

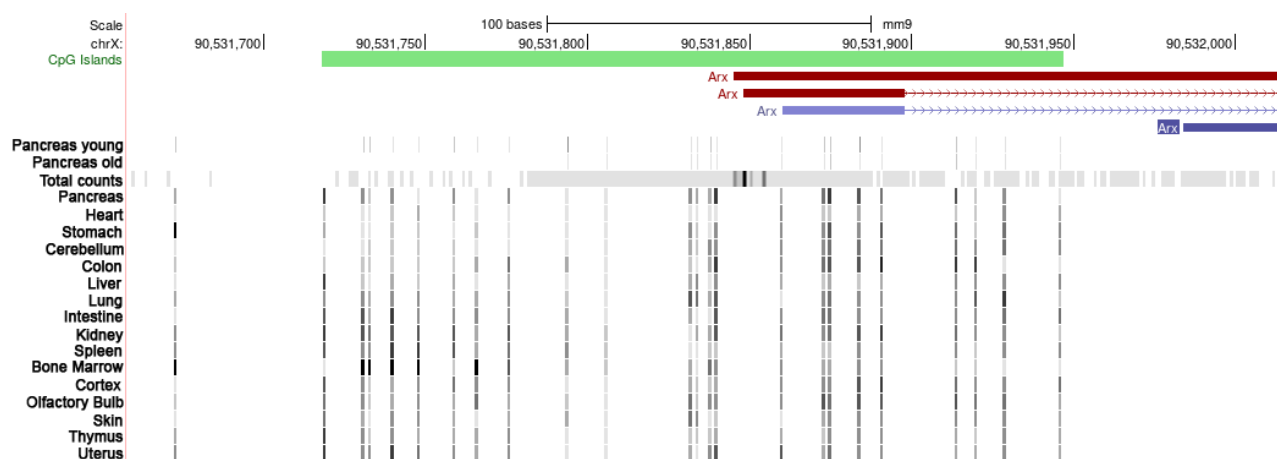
## Supplementary Material

Article

# EpiCRISPR targeted methylation of Arx gene initiates transient switch of mouse pancreatic alpha to insulin-producing cells

Marija Đorđević<sup>1</sup>, Peter Stepper<sup>2</sup>, Clarissa Feuerstein-Akgoz<sup>3</sup>, Clarissa Gerhauser<sup>3</sup>, Verica Paunović<sup>4</sup>, Anja Tolić<sup>1</sup>, Jovana Rajić<sup>1</sup>, Svetlana Dinić<sup>1</sup>, Aleksandra Uskoković<sup>1</sup>, Nevena Grdović<sup>1</sup>, Mirjana Mihailović<sup>1</sup>, Renata Z. Jurkowska<sup>5</sup>, Tomasz P. Jurkowski<sup>5\*</sup>, Jelena Arambašić Jovanović<sup>1\*</sup>, Melita Vidaković<sup>1‡</sup>

Correspondence: [jurkowskit@cardiff.ac.uk](mailto:jurkowskit@cardiff.ac.uk); TJ, [jelena.arambasic@ibiss.bg.ac.rs](mailto:jelena.arambasic@ibiss.bg.ac.rs); JAJ., [melita@ibiss.bg.ac.rs](mailto:melita@ibiss.bg.ac.rs); MV.



**Supplementary Figure 1.** UCSC genome browser views of murine *Arx* gene's DNA methylation sites in the pancreas and other tissues. If compared with other tissues, *Arx* gene in pancreatic tissue (young and old) exhibits low DNA methylation. CpG island was shown as a green box.

## Supplementary Tables

**Table 1. Targeted sequences for sgRNAs**

Targeting sequences of sgRNAs		
Arx sgRNA	1	GCAAAGCGCAAAGCGCGAAC
	2	TAACAAGTGTAGTGAGCCGC
	3	AGGGTGGGAGCCCGCAACCG
	4	GATGCTGTCGATGCAGTAGG

**Table 2. Primers used for RT-qPCR**

Gene		Primer sequence (5'-3')
REEP 5	fw	TCATCGGACTGGTGGCTTTG
	rev	GTTGGGACTCTCGATGGCTT
Cas 9	fw	TCAGGCGGCAAGAGGATTC
	rev	AGTCATCCACGCGAATCTGG
Arx	fw	CGGAGAAGCCCATGCAAAAT
	rev	TGCAGCTCAGCCTCGAAC
Pax 4	fw	GCTCGAATTGCCAGCTAAAG
	rev	TTACTGTGGGGACTGGGAAGA
Ins 2	fw	GGAGCGTGGCTTCTTCTACA
	rev	TTCATTGCAGAGGGGTAGGC
Slc2a2	fw	TGCACCATCTTCATGTCGGT
	rev	ATTGCAGACCCAGTTGCTGA
Maf A	fw	TTCTGGAGAGCGAGAAGTGC
	rev	ACAGAAAGAAGTCGGGTGCG
Pax6	fw	TGTCTACCAGCCAATCCCAC
	rev	CATCACTGACGGGCTGGT
Pdx1	fw	CCTTCCCGAATGGAACCGA
	rev	GGGCCGGGAGATGTATTTGT
Nkx2-2	fw	TTCCGGACACCAACGATGAA
	rev	CCGTGCAGGGAGTATTGGA
Nkx6-1	fw	GAGAGCACGCTTGGCCTATT
	rev	TCGTCGTCATCCTCCTCATTC

**Table 3. Primers used for HRM**

Primers		Sequence (5'-3')		Primer length	Amplicon length
<b>R1</b>	M	Fw	AATTTTAAGGATAAGAAGGAGCGATA	26	198
		Rev	CGAACTACGTAAACCTACCG	20	
	U	Fw	TTTTAAGGATAAGAAGGAGTGATA	24	200
		Rev	AAACCAAACCTACATAAACCTACCAA	25	
<b>R2</b>	Fw	GTATTAGGAAGAGGGTTGTTTTGAG	25	287	
	Rev	ATATCTCCAACCTAACAAATCCCAA	25		

**Table 4. Touchdown PCR program for amplification of bisulfite converted DNA, starting at 55 °C.**

bisulfite PCR program		
95	15 min	
94 °C	30 s	6 x
x °C	30 s	10 x
72 °C	40 s	
94 °C	30 s	
x-5 °C	30 s	35 x
72 °C	40 s	
94 °C	30 s	
x-10 °C	30 s	
72 °C	40 s	
72 °C	4 min	
4 °C	hold	

**Table 5. Primers for NGS library preparation**

Primers	Sequence (5'-3')
mArx_bis_1_f	TTT TTT GTT TTT ATA TTT ATA GGT TTT TTT T
mArx_bis_1_r	TTA CTC ATA ACT AAT ACT TTT TCC TTA AAC
mArx_bis_2_f	GTT TAA GGA AAA AGT ATT AGT TAT GAG TAA
mArx_bis_2_r	TAT CTC CAA CTT AAC AAA TCC CA
mArx_biscg2_3_f	AGG TTG AGT TGT ATT TGT TAT TTA AGT
mArx_biscg2_3_r	CTA ACT AAT CTT AAA CRT ATC CCA AAC
mArx_biscg2_4_f	GTT TGG GAT AYG TTT AAG ATT AGT TAG
mArx_biscg2_4_r	AAT ACA ACA ACA ACT CTT CCT TAA A

**Table 6. Antibodies used for Immunoblot analysis (IBA) and Immunocytochemistry (ICC)**

	<b>Antibody</b>	<b>Host species</b>	<b>Dilution IBA/ICC</b>	<b>Supplier</b>
<b>Primary antibody</b>	anti-glucagon	Goat	1:500 / 1:50	C-18 / Santa Cruz Biotechnology
	anti-insulin	Rabbit	1:500 / 1:200	H-86 / Santa Cruz Biotechnology
	anti-Arx	Rabbit	1:1000 / 1:100	ab48856 / Abcam
	anti-beta Actin	Rabbit	1:2000 / 1: -100	ab8227 / Abcam
<b>Secondary antibody</b>	anti-rabbit IgG-HRP	Cow	1:2000 / -	sc-2379 / Santa Cruz Biotechnology
	anti-goat IgG-HRP	Donkey	1:2000 / -	sc-2020 / Santa Cruz Biotechnology
	anti-goat IgG, F(ab') <sub>2</sub> -TRITC	Donkey	1:100	sc-3855 / Santa Cruz Biotechnology
	anti-rabbit IgG (H+L), Alexa Fluor 555	Donkey	1:4000	A-31572 / Invitrogen

**Table 7. Primers for PCR reaction after ChIP**

		<b>Sequence (5'-3')</b>	<b>Amplicon length</b>
<b>ChIP primers</b>	fw	TTAGGGTTTGCCACCCCATCTA	208
	rev	CAATCAGCCTCCCAAATGAAG	