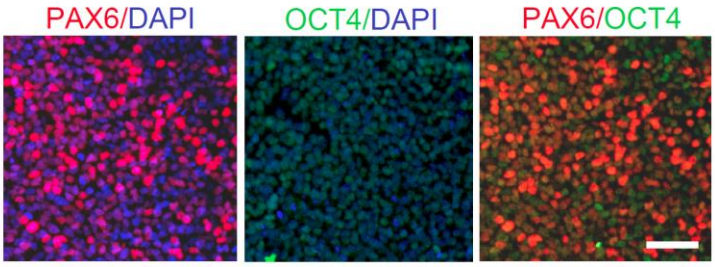
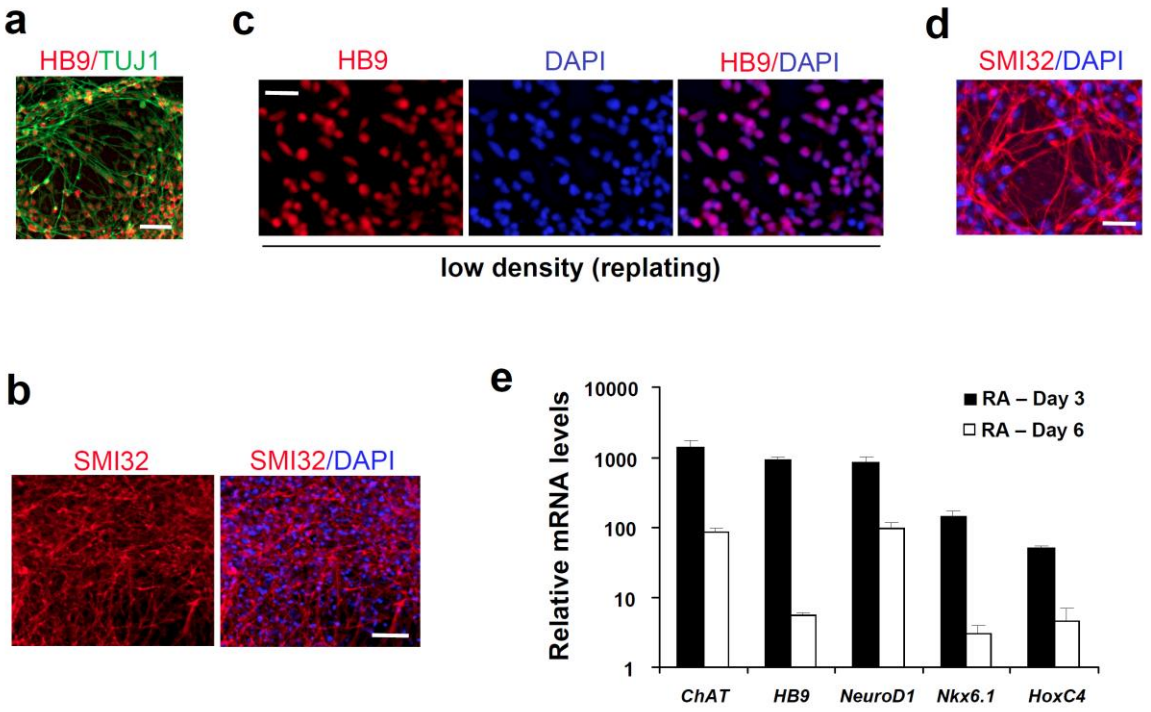
a

	Day 1	Day 3	Day 6
PAX6⁺	1.9±0.3%	68.7±4.1%	81.9±3.1%
SOX1⁺	0.10±0.08%	19.2±2.3%	45.4±2.9%
PAX6⁺/SOX1⁺	0.10±0.08%	17.8±1.7%	43.7±2.8%

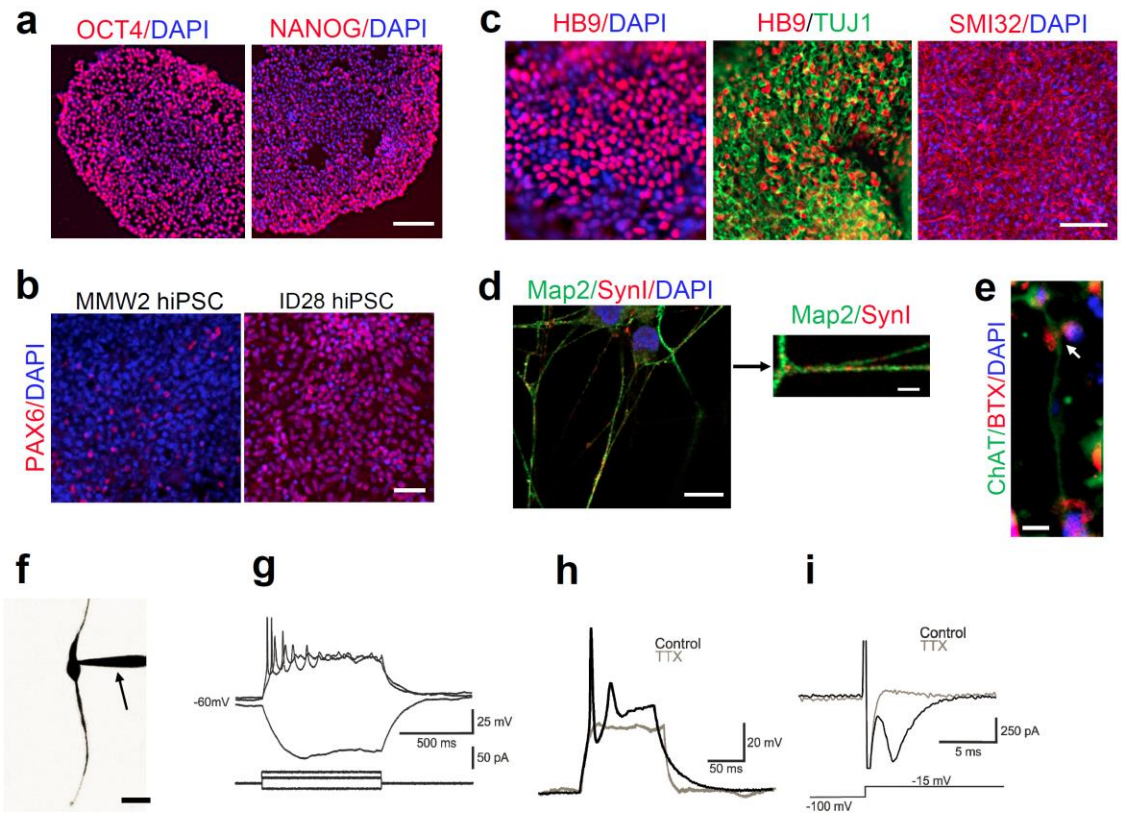
b



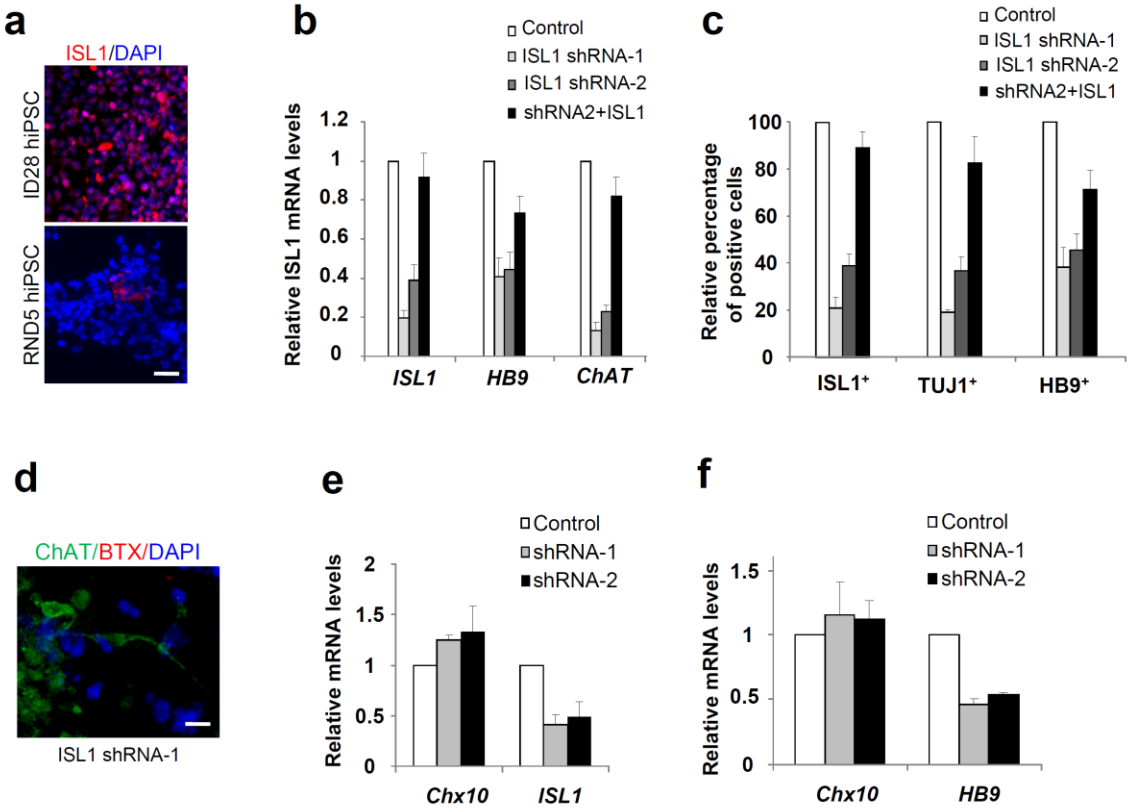
Supplementary Figure 1. Differential expression of neural and pluripotency markers during neural induction. (a) The percentage of PAX6-positive, SOX1-positive and PAX6/SOX1 double-positive cells at day 1, 3 and 6 of compound C induction. Data are mean ± SD of five separate experiments. (b) Immunofluorescence of PAX6 (red) and OCT-4 (green) in H1 hESCs at day 3 of compound C induction. Cell nuclei were stained with DAPI (blue). Bar, 50 μm.



Supplementary Figure 2. Rapid and high-efficiency MN differentiation from hESCs. (a) Merged fluorescence image of HB9/TUJ1 staining of cells after 20-day differentiation from H1 hESCs. Bar, 50 μm. (b) Immunofluorescence of SMI32 (red) in cells after 20-day differentiation from H1 hESCs. Cell nuclei were stained with DAPI (blue). Bar, 60 μm. (c) Fluorescence images of HB9, DAPI and HB9/DAPI staining of differentiated cells replated at a low density for 3-4 days after 20-day differentiation from H1 hESCs. Bar, 30 μm. (d) Immunofluorescence of SMI32 (red) in cells replated at a low density for 3-4 days after 20-day differentiation from H1 hESCs. Bar, 30 μm. (e) Relative mRNA levels of neuronal differentiation markers in cells after 20-day differentiation, assessed with real-time PCR. All values were normalized to the level (=1) of mRNA in cells prior to differentiation. Patterning was initiated at day 3 (RA-Day 3) or day 6 (RA-Day 6). Four separate experiments were conducted, and quantification of three replicates of a typical experiment is shown. Each bar represents the mean ± SEM (error bars). For (a)-(d), neural patterning was initiated at day 3 after neural induction.

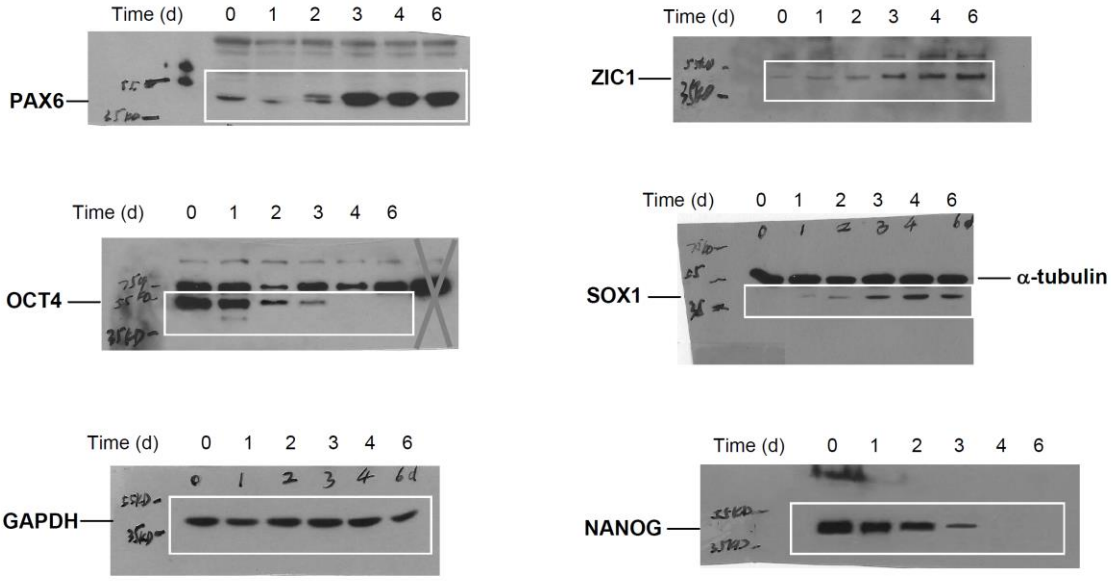


Supplementary Figure 3. Rapid and efficient MN derivation from hiPSCs. (a) Immunofluorescence of OCT-4 and NANOG (red) in ID28 hiPSCs cultured under self-renewal conditions. Cell nuclei were stained with DAPI (blue). Bar, 100 μm. (b) Immunofluorescence of PAX6 (red) in MMW2 and ID28 hiPSCs at day 6 of compound C induction. Cell nuclei were stained with DAPI (blue). Bar, 50 μm. (c) Immunofluorescence of HB9 (red), TUJ1 (green) and SMI32 (red) of ID28 hiPSCs after 18-day differentiation. Cell nuclei were stained with DAPI (blue). Merged images of HB9/DAPI (left), HB9/TUJ1 (middle) and SMI32/DAPI (right) are shown. Bar, 50 μm. (d) Confocal fluorescence microscopy images showing Map2, synapsin I (SynI) and DAPI (merged) staining in cells after 20-day differentiation from ID28 hiPSCs (left). Bar, 10 μm. The arrow points to synapsin-positive synaptic structures, which are further shown in an enlarged image (right, with Map2 and SynI merged). Bar, 5 μm. (e) ID28 hiPSC-derived MNs, stained with ChAT antibody (green), form neuromuscular junctions, labeled with bungarotoxin (BTX, red), when co-cultured with differentiated C2C12 myotubes. Arrow shows co-localization of ChAT-positive neurites with BTX. Cell nuclei were stained with DAPI (blue). Myotubes were differentiated from mouse C2C12 cells. Bar, 10 μm. (f) Image of recorded neuron filled with fluorescent dye (Alexa594). The arrow indicates the patch pipette. Bar, 20 μm. (g) Representative voltage responses following current step injection. Note the multiple action potentials evoked by depolarizing current steps. (h) The action potentials evoked by depolarizing current step was completely blocked by TTX (1 μM). (i) An inward transient sodium current was evoked by depolarizing voltage steps. This inward current was abolished by TTX.



Supplementary Figure 4. The effects of ISL1 depletion on MN differentiation. (a) Immunofluorescence of ISL1 (red) in cells differentiated for 15 days from ID28 and RND5 hiPSCs. Cell nuclei were stained with DAPI (blue). Bar, 50 μm. (b) Relative mRNA levels of *ISL1, HB9* and *ChAT* in H1 hESCs cells infected with lentivirus containing non-targeting (NT) shRNA (Control) and ISL1-targeting shRNAs (shRNA-1 and shRNA-2). Full-length human ISL1 was ectopically expressed in cells containing shRNA-2 (shRNA-2+ISL1). The mRNA levels were assessed with real-time PCR. All values were normalized to the level (=1) of mRNA in cells containing NT shRNA. Four separate experiments were conducted, and quantification of three replicates of a typical experiment is shown. Each bar represents the mean ± SEM (error bars). (c) Relative percentages of ISL1, TUJ1 and HB9-positive cells under various conditions for ISL1 depletion and depletion/rescue. Each bar represents mean±SD (error bars) of at least four separate experiments. All values were normalized to the number (=100%) of cells containing NT shRNA. (d) H1 hESCs infected with lentivirus containing ISL1-targeting shRNA1 were induced to undergo MN differentiation and co-cultured with differentiated C2C12 myotubes. Cells were stained with ChAT antibody (green) and bungarotoxin (BTX, red). Cell nuclei were stained with DAPI (blue). Cells containing NT shRNA were similar to non-infected hESCs (Fig. 3d). Bar, 10 μm. (e) Relative mRNA levels of *Chx10* and *ISL1* in H1 hESCs cells infected with lentivirus containing non-targeting (NT) shRNA (Control) and ISL1-targeting shRNAs (shRNA-1 and shRNA-2). The mRNA levels were assessed with real-time PCR. Cells were analyzed after 15-day differentiation.

All values were normalized to the level (=1) of mRNA in cells containing NT shRNA. Four separate experiments were conducted, and quantification of three replicates of a typical experiment is shown. Each bar represents the mean ± SEM (error bars). (f) Relative mRNA levels of *Chx10* and *HB9* in H1 hESCs cells infected with lentivirus containing non-targeting (NT) shRNA (Control) and ISL1-targeting shRNAs (shRNA-1 and shRNA-2). The mRNA levels were assessed with real-time PCR. Cells were analyzed after 20-day differentiation. All values were normalized to the level (=1) of mRNA in cells containing NT shRNA. Four separate experiments were conducted, and quantification of three replicates of a typical experiment is shown. Each bar represents the mean ± SEM (error bars).



Supplementary Figure 5. Western blot analysis of neural progenitor and pluripotency markers at various time points after neural induction of H1 hESCs with compound C (from day 0 to day 6). The corresponding cropped blots (within the rectangular region) are shown in Figure 2a.

Supplementary Table 1. Sources and dilutions of antibodies

Antibody	Source	Catalogue #	Dilution
OCT-3/4	Santa Cruz	SC-9081	1:1000 (I) /1:2,000 (W)
ChAT	Millipore	AB144p	1:100 (I)
GAPDH	GenScript	A00191	1:10,000 (W)
NANOG	Cell Signaling	#3580	1:500 (I)
Islet-1	Millipore	AB4326	1:100 (I)
SV2	DSHB	SV2	1:200 (I)
PAX6	COVANCE	PRB-278P	1:1000 (I)/ 1:2,000 (W)
TUJ1	COVANCE	MMS-435P	1:500 (I)
HB9	DSHB	81.5C10	1:100 (I)
Synapsin I	Millipore	574777	1:500 (I)
MAP2	Sigma	M9942	1:1000 (I)
SOX1	R&D System	AF3369	1:1000 (W)
ZIC1	Novus Bio	NB600-488	1:1000 (W)
SMI-32	COVANCE	SMI-32R	1:1000 (I)
Chx10	Sigma	HPA003436	1:100 (I)

(I: immunofluorescence; W: western blotting)

Supplementary Table 2. The sequences of primers

	5'-Forward primers	3'-Reverse primers
ChAT	ccctgatgccttcatcca	gtaggtgggcaccagtcttc
HB9	tgcctaagatgcccgactt	agctgctggctggtgaag
NeuroD1	ctgctcaggacctactaacaacaa	gtccagcttggaggacctt
Nkx6.1	gagatgaagaccccgctgta	gacgacgacgaggacgag
HoxC4	acgagaaagagagtgggagaga	ggaggtctgggggttgag
RARA	gaatcctgaatcgagctgaga	gggccatgtcctgtgatg
RARB	tcggcacactgctcaatc	gaagcagggtttgtacactcg
RARG	ggagatggcctctctgtcg	ggcttgtagacccgaggag
RXRA	cccatttatggaggggaaac	caccttcatgcaccactcag
RXRB	cggaggccttccctttac	gtgtccccagcctatgctat
RXRG	aagtttcccgcaggctatg	tccattggcttccctgtg
NCOA1	gcagatggaacccagcag	cccgcctaccagattcact
NCOA2	aaacagcactgcgaatttca	tggtaaattctggtttggcaat
NCOA3	agctgagctgcgaggaaa	gagtccaccatccagcaagt
EP300	tcttcagcaccatggacagt	gttgcatacgaggcccatag
KAT2B	cccttcatggaacctgtga	ggcgttcactcatggttttc
CREBBP	acaagcgaaaccaacaaacc	aaagaagtggcattctgttgc
RAX	ttcgagaagtcccactaccc	acttagcccgtcggttctg
OTX1	acccatccgtgggctatc	tgtgaacgcgtgaaggtg
OTX2	gggtatggacttgctgcac	ccgagtgaacgtcgtcct
NR2F1	atcgtgctgttcacgtcaga	gctcctcacgtactcctcca
HESX1	ctggaccagaagaaagactgtgt	actgggaggatttgggacat
NR2F2	ccatagtcctgttcacctcaga	aatctcgtcggctggttg
POU3F2	aataaggcaaaaggaaagcaact	caaaacacatcattacacctgct
FEZF1	ctgtggcaaagggtttcatc	tgttgcagatattgcacttgaa
SOX3	tgggctcggtagtgaagtct	tgagagtgcgatgcgatg
LHX2	ccaaggacttgaagcagctc	aagaggttgcgcctgaact
ZEB2	aggagctgtctcgccttg	ggcaaaagcatctggagttc
EMX2	aggaagcagctggcacac	tcttcggttctgaaaccatactt
POU3F1	tgggaaccatgtaaatatgtgaga	ccaaaaataaaaaccaagcacaa
SIX3	ctcctcctggtcctcatcg	gagaggagaaaattcagggagag
LEF1	cagtcgacacttccatgtcc	gagggatgccagttgtgtg
EVI1	cattgggaacagcaaccat	ggtcaccaaagccttttcat
WNT7B	tggcgtcctgtacgtgaa	tcttgttgcagatgatgttgg
ARX	gcaccacgttcaccagcta	cagcctcatggccagttc
FEZF2	aagcccaaaaacttcacctg	cagactttgcacacgaacg
CNTAP2	ccaaatcgatatttcctcaggt	cttggctaggaagcgaacc
FOXA2	cgttccgggtctgaactg	tgcccttccatcttcacc
HES3	ccgctgatggagaaaaagc	acgctcaactccaggatgtc
NeuroD1	atgaccaaatcgtacagcgag	gttcatggcttcgaggtcgt
GBX2	aaagagggctcgctgctc	atcgctctccagcgagaa
MAFB	agggaagctgccaagctc	atttgaccataagacaaggctgt
CTNNA2	gcctctccagtcccttctg	ggtgaagttgccgaagtcat

EGR2	ttgaccagatgaacggagtg	tggtttctaggtgcagagacg
CEP290	aagcaaaagaatgaattgttgtca	ttggagagctgcaatcttga
EGFL7	cgggggatgactgattctc	gcagcacctcctgagagc
CACNA1A	ctgaccctcttcaccgtgtc	tccaccgaatgcttgagg
RORA	gcattattttctgcatttgtactga	tgcagtttttcaatttttacctttc
MTPN	ggagacttggatgaggtgaaag	caccttctagtgtccggttga
ULK1	cagacagcctgatgtgcagt	cagggtggggatggagat
GAS1	tctcgacagctgttcatttcc	gcagaaggtccccttcg
GNPAT	ggtttctcatttggcctgata	gcaatgttggaatgcagaag
SDF4	catcaggctcaacgaggaac	tggtaccagcggtccttc
SMO	gcttccgggactatgtgcta	gcgattcttgatctcacagtca
BCL2	agtacctgaaccggcacct	gccgtacagttccacaaagg
HSPA5	agctgtagcgtatggtgctg	aaggggacatacatcaagcagt
Chx10	tgccggaagacaggatacag	catactccgccatgacactg