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Supplemental information

A dual conditional CRISPR-Cas9 system to activate gene editing and reduce off-target effects in human stem cells

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Supplemental Information

Table S1. Summary of sgRNAs sequences

Gene	Sequence (5’ to 3’)
GFP	GGGCGAGGAGCTGTTCACCG
p53	GAATGAGGCCTTGGAACTCA
PTEN	AGATCGTTAGCAGAAACAAA
APC	GGCAACTTCTGGTAATGGTC
EMX1	GAGTCCGAGCAGAAGAAGAA
HBB	CTTGCCCCACAGGGCAGTAA
FANCF	GGAATCCCTTCTGCAGCACC
Nanoluciferase	TGTCCGTAACTCCGATCCAA
Nanoluciferase (L)	GGTCCGTAACTCCGATCCAA
Nanoluciferase (M)	TGTCCGTAATTCCGATCCAA
Nanoluciferase (R)	TGTCCGTAACTCCGATCCAC

Table S2. Summary of primer sequences

Gene	Sequence (5' to 3')
Cas9 Forward	GGCTGAGAACGGGAAGCTTGTCAT
Cas9 Reverse	CAGCCTTCTCCATGGTGGTGAAGA
GAPDH Forward	CCCAAGAGGAACAGCGATAA
GAPDH Reverse	TTGGCTTCCAGAAAGTCGAT
TIDE *p53* On-target Forward	CTGTATAGGTACTTGAAGTGCAG
TIDE *p53* On-target Reverse	CTCTGGCTGTATTCAGTATTAC
TIDE *p53* Off-target Forward	GGCAGAATGAGTTTGCACAG
TIDE *p53* Off-target Reverse	CGCTTGGGTCCCTTTCTATT
TIDE *PTEN* Forward	GTTCGGAGGATTATTCGTCTTC
TIDE *PTEN* Reverse	GCACTATTGACTTCAAACTAC
TIDE *APC* Forward	CAGAAGCTAAGAGCCTATC
TIDE *APC* Reverse	CTTTACATTGAAGGTCTTAGTG
TIDE *EMX1* On-target Forward	CCACTCTGTGAAGAAGCGATTA
TIDE *EMX1* On-target Reverse	CTTCCCTATGTCTAGCCTGTTTC
TIDE *EMX1* Off-target Forward	GCTACTCTCTCTCCTTCAACTC
TIDE *EMX1* Off-target Reverse	CTACTGTGGGCACTACACTATAA
TIDE *HBB* On-target Forward	AAACATCAAGCGTCCCATAGA
TIDE *HBB* On-target Reverse	GTACGGCTGTCATCACTTAGAC
TIDE *HBB* Off-target Forward	CCCATTGCCTCCTCTGTTATC
TIDE *HBB* Off-target Reverse	GAGGTTGCTCAGCTTCTTGTA
TIDE *FANCF* On-target Forward	GGCCTGGAAGTTCGCTAAT

TIDE *FANCF* On-target Reverse	ATCTGCTCTCCCTCCACTAA
TIDE *FANCF* Off-target Forward	GCAAGCTGAAGCTCAGTAGA
TIDE *FANCF* Off-target Reverse	TCACCACCATGCACCTTAAA
p53 sgRNA Forward	GAATGAGGCCTTGGAACTCA
p53 sgRNA Reverse	CGACTCGGTGCCACTTT

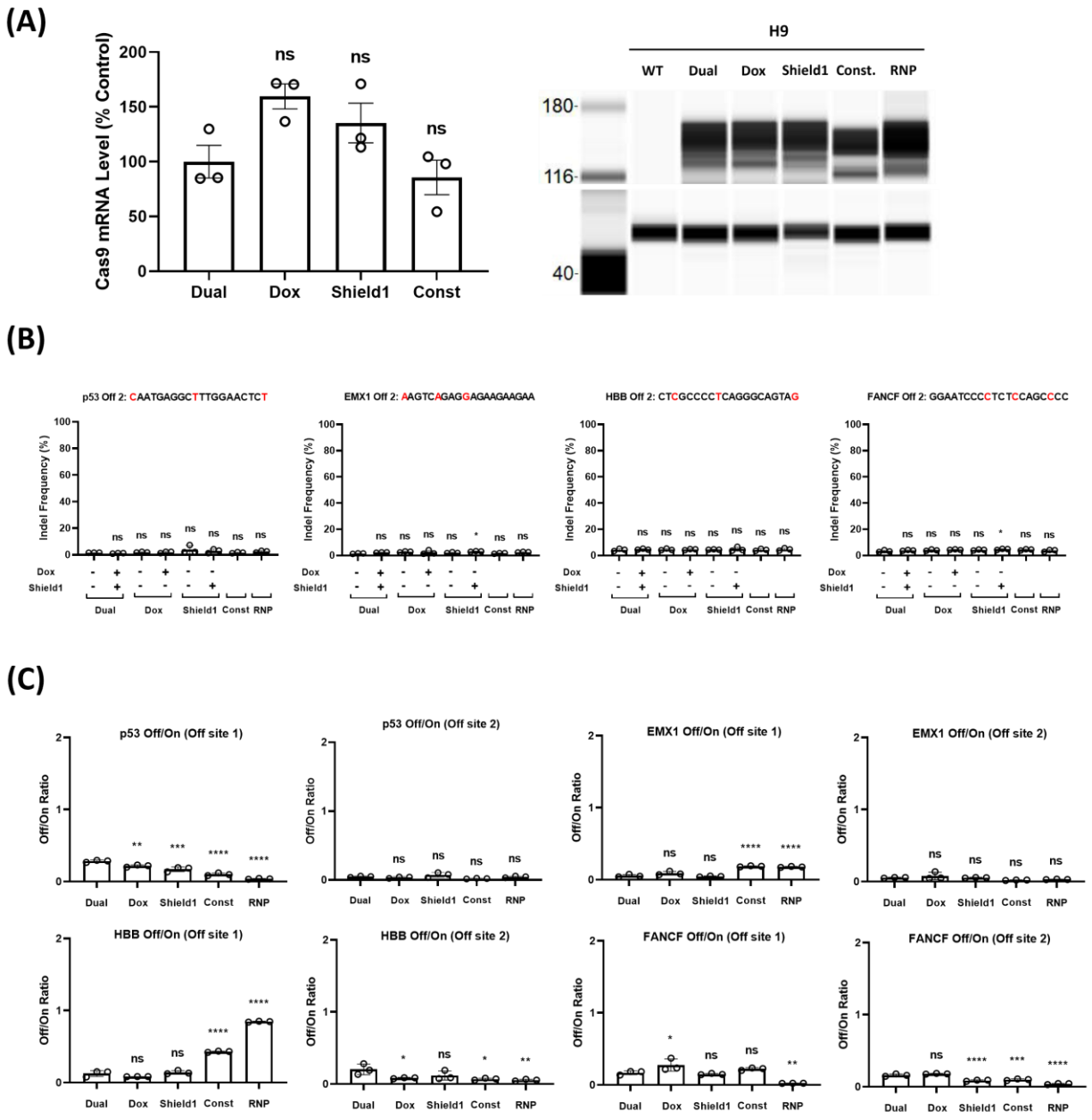


Figure S1. Dual conditional CRISPR/Cas9 system reduces off-target effects.

(A) To compare Cas9 expression level between different Cas9 systems, RNA and protein from each Cas9 system were isolated and were performed by qRT-PCR and Wes assay, respectively. For the inducible systems, the H9 Cas9 pooled clones were treated with 300 ng/mL of doxycycline and/or 250 nM of Shield1 for 3 days followed by RNA/protein extraction and qRT-PCR/Wes assay. The Dual conditional system was set as 100% (control) for mRNA level comparison. (B)

H9 Cas9 pooled clones were used to compare off-target gene editing efficiencies for our dual conditional Cas9 system (Dual), doxycycline inducible Cas9 system (Dox), Shield1 regulated Cas9 system (Shield1), constitutive Cas9 expressing system (Const). For Cas9 ribonucleoprotein delivery system (RNP), H9 cells were electroporated with 3.75 pmol of Cas9 protein and sgRNAs. For the inducible systems, the cells were treated with 300 ng/mL of doxycycline and/or 250 nM of Shield1 for 3 days to knock-out p53, *EMX1*, *HBB*, or *FANCF* gene. After DNA extraction, 500 ~ 1,000 bp sequences that contain the knock-out sites were amplified by PCR and performed subsequent TIDE assay. The data are expressed as indel frequency (%) (0% as no editing and 100% near-complete editing). *P* values were calculated by comparing to the uninduced dual conditional system (-/-). (C) Off/on site ratios were determined by dividing the indel frequency of the off-target site by that of the on-target site. When measuring off/on site ratios, Cas9 induced groups were compared along with constitutive and RNP systems. Data are shown as mean values ± SEM of triplicate experiments (ns: not significant).

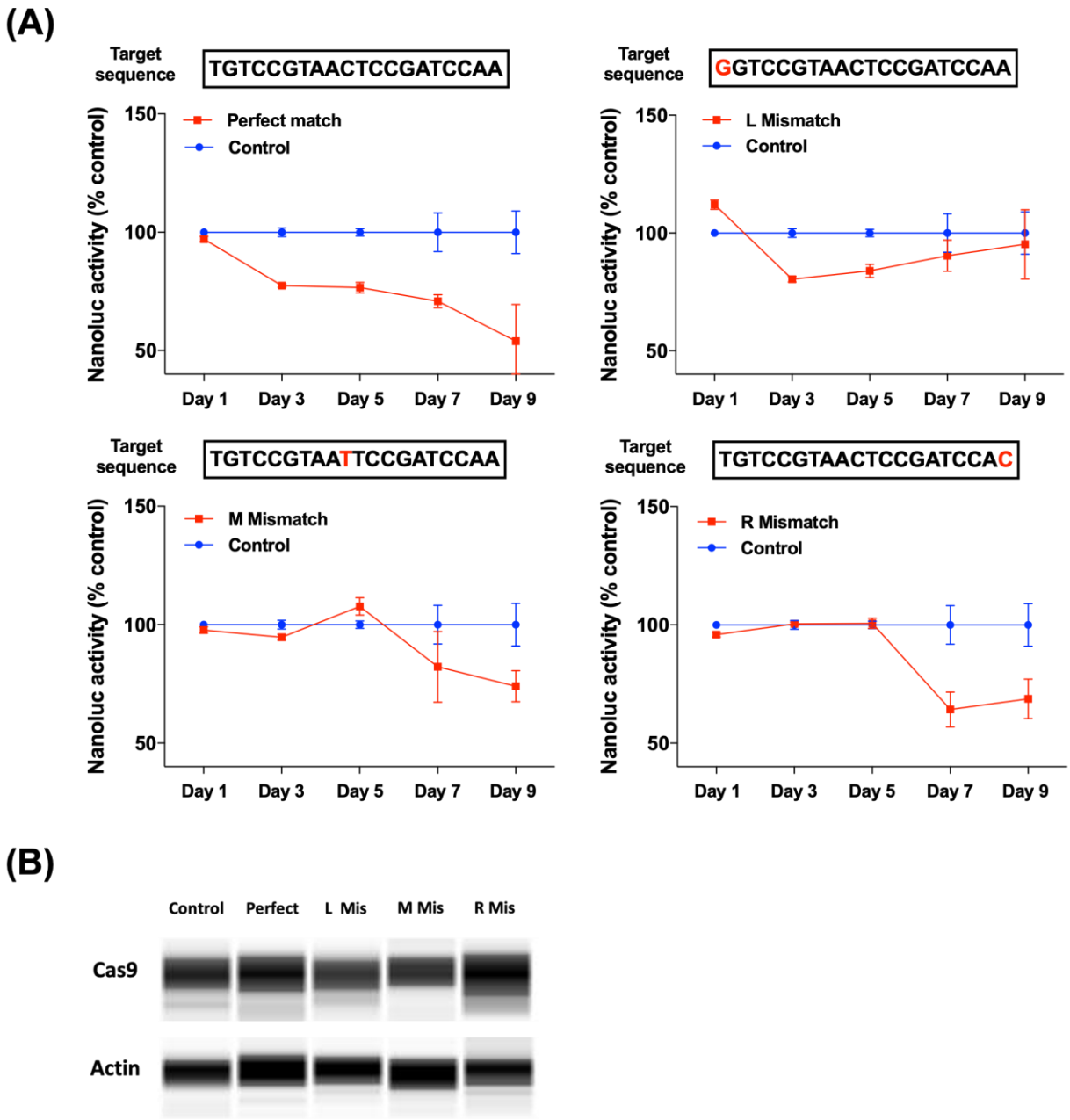


Figure S2. Dual conditional CRISPR/Cas9 system reduces off-target effects.

(A) Using our SW-Cas9 clone which expresses Nanoluciferase (Nanoluc) activity, knock-out efficiency was measured by designing one nucleotide mismatch sgRNA sequences for Nanoluciferase gene. Each SW-Cas9 clone was transduced by different lentivirual sgRNA

sequences that have one nucleotide mismatch. The cells were seeded in white 96 well plates 24 hr before Nanoluciferase measurement. The cells were treated with doxycycline and Shield1 throughout the experiement to induce Nanoluciferase gene knock-out. Nanoluciferase assay was performed on day 1, 3, 5, 7 and 9. (B) Knocking-out of Nanoluciferase gene does not change Cas9 protein level. Perfect, perfect match; L Mis, 5' mismatch; M Mis, mismatch in the middle; R Mis, 3' mismatch.

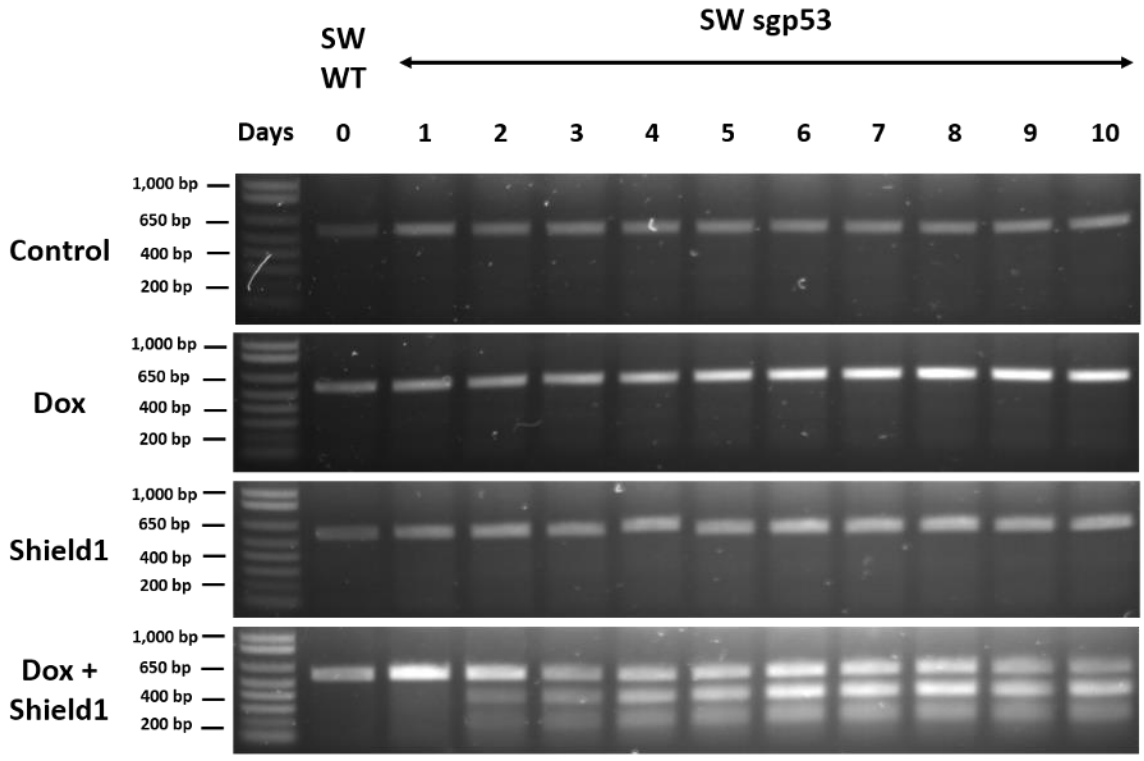


Figure S3. Time course experiment for knock-out efficiency.

Knock-out efficiency was measured by T7 endonuclease assay in SW-sg*p53* cells. SW wild-type and SW sg*p53* cells were seeded in 12 well plates and treated with or without doxycycline and/or Shield1 ligand throughout the experiment. The cells were collected at different time points followed by T7 endonuclease assay.

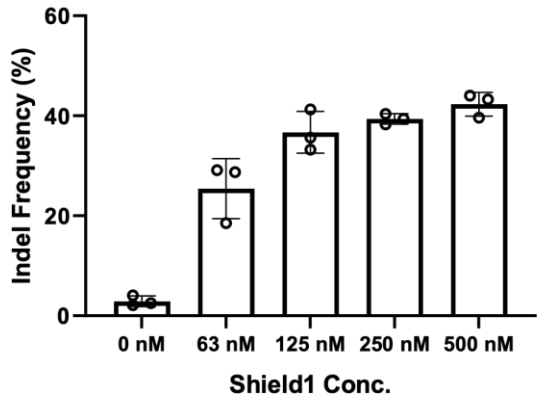


Figure S4. Dose-response change of gene editing efficiency by Shield1 ligand.

Using H9-Cas9-sg*p53* pooled clone, TIDE assay was performed to test knock-out efficiency according to the different concentrations of Shield1 ligand. The H9-Cas9-sg*p53* pooled clone was treated with doxycycline and 5 different concentrations (0 nM, 63 nM, 125 nM, 250 nM, and 500 nM) of Shield1 for 3 days to knock-out *p53*. After DNA extraction, about 500 bp sequences that comprises knock-out site were amplified by PCR and performed subsequent TIDE assay. The results are representative of three independent experiments.

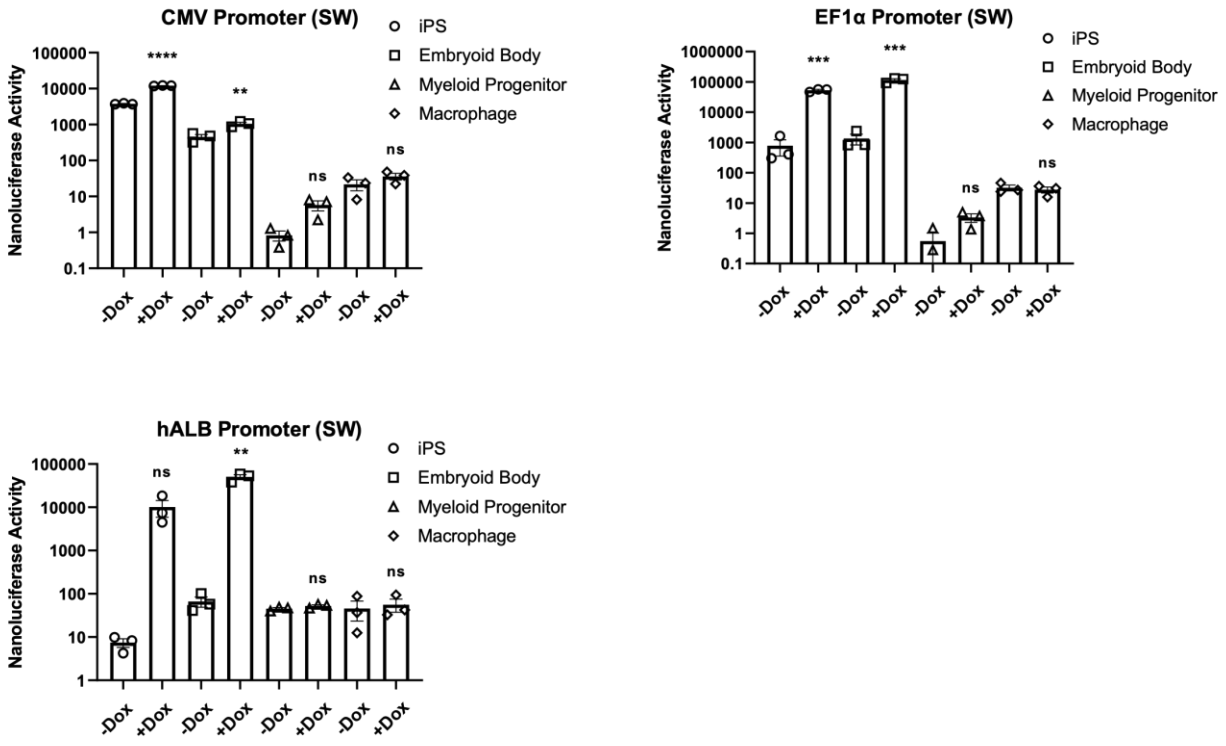


Figure S5. Conditional CRISPR/Cas9 system and human stem cell macrophage differentiation.

SW-Cas9 clones that are replaced with EF1α and human albumin (*hALB*) promoters were differentiated into macrophage cells. CMV, EF1α, and *hALB* promoter activities in the conditional CRISPR/Cas9 system, as shown by the Nanoluciferase activities, were compared during macrophage differentiation of SW-Cas9 clones. Data are shown as mean values ± SEM of triplicate experiments (ns: not significant, **p* <0.05, ***p* <0.01, ****p* <0.001, *****p* <0.0001).

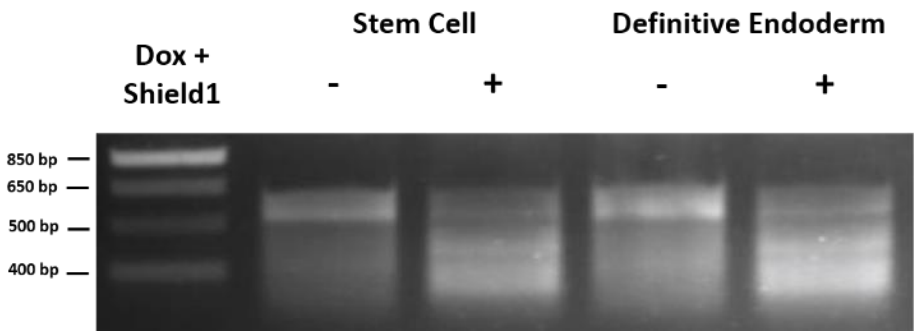


Figure S6. EF1α promoter-driven CRISPR/Cas9 knock-out efficiency after 4 days of definitive endoderm differentiation.

Knock-out efficiency was measured by T7 endonuclease assay in H9-sg*p53* cells which were differentiated with 4 days of definitive endoderm induction. H9 sg*p53* cells were seeded in 6 well plates and were treated with doxycycline and Shield1 ligand along with definitive endoderm differentiation.

WES raw data

Fig. 2A Cas9

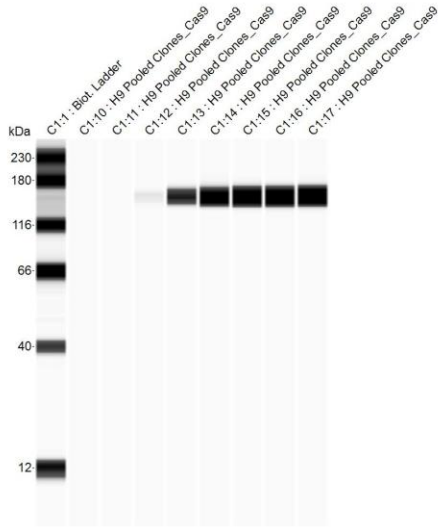


Fig. 2A Actin

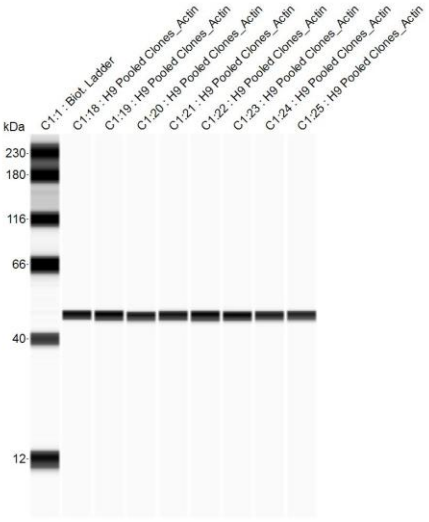


Fig. 2B Cas9

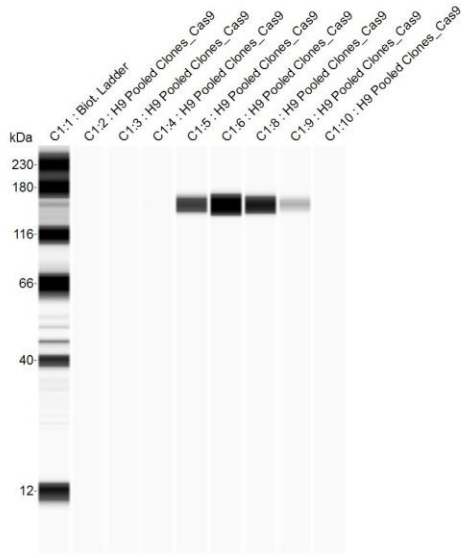


Fig. 2B Actin

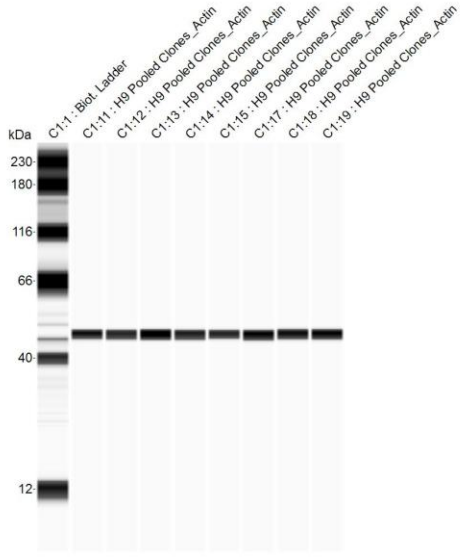
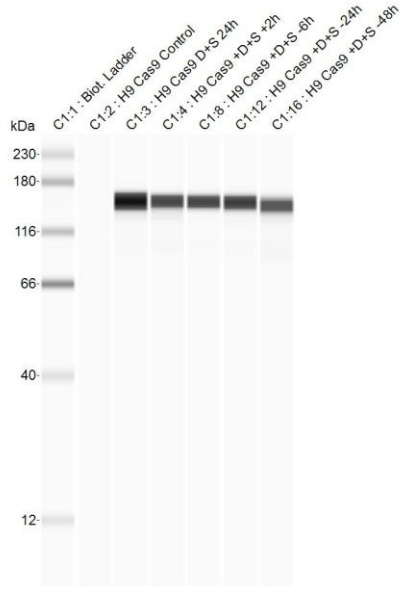


Fig. 2C No medium change Cas9



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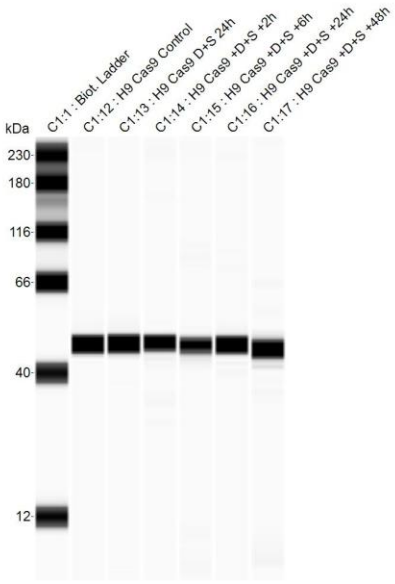
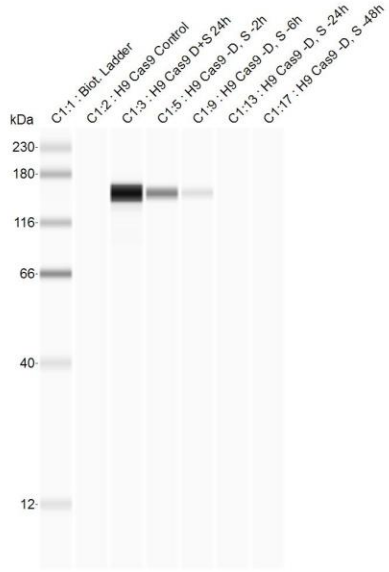


Fig. 2C No Doxycycline and Shield1 Cas9



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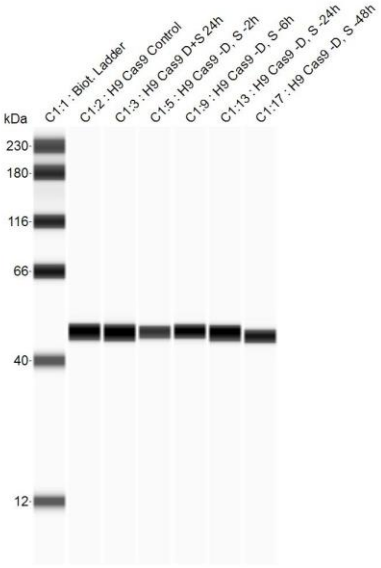
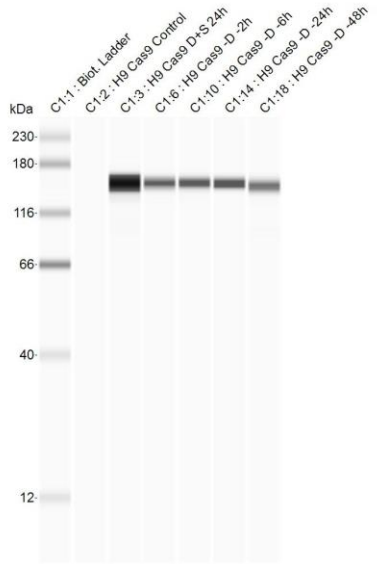


Fig. 2C No Doxycycline Cas9



No Doxycycline Actin

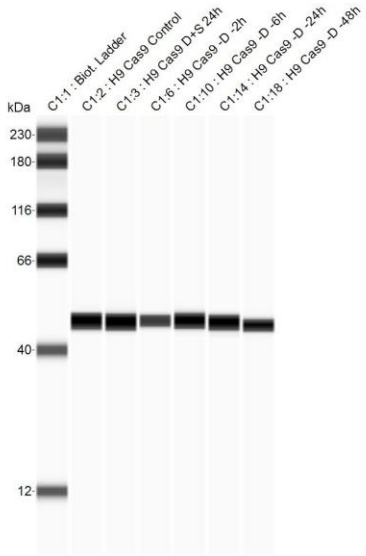
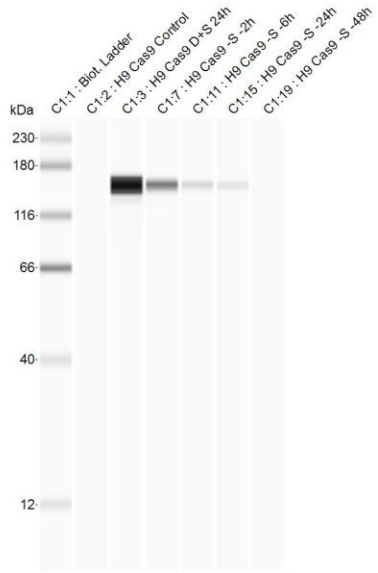


Fig. 2C No Shield1 Cas9



No Shield1 Actin

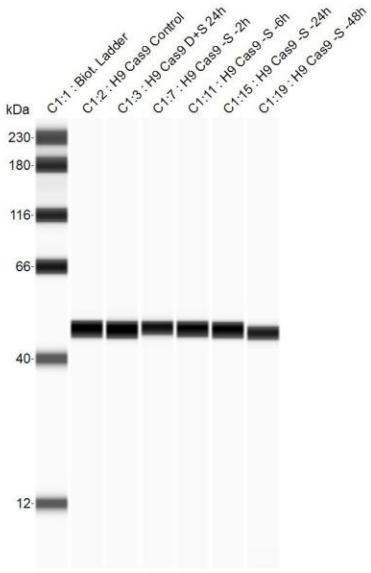


Fig. 3B Cas9

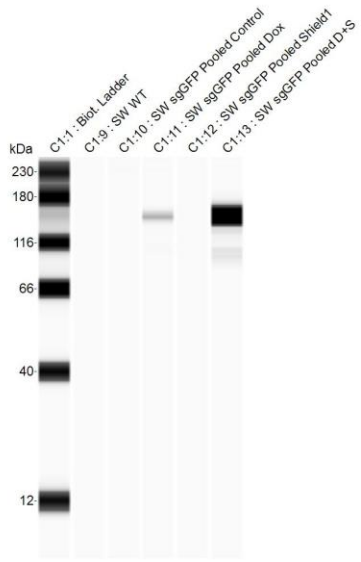


Fig. 3B Actin

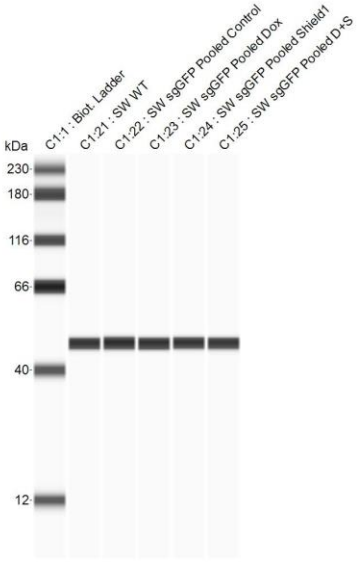


Fig. 4C p53

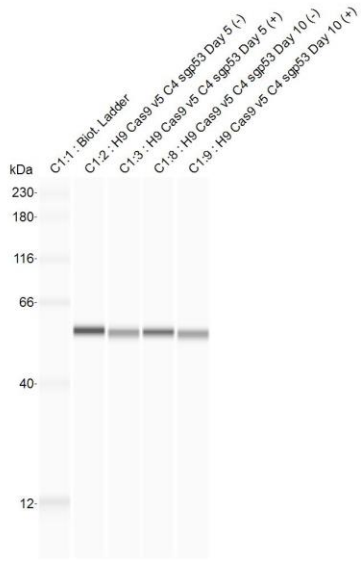


Fig. 4C Actin (p53)

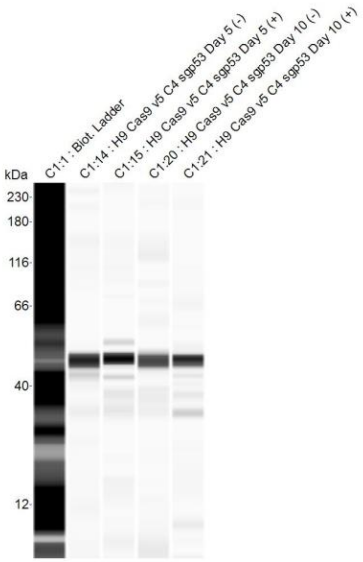
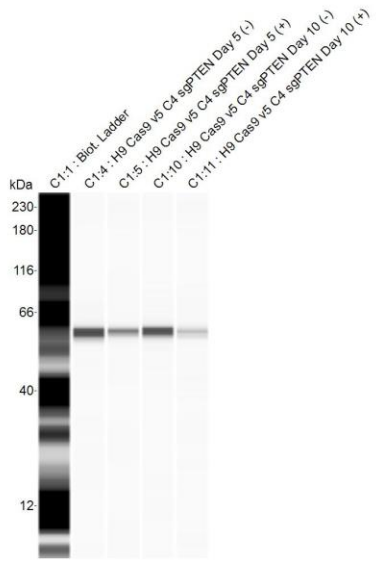


Fig. 4C PTEN



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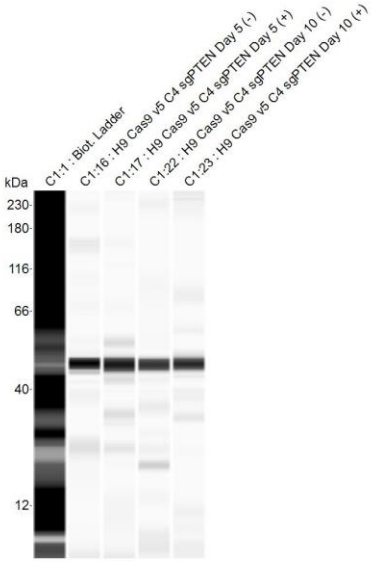
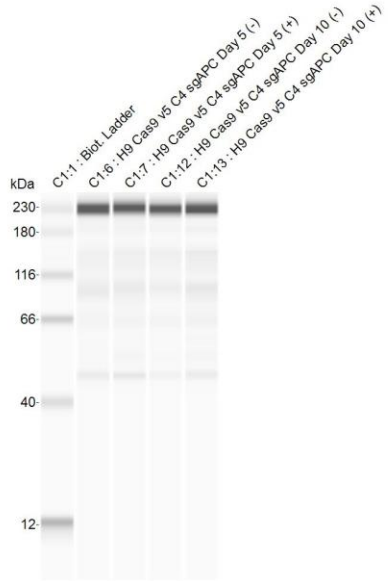


Fig. 4C APC



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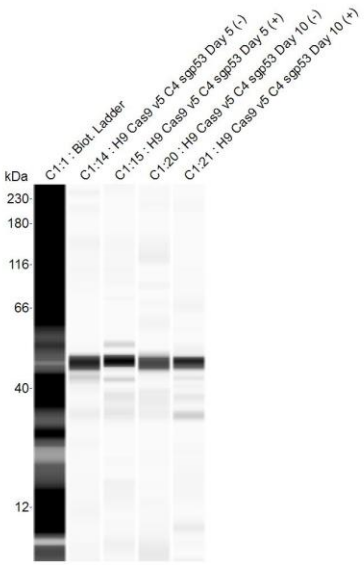
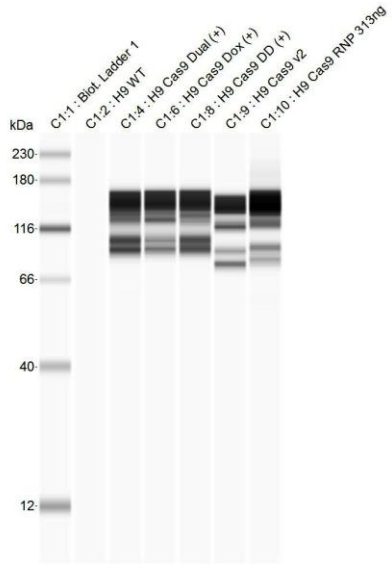


Fig. S1A Cas9



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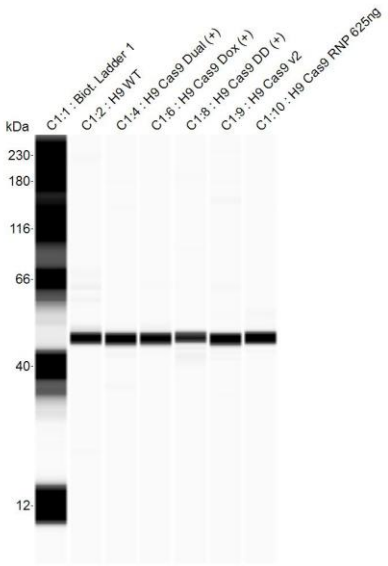

Fig. S2B Cas9

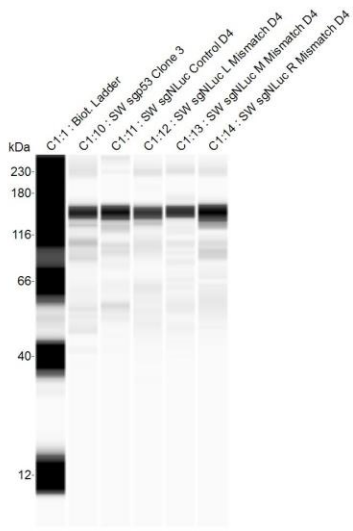

Fig. S2B Actin