The type 1 diabetes gene *IFIH1* regulates anti-viral responses differentially in pancreatic alpha and beta cells

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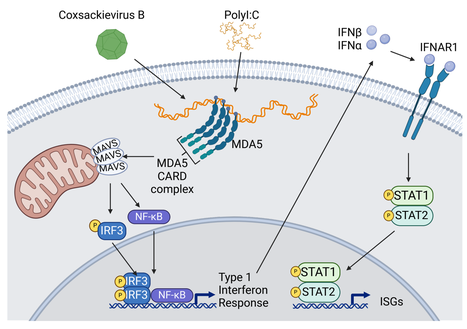
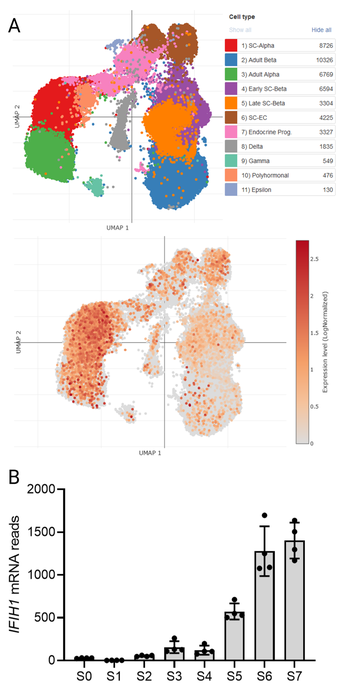
INTRODUCTION



- Recent evidence suggests that direct enteroviral infection of islet cells could be an important factor in T1D pathogenesis.
- Melanoma differentiation associated protein 5 (MDA5), encoded by *IFIH1*, is responsible for the detection of cytosolic viral dsRNA to initiate an antiviral response.
- Loss-of-function variants in *IFIH1* confer protection against T1D while gain-of-function have been linked with increased risk.

We have generated two human iPSC lines, one with an *IFIH1*-knock-out and one with an *IFIH1* gain-of-function mutation (E444G) KI, and differentiated them to the pancreatic endocrine lineage to generate stem cell derived islets (SC-islets).

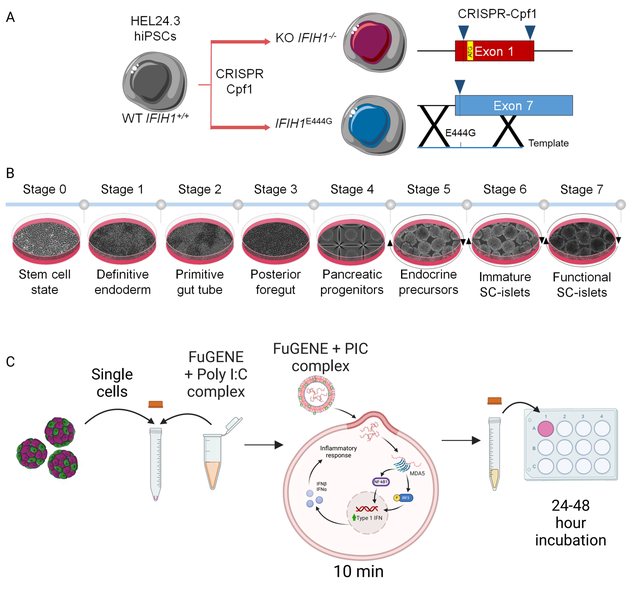
Expression pattern of *IFIH1* in SC-islets



(A) Single cell RNAseq of SC-islets showed enrichment of *IFIH1* in the alpha cell population compared to beta cells, and

(B) throughout SC-islet maturation.

METHODS

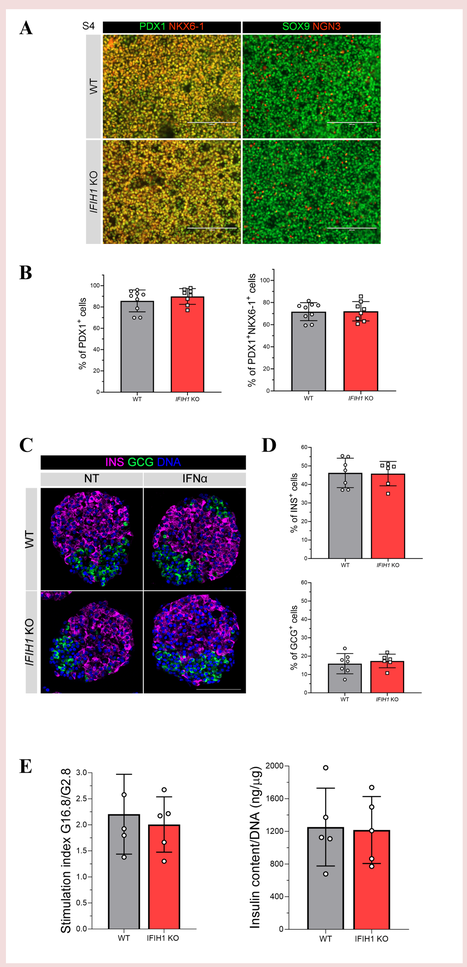


CONCLUSION

- Our results suggest that absence of MDA5 does not compromise the generation of SC-islets or their glucose responsiveness.
- Stimulation of the KO and WT SC-islets with PolyI:C demonstrated differential MDA5 mediated inflammatory responses.
- Results from the KI (E444G) model suggest a spontaneous hyper IFN signalling which was ameliorated by TYK2 inhibition.
- scRNAseq indicates decrease IFN signalling in *IFIH1*-KO alpha and beta-cells while eukaryotic translation elongation pathways enriched in the alpha-cells.
- Our model will make it possible to further elucidate the differences in the antiviral responses between alpha and beta cells.

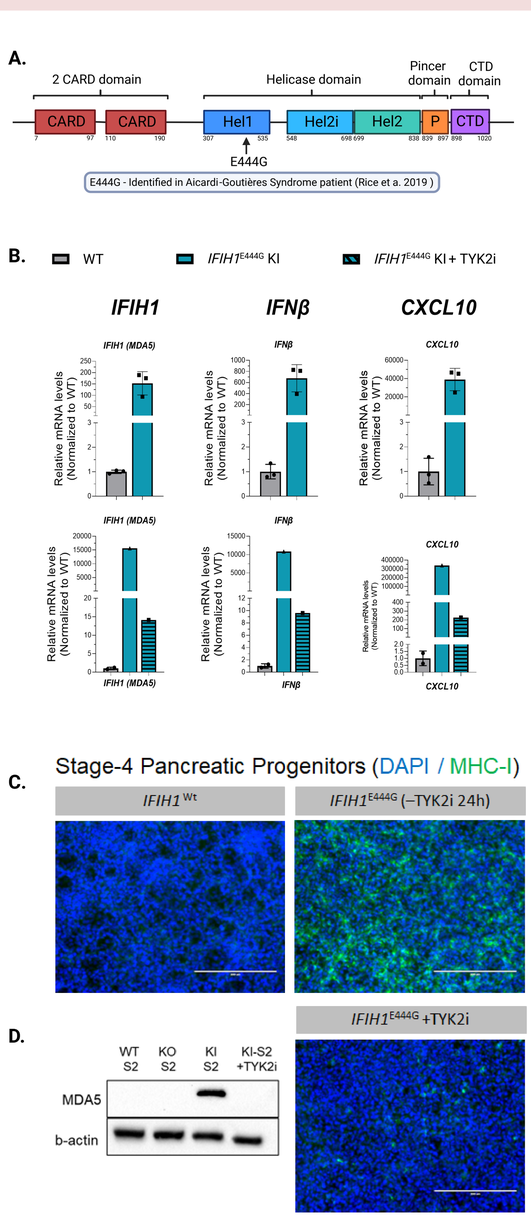
RESULTS

Fig. 1. Pancreatic lineage differentiation of WT and *IFIH1* KO clones



Analysis of differentiation markers in pancreatic progenitor using
A) immunocytochemical analysis and
B) flow cytometry.
Analysis of insulin and glucagon expression in mature SC-islets using
C) immunohistochemistry and
D) flow cytometry.
E) Functional analysis of SC-islets using static GSIS.

Fig 3. Pancreatic lineage differentiation of gain-of-function *IFIH1*^{E444G} KI clone



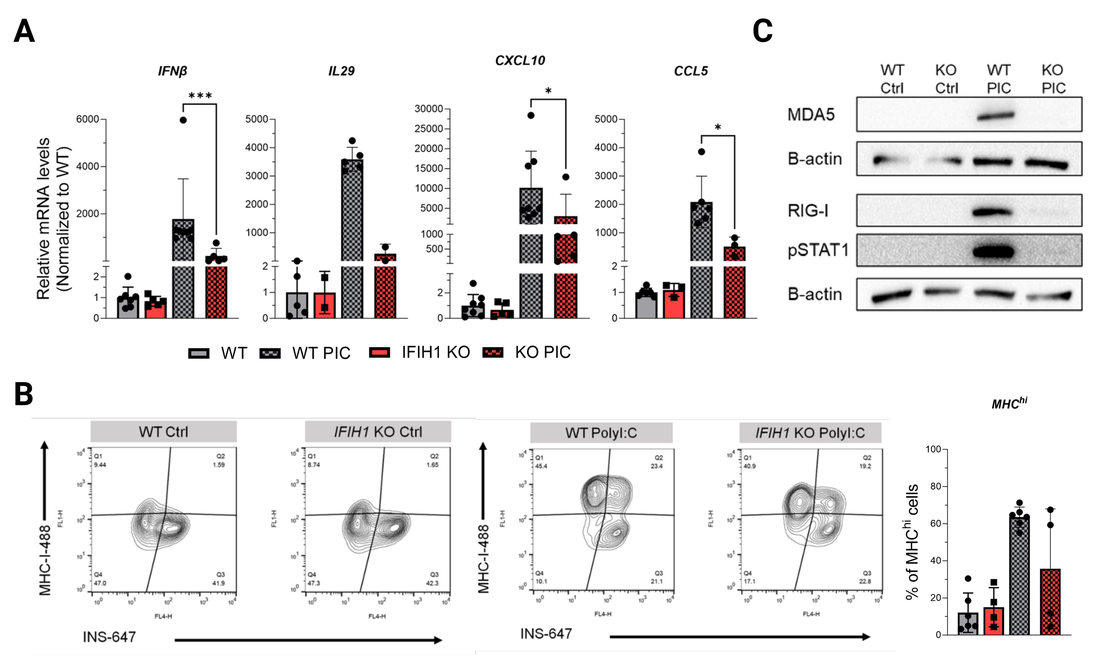
A. An overview of the IFIH1 gene and gain-of-function mutation KI (E444G).

B. Relative transcripts levels of *IFIH1*, *IFNb* and *CXCL10* in the KI at stage 0 (hiPSCS) and in S2-Definitive endoderm stage +/- TYK2i.

C. Immunocytochemistry of S4 (Panc. Prog.) KI cells for the expression of MHC-I when TYK2i was temporarily withdrawn for 24h.

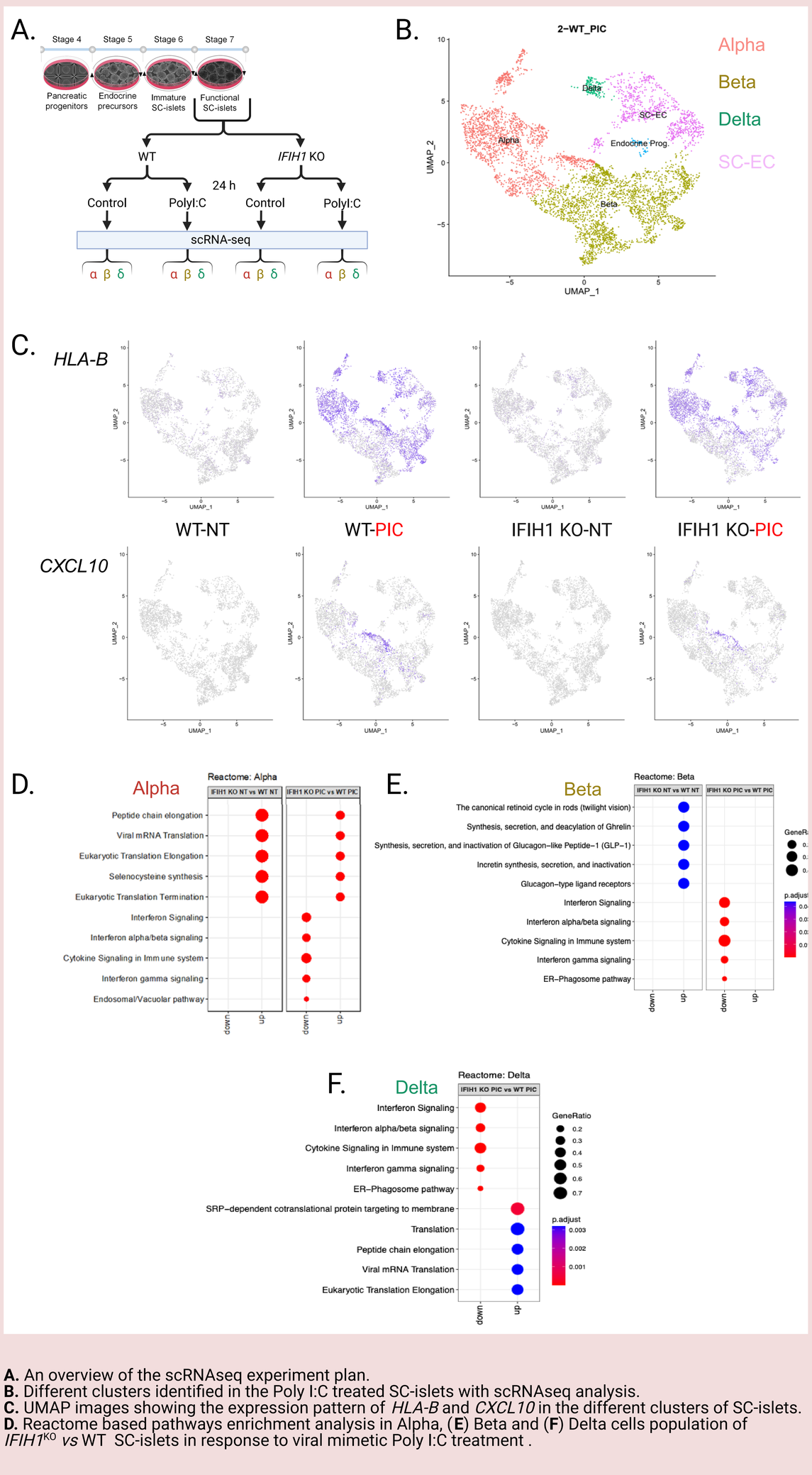
D. Western blot analysis for the expression of MDA5 in the KI cells at S2 of pancreatic differentiation.

Fig. 2. Treatment with a dsRNA viral mimetic (poly I:C) revealed MDA5 dependent STAT1-mediated induction of *CXCL10* in the SC-islets.



Analysis of PolyI:C treated SC-islets **A**) mRNA expression **B**) Quantification of MHC-I+ and Insulin+ cells using flow cytometry **C**) Western blot

Fig 3. scRNAseq analysis of viral-mimetic (poly I:C) treated WT and *IFIH1*^{KO} SC-islets



A. An overview of the scRNAseq experiment plan.
B. Different clusters identified in the Poly I:C treated SC-islets with scRNAseq analysis.
C. UMAP images showing the expression pattern of *HLA-B* and *CXCL10* in the different clusters of SC-islets.
D. Reactome based pathways enrichment analysis in Alpha, (**E**) Beta and (**F**) Delta cells population of *IFIH1*^{KO} *vs* WT SC-islets in response to viral mimetic Poly I:C treatment .

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