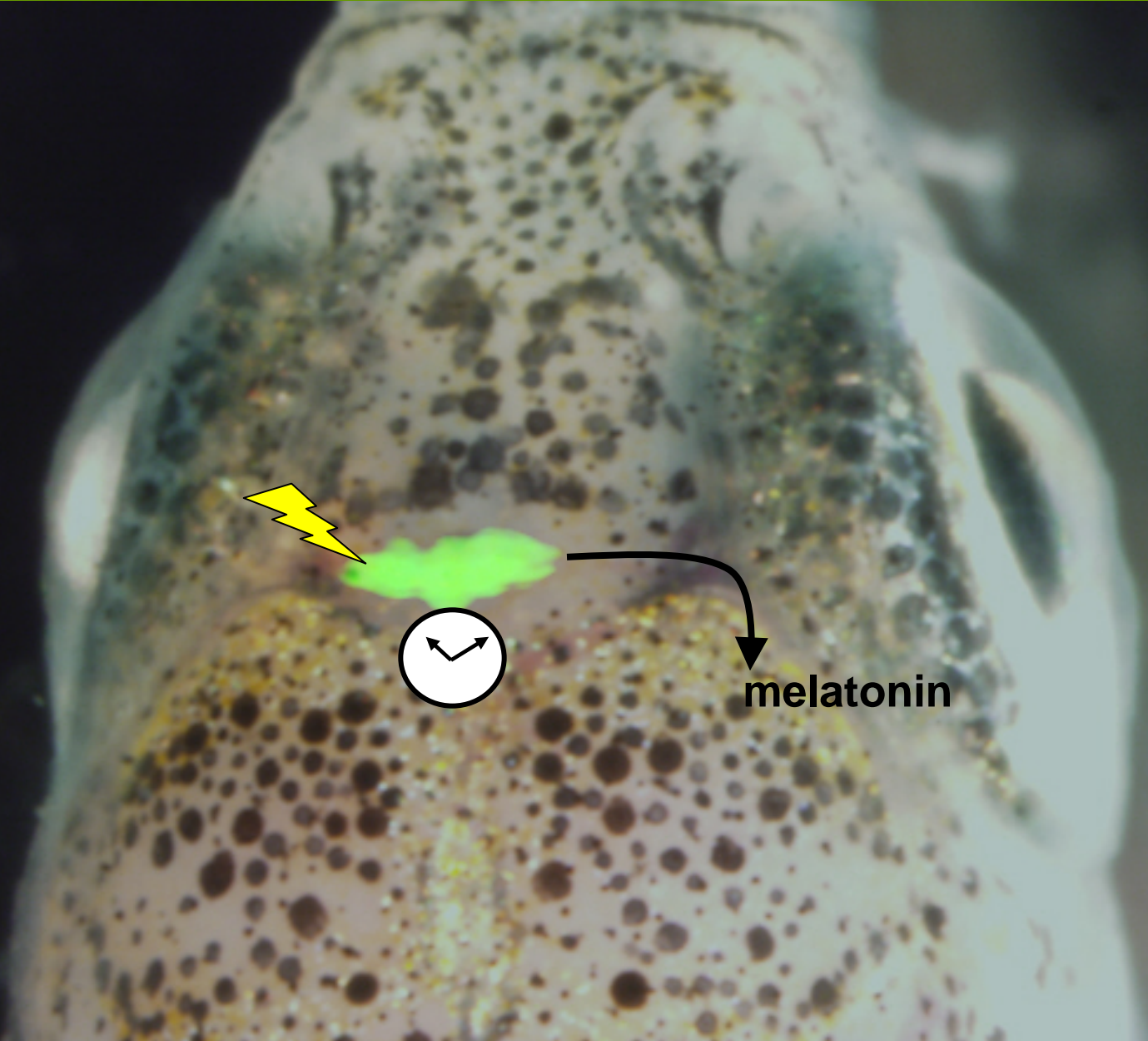


# Appetite control and the AgRP neuronal system in zebrafish

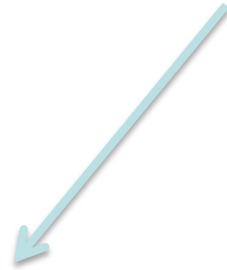


- Photoreception
- Circadian clock
- Melatonin synthesis

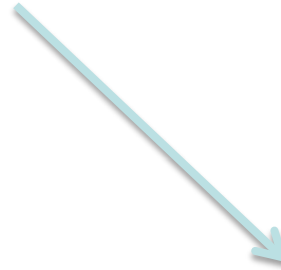
**Table 1.** A list of transcripts with high pineal expression, the 40 transcripts presented have the highest MFD.

Gene Symbol	MFD	ISH Image
*BG305792	260	
†Pdc2	215	** (Kobayashi <i>et al.</i> , 2002)
*BI671344	200	
†Exorh	200	Pineal (Mano <i>et al.</i> , 1999)
†Rbp4	185	
†Pde6a	170	** (Vihtelic <i>et al.</i> , 2005)
†Gnat1	165	Pineal, Retina (Thisse <i>et al.</i> , 2004)
†Pde6c	150	** (Vihtelic <i>et al.</i> , 2005)
*BI671149	140	
*BI879853	120	
†Guk1	115	Pineal, Retina (Thisse <i>et al.</i> , 2004)
†Zgc:92682	115	Pineal, Retina (Thisse <i>et al.</i> , 2004)
†Opn1lw1	110	Pineal, Retina (Thisse <i>et al.</i> , 2004)
Gucy2f	105	** (Brockerhoff <i>et al.</i> , 2003)
†Aanat2	105	Pineal, Retina (Thisse <i>et al.</i> , 2004)
†GngT1	90	Pineal, Retina (Thisse <i>et al.</i> , 2004)
*AW826706	85	
†Zgc:73075	80	
†Tph1	80	Pineal (Thisse <i>et al.</i> , 2004)
†Arr3	80	Pineal, Retina (Thisse <i>et al.</i> , 2004)
†Zgc:73213	75	Pineal, Retina (Thisse <i>et al.</i> , 2004)
*BG308558	75	
†Tph2	70	Pineal, Brain (Rauch <i>et al.</i> , 2003)
*BI880166	70	
Elovl4	70	Pineal, Retina (Thisse <i>et al.</i> , 2004)
†Slc25a3l	70	Pineal, Retina, Background (Thisse <i>et al.</i> , 2004)
Rlbp11	70	Pineal, Retina (Thisse <i>et al.</i> , 2004)
LOC563645	70	
†Arl3l2	65	Pineal, Retina (Thisse <i>et al.</i> , 2004)
*BI671344	60	
†Crx	60	Pineal, Retina (Thisse <i>et al.</i> , 2004)
*BI670871	55	
†Zgc:73310	50	** (Vihtelic <i>et al.</i> , 2005)
*BI671248	45	
†Rcv1	45	Pineal, Retina (Thisse <i>et al.</i> , 2004)
Ddc	45	Diencephalons, Midbrain (Thisse <i>et al.</i> , 2004)
*BI881745	40	
zgc:73359	40	Pineal, Retina (Thisse <i>et al.</i> , 2004)
nme2l	35	Pineal, Retina (Thisse <i>et al.</i> , 2004)
*BG738656	35	

# Zebrafish AgRPs:



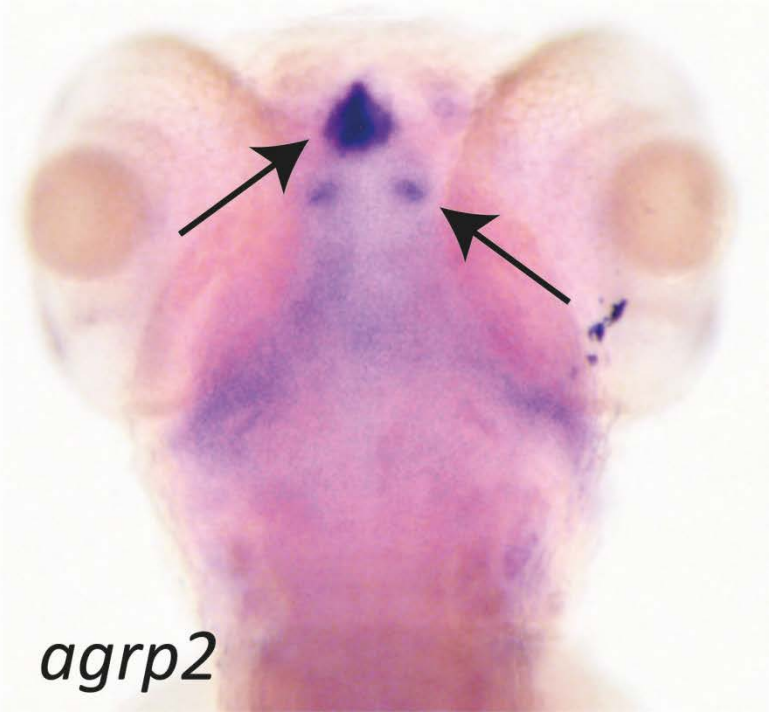
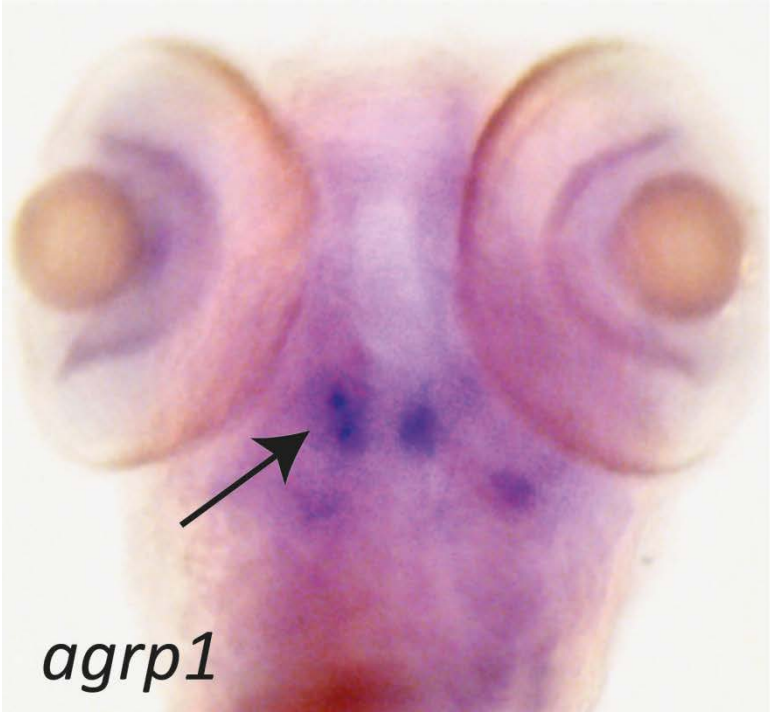
AgRP1



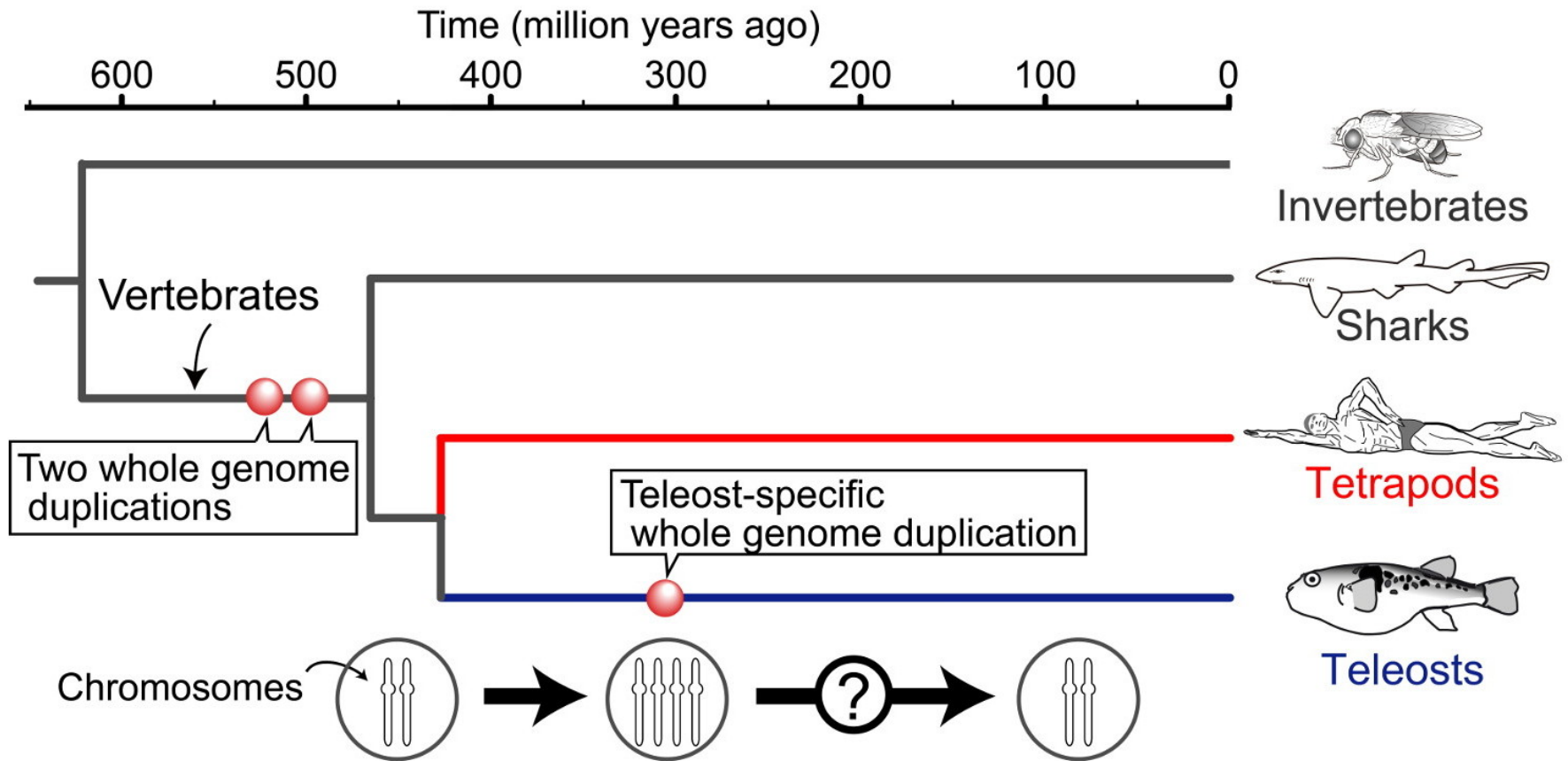
AgRP2

Hypothalamic specific

Pineal enhanced

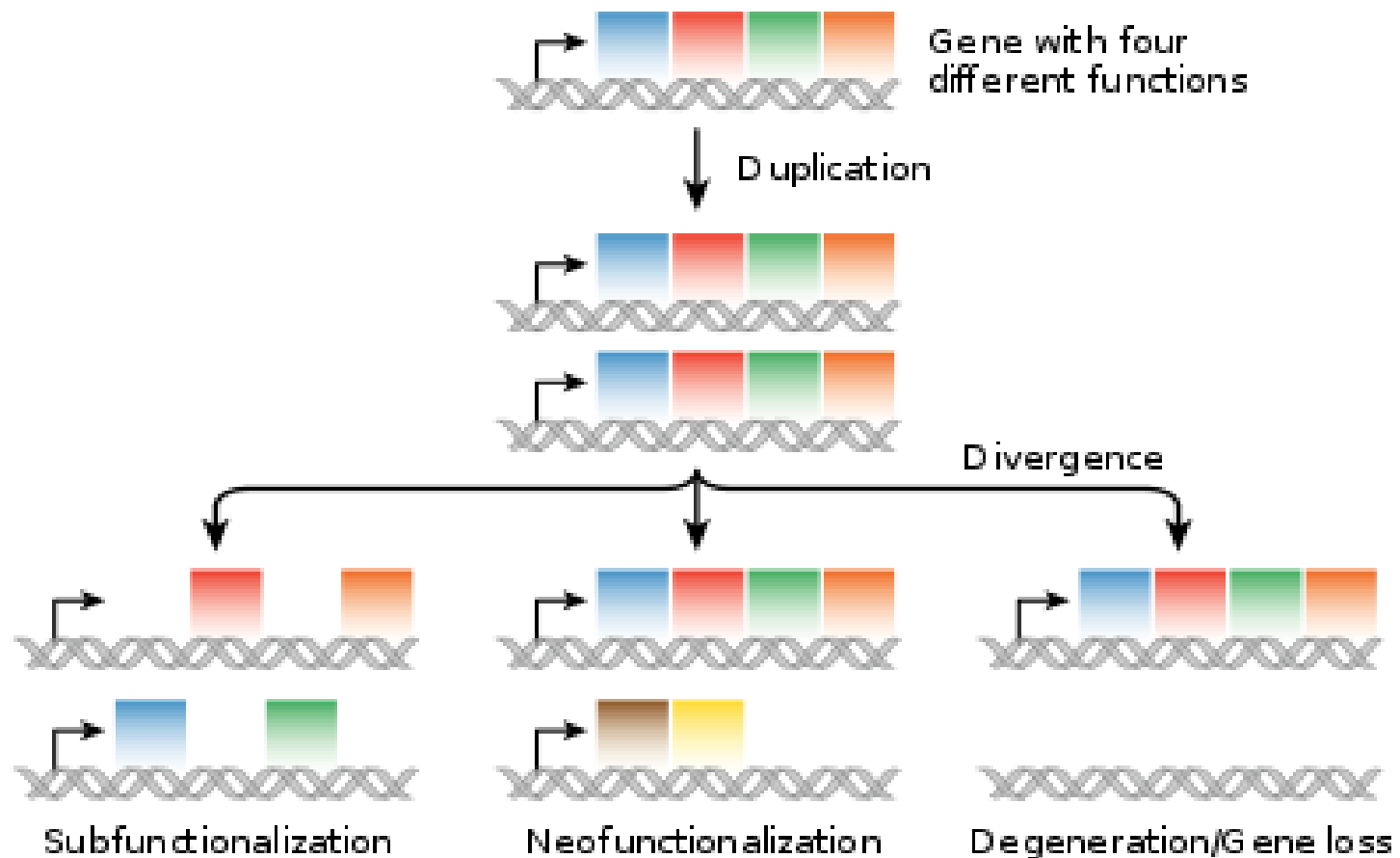




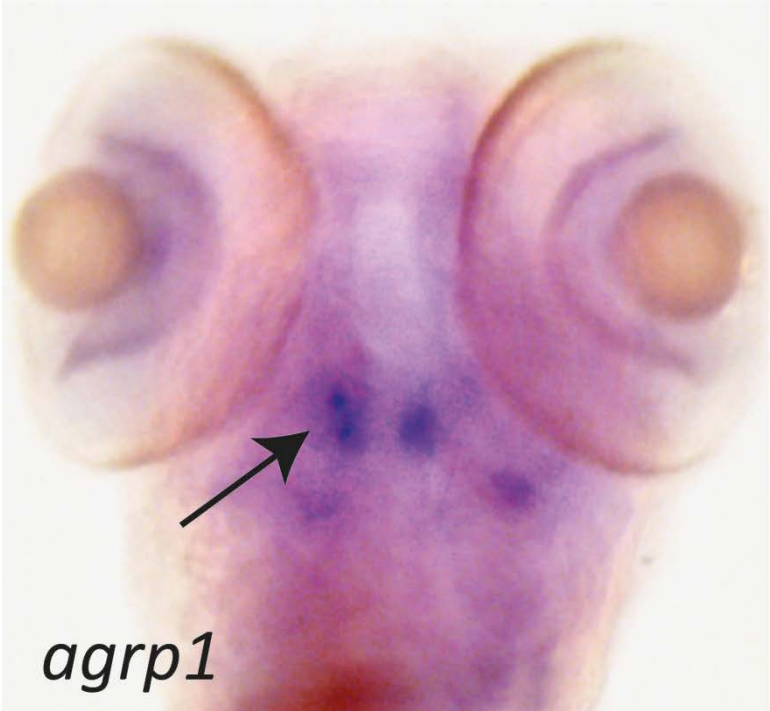


Whole genome duplication is a rare evolutionary event that has played a dramatic role in diversification

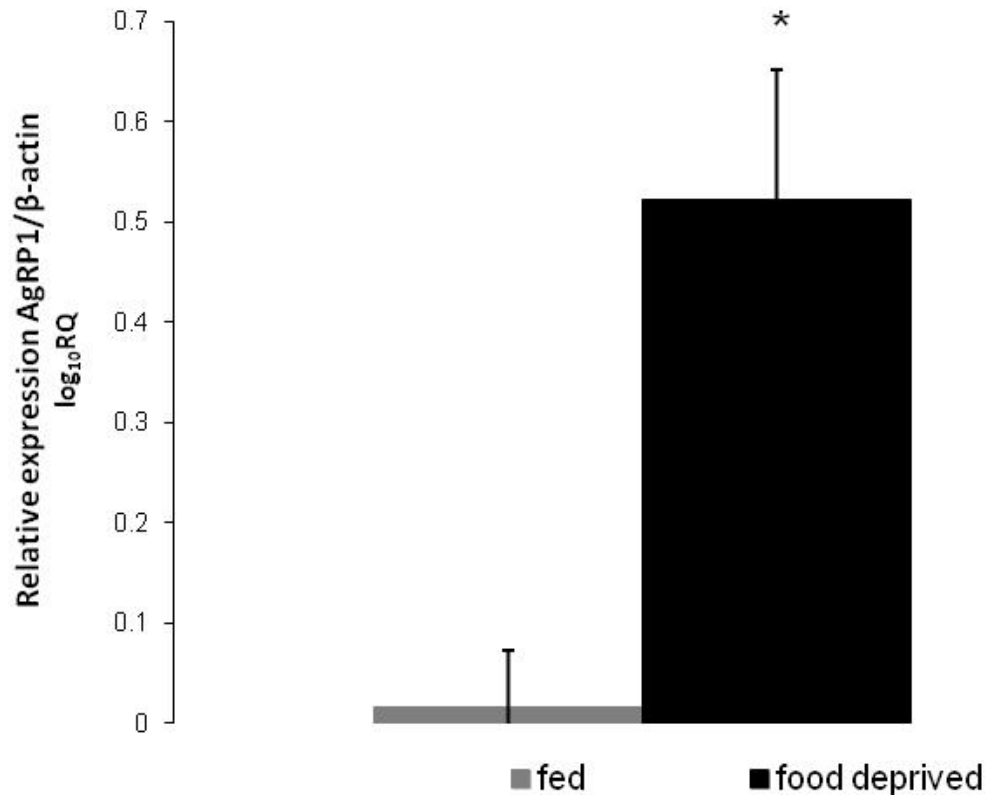
# Whole genome duplication: What could be the consequence and why is this a consideration?



Hypothalamic specific



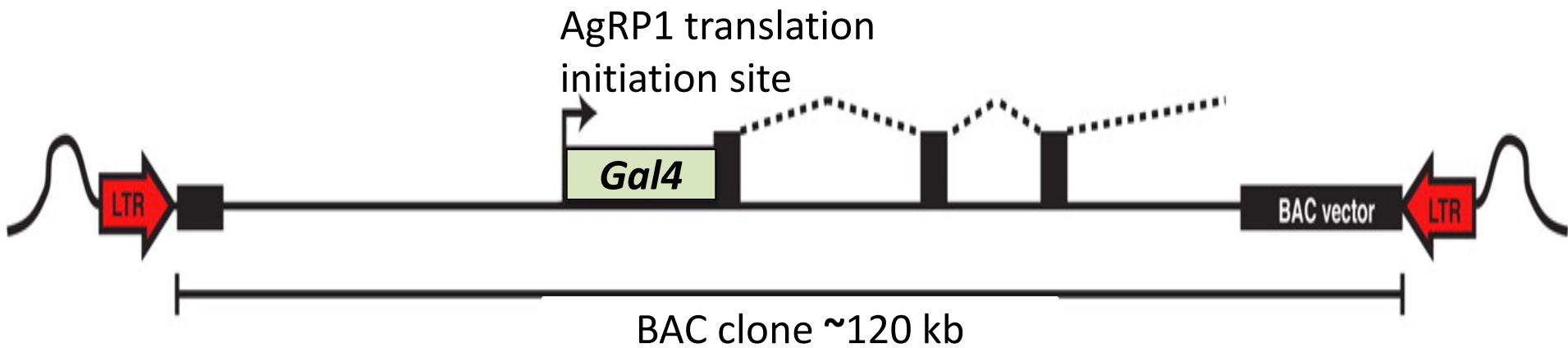
# Zebrafish's AgRP<sub>1</sub> increases in response to food deprivation

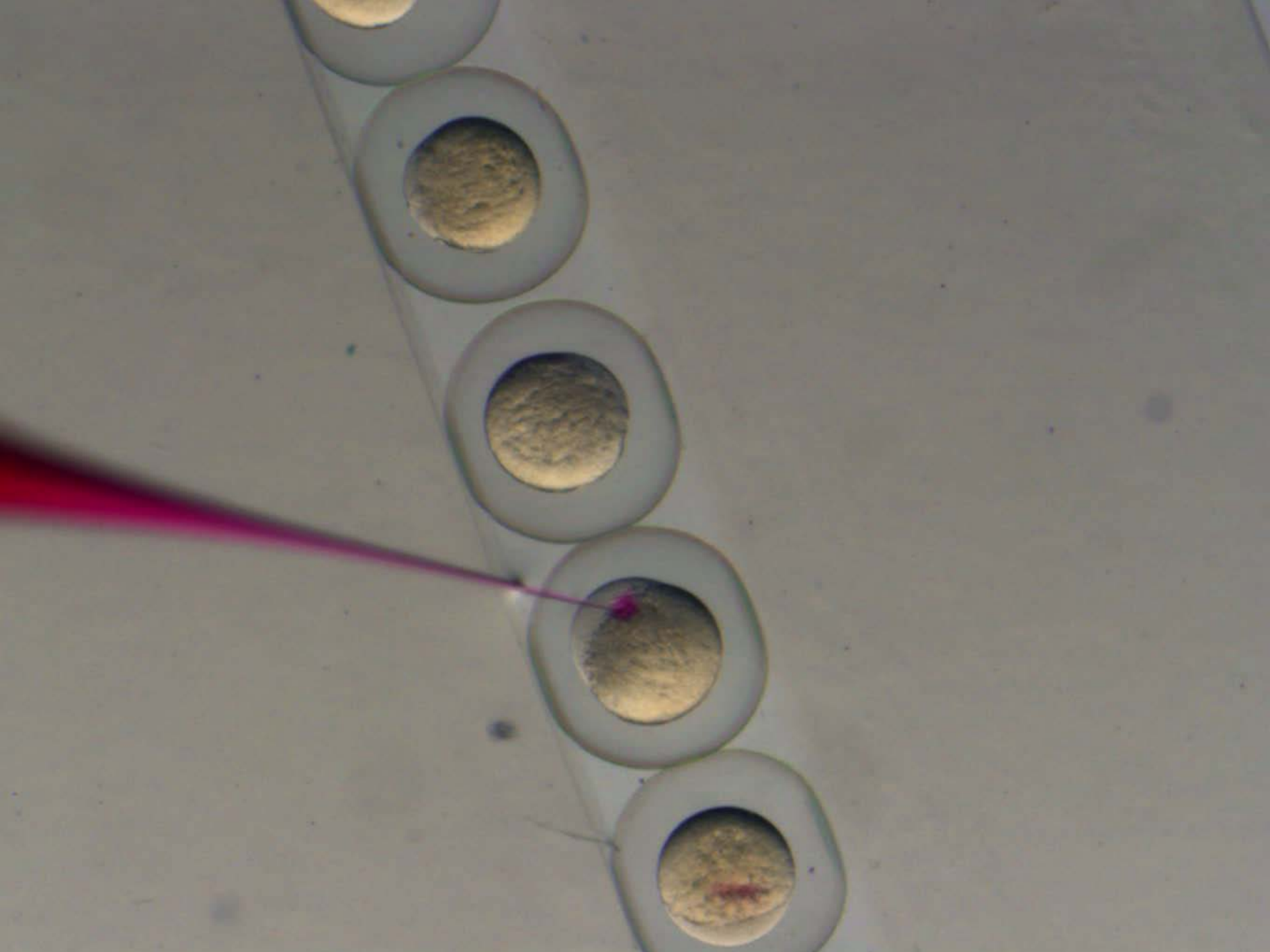


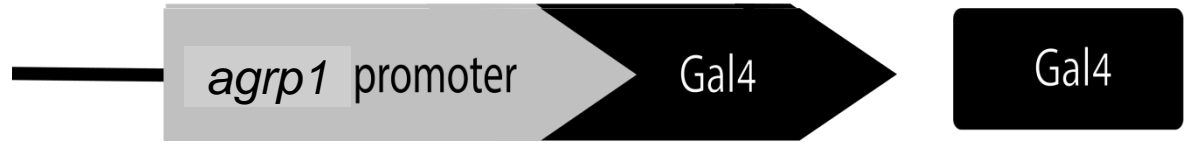
Quantitative real-time PCR analysis. Under food deprivation conditions, AgRP<sub>1</sub> (mRNA expression levels are elevated (p-value=0.0015)).



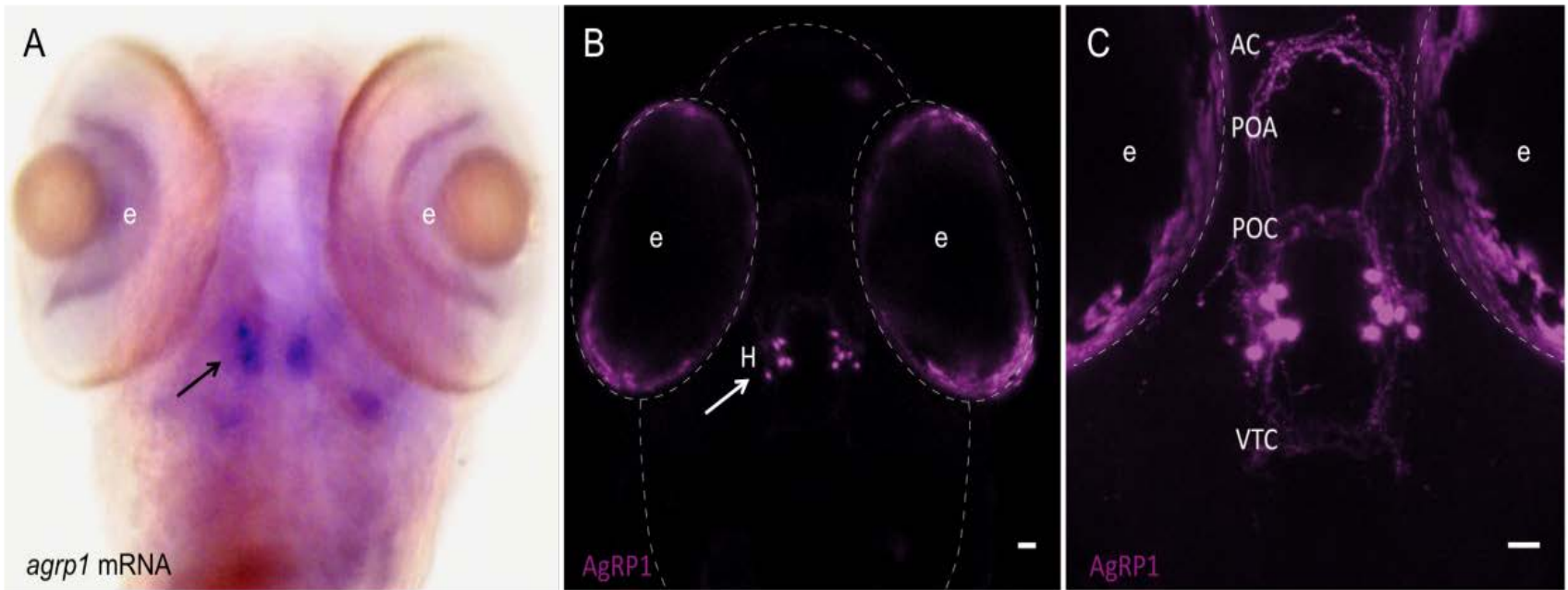
# AgRP1 BAC transgenesis





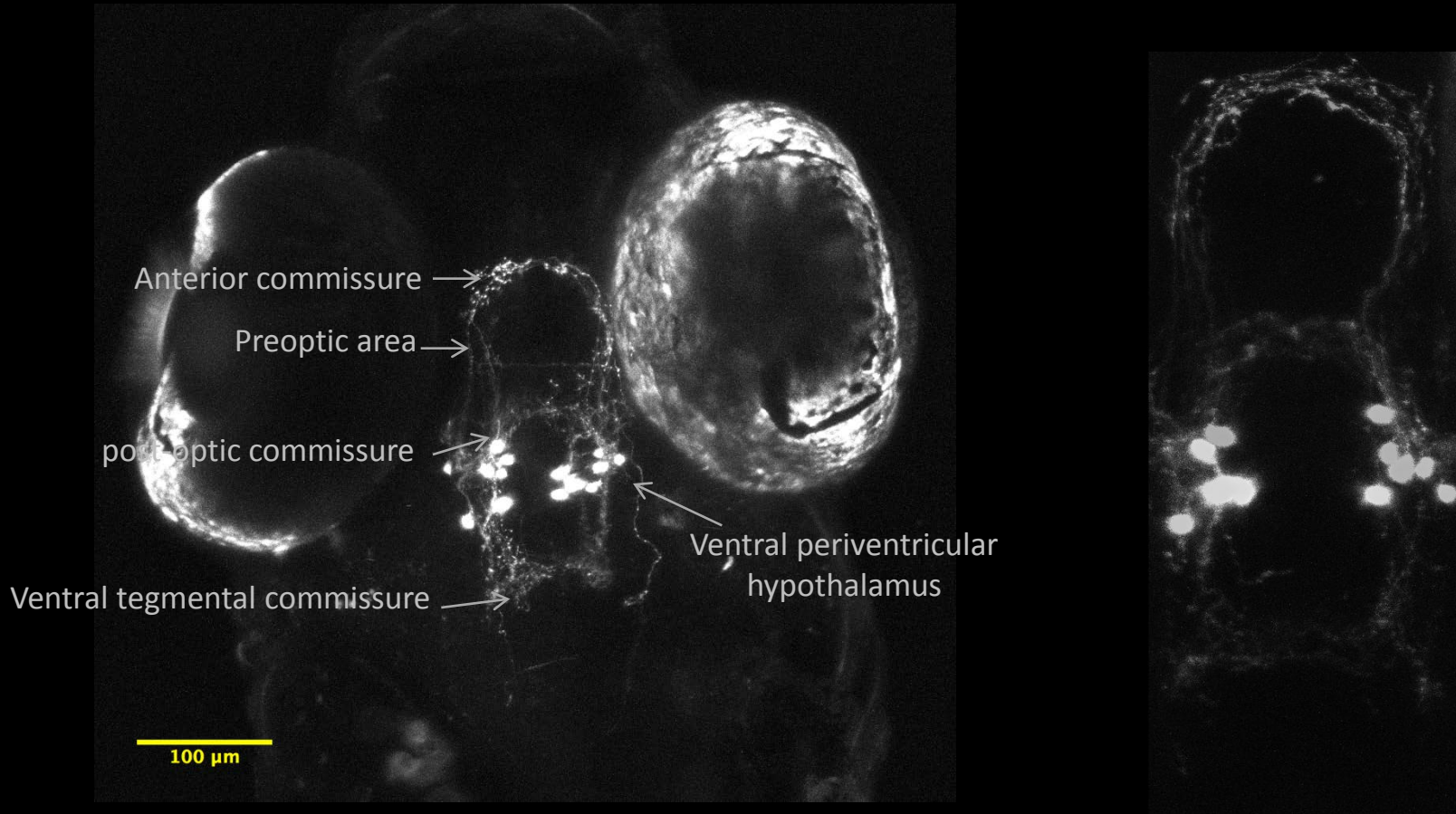


# The *agrp1* transgenic line

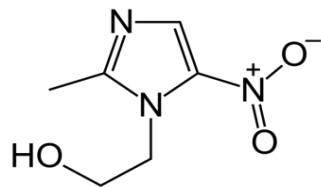
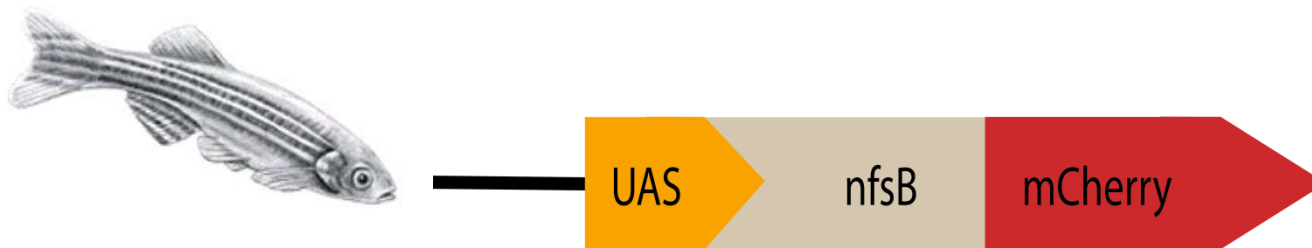




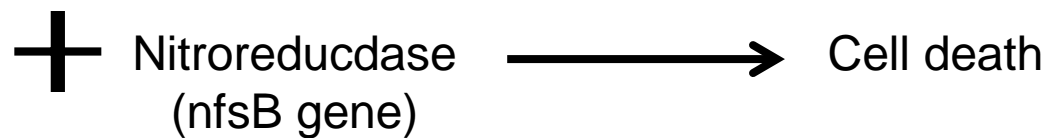
# TgBAC(*agrp1*:Gal4-VP16), 6dpf dorsal view

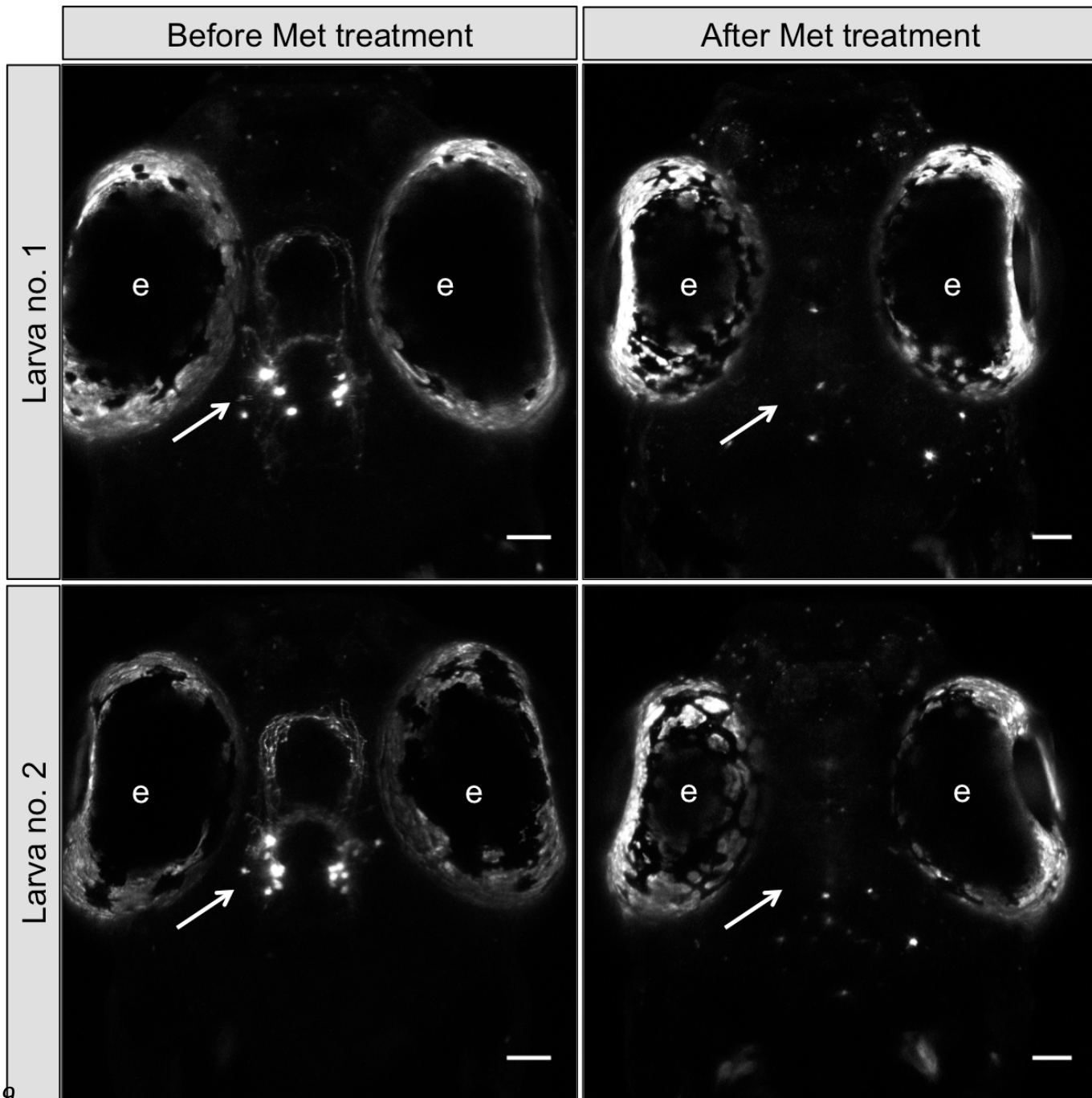


# Neuronal ablation



Metronidazole



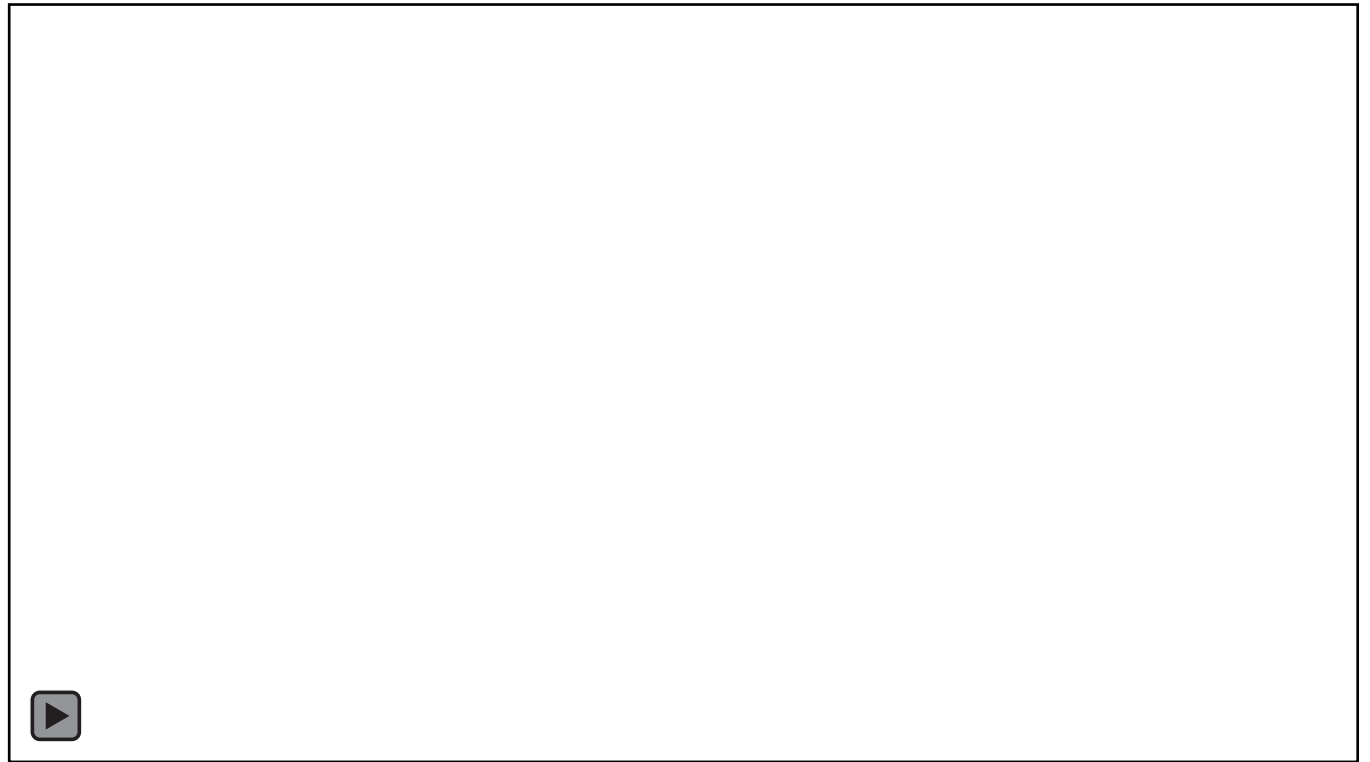




סנדלית

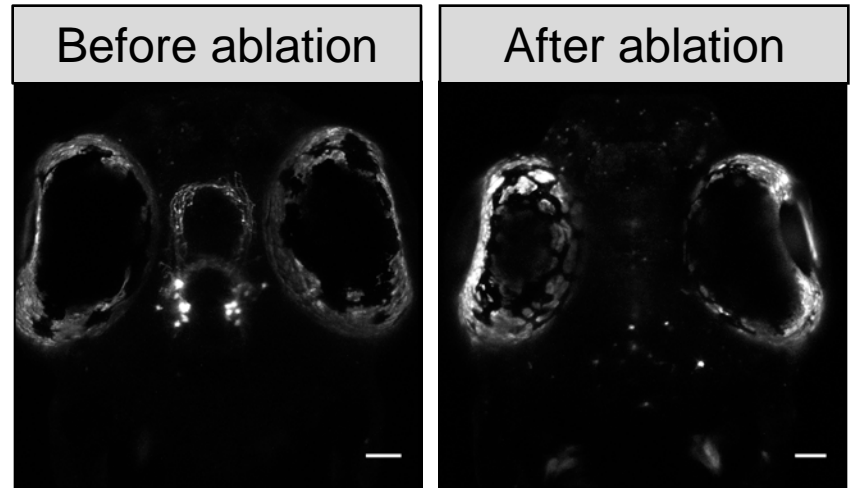


Image by  
Idit Aviram

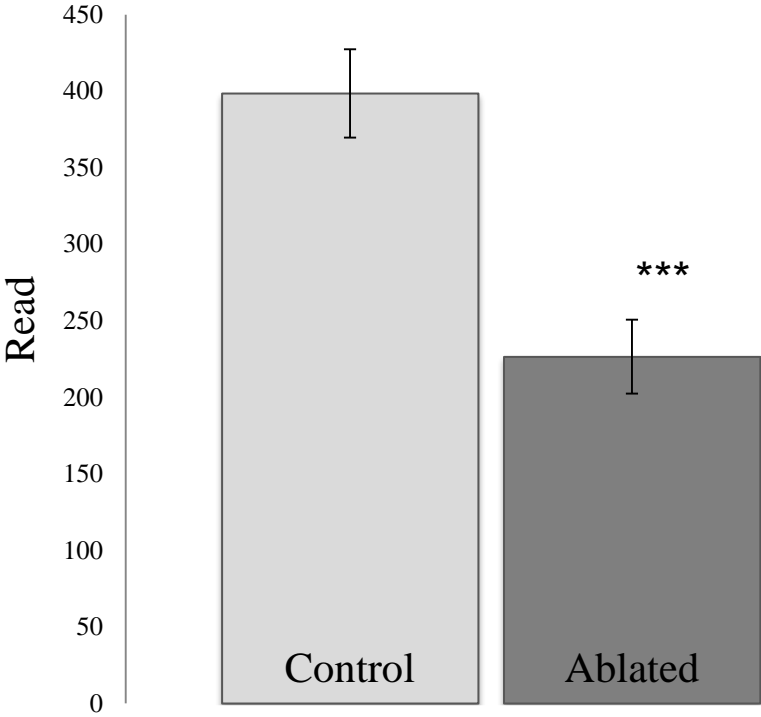




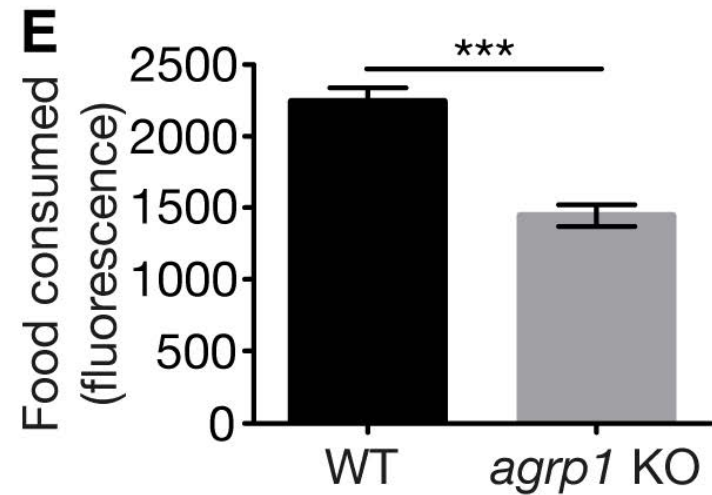
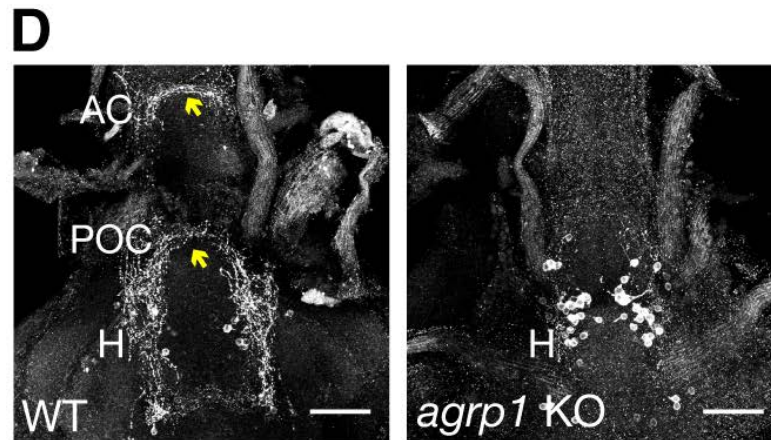
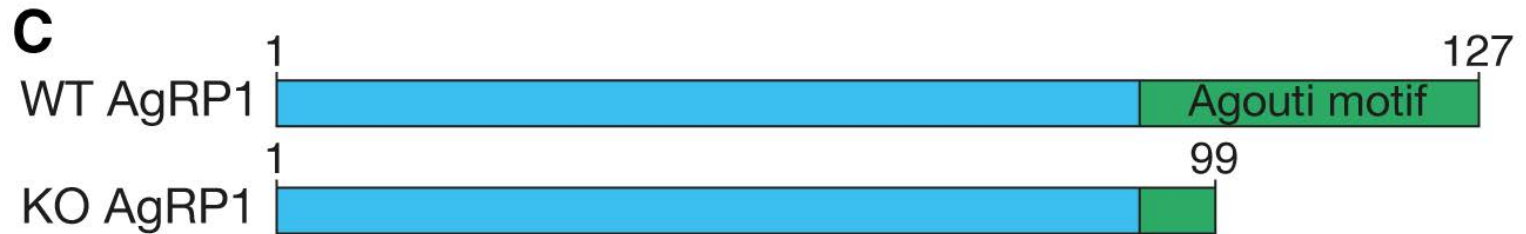
# Food consumption assay



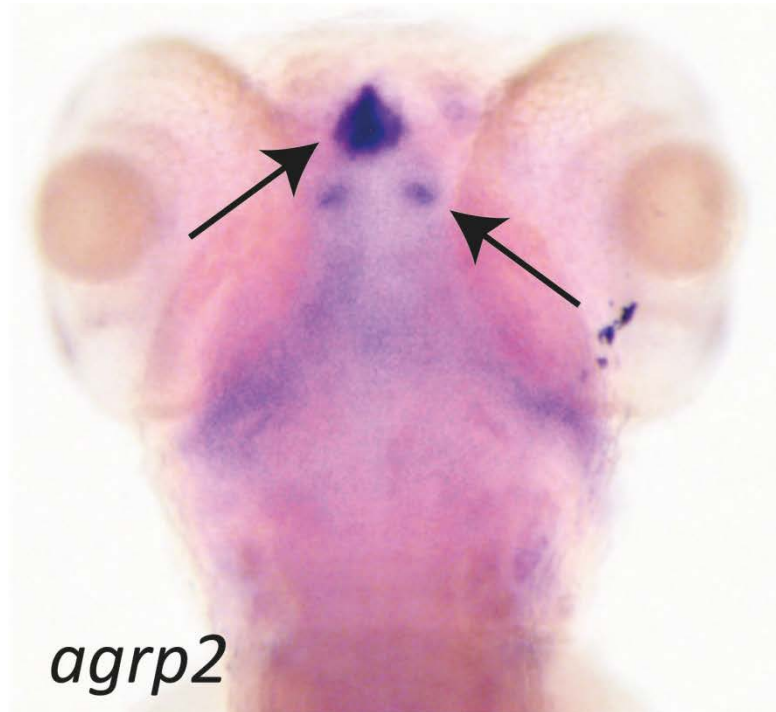
# AgRP1 neuron ablation reduces food consumption



# AgRP1 gene knockout reduces food consumption

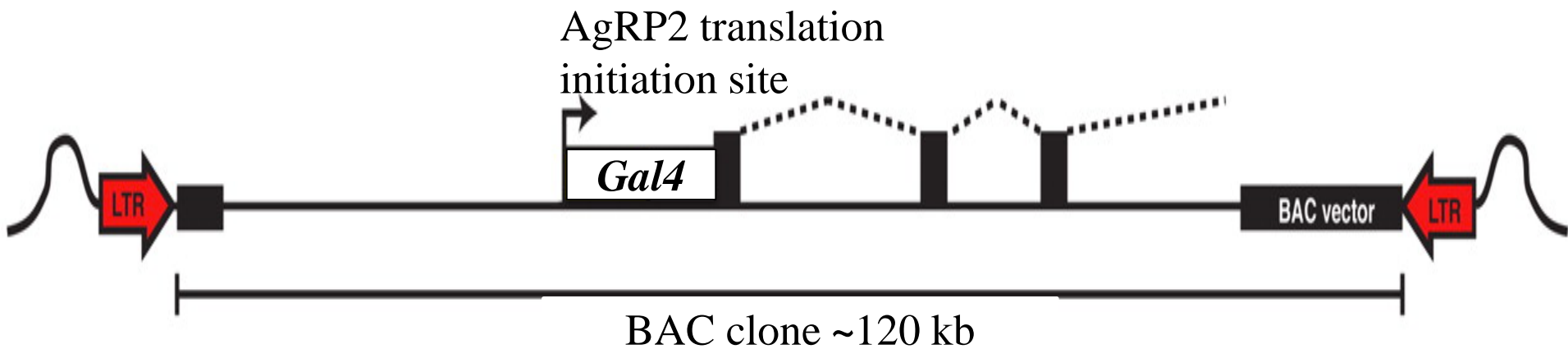


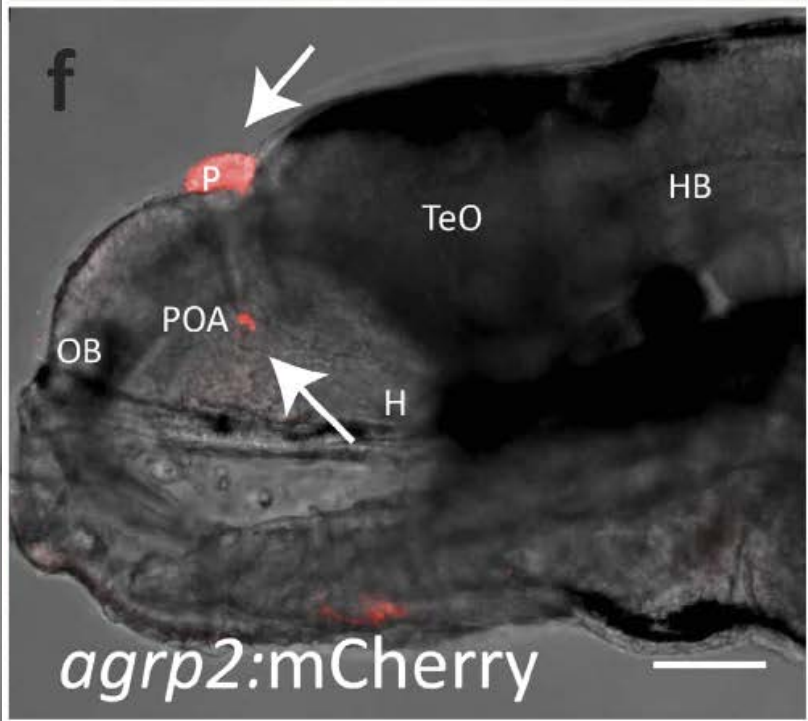
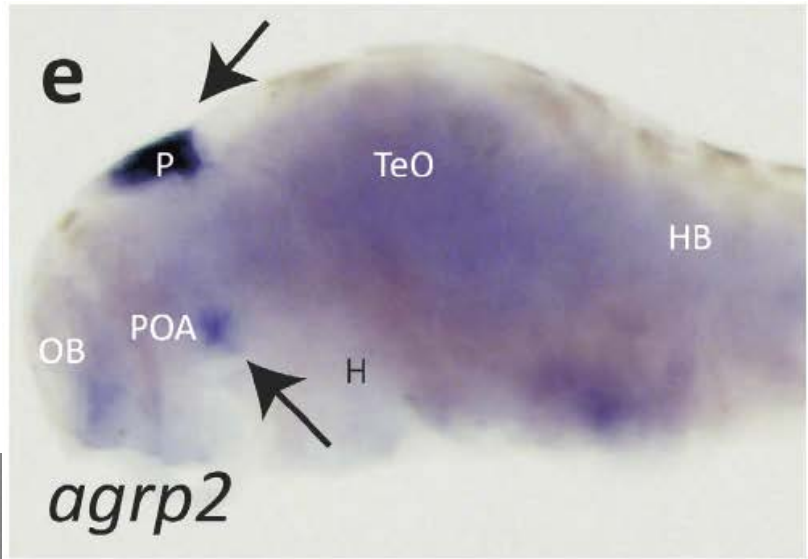
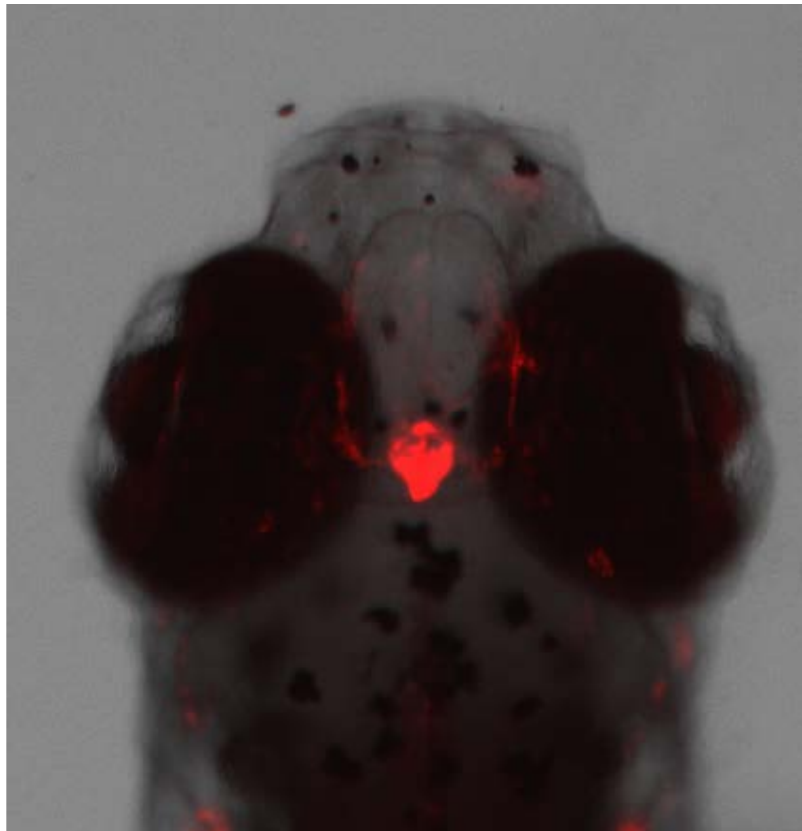
Pineal enhanced

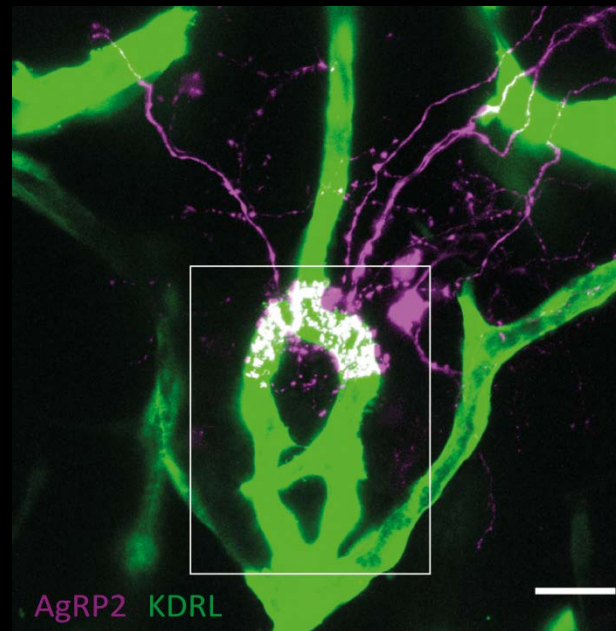
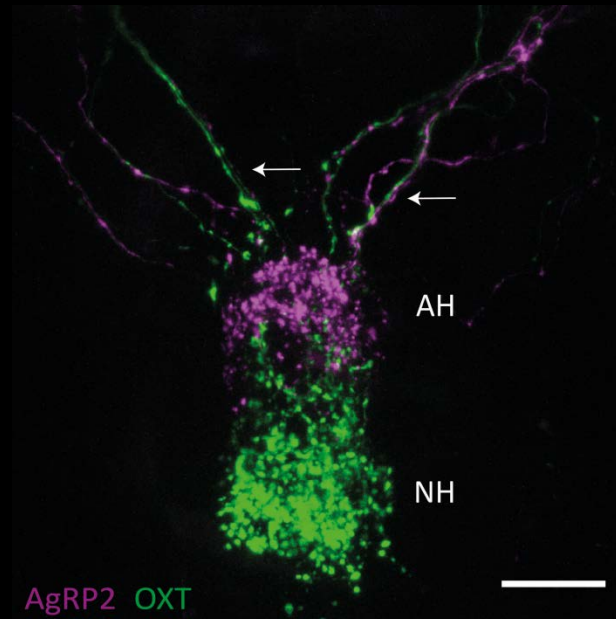
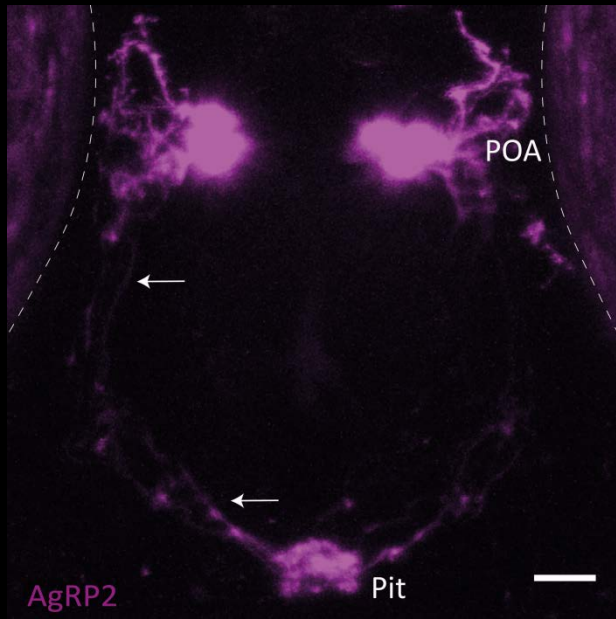


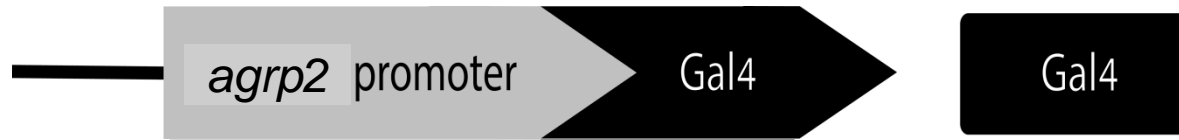


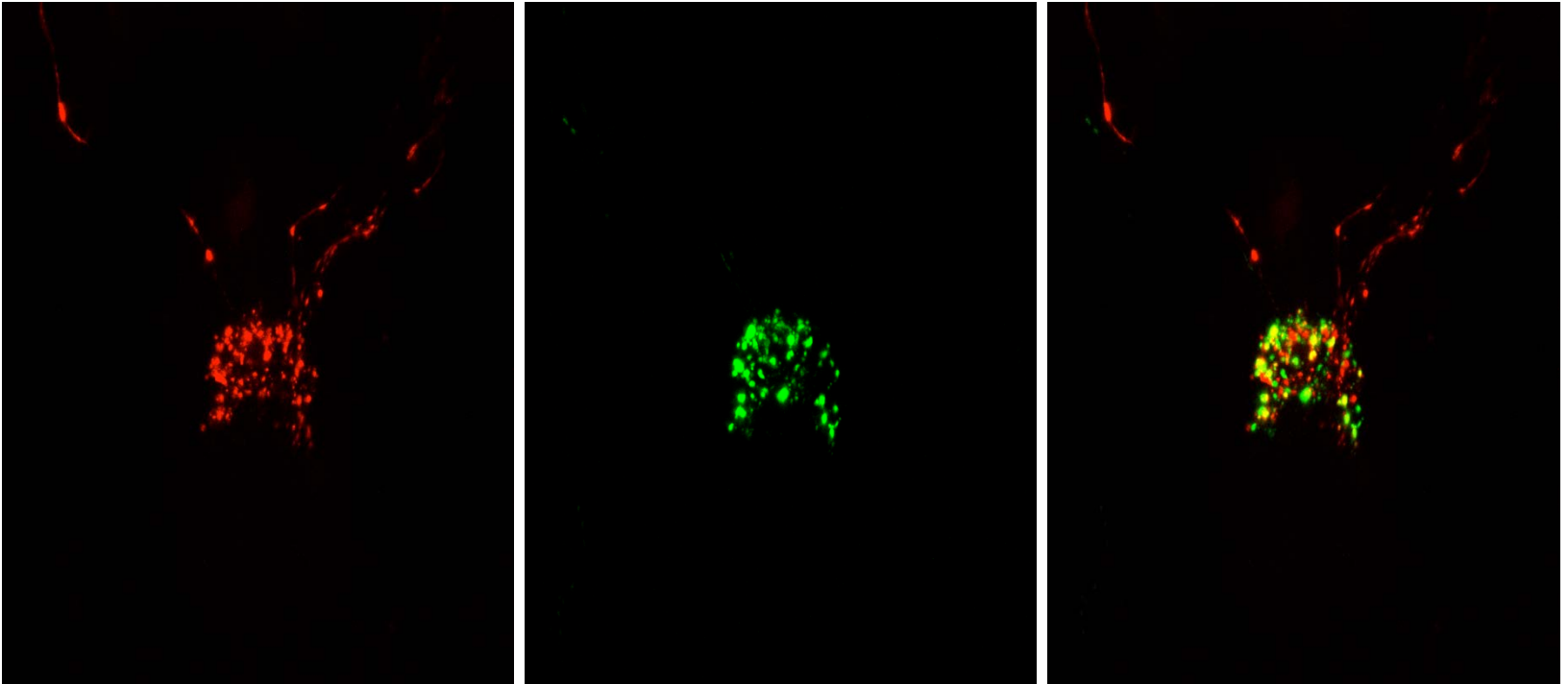
# AgRP2 BAC transgenesis

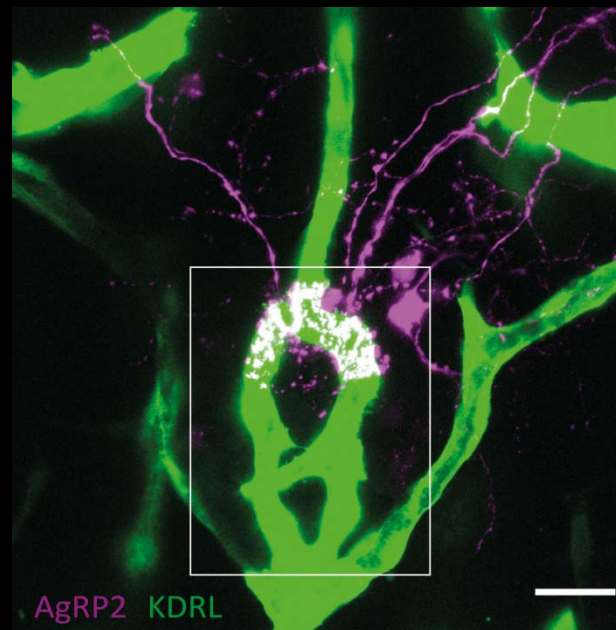
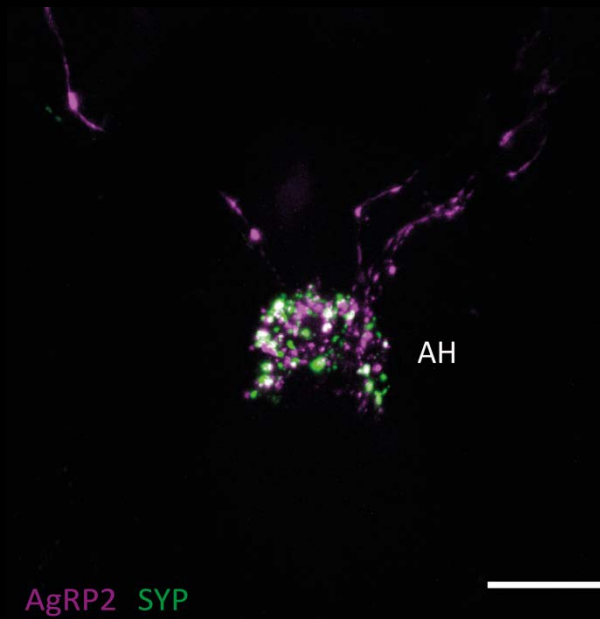
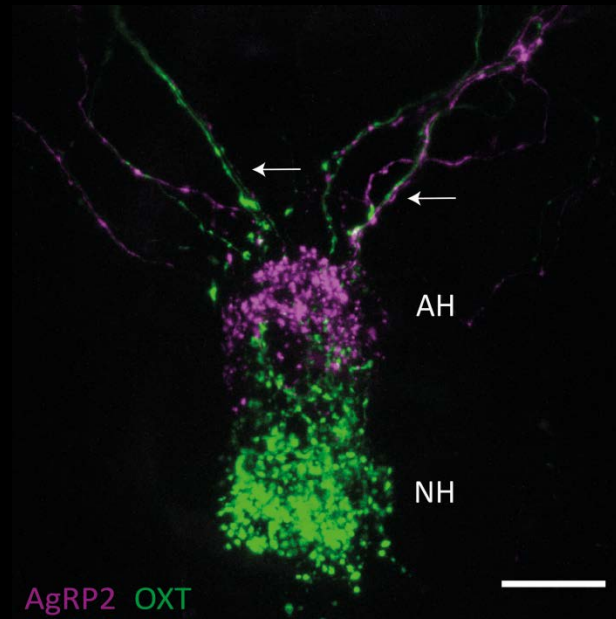
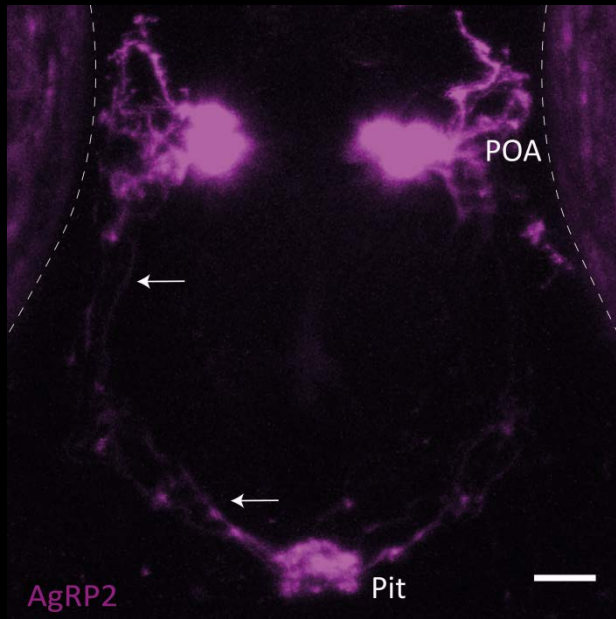








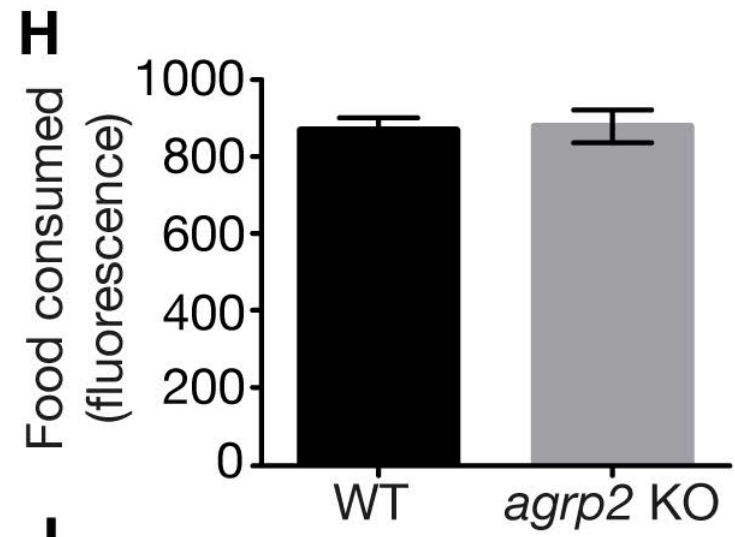
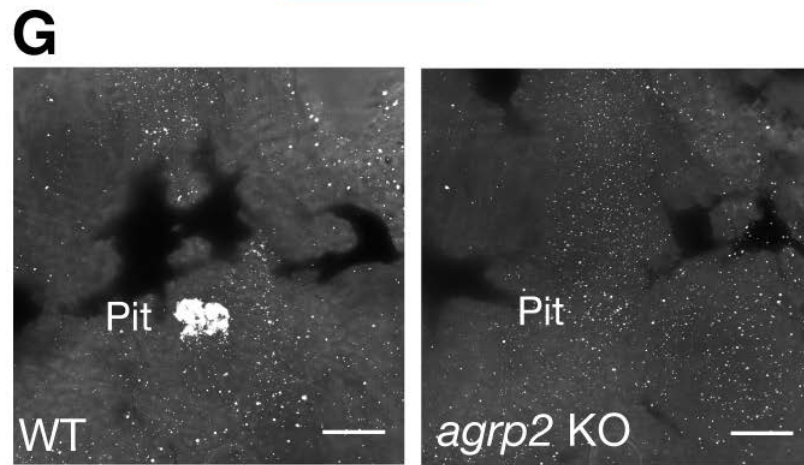


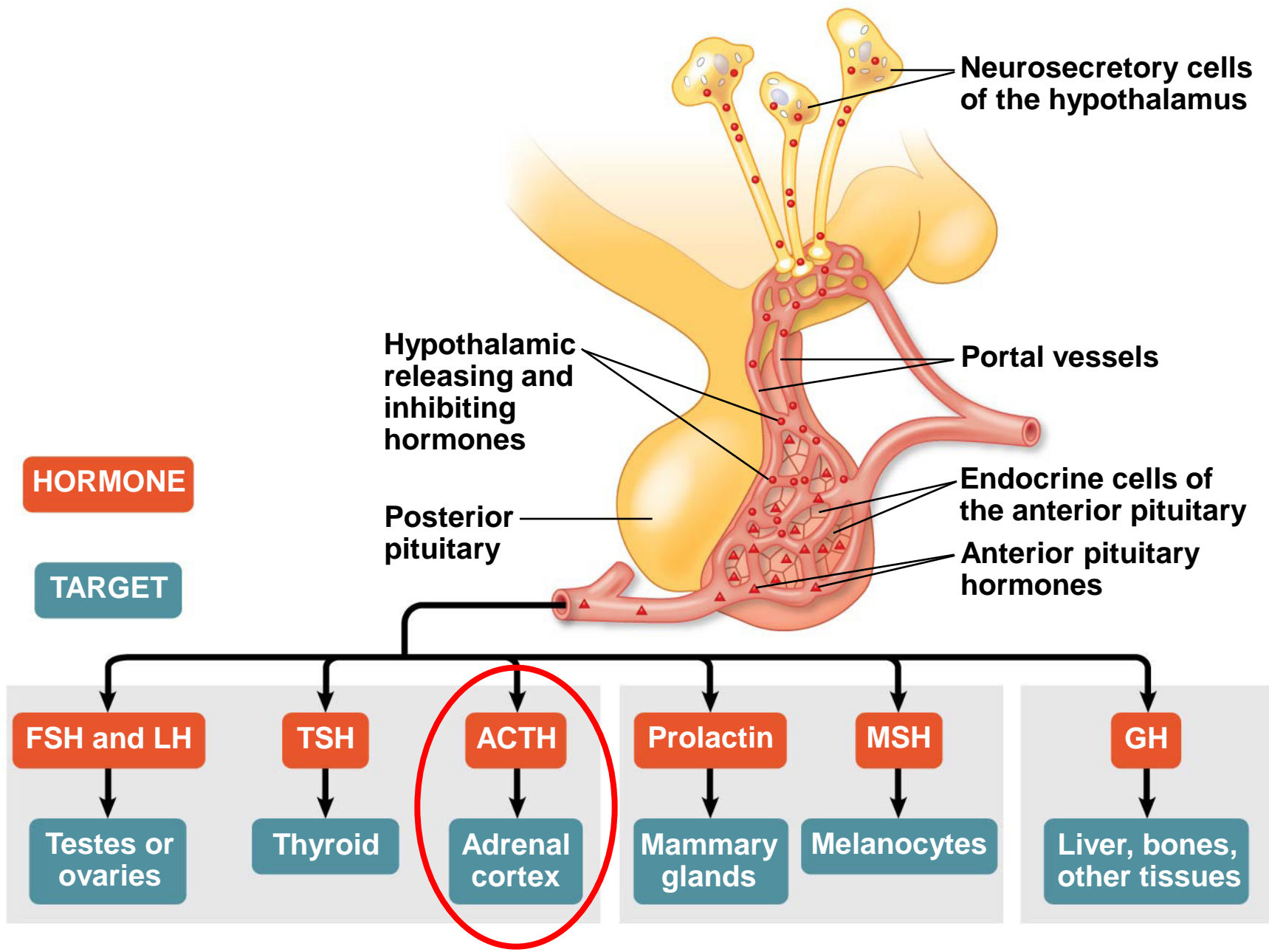


M	T	T	A	V	L	K	G	F	V	L	F	I	C	L	F	F	T	V	V	Q	Amino acid sequence		
5'	ATG	ACG	ACG	GCG	GTG	CTG	AAA	GGA	TTT	GTG	CTC	TTC	ATC	TGC	TTG	TTC	TTC	ACA	GTA	GTT	CAG	3'	Wild type sequence
5'	ATG	ACG	ACG	GCG	GTG	CTG	AAA	GGA	Tca	---	-TC	TTC	ATC	TGC	TTG	TTC	TTC	ACA	GTA	GTT	CAG	3'	Δ4 bp (frameshift)
M	T	T	A	V	L	K	G	S				S	S	S	A	C	S	S	Q	Stop	Predicted mutated protein		

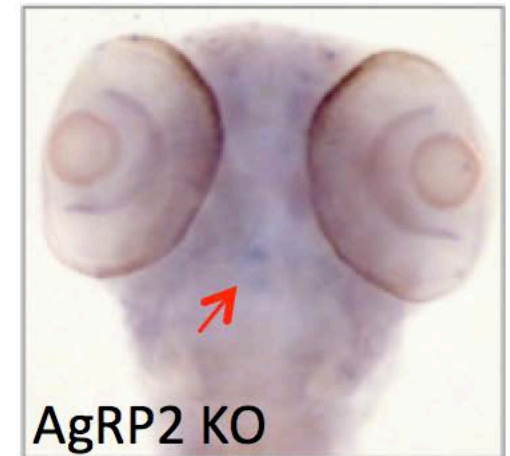
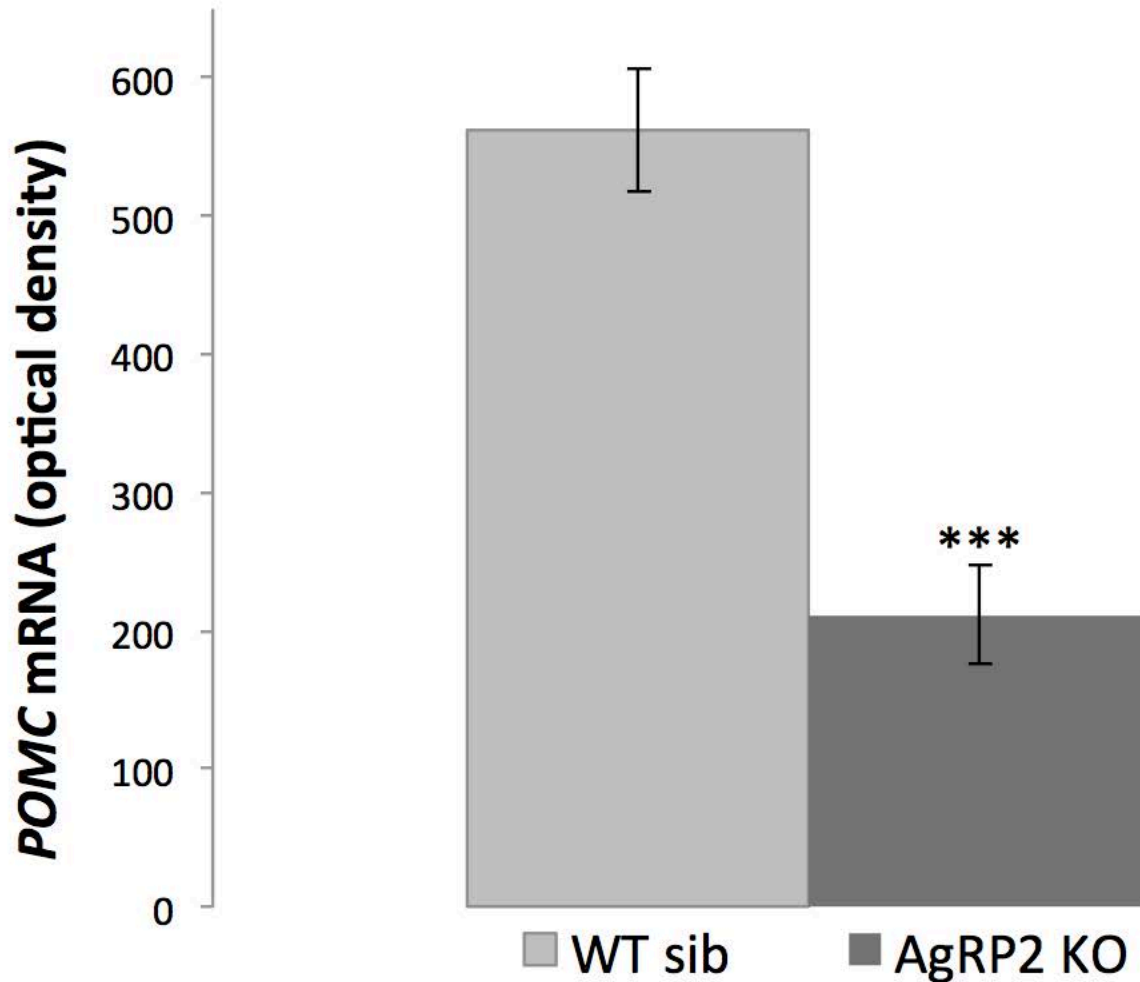
**Figure 5. The *agrp2* knockout line.** The first 20 amino acid and coding sequence of the 136-amino-acid AgRP2 are shown at the top. A 4-bp deletion in the mutant and the resulting frameshift, resulting in a 17-amino-acid peptide, are shown at the bottom.



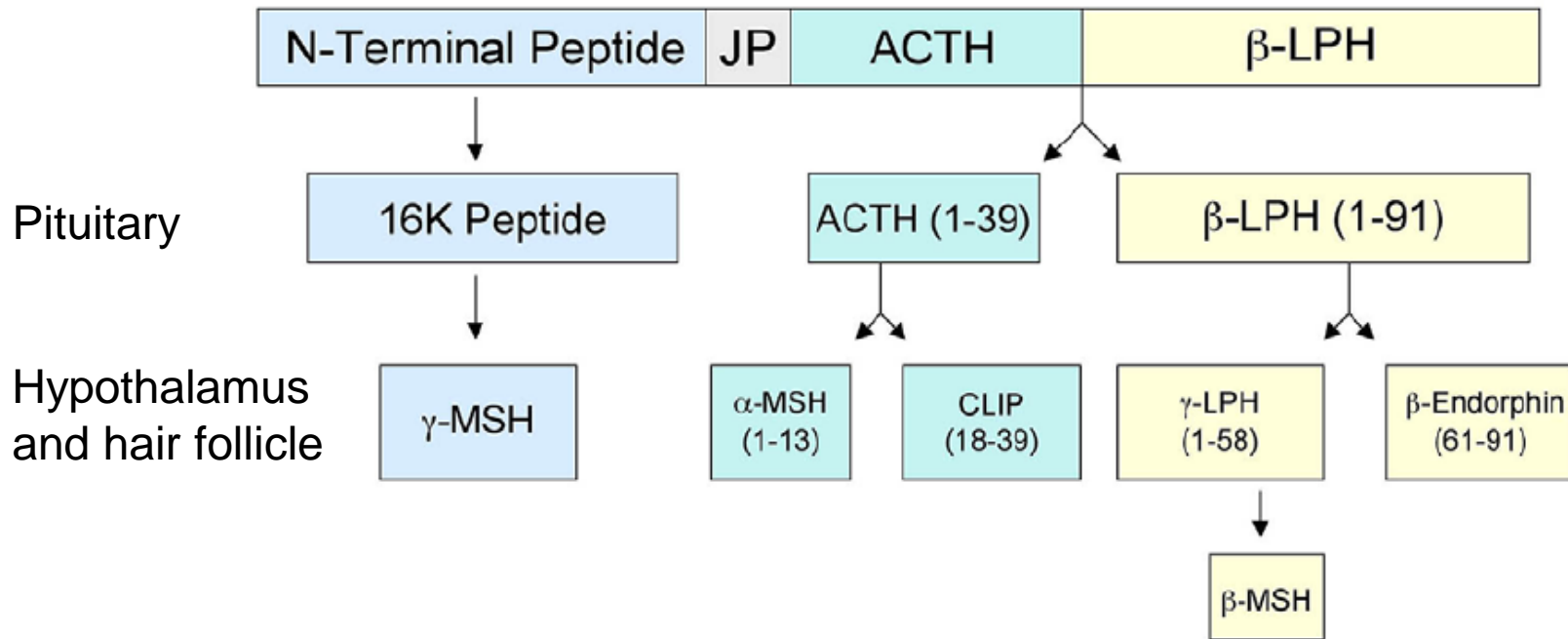




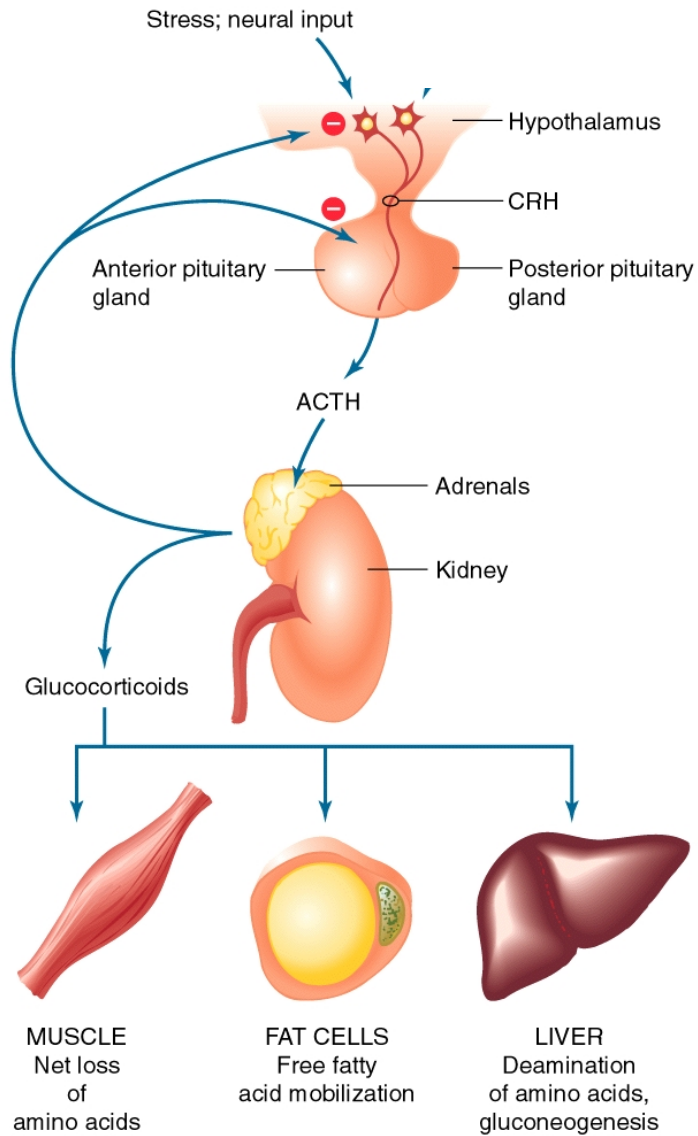
# AgRP2 gene knockout reduces pituitary gland POMC expression



## PROOPIOMELANOCORTIN (POMC)



**Figure 2.** Schematic diagram of the POMC precursor molecule and the major peptide products which are derived from this precursor by endoproteolytic cleavage. (JP = Joining peptide; LPH= Lipotropin; CLIP= corticotropin-like-intermediate lobe peptide).



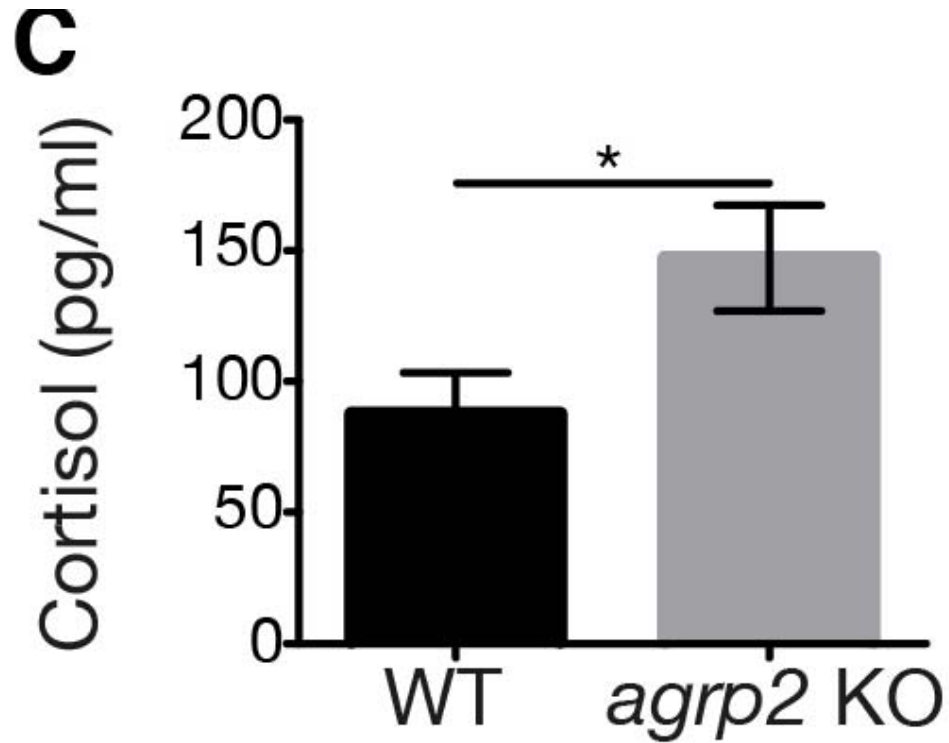
CRH – corticotropin releasing hormone

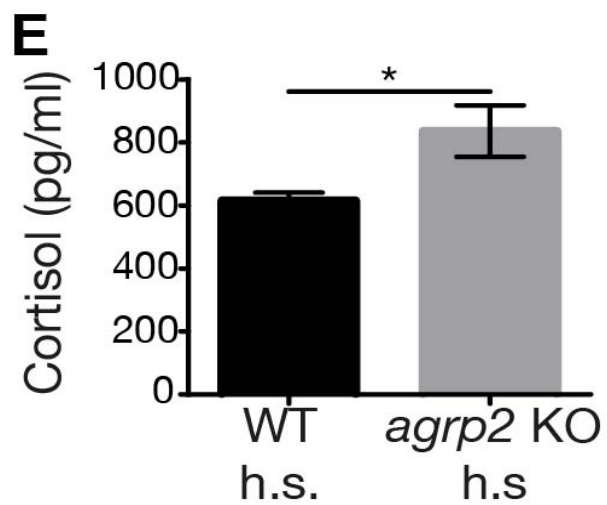
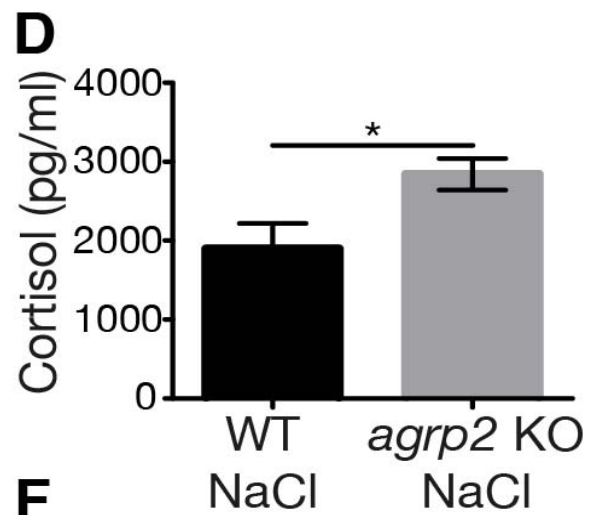
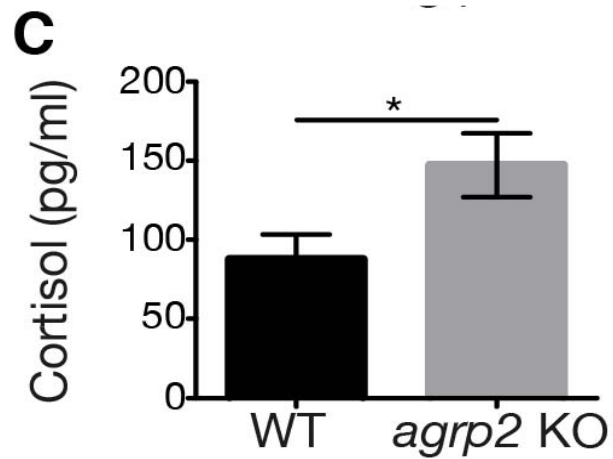
POMC

ACTH – corticotropin; adrenocorticotropic hormone

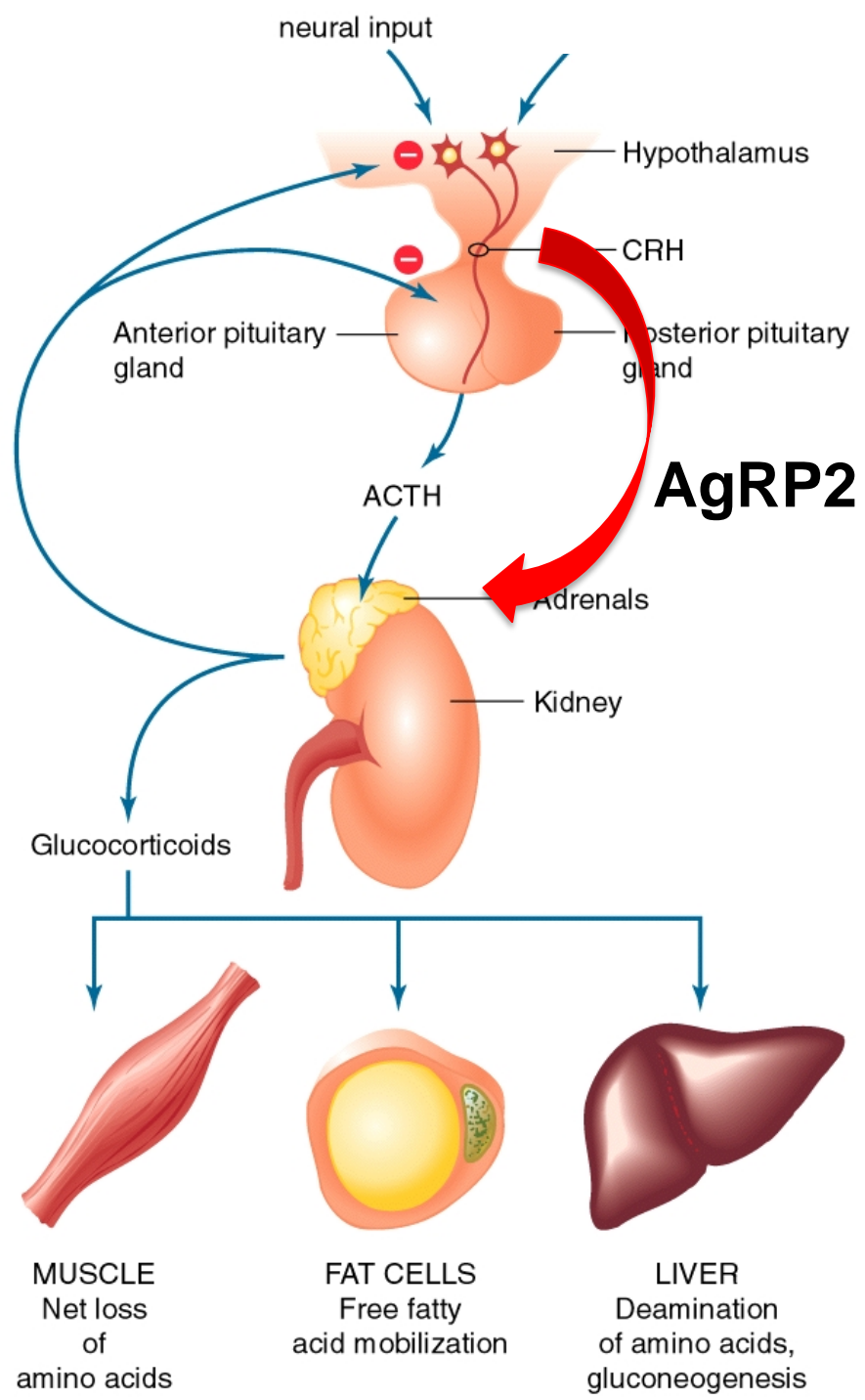
Glucocorticoids, cortisol or corticosterone

## High cortisol levels in agrp2 KO fish

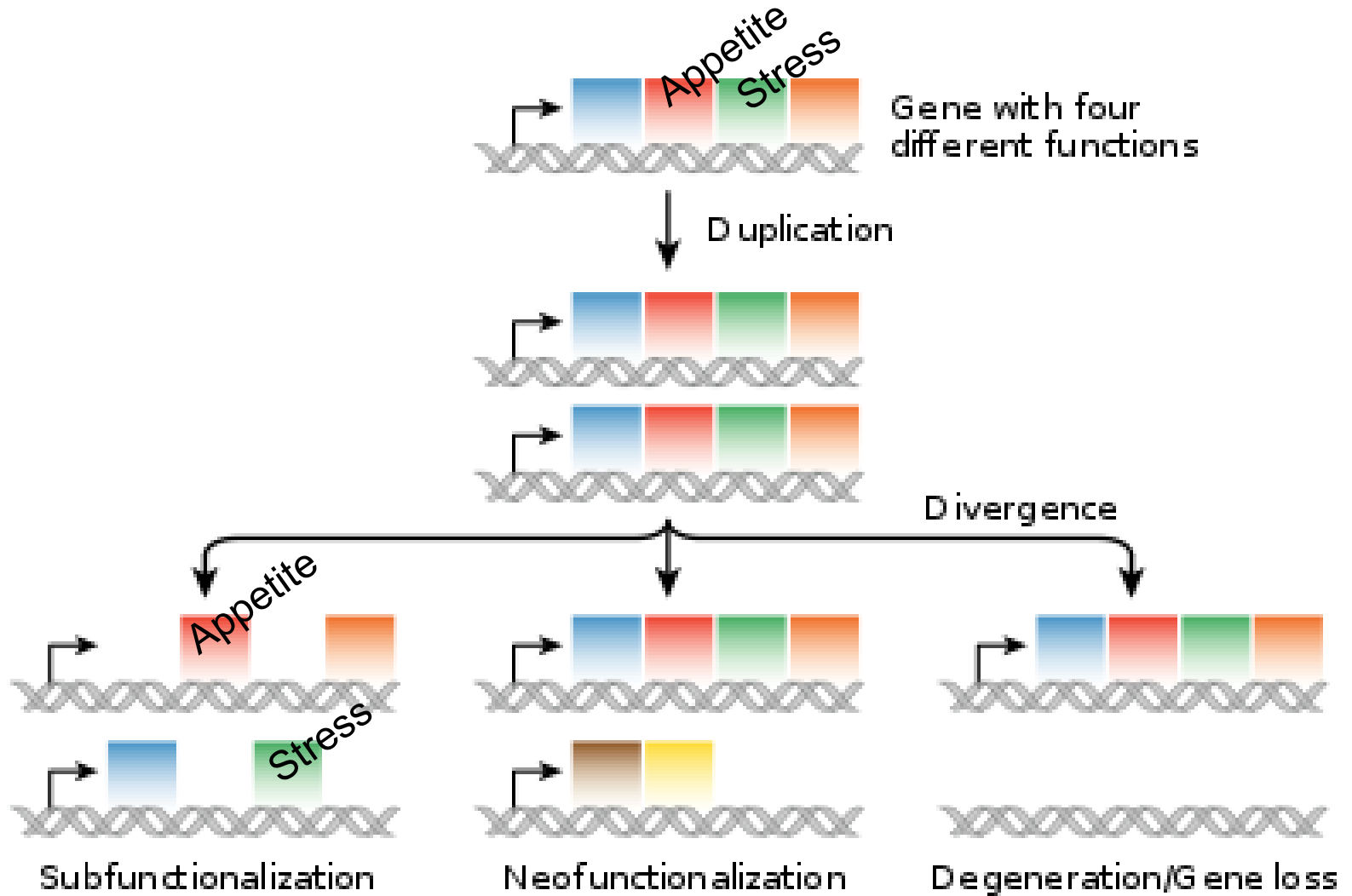




**F**



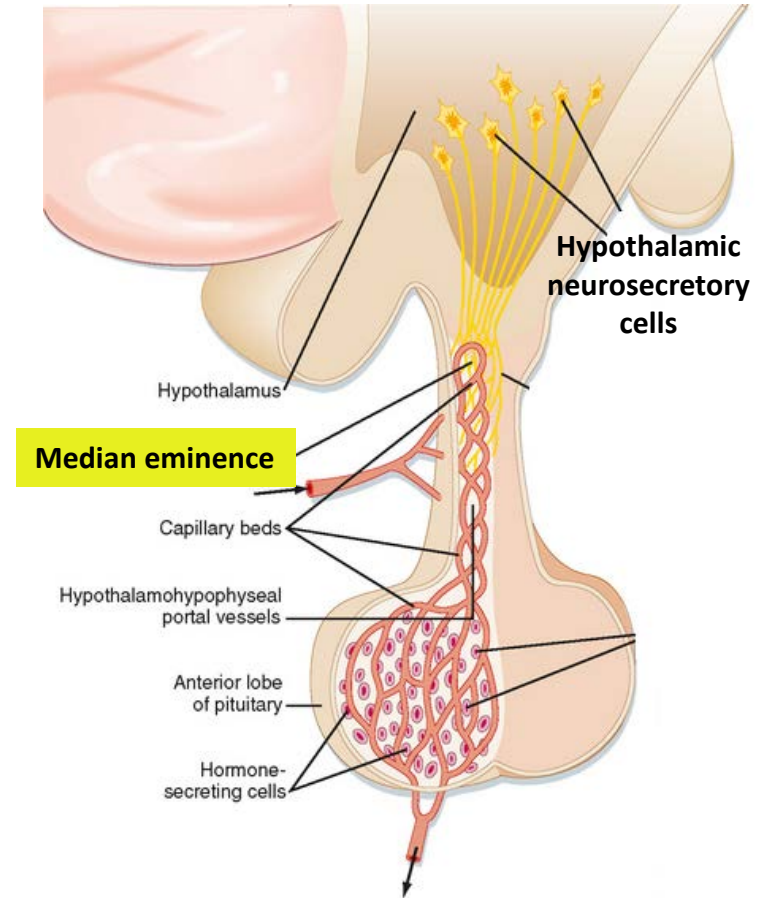




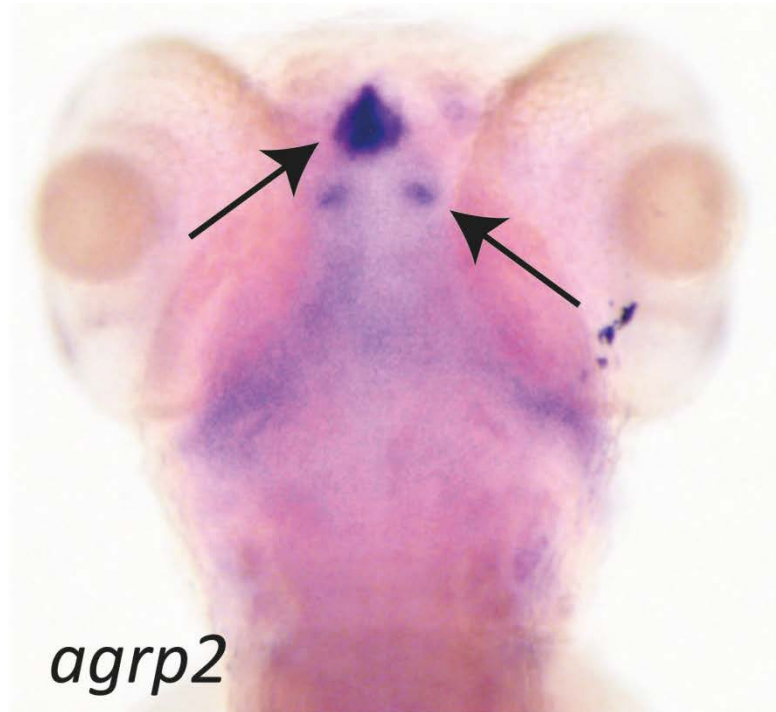
# AgRP neurons in mice also innervate the pituitary

**Table 1. Distribution and relative abundance of AGRP-immunoreactive fibers and terminals in the rat CNS**

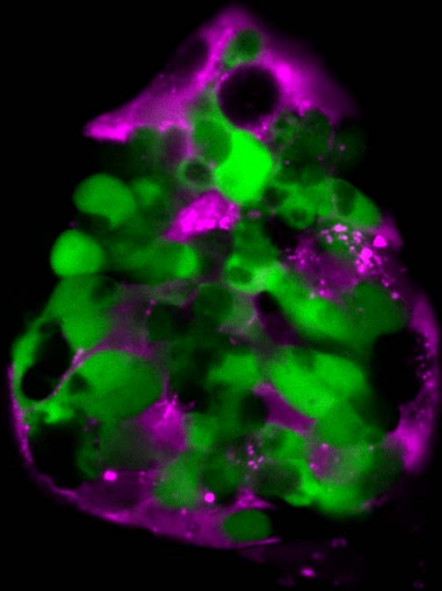
Anatomical sites	Agrp
Compact	–
Ventral part	+++
Dorsal hypothalamic area	++
Lateroanterior hypothalamic nucleus	+
Lateral hypothalamic area	+++
Ventrolateral hypothalamic nucleus	++
Perifornical nucleus	++++
Posterior hypothalamic area	+
Arcuate nucleus	+++++
Median eminence, internal part	+++
Median eminence, external part	+
Medial tuberal nucleus	++
Supramammillary nucleus	+



Pineal enhanced

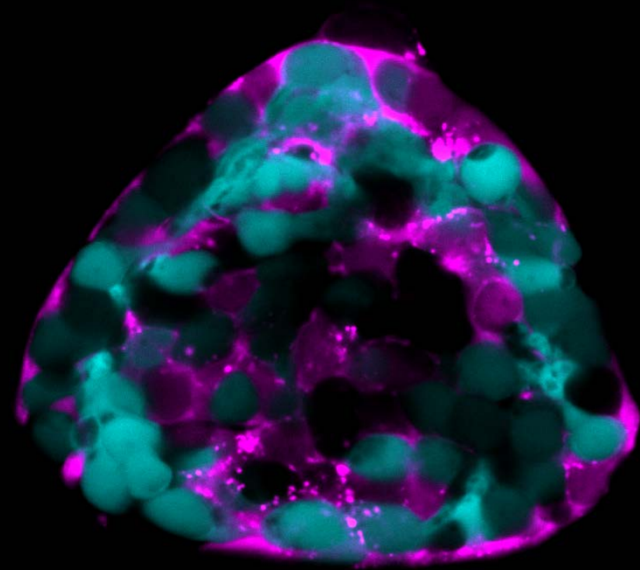


Pineal AgRP2 cells are not photoreceptors nor neurons.  
Newly characterized neuropeptide-expressing pineal cells?



10  $\mu$ m

Photoreceptors  
*Tg(aanat2:EGFP)*  
*Tg(agr2:mCherry)*



10  $\mu$ m

Projection neurons  
*Tg(foxd3:EGFP)*  
*Tg(agr2:mCherry)*

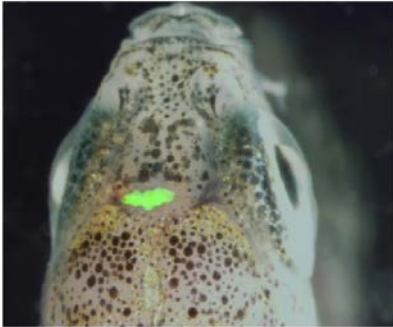
At later stage of development the signal extends beyond the pineal gland



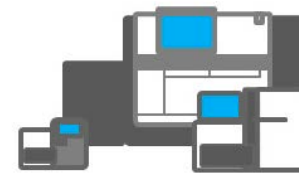
Dorsal view, 21dpf

# Single-cell sequencing of the zebrafish pineal gland

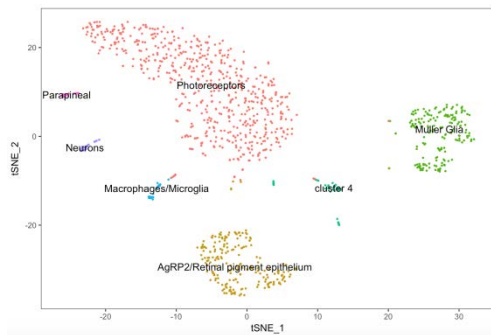
Pineal dissections



Single-cell barcoding and library prep

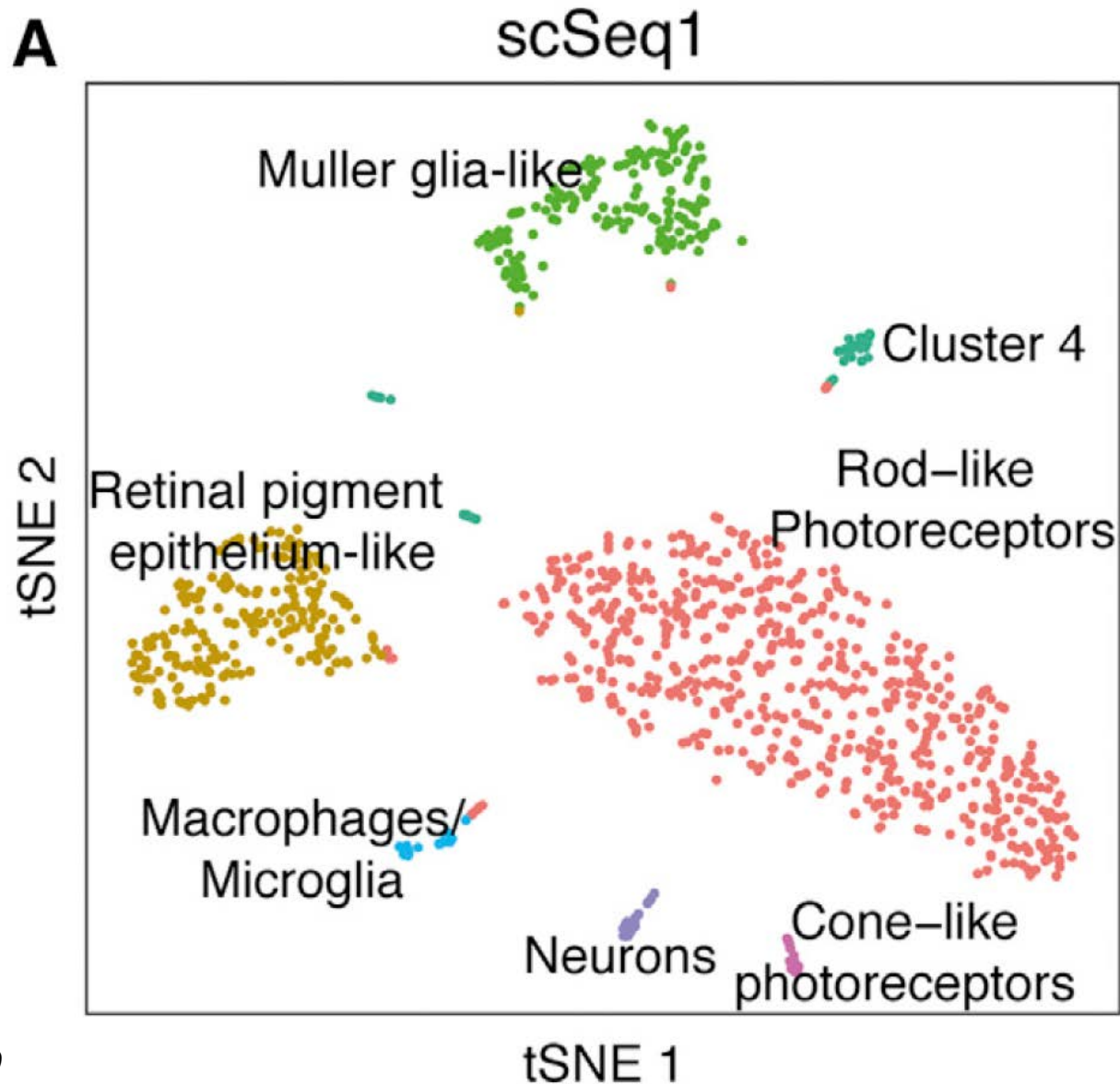


Sequencing



Data analysis

# Single-cell sequence analysis of zebrafish pineal gland 2D visualization of cell clustering





# **AgRP2 is expressed in Retinal Pigment Epithelium (RPE)-like cells.**

**RPE-like cells express genes that suggest involvement in the retinoid cycle in the pineal gland:**

rbp5 (retinol binding protein 5)

rgra (retinal G-protein coupled receptor)

stra6 (stimulated by retinoic acid 6)

rdh5 (retinol dehydrogenase 5)

rpe65 (retinal pigment epithelium-specific 65 kDa protein)

CD63 (exosome protein)

# Pineal signals

- The melatonin signal
  - Neuronal signal
  - AgRP2?
  - Exosomal signal?
- 
- What are their targets?
  - What are their roles?

