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Supplementary Information for

Direct delivery of adenoviral CRISPR/Cas9 vector into the blastoderm for generation of targeted gene knockout in quail

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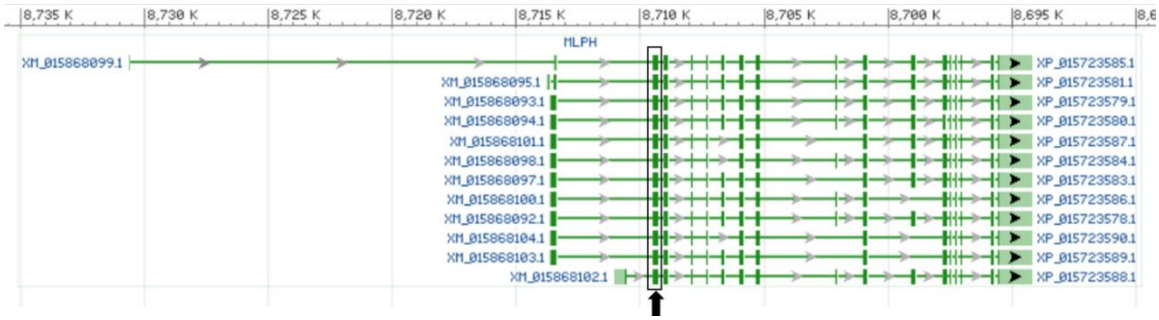

Fig. S1. Diagrammatic representation of multiple transcripts of the Japanese quail *MLPH* gene and *MLPH* gRNA region. A part of the 'Genomic regions, transcripts, and products' section was acquired from the NCBI Gene record for Japanese quail *MLPH* (Gene ID 107316507). The exons containing gRNA target sequences are boxed and directed by an arrow. Dark and light green boxes indicate translated and untranslated regions, respectively.

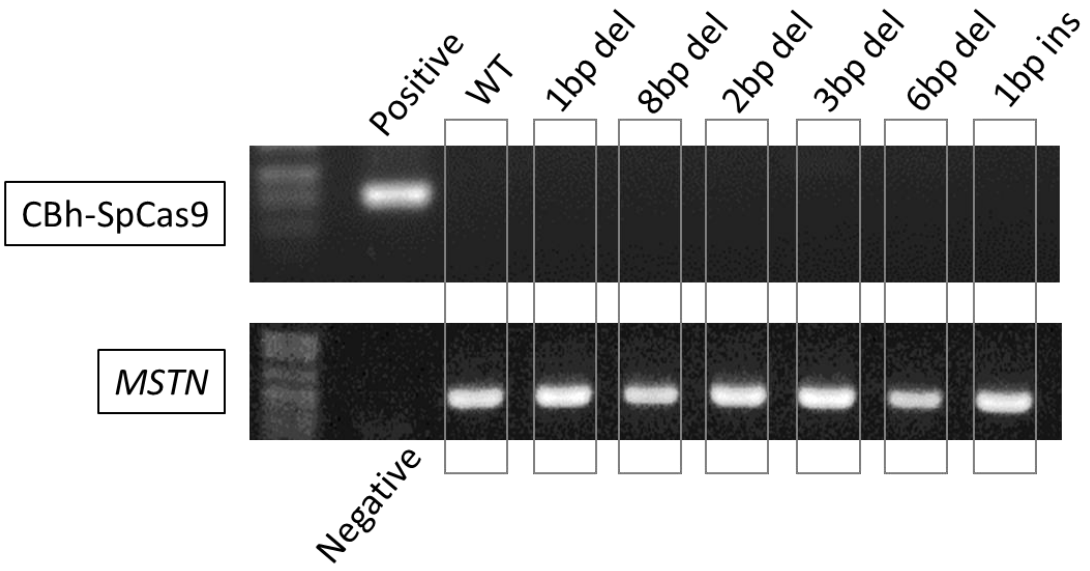

Fig. S2. Detection of chromosomal integration of the viral vector into the host genome. PCR amplification of a portion of the viral sequences, CBh promoter-Cas9, and the myostatin (*MSTN*) gene in all mutant quail genomic DNAs are shown. The adenoviral shuttle vector was used as a positive control and PCR master mix without genomic DNA was used as a negative control. Genomic DNA from wild-type quail (WT) was included to so that there was a negative control in viral vector detection and a positive control in *MSTN* gene amplification.

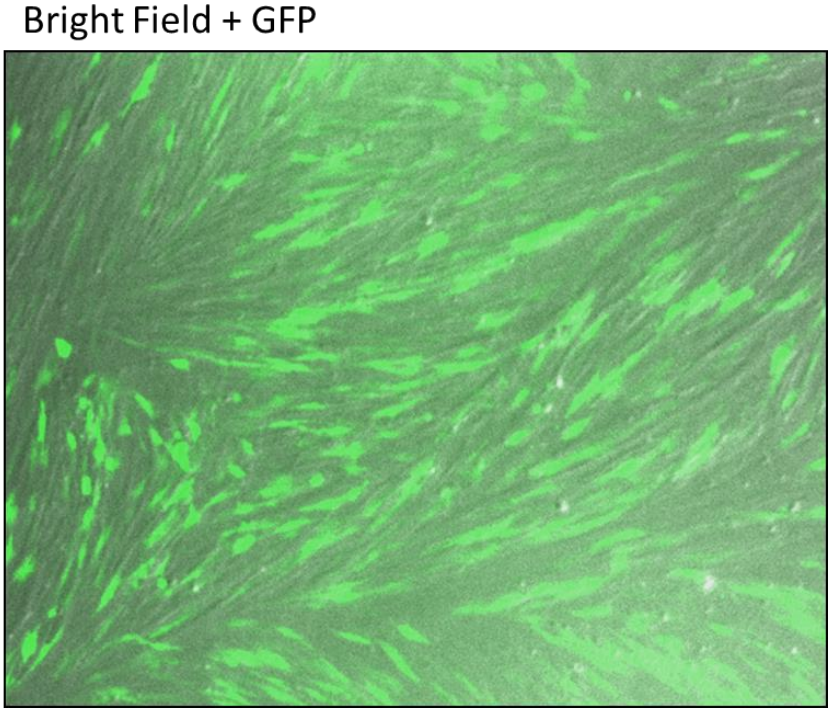

Fig. S3. Adenovirus-mediated green fluorescent protein (GFP) gene expression in the primary turkey muscle cells. Both GFP negative and positive cells are shown using a bright field with a fluorescent light microscopy.

Table S1. Potential off-target sites of *MLPH* in Japanese quail genome.

	Chromosome	Locus	Score	Sequence*	PAM	Direction
MLPH	7	8,709,348	46.1	AGGTGTAGAAGCGGCAATCC	AGG	−
off-target 1	3	51,763,707	30.2	CCTGAACTAAGCGGCAATCC	AGG	+
off-target 2	18	17,397	30.2	TGATTGCTAAGCGGCAATCC	TGG	+
off-target 3	12	17,221,856	30.2	TGATTGCTAAGCGGCAATCC	TGG	+
off-target 4	unplaced genomic scaffold	7,095	30.2	TGATTGCTAAGCGGCAATCC	TGG	+
off-target 5	18	1,841,485	28.2	CTCTTTAGAAGCGGCAATAC	AGG	−
off-target 6	Z	402,850	28.2	CTAAATCACAGCGGCAATCC	AGG	−

*Identical nucleotides in potential off-target sites are underlined.

Table S2. List of primers used in the present study.

Purpose	Forward (5'-3')	Reverse (5'-3')
MLPH	GACCTGAAGTGCAAGATAGACCA	CTAGAAGAGCTGAATTCCCCTTC
	CACCAGTCCCACCTGAATGAA	AGCTTGCAGGACAGCAGAAA
Off-target 1	CTCCCTCATTTTCTTCTGCTGTCTT	TCACTTGTAGGAAAGGCATTCTAGGA
Off-target 2	GAGGACTTCACCTATTTGGGACTTGATATAT	ACGTGAAGCCAACCACTTCA
Off-target 3	GACGTAGCCCCGGGGTTT	CCGCCCCACCCCAAAAT
Off-target 4	GGTGACGTAGCCCCATGGTCTT	GCTTCTATTTATTGCCCCGCCCACA
Off-target 5	CACATCCCTCACAACTCCCTCTT	GAACCTCCAGGCCTCTGCTATTT
Off-target 6	GGGAAGACAGCGCACTTATATTTATCAA	GAGCACTCAGAGGTTGGTGAAA
MSTN	TGTGATCGACAGGGCTTTAAC	CGCAGTTTGCTGAGGATTTGAA
CBh promoter-Cas9	GCTCTGACTGACCGCGTTACT	GTTGCCCAGCACCTTGAATT