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Supplemental Information

Regional Molecular Mapping of Primate Synapses during Normal Healthy Aging

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A. NHP ageing timecourse enrichment analysis

PANTHER GO-Slim Cellular Component	Fold Enrichment	P-value
Synapse	5.72	4.05E-03
Vesicle coat	4.57	2.3E-04
Mitochondrial inner membrane	3.63	6.73E-09
Presynaptic membrane	3.48	2.21E-02
Heterotrimeric G-protein complex	3.43	1.33E-02
SNARE complex	3.3	9.26E-03
Postsynaptic membrane	3.2	1.06E-02
Cytoplasmic membrane-bound vesicle	3.05	4.16E-08
Endosome	2.91	3.2E-05

B. Human patient ageing timecourse enrichment analysis

PANTHER GO-Slim Cellular Component	Fold enrichment	P-value
Postsynaptic membrane	10.48	3.97E-02
Mitochondrial inner membrane	5.69	4.06E-09
Ribosome	3.81	2.7E-10
Actin cytoskeleton	3.57	1.02E-12
Cytosol	3.4	2.07E-21
Cell junction	2.89	8.1E-03
Microtubule	2.55	5.63E-03
Cytoskeleton	1.96	1.53E-05
Cytoplasm	1.93	3.66E-29

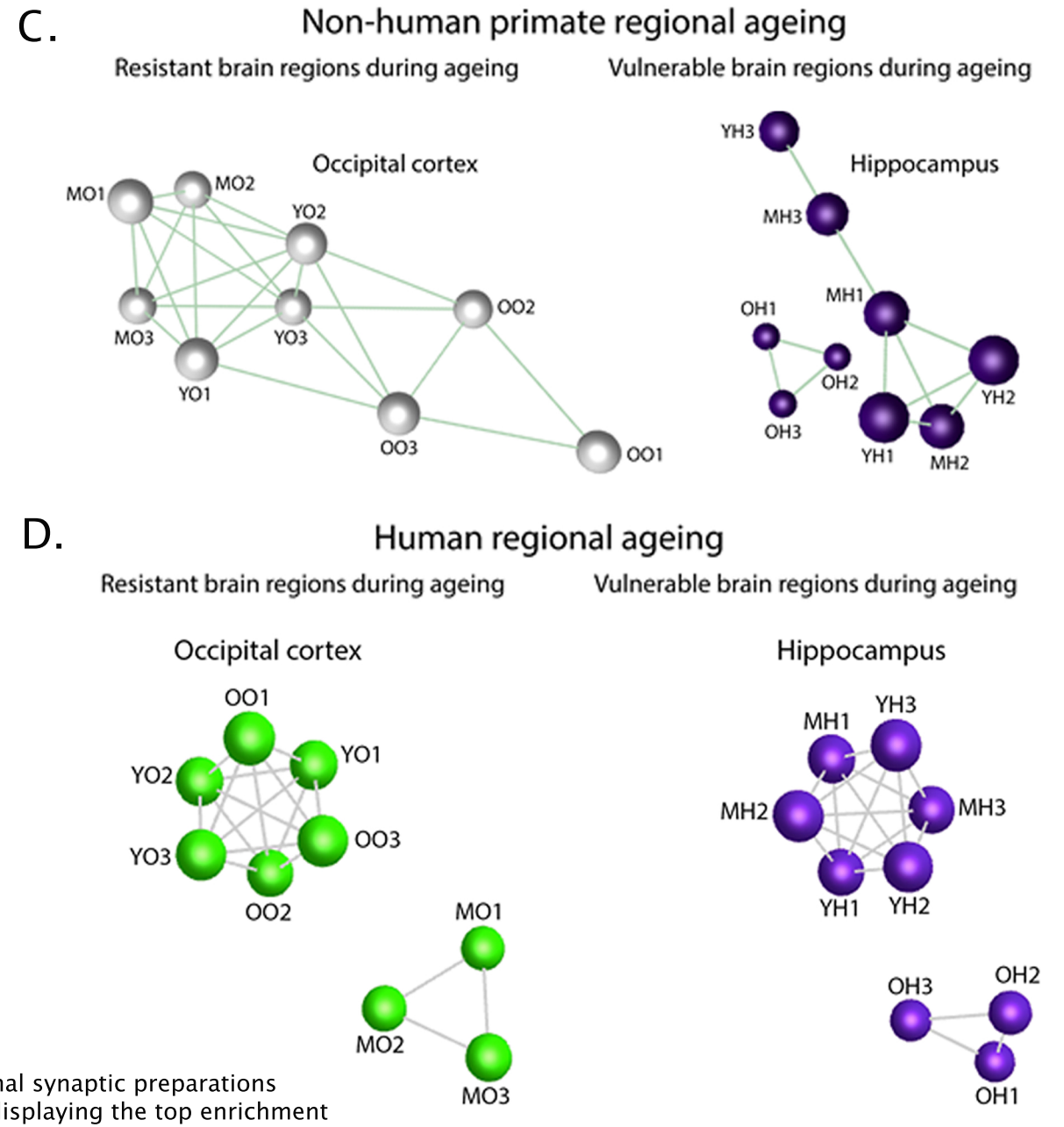
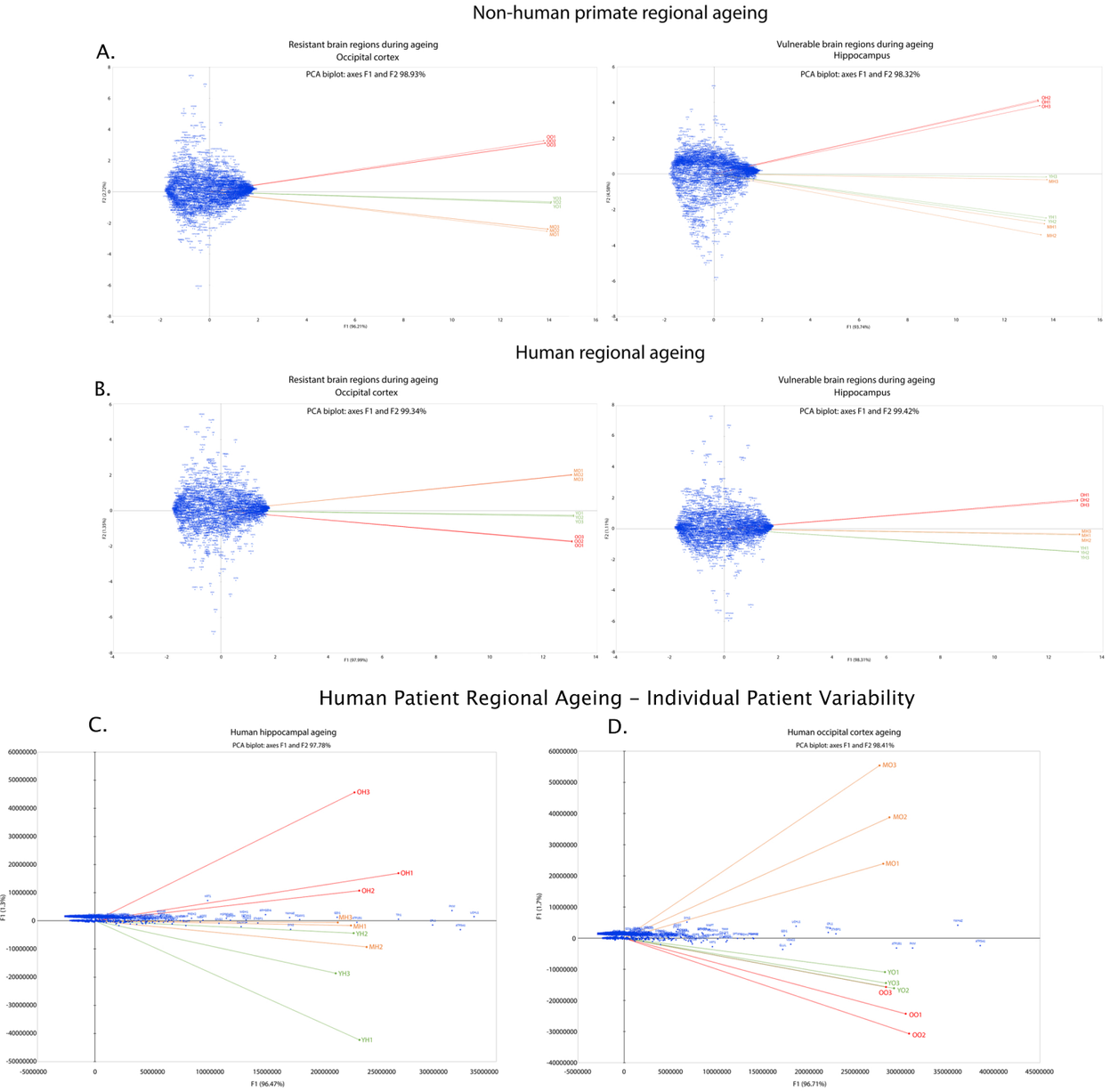


Figure S1 Enrichment analyses demonstrating purity of NHP and human regional synaptic preparations (A&B related to Figure 1): PANTHER Gene ontology (GO) enrichment analyses displaying the top enrichment terms in both the young NHP synaptosomal preparations (A.) and the young human patient synaptic isolates (B.). Synaptic ageing is regionally heterogeneous. (C&D related to Figure 2): Unbiased sample-sample Pearson correlation analyses generated from BioLayout Express3D software. C. NHP hippocampal and occipital cortex synaptic timecourse Pearson correlation analyses. DA.. NHP hippocampal and occipital cortex synaptic timecourse Pearson correlation analyses. Nodes signify individual samples and edges reflect the strength of correlation of expression. Increased edge length between nodes represents reduced correlative value between sample nodes; reduced edge length between nodes represents increased correlative value between sample nodes; detachment of nodes from primary cluster illustrates significant variability between sample populations. Graphs clustered using Pearson r=0.98.



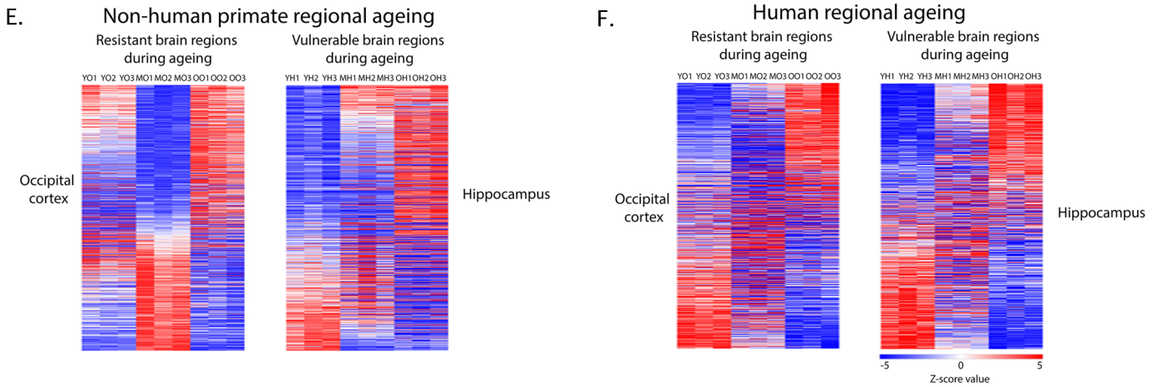


Figure S2: PCA biplots of sample variability (technical replicates – A&B; Example biological replicates – C&D) and heat maps of temporal expression (E&F): PCA biplots conveying global variability in the NHP and human patient brain regional proteomic datasets (related to Figure 2): PCA biplots display identified proteins from the NHP (A) and human patient (B) occipital cortex and hippocampal proteomic datasets. Individual sample technical replicates are indicated by filled circles and grouped by colour. Patient sample technical replicates are indicated by filled circles and grouped by colour: green = young patients; orange = mid-age patients; red = aged patients. Sample variability is dictated by the location of the representative markers in space: proximate variables demonstrate a significant positive correlation (r value is close to or equal to 1); variables opposing the centre line display a significant negative correlation (r value is close to or equal to -1). YO = young occipital cortex; MO = mid-age occipital cortex; OO = old occipital cortex; YH = young hippocampus; MH = mid-age hippocampus; OH = old hippocampus; numbers refer to technical replicates. Blue = active observations in the PCA space (proteins identified by gene name); green, orange and red = active variables observed in the PCA space (green = young time point; orange = mid-age time point; red = aged time point). All PCA biplots generated using Pearson correlation. C&D (related to Figure 2): PCA biplots displaying inter-patient variability in the human ageing regional proteomic datasets. PCA biplots display 630 identified proteins from the human patient hippocampal (C.) and occipital cortex (D.) proteomic datasets. See above for colour code, abbreviations and generation. Numbers refer to biological replicates. E&F (related to Figure 2): Heat maps illustrating temporal protein expression in differentially vulnerable populations of synapses derived from the NHP and human patient brain. E. Heat maps displaying temporal protein expression in NHP occipital cortex (left panel) and hippocampal (right panel) synaptic populations across ageing. F. Temporal protein expression in human patient occipital cortex (left panel) and hippocampal (right panel) synapses during advancing age. Heat maps display Z-score transformed normalized abundance values derived from the raw proteomic data. Blue = minimum Z-score value (-5); red = maximum Z-score value (5). YO = young occipital cortex; MO = mid-age occipital cortex; OO = old occipital cortex; YH = young hippocampus; MH = mid-age hippocampus; OH = old hippocampus; numbers refer to technical replicates.

A. Correlates of ageing

B. Correlates of vulnerability

Steady increase during ageing

NHP Hippocampus

PANTHER GO Molecular Function	Fold Enrichment	P-value
Phosphatidylcholine-sterol activity	> 100	1.07E-05
Low-density lipoprotein particle binding	33.53	1.64E-04
Amyloid-beta binding	22.01	5.83E-06
Cholesterol binding	20.76	7.56E-06
Calcium-dependent protein binding	19.63	9.67E-06
Oxidoreductase activity	11.18	1.03E-06
Cadherin binding	6.72	3.55E-05
Cell adhesion molecule binding	6.05	3.11E-06
Actin binding	5.04	1.64E-05

NHP Occipital cortex

PANTHER GO Molecular Function	Fold Enrichment	P-value
Very-low-density lipoprotein particle binding	> 100	3.28E-05
Phosphatidylcholine-sterol activity	> 100	3.28E-05
Amide binding	15.07	1.49E-04
Antioxidant activity	30.13	1.52E-04
Low-density lipoprotein particle binding	> 100	2.28E-04
Cholesterol transporter activity	63.13	5.45E-04
Sterol transporter activity	57.64	6.45E-04
Sulfur compound binding	18.08	6.50E-04
Oxidoreductase activity	5.49	6.85E-04

Human Hippocampus

PANTHER GO Molecular Function	Fold Enrichment	P-value
Myosin II binding	54.80	4.88E-05
Aldehyde dehydrogenase (NAD) activity	29.23	2.33E-04
Fatty acid binding	16.70	1.40E-04
Calcium-dependent protein binding	14.14	6.87E-06
Amyloid-beta binding	12.82	6.41E-05
NAD binding	12.38	7.47E-05
Oxidoreductase activity	10.74	6.19E-08
Magnesium ion binding	6.99	2.61E-06
Cadherin binding	4.65	7.67E-05

Human Occipital cortex

PANTHER GO Molecular Function	Fold Enrichment	P-value
Antioxidant activity	19.04	8.51E-06
Oxidoreductase activity	4.70	2.08E-05
Identical protein binding	2.74	2.77E-04
Coenzyme binding	6.65	3.09E-04
Chaperone binding	12.82	3.10E-04
NAD binding	16.46	9.26E-04
Copper ion binding	15.66	1.06E-03
Cofactor binding	4.37	1.15E-03
Catalytic activity	1.67	1.32E-03

Steady decrease during ageing

PANTHER GO Molecular Function	Fold Enrichment	P-value
Malate dehydrogenase activity	> 100	1.49E-04
Oxidoreductase activity, acting on NAD(P)H	17.86	7.01E-04
NADH dehydrogenase activity	34.44	1.76E-03
Oxidoreductase activity	3.99	3.76E-03
Ferrochelatase activity	> 100	4.14E-03
Citrate synthase activity	> 100	4.14E-03
Lactoylglutathione lyase activity	> 100	4.14E-03
Methionine-tRNA ligase activity	> 100	6.20E-03
3-oxoacid CoA-transferase activity	> 100	6.20E-03

PANTHER GO Molecular Function	Fold Enrichment	P-value
Electron transporter	> 100	1.08E-05
Proton transmembrane transporter activity	20.90	4.22E-05
Transmembrane transporter activity	5.54	2.05E-04
Nucleoside monophosphate kinase activity	73.14	4.09E-04
Inorganic molecule transporter activity	6.00	4.13E-04
Transporter activity	4.82	4.74E-04
Phosphotransferase activity	43.03	1.10E-03
Monocarboxylic acid transporter activity	40.64	1.23E-03
Cation transmembrane transporter activity	8.48	1.23E-03

PANTHER GO Molecular Function	Fold Enrichment	P-value
NADH dehydrogenase (quinone) activity	49.72	4.46E-14
Oxidoreductase activity	38.12	4.73E-13
Catalytic activity	1.95	8.07E-08
Electron transfer activity	14.17	9.38E-07
Cytochrome-c oxidase activity	33.88	9.67E-06
Heme-copper terminal oxidase activity	33.88	9.67E-06
Proton transmembrane transporter activity	10.02	3.82E-05
Metal cluster binding	13.86	2.48E-04
Structural constituent of presynapse	> 100	2.78E-04

PANTHER GO Molecular Function	Fold Enrichment	P-value
Oxidoreductase activity	7.33	8.53E-12
NADH dehydrogenase activity	43.53	6.20E-10
Catalytic activity	1.95	2.33E-06
Electron transfer activity	15.52	3.26E-06
Cofactor binding	5.63	1.19E-05
Glutamate:sodium symporter activity	> 100	2.38E-04
Pyruvate dehydrogenase activity	97.42	3.17E-04
Coenzyme binding	6.01	5.38E-04
Glutamate receptor binding	18.27	7.04E-04

Late stage increase during ageing

PANTHER GO Molecular Function	Fold Enrichment	P-value
Cadherin binding	21.51	5.29E-11
Cell adhesion molecule binding	14.10	2.71E-09
Phospholipase A2 inhibitor activity	> 100	4.22E-05
S100 protein binding	> 100	1.05E-04
Calcium ion binding	5.31	1.30E-04
Calcium-dependent phospholipid binding	31.90	1.44E-04
Phospholipase inhibitor activity	> 100	1.47E-04
Water channel activity	> 100	2.51E-04
Water transmembrane transporter activity	93.04	3.13E-04

PANTHER GO Molecular Function	Fold Enrichment	P-value
Catalytic activity	1.75	9.21E-05
Oxidoreductase activity	11.21	1.05E-04
Transferase activity	> 100	1.08E-04
ATPase regulator activity	34.96	1.26E-04
Cytoskeletal protein binding	3.63	4.71E-04
Ribose phosphate diphosphokinase activity	58.27	7.94E-04
Protein binding	1.69	1.23E-03
Peptidase activity	3.44	1.30E-03
ATPase activator activity	38.85	1.59E-03

PANTHER GO Molecular Function	Fold Enrichment	P-value
Phospholipase inhibitor activity	43.12	8.08E-05
S100 protein binding	34.50	1.43E-04
Lipase inhibitor activity	30.44	1.98E-04
Calcium-dependent protein binding	13.91	4.25E-05
Cadherin binding	8.79	7.86E-11
Actin binding	7.27	9.23E-11
Cell adhesion molecule binding	7.16	9.84E-12
Calcium ion binding	4.39	1.83E-07
Cytoskeletal protein binding	4.05	2.71E-08

PANTHER GO Molecular Function	Fold Enrichment	P-value
Structural constituent of ribosome	9.33	9.21E-10
RNA binding	2.77	2.80E-09
Identical protein binding	2.59	1.77E-08
Catalytic activity	1.68	2.31E-08
Cadherin binding	5.35	2.59E-07
Structural molecule activity	3.30	4.31E-07
Protein homodimerization activity	3.16	8.74E-07
Oxidoreductase activity	8.19	2.75E-06
Ion binding	1.49	2.81E-05

Late stage decrease during ageing

PANTHER GO Molecular Function	Fold Enrichment	P-value
Dynein intermediate chain binding	41.92	1.23E-03
Protein binding	1.93	3.23E-03
Guanine deaminase activity	> 100	4.32E-03
Prenylcysteine oxidase activity	> 100	6.48E-03
Calcium ion binding	4.11	7.33E-03
Glutaminase activity	> 100	8.63E-03
Ganglioside GT1b binding	> 100	8.63E-03
Sialic acid binding	> 100	8.63E-03
Oxidoreductase activity	> 100	8.63E-03

PANTHER GO Molecular Function	Fold Enrichment	P-value
Calmodulin-dependent protein kinase activity	42.54	6.83E-05
Rac GTPase binding	25.29	2.83E-04
Clathrin binding	20.80	4.87E-04
Rho GTPase binding	9.82	8.24E-04
Cytoskeletal protein binding	3.88	1.08E-03
Spectrin binding	36.70	1.66E-03
ATP-dependent microtubule motor activity	28.36	2.65E-03
Dynein intermediate chain binding	28.36	2.65E-03
Glutamate receptor activity	24.00	3.60E-03

PANTHER GO Molecular Function	Fold Enrichment	P-value
Protein binding	1.54	2.10E-12
Cytoskeletal protein binding	3.90	1.09E-07
ATP-dependent protein binding	65.21	2.91E-05
Structural constituent of ribosome	7.25	6.71E-05
Tubulin binding	4.74	1.46E-04
Glutamate receptor binding	14.49	2.23E-04
Glutaminase activity	> 100	3.21E-04
Chaperone binding	8.61	3.60E-04
Protein self-association	11.79	4.67E-04

PANTHER GO Molecular Function	Fold Enrichment	P-value
Cytoskeletal protein binding	5.37	9.97E-11
Structural constituent of synapse	57.18	1.54E-06
Protein binding	1.42	4.01E-06
Catalytic activity	1.76	1.41E-05
Actin filament binding	8.43	2.47E-05
Calmodulin binding	8.38	2.55E-05
Proline-rich region binding	34.31	1.33E-04
Clathrin binding	15.51	1.65E-04
Structural molecule activity	3.37	2.44E-04

Figure S3 (related to Figure 3): Enrichment analyses displaying the predominant molecular functions of identified Biolayout Express3D clusters. PANTHER GO Enrichment tables demonstrating the primary molecular functions of identified clusters termed “biomarkers of ageing” (A.) or “biomarkers of vulnerability” (B.) in synaptic isolates derived from both the NHP and human patient hippocampus and occipital cortex. Note the difference in enrichment terms between resistant (occipital cortex) and vulnerable (hippocampus) brain regions within species. Additionally, note the identification of similar enrichment terms in analogous brain regions independent of species.

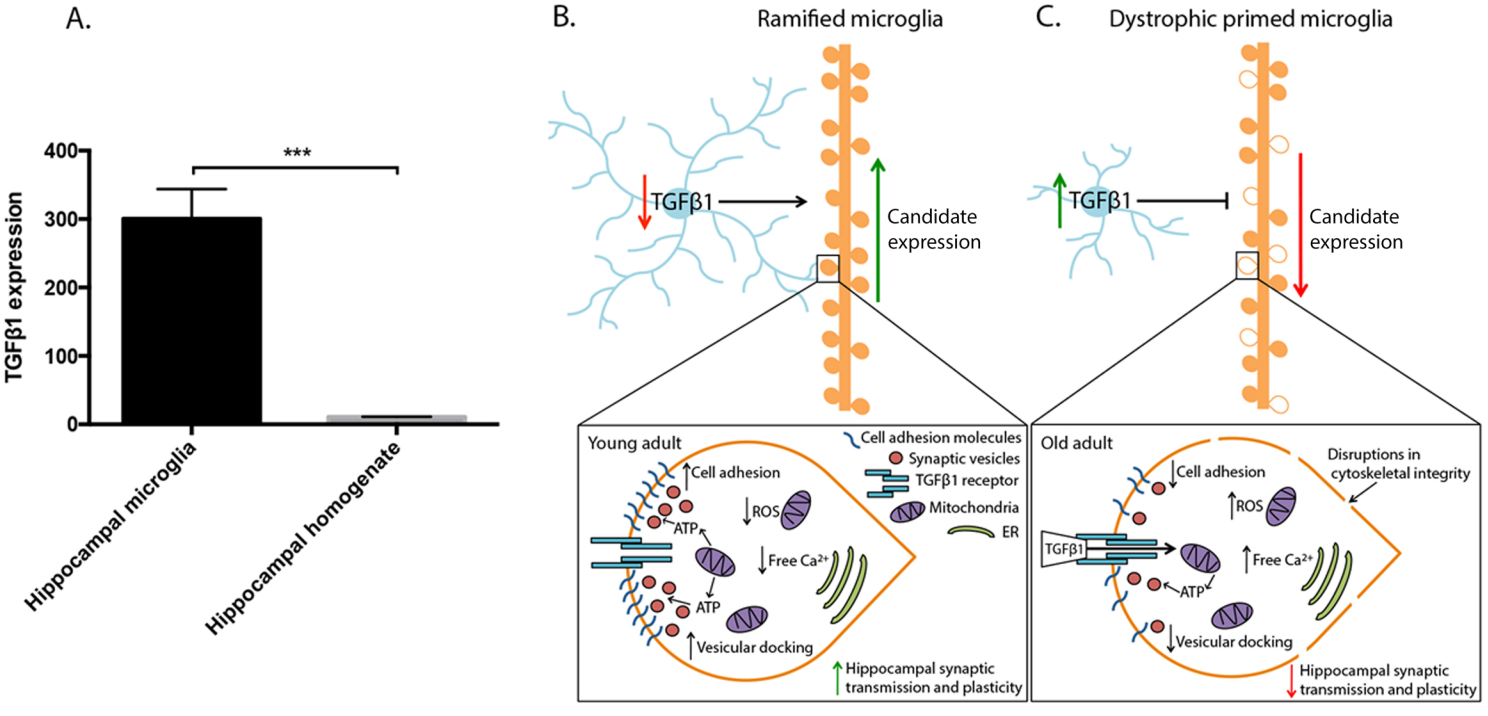


Figure S4 (related to Figure 4): TGFβ1 is expressed exclusively in microglial isolates. A. Bar chart displaying TGFβ1 transcript expression in isolated hippocampal microglia and hippocampal homogenate. The homogenate exhibits relatively little TGFβ1 expression versus the isolated microglia (unpaired two-tailed student's t-tests, p=0.0009; data represent mean SEM). B&C. Summary of synaptic-microglial interactions and regulation of the cellular milieu during ageing. Young adult microglia (B.) demonstrate ramified morphology and inhibition of the TGFβ1 signalling cascade, promoting homeostatic regulation of the subcellular machinery within the synaptic compartment. Intracellular processes are illustrated in the black box. Conversely, resident microglia in an aged brain display a dystrophic, primed phenotype (C.). Activation of the TGFβ1 signalling cascade, by binding of TGFβ1 to the TGFβ1 synaptic receptor, promotes injurious events within the synaptic terminal facilitating synapse instability. Orange colour represents dendritic arbour with synaptic terminals; pale blue indicates microglia; white synaptic terminals symbolises dysfunction. See key in black box for intracellular information. YH – young hippocampus; MH – mid-age hippocampus; OH – old hippocampus; YO – young occipital; MO – mid-age occipital; OO – old age occipital.