Supplementary materials

of

Partial Somatic to Stem Cell Transformations Induced By Cell-Permeable Reprogramming Factors

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Supporting Information

Figure S1. FACS analysis of MTD-mediated protein uptake by cultured cells. RAW 264.7 cells were treated for 1 hr at 37°C with buffer alone (gray, cells only), with 10 μM unconjugated FITC (black, FITC only) or with 10 μM FITC-conjugated enhanced green fluorescent proteins (EGFP) containing a random peptide (blue line, FITC-HSE), the Fibroblast Growth Factor 4-derived membrane translocating sequence (FGF4 MTS, red line, FITC-HM_mE) or one of the following MTDs (green line, FITC-HME) counter clockwise from top left: MTD84, MTD86, MTD47, MTD132, MTD181, MTD173 and MTD52. The cells were then washed 3 times and analyzed by Flow cytometry.

Figure S2. Fluorescence confocal laser scanning micrographs of macromolecule transduction domain-mediated protein uptake. NIH3T3 cells were treated for 1 hr at 37°C with buffer alone (cells only), 10 μM unconjugated FITC (FITC only), or with 10 μM FITC-conjugated EGFPs containing a random peptide (FITC-HSE), the FGF4 MTS (FITC-HM_mE) or a MTD designated with the numbered subscript. Green fluorescence results from internalized protein resistant to extensive washing and protease treatment. Nuclei were stained (red) with propidium Iodide (PI). Bottom panels show Nomarski images of the same cells.

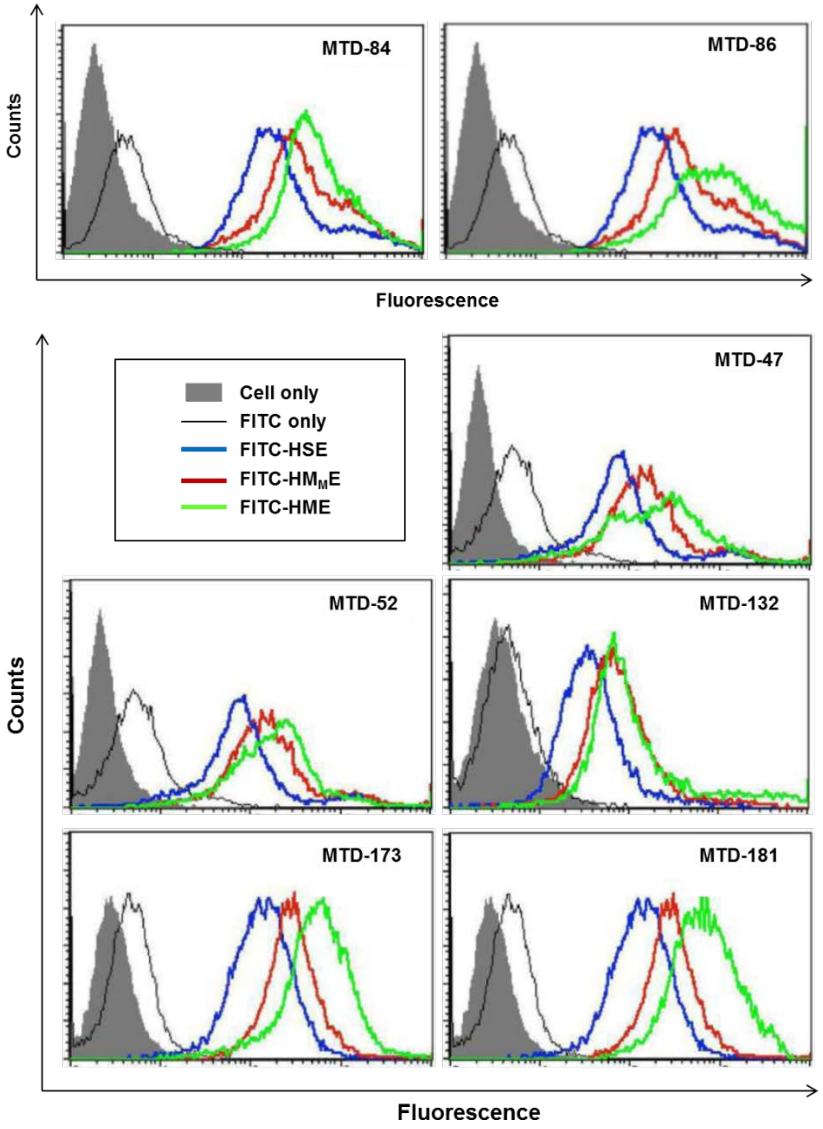
Figure S3. Transactivation of reporter gene expression by cell-permeable reprogramming factors. Luciferase reporter plasmids under the control of OCT4-, SOX2-, KLF4A- and CMYC-response elements were introduced by lipofection into HEK293 cells. After 12 hrs, the cells were treated either with the appropriate CP-RF or with cell-permeable Cre recombinase (CP-

Cre), which served as a negative control, i.e., a protein without transactivation activity. Relative luciferase activities are plotted relative to CP-Cre treatment, expressed as means ± s.d. for triplicate values from three independent experiments. *p* values were determined by an unpaired Student's *t*-test.

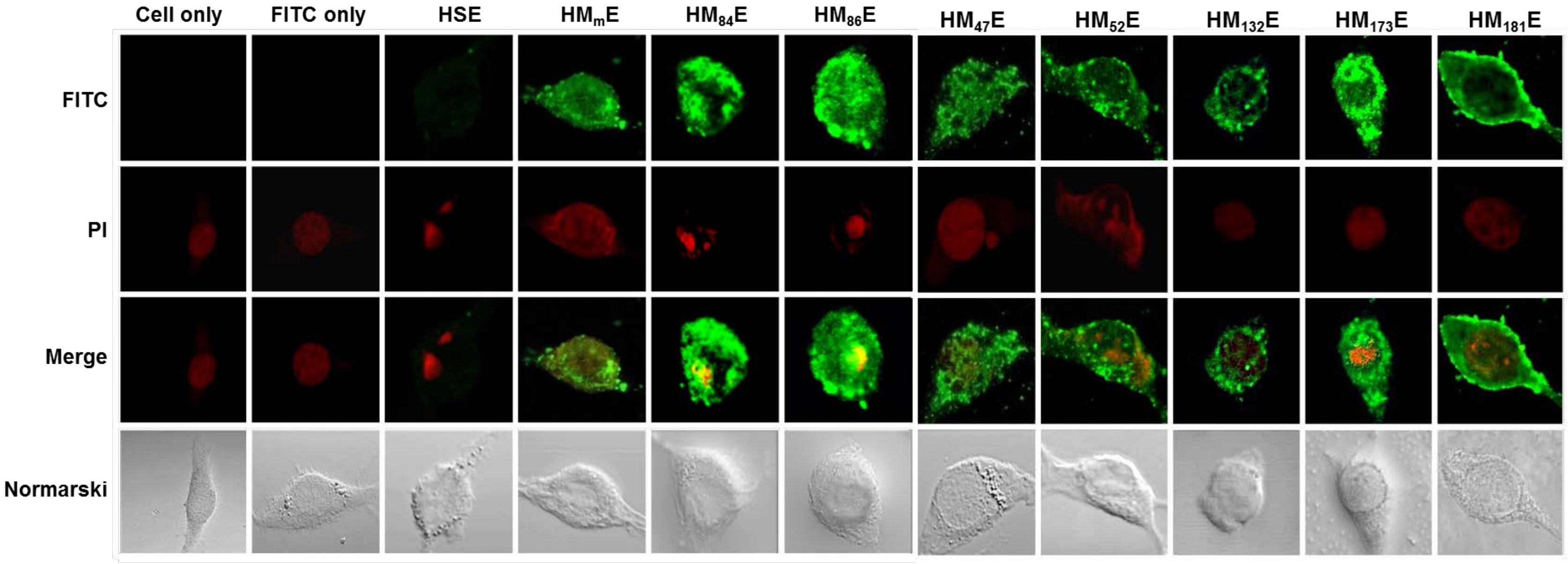
Table S1. Macromolecule transduction domains (MTDs). The 7 MTDs used in the present study were identified while screening 1,500 potential signal peptides for sequences capable of enhancing protein uptake by cultured cells. The final MTD sequences were shortened and modified from the original primarily to enhance their predicted α-helical structure.

Table S2. PCR primers used to construct recombinant cell-permeable reprogramming factors. Coding sequences for EGFP (E) and reprogramming factors (OCT4, SOX2, KLF4, CMYC, NANOG and LIN28; abbreviated O, S, K, M, N, and L, respectively) were cloned into pET-28a(+) from DNA segments PCR amplified using 5' and 3' flanking primers.

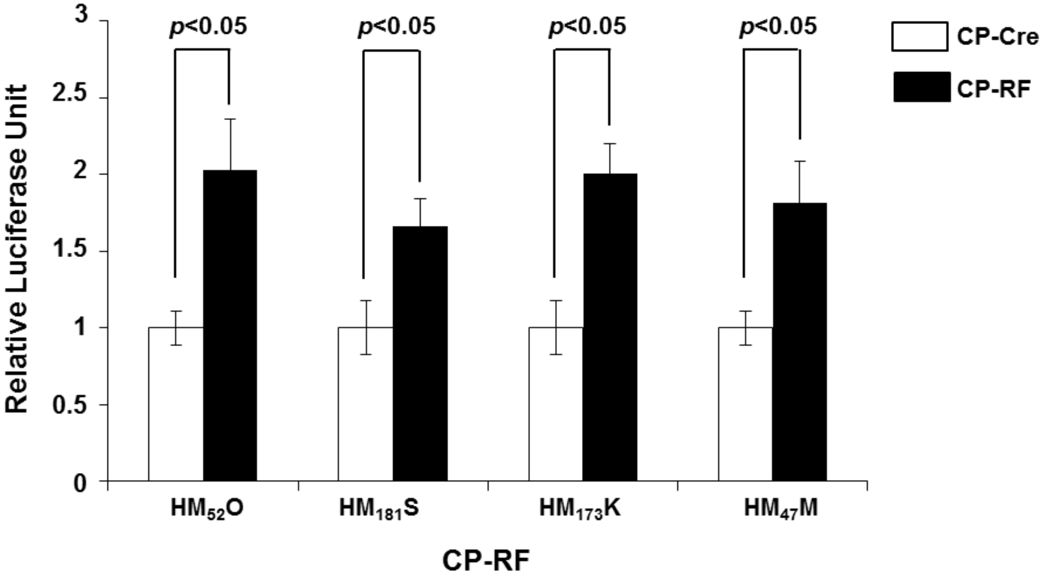
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



Supplementary Table S1

A

Information	MTD-84	MTD-86
Origin	*Phytophthora cactorum*	*Streptomyces coelicolor*
Protein	Phytotoxic portein PcF precursor	Peptide Transport system secreted peptide binding protein
Accession Number	AAK63068	NP_629842
Original Sequence	^{2}NFKTCPAVALVAVVATVATAEDP^{24}	^{20}RLLAAAGAGALLLASGAVAPSVA^{42}
Final Sequence	AVALVAVVAVA	LLAAAAALLLA

B

Information	MTD-47	MTD-52	MTD-132	MTD-173	MTD-181
Origin	*StrePtomyces coelicolor*	*Homo sapiens*	*StrePtomyces coelicolor*	*StrePtomyces coelicolor*	*Neisseria meningitidis Z2491*
Protein	Secreted Protein	Ficlin 3 isoform 2 precursor	P60-family secreted protein	Secreted Protein	Putative secreted protein
Accession Number	NP_627512	NP_775628	NP_628377	NP_624384	CAB84257.1
Original Sequence	^{23}VLVGAAAVPVMLVAAGC^{39}	^{1}MDLLWILPSLWLLLLGGPACLK^{22}	^{7}VLTTTAVTVVCAITVLAAPG^{26}	^{4}LGLSAVMISILAVTGCGG^{21}	^{4}MFLSAVLLLSAAAQTVWADTVF^{25}
Final Sequence	AAAVPVLVAA	PLLLLLPAL	AVVVPAIVLAAP	AVIPILAVP	AVKKKPAAA

Supplementary Table S2

Primer	Sequence
HO-5’ (45nts)	CCGCATATGAAGAAGAAGAGGAAGGCGGGACACCTGGCT TCGGAT
HO-3’ (36nts)	CCGCATATGTCAGTTTGAATGCATGGGAGAGCCCAG
HS-5’ (45nts)	CCGCATATGAAGAAGAAGAGGAAGTACAACATGATGGAG ACGGAG
HS-3’ (36nts)	CCGCATATGTCACATGTGTGAGAGGGGCAGTGTGCC
HK-5’ (45nts)	CCGCATATGAAGAAGAAGAGGAAGGCTGTCAGCGACGCG CTGCTC
HK-3’ (36nts)	CCGCATATGTTAAAAATGCCTCTTCATGTGTAAGGC
HM-5’ (45nt)	CCGCATATGAAGAAGAAGAGGAAGGATTTTTTTCGGGTA GTGGAA
HM-3’ (36nts)	CCGCATATGTTACGCACAAGAGTTCCGTAGCTGTTC
HN-5’ (45nts)	CCGCATATGAAGAAGAAGAGGAAGAGTGTGGATCCAGCTTGTCCC
HN-3’ (36nts)	CCGCATATGTCACACGTCTTCAGGTTGCATGTTCAT
HOM$_{84}$-3’ (63nts)	CCGCATATGTCACAGCACCGCCAGCAGCGCCGCCACCAGGTTTGAATGCATGGGAGAGCCCAG
HM$_{86}$SM$_{86}$-5’ (72nts)	CCGCATATGAAGAAGAAGAGGAAGCTGGCGGTGCTGGCGGCGGCGCCGTACAACATGATGGAGACGGAGCTG
HM$_{86}$SM$_{86}$-3’ (60nts)	CCGCATATGTCACGGCGCCGCCGCCAGCACCGCCAGCATGTGTGAGAGGGGCAGTGTGCC
HM$_{86}$KM$_{86}$-5’ (72nts)	CCGCATATGAAGAAGAAGAGGAAGCTGGCGGTGCTGGCGGCGGCGCCGGCTGTCAGCGACGCGCTGCTCCCA
HM$_{86}$KM$_{86}$-3’ (60nts)	CCGCATATGTTACGGCGCCGCCGCCAGCACCGCCAGAAAATGCCTCTTCATGTGTAAGGC
HM$_{86}$M-5’ (72nts)	CCGCATATGAAGAAGAAGAGGAAGCTGGCGGTGCTGGCGGCGGCGCCGGATTTTTTTCGGGTAGTGGAAAAC
HM$_{86}$N-5’ (72nts)	CCGCATATGAAGAAGAAGAGGAAGCTGGCGGTGCTGGCGGCGGCGCCGAGTGTGGATCCAGCTTGTCCCCAA