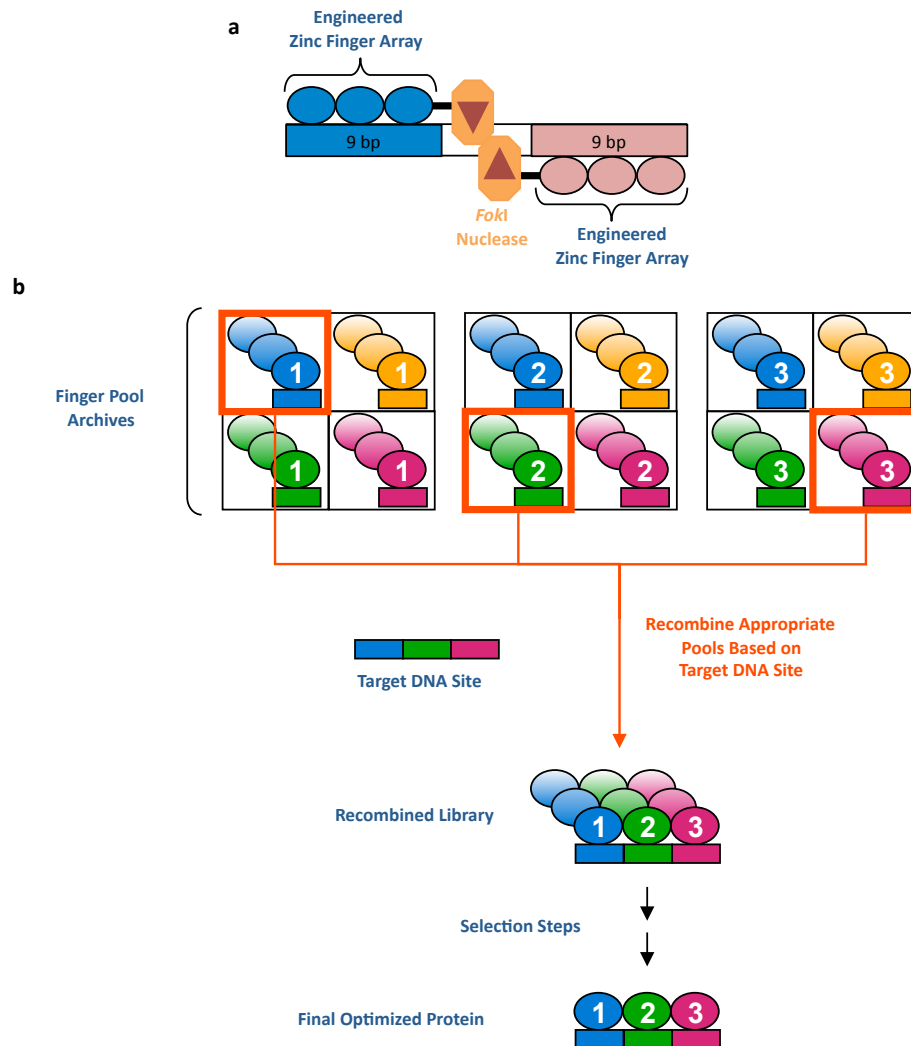
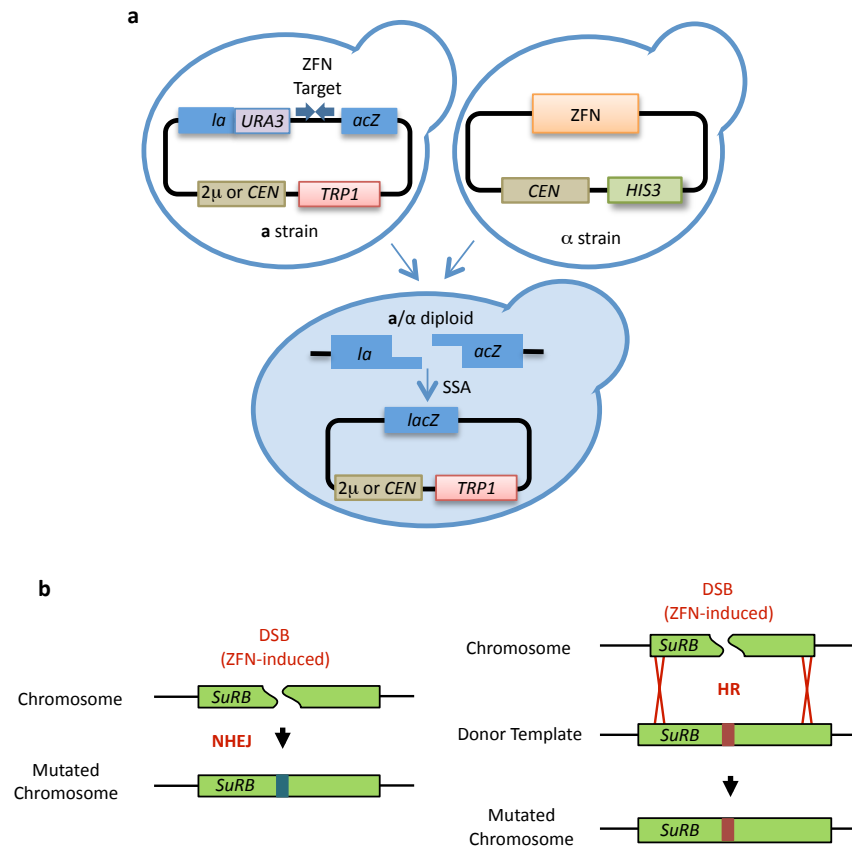


Supplementary Figure 1
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Legend. A) ZFN architecture. Two engineered zinc finger arrays (ZFAs) recognize two 9 bp target sequences. The target sites are separated by a spacer of 5-7 bp. Binding of the ZFAs to the target sequences enables the *FokI* nuclease monomers to dimerize and cleave within the spacer. B) The OPEN (Oligomerized Pool Engineering) platform, a combinatorial-based selection method for making zinc finger arrays (Maeder *et al.*, Mol. Cell, 31:294). OPEN selections use an archive of pre-selected zinc finger pools, each containing a collection of fingers targeted to a different three base pair subsite at a defined position within the context of a three-finger protein. A total of 192 finger pools (64 potential three bp subsites for each position in a three-finger protein) are required to fully enable OPEN. To date, the Zinc Finger Consortium has created pools (each containing a maximum of 95 different fingers) targeted to 66 subsites (48 GNN subsites and 18 TNN subsites (Maeder *et al.*, Mol. Cell, 31:294)). To perform an OPEN selection for a target site, appropriate finger pools from the archive are recombined to create a small library of variants ($95^3 = 8.6 \times 10^5$ members for a three-finger domain). This library is then interrogated using a bacterial two-hybrid (B2H) selection system in which binding of a zinc-finger domain to its cognate site activates expression of selectable marker genes (Hurt *et al.*, Proc. Natl. Acad. Sci. USA, 100:12271; 2003; Joung *et al.*, Proc. Natl. Acad. Sci. USA, 97:7382; Maeder *et al.*, Mol. Cell, 31:294).

Supplementary Figure 2
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Legend. A) Schematic of the yeast assay for testing ZFN function. Details are provide in the text and in the Supplementary Materials and Methods provided online. SSA, single-strand annealing. B) Gene modification with ZFNs. The left figure depicts the use of ZFNs to introduce mutations through imprecise repair of chromosome breaks by non-homologous end-joining (NHEJ). The right figure depicts the use of a donor template to introduce mutations at the cleavage site through repair by homologous recombination (HR).

Supplementary Table 1: Zinc finger arrays selected by modular assembly.

Site Name	ZFA Name	Target Sequence	F1 subsite & RH sequence	F2 subsite & RH sequence	F3 subsite & RH sequence	EMSA
815L	pDW1598	tGACGTGGTA _g	GTA	GTG	GAC	-
815L	pDW1598CF	tGACGTGGTA _g	QSGALTR	RSDALSR	DRSNLTR	-
815L	pDW1598DF	tGACGTGGTA _g	QSGELVR	RSDALSR	DRSTLER	-
815L	pDW1598CE	tGACGTGGTA _g	QSSSLVR	RSDALSR	DRSTLER	-
815L	pDW1598DE	tGACGTGGTA _g	QSGELVR	RSDALSR	DRSTLER	-
815L	*pRW128	tGACGTGGTA _g	QSSSLVR	RSDALSR	DPGNLVR	-
815L		tGACGTGGTA _g	QSSSLVR	RSDELVR	DPGNLVR	+
815R	pDW1596	cAGGGTGGT _{Gt}	GTG	GTG	AGG	-
815R	pDW1596A	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLSQ	-
815R	pDW1596B	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLTN	-
815R	*pDW1596Q3	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLAE	-
815R	*pDW1596R6	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLSQ	-
815R	*pDW1596S4	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLTN	-
815R	pDW1683	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLAE	-
815R	pDW1684	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDKLNLR	-
815R	pDW1687	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLKT	-
815R	pDW1688	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLTQ	-
815R	pDW1689	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDHLKT	-
815R	*pRW129	cAGGGTGGT _{Gt}	RSDALTR	RSDALSR	RSDKLNLR	-
815R		cAGGGTGGT _{Gt}	RSDELVR	RSDELVR	RSDHLTN	+
1071L	pPJ1	tAACGAGATA _a	ATA	GAG	AAC	-
1071L		tAACGAGATA _a	QKSSLIA	RSDNLVR	DSGNLRV	-
1071R	pPJ2	cGTAGAGGATA _a	GAT	GAG	GTA	-
1071R		cGTAGAGGATA _a	TSGNLVR	RSDNLVR	QSSSLVR	-
1853L	pDW1597	tGGTGCTGCC _c	GCC	GCT	GGT	-
1853L	pDW1597GI	tGGTGCTGCC _c	ERGTAR	QSSDLTR	TSGHLTR	-
1853L	pDW1597GJ	tGGTGCTGCC _c	DCRDLAR	QSSDLTR	TADKLSR	-
1853L	pDW1597GK	tGGTGCTGCC _c	DCRDLAR	QSSDLTR	TQGGLAR	-
1853L	pDW1597HI	tGGTGCTGCC _c	DCRDLAR	QSSDLTR	TSGSLAR	-
1853L	pDW1597HJ	tGGTGCTGCC _c	GCRELSR	QSSDLTR	TADKLSR	-
1853L	pDW1597HK	tGGTGCTGCC _c	GCRELSR	QSSDLTR	TQGGLAR	-
1853L	*pRW130	tGGTGCTGCC _c	GCRELSR	QSSDLTR	TSGSLAR	-
1853L		tGGTGCTGCC _c	DCRDLAR	TSGELVR	TSGHLVR	-
1853R	pDW1599	gTGGGCTGCT _c	GCT	GCT	TGG	-
1853R	*pRW131	gTGGGCTGCT _c	QSSDLTR	QSSDLTR	RSDHLTT	-
1853R		gTGGGCTGCT _c	TSGELVR	TSGELVR	RSDHLTT	-
1947L	*pFF24	aGCAGCGGGCa	GGC	GCG	GCA	+
1947L	pFF32	aGCAGCGGGCa	DPGHLVR	RSDDLVR	QSGDLRR	-
1947L		aGCAGCGGGCa	DRSHLTR	RSDDLQR	QSGDLTR	-

ZFAs used for *in vivo* experiments are in bold. The SP1 backbone (Liu *et al.*, J. Biol. Chem., 277:3850) was used for all ZFAs with the exception of those marked with an asterisk, for which the SP1C backbone was used (Segal *et al.*, Proc. Natl. Acad. Sci. USA, 96:2758). RH, recognition helix; EMSA, Electrophoretic Mobility Shift Assay.

Supplementary Table 2: Zinc finger arrays selected by OPEN.

Site Name	ZFA Name	Target Sequence	F1 subsite & RH sequence	F2 subsite & RH sequence	F3 subsite & RH sequence	Mean B2H fold-activation
865L	OZ442	aGCCGGTGGCg	GGC RPDHLAR	GGT LGHHLVR	GCC DPSNLRR	7.49 ± 0.48
865L	OZ443	aGCCGGTGGCg	VPSKLKR	EAHHLSR	DPSNLRR	7.40 ± 0.86
865L	OZ444	aGCCGGTGGCg	APSKLDR	LPHHLQR	DPSNLRR	7.53 ± 0.38
865L	OZ445	aGCCGGTGGCg	APSKLDR	EAHHLSR	DPSNLRR	7.18 ± 0.31
865L	OZ446	aGCCGGTGGCg	SKHKLER	LPHHLQR	DPSNLRR	7.33 ± 0.67
865R	OZ447	cGGCGTTTGCa	TGC RHRNLQL	GTT HESSLVR	GCC DPSHLPR	0.78 ± 0.02
865R	OZ448	cGGCGTTTGCa	RTRNLTI	TNSSLTR	KNISLNH	0.71 ± 0.03
865R	OZ449	cGGCGTTTGCa	RTRNLVL	HASSLTR	KNVSLTH	0.84 ± 0.13
865R	OZ450	cGGCGTTTGCa	RQRNLAL	TAHSLMR	KNISLNH	0.87 ± 0.04
865R	OZ451	cGGCGTTTGCa	RARNLTL	VASSLVR	DPSHLPR	0.84 ± 0.17
865R	OZ452	cGGCGTTTGCa	RHRNLQL	HAESLTR	KKMTLRN	0.84 ± 0.03
1853L	OZ402	tGGTGCTGCCc	GCC HRRDLDR	GCT LSQTLKR	GGT LTQGLRR	6.63 ± 0.11
1853L	OZ403	tGGTGCTGCCc	VRKDLDR	LSQTLKR	LVHGLNR	5.75 ± 0.38
1853L	OZ404	tGGTGCTGCCc	DGSTLNR	LSQTLNR	IKHHLGR	6.98 ± 0.36
1853L	OZ405	tGGTGCTGCCc	HRRDLDR	LSQTLNR	MNHGLAR	8.31 ± 0.68
1853L	OZ406	tGGTGCTGCCc	VRKDLVR	LSQTLKR	VKHGLTR	4.88 ± 0.05
1853L	OZ407	tGGTGCTGCCc	KSRDLAR	LSQTLKR	IKHHLGR	4.99 ± 0.26
1853L	OZ408	tGGTGCTGCCc	DLSTLRR	LSQTLKR	HTHHLGR	10.13 ± 0.26
1853L	OZ409	tGGTGCTGCCc	DESTLRR	LSQTLKR	VKHGLGR	7.80 ± 0.43
1853L	OZ410	tGGTGCTGCCc	LGKDLVR	LSQTLKR	LSQGLAR	2.22 ± 0.17
1853L	OZ411	tGGTGCTGCCc	HSRDLRR	LSQTLRR	VKHGLTR	6.06 ± 0.36
1853L	OZ412	tGGTGCTGCCc	DGSTLRR	LSQTLKR	MNHGLAR	7.85 ± 0.53
1853L	OZ413	tGGTGCTGCCc	HSRDLHR	LSQTLKR	IRHHLKR	7.28 ± 0.56
1853R	OZ414	gTGGGCTGCTc	GCT TKQVLDR	GCT LSQTLNR	TGG RVDHLGG	5.49 ± 0.51
1853R	OZ415	gTGGGCTGCTc	TAQTLTR	LSQTLKR	RMDHLAG	6.68 ± 0.61
1853R	OZ416	gTGGGCTGCTc	TKQVLDR	LSQTLKR	RMDHLAG	6.70 ± 0.34
1853R	OZ417	gTGGGCTGCTc	TKQVLDR	LSQTLKR	RVDHLGG	6.58 ± 0.12
1947L	OZ418	aGCAGCGGGCa	GGC APSKLTR	GCG REDTLTR	GCA QGNTLTR	7.40 ± 1.27
1947L	OZ419	aGCAGCGGGCa	DRAKLTM	RPDVLGR	QKGDLAG	1.43 ± 0.33
1947L	OZ420	aGCAGCGGGCa	SKHKLER	RDDTLVR	QGGVLTR	6.14 ± 0.59
1947L	OZ421	aGCAGCGGGCa	SPSKLAR	RADSLGR	QGGVLTR	6.71 ± 0.15
1947L	OZ422	aGCAGCGGGCa	APSKLKR	RTDVLTR	QGGILTR	8.00 ± 0.57
1947L	OZ423	aGCAGCGGGCa	APSKLMR	RMDTLGR	QGGILTR	8.30 ± 1.50
1947L	OZ424	aGCAGCGGGCa	SPSKLAR	RRDTLER	QGGTLMR	5.74 ± 0.44
1947L	OZ425	aGCAGCGGGCa	APSKLDR	RADSLGR	QTATLKR	5.31 ± 0.67
1947L	OZ426	aGCAGCGGGCa	APSKLDR	RPDVLTR	QGGTLRR	7.08 ± 0.65
1947L	OZ427	aGCAGCGGGCa	APSKLDR	RMDTLGR	QTATLKR	6.74 ± 0.41
1947L	OZ428	aGCAGCGGGCa	APSKLDR	REDTLTR	QTATLKR	5.75 ± 0.39

Supplementary Table 2 (continued).

Site Name	ZFA Name	Target Sequence	F1 subsite & RH sequence	F2 subsite & RH sequence	F3 subsite & RH sequence	Mean B2H fold-activation
1947R	OZ429	gTGCGGCTGTt	TGT RRQHLLT	GGC LKEHLTR	TGC ANRTLGH	0.89 ± 0.03
1947R	OZ430	gTGCGGCTGTt	TRQKLTL	EQQLKR	ANRSLGR	1.00 ± 0.04
1947R	OZ431	gTGCGGCTGTt	RKQHLLL	EKGHLTR	QQRSLVG	1.00 ± 0.05
1947R	OZ432	gTGCGGCTGTt	RKQHLVL	GNRVLAA	ANRTL VH	1.20 ± 0.07
1947R	OZ433	gTGCGGCTGTt	KRQHLVL	ERQHLVR	QQRSLVG	1.21 ± 0.18
1947R	OZ434	gTGCGGCTGTt	RNQHLMI	ERGHLTR	ANRTL VH	0.93 ± 0.08
1947R	OZ435	gTGCGGCTGTt	TRQKLTT	QGGNLVR	QQRSLVG	1.03 ± 0.18
1947R	OZ436	gTGCGGCTGTt	RQRNLAI	EQSKLTR	ANRTL VH	0.91 ± 0.07
1947R	OZ437	gTGCGGCTGTt	RKPLLIV	ESSKLKR	SARGLGV	2.04 ± 0.30
1947R	OZ438	gTGCGGCTGTt	RKQHLSL	ERSKLQR	QQRSLVG	1.18 ± 0.02
1947R	OZ439	gTGCGGCTGTt	RRQHLLT	LPEHLKR	QQRSLVG	1.07 ± 0.03
1947R	OZ440	gTGCGGCTGTt	SNRNLKI	AASGLAG	ANRTL VH	1.20 ± 0.15
1947R	OZ441	gTGCGGCTGTt	TKQVLDR	LSQTLKR	RTDGLGH	2.14 ± 0.29
2163L	OZ391	aGAAGGATT Cc	TTC RPNHLAI	GGA QSPHLKR	GAA QSNLTR	8.40 ± 0.29
2163L	OZ392	aGAAGGATT Cc	RPNHLTA	QSAHLKR	LGENLRR	5.74 ± 0.34
2163L	OZ393	aGAAGGATT Cc	RPNHLAI	QSPHLKR	QSNLTR	8.10 ± 0.05
2163L	OZ394	aGAAGGATT Cc	RANHLTI	QSAHLKR	LGENLRR	6.41 ± 0.87
2163R	OZ395	aGGCGGAGATc	GAT SQQALGV	GGA QSAHLKR	GGC ESGHLKR	1.70 ± 0.23
2163R	OZ396	aGGCGGAGATc	TKQH LAV	QSAHLKR	ESGHLRR	1.89 ± 0.04
2163R	OZ397	aGGCGGAGATc	SKQALAV	QSAHLKR	ENSKLRR	1.89 ± 0.13
2163R	OZ398	aGGCGGAGATc	TNQRLDV	QNPHTN	KNVSLVG	0.89 ± 0.02
2163R	OZ399	aGGCGGAGATc	SKQALAV	QSAHLKR	ENSKLRR	1.74 ± 0.07
2163R	OZ400	aGGCGGAGATc	TKQH LAV	QSAHLKR	ESGHLRR	2.08 ± 0.25
2163R	OZ401	aGGCGGAGATc	SKQALAV	QSAHLKR	ESGHLKR	1.75 ± 0.15

ZFAs used for *in vivo* experiments are in bold. The Zif268 F2 backbone was used for each of the fingers in the OPEN ZFAs (Maeder *et al.*, Mol. Cell, 31:294). RH, recognition helix; B2H, bacterial two-hybrid assay. Standard deviation among three replicates is shown for B2H data. The 2163R B2H reporter gave high levels of background making it difficult to estimate the activity of the 2163R arrays.

Supplementary Table 3. Frequency of insertion/deletion polymorphism introduced at the ZFN target sites.

SuRA						
ZFN¹	Total seq. reads	Seq. reads with insertions	Seq. reads with deletions	Insertions + deletions	Insertions + deletions/total seq. reads	Rep Ratio minus Zif268 Ratio²
815 Rep1	2693	56	89	145	0.0538	0.0459
815 Rep2	4566	110	165	275	0.0602	0.0523
815 Rep3	3067	64	101	165	0.0538	0.0459
815 Rep4	2913	59	81	140	0.0481	0.0401
Zif268	20224	95	65	160	0.0079	
					Normalized Ratio:	0.0461 +/- 0.0049
1853 Rep1	1318	48	91	139	0.1055	0.0338
1853 Rep2	1386	60	53	113	0.0815	0.0099
1853 Rep3	1234	39	68	107	0.0867	0.0151
1853 Rep4	1680	42	68	110	0.0655	-0.0061
Zif268	8057	341	236	577	0.0716	
					Normalized Ratio:	0.0132 +/- 0.0162
2163 Rep1	2736	25	36	61	0.0223	0.0083
2163 Rep2	2481	23	23	46	0.0185	0.0045
2163 Rep3	2503	16	29	45	0.0180	0.0040
2163 Rep4	2770	16	19	35	0.0126	-0.0014
Zif268	15495	82	135	217	0.0140	
					Normalized Ratio:	0.0039 +/- 0.0039
SuRB						
815 Rep1	33946	286	371	657	0.0194	0.0117
815 Rep2	27370	205	207	412	0.0151	0.0074
815 Rep3	36676	298	367	665	0.0181	0.0104
815 Rep4	36354	265	334	599	0.0165	0.0088
Zif268	22224	96	75	171	0.0077	
					Normalized Ratio:	0.0096 +/- 0.0019
1853 Rep1	14388	838	451	1289	0.0896	0.0410
1853 Rep2	15238	736	329	1065	0.0699	0.0213
1853 Rep3	13322	630	335	965	0.0724	0.0238
1853 Rep4	16670	693	396	1089	0.0653	0.0167
Zif268	6502	212	104	316	0.0486	
					Normalized Ratio:	0.0257 +/- 0.0104
2163 Rep1	30152	202	337	539	0.0179	-0.0004
2163 Rep2	31526	252	328	580	0.0184	0.0001
2163 Rep3	29409	231	351	582	0.0198	0.0015
2163 Rep4	17409	143	183	326	0.0187	0.0005
Zif268	16698	98	207	305	0.0183	
					Normalized Ratio:	0.0004 +/- 0.0008

¹Rep' refers to a replicate sample. For each replicate, 10⁶ protoplasts were transformed by electroporation with 30 µg of DNA encoding a given ZFN.

²Rep ratio' refers to the number of sequences with insertions and deletions divided by the total sequence reads obtained for a given sample. This ratio was then subtracted from the ratio obtained for the Zif268 control.

Supplementary Table 4. Sequence analysis of *SuRA* and *SuRB* from resistant calli resulting from treatment with ZFN 815 and donor DNAs with mutations that confer herbicide resistance at varying distances from the 815 cut site.

Callus¹	Donor	<i>SuRA</i> at ZFN target site	<i>SuRA</i> at site of mutation	<i>SuRB</i> at ZFN target site	<i>SuRB</i> at site of mutation
95.3.1	P191A pDW1963	Both alleles WT	Both alleles WT	Both alleles WT	Both alleles WT
*95.3.2	P191A pDW1963	Both alleles WT	Both alleles WT	Both alleles WT	HR at one allele
95.3.3	P191A pDW1963	Both alleles WT	HR at one allele	4 bp deletion at 815 cut site in one allele	Both alleles WT
95.3.4	P191A pDW1963	Both alleles WT	HR at one allele	3 bp deletion at cut site at one allele and a 1 bp insertion (C) at the other	Both alleles WT
95.3.5	P191A pDW1963	Both alleles WT	HR at one allele	One allele has an insertion at the 815 cut site	Both alleles WT
95.3.6	P191A pDW1963	Both alleles WT	HR at one allele	Both alleles WT	Both alleles WT
95.3.7	P191A pDW1963	Both alleles WT	Both alleles WT	The allele without an HR event has a 4 bp deletion at the 815 cut site	HR at one allele
95.3.8	P191A pDW1963	Both alleles WT	Both alleles WT	Both alleles WT	HR at one allele
95.3.9	W568L pDW1963	Both alleles WT	Both alleles WT	The allele without the HR event has a 5 bp deletion at the 815 cut site	HR at one allele
95.3.10	P191A pDW1963	Both alleles WT	HR at one allele	1 bp insertion (C or A) at the 815 cut site in one allele and an uncharacterized insertion at the 815 cut site in the other allele	Both alleles WT
95.3.11	P191A pDW1963	Both alleles WT	Both alleles WT	Both alleles WT	Both alleles WT
95.3.12	P191A pDW1963	Both alleles WT	Both alleles WT	Both alleles WT	Both alleles WT
95.6.1	W568L pDW1927	Both alleles WT	Both alleles WT	Both alleles WT	Both alleles WT
95.6.2	W568L pDW1927	Both alleles WT	HR at one allele	2 bp insertion (AG) at 815 site in one allele	Both alleles WT
95.6.3	W568L pDW1927	Both alleles WT	HR at one allele	Both alleles WT	Both alleles WT
*95.6.4	W568L pDW1927	Both alleles WT	Both alleles WT	The allele without an HR event has an 11 bp deletion at the 815 cut site	HR at one allele
95.6.5	W568L pDW1927	Both alleles WT	Both alleles WT	Both alleles WT	HR at one allele
95.6.6	W568L pDW1927	Both alleles WT	Both alleles WT	Both alleles WT	HR at one allele
*95.6.7	W568L pDW1927	Both alleles WT	Both alleles WT	The other allele has a 1 bp insertion (G) at the 815 cut site	HR at one allele
95.6.8	W568L pDW1927	Both alleles WT	HR at one allele	Both alleles WT	Both alleles WT
*95.6.9	W568L pDW1927	Both alleles WT	HR at one allele	Both alleles WT	Both alleles WT
95.6.10	W568L pDW1927	Both alleles WT	Both alleles WT	Both alleles WT	Both alleles WT
95.6.11	W568L pDW1927	Both alleles WT	Both alleles WT	Both alleles WT	Both alleles WT
*95.6.12	W568L pDW1927	Both alleles WT	Both alleles WT	Both alleles WT	HR at one allele

Supplementary Table 4 (continued).

Callus¹	Donor	<i>SuRA</i> at ZFN target site	<i>SuRA</i> at site of mutation	<i>SuRB</i> at ZFN target site	<i>SuRB</i> at site of mutation
95.7.1	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	S647N spontaneous mutation
95.7.2	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	S647N spontaneous mutation
95.7.3	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	Both alleles WT
95.7.4	S647T pDW1969	Both alleles WT	Both alleles WT	2 bp insertion (AG) at 815 cut site in one allele	Both alleles WT
95.7.5	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	A199V spontaneous mutation
95.7.6	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	A199V spontaneous mutation
95.7.7	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	A199V spontaneous mutation
95.7.8	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	S647N spontaneous mutation
95.7.9	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	S647N spontaneous mutation
95.7.10	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	S647N spontaneous mutation
95.7.11	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	S647N spontaneous mutation
95.7.12	S647T pDW1969	Both alleles WT	HR at one allele	2 bp deletion in one allele	Both alleles WT
97.2.1	P191A pDW1963	Both alleles WT	Both alleles WT	The allele without the HR event has a 5 bp deletion at the 815 cut site	HR at one allele
*97.4.1	P191A pDW1963	Both alleles WT	HR at one allele	Both alleles WT	Both alleles WT

¹The 95.3 series was generated with the P191A donor; the 95.6 series was generated with the W568L donor; the 95.7 series was generated with the S647T donor. The 97 series differs from the others in that transformed cells were first identified using an NPTII reporter gene, which was introduced along with ZFN 815 and the W568L donor. The transformed cell population was then subjected to herbicide selection. Asterisks denote those calli from which whole plants were regenerated and shown to carry mutations introduced by gene targeting.

Supplementary Table 5. Sequence analysis of *SuRA* and *SuRB* from resistant calli resulting from treatment with ZFNs 815, 1853, 2163 and donor DNAs with mutations in the ZFN recognition site that prevent cleavage.

Callus	ZFN	Donor	<i>SuRA</i> at ZFN target site	<i>SuRA</i> at site of mutation	<i>SuRB</i> at ZFN target site	<i>SuRB</i> at site of mutation
88.2.1	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
88.2.2	815	P191A-m pDW1964	One <i>SuRA</i> allele has a 4 bp deletion and an 'A' insertion at the 815 break site	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
*88.2.3	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
88.2.4	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site; other allele has a 5 bp deletion at the 815 target site	HR at one allele
*88.2.5	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
88.2.6	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
*88.2.7	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
88.2.8	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
88.2.9	815	P191A-m pDW1964	One <i>SuRA</i> allele has a 1 bp insertion (G) at 815 break site	Both alleles WT	Recombinant alleles have mutations from donor at the 815 target site; one <i>SuRB</i> allele also has a 'G' insertion at the 815 target	HR at both alleles
88.2.10	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
86.2-11	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Both alleles WT	Spontaneous missense mutation (P196Q)
86.2-12	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
86.2-13	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
86.2-14	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
86.2-15	815	P191A-m pDW1964	Seven bp deletion in <i>SuRA</i> (CACGAGC)	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site; the other allele has a 1 bp deletion	HR at one allele
86.2-16	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at both alleles
86.2-17	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at one allele
86.2-18	815	P191A-m pDW1964	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 815 target site	HR at both alleles

Supplementary Table 5 (continued).

Callus	ZFN	Donor	SuRA at ZFN target site	SuRA at site of mutation	SuRB at ZFN target site	SuRB at site of mutation
86.5.1	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site	HR at one allele
86.5.2	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site; other allele has a 5 bp deletion	HR at one allele
86.5.3	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site	HR at one allele
*86.5.4	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site	HR at one allele
86.5.5	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site	HR at one allele
86.5.6	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site; other allele has a 5 bp deletion	HR at one allele
86.5.7	1853	W568L-m pDW1968	Both alleles WT	One allele has a V193L spontaneous mutation	Both alleles WT	Both alleles WT
86.5.8	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site	HR at one allele
86.5.9	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Both alleles WT	One allele has a V190L spontaneous mutation
86.5.10	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site	HR at one allele
86.5.24	1853	W568L-m pDW1968	Both alleles WT	Both alleles WT	Recombinant allele has mutations from donor at the 1853 target site	HR at one allele
86.7.1	2163	S647T pDW1969	Both alleles WT	Both alleles WT	Both alleles WT	One allele has a S647N spontaneous mutation
86.10.1	2163	S647T-m pDW1972	Both alleles WT	Both alleles WT	Both alleles WT	One allele has a W568L spontaneous mutation

¹The 88.2 and 86.2 series were generated with the P191A-m donor; the 86.5 series was generated with the W568L-m donor. The 86.7 and 86.10 series were generated with the S647T and S647T-m donors, respectively. Asterisks denote those calli from which whole plants were regenerated and shown to carry mutations introduced by gene targeting.

Supplementary Table 6. Summary of ZFAs and functional tests performed prior to experiments in plants.

ZFN	Site Name	ZFA Name	Engineering Method	Target Sequence	F1 subsite/ RH sequence	F2 subsite/ RH sequence	F3 subsite/ RH sequence	Mean B2H fold-activation ¹	EMSA ²	Yeast Assay ³
815	815L	pRW128	Modular Assembly	tGACGTGGTAG	GTA/ QSSSLVR	GTG/ RSDELVR	GAC/ DPGNLVR	NT	+	6.12 ± 0.82
	815R	pRW129	Modular Assembly	cAGGGTGGTGt	GTG/ RSDELVR	GTG/ RSDELVR	AGG/ RSDHLTN	NT	+	10.74 ± 1.10
1853	1853L	OZ408	OPEN	tGGTGCTGCCc	GCC/ DLSTLRR	GCT/ LSQTLKR	GGT/ HTHHLGR	10.13 ± 0.26	NT	5.43 ± 0.60
	1853R	OZ414	OPEN	gTGGGCTGCTc	GCT/ TKQVLDR	GCT/ LSQTLNR	TGG/ RVDHLGG	5.49 ± 0.51	NT	6.63 ± 0.67
2163	2163L	OZ391	OPEN	aGAAGGATTCC	TTC/ RPNHLAI	GGA/ QSPHLKR	GAA/ QSNLTR	8.40 ± 0.29	NT	2.70 ± 0.33
	2163R	OZ396	OPEN	aGGCGGAGATc	GAT/ TKQHIAV	GGA/ QSAHLKR	GGC/ ESGHLRR	1.89 ± 0.04	NT	2.07 ± 0.22

¹Data from Supplementary Table 2.

²Data from Supplementary Table 1.

³Data from Figure 2. Values are for the low copy target plasmids.

Supplementary Table 7. Effect of nuclease architecture on recovery of herbicide resistance.

Row	ZFN ¹	Donor DNA	Distance to mutation	Plating efficiency ²	Number of herbicide resistant cells in 2 X 10 ⁶ treated protoplasts ³
1	815 homodimer	P191A	188 bp	14.3%	0
2	815 heterodimer	P191A	188 bp	29.5%	168
3	815 homodimer	W568L	1319 bp	13.6%	0
4	815 heterodimer	W568L	1319 bp	24.7%	100
5	815 homodimer	S647T	1541 bp	13.4%	1
6	815 heterodimer	S647T	1541 bp	29.7%	41

¹The term homodimer denotes the wild type *FokI* nuclease; heterodimer denotes a modified nuclease architecture that requires heterodimerization for activity (Szczeppek et al., Nature Biotechnol. 25:786-93).

²Plating efficiency refers to the frequency with which calli are formed in the absence of selection relative to the total number of protoplasts treated.

³Note that no homologous recombination events were recovered with ZFN constructs expressing the *FokI* nuclease homodimer architecture. The single resistant cell line recovered with the S647T donor was a spontaneous herbicide resistance mutation (data not shown). Heterodimeric ZFN pairs were used for all gene targeting experiments described in the manuscript.