SUPPLEMENTARY INFORMATION

Hamartin confers neuroprotection against ischemia by inducing autophagy

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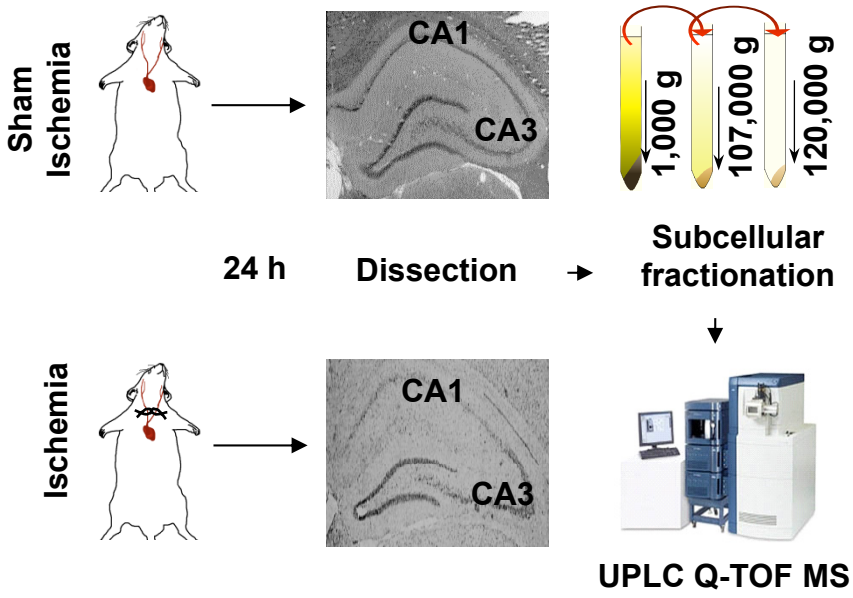
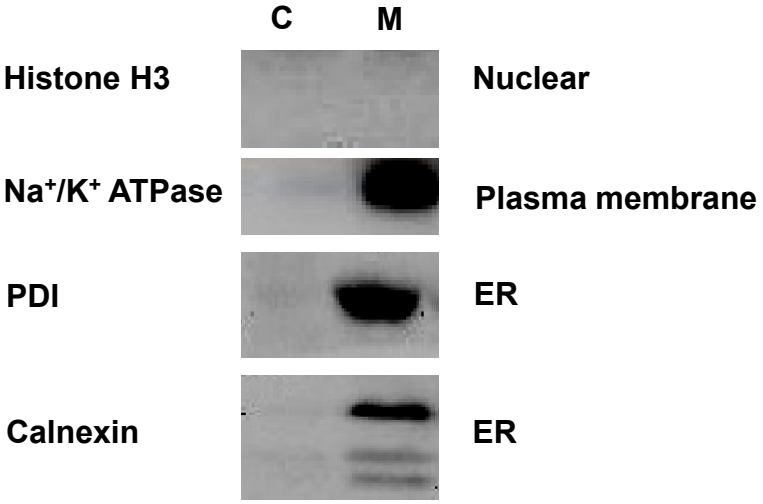
Supplementary Figure 1



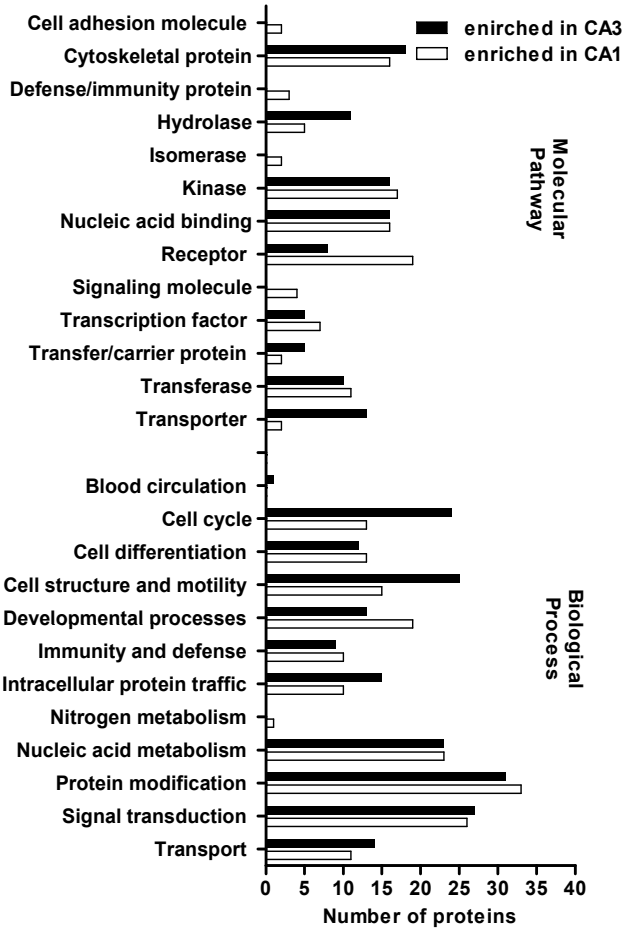
Experimental design of proteomic experiments. Rats were subjected to either sham ischemia or 10 min ischemia. Following 24 h of reperfusion, the CA1 and CA3 regions were microdissected and each region was subcellularly fractionated by differential centrifugation to generate a cytoplasmic and a membrane fraction. Each fraction was quantitatively analysed to define its proteome using UPLC-QTOF MS.

Supplementary Figure 2



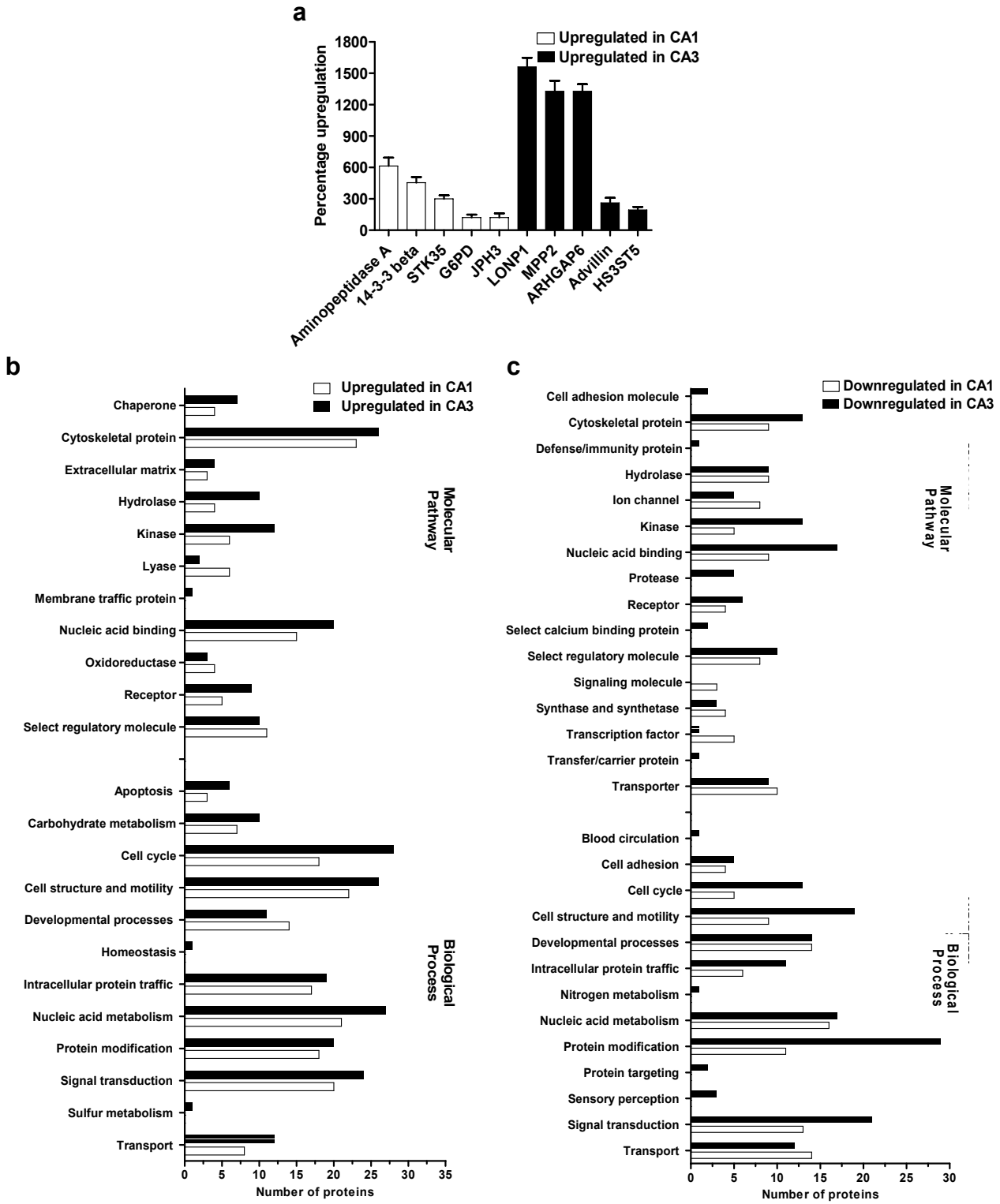
Purity of subcellular fractions. Microdissected hippocampal tissue was subjected to subcellular fractionation to derive a cytoplasmic (C) and a membrane (M) fraction. The purity of the fractions was assessed by immunobloting using nuclear (Histone H3), plasma membrane (Na^+/K^+ ATPase), and endoplasmic reticulum (ER; protein disulfide isomerase (PDI), Calnexin) markers. The figure shows representative immunoblots from n=3, demonstrating that neither the cytoplasmic nor the membrane fraction was contaminated with nuclear proteins. In addition, the immunoreactivity of the plasma membrane and organelle markers was limited to the membrane fraction.

Supplementary Figure 3



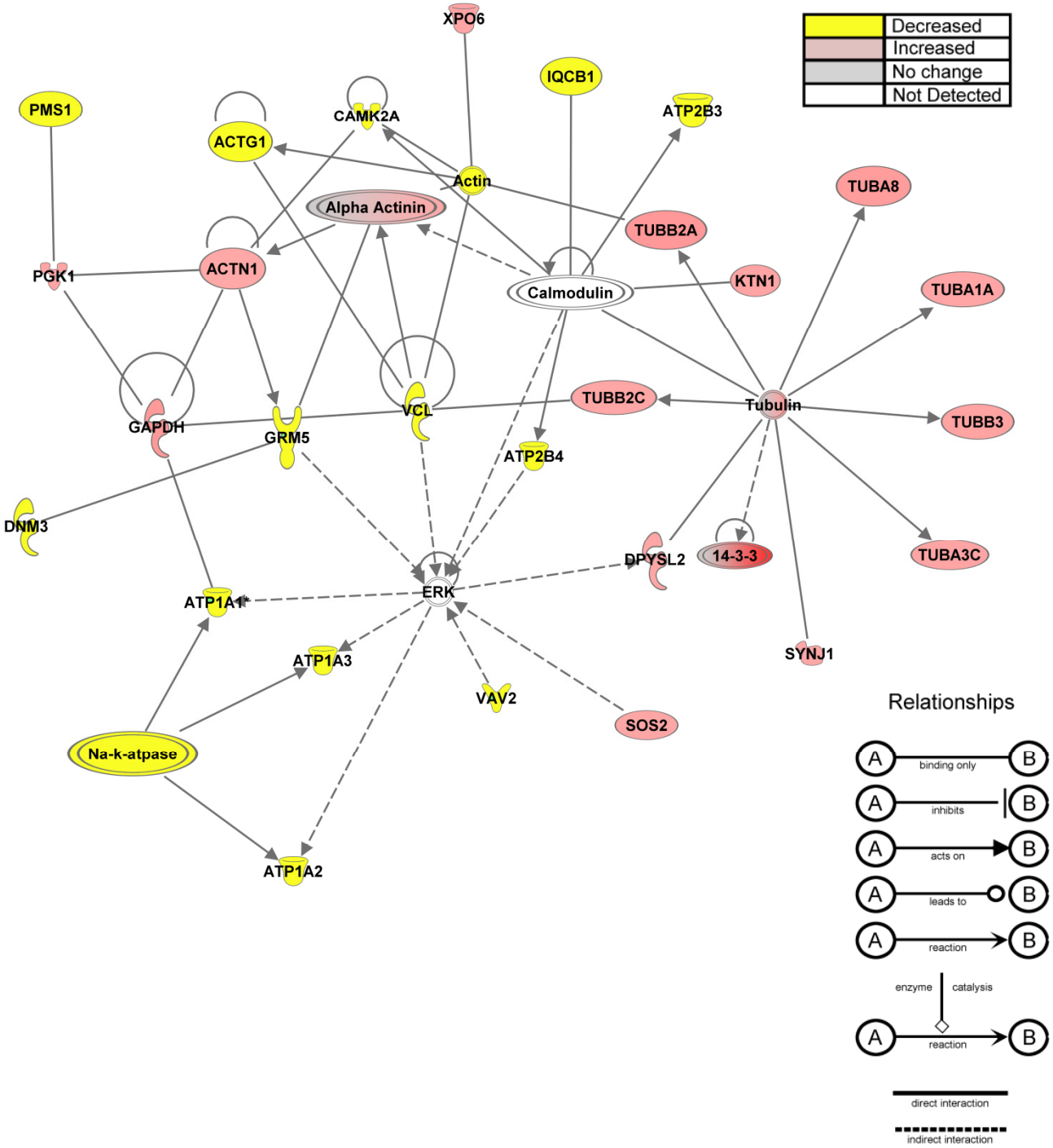
Ontological analysis of enriched proteins in the CA1 and CA3 regions of sham ischemic animals. Using the proteomic datasets, proteins that were overexpressed in the CA1 or the CA3 region of sham ischemic rats were ontologically analyzed using the panther classification system according to both the biological process and the molecular pathway they participate. The bar graph shows the number of protein that belong to each group for the CA3 (black-filled bars) or the CA1 (white-filled bars) region. The ten biological processes and ten molecular pathways containing most proteins and those that were selectively affected either in the CA1 or CA3 region are displayed.

Supplementary Figure 4



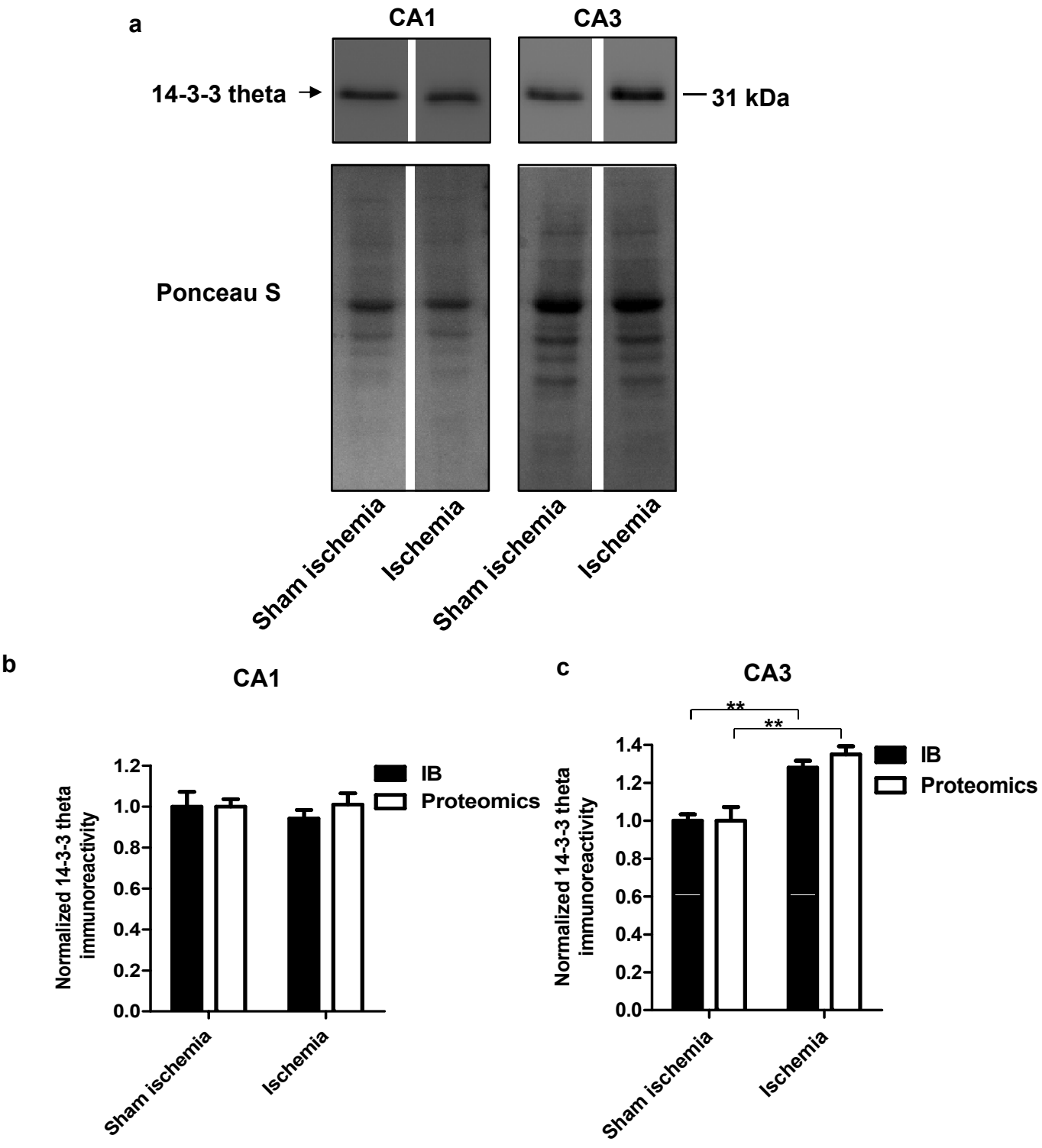
Ontological analysis of proteins with altered expression within the CA1 or CA3 by ischemia and reperfusion. (**a**) Bar graph shows the percentage increase of the five mostly upregulated proteins within the CA1 or CA3 regions, after ischemia and reperfusion, as determined by the proteomic analysis. (**b**,**c**) The proteins whose expression was increased (**b**) or decreased (**c**) by ischemia within the CA1 (white-filled columns) or the CA3 (black-filled columns) region were analyzed with the panther classification system, and were grouped according to both their biological process and their molecular pathway. Both bar graphs show the number of proteins belonging to each group. The ten biological processes and ten molecular pathways containing most proteins and those that were selectively affected either in the CA1 or CA3 region are displayed.

Supplementary Figure 5



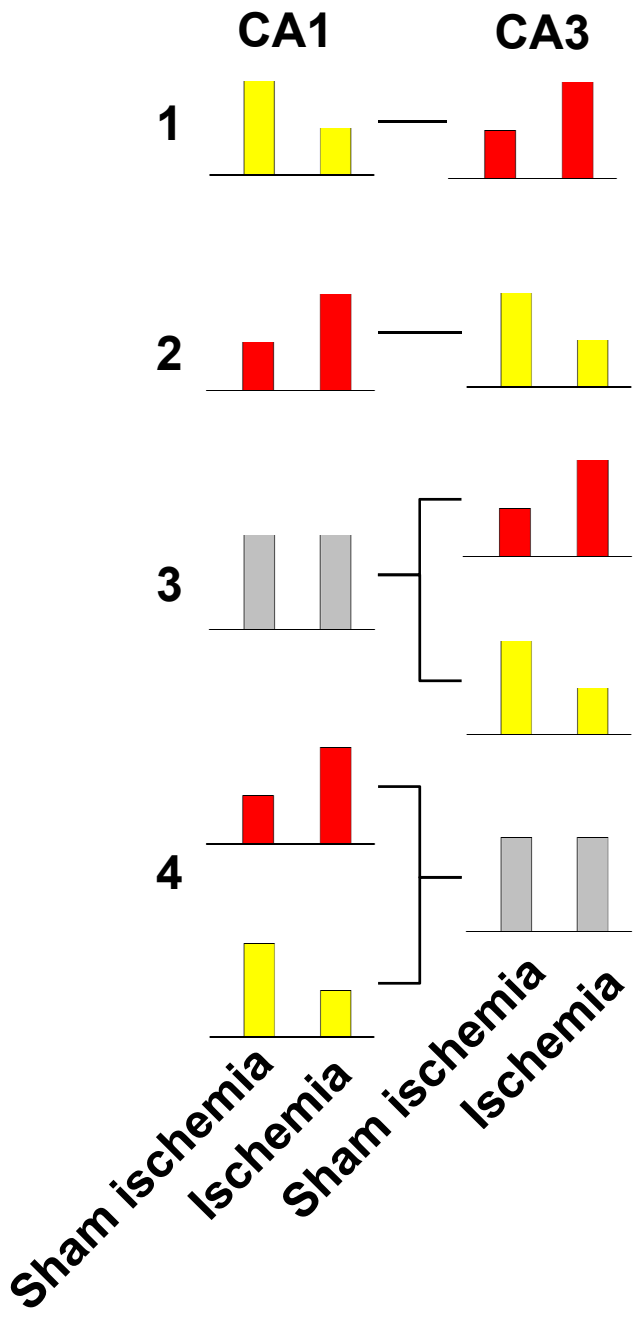
Network of genes affected in the CA1 after ischemia. Diagram of a protein network, which was significantly associated with the expression level changes taking place by ischemia in the CA1 region. Proteins in red and yellow were upregulated and downregulated, respectively, by ischemia. Proteins in grey were detected by the proteomic analysis, but their expression was unaffected. Proteins in white participate in the network, but were not detected by the proteomic experiments. Images were created using the IPA software. The relationships between the displayed proteins are indicated.

Supplementary Figure 6



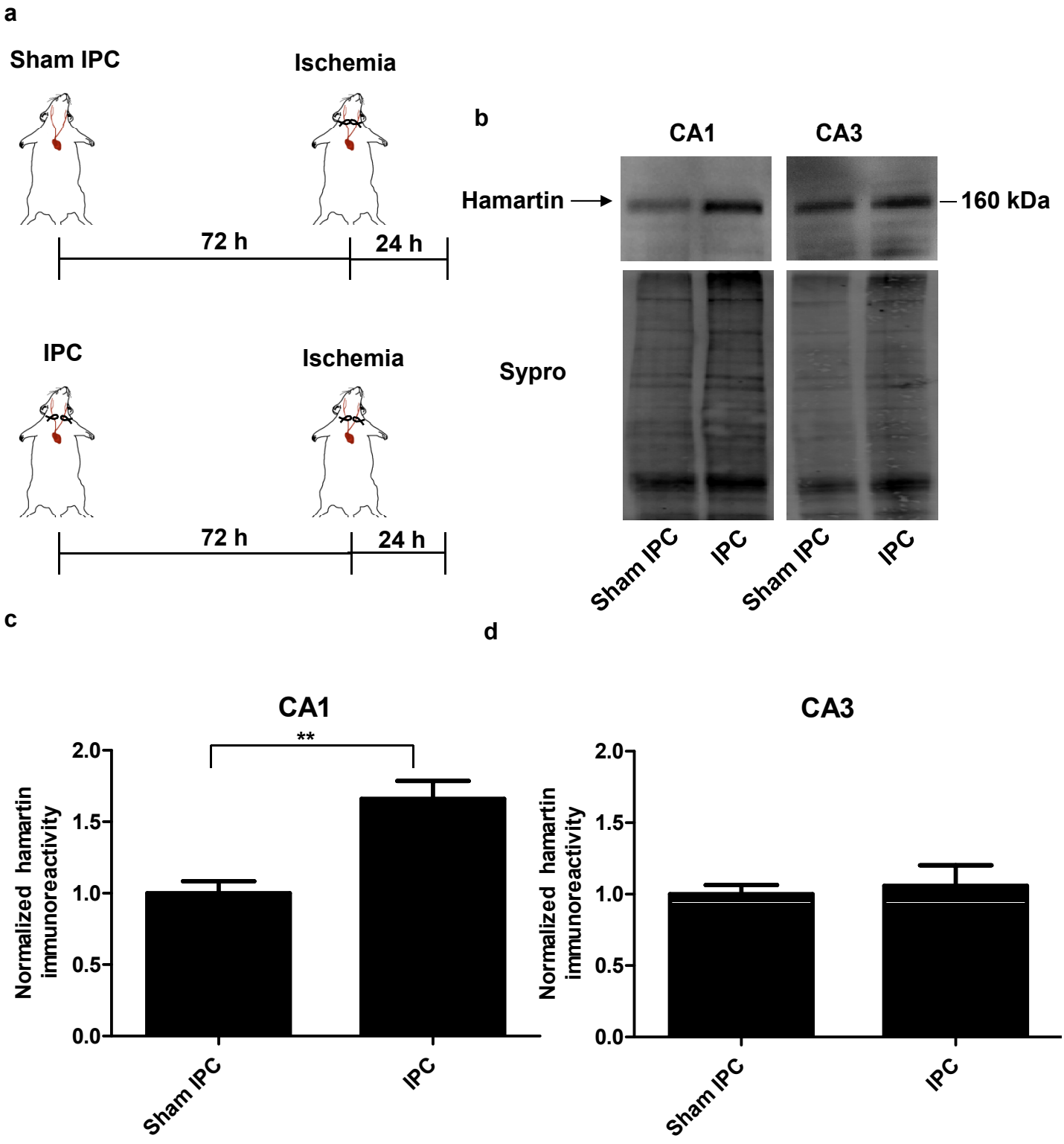
Selective induction of 14-3-3 theta expression in the CA3 region after ischemia. (**a**) Representative immunoblots illustrating the protein expression levels of 14-3-3 theta in CA1 and CA3 cytoplasmic fractions from both sham ischemic and ischemic animals. (**b**,**c**) Bar graphs summarizing the immunoblotting (IB) and proteomic results for 14-3-3 theta in the CA1 (**b**) and CA3 (**c**) region. The values were corrected to total protein content, determined by Ponceau S staining and normalized such that the expression levels from sham ischemic samples were 1 and are means ± S.E.M. for n=6 per group (**p<0.01, two-tailed t-test).

Supplementary Figure 7



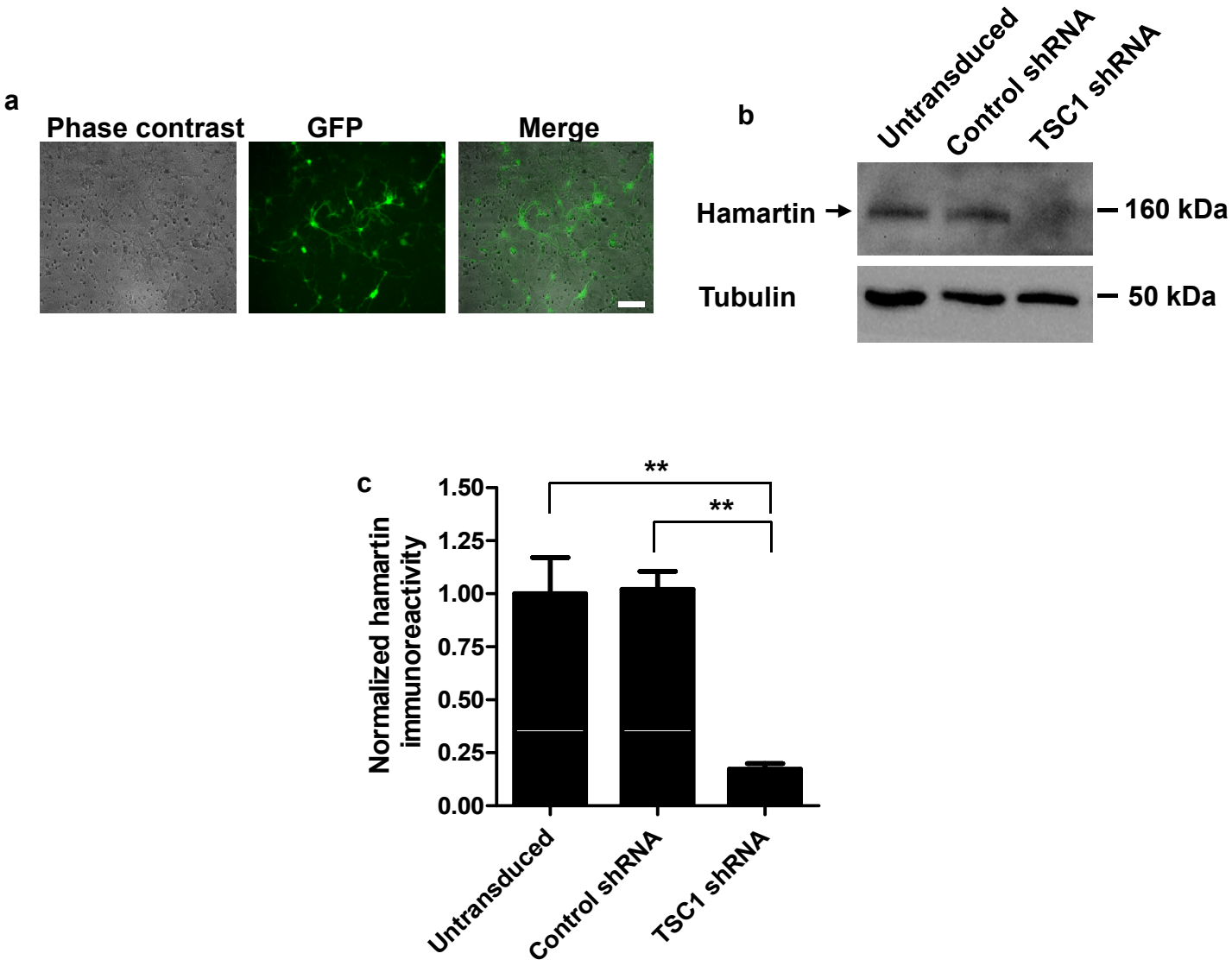
Selection criteria for the identification of individual proteins associated with the resistive properties of CA3. Panel shows the selection criteria applied to the proteomic datasets for the identification of individual proteins that were differentially regulated after ischemia in CA1 compared to CA3. (**1**) downregulated following ischemia in the CA1, but upregulated in the CA3 or (**2**) vice versa; (**3**) unaffected following ischemia in the CA1, but either upregulated or downregulated in the CA3 or (**4**) vice versa.

Supplementary Figure 8



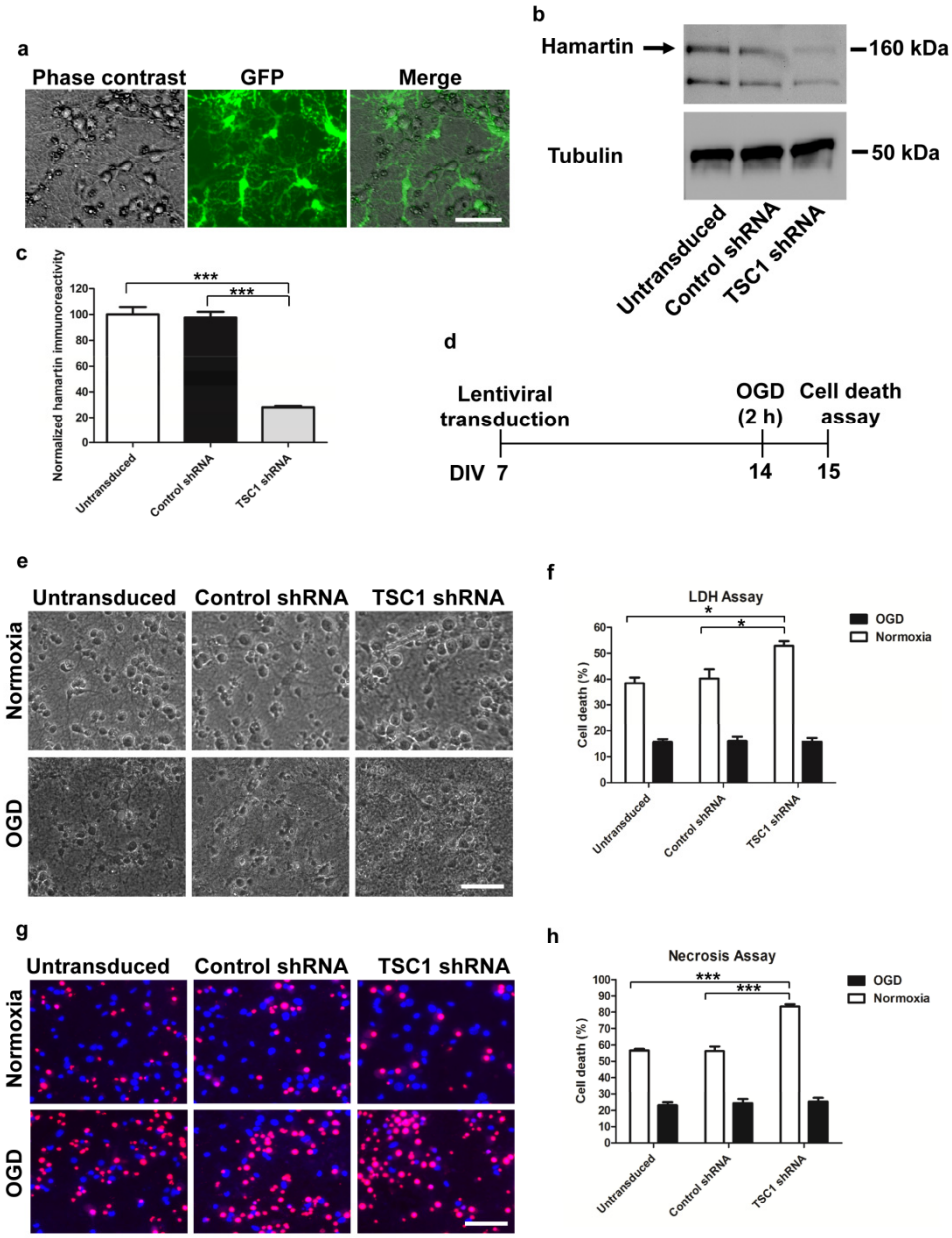
Hamartin induction in the CA1 following IPC and ischemia. (**a**) Experimental design showing rats subjected to either sham IPC or 2 min IPC, followed by 72 h of reperfusion, 10 min injurious ischemia and 24 h of reperfusion. In (**b–d**), the CA1 and CA3 membrane fractions were analyzed by immunoblotting using anti-hamartin antibodies. (**b**) Representative immunoblots showing hamartin expression. (**c**,**d**) Quantification of hamartin expression from the immunoblotting data for the CA1 (**c**) and CA3 (**d**) regions. The values were corrected to total protein content, determined by Sypro staining and were normalized such that expression levels from sham IPC samples was 1 and are means ± S.E.M. for n=4 per group (**p<0.01, two-tailed t test).

Supplementary Figure 9



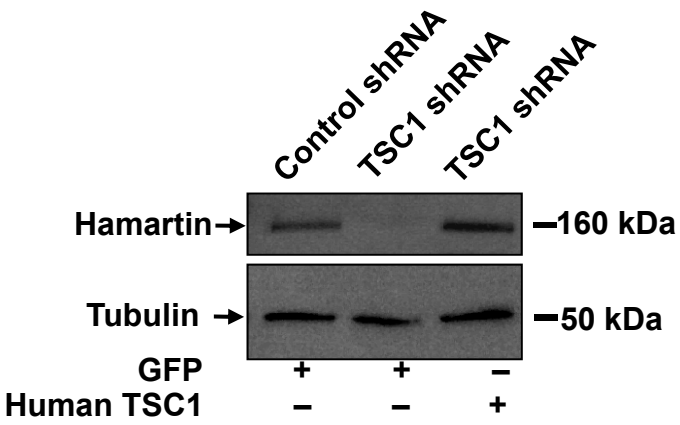
Suppression efficiency of TSC1 shRNA treated cultures. (**a**) Phase contrast and fluorescent images of primary hippocampal neurons transduced with a control pLKO.1 TurboGFP vector (n=2). Merge shows the phase contrast and GFP fluorescence images. Scale bar is 50 µm. (**b**) Representative immunoblots showing hamartin expression in untransduced, control shRNA- and TSC1 shRNA-treated cultures. (**c**) Densitometric quantification of hamartin expression levels from (**b**). Tubulin was used as a loading control and expression was normalised so that hamartin immunoreactivity in untransduced cultures was 1 and are means ± S.E.M (n=4; one-way ANOVA with Bonferonni post-test, $^{**}p<0.01$).

Supplementary Figure 10



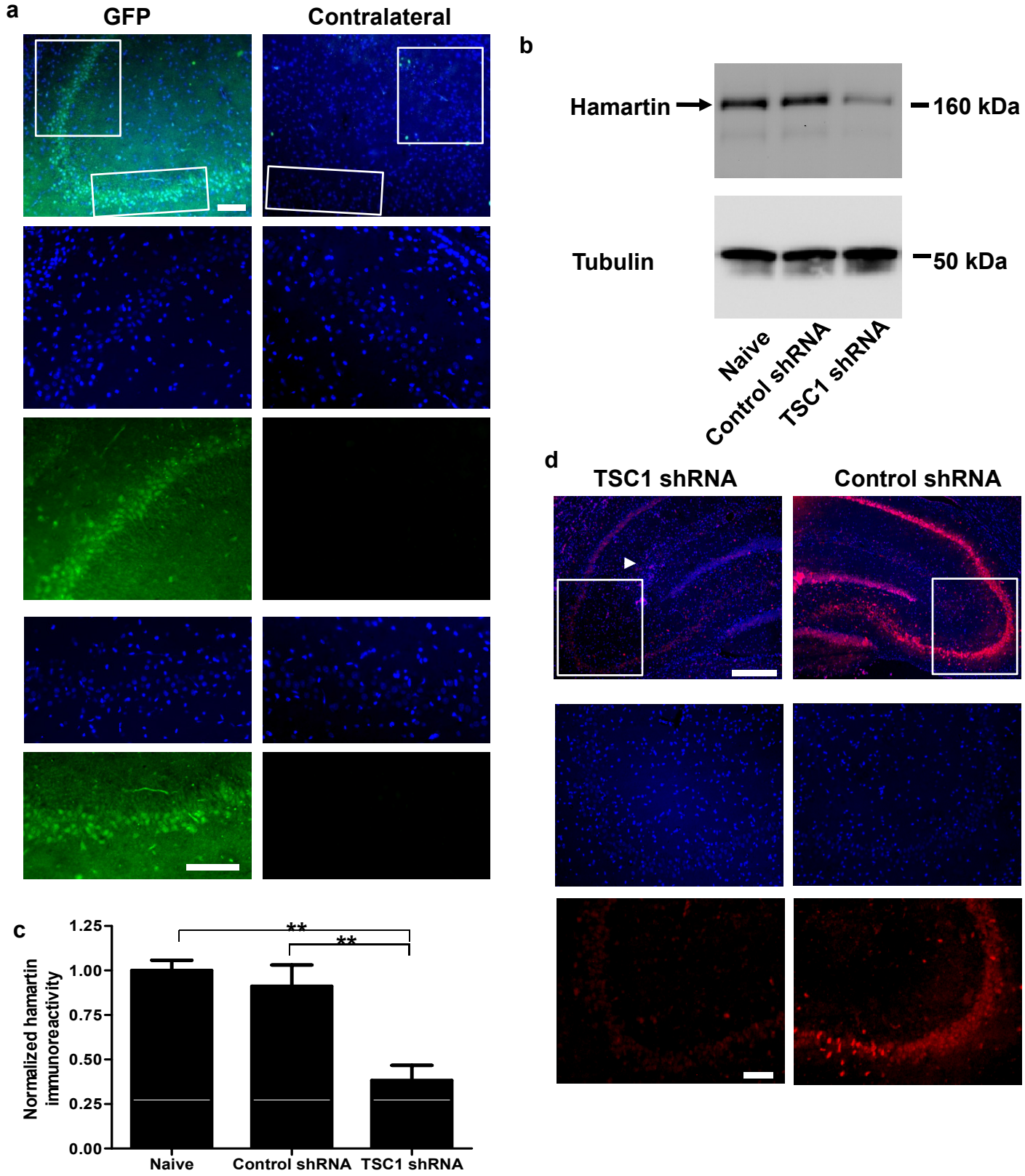
Increased vulnerability to OGD in primary cortical neurons in which hamartin expression was suppressed. (**a**) Phase contrast and immunofluorescent images of primary cortical neurons transduced with pLKO.1 TurboGFP vectors. Merge shows the phase contrast and GFP fluorescence images. (**b**) Representative immunoblots of hamartin expression from untreated, control shRNA - and TSC1 shRNA-treated cultures. (**c**) Bar graph summarizing the immunoblotting results. Expression is normalized so that hamartin immunoreactivity in untransduced cultures was 100%. (**d–h**) Cortical cultures were transduced 7 DIV with lentiviral vectors or remained untreated. At 14 DIV cultures were subjected to 2 h of either OGD or normoxia and cell death was quantified at 24 h of reperfusion. (**d**) Illustration of the experimental design. (**e**) Representative phase contrast images of cortical cultures transduced with TSC1 shRNA after 2 h OGD and 24 h of reperfusion. (**f**) Quantification of cell death by LDH assays showing % cell death in cultures treated with TSC1 shRNA, control shRNA and untransduced cultures. (**g**) Merged fluorescent images from cultures stained with Hoechst 33342 (blue) and ethidium homodimer III (red). (**h**) Quantification of cell death expressed as the percentage of neurons in (**g**) stained with ethidium homodimer III to stained with Hoechst 33342. Scale bars are 50 µm. All data are expressed as means ± S.E.M. from n=3 (one-way ANOVA with Bonferroni post-test, *p<0.05, ***p<0.001).

Supplementary Figure 11



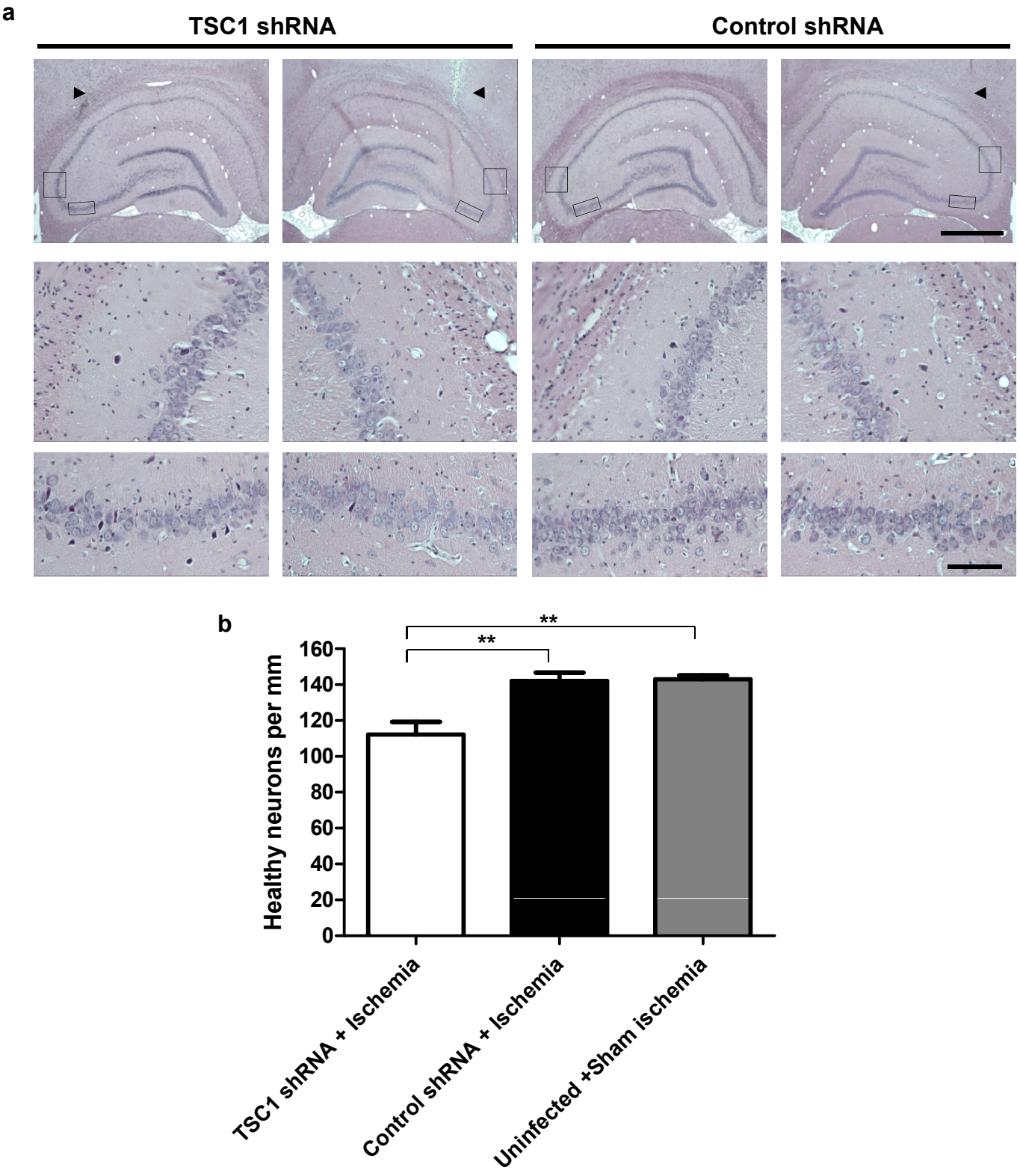
Rescue of hamartin expression in hippocampal cultures. Representative immunoblot showing hamartin expression from hippocampal cells treated with both TSC1 shRNA and pEZ-Lv201 human TSC1 lentiviral vectors. GFP is pEZ-LV201 eGFP. Tubulin is loading control.

Supplementary Figure 12



Attenuation of hamartin expression in CA3 cells. (**a**) Representative fluorescence images from coronal sections immunostained with an anti-GFP antibody (green) and counterstained with DAPI (blue) following unilateral injection of control pLKO.1 TurboGFP vectors (2.5 x 10^6 particles) in CA3, which produced ipsilateral GFP expression. (**b**) Representative immunoblot from CA3 whole cell homogenates showing hamartin expression and (**c**) bar graph summarizing the densitometric data. Data were normalised to hamartin immunoreactivity from naïve CA3 cell homogenates. (**d**) Immunofluorescent images from brain sections immunostained with an anti-hamartin antibody (red) and DAPI (blue) (n=3). For (**a**) scale bars are 0.1 mm for first row panels and 0.01 mm for lower panels. For (**d**) scale bars are 0.5 mm for first row panels and 0.1 mm for lower panels. Boxes indicate the CA3 regions magnified in the lower panels.

Supplementary Figure 13



Reduction of resistance to ischemia in CA3 cells in which hamartin expression was suppressed. (**a**) Hematoxylin and eosin stained hippocampal sections from rats administered bilaterally with either TSC1 shRNA (n=6) or Control shRNA (n=4) and after 14 d subjected to 10 min ischemia and 7 d reperfusion. Arrow heads show the needle trace. Boxes indicate the magnified regions displayed in the middle and lower panels. Scale bar for upper panels is 1 mm and for middle and lower panels is 0.01 mm. (**b**) Quantification of the number of healthy neurons per mm surviving ischemia in the dorsal CA3 pyramidal layer, treated with TSC1 shRNA, compared to control shRNA-treated or uninfected hippocampi subjected to sham ischemia. Data are mean ± S.E.M. (one-way ANOVA with Bonferonni post-test, $^{**}p<0.01$)

Supplementary Table 1. Enhanced proteins in the CA1 region compared to the CA3 region of sham ischemic animals.

Accession Number	Protein Name	CA1 Sham: CA3 Sham Ratio	p-value
O95479	hexose-6-phosphate dehydrogenase	4.762	0
Q9Y3Q4	hyperpolarization activated cyclic nucleotide-gated potassium channel 4	3.571	0.01
Q8WZA1	protein O-linked mannose beta1,2-N-acetylglucosaminyltransferase	3.333	0
Q96NW4	ankyrin repeat domain 27	2.941	0
Q58EX7	Puratrophin-1	2.632	0
Q15021	non-SMC condensin I complex, subunit D2	2.564	0
Q86UL8	membrane associated guanylate kinase, WW and PDZ domain containing 2	2.381	0.02
O14746	telomerase reverse transcriptase	1.887	0
Q8TEX9	importin 4	1.818	0.02
P08779	keratin 16 (focal non-epidermolytic palmoplantar keratoderma)	1.754	0
Q86YN6	peroxisome proliferator-activated receptor gamma, coactivator 1 beta	1.724	0.01
O76094	signal recognition particle 72kDa	1.695	0
P16435	P450 (cytochrome) oxidoreductase	1.639	0.01
Q9BPU6	dihydropyrimidinase-like 5	1.613	0
Q14137	block of proliferation 1	1.587	0.01
Q14679	tubulin tyrosine ligase-like family, member 4	1.587	0.02
P54132	Bloom syndrome	1.562	0
Q8NHU6	tudor domain containing 7	1.562	0.02
P17812	CTP synthase	1.562	0.03
Q92526	chaperonin containing TCP1, subunit 6B (zeta 2)	1.515	0
O95202	leucine zipper-EF-hand containing transmembrane protein 1	1.471	0
Q9UPT6	mitogen-activated protein kinase 8 interacting protein 3	1.471	0
Q96G03	phosphoglucomutase 2	1.471	0
Q9HCK4	roundabout, axon guidance receptor, homolog 2	1.471	0
Q92540	Smg-7 homolog, nonsense mediated mRNA decay factor	1.449	0
O75197	low density lipoprotein receptor-related protein 5	1.429	0
P10721	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	1.429	0.04
Q9NRL2	bromodomain adjacent to zinc finger domain, 1A	1.408	0
Q9H3U1	unc-45 homolog A	1.408	0.01
O76074	phosphodiesterase 5A, cGMP-specific	1.408	0.02
Q9Y3Q0	N-acetylated alpha-linked acidic dipeptidase 2	1.408	0.04
O75473	leucine-rich repeat-containing G protein-coupled receptor 5	1.389	0.02
Q8TEW8	par-3 partitioning defective 3 homolog B	1.37	0.01
Q5JTH9	ribosomal RNA processing 12 homolog	1.37	0.01
Q8TEL6	transient receptor potential cation channel, subfamily C,	1.351	0
Q9Y613	formin homology 2 domain containing 1	1.316	0
Q8TCU5	glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	1.316	0
Q92839	hyaluronan synthase 1	1.316	0
O95025	sema domain, immunoglobulin domain (Ig)	1.316	0
Q14324	myosin binding protein C, fast type	1.316	0.01
O94953	jumonji domain containing 2B	1.299	0
Q96CW5	tubulin, gamma complex associated protein 3	1.282	0
O15439	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.266	0
P61158	ARP3 actin-related protein 3 homolog	1.266	0
O60477	deleted in bladder cancer 1	1.266	0
O76041	nebulette	1.266	0
P46934	neural precursor cell expressed, developmentally down-regulated 4	1.266	0
Q9BRZ2	tripartite motif-containing 56	1.266	0

Q13620	cullin 4B	1.266	0.01
O75820	zinc finger protein 189	1.266	0.02
O75746	solute carrier family 25 (mitochondrial carrier, Aralar), member 12	1.266	0.04
P43405	spleen tyrosine kinase	1.266	0.04
P07996	thrombospondin 1	1.266	0.04
O60879	diaphanous homolog 2	1.25	0
P41594	glutamate receptor, metabotropic 5	1.25	0
Q12772	sterol regulatory element binding transcription factor 2	1.25	0
O94973	adaptor-related protein complex 2, alpha 2 subunit	1.25	0.01
O00391	quiescin Q6 sulfhydryl oxidase 1	1.25	0.02
Q96NW7	leucine rich repeat containing 7	1.25	0.03
Q92696	Rab geranylgeranyltransferase, alpha subunit	1.25	0.03
Q12931	TNF receptor-associated protein 1	1.235	0.02
Q8TD31	coiled-coil alpha-helical rod protein 1	1.235	0.03
P02538	keratin 6A	1.235	0.03
Q8NE62	choline dehydrogenase	1.22	0.01
Q08211	DEAH (Asp-Glu-Ala-His) box polypeptide 9	1.22	0.01
Q6VAB6	kinase suppressor of ras 2	1.22	0.03
P17948	fms-related tyrosine kinase 1	1.22	0.04
Q13619	cullin 4A	1.205	0
P52306	RAP1, GTP-GDP dissociation stimulator 1	1.205	0
Q13751	laminin, beta 3	1.205	0.02
Q86UU1	pleckstrin homology-like domain, family B, member 1	1.205	0.02
Q9Y6K5	2'-5'-oligoadenylate synthetase 3, 100kDa	1.205	0.04
Q9UNM6	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	1.205	0.04
Q7Z3E2	chromosome 10 open reading frame 118	1.19	0
P09104	enolase 2 (gamma, neuronal)	1.19	0
Q96PE3	inositol polyphosphate-4-phosphatase, type I, 107kDa	1.19	0
O95819	mitogen-activated protein kinase kinase kinase kinase 4	1.19	0
P07949	ret proto-oncogene	1.19	0
Q13464	Rho-associated, coiled-coil containing protein kinase 1	1.19	0
P54098	polymerase (DNA directed), gamma	1.19	0.01
P18206	vinculin	1.19	0.01
O60645	exocyst complex component 3	1.19	0.03
P00338	lactate dehydrogenase A	1.19	0.03
O00206	toll-like receptor 4	1.19	0.03
Q8N4X5	actin filament associated protein 1-like 2	1.19	0.04
Q9HCS7	XPA binding protein 2	1.19	0.04
Q9H7Z3	chromosome 14 open reading frame 102	1.176	0.01
P51523	zinc finger protein 84	1.176	0.01
Q13618	cullin 3	1.176	0.02
O95461	like-glycosyltransferase	1.176	0.04
Q9BXL6	caspase recruitment domain family, member 14	1.163	0
Q9UJA3	minichromosome maintenance complex component 8	1.163	0
Q9UM54	myosin VI	1.163	0
Q9H2G2	STE20-like kinase	1.163	0
P29322	EPH receptor A8	1.163	0.01
P20702	integrin, alpha X (complement component 3 receptor 4 subunit)	1.163	0.01
Q9Y239	nucleotide-binding oligomerization domain containing 1	1.163	0.01
O95153	benzodiazapine receptor (peripheral) associated protein 1	1.163	0.02

Q9Y2I6	ninein-like	1.163	0.02
P16234	platelet-derived growth factor receptor, alpha polypeptide	1.163	0.03
Q01118	sodium channel, voltage-gated, type VII, alpha	1.163	0.03
O00418	eukaryotic elongation factor-2 kinase	1.163	0.04
P12277	creatine kinase, brain	1.149	0
Q13948	cut-like homeobox 1	1.149	0
Q12768	KIAA0196	1.149	0
O00160	myosin IF	1.149	0
P36956	sterol regulatory element binding transcription factor 1	1.149	0.01
P78524	suppression of tumorigenicity 5	1.149	0.02
P22314	ubiquitin-like modifier activating enzyme 1	1.149	0.02
P35913	phosphodiesterase 6B, cGMP-specific, rod, beta	1.149	0.03
Q6IQ26	RAB6 interacting protein 1	1.149	0.03
Q9HBT7	zinc finger protein 287	1.149	0.03
Q10567	adaptor-related protein complex 1, beta 1 subunit	1.136	0
Q9Y597	potassium channel tetramerisation domain containing 3	1.136	0.02
P29474	nitric oxide synthase 3 (endothelial cell)	1.136	0.02
Q9UNY4	transcription termination factor, RNA polymerase II	1.136	0.03
P14868	aspartyl-tRNA synthetase	1.136	0.04
P30153	protein phosphatase 2 (formerly 2A), regulatory subunit A , alpha isoform	1.136	0.04
P46020	phosphorylase kinase, alpha 1	1.136	0.04
Q14644	RAS p21 protein activator 3	1.136	0.04
P43304	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	1.124	0
Q14146	KIAA0133	1.124	0
Q96FN5	kinesin family member 12	1.124	0.04
Q99707	5-methyltetrahydrofolate-homocysteine methyltransferase	1.124	0.04
Q9BXT6	Mov10l1, Moloney leukemia virus 10-like 1, homolog	1.124	0.04
P63010	adaptor-related protein complex 2, beta 1 subunit	1.111	0.01
O14976	cyclin G associated kinase	1.111	0.01
Q05586	glutamate receptor, ionotropic, N-methyl D-aspartate 1	1.111	0.01
P60174	triosephosphate isomerase 1	1.111	0.01
Q8WTW3	component of oligomeric golgi complex 1	1.111	0.02
Q9Y4D1	dishevelled associated activator of morphogenesis 1	1.111	0.02
Q9BWT7	caspase recruitment domain family, member 10	1.111	0.03
Q9BZ76	contactin associated protein-like 3	1.111	0.03
Q9P2S2	neurexin 2	1.111	0.03
Q9UKV0	histone deacetylase 9	1.111	0.04
Q12965	myosin IE	1.111	0.04

Supplementary Table 2. Enhanced proteins in the CA3 region relative to the CA1 region of sham ischemic animals.

Accession Number	Protein Name	CA3 Sham: CA1 Sham Ratio	p-value
P31946	14-3-3 beta	6.82	0
Q07075	glutamyl aminopeptidase	5.87	0
O75717	WD repeat and HMG-box DNA binding protein 1	3.1	0
O43566	regulator of G-protein signaling 14	2.46	0
Q99665	interleukin 12 receptor beta 2	2.34	0
P19013	keratin 4	1.99	0
Q96IZ5	RNA binding motif protein 41	1.95	0
Q8WUM0	nucleoporin 133kDa	1.9	0.01
Q8WXD9	CASK interacting protein 1	1.86	0.04
Q9HBL0	*tensin 1*	1.7	0.01
P78332	RNA binding motif protein 6	1.57	0
Q9ULE6	KIAA1274	1.55	0.01
Q9P202	deafness, autosomal recessive 31	1.54	0
P13645	keratin 10	1.54	0
Q9UIF3	tektin 2 (testicular)	1.54	0
Q9Y2X7	G protein-coupled receptor kinase interactor 1	1.51	0
P04406	glyceraldehyde-3-phosphate dehydrogenase	1.49	0
Q7Z4K8	tripartite motif-containing 46	1.49	0
O60231	DEAH (Asp-Glu-Ala-His) box polypeptide 16	1.48	0.01
Q6PL18	ATPase family, AAA domain containing 2	1.48	0.03
Q9NY65	tubulin, alpha 8	1.46	0
O00159	myosin IC	1.42	0
O14924	regulator of G-protein signaling 12	1.39	0.01
Q16720	ATPase, Ca++ transporting, plasma membrane 3	1.38	0
O94909	microtubule associated monoxygenase 3	1.38	0
Q07157	tight junction protein 1	1.35	0
P49588	alanyl-tRNA synthetase	1.35	0.04
O43242	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3	1.34	0.01
Q86Y38	xylosyltransferase I	1.34	0.02
P00367	glutamate dehydrogenase 1	1.32	0
Q96RT1	erbb2 interacting protein	1.31	0
Q9NYY8	FAST kinase domains 2	1.31	0
Q96JC1	vacuolar protein sorting 39 homolog (S. cerevisiae)	1.31	0.01
Q92523	carnitine palmitoyltransferase 1B (muscle)	1.31	0.02
Q5W0U4	B-box and SPRY domain containing	1.31	0.03
Q02763	TEK tyrosine kinase, endothelial	1.3	0.02
P23677	inositol 1,4,5-trisphosphate 3-kinase A	1.3	0.03
Q9BY66	jumonji, AT rich interactive domain 1D	1.28	0
P08581	met proto-oncogene (hepatocyte growth factor receptor)	1.28	0
Q9H2T7	RAN binding protein 17	1.27	0.01
Q96BY7	ATG2 autophagy related 2 homolog B	1.27	0.02
P39900	matrix metallopeptidase 12	1.27	0.04
Q6PI48	aspartyl-tRNA synthetase 2, mitochondrial	1.26	0
O75533	splicing factor 3b, subunit 1, 155kDa	1.26	0
O75093	slit homolog 1 (Drosophila)	1.26	0
Q16822	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	1.26	0.01
P17858	phosphofructokinase	1.25	0.01
P19012	keratin 15	1.25	0
Q13885	tubulin, beta 2A	1.25	0

Q9BVA1	tubulin, beta 2B	1.25	0
Q05639	eukaryotic translation elongation factor 1 alpha 2	1.25	0.04
Q9H0K1	SNF1-like kinase 2	1.23	0
Q13233	mitogen-activated protein kinase kinase kinase 1	1.23	0.01
Q15020	squamous cell carcinoma antigen recognized by T cells 3	1.23	0.01
Q96Q42	amyotrophic lateral sclerosis 2 (juvenile)	1.22	0
Q8IY63	angiomotin like 1	1.22	0
Q9Y2G3	ATPase, class VI, type 11B	1.22	0
Q00341	high density lipoprotein binding protein (vigilin)	1.22	0
P00558	phosphoglycerate kinase 1	1.22	0
Q13972	Ras protein-specific guanine nucleotide-releasing factor 1	1.22	0
Q9NVE7	pantothenate kinase 4	1.22	0.01
O00411	polymerase (RNA) mitochondrial (DNA directed)	1.22	0.01
Q16706	mannosidase, alpha, class 2A, member 1	1.22	0.04
P39086	glutamate receptor, ionotropic, kainate 1	1.21	0
P23458	Janus kinase 1 (a protein tyrosine kinase)	1.21	0
P12235	solute carrier family 25 member 4	1.21	0
O75334	protein tyrosine phosphatase, alpha 2	1.21	0.02
Q9NPG3	ubinuclein 1	1.21	0.03
O94983	calmodulin binding transcription activator 2	1.2	0.01
Q9UIA9	exportin 7	1.2	0.01
Q9BZZ5	apoptosis inhibitor 5	1.2	0.02
Q5VUB5	chromosome 10 open reading frame 38	1.2	0.02
Q12882	dihydropyrimidine dehydrogenase	1.2	0.02
P50416	carnitine palmitoyltransferase 1A (liver)	1.2	0.04
Q9UM73	anaplastic lymphoma receptor tyrosine kinase	1.19	0
Q86UW7	Ca2+-dependent activator protein for secretion 2	1.19	0
O15455	toll-like receptor 3	1.19	0
Q9BZF9	uveal autoantigen with coiled-coil domains and ankyrin repeats	1.19	0
Q14587	zinc finger protein 268	1.19	0.01
Q96J65	ATP-binding cassette, sub-family C (CFTR/MRP), member 12	1.19	0.02
Q8TEK3	DOT1-like, histone H3 methyltransferase (S. cerevisiae)	1.19	0.03
Q8TEQ6	gem (nuclear organelle) associated protein 5	1.19	0.04
P33176	kinesin family member 5B	1.19	0.04
P13929	enolase 3 (beta, muscle)	1.17	0
P20594	natriuretic peptide receptor B/guanylate cyclase B	1.17	0
O43295	SLIT-ROBO Rho GTPase activating protein 3	1.17	0
P61981	tyrosine 3-monooxygenase gamma polypeptide	1.17	0
Q05481	zinc finger protein 91	1.17	0.02
Q96P47	centaurin, gamma 3	1.17	0.04
P11142	heat shock 70kDa protein 8	1.16	0
Q9UGL1	jumonji, AT rich interactive domain 1B	1.16	0
Q7Z2Y5	Nik related kinase	1.16	0
Q13748	tubulin, alpha 3c	1.16	0
Q13509	tubulin, beta 3	1.16	0
Q9BZF3	oxysterol binding protein-like 6	1.16	0.01
Q16352	internexin neuronal intermediate filament protein, alpha	1.16	0.02
Q96RT8	tubulin, gamma complex associated protein 5	1.16	0.04
Q70CQ4	ubiquitin specific peptidase 31	1.16	0.04
P08183	ATP-binding cassette, sub-family B (MDR/TAP), member 1	1.15	0
P25705	ATP synthase, H+ transporting, alpha subunit 1	1.15	0
Q9BYP7	WNK lysine deficient protein kinase 3	1.15	0
O15438	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	1.15	0.01
Q8N1F7	nucleoporin 93kDa	1.15	0.01
Q15431	synaptonemal complex protein 1	1.15	0.01
P68371	tubulin, beta 2C	1.15	0.01
O60706	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	1.15	0.02

Q9NTI2	ATPase, aminophospholipid transporter-like, class I, type 8A, member 2	1.15	0.02
O75747	phosphoinositide-3-kinase, class 2, gamma polypeptide	1.15	0.02
P11216	phosphorylase, glycogen; brain	1.15	0.02
Q8TEQ8	phosphatidylinositol glycan anchor biosynthesis, class O	1.15	0.03
Q9H267	vacuolar protein sorting 33 homolog B (yeast)	1.15	0.04
P33992	minichromosome maintenance complex component 5	1.15	0.04
Q7L2E3	DEAH (Asp-Glu-Ala-His) box polypeptide 30	1.14	0
Q5JR59	KIAA0774	1.14	0
Q9BUF5	tubulin, beta 6	1.14	0
Q7Z460	cytoplasmic linker associated protein 1	1.14	0.01
Q8WXW3	progesterone immunomodulatory binding factor 1	1.14	0.01
Q9NZL3	zinc finger protein 224	1.14	0.01
P14616	insulin receptor-related receptor	1.14	0.02
Q96DY2	IQ motif containing D	1.14	0.02
Q5UE93	phosphoinositide-3-kinase, regulatory subunit 6	1.14	0.02
Q9ULE0	WWC family member 3	1.14	0.03
O14980	exportin 1 (CRM1 homolog, yeast)	1.14	0.04
O00754	mannosidase, alpha, class 2B, member 1	1.13	0.01
Q8N4C8	misshapen-like kinase 1 (zebrafish)	1.13	0.02
Q9H3S7	protein tyrosine phosphatase, non-receptor type 23	1.13	0.03
Q9BW92	threonyl-tRNA synthetase 2, mitochondrial (putative)	1.13	0.04
Q6ZW49	PAX interacting (with transcription-activation domain) protein 1	1.13	0.04
Q16555	dihydropyrimidinase-like 2	1.12	0
Q96RY7	intraflagellar transport 140 homolog (Chlamydomonas)	1.12	0.01
Q9C000	NLR family, pyrin domain containing 1	1.12	0.01
O75122	cytoplasmic linker associated protein 2	1.12	0.03
P06576	ATP synthase, H+ transporting beta polypeptide	1.11	0
O75417	polymerase (DNA directed), theta	1.11	0
Q14152	eukaryotic translation initiation factor 3, subunit A	1.11	0.02
O60674	Janus kinase 2 (a protein tyrosine kinase)	1.11	0.03
Q6PEY2	tubulin, alpha 3e	1.11	0.03
Q9NUT2	ATP-binding cassette, sub-family B (MDR/TAP), member 8	1.11	0.04
Q9UL54	TAO kinase 2	1.11	0.04

Supplementary Table 3. Proteins upregulated within the CA1 region following ischemia and reperfusion.

Accession Number	Protein Name	CA1 ischemia: CA1 Sham Ratio	p-value
Q07075	aminopeptidase A	7.143	0
P31946	14-3-3 beta	5.556	0
Q8TDR2	serine/threonine kinase 35	4	0.01
P11413	glucose-6-phosphate dehydrogenase	2.273	0.02
Q8WXH2	junctophilin 3	2.222	0.04
Q13835	plakophilin 1	2.083	0.03
Q99665	interleukin 12 receptor, beta 2	2	0
Q96IZ5	RNA binding motif protein 41	1.923	0
P32926	desmoglein 3 (pemphigus vulgaris antigen)	1.754	0.01
Q13017	Rho GTPase activating protein 5	1.754	0
P51841	guanylate cyclase 2F, retinal	1.724	0
Q8WXD9	CASK interacting protein 1	1.695	0.04
P16118	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	1.695	0
Q7Z4K8	tripartite motif-containing 46	1.639	0
P04264	keratin 1 (epidermolytic hyperkeratosis)	1.562	0
Q9HAQ2	kinesin family member 9	1.515	0
O00159	myosin IC	1.515	0
P78332	RNA binding motif protein 6	1.493	0
Q96JC1	vacuolar protein sorting 39 homolog	1.493	0
Q8TDC3	BR serine/threonine kinase 1	1.408	0.04
Q8N1G1	REX1, RNA exonuclease 1 homolog	1.37	0.03
P00367	glutamate dehydrogenase 1	1.37	0
Q6SZW1	sterile alpha and TIR motif containing 1	1.37	0
Q9UBB9	tuftelin interacting protein 11	1.37	0
Q9UIF3	tektin 2	1.351	0.04
Q6XZF7	dynamin binding protein	1.351	0.02
Q9P202	deafness, autosomal recessive 31	1.333	0.01
P11498	pyruvate carboxylase	1.333	0
Q9UJF2	RAS protein activator like 2	1.333	0
Q9NSC2	sal-like 1	1.316	0.02
Q13885	tubulin, beta 2A	1.316	0
P51788	chloride channel 2	1.299	0.04
P04406	glyceraldehyde-3-phosphate dehydrogenase	1.299	0
Q9BXR5	toll-like receptor 10	1.282	0.04
Q9BYX4	interferon induced with helicase C domain 1	1.282	0.03
Q8IWV2	contactin 4	1.282	0
P19012	keratin 15	1.282	0
Q8NEV4	myosin IIIA	1.282	0
O75334	protein tyrosine phosphatase, alpha 2	1.266	0.02
Q9NY65	tubulin, alpha 8	1.266	0
O94776	metastasis associated 1 family, member 2	1.25	0.04
Q8IWY9	congenital dyserythropoietic anemia, type I	1.25	0.02
Q15031	leucyl-tRNA synthetase 2, mitochondrial	1.235	0.02
P48681	nestin	1.235	0.02

Q05639	eukaryotic translation elongation factor 1 alpha 2	1.22	0.04
P07101	tyrosine hydroxylase	1.22	0.03
Q86UW7	Ca2+-dependent activator protein for secretion 2	1.22	0
Q8N1F7	nucleoporin 93kDa	1.22	0
Q12884	fibroblast activation protein, alpha	1.205	0.01
Q96NY9	MUS81 endonuclease homolog	1.205	0.01
Q9BZ72	phosphatidylinositol transfer protein, membrane-associated 2	1.205	0
P12236	solute carrier family 25, member 6	1.205	0
Q13507	transient receptor potential cation channel, subfamily C	1.19	0.04
Q00341	high density lipoprotein binding protein	1.19	0.04
Q9BY66	jumonji, AT rich interactive domain 1D	1.19	0.04
Q29RF7	PDS5, regulator of cohesion maintenance, homolog A	1.19	0.04
O15164	tripartite motif-containing 24	1.19	0.03
P20742	pregnancy-zone protein	1.19	0.02
O94983	calmodulin binding transcription activator 2	1.19	0.01
Q9BYZ6	Rho-related BTB domain containing 2	1.19	0.01
Q12860	contactin 1	1.19	0
P13929	enolase 3 (beta)	1.19	0
O75093	slit homolog 1	1.176	0.01
P11142	heat shock 70kDa protein 8	1.176	0
Q13509	tubulin, beta 3	1.176	0
Q9BUF5	tubulin, beta 6	1.176	0
Q7KZF4	staphylococcal nuclease and tudor domain containing 1	1.163	0.04
Q96R06	sperm associated antigen 5	1.163	0.03
P42229	signal transducer and activator of transcription 5A	1.163	0.02
Q86UP2	kinectin 1 (kinesin receptor)	1.163	0.01
Q70J99	unc-13 homolog D	1.163	0.01
P08238	heat shock protein 90kDa alpha (cytosolic), class B member 1	1.163	0
P20594	natriuretic peptide receptor B/guanylate cyclase B	1.163	0
Q99797	mitochondrial intermediate peptidase	1.149	0.04
Q96QU8	exportin 6	1.149	0.04
O94851	microtubule associated monoxygenase	1.149	0.03
P00558	phosphoglycerate kinase 1	1.149	0.02
P29590	promyelocytic leukemia	1.149	0.02
O60229	kalirin, RhoGEF kinase	1.149	0.01
Q9H0X9	oxysterol binding protein-like 5	1.149	0.01
Q9P0K7	retinoic acid induced 14	1.149	0
Q9UL54	TAO kinase 2	1.149	0
Q71U36	tubulin, alpha 1a	1.149	0
Q13748	tubulin, alpha 3c	1.149	0
Q6PKC3	thioredoxin domain containing 11	1.136	0.04
Q92841	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	1.136	0.03
Q9UBJ2	ATP-binding cassette, sub-family D (ALD), member 2	1.136	0
Q14999	cullin 7	1.136	0
Q16555	dihydropyrimidinase-like 2	1.136	0

O60343	TBC1 domain family, member 4	1.136	0
P08581	met proto-oncogene (hepatocyte growth factor receptor)	1.124	0.04
O60503	adenylate cyclase 9	1.124	0.04
Q07890	son of sevenless homolog 2	1.124	0.03
Q6P0Q8	microtubule associated serine/threonine kinase 2	1.124	0.02
P68371	tubulin, beta 2C	1.124	0.02
P61981	tyrosine 3-monooxygenase/ gamma polypeptide	1.124	0.02
O75122	cytoplasmic linker associated protein 2	1.124	0
P07437	tubulin, beta	1.124	0
O75146	huntingtin interacting protein 1 related	1.111	0.04
Q7Z4S6	kinesin family member 21A	1.111	0.04
O43426	synaptojanin 1	1.111	0.04
P98171	Rho GTPase activating protein 4	1.111	0.03
O00429	dynamin 1-like	1.111	0.03
Q7RTR2	NLR family, CARD domain containing 3	1.111	0.03
Q96MT8	centrosomal protein 63kDa	1.111	0.02
Q8IYF3	testis expressed 11	1.111	0.02
Q6PEY2	tubulin, alpha 3e	1.111	0.02
P12814	actinin, alpha 1	1.111	0.01
Q96M83	coiled-coil domain containing 7	1.111	0.01
Q66GS9	centrosomal protein 135kDa	1.111	0.01
P56524	histone deacetylase 4	1.111	0.01
O43166	signal-induced proliferation-associated 1 like 1	1.111	0.01
Q9NR96	toll-like receptor 9	1.111	0.01
O43592	exportin, tRNA	1.111	0.01

Supplementary Table 4. Proteins upregulated within the CA3 region following ischemia and reperfusion.

Accession Number	Protein Name	CA3 ischemia: CA3 Sham Ratio	p-value
P36776	lon peptidase 1, mitochondrial	16.667	0
O43182	Rho GTPase activating protein 6	14.286	0
Q14168	membrane protein, palmitoylated 2	14.286	0
O75366	advillin	3.613	0.02
Q8IZT8	heparan sulfate (glucosamine) 3-O-sulfotransferase 5	2.941	0
Q15021	non-SMC condensin I complex, subunit D2	2.778	0
Q86UL8	membrane associated guanylate kinase	2.632	0.03
Q96NW4	ankyrin repeat domain 27 (VPS9 domain)	2.273	0.04
P17858	phosphofructokinase, liver	1.887	0
O14746	telomerase reverse transcriptase	1.887	0
Q9NZM3	intersectin 2	1.852	0
P16435	P450 (cytochrome) oxidoreductase	1.754	0
O75473	leucine-rich repeat-containing G protein-coupled receptor 5	1.695	0
O94776	metastasis associated 1 family, member 2	1.695	0
Q9UPT6	mitogen-activated protein kinase 8 interacting protein 3	1.587	0
P54132	Bloom syndrome	1.538	0
O60832	dyskeratosis congenita 1, dyskerin	1.538	0
P11413	glucose-6-phosphate dehydrogenase	1.515	0
P53355	death-associated protein kinase 1	1.493	0
Q12769	nucleoporin 160kDa	1.493	0
O14841	5-oxoprolinase (ATP-hydrolysing)	1.493	0
Q96NW7	leucine rich repeat containing 7	1.471	0
Q14164	inhibitor of kappa light polypeptide gene enhancer in B-cells	1.429	0.01
P22059	oxysterol binding protein	1.429	0
Q96PV0	synaptic Ras GTPase activating protein 1 homolog	1.429	0
Q9H3U1	unc-45 homolog A	1.429	0
Q8TEW8	par-3 partitioning defective 3 homolog B	1.389	0.01
P54652	heat shock 70kDa protein 2	1.389	0
P00338	lactate dehydrogenase A	1.389	0
Q9Y6N6	laminin, gamma 3	1.37	0.01
P07996	thrombospondin 1	1.37	0
Q9H078	ClpB caseinolytic peptidase B homolog	1.351	0.01
Q9Y281	cofilin 2	1.351	0
P27348	14 3 3 theta	1.351	0
Q9BPU6	dihydropyrimidinase-like 5	1.333	0.03
P02538	keratin 6A	1.333	0.02
Q96G03	phosphoglucomutase 2	1.333	0.02
Q9HCK4	roundabout, axon guidance receptor, homolog 2	1.316	0.02
P16234	platelet-derived growth factor receptor, alpha polypeptide	1.316	0.01
P54756	EPH receptor A5	1.299	0.03
Q9Y6U3	scinderin	1.299	0.01
P59046	NLR family, pyrin domain containing 12	1.282	0.04
Q7L8L6	FAST kinase domains 5	1.266	0.04
Q96JM2	zinc finger protein 462	1.266	0.02
Q96KQ7	euchromatic histone-lysine N-methyltransferase 2	1.266	0
Q96CN9	GRIP and coiled-coil domain containing 1	1.266	0
Q9NPP4	NLR family, CARD domain containing 4	1.266	0
Q92574	Hamartin	1.266	0
P68363	tubulin, alpha 1b	1.266	0

Q9BQE3	tubulin, alpha 1c	1.266	0
P12236	solute carrier family 25 , member 6	1.25	0.03
Q8NFM4	adenylate cyclase 4	1.25	0.02
Q92526	chaperonin containing TCP1, subunit 6B (zeta 2)	1.25	0.01
O00160	myosin IF	1.25	0
P04350	tubulin, beta 4	1.25	0
Q96CW5	tubulin, gamma complex associated protein 3	1.25	0
Q02241	kinesin family member 23	1.235	0.04
Q14160	scribbled homolog	1.235	0.01
P07900	heat shock protein 90kDa alpha (cytosolic), class A member 1	1.235	0
Q96LB3	intraflagellar transport 74 homolog	1.235	0
Q8TEL6	transient receptor potential cation channel, subfamily C	1.235	0
Q71U36	tubulin, alpha 1a	1.235	0
Q15393	splicing factor 3b, subunit 3, 130kDa	1.22	0.04
P56524	histone deacetylase 4	1.22	0.01
Q6XUX3	receptor interacting protein kinase 5	1.22	0.01
Q9UQL6	histone deacetylase 5	1.22	0
Q14139	ubiquitination factor E4A (UFD2 homolog)	1.22	0
O76041	nebulette	1.205	0.04
Q7KZF4	staphylococcal nuclease and tudor domain containing 1	1.205	0.04
P55786	aminopeptidase puromycin sensitive	1.205	0.02
O15164	tripartite motif-containing 24	1.205	0.02
P08238	heat shock protein 90kDa alpha (cytosolic), class B member 1	1.205	0.01
O95819	mitogen-activated protein kinase kinase kinase kinase 4	1.205	0.01
P13497	bone morphogenetic protein 1	1.205	0
Q29RF7	PDS5, regulator of cohesion maintenance, homolog A	1.19	0.04
Q14587	zinc finger protein 268	1.19	0.04
Q9NRL2	bromodomain adjacent to zinc finger domain, 1A	1.19	0.04
Q92896	golgi apparatus protein 1	1.19	0.02
P06213	insulin receptor	1.19	0.02
P26232	catenin (cadherin-associated protein), alpha 2	1.19	0.01
Q16706	mannosidase, alpha, class 2A, member 1	1.19	0.01
Q9UKW4	vav 3 guanine nucleotide exchange factor	1.19	0.01
P60709	actin, beta	1.19	0
P63261	actin, gamma 1	1.19	0
Q05707	collagen, type XIV, alpha 1	1.19	0
Q92841	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	1.19	0
Q8IYF3	testis expressed 11	1.19	0
Q9BVA1	tubulin, beta 2B	1.19	0
P62258	tyrosine 3-monooxygenase, epsilon polypeptide	1.19	0
O60391	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	1.176	0.04
Q12772	sterol regulatory element binding transcription factor 2	1.176	0.02
Q8NBJ4	golgi membrane protein 1	1.176	0.01
O15344	midline 1 (Opitz/BBB syndrome)	1.176	0.01
Q86UP2	kinectin 1 (kinesin receptor)	1.176	0
O75150	ring finger protein 40	1.163	0.04
Q8WVM7	stromal antigen 1	1.163	0.03
Q6PEY2	tubulin, alpha 3e	1.163	0.02
O94887	FERM, RhoGEF and pleckstrin domain protein 2	1.163	0.01
Q9UJF2	RAS protein activator like 2	1.163	0.01

Q5VWQ8	DAB2 interacting protein	1.163	0
O60264	SWI/SNF related, actin dependent regulator of chromatin, alpha 5	1.163	0
Q13509	tubulin, beta 3	1.163	0
P50416	carnitine palmitoyltransferase 1A	1.149	0.04
O95786	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	1.149	0.02
Q6BDS2	UHRF1 (ICBP90) binding protein 1	1.149	0.02
Q96HN2	S-adenosylhomocysteine hydrolase-like 2	1.149	0.01
Q9ULK4	mediator complex subunit 23	1.149	0.01
Q14525	keratin 33B	1.149	0
Q7RTR2	NLR family, CARD domain containing 3	1.149	0
Q9BUF5	tubulin, beta 6	1.149	0
P54198	HIR histone cell cycle regulation defective homolog A	1.136	0.04
Q9Y2K2	KIAA0999 protein	1.136	0.04
Q9UNY4	transcription termination factor, RNA polymerase II	1.136	0.04
Q9Y5Q8	general transcription factor IIIC, polypeptide 5, 63kDa	1.136	0.04
Q9HBT7	zinc finger protein 287	1.136	0.03
P09104	enolase 2 (gamma, neuronal)	1.136	0.02
Q13029	PR domain containing 2, with ZNF domain	1.136	0.02
Q70CQ4	ubiquitin specific peptidase 31	1.136	0.02
O15438	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	1.136	0.01
Q66GS9	centrosomal protein 135kDa	1.136	0.01
Q05586	glutamate receptor, ionotropic, N-methyl D-aspartate 1	1.136	0.01
P61981	tyrosine 3-monooxygenase, gamma polypeptide	1.136	0
Q9BX63	BRCA1 interacting protein C-terminal helicase 1	1.124	0.04
P54707	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	1.124	0.02
Q9UJA3	minichromosome maintenance complex component 8	1.124	0.02
P29590	promyelocytic leukemia	1.124	0.02
P12277	creatine kinase, brain	1.124	0.01
P31150	GDP dissociation inhibitor 1	1.124	0.01
P07437	tubulin, beta	1.124	0
Q13885	tubulin, beta 2A	1.124	0
P46020	phosphorylase kinase, alpha 1	1.111	0.04
O94759	transient receptor potential cation channel, subfamily M, member 2	1.111	0.04
Q9BQG0	MYB binding protein (P160) 1a	1.111	0.03
Q12768	KIAA0196	1.111	0.02
P48681	nestin	1.111	0.02
Q99758	ATP-binding cassette, sub-family A (ABC1), member 3	1.111	0.01
P60174	triosephosphate isomerase 1	1.111	0
Q8N3P4	vacuolar protein sorting 8 homolog	1.111	0

Supplementary Table 5. Proteins downregulated within the CA1 region after ischemia and reperfusion.

Accession Number	Protein Name	CA1 Sham: CA1 ischemia Ratio	p-value
P47989	xanthine dehydrogenase	9.03	0
Q58EX7	Puratrophin-1	2.92	0
Q92526	chaperonin containing TCP1, subunit 6B	2.32	0
O75366	advillin	2.23	0.01
O76094	signal recognition particle 72kDa	1.48	0
Q9NRL2	bromodomain adjacent to zinc finger domain, 1A	1.43	0
Q9Y613	formin homology 2 domain containing 1	1.42	0
Q9BQN1	family with sequence similarity 83, member C	1.4	0.03
Q9Y2J4	angiomotin like 2	1.39	0
P17812	CTP synthase	1.36	0
P43243	matrin 3	1.35	0.03
O95025	Semaphorin 3D	1.35	0
Q9P253	vacuolar protein sorting 18 homolog	1.34	0
P61158	ARP3 actin-related protein 3 homolog	1.32	0
O60462	neuropilin 2	1.32	0
Q12872	splicing factor, arginine/serine-rich 8	1.32	0.04
Q9UIG0	bromodomain adjacent to zinc finger domain, 1B	1.31	0
Q14527	helicase-like transcription factor	1.3	0.01
O15439	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.28	0
P50213	isocitrate dehydrogenase 3 (NAD+) alpha	1.28	0.04
P34896	serine hydroxymethyltransferase 1 (soluble)	1.27	0.02
Q96ED9	hook homolog 2	1.26	0.04
P35908	keratin 2	1.26	0.01
P13637	ATPase, Na+/K+ transporting, alpha 3 polypeptide	1.25	0
Q16720	ATPase, Ca++ transporting, plasma membrane 3	1.23	0
Q9Y2T1	axin 2 (conductin, axil)	1.23	0.04
Q9H2K2	tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2	1.23	0.02
Q9Y5H6	protocadherin alpha 8	1.22	0
Q6P996	pyridoxal-dependent decarboxylase domain containing 1	1.22	0.02
Q16825	protein tyrosine phosphatase, non-receptor type 21	1.22	0
P42658	dipeptidyl-peptidase 6	1.21	0
P14923	junction plakoglobin	1.21	0.02
P54277	PMS1 postmeiotic segregation increased 1	1.21	0.03
Q9UNM6	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	1.21	0
Q5JTH9	ribosomal RNA processing 12 homolog	1.21	0
Q92839	hyaluronan synthase 1	1.2	0.03
Q8WTR7	zinc finger protein 473	1.2	0.01
Q9UQ16	dynamin 3	1.19	0.01
Q15051	IQ motif containing B1	1.19	0
Q9H0B3	KIAA1683	1.19	0
Q96G03	phosphoglucomutase 2	1.19	0.02
Q8TEL6	transient receptor potential cation channel, subfamily C	1.19	0.01
Q9UQM7	calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha	1.17	0
P14868	aspartyl-tRNA synthetase	1.17	0
Q01118	sodium channel, voltage-gated, type VII, alpha	1.17	0.02
Q12772	sterol regulatory element binding transcription factor 2	1.17	0.04

P52735	vav 2 guanine nucleotide exchange factor	1.17	0.04
O95342	ATP-binding cassette, sub-family B (MDR/TAP), member 11	1.16	0.03
P98196	ATPase, class VI, type 11A	1.16	0.01
P06576	ATP synthase, H+ transporting, beta polypeptide	1.16	0
Q8TCU5	glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	1.16	0.04
Q08J23	NOL1/NOP2/Sun domain family, member 2	1.16	0.04
P05023	ATPase, Na+/K+ transporting, alpha 1 polypeptide	1.15	0
O15083	ELKS/RAB6-interacting/CAST family member 2	1.15	0.02
Q99707	5-methyltetrahydrofolate-homocysteine methyltransferase	1.15	0.04
P18206	vinculin	1.15	0.01
Q08AN1	zinc finger protein 616	1.15	0.03
P29322	EPH receptor A8	1.14	0.01
P41594	glutamate receptor, metabotropic 5	1.14	0.01
Q6VAB6	kinase suppressor of ras 2	1.14	0.03
P55160	NCK-associated protein 1-like	1.14	0.02
O60312	ATPase, class V, type 10A	1.13	0.03
P50993	ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide	1.13	0
P23634	ATPase, Ca++ transporting, plasma membrane 4	1.13	0.03
O60231	DEAH (Asp-Glu-Ala-His) box polypeptide 16	1.13	0.04
Q5UE93	phosphoinositide-3-kinase, regulatory subunit 6	1.13	0.04
Q6IQ26	RAB6 interacting protein 1	1.13	0.01
Q9H2G2	STE20-like kinase	1.13	0
P51523	zinc finger protein 84	1.13	0.04
Q9P232	contactin 3 (plasmacytoma associated)	1.12	0
P11215	integrin, alpha M (complement component 3 receptor 3 subunit)	1.12	0.01
Q14687	KIAA0182	1.12	0.04
Q8TCG1	KIAA1524	1.12	0.03
Q13201	multimerin 1	1.12	0.03
Q86SQ0	pleckstrin homology-like domain, family B, member 2	1.12	0.01
Q8NDV3	structural maintenance of chromosomes 1B	1.12	0
Q13049	tripartite motif-containing 32	1.12	0.04
P63261	actin, gamma 1	1.11	0.02
Q96F07	cytoplasmic FMR1 interacting protein 2	1.11	0
O00418	eukaryotic elongation factor-2 kinase	1.11	0.04
P36956	sterol regulatory element binding transcription factor 1	1.11	0.03

Supplementary Table 6. Proteins downregulated within the CA3 region following ischemia and reperfusion

Accession Number	Protein Name	CA3 Sham: CA3 ischemia Ratio	p-value
O95153	benzodiazapine receptor (peripheral) associated protein 1	13.6	0
Q5T9A4	ATPase family, AAA domain containing 3B	5.26	0.01
Q8IWY9	congenital dyserythropoietic anemia, type I	3.63	0
P51788	chloride channel 2	2.51	0.01
Q96IZ5	RNA binding motif protein 41	2.08	0
O43566	regulator of G-protein signaling 14	1.88	0
Q8WTU0	DDI1, DNA-damage inducible 1, homolog 1	1.86	0.03
Q9P202	deafness, autosomal recessive 31	1.72	0
O14678	ATP-binding cassette, sub-family D (ALD), member 4	1.68	0.03
Q16549	proprotein convertase subtilisin/kexin type 7	1.68	0
Q9UKV0	histone deacetylase 9	1.67	0.02
Q16720	ATPase, Ca++ transporting, plasma membrane 3	1.63	0
P49916	ligase III, DNA, ATP-dependent	1.62	0
P78332	RNA binding motif protein 6	1.6	0.01
P35228	nitric oxide synthase 2A (inducible)	1.54	0
Q5VUB5	chromosome 10 open reading frame 38	1.49	0
Q9P1Y5	KIAA1543	1.48	0
Q8WXD9	CASK interacting protein 1	1.46	0.01
Q9H0K1	SNF1-like kinase 2	1.45	0
Q9UQM7	calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha	1.43	0
Q7Z7B0	filamin A interacting protein 1	1.43	0
Q9UI12	ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H	1.42	0.03
Q9P2M7	cingulin	1.42	0
P14780	matrix metallopeptidase 9	1.4	0.04
Q9H9E3	component of oligomeric golgi complex 4	1.4	0.01
O95757	heat shock 70kDa protein 4-like	1.39	0
O15394	neural cell adhesion molecule 2	1.38	0.01
O94874	KIAA0776	1.36	0.02
P19012	keratin 15	1.36	0
Q7Z3E2	chromosome 10 open reading frame 118	1.35	0.03
Q96MW5	component of oligomeric golgi complex 8	1.35	0.01
Q9BQN1	family with sequence similarity 83, member C	1.35	0.01
Q5UE93	phosphoinositide-3-kinase, regulatory subunit 6	1.35	0
Q07075	glutamyl aminopeptidase (aminopeptidase A)	1.34	0.04
Q6PI48	aspartyl-tRNA synthetase 2, mitochondrial	1.32	0
P11216	phosphorylase, glycogen; brain	1.32	0
O15455	toll-like receptor 3	1.32	0
Q9BST9	rhotekin	1.31	0.04
P22102	phosphoribosylglycinamide formyltransferase	1.31	0
P50991	chaperonin containing TCP1, subunit 4 (delta)	1.3	0.02
Q04695	keratin 17	1.3	0.01
P08581	met proto-oncogene	1.3	0.01
Q70J99	unc-13 homolog D	1.3	0

Q9Y2H9	microtubule associated serine/threonine kinase 1	1.28	0.04
O43295	SLIT-ROBO Rho GTPase activating protein 3	1.28	0
Q07157	tight junction protein 1 (zona occludens 1)	1.28	0
Q8TB22	spermatogenesis associated 20	1.27	0.02
P60228	eukaryotic translation initiation factor 3, subunit E	1.26	0.04
P55201	bromodomain and PHD finger containing, 1	1.26	0.01
Q13796	shroom family member 2	1.26	0
Q9Y6L7	tolloid-like 2	1.26	0
Q16531	damage-specific DNA binding protein 1, 127kDa	1.25	0.04
Q9NYA3	golgi autoantigen, golgin subfamily a, 6	1.25	0
Q9BZZ5	apoptosis inhibitor 5	1.23	0.04
Q01814	ATPase, Ca++ transporting, plasma membrane 2	1.23	0.04
Q9BZF3	oxysterol binding protein-like 6	1.23	0.03
Q13972	Ras protein-specific guanine nucleotide-releasing factor 1	1.23	0.02
O00231	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	1.22	0.01
Q7Z460	cytoplasmic linker associated protein 1	1.22	0
P21399	aconitase 1, soluble	1.21	0.01
O95782	adaptor-related protein complex 2, alpha 1 subunit	1.21	0.01
Q9H0B6	kinesin light chain 2	1.21	0.01
O75747	phosphoinositide-3-kinase, class 2, gamma polypeptide	1.21	0.01
P04259	keratin 6B	1.2	0.02
Q9UL54	TAO kinase 2	1.2	0.02
Q96M96	FYVE, RhoGEF and PH domain containing 4	1.2	0.01
O75083	WD repeat domain 1	1.2	0
P98171	Rho GTPase activating protein 4	1.19	0.04
P43243	matrin 3	1.19	0.01
Q8N4C8	misshapen-like kinase 1	1.19	0
Q16825	protein tyrosine phosphatase, non-receptor type 21	1.19	0
Q02880	topoisomerase (DNA) II beta 180kDa	1.17	0.03
Q96NH3	chromosome 6 open reading frame 170	1.17	0.02
Q9NTJ3	structural maintenance of chromosomes 4	1.17	0
P12035	keratin 3	1.16	0.03
Q92503	SEC14-like 1	1.16	0.03
O75094	slit homolog 3	1.16	0.03
Q92805	golgi autoantigen, golgin subfamily a, 1	1.16	0.02
O60282	kinesin family member 5C	1.16	0.02
P52306	RAP1, GTP-GDP dissociation stimulator 1	1.16	0
O14924	regulator of G-protein signaling 12	1.15	0.04
Q8TEK3	DOT1-like, histone H3 methyltransferase	1.15	0.04
Q14764	major vault protein	1.15	0.04
O95155	ubiquitination factor E4B (UFD2 homolog)	1.15	0.04
Q9P2Y5	UV radiation resistance associated gene	1.15	0.03
P23634	ATPase, Ca++ transporting, plasma membrane 4	1.15	0.02
Q7Z478	DEAH (Asp-Glu-Ala-His) box polypeptide 29	1.15	0.02

Q8TEQ6	gem (nuclear organelle) associated protein 5	1.15	0.02
P46940	IQ motif containing GTPase activating protein 1	1.15	0.02
P55160	NCK-associated protein 1-like	1.15	0.02
P33176	kinesin family member 5B	1.15	0.01
P82094	TATA element modulatory factor 1	1.15	0.01
P09884	polymerase (DNA directed), alpha 1	1.15	0
P30153	protein phosphatase 2 (formerly 2A), regulatory subunit A	1.15	0
Q9ULU8	Ca2+-dependent secretion activator	1.14	0.04
Q9BYP7	WNK lysine deficient protein kinase 3	1.14	0.04
Q9UQ16	dynamin 3	1.14	0.02
Q8IWB4	family with sequence similarity 75, member A7	1.14	0.02
Q12965	myosin IE	1.14	0.02
Q9UIG0	bromodomain adjacent to zinc finger domain, 1B	1.14	0.01
O60674	Janus kinase 2 (a protein tyrosine kinase)	1.14	0.01
O75417	polymerase (DNA directed), theta	1.14	0
Q8N4X5	actin filament associated protein 1-like 2	1.13	0.04
P28340	polymerase (DNA directed), delta 1, catalytic subunit 125kDa	1.13	0.04
P06737	phosphorylase, glycogen; liver	1.13	0.03
O14795	unc-13 homolog B	1.13	0.02
Q9UL18	eukaryotic translation initiation factor 2C, 1	1.13	0.01
O75116	Rho-associated, coiled-coil containing protein kinase 2	1.13	0.01
Q9UKX5	integrin, alpha 11	1.12	0.04
P11137	microtubule-associated protein 2	1.12	0.04
P55072	valosin-containing protein	1.12	0.04
O60423	ATPase, class I, type 8B, member 3	1.12	0.04
Q8N122	raptor	1.12	0.04
P08183	ATP-binding cassette, sub-family B (MDR/TAP), member 1	1.12	0.03
Q76N89	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1	1.12	0.03
Q9ULE0	WWC family member 3	1.12	0.02
O43432	eukaryotic translation initiation factor 4 gamma, 3	1.11	0.04
P21439	ATP-binding cassette, sub-family B (MDR/TAP), member 4	1.11	0.04
Q6DT37	CDC42 binding protein kinase gamma (DMPK-like)	1.11	0.03
P19367	hexokinase 1	1.11	0.03
P14616	insulin receptor-related receptor	1.11	0.03
Q8WTW3	component of oligomeric golgi complex 1	1.11	0.02
Q9BZF9	uveal autoantigen with coiled-coil domains and ankyrin repeats	1.11	0.02
Q8IWV7	ubiquitin protein ligase E3 component n-recognin 1	1.11	0.02
Q08AN1	zinc finger protein 616	1.11	0.02
O15061	desmuslin	1.11	0.01
Q9P2H0	KIAA1377	1.11	0

Supplementary Table 7. Proteins composing the pathway of Figure 1a

Abbreviation	Protein Name
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1
14-3-3	14-3-3
14-3-3 beta	14-3-3 beta
14-3-3 epsilon	14-3-3 epsilon
14-3-3 gamma	14-3-3 gamma
14-3-3 theta	14-3-3 theta
Akt-Calmodulin-Hsp90-Nos3	Akt-Calmodulin-heat shock protein 90-nitric oxide synthase 3
ASK1	Apoptosis signal-regulating kinase 1
AKT	Protein Kinase B
BAD	Bcl2 antagonist of cell death
Bcl-2	B-cell lymphoma 2
Bcl-XL	B-cell lymphoma-extra large
Calmodulin-Hsp90-Nos3	Calmodulin-heat shock protein 90-nitric oxide synthase 3
c-Raf	proto-oncogene c-RAF
Cot	Cancer Osaka Thyroid oncogene
eIF4E	Eukaryotic translation initiation factor 4E
eNOS	Endothelial nitric oxide synthase
ERK 1/2	Extracellular signal-regulated kinase 1/2
GRB2	Growth factor receptor-bound protein 2
Hamartin	Hamartin
Hsp90	heat shock protein 90kDa
HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1
HSP90AB1	heat shock protein 90 alpha (cytosolic), class B member 1
I κB	Inhibitor of NF-κB
IKK	Complex of two subunits, IKKα and IKKβ
JAK	Tyrosine protein kinase JAK3
MEK 1/2	Mitogen-activated protein kinase kinase 1/2
mTORC1	Mammalian target of rapamycin
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells)
P70S6K	p70S6 kinase (p70S6K) is a serine/threonine kinase
p27Kip1	A cyclin-dependent kinase inhibitor which binds to cyclinE/cdk2
PDK1	An activator of Akt
PI3K p85	Phosphatidylinositide 3-kinase p85 subunit
PI3K p110	Phosphoinositide 3-kinase [PI3K(p110 alpha)]
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P3)
PP2A	Protein phosphatase 2A
Ras	Oncogene
Rheb	Ras homolog enriched in brain
RTK	Receptor tyrosine kinase
SHC	SHC-transforming protein 1
SHIP	SH2 domain-containing inositol 5'-phosphatase
SOS	Son of Sevenless
Tuberin	Tuberin

Supplementary Table 8. Proteins composing the network of Figure 1b

Abbreviation	Protein Name
14-3-3 beta	14-3-3 beta
14-3-3 epsilon	14-3-3 epsilon
14-3-3 gamma	14-3-3 gamma
14-3-3 theta	14-3-3 theta
ATP2B2	Plasma membrane calcium-transporting ATPase 2
ATP2B3	Plasma membrane calcium-transporting ATPase 3
ATP2B4	Plasma membrane calcium-transporting ATPase 4
Ca^{2+}ATPase	Ca^{2+}ATPase
Calmodulin	Calmodulin
CLASP1	Cytoplasmic linker associated protein 1
ERK	Extracellular signal-regulated kinases
Hamartin	Hamartin
KIAA1383	Microtubule regulator 120 KDa; uncharacterized protein KIAA1383
KIAA1543	Calmodulin regulated spectrin-associated protein family, member 3 (CAMSAP3)
KIF5B	Kinesin heavy chain isoform 5B
KIF5C	Kinesin heavy chain isoform 5C
KIF23	Kinesin heavy chain isoform 23
KLC2	Kinesin light chain 2
KTN1	Kinectin
KRT33B	Keratin, type I cuticular Ha3-II
MAP2	Microtubule-associated protein 2
MVP	Major vault protein
PARD3B	Partitioning defective 3 homolog B
PMCA	The plasma membrane Ca2+ ATPase
RASAL2	Ras GTPase-activating protein nGAP
RASGRF1	Ras protein-specific guanine nucleotide-releasing factor 1
SHROOM2	Shroom family member 2
TRPM2	Transient receptor potential cation channel, subfamily M, member 2
TUBA1A	Tubulin alpha-1A chain
TUBB2A	Tubulin beta-2A chain
TUBB3	Tubulin beta-3 chain
TUBB4	Tubulin beta-4 chain
Tubulin	Tubulin
UNC13B	Protein unc-13 homolog B
VAV3	Guanine nucleotide exchange factor VAV3

Supplementary Table 9. Differentially expressed proteins in CA1 compared to CA3 region after ischemia and reperfusion.

	Decreased
	Increased
	No change

Protein Name	CA1 S: CA1 I	CA3 S: CA3 I
Sodium potassium transporting ATPase subunit alpha 3		
Zinc finger protein 214		
Protein EMSY		
Catenin alpha 1		
Uncharacterized protein C15orf33		
FYVE RhoGEF and PH domain containing protein 5		
RNA polymerase II associated protein 1		
Actin like protein 3		
Chaperonin subunit 6a		
FH1 FH2 domain containing protein 1		
Tubulin alpha 1A chain		
Actin cytoplasmic 1 Beta actin		
ATP binding cassette sub family B member 8		
Advillin p92		
Actin cytoplasmic 2 Gamma actin		
Ubiquitin protein ligase E3B		
RRP12 like protein		
Neurobeachin like		
Ran binding protein 17		
Caskin 1		
RNA binding protein 41		
Alpha actinin 1		
Jouberin Abelson helper integration site 1 protein homolog		
Serine threonine protein kinase TAO2		
Zinc finger protein 780B		
Unc 13 homolog D		
Codanin 1		
Centrosomal protein of 135 kDa		
Tubulin beta chain Tubulin beta 5 chain		
Vam6 Vps39 like protein		

Thioredoxin domain containing protein 11		
FERM RhoGEF and pleckstrin domain containing protein 1		
Interferon induced helicase C domain containing protein 1		
Kinesin like protein KIF21A		
Myosin IIIA EC 2 7 11 1		
Pyruvate carboxylase mitochondrial precursor		
Synaptojanin 1		
Tubulin alpha 8 chain		
DNA cytosine 5 methyltransferase 1		
Bromodomain containing protein 1		
Zinc finger protein 228		
Protein Jumonji		
FH1 FH2 domain containing protein 1		
Mannosyl oligosaccharide glucosidase		
Tyrosine protein kinase JAK3		
PMS1 protein homolog 1		
Dipeptidyl aminopeptidase like protein 6		
Coiled coil domain containing protein 22		
Centrosomal protein of 170 kDa		
Succinate dehydrogenase ubiquinone flavoprotein subunit mitochondrial precursor		
Ras GTPase activating protein 4		
Tubulin beta 2A chain		
Neuropilin 2 precursor		
DNA directed RNA polymerase III subunit RPC1		
Alpha aminoadipic semialdehyde dehydrogenase		
Slit homolog 1 [precursor]		
Vacuolar protein sorting associated protein 18 homolog		
Dynactin subunit		
Hook homolog 2		
ProSAAS		
Lysyl oxidase homolog 4 precursor		

Aspartate aminotransferase cytoplasmic		
UNC45 homolog A		
Leucine rich repeat containing G protein coupled receptor 5 precursor		
Hamartin		
Zinc finger protein 658		
Tubulin alpha 1C chain		
2 5 oligoadenylate synthetase 3		
Intersectin 2		
Tubulin alpha 1B chain		
GRIP and coiled coil domain containing protein		
Ecotropic viral integration site 5 protein homolog		
Protein glutamine gamma glutamyltransferase 6		
SET and MYND domain containing protein 4		
Oxysterol binding protein 1		
5 oxoprolinase		
14 3 3 protein theta		
Intraflagellar transport 74 homolog		
Kinesin like protein KIF24		
AFG3 like protein 2		
Toll like receptor 3 precursor		
Trifunctional purine biosynthetic protein adenosine 3		
SLIT ROBO Rho GTPase activating protein 3		
Histone deacetylase 9		
Formin like protein 1		
Uncharacterized protein C10orf118		
Tryptophan 2 3 dioxygenase		
Probable ATP dependent RNA helicase		
Zinc finger protein 462		
UDP N acetylhexosamine pyrophosphorylase		
Polypeptide N acetylgalactosaminyltransferase 5		

Zinc finger protein 234		
Progesterone induced blocking factor 1		
Cingulin		
Conserved oligomeric Golgi complex component 4		
Deleted in lung and esophageal cancer protein 1		
Golgin subfamily A member 6		
Twinkle protein mitochondrial precursor		
Protein Shroom2		
Tolloid like protein 2 precursor		
UHRF1-binding protein 1-like		
ATPase family AAA domain containing protein 3B		
CRSP complex subunit 6		
UHRF1-binding protein 1-like		
Filamin A interacting protein 1 FILIP		
Dual specificity tyrosine phosphorylation regulated kinase 4		
Uncharacterized protein KIAA0562		
Conserved oligomeric Golgi complex component 8		
DENN domain containing protein 2C		
Chromosome associated protein G		
T complex protein 1 subunit delta		
MICAL like protein 2		
Peregrin		

Table showing a list of proteins shortlisted from the proteomic datasets using the criteria described in **Supplementary Fig. 7**. CA1 S is CA1 sham ischemia; CA1 I is CA1 ischemia; CA3 S is CA3 sham ischemia; CA3 I is CA3 ischemia. Red boxes show increase, yellow boxes indicate decrease, whereas, grey boxes represent no change in the expression levels between the groups.

Supplementary Results and Discussion

Protein expression profile within the CA1 and CA3 regions under sham conditions

Proteomic analysis of the cytoplasmic and membrane fractions from sham ischemic animals identified 1,066 proteins that were present in both the CA1 and CA3 regions of the hippocampus. From those, 137 (13%) had higher expression in the CA1 region compared to the CA3 region **(Supplementary Table 1)** and 139 (13%) were enriched within the CA3 relative to the CA1 region **(Supplementary Table 2)**.

The datasets were analyzed ontologically using the panther classification system (www.pantherdb.org). For each region, the proteins with enhanced expression were grouped according to the molecular pathway or the biological process in which they participate. The number of proteins present in each molecular pathway or biological process was compared between the two regions. As a result, we identified pathways and processes that had a different number of enhanced proteins in the CA1 compared to the CA3 region.

Thirteen transporter proteins showed higher expression levels in the CA3 region, whereas in the CA1 there were 2 transporter proteins with enhanced expression relative to the CA3 region (**Supplementary Fig. 3**). Interestingly, one of these 13 transporters was the Ca^{2+} transporting ATPase, which is involved in calcium homeostasis[1, 2]. Its expression was enhanced 38±14% in the CA3 region compared to the CA1 region ($p<0.05$). This probably indicated an enhanced innate ability of CA3 cells to regulate Ca^{2+} levels and limit excitotoxicity. Overall, in both groups a similar number of proteins was enhanced between the two regions.

The effect of ischemia and reperfusion on the proteome profile of the CA1 and CA3 regions

In the CA1 region, 1,101 proteins were found to be present in both sham ischemic and ischemic rats, and 1,062 proteins were present the CA3 region of both sham ischemic and ischemic rats.

Profile of upregulated proteins following ischemia and reperfusion

The profile of the proteins that were either upregulated or downregulated within the CA1 region following 10 min ischemia and 24 h of reperfusion was compared to the corresponding profile of the CA3 region using ontological analyses.

The number of proteins upregulated by ischemia and reperfusion was 114 (10%) in the CA1 (**Supplementary Table 3**) and 137 (13%) in the CA3 region (**Supplementary Table 4**). In the CA1 region, proteins with the highest upregulation included serine threonine protein kinase 35 (STK35; 300±34%; $p<0.05$) and glucose-6-phosphate dehydrogenase (G6PD; 127±27%; $p<0.05$) (**Supplementary Fig. 4a**).

In the CA3 region, proteins that were most highly upregulated as a consequence of ischemia were lon protease homolog (LONP1; 1,566±87%; $p<0.05$), Rho GTPase-activating protein 6 (ARHGAP6; 1,326±67%; $p<0.05$) and advillin (261±48%; $p<0.05$) (**Supplementary Fig. 4a**). LONP1 is a component of the proteolytic mitochondrial system which is inactivated by peroxynitrite[3]. It may be that CA3 cells are able to degrade abnormal proteins within the mitochondria more efficiently than the CA1 cells, preserving the vital integrity of mitochondria. ARHGAP6 is a Rho GTPase-activating protein which regulates cytoskeletal organization[3]. Finally, advillin is a calcium-regulated acting-binding protein that is implicated in neuronal cell morphogenesis[4].

An ontological analysis was carried out using the panther classification to compare the profile of proteins upregulated by ischemia and reperfusion between the CA1 and the CA3 region (**Supplementary Fig. 4b**). In the CA3 region, 6 of the proteins upregulated were involved in apoptosis, with 4 being inhibitors of apoptosis**,** while 2 apoptosis inhibitors were found upregulated in the CA1 region. Additionally, Na^+/K^+ ATPase was the only protein involved in homeostasis that was upregulated by ischemia in the CA3 region (12±3.4%, $p<0.05$). This protein regulates cellular homeostasis by restoring the resting potential[5], and might constitute a mechanism which allows the CA3 region to prevent overexcitability and subsequent excitotoxicity following ischemia.

Profile of downregulated proteins following ischemia and reperfusion

Ischemia and reperfusion resulted in the significant downregulation of 81 (7%) proteins in the CA1 (**Supplementary Table 5**) and 127 (12%) proteins in the CA3 region (**Supplementary Table 6**). According to the ontological analysis, the biological processes encompassing the largest number of proteins downregulated selectively in the CA3 region were "protein modification" (29 proteins) and "signal transduction" proteins (21 proteins). In addition, the molecular pathways which had the largest number of downregulated proteins in the CA1 region were transporters (10 proteins) and hydrolases (9 proteins), whereas in the CA3 region they were nucleic acid binding proteins (17 proteins) and kinases (13 proteins) (**Supplementary Fig. 4c**).

Protein network associated with the vulnerability of CA1 to ischemia

To further identify targets for future studies we investigated the protein network most significantly associated with the events induced in the CA1 after ischemia (**Supplementary Fig. 5**). Examination of this protein network revealed important insights and complemented

previous studies explaining the vulnerability of CA1 cells to ischemia[6, 7]. In particular, many of the proteins comprising this network regulate ionic and calcium homeostasis dictating the outcome of excitotoxicity. ATP1A1, ATP1A2 and ATP1A3 are Na^+/K^+ ATPases that help maintain the resting potential[8]. Interestingly, all three were found downregulated in the CA1 by ischemia probably contributing to hyperexcitability. It has been suggested that the reduction in the expression levels of the glutamate transporter, GLT-1 in astrocytes exacerbates excitotoxicity due to accumulation of synaptic glutamate as uptake is suppressed. Further evidence for excitotoxic injury was provided by the downregulation of ATP2B3, ATP2B4 and calcium/calmodulin-dependent kinase II (CaMKII). ATP2B3 and ATP2B4 are Ca^{2+} ATPases that are essential to rectify changes in calcium levels resulting from excitotoxicity[9]. CaMKII inhibition enhances neuronal activity and increases sensitivity to excitotoxic glutamate signaling following ischemia [10]. Impairment of all these processes leads to the generation of free radicals, well established mediators of ischemic cell death, with superoxide mediating oxidative stress in the CA1 region by ischemia[6]. Protection from reactive oxygen species can be conferred by G6PD, which can act as a reducing agent[11]. We found GP6D to be one of the mostly upregulated proteins in the CA1 following ischemia possibly denoting that the vulnerable CA1 neurons have to cope with oxidative stress overload after ischemia and reperfusion. Ultimately, induction of apoptosis is the end-executor of cell death. Activation of caspases has been demonstrated as a detrimental step in the CA1 region[12]. Although we did not detect activated caspases in our proteomic analysis, primarily since we did not analyze the nuclear fraction, their activity after ischemia has been shown to cleave Ca^{2+} ATPases resulting in calcium overload and cell death[9]. Overall, the biological importance of this network is in agreement with current knowledge explaining the vulnerability of CA1 neurons to ischemia.

Validation of the proteomic datasets by immunoblotting and comparison with current knowledge

We carried out immunoblotting analysis to validate 14-3-3 theta expression levels in the cytoplasmic fraction (**Supplementary Fig. 6a**). In agreement with the proteomic data, 14-3-3 theta expression levels were unaffected following ischemia in the CA1 region (**Supplementary Fig. 6b**), but were significantly upregulated in the CA3 region (**Supplementary Fig. 6c**).

In addition, our proteomic datasets are in keeping with previously published proteomic studies. Gozal and colleagues[13] used 2D electrophoresis to characterize the CA1 and CA3 proteome from rats exposed to 6 h of intermittent hypoxia. Although the effect of hypoxia rather than ischemia was studied, a significant number of proteins exhibited an expression profile similar to the one shown in our study. For instance, beta tubulin, gamma enolase, and creatine kinase B were upregulated in the CA3 region, after either ischemia or intermittent hypoxia. Similarly, triose phosphate isomerase expression was found to be higher in the CA3 compared to the CA1 of control animals in both studies. The differences that exist between our study and the Gozal et al.[13] study may be attributed mainly to the difference in the insult (intermittent hypoxia versus ischemia) and in the analytical techniques used (2D electrophoresis versus LC-based label-free quantitation).

Results from studies showing the expressed genome of CA1 and CA3 cells after ischemia in rats[14] do not correlate closely with our results, with many of the entries identified in the genomic analyses not present in our data and vice versa. These differences could be a result of the experimental limitations of the proteomics as compared to the microarray studies. However, the current study shows similarities to genomic studies of human post-mortem tissue that compared the gene expression between the CA1 and the CA3[15]. For example, cytoskeletal proteins, such as actin, were found to be enriched in the CA3 region compared to

the CA1 in both studies. In agreement with our findings, microarray studies in mice[16] revealed overexpression of phosphofructokinase and Ca^{2+} transporting ATPase in the CA3 region compared to the CA1 region.

Proteins associated with the CA3 resistance to ischemia

To identify individual protein candidates associated with the ischemia-resistive properties of CA3 neurons, we hypothesized that in order for a protein to be functionally relevant to the differential response of the CA1 and the CA3 to ischemia, its expression profile should differ between the two regions after ischemia. We used the proteomic datasets and applied the selection criteria illustrated in **Supplementary Fig. 7** (see **Supplementary Table 9**)**.** Critically, hamartin was selected using these criteria.

Methodology

Employing a label-free quantification of the proteome expression levels using groups of 5 animals allowed the detection of small expression changes with significant confidence. This relied upon the ability of the system to derive individual quantitative data for a particular protein from all the detected peptides corresponding to this protein and integrating them to overall protein levels.

Although we tried to optimize our experimental design both for the proteomics experiments and the subsequent analysis, we acknowledge the associated limitations. Group sizes of 5 were a limitation imposed by the capacity of the label-free quantitation system and by the vast amount of data generated from 20 samples (5 CA1 and 5 CA3 fractions from sham ischemic and ischemic samples). In addition, in our analysis we did not consider any post-translational modifications since this would have increased the complexity of the analysis tremendously. Interpretation of proteomic data can often give rise to false positive

identification despite the use of rigorous filtering algorithms. However, the validation of our datasets by independent experiments and by comparison with published data provided additional confidence about the accuracy of our findings. In addition, proteomic analysis favors high copy number proteins, since protein abundance is a factor that determines the proteins identified[17, 18]. This may have resulted in the failure to detect proteins that were expressed at very low levels.

Crude microdissection of the CA1 and CA3 regions resulted to mixed cell populations present within these regions. Unfortunately, isolating single cell subset does not provide enough material to run proteomics experiments of this magnitude and requires tissue post-processing that can introduce artefactual changes in protein expression levels. One advantage of looking at all cell types is the creation of a more complete picture of the events of interest.

Our study examined only the cytoplasmic and membrane fractions, and not the nuclear fraction, as we were unable to avoid significant contamination in our attempts to obtain a pure nuclear fraction.

Finally, all our data were generated from animals killed at a single time point following reperfusion. The reasoning is that after 24 h of reperfusion, although the CA1 cells are still viable, the ischemic cascade has already been activated, allowing the investigation of the effects of ischemia on morphologically intact cells.

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