Title: *Wnt4* is heterogeneously activated in maturing β-cells to control calcium signaling, metabolism and function

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Supplementary Fig. 1: Gene ontology analysis of P1 Wnt4eGFPCre; mT/mG islets.

a-d, Gene ontology analysis results of P1 Wnt4eGFPCre; mT/mG islets based on DAVID Bioinformatics Resources 6.8. Results with the modified Fisher exact p-value (EASE score) < 0.05 were considered to be significantly enriched. Selected significant enriched terms of gene ontology. Enriched terms of KEGG Pathway (a) and Biological Process (b) from up-genes of Wnt4eGFPCre Tg+; mTMG Tg+. Enriched terms of KEGG Pathway (c) and Biological Process (d) from up-genes of Wnt4eGFPCre Tg-; mTMG Tg+. Source data are provided as a Source Data file.
Supplementary Fig. 2: Canonical and non-canonical Wnt signal in P1 islets.

**a-d**, Expression pattern of insulin (**a**), GFPCre (**b**), active beta-catenin (**c**) and merge image including DAPI staining (**d**) in P1 Wnt4eGFPCre Tg+ islets. **e-h**, Expression pattern of insulin (**e**), GFPCre (**f**), pMLC (**g**) and merge image including DAPI staining (**h**) in P1 Wnt4eGFPCre Tg+ islets. **d’** **d”** **h’**, High magnification of yellow squares in corresponding panels. Scale bar 50 μm in (**a-d, e-h**), 5 μm in (**d’, d”, h’**). Representative images are from three independent samples.
Supplementary Fig. 3: Expression pattern of WNT4 in Wnt4\textsuperscript{\textless}KO islets.

a-d, Expression pattern of Wnt4 (a), mGFP (b), DAPI staining (c) and merge image (d) in control 3 months Wnt4\textsuperscript{eGFPCre Tg\textsuperscript{-}; mTmG Tg\textsuperscript{+}} islet. e-h, Expression pattern of Wnt4 (e), mGFP (f), DAPI staining (g) and merge image (h) in mutant 3 months Wnt4\textsuperscript{eGFPCre Tg\textsuperscript{+}; mTmG Tg\textsuperscript{+}} islet. h', High magnification of yellow squares in corresponding panels. Scale bar 100 μm in (a-h), 20 μm in (h'). Representative images are from three independent samples.
Supplementary Fig. 4: Glucose tolerance and basal glucose in \( \text{Wnt}^{4\text{K/O}} \) females.

a, \( \text{Wnt}4 \) qPCR results of 7 weeks \( \text{Wnt}^{4\text{K/O}} \) islets. Control: 4 mice, \( \text{Wnt}^{4\text{K/O}} \): 6 mice. b, Illustration of experimental design for tamoxifen-induced inactivation of \( \text{Wnt}4 \) in \( \beta \)-cells in female mice for intraperitoneal glucose tolerance test (IPGTT). c-f, Results of IPGTT of \( \text{Wnt}^{4\text{K/O}} \) female mice at 7 weeks (c), 2 months (d), 3 months (e) and 10 months (f). g-j, Basal blood glucose levels of \( \text{Wnt}^{4\text{K/O}} \) female mice at 7 weeks (g), 2 months (h), 3 months (i) and 10 months (j). Data in graph of a, c-j are presented as mean values ± SD. Statistical analyses are two-tailed unpaired student t-test. a, \( p = 0.0027 \), d, \( p = 0.0439 \) (30min), \( p = 0.0414 \) (60min), e, \( p = 0.0005 \) (15min), \( p = 0.0010 \) (30min), \( p = 0.0002 \) (60min), \( p = 0.0147 \) (120min), f, \( p = 0.0371 \) (15min), \( p = 0.0078 \) (30min), \( p = 0.0055 \) (60min), \( p = 0.0127 \) (120min). j, \( p = 0.0006 \). * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \) and NS; not significant. Source data are provided as a Source Data file.
**Supplementary Fig. 5:** Body mass in Wnt4βKO females and islet area and insulin content in males.

a, Illustration of experimental design: after the tamoxifen-induced inactivation of Wnt4 in β-cells in females, the body mass was measured in the mice submitted to IPGTT reported in Supplementary Fig. 4 at 7 weeks (b), 2 months (c), 3 months (d) and 10 months (e). f, Experimental design relative to the islet histology quantifications presented in (g) and the insulin content measurements shown in (h). g, Average islet area in analysis of insulin+ and glucagon+ cell ratio and insulin+ and glucagon+ average cell area (Fig. 4d,e). h, Total insulin amount of islet assessed by ELISA, relative to the insulin secretion shown in Fig. 4g. Data in graph of b-e, g-h are presented as mean values ± SD. Statistical analyses are two-tailed unpaired student t-test. b, p=0.0399, e, p=0.0322. *p<0.05 and NS; not significant. Source data are provided as a Source Data file.
Supplementary Fig. 6: Insulin tolerance test and fasted insulin and glucagon amount in Wnt4<sup>ΔKO</sup> mice.

**a**, Experimental design for basal glucose (b), body mass (d) and insulin tolerance test (ITT) (f,g) at 2 months and 6 hours fasted glucose (c), body mass (e), ELISA measurement of insulin (h) and glucagon (i) at 3 months in tamoxifen-induced Wnt4 inactivation in β-cells in male mice. **b**, Basal blood glucose level at 2 months. **c**, 6 hours fasted blood glucose level at 3 months. **d,e**, Body mass of Wnt4<sup>ΔKO</sup> male mice at 2 months (d) and 3 months (e). **f,g**, Result of insulin tolerance test (ITT) of Wnt4<sup>ΔKO</sup> male mice at 2 months (f) and glucose level was normalized to 0 time (g). **h,i**, ELISA for insulin (h) and glucagon (i) in 6 hours fasted blood samples from 3 months Wnt4<sup>ΔKO</sup> male mice. Data in graph of **b-i** are presented as mean values ± SD. Statistical analyses are two-tailed unpaired student t-test. **b, p=0.0108, c, p=0.0389, f, p=0.0169 (0 time), *p<0.05 and NS; not significant. Source data are provided as a Source Data file.
Supplementary Fig. 7: Gene ontology analysis of 7-weeks Wnt4\textsuperscript{BKO} islets.

a-d. Gene ontology analysis results of 7-weeks Wnt4\textsuperscript{BKO} islets based on DAVID Bioinformatics Resources 6.8. Results with the modified Fisher exact p-value (EASE score) < 0.05 were considered to be significantly enriched. Selected significant enriched terms of gene ontology. Enriched terms of KEGG Pathway (a) and Biological Process (b) for down-regulated genes in Wnt4\textsuperscript{BKO} islets. Enriched terms of KEGG Pathway (c) and Biological Process (d) for up-regulated genes in Wnt4\textsuperscript{BKO} islets. Source data are provided as a Source Data file.
Supplementary Fig. 8: WNT4 regulates influx of calcium in mouse islets together with glucose.

a-d, Area Under Curve (AUC) of calcium fluorescence traces in 2 months Wnt4Δ^PKO islets (Related to Fig. 6b) at 5 min in high glucose (a), 10 min in high glucose (b), 15 min in high glucose (c) and 5 min of KCl (d). e-h, AUC of calcium fluorescence trace in the absence or presence of WNT4 (Related to Fig. 6e) at 5 min in high glucose (e), 10 min in high glucose (f), 15 min in high glucose (g) and 5 min of KCl (h). i-r, Imaging calcium in islets after in vitro Wnt4 inactivation. i, Experimental design for in vitro calcium imaging. Single cell imaging influx of calcium in control (j) and Wnt4 inactivation (4-OHT, k). (l-r), Islets imaging influx of calcium in control (black) and Wnt4 inactivation (4-OHT, Red). AUC of calcium trace after in vitro inactivation of Wnt4 in islets after 5 min in high glucose (l), 10 min in high glucose (m), 15 min in high glucose (n), 20 min in high glucose (o), and 5 min in KCl (p), 1st phase response peak (q) and peak in KCl (r). Data in graph of a-h, l-r are presented as mean values ± SD. Statistical analyses are two-tailed unpaired student t-test. a, p=0.0002, b, p=0.0006, c, p=0.0002, d, p=0.0029, e, p=0.0024, f, p=0.0241, g, p=0.0302, h, p=0.0392, i, p=0.0073, n, p=0.0480, q, p=0.0048. *p<0.05, **p<0.01, ***p<0.001 and NS; not significant. Source data are provided as a Source Data file.
Supplementary Fig. 9: WNT4 alone positively regulate influx of calcium in mouse islets.

a. Calcium signaling in the presence of 600 ng WNT4 in low glucose. Red broken lines show standard deviations. b-k, AUC of calcium fluorescence trace in 600 ng WNT4 in low glucose (a) at 30 sec (b), at 1 min (c), at 1.5 min (d), at 2 min (e), at 2.5 min (f), at 3 min (g), at 3.5 min (h), at 4 min (i), at 4.5 min (j) and 5 min (k). a-k, 34 islets from 3 mice were analyzed. Data in graph of a-k are presented as mean values ± SD. Statistical analyses are two-tailed paired student t-test. b, p=9.54e-11, c, p=5.92e-10, d, p=1.11e-09, e, p=1.9e-09, f, p=4.07e-09, g, p=1.11e-08, h, p=2.65e-08, i, p=5.4e-08, j, p=1.03e-07, k, p=1.68e-07. ***p<0.001.

l. AUC of single cell imaging influx of calcium in Zebrafish in Fig. 6f-i. 45 cells, n=4 larvae (12.8 mM WNT4), 39 cells, n=4 larvae (25 mM Glucose), 50 cells, n=5 larvae (25 mM Glucose + 12.8 mM WNT4). Data are mean ± s.d (1-way –ANOVA with Tukey’s multiple comparison correction, NS, not significant). Source data are provided as a Source Data file.
Supplementary Fig. 10: Gating Strategy of Flow Cytometry.
a-h, Gating strategy of mGFP+ islet cells and mGFP- islet cells in cell proliferation analysis (Fig. 2f-i). a-d, Control islets. e-h, Mutant islets. Cell population (a,e), single cells (b,f), mRFP+ islet cells (c,g) and mGFP+ islet cells and mGFP- islet cells (d,h). i-r, Gating strategy of mGFP+ islet cells and mGFP- islet cells in mitochondrial mass analysis (Fig. 2j-n). i-m, Control islets. n-r, Mutant islets. Cell population (i,n), single cells (j,o), live cells (k,p), mRFP+ islet cells (l,q) and mGFP+ islet cells and mGFP- islet cells (m,r).