



# MODEL GENERATION TECHNICAL REPORT

## Nr2f2-Cre-F3-ERT2-F3 BAC transgenic line

**C57BL/6N-Tg(Nr2f2-cre/ER<sup>T2</sup>)40.Ics; ICS internal name Ras6261-40**

**C57BL/6N-Tg(Nr2f2-cre)40.ICS: ICS internal name Rs6261-40\*Flp**

Report finalized the 16/05/23

By Marie-Christine Birling (PhD)

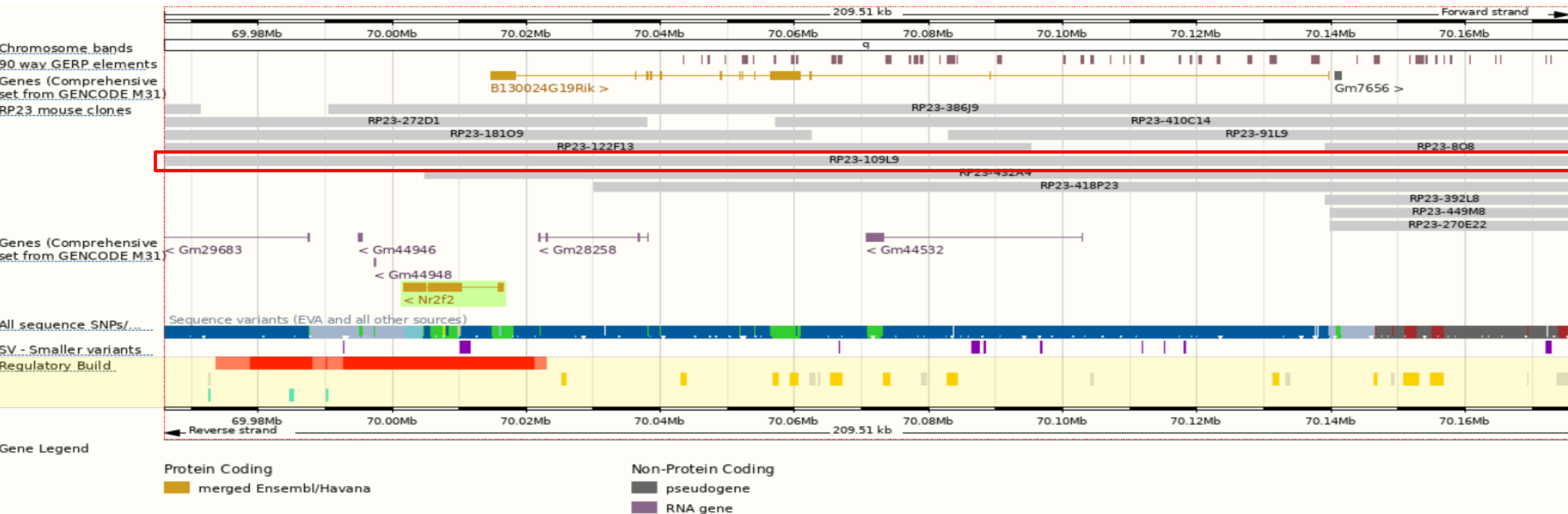
[birlingm@igbmc.fr](mailto:birlingm@igbmc.fr)

## Strategy undertaken



# Nr2f2 genomic locus and BACs

Nr2f2 ENSMUSG00000030551



BAC selected for modification:

RP23-109L9 (**Chromosome 7: 69,965,961-70,175,471, GRCm39**): this BAC is suitable for BAC transgenesis (see Gensat comments next slide). It also contains passenger genes (Gm28258; Gm44532; B130024G19Rik; Gm7656).

# Same BAC used by Gensat

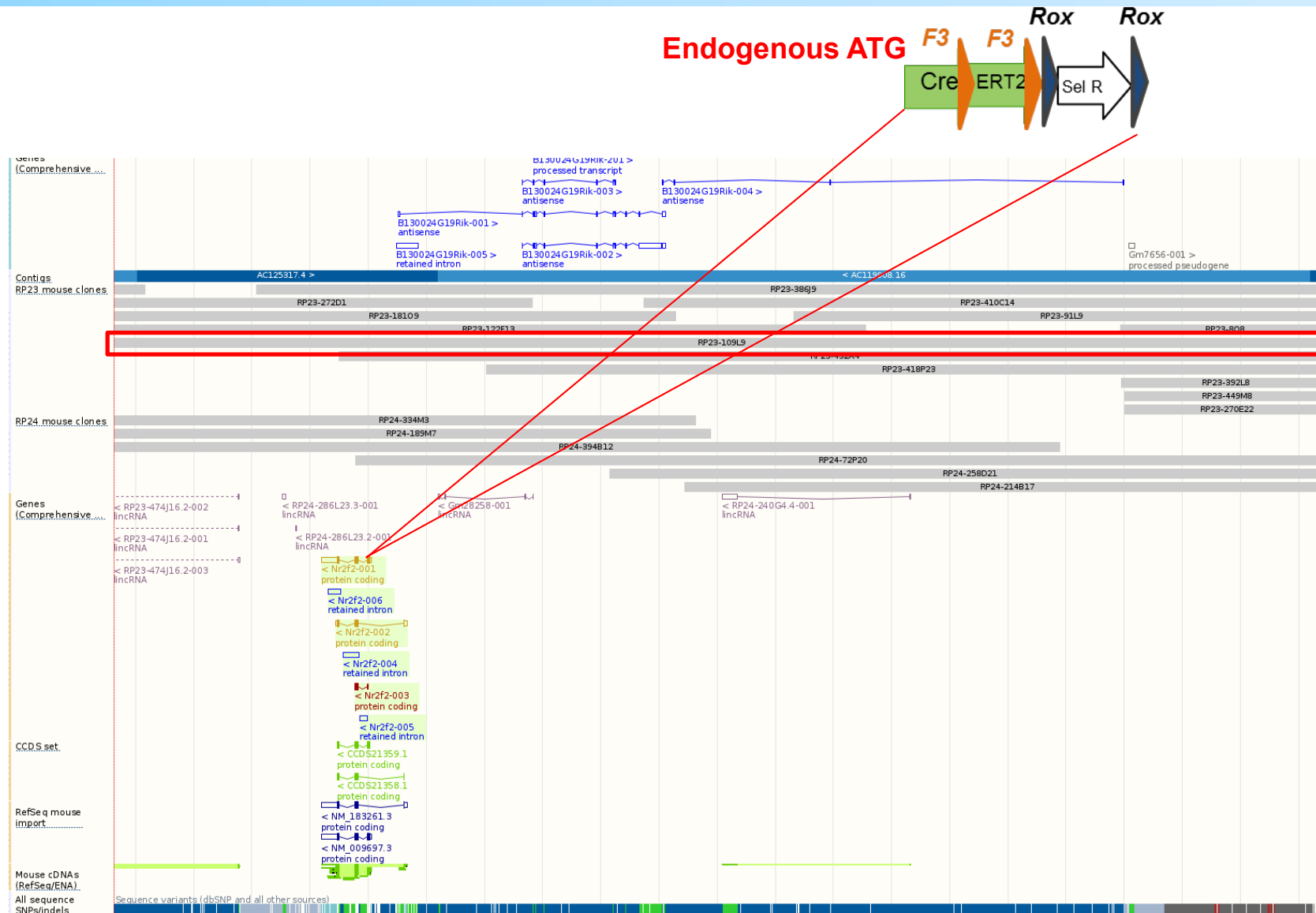
Gene Symbol	Gene name	Images	Aliases	BAC Address	Marker	Comment
<a href="#">Nr2f2</a>	nuclear receptor subfamily 2, group F, member 2	<a href="#">adult</a> <a href="#">P7</a> <a href="#">E15.5</a> <a href="#">All</a>	2700033K02Rik 9430015G03Rik Aporp1 ARP-1 COUP-TF2 COUP-TFII COUPTFB SVP40 Tfcoup2 <i>apolipoprotein regulatory protein 1</i>	<a href="#">RP23-109L9</a>	EGFP	Two BAC lines have very similar expression at P7. Our data is consistent with the literature and the in situ data. However, the choroid plexus and ventrolateral thalamic area are not stained in adult BAC mice. The absence of expression in choroid plexus contrasts with the literature, but is consistent with the in situ data. In addition, our BAC reveals extra expression sites in cortex and olfactory bulb. These sites are confirmed by the in situ data. The P7 in situ data detects stronger hybridization signals in thalamus.

ATG of isoform Nr2f2-001 ENSMUST00000032768 was targeted

# Nr2f2 mRNAs and proteins

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq
Nr2f2-001	<a href="#">ENSMUST00000032768</a>	4222	<a href="#">414aa</a>	Protein coding	<a href="#">CCDS21359</a>	<a href="#">P43135</a> <a href="#">Q3UST6</a>	<a href="#">NM_009697</a> <a href="#">NP_033827</a>
Nr2f2-002	<a href="#">ENSMUST00000089565</a>	1664	<a href="#">281aa</a>	Protein coding	<a href="#">CCDS21358</a>	<a href="#">D3YYP4</a>	<a href="#">NM_183261</a> <a href="#">NP_899084</a>
Nr2f2-003	<a href="#">ENSMUST00000208081</a>	636	<a href="#">170aa</a>	Protein coding	-	-	-
Nr2f2-004	<a href="#">ENSMUST00000208474</a>	2715	No protein	Retained intron	-	-	-
Nr2f2-006	<a href="#">ENSMUST00000207153</a>	2164	No protein	Retained intron	-	-	-
Nr2f2-005	<a href="#">ENSMUST00000208681</a>	1275	No protein	Retained intron	-	-	-

# Proposal : BAC transgenesis



# Proposal 1: BAC transgenesis

- Assembly of the targeting vector for homologous recombination in the BAC
- Validation of a well recombined BAC (2 in 1 cassette at the ATG of the Nr2f2 gene)
- Microinjection in C57BL/6N fertilized eggs
- Genotyping of potential founders
- Founder breeding to germ line transmission
- Global characterization of the line(s)

# Nr2f2 het KO has already a phenotype

See <http://www.informatics.jax.org/marker/MGI:1352452>

- Homozygotes for a targeted null mutation exhibit impaired angiogenesis and heart development with hemorrhagic brains and hearts, and die around embryonic day 10.
- About 5% of heterozygotes share the hemorrhagic phenotype at embryonic day 9.5.

=> A CreER<sup>T2</sup> KI is not advised



## Data



### 3 MICROINJECTION in C57BL/6N fertilized oocytes

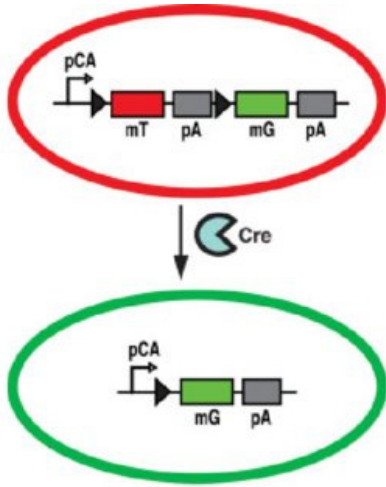


Concentration of modified BAC	Nb oocysts reimplanted	Nb pups born	Positive pups (=founders)
2 ng/ $\mu$ l	397	46	6 (1 to 4 copies detected)

- All 6 founders were bred in order to obtain germ line transmission.
- The line issued from founder #40 is described in this report.
- The characterization was done on the Cre line (after excision of the F3-ER<sup>T2</sup>-F3 cassette)

# Characterization of the C57BL/6N-Tg(Nr2f2-cre)40.ICS line (Ros6261-40 \*flp ): Cre expression under the Nr2f2 promoter

## mT/mG reporter line



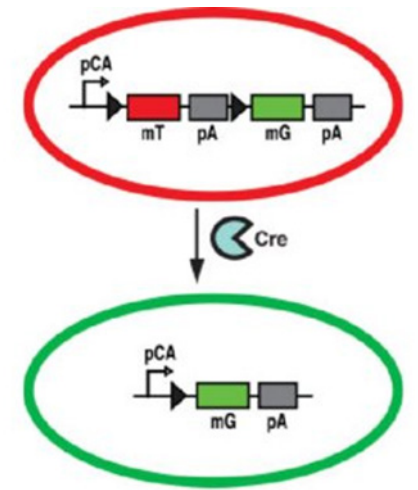
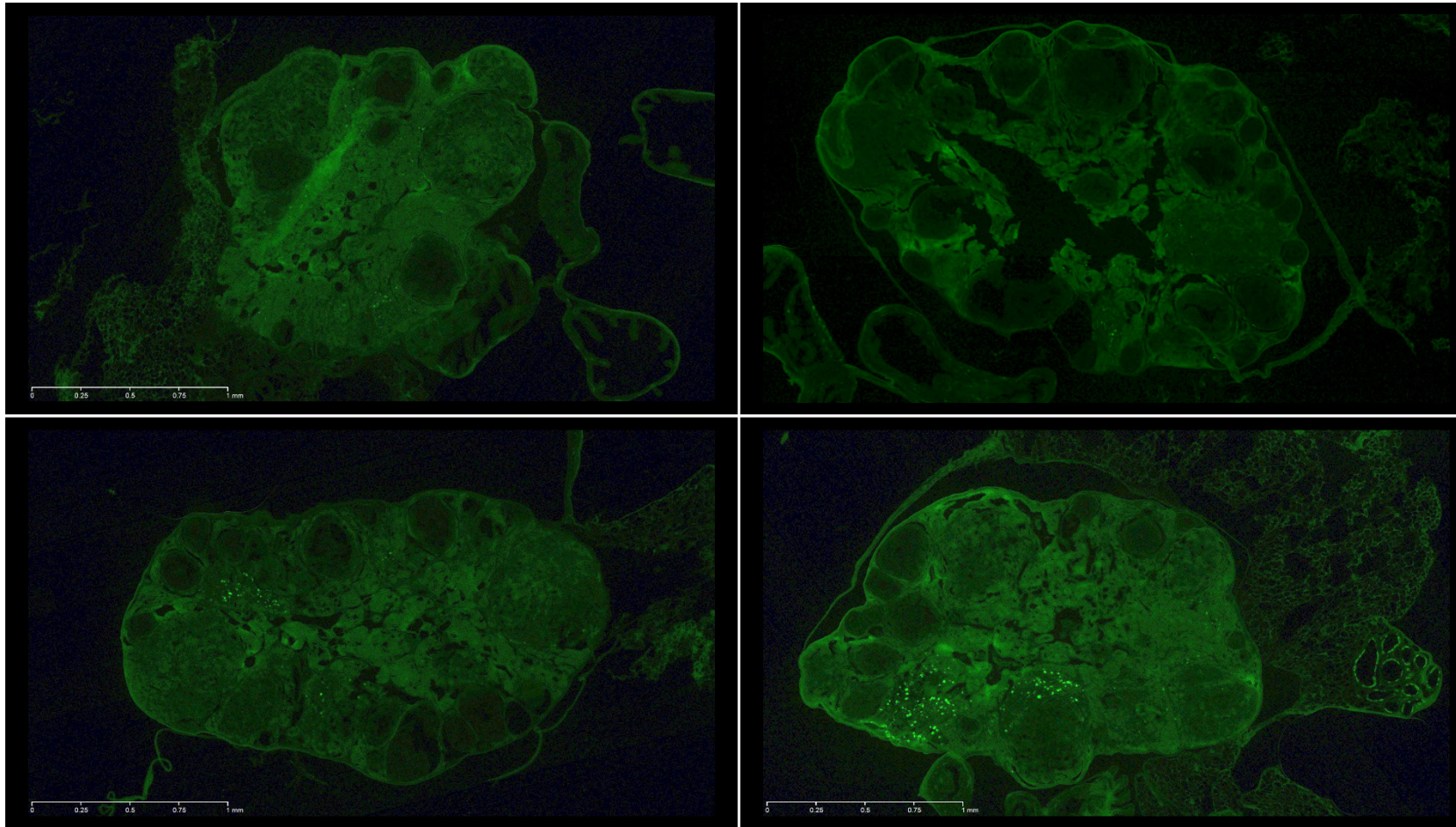
Histological characterization after breeding with the mT/mG reporter line  
Line Ras6261-40 \*flp \*mTmG

This nuclear receptor is an important transcriptional fate regulator in several systems (cardiovascular, pancreas, nervous system, reproduction, etc) and participate in mesenchymal-epithelial interactions required for organogenesis. What is remarkable, is that the gene is expressed in restricted expression patterns and in specific cell populations during early during development. We are very keen in using it for dorsal and ventral telencephalic fate mapping and conditional knock-outs.

By Michèle Studer (laureate of the PHENOMIN CreERT2 call)

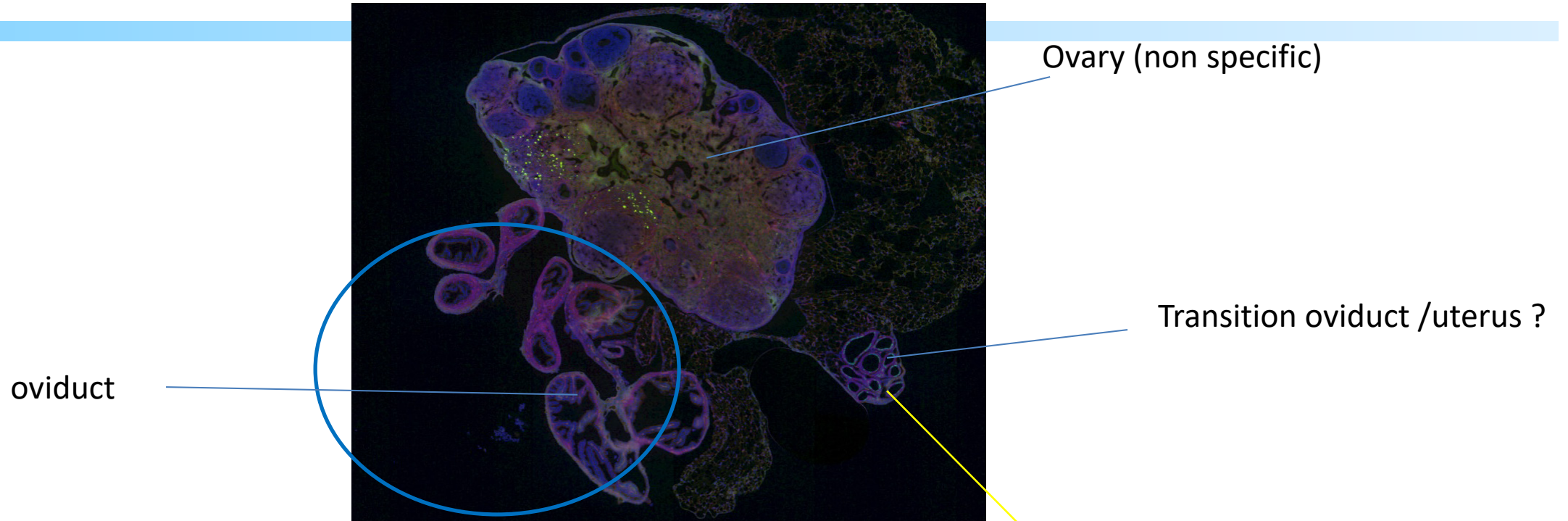
# Ovary

F21 contro WT; mT/mG

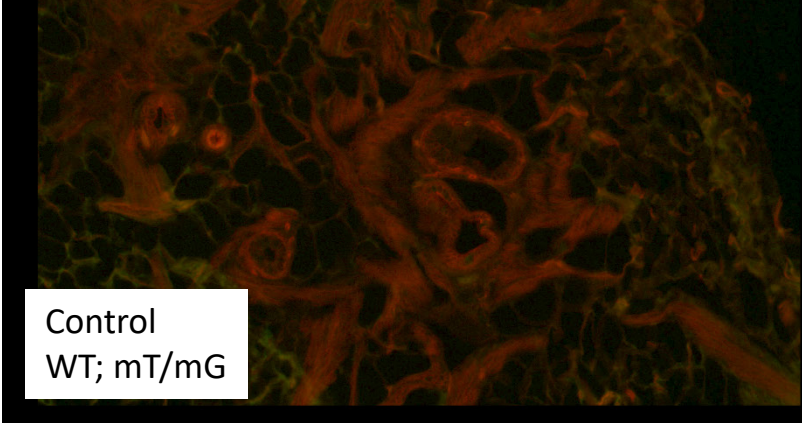


GFP fluorescence present in the stroma but also present in the control animal (= non specific)

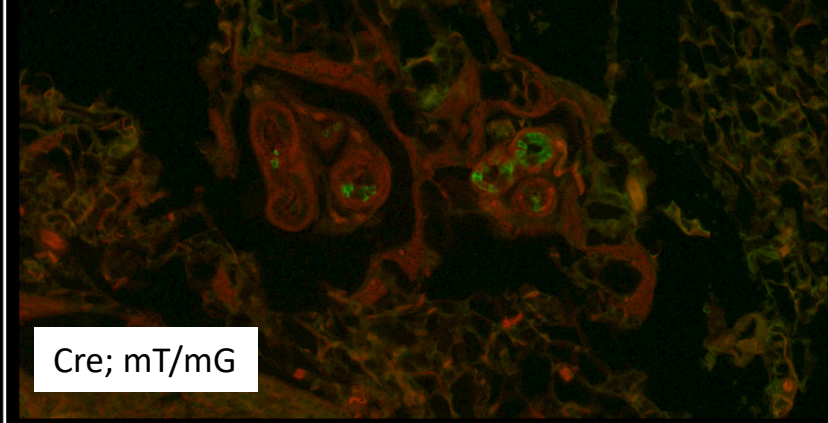
# Ovary



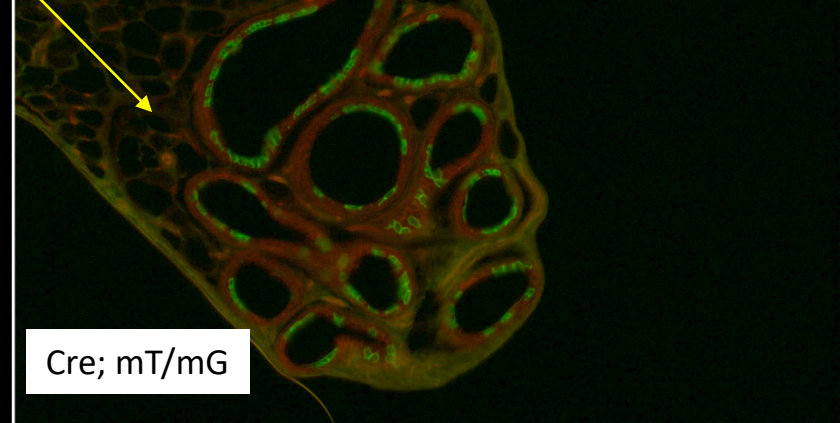
IP6796h\_F21\_wt\_I2 \_\_x20.jpg



IP6796h\_F19\_CREmtmg\_I2 x20\_.jpg



IP6796h\_F20\_CREmtmg\_I2 \_.jpg



Staining in a structure beside the ovary (could be the transition oviduct/uterus; no staining in the oviduct)

# Heart (X10)

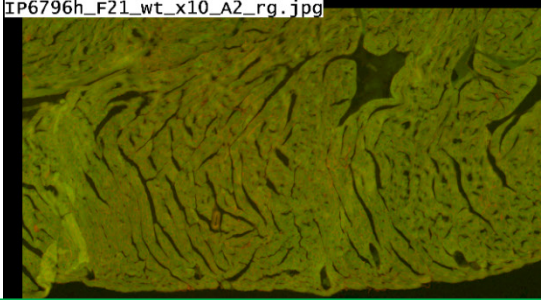
Mix fluo

mTomato

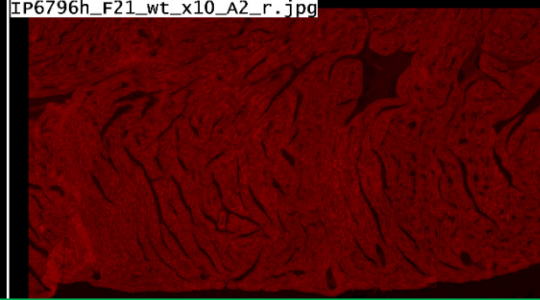
mGFP

Control  
WT; mT/mG

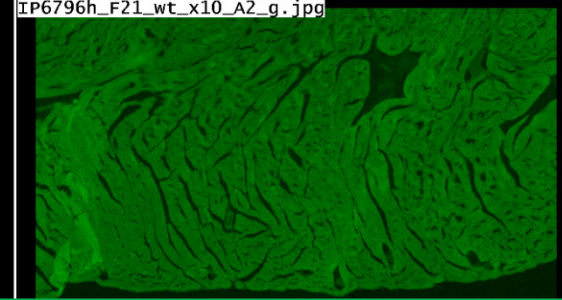
IP6796h\_F21\_wt\_x10\_A2\_rg.jpg



IP6796h\_F21\_wt\_x10\_A2\_r.jpg

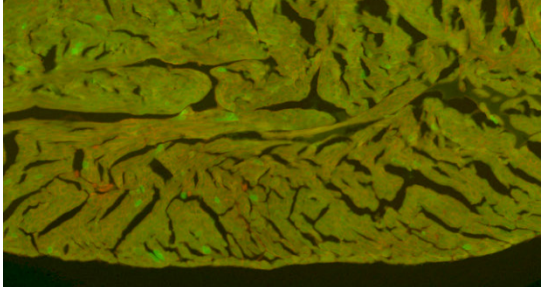


IP6796h\_F21\_wt\_x10\_A2\_g.jpg

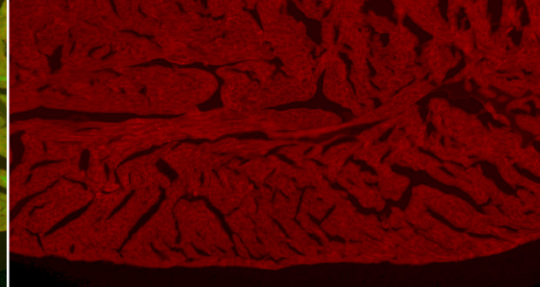


Nf2f2 Cre; mT/mG

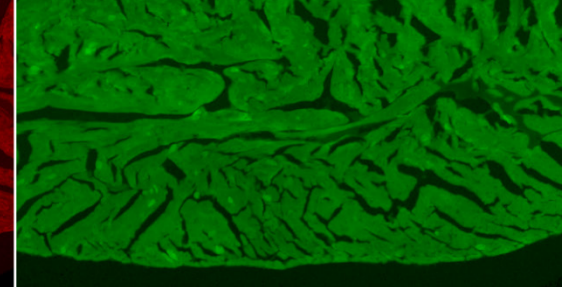
IP6796h\_F20\_CREmtg\_A2\_x10\_rg.jpg



IP6796h\_F20\_CREmtg\_A2\_x10\_r.jpg

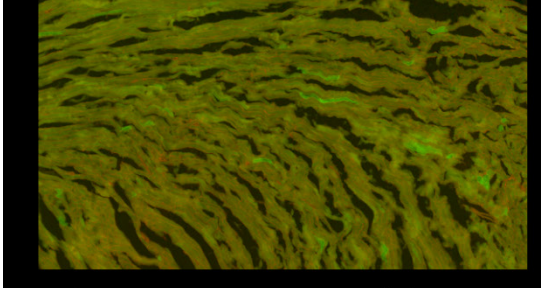


IP6796h\_F20\_CREmtg\_A2\_x10\_g.jpg

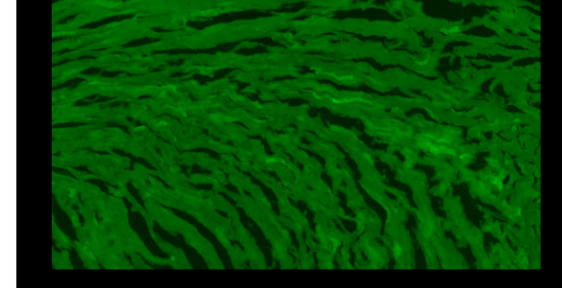


Nf2f2 Cre; mT/mG

IP6796h\_F19\_CREmtg\_A2\_x10\_gr.jpg

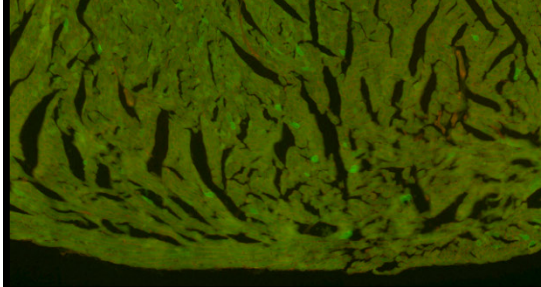


IP6796h\_F19\_CREmtg\_A2\_x10\_g.jpg

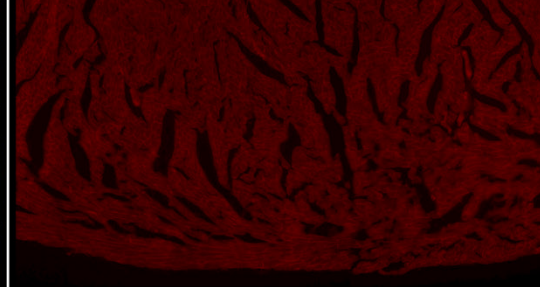


Nf2f2 Cre; mT/mG

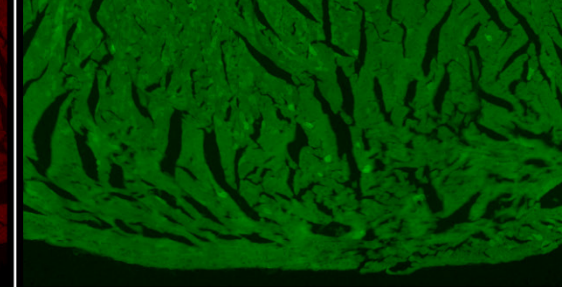
IP6796h\_F18\_CREmtg\_L1A2 \_rg\_x10.jpg



IP6796h\_F18\_CREmtg\_L1A2 \_r\_x10.jpg



IP6796h\_F18\_CREmtg\_L1A2 \_g\_x10.jpg

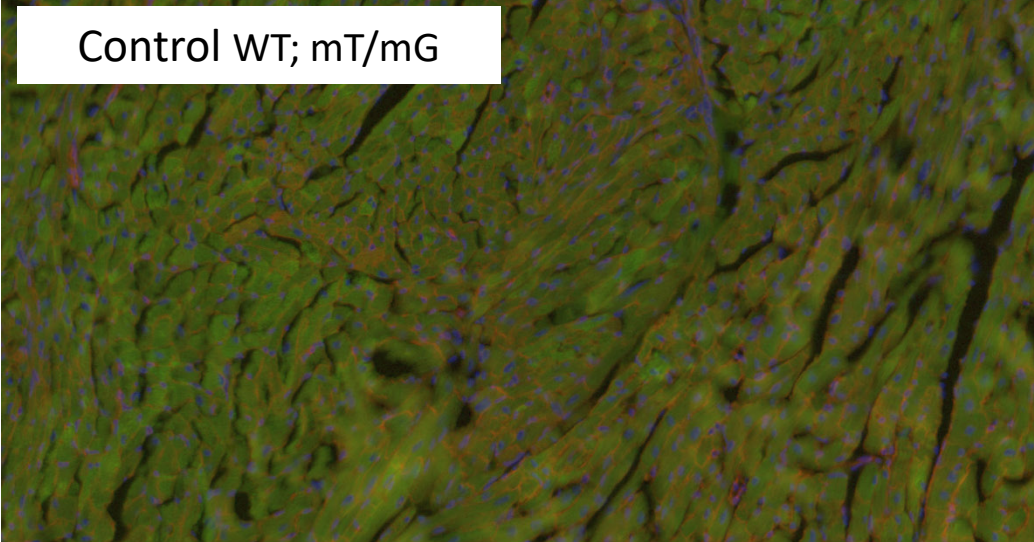


Staining in some  
cardiomyocytes  
(for more details,  
see next slide)

# Heart (X20)

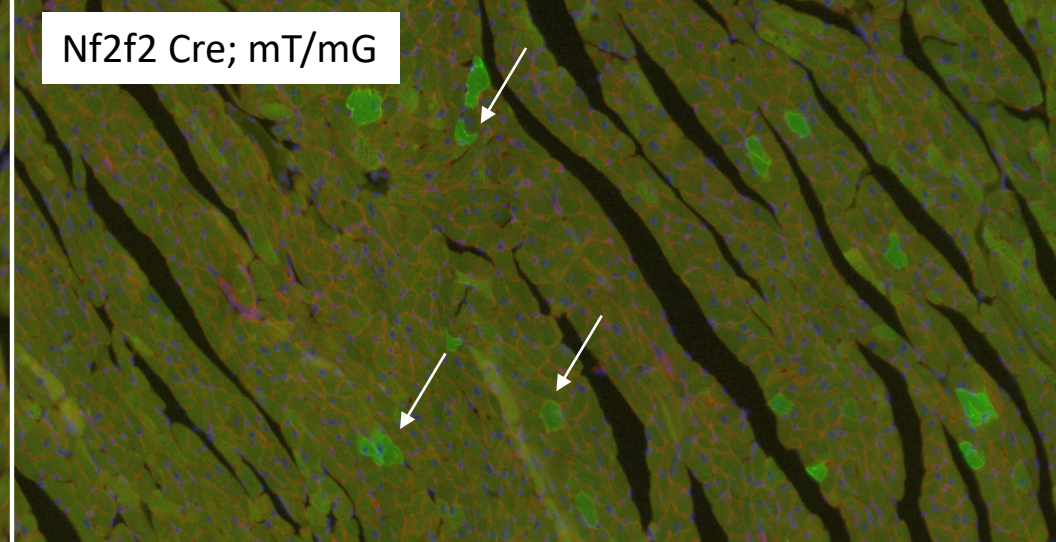
IP6796h\_F21\_wt\_A2\_x20.jpg

Control WT; mT/mG



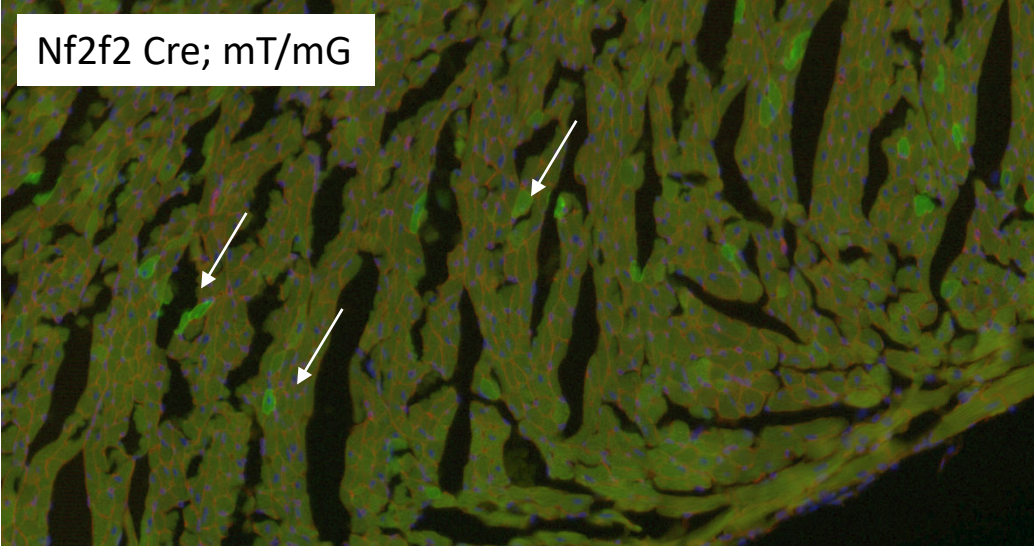
IP6796h\_F18\_CREmtmg\_A2\_x20.jpg

Nf2f2 Cre; mT/mG



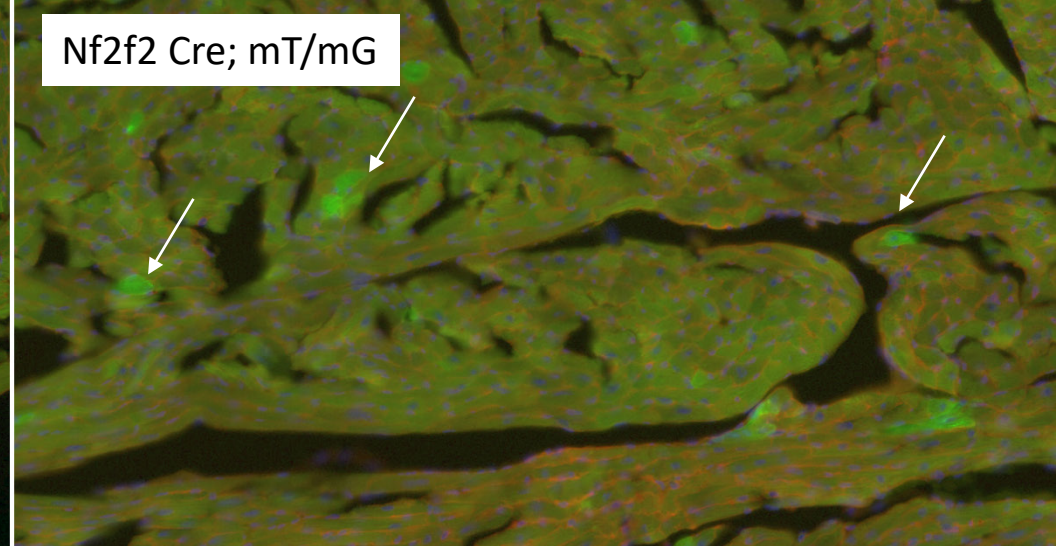
IP6796h\_F19\_CREmtmg\_A2\_x20.jpg

Nf2f2 Cre; mT/mG



IP6796h\_F20\_CREmtmg\_A2\_x20.jpg

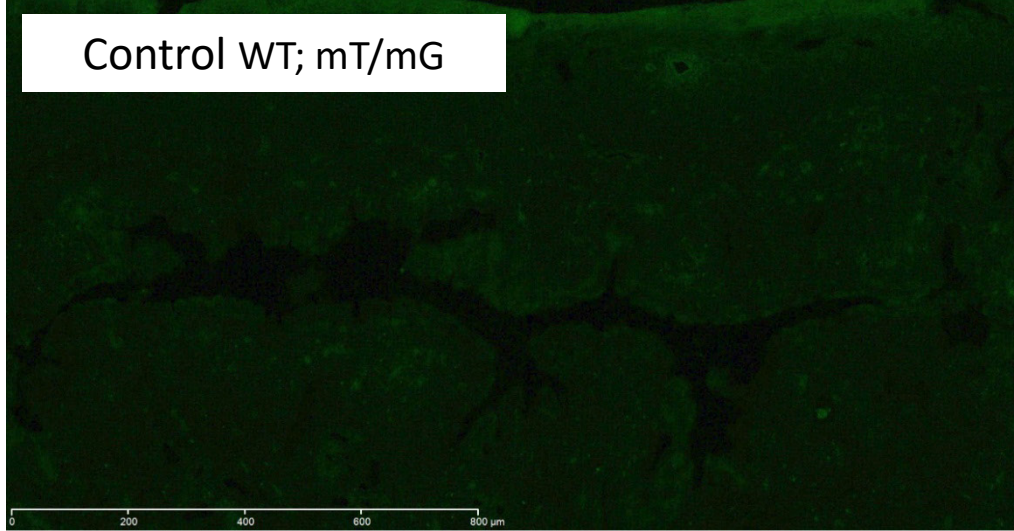
Nf2f2 Cre; mT/mG



# Uterus

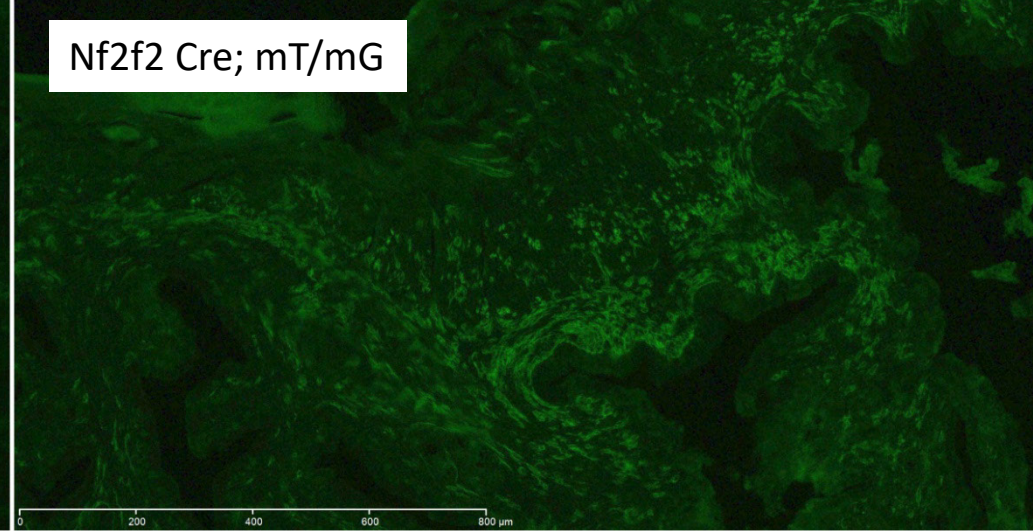
IP6796h\_F21\_wt\_x10\_I3.jpg

Control WT; mT/mG



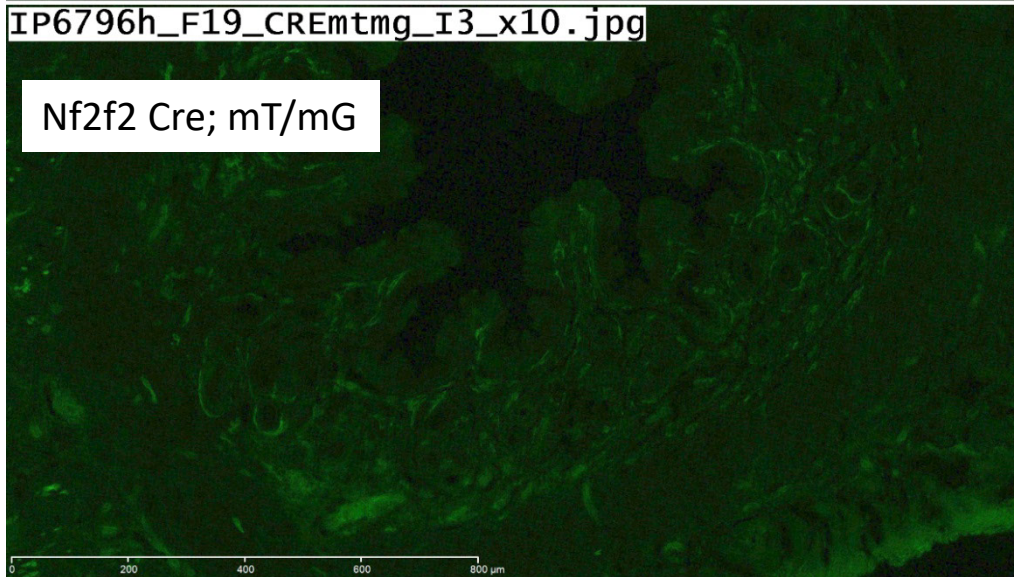
IP6796h\_F18\_CREmtmg\_I3.jpg

Nf2f2 Cre; mT/mG



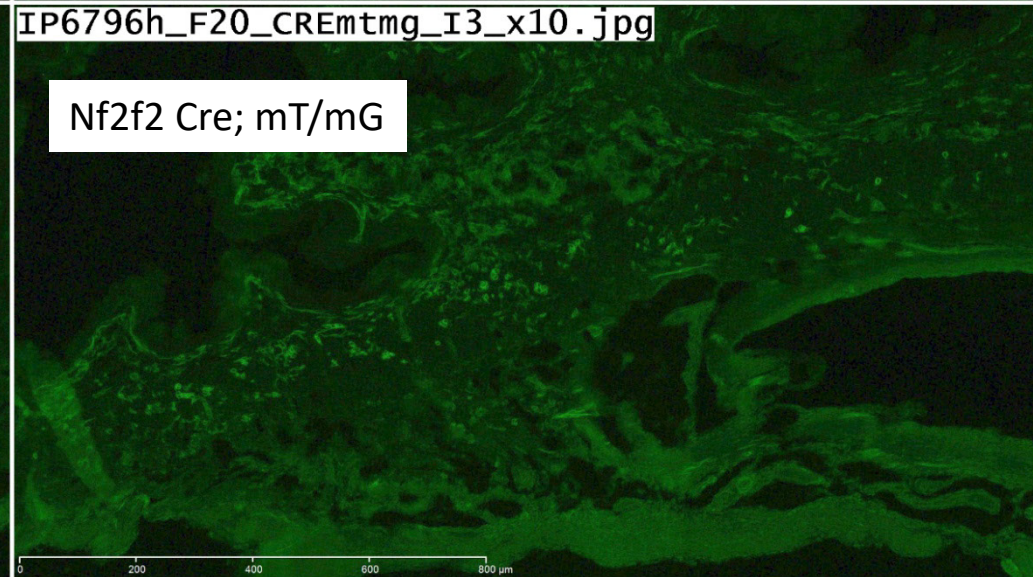
IP6796h\_F19\_CREmtmg\_I3\_x10.jpg

Nf2f2 Cre; mT/mG



IP6796h\_F20\_CREmtmg\_I3\_x10.jpg

Nf2f2 Cre; mT/mG



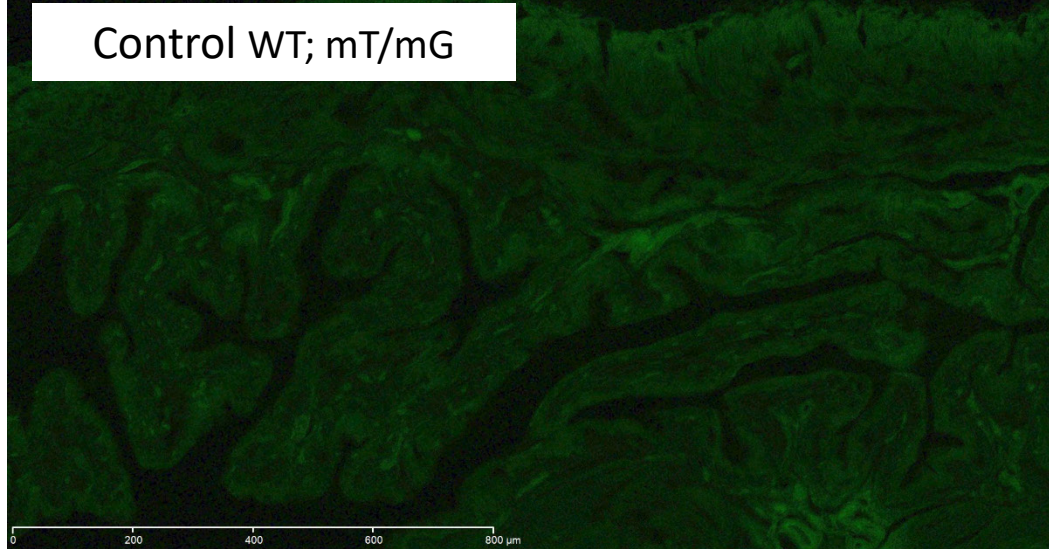
Staining in the smooth muscle of the uterus



# Bladder

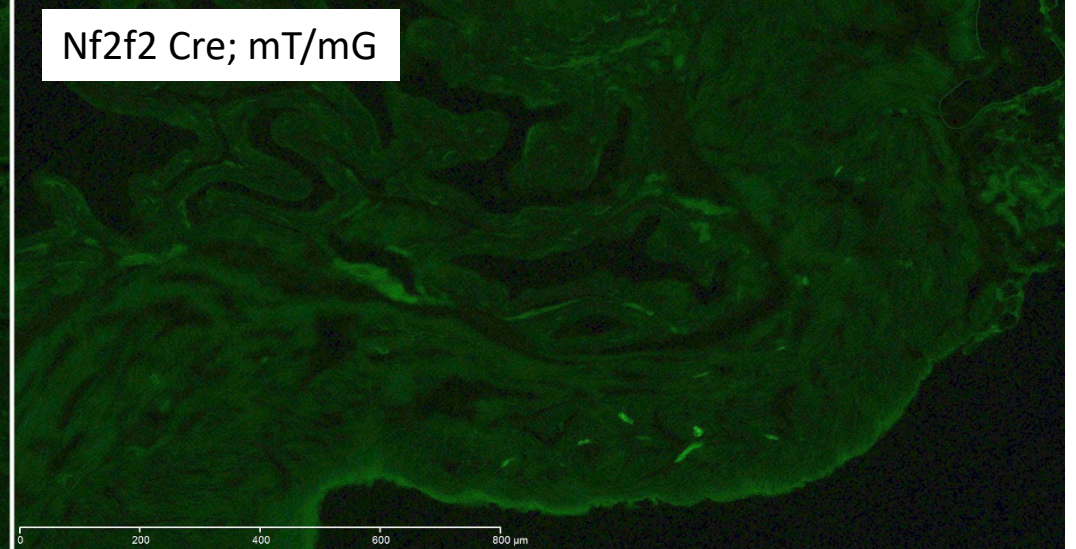
IP6796h\_F21\_wt\_x10\_L2.jpg

Control WT; mT/mG



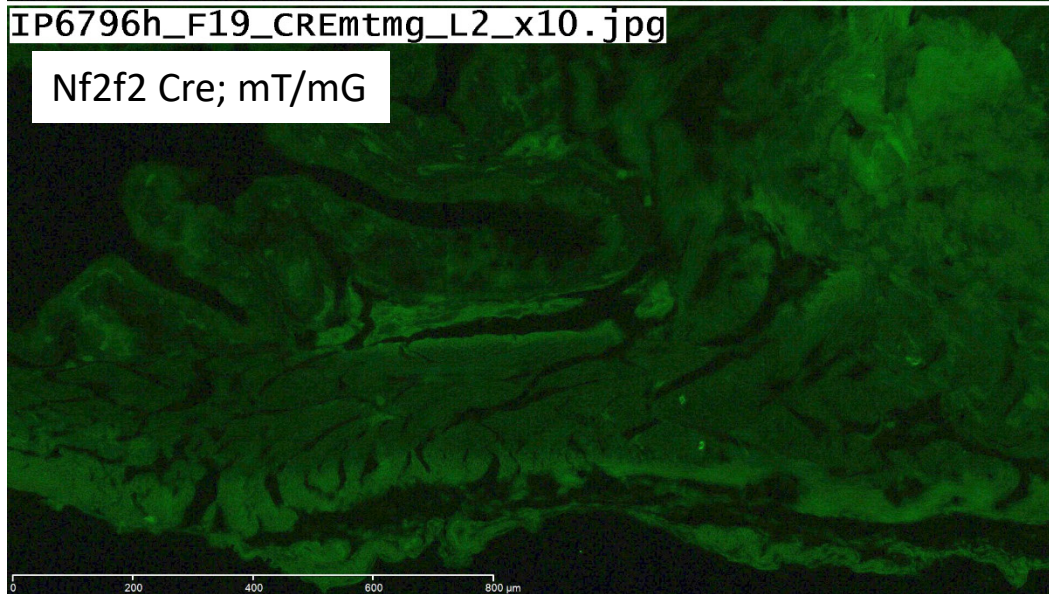
IP6796h\_F18\_CREmtmg\_L2\_x10.jpg

Nf2f2 Cre; mT/mG



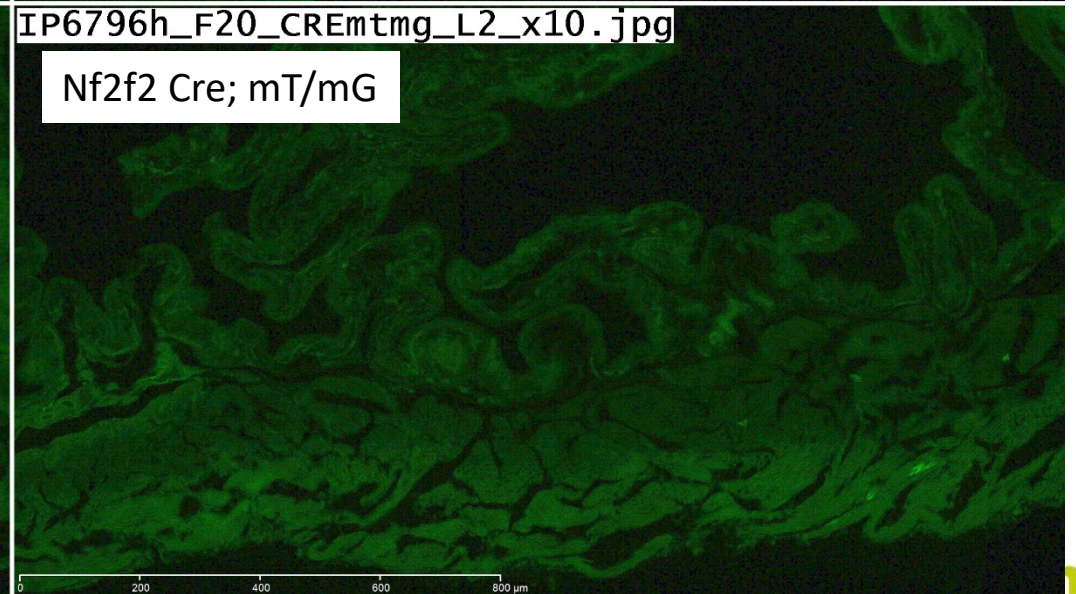
IP6796h\_F19\_CREmtmg\_L2\_x10.jpg

Nf2f2 Cre; mT/mG



IP6796h\_F20\_CREmtmg\_L2\_x10.jpg

Nf2f2 Cre; mT/mG

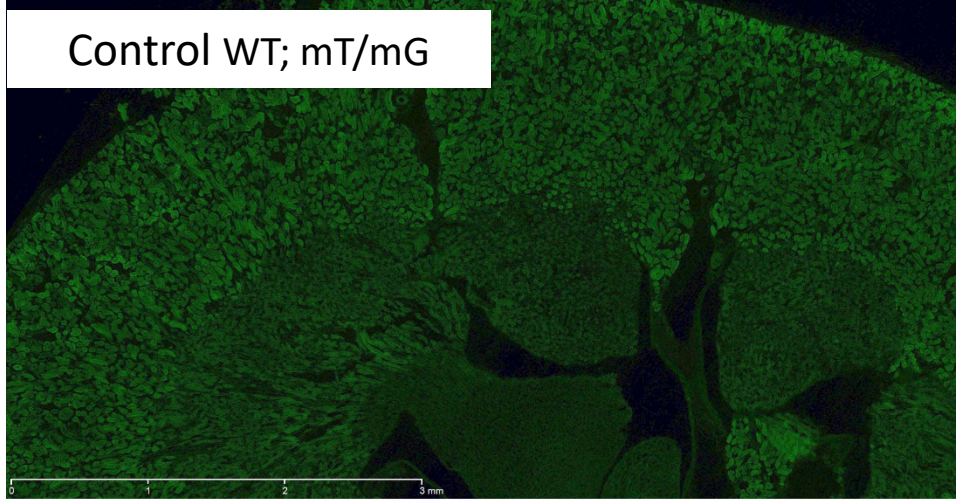


Very few cells stained

# Kidney

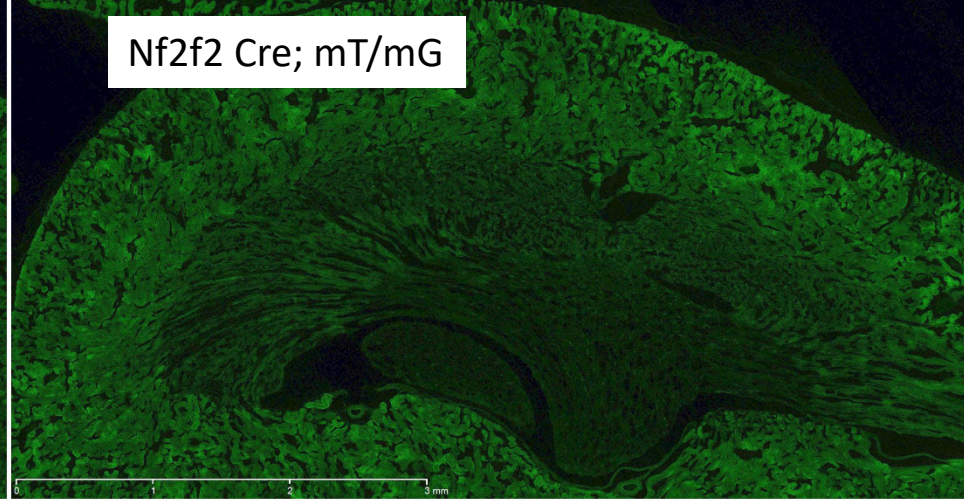
IP6796h\_F21\_wt\_x2\_50\_L1.jpg

Control WT; mT/mG



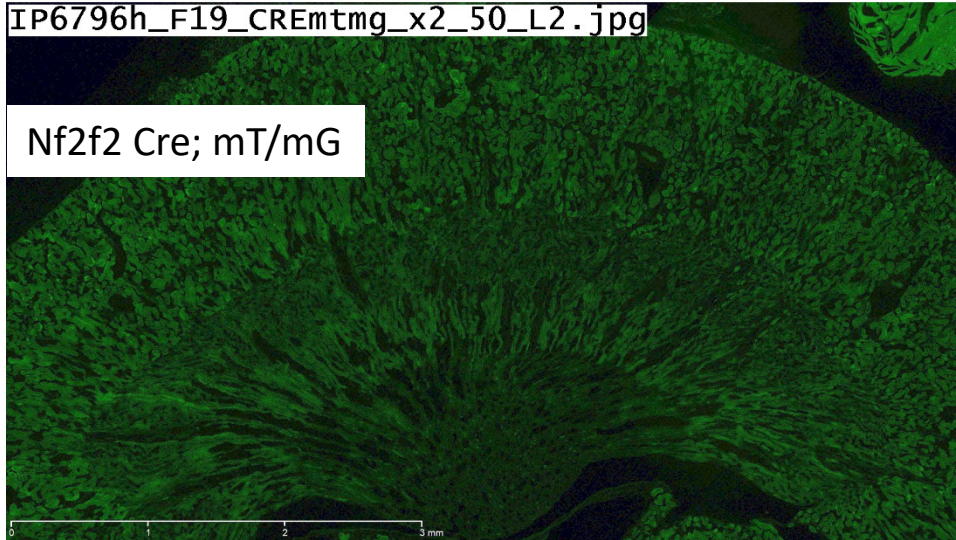
IP6796h\_F18\_CREmtmg\_x2\_50\_L2.jpg

Nf2f2 Cre; mT/mG



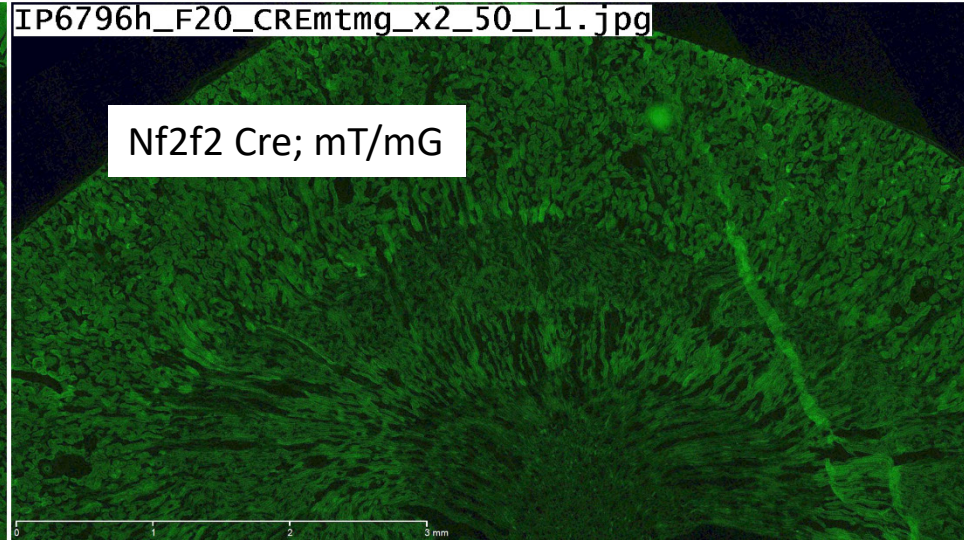
IP6796h\_F19\_CREmtmg\_x2\_50\_L2.jpg

Nf2f2 Cre; mT/mG



IP6796h\_F20\_CREmtmg\_x2\_50\_L1.jpg

Nf2f2 Cre; mT/mG



Too much background in the control.



## REPORT REDACTION & VALIDATION

Report finalized on 2023/05/16

by Marie-Christine BIRLING, PhD

## CONTACT US

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