Supplementary information

Title: High glucose-mediated PICALM and mTORC1 modulate processing of amyloid precursor protein via endosomal abnormalities

Running title: Effect of high glucose on APP-processing endosome

Chang Woo Chae[1], Hyun Jik Lee[4,5], Gee Euhn Choi[1], Young Hyun Jung[1], Jun Sung Kim[1], Jae Ryong Lim[1], Seo Yihl Kim[1], In Koo Hwang[2,3], Je Kyung Seong[2,3] and Ho Jae Han[1,*]

[1]Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Program for Creative Veterinary Science Research, Seoul National University, Seoul 08826, Republic of Korea.

[2]BK21 PLUS Program for Creative Veterinary Science Research, and Research Institute for Veterinary Science;
Seoul National University and Korea Mouse Phenotyping Center (KMPC), Seoul, Korea.

[3]Department of Anatomy and Cell Biology, College of Veterinary Medicine, and Research Institute for Veterinary Science, Seoul National University, Seoul 08826, South Korea.

[4]Laboratory of Veterinary Physiology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 28644, South Korea.

[5]Institute for Stem Cell & Regenerative Medicine (ISCRM), Chungbuk National University, Cheongju, 28644, Chungbuk, Korea.

[*]Corresponding author: Ho Jae Han, D.V. M, Ph.D
Professor of Department of Veterinary Physiology
College of Veterinary Medicine, Seoul National University, Seoul 08826, Republic of Korea
E-mail address: hjhan@snu.ac.kr
Tel / Fax: +82-2-880-1261 / +82-2-880-2732

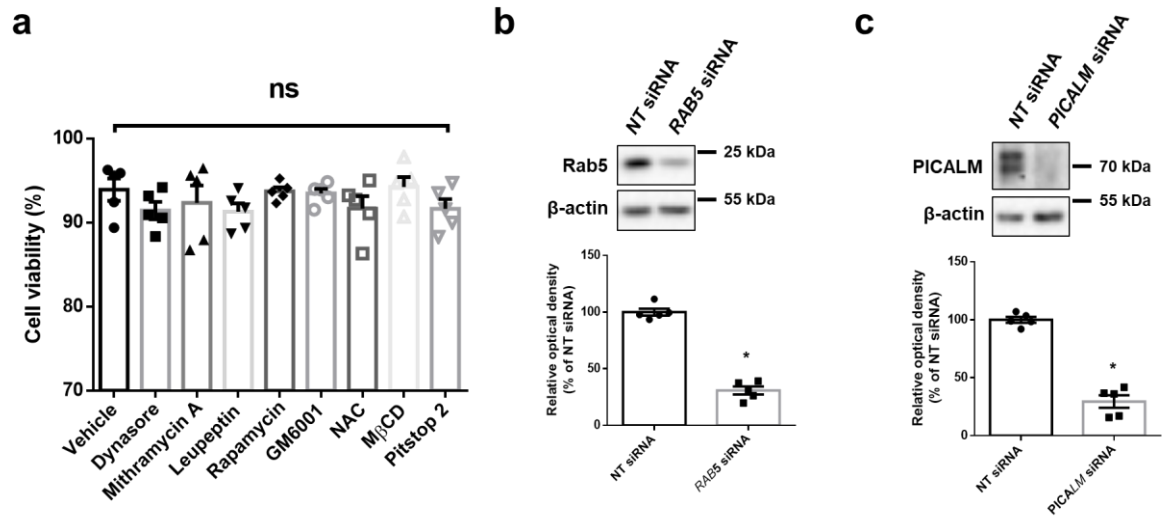

Figure S1. The efficacy of siRNAs and toxic effects of drugs used in experiments. a The cells were treated with vehicle or drugs, Dynasore (25 μM), Mithramycin A (25 nM), Leupeptin (100 nM), Rapamycin (200 nM), GM6001 (20 μM), NAC (4 mM), MβCD (1 mM), and Pitstop 2 (30 μM), respectively for 24 h. Cell viability was measured by trypan blue exclusion cell viability assay. $n = 5$ from independent experiments. **b** The SK-N-MCs were transfected with NT siRNA or *RAB5* siRNA for 12 h and incubated in medium for 24 h. Rab5 were detected by western blot. $n = 5$ from independent experiments. **c** The cells were transfected with NT siRNA or *PICALM* siRNA for 12 h and incubated in medium for 24 h. PICALM were detected by western blot. $n = 5$ from independent experiments. $^*p < 0.05$ vs. NT siRNA transfection. Quantitative data are presented as a mean ± S.E.M. All blots are representative.

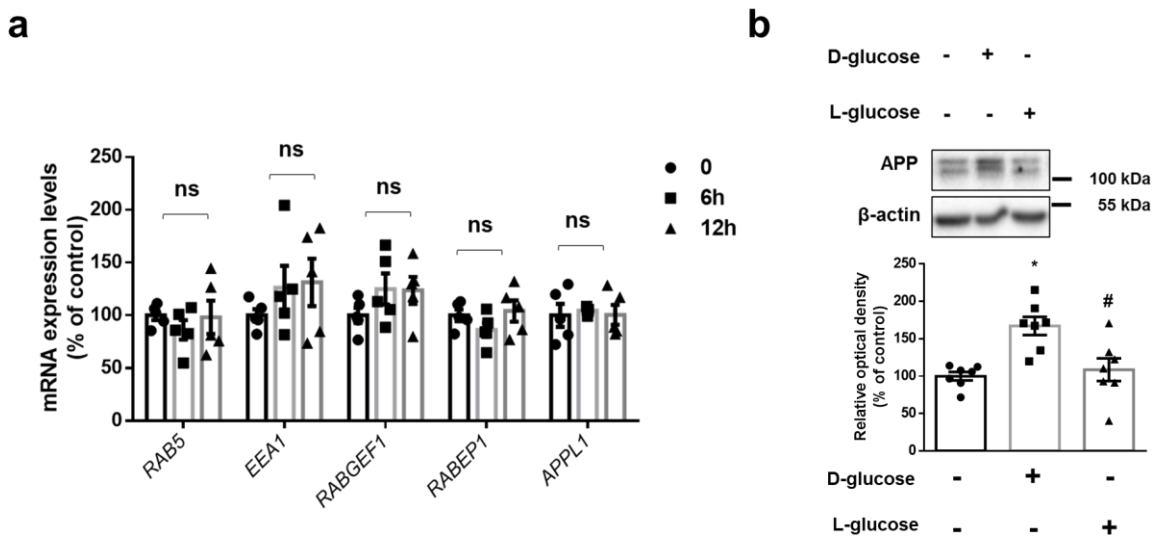

Figure S2. High glucose has no significant alterations on the mRNA expression of Rab5 and its effector proteins. a The SK-N-MCs were treated with high glucose (25 mM) in a time dependent manner. The mRNA expression levels of *RAB5*, *EEA1*, *RABGEF1*, *RABEP1*, and *APPL1* were analyzed by quantitative real-time PCR. $n = 5$ from independent experiments with 2 technical replicates each. **b** The cells were treated with D-glucose (25 mM) or L-glucose (25 mM) for 24 h. Then, APP and β-actin were detected by western blot. $n = 7$ from independent experiments. $^{*}p < 0.05$ vs. control, $^{\#}p < 0.05$ vs. D-glucose. Quantitative data are presented as a mean ± S.E.M. All blots are representative.

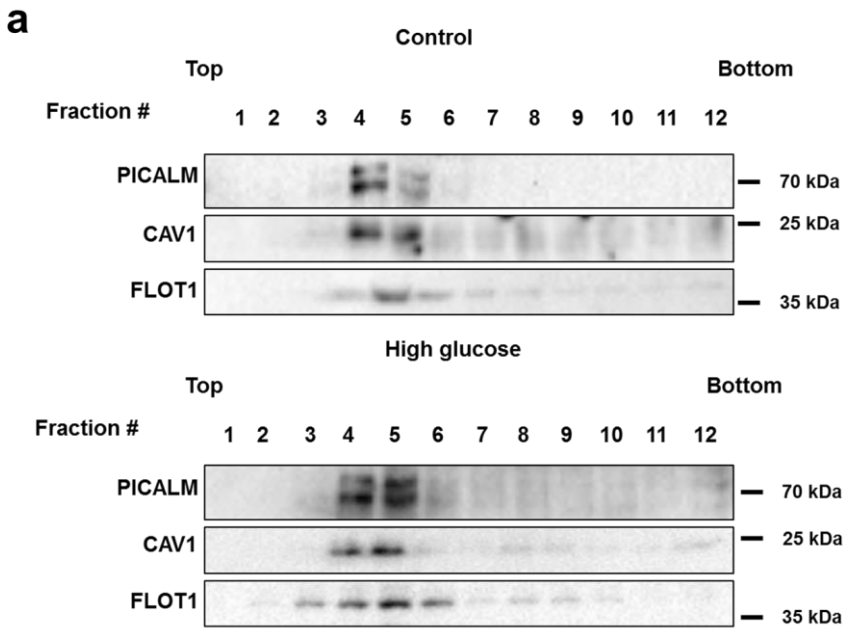

Figure S3. PICALM is located in lipid rafts under both normal- and high glucose-conditions. a High glucose (25 mM) were treated for 24 h in the cells. Sucrose gradient-fractionized lysates were subjected to western blot. PICALM, CAV1, and FLOT1 were detected. Exploratory data.

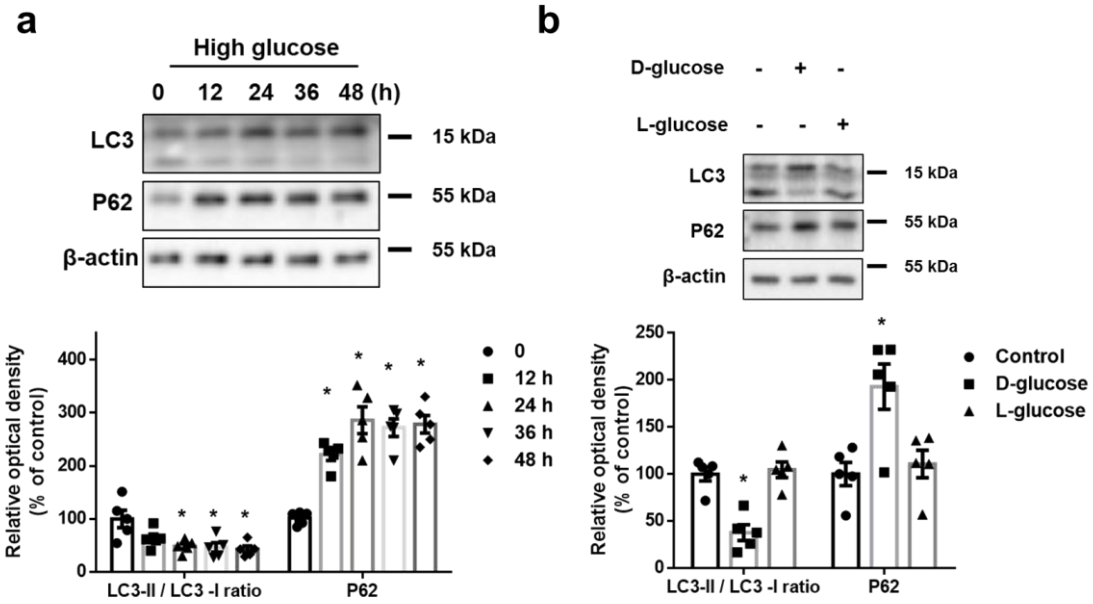

Figure S4. High glucose impairs autophagy in a time dependent manner. a The SK-N-MCs were treated with high glucose (25 mM) in a time dependent manner. LC3, P62, and β-actin were subjected to western blot. $n = 5$ from independent experiments. **b** The cells were treated with D-glucose (25 mM) or L-glucose (25 mM) for 24 h. Then, LC3, P62, and β-actin were detected by western blot. $n = 5$ from independent experiments. $^*p < 0.05$ vs. control. Quantitative data are presented as a mean ± S.E.M. All blots are representative.

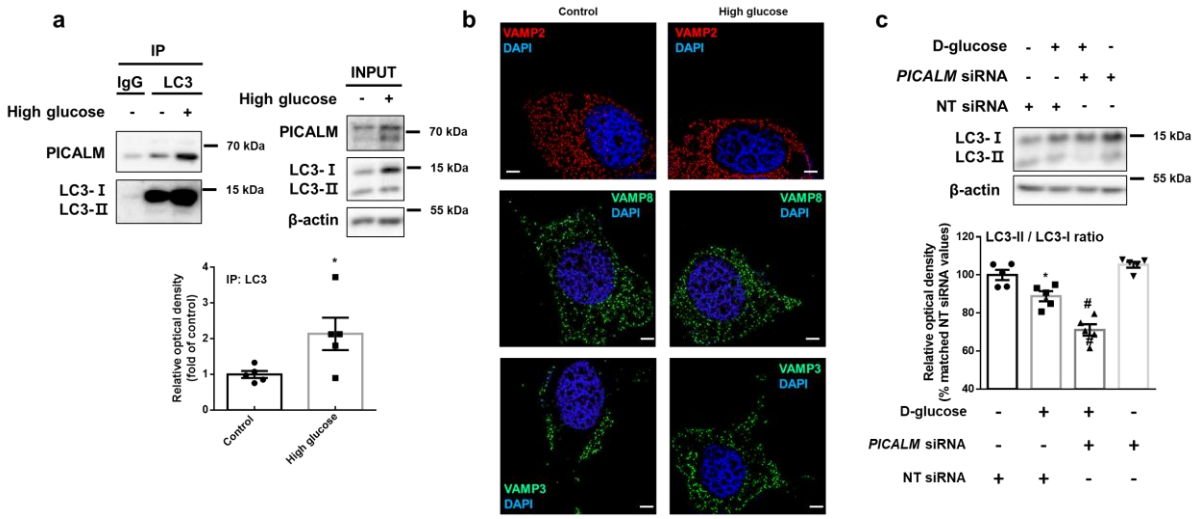

Figure S5. PICALM induces autophagy under high glucose conditions. a, b The SK-N-MCs were treated with high glucose (25 mM) for 24 h. **a** Co-immunoprecipitation of PICALM with IgG and LC3 antibodies were shown in left panel. Total protein expressions in lysate were shown in right panel. $n = 5$ from independent experiments. *$p < 0.05$ vs. Control. **b** The cells were immunostained with VAMP2 or VAMP8 or VAMP3-specific antibodies and counterstained with DAPI. Scale bars, 8 μm (magnification, ×1,000). $n = 5$ from independent experiments. Exploratory data. **c** The cells were transfected with NT siRNA or *PICALM* siRNA for 12 h prior to high glucose (25 mM) treatment for 24 h. LC3 and β-actin were detected by western blot. $n = 5$ from independent experiments. Logarithmic transformations were performed for homogeneity of the sample variance. *$p < 0.05$ vs. NT siRNA transfection, #$p < 0.05$ vs. NT siRNA transfection + high glucose. Quantitative data are presented as a mean ± S.E.M. All blots and immunofluorescence images are representative.

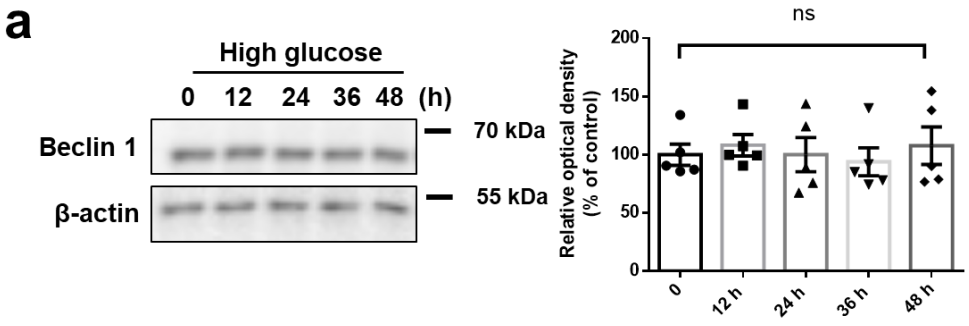

Figure S6. High glucose has no significant alterations on the protein expression of Beclin1. The SK-N-MCs were treated with high glucose (25 mM) in a time dependent manner. The Beclin1 were detected by western blot. $n = 5$ from independent experiments. Quantitative data are presented as a mean ± S.E.M. All blots are representative.

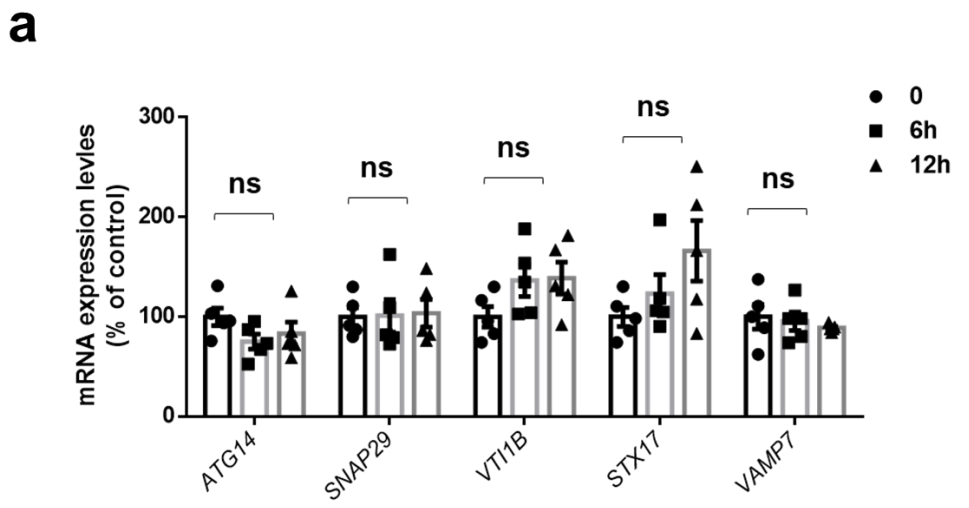

Figure S7. High glucose has no significant alterations on the mRNA expression of endo-lysosomal fusion related proteins. **a** The SK-N-MCs were treated with high glucose (25 mM) in a time dependent manner. The mRNA expression levels of *ATG14*, *SNAP29*, *VTI1B*, *STX17*, and *VAMP7* were analyzed by quantitative real-time PCR. $n = 5$ from independent experiments with 2 technical replicates each. Quantitative data are presented as a mean ± S.E.M.

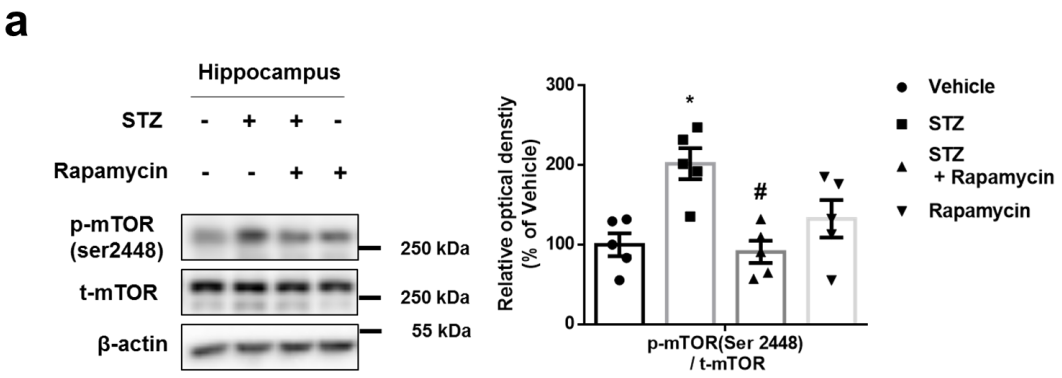

Figure S8. The treatment of rapamycin attenuated STZ-induced phosphorylation of mTORC1. a Hippocampal sample was obtained from vehicle-, STZ-, STZ + rapamycin-, or rapamycin-treated mice. Then, p-mTOR (Ser 2448), t-mTOR, and β-actin were subjected to western blot. $n = 5$ in each animal. *$p < 0.05$ vs. vehicle-treated mice, $^{\#}p < 0.05$ vs. STZ-treated mice. Quantitative data are presented as a mean ± S.E.M. All blots are representative.

Table S1. Body weight and blood glucose levels in ZLC and ZDF rats. Blood glucose and body weight were determined with a portable glucose monitor at 6 and 33 weeks of age. Data are presented as a mean ± S.E.M.

	ZLC	ZDF
Body weight (g), 6 weeks	119 ± 1.82	144.4 ± 5.22
Body weight (g), 33 weeks	394.2 ± 9.05	355.2 ± 6.77
Blood glucose (mg/dl), 6 weeks	102.8 ± 5.38	153.3 ± 15.85
Blood glucose (mg/dl), 33 weeks	124.5 ± 5.05	575.9 ± 25.53

Table S2. Body weight and blood glucose levels in experimental mice. Blood glucose and body weight were determined with a portable glucose monitor at 9 and 18 weeks of age. Data are presented as a mean ± S.E.M.

	Vehicle	Dynasore	STZ	STZ + Dynasore
Body weight (g), 9 weeks	38.5 ± 0.42	37.17 ± 0.7	37.5 ± 1.58	39 ± 0.63
Body weight (g), 18 weeks	42.33 ± 1.45	44.83 ± 0.87	34.83 ± 1.62	42.6 ± 1.20
Blood glucose (mg/dl), 9 weeks	116.3 ± 4.64	137.7 ± 7.08	120.3 ± 15.02	135.7 ± 7.24
Blood glucose (mg/dl), 18 weeks	126.7 ± 6.29	131.8 ± 4.64	497.7 ± 20.49	439.6 ± 35.16

	Vehicle	Rapamycin	STZ	STZ + rapamycin
Body weight (g), 9 weeks	37.33 ± 0.33	36.33 ± 1.08	37 ± 1.09	37.67 ± 0.55
Body weight (g), 18 weeks	44.83 ± 1.24	43.5 ± 0.92	40.67 ± 1.47	46.67 ± 1.145
Blood glucose (mg/dl), 9 weeks	126.7 ± 7.37	116.5 ± 7.85	118.2 ± 9.26	118.5 ± 5.38
Blood glucose (mg/dl), 18 weeks	127.3 ± 8.78	120.8 ± 6.39	484.7 ± 5.94	503.7 ± 10.56

Table S3. Human gene primers for real-time qPCR.

Gene symbol	Alias/Common name	Primer sequence (5'-3')
PICALM	Phosphatidylinositol Binding Clathrin Assembly Protein	F-TTC CTG TTG CCA AAC TCC CA
		R-TGG TTC CAT TTC CGA TGC CA
AP2A1	Adaptor Related Protein Complex 2 Subunit Alpha 1	F- TCC TGC TTG GCC ATG ACA TT
		R-AGT TCG AGT TCA CCA GCA CA
RAB5A	RAB5A, Member RAS Oncogene Family	F- CAT TGG GGC TGC TTT TCT AA
		R- GGA CTT GCT TGC CTC TGA AG
EEA1	Early Endosome Antigen 1	F- TGC ATC TGA AAC CTC ACT GC
		R- CTA GTT GGC GCT CTG TCT CC
RABGEF1	RAB Guanine Nucleotide Exchange Factor 1	F-AAG CCT CCG AAT CAA CCG TT
		R-TGC AGT GGT GGA GGA AGT TT
RABEP1	Rabaptin, RAB GTPase Binding Effector Protein 1	F-TCC AGA TGC CAA GTG GGT TT
		R-TGT TCT GGT GTC ATC GCC TT
APPL1	Adaptor Protein, Phosphotyrosine Interacting With PH Domain And Leucine Zipper 1	F-AGC GTT TTC CAT TGG GAG GT
		R-AGC ACT GCA TGA CAA GAG CT
ATG14	Autophagy Related 14	F-ACA ACG GAG ACA CCA GCA TT
		R-ACC AGC TGA GTT GCA TAG CA
SNAP29	Synaptosome Associated Protein 29	F-ACC CAA AGA ACC CAC ACC TT
		R-TGT CAG CCG GTC AAG AAT GT
VTI1B	Vesicle Transport Through Interaction With T-SNAREs 1B	F-TTT CGA GAA GCT GCA CGA GA
		R-TGC CAG CGT TTC ATT TGC TT
STX17	Syntaxin 17	F-AAA TGC TGC AGA ATC GTG GG
		R-AAG TCA GTG ACC AGT TGG CT
VAMP7	Vesicle Associated Membrane Protein 7	F-TGA ACG TTC CCG AGC CTT TA
		R-ATG GAA GTG CTG TCT GTG CT
ACTB	Actin Beta	F-AAC CGC GAG AAG ATG ACC CAG ATC ATG TTT
		R-AGC AGC CGT GGC CAT CTC TTG CTC GAA GTC