SUPPLEMENTARY MATERIAL

PfA-M1 Pv-M1 Pb-M1	1	MKLTKGCAYKYIIFTVLILANILYDNKKRCMIKKNLRISSCGIISRLLKSNSNYNSFN MIRKKMLNFHFLFITVLVALANYTPVDYQNTCMISKSCRKNSCGITSRVLSGIHVNKTSA MVLKKLLCFNLFLIIILTFENLSFDKKNTCMINNTIRSNSCCIVNRVLREKTHHYSSK * .* :: * . * :: * .* :: * .* :: * .* :* :* :* :* :* :* :* :* :* :* :* :* :*	58 60 58	PfA-M1 Pv-M1 Pb-M1	592	SEVMRMYLTILGEEYYKKGFDIYIKKNDGNTATCEDFNYAMEQAYKMKKADNSANLNQYL SEVMRMYQTILGDEYYKKGMDIYIKKNDGGTATCEDFNDAMNEAYKMKKGDKTANLDQYL SEVMRMYQTILGDDYYKKGIDIYLKKHDGGTATCEDFNDAMNEAYQMKKGNTDENLDQYL ****** ***::****:***:*****************	651
PfA-M1 Pv-M1 Pb-M1	61	KNYNFTSAISELQFSNFWNLDILQKDIFSNIHNNKNKPQSYII-HKRLMSEKGDN RAKALISLSSLIYHLQLPKLVSLDLFRRDLFTGVKQKGKRSPVPSYIIQ-NRLMSENGDS ISKSIPFIQNFSLEKYFTGESLQKNILNNINKLGGAHLFHISKSHLTAKSG *: : .:::::::::::::::::::::::::::::	112 119 109	PfA-M1 Pv-M1 Pb-M1	652	Domain III LWFSQSGTPHVSFKYNYDAEKKQYSIHVNQYTKPDENQKEKKPLFIPISVGLINPENGKE LWFSQSGTPHVTAEYSYDAGKKEFVIEVTQVTNPDPNQKEKKALFIPIRVGFINPKNGQD LWFSQSGTPHVTAEYIYDENEKTFTINLSQITYPDDNQKEKYPLFIPVKVGFISPKDGKD *****	711 678
PfA-M1 Pv-M1 Pb-M1	113 120 110	NNNNHQNNNGNDNKKRLGSVVNNEENTCSDKRMKPFEEGHGITQVDKMNNNSD GSTNMSVTA-NQEKKPGTGDASEGNNQSGISAAAQDKRMKGDQSEEVSNVSG -NKNTEFGINMTENKQTNIG	172	PfA-M1 Pv-M1 Pb-M1	712	Domain IV MISQTTLELTKESDTFVFNNIAVKPIPSLFRGFSAPVYIEDNLTDEERILLLKYDSDAFV VIPEVTLEFKKDKEKFIFNNVNEKPIPSLFRGFSAPVYIKDNLTDSERILLLKYDTDAFV VIPETVLELKKDKESFVFQNVSEKPIPSLFREFSAPVYIKDNLTDEERIALLKYDSDAFV :* :**:.*::*:*:*	771 738
PfA-M1 Pv-M1 Pb-M1	166 173 144	N-terminal truncation site↓Domain I HLQQNGVMNLNSNNVENNNNNNSVVVKKNEPKIHYRKDYKPSGFIINNVTLNINIHDN STNA-AMTNGASSTTEGGDNNNGSGNDGKNEPKIHYRKDYKPSGFVIDNVTLNINIFDN RMLCENDNNNGGEDTSTEKAIFKKSKDSQIHYRTDYKPSGFTIDNVTLNINIFDN : :: : : : : ****.*************	223 231 198	PfA-M1 Pv-M1 Pb-M1	772	Connecting loop RYNSCTNIYMKQILMNYNEFIKAKNEKLESFNLTPVNAQFIDAIKYLLEDPHADAG RYNVCVDLYMKQILKNYQELLQAKSENKQESAEMPSLTPVSEDFINAIKYLMEDPHADAG RYNVCIDLYMKQIIKNYNELISQM <u>TKENNVLE</u> LSLTPVNDEFINAIKHLLEDKHADPG *** * ::*****: **::*: *:: *:: *:: *::	831
PfA-M1 Pv-M1 Pb-M1	232	ETIVRSVLDMDISKHNVGEDLVFDGVGLKINEISINNKKLVEGEEYTYDNEFLTIFSKFV ETSVRSTLDMKLSEHYGGEDLIFDGVSLEIKEISIDNNKLMEGEHYKVDNEFLTIYSKFI ETIVRSSLNMCTNENYADEDLVFDGVGLSIKEISINNNKLTEGEDYTYDNEFLTIFAKNV ** *** *: .: .***:****.*	291	PfA-M1 Pv-M1 Pb-M1	832	FKSYIVSLPQDRYIINFVSNLDTDVLADTKEYIYKQIGDKLNDVYYKMFKSLEAKADDLT FKSYIITLPRDRFILNYIKNVDTDVLADTKDFIYKQLGDKLNDLYFQMFKSLQAKADDMT FKSYIIALPRDRYIMNYIKEVDPIVLADTKDYIYKQIGSRLNPVLFSIFQNTESKANDMT *****::**:**:*::*::*	891
PfA-M1 Pv-M1 Pb-M1	284 292 259	Domain II PKSKFAFSSEVIIHPETNYALTGLYKSKNIIVSQCEATGFRRITFFIDRPDMMAKYDVTV PKGKFTFGSEVIIHPETNYALTGLYKSKNIIVSQCEATGFRRITFFIDRPDMMAKYDVTI PKENFVFLSEVVIHPETNYALTGLYKSKDIIVSQCEATGFRRITFFIDRPDMMAKYDVTL ** :*.* ***:***************************	343 351 318	PfA-M1 Pv-M1 Pb-M1	892	YFNDESHVDFDQMNMRTLRNTLLSLLSKAQYPNILNEIIEHSKSPYPSNWLTSLSVSAYF HFEDESYVDFEQLNMRKLRNTLLTLLSRAKYPNMLDQIMEHSKSPYPSNWLASLAVSAYY HFKDESYIDFDQLNMRKLRNSILMMLSKAQYPHMLKYIKEQSNSPYPSNWLASLSASSYF :*:***::**:************	951
PfA-M1 Pv-M1 Pb-M1	344 352 319	TADKEKYPVLLSNGDKVNEFEIPGGRHGARFNDPHLKPCYLFAVVAGDLKHLSATYITKY TADKEKYPVLLSNGDKLNEFEIPGGRHGARFNDPYLKPCYLFAVVAGDLKHLSDNYVTKF TADKKKYPVLLSNGDKLNEFDIPGGRHGARFNDPHLKPCYLFAVVAGDLKHLSDNYVTKY ****:********************************	403 411 378	PfA-M1 Pv-M1 Pb-M1	952	DKYFELYDKTYKLSKDDELLLQEWLKTVSRSDRKDIYEILKKLENEVLKDSKNPNDIR DKYFDLYEKTYNQSKDDELLQEWLKTVSRSDRKDIYDIIKKLETEVLKDSKNPNEIR SGDDYYDLYDKTYKLSKNDELLLQEWLKTVSRSDRSDIYSIIKKLEVEILKDSKNPNNIR *.*::**:**: **:****	1009
PfA-M1 Pv-M1 Pb-M1	404 412 379	X TKKKVELYVFSEEKYVSKLQWALECLKKSMAFDEDYFGLEYDLSRLNLVAVSDFNVGAME SKKNVELYVFSEEKYVSKLKWALECLKKAMKFDEDYFGLEYDLSRLNLVAVSDFNVGAME TKKPVELYVYSEAKYVSKLKWALECLKKAMKFDEDYFGLEYDLSRLNLVAVSDFNVGAME :** *****:** ******	463 471 438	PfA-M1 Pv-M1 Pb-M1	1010	AVYLPFTNNLRRFHDISGKGYKLIAEVITKTDKFNPMVATQLCEPFKLWNKLDTKRQELM AVYLPFTYNLRYFNDISGKGYKMMADIIMKVDKFNPMVATQLCDPFKLWNKLDQRRQDMM AVYLPFTSNLRAFNDISGKGYKLMANVIMKVDKFNPMVATQLCDPFKLWNKLDLKRQALM ****** *** *:*************************	1069
PfA-M1 Pv-M1 Pb-M1	472	XX X X X X X X X X X X X X X X X X X X	523 531 498	PfA-M1 Pv-M1 Pb-M1		LNEMNTMLQEPNISNNLKEYLLRLTNKL LNEMNRMLSMENISNNLKEYLLRLTNKL HDEMNRMLNMENISPNLKEYLLRLTNKM :*** **. *** **********	1085 1097 1064
PfA-M1 Pv-M1 Pb-M1	524 532 499	HRENLFSEEMTKTVTTRLSHVDLLRSVQFLEDSSPLSHPIRPESYVSMENFYTTTVYDKG HRENLFSEQTTKTATFRLDHVDILRSVQFLEDSSPLAHPIRPESYVSMENFYTTTVYDKG HRENLFSEETTKTATFRLTHIDLLRSVQFLEDSSPLSHPIRPESYISMENFYTNTVYDKG *******: ***.* ** *:*:*****************	583 591 558			C-terminal hexahistidine tag	

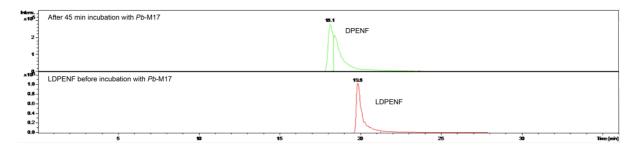
Supplementary Figure S1. Sequence alignment of *PfA-M1*, *Pv-M1* and *Pb-M1*, colored by domain. Amino acid sequence alignment of *PfA-M1*, *Pv-M1* and *Pb-M1*. Domain I highlighted in blue, domain II in yellow, domain III in green and domain IV in pink. N-terminal truncation sites and C-terminal histidine site are labeled, conserved metal-binding residues are marked with 'x' and conserved active site residues boxed.

PfA-M17 Pv-M17	1 1	MYFSSLCKFLPISEKEKIYLNIVKKRFCMPLLRSSQHIKNTYWNIPKKSFRTGVPQFAE	36 31	PfA-M17 Pv-M17	538 554	NSKYADINNISS NSKYADLNNISS
Pb-M17	1	MYLTRLNINFVDKIFENKFYKNIIKRYYFYNIKHSQKKFFFSFFISNSIKNKNNYNS	57	Pb-M17	568	NSKYADINNISS
		N-terminal truncation site				
PfA-M17	37	NNNNIINYNKRGLKFYPFCNNLKKNINFVNINNKKGINFHSINKERKMASEV	88	PfA-M17	598	EFVLNDAL
Pv-M17	32	SKKTRILHLHPLCKSASGVESPPFFDSQTFSSISNRKEFRKMATTV	77	Pv-M17	614	EFVLNDAV
Pb-M17	58	DCREFLTFNTYHYQKTPKYKIESLNFSKTIKKFYSTNNIK-NFNISFSKFEKMSLKV	113	Pb-M17	628	EFILNHSI
		······································				**:**.::
	89	N-terminal domain POVVSLDPTSIPIEYNTPIHDIKVOVYDIK-GGCNVEEGLTIFLVNNPGK	100			C-te
PfA-M17 Pv-M17	78	POVVSLDPTSIPIEINIPIHDIKVQVIDIK-GGCNVEEGLIIFEVNNPGKPOVVSLDPTTIPIDYHTPIDDLSIEVKDISAEACPADEGLIVFLLNSAPKHSSSGGSGGN	137 137			
PD-M17 Pb-M17	114	POVINLDPTVLPVNYTTPIDDIEIVLKDGIKESCNFDDGLAIFLVNSKSEK	164			
10 111 /		***:.**** :*::* ***.*::: * .* ::** :**:*. :	104			
PfA-M17	138	ENGPVKISSKVNDKNVSEFLKDENMEKFNVKLGTSKHFYMFNDNKNSVA	186			
Pv-M17	138	GGSAGSSGNGEGGAQIKINSSVKDNTINEFLKEGNMENFTGKLGTSKSFYIANDQKKYVS	197			
Pb-M17	165	DNGNTKISSQIKDSKINEFLSK-NDDIFNGKLGTFKSFYMANEKNKYIN	212			
		··· **.*.::*··· * : *. **** * **: *:::: :				
PfA-M17	187	VGYVGCGSVAD-LSEADMKRVVLSLVTMLHDNKLSKLTVVFEINVDKNLFRFFLETLFYE	245			
Pv-M17	198	LAYVGCGPANE-ETELEIRKVAYALVTLLHDSKHKKVSIIFEIKIEEALFRFFLEHLFYE	256			
Pb-M17	213	LSFIRCGTIDEEMTEFEIRKIVSSLVQILHDNKSVSTSIIFEIGINESLFRFFLETVFYE	272			
		····· ** · ·* ······ ·** ·*** ·* · ····*** ···· ******				
PfA-M17	246	Helical linker domain	302			
Pv-M17	257	YVTDERFKSADKSTETDFIKNLSLHIAN-ADAYKGQIDKARVYFYGTYYAAQLIAAPS	313			
Pb-M17	273	YVVDERFKSNDNKSSGNSENMKNLQIFLRNYNNNYNKQVKKSRTYFMGTYFASQLISAPS	332			
		*:.*******:. :::*:*:*:*****************				
PfA-M17	303	C-terminal domain NYCNPVSLSNAAVELAOKLNLEYKILGVKELEELKMGAYLSVGKGSMYPNKFIHLTYKSK	362			
PV-M17	314	NYCNPVSLSNAAVELAOKVNLECKILDVKELEELKMGAYLSVGKGSMYPNKFIHLTYKGA	373			
Pb-M17	333	NYCNPVSLANVSVELAEKLNLECKILGIKELENLKMGAYLSVGKGSMYPNRFIHLTYKGK	392			
		***************************************				
DEA M17	363	GDVKKKIALVGKGITFDSGGYNLKAAPGSMIDLMKFDMSGCAAVLGCAYCVGTLK	417			
PfA-M17 Pv-M17	374	OTGASONEKKKIALIGKGITFDSGGYNLKAAPGSMIDLMKFDMSGCAAVLGCAYCIGTIK	417			
Pb-M17	393	GDIKKKIALVGKGITFDSGGYNLKASPGSMIDLMKFDMSGCAAVLGCAYCIGSIK	447			
		: ******:* <u>*</u> **** <u>*</u> ********************				
PfA-M17	418	X X PENVEIHFLSAVCENMVSKNSYRPGDIITASNGKTIEVGNTDAEGRLTLADALVYAEKLG	477			
PIA-MI/ Pv-M17	434	PDNVEVHFLSAVCENMVSKNSYRPGDIITASNGKTIEVGNTDAEGRLTLADALVYAEKLG	493			
Pb-M17	448	PENVEVHFLSAVCENMVSKNSYRPGDIITASNGKTIEVGNTDAEGRLTLADALVYAENIG	507			
		*:***:*********************************				
PfA-M17	478	VDYIVDIATLTGAMLYSLGTSYAGVFGNNEELINKILNSSKTSNEPVWWLPIINEYRATL	537			
Pv-M17	494	VDYIVDIATLTGAMLYSLGTSYAGVFGNNDQLINKILSSSKTSNEPVWWLPIINEYRSSL	553			
Pb-M17	508	VDHIIDIATLTGAMLYSLGTSYAGVFGNDNKLINKILESSKSSNEPVWWLPIIKEYRASL	567			
		**:*:**********************************				

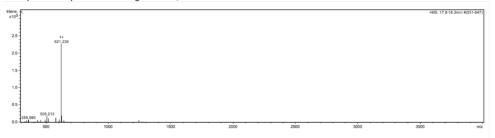
PfA-M17 Pv-M17 Pb-M17	538 554 568	NSKYADLNN NSKYADINN	ISSSVKASSIVASLFLKEFVQNTAWAHIDIAGVSWNFKARKPKGFGVRLLT ISSSVKASSVVASLFLKEFIENTPWAHIDIAGVSWNFKARKPKGFGVRLLT ISSNVKASSVVASLFLNEFIQSTSWAHIDIAGVAWNFKDRKPKGFGVRLLS ***.*****	597 613 627
PfA-M17 Pv-M17 Pb-M17	598 614 628	EFVLNDAL EFVLNDAV EFILNHSI **:**.::1		605 621 635

terminal hexahistidine tag

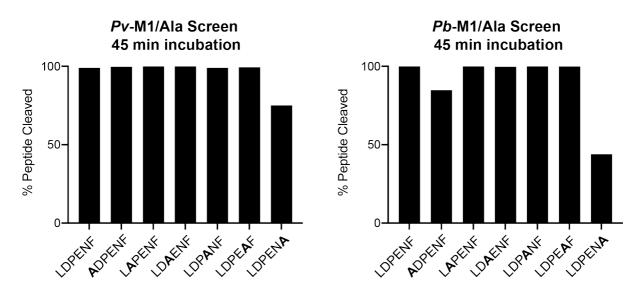
Supplementary Figure S2. Sequence alignment of *PfA*-M17, *Pv*-M17 and *Pb*-M17, colored by domain. Amino acid sequence alignment of *PfA*-M17, *Pv*-M17 and *Pb*-M17. Domain I highlighted in blue, domain II in yellow, and domain III in green. N-terminal truncation sites and C-terminal histidine site are labeled, conserved metal-binding residues are marked with 'x' and conserved active site residues boxed.



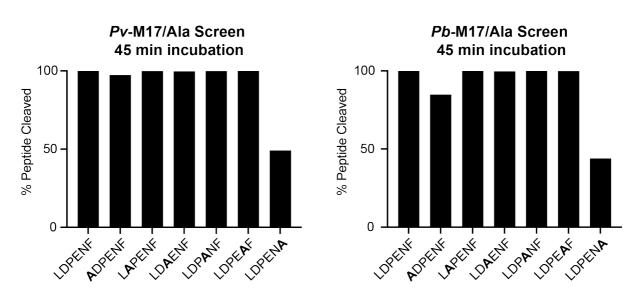
Spectrum for peak observed in green trace, after incubation with Pb-M17



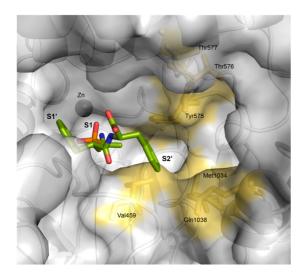
**Supplementary Figure S3. Representative mass spectrometry trace of LDPENF digestion by** *Pb*-M17. After 45 min of incubation, only one product at a molecular weight of 621 Da is observed. This correlated to the removal of the single amino acid residue Leu from the N-terminus of hexapeptide LDPENF, producing pentapeptide DPENF. Trace is representative of cleavage results obtained for each aminopeptidase – in each experiment only peptides of molecular weights 734 Da (LDPENF) and 621 Da (DPENF) were observed.



Supplementary Figure S4. Digestion of Ala screen peptides by *Pv*-M1 and *Pb*-M1 in 45-min incubation period. Template hexapeptide (LDPENF) and Ala screen peptides were digested to almost 100% in the 45-min incubation period at 37°C. *Pv*-M1 and *Pb*-M1 showed decreased activity levels against peptides ADPENF and LDPENA.



Supplementary Figure S5. Digestion of Ala screen peptides by *Pv*-M17 and *Pb*-M17 in 45-min incubation period. Template hexapeptide (LDPENF) and Ala screen peptides were digested to almost 100% in the 45-min incubation period at 37°C. *Pv*-M17 and *Pb*-M17 showed decreased activity levels against peptides ADPENF and LDPENA compared to LDPENF.



Supplementary Figure S6. APN inhibitor PL250 docked into *Pf*A-M1 X-ray crystal structure with P2' subsite highlighted.

*E. coli* APN X-ray crystal structure in complex with PL250 (PDB ID: 2ZXG) used to dock PL250 (green sticks) into *Pf*A-M1 X-ray crystal structure (PDB ID: 3EBG). S1, S1' and S2' pockets labelled. Residues postulated