Product feedback regulation implicated in translational control of the *Trypanosoma brucei S*-adenosylmethionine decarboxylase regulatory subunit prozyme

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Supplementary Material
Figures.

Figure S1. Cloned Prozyme mRNA 3'UTR RACE products. Primers used to generate PCR fragments from cDNA are displayed in Table S1. All clones were sequenced in their entirety in both directions. The sequence is number from the first gene specific base after the spliced leader sequence in the 5'UTR.

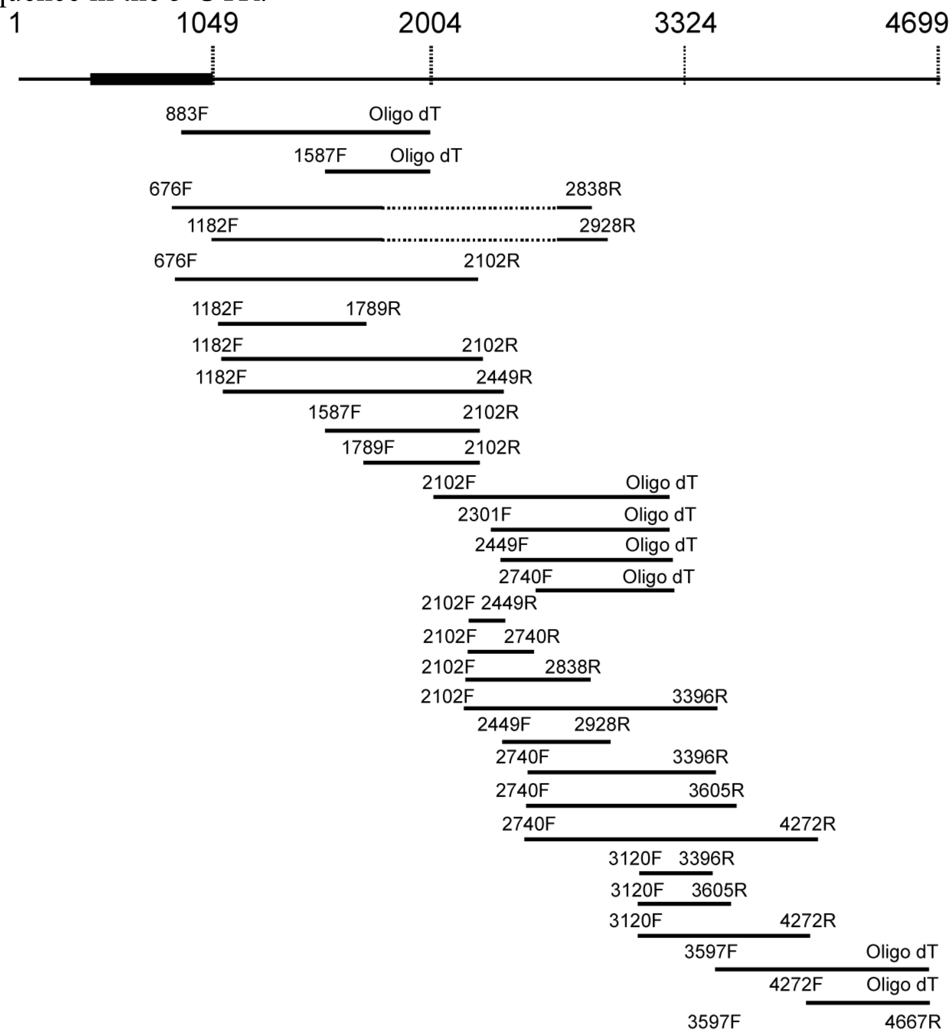

Figure S2. A. Sequence of the prozyme gene (Tb927.6.4470). The mRNA 5'UTR is shown in purple, the coding sequence is shown in red, the 3'UTR is shown in black and the region of secondary structure is shown in blue. The sequence adjacent to the polyadenylation (poly(A)) site of the 4.7 kb message is boxed in turquoise. The genome encoded poly (A) tracks at positions 2004 and 3324 leading to stops in the RACE analysis are shown in italics and highlighted in yellow. The poly pyrimidine (poly(Y)) track and possible AG splice sites that could signal splicing/polyadenylation of the shorter messages are underlined. Sequences in bold italics represent the region of secondary structure providing an exact complementary base pair match that led to loop out of the turquoise sequence when using Superscript III reverse transcriptase. Bold red sequence represents Northern probe 1 and the bold turquoise sequence represents Northern probe 2. Sequences in black bold represent primers used to generate PCR fragments in Figure 1C. The sequence has been numbered from the start of the 5'UTR absent the splice leader sequence (shown in small letters). The sequence data were obtained from sequencing the clones described in Figure S1 in their entirety in both directions as described in materials and methods.

(-30)-cgctattattagaacagtttctctatattg 1-
TGCGTTACTTCTGCTGCACGGCGAGTTGAGGGGAGGCAAACTGGTTTTACACCAGAGGGAAACGGTAAGGATGTC
GGTCACGCGGATTAACCAGCAAACCGAGTGCCCAAGTTCGGTGCATGACCTTGTGTCATGTTGGGGAGGTTGCAC
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TTCAGTGATGCTACACGAAGCGGTACACCCGATGCTTGAGCTCATGCGCTCCCACAACATTATTGTGGATTGGGC
G**TCTTTCATGCGGGTCAACTACGGCTCGCCTTGGGACATGACATCGGAAACGAGTGATATTATGGCTCATGAATA**
CGCCGAGTTGAAGTCCGCGTTCCCAACGGGCCACCCGTACCTAGCTGGTCCTGTTGACCGTGATCACTGTTTTTA
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CGCGGGGGAGTACGAAACGTTGCGAGTGTCAACATATGGGTCCACGCATCCCTTTGCCTCCTTCGAAACGAATGC
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TCCAGTACAGATGCTGAAACGCTTCTGAAGGTTTTTTTAATGGTCACCCTGGTCAGCGCGAAAACACACCTCAG
CTTTTTAGGTATTTTGGGTGCCATACAAGTATATATATATATATATATATATATGT**ATATGGATACTCTCCCCCT**
AATTTTTACAGTTATTCTATGGTAGTCGGTTTTAAAGATGAGGAGTAAAACGGAGTCATTCCATTGTGCGTAAAT
CGGTTTATGTATATTTGTTGATTTGTCGTTTTTATCTGCAATGGAGGGCTCTTTTTTTTTTTTAACATCCGCTTA
TTAAGCATCATCACTGTCAAAGGTTCGAGAGTTTTAATGCCTCTTTTGTCATTTCATGTGCCAGTTTATTGAGTT
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GTGTCCAACGGTGCGAAGAAAGAAAATATTTTAACTGCTGAACCCGCTTCTTCCCCTGTGATTCTTTCCCGCCCG
TCCTCATGTTTCCACAGCAGCCGCCAAGAGGCACACATTTTCCTGTTTTTTGTTTTCTGGTGTTTTTCATTCATA
CGCATACGCGCCTTTCAATTATCTCTATTAGGCGCGTGCGAAGGGGTGTCAGCAGGCAAGTGACTGTGTTTTTTG
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TGCAGTGGTTCTGTTCATCAACAAAAGCGAATATTGTGGTTGTGGTAAAACCTTTTCGGGGGGCA***TTCACCCCTC***
CCTCCTGTAGCCAACGCCCACGGACACGCGGGCAGCTCGAACTGGCGGATAGGCGCACACAACAGCAGCTAAAAT
GGCGGCGCTCACGACGAACGATGAGTACCCGCACGCTTCAGATTTTTCTTCCTG*AAAAAAAAAAGGAAAAATA*GA
AAAAGAAATCTGGTTCGCTTGCCTGAGCATTTCTTTTTTTTTAATTTTTTGTTTTAAAATAATTCTAGCACTGTA
TCGAACCTTGTTCAATACATTAGGTAATAAACGCGGGACAACTGCTTTTCCTTTACTCTACCCCACAAAACCCTA
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ACTTCAGACGTCTCCGTTGCCCCGTTTCGTTTCATAATCACTAAACAAGTGGAGCGTGATTTGGTTTGCACGAAA
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AGGCATTAAAAGAGAACAGGCTAATGAACAGCTGGGAAAGAAAACCAAATCGAGAAGCGGCGTGTTACGTGAAGT
GG**CTTACCTTCGTTCAAATTAGTGGTGTCTC**ATCCCCTTCATTTTCGTTCTTTCTTTTTTCCTCCCCGTATCATT
TCACTATTTTTTCCAACCTTGTTTCGCTTTCATGGTTCCCACCACCAGCACCTCTTGGTAATGCCTATTTTCCTT
TTCTTTTTTTGTCCAGCTGTACAGCTGGTTTAAAACTATTCGATGGACAACGGCACAGGAATGACGGAACAGTAA
CGGAAACAAAGATGTGAGTATTTGTGGGTGGGAGCTTGAAAATGAATACGCGCGCGTCCGTACGTTTGTATTTGC
GATTTGAAAGGTGGATAGAGCCGCTAATTTACGTTACTGAGCGAAAGCCTTAACAGACTTTCAGCAGCGGGGTTT
TTGTTGCATACAAACTTATTTATC*AAAAAGAAAAAAAAAGAAA*GAACCATTTGTCGCCGTTACGTATTGCTTTTCC
TGCCCCTCGCCCTTCTTGTTCACTTCTACTTCCTTTCCTTACCATCACTGGGGGCGGAAGATCATATGGTTATAT
ACCAAGCAGCGGTGGTATTGAAAGGAAGTAGCTGAAGTTATGTCATCCGACAGGAAGCGAGGTGACGGTTAAAGA
GAAACGCAACAGGCCGACACAAGCACAAGTCAAGAGAAGTTGAAACAACAGAAGCGAAAGAGGTGATTAGACCTT
CAAGCAGGTATAAGGAAGAGGATCAAGAGTAATATGAAAGAAAAAGAATGAAATGAAGTGTAGAGTTGATGAATT
ATCGAATAATAGTAACAAAATATCTACGACTTTCTCCCTCTGCTTTCGTGTGTGGCTGGAGAAATGCTGCATTAA
CATGTGTTATGAGGTATAGCACTGGAAGGAAAAGAATCAAGTAGCGTTTTAGAAGAAGAGAAATGATGCCGATCG
GAAGGGAGAAGGTGATACGATGTCGGAGGGGTGACGCTGCCAAATACATGTACCTGAGTCTGTGTGTTTTAAGGA
TAGATATGCGTAACATACGAGTCTGGAAACCAGAGGCTGATGGTGATGTGAGGGGTGATTGAGACGTATGAGAAT
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GAGAGCGCATGATAATGTGAGGAACTACCAGCATGTTTCTGCTCCTCTCAGTTTTGTGATGTTGCTGTTGTTGCC
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AAGGGAGAGTGAAGGATAATAGTCAGCTGAGGTGACGGGAGAGGAAGACATATTTGTATACATACCTACACATAT
CAATATATATGTGTATATGTATACGTTCCTCCCCAAATTAGTATCACCTGGGGTTTCTCCTGTGTGACGAGGTCT
AACTCAAAAGGGTTAAAGGAAGACGCAAAAGAGATTAATTCTCTTGGCTTCATTTTTGGTTGAGCTTCACAACCT
CCGTGTGCCGAGTTTCCATAAATGCCTTTTTGTCGACTCGCACTTGTGA – 4699

Figure S2B. Downstream gene and 3' splice site for the 4699 kb prozyme mRNA. Data were taken from tritrypdb.org/. The downstream gene is AdoMetDC (Tb927.6.4460) and the sequence in blue represents the end of the prozyme 3'UTR from the 4.7kb mRNA as mapped by RACE (the polyadenylation site) and yellow represents the start of the 5'UTR of the downstream AdoMetDC gene. The AG splice site is in bold, the upstream poly(Y) track is underlined and in bold and the ATG start site of the AdoMetDC gene is in italics and underlined.

GCTTCACAACCTCCGTGTGTCAAGTTTCCATAAATGCCTTTTTGTCGA*C*TCGCACTTGTGATGTAGGCACCACTAATA
CCTG**CCCTTTCCCTTCTCTTTCTTTTCTCTTCTCCCT**ATTCTATCTTATTCATATTTTATTTCCCTCGTTTCGGATTT
TTGACCTTTTTCTGTGG**AG**TTTGTACGCGTATCCTCGAGCACAAAAAGTACATTGTTTGTTGAGCTTGCACTCTTAGG
AAGCGCACCATTCCTTTGTGGGCCTGCGTGTGTGCGTGTCTGGTTTGGTTATTTAATCCGTAAGGTGCTATATTTGAC
CGCACACTGACTGAAGTACTAGTC*ATG*T*CC*TCTTGCAAGGACTCTCTTTCGCTCATGGCGATGTGGGGTTCCATCGCT
CGTTTTGACCCAAAGCACGAGCGAAGCTTCG

Figure S3. RNase protection assay (RPA) of RNA isolated from BSF *T. brucei*. A. RPA probe spanning 94 bases of sequence 5' to the region of secondary structure in the prozyme cDNA and 114 bases down stream of the 3' region of secondary structure. A 41-base tag was included on the 5'end to prime T7 polymerase. **B.** RPA of total RNA extracted from BSF 427 cells and from BSF 90-13 cells transiently transfected with construct 4a or 4b to serve as controls. Arrows indicate bands at the expected sizes of either the fully protected probe (208 nt) or of the two fragments (114 and 94 nt). MspI digested pBR322 DNA (New England Biolabs) was used as markers (M).

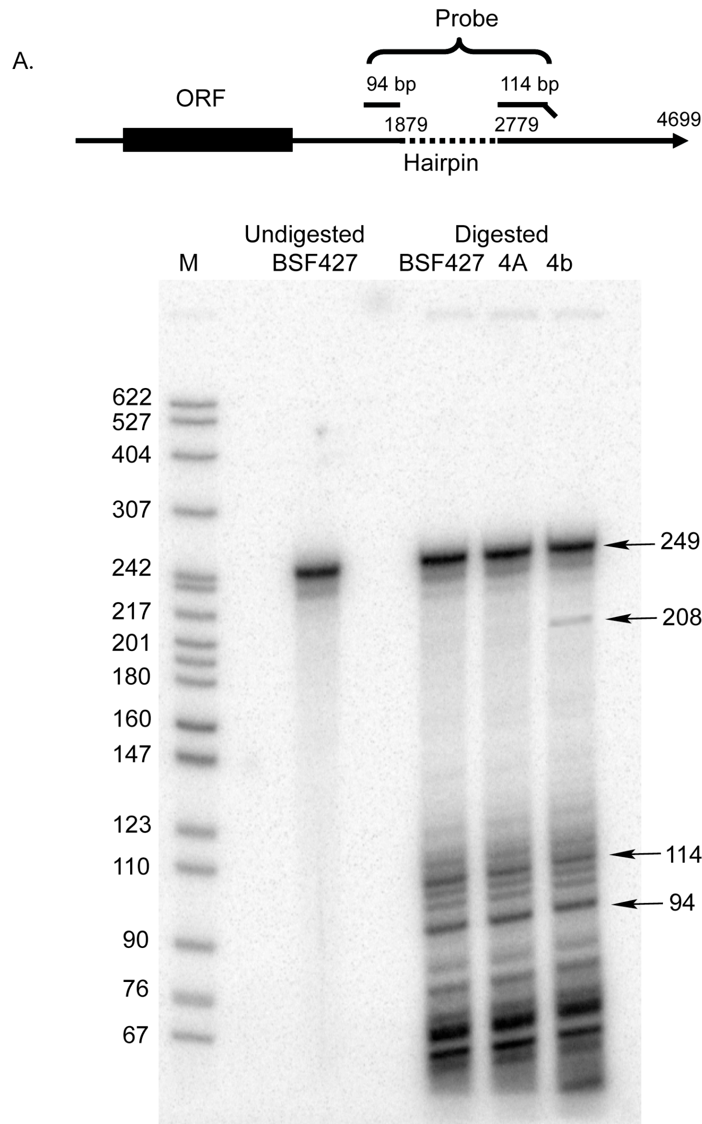
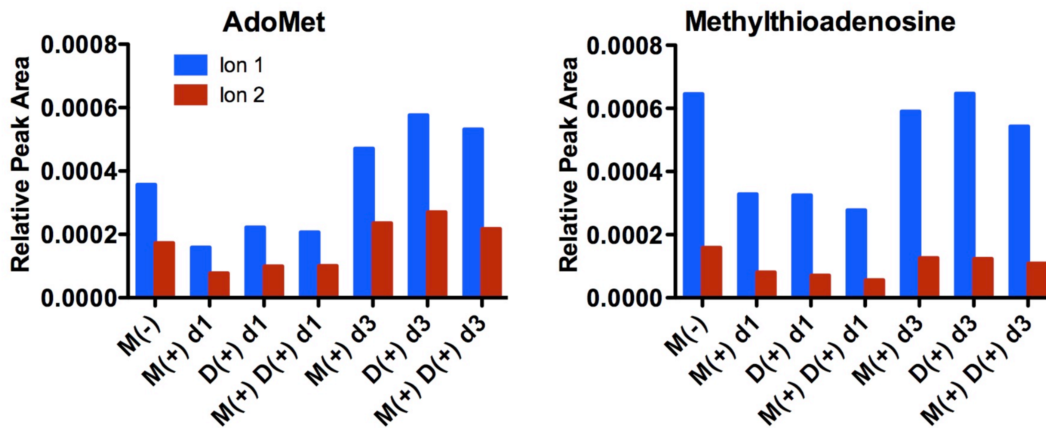


Figure S4. LC-MS/MS analysis of polyamine and AdoMet pools after knockdown or chemical inhibition of AdoMetDC. A. The effects of chemical inhibition of AdoMetDC and/or ODC on the intracellular AdoMet and methylthioadenosine (MTA) pools. BF427 cells were treated with MDL 73811 (75 nM) (M) and/or DMFO (12.5 μM) (D) for either 1 (d1) or 3 (d3) days before harvesting (n = 1). After acid extraction from cell lysates, intracellular AdoMet and MTA levels were analyzed by LC-MS/MS. Data complements that shown in Figure 3C. **B.** The effect of knockdown of AdoMetDC by RNAi on polyamine and AdoMet pools. RNAi (*T. brucei* AdoMetDC RNAi cell line) and RNAi-C (*T. brucei* AdoMetDC RNAi cell line complemented with human AdoMetDC) cells lysates were analyzed by LC/MS three days post Tet induction. Data were collected for MDL 73811 in parallel and are shown. Errors represent the standard error of the mean for n=3 independent biological replicates. For each compound two separate daughter ions (represented by the red and blue bars) were followed by MS to provide additional conformation of the data. For dcAdoMet a relative peak area of 1 x10^{-6} for the more abundant ion represents the lower limit of quantitation. Daughter ion pairs were as follows: AdoMet (ion 1, 355/250; ion 2, 399/136), dcAdoMet (ion 1, 355/250; ion 2, 355, 136), MTA (ion 1, 298/136; ion 2, 298/119), putrescine (ion 1, 89/72; ion 2, 89/71) and spermidine (ion 1, 146/72; ion 2, 146/84).

A.



B.

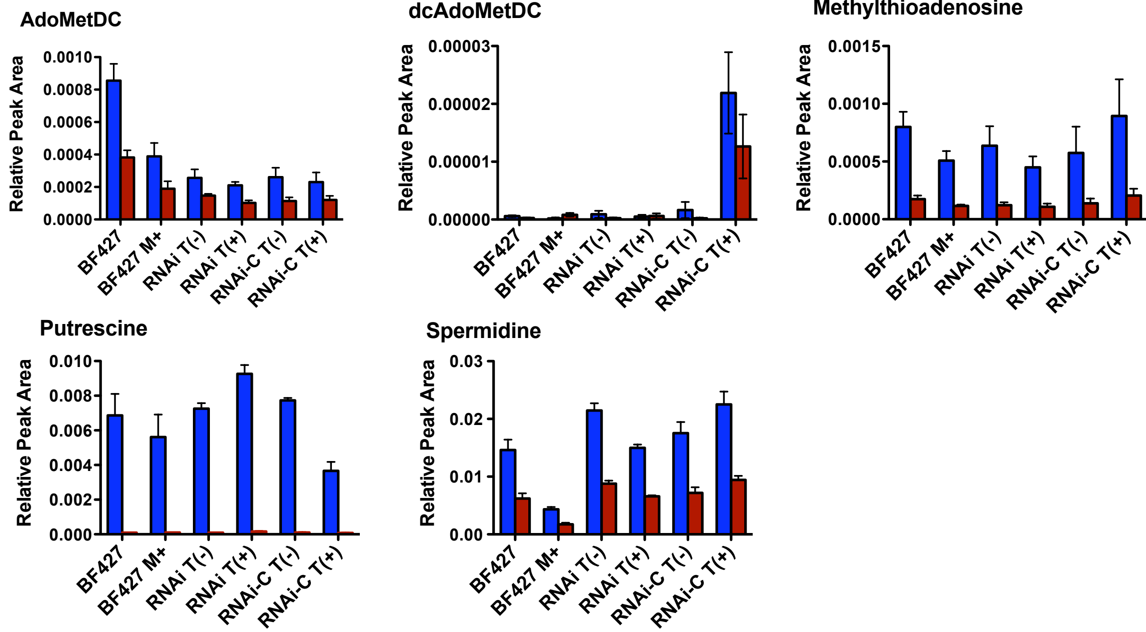


Figure S5. Exogenous dcAdoMet does not rescue the cell growth arrest caused by AdoMetDC inhibition or depletion by RNAi. A. BSF *T. brucei* 427 cells were treated with MDL 73811 (225 nM) with and without the addition of exogenous dcAdoMet (50 µM). B. *T. brucei* AdoMetDC RNAi cells were treated with and without Tet (1 µg/ml) to induce AdoMetDC RNAi both in the absence and presence of exogenous dcAdoMet (50 µM). Similarly dcAdoMet was unable to rescue the growth defect caused by treatment with 75 nM MDL 73811 (data not shown).

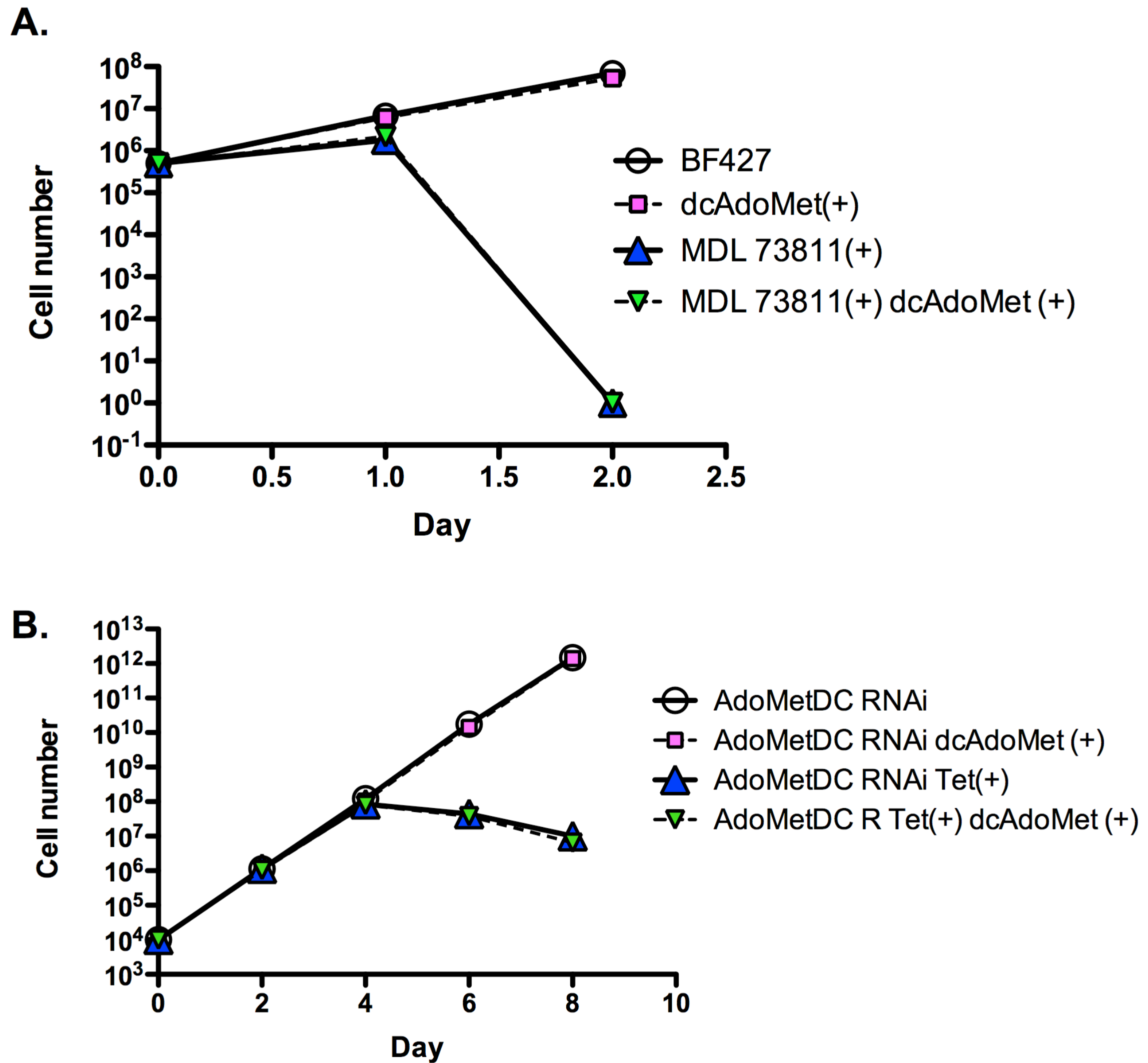


Table S1. PCR and sequencing primers (mismatches between our sequence (Figure 2) and the sequence in the TriTrypDB used to generate primers are shaded in yellow)

Primer name	Sequence
Forward Primers	
676F	5'- GTA CAA CGT ACA AGC AGA TGA TGA GTA TG -3'
883F	5'- CCC GGA GAG AGT TCT GCT CGT GCT GCT GCA GGA C -3'
1182F	5'- ATA TGG ATA CTC TCC CCC TAA TTT TTA CAG -3'
1587F	5'- CCA TAG CAG CCG TCA AGA GGC ACA CAT TTT CC -3'
1789F	5'- GCT TGC GCC TGA TGC AGT GGT TCT GTT CAT CAA C -3'
2102F	5'- CGA ACC TTG TTC AAT ACA TTA GGT AAT AAA CGC GGG -3'
2301F	5'- GGA GCG TGA TTT GGT TTG CAC GAA AGT GG -3'
2449F	5'- GCT GGA AGT GAA AGG TGC TGC CTA ATA GT -3'
2740F	5'- CTG CCG TAA TGG TGT ATG TGT CTT GTG GGT -3'
3120F	5'- GGA CAA CGG CAC AGG AAT GAC GGA ACA GTA ACG -3'
3597F	5'- CCT TCA AGC AGG TAT AAG GAA GAG GAT CAA GAG TA -3'
4272F	5'- GTT CTC AGT TTA TCT TAC CCA CTT TCC AGG -3'
Reverse Primers	
1789R	5'- GTT GAT GAA CAG AAC CAC TGC ATC AGG CGC AAG C -3'
2102R	5'- CCC GCG TTT ATT ACC TAA TGT ATT GAA CAA GGT TCG -3'
2449R	5'- ACT ATT AGG CAG CAC CTT TCA CTT CCA GC -3'
2740R	5'- ACC CAC AAG ACA CAT ACA CCA TTA CGG CAG -3'
2838R	5'- CTC TTT TAA TGC CTG ATA GGA ACT GCC -3'
2928R	5'- GAG ACA CCA CTA ATT TGA ACG AAG GTA AG -3'
3396R	5'- GAT GGT AAG GAA AGG AAG TAG AAG TG -3'
3605R	5'- TAC TCT TGA TCC TCT TCC TTA TAC CTG -3'
4272R	5'- GGA AAG TGG GTA AGA TAA ACT GAG AAC -3'
4667R	5'- TCA CAA GTG CGA GTC GAC AAA AAG GCA TTT ATG -3'

Table 2S. Cloning primers for CAT reporter constructs (sequences underlined are restriction enzyme sites, mismatches between Figure 2 and the TriTrypDB sequence are shaded in yellow)

	Sequence
Fragment 1	Forward 5'- ATC AGC GGA TCC TTT CCA GTA CAG ATG CTG AAA C-3'
	Reverse 5'- CAA AGT ACG CGT GAG GGA GGG GTG AAT GCC CCC C-3'
Fragment 2	Forward 5'- ATA AGA GGA TCC CTG TAG CCA ACG CCC ACG GAC -3'
	Reverse 5'- CAA ATT ACG CGT TGC CCG CGC ACC CAC AAG ACA CAT-3'
Fragment 3	Forward 5'- ATC AGC GGA TCC TTT CCA GTA CAG ATG CTG AAA C-3'
	Reverse 5'- CAA ATT ACG CGT TGC CCG CGC ACC CAC AAG ACA CAT-3'
Fragment 5	Forward 5'- ATC AGC GGA TCC TTT CCA GTA CAG ATG CTG AAA C-3'
	Reverse 5'- GAT CGT ACG CGT CTC CTT TAT AAG ACG AAA AAG AGG -3'
Fragment 6BM	Forward 5'- AAT ATT GGA TCC CGC GGG CAG GGA GGG GTG AAA AAG -3'
	Reverse 5'- CGC AGT ACG CGT TCA CAA GTG CGA GTC GAC AAA AAG GCA TTT ATG -3'
Fragment 6MS	Forward 5'- CAA GTG ACG CGT CGC GGG CAG GGA GGG GTG AAA AAG -3'
	Reverse 5'- CGC AGG GTC GAC TCA CAA GTG CGA GTC GAC AAA AAG GCA TTT ATG -3'
Fragment 8	Forward 5'- CAA TCT GGA TCC TGG AGA AAT GCT GCA TTA AC -3'
	Reverse 5'- CGC AGT ACG CGT TCA CAA GTG CGA GTC GAC AAA AAG GCA TTT ATG -3'
Fragment 9BM	Forward 5'- AAT ATT GGA TCC CGC GGG CAG GGA GGG GTG AAA AAG -3'
	Reverse 5'- CGC AGT ACG CGT GAT AAA TAA GTT TGC ATG CAA C -3'
Fragment 9MS	Forward 5'- CAA GTG ACG CGT CGC GGG CAG GGA GGG GTG AAA AAG -3'
	Reverse 5'- CGC AGG GTC GAC GAT AAA TAA GTT TGC ATG CAA C -3'

Table 3S. Primers employed for PCR, real time PCR, and synthesis of RNA probes for northern blot analysis and RNase protection assay. Sequences in blue represent the T7 promoter, the underlined sequence in the RPA probe represents added random sequence to extend the probe length, and mismatches between Figure 2 and the TriTrypDB sequence are shaded in yellow.

		Sequence
Primer set 1	Forward	5'- GTA CAA CGT ACA AGC AGA TGA TGA GTA TG -3'
	Reverse	5'- CTC TTT TAA TGC CTG ATA GGA ACT GCC -3'
Primer set 2	Forward	5'- ATA TGG ATA CTC TCC CCC TAA TTT CTA CAG -3'
	Reverse	5'- GAG ACA CCA CTA ATT TGA ACG AAG GTA AG -3'
Probe 1	Forward	5'- TCT TTC ATG CGG GTC AAC TAC GGC TCG -3'
	Reverse	5'- TAA TAC GAC TCA CTA TAG GGG TGA AAC CTT CCA ATC TAT C-3'
Probe 2	Forward	5'- GGA CAG GAA GAA GGC ACT GGT ACT TCA GAC GTC TC-3'
	Reverse	5'- TAA TAC GAC TCA CTA TAG GGA CAC CAT TAC GGC AGC TCT T -3'
Human AdoMetDC	Forward	5'- GGC ATA TGG AAG CTG CAC ATT TTT TCG AAG G-3'
	Reverse	5'- GGG GAT CCT CAA CTC TGC TGT TGT TGC TGC TTC-3'
CAT	Forwrad	5'- CAT TTT GAG GCA TTT CAG TCA G -3'
	Reverse	5'- TAA AGG CCG GAT AAA ACT TGT G -3'
TERT	Forwrad	5'- GAG CGT GTG ACT TCC GAA GG -3'
	Reverse	5'- AGG AAC TGT CAC GGA GTT TGC -3'
RPA probe		5'-TAATACGACTCACTATAGGGCTCTGCTCTTCTCGCACCGCATTCTTTCCCAGCTGT TCATTAGCCTGTTCTCTTTTAATGCCTGATAGGAACTGCCGCCGCCGTCCATCTACTATCCTCAATAGCGGGTAA CCACACAACTTTTTCACCCCTCCCGAGGGAGGGGTGAATGCCCCCCGAAAAGGTTTTACCACAACCACAATATTC GCTTTTGTTGATGAACAGAACCACTGCATCAGGCGCAAGCTGG -3'

Table 4S. 3'UTR CAT reporter constructs

	Start	End
Construct 1	1049	1879
Construct 2	1880	2778
Construct 3	1049	2778
Construct 4a	1049	3324
Construct 4b (minus region of secondary structure)	1049 2779	1879 3324
Construct 5	1049	2555
Construct 6	2779	4699
Construct 7	1049	4699
Construct 8	3731	4699
Construct 9	2779	3324
Construct 10	1049	2004

Table 5S. Summary table of CAT enzyme activity and mRNA level in cells transfected with prozyme mRNA 3'UTR reporter constructs. Data shown are averages and the standard deviation of the mean for n=3-4 independent biological replicates and represent the fold change when cells were culture with MDL 73811 (M) versus without the compound (M+/M-). The average data are plotted in Figure 2.

	Protein Fold change	mRNA Fold change
Negative control	1.2 ± 0.22	1.0 ± 0.14
Fragment 1	1.4 ± 0.30	1.3 ± 0.59
Fragment 2	0.42 ± 0.02	1.0 ± 0.26
Fragment 3	7.9 ± 1.79	1.3 ± 0.35
Fragment 4a	7.7 ± 4.5	1.1 ± 0.21
Fragment 4b	4.8 ± 1.3	1.1 ± 0.23
Fragment 5	3.4 ± 1.4	1.8 ± 0.95
Fragment 6	4.0 ± 2.8	0.91 ± 0.16
Fragment 7	2.5 ± 1.3	1.4 ± 0.50
Fragment 8	1.2 ± 0.84	1.3 ± 0.64
Fragment 9	1.5 ± 0.33	0.79 ± 0.17
Fragment 10	1.1 ± 0.22	0.91 ± 0.13