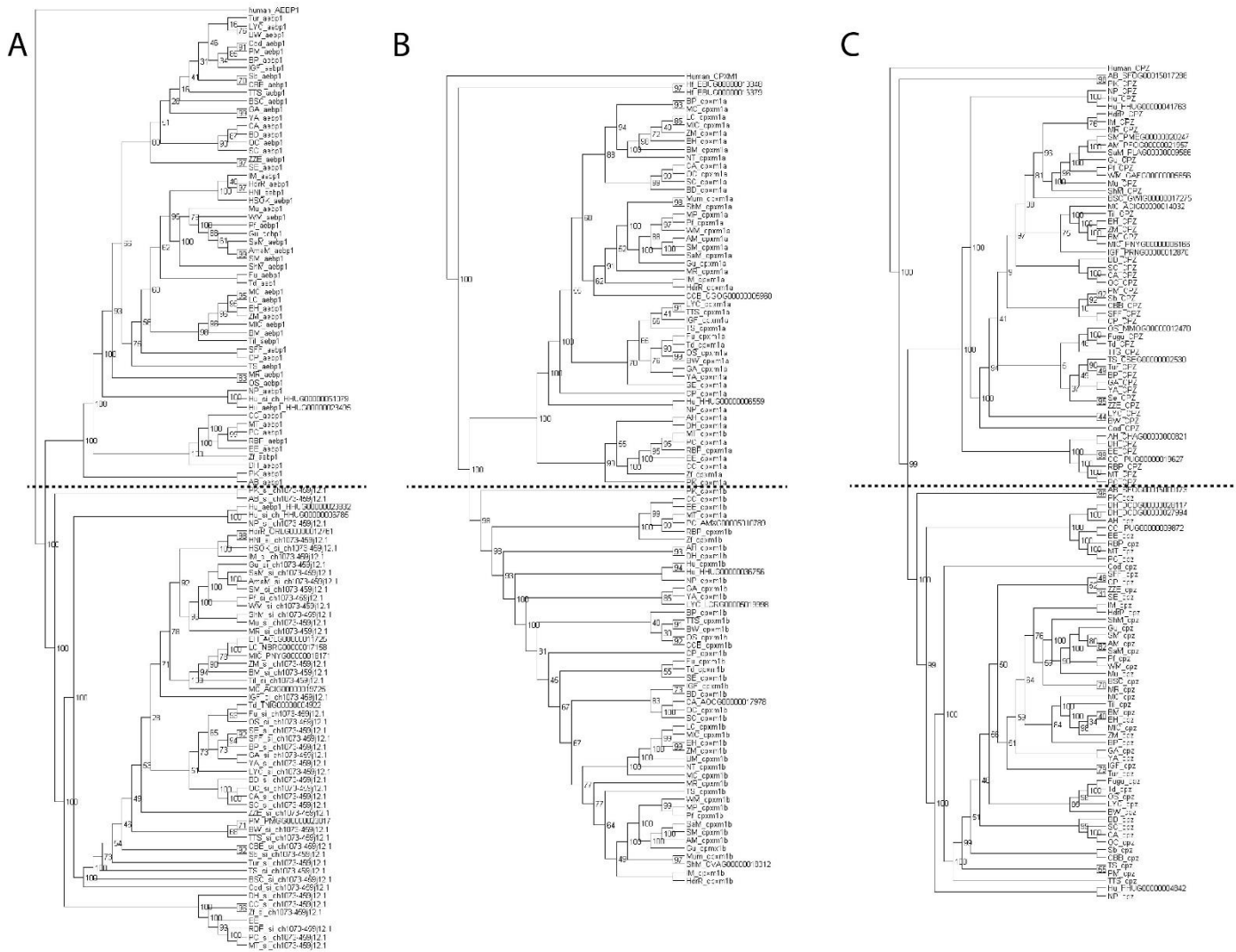


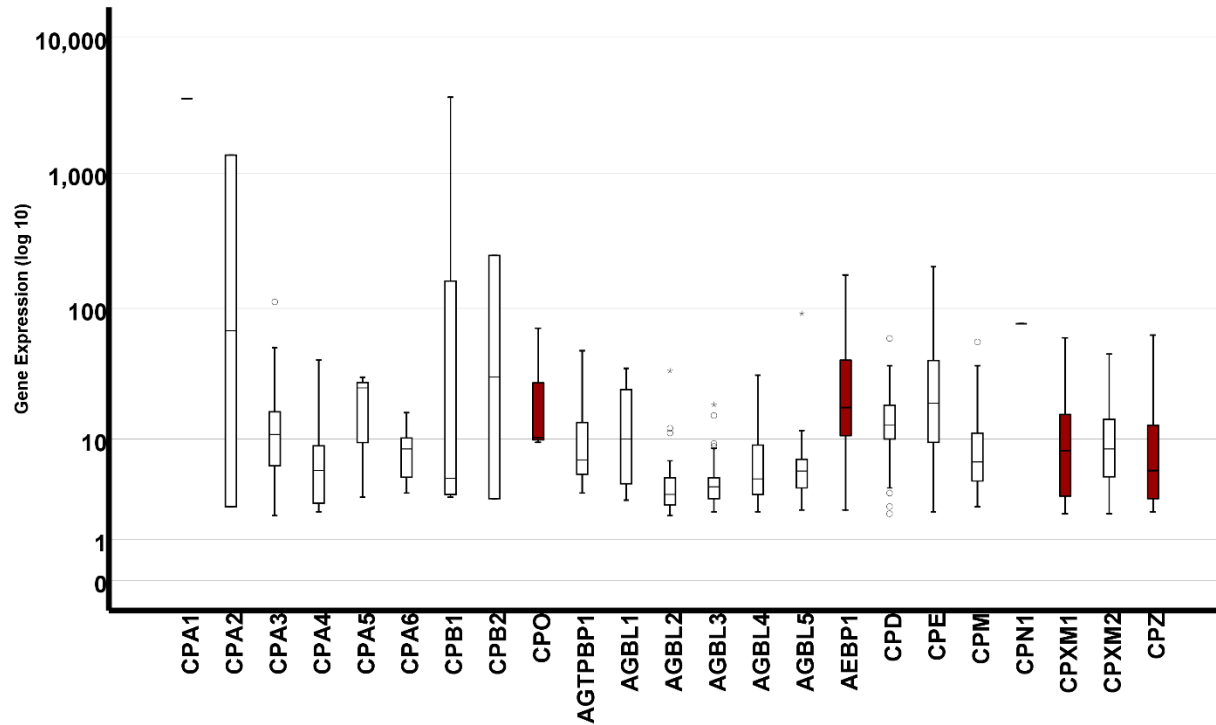
Supplementary Information

Acquisition of new function through gene duplication in the metallocarboxypeptidase family

Daniel Fajardo, Ritchie Saint Jean, and Peter J. Lyons

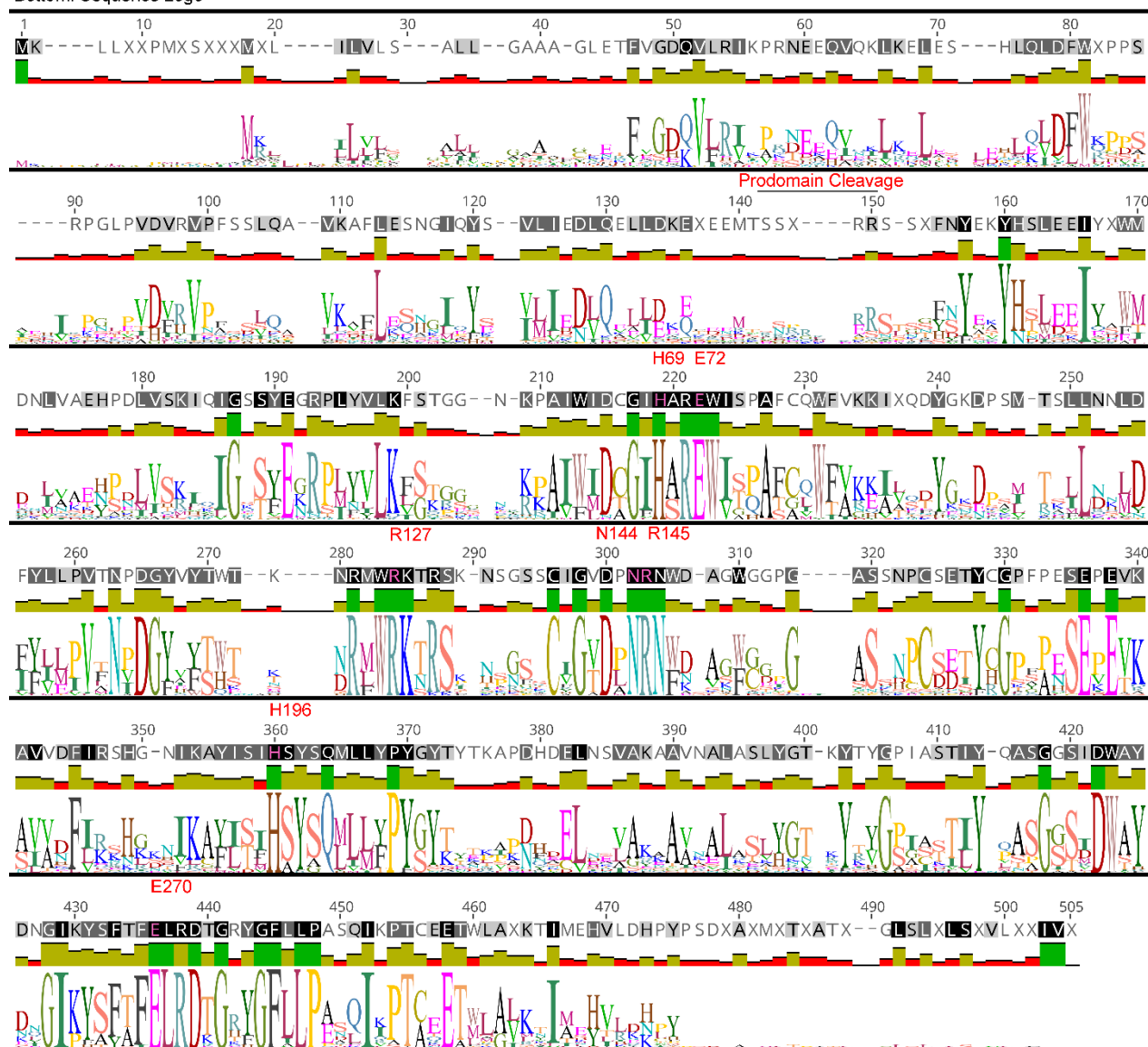


Supplementary Figure S2. Phylogenetic trees showing relationships among all AEBP1, CPXM1 and CPZ gene duplicates.



Supplementary Figure S3. Gene expression levels do not impact rates of gene duplication within the CP family. Expression levels for the indicated human genes were curated from proteomics.org. Expression levels of all tissues were averaged for each gene. An independent sample t-test was performed comparing all frequently duplicated genes (red) to all others (white; $p=0.657$).

Top: Pan-CPA/B Consensus
 Middle: Mean Pairwise Identity
 Bottom: Sequence Logo



Supplementary Figure S4. In order to categorize predicted proteins as pseudogenes, pseudoenzymes, or active enzymes, a multiple alignment was created using Clustal Omega, consisting of a broad range of vertebrate carboxypeptidase sequence representatives (CPA1, CPA2, CPA3, CPA4, CPA5, CPA6, CPB1, CPB2, and CPO) typically including five mammal sequences (including one afrotherian, one marsupial, and one monotreme), four sauropsid sequences (two bird and two reptile), one amphibian sequence, and two fish sequences.

Supplementary Figure S5. A multiple alignment of amino acid sequences for human CPO, *Xenopus tropicalis* Cpo.1, Cpo.2, Cpo.3, Cpo.4, and bovine CPA1. Predicted ER signal peptides are shown in blue, predicted prodomains are shown in red, enzymatic domains are shown in black, and predicted GPI signal peptides are shown in green. Key active site residues are highlighted and numbered below by their position in bovine CPA1, the convention in this field.

predicted ER signal peptide; predicted prodomain; enzymatic domain;
predicted GPI signal peptide; key active site residues

hCPO: human CPO
xCpo: *Xenopus* CPO
bCPA1: Bovine CPA1

	10	20	30	40	50	60
hCPO	MKPLLETLYL	LGMLVPGGLG	-----	-----	-----	-----
xCpo.1	MKRILLCICI	LGILTQVGAY	LKVQYNGDQV	LKLTCLKTTDD	CEHMQNI--C	KQLQLDLWKP
xCpo.2	MIFLLGTIFL	LGVQVYEGSC	LTVQYNGDNV	FKITPETSEH	AQYLQTL--A	NEWLLDLWRP
xCpo.3	-MHHLWIVWT	FGTLTLQVCC	TGTEYDGGNI	LEITPESEKQ	VQCLQNI--L	QSWLLDLLKP
xCpo.4	MKLFNLWFCL	LGILVYEGFC	MKVKYDGDQV	LKMIPQTLKH	AQFMQGL--I	QEWMLDLWKP
bCPA1	MQGLLILSVL	LGAALG----	-KEDFVGHQV	LRITAADAEAE	VQTVKELEDL	EHLQLDFWRG
	70	80	90	100	110	120
hCPO	-----	-----	-----	-----Y	DRSLAQHRQE	I--VDKSVSP
xCpo.1	SRIEDIQPGK	EMHVRIPFPL	LQKFKENLHQ	YSIPFEVMIK	DVQKLIDSSN	V--GDYRRQK
xCpo.2	QTVEQIHEGS	DIHVQIPFAY	MEQMKGKLLQ	HSIPYEVLIN	DVQKLIDSNT	V--SAPKIQK
xCpo.3	LQPEDINVKT	TVHVRIPSTA	LQLVKEDLLH	CSQSLEILTG	NVKYIEE--D	K--IDTKETR
xCpo.4	VMVEQIQAGR	EMHVRVPFSH	LQEIKEKLLQ	NMLPYQILIS	DVQELVNRNT	P--IETKMOK
bCPA1	P----GQPGS	PIDVRVPFPS	LQAVKVFLEA	HGIRYRIMIE	DVQSLLDEEQ	EQMFASQSRA
	130	140	150	160	170	180
hCPO	WSLETYSYNI	YHPMGEIYEW	MREISEKYKE	VVTQHFLGVT	YETHPMYYLK	ISQPSGNPKK
xCpo.1	KILAEFDYTT	YHPMDEIYQW	MDQVKEAYS	DVSMHYLGST	YELRPIYYFK	IGWPSDKQKK
xCpo.2	ASLENYDYTK	YHPMDEIYNW	MDLMKEKHSE	IVSQHYIGCT	YELRPMYYLK	IGWPSDKQKK
xCpo.3	KTINEYNYTT	YHPMNEIYDW	INGIAKKHSQ	FVTQHLLGLT	YESRPMQYLY	ISQPSSENHKK
xCpo.4	ISLDNYDYTK	YHPMDEIYDW	MEQIQQLKHRD	LVTKHFMGST	YELRPIYYFK	IGWPSDKPKK
bCPA1	RSTNTFNYAT	YHTLDEIYDF	MDLLVAEHPQ	LVSKLQIGRS	YEGRPIYVLK	FSTGG-SNRP
	190	200	210	220	230	240
hCPO	IIWMDCGIHA	REWIAPAFQC	WVFKEILQNH	KDNSSIRKLL	RNLDFYVLPV	LNIDGYIYTW
xCpo.1	IIWMDCGIHA	REWIAYAYCQ	WVFKEILETH	KTNPLLQKVL	HNIDFYIVPV	LNIDGFVYSW
xCpo.2	IFFIDCGFHA	REWISVAFQC	WVFNEIVSHY	KTDAILANVL	KQVDFYVLPV	MNIDGYVYTW
xCpo.3	IVWIDCGIHA	REWIAPAFQC	WVFKEIVQNY	QNDQRIRKIL	QNLDIYVLPV	LNIDGYIYSW
xCpo.4	IIFMDCGIHA	REWIAYAYCQ	WVFKEILSSH	SNNKLLTNVL	KQVDFYVVPV	FNIDGYIYSW
bCPA1	AIWIDLGIHS	REWITQATGV	WFAKKFTEDY	GQDPSFTAIL	DSMDIFLEIV	TNPDGFATFH
	H69	E72				

	250	260	270	280	290	300
hCPO	TTDRLW R KSR	SPHNNGTCFG	TDL N RNFNAS	WCSIGASRNC	QDQTFCGTGP	VSEPETKAVA
xCpo.1	NVNRLW R KSR	SPHNNGSCYG	VDL N RNFNSK	WGSIGASNNC	RDETYCGTGP	ASEPEVNAVS
xCpo.2	TTNRLW R KNR	SPHENGTCYG	VDL N RNFDSQ	WCSIGASRDC	NSNTFCGPEA	ASEPETKALS
xCpo.3	TKERLW R KNR	SQYGNGTCYG	VDL N RNFNVS	WCTHRSSTNC	SSNSFCGSSP	VSEPETRAVV
xCpo.4	TTERLW R KNR	SPHNNATCYG	VDL N RNFNSS	WCSVGASRDC	NSQTFCGSAP	ASEPETQAVA
bCPA1	SQNRLW R KTR	SVTSSSLCVG	VDAN R NWDAG	FGKAGASSSP	CSETYHGKYA	NSEVEVKSIIV
	R127		N144R145			
	310	320	330	340	350	360
hCPO	SFIESKKDDI	LCFLTM H SYG	QLILTPYGYT	KNKSSNHPEM	IQVGQKAANA	LKAKYGTNYR
xCpo.1	KLLGSLKSDV	LCFLTI H SYG	QLLLLPYGYT	KDPSINHEEM	INVAQKAAAK	LQEKHGTEYR
xCpo.2	GLIEKTKSDI	LCYLT I HSYG	QMILLPYGYK	KDPSPNHDEM	MLVAKNAVAK	MKEKHNNEYE
xCpo.3	EFVESRKADI	VCFLTM H SYG	QLILTAYGYS	TGLSRNYNEI	FKVAEMAASA	MEKIHGTYR
xCpo.4	NLMERTKSQI	LFYLT I HSYG	QYILLPYGST	TNPSVNHVEM	TKVAEAAAAK	MKEKHNVYIT
bCPA1	DFVKD-HGNF	KAFLSI H SYG	QLLLPYGYT	TQSIPDKTEL	NQVAKSAVEA	LKSLYGTSYK
		H196				
	370	380	390	400	410	420
hCPO	VGSSADILYA	SSGSS R DWAR	DIGIPFSYTF	E LRDSGTYGF	VLPEAQIQPT	CEETMEAVLS
xCpo.1	VGSTSHLLYS	NSGSS R DWAT	DLGINFSYTF	E LRDTGAHGF	ILPANQIRPT	CEETMAGVMT
xCpo.2	YESSAVILYY	DSGSS G DWTV	ELGIQLSYTL	E LRDNGTYGF	VLPPDQIKPT	CEETTTAVMS
xCpo.3	AGPFSKLLYE	ASGTS Q DWVH	DLGIDFSFTF	E LRDNGSHKF	TLPEDQIQPT	CEETMAGVMT
xCpo.4	VGSSSVVLYE	NSGSS C DWAG	DIGIKFSYTF	E LRDNGTYGF	QLPAELIKPT	CEETMTAVIS
bCPA1	YGSIIITTIYQ	ASGGS I DWSY	NQGIKYSFTF	E LRDTGRYGF	LLPASQIIPT	AQETWLGVLV
		X255		E270		
	430	440	450	460		
hCPO	VLDDVYAKHW	H SDSAGR V TS	A TML L G L L V S	C MS L L-----	-	
xCpo.1	IVEHVDAKFF	N SAI--S- I F	S SN--LV S L S	L IIG L Y A I F	-	
xCpo.2	MVEYINEEYL	ENSG--V- T T	TSFWLN V FL S	FTVCIY G I A	K	
xCpo.3	IIEYVNEKYF	PNK A S-- T T V	F NC W IN I L I F	N TF M Q V S V L F	F	
xCpo.4	MMEYANEKYL	EN S A-- K - T V	T FM W LN V L L S	C AV C M Y Y A L L	H	
bCPA1	IMEHTLNNLY	-----	-----	-----	-	

Supplementary Data: Raw data for Fig. 6

Fig. 6a

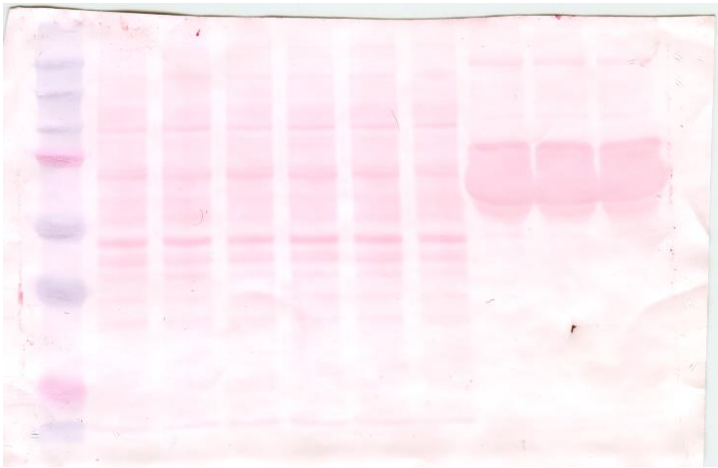
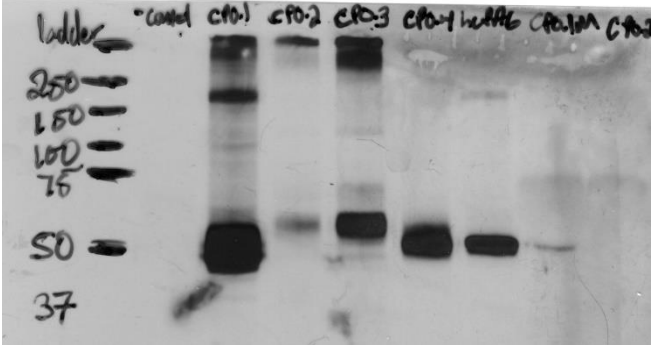


Fig. 6c

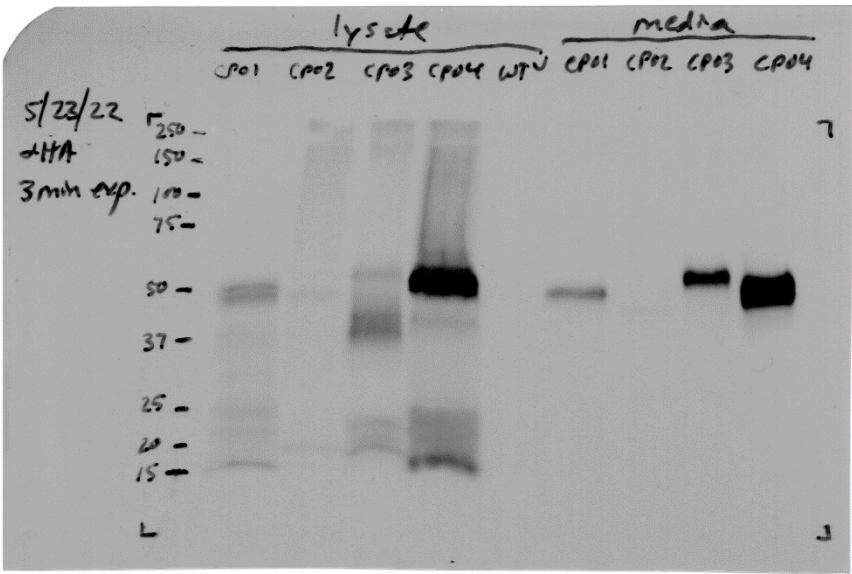


Fig. 6d

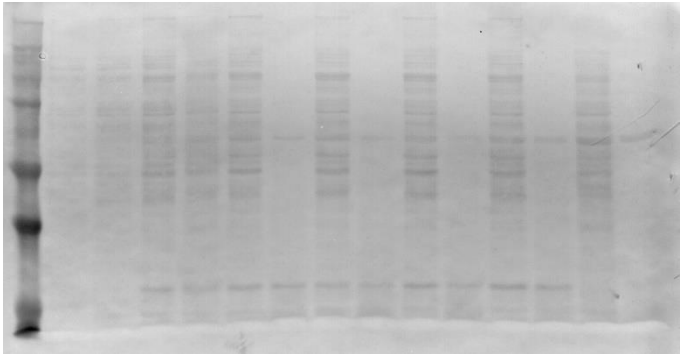
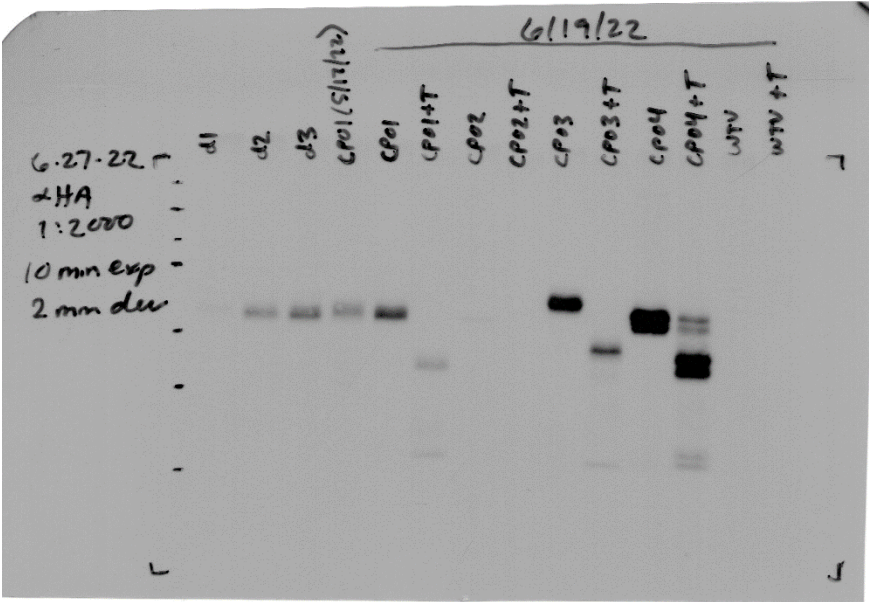


Fig. 6e

Rate of reaction (slope)		Trypsinized Media						
		WTV	CPO1	CPO2	CPO3	CPO4		
	FA-EE	0.049	-0.186	-0.004	-0.099	-0.161		
	FA-EE	0.120	-0.245	-0.034	-0.096	-0.317		
	FA-EE	0.068	-0.184	0.041	-0.086	-0.257		
	FA-EE	-0.080	-0.185	-0.313	-0.299			
	FA-EE		-0.164		-0.195			
	FA-EE		-0.125		-0.152			
average		0.039	-0.182	-0.078	-0.155	-0.245		
stdev		0.085	0.039	0.160	0.082	0.079		
	FA-FA	0.131	-0.195	-0.214	-0.293	-1.346		
	FA-FA	0.033	-0.076	-0.252	-0.218	-1.397		
	FA-FA	0.118	0.043	-0.285	-0.165	-1.355		
	FA-FA	-0.072	-0.110					
	FA-FA	-0.083						
	FA-FA	-0.257						
average		-0.022	-0.085	-0.250	-0.225	-1.366		
stdev		0.147	0.099	0.036	0.064	0.027		
	FA-FF	-0.162	-0.149	-0.294	-0.229	-0.462		
	FA-FF	-0.244	-0.090	-0.433	-0.082	-0.426		
	FA-FF	-0.134	-0.183	-0.098	-0.270	-0.481		
	FA-FF		-0.110			-0.646		
	FA-FF					-0.707		
average		-0.180	-0.133	-0.275	-0.194	-0.544		
stdev		0.057	0.041	0.168	0.099	0.124		
Raw Data								
average					stdev			
	FA-EE	FA-FA	FA-FF			FA-EE	FA-FA	FA-FF
WTV	0.039	-0.022	-0.180		WTV	0.085	0.147	0.057
CPO1	-0.182	-0.085	-0.133		CPO1	0.039	0.099	0.041
CPO2	-0.078	-0.250	-0.275		CPO2	0.160	0.036	0.168
CPO3	-0.155	-0.225	-0.194		CPO3	0.082	0.064	0.099
CPO4	-0.245	-1.366	-0.544		CPO4	0.079	0.027	0.124
Data Inverted and WTV subtracted								
average					stdev			
	FA-EE	FA-FA	FA-FF			FA-EE	FA-FA	FA-FF
WTV	0.000	0.000	0.000		WTV	0.085	0.147	0.057
CPO1	0.221	0.063	-0.047		CPO1	0.039	0.099	0.041
CPO2	0.117	0.229	0.095		CPO2	0.160	0.036	0.168
CPO3	0.194	0.204	0.014		CPO3	0.082	0.064	0.099
CPO4	0.284	1.344	0.364		CPO4	0.079	0.027	0.124

Supplementary Methods: *Xenopus tropicalis* CPO sequences

cpo.1 cDNA, HA tag inserted

```
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(cpo.1), transcript variant X1, mRNA
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TCCAAGAGAAGCATGGCACCGAGTACAGAGTTGGATCTACATCACACTTACTATATAGCAATTCTGGGTC
ATCTCGAGACTGGGCCACAGATCTAGGAATTAATTTTCTTATACATTTGAGCTGAGAGACACTGGTGCA
CATGGATTTTATACTTCTGCGAATCAGATCAGACCTACTTGTGAAGAAACCATGGCTGGAGTAATGACCA
TTGTTGAACATGTTGATGCA TAC CCA TAC GAT GTT CCA GAT TAC GCT
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ATGCATTGTTTGCAGTGGATAAATGAGAAGCATCCATCTATTTACCCGTGTTGCCTTTTATTGTGTTGTT
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TTTTCTTCACATTACCAAGTAAAGCATATGTTTGTGTCATGCA
```

Cpo.1 protein, HA tag inserted

```
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```

cpo.1 cDNA for cloning

```
>cpo.1_cloning
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cpo.2 cDNA, HA tag inserted

>XM_002937111.4 PREDICTED: *Xenopus tropicalis* carboxypeptidase O gene 2
(*cpo.2*), mRNA

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Cpo.2 protein, HA tag inserted

>XP_002937157.1 carboxypeptidase O [*Xenopus tropicalis*]

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cpo.2 cDNA for cloning

>*cpo.2*_cloning

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TGAAGAACTACTACTGCTGTTATGTCCATGGTTGAATATATAAATGAAGAATACCTTGAAAATAGTGGAGTGACAA
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cpo.3 cDNA, HA tag inserted

>XM_018097644.2 PREDICTED: *Xenopus tropicalis* carboxypeptidase O
(LOC100490370), transcript variant X2, mRNA

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TAC GAT GTT CCA GAT TAC GCT CCAA

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Cpo.3 protein, HA tag inserted

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 EEDKIDTKETRKITINEYNTTYHPMNEIYDWINGIAKKHSQFVTQHLLGLTYESRPMQYLKISQPSENHK
 KIVWIDCGIHAREWIAPAFCQWVKEIVQNYQNDQIRKILQNLDIYVLPVLNIDGYIYSWTKERLWRKN
 RSQYGNGTCTGYVDLNRNFNVSWCTHRSTNCSNSFCGSSPVSEPETRAVVEFVESRKADIVCF LTMHSY
 SQLILTAYGYSTGLSRNYNEIFKVAEMAASAMEKIHGTYRAGPFSKLLYEASGTSQDWVHDLGIDFSFT
 FELRDNGSHKFTLPEDQIQPTCEETMAGVMTII EYVNEKYF **YPYDVDPDYA** PNKASTTVFNCWINILIFNTFMQVSVL
 FF

cpo.3 cDNA for cloning

>cpo.3_cloning

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TGGCTGGTGTGATGACCATTATAGAATATGTCAATGAAAAATACTTTT**TACCCATACGATGTTCCAGATTACGCT**CCA
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cpo.4 cDNA, HA tag inserted

>XM_018097354.2 PREDICTED: *Xenopus tropicalis* carboxypeptidase O
(LOC100489361), mRNA

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CCAATTCTGAAAAATTTGTTTTTAACAAGATTGTCTTCTTTTTTAAAGAAAATTAAGTGTCTTGTAACTG
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Cpo.4 protein, HA tag inserted

>XP_017952843.2 carboxypeptidase O [*Xenopus tropicalis*]
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FCGSAPASEPETQAVANLMERTKSQILFYLTIHSGYQYILLPYGSTTNPSVNHVEMTKVAEAAAAMKMEK
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cpo.4 cDNA for cloning

>*cpo.4*_cloning

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