	Embryos	Blastula after O.N.	Enclosed in capillary	Gastrulating	Late trochophre	Late veliger	Juvenile snail	Survived to adult snail
Exp. 1								
Injection	20	20	8	7	7	7	7	4
Control	7	7	2	1	1	1	1	0
Exp. 2								
Injection	16	16	10	8	7	7	6	4
Control	28	28	3	3	3	3	3	1
Exp. 3								
Injection	18	18	12	7	1	0	—	—
Control	5	5	3	3	1	1	—	—
Exp. 4								
Injection	17	13 Abnormal 3 Broken 1	9	7	4	4	3	2
Control	21	21	2	2	1	1	1	1 12

Fig. S1. Targeting by CRISPR/Cas9 and follow up breeding experiments.
(**A**) PAM and target sequence (20 bp, underlined in red) to discriminate *Lsdia1* from the *Lsdia2* gene. The PCR primers for HMA, D1-MA4 detection Fw and D1-MA4 detection Rv, are boxed. The PCR primer for genotyping, D1-MA4 seq Rv, is also indicated. (**B**) Summary table of CRISPR/Cas9 experiments. Some of the CRISPR/Cas9-injected and control embryos were encapsulated individually in a glass capillary tube at the blastula stage and bred to the juvenile stage, a step required for the normal growth of embryos. Ten injected and two control adult snails were obtained out of four independent CRISPR/Cas9 - breeding experiments, and they were named as snails 1 - 12. (**C**) The rest of the embryos not used for the breeding experiments were used for embryo-direct PCR for HMA on 2% agarose gel (left) and 10% polyacrylamide gel (right) electrophoresis. Lane M is the 1kb DNA Ladder size marker (FastGene).

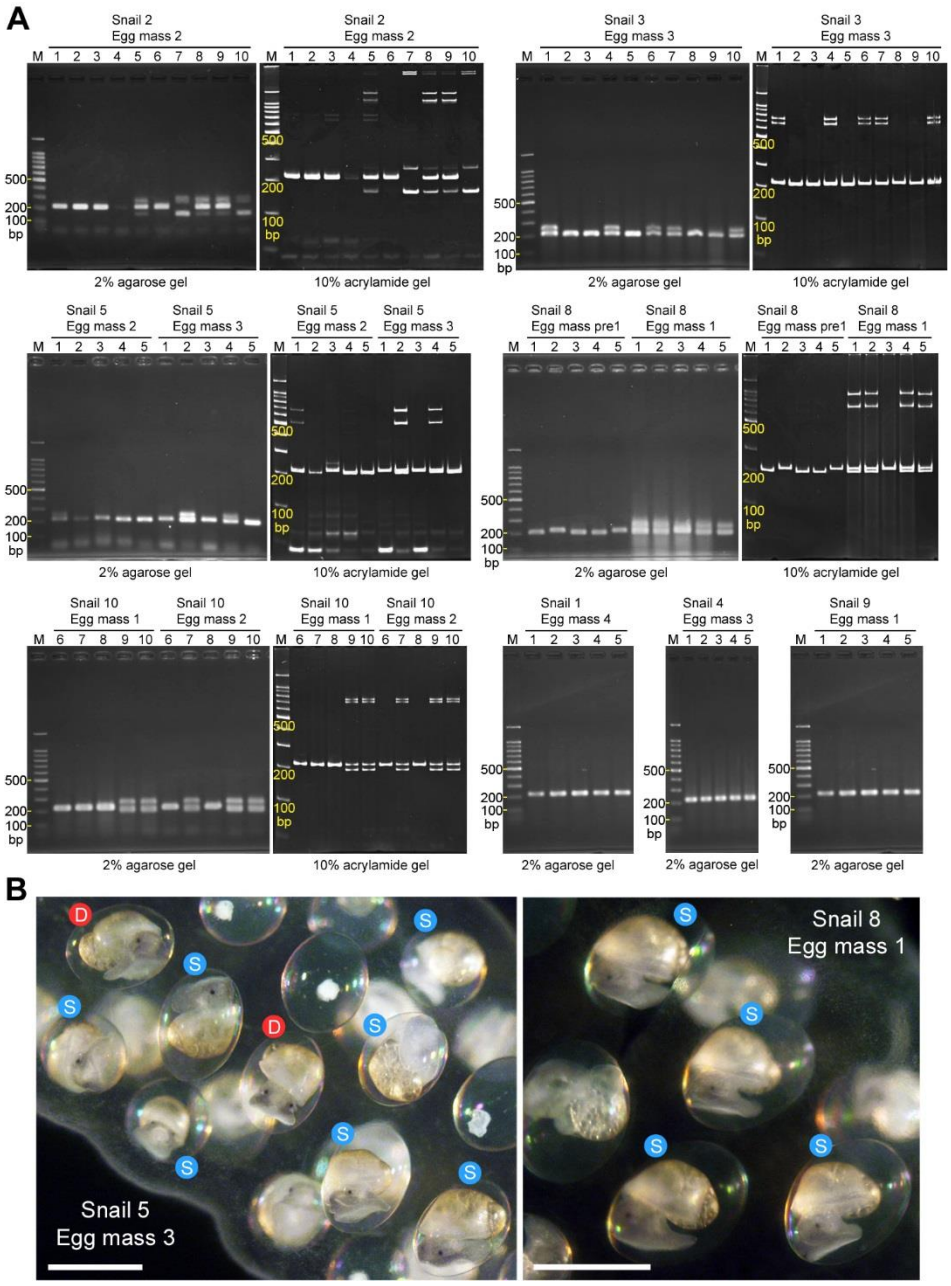


Fig. S2. Efficient germline transmission.

(**A**) Embryos oviposited by F0 were subjected to embryo-directed PCR and HMA by 2% agarose gel (left) and 10% polyacrylamide gel (right) electrophoresis for snails 2, 3, 5, 8 and 10, which showed germline transmission to F1. Germline transmission was not observed in snails 1, 4, 9 as shown by 2% agarose gel electrophoresis (Extended Data Table 1, External Data S1). Lane M is the 100bp DNA Ladder size marker (Promega). (**B**) Photographs of an egg mass containing both dextral and sinistral juveniles in the case of snail 5 (left) and only the sinistral juveniles for snail 8 (right). D in a red circle and S in a blue circle indicate dextral and sinistral snails, respectively. Scale bar: 1 mm.

A

Mother snail (F1)		F2 snails used for phenotype check		Total egg masses laid	No. of offspring for each phenotype				Total No. of embryos checked
					Dextral juvenile	Sinistral juvenile	Abnormal embryo	Arrested before Late veliger	
Snail 2-F1-20	del57/del57	del57/del57	4	12	1028	0	17	83	1128
Snail 3-F1-7	del5/del5	del5/del5	3	10	0	146	70	216	432
Snail 3-F1-17	del7+ins1/del5	del5/del5	2	8	2	257	116	298	673
		del7+ins1/del5	3	10	537	0	1	33	571
		del7+ins1/del7+ins1	1	5	424	0	1	53	478
Snail 3-F1-5	wt/del5	del5/del5	1	5	0	124	69	126	319
		wt/del5	2	6	582	0	2	30	614
Snail 5-F1-S68	del4/del4	del4/del4	2	5	0	167	59	110	336
Snail 5-F1-D33	del3/del4	del4/del4	3	10	1	397	141	279	818
		del3/del4	3	9	1039	0	9	58	1106
		del3/del3	1	3	340	0	7	12	359
Snail 8-F1-16	ins1/ins1	ins1/ins1	2	2	0	23	6	15	44
			27	85					6878

B

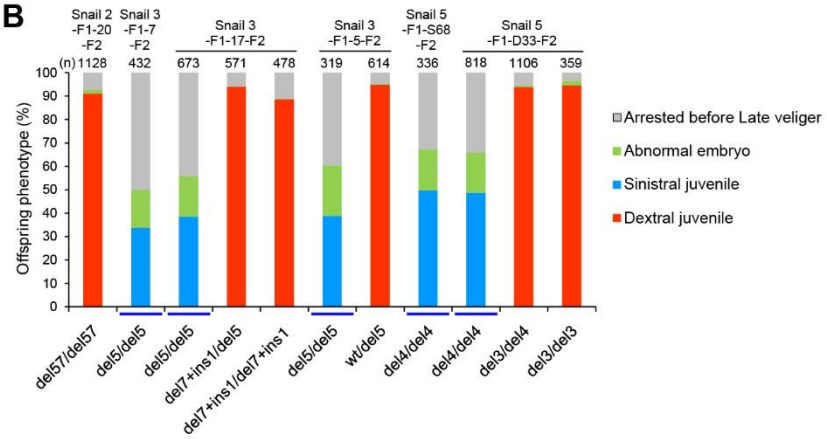


Fig. S3. Gene-editing continues to alter the phenotype at the F3 generation.
(**A,B**) Summary table and bar chart of offspring phenotype for the gene-edited snails (F2). Snail 8-F1-16-F2 was omitted from the chart due to insufficient data size. The offspring (F3) phenotype clearly depends on the F2 genotype. Homozygous knockout mutants (blue line; $p<0.0001$, compared with the wild-type dextral in Fig. 2G).

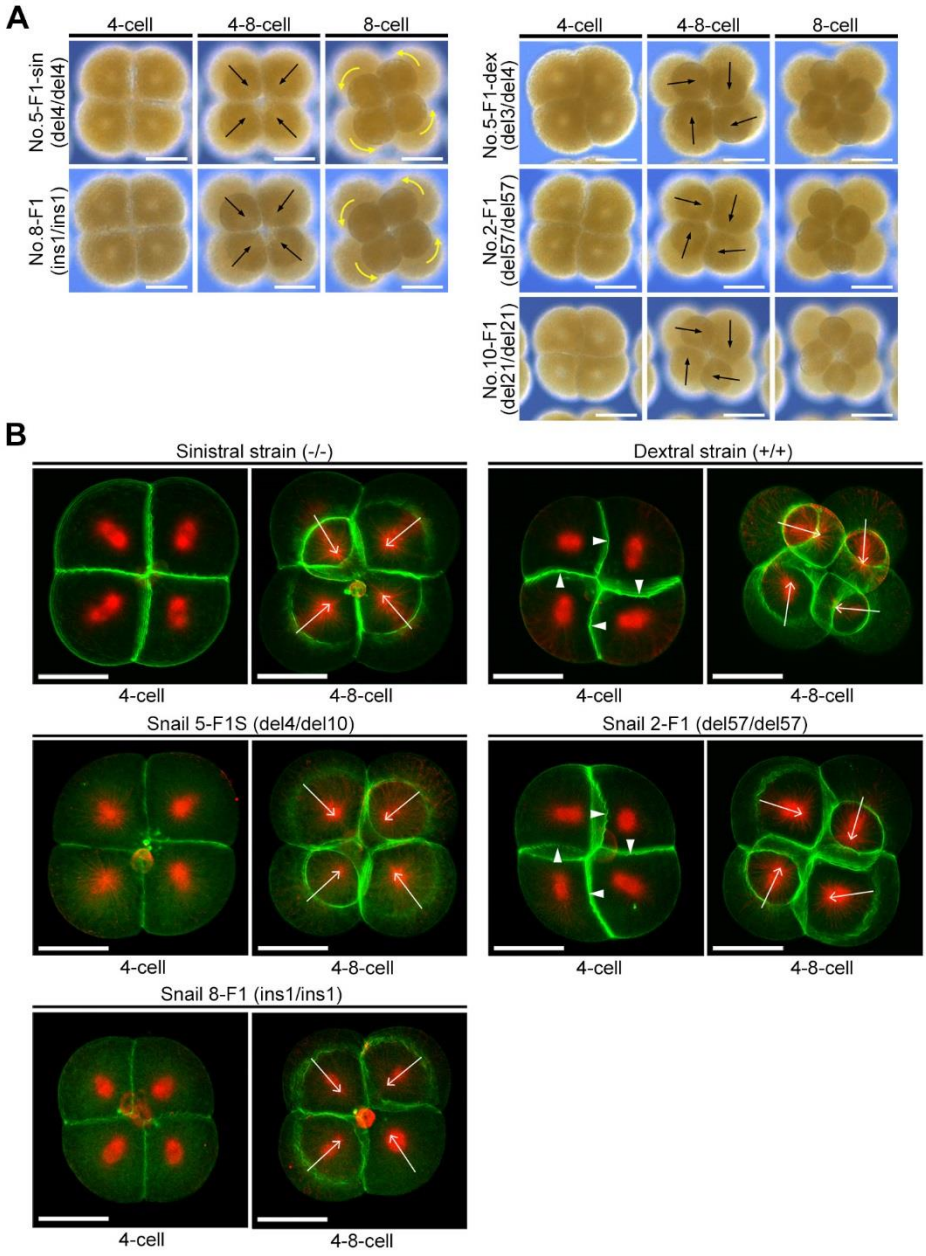
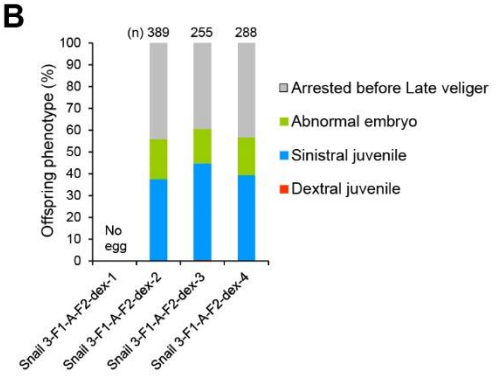


Fig. S4. Cytoskeletal dynamics at the third cleavage are dictated by the gene-edited genotype.
(**A**) The micromeres rotated to the clockwise direction with SD and SI for F2 embryos oviposited by the biallelic non-frameshift mutant, 2-F1 (del57/del57) and 10-F1 (del21/del21) as well as by the heterozygous knockout, 5-F1 (del3/del4), whereas the micromeres rotated to the anti-clockwise direction without SD or SI for the homozygous knockout, 5-F1-sin (del4/del4) and 8-F1 (ins1/ins1). Scale bar: 50 µm. (**B**) Embryos at the 4-cell and 4- to 8- cell stages (metaphase-anaphase of the third cleavage) were double stained for filamentous actin with Alexa Fluor 488-labeled phalloidin (green) and ß-tubulin with Cy3-labeled anti-ß-Tubulin antibody (red). White arrows point the direction of spindles and white arrowheads indicate the direction of SD for the dextral embryos. Scale bar: 50µm.

A

F2 snail ID	Genotype	Total egg masses laiid	No. of offspring for each phenotype				Total No. of embryos checked
			Dextral juvenile	Sinistral juvenile	Abnormal embryo	Arrested before Late veliger	
Snail 3-F1-A-F2-dex-1	del5/del5	0	–	–	–	–	No egg
Snail 3-F1-A-F2-dex-2	del5/del5	4	0	146	71	172	389
Snail 3-F1-A-F2-dex-3	del5/del5	4	1	113	40	101	255
Snail 3-F1-A-F2-dex-4	del5/del5	4	0	113	50	125	288
		12					932

B



C

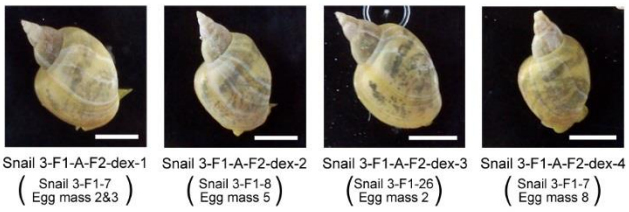


Fig. S5. The very rare dextrally-coiled adults born from the homozygous knockout snails gave birth to sinistral offspring according to its own genotype.

(**A,B**) Summary table and bar chart of offspring phenotype oviposited by the rare dextrally-coiled snails with homozygous knockout genotype ($p<0.0001$, compared with the wild-type dextral in Fig. 2G). (**C**) Photographs of the very rare four dextral snails (F2) born from the homozygous knockout mother (F1). Scale bar: 1 cm.

Table S1. Summary of the CRISPR/Cas9 injection experiments with the number of egg masses laid by F0 and the number of the respective phenotype of their offspring (F1).

F0 snail	Egg masses laid by F0	Germline transmission[a]	No. of offspring for each phenotype				Total No. of embryos checked
			Dextral juvenile	Sinistral juvenile	Abnormal embryo	Arrested before Late veliger	
CRISPR/Cas9-injected							
Snail 1	8	N.D.	759	0	21	68	848
Snail 2	8	D	446	0	2	14	462
Snail 3	7	D	413	0	1	17	431
Snail 4	5	N.D.	244	0	0	22	266
Snail 5	20	D	107	640	179	527	1453
Snail 6	0	No egg	—	—	—	—	—
Snail 7	0	No egg	—	—	—	—	—
Snail 8	5	D	0	117	21	53	191
Snail 9	3	N.D.	262	0	2	7	271
Snail 10	20	D	2135	0	11	142	2288
Control							
Snail 11	3	N.D.	187	0	0	6	193
Snail 12	0	No egg	—	—	—	—	—
	79						6403

a: Germline transmission was studied by HMA and genotyping of F1. D: detected; N.D.: not detected.

Table S2. Summary of the CRISPR/Cas9 experiments with F1 genotype, number of F1 snails studied and egg masses laid by the F1 snails, as well as the number of the respective phenotype of their offspring (F2).

Line	Snails studied for genotyping[a]	F1 genotype and the no. of snails		F1 snails used for phenotype check[b]	Total egg masses laid	No. of offspring for each phenotype				Total No. of embryos checked
						Dextral juvenile	Sinistral juvenile	Abnormal embryo	Arrested before Late veliger	
Dex	—	(+/+)		arbitrarily	26	2346	0	5	38	2389
Sin	—	(-/-)		arbitrarily	32	2	1873	139	385	2399
Snail 11-F1	8	wt/wt	8	5	31	2287	0	6	109	2402
Snail 1-F1	20	wt/wt	20	5	19	1750	0	2	85	1837
Snail 4-F1	14	wt/wt	14	4	17	1619	0	4	56	1679
Snail 9-F1	14	wt/wt	14	5	19	1884	0	7	68	1959
Snail 2-F1	81	del57/del57	20	6	30	2256	0	10	139	2405
		wt/del57	32	5	19	1479	0	2	27	1508
		wt/wt	29	5	22	1625	0	9	69	1703
Snail 3-F1	54	del5/del5	15	6	33	10	1308	548	1334	3200
		wt/del7+ins1	4	4	14	1140	0	18	75	1233
		wt/del5	23	5	25	1787	0	18	128	1933
		del7+ins1/del5	4	4	15	1294	0	8	51	1353
		wt/wt	8	5	24	1872	0	11	104	1987
Snail 5-F1-sin	102	del4/del4	43	6	25	0	950	300	777	2027
		del4/del10	57	6	24	1	1020	275	601	1897
		del3/del10	2	2	7	160	0	3	7	170
Snail 5-F1-dex	35	del4/del10	14	5	18	0	737	218	651	1606
		del3/del10	8	4	17	1407	0	12	87	1506
		del3/del4	13	7	27	2354	0	15	146	2515
Snail 8-F1	67	ins1/ins1	18	6	19	0	727	169	333	1229
		del13/del13	13	7	26	0	810	213	388	1411
		ins1/del17	2	2	11	0	430	103	208	741
		ins1/del4	2	1	7	0	310	34	93	437
		ins1/del13	27	4	16	0	661	122	260	1043
		del13/del17	2	1	6	0	124	54	83	261
		del4/del13	3	2	13	1	435	89	239	764
Snail 10-F1	70	del21/del21	10	5	17	1176	0	7	49	1232
		wt/del21	33	5	17	1297	0	6	54	1357
		wt/wt	27	5	19	1259	0	8	39	1306
	465			>127	595					47489

a: Number of individual snails for each line, whose DNA was extracted from foot clips for genotyping.
b: Number of individual snails used for the offspring phenotype analysis. Total number of egg masses laid is given in the next right column. In the cases of wild-type snails, fresh egg masses were collected arbitrarily from the mass-breeding aquarium.

Movies

Movie 1. The third cleavage pattern of the wild-type dextral and wild-type sinistral embryos.
Time-lapse images in this movie were obtained every 30 s.

Movie 2. The third cleavage pattern of the homozygous knockout snail embryos.
Time-lapse images in this movie were obtained every 30 s.

Movie 3. The third cleavage pattern of the heterozygous knockout snail embryos.
Time-lapse images in this movie were obtained every 30 s.

Movie 4. The first cleavage pattern of the trypsin treated 1-cell stage embryos.
Time-lapse images in this movie were obtained every 20 s.