

Single molecule-based fliFISH validates radial and heterogeneous gene expression patterns in pancreatic islet β cells

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SUPPLEMENTAL TABLE AND FIGURS

	<i>Ins2 - Atto488</i>
Primary probes	GTGCTGCTTGACAAAAGCCT <u>CCAGCTTCTAGCATCCATGCCCTATAAG</u> CCACCA <u>GATGGGAACCACAACCAGCTTCTAGCATCCATGCCCTATAAG</u> CATGGCGTGTAGAAGAAG <u>CCCAGCTTCTAGCATCCATGCCCTATAAG</u> CAAGGTCTGAAGGT <u>CACCTGCCAGCTTCTAGCATCCATGCCCTATAAG</u> CAGCACTGATCTACAA <u>TGCCCCAGCTTCTAGCATCCATGCCCTATAAG</u> TAGTTGCAGTAGTTCT <u>CCAGCCAGCTTCTAGCATCCATGCCCTATAAG</u> GCCATGTTGAA <u>ACAATAACCCAGCTTCTAGCATCCATGCCCTATAAG</u> CCACACACCAGGTAGAG <u>AGAGCCAGCTTCTAGCATCCATGCCCTATAAG</u>
Secondary probe	5'-/5Atto488N/CTTATAGGGCATGGATGCTAGAACGCTGG/3ATTO488N/-3'
	<i>Unc3 - Alexa594</i>
Primary probes	CCAGCTAATTCTAGCCTCTTGT <u>GACTCAGGTCGAGCTTGCTAACCTC</u> TGGAAATGCT <u>CAGGTGAGGGTGACTCAGGTCGAGCTTGCTAACCTC</u> TGGCAGCAGGA <u>AGTAGGTTGGTGACTCAGGTCGAGCTTGCTAACCTC</u> GCGTTGGTT <u>GTCATCTTCTGTGACTCAGGTCGAGCTTGCTAACCTC</u> CCTTGTCT <u>GATGTGCCACCGTGACTCAGGTCGAGCTTGCTAACCTC</u> GGCTGAG <u>CAATACTGCGCTTGTGACTCAGGTCGAGCTTGCTAACCTC</u> CGAGGCT <u>CAGACCAATGTGGTGACTCAGGTCGAGCTTGCTAACCTC</u> AATCCAGAT <u>GAGGAAGTAGGGTGACTCAGGTCGAGCTTGCTAACCTC</u>
Secondary probe	5'-/5Alex594N/GAGGTTAGCAAAGCTCGACCTGAGTCAC/3AlexF594N-3'
	<i>Rgs4 - TAMRA</i>
Primary probes	TCAGAAC <u>TCCTCTGAGCCTCTTACACTCGGACCTCGTCGACATGCATT</u> AGCCCG <u>GGTGGTTCTATTTCACACTCGGACCTCGTCGACATGCATT</u> CCAGCC <u>GATGTTCATGTCCACACTCGGACCTCGTCGACATGCATT</u> CTACTT <u>GTCCCTTCTTGCTGTTACACTCGGACCTCGTCGACATGCATT</u> TGACTT <u>CTTCTTGCTTACCCACACTCGGACCTCGTCGACATGCATT</u> TGAGCAC <u>CTAGGATCTTCCACACTCGGACCTCGTCGACATGCATT</u> GAAC <u>TTCCCTCCTCCTGATAACACTCGGACCTCGTCGACATGCATT</u> TCCT <u>CTCTCCTTCAATTGGTTACACTCGGACCTCGTCGACATGCATT</u>
Secondary probe	5'-/56-TAMN/AATGCATGTCGACGGGTCCGAGTGTA <u>A/36-TAMSp/-3'</u>
	<i>Mafa - Alex647</i>
Primary probes	CTTCACCTCGAAC <u>TTCATCACCTCTAAAGTTCGAGCTGGACTCAGTG</u> GCT <u>TCATCCAGTACAGATCCTCCTCTAAAGTTCGAGCTGGACTCAGTG</u> CCA <u>ACTTCTCGTATTCTCCCTCTAAAGTTCGAGCTGGACTCAGTG</u> CAGAAAG <u>AGTCGGGTGCGCCCTCTAAAGTTCGAGCTGGACTCAGTG</u>

	GAGGTTGGGACGCAGAACTGCCTCCTAAGTTCGAGCTGGACTCAGTG GTAAGTTCCCTCGGCTTCCTC <u>CCTCCTAAGTTCGAGCTGGACTCAGTG</u> CTCTCTTCCGGCTAGTCAA <u>CCTCCTAAGTTCGAGCTGGACTCAGTG</u> CGATTGTCTAGCGTCCTGT <u>CCTCCTAAGTTCGAGCTGGACTCAGTG</u>
Secondary probe	5'-/5Alex647N/CACTGAGTCCAGCTCGAAACTTAGAGG/3AlexF647N/-3'

Table S1: The primary and secondary probe sequences used in this study. Underlined parts of each probe are the overhang sequences designed to hybridize with the secondary probes.

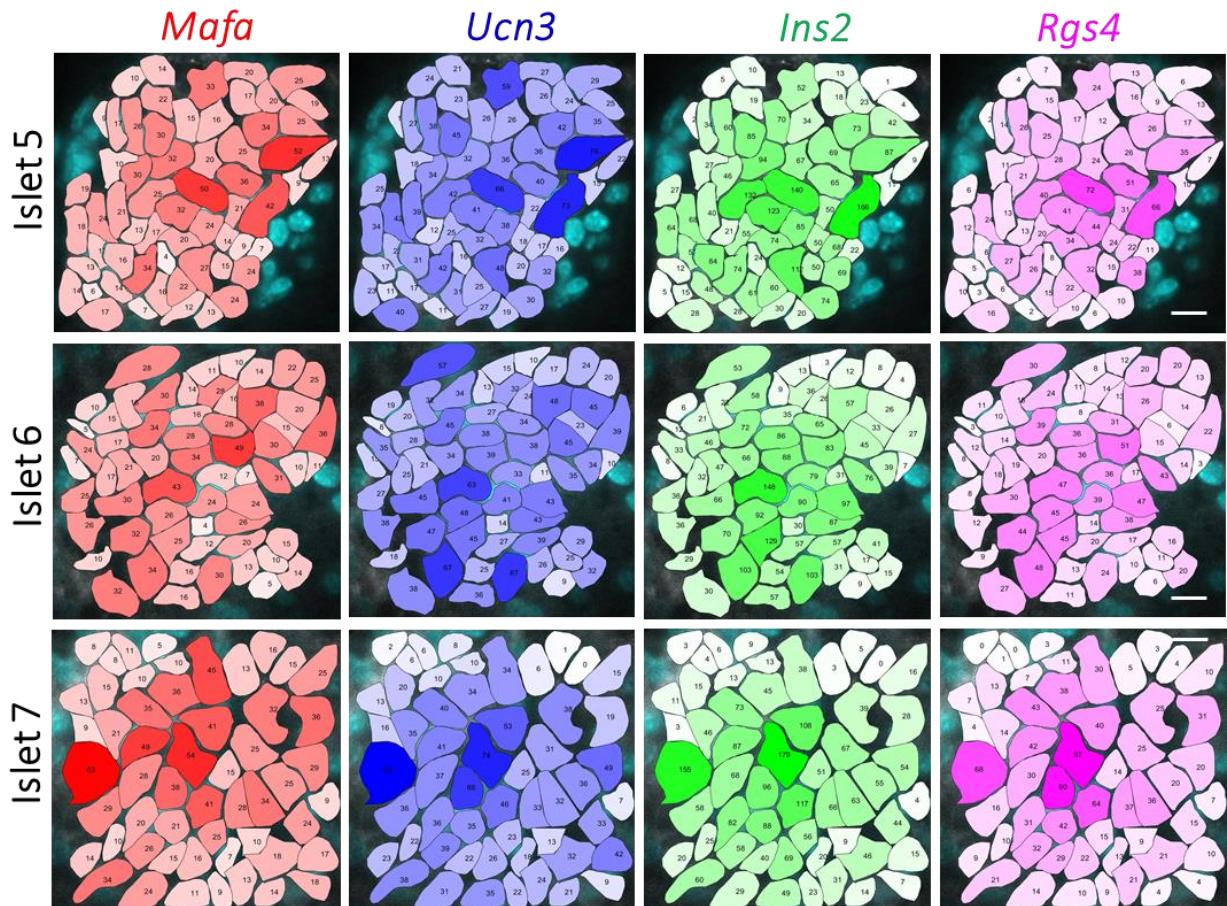


Figure S1: Supplement to Figure 2: Mapping gene expression levels in single islet cells. Each horizontal panel shows the same islet, where transcript counts for *Mafa* (red), *Ucn3* (blue), *Ins2* (green) and *Rgs4* (magenta) are indicated by the numbers placed over each cell. Each cell was assigned an intensity level or shade of the respective gene colors based on the ratios between the transcript counts in the cell and the highest count found in that islet per cell. Thus, cells with high transcript counts are shown in intense or bright colors while cells with low transcript counts are shown in faint or light colors. Scale bars equal 10 μ m.

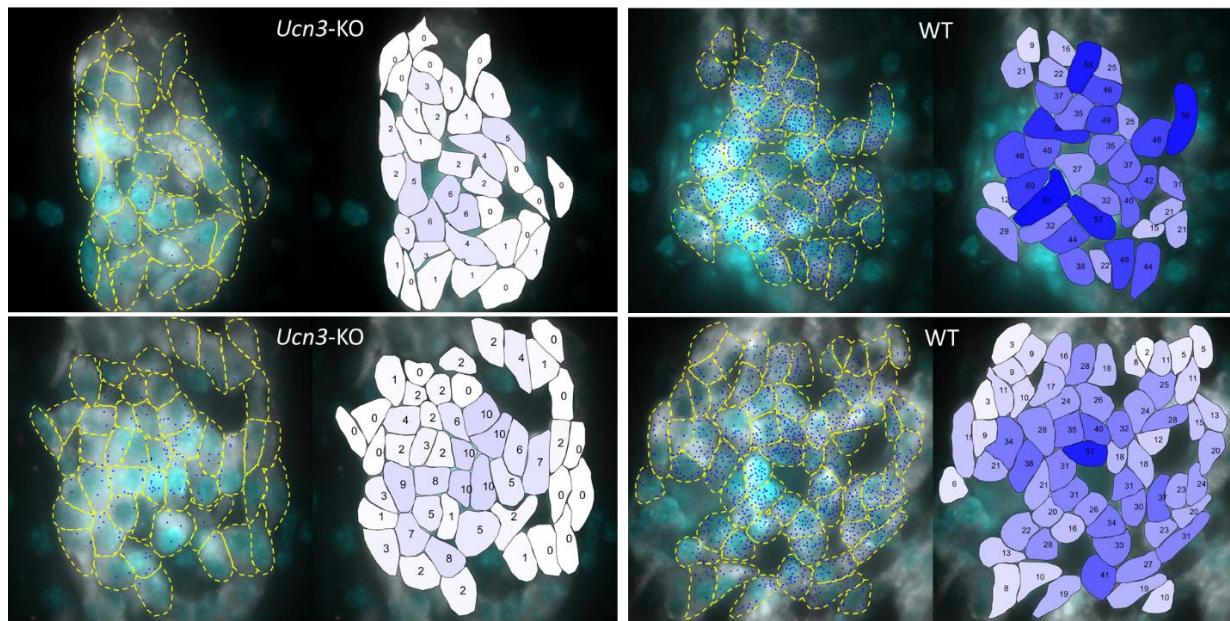


Figure S2: Validation of the fliFISH specificity by comparing *Ucn3* transcripts in pancreatic islets from *Ucn3* knockout (KO, left panel) and wildtype (WT, right panel) mice. In the *Ucn3*-KO islets, very low counts are detected toward the islet edge (~0-4 transcripts per cell) and slightly higher counts are detected in cells closer to the center (~5-10 transcripts per cell). These differences are also shown by the shade of the blue color, assigned to each cell based on the number of detected transcripts using the same scale as in Figures 2 and S1. These values are significantly lower than the values found in wildtype islets and likely originate from nonspecific probe binding. If these low counts reflect residual *Ucn3* expression, the slightly lower counts in cells at the islet edge could potentially result from their proximity to the acinar RNases. However, Figure S4 shows that cells with very high GAPDH counts or even the highest counts can appear at the islet edge, ruling out the involvement of acinar RNases.

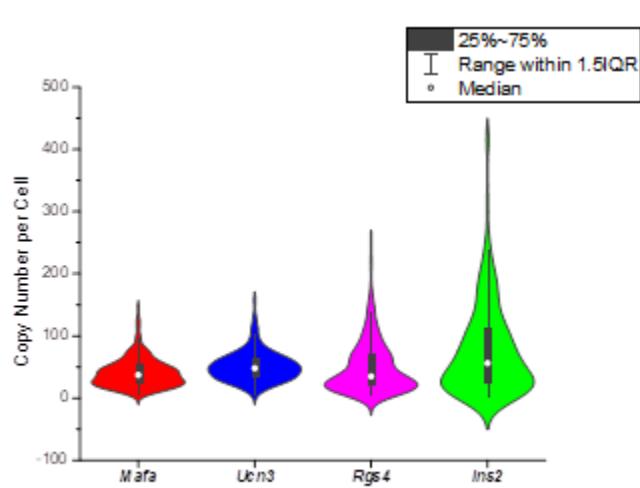


Figure S3: The distribution of the number of transcripts per cell for each gene over the 7 islets shown in Figure 2 and Figure S1.

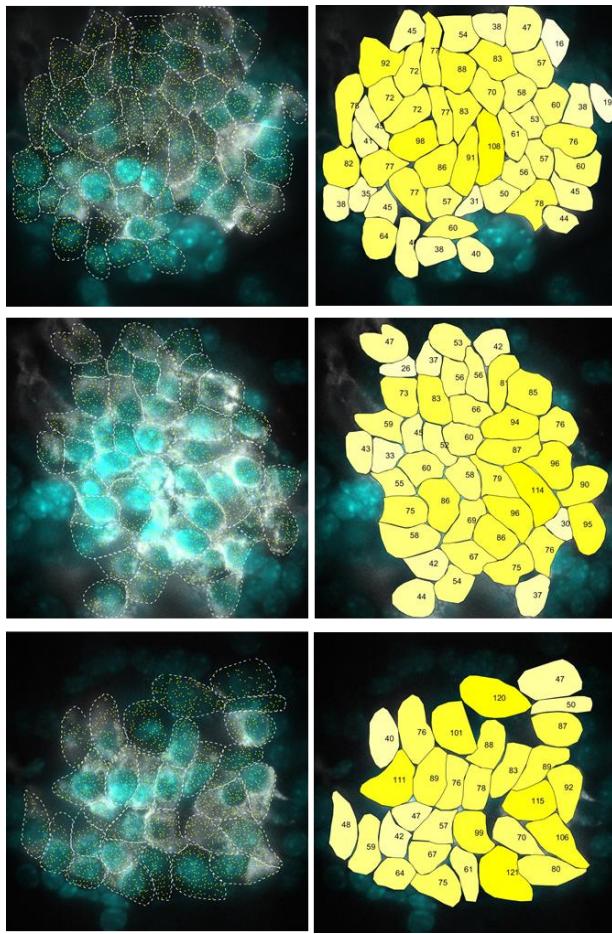


Figure S4: No radial pattern is observed in the number of transcripts per cell for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a house-keeping gene expected to be expressed relatively uniformly in all cells. Three representative islets are shown. In the images on the left panel, the nuclei are stained with DAPI (light blue), GAPDH transcripts are shown as yellow dots, and insulin protein is detected by the specific antibody (gray). The right panel shows the same islets as on the left panel, where the number of transcripts is indicated for each cell and the relative expression level per cell is indicated by the yellow shade (the brighter the yellow the higher the number of transcripts).

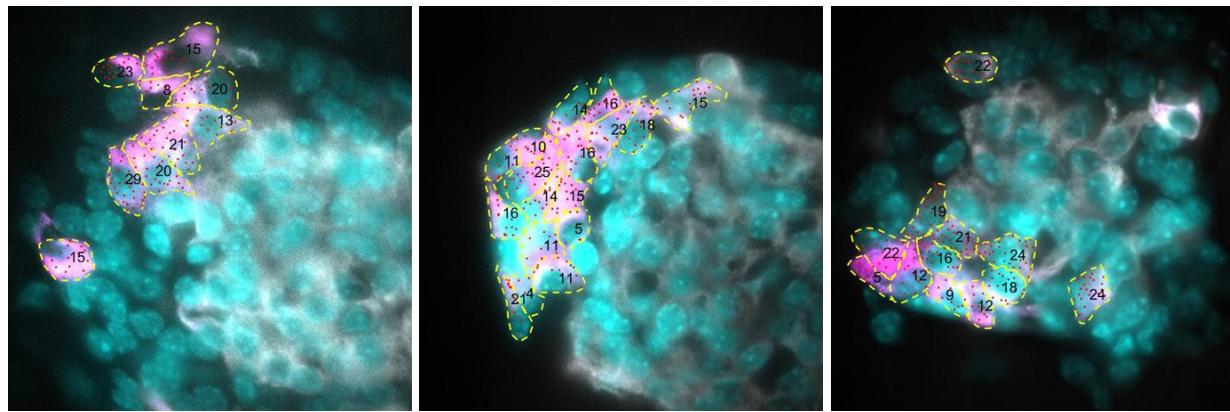


Figure S5: Representative images of pancreatic islets, where nuclei are stained with DAPI (light blue), β cells are identified by insulin antibody (white/gray), α cells are recognized by glucagon antibody (magenta), and *Rgs4* transcripts are marked with red dots. The number of transcripts per cell is shown by the number placed over each cell.