

Supplementary Materials

Supplementary Table 1. Enriched biological processes in the young mouse brain insoluble proteome.

Analysis was carried out using the Database for Annotation, Visualization and Integrated Discovery (DAVID)

v6.8. EASE score p value: modified Fisher exact p value ($p \leq 0.05$).

| Term | P-Value | Fold Enrichment |
|---|-----------------|------------------------|
| Neuron projection development | 9.20E-03 | 19.5 |
| Calcium-mediated signaling | 1.80E-02 | 106.4 |
| Protein targeting to mitochondrion | 2.30E-02 | 82.2 |
| Mitochondrion morphogenesis | 3.30E-02 | 56.5 |
| Nervous system development | 5.90E-02 | 7.2 |

Supplementary Table 2; List of all insoluble proteins in the aging proteome

| Accession | Description |
|------------------|--|
| Q11011 | Puromycin-sensitive aminopeptidase |
| Q3TZ32 | Putative uncharacterized protein |
| Q8R0Y6 | Aldehyde dehydrogenase family 1 member L1 |
| Q8C0M9 | L-asparaginase |
| E9Q6Q4 | Uncharacterized protein |
| Q9R0P5 | Dextrin |
| O08709 | Peroxiredoxin-6 |
| Q3TED3 | Putative uncharacterized protein |
| Q3TP62 | Pleckstrin homology domain containing, family B (Evectins) member 1, isoform CRA_b |
| Q9R0P9 | Ubiquitin carboxyl-terminal hydrolase isozyme L1 |
| Q8BL66 | Early endosome antigen 1 |
| A3KME3 | Kbtbd11 protein |
| A2AGT5 | Cytoskeleton-associated protein 5 |
| P16054 | Protein kinase C epsilon type |
| Q05DT0 | Add3 protein (Fragment) |
| O55023 | Inositol monophosphatase 1 |
| Q9ES97 | Reticulon-3 OS=Mus musculus GN=Rtn3 PE=1 SV=2 - [RTN3_MOUSE] |
| Q9WV34 | MAGUK p55 subfamily member 2 |
| Q3TEU8 | Putative uncharacterized protein |
| Q3TPI3 | Putative uncharacterized protein (Fragment) |
| Q5BL10 | Kinesin family member 5B |
| A2A7X3 | Ubiquitin-conjugating enzyme |
| Q6ZQ38 | Cullin-associated NEDD8-dissociated protein 1 |
| Q6P5F9 | Exportin-1 |
| Q8K1M6 | Dynamin-1-like protein |
| P10637 | Microtubule-associated protein tau |
| Q9CS42 | Ribose-phosphate pyrophosphokinase 2 |
| Q91V12 | Cytosolic acyl coenzyme A thioester hydrolase |
| Q80TL0 | Protein phosphatase 1E |
| Q3TE06 | Putative uncharacterized protein (Fragment) |
| B1AQW3 | Microtubule-associated protein |
| P28738 | Kinesin heavy chain isoform 5C |
| Q8R3V5 | Endophilin-B2 |
| Q4KMM3 | Oxidation resistance protein 1 |
| E9PUD2 | Uncharacterized protein |
| Q5EBJ4 | Ermin |
| Q99P72 | Reticulon-4 |
| Q76MZ3 | Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform |
| Q9Z0E0 | Neurochondrin |
| Q60829 | Protein phosphatase 1 regulatory subunit 1B |

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|--------|---|
| Q8C437 | PEX5-related protein |
| P51410 | 60S ribosomal protein L9 |
| E9QM49 | Uncharacterized protein |
| Q8BGT8 | Phytanoyl-CoA hydroxylase-interacting protein-like |
| Q547J4 | Microtubule-associated protein |
| Q9R0Y5 | Adenylate kinase isoenzyme 1 |
| E9QP92 | Uncharacterized protein |
| Q9CZC8 | Secernin-1 |
| Q8K0S0 | Phytanoyl-CoA hydroxylase-interacting protein |
| Q61553 | Fascin |
| O70161 | Phosphatidylinositol-4-phosphate 5-kinase type-1 gamma |
| Q3TJ97 | Putative uncharacterized protein |
| Q3UZJ4 | Putative uncharacterized protein |
| Q52KE3 | Gna1 protein (Fragment) |
| Q6NZL0 | Uncharacterized protein C6orf174 homolog |
| E9Q2A6 | Uncharacterized protein |
| Q5SQB0 | Nucleophosmin 1 |
| Q9CYT6 | Adenylyl cyclase-associated protein |
| Q00PI9 | Heterogeneous nuclear ribonucleoprotein U-like protein 2 |
| Q3TT18 | Putative uncharacterized protein |
| P14106 | Complement C1q subcomponent subunit B |
| Q7TQD2 | Tubulin polymerization-promoting protein |
| P61922 | 4-aminobutyrate aminotransferase, mitochondrial |
| Q99PU5 | Long-chain-fatty-acid--CoA ligase ACSBG1 |
| P58252 | Elongation factor 2 |
| E9Q2W9 | Uncharacterized protein |
| Q3UF82 | Putative uncharacterized protein |
| Q02053 | Ubiquitin-like modifier-activating enzyme 1 |
| Q3U8N1 | Putative uncharacterized protein |
| Q60668 | Heterogeneous nuclear ribonucleoprotein D0 |
| Q99LC5 | Electron transfer flavoprotein subunit alpha, mitochondrial |
| Q8K0T0 | Reticulon-1 |
| Q3TFD9 | Putative uncharacterized protein |
| Q3TI27 | Ribose-phosphate pyrophosphokinase |
| P70296 | Phosphatidylethanolamine-binding protein 1 |
| E9PZV5 | Uncharacterized protein |
| E9QAC7 | Uncharacterized protein |
| P03995 | Glial fibrillary acidic protein |
| A2AN08 | E3 ubiquitin-protein ligase UBR4 |
| Q9D6R2 | Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial |
| Q9D394 | Protein RUFY3 |
| Q80SW1 | Putative adenosylhomocysteinase 2 |
| E9PZ90 | Uncharacterized protein |

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|--------|---|
| P09528 | Ferritin heavy chain |
| Q9Z140 | Copine-6 |
| P47738 | Aldehyde dehydrogenase, mitochondrial |
| O35098 | Dihydropyrimidinase-related protein 4 |
| E9Q7H5 | Uncharacterized protein |
| P19096 | Fatty acid synthase |
| Q9QYG0 | Protein NDRG2 |
| P62196 | 26S protease regulatory subunit 8 |
| Q8C522 | Endonuclease domain-containing 1 protein |
| Q8C5R8 | Ribose-phosphate pyrophosphokinase |
| P61161 | Actin-related protein 2 |
| Q99JY0 | Trifunctional enzyme subunit beta, mitochondrial |
| P57780 | Alpha-actinin-4 |
| Q5FW75 | Actinin alpha 2 |
| Q7M6W1 | RTN1-C |
| Q9Z0H8 | CAP-Gly domain-containing linker protein 2 |
| E9PUE7 | Uncharacterized protein |
| E9QK48 | Uncharacterized protein |
| P80318 | T-complex protein 1 subunit gamma |
| Q8K232 | Adducin 1 (Alpha) |
| Q99PT1 | Rho GDP-dissociation inhibitor 1 |
| P63005 | Platelet-activating factor acetylhydrolase IB subunit alpha |
| Q3TGW0 | Putative uncharacterized protein |
| Q8BH66 | Atlastin-1 |
| Q3UYN2 | Putative uncharacterized protein |
| P00920 | Carbonic anhydrase 2 |
| Q3TI61 | Putative uncharacterized protein |
| Q9JME5 | AP-3 complex subunit beta-2 |
| Q7TT10 | Adaptor-related protein complex 2, sigma 1 subunit |
| Q9QZE5 | Coatomer subunit gamma |
| Q3U0C4 | Putative uncharacterized protein |
| E9Q105 | Uncharacterized protein |
| E9Q3M3 | Uncharacterized protein |
| Q9QUM9 | Proteasome subunit alpha type-6 |
| Q3TED2 | Programmed cell death 6 interacting protein |
| P62204 | Calmodulin |
| P27546 | Microtubule-associated protein 4 |
| P62748 | Hippocalcin-like protein 1 |
| A2ALS5 | Rap1 GTPase-activating protein |
| Q61699 | Heat shock protein 105 kDa |
| Q9QYB8 | Beta-adducin |
| E9QM56 | Uncharacterized protein |
| Q5SUT0 | Ewing sarcoma breakpoint region 1 |

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|--------|---|
| D3Z0M7 | Dynein cytoplasmic 1 intermediate chain 1, isoform CRA_a |
| Q8BH80 | Vesicle-associated membrane protein, associated protein B and C |
| Q9R0Q6 | Actin-related protein 2/3 complex subunit 1A |
| P80313 | T-complex protein 1 subunit eta |
| Q3TFE8 | Putative uncharacterized protein |
| A1BN54 | Alpha actinin 1a |
| O35954 | Membrane-associated phosphatidylinositol transfer protein 1 |
| Q3UCX7 | Putative uncharacterized protein |
| P62830 | 60S ribosomal protein L23 |
| P35278 | Ras-related protein Rab-5C |
| Q9EQF6 | Dihydropyrimidinase-related protein 5 |
| P35700 | Peroxiredoxin-1 |
| Q7TPR4 | Alpha-actinin-1 |
| P62137 | Serine/threonine-protein phosphatase PP1-alpha catalytic subunit |
| P28663 | Beta-soluble NSF attachment protein |
| Q9EPN1 | Neurobeachin |
| Q3TTF6 | Putative uncharacterized protein |
| P70333 | Heterogeneous nuclear ribonucleoprotein H2 |
| P49615 | Cyclin-dependent kinase 5 |
| Q8R2D4 | MCG140223] |
| Q3UNH4 | G protein-regulated inducer of neurite outgrowth 1 |
| E9Q7S0 | Uncharacterized protein |
| Q3U781 | MCG21131, isoform CRA_a |
| Q9D7X3 | Dual specificity protein phosphatase 3 |
| A2AD25 | MCG49690 |
| O70435 | Proteasome subunit alpha type-3 |
| Q9D6C8 | Putative uncharacterized protein |
| Q3THL2 | Putative uncharacterized protein |
| E9QKZ6 | Uncharacterized protein |
| Q9CYH2 | UPF0765 protein C10orf58 homolog |
| Q5SXY1 | Cytospin-B |
| A2AKX3 | Probable helicase senataxin |
| Q9JJI8 | 60S ribosomal protein L38 |
| Q9CXS4 | Centromere protein V |
| E9QNW9 | Uncharacterized protein |
| E9QMF3 | Uncharacterized protein |
| D4N6R6 | Beta-globin |
| A2AMH3 | Solute carrier family 44, member 1 |
| Q9CX86 | Putative uncharacterized protein |
| P63330 | Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform |
| Q9DBJ1 | Phosphoglycerate mutase 1 |
| Q3TKM5 | Isocitrate dehydrogenase 3 (NAD+), gamma, isoform CRA_c |
| Q62348 | Translin |

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|--------|---|
| P40240 | CD9 antigen |
| E9QA32 | Uncharacterized protein |
| Q8CGA0 | Protein phosphatase 1F |
| D3Z7I9 | Oxysterol-binding protein |
| Q8C550 | Putative uncharacterized protein |
| Q3UNZ8 | Quinone oxidoreductase-like protein 2 |
| Q9R1R9 | UBE-1a |
| Q8VCW8 | Acyl-CoA synthetase family member 2, mitochondrial |
| Q32MV2 | Keratin 82 |
| B2RWW1 | Microtubule-associated protein 1S |
| B1AXF2 | Solute carrier family 24 (Sodium/potassium/calcium exchanger), member 2 |
| O54984 | ATPase Asna1 |
| E9QNK4 | Uncharacterized protein |
| Q80XK8 | Nbas protein (Fragment) |
| B1B0C7 | Perlecan (Heparan sulfate proteoglycan 2) |
| A2BI13 | PC4 and SFRS1 interacting protein 1 (Fragment) |
| Q9CU60 | Putative uncharacterized protein (Fragment) |
| Q99LD4 | COP9 signalosome complex subunit 1 |
| Q8CE29 | Ubiquitin protein ligase E3A |
| Q9D1P4 | Cysteine and histidine-rich domain-containing protein 1 |
| Q9CVY8 | Putative uncharacterized protein |
| B2KFD4 | Aspartoacylase (Aminoacylase) 2 |
| E9Q197 | Uncharacterized protein |
| O88543 | COP9 signalosome complex subunit 3 |
| E9PX61 | Uncharacterized protein |
| Q8CCS7 | Calbindin 2, isoform CRA_a |
| Q3TA40 | Putative uncharacterized protein |
| E9Q460 | Uncharacterized protein |
| Q99K51 | Plastin-3 |
| Q9DAS9 | Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12 |
| B8JJ84 | Syntaxin binding protein 6 (Amisyn) (Fragment) |
| Q5M8N4 | Epimerase family protein SDR39U1 |
| E9Q5Z3 | Uncharacterized protein |
| Q99J09 | Methylosome protein 50 |
| Q05BD4 | Prpsap2 protein |
| Q9CRY7 | Glycerophosphodiester phosphodiesterase domain-containing protein 1 |
| Q9CRE9 | Putative uncharacterized protein (Fragment) |
| O35449 | Proline-rich transmembrane protein 1 |
| Q61081 | Hsp90 co-chaperone Cdc37 |
| Q3TDM2 | Putative uncharacterized protein |
| E9PVZ5 | Uncharacterized protein |
| P97390 | Vacuolar protein sorting-associated protein 45 |
| P46664 | Adenylosuccinate synthetase isozyme 2 |

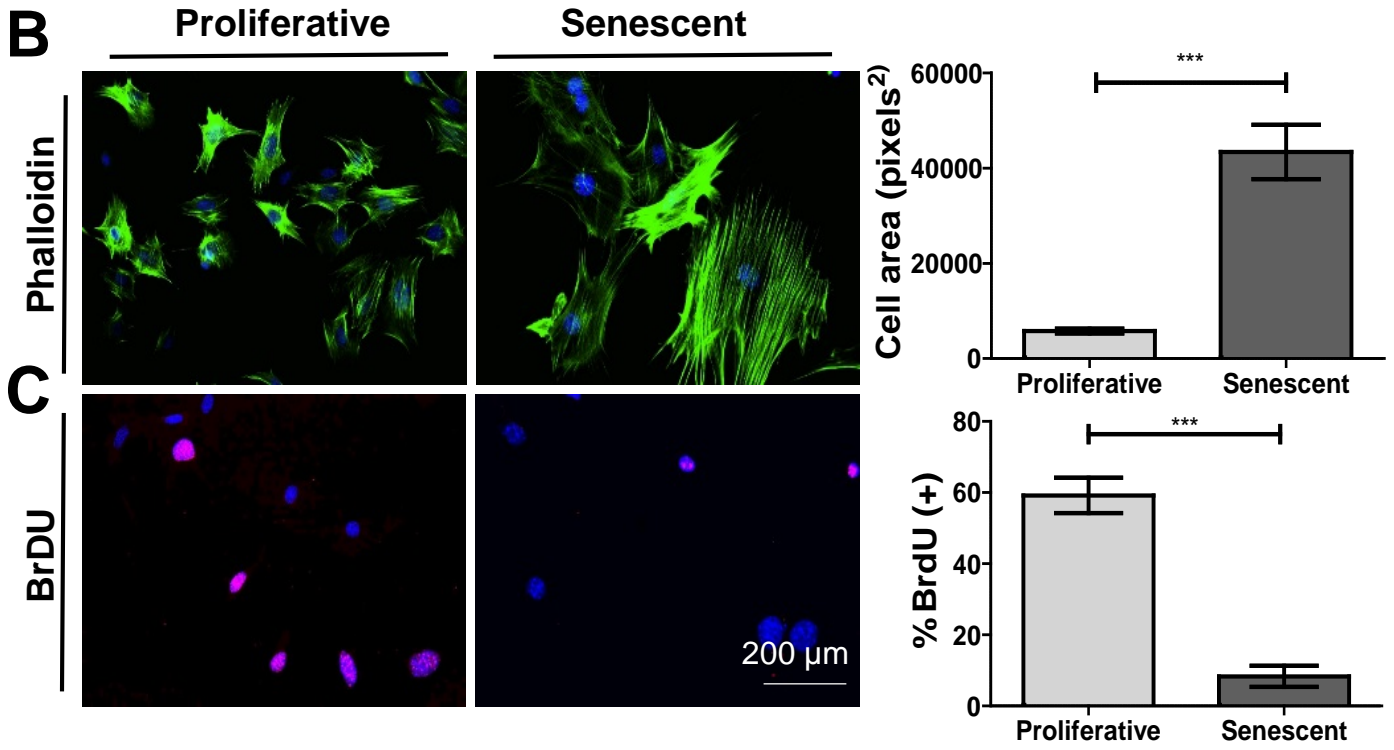
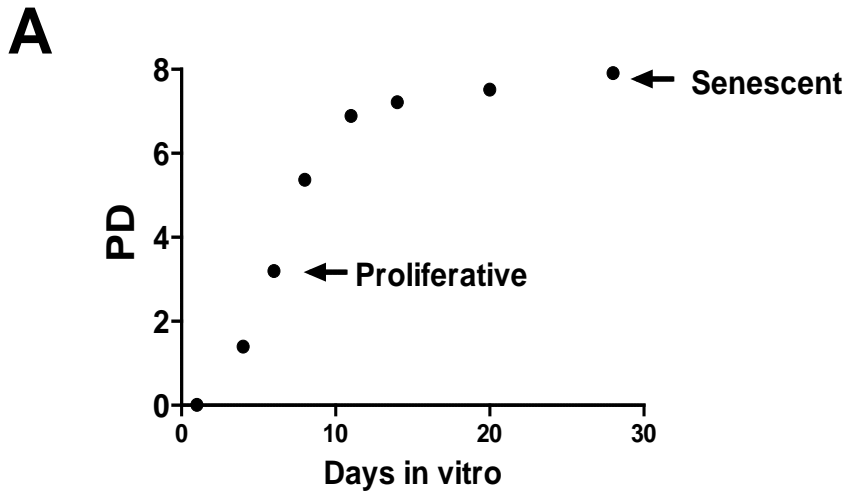
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|--------|--|
| E9QPD4 | Uncharacterized protein |
| B2KFH8 | Parkinson disease (Autosomal recessive, early onset) 7 |
| O09172 | Glutamate--cysteine ligase regulatory subunit |
| Q3THK7 | GMP synthase [glutamine-hydrolyzing] |
| Q99KC8 | von Willebrand factor A domain-containing protein 5A |
| Q3V1M8 | Putative uncharacterized protein |
| D3YWK1 | Uncharacterized protein |
| Q99KJ8 | Dynactin subunit 2 |
| Q8BVG8 | Beta-arrestin-1 |
| Q60676 | Serine/threonine-protein phosphatase 5 |
| Q91ZP9 | N-terminal EF-hand calcium-binding protein 2 |
| Q3TEA8 | Heterochromatin protein 1-binding protein 3 |
| Q3TDN2 | FAS-associated factor 2 |
| P12658 | Calbindin |
| Q9D358 | Low molecular weight phosphotyrosine protein phosphatase |
| Q8K2U0 | Map2k4 protein |
| Q8BN05 | Putative uncharacterized protein (Fragment) |
| Q3TW74 | Putative uncharacterized protein |
| Q6DI95 | Transportin 3 |
| P19157 | Glutathione S-transferase P 1 |
| Q8BH95 | Enoyl-CoA hydratase, mitochondrial |
| E9PV04 | Uncharacterized protein |
| D3YYM6 | Uncharacterized protein |
| Q3U6U7 | Putative uncharacterized protein |
| Q80Y17 | Lethal(2) giant larvae protein homolog 1 |
| O55091 | Protein IMPACT |
| Q3UTI7 | Putative uncharacterized protein |
| Q8CAA7 | Glucose 1,6-bisphosphate synthase |
| Q91VB8 | Alpha globin 1] |
| B9EHZ5 | Membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6) |
| P84086 | Complexin-2 |
| Q8R4V2 | Dual specificity protein phosphatase 15 |
| Q8C7C4 | Putative uncharacterized protein (Fragment) |
| Q9DBP5 | UMP-CMP kinase |
| Q3UM23 | Putative uncharacterized protein |
| Q99K69 | RAS protein activator like 1 (GAP1 like) |
| Q8K1J7 | Acyl-CoA synthetase long-chain family member 3 |
| P48758 | Carbonyl reductase [NADPH] 1 |
| A3KGQ6 | Actin-related protein 2/3 complex subunit 5 |
| Q5UE59 | Kinesin light chain-1 |
| P15331 | Peripherin |
| P35235 | Tyrosine-protein phosphatase non-receptor type 11 |
| P28474 | Alcohol dehydrogenase class-3 |

Supplementary Figure 1. MEFs senescence characterization. **A)** MEFs population doubling (PD) curve, arrows indicate the samples used in this study; **B)** Phalloidin staining and cellular size quantification in proliferative and senescent MEFs. DAPI (blue), phalloidin (green), n= 30 cells; **C)** 5-Bromo-2'-deoxyuridine (BrdU) incorporation in proliferative and senescent MEFs. DAPI (blue), BrdU (red), n=100 cells. T test, ns $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Average \pm SEM. Scale bar 200 μm .

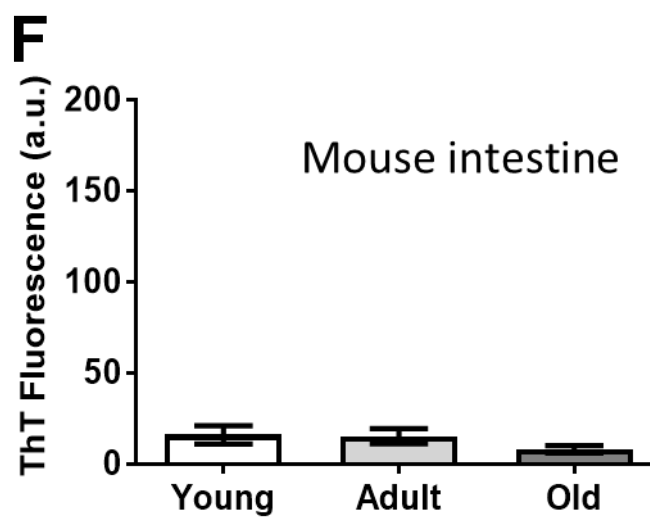
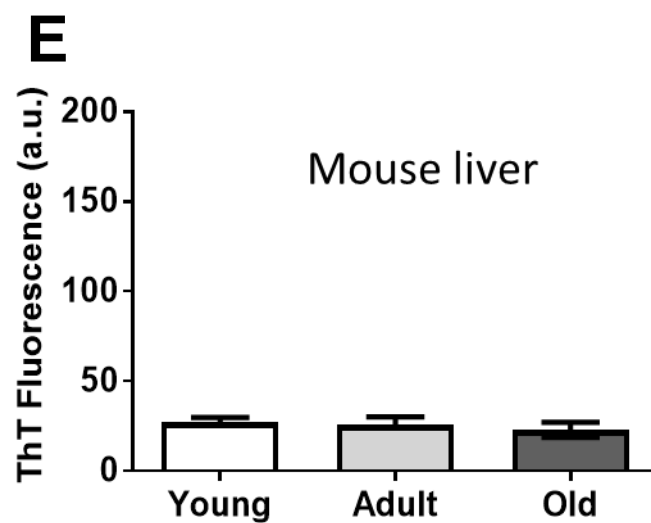
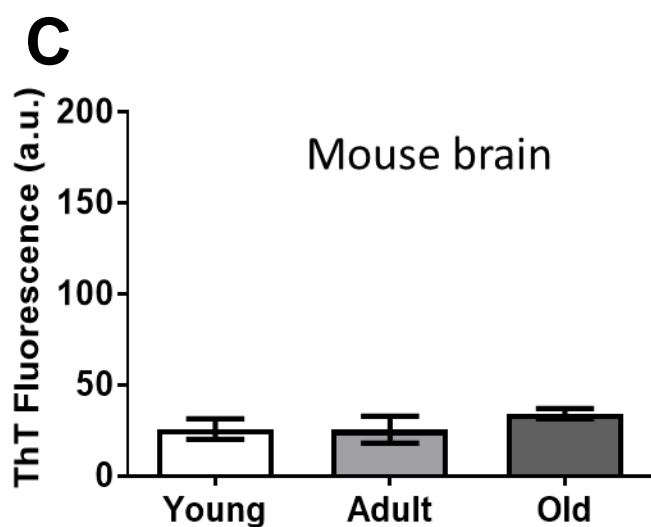
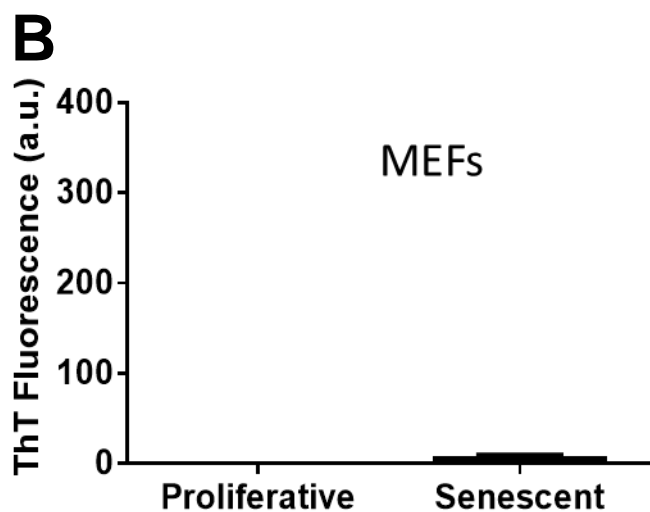
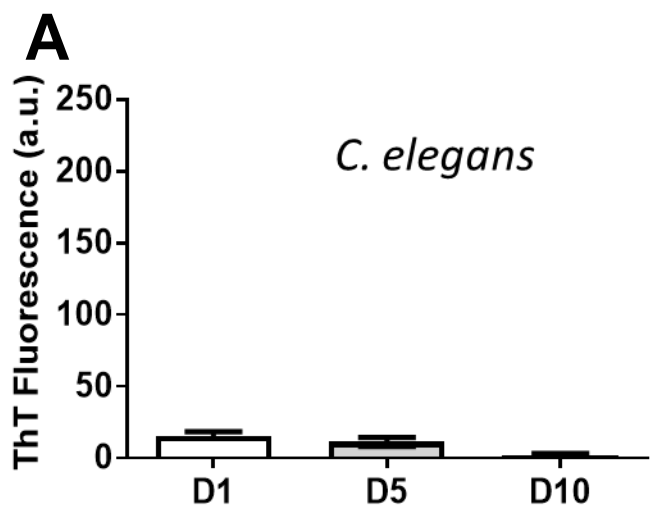
Supplementary Figure 2. Thioflavin T binding was eliminated by harsh proteolytic degradation. Samples of insoluble proteins were treated with a high concentration of PK (1mg/ml) for 1h at 37 °C. Thioflavin T units of fluorescence was measured as described in methods of: **A)** *C. elegans*, day 1 (D1), day 5 (D5) and day 10 (D10) of adult life, n=3; **B)** MEFs, proliferative (PD=3) and senescent (PD=8), n=3; **C)** Brain; **D)** Heart; **E)** Liver and **F)** Intestine of mouse, young (3 m.o., n=8), adult (12 m.o., n=8) and old (22 m.o., n=4). One way ANOVA, Tukey post-hoc test. Average \pm SEM.

Supplementary Figure 3. A) Confocal microscopy of histological sections of old mouse brain (22 m.o.), co-stained with Thioflavin S (green), aggresome dye (red) and thioflavine analog BTA-1 (blue); **B)** Co-localization of Thioflavin S, aggresome dye and BTA-1 in old (22 m.o.) mouse brain. Scale bar: 10 μm .

Supplementary Figure 4. Confocal microscopy of non-stained histological sections of young (3 m.o.), adult (12 m.o.) and old (22 m.o.) mouse brain. Scale bar: 10 μm



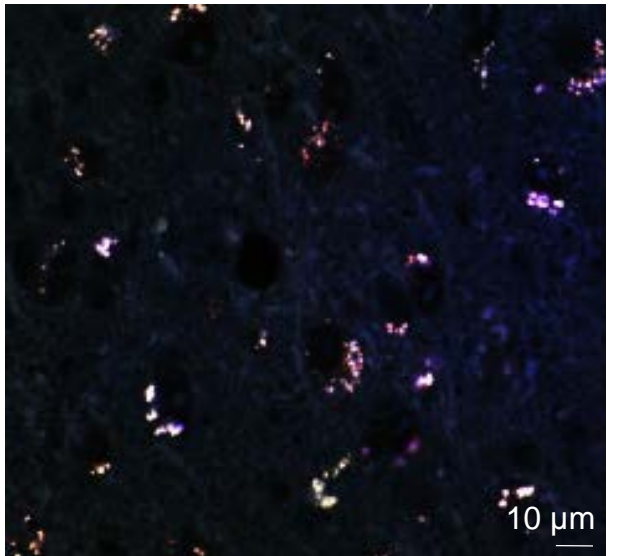
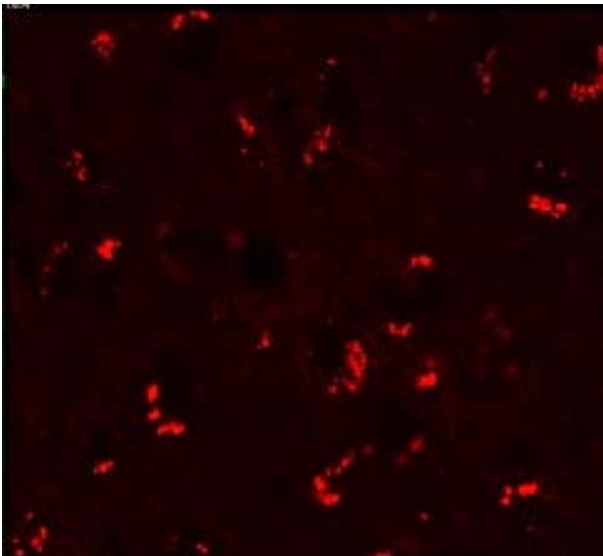
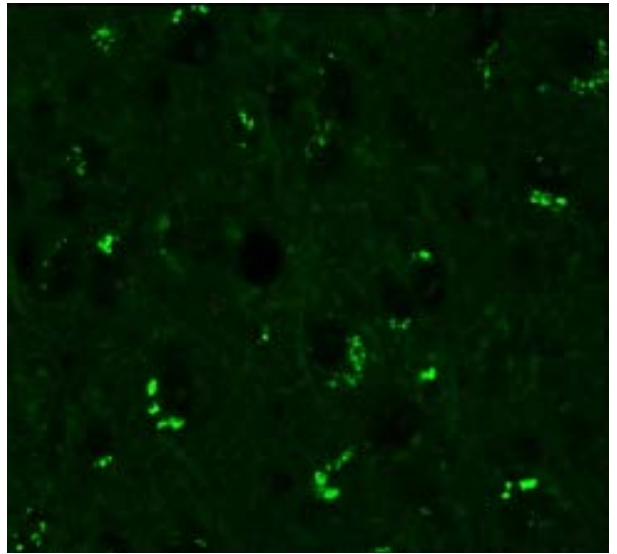
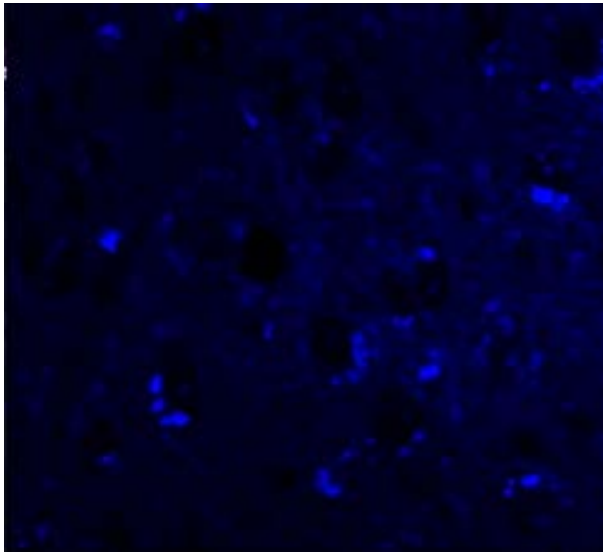
Supplementary Figure 1



Supplementary Figure 2

BTA

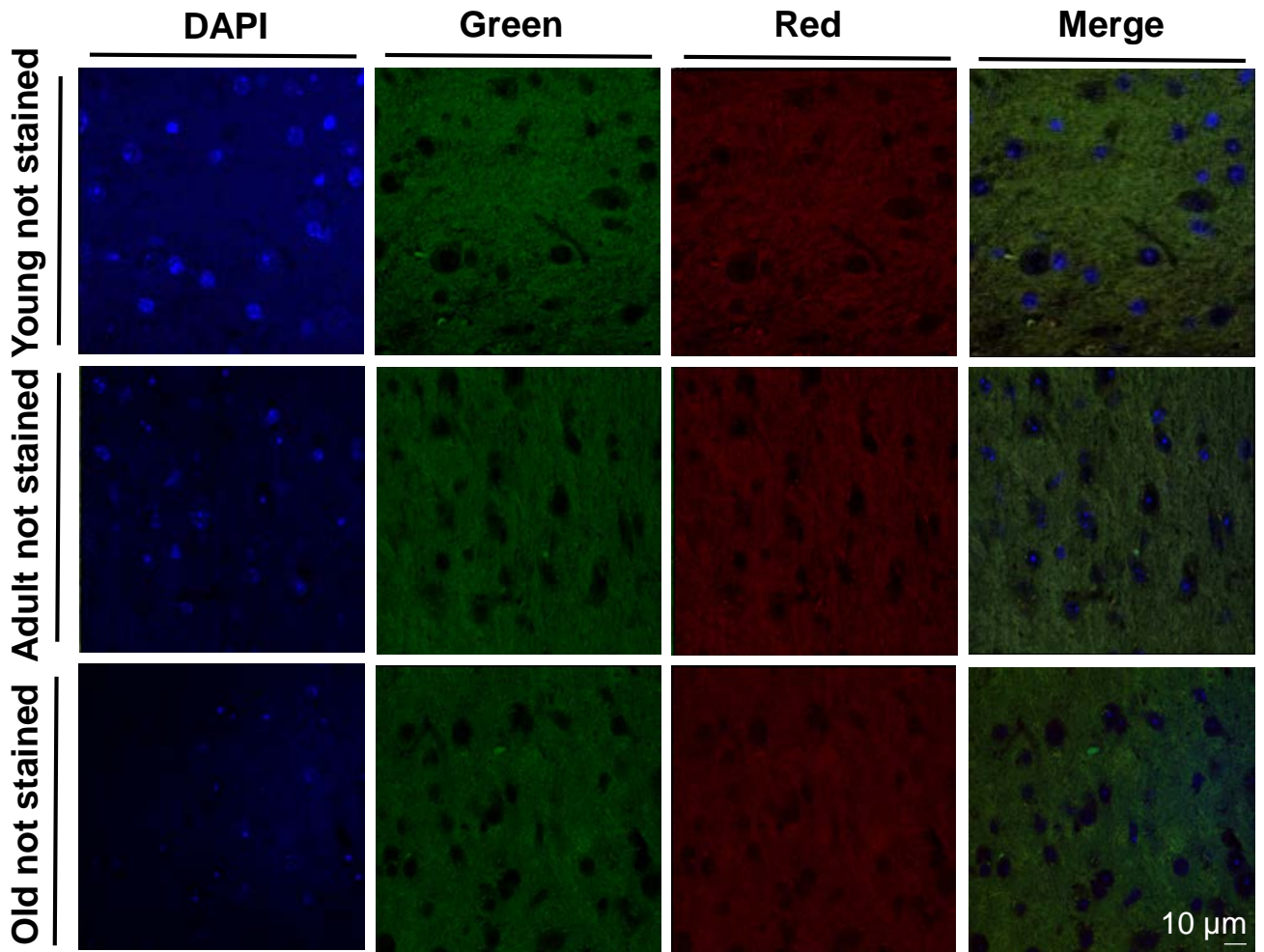
ThS



Aggresome

Merge

Supplementary Figure 3



Supplementary Figure 4