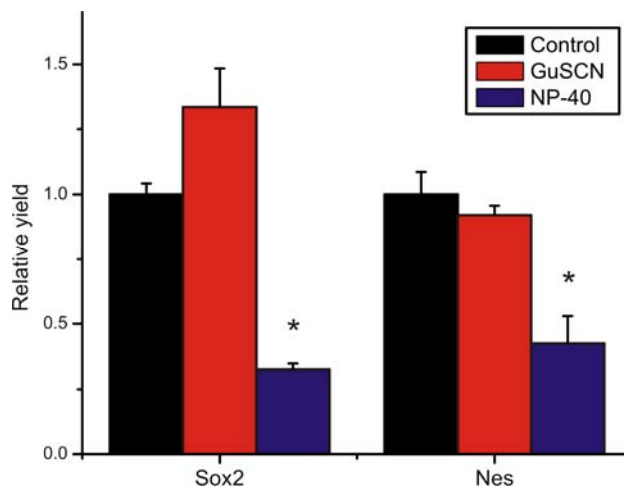
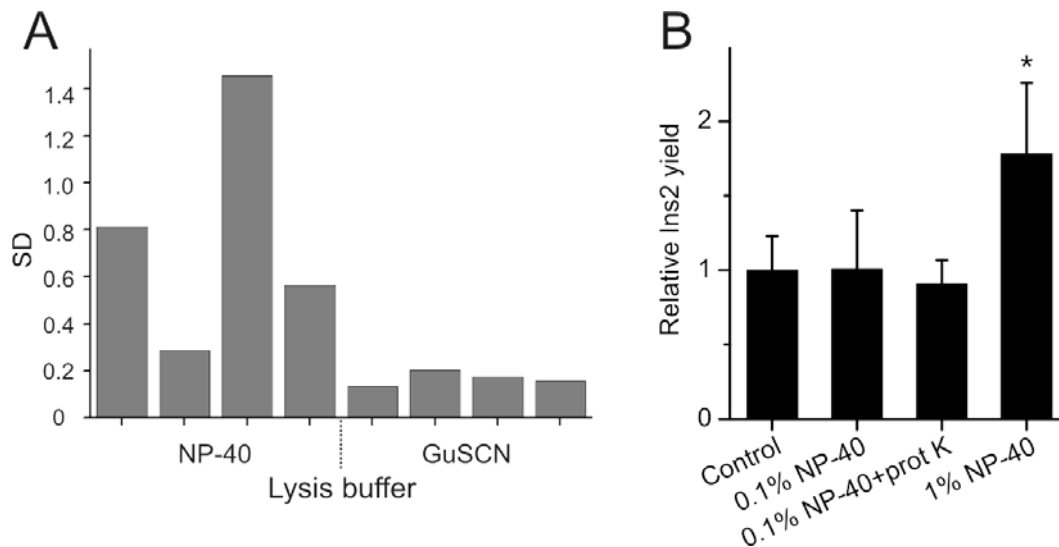


**Additional file 1: Supplementary figures and tables for**  
***Quantification of mRNA in single cells and***  
***modelling of RT-qPCR induced noise***

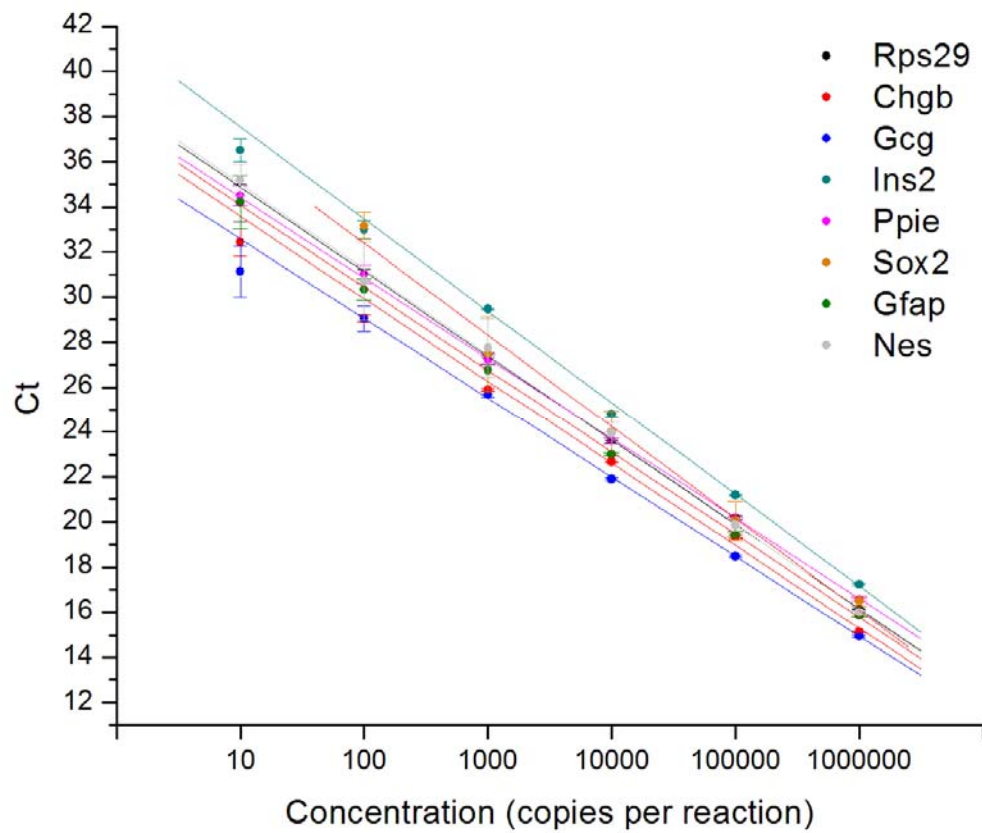
*Martin Bengtsson, Martin Hemberg, Patrik Rorsman and Anders Ståhlberg*



**Additional File 1, Figure 1:** Evaluation of lysis buffers on primary astrocytes. Primary astrocytes were dissociated into single cells by Trypsin/EDTA treatment. Trypsin was inactivated by cell medium and washed once in PBS. Each bar indicates relative yield of Sox2 and Nes from equally pooled astrocytes as starting material. Each sample was treated with indicated concentrations of either mQ-water (control), 0.5 M guanidine thiocyanate (GuSCN) or 0.5% NP-40 all supplemented with RNase inhibitor (2U/ $\mu$ L). The value of the control was arbitrarily set to 1. Values are mean  $\pm$  SEM for 4 separate experiments (\*  $p < 0.01$  compared to control).

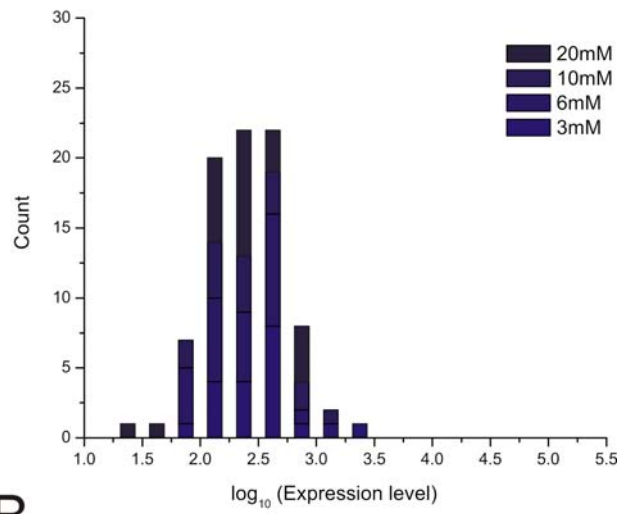


**Additional file 1, Figure 2:** (A) Eight single-cell samples analysed in triplicate RT-reactions; four samples with lysis buffer containing 0.5% NP-40 and four with 0.5 M GuSCN. Intra-assay variation for each sample is shown. (B) Effect of lysis buffers on RT reaction yield. Identical amounts of purified islet total RNA were used as starting material. Relative yield of reverse transcribed *Ins2* are shown for samples supplemented with NP-40 and proteinase K (prot K). The value of the control (water) was arbitrarily set to 1. Values are mean  $\pm$  SEM for 3 separate experiments (\*  $p < 0.01$  compared to control).

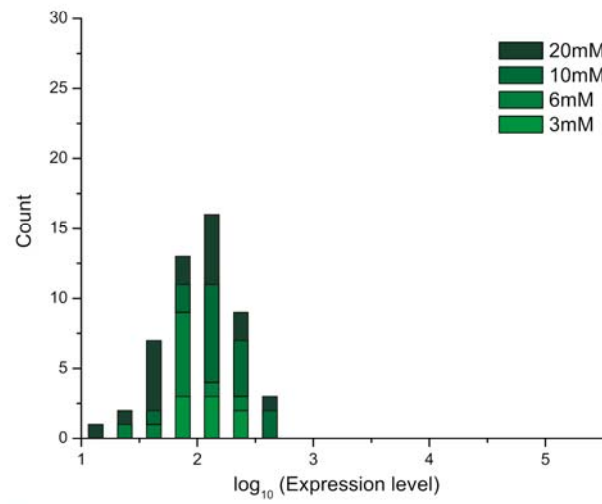


**Additional file 1, Figure 3:** Standard curves based on purified PCR-products. Each data point is the average of triplicate reactions with SD indicated with bars. The standard curve is generated by linear regression ( $R > 0.99$  for all assays). The slope ( $a$ ) is used to derive the PCR efficiencies, using the equation  $E = 10^{(-1/a)} - 1$  (*Rps29*: 0.86; *Chgb*: 0.95; *Gcg*: 0.92; *Ins2*: 0.81; *Ppie*: 0.90; *Sox2*: 0.77; *Gfap*: 0.88; and *Nes*: 0.85).

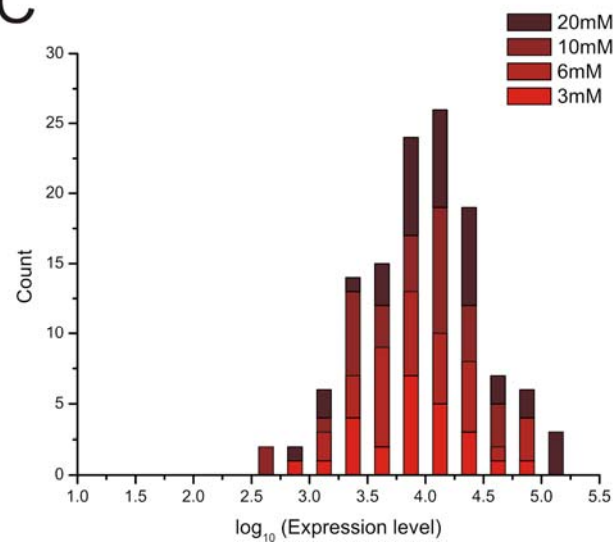
A



B



C



**Additional file 1, Figure 4:** Histograms showing expression levels of *Rps29* (A), *Chgb* (B) and *Ins2* (C) in single pancreatic  $\beta$ -cells at four different glucose concentrations (3, 6, 10 and 20 mM).

**Additional file 1, Table 1: Effect of contaminants from cell collection on downstream reaction yields**

| Gene        | Control    | 1) EC       | 2) one cell | 3) ten cells |
|-------------|------------|-------------|-------------|--------------|
| <i>Ins2</i> | 1.0 (0.15) | 0.98 (0.21) | 1.9* (0.67) | 2.4* (1.1)   |
| <i>Gcg</i>  | 1.0 (0.20) | 1.1 (0.21)  | 2.2* (0.71) | 2.9* (1.4)   |
| <i>Hprt</i> | 1.0 (0.18) | 1.0 (0.24)  | 1.8* (0.20) | 1.5 (0.30)   |

Fold difference (SD) in qRT-PCR yield compared to control conditions (water) when complementing the reverse transcription reaction with: 1) 0.2 µl extracellular solution (EC); 2) one or 3) ten cell/s collected in EC and transferred to 1 µl lysis buffer containing 0.5 M GuSCN. All tubes were complemented with identical amounts of purified total islet RNA prior the reverse transcription reaction. \* P<0.05 compared to corresponding control (n=8).

**Additional file 1, Table 2: Mitigation of PCR-inhibition by reduction of RT-enzyme concentration and increase in Taq polymerase-concentration**

| cDNA load into PCR:            | 5% cDNA    | 20% cDNA       | 20% cDNA   |
|--------------------------------|------------|----------------|------------|
| Enzyme units (U) per reaction: | 0.25 U Taq | 0.25 U Taq     | 2.75 U Taq |
| 100 U RT                       | 21.0 (20)  | No signal (80) | 24.5 (7.3) |
| 40 U RT                        | 20.1 (8)   | No signal (32) | 22.1 (2.9) |
| 10 U RT                        | 17.2 (2)   | 19.7 (8)       | 15.6 (0.7) |

Average Ct-values of *Ins2* and (in parenthesis) RT:Taq ratio (U/U) in the PCR-reaction. Italic style indicates inhibited PCR. Both RT- and PCR-reactions were 10 µl. Taq = Taq polymerase, RT = reverse transcriptase.

**Additional file 1, Table 3: PCR primer sequences**

| Gene abbrev. | Full name                                       | Fwd primer (5'-3')                                | Rev primer (5'-3')    | Intron spanning | NCBI Gene ID |
|--------------|-------------------------------------------------|---------------------------------------------------|-----------------------|-----------------|--------------|
| <i>Ins1</i>  | Insulin I                                       | TAGTGACCAGCTATAATCAGAG                            | ACGCCAAGGTCTGAAGGTCC  | yes             | 16333        |
| <i>Ins2</i>  | Insulin II                                      | CCCTGCTGGCCCTGCTCTT                               | AGGTCTGAAGGTCACCTGCT  | yes             | 16334        |
| <i>Gcg</i>   | Glugacon                                        | CTACACCTGTTCGCACTCA                               | CTGGGGTTCTCCTCTGTGTC  | yes             | 14526        |
| <i>Rps29</i> | Ribosomal protein S29                           | GGAGTCACCCACGGAAGT                                | TCCATTCAAGGTCGCTTAGTC | yes             | 20090        |
| <i>Chgb</i>  | Chromogranin B                                  | CAAATGCCCTATCCAAGTCC                              | TCACCTTTGACCTCTTTTCCA | yes             | 12653        |
| <i>Hprt</i>  | hypoxanthine guanine phosphoribosyl transferase | AGCCCCAAAATGGTTAAGGT                              | CAAGGGCATATCCAACAACA  | yes             | 15452        |
| <i>Sst</i>   | Somatostatin                                    | CCACCGGGAAACAGGAAC                                | GCTCCAGCCTCATCTCGTC   | yes             | 20604        |
| <i>Gapdh</i> | Glyceraldehyde-3-phosphate dehydrogenase        | AGCGAGACCCCACTAACATC                              | GGTTCACACCCATCACAAAC  | yes             | 407972       |
| <i>Sox2</i>  | SRY-box containing gene 2                       | AAAAACCACCAATCCCATCC                              | AGTCCCCCAAAAAGAAGTCC  | no <sup>1</sup> | 20674        |
| <i>Gfap</i>  | Glial fibrillary acidic protein                 | AACCGCATCACCATTCTT                                | CGCATCTCCACAGTCTTTACC | yes             | 14580        |
| <i>Nes</i>   | Nestin                                          | AGCAACTGGCACACCTCAA                               | GGTATTAGGCAAGGGGAAG   | yes             | 18008        |
| <i>Ppie</i>  | Cyclophilin E (control)                         | CAACGGCTCCCAAGTTCTTC                              | TCTGCTTTGGCTTCCCATCC  | N.A.            | 56031        |
| <i>Ppie</i>  | Extended forward primer including T7 promoter   | ATTAATACGACTCACTATAGGG<br>AGACAACGGCTCCCAAGTTCTTC |                       |                 |              |

<sup>1</sup>single-exon gene; intron-spanning primer design not possible. N.A. = not applicable.