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Supplemental Information

Cpmer: A new conserved eEF1A2-binding partner that regulates *Eomes* translation and cardiomyocyte differentiation

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Supplemental Information

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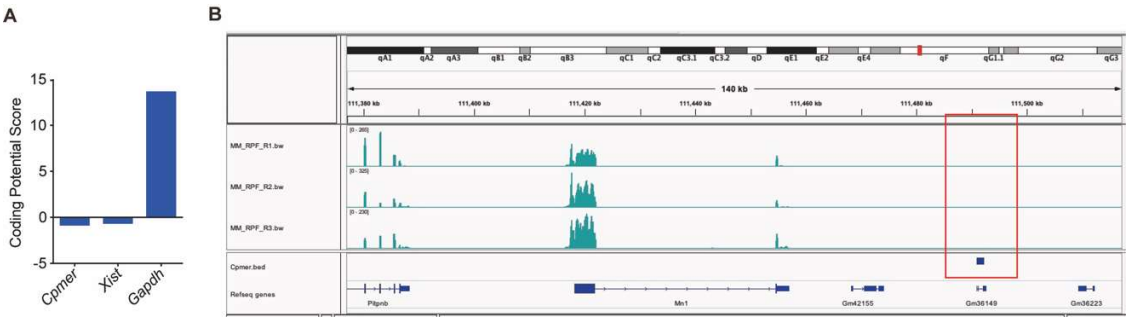


Figure S1. Identification of *Cpmer* as a non-coding RNA. Related to figure 1.

(A) The coding potential prediction of *Cpmer*, *Xist*, and *Gapdh* by Coding Potential Calculator (http://cpc.gao-lab.org).

(B) The ribosome occupancy analysis of the *Cpmer* and *Mn1* mRNA in mesoderm cells (GSE86467).

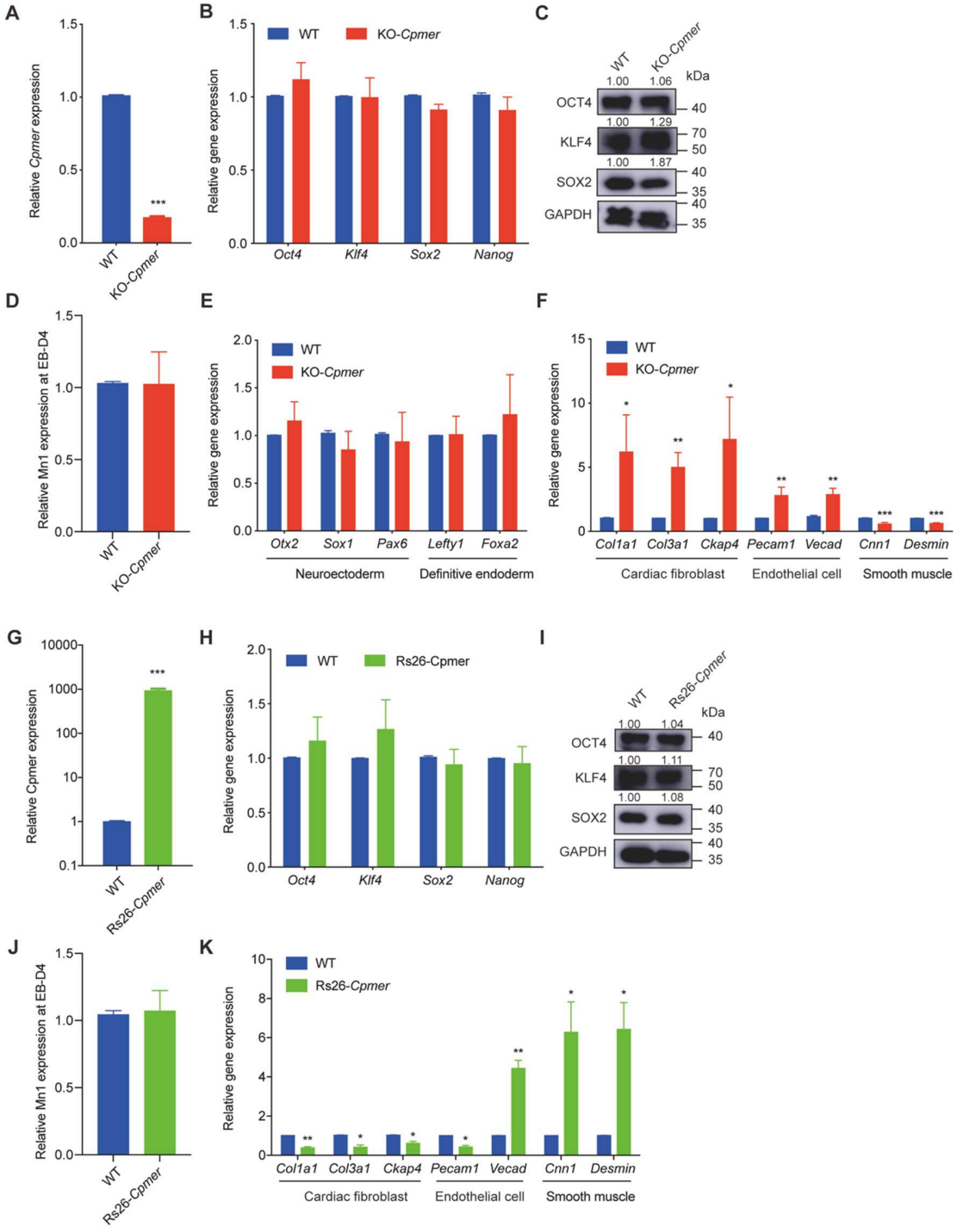


Figure S2. The effect of *Cpmer* on the expression of pluripotent genes, neighbor gene, and lineage genes. Related to figure 2.

(A-B) qRT-PCR analysis of the expression of *Cpmer* and pluripotency-related genes in KO-*Cpmer* ESCs compared with WT ESCs. Data shown are the mean ± SEM, n=3 independent experiments.

(C) Western blotting analysis of pluripotency-related gene expression in WT and KO-*Cpmer* ESCs.

(D) qRT-PCR analysis of *Mn1* expression in KO-*Cpmer* MES cells compared with WT cells. Data shown are the mean ± SEM, n=3 independent experiments.

(E) qRT-PCR analysis of the expression of neuroectoderm markers (*Otx2*, *Sox1*, and *Pax6*) and definitive endoderm markers (*Lefty1* and *Foxa2*) in KO-*Cpmer* cells compared with WT cells. Data shown are the mean ± SEM, n=3 independent experiments.

(F) qRT-PCR analysis of the expression of cardiac fibroblast markers (*Col1a1*, *Col3a1*, and *Ckap4*), endothelial cell markers (*Pecam1* and *Vecad*), and smooth muscle markers (*Cnn1* and *Desmin*) in KO-*Cpmer* cells compared with WT cells. Data shown are the mean ± SEM, n=3 independent experiments.

(G-H) qRT-PCR analysis of the expression of *Cpmer* or pluripotency-related genes in Rs26-*Cpmer* ESCs compared with WT ESCs. Data shown are the mean ± SEM, n=3 independent experiments.

(I) Western blotting analysis of pluripotency-related gene expression in WT and Rs26-*Cpmer* ESCs.

(J) qRT-PCR analysis of *Mn1* expression in Rs26-*Cpmer* MES cells compared with WT cells. Data shown are the mean ± SEM, n=3 independent experiments.

(K) qRT-PCR analysis of the expression of cardiac fibroblast markers, endothelial cell markers, and smooth muscle markers in Rs26-*Cpmer* cells compared with WT cells. Data shown are the mean ± SEM, n=3 independent experiments.

*P < 0.05, **P < 0.01, and ***P < 0.001 (versus WT) by Student`s t-test.

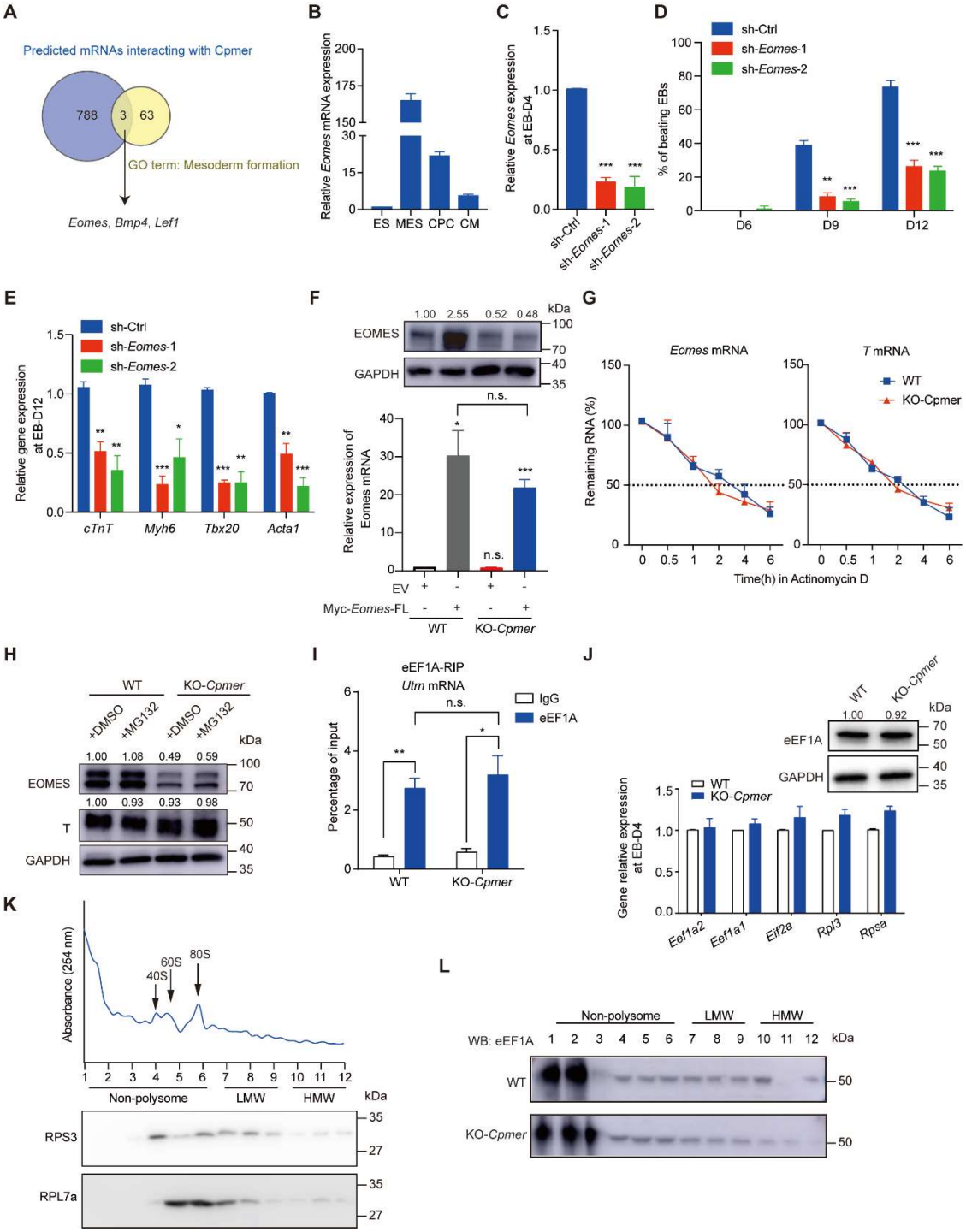


Figure S3. *Cpmer* specifically affects the translation of Eomes, which is critical for the CM differentiation, independent of the eEF1A recruitment in ribosome complex. Related to figure 4.

(A) Overlapping of the predicted mRNAs (791) interacted with *Cpmer* and the genes (66) associated with mesoderm formation (GO_ID: 00010707).

(B) The expression pattern of *Eomes* during CM differentiation. Data shown are mean ± SEM, n=3 independent experiments.

(C) The expression of *Eomes* at day 4 of EB differentiation after *Eomes* knockdown (sh-*Eomes*-1 and sh-*Eomes*-2) in ESCs. Data shown are mean ± SEM, n=3 independent experiments.

(D) Percentage of spontaneously contracting EBs determined at days 6, 9, and 12 of EB differentiation in *Eomes* knockdown cells compared with control cells. Data shown are mean ± SEM, n=3 independent experiments.

(E) qRT-PCR analysis of the CM marker genes in *Eomes* knockdown cells compared with control cells at day 12 of EB differentiation. Data shown are mean ± SEM, n=3 independent experiments.

(F) qRT-PCR and western blot analysis of *Eomes* mRNA and protein levels in the WT and KO-*Cpmer* cells transfected with full-length (FL) *Eomes* at the mesoderm stage. Data shown are mean ± SEM, n=3 independent experiments.

(G) The half-lives of *Eomes* and *T* mRNA were quantified after exposure to actinomycin D at different time points (0, 0.5, 1, 2, 4, and 6 h). Data shown are the mean ± SEM, n=3 independent experiments.

(H) Western blotting analysis of EOMES and T protein levels in the WT and KO-*Cpmer* mesoderm cells after treatment with MG132 (5 μM) for 6 h.

(I) Endogenous RIP analysis of the interaction of eEF1A with *Utrn* mRNA (as positive control) in the WT and KO-*Cpmer* cells at the mesoderm stage. Data shown are mean ± SEM, n=3 independent experiments.

(J) qRT-PCR and western blotting analysis of translation-related genes in the WT and KO-*Cpmer* cells at day 4 of EB differentiation (EB-D4). Data shown are mean ± SEM, n=3 independent experiments.

(K) Polysome trace analysis exhibited the presence of absorption peaks for free RNA, pre-polysome (40S, 60S, and 80S), and polysomes (LMW and HMW) (up) and the accompanying western blotting of RPS3 (40S ribosomal protein S3) and RPL7a (60S ribosomal protein L7a) (down).

(L) Western blotting analysis of eEF1A distribution in polysome fractions of the WT and KO-*Cpmer* cells at EB-D4.

*P < 0.05, **P < 0.01, and ***P < 0.001 (versus sh-Ctrl) by Student`s t-test.

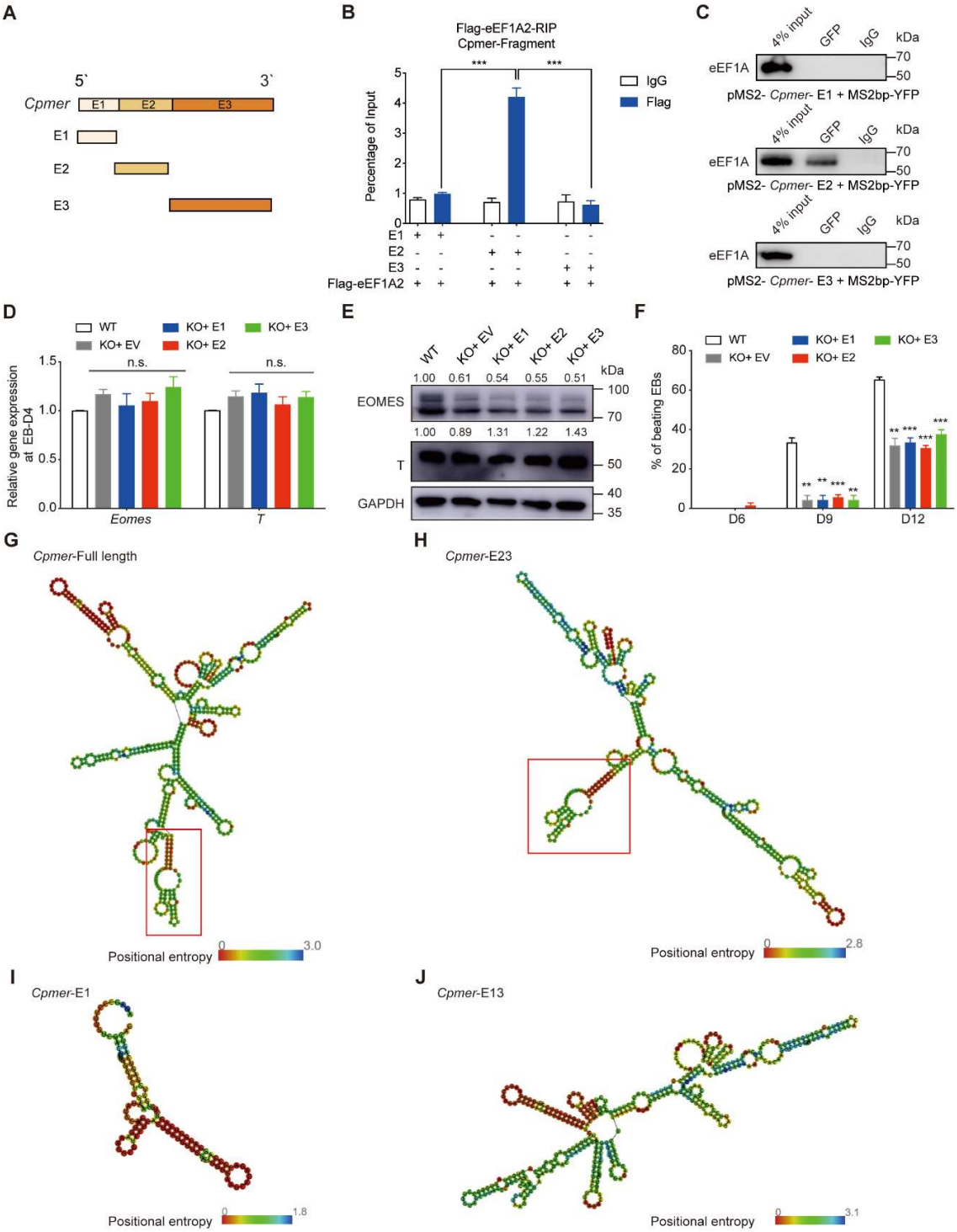


Figure S4. Single exon of *Cpmer* is not able to rescue the EOMES expression or CM differentiation.

Related to figure 5.

(A) Schematic representation of the full-length *Cpmer* and three mutants (single exons: E1, E2, and E3).

(B) RIP analysis for the exogenous expression of *Cpmer* mutants and Flag-eEF1A2 in 293T cells, as determined by anti-Flag antibody.

(C) MS2bp-YFP RNA pull-down analysis for mesoderm cells co-transfected with MS2-*Cpmer* mutants and MS2bp-YFP.

(D-E) qRT-PCR and western blotting analysis of *Eomes* and *T* expression in the KO-*Cpmer* cells transfected with different *Cpmer* mutants at mesoderm stage.

(F) Percentage of spontaneously contracting EBs in the WT and KO-*Cpmer* cells transfected with different *Cpmer* mutants.

Data shown are the mean ± SEM (n=3). **P < 0.01 and ***P < 0.001 (versus WT) by Student`s t-test.

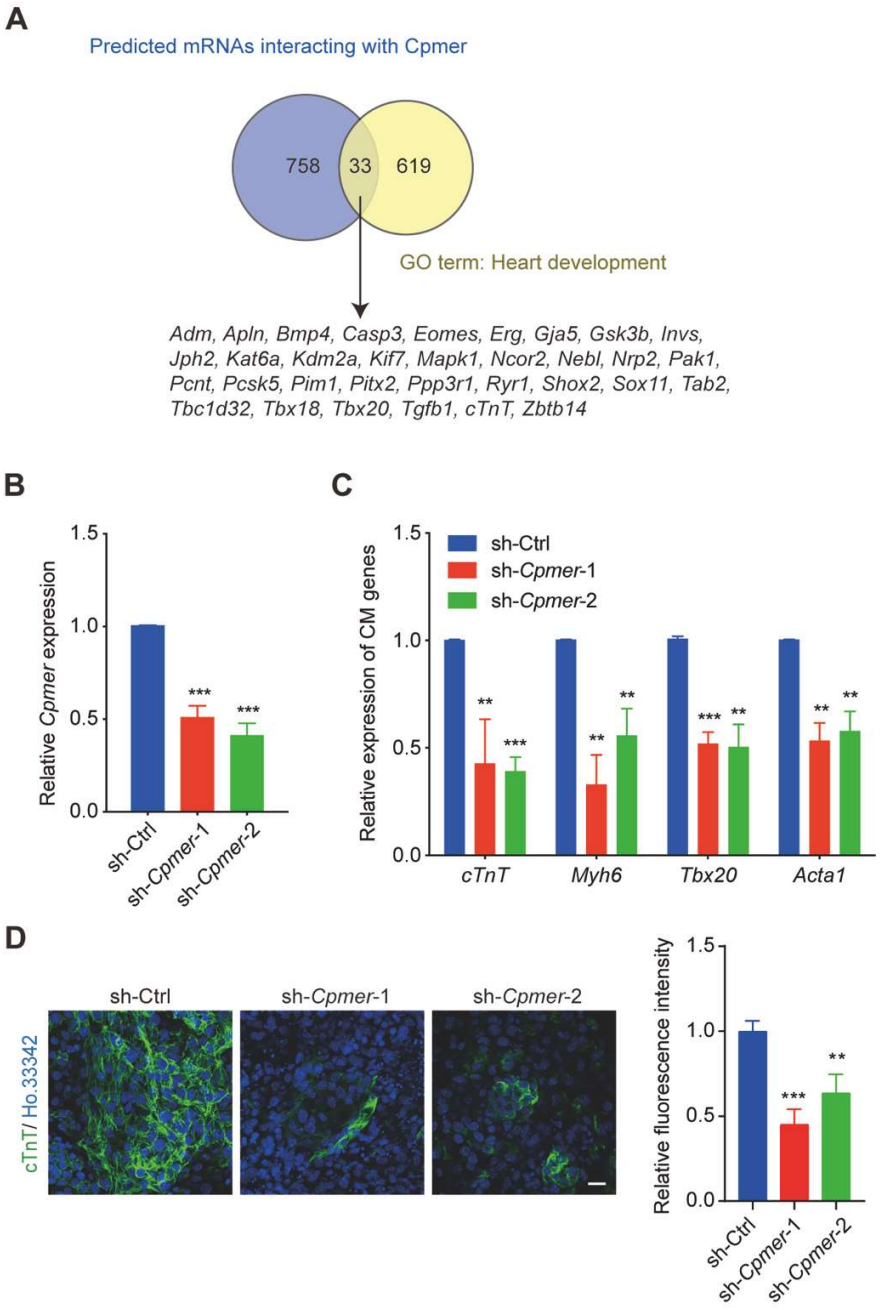


Figure S5. Knockdown of *Cpmer* after mesoderm stage also inhibits the CM differentiation. Related to figure 5.

(A) Overlapping of the predicted mRNAs (791) interacted with *Cpmer* and the genes (622) associated with heart development (GO_ID:0007507).

(B) The expression detection of *Cpmer* knockdown with shRNA virus (sh-*Cpmer*-1 and sh-*Cpmer*-2) after mesoderm stage. Data shown are mean ± SEM, n=3 independent experiments.

(C) qRT-PCR analysis of the CM marker genes in the *Cpmer* knockdown cells compared with control cells at day 8 of CM differentiation. Data shown are mean ± SEM, n=3 independent experiments.

(D) cTnT immunostaining (green) in the *Cpmer* knockdown and control cells at day 8 of CM differentiation (left). The statistics for relative fluorescence intensity was shown (right). Scale bar, 20 μm. Data shown are the mean ± SEM (n= 6 fields from 3 independent experiments).

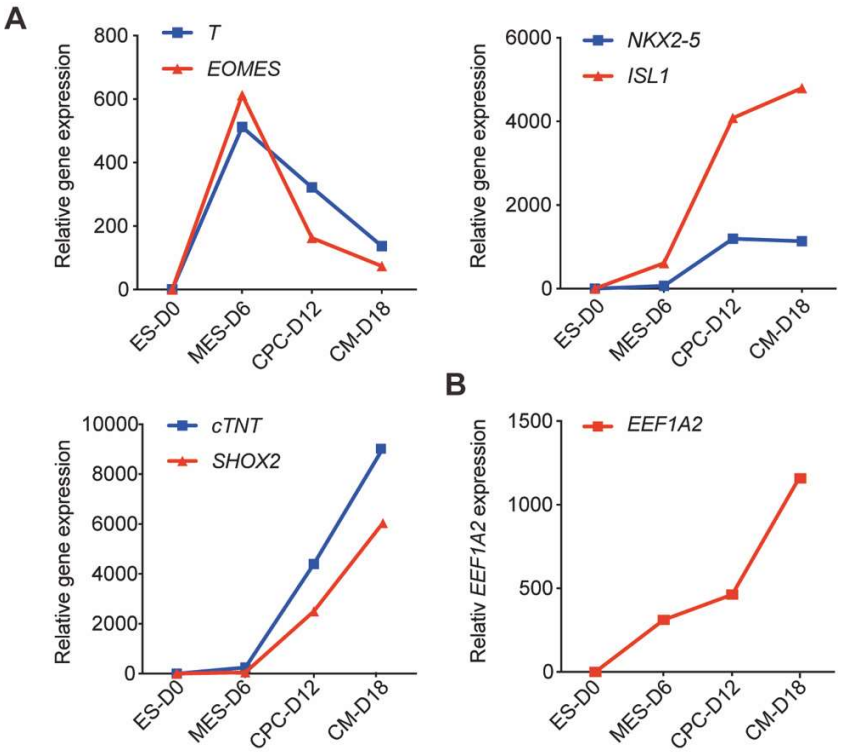


Figure S6. Expression patterns of stage-specific markers and *EEF1A2* gene during hESC-derived CM differentiation. Related to figure 6.

(A) qRT-PCR analysis of marker genes at the indicated stages (ES, MES, CPC, and CM) of hESC-derived CM differentiation.

(B) Expression pattern of *EEF1A2* during hESC-derived CM differentiation.

Supplemental Experimental Procedures

Cell culture and differentiation

For EB differentiation, mESCs were plated at a density of 4×10^4 cells/mL in ultralow-attachment plates in 5 mL of mESC medium without LIF. The medium was changed every 2 days. For beating EB analysis, single EBs were picked and reseeded onto gelatin-coated 48-well plates on the 4th day. Beating EBs were observed under a light microscope from the 6th day to the 12th day.

For mouse CM differentiation, mESCs were cultured in serum-free medium (DMEM: Neurobasal=1:1 (v/v) (Gibco), 0.05% BSA, 2 mM GlutaMAX, 0.5 × N2 supplement (Gibco), 0.5×B27 supplement (Gibco), 1.5×10^{-4} M monothioglycerol, 10 ng/mL hBMP4 (R&D), and LIF (Millipore; 10000×)) for 2 passages. The medium was changed every day. Then, EBs were aggregated at a density of 6×10^4 cells/mL for 48 h in serum-free medium (DMEM: DMEM/Ham's F12=1:1 (v/v) (Gibco), 0.05% BSA, 2 mM GlutaMAX, 1 × N2 supplement, 1×B27 supplement, 4.5×10^{-4} M monothioglycerol, and 25 μg/ml L-ascorbic acid (Sigma-Aldrich)). EBs were dissociated with 0.125% trypsin and reaggregated in the presence of 0.2 ng/mL hBMP4, 5 ng/mL hVEGF (R&D), 5 ng/mL hActivin A (R&D), and 25 μg/mL L-ascorbic acid for 40 h. EBs were dissociated and plated in gelatin-coated 12-well plates at a density of 2.5×10^5 cells/well with StemPro-34 medium (Gibco) supplemented with 5 ng/mL hVEGF, 10 ng/mL hbFGF (R&D), 25 ng/mL hFGF10 (R&D), and 25 μg/ml L-ascorbic acid. The medium was changed every day until contracting CMs were observed.

For hESCs induced CM differentiation, hESCs were incubated with dispase (Gibco) at 37°C for 5 mins. Then, colonies were cultured in suspension as EBs in basic StemPro-34 (Gibco) media for 18 h in ultra-low attachments dishes. At day 1, EBs were cultured in pacemaker mesoderm induction media. At day 3, EBs were harvested and washed once then cultured in cardiac induction media. At day 6, media was changed to cardiac maintenance media, and the media was changed every 3 days until day 18.

RNA-sequencing and bioinformatic analysis

The whole heart samples were collected from embryos at E10.5, E14.5, E17.5, as well as the newborn and adult mice. Total RNA for RNA-seq analysis was isolated from the heart samples using RNAiso Reagent (TaKaRa) and paired-end RNA-seq for developing mouse hearts was performed. The RNA-seq reads of developing hearts were aligned to the mm9 UCSC reference gene GTF using TopHat2 with default parameters. The transcript assembly and differential expression analysis with the mm9 UCSC reference gene GTF and reference genome GSE52313 were performed through Cufflinks2 and Cuffdiff2 with default parameters, respectively. The upregulated lncRNAs were sorted by the changes in FPKM during heart development.

Generation of Cpmer-knockout cell lines

A dual guide RNA (gRNA) knockout strategy was used to completely knock out *Cpmer*. The gRNAs targeted to the 5' and 3' regions of *Cpmer* were designed by using the CRISPR DESIGN website (https://zlab.bio/guide-design-resources), and the detailed sequences are shown in Table S4. The gRNAs were inserted into the pX330 vector. Two gRNAs and Cas9 plasmids of 5 μg each were mixed in P3 primary Cell Solution Box (PBP3-00675, Lonza), electroporated into mESCs with the Gene Pulser X Cell System (Bio-Rad) at 250 V and 500 μF in a 0.4 cm Gene Pulser cuvette (Bio-Rad), and then seeded on feeder cells. 24 h later, 0.5 μg/mL puromycin was added for 2 days. Individual colonies were picked and expanded in 24-well plates with feeder cells. The genomic DNA from each colony was extracted by a TIANamp Genomic DNA Kit (DP304-03) and further verified by PCR.

RACE

To obtain the full-length sequence of the *Cpmer* transcript, 5 prime and 3 prime RACE methods were employed to amplify the cDNA of mouse heart tissue using the SMARTer RACE 5'/3' Kit (TaKaRa). cDNA with adapter addition was cloned into the pPLB vector (TIANGEN) and verified by sequencing. The sequences of gene-specific primers are listed in Table S4.

Flow cytometry and cell sorting

EBs generated from CM differentiation experiments at day 4 were dissociated with 0.125% trypsin (Gibco) and washed with 1× PBS. The dissociated cells were stained with 1 μL of CD140a (Pdgfrα)-APC antibody (130-109-784, Miltenyi Biotec.) and 1 μL of CD309 (Flk1)-PE antibody (130-102-559, Miltenyi Biotec.) for 30 min at room temperature. Then, the cells were washed with 1×PBS and analyzed by BD FACSVerse and FlowJo software.

Biotin-RNA pull-down and LC-MS

Biotin-labeled *Cpmer* was obtained using RNA Labeling Mix (11685597910, Roche) with T7 (10881767001, Roche) or T3 RNA polymerase (11031171001, Roche). 3 μg of total RNA was heated to 90°C for 2 min, held on ice for 2 min, and incubated in RNA structure buffer (10 mM Tris-HCl pH 7.0, 100 mM KCl, and 10 mM $MgCl_2$) to allow the formation of the proper secondary structure. Cells at EB-D4 were lysed with RIP lysis buffer for 30 min on ice. IP was performed using streptavidin beads coated with biotin-labeled sense *Cpmer* or antisense *Cpmer*. The RNA-binding proteins were analyzed by LC-MS as described previously (Shevchenko et al., 2006). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2022)partner repository with the dataset identifier PXD031814.

RIP assay

RIP was performed as previously described (Ng et al., 2012). Briefly, 5×10^6 cells were lysed with RIP buffer (100 mM KCl, 5 mM MgCl2, 10 mM HEPES pH 7.0, 0.5% NP-40, and 1 mM dithiothrectol) for 30 min on ice to facilitate lysis. Three micrograms of anti-Flag (GNI4110, GNI) antibody was used to bind *Cpmer* or its fragments after Flag-eEF1A2 was ectopically expressed in 293T cells. Coimmunoprecipitated RNAs were extracted and analyzed by qRT-PCR. The enrichment was calculated relative to the percentage of input.

MS2bp-YFP RNA pull-down

Briefly, cells were cotransfected with 4 μg of MS2bp-YFP overexpression plasmid, 4 μg of pMS2-*Cpmer* (or its fragments) or control vector with Renilla luciferase inserts (pMS2-Renilla) mixed with 24 μL Lipofectamine 3000 (Invitrogen). After 48 h, the cells were lysed with RIP buffer on ice for 30 min. The proteins were immunoprecipitated using control IgG (CST) or anti-GFP (ab290, Abcam). The RNA-protein complex was treated with RNAiso to purify RNA or SDS lysis buffer for western blotting.

Polysome profile analysis

Cells were treated with 100 μg/mL cycloheximide (CHX) (S7418, Selleck) and incubated for 10 min at 37°C before harvest. The cells were washed twice with ice-cold 1×PBS supplemented with 100 μg/mL CHX. After centrifugation for 5 min at 1000 rpm, the cells were lysed with 1 mL polysome extraction buffer (20 mM Tris-HCl pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, and 0.5% NP-40) to which 100 μg/mL CHX, protease inhibitors, RNase inhibitor were added before use. The samples were incubated on ice for 10 min and centrifuged at 13,000 rpm for 10 min. The supernatant was loaded onto the top of a 10-50% sucrose gradient in a 13.2-mL tube (331372, Beckman Coulter) and centrifuged for 90 min in an SW41Ti swinging bucket rotor (3331336, Beckman Coulter) at 39,000 rpm at 4°C with maximum acceleration and braking. The gradient was pumped out and fractions were collected into 12 1.5-mL tubes, and RNA was extracted from each tube. The polysome profile was analyzed by qRT-PCR. Eomes and T mRNA distributions across the polysome profile are presented as percentages. For the analysis of co-sedimenting proteins, trichloro-acetic acid (TCA) was added to each fraction (final 10% v/v). Then proteins were precipitated overnight at 4°C and centrifuged at 13,000 rpm at 4°C for 20 min. Pellets were washed with ice cold acetone, then dissolved in 100 μL 2 × SDS loading buffer and heated at 95°C for 10 min.

RNA FISH

Cells were seeded on slides, washed once with 1×PBS, fixed with 4% paraformaldehyde at room temperature for 10 min, and then permeabilized with 0.2% Triton X-100 for 5 min. The cells were washed twice with 1×PBS and incubated with prehybridization buffer for 30 min at 37°C. The FISH

probe was added to the hybridization buffer at a dilution of 1:1000, and the cells were incubated in a humidified chamber in the dark at 37°C for 14 h. After hybridization, slides were washed 3 times with Wash Buffer I (4×SSC with 0.1% Tween-20), once each with Wash Buffer II (2×SSC) and Wash Buffer III (1×SSC) at 42°C for 5 min in the dark, and once with 1×PBS at room temperature. Then, the cells were stained with Hoechst 33342 for 10 min in the dark. Images were captured with a confocal microscope (Nikon). *Cpmer*-as-cy3 and *Cpmer*-s-cy3 probes were designed and synthesized by RiboBio Co., Ltd. Mouse U6 probes (LNC110103, RiboBio) and 18S probes (LNC110104, RibiBio) were used as the nuclear and cytoplasmic controls, respectively.

mRNA stability

Cells were treated with actinomycin D (S8964, Selleck) at a concentration of 100 μg/mL for different time intervals (0, 0.5, 1, 2, and 4 h). The cells were harvested, and RNA was extracted and analyzed by qRT-PCR. The remaining RNA levels of interest at each time point were normalized to those at the beginning.

Quantitative real-time PCR

Total RNA was extracted with RNAiso Reagent (TaKaRa) according to the manufacturer`s instructions. cDNA was synthesized with the PrimeScript RT reagent Kit (TaKaRa) by reverse transcribing 500 ng of total RNA. The resultant cDNA was diluted in double-distilled water, and 5 ng was used in each reaction. Quantitative real-time PCR was performed on an Mx3000 instrument (Agilent) using SYBR® Premix Ex Taq (TaKaRa, RR420A) and gene-specific primers. Samples were run in biological triplicate. Gene expression was normalized to the housekeeping gene GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicate and repeated 3 times. The primer sequences are listed in Table S5.

Immunofluorescence staining

Cells were washed once with 1×PBS and fixed with 4% paraformaldehyde at room temperature for 20 min. Then, the cells were permeabilized with 0.2% Triton X-100 for 8 min. The cells were washed twice with 1×PBS, blocked in 10% FBS for 1 h, and incubated overnight with primary antibodies at the appropriate dilution at 4°C. Then, the cells were washed twice and incubated with AlexaFluor-488- or AlexaFluor-594-conjugated secondary antibody (Invitrogen) for 2 h at 4°C and counterstained with Hoechst 33342. Finally, the cells were examined under a fluorescence microscope to capture fluorescent images. Details of antibodies are listed in Table S6.

Western blotting

Cells were collected and washed in 1×PBS and incubated in 1×SDS lysis buffer supplemented with 1×Protease Inhibitor Cocktail (Roche) for 10 min on ice. The cells were centrifuged at 12,000 rpm for 15 min at 4°C, and equal amounts of cell lysates were resolved by 10% Bis-Tris SDS-PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Nonspecific binding was blocked by incubating in 3% BSA in TBST at 4°C for 2 h. Then, the blots were incubated with various primary antibodies in TBST at 4°C overnight. After washing with TBST 3 times, the blots were incubated with the appropriate secondary antibody for 2 h at 4°C. Signals were visualized by adding SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) in enhanced chemiluminescence (ECL, ImageQuant LAS 4000 mini). GAPDH was used as the loading control. The anti-eEF1A antibody was unable to distinguish eEF1A1 and eEF1A2 protein. Details of antibodies are listed in Table S6.

References:

Ng, S.Y., Johnson, R., and Stanton, L.W. (2012). Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. EMBO J *31*, 522-533.

Perez-Riverol, Y., Bai, J., Bandla, C., Garcia-Seisdedos, D., Hewapathirana, S., Kamatchinathan, S., Kundu, D.J., Prakash, A., Frericks-Zipper, A., Eisenacher, M., et al. (2022). The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. Nucleic Acids Res *50*, D543-D552.

Shevchenko, A., Tomas, H., Havlis, J., Olsen, J.V., and Mann, M. (2006). In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc *1*, 2856-2860.

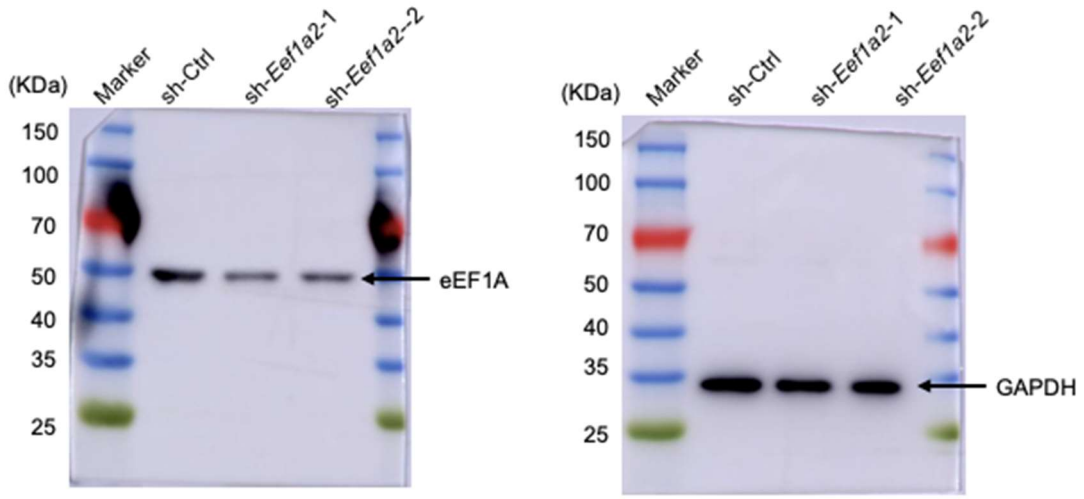
Table S4. Primers used for vector construction.			
Primer Name	**Sequence**		**Product Size**
Cpmer-KO-gRNA-1	CACCGAGCCTCTTGTGACTCGGGAC	S	N/A
	AAACGTCCCGAGTCACAAGAGGCTC	AS	
Cpmer-KO-gRNA-2	CACCGAGTGGGCCTGGTAGCTTACC	S	N/A
	AAACGGTAAGCTACCAGGCCCACTC	AS	
Rosa26-Cpmer-BglII	GGAAGATCTAGAGCCCTTTGTCGACTCTTT	F	586 bp
Rosa26-Cpmer-MfeI	CCGCAATTGCTTTTGTTTTTTGTTTCATGTG	R	
pBSK-Cpmer-EcoRI	CCGGAATTCAGAGCCCTTTGTCGACTCTTT	F	590 bp
pBSK-Cpmer-NotI	TTTATAGCGGCCGCTTTTGTTTTTTGTTTCATGT	R	
m-*Eef1a2*-sh1	CCGGGTCGGGTTCAATGTGAAGAATCTCGAGATTCTTCACATTGAACCCGACTTTTTG	S	N/A
	AATTCAAAAAGTCGGGTTCAATGTGAAGAATCTCGAGATTCTTCACATTGAACCCGAC	AS	
m-*Eef1a2*-sh2	CCGGGAGCGTAAGGAAGGAAATGCACTCGAGTGCATTTCCTTCCTTACGCTCTTTTTG	S	N/A
	AATTCAAAAAGAGCGTAAGGAAGGAAATGCACTCGAGTGCATTTCCTTCCTTACGCTC	AS	
Cpmer-sh1	CCGGGGATGATTCCAGACAGCAACCCTCGAGGGTTGCTGTCTGGAATCATCCTTTTTG	S	N/A
	AATTCAAAAAGGATGATTCCAGACAGCAACCCTCGAGGGTTGCTGTCTGGAATCATCC	AS	
Cpmer-sh2	CCGGGCAACCTCAAGGCTCTACATGCTCGAGCATGTAGAGCCTTGAGGTTGCTTTTTG	S	N/A
	AATTCAAAAAGCAACCTCAAGGCTCTACATGCTCGAGCATGTAGAGCCTTGAGGTTGC	AS	
h-CPMER-sh1	CCGGGTGATGTCTGCCACGAATATACTCGAGTATATTCGTGGCAGACATCACTTTTTG	S	N/A
	AATTCAAAAAGTGATGTCTGCCACGAATATACTCGAGTATATTCGTGGCAGACATCAC	AS	
h-CPMER-sh2	CCGGACCTGACCCCAGCGCTTAATTCTCGAGAATTAAGCGCTGGGGTCAGGTTTTTTG	S	N/A
	AATTCAAAAAACCTGACCCCAGCGCTTAATTCTCGAGAATTAAGCGCTGGGGTCAGGT	AS	
h-*EEF1A2*-sh1	CCGGGCTGGAAGGTGGAGCGTAAGGCTCGAGCCTTACGCTCCACCTTCCAGCTTTTTG	S	N/A
	AATTCAAAAAGCTGGAAGGTGGAGCGTAAGGCTCGAGCCTTACGCTCCACCTTCCAGC	AS	
h-*EEF1A2*-sh2	CCGGGCAGGACGTGTACAAGATTGGCTCGAGCCAATCTTGTACACGTCCTGCTTTTTG	S	N/A
	AATTCAAAAAGCAGGACGTGTACAAGATTGGCTCGAGCCAATCTTGTACACGTCCTGC	AS	

Fugw-Flag-*Eef1a2*-BamHI	CGCGGATCCATGGATTACAAGGATGACGACGATAAGGGCAAGGAGAAGACACACATCA	F	1434 bp
Fugw-*Eef1a2*-EcoRI	CCGGAATTCTCACTTGCCCGCTTTCTGAGCCTTCT	R	
Cpmer-AgeI	AAATATACCGGTAGAGCCCTTTGTCGACTC	F	588 bp
Cpmer-EcoRI	CCGGAATTCTTTTGTTTTTTGTTTCATGTG	R	
Cpmer-Exon2-AgeI	AAATATACCGGTGTCGAGGAGTCACCAGATGTGA	F	177 bp
Cpmer-Exon2-EcoRI	CCGGAATTCCTGTGGAGTAGGCTGACAAC	R	
Cpmer-Exon3-AgeI	AAATATACCGGTTGCTAGTGGGCCTGGTAGCT	F	316 bp (RP: Cpmer-EcoRI)
pMS2-Cpmer-HindIII-F	CCGGAAGCTTAGAGCCCTTTGTCGACTCTTTGGCT	F	587 bp
pMS2-Cpmer-EcoRI-R	CCGGGAATTCTTTTGTTTTTTGTTTCATGTGTGTGGCATG	R	
pMS2-*Renilla*-HindIII-F	CCGGAAGCTTATGACTTCGAAAGTTTATGATCCAG	F	956 bp
pMS2-*Renilla*-EcoRI-R	CCGGGAATTCTTATTGTTCATTTTTGAGAACTC	R	
Fugw-ms-*Eomes*-BamHI	GGCCGGATCCATGCAGTTGGGAGAGCAGCTCC	F	2267 bp
Fugw-ms-*Eomes*-EcoRI	CCGGGAATTCCTAGGGACTTGTGTAAAAAGCAT	R	
5` RACE primer	CAGTGGAAGATCAGTGAG	F	N/A
3` RACE primer	GGCTGAGATAACCAAGTC	R	
Fugw-Myc-Eomes-FL-5'	GGCCGGATCCATGGAGCAGAAACTCATCTCTGAAGAGGATCTGCAGTTGGGAGAGCAGCTCC	F	2118 bp
Fugw-Myc-Eomes-FL-3'	CCGGGAATTCCTAGGGACTTGTGTAAAAAGCAT	R	
Fugw-Myc-Eomes-⊿BS-R	CGGGGAGGAGGCTGACGCTCGAGTAGAAGTGCGCGCCGGG	R	125 bp (FP: Fugw-Myc-Eomes-FL-5')
Fugw-Myc-Eomes-⊿BS-F	CCGGCGCGCACTTCTACTCGAGCGTCAGCCTCCTCCCCGG	F	1974 bp (RP: Fugw-Myc-Eomes-FL-3')
Fugw-Myc-Eomes-⊿ELS-R	CTGCCCGGAAACTTCTTGGACCCTCCGCCTCCCCCTCCTCCTCCGCCTCCTCC	R	153 bp (FP: Fugw-Myc-Eomes-FL-5')
Fugw-Myc-Eomes-⊿ELS-F	GGAGGAGGAGGGGGAGGCGGAGGGTCCAAGAAGTTTCCGGGCAGTCTC	F	1938 bp (RP: Fugw-Myc-Eomes-FL-3')
Fugw-h-EOMES-BamHI	CCGGACCGGTATGCAGTTAGGGGAGCAGCTCT	F	2261 bp
Fugw-h-EOMES-EcoRI	CCGGGAATTCTTAGGGAGTTGTGTAAAAAGCATAA	R	
Fugw-Flag-h-*EEF1A2*-AgeI	CCGGACCGGTATGGATTACAAGGATGACGACGATAAGGGCAAGGAGAAGACCCAC	F	1436 bp
Fugw-h-*EEF1A2*-EcoRI	CCGGGAATTCTCACTTGCCCGCCTTCTGCG	R	
Abbreviation: RP: Reverse Primer; FP: Forward Primer			

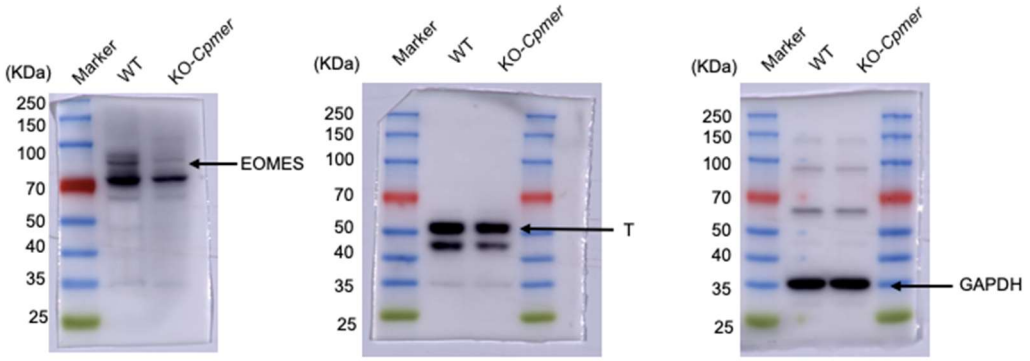
Table S5. Primers used in RT-qPCR.				
Gene Name	**Forward Sequence**	**Reverse Sequence**	**Species**	**Product Size**
Gapdh	GTGTTCCTAACCCCAATGTGTC	ATTGTCATACCAGGAAATGAGCTT	mouse/ human	248 bp
Nkx2.5	ACATTTTACCCGGGAGCCTA	CGCTCCAGCTCGTAGACC	mouse	194 bp
cTnT	CGTGGAGAAGGACCTGAATG	CCCGCTCATTGCGAATAC	mouse	152 bp
Eomes	CCCTATGGCTCAAATTCCAC	CCAGAACCACTTCCACGAAA	mouse	141 bp
T	GCTCTAAGGAACCACCGGTCATC	ATGGGACTGCAGCATGGACAG	mouse	111 bp
Cpmer	GTCGAGGAGTCACCAGATGT	GGAGTAGGCTGACAACCATGA	mouse	152 bp
Eef1a2	ACTCCACGGAACCAGCCTA	GGGCAGGATTGTGTCCAGG	mouse	245 bp
Rpsa	TGCGGGAACCCACTTAGGT	AGGATTCTCGATGGCAACAATAG	mouse	154 bp
Rpl3	GGAAAGTGAAGAGCTTCCCTAAG	CTGTCAACTTCCCGGACGA	mouse	106 bp
Eef1a1	CAACATCGTCGTAATCGGACA	GTCTAAGACCCAGGCGTACTT	mouse	160 bp
Eif2s	CACCGCTGTTGACAGTCAGAG	GCAAACAATGTCCCATCCTTACT	mouse	142 bp
Tbx5	ATGGCCGATACAGATGAGGG	TTCGTGGAACTTCAGCCACAG	mouse	207 bp
Myh6	GACGCCCAGATGGCTGACTT	GGTCAGCATGGCCATGTCCT	mouse	276 bp
Tbx20	AAACCCCTGGAACAATTTGTGG	CATCTCTTCGCTGGGGATGAT	mouse	171 bp
Acta1	CCCAAAGCTAACCGGGAGAAG	CCAGAATCCAACACGATGCC	mouse	134 bp
Cpmer-E1	GCCCTTTGTCGACTCTTTGG	CCAACAGAGACTGACCCTCCT	mouse	103 bp
Cpmer-E3	AGCTCTGGGTTGGACTGAGA	TTTCATGTGTGTGGCATGTG	mouse	191 bp
Col1a1	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG	mouse	103 bp
Col3a1	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC	mouse	144 bp
Ckap4	TCCCGTCAGAGGGATGAGC	GCTGGGAGTTTCTCAGGAGG	mouse	109 bp
CPMER	CCAAATGTGAAGGTGTAA	GCCAATCTTAATCTGACA	human	94 bp
CTNT	ACAGAGCGGAAAAGTGGGAAG	TCGTTGATCCTGTTTCGGAGA	human	230 bp
TBX20	TCGTCCCTGTGGACAACAAG	TTCAGGTTGAGCAATGAGGCT	human	286 bp
MYH6	CGGTGCTTTTCAACCTCAAGG	GGACTGGTTCTCCCGATCTGT	human	221 bp
ACTA1	GGCATTCACGAGACCACCTAC	CGACATGACGTTGTTGGCATAC	human	84 bp
EEF1A2	TCGTGGGCGTGAACAAAATG	GCTGACTTCCTTGACGATCTC	human	80 bp
EOMES	CTGCCCACTACAATGTGTTCG	GCGCCTTTGTTATTGGTGAGTTT	human	211 bp
T	TATGAGCCTCGAATCCACATAGT	CCTCGTTCTGATAAGCAGTCAC	human	109 bp

Table S6. Antibodies used in the study.						
Antibodies	**Company**	**Catalog**	**Species**	**Dilution WB**	**Dilution IF**	**Dilution FACS**
Anti-cTnT	Abcam	ab8295	Mouse	N/A	1:1000	N/A
Anti-Flk1	Miltenyi Biotec	130-102-559	Mouse	N/A	N/A	1:250
Anti-Pdgfrα	Miltenyi Biotec	130-109-784	Mouse	N/A	N/A	1:250
Anti-eEF1A	Santa Cruz	sc-377439	Mouse	1:500	N/A	N/A
Anti-GAPDH	Bioworld	AP0063	Rabbit	1:3000	N/A	N/A
Anti-EOMES	Abcam	ab23345	Rabbit	1:2000	1:1000	N/A
Anti-T	Abcam	ab209665	Rabbit	1:2000	1:1000	N/A
Anti-GFP	Abcam	ab290	Rabbit	N/A	1:1000	N/A
Anti-Myc tag	Abcam	ab9132	Mouse	1:3000	N/A	N/A
Anti-Sox2	CST	#23064	Rabbit	1:1000	N/A	N/A
Anti-Oct4	Abcam	ab19857	Rabbit	1:2000	N/A	N/A
Anti-Klf4	Santa Cruz	sc-20691	Rabbit	1:2000	N/A	N/A
Anti-mouse IgG, HRP	CST	7076s	N/A	1:3000	N/A	N/A
Anti-Rabbit IgG, HRP	CST	7074s	N/A	1:3000	N/A	N/A

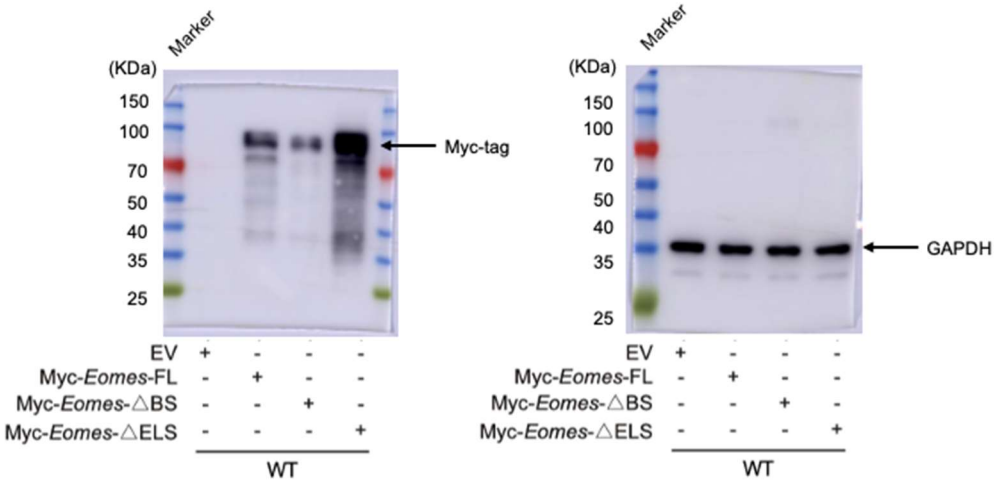
Supplementary Data



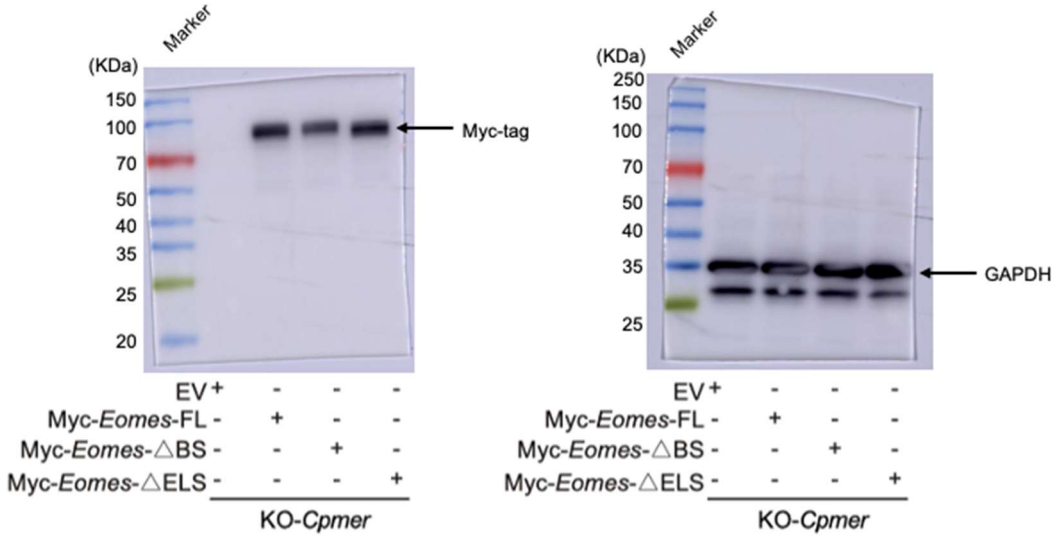
Whole membrane image 1. The efficiency of *Eef1a2* knockdown at mesoderm stage. Related to Figure 3F.



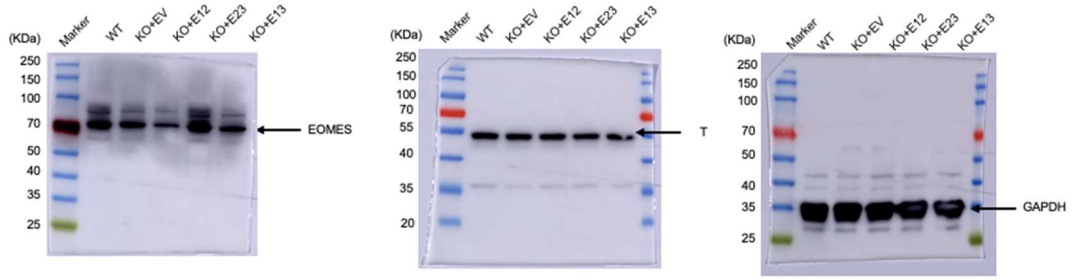
Whole membrane image 2. The EOMES and T protein levels at mesoderm stage in the WT and KO-*Cpmer* cells. Related to Figure 4B.



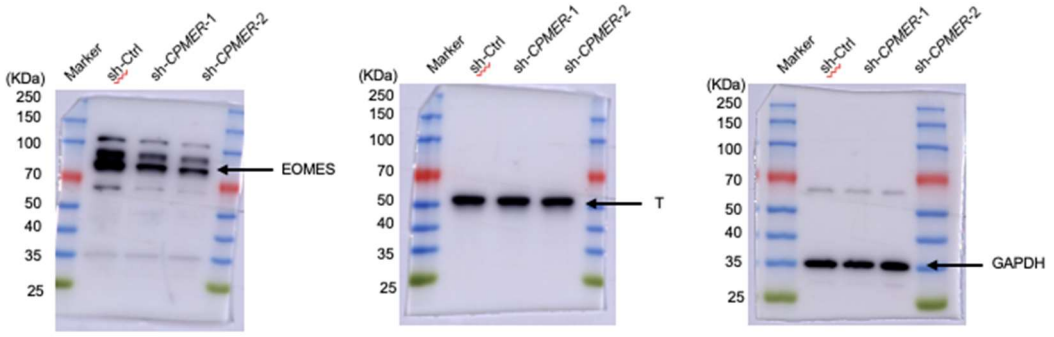
Whole membrane image 3. The exogenous Eomes expression in the WT cells transfected with full-length (FL) or deletion mutant *Eomes* at mesoderm stage. Related to Figure 4G.



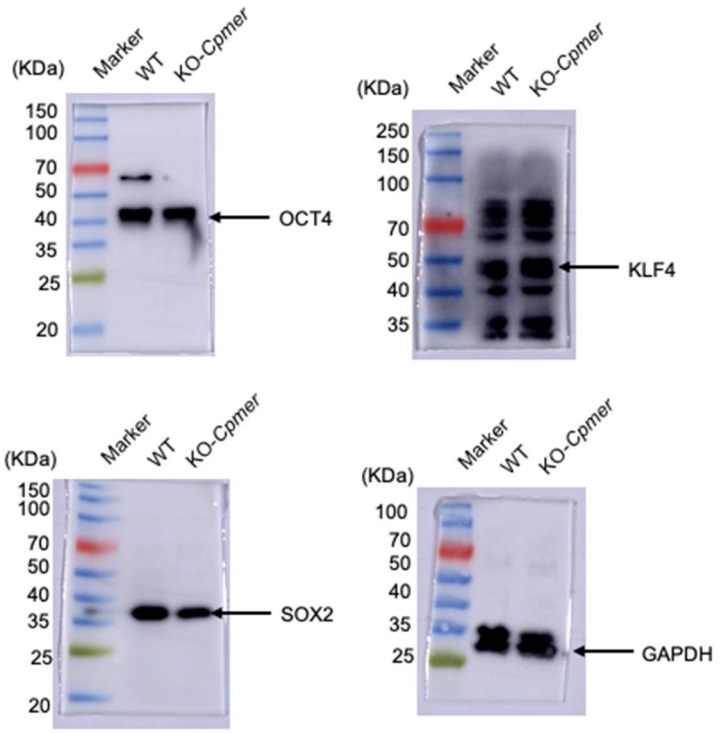
Whole membrane image 4. The exogenous Eomes expression in the KO-*Cpmer* cells transfected with FL or deletion mutant *Eomes* at mesoderm stage. Related to Figure 4H.



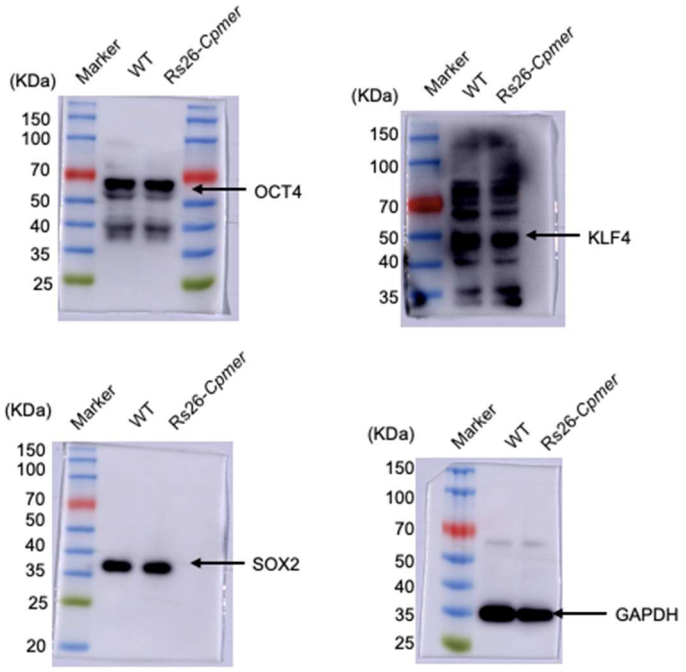
Whole membrane image 5. The EOMES and T protein levels in the WT and KO-Cpmer cells transfected with the double-exon mutants of *Cpmer* at mesoderm stage. Related to Figure 5E.



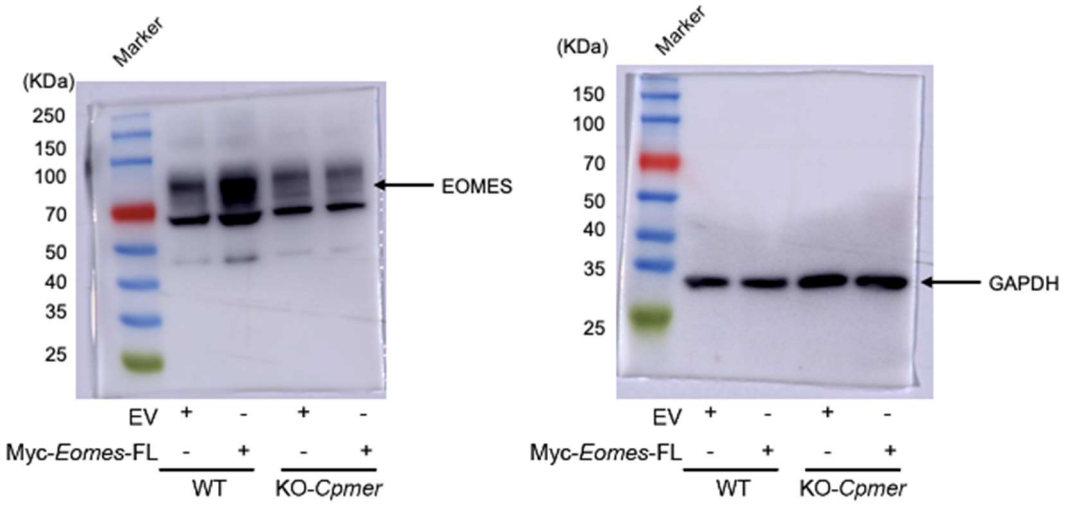
Whole membrane image 6. The EOMES and T protein levels in *CPMER*-knockdown cells at mesoderm stage. Related to Figure 6C.



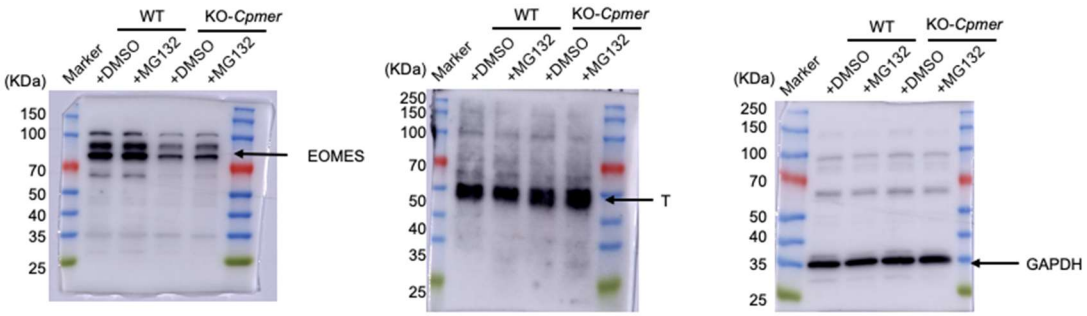
Whole membrane image 7. The expression level of pluripotency markers in the WT and KO-*Cpmer* cells. Related to Figure S2C.



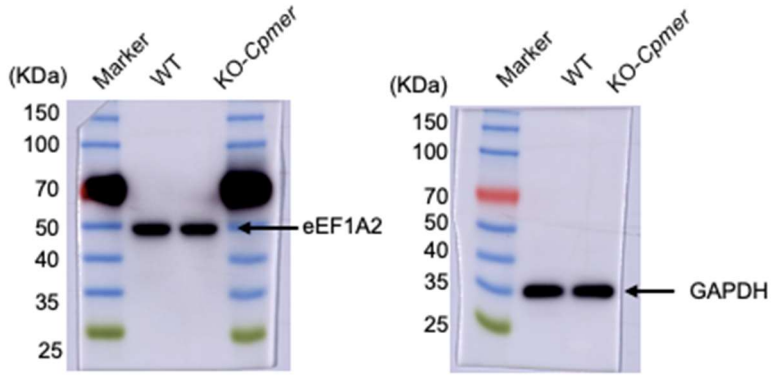
Whole membrane image 8. The expression level of pluripotency markers in the WT and Rs26-*Cpmer* cells. Related to Figure S2I.



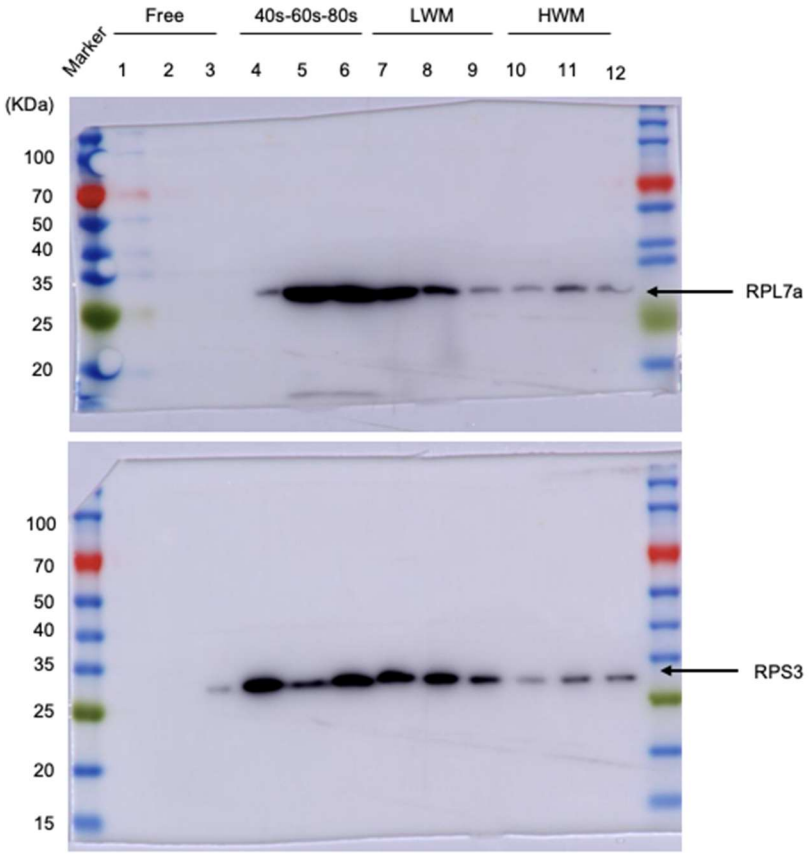
Whole membrane image 9. The EOMES levels in the WT and KO-Cpmer cells transfected with Myc-*Eomes*-FL at mesoderm stage. Related to Figure S4A.



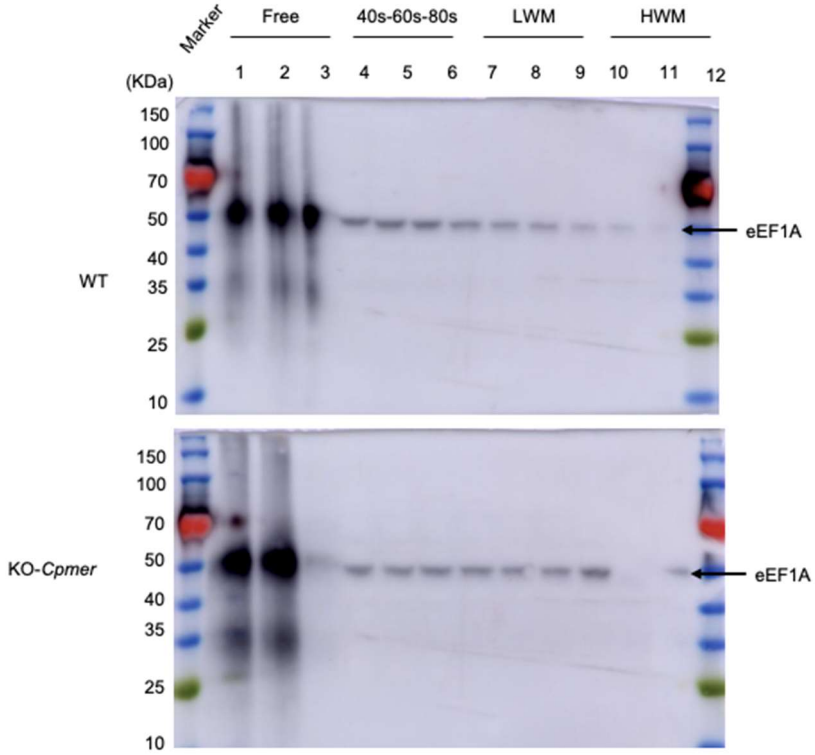
Whole membrane image 10. The EOMES and T levels in the WT and KO-*Cpmer* cells treated with MG132 at mesoderm stage. Related to Figure S4C.



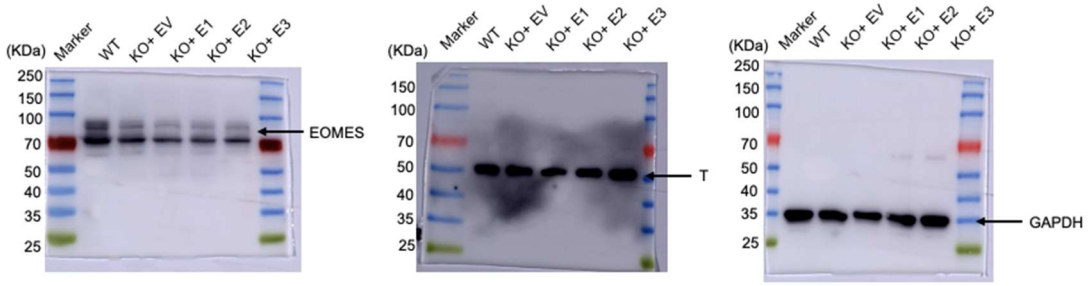
Whole membrane image 11. The eEF1A levels in the WT and KO-*Cpmer* cells. Related to Figure S4F.



Whole membrane image 12. Western blotting analysis of polysome profile with RPL7a and RPS3 at mesoderm stage. Related to Figure S4G.



Whole membrane image 13. The eEF1A distribution in polysome fractions of the WT and KO-*Cpmer* cells at mesoderm stage. Related to Figure S4H.



Whole membrane image 14. The EOMES and T expression levels in the KO-Cpmer cells transfected with different single-exon mutants of *Cpmer* at mesoderm stage. Related to Figure S5E.