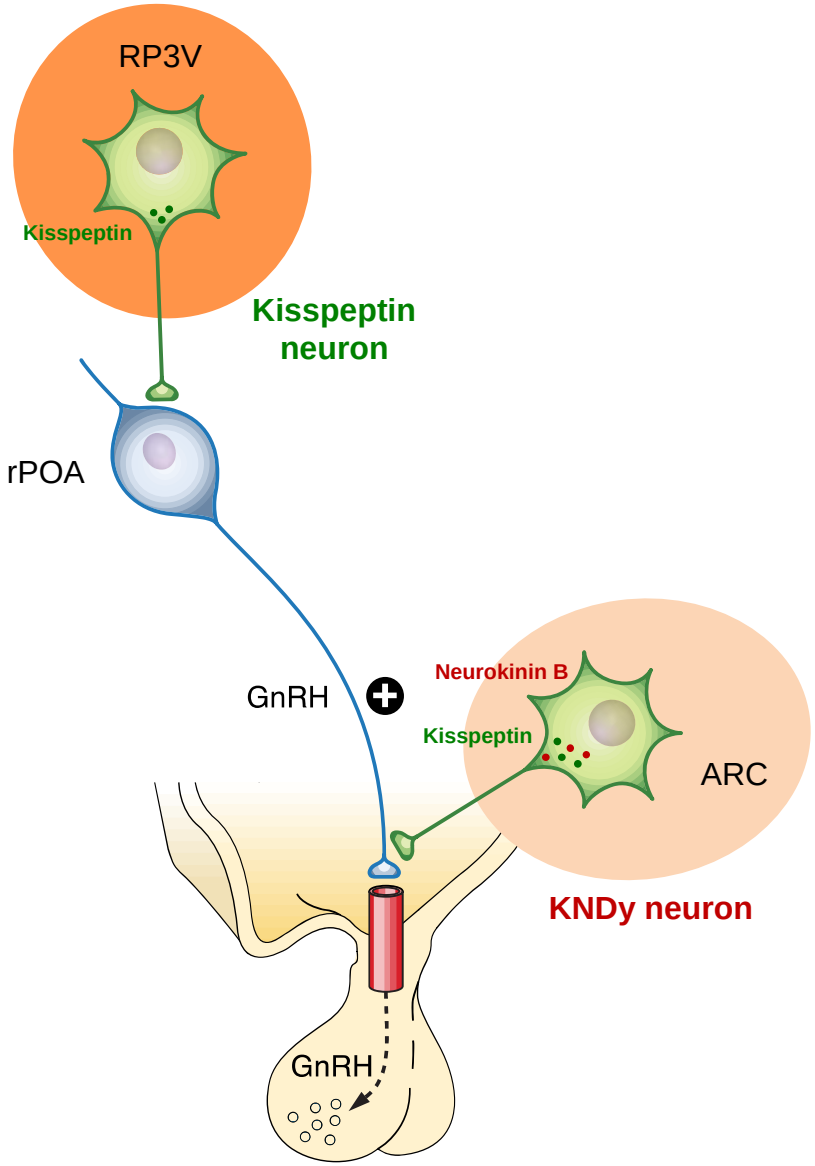


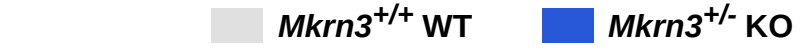
Kiss1 expression in the arcuate nucleus (ARC)



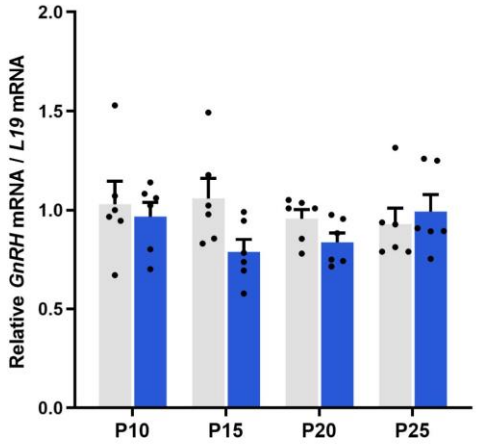
Naulé et al., In press

MECHANISMS OF ACTION OF MKRN3 IN PUBERTY INITIATION?

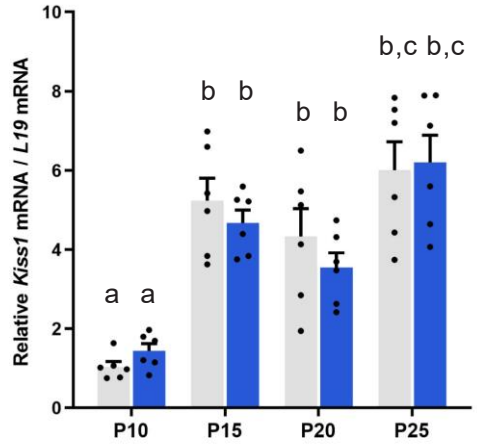




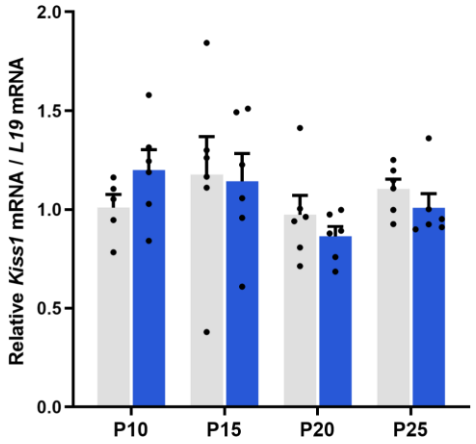
GnRH expression in the preoptic area (rPOA)



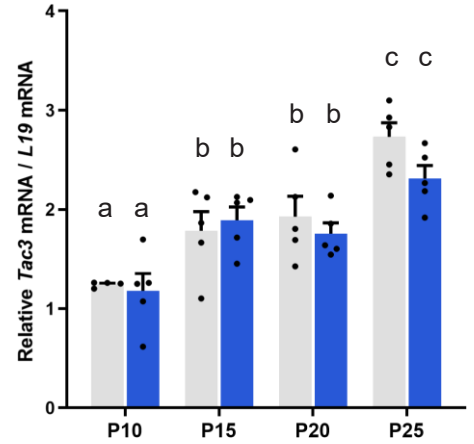
Kiss1 expression in the preoptic area (POA)



Kiss1 expression in the arcuate nucleus (ARC)

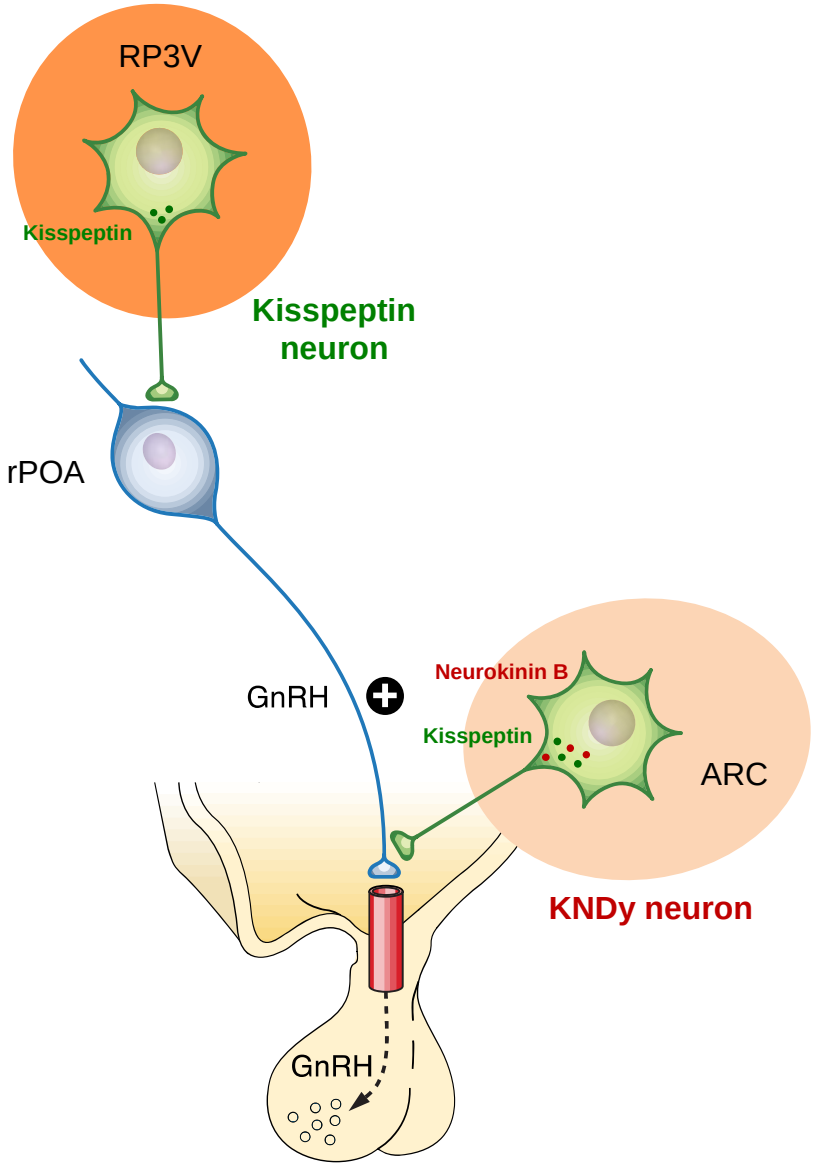


Tac3 expression in the arcuate nucleus (ARC)



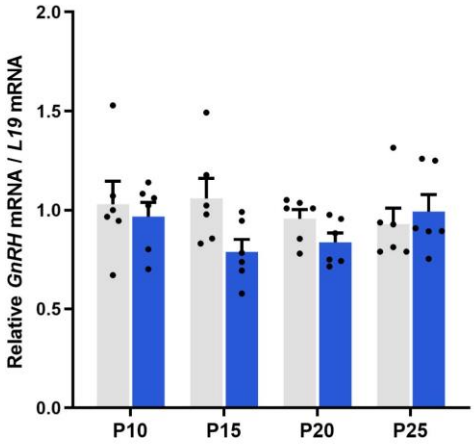
Naulé et al., 2023

MECHANISMS OF ACTION OF MKRN3 IN PUBERTY INITIATION?



$Mkrn3^{+/+}$ WT $Mkrn3^{+/-}$ KO

GnRH expression in the preoptic area (rPOA)



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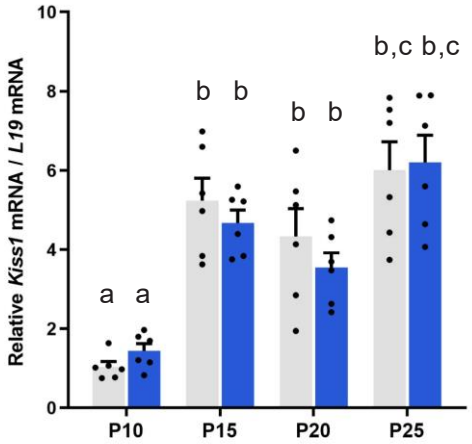

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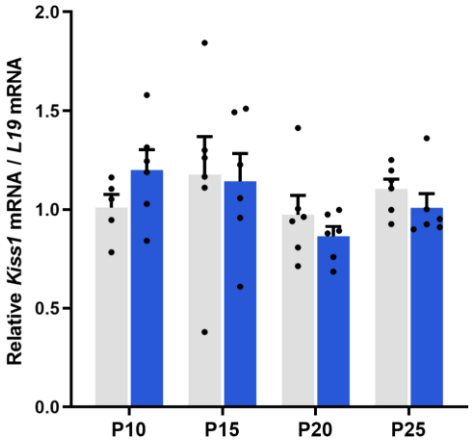

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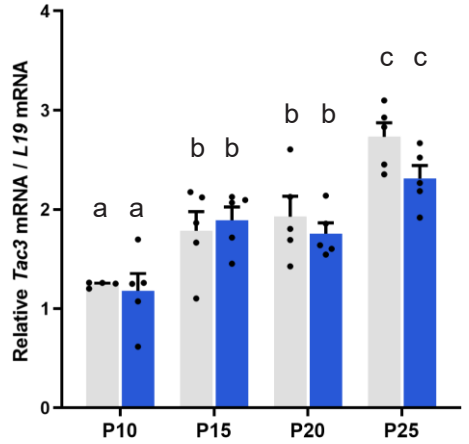
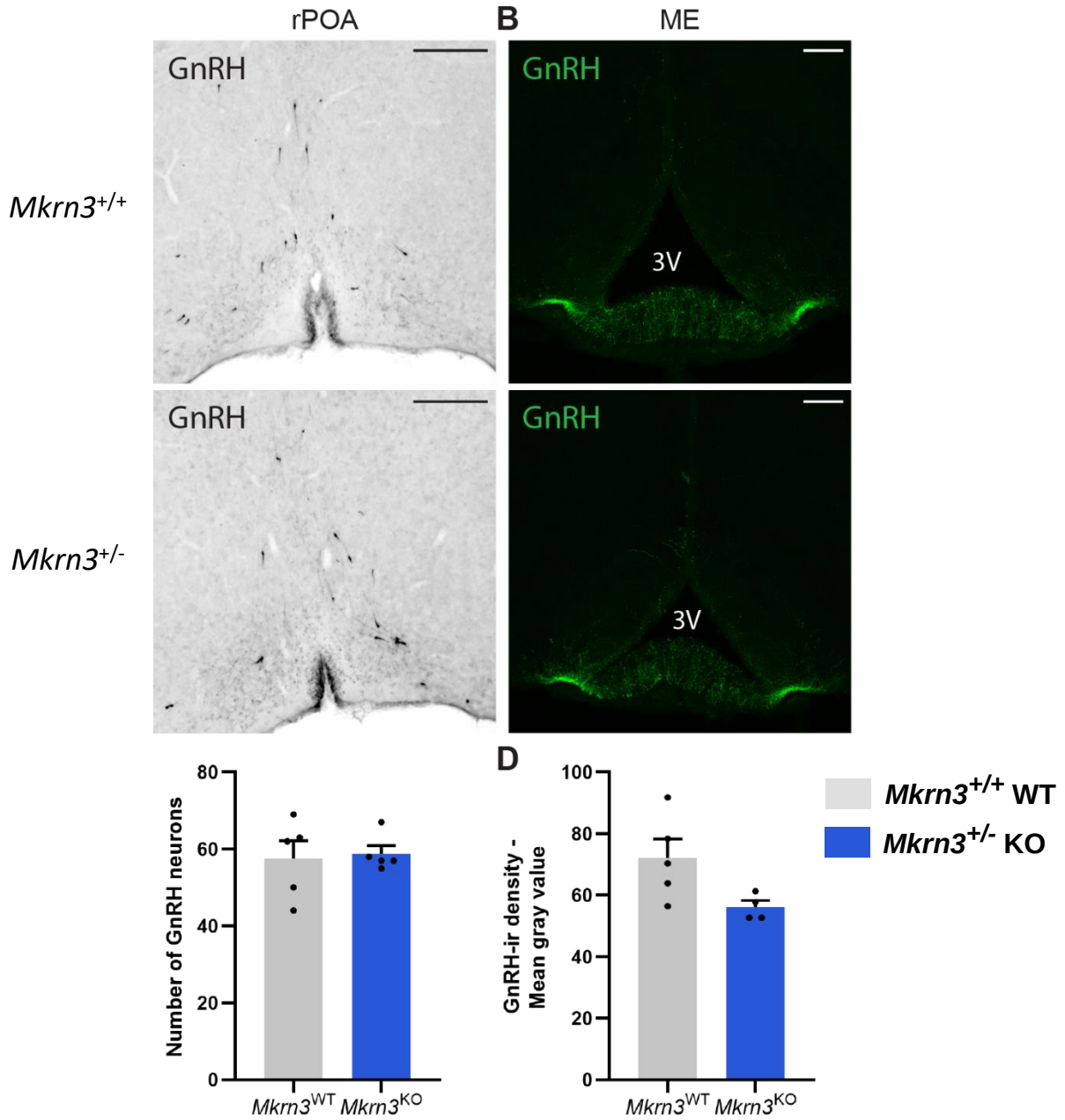


***Mkrn3* deletion in *Mkrn3*$^{+/-}$ female mice is not associated with change of *GnRH*, *Kiss1 and Tac3* mRNA expression in the POA and/or ARC.**

Naulé et al., 2023

PROTEIN EXPRESSION OF KEY REGULATORS OF PUBERTY INITIATION

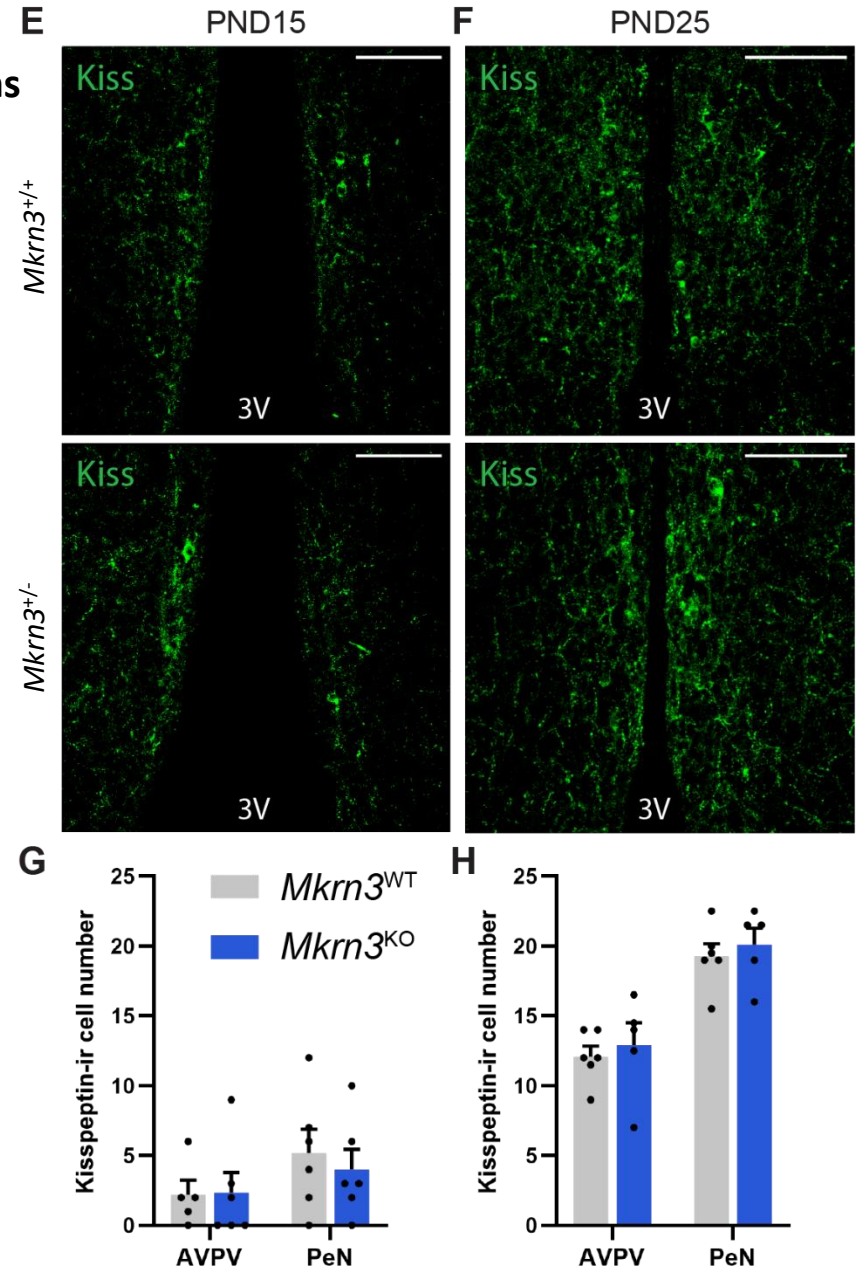
GnRH neurons – PND10



Naulé et al., 2023

PROTEIN EXPRESSION OF KEY REGULATORS OF PUBERTY INITIATION

Kisspeptin neurons AVPV/PeN



Naulé et al., 2023

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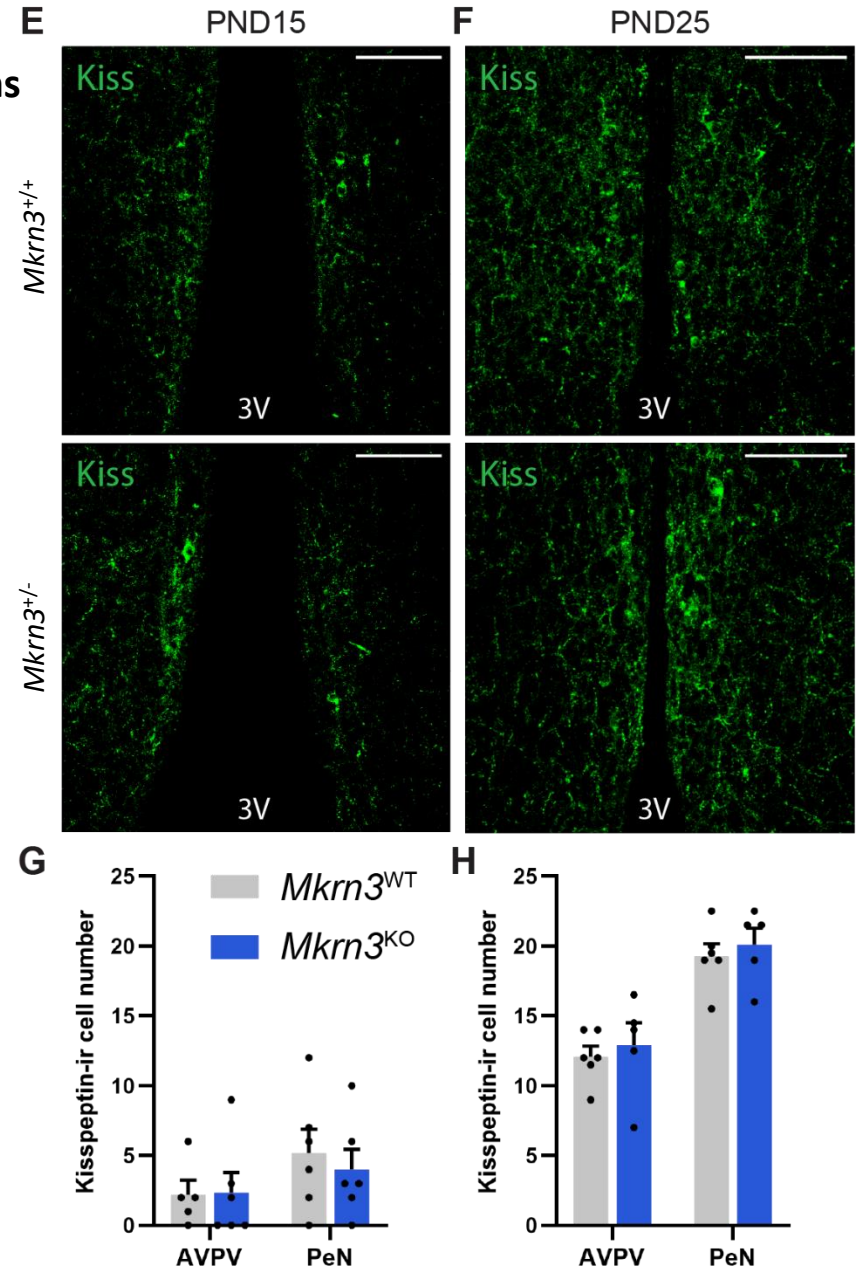
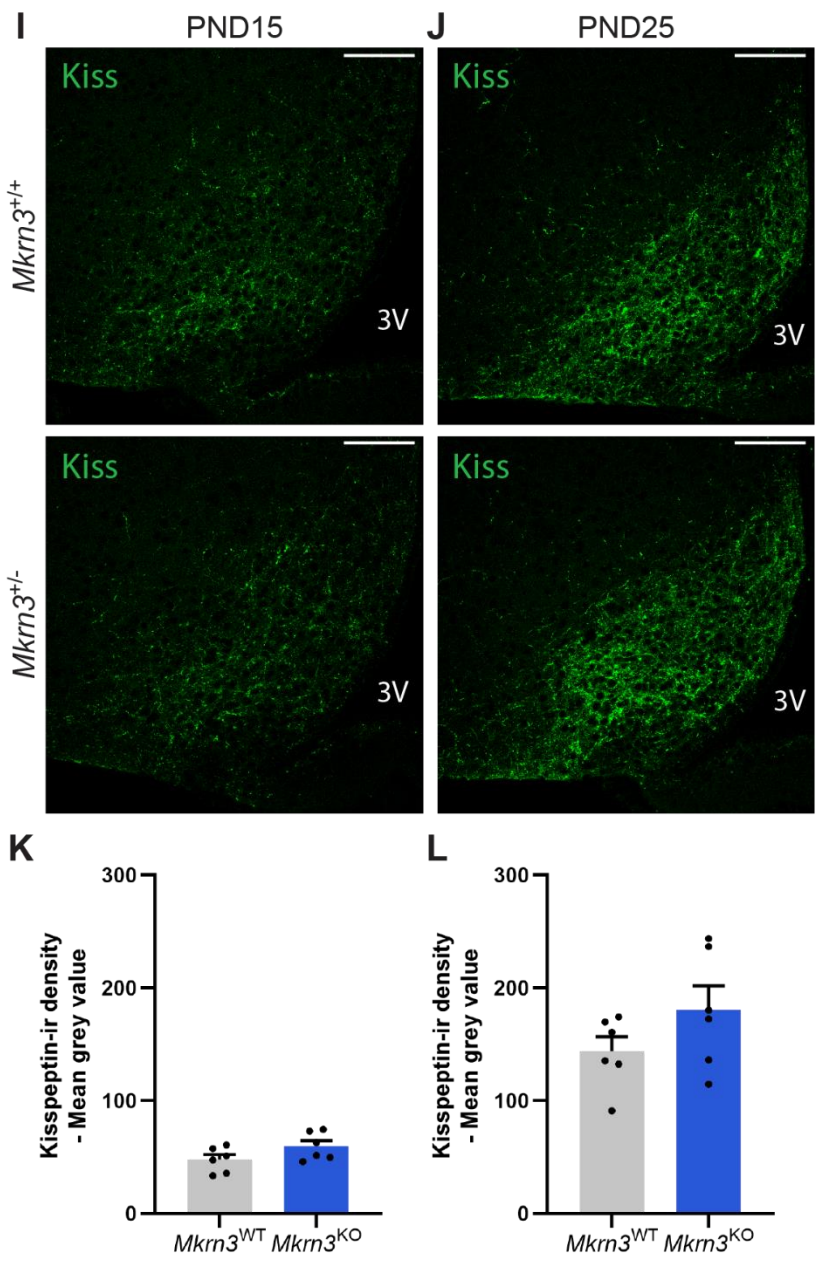


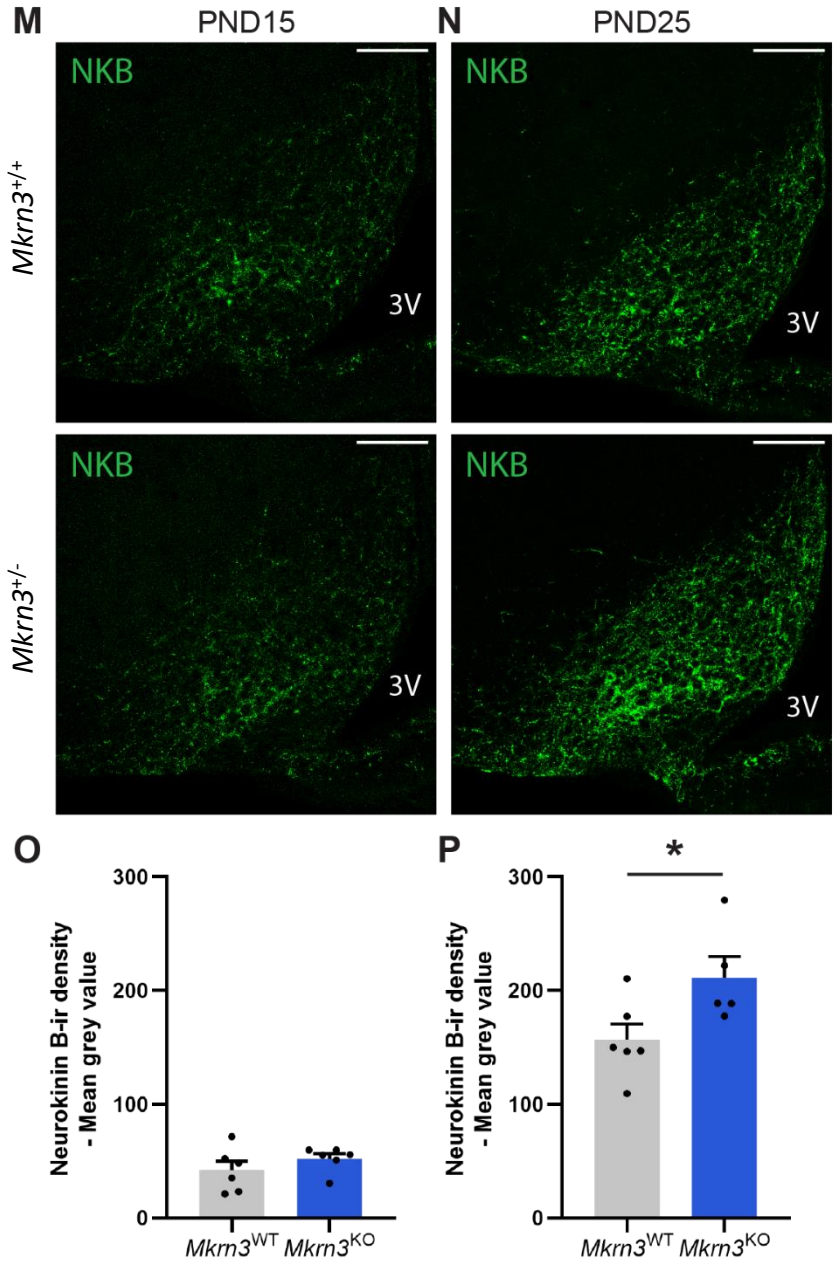
Kisspeptin neurons ARC



Naulé et al., 2023

PROTEIN EXPRESSION OF KEY REGULATORS OF PUBERTY INITIATION

Neurokinin B
ARC



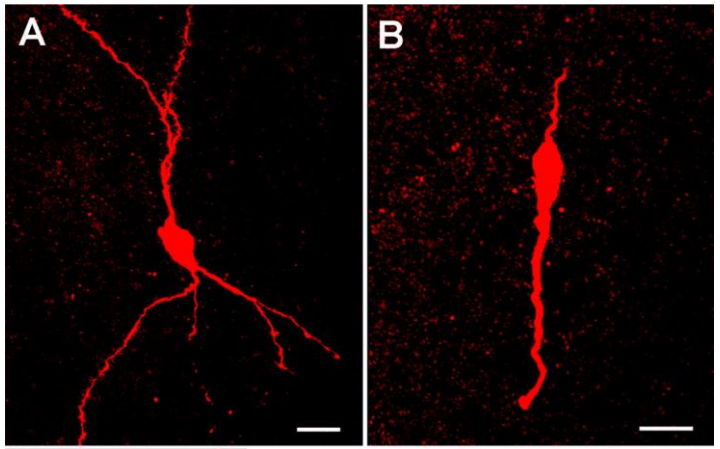
Mkrn3 **deletion is associated with an increase in the expression of Neurokinin B in the ARC.**

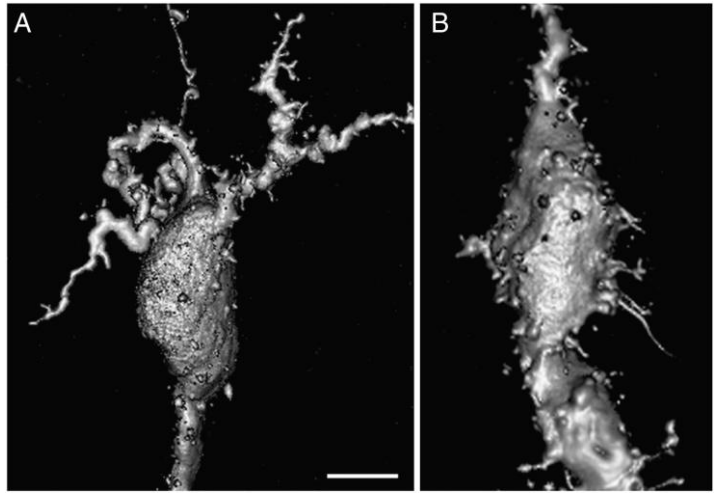
Naulé et al., 2023

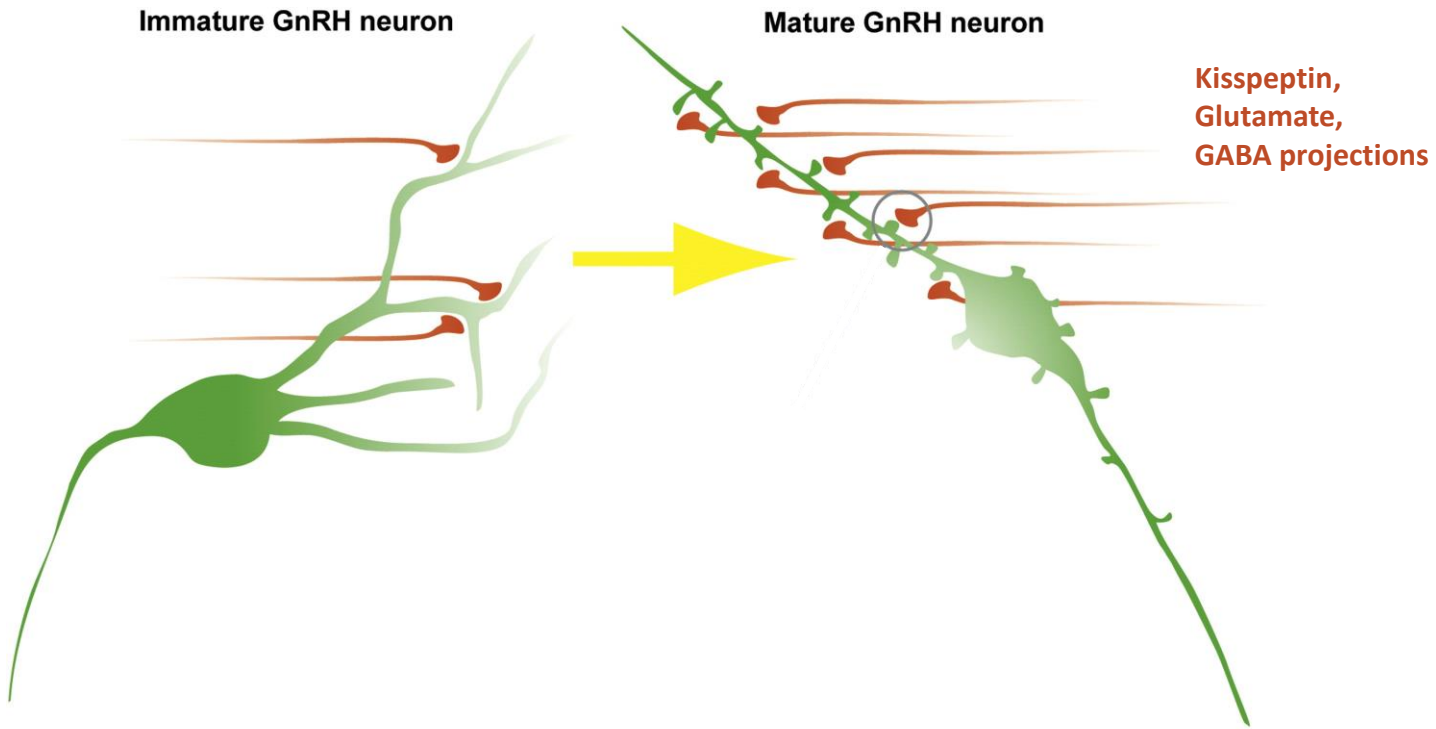
CHANGES IN GNRH NEURONAL MORPHOLOGY ACROSS PUBERTY

Prepubertal GnRH neuron

Adult GnRH neuron







- Prepubertal GnRH neurons have a much more complex dendritic arbor compared to GnRH neurons from mature mice.

- GnRH neurons from mature mice have approximately twice as many somatic/dendritic spines compared to prepubertal GnRH cells

Cottrell et al., 2006

CHANGES IN GNRH NEURONAL MORPHOLOGY ACROSS PUBERTY

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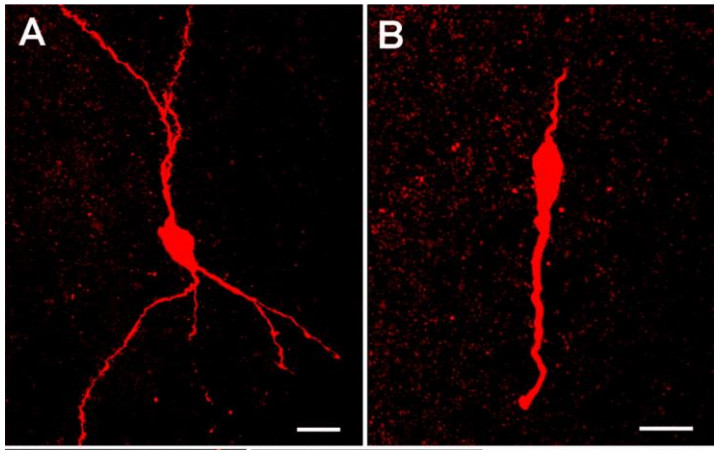
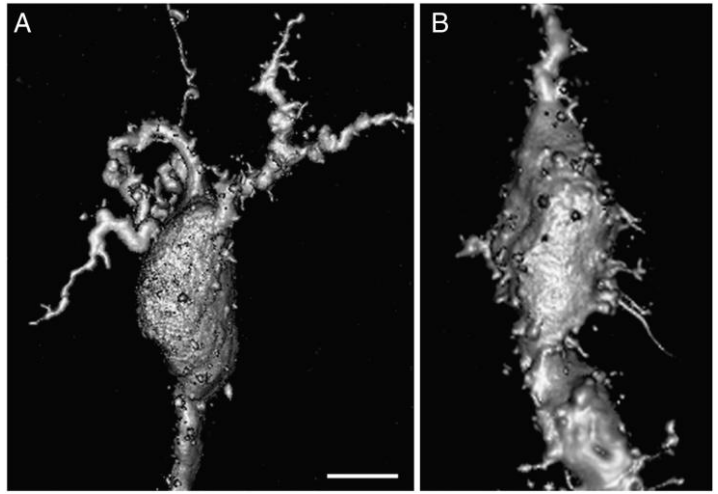
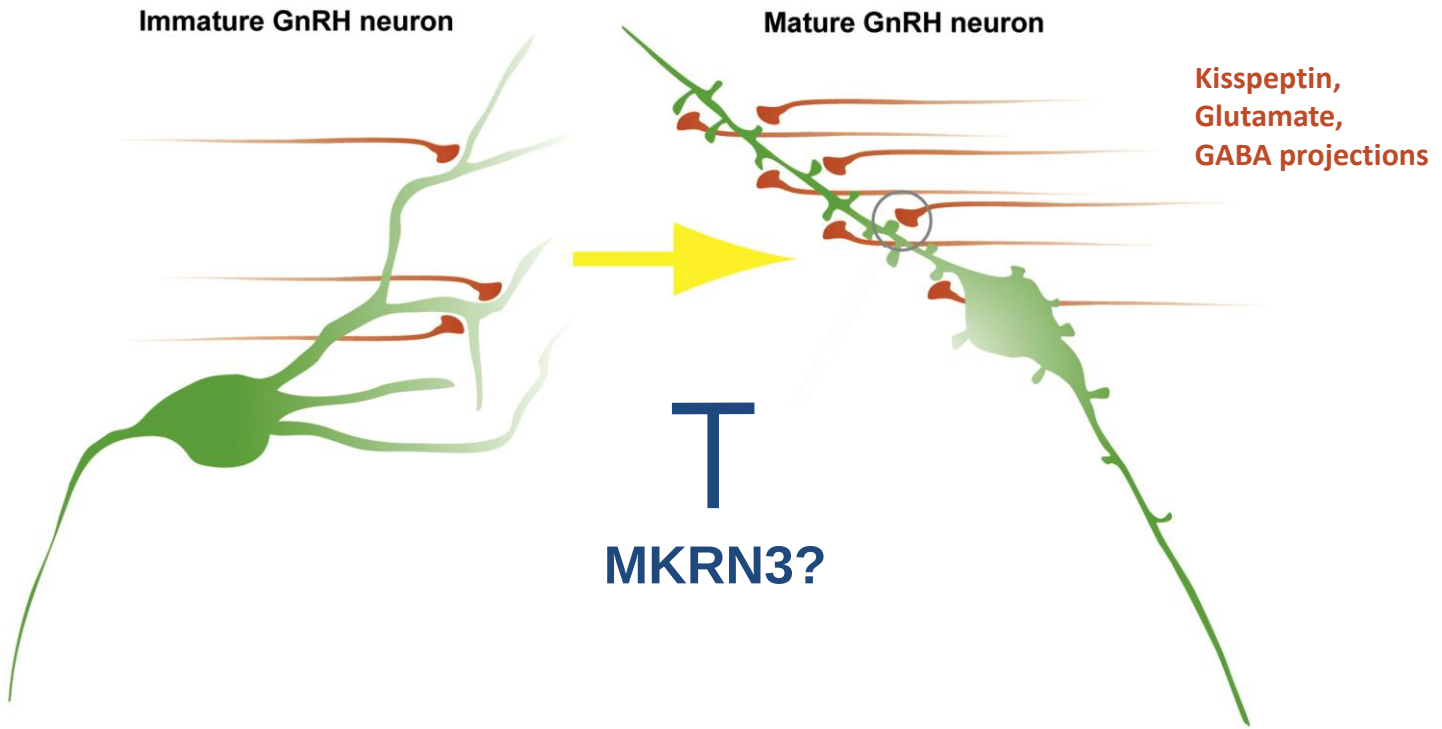
Adult GnRH neuron







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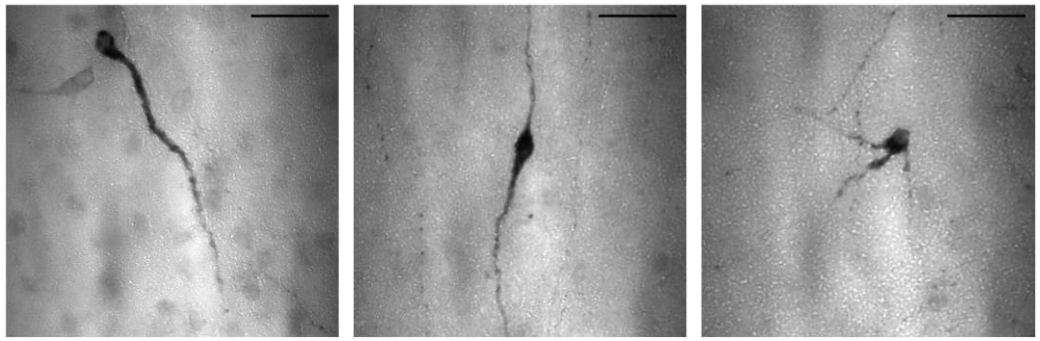
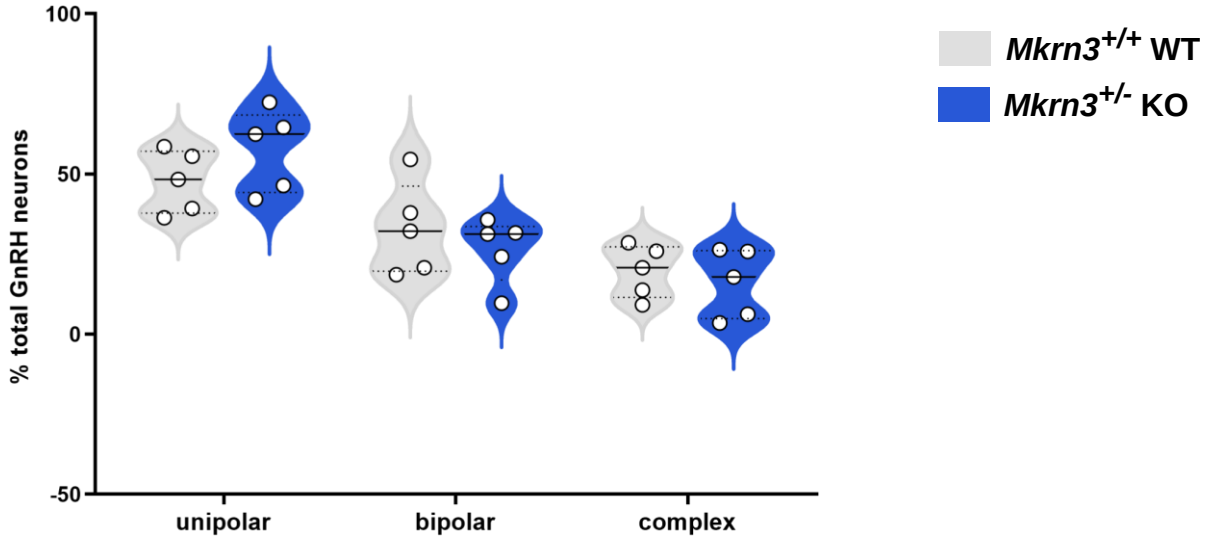
Cottrell et al., 2006

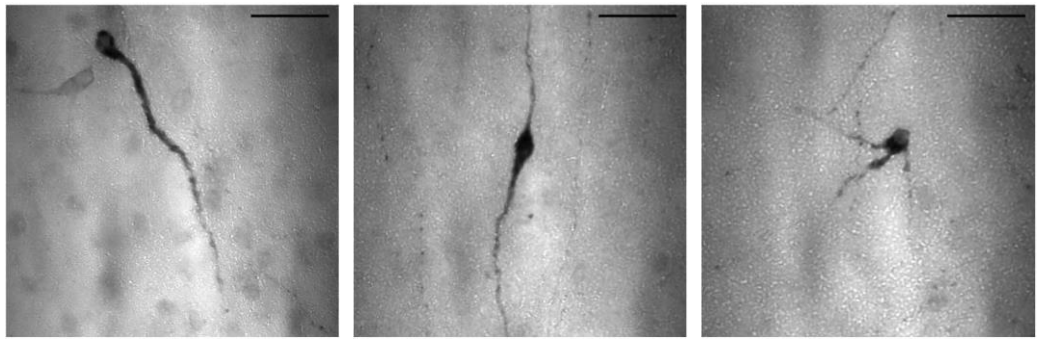
GnRH neuron morphology (PND15)

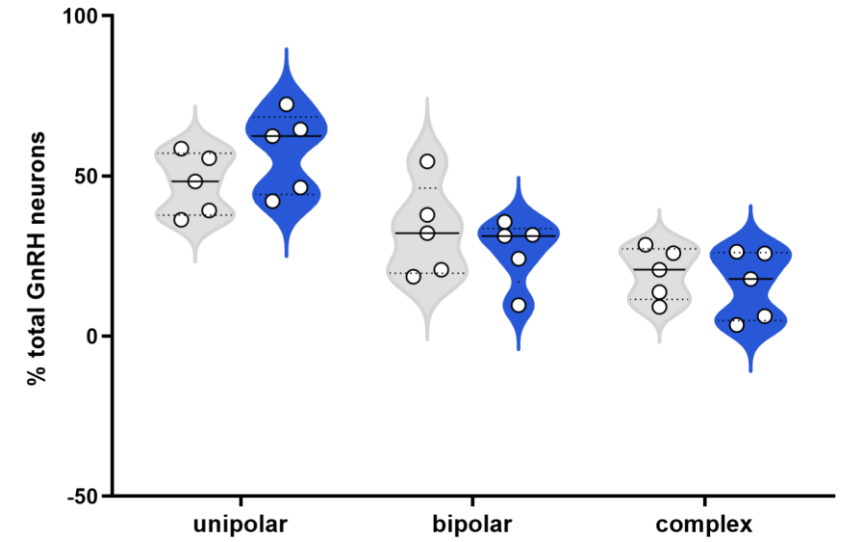




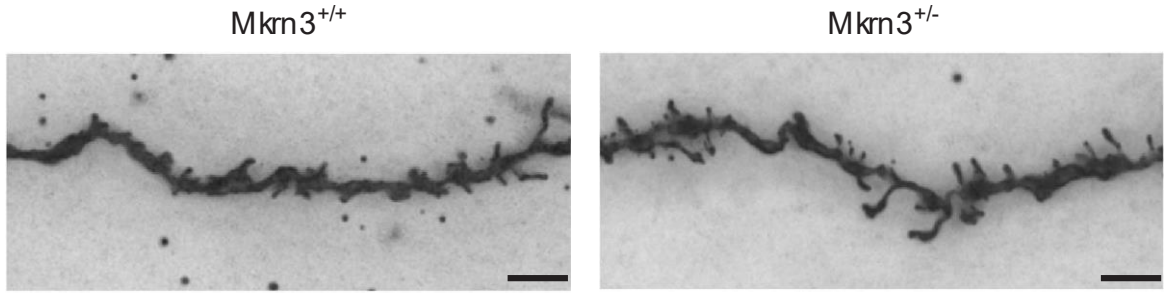
Naulé et al., 2023

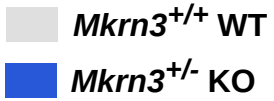
GnRH neuron morphology (PND15)

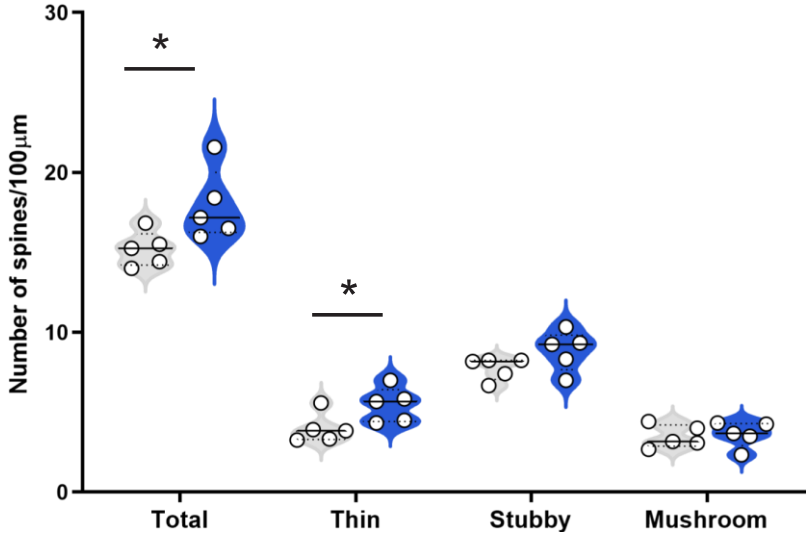




Spine density - ARC (PND15)

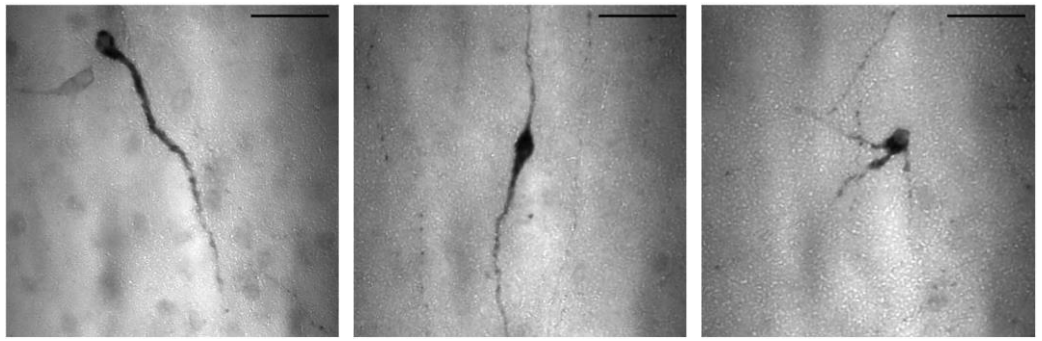




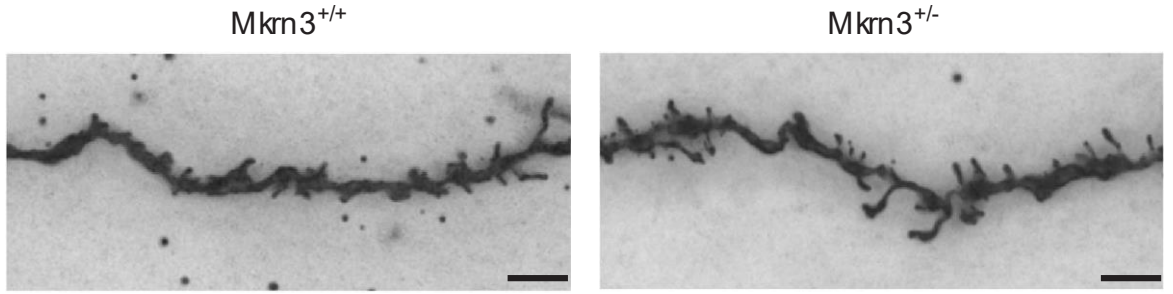


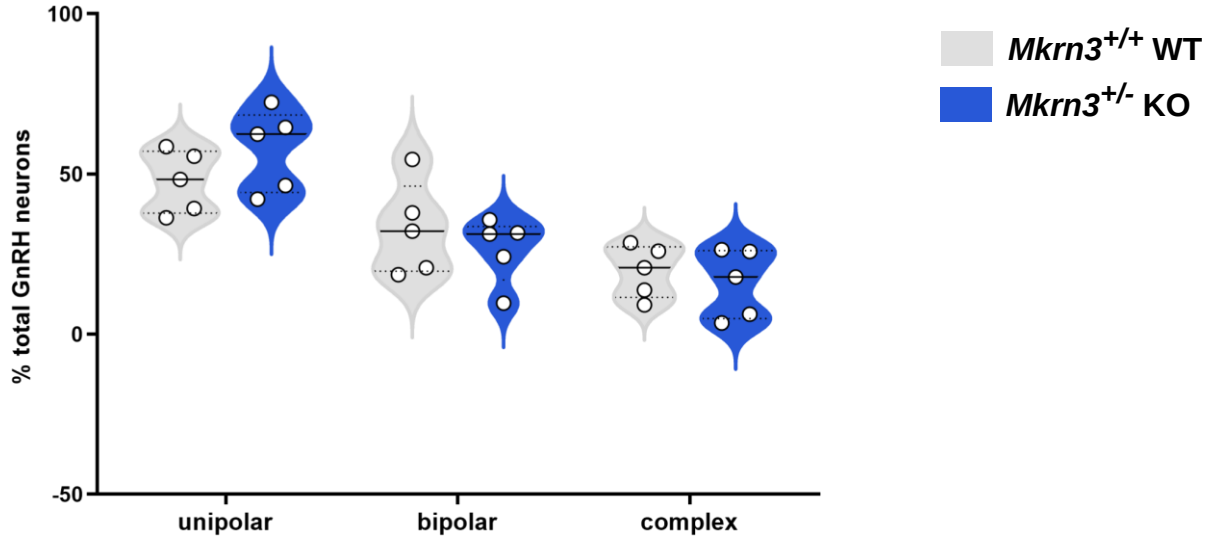
Naulé et al., 2023

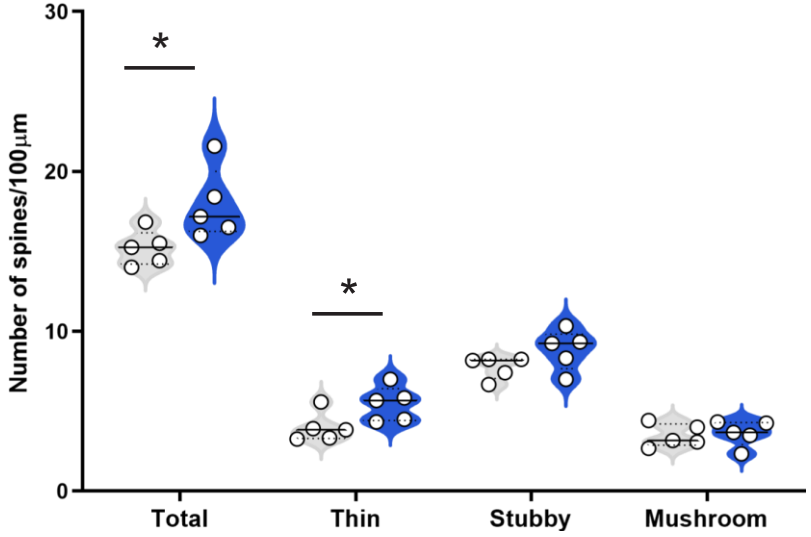
GnRH neuron morphology (PND15)



Spine density - ARC (PND15)





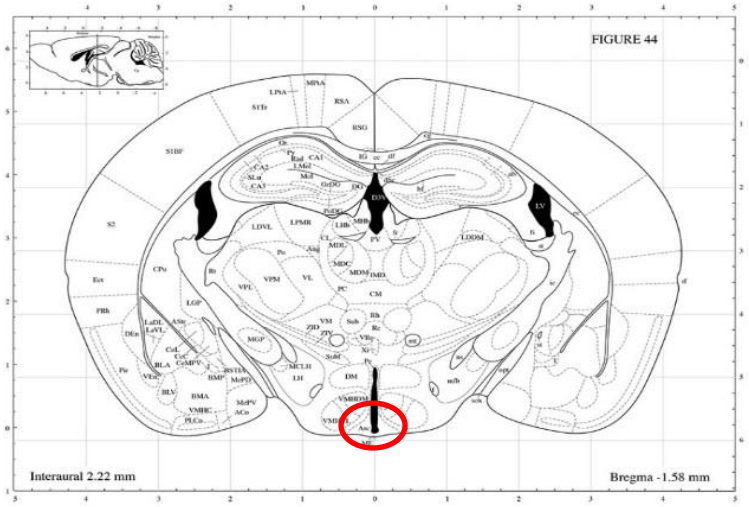


***Mkrn3* deletion is associated with an increase in dendritic spine density in the arcuate nucleus during postnatal development.**

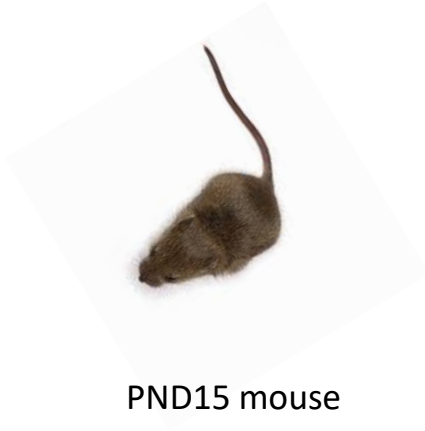
Naulé et al., 2023

IDENTIFICATION OF OTHER TARGETS OF MKRN3 ACTION – PROTEOMIC ANALYSIS

Proteomic analysis of the hypothalamic arcuate nucleus of *Mkrn3*$^{+/-}$ KO and *Mkrn3*$^{+/+}$ WT P15 male and female mice.



Arcuate nucleus



PND15 mouse

TNT10plex Mass Tag Labeling technique

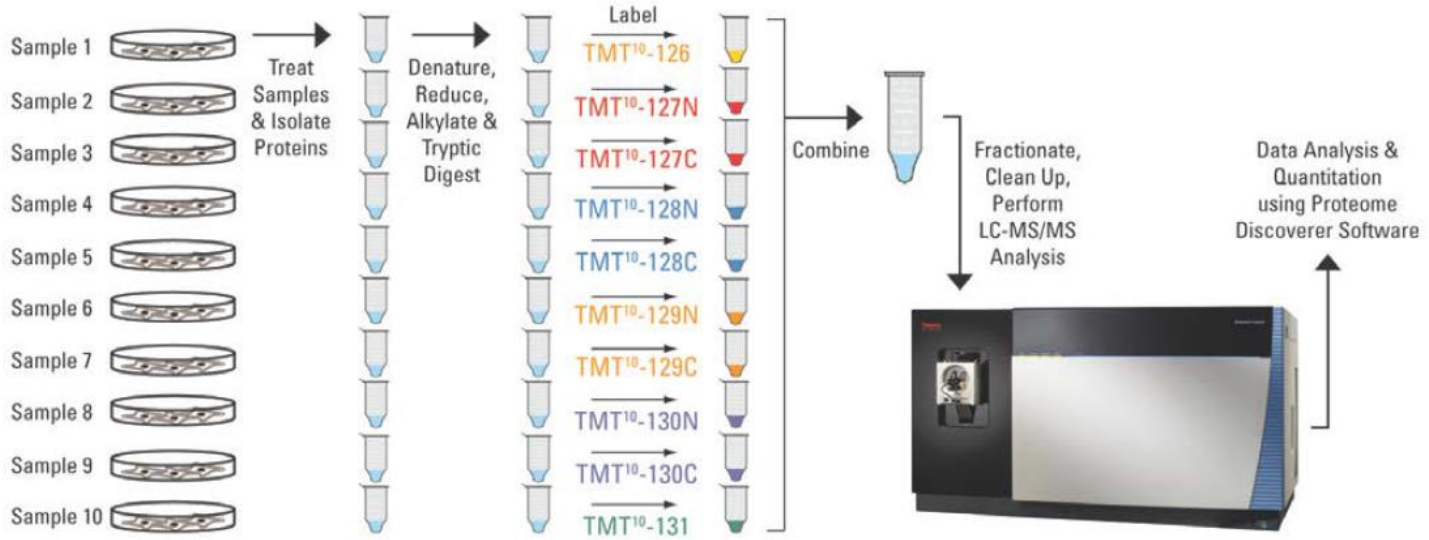
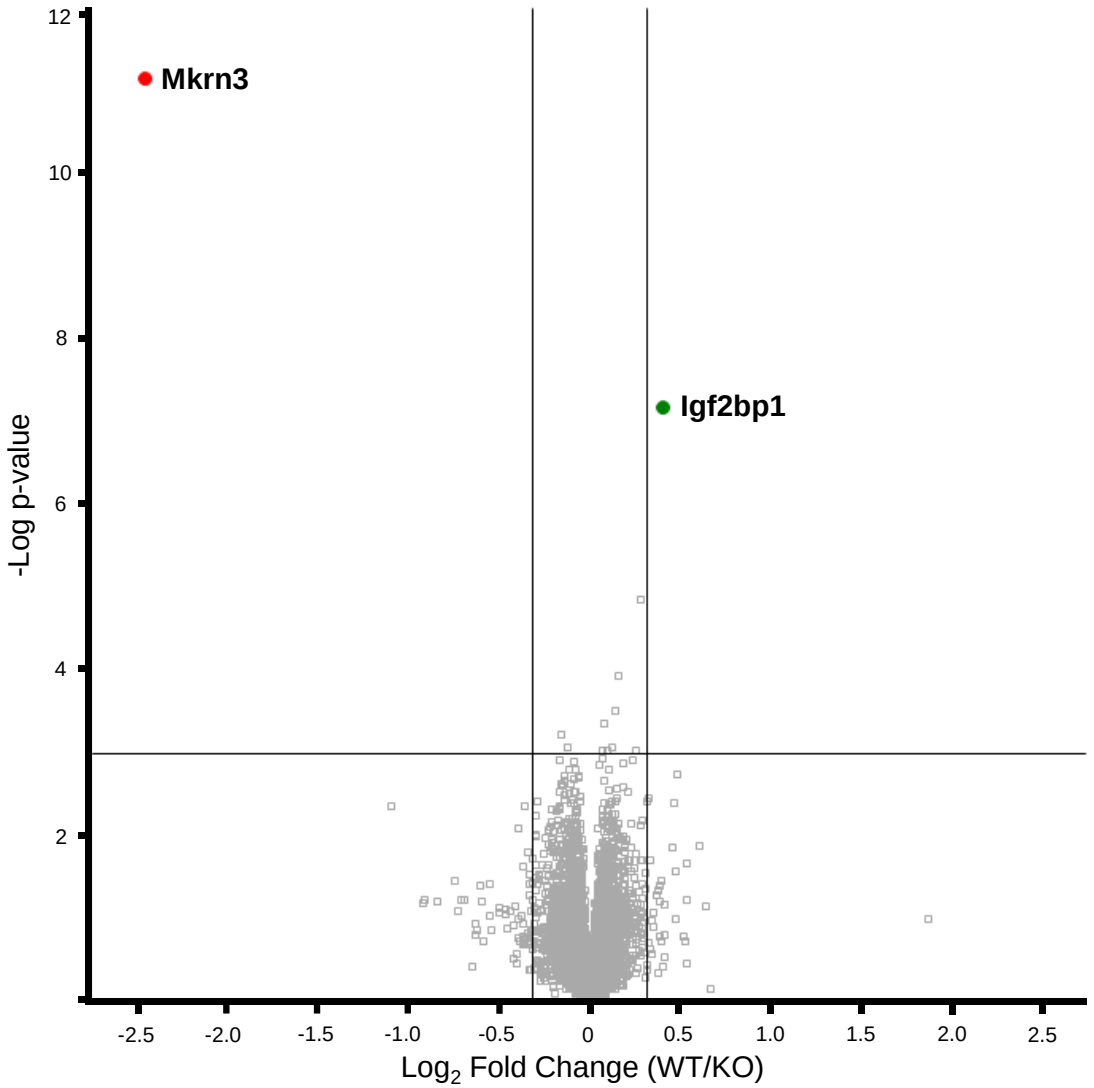


Figure 1. Procedure schematic for using the Thermo Scientific TMT10plex Label Reagents.

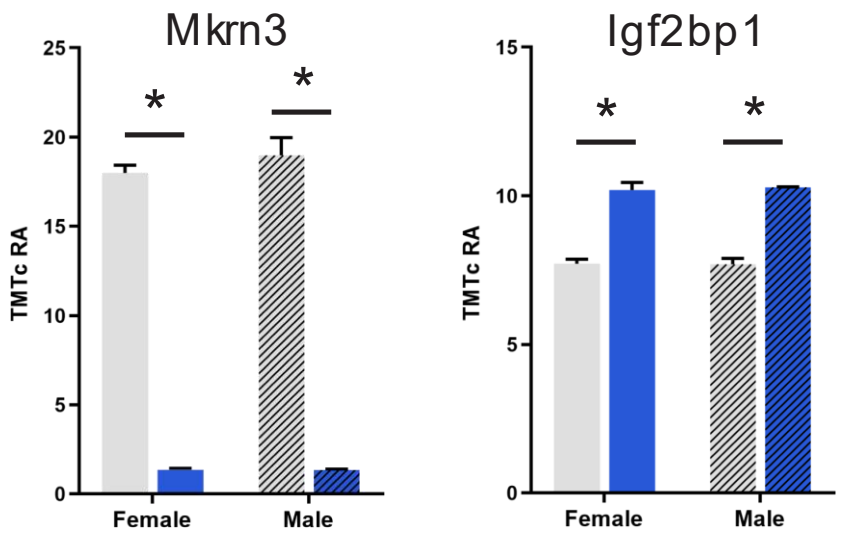
In collaboration with
Dr. Steven Gygi laboratory

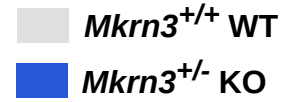
IDENTIFICATION OF OTHER TARGETS OF MKRN3 ACTION – PROTEOMIC ANALYSIS

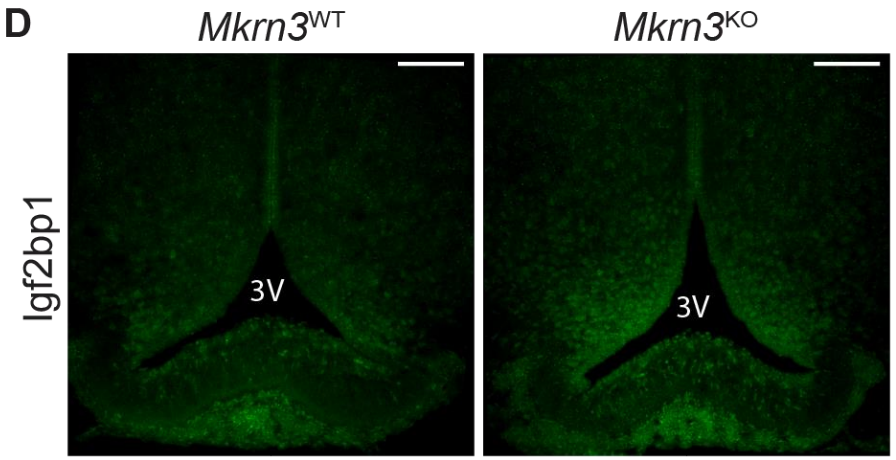


Volcano plot indicating significantly altered proteins identified according to their statistical P-value (y-axis) and their relative abundance ratio (log2 fold change) between Mkrn3 WT and Mkrn3 KO animals.

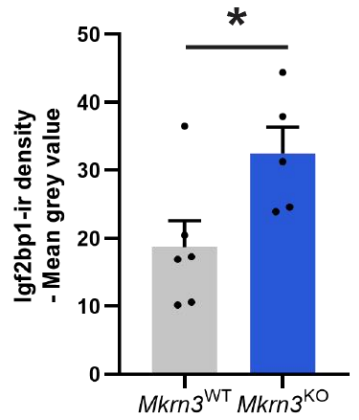
Igf2bp1 = Insulin growth factor 2 binding protein 1





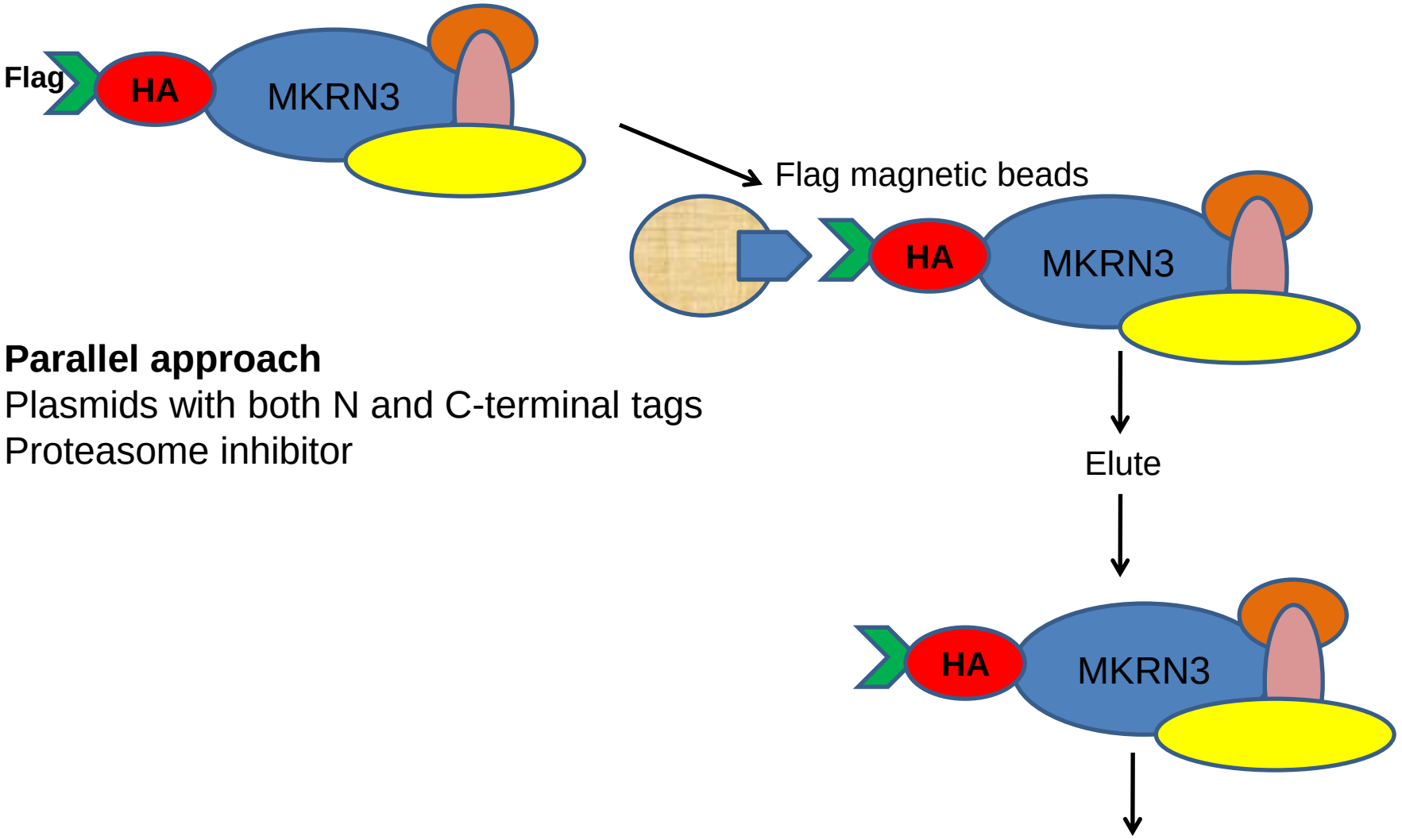


PND15



Naulé et al., 2023

IGF2BP1 PROTEIN INTERACTS WITH MKRN3 – INTERACTOME STUDIES



Parallel approach

Plasmids with both N and C-terminal tags

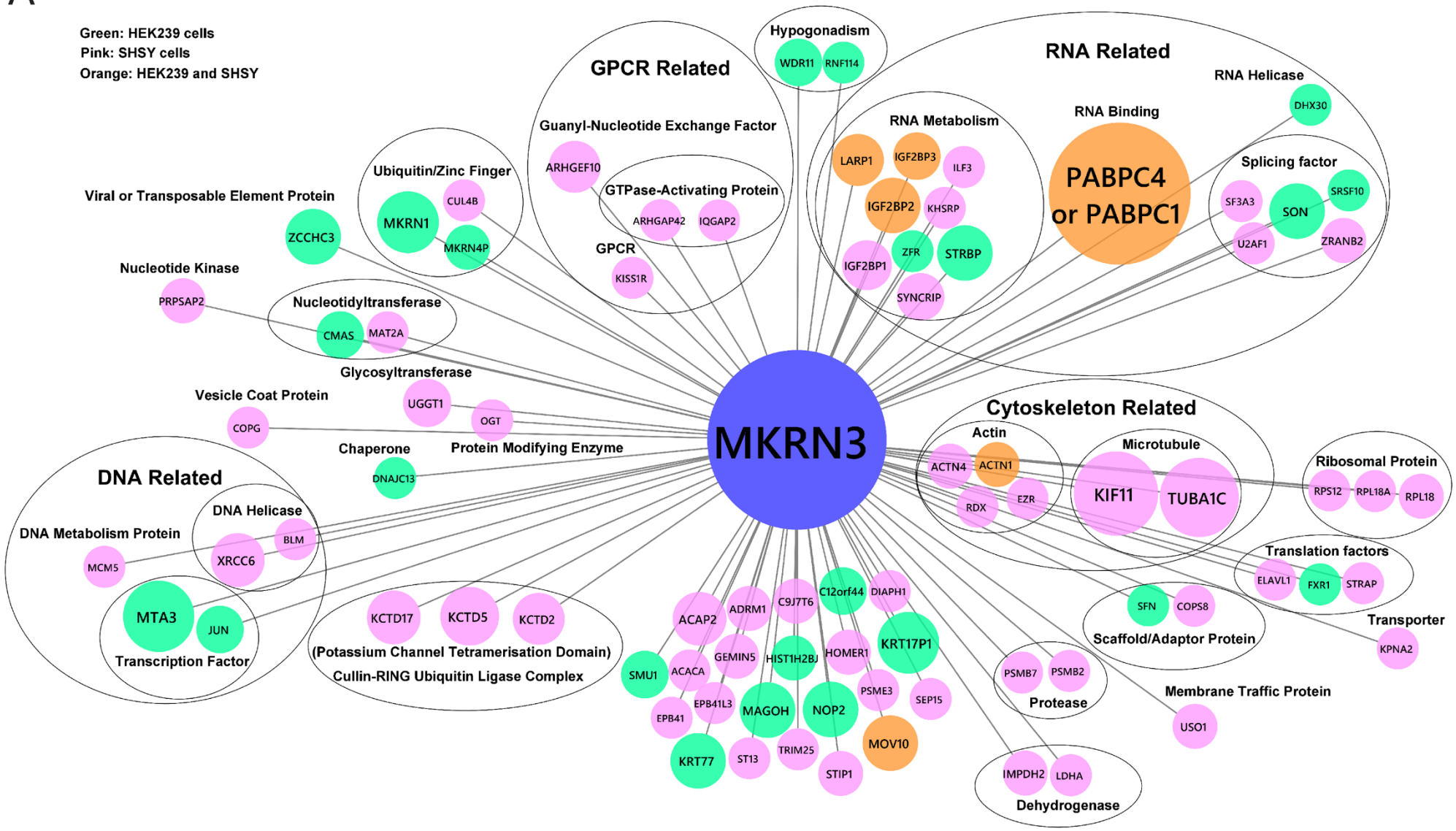
Proteasome inhibitor

In collaboration with Dr. Wade Harper

IGF2BP1 PROTEIN INTERACTS WITH MKRN3 – INTERACTOME STUDIES

Identification of 85 high-confidence candidate interacting proteins (HCIPs) that interact with MKRN3.

A



Mkrn3 interacts with Igf2bp1 and inhibits its protein expression in the arcuate nucleus during postnatal development.

Naulé et al., 2023

IGF2BP1 PROTEIN INTERACTS WITH MKRN3 – INTERACTOME STUDIES

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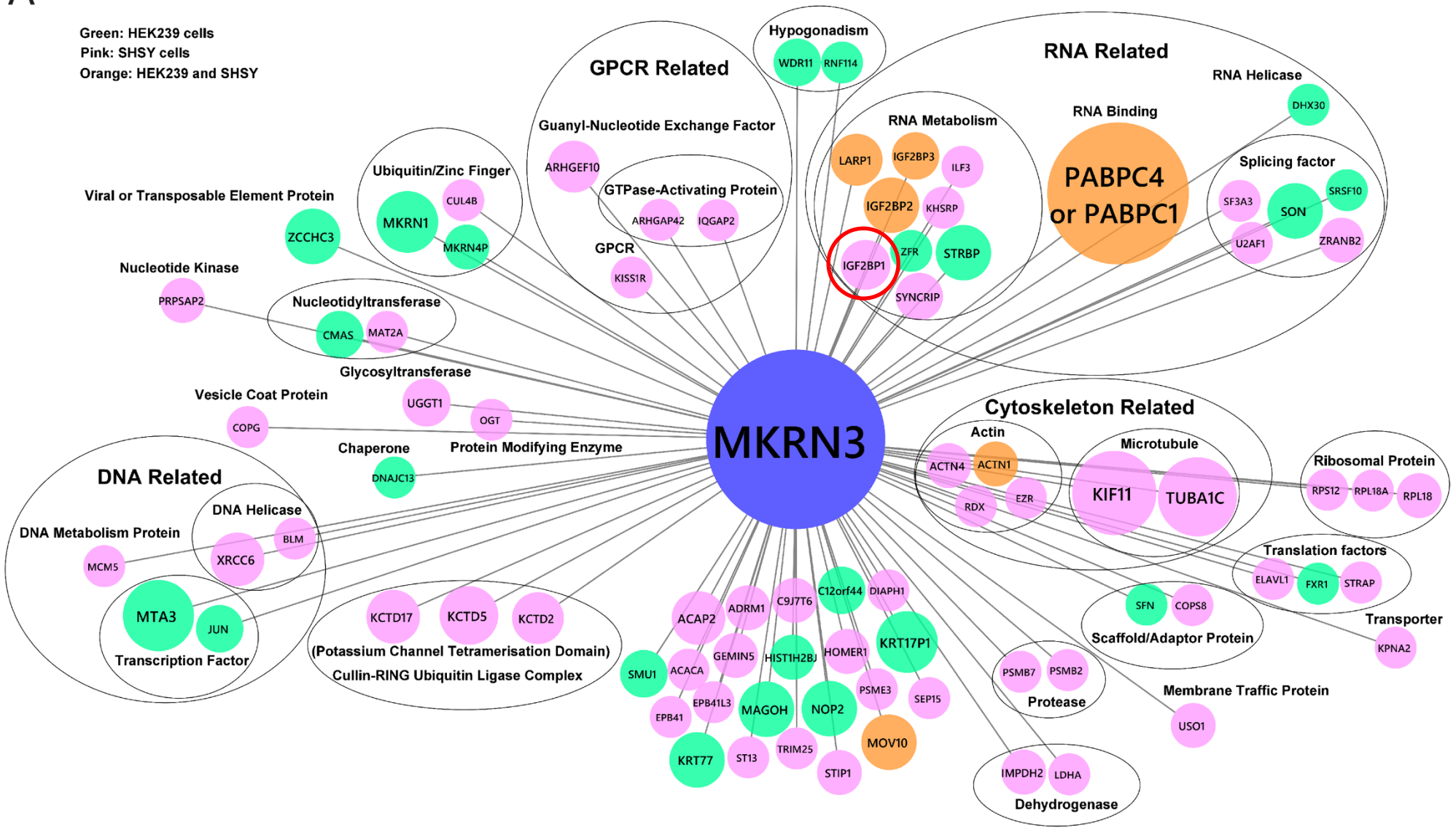


Mkrn3 interacts with Igf2bp1 and inhibits its protein expression in the arcuate nucleus during postnatal development.

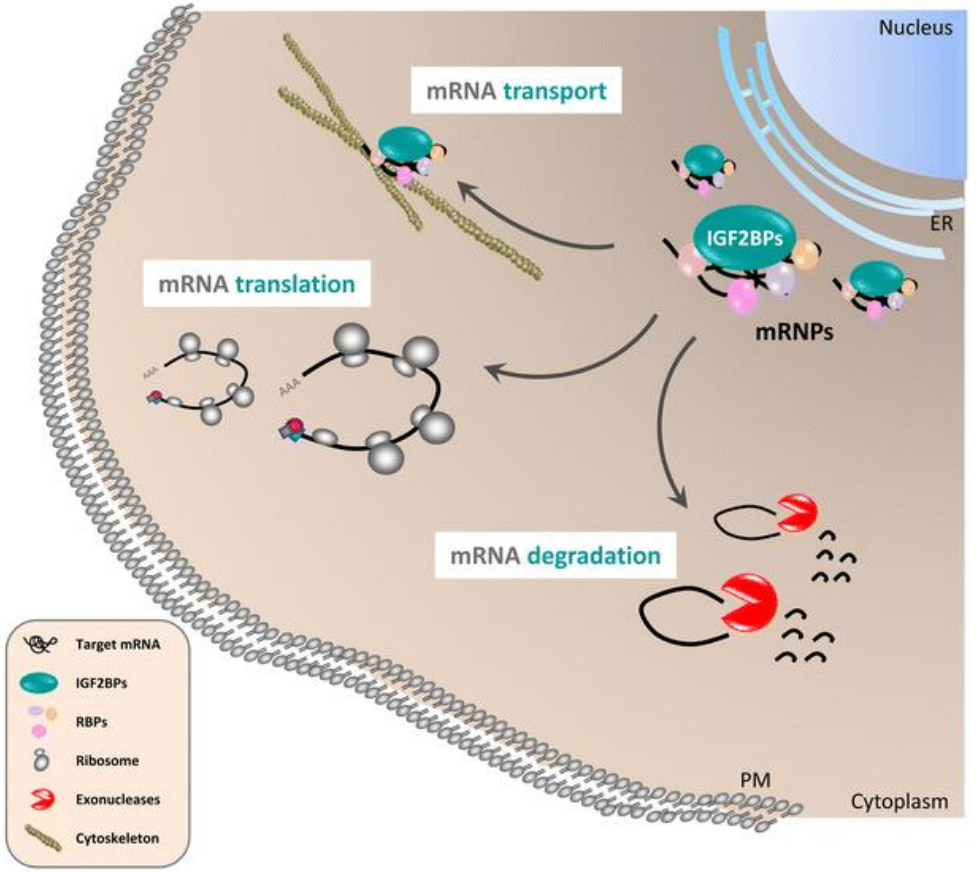
Naulé et al., 2023

IGF2BP1, A TARGET OF MKRN3 ACTION

IGF2BP1 belong to a conserved family of **mRNA binding (RBPs) protein.**

IGF2BP1 required for the transport of certain mRNA that play essential roles in **embryogenesis, carcinogenesis** and chemoresistance, by affecting their stability, translatability or localization.

IGF2BP1 was identified as a key player in the spatiotemporal control of mRNA localization, a key determinant of **neuronal development**, **cytoskeletal remodeling, cell adhesion** and **synaptic function**.



Bell et al., 2013 ; Huang et al., 2018

CONCLUSIONS

- *MRKN3* deletion in hiPS-derived hypothalamic neuron is associated with differences in gene expression between MKRN3-deficient and WT cells in factors involved in **extracellular matrix organization**, **cell adhesion**, and **axon guidance pathways**, which together control **hypothalamic development and plasticity.**

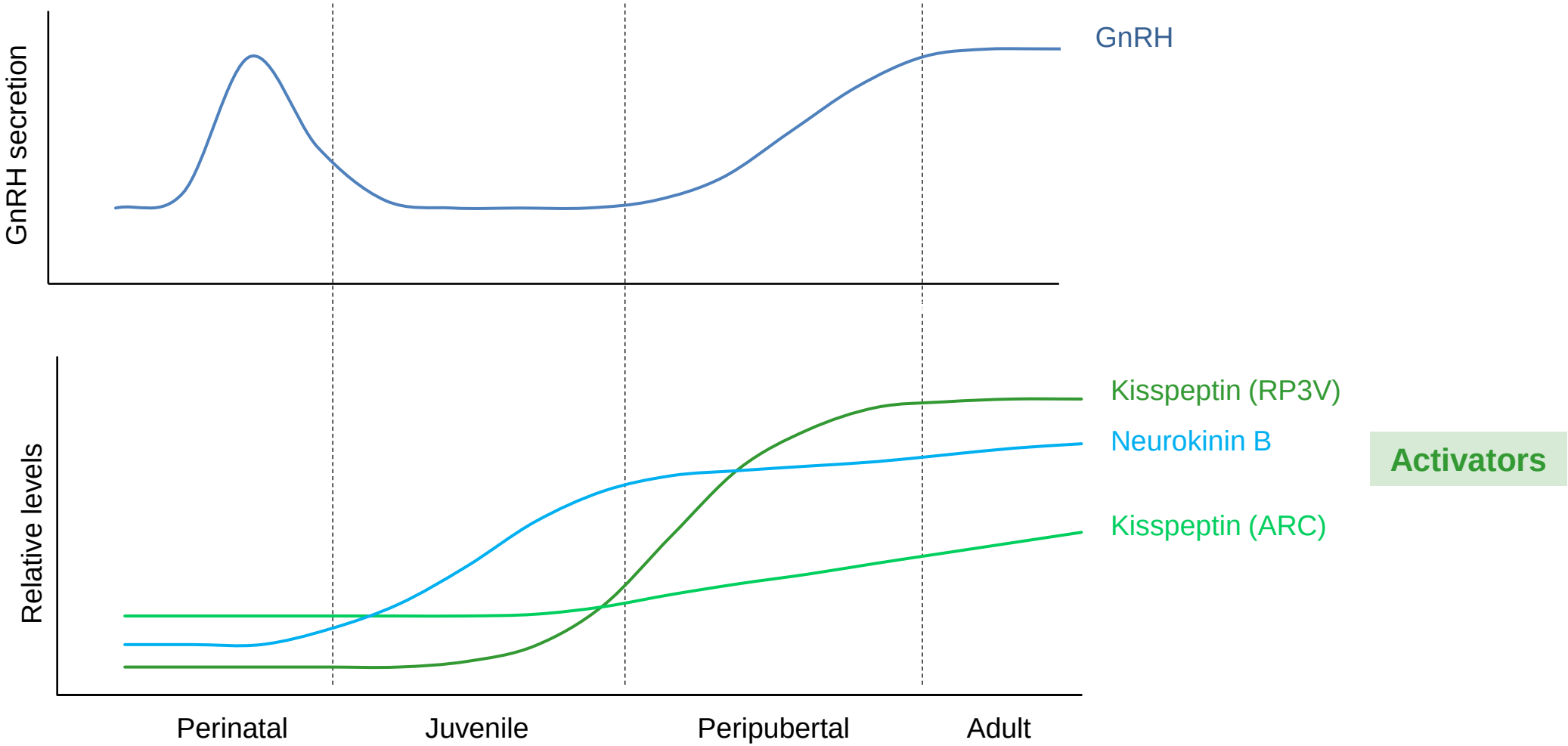
- *Mkrn3* deletion is associated with **accelerated puberty onset in female mice** and a **tendency towards early puberty in male mice**.

- *Mkrn3* deletion in *Mkrn3*$^{+/-}$ KO female mice is associated with an **increase in expression of Neurokinin B** in the ARC at PND25.

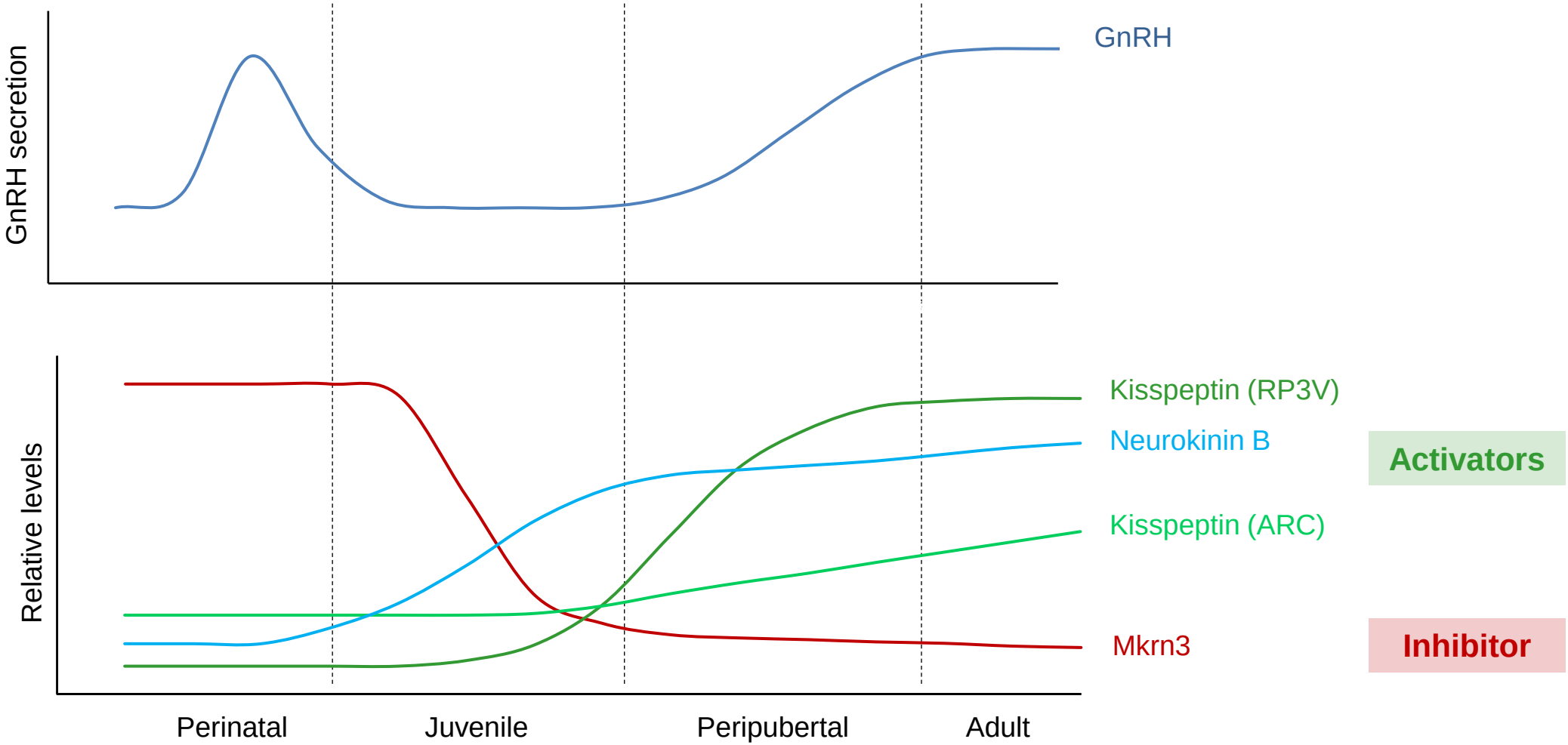
- *Mkrn3* deletion in *Mkrn3*$^{+/-}$ KO female mice is associated with an **increase in dendritic spine density** in the arcuate nucleus during postnatal development.

- Mkrn3 **interacts with Igf2bp1** and inhibits its protein expression in the arcuate nucleus during postnatal development.

SUMMARY


GnRH secretion
GnRH
Relative levels
Kisspeptin (RP3V)
Neurokinin B
Kisspeptin (ARC)
Activators
Perinatal
Juvenile
Peripubertal
Adult

SUMMARY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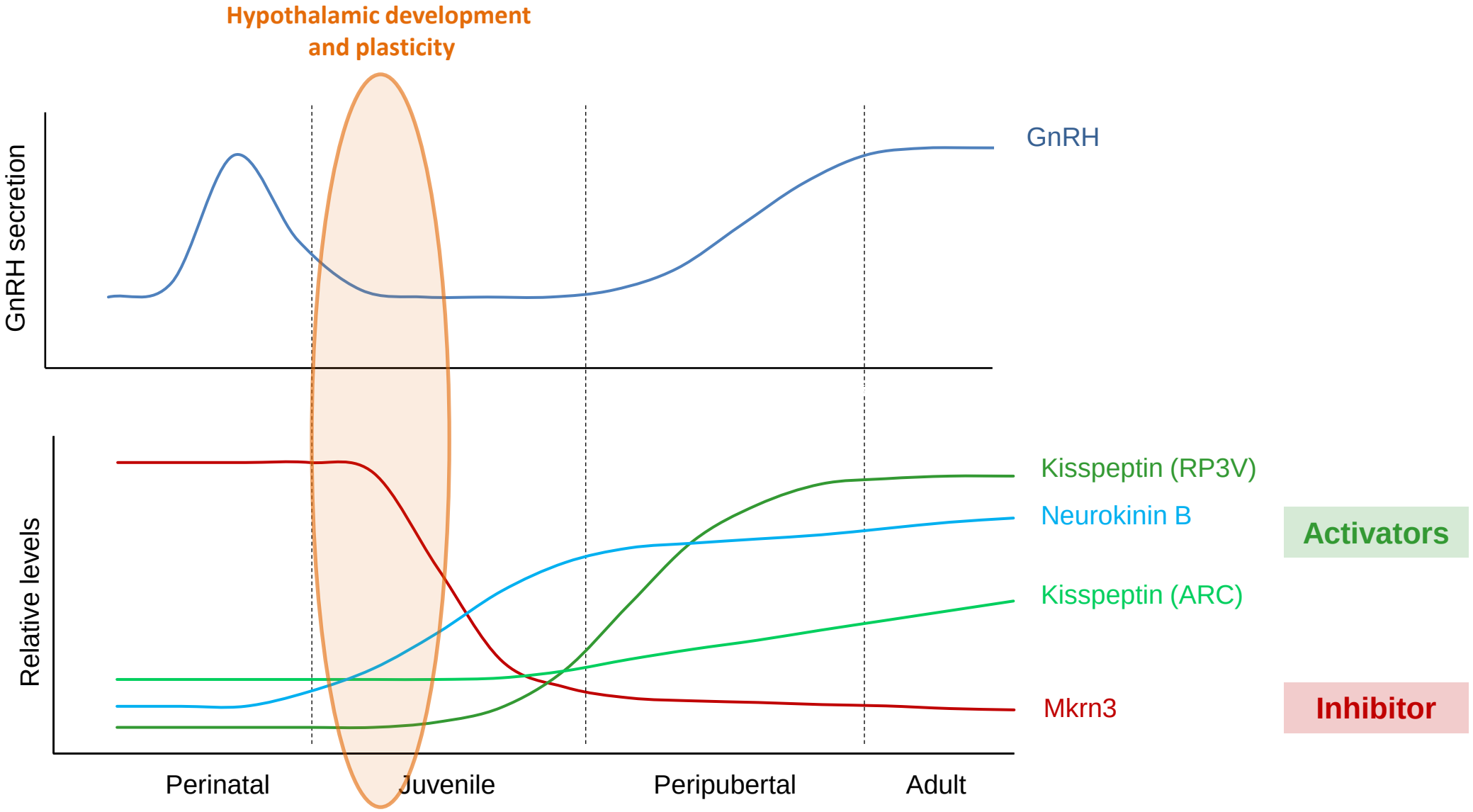
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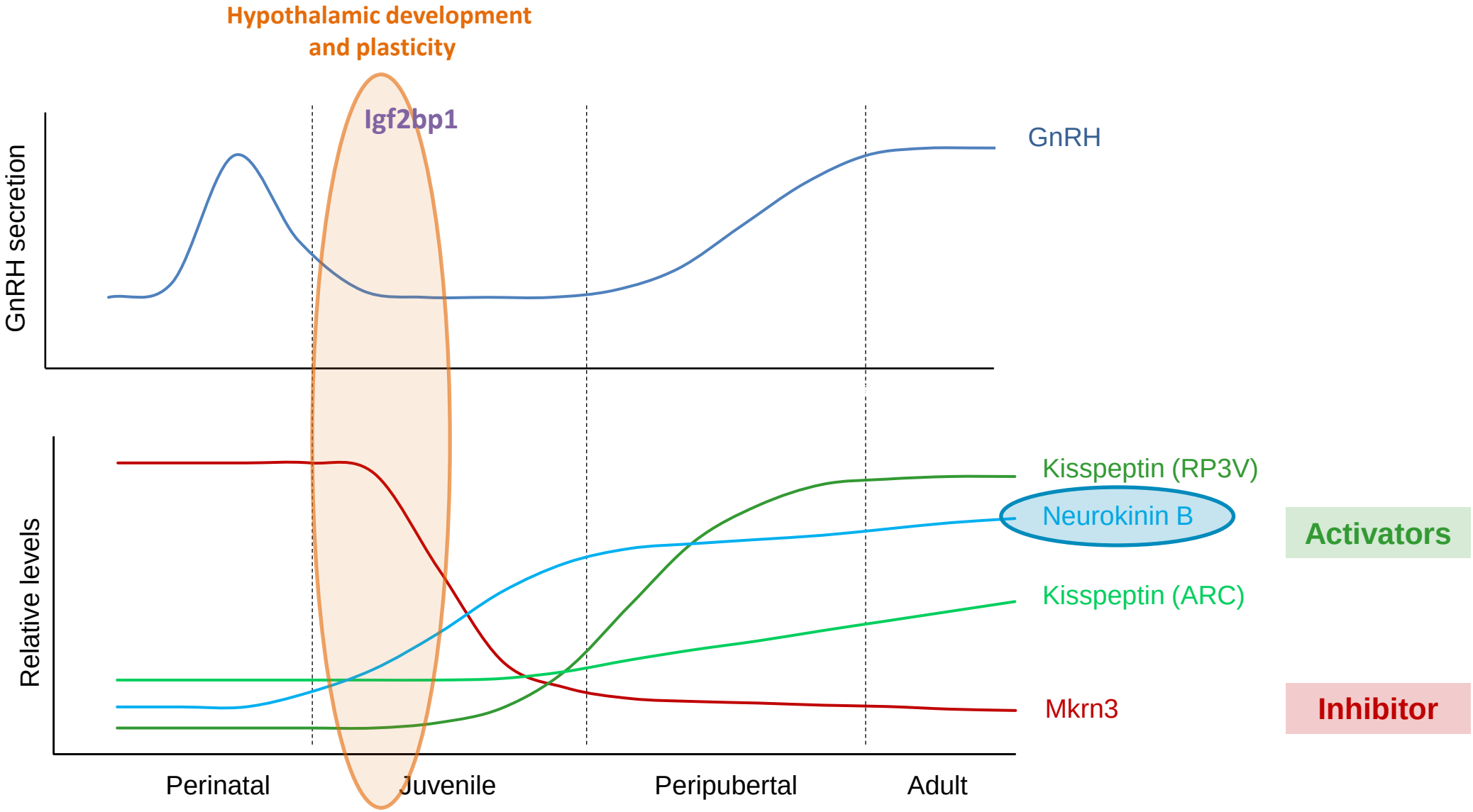
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