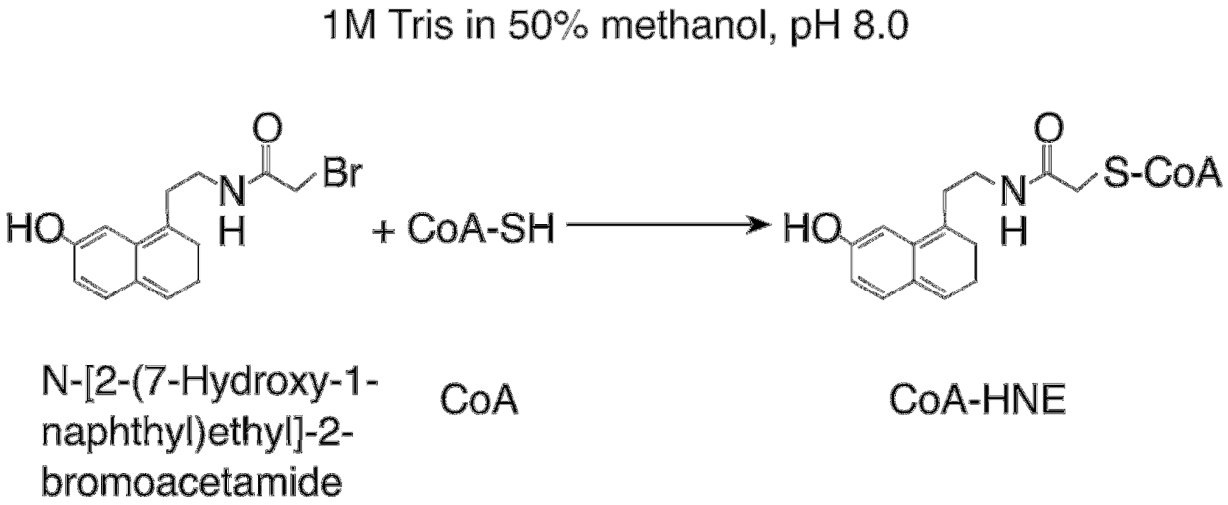
Supplementary Fig. 1. **Synthesis of fluorescent probe.** The schema is shown of the synthesis of bisubstrate inhibitor CoA-HNE used as a fluorescent probe for the studies of active center of AANAT forms. Synthesis was based on a published procedure (18).

Supplementary Fig. 2. **Characterization of synthesized probe using LC-MS.** Panel *A* shows elution peak from HPLC, characterizing the purity of the sample, panel *B* shows expected m/z value of the ion characterizing the final product of the chemical synthesis.

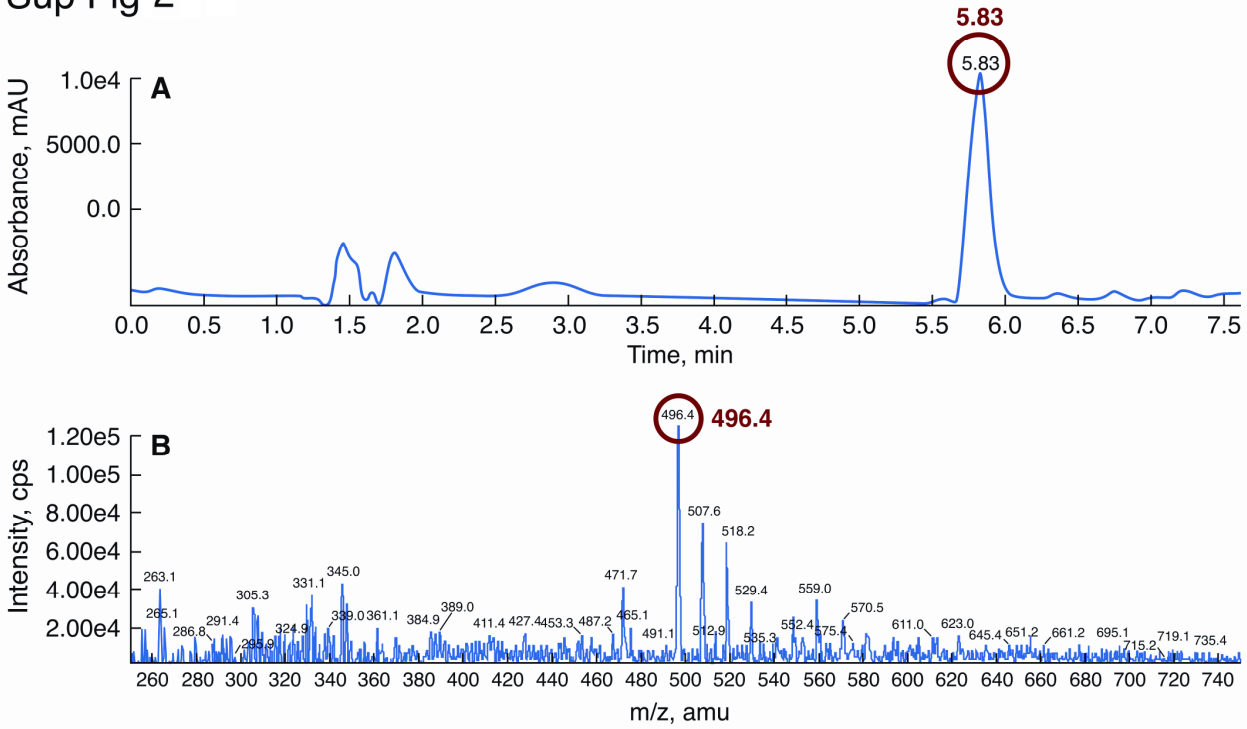
Supplementary Fig. 3. **Sequence alignment of studied proteins.** Sequence alignment of ovine wildtype AANAT, ovine mutant AANATs and yeast wild-type AANAT. Sequences have been aligned using CLUSTALW, and then manually adjusted. Residues highlighted in grey are identical between yeast and sheep. Residues inversely highlighted bold and black in the Loop 1 area are those which have been mutated; asterisks denote the deletions in mutant.

Supplementary Fig. 4. **SDS-PAGE of studied protein samples.** Coomassie Blue-stained SDS-PAGE with analysis of all studied proteins on a 14% gel. The analysis indicates the quality of prepared GSTfusion protein samples. Molecular weight marker High-Range Rainbow (Amersham Biosciences) has been used.

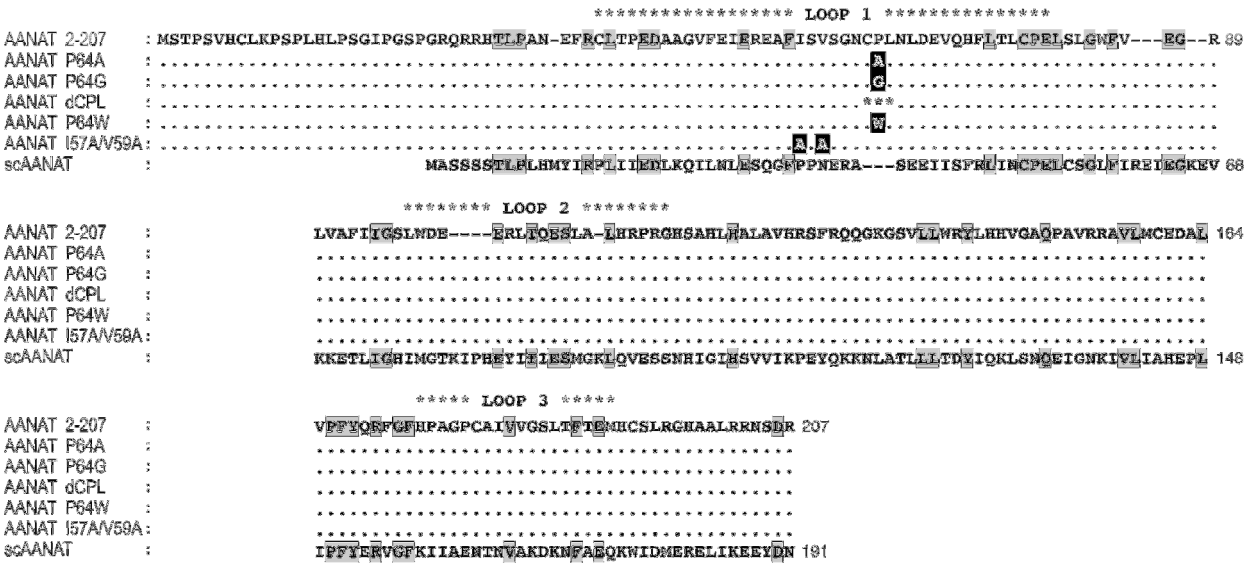
Sup Fig 1


1M Tris in 50% methanol, pH 8.0
HO
N
H
O
Br
+ CoA-SH
HO
N
H
O
S-CoA
N-[2-(7-Hydroxy-1-naphthyl)ethyl]-2-bromoacetamide
CoA
CoA-HNE

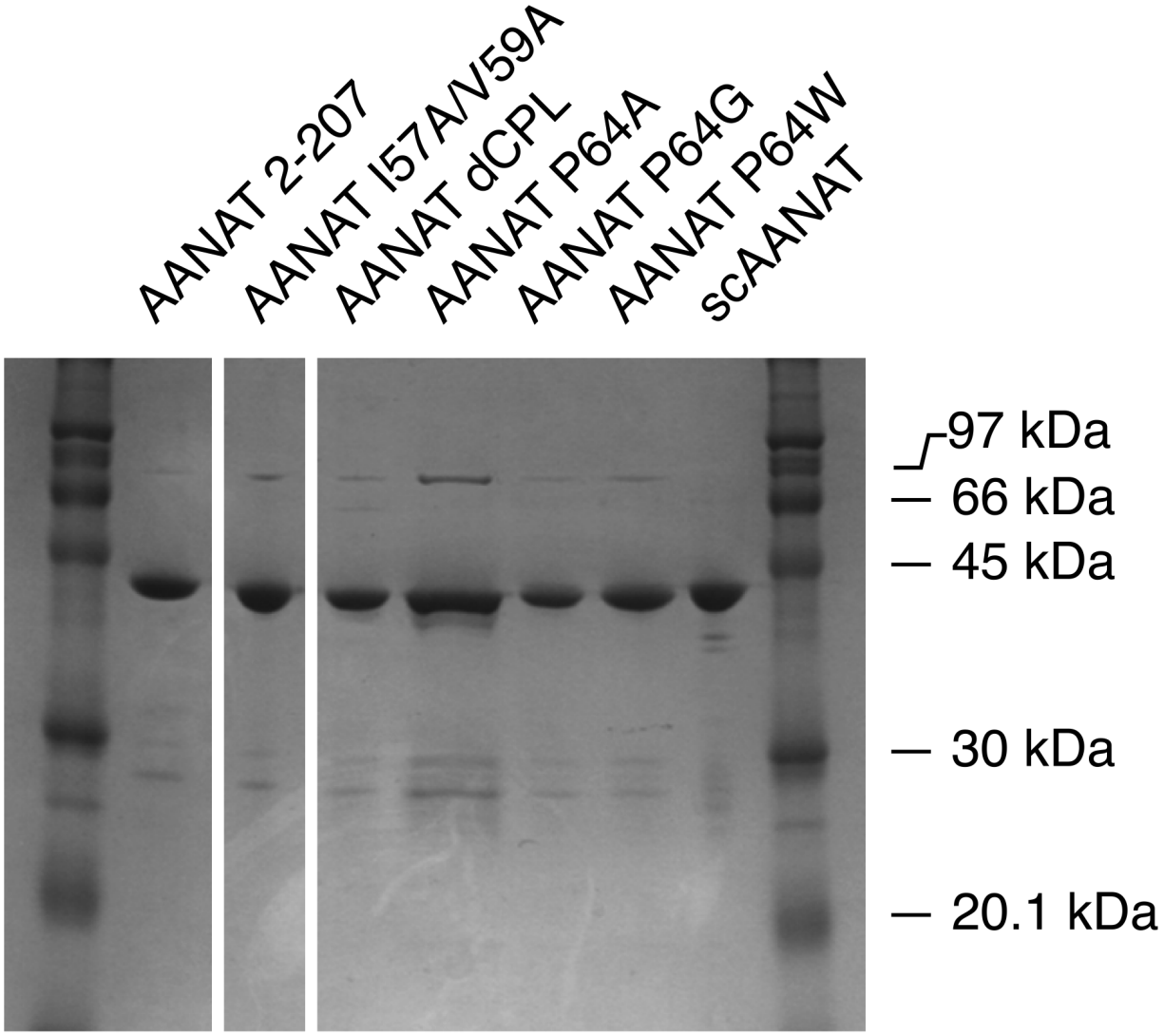
Sup Fig 2


A
5.83
5.83
Absorbance, mAU
1.0e4
5000.0
0.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5
Time, min
B
496.4
496.4
Intensity, cps
1.20e5
1.00e5
8.00e4
6.00e4
4.00e4
2.00e4
263.1
265.1
286.8
291.4
295.9
305.3
324.9
331.1
339.0
345.0
361.1
384.9
389.0
411.4
427.4
453.3
471.7
487.2
491.1
507.6
512.9
518.2
529.4
532.4
535.3
559.0
570.5
575.4
611.0
623.0
645.4
651.2
661.2
695.1
715.2
719.1
735.4
260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740
m/z, amu

Sup Fig 3


********** LOOP 1 **********
AANAT 2-207 : MSTPSVHCLKPSPLHLPSGIPGSPGRQRRHTLPAN-EFRCLTPEDAAGVFEIEREAFISVSGNCPLNLDEVQHFLTLCPELSLGWFV---EG--R 89
AANAT P64A :
AANAT P64G :
AANAT dCPL :
AANAT P64W :
AANAT I57A/V59A :
scAANAT : MASSSSTLPLHMYIRPLIIEDLKQILNLESQGFPPNERA---SEEIISFRLIMCPELCSGLFIREIEGKEV 68
******** LOOP 2 ********
AANAT 2-207 : LVAFIIGSLWDE----ERLTQESLA-LHRPRGHSAHLHALAVERSFRQQGKGSVLLWRYLHHVGAQPAVRRAVLMCEDAL 164
AANAT P64A :
AANAT P64G :
AANAT dCPL :
AANAT P64W :
AANAT I57A/V59A :
scAANAT : KKETLIGHIMGTKIPHEYIFIESMGKLQVESSNHIGIHSVVIKPEYQKKNLATLLLTDYIQKLSNQEIGNKIVLIAHEPL 148
***** LOOP 3 *****
AANAT 2-207 : VPFYQRFGFHPAGPCAIMVGSLTETEMHCSLRGHAALRRNSDR 207
AANAT P64A :
AANAT P64G :
AANAT dCPL :
AANAT P64W :
AANAT I57A/V59A :
scAANAT : IPFYERVGFKIIAEMTNVAKDKNFAEQKWIDMERELIKEEYDN 191

Sup Fig 4


AANAT 2-207
AANAT I57A/V59A
AANAT dCPL
AANAT P64A
AANAT P64G
AANAT P64W
scAANAT
97 kDa
66 kDa
45 kDa
30 kDa
20.1 kDa