

The RB611 recombinant antibody recognizes human and murine pancreatic polypeptide by immunofluorescence

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Abstract

A new recombinant antibody, RB611, detects human and murine pancreatic polypeptide (PPY) by immunofluorescence on histological sections.

Introduction

Pancreatic polypeptide (UniProt P10601/P01298, encoded by the *PPY* gene) is a peptidic hormone secreted by gamma cells (also known as PP cells), located in pancreatic islets. It regulates pancreatic and gastrointestinal functions (Yonekura *et al.*, 1988). Here we describe the ability of the antibody RB611 to recognize human and murine pancreatic polypeptide on paraffin sections by immunofluorescence.

Materials & Methods

Antibodies: ABCD_RB608, ABCD_RB609, ABCD_RB610, ABCD_RB611 and ABCD_RB612 antibodies (ABCD nomenclature, <https://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (<https://www.unige.ch/medecine/antibodies/>) as described (minibody format with the antigen-binding domain fused to a mouse IgG2a Fc) (Perez-Frances *et al.*, 2021).

Antigen: Human pancreatic histologic samples were obtained from anonymized deceased patients through the nPOD (Network for Pancreatic Organ Donors with Diabetes), supported by JDRF (Juvenile Diabetes Research Foundation International) at the University of Florida. Donor information and consent from the donor family were obtained for all nPOD samples. Mouse (WT and *Ppy*-knockout strains) and human ventral pancreata were fixed with 4% paraformaldehyde (Santa Cruz Biotechnology, sc-281692) in PBS overnight. Pancreases were embedded in paraffin and sectioned at 5 μ m-thickness.

Protocol: Antibodies RB608, RB609, RB610, RB611 and RB612 were used to recognize PPY in human and mouse paraffin-embedded sections. The immunofluorescence protocol was carried out at room temperature. After each incubation step, sections were washed 3 times for 5 min with PBS. Paraffin sections were deparaffinized and rehydrated in Neoclear (Sigma-Aldrich, 1098435000) two times, 100% ethanol 2 times, 95% ethanol, 70% ethanol, 50% ethanol and 30% ethanol solution for 5 minutes each. Then, sections were rinsed with PBS before 20 min permeabilization with 0.1% TritonX-100 (AppliChem A1388.0500) in PBS. Blocking was performed with 3% bovine serum albumin (Sigma-Aldrich A3912-100G) and

0.1% Tween20 (AppliChem A1389.0500) in PBS for 30 min. The sections were incubated for either 2h (for mouse sections) or overnight (for human sections) with the mouse recombinant antibodies RB608, RB609, RB610, RB611 and RB612 (10 μ g/ml for mouse sections and 20 μ g/ml for human sections) plus rabbit anti-insulin (1/1000; ThermoFisher, 19H4L12). After washing, the sections were incubated for 45 min in PBS containing the secondary antibodies anti-mouse TRITC (1/500, Southern Biotech 1070-03), anti-rabbit Alexa 488 (1/500, Life Technologies A11034) and DAPI (1/500, Life Technologies D3571). Samples were washed and mounted on SuperFrost Plus slides (Thermo Scientific, J1800AMNZ) using a DAPI Fluoromount-G media (Southern Biotech 0100-20). All sections were examined with a confocal microscope (Leica TCS SPE).

Results

Out of the five anti-PPY antibodies tested, only RB611 efficiently detected PPY hormone in murine (Fig. 1A, right panel, in red) and human (Fig. 1B, right panel, in red) paraffin-embedded sections of the ventral pancreas. No signal above background could be detected with RB608, RB609, RB610 or RB612 antibodies (shown for RB608 in murine sections, Fig. 1A, left panel; and for RB612 in human sections, Fig. 1B, left panel).

Moreover, no PPY staining was detected with RB611 on pancreatic sections from *Ppy* knock-out (KO) mice (Fig. 1C), which confirms the antibody's specificity.

References

Perez-Frances M, Thorel F, Herrera PL. RB608, RB609, RB610, RB611 and RB612 antibodies recognize murine pancreatic polypeptide by ELISA. *Antibody Reports*, 2021, 4:e537. doi:10.22450/journals/abrep.2021.e537

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Conflict of interest

The authors declare no conflict of interest.

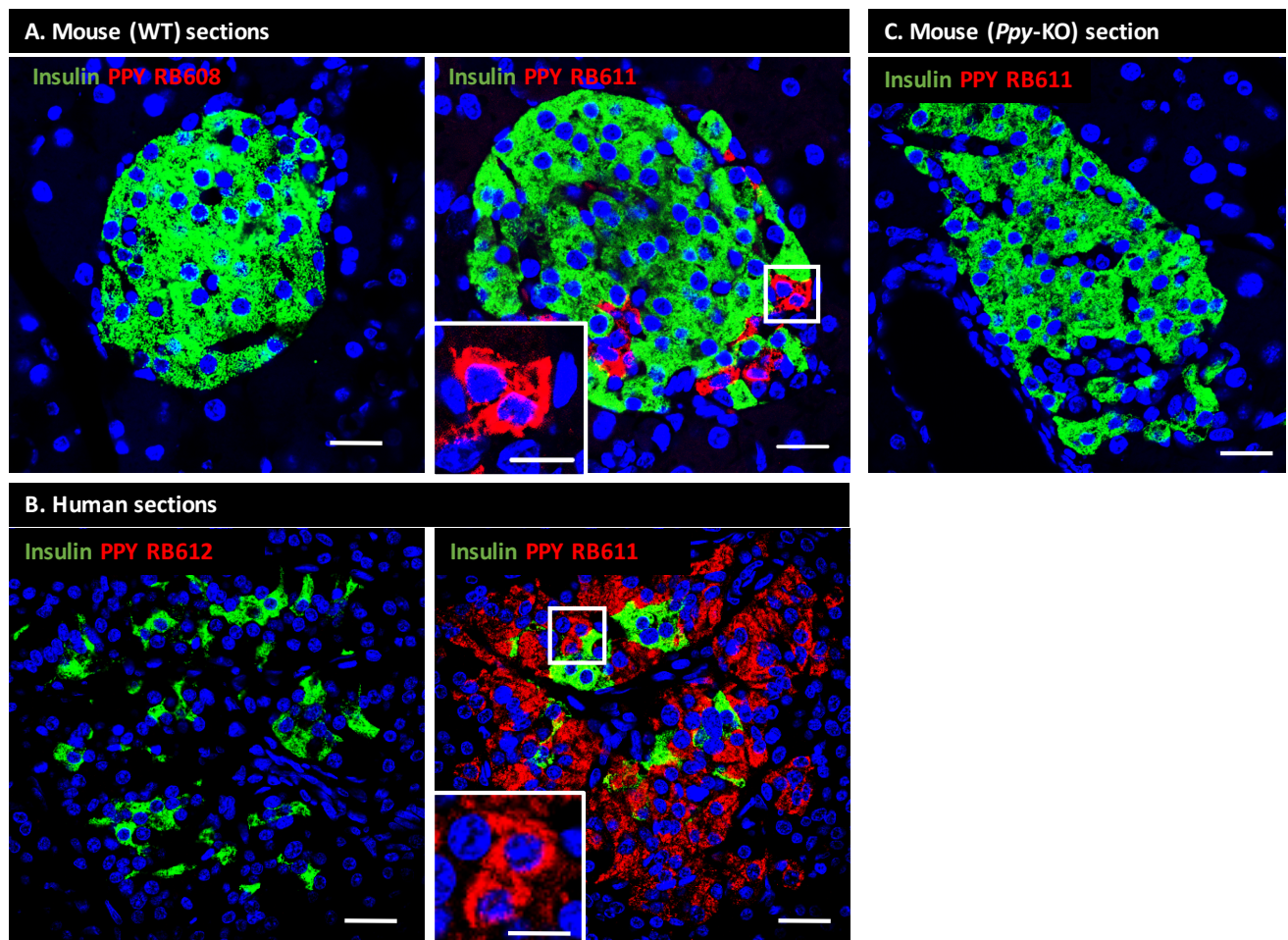


Fig. 1. The anti-PPY RB611 antibody efficiently recognizes PPY hormone in mouse and human islets. (A) Immunofluorescence on pancreatic sections from 2-month-old wild-type (WT) mice (RB608 on the left and RB611 on the right). (B) Immunostaining of human islet cells (RB612 on the left and RB611 on the right). (C) Immunofluorescence on a pancreatic section from a 2-month-old *Ppy* knock-out mouse; no labeling detected with RB611. Scale bars: 20 μm or 10 μm (inset). Region of the pancreas: ventral, corresponding to the head of the pancreas.