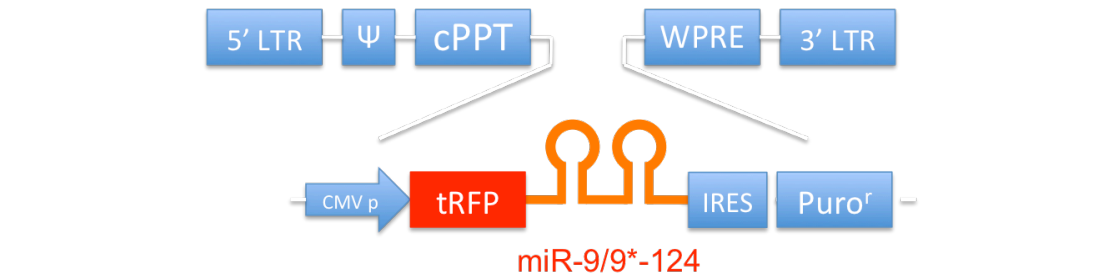
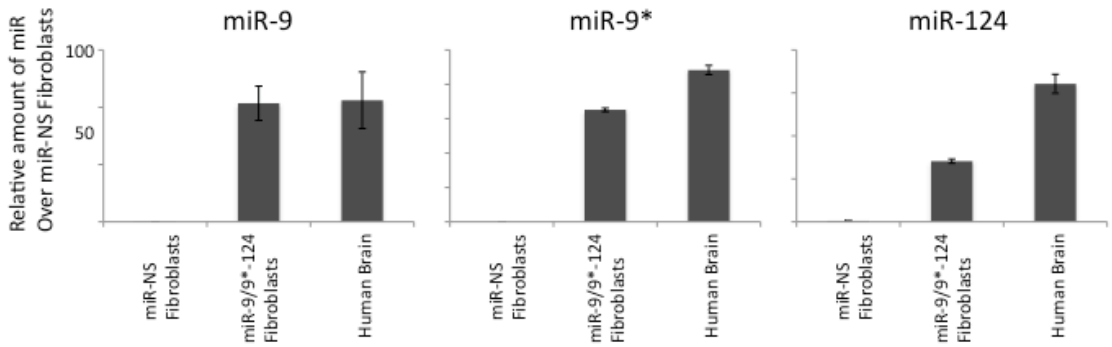
doi:10.1038/nature10323

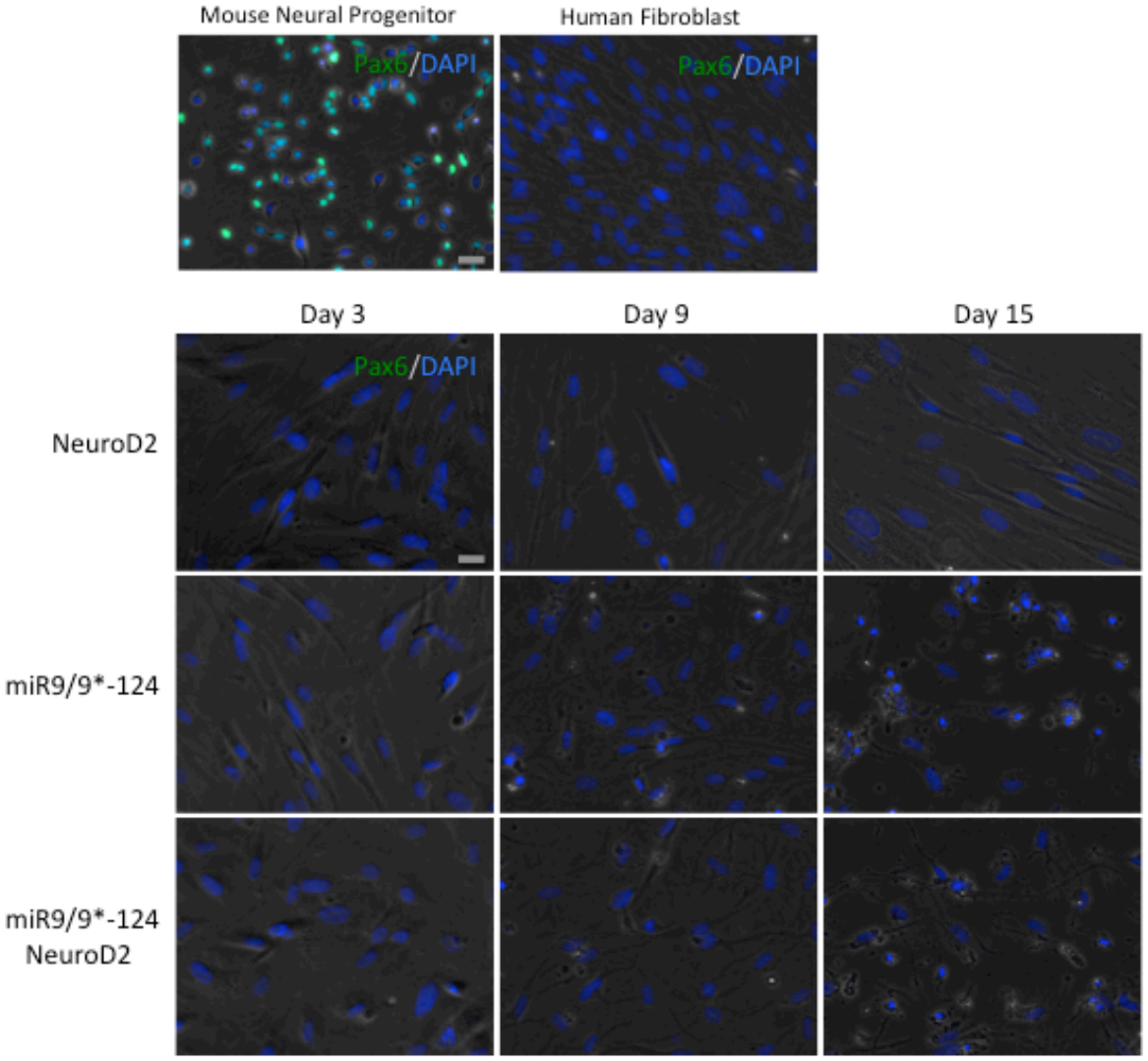
Supplementary Figure 1





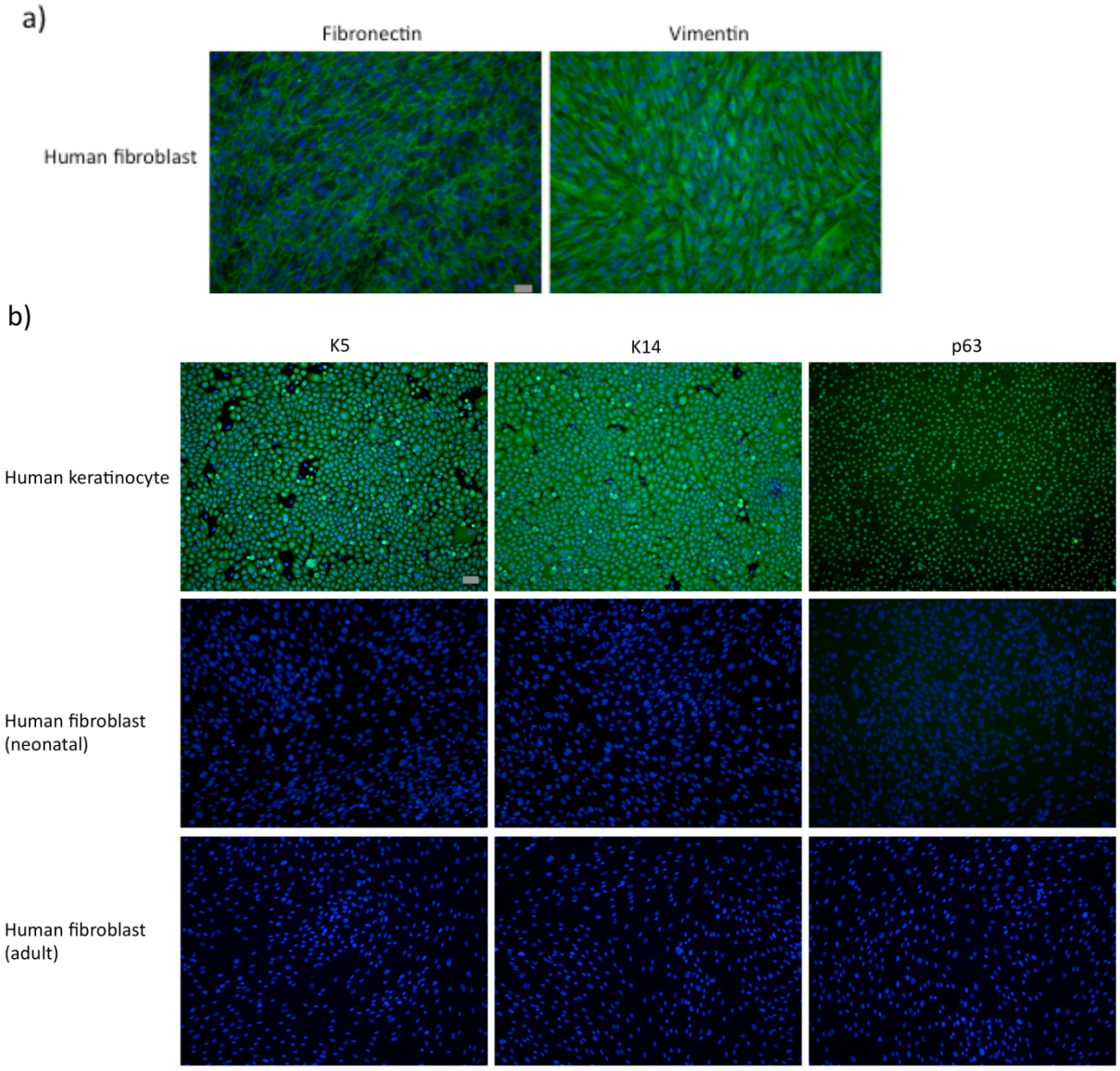
Relative amount of microRNAs expressed in human fibroblasts in comparison to human brain. The top diagram illustrates the lentiviral construct used to express miR-9, -9* and -124. Quantitative real time PCR was performed from human fibroblasts expressing non-specific microRNA (miR-NS) or the synthetic cluster of miR-9/9* and miR-124 (miR-9/9*-124) to estimate how much miR-9/9* and miR-124 are expressed compared to the level found in human brains (bottom graphs).

Supplementary Figure 2



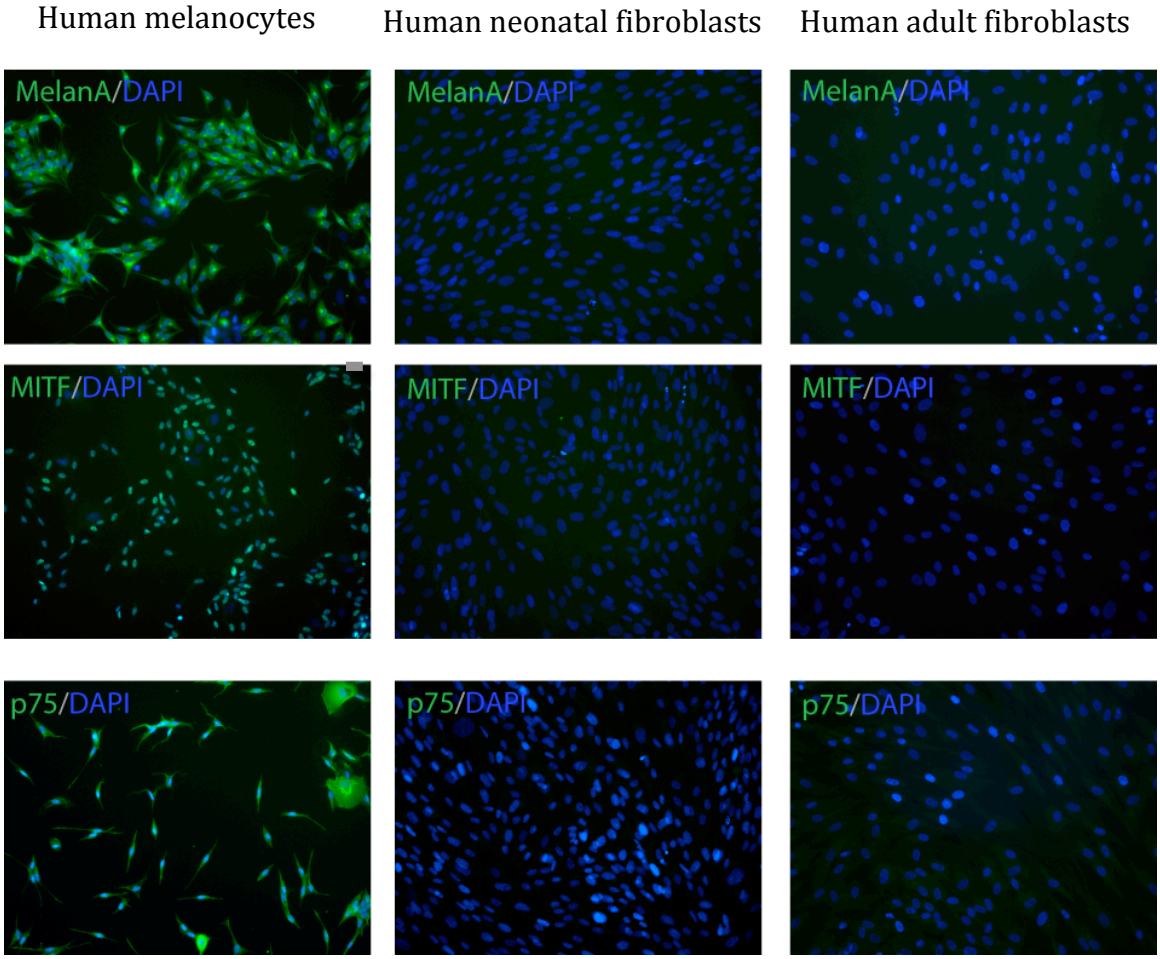
Immunostaining for progenitor markers during the time-course of conversion. The top left panel shows the positive control for Pax6 antibody staining (mouse neural progenitors). The top right photo shows human fibroblast stained for Pax6. The bottom pictures shows human fibroblasts expressing NeuroD2, miR-9/9*-124 and miR-9/9*-124-NeuroD2 stained for Pax6. We sampled every three days for 3 weeks. Here we show examples of the cells sampled on Day 3, 9, and 15. We did not observe any Pax6 expression during the conversion. Similar results were obtained for Sox2 and Tbr2. Note that NeuroD2 only did not produce any MAP2-positive cells (data not shown). Scale bar = 20μm.

Supplementary Figure 3



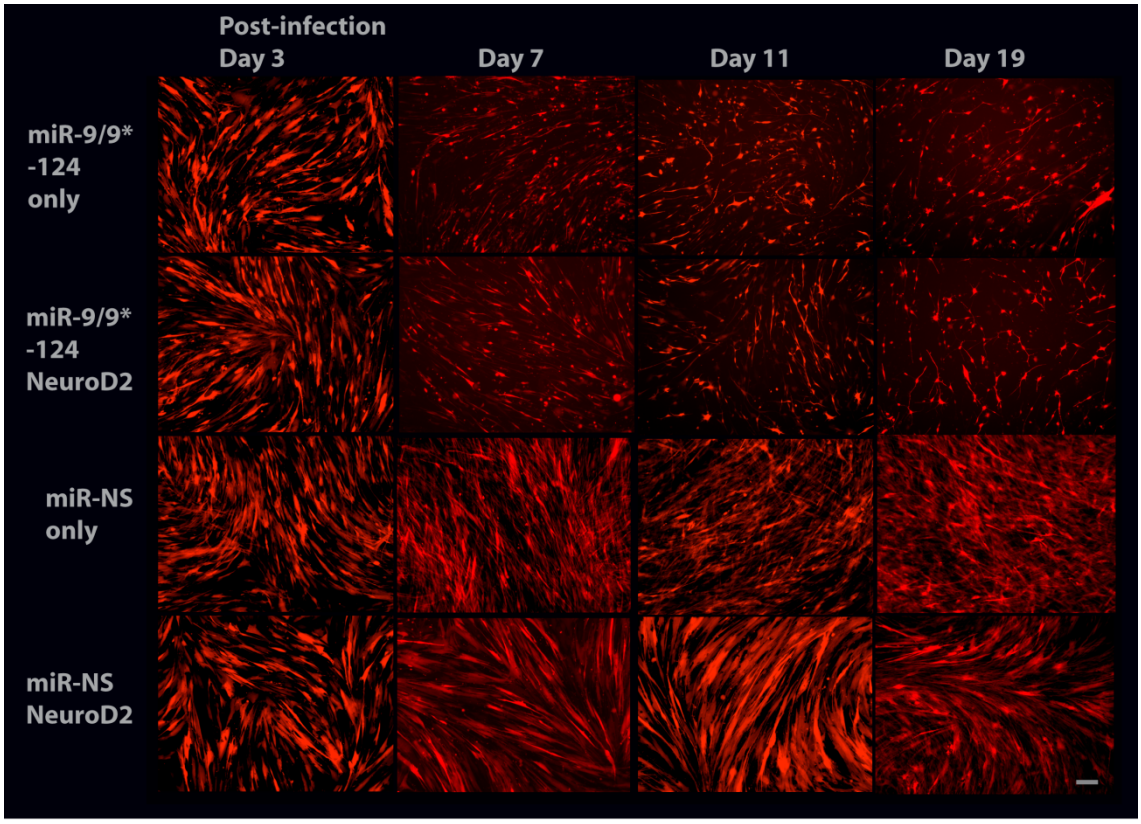
Immunostaining for keratinocytes in the starting culture of human fibroblasts. a) Top pictures show the starting culture of fibroblasts stained with Fibronectin and Vimentin. We observed a homogenous population of fibroblasts characterized by high expression of both Fibronectin and Vimentin. b) The starting culture of fibroblasts (neonatal and adult) was stained with 3 different markers for keratinocytes:K5, K14, and p63. The top row shows the positive control stain for human keratinocytes using K5, K14, and p63. These markers were absent in the starting culture of human fibroblasts, as shown in the bottom panels. Scale bar = 20μm.

Supplementary Figure 4



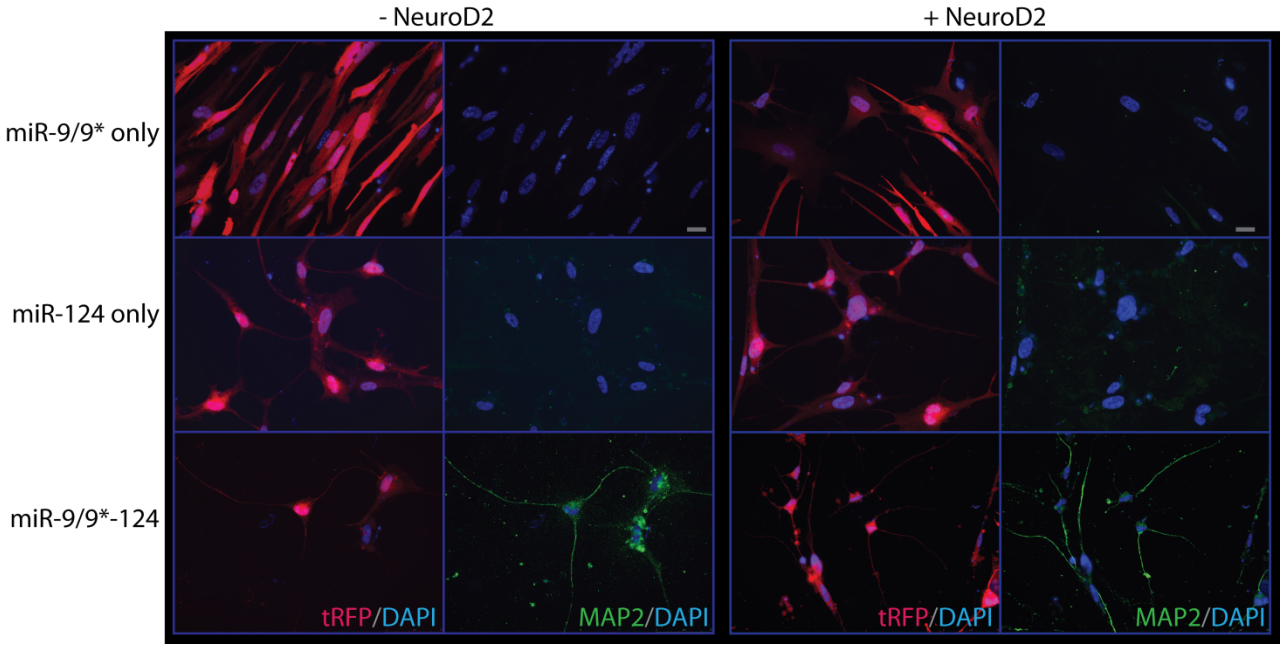
Immunostaining for melanocytes in the starting culture of human fibroblasts. The first column shows human melanocytes stained with antibodies against MelaninA, MITF, and p75 as positive controls. The second and third column shows the respective human neonatal fibroblasts and human adult fibroblasts also stained for MelanA, MITF, and p75. We did not observe melanocytes in the fibroblast cultures used in this study. Scale bar = 20 μm.

Supplementary Figure 5



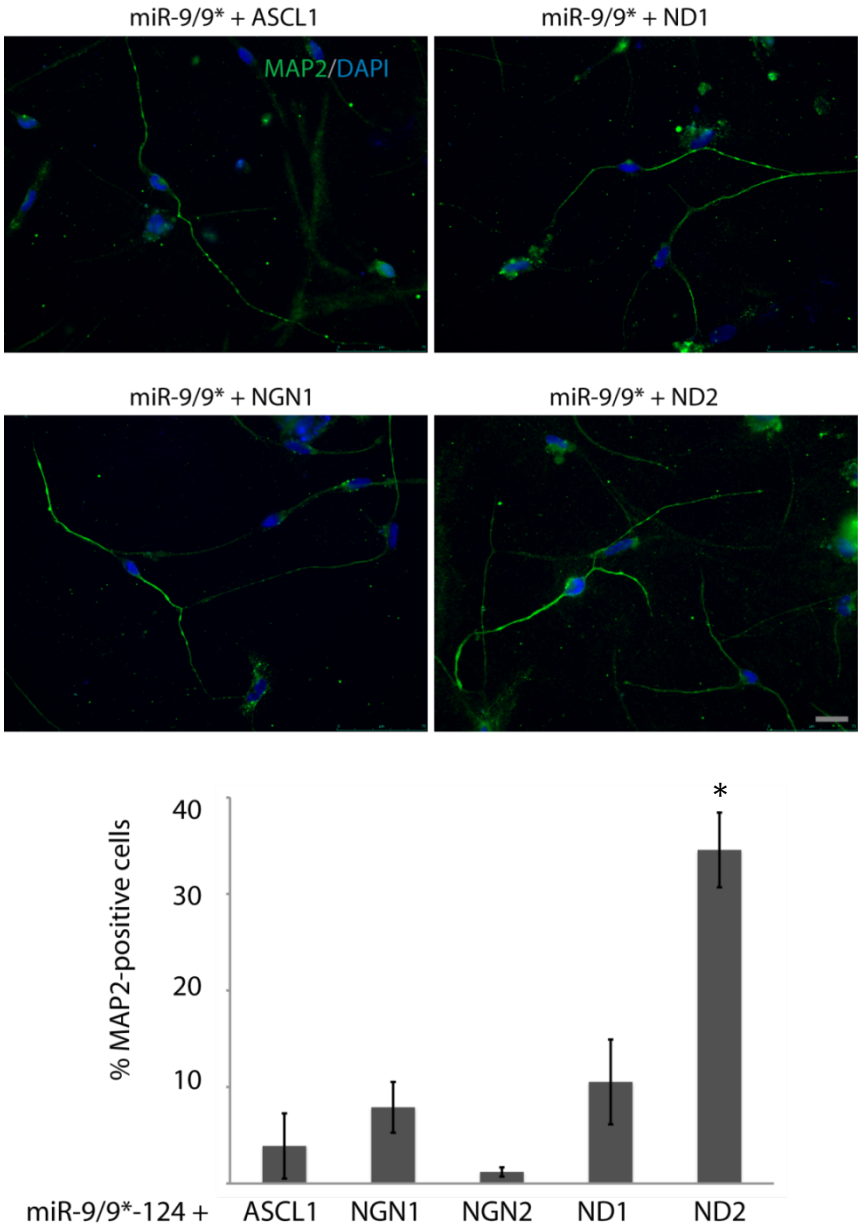
Morphological changes of fibroblasts induced by microRNAs. Neonatal foreskin fibroblasts infected with lentivirus to express either miR-9/9*and -124 (miR-9/9*-124) or a non-specific microRNA (miR-NS) are marked by RFP in order to track morphological changes. The images show the transition of the infected cells from the same coverslip over 20 days post-infection. The two top rows show miR-9/9*-124 overexpression in the absence or presence of NeuroD2, respectively. The bottom two rows display cells expressing miR-NS in the absence or presence of NeuroD2, respectively. Scale bar = 20 μm.

Supplementary Figure 6



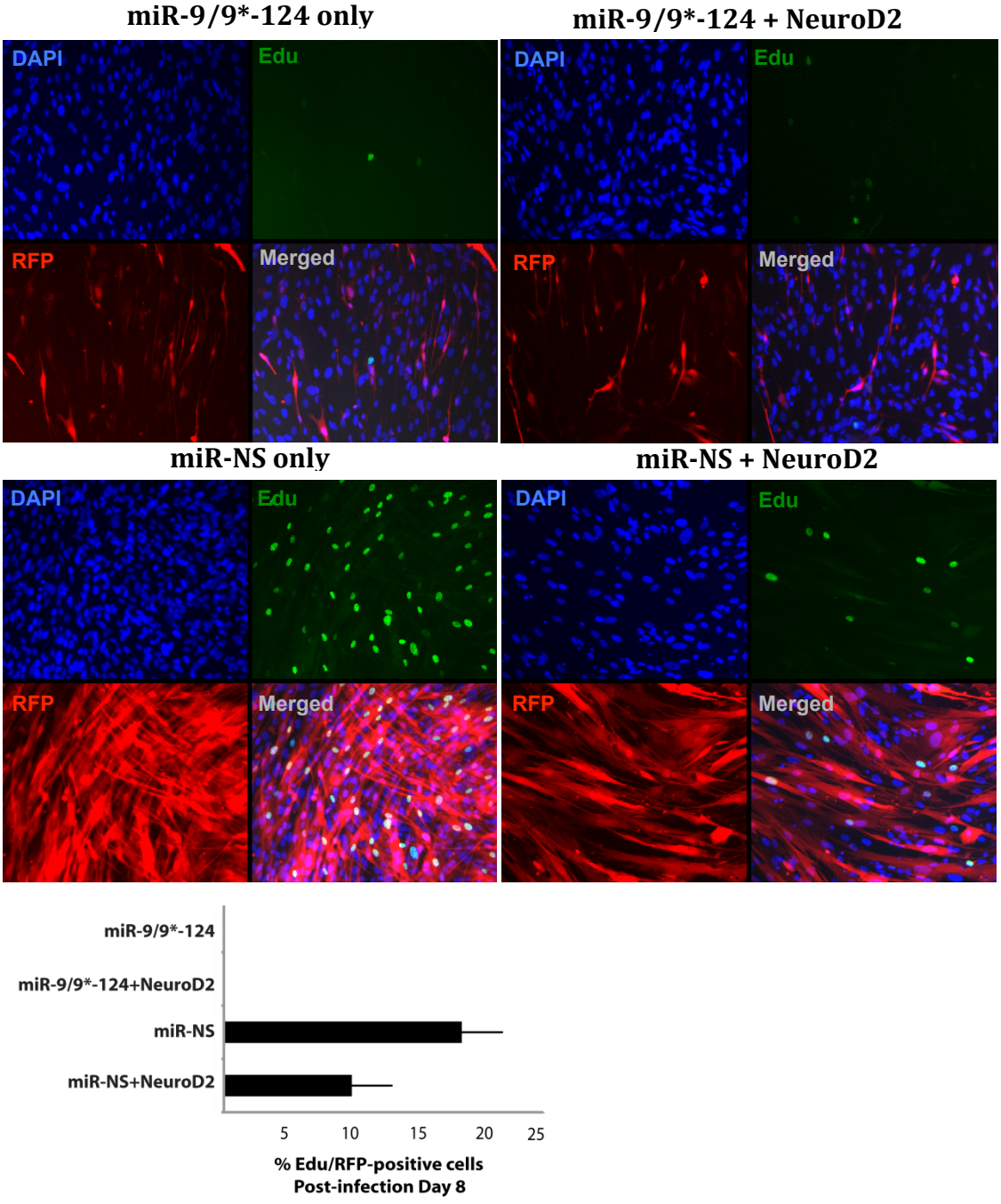
Synergistic effect of miR-9/9* and miR-124 on transformation. The panel of images on the left shows human fibroblasts expressing miR-9/9* only (top), miR-124 only (middle), and miR-9/9*-124 together (bottom) in the absence of NeuroD2. tRFP marks microRNA-expressing cells. The panel of images on the right shows microRNA-expressing cells in the presence of NeuroD2. MAP2-positive cells appear only when miR-9/9* and miR-124 are expressed together, independent of NeuroD2, demonstrating the synergistic effect of miR-9/9* and -124 on neuronal transformation of human fibroblasts. Scale bar = 20μm.

Supplementary Figure 7



Effect of neurogenic factors on miR-9/9*-124-mediated conversion of human fibroblasts. NGN1=Neurogenin1, NGN2=Neurogenin2, ND1=NeuroD1, ND2=NeuroD2. Images on top show MAP2-positive cells expressing miR-9/9*-124 with the selected neurogenic factors. Bottom bar graph shows the quantification. The actual figures for MAP2-positive cells in each category are as follows: ASCL1 - 7/180, NGN1- 6/76, NGN2 - 1/84, ND1 - 6/57, ND2 - 28/81. Asterisk (*) indicates $p < 0.01$ by Student's t-test of ASCL1, NGN1, NGN2 or ND1 compared to ND2. Scale bar = 20μm.

Supplementary Figure 8



Representative images of EdU-incorporation 8 days after infection. EdU-positive cells are shown in green in four conditions: miR-9/9*-124 overexpression (top left), miR-9/9*-124 with NeuroD2 overexpression (top right), non-specific microRNA, miR-NS overexpression (lower left), and miR-NS with NeuroD2 overexpression (lower right panel). The graph represents quantification of EdU-positive cells/RFP-positive cells. EdU pulse is for two hours.

Supplementary Figure 9

Intrinsic properties of induced neurons

Fibroblast	Factors	N_T	N_{AP}	%	N_R	%	RMP, mV	R_m, GΩ	C, pF	AP_{amp}, mV	AP_{th}, mV	I_{Na}, nA
Neonatal	miR+D2	22	14	64	2	14	-34.1±1.7	4.8±1.6	17.3±1.3	33.3±2.3	-23.8±1.3	-0.5±0.3
	miR+DAM	27	23	85	21	91	-42.0±1.8*	2.6±0.2	16.1±1.8	58.3±3.3**	-26.7±1.3	-1.1±0.2
Adult	miR+DAM	15	9	60	5	56	-31.4±3.2	2.8±0.7	22.9±3.7	46.0±3.9	-24.1±2.2	-1.0±0.3

Abbreviations: N_T – total number of recorded cells; N_{AP} – total number of cells with action potentials (APs); N_R – total number of cells with repetitive APs; RPM – resting membrane potential; R_{in} – input resistance; C – capacitance; AP_{amp} – action potential amplitude; AP_{th} – action potential threshold; I_{Na} –maximum amplitude of sodium current.

RPM was measured right after breaking into the cell;$R_{in}$was measured from the linear voltage deflections in response to negative and positive current injections around V_{hold} = -60 – -75 mV;C_m was calculated as a ratio of $\tau_{on}$and R_{in};$AP_{th}$was measured from the phase-plane plot;$AP_{amp}$was calculated as a difference between the threshold and the peak of AP.

One-way ANOVA was performed on each parameter and Tukey's post-hoc test was then performed for significant ($p<0.05$) parameters. * indicates $p<0.05$ when neonatal miR+DAM group is compared to either of the other two groups. ** indicates $p<0.05$ when neonatal miR+DAM group is compared to neonatal miR+D2 group.

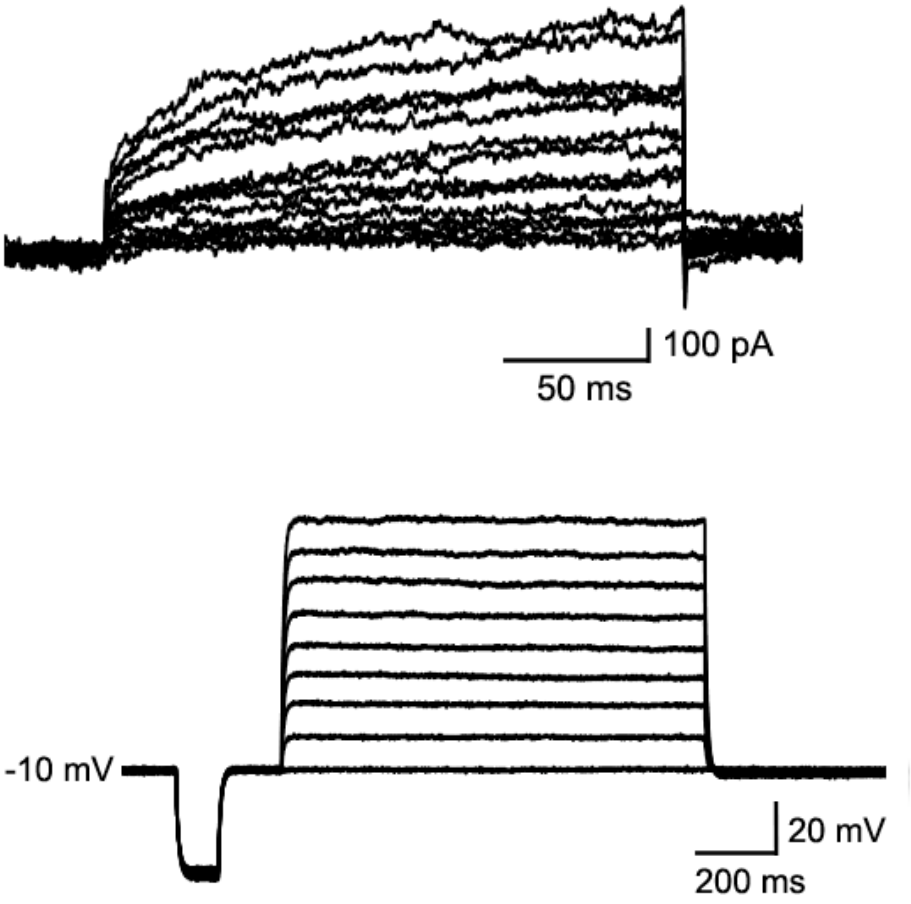
Synaptic properties of induced neurons (miR+DAM)

Fibroblast	Spontaneous EPSCs					Evoked EPSC				Evoked IPSC			
	N_T	N_R	%	Ampl, pA	Freq, Hz	N_T	N_R	%	Ampl, pA	N_T	N_R	%	Ampl, pA
Neonatal	14	10	71	-7.0±0.5	1.9±0.4	12	5	42	71.1±19.3	12	6	50	57.1±22.1
Adult	12	10	83	-7.7±0.7	0.6±0.4	10	5	50	59±17	11	5	45	45±8

Abbreviations: N_T – total number of recorded cells; N_R – total number of cells with responses; Ampl – amplitude; Freq - frequency

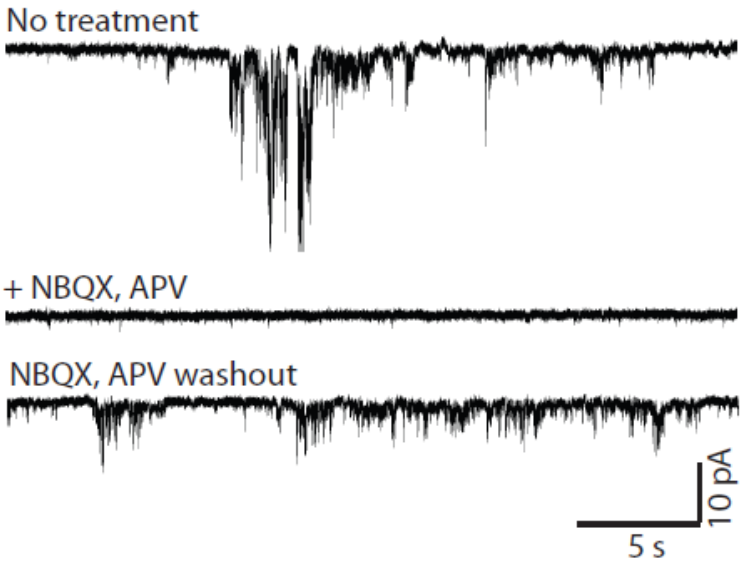
Spontaneous EPSCs were recorded for 3 min at -70 mV with intracellular solution containing 5 mM QX-314 to block sodium channels, and extracellular solution containing 50 μM APV to block NMDA receptors. Reversal potential for Cl^- is ~ -79 mV. EPSC template was created in Clampfit 10 by averaging at least 20 events, and then used to detect the spontaneous EPSCs. Evoked postsynaptic responses were recorded in response to locally delivered stimuli (0.1 – 0.7 mA) at -70 mV (evoked EPSC) and 0 mV (evoked IPSC), respectively.

Supplementary Figure 10



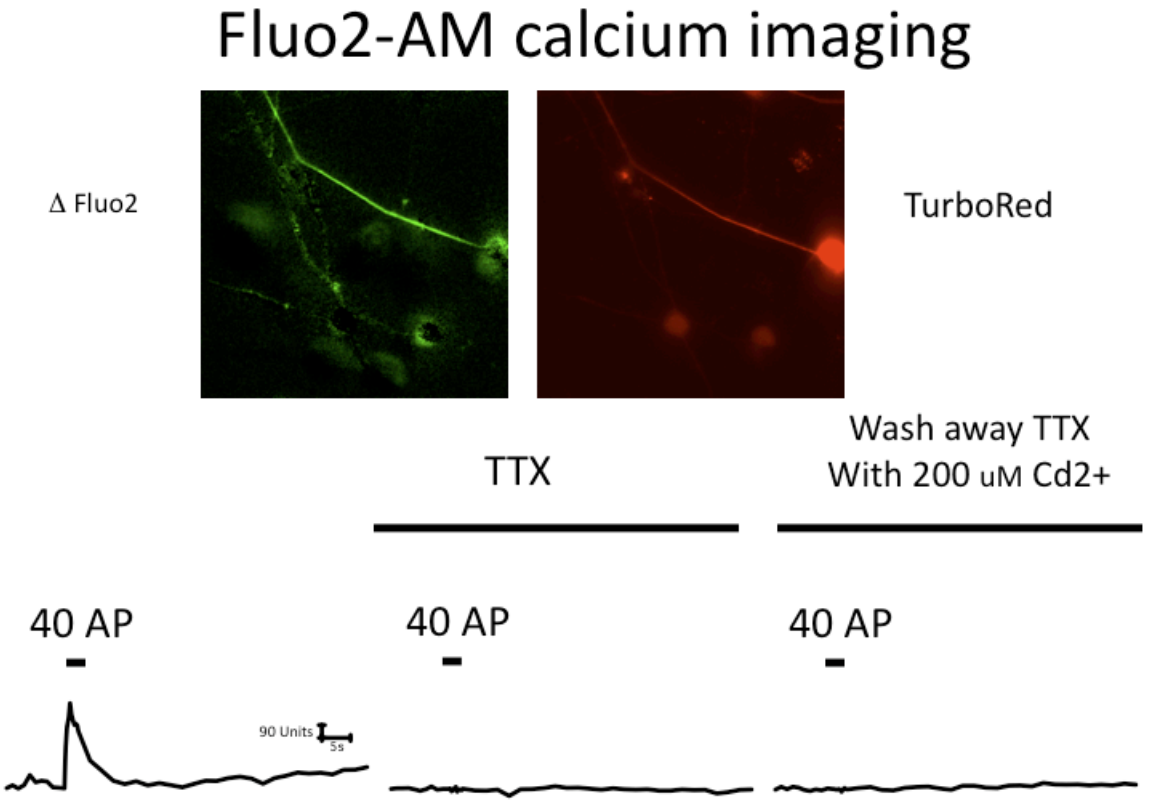
Electrophysiological properties of fibroblasts expressing non-specific microRNA. Top diagram is a representative voltage-clamp recording in a fibroblast. Note that there is an absence of inward current. The bottom diagram shows a representative current clamp recording in a fibroblast. Note that the fibroblast does not have any action potential-like events.

Supplementary Figure 11



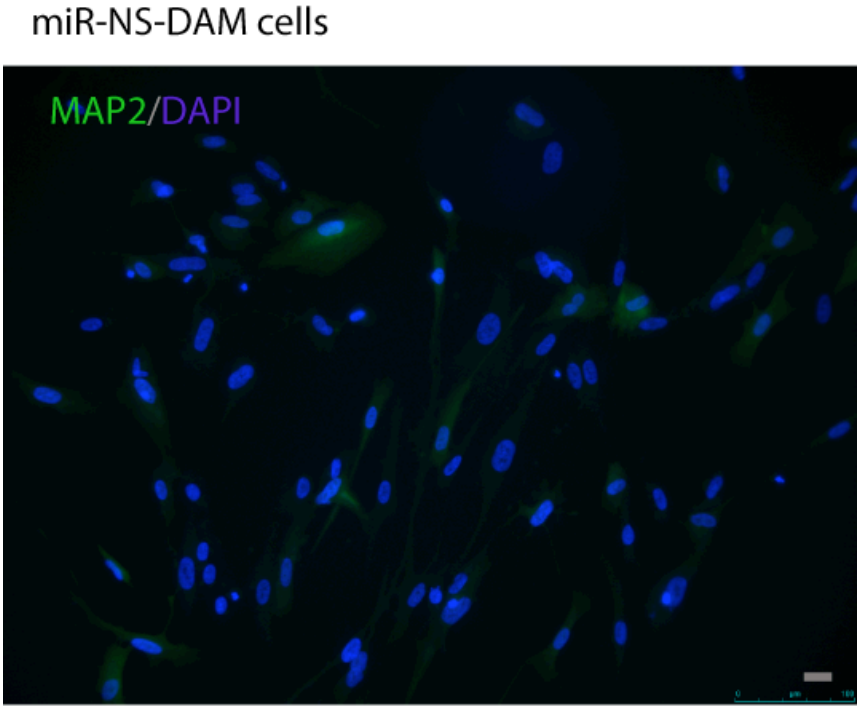
Representative trace of synaptic events in miR-9/9*-124-NeuroD2-induced neurons. The induced neuron was held at -70 mV. Postsynaptic currents that were observed in untreated condition (top trace), were abolished in the presence of 10 μM NBQX and 50 μM APV (middle trace), and reappeared after washing out the synaptic blockers (bottom trace).

Supplementary Figure 12



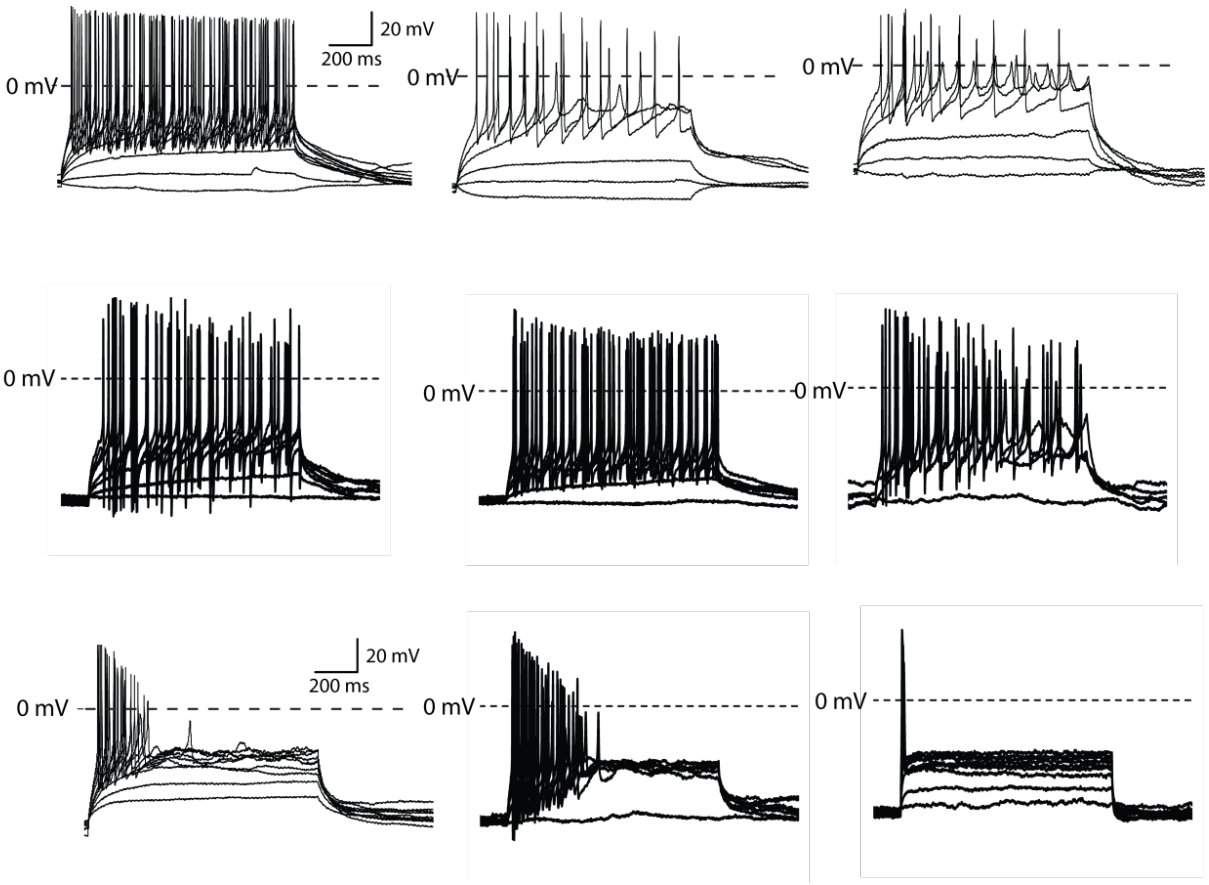
An example of Fluo2-AM calcium imaging displaying action potential (AP)-dependent Ca^{2+} influx. The pictures show the increase in Fluo2 signal during stimulation, which was blocked by TTX. After TTX was washed away, the same cell was treated with Cd^{2+},which also blocked the Ca^{2+} influx.

Supplementary Figure 13



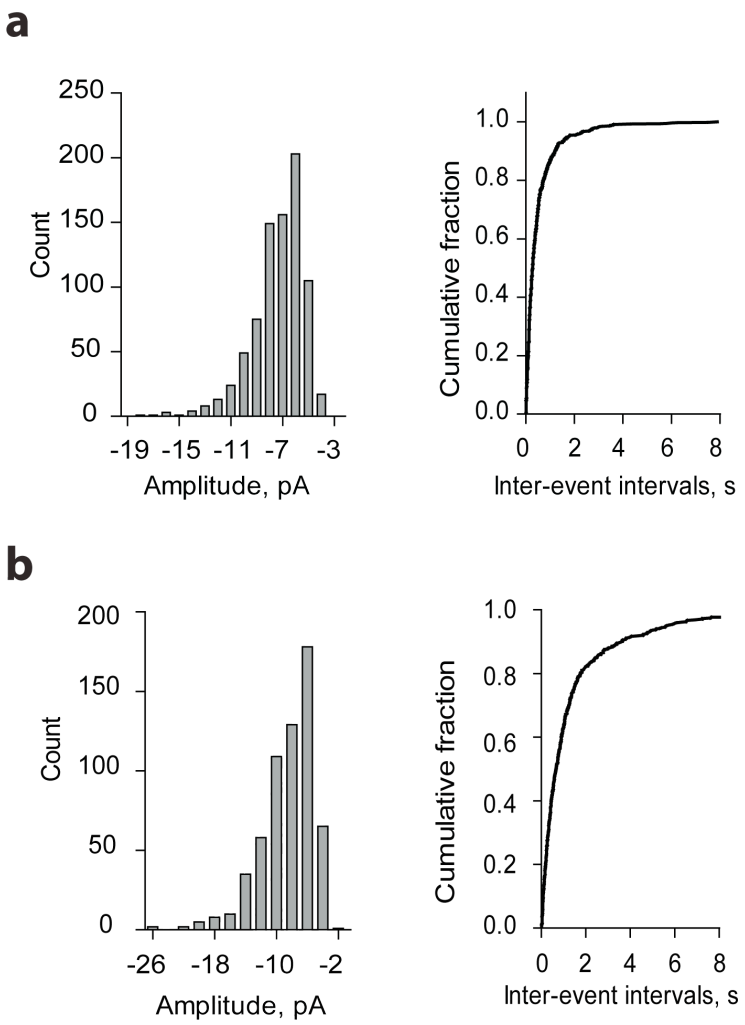
Non-specific microRNA and DAM factors do not convert fibroblasts to neurons. Cells infected with non-specific microRNA (miR-NS) and DAM factors were stained by antibodies against MAP2. miR-NS-DAM did not lead to induction to MAP2- positive neurons (more than 500 cells analyzed). Scale bar = 20μm.

Supplementary Figure 14



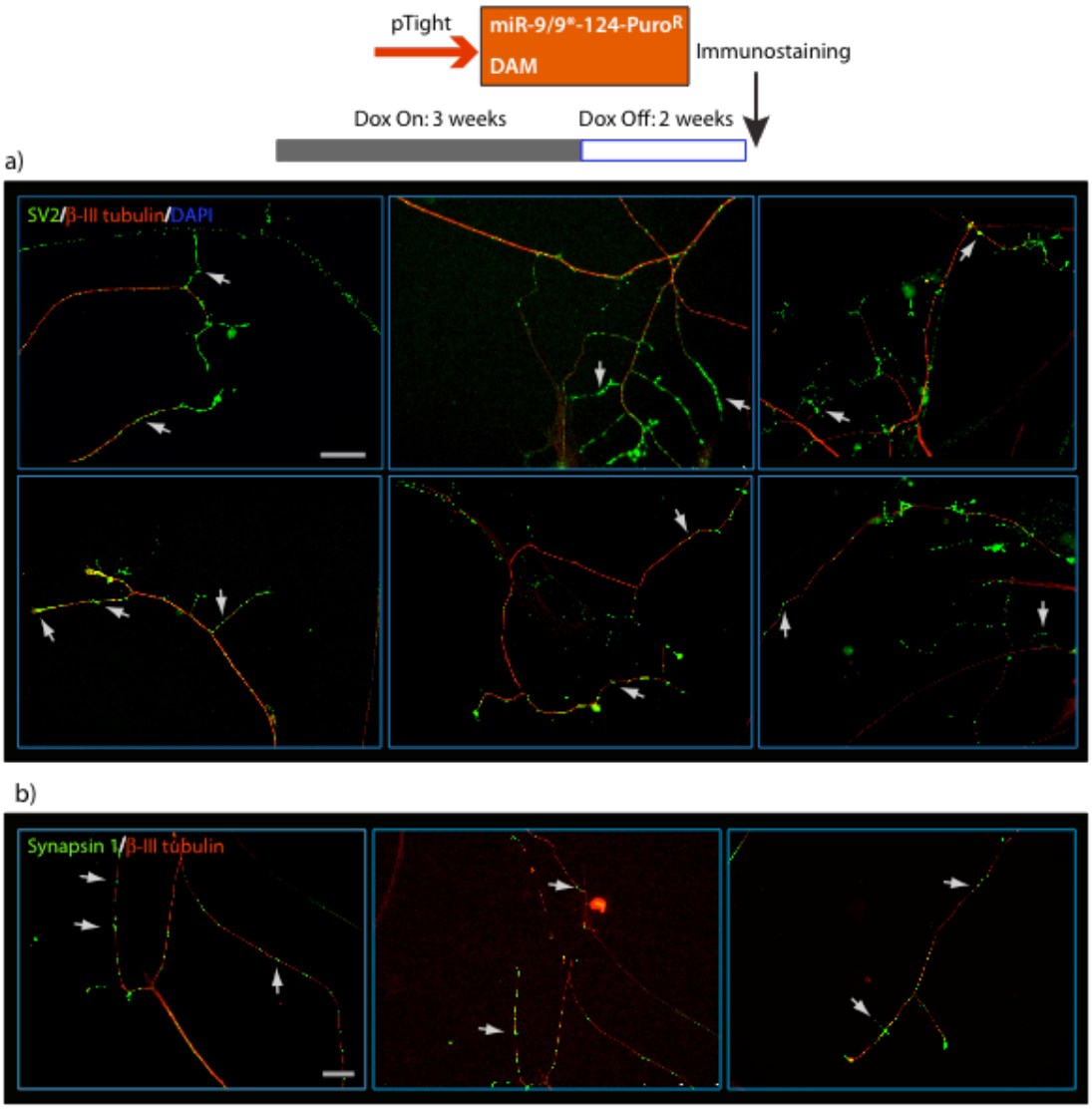
Representative traces of action potentials displayed in miR-9/9*-124-DAM-induced neurons. Current was injected into the induced neurons to target a V_{hold} ~-60 – -75 mV, and a series of injection steps were subsequently applied to elicit the firing of action potentials. We observed cells that exhibit persistent repetitive firing during the depolarizing step, repetitive firing that attenuates during the depolarization step, and single action potential firing.

Supplementary Figure 15



Amplitude (left) and inter-event interval (right) distributions of spontaneous EPSCs in neonatal (a) and adult (b)-converted cells.

Supplementary Figure 16



Stable transformation of human fibroblasts using doxycycline (Dox)-inducible promoter to express miR-9/9*-124 and DAM factors. After 3 weeks of induction, Dox was removed (Dox off) for two weeks, and the cells were analyzed for a) synaptic vesicle 2 (SV2) and b) Synapsin 1. In fibroblasts, we found 3 days of Dox removal was sufficient to turn off transgene expression as monitored by uniform sensitivity to puromycin (data not shown). Arrows indicate exemplary staining of putative presynaptic boutons. Scale bar = 20 μm

Supplementary Figure 17

List of primers used for single cell analysis

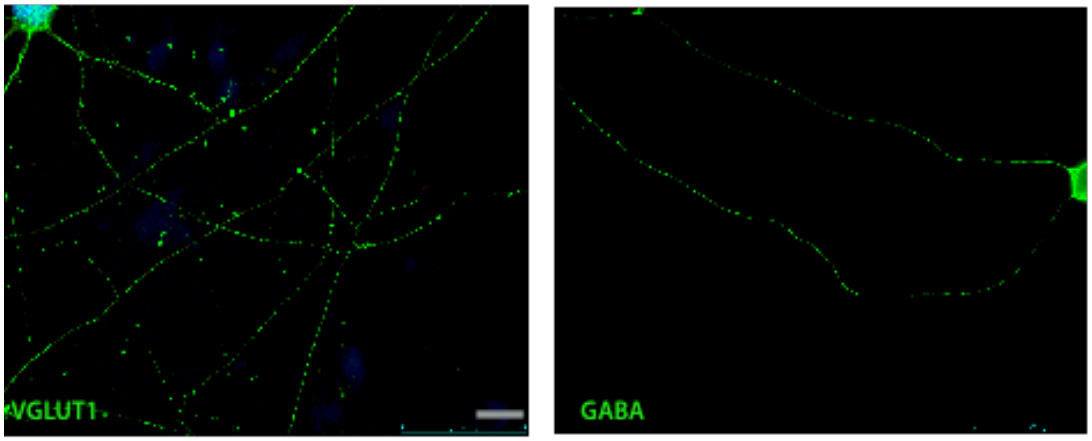
Name	Sequence	Primerbank ID http://pga.mgh.harvard.edu/primerbank
h18S-F	GATGGGCGGCGGAAAATAG	11968182a1
h18S-R	GCGTGGATTCTGCATAATGGT	11968182a1
h5HT-2C-F	TCTTAATGTCCCTAGCCATTGCT	4504541a3
h5HT-2C-R	TACCGATCCAGCGATATAGCG	4504541a3
hBDNF-F	GATGCTCAGTAGTCAAGTGCC	25306253a2
hBDNF-R	GCCGTTACCCACTCACTAATAC	25306253a2
hBSN-F	CCACATCACCCTACTCCGTC	4508019a1
hBSN-R	TTGCAGACCTTGTTGTGACAC	4508019a1
hCACNA1C-F2	TCCGCTGCTTCTGAAGATGA	
hCACNA1C-R2	GGCCGTCGCTTTGGTAGTA	
hCRIM1-F	GCGTTTGCGAAGATGAGAACT	10092639a1
hCRIM1-R	TGGTGTTACATTCACATTTCCCA	10092639a1
hCTIP2-BCL11B-F	TGGGTGCCTGCTATGACAAG	12597635a1
hCTIP2-BCL11B-R	GGCTCGGACACTTTCCTGAG	12597635a1
hCUX1-F	GCTCTCATCGGCCAATCACT	
hCUX1-R	TCTATGGCCTGCTCCACGT	
hDCX-F	CCTTGGCTAGCAGCAACAGT	
hDCX-R	CCACTGCGGATGATGGTAA	
hDDC-F	ACTGGCTCGGGAAGATGCT	4503281a1
hDDC-R	CCGATGGATCACTTTGGTCC	4503281a1
hDKK3-F	TGGGGTCACTGCACCAAAAT	27735014a1
hDKK3-R	GAAGGTCGGCTTGCACACATA	27735014a1
hDLX1-f	CCATGCCAGAAAGTCTCAACA	31418473a3
hDLX1-r	GGCCCAAACTCCATAAACACC	31418473a3
hDLX5-F	AGCTCCTACCACCAGTACGG	4885187a3

hDLX5-R	GTTTGCCATTCACCATTCTCAC	4885187a3
hETV1-F	CTGGATGACCCGGCAAATTCT	1045061a3
hETV1-R	CCTCTTCAGGCTCAATCAGTTT	1045061a3
hFOXP1-F	AGACAAAAAGTAACGGTTCAGCC	21750965a3
hFOXP1-R	CGCACTCTAGTAAGTGGTTGC	21750965a3
hFOXP2-F	TTTCTAAAGAACGCGAACGTCT	21518701a2
hFOXP2-R	GCAATATGCACTTACAGGTTTGG	21518701a2
hGAD67-F	GCCAGACAAGCAGTATGATGT	4503873a2
hGAD67-R	CCAGTTCCAGGCATTTGTTGAT	4503873a2
hGAPDH-f2	CATGAGAAGTATGACAACAGCCT	7669492a3
hGAPDH-r2	AGTCCTTCCACGATACCAAAGT	7669492a3
hGRP-F	GTGGGGCACTTAATGGGGAAA	31542860a1
hGRP-R	CTATGAGACCCAGCAAATTCCTT	31542860a1
hLXN-F	AACGGGACAAGAAACTGCAC	21359933a3
hLXN-R	CTAGCGGTTCCTTCATGGACT	21359933a3
hMAPT-Tau-f	TACAAACCAGTTGACCTGAGCA	8400715a3
hMAPT-Tau-r	ATGGATGTTGCCTAATGAGCC	8400715a3
hMEF2c-f	ATGCCATCAGTGAATCAAAGGAT	298698a1
hMEF2c-r	CTGGTAAAGTAGGAGTTGCTACG	298698a1
hNCAM-F	ACATCACCTGCTACTTCCTGA	10834990a1
hNCAM-R	CTTGGACTCATCTTTCGAGAAGG	10834990a1
hNR4A3-F	CTGAGCATGTGCAACAATTCTAC	1311505a2
hNR4A3-R	ACAGCTCCAAAAAGGCTGATTC	1311505a2
hNSE-F	GGAGTTGGATGGGACTGAGAA	5803011a3
hNSE-R	CTGAGCAATGTGGCGATACAG	5803011a3
hOMA1-F	TAGGCAGGGGCATAAGGAAAT	21686999a3
hOMA1-R	CTCAAACCAAGGAATAGCTTCCA	21686999a3
hPCLO-F	CAGACACTTTCAGGTCAGAGC	6433936a2
hPCLO-R	AGGCATCATACTAGACTTGTGCT	6433936a2
hPCP2-F	AGAGGCCAGCAGAAAAGTGACT	
hPCP2-R	GTGGCTCAGCAGATTGAAGAA	

hPERIPHERIN-F	CCAAGTACGCGGACCTGTC	21264345a1
hPERIPHERIN-R	CTCGCACGTTAGACTCTGGA	21264345a1
hPLXND1-F	CATGGAGATGGCCTGTGACTA	3327054a1
hPLXND1-R	GGAAGGGCGGAAACTGGTC	3327054a1
hPPP1R1B-DARPP32F	AGTCTGCTGGGCAAAAGACAA	21735492a2
hPPP1R1B-DARPP32R	AGGCTCACTTAGTGCTGGGT	21735492a2
hS100A10-F	GGACCAGTGTAGAGATGGCAAA	
hS100A10-R	ATGGTGAGGCCCGCAATTA	
hSATB2-F	TCTCCCCCTCAGTTATGTGAC	5689405a3
hSATB2-R	AGGCAAGTCTTCCAACTTTGAA	5689405a3
hSCN1A-RD1	TGGGGAGTGGATAGAGACCA	
hSCN1A-RD2	GAAAGAGATTCAGGACCACTAGG	
hSCN2A-RD10	GGTGATTGGAAATCTAGTGGTTC	
hSCN2A-RD11	CATCCTTCCCACAGCAATCT	
hSCN3A-RD16	AGTAGTGGTGCATTGGCCTT	
hSCN3A-RD17	GCAACCCATTTGAGAAGCAT	
hSCN8A-RD19	ACAGGAAGAGGCACAGGC	
hSCN8A-RD20	CCCCTCCTTCTTCACCTTCT	
hSEMA3E-F	ATTGTTTGCTGGACTCTACAGTG	6912650a3
hSEMA3E-R	CTTTCAACAGACGCTCATCGT	6912650a3
hSHANK3-F	GGAGAGCGGGGAACTCACT	13359173a1
hSHANK3-R	CTGTCCGAGGACTGCTTCAG	13359173a1
hSLC1A2-f	AAGTGCGAATGCCAGACAGTC	4759124a1
hSLC1A2-r	CAGGATGACACCAAACACCG	4759124a1
hSOX5-F	CAGAGTGGCGAGTCCTTGTC	23308715a3
hSOX5-R	TTTCTTCCGGCTCGTTTTTGA	23308715a3
hSYNAPSIN-1-F	TGAAGCCGGATTTTGTGCTGA	19924097a2
hSYNAPSIN-1-R	GACCAAACTGCGGTAGTCTCC	19924097a2
hSYT9-F	TGGCAGACGACTGAAGAAGAG	28376627a2
hSYT9-R	GGATTTGGTCAATGTTCTCGGG	28376627a2

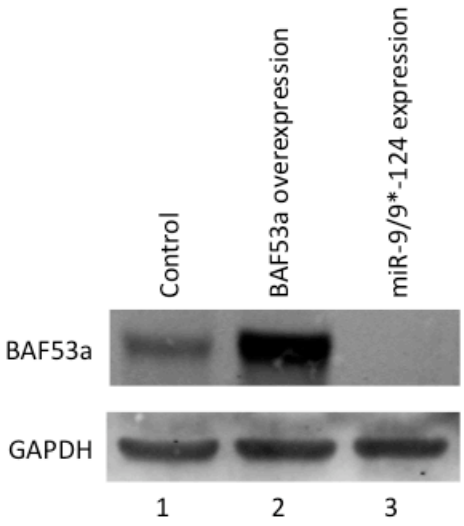
hTBR1-f2	CATTATCTCGACCACTGACAACC	5730081a2
hTBR1-r2	AGACCCCGTCCAAGACAGG	5730081a2
hTH-F	GCCCTACCAAGACCAGACGTA	37127a2
hTH-R	CGTGAGGCATAGCTCCTGA	37127a2
hTIS21-BTG2-f	CAGAGCACTACAAACACCACTG	5802988a1
hTIS21-BTG2-r	CTGAGTCCGATCTGGCTGG	5802988a1
hTLE1-f	AAGTTCACTATCCCGGAGTCC	21541824a1
hTLE1-r	TCTGTCTTTTCACTTGCCAGTTT	21541824a1
hTLE4-F	ACAAGCAGGCAGAGATTGTCA	6330948a1
hTLE4-R	TCCATGTGATAAATGCTGGGC	6330948a1
hTPM2-F	CTGAGACCCGAGCAGAGTTTG	4507649a1
hTPM2-R	TGAATCTCGACGTTCTCCTCC	4507649a1
hTUBB3-f2	CGGTGGTGGAACCCTACAAC	5174737a1
hTUBB3-r2	AGGTGGTGACTCCGCTCAT	5174737a1
hu-GRM5-f	TCCAATCTCCCGATGTCAAGT	4504143a3
hu-GRM5-r	TCGGCACTGAAAACGATGCT	4504143a3
hUNC5D-F	AAGCCCTTCCCGAATCCATC	18254472a1
hUNC5D-R	AGTGCAATAGGGTTGCTCTTG	18254472a1
hVGLUT1-F	CGACGACAGCCTTTTGTGGT	9945322a2
hVGLUT1-R	GCCGTAGACGTAGAAAACAGAG	9945322a2

Supplementary Figure 18



Human neurons derived from expressing miR-9/9* and DAM factors were immunostained for VGLUT1 and GABA. We detected a heterogeneous population of cells containing VGLUT1- or GABA-positive cells. Scale bar = 20 μm

Supplementary Figure 19



Western blot analysis of BAF53a expression in human fibroblasts. Lane1 is the native human fibroblast control showing the presence of BAF53a. Lane 2 shows BAF53a overexpression in fibroblasts; the increased detection demonstrates the specificity of the antibody. Lane 3 represents fibroblasts expressing miR-9/9*-124, showing the reduced expression of BAF53a, demonstrating that miR-9/9*-124 leads to the downregulation of BAF53a.