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Supplementary Information for

The ribonuclease activity of MARF1 controls oocyte RNA homeostasis and genome integrity

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Supplementary Information Text

Materials and Methods

Generation of knock in mice expressing D272A-MARF1 mutant protein

Cas9 mRNA and sgRNA were produced as described previously(1). Briefly, T7 promoter was inserted into the PX330 vector, and the fragment harboring T7-Cas9 sequences was then amplified by PCR using the primers shown in Supplementary Table 2 to serve as the template for in vitro transcription (IVT). Specific sgRNAs targeting the codon D272 of *Marf1* gene were designed using the online tool for design of sgRNAs (http://crispr.mit.edu/), and cloned into the PX330 vector to serve as the template for IVT . IVT of Cas9 mRNA and sgRNA was carried out using the mMESSAGE mMACHINE T7 ultra transcription kit (Ambion, USA) and MEGA short script T7 kit (Ambion, USA), respectively. The resultant RNA was then purified by phenol:chloroform extraction and ethanol precipitation, and dissolved in DNase/RNase-free water (Life Technologies, USA) for microinjection. The specificity of the sgRNA was first verified in vitro by microinjection of the sgRNA and Cas9 mRNA into zygotes and test the incision activity in the blastocysts derived from them. The sgRNA with the sequence of AGTGATCTTAGGCACCGGCA was proved to have the optimal specificity. Donor DNA for HDR (homology directed repair) was synthesized by Sangon Biotech (Shanghai, China), in which the codon D272 of *Marf1* was mutated into A272.

Its sequence reads: 5'-ATGCTTGTATTTTTCCTTTCAGCTGCTGTCAATTTTGCCTTGGAACT

CAGTGATCTTAGGCATCGGCACGGTTTCCACATCATTTTGGTACATAAAAACCAGGCCT CTGAAGCCTTGTTACATCATGCTA-3'. The mixture of Cas9 mRNA (100 ng/μl), sgRNA (50 ng/μl) and donor DNA (10–20 ng/μL) were then microinjected into the cytoplasm of the zygotes that are on a B6DBA2 genetic background in CZB (Chatot–Ziomek–Bavister) medium. After the injected zygotes reached the 2-cell stage in culture, they were transferred into the oviduct of the surrogate pseudopregnant ICR female mice (18-20 embryos/per recipient) that were prepared by mating with vasectomized males. Mice carrying this knock in allele (hereafter referred to as $Marf1^{D272A}$) were identified by PCR followed by sequencing using primers shown in Supplementary Table 2.

Fertility and allelic complementation test

Fertility test was carried out by mating the 6-week old female mice (three of each genotype) with normal adult C57BL/6J X DBA2 (B6D2) F1 males with proven fertility for 8-10 months. The number of pups for each litter was recorded at birth, and the average number of pups per litter was calculated at the end of the testing period.

For allelic complementation test, mice carrying both the gene trap allele and D272A mutant allele (hereafter referred to as $Marf1^{D272A/GT}$) were obtained by breeding the $Marf1^{D272A/+}$ female

mice with *Marf1*$^{GT/GT}$ males. The resultant *Marf1*$^{D272A/GT}$ females were then subjected to either fertility test or superovulation and histology analyses.

Histology

These were carried out exactly as described previously (2). Briefly, the ovaries and the oviductal ampulla containing the ovulated cumulus oocyte-complexes (COCs) inside were first fixed for 2-3 h at room temperature in Bouin's fixative, and then dehydrated in ethanol and xylene, and embedded in paraffin. The blocks were serially sectioned at 5-μm thickness, and stained with periodic acid-Schiff reagent and Lillie-Mayer hematoxylin.

Cloning, expression and purification of recombinant MARF1

The gene encoding MmMARF1(residues 158–1381, referred as MARF1) was cloned into pfastBacHTB vector (Thermo Fisher Scientific Inc., Waltham, MA, USA). Bacmids and baculoviruses were generated following the instructions for the Bac-to-Bac baculovirus expression system (Thermo Fisher Scientific Inc.). Sf9 insect cells were cultured in SIM SF medium (Sino Biological Inc.) at 27°C for 48 h after infection with 1% P2 baculoviruses. The insect cells were harvested, and then resuspended in buffer 1(20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 10 mM imidazole, 5% glycerol and 1mM PMSF), lysed by sonication, and centrifuged. The supernatant was picked out and incubated with 2 ml Ni-NTA Beads (Qiagen, Germantown, MD, USA) for 1 hours at 4°C. After wash by 10 ml Buffer 1, proteins were eluted by the buffer 2 (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 500 mM imidazole, 5% glycerol). The protein was further purified by Hiload Superdex 200 16/600 (GE Healthcare) and concentrated to about 10 mg/ml in a storage buffer of 20 mM Tris, pH 7.5, 300 mM NaCl and 2 mM DTT.

The coding sequence of MmMARF1 (residues 158-320, residues 158-407 and residues 158-690) were inserted into a modified pET28a vector (Merck, Darmstadt, Germany) with N-terminal His_6-SUMO tag and the MmMARF1 (residues 687-1381) was cloned into a pET28a vector (Merck, Darmstadt, Germany) with C-terminal His_6 tag. These plasmids were transformed into the *Escherichia coli* strain BL21 (DE3) (Agilent Technologies, Santa Clara, CA, USA). The cells were grown at 37 °C until OD600 reached 0.6-0.8, and then cultured overnight at 16°C after adding 0.2 mM Isopropyl β-D-1-thiogalactopyranoside (J&K) to induce protein expression. Cells were harvested and then lysed by high pressure cell cracker. These His_6-SUMO-tagged proteins were purified using HisTrap FF columns (GE Healthcare, Beijing, China). And then his_6-SUMO tags were cleaved by Ulp1 protease during dialysis against buffer S (20 mM Tris-HCl, pH 8.0, 500 mM NaCl) and further removed by a second step HisTrap FF column (GE Healthcare) purification. The His_6-SUMO tag proteins were purified by size-exclusive columns Hiload Superdex 75 16/60 or Hiload Superdex 200 16/600 (GE Healthcare) in buffer GF (10 mM Tris-HCl, pH 8.0, 100 mM NaCl). The MmMARF1 (residues 687-1381) was purified by HisTrap FF column, HiTrap Q FF

column and Hiload Superdex 200 16/600 column (GE Healthcare), and stored in the buffer of 20 mM Tris, pH 7.5, 500 mM NaCl and 2 mM DTT.

Amino acid substitution

L253M, L303M, D178A, D215A, D246A and D272) were introduced into the plasmids by site-directed mutagenesis using the QuikChange II XL kit (Catalog # 200517, Agilent Technologies, Beijing, China) according to the manufacturer's instructions. Mutant proteins were expressed and purified in the same manner as the wild-type protein.

Crystallization

Crystals were grown by hanging drop vapour diffusion at 18°C. SeMet-LOTUS1 proteins were concentrated to 20 mg/ml. Crystals of SeMet-LOTUS1 were obtained by mixing 1 μl protein and 1 μl reservoir solution (1.5 M $(NH_4)_2SO_4$, 0.1 M Citrate pH5.5). SeMet-NYN L253M/L303M proteins were concentrated to 16 mg/ml. Crystals of SeMet-NYN were obtained by mixing 1 μl protien and 1 μl reservoir solution (22% PEG-3350, 100 mMTris-HCl pH 8.5, and 200 mM Li_2SO4). The crystals were soaked into the reservoir solution supplemented with 25% glycerol before flash-cooling into liquid nitrogen for diffraction data collection.

Data collection, structure determination and refinement

SeMet-LOTUS1 crystal diffraction data was collected at BL-19U1 (SSRF, China) and data sets were processed and scaled using the HKL3000 package (3). SeMet-NYN crystal diffraction data was collected at BL-17U1 (SSRF, China) and data sets were processed and scaled using the iMOSFLM package (4). The SeMet-LOTUS1and SeMet-NYN structure were solved using SAD method with the Autosol program embedded in the Phenix suit (5-7).The initial models were adjusted with the program COOT (8). All refinements were carried out using the REFMAC program (9). The crystallographic data and refinement statistics for the structure were shown in Supplementary Table 1. Figures were prepared using PyMol (Pymol Molecular Graphics System, LLC). Atomic coordinates and structure factors for the reported crystal structures have been deposited at the Protein Data Bank under accession codes 5YAA (NYN domain) and 5YAD (LOTUS1 domain).

Nucleic acid Preparation

The ssRNA H49 and H49AS were obtained by in vitro transcription. The templates of H49 and H49AS were obtained by PCR using primers shown in Supplementary Table 2, and were transcribed with T7 RNA polymerase. The

RNA transcripts were urea gel purified. The 5' terminus of unlabelled H49 was dephosphorylated by CIP (New England Biolabs, Ipswich, MA, USA) for 1 h at 37°C, and then purified by using standard procedures. Forty-nine nt ssRNA treated with CIP were 5′-radiolabeled with ^{32}P-γ-ATP (Perkin Elmer Health Sciences, Shanghai, China) and T4 polynucleotide kinase (New England

Biolabs). Free ATP was removed and the buffer was exchanged using MicroSpin G-25 Columns (Roche, Shanghai, China). To prepare the dsRNA substrates, the radiolabeled strands were mixed with 2-fold excess of the non-labeled, complementary strands in buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl), heated at 95°C for 5 min, and then gradually cooled to 25°C. To prepare the circular ssRNA substrate, the 5' terminal radiolabeled H49 was incubated at 37°C for 15 min with ATP and T4 RNA ligase 1 (New England Biolabs) and then purified by 20% denaturing PAGE.

Nuclease activity assay

Recombinant proteins and 5' ^{32}P-labelled substrates were incubated at 37 °C for 5 min in the reaction buffer containing 20 mM Tris-HCl, pH 7.5,150 mM NaCl, 1 mM DTT and 1 mM $MgCl_2$. The concentration of the substrates was 20 nM, the final concentration of the recombinant MARF1 proteins were indicated in the figures of the corresponding experiments. The reactions were terminated by addition of 2×loading buffer (90% formamide, 20 mM EDTA, 0.1% SDS and 0.02% bromophenol blue). Samples were heated at 95 °C for 5 minutes, centrifuged and loaded onto 16% urea PAGE followed by electrophoresis. Products were detected by autoradiography using Typhoon FLA-9000 imaging system (GE Healthcare).

Electrophoretic mobility shift assay

Recombinant proteins and 5'^{32}P-labelled substrates were incubated at 25 °C for 30 min in the reaction buffer containing 20 mM Tris-HCl, pH 7.5 and 150 mM NaCl. The final concentration of the recombinant MARF1 proteins were indicated in the figures of the corresponding experiments. After adding 6×loading buffer (12% ficoll 400, 0.012% bromophenol blue and 0.012% xylene cyanol), samples were loaded onto 8% native PAGE, then separated by electrophoresis. Products were detected by autoradiography using Typhoon FLA-9000 imaging system (GE Healthcare).

Oocyte isolation, in vitro fertilization and embryo culture

Fully-grown oocytes (FGOs) were isolated from the large antral follicles of equine chorionic gonadotropin (eCG, Ningbo A Second Hormone Factory, Cixi, China) primed (46 h) 22-d old mice as described previously (10). Ovulated COCs were obtained from the ampulla of oviducts following a super-ovulation regimen for mice (2) . For IVF, the ovulated oocytes with visible first polar bodies were inseminated with normal sperm isolated from B6D2F1 adult males. 2-cell stage embryos were collected 24 h after IVF. Media used for oocyte collection and culture were bicarbonate-buffered MEM-alpha (Thermo Fisher Scientific Inc.) with Earles' salts, supplemented with 75 μg/ml penicillin G, 50 μg/ml streptomycin sulfate, 0.23 mM pyruvate, and 3 mg/ml bovine serum albumin. Oocytes and embryos cultures were carried out at 37 ºC in an E*ppendorf New Brunswick* Galaxy170R incubator (Hamburg, Germany) infused with 5% O_2, 5% CO_2 and 90% N_2.

Cloning and site-directed mutagenesis of *Marf1*

The *Marf1* coding sequence was cloned into the pCMV6-AC-3DDK plasmid (Catalog# PS1000057, OriGene Technologies, Rockville, MD, USA) using the In-Fusion HD Cloning Kit (Catalog# 121416, Takara Biomedical Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions and primers shown in Supplementary Table 2. D272A-mutation of *Marf1* was then achieved through site-directed mutagenesis using QuikChange XL Site-Directed Mutagenesis Kit (Catalog # 200517, Agilent Technologies) according to the manufacturer's instructions and primers shown in Supplementary Table 2. These plasmid DNAs were then linearized and transcribed *in vitro* using the Ambion's mMESSAGE mMACHINE kit (Applied Biosystems/Ambion, Austin, TX), and the resultant mRNA were then polyadenylated *in vitro* using the Poly (A) Tailing Kit (Applied Biosystems/Ambion) and purified using the RNeasy micro kit (Qiagen, Valencia, CA) as described previously (2).

Microinjection of oocytes with mRNA

The purified mRNAs of WT- and D272A-mutant *Marf1* were adjusted into a final concentration of ~500 ng/μl, and were then microinjected into the cytoplasm of *Marf1*$^{GT/GT}$ GV-stage FGOs in M2 medium containing 10% fetal bovine serum. Approximately 10 pl of the mRNA was injected into one oocyte. After injection, the oocytes were cultured for 40 h in 5 μM milrinone-containing medium to maintain GV-arrest, and then collected for RNA-Seq or real-time RT-PCR analyses.

RNA-Seq analysis

Four sets of samples with each containing 80 WT-, *Marf1*$^{GT/GT}$ and *Marf1*$^{D272A/D272A}$ GV-stage FGOs, or 80 *Marf1*$^{GT/GT}$ GV-stage FGOs after receiving the microinjection with WT- and D272A-mutant *Marf1* mRNAs were collected for RNA-Seq analysis. Total RNA was extracted using the RNeasy Micro Kit (Qiagen, Germantown, MD, USA) according to manufacturer's instructions, and then subjected to mRNA isolation using NEB Next Poly (A) mRNA Magnetic Isolation Module (NEB). Construction of the mRNA library, which includes sequential RNA fragmentation, reverse transcription using random primers, second strand cDNA synthesis, end repair, dA-tailing, adapter ligation, U excision, and PCR enrichment, was carried out using NEB Next Ultra^{TM} RNA Library Prep Kit for Illumina (NEB, Ipswich, MA) according to the instruction manual. The libraries were sequenced on an Illumina HiSeq X Ten platform with 150bp pair-end reads. All reads passed filter were trimmed to remove adaptor sequences and low-quality bases, and the FPKMs were calculated and normalized using cufflinks (v2.2.1). Reads were aligned to the mm10 genome reference using tophat2 (v2.0.13), and the differentially expressed genes were calculated using default parameter of cuffdiff (v2.2.1). RNA-Seq data are deposited in the Gene Expression Omnibus (datasets GSE109213).

Gene enrichment analysis of differentially expressed transcripts was carried out using Metascape (http://metascape.org), a gene annotation and analysis resource, as described previously (11).

qRT-PCR analysis

Three sets of samples with each sample containing 100 WT-, *Marf1*$^{GT/GT}$ and *Marf1*$^{D272A/D272A}$ GV-stage FGOs, or 100 *Marf1*$^{GT/GT}$ GV-stage FGOs after receiving the microinjection with WT- and D272A-mutant *Marf1* mRNAs were collected for this analysis. Total RNA isolation and reverse transcription, as well as real-time PCR quantification of the steady-state levels of mRNA in oocytes, were carried out as described previously (12) . Primer pairs used for this analysis were shown in Table S2.

Western blot analysis

Eighty WT- and mutant *Marf1* GV-stage FGOs were collected and subjected to Western blot analysis for MARF1 and ACTB using the same antibodies as described previously (12) (2, 13) .

Detection and confocal microscopic imaging of DNA double-stranded breaks in oocytes

These were carried out as described previously using the anti-γH2AX primary antibody (catalog no. BS4760; 1:100 dilution; Bioworld technology, St. Louis Park, MN) and the Alexa fluor 594-conjugated secondary antibodies (Thermo Fisher Scientific) (2). The DNA was counterstained with DAPI. The staining was examined and imaged under a LSM 710 confocal laser scanning microscope (Zeiss). Images for WT and *Marf1*$^{D272A/D272A}$ oocytes were taken under exactly the same sets of parameters, and number of positively stained foci were counted and calculated.

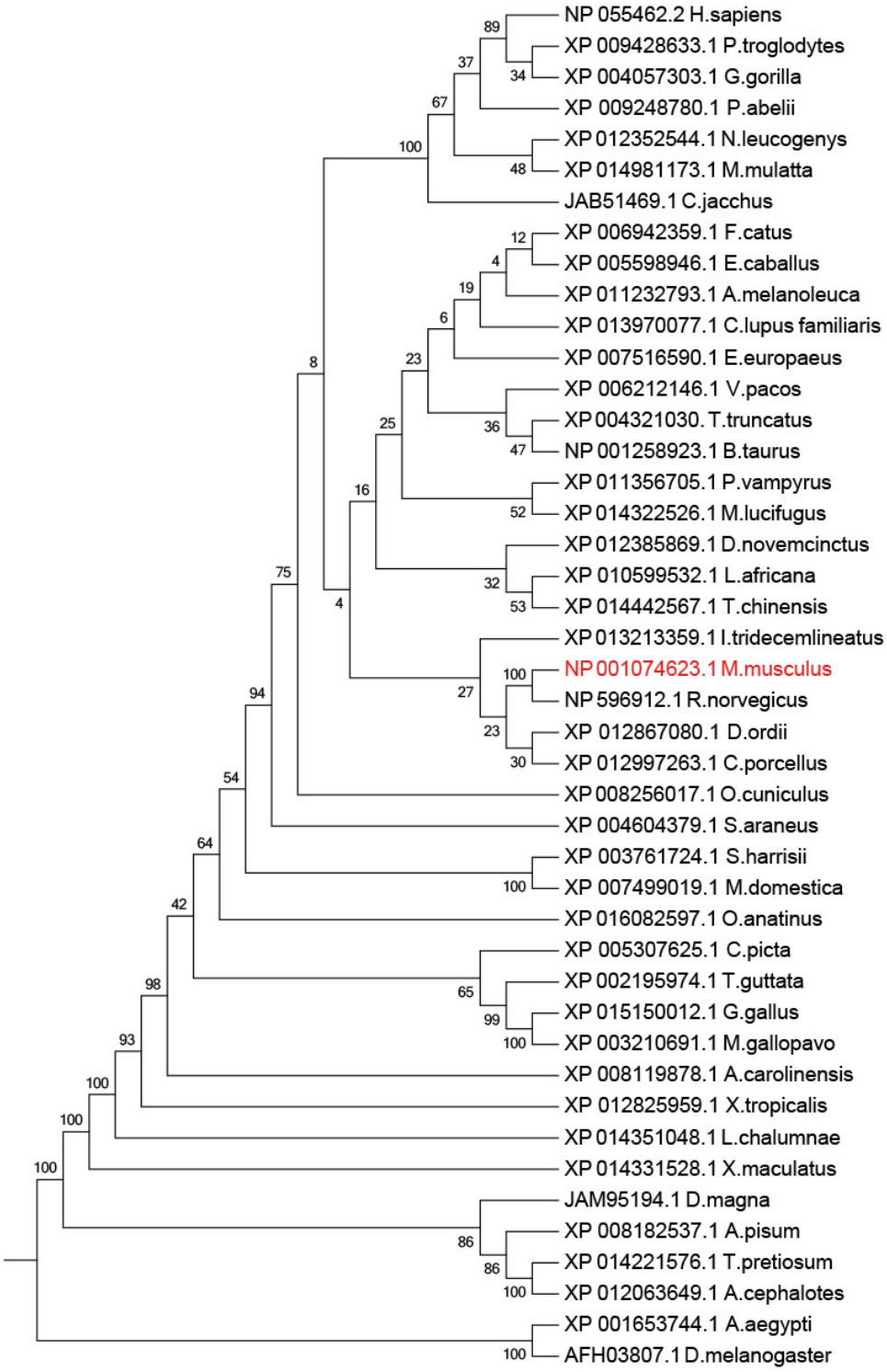

Fig. S1. Phylogenetic analysis of MARF1 in animal lineages. The phylogenic tree was constructed with MEGA (ver.5.05) using the Maximum Likelihood method. Bootstrap percentage values (500 replicates) are shown at the nodes. *M. musculus* MARF1 are highlighted in red.

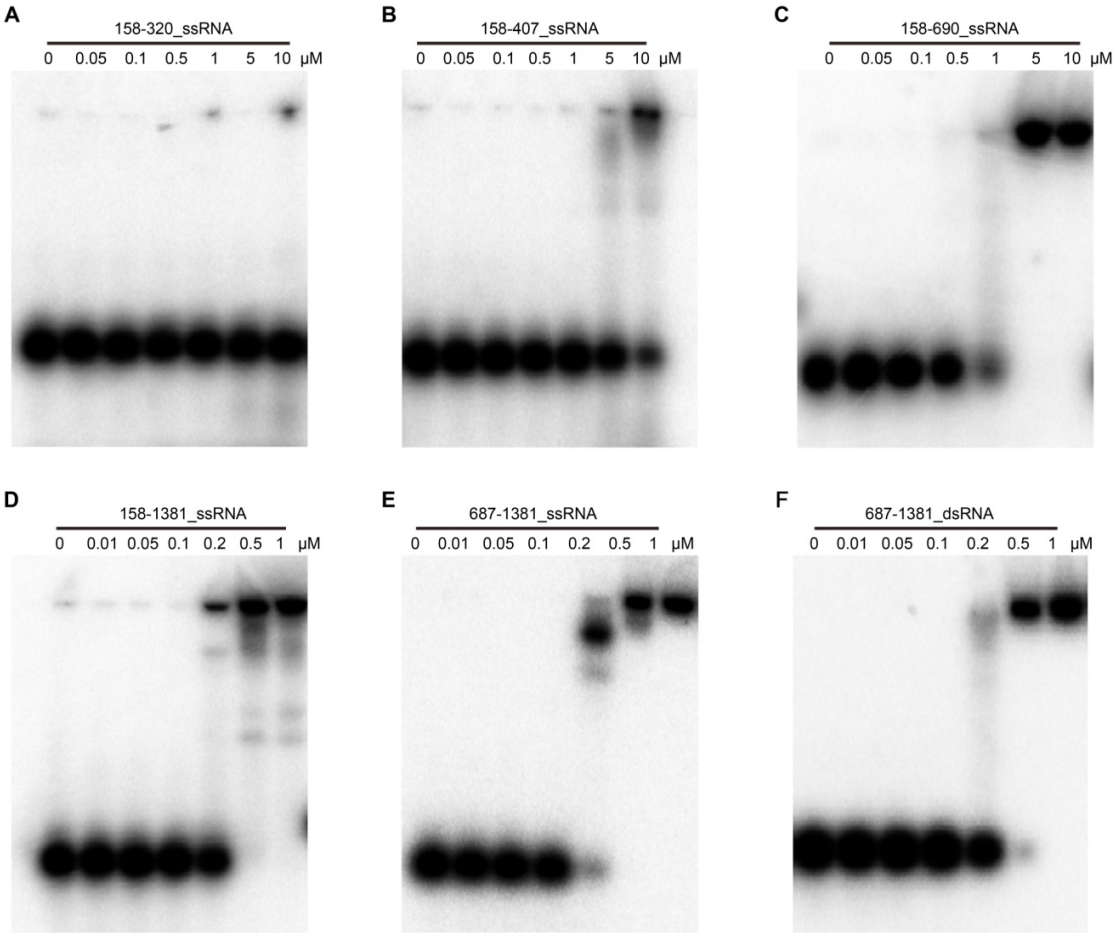

Fig. S2. ***In vitro*** **assay of MARF1 RNA binding activity. (A)** RNA binding analysis of MARF1 (residues 158-320) with 49 nt ssRNA. **(B)** RNA binding analysis of MARF1 (residues 158-407) with 49 ntssRNA. **(C)** RNA binding analysis of MARF1 (residues 158-690) with 49 ntssRNA. **(D)** RNA binding analysis of MARF1 (residues 158-1381) with 49 ntssRNA. **(E)** RNA binding analysis of MARF1 (residues 687-1381) with 49 ntssRNA. **(F)** RNA binding analysis of MARF1 (residues 687-1381) with 49 bp dsRNA.

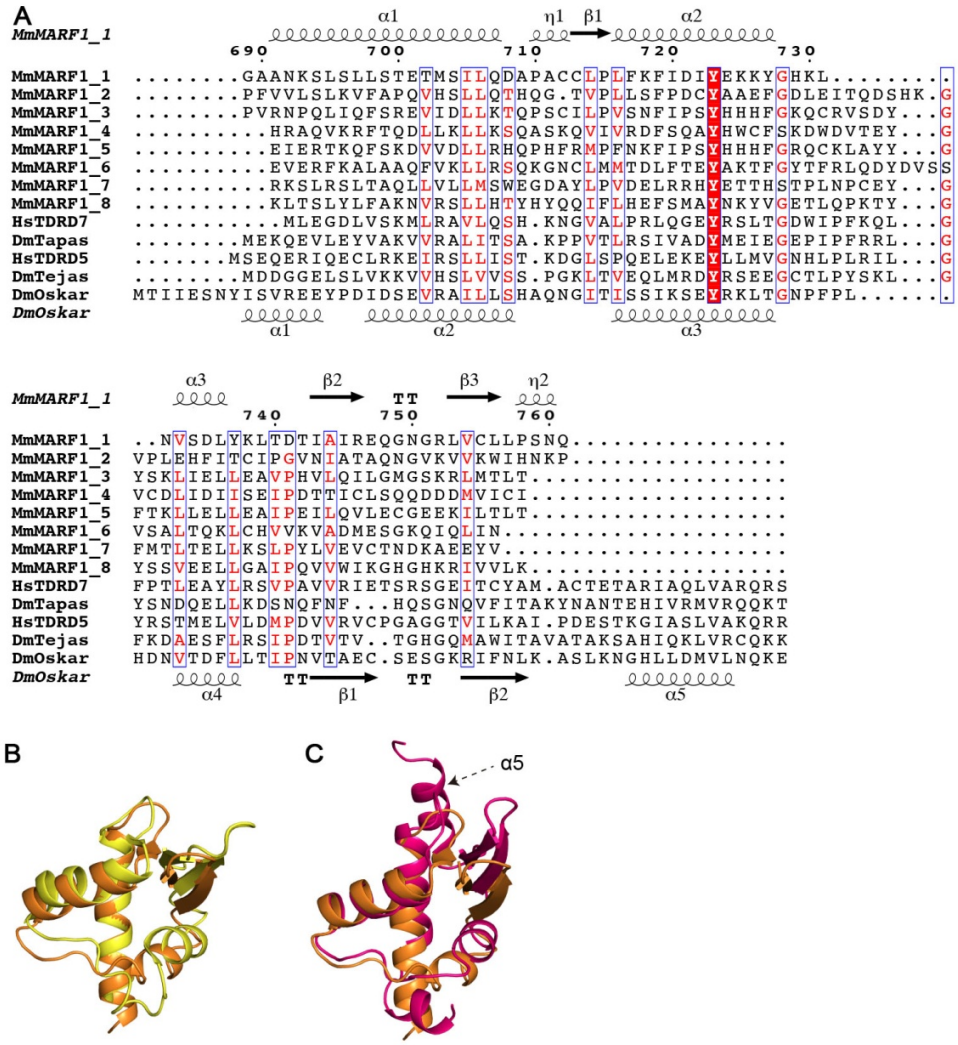

Fig. S3. Sequence alignment and structural superimposition of the LOTUS domains from MARF1 and other proteins. (A) Sequence alignment of the mouse MARF1-LOTUS domains (repeat 1-8) with that of the other proteins. **(B)** Superimposition of the structure of MARF1-LOTUS1 (colored in orange) on the LOUTS domain of human TDRD7 (yellow). (C) Superimposition of the structure of MARF1-LOTUS1 (orange) on the LOUTS domain of fruit fly Oskar (hot pink). The dashed arrow points to the extended helix α5 in Oskar-LOTUS.

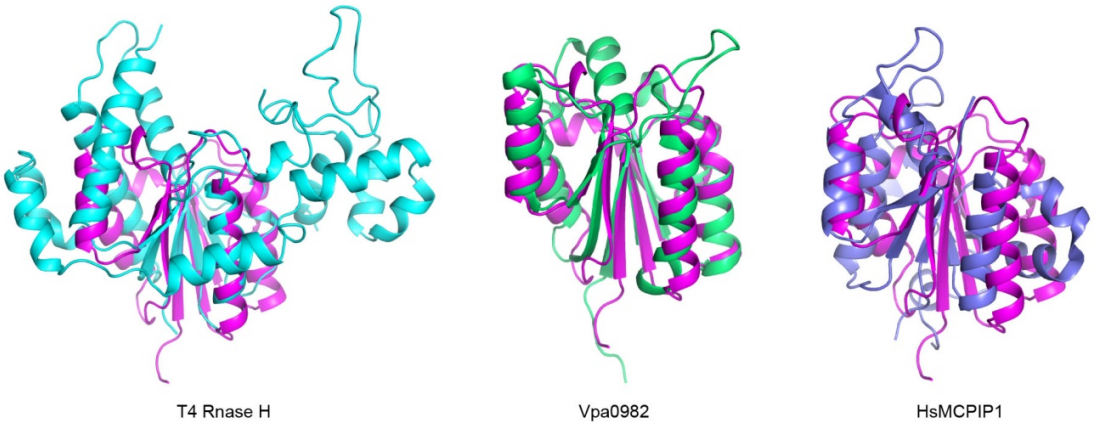

Fig. S4. Superimposition of the MARF1-NYN structure on the PIN domain of T4 RNase H, VPa0982 and MCPIP1. MARF1-NYN is shown in magenta, while T4RNase H, Vpa0982 and HsMCPIP1 are marked in cyan, green, and slate.

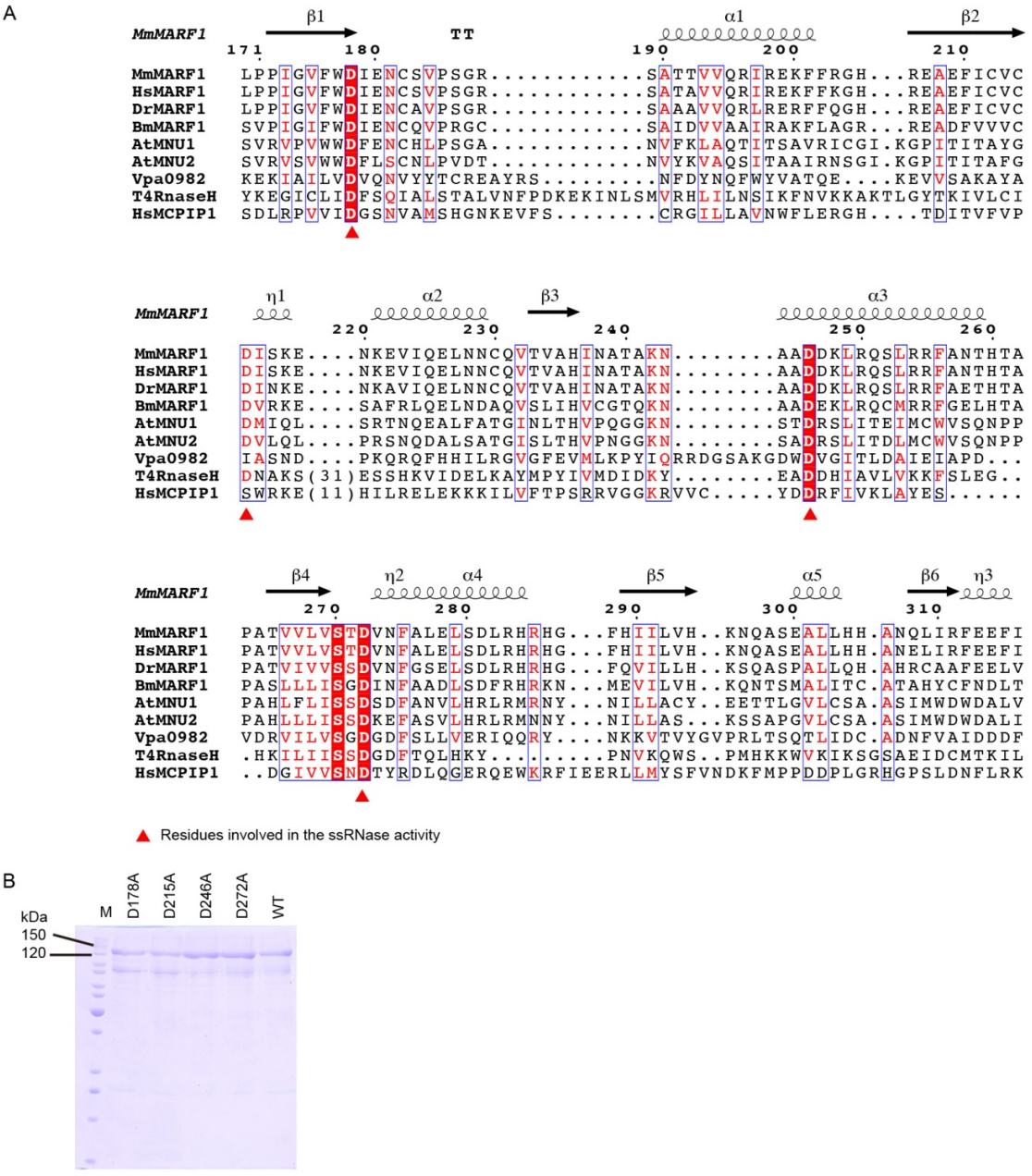

Fig. S5. Conserved amino acids in MARF1-NYN domain that compose the potential active sites of the RNase. (A) Sequence alignment of the mouse MARF1-NYN domain with that from different species and the PIN-like domains in other proteins. **(B)** Superimposition of the conserved amino acids of MARF1-NYN structure (marked in magenta) on the PIN domain of T4 Rnase H (cyan), VPa0982 (green) and MCPIP1(slate). **(C)** SDS-PAGE image showing the purified wild type (WT) and mutant (D178A, D215A, D246A, D272A) MARF1 proteins.

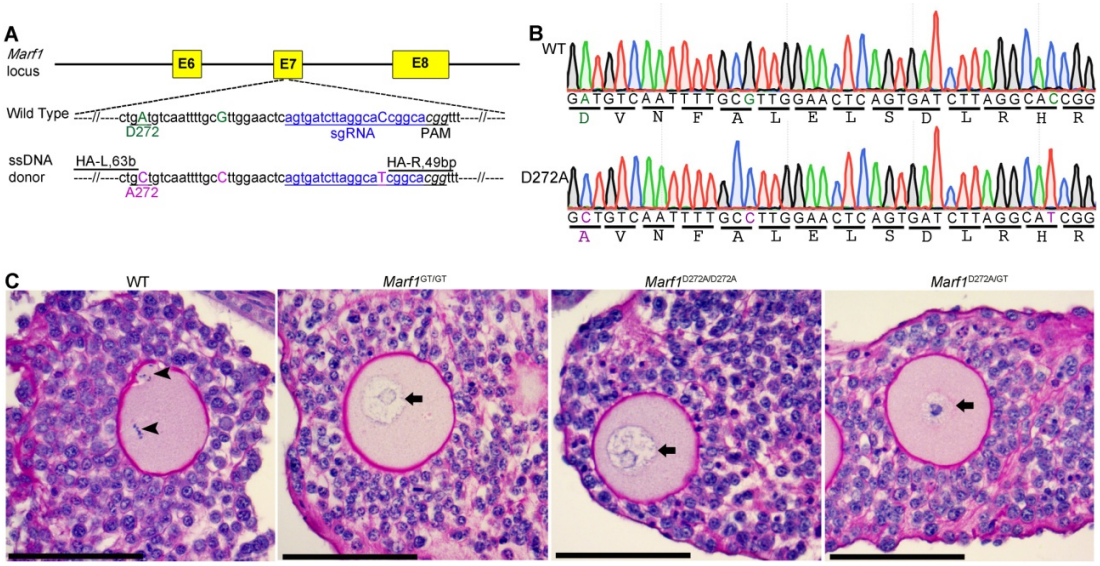

Fig. S6. Knock-in mutation of MARF1-D272 causes ovulation of immature oocytes in mice. (A) Schematic illustration of the design for creation of the D272A-*Marf1* knock in allele. A 150-bp single-stranded donor DNA (ssDNA) harboring the point mutation for D272A and the other two synonymous point mutations for a new site for restricting enzyme was designed. The points mutations were marked in magenta. The sgRNA sequence was written in blue. **(B)** Sequencing chromatograms showing the correct creation of the point mutations (marked in magenta) in the D272A-*Marf1* knock-in homozygous mice. **(C)** Microphotographs showing the PAS and hematoxylin-stained histological sections of the cumulus-oocyte-complexes ovulated into the oviducts. Scale bars indicate 100 μm. The arrowheads point to the first polar body and MII-spindle, respectively, and arrows indicate GV.

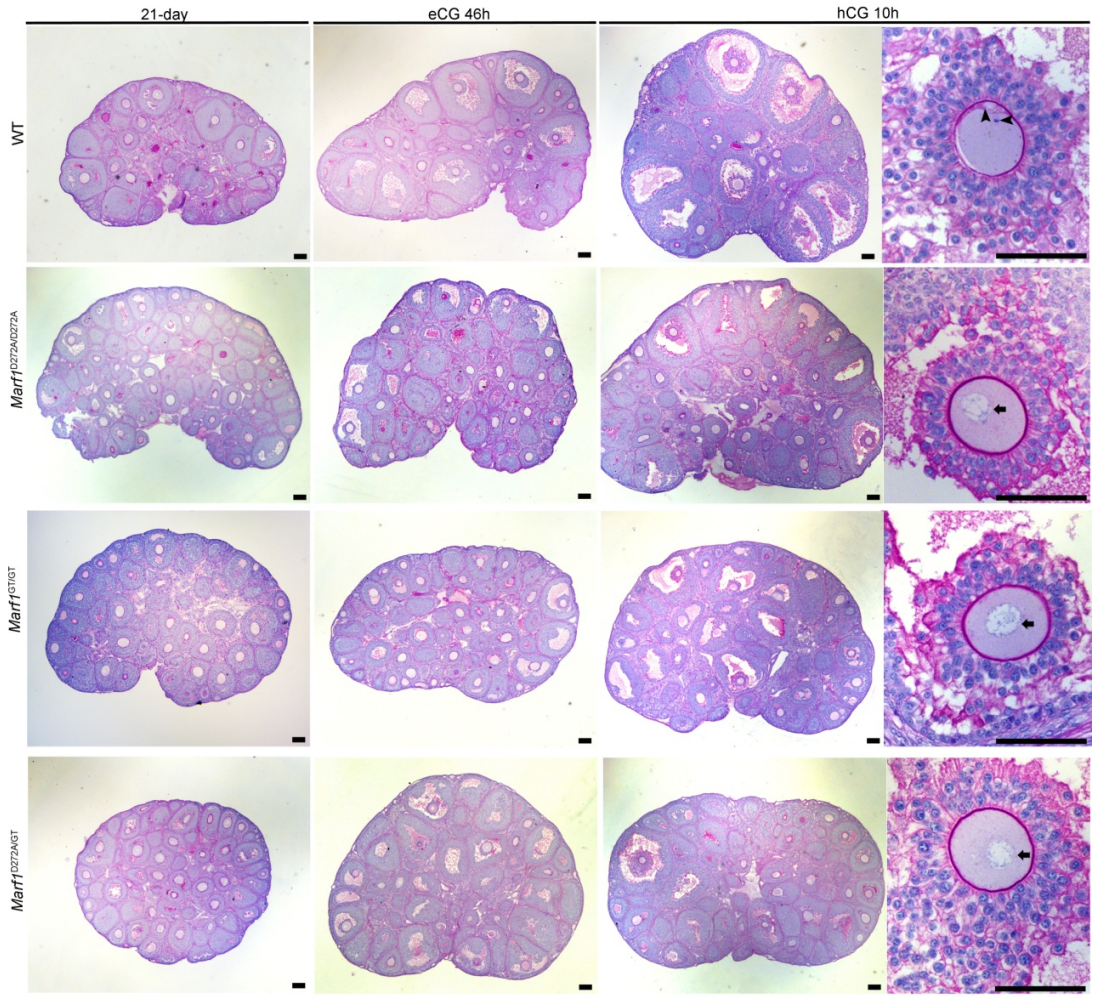

Fig. S7. Histological analysis of the *Marf1*- wild type and mutant ovaries before and after receiving the super ovulation treatment. Ovaries were collected from 21-day old wild type (WT) and various types of *Marf1* mutant (*Marf1*$^{D272A/D272A}$, *Marf1*$^{GT/GT}$ and *Marf1*$^{D272A/GT}$) mice without hormonal treatment (referred to as 21-day), or being treated with eCG for 46 h (referred to as eCG 46 h), or being treated with eCG for 46 h followed by hCG for another 10 h (referred to as hCG 10 h). The magnified view of the ovulating follicles in the hCG 10 h-group were shown on the right side of the whole ovaries. Arrowheads indicate the MII spindle and first polar body, arrows indicate GV. Scale bars indicate 100 μm.

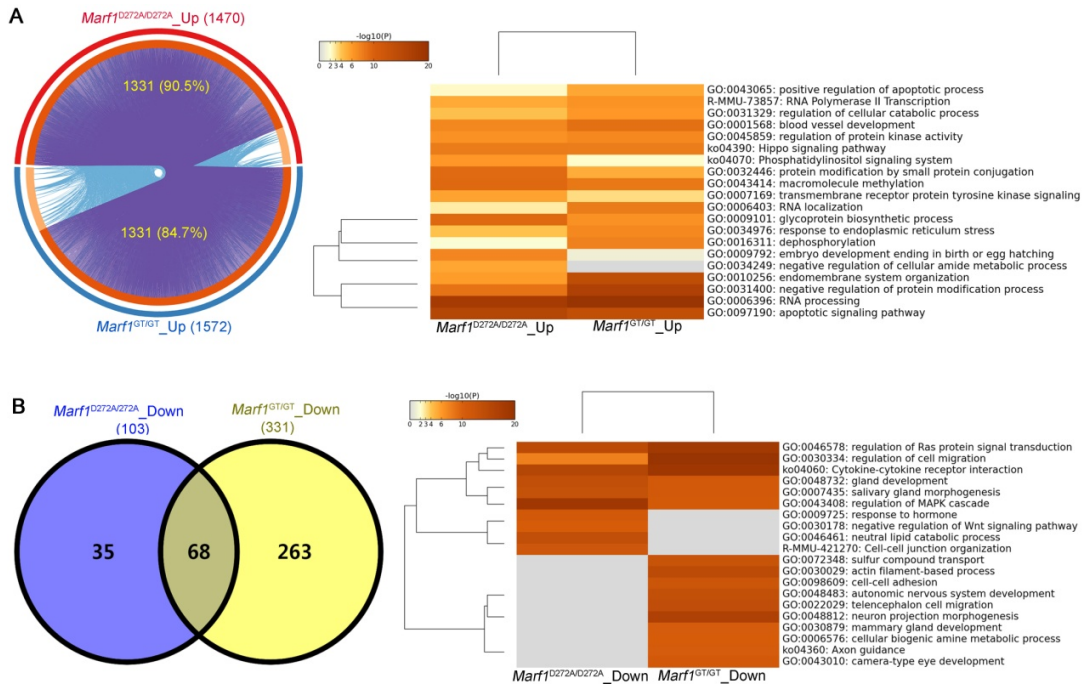

Fig. S8. Bioinformatics analysis of the changed transcripts in *Marf1* mutant oocytes. (A) Left graph is the Circos plot showing the overlap between the transcripts upregulated in *Marf1*$^{D272A/D272A}$ (referred to as *Marf1*$^{D272A/D272A}$ _Up) and those upregulated *Marf1*$^{GT/GT}$ (referred to as *Marf1* $^{GT/GT}$ _Up) oocytes. On the outside, the red and blue arcs represent the identity of the upregulated transcript lists of *Marf1*$^{D272A/D272A}$ and *Marf1*$^{GT/GT}$ oocytes, respectively, with the number of transcripts in each list shown in the parentheses. On the inside, each arc represent a transcript list, where each transcript has a spot on the arc. Dark orange color represents the transcripts that appear in both transcript lists and light orange color represents transcripts that are unique to that transcript list. Purple lines link the same transcript that are shared by both transcript lists. The greater the number of purple links and the longer the dark orange arcs implies greater overlap among the input transcript lists. Blue lines link the different transcripts where they fall into the same ontology term. Blue links indicate the amount of functional overlap among the input transcript lists. Right graph is the Heatmaps illustrating the enriched terms (GO/KEGG terms or canonical pathways) associated significantly with the transcripts upregulated in *Marf1*$^{D272A/D272A}$ and *Marf1*$^{GT/GT}$ oocytes, respectively. **(B)** Left graph is the Venn diagrams illustrating the relationship of the transcripts downregulated in *Marf1*$^{D272A/D272A}$ and *Marf1*$^{GT/GT}$ oocytes. Right graph is the Heatmaps illustrating the enriched terms (GO/KEGG terms or canonical pathways) associated significantly with the transcripts downregulated in *Marf1*$^{D272A/D272A}$ and *Marf1*$^{GT/GT}$ oocytes, respectively.

Table S1. The crystallographic collection and refinement statistics for the LOTUS1 and NYN structures

	NYN(5YAA)	LOTUS(5YAD)
Data collection		
Space group	H3	$P2_12_12_1$
Cell dimension		
a, b, c (Å)	163.47, 163.47, 56.15	54.97, 53.96, 67.45
α, β, γ (°)	90.00, 90.00, 120.00	90.00, 90.00, 90.00
Wavelength (Å)	0.97916	0.97853
Resolution (Å)	30-1.75(1.84-1.75)	50-1.76(1.82-1.76)
*R*merge	0.105	0.059
$I/\sigma I$	11.1 (2.8)	38.95(2.55)
Completeness (%)	99.8(99.9)	95.9(77.4)
Redundancy	7.6	6.5
Refinement		
Resolution (Å)	30.00-1.75	30.00-1.76
No. reflections	53406	18762
R_{work}/R_{free}	23.19/28.25	0.1816/0.2162
No. atoms	5140	1250
Protein	4792	1100
Water	277	128
Ligand	71	22
Average *B*-factor (Å)	17.97	33.022
R.m.s. deviations		
Rmsd bonds (Å)	0.009	0.007
Rmsd angle (°)	1.064	1.262
Ramachandran plot		
Favored/allowed/disallowed (%)	97.47/2.53	96.97/3.03

Table S2. Primers used in this study

Gene symbol	Forward primer	Reverse primer	Application
Marf1-3xDDK	AGATCTGCCGCCGCGATCGCATGGA AGGGAAAGGAACTGA	GCGGCCGCGTACGCGTAAGCTTGGT TACAGGTGCAAA	Cloning
MARF1-D272A	GTGGTTCTTGTGTCAACTGCTGTCAA TTTTGCGTTGGAA	TTCCAACGCAAAATTGACAGCAGTT GACACAAGAACCAC	Cloning
T7-sgRNA	TAATACGACTCACTATAGGGAGTGA TCTTAGGCACCGGCAGTTTTAGAGCT AGAAATAGC	AAAAGCACCGACTCGGTGCC	Cloning
T7-Cas9	TAATACGACTCACTATAGGGAGAAT GGACTATAAG GACCACGAC	GCGAGCTCTAGGAATTCTTAC	Cloning
Marf1	GCGGAGTTTGGTGACCTAGAAA	TCACTACTTTGACGCCATTCTGA	qPCR
Iap	ACAAGAAAAGAAGCCCGTGA	GCCAGAACATGTGTCAATGG	qPCR
Line1	GAGACATAACAACAGATCCTGA	AACTTTGGTACCTGGTATCTG	qPCR
Ppp2cb	AGGGCCCAATGTGTGATCTC	TGGCATGGTTAAATGTTTCAGAA	qPCR
Ppp1r8	CGATCTGTGTGACTTCACTATCGA	AAAGTGCCATGTGTGCTGTTG	qPCR
Wdr45b	TGTCCTGAGCTGTATTGCTCTCAA	ATGCCCTGATGAAGTATCAAATATTCT	qPCR
Polr3e	GACACCCTGAATCCCAACTACTG	CAGCTTTGAGGAATATGTGCTAGTCT	q-RT-PCR
Tbpl2	CCCAAAGAGGTTTGCTGCAGTA	GCCAGCCGAGACTCCTCTTC	qPCR
Riox1	TCACCAAGCTGAATGTCAGGAT	AGGCAGTACAGCCTCCAGGAA	qPCR
Rpl19	CCGCTGCGGGAAAAAGAAG	CAGCCCATCCTTGATCAGCTT	qPCR
Marf1^{GT}	GCCATATCTTTACTGCGCATGG	ATATGGTGCCGTGCAGGAAA	Genotyping
Marf1-WT	GCCATATCTTTACTGCGCATGG	TACCTGCTGACCCCCAATAAAGT	Genotyping
Marf1 ^{D272A}	TGTGATTGCCCTGTCATGCT	AGAGAGGCTCAAAAGGCTGC	Genotyping
MARF1 158-1381	ATCGGGATCCCCAGAAGTTGCAGTC ACTGGACAGG	AGCCCTCGAGTTATTTCATGTCATTT TTTAACACTACAATTCTCTTATGAC	Protein expression
MARF1 158-320	ATCGGGATCCCCAGAAGTTGCAGTC ACTGGACAGG	AGCCCTCGAGTTAGGGGAGATCAGA AATGAACTCTTCAAATC	Protein expression
MARF1 158-407	ATCGGGATCCCCAGAAGTTGCAGTC ACTGGACAGG	AGCCCTCGAGTTATTCTCTATGTTTT GGAGTAAAGGACACGATG	Protein expression
MARF1 158-690	ATCGGGATCCCCAGAAGTTGCAGTC ACTGGACAGG	AGCCCTCGAGTTAAGCTCCTGTGGA GAGTGAGACCAGTATC	Protein expression
MARF1 687-761	ATCGGGATCCACAGGAGCTGCCAAC AAGTCACTCTC	AGCCCTCGAGTTACTGATTACTAGGT AAGAGACACACCAATCGC	Protein expression
MARF1 687-1381	ATTCCCATGGGCACAGGAGCTGCCA ACAAGTCACTCTC	AGCCCTCGAGTTTCATGTCATTTTTT AACACTACAATTCTCTTATGAC	Protein expression
MARF1-L253M	ATAAACTCCGTCAGAGCATGCGAAG GTTCGCAAAC	GTTTGCGAACCTTCGCATGCTCTGAC GGAGTTTAT	Protein expression
MARF1-L303M	CAGGCCTCTGAAGCCTTGATGCATC ATGCTAACCAGCTG	CAGCTGGTTAGCATGATGCATCAAG GCTTCAGAGGCCTG	Protein expression
MARF1-D178A	CCATTGGCGTTTTCTGGGCTATTGAA AACTGCTCAGT	ACTGAGCAGTTTTCAATAGCCCAGA AAACGCCAATGG	Protein expression

MARF1-D215A	TCCTTGTTTTCTTTACTGATGGCACA CACACAGATAAACTCTG	CAGAGTTTATCTGTGTGTGTGCCATC AGTAAAGAAAACAAGGA	Protein expression
MARF1- D246A	CTGCAAAGAATGCTGCTGCTGATAA ACTCCGTCAGAG	CTCTGACGGAGTTTATCAGCAGCAG CATTCTTTGCAG	Protein expression
MARF1 D272A	GTGGTTCTTGTGTCAACTGCTGTCAA TTTTGCGTTGGAA	TTCCAACGCAAAATTGACAGCAGTT GACACAAGAACCAC	Protein expression
H49	TAATACGACTCACTATAGGGATTTA GATGAACAAAAAGAAAAAGAAAAT TG	AGTAAATGACCAAAACTCAATTTTCT TTTTCTTTTTGTTCATC	Transcription
H49AS	TAATACGACTCACTATAGGAGTAAA TGACCAAAACTCAATTTTCTTTTTC	GATTTAGATGAACAAAAAGAAAAAG AAAATTGAGTTTTGGTC	Transcription

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Captions for SI Datasets 1 to 4

SI Dataset 1. List of the transcripts that are downregulated in *Marf*$^{GT/GT}$ oocytes after microinjection with mRNA for D272A-MARF1.

SI Dataset 2. List of the transcripts that are downregulated in *Marf*$^{GT/GT}$ oocytes after microinjection with mRNA for WT-MARF1

SI Dataset 3. List of the transcripts that are differentially expressed in *Marf*$^{D272A/D272A}$ oocytes as compared with the WT-oocytes.

SI Dataset 4. List of the transcripts that are differentially expressed in *Marf*$^{GT/GT}$ oocytes as compared with the WT-oocytes.