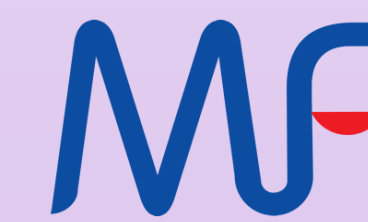


Pancreatic islet surface and share of insulin granules after streptozotocin treatment in wild-type and Tff3 knock-out mice

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Introduction

Trefoil Factor 3 (Tff3) is a small peptide mainly expressed in gastrointestinal epithelial cells with a known role in the protection and repair of the mucosal barrier. It promotes cellular migration and angiogenesis. Tff3 is also found expressed in pancreas islet cells where it is localized in insulin and some glucagon-expressing cells [1]. Some studies indicate that Tff3 could be involved in islet cell proliferation [2].

Materials & methods

To investigate the effect of Tff3 deficiency on the integrity of the pancreas we have performed a study on a novel congenic Tff3^{-/-}-C57Bl6NCrI mouse strain using a multiple low-dose streptozotocin (STZ) protocol [3]. STZ is selectively toxic to insulin-producing pancreatic β cells and is commonly used to induce Type 1 diabetes (T1D) in animal models. 7-week-old Tff3^{-/-} male and female mice and wild type controls were administered low 50 mg/kg, multiple intraperitoneal STZ injections (for 5 consecutive days) We have monitored weight and blood glucose levels during the experiment and after 6 weeks of STZ induced hyperglycemia, mice were sacrificed.

Pancreases were collected and tissue was fixed in 4% paraformaldehyde and embedded into paraffin. The organs were cut into 6 μ m sections. 3 different sections through the whole pancreas, up to 1 mm apart were taken for each animal, and 2 largest pancreatic islets found on the section were used for measurements. Sections were deparaffinized and stained using Gomori's aldehyde fuchsin for pancreatic beta-granules. 6 digital photographs were taken per animal, islets were separated from the rest of the tissue in GIMP software, and islet surface and beta granule surface were measured using FIJI software.

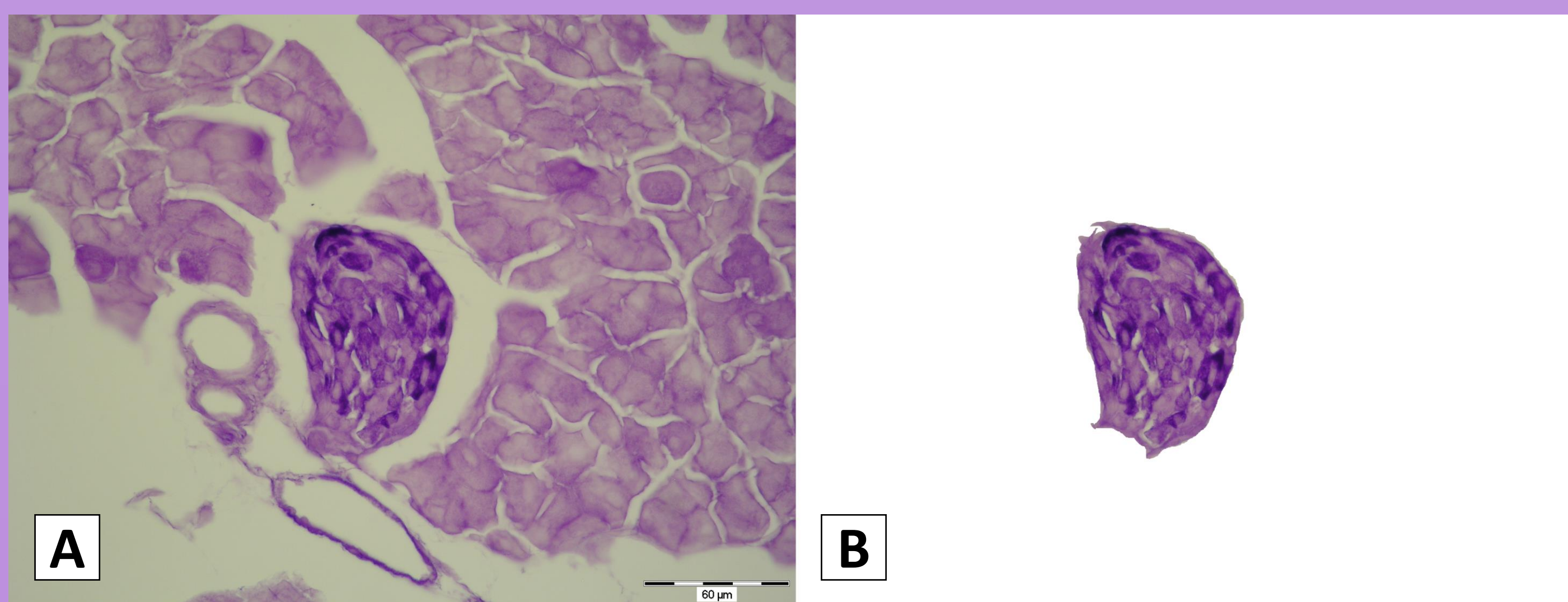


Figure 1. A: Aldehyde fuchsin-stained pancreatic tissue photographed under a high magnification (40x) objective. A pancreatic islet is visible in the middle of the image, surrounded by pancreatic acini and small blood vessels. Insulin-containing granules are markedly darker than other tissue details. Elastin in blood vessels also stained dark violet. **B:** The same pancreatic islet isolated manually in Gimp software, prepared for measurement of islet surface and surface of insulin granules in FIJI.

References

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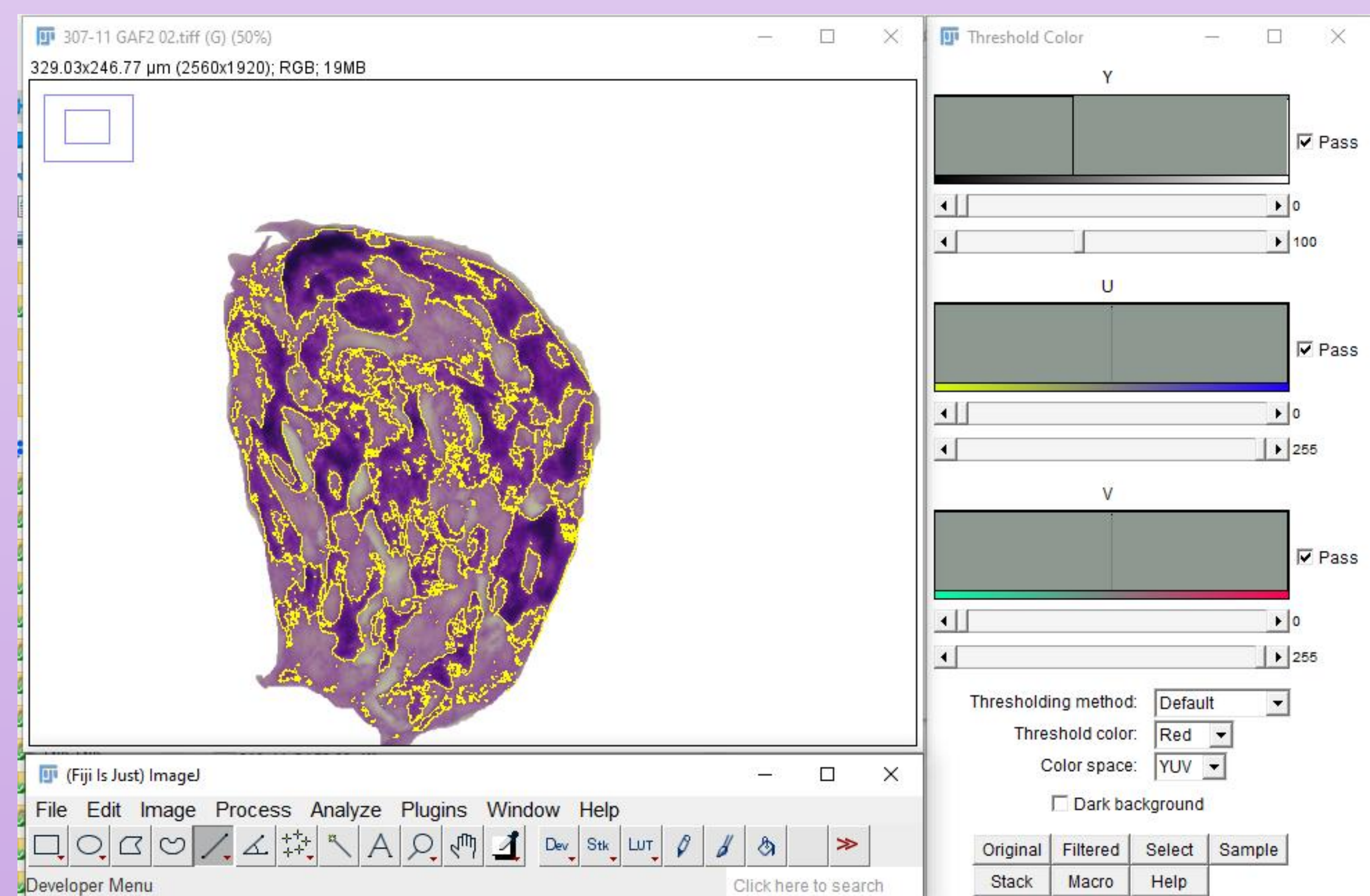


Figure 2. Aldehyde fuchsin-stained pancreatic islet from Figure 1 opened in FIJI software. Using Color Thresholding tool with YUV color space, dark violet-stained areas occupied by insulin-containing granules were selected and their surface was measured. The method allowed for manual adjusting of the selection to encompass all the granules.

Results & Conclusion

Brightfield microscopy showed only a small number of detectable islets in pancreatic tissue. There was no significant difference in average pancreatic islet surface, nor in the share of beta granules in the islets among the examined groups.

Although there were no differences in pancreas tissue between groups, during the experiment Tff3^{-/-} male and female mice had statistically reduced body weight and higher mortality rate compared to WT animals. These results indicate a role of Tff3 in the response to STZ treatment, but its exact nature remains yet to be determined.

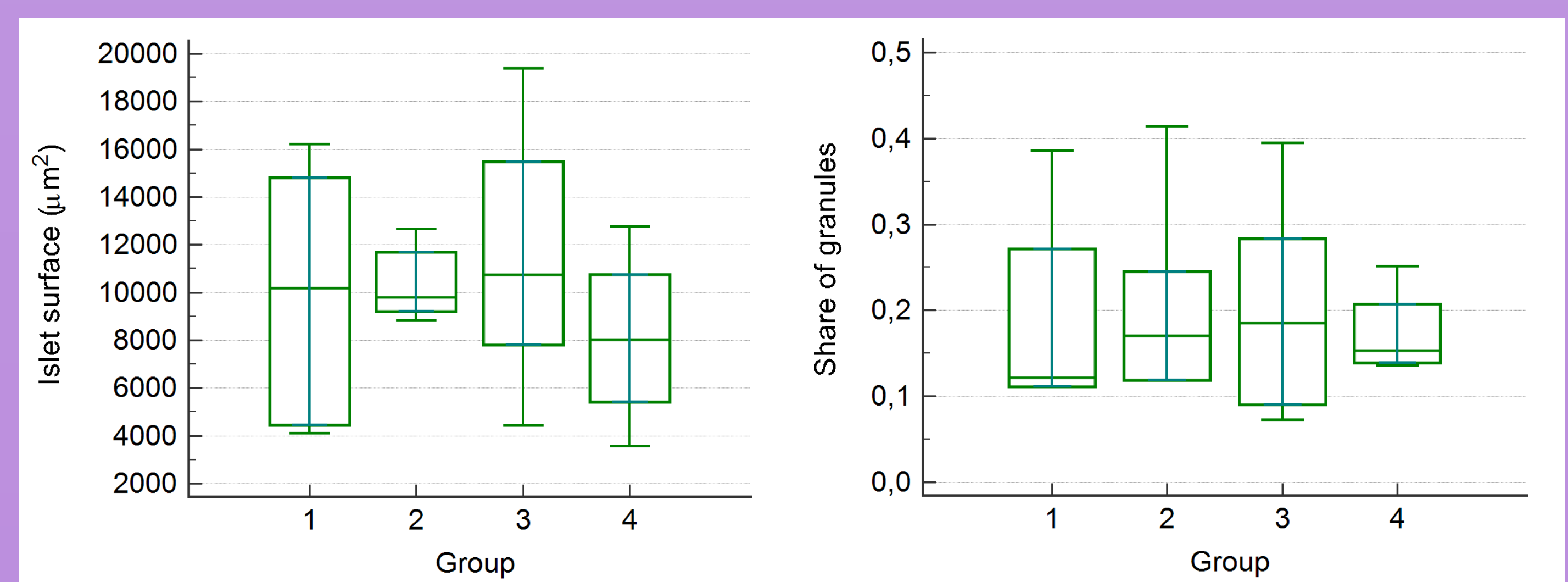


Figure 3. Left: Comparison of islet surface between the groups. No significant differences were found (Kruskal-Wallis test). **Right:** Comparison of the share of insulin granules in islet surface between the groups. No significant differences were found (Kruskal-Wallis test). Groups: 1) wild-type male; 2) wild-type female; 3) Tff3^{-/-} male; 4) Tff3^{-/-} female.