

Appendix

REST suppression mediates neural conversion of adult human fibroblasts via microRNA dependent and independent pathways

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Appendix Table S1. Summary of the electrophysiological properties

Intrinsic properties	Adult iN in vitro	Adult iN in vivo
Resting membrane potential (mV)	-47.28 ± 4.05 (n=18)	-71.14 ± 3.93 (n=7)
Cell capacitance (pF)	21.78 ± 7.55 (n=22)	60.88 ± 11.99 (n=8)
Membrane resistance (MΩ)	1808 ± 308.3 (n=20)	76.63 ± 33.89 (n=8)
Number of AP able to evoke	1.35 ± 0.65 (n=20)	3.25 ± 1.35 (n=8)
N of cells with post synaptic activity	1/20	6/8

Appendix Table S2. Demographic information on the biopsy donors

Cells source	Source	Disease	Sex	Age	Disease Duration (years)	Mutation
Dermal biopsy	John van Geest Centre for Brain Repair	None	F	52		
Dermal biopsy	John van Geest Centre for Brain Repair	None	F	61		
Dermal biopsy	John van Geest Centre for Brain Repair	None	F	67		
Dermal biopsy	John van Geest Centre for Brain Repair	None	M	69		
Dermal biopsy	John van Geest Centre for Brain Repair	None	F	70		
Dermal biopsy	John van Geest Centre for Brain Repair	None	M	71		
Lung biopsy	Lund University, Lund	None	F	45-65		
Dermal biopsy	Lund University, Lund	None	F	74		
Dermal biopsy	Karolinska Institute, Stockholm	Genetic Alzheimer's disease	F	58	4	<i>APP</i> KM670/671NL
Dermal biopsy	John van Geest Centre for Brain Repair	Huntington's disease (41 CAG repeats)	M	61		<i>HTT</i> - 41 CAG repeats
Dermal biopsy	John van Geest Centre for Brain Repair	Sporadic Parkinson's disease	M	77	4	
Dermal biopsy	John van Geest Centre for Brain Repair	Genetic Parkinson's disease	F	55	8	<i>LRRK2</i> c.6055G>A mutation

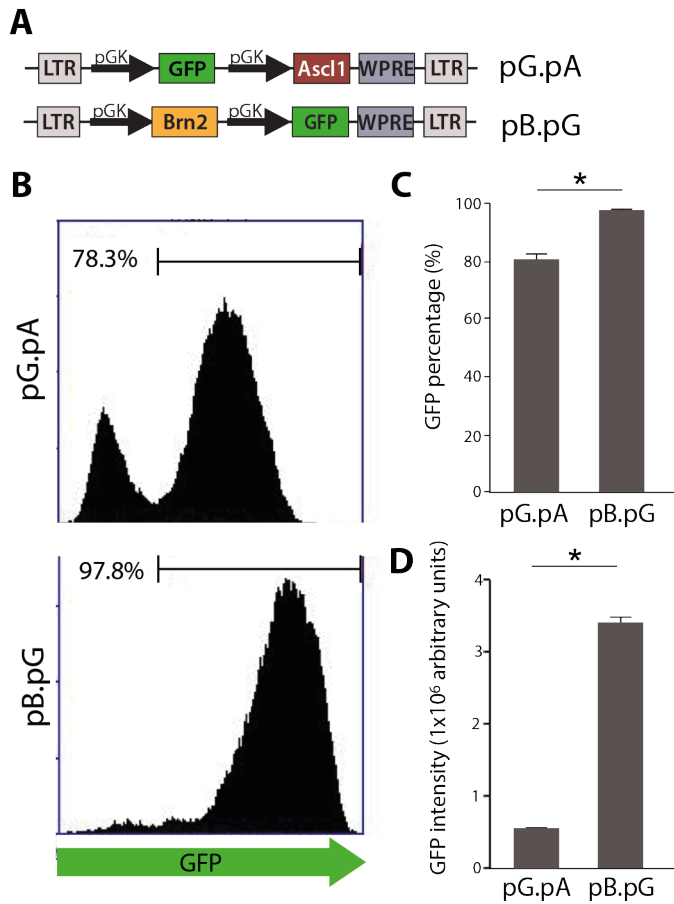
Appendix Table S3. List of the primers used in this study

Gene	Direction	Sequence (5' → 3')
<i>ACTB</i>	Forward	CCTTGCACATGCCGGAG
	Reverse	GCACAGAGCCTCGCCTT
<i>GAPDH</i>	Forward	TTGAGGTCAATGAAGGGGTC
	Reverse	GAAGGTGAAGGTCGGAGTCA
<i>HPRT1</i>	Forward	ACCCTTTCCAAATCCTCAGC
	Reverse	GTTATGGCGACCCGCAG
<i>NCAM1</i>	Forward	GTCAGAGGCCACCGTCAACGTG
	Reverse	CTTCCCCCTCCCGGAACCTCCTG
<i>MAP2</i>	Forward	CCGTGTGGACCATGGGGCTG
	Reverse	GTCGTCGGGGTGATGCCACG
<i>REST</i>	Forward	AAATGTGGCCTTAACTGGGGAA
	Reverse	TCTGTCTTTCTTCACCGACCAG
<i>SNCA</i>	Forward	GAGGGAGTGGTGCATGGT
	Reverse	TGCTGTCACACCCGTCAC
<i>SYNAPSIN</i>	Forward	CCCGTGGTTGTGAAGATGGGGC
	Reverse	TGCCACGACACTTGCGATGTCC
<i>SYNAPTOPHYSIN</i>	Forward	ACCTCGGGACTCAACACCTCGG
	Reverse	GAACCACAGGTTGCCGACCCAG
<i>MAPT</i>	Forward	CTCCAAAATCAGGGGATCGC
	Reverse	TTTTTATTTCTCCGCCAG

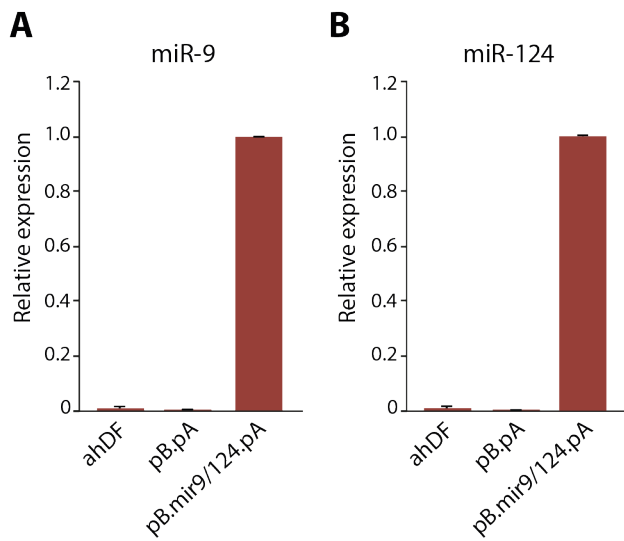
Appendix Table S4. Exact p-values derived from statistical tests.

Figure	Comparison	p-values	Category	Test
Figure 1E	ABM vs. pBpA MOI 5	0.0063	**	Kruskal-Wallis test, Conover post-hoc
	ABM vs. pBpA MOI 10	0.0002	***	Kruskal-Wallis test, Conover post-hoc
	ABM vs. pBpA MOI 20	>0.0001	***	Kruskal-Wallis test, Conover post-hoc
	pBpA MOI 5 vs. pBpA MOI 10	0.0063	**	Kruskal-Wallis test, Conover post-hoc
	pBpA MOI 10 vs. pBpA MOI 20	0.0063	**	Kruskal-Wallis test, Conover post-hoc
Figure 2A	hFL1 vs. aHDF -shREST	0.0106	*	Kruskal-Wallis test, Conover post-hoc
	aHDF -shREST vs. aHDF +shREST	0.0032	**	Kruskal-Wallis test, Conover post-hoc
Figure 2D	Purity: p7 vs p10	0.0051	**	Kruskal-Wallis test; Dunn's multiple comparisons test
Figure 3F	% of TAU+ cells: CTR vs. miR-9 KD	0.0018	**	T-test
Figure EV1C	PGPA vs PBPG GFP%	0.0044		T-test
Figure EV1D	PGPA vs PBPG GFP intensity	0.0001		T-test

Appendix Figures



Appendix Figure S1. Validation of the transgene expression in relation to its position from WPRE. (A) Vector maps of constructs containing GFP as well as the transcription factors *Ascl1* or *Brn2* at different position from the woodchuck hepatitis post-transcriptional element (WPRE) at different positions. (B) Quantitative analysis of GFP showing in hFL1 transduced with the different constructs. (C) Quantifications of the percentage of cells expression GFP (D) and of the GFP fluorescence intensity in the two constructs.



Appendix Figure S2. High miRNA-9 and miRNA-124 expression following transduction with pB.mir9/124.pA. (A, B) Quantitative PCR analysis of miR-9 (A) and miR-124 (B) three days following the transduction with either pB.pA or pB.mir9/124.pA as compared to fibroblast levels. Abbreviations: ahDF: adult human dermal fibroblasts.