SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1. NF-κB activation potentiates IFN-γ-induced IRF-1 up-regulation. (A-B) Primary β-cells were transfected with siCtrl, siIRF-1 or siSTAT1. After 24h, cells were exposed to IL-1β+IFN-γ for 12 or 24h. STAT1 (A) and IRF-1 (B) mRNA expressions were assayed by real time PCR and normalized for the housekeeping gene GAPDH. (C-D) INS-1E cells were left untreated, or exposed to IFN-γ, IL-1β+IFN-γ or TNF-α+IFN-γ for 30 min, 1h, 2h, 4h, 8h or 24h. (C) IRF-1 and α-tubulin protein expression were evaluated by Western blot. (D) Densitometry of IRF-1 corrected by α-tubulin. (E) INS-1E cells were left uninfected, or infected with Ad-Luc or Ad-srIκB and subsequently treated with cytokines as indicated. IRF-1, IκBα and α-tubulin expressions were probed. §: $p<0.05$, §§: $p<0.01$ vs IFN-γ-treated at the same time point, ANOVA followed by Student's *t* test with Bonferroni correction.

Supplementary Fig. 2. STAT1 knockdown by a second siRNA also protects INS-1E cells against cytokine-induced apoptosis. INS-1E cells were left untransfected (NT), or transfected either with 30 nM (unless indicated otherwise) of siCtrl, siSTAT1 or siSTAT1 #2, siIRF-1 or siIRF-1 #2. After 24h of recovery, cells were left untreated or exposed to IFN-γ 100 U/ml, IL-1β 10 U/ml + IFN-γ 100 U/ml or TNF-α 1000 U/ml + IFN-γ 100 U/ml for 24h as indicated. (A, B & C) STAT1, IRF-1 and α-tubulin protein expression were evaluated by Western blot. Results are representative of 3-5 independent experiments. (D & E) Apoptosis was evaluated using HO/PI staining. Results are mean ± SEM of 3-5 independent experiments. ***: $p<0.001$ vs untreated (i.e. not treated with cytokines) or untreated transfected with the same siRNA; §§: $p<0.01$ and §§§: $p<0.001$ vs NT & siCtrl treated with cytokines at the same time point, ANOVA followed by Student's *t* test with Bonferroni correction.

Supplementary Fig. 3. Cytokine-modified functions and canonical pathways regulated by IRF-1 and STAT1 in insulin-producing INS-1E cells. The figure represents top selected functions (A) and canonical pathways (B) identified using the IPA software that were significantly modified by cytokine treatment alone (black bars), by combined cytokine treatment and IRF-1 silencing (dark grey bars) or by combined cytokine exposure and STAT1 silencing (light grey bars). The selection of the represented functions and pathways were based on higher statistical significance and potential relevance in the context of insulitis or β-cell function and survival upon cytokine treatment.

Supplementary Fig. 4. STAT1 overexpression exacerbates cytokine-induced DP5 up-regulation. (A-C) INS-1E cells were transfected with pCMV-Ctrl or pCMV-STAT1. After overnight incubation, the cells were left untreated (time 0) or exposed to IL-1β 10 U/ml + IFN-γ 100 U/ml for 2, 4, 8 16 or 24h as indicated. (A & C) STAT1 and DP5 mRNA expressions were assayed by real time RT-PCR and normalized for the housekeeping gene GAPDH. Results are mean ± SEM of 4 independent experiments. (B) STAT1 and α-tubulin protein expression were evaluated by Western blot in untreated pCMV-Ctrl- and pCMV-STAT1-transfected cells 24h after transfection. (D & E) INS-1E cells were left untransfected (NT), or transfected with 30 nM of siCtrl, siDP5, siSTAT1 or siDP5 + siSTAT1. After 24h of recovery, cells were left untreated or exposed to IL-1β 10 U/ml + IFN-γ 100 U/ml for 24h as indicated. (E) Apoptosis was evaluated using HO/PI staining. (E) Nitrite concentrations in supernatants were evaluated as described in Methods. Results are mean ± SEM of 4 independent experiments. *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ vs respective untreated control; §: $p<0.05$, §§§: $p<0.001$ vs respective control treated with cytokines at the same time point, ###: $p<0.001$ vs siDP5 treated with cytokines at the same time point; +++ $p<0.001$ vs siSTAT1 treated with cytokines at the same time point , ANOVA followed by Student's *t* test with Bonferroni correction.

Supplementary Fig. 5. IRF-1 overexpression hampers cytokine-induced STAT1 induction and chemokine production. (A) INS-1E cells were left untransfected (NT), or transfected with 30 nM of

siCtrl, siIRF-1 or siIRF-1 #2. After 24h of recovery, cells were left untreated or exposed to IL-1β 10 U/ml + IFN-γ 100 U/ml for 2, 4, 8, 16 or 24h as indicated. (A) Mean optical density measurements of phospho-STAT1 corrected for α-tubulin from Figure 6A. Results are mean fold variation ± SEM of 5 independent experiments. (B) INS-1E cells were co-transfected an NF-κB reporter + pRL-CMV alone (NT) or in combination with siCtrl, siIRF-1 or siIRF-1 #2. After 1 day of recovery, cells were left untreated or exposed to IL-1β 10 U/ml + IFN-γ 100 U/ml for 24h as indicated. Results are mean Relative Luciferase Unit (R.L.U.) ± SEM of 5 independent experiments. (C-F) INS-1E cells were transfected with pCMV-Ctrl or pCMV-IRF-1. After overnight incubation, the cells were left untreated (time 0) or exposed to IL-1β 10 U/ml + IFN-γ 100 U/ml for 2, 4, 8 16 or 24h as indicated. IRF-1, STAT1, CXCL1 and CXCL9 mRNA expressions were assayed by real time RT-PCR and normalized for the housekeeping gene GAPDH. Results are mean ± SEM of 4 independent experiments. (G) INS-1E cells were transfected with 30 nM of siCtrl, siSOCS-1 or siSOCS-1 #2. After 24h of recovery, cells were left untreated or exposed to IL-1β 10 U/ml + IFN-γ 100 U/ml for 2, 4, 8, 16 or 24h as indicated. Phospho-STAT1and α-tubulin protein expressions were evaluated by Western blot. Pictures are representative of 3 independent experiments. *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ vs respective untreated control; §: $p<0.05$, §§: $p<0.01$ and §§§: $p<0.001$ vs respective control treated with cytokines at the same time point, ANOVA followed by Student's *t* test with Bonferroni correction.

SUPPLEMENTARY TABLES

Suppl. Table 1. Effects of cytokine treatment and IRF-1- or STAT1 silencing on the expression of selected genes detected by array analysis (online appendix).

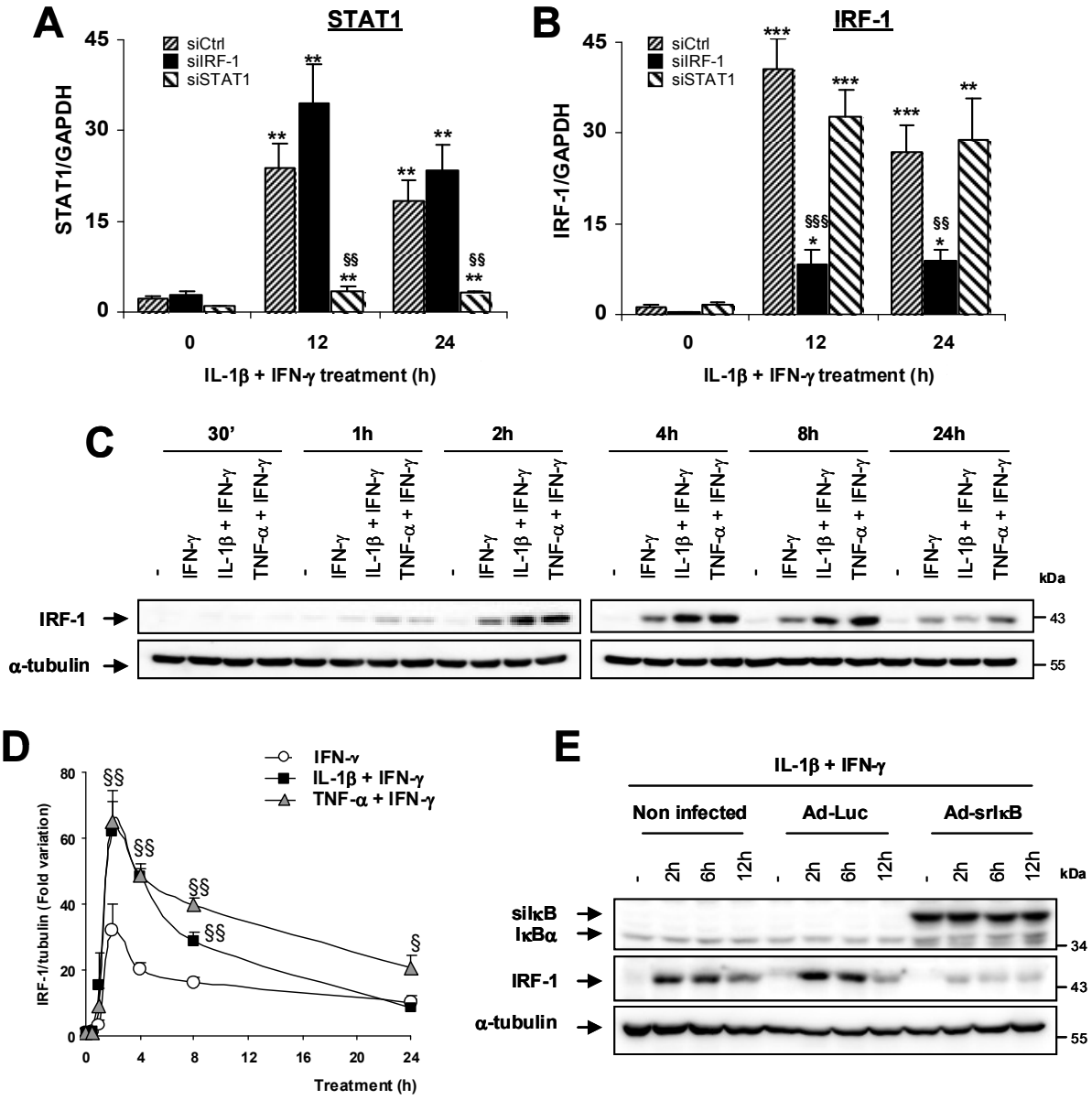
Suppl. Table 2. List of all transcripts detected as present by array analysis in INS-1E cells exposed to IL-1β + IFN-γ for 2h (online appendix).

Suppl. Table 3. List of all transcripts detected as present by array analysis in INS-1E cells exposed to IL-1β + IFN-γ for 12h (online appendix).

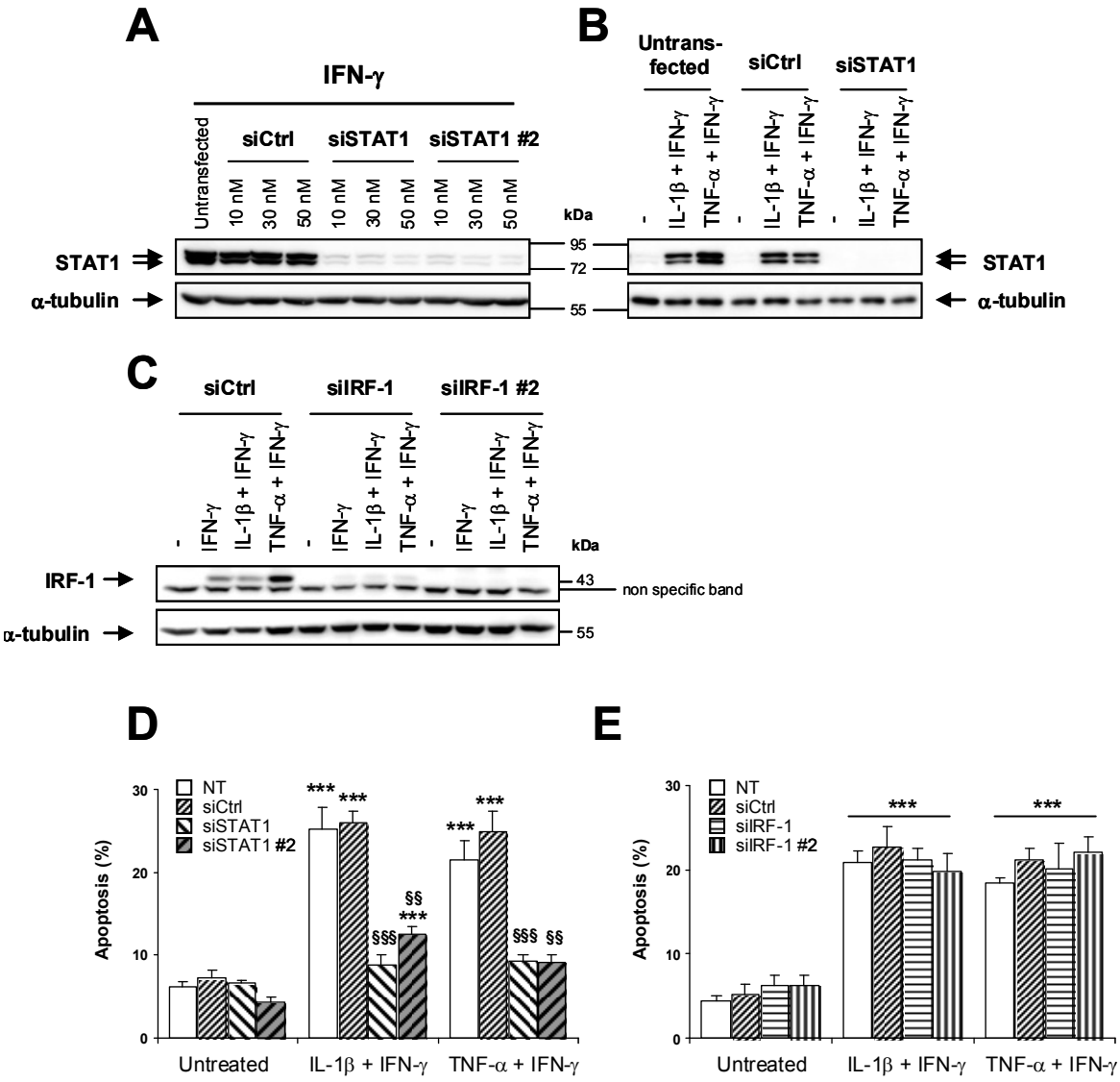
Suppl. Table 4. List of all transcripts detected as present by array analysis in INS-1E cells exposed to IL-1β + IFN-γ for 24h (online appendix).

Suppl. Table 5. List of transcripts that are differentially regulated after STAT1 silencing in untreated INS-1E cells (online appendix).

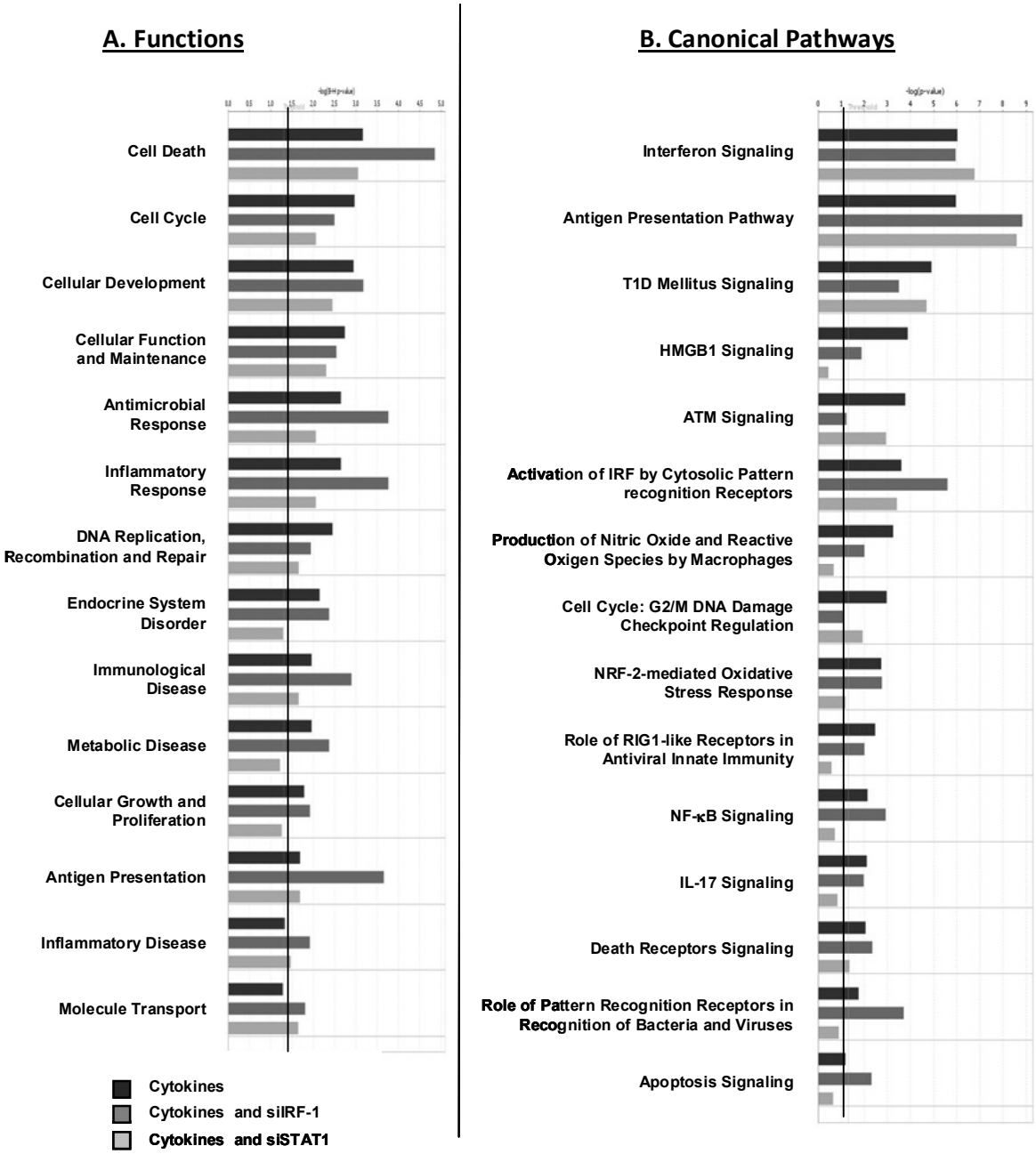
Supplementary Figure 1



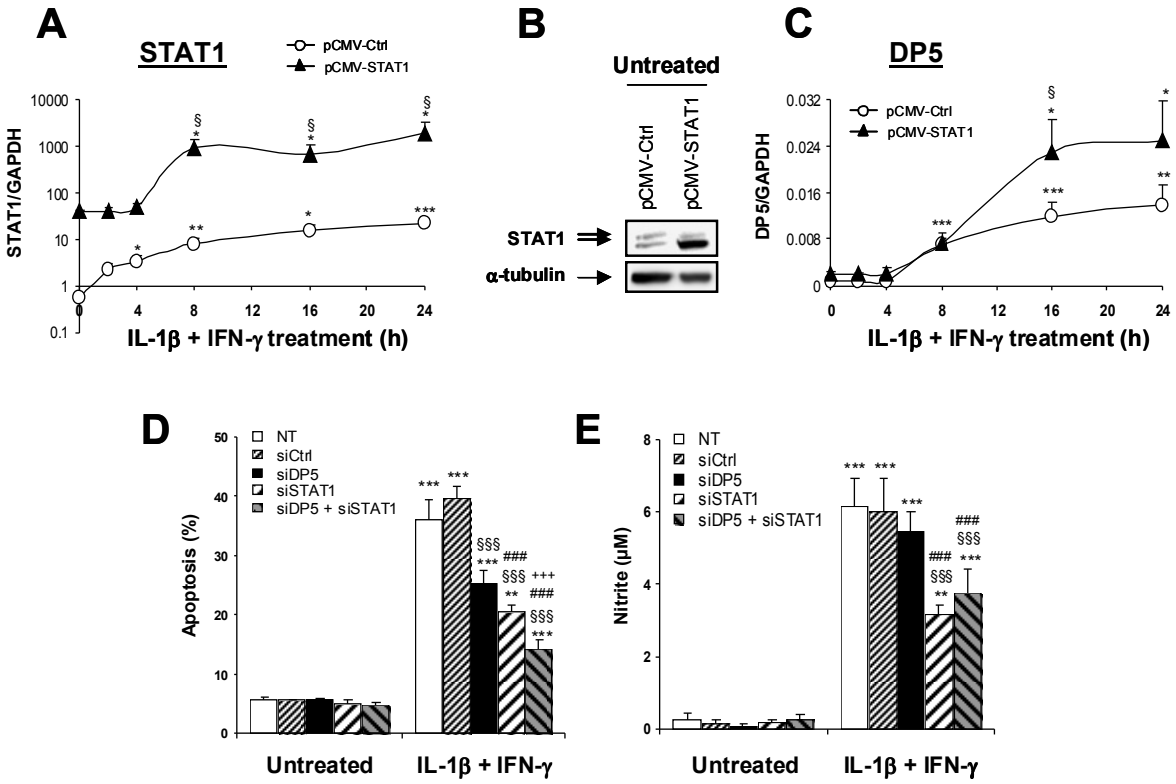
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5