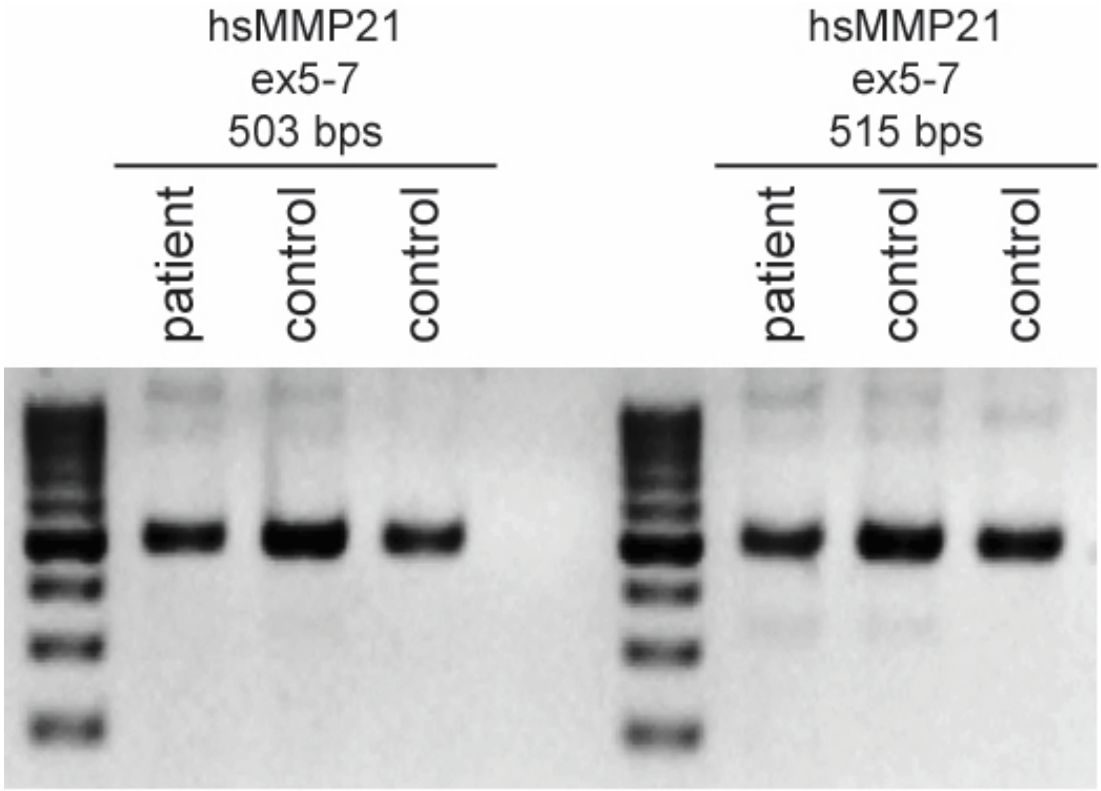
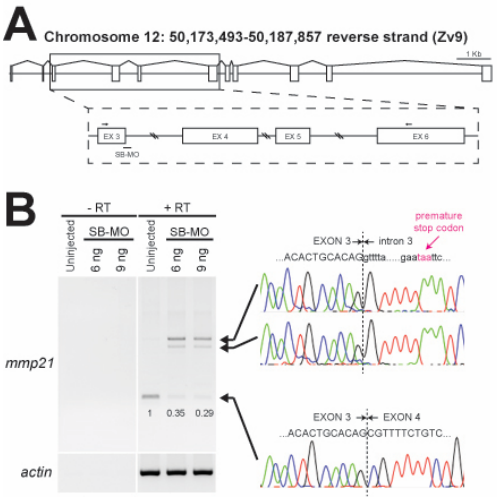
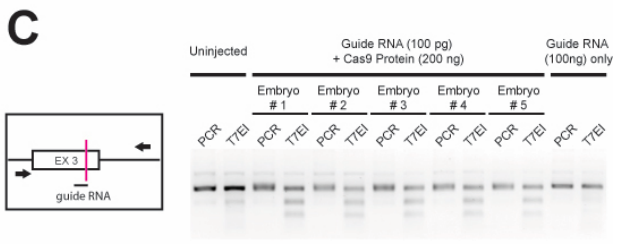
Supplementary Fig 1. RT-PCR analysis using RNA extracted from patient lymphocytes. RT-PCR data suggested that mRNA harboring MMP21 c.1024_1025delAA was detectable, as shown by two primer sets in Exon 5 and Exon 7.



Supplementary Fig 2. (A) Schematic of genomic region on zebrafish *mmp21* gene according to zebrafish genome database version 9 (Zv9). Dashed box showed the regions for SB-MO as well as primer sets used for RT-PCR. (B) RT-PCR experiment for testing *mmp21* SB-MO efficiency. Only morphants showed larger sized PCR amplicons, compared to control embryos. The intensity of the PCR product from WT transcript was decreased in dosedependent manner. Sanger sequencing confirmed that the high molecular weight PCR products corresponded to the inclusion of intronic region, leading to a premature stop codon. (C) T7 endonuclease I (T7EI) assay revealing heteroduplex formation of PCR amplicon. Embryos injected with guide RNA and Cas9 protein showed the presence of extra size of bands by T7 endonuclease I, indicating putative genomic edition sites. Sanger sequencing from TA cloning on the PCR amplicon (embryo #1) showed a variety of insertions or deletions and both, suggesting the genome editing on exon 3. Box in (C) shows a targeting region of guide RNA as well as primer sets used for RT-PCR (line in magenta indicates potential genomic edition site)

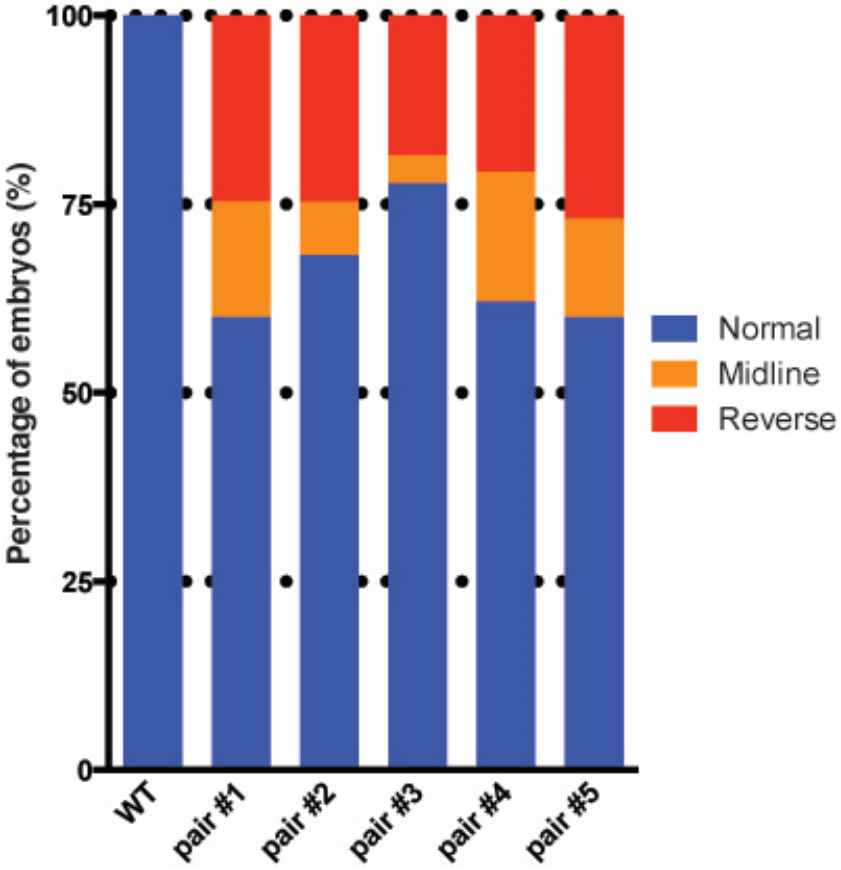




Mutations found in Embryo #1 by TA cloning

---AGGCGGAGCTGATCACTGACACGGACACTGCACAG---	Reference
---AGGCGGAGCTGATCACTGTGTTTTTATCATCAGTCAGTACTGCACAG---	Ins (3/11)
---AGGCGGAGCTGATCACTGATCATCAGTGGTTCTGATCACACGGGACACTGCACAG---	Ins (1/11)
---AGGCGGAGCTGATCACTGATCATCAGTGGTTCTGATCACACGGACACTGCACAG---	Ins (1/11)
---AGGCGGAGCTGATCT - - - ACACGGACACTGCACAG---	Ins/Del (1/11)
---AGGA- - - - - - - - - - - - - - - ACACGGACACTGCACAG---	Ins/Del (1/11)
---AGGCGGAGCTGATCAC - - - - - - - - - - - - - - - CACAG---	Del (1/11)
---AGGCGGAGCTGATC - - - - ACACGGACACTGCACAG---	Del (2/11)
---AGGCGGAGCTGATCAC - - - - ACGGACACTGCACAG---	Del (1/11)

Supplementary Fig 3 Intercross between F0 founders. Progenies from five pairs of F0 founders generated by CRIPR/Cas9 method displayed heart looping defect whereas progenies from WT mating pair did not show heart looping defect.



Supplementary Fig 4 (A) Testing knockdown efficiency by co-transfecting shRNA and GFP-tagged human MMP21. Compared to control shRNA, GFP signal was dramatically reduced in HEK293 cells transfected with *MMP21* shRNA, suggesting robust knockdown of *MMP21*. *Scale bar*, 50 ⌈m. (B) Quantification of GFP-positive cells to show knockdown efficiency. Percentage of cells expressing GFP on the 5 images from single experiment indicated overall knockdown efficiency in *MMP21* shRNA (6%, 73/1225), compared to control shRNA (29%, 354/1217). (C) Immunoblotting against GFP and Actin using protein extract from HEK293 cell transiently transfected with GFP-MMP21 and control shRNA or with GFP-MMP21 and MMP21 shRNA. The level of GFP was greatly reduced, indicating efficient knockdown of *MMP21*. Statistical significance is depicted as ***, $p < 0.001$ by student's t-test.