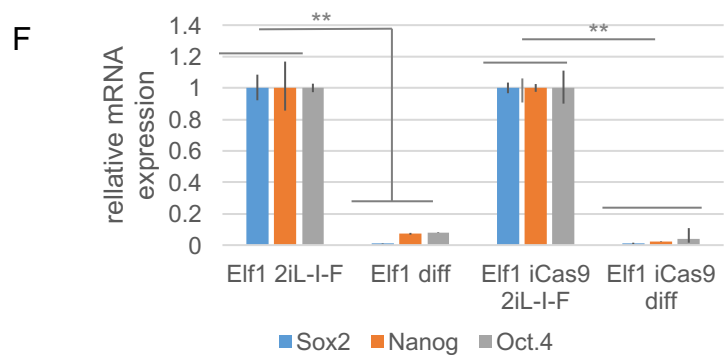
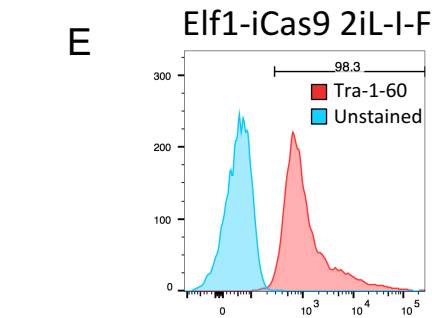
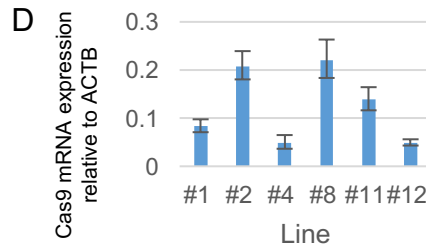
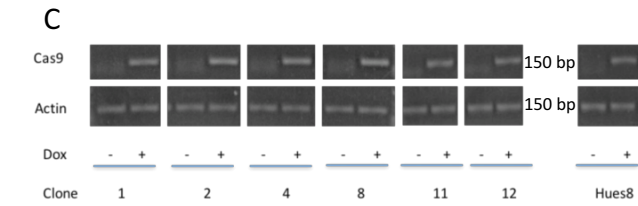
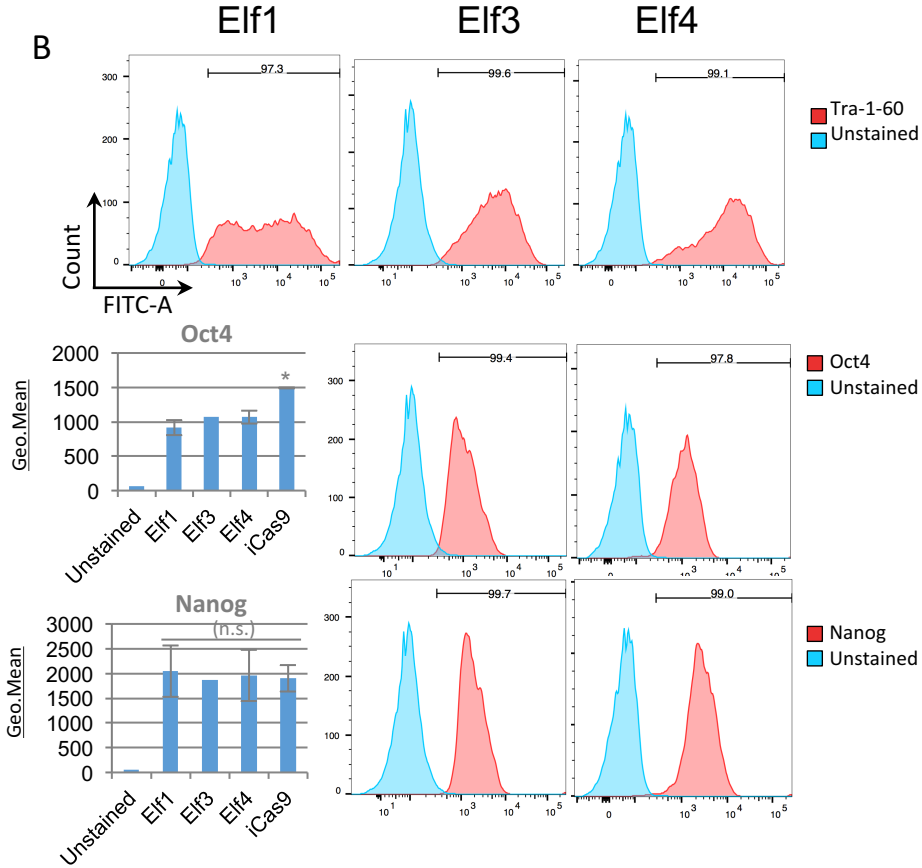
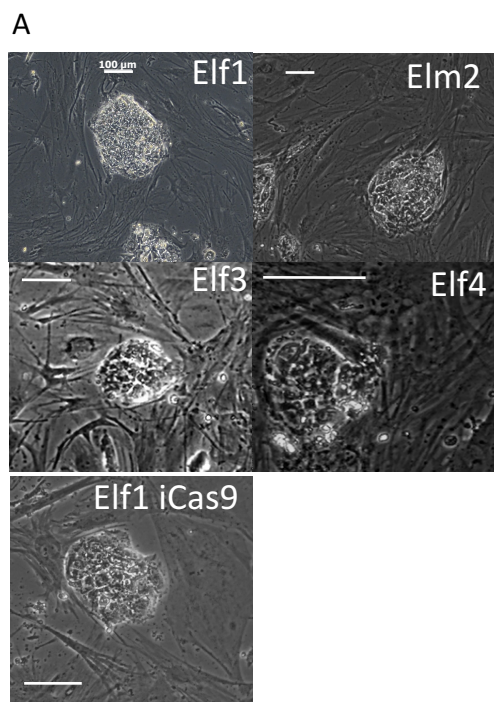


Supplemental Figure 1



Supplemental Figure 1: (A) Elf1, Elm2, Elf3, Elf4 and Elf1 iCas9 cell lines exhibit typical naïve compact colony morphology. (B) Elf1, Elf3, and Elf4 cell lines express pluripotent stem cell markers, analyzed by flow cytometry (Oct4, Nanog, TRA-1-60). The blue peak indicates unstained cells and red peaks stained cells. Graphs show comparison of GeoMean values for OCT4 and NANOG among naïve hESC lines Elf1, Elf1-iCas9, Elf3 and Elf4. The flow-cytometry histograms for OCT4 and NANOG are presented in Fig.1H. (C) RT-PCR was carried out to detect Cas9 expression following electrophoresis on an agarose gel. Elf1-iCas9 lines do not express Cas9 without doxycycline induction. (D) Level of expression of Cas9 after 3 days of Doxycycline induction varies among clones, as analyzed by RT-qPCR. (E) Elf1-iCas9 stain positive for TRA-1-60 as shown by flow cytometry. (F) Elf1 and Elf1-iCas9 cell lines lose pluripotency markers after differentiation in 20% Serum with 0.1mM RA for 5 days analyzed by RT-qPCR.

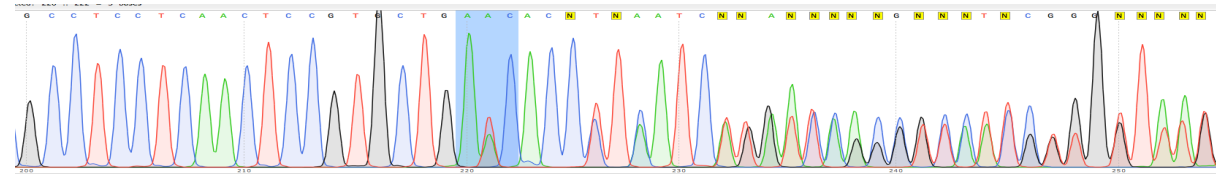
Supplemental Figure 2

PSEN2 Sequence:

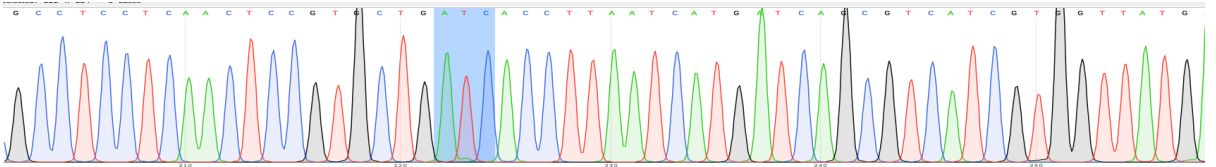
WT GCCTCCTCAACTCCGTGCTGAACACCCTCATCATGATCAGCGTCATCGTGGTTATG
Mutant GCCTCCTCAACTCCGTGCTGATCACCCTTAATCATGATCAGCGTCATCGTGGTTATG

Index:
N141: AAC
N141I: ATC
MseI: (TTAA)

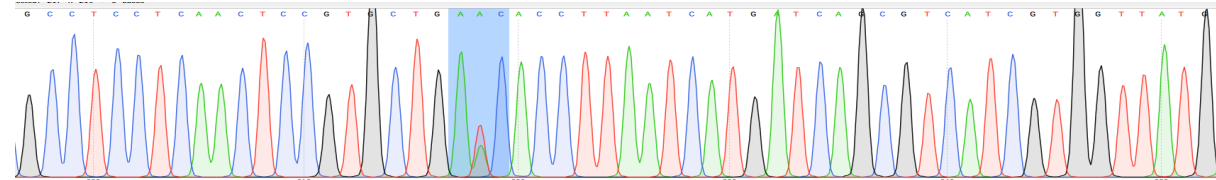
Clone 1:
indels



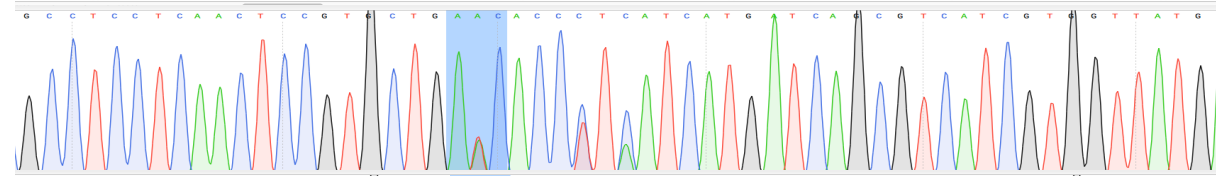
Clone 2:
N141I(++)
Mse(++)



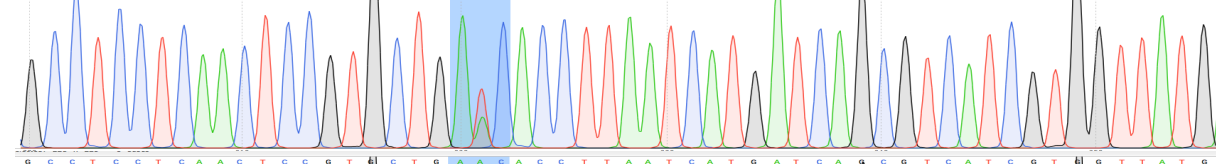
Clone 3:
N141I(+)
Mse(++)



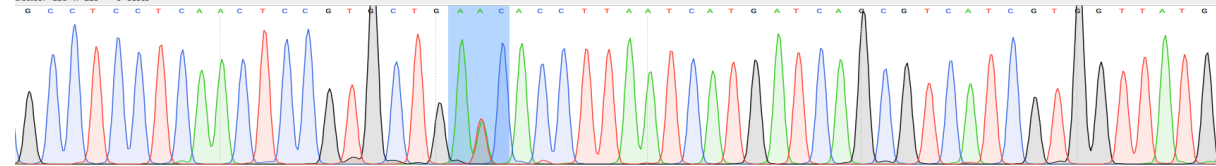
Clone 4:
N141I(+)
Mse(+)



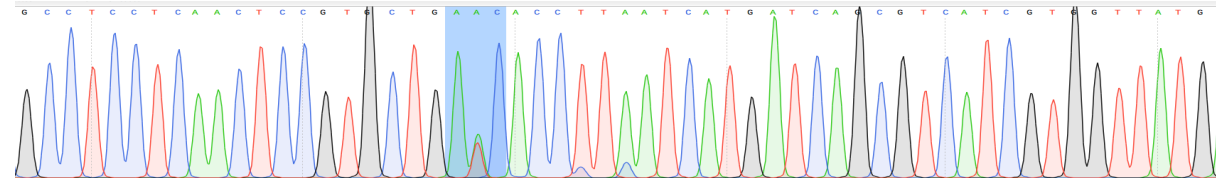
Clone 5:
N141I(+)
Mse(++)



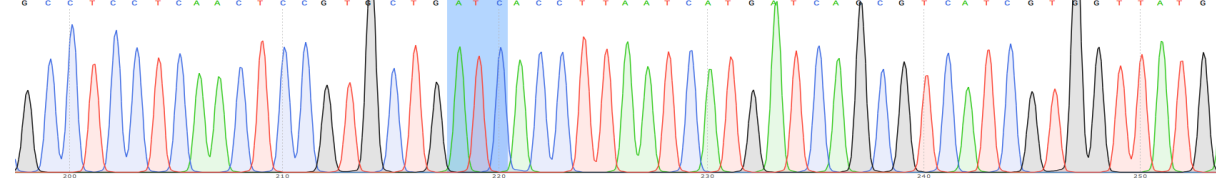
Clone 6:
N141I(+)
Mse(++)



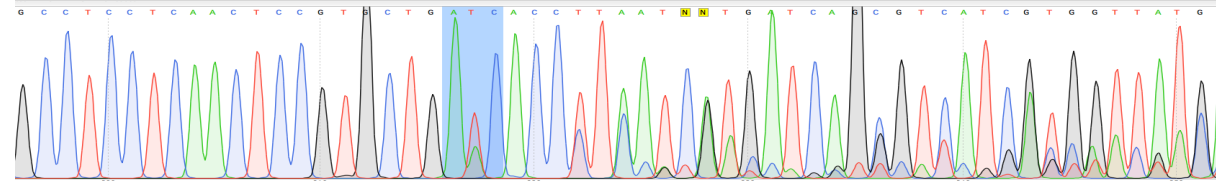
Clone 9:
N141I(+)
Mse(+)



Clone 10:
N141I(++)
Mse(++)



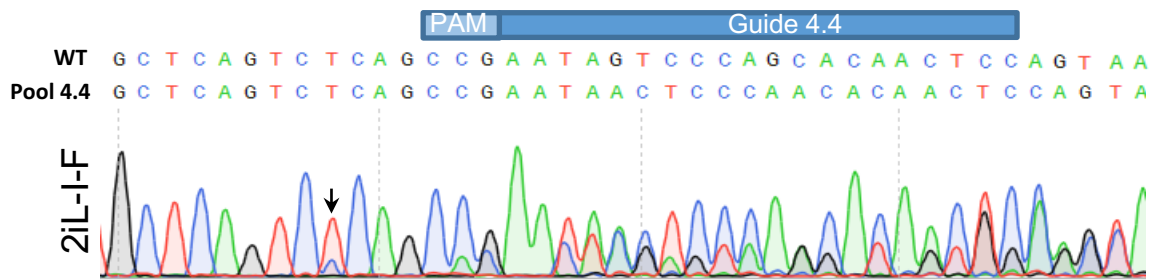
Clone 11:
indels



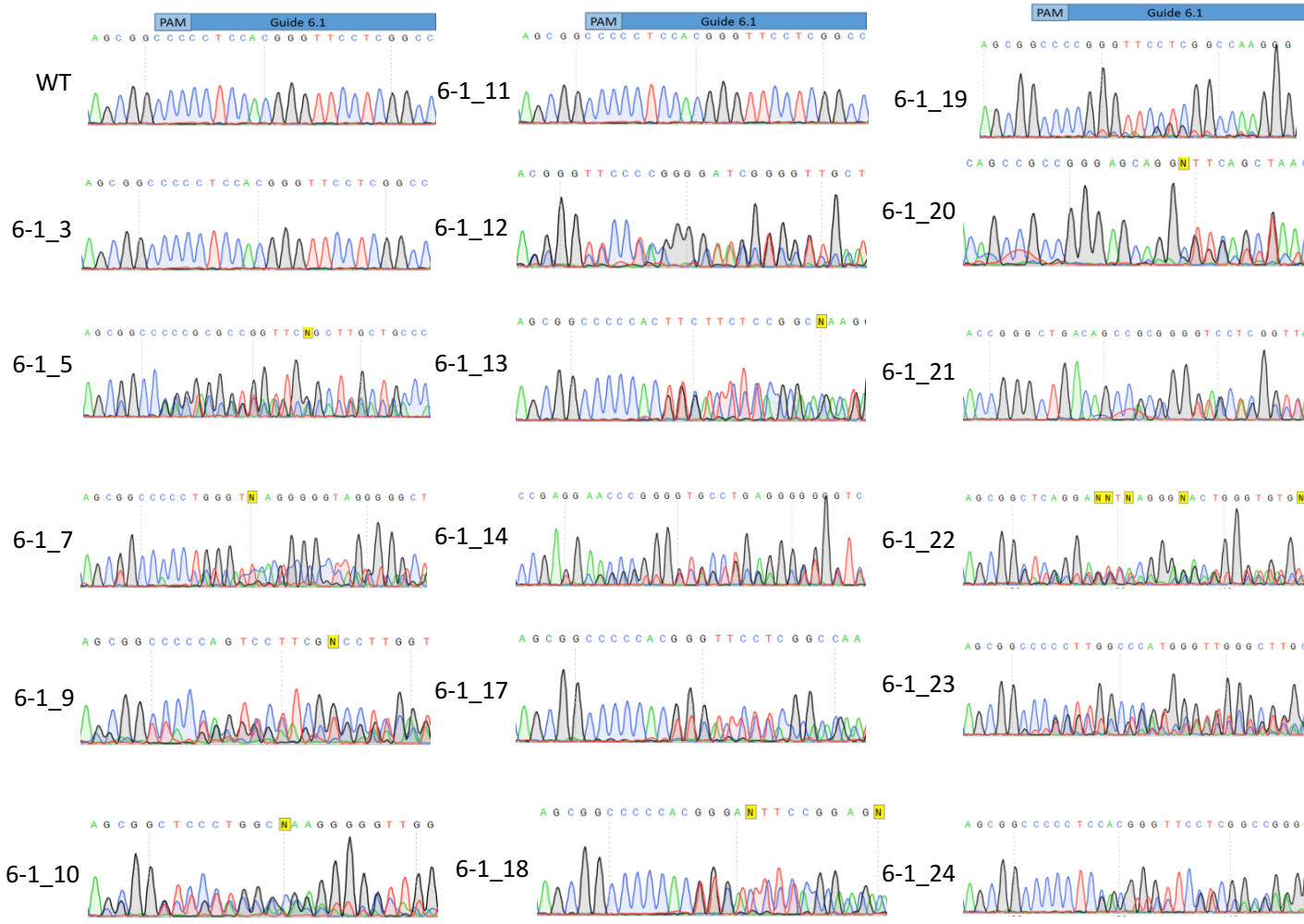
Supplemental Figure 2: Insertion sequences of selected clones. Elf1- iCas9 cells were induced with doxycycline, transfected with guide RNA plus DNA repair template, stained for TRA-1-81, and then FAC sorted into a 96 well plate (15 cells/well). DNA was analyzed, and pools 3, 9, and 35 were combined. A second TRA-1-81 FAC sort was done and plated in a 96 well plate (1 cell/well). Clones were analyzed by PCR around the insertion site followed by digestion of the MseI sites.

Supplemental Figure 3

A



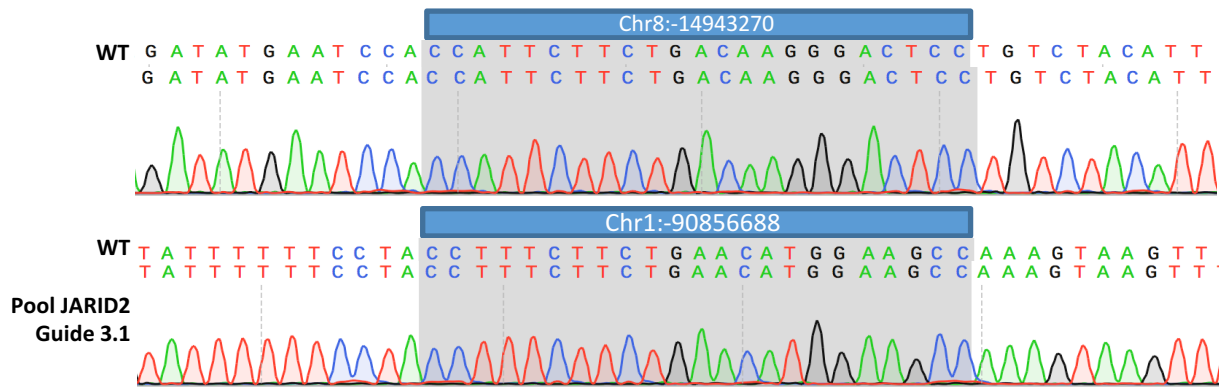
B



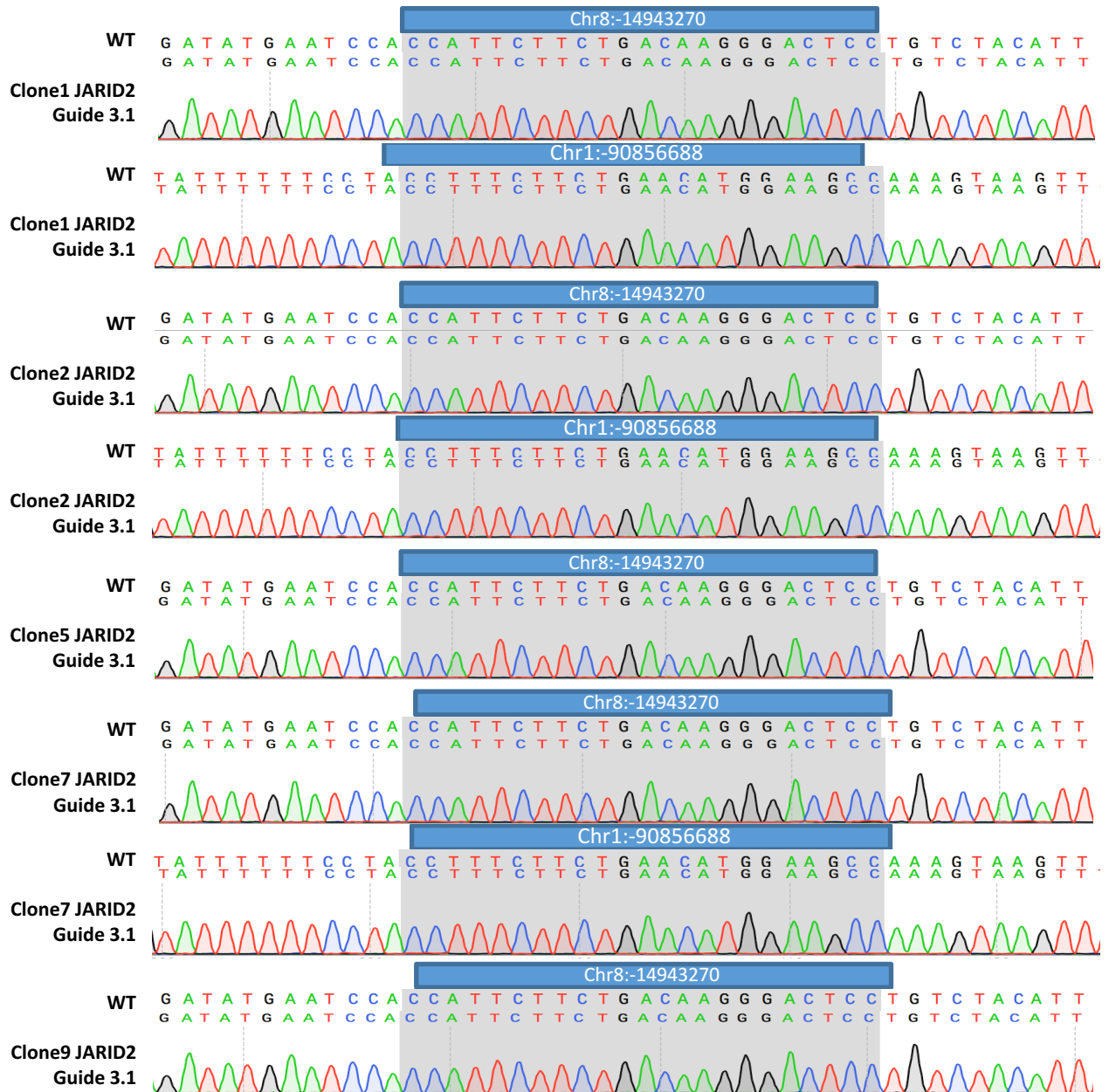
Supplemental Figure3: DNA sequence for JARID2 guide 4.4 pool and guide 6.1 clones. (A) Sanger sequencing trace file of the pool of Elf1-iCas9 cells transfected with JARID2 sgRNA 4.4 Black arrow indicates start of mutated region. (B) Sequencing results for the clones isolated after treatment of Elf1-iCas9 with gRNA 6.1 targeting JARID2. The area covered by the guide is showed in dark blue. Both mutant and wild-type sequences are shown.

Supplemental Figure 4

A



B



Supplemental Figure 4: DNA sequence of predicted Off-Target regions for JARID2 guide 3.1. (A-B) Sanger sequencing results show no mutations on the top predicted off-target regions for JARID2 Guide 3.1 in pool DNA (A) and on the selected JARID2 KO clones (B).

Supplemental Figure 5

Guide 3.1

WT GAT GGG ATT CCG TGG TCA GAA GAA CGG GTG GTA CGT AAA GTC CTT TAT
3.1-9 GAT GGG ATT CCG TGG TCA GAA CGG --- GTG GTA CGT AAA GTC CTT TAT

WT GAT GGG ATT CCG TGG TCA GAA GAA CGG...(x4 AA) GTC CTT TAT TTG TCT CTG
3.1-5 GAT GGG ATT CCG GGG GCA AAG AAC AGN...(x4 AA) TCC TAT AGA TGT CTC **TAA**

WT GAT GGG ATT CCG TGG TCA GAA GAA CGG...(x19 AA) CAG CAT GCG GAA GGC ATT
3.1-7 GAT GGG ATT CCG TGG CAN GAA TTN NTC...(x19 AA) CTC AGG CCC GAN GTG **TAG**

WT GAT GGG ATT CCG TGG TCA GAA GAA CGG...(x6 AA) TAT TTG TCT CTG AAG GAG
3.1-1 GAT GGG ATT CCG TGG TCA GAA AGA ANG...(x6 AA) TTG TTT CTC TCT GAN **TGA**

WT GAT GGG ATT CCG TGG TCA GAA GAA CGG GTG GTA CGT AAA GTC CTT TAT
3.1-2 GAT GGG ATT CCG TGG TCA GAA **TGA** ACG GGT GGT ACT TTT AGT CCT TTA

Supplemental Figure 5: Sequence of cloned PCR products after amplifying the genomic region around guide 3.1 targeting Jarid2. Early stop codons were observed in Elf1-iCas9 protein null clones 5, 7, 1 and 2, but not in clone 9 that still showed JARID2 protein expression (Fig.6A).

Supplemental Table 1. Primers for target gene

Gene	5' Forward	3' Reverse
Jarid2 (Exon 6) - Guide 6.1	TTCAATGGTTCCAGCAGGTCA	AAGGTGCGCTCTGTCTGTC
Jarid2 (Exon 2) - Guide 3.1	TTGAGAACTGGGTCGTGGTC	ACAGACTGCCTTTAGGTGCC
Jarid2 (Exon 4) - Guide 4.4	TGTGACCCCGTTAGATGGAC	GATGGTTCAAAAGCCACACCC
Mel-18 (Exon 1) - Guide 1.1	CTGGTGTCTCTCCCGACCAT	GCTACGTCCCTGACGCC
TCTN-2 (Exon 6) - Guide 6.1	GGTCACAGCTCCTGCCTTTA	GGTTGGGAAAAACGTGACCG
HIF-1 (Exon 2) - Guide 6.2	GCCAGATCTCGGCGAAGTAA	TCAAAACATTGCGACCACCT
HIF-2 (Exon 3) - Guide 4	GTATGCGTTTCCAGAAAAGTCC	GTCACCTGTGTAAGTCCCATGA
HIF-2 (Exon 2) - Guide 5	CCACTTAGGAGTAGCTCGGAGA	CTTACCTGAGGAGAGGAGCTTG
NNMT (Exon 2) - Guide 6.2	TCTTTGAGGATCGCCAGCAC	TGCCAACCATCACTCAGGTA
Off Target Jarid2 Guide 3.1 #1	GACTGCAGTACACGATGGGT	TACCCAACAGGAGGGTAGGT
Off Target Jarid2 Guide 3.1 #2	ACTGTGGCATGTTTCAGGGAAA	ACTCCATGCTACCGTGACTC

Supplemental Table 2. Primers for qPCR

Gene	5' Forward	3' Reverse
β -Actin	TCCCTGGAGAAGAGCTACG	GTAGTTTCGTGGATGCCACA
Cas9	CCGAAGAGGTCTGTAAGAAG	GCCTTATCCAGTTCGCTCAG
4-Oct	AGTTTGTGCCAGGGTTTTTG	ACTTCACCTTCCCTCCAACC
Sox2	GTATCAGGAGTTGTCAAGGCAGAG	TCCTAGTCTTAAAGAGGCAGCAAC
Nanog	CCTGGGATTTGTGGGCCTG	GACAGTCTCCGTGTGAGGCAT

Supplemental Table 3. Guide RNA

Gene	Sg RNA
Jarid2 (Exon 6) - Guide 6.1	CCTCCACGGGTTCTCGGCC
Jarid2 (Exon 2) - Guide 3.1	GGATTCCGTGGTCAGAAGAA
Jarid2 (Exon 4) - Guide 4.4	GGAGTTGTGCTGGGACTATT
Mel-18L (Exon 1) - Guide 1.1	CCTCCTGTCTCCCCGCCCGC
TCTN-2 (Exon 6) - Guide 6.1	CCCTTGCCAACACACCCTTC
HIF-1 (Exon 2) - Guide 6.2	CCTCACACGCAAATAGCTGA
HIF-2 (Exon 3) - Guide 4	CCCCCCCCCTTTCCAGTTTGCTC
HIF-2 (Exon 2) - Guide 5	GGGATGCTGCGCGGTGCCGG
NNMT (Exon 2) - Guide 6.2	AGTGACGACGATCTCCTTAA