The type 1 diabetes gene *TYK2* regulates β-cell development and its responses to interferon-α

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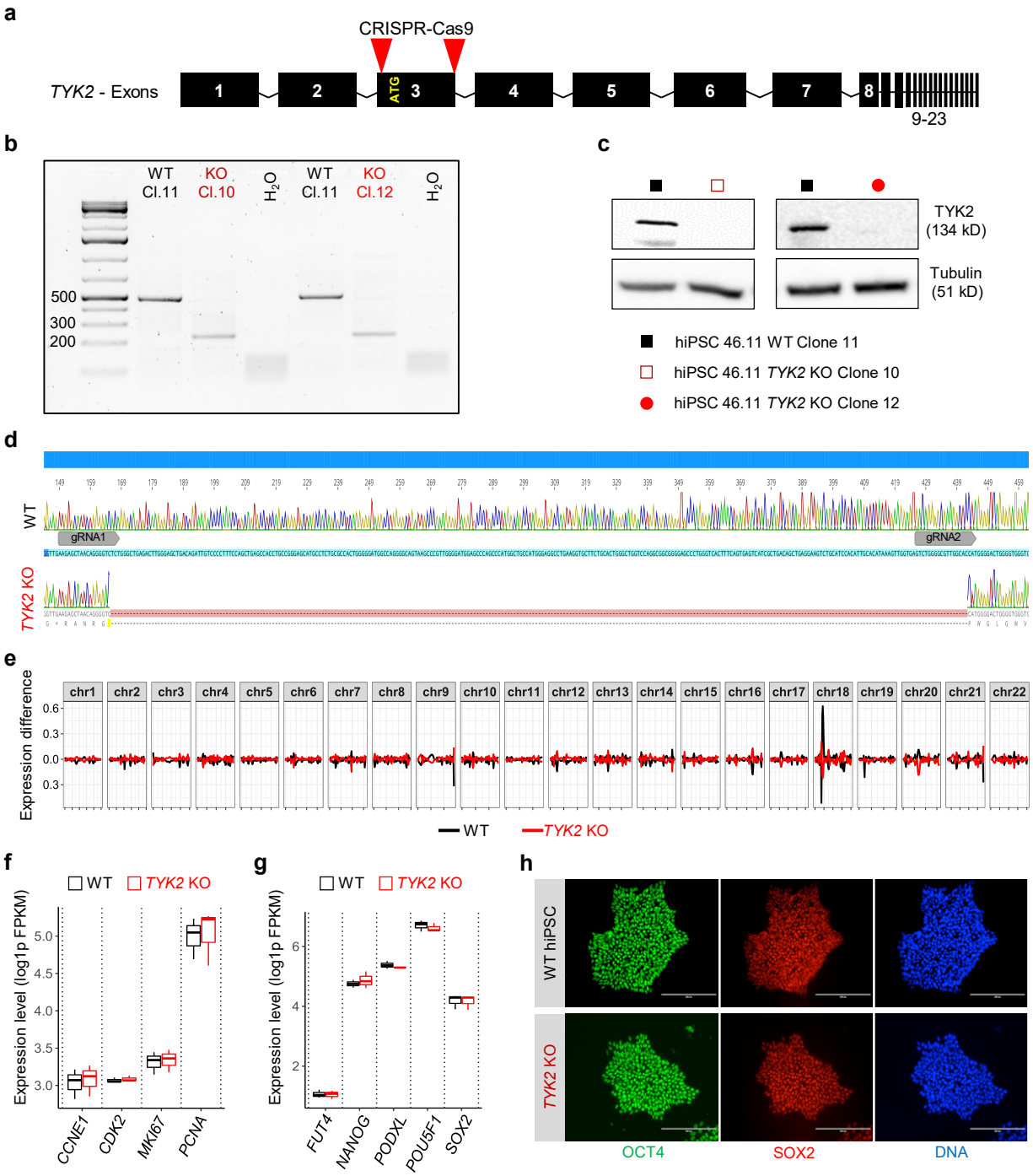
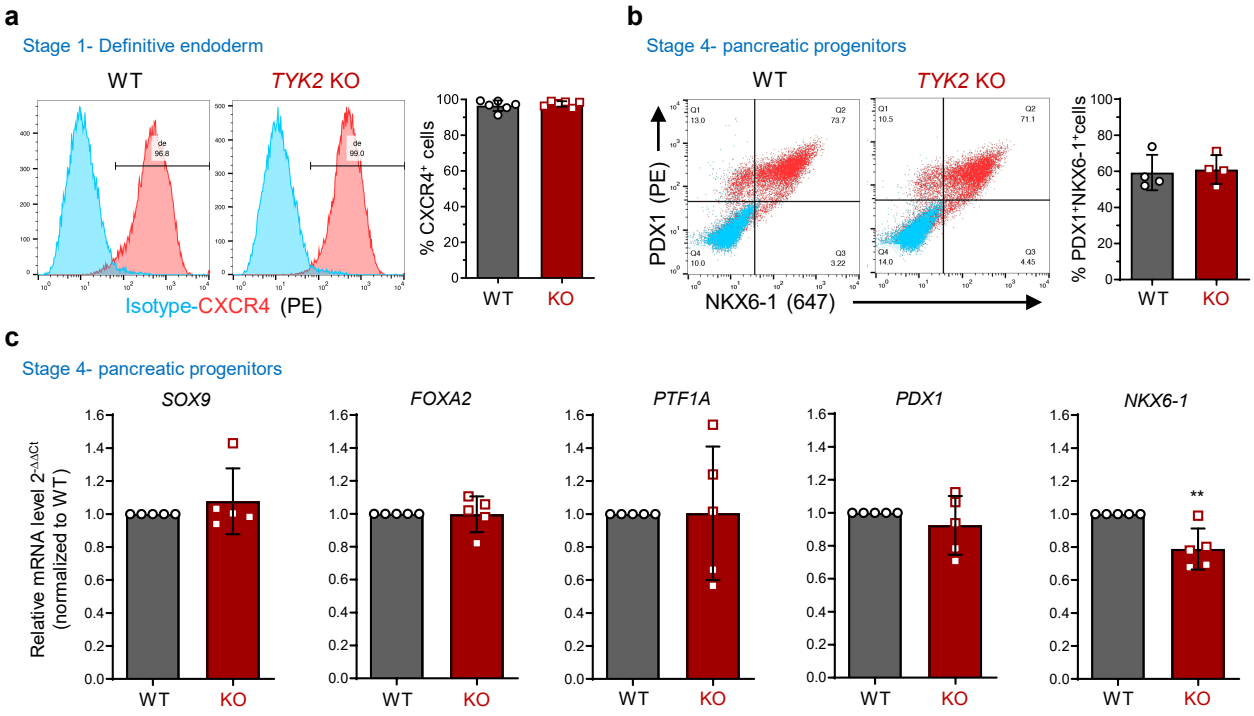
Supplementary Figures

Supplementary Fig. 1



Supplementary Fig. 1: Generation and characterization of *TYK2* knockout hiPSCs clones. **(a)** Schematic for the strategy to generate *TYK2* knockout by removing the ATG-containing third exon in the *TYK2* gene of hiPSCs line HEL46.11. **(b)** A 504 bp PCR amplicon of *TYK2* gene showing a 277 bp deletion in KO clones (n = 3). **(c)** Immunoblot analysis for the expression of TYK2 protein in WT and *TYK2* KO hiPSC clones; tubulin protein expression shown for the loading control (n = 3). **(d)** Sanger sequencing confirming the exact 277 bp deletion in the KO C10 clone compared to the unedited WT clone C11 sequence. **(e)** Global gene expression-based e-karyotyping analysis for WT and *TYK2* KO hiPSCs lines (n = 12). **(f)** Boxplot showing the normalized FPKM values for the expression levels of proliferation markers, *CCNE1*, *CDK2*, *MKI67*, *PCNA* and **(g)** pluripotency factors, *FUT4*, *NANOG*, *PODXL*, *POU5FI*, *SOX2* utilizing whole transcriptome data of WT and *TYK2* KO hiPSCs cell-lines. The boxplots showing the median with lower and upper hinges corresponding to the first and third quartiles (the 25th and 75th percentiles), the whiskers extend from min to max values (n = 3). **(h)** Representative image of immunocytochemistry analysis for the expression of pluripotency factors OCT4 and SOX2 in WT and *TYK2* KO hiPSCs (n = 3). Scale bar = 200 μm. Source data are provided as a Source Data file.

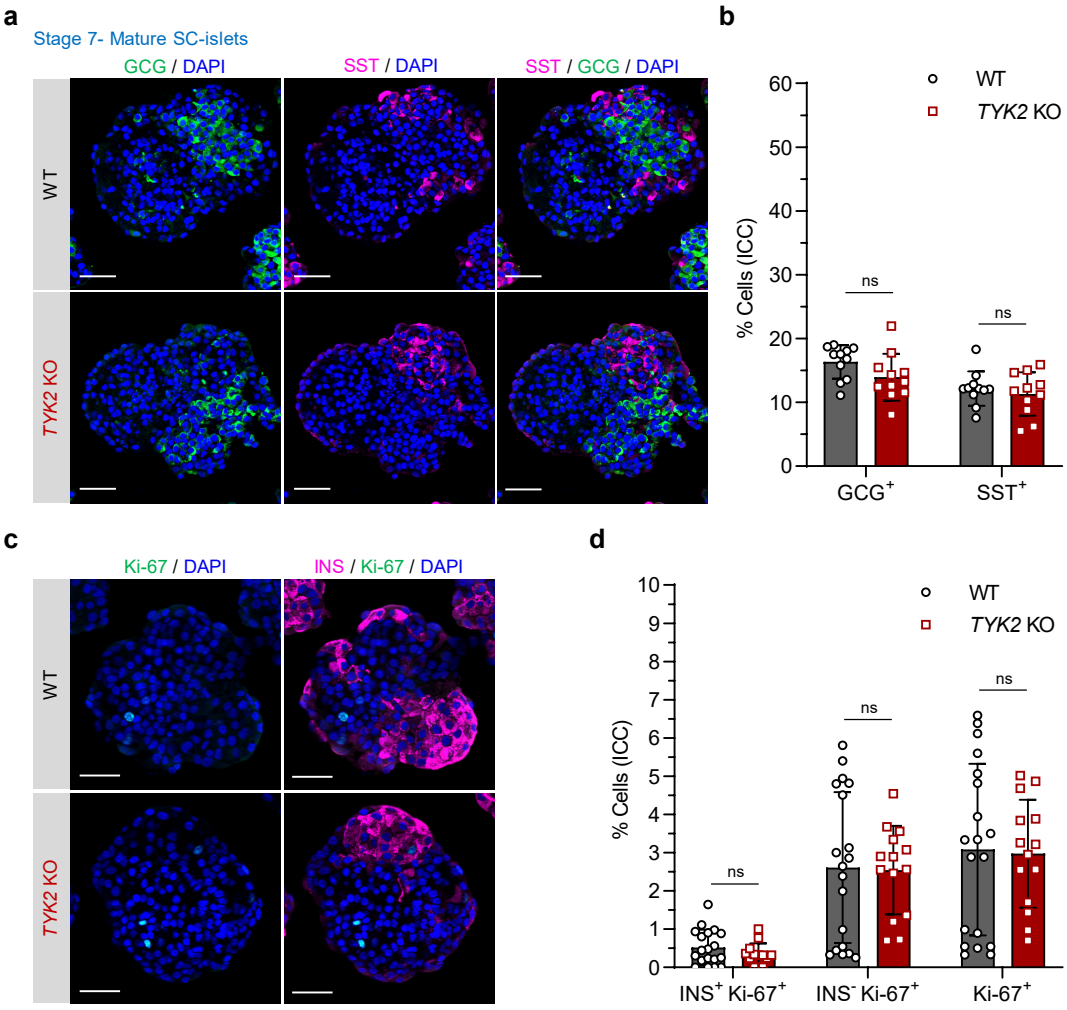
Supplementary Fig. 2



Supplementary Fig. 2: *In vitro* characterization of WT and *TYK2* KO hiPSCs at early stages of pancreatic differentiation.

(a) Flow cytometry analysis for the expression of $CXCR4^+$ definitive endoderm cells at the end of stage-1 and quantification for WT and *TYK2* KO cells (n = 6). **(b)** Flow cytometry analysis for $PDX1^+$ and $NKX6\text{-}1^+$ double positive cells at the end of stage-4 and quantification for WT and *TYK2* KO cells (n = 4). **(c)** Relative transcript expression of stage-4 pancreatic progenitor markers *SOX9*, *FOXA2*, *PTF1A*, *PDX1* and *NKX6-1* with qRT-PCR (n = 5). Two-tailed unpaired t-test was performed to determine the significance levels $^{**}p < 0.01$. Source data are provided as a Source Data file.

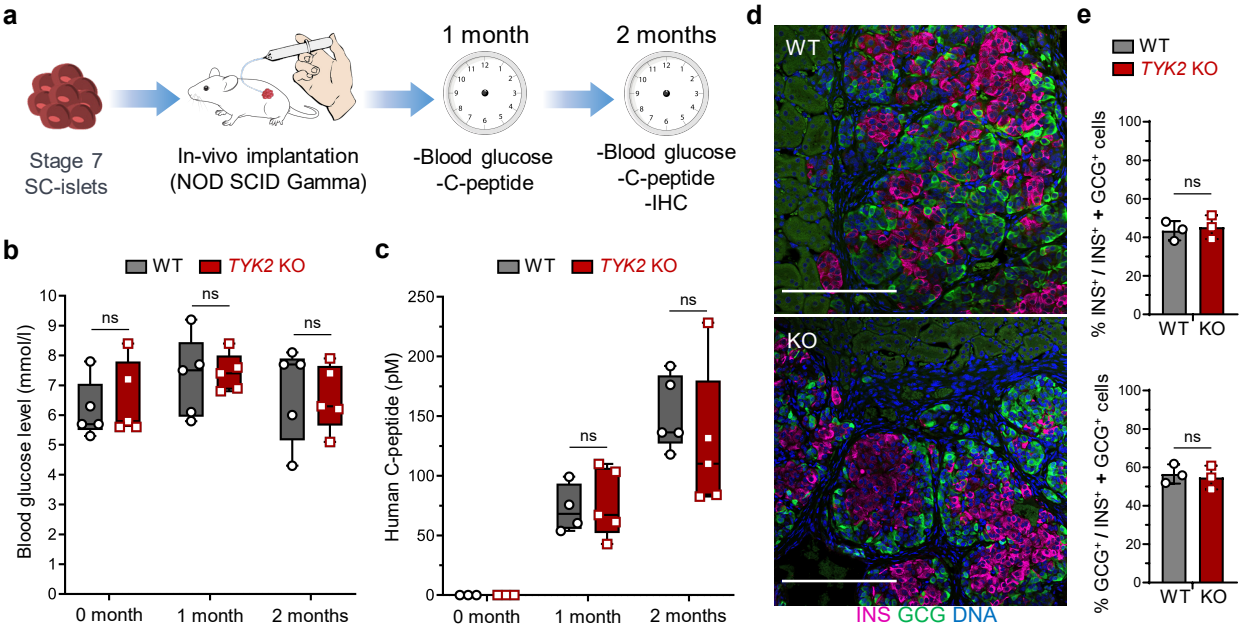
Supplementary Fig. 3



Supplementary Fig. 3: *In vitro* characterization of WT and *TYK2* KO S7 SC-islets for α-like cells (GCG^+), δ-like cells (SST^+) and proliferating cells ($Ki\text{-}67^+$).

(a) Representative image of immunocytochemistry for GCG and SST in S7 SC-islets (scale bar = 50 μm) and **(b)** their quantification from 2 experiments with 5-6 images each (n = 11). **(c)** Representative image of immunocytochemistry for Ki-67 and INS in S7 SC-islets (scale bar = 50 μm) and **(d)** their quantification from 2 experiments with 7-10 images each (n =14-19). Two-tailed unpaired t-test was performed to determine the significance levels, ns – non-significant. Data are presented as means ± S.D. Source data are provided as a Source Data file.

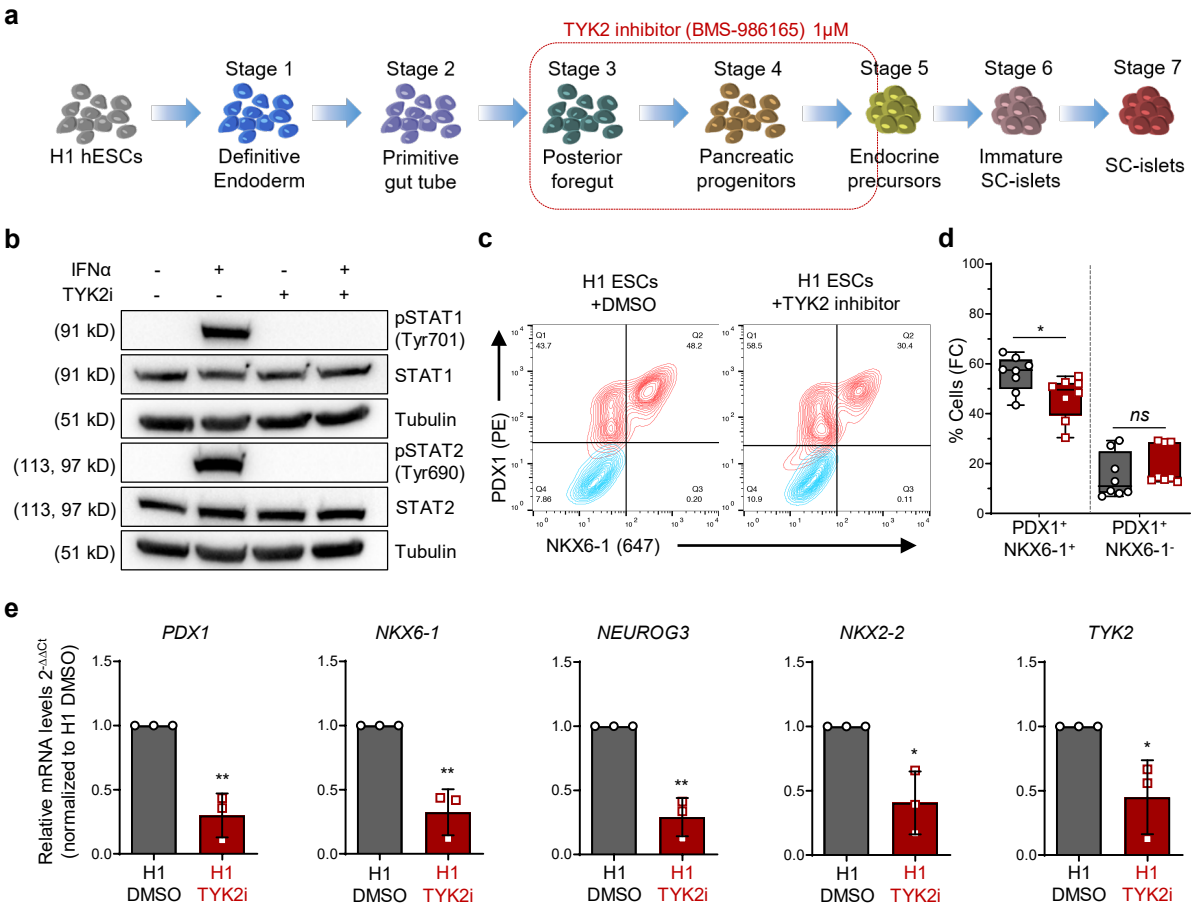
Supplementary Fig. 4



Supplementary Fig. 4: *In vivo* functional assessment of implanted WT and *TYK2* KO S7 SC-islets.

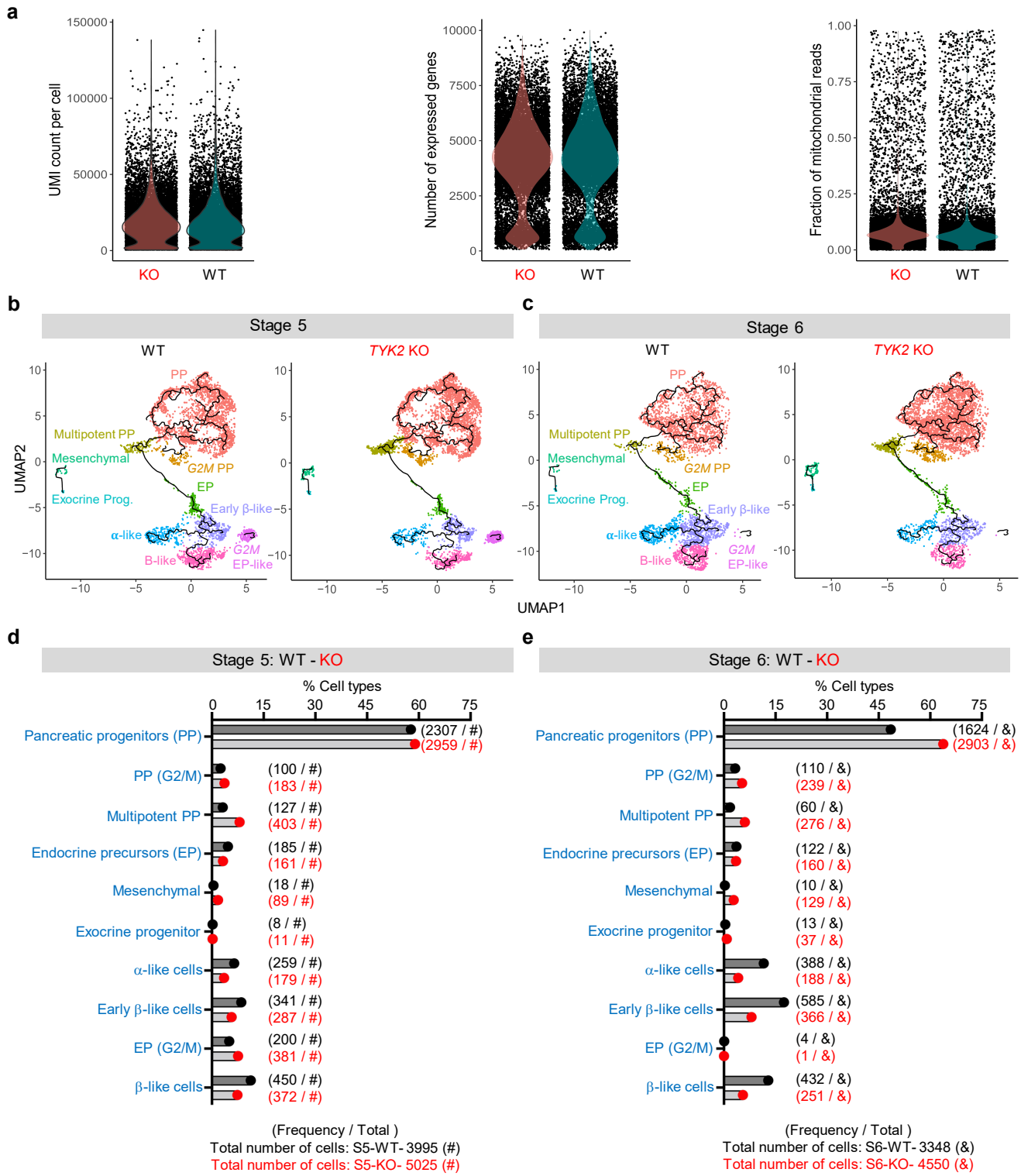
(a) Schematic of *in vivo* implantation study in NOD/SCID-γ mice. S7 WT and *TYK2* KO SC-islets were implanted in the mice (n = 5). The figure was partly generated using Servier Medical Art. **(b)** Mouse blood glucose levels measured at day 0, 1- and 2-months following implantation (n = 5). **(c)** Human C-peptide levels measured in the mice sera at day 0, 1- and 2-months following implantation (n = 3-5). Box and whiskers plots showing the median with min to max whiskers with all the points. **(d)** Immunohistochemistry of grafts after 2 months of implantation showing insulin (INS) and glucagon (GCG), scale bar = 200 μm and **(e)** their quantification for relative abundance (INS^+ or GCG^+ / INS^+ and GCG^+) in the graft sections (n = 3). Two-tailed unpaired t-test was performed to determine the significance levels. Data are mean ± S.D, ns – non-significant. Source data are provided as a Source Data file.

Supplementary Fig. 5



Supplementary Fig. 5: TYK2 inhibitor (TYK2i; BMS-986165) treatment during early pancreatic differentiation compromised endocrine precursors formation in hESCs H1 cell-line. **(a)** Human ES cell-line H1 differentiated with TYK2i as per schematic of the experiment plan. The figure was partly generated using Servier Medical Art. **(b)** Phosphorylation of STAT1 and STAT2 following 30 min IFNα treatments in DMSO or TYK2i treated differentiated cells (n = 3) at stage 5. **(c-d)** Representative contour plots of flow cytometry analysis following TYK2i treatment for the determination of $PDX1^{+}NKX6\text{-}1^{+}$ and $PDX1^{+}NKX6\text{-}1^{-}$ cells and their quantification (n = 8). Box and whiskers plot showing the median with min to max whiskers with all the points. **(e)** Relative transcript expression of *PDX1*, *NKX6-1*, *NEUROG3*, *NKX2-2* and *TYK2* with qRT-PCR (n = 3). Two-tailed unpaired t-test was performed to determine the significance levels. Data are mean ± S.D. *$p < 0.05$; **$p < 0.01$; ns – non-significant. Source data are provided as a Source Data file.

Supplementary Fig. 6

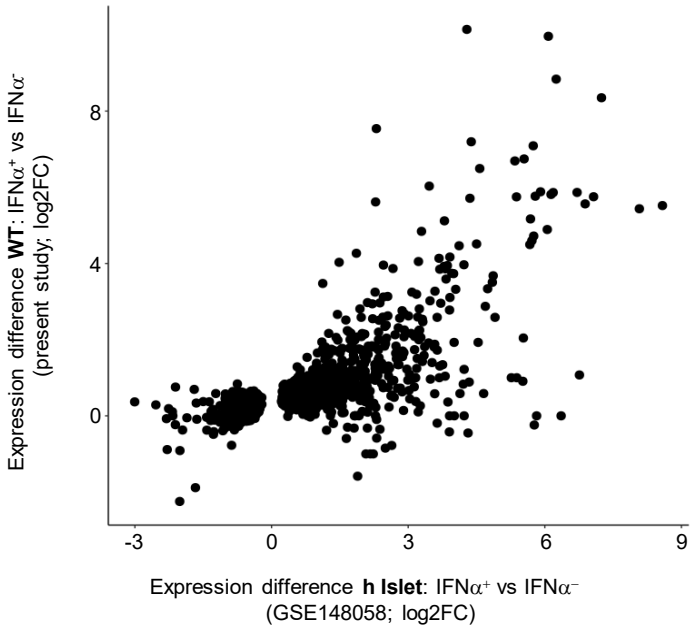


Supplementary Fig. 6: Quality assessment of scRNA-seq analysis and relative percentage of cells in noted clusters.

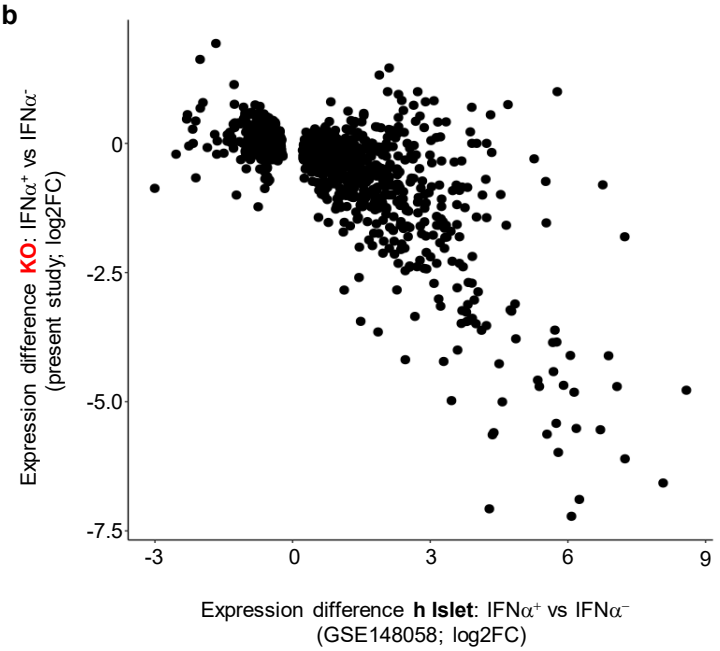
(a) Average unique molecular identifier (UMI) counts, number of expressed genes and mitochondrial reads per cell were plotted for the scRNA-seq analysis. Cells with less than 5000 UMI counts or 1700 expressed genes or with unusually high levels of mitochondrial reads (>20% of counts) were excluded. **(b)** Various noted clusters are indicated in different colour codes and presented as Uniform Manifold Approximation and projection (UMAP) with pseudotime trajectories for S5 and **(c)** S6 of WT and *TYK2* KO samples. **(d)** Relative percentage of cells in all noted clusters at S5 and **(e)** S6. Source data are provided as a Source Data file.

Supplementary Fig. 7

a



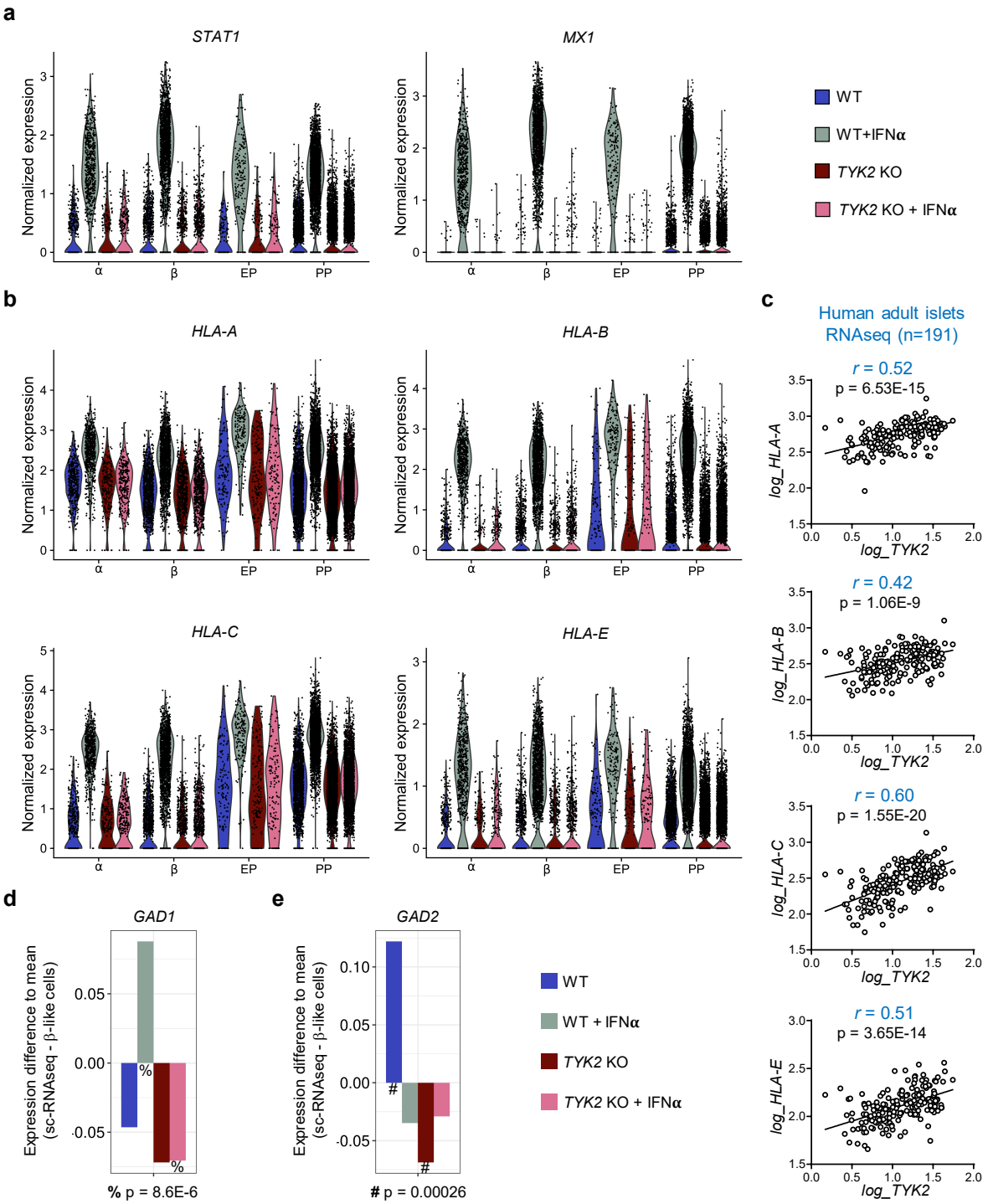
b



Supplementary Fig. 7: SC-islets exposed to IFNα show similar global transcriptomic changes compared to human islet samples under similar treatment.

Log2FC of the differentially expressed genes from a previous study (GSE148058) of human islets treated with or without IFNα (-/+) for 18h at X-axis versus the values of **(a)** WT or **(b)** *TYK2* KO SC-islets, treated for 24h with IFNα in the present study at Y-axis, respectively.

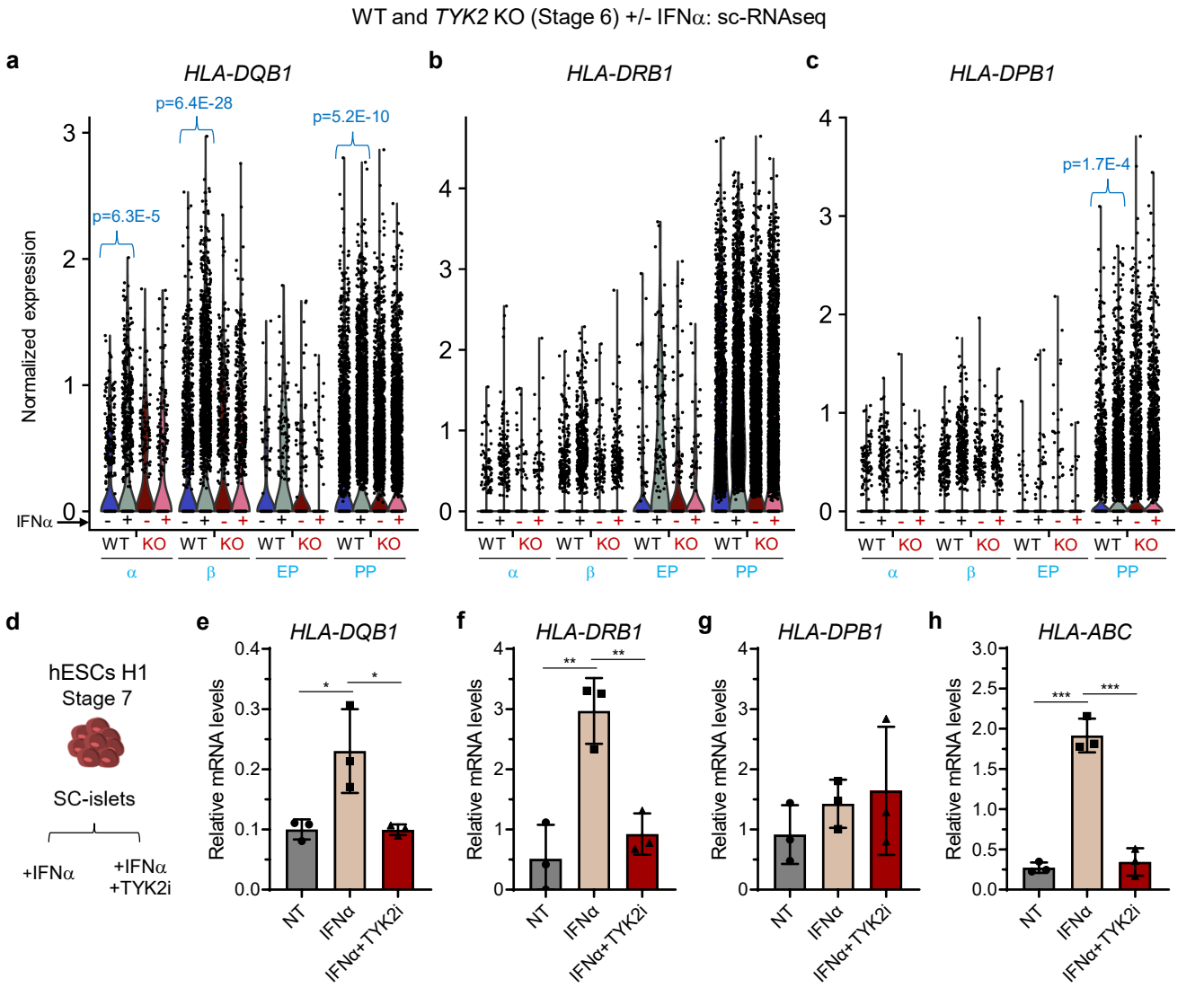
Supplementary Fig. 8



Supplementary Fig. 8: TYK2 regulates IFNα stimulated genes and autoantigen (*GAD1* and *GAD2*) in SC-islets.

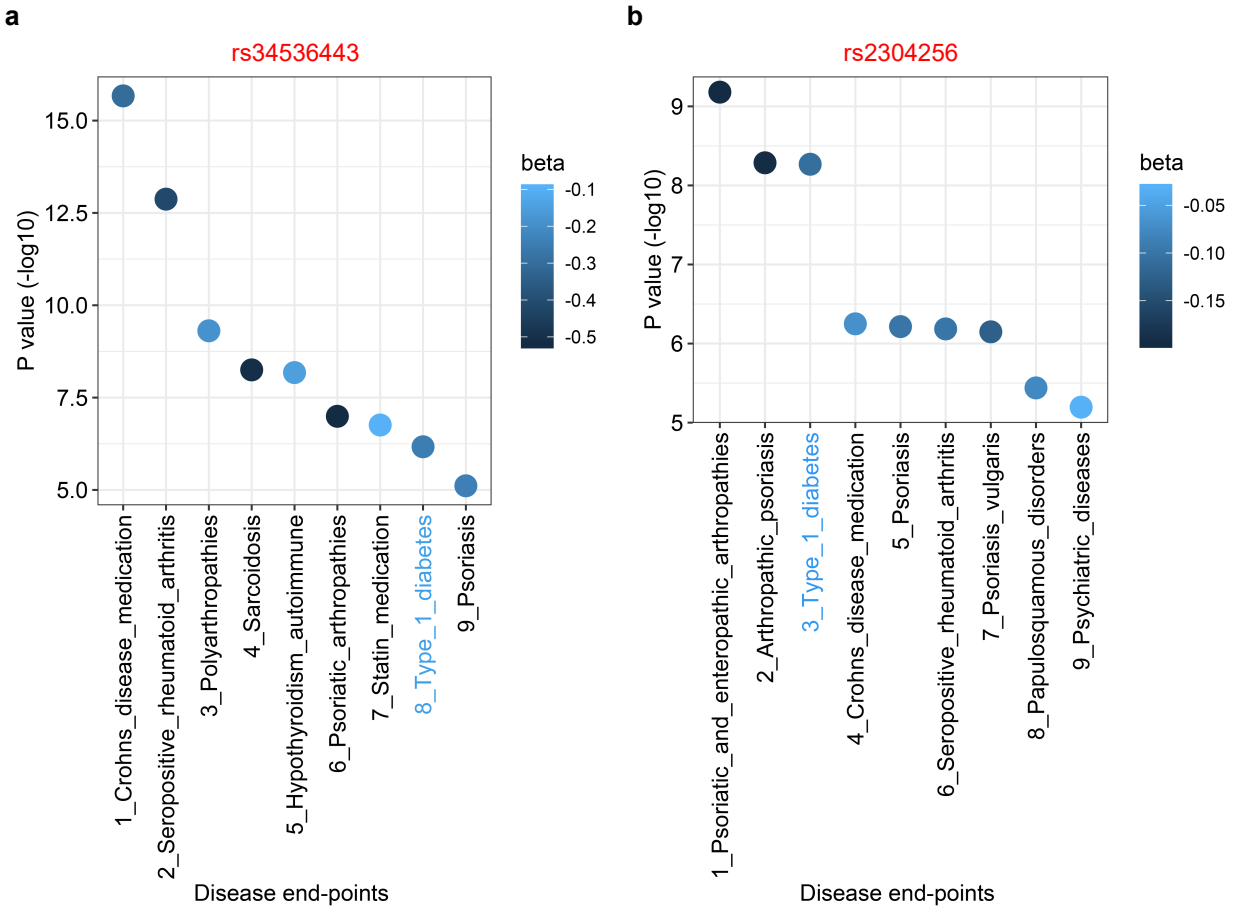
Single cell transcriptomics performed on the WT and *TYK2* KO S6 SC-islets following 24h -/+ IFNα treatment. Violin plots showing the normalized expression of IFNα stimulated genes **(a)** *STAT1* and *MX1*, **(b)** *HLA-A*, *HLA-B*, *HLA-C* and *HLA-E* in the clusters of α-, β-, endocrine precursor (EP)- and pancreatic progenitor (PP)- like cells. **(c)** Gene expression correlation between *TYK2* and *HLA-A*, *HLA-B*, *HLA-C*, *HLA-E* with human islets RNA-seq samples (n = 191). Pearson's correlations *r* after log normalization of counts and significance levels *p* are indicated in the panel. **(d-e)** The Bar plots showing the expression pattern for the autoantigens *GAD1* and *GAD2* in different genotypes indicated by colour codes. Source data are provided as a Source Data file.

Supplementary Fig. 9



Supplementary Fig. 9: TYK2 regulates MHC Class II expression in IFNα induced SC-islets. **(a)** scRNA-seq based analysis for the expression of MHC Class II genes *HLA-DQB1* **(b)** *HLA-DRB1*, **(c)** *HLA-DPB1* upon IFNα treatment in S6 WT and *TYK2* KO cells. **(d)** Schematic of qRT-PCR based experiment to assess the expression of MHC Class II genes **(e)** *HLA-DQB1*, **(f)** *HLA-DRB1*, **(g)** *HLA-DPB1* and MHC Class I gene **(h)** *HLA-ABC* in matured S7 H1 SC-islets in presence of IFNα or IFNα+TYK2i (BMS-986165). Bar plots are means ± S.D. Ordinary one-way ANOVA with Tukey's multiple comparison (n = 3). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Source data are provided as a Source Data file.

Supplementary Fig. 10



Supplementary Fig. 10: rs34536443 and rs2304256 SNPs protect against autoimmune diseases in the Finnish population.

The association analysis of **(a)** rs34536443 and **(b)** rs2304256 with 3095 clinical endpoints obtained from electronic health record data (ICD codes and drug purchase data) of 309,154 Finnish individuals. The figure depicts the effect size (beta) and the corresponding significance of association for the top ($p < 1.6x10^{-5}$) associated unique clinical endpoints (Supplementary Table 1). The association analysis was done using mixed model logistic regression adjusted for age, sex, 10 PCs and genotype batch. Source data are provided as a Source Data file.

Supplementary Tables

Supplementary Table 1: Cases and controls of *TYK2* rs34536443 and rs2304256 SNPs in R7 FinnGen dataset

	Phenotype	P_value (-log10)	beta	n_case	n_control	SNP
1	Crohn's disease medication	15.6687003	-0.303929	15801	293353	rs34536443
2	Seropositive rheumatoid arthritis	12.8730994	-0.415097	7294	238484	
3	Polyarthropathies	9.3086497	-0.187344	24310	202617	
4	Sarcoidosis	8.25059997	-0.505354	3103	304494	
5	Hypothyroidism autoimmune	8.17758999	-0.150063	33422	227415	
6	Psoriatic arthropathies	6.99376079	-0.520263	2430	202617	
7	Statin medication	6.75800054	-0.0972771	100639	208515	
8	Type 1 diabetes	6.16889972	-0.248286	8671	255466	
9	Psoriasis	5.11218023	-0.241221	6995	299128	
1	Psoriatic and enteropathic arthropathies	9.17987016	-0.195162	2818	238484	rs2304256
2	Arthropathic psoriasis	8.28646998	-0.192696	2571	299128	
3	Type 1 diabetes	8.26789989	-0.107933	8671	255466	
4	Crohn's disease medication	6.24964977	-0.067565	15801	293353	
5	Psoriasis	6.21471004	-0.099716	6995	299128	
6	Seropositive rheumatoid arthritis	6.18526975	-0.0991523	7294	238484	
7	Psoriasis vulgaris	6.14856002	-0.125926	4321	299128	
8	Papulosquamous disorders	5.43886998	-0.0775879	10026	299128	
9	Psychiatric diseases	5.19538018	-0.0318792	79207	229947	

The association analysis was done using mixed model logistic regression adjusted for age, sex, 10 PCs and genotype batch.

Supplementary Table 2: Guides and primers used for hiPSCs genome editing

Primers	Forward	Reverse
TYK2 PCR amp	GAGAGGAGGGTTCAGTGTTATTG	AGCTGCTGCTTGCACAC
TYK2 G1 OFF-TARGET1	CTTGGGCTAGTAGGCGTGGC	GGGACACCCTGATCCCCAGT
TYK2 G1 OFF-TARGET2	GAGGCAGGCAAGAAACCCCA	TGGGAGCCAGGAAGACTCACA
TYK2 G1 OFF-TARGET3	TCACCGTGGAGCCAAAGTGT	AGGTGGGGCCAATCATCAGG
TYK2 G2 OFF-TARGET1	GCAAAGTCAGAATCTGAGGGCA	TGTGAGAAAGCTGCTGGGTGA
TYK2 G2 OFF-TARGET2	AGTTTGAAGTGGCTCTGGACCA	TGCACACCTTTATACTGGAAAAGAG
TYK2 G2 OFF-TARGET3	GCCTTTGGCCAGCTCAAATG	TGGCCAATCCAGATGTCCCC

Supplementary Table 3: Differentiation protocol and media formulations

Stage	Reagent	Medium
Stage 1 3 days	d0: 100 ng/ml human Activin A, 3 μM CHIR d1: 100 ng/ml human Activin A, 0.3 μM CHIR d2: 100 ng/ml human Activin A	Basal 1: MCDB131 (10372-019, Life Technologies), 2 mM Glutamax, 1.5 g/L NaHCO3 (Sigma-Aldrich), 0.5% BSA fraction V Fatty acid free (Sigma-Aldrich), 10 mM glucose (Sigma-Aldrich)
Stage 2 3 days	0.25 mM Ascorbic acid, 50 ng/ml FGF7	
Stage 3 2 days	0.25 mM Ascorbic acid, 50 ng/ml FGF7, 0.25 μM SANT1, 1 μM Retinoic Acid, 100 nM LDN, 200 nM and TPB	Basal 2: MCDB131, 2 mM Glutamax (35050038, Life Technologies), 2.5 g/L NaHCO3, 2% BSA fV, 10 mM glucose, 1:200 ITS-X (51500-056, Life Technologies)
Stage 4 4 days	0.25 mM Ascorbic acid, 50 ng/ml FGF7, 0.25 μM SANT1, 0.1 μM Retinoic Acid, 200 nM LDN, 100 ng/ml EGF and 10 mM Nicotinamide	
Stage 5 4 days	0.25 μM SANT1, 0.05 μM Retinoic acid, 100 nM LDN, 10 μM ALK5inhII, 1 μM GC1, 20 ng/mL Betacellulin and 100 nM GSiXX	Basal 3: MCDB131, 2 mM Glutamax, 1.5 g/L NaHCO3, 2% BSA fV, 20 mM glucose, 1:200 ITS-X, 10 μg/mL Heparin (H3149, Sigma-Aldrich), 10 μM Zinc Sulfate (Z0251, Sigma-Aldrich)
Stage 6 8 days	100 nM LDN, 10 μM ALK5inhII, 1 μM GC1 and 100 nM GSiXX	
Stage 7 21 days	1 mM N-Acetylcysteine, 10 nM Tri-iodothyronine (T3) and 0.5 μM Aurora kinase inhibitor ZM447439	CMRL-modified: CMRL 1066 (15-110-CVR, Corning), 2% BSA fV, 2 mM Glutamax, 1:200 ITS-X, Heparin 10 μg/ml, 10 μM Zinc Sulfate, 5 mM Sodium Pyruvate (Lonza), 1:2000 chemically defined lipid concentrate (11905-031, Invitrogen), 1:2000 medium trace elements A (25-021-CI,Cellgro), 1:2000 medium trace elements B (99-175-CI, Cellgro)

Supplementary Table 4: Chemicals and Reagents used in the study

Chemical/Reagent	Company name	Catalogue#
Activin A	QKine Ltd, Cambridge, UK	Cat# Qk001
CHIR	Tocris	Cat# 4423
Ascorbic acid	Sigma-Aldrich	Cat# A4544
FGF7	Genscript	Cat# Z03047
SANT1	Sigma-Aldrich	Cat# 4572
LDN	Selleckchem	Cat# S2618
Retinoic acid	Sigma-Aldrich	Cat# R2625
TPB	Santa Cruz	Cat# sc-204424
EGF	Peprotech	Cat# AF-100-15
Nicotinamide	Sigma-Aldrich	Cat# N0636
ALK5inhII	Selleckchem	Cat# S7233
GC1	Tocris	Cat# 4554
GSiXX	Millipore	Cat# 56578
N-Acetylcysteine	Sigma-Aldrich	Cat# A9165
Tri-iodothyronine (T3)	Sigma-Aldrich	Cat# T6397
Aurora kinase inhibitor ZM447439	Selleckchem	Cat# S1103
Glutamax	Life Technologies	Cat# 35050038
Heparin	Sigma-Aldrich	Cat# H3149
Zinc Sulfate	Sigma-Aldrich	Cat# Z0251
Chemically defined lipid concentrate	Invitrogen	Cat# 11905-031
Medium trace elements A	Cellgro	Cat# 25-021-CI
Medium trace elements B	Cellgro	Cat# 99-175-CI
TYK2 inhibitor (BMS-986165)	MCE-MedChemExpress	Cat# HY-117287
Human Interferon α 2B	Sigma-Aldrich	Cat# H6166
Recombinant human IFNγ	R&D	Cat# 285-IF

Supplementary Table 5: Antibodies used for western blot

Antibody	Company	Catalog# and clone	Dilution
pSTAT1 (Tyr701) Rabbit mAb	Cell Signaling Technology	Cat# 7649; (D4A7)	1:1000
STAT1 Mouse mAb	Santa-Cruz	Cat# sc-464; (C-136)	1:500
pSTAT2 (Tyr690) Rabbit mAb	Cell Signaling Technology	Cat# 88410; (D3P2P)	1:1000
STAT2 Rabbit mAb	Cell Signaling Technology	Cat# 72604; (D97JL)	1:1000
pSTAT3 (Tyr705) Rabbit mAb	Cell Signaling Technology	Cat# 9145; (D3A7)	1:1000
STAT3 Mouse mAb	Cell Signaling Technology	Cat# 9193; (124H6)	1:1000
TYK2 Mouse mAb	Abcam	Cat# ab57678	1:500
K-RAS Mouse mAb	Santa-Cruz	Cat# sc-517599; (3B10-2F2)	1:250
Tubulin Mouse mAb	Sigma-Aldrich	Cat# T5168; (B-5-1-2)	1:2000
β-actin-HRP Mouse mAb	Santa Cruz	Cat# sc-47778; (C4)	1:1000

Supplementary Table 6: Antibodies used for flow cytometry and immunofluorescence

Antibody	Company	Catalog#	Dilution
Mouse Anti-CD184 (CXCR4) Monoclonal Antibody	BD Biosciences	Cat# 555974; RRID: AB_396267	1:10
Mouse IgG2a, kappa Isotype Control, Phycoerythrin Conjugated	BD Biosciences	Cat# 5555749; RRID: AB_396091	1:10
Rabbit anti-OCT4 Rabbit	Santa Cruz Biotechnology	Cat# sc-9081; RRID: AB_2167703	1:250
Rabbit anti-SOX2 mAb	Cell Signaling Technology	Cat# 3579; RRID: AB_2195767	1:500
Sheep anti Human Neurogenin-3 antibody	R&D systems	Cat# AF3444; RRID: AB_2149527	1:400
PE-mouse anti PDX1	BD Biosciences	Cat# 562161; RRID: AB_10893589	1:80
Alexa Fluor 647 Mouse anti NKX6-1	BD Biosciences	Cat# 563338; RRID: AB_2738144	1:80
Alexa Fluor 647 Mouse IgG1 k isotype control	BD Biosciences	Cat# 557714; RRID: AB_396823	1:80
Insulin (C27C9) Rabbit Antibody (Alexa Fluor 647 Conjugate)	Cell Signaling Technology	Cat# 9008; RRID: AB_2687822	1:80
Rabbit IgG Isotype Control (Alexa Fluor 647 Conjugate)	Cell Signaling Technology	Cat# 3452S; RRID: AB_10695811	1:80
Mouse anti-GCG antibody	Sigma-Aldrich	Cat# G2654; RRID: AB_259852	FC-1:160 ICC- 1:500
Guinea pig anti-INS	Dako	Cat# A0564; RRID: AB_10013624	1:1000
Rabbit anti-Ki67	Leica Microsystems	Cat# NCL-Ki67p; RRID: AB_442102	1:500
Mouse anti-MHC Class I monoclonal antibody (W6/32)	Enzo Life Sciences	Cat# ALX-805-711-C100; RRID: AB_11179235	FC- 1:80 ICC- 1:200
Rabbit anti-SLC18A1	Sigma-Aldrich	Cat# HPA063797; RRID: AB_2685125	1:250
Rabbit anti-Somatostatin	Dako	Cat# A0566; RRID: AB_2688022	1:500

Brilliant violet 605 anti-human HLA-A, B, C (W6/32)	BioLegend	Cat# 311432; RRID: AB_2566151	1:100
PE anti-human CD274 (B7-H1, PD-L1) (29E.2A3)	BioLegend	Cat# 329706; RRID: AB_940368	1:100
Alexa FluoR 488 Donkey anti-Mouse IgG secondary antibody	ThermoFisher Scientific	Cat# A-21202; RRID: AB_141607	1:500
Alexa FluoR 594 Goat anti-Guinea Pig IgG secondary antibody	ThermoFisher Scientific	Cat# A-11076; RRID: AB_2534120	1:500
Alexa FluoR 594 Donkey anti-Sheep IgG secondary antibody	ThermoFisher Scientific	Cat# A- 11016; RRID: AB_2534083	1:500
Alexa FluoR 488 Donkey anti-Rabbit IgG secondary antibody	ThermoFisher Scientific	Cat# A-21206; RRID: AB_2535792	1:500
Alexa FluoR 594 Donkey anti-Rabbit IgG secondary antibody	ThermoFisher Scientific	Cat# A-21207; RRID: AB_141637	1:500
Alexa FluoR 488 Donkey anti-Mouse IgG secondary antibody	ThermoFisher Scientific	Cat# A-21203; RRID: AB_141633	1:500