

**SUPPLEMENTARY MATERIAL**

<b>A)</b>	1	MRATDRMGAR	AVSELRLALL	FVLVLGTPRL	GVQGEDGLDF	PEYDGVORVI
	51	NVNAKNYKNV	FKKYEVLLALL	YHEPPEDDKA	SQRQFEMEEL	ILELAAQVLE
	101	DKGVGFGLVD	SEKDAAVAKK	LGLTEEDSVY	VFKGDEMEY	DGEFSADTLV
	151	EFLLDVLEDP	VELIEGEREL	QAFENIEDEI	KLIGYFKSKD	SEHYKAYEDA
	201	AEEFHPYIPF	FATFDSKVAK	KLTLKLEID	FYEAFMEEPIM	TIPDKPNSEE
	251	EIVSFVEEHR	RSTLRKLPKE	SMYETWEDDL	DGIHIVAFAE	EADPDGYEFL
	301	ETLKAVAQDN	TENPOLSIMW	IDPDDFPLL	PYWEKTFDID	LSAPQIGVWI
	351	VTDADSIWME	MDNEEDLPSA	DELEDWLEDV	LEGEINTEDD	DDDDDDDDDD
	401	DDDDD				
<b>B)</b>	1	MAVPPTYADL	GKSARDVFTK	GYGFGLIKLD	LTKKSENGLE	FTSSGSANTE
	51	TTKVNGSLET	KYRWTEYGLT	FTEKWNTDNT	LGTEITVEDQ	LARGKLTFD
	101	SSFSPNTGKK	NAKIKTGYKR	EHINLGCDDV	FDIAGPSIRG	ALVLGYEGWL
	151	AGYQMNFFETS	KSRVTQSNFA	VGYKTDEFQL	HTMNDGTEF	GGSIYQKVNK
	201	KLETAVNLAW	TAGNSNTRFG	IAAKYQVDPD	ACFSAKVNNS	SLIGLGYTQT
	251	LKPGIKLTL	ALLDGKNVNA	GGHKLGLGLE	FOA	
<b>C)</b>	1	MAAPAASGLS	RQVRSFSTSV	VRPFAKLRVP	PVQVYGIEGR	YATALYSAAS
	51	KEKKLDQVEK	ELLRVGQLLK	DPKVSALVLN	PYIKRTVKVK	SLNDITKREK
	101	FSPLTANLJMN	LLAENGRIGN	TQGIISAFST	IMSVHRGEVP	CTVTTASPLD
	151	DAVLSELKTV	LKSFLSPNQI	LKLEIKTDP	IMGGMIVRIG	EKYVDMSAKS
	201	KIQKLSKAMR	EML			
<b>D)</b>	1	MASKRALVIL	AKGAEEMETV	IPVDVMRRAG	IKVTVAGLAG	KDPVQCSRDV
	51	MICPDTSLED	AKTQGPYDVV	VLPGGNLGAQ	NLSESPMVKE	ILKEQESRKG
	101	LIAAICAGPT	ALLAHEVGFG	CKVTTHPLAK	DKMMNGSHYS	YSESRVEKDG
	151	LILTSRGPPT	SFEFALAIVE	ALVGKDMANQ	VKAPLVLKD	
<b>E)</b>	1	MAKEWGYASH	NGPDHWHELY	PIAKGDNOSP	IELHTKDIKH	DPSLQPWSAS
	51	YDPGSAKTIL	NNGKTCRVVF	DDTYDRSMLR	GGPLSGPYRL	RQFHLHWGSS
	101	DDHGSEHTVD	GVKYAAELHL	VHWNPKYNTF	GEALKQPDGI	AVVGIFLKIG
	151	REKGEFQILL	DALDKIKTKG	KEAPFTHFDP	SCLFPACRDY	WTYHGSFTTP
	201	PCEECIVWLL	LKEPMTVSSD	QMAKLRSLFS	SAENEPPVPL	VGNWRPPQPW
	251	KGRVVRASFK				
<b>F)</b>	1	MVLSADDKAN	IKATWEKIGG	HGAEYGAEL	ERMFASFPTT	KTYFPHFDVS
	51	HGSAQVKSHG	KKVADALANA	AHLDLPGA	LSALSOLHAH	KLRVDPVNFK
	101	LLGHCLLVTL	ATHLQAGLTP	AAHASLDFL	ASVSTVLTSK	YR

**Supplementary Figure 1.** (A) The peptides identified from spot 6 matched the peptide sequence of the protein calsequestrin. The peptides found in spot 6 are highlighted in red and cover 9.5% of the peptide sequence for calsequestrin. (B) The peptides in spot 26 matched the peptide sequence of voltage-dependent anion-selective channel protein 1 (VDAC 1). The peptides found in spot 26 are highlighted in red and covers 26% of VDAC 1 peptide sequence. (C) The peptides in spot 47 matched the peptide sequence of ATP synthase subunit O. The peptides found in spot 47 are highlighted in red and covers 44% of ATP synthase subunit O peptide sequence. (D) The peptides in spot 47 also matched the peptide sequence of protein DJ-1. The peptides found in spot 47 are highlighted in red and covers 24% of protein DJ-1 peptide sequence. (E) The peptides in spot 29 matched the peptide sequence of protein carbonic anhydrase III. The peptides found in spot 47 are highlighted in red and covers 19% of protein carbonic anhydrase III peptide sequence. F) The peptides in spot 60 matched the peptide sequence of protein haemoglobin subunit alpha. The peptides found in spot 60 are highlighted in red and covers 21% of protein haemoglobin subunit alpha peptide sequence.

A)					
1	MSHHWGYSKH	NGPENWHKDF	PIANGDRQSP	VDIDTATAQH	DPALQPLLIS
51	YDKAASKSIV	NNGHSFNVEF	DDSQDNAVLK	GGPLSDSYRL	IQFHFWGSS
101	DGQGSEHTVN	KKKYAAELHL	VHWNKYGDF	GKAVQQPDGL	AVLIGIFLKIG
151	<b>PASQGLQKVL</b>	<b>EALHSIKTKG</b>	KRAAFANFDP	CSLLPGNLDY	WIYFGSLTIP
201	PLLECIVIV	LREPITVSE	QMSHFRTLNF	NEEGDAEEM	VDNWRPAQPL
251	KNRKIKASFK				

  

B)	
1	mavvaglvrgplrqsagllkrrfhrsapaavqltvreainqgmdeelerdekvfllgeev
61	aqydgaykvsrglwkkygdkriidtpisemgfagiavgaamaglrpicefntfnsmqai
121	dqvinsaaktyymsaglpvqvfrgpngasagvaahsqcfaawygchcpglkvvapwms
181	edakglikasairdnnpvmlenelmygvafelpaaqskdflipigkakierqgthitvv
241	ahsrpvgchleaaaavlskegiecevinlxtirpmdieaieasvmktnhlvtvegwpqfg
301	vgaeicarimegpaflndapavrvtgadvmpmyakvlednsvpqvkdiiifavkktlni

  

C)					
1	MSILRIHARE	IFDSRGNPTV	EVDLYTAKGL	<b>FRAAVPSGAS</b>	<b>TGIYEALCLR</b>
51	DNDKTRFMGK	<b>GVSQAVEHIN</b>	KTIAPALVSK	<b>KVNWEQEKI</b>	DKLMIEMDGT
101	ENKSKFGANA	ILGVSLAVCK	AGAVEKGVPL	YRHIADLAGN	PEVILPVPAF
151	NVINGGSHAG	NKLAMQEFMI	LPVGASSFRE	AMRIGAEVYH	NLKNVIKEY
201	GKDATNVGDE	GGFAPNILEN	KEALELLKTA	IAKAGYTDQV	VIGMDVAASE
251	FYRSGKYDLD	FKSPDDPSRY	<b>ITPDQLADLY</b>	<b>KSFVQNYFVV</b>	SIEDPFDQDD
301	NGAWQKFTAS	AGIQVVGDDL	TVINPKRIAK	AASEKSCNCL	LLKVNQIGSV
351	TESLQACKLA	QSNWGVVMS	HRSGETEDTF	IADLVVGLCT	GQIKTGAPCR
401	SERLAKYNQI	<b>LRIEEELGSK</b>	AKFAGRSFRN	PLAK	

  

D)					
1	MFSLALRARA	TGLAAQWGRH	ARNLHKTAVH	NGAGGALFVH	RDPENNPDT
51	PFDFTPENYK	RIEAIVKNYP	<b>EGHQAAAVLP</b>	<b>VLDLAQRQNG</b>	WLPISAMNKV
101	AEVLQVPPMR	VYEVATFYIM	YNRKFVGKYH	IQVCITTPCM	<b>LRSDSILET</b>
151	<b>LQRKLGKIVG</b>	ETTPDKLFTL	IEVECLGACV	NAPMVQINDN	YYEDLTPKDI
201	EEIIDELKAG	KVPKGPFRSG	RFCCEPAGGL	TSLTEPFKGP	GFGVQAGL

**Supplementary Figure 2.** (A) The peptides in spot 133 matched the peptide sequence of carbonic anhydrase II. The peptides found in spot 133 are highlighted in red and covers 7% of carbonic anhydrase II peptide sequence. Carbonic anhydrase II was found to increase in abundance in the aged brain mitochondrial proteome. (B) In addition to using the MASCOT database peptide de novo sequencing was also used to identify protein spot 108. The peptides identified in protein spot 108 matched 14% of the sequence for the protein pyruvate dehydrogenase E1 subunit  $\beta$  (matching peptides are highlighted in blue, purple and green). (C) The peptides in spot 75 matched the peptide sequence of alpha enolase. The peptides found in spot 75 are highlighted in red and covers 13% of alpha enolase peptide sequence. Alpha enolase was found to increase in abundance in the aged mitochondrial proteome. (D) The peptides in spot 153 matched the peptide sequence of NADH dehydrogenase flavoprotein 2. The peptides found in spot 153 are highlighted in red and covers 12% of alpha enolase peptide sequence. NADH dehydrogenase flavoprotein 2 was found to increase in abundance in the aged mitochondrial proteome.