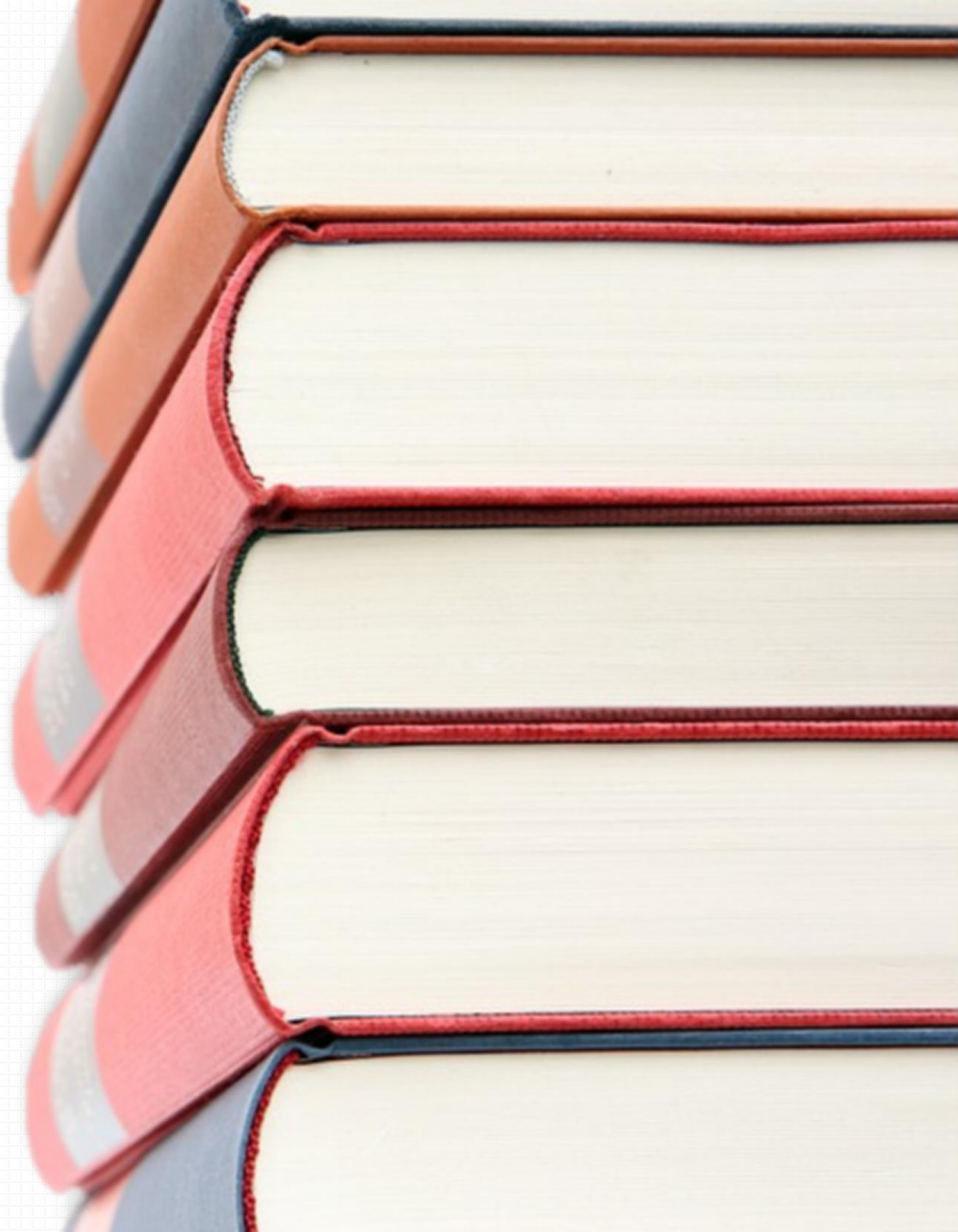
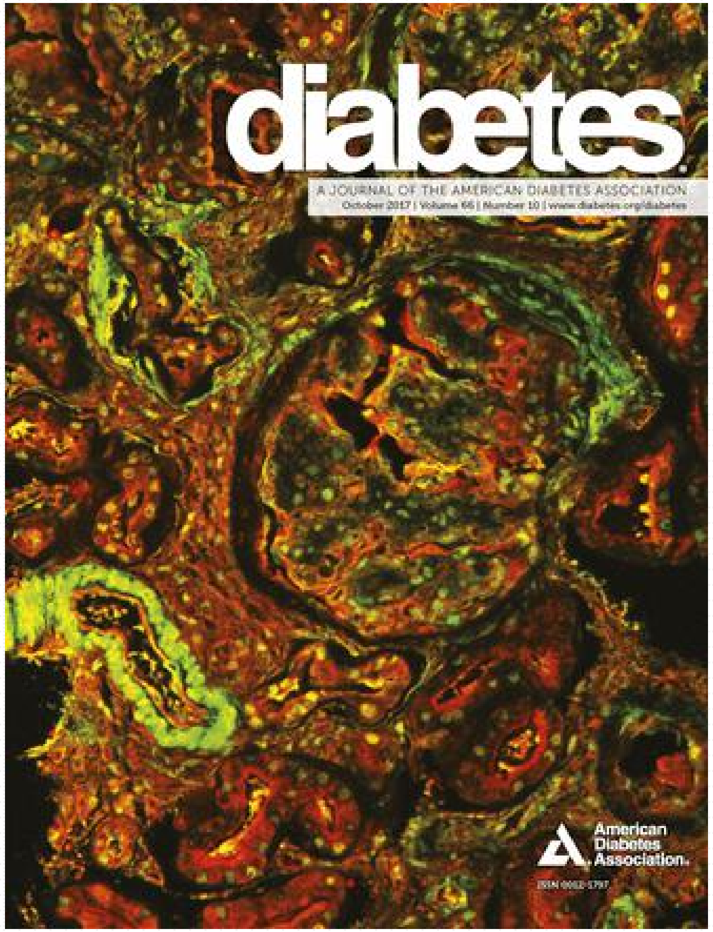
读书报告

■ 汇报时间：2018年8月18日

■ 汇报人：赵文丽





Thyroid Hormone Coordinates Pancreatic Islet Maturation During the Zebrafish Larval-to-Juvenile Transition to Maintain Glucose Homeostasis

Hiroki Matsuda[1], Sri Teja Mullapudi[1], Yuxi Zhang[2], Daniel Hesselson[2,3] and Didier Y.R. Stainier[1]

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Diabetes 2017 Oct; 66(10): 2623-2635.
https://doi.org/10.2337/db16-1476

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在斑马鱼仔鱼向幼鱼过渡期间甲状腺激素协调胰岛成熟以维持葡萄糖稳态

IF=7.273

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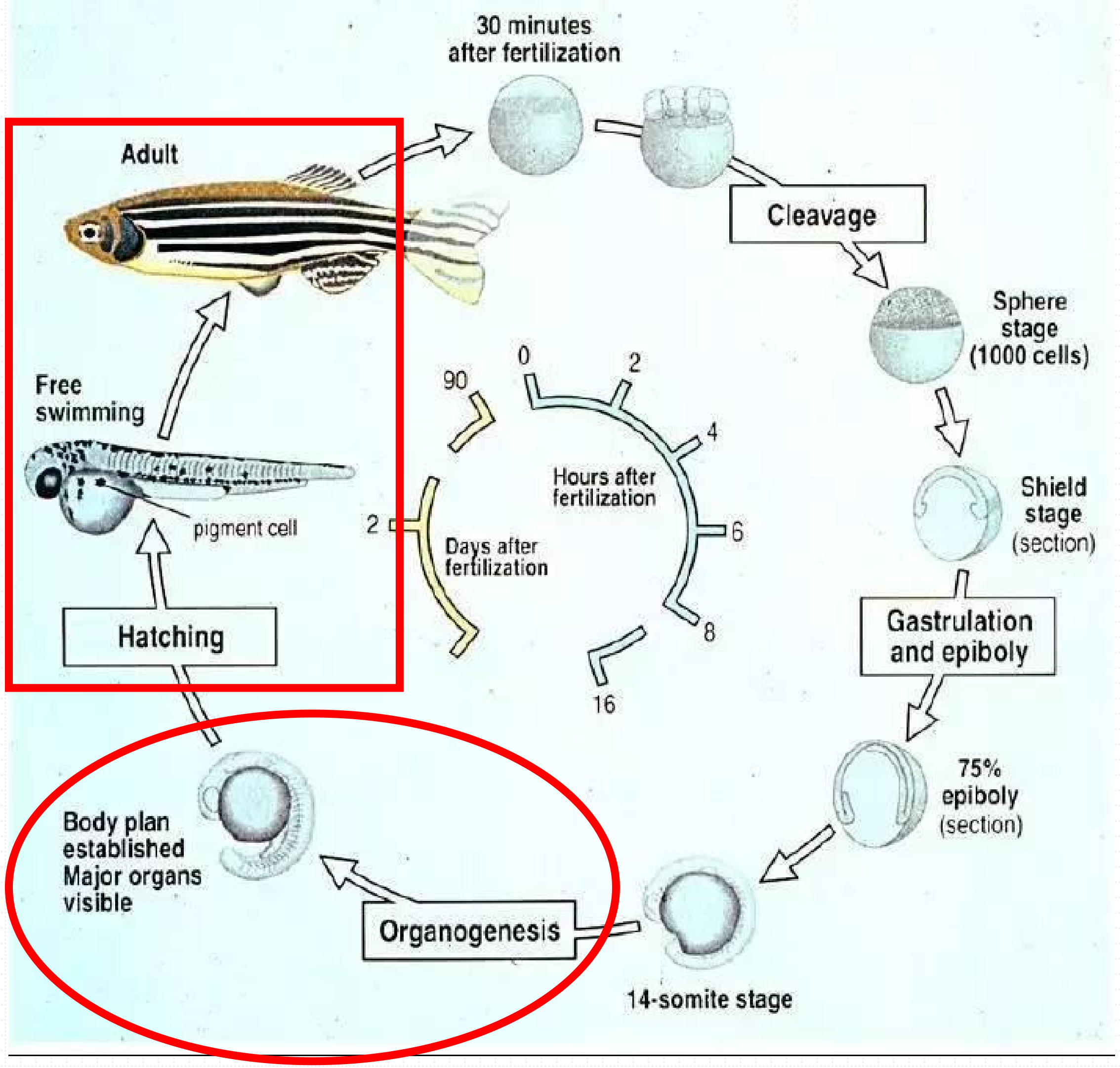
PART ONE

Introduction

PART ONE Introduction

Organ development : the formation of a functionally immature organ during embryogenesis followed by maturation into the adult form. This second step most often takes place during the postembryonic/postnatal (胚后期/出生后) developmental period, when plasma thyroid hormone (TH) concentrations are high. these major developmental changes occur during the **suckling to weaning transition(婴儿断奶过渡)**.

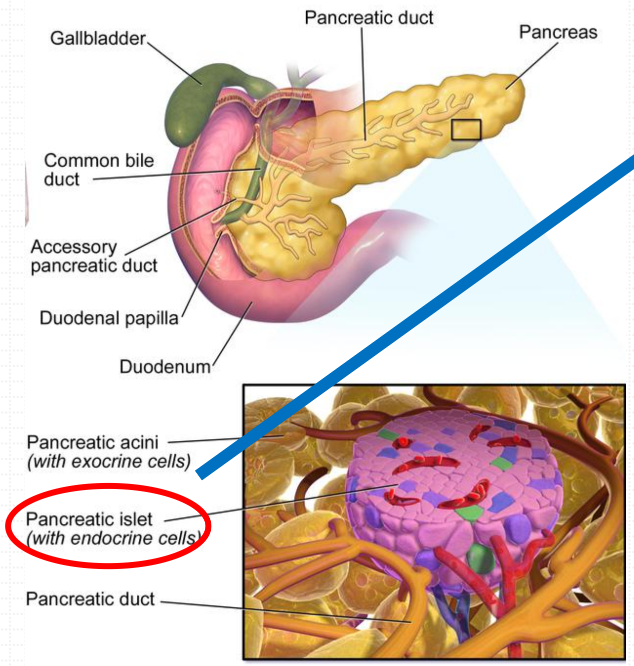
In zebrafish, these major developmental changes occur during the **larval-juvenile transition（仔鱼—幼鱼过渡）**, and their digestive organs undergo functional and morphological changes during this period.



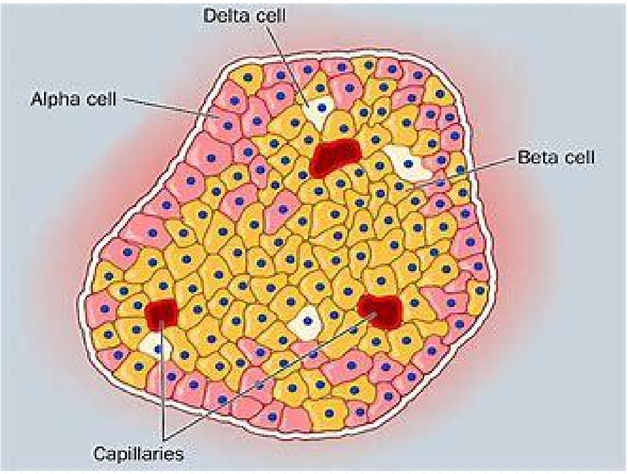
PART ONE Introduction

The **pancreas(胰腺)** is an organ in the digestive system that undergoes morphological and functional changes during the suckling to weaning transition.

In the endocrine pancreas, **β cell** mass increases (8), and more organized, functional islets appear during this period (8-13) as β cells acquire the ability to secrete insulin in response to glucose around the second week after birth (9).



Pancreatic Tissue



Hormones produced in the pancreatic islets are secreted directly into the blood flow by (at least) five types of cells.

- Alpha cells producing glucagon (20% of total islet cells)
- Betacells producing insulin and amylin (≈70 %)

PART ONE Introduction

thyroid hormone **T3** （甲状腺激素）signals through the nuclear **THR**(甲状腺激素受体). It has been reported that ligand bound THR can induce glucose-responsive insulin secretion in the rat pancreas .

It has therefore been hypothesized that TH regulates multiple aspects of pancreas development, including endocrine cell differentiation(内分泌细胞分化), β cell maturation(**β细胞成熟**), and the size of the exocrine compartment(外分泌区室的大小). However, it remains unclear exactly when and where TH signaling is activated during pancreas development.（**然而，胰腺发育过程中TH信号如何作用目前尚不清楚**）

PART ONE Introduction

- The zebrafish (*Danio rerio*) （斑马鱼）has emerged as a powerful model to study mechanisms of pancreas development and β cell differentiation. （**胰腺发育和β细胞分化机制的有力模型**）

研究目的和内容：

发育转变改变了生物体的代谢需求，而发育和代谢需求是如何协调的尚不清楚，本研究以斑马鱼作为模型， 在**larval-juvenile**过渡时期，测试**TH**是否和如何影响胰岛素和胰高血糖素的表达，进而影响葡萄糖稳态。

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PART TWO

Research Design and Methods

甲状腺激素

- 甲状腺激素对胰岛素和胰高血糖素的影响
- 对insulin、gcga、gcgb上游转录因子的影响
- 胰腺α cell和β cell是否是T3的靶器官
- larval-juvenile过渡期间对胰岛素和胰高血糖素的影响
- 在成熟时期，对胰岛素和胰高血糖素的影响

PART TWO Research Design and Methods

- 甲状腺激素对**胰岛素**和**胰高血糖素**的影响
  - 甲状腺激素T3对胰岛素启动子活性的影响和血糖水平的变化。
    - T3对胰岛素启动子的活性的影响
    - 试剂盒检测T3处理后，血糖水平的变化
  - T3对胰岛素表达和β cell 的影响
    - QPCR测定insulin的表达
    - 原位杂交检测TH是否改变内源insulin的表达模式
    - *ins:eGFP* reporter fish检测β cell的细胞群数目变化
  - T3对胰高血糖素和α cell 的影响
    - QPCR测定gcga和gcgb的表达
    - 原位杂交检测TH是否改变内源gcga、gcgb的表达模式
    - *gcga:eGFP* reporter fish检测β cell的细胞群数目变化
    - 用ins突变体斑马鱼，QPCR检测gcga和gcgb的表达水平
      - 排除insulin的旁分泌对胰高血糖素分泌的刺激

PART TWO Research Design and Methods

T3对insulin、gcga、gcgb上游转录因子基因(*pax6b,neurod1,mnx1* , *pax4* , *arxa*)的影响

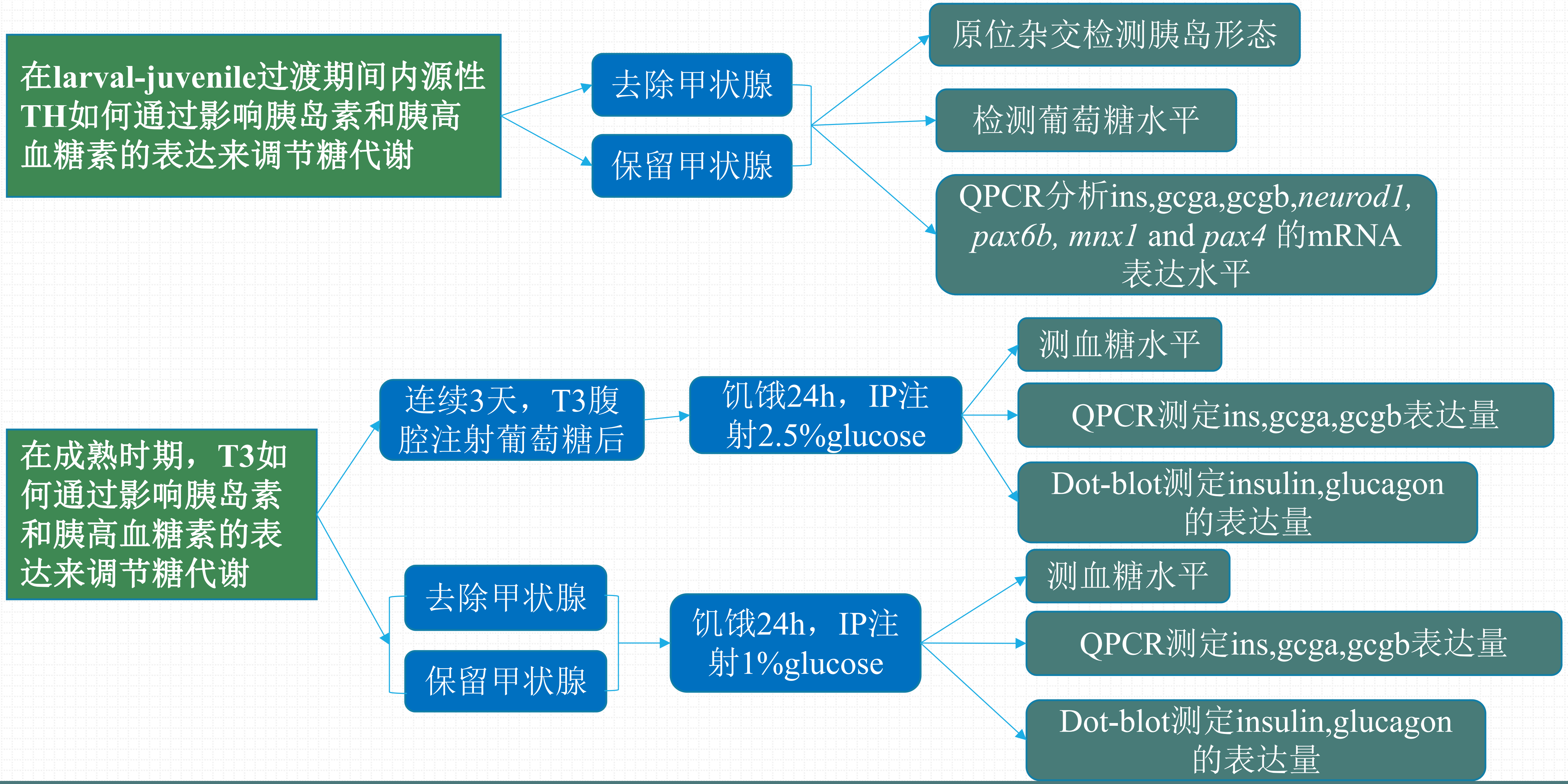
- *pax6b***:*eGFP,*** *neurod1***:*eGFP,*** ,*mnx1***:*eGFP,*转基因斑马鱼，检测三种因子的表达量**
- **QPCR测定*pax6b,neurod1,mnx1* , *pax4* , *arxa*的mRNA表达量**

α cell和β cell是否是T3作用的靶器官

可视化T3的目标组织

- **构建T3响应的转基因斑马鱼**
- **免疫荧光和荧光成像检测*eGFP*，*insulin*，*Glucagon*的阳性细胞**

PART TWO Research Design and Methods



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PART THREE

Results and Discussion

PART THREE Results and Discussion

甲状腺激素

- 甲状腺激素对胰岛素和胰高血糖素的影响
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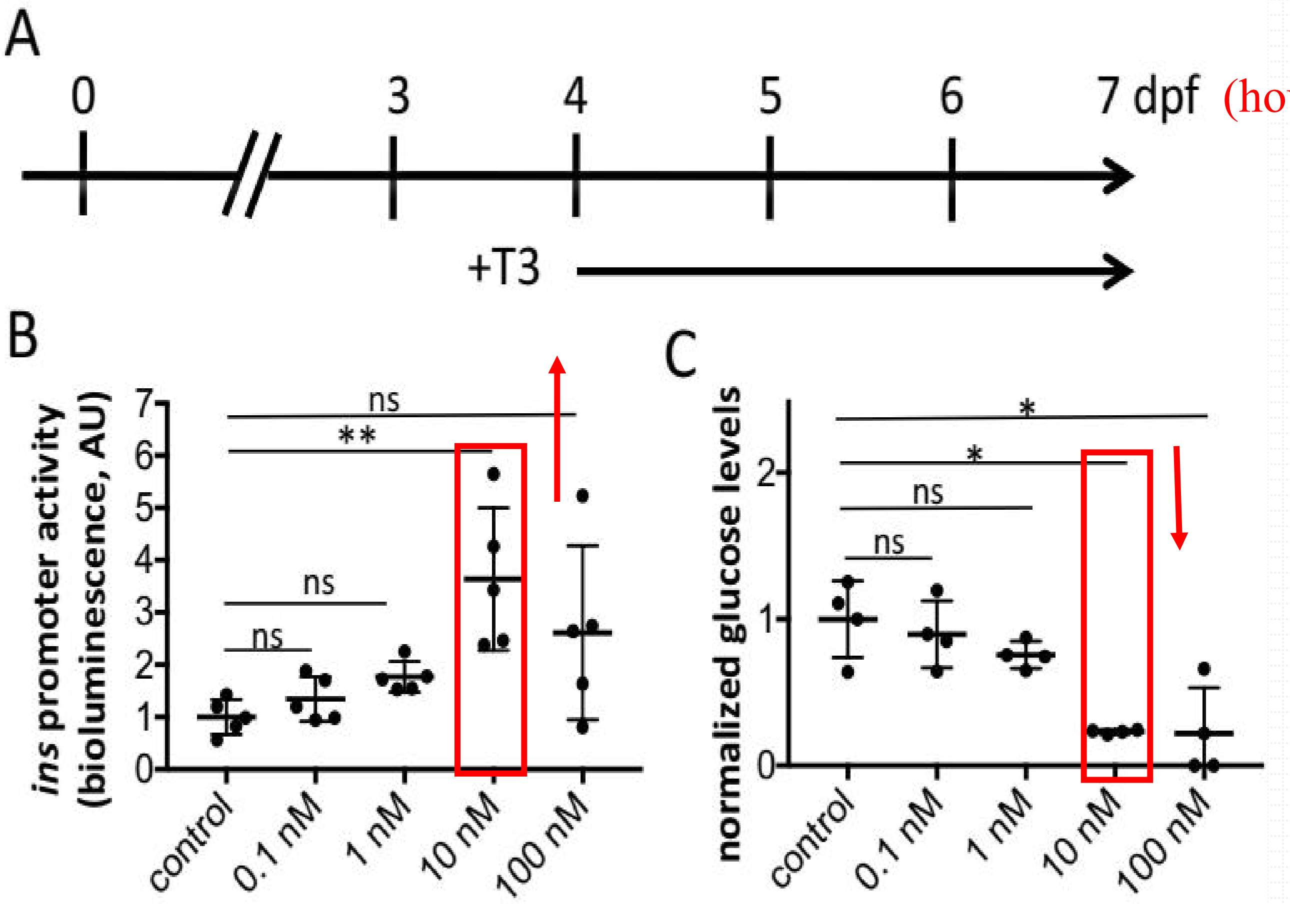
甲状腺激素T3对胰岛素启动子活性的影响和血糖水平的变化



(hours post fertilization 受精后的天数)

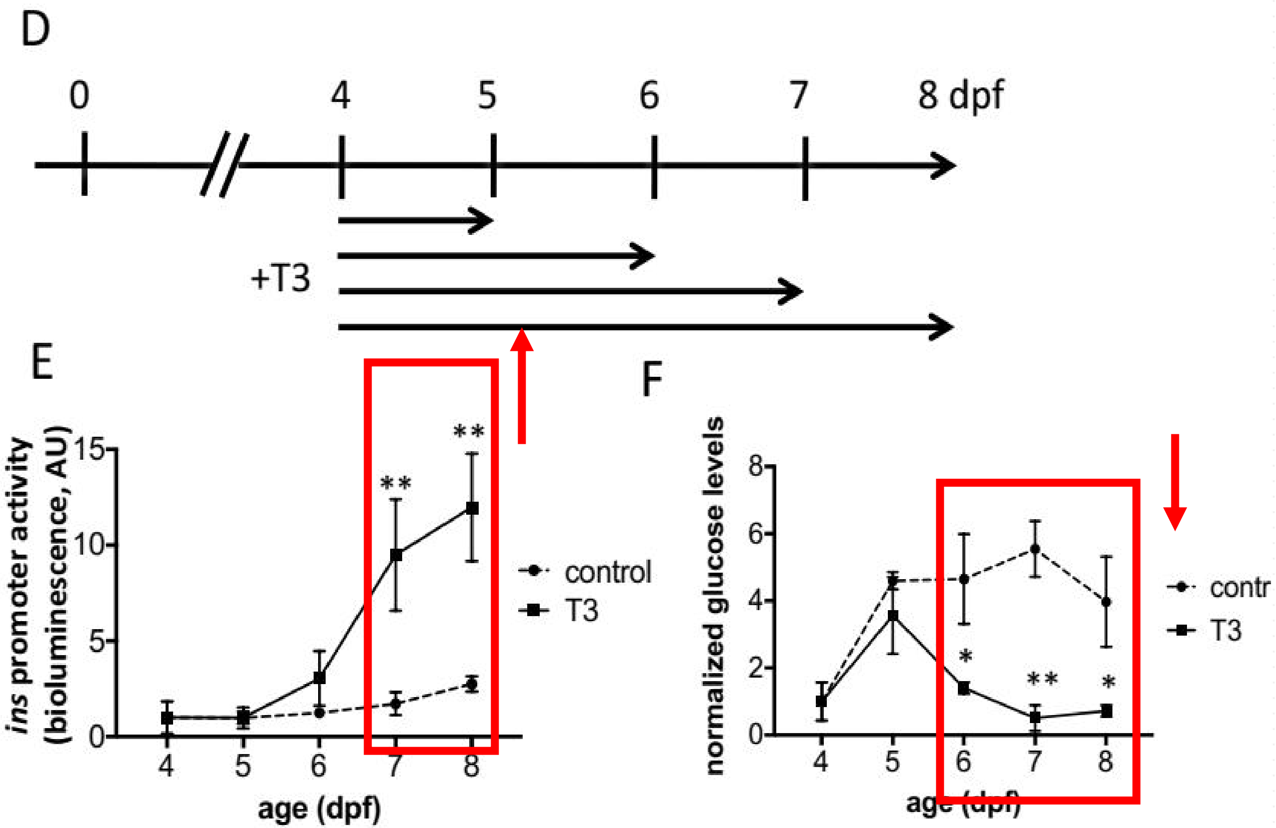
胰岛素启动子的活性

葡萄糖水平

10 nM T3 was the optimal concentration for activation of *insulin* expression and glucose reduction.

PART THREE Results and Discussion

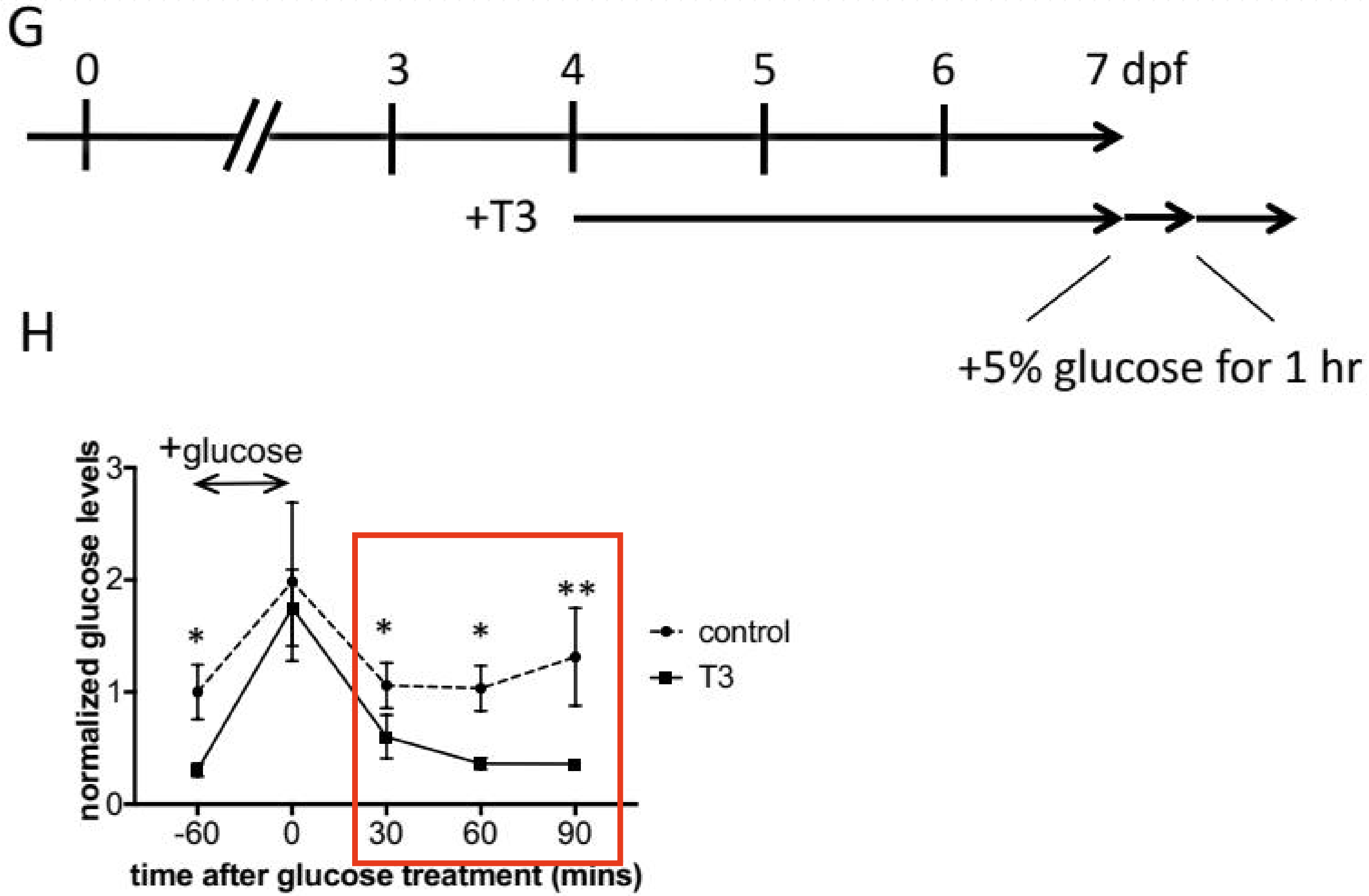
T3在什么时候对胰岛素启动子的活性起作用呢？



These results indicate that *insulin* expression is T3 responsive by 7 dpf and that our 4 to 7 dpf treatment conditions induce a hypoglycemic phenot（低血糖）.

PART THREE Results and Discussion

T3处理后对仔鱼进行葡萄糖耐量实验研究T3对仔鱼葡萄糖清除率的影响。



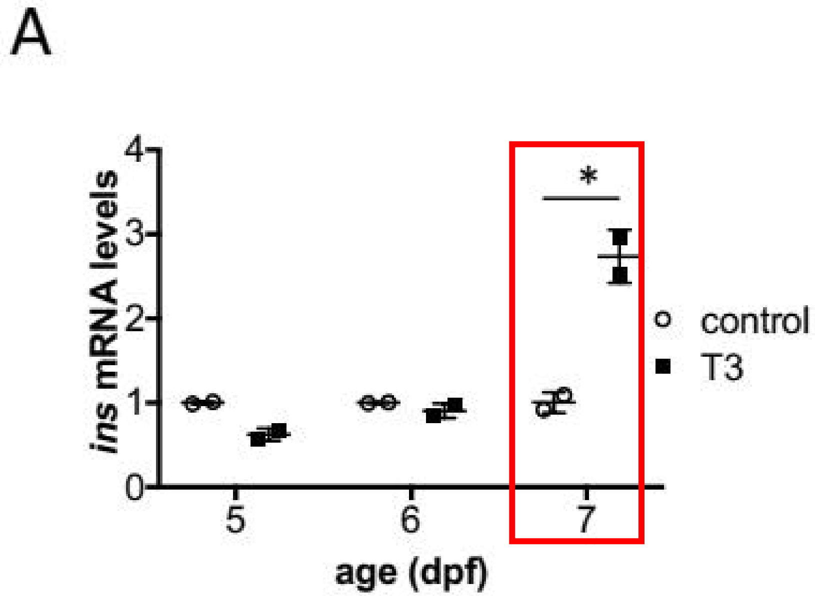
These data indicate that T3 increases the glucose disposal capacity of zebrafish larvae.

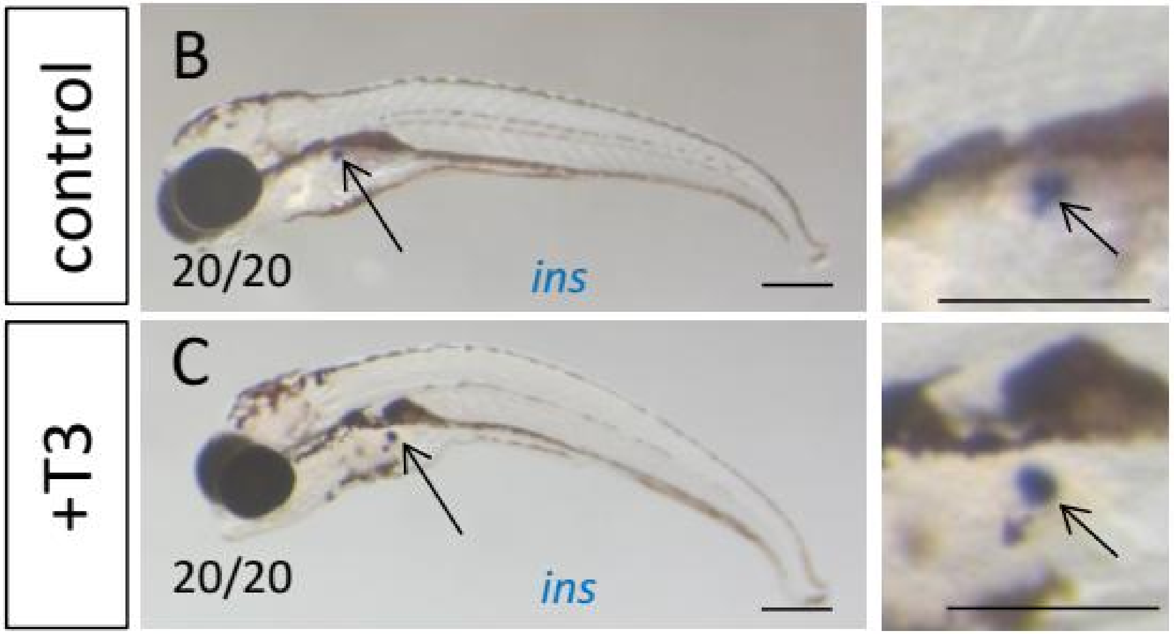
Brief summary 1

T3 stimulates insulin promoter activity, reduces glucose levels and improves glucose tolerance.

PART THREE Results and Discussion

T3对胰岛素表达和β cell的影响





原位杂交

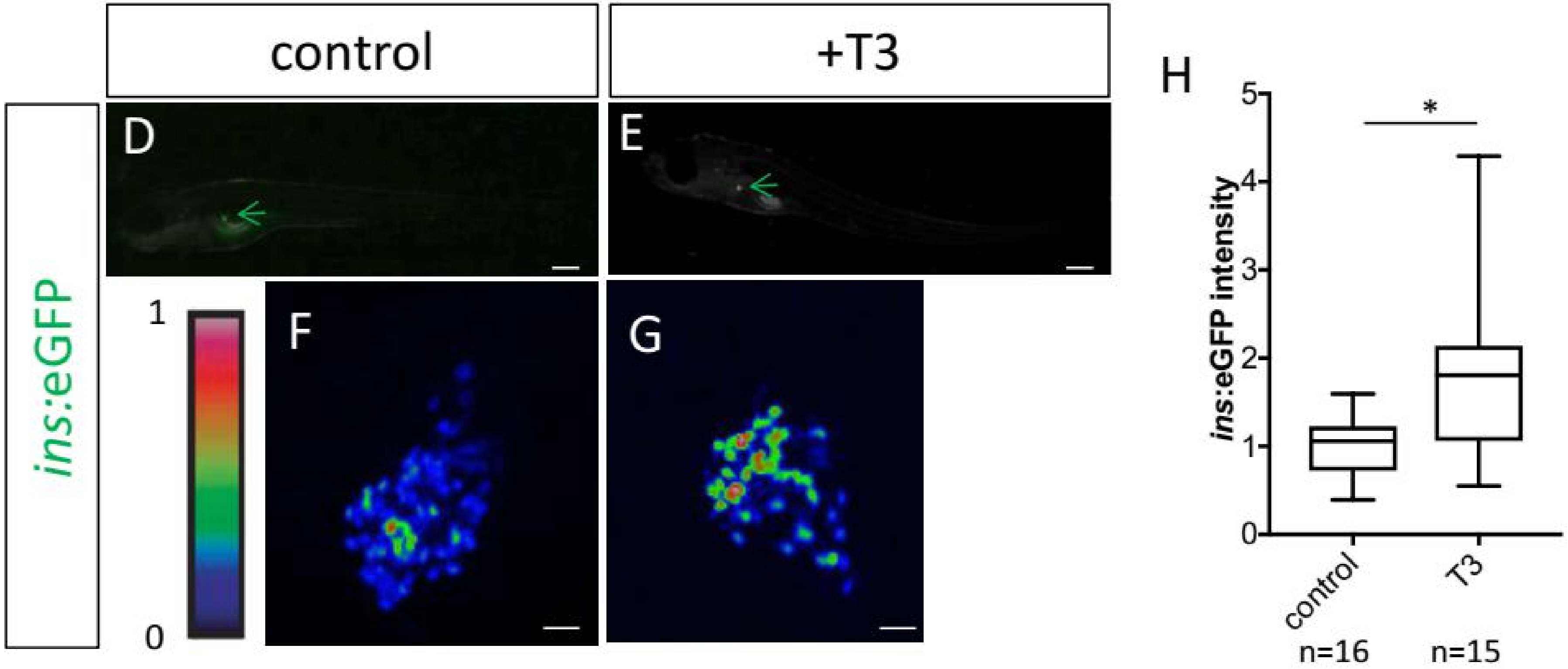
Starting T3 treatment at 4 dpf,*insulin* expression was analyzed at 5, 6 and 7 dpf.

在4 dpf,用T3进行处理， 5，6，7 dpf,进行insulin表达分析

Zebrafish larvae were treated with T3 from 4 dpf and analyzed at 7 dpf

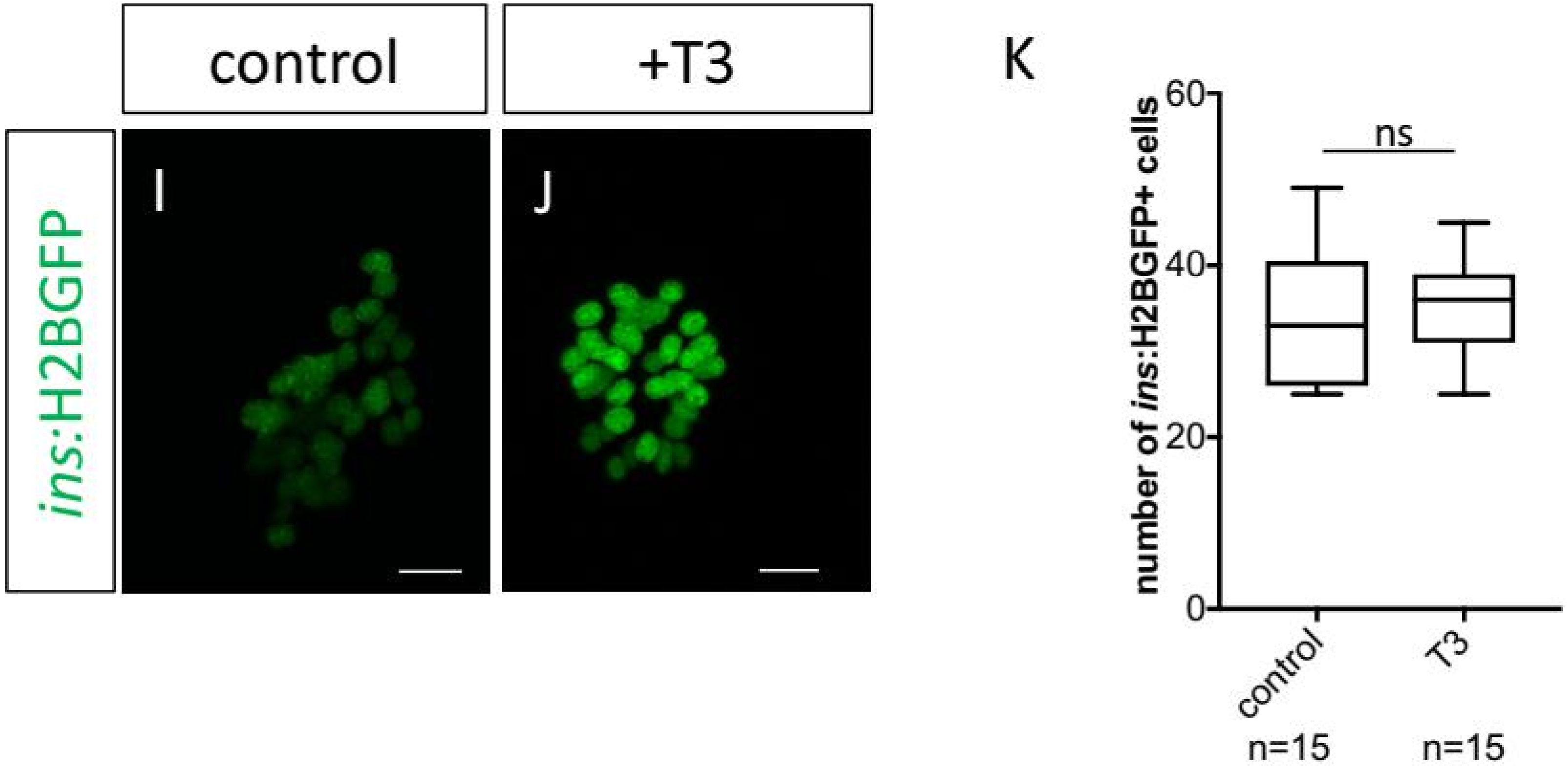
在4 dpf,用T3进行处理， 7 dpf,进行分析

PART THREE Results and Discussion



T3 increased *ins*:eGFP expression intensity in the principal islet compared to controls .

PART THREE Results and Discussion



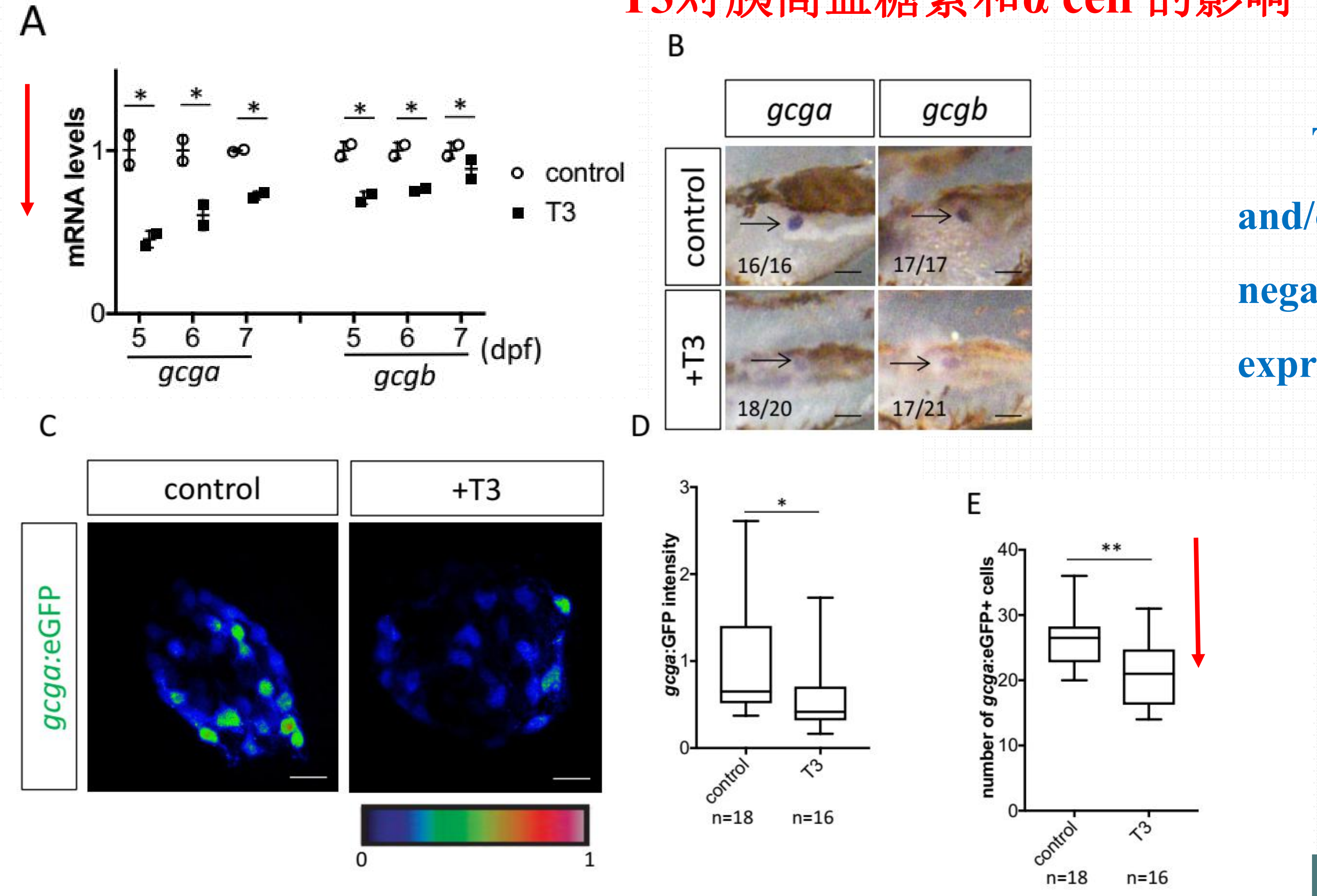
T3 did not alter the number of β cells .

Brief summary 2

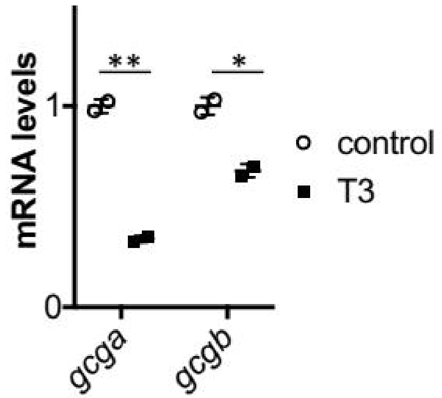
T3 treatments of zebrafish larvae enhance insulin expression without increasing β cell number in zebrafish.

PART THREE Results and Discussion

T3对胰高血糖素和α cell 的影响



TH impairs α cell differentiation and/or proliferation in addition to negatively regulating *glucagon* expression



Ins突变斑马鱼，T3处理后，6dpf时的gcga，gcgb的表达量

Brief summary 3

T3 represses glucagon expression and reduces α cell number.

PART THREE Results and Discussion

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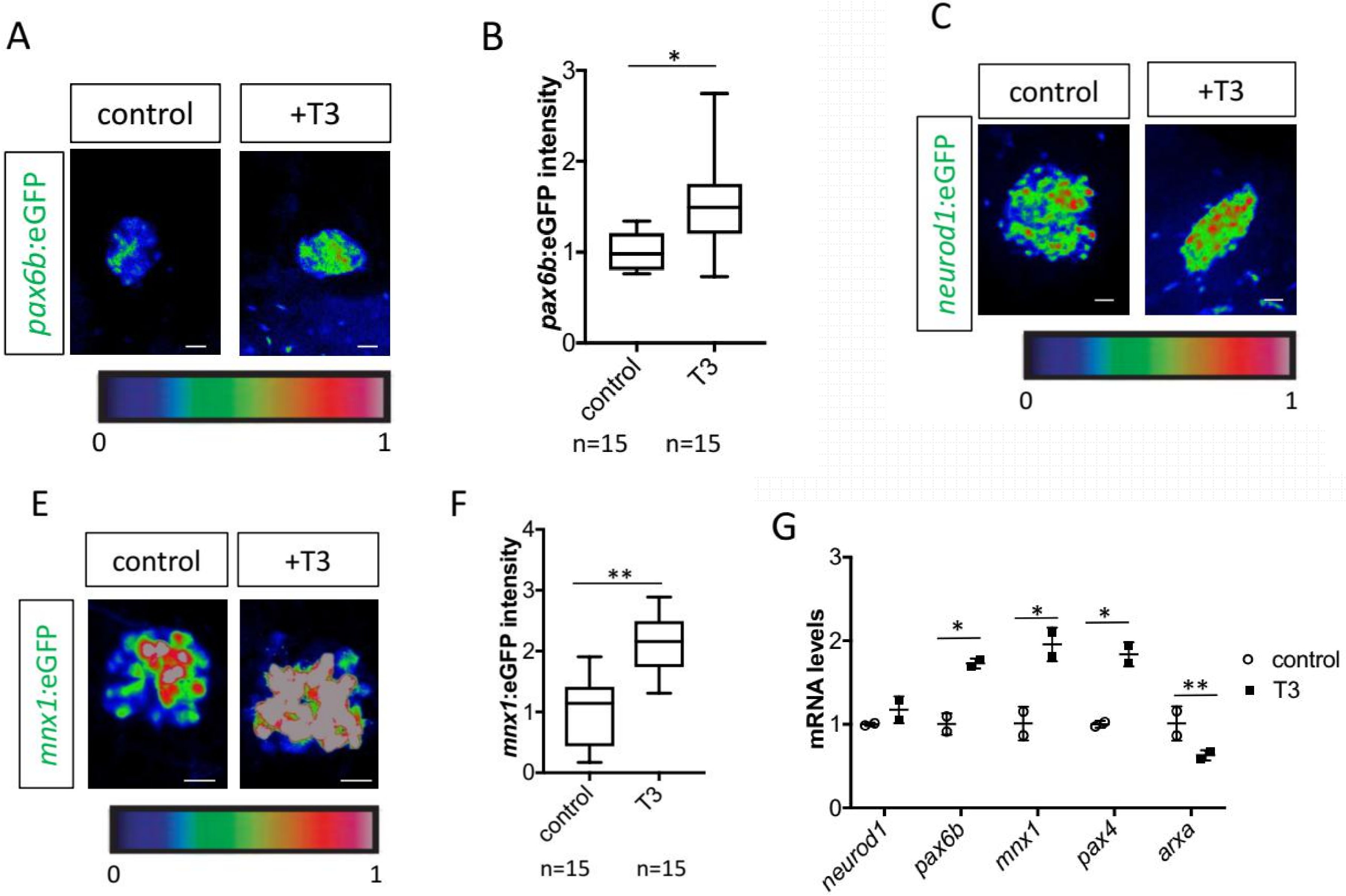
T3对insulin、gcga、gcgb上游转录因子基因的影响



- ✓ **Neurod1，Pax6b，mnx1** 可刺激胰岛素的表达。
- ✓ **Arxa** 可刺激胰高血糖素的表达。
- ✓ **Arxa和pax4**相互拮抗，调节α cell 分化和胰高血糖素的表达

Brief summary 4

T3 stimulates pax6b, mnx1 and pax4 expression and represses arxa expression.

PART THREE Results and Discussion

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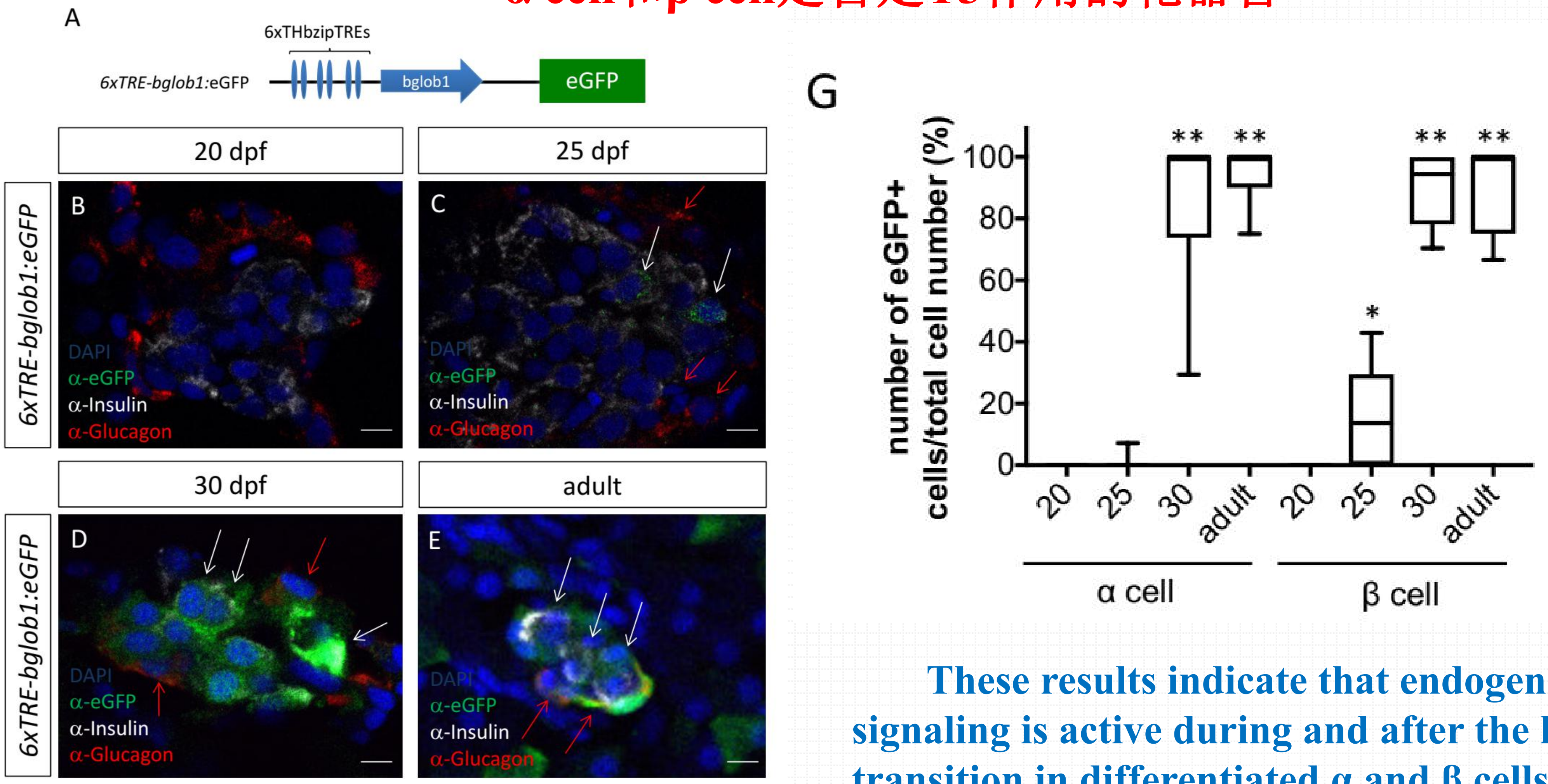
PART THREE Results and Discussion

α cell和β cell是否是T3作用的靶器官



These results indicate that endogenous TH signaling is active during and after the larval-juvenile transition in differentiated α and β cells .

Brief summary 5

Pancreatic α and β cells are targets of thyroid hormone during and after larval development

PART THREE Results and Discussion

- 甲状腺激素
  - 甲状腺激素对胰岛素和胰高血糖素的影响
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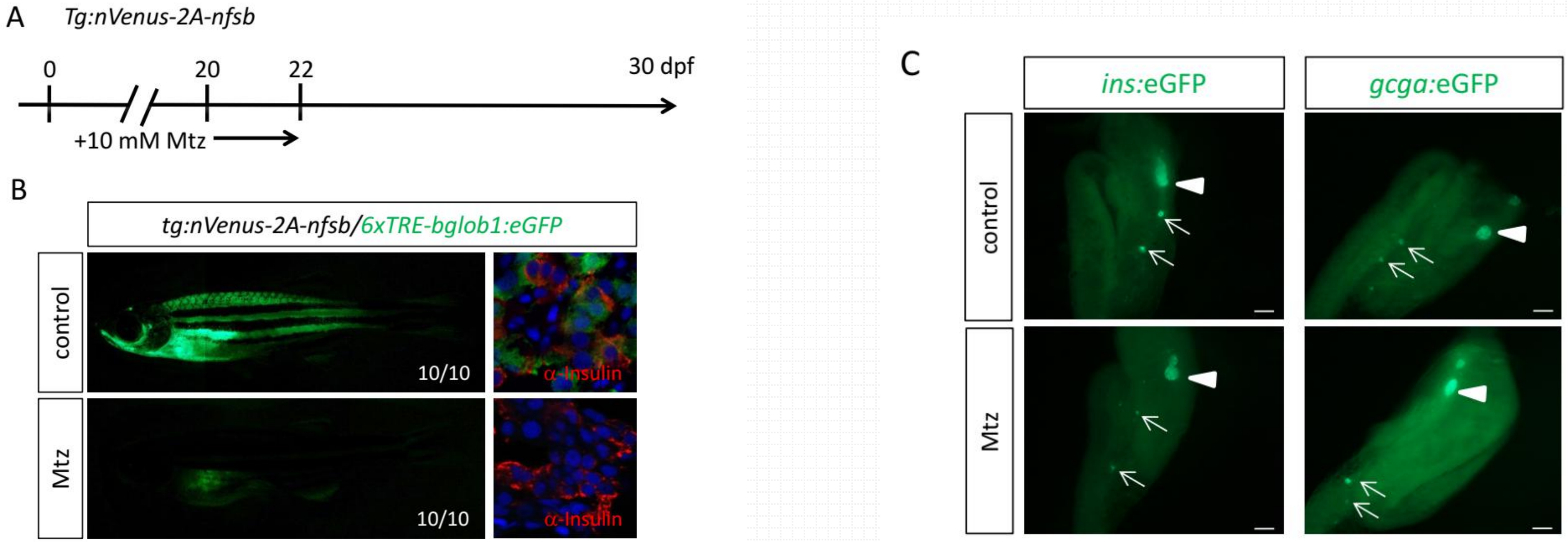
  - 在成熟时期，对胰岛素和胰高血糖素的影响

PART THREE Results and Discussion

在larval-juvenile过渡期间内源性TH如何通过影响胰岛素和胰高血糖素的表达来调节糖代谢

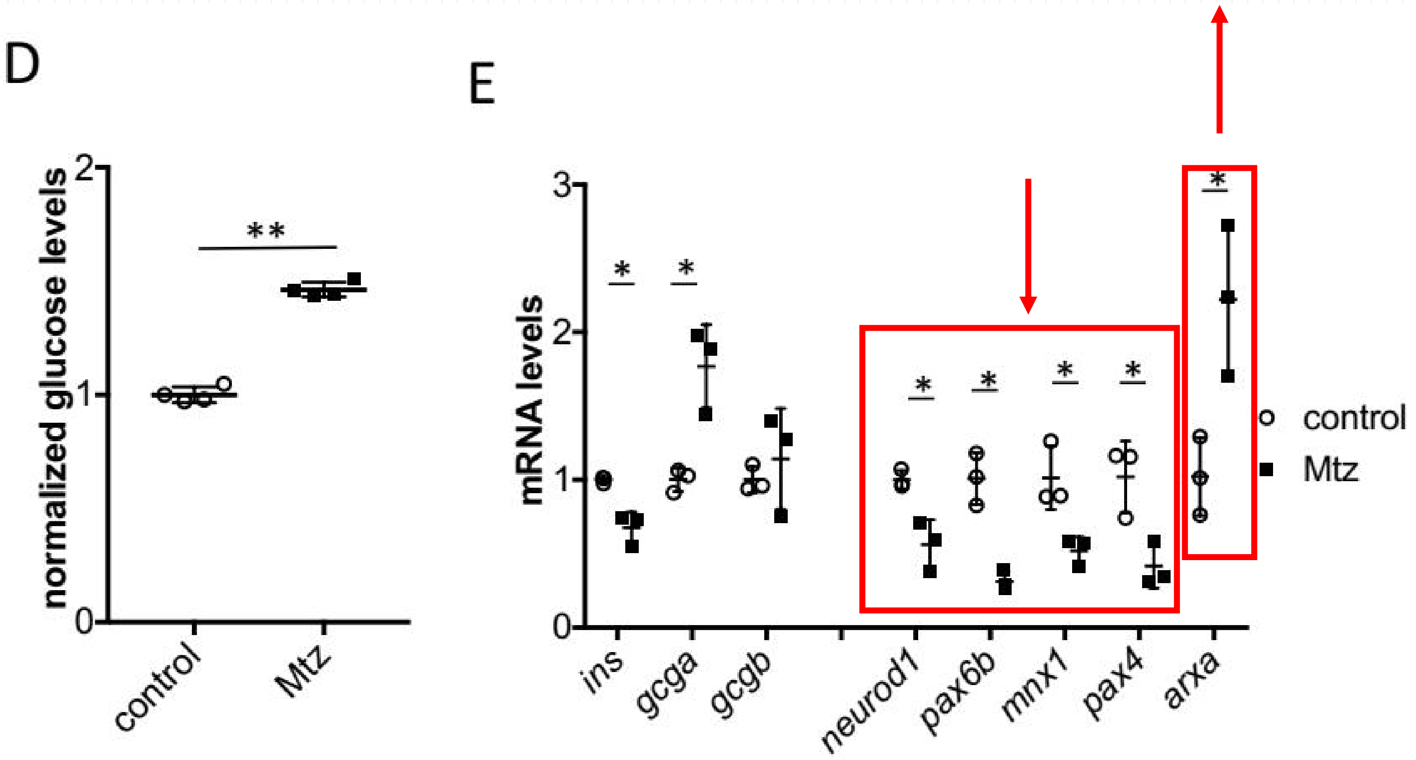


胰岛　　　　　　　　　　　　胰岛形态

Overall eGFP expression of the TH reporter was reduced, and TH signaling was not detected in the islets of Mtz treated animals at 30 dpf.

Islet morphology was unaltered in thyroid-ablated zebrafish.

PART THREE Results and Discussion



These results suggest that endogenous TH regulates glucose homeostasis during the larval-juvenile transition by modulating the expression of endocrine genes including insulin and glucagon（包括胰岛素和胰高血糖素在内的内分泌基因）.

Brief summary 6

Thyroid hormone regulates glucose metabolism by stimulating insulin expression and suppressing glucagon expression during the larval-juvenile transition.

PART THREE Results and Discussion

甲状腺激素

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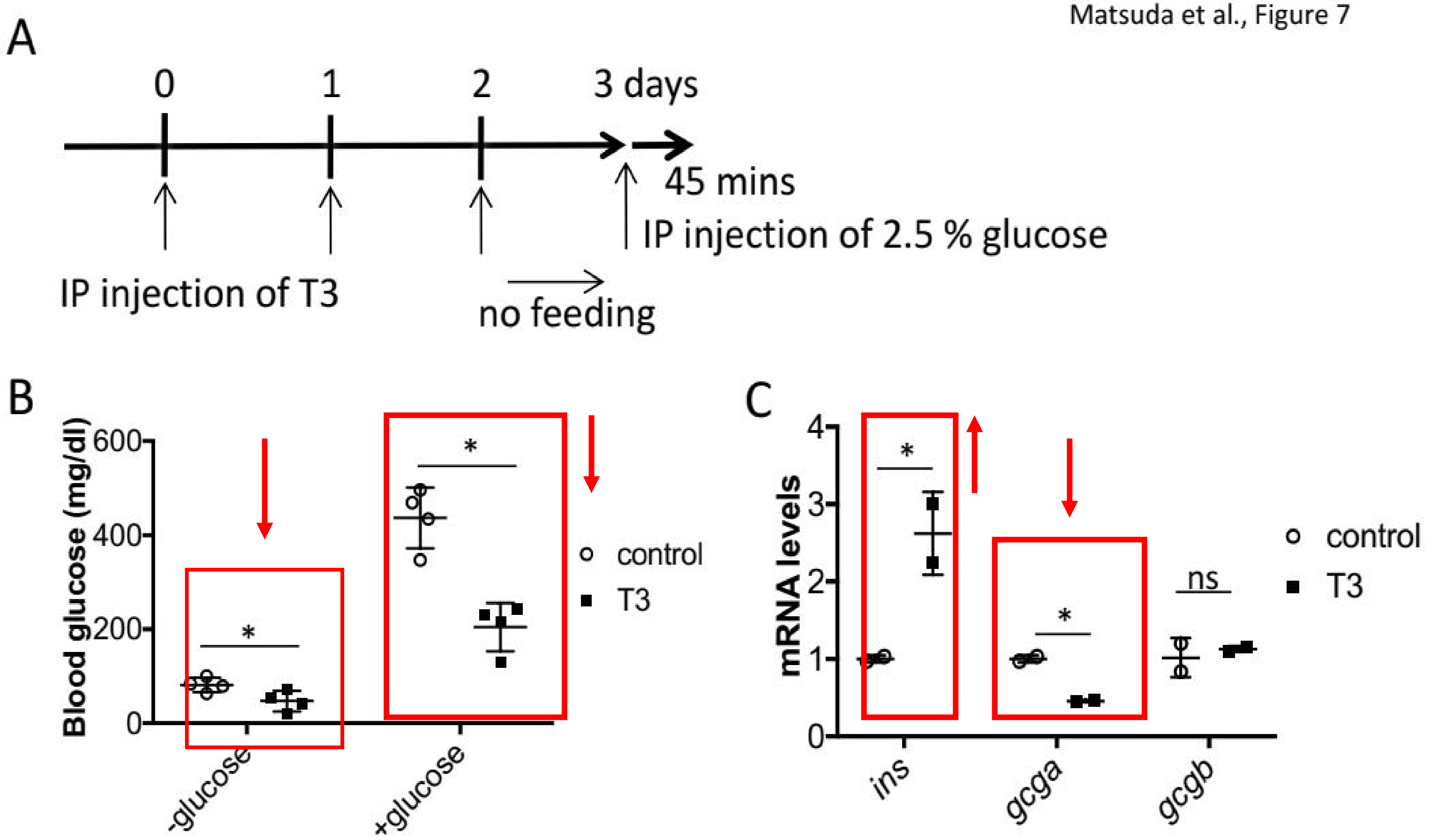
- 在成熟时期，对胰岛素和胰高血糖素的影响

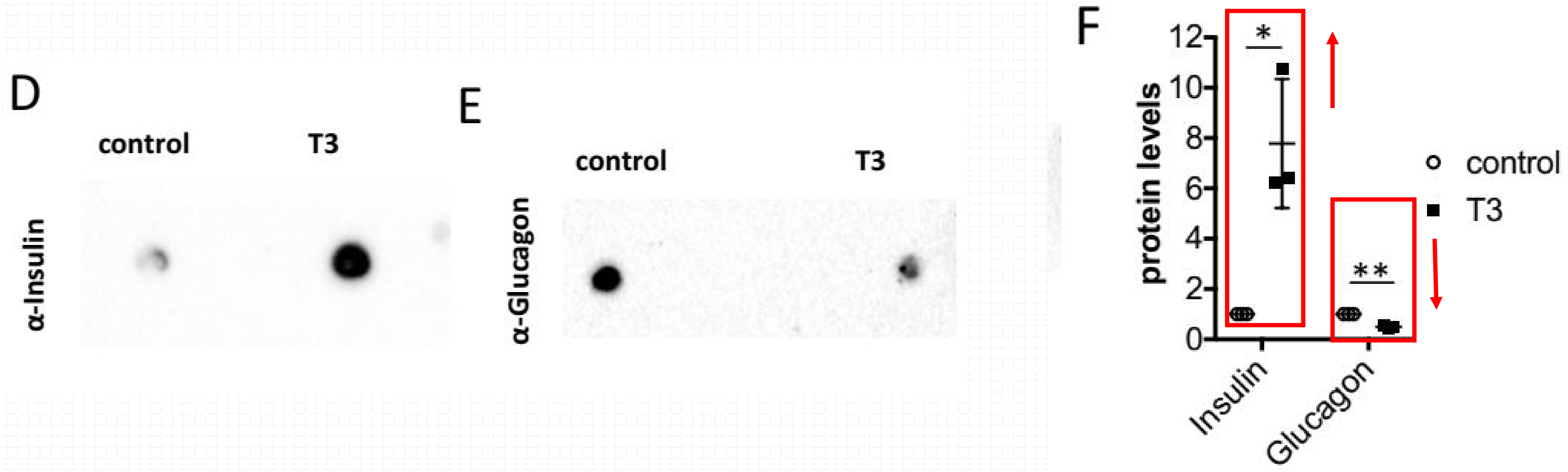
在成熟时期，T3如何通过影响胰岛素和胰高血糖素的表达来调节糖代谢

Matsuda et al., Figure 7



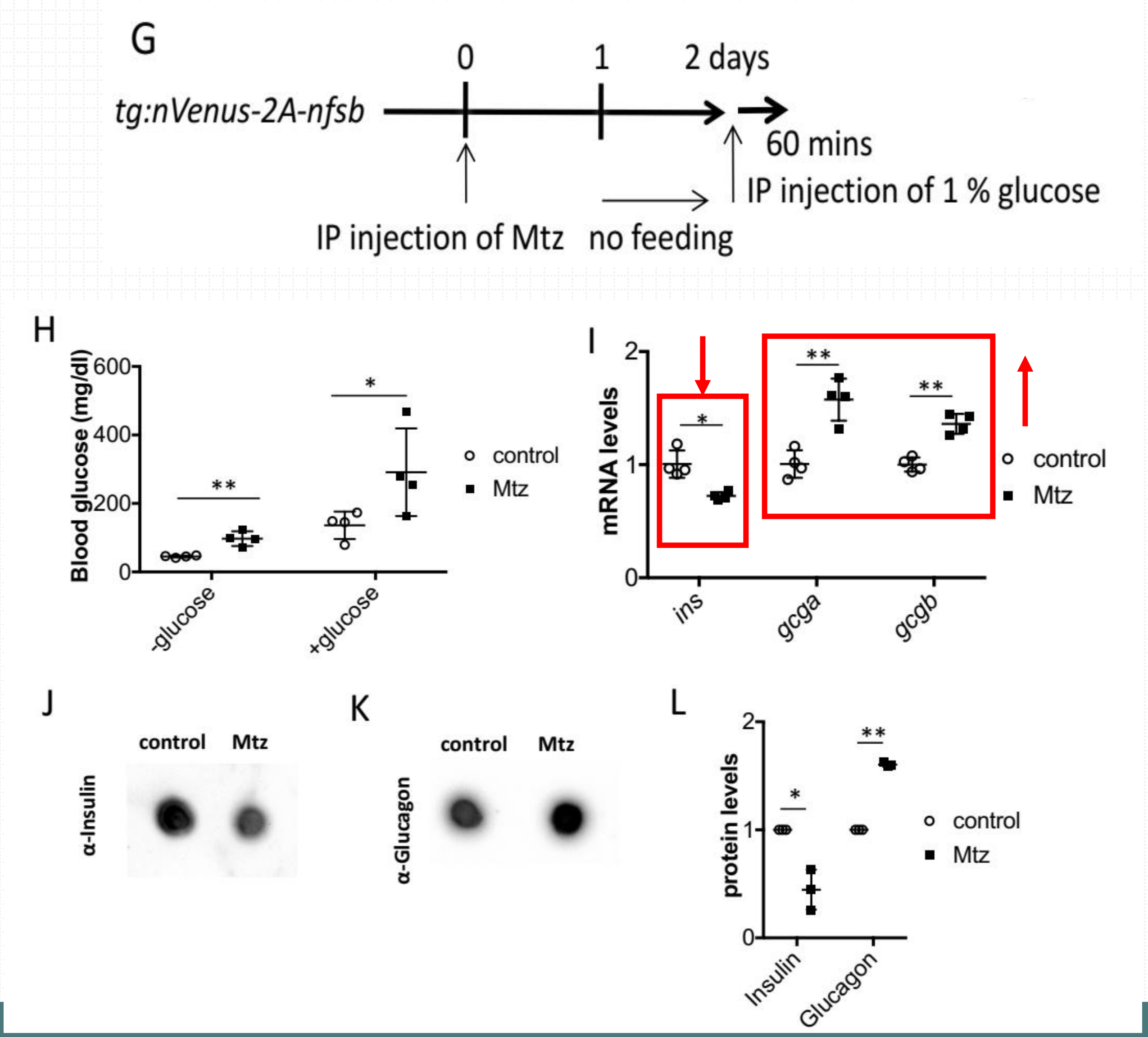
mRNA表达水平

Dot-blot



蛋白质表达水平

PART THREE Results and Discussion



TH mediated effects on pancreatic endocrine hormone expression induce an anti-hyperglycemic(抗高血糖) islet secretion profile that improves glucose tolerance.

Brief summary 7

T3 regulates glucose metabolism by stimulating Insulin secretion and suppressing Glucagon secretion in adults

summary

TH induced stimulation of Insulin secretion and inhibition of Glucagon secretion led to reduced fasting glucose levels and increased glucose tolerance during larvae-juwenile transition and adults.

TH通过刺激胰岛素的表达和胰高血糖素的表达，而导致葡萄糖清除率的升高，增加了larval-juvenile transition时期和成年时期的葡萄糖耐受力。

请各位老师同学批评指正！

- 汇报时间：2018年8月18日
- 汇报人：赵文丽