SUPPLEMENATRY FIGURES

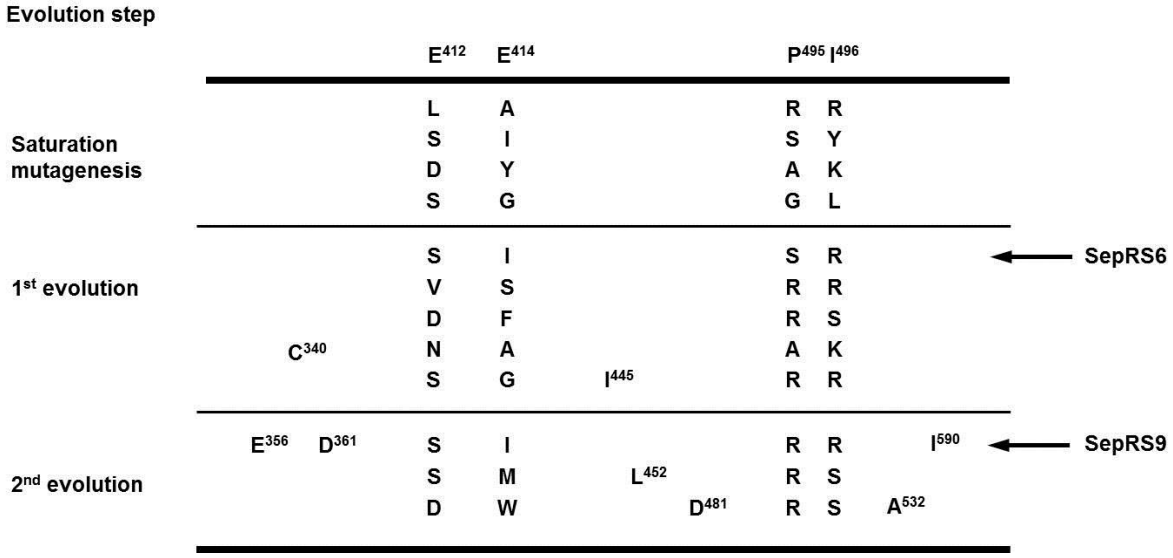

Figure S1. The amino acid sequences of representative Mmp SepRS variants from each evolutionary step. The best clones of each step, SepRS6 and SepRS9, are indicated.

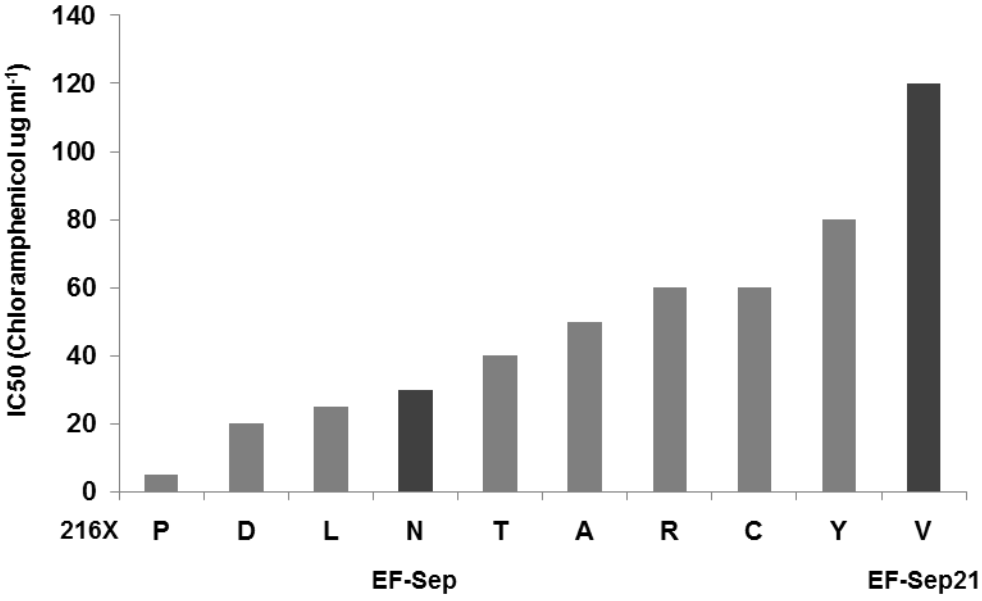

Figure S2. In vivo aminoacylation activities of various EF-Sep variants selected from EF-Sep N216X library. Amino acid sequence of the residue 216 in EF-Sep is shown. The original EF-Sep (N216) and the newly selected variant EF-Sep21 (V216) are indicated.

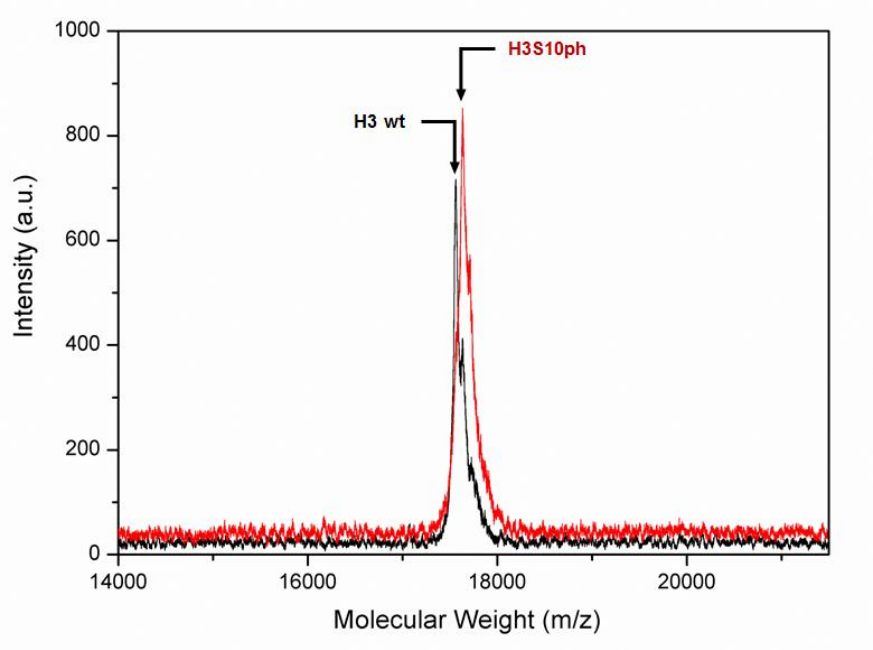

Figure S3. MALDI-TOF mass spectra of H3 wild type and H3S10ph carrying His6-tag. The calculated mass of H3 wild type is 17,664.4 Da and the detected mass is 17,662.6 Da. H3S10ph is determined to be 17,742.4 Da, which is 80 Da higher than the wild type. The mass difference agrees well with the added phosphate group in H3S10ph.

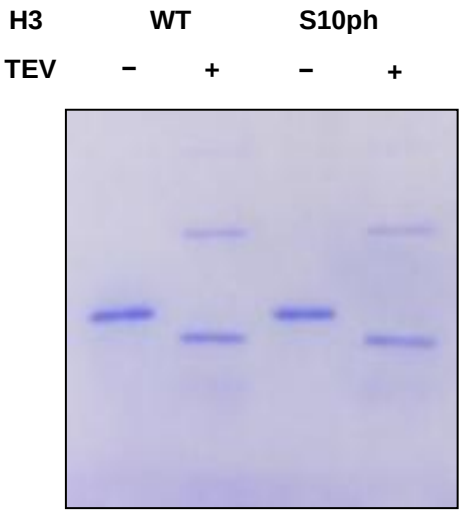

Figure S4. TEV protease digestion of H3 wt and H3S10ph

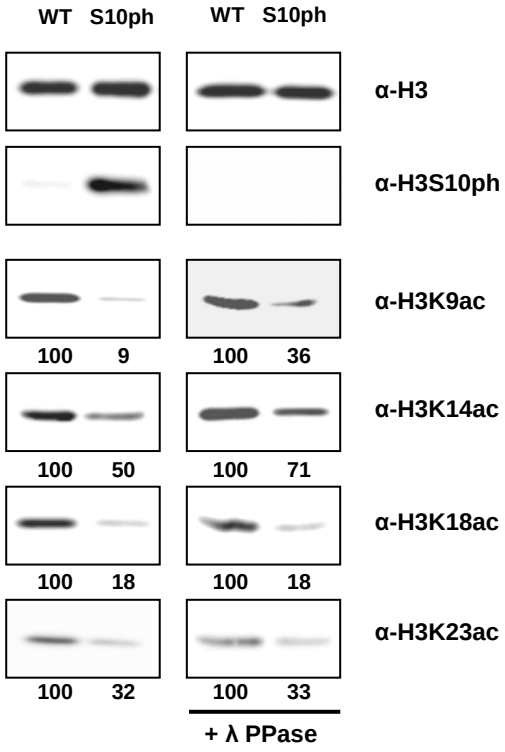

Figure S5. Western blotting before (Left) and after (Right) alkaline phosphatase treatment.

SUPPLEMENATRY METHODS

1. Evolution of SepRS

To improve in vivo phosphoserine (Sep) incorporation in *Escherichia coli* [1], molecular evolution of SepRS was attempted. Based on the crystal structure of the *Archeaglobus fulgidus* SepRS:tRNA^{Cys} complex[2], four residues (Glu412, Glu414, Pro495 and Ile496) in the anticodon-binding region of *Methanococcus maripaludis* SepRS (Mmp SepRS) were selected. For efficient PCR-based random mutation, nucleotides around these residues of Mmp SepRS were changed from AT-rich to GC-rich codons using the following primers: SP418GCF1, 5'-GAA GAG GGC AAG AAC CTG CTC GGA CCT TCA ATT TTA AAC G-3'; SP418GCF2, 5'-CGT GAA GAT CAA CAT CTT CGA AAA AGA AGA GGG CAA GAA CCT G-3'; SP418GCR, 5'-GAA GAT GTT GAT CTT CAC GTT TTT CTT GGT TTT TCC AAA TG-3'; SP130F, 5'-CGA TGG AAA TGT AAT TGG CAT TCC TGA AAG CTT TGA CG-3'; SP130R, 5'-CCA ATT ACA TTT CCA TCG-3'; SP492GCF, 5'-GAG TTC AAG GTC AAG GTG CCA ATT GTC AGA AGT TTA AGC G-3'; SP492GCR, 5'-CAC CTT GAC CTT GAA CTC AGT AGT GTT TGA CAC AAA TGC-3.

For library construction, *Eco*RI restriction site was inserted into Mmp SepRS (using the primer: SP263F, 5'-CAA TTT GGC TTT ACA AAC TTT GAA TTC ATT CCT GAT GAA AAG-3'). And 67R of the EF-Sep was changed to Ser using the primers: EF67SF, 5'-GTA TCA CCA TCA ACA CTT CTT CCG TTG AAT ACG ACA CCC CG-3'; EF67R, 5'-AGA AGT GTT GAT GGT GAT AC-3. The resulting plasmid pKD-SepRS-EFSep67S was used as a template to generate a library of Mmp SepRS mutants. For random mutation of the four residues of MmpSepRS, the following primers were used: SP418X420XF, 5'-GTG AAG ATC AAC ATC TTC NNS AAA NNS GAG GGC AAG AAC CTG CTC-3'; SP418420R, 5'-GAAGATGTTGATCTTCACG-3'; SP492X493XF, 5'-GAG TTC AAG GTC AAG GTG NNS NNS GTC AGA AGT TTA AGC GAC-3'; SP492493R, 5'-CAC CTT GAC CTT GAA CTC-3'; SP263F, 5'-CAA TTT GGC TTT ACA AAC TTT GAA TTC ATT CCT GAT GAA AAG-3'; EF67R, 5'-AGA AGT GTT GAT GGT GAT AC-3'. The final overlapped PCR product was gel extracted, digested with *Eco*RI and *Sal*I, ligated into pKD-SepRS-EFSep67S, and transformed into *Escherichia coli* Top10*ΔSerB* containing the plasmid pCAT112TAG-SepT for CAT-based

positive selection[1]. A library of $1.6X10^6$ SepRS variants was spread onto LB plates supplemented with 30μg/ml chloramphenicol (Cm), 25μg/ml kanamycin (Kan), 10μg/ml tetracycline (Tc) and 0.05 mM isopropyl-β-D-thiogalactopyranoside (IPTG). After 60h at 30℃, around 4,000 colonies were collected from the plates and their plasmids were isolated. The pKD-SepRS-EFSep67S plasmid was separated from the pCAT112TAG-SepT plasmid by agarose gel extraction. Individual clones were then tested for their ability to grow on Cm ranging from 5 to 100 μg/ml.

Two rounds of molecular evolution of the selected SepRS variants were conducted via error-prone PCR and DNA shuffling[3, 4]. The same CAT-based system was applied for positive selection[1]. Error-prone PCR was performed using pKD-SepRS-EFTu as a template, 0.5 nM primers, 0.2 mM dATP and GTP, 1 mM dCTP and dTTP, 0.025 U μl^{-1} *Taq* DNA polymerase and *Taq* buffer containing 7 mM $MgCl_2$ and 0.5 mM $MnCl_2$. PCR primers were SP263F (5'-CAA TTT GGC TTT ACA AAC TTT GAA TTC ATT CCT GAT GAA AAG-3') and EF67R (5'-AGA AGT GTT GAT GGT GAT AC-3'). For DNA shuffling, DNaseI-treated fragments between 50-150 base pairs were gel-extracted and reassembled through primerless PCR. Reassembled DNA (around 0.8kb) was isolated and used for PCR using primers SP263F and EF67R. The reassembled DNA was digested with *Eco*RI and *Sac*I, cloned into pre-digested pKD-SepRS-EFSep67S, and transformed into *E. coli* Top10*ΔSerB* by electroporation. Around 300 and 100 positive clones were obtained from 1st and 2nd evolution. The Sep incorporation activity of individual colony was checked using CAT-based in vivo aminoacylation assay.

The nucleotide sequence of the best mutant MmpSepRS9 is as follows:

ATGTTTAAAAGAGAAGAAATCATTGAAATGGCCAATAAGGACTTTGAAAAAGCAT
GGATCGAAACTAAAGACCTTATAAAAGCTAAAAAGATAAACGAAAGTTACCCAAGA
ATAAAACCAGTTTTTGGAAAAACACACCCTGTAAATGACACTATTGAAAATTTAAGA
CAGGCATATCTTAGAATGGGTTTTGAAGAATATATAAACCCAGTAATTGTCGATGAA
AGAGATATTTATAAACAATTCGGCCCAGAAGCTATGGCAGTTTTGGATAGATGCTTT
TATTTAGCGGGACTTCCAAGACCTGACGTTGGTTTGAGCGATGAAAAAATTTCACAG
ATTGAAAAACTTGGAATTAAAGTTTCTGAGCACAAAGAAAGTTTACAAAAAATACT
TCACGGATACAAAAAAGGAACTCTTGATGGTGACGATTTAGTTTTAGAAATTTCAAA

TGCACTTGAAATTTCAAGCGAGATGGGTTTAAAAATTTTAGAAGATGTTTTCCCAGA
ATTTAAGGATTTAACCGCAGTTTCTTCAAAATTAACTTTAAGAAGCCACATGACTTC
AGGATGGTTCCTTACTGTTTCAGACCTCATGAACAAAAAACCCTTGCCATTTAAACT
CTTTTCAATCGATAGATGTTTTAGAAGAGAACAAAAAGAAGATAAAAGCCACTTAA
TGACATACCACTCTGCATCCTGTGCAATTGCAGGTGAAGGCGTGGATATTAATGATG
GAAAAGCAATTGCAGAAGGATTATTATCCCAATTTGGCTTTACAAACTTTGAATTCA
TTCCTGATGAAAAGAAAAGTAAATACTACACCCCTGAAACACAGACTGAAGTTTAC
GCATACCACCCAAAATTAAAAGAATGGCTCGAAGTTGCTACATTTGGAGTATATTCG
CCAGTTGCATTAAGCAAATACGGAATAGATGTACCTGTAATGAATTTGGGTCTTGGT
GTTGAAAGACTTGCAATGATTTCTGGAAATTTCGCAGATGTTCGAGAAATGGTATAT
CCTCAGTTTTACGAACACGAACTTAATGACCGGGATGTCGCTTCAATGGTAAAACTC
GATAAAGTTCCAGTAATGGATGAAATTTACGATTTAACAAAAGAATTAATTGAGTCA
TGTGTTAAAAACAAAGATTTAAAATCCCCTTGTGAATTAGCTATTGAAAAAACGTTT
TCATTTGGAAAAACCAAGAAAAACGTGAAGATCAACATCTTCAGCAAAATCGAGGG
CAAGAACCTGCTCGGACCTTCAATTTTAAACGAAATCTACGTTTACGATGGAAATGT
AATTGGCATTCCTGAAAGCTTTGACGGAGTAAAAGAAGAATTTAAAGACTTCTTAGA
AAAAGGAAAATCAGAAGGGGTAGCAACAGGCATTCGATATATCGATGCGCTTTGCT
TTAAAATTACTTCAAAATTAGAAGAAGCATTTGTGTCAAACACTACTGAGTTCAAGG
TCAAGGTGCGGCGCGTCAGAAGTTTAAGCGACATTAACTTAAAAATCGATGATATC
GCAATAAAACAGATCATGAGCAAAAATAAAGTAATCGACGTTAGAGGCCCAGTCTT
TTTAAATGTCGAAGTAAAAATTGAATAA

2. Evolution of EFTu

To check the role of mutations found in EF-Sep (H67R, E216N, D217G, F219Y, T229S, and N274W)[1] in the binding of Sep, all the mutated residues except D217G were individually changed to Ala to generate following EF-Sep derivatives, EF-Sep1 (R67A), EF-Sep2 (N216A), EF-Sep3 (Y219A), EF-Sep4 (S229A), and EF-Sep5 (W274A). pKD-SepRS-EF-Sep plasmid containing each EF-Sep variant was transformed into *E. coli* Top10*ΔserB* carrying plasmid pCAT112TAG-SepT and the Sep binding ability was checked via CAT-based assay. Since mutation N216A in EF-Sep2 exhibited a dramatic improvement in Sep incorporation efficiency,

position 216 was randomly mutated and checked for further improvement. The plasmid carrying mutated EF-Tu (pKD-SepRS-EFSep216X) was transformed into *E. coli* Top10*ΔserB* containing pCAT112TAG-SepT. The transformed cell was plated onto LB agar containing 50 μg/mL Kan, 10 μg/mL Tc, and different Cm concentrations of 0-160 μg/mL. Among 500 colonies plated on one plate, 20 colonies survived at Cm concentration of 20 μg/mL. DNA sequencing revealed that the selected colonies have following amino acids at the position 216; Arg, Val, Asp, Cys, Thr, Tyr, Ala, Leu, and Pro. To evaluate Sep binding ability of the EF-Sep variants, CAT-based in vivo aminoacylation assay was performed (see **Figure S2**). EF-Sep21 carrying N216V mutation exhibited the highest IC_{50} value.

3. H3S10ph expression and purification

pCDFDuet-H3 wt was constructed by cloning *Xenopus laevis* histone H3 gene containing N-terminal His6-tag and TEV protease recognition site between *Bam*HI and *Asc*I of pCDFDuet (Novagen). The following PCR primers were used: H3BamTEVF (5'-CCT GGA TCC GGA GAA CCT GTA CTT CCA GGG CCG TAC CAA GCA GAC CGC C-3') and H3AscR (5'GCC AGA TCT TTA AGC CCT CTC GCC TCG GAT TC-3'). To construct plasmid pCDFDuet-H3S10TAG, an amber stop codon was introduced to the histone H3 gene at position Ser10 by nested PCR using following primers: H3S10TAGF(5'-CGT ACC AAG CAG ACC GCC CGT AAA TAG ACC GGA GGG AAG GCT CCC-3') and H3AscR (5'GCC AGA TCT TTA AGC CCT CTC GCC TCG GAT TC-3') for 1st PCR; H3BamTEVF (5'-CCT GGA TCC GGA GAA CCT GTA CTT CCA GGG CCG TAC CAA GCA GAC CGC C-3') and H3AscR (5'GCC AGA TCT TTA AGC CCT CTC GCC TCG GAT TC-3') for 2nd PCR. pETDuet-SepRS9-SepT was created by adding the SepRS9 gene between *Nco*I and *Sac*I and ligating with overlap PCR product containing a $tRNA^{Sep}$ expression cassette between *Not*I and *Bgl*II into pETDuet (Novagen). Following primers were used; DuetF (5'- GGG ATC TCG ACG CTC TCC C'), DuetSepTR(5'- CCC CTA GAC TAC CCC GGC CTT AAC TAA TAT ACT AAG ATG -3'), SepTF (5'- GCC GGG GTA GTC TAG GGG -3'), and SepTBglR (5'- TGC CTG AAC TAG ATC TTG GAG CCG GGG GTG GGA T-3').

To expression *Xenopus laevis* histone H3 containing Sep at position Ser10, *E. coli* BL21 (DE3) cells were transformed with plasmid pKD-SepRS9-EFSep21, pETDuet-SepRS9-sepT, and

pCDFDuet-H3S10TAG. The cells were grown overnight in 2xYT medium supplemented with 50 μg/ml Kan, 100μg/ml ampicillin (Amp) and 50 μg/ml streptomycin (Sm). One liter of 2xYT medium was inoculated and incubated at 30°C. At A600 of 0.5, the culture was induced by adding 0.5 mM IPTG. Temperature was changed to 37℃ and incubation continued for 12h. Since rich medium contains considerable amounts of Sep, additional Sep was not provided in the medium. Little difference was observed whether or not *serB* (encoding phosphoserine phosphatase) gene was deleted when rich medium was used. After harvesting, the pellet was resuspended in 50 ml of lysis buffer (6 M guanidinium chloride, 100 mM NaH_2PO_4 (pH 8.0), 10 mM Tris-HCl, and 3 mM β-mercaptoethanol). Cell debris was removed from the lysate by centrifugation, and supernatant was loaded onto a 0.5ml pre-equilibrated Ni^{2+}-NTA agarose column. The column was washed with washing buffer (8 M urea, 100 mM NaH_2PO_4 (pH 6.3), 10mM Tris-HCl, and 3 mM β-mercaptoethanol). The protein was eluted with elution buffer (8 M urea, 100 mM NaH_2PO_4 (pH 4.5), 10mM Tris-HCl, and 3 mM β-mercaptoethanol). The eluted protein was collected and dialyzed at 4℃ against distilled water with 3mM β-mercaptoethanol. In order to remove His-tag, histone H3 was treated with 2 mg/ml TEV (1:100 ratio) for 3hr at 30℃ followed by running the SDS-PAGE gel to confirm complete digestion (see **Figure S4**). The digested sample was dialyzed and lyophilized. Histone H3 wt sample was also prepared using *E. coli* BL21 (DE3) transformed with plasmid pCDFDuet-H3wt. The protein was purified using the same method as written above.

The amino acid sequences of wild type histone H3 and Sep-inserted histone H3S10Sh containing N-terminal His6-tag and TEV protease recognition site are as follows:

H3 wild type
MGSSHHHHHHSQDPENLYFQGRTKQTARKSTGGKAPRKQLATKAARKSAP
ATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQSS
AVMALQEASEAYLVALFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA

H3S10ph

MGSSHHHHHHSQDPENLYFQGRTKQTARK(Sep)TGGKAPRKQLATKAARKSAP
ATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQSS
AVMALQEASEAYLVALFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA

4. Mass spectrometric analysis of H3 wild type and H3S10ph

Molecular weight of H3 wild type and H3S10ph carrying N-terminal His6-tag was analyzed by matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The protein sample was mixed in the ratio 1:1 with saturated sinapinic acid matrix solution in 0.1 % TFA, 50% acetonitrile, and 1 uL of the mixture was spotted on a ground steel MTP384 for analysis. Linear, positive ion mode of mass spectrometer was used to confirm the molecular weight of the histone.

5. Histone octamer assembly and nucleosomal array reconstitution

Equivalent amounts of lyophilized histones (H4, H2A, H2B, and H3wt or H3S10ph) were dissolved and mixed in unfolding buffer (7 M guanidinium chloride in 20 mM Tris-HCl (pH7.5), and 10 mM DTT). After 3hr incubation at room temperature with gentle agitation, the histones were dialyzed against refolding buffer (2 M NaCl, 10mM Tris-HCl (pH8.0), 1 mM EDTA, and 5 mM β-mercaptoethanol) 3 times at 4℃. Octamers were then separated by glycerol gradient centrifugation[5]. For reconstitution of nucleosomal array, 35 μg of DNA templates (pGEM-3z/601 or pG5E4T) was incubated with histone octamers (1:1.1 mass ratio) and 2 M NaCl concentration of mixture were gradually decreased to 0.1 M by salt step dilution methods[6, 7]. Nucleosomal arrays were separated from remaining histone octamers and DNA templates by glycerol gradient centrifugation[8]. The reconstituted nucleosomal arrays were analyzed by 0.8% agarose gel.

6. Histone acetyltransferase (HAT) assay

Saccharomyces cerevisiae GCN5 with His6-tag was expressed in *E. coli* BL21 (DE3) and purified via Ni^{2+}-NTA agarose. The SAGA complex was purified by using the tandem affinity purification (TAP) protocol[9]. In liquid HAT assays, 60 pmol of histone H3, histone octamers and nucleosomal array were reacted with 0.6 pmol of recombinant Gcn5 protein or 0.3 pmol of the SAGA complex in HAT buffer (50 mM Tris-HCl (pH7.5), 5% glycerol, 0.125 mM EDTA,

50 mM KCl, 1mM DTT, 1 mM PMSF, 10 mM sodium butyrate, 2.5 μM [^{3}H]Acetyl-CoA (3.3Ci/mmol), 1 mM Na_3Vo_4, and 5 mM NaF) at 30℃[10]. Reactions were stopped by pipetting reaction mixtures onto P81 filter paper (Whatman). The filter papers were washed four times in 50 mM $NaHCO_3$/Na_2CO_3 (pH 8.5) and one time in acetone. The extent of acetylation was determined by scintillation counter.

7. Western blotting

After histone acetyltransferase reaction in the HAT buffer (50 mM Tris-HCl, pH7.5, 1 mM PMSF,1 mM DTT, and 0.2 mM Acetyl-CoA), the reaction mixtures were loaded onto a 15% SDS-PAGE gel, transferred to nitrocellulose membrane, and analyzed by Western blotting using following antibodies; 1:1000 dilution of anti-H3 K9Ac antisera, 1:1,000 dilution of anti-H3 K14Ac antibody (Millipore; Cat# 06-911), 1:1,000 dilution of anti-H3 K18Ac antibody (Millipore; Cat# 07-354), 1:100,000 dilution of anti-H3 K23Ac antibody (Millipore; Cat# 07-355), 1:1,000 dilution of anti-H3 S10Pi antibody (Millipore; Cat# 04-817), 1:1,000 dilution of anti-H3 antisera. To prevent occlusion effect by phosphorylation of H3S10 and more precisely analyze acetylation at H3K9 and H3K14 by, HAT reaction mixtures were treated with lambda phosphatase (New England Biolabs; Cat# P0753) for 30 min at 30 ℃ before Western blotting (see **Figure S5**)[11].

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