

Enhancement of Transgene Expression by Mild Hypothermia is Promoter-Dependent in HEK293 Cells

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Supplementary Materials

Table S1. List of common transcription factors identified from comparison of *in silico* analysis of CMV-enhancer specific transcription factors and significant DEG lists from RNA-seq analysis.

Gene Symbol	Gene Description	Fold Change (32 °C versus 37 °C)
<i>FOS</i>	Fos proto-oncogene, AP-1 transcription factor subunit	−3.86 (3.25×10^{-34})
<i>RFX3</i>	regulatory factor X3	−2.22 (8.52×10^{-6})
<i>PRRX2</i>	paired related homeobox 2	−2.03 (5.42×10^{-3})
<i>FOSL1</i>	FOS like 1, AP-1 transcription factor subunit	−4.51 (1.94×10^{-10})
<i>LBX2</i>	ladybird homeobox 2	−3.00 (3.23×10^{-6})
<i>JDP2</i>	Jun dimerization protein 2	−4.31 (1.80×10^{-22})
<i>NEUROG2</i>	neurogenin 2	2.17 (3.71×10^{-7})
<i>EBF1</i>	EBF transcription factor 1	−2.06 (1.97×10^{-3})
<i>ETV1</i>	ETS variant 1	−2.67 (8.10×10^{-8})
<i>ETV2</i>	ETS variant 2	2.61 (2.35×10^{-4})
<i>NKX3-1</i>	NK3 homeobox 1	2.59 (6.02×10^{-18})
<i>ONECUT3</i>	one cut homeobox 3	−3.08 (3.41×10^{-6})
<i>HOXD9</i>	homeobox D9	−2.25 (1.59×10^{-13})
<i>INSM1</i>	INSM transcriptional repressor 1	−3.03 (1.68×10^{-12})

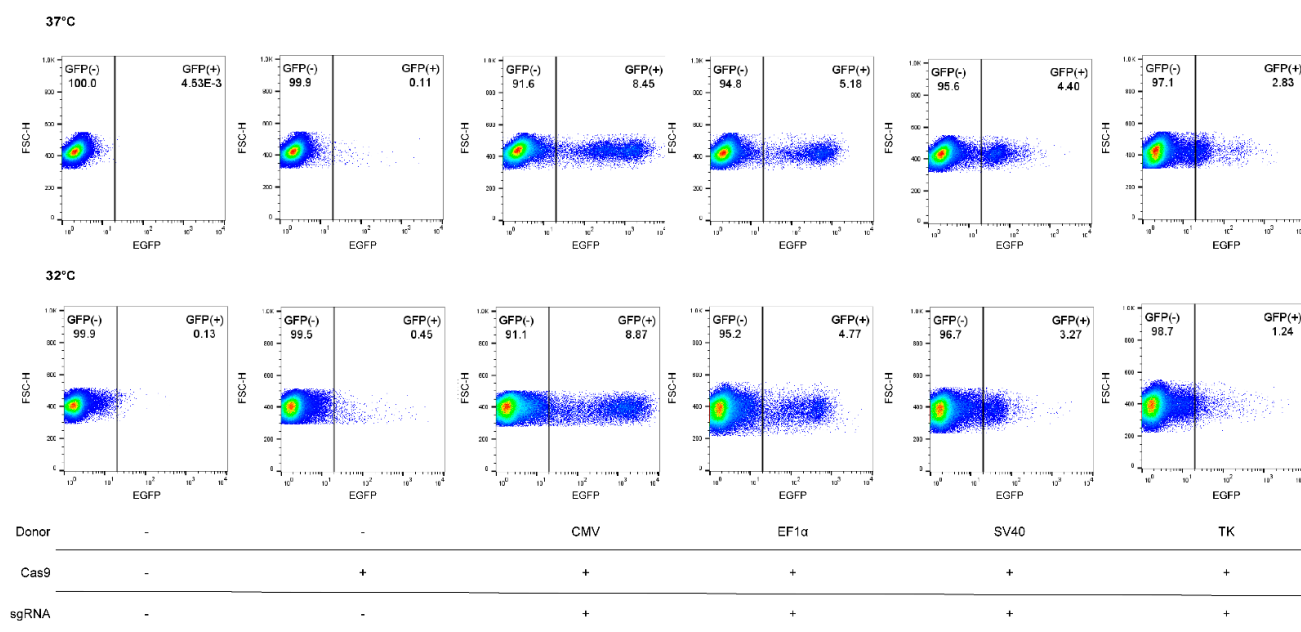
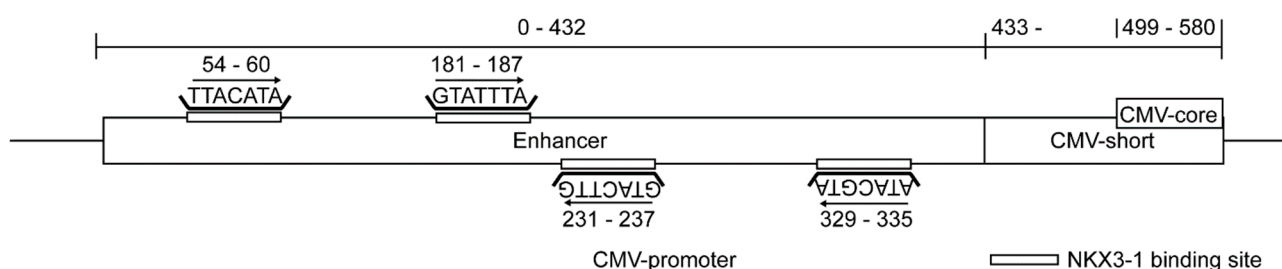
Table S2. Primer sequences used for RT-PCR and RT-qPCR.

Gene	Direction	Sequence (5'-3')
<i>ACTB</i>	Forward	CTGGAACGGTGAAGGTGACA
	Reverse	AAGGGACTTCCTGTAACAACGCA
<i>ETV2</i> isoform 1	Forward	AGCAGAGACATGCTGGAAAG
	Reverse	GGAACCTTCTGGGTGCAGTAA
<i>ETV2</i> isoform 2	Forward	CGCACGGACTGTACCATTT
	Reverse	GCTCTGGTACCGCTTCAAA
<i>NEUROG2</i>	Forward	CTGGGTCTGGTACACGATTG
	Reverse	CAGTCTACGGGTCTTCTTGATG
<i>NKX3-1</i>	Forward	CGGAGACCCAAGTGAAGATATG
	Reverse	CAAAGAGGAGTGCTTCTCCAA

Table S3. Comparison of fold changes in DEGs identified by RNA-seq and RT-qPCR.

Gene	Fold Change of DEGs (32 °C versus 37 °C)	
	RNA-seq (raw p-value)	RT-qPCR ^a
<i>ETV2</i>	2.61 (2.35×10^{-4})	1.36/1.12 ^b
<i>NEUROG2</i>	2.17 (3.71×10^{-7})	1.24
<i>NKX3-1</i>	2.59 (6.02×10^{-18})	2.02

^a Human *ACTB* gene was used as a reference gene. Average values obtained in triplicate experiments are shown. ^b *ETV2* isoform 1 / *ETV2* isoform 2.

**Figure S1.** Representative flow cytometry plots and gates of CRISPR/Cas9-mediated targeted integration of promoter sequences at the AAVS1 locus shown in Figure 1B.**Figure S2.** Schematic representation of the short variants of the CMV promoter. Predicted binding sites of NKX3-1 on the CMV-enhancer region are shown.

