Supporting Information for:

Citrullination Inactivates Nicotinamide-*N*-methyltransferase

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Running Title: Citrullination Inactivates NNMT

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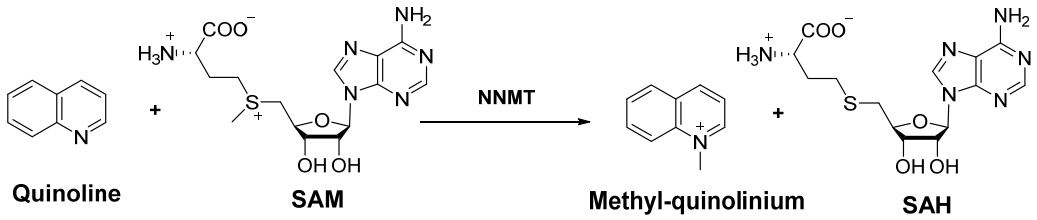
A



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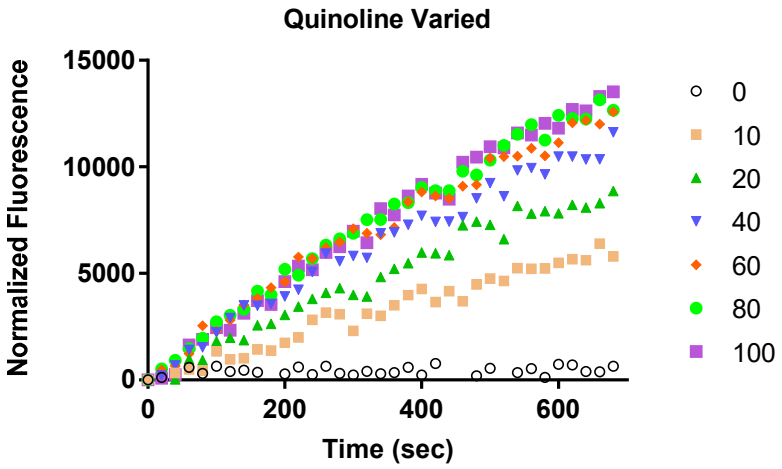
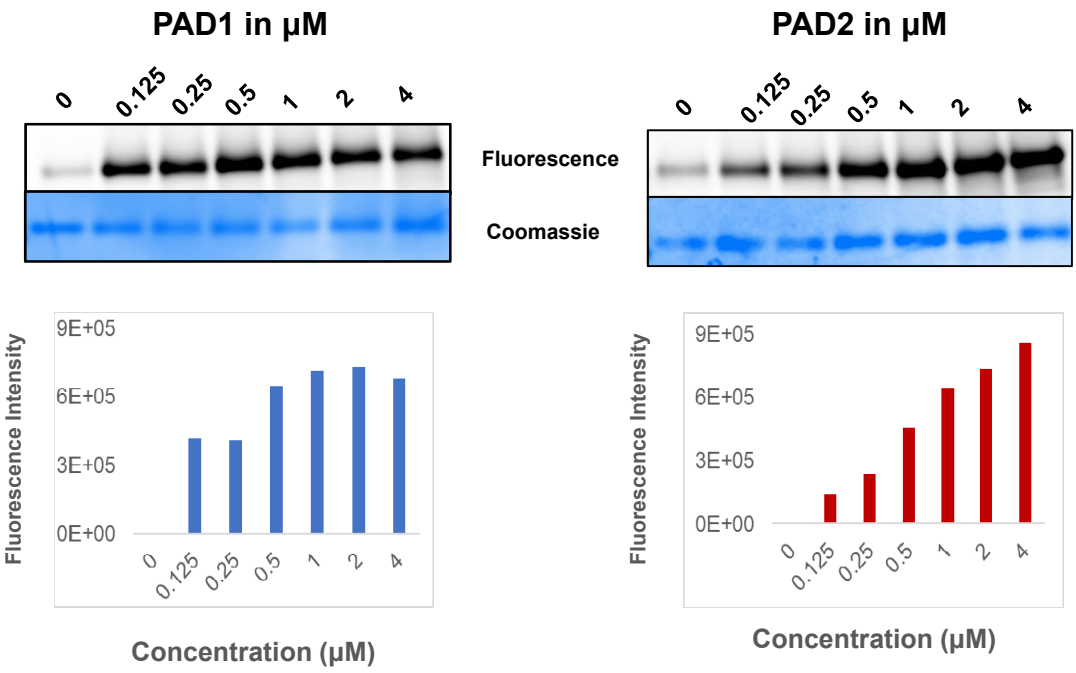


Figure S1. (**A**) Methyltransferase reaction catalyzed by NNMT with quinoline as a substrate and SAM as the methyl donor. (**B**) Progress curves of the reaction catalyzed by NNMT. The time courses are shown for various concentrations of quinoline at a fixed SAM concentration. (λ ex = 330 nm; λ em = 405 nm)

A



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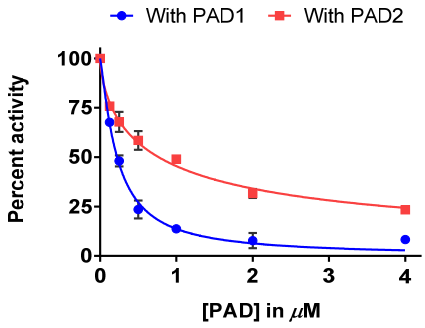
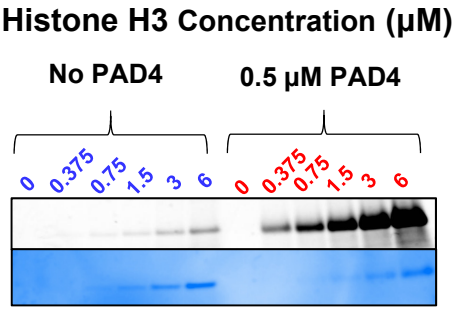


Figure S2. (**A**) NNMT citrullination as a function of increasing PAD1 and PAD2 concentrations. Fluorescence values are quantified and plotted as a bar graph in the bottom. (**B**) Loss of activity of citrullinated NNMT measured as a function of PAD concentration.

A



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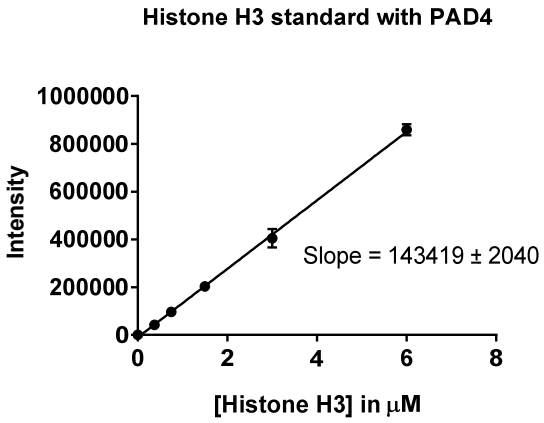

Figure S3. (**A**) Citrullination of Histone H3 by PAD4 visualized by Rh-PG. (**B**) Normalized fluorescence values are plotted against H3 concentration to obtain a standard curve. Experiments were performed in duplicate.

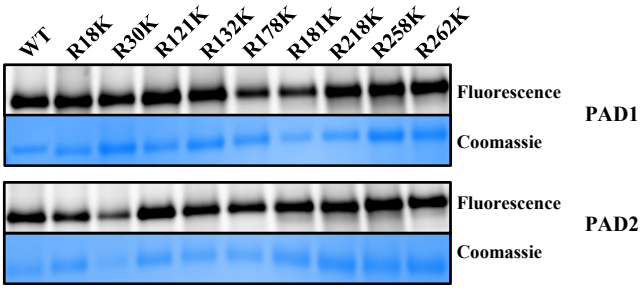

Figure S4. Citrullination of NNMT mutants by PAD1 and PAD2 visualized by RhPG labeling.

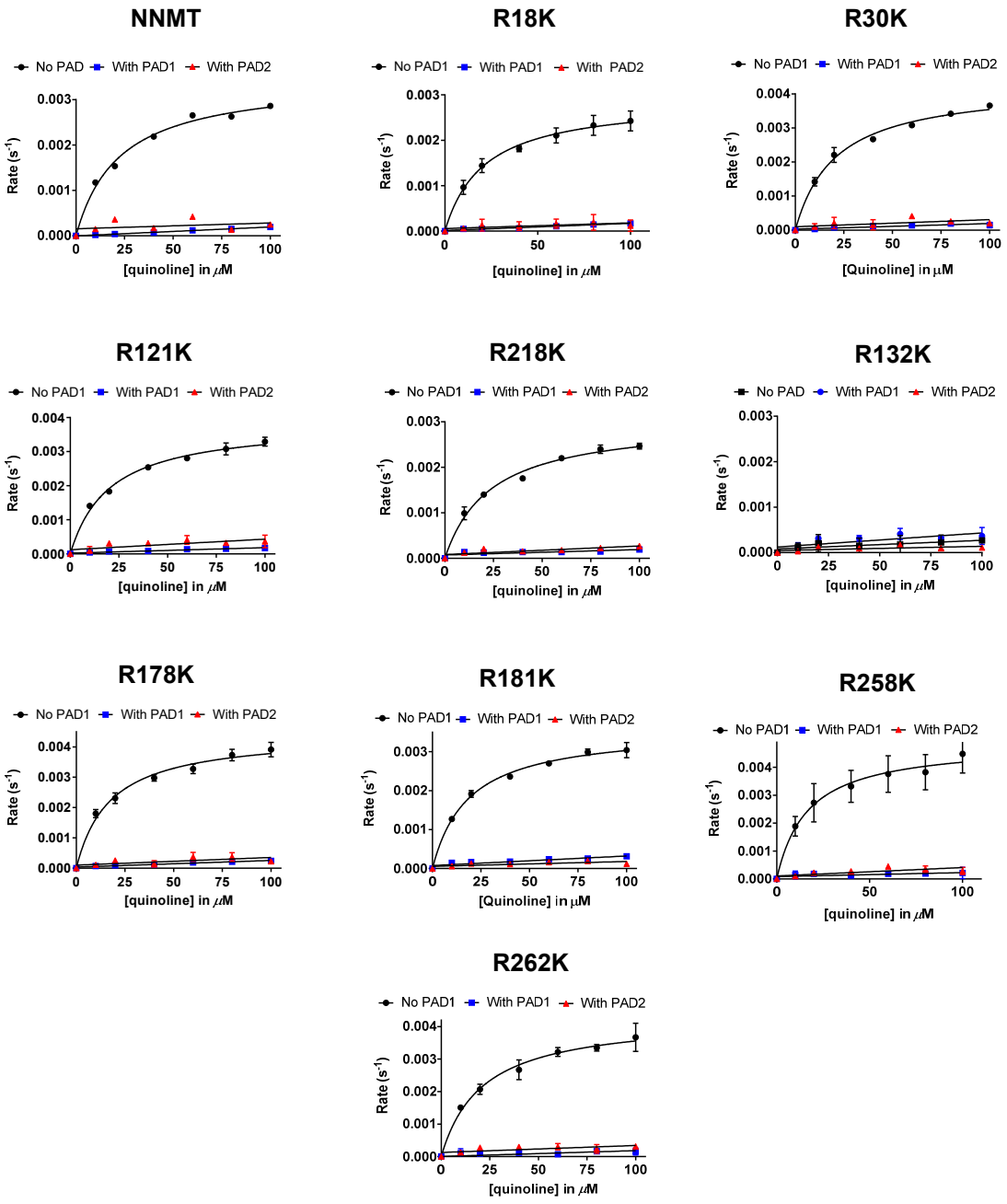

Figure S5. Michaelis-Menten curves of the methyltransferase reaction catalyzed by citrullinated and uncitrullinated NNMT mutants at varying quinoline and fixed SAM concentrations

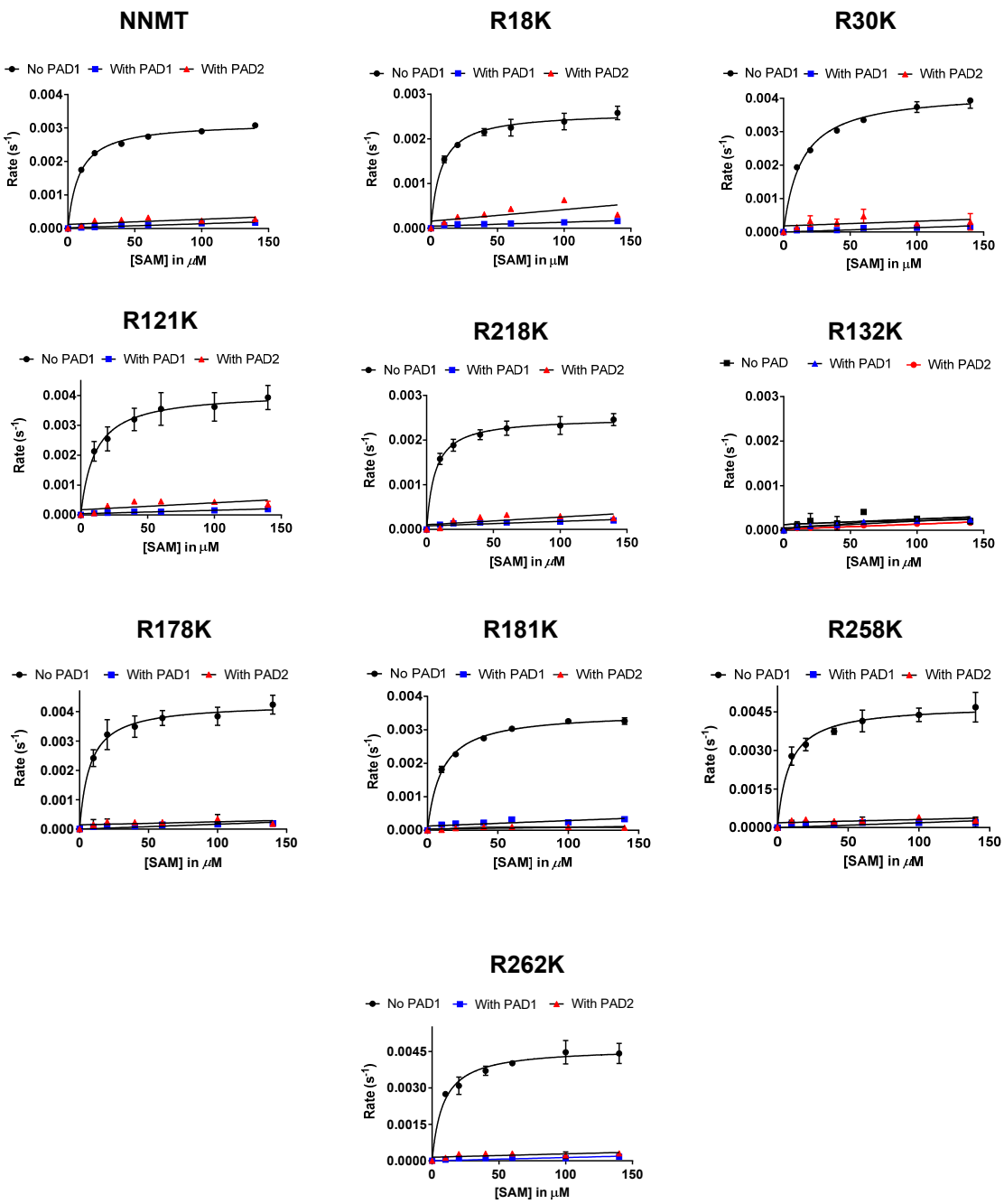

Figure S6. Michaelis-Menten curves of the methyltransferase reaction catalyzed by citrullinated and uncitrullinated NNMT mutants at varying SAM and fixed quinoline concentrations

Table S1. Steady-state kinetic parameters for citrullinated and uncitrullinated NNMT mutants at varying quinoline and fixed SAM concentrations[a]

	Without PAD	With PAD1	With PAD2
Proteins	k_{cat}/ K_m (M^{-1} s^{-1})	k_{cat}/ K_m (M^{-1} s^{-1})	k_{cat}/ K_m (M^{-1} s^{-1})
NNMT	180 ± 20	2.0 ± 1.0	2.5 ± 1.0
R18K	140 ± 30	2.0 ± 0.3	2.0 ± 1.0
R30K	200 ± 50	3.0 ± 0.5	2.0 ± 0.5
R121K	200 ± 50	3.0 ± 0.5	2.0 ± 0.8
R132K	2.0 ± 1.0	1.0 ± 0.5	1.0 ± 0.5
R178K	280 ± 40	3.0 ± 0.5	2.5 ± 1.0
R181K	200 ± 40	3.5 ± 0.8	2.0 ± 0.6
R218K	180 ± 30	2.0 ± 1.0	2.4 ± 1.0
R258K	280 ± 70	2.5 ± 1.0	3.0 ± 1.0
R262K	250 ± 40	3.0 ± 1.0	2.0 ± 1.0

[a]SAM concentration = 100 μM

Table S2. Steady-state kinetic parameters for citrullinated and uncitrullinated NNMT mutants at varying SAM and fixed quinoline concentrations[a]

	Without PAD	With PAD1	With PAD2
Proteins	k_{cat}/ K_m (M^{-1} s^{-1})	k_{cat}/ K_m (M^{-1} s^{-1})	k_{cat}/ K_m (M^{-1} s^{-1})
NNMT	350 ± 70	2.0 ± 1.0	2.0 ± 1.0
R18K	340 ± 50	2.5 ± 0.8	2.5 ± 0.8
R30K	470 ± 80	2.3 ± 1.0	2.3 ± 1.0
R121K	410 ± 40	2.1 ± 0.8	2.1 ± 0.8
R132K	2.0 ± 1.0	1.0 ± 0.3	1.0 ± 0.5
R178K	470 ± 80	3.0 ± 0.5	2.0 ± 1.0
R181K	350 ± 40	3.0 ± 0.8	2.0 ± 1.0
R218K	410 ± 60	2.3 ± 1.0	2.0 ± 1.0
R258K	450 ± 60	3.0 ± 1.0	3.0 ± 1.0
R262K	450 ± 60	2.6 ± 1.0	3.0 ± 1.0

[a]Quinoline concentration = 100 μM

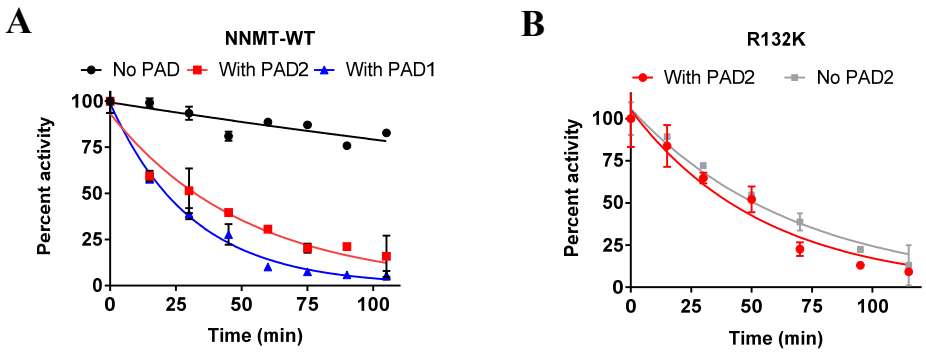

Figure S7. (**A**)) Time dependent inactivation of WT NNMT by PAD1 (blue) and PAD2 (red). The loss in activity upon citrullination was measured using the quinoline assay. Time dependent change in the activity of uncitrullinated NNMT is shown as black circles. **(B)** Time dependent inactivation of the NNMT R132K mutant as a result of PAD2 (red). Time dependent change in the activity of uncitrullinated NNMT R132K is shown in grey.

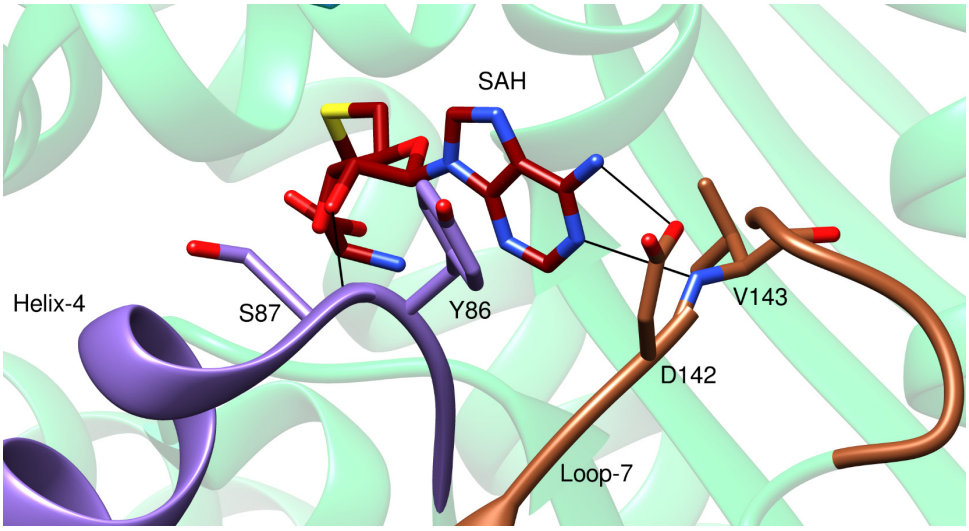

Figure S8 Interactions of SAH with residues from helix-4 and loop-7. The phenyl ring of Y86 stacks with the adenine ring of SAH. 2'-OH of the ribose moiety interacts with the backbone NH of S87. D142 and V143 of loop-7 interacts with the adenine ring by making hydrogen bonding interactions.

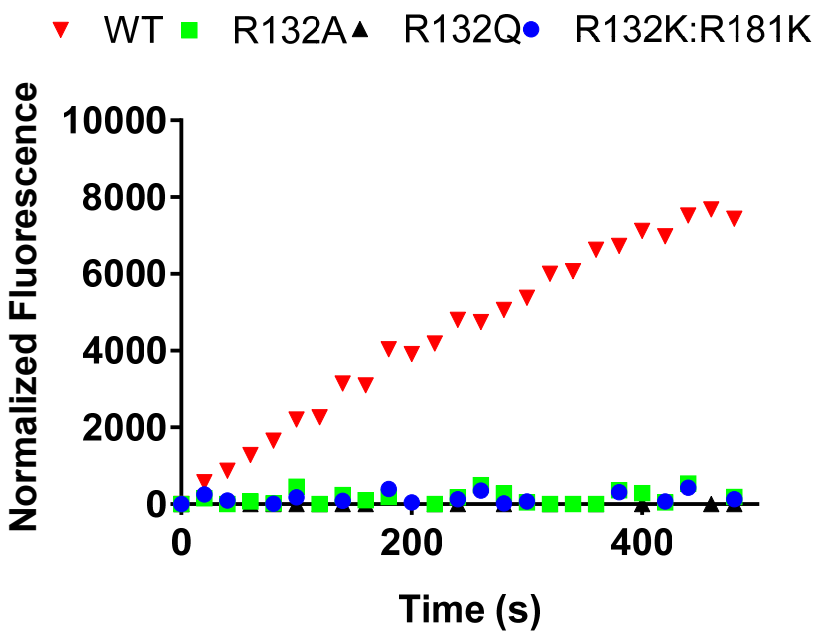

Figure S9. Time course of NNMT reaction at 100 μM quinoline and 100 μM SAM concentration. R132A, R132Q, and R132K:R181K are inactive.

Table S3. Primers for mutagenesis

R18K	Forward - GCCATTTTAACCCT*AAG*GATTACCTA Reverse - TAGGTAATC*CTT*AGGGTTAAAATGGC
R30K	Forward - AGTTTGGTTCT*AAG*CACTCTGCAGAAAG Reverse - CTTTCTGCAGAGTG*CTT*AGAACCAAACT
R121K	Forward - CTTGAAGGGAAC*AAG*GTCAAGGGTCCA Reverse - TGGACCCTTGAC*CTT*GTTCCCTTCAAG
R132K	Forward - GGAGGAGAAGTTGAAACAGGCGGTCAAGCAGGTGCTG Reverse - CAGCACCTGCTTGACCGCCTGTTTCAACTTCTCCTCC
R178K	Forward - CAGACCTCCCCACCTACTGC*AAA*GCGCTCAGGAACC Reverse - GGTTCCTGAGCGC*TTT*GCAGTAGGTGGGGAGGTCTG
R181K	Forward – CTGCAGGGCGCTC*AAG*AACCTCGGCAGCCTACTGAAG Reverse – CTTCAGTAGGCTGCCGAGGTT*CTT*GAGCGCCCTGCAG
R218K	Forward – GCCTCCCCCTGGGC*AAA*GAGGCAGTAGA Reverse - TCTACTGCCTC*TTT*GCCCAGGGGGAGGC
R258K	Forward - CTTTTCTCCCTGGTGGCG*AAG*AAGCTGAGCAGAC Reverse - GTCTGCTCAG*CTT*CTTCGCCACCAGGGAGAAAAG
R262K	Forward - CTCCCTGGTGGCGAGG*AAG*CTGAGCAAACCCCTG Reverse - CAGGGGTTTGCTCAG*CTT*CCTCGCCACCAGGGAG
R132A	Forward - GAGGAGAAGTTG*GCA*CAGGCGGTCAAGCAG Reverse - CTGCTTGACCGCCTG*TGC*CAACTTCTCCTC
R132Q	Forward - GAGGAGAAGTTG*CAA*CAGGCGGTCAAGCAG Reverse - CTGCTTGACCGCCTG*TTG*CAACTTCTCCTC