Supporting Information for:

Initial Kinetic Characterization of Sterile Alpha and Toll/Interleukin Receptor Motif–Containing Protein 1

Heather S. Loring[1,2], Janneke D. Icso[1,2], Venkatesh V. Nemmara[1,2], and Paul R. Thompson[1,2]*

[1]Department of Biochemistry and Molecular Pharmacology, UMass Medical School, 364 Plantation Street, Worcester, MA 01605, USA

[2]Program in Chemical Biology, UMass Medical School, 364 Plantation Street, Worcester, MA, 01605, USA.

*To whom correspondence should be addressed: Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, LRB 826, 364 Plantation Street, Worcester MA 01605 tel: 508–856–8492; fax: 508–856–6215; e–mail: paul.thompson@umassmed.edu.

Running Title: Kinetic Characterization of SARM1

Table S1: Key Resources.

Reagent or Resource	Source	Identifier
4–Chloronicotinamide	Matrix Scientific	7418–70–4
6–Chloropyridine–3–carboxamide	Ark Pharma	AK–44668
96–well Half Area Black Flat Bottom Polystrene NBS™ plate	Corning®	3993
ADPR	Sigma Aldrich	A0752
ADP-ribosylcyclase	Sigma Aldrich	A9106, C7344
BamH1	NEB	R0136S
Gel Extraction Kit	Qiagen	28704
Kanamycin	Research Products International	25389–94–0
Lysozyme	Sigma Aldrich	L6876
MTase–Glo™ Methyltransferase Assay	Promega	V7601
Nicotinamide	Sigma Aldrich	72340
Nicotinamide 1,N^6–ethenoadenine dinucleotide	Sigma Aldrich	N2630
Nicotinamide guanine dinucleotide	Sigma Aldrich	5624-35-1
PCR Purification Kit	Qiagen	28104
Pierce™ EDTA–free protease inhibitor tablets	Thermo Fisher Scientific	A32955
Streptacin XT	IBA Life Sciences	2–4010–025
Streptavidin Antibody	LI–COR Biosciences IR Dye 680RD	976–68079 Lot #C60504–02
T4 Polynucleotide Kinase (PNK)	NEB	M0201S
T4 DNA Ligase	NEB	M0202S
Talon Metal Affinity Resin	Takara	635503
C43 (DE3)	Sigma	CMC0020

SARM1 pET30a+ vector	Genscript	
Streptavidin	LI–COR Biosciences IR Dye 680RD	976–68079 Lot #C60504–02
XhoI	NEB	R0146S
Oligonucleotides		
PolyHistidine Tag Removal For	IDT	GGCAGCCTCGAGTCG**TGA**CACCACCACCAC
PolyHistidine Tag Removal Rev	IDT	CCGTCGGAGCTCAGCACTGTGGTGGTGGTG
Strep–SAM$^{1–2}$ TIR For	IDT	GGGGGCGGTGCTAGCGTGCCGAGCTGGAAG
Strep–SAM$^{1–2}$ TIR Rev	IDT	CCCCCGCCACGATCGCACGGCTCGACCTTC
Strep–TIR For	IDT	GGGGGCGGTGCTAGCACCCCGGATGTGTTC
Strep–TIR Rev	IDT	CCCCCGCCACGATCGTGGGGCCTACACAAG
TIR For	IDT	GGAGATATACATATGACCCCGGATGTGTTC
TIR Rev	IDT	CCTCTATATGTATACTGGGGCCTACACAAG
C. Elegans SAMTIR (CeSAMTIR) For	IDT	AAAAAAGGATCCATGGTGCCGGGTTGGACC
C. Elegans SAMTIR (CeSAMTIR) Rev	IDT	AAAAAACTCGAGTTAGTTACGGTCGCTGGTGG
C. Elegans TIR (CeTIR) For	IDT	AAAAAAGGATCCATGCAGATCGATGTGTTTATTAG
C. Elegans TIR (CeTIR) Rev	IDT	AAAAAACTCGAGTTAGTTACGGTCGCTGGTGG

Table S2. The Steady-State Kinetic Parameters for ceSARM

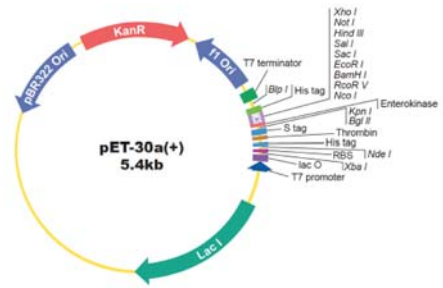
	ceSARM	ceSAMTIR	ceTIR
K_m (μM)	24 ± 4	10 ± 1	13 ± 6
k_{cat} (s^{-1})	0.05 ±0.004	0.007 ± 0.003	0.005 ± 0.0006
k_{cat}/K_m ($M^{-1}s^{-1}$)	2000 ± 1000	700 ± 200	400 ± 200

*Average of values calculated from 2 separate experiments run in duplicate

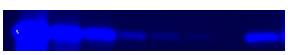
A.

```
ATGGTTCTGACCCTGCTGCTGAGCGCGTATAAGCTGTGCCGTTTCTTTGCGATGAGCGGTCCGCGTCCGGGTGCG
GAGCGTCTGGCTGTGCCGGGTCCGGACGGTGGTGGTGGCACCGGCCCGTGGTGGGCTGCGGGTGGTCGTGGTCCG
CGTGAAGTTAGCCCGGGTGCTGGCACCGAGGTGCAGGACGCGCTGGAACGTGCGCTGCCGGAGCTGCAACAGGCG
CTGAGCGCTCTGAAGCAAGCGGGTGGCGCGCGTGCTGTGGGTGCGGGCCTGGCTGAAGTGTTCCAGCTGGTTGAG
GAAGCGTGGCTGCTGCCGGCTGTGGGTCGTGAGGTTGCTCAGGGCCTGTGCGATGCGATCCGTCTGGACGGTGGC
CTGGATCTGCTGCTGCGTCTGCTGCAGGCTCCGGAACTGGAAACCCGTGTGCAGGCGGCTCGTCTGCTGGAACAG
ATCCTGGTGGCTGAGAACCGTGACCGTGTTGCGCGTATCGGTCTGGGCGTGATCCTGAACCTGGCGAAGGAACGT
GAGCCGGTGGAACTGGCGCGTAGCGTTGCTGGCATCCTGGAGCACATGTTCAAACACAGCGAGGAAACCTGCCAA
CGTCTGGTTGCGGCGGGTGGTCTGGACGCTGTGCTGTACTGGTGCCGTCGTACCGACCCGGCTCTGCTGCGTCAC
TGCGCTCTGGCTCTGGGTAACTGCGCTCTGCACGGTGGCCAGGCTGTGCAGCGTCGTATGGTTGAAAAGCGTGCT
GCTGAGTGGCTGTTCCCGCTGGCGTTCAGCAAAGAAGACGAGCTGCTGCGTCTGCACGCTTGCCTGGCGGTGGCT
GTTCTGGCGACCAACAAGGAAGTGGAGCGTGAAGTTGAACGTAGCGGCACCCTGGCTCTGGTGGAGCCGCTGGTT
GCGAGCCTGGACCCGGGTCGTTTCGCTCGTTGCCTGGTGGATGCTAGCGATACCAGCCAAGGTCGTGGTCCGGAT
GATCTGCAGCGTCTGGTTCCGCTGCTGGACAGCAACCGTCTGGAAGCGCAGTGCATCGGTGCTTTCTACCTGTGC
GCGGAGGCGGCGATCAAGAGCCTGCAGGGTAAAACCAAAGTGTTCAGCGACATCGGTGCGATCCAGAGCCTGAAA
CGTCTGGTTAGCTACAGCACCAACGGCACCAAGAGCGCGCTGGCTAAACGTGCTCTGCGTCTGCTGGGTGAGGAA
GTTCCGCGTCCGATCCTGCCGAGCGTGCCGAGCTGGAAGGAAGCGGAAGTGCAGACCTGGCTGCAGCAGATCGGC
TTCAGCAAATACTGCGAGAGCTTCCGTGAGCAGCAGGTGGACGGTGACCTGCTGCTGCGTCTGACCGAGGAAGAG
CTGCAGACCGACCTGGGTATGAAGAGCGGCATCACCCGTAAACGTTTCTTCCGTGAACTGACCGAGCTGAAGACC
TTCGCTAACTACAGCACCTGCGACCGTAGCAACCTGGCTGATTGGCTGGGTAGCCTGGACCCGCGTTTCCGTCAG
TACACCTACGGTCTGGTGAGCTGCGGCCTGGATCGTAGCCTGCTGCACCGTGTTAGCGAACAGCAGCTGCTGGAG
GACTGCGGTATCCACCTGGGCGTGCACCGTGCTCGTATCCTGACCGCTGCTCGTGAGATGCTGCACAGCCCGCTG
CCGTGCACCGGCGGCAAGCCGAGCGGCGACACCCCGGATGTGTTCATCAGCTACCGTCGTAACAGCGGTAGCCAG
CTGGCTAGCCTGCTGAAGGTTCACCTGCAGCTGCACGGCTTCAGCGTGTTCATCGATGTTGAAAAACTGGAGGCG
GGCAAGTTCGAGGACAAACTGATCCAGAGCGTGATGGGCGCTCGTAACTTCGTGCTGGTTCTGAGCCCGGGTGCG
CTGGATAAGTGTATGCAGGACCACGATTGCAAGGACTGGGTGCACAAAGAAATCGTTACCGCTCTGAGCTGCGGC
AAAAACATCGTTCCGATCATCGATGGTTTCGAATGGCCGGAGCCGCAGGTGCTGCCGGAAGACATGCAGGCTGTT
CTGACCTTCAACGGTATCAAGTGGAGCCACGAATACCAGGAAGCGACCATCGAGAAAATCATCCGTTTCCTGCAA
GGCCGTAGCAGCCGTGATAGCAGCGCTGGTAGCGACACCAGCCTGGAAGGTGCTGCTCCGATGGGCCCGACCCTG
GTTCCGCGTGGCAGC
```

B.



C.



D.

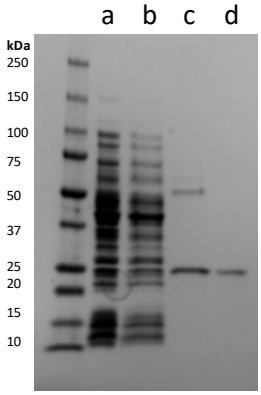


Figure S1. SARM1 Construct Design. (A) Codon optimized sequence of the human SARM1 gene. (B) Map of the pET30a^{+} construct. (C) An example western blot with streptavidin secondary depicting standard curve on the left (2, 1, 0.5, 0.2, 0.1, 0 µM) with sample prep for quantification on the right. (D) Coomassie gel of Strep-tagged TIR domain purification, Lanes: (a) Strep FT, (b) strep wash, (c) strep elution, and (d) after size-exclusion chromatography

A.

```
ATGAGCGCGTGGAGCCATCCGCAGTTTGAAAAGGGTGGTGGTAGCGGTGGTGGTAGCGGTGGTAGCGCGTGGAGC
CATCCGCAGTTCGAGAAGGGTGGCGGTAGCAGCGGCGGTGGCGCGAGCGGTAGCATGGGCGAGGAAATCCTGACC
GAACGTAACCAGCTGAGCCGTCAAGATGCGAGCTGCTTTCGTAGCCCGCGTAGCCCGCTGGGTCGTACCGACGAT
GCGTTCTTTGAGCTGGACGATGATGATGATCTGAGCAGCCCGAGCGTGCCGGGCAGCCCGGTTGACCCGCCGAGC
ATTAGCGTGCCGCTGCCGCCGAAAAGCGCTCCGCCGTGCCCGACCCAACCGGCGCCGCTGACCAACGGTGATCTG
TACCCGACCATCCTGAGCAACGGTACCCCGATCCCGAACGGCCGTATTACCCCGGCGCTGAGCACCGTGAGCGTT
AGCCTGATTCACGAGGCGCGTCTGCAGCAAAGCCTGAGCACCCCGTGCAACGGTAGCGAGGAAGAGATGCACAAC
GGCCAGGTTCGTAAGGAAAGCGAGTATCGTCGTTTCAAAAGCGAAGGTAGCACCGCGGGTGCGAGCCTGCCGGCG
GCGGAAAAGACCCACATGGACGAGCTGAGCCCGGTGGATCAACGTAGCACCAGCGGTACCGCGCGTTTTCTGATC
CAGCAAGACAGCGTGGTTAACCCGAGCACCAAAATGAGCAACACCGAGCAGGTTGCGATGATGCACACCCTGAAA
ACCAAGCTGAGCAAATACCAAGCGATGATGGACAAGGCGTTCGAAGAGATCGCGAAAGTGGAAGATGCGAACATC
ATTGAGGGTTGCACCATTGTTCGTAAACTGATGCGTAAAGTGTGGAACACCCCGAAGGTTAGCGCGGACCTGGCG
AACGCGCTGTGCGATTACCTGCGTGATCGTGACTACTTCGACAAGCTGATCAAGATGTTCATCAGCCCGAACACC
GCGGCGTGCGATCAAGTGCGTATGGAGTGCGGCAAGGTTCTGGAAGAGTGCACCAGCAGCGCGAACCTGGAATAC
ATCGTGAACAAGAGCTACACCAAGAAAATCATGATCGTTGCGATGAAACTGAACAAGACCCCGGATCAGCAACGT
CTGAGCCTGAGCCTGATTGGCAACCTGTTCAAGCACAGCAACGCGGTGAGCCTGAGCCTGATCGAAACCGATGTT
ATTGACCACATCATTCTGACCTTTAAACGTGCGCCGGAATGCCCGGACATCCTGCGTCATGCGGCGCTGGCGCTG
GCGAACATTCTGCTGTACACCTGCTTCGAGGGTAAGAAAAAGATCATTCAGAAAAAGATCCCGGAATGGCTGTTC
TTTCTGGCGAGCCAAGCGGATGACGTGACCCGTTACTATGCGTGCATCGCGGTTTGCACCATTGTGAGCGTTAAG
GAATTTGAGCCGCTGGTGCGTAAAAGCGATACCATGAAACTGGTGGAGCCGTTCCTGCAGGTTCACGACCCGGCG
ACCTTTGCGCGTGATTACCACAAGTATGCGCAGGGTAACACCAAAGAATGGCTGGAGCGTCTGCTGCCGATGCTG
CAACCGAGCCGTCGTCGTGAAGCGCGTAGCGTGGCGGCGTTCCACTTTACCCTGGAAGCGACCATCAAAAAGGAG
CAAAACAAGCTGGACGTTTTCCAGGAGATCGGCGCGATTCAAGCGCTGAAAGAGGTGGCGAGCAGCCCGGATGAA
GTTGCGGCGAAATTTGCGAGCGAAGCGCTGACCGTGATCGGTGAAGAGGTTCCGTACAAACTGGCGCAGCAAGTG
CCGGGTTGGACCTGCGCGGACGTGCAGTACTGGGTTAAAAAGATTGGTTTCGAAGAGTATGTGGAGAAATTTGCG
AAGCAGATGGTTGATGGCGACCTGCTGCTGCAACTGACCGAAAACGATCTGAAACACGACGTTGGTATGATCAGC
GGCCTGCACCGTAAGCGTTTCCTGCGTGAGCTGCAGACCCTGAAAGTGGCGGCGGATTATAGCAGCGTTGACGAG
AGCAACCTGGATAACTTTCTGATGGGTCTGAGCCCGGAACTGAGCGTGTACACCTATCAAATGCTGACCAACGGC
GTTAACCGTAGCCTGCTGAGCAGCCTGACCGACGAAATGATGCAGAACGCGTGCGGTATCACCAACCCGATTCAC
CGTCTGAAGCTGACCCAAGCGTTCGAAACCGCGAAACACCCGGATGACGTGGAAGTTGCGATGCTGAGCAAGCAG
ATCGATGTGTTTATTAGCTACCGTCGTAGCACCGGCAACCAGCTGGCGAGCCTGATCAAAGTTCTGCTGCAACTG
CGTGGTTACCGTGTGTTCATTGATGTTGACAAACTGTATGCGGGCAAGTTTGACAGCAGCCTGCTGAAAAACATC
CAAGCGGCGAAGCACTTCATTCTGGTGCTGACCCCGAACAGCCTGGACCGTCTGCTGAACGATGACAACTGCGAA
GATTGGGTTCACAAAGAGCTGAAGTGCGCGTTCGAACACCAGAAGAACATCATTCCGATCTTTGACACCGCGTTC
GAATTTCCGACCAAAGAGGACCAAATTCCGAACGATATCCGTATGATTACCAAATACAACGGTGTGAAGTGGGTT
CACGATTATCAGGACGCGTGCATGGCGAAAGTGGTTCGTTTTATCACCGGCGAGCTGAACCGTACCACCCCGACC
ACCAAGGAAATGCCGAGCATTAGCCGTAAAACCACCCAGCAACGTTGGCAGACCACCAACACCGTGAGCCGTACC
GGTCCGAGCCGTAGCATCGGTGGCCCGCGTATGGAGCCGCCGACCCCGACCTTCTTTAGCGTTACCCCGACCGGC
```

B.

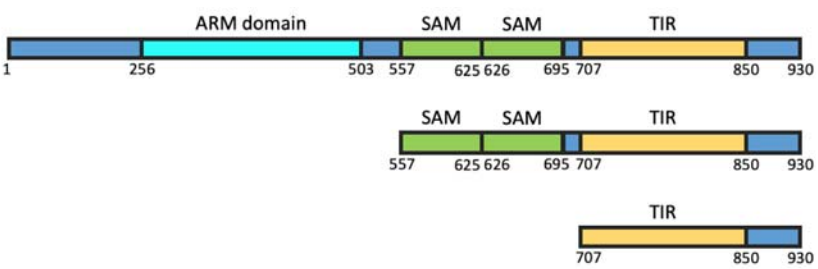


Figure S2. CeSARM Construct Design. (A) Codon optimized sequence of full length Tir-1 (*C. elegans* SARM1 ortholog) (B) The domain architecture of full length CeSARM, CeSAM$^{1\text{-}2}$TIR, and CeTIR constructs used in this study.

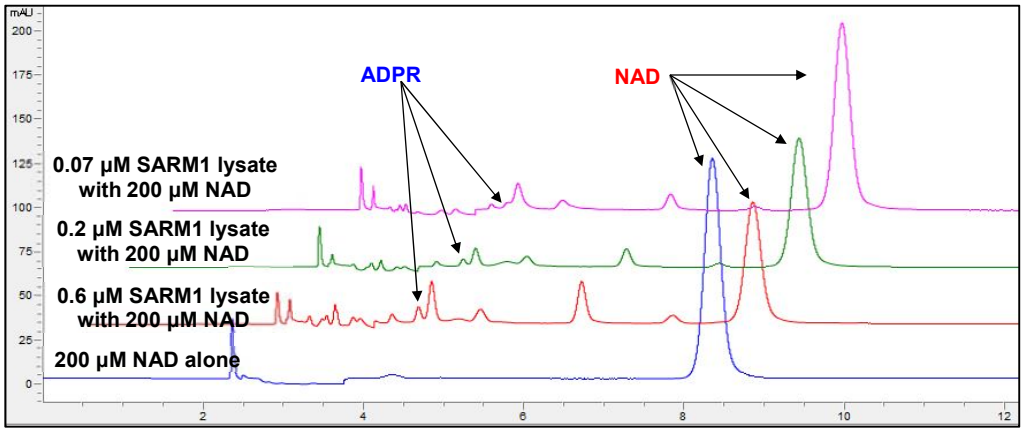


Figure S3. SARM1 Activity is Dose Dependent. Varying concentrations of SARM1 lysate treated with NAD^+. A decrease in NAD^+ peak area and increase in ADPR peak area can be seen with increasing SARM1 concentration.

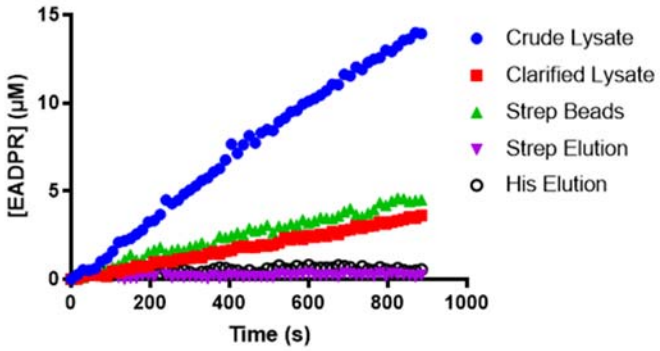


Figure S4. Activity of CeTIR during Purification. NAD^+ hydrolase activity at different steps of the *C. elegans* TIR domain purification.

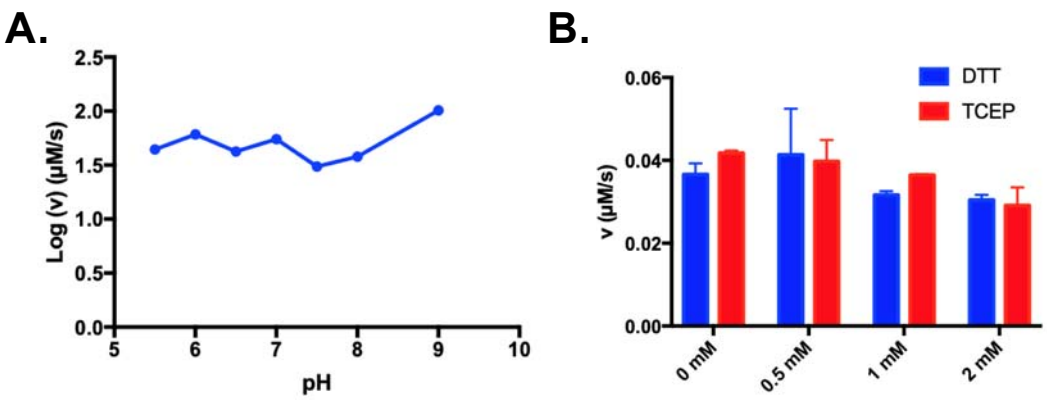


Figure S5. Determination of Effect of pH and Reducing Agents. (A) Effect of pH on SARM1 activity at 50 µM ENAD. (B) Effect of reducing agents on activity at 50 µM ENAD.

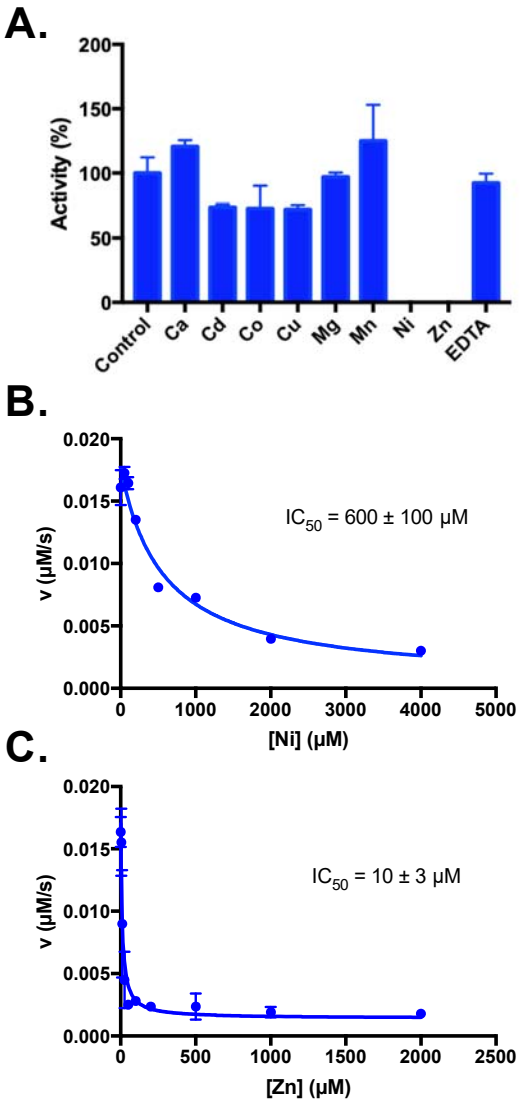


Figure S6. Determination of Effect of Metals on Catalysis. (A) Effect of metals on SARM1 activity. (B) $NiCl_2$ and (C) $ZnCl_2$, inhibit SARM1 with IC_{50}s of 600 ± 100 µM and 10 ± 3 µM, respectively.