Supplemental Figure Legends

Supplemental Figure 1. T7EI lesion rate analysis for each TALEN within the *apoea* locus. Schematic of the *apoea* locus with the positions of the TALEN binding sites indicated at the top of the page, where each site is numbered and the distance between them is indicated in basepairs. The TALEN-induced lesion rates were calculated using the T7 Endonuclease I (T7EI) assay by comparing T7EI activity in TALEN-injected embryos and uninjected embryos at 24 hpf. Filled triangles indicate the position of the uncleaved PCR product and open triangles denote the position of bands consistent with T7EI cleavage at the expected position within the PCR amplicon. Lesion rates, which were calculated as previously described, are indicated at the bottom of each lane (Guschin et al. 2010).

Supplemental Figure 2. Quantification of the deletion rate in zebrafish embryos. A standard curve was generated by serially diluting the genomic DNA of a zebrafish embryo that was heterozygous for the 500 bp deletion in the *apoea* locus with the genomic DNA from a wild-type embryo (Expected frequency: percentages indicated above the gel image; Calculated frequency: percentages indicated below the gel image). A linear correlation was established for the formation of PCR amplicons spanning this deletion relative to the wild-type sequence (Lee et al. 2010). Using this standard curve, we can estimate the deletion rate in pools of genomes from TALEN-injected embryos. For the *apoea*

500 bp segmental deletion, we estimate the frequency of occurrence is 6.7 and 15% for the 100 pg and 250 pg dose, respectively.

Supplemental Figure 3. (Top) Schematic diagram of the *apoea* locus demarcating the position of the two proximal TALEN binding sites following an inversion at this locus. Each TALEN half-site is differentially colored (cyan, blue, orange, yellow), as are the spacers between the target sites (red, green), which are shown as hybrids due to the expected heterogeneous joining of these regions following inversion. Primer position and directionality following the inversion is indicated by arrows within the schematic. (below) Junction sequences for the 500 bp segmental inversions at the *apoea* locus. The TALEN-1 and TALEN-2 recognition sequences are indicated above the junction sequences, where the individual TALEN half-sites are differentially colored. PCR products spanning each junction region were shotgun cloned from 24 hpf embryos and sequenced to define the breakpoints associated with segment inversion. The resulting inversion creates two new junctions between the TALEN cleavage sites within the locus. Insertions or mutations within the recovered sequences are shown in uppercase regular font.

Supplemental Figure 4. A) Schematic diagram of the unequal exchange between homologous chromosomes containing the *apoea* locus with the positions of the TALEN-1 and TALEN-2 binding sites denoted. Red arrows indicate the position of a single DSB within each locus at different positions on

homologous chromosomes. Following cleavage at each position unequal exchange occurs between homologous chromosomes resulting in the generation of a segmental deletion and a segmental duplication. Each primer position and directionality is indicated by arrows within the schematic. B) Segmental duplication is detected in TALEN-treated embryos when two TALENs are co-injected, where the primer pair used for detection is listed to the left of the figure. The presence or absence of each TALEN component (5' and 3' recognition unit) is indicated above each gel lane, as is the dose of each TALEN component. PCR products corresponding to the segmental duplication (~135 bp) are present only in embryos injected with both TALENs. Estimated duplication rate is indicated below the positive lane. C) Junction sequences for the segmental duplication at the *apoea* locus. The TALEN-1 and TALEN-2 recognition sequences are indicated above the junction sequences, where the individual TALEN recognition sites are differentially colored. PCR products spanning this region were shotgun cloned and sequenced to define the breakpoints associated with segment duplication. Each fusion point creates a set of heterogeneous junction sequences between the TALEN binding sites.

Supplemental Figure 5. A) Schematic diagram of the *apoea* locus demarcating the positions of all four TALEN binding sites and the distance separating them. B) Junction sequences for the 750 bp segmental deletion at the *apoea* locus. The TALEN-1 and TALEN-3 recognition sequences are indicated above the junction sequences, where the individual TALEN half-sites are differentially colored. In

the recovered junction sequences there are variable length deletions as well as evidence of insertions between some of the target sites, where the latter are indicated in plain text. C) Junction sequences for the 4.2 kb segmental inversions at the *apoea* locus. The TALEN-1 and TALEN-4 recognition sequences are indicated above the junction sequences, where the individual TALEN half-sites are differentially colored. PCR products spanning each junction region were shotgun cloned from 24 hpf TALEN-injected embryos and sequenced to define the breakpoints associated with segment inversion. Each fusion point creates a set of heterogeneous junction sequences between the TALEN binding sites. Insertions or mutations within the recovered sequences are shown in uppercase regular font.

Supplemental Figure 6. A) Segmental duplication is readily detected at the *apoea* locus in TALEN-treated embryos when TALEN-1 and TALEN-4 are co-injected. The presence or absence of each TALEN component (5' and 3' recognition unit) is indicated above each gel lane, as is the dose of each TALEN component. PCR products corresponding to the segmental duplication (~156 bp) are present only in embryos injected with both TALENs. The estimated duplication rate is indicated below the positive lane. B) Junction sequences for the segmental duplication at the *apoea* locus. The TALEN-1 and TALEN-4 recognition sequences are indicated above the junction sequences, where the individual half-sites are differentially colored. PCR products spanning the potential duplication junction were shotgun cloned and sequenced to define the

associated breakpoints. Each fusion point creates a set of heterogeneous junction sequences between the TALEN binding sites. Insertions or mutations within the recovered sequences are shown in uppercase regular font.

Supplemental Figure 7. T7EI lesion rate analysis for each TALEN within the *flt4* locus. Schematic of the *flt4* locus with the positions of the TALEN binding sites indicated at the top of the page, where the sites are numbered and the distance between them is indicated in basepairs. The TALEN-induced lesion rates were calculated using the T7 Endonuclease I (T7EI) assay by comparing T7EI activity in TALEN-injected embryos and uninjected embryos at 24 hpf. Filled triangles indicate the position of the uncleaved PCR product and open triangles denote the position of bands consistent with T7EI cleavage at the expected position within the PCR amplicon. Lesion rates, which were calculated as previously described, are indicated at the bottom of each lane (Guschin et al. 2010).

Supplemental Figure 8. A) Segmental duplication is detected at the *flt4* locus in TALEN-treated embryos when TALEN-1 and TALEN-3 are co-injected. The presence or absence of each TALEN component (5' and 3' recognition unit) is indicated above each gel lane, as is the dose of each TALEN component. PCR products corresponding to the segmental duplication (~235 bp) are present only in embryos injected with both TALENs. Estimated duplication rate is indicated below the positive lane. B) Junction sequences for the segmental duplication at the *flt4* locus. The TALEN-1 and TALEN-3 recognition sequences are indicated

above the junction sequences, where the TALEN recognition sites are differentially colored. PCR products spanning the potential duplication junction were shotgun cloned and sequenced to define the associated breakpoints. Each fusion point creates a set of heterogeneous junction sequences between the TALEN binding sites. Insertions or mutations within the recovered sequences are shown in uppercase regular font.

Supplemental Figure 9. T7EI lesion rate analysis for the ZFN within the *gol* locus and the TALEN within the *lepa* locus. Schematic of the region of chromosome 18 spanned by these nucleases with the positions of the nuclease binding sites indicated at the top of the genomic diagram, where the distance between the sites is indicated. The TALEN-induced lesion rates were calculated using the T7 Endonuclease I (T7EI) assay by comparing T7EI activity in TALEN-injected embryos and uninjected embryos at 24 hpf. Filled triangles indicate the position of the uncleaved PCR product and open triangles denote the position of bands consistent with T7EI cleavage at the expected position within the PCR amplicon. Bars across the top of the panels indicate the amplification of a common genomic region for testing two TALENs (e.g. TAL-3 and TAL-4 we assessed using a common primer set). The highest mobility band on the 2-log ladder lane in each panel is 100 bp. Lesion rates, which were calculated as previously described, are indicated at the bottom of each lane (Guschin et al. 2010).

Supplemental Figure 10. T7EI lesion rate analysis for each TALEN targeting the major *globin* LCR control region. Schematic of the *nrpl3* locus with the positions of the TALEN binding sites indicated at the top of the page, where the TALEN target sites are numbered and the distance between these sites in basepairs is indicated. The TALEN-induced lesion rates were calculated using the T7 Endonuclease I (T7EI) assay by comparing T7EI activity in normal-appearing TALEN-injected embryos and uninjected embryos at 24 hpf. Filled triangles indicate the position of the uncleaved PCR product and open triangles denote the position of bands consistent with T7EI cleavage at the expected position within the PCR amplicon. Lesion rates, which were calculated as previously described, are indicated at the bottom of each lane (Guschin et al. 2010).

Supplemental Figure 11. T7EI lesion rate analysis for each TALEN targeting the *megamind* lincRNA. Schematic of the *birc6* locus with the positions of the TALEN sites flanking the *megamind* locus indicated at the top of the page, where the TALEN target sites are numbered and the distance between the most distant pair of sites flanking this element is indicated. The TALEN-induced lesion rates were calculated using the T7 Endonuclease I (T7EI) assay by comparing T7EI activity in normal-appearing TALEN-injected embryos and uninjected embryos at 24 hpf. Filled triangles indicate the position of the uncleaved PCR product and open triangles denote the position of bands consistent with T7EI cleavage at the expected position within the PCR amplicon. Lesion rates, which were calculated

as previously described, are indicated at the bottom of each lane (Guschin et al. 2010).

Supplemental Figure 12. Founder analysis for the germline transmission of segmental deletions and inversions at the *apoea* locus for animals treated with TALEN-1 and TALEN-2 (~500 bp separation). A) Potential founders (16 in total, identified by ".#") were outcrossed and progeny (pool of at least 20 embryos) from each founder was assayed for the presence of segmental deletion and inversion using PCR analysis. Genomic DNA from TALEN-injected embryos was used as the positive control (+ve) and from uninjected embryos as the negative control (-ve). Positive founders are indicated by green text above each lane. Animals displaying positive transmission of deletion or inversion were outcrossed again and the PCR analysis was repeated on single embryos (except for founder .16 for which no additional off-spring were obtained in subsequent matings) to determine the frequency of transmission where these numbers are indicated below positive lanes in parenthesis as (number of positive embryos / number of embryos screened). The lower two panels display T7EI analysis for detecting germline transmission of lesions at each TALEN (TALEN-1 & TALEN-2) target site in the pool of embryos from each of the 16 potential founders. Calculated lesion frequencies are indicated below each lane. B) Junction sequences for the 500 bp segmental deletions and one inversion at the *apoea* locus. The TALEN-1 and TALEN-2 recognition sequences are indicated above the junction sequences, where the TALEN recognition sites are differentially colored. PCR products spanning the segmental deletion from individual embryos

of each founder were sequenced to define the breakpoints associated with segment deletion, where the founder ID is indicated to the left of each sequence. For some founders more than one segmental deletion was transmitted to their offspring. In the case of founder .11, the second segmental deletion junction contains an additional insertion 'AGTCCTTTGGA' ~50 bp away from the junction. For the inversion founder, PCR product spanning the TALEN-1 and TALEN-2 binding sites from the individual embryo was shotgun cloned and sequenced. The 5' and 3' junction sequences are shown.

Supplemental Figure 13. Founder analysis for the transmission of segmental deletions or inversions at the *apoea* locus for animals treated with TALEN-1 and TALEN-4 (4.2 kb separation). A) Potential founders (17 in total, identified by ".#") were outcrossed and progeny (pool of at least 20 embryos) from each founder was assayed for the presence of segmental deletion and inversion using PCR analysis. Genomic DNA from TALEN-injected embryos was used as the positive control (+ve) and from uninjected embryos as the negative control (-ve). Positive founders are indicated by green text above each lane. Animals displaying positive transmission of deletion or inversion were outcrossed again and the PCR analysis was repeated on single embryos to determine the frequency of transmission where these numbers are indicated below positive lanes in parenthesis as (number of positive embryos / number of embryos screened). The lower two panels display T7EI analysis for detecting germline transmission of lesions at each TALEN (TALEN-1 & TALEN-4) target site in the pool of embryos from each of the 16 potential founders. Calculated lesion frequencies are

indicated below each lane. B) Junction sequences for the 4.2 kb segmental deletions. The TALEN-1 and TALEN-4 recognition sequences are indicated above the junction sequences, where the TALEN recognition sites are differentially colored. PCR products spanning the segmental deletion from individual embryos of each founder were sequenced to define the breakpoints associated with segment deletion, where the founder ID is indicated to the left of each sequence. Insertions or mutations within the recovered sequences are shown in uppercase regular font.

Supplemental Figure 14. Linked lesions at the TALEN-1 and TALEN-2 sites in the founder progeny. PCR amplicon spanning the *apoea* TALEN-1 and TALEN-2 sites from the apoea-500bp founder '.13' was shotgun cloned and sequenced to determine lesions at the TALEN sites. TALEN binding sites were aligned to the expected sequences. Monomeric TALEN sites and spacers are differentially colored. Insertions and mutations are displayed in regular text.

Supplemental Figure 15. Sequences of the *golden* ZFNs. The ZFNs employ the DD/RR versions of FokI nuclease.

Supplemental Table 1: Summary of activities of two nucleases at various loci in zebrafish. At each loci, individual nuclease activity as well as frequencies of deletions, inversions and duplications induced by two nucleases are given. Where available, the rate of founder identification and germline transmission

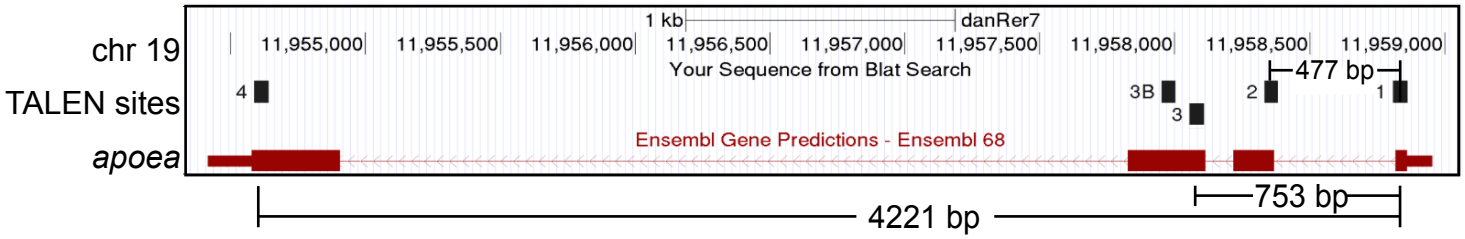
frequencies obtained from outcrossing TALEN-treated animals are provided. N.A. indicates Not Assayed.

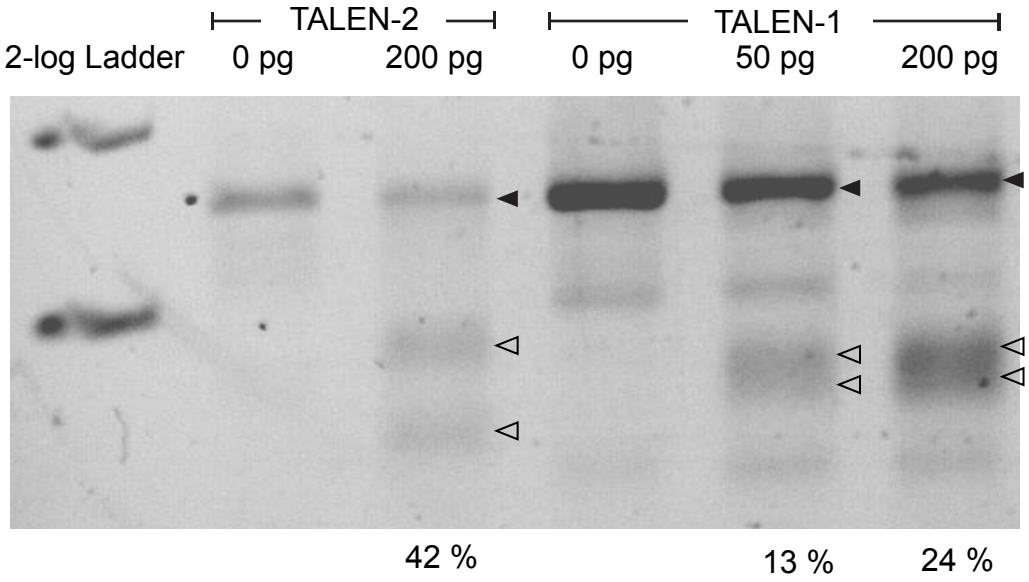
Supplemental Table 2: List of TALENs and their target sites tested in this study. The table provides the sequences for the TALEN-half sites and their lengths along with the spacer lengths. Base composition of the target sites is indicated where the '0^{th}-T' was not included in the analysis. Also, the activity of each TALEN at its target site, as determined by the T7EI assay, is provided.

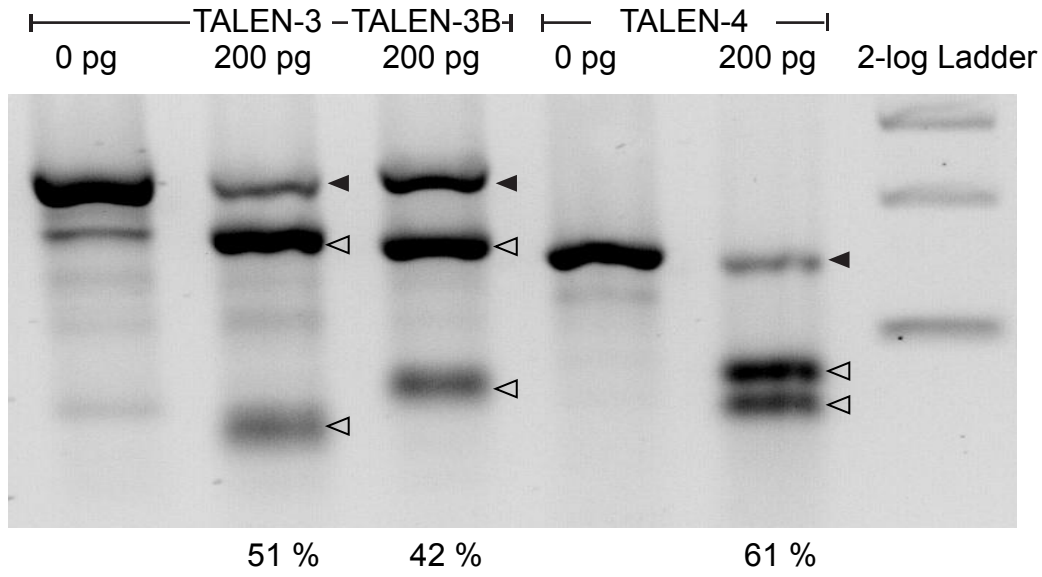
Supplemental Table 3: List of primer sequences used for lesion analysis.

Supplemental Table 4: List of primer pairs used for amplifying the region of interest for each application.

Supplemental Figure 1







Supplemental Figure 2

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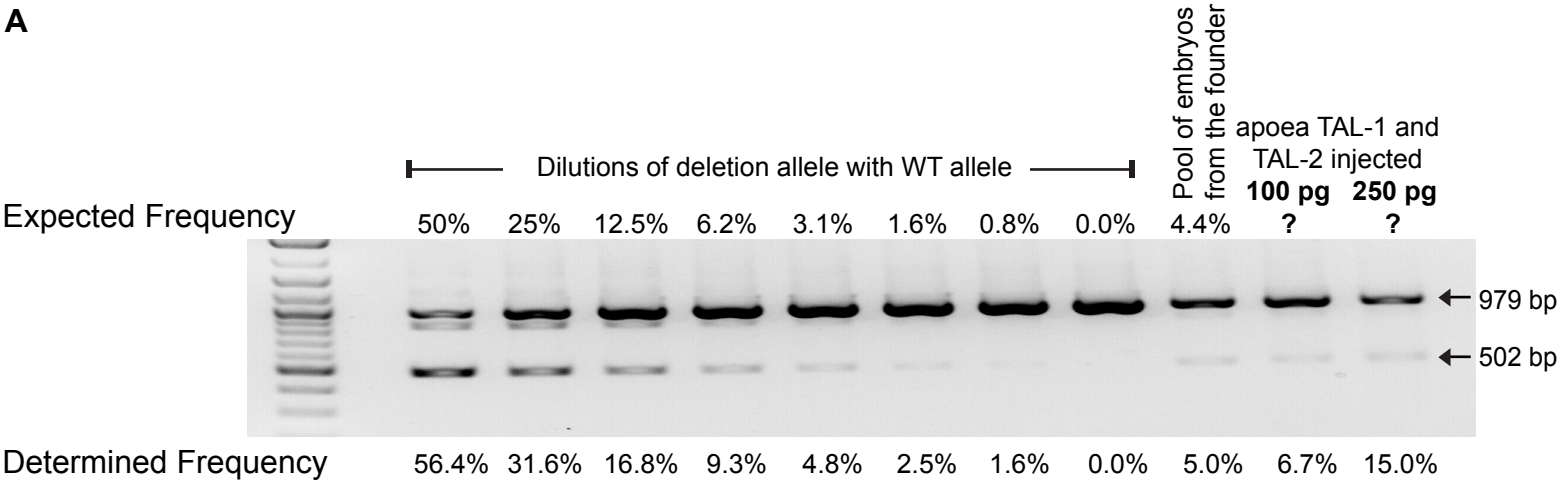

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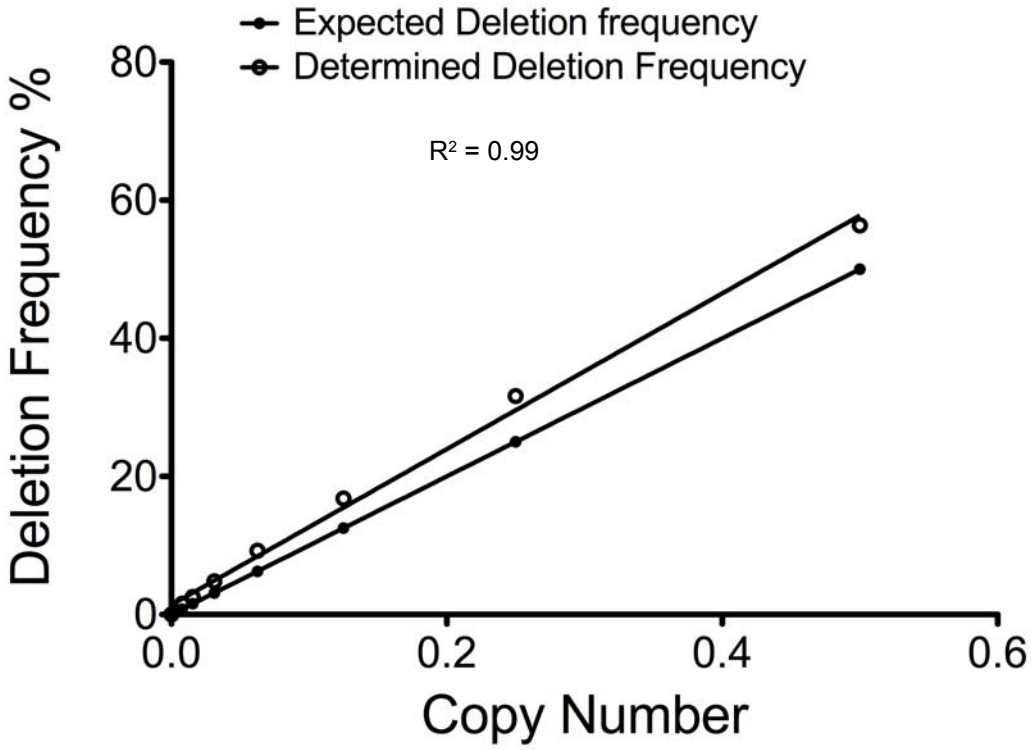
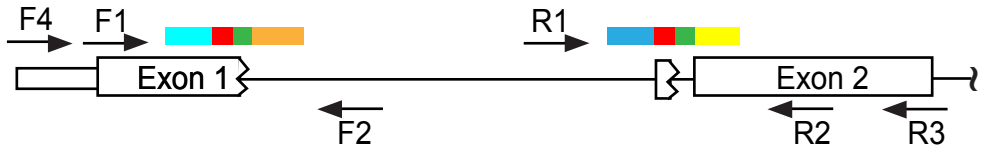


Supplemental Figure 3



5' Junction sequences

```
                    5' half-site                                  3' half-site
TALEN-1 Site        TCATGAAGTTTGTGGCTGTaattgttgcgctcgccGTCATTTCAGGTAGGAAA
TALEN-2 Site          TTTTGACTTTGCAGGCTgccaggggcgattcctGTTTCAGGATGAGCCAA
Junction 1          TCATGAAGTTTGTGGCTGTaattgttg-ccctggcAGCCTGCAAAGTCAAAA
Junction 2          TCAAGAAGTTTGTGGCTGTaattg------ctggcAGCCTGCAAAGTCAAAA
Junction 3          TCATGAAGTTTGTGGCTGTaattgtcgcccctggcAGCCTGCAAAGTCAAAA
Junction 4          TCATGAAGTTTGTGGCTGTaatt--cgcccctggcAGCCTGCAAAGTCAAAA
Junction 5          TCATGAAGTTTGTGGCTGTaattgttgcccctggcAGCCTGCAAAGTCAAAA
Junction 6          TCATGAAGTTTGTGGCTGTaattgttgcccctggcAGCCTGCAAAGTCAAAA
Junction 7          TCATAAAGTTTGTGGCTGTaattgttgcccctggcAGCCTGCAAAGTCAAAA
                                                 CGCC
Junction 8          TCATGAAGTTTGTGGCTGTaattgtcgcccctggcAGCCTGCAAAGTCAAAA
                                                  C
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3' Junction sequences

```
                    5' half-site                                  3' half-site
TALEN-1 Site        TCATGAAGTTTGTGGCTGTaattgttgcgctcgccGTCATTTCAGGTAGGAAA
TALEN-2 Site          TTTTGACTTTGCAGGCTgccaggggcgattcctGTTTCAGGATGAGCCAA
Junction 1          TTTCCTACCTGAAATGACgg----------cctGTTTCAGGATGAGCCAA
Junction 2          TTTCCTACCTGAAATGACggcgag----attcctTTTTCAGGATGAGCCAA
Junction 3          TTTCCTACCTGAAATGACggcgagcgcaattcctTTTTCAGGATGAACCAA
Junction 4          TTTCCTACCTGAAATGAT---gagc-c-at-cc---TTCAGGATGAGCCAA
Junction 5          TTTCCTACCTGAAATGACggc----------tTTTTCAGGATGAGCCAA
Junction 6          TTTCCTACCTGAAATGACggcgagggcgattcctGTTTCAGGATGAGCCAA
Junction 7          TTTCCTACCTGAAATGACggcgagcgcgattcctGTTTCAGGATGAGCCAA
                                                  G
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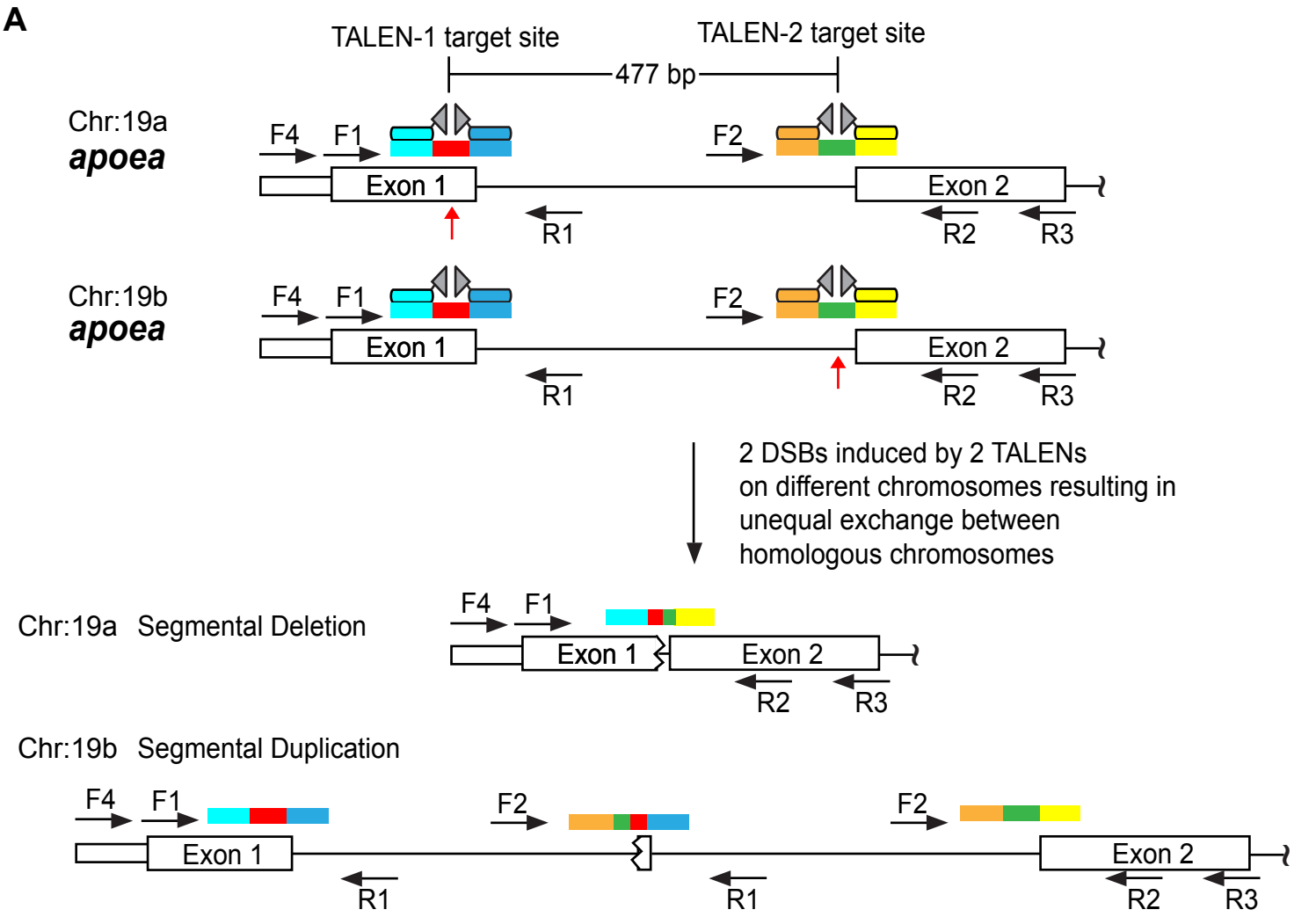
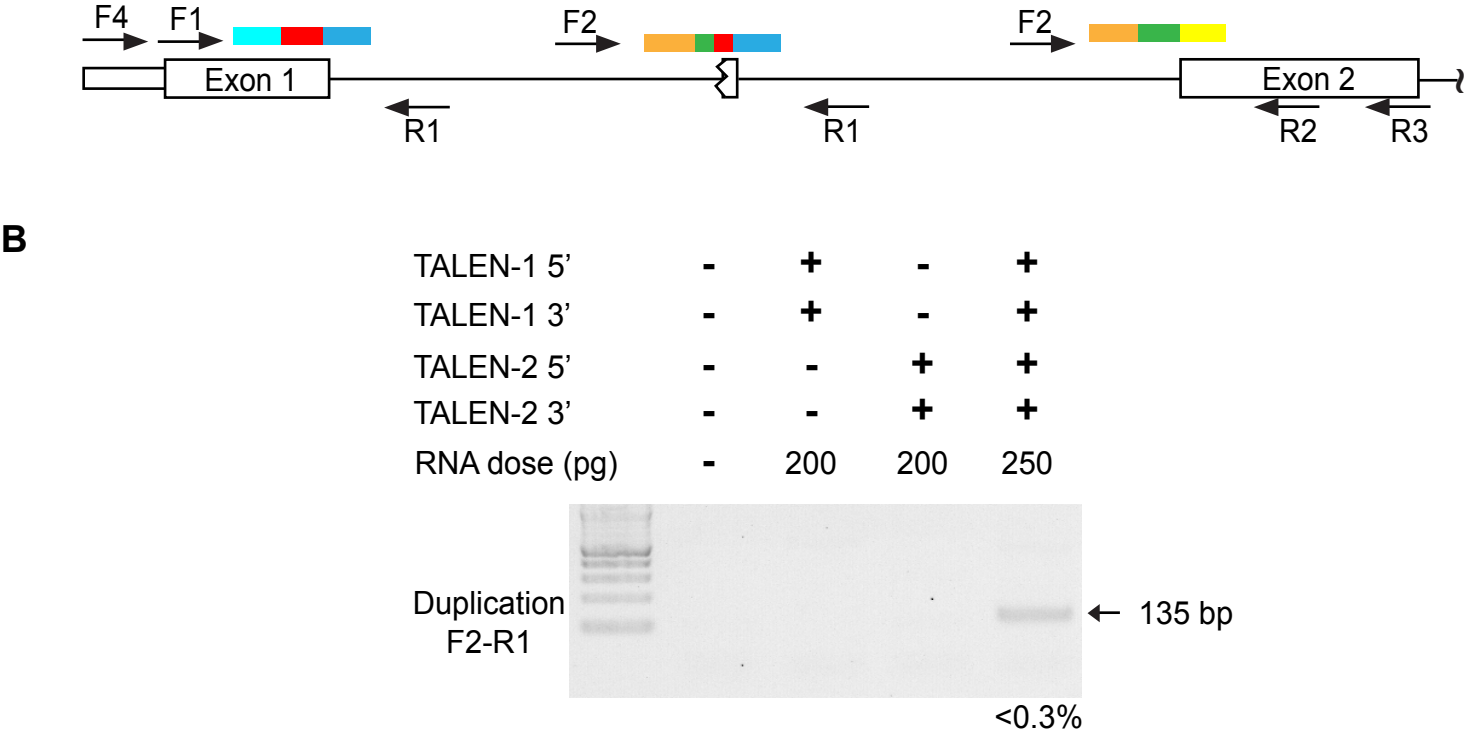
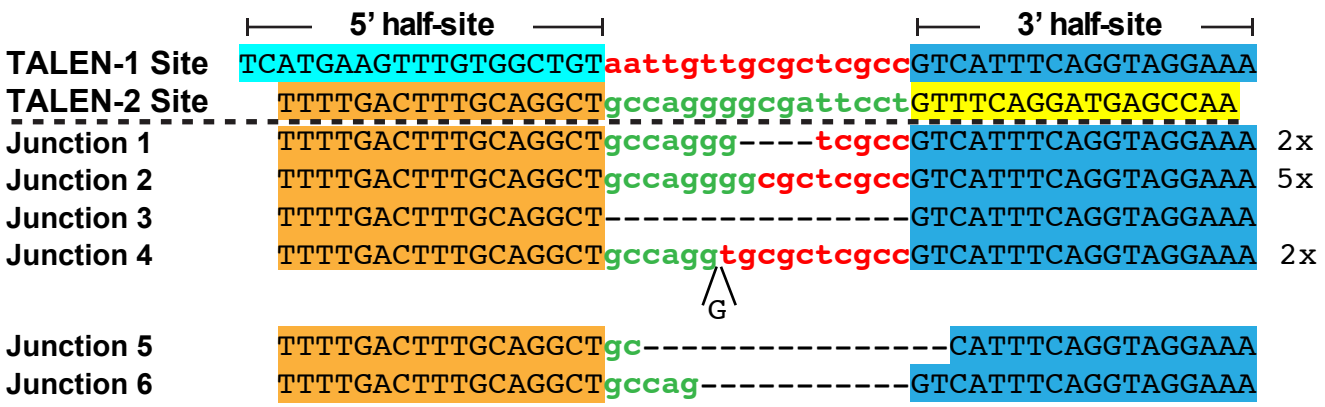
Supplemental Figure 4







Supplemental Figure 5

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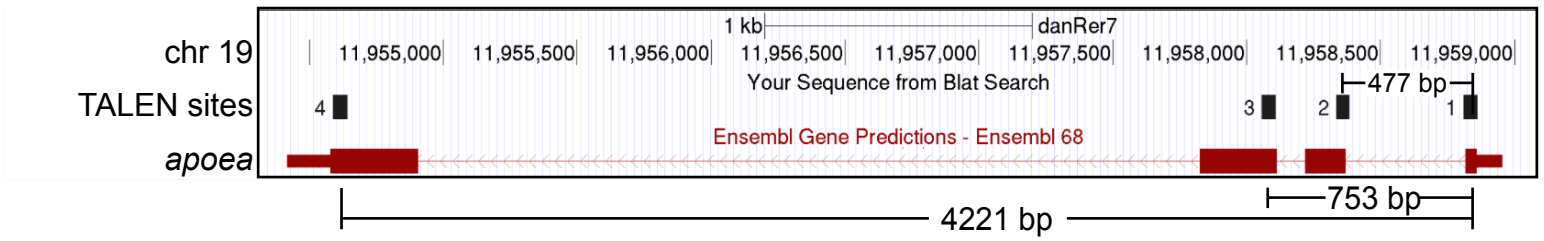

B **Junction sequences for 753 bp deletions**

	5' half-site	3' half-site
TALEN-1 Site	TCATGAAGTTTGTGGCTGTaattgttgcgctcgccGTCATTTCAGGTAGGAAA	
TALEN-3 Site	TGATCTCTGACAGCATGATggagctgcaaatgtacAGAGATGATCTGCACTCCA	
Junction 1	TCATGAAGTTTGTGGCTGTaattgttg-----tgg---------CTGCACTCCA	
	T	
Junction 2	TCATGAAGTTTGTGGCTGTaat--------tgtacAGAGATGATCTGCACTCCA	
Junction 3	TCATGAAGTTTGTGGCTGTaattgt----------AGAGATGATCTGCACTCCA	
Junction 4	TCATGAAGTTTGTGGCTGTaattg---CAAACTTGGCCCCAAACTGGCC--CCA	

C **Junction sequences for 4221 bp inversions**

5' Junction sequences

	5' half-site	3' half-site
TALEN-1 Site	TCATGAAGTTTGTGGCTGTaattgttgcgctcgccGTCATTTCAGGTAGGAAA	
TALEN-4 Site	TGGTTTGAGCCCTACGTCtctcacatccagaagcAAATGGAGCCAGTCTGGGA	
Junction 1	TCATGAAGTTTGTGGCTGTaat--------tgagaGACGTAGGGCTCAAACCA	
Junction 2	TCATGAAGTTTGTGGCTGTaattgttgatgtgagaGACGTAGGGCTCAAACCA	
	G	
Junction 3	TCATGAAGTTTGTGGCTGTaat------tgtgagaGACGTAGGGCTCAAACCA	
Junction 4	TCATGAAGTTTGTGGCTGTaattgt------gagaGGCGTAGGGCTCAAACCA	
Junction 5	TCATGAAGTTTGTGGC----------tgagaGACGTAGGGCTCAAACCA	
Junction 6	TCATGAAGTTTGTGGCTGTaat------tgtgagaGACGTAGGGCTCAAACCA	
Junction 7	TCATGAAGTTTGTGGCTGTaattgtt tgagaGACGTAGGGCTCAAACCA	
	CCATTCCAAA	

3' Junction sequences

	5' half-site	3' half-site
TALEN-1 Site	TCATGAAGTTTGTGGCTGTaattgttgcgctcgccGTCATTTCAGGTAGGAAA	
TALEN-4 Site	TGGTTTGAGCCCTACGTCtctcacatccagaagcAAATGGAGCCAGTCTGGGA	
Junction 1	TTTCATACCTGAAATTACggc---------aagcAAATGGAGCCAGTCTGGGA	
Junction 2	TTTCCTACCTGAAATGACggcgagcg-cagaagcAAATGGAGCCAGTCTGGGA	
Junction 3	TTTCCTACCTGAAATGACg----------gaagcAAATGGAGCCAGTCTGGGA	
Junction 4	TTTCCTACCTGAAATGACggcgag------aagcAAATGGAGCCAGTCTGGGA	
Junction 5	TTTCCTACCTGAAATGACggcg-------gaagcAAATGGAGCCAGTCTGGGA	
Junction 6	TTTCCTACCTGAAATGACggcgagcgc-agaagcAAATGGAGCCAGTCTGGGA	
Junction 7	TTTCCTACCTGAAATGACggcgagcgccagaagcAAATGGAGCCAGTCTGGGA	
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Supplemental Figure 6

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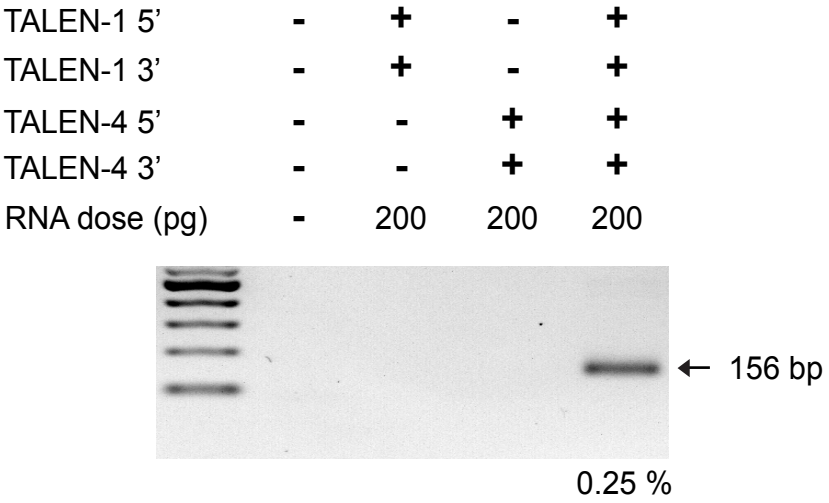

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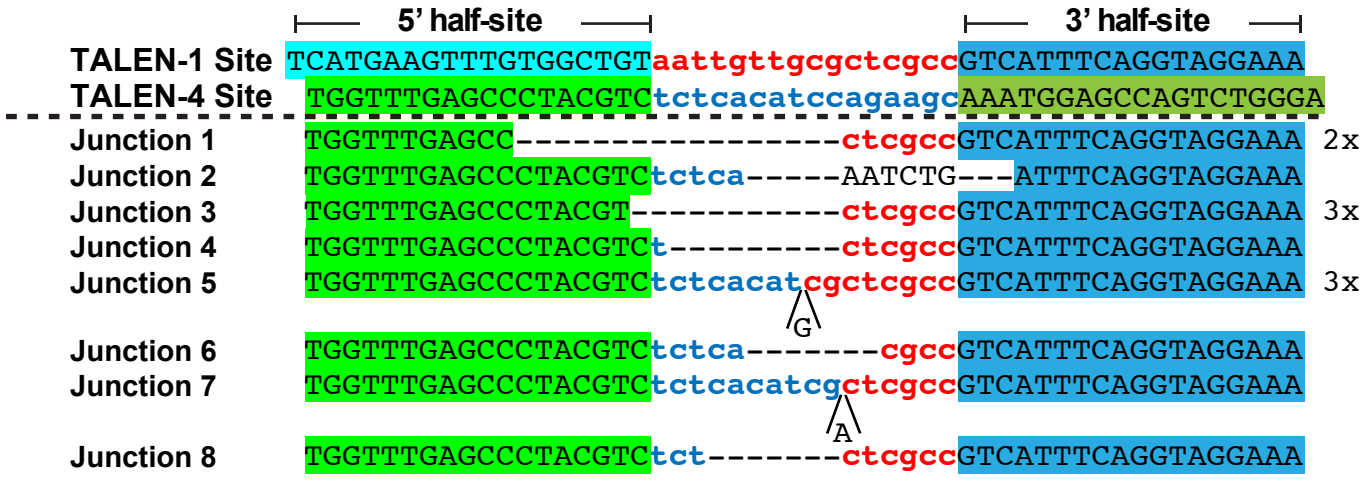
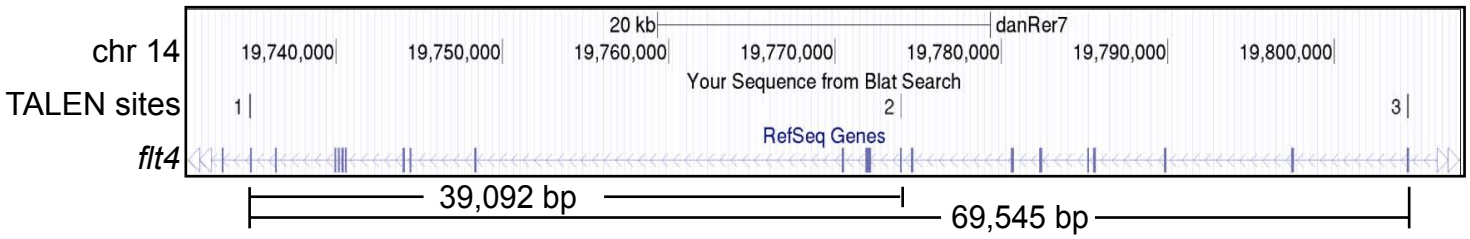
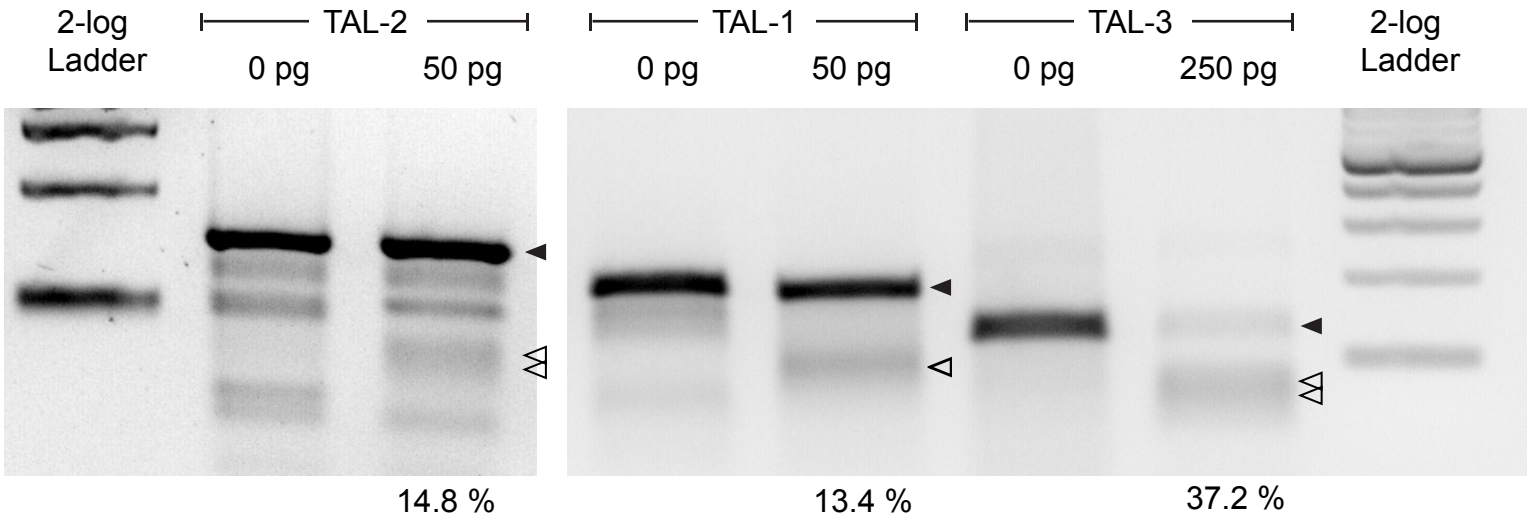


Supplemental Figure 7





Supplemental Figure 8

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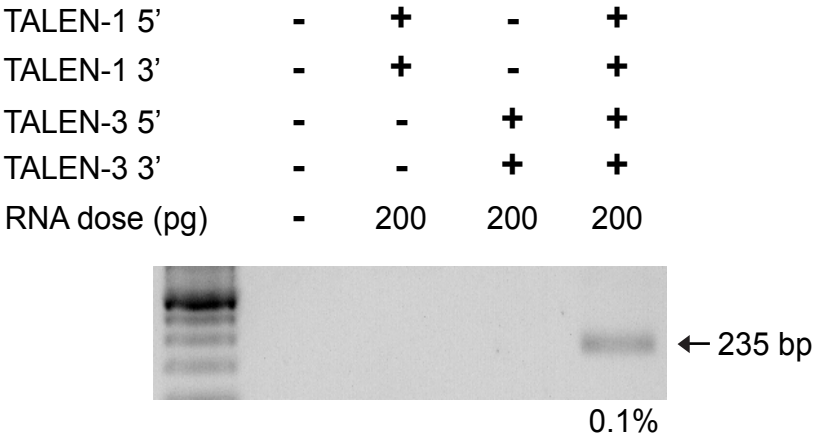

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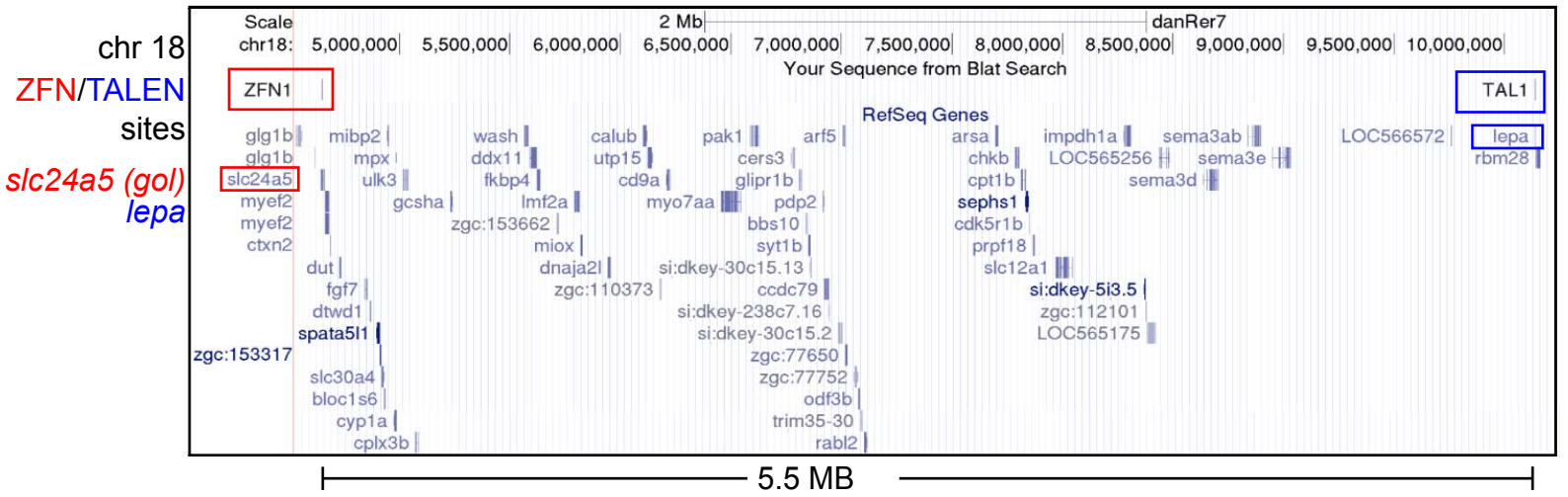
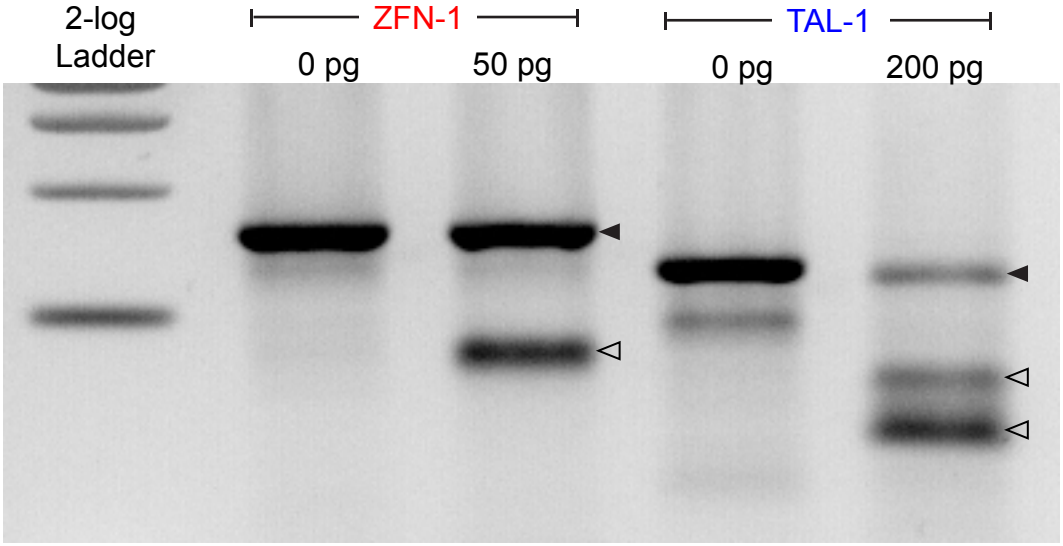
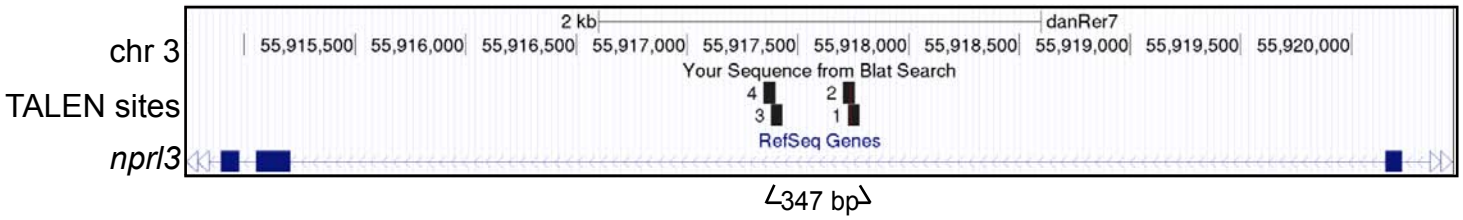
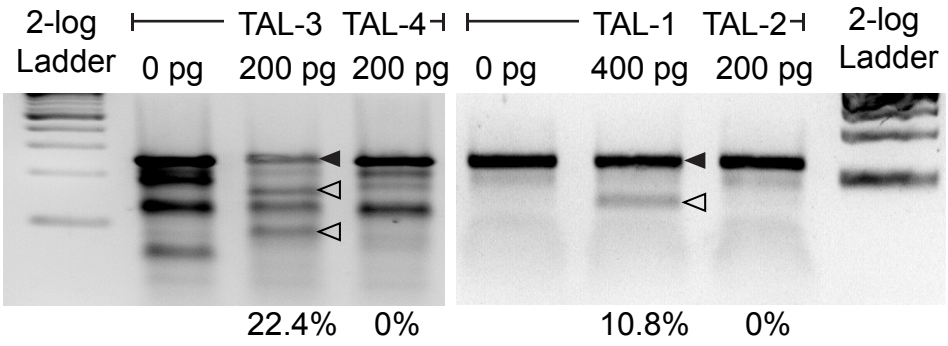
Supplemental Figure 9



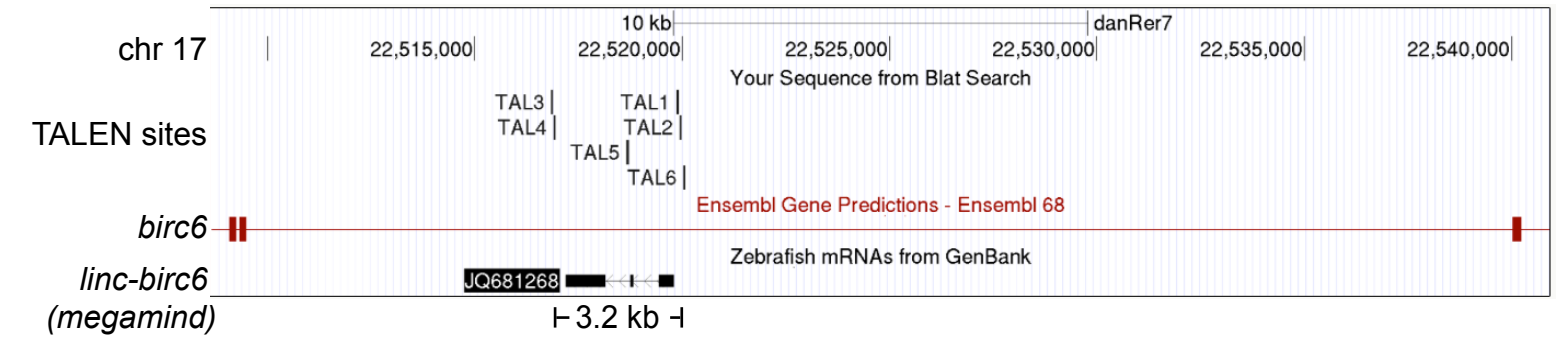


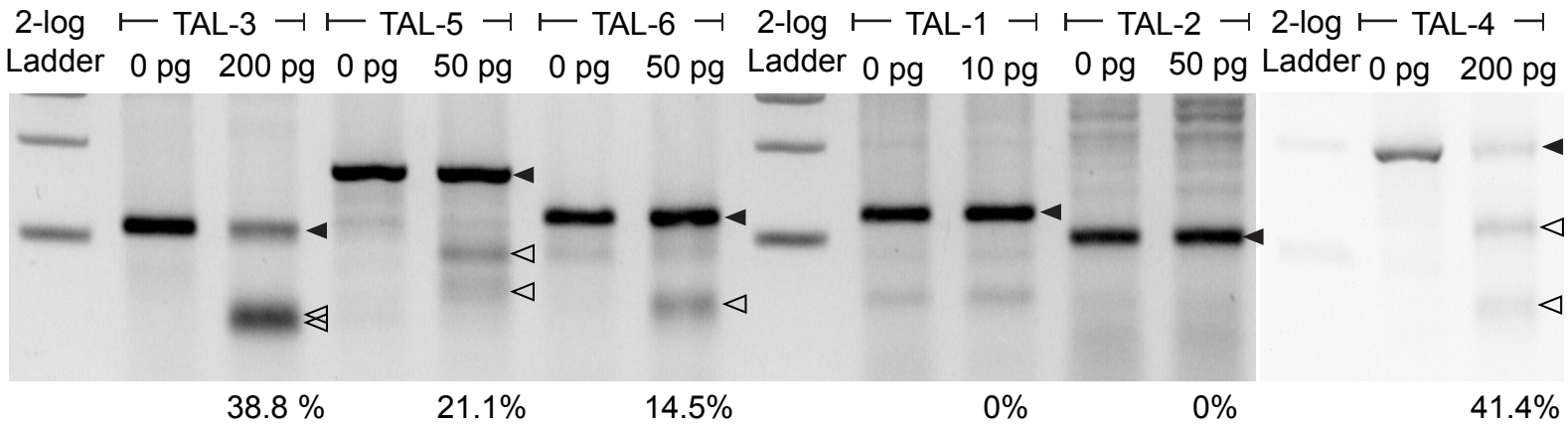
Supplemental Figure 10





Supplemental Figure 11





Supplemental Figure 12

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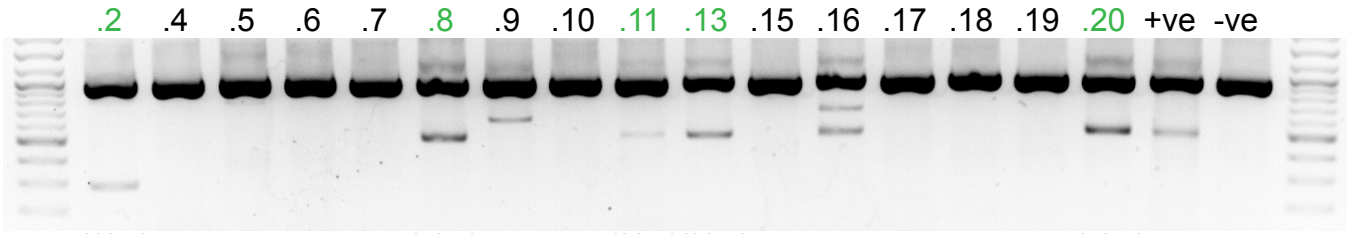
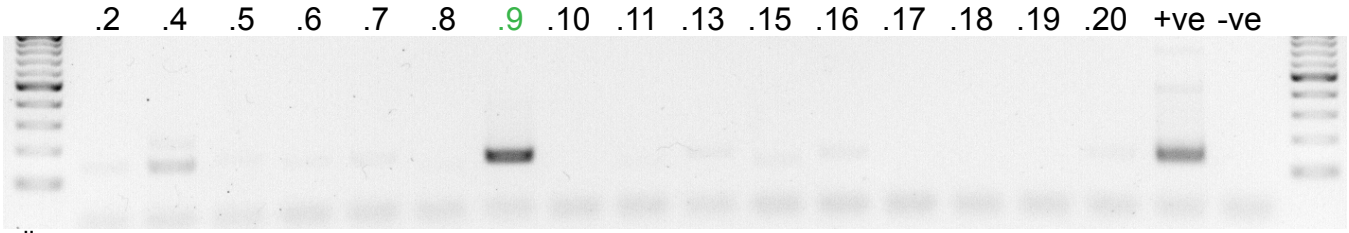
Segmental Deletion



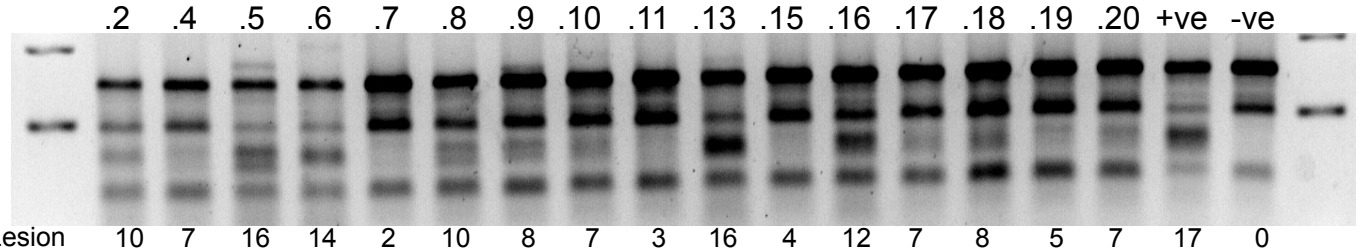
Germline transmission frequency (1/45) (4/45) (3/45)(3/45) (5/45)

Segmental Inversion



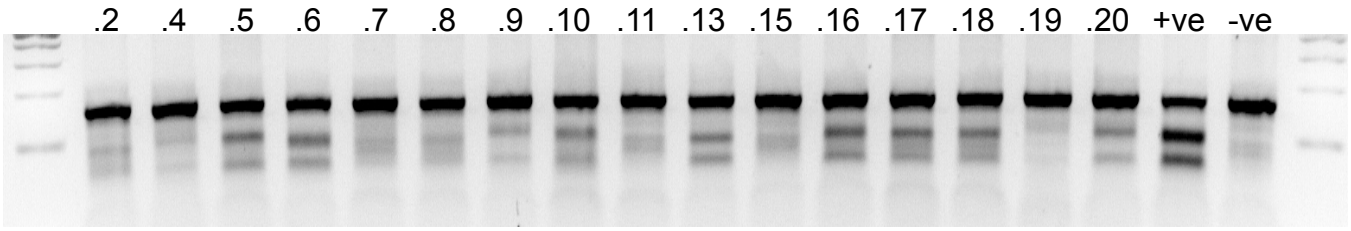
Germline transmission frequency (1/45)

TALEN-1 T7 Endonucleasel Assay



Lesion Frequency (%)	.2	.4	.5	.6	.7	.8	.9	.10	.11	.13	.15	.16	.17	.18	.19	.20	+ve	-ve
	10	7	16	14	2	10	8	7	3	16	4	12	7	8	5	7	17	0

TALEN-2 T7 Endonucleasel Assay



Lesion Frequency (%)	.2	.4	.5	.6	.7	.8	.9	.10	.11	.13	.15	.16	.17	.18	.19	.20	+ve	-ve
	7	3	17	19	4	4	8	14	0	15	1	21	17	16	0	13	36	0

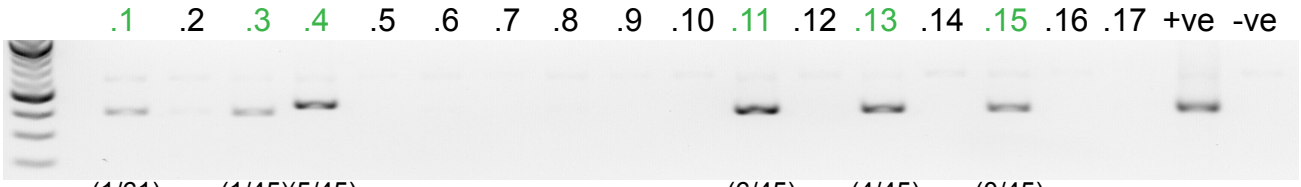
B Founder Deletion and Inversion Sequences

	5' half-site		3' half-site
TALEN-1 Site	TCATGAAGTTTGTGGCTGT	aattgttgcgctcgcc	GTCATTTCAGGTAGGAAA
TALEN-2 Site	TTTTGACTTTGCAGGCT	gccaggggcgattcct	GTTTCAGGATGAGCCAA
Deletion Sequences			
.2 Junction	706 bp deletion		GTCCAACGCATTCCTTTTTCAGGATGAGCCAA
.8 Junction A	TCATGAAGT	----------gcgattcct	GTTTCAGGATGAGCCAA
.8 Junction B	TCATGAAGTTTGTGGCTGT	aattgt-----ttcct	GTTTCAGGATGAGCCAA
.11 Junction A	TCATGAAGTTTGTGGCTGT	a---------attcct	GTTTCAGGATGAGCCAA
.11 Junction B^	TCATGAAGTTTGTGGCTGT	aatt-----gattcct	GTTTCAGGATGAGCCAA
.13 Junction	TCATGAAGTTTGTGGCTGT	aatt---------cct	ATTTCAGGATGAGCCAA
.20 Junction A	TCATGAAGTTTGTGGCTGT	aatt-----gattcct	GTTTCAGGATGAGCCAA
.20 Junction B	TCATGAAGTTTGTGGCTGT	aattgtt----ttcct	GTTTCAGGATGAGCCAA
Inversion Sequences			
.9 5' Junction	TCATGAAGTTTGTGGATGT	aattgtcgcccctggc	AGCCTGCAAAGTCAAAA
.9 3' Junction	TTTCCTACCTGAAATGAC	ggcgagcgcgattcct	GTTTCAGGATGAGCCAA

Supplemental Figure 13

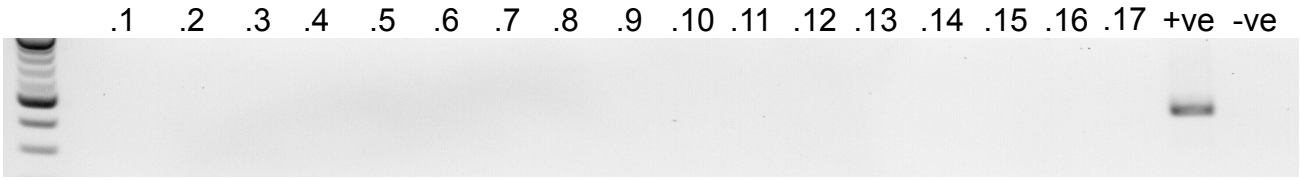
A

Segmental Deletion

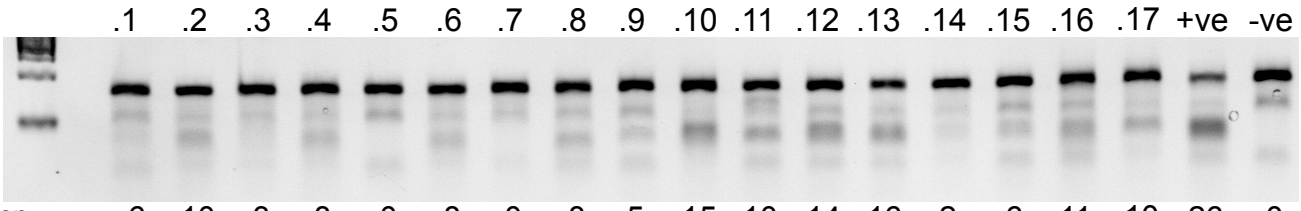


Germline transmission frequency: (1/61) (1/45) (5/45) (6/45) (4/45) (0/45)

Segmental Inversion

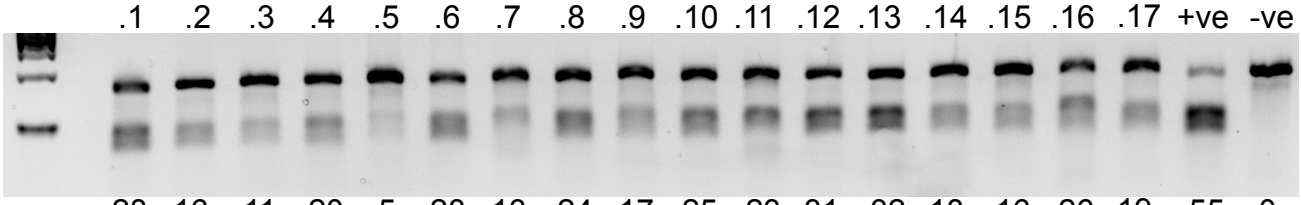


TALEN-1 T7 Endonucleasel Assay



Lesion Frequency (%)	.1	.2	.3	.4	.5	.6	.7	.8	.9	.10	.11	.12	.13	.14	.15	.16	.17	+ve	-ve
	6	10	3	8	0	8	0	8	5	15	10	14	16	2	6	11	10	26	0

TALEN-4 T7 Endonucleasel Assay



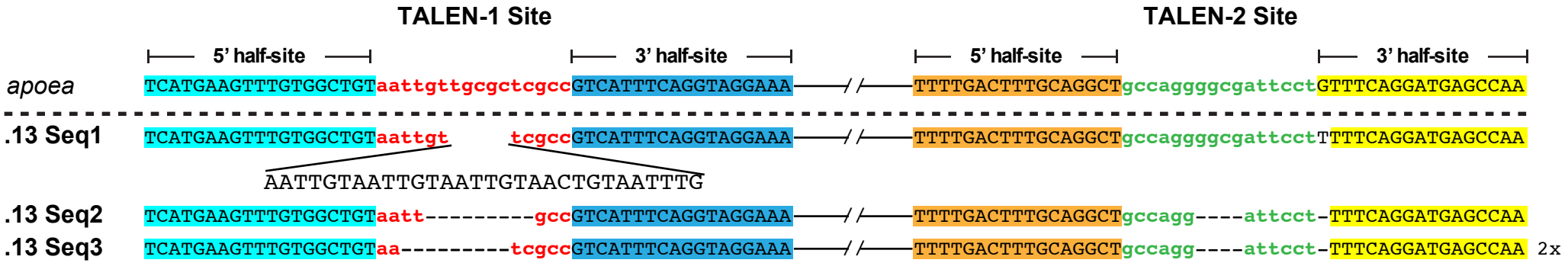
Lesion Frequency (%)	.1	.2	.3	.4	.5	.6	.7	.8	.9	.10	.11	.12	.13	.14	.15	.16	.17	+ve	-ve
	28	16	11	20	5	28	13	24	17	25	23	31	32	18	16	26	19	55	0

B Founder Deletion Sequences

5' half-site — 3' half-site

```
TALEN-1 Site   TCATGAAGTTTGTGGCTGTaattgttgcgctcgccGTCATTTCAGGTAGGAAA
TALEN-4 Site    TGGTTTGAGCCCTACGTCtctcacatccagaagcAAATGGAGCCAGTCTGGGA
.1 Junction    TCATGAAGTTTGTGGCTGTaattgttCc--gaagcAAATGGAGCCAGTCTGGGA
.3 Junction    TCATGAAGTTTGTGGCTGTaattgt--------gcAAATGGAGCCAGTCTGGGA
.4 Junction    TCATGAAGTTTGTGGCTGTaattgt      gaagcAAATGGAGCCAGTCTGGGA
                                  GGCTGTAAAATGTAAAAAATTTGTGGCTGTAATT
.11 Junction   TCATGAAGTTTGTGGCTGTaattgt-----gaagcAAATGGAGCCAGTCTGGGA
.13 Junction   TCATGAAGTTTGTGGCTGTaattgt-----CAGCcAAATGGAGCCAGTCTGGGA
```

Supplemental figure 14



Supplemental Figure 15.

5’ golden ZFN (FokI-RR in italics)
GTQPYKCPECGKSFSLKGNLTRHQRTHTGEKPYACPVESCDRRFSTSGNLTR
HIRIHTGQKPFQCRICMRNFSDRSHLARHIRTHTGEKPFACDICGRKFAVASNLT
RHTKIHTGGS*QLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVM*
EFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQAR
EMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRL
NHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

3’ golden ZFN (FokI-DD in italics)
GTERPYKCPECGKSFSYRQSLTRHQRTHTGEKPYACPVESCDRRFRQSSHLK
QHIRIHTGQKPFQCRICMRNFSERTNLIHHIRTHTGEKPFACDICGRKFALSFNL
TRHTKIHTGGS*QLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKV*
MEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQA
DEMQDYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

Supplemental Figure 16

TALEN Scaffold Sequence:

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHRGVPMVDLRTLGYSQQQ
QEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALP
EATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAV
EAVHAWRNALTGAPLPLN
(LTPDQVVAIAS**XX**GGKQALETVQRLLPVLCQDHG)$_{n-1}$
(LTPDQVVAIAS**XX**GGRPALE)
SIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIP
ERTSHRVAGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKV
MEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQA
DEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF*

Key:
3X Flag Tag
Nuclear Localization Signal
TAL N-terminal Domain
TAL Repeats (**XX** represents the RVD where NI recognizes Adenine, NN recognizes Guanine, NG recognizes Thymidine, and HD recognizes Cytosine). 'n' is the length of the TALEN monomer binding site where the 1st base (T) is ignored for calculating 'n'.
TAL half repeat (this is the terminal nth TAL repeat where **XX** is the same as described above)
TAL C-terminal Domain
Wild type *FokI* nuclease domain

Supplemental Table 1

Locus	Deletion length	Nuclease-A Activtiy (%)	Nuclease-B Activity (%)	Deletion frequency (%)	Inversion Frequency (%)	Duplication Frequency (%)	Founder Rate (# of positives / # of animals screened)	Germline Transmission frequency
apoea	477 bp	42	24	15	0.3	< 0.3	5/16	2% - 11%
apoea	753 bp	42	51	10	1.1	N.A.	N.A.	N.A.
apoea	4221 bp	42	61	2.7	1.8	0.25	5/17	1% - 13%
flt4	39 kb	13	15	3.2	0.4	N.A.	N.A.	N.A.
flt4	69 kb	13	37	4.9	0.2	0.1	N.A.	N.A.
slc24a5/lepa	5.5 MB	24	53	0.7	N.A.	N.A.	N.A.	N.A.
globin LCR	347 bp	11	22	1.1	N.A.	N.A.	N.A.	N.A.
linc-birc6	3.2 kb	39	15	1.2	N.A.	N.A.	N.A.	N.A.

Supplemental Table 2

Locus	Name	TALEN 5p site	TALEN 3p site	5p TALEN site length	3p TALEN site length	Spacer length	%A	%C	%G	%T	Lesion frequency (%)
apoea	TALEN-1	TTTCCTACCTGAAATGAC	TCATGAAGTTTGTGGCTGT	17	18	16	22.9	20.0	22.9	34.3	42
apoea	TALEN-2	TTGGCTCATCCTGAAAC	TTTTGACTTTGCAGGCT	16	16	16	18.8	25.0	21.9	34.4	24
apoea	TALEN-3	TGGAGTGCAGATCATCTCT	TGATCTCTGACAGCATGAT	18	18	16	25.0	22.2	25.0	27.8	51
apoea	TALEN-3b	TGACACGATCCTTCGCCT	TTGTTGGTCACCAAGCTC	17	17	16	17.6	35.3	20.6	26.5	42
apoea	TALEN-4	TCCCAGACTGGCTCCATTT	TGGTTTGAGCCCTACGTC	18	17	16	14.3	34.3	22.9	28.6	61
Flt4	TALEN-1	TAAGGGTTCCTCTTACATT	TCTACAAAGACCCTGACT	18	17	14	28.6	28.6	14.3	28.6	13
Flt4	TALEN-2	TCTTTTCCCACTTTGTTCT	TGCCAGTGTGCCAGCTAT	18	17	17	11.4	31.4	17.1	40.0	15
Flt4	TALEN-3	TCTGAAACCAGACATGGT	TCTTCATAACAGACTCT	17	16	17	33.3	27.3	15.2	24.2	37
lepa	TALEN-1	TATACAGGAAGTATGCGTT	TGCTCAAAATACAGGTT	18	16	16	35.3	14.7	23.5	26.5	53
globin LCR	TALEN-1	TTAAAAGTGATGAGTCAGC	TTTCTGAGAAACTGAAGA	18	17	16	40.0	11.4	25.7	22.9	11
globin LCR	TALEN-2	TTGTCTGTGAGGGTCTTCA	TTTGGTTTATCAGCGCCGC	18	18	16	11.1	22.2	30.6	36.1	0
globin LCR	TALEN-3	TAATTGAGTATGCCCTAT	TCCCTGAAAATTGGATACA	17	18	16	34.3	20.0	17.1	28.6	22
globin LCR	TALEN-4	TTTGTATCCAATTTTCAGG	TTGTTCAAATGCACCAAAA	18	18	16	33.3	19.4	13.9	33.3	0
linc-birc6	TALEN-1	TACAAAAGACAGTCATATTT	TCACACACAGGCATTTAATA	19	19	16	44.7	21.1	18.5	23.7	0
linc-birc6	TALEN-2	TGTACATGTGCATGGGTG	TACAACTCTAGTATGAAA	17	17	16	32.4	14.7	26.5	26.5	0
linc-birc6	TALEN-3	TCATGAAGCTCCCATCAG	TCCTGTCACATGTGATAG	17	17	16	26.5	29.4	20.6	23.5	39
linc-birc6	TALEN-4	TGCATTCTCAGAGGGTT	TCTGTCTGTACCAGTGGT	16	17	16	15.2	21.2	30.3	33.3	41
linc-birc6	TALEN-5	TGGACAACAAGCCTTGT	TAGTGTTCCCTCCACTGT	16	17	16	21.2	30.3	21.2	27.3	21
linc-birc6	TALEN-6	TGTAGAGGCATCCATTG	TTGAACAGCCCAGAATGT	16	17	16	30.3	21.2	27.3	21.2	15

Supplemental Table 3

Locus	Name	TALEN monomer binding site and RVD sequence
apoea	TALEN-1-5p	T T T C C T A C C T G A A A T G A C
		NG NG HD HD NG NI HD HD NG NN NI NI NI NG NN NI HD
	TALEN-1-3p	T C A T G A A G T T T G T G G C T G T
		HD NI NG NN NI NI NN NG NG NG NN NG NN NN HD NG NN NG
apoea	TALEN-2-5p	T T G G C T C A T C C T G A A A C
		NG NN NN HD NG HD NI NG HD HD NG NN NI NI NI HD
	TALEN-2-3p	T T T T G A C T T T G C A G G C T
		NG NG NG NN NI HD NG NG NG NN HD NI NN NN HD NG
apoea	TALEN-3-5p	T G G A G T G C A G A T C A T C T C T
		NN NN NI NN NG NN HD NI NN NI NG HD NI NG HD NG HD NG
	TALEN-3-3p	T G A T C T C T G A C A G C A T G A T
		NN NI NG HD NG HD NG NN NI HD NI NN HD NI NG NN NI NG
apoea	TALEN-3b-5p	T G A C A C G A T C C T T C G C C T
		NN NI HD NI HD NN NI NG HD HD NG NG HD NN HD HD NG
	TALEN-3b-3p	T T G T T G G T C A C C A A G C T C
		NG NN NG NG NN NN NG HD NI HD HD NI NI NN HD NI HD
apoea	TALEN-4-5p	T C C C A G A C T G G C T C C A T T T
		HD HD HD NI NN NI HD NG NN NN HD NI HD HD NI NG NG NG
	TALEN-4-3p	T G G T T T G A G C C C T A C G T C
		NN NN NG NG NG NN NI NN HD HD HD NG NI HD NN NG HD
Flt4	TALEN-1-5p	T A A G G G T T C C T C T T A C A T T
		NI NI NN NN NN NG NG HD HD NI HD NG NG NI HD NI NG NG
	TALEN-1-3p	T C T A C A A A G A C C C T G A C T
		HD NG NI HD NI NI NI NN NI HD HD HD NG NN NI HD NG
Flt4	TALEN-2-5p	T C T T T T C C C A C T T T G T T C T
		HD NG NG NG NG HD HD HD NI HD NG NG NG NN NG NG HD NG
	TALEN-2-3p	T G C C A G T G T G C C A G C T A T
		NN HD HD NI NN NG NN NG NN HD HD NI NN HD NG NI NG
Flt4	TALEN-3-5p	T C T G A A A C C A G A C A T G G T
		HD NG NN NI NI NI HD HD NI NN NI HD NI NG NN NN NG
	TALEN-3-3p	T C T T C A T A A C A G A C T C T
		HD NG NG HD NI NG NI NI HD NI NN NI HD NG HD NG
lepa	TALEN-1-5p	T A T A C A G G A A G T A T G C G T T
		NI NG NI HD NI NN NN NI NI NN NG NI NG NN HD NN NG NG
	TALEN-1-3p	T G C T C A A A A T A C A G G T T
		NN HD NG HD NI NI NI NI NG NI HD NI NN NN NG NG
globin LCR	TALEN-1-5p	T T A A A A G T G A T G A G T C A G C
		NG NI NI NI NI NN NG NN NI NG NN NI NN NG HD NI NN HD
	TALEN-1-3p	T T T C T G A G A A A C T G A A G A
		NG NG HD NG NN NI NN NI NI NI HD NG NN NI NI NN NI

Supplemental Table 3 contd.

Locus	Name	TALEN monomer binding site and RVD sequence
globin LCR	TALEN-2-5p	T T G T C T G T G A G G G T C T T C A
		NG NN NG HD NG NN NG NN NI NN NN NN NG HD NG NG HD NI
	TALEN-2-3p	T T T G G T T T A T C A G C G C C G C
		NG NG NN NN NG NG NG NI NG HD NI NN HD NN HD HD NN HD
globin LCR	TALEN-3-5p	T A A T T G A G T A T G C C C T A T
		NI NI NG NG NN NI NN NG NI NG NN HD HD HD NG NI NG
	TALEN-3-3p	T C C C T G A A A A T T G G A T A C A
		HD HD HD NG NN NI NI NI NI NG NG NN NN NI NG NI HD NI
globin LCR	TALEN-4-5p	T T T G T A T C C A A T T T T C A G G
		NG NG NN NG NI NG HD HD NI NI NG NG NG NG HD NI NN NN
	TALEN-4-3p	T T G T T C A A A T G C A C C A A A A
		NG NN NG NG HD NI NI NI NG NN HD NI HD HD NI NI NI NI
linc-birc6 (Megamind)	TALEN-1-5p	T A C A A A A G A C A G T C A T A T T T
		NI HD NI NI NI NI NN NI HD NI NN NG HD NI NG NI NG NG NG
	TALEN-1-3p	T C A C A C A C A G G C A T T T A A T A
		HD NI HD NI HD NI HD NI NN NN HD NI NG NG NG NI NI NG NI
linc-birc6 (Megamind)	TALEN-2-5p	T G T A C A T G T G C A T G G G T G
		NN NG NI HD NI NG NN NG NN HD NI NG NN NN NN NG NN
	TALEN-2-3p	T A C A A C T C T A G T A T G A A A
		NI HD NI NI HD NG HD NG NI NN NG NI NG NN NI NI NI
linc-birc6 (Megamind)	TALEN-3-5p	T C A T G A A G C T C C C A T C A G
		HD NI NG NN NI NI NN HD NG HD HD HD NI NG HD NI NN
	TALEN-3-3p	T C C T G T C A C A T G T G A T A G
		HD HD NG NN NG HD NI HD NI NG NN NG NN NI NG NI NN
linc-birc6 (Megamind)	TALEN-4-5p	T G C A T T C T C A G A G G G T T
		NN HD NI NG NG HD NG HD NI NN NI NN NN NN NG NG
	TALEN-4-3p	T C T G T C T G T A C C A G T G G T
		HD NG NN NG HD NG NN NG NI HD HD NI NN NG NN NN NG
linc-birc6 (Megamind)	TALEN-5-5p	T G G A C A A C A A G C C T T G T
		NN NN NI HD NI NI HD NI NI NN HD HD NG NG NN NG
	TALEN-5-3p	T A G T G T T C C C T C C A C T G T
		NI NN NG NN NG NG HD HD HD NG HD HD NI HD NG NN NG
linc-birc6 (Megamind)	TALEN-6-5p	T G T A G A G G C A T C C A T T G
		NN NG NI NN NI NN NN HD NI NG HD HD NI NG NG NN
	TALEN-6-3p	T T G A A C A G C C C A G A A T G T
		NG NN NI NI HD NI NN HD HD HD NI NN NI NI NG NN NG

Supplemental Table 4

Primer Name	Sequence
apoea-R3	GTTCTGTTGAGATGTTTCAAAGC
apoea-F4	CATTCTGTGCTTATCTCTCTGAC
apoea-F1	CCAAAGGACTTCCTTACAGAG
apoea-F2	GTGAATATTCCACACACTAAAG
apoea-R1	GGTTATAACAGGTAAAAAAAGCAAAGG
apoea-R2	CTCTAGTGGTCAGTTCAGAAAC
apoea-1kb-F	AAGAAGACTCAAATGGCGTTC
apoea-1kb-R	CTTGAGCTCATCAGAGTTCTG
apoea-5kb-F	GAGAAGACCAAAGAGAATGTG
apoea-5kb-R	AGTGGAAAAAGTCTTGAATGAG
flt4-TAL2-F	TTGAAAGAGTTTCTGTTGTTCC
flt4-TAL2-R	AGCATGAAACTCACTGGTTAC
flt4-TAL1-F	GAAACATCCTCCTGTCCGAGAACAATG
flt4-TAL1-R	CAGTTCCTGTGAGATTCACCCAG
flt4-TAL3-F	ACCCAGAGCATCCATTCATC
flt4-TAL3-R	CGAGAAGAGAGTGACTTTTAGGTCA
flt4-TAL1-R2	TTTAGTCTTCAACTTTTTAGCAC
flt4-TAL1-F2	GAAGATCTGTGACTTTGGTTTAG
flt4-TAL3-R2	CTGTAACAGTCCTGAGTTAAAC
flt4-TAL3-F2	TAGGTTTCAGAACTTATAGGCAG
golden-F2	GCTGATCTGACATGATGTTTATG
lepa-R2	CAGCGGGAATCTCTGGATAA
golden-F	GGTCGTCCGAGCTGCTGGCCGTTG
golden-R	CAGTCATTACTGTGAGATACAAAGTATTC
lepa-TAL-F	CTCACAGGTATTGCTGAACTATTG
lepa-TAL-R	TTCCGGTCATGCTGATGAACG
lcr-TAL3/4-F	ATTTATTTCAACCATATTTCGATGG
lcr-TAL3/4-R	CAGTTGTTGCATTTTTAGGAATG
lcr-TAL1-F2	TGATGTGTGGTGAGCTGCATG
lcr-TAL1-R1	GTTGTAGTTTAAAAGTGATGAGTC
megamind-TAL3/4-F	CTTTAGGGCACGTTCTGGTG
megamind-TAL6-R1	GAATCGGTGAAAAGGGTAGTC
megamind-TAL3/4-R	CAGAGGATGTGGTAGCTGAG
megamind-TAL3-R	AAACCCTCTGAGAATGCAGAG
megamind-TAL1/2-F	GATGATCCCATTGATCTCACAG
megamind-TAL1-R	GGACACCCATGCACATGTACAC
megamind-TAL2-F	GTGTGAAAGGACTAATGTGTACGTGTGGATGG
megamind-TAL1/2-R	GAGTAAAGAGATTAGAAGGTACC
megamind-TAL6-F	CCTTCTAATCTCTTTACTCAATCTC
megamind-TAL6-R1	GAATCGGTGAAAAGGGTAGTC
megamind-TAL5-F1	GAAGTGTCTGCTTCCAGATAC
megamind-TAL5-R1	ACGCCGTAGGGTAAGACTCC

Supplemental Table 5

Application	Primer-A	Primer-B
apoea 477 bp deletion	apoea-R3	apoea-F4
apoea 477 bp inversion 5p junction	apoea-F2	apoea-F1
apoea 477 bp inversion 3p junction	apoea-R1	apoea-R3
apoea 477 bp duplication	apoea-F2	apoea-R1
apoea 753 bp deletion	apoea-1kb-R	apoea-F4
apoea 753 bp inversion 5p junction	apoea-1kb-R	apoea-R1
apoea 753 bp inversion 3p junction	apoea-1kb-F	apoea-F4
apoea 4.2 kb deletion	apoea-5kb-R	apoea-F4
apoea 4.2 kb inversion 5p junction	apoea-5kb-R	apoea-R1
apoea 4.2 kb inversion 3p junction	apoea-5kb-F	apoea-F4
apoea 4.2 kb duplication	apoea-5kb-F	apoea-R1
apoea TAL-1 T7E1	apoea-F1	apoea-R1
apoea TAL-2 T7E1	apoea-F2	apoea-R2
apoea TAL-3 and 3b T7E1	apoea-1kb-F	apoea-1kb-R
apoea TAL-4 T7E1	apoea-5kb-F	apoea-5kb-R
flt4 39 kb deletion	flt4-TAL2-F	flt4-TAL1-R2
flt4 39 kb Inversion 5p junction	flt4-TAL2-F	flt4-TAL1-F2
flt4 39 kb Inversion 3p junction	flt4-TAL2-R	flt4-TAL1-R2
flt4 69 kb deletion	flt4-TAL1-R	flt4-TAL3-F2
flt4 69 kb Inversion 5p junction	flt4-TAL1-R	flt4-TAL3-R2
flt4 69 kb Inversion 3p junction	flt4-TAL3-F2	flt4-TAL1-F2
flt4 69 kb duplication	flt4-TAL1-F	flt4-TAL3-R2
flt4 TAL-1 T7E1	flt4-TAL1-F	flt4-TAL1-R
flt4 TAL-2 T7E1	flt4-TAL2-F	flt4-TAL2-R
flt4 TAL-3 T7E1	flt4-TAL3-F	flt4-TAL3-R
slc24a5 (gol)/lepa deletion	golden-F2	lepa-R2
scl24a5 (gol) T7E1	golden-F	golden-R
lepa T7E1	lepa-TAL-F	lepa-TAL-R
LCR deletion	lcr-TAL3/4-F	lcr-TAL1-R1
LCR TAL-1 T7E1	lcr-TAL1-F2	lcr-TAL1-R1
LCR TAL-2 T7E1	lcr-TAL1-F2	lcr-TAL1-R1
LCR TAL-3 T7E1	lcr-TAL3/4-F	lcr-TAL3/4-R
LCR TAL-4 T7E1	lcr-TAL3/4-F	lcr-TAL3/4-R
linc-birc6 (Megamind) Deletion	megamind-TAL3/4-F	megamind-TAL6-R1
linc-birc6 (Megamind) TAL-1 T7E1	megamind-TAL1/2-F	megamind-TAL1-R
linc-birc6 (Megamind) TAL-2 T7E1	megamind-TAL2-F	megamind-TAL1/2-R
linc-birc6 (Megamind) TAL-3 T7E1	megamind-TAL3/4-F	megamind-TAL3-R
linc-birc6 (Megamind) TAL-4 T7E1	megamind-TAL3/4-F	megamind-TAL3/4-R
linc-birc6 (Megamind) TAL-5 T7E1	megamind-TAL5-F1	megamind-TAL5-R1
linc-birc6 (Megamind) TAL-6 T7E1	megamind-TAL6-F	megamind-TAL6-R1