Table S1.

Site-directed Mutagenesis Primers

| Mutant | Forward or Reverse Primer | Primer Sequence |
|---|---|---|
| E47A | Forward | CTT TTT CTT CAA GGA TGC ACC GCT GAA CAC CTT CC |
| | Reverse | GGA AGG TGT TCA GCG GTG CAT CCT TGA AGA AAA AG |
| P48A | Forward | AGG TGT TCA GCG CTT CAT CCT TGA AGA AAA AGG TCT |
| | Reverse | AGA CCT TTT TCT TCA AGG ATG AAG CGC TGA ACA CCT |
| Y64A | Forward | GAA GCG GTT TCA GGG AGG CCT TCT CCA GCT CCT TGC |
| | Reverse | GCA AGG AGC TGG AGA AGG CCT CCC TGA AAC CGC TTC |
| D142A | Forward | ACA GGA TCT TGC CGG CCA GGA TGA GCT CC |
| | Reverse | GGA GCT CAT CCT GGC CGG CAA GAT CCT GT |
| R153A | Forward | GTG GAC ACC AAC TAC GCG GGC CTG GGC A |
| | Reverse | TGC CCA GGC CCG CGT AGT TGG TGT CCA C |
| H178A | Forward | GGC TGG AAC AGA GAA CGG CGT ACA CAT TGA TGC CGT |
| | Reverse | ACG GCA TCA ATG TGT ACG CCG TTC TCT GTT CCA GCC |
| C181A | Forward | ATG TGT ACC ACG TTC TCG CTT CCA GCC ACT ACT CCG |
| | Reverse | CGG AGT AGT GGC TGG AAG CGA GAA CGT GGT ACA |
| S186A | Forward | CCA GCC ACT ACG CCG CCC GGG TG |
| | Reverse | CAC CCG GGC GGC GTA GTG GCT GG |
| S182A/S186A | Forward | TCA ATG TGT ACC ACG TTC TCT GTG CCA GCC ACT ACG |
| | Reverse | CGT AGT GGC TGG CAC AGA GAA CGT GGT ACA |
| H220A | Forward | CCG GCG GCC CCG GCC GTA GGC ATC CA |
| | Reverse | TGG ATG CCT ACG GCC GGG GCC GCC GG |

Table S2.

AANATA product characterization.

| Sample | Retention Time | Mass/Charge |
|---|---|---|
| | (min) | [M + H] (*m/z*) |
| *N*-acetylserotonin Standard | 0.78 | 219.1132 |
| AANAT A Enzyme Assay [a] | 0.78 | 219.1133 |

[a] Reaction condition – 300 mM Tris-HCl pH 8.0, 500 µM acetyl-CoA, 1 mM serotonin, 15 µg AANATA.

Table S3.

Conserved *D. melanogaster* AANATA Arg153 Found in other GNAT Enzymes

| Organism | Enzyme | PDB Data Base ID for Crystal Structure | Conserved Arginine |
|---|---|---|---|
| Drosophila | AANATA | 3TE4 | R153 |
| Sheep | SNAT | 1CJW | R131 |
| Human | SSAT | 2JEV | R101 |
| Tuberculosis | AgNAT | 1M4G | R91 |
| Yeast | HAT (Hpa2) | 1QSM | R100 |
| Human | NMT | 3JTK | R255 |

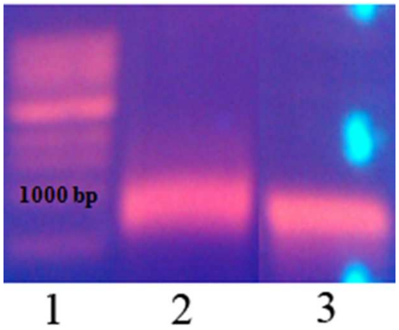

Figure S1. Cloning of *AANATA* and *AANATB* from *D. melanogaster*. (Lane 1) 1 kb DNA ladder (New England Biolabs), (Lane 2) *AANATB*; 828 bp band, (Lane 3) *AANATA*; 723 bp band.

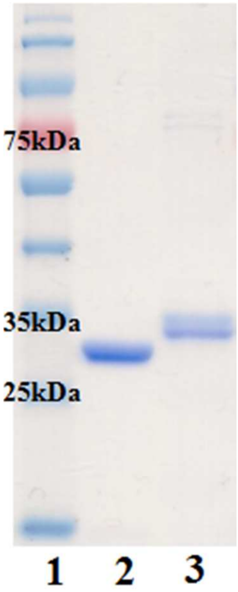

Figure S2. SDS-PAGE gel for purified AANATA and AANATB. (Lane 1) Precision Plus Protein™ Kaleidoscope™ Standards (Biorad), (Lane 2) AANATA; 27 kDa, (Lane 3) AANATB; 31 kDa.

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AANATA                      LIQPEDGEAVIAMLKTFFFKDEPLNTFLDL-----GECKELEKYSLKPLPDNCSYKAVNK 80
AANATB                      LIQPEDGEAVIAMLKTFFFKDEPLNTFLDL-----GECKELEKYSLKPLPDNCSYKAVNK 115
AANATL2 (CG9486)            AMTIGDYEEVEAFLAVHFFKQEPLMLIPQEDPKQSEVSSAEAELHRSLIPQDLSLVAVD- 66
AANATL3 (CG10659)           TMTKEDYPSVKAFLKDNFFQSEPLCQSTSE-NVQSQNEKENDEYHLSMIAQGTCLVAIDE 71
AANATL4 (CG18607)           IMRPEDYAQVKAYMEAEYYTSEPLCQSSGE-PVHQQNEEINDAFNQSIIAEGTSLLALDE 71
AANATL5 (CG10476)           VMKEDDYPRVKTFMTDYFHYDEPMGMGLEE-PIHLQHEEEVDRQYLAVIRQGLSIVALDD 71
AANATL6 (CG18606)           VMKEDDYPRVKTFMTDYFHYDEPMGMGLEE-PIHLQHEEEVDRQYLAVIRQGLSIVALDD 71
AANATL7 (CG13759)           MIAPEHSEQVMEHLRRNFFADEPLNKAAGL-CQNGSSCPALEAHCAEAIQHRMSVMAVDA 63
AANATL8 (CG15766)           QVDVGETEQLMTFLLAHYYPEEPLTAGTHP----PEPEAADKEFLLSNVPFGTCFVALH- 67
                             :   .   :   :   :. .**:                        :    .  *:.

AANATA                      PDEELILDGKILSVDTNYRGLGIAGRLTERAYEYMRENGINVYHVLCSSHYSARVMEKLG 194
AANATB                      PDEELILDGKILSVDTNYRGLGIAGRLTERAYEYMRENGINVYHVLCSSHYSARVMEKLG 229
AANATL2 (CG9486)            G-VERALYLYMLGVDVSIRRQRVGTRLVEATIELGRQRGFPVVTSTCSNQNSKRLMTALN 179
AANATL3 (CG10659)           G-ISKLLYSHITSVESSMRGKGLGSRLAATLMDVGRAKGFPAMTAYCTSFYSARQKEALG 185
AANATL4 (CG18607)           D-IPKALYSHVTSVASWKRGKGLGSRLAATLMELGRSNGFPLMMAFCTSFYSARQKGALG 187
AANATL5 (CG10476)           G-VSSYLSLLVISVHPSMRQRGILVILSKCLFKLGRLRGLRLFIGSGTNHYSSRSAMKAG 185
AANATL6 (CG18606)           G-VSSYLSLLVISVHPSMRQRGILVILSKCLFKLGRLRGHTLFITSGTNHYSSRSAMKAG 185
AANATL7 (CG13759)           D-VDCMFDVRILSVDSCYRGQGIANELVKRSVAVAKKNGFRLLKADATGIFSQKIFRSHG 179
AANATL8 (CG15766)           S-VPSCLHVHALGVDPQLRGRNLGGRLMETVAQRGRDLGHQLVSVDCTSVYSARLVQRLG 179
                                  :     .*    *   :   *        :  *        :.  * :     .

AANATA                      FHEVFRMQFADYK-PQGEVVFKPAAPHVGIQVMAKEVGPAKAAQTKL-- 240
AANATB                      FHEVFRMQFADYK-PQGEVVFKPAAPHVGIQVMAKEVGPAKAAQTKL-- 275
AANATL2 (CG9486)            MECILTKDYADYKDEHGEIVLRASEPHTSASVVAIRL------------ 216
AANATL3 (CG10659)           MKCVHSLPYADYKDDQGRPIFTPAEPHTMARIMFIKL------------ 222
AANATL4 (CG18607)           MECIYSIDYADYKDDEGRVIFTPAAPHTKLRVMAIKL------------ 224
AANATL5 (CG10476)           CECIHSVAYADYKDEQGRPIYNPPAPHTHIRVLASKL------------ 222
AANATL6 (CG18606)           CECIHSVAYADYKDELGRPIYNPPAPHTHIRVLASKM------------ 222
AANATL7 (CG13759)           FEVFSEQPYSKYTDENGKVILPVEAPHIKLQQLYKAICADDQDEKKQSL 228
AANATL8 (CG15766)           YQLINTLRYVDHLDASGQQVIRPPPPHESVQTFVLHL------------ 216
                             . .    : .:    *. :     **     .   :
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Figure S3. Primary sequence alignment of AANATA with putative AANATL enzymes found in *D. melanogaster* using clustalW2.

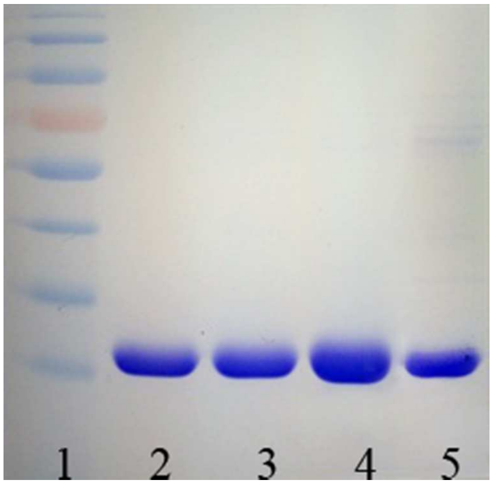

Figure S4. SDS-PAGE gel of purified AANATA mutants. (Lane 1) PageRuler™ Prestained protein ladder (Thermo Scientific), (Lane 2) E47A, (Lane 3) R153A, (Lane 4) S186A, (Lane 5) H220A.

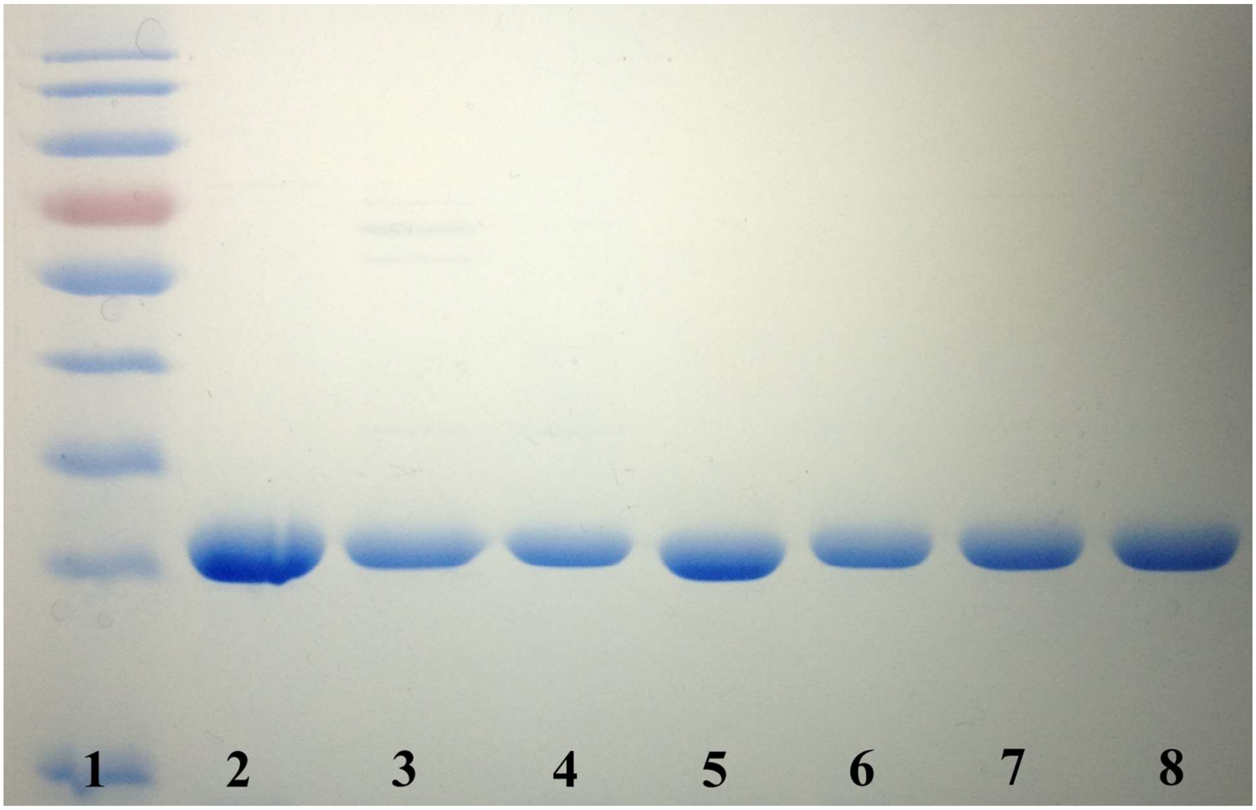

Figure S5. SDS-PAGE gel of purified AANATA mutants. (Lane 1) PageRuler™ Prestained protein ladder (Thermo Scientific), (Lane 2) P48A, (Lane 3) Y64A, (Lane 4) D142A, (Lane 5) H178A, (Lane 5) C181A, (Lane 5) S182A/S186A.

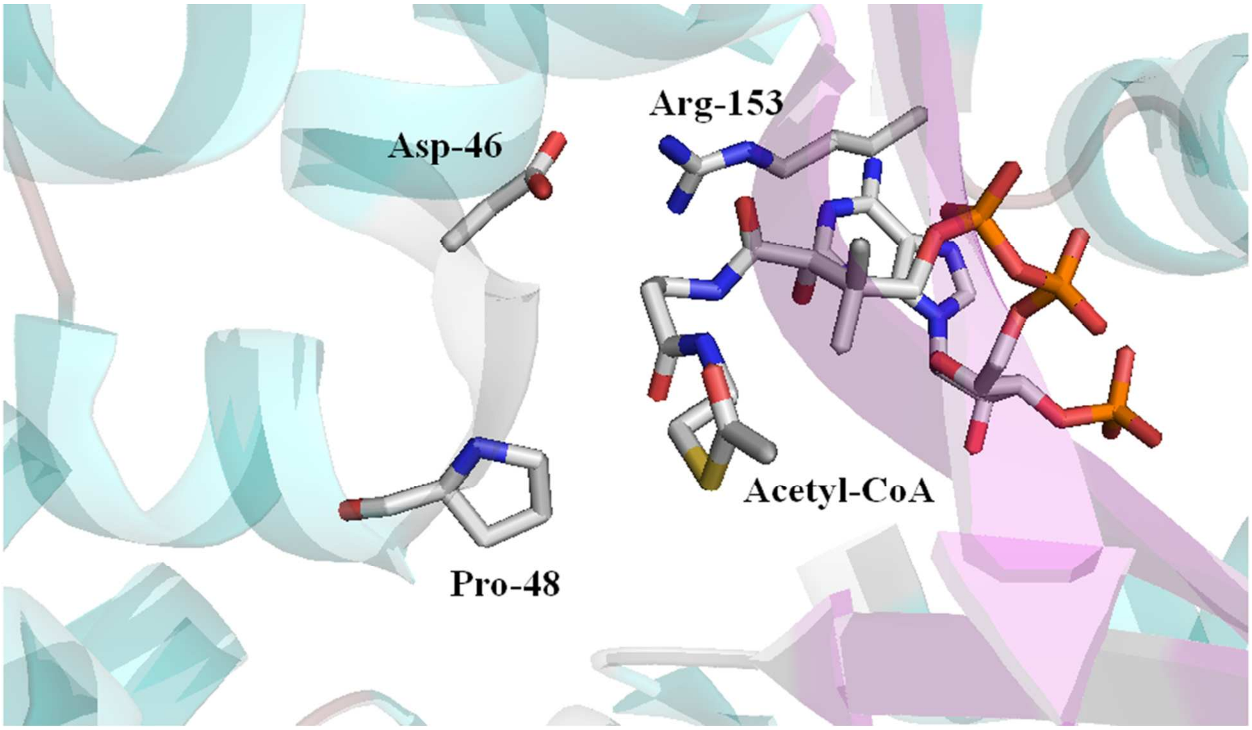

Figure S6. *D. melanogaster* AANATA Pro-48 (PDB: 3TE4) showing general location with respect to amine binding pocket and bound acetyl-CoA.