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## Data Article

# Tetramer organizing polyproline-rich peptides identified by mass spectrometry after release of the peptides from Hupresin-purified butyrylcholinesterase tetramers isolated from milk of domestic pig (*Sus scrofa*)



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## ABSTRACT

Milk of the domestic pig has 10 times more butyrylcholinesterase (BChE) per mL than porcine serum. We purified BChE from porcine milk by affinity chromatography on Hupresin-Sepharose. The pure porcine BChE (PoBChE) was a tetramer with a molecular weight of 340,000, similar to that of human BChE tetramers. The C-terminal 40 residues of PoBChE constitute the tetramerization domain. The glue that holds the 4 BChE subunits together is a polyproline-rich peptide. Mass spectrometry analysis of trypsin-digested PoBChE identified a variety of polyproline-rich peptides originating from 12 different proteins. The donor proteins exist in the nucleus or cytoplasm of cells and contribute their polyproline-rich peptides after a cell is degraded. The secreted PoBChE scavenges the polyproline-rich peptides and incorporates one polyproline peptide per PoBChE tetramer, where the polyproline peptide is bound noncovalently but very tightly with an estimated dissociation

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constant of  $10^{-12}$  M. The most abundant polyproline-rich peptides were derived from acrosin, homeobox protein HoxB4, lysine-specific demethylase 6B, proline-rich protein 12, and proline-rich membrane anchor 1 (PRiMA). The research article associated with the data in this report can be found in Saxena et al. (2018). The Data in Brief report lists all the polyproline-rich peptides identified in PoBChE tetramers.

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## Specifications table

Subject area	Biology
More specific subject area	Tetramer organizing polyproline-rich peptides of butyrylcholinesterase
Type of data	Amino acid sequences of polyproline-rich peptides released from pure PoBChE
How data was acquired	Liquid chromatography tandem mass spectrometry (LC-MS/MS) on the 6600 Triple-TOF mass spectrometer (AB Sciex).
Data format	Analyzed
Experimental factors	Pure PoBChE tetramers were deglycosylated with PNGaseF, denatured in a boiling water bath to release noncovalently bound polyproline-rich peptides, and digested with trypsin. Peptides were separated by Ultra High Performance Liquid Chromatography and analyzed by MS/MS. Peptide data were searched against the NCBI Inr 15Sep2014 database for <i>Sus scrofa</i> proteins using the Paragon algorithm from Protein Pilot v 5.01 (AB Sciex).
Experimental features	The amino acid sequence of the 574 amino acid PoBChE protein was determined by LC-MS/MS of trypsin-digested PoBChE.
Data source location	Omaha, Nebraska, USA
Data accessibility	The NCBI accession number for the PoBChE protein amino acid sequence is NP_001344438.1. <a href="https://www.ncbi.nlm.nih.gov/protein/NP_001344438.1">https://www.ncbi.nlm.nih.gov/protein/NP_001344438.1</a>
Related research article	Saxena A, Belinskaya T, Schopfer LM, Lockridge O. Characterization of butyrylcholinesterase from porcine milk. Arch Biochem Biophys 2018, 652:38-49

## Value of the data

- The finding that PoBChE is abundant in porcine milk [1] and in human milk [2,3] leads to new questions. What is the function of BChE in milk? Does the infant benefit from BChE in mother's milk as a consequence of the inactivation of octanoyl-ghrelin by BChE, thus reducing the infant's anxiety and stress [4]? Ghrelin has a role in regulation of neural circuits and body growth during neonatal development [5]. Is BChE in milk involved in these ghrelin-related effects?
- The set of polyproline-rich peptides in PoBChE tetramers is different from the set in human BChE tetramers [6,7]. PoBChE was purified from milk. Human BChE was purified from plasma. Porcine plasma has a very low amount of BChE. Since incorporation of a polyproline-rich peptide stabilizes the BChE tetramer, is the limiting factor for the level of BChE the availability of polyproline-rich peptides? Does it mean the cells in the porcine mammary gland (source of milk BChE) undergo a higher rate of degradation and therefore produce more polyproline-rich peptides than cells in porcine liver (source of plasma BChE)?

- Are excess polyproline-rich peptides toxic to cells? Does BChE incorporate polyproline peptides from degraded cells because degraded cells are a convenient source? If excess polyproline-rich peptides are toxic to cells, BChE might be protecting the organism by scavenging these peptides.
  - The BChE tetramer incorporates not only short polyproline-rich peptides, but also long proteins that contain a polyproline-rich region. An example is the C5 variant of human BChE whose tetrameric structure includes a 60 kDa lamellipodin protein [8]. Does the BChE tetramer, and by implication the AChE tetramer, serve as a carrier of proteins that confer the observed non-cholinergic functions of BChE and AChE in bone development [9]? Examples of polyproline-rich donor proteins that could account for non-cholinergic functions of BChE and AChE include the Homeobox protein Hox-B4 which is a transcription factor involved in development, and the Formin-homology domain containing protein 1 which is involved in cell migration and adhesion.
  - The ability of BChE subunits to assemble into stable, long-lived tetramers by binding the polyproline-rich region of a protein, suggests that BChE could serve as a delivery vehicle for any protein that has been engineered to include a polyproline-rich tag.
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## 1. Data overview

We present all the polyproline-rich peptides associated with PoBChE tetramers that were identified by mass spectrometry. In addition we show the complete amino acid sequence of the full-length protein that donated each polyproline-rich peptide, the location of the polyproline-rich peptide within the full-length protein sequence, and the abundance of the polyproline-rich peptide relative to PoBChE peptides. A brief description is given of the function of the donor protein and its location within a cell.

## 2. Experimental design, materials, and methods

### 2.1. Purification of PoBChE

PoBChE was purified from defatted porcine milk (1200 mL) by affinity chromatography on procainamide-Sepharose [10]. A side fraction was purified to homogeneity on 16 mL of Hupresin-Sepharose (CHEMFORASE, Mont-Saint-Aignan, France). Contaminating proteins were washed off with 20 mM Tris.HCl pH 7.5, 0.05% azide followed by 0.3 M NaCl in 20 mM Tris.HCl pH 7.5, 0.05% azide. PoBChE was eluted with 0.1 M tetramethylammonium bromide in 20 mM Tris.HCl pH 7.5, 0.05% azide at room temperature.

### 2.2. Sample preparation for LC–MS/MS

The Hupresin-purified PoBChE was reduced in volume from 7.3 mL to 0.17 mL in a Centricon YM-30 spin filter. After the buffer was changed to 10 mM ammonium bicarbonate pH 8, the PoBChE was deglycosylated with 1  $\mu$ L of PNGaseF for 1 h. Noncovalently bound polyproline-rich peptides were released from PoBChE by denaturing the protein in a boiling water bath for 5 min. The denatured 170  $\mu$ g of PoBChE protein in 170  $\mu$ L of 10 mM ammonium bicarbonate pH 8 was digested with 2  $\mu$ g of trypsin (Promega V511C, 2  $\mu$ g in 5  $\mu$ L) for 20 h at 37 °C in a humidified chamber. Particles that could clog the small diameter tubing in the Ultra High Pressure Liquid Chromatography column were removed by centrifuging the digest for 30 min at 14,000 rpm in a microfuge. A 10  $\mu$ L aliquot from the top of the centrifuged digest was transferred to an autosampler vial. The protein concentration in the digest was estimated at 1  $\mu$ g/ $\mu$ L.

### 2.3. LC–MS/MS

The protocol for liquid chromatography tandem mass spectrometry (LC–MS/MS) is described in detail in [11]. In brief, peptides in a 5  $\mu$ L volume were separated on a cHiPLC Nanoflex microchip

column (Eksigent, Dublin, CA) packed with ChromXP C18. The eluted peptides were electrosprayed into the 6600 Triple-TOF mass spectrometer (AB Sciex) where mass spectra were collected in positive mode. Peptides were fragmented by collision-induced dissociation with nitrogen gas. The Triple-TOF data were searched against the NCBI nr 15Sep2014 database for *Sus Scrofa* proteins using the Paragon algorithm from Protein Pilot (AB Sciex) (Tables 1–12).

**Table 1**

Acrosin peptides in PoBChE tetramers.

Name	Gene	Gi #	peptide	length	count
Acrosin	ACR	47522886 P08001	APPPPPPPPP	12	12
			PAPPPPPPPPP	12	12
			PPAPPPPPPP	12	6
			PPAPPPPPPPPPPP	16	3
			PPAPPPPPPPPPPP	16	9
			APPPPPPPPPPPPP	16	3
			PAPPPPPPPPPPPPP	16	3
			PPPPPPPPPPPPPPQ	17	4
			APPAPPPPPPP	14	2
			PAPPPAPPPPPPP	14	2
			PPAPPPPPPP	12	7
			APPPPPPPPPPP	14	1
			PAPPPPPPPPPPP	14	2
			PPAPPPPPPPPP	14	2
			APPPPPPPPPPP	13	2
			PAPPPPPPPPPPP	13	2
			PPAPPPPPPP	11	1
			PPPPPPPPPPPP	12	5
			PPPPPPPPPPPP	13	11
			PPPPPPPPPPPPPP	14	7
PPPPPPPPPPPPPP	15	1			
PPPPPPPPPPPPPPPP	17	20			
PPPPPPPPPPPPPPPPPP	18	13			
PPPPPPPPPPPPPPPPPP	19	6			
PPPPPPPPPPPPPPPPQ	18	2			

Total peptide count 138.

138 peptides for acrosin compared to 10978 peptides for PoBChE calculates to 1.25%.

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> sp|P08001|ACRO_PIG Acrosin OS=Sus scrofa GN=ACR PE=1 SV=5 MLPTAVLLVLAVSV
AARDNATCDGPGCLRFRQKLESGMRVVGMSAEPGAWPVMVSLQIFMYHNNRRYHTCGGILLNSHWLTAACHCFKN
KKKVTDWRLIFGANEVVGWGSNKPKPLQERFVEEII IHEKYVSGLEINDIALIKITPPVPCGPF IGPGCLPQFKA
GPPRAPQTCVWTGWGYLKEKGPRTSPTLQEARVALIDLLELCNSTRWYNGRIRSTNVACAGYPRGKIDTCQGDSSGGL
MCRDRAENTFVVGITSWGVCARAKRPGVYTSTWPYLNWIASKIGSNALQMVLGTTPRPSTPAPPVVRPPSVQTP
VRPPWYFQRPPGPSQQPGSRPRP[PAPPPAPPPPPPPPPPPPPPPPPPPPPPPQ]VSAKPPQALSFAKRLQQLIE
ALKGTAFSSGRSYYETETDQLQELPAS.
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27 residues PAPPPAPPPPPPPPPPPPPPPPPPPPPQ MW 2648.4.

Acrosin is the major proteinase present in the acrosome of mature spermatozoa. It is a typical serine proteinase with trypsin-like specificity. It is stored in the acrosome in its precursor form, proacrosin. The active enzyme functions in the lysis of the zona pellucida, thus facilitating penetration of the sperm through the innermost glycoprotein layers of the ovum. The mRNA for proacrosin is synthesized only in the postmeiotic stages of spermatogenesis. In humans proacrosin first appears in the haploid spermatids. <https://www.gtexportal.org/home/gene/ACR> The human acrosin gene is expressed in testis and to a small extent in mammary breast tissue, lung, spleen, adipose tissue, and tibial artery.

**Table 2**  
Homeobox protein Hox-B peptides in PoBChE tetramers.

Name	Gene	Gi #		Length	count
Homeobox protein Hox-B4	HOXB4	311267494	RDPGPPPPPPPPPPPPPPGL	21	32
			DPGPPPPPPPPPPPPPPGL	20	9
			GPPPPPPPPPPPPPPGL	18	10
			PGPPPPPPPPPPPPPPGL	19	24
			GPPPPPPPPPPP	13	8
			PGPPPPPPPPPPP	13	8
			PGPPPPPPPPPPPPP	17	1
			PPPPPPPPPPP	12	5
			PPPPPPPPPPP	13	11
			PPPPPPPPPPPPP	14	7
			PPPPPPPPPPPPP	15	1

Total peptide count 116.

116 homeobox peptides/ 10978 PoBChE peptides = 1.0%.

> XP\_003131596.1 PREDICTED: homeobox protein Hox-B4 [Sus scrofa] MAMSSFLINSNYVDP  
KFPPCEEYSQSDYLPDSDHSPGYAGGQRRESSFQPEAGFGRRAACTVQRYAAC RDPGPPPPPPPPPPPPPPPPGL  
SPRAPAPPPSGALLPEPGQRC EAVSSPPPPPCAQNPLHPSHSAKCEPVVYPWMRKVHVSTVNPNYAGGEPKRSRTAY  
TRQQVLELEKEFHYNRYLTRRRRVEIAHALCLSERQIKIWFQNRMRKWKKDHLKLPNTKIRSGGPASAAGGPPG  
RPNGGPPAL.

21 residues RDPGPPPPPPPPPPPPPPGL MW 2069.1.

The HOXB4 gene is a member of the Antp homeobox family and encodes a nuclear protein with a homeobox DNA-binding domain. It is included in a cluster of homeobox B genes located on human chromosome 17. The encoded protein functions as a sequence-specific transcription factor that is involved in development. Intracellular or ectopic expression of this protein expands hematopoietic stem and progenitor cells in vivo and in vitro, making it a potential candidate for therapeutic stem cell expansion.

**Table 3**  
Lysine – specific demethylase 6B peptides in PoBChE tetramers.

Name	Gene	Gi #	peptide	Length	count
Lysine-specific demethylase 6B	KDM6B	545860221	PPPPPPPPPPPPPLPLGLAT	21	1
			PPPPPLPPPPPPPPPPP	17	1
			LPPPPPPPPPPP	12	17
			LPPPPPPPPPPP	13	17
			LPPPPPPPPPPPPP	16	5
			PLPPPPPPPPPPP	12	16
			PLPPPPPPPPPPP	13	15
			PLPPPPPPPPPPPPP	16	4
			PLPPPPPPPPPPPPP	17	1
			PPLPPPPPPPPP	12	7
			PPLPPPPPPPPP	13	9
			PPLPPPPPPPPPPP	16	4
			PPPLPPPPPPPPP	12	21
			PPPLPPPPPPPPP	13	19
			PPPLPPPPPPPPPPP	16	9
			PPPLPPPPPPPPP	12	6
			PPPLPPPPPPPPP	13	26
			PPPLPPPPPPPPPPP	16	7
			PPPPPPPPPPP	12	5
			PPPPPPPPPPP	13	11
			PPPPPPPPPPPLPGL	17	1
			PPPPPPPPPPPPP	14	7
			PPPPPPPPPPPPP	15	1

Total peptide count 210.

210 Lysine-specific demethylase 6B peptides/10978 PoBChE peptides = 1.9%.

> XP\_005657086.1 PREDICTED: LOW QUALITY PROTEIN: lysine-specific demethylase 6B [Sus scrofa] MHRAVDPPGARTAREAFALGGLSCAGAWSSCPPHPPPPRSAWLPGGRCSASIGQPPLSAPLPPSH GSSSGHPNKLIFAPGTPNPRPLHGKLES LHGCVQALLREPAQPLWEQLGQLYESEHDSEEAIRCYHSALRYGSSL AELGPRIGRLQQAQLWNFHAGSCQHRPKVLPPLQVWNLHLHEHKRNYGAKRGGPPVKRAAEPVQVPPAALSG PSGEGLSPGGKRRRCNSEQTGLPPGL[PLPPPPPLPPPPPPPPPPPPPPPPPLPGLAT]SPPFQLTKPGLWST LHGDAGWPERKGTAPPERQEQRHSLPRHPYPYPAPAYPSRTPWPRLVPAAPPGGPXPPGAESHGCPPATRPFGSD LRESRVQRSRMDSVSPAATTACVPYAPSRPPALPGTTTSSSSSSSNTGLRGVEPSGIPGADHYQTPALEVSSHQ GRLGPSAHSRRKFLAAPAATPHLSLPPGPPSPPPPCPRLLRPPPPAWLKGPAACRAAREDEGEILEELFFGAEGR PRPPPPPLPHREGFLGPPAPRFSVGTQDSHTPPTPPTTSSSSSSNNGSHSSSPTGVSFPPPPYLARSMDPLPRPPS PTLSPQDPLPLALPLSLALPPAPPSSCHQNTSGSFRRPESPRPRVSFPKTPPEVGGPSPGPLNKAPQVPVSRVGGELPA RGRPLDFPPTPLEDQFEPAEFKILPDGLANIMKMLDESIRKEEQQQEQAGVPPPPPLKEPFASLQPPFPPTDTA PATTAATTAATTTATQEEEEKKPPALPPPPPLAKFPPPPQPPPPPLPPPPASPASLLKSLASVLEGQKYCYRGTG AAVATRPGPLPTTQYSPGPPSGATAPPTSAAPSAQGSFPQPSASSSQFSTSGGPWARERRAGEEPAGPPTTAPP PPPLPLPPARSESEVLEEISRACETLVERVGRGATDPADPADTADPVDTGAERLLPPAQAKEEAGGASAVAAAAAG PGSSKRRQKEHQKEHRRHRACKDSVGRRPREGRAKAKAKAPKEKSRVNLGNLDLQSEEIQGREKARPDLGASKA KPPTAPAPLPAPAPSTQSTPPSAPVPGKKAREEAPGPPGVSADMLKRLSLSEGPPKELKIRLIKVESGDKETFIA SEVEERLRMLADLTI SHCAADVVRASKNAKVKGKFRESYLSPAQSVKPKINTEEKLPREKLNPTPSIYLESKRDA FSPVLLQFCTDPRNPITVIRGLAGSLRLNLGLFSTKTLVEASGEHTVEVRTQVQQPSDENWDLTGTRQIWPCESSR SHTTIIAKYAQYQASSFQESLQEEKESEDEESEEPDSTTETPPSSAPDPKNNHIIKFGTNIIDLSDAKRWKQLQELL KLPAPMRVTSTGNMLSHVGHITILGMNTVQLYMKVPGSRTPGHQENNNFCSVNIINIGPGDCEWFVHEHYWETISAF CDRHGVDYLTGSWWPILDDLYASNIPVYRFVQRPGDLVWVWVQATGWCNNIAWNVGPLTAYQYQLALERY EWNEVKNVKSIIVPMIHVSWNVARTVKISDPDLFKMIKFCLLQSMKHCQVQRESLVRAGKKIAYQGRVKDEPAYCNC ECDVEVFNILFVTSSENGSRNTYLHCEACARRRSAGLQGVVLEQYRTEELAQAQYDAFTLAPASTSR.

28 residues PLPPPPPLPPPPPPPPPPPPPLPGLAT MW 2737.5.

Histone demethylase specifically demethylates 'Lys-27' of histone H3, thereby playing a central role in histone code (PubMed:17825402, PubMed:17851529, PubMed:17713478, PubMed:18003914). Demethylates trimethylated and dimethylated H3 'Lys-27' (PubMed:17825402, PubMed:17851529, PubMed:17713478, PubMed:18003914). Plays a central role in regulation of posterior development, by regulating HOX gene expression (PubMed:17851529). Involved in inflammatory response by participating in macrophage differentiation in case of inflammation by regulating gene expression and macrophage differentiation (PubMed:17825402). Plays a demethylase-independent role in chromatin remodeling to regulate T-box family member-dependent gene expression by acting as a link between T-box factors and the SMARCA4-containing SWI/SNF remodeling complex.

**Table 4**  
Zinc finger homeobox protein 4 peptides in PoBChE tetramers.

Name	Gene	Gi #	peptide	Length	Count
Zinc finger homeobox protein 4	ZFHX4	545821241	TPPPPPPPPPPPPPPPPPSA	22	2
			TPPPPPPPPPPPPPSSL	18	1
			PPPPPPPPPPPP	12	10
			PPPPPPPPPPPP	13	11
			PPPPPPPPPPPP	14	7
			PPPPPPPPPPPP	15	1
			PPPPPPPPPPPPPP	17	20
			PPPPPPPPPPPPPP	18	13
			PPPPPPPPPPPPPP	19	6

Total peptide count 71.  
69 Zinc finger homeobox protein 4 peptides/10978 PoBChE peptides = 0.6%.

> XP\_005663076.1 PREDICTED: LOW QUALITY PROTEIN: zinc finger homeobox protein 4 [Sus scrofa] METCDSPPISRQENGQSTSKLCGTAQLDNEVPEKVAGMEPDRENSSTDDNLKTDERKSEVLLGF SVENAAATQVTSAKEIPCNECATSFP SLQKYMHEHCNARLPVLKDDNESEISELESDVENLTGEIVYQPDGSAY IIEDSKESGQNAQTGANSKLFSTAMFLDSLASAGEKSDQSASAPMSFY PQIINTFHIIASSLGKPFPTADQAFPNTSA

LAGVGPVLHFSFRVYDLRHKREKDYLTSDGSAKNSCVSKDVPNNVDLSKFDGCVSDGKRKPVLMCFCLKLSFGYIRS  
 FVTHAVHDHRMTLNEEEEQKLLSNKCVSAI IQIGKDKLEPLISFLEPKKSTSVYPHFSTTNLIGDPDPTFRGLWSAFH  
 VENGDSLPAAGFAFLKGSAGTSGSAEQPLGITQMPKAEVTLGGLSSLVVNTPIITSVLSLNASSESSKMSSEKQDEND  
 CERPKESNALHPNGECPVKSEPTAGEDEDEEDAYSNELEDEEVLGELTDSIGNKDFPLLNQSI SPLSSSVLKFIEK  
 GPSSSSASVTDDAEKKKPTAAVRASGGVANSYGIGGKDFAEASASKDGATAAHSSEPARGEDSSATPHQHGFTPS  
 APGTPGPGDGS PGSGIECPKCDTVL GSSRSLGGHMTMMHSRNSCKTLKC PKCNWHYKYQQTLEAHMKEKHPEPGG  
 SCVYCKTGQPHPRLARGESYTCGYKPFRCVNCYSTTTKGNLSIHMQSDKHLNNVQNLQNGNGEQVFGHSAPAPNT  
 SLSGCGTSPSPKPKQKPTWRCEVCDYETNVARNLRIHMTSEKHMHNMLLQQNMKQIQHNHLGLAPAEAELYQYY  
 LAQNI GLTG MKLENPGDPQLMLNPFQLDPATAAALAPGLVNNELPPEIRL ASGQLMGDDLSLLTAGELSPYISDPA  
 LKLFQCAVCNKFTSDSLEALS VHVSSERSLPEE EWRAVIGDIYQCKLCNYNTQLKANFQLHCKT DKHMKYQLVAH  
 IKEGGKSNWRKLCIAIGNPVHLKCNACDYITNSVDKLRDLRHTNHRHEAALKLYKHLQKQEGAVNPPESCCYYCAVC  
 DYSTKVKLNLVQHVRSVKHHQTEGLRKLQLHQGLAPBEDNLSEIFFVKDCPPNELETASLGARTCEDDLLLEQQLR  
 APSEEQSEETEGASRPTAAEDDEKDTSERDNNEGKNSNKDTGTIITPEKELKVS VAGGTQPLLLAKEEDVATKRSK  
 PTEDSKFCHEQFYQCPYCNYSRDQSRIQMHVLSQHSVQPVICCPQCQDVL SNKMHLQLHLTHLSVSPDCVEKLL  
 MTPVPVDMMPNSLLL PAAASEKSERDTPAAITAE GPGKYSGES PMDDKSMAGLDDSKAIMEIKSEEQKPTKEPTE  
 ASEWNKNSKDGKISDPLQDQLEQQKRQPLSVSDRHVYKYRCNHC SLAFKTMQKLQIHSQYHAI RAATMCNLCQR  
 SFRTFQALKKHL EAGHPLESEAELQQLYASL PVNGELWAESETMAQDDHALEQEMEREYEV DHEGKAS PVGSDSSS  
 IPDDMGSEPKRTLPFRKGNFTMEKFLDPSRPYKCTVCKESFTQKNILLVHNSVSHLHKLKVV LQEASSVPQET  
 NSSTDNKPYKCSICNAVYSQSSTLEIHMRSVLHQTKARA AKLEPSSHVVSGHSAANVSSPGQGLDMSLAGVSSK  
 DTHLDAKELNKKQTPELISAQPAHHP PQSPAQIQMQLQHELQQQA AFFQPQFLNPAFLPHFPMTPEALLQFQQPQF  
 LFPFYIPGTEFSLGPDGLPGSAAFMPGTMGAGSLEDLQKQIQTQHVVGQTQLQILQQQAQQYQATQPQLQSQ  
 KPQQQPQPQQQASKLLKQEQTTLASAECP I VKDI PSFKEAEEMAKKQDKPKQEVXSEGEGLKEGKDEKKQKSS  
 EPSILPPRIASGARGNAAKALLENF GFELVIQYENRQKVQKKGKSGEGESTEKLECGTCGKLF SNVLI LKSH  
 QEHVHGQFFPYGALEK FARQYREAYDKLYPI SPSSPETPPPPPPPPPLPAPPQPASLGPVKLPSTVSTPIQA  
 PPF TPPPPPPPPPPPPPPPPPPPPSA PPRVQLPVSLDLPLFP SIMMQPVQHPALPQLALQLPQMDTLSADLT  
 QLCQQQLGLDPNFLRHSQFKRPRTRITDDQLKILRAYFDINNSPSEEQIQEMAESGLSQKVIKHWFRNTL FKER  
 QRNKDS PYNFSNPPI TVLEDIRIDPQPSLEHYKSDASF SKRSRTRFTDYQLRVLQDFFDTNAYPKDDEIEQLST  
 VLNLPTRVIVVWFQ NARQKARKSYENQAEAKDNEKREL TNERY IRTSNMQYQCKKCNVVFPRIFDLITHQKKQCYK  
 DEDDDA QDESQTEDSMDATDQVVYKHCTVSGQTEAAKNAPVAAA SSGSGASTPLLPSPKPEPEKTS PKPEYPTKEP  
 KYSDPSPPSQGTK PALPLASTSSEPPQAAA QPQPQPPKQPQLIGRPPSASQTPIPSSPLQISMTSLQNSLPPQLL  
 QYQCDQCTVAFPTLELWQEHQHMHFLAAQNQFLHSPFLERPM DMPYMI FDPNNPLMTGQLLSGFLTQMP PQNASSQ  
 TPASATVAASL KGNWDDKEDTNCSEKEGGNSGEDQHRDKRXTTITPDKLEILYEKYL LDSNPTRKMLDHIAREVG  
 LKRRVVQVWFQ NTRARERKQFRAVGAQSHKRC PFCRALFKAKSALESHIRSRHWNEGKQAGYSLPPSPLIATED  
 GGESPQKYIYFDYPSLPLTKIDL SSENELASTVSTPVS KTAELSPKNLLSPSSFKAECSEDVENLNAPAEAGYDQ  
 NKPPDFETSSINTAISDAT TGDENAEEMESTTGSSGDVKPALS PKEPKTLDTLAKTATTPTTEVCDEKFLFSLTSP  
 SIPFNDKDGHDQSFYITDDPDDNADRSETSSIADPSSPNPFGSSNPFKSKSNDRPGHKRFR TQMSNLQLKVLKAC  
 FSDYRTPMQECEMLGNEIGL PKRVVQVWFQ NARAKEKFKINIGKPFMINQSGTEGKTP ECTLCGVKYSARLSIRDHIF  
 SKQHISKVRET VGSQLDREKDYLAPTTVRQLMAQQELDR IKKASDVLGLAVQQPSMMDSSSLHGISLPAAYPGLPGLPV  
 LLPGMNGPSSLPGFQNSNTLTPPGAGMLGFPTSATSSPALSLS SAPTKPLLQ TPPPPPPPPPPPPPSSSL SGQQ  
 TEPQNKESKQTKPNKVKKIKEEEL EATKPEKHPKKEEKISSALSVLGKVVGETHVDP SQLQALQNAIAGDPASF  
 LGGQLPYFIPGFASYFTPLPGTVQGGYLPVCGMESLFPYGP TMPQTLAGLSGALLQYQQYQQLQDSDLQKQ  
 QKQQQQEQPQKPGQA KTSKGESEPPQNASDASETKEDKSTATESTKEEPQLESKSA DFDYVVPVFKYEFICRKC  
 QMMFTDEDAAVNHQKSFYFGQPLIDPQETVLRVPV SRYQCLACDVAISGNEALSQHLQSSLHKEKTIKQAMRNAK  
 EHVRLLPHSVCSNPNTTSTSQSAASNTYPHLSCFSMKSWPNILFQASARRAASSPSSPSSLPSTVTS SLCST  
 SGVQTS LPTESCSDSELSQKLELDLNSLEVKAKPASGLDGNFNSIRMDMFSV.

22 residues TTPPPPPPPPPPPPPPPPSA MW 2121.1.

18 residues TTPPPPPPPPPPPSSSL MW 1764.9.

May play a role in neural and muscle differentiation. May be involved in transcriptional regulation.





KGPGPMIRIKARYQTITILPMEMYKEFAEHI TNHYLGLCAALEPILSAKTKEMASALVHILQSTGKVKDFLTDLM  
MSEVDRCGENEHLIFRENTLATKAI EEYLKLVGQKYLQDALGEFIKALYESDENCEVDPSKCSAADLPEHQNLKM  
CCELAFCKI INSYCVFPRELKEVFASWRQECSSRRGRPD I SERLISASLFLRFLCPAIMSPSLFHLLQEYPPDRRTAR  
TLTLIAKVTONLANFAKFGSKEEYMSFMNQFLEHEWTNMQRFLLEISNPETISNTAGFEYIDLGRELSLHSLLLW  
EAVSQLEQSI VSKLGLPLPRILRDVHTALSTPGSGQLTGTNDLASTPGSGSSSISAGLQKMVIENDLSGLIDFTRLP  
SPTPENKDLFFVTRSSGVQPS PARSSSYSEANEPDLQMANGGKSLSMVDLQDARALDGEAGSPAGPDALAADGQ  
VPTAQLVAGWPARAAPVSLAGLATVRRAGQTPTTPGTSEGAPGRPQLLAPLSFQNPVYQMAAGLPLSPRGLGDS  
GSEGHSSLSHNSSEELAAA AKLGSFSS ; AAAAAEDLGRPPGELARRQMSL TEKGGQPTVPRQNSAGPQRR  
IDQPPPPPPPPPPAPR GRTPPTLLSTLQYPRPSSGTLASAPDWAGPGARLRQSSSSKGDSPELKPRAVHK  
QGSPVSEPNALDRTAAWLLTMNAQLLEDEALGPDPPHRDRLRKEELSAEKDLAVLQDKLRI STKKLEEYETLF  
KCQEETTQKLVLEYQARLEEGERLRQEDKDI QMKGI I SRLMSVEEELKKDHAEMQAAVDSKQKI IDAQEKRI  
ASLDAANARLMSALTQLKERYSMQARNGI SPTNPTKLQITENGEFRNSSNC.

16 residues IDQPPPPPPPPPPAPR MW 1668.9.

Functions as a scaffold protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways.

**Table 7**  
Protein FAM171A2 peptides in PoBChE tetramers.

Name	Gene	Gi #	peptide	Length	Count
Protein FAM171A2	FAM171A2	545857706	AAAPPPPPPPPPAPR	17	4

Total peptide count 4.

4 Protein FAM171A2 peptides/10987 PoBChE peptides = 0.03%.

> XP\_005668832.1 PREDICTED: protein FAM171A2 [Sus scrofa] MPPPSGPSVLARLLPLL  
GLLLGGASRAPGKSPPEPPSPQEILIKVQVYVSGELVPLARASVDVFGNRMLLAAGTTDSEGVATLPLSYRLGTWV  
LVTAARPGFLLTNSVPWRVDKLPYASVSLYLLPERPATLILYEDLVHILLGSPGAR SQPWVQFQRRARLPVSSTY  
SQLWASLTASTQQEMRAFFAFLGTDASSNGSWLELMPVA AVSVHLLAGNGTEVPLSGPIHLSLVPSEPRALA  
VGTSI PAWRFPDKSGLWRNGTGVIRKEGRQLYWTFISPQLGYAAAMASPTSGLVTITSGIQDIGTYHTIFLLTI  
LAALALLVLILLCLLIYCRRLKPRQQRHLKQLSGPSDGNKRDQATSMSQLHLICGGPLEPAASGDPEAPPPGP  
LHSAFSSSRDLAASRDDFFRAKPRASARPAEAPAGARGGEGAGLKGARSVEGPGGLEPGLLEEYRRGPPGTATFLQE  
PPSPPPFEHYLGHKGAESKTPDFLLSQSVDQLERPPSLSQAGQLIFCGSIDHHSQVRHSYIDLQAGGGGRSTDA  
LLDSGVVHEARPARRRRLREERERAA AAAPPPPPPPPPPPAPR LALSEDTEPSSSESRTGLCSPEDNSLTP  
LLDEVAPEGRAATVPRGRGRSGDSSRSASELRRDLSLTSPEDELGAEVGDEAGDKKSPWQRREERPLMVFNVK.

17 residues AAAPPPPPPPPPAPR MW 1622.9.

**Table 8**  
FH2 domain-containing protein 1 peptides in PoBChE tetramers.

Name	Gene	Gi #	peptide	Length	Count
FH2 domain-contain- ing protein 1	FHDC1	545845605	PPPPSPPPPPPP	13	1
			PPPPSPPPPPPP	12	4
			PPPPSPPPPPPP	12	4
			PPSPPPPPPPPP	12	1

Total peptide count 10.

10 FH2 domain-containing protein 1 peptides/10987 PoBChE peptides = 0.09%.

> XP\_005666867.1 PREDICTED: FH2 domain-containing protein 1 [Sus scrofa]  
MHVMNCVSSVSDKNGNIAPAAGFMIGQT PPPSPPPPPPPPP CPYSGAGFPAPPPPPPLPGGPPVPPPP  
PGLPPPSHLNGYSHLGKKKMRMSFFWKIPEEQVRGKTNIWTLAARQQHHYQIDTKTIEELFGQQEESAKSSPSRR  
GGPLNSSFREAREEITILDAKRSMNIGIFLKQFKKSPSIVEDIHQKSEHYGSETLREFLKLPESEI IKKLFKAF  
SGDVAKLSLADSLHCLIQVNPYSLRIEAMVLKKEFLPSCSSLYTDMTILRTATKELMSCEELHSILHLVLQAGNI

MNAGGYAGNAVGFKLSSLLKLADTKANKPGMNLHFVAQEAQKKDAVLLNFKSEKLHHVQEAARLSLDNTEAELHSL  
 FVTRSRSLKENIQRDGLCQQMEDFLQFAVEELSELERWKQELLAEHTLIDFFCEDKDTVKLDECLQIFRDFCIKF  
 NKAVKDNHDREVQELKQLQRLKEQEQRSSWAAGELGFSRSSSENDVELLTKRGAEDPFLHSRPI SPHSRPPNTRR  
 SRLSLGASADRELLTFLESSTGNPEELKFNSLPRSCPRQAPPSRAWMESGEQRDQDSSQAHRLPASKDQEATDPP  
 STWQSQLLAPRLEEPATALPRVRRSGVSI LRKRNSEPLGLGPVRSFPLSPALALGIKEHELVTGLAQFDLQAPKGP  
 EPARLTMNDFSPMELMSVVGESPOAPRAPNDHRCEGLIPPCFSNEDLGNILLYVRAHAASRPYRESRAPSRSSFRK  
 PSVKPLRNVPKPEDDKMCRSSSQGPESPEEAPRAPAAPAPRGPAPVPSFARNTVASSSRCLRTDSPAVARPPG  
 LTRTVSQRLRAKGGPEEAAPKDGGALRRASSARGPRKGPPELPEGPRAGSEASPKGRGAGERASVRLKDASRPALG  
 KGLHPLRK.

14 residues PPPSPPPPPPP MW 1366.7.

Formin-homology-domain-containing protein FHOD1 is involved in cell migration and adhesion, acting as a regulator of stress fibers organization, maturation of integrin-based adhesion sites, and podosome-associated contractility.

**Table 9**  
 Proline-rich protein 12 peptides in PoBChE tetramers.

Name	Gene	Gi #	peptide	Length	Count
Proline-rich protein 12	PRR12	335290066	APPPPPPPPPPPASEPK	19	2
			APPPPPPPPPPP	12	12
			APPPPPPPPPPP	13	2
			APPPPPPPPPPPPP	14	2
			LPPPPPPPPPP	12	17
			LPPPPPPPPPP	13	17
			LPPPPPPPPPPPP	16	5
			LPPPPPPPPPPPPPP	17	1
			PPPPPPPPPP	12	5
			PPPPPPPPPPPP	13	11
			PPPPPPPPPPPP	14	7
			PPPPPPPPPPPPPP	15	1
			PPPPPPPPPPPPPPPP	17	21
			PPPPPPPPPPPPPPPP	18	14
PPPPPPPPPPPPPPPPPP	19	6			

Total peptide count 123.  
 123 Proline-rich protein 12 peptides/10987 PoBChE peptides = 1.1%.

> XP\_003127395.2 PREDICTED: proline-rich protein 12 isoform X1[Sus scrofa]  
 MDRNYP SAGFDPLGAGAGWSYERSAKASLVGSSRTSHPETDILHRQAYAAPHP LQSYATNHH PAGLSGLFDTGL  
 HHAGSAGPDASVMNLI SALESRGPQPGPSASLLSQFRSPSWQTAMHTPGPTELFISGALPGSSTFPSSALSAYQ  
 HPASFGSRFPVPSSLSLQDPPFSPANGLLSPHDVHLHKPSQAPTVPSSLGFERLAGGGVLPAGLGPAQTTPYR  
 PGPPDPPPPRHLPTQFNLLASSSAAAAAAEQSSPQLYNFSGAAPGPPPERALPRQDTVIKHYQRPA SAQPPPP  
 PPAHALQH YLSCGGSYPSMGHRANLACSP LGGGEPSPGAGEPSKAGPSGATAGASGRAAGPEAAGGGAGGGGG  
 YRPIIQSPGYKTKGGYAAAGANRPPPRSTATPKCQSLGGPAAAYATGKASGAGGAGGQAYSPGQPQLLGPQ  
 AYQGQFGGGQAQDLSKGPSYSGPQQPPNPPPPGLATCQSYSPDQLQGQLYGVQGEFYPGPA AHSQGLPTASPSL  
 SYSTGHSPALSGHGGGWGSSLLGGGGEASPSHIIRPLQSPAPGRPPGVGSPGAPGKYLSSV LASAPFLAPPGAGS  
 YAAGAGGYKKG DGSSELLAGPGGPAERTEDEEFLIQHLLQAPSPPRTSGADGLVGEDGAADASKLGGSGGAGGP  
 PGTPEYELAKEDPQRYHLQSVIRTSASLDEGATAALELGLGRLKEKKKGPERGGETPEGLATSVVHYGAGAKELGAF  
 LQKSPPPPPPTAQA SAQPTPHGLLLEAGGPD LPLVLP PPPPQLLPSVLSHAPS SSSAPKVG VHLLPEAARDGAPP  
 PPPPPPPMPLQLEAHLRSHGLEPGAPSRLRPEESLEPPGAMQELLGALEPLPGPGD TGVPPTAEGKDP SGAY  
 RSPSPQGTAKARFVPLT SICFPD SLLQDEERSFFPTMEEMFGGGPADDY GKAGPPPEDEGDPKAGAGPPPGFPAYDP  
 YGPYCSRASGAGPETPGLGLDPSKPELPSTVNAEPLGLIQSGPHQA **APPPPPPPPPPPPPPPASEPK** GGLTS  
 PIFCSTKPKKLLKTS SFHLLRRRDPPFQTPKKLYAQEYEFEADEKADV PADIRLNPRLPDLVSSCSRSPALSPL  
 GDIDFCPPNPGDPGPRRRGRKPTKAKRDGPPRGRPRIRPLEGPATAGPALASTPTDGAKKPRGRGRGRKAE  
 AGGTRLEPLKPLKIKLSVPKAGEGLGASSGEAVSGADPNLSSLTREKIEAKI KEVEEKQPEMKS GFMA SFLDFL

KSGKRHPPLYQAGLTPPLSPPKSVPPSVPARGLQPPSTPAVPHPPPAGAFGLGGALEAAESEGLGLGCPSPCR  
 LDEELKRNLETLPSPSSDEEDSVAKNRDLQESISSAISALDDPPLAGPKDSTPDGPPLAADAAPVGPPLPLGLPS  
 ASSNGTPEPPLLEEKPPSPPPVPTQPPPPPALPSPPLVAPAPSSPPQ[LPPPPPPPPPPPPPPPPPPPP]  
 PPAPAPAPAPPALPSPAPPAAAAAPPPEPAAPSPDDSEPPDARPLHLAKKQETAAVCGETDEEAGESGGEG  
 IFRERDEFVIRAEDI PSLKLALQTGREPPPIWRVQKALLQKFTPEIKDGRQFCATSNYLGYPGDAKNRYQRLYVK  
 FLENVNKKDYVRVCARKPWHRPPVVRRSQQAKGPPSSSGSSAPPKAPAPPKPETPKMASEKPPPETAVP  
 EPPAPEKPSPPRLVEKEKEKERTPRGERPLRGERGTGGRQIRPDRGLTTGQPATSRLPKSRPTKVKAEPKRRKK  
 WLKEAAGNASAGGGPPGSSSDSESSPGAPSEDERAVPGRLLKTRAMREMYRSYVEMLVSTALDPDMIQALEDTHDE  
 LYLPPMRKIDGLLNEHKKVKLRLSLSPALQDALHTFPQLQVEQSGEGSPPEGAVRLRPAGEPYNRKTLSKLRKRSV  
 VRAQEFKVELDKSGYYTLVHSLHHYKYHTFLRCRDQTLAIEGGAEDLGQEEVVQQCMRNQPWLEQLFDSFSDLLAQ  
 AQAHSRCG.

19 residues APPPPPPPPPPPPASEPK MW 1863.0.

20 residues LPPPPPPPPPPPPPPPP MW 1975.1.

**Table 10**

WAS/WASL-interacting protein family member 2 isoform X1 peptide in PoBChE.

Name	Gene	Gi #	peptide	Length	Count
WAS/WASL-interacting protein family member 2 isoform X1	WIPF2	346716187	PIPPPPPPPGPPPPPTF	18	5
			MPIPPPPPPPGPPPPPTF	19	1

Total peptide count 6.

6 WAS/WASL-interacting protein family member 2 isoform X1 peptides/10987 PoBChE peptides = 0.05%.

> NP\_001231241.1 WAS/WASL-interacting protein family member2 Sus scrofa  
 [MPIPPPPPPPGPPPPPTF]NQANTELPKLSRDEQRGRGALLQDICKGTCLKKVTNINDRSAPILEKPKGSSG  
 GYGPAAALQPKGGLFQGGVPKLRPVGAKDGSNENLAGKPALQVSSRAAARPPVSTASGRPQDDTDSNRASLP  
 ELPRTQRPSPDLRSRPHATSSTGMKHSSSAPPPIPPGRRANAPPTPLAMHSNKAPAYNREKPLPPTPGQRLHPGRE  
 GPSAPPVVKPPSPVNI RTGSPGQSLAPPPPPYRQPPGVNPGSSPTNESAPELPQRHNSLHRKTPGPVVRGLAPPP  
 PTSASPSLQSNRPPPPARDPPSRGAAPPPPPMIRNGARDAPPPPPYRMHGEPLSRGKPPPPSRTPAGPPPP  
 PPLRNHRDSTITVRSFLDDFESKYSFHPVEDFPAPEEYKHFQRVYPSKTNRAARGAPPLPPIILR.

19 residues MPIPPPPPPPGPPPPPTF MW 1926.0.

Plays an active role in the formation of cell surface protrusions downstream of activated PDGFB receptors. Plays an important role in actin-microspike formation through cooperation with WASL. May cooperate with WASP and WASL to induce mobilization and reorganization of the actin filament system.

**Table 11**

Proline-rich protein 16 peptide in PoBChE tetramers.

Name	Gene	Gi #	peptide	Length	Count
Proline-rich protein 16	PRR16	545812042	PNPPPPPR	9	1

Total peptide count 1.

1 Proline-rich protein 16 peptide/10987 PoBChE peptides = 0.009%.

> XP\_005655053.1 PREDICTED: proline-rich protein 16 [Sus scrofa] MTDSSKTD  
 LNSSSSGTTASSIEKIKVQANAPLIKPPAHPSAILTCLR[ENPPPPPR]LTPVKCEDPQRVVPTVNPVKNTGL  
 LRNGGFPGAPNKIPNGDICCKPGSIVDKAPVQPLMHRPEKDRCPQAGPRERVRFNKQVYHGYCPDCDTRYNIKNR  
 EVHLHSEFVHPPGKLPQGHPPPHLPPFLENGGLGISHSNSFPPLRPATVPPPTAPKPKQKTIILRKSTTTTV.

9 residue PNPppppppr MW 967.5.

Regulator of cell size that promotes cell size increase independently of mTOR and Hippo signaling pathways. Acts by stimulating the translation of specific mRNAs, including those encoding proteins affecting mitochondrial functions. Increases mitochondrial mass and respiration.

**Table 12**

Proline-rich membrane anchor 1 peptides in PoBChE tetramers.

Name	Gene	Gi #	peptide	Length	Count
Proline-rich membrane anchor 1	PRIMA1	350587156	PPLPPPPPPPPPR	14	3
			PLPPPPPPPPPP	12	23
			PLPPPPPPPPPP	13	9
			PPPLPPPPPPPP	12	21
			PPPLPPPPPPPP	13	19
			PPPPLPPPPPP	12	6
			PPPPLPPPPPPPP	13	26

Total peptide count 107.

107 Proline-rich membrane anchor 1 peptides/10987 PoBChE peptides = 1%.

>XP\_020955308.1 proline-rich membrane anchor 1 isoform X2 [Sus scrofa] MLLRDLVLRGGCCWPSLLLHLCALHPLWGFVQVAHGEPQKSCSKVTDSCQHICQCR[PPPPPLPPPPPPPPPPPR]LL SAPAPNATSCPAEESWWSGLAIVIAVCCASLVFLTVLVIICYKAIKRKPLRKEENGTSVAEYPMTSSQSNKGVDVN SAVV.

16 residues PPPPLPPPPPPPPPR MW 1645.9.

Required to anchor acetylcholinesterase (ACHE) to the basal lamina of the neuromuscular junction and to the membrane of neuronal synapses in brain. Also able to organize ACHE into tetramers.

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## Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2018.08.109>.

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