Supplemental data for

Title: Stabilization of RNT-1, the RUNX homolog of *Caenorhabditis elegans*, by oxidative stress through a MAP kinase pathway

Authors: Kiho Lee[1], Jiwon Shim[1], Jaebum Bae[2], Young-Joon Kim[2] and Junho Lee[1] *

[1]From Research Center for Cellulomics, Department of Biological Sciences, WCU Department of Biophysics and Chemical Biology, Seoul National University, Seoul, 151-742, South Korea

[2]Department of Biochemistry, College of Life Science and Technology, Yonsei University, Seoul 120-749, South Korea

Supplemental Table 1. Primers used in this study

Purpose	Name	Sequence
To detect mRNA of *rnt-1* by *in situ* hybridization	rnt-1 in situ -1	TTTGACAATCGTTATACATTTGGCG CCGATGATGGTGGCC
To detect mRNA of *rnt-1* by *in situ* hybridization	rnt-1 in situ -2	GGCCACCATCATCGGCGCCGAATG TATAACGATTGTCAA
To detect mRNA level of *rnt-1* by qPCR	rnt-1 Q -1	GACCAAGGGATGCGAGAATA
To detect mRNA level of *rnt-1* by qPCR	rnt-1 Q -2	GAAGGATGTGGTCCTGGAGA
To clone *pmk-1* into L4440 vector for RNA*i*	pmk-1 C -1	AACCATGGATGTTTCCACAGACAA CAATG
To clone *pmk-1* into L4440 vector for RNA*i*	pmk-1 C -2	AAAGTCGACCTACGATTCCATTTTC TCCT
To clone *pmk-1* into pcDNA3.1 for expressing in HEK293T cell line	pmk-1 flag-1	AGGATCCATGTTTCCACAGACAAC AATGGA
To clone *pmk-1* into pcDNA3.1 for expressing in HEK293T cell line	pmk-1 flag-2	AAACTCGAGCGATTCCATTTTCTCC TCATC
To clone *sek-1* into pcDNA3.1 for expressing in HEK293T cell line	sek-1 ha-1	AGGATCCATGGAGCGAAAAGGACG TGAG
To clone *sek-1* into pcDNA3.1 for expressing in HEK293T cell line	sek-1 ha-2	AAACTCGAGTCGTCGCCAAACAGT GTCG
To clone *sek-1* into pcDNA3.1 for expressing in HEK293T cell line	rnt-flag-1	ATGACCAACGTCTTCCATCAC
To clone *sek-1* into pcDNA3.1 for expressing in HEK293T cell line	rnt-flag-2	AAAAGGCCTCCAAATAGTCGG
To express GST-fused *rnt-1* in *E. coli*	rnt-S200-1	AGGATCCATGACCAACGTCTTCCAT CAC
To express GST-fused *rnt-1* in *E. coli*	rnt-S200-2	AAACTCGAGAGAAGGATGTGGTCC TGGAGA
To express GST-fused *rnt-1*	rnt-S250-1	AGGATCCATCTCAGCTGCACTTTGG

in *E. coli*		AAA
To express GST-fused *rnt-1* in *E. coli*	rnt-S250-2	AAACTCGAGTAACTTCGGATCATC AGAAGT
To express GST-fused *rnt-1* in *E. coli*	rnt-S301-1	AGGATCCAAACGACCATCTTCTCCT CGT
To express GST-fused *rnt-1* in *E. coli*	rnt-S301-2	AAACTCGAGAAAAGGCCTCCAAAT AGTCGG
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 T215A-1	CAC AGC GAA TCG ATG AAA GCT CCG ATT AAA CAA AAA GTT GAA CAG
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 T215A-1	CTG TTC AAC TTT TTG TTT AAT CGG AGC TTT CAT CGA TTC GCT GTG
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 T236A-1	GTA TCC CTT AAT ACG TCT GCA TGC CTA TCA TCT
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 T236A-2	AGA TGA TAG GCA TGC AGA CGT ATT AAG GGA TAC
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 S242A -1	TGC CTA TCA TCT CCA GCA ATT TTT ATA ACT CCA ACT TCT GAT
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 S242A-2	ATC AGA AGT TGG AGT TAT AAA AAT TGC TGG AGA TGA TAG GCA
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 S255A -1	AAA CGA CCA TCT GCT CCT CGT TCA ATC ACA AAA TCA
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 S255A-2	TGA TTT TGT GAT TGA ACG AGG AGC AGA TGG TCG TTT
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 T273A -1	TTA ATT CAA GAA GCT CCG GAA TCT GTA GAA TCA AAA AGA CGA
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 T273A-2	TCG TCT TTT TGA TTC TAC AGA TTC CGG AGC TTC TTG AAT TAA
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 S294A -1	ACT TCA TCG AAT AGT TCT GCT CCG ACT ATT TGG AGG
To mutate RNT-1 threonine serine 215 to alanine	RNT-1 S294A-2	CCT CCA AAT AGT CGG AGC AGA ACT ATT CGA TGA AGT
To detect DNA level of *vhp-1* promoter region A	vhp-1 A Q -1	GGGGATCATTCCATTTTCCT
To detect DNA level of *vhp-1* promoter region A	vhp-1 A Q -2	CCTGTTTGCATCCAGGATCT
To detect DNA level of *vhp-1* promoter region B	vhp-1 B Q -1	TTGCCGTATTTTCTCTCATTCA
To detect DNA level of *vhp-1* promoter region B	vhp-1 B Q -2	GAAGACCTCGGCCATCTGTA
To detect DNA level of *vhp-1* promoter region C	vhp-1 C Q -1	TAATCCTGGGTTCCCATCCT
To detect DNA level of *vhp-1* promoter region C	vhp-1 C Q -2	GGATTTGCTTCTCGGAAGTG
To detect DNA level of *vhp-1* promoter region D	vhp-1 D Q -1	ACCTACCTGCCTGCCTACCT
To detect DNA level of *vhp-1* promoter region D	vhp-1 D Q -2	AACCACTTACGTGCCTACGG
To detect mRNA level of *vhp*-1 by qPCR	vhp-1 Q -1	CTTCGATCTCGCCAAACTTC
To detect mRNA level of *vhp*-1 by qPCR	vhp-1 Q -2	TGGATGATGCACTTTTTGGA

Supplemental Table 2. A list of candidate genes from ChiP-seq experiments

Gene	Description	RNT-1 binding sequence	References
vhp-1	MAP kinase phosphatase	-9737, -9731	Mizuno, T. *et al.*(1)
tir-1	ortholog of the human TIR domain-containing protein SARM	-5979, -5967	Liberati, N.T. *et al.*(2)
nas-15	astacin-like metalloprotease	-1195, -1066	Mohrlen, F., Hutter, H. & Zwilling, R. (3)
nhr-274	nuclear hormone receptor family	-4547, -4439	Okkema, P.(4)
clec-151	C-type LECtin	-3921, -3914	Wormbase
tag-260	Predicted E3 ubiquitin ligase	-2567, -2301	Wormbase
eat-20	Fibrillins and related proteins containing Ca2+-binding EGF-like domains	-4698, -4475	Shibata *et al.*, (5)
ell-1	RNA polymerase II elongation factor	-581, -291	Wormbase
mvk-1	Mevalonate kinase	504, 597	Wormbase(Kuwabara PE, O'Neil N)

Supplemental references

1. Mizuno, T., Hisamoto, N., Terada, T., Kondo, T., Adachi, M., Nishida, E., Kim, D. H., Ausubel, F. M., and Matsumoto, K. (2004) *The EMBO journal* **23**, 2226-2234
2. Liberati, N. T., Fitzgerald, K. A., Kim, D. H., Feinbaum, R., Golenbock, D. T., and Ausubel, F. M. (2004) *Proceedings of the National Academy of Sciences of the United States of America* **101**, 6593-6598
3. Mohrlen, F., Hutter, H., and Zwilling, R. (2003) *European journal of biochemistry / FEBS* **270**, 4909-4920
4. Okkema, P. (2005) *WormBook*
5. Shibata, Y., Fujii, T., Dent, J. A., Fujisawa, H., and Takagi, S. (2000) *Genetics* **154**, 635-646

Supplemental figures

Supplemental figure 1. Stabilization of RNT-1 in worms after the MG132 treatment. The photo shows the anti-GFP western blot data using the N2; Ex[GFP::RNT-1] worm lysate. Same amounts of the worm lysates were used as confirmed by the actin western analysis.

Supplemental figure 2. Transcription level of *rnt-1* in the oxidative stress condition. The mRNA level of *rnt-1* was detected by quantitative PCR at each time point with 0.1 M paraquat treatment.

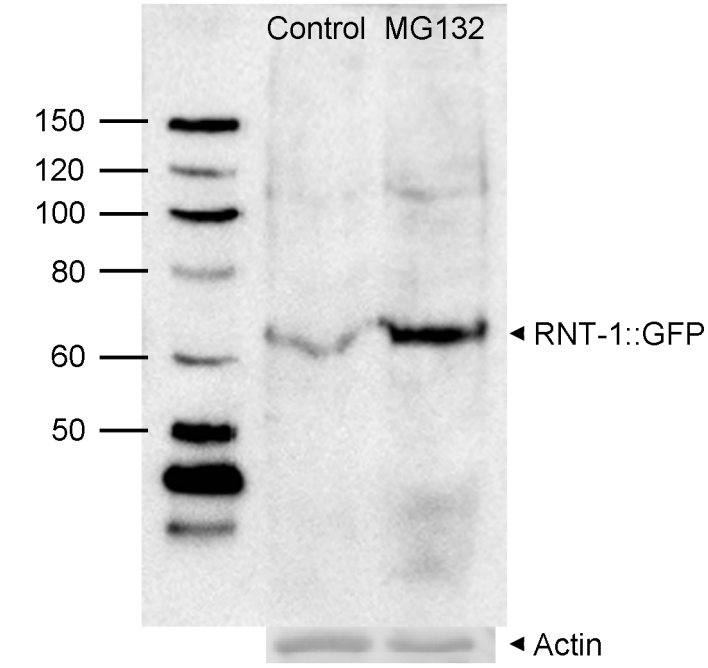
Supplemental figure 3. *rnt-1* mutants alleles show defects in oxidative stress response. The defects were rescued by RNT-1 expression under its own promoter or under an intestine-specific promoter, *act-5*, in the *rnt-1*(ok351) background.

Supplemental figure 4. Partial involvement of *kgb-1* and *mek-1* in the stabilization of RNT-1. RNA*i* of *kgb-1* and *mek-1* partially decreased the stabilization of RNT-1.

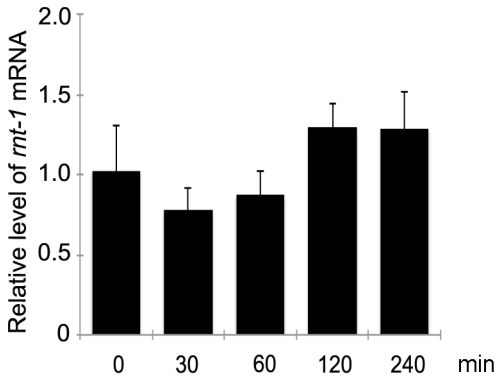
Supplemental figure 5. (A) The amino acid sequence of RNT-1. The red underline represents the conserved MAPK target sequence, Pro-X-Ser/Thr-Pro in RNT-1. The blue underlines represent the minimal MAPK target sequences in 201 to 250 amino acids of RNT-1. (B) Kinase assay of 201 to 250 amino acids of RNT-1 and mutations of putative MAPK target sequences in 201 to 250 amino acids of RNT-1.

Supplemental figure 6. Additional survival experiments of *vhp-1* RNAi in wild type N2 and *rnt-1*(tm491) animals in the 0.1 M paraquat background.

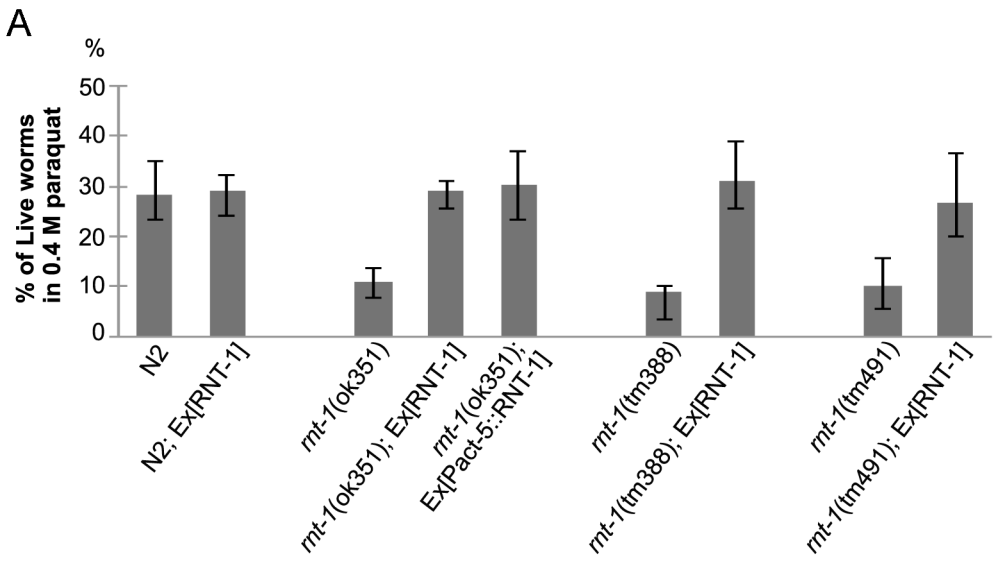
Lee *et al.*, Supplemental Figure 1



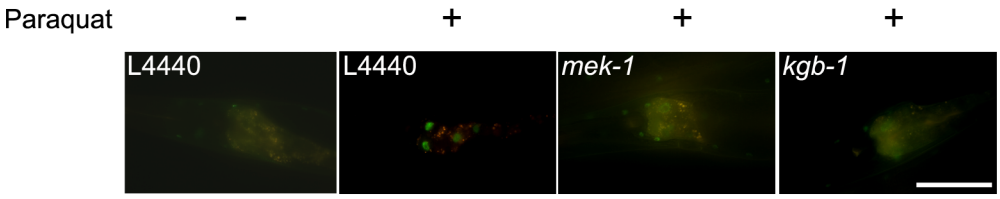
Lee *et al.*, Supplemental Figure 2



Lee *et al.*, Supplemental Figure 3

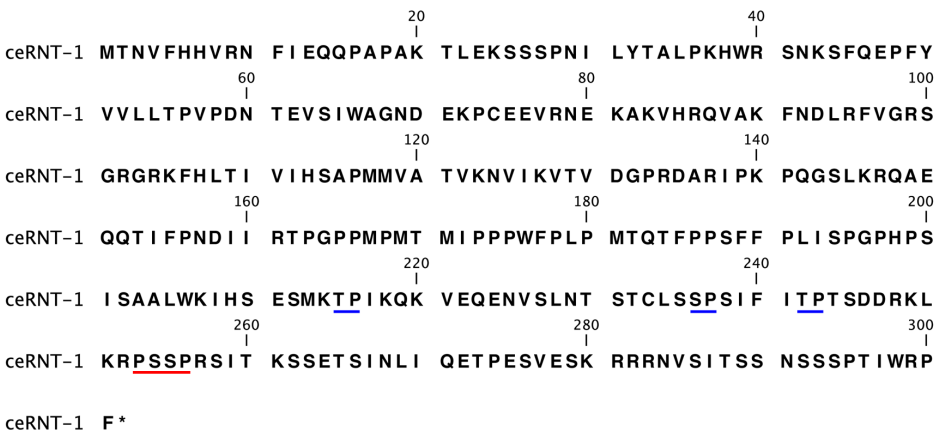


Lee *et al.*, Supplemental Figure 4

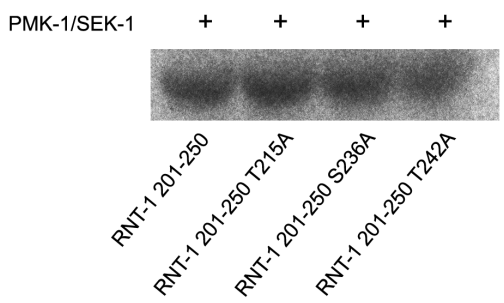


Lee *et al.*, Supplemental Figure 5

A



B



Lee *et al.*, Supplemental Figure 6