Stem Cell Reports

Supplemental Information

Cloning-free CRISPR

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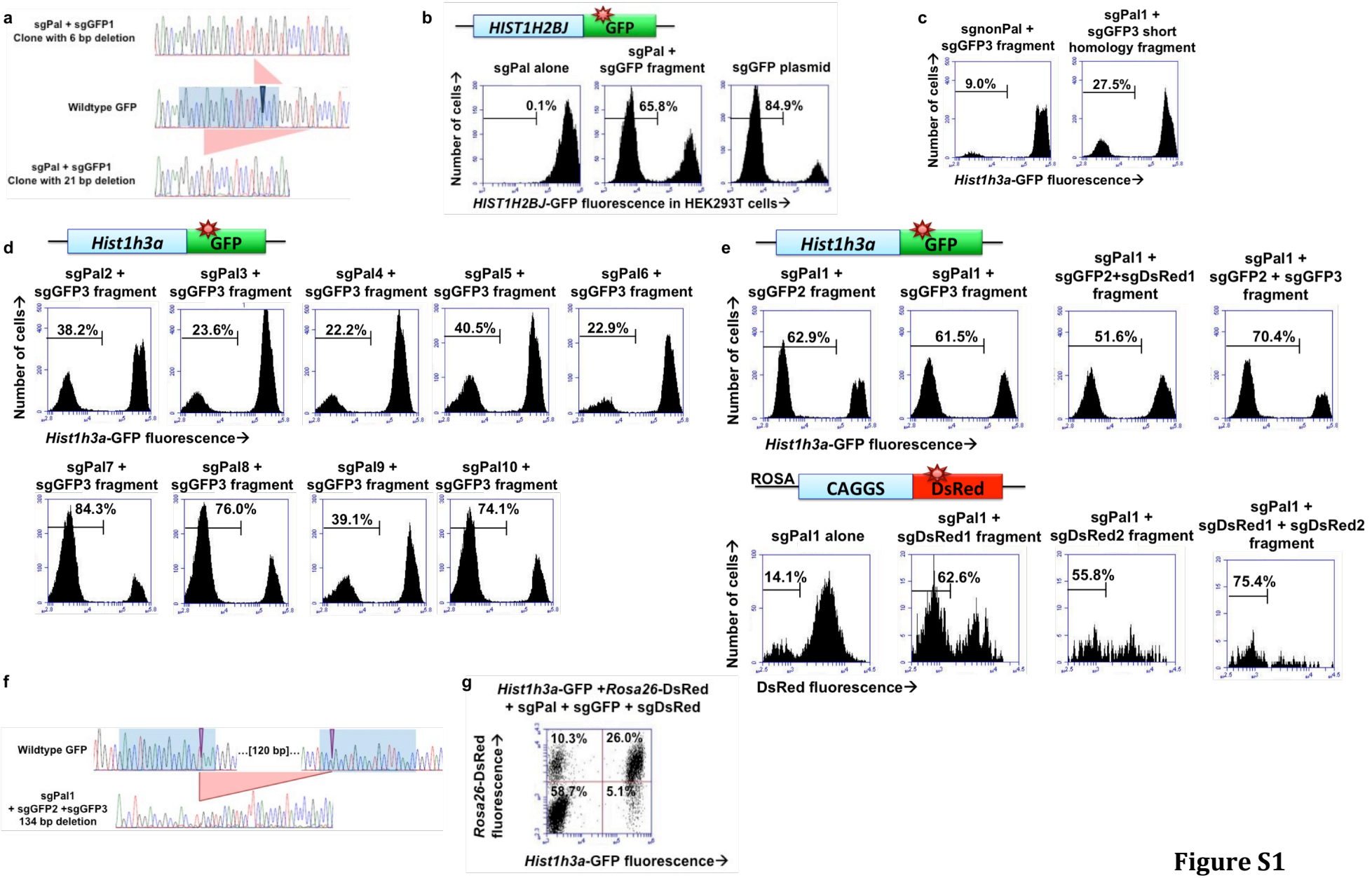

a
sgPal + sgGFP1
Clone with 6 bp deletion
Wildtype GFP
sgPal + sgGFP1
Clone with 21 bp deletion
b
HIST1H2BJ
GFP
sgPal alone
sgPal + sgGFP fragment
sgGFP plasmid
Number of cells→
0.1%
65.8%
84.9%
HIST1H2BJ-GFP fluorescence in HEK293T cells→
c
sgnonPal + sgGFP3 fragment
sgPal1 + sgGFP3 short homology fragment
Number of cells→
9.0%
27.5%
Hist1h3a-GFP fluorescence→
d
Hist1h3a
GFP
sgPal2 + sgGFP3 fragment
sgPal3 + sgGFP3 fragment
sgPal4 + sgGFP3 fragment
sgPal5 + sgGFP3 fragment
sgPal6 + sgGFP3 fragment
Number of cells→
38.2%
23.6%
22.2%
40.5%
22.9%
Hist1h3a-GFP fluorescence→
sgPal7 + sgGFP3 fragment
sgPal8 + sgGFP3 fragment
sgPal9 + sgGFP3 fragment
sgPal10 + sgGFP3 fragment
84.3%
76.0%
39.1%
74.1%
e
Hist1h3a
GFP
sgPal1 + sgGFP2 fragment
sgPal1 + sgGFP3 fragment
sgPal1 + sgGFP2+sgDsRed1 fragment
sgPal1 + sgGFP2 + sgGFP3 fragment
Number of cells→
62.9%
61.5%
51.6%
70.4%
Hist1h3a-GFP fluorescence→
ROSA
CAGGS
DsRed
sgPal1 alone
sgPal1 + sgDsRed1 fragment
sgPal1 + sgDsRed2 fragment
sgPal1 + sgDsRed1 + sgDsRed2 fragment
Number of cells→
14.1%
62.6%
55.8%
75.4%
DsRed fluorescence→
f
Wildtype GFP
…[120 bp]…
sgPal1
+ sgGFP2 +sgGFP3
134 bp deletion
g
Hist1h3a-GFP +Rosa26-DsRed
+ sgPal + sgGFP + sgDsRed
Rosa26-DsRed fluorescence→
10.3%
26.0%
58.7%
5.1%
Hist1h3a-GFP fluorescence→

Figure S1

Figure S1

a. Sanger sequencing analysis of genomic DNA from two *Hist1h3a*-GFP^{-} clones produced through scCRISPR with sgPal and sgGFP1, showing short deletions surrounding the expected CRISPR cut site. b. Histograms showing flow cytometric *HIST1H2BJ*-GFP fluorescence (x-axis) after electroporation with Cas9 and sgPal alone (left panel), sgPal and sgGFP homology fragment (middle panel), or sgGFP plasmid (right panel). c. Histograms showing flow cytometric *Hist1h3a*-GFP fluorescence (x-axis) after electroporation with Cas9, sgRNA plasmid, and homology fragment. Co-electroporation of a non-palindromic sgRNA plasmid (sgnonPal) exhibits substantially less GFP loss than sgPal1 (Figure 1), indicating that self-cleavage is an important factor in scCRISPR efficiency. A homology fragment with 30 bp of homology shows substantially less GFP loss than the standard homology arms (Figure 1), indicating that homology arm length is important in scCRISPR efficiency. Taken together, these results argue that scCRISPR functions through homologous recombination. d Histograms showing flow cytometric *Hist1h3a*-GFP fluorescence (x-axis) after electroporation with Cas9, palindromic sgRNA plasmids, and homology fragment. Palindromic sgRNA plasmids (sgPal2-10) all exhibit substantial GFP loss, but differ widely in their relative efficiency. sgPal7 induces the highest degree of GFP loss among the ten palindromic sgRNA's. e. Histograms showing flow cytometric *Hist1h3a*-GFP fluorescence (x-axis, a) or DsRed fluorescence (x-axis, b) after electroporation with Cas9, sgRNA plasmid, and homology fragment. In these plots, sgPal is combined with homology fragments targeting two additional sites within the GFP gene (sgGFP2 and sgGFP3) as well as two locations within the dsRed gene (sgDsRed1 and sgDsRed2), producing >55% loss of fluorescence in all cases. Additionally, multiplexing sgGFP2 and sgGFP3 in *Hist1h3a*-GFP cells, or sgDsRed1 and sgDsRed2 in dsRed positive cells increases the fraction of cells with loss of fluorescence. Multiplexing sgGFP2 and sgDsRed1 in single positive *Hist1h3a*-GFP mouse ESCs cells only minimally decreases the fraction of cells with loss of GFP fluorescence, indicating that scCRISPR can lead to efficient and specific multiplexed mutation.f. Sanger sequencing analysis of a gel-isolated deletion band from bulk genomic DNA of *Hist1h3a*-GFP cells after multiplexed scCRISPR with sgPal1, sgGFP2, and sgGFP3. This band shows a 134 bp deletion with junctions at the predicted CRISPR cut sites.g. Multiplexed mutation of GFP (x-axis) and dsRed (y-axis) in *Hist1h3a*-GFP Rosa26-dsRed mouse ESCs (left panel) after co-introduction of Cas9, sgPal plasmid, and sgGFP2 and sgDsRed1 standard length homology fragments.

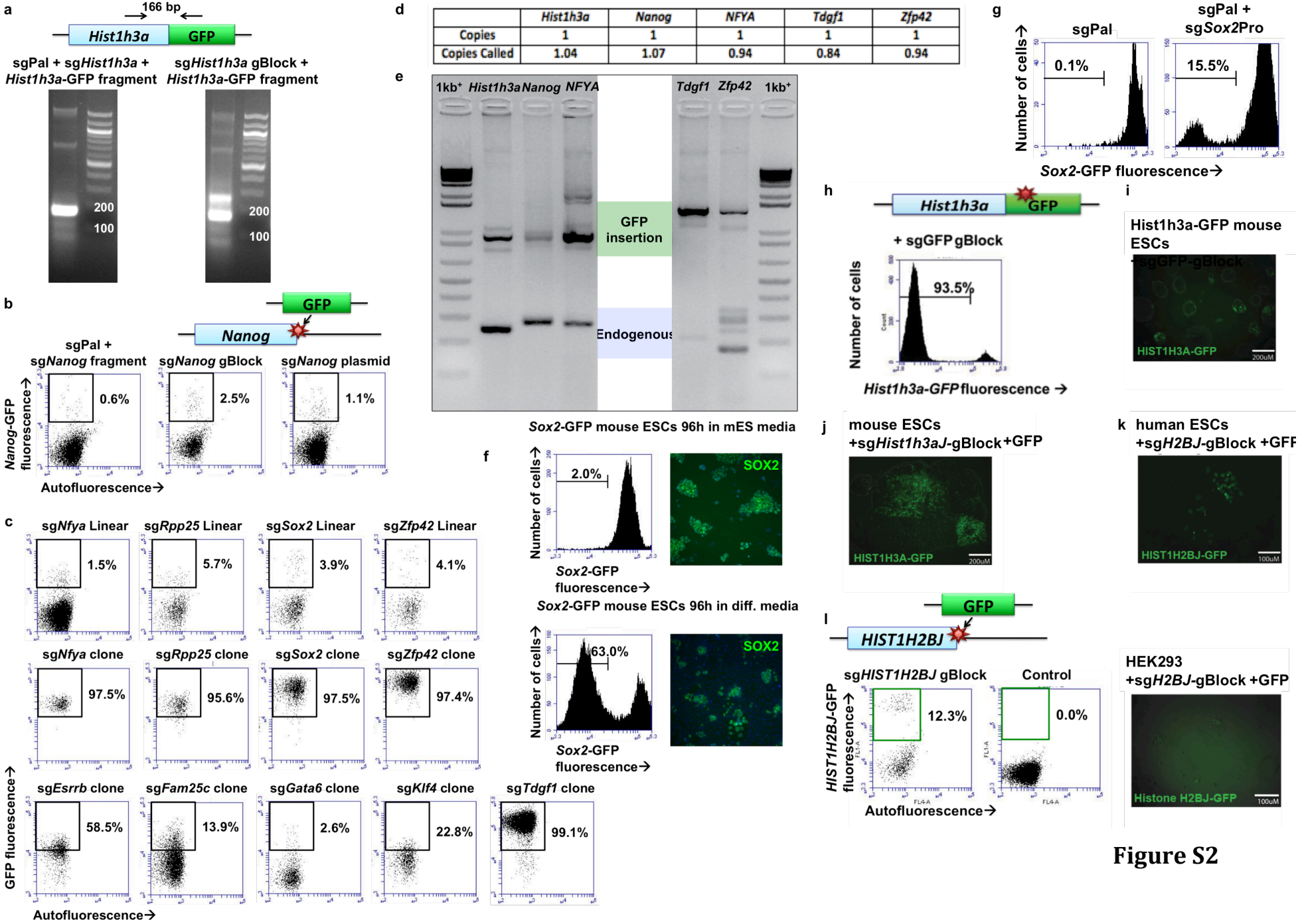
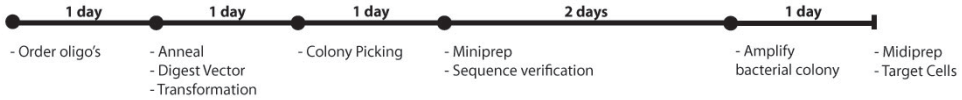

a
166 bp
Hist1h3a
GFP
sgPal + sgHist1h3a + Hist1h3a-GFP fragment
sgHist1h3a gBlock + Hist1h3a-GFP fragment
200
100
200
100
b
GFP
Nanog
sgPal + sgNanog fragment
sgNanog gBlock
sgNanog plasmid
0.6%
2.5%
1.1%
Nanog-GFP fluorescence→
Autofluorescence→
c
sgNfya Linear
sgRpp25 Linear
sgSox2 Linear
sgZfp42 Linear
1.5%
5.7%
3.9%
4.1%
sgNfya clone
sgRpp25 clone
sgSox2 clone
sgZfp42 clone
97.5%
95.6%
97.5%
97.4%
sgEsrrb clone
sgFam25c clone
sgGata6 clone
sgKlf4 clone
sgTdgf1 clone
58.5%
13.9%
2.6%
22.8%
99.1%
GFP fluorescence→
Autofluorescence→
d
Hist1h3a
Nanog
NFYA
Tdgf1
Zfp42
Copies
1
1
1
1
1
Copies Called
1.04
1.07
0.94
0.84
0.94
e
1kb+
Hist1h3a
Nanog
NFYA
Tdgf1
Zfp42
1kb+
GFP insertion
Endogenous
Sox2-GFP mouse ESCs 96h in mES media
f
Number of cells→
2.0%
Sox2-GFP fluorescence→
SOX2
Sox2-GFP mouse ESCs 96h in diff. media
Number of cells→
63.0%
Sox2-GFP fluorescence→
SOX2
g
sgPal
sgSox2Pro
Number of cells→
0.1%
15.5%
Sox2-GFP fluorescence→
h
Hist1h3a
GFP
+ sgGFP gBlock
Number of cells
93.5%
Hist1h3a-GFP fluorescence →
i
Hist1h3a-GFP mouse ESCs
+sgGFP-gBlock
HIST1H3A-GFP
200uM
j
mouse ESCs
+sgHist1h3aJ-gBlock+GFP
HIST1H3A-GFP
200uM
k
human ESCs
+sgH2BJ-gBlock +GFP
HIST1H2BJ-GFP
100uM
l
GFP
HIST1H2BJ
sgHIST1H2BJ gBlock
Control
HIST1H2BJ-GFP fluorescence→
12.3%
0.0%
Autofluorescence→
HEK293
+sgH2BJ-gBlock +GFP
Histone H2BJ-GFP
100uM

Figure S2

Figure S2

a. Flow cytometric analysis shows efficient generation of *Nanog*-GFP knock-in mouse ESCs (y-axis) after scCRISPR, gBlock-CRISPR, and plasmid-based CRISPR using a PCR-amplified GFP fragment with 80 bp *Nanog* homology arms. b. Genomic DNA PCR analysis using a forward primer in the *Hist1h3a* coding region and a reverse primer in the GFP coding region that will produce a 166 band only if GFP is inserted into the *Hist1h3a* locus. scCRISPR and gBlock-CRISPR-based knock-in using PCR-amplified GFP fragments with 80 bp *Hist1h3a* homology arms show robust bands indicating successful knock-in. c. Flow cytometric analysis shows efficient generation of GFP knock-in at four loci in mouse ESC (y-axis) using a PCR-amplified GFP fragment with 80 bp homology arms. Top row *Nfya*, *Rpp25*, and *Sox2* lines are C-terminal GFP fusion proteins and *Zfp42* is a GFP replacement. Middle and bottom row show flow cytometric analysis of nine clonal mouse ESC knock-in lines, all generated using PCR-amplified 80 bp homology arms. All are C-terminal GFP fusion cell lines except *Tdgf1* and *Zfp42*, which are GFP replacements. All lines have clonal knock-in in every cell, but GFP fluorescence intensities vary based on the native gene expression levels. Bulk measurements of GFP fluorescence were only performed for the four loci in the top row. d. Copies of GFP called by quantitative-PCR using Taqman Copy Number Assay. GFP integrations were assed by comparing CT amplification values of GFP and *Tfrc* as a reference gene, using eGFP FAM-labeled probes and VIC-labeled TAMRA probes, respectively. Copies per cell were calculated using CopyCaller software.e. PCR amplification spanning the appropriate GFP integration sites for *Hist1h3a*, *Nanog*, *Nfya*, *Tdgf1*, and *Zfp42* heterozygous GFP cell lines. Upper bands in *Hist1h3a*, *Nanog*, and *Nfya* cell lines indicate insertion of ±750bp GFP sequence. The integrated sequence in *Tdgf1* and *Zfp42* cell lines encodes GFP with an extended poly-A tail, and is ±1150bp long. The lower bands show amplification of the endogenous loci.f. Fluorescence imaging of *Sox2*-GFP cells and histograms showing flow cytometry of *Sox2*-GFP fluorescence (x-axis) prior to, and 96 hours after mouse ESC incubation with serum-reduced media. Stem cell differentiation leads to substantial loss of SOX2 expression and a loss of GFP fluorescence in 63% of cells is observed accordingly, indicating GFP reports faithfully on *Sox2* gene expression. g. Histograms showing flow cytometric *Sox2*-GFP fluorescence (x-axis) after electroporation with Cas9 with sgPal1 alone, or in combination with an sgRNA fragment targeted to the *Sox2* promoter region (sg*Sox2*Pro). sgPal1 alone does not affect *Sox2*-GFP expression while combined sgPal1 with sg*Sox2*Pro fragment induces a loss of measurable GFP fluorescence, indicating GFP signal is regulated by *Sox2* regulatory elements. h-i. Flow cytometric and fluorescence microscopy analysis show efficient loss of *Hist1h3a*-GFP after co-electroporation of Cas9 with GFP-targeting gBlock sgRNA. j-k. Fluorescence microscopy of nuclear GFP expression in mouse and human ESCs after targeted GFP insertion into *Hist1h3a* or *HIST1H2BJ* loci, respectively. Cells were targeted by co-electroporation of Cas9, sg*Hist1h3a* or sg*HIST1H2BJ* PCR amplified gBlocks, along with PCR-amplified GFP fragments with 80 bp overhangs homologous to the respective regions.l. Flow cytometric analysis of HEK293T shows efficient generation of *HIST1H2BJ*-GFP knock-in cells (y-axis) after, gBlock-CRISPR using a PCR-amplified GFP fragment with 80 bp *HIST1H2BJ* homology arms.

Supplemental Table 1: Comparison of time, cost, and efficiency of CRISPR/Cas9 mutation and gene knock-in using different methods of sgRNA introduction

	Cost in USD	Mutation Rate	Gene Knock-In
sgPal 	± 15	≥ 85%	± 0.5%
gBlock sgRNA 	± 90	≥ 90%	± 3%
conventional plasmid sgRNA 	± 85 - 100	≥ 90%	± 1%

Experimental Procedures Supplemental Table:
Predicted off-target effects of scCRISPR palindromic sgRNAs

Name	Sequence	BLAST hits	Missmatches		3' nt
			Overall	Last 11nt	
sgPal1	**GCTCTGTGACT AGTCACAGAG**	Human cr.2	4	2	…GGG
		Human cr.8	4	1	…AGG
		Human cr.10	4	2	…GGG
		Human cr.20	4	2	…AGG
		Human cr.22	4	2	…GGG
		Mouse cr.11	4	2	…GGG
		Mouse cr.9	4	2	…AGG
sgPal2	**GCGGAACACA TGTGTTCCG**	Human cr.22	4	2	…GGG
		Mouse cr.5	4	2	…GGG
		Mouse cr.17	4	1	…CGG
sgPal3	**TCGATCGTCG CGACGATCGA**	none	-	-	-
sgPal4	**CGACGATCGA TCGATCGTCG**	none	-	-	-
sgPal5	**GCAGTACTTTG CAAAGTACTG**	Human cr.1	4	2	…GGG
		Human chr.2	4	2	…TGG
		Mouse chr.2	3	2	…TGG
		Mouse chr.6	4	1	…TGG
		Mouse chr.15	4	2	…GGG
		Mouse chr.19	4	1	…GGG
sgPal6	**GTCCCATCCTT AAGGATGGGA**	Human cr.6	4	2	…TGG
		Human chr.9	4	2	…TGG
		Mouse chr.2	4	2	…CGG
		Mouse chr.5	4	2	…AGG
		Mouse chr.11	4	2	…GGG
		Mouse chr.11	4	2	…TGG
		Mouse chr.12	4	2	…TGG
		Mouse chr.16	4	2	…AGG
sgPal7	**GGCTTAGTACT AGTACTAAGC**	Human cr.16	4	1	…AGG
		Mouse chr.1	4	1	…AGG
sgPal8	**GGCCTTTCGAC GTCGAAAGGC**	Human chr.2	4	2	…CGG
		Human chr.2	4	2	…GGG
		Human chr.4	4	1	…AGG
		Mouse chr.1	4	2	…AGG
sgPal9	**GCGACCTGCAT ATGCAGGTCG**	Human chr.1	4	1	…GGG
		Human chr.17	4	2	…AGG
		Human chr.18	4	2	…GGG
		Mouse chr.11	4	2	…TGG
sgPal10	**GAATCTGCCAG CTGGCAGATT**	Human chr.1	4	2	…GGG
		Human chr.9	4	2	…AGG
		Human chr.11	4	2	…GGG
		Human chr.15	4	2	…AGG
		Human chr.22	4	1	…AGG
		Human chr.22	4	2	…TGG
		Mouse chr.2	4	2	…GGG
		Mouse chr.12	4	2	…GGG
		Mouse chr.13	4	1	…TGG
		Mouse chr.17	4	2	…TGG

Experimental Procedures Supplemental Tables: Oligonucleotides used in this work

Palindromic sgRNA sequences		
sgPal1	GCTCTGTGACTAGTCACAGAG	Among most efficient, used for the majority of experiments
sgPal2	GCGGAACACATGTGTTCCG	
sgPal3	GTCGATCGTCGCGACGATCGA	
sgPal4	GCGACGATCGATCGATCGTCG	
sgPal5	GCAGTACTTTGCAAAGTACTG	
sgPal6	GTCCCATCCTTAAGGATGGGA	
sgPal7	GGCTTAGTACTAGTACTAAGC	Most efficient
sgPal8	GGCCTTTCGACGTCGAAAGGC	Among most efficient
sgPal9	GCGACCTGCATATGCAGGTCG	
sgPal10	GAATCTGCCAGCTGGCAGATT	Among most efficient

sgRNA sequences for plasmid control tests		
sgGFP3	GCTGAAGCACTGCACGCCGT	
sg*Hist1h3a*	GTTAATTCCGTAGAACTGTA	
sg*Nanog*	GTATGAGACTTACGCAACATC	

scCRISPR primer sequences		
scCRISPR_homology_fw	TGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC	Use in first PCR of all scCRISPR oligos
scCRISPR_homology_rv	GTTGATAACGGACTAGCCTTATTTAAACTTGCTATGCTGTTTCCAGCATAGCTCTTAAAC	Use in first PCR of all scCRISPR oligos
scCRISPR_homology_extension_fw	GTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACC	Use in second PCR of all scCRISPR oligos
scCRISPR_homology_extension_rv	ATTTTAACTTGCTATTTCTAGCTCTAAAACAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTAAAC	Use in second PCR of all scCRISPR oligos
scCRISPR_homology_doubleextension_fw	CGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAA	Use to produce long scCRISPR homology arms
scCRISPR_homology_doubleextension_rv	TCAATGTATCTTATCATGTCTGCTCGATTTTAACTTGCTATTTCTAGCTCTAAAACAAAA	Use to produce long scCRISPR homology arms

scCRISPR sgRNA sequences		
All scCRISPR oligonucleotides were ordered using format described in the methods		
sgGFP1	GGGCGAGGAGCTGTTCACCG	
sgGFP2	GAGCTGGACGGCGACGTAAA	
sgGFP3	GCTGAAGCACTGCACGCCGT	
sg*Hist1h3a*	GTTAATTCCGTAGAACTGTA	
sg*Nanog*	GTATGAGACTTACGCAACATC	
sgDsRed1	GAACTCCTTGATGACGTCCT	
sgDsRed2	GCCAAGCTGAAGGTGACCAA	
sg*HIST1H2BJ*	GCGCTAAGTAAACAGTGAGT	
sg*Esrrb*	GTGATGGCCCAGCACATGGA	
sg*Fam25c*	GGCCAGCCATGCTGGTAGGC	
sg*Gata6*	GCCTGAGCTGGTGCTACCAAG	
sg*Klf4*	GCACTTTTAAATCCCACGTAG	
sg*Nfya*	GTTTCCTAACCACAGGAGGG	
sg*Rpp25*	GCTCAGAGGCGAGAATTCTC	
sg*Sox2*	GTGAGGGCTGGACTGCGAAC	
sg*Tdgf1*	GAGATGGGGTACTTCTCATCC	
sg*Zfp42*	GAATGAACAAATGAAGAAAA	
sg*Xrcc6*	GGGCGAGGAAGAGGAAGAGG	
sg*Xrcc5*	GCATCACCCTTGTCACCATG	
sg*Xrcc4*	GATGACATGGCTATGGAGAA	
sg*Prkdc*	GAGAGTAATGCATAACCTTC	
sg*Dclre1c*	GCAGATATCTTTAGTTGATG	
sg*Lig4*	GAAAGAGAGAGGATGGCTTA	
sg*Parp1*	GTGGCCCACCTTCCAGAAGC	
sg*Xrcc1*	GTCCGCCTTGAGAAGATTTT	
sg*Nhej1*	GCCAGGCTCACACCCATCAG	
sg*Pnkp*	GACACTGTCCTCTACCGAGA	
sg*Rif1*	GAGACAAACCTTTATTACTT	
sg*Tdp1*	GTCAGCATCCTCTCACCCCC	
sg*Trp53bp1*	GAATCTTCTATTATCAGGCA	

gBlock-CRISPR		
gBlock-CRISPR_fw	TGAGTATTACGGCATGTGAGGGC	Use in PCR of all gBlock-CRISPR gBlocks
gBlock-CRISPR _rv	TCAATGTATCTTATCATGTCTGCTCGA	Use in PCR of all gBlock-CRISPR gBlocks
Homologous recombination primers		
*Hist1h3a*HDR_GFP_fw	GGACATCCAACTGGCCCGCCGCATCCGCGGGGAGAGGGCG GTGAGCAAGGGCGAGGAGCT	
*Hist1h3a*HDR_GFP_rv	AAATCGTGTGTGGCTCTGAAAAGAGCCTTTGGTTAATTCC TGAGGAGTGAATTGCGGCCG	
*Hist1h3a*HDR_Extension_fw	TGTGCGCCATCCACGCCAAGCGTGTCACCATCATGCCCAAGGACA TCCAACTGGCCCGCC	
*Hist1h3a*HDR_Extension_rv	TTCGTTTAAGGATGGAGTAAATTACAGCCATTTTACTTGAAATC GTGTGTGGCTCTGAAA	
*Nanog*HDR_GFP_fw	ATTATTCCTGAACTACTCTGTGACTCCACCAGGTGAAATA GTGAGCAAGGGCGAGGAGCT	
*Nanog*HDR_GFP_rv	GAAGGAACCTGGCTTTGCCCTGACTTTAAGCCCAGATGTT TGAGGAGTGAATTGCGGCCG	
*Nanog*HDR_Extension_fw	CCATGCGCATTTTAGCACCCCACAAGCCTTGGAATTATTCCTGAA CTACTCTGTGACTCC	
*Nanog*HDR_Extension_rv	aataaatctttaaaaaaaaTATGAAAATATTTGGAAGAAGGAAGGAACC TGGCTTTGCCC	
*HIST1H2BJ*HDR_GFP_fw	CGAGGGTACTAAGGCCGTCACCAAGTACACCAGCGCTAAG GTGAGCAAGGGCGAGGAGCT	
*HIST1H2BJ*HDR_GFP_rv	GGTGGCTCTTAAAAGAGCCGTTAGGGTTGAGAGTTTGCAA TGAGGAGTGAATTGCGGCCG	
*HIST1H2BJ*HDR_Extension_fw	CCTGCTGCTGCCTGGGGAGTTGGCCAAGCACGCCGTGTCCGAGGG TACTAAGGCCGTCAC	
*HIST1H2BJ*HDR_Extension_rv	AGGAGGAATACAAGCACCAGCTCTTTCTTTGAGAACATGGGTGG CTCTTAAAAGAGCCGT	
*Esrrb*_GFP_fw	CATGCACAAACTCTTCCTGGAGATGCTGGAGGCCAAGGTGGTGA GCAAGGGCGAGGAGCT	
*Esrrb*_GFP_rv	CGAGGCTGGTGGCTGTGGAGGTCTCCACTTGGATCGTGTC TGAGGAGTGAATTGCGGCCG	
*Esrrb*_Extension_fw	ACACTTCTACAGTGTGAAACTGCAGGGCAAGGTGCCCATGCACA AACTCTTCCTGGAGAT	
*Esrrb*_Extension_rv	CTGGGACAGCTCAGAGCCCCGATGCGGGTGTGAAAAAAGTCGAG GCTGGTGGCTGTGGAG	
*Fam25c*_GFP_fw	TGTTACCCATGCGGCAGAAGGCCTGGGAAGACTGGGACAG GTGAGCAAGGGCGAGGAGCT	
*Fam25c*_GFP_rv	TCACGTTTCACACTCTTTATTGACCTTCAGGAAGGGCCAG TGAGGAGTGAATTGCGGCCG	
*Fam25c*_Extension_fw	AGGAGGTCACTGAGAAGGTCACCCACACCATCACTGATGCTGTT ACCCATGCGGCAGAAG	
*Fam25c*_Extension_rv	ATTCCATCCAAACAGAGGTAAACTCAGGACTCTGTTCACGTTTC ACACTCTTTATTGACC	
*Gata6*_GFP_fw	CTCCGTGCGACAGGATTCTTGGTGTGCTCTGGCCCTGGCC GTGAGCAAGGGCGAGGAGCT	
*Gata6*_GFP_rv	AATATCAGACACAAGTGGTATGAGGCCTTCAGAGCCCTCC TGAGGAGTGAATTGCGGCCG	

*Gata6*_Extension_fw	CATAGGTGTCAGTCTGTCCTCCCCTGCCGAAGTCACATCCTCCGT GCGACAGGATTCTTG	
*Gata6*_Extension_rv	GTCTGCATTTTTGCTGCCATCTGGACTGCTGGACAATATCAGAC ACAAGTGGTATGAGGC	
*Klf4*_GFP_fw	CAGGTCGGACCACCTTGCCTTACACATGAAGAGGCACTTTGTGA GCAAGGGCGAGGAGCT	
*Klf4*_GFP_rv	AAAAAAAATACTGAACTCTCTCTCCTGGCAGTGTGGGTCA TGAGGAGTGAATTGCGGCCG	
*Klf4*_Extension_fw	ACCGGCCCTTTCAGTGCCAGAAGTGTGACAGGGCCTTTTCCAGGT CGGACCACCTTGCCT	
*Klf4*_Extension_rv	TCCCCTCGTGGGAAGACAGTGTGAAAGGTTAGAAAAAAAAAATAC TGAACTCTCTCTCCTG	
*Nfya*_GFP_fw	AGCTGACGAAGAAGCCATGACACAGATCATCCGAGTTTCCGTGA GCAAGGGCGAGGAGCT	
*Nfya*_GFP_rv	CCATTTCCAGAACAGTGGAGAGGACCGTGACTGATCAGCT TGAGGAGTGAATTGCGGCCG	
*Nfya*_Extension_fw	AGGACTGTTGTGCTGTCTCTCTCTGTAGGATCCAAACCAAGCTG ACGAAGAAGCCATGAC	
*Nfya*_Extension_rv	AGTGAGACTGTCAGTGCCCCACTGGAAGTCAGTCCATTTCCAGA ACAGTGGAGAGGACCG	
*Rpp25*_GFP_fw	TCAGCCTGAGCCAGAGGCTGAGAATGAGGACAGGACCGCC GTGAGCAAGGGCGAGGAGCT	
*Rpp25*_GFP_rv	GTGTTGAAGATATATGATTCAGTCGGTCTGGGTGGCTCAG TGAGGAGTGAATTGCGGCCG	
*Rpp25*_Extension_fw	TGGGGGAATCTGCTGCTGAAGAAGGCACCGCTAAGCGGTCTCAG CCTGAGCCAGAGGCTG	
*Rpp25*_Extension_rv	TATGAAAGGTGCGTGTGTTGAAAGGTATGCAGGAGTGTTGAAGA TATATGATTCAGTCGG	
*Sox2*_GFP_fw	CGGCACGGCCATTAACGGCACACTGCCCCTGTCGCACATG GTGAGCAAGGGCGAGGAGCT	
*Sox2*_GFP_rv	CCTCCCAATTCCCTTGTATCTCTTTGAAAATCTCTCCCCT TGAGGAGTGAATTGCGGCCG	
*Sox2*_Extension_fw	GACTGCACATGGCCCAGCACTACCAGAGCGGCCCGGTGCCCGGCA CGGCCATTAACGGCA	
*Sox2*_Extension_rv	ATTATCAGATTTTTCCTACTCTCCTCTTTTTGCACCCCTCCCAAT TCCCTTGTATCTCTT	
*Tdgf1*_GFP_fw	TTGTCTTTTCCTCCAACGTTTTTACGAGCCGTCGAAGATG GCTAGCAAAGGAGAAGAACT	
*Tdgf1*_GFP_rv	AAGTGGCTATCTCCAGCAACCAAAAAGTCAAGGTTA TCGCGATTTTACCACATTTGTAGA	
*Tdgf1*_Extension_fw	TGGCTTTATGAACTAAAGCCATCTGCTAATATTGTGTTTCTTGT CTTTTCCTCCAACGTT	
*Tdgf1*_Extension_rv	GCAAGACAAAAATCAGAGCGTCATAGAACGTGATTTTCCGAAGT GGCTATCTCCAGCAAC	
*Zfp42*_GFP_fw	AGGAAGCAGCTAAGACAACATGAATGAACAAAAAATGAAT GTGAGCAAGGGCGAGGAGCT	
*Zfp42*_GFP_rv	GGGCTCTTCCGCCCGGCCCTTTCTGGCCACTTGTCT TCGCGATTTTACCACATTTGTAGA	
*Zfp42*_Extension_fw	GATCAGTGCCCCCTGGAAGTGAGTCATAGGCATTGTTCAAGAAG GAAGCAGCTAAGACAA	
*Zfp42*_Extension_rv	ACTGGCCTTGCCTCGTCTTGCTTTAGGGTCAGTCTGTCGAGGGCT CTTCCGCCCGGCCCT	

Primers for sequencing		
Hist1h3a-GFP_fw	CCTTGTGGGTCTGTTTGAGGA	
GFP_rv	GTCTTTGCTCAGGGCGGACT	