http://dergipark.org.tr/trkjnat
Trakya University Journal of Natural Sciences, 26(1): 49-59, 2025
ISSN 2528-9691
DOI: 10.23902/trkjnat.1622077

Supplementary Material

SUPPLEMENTARY MATERIAL TO

Akçakale Kaba F., Akıncı E. , Cengiz M.F. & Kaba A. 2025. Comparison of DCAS9-activator complexes for the activation of PDX1 and NGN3 pancreatic genes using the CRISPR system. *Trakya Univ J Nat Sci*, 26(1): 49-59, DOI: 10.23902/trkjnat.1622077

Table S1. The gRNA sequences designed for the Pdx1 gene were ligated into the pSPgRNA plasmid after cutting with the *BbsI* enzyme, and the BbsI cut sites were highlighted in red to complement the sticky ends.

gRNA		Sequence (5′-3′)	Chain	Distance from TSS
1	Forward	CACCGGAACGGGCAGCTGGCGGTGC	-	-240
	Reverse	AAACGCACCGCCAGCTGCCCGTTCC		
2	Forward	CACCGGCGAGCACCTGCTTTTGTTC	-	-183
	Reverse	AAACGAACAAAAGCAGGTGCTCGCC		
3	Forward	CACCGGGCTGGCCGCACTAAGAGGC	-	-148
	Reverse	AAACGCCTCTTAGTGCGGCCAGCCC		
4	Forward	CACCGGCTCGCTTTGACAGCTCCGC	-	-22
	Reverse	AAACGCGGAGCTGTCAAAGCGAGCC		
5	Forward	CACCGATTTTCTCTCTCAGCTGAGT	-	-205
	Reverse	AAACACTCAGCTGAGAGAGAAAATC		
6	Forward	CACCGCTGGCGGTGCTCCCCAAAAT	+	-250
	Reverse	AAACATTTTGGGGAGCACCGCCAGC		
7	Forward	CACCGGCCGGGGGCCGTGATTGGCC	+	-127
	Reverse	AAACGGCCAATCACGGCCCCCGGCC		
8	Forward	CACCGAGGCTCCGCGGGGCCCCACG	+	-100
	Reverse	AAACCGTGGGGCCCCGCGGAGCCTC		
9	Forward	CACCGGCGGGCCGGCCGCCGCACCA	-	-79
	Reverse	AAACTGGTGCGGCGGCCGGCCCGCC		
10	Forward	CACCGAACCCACAGCCAGCGCGGAC	+	-57
	Reverse	AAACGTCCGCGCTGGCTGTGGGTTC		

Table S2. The gRNAs designed for the *Ngn3* gene (BbsI cutting sites in red color were added to the gRNA sequences to complement the sticky ends generated by cutting the pSPgRNA plasmid with *BbsI* enzyme)

gRNA		Sequence (5′-3′)	Chain	Distance from TSS
1	Forward	CACCGGGCCTGACCAGAGCCACACG	+	-239
	Reverse	AAACCGTGTGGCTCTGGTCAGGCCC		
2	Forward	CACCGGCTAGGAGCAAAGCCGTCTG	+	-263
	Reverse	AAACCAGACGGCTTTGCTCCTAGCC		
3	Forward	CACCGAGAGTTGCTGGGACCCAGCC	-	-182
	Reverse	AAACGGCTGGGTCCCAGCAACTCTC		
4	Forward	CACCGGGCGCGGGCAGCAGCCGGGC	+	-27
	Reverse	AAACGCCCGGCTGCTGCCCGCGCCC		
5	Forward	CACCGGGGCAGGCACGCTCCTGGCC	+	-11
	Reverse	AAACGGCCAGGAGCGTGCCTGCCCC		
6	Forward	CACCGGGGCCCCGGCGCTGATTGGC	+	-51
	Reverse	AAACGCCAATCAGCGCCGGGGCCCC		
7	Forward	CACCGGTGCCCTGCGGGGGAGGAGC	+	-93
	Reverse	AAACGCTCCTCCCCCGCAGGGCACC		
8	Forward	CACCGGCGCTCCCCTCCCCCGACCC	+	-137
	Reverse	AAACGGGTCGGGGGAGGGGAGCGCC		

Table S3. The primer sequences used for the determination of endogenous gene expression by RT-qPCR upon *Pdx1* and *Ngn3* gene activation.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Product Length
hINS	GCAGCCTTTGTGAACCAACAC	CCCCGCACACTAGGTAGAGA	67 bp
hMAFA	TTGTACAGGTCCCGCTCTTT	AGCGAGAAGTGCCAACTCC	83 bp
hPDX1	GGAACTCCTTCTCCAGCTCTA	CCTTTCCCATGGATGAAGTC	145 bp
hNGN3	AGTTGGCACTGAGCAAGC	AGTGCCGAGTTGAGGTTG	88 bp
hNKX2.2	CGAGGGCCTTCAGTACTCC	GGGGACTTGGAGCTTGAGT	72 bp
hNKX6.2	TTCCGTTTTCCCGCTTTGG	ATGCGCAGAGGGACTTTGG	103 bp
hPAX4	ACCCCACCTAAAGCCTGTCT	AGGCAAAGCAGTCCTGAGTC	83 bp
hGLUT2	TACATTGCGGACTTCTGTGG	AGACTTTCCTTTGGTTTCTGG	108 bp
hKIR6.2	TGTGTCACCAGCATCCACTC	CACTTGGACCTCAATGGAGAA	60 bp
hGAPDH	AGGGCTGCTTTTAACTCTGGT	CCCCACTTGATTTTGGAGGGA	152 bp

Table S4. Nucleic acid stain, antibodies and dilution ratios used in immunofluorescence staining.

Antibody	Brand / Catalog no	Dilution
Rb polyclonal anti-Pdx1	Abcam/ Ab47267	1: 2000
Rb polyclonal anti-Ngn3	Abcam/ Ab38548	1: 200
Rabbit monoclonal anti-Insulin	Abcam/ EPR17359	1:300
Goat Anti-Rabbit IgG, DyLight 488	Thermo Scientific / 35552	1:400
Hoechst 33342 Solution	Thermo Fisher Scientific / 62249	1: 400

To demonstrate the successful transfection of gRNA plasmids along with dCas9-VPR, dCas9-p300, or dCas9-VP64 plasmids into cells via lipofection, cells were also treated with a dCas9-VP64-GFP plasmid. The success of the transfection was visualized using fluorescence microscopy. Transfection efficiency was determined by counting GFP-positive cells, revealing a transfection efficiency of 70%.

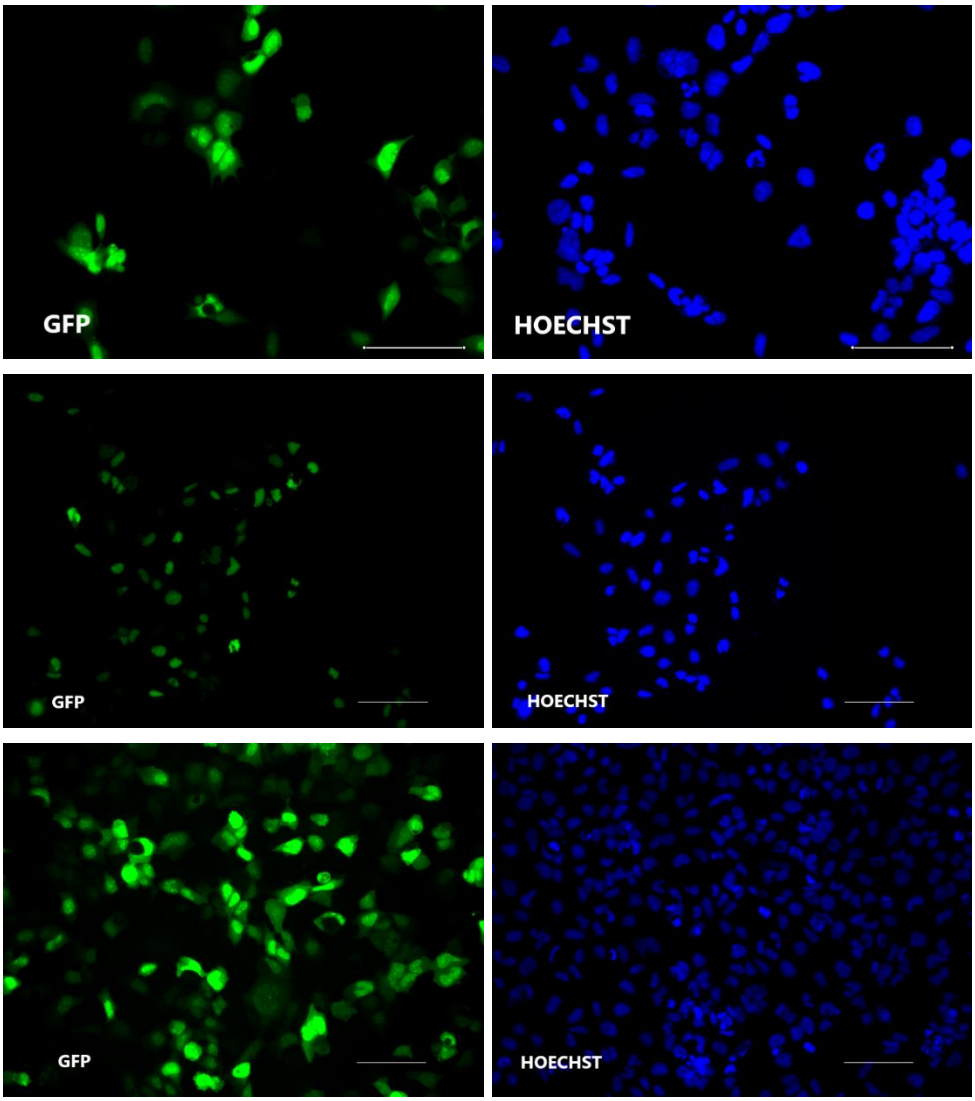

Fig. S1. Immunocytochemical analysis of GFP in HEK293 cells 48 hours after transfection with 200ng dCas9-VP64-GFP plasmid. GFP (green), Hoechst (blue) and scale bars represent 100 µm.