

**Supplementary Table 1. Primer and crRNA/tracrRNA sequences**

Name of sequence	Forward (5'-3')	Reverse (5'-3')
<b>crRNA/tracrRNA</b>		
crRNA1	CUAUCCAUCAUCAGAUCAUUGUUUUUAGAGCUAUGCUGUUUUUG	
crRNA2	GCUGGCAGGGCAGUAAAAACGUUUUAGAGCUAUGCUGUUUUUG	
crRNA3	ACUACUCAGCAGUAUGUUCUGUUUUUAGAGCUAUGCUGUUUUUG	
crRNA4	GUGGGCAGGAAAGGAAGAGGGUUUUUAGAGCUAUGCUGUUUUUG	
tracrRNA	AAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCU	
<b>Primers for PCR</b>		
T7-Cas9	TAATACGACTCACTATAGGGAGAATGGACAAGAAGTACTCCATTGG	TCACACCTTCCTCTTCTTC
<i>Bcr</i>	CAGGGAGGCAGGAGTGTTTAC	TCAAAAGATTCGGTTTCCTG
<i>Abl1</i>	CAGCCAGCCCTTTACTTTTG	ATGCATACACACAGGCAGGA
crRNA1_OT1	GCACTCAGGTCCCCACTAGA	TGCAACCAGAAGCAAGTTGT
crRNA1_OT2	CCCATTTCTGTTCTCTCCA	CCAGGCTGGATCACATAACA
crRNA1_OT3	TGGCAGGTAATGGCTAGGTC	TCACCAGTTCCAAGGAGAGG
crRNA2_OT1	TCTTCCTTCAGTTCTTAGCTTATTCC	AGAGTTCAAGAACAGCCAAGG
crRNA2_OT2	TGGCAAGGTGAGAGATAATCAG	AGGCTTCGGTAACACAGAGG
crRNA2_OT3	CAGAAACCCAAGGGACCATA	TGTTCCAGCAGAGTCGAATG
crRNA3_OT1	TAAGCCCATCCAGTCCAAGT	GCACCGTCTTGTCTCCTAGC
crRNA3_OT2	CCTCTTCAGCCTCCCTCTCT	CAGGCCACAACACTCTTTT
crRNA4_OT1	CGCCATCCAGTCCTGTAAGT	CGACACCACTTGTCCATCCAC
crRNA4_OT2	ACCCTGACCCATGCTTACTG	GCCACCAGATGGGCTAGATA
crRNA4_OT3	GGCAACACGCTCTCGATT	CATCAACTTGCAGGTCCAGA

**Supplementary Table 2. Germline transmission of mutations at *Ab1* locus**

Mutant line	Genotype of F0	No. of F1 newborn	Genotype of F1	No. of genotype (%)
#5	$\Delta 5/\Delta 8$	9	$\Delta 5/+$	5 (55.6)
			$\Delta 8/+$	4 (44.4)
#8	$\Delta 22/+$	10	$\Delta 26/+$	3 (30.0)
			wild-type	7 (70.0)
#9	$\Delta 17/\Delta 8$	7	$\Delta 17/+$	4 (57.1)
			$\Delta 8/+$	3 (42.9)
#11	$\Delta 31/+$	5	$\Delta 31/+$	3 (60.0)
			wild-type	2 (40.0)

**Supplementary Table 3. Off-target analysis of mutant embryos injected with crRNA/tracrRNA with Cas9**

target site	Sequence	Chr.	Position (mm9)	Strand	crRNA Cas9	mutation rate (%)				
						1 WT	1+2 nCas9	1+2 fCas9	3+4 nCas9	3+4 fCas9
crRNA1	CTATCCATCATCAGATCATTAGG	10	74561466–74561488	-		on-target				
crRNA1_OT1	CAAGCCAACATCAGATCATTCAG	1	84239159–84239181	+		0/38 (0)	0/13 (0)	0/17 (0)		
crRNA1_OT2	CAATACATCGTCAGATCATTCAG	3	112969795–112969817	-		0/38 (0)	0/13 (0)	0/17 (0)		
crRNA1_OT3	CTATCCATCATGAGATCATATGG	4	13191165–13191187	-		0/38 (0)	0/13 (0)	0/17 (0)		
crRNA2	GCTGGCAGGGCAGTAAAAACAGG	10	74561505–74561527	+		on-target				
crRNA2_OT1	GAAGGCAAGGCAGTAAAAACGAG	X	101766855–101766877	-		N/A	0/13 (0)	0/17 (0)		
crRNA2_OT2	ACTGGCAAGGCAGTAAAAAGAGG	X	83169655–83169677	-			0/13 (0)	0/17 (0)		
crRNA2_OT3	GCAGGCATTGCAGTAAAAACAGG	13	98653829–98653851	-			0/13 (0)	0/17 (0)		
crRNA3	ACTACTCAGCAGTATGTTCTAGG	2	31621244–31621266	-		on-target				
crRNA3_OT1	ACTACTGAGCAGCATGTTCTGAG	8	117086071–117086093	-					0/9 (0)	0/10 (0)
crRNA3_OT2	ACTCTCCTCAGTATGTTCTGAG	6	49304224–49304246	+					0/9 (0)	0/10 (0)
crRNA4	GTGGGCAGGAAAGGAGAGGAGG	2	31621283–31621305	+		on-target				
crRNA4_OT1	GTGGGCAGGACAGGAAAGAGGGG	5	33376892–33376914	-					0/9 (0)	0/10 (0)
crRNA4_OT2	GGGGCAGGAAAGGAAACAGGAGG	16	4912768–4912790	-					0/9 (0)	0/10 (0)
crRNA4_OT3	GAGGGCGGAAAGGAAAGAGGAGG	9	108392953–108392975	-					0/9 (0)	0/10 (0)

fCas9 (FokI-dCas9), nCas9 (Cas9 D10A), WT (Cas9 WT).