

**Enrichment of G2/M cell cycle phase in human pluripotent stem cells enhances HDR-mediated gene repair with customizable endonucleases.**

Diane Yang<sup>1, 7</sup>, Marissa Scavuzzo<sup>2, 7</sup>, Jolanta Chmielowiec<sup>3, 4</sup>, Robert Sharp<sup>3, 4</sup>, Aleksandar Bajic<sup>6</sup>, Malgorzata Borowiak<sup>1-5\*</sup>

<sup>1</sup> Molecular and Cellular Biology Department, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

<sup>2</sup> Program in Developmental Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

<sup>3</sup> Stem Cells and Regenerative Medicine Center, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

<sup>4</sup> Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital and Houston Methodist Hospital, Houston, TX 77030, USA

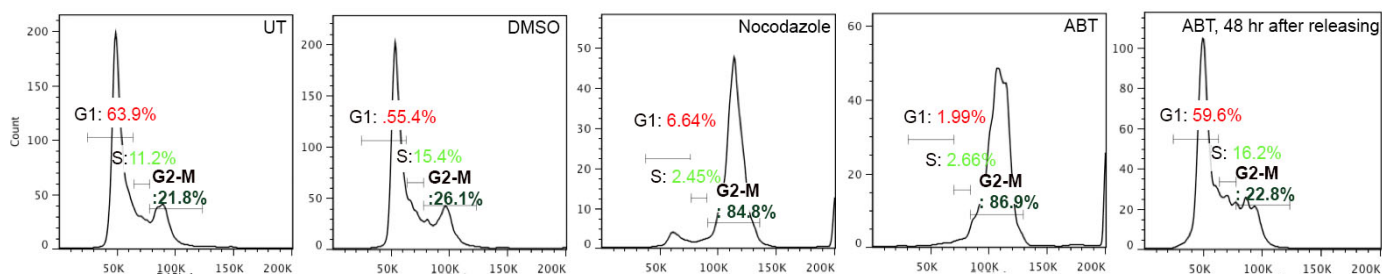
<sup>5</sup> McNair Medical Institute, Houston, TX 77030, USA

<sup>6</sup> Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, 1250 Moursund Street, Houston, TX 77030

<sup>7</sup> equal contribution

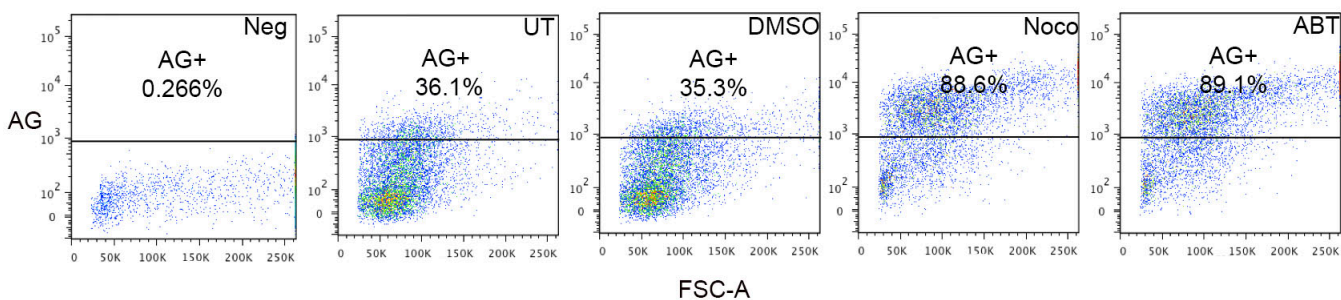
\* Corresponding author: [borowiak@bcm.edu](mailto:borowiak@bcm.edu), phone +1 713 798 7771

Running title: Enhancing gene targeting in human PSCs by cell cycle manipulation



	UT	DMSO	Nocodazole	ABT
G1 (%)	59.13±4.88	63.83±9.08	4.27±1.89	4.37±3.11
S (%)	11.67±1.15	10.87±1.97	1.6±0.36	1.57±0.35
G2/M (%)	28.8±4.45	24.7±8.22	90.73±1.56	91.43±3.29

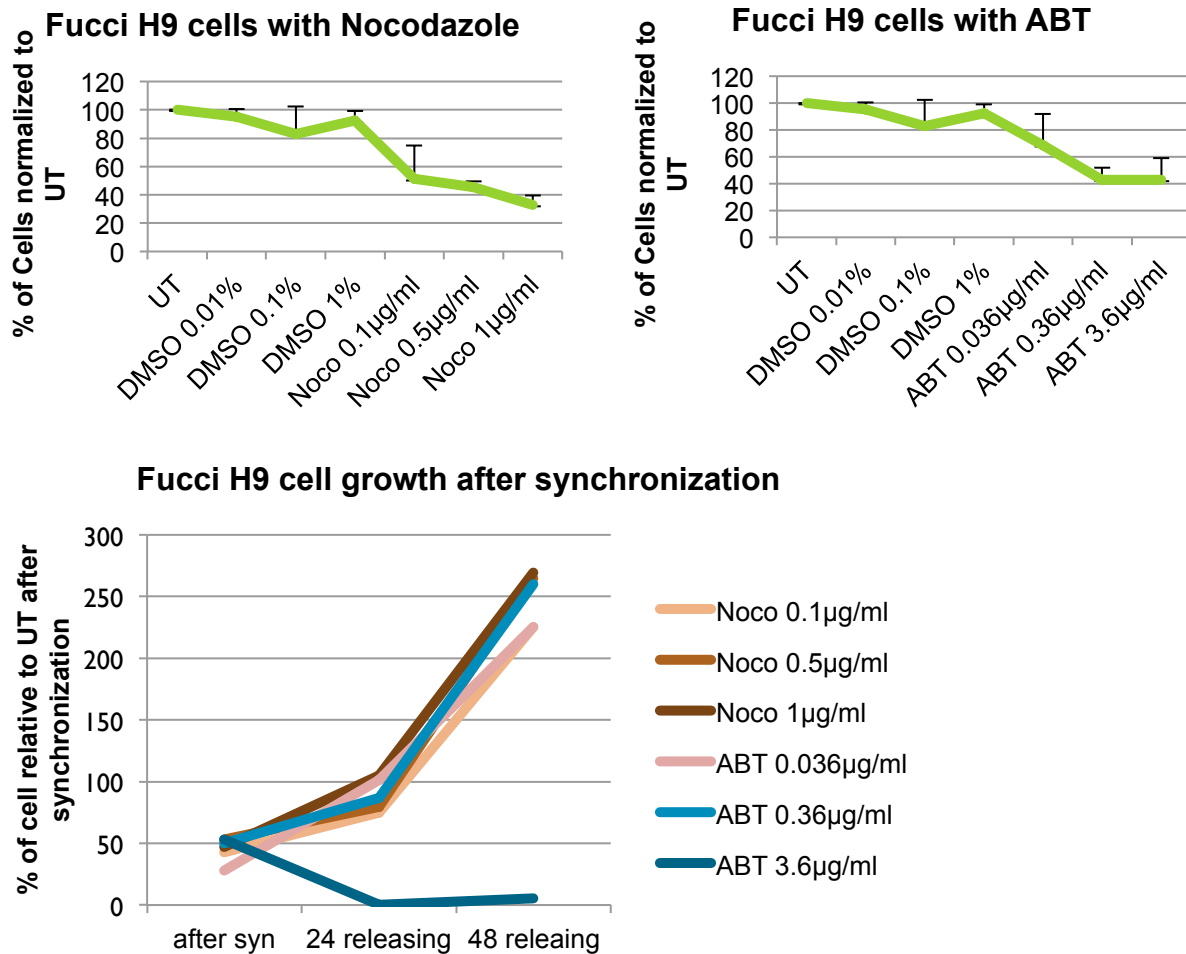
**Supplementary Figure 1: Synchronized Fucci H9 cells detected measured by DNA content and flow cytometry.** Representative FACS plots of synchronized Fucci H9 cells stained with Hoechst show efficient G2/M arrest in ABT or Nocodazole treated cells as compared to untreated and DMSO vehicle control cells (Top panel). ABT treatment shows a reversible effect of cell cycle after 48 hours of releasing. Lower panel shows Mean ± standard deviation for percentage of each cell cycle phase ( $n=3$  biological replicates).



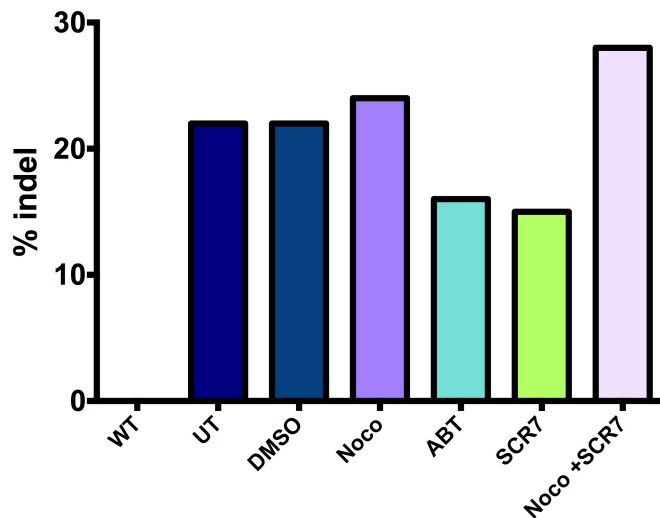
	UT	DMSO	Nocodazole	ABT
DN (%)	45.07±27.43	59.93±1.01	7.33±0.67	7.4±0.61
KO+(%)	9.4±15.07	0.67±0.06	3.37±0.67	2.03±1.59
AG+(%)	38.23±8.41	34.4±1.31	88.43±0.38	88.6±0.7

**Supplementary Figure 2: G2/M cell cycle phase of Fucci H9 cells measured by AG fluorescence with and without synchronization using flow cytometry.** Representative FACS plots of synchronized Fucci H9 cells show efficient G2/M arrest by ABT or Nocodazole treated cells as compared to untreated and DMSO vehicle control cells (Top panel). Lower panel shows Mean ±

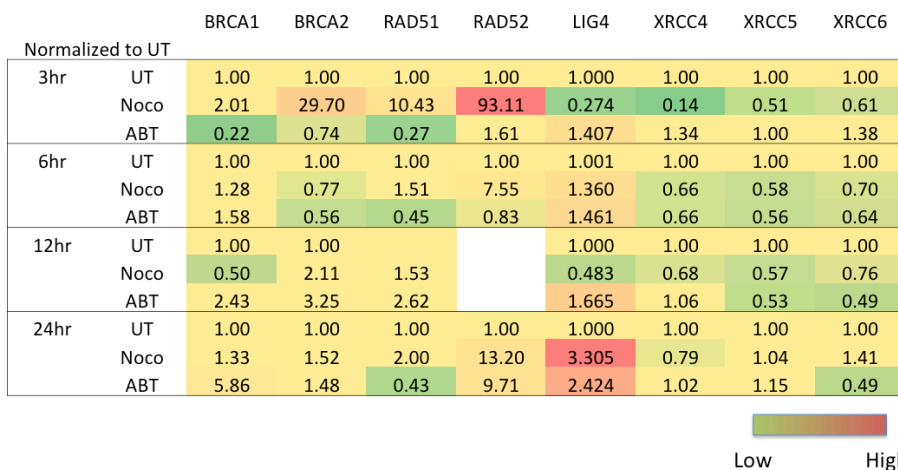
standard deviation for percentage of each cell cycle phase ( $n=3$  biological replicates). DN, double negative. KO, Kusabira orange. AG, Azami Green.



**Supplementary Figure 3: Cell survival rate after synchronization with Nocodazole and ABT.** Fucci H9 cell counts immediately after synchronization normalized to untreated cells show a decrease after treatment (Top panel,  $n=3$  biological replicates). Nocodazole and ABT induce reversible growth arrest and cells recover their number around 30hrs post synchronization as shown by survival and recover cure normalized to untreated cell number at 0 hours post synchronization (Lower panel). The high concentration of ABT (3.6  $\mu\text{g/ml}$ ) inhibits cell number recovery.



**Supplementary Figure 4: Quantification of indel formation after targeting of Oct4 using CRISPR/Cas9 in HEK293t cells.** CRISPR/Cas9 targeted region was amplified and indels were analyzed by T7 endonuclease assay in HEK293t untreated, DMSO vehicle treated, synchronized, and control untargeted cells.



**Supplementary Figure 5: HR related (*BRCA1*, *BRCA2*, *RAD51*, *RAD52*) and NHEJ related (*LIG4*, *XRCC4*, *XRCC5* and *XRCC6*) gene expression detected by qPCR.** H1 synchronized cell gene expression analyzed by qPCR at 3, 6, 12, and 24 hours post nucleofection normalized to untreated cells ( $n=2$  technical replicates).

Treatment	G0/G1 (%)	S (%)	G2/M (%)
UT	46.73±5.66	21.4±6.95	30.57±4.92
DMSO 1%	45.2±7.13	18±0.79	35.97±6.74
DMSO 0.1%	47.77±6.56	18.9±0.7	32.67±6.82
ABT 3.6µg/ml	5.87±2.57	16.93±6.45	76.6±9.4

ABT 0.36µg/ml	6.5±4.16	15.97±8.08	77±12.99
Noco 1µg/ml	5.07±2.23	13.5±2.65	81.43±5.25
Noco 0.5µg/ml	5.77±4.41	16.5±9.23	76.77±14.01

**Supplementary Table 1-** H1 hPSCs synchronized using different concentrations of Nocodazole and ABT (*n*=3 biological replicates). Data presented as Mean ± Standard deviation.

Treatment /Releasing time (hr)	Treatment	G1 (%)	S (%)	G2/M (%)
	UT	52±8.66	27.6±1.67	18.07±7.04
	DMSO	47.23±3.9	26.27±2.6	25.9±4.84
4/3	Nocodazole	25.53±1.1	26.83±1.15	48.83±1.81
	ABT	24.37±1.63	27.47±1.35	46.57±0.21
4/1	Nocodazole	33.63±1.79	23.47±1.98	41.36±0.35
	ABT	32.27±3.26	25.23±2.63	40.7±2.67
4/0	Nocodazole	34.6±2.05	23.2±0.87	40.13±2.05
	ABT	38.07±0.86	22.8±0.72	36.73±1.18
16/3	Nocodazole	23.9±15.25	6.41±1.36	68.37±17.55
	ABT	30.77±26.72	5.05±2.21	62.47±25.61
<b>16/1</b>	<b>Nocodazole</b>	<b>5.7±1.18</b>	<b>2.17±0.46</b>	<b>91±1.25</b>
	ABT	14.76±9.87	3.55±2.36	80.13±11.18
<b>16/0</b>	Nocodazole	6.41±1.59	2.77±0.59	89.8±2.88
	<b>ABT</b>	<b>6.51±3.2</b>	<b>2.86±0.99</b>	<b>89.23±4.42</b>
12/3	Nocodazole	11.64±8.83	2.75±0.66	84.43±9.79
	ABT	21.82±24.49	2.32±0.66	74.73±25.69
12/1	Nocodazole	5.54±0.23	2.6±1.3	61.72±48.61
	ABT	10.93±6.5	2.74±0.58	84.77±7.62
12/0	Nocodazole	8.34±2.41	3.27±1.58	84.53±2.54
	ABT	6.23±2.94	3.23±1.38	89.13±4.52
18/3	Nocodazole	9.7± 6.29	3.4±1	86.1±7.31
	ABT	14.13±5.23	5.72±2.45	79.47±7.48
18/1	Nocodazole	5.95±2.19	1.43±0.27	91.87±2.57
	ABT	5.95±1.35	1.6±0.48	91.47±1.52
18/0	Nocodazole	6.83±3.85	2.04±0.82	90.37±4.7
	ABT	8.25±3.01	1.74±0.23	89.27±2.68

**Supplementary Table 2-** H1 hPSCs with different synchronization and releasing time. Treatment and releasing time are annotated as Treatment/Releasing time (hr) ( $n=3$  biological replicates). Data presented as Mean  $\pm$  Standard deviation.

Program	GFP+(%)	Viability
CA137	67.6	~60%
CB150	37.4	~60%
CD118	29.2	~80%
CE118	28.6	~80%
CM113	38.6	~80%
DC100	66.3	~30%
DN100	84.4	~20%

**Supplementary Table 3:** Various nucleofection programs were tested using 10ug pMAXGFP of  $2.5 \times 10^5$  of H1. GFP and cell viability was measured by FACS after 48 hours of nucleofection.

Gene target	Targeting method	Plasmid
<i>WNT5A</i>	CRISPR; D10A Cas9; <i>S. pyogenes</i>	Targeting: pX335 Donor(s): PL452
<i>NEUROD1</i> 1-3	CRISPR; Cas9; <i>S. pyogenes</i>	Targeting: pX459 Donor(s): OCT4-2A-eGFP-PGK-Puro; Kume_mKO1_pPL452loxP_EM7_PGK_neo
<i>NEUROD1</i> 4-5	CRISPR; Cas9; <i>N. meningitides</i>	Targeting: pSimpleII-U6-tracr-U6-BsmBI-NLS-NmCas9-HA-NLS Donor(s): OCT4-2A-eGFP-PGK-Puro; Kume_mKO1_pPL452loxP_EM7_PGK_neo
NKX6.1	ZFNs	Targeting: PZFN1_Nkx6.1 and PZFN2_Nkx6.1 Donor(s): OCT4-2A-eGFP-PGK-Puro
<i>INS</i>	CRISPR; Cas9 TALENs	Targeting: 13ABPZVC_INS_TAL_3R_TALtrunc_FokI Donor(s): Kume_mKO1_pPL452loxP_EM7_PGK_neo
<i>OCT4</i>	CRISPR; Cas9; <i>S. pyogenes</i>	Targeting: pX330 Donor(s): CMV-Brainbow-1.1M and Oct4-eGFP-PGK-Puro

**Supplementary Table 4:** Plasmids for gene targeting method and donor plasmids for HDR. *NEUROD1* CRISPR/Cas9 are separated by species with sgRNA numbers corresponding to Supplementary Table 5.

Gene target	Targeting strategy	Guide set	Direction	Sequence (5' to 3')
	CRISPR nickase	1	F	CACCGTGCAGTTCCACCTTCGAT GTCCG
	CRISPR nickase	1	R	AAACCCCGACATCGAAGGTGGA ACTGCAC

WNT5A	CRISPR nickase	2	F	CACCGTTTGGCAGGGTGATGCA GATAGG
	CRISPR nickase	2	R	AAACCCTATCTGCATCACCTGC CAAAC
	CRISPR nickase	3	F	CACCGTTCGATGTCGGAATTGAT ACTGG
	CRISPR nickase	3	R	AAACCCAGTATCAATTCCGACAT CGAAC
	CRISPR nickase	4	F	CACCGGTGGATAACACCTCTGTT TTTGG
	CRISPR nickase	4	R	AAACCCAAAAACAGAGGTGTTAT CCACC
NEUR OD1	CRISPR	1	F	CACCGAGCCACGGATCAATCTT CTC
	CRISPR	1	R	AAACGAGAAGATTGATCCGTGGT CC
	CRISPR	2	F	CACCGGCACTATCCTGCAGCGA CAC
	CRISPR	2	R	AAACGTGTGCTGCAGGATAGT GCC
	CRISPR	3	F	CACCGGCACTATCCTGCAGCGA CAC
	CRISPR	3	R	AAACGTGTGGCTGCAGGATAGT GCC
	CRISPR	4	F	GAGCCCTTCTTTGAAAGCCCTCT
	CRISPR	4	R	AGAGGGCTTTCAAAGAAGGGCT C
	CRISPR	5	F	AGTGCCAGCTCAATGCCATATT
	CRISPR	5	R	AATATGGCATTGAGCTGGGCACT
NKX6.1	ZFN	1	-	CTCTACTTCAGCCCCAGnnCCGC GGCCGTGGCCGCCGG
	ZFN	2	-	GGACGCCCATCTTCTGnCCGG AGTGATGCAGAGCC
	ZFN	3	-	<b>CTGGCCGGACGCCCATCTTcNnG GCCCGGAGTGATGCAGAGCC</b>
	ZFN	4	-	CCGGTACCCCAAGCCGCnnGCT GAGCTGCCTGGCCGGC
	ZFN	5	-	CGGACGCCCATCTTCTGnnCCG GAGTGATGCAGAGCC
	ZFN	6	-	TACTTCAGCCCCAGCGCCGCnnC CGTGGCCGCCGTGGGCCGG
	ZFN	7	-	CTGGCCGGACGCCCATCTTCTn GCCCGGAGTGATGCA
	ZFN	8	-	CGCCGTGGGCCGGTACCCCAAn nCGCTGGCTGAGCTG
	ZFN	9	-	CCGGTACCCCAAGCCGCTGGnn GAGCTGCCTGGCCGGC
	ZFN	10	-	CGTGGGCCGGTACCCCAAGCnn CTGGCTGAGCTGCCTG
	ZFN	11	-	GGCCGCCGTGGGCCGGTACCnn AAGCCGCTGGCTGAGT

	ZFN	12	-	CCTGGCCGGACGCCCATCTTnT GGCCCGGAGTGATGCAGAG
	ZFN	13	-	GGCCGCCGTGGGCCGGTACCCn AAGCCGCTGGCTGAGT
	ZFN	14	-	GACGCCCATCTTCTGGCCCCn GTGATGCAGAGCCCCGCC
	ZFN	15	-	CTGGCCGGACGCCCATCnnCTG GCCCGGAGTGATGCAG
	ZFN	16	-	CGGACGCCCATCTTCTGGCCn GAGTGATGCAGAGCCCCGCCCT
INS	TALENs	1	F	TCTGCTCCCTCTACCAGCT
	TALENs	1	R	TAGACGCAGCCCGCAGGCA
OCT4	CRISPR	1	F	CACCGCTCTCCCATGCATTCAA CTGAGG
	CRISPR	1	R	AAACCCTCAGTTTGAATGCATGG GAGAGC

**Supplementary Table 5:** Guide sequences for gene targeting.

Gene target	Chromosomal location	Sequence
<i>WNT5A</i> first constitutive exon	chr3:55,513,341-55,513,466	atgcagtacatcggagaaggcgcaagacag gcatcaaagaatgccagatcaatt <b>ccgacatcg</b> <b>aagggtggaactgca</b> gactgtggataaacacctt gttt <b>ttggcagggtgatgcagatagg</b> cagccgag agacggccttcacatacgcggtgagcgcagca ggggtggtgaacgcatgagccgggctgccc cgaggggcagctgtccacctgaggctg
<i>NEUROD1</i> , exon 1	chr2:182,540,833-182,545,392	<b>acgcctacagcgcagcgtggagcccttctt</b> <b>tgaagccctctgactgattgcaccagccctt</b> <b>cctttgatggaccctcagccgcccgtcag</b> <b>catcaatggcaacttctcttcaaacacgaac</b> <b>cgccgcccagcttgagaaaattatgccttta</b> <b>ccatgcaactcctgcagcgcactggcagg</b> <b>ggcccaaagccacggatcaatctctcaggc</b> <b>accgctgcccctcgtgcgagatcccatag</b> <b>acaatattatgtccttcgatagccattcacatc</b> <b>atgagcagctcatgagtgccagctcaatgc</b> <b>catattcatgattag</b>
<i>NKX6.1</i> , 3' of exon 1	chr4:85,403,140-85,429,603	gcgccgctggccgcccgtggccggtaccccaa gccgctggctgagctgc <b>ctggtggccggacgcc</b> <b>catcttctggcccgg</b> agtgatgcagagcccgcc tgaggggacgcacgcctggcctgtaccctcgtg agtac
INS	chr11:2,171,009-2,192,571	tggggcaggtggagctggcgggggccctggt gcaggcagcctgcagccctggccctggaggg gtccctgcagaagcgtggcattgtggaacaatgc tgtaccagca <b>tctgctcccttaccagct</b> ggagaa ctactgcaact <b>tagacgcagcccgcaggcagcc</b> ccacaccgcccctcctgcaccgagagagat ggaataaagccctgaaccagc



OCT4	chr6_ssto_hap7:2,4 67,051-2,473,404	cctcggtcctttccctgagggggaagccttccc cctgtctctgtcaccactctgggcttccatgcatt caactgagggcctgcccttaggaatggggg acagggggaggggaggagctagggaaag
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**Supplementary Table 6:** Genomic location of targeting. Red sequence shows complementary sequence binding targeting guides.

Homologous arms of donor	Sequence	Mutated sgRNA binding site
<i>WNT5a</i> left homology arm, guides 1 & 2; 3 & 4	CGTCATGGTACCCCTCACAATCACATAAGGA GCCCTTTTAAAATGTCAAGAAACATGGTGAAT TTTCTCCAGTCTGTATACTTGTCCCTCTGTTC TTCCATCAGTCAAAGGAACTGCTATCTCCTA CCCGTATCTAATTGTGTCTACTTTCAGCATT ATAAGTTTTCAAGGCAGATTGGTCCAATTTGT GGGGCAAAGTGGGTCACTTTCTCTAATGTCA GAGGCCTGTCCCTCAAAGTCTGTTTCCTCC TTTTCTGAGTGGATTTTGGAAATACTGGTTCA GGTATATGGTGACAGGGGCTAGGAAGGGAG TGAAAATACTAACTGAGGAGCCAGATGGCAG GATGGGGACTGCACAATACCTCCGTTTTCTG AGTGCAGTTTAAACAGAGGAGTTTTATGAAG CCTTTCTCATACTTTGTCATGAGGACAAGCA GGAGAGAAAAAAGCATCTGTGTGCTTAAAC TATGTATTGCTGACATGCTTTCAGGGGGCCC ACCTCGAGATTTACGTAATCTTGAATGCAC ATCTCCCCCCTTGCAGGTCGCTAGGTATGA ATAACCCTGTTTCAGATGTCAGAAGTATATATT ATAGGAGCACAGCCTCTCTGCAGCCAAGTGG CAGGACTTTCTCAAGGACAGAAGAACTGTG CCACTTGTATCAGGACCACATGCAGTACATC GGAGAAGGCGCGAAGACAGGCATCAAAGAA TGAATGTTTTCAATTATCACATCCTTGGTGGAA CTGCAGCAGTCGACCTAGCTG	ATCACATC CTTGGTGG AACTGCA
<i>WNT5a</i> right homology arm, guides 1 & 2; 3 & 4	GTCAGTAAGCGGCCCGCCTGTGGATAACACC GTGGTTTTGAACAGGGTGTGCTTATTAATAG GAAAACATTTAAAATATTTTTTTAAAACATTAA CTTTTTTTCCTAGAAGAAATCCTTAGCATTAC TTCATGCTTGATAGAAAGCATGAAGGAGGAT GCCTTCCAGCTCGGGTGGTGGCTTGGGCATT TTTGTCAATTATAGCAAATGATGTTAATTCCTGT CCCTACACCTGATGATAAGTTTTGGATTTTAA TCAAATCAGGAGGCAAGTATCACAGACACCT TCATTTTTGCAAACACTTAATTTTTCTATAAAG CATTTTGTTTAAAATAGAAGCAGAACACTTAT TTTGGTTTCTTAAGAGTAGGTGGGGGAAGCA AGTCATGTATATGCTTTTAAACGTGAACTC TCTTGCCTGTTAGTTTTTAAAGCCACAATTGA AACGGCCCAGCATCGAACTAAAAGTTGCCG	TTGAACAG GGTGATGC TTATTAA

	<p>TCTTTTAGAATCCTGGATATATTGAAAGTGAA  CAAAGTTTTAATTTTCCTAATAGAACTATTTCC  TGAGGCTATAAAGATATAGACGTTAAAAGCTT  TATTTTTAGTCCCAGAAAGAATTTAATTA  GTGATCCAATTTATGTTTGTGATGTCAGCAAT  TGATATAATAAAAGCACATGCTTGGGATGTCA  ACCTAGGAACCTGAAAAAAAAAATTAAGAGTAT  TGCTCACAGTCCGATTCTTCATTTTAAATACG  TTGTTGAATGTCTTACTTCCCCATTCAAAGAG  ATAGCATTTCAAATGGGCTAAAGGCAAAGATT  TTAAAAATCTAGTAATGATAGTGAGATCCAG  ACATGATAAGATACATTGATGAGTTTGGACAA  ACCACAACCTAGAATGCAGTGAAAAAATGCTT  TATTTGTGAAATTTGTGATGCTATTGCTTTATT  TGTAACCATTATAAGCTGCAATAACAAGTTC  CGCGGCTATGAC</p>	
<p><i>NEUROD1</i>  left homology  arm, guide 1</p>	<p>TTAATTggatccATGAAGGCTAACGCCCGGGAG  CGGAACCGCATGCACGGACTGAACGCGGCG  CTAGACAACCTGCGCAAGGTGGTGCCTTGCT  ATTCTAAGACGCAGAAGCTGTCCAAAATCGA  GACTCTGCGCTTGGCCAAGA ACTACATCTGG  GCTCTGTGCGGAGATCCTGCGCTCAGGCAAAA  GCCCAGACCTGGTCTCCTTCGTT CAGACGCT  TTGCAAGGGCTTATCCCAACCCACCACCAAC  CTGGTTGCGGGCTGCCTGCAACTCAATCCTC  GGACTTTTCTGCCTGAGCAGAACCAGGACAT  GCCCCCCACCTGCCGACGGCCAGCGCTTC  CTTCCCTGTACACCCCTACTCCTACCAGTCG  CCTGGGCTGCCAGTCCGCCTTACGGTACCA  TGGACAGCTCCCATGTCTTCCACGTTAAGCC  TCCGCCGCACGCCTACAGCGCAGCGCTGGA  GCCCTTCTTTGAAAGCCCTCTGACTGATTGC  ACCAGCCCTTCCTTTGATGGACCCCTCAGCC  CGCCGCTCAGCATCAATGGCAACTTCTCTTT  CAAACACGAACCGTCCGCCGAGTTTGAGAAA  AATTATGCCTTTACCATGCACTATCCTGCAGC  GACACTGGCAGGGGCCCAAAGCCACGGATC  AATCTTtgctagcTTAATT</p>	<p>AGCCACGG  ATCAATCTT  tgc</p>
<p><i>NEUROD1</i>  right  homology  arm, guide 1</p>	<p>TTAATTggcgcgccCTCAGGCACCGCTGCCCT  CGCTGCGAGATCCCCATAGACAATATTATGT  CCTTCGATAGCCATTCACATCATGAGCGAGT  CATGAGTGCCCAGCTCAATGCCATATTTT  GATTAGAGGCACGCCAGTTTCACCATTTCCG  GGAACGAACCCACTGTGCTTACAGTGACTG  TCGTGTTTACAAAAGGCAGCCCTTTGGGTAC  TACTGCTGCAAAGTGCAAATACTCCAAGCTTC  AAGTGATATATGTATTTATTGTCATTACTGCCT  TTGGAAGAAACAGGGGATCAAAGTTCCTGTT  CACCTTATGTATTATTTTCTATAGCTCTTCTAT  TTAAAAATAAAAAAATACAGTAAAGTTTAAAA  AATACACCACGAATTTGGTGTGGCTGTATTCA  GATCGTATTAATTATCTGATCGGGATAACAAA</p>	<p>ttaattggcgcg  ccCTC</p>

	<p>ATCACAAGCAATAATTAGGATCTATGCAATTT  TTAAACTAGTAATGGGCCAATTTAAAATATATA  TAAATATATATTTTTCAACCAGCATTTTACTAC  TTGTTACCTTTCCCATGCTGAATTATTTTGTTG  TGATTTTGTACAGAATTTTAAATGACTTTTAT  AATGTGGATTTCCCTATTTTAAAACCATGCAGC  TTCATCAATTTTATACATATCgcggccgcTTAAT  T</p>	
<p><i>NEUROD1</i>  left homology  arm, guide 2</p>	<p>TTAATTggatccACTAAGGCTCGCCTGGAGCGT  TTTAAATTGAGACGCATGAAGGCTAACGCCC  GGGAGCGGAACCGCATGCACGGACTGAACG  CGGCGCTAGACAACCTGCGCAAGGTGGTGC  CTTGCTATTCTAAGACGCAGAAGCTGTCCAA  AATCGAGACTCTGCGCTTGGCCAAGA ACTAC  ATCTGGGCTCTGTTCGGAGATCCTGCGCTCAG  GCAAAGCCCAGACCTGGTCTCCTTCGTTCA  GACGCTTTGCAAGGGCTTATCCCAACCCACC  ACCAACCTGGTTGCGGGCTGCCTGCAACTCA  ATCCTCGGACTTTTCTGCCTGAGCAGAACCA  GGACATGCCCCCCCACCTGCCGACGGCCAG  CGCTTCCTTCCCTGTACACCCCTACTCCTAC  CAGTCGCCTGGGCTGCCAGTCCGCCTTAC  GGTACCATGGACAGCTCCCATGTCTTCCACG  TTAAGCCTCCGCCGCACGCCTACAGCGCAG  CGCTGGAGCCCTTCTTTGAAAGCCCTCTGAC  TGATTGCACCAGCCCTTCCTTTGATGGACCC  CTCAGCCCGCCGCTCAGCATCAATGGCAACT  TCTCTTTCAAACACGAACCGTCCGCCGAGTT  TGAGAAAAATTATGCCTTTACCATGCACTATC  CTGCAGCGAtggctagcTTAATT</p>	<p>GCACTATC  CTGCAGCG  Atgg</p>
<p><i>NEUROD1</i>  right  homology  arm, guide 2</p>	<p>TTAATTggcgccgcccACTGGCAGGGGGCCCAAAG  CCACGGATCAATCTTCTCAGGCACCGCTGCC  CCTCGCTGCGAGATCCCCATAGACAATATTA  TGTCCTTCGATAGCCATTCACATCATGAGCG  AGTCATGAGTGCCCAGCTCAATGCCATATTT  CATGATTAGAGGCACGCCAGTTTCACCATTT  CCGGGAAACGAACCCACTGTGCTTACAGTGA  CTGTCTGTTTTACAAAAGGCAGCCCTTTGGG  TACTACTGCTGCAAAGTGCAAATACTCCAAG  CTTCAAGTGATATATGTATTTATTGTCATTACT  GCCTTTGGAAGAAACAGGGGATCAAAGTTCC  TGTTACCTTATGTATTATTTTCTATAGCTCTT  CTATTTAAAAAATAAAAAAATACAGTAAAGTTT  AAAAAATACACCACGAATTTGGTGTGGCTGTA  TTCAGATCGTATTAATTATCTGATCGGGATAA  CAAATCACAGCAATAATTAGGATCTATGCA  ATTTTTAACTAGTAATGGGCCAATTTAAAATAT  ATATAAATATATATTTTTCAACCAGCATTTTAC  TACTTGTTACCTTTCCCATGCTGAATTATTTG  TTGTGATTTTGTACAGAATTTTAAATGACTTTT  TATAATGTGGATTTCCCTATTTTAgcggccgcTTAA  TT</p>	<p>ttaattggcgcg  ccCAC</p>

<p><i>NEUROD1</i> left homology arm, guide 3</p>	<p>TTAATTggatccGATGAGGACCTGGAAGAGGAG GAAGAAGAGGAAGAGGAGGATGACGATCAA AAGCCCAAGAGACGCGGCCCCAAAAAGAAG AAGATGACTAAGGCTCGCCTGGAGCGTTTTA AATTGAGACGCATGAAGGCTAACGCCCGGGA GCGGAACCGCATGCACGGACTGAACGCGGC GCTAGACAACCTGCGCAAGGTGGTGCCTTGC TATTCTAAGACGCAGAAGCTGTCCAAAATCG AGACTCTGCGCTTGGCCAAGAACTACATCTG GGCTCTGTCGGAGATCCTGCGCTCAGGCAAA AGCCAGACCTGGTCTCCTTCGTTCAGACGC TTTGCAAGGGCTTATCCCAACCCACCACCAA CCTGGTTGCGGGCTGCCTGCAACTCAATCCT CGGACTTTTCTGCCTGAGCAGAACCAGGACA TGCCCCCCCACCTGCCGACGGCCAGCGCTT CCTTCCCTGTACACCCCTACTCCTACCAGTC GCCTGGGCTGCCAGTCCGCCTTACGGTAC CATGGACAGCTCCCATGTCTTCCACGTTAAG CCTCCGCCGCACGCCTACAGCGCAGCGCTG GAGCCCTTCTTTGAAAGCCCTCTGACTGATT GCACCAGCCCTTCTTTGATGGACCCCTCAG CCCGCCGCTCAGCATggctagcTTAATT</p>	<p>AGCCCGCC GCTCAGCA Tggc</p>
<p><i>NEUROD1</i> right homology arm, guide 3</p>	<p>TTAATTggcgcgccCAATGGCAACTTCTCTTTCA AACACGAACCGTCCGCCGAGTTTGAGAAAAA TTATGCCTTTACCATGCACTATCCTGCAGCGA CACTGGCAGGGGCCCAAAGCCACGGATCAA TCTTCTCAGGCACCGCTGCCCTCGCTGCGA GATCCCATAGACAATATTATGTCCTTCGATA GCCATTCACATCATGAGCGAGTCATGAGTGC CCAGCTCAATGCCATATTTTCATGATTAGAGGC ACGCCAGTTTCACCATTTCCGGGAAACGAAC CCACTGTGCTTACAGTGACTGTCGTGTTTACA AAAGGCAGCCCTTTGGGTACTACTGCTGCAA AGTGCAAATACTCCAAGCTTCAAGTGATATAT GTATTTATTGTCATTACTIONGCTTTGGAAGAAA CAGGGGATCAAAGTTCCTGTTACCTTATGTA TTATTTTCTATAGCTCTTCTATTTAAAAAATAA AAAAATACAGTAAAGTTTAAAAAATACACCAC GAATTTGGTGTGGCTGTATTCAGATCGTATTA ATTATCTGATCGGGATAACAAAATCACAAGCA ATAATTAGGATCTATGCAATTTTTAACTAGTA ATGGGCCAATTAATAATATATAAATATATATT TTTCAACCAGCATTACTACTTGgcgggccgcTT AATT</p>	<p>ttaattggcgcg ccCAA</p>
<p><i>NEUROD1</i> left homology arm, guide 4</p>	<p>TTAATTggatccGAGGACGACCTCGAAACCATG AACGCAGAGGAGGACTCACTGAGGAACGGG GGAGAGGAGGAGGACGAAGATGAGGACCTG GAAGAGGAGGAAGAAGAGGAAGAGGAGGAT GACGATCAAAAGCCCAAGAGACGCGGCCCC AAAAAGAAGAAGATGACTAAGGCTCGCCTGG AGCGTTTTAAATTGAGACGCATGAAGGCTAA CGCCCGGGAGCGGAACCGCATGCACGGACT</p>	<p>GAGCCCTT CTTTGAAA GCCCGgc</p>

	<p>GAACGCGGGCGCTAGACAACCTGCGCAAGGT  GGTGCCTTGCTATTCTAAGACGCAGAAGCTG  TCCAAAATCGAGACTCTGCGCTTGGCCAAGA  ACTACATCTGGGCTCTGTGCGGAGATCCTGCG  CTCAGGCCAAAAGCCCAGACCTGGTCTCCTTC  GTTCAGACGCTTTGCAAGGGCTTATCCCAAC  CCACCACCAACCTGGTTGCGGGCTGCCTGC  AACTCAATCCTCGGACTTTTCTGCCTGAGCA  GAACCAGGACATGCCCCCCCACCTGCCGAC  GGCCAGCGCTTCCTTCCCTGTACACCCCTAC  TCCTACCAGTCGCCTGGGCTGCCCAGTCCG  CCTTACGGTACCATGGACAGCTCCCATGTCT  TCCACGTTAAGCCTCCGCCGCACGCCTACAG  CGCAGCGCTGGAGCCCTTCTTTGAAAGCCCg  gctagcTTAATT</p>	
<p><i>NEUROD1</i>  right  homology  arm, guide 4</p>	<p>TTAATTggcgcgccTCTGACTGATTGCACCAGCC  CTTCCTTTGATGGACCCCTCAGCCCGCCGCT  CAGCATCAATGGCAACTTCTCTTTCAAACACG  AACCGTCCGCCGAGTTTGAGAAAAATTATGC  CTTTACCATGCACTATCCTGCAGCGACTG  GCAGGGGCCCAAAGCCACGGATCAATCTTCT  CAGGCACCGCTGCCCTCGCTGCGAGATCC  CCATAGACAATATTATGTCCTTCGATAGCCAT  TCACATCATGAGCGAGTCATGAGTGCCCAGC  TCAATGCCATATTTTCATGATTAGAGGCACGCC  AGTTTCACCATTTCCGGGAAACGAACCCACT  GTGCTTACAGTGAAGTGTGTTTACAAAAG  GCAGCCCTTTGGGTACTACTGCTGCAAAGTG  CAAATACTCCAAGCTTCAAGTGATATATGTAT  TTATTGTCATTACTGCCTTTGGAAGAAACAGG  GGATCAAAGTTCCTGTTACCTTATGTATTAT  TTTCTATAGCTCTTCTATTTAAAAAATAAAAAA  ATACAGTAAAGTTTAAAAAATACACCACGAAT  TTGGTGTGGCTGTATTCAGATCGTATTAATTA  TCTGATCGGGATAACAAAATCACAAGCAATAA  TTAGGATCTATGCAATTTTTAAACTAGTAATG  GGCCAATTAATAATgcggccgcTTAATT</p>	<p>ttaattggcgcg  ccTCT</p>
<p><i>NEUROD1</i>  left homology  arm, guide 5</p>	<p>TTAATTggatccAGACGCAGAAGCTGTCCAAAA  TCGAGACTCTGCGCTTGGCCAAGAACTACAT  CTGGGCTCTGTGCGGAGATCCTGCGCTCAGG  CAAAGCCCAGACCTGGTCTCCTTCGTTTCAG  ACGCTTTGCAAGGGCTTATCCCAACCCACCA  CCAACCTGGTTGCGGGCTGCCTGCAACTCAA  TCCTCGGACTTTTCTGCCTGAGCAGAACCAG  GACATGCCCCCCCACCTGCCGACGGCCAGC  GCTTCCTTCCCTGTACACCCCTACTCCTACCA  GTCGCCTGGGCTGCCCAGTCCGCCTTACGG  TACCATGGACAGCTCCCATGTCTTCCACGTT  AAGCCTCCGCCGCACGCCTACAGCGCAGCG  CTGGAGCCCTTCTTTGAAAGCCCTCTGACTG  ATTGCACCAGCCCTTCTTTGATGGACCCCT  CAGCCCGCCGCTCAGCATCAATGGCAACTTC</p>	<p>AGTGCCCA  GCTCAATG  CCATagc</p>

	<p>TCTTTCAAACACGAACCGTCCGCCGAGTTTG  AGAAAAATTATGCCTTTACCATGCACTATCCT  GCAGCGACACTGGCAGGGGCCAAAGCCAC  GGATCAATCTTCTCAGGCACCGCTGCCCTC  GCTGCGAGATCCCATAGACAATATTATGTC  CTTCGATAGCCATTCACATCATGAGCGAGTC  ATGAGTGCCCAGCTCAATGCCATagctagcTTA  ATT</p>	
<p><i>NEUROD1</i>  right  homology  arm, guide 5</p>	<p>TTAATTggcgcgccATTTTCATGATTAGAGGCACG  CCAGTTTCACCATTTCGGGAAACGAACCCA  CTGTGCTTACAGTGA CTGTGTTTACAAAA  GGCAGCCCTTTGGTACTACTGCTGCAAAGT  GCAAATACTCCAAGCTTCAAGTGATATATGTA  TTTATTGTCATTACTGCCTTTGGAAGAAACAG  GGGATCAAAGTTCCTGTTACCTTATGTATTA  TTTTCTATAGCTCTTCTATTTAAAAAATAAAAA  AATACAGTAAAGTTTAAAAAATACACCACGAA  TTTGGTGTGGCTGTATTCAGATCGTATTAATT  ATCTGATCGGGATAACAAAATCACAAGCAATA  ATTAGGATCTATGCAATTTTTAACTAGTAAT  GGGCCAATTAATAATATAAATATATATTTT  TCAACCAGCATTTTACTACTTGTACCTTTCC  CATGCTGAATTATTTGTTGTGATTTTGTACA  GAATTTTAAATGACTTTTTATAATGTGGATTTC  CTATTTTAAACCATGCAGCTTCATCAATTTT  ATACATATCAGAAAAGTAGAATTATATCTAATT  TATACAAAATAATTTAACTAATTTAAACCAGCA  GAAAAGTGCTTAGAAAGTTATTGTGTTGCCTT  AGCACTTCTTTCCTCTCCAATTGTAAAAAAA  gcgccgcTTAATT</p>	<p>ttaattggcgcg  ccATT</p>
<p><i>NKX6.1</i>, left  homology  arm, ZFN 3</p>	<p>AGCCAGGGATCGAATCTAGGACTCGCGGAA  CGAAAGGACTGCCTAGCCCGCCGGGACGCC  TGCTTTTCTCGGCGAGCTGCCGCCTCCCGCG  TGGAGGGTTTGGACATCTCTGCTGCGCAGCT  AGGCGAGCAACTCCCGGCAGCGGCATTTTTG  GTTTCAAGTTGGCAGCTCGCCTCCGGGCGCGC  CGAGTGCCTCTCCGCTCGCGCCCTCGGCGC  TTCCGGCTCCTCTGAGCCCCGCGGGGGGCA  CCAGCCAGCGCCCTCGCTGCAAGGCTACGG  TCTCCGGCGTGGCCGTGGGATGTTAGCGGT  GGGGGCAATGGAGGGCACCCGGCAGAGCG  CATTCTGCTCAGCAGCCCTCCCCTGGCCGC  CCTGCACAGCATGGCCGAGATGAAGACCCC  GCTGTACCCTGCCGCGTATCCCCCGCTGCCT  GCCGGCCCCCCTCCTCCTCGTCTCGTCTGT  CGTCTCCTCGTCCGCTCCCGCCTCTGG  GCACCCACAACCCAGGCGGCCTGAAGCCCC  CGGCCACGGGGGGGCTCTCATCCCTCGGCA  GCCCCCGCAGCAGCTCTCGGCCGCCACCC  CACACGGCATCAACGATATCCTGAGCCGGCC  CTCCATGCCCGTGGCCTCGGGGGCCGCCCT  GCCCTCCGCTCGCCCTCCGGTTCCTCCTCC</p>	<p>CTGGCCGG  ACGCCCAT  CTtctggCCC  GGAGTGAT  GCAGAGCC</p>

	<p>TCCTCTTCCTCGTCCGCCTCTGCCTCCTCCG  CCTCTGCCGCCGCCGCCGGCTGCTGCCGCCGG  CCGCAGCCGCCGCCCTCATCCCCGGCCGGGC  TGCTGGCCGGACTGCCACGCTTTAGCAGCCT  GAGCCCGCCGCCGCCGCCGCCGGGCTCTA  CTTCAGCCCCAGCGCCGCCGCCGTGGCCGC  CGTGGGCCGGTACCCCAAGCCGCTGGCTGA  GCTGCCTGGCCGGACGCCCATCTtctggGCTA  GC</p>	
<p>NKX6.1, right  homology  arm, ZFN 3</p>	<p>CCCGGAGTGATGCAGAGCCCCGCCCTGGAGG  GACGCACGCCTGGCCTGTACCCCTCGTGAG  TACTACCACCCGCGCCCCGATGCCTGCCTGC  CGTGCCTGTTCTGCCACTCCCGGGTCCGC  GCCCTGGTGTGCATGCCGCTCAGTCCATCC  TGTGGCCCGCCCCAGTAGTTTGACAGCGCC  GGAATCACACCAGTTAGGTTGGGCCAGGGCT  TCCAGAAAGGGATTCTCGCTTTTCTGAGCTC  GCGTGGCTGTATCCAAGCGCCTCCCTCTCTC  TAAGTCTTGAGTACGCCTGGGCTGAGCCGGT  GTGTGTGTGTTTCGTGTGGACCACAACGCGT  GAGGGACGTGTGTCTCGTCCGAGGCCCTGG  CTGTTCCCTGAAACGGCCTGGCCAGGACCGC  AGGCCTGGCCTGGATTAGAAAGATGGGGAG  GAGAGGGGAGGAGAGGCCACTGCATCTCTG  CTTTCCCGGCTCCTCCCTGTCCGAGCGCCC  CATTCCGCCTTCGCCAGCCTAACCGCTGCCT  CTCTTGAAGCAGTGAGGCCCGGCAGCGGT  GAGCCCTCCACCGCCCCGGGAAGTGAGTGTA  TTTGCATGCACGTCTCCGCCTGGGGTGCCCC  ACCTCACCTCTTCGCCCTCGCCGCCCGAGTA  CACTCGGCCTGGGTGAGCCCATTGAAGACA  GTCCAGCGTCCCCAGGGCAGGGCGAGCGCT  TCCAGCCGCTCACCAGCGCTGGGCCCGCGC  TTTCCACCTGCCTCCACTCTGGGGGCTGGC  ACACGCTGCGTCCGGGCA</p>	<p>CTGGCCGG  ACGCCCAT  CTtctggCCC  GGAGTGAT  GCAGAGCC</p>
<p>INS left  homology  arm</p>	<p>GCCGCCCCAGCCACCCCTGCTCCTGGCG  CTCCCACCCAGCATGGGCAGAAGGGGGCAG  GAGGCTGCCACCCAGCAGGGGGTCAGGTGC  ACTTTTTTAAAAAGAAGTTCTCTTGGTCACGT  CCTAAAAGTGACCAGCTCCCTGTGGCCAGT  CAGAATCTCAGCCTGAGGACGGTGTTGGCTT  CGGCAGCCCCGAGATACATCAGAGGGTGGG  CACGCTCCTCCCTCCACTCGCCCCTCAAACA  AATGCCCCGCAGCCATTTCTCCACCCTCAT  TTGATGACCGCAGATTCAAGTGTTTTGTAAAG  TAAAGTCCTGGGTGACCTGGGGTCACAGGGT  GCCCCACGCTGCCTGCCTCTGGGCGAACAC  CCCATCACGCCCGGAGGAGGGCGTGGCTGC  CTGCCTGAGTGGGCCAGACCCCTGTCGCCA  GGCCTCACGGCAGCTCCATAGTCAGGAGAT  GGGAAGATGCTGGGGACAGGCCCTGGGGA  GAAGTACTGGGATCACCTGTTCAAGGCTCCCA</p>	-

	<p>CTGTGACGCTGCCCCGGGGCGGGGGAAGGA  GGTGGGACATGTGGGCGTTGGGGCCTGTAG  GTCCACACCCAGTGTGGGTGACCCTCCCTCT  AACCTGGGTCCAGCCCGGCTGGAGATGGGT  GGGAGTGCGACCTAGGGCTGGCGGGCAGG  CGGGCACTGTGTCTCCCTGACTGTGTCCTCC  TGTGTCCCTCTGCCTCGCCGCTGTTCCGGAA  CCTGCTCTGCGCGGCACGTCCTGGCAGTGG  GGCAGGTGGAGCTGGGCGGGGGCCCTGGT  GCAGGCAGCCTGCAGCCCTTGGCCCTGGAG  GGGTCCCTGCAGAAGCGTGGCATTGTGGAA  CAATGCTGTACCAGCATCTGCTCCCTCTACC  AGCTGGAGAACTACTGCAAC</p>	
<i>INS</i> right homology arm	<p>TAGACGCAGCCCGCAGGCAGCCCCACACCC  GCCGCCTCCTGCACCGAGAGAGATGGAATAA  AGCCCTTGAACCAGCCCTGCTGTGCCGTCTG  TGTGTCTTGGGGGCCCTGGGCCAAGCCCCA  CTTCCCGGCACTGTTGTGAGCCCCTCCCAGC  TCTCTCCACGCTCTCTGGGTGCCACAGGTG  CCAACGCCGGCCAGGCCCAGCATGCAGTGG  CTCTCCCCAAGCGGCCATGCCTGTCGGCTG  CCTGCTGCCCCCACCCTGTGGCTCAGGGTC  CAGTATGGGAGCTGCGGGGGTCTCTGAGGG  GCCAGGGGTGGTGGGGCCACTGAGAAGTGA  CTTCTTGTTCACTAGCTCTGGACTCTTGGAGT  CCCCAGAGACCTTGTTCAAGAAAGGGAATGA  GAACATTCCAGCAATTTTCCCCCACCTAGC  CCTCCAGGTTCTATTTTTAGAGTTATTTCTG  ATGGAGTCCCTGTGGAGGGAGGAGGCTGGG  CTGAGGGAGGGGGTCTGCAGGGCGGGGG  GCTGGGAAGGTGGGGAGAGGCTGCCGAGAG  CCACCCGCTATCCCCAGCTCTGGGCAGCCC  CGGGACAGTCACACACCCTGGCCTCGCGGC  CCAAGCTGGCAGCCGTCTGCAGCCACAGCTT  ATGCCAGCCCAGGTCCAGCCAGACACCTGA  GGGACCCACTGGTGCCTTGGAGGAAGCAGG  AGAGGTCAGATGGCACCATGAGCTGGGGCA  GGTGCAGGGACCGTGGCAGCACCTGGCAGG  GCCTCAGAACCCATGCCTTGGGCACCCCGG  CCATGAGGCCCTGAGGATTGCAGCCCAGGA  GAAGCAGGGAACCGCCAGGGCCACAGGGGC  AGAGACCAGGGCCAGGGTCCCCCTGCAGCC  CCTTAGCCCACCCCTCCCAGTAAGCAGGGC  TGCTTGGCTGGCTTCCTTTGCTACAGACCTG  CTGCT</p>	-
<i>OCT4</i> left homology arm	<p>ACTAGTAACGGCCGCCAGTGTGCTGGAATTC  GCCCTTTTCTGCAGGTCCCACCTGCACAGA  TATGCAAAGCAGAAACCCTCGTGCAGGCCCG  AAAGAGAAAGCGAACCAGTATCGAGAACCGA  GTGAGAGGCAACCTGGAGAATTTGTTCTTGC  AGTGCCCGAAACCCACACTGCAGCAGATCAG  CCACATCGCCAGCAGCTTGGGCTCGAGAA</p>	<p>GCTCTCCC  ATGCATTC  AAAC/tgagg</p>



	GGATGTGAGTGCCATGTCTCTCTGCGGGCTC CATCTCTTTCCCCTGTCACCACCTCGCTTTCC CTAGCTCTGGCTCCTCCAAGTCTCTAGGGC TGTTGGCTTTGGACAGAATGTCCAAGCAGTC AGGCCTGTCTCAGCTCATTCTCTAATGTCTC CTCTAACTGCTCTAGGGCTGTTGGCTTTGGA TAGAATGTCCAAGCAGAGTCAGGCCCGTCTC AGCTCATTGTCTAATGTCATTCTCCTTTCTGT CATTCACTTGCAGGTGGTCCGAGTGTGGTTC TGTAACCGGCGCCAGAAGGGCAAGCGATCA AGCAGCGACTATGCACAACGAGAGGATTTTG AGGCTGCTGGGTCTCCTTTCTCAGGGGGACC AGTGTCTTTCTCTGGCCCCAGGGCCCCAT TTTGGTACCCAGGCTATGGGAGCCCTCACT TCACTGCACTGTA CTCTCGGTCCCTTTCCCT GAGGGGGAAGCCTTTCCCCCTGTCTCCGTCA CCTCTGGGCTCTCCCATGCATTCAAAC	
OCT4 right homology arm	GGTGCCTGCCCTTCTAGGAATGGGGGACAG GGGGAGGGGAGGAGCTAGGGAAAGAAAACC TGGAGTTTGTGCCAGGGTTTTTGGGATTAAG TTCTTCATTACTAAGGAAGGAATTGGGAACA CAAAGGGTGGGGGCAGGGGAGTTTGGGGCA ACTGGTTGGAGGGGAAGGTGAAGTTCAATGAT GCTCTTGATTTAATCCACATCATGTATCAC TTTTTTCTTAAATAAAGAAGCCTGGGACACAG TAGATAGACACACTTATCTTGGTTTGTCTTC AGTTACTGAGGTAGGGATGGGAATATCCAAT GCTCATACCCAAGTGACCCTGAAACTAAGGT GCCATTTACACTCCTTAAGGTCACACAACATC AGAGGGAGAGCTGGGATTGCAGCCAAGTTTA TTTGTACAGGGCCCTGTGATAGGCTAGTTCC CAAAGCCTGTGATGCAAGAACTTTTGCCCA TAGACTCAGTCACCATGTAGCTGTTACCTGTT CAGAGCTGGCTTTTTGCTTTCCACCCCTACTC TGGAATTCTTAAATGGCTTTATACTTAGAAAT CATCTTATTTCTGTTGAACCTAGATCACCCCA ACCAGAACTTCTATTAATACTTTGTGCTTTCT TGATACCAGGGTCTATTTGGTTTCCACTTAAG GTTTTTGCATACTCTGCCATAAGTGA CT CAT TAGTTACGGCCGCCAAAAGGGCGAATTCTG CAGATATCCATCACACTG	gctctccatgc attcaaactga GG

**Supplementary Table 7:** Homology arm sequences for donor integration. Guide sequences are mutated or interrupted by the donor cassette to prevent binding to homologous donors after integration. Mutated sequences and interrupted sequences are shown in lower case.

Gene target	Primer	Region of amplification	Size	Sequence
WNT5A	F	5'		GCAGAAGTATTGGGGCTTGA
WNT5A	R	5'		GCTTGGCTGGACGTAAACTC
WNT5A	F	3'		ATTGCATCGCATTGTCTGAG

<i>WNT5A</i>	R	3'		CAAAAGCAGAGGCAAACAAA
<i>NKX6.1</i>	F	Cut site		CACGCTTTAGCAGCCTGAG
<i>NKX6.1</i>	R	Cut site		CTAACTGGTGTGATTCCGGC
<i>INS</i>	R	3'		TTGCTACAGACCTGCTGCTC
<i>OCT4</i>	F	Cut site		GTA CTCTCGGTCCCTTTCC
<i>OCT4</i>	R	Cut site		CAAAAACCCTGGCACAACT
<i>eCFP</i>	F	Cloning		CTAGGGAATTAATTCACAGCCACC
<i>eCFP</i>	R	Cloning		CGCGTAGAGTCGCGGTGATCTAGA

**Supplementary Table 8:** List of primers used for NHEJ and integration assays. (eCFP: enhanced Cerulean fluorescent protein).

Gene	Forward primer	Reverse primer
<i>OCT3/4//POU5F1</i>	tgggctcgagaaggatgtg	gcatagtcgctgcttgatcg
<i>NANOG</i>	ttgggactggtggaagaatc	gatttgtggcctgaagaaa
<i>REX1/ZFP42</i>	aggatctcccacctttccaa	caggtagcacacctctctgc
<i>LIN8</i>	acccttccatgtgcagctta	tgtaagtggttcaacgtgcg
<i>SOX2</i>	agaaaaacgagggaaatggg	gtcatttgctgtgggtgatg
<i>TERT</i>	atcagccagtgccaggaactt	agctgacgtggaagatgagc
<i>FOXA2/HNF3B</i>	gctactcctccgtgagcaac	gggctcatggagttcatgtt
<i>SOX17</i>	ggcgcagcagaatccaga	ccacgactgcccagcat
<i>GATA4</i>	caggcgtgacagatagtg	cccgacaccccaatctc
<i>HNF4A</i>	catagcttgaccttcgagtg	cgtggtggacaagacaaga
<i>CXCR4</i>	caccgcatctggagaacca	gcccatttctcggttagtt
<i>BRA/TBX1</i>	gctgaagtgcacccctgc	agcgaggaggaaggggaac
<i>GATA2</i>	gccataagggtggtggtg	cacaagatgaatgggcagaa
<i>RUNX1</i>	caatggatcccaggattgg	ccactccactgccttaacc
<i>HAND1</i>	aatccttctcgcactgggc	cctcaaggctgaactcaaga
<i>BRCA1</i>	gaaggcccttctctggtt	agagtgcccattctgtctgga
<i>BRCA2</i>	acaaatagacgaaaggggca	gccccttcactcagcaaat
<i>TUBB3</i>	agtcgcccacgtagttgc	cgcccagtatgaggagat
<i>NESTIN</i>	tctttgctcccagtcctgag	gggctctgatctctgcatct
<i>PAX6</i>	tgtgtgctctgaaggctcagg	cctggagctctggttgaag
<i>RAD51</i>	ctggtggtctgtgtgaacg	ctgagggtacctttaggcca
<i>RAD52</i>	gctgtcacgtcctccaaga	cttcccctgtccatagcct
<i>XRCC4</i>	tggactgggacagtttctga	tcagttaccaacatattccc
<i>XRCC5</i>	tgaggaagcgagtaaccagctcataaat cacatc	atgcagtctatgctcttataaaata cg
<i>XRCC6</i>	agcactcagcaggttaaagctgaagctc aac	gattataaatgcccacagagatca ctat
<i>LIG4</i>	agcttgcccaggccaggttaaacga	aaaaggaacgtgagatgcaaca
<i>TBP</i>	gttctgaataggctgtgggg	acaacagcctgccaccttac
<i>ACTB</i>	ccttgacatgccggag	gcacagagcctcgctt
18s rRNA	tcggaactgaggccatgatt	ctttcgctctggtccgtctt

**Supplementary Table 9:** List of primers used for qRT-PCR shown in Figure 5 and Supplementary Figure 5.

Antigen	Supplier, species, catalog number	Dilution
OCT3/4	SCBT, mouse, sc-5279	1:100
SOX2	SCBT, goat, sc-17320	1:100

TRA1-81	Abcam, mouse, 16289	1:200
SSEA-4	DSHB, mouse, MC-813-70	1:50
SOX17	R&D Systems, goat, AF1924	1:500
FOXA2/HNF3B	Millipore, rabbit, 07-633	1:250
PDX1	R&D Systems, goat, AF2419	1:500
CTNT	Abcam, rabbit, ab45932	1:500
NKX2.5	DSHB, mouse, PCRP-NKX2-5-3B4	1:100

**Supplementary Table 10:** List of antibodies and dilutions used in Figure 5.

Gene name	#	Category	H1 UT	H1 DMSO	H1 Noco	H1 ABT	HUES8 UT	HUES8 DMSO	HUES8 Noco	HUES8 ABT	DE	CP	NP
<b>OCT4</b>	1	pluripotency	10.2	11.5	9.8	10.5	13.4	12	10.1	11			
	2	pluripotency	10.64	11.68	9.9	10.1	14.15	12.5	10.3	10.95			
	3	pluripotency	10.04	11.2	9.23	9.89	13.89	11.67	10.44	12.05			
<b>NANOG</b>	1	pluripotency	7.3	5.6	5.85	6.2	9.5	7.1	7.45	8.8			
	2	pluripotency	6.8	5	5.57	6.2	9.15	7.93	8.22	8.73			
	3	pluripotency	6.56	4.8	5.72	6.89	8.66	7.44	7.72	8.16			
<b>REX1</b>	1	pluripotency	4.1	4.56	3.99	4.53	4.6	4.4	4.73	4.21			
	2	pluripotency	4.22	4.67	4.13	4.38	4.17	4.84	5.01	4.44			
	3	pluripotency	4.78	4.8	4.42	5.01	4.06	4.25	4.66	4.92			
<b>GDF3</b>	1	pluripotency	7.28	7.8	7.94	7.1	6.54	6.32	7.04	6.22			
	2	pluripotency	6.9	8.12	7.28	6.92	6.93	7.93	8.11	8.73			
	3	pluripotency	6.67	7.74	7.92	6.19	7.05	7.44	7.72	8.16			
<b>LIN28</b>	1	pluripotency	9.3	9.6	8.85	10.2	9.5	9.1	8.45	8.8			
	2	pluripotency	9.8	9	8.97	11.1	9.15	8.93	8.11	8.73			
	3	pluripotency	8.56	8.4	7.52	8.89	8.66	8.77	9.72	8.16			
<b>FOXA2</b>	1	endoderm	1.23	1.5	1.2	1.74	1.45	1.67	1.74	1.85	159.1		
	2	endoderm	1.11	1.22	1.05	1.64	1.43	1.84	1.6	1.65	147		
	3	endoderm	1.37	1.56	1.15	1.8	1.69	1.62	1.63	1.48	161		
<b>GATA4</b>	1	endoderm	1.6	1.32	1.3	1.1	1.1	0.96	0.76	1.03	53.8		
	2	endoderm	1.41	1.25	1.45	1.02	0.98	0.88	0.64	1.14	58.1		
	3	endoderm	1.66	1.28	1.27	1.16	1.24	1.19	0.75	0.99	56.7		
<b>CXCR4</b>	1	endoderm	2.4	3	2.68	2.11	1.43	1.77	1.94	1.88	66.1		
<b>SOX17</b>	1	endoderm	1.8	2.5	2.89	1.5	2.4	2.71	2.89	2.16	127.8		
	2	endoderm	1.96	2.08	2.89	1.75	2.2	2.65	2.51	2.37	144.3		
	3	endoderm	2.08	2.89	2.41	1.88	2.11	2.43	2.18	2.02	125		
<b>BRA(T)</b>	1	mesoderm	1.96	2.28	2.14	2.35	1.67	1.99	2.12	2.11		43	
	2	mesoderm	1.85	2.49	2.15	2.22	1.59	2.11	2.36	2.18		48.11	
	3	mesoderm	2.15	2.51	1.99	2.21	1.42	1.94	2.56	2.74		51.95	
<b>TPNT</b>	1	mesoderm	1.45	1.85	1.77	2.19	2.05	1.78	1.81	1.99		85.64	
	2	mesoderm	1.84	2.00	2.14	1.68	1.93	2.39	2.3	1.73		81.5	
	3	mesoderm	1.42	1.67	1.94	1.99	1.72	2.19	2.3	2.16		81.44	
<b>NKX2.5</b>	1	mesoderm	0.78	1.29	1.11	1.21	0.93	1.25	0.72	1.33		93.6	
	2	mesoderm	1.15	1.37	1.25	1.34	1.17	1.03	0.69	1.19		99.7	
	3	mesoderm	1.02	1.56	1.73	0.69	0.97	0.85	1.13	1.41		98.1	
<b>PAX6</b>	1	ectoderm	2.11	2.50	2.74	2.91	2.60	3.15	2.74	3.01			171.9
	2	ectoderm	2.84	3.07	3.11	2.67	3.50	2.93	2.93	2.45			168.5
	3	ectoderm	2.88	3.67	3.21	3.00	3.24	2.86	2.61	2.88			170.2
<b>SOX1</b>	1	ectoderm	1.67	1.84	1.93	1.52	1.67	1.89	2.15	2.28			72.4
	2	ectoderm	2.51	1.11	1.78	1.46	2.02	2.06	1.83	2.51			72.1
	3	ectoderm	2.55	1.44	1.72	1.62	2.04	2.23	2.33	1.85			67.8

**Supplementary Table 11: Synchronized cells have similar gene expression patterns to unsynchronized cells.** qPCR values shown for three replicates.