

## SUPPLEMENTARY DATA (Küster et al. Cathepsins in zebrafish embryos)

### 1. ESI-MS/MS based identification of upregulated protein bands from 1D SDS-Experiment (Figure 2B)

#### 1.1 Method

The two protein bands (fragment a and fragment b from 0.2 mM leupeptin exposure [Figure 2B]) of interest were excised from stained gels, destained, and prepared for nano-HPLC/nano-ESI-MS/MS analysis by reduction, alkylation, tryptic digestion, peptide extraction, and drying. Peptides were reconstituted in 0.1% formic acid. The measurements were performed using a nano-HPLC system (nanoAcquity, Waters) online coupled to a linear ion trap/orbitrap hybrid mass spectrometer (LTQ orbitrap XL ETD, Thermo Fisher Scientific). Briefly, samples were injected by the autosampler and concentrated on a trapping column (nanoAcquity UPLC column, C18, 180  $\mu\text{m}$  x 20 mm, 5  $\mu\text{m}$ , Waters) with 0.1% formic acid at a flow rate of 15  $\mu\text{L}/\text{min}$ . After 4 min, the peptides were eluted onto the separation column (nanoAcquity UPLC column, C18, 75  $\mu\text{m}$  x 150 mm, 1.7  $\mu\text{m}$ , Waters). Chromatography was performed using 0.1% formic acid in solvents A (100% water) and B (100% ACN) with peptides eluting over 30 min with 8–40% solvent B gradient. Continuous scanning of eluted peptide ions was carried out between  $m/z$  350–2000 $m/z$ , automatically switching to MS/MS CID mode on ions exceeding an arbitrary intensity of 2000 counts. Raw MS/MS spectra were converted to Mascot generic files (mgf-files) and database searches by MASCOT search engine were performed using the ProteomDiscoverer 1.0 software (Thermo Fisher Scientific). Searches were conducted on NCBI database against all *Danio rerio* sequences, tolerating up to one tryptic missed cleavage, a mass tolerance of 10 ppm for precursor ions and 0.5 Da for MS/MS product ions allowing for methionine oxidation (dynamic modification), and cysteine carbamidomethylation (static modification).

<i>Band Name</i>	<i>Protein Name</i>	<i>Species</i>	<i>MASCOT Score</i>	<i>Database</i>	<i>NCBI ID / GenBank ID</i>	<i>MW (kDa) obs/cal</i>
0.2 mM Leupeptin fragment a	Vitellogenin 1 fragment a	<i>Danio rerio</i>	5317	NCBI	gi 94733730 / CAK03614	100/149.8
0.2 mM Leupeptin fragment b	Vitellogenin1 fragment b	<i>Danio rerio</i>	4399	NCBI	gi 94733730 / CAK03614	66/149.8

#### 1.2 Results

##### Mascot Search Results

## Vitellogenin 1 fragment a:

Match to: [gi|94733730](#) Score: 5317  
vitellogenin 1 [Danio rerio]

Nominal mass ( $M_r$ ): 149825; Calculated pI value: 8.74  
NCBI BLAST search of [gi|94733730](#) against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Danio rerio](#)

Fixed modifications: Carbamidomethyl (C)  
Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 46%

Matched peptides shown in **Bold Red**

1 MRVVLLALTV ALVACQQFNL VPEFAHDKTY **VYKYEALLLG GLPQEGLARA**  
51 GIKVSSK**VLI SATTENTYLM KLMDPLLYEY AGTWPKDPFV PATKLTSA**  
101 **AQLQIPIKFE YANGVVGKVF APAGVSPTVM NLHRGILNIL QLNKKTQNI**  
151 YEMQEAGAQQ VCR**THYVINE DPKANHIIVT KSKDLSHCQE RIMKDVGLAY**  
201 **TERCAECTER** VKSLIETATY NYIMKPADNG ALIAEATVEE VYQFSPFNEI  
251 HGAAMMEAK**Q TLAFFEIEKT PVVPIKADYM PRGSLQYEFATEILQTP**  
301 **MKISDAPAQI VEVLKHLVSN NKDMVHDDAP FKFVQLVQLL R**  
351 **IWSQFKDKPV YRRWLLDALP AVGTPVVIKF IKEKFLAGEF TTPEFIQTLV**  
401 **IALQVMTADP ETIKMTASLA THEKFATIPA LREVVMVLYG SLIAKYCVAV**  
451 PTCPAELLRP IHEIATEAIS **KNDIPEITLA LKVMGNAGHP SSLKPIMKLL**  
501 **PGLRRTAANAL PIRVQVDAIL ALRNIAKKEP KLVQPVVALQL VLDRALHPEV**  
551 **RMVACIVLFE AEPVALVSS LAGALRIEPN MHVAFAYSH IKSLTRITAP**  
601 **DMAVAGAAAN VAIKLMRKL DRLNYRYSRA FQMDYYYTPL MIGAAGSAYM**  
651 **INDAATILPR AVVAKARAYL AGAAADVIEF GVRTGGIHEA LLKSPADES**  
701 **ADRIKIKRT LRALTNWKAL PTDKPLASAY LKVFQGEVAY VNFDKTIEE**  
751 **AIPMATGPKP RALLKEALKA LQEGVAFQYA KPLLAEEVRR ILPTAVGVPM**  
801 EFSWYTAAVA AASVNVQATI TPALPEK**LES MTYEQLKKT** VQFQAEARPS  
851 VALQTFAVMG VNTAFIQAAV MARGKIRTIA PGKVAAR**ADI LKGNKYVEAL**  
901 **PVELPEHIAS ASFETYAVVR NIEDHSAERS VPLVPELSLQ NSQASYAGDL**  
951 SSEMSSVASV RAPAPFDRTL CYAVPYIEIK GCVEVHSHNA AFIRNSTLFY  
1001 IIGHHSVRAA VARAEGPAVE RLEFEVQVGP RAAERLVKQI NIIDDDTPEG  
1051 QAFLLKLREI LDTEAKNAPV SSSSSSRNS RSSSSRSTST STSSSSSSSS  
1101 SSSSSSSSSM SSSRMSKTAT IIEPFRKFHK DRYLAHHSAT KDTSSGSAAA  
1151 SFEQMOKQNR **FLGNDIPPVF AIIARAVRAD QKLLGYQLAA YFDKPTARVQ**  
1201 LIVSSIAEND NMKICADGAL LSKHKVTGKF SWGAECKQYA VFAKAEAGVL  
1251 GEFPAARLEV EWER**LPIIVT TYAKKLGKHI LTAAYDTGFR FERATNSEKE**  
1301 IELTAALPSQ RSLNIIARIP EITMSKRDIY LPVAVPINPD GTFSIETYED  
1351 FLAWIQKYIK EE

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## Vitellogenin 1 fragment b:

Match to: [gi|94733730](#) Score: 4399  
vitellogenin 1 [Danio rerio]

Nominal mass ( $M_r$ ): 149825; Calculated pI value: 8.74  
NCBI BLAST search of [gi|94733730](#) against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: Danio rerio

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: **46%**

Matched peptides shown in **Bold Red**

1	MRAVVLALTV	ALVACQQFNL	VPEFAHDK <b>TY</b>	<b>VYKYEALLLG</b>	<b>GLPQEGLARA</b>
51	GIKVSSK <b>VLI</b>	<b>SATTENTYLM</b>	<b>KLMDPLLYEY</b>	<b>AGTWPKDPFV</b>	<b>PATKLTSALA</b>
101	<b>AQLQIPIKFE</b>	<b>YANGVVGKVF</b>	<b>APAGVSPTVM</b>	<b>NLHRGILNIL</b>	<b>QLNLKKTQNI</b>
151	YEMQEAGAQQ	VCR <b>THYVINE</b>	<b>DPKANHIIVT</b>	KSKDLSHCQE	RIMKDVGLAY
201	<b>TER</b> CAECTER	VKSLIETATY	NYIMKPADNG	ALIAEATVEE	VYQFSPFNEI
251	HGAAMMEAK <b>Q</b>	<b>TLAFVEIEKT</b>	<b>PVVPKADYM</b>	<b>PRGSLQYefa</b>	<b>TEILQTPIQl</b>
301	<b>MKISDAPAQI</b>	<b>VEVLKHLVSN</b>	<b>NKDMVHDDAP</b>	<b>FKFVQLVQLL</b>	<b>RVASLEKIEA</b>
351	<b>IWSQFKDKPV</b>	<b>YRRWLLDALP</b>	<b>AVGTPVLIKf</b>	<b>IKEKFLAGEF</b>	<b>TTPEFIQTLV</b>
401	<b>IALQMVTADP</b>	<b>ETIKMTASLA</b>	<b>THEKFATIPA</b>	<b>LREVVMLGYG</b>	<b>SLIAKYCVAV</b>
451	PTCPAELLRP	IHEIATEAIS	KNDIPEITLA	LKVMGNAGHP	SSLKPIMKLL
501	<b>PGLRTAANAL</b>	<b>PIRVQVDAIL</b>	<b>ALRNIAKKEP</b>	<b>KLVQPVALQL</b>	<b>VLDRALHPEV</b>
551	<b>RMVACIVLFE</b>	<b>AEPSVALVSS</b>	<b>LAGALRIEPN</b>	<b>MHVASFAYSH</b>	<b>IKSLTRITAP</b>
601	<b>DMASVAGAAN</b>	<b>VAIKLMSRKL</b>	<b>DRLNYRYSRA</b>	<b>FQMDYYYYTPL</b>	<b>MIGAAGSAYM</b>
651	<b>INDAATILPR</b>	<b>AVVAKARAYL</b>	<b>AGAAADVIEF</b>	<b>GVRTGGIHEA</b>	<b>LLKSPADES</b>
701	<b>ADRITKIKRT</b>	<b>LRALTNWKAL</b>	<b>PTDKPLASAY</b>	<b>LKVFQGEVAY</b>	<b>VNFDKTIIEE</b>
751	<b>AIPMATGPKP</b>	<b>RALLKEALKA</b>	<b>LQEGVAFQYA</b>	<b>KPLLAAEVRR</b>	<b>ILPTAVGVPM</b>
801	EFQWYTAAVA	AASVNVQATI	TPALPEK <b>LES</b>	<b>MTYEQLKKTd</b>	<b>VQFQAEARPS</b>
851	VALQTFAVMG	VNTAFIQAAV	MARGKIRTIA	PGKVAARADI	LKGNKY <b>VEAL</b>
901	<b>PVELPEHIAS</b>	<b>ASFETYAVVR</b>	<b>NIEDHSAERS</b>	<b>VPLVPELSLQ</b>	<b>NSQASYAGDL</b>
951	SSEMSSVASV	RAPAPFDRTL	CYAVPYIEIK	GCVEVHSHNA	AFIRNSTLFY
1001	IIGHHSVRAA	VARAEGPAVE	RLEFEVQVGP	RAAERLVKQI	NIIDDDTPEG
1051	QAFLLKLREI	LDTEAKNAPV	SSESSSRNS	RSSSSRSTST	STSSSSSSSS
1101	SSSSSSSSSM	SSSRMSKTAT	IIIEPFRKFHK	DRYLAHHSAT	KDTSSGSAAA
1151	SFEQMOKQNR	FLGNDIPPVF	AIIARAVRAD	QKLLGYQLAA	YFDKPTARVQ
1201	LIVSSIAEND	NMKICADGAL	LSKHKVTGKF	SWGAECKQYA	VFAKAEAGVL
1251	GEFPAAR <b>LEV</b>	<b>EWERLPIIVT</b>	<b>TYAKKLGKHI</b>	<b>LTAAYDTGFR</b>	<b>FERATNSEKE</b>
1301	IELTAALPSQ	RSLNIIAR <b>IP</b>	<b>EITMSKRDIY</b>	<b>LPVAVPINPD</b>	<b>GTFSIETYED</b>
1351	FLAWIQKYIK	EE			

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