Functional and Proteomic Investigations Reveal Major Royal Jelly Protein 1 Associated with Anti-hypertension Activity in Mouse Vascular Smooth Muscle Cells

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Supplemental Figures

Fig.S1 EGFP expression shows the successful lentiviral transduction for mouse VSMCs. (a). is the mode chart of lentiviral vector harboring *mrjp1* gene. **(b).** is the EGFP expression observed in both control and MRJP1 expressing VSMCs after lentiviral transduction.

Fig.S2 Primers for PCR amplification and real time RT-PCR analysis. (a). shows the forword and revered primers for PCR amplification of *mrjp1*. **(b).** displays the forword and revered primers for real time RT-PCR analysis of *mrjp1* RNA.

Fig.S3 Immunofluenscent analysis shows reduced αSMA expression by MRJP1 in VSMCs. Immunofluenscent analysis of α SMA expression between control and MRJP1 expressed VSMCs. The expression level is shown as α SMA /DAPI from three independent experiments (n=3, mean ±S.D.) and * indicates $p < 0.05$.

Fig.S4 Differentially expressed proteins visulized in the enriched pathway of RNA transport. Green labeled boxes represent the proteins annotated in the significantly enriched pathway of RNA transport. Highlighted pink boxes indicate the differentially expressed proteins mapped into the pathway.

Fig.S5 Differentially expressed proteins visulized in the enriched pathway of Splicesome. Proteins in green labeled boxes are the proteins invovled in the significantly enriched pathway of Splicesome. Proteins in highlighted pink boxes are the differentially expressed ones mapped into the pathway.

Fig.S6 Differentially expressed proteins visulized in the enriched pathway of Citrate cycle. Green labeled boxes represent the protein references annotated in the significantly enriched pathway of Citrate cycle. The highlighted pink boxes are the entries of differentially expressed proteins mapped into the pathway.

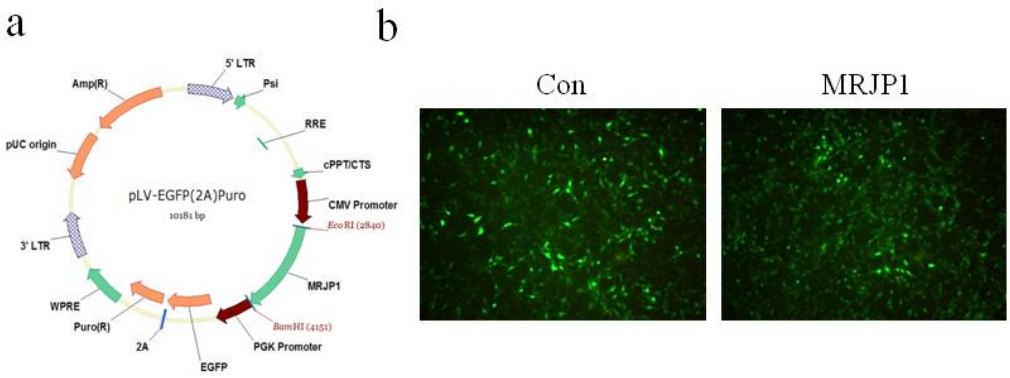

Fig.S1

a

Primers for PCR amplification	MRJP1-F: ATGACAAGATTGTTTATGCTGG
	MRJP1-R: TTACAAATGGATTGAAATTTTG

b

Primers for Real time RT-PCR analysis	MRJP1-F: GTCCTGTAGCTTCCACCAGT
	MRJP1-R: ATCCGAAGAAGAGAACGCCA
	GFP F1: TATGGAGTGCAATGCTTCGC
	GFP R1: TACTCCAGCTTGTGTCCCAG

Fig.S2

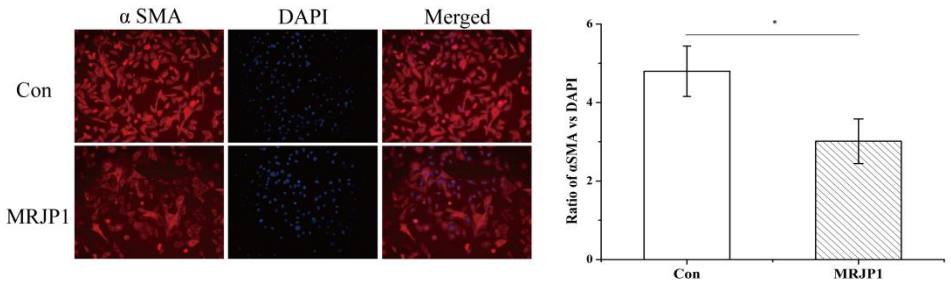

Fig.S3

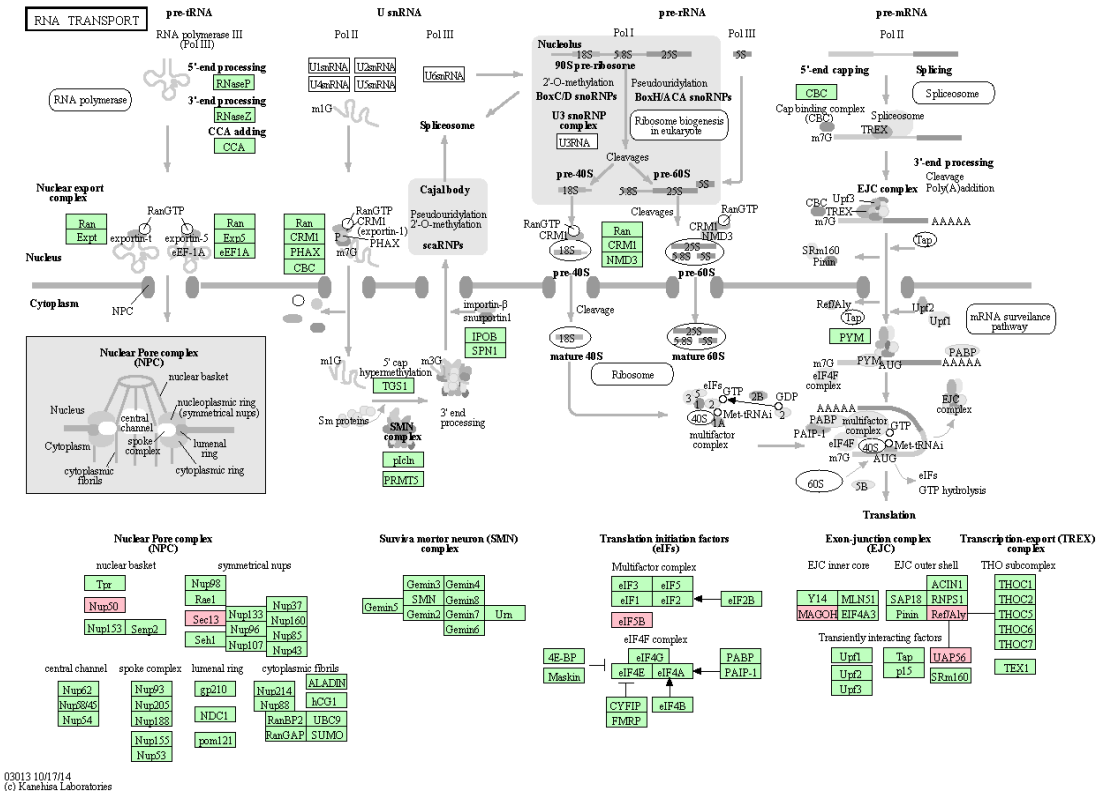
RNA TRANSPORT
pre-tRNA
RNA polymerase III (Pol III)
5'-end processing
RNaseP
3'-end processing
RNaseZ
CCA adding
CCA
RNA polymerase
U snRNA
Pol II
Pol III
U1snRNA
U2snRNA
U4snRNA
U5snRNA
U6snRNA
m1G
Spliceosome
Cajal body
Pseudouridylation
2'-O-methylation
scaRNPs
pre-rRNA
Pol I
Pol III
Nucleolus
18S
5.8S
25S
5S
90S pre-ribosome
2'-O-methylation
BoxC/D snoRNPs
Pseudouridylation
BoxH/ACA snoRNPs
U3 snoRNP complex
U3RNA
Ribosome biogenesis in eukaryote
Cleavages
pre-40S
pre-60S
pre-mRNA
Pol II
5'-end capping
CBC
Cap binding complex (CBC)
m7G
Splicing
Spliceosome
TREX
3'-end processing
Cleavage
Poly(A)addition
EJC complex
CBC
Upf3
TREX
m7G
AAAAA
Tap
SRm160
Pinin
Nuclear export complex
Ran
Expt
exportin-t
RanGTP
exportin-5
eEF-1A
Ran
Exp5
eEF1A
Nucleus
Cytoplasm
NPC
Ran
CRM1
PHAX
CBC
RanGTP
CRM1 (exportin-1)
PHAX
m7G
Pseudouridylation
2'-O-methylation
scaRNPs
RanGTP
CRM1
18S
pre-40S
Ran
CRM1
NMD3
RanGTP
CRM1
NMD3
28S
5.8S
5S
pre-60S
importin-β
enuportin1
IPOB
SPN1
Cleavage
18S
mature 40S
28S
5.8S
5S
mature 60S
Ribosome
RefIAly
Tap
Upf2
Upf1
mRNA surveilance pathway
PYM
PABP
AAAAA
m7G
eIF4F complex
PYM
AUG
eIFs
GTP
3
2
2B
GDP
40S
1A
Met-tRNAi
2
multifactor complex
AAAAA
PABP
multifactor complex
PAIP-1
eIF4F
40S
AUG
Met-tRNAi
m7G
60S
5B
eIFs
GTP hydrolysis
Translation
Nuclear Pore complex (NPC)
nuclear basket
Nucleus
central channel
spoke complex
lumenal ring
cytoplasmic fibrils
cytoplasmic ring
nucleoplasmic ring (symmetrical nups)
Cytoplasm
m1G
5' cap hypermethylation
TGS1
m3G
3' end processing
Sm proteins
SMN complex
pICln
PRMT5
m1G
Nuclear Pore complex (NPC)
nuclear basket
Tpr
Nup50
Nup153
Senp2
symmetrical nups
Nup98
Rae1
Sec13
Seh1
Nup133
Nup96
Nup107
Nup37
Nup160
Nup85
Nup43
central channel
Nup62
Nup58/45
Nup54
spoke complex
Nup93
Nup205
Nup188
Nup155
Nup53
lumenal ring
gp210
NDC1
pom121
cytoplasmic fibrils
Nup214
Nup88
RanBP2
RanGAP
ALADIN
hCG1
UBC9
SUMO
Survival motor neuron (SMN) complex
Gemin3
Gemin4
Gemin5
SMN
Gemin8
Gemin2
Gemin7
Unr
Gemin6
Translation initiation factors (eIFs)
Multifactor complex
eIF3
eIF5
eIF1
eIF2
eIF2B
eIF5B
eIF4F complex
4E-BP
Maskin
eIF4G
eIF4E
eIF4A
PABP
PAIP-1
CYFIP
FMRP
eIF4B
Exon-junction complex (EJC)
EJC inner core
Y14
MAGOH
MLN51
EIF4A3
EJC outer shell
SAP18
Pinin
ACIN1
RNPS1
RefIAly
Transiently interacting factors
Upf1
Upf2
Upf3
Tap
p15
UAP56
SRm160
Transcription-export (TREX) complex
THO subcomplex
THOC1
THOC2
THOC5
THOC6
THOC7
TEX1
03013 10/17/14
(c) Kanehisa Laboratories

Fig.S4

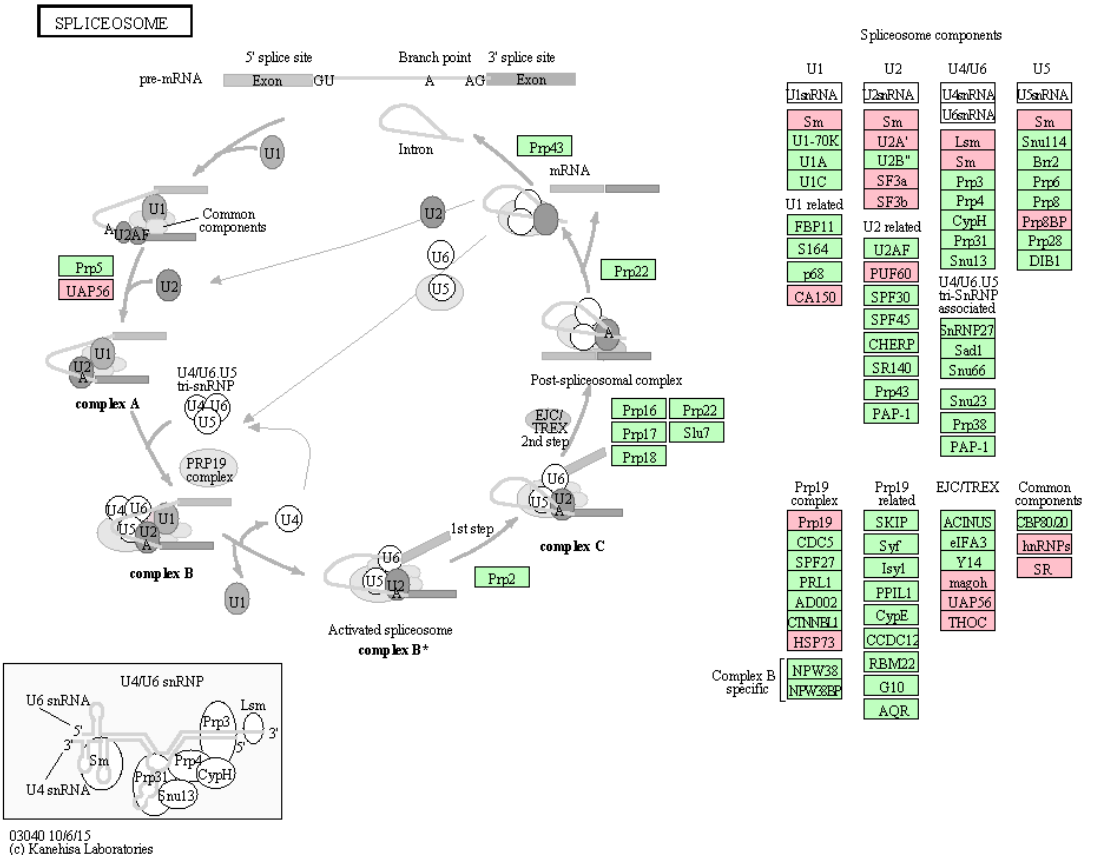
SPLICEOSOME
5' splice site
Branch point
3' splice site
pre-mRNA
Exon
GU
A
AG
Exon
Intron
mRNA
Prp43
U1
U2
U6
U5
A
Common components
U2AF
Prp5
UAP56
Prp22
complex A
U4/U6.U5 tri-snRNP
Post-spliceosomal complex
Prp16
Prp22
Prp17
Slu7
Prp18
EJC/TREX 2nd step
PRP19 complex
U4
1st step
complex B
complex C
Prp2
Activated spliceosome
complex B*
U4/U6 snRNP
U6 snRNA
U4 snRNA
Lsm
Prp3
Sm
Prp31
Prp4
CypH
Snu13
Spliceosome components
U1
U1snRNA
Sm
U1-70K
U1A
U1C
U1 related
FBP11
S164
p68
CA150
U2
U2snRNA
Sm
U2A'
U2B"
SF3a
SF3b
U2 related
U2AF
PUF60
SPF30
SPF45
CHERP
SR140
Prp43
PAP-1
U4/U6
U4snRNA
U6snRNA
Lsm
Sm
Prp3
Prp4
CypH
Prp31
Snu13
U4/U6.U5 tri-snRNP associated
SnRNP27
Sad1
Snu66
Snu23
Prp38
PAP-1
U5
U5snRNA
Sm
Snu114
Brr2
Prp6
Prp8
Prp8BP
Prp28
DIB1
Prp19 complex
Prp19
CDC5
SPF27
PRL1
AD002
CTNNBL1
HSP73
Complex B specific
NPW38
NPW38BP
Prp19 related
SKIP
Syf
Isy1
PPIL1
CypE
CCDC12
RBM22
G10
AQR
EJC/TREX
ACINUS
eIFA3
Y14
magoh
UAP56
THOC
Common components
CBP80/20
hnRNPs
SR
03040 10/6/15
(c) Kanehisa Laboratories

Fig.S5

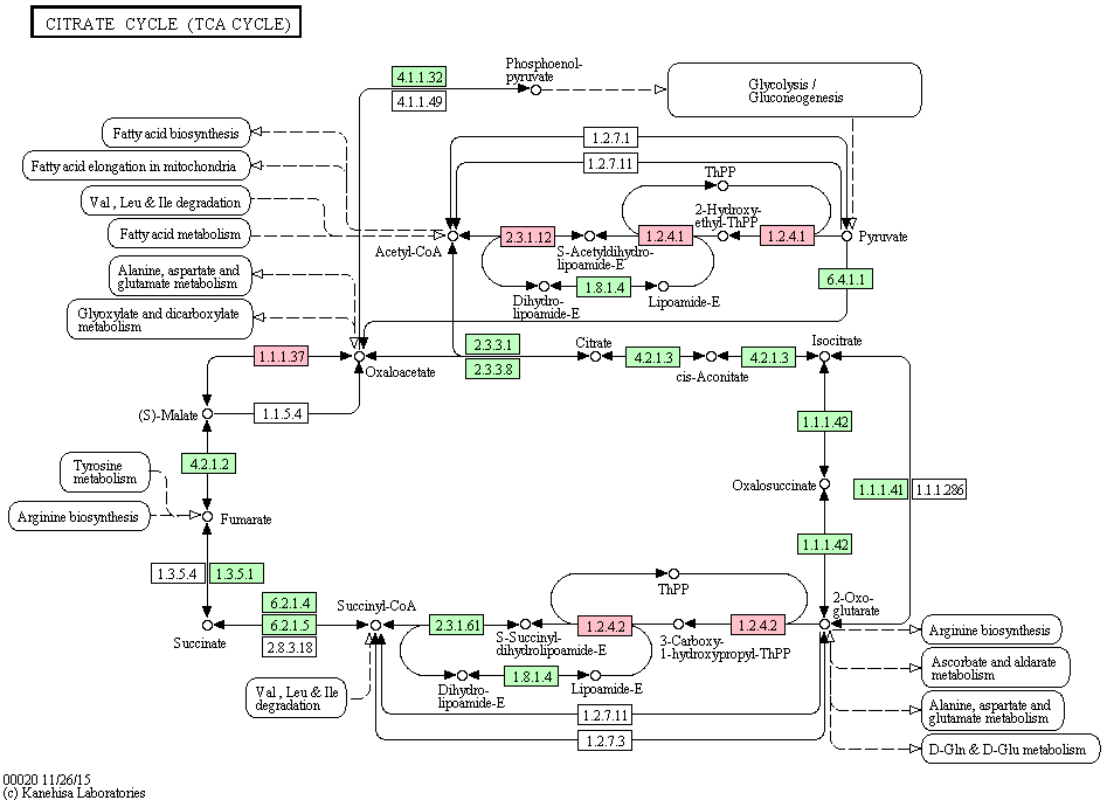
CITRATE CYCLE (TCA CYCLE)
4.1.1.32
4.1.1.49
Phosphoenol-pyruvate
Glycolysis / Gluconeogenesis
Fatty acid biosynthesis
Fatty acid elongation in mitochondria
Val, Leu & Ile degradation
Fatty acid metabolism
Alanine, aspartate and glutamate metabolism
Glyoxylate and dicarboxylate metabolism
1.2.7.1
1.2.7.11
ThPP
2-Hydroxy-ethyl-ThPP
2.3.1.12
1.2.4.1
1.2.4.1
Pyruvate
Acetyl-CoA
S-Acetyldihydro-lipoamide-E
1.8.1.4
Dihydro-lipoamide-E
Lipoamide-E
6.4.1.1
1.1.1.37
2.3.3.1
2.3.3.8
Oxaloacetate
Citrate
4.2.1.3
4.2.1.3
cis-Aconitate
Isocitrate
(S)-Malate
1.1.5.4
1.1.1.42
Tyrosine metabolism
4.2.1.2
Arginine biosynthesis
Fumarate
Oxalosuccinate
1.1.1.41
1.1.1.286
1.1.1.42
1.3.5.4
1.3.5.1
ThPP
6.2.1.4
6.2.1.5
2.8.3.18
Succinyl-CoA
2.3.1.61
1.2.4.2
1.2.4.2
2-Oxo-glutarate
Succinate
S-Succinyl-dihydrolipoamide-E
3-Carboxy-1-hydroxypropyl-ThPP
Arginine biosynthesis
Ascorbate and aldarate metabolism
Alanine, aspartate and glutamate metabolism
D-Gln & D-Glu metabolism
Val, Leu & Ile degradation
1.8.1.4
Dihydro-lipoamide-E
Lipoamide-E
1.2.7.11
1.2.7.3
00020 11/26/15
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Fig.S6