**SUPPLEMENTARY MATERIAL**

Proinflammatory cytokines perturb mouse and human pancreatic islet circadian rhythmicity and induce uncoordinated β-cell clock gene expression via nitric oxide, lysine deacetylases and immunoproteasomal activity

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**Table S1: Human islet donor characteristics**

|  |  |  |  |
| --- | --- | --- | --- |
| Donor  no. | Sex | Age  (years) | BMI (kg/m2) |
| 11 | M | 46 | 27.2 |
| 22 | M | 66 | 27.0 |
| 32 | M | 25 | 27.3 |
| 41 | F | 55 | 21.9 |

M, male sex; F, female sex.

1Donor provided by Islet Transplantation Center of Geneva University Hospital;

2Donors provided by Prodo Laboratories (California, US).

**Table S2: Primer sequences**

|  |  |  |
| --- | --- | --- |
| *Target gene* |  | *Primer sequence* |
| 5S rRNA | *Forward* | 5'-TCTTTGGGAAATGGAGCACT-3' |
| *Reverse* | 5'-ATGAGCTTCTTGCCGTTGTT-3' |
| *Arg-1* | *Forward* | 5'-TATCGGAGCGCCTTTCTCTA-3' |
| *Reverse* | 5'-ACAGACCGTGGGTTCTTCAC-3' |
| *β-Actin* | *Forward* | 5'-CACCCGCGAGTACAACCTTC-3' |
| *Reverse* | 5'-CCCATACCCACCATCACACC-3' |
| *Bmal1* | *Forward* | 5'-TAAACTCACCGTGCTCAGGA-3' |
| *Reverse* | 5'-CGGTCACATCCTACGACAAA-3' |
| *Chop* | *Forward* | 5'-CAGCGACAGAGCCAAAATAAC-3' |
| *Reverse* | 5'-TGTGGTGGTGTATGAAGATGC-3' |
| *Clock* | *Forward* | 5'-ACTATACAGCGCACACACAGG-3' |
| *Reverse* | 5'-TGTGAACTCTTCATTCGGTTCT-3' |
| *Cry1* | *Forward* | 5'-CCTTGCCTCAGTCCCTTCTA-3' |
| *Reverse* | 5'-GTGCGTCCTCTTCCTGACTT-3' |
| *Cry2* | *Forward* | 5'-CCTCTTCTACTACCGCCTGTG-3' |
| *Reverse* | 5'-ATTCTCGCCATAGGAGTTGTC-3' |
| Fas | *Forward* | 5’-TGAGGGTTTGGAGTTGAAGAG-3’ |
| *Reverse* | 5’-CCACTTGTTGTGCAGTCCTTA-3’ |
| *Hprt1* | *Forward* | 5'-GCAGACTTTGCTTTCCTT-3' |
| *Reverse* | 5'-CCGCTGTCTTTTAGGCTT-3' |
| *Inos* | *Forward* | 5'-CACCACCCTCCTTGTTCAACA-3' |
| *Reverse* | 5'-CAATCCACAACTCGCTCCAA-3' |
| *Ins-1* | *Forward* | 5'-GGGGAACGTGGTTTCTTCTAC-3' |
| *Reverse* | 5'-CCAGTTGGTAGAGGGAGCAG-3' |
| *Ins-2* | *Forward* | 5'-CAGCACCTTTGTGGTTCTCA-3' |
| *Reverse* | 5'-CACCTCCAGTGCCAAGGT-3' |
| Nfkbia  (IκBα) | *Forward* | 5’-ATTACGAGCAGATGGTGAAGG-3’ |
| *Reverse* | 5’-GGTCAGTGTCTTCTCTTCATGG-3’ |
| *Per1* | *Forward* | 5'-GCTCCATTGCCTATAGTCTCCT-3' |
| *Reverse* | 5'-AAGTGCGGTCATGAGTTCTTT-3' |
| *Per2* | *Forward* | 5'-GTGACTGTGACGACAGTGGAA-3' |
| *Reverse* | 5'-CTTGTGGAGGGGTTATGCTC-3' |
| *Ppia* | *Forward* | 5'-AGCACTGGGGAGAAAGGATT-3' |
| *Reverse* | 5'-GATGCCAGGACCTGTATGCT-3' |
| *Rev-erbα* | *Forward* | 5'-TTTGGACGTATCCCCAAGAG-3' |
| *Reverse* | 5'-CTGAGAGAAGCCCACCAAAG-3' |



**Figure S1: Cytokines do not cause apoptosis in synchronized mouse or human Per2-luc reporter islets.** Islet cell apoptosis was measured using Cell Death ELISA Kit (Roche) at the end of the bioluminescence recording experiments from mouse (**A**, n = 2 islet isolations with 2-3 animals per isolation) and human (**B**, n = 3 human donors) islets. Values are means ± SEM.



**Figure S2:** **Time-dependent differential responses in clock gene expression to proinflammatory cytokines in non-synchronized INS-1 cells and lack of effects of cytokines on response to forskolin.** (**A**) INS-1 cells were exposed to 150 pg/ml mouse IL-1β + 0,1 ng/ml rat IFN-γ (Cyt) at 4 hours intervals at 8-36 hours. Relative mRNA expression is calculated using *Hprt1* as reference gene (n = 5). (**B, C**) INS-1 cells were treated with 10 µM forskolin, for either one-hour pulse with or without cytokines, following one-hour exposure to control media or cytokines (**B**) or 12-hours preincubation with cytokines following one-hour forskolin pulse in normal media (**C**). Samples were collected one hour after the one-hour forskolin pulse. Relative mRNA expression is calculated using *Hprt1* and 5S rRNA as reference genes (n = 3-4). Values are means ± SEM. Statistics are paired Student’s t-test. ns: not significant.



**Figure S3:** **SR9009 does not sensitize non-synchronized INS-1 cells to cytokine-mediated cytotoxicity.** INS-1 cells were exposed to increasing concentrations of SR9009 in combination with 150 pg/ml mouse IL-1β + 0,1 ng/ml rat IFN-γ (Cyt) for 12 or 24 hours to assess the effect on cell viability. Values are means ± SEM (n = 3). Statistics are one-way ANOVA with p-values represented by symbols above the line and with Dunnett’s corrected multiple comparisons to Ctrl (black bars) or to Cyt (grey bars).



**Figure S4:** **Glucolipotoxicity increases apoptosis and perturb the clockwork in human or mouse Per2-luc reporter islets.** (**A**) Levels of apoptosis in human islets (n = 3) exposed to a combination of 500 nM palmitate and 20 mmol glucose (GLT); to 20 mmol glucose (GLU), or to BSA alone, were measured by Cell Death ELISA Kit (Roche) at the end of the bioluminescence recording experiments. (**B**) Average raw (left panel) and detrended (right panel) Per2-luc oscillatory profiles of forskolin-synchronized mouse islets (n = 2 independent isolations from 2-3 animals each) exposed to GLT. (**C**) Levels of apoptosis in mouse islets (n = 2 experiments). Values are means or means ± SEM.



**Figure S5: Glucolipotoxicity time-dependently and differentially affects clock gene expression in non-synchronized INS-1E cells.** INS-1E cells were exposed to 0.5 mM palmitate+ 25mM glucose (GLT) for 6, 12, 18 or 24 hours. Relative mRNA expression is calculated using *Hprt1* as reference gene. Values are means ± SEM (n = 4). Statistics are repeated measures one-way ANOVA with Bonferroni corrected multiple comparisons between Ctrl and GLT at similar timepoints.



**Figure S6:** **iNOS or KDAC inhibition does not affect cytokine-perturbation of the clockwork in synchronized mouse Per2-luc reporter islets**. (**A-B**) Average raw (left panel) and detrended (right panel) Per2-luc oscillatory profiles of forskolin-synchronized mouse islets exposed to a combination of 300 pg/ml IL-1β + 0.2 ng/ml IFN-γ (Cyt) in the presence of Givinostat (**A**, n = 3 experiments) or iNOS inhibitor NG-methyl-L-arginine (NMA, **B**, n = 3 experiments) during the entire recording. (**C**) Average raw (left panel) and detrended (right panel) Per2:Luc bioluminescence profiles of forskolin-synchronized mouse islets exposed to GLT in the presence or absence of NMA during the entire recording (n = 2 experiments). Values are means or means ± SEM.



**Figure S7: Class I** **KDAC inhibition reduces cytokine mediated changes in clock gene expression in non-synchronized INS-1 cells.** INS-1 cells were treated with 1 μM MS-275 for 1 hour pre-incubation followed by 12 hour co-incubation with or without 150 pg/ml mouse IL-1β + 0,1 ng/ml rat IFN-γ (Cyt). Relative mRNA expression is calculated using *Ppia* as reference gene. Values are means ± SEM (n = 6). Statistics are paired Student’s t-test.



**Figure S8: NAC dose-dependently lowers ROS and superoxide production in INS-1 cells**. INS-1 cells were treated with different doses of NAC for 12 hours with or without or without 150 pg/ml mouse IL-1β + 0,1 ng/ml rat IFN-γ (Cyt). Florescent values from the two probes using ROS-ID® Total ROS/Superoxide detection kit are normalized to the viability of the cells. Values are means ± SEM of 5-6 technical replicates.



**Figure S9: Effects of SR9009 on NO synthesis in non-synchronized INS-1 cells.** INS-1 cells were exposed to increasing concentrations of SR9009 alone or in combination with 150 pg/ml mouse IL-1β + 0,1 ng/ml rat IFN-γ (Cyt). Accumulated nitrite was assessed after 12 (**A**) or 24 (**B**) hours exposure. Relative expression of *Inos* (**C**) and *Arg-1* (**D**) were assessed following 12 hours exposure, using *Hprt1* as reference gene. Values are means ± SEM (n = 3). Statistics are one-way ANOVA with p-values represented by symbols above the line and with Dunnett’s corrected multiple comparisons to Ctrl (black bars) or to Cyt (grey bars).