Supporting Information for:

Reconstitution and substrate specificity for isopentenyl pyrophosphate of the antiviral radical SAM enzyme viperin

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Score              Expect        Identities     Positives Gaps
47.0 bits(110) 1e-05          52/223(23%)    99/223(44%)    21/223(9%)
HsaV  80   TRQCNYKCGFCF--HTAKTSFV-------LPLEEAKRGLLLLKEAGMEKINFSGGEPFL-  129
           T +CN++C +C          FV       L  +E  R   +  E G++KI  +GGEP +
SauM  21   TDRCNFRCDYCMPKEVFGDDFVFLPKNELLTFDEMARIAKVYAELGVKKIRITGGEPLMR  80

HsaV  130  QDRGEYLGKLVRFCKVELRLPSVSIVSNGSLIRERWFQNYGEYLDILAISCDSFDEEVNV  189
           +D    + KL +   +E     + + +NG L+++   + Y   L  + +S D+ D+ +
SauM  81   RDLDVLIAKLNQIDGIE----DIGLTTNGLLLKKHGQKLYDAGLRRINVSLDAIDDTLFQ  136

HsaV  190  LIGRGQGKKNHVENLQKLRRWCRDYRVAFKINSVINRFNVEEDMTEQIKALNPVRWKVFQ  249
            I     K   +  L+++  +     +  K+N VI +  + +D  + I  L   + K  +
SauM  137  SINNRNIKATTI--LEQI-DYATSIGLNVKVNVVIQK-GINDD--QIIPMLEYFKDKHIE  190

HsaV  250  CLLIEGENCGEDALREAERFVIGDEEFERFLERHKEVSCLVPE  292
              IE  + G D   +  + V  DE     +E+H E+  + P+
SauM  191  IRFIEFMDVGNDNGWDFSKVVTKDEMLT-MIEQHFEIDPVEPK  232
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Figure S1. Conservation between *Hsa*Viperin and *Sau*MoaA. Before the availability of the recent crystal structure of *Mmu*Viperin, Blastp of *Hsa*Viperin (labeled as *Hsa*V) against the PDB database using the default search setting at NCBI revealed that the structure of *Sau*MoaA (labeled as *Sau*M) was the only positive result. Seven of the eight *Sau*MoaA residues involved in recognition of the triphosphate motif of GTP are highlighted in red, and three of them (in bold) are conserved in *Hsa*Viperin.

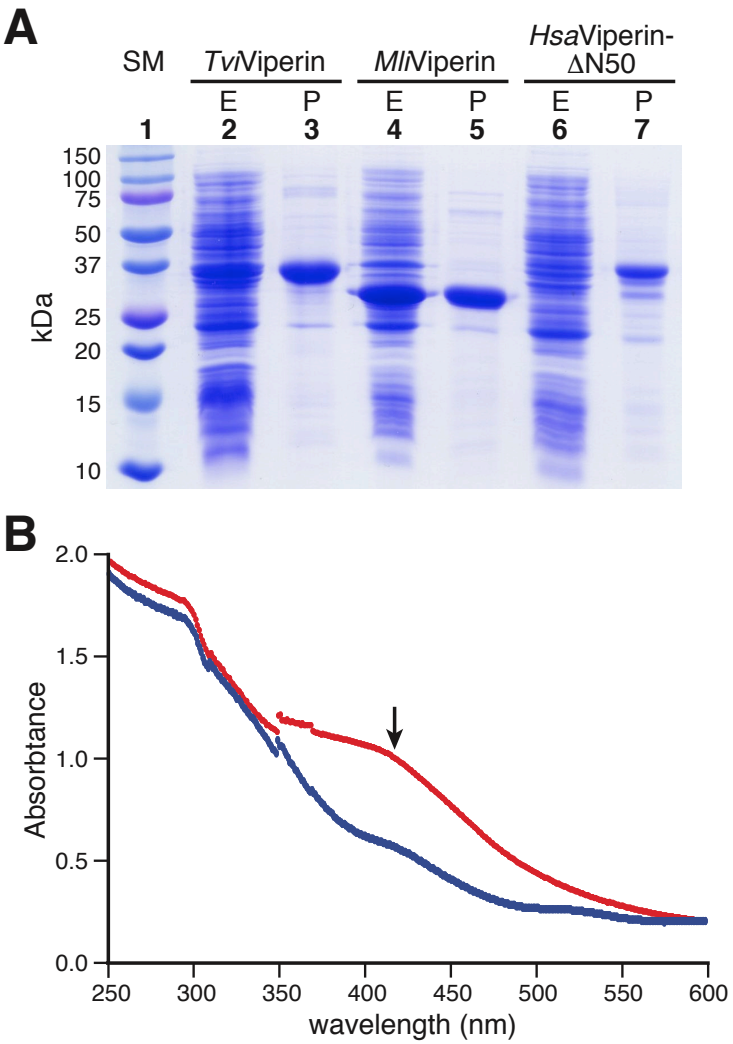

Figure S2. Expression and purification of the recombinant viperin. *A,* SDS gel analysis of three viperin expressed in *E. coli* and purified via Ni-NTA column in an anaerobic chamber. Three μL of each purified recombinant viperin, with a calculated concentration of 50 μM of the total protein, was used for SDS gel analysis in lanes 3, 5, and 7. SM, size marker; E, expressed; P, purified. *B*, UV-vis spectrum of the purified *Tvi*Viperin (200 μM) before (red) and after (blue) overnight incubation at 4 °C under aerobic conditions. The shoulder marked by an arrow indicates the presence of [4Fe-4S].

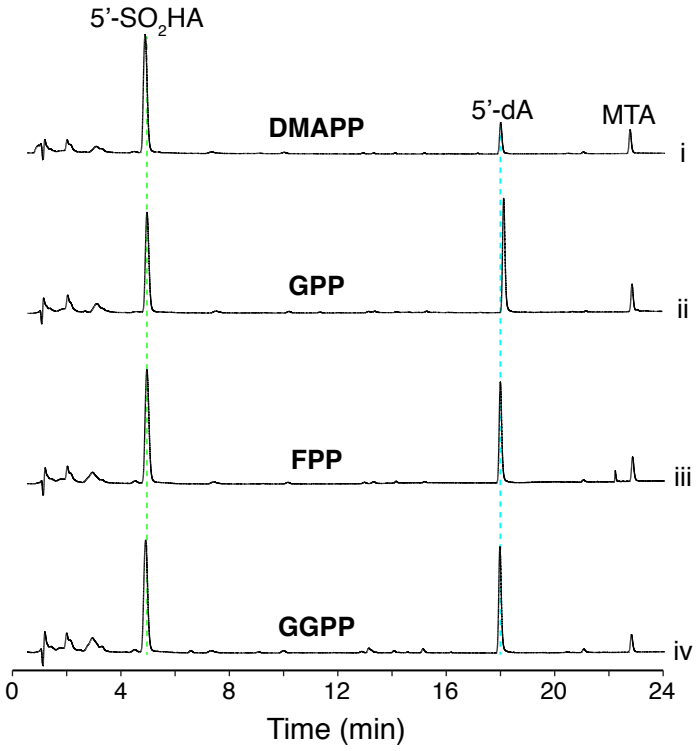

Figure S3. GPP, FPP, and GGPP are not substrates of viperin. UPLC analysis of the *Tvi*Viperin-catalyzed reactions using GPP, FPP, and GGPP as the substrates. The same analysis of the reaction with DMAPP was used for comparison. The reaction condition was the same as ones shown in Fig. 2*B* (50 μM of *Tvi*Viperin, 100 μM of SAM, 100 μM of each substrate, 0.5 mM of $Na_2S_2O_4$, and 1h incubation at room temperature). Even for the reactions carried out by the same enzyme, the ratio of 5'-SO_2HA and 5'-dA could differ greatly depending on the specific compound used as the substrate (also see Fig. S10).

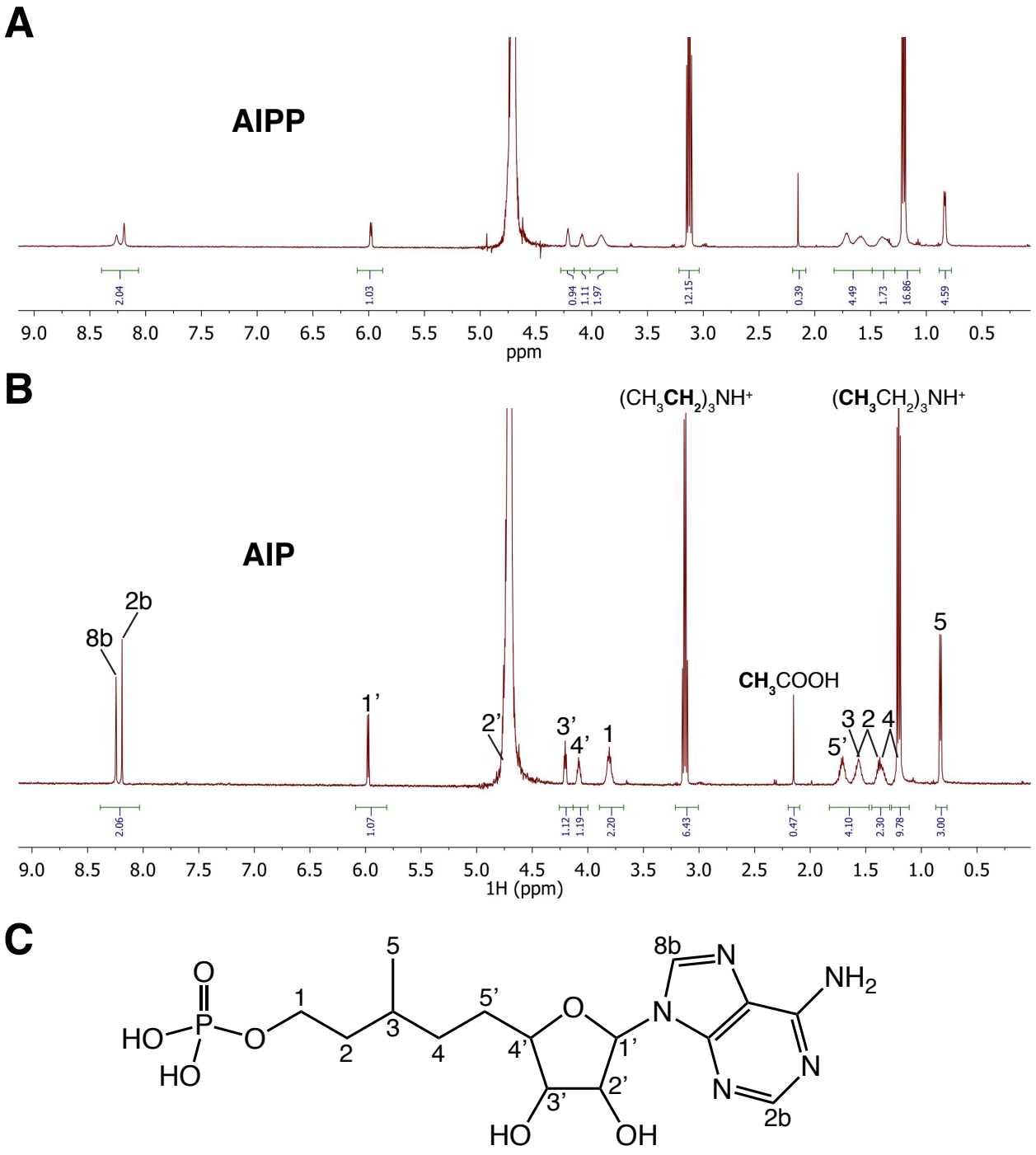

Figure S4. 1D ^{1}H NMR (500 MHz, D_2O) of AIPP and AIP. *A* and *B*, ^{1}H NMR of the purified AIPP and AIP. Because both AIPP and AIP were purified via HPLC using 1 mM $(Et)_3NHCO_3$ as buffer A, $(Et)_3NH^+$ is present in the final NMR samples as the counter ion of AIPP and AIP (2-to-1 ratio in AIPP and 1-to-1 ratio in AIP). Despite approximately similar concentration (1.6 vs. 1.5 mM) of both AIPP and AIP samples used for NMR, proton peaks in the spectrum of AIP are significantly sharper. Therefore, subsequent acquisition of 2D NMR spectra were based on AIP sample. *C*. Chemical structure of AIP with protons numbered according to the spectrum in panel (B). The assignments were assisted by 2D NMR spectra of AIP (Fig. 3*C* and Fig. S5).

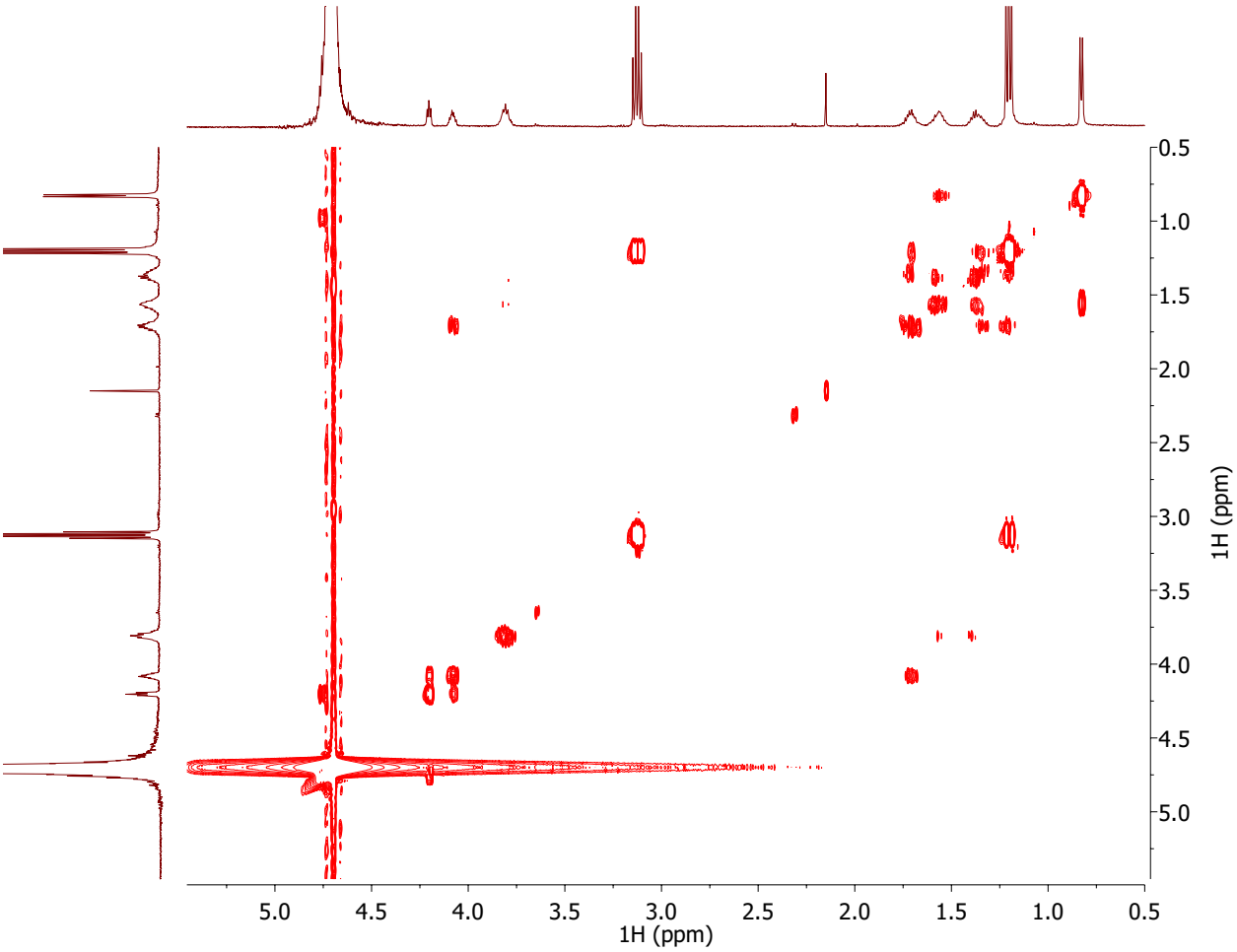

Figure S5. 2D ^{1}H-^{1}H COSY spectrum of AIP. Only the region between 0.5 and 5.5 ppm is shown.

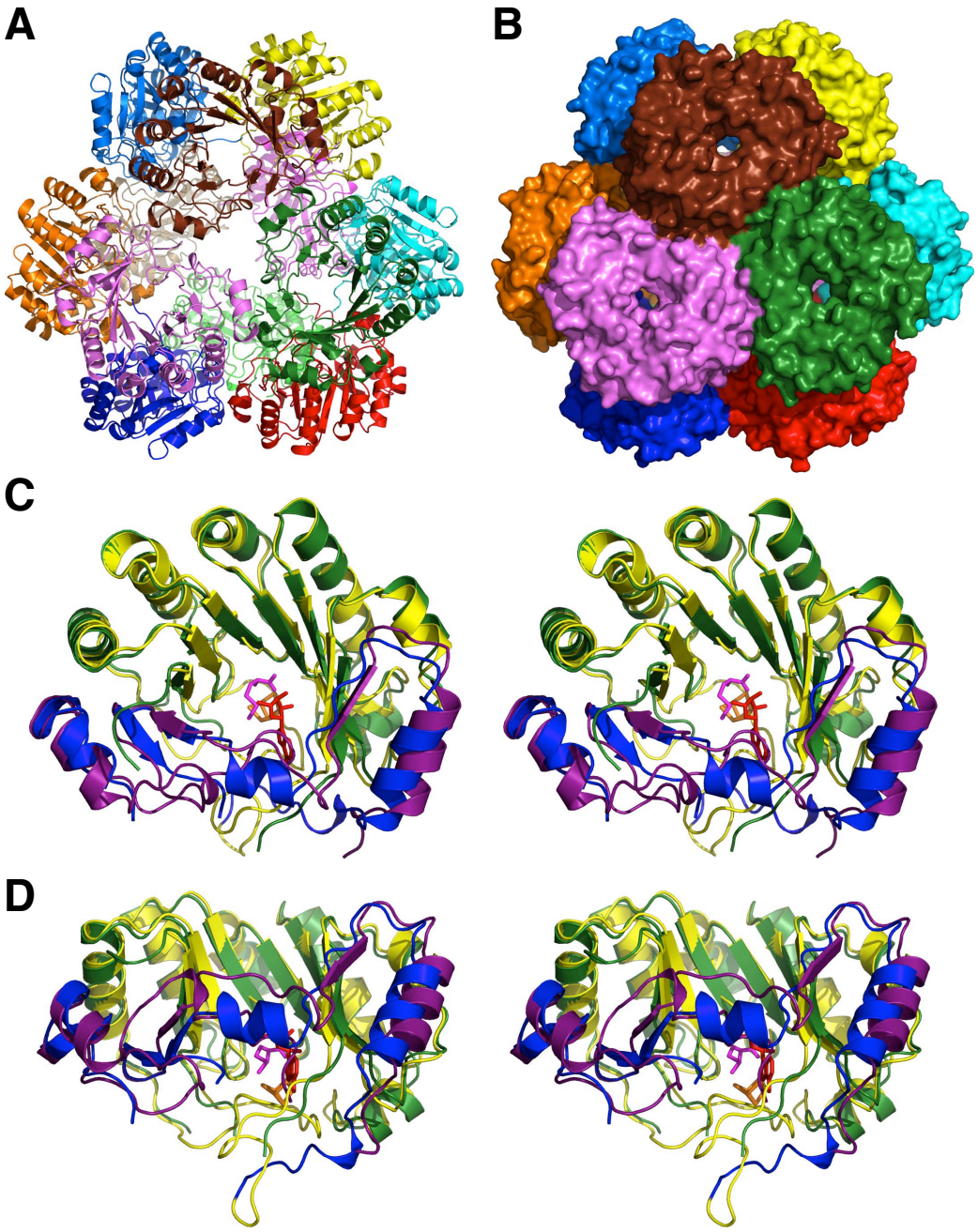

Figure S6. Crystal structure of *Tvi*Viperin and its comparison to the structure of *Mmu*Viperin. *A* and *B*, Cartoon and surface representations of 12 copies of *Tvi*Viperin in an asymmetric unit, forming a spherical complex reminiscent of a soccer ball. *C* and *D*, Stereoview of the structural alignment of *Tvi*Viperin and *Mmu*Viperin viewed from the top (C) and the side (D). The structure of *Tvi*Viperin is colored the same as in Fig. 1*C*, and the corresponding radical SAM and the C-terminal extension of *Mmu*Viperin are colored yellow and blue, respectively. [4Fe-4S], Met, and 5'-dA are in sticks and colored orange, magenta, and red, respectively. *Tvi*Viperin and *Mmu*Viperin share 52% sequence identities, and the rmsd of the two structures is 1.3 Å. However, the rmsd of the radical SAM domain (colored green and yellow) is 0.9 Å, and the number for the C-terminal extension (colored purple and blue) is 1.9 Å.

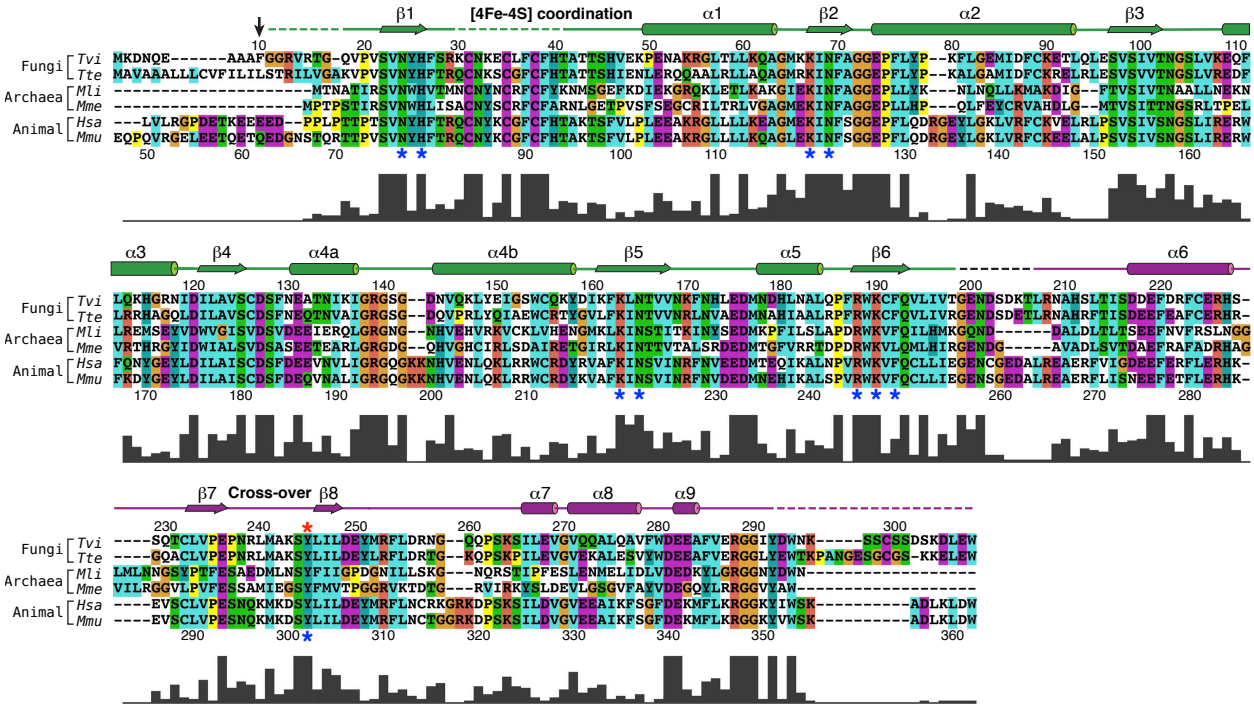

Figure S7. Conservation of Viperin. Sequences of six viperin (two representatives each from fungi, archaea, and animal) were aligned using Clustal X. The residues were colored using the default setting of software. The secondary structure of *Tvi*Viperin is depicted above the primary sequence, with α-helices highlighted as cylinders, β-strands as arrows, loops as solid lines, and disordered residues as dotted lines. The numbers above and below the sequences correspond to *Tvi*Viperin and *Mmu*Viperin, respectively. Y245 in *Tvi*Viperin, marked with a red asterisk and located in the cross-over loop, was mutated to F245 to evaluate its potential role of providing the hydrogen for Step 2 reaction. Ten residues in *Mmu*Viperin that are displayed in Movie S1 are marked with blue asterisks. Seven of them are also shown in Fig. 7*A*. *Mme*, *Methanococcoides methylutens.*

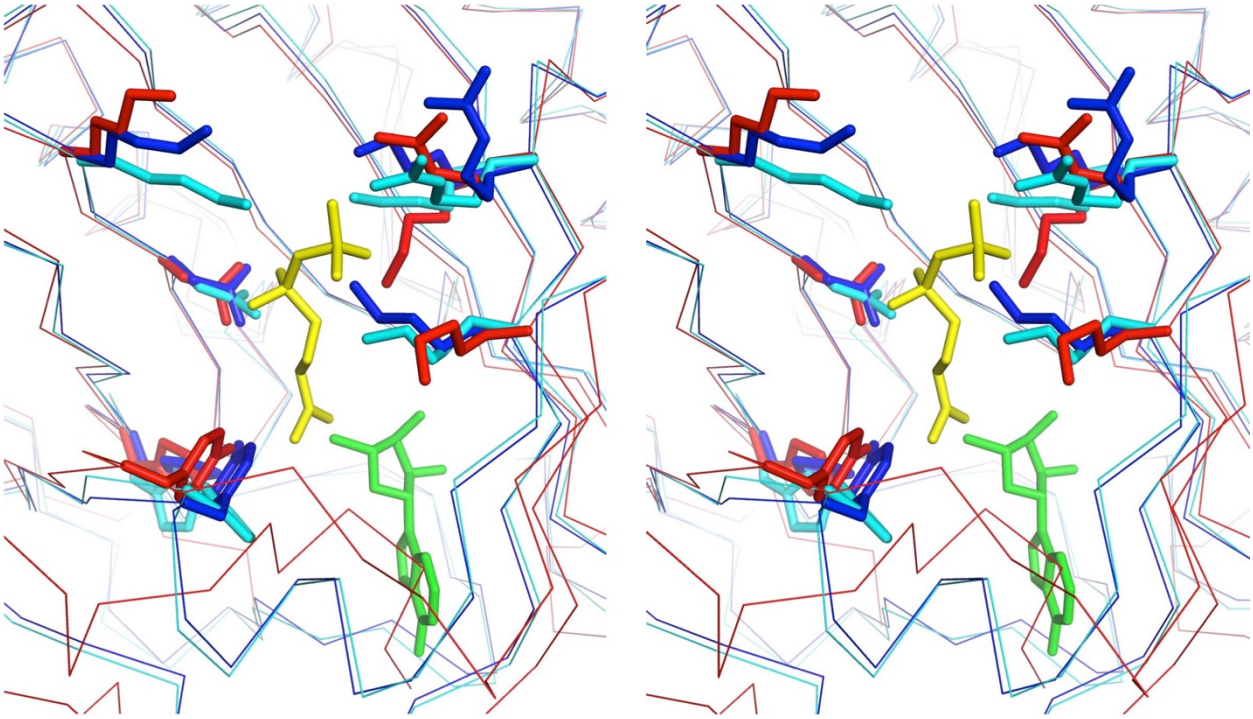

Figure S8. Side-chain conformational changes resulting from viperin-IPP interaction revealed by MD simulations. Stereoview of the aligned structures of *Tvi*Viperin (red), *Mmu*Viperin (blue), and the frame from the MD simulations for Fig. 7*A* (cyan). The view is the same as the one shown in Fig. 7*A*. IPP and 5'-dA are colored yellow and green, respectively.

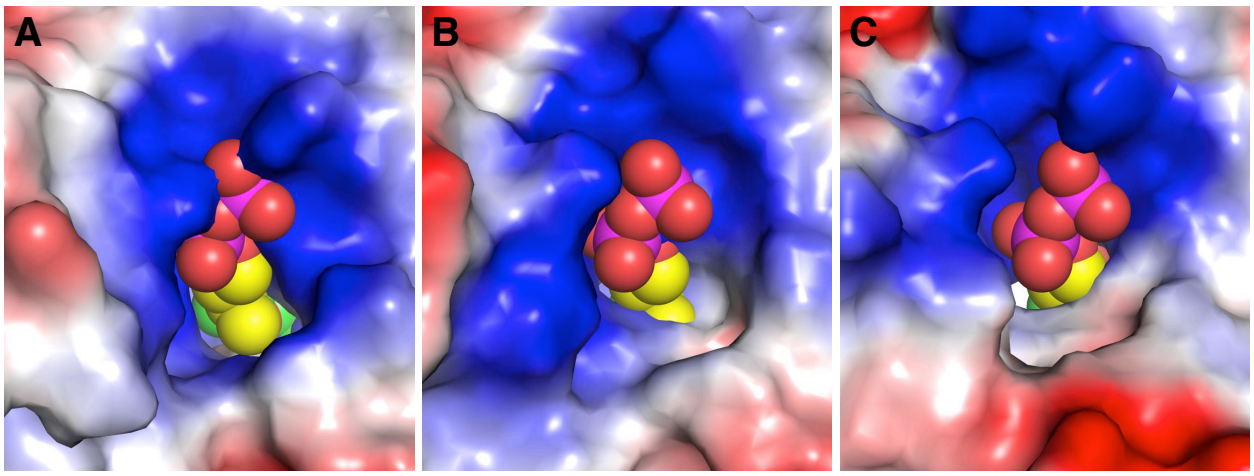

Figure S9. Surface view of the structural changes near the entrance of the active site resulting from association of IPP with viperin. *A*. The same as the one in Fig. 7*C*. *B*. The same as in panel (A) except the surface is based on the structure of *Mmu*Viperin. *C*. The same as in panel (B) except the surface is based on the structure of *Tvi*Viperin.

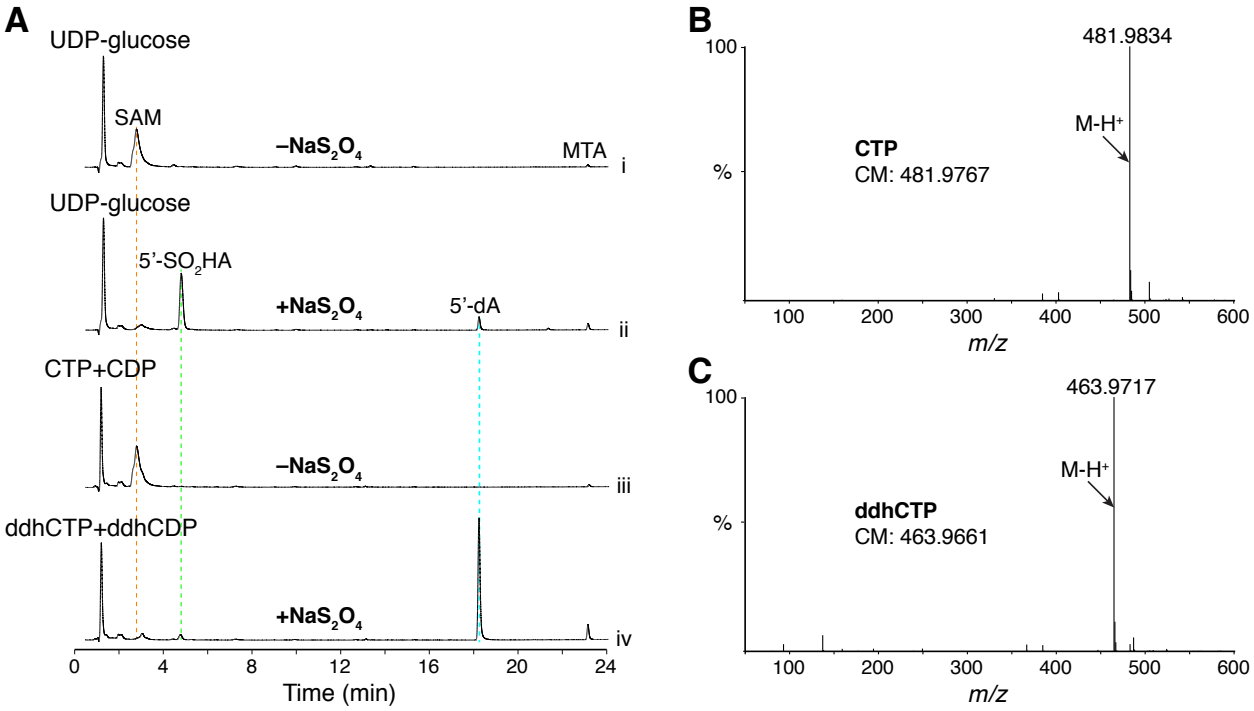

Figure S10. UDP-glucose is not a substrate of *Tvi*Viperin but CTP is. *A.* UPLC analysis of the *Tvi*Viperin-catalyzed reactions using UDP-glucose and CTP as substrates in the absence (traces i and iii) and presence (traces ii and iv) of the reducing agent $Na_2S_2O_4$. The reaction condition was the same as ones shown in Fig. 2*B* (50 μM of *Tvi*Viperin, 100 μM of SAM, 100 μM of each substrate, 0.5 mM of $Na_2S_2O_4$, and 1h incubation at room temperature). *B.* High resolution LC-MS analysis (in negative mode) of CTP from the sample for trace iii in (A). *C.* High resolution LC-MS analysis (in negative mode) of ddhCTP from the sample for trace iv in (A).

Legend for Movie S1. Structural based MD simulations of viperin interacting with IPP. The simulations were based on the structure of *Mmu*Viperin (PDB ID code: 5VSM). Only ten strictly conserved residues of viperin, N77, H79, K120, N122, K220, N222, R245, K247, F249, and Y302 (marked with blue asterisks in Fig. S7), are displayed in sticks. The carbon atoms of the residues from viperin, IPP, and 5'-dA are colored gray, yellow, and green, respectively. The heteroatoms are colored individually, with nitrogen in blue, oxygen in red, and phosphate in magenta. The hydrogen atoms are omitted for clarity.

Table S1. Summary of X-ray Crystallography Data Collection and Refinement

***Tvi*Viperin**	
Data Collection	
Space Group	$P2_12_12_1$
Cell dimensions a, b, c (Å) α, β, γ (°)	 173.9, 209.6, 233.9 90.0, 90.0, 90.0
Resolution (Å)	50.0-2.8 (2.85-2.8)
R_{merge} (%)	8.2
I/σ	9.0 (2.4)
Completeness (%)	99.9 (99.5)
Redundancy	6.7
Refinement	
Resolution (Å)	45.7-2.8
No. reflections	209,265 (19,607)
R_{work}/R_{free} (%)	19.0/21.1 (27.8/29.9)
No. atoms Protein Ligand/ion	25,749 24,912 276
Average B factors (Å^2) Protein Ligand/ion	 43.8 62.1
R.m.s. deviations Bond lengths (Å) Bond angles (°)	 0.015 1.42
Ramachandran statistics (%) Favored Allowed Outliers	 94.0 4.6 1.6

*Values in parenthesis are for highest resolution shell.