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3 **Title: Casein kinase 2 promotes the TGF-β-induced activation of α-tubulin**
4 **acetyltransferase 1 in fibroblasts cultured on a soft matrix**

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6 **Authors' names:** Eunae You[1§], Jangho Jeong[1§], Jieun Lee[1], Seula Keum[1], Ye Eun
7 Hwang[1], Jee-Hye Choi[1*] and Sangmyung Rhee[1*]

8

9 **Affiliation:** [1]Department of Life Science, Chung-Ang University, Seoul, 06974,
10 Republic of Korea

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12 [§]These authors contributed equally to this work.

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14 **Running Title:** Regulation of α-TAT1 activity by CK2

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19 **Corresponding Authors' Information:**
20 Sangmyung Rhee
21 Tel: +82-2-820-5818; Fax: +82-2-825-5206; E-mail:
22 sangmyung.rhee@cau.ac.kr

23

24 Jee-Hye Choi
25 Tel: +82-2-814-3997; Fax: +82-2-825-5206; E-mail: choijh97@cau.ac.kr

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## MATERIALS AND METHODS

### *Antibodies and reagents*

Primary antibodies were obtained against the following proteins: acetylated $\alpha$-tubulin (#5335), phospho-STAT3$^{Y705}$ (#9131), and phopho-Smad2$^{S465/467}$/Smad3$^{S423/425}$ (#8828; Cell signaling Technology, MA, USA); GFP (sc-9996), $\alpha$-tubulin (#sc-12462), and CK2$\alpha$ (#sc-12738; Santa Cruz Biotechnology, CA, USA). Moreover, Flag M2 antibody (#3165) was purchased from Sigma-Aldrich (MO, USA), whereas horseradish peroxidase (HRP) - conjugated goat anti-mouse (115-035-006) and HRP-conjugated goat anti-rabbit (111-035-006) antibodies were purchased from Jackson ImmunoResearch Laboratories (PA, USA). We also purchased blebbistatin (#B592500; Toronto Research Chemicals, ON, Canada), TGF-$\beta$1 (#240B; R&D systems, MN, USA), TBB (#218697; Calbiochem, CA, USA), SB202190 (#S7067; Sigma-Aldrich), SP600125 (#S5567; Sigma-Aldrich), and PD98059 (#1213, MEK inhibitor; Tocris, Bristol, UK).

### *Plasmid constructs*

Various truncated mutants of $\alpha$-TAT1 (*Mus musculus*, isoform 1) tagged with GFP or Myc were generated by polymerase chain reaction (PCR) using specific forward and reverse primers complementary to the defined sequences, using GFP-$\alpha$-TAT1 as a template. $\alpha$-TAT1$^{\Delta C}$, $\alpha$-TAT1$^{\Delta N}$, $\alpha$-TAT1$^{\Delta C1}$, $\alpha$-TAT1$^{\Delta C1-2}$, and $\alpha$-TAT1$^{\Delta C3}$ were cloned between XhoI and BamHI restriction sites and inserted into the pEGFP-C1 vector (Clontech, CA, USA). $\alpha$-TAT1$^{\Delta N}$ was cloned between BamHI and XbaI restriction sites and inserted into the pcDNA6/Myc-His A vector (Invitrogen). *Csnk2a1* (CK2$\alpha$) cDNA was amplified from XE242 mouse CK2$\alpha$ CS2p+ (#16730; Addgene, MA, USA) via PCR and cloned into the p3xFLAG-CMV™-10 expression vector (#E4401; Sigma-Aldrich). The primer sequences used for plasmid construction are listed in Supplementary Table 1. Point mutations were introduced via PCR using the *PfuUltra* High-Fidelity DNA polymerase (#600380; Agilent, CA, USA). The sequences of the primers used for site-directed mutagenesis are listed in Supplementary Table 2.

### *Cell culture*

WT and α-TAT1 KO MEFs were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Grand Island, NY, USA) supplemented with 1% GlutaMAX, 1% minimum essential medium (MEM) containing non-essential amino acids, 10% fetal bovine serum (FBS) (Youngin Frontier, Seoul, Korea), 100 unit/mL penicillin, and 100 ug/mL streptomycin (Welgene, Seoul, Korea). The cells were maintained at 37 °C and 5% carbon dioxide in a humidified incubator. HEK293T cells were obtained from American Type Culture Collection (Manassas, VA, USA). HEK293T cells were cultured in DMEM supplemented with 10% FBS.

*Generation of CK2α KO cell line using CRISPR/Cas9*

A 20-bp guide RNA (gRNA) sequence (5′-AGACATTGTAAAAGACCCTG-3′) targeting genomic DNA within exon 3 of *CK2α* was selected from the GenBank database (Accession number NC_000068; region: 152067851 - 152123772) of predicted high-specificity protospacer adjacent motif sequences in the mouse exome. gRNA was cloned into LentiCRISPR V2 (#52961; Addgene) and WT MEFs were infected with lentivirus containing the gRNA sequence with polybrene (0.8 μg/mL) for 48 h. Cells were selected using puromycin (2 μg/mL) and single-cell colonies were acquired. *CK2α* KO cell line was verified by genomic DNA sequencing and western blotting.

*Transfection*

The cells ($3 \times 10^5$) were plated on 60-mm dishes and then transfected with 2.5 μg of DNA after 24 h using Lipofectamine 2000 (#11668019; Thermo Fisher Scientific, NH, USA), according to the manufacturer's instructions. Later, the cells ($7 \times 10^5$) were plated in a 6-well plate and then transfected with 500 ng of DNA after 24 h using TransFectin™ Lipid Reagent (#1703351; Bio-Rad, CA, USA).

*Co-IP and western blotting*

For western blotting, the cells were lysed with the lysis buffer containing 1% Nonidet P-40 (NP-40), 1% sodium dodecyl sulfate, 150 mM NaCl, 6 mM $Na_2HPO_4$, 4 mM $NaH_2PO_4$, 2 mM ethylenediaminetetraacetic acid (EDTA), 50 mM NaF, 1 mM $Na_3VO_4$, 1 mM 1,4-dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride (PMSF). Protein concentrations were measured using the bicinchoninic acid assay (Thermo Fisher

Scientific). For IP, the cells were lysed with IP buffer (50 mM Tris-Cl (pH 7.4), 150 mM NaCl, 1% NP-40, 0.5% Triton X-100, 1 mM $Na_3VO_4$, 50 mM NaF, 1 mM PMSF, 10% glycerol, and 2 mM EDTA) for 1 h on ice and centrifuged at 4 ˚C and 13,000 rpm for 15 min to collect the supernatant. Protein concentrations were measured using Bradford assay (Bio-Rad, Hercules, CA, USA). The proteins (1 mg) were incubated with primary antibodies at 4 ˚C overnight. Following incubation, protein A/G beads were incubated at 4 ˚C for 2 h. Finally, the beads were washed thrice with the IP buffer, boiled, and analyzed by western blotting. Signals were developed using enhanced chemiluminescence reagents (Bio-Rad) and band density was measured using the Quantity One system (Bio-Rad).

*Statistical analyses*

Statistical analyses were performed using GraphPad Prism 7 software (GraphPad Software, CA, USA). We used the Student's *t*-test to compare two groups and one-way analysis of variance (ANOVA) to compare more than two groups. Tukey's multiple comparison test was applied to all ANOVA post hoc tests. ANOVA *F* values are described in each figure legend as $F_{(DFn, Dfd)}$, where DFn is the df numerator, and Dfd is the df denominator. Statistical significance was set at $p < 0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$). All data are presented as the mean ± standard deviation.

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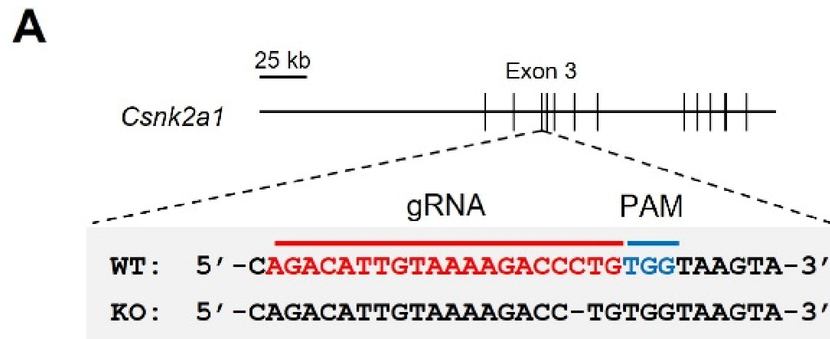


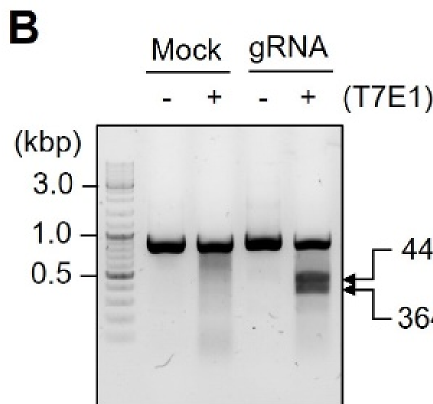


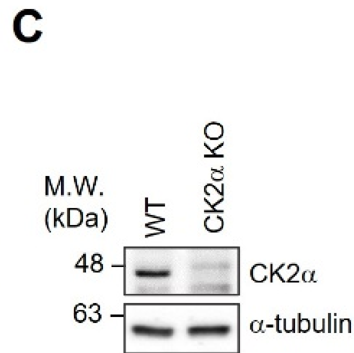


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5 **Supplementary Fig. 1. CK2α knockout (KO) cells were generated using the clustered**
6 **regularly interspaced palindromic repeat-caspase 9 (CRISPR-Cas9) system. (A)**
7 Single guide RNA (gRNA) targeting exon 3 of *Csnk2a1* was selected by the
8 GeneScript software. CK2α KO MEFs were verified via DNA sequencing (single
9 nucleotide deletion) **(B)** T7 endonuclease 1 assay was performed to determine the
10 efficiency of gRNA. **(C)** Protein extracts obtained from the wild-type (WT) and CK2a
11 KO MEFs were subjected to western blotting to assess the protein expression levels
12 of CK2α.
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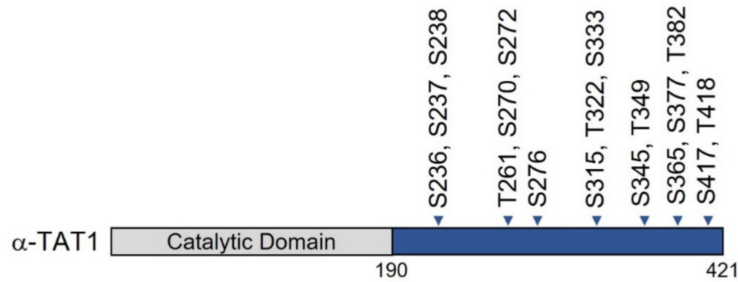
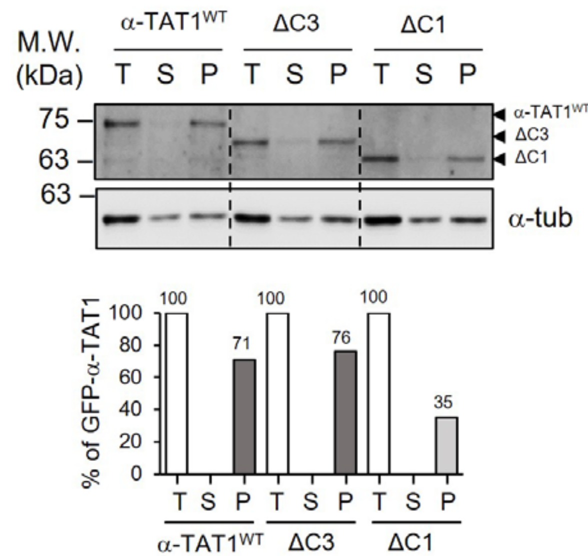



**Supplementary Fig. 2. Putative phosphorylation sites in the C-terminal domain of α-tubulin acetyltransferase 1 (α-TAT1). Schematic representation of α-TAT1 of** ***Mus musculus*****.** Putative serine/threonine phosphorylation sites in the C-terminal domain are indicated. These sites were obtained from PhosphoSitePlus and Netphos 3.1.




**Supplementary Fig. 3. Deletion of the Cluster 3 domain of α-TAT1 maintained the binding affinity to polymerized microtubules.** α-TAT1 KO MEFs were transfected with the indicated plasmids, and a microtubule sedimentation assay was conducted. Each fraction was assessed via western blotting to detect the levels of α-TAT1 constructs (WT, ⊿C3, and ⊿C1), supernatant (S) (depolymerized tubulin), and pellet (P) (polymerized tubulin).

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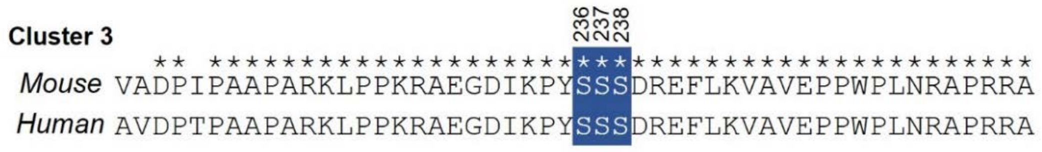


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5 **Supplementary Fig. 4. Amino acid sequence alignment of Cluster 3 in α-TAT1**
6 **between mice (*M. musculus*) and humans (*Homo sapiens*).** Cluster 3 domain in α-
7 TAT1 was highly conserved in both mice and humans.
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**Supplementary Table 1. Primers used for plasmid construction**

| Plasmid | Direction (Restriction enzyme) | Sequence |
|---|---|---|
| GFP-$\alpha$-TAT1$^{\Delta C1}$ | F (XhoI) | CCGCTCGAGGAATGGAGTTCCCGTTCGAT |
| | R (BamHI) | CGGGATCCGCCTCCAGGGTCAGTGGC |
| GFP-$\alpha$-TAT1$^{\Delta C1,2}$ | F (XhoI) | CCGCTCGAGGAATGGAGTTCCCGTTCGAT |
| | R (BamHI) | CGGGATCCGGCACGCCGAGGGGCCCTGTT |
| GFP-$\alpha$-TAT1$^{\Delta C}$ | F (XhoI) | CCGCTCGAGGAATGGAGTTCCCGTTCGAT |
| | R (BamHI) | CGGGATCCCACAGCAGCACGAGAGTG |
| GFP-$\alpha$-TAT1$^{\Delta N}$ | F (XhoI) | CCGCTCGAGGAATGGCCGATCCCATACCTGCTGCT |
| | R (BamHI) | CGGGATCCTTACCAAGGCCTGGTGCTGCG |
| $\alpha$-TAT1$^{\Delta N}$-Myc | F (BamHI) | CGGGATCCATGGCCGATCCCATACCTGCTGCT |
| | R (XbaI) | GCTCTAGACCAAGGCCTGGTGCTGCG |
| GFP-$\alpha$-TAT1$^{\Delta C3}$ | 1-F (XhoI) | CCGCTCGAGGAATGGAGTTCCCGTTCGAT |
| | 2-R | GTGGGCTGGAGGTGTCACAGCAGCACGAGA |
| | 3-F | TCTCGTGCTGCTGTGACACCTCCAGCCCAC |
| | 4-R (BamHI) | CGGGATCCTTACCAAGGCCTGGTGCTGCG |
| Flag-CK2$\alpha$ | F (NotI) | AAGGAAAAAAGCGGCCGCGATGTCGGGACCCGTGCCAAG |
| | R (KpnI) | CGGGGTACCTTACTGCTGAGCGCCAGC |

1 **Supplementary Table 2. Primers used for site-directed mutagenesis of α-TAT1**

| Plasmid | Direction | Sequence |
|---|---|---|
| GFP-α-TAT1 | F | GGAGACATTAAGCCATACGCTTCCAGTGACAGAGAATTC |
| (S236A) | R | GAATTCTCTGTCACTGGAAGCGTATGGCTTAATGTCTCC |
| GFP-α-TAT1 | F | GACATTAAGCCATACTCTGCCAGTGACAGAGAATTCCTGAAG |
| (S237A) | R | CTTCAGGAATTCTCTGTCACTGGCAGAGTATGGCTTAATGTC |
| GFP-α-TAT1 | F | GACATTAAGCCATACTCTTCCGCTGACAGAGAATTCCTGAAG |
| (S238A) | R | CTTCAGGAATTCTCTGTCAGCGGAAGAGTATGGCTTAATGTC |
| GFP-α-TAT1 | F | GACATTAAGCCATACTCTGCCGCTGACAGAGAATTCCTGAAG |
| (S237/238A) | R | CTTCAGGAATTCTCTGTCAGCGGCAGAGTATGGCTTAATGTC |

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