SUPPLEMENTARY DATA

"Engineered Zinc Finger Nickases Induce Homology-Directed Repair with Reduced Mutagenic Effects"
Ramirez & Certo et al.

Supplementary Tables 1-3 and Supplementary Figure 1.

Supplementary Table 1: Sequences of oligonucleotides used in this study.

Name	**5' to 3' Sequence**
OC152	ATCTGTCATCCTCATCCTGATAAACTGCAAAAG
OC153	TAATCTTTTGCAGTTTATCAGGATGAGGATGAC
OC213	GACGCCCGCCATAAACTG
OC215	GCACGTTCCTTATATGTAGCTTTCG
OC417	CGGTGTGAAATACCGCACAG
OC418	CAGCAGCAGCAGACCATTTTC
OC665	GATCGAGCAGCGTCTTCGAGAGTGAGGAC
OC666	CTAGGTCCTCACTCTCGAAGACGCTGCTC
OC667	GATCCAGCGTCTTCGAGAGTGAGGACGTGT
OC668	CTAGACACGTCCTCACTCTCGAAGACGCTG
OC671	GATCGAGACTCCCACGGCCGGGGAAGAGT
OC672	CTAGACTCTTCCCCGGCCGTGGGAGTCTC
ams1228	GGTCGAGCAGCGTCTTCGAGAGTGAGGACGTGTA
ams1229	CTAGTACACGTCCTCACTCTCGAAGACGCTGCTCGACCTGCA
ams1230	GGGGTCATCCTCATCCTGATAAACTGCAAAAGGA
ams1231	CTAGTCCTTTTGCAGTTTATCAGGATGAGGATGACCCCTGCA

Supplementary Table 2: ZFN/ZFNickase target sites.

Target site	**Full site**	**LEFT half-site (5'to3')**	**RIGHT half-site (5'to3')**
CCR5	gGTCATCCTCATCctgatAAACTGCAAAAGg cCAGTAGGAGTAGgactaTTTGACGTTTTCc	gGATGAGGATGACc	tAAACTGCAAAAGg
HX735	gAGACTCCCAcggccGGGGAAGAGt cTCTGAGGGTgccggCCCCTTCTCa	gTGGGAGTCTc	cGGGGAAGAGt
VF2468	gAGCAGCGTCttcgaGAGTGAGGAc cTCGTCGCAGaagctCTCACTCCTg	aGACGCTGCTc	aGAGTGAGGAc
VF2471	cAGCGTCTTCgagagtGAGGACGTGt gTCGCAGAAGctctcaCTCCTGCACa	cGAAGACGCTg	tGAGGACGTGt

Supplementary Table 3: Numerical data used to create Figure 4c-h.

VF2468

FokI Catalysis	Avg HDR (%)	Avg mutNHEJ (%)	Ratio (HDR:mutNHEJ)
+/+	1.279	3.620	0.353
-/+	0.112	0.087	1.289
+/-	0.142	0.036	3.913
-/-	0.000	0.017	0.000

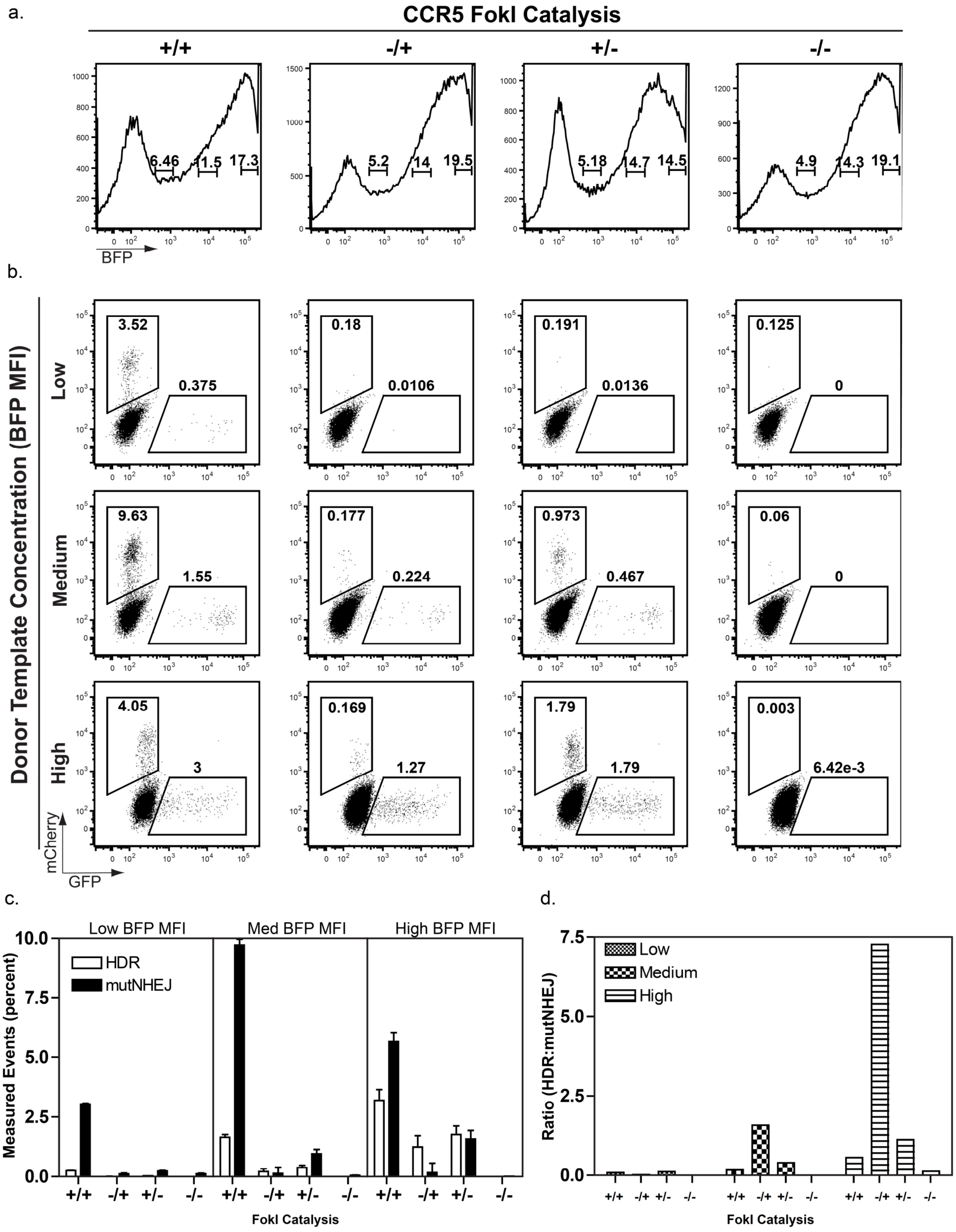
VF2471

FokI Catalysis	Avg HDR (%)	Avg mutNHEJ (%)	Ratio (HDR:mutNHEJ)
+/+	1.280	6.684	0.192
-/+	0.019	0.035	0.544
+/-	0.005	0.040	0.117
-/-	0.002	0.024	0.080

CCR5

FokI Catalysis	Avg HDR (%)	Avg mutNHEJ (%)	Ratio (HDR:mutNHEJ)
+/+	2.696	8.660	0.311
-/+	0.641	0.174	3.694
+/-	0.963	1.572	0.612
-/-	0.000	0.019	0.015

Supplementary Figure 1: Effect of donor template concentration on ZFNickases (illustrated with the CCR5 site) as assessed by gating on different mean fluorescence intensities (MFI) of the BFP-linked donor template. **(a)** Cell populations were gated to analyze cells with low, medium, and high BFP MFIs for each combination of active and/or inactive CCR5 monomers. A set of representative plots is shown. **(b)** As indicators of mutagenic NHEJ and HDR rates, respectively, the percent of mCherry positive (y-axis) and EGFP-positive (x-axis) cells are noted for each cell population analyzed in (**a**). **(c)** Bar graphs show mean percentages of EGFP-positive and mCherry-positive cells, with results derived from 3 independent experiments. Error bars indicate SEM. **(d)** Ratios of percentage of EGFP-positive cells to percentage of mCherry-positive cells for the CCR5 ZFNs and ZFNickases using the data from (**c**).


a.
CCR5 FokI Catalysis
+/+
-/+
+/-
-/-
6.46
11.5
17.3
5.2
14
19.5
5.18
14.7
14.5
4.9
14.3
19.1
BFP
b.
Donor Template Concentration (BFP MFI)
Low
Medium
High
3.52
0.375
0.18
0.0106
0.191
0.0136
0.125
0
9.63
1.55
0.177
0.224
0.973
0.467
0.06
0
4.05
3
0.169
1.27
1.79
1.79
0.003
6.42e-3
mCherry
GFP
c.
Low BFP MFI
Med BFP MFI
High BFP MFI
HDR
mutNHEJ
Measured Events (percent)
FokI Catalysis
d.
Low
Medium
High
Ratio (HDR:mutNHEJ)
FokI Catalysis