



# Identifying the beta-site amyloid precursor protein cleaving enzyme 1 interactome through the proximity-dependent biotin identification assay

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## ABSTRACT

Beta-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) is a key drug target against Alzheimer's Disease however, due to its promiscuous proteolytic activity, little is known about its physiological functions. Previous studies have analysed BACE1 cleavage products to examine BACE1 interactions and determine substrates, but these studies cannot establish non-enzymatic (and potentially functional) associations. This study used the biotin identification proximity assay to establish the BACE1 interactome in healthy neuronal cells and identified interactions involved in BACE1 trafficking, post-translational modification and substrates. Furthermore, this method has identified a putative novel role for BACE1 in sex hormone signalling and haem regulation through interaction with the progesterone receptor membrane component 2 (PGR2). Data are available via ProteomeXchange with identifier PXD021464.

## 1. Introduction

One of the first pathological markers in Alzheimer's Disease (AD) is increased production of neurotoxic amyloid beta (A $\beta$ ) peptides. A $\beta$  is produced from sequential cleavage of the amyloid precursor protein (APP) and aggregates within the brain leading to the hallmark amyloid plaque deposits that are used as a marker for AD diagnosis [1]. Whilst APP processing occurs in healthy and diseased individuals, it is thought that changes in the initial cleavage step of APP determines whether the toxic forms of A $\beta$  are produced. In healthy individuals this cleavage is thought to be predominantly performed by the  $\alpha$ -secretase enzymes (members of the 'a disintegrin and metalloprotease domain' family; e.g. ADAM10), whereas in AD this cleavage is largely performed by the beta-site APP cleaving enzyme 1 (BACE1) [2].

The suspected role of BACE1 as the gatekeeper to amyloidogenesis has made it a key drug target in the treatment of AD. Many BACE1 inhibitors have been developed but none have currently passed clinical trials, with safety concerns and non-significant physiological outcomes predominating [3]. Whilst it has been shown consistently through cell, animal and clinical studies that BACE1 expression is increased in AD, inhibiting it has yet to prove a viable treatment for this dementia and we

have little understanding of the role of BACE1 in healthy physiology. This has made it difficult to predict potential on-target as well as off-target side-effects to its inhibition.

Thus far, the BACE1/APP association has been studied intensively as the predominant role for BACE1 in neurodegeneration and recent proteomic methods have allowed for large scale identification of numerous BACE1 substrates. Many studies have used secretome analysis to identify fragments of putative BACE1 substrates leading to a library of plausible and in some cases independently validated protein interactions and suggesting that BACE1 substrate targeting is highly non-specific [4–10]. Whilst this provides valuable information into the role of BACE1 as an aspartyl protease, there is also evidence indicating that BACE1 may have additional, alternative non-enzymatic roles within the brain such as acting as a putative interaction partner of various post- and pre-synaptic neuronal voltage-gated potassium channels, resulting in modifications of their expression and function [11,12].

With these issues in mind, we utilised the BioID proximity assay developed by Roux et al [13] to create an assay system that identifies all BACE1 interactors including non-enzymatic. The advantage in adopting this assay is that results are not dependent upon the proteolytic activity of BACE1, in principle enabling the identification of all interactors

**Abbreviations:** BACE1, Beta-site APP cleaving enzyme 1; APP, amyloid precursor protein; PGR2, progesterone receptor membrane component 2; A $\beta$ , amyloid beta.

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throughout the BACE1 life cycle from translation to degradation. The aim was to create and optimise an assay to enable the study of BACE1 processing, transport, substrate specificity and degradation in neuronal cells. The assay was designed as an inducible system, enabling future studies to differentiate neuronal cultures and study the effects of chronic stressors on the interactome of BACE1 in neurons. Here, the authors describe the results of preliminary experiments in a proof of principle study.

A BACE1-BirA(R118A) fusion protein was expressed in the mouse HT-22 neuronal cell line. The BirA tag biotinylated proteins proximal to the BACE1 C-terminus were then purified through streptavidin pull-down and identified by mass spectrometry. The results not only show proof of principle, identifying BACE1 within key sub-cellular localisations but validate the model by identifying known BACE1 protein associations, modifiers and substrates. Furthermore, in a novel finding the data suggest BACE1 interacts with the progesterone receptor membrane component 2, PGR2 (also known as PGRMC2) in non-diseased conditions.

## 2. Experimental Procedures

### 2.1. Cell culture

The mouse hippocampal neuronal cell line HT-22 (gifted by Prof. Frank Gunn-Moore, St. Andrews University) was maintained in Dulbecco's Modified Eagle Medium (DMEM; ThermoFisher Scientific) containing 25 mM glucose with 10% Fetal Bovine Serum, 4 mM glutamine and 50U/ml penicillin–streptomycin at 37°C in a humidified atmosphere of 95% air and 5% CO<sub>2</sub>. Cells were passaged every 3–4 days. Prior to transfection cells were seeded at  $0.5 \times 10^6$  cells in 10 cm dishes and left to adhere overnight.

### 2.2. Fusion protein expression

A C-terminal modified biotin protein ligase BirA(R118A) fusion protein of BACE1 was generated synthetically (Sigma Aldrich) with a 18nt linker between the C-terminus of BACE1 and the N-terminus of the BirA gene (nt sequence: GGGGGCTCAGGAGGTAGT; aa sequence: GSGSGS) This insert was cloned into a pcDNA5D FRT vector creating the pcDNA5D FRT BACE1-BirA(R118A)-HA plasmid (University of Dundee cloning facility). The plasmid was linearised by overnight SspI-HF (New England Biolabs) digest and 1.8 µg of DNA transfected into undifferentiated HT-22 cells using FuGene HD (Promega). Control cells were subjected to a sham transfection procedure without DNA.

### 2.3. Biotin loading

24 h after transfection, cells were returned to normal culture medium and treated with 10 µM biotin (resuspended in DMSO and diluted to 50 mM stock in nuclease free H<sub>2</sub>O in a 50:50 solution) and incubated for 24 or 48 h. Control cells were incubated in normal culture medium for 48 h. After biotin load, cells were washed and lysed in 0.1% SDS, 1% Triton X-100 PBS with protease inhibitors.

### 2.4. Protein immunoprecipitation

Each lysate was incubated with 30 µl of equilibrated streptavidin-sepharose medium (GE healthcare) overnight at 4 °C. The unbound fraction was removed, and the streptavidin bound fraction washed prior to digestion in 500 µM Trypsin gold (Promega). The digests were incubated overnight at 37 °C.

### 2.5. Western blot

15 µl of crude lysate, unbound fraction and digested fractions were retained for western blot. 5 µl of sample buffer was added to each and

run on 10% acrylamide tris–glycine gels under denaturing conditions. Gels were transferred onto nitrocellulose using wet transfer and blocked in 10% skimmed milk in TBS. Blots were analysed for biotinylated proteins using streptavidin-HRP conjugated antibody, BACE1 and the HA tag in 5% BSA TBST (all Cell Signalling Technology). Blots were visualised using ECL (Pierce™).

### 2.6. Protein identification

Peptide identification was performed using the Ultimate 3000 RSLCnano system (Thermo Scientific) coupled to a LTQ OrbiTrap Velos Pro (Thermo Scientific).

The protein digests were cleaned to remove polymers using a C18 ZipTip (Millipore) followed by High Protein and Peptide Recovery (HiPPR) Detergent Removal spin column (ThermoFisher). Following this, samples were re-suspended to 50 µl with 1% formic acid and 10 µl injected onto an Acclaim PepMap 100 (C18, 100 µM × 2 cm) trap (Thermo Scientific) and washed with 0.1% formic acid. After 5 min wash gradient formed with buffers 0.1% formic acid and 80% acetonitrile in 0.08% formic acid over 105 mins at 0.3 µl/min. Peptides were separated onto Easy-Spray PepMap RSLC C18 column (75 µM × 50 cm) (Thermo Scientific) and transferred to mass spectrometer with temperature set at 50°C and source voltage of 2.6 kV. Mass spectrometry was conducted using Top 15 Method: FT-MS plus 15 IT-MS/MS (100 min acquisition) operating in data dependent acquisition mode.

MS/MS data were analysed for protein identifications using MaxQuant 1.6.14 [14] with the in-built Andromeda search engine [15]. The raw files were searched against the UniProt [16] mouse uniprot proteome UP000000589, last modified on June 28, 2020 supplemented with the sequence of the BACE1-BirA construct. The mass tolerance was set to 4.5 ppm for precursor ions and trypsin set as the proteolytic enzyme with two missed cleavages permitted. Carbamidomethyl on cysteine, was set as fixed modifications. Oxidation of methionine, Acetylation of Protein N-term, Biotinylation, Deamidation of Asparagine and Glutamine, Dioxidation of Methionine and Tryptophan, and N-terminal Glutamate to Pyroglutamate Conversion were set as variable modifications. The false-discovery rate for protein and peptide level identifications was set at 1%, using a target-decoy based strategy. The minimum peptide length was set to seven amino acids and protein quantification was performed on unique plus razor peptides [17]. “Reverse Hits”, “Only identified by site” and “Potential contaminant” identifications were filtered out. Only protein groups with at least two unique peptide sequences and Andromeda protein score greater than 1 were selected for further quantification. For the differential protein expression analysis, the iBAQ values were analysed with the ProtRank package [18] by comparing the three control samples versus the three 24 h and 48 h treatment samples. ProtRank analyses the comparisons that involve a missing value separately from those that do not involve a missing value. The logarithmic fold changes and their magnitude relative to other genes' fold changes are computed for comparisons without missing values. Then, fold changes where a zero-value control is compared to a positive experimental value are assigned the same relatively high virtual rank, and all comparisons where a positive value control is compared to a zero experimental value are assigned the same relatively low rank [18]. These values are then used to calculate a rank score.

The analysis pipeline was implemented in python using the SciPy packages (<https://www.scipy.org/>) [19] and Jupyter notebook (<http://jupyter.org/>).

### 2.7. Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [20] partner repository with the dataset identifier PXD021464.

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Password: osTJnVnw

The analysis pipeline is available in GitHub ([https://github.com/mtinti/BACE1\\_pulldown](https://github.com/mtinti/BACE1_pulldown)) and it is archived in Zenodo (<https://zenodo.org/badge/latestdoi/283470327>). The analysis pipeline can be reproduced using the mybinder app at ([https://mybinder.org/v2/gh/mtinti/BACE1\\_pulldown/master](https://mybinder.org/v2/gh/mtinti/BACE1_pulldown/master)).

The code to run the webserver for exploring the BACE1 interactome is available in GitHub ([https://github.com/mtinti/jennifer\\_bace1](https://github.com/mtinti/jennifer_bace1)) and it is archived in Zenodo (<https://zenodo.org/badge/latestdoi/290785628>).

### 3. Results

#### 3.1. Transfection of HT-22 cells and pulldown efficiency

The BACE1-BirA fusion protein (Fig. 1) was transfected into the mouse hippocampal cell line HT-22. Successful transfection was determined by western blot of cell lysates at both the 24hr and 48hr timepoints compared to an untransfected control. Western blots for both BACE1 (Fig. 2A) and the c-terminal HA tag (Fig. 2B) reveal a strong band at approximately 98 kDa with the estimated mass of the fusion protein being 92 kDa. Western blot using a streptavidin-HRP antibody on both lysates and unbound fractions show that no proteins were biotinylated in the control and that the pulldown successfully removed all biotinylated proteins from the lysate (Fig. 2C).

#### 3.2. Identification of BACE1 interactors

1598 proteins were identified in both the 24 h and 48 h assays (Supplementary Tables S1 and S2 respectively; data can be browsed at both timepoints by the following links: 24hr timepoint <https://bace1-pulldown-7pkw.onrender.com/exp24h.html> 48 h timepoint <https://bace1-pulldown-7pkw.onrender.com/exp48h.html>). Analysis was conducted using an FDR cut-off at both 0.01 and 0.1. At FDR < 0.01, 7 protein interactors were identified at 24hr as well as BACE1-BirA due to self-biotinylation. IFM3, SEPT11, VAMP3, CAV1, YKT6, PGRC2 and STEAP3 were significantly over-represented at both 24 hr (Table 1) and 48hr time points whilst CAVN1, BAS1 and DLG1 were also significant at 48 hr (Table 2). These represent the most likely BACE1 interactors from this study. Reducing the stringency of analysis to FDR < 0.1 identified 33 hits at 24 hrs and 27 hits at 48 hrs including BACE1-BirA self-biotinylation (Tables 1 and 2 respectively). 10 hits at 24 hrs were unique to this time point and 5 unique to the 48 hr time point. The BACE1 interactor FLOT2 was identified but not significant at 24 hr (rank = 23.44, FDR = 0.12; Supplementary Table S1) and 48 hr (rank = 15.16, FDR = 0.26) (Supplementary Table S2).

### 4. Discussion

Many of the proteins identified as unique to one timepoint may be attributed to the processing and movement of the BACE1-BirA protein through the various cell compartments. The presence of biotinylated RPS11, Eif5a, Polr2h and RPL5 at 24 hr but not 48 hr may be explained by tagging of the RNA translational machinery soon after protein synthesis. Due to the transient nature of the transfection method, BACE1-BirA protein production will have diminished after 48 h; therefore,

BirA expression and consequently, biotinylation of these proteins will be reduced at this later timepoint. The identification of CAV1 and CAVN1 as BACE1 interactors is unsurprising as vesicular transport of the BACE1-BirA construct out of the ER and into lipid rafts in the plasma membrane is expected, as shown for BACE1 [21]. This indicates that transport of the BACE1-BirA fusion protein follows known BACE1 transport routes and that the presence of the BirA tag is not hindering movement of BACE1 within the cell. Interestingly, CAV2 and CAVN2 were identified as interacting proteins at 24 hrs but not 48 hrs, perhaps indicating a change in caveolae structure between these two timepoints. The identification of Kif5b at 48 hr but not 24 hr may be indicative of greater axonal transport at this timepoint and offer further confirmation that the BACE1 fusion protein is behaving as previously identified for BACE1 [22]. Furthermore, the presence of two actin stabilising proteins TagL2 and TrioBP also suggests greater trafficking at this later timepoint.

It has been previously shown that BACE1 is glycosylated to produce mature BACE1 [23]. However, there is also evidence to suggest that BACE1 glycosylation, and therefore maturation, is inhibited by TMEM59 [24]. The biotinylation of TMEM59 in this assay suggests that, whilst the fusion protein is undergoing post-translational modifications as expected, the amount of mature BACE1-BirA may be reduced by association with this protein. This interaction may also explain the presence of two distinct BACE1 and HA bands around 68 kDa by western blot indicative of both mature and immature protein. Other proteins identified are largely membrane associated and may represent interactors in which BACE1 may be acting as an auxiliary subunit to influence their function. These include transporter systems such as ANO6, a Ca<sup>2+</sup> activated Cl<sup>-</sup> channel, the amino-acid transporters Slc7a1 and Slc1a5 and the Na<sup>2+</sup> and Cl<sup>-</sup> uptake transporter Slc12a2.

Many of the top hits from this assay are associated with vesicle transport, indeed 4 of the top 5 (YKT6, VAMP3, CAV1 and IFM3) have been shown to play a role in endocytosis, protein trafficking and vesicle formation, and dysregulated vesicle organelle function is associated with neurodegenerative diseases [25–29]. However, the most over-represented protein at both timepoints was the membrane-associated progesterone receptor component 2 (PGRC2) (Fig. 3). As a single-pass transmembrane protein, PGRC2 also represents a potential substrate for BACE1. mRNA for PGRC2 has been previously identified within the hippocampus spanning Taenia tecta, Dentate gyrus and all CA regions indicating a widespread role within this brain region [30]. Progesterone exhibits properties of neuroprotection and a reduced progesterone level with aging has been linked with increased risk of AD development and altered tau expression and phosphorylation [31,32]. However, the relative influence of membrane bound progesterone receptors such as PGRC 1 and 2 (as opposed to canonical nuclear progesterone receptors) in the brain is presently unclear. Whilst little is known about the function of PGRC2 within the hippocampus, recent studies have shown that it is highly expressed in fat tissue, where it plays a vital role in haem trafficking. Of particular interest is the discovery that PGRC2 acts as a chaperone for haem and is a requirement for adipogenesis and maintaining mitochondrial function in these fat cells [33]. Indeed, deletion of PGRC2 in adipose tissue resulted in more severe glucose intolerance and insulin resistance in mice fed a high fat diet. This may tie-in with data showing that BACE1-null mice are protected from diet-induced obesity, glucose intolerance and associated co-morbidities [34,35]. In neurons, dysfunctional haem metabolism has been linked to neurodegeneration

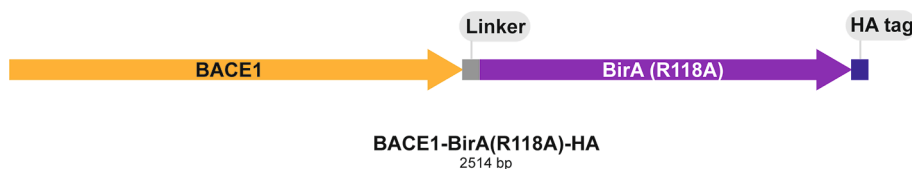
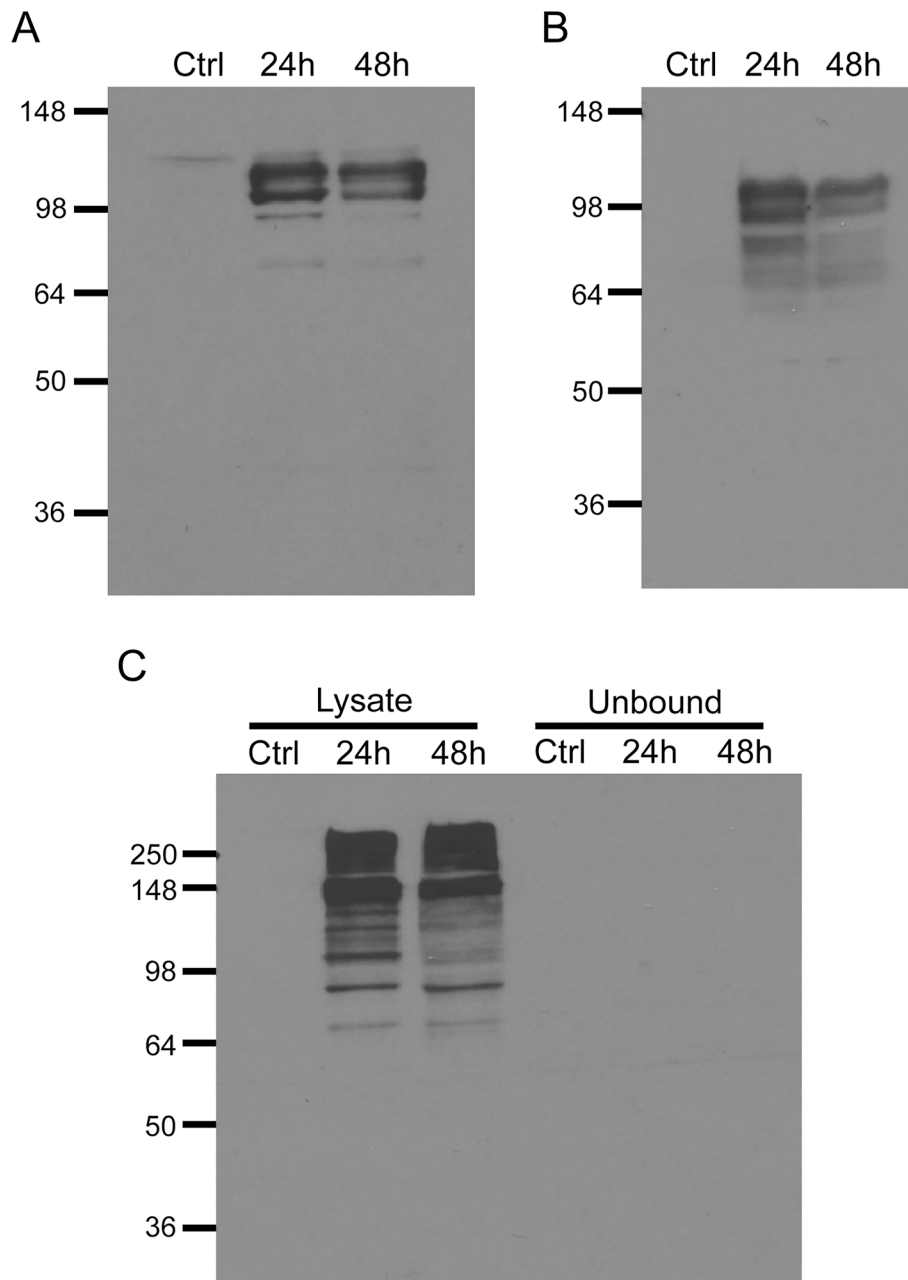


Fig. 1. Map of the BACE1-BirA fusion protein used within the BioID assay. The BirA gene is separated from BACE1 by a short linker sequence and completed with a C-terminal HA tag.



**Fig. 2.** Western blots of BACE1 (A) and HA (B) in HT-22 lysates after transfection. Streptavidin western blot of lysate and unbound fraction show that biotinylation did not occur within the control cells and that pull-down of biotinylated proteins was successful (C).

and to many of the putative underlying causes including oxidative stress, ER stress and mitochondrial dysfunction [36]. Thus, the identification of a BACE1-PGCR2 interaction provides a new avenue of research that suggests an alternative role for BACE1 in neurodegeneration outside of APP cleavage.

This assay identified 2 previously published putative substrates of BACE1; BAS1 and EPHA2 [6] (Fig. 3). These hits, identified through secretome analysis provide further evidence for the importance of these interactions, though further study is required to determine the physiological effects of BACE1-dependent cleavage of these membrane proteins. The palmitoyltransferase ZDH20, identified in this study, is suggested to palmitoylate BACE1 indicating that this post-translational modification of BACE1 has not been affected by the assay and that the BACE1-BirA fusion protein is undergoing plasma membrane targeting [37]. The identification of Flotillin 2 (FLOT2) in this assay, whilst not significant is further evidence that the BACE1-BirA fusion protein is

being processed as expected with previous studies having identified this interaction by co-immunoprecipitation (Supplemental Tables S1 and S2) [38].

A surprising and interesting outcome is the lack of identification of AD associated BACE1 substrates. For example, neither APP nor APLP proteins were identified in this assay. Indeed, no AD associated BACE1 substrates were identified. Consequently, we acknowledge that this study provides only preliminary evidence of the BACE1 interactome and there are limitations to what can be concluded. It cannot be ignored that few of the very many putative BACE1 substrates were identified in this study. This may be attributed to differences in physiological and diseased conditions, the lack of functional synapses or the length of the linker sequence being inadequate for the short-term nature of some interactions. Without the presence of a suitable control fusion protein, the presence of false positives within the data cannot be ruled out, however the stringency of statistical analysis was designed to compensate for this

**Table 1**

Proteins significantly over-represented in assay. FDR < 0.1 at 24hr. The Enrichment rank is computed by the ProtRank software as reported in Experimental Procedures section. Proteins in italics are those unique to the 24hr time point.

Uniprot accession number	Gene	Protein	Enrichment rank	FDR
	BACE1-BirA		269.452724	0
Q80UU9	PGRC2	Membrane-associated progesterone receptor component 2	363.412516	0
P49817	CAV1	Caveolin-1	228.880976	0
Q9CQW9	IFM3	Interferon-induced transmembrane protein 3	217.770158	0
P63024	VAMP3	Vesicle-associated membrane protein 3	189.832221	0
Q9CQW1	YKT6	Synaptobrevin homolog YKT6	183.34534	0
A0A0J9YTY0	Septin 11	Septin-11	128.119252	0.00857
A0A0R4J1G9	Steap 3	Metalloreductase STEAP3	124.59724	0.00857
O09044	SNP23	Synaptosomal-associated protein 23	102.231063	0.01
F7DBB3	Ahnak2	AHNAK nucleoprotein 2	97.011204	0.01
O54724	CAVN1	Caveolae-associated protein 1	91.285849	0.01
<i>D3YVM2</i>	<i>TMEM59</i>	<i>Transmembrane protein 59</i>	<i>75.291466</i>	<i>0.01333</i>
Q5Y5T1	ZDHHC20	Palmitoyltransferase DHHC20	74.73822	0.01333
Q811D0	DLG1	Disks large homolog 1	73.69508	0.01357
Q80Z96	VANG1	Vang-like protein 1	66.950722	0.01688
<i>Q6P9J9</i>	<i>ANO6</i>	<i>Anoctamin-6</i>	<i>64.560757</i>	<i>0.01824</i>
<i>Q63918</i>	<i>CAVN2</i>	<i>Caveolae-associated protein 2</i>	<i>63.738571</i>	<i>0.01889</i>
<i>Q924U4</i>	<i>CAV2</i>	<i>Caveolin -2</i>	<i>60.171518</i>	<i>0.01947</i>
Q03145	EPHA2	Ephrin type-A receptor 2	58.02899	0.01952
P18572	BASI	Basigin	55.245989	0.02455
Q62371	DDR2	Discoidin domain-containing receptor 2	47.3587	0.04783
<i>P47738</i>	<i>ALDH2</i>	<i>Aldehyde dehydrogenase, mitochondrial</i>	<i>46.714983</i>	<i>0.04783</i>
E9Q9C3	AFDN	Afadin	43.759178	0.0548
<i>A0A1B0GRR3</i>	<i>Rps11</i>	<i>40S ribosomal protein S11</i>	<i>42.570978</i>	<i>0.0563</i>
<i>A0A0A0MQM0</i>	<i>Eif5a</i>	<i>Eukaryotic translation initiation factor 5A</i>	<i>41.435449</i>	<i>0.0575</i>
E9Q616	AHNAK	AHNAK nucleoprotein	40.450935	0.05897
Q8CIB5	FERM2	Fermitin family homolog 2	39.722675	0.05897
Q9ESU7	Slc1a5	Amino acid transporter Slc1a5	39.203897	0.05897
<i>A0A338P6B4</i>	<i>Polr2h</i>	<i>RPABC3</i>	<i>38.593471</i>	<i>0.05897</i>
P23242	Gja1	Gap junction alpha-1 protein	34.592963	0.07194
<i>E9Q3N1</i>	<i>Slc7a1</i>	<i>High affinity cationic amino acid transporter 1</i>	<i>33.123642</i>	<i>0.0773</i>
<i>P47962</i>	<i>RPL5</i>	<i>60S ribosomal protein L5</i>	<i>30.138254</i>	<i>0.094</i>
A2A6U3	Septin 9	Septin-9	28.017551	0.09909

somewhat.

This study offers a novel method through which the BACE1 interactome may be characterised. Uniquely, the assay does not rely on secretion of cleavage fragments and can identify protein-protein interactions without BACE1-dependent proteolysis. The assay can provide valuable insight into BACE1 trafficking, post-translational modification and protein substrate interaction and will enable future studies to identify how neuronal stressors such as oxidative stress, inflammation

**Table 2**

Proteins significantly over-represented in assay. FDR < 0.1 at 48hr. The Enrichment rank is computed by the ProtRank software as reported in Experimental Procedures section. Proteins in italics are those unique to the 48hr time point.

Uniprot accession number	Gene	Protein	Enrichment rank	FDR
	BACE1-BirA		135.382127	0.00333
Q80UU9	PGRC2	Membrane-associated progesterone receptor component 2	453.949698	0
Q9CQW1	YKT6	Synaptobrevin homolog YKT6	203.96369	0
P63024	VAMP3	Vesicle-associated membrane protein 3	203.96369	0
P49817	CAV1	Caveolin-1	202.757274	0
Q9CQW9	IFM3	Interferon-induced transmembrane protein 3	157.307093	0.002
A0A0J9YTY0	Septin 11	Septin-11	139.068223	0.00333
O54724	CAVN1	Caveolae-associated protein 1	128.586892	0.00333
A0A0R4J1G9	Steap 3	Metalloreductase STEAP3	113.182243	0.00333
P18572	BASI	Basigin	88.741372	0.008
Q811D0	DLG1	Disks large homolog 1	85.434238	0.00818
O09044	SNP23	Synaptosomal-associated protein 23	74.299707	0.01167
Q5Y5T1	ZDHHC20	Palmitoyltransferase DHHC20	71.837571	0.01167
E9Q616	AHNAK2	AHNAK nucleoprotein 2	63.478337	0.02143
A2A6U3	Septin 9	Septin-9	54.554429	0.03667
<i>Q9WVA4</i>	<i>TagL2</i>	<i>Transgelin-2</i>	<i>49.300921</i>	<i>0.04437</i>
Q8CIB5	FERM2	Fermitin family homolog 2	48.044028	0.04647
Q03145	EPHA2	Ephrin type-A receptor 2	47.63482	0.04647
Q80Z96	VANG1	Vang-like protein 1	46.971202	0.04647
<i>A0A2U3TZ82</i>	<i>TrioBP</i>	<i>TRIO and F-actin-binding protein</i>	<i>44.341641</i>	<i>0.0515</i>
E9Q9C3	AFDN	Afadin	44.198148	0.0515
Q9ESU7	Slc1a5	Amino acid transporter Slc1a5	43.167692	0.05273
Q62371	DDR2	Discoidin domain-containing receptor 2	42.212883	0.05304
<i>Q61768</i>	<i>Kif5b</i>	<i>Kinesin-1 heavy chain</i>	<i>41.349599</i>	<i>0.05304</i>
E9Q616	AHNAK	AHNAK nucleoprotein	40.027371	0.0552
<i>E9QM38</i>	<i>Slc12a2</i>	<i>Solute carrier family 12 member 2</i>	<i>36.189551</i>	<i>0.0737</i>
<i>P08752</i>	<i>GNAI2</i>	<i>Guanine nucleotide-binding protein G(i) subunit alpha-2</i>	<i>32.030791</i>	<i>0.08906</i>

and nutrient overload or deprivation may influence BACE1 behaviour within the cell. Furthermore, this study provides preliminary evidence for the interaction of BACE1 with PGCR2, a previously unidentified association, indicating a potential role for BACE1 in hormone signalling.

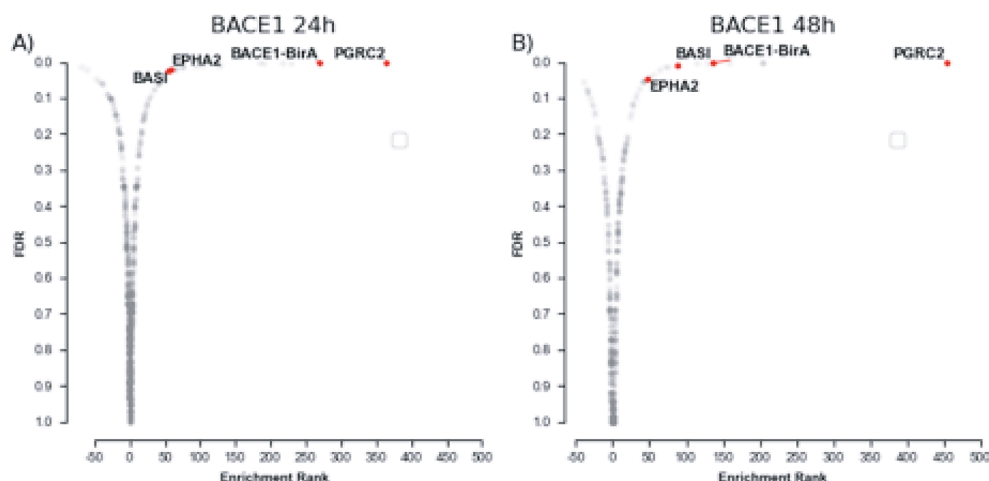
#### CRediT authorship contribution statement

**Jennie L. Gabriel:** Methodology, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition, Visualization. **Michele Tinti:** Formal analysis, Visualization. **William Fuller:** Methodology, Resources. **Michael L.J. Ashford:** Supervision, Writing – review & editing, Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence





**Fig. 3.** Volcano plot identifying significantly over-represented proteins in BACE1-BirA pulldown at 24 h (A) and 48 h (B). Proteins significantly over-represented in assay have the greatest likelihood of being BACE1 interactors. With an FDR < 0.01, the greatest interaction was with the progesterone receptor membrane component 2 (PGRC2). The BACE1-BirA fusion protein was also identified as a product of self-biotinylation. With an FDR < 0.1, two previously published BACE1 substrates were identified: ephrin type-A receptor 2 (EPHA2) and Basi-gin (BAS1/BSG).

the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neulet.2021.136302>.

### References

- [1] J. Hardy, D. Allsop, Amyloid deposition as the central event in the aetiology of Alzheimer's disease, *Trends Pharmacol Sci.* 12 (10) (1991) 383–388, [https://doi.org/10.1016/0165-6147\(91\)90609-v](https://doi.org/10.1016/0165-6147(91)90609-v).
- [2] S.L. Cole, R. Vassar, BACE1 structure and function in health and Alzheimer's disease, *Curr Alzheimer Res.* 5 (2) (2008) 100–120, <https://doi.org/10.2174/156720508783954758>.
- [3] B. Das, R. Yan, A close look at BACE1 inhibitors for Alzheimer's Disease treatment, *CNS Drugs* 33 (3) (2019) 251–263, <https://doi.org/10.1007/s40263-019-00613-7>.
- [4] B. Dislich, F. Wohlrab, T. Bachhuber, S.A. Müller, P.-H. Kuhn, S. Hög, M. Meyer-Luehmann, S.F. Lichtenthaler, Label-free Quantitative Proteomics of Mouse Cerebrospinal Fluid Detects  $\beta$ -Site APP Cleaving Enzyme (BACE1) Protease Substrates In Vivo, *Mol Cell Proteomics.* 14 (10) (2015) 2550–2563, <https://doi.org/10.1074/mcp.M114.041533>.
- [5] M.L. Hemming, J.E. Elias, S.P. Gygi, D.J. Selkoe, S. Maas, Identification of  $\beta$ -Secretase (BACE1) substrates using quantitative proteomics, *PLoS One* 4 (12) (2009) e8477, <https://doi.org/10.1371/journal.pone.0008477>.
- [6] J. Herber, J. Njavro, R. Feederle, U. Schepers, U.C. Müller, S. Bräse, S.A. Müller, S. F. Lichtenthaler, Click Chemistry-mediated Biotinylation Reveals a Function for the Protease BACE1 in Modulating the Neuronal Surface Glycoproteome, *Mol Cell Proteomics* 17 (8) (2018) 1487–1501, <https://doi.org/10.1074/mcp.RA118.000608>.
- [7] P.-H. Kuhn, K. Koroniak, S. Hög, A. Colombo, U. Zeitschel, M. Willem, C. Vollbracht, U. Schepers, A. Imhof, A. Hoffmeister, C. Haass, S. Roßner, S. Bräse, S. F. Lichtenthaler, Secretome protein enrichment identifies physiological BACE1 protease substrates in neurons, *EMBO J* 31 (14) (2012) 3157–3168, <https://doi.org/10.1038/emboj.2012.173>.
- [8] L. Zhou, S. Barão, M. Laga, K. Bockstael, M. Borgers, H. Gijzen, W. Annaert, D. Moechars, K. Mercken, K. Gevaert, B. De Strooper, The neural cell adhesion molecules L1 and CHL1 are cleaved by BACE1 protease in vivo, *J Biol Chem* 287 (31) (2012) 25927–25940, <https://doi.org/10.1074/jbc.M112.377465>.
- [9] T. Tüshaus, S.A. Mülle, E.S. Kataka, J. Zauha, L.S. Monasor, M. Su, G. Güner, G. Joher, S. Tahirovic, D. Frishman, M. Simons, S.F. Lichtenthaler, An optimized quantitative proteomics method establishes the cell type-resolved mouse brain secretome, *EMBO J* 39 (20) (2020), e105693. <https://doi.org/10.15252/emboj.202105693>.
- [10] I. Stützer, N. Selevsek, D. Esterházy, A. Schmidt, R. Aebersold, M. Stoffel, Systematic proteomic analysis identifies  $\beta$ -Site amyloid precursor protein cleaving enzyme 2 and 1 (BACE2 and BACE1) substrates in pancreatic  $\beta$ -Cells, *J Biol Chem* 288 (15) (2013) 10536–10547, <https://doi.org/10.1074/jbc.M112.444703>.
- [11] S. Lehnert, S. Hartmann, S. Hessler, H. Adelsberger, T. Huth, C. Alzheimer, Ion channel regulation by  $\beta$ -secretase BACE1 – enzymatic and non-enzymatic effects beyond Alzheimer's disease, *Channels* 10 (5) (2016) 365–378, <https://doi.org/10.1080/19336950.2016.1196307>.
- [12] S. Hartmann, F. Zheng, M.C. Kyncl, S. Karch, K. Voelkl, B. Zott, C. D'Avanzo, S. Lomoio, G. Tesco, D.Y. Kim, C. Alzheimer, T. Huth,  $\beta$ -secretase BACE1 promotes surface expression and function of Kv3.4 at hippocampal mossy fiber synapses, *J Neurosci* 38 (14) (2018) 3480–3494, <https://doi.org/10.1523/JNEUROSCI.2643-17.2018>.
- [13] K.J. Roux, D.I. Kim, M. Raida, B. Burke, A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells, *J Cell Biol* 196 (6) (2012) 801–810, <https://doi.org/10.1083/jcb.201112098>.
- [14] J. Cox, M. Mann, MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification, *Nat Biotechnol* 26 (12) (2008) 1367–1372, <https://doi.org/10.1038/nbt.1511>.
- [15] J. Cox, N. Neuhauser, A. Michalski, R.A. Scheltema, J.V. Olsen, M. Mann, Andromeda: A peptide search engine integrated into the MaxQuant environment, *J Proteome Res* 10 (4) (2011) 1794–1805, <https://doi.org/10.1021/pr101065j>.
- [16] The UniProt Consortium, UniProt: The universal protein knowledgebase, *Nucleic Acids Research* 45 (D1) (2017) D158–D169, <https://doi.org/10.1093/nar/gkw1099>.
- [17] J. Cox, I. Matic, M. Hilger, N. Nagaraj, M. Selbach, J.V. Olsen, M. Mann, A practical guide to the maxquant computational platform for silac-based quantitative proteomics, *Nature Protocols* 4 (5) (2009) 698–705, <https://doi.org/10.1038/nprot.2009.36>.
- [18] M. Medo, D.M. Aebersold, M. Medová, ProtRank: bypassing the imputation of missing values in differential expression analysis of proteomic data, *BMC Bioinformatics* 20 (1) (2019) 563, <https://doi.org/10.1186/s12859-019-3144-3>.
- [19] Eric, J., Travis, O., Pearu, P. (2001). SciPy: Open source scientific tools for Python.
- [20] Perez-Riverol, Y., Csordas, A., Bai, J., Bernal-Llinares, M., Hewapathirana, S., Kundu, D. J., Inuganti, A., Griss, J., Mayer, G., Eisenacher M., Pérez, E., Uszkoreit J., Pfeuffer, J., Sachsenberg, T., Yilmaz, S., Tiwary, S., Cox, J., Audain, E., walzer, M., Jarnuczak, A. F., Ternent, T., Brazma A., Vizcaíno, R. A. (2019). The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Res.* 47(D1), D442–D450. <https://doi.org/10.1093/nar/gky1106>.
- [21] R. Ehehalt, P. Keller, C. Haass, C. Thiele, K. Simons, Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts, *J Cell Biol* 160 (1) (2003) 113–123, <https://doi.org/10.1083/jcb.200207113>.
- [22] A. Kamal, A. Almenar-Queral, J.F. LeBlanc, E.A. Roberts, L.S. Goldstein, Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP, *Nature* 414 (6864) (2001) 643–648, <https://doi.org/10.1038/414643a>.
- [23] M. Haniu, P. Denis, Y. Young, E.A. Mendiaz, J. Fuller, J.O. Hui, B.D. Bennett, S. Kahn, S. Ross, T. Burgess, V. Katta, G. Rogers, R. Vassar, M. Citron, Characterization of Alzheimer's beta-secretase protein BACE: A pepsin family member with unusual properties, *J Biol Chem* 275 (28) (2000) 21099–21106, <https://doi.org/10.1074/jbc.M002095200>.
- [24] S. Ullrich, A. Münch, S. Neumann, E. Kremmer, J. Tatzelt, S.F. Lichtenthaler, The novel membrane protein TMEM59 modulates complex glycosylation, cell surface expression, and secretion of the amyloid precursor protein, *J Biol Chem* 285 (27) (2010) 20664–20674, <https://doi.org/10.1074/jbc.M109.055608>.
- [25] D.E. Gordon, J. Chia, K. Jayawardena, R. Antrobus, F. Bard, A.A. Peden, G. P. Copenhaver, VAMP3/Syb and YKT6 are required for the fusion of constitutive secretory carriers with the plasma membrane, *PLOS Genet* 13 (4) (2017) e1006698, <https://doi.org/10.1371/journal.pgen.1006698>.
- [26] J.A. McNew, M. Søgaard, N.M. Lampen, S. Machida, R.R. Ye, L. Lacomis, P. Tempst, J.E. Rothman, T.H. Söllner, Ykt6p, a prenylated SNARE essential for endoplasmic

- reticulum-Golgi transport, *J Biol Chem* 272 (28) (1997) 17776–17783, <https://doi.org/10.1074/jbc.272.28.17776>.
- [27] K.G. Rothberg, J.E. Heuser, W.C. Donzell, Y.S. Ying, J.R. Glenney, R.G. Anderson, Caveolin, a protein component of caveolae membrane coats, *Cell* 68 (4) (1992) 673–682, [https://doi.org/10.1016/0092-8674\(92\)90143-z](https://doi.org/10.1016/0092-8674(92)90143-z).
- [28] G. Tai, L. Lu, T.L. Wang, B.L. Tang, B. Goud, L. Johannes, W. Hong, Participation of the syntaxin 5/Ykt6/GS28/GS15 SNARE complex in transport from the early/recycling endosome to the trans-Golgi network, *Mol Biol Cell* 15 (9) (2004) 4011–4022, <https://doi.org/10.1091/mbc.e03-12-0876>.
- [29] Y.S. Wee, K.M. Roundy, J.J. Weis, J.H. Weis, Interferon-inducible transmembrane proteins of the innate immune response act as membrane organizers by influencing clathrin and v-ATPase localization and function, *Innate Immun* 18 (6) (2012) 834–845, <https://doi.org/10.1177/1753425912443392>.
- [30] K.A. Intlekofer, S.L. Petersen, Distribution of mRNAs encoding classical progesterin receptor, progesterone membrane components 1 and 2, serpine mRNA binding protein 1, and progesterin and ADIPOQ receptor family members 7 and 8 in rat forebrain, *Neuroscience* 172 (2011) 55–65, <https://doi.org/10.1016/j.neuroscience.2010.10.051>.
- [31] D. Muñoz-Mayorga, C. Guerra-Araiza, L. Torner, T. Morales, Tau phosphorylation in female neurodegeneration: Role of estrogens, progesterone, and prolactin, *Front Endocrinol.* 28 (9) (2018) 133, <https://doi.org/10.3389/fendo.2018.00133>.
- [32] M. Singh, C. Su, Progesterone and neuroprotection, *Horm Behav.* 63 (2) (2013) 284–290, <https://doi.org/10.1016/j.yhbeh.2012.06.003>.
- [33] A. Galmozzi, B.P. Kok, A.S. Kim, J.R. Montenegro-Burke, J.Y. Lee, R. Spreafico, S. Mosure, V. Albert, R. Cintron-Colon, C. Godio, W.R. Webb, B. Conti, L.A. Solt, D. Kojetin, C.G. Parker, J.J. Peluso, J.K. Pru, G. Siuzdak, B.F. Cravatt, E. Saez, PGRMC2 is an intracellular haem chaperone critical for adipocyte function, *Nature* 576 (7785) (2019) 138–142, <https://doi.org/10.1038/s41586-019-1774-2>.
- [34] P.J. Meakin, A.J. Harper, D.L. Hamilton, J. Gallagher, A.D. McNeilly, L.A. Burgess, L.M. Vaanholt, K.A. Bannon, J. Latcham, I. Hussain, J.R. Speakman, D.R. Howlett, M.L.J. Ashford, Reduction in BACE1 decreases body weight, protects against diet-induced obesity and enhances insulin sensitivity in mice, *Biochem J* 441 (1) (2012) 285–296, <https://doi.org/10.1042/BJ20110512>.
- [35] P.J. Meakin, S.M. Jalicey, G. Montagut, D.J.P. Allsop, D.L. Cavellini, S.W. Irvine, C. McGinley, M.K. Liddell, A.D. McNeilly, K. Parmionova, Y.-R. Liu, C.L.S. Bailey, J. K. Dale, L.K. Heisler, R.J. McCrimmon, M.L.J. Ashford, Bace1-dependent amyloid processing regulates hypothalamic leptin sensitivity in obese mice, *Sci Rep* 8 (1) (2018), <https://doi.org/10.1038/s41598-017-18388-6>.
- [36] D. Chiabrando, V. Fiorito, S. Pettillo, E. Tolosano, Unraveling the role of heme in neurodegeneration, *Front Neurosci* 12 (2018) 712, <https://doi.org/10.3389/fnins.2018.00712>.
- [37] K.S. Vetrivel, X. Meckler, Y. Chen, P.D. Nguyen, N.G. Seidah, R. Vassar, P.C. Wong, M. Fukata, M.Z. Kounnas, G. Thinakaran, Alzheimer disease Abeta production in the absence of S-palmitoylation-dependent targeting of BACE1 to lipid rafts, *J Biol Chem* 284 (6) (2009) 3793–3803, <https://doi.org/10.1074/jbc.M808920200>.
- [38] B.A. John, M. Meister, A. Banning, R. Tikkanen, Flotillins bind to the dileucine sorting motif of  $\beta$ -site amyloid precursor protein-cleaving enzyme 1 and influence its endosomal sorting, *FEBS J* 281 (8) (2014) 2074–2087, <https://doi.org/10.1111/febs.12763>.