Supplemental Information – Lysy et al.

MAFA smRNA drives reprogramming of human pancreatic duct-derived cells into insulin-secreting cells

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Supplemental Information inventory:

Figure S1 shows EGFP protein expression kinetics after one transfection of βHDDCs using EGFP smRNA. Highest protein expression levels were observed 48-72 hours after transfection.

Figure S2 shows negligible effects of B18R addition to transfection media on cell viability and absence of transdifferentiation induced in HDDCs by EGFP smRNA transfection and IFNα incubation.

Figure S3 shows phenotypic changes of HDDCs during differentiation after MAFA overexpression and absence of strong activation of the cellular innate immune response after smRNA transfection. A global decrease of mesenchymal marker expression was observed β-HDDCs**.**

Figure S4 shows morphological changes of HDDCs during differentiation after MAFA overexpression.

Table S1 shows primers used for real-time RT-PCR analysis. This table supports data presented in main Figure 2, Figure 3, Figure 4, Figure 5 and Supplementary Figure 1 and 2.

Table S2 shows antibodies used for immunostaining. This table supports data presented in main Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure 7 and Supplementary Figures 3 and 5.

Table S3 describes the estimation of cell ratios in staining assays.

Figure S1

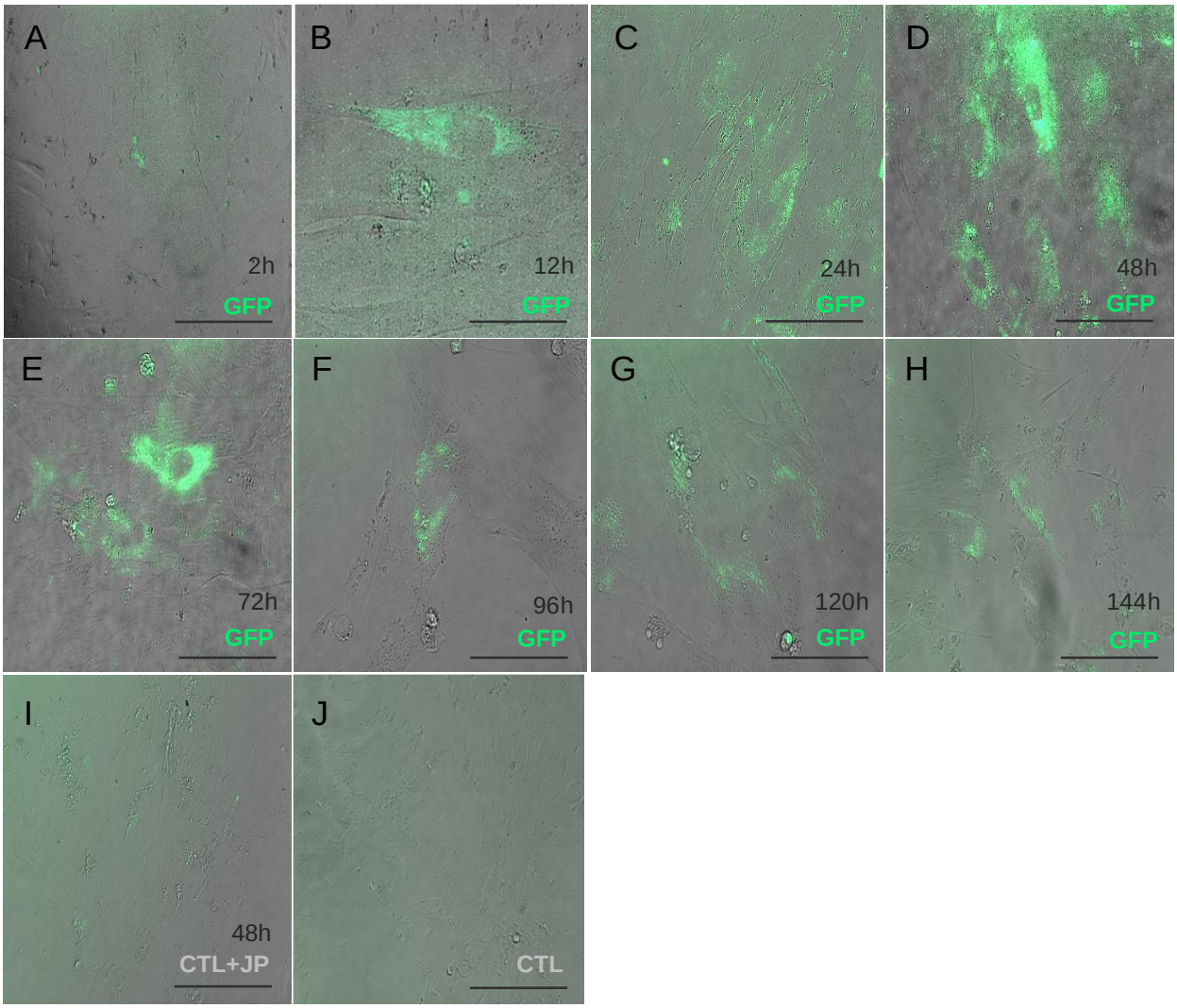
A
2h
GFP
B
12h
GFP
C
24h
GFP
D
48h
GFP
E
72h
GFP
F
96h
GFP
G
120h
GFP
H
144h
GFP
I
48h
CTL+JP
J
CTL

Figure S1: EGFP protein expression kinetics after single EGFP smRNA transfection

(**A**) EGFP protein expression observed 2 hours, (**B**) 12 hours, (**C**) 24 hours, (**D**) 48 hours, (**E**) 72 hours, (**F**) 96 hours, (**G**) 120 hours and (**H**) 144 hours after single EGFP smRNA transfection on β-HDDCs. (**I-J**) Fluorescence observed in control groups, 48 hours from the transfection with the JetPEI reagent only (CTL+JP) and in untransfected HDDCs (CTL). These data are representative of three different experiments. Magnification bars: 50 µm (**A**, **C**-**J**); 25 µm (**B**).

Figure S2

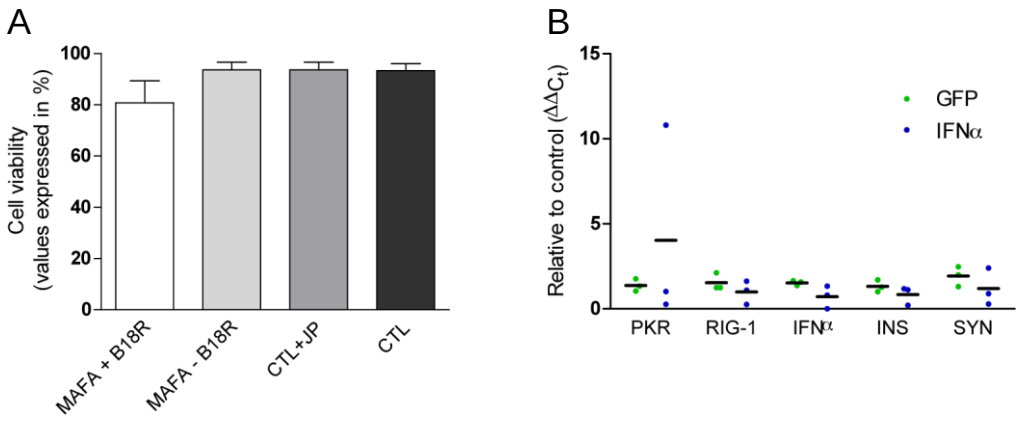
A
Cell viability
(values expressed in %)
100
80
60
40
20
0
MAFA + B18R
MAFA - B18R
CTL+JP
CTL
B
Relative to control (ΔΔCt)
15
10
5
0
GFP
IFNα
PKR
RIG-1
IFNα
INS
SYN

Figure S2: Additional evidence of the specificity of MAFA smRNA-induced HDDCs differentiation

(**A**): The addition of B18R molecule to reprogramming media did not significantly alter cell viability (*n=3-6*). (**B**): Absence of significant β-cell differentiation of HDDCs after 7 daily consecutive transfections with *EGFP* smRNA or after 72-hour incubation with 200 U/mL IFNα according to insulin and synaptophysin gene expression (*n*=3).

Figure S3

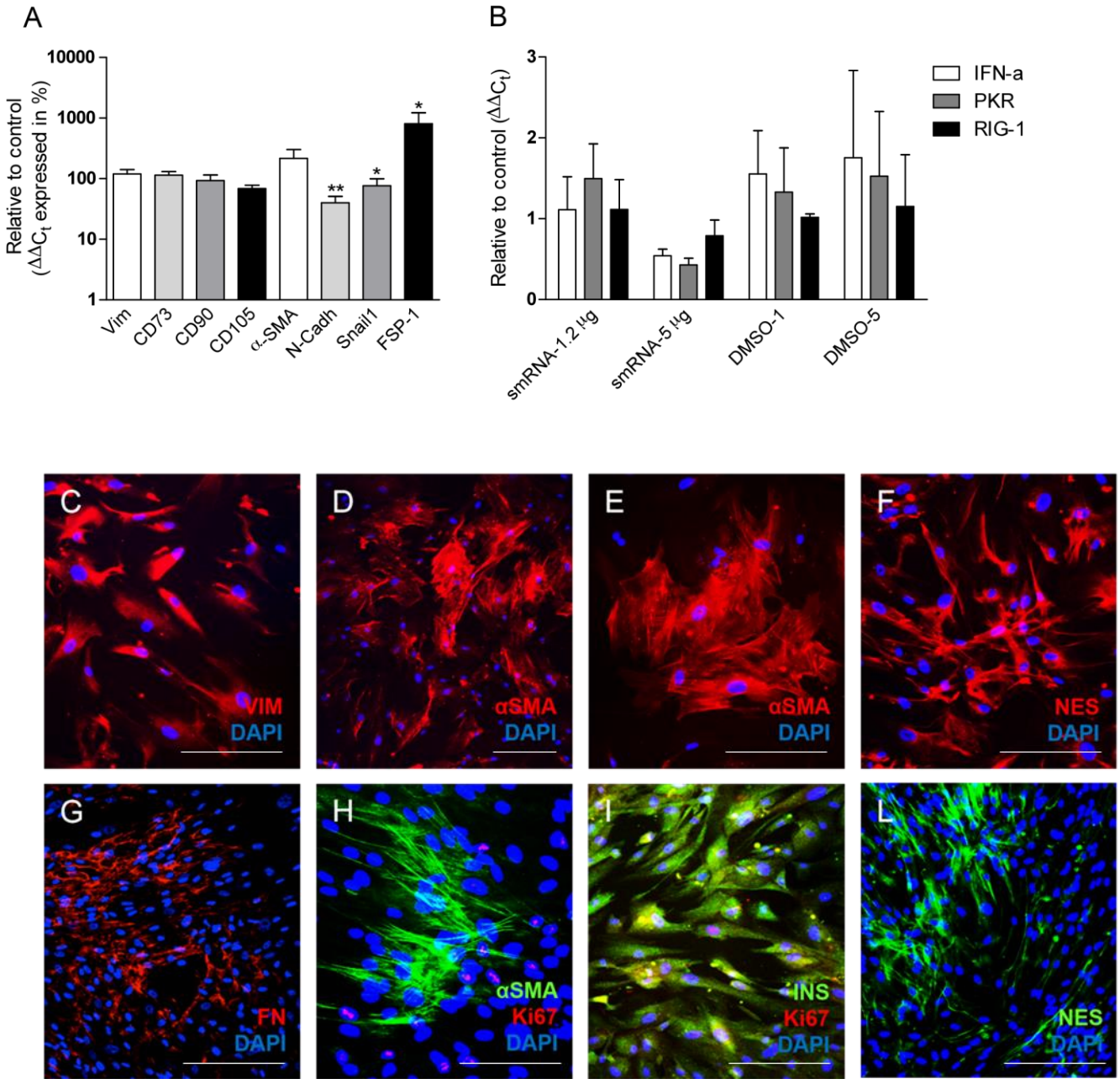
A
Relative to control (ΔΔCt expressed in %)
10000
1000
100
10
1
Vim
CD73
CD90
CD105
α-SMA
N-Cadh
Snail1
FSP-1
**
*
*
B
Relative to control (ΔΔCt)
3
2
1
0
IFN-a
PKR
RIG-1
smRNA-1.2 μg
smRNA-5 μg
DMSO-1
DMSO-5
C
VIM
DAPI
D
αSMA
DAPI
E
αSMA
DAPI
F
NES
DAPI
G
FN
DAPI
H
αSMA
Ki67
DAPI
I
INS
Ki67
DAPI
L
NES
DAPI

Figure S3: Additional evidence of HDDC differentiation

(**A**): After 7 consecutive transfections with MAFA smRNA, HDDCs showed downregulation of EMT transcripts (*i.e., N-cadherin, Snail1*), maintenance of mesenchymal transcripts (*i.e., vimentin, CD73/90/105, α-SMA*) and upregulation of FSP-1 gene expression (n=3-4). (**) P<0.01 and (*) P<0.05 compared to control condition. (**B**): Quantitative real-time PCR shows no significant differences in *IFNα*, *PKR* and *RIG-1* gene expression levels between HDDCs transfected with 1.2 or 5 µg of smRNA and HDDCs treated with 1-5 % of DMSO (n=3). (**C**-**L**): β-HDDCs decreased their protein expression of αSMA, fibronectin, and nestin mesenchymal markers (**G**-**H**, **L**) as compared to undifferentiated HDDCs (**C**-**F**), with a complete loss of vimentin expression (data not shown). (**I**): About 50% of insulin^{+} β-HDDCs showed co-expression with Ki67, confirming cell proliferation in these cells during *MAFA*-induced endocrine reprogramming. Staining data are representative of three independent experiments. Magnification bars: 200 µm (**C**-**G, I**, **L**), 100 µm (**H**).

Figure S4

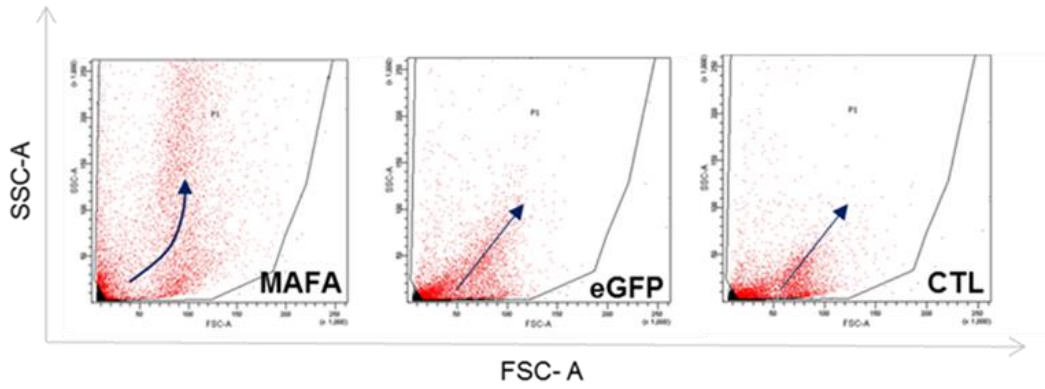

Figure S4: Morphological and phenotypic changes of MAFA-differentiated HDDCs

Flow cytometry data showing an increased cell size and cytoplasmic granularity on HDDCs after MAFA overexpression as compared to EGFP-transfected HDDCs and control conditions (*n*=3).

Table S1: Primers for real-time RT-PCR analysis

Transcript	Forward Primer	Reverse Primer
TBP	CCGCCGGCTGTTTAACTTC	GCTGGGTCACTGCAAAGATCA
MAFA (exo)	TCAACGACTTCGACCTGATG	GGTTGAGGTGATGCTGGTAG
NGN3 (exo)	TCGGAAGACGAAGTGACCTG	GTACAAGCTGTCGTGGTCCGCTATG
PDX1 (exo)	CGCAGCTTTACAAGGACCCAT	ATCTTGATGTGTCTCTCGGTC
PKR	TCGCTGGTATCACTCGTCTG	GATTCTGAAGACCGCCAGAG
IFNα	ACCCACAGCCTGGATAACAG	ACTGGTTGCCATCAAACTCC
RIG-1	GTTGTCCCCATGCTGTTCTT	GCAAGTCTTACATGGCAGCA
Insulin	GCAGCCTTTGTGAACCAACA	TTCCCCGCACACTAGGTAGAGA
Synaptophysin	ACTCCTCGTCAGCCGAATTCT	GCCCCCATGGAGTAGAGGAA
NeuroD	CAAGGTGGTGCCTTGCTATTC	GCGCAGAGTCTCGATTTTGG
NKX2.2	GGAGCGCCACGAATTGAC	TTCGAGACCCCAAAATTTATGTC
GATA4	AATTGGGATTTTCCGGAGTAAAC	CGTATTAAATCCAGCATTGAGCAA
PAX6	CAGACACAGCCCTCACAAACAC	AGGTTATTTGCCATGGTGAAGCT
Glucokinase	GAAGCCCCCCACCTTTCTC	CCTGAGTGAGCAACTCCCTTCT
GLUT-2	TTTTTCAGACGGCTGGTATCAG	CCATGTTTACAGCGCCAACTC
FOXA2	TTCAGGCCCGGCTAACTCT	ACCCCCACTTGCTCTCTCACT
MAFA (endo)	GCCATCGAGTACGTCAACGA	CGGGAGGCTCCTTCTTCAC
NGN3 (endo)	GCTGCTCATCGCTCTCTATTCTT	CGAGGGTTGAGGCGTCAT
PDX1 (endo)	TTTCTATTTAGGATGTGGACGTAATTCC	GGCCACTGTGCTTGTCTTCA
NKX6.1	ACCCCTCATCAAGGATCCATT	TGGGTCTCGTGTGTTTTCTCTTC
Pc	AGAAGTGGTCCGCAAGATGG	GTAATTCTCCTCCAGCTCCT
Prlr	CTCCACCTACCCTGATTG	CTCCATGCACTCCAGTATCC
Glucagon	CAAGGCAGCTGGCAACGT	CTGGTGAATGTGCCCTGTGA
Somatostatin	CAACCAGACGGAGAATGATG	CAGCCAGCTTTGCGTTCT
Pancreatic polypeptide	AGAGCAGATGGCCCAGTATG	CTGCTCATGGAGTCGTAGGA
Vimentin	GCAGGCTCAGATTCAGGAACA	GTGAGGTCAGGCTTGGAAACA
N-cadherin	TGGGAATCCGACGAATGG	GCAGATCGGACCGGATACTG
α-SMA	CCTCCTCCCTGGAAAAGAGCTA	TCGTTGCCGATGGTGATG
FSP-1	GCTTGCACACGCTGTTGCTA	GAGGGCACGCCATGACA
CD73	CACTGGGACATTCGGGTTTT	CGTCCACACCCCTCACTTTC
CD90	CGAACCAACTTCACCAGCAAAT	CCTTGCTAGTGAAGGCGGATA
CD105	CCGCGCTTCAGCTTCCT	GAGGGTGCCGGTTTTGG
Snail1	GTTTCCCGGGCAATTTAACA	CCCGACAAGTGACAGCCATT

Table S2: Antibodies for immunostaining

Primary Antibodies (Immunohistochemistry)			
Antigen	**Species**	**Dilution**	**Source**
PKR	Rabbit	1:200	Abcam
Insulin	Guinea Pig	1:100	Dako
Insulin	Rabbit	1:50	Santa Cruz
MAFA	Rabbit	1:100	Abcam
MAFA	Goat	1:50	Santa Cruz
PDX1	Goat	1:6	R&D systems
GLUT-2	Rabbit	1:100	Abcam
Somatostatin	Rabbit	1:200	Vision Biosystems Novocastra
Pancreatic polypeptide	Rabbit	1:200	Vision Biosystems Novocastra
Glucagon	Rabbit	1:50	Vision Biosystems Novocastra
Secondary Antibodies (Immunohistochemistry)			
Target	**Conjugate**	**Dilution**	**Source**
Guinea-Pig	Biotin	1:400	Jackson ImmunoResearch
Biotin	AF488	1:200	Molecular Probes
Rabbit	Alexa594	1:200	Jackson ImmunoResearch
Goat	FITC	1:200	Jackson ImmunoResearch

Table S3

Number of insulin positive cells after *in vitro* differentiation

Donor	*Conditions*	*Random fields*	*Cells counted*
Hp1111	Control group	6	7723 (total cells)
Hp1116	MAFA group	6	2564 (positive cells)
Hp1117			
Hp1			
Hp2			

Number of MAFA positive cells after *in vitro* differentiation

Donor	*Conditions*	*Random fields*	*Cells counted*
Hp1111	Control group	6	5138 (total cells)
Hp1116	MAFA group	6	1762 (positive cells)
Hp1117			
Hp1			
Hp2			

Number of PKR positive cells after *in vitro* differentiation

Donor	*Conditions*	*Random fields*	*Cells counted*
Hp1116	Control group	4	6040 (total cells)
Hp1117	MAFA group	4	2669 (positive cells)
Hp1			

Number of EGFP positive cells after *in vitro* differentiation

Donor	*Conditions*	*Random fields*	*Cells counted*
Hp1080	Control group	4	4701 (total cells)
Hp1116	MAFA group	5	2218 (positive cells)
Hp2			