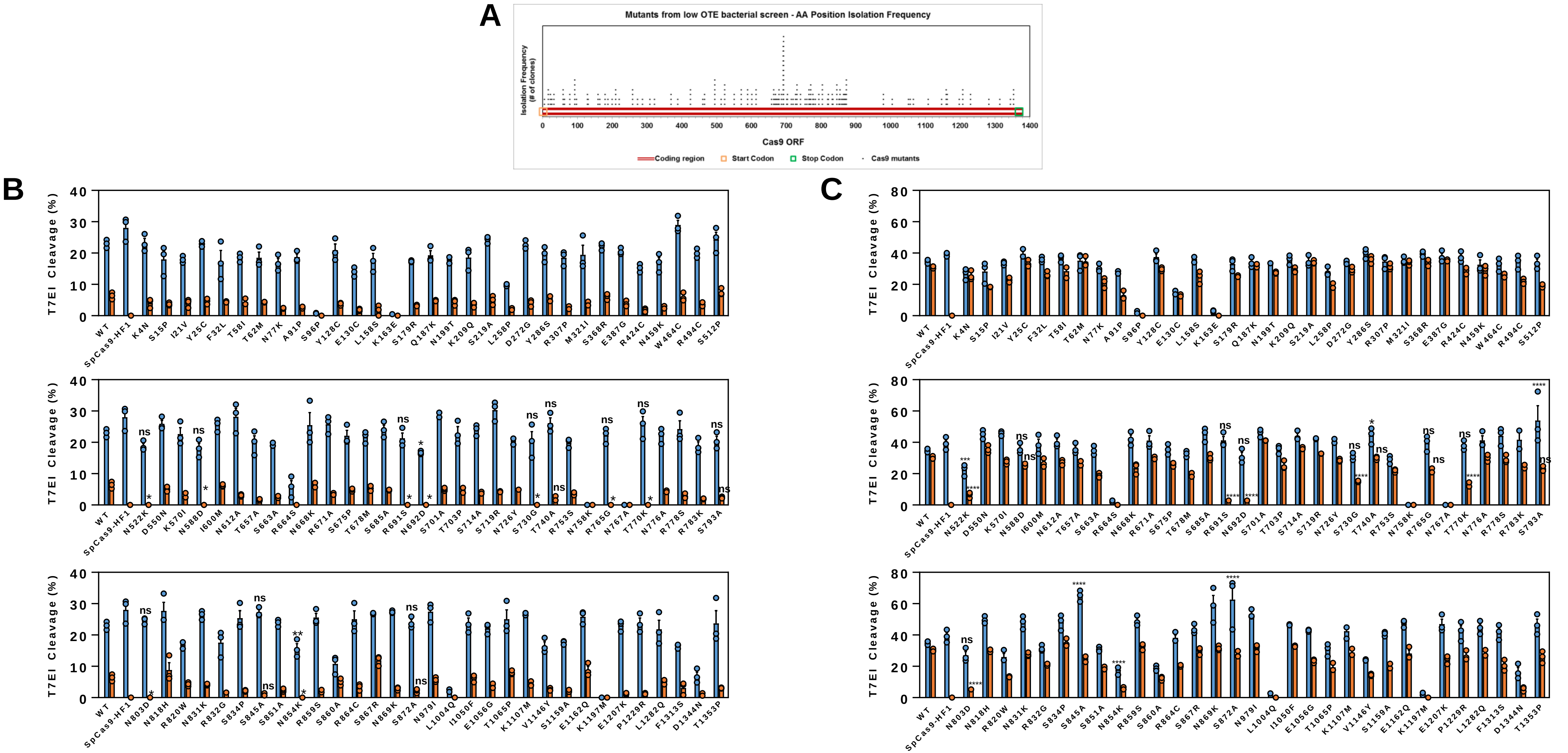
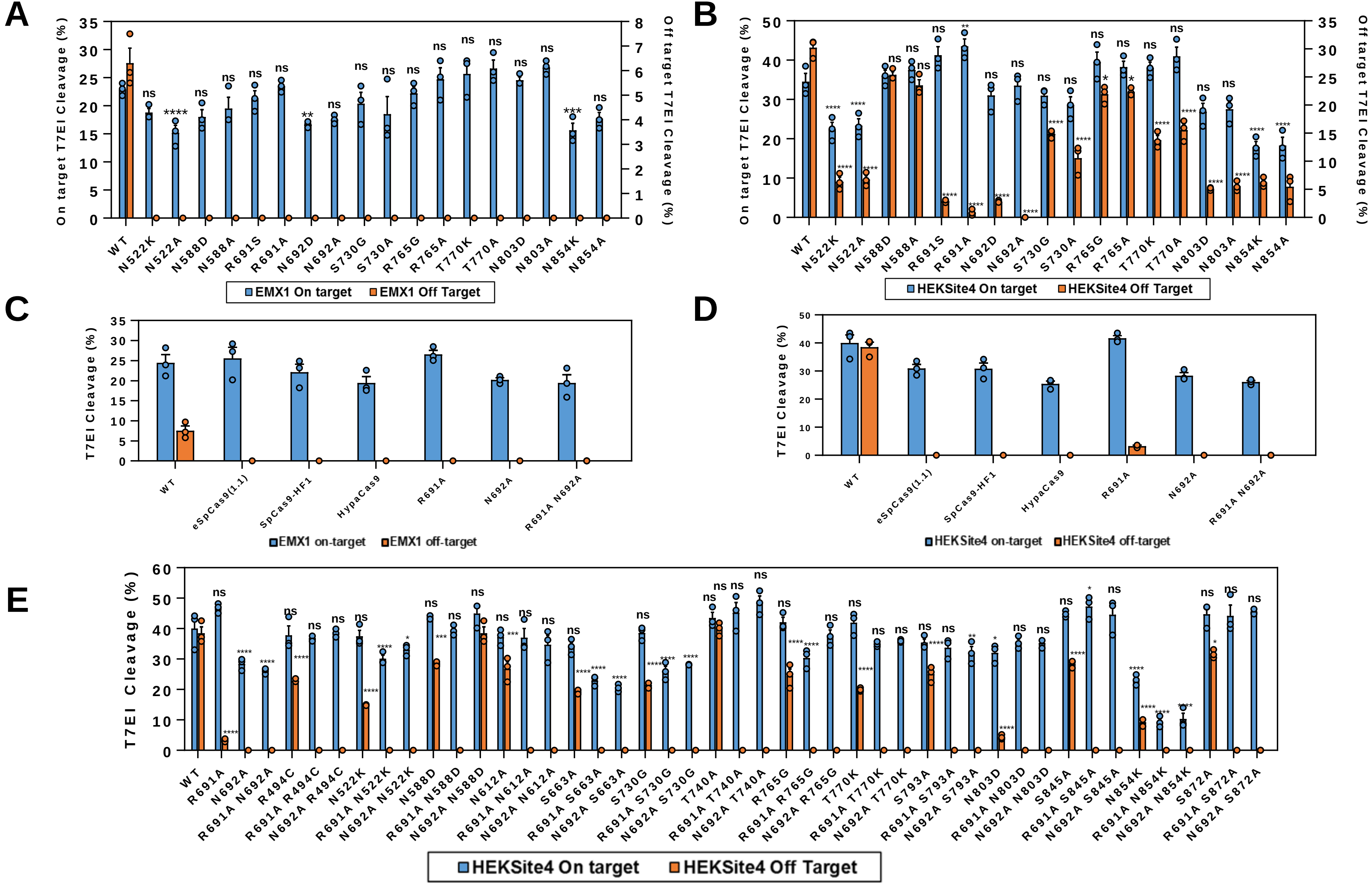
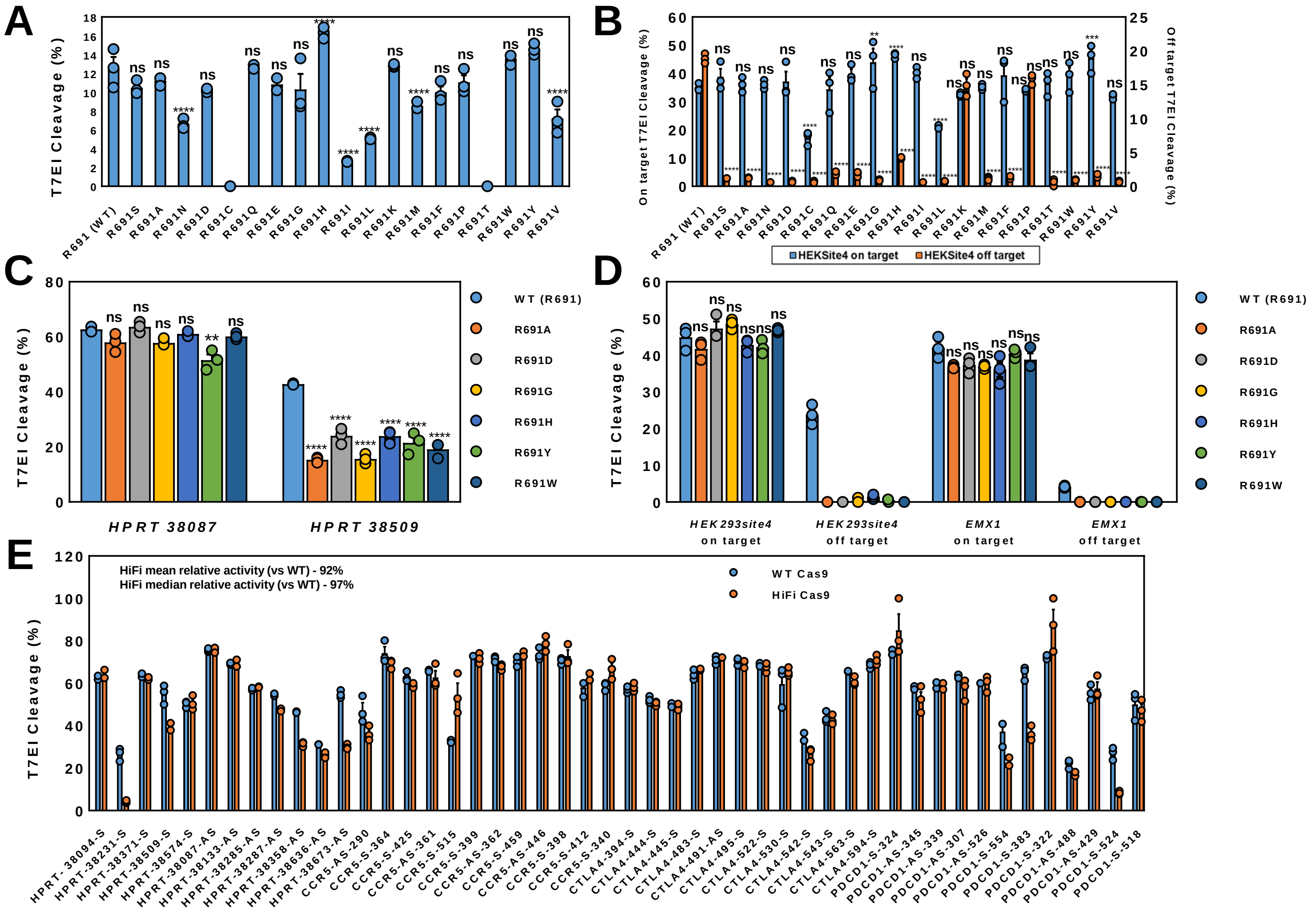


Supplementary Figure 1 – Bacterial dual selection screen identifies point mutations in Cas9 that reduce off-target editing. (**A**) Location and frequency of Cas9 amino acid positions where selected mutants maintained on-target potency and reduced off-target editing. Only substitution-bearing positions that were isolated ≥ 2 times were plotted. The Cas9 open reading frame is plotted on the x-axis and the frequency of mutation at each position was plotted on the y-axis. In total, 250,000 clones were screened from a mutant library using guides and target sites first with *VEGFA3*, and secondarily with *EMX1*. From the primary screen (*VEGFA3*), 875 clones were isolated and subsequently screened with *EMX1*. Plasmids were purified from 163 surviving colonies and the *cas9* was sequenced revealing 94 positions where substitution mutations were isolated at least twice. (**B-C**) On- and off-target editing efficiencies of plasmid-borne Cas9 mutations delivered into HEK293 cells with gRNA complexes targeting the EMX1 (**B**) or *HEKSite4* (**C**) loci. Each of 94 low off-target substitution mutations isolated from the bacterial selection screen were created in the context of a Cas9 human expression plasmid using site-directed mutagenesis. The most frequently-isolated mutation at each position was carried forward for testing into human cells. Blue bars represent editing at the intended on-target sites for *EMX1* (B - GAGTCCGAGCAGAAGAAGAAGGG) and *HEKSite4* (C - GGCACTGCGGCTGGAGGTGGGGG), whereas orange bars represent editing at known, problematic off-target sites for the *EMX1* (B – GAGTTAGAGCAGAAGAAGAAAGG) and *HEKSite4* (C – GGCACGACGGCTGGAGGTGGGGG) loci. DNA was extracted 48 hrs post-transfection and editing efficiencies were calculated using PCR followed by T7EI mismatch detection. Error bars represent mean ± s.e.m., n=3 independent experiments. Statistical comparisons were made to WT Cas9 for On and Off target activity. *P<.05, **P<.01, ***P<.001, ****P < 0.0001, NS (not significant) = P ≥ 0.05, two-way analysis of variance (ANOVA) and Tukey's multiple comparison test

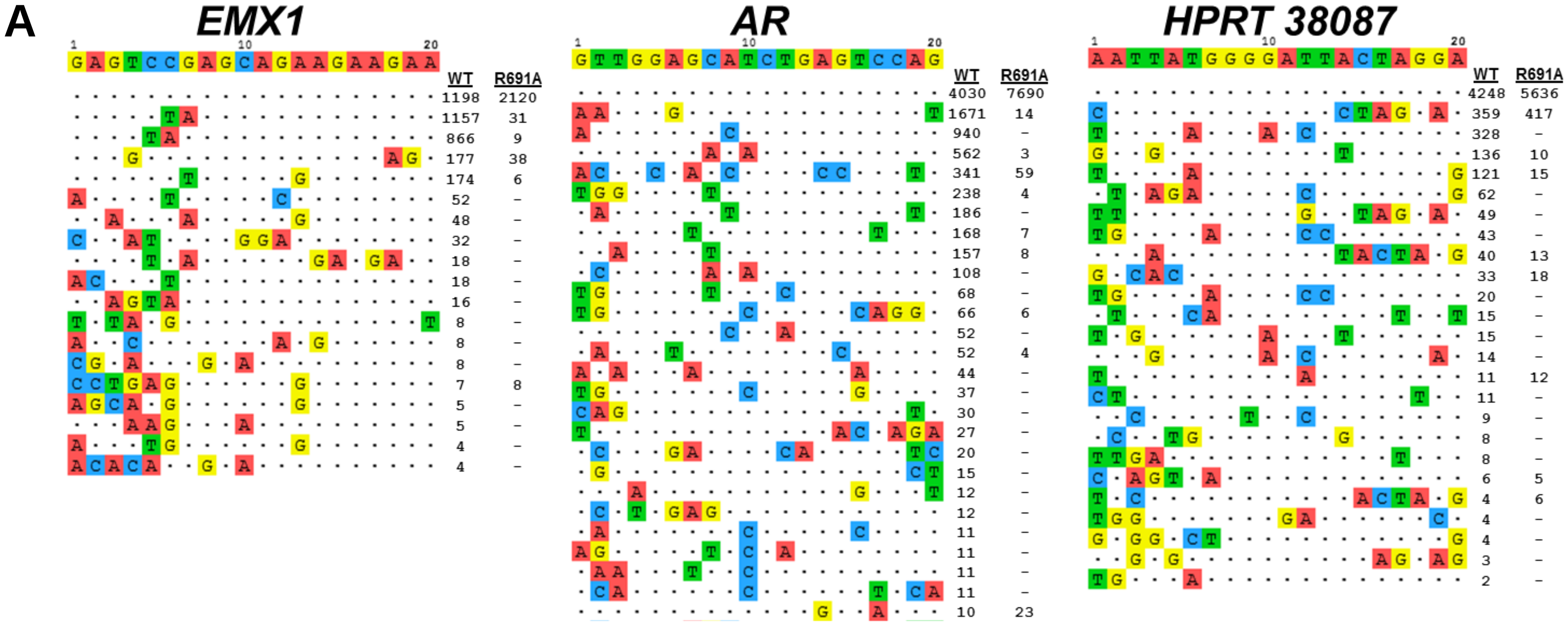


Supplementary Figure 2 – On- and off-target performance of alanine-substituted and combined Cas9 mutations in human cells. Previously described literature high-fidelity Cas9 mutants were also compared to R691A and N692A in the context of plasmid-based protein expression with the EMX1 (**A**) and HEKSite4 (**B**) guides. Alanine substitutions and mutant combinations were isolated by site-directed mutagenesis, and mutant plasmids were delivered into HEK293 cells by lipofection using crRNAs that target the *EMX1* (**A, D**) or *HEKSite4* **(B-C, E**) loci. Blue bars represent editing at the intended on-target sites for *EMX1* (A,D - GAGTCCGAGCAGAAGAAGAAGGG) and *HEKSite4* (B-C, E - GGCACTGCGGCTGGAGGTGGGGG), whereas orange bars represent editing at known, problematic off-target sites for the *EMX1* (A, D – GAGTTAGAGCAGAAGAAGAAAGG) and *HEKSite4* (B-C, E – GGCACGACGGCTGGAGGTGGGGG) loci. DNA was extracted 48 hrs post-transfection and editing efficiencies were calculated using PCR followed by T7EI mismatch detection. Error bars represent mean ± s.e.m., n=9 independent experiments. Statistical comparisons were made to WT Cas9 for on and off target activity. *P<.05, **P<.01, ***P<.001, ****P < 0.0001, NS (not significant) = P ≥ 0.05, two-way analysis of variance (ANOVA) and Tukey's multiple comparison test.



Supplementary Figure 3 On- and off-target analysis of every possible amino acid substitution at the R691 position and overall performance of the R691A substitution by RNP. (A-B) Alanine substitutions were isolated by site-directed mutagenesis, and mutant plasmids were delivered into HEK293 cells by lipofection using crRNAs that target the *HPRT-38509* (A) or *HEKSite4* (B) loci. Blue bars represent editing at the intended on-target sites for *HPRT-38509* (A - TTGACTATAATGAATACTTCAGG) and *HEKSite4* (B - GGCACTGCGGCTGGAGGTGGGGG), whereas orange bars represent editing at a known, problematic off-target site for the *HEKSite4* (B – GGCACGACGGCTGGAGGTGGGGG) locus. (C-D) On- and off-target analysis of the most promising R691 position substitutions delivered as RNP. WT and the indicated Cas9 proteins were complexed with gRNAs that target the *HPRT 38087* (C) , *HPRT 38509* (C), *HEKSite4* (D), and *EMX1* (D) loci, and RNP complexes (10 nM) were delivered into HEK293 cells by lipofection. Total editing was assessed at the *HPRT 38087* (C - AATTATGGGGATTACTAGGAAGG), *HPRT 38509* (C - TTGACTATAATGAATACTTCAGG), *HEKSite4* (D - GGCACTGCGGCTGGAGGTGGGGG) and *EMX1* (D - GAGTCCGAGCAGAAGAAGAAGGG) on-target sites, as well as at known, problematic off-target sites for the *HEKSite4* (D - GGCACGACGGCTGGAGGTGGGGG) and *EMX1* (D - GAGTTAGAGCAGAAGAAGAAAGG) guides. (E) Comparison of on-target editing between WT and HiFi (R691A) Cas9 delivered as RNP with guides that target 48 different sites. DNA was extracted 48 hrs post-transfection and editing efficiencies were calculated using PCR followed by T7EI mismatch detection. Error bars represent mean ± s.e.m., n=3 independent experiments. Statistical comparisons were made to WT Cas9 for On and Off target activity. **P<.01, ****P < 0.0001, NS (not significant) = P ≥ 0.05, two-way analysis of variance (ANOVA) and Tukey's multiple comparison test.

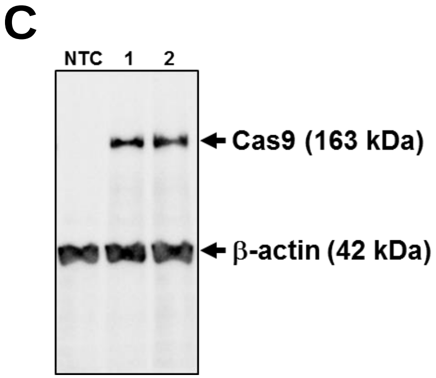
A



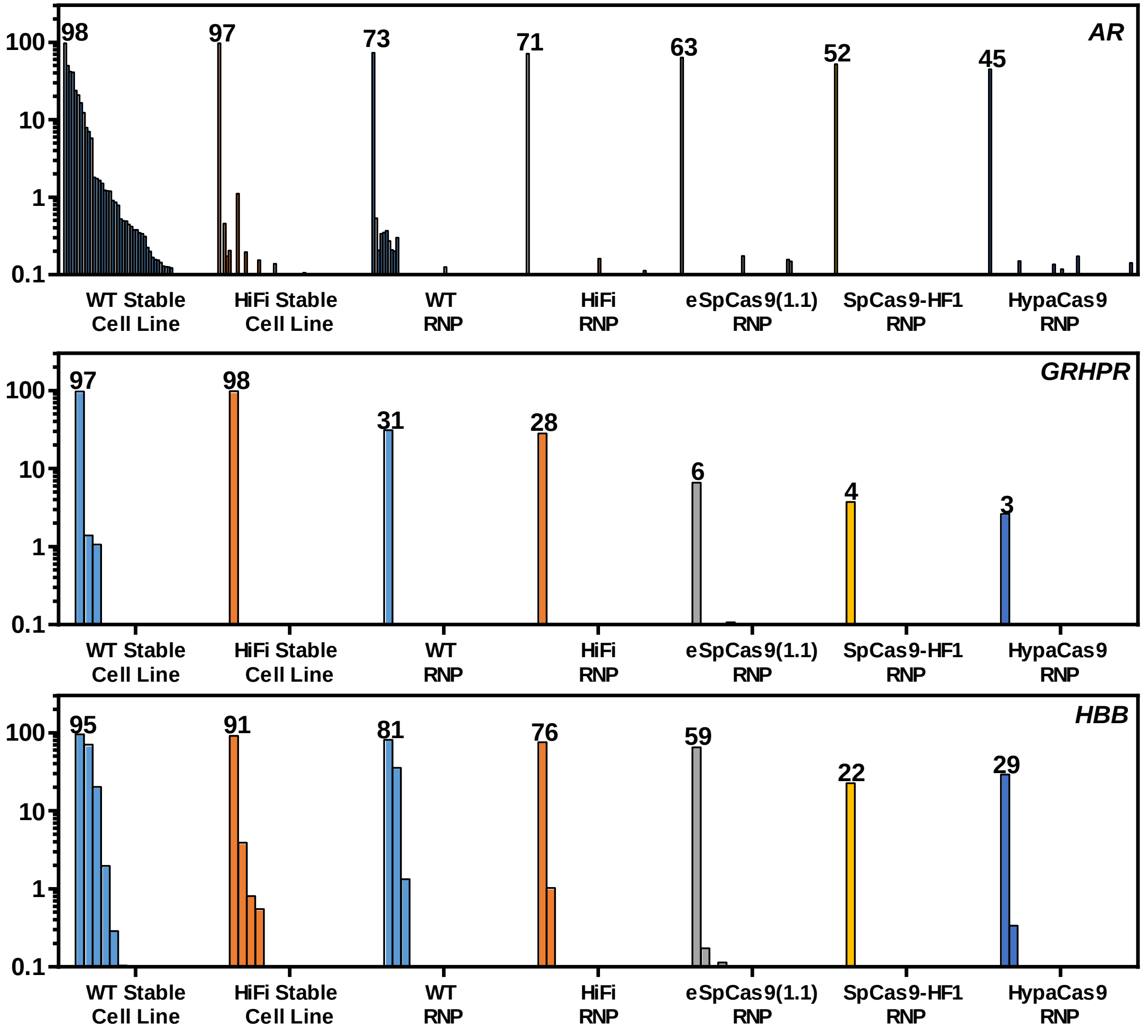
B

Site	Sequence	Closest Gene	Distance (kb)	Feature	hg19 Location
ON target	CTTGCCCCACAGGGCAGTAANGG	HBB	n/a	Exon	Chr11:5248198-5248220
Off Target 1	TCAGCCCCACAGGGCAGTAAGGG	GRIN3A	95.004	Intergenic	Chr9:104595866-104595888
Off Target 2	GTGGCCCCACAGGGCAGGAANGG	MAGEE2	1.209	Intergenic	ChrX:75006240-75006262
Off Target 3	GCTGCCCCACAGGGCAGCAANGG	FAM101A	3.258	Intergenic	Chr12:124803828-124803850
Off Target 4	GATGCCATTCATAGCAGTCANCG	C22orf34	225.248	Intergenic	Chr22:49582904-49582926
Off Target 5	CTCGCCCCTCAGGGCAGTAGTGG	GREB1	n/a	Intron	Chr2:11777795-11777817
Off Target 6	CCTCTCCCACAGGGCAGTAAAGG	LINC01482	0.034	Intergenic	Chr17:66624239-66624261
Off Target 7	TTTTCCCCAAAGGGCAGTAATAG	MYO16	n/a	Intron	Chr13:109818336-109818358

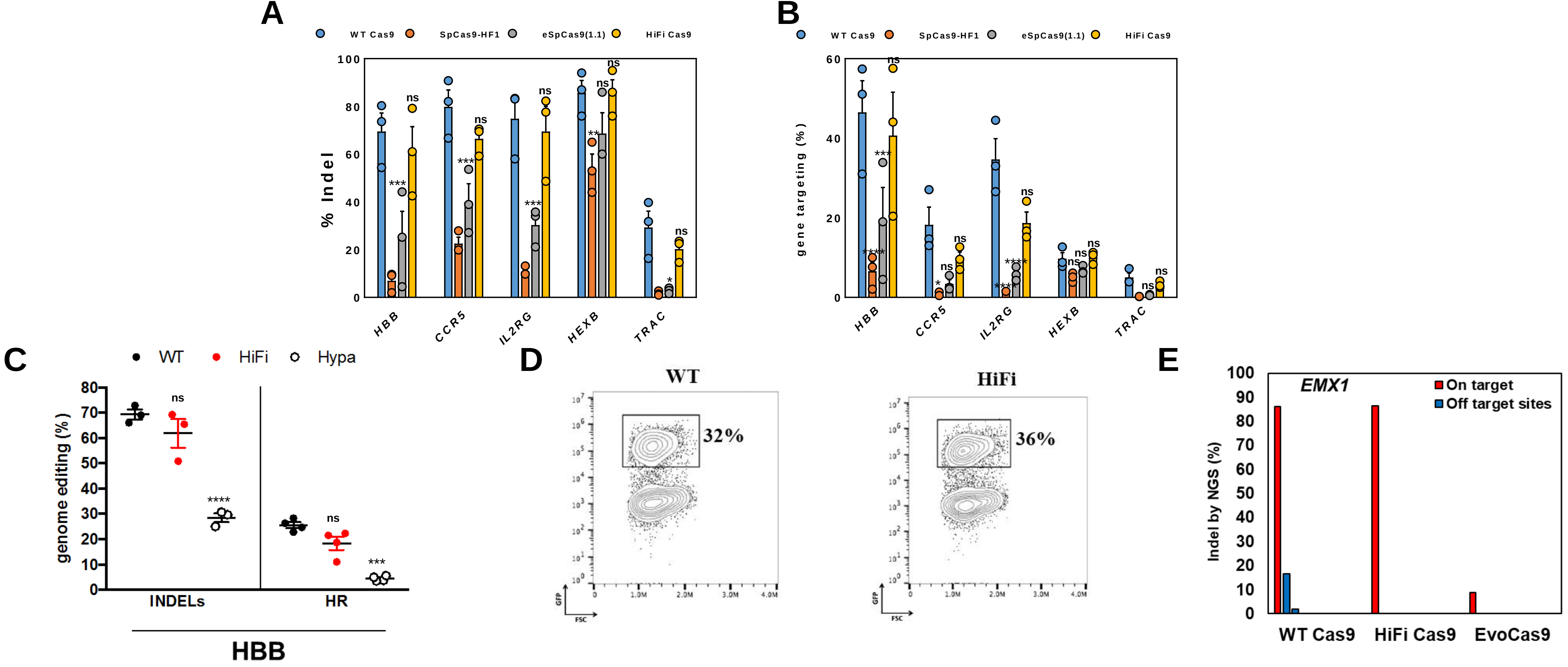
C



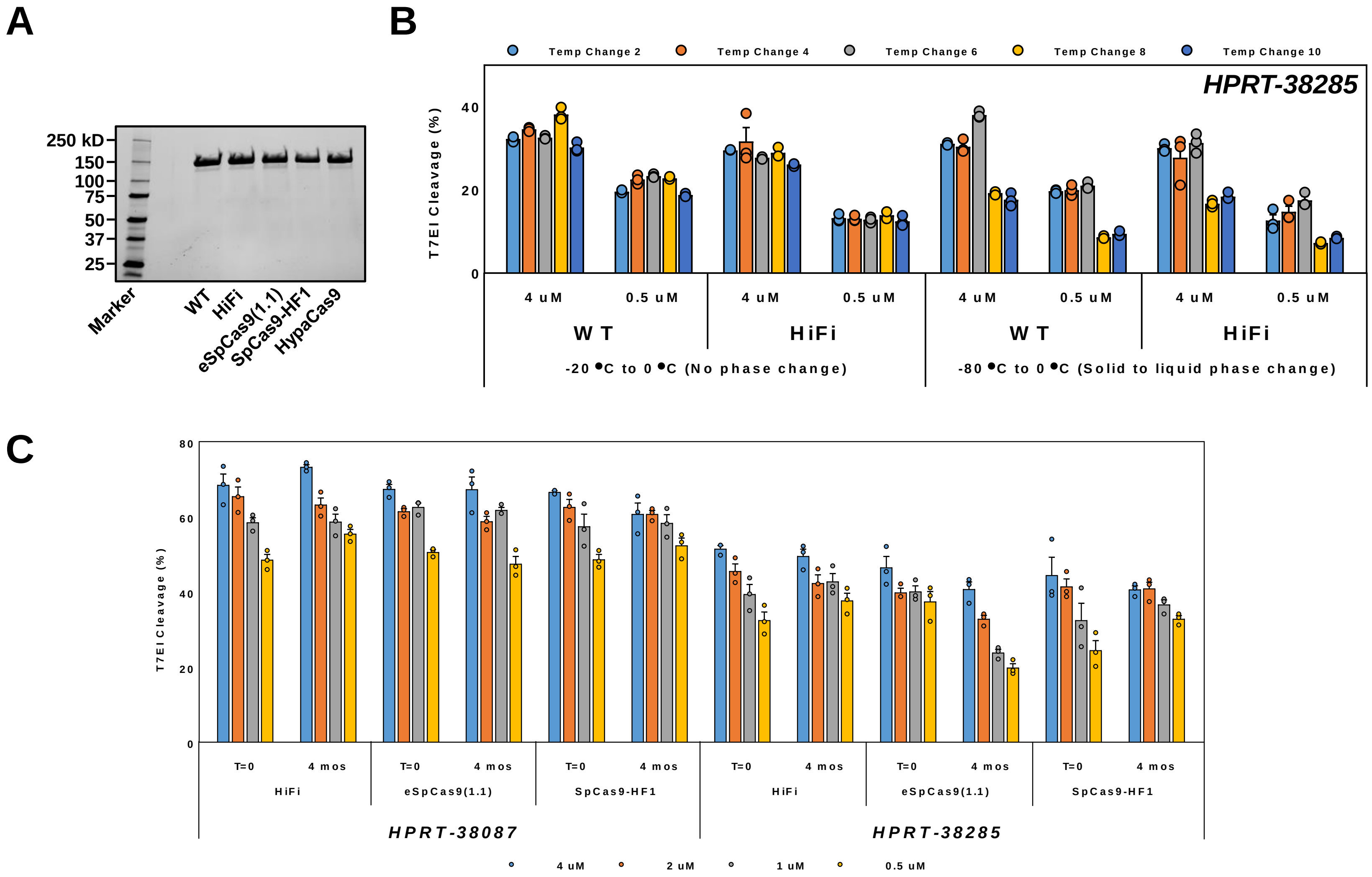
Supplementary Figure 4 Identification of Cas9 off-target sites used for amplicon-based sequencing. (A) Sequences of off-target sites identified by GUIDE-seq for *EMX1*, *AR*, and *HPRT 38087*. For each experiment the guide sequence is listed on top with off-target sites shown underneath and with mismatches to the on-target site indicated in color. The number of sequencing reads obtained for each site for either WT Cas9 or R691A "HiFi" Cas9 are indicated. Experiments were performed with HEK293-Cas9 or HEK293-Cas9-HiFi stable cell lines and 4 μM gRNA complexes delivered by electroporation. (B) List of *HBB* off-target sites investigated by next generation sequencing. Presented is a list of sites investigated in **Figure 5C** that detail the mismatches, closest gene, distance to the closest gene, and the location in the reference human genome. Off-targets 1-4 are the top 4 sites identified using GUIDE-Seq (data not shown). Off-target 5 was the top hit using the MIT CRISPR design tool. Off-targets 6 and 7 are two of the top three sites identified using the COSMID algorithm. The top hit identified in COSMID is off-target 1, which was the top hit identified by GUIDE-Seq. The nucleotides highlighted in red are mismatches compared to the on-target guide sequence targeting *HBB* exon 1. (C) The R691A HiFi mutation does not influence steady-state Cas9 expression levels in a HEK293 Cas9 stable cell line. A monoclonal HEK293 cell line that stably expresses WT Cas9 was isolated and determined to have a single copy of cas9 on the X chromosome. The R691A mutation was introduced by homology-directed repair (HDR) following Cas9 cleavage, and a monoclonal population was isolated and confirmed to have the correct sequence. Standard HEK293 (NTC), WT Cas9 (1), and HiFi Cas9 (2) cell lines were grown to confluency, and total protein samples were subjected to SDS-PAGE. Western blots were performed using a monoclonal Cas9 antibody and a β-actin antibody which served as a loading control. This experiment was repeated three times with similar results.



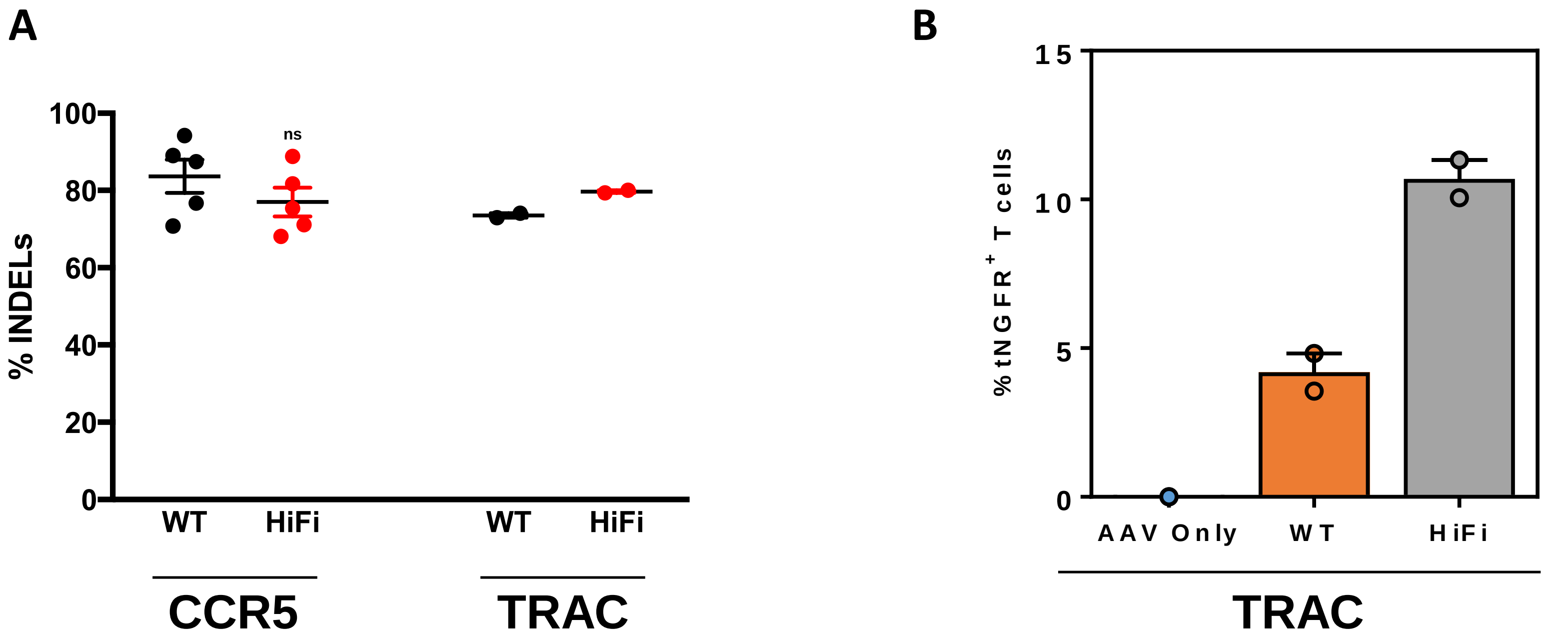
Supplementary Figure 5 Comparison of global off-target editing facilitated by WT and high-fidelity mutant Cas9 proteins. Total editing as determined by NGS for the *AR*, *GRHPR*, and *HBB* (top to bottom) gRNAs (4 µM) delivered into HEK293 cells that express WT (blue) or HiFi (orange) Cas9, or complexed to WT (blue), HiFi (orange), eSpCas9(1.1) (gray), SpCas9-HF1 (yellow), or HypaCas9 (dark blue) and delivered as RNP (4 µM) into standard HEK293 cells (left to right). INDEL formation percentages at the on-target loci are indicated directly above the on-target bars, other bars represent off-target amplicons. All amplicons are rank-ordered (highest to lowest) by INDEL formation percentage as determined for the WT Cas9 stable cell line. The Y-axis is plotted as log-10 scale.



Supplemental Figure 6 HiFi Cas9 exhibits superior INDEL formation and gene targeting in human CD34$^+$ HSCPs. (**A**) The raw data, which was presented normalized to WT Cas9 in **Figure 5a.** Data shows that the HiFi version consistently induces more INDELs compared to all other high fidelity mutants. (n=3, number of data points within each group, all from different HSPC cord blood donors). Bars represent mean. (**B**) Data shows the raw gene targeting frequencies that are shown in main text **Fig 5b**. HR for HBB and CCR5 was measured by GFP^{high} population, for HEXB and IL2RG HR was measured via ddPCR and for TRAC HR was measured by the tNGFR^{high} population. (n=3, number of data points within each group, all from different HSPC cord blood donors). Bars represent mean. (**C**) CD34$^+$ HSPCs were targeted with either WT, HiFi, or Hypa Cas9 RNPs and then half of the cells were transduced with a AAV6 *HBB* homologous UbC-GFP donor vector. Three days after electroporation, gDNA was harvested for INDEL analysis by TIDE and the cells that were transduced with AAV6 were then harvested for FACS analysis of targeted integration of UbC-GFP into the *HBB* locus. Bars represent mean, n=3 different HSPC cord donors. (**D**) Representative FACS image showing HSPCs with HR of a UbC-GFP donor into the HBB gene. (n=3-5, number of data points within each group, all from different HSPC cord blood donors). (**E**) Data shows the on-target performance of the EvoCas9 mutant as compared to WT and HiFi Cas9 with a crRNA that targets the *EMX1* locus. Statistical comparisons were made to WT Cas9 for on target NHEJ and HR activity. *P<.05, **P<.01, ***P<.001, ****P < 0.0001, NS (not significant) = P ≥ 0.05, two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. Bars represent mean.



Supplemental Figure 7. Characterization of recombinant Cas9 proteins and mutants. (A) SDS-PAGE gel demonstrating equivalent purity (>95%) between WT Cas9 and the indicated high-fidelity mutant. This experiment was performed three times with similar results. (B) Repeated phase changes begins to reduce Cas9 activity with either WT or HiFi after 8 phase change events. Storage under conditions that avoid phase change shows no difference in activity after 10 temperature changes. (C) The HiFi, eSpCas9(1.1), and SpCas9-HF1 Cas9 mutants show no evidence of loss in activity after 4 months of storage at -20 °C in 25 mM Tris-HCl (pH 7.4), 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, and 50% glycerol. Error bars represent mean + s.e.m., n=3 independent experiments.



Supplemental Figure 8. Equivalent double strand break activity of WT and HiFi Cas9 in primary human T-cells. **A**. T-cells were harvested from buffy coats as described in the materials and methods. T-cells were activated with anti-CD3/28 beads 3 days before electroporation. The cells were resuspended in electroporation solution, mixed with the pre-complexed RNP (either targeting CCR5 or TRAC loci) and then were electroporated using a 4D-Nucleofector with program EO-115. Genomic DNA was harvested 4 days after electroporation and INDELs were calculated for each locus using TIDE software (n=2-5, number of data points within each group, all from different healthy T-cell donors). Error bars represent mean + s.e.m. NS (not significant) = P $\geq$ 0.05, one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. Statistical comparisons were made to WT Cas9 for on target NHEJ activity. **B**. Activated T-cells were targeted at the endogenous TRAC locus with an AAV6 homologous donor vector that intended to knock-in frame T2A-CD19-T2A-tNGFR. Targeted cells were harvested 4 days post electroporation and stained for CD3 and tNGFR and then were analyzed on a Cytoflex flow cytometer (n=2, number of data points within each group, all from different T-cell donors). Statistical comparisons were made to WT Cas9 for on target NHEJ and HR activity. NS (not significant) = P ≥ 0.05, one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. Bars represent mean.

Plasmid	Description	Primers / Fragments
pUC19	High copy routine cloning vector – Cb^R	N/A
pIDTV-CcdB	pUC19 with lacYA177C and araC-PBAD-ccdB – Cb^R	lacYA177C and araC-PBAD-ccdB
pIDTV-CcdB-V3	pIDTV-CcdB with the *VEGFA3* on target site introduced by SDM – Cb^R	VEGFA3top-VEGFA3btm
pIDTV-CcdB-EMX1	pIDTV-CcdB with the *EMX1* on-target site introduced by SDM - Cb^R	ccdBemx1top-ccdBemx1btm
pACYCduet-1	Dual T7 promoter expression plasmid based on pACYC184 from Novagen - Cm^R	N/A
pIDTV-V3sg-off1	Cas9, *VEGFA3* sgRNA, and *VEGFA3* off target site cloned into NcoI/SacI/PstI of pACYCduet-1 – Cm^R	usecncocas-dsecsaccas, V3off1
pIDTV-EXsg-off1	Cas9, *EMX1* sgRNA, and *EMX1* off target site cloned into NcoI/SacI/PstI of pACYCduet-1 – Cm^R	usecncocas-dsecsaccas, EXoff1
Alt-R® S.p. Cas9 Expression Plasmid	Commercial Cas9 expression plasmid with mammalian promoter and NLS sequences – Cb^R	N/A
GeneArt® CRISPR Nuclease Vector	Commercial Cas9 expression plasmid with mammalian promoter and NLS sequences – Cb^R	N/A
pCDNA™3.1$^{(-)}$	Commercial expression plasmid with mammalian promoter and NLS sequences – Cb^R, Neo^R	N/A
pIDTG-Hs-Int-SpCas9	pCDNA3.1$^{(-)}$ with Cas9 ORF and NLS sequences for Hs integration – Cb^R, Neo^R	N/A
pET28b	*E. coli* protein expression plasmid – T7 Promoter – CTD His6 – Kn^R	N/A
pIDTV-NLS-C9-2NLS-H	T7 driven NLS-Cas9-NLS-NLS-HIS6 pET28 plasmid - Kn^R	NLS-Cas9-NLS-NLS

Supplemental Table 1 Description of plasmids

Oligo Name	Oligo Sequence	T7EI Annealing Temp (°C)
VEGFA3top	ATGTTCTGGGGAATATAATCTAGAGGTGAGTGAGTGTGTGCGTGTGGAAGCTTGGCTGTTTTGGCGGATGAG	64
VEGFA3btm	CTCATCCGCCAAAACAGCCAAGCTTCCACACGCACACACTCACTCACCTCTAGATTATATTCCCCAGAACAT	
ccdBemx1top	ATGTTCTGGGGAATATAATCTAGAGAGTCCGAGCAGAAGAAGAAGGGAAGCTTGGCTGTTTTGGCGGATGAG	N/A
ccdBemx1btm	CTCATCCGCCAAAACAGCCAAGCTTCCCTTCTTCTTCTGCTCGGACTCTCTAGATTATATTCCCCAGAACAT	
usecncocas	ACATCAGCACCATGGACAAAAAGTACTCTATTGGCCTGGATATC	N/A
Dsecsaccas	CAGTCACAGGAGCTCTTAATCCCCACCTAATTGGCTAAGGTCGATACG	N/A
HiFi HDR Donor	AAGGTCAAGCTGTCATCGTGAATCAGCTGCATGAAGTTGGCGTTGGCGAAGCCGTCGGACTTCAGGAAATCCAGGATGGT	N/A
HPRT T7EI FWD	AAGAATGTTGTGATAAAAGGTGATGCT	67
HPRT T7EI REV	ACACATCCATGGGACTTCTGCCTC	
CTLA4 T7EI FWD	AGAGCCAGGTCTTCTGTTTGTC	67
CTLA4 T7EI REV	GTTAGCACTCCAGAGCGAGAG	
PDCD1 T7EI FWD	AGGCTCTTAGTAGGAAATCAGGG	67
PDCD1 T7EI REV	ACCACCAGGGTTTGGAACTG	
EMX1 ontrgt T7EI FWD	CCACTCTGTGAAGAAGCGATTA	64
EMX1 ontrgt T7EI REV	CTTCCCTATGTCTAGCCTGTTTC	
EMX1 offtrgt1 T7EI FWD	TCACGAGCTGTCTATGAGGT	64
EMX1 offtrgt1 T7EI FWD	TGTCTTCCAGGAATGTGAAGTG	
VEGFA3 ontrgt T7EI FWD	CCAGATGGCACATTGTCAGA	64
VEGFA3 ontrgt T7EI REV	GGAGCAGGAAAGTGAGGTTAC	
VEGFA3 offtrgt1 T7EI FWD	AGGACTCACGTCGCTCTC	64
VEGFA3 offtrgt1 T7EI FWD	GGTCTGCGGACTACGACT	
HEKSite4 ontrgt T7EI FWD	CTGAGATCCTGTCCTTAGTTTACTG	64
HEKSite4 ontrgt T7EI REV	TTTCAACCCGAACGGAGAC	
HEKSite4 offtrgt3 T7EI FWD	GGGGAGCCTGAGAGGCCATTGTCAC	68
HEKSite4 offtrgt3 T7EI FWD	TACGGGGCCACCCTGAGCGCTGACT	

Supplemental Table 2 – Cloning and HDR oligos

Oligo Name	Oligo Sequence	Oligo Name	Oligo Sequence
Hs_K4N_top	GTGCCTGCGGCCGACAAAAATTACAGCATCGGCCTTGAT	Hs_K4N_btm	ATCAAGGCCGATGCTGTAATTTTTGTCGGCCGCAGGCAC
Hs_S15P_top	CTTGATATCGGCACCAATCCCGTGGGCTGGGCCGTTATC	Hs_S15P_btm	GATAACGGCCCAGCCCACGGGATTGGTGCCGATATCAAG
Hs_I21V_top	AGCGTGGGCTGGGCCGTTGTCACAGACGAATACAAGGTA	Hs_I21V_btm	TACCTTGTATTCGTCTGTGACAACGGCCCAGCCCACGCT
Hs_Y25C_top	GCCGTTATCACAGACGAATGCAAGGTACCCAGCAAGAAG	Hs_Y25C_btm	CTTCTTGCTGGGTACCTTGCATTCGTCTGTGATAACGGC
Hs_F32L_top	AAGGTACCCAGCAAGAAGTTGAAGGTGCTGGGGAATACA	Hs_F32L_btm	TGTATTCCCCAGCACCTTCAACTTCTTGCTGGGTACCTT
Hs_T58I_top	CTGTTTGACTCAGGCGAGATCGCCGAGGCCACCAGGTTG	Hs_T58I_btm	CAACCTGGTGGCCTCGGCGATCTCGCCTGAGTCAAACAG
Hs_T62M_top	GGCGAGACCGCCGAGGCCATGAGGTTGAAGAGGACCGCA	Hs_T62M_btm	TGCGGTCCTCTTCAACCTCATGGCCTCGGCGGTCTCGCC
Hs_N77K_top	AGGTACACCCGGAGGAAGAAAAGGATCTGCTATCTGCAG	Hs_N77K_btm	CTGCAGATAGCAGATCCTTTTCTTCCTCCGGGTGTACCT
Hs_A91P_top	ATCTTCAGCAACGAGATGCCCAAGGTGGACGACAGCTTC	Hs_A91P_btm	GAAGCTGTCGTCCACCTTGGGCATCTCGTTGCTGAAGAT
Hs_S96P_top	ATGGCCAAGGTGGACGACCCCTTCTTCCACAGGCTGGAG	Hs_S96P_btm	CTCCAGCCTGTGGAAGAAGGGGTCGTCCACCTTGGCCAT
Hs_Y128C_top	ATAGTCGACGAGGTCGCTTGTCACGAGAAGTACCCCACC	Hs_Y128C_btm	GGTGGGGTACTTCTCGTGACAAGCGACCTCGTCGACTAT
Hs_E130C_top	GACGAGGTCGCTTATCACTGCAAGTACCCCACCATCTAC	Hs_E130C_btm	GTAGATGGTGGGGTACTTGCAGTGATAAGCGACCTCGTC
Hs_L158S_top	CGACTTATCTACTTGGCTTCGGCGCACATGATTAAGTTC	Hs_L158S_btm	GAACTTAATCATGTGCGCCGAAGCCAAGTAGATAAGTCG
Hs_K163E_top	GCTCTGGCGCACATGATTGAGTTCAGGGGCCACTTCCTG	Hs_K163E_btm	CAGGAAGTGGCCCCTGAACTCAATCATGTGCGCCAGAGC
Hs_S179R_top	GACCTTAACCCCGACAACAGGGACGTAGACAAATTGTTC	Hs_S179R_btm	GAACAATTTGTCTACGTCCCTGTTGTCGGGGTTAAGGTC
Hs_Q187K_top	GTAGACAAATTGTTCATCAAGCTTGTACAGACCTATAAC	Hs_Q187K_btm	GTTATAGGTCTGTACAAGCTTGATGAACAATTTGTCTAC
Hs_N199T_top	AACCAGCTGTTCGAGGAAACCCCTATTAACGCCAGCGGG	Hs_N199T_btm	CCCGCTGGCGTTAATAGGGGTTTCCTCGAACAGCTGGTT
Hs_K209Q_top	GCCAGCGGGGTGGATGCGCAGGCCATACTTAGCGCCAGG	Hs_K209Q_btm	CCTGGCGCTAAGTATGGCCTGCGCATCCACCCCGCTGGC
Hs_S219A_top	AGCGCCAGGCTGAGCAAAGCCAGGCGCTTGGAGAACCTG	Hs_S219A_btm	CAGGTTCTCCAAGCGCCTGGCTTTGCTCAGCCTGGCGCT
Hs_L258P_top	TTCAAGAGCAACTTCGACCCGGCAGAAGATGCCAAGCTG	Hs_L258P_btm	CAGCTTGGCATCTTCTGCCGGGTCGAAGTTGCTCTTGAA
Hs_D272G_top	TTGAGTAAGGACACCTATGGCGACGACTTGGACAATCTG	Hs_D272G_btm	CAGATTGTCCAAGTCGTCGCCATAGGTGTCCTTACTCAA
Hs_Y286S_top	GCCCAAATCGGCGACCAGTCCGCTGACCTGTTCCTCGCC	Hs_Y286S_btm	GGCGAGGAACAGGTCAGCGGACTGGTCGCCGATTTGGGC
Hs_R307P_top	CTGCTTAGCGATATCCTTCCAGTGAACACAGAGATCACC	Hs_R307P_btm	GGTGATCTCTGTGTTCACTGGAAGGATATCGCTAAGCAG
Hs_M321I_top	GCCCCCCTGAGCGCCAGCATCATCAAGAGGTACGACGAG	Hs_M321I_btm	CTCGTCGTACCTCTTGATGATGCTGGCGCTCAGGGGGGC
Hs_S368R_top	TACATCGACGGCGGAGCCAGGCAAGAGGAGTTCTACAAG	Hs_S368R_btm	CTTGTAGAACTCCTCTTGCCTGGCTCCGCCGTCGATGTA
Hs_E387G_top	GAGAAGATGGATGGCACCGGGGAGCTGCTGGTGAAGCTG	Hs_E387G_btm	CAGCTTCACCAGCAGCTCCCCGGTGCCATCCATCTTCTC
Hs_R424C_top	GAGCTGCACGCAATACTGTGCCGACAGGAGGATTTCTAC	Hs_R424C_btm	GTAGAAATCCTCCTGTCGGCACAGTATTGCGTGCAGCTC
Hs_N459K_top	GGCCCTCTTGCCAGGGGCAAAAGCCGATTCGCTTGGATG	Hs_N459K_btm	CATCCAAGCGAATCGGCTTTTGCCCCTGGCAAGAGGGCC
Hs_W464C_top	GGCAACAGCCGATTCGCTTGTATGACAAGAAAGAGCGAG	Hs_W464C_btm	CTCGCTCTTTCTTGTCATACAAGCGAATCGGCTGTTGCC
Hs_R494C_top	GCGCAGTCTTTCATCGAATGTATGACCAATTTCGACAAA	Hs_R494C_btm	TTTGTCGAAATTGGTCATACATTCGATGAAAGACTGCGC
Hs_S512P_top	AAGGTGCTGCCCAAGCACCCCCTGCTTTACGAGTACTTC	Hs_S512P_btm	GAAGTACTCGTAAAGCAGGGGGTGCTTGGGCAGCACCTT
Hs_N522K_top	GAGTACTTCACCGTGTACAAAGAGCTCACCAAGGTGAAA	Hs_N522K_btm	TTTCACCTTGGTGAGCTCTTTGTACACGGTGAAGTACTC
Hs_D550N_top	CAGAAGAAGGCCATCGTGAACCTGCTGTTCAAGACCAAC	Hs_D550N_btm	GTTGGTCTTGAACAGCAGGTTCACGATGGCCTTCTTCTG
Hs_K570I_top	CTGAAGGAGGACTACTTCATAAAGATCGAGTGCTTTGAT	Hs_K570I_btm	ATCAAAGCACTCGATCTTTATGAAGTAGTCCTCCTTCAG
Hs_N588D_top	GGCGTGGAGGACAGGTTCGACGCCAGCCTGGGCACCTAC	Hs_N588D_btm	GTAGGTGCCCAGGCTGGCGTCGAACCTGTCCTCCACGCC
Hs_I600M_top	CACGACTTGTTGAAGATAATGAAAGACAAGGATTTCCTG	Hs_I600M_btm	CAGGAAATCCTTGTCTTTCATTATCTTCAACAAGTCGTG
Hs_N612A_top	TTCCTGGATAATGAGGAGGCCGAGGATATACTCGAGGAC	Hs_N612A_btm	GTCCTCGAGTATATCCTCGGCCTCCTCATTATCCAGGAA
Hs_T657A_top	CTGAAGAGACGAAGATACGCCGGCTGGGGCAGACTGTCC	Hs_T657A_btm	GGACAGTCTGCCCCAGCCGGCGTATCTTCGTCTCTTCAG
Hs_S663A_top	ACCGGCTGGGGCAGACTGGCCAGGAAGCTCATCAACGGC	Hs_S663A_btm	GCCGTTGATGAGCTTCCTGGCCAGTCTGCCCCAGCCGGT
Hs_R664S_top	GGCTGGGGCAGACTGTCCAGCAAGCTCATCAACGGCATT	Hs_R664S_btm	AATGCCGTTGATGAGCTTGCTGGACAGTCTGCCCCAGCC
Hs_N668K_top	CTGTCCAGGAAGCTCATCAAAGGCATTAGGGACAAGCAG	Hs_N668K_btm	CTGCTTGTCCCTAATGCCTTTGATGAGCTTCCTGGACAG
Hs_R671A_top	AAGCTCATCAACGGCATTGCGGACAAGCAGAGCGGCAAG	Hs_R671A_btm	CTTGCCGCTCTGCTTGTCCGCAATGCCGTTGATGAGCTT
Hs_S675P_top	GGCATTAGGGACAAGCAGCCCGGCAAGACCATCCTGGAT	Hs_S675P_btm	ATCCAGGATGGTCTTGCCGGGCTGCTTGTCCCTAATGCC
Hs_T678M_top	GACAAGCAGAGCGGCAAGATGATCCTGGATTTCCTGAAG	Hs_T678M_btm	CTTCAGGAAATCCAGGATCATCTTGCCGCTCTGCTTGTC
Hs_S685A_top	ATCCTGGATTTCCTGAAGGCCGACGGCTTCGCCAACCGA	Hs_S685A_btm	TCGGTTGGCGAAGCCGTCGGCCTTCAGGAAATCCAGGAT
Hs_R691S_top	TCCGACGGCTTCGCCAACAGTAACTTCATGCAGCTGATT	Hs_R691S_btm	AATCAGCTGCATGAAGTTACTGTTGGCGAAGCCGTCGGA
Hs_N692D_top	GACGGCTTCGCCAACCGAGACTTCATGCAGCTGATTCAC	Hs_N692D_btm	GTGAATCAGCTGCATGAAGTCTCGGTTGGCGAAGCCGTC
Hs_S701A_top	CAGCTGATTCACGATGACGCTTTGACCTTCAAGGAGGAC	Hs_S701A_btm	GTCCTCCTTGAAGGTCAAAGCGTCATCGTGAATCAGCTG
Hs_T703P_top	ATTCACGATGACAGCTTGCCCTTCAAGGAGGACATCCAG	Hs_T703P_btm	CTGGATGTCCTCCTTGAAGGGCAAGCTGTCATCGTGAAT
Hs_S714A_top	ATCCAGAAGGCCCAGGTTGCTGGCCAGGGCGACTCCCTG	Hs_S714A_btm	CAGGGAGTCGCCCTGGCCAGCAACCTGGGCCTTCTGGAT
Hs_S719R_top	GTTAGCGGCCAGGGCGACAGGCTGCACGAACATATTGCA	Hs_S719R_btm	TGCAATATGTTCGTGCAGCCTGTCGCCCTGGCCGCTAAC
Hs_N726Y_top	CTGCACGAACATATTGCATACCTGGCAGGCTCCCCTGCG	Hs_N726Y_btm	CGCAGGGGAGCCTGCCAGGTATGCAATATGTTCGTGCAG
Hs_S730G_top	ATTGCAAACCTGGCAGGCGGCCCTGCGATCAAGAAGGGC	Hs_S730G_btm	GCCCTTCTTGATCGCAGGGCCGCCTGCCAGGTTTGCAAT
Hs_T740A_top	AAGAAGGGCATACTGCAGGCCGTTAAGGTTGTGGACGAA	Hs_T740A_btm	TTCGTCCACAACCTTAACGGCCTGCAGTATGCCCTTCTT
Hs_R753S_top	TTGGTCAAGGTCATGGGCAGCCACAAGCCCGAAAACATA	Hs_R753S_btm	TATGTTTTCGGGCTTGTGGCTGCCCATGACCTTGACCAA
Hs_N758K_top	GGCAGGCACAAGCCCGAAAAAATAGTTATAGAGATGGCC	Hs_N758K_btm	GGCCATCTCTATAACTATTTTTTCGGGCTTGTGCCTGCC
Hs_R765G_top	ATAGTTATAGAGATGGCCGGAGAGAACCAGACCACCCAA	Hs_R765G_btm	TTGGGTGGTCTGGTTCTCTCCGGCCATCTCTATAACTAT
Hs_N767A_top	ATAGAGATGGCCAGAGAGGCCCAGACCACCCAAAAGGGC	Hs_N767A_btm	GCCCTTTTGGGTGGTCTGGGCCTCTCTGGCCATCTCTAT
Hs_T770K_top	GCCAGAGAGAACCAGACCAAACAAAAGGGCCAGAAGAAC	Hs_T770K_btm	GTTCTTCTGGCCCTTTTGTTTGGTCTGGTTCTCTCTGGC
Hs_N776A_top	ACCCAAAAGGGCCAGAAGGCCAGCCGGGAGCGCATGAAA	Hs_N776A_btm	TTTCATGCGCTCCCGGCTGGCCTTCTGGCCCTTTTGGGT
Hs_R778S_top	AAGGGCCAGAAGAACAGCAGCGAGCGCATGAAAAGGATC	Hs_R778S_btm	GATCCTTTTCATGCGCTCGCTGCTGTTCTTCTGGCCCTT
Hs_R783K_top	AGCCGGGAGCGCATGAAAAAGATCGAGGAGGGTATCAAG	Hs_R783K_btm	CTTGATACCCTCCTCGATCTTTTTCATGCGCTCCCGGCT
Hs_S793A_top	GGTATCAAGGAACTCGGAGCCCAGATCCTCAAAGAGCAC	Hs_S793A_btm	GTGCTCTTTGAGGATCTGGGCTCCGAGTTCCTTGATACC
Hs_N803D_top	AAAGAGCACCCCGTGGAGGACACCCAGCTCCAGAACGAG	Hs_N803D_btm	CTCGTTCTGGAGCTGGGTGTCCTCCACGGGGTGCTCTTT
Hs_N818H_top	TACCTGTACTACCTGCAGCACGGCAGGGACATGTACGTT	Hs_N818H_btm	AACGTACATGTCCCTGCCGTGCTGCAGGTAGTACAGGTA
Hs_R820W_top	TACTACCTGCAGAACGGCTGGGACATGTACGTTGACCAG	Hs_R820W_btm	CTGGTCAACGTACATGTCCCAGCCGTTCTGCAGGTAGTA
Hs_N831K_top	GACCAGGAGTTGGACATCAAAAGGCTTTCAGACTATGAC	Hs_N831K_btm	GTCATAGTCTGAAAGCCTTTTGATGTCCAACTCCTGGTC
Hs_R832G_top	CAGGAGTTGGACATCAACGGGCTTTCAGACTATGACGTG	Hs_R832G_btm	CACGTCATAGTCTGAAAGCCCGTTGATGTCCAACTCCTG
Hs_S834P_top	TTGGACATCAACAGGCTTCCAGACTATGACGTGGATCAC	Hs_S834P_btm	GTGATCCACGTCATAGTCTGGAAGCCTGTTGATGTCCAA
Hs_S845A_top	GATCACATAGTGCCCCAGGCCTTTCTTAAAGACGATAGC	Hs_S845A_btm	GCTATCGTCTTTAAGAAAGGCCTGGGGCACTATGTGATC
Hs_S851A_top	AGCTTTCTTAAAGACGATGCCATCGACAACAAGGTCCTG	Hs_S851A_btm	CAGGACCTTGTTGTCGATGGCATCGTCTTTAAGAAAGCT
Hs_N854K_top	AAAGACGATAGCATCGACAAAAAGGTCCTGACCCGCTCC	Hs_N854K_btm	GGAGCGGGTCAGGACCTTTTTGTCGATGCTATCGTCTTT
Hs_R859S_top	GACAACAAGGTCCTGACCAGCTCCGACAAAAACAGGGGC	Hs_R859S_btm	GCCCCTGTTTTTGTCGGAGCTGGTCAGGACCTTGTTGTC
Hs_S860A_top	AACAAGGTCCTGACCCGCGCCGACAAAAACAGGGGCAAA	Hs_S860A_btm	TTTGCCCCTGTTTTTGTCGGCGCGGGTCAGGACCTTGTT
Hs_R864C_top	ACCCGCTCCGACAAAAACTGTGGCAAAAGCGACAACGTG	Hs_R864C_btm	CACGTTGTCGCTTTTGCCACAGTTTTTGTCGGAGCGGGT
Hs_S867R_top	GACAAAAACAGGGGCAAAAGGGACAACGTGCCAAGCGAA	Hs_S867R_btm	TTCGCTTGGCACGTTGTCCCTTTTGCCCCTGTTTTTGTC
Hs_N869K_top	AACAGGGGCAAAAGCGACAAAGTGCCAAGCGAAGAGGTG	Hs_N869K_btm	CACCTCTTCGCTTGGCACTTTGTCGCTTTTGCCCCTGTT
Hs_S872A_top	AAAAGCGACAACGTGCCAGCCGAAGAGGTGGTTAAAAAG	Hs_S872A_btm	CTTTTTAACCACCTCTTCGGCTGGCACGTTGTCGCTTTT
Hs_N979I_top	TACAAGGTGAGGGAGATCATCAACTACCACCATGCCCAC	Hs_N979I_btm	GTGGGCATGGTGGTAGTTGATGATCTCCCTCACCTTGTA
Hs_L1004Q_top	ATTAAGAAGTATCCAAAGCAGGAGTCCGAATTTGTCTAC	Hs_L1004Q_btm	GTAGACAAATTCGGACTCCTGCTTTGGATACTTCTTAAT
Hs_I1050F_top	AACTTTTTCAAGACCGAGTTCACACTTGCCAACGGCGAA	Hs_I1050F_btm	TTCGCCGTTGGCAAGTGTGAACTCGGTCTTGAAAAAGTT
Hs_E1056G_top	ATCACACTTGCCAACGGCGGAATCAGGAAGAGGCCGCTT	Hs_E1056G_btm	AAGCGGCCTCTTCCTGATTCCGCCGTTGGCAAGTGTGAT
Hs_T1065P_top	AAGAGGCCGCTTATCGAGCCCAACGGTGAGACCGGCGAG	Hs_T1065P_btm	CTCGCCGGTCTCACCGTTGGGCTCGATAAGCGGCCTCTT
Hs_K1107M_top	CAGACAGGCGGCTTTAGCATGGAATCCATCCTGCCCAAG	Hs_K1107M_btm	CTTGGGCAGGATGGATTCCATGCTAAAGCCGCCTGTCTG
Hs_V1146Y_top	GCCTACAGCGTGCTGGTGTACGCGAAGGTAGAGAAGGGG	Hs_V1146Y_btm	CCCCTTCTCTACCTTCGCGTACACCAGCACGCTGTAGGC
Hs_S1159A_top	AAGAGCAAGAAACTGAAGGCCGTGAAGGAGCTGCTCGGC	Hs_S1159A_btm	GCCGAGCAGCTCCTTCACGGCCTTCAGTTTCTTGCTCTT
Hs_E1162Q_top	AAACTGAAGAGCGTGAAGCAGCTGCTCGGCATAACCATC	Hs_E1162Q_btm	GATGGTTATGCCGAGCAGCTGCTTCACGCTCTTCAGTTT
Hs_K1197M_top	AAAAAGGACCTGATCATCATGCTCCCCAAGTACTCCCTG	Hs_K1197M_btm	CAGGGAGTACTTGGGGAGCATGATGATCAGGTCCTTTTT
Hs_E1207K_top	TACTCCCTGTTTGAATTGAAGAACGGCAGAAAGAGGATG	Hs_E1207K_btm	CATCCTCTTTCTGCCGTTCTTCAATTCAAACAGGGAGTA
Hs_P1229R_top	GGCAACGAACTGGCGCTGCGCAGCAAGTACGTGAATTTT	Hs_P1229R_btm	AAAATTCACGTACTTGCTGCGCAGCGCCAGTTCGTTGCC
Hs_L1282Q_top	TTCAGCAAGAGGGTGATTCAGGCCGACGCGAACCTGGAT	Hs_L1282Q_btm	ATCCAGGTTCGCGTCGGCCTGAATCACCCTCTTGCTGAA
Hs_F1313S_top	GAGAATATCATACACCTGTCCACCCTGACAAATCTGGGC	Hs_F1313S_btm	GCCCAGATTTGTCAGGGTGGACAGGTGTATGATATTCTC
Hs_D1344N_top	AGCACTAAGGAGGTGCTGAATGCCACCTTGATCCACCAG	Hs_D1344N_btm	CTGGTGGATCAAGGTGGCATTCAGCACCTCCTTAGTGCT
Hs_T1353P_top	TTGATCCACCAGTCCATTCCCGGCCTGTATGAGACCAGG	Hs_T1353P_btm	CCTGGTCTCATACAGGCCGGGAATGGACTGGTGGATCAA
Hs_N522A_top	GAGTACTTCACCGTGTACGCGGAGCTCACCAAGGTGAAA	Hs_N522A_btm	TTTCACCTTGGTGAGCTCCGCGTACACGGTGAAGTACTC
Hs_N588A_top	GGCGTGGAGGACAGGTTCGCGGCCAGCCTGGGCACCTAC	Hs_N588A_btm	GTAGGTGCCCAGGCTGGCCGCGAACCTGTCCTCCACGCC
Hs_R691A_top	TCCGACGGCTTCGCCAACGCGAACTTCATGCAGCTGATT	Hs_R691A_btm	AATCAGCTGCATGAAGTTCGCGTTGGCGAAGCCGTCGGA
Hs_N692A_top	GACGGCTTCGCCAACCGAGCGTTCATGCAGCTGATTCAC	Hs_N692A_btm	GTGAATCAGCTGCATGAACGCTCGGTTGGCGAAGCCGTC
Hs_S730A_top	ATTGCAAACCTGGCAGGCGCGCCTGCGATCAAGAAGGGC	Hs_S730A_btm	GCCCTTCTTGATCGCAGGCGCGCCTGCCAGGTTTGCAAT
Hs_R765A_top	ATAGTTATAGAGATGGCCGCGGAGAACCAGACCACCCAA	Hs_R765A_btm	TTGGGTGGTCTGGTTCTCCGCGGCCATCTCTATAACTAT
Hs_T770A_top	GCCAGAGAGAACCAGACCGCGCAAAAGGGCCAGAAGAAC	Hs_T770A_btm	GTTCTTCTGGCCCTTTTGCGCGGTCTGGTTCTCTCTGGC
Hs_N803A_top	AAAGAGCACCCCGTGGAGGCGACCCAGCTCCAGAACGAG	Hs_N803A_btm	CTCGTTCTGGAGCTGGGTCGCCTCCACGGGGTGCTCTTT
Hs_N854A_top	AAAGACGATAGCATCGACGCGAAGGTCCTGACCCGCTCC	Hs_N854A_btm	GGAGCGGGTCAGGACCTTCGCGTCGATGCTATCGTCTTT
Hs_R691A-N692A_top	AAGTCCGACGGCTTCGCCAACGCGGCGTTCATGCAGCTGATTCACGAT	Hs_R691A-N692A_btm	ATCGTGAATCAGCTGCATGAACGCCGCGTTGGCGAAGCCGTCGGACTT
Hs_R691N_top	TCCGACGGCTTCGCCAACAACAACTTCATGCAGCTGATT	Hs_R691N_btm	AATCAGCTGCATGAAGTTGTTGTTGGCGAAGCCGTCGGA
Hs_R691D_top	TCCGACGGCTTCGCCAACGATAACTTCATGCAGCTGATT	Hs_R691D_btm	AATCAGCTGCATGAAGTTATCGTTGGCGAAGCCGTCGGA
Hs_R691C_top	TCCGACGGCTTCGCCAACTGCAACTTCATGCAGCTGATT	Hs_R691C_btm	AATCAGCTGCATGAAGTTGCAGTTGGCGAAGCCGTCGGA
Hs_R691Q_top	TCCGACGGCTTCGCCAACCAGAACTTCATGCAGCTGATT	Hs_R691Q_btm	AATCAGCTGCATGAAGTTCTGGTTGGCGAAGCCGTCGGA
Hs_R691E_top	TCCGACGGCTTCGCCAACGAAAACTTCATGCAGCTGATT	Hs_R691E_btm	AATCAGCTGCATGAAGTTTTCGTTGGCGAAGCCGTCGGA
Hs_R691G_top	TCCGACGGCTTCGCCAACGGCAACTTCATGCAGCTGATT	Hs_R691G_btm	AATCAGCTGCATGAAGTTGCCGTTGGCGAAGCCGTCGGA
Hs_R691H_top	TCCGACGGCTTCGCCAACCATAACTTCATGCAGCTGATT	Hs_R691H_btm	AATCAGCTGCATGAAGTTATGGTTGGCGAAGCCGTCGGA
Hs_R691I_top	TCCGACGGCTTCGCCAACATTAACTTCATGCAGCTGATT	Hs_R691I_btm	AATCAGCTGCATGAAGTTAATGTTGGCGAAGCCGTCGGA
Hs_R691L_top	TCCGACGGCTTCGCCAACCTGAACTTCATGCAGCTGATT	Hs_R691L_btm	AATCAGCTGCATGAAGTTCAGGTTGGCGAAGCCGTCGGA
Hs_R691K_top	TCCGACGGCTTCGCCAACAAAAACTTCATGCAGCTGATT	Hs_R691K_btm	AATCAGCTGCATGAAGTTTTTGTTGGCGAAGCCGTCGGA
Hs_R691M_top	TCCGACGGCTTCGCCAACATGAACTTCATGCAGCTGATT	Hs_R691M_btm	AATCAGCTGCATGAAGTTCATGTTGGCGAAGCCGTCGGA
Hs_R691F_top	TCCGACGGCTTCGCCAACTTTAACTTCATGCAGCTGATT	Hs_R691F_btm	AATCAGCTGCATGAAGTTAAAGTTGGCGAAGCCGTCGGA
Hs_R691P_top	TCCGACGGCTTCGCCAACCCGAACTTCATGCAGCTGATT	Hs_R691P_btm	AATCAGCTGCATGAAGTTCGGGTTGGCGAAGCCGTCGGA
Hs_R691T_top	TCCGACGGCTTCGCCAACACCAACTTCATGCAGCTGATT	Hs_R691T_btm	AATCAGCTGCATGAAGTTGGTGTTGGCGAAGCCGTCGGA
Hs_R691W_top	TCCGACGGCTTCGCCAACTGGAACTTCATGCAGCTGATT	Hs_R691W_btm	AATCAGCTGCATGAAGTTCCAGTTGGCGAAGCCGTCGGA
Hs_R691Y_top	TCCGACGGCTTCGCCAACTATAACTTCATGCAGCTGATT	Hs_R691Y_btm	AATCAGCTGCATGAAGTTATAGTTGGCGAAGCCGTCGGA
Hs_R691V_top	TCCGACGGCTTCGCCAACGTGAACTTCATGCAGCTGATT	Hs_R691V_btm	AATCAGCTGCATGAAGTTCACGTTGGCGAAGCCGTCGGA
Hs_N497A_top	TTCATCGAACGGATGACCGCGTTCGACAAAAACCTGCCT	Hs_N497A_btm	AGGCAGGTTTTTGTCGAACGCGGTCATCCGTTCGATGAA
Hs_R661A_top	AGATACACCGGCTGGGGCGCGCTGTCCAGGAAGCTCATC	Hs_R661A_btm	GATGAGCTTCCTGGACAGCGCGCCCCAGCCGGTGTATCT
Hs_Q695A_top	GCCAACCGAAACTTCATGGCGCTGATTCACGATGACAGC	Hs_Q695A_btm	GCTGTCATCGTGAATCAGCGCCATGAAGTTTCGGTTGGC
Hs_Q926A_top	CAGCTGGTCGAGACCCGAGCGATCACGAAGCACGTGGCC	Hs_Q926A_btm	GGCCACGTGCTTCGTGATCGCTCGGGTCTCGACCAGCTG

Supplemental Table 2 (Continued)
Hs-optimized Cas9 SDM oligos

Oligo Name	Oligo Sequence	Oligo Name	Oligo Sequence
Ec_R691A_top	TCGGACGGATTTGCTAATGCGAACTTCATGCAGTTGATC	Ec_R691A_btm	GATCAACTGCATGAAGTTCGCATTAGCAAATCCGTCCGA
Ec_N692A_top	GACGGATTTGCTAATCGCGCGTTCATGCAGTTGATCCAT	Ec_N692A_btm	ATGGATCAACTGCATGAACGCGCGATTAGCAAATCCGTC
Ec_T740A_top	AAAAAGGGGATCCTCCAGGCGGTGAAAGTTGTAGATGAG	Ec_T740A_btm	CTCATCTACAACTTTCACCGCCTGGAGGATCCCCTTTTT
Ec_S845A_top	GATCATATCGTCCCGCAGGCGTTCCTCAAAGACGATTCT	Ec_S845A_btm	AGAATCGTCTTTGAGGAACGCCTGCGGGACGATATGATC
Ec_S872A_top	AAGTCGGACAACGTGCCCGCGGAAGAGGTTGTGAAAAAG	Ec_S872A_btm	CTTTTTCACAACCTCTTCCGCGGGCACGTTGTCCGACTT
Ec_R691A-N692A_top	TCGGACGGATTTGCTAATGCGGCGTTCATGCAGTTGATCCAT	Ec_R691A-N692A_btm	ATGGATCAACTGCATGAACGCCGCATTAGCAAATCCGTCCGA
Ec_R691D_top	TCGGACGGATTTGCTAATGATAACTTCATGCAGTTGATC	Ec_R691D_btm	GATCAACTGCATGAAGTTATCATTAGCAAATCCGTCCGA
Ec_R691G_top	TCGGACGGATTTGCTAATGGCAACTTCATGCAGTTGATC	Ec_R691G_btm	GATCAACTGCATGAAGTTGCCATTAGCAAATCCGTCCGA
Ec_R691H_top	TCGGACGGATTTGCTAATCATAACTTCATGCAGTTGATC	Ec_R691H_btm	GATCAACTGCATGAAGTTATGATTAGCAAATCCGTCCGA
Ec_R691Y_top	TCGGACGGATTTGCTAATTATAACTTCATGCAGTTGATC	Ec_R691Y_btm	GATCAACTGCATGAAGTTATAATTAGCAAATCCGTCCGA
Ec_R691W_top	TCGGACGGATTTGCTAATTGGAACTTCATGCAGTTGATC	Ec_R691W_btm	GATCAACTGCATGAAGTTCCAATTAGCAAATCCGTCCGA
Ec_N497A_top	TTTATTGAACGTATGACGGCGTTCGATAAAAACTTGCCC	Ec_N497A_btm	GGGCAAGTTTTTATCGAACGCCGTCATACGTTCAATAAA
Ec_R661A_top	CGCTACACAGGATGGGGCGCGTTGAGTCGCAAACTTATT	Ec_R661A_btm	AATAAGTTTGCGACTCAACGCGCCCCATCCTGTGTAGCG
Ec_Q695A_top	GCTAATCGCAACTTCATGGCGTTGATCCATGATGACTCC	Ec_Q695A_btm	GGAGTCATCATGGATCAACGCCATGAAGTTGCGATTAGC
Ec_Q926A_top	CAGTTAGTGGAGACTCGTGCGATCACCAAACATGTCGCG	Ec_Q926A_btm	CGCGACATGTTTGGTGATCGCACGAGTCTCCACTAACTG
Ec_K848A_top	GTCCCGCAGAGCTTCCTCGCGGACGATTCTATTGACAAT	Ec_K848A_btm	ATTGTCAATAGAATCGTCCGCGAGGAAGCTCTGCGGGAC
Ec_K1003A_top	CTTATCAAGAAATATCCTGCGCTGGAGTCGGAGTTTGTC	Ec_K1003A_btm	GACAAACTCCGACTCCAGCGCAGGATATTTCTTGATAAG
Ec_R1060A_top	AACGGGGAGATTCGCAAAGCGCCGTTGATCGAAACAAAC	Ec_R1060A_btm	GTTTGTTTCGATCAACGGCGCTTTGCGAATCTCCCCGTT
Ec_hypa_top	TTCTTGAAGTCGGACGGATTTGCTAATCGCGCCTTCGCCGCCTTGATCGCCGATGACTCCCTGACTTTTAAAGAGGATATT	Ec_hypa_btm	AATATCCTCTTTAAAAGTCAGGGAGTCATCGGCGATCAAGGCGGCGAAGGCGCGATTAGCAAATCCGTCCGACTTCAAGAA

Supplemental Table 2 (Continued) – Ec-optimized Cas9 SDM oligos

gBlock name	Sequence (restriction sites in bold)
lacYA177C	ACGCGATAC**GAATTC**CCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGAATCACACAGGAAACAGAATTATGTACTATTTAAAAAACACAAACTTTTGGATGTTCGGTTTATTCTTTTTCTTTTACTTTTTTATCATGGGAGCCTACTTCCCGTTTTTCCCGATTTGGCTACATGACATCAACCATATCAGCAAAAGTGATACGGGTATTATTTTTGCCGCTATTTCTCTGTTCTCGCTATTATTCCAACCGCTGTTTGGTCTGCTTTCTGACAAACTCGGGCTGCGCAAATACCTGCTGTGGATTATTACCGGCATGTTAGTGATGTTTGCGCCGTTCTTTATTTTTATCTTCGGGCCACTGTTACAATACAACATTTTAGTAGGATCGATTGTTGGTGGTATTTATCTAGGCTTTTGTTTTAACGCCGGTGCGCCAGCAGTAGAGGCATTTATTGAGAAAGTCAGCCGTCGCAGTAATTTCGAATTTGGTCGCGCGCGGATGTTTGGCTGTGTTGGCTGGGCGCTGTGTGCCTCGATTGTCGGCATCATGTTCACCATCAATAATCAGTTTGTTTTCTGGCTGGGCTCTGGCTGTTGCCTCATCCTCGCCGTTTTACTCTTTTTCGCCAAAACGGATGCGCCCTCTTCTGCCACGGTTGCCAATGCGGTAGGTGCCAACCATTCGGCATTTAGCCTTAAGCTGGCACTGGAACTGTTCAGACAGCCAAAACTGTGGTTTTTGTCACTGTATGTTATTGGCGTTTCCTGCACCTACGATGTTTTTGACCAACAGTTTGCTAATTTCTTTACTTCGTTCTTTGCTACCGGTGAACAGGGTACGCGGGTATTTGGCTACGTAACGACAATGGGCGAATTACTTAACGCCTCGATTATGTTCTTTGCGCCACTGATCATTAATCGCATCGGTGGGAAAAACGCCCTGCTGCTGGCTGGCACTATTATGTCTGTACGTATTATTGGCTCATCGTTCGCCACCTCAGCGCTGGAAGTGGTTATTCTGAAAACGCTGCATATGTTTGAAGTACCGTTCCTGCTGGTGGGCTGCTTTAAATATATTACCAGCCAGTTTGAAGTGCGTTTTTCAGCGACGATTTATCTGGTCTGTTTCTGCTTCTTTAAGCAACTGGCGATGATTTTTATGTCTGTACTGGCGGGCAATATGTATGAAAGCATCGGTTTCCAGGGCGCTTATCTGGTGCTGGGTCTGGTGGCGCTGGGCTTCACCTTAATTTCCGTGTTCACGCTTAGCGGCCCCGGCCCGCTTTCCCTGCTGCGTCGTCAGGTGAATGAAGTCGCTTAAGCAATCAATGTCGGATGCGGACCGGGAGCGCTGTGAATACAGTGCTCCCTTTTTTTATT**GAGCTC**ACTTCACAC
araC-PBAD-ccdB	CGTTACCAA**GGTACC**TTATGACAACTTGACGGCTACATCATTCACTTTTTCTTCACAACCGGCACGGAACTCGCTCGGGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAATAGAGTTGATCGTCAAAACCAACATTGCGACCGACGGTGGCGATAGGCATCCGGGTGGTGCTCAAAAGCAGCTTCGCCTGGCTGATACGTTGGTCCTCGCGCCAGCTTAAGACGCTAATCCCTAACTGCTGGCGGAAAAGATGTGACAGACGCGACGGCGACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATTGCTGTCTGCCAGGTGATCGCTGATGTACTGACAAGCCTCGCGTACCCGATTATCCATCGGTGGATGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAACAATTGCTCAAGCAGATTTATCGCCAGCAGCTCCGAATAGCGCCCTTCCCCTTGCCCGGCGTTAATGATTTGCCCAAACAGGTCGCTGAAATGCGGCTGGTGCGCTTCATCCGGGCGAAAGAACCCCGTATTGGCAAATATTGACGGCCAGTTAAGCCATTCATGCCAGTAGGCGCGCGGACGAAAGTAAACCCACTGGTGATACCATTCGCGAGCCTCCGGATGACGACCGTAGTGATGAATCTCTCCTGGCGGGAACAGCAAAATATCACCCGGTCGGCAAACAAATTCTCGTCCCTGATTTTTCACCACCCCCTGACCGCGAATGGTGAGATTGAGAATATAACCTTTCATTCCCAGCGGTCGGTCGATAAAAAAATCGAGATAACCGTTGGCCTCAATCGGCGTTAAACCCGCCACCAGATGGGCATTAAACGAGTATCCCGGCAGCAGGGGATCATTTTGCGCTTCAGCCATACTTTTCATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACCCCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTTTGGGCTAGCGATTGAAAACGGTGCAGTTCAAGGTTTACACCTATAAAAGAGAGAGCCGCTATCGCCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCCAGATGGTCAGTGTGCCGGTCTCCGTCATCGGAGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAATCTGATGTTCTGGGGAATATAA*TCTAGA*GGGCACGGGCAGCTTGCCGGTGGAAGCTTGGCTGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATACAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACAGAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGACCCCATGCCGAACTCAGAAGTGAAACGCCGTAGCGCCGATGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTT**CTGCAG**TGCGTTTCT

Supplemental Table 3 – Sequence of gBlocks employed to make plasmid clones

gBlock® name	Sequence (restriction sites in bold)
V3off1	CAGGACCAT**GAGCTC**AGTGAGTGAGTGTGTGTGTGGGGTAATACGACTCACTATAGGTGAGTGAGTGTGTGCGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTCCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGAA**CTGCAG**CAGCAGTAC
EXoff1	CAGGACCAT**GAGCTC**GAGTTAGAGCAGAAGAAGAAAGGTAATACGACTCACTATAGGAGTCCGAGCAGAAGAAGAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTCCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGAA**CTGCAG**CAGCAGTAC
NLS-Cas9-NLS-NLS	AGCCAGTTA**CCATGG**GCAGCAGCGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCATGGACAAAAAGTACTCTATTGGCCTGGATATCGGGACCAACAGCGTCGGGTGGGCTGTTATCACCGACGAGTATAAAGTACCTTCGAAAAAGTTCAAAGTGCTGGGCAACACCGATCGCCATTCAATCAAAAAGAACTTGATTGGTGCGCTGTTGTTTGACTCCGGGGAAACCGCCGAGGCGACTCGCCTTAAACGTACAGCACGTCGCCGGTACACTCGGCGTAAGAATCGCATTTGCTATTTGCAGGAAATCTTTAGCAACGAGATGGCAAAAGTCGATGACTCGTTTTTCCACCGCCTCGAGGAAAGCTTTCTGGTGGAGGAAGACAAAAAGCATGAGCGTCACCCGATCTTCGGCAACATTGTCGATGAAGTAGCGTATCATGAAAAATACCCAACCATTTACCACTTACGCAAAAAGCTGGTGGACAGCACTGACAAAGCTGATTTGCGCCTTATCTATTTAGCCCTGGCACATATGATTAAGTTTCGTGGTCACTTCCTGATCGAAGGAGACTTAAATCCCGACAACAGTGATGTTGATAAATTGTTTATTCAGCTTGTCCAAACTTACAATCAACTGTTCGAGGAAAACCCGATCAATGCCTCCGGTGTGGATGCAAAAGCCATTTTAAGTGCACGCCTTAGCAAGTCCCGTCGCTTAGAAAACCTTATCGCGCAGCTGCCCGGCGAGAAAAAGAATGGTTTGTTTGGGAACCTTATTGCCTTGAGCTTAGGCCTCACCCCGAATTTCAAAAGTAATTTCGATCTTGCAGAAGACGCCAAATTACAACTGTCGAAGGATACTTATGATGACGATCTCGATAATCTGTTAGCGCAGATTGGTGACCAATACGCCGATCTTTTTCTGGCGGCTAAAAATCTGAGCGACGCCATCTTGCTTTCGGATATTCTCCGCGTTAACACCGAAATCACGAAAGCGCCTCTTAGTGCCAGCATGATTAAACGTTATGATGAACACCACCAGGACCTGACCTTACTCAAAGCGTTGGTTCGCCAGCAACTGCCAGAGAAGTACAAAGAAATCTTCTTTGATCAGTCAAAGAATGGTTATGCCGGCTATATTGACGGGGGTGCAAGCCAAGAGGAATTCTACAAATTTATCAAGCCTATTCTGGAGAAAATGGATGGCACCGAAGAGTTATTGGTGAAGCTTAACCGTGAAGACCTCCTGCGGAAACAGCGCACATTCGATAATGGTTCGATCCCACACCAAATCCATTTGGGGGAGTTACACGCTATTTTGCGTCGCCAGGAAGACTTTTACCCTTTCCTGAAGGATAACCGGGAGAAAATTGAGAAGATCCTTACCTTTCGTATTCCGTATTACGTAGGCCCCTTAGCACGGGGTAATAGCCGTTTCGCGTGGATGACACGGAAGTCGGAAGAGACGATCACCCCGTGGAACTTCGAAGAGGTAGTCGACAAGGGCGCATCAGCGCAGTCTTTTATTGAACGTATGACGAATTTCGATAAAAACTTGCCCAATGAGAAGGTGCTTCCGAAACATTCCTTGTTATATGAATATTTTACAGTTTACAACGAGCTGACCAAGGTTAAATACGTGACGGAAGGAATGCGCAAGCCCGCTTTTCTTAGCGGTGAGCAAAAAAAGGCGATCGTCGACCTGTTATTCAAAACGAATCGTAAGGTGACTGTAAAGCAACTCAAAGAAGATTACTTCAAAAAGATTGAGTGCTTCGACAGCGTCGAAATCTCTGGGGTAGAGGATCGGTTTAACGCAAGTTTAGGTACCTACCATGACCTGCTTAAAATCATTAAGGATAAAGACTTCTTAGATAATGAAGAGAACGAAGATATTCTCGAGGACATCGTCTTGACGTTAACCTTATTTGAGGATCGTGAAATGATTGAGGAACGCCTCAAAACTTATGCCCACCTGTTCGACGATAAGGTGATGAAGCAGCTGAAACGTCGGCGCTACACAGGATGGGGCCGCTTGAGTCGCAAACTTATTAACGGAATCCGTGACAAGCAATCCGGCAAAACGATTCTGGATTTCTTGAAGTCGGACGGATTTGCTAATCGCAACTTCATGCAGTTGATCCATGATGACTCCCTGACTTTTAAAGAGGATATTCAAAAGGCGCAGGTTAGTGGTCAAGGCGACAGCTTACACGAACACATCGCAAATTTGGCTGGTTCGCCGGCCATTAAAAAGGGGATCCTCCAGACCGTGAAAGTTGTAGATGAGCTTGTTAAGGTCATGGGTCGTCATAAGCCCGAAAACATCGTGATTGAAATGGCGCGGGAGAATCAAACGACCCAGAAAGGACAAAAGAATAGCCGTGAACGGATGAAGCGGATCGAGGAAGGCATTAAAGAGCTGGGGTCTCAAATCTTGAAGGAACACCCTGTGGAGAACACTCAGCTCCAAAATGAAAAACTTTACCTGTACTATTTGCAGAACGGACGCGATATGTACGTGGACCAAGAGTTGGATATTAATCGGCTGAGTGACTACGACGTTGATCATATCGTCCCGCAGAGCTTCCTCAAAGACGATTCTATTGACAATAAGGTACTGACGCGCTCTGATAAAAACCGTGGTAAGTCGGACAACGTGCCCTCCGAAGAGGTTGTGAAAAAGATGAAAAATTATTGGCGCCAGCTTTTAAACGCGAAGCTGATCACACAACGTAAATTCGATAATTTGACCAAGGCTGAACGGGGTGGCCTGAGCGAGTTAGATAAGGCAGGATTTATTAAACGCCAGTTAGTGGAGACTCGTCAAATCACCAAACATGTCGCGCAGATTTTGGACAGCCGGATGAACACCAAGTACGATGAAAATGACAAACTGATCCGTGAGGTGAAAGTCATTACTCTGAAGTCCAAATTAGTTAGTGATTTCCGGAAGGACTTTCAATTCTACAAAGTCCGTGAAATTAATAACTATCATCACGCACATGACGCGTACCTGAATGCAGTGGTTGGGACCGCCCTTATCAAGAAATATCCTAAGCTGGAGTCGGAGTTTGTCTATGGCGACTATAAGGTATACGATGTTCGCAAAATGATTGCGAAATCTGAGCAGGAGATCGGTAAGGCAACCGCAAAATATTTCTTTTACTCAAACATTATGAATTTCTTTAAGACAGAAATCACTCTGGCCAACGGGGAGATTCGCAAACGTCCGTTGATCGAAACAAACGGCGAGACTGGCGAAATTGTTTGGGACAAAGGGCGTGATTTCGCGACGGTGCGCAAGGTACTGAGCATGCCTCAAGTCAATATTGTTAAGAAAACCGAAGTGCAGACGGGCGGGTTTTCCAAGGAAAGCATCTTACCCAAACGTAATTCAGATAAACTTATTGCACGCAAAAAGGACTGGGATCCGAAAAAGTATGGAGGCTTCGACAGTCCAACCGTAGCCTACTCTGTTCTCGTTGTAGCGAAAGTAGAAAAGGGTAAATCCAAGAAACTGAAATCTGTCAAGGAGTTGCTTGGAATCACCATTATGGAGCGTAGCTCCTTCGAGAAGAACCCGATTGACTTTCTGGAAGCCAAAGGATATAAAGAGGTCAAGAAAGATCTTATCATTAAGCTGCCTAAGTATTCACTCTTCGAGCTGGAAAATGGTCGTAAACGCATGCTCGCTTCTGCCGGCGAGTTGCAGAAGGGCAATGAATTAGCACTTCCATCAAAGTACGTTAACTTCCTGTATTTGGCCAGCCATTACGAGAAACTGAAGGGGTCTCCAGAGGACAACGAACAGAAACAATTATTTGTAGAGCAGCACAAGCATTATCTTGATGAAATCATTGAGCAAATTTCCGAATTCAGTAAACGCGTAATCCTGGCCGATGCAAACCTCGACAAGGTGCTGAGCGCTTACAATAAGCATCGCGACAAACCTATCCGTGAGCAGGCTGAAAATATCATTCACCTGTTCACATTAACGAACCTGGGCGCTCCGGCCGCTTTTAAATATTTCGACACGACAATCGACCGTAAGCGCTATACCAGTACGAAAGAAGTGTTGGATGCGACCCTTATTCACCAGTCAATTACAGGATTATATGAGACCCGTATCGACCTTAGCCAATTAGGTGGGGATGCGGCCCCGAAGAAAAAACGCAAAGTGGATCCGAAGAAAAAACGCAAAGTG**GCGGCCGC**CAGGACCTA

Supplemental Table 3 (continued)

crRNA name	**20 nt targeting crRNA sequence (**DNA sequence listed absent proprietary universal sequence and chemical modifications**)**
EMX1	GAGTCCGAGCAGAAGAAGAA
HEKSite4	GGCACTGCGGCTGGAGGTGG
VEGFA3	GGTGAGTGAGTGTGTGCGTG
HPRT-S-38094	TCCATTTCATAGTCTTTCCT
HPRT-S-38231	TTTTGTAATTAACAGCTTGC
HPRT-S-38371	CTTAGAGAATATTTGTAGAG
HPRT-S-38509	TTGACTATAATGAATACTTC
HPRT-S-38574	CAAAACACGCATAAAAATTT
HPRT-AS-38087	AATTATGGGGATTACTAGGA
HPRT-AS-38133	GGTCACTTTTAACACACCCA
HPRT-AS-38285	CTTATATCCAACACTTCGTG
HPRT-AS-38287	GGCTTATATCCAACACTTCG
HPRT-AS-38358	ATTTCACATAAAACTCTTTT
HPRT-AS-38636	TCAAATTATGAGGTGCTGGA
HPRT-AS-38673	TACAGCTTTATGTGACTAAT
CTLA4-S-542	AGGACTGAGGGCCATGGACA
CTLA4-S-543	GGACTGAGGGCCATGGACAC
PDCD1-S-383	CGGAGAGCTTCGTGCTAAAC
PDCD1-AS-429	CTGGCTGCGGTCCTCGGGGA
AR-S-1893	GTTGGAGCATCTGAGTCCAG
HBB	CTTGCCCCACAGGGCAGTAA
HiFi-Cell-Line	CAGCTGCATGAAGTTTCGGT

Supplemental Table 4 – crRNA protospacer sequences

Multiplex NGS Off-target Primers
crRNA – *AR*
Note - Oligo sequences are absent proprietary 5' tails and chemical modifications
"iGS" – Amplicons for GUIDE-seq identified targets
"picks" – Amplicons for CRISPOR predicted targets

Forward		Reverse	
A_iGS_10.1410_FO	AAGACAGGGAACCCAGCGCAGAA	A_iGS_10.1410_EV	GCAAGGGAGATTCAGCCACGAAAG
A_iGS_11.4097_FO	GTGGGTATTTAGGGAACTGTGAGCAT	A_iGS_11.4097_EV	AGCAAATTTGAAGCAAGTCTGGCCTCG
A_iGS_12.80_FO	GTCAGAAGTCACACCTAATCACCCTCTG	A_iGS_12.80_EV	CTTATAGTAAGTTTAGGTGTGAGGGGAAGCT
A_iGS_13.2373_FO	CTATGGAGTCCCTAAAACCTCAGATTTC	A_iGS_13.2373_EV	GTCTAATACACTCACAAGTCCAGCCAAAC
A_iGS_14.2155_FO	CACATCTGACCAAGTCTGAAACAAAGT	A_iGS_14.2155_EV	GTTTTCCTGGAAACTCCGTTTTCCTAAC
A_iGS_15.349_FO	TAAAAGTAGTGCATCCACCCACGGTAG	A_iGS_15.349_EV	TTGTGTAGAAAGAACAGACTGGCAGGGC
A_iGS_16.939_FO	TGTGGATGAAGGACAGCAGAGAAT	A_iGS_16.939_EV	GTGGGCTATGCTGGATGAATATCT
A_iGS_17.1071_FO	ACTGAAGTACAGGTGACCTCAGAGC	A_iGS_17.1071_EV	ACACTCTCCACCCTAGCCCTACA
A_iGS_18.460_FO	CACTAGTTTCAGCAGCAAGACATAGA	A_iGS_18.460_EV	TCTTTAGGATGGGTTTCGTGACAGTTT
A_iGS_19.3546_FO	CGTTACTGTGCCTCCAAGCATCCG	A_iGS_19.3546_EV	GCAACAAGAAACCCTCCTGTCTCA
A_iGS_1.1013_FO	ACCATTTCTGACAACGCCAAGGAGTA	A_iGS_1.1013_EV	CAGAGAACCTTTGCATTCGGCCAATC
A_iGS_20.1461_FO	GCATTTGTGTTTCCTGCCGAGAGC	A_iGS_20.1461_EV	CATTTTCAGCAGGGCATCCAGCAG
A_iGS_21.2084_FO	GGCAATAGTTCCTGCTGTTGGGAGT	A_iGS_21.2084_EV	TGTGAGAAGTGATTGCAGCCTGGCT
A_iGS_22.1004_FO	AAGATGACCTGTATGCTTGGCACTGC	A_iGS_22.1004_EV	ATTCTTTCCCTACCAGGTCAGTCACG
A_iGS_23.536_FO	GTGGTTCTTGAAAATGAGTCACACACT	A_iGS_23.536_EV	AGCAGGGTCTTACTAAGTTGCCCAAC
A_iGS_24.745_FO	TCACCCTCCTACACGATATCACTGGTC	A_iGS_24.745_EV	ATACTTTGGGTTCTACCCCATCGGCTC
A_iGS_25.1019_FO	GCCTAGACAACATGGTCAAACCCTTTG	A_iGS_25.1019_EV	CTTTTGCCCAGGCTGAAATACAGTGC
A_iGS_26.321_FO	TACACACACTTCATACACCACACCTT	A_iGS_26.321_EV	ATTGTGGGAGAATGAGAGAAAGCTTCT
A_iGS_27.4760_FO	CTTAGTGTGATGGTCAAAGACCGCTCTA	A_iGS_27.4760_EV	GAAACAAGGGAACATTTAGGCAGGATGT
A_iGS_28.731_FO	CTCACTGTGGAGTCTAACCAGCCTAAC	A_iGS_28.731_EV	ATCTAGTAATCCCTGGCAGTCCGCATC
A_iGS_29.890_FO	ATCTCTCCTGATGTGCTGCTGGAT	A_iGS_29.890_EV	CAATCCAGAGTCCATCCTCCCAACA
A_iGS_2.2642_FO	GGTTTTTCTCTTTGCAATTGTTATGTGGTCTG	A_iGS_2.2642_EV	GAACATTCAGAAACTGTCTTGACTACGTCTC
A_iGS_30.352_FO	GGGCCTCCATTTTCTCATCTGTAT	A_iGS_30.352_EV	GACTCCTGGTACCAGTACTGAGATC
A_iGS_32.120_FO	ATCTCGCACCCTTCCGCGTTGC	A_iGS_32.120_EV	TGAGCGCTGCTTGGTGCATGGC
A_iGS_33.1128_FO	CCAGCCAGCATTTACTCTTAATATTCCACTAG	A_iGS_33.1128_EV	GTACCTATCCATAAGACTGGTTTGAAATAAGA
A_iGS_34.739_FO	ATGGCAAAATCAGATTCAGGATTGGAGAT	A_iGS_34.739_EV	GTCTTCAGTGTTCAGAACAGAATACTTGA
A_iGS_35.908_FO	CTCCTAGGACTTCATAGTTGCTGGTAAT	A_iGS_35.908_EV	TTCTGGCTCCATCTACCTTAGGGACTC
A_iGS_36.1232_FO	TGTTACGAGCTTTGCCTGAGGTCACT	A_iGS_36.1232_EV	ATGGGCTTTTGTACCATCCAGGAAGT
A_iGS_37.1106_FO	TGAGAACAGCCTTTTCCAGACCTG	A_iGS_37.1106_EV	AAGCCTCTCCAAACCATCTGCTC
A_iGS_38.828_FO	CAGTGGCTTATGCCTGTAATACTAGCT	A_iGS_38.828_EV	GACAAGGTTTCGTCATGTTACCCAGAG
A_iGS_39.400_FO	GTGAATTTCTGAGGGACACATTCAAGCG	A_iGS_39.400_EV	CCACTCACCTTCTCATTTGATTTGAGCAT
A_iGS_3.3423_FO	GGTATGTGTCACCTCCTCTCCATGT	A_iGS_3.3423_EV	CACAAAGCCAATAGGTGCTGTACGT
A_iGS_40.472_FO	TCAAAACAAACAAAAGTAACCCAGCTGTA	A_iGS_40.472_EV	CCACGCCTTGCTAATTTTAAAATGTTTTA
A_iGS_41.1521_FO	CAATGTATATTGGGAATTCTTCTCCCCCTCTT	A_iGS_41.1521_EV	CCTATGATTTTAGGTTGTGTTCCTTTGAGGCG
A_iGS_42.3774_FO	TGTTATAACCTGAGGACACCAGCAAGT	A_iGS_42.3774_EV	TCGTTAAACACCAAGAGTCACCCAAGA
A_iGS_43.5085_FO	CCTTTTCTCATACTACTCCAGCTCTGACA	A_iGS_43.5085_EV	ATGCGTAGACTCAACTGATGTGCAGTGA
A_iGS_44.2328_FO	CTAAAAGGGAGTTGTCACTGGGTATGT	A_iGS_44.2328_EV	AGTAGCTGGGATTATGGAGACAGGAA
A_iGS_45.347_FO	GAGTCTGACACCTTTTAAGATCTGACAGT	A_iGS_45.347_EV	GAAAACTCAAAGAAATGCCCAATCATTGT
A_iGS_46.31_FO	GTGTGAACTCTCATGGCGCTCCAG	A_iGS_46.31_EV	GCTCGACTGTTTCCCAGACCTCAC
A_iGS_4.415_FO	CCCTCCATCTTCCTAACTGTAACTTGGC	A_iGS_4.415_EV	AGTAAGCCTCTACCATGAGCCAGCAA
A_iGS_5.889_FO	AACTGAGTACTCTCTTGCCGTCTCACG	A_iGS_5.889_EV	GTAGTTACAGCTACTTGGGAGCCCAAGC
A_iGS_6.1573_FO	CCCTTCAGCTGCAGAATAAACAGGAG	A_iGS_6.1573_EV	AGCCCATAAGTCTTTCAGAAGCAGCCT
A_iGS_7.3052_FO	ACTTCAGGCAAGACAAGAAGATACAGGGT	A_iGS_7.3052_EV	CACTCCACCTGATTCAAACATGCAGCTG
A_iGS_8.435_FO	GCTAATGGGTCCAGGGTTTCTATCA	A_iGS_8.435_EV	TTACAGGCACCCACGATCACTCG
A_iGS_9.602_FO	GCTCAGCAAATCTCATACTCCTACTCAGT	A_iGS_9.602_EV	CATGGAAACTGAAGAAAGGTAGAACACAGG
A_picks_10.331_FO	CAACTTCTTGCTTGTTCCATGCAGACA	A_picks_10.331_EV	GATTGCAGCTCTTCAGTAAAAAGGGTTA
A_picks_1.106_FO	CGCAACCACTTTCCCAAACATCCG	A_picks_1.106_EV	CCAGGCTAGAGAGGGATGACCTC
A_picks_2.59_FO	CACATGATGGATCTGTGCAATAGTCATT	A_picks_2.59_EV	TCATGATAAATACCTGGCAGTGGACAGT
A_picks_3.868_FO	GGTAGAAAAGGGATCTAGCTCTGTTGCG	A_picks_3.868_EV	CGATCCAGTATTGAGCACCTACTGCTA
A_picks_4.1283_FO	GGGAATTCCACTCCTTTTGTCTTGTC	A_picks_4.1283_EV	TCCCAATGGCAAGTGATATTTCCACAA
A_picks_5.560_FO	CTGAAAGAAGCAGTACACTTGGCATGAA	A_picks_5.560_EV	TCAGGGCCTAGAGTTTTATGAGCATTA
A_picks_6.155_FO	AGCAAAACTAATACATACACAGAACAAGA	A_picks_6.155_EV	GAAATGTTCCCTCCTTTAGTATTCTCCAC
A_picks_7.2454_FO	AGGCTGAGAAGTTCTACAAGATGCTCTCA	A_picks_7.2454_EV	TCACTCTTTGAAGGAGTTTGCTGCAACTG
A_picks_8.936_FO	CCAAGCTGACTAAGACACTCTGATCCA	A_picks_8.936_EV	CTTCTTTGTCTGAGACAGCTGGGCTTC
A_picks_9.1770_FO	CAACTTTTAAAACCCATCATGCATTCTTCAC	A_picks_9.1770_EV	GTGAAGCTTGTGAAAAACTGATTGTAGCATAA

Supplemental Table 5 – Primer sequences

Multiplex NGS Off-target Primers
crRNA – *HPRT 38087*
Note - Oligo sequences are absent proprietary 5’ tails and chemical modifications
“iGS” – Amplicons for GUIDE-seq identified targets
“picks” – Amplicons for CRISPOR predicted targets

Forward		Reverse	
HPT38087_iGS_10.2441_FO	CTCACTTACACGTAGGAGCTAAACACTAC	HPT38087_iGS_10.2441_EV	GGTAGTGAACACAGTACCTACACAATGC
HPT38087_iGS_11.728_FO	GAACAGTGTGACAAGTGTCCCAATGC	HPT38087_iGS_11.728_EV	CAAATGTTGAACCAATCCGTGCCATCA
HPT38087_iGS_12.2204_FO	CATTCAGGAAACAGCTGTAGTATCTGCAT	HPT38087_iGS_12.2204_EV	CCTCTTGGATGATCTTGTAACTCGAGAGT
HPT38087_iGS_13.110_FO	ATTATTTCAAAGAATGCTGCCAGAGACC	HPT38087_iGS_13.110_EV	AAAACTGTTAGACTGTCAATAGTAAGTGA
HPT38087_iGS_14.1291_FO	ATCACAAGTGCTATGAAGGAAATGAAG	HPT38087_iGS_14.1291_EV	CTATGAAGCACAGGGAGTTCAAGTCG
HPT38087_iGS_15.2869_FO	CTGAGAGTTTCTGCTGGTCACTTG	HPT38087_iGS_15.2869_EV	TCACCTGGAAAGAAGCAACCACTC
HPT38087_iGS_16.3205_FO	CCACTGTATCCAGCACTTTGTCAAATC	HPT38087_iGS_16.3205_EV	TTCCATACCAGAGAACAGCAAGCATTA
HPT38087_iGS_17.647_FO	ACTCTGAATAAAACAGACATGGTCCCTGG	HPT38087_iGS_17.647_EV	TTCATTGAGGTTTCTGGTATGATAAACAA
HPT38087_iGS_18.206_FO	TTTACCCAATACCTGTACCCACATTGA	HPT38087_iGS_18.206_EV	CACAGCCAAACCATATCATACCACTCG
HPT38087_iGS_19.1052_FO	CCTTCACACAAACGCCATCCAAAC	HPT38087_iGS_19.1052_EV	AGAGTTTTCAGGGCAAGGGCCAAC
HPT38087_iGS_1.2824_FO	CAGGTCTCAGAACTGTCCTTCAGGTA	HPT38087_iGS_1.2824_EV	CATTTCATCCGTGCTGAGTGTACCT
HPT38087_iGS_20.1010_FO	AACAATTCCGACACACACTTCCCAGGC	HPT38087_iGS_20.1010_EV	AATCTGGTCTCTGGTCTCTTTCTGGCA
HPT38087_iGS_21.4345_FO	TCCCAGAATAATCGTAAAGCAAATGACCAT	HPT38087_iGS_21.4345_EV	CAAGTTTCCTCACCTAAGAAGTGGAGATT
HPT38087_iGS_22.1702_FO	CCCTCAGACTCAAGCAAGCATGG	HPT38087_iGS_22.1702_EV	AGCTGCTGGAGCATTCCTGGGA
HPT38087_iGS_23.1695_FO	AGGATAGAGCTTCGACTCATATTTTCCACGA	HPT38087_iGS_23.1695_EV	GATGTTTCTTTCTTCCCACCAAACTCAGG
HPT38087_iGS_24.2707_FO	GAACTCCTGGGCTCATGCAGTCG	HPT38087_iGS_24.2707_EV	CCCTCCTCCGAGTTGTTACAACCT
HPT38087_iGS_25.1554_FO	CAGAGTACTCACCCACCTCCTAAA	HPT38087_iGS_25.1554_EV	TGAACAGACCCACTCACAAGACAAG
HPT38087_iGS_2.2713_FO	GCAATATGCCTCTGATAAGGAATTAGAGATGAA	HPT38087_iGS_2.2713_EV	GGGATTTTGTCACTAGAAAGTTTAGAAAGTACA
HPT38087_iGS_3.998_FO	TGCTGTTTCCTAGGATTCCAGGACCAC	HPT38087_iGS_3.998_EV	CAAATGGACGTGTGTAGAGGCAGACT
HPT38087_iGS_4.2924_FO	CTCAACAAGATATTTCCTCCTACTTCTAGCT	HPT38087_iGS_4.2924_EV	GATTAATGTCTCTGCTTTTCTGAGTGGATTG
HPT38087_iGS_5.672_FO	CCACACCTGAACAGATTCTTTCACAAC	HPT38087_iGS_5.672_EV	TACACAAGGAGAACCACAGACTGACG
HPT38087_iGS_6.215_FO	AGGCCATTGTTCTATTTGGGACAGCTG	HPT38087_iGS_6.215_EV	GGCTCCCGAGAAATCATCAAGTCAAT
HPT38087_iGS_7.2963_FO	GAATTTCTGCAGGTTGACCAAATACCTTTG	HPT38087_iGS_7.2963_EV	TACTAGGTCAAGAAGCATCAGTCCCAAGT
HPT38087_iGS_8.1896_FO	AAAATTTGAACCAATCCCTACCATCTCCTA	HPT38087_iGS_8.1896_EV	ACATGGTTAGGAAAATTGGTACTCTGATGAT
HPT38087_iGS_9.136_FO	GAATGAACTATTGGTCCATGTCACAACAA	HPT38087_iGS_9.136_EV	ATACACCAGTGCAATCCACAGTCAAC
HPT38087_picks_10.479_FO	AGATATTCTCATATGCTGCCAATGGGT	HPT38087_picks_10.479_EV	TATTTCAAAGAATGCTGCCAGAGACGC
HPT38087_picks_1.3647_FO	CACTGTTGGTTTAATGACATGCTCACATA	HPT38087_picks_1.3647_EV	TTGAGAGGCTTTACTACCACATAAGGAGAA
HPT38087_picks_2.1416_FO	GATGTTGTTCTGCCTGTTTAGCCTCTGA	HPT38087_picks_2.1416_EV	GACGCATTGAGAAAGAAGATCCCACCAA
HPT38087_picks_3.1479_FO	CATGATTCAATTACCTCCACTTGGTCCCG	HPT38087_picks_3.1479_EV	TCCTACAATTAACCAATAGAGCAGTGGCT
HPT38087_picks_4.2023_FO	TCTCTGTGTGAATCAGTAAGGAAATAGGAAGG	HPT38087_picks_4.2023_EV	CTGTCCTTCTTCTATAGTTATAAGCAACCTGTC
HPT38087_picks_5.427_FO	GACTCCATTTGGATGACAGTAAGGGAAC	HPT38087_picks_5.427_EV	GAGACATTTTGTGTTTGGAAACAAGAAGGA
HPT38087_picks_6.393_FO	GTGAGAATTCAGGGCTCTCTTGAC	HPT38087_picks_6.393_EV	CACAGGACCTTGCCTTTCCTCTAT
HPT38087_picks_7.1006_FO	CATGATTCAATTACCTCCAACTGGTCCCT	HPT38087_picks_7.1006_EV	GGTCAGACAGAATAATATGGTTTGGCTTA
HPT38087_picks_8.1290_FO	CGAGACTCCAGCTCAAAATAATAAAATAT	HPT38087_picks_8.1290_EV	ACAATTTCAGGTCAAAATGAACTTGGGAAGG
HPT38087_picks_9.1585_FO	CAACATTGTAATTCCCGATAGTGACCCAAA	HPT38087_picks_9.1585_EV	CTGAAGATGAGAGCATATTCCTTATGGCACA

Supplemental Table 5 (continued)

Multiplex NGS Off-target Primers
crRNA – *EMX1*
Note - Oligo sequences are absent proprietary 5’ tails and chemical modifications
“iGS” – Amplicons for GUIDE-seq identified targets
“picks” – Amplicons for CRISPOR predicted targets

Forward		Reverse	
EMX1_aGS_1.510_FO	GGTACTTCATGTCCGTGCTCTTGAC	EMX1_aGS_1.510_EV	CACTTGGAGAGTCAGAGGTCACAAT
EMX1_aGS_2.1432_FO	GGAATAAAGGCGAGGAAGCGGGAGG	EMX1_aGS_2.1432_EV	CTGTCTGCCTCTGACGACGAGCAT
EMX1_aGS_3.181_FO	GGGATGTTCTTCTTGCCTTGGTAGC	EMX1_aGS_3.181_EV	ACCATTCACTCCACCTGATCTCGGC
EMX1_iGS_10.1047_FO	CACACCAGCAATGCTCTCGTCTTG	EMX1_iGS_10.1047_EV	CTTGGCCCTTCCTCTGTACTCTAA
EMX1_iGS_11.688_FO	CATCAGCTGTTCGTAGGACATGTG	EMX1_iGS_11.688_EV	GGTTAGCCATTTACGCTCACATCTC
EMX1_iGS_12.3334_FO	AGTGGGTGAGGAAATGTTCAGGGAAA	EMX1_iGS_12.3334_EV	CTTAGCATTGGAAAGCCTGTCTCTGT
EMX1_iGS_13.2205_FO	AAGTGAGCTGTGATCACGTGACTGG	EMX1_iGS_13.2205_EV	GGCTGAAGCAATCCTCCTACTTCAC
EMX1_iGS_14.767_FO	GGTTATTGTAGCTTCCTAAGTGGTCCTT	EMX1_iGS_14.767_EV	AAGGAAGTCGTTAGAAGACTCTACAGACT
EMX1_iGS_15.1713_FO	AGCCCAGAAGTTTCCTGAGCCCAAA	EMX1_iGS_15.1713_EV	TCTTCGAAAGAGGCAGTGTATGCGC
EMX1_iGS_17.1586_FO	CTACCATCACAGTCTATGGCGCTTCA	EMX1_iGS_17.1586_EV	TGCAGGATTTGGAAACAGATGCCAGAG
EMX1_iGS_18.1680_FO	TCTAACATTATCTCACTCAGAAGCTCCATGTT	EMX1_iGS_18.1680_EV	GATTTCTTGCTAAATCCTAGAACAGTGGTTTA
EMX1_iGS_19.1953_FO	CGAAAAAGCGAGACTCTGGAGTCTG	EMX1_iGS_19.1953_EV	TTGTAAGGGAGAAGCTGGTGAGGAT
EMX1_iGS_1.2537_FO	AGGACAAAGTACAAACGGCAGAAGCA	EMX1_iGS_1.2537_EV	TTGCCCACCCTAGTCATTGGAGGTC
EMX1_iGS_20.1374_FO	CAGGCAGAGGGATCTTCATATGCTAAC	EMX1_iGS_20.1374_EV	AAGTGCAGACCTAATGTTGCCACCAG
EMX1_iGS_2.3261_FO	AATGTGCTTCAACCCATCACGGCCTA	EMX1_iGS_2.3261_EV	CATCAGTGTTGGCTTTCACAAGGATGG
EMX1_iGS_3.347_FO	GAGTCTGACACCTTTTAAGATCTGACAGT	EMX1_iGS_3.347_EV	GAAAACTCAAAGAAATGCCCAATCATTGT
EMX1_iGS_4.113_FO	CCCGGCTTAGGTACAAAGCTCTCG	EMX1_iGS_4.113_EV	TATCCCTCACAAACCGTCCCCTCG
EMX1_iGS_5.2553_FO	GAAGACCAGACTCAGTAAAGCCTGGAC	EMX1_iGS_5.2553_EV	CCCCAGTCTCTCTTCTATGTGCACTTG
EMX1_iGS_6.239_FO	GATGTAGTTCTGACATTCCTCCTGAGC	EMX1_iGS_6.239_EV	TTTTGGTCAATATCTGAAAGGTTTATTTGA
EMX1_iGS_7.49_FO	GGGAATAAACTTGTGCTTATTTGTTGGAAGAC	EMX1_iGS_7.49_EV	TGTTTTAAATTCTCCACAGTAAAAGAGATATAAAT
EMX1_iGS_8.466_FO	GCTGCTAAAGTGTTAAGAACTCTGAGAAT	EMX1_iGS_8.466_EV	TCTGGGACTTTTCTTTGATAAGCCCTTCT
EMX1_iGS_9.1740_FO	CTAGCTCCCCTTATGTCACTCACACT	EMX1_iGS_9.1740_EV	CAGTCAAATGTGAGCAGAAGGCCTCAG
EMX1_picks_10.2296_FO	GATTCTGTCAAAGTCTCCCTGGCCAAG	EMX1_picks_10.2296_EV	GTTCCTCCCTGCATTTCTTATCACACA
EMX1_picks_1.827_FO	AGAGGCAAACTAGGACAACCACTTACT	EMX1_picks_1.827_EV	CCGGTCTGAGTGATGGAAGGAACCA
EMX1_picks_2.1783_FO	GCATCTGAAATCATAACCTCTCACTGACG	EMX1_picks_2.1783_EV	TTCTGTCTTTCAAGACAGAAGTGCACCTC
EMX1_picks_3.1232_FO	GTCATGCCTGATCAAACCAATCTGTGT	EMX1_picks_3.1232_EV	CAGACACACACAAGGAGTTTTGGAGCAC
EMX1_picks_4.2880_FO	CAAATCCACTCTGAAGATCAAAGTCATCAT	EMX1_picks_4.2880_EV	GAGAAAACTGAGGGAATGGTAGACTAAGC
EMX1_picks_5.71_FO	TGTAATGATTCTGCCTTAGAGTCCCAGGA	EMX1_picks_5.71_EV	CAGTACTATATTATTTATGGCAGGGCATGGC
EMX1_picks_6.14_FO	TCCTCAGATTTGGAAATTTTTCTGCTATA	EMX1_picks_6.14_EV	CATTAACAGCAGACTTGATGAAGCAGAAC
EMX1_picks_7.1815_FO	CAAGTCCAAAGGCTGAAGAAAACCAATC	EMX1_picks_7.1815_EV	TGGACCCAGTAGATTGAGTAAAGCAGACA
EMX1_picks_8.254_FO	GCAAGAGAATCGCTTGAAACCGGAAGC	EMX1_picks_8.254_EV	GGATGTTTCCACACACAACAGCCTTG
EMX1_picks_9.525_FO	TTGCACACTCTGAAGCTATGGCCCAAC	EMX1_picks_9.525_EV	GGGAAAAATGTCTCCAGGGAATGCTAGT

Supplemental Table 5 (continued)

Multiplex NGS Off-target Primers

crRNA – *HBB*

Note - Oligo sequences are absent proprietary 5’ tails and chemical modifications

“iGS” – Amplicons for GUIDE-seq identified targets

“picks” – Amplicons for CRISPOR predicted targets

Forward		Reverse	
HBB_picks_10.706_FO	CTGACCCTGATTCTACTCATCATATCTTCTG	HBB_picks_10.706_EV	AATTCTTAGGTCCCATAAATTCCAAACAGGTA
HBB_picks_1.3758_FO	GAGCTGAACTAAGTCTGACCGGTTCA	HBB_picks_1.3758_EV	AGGGAGACTTACCAGCTTCCCGTAA
HBB_picks_2.1025_FO	GACATAATCAAAGATGTTCAAACTCCACCAAAT	HBB_picks_2.1025_EV	ATTTTCTATGTGGACAAGCATTATGCCAAGCG
HBB_picks_3.456_FO	TGTATGTAACCTGTGATCCCTTCGAAGT	HBB_picks_3.456_EV	CTAGAAGGAGGTGTCTGTGAGGTGAAC
HBB_picks_4.1364_FO	CTGAGTGACACAAGCTCCCATGCA	HBB_picks_4.1364_EV	GCAGAGTTATCAGACACCTGCACGC
HBB_picks_5.2922_FO	TGTCAGTCCTTACTCCTCATCTCTGA	HBB_picks_5.2922_EV	GATGATCCTGCCACAGTTCCAGTGG
HBB_picks_6.125_FO	TACGACTTAATCACCTCCCACCAGGCG	HBB_picks_6.125_EV	TTGGTTTCCTATTACACCTCTCTGGAAGT
HBB_picks_7.304_FO	TGCAAAACCCCATTTCTAGGCCACAT	HBB_picks_7.304_EV	CACATTGCCCACTTACACCCCAGAT
HBB_picks_8.1776_FO	TAATGTGCTATTTGTGTGGGATGCTGAGT	HBB_picks_8.1776_EV	AATACTTTTTAAGCTACCACGGTGACAGA
HBB_picks_9.2245_FO	CTCCCAGAACACTCAGGAAACTTCTG	HBB_picks_9.2245_EV	GCACGAGCTGATTGTCATTCGGAGAT
HBB_SGS_1.1557_FO	CATGCCCAGTTTCTATTGGTCTCCTA	HBB_SGS_1.1557_EV	CTAGCAACCTCAAACAGACACCATGC
HBB_SGS_2.2769_FO	GAAGATCCCAGAGAACTTGGATAGGAAAC	HBB_SGS_2.2769_EV	CAGTATGTCCAACTCCCAAATTGAAAGG
HBB_SGS_3.2533_FO	GATGAAGGTTGCTAGACAGTAAGGATTGC	HBB_SGS_3.2533_EV	CACGCATACACATACATAAACCCTCTGT
HBB_SGS_4.3843_FO	TATCTGTGTCATCCCAGGAAAGCTTGA	HBB_SGS_4.3843_EV	GTGCACAAAAGTCTCTGTAGAAGGAGAG

Supplemental Table 5 (continued)