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SUPPLEMENTARY INFORMATION

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Senolytic therapy alleviates Aβ-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model

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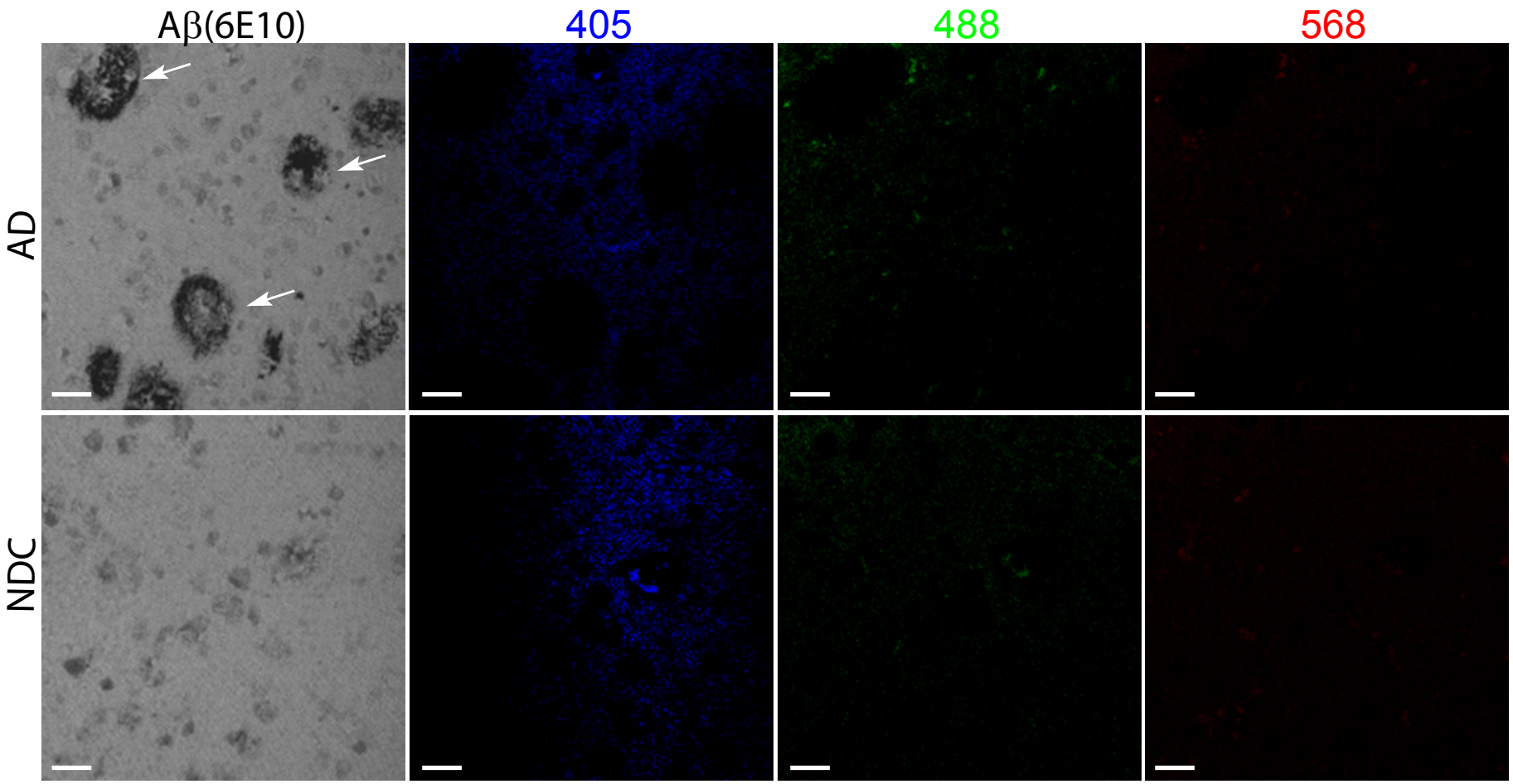
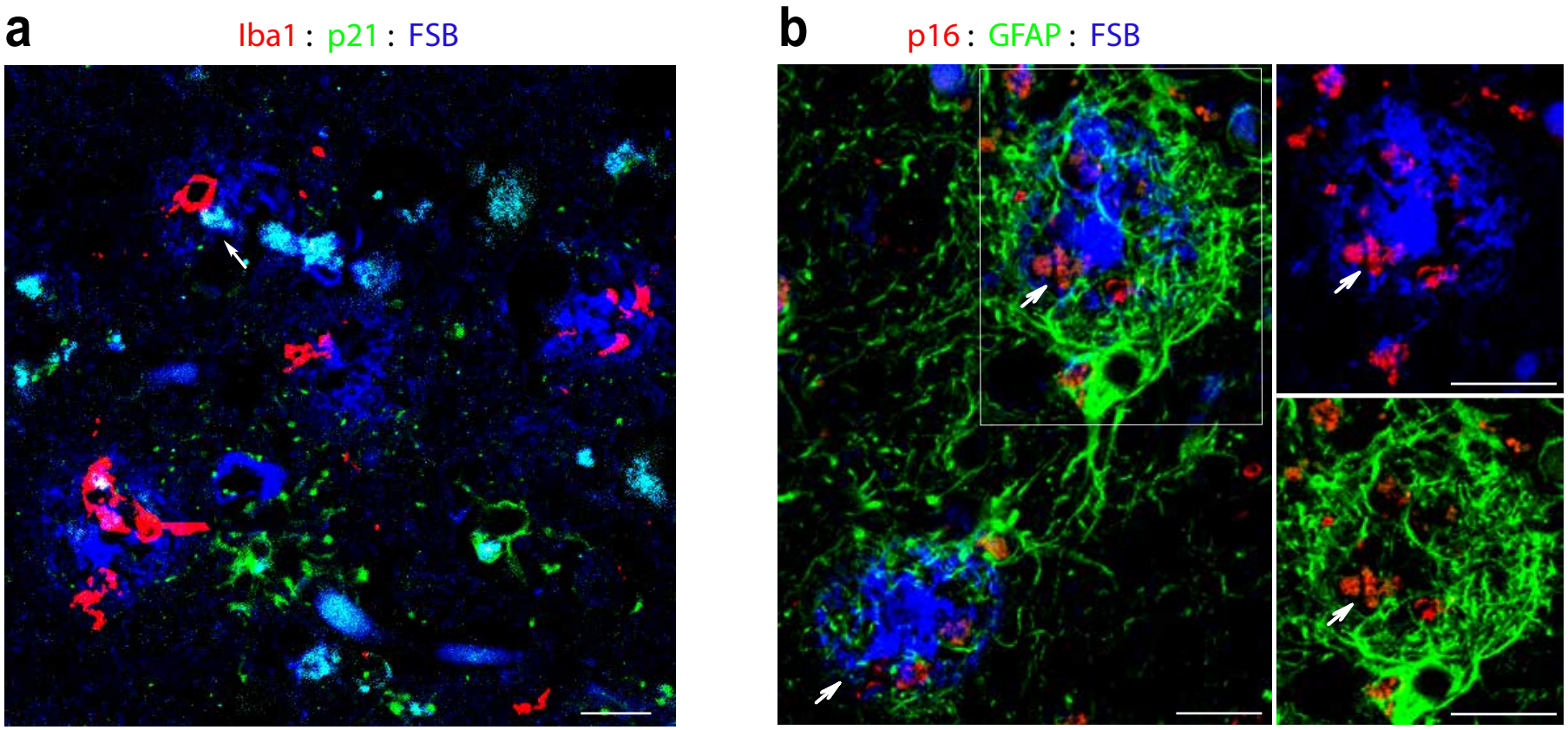
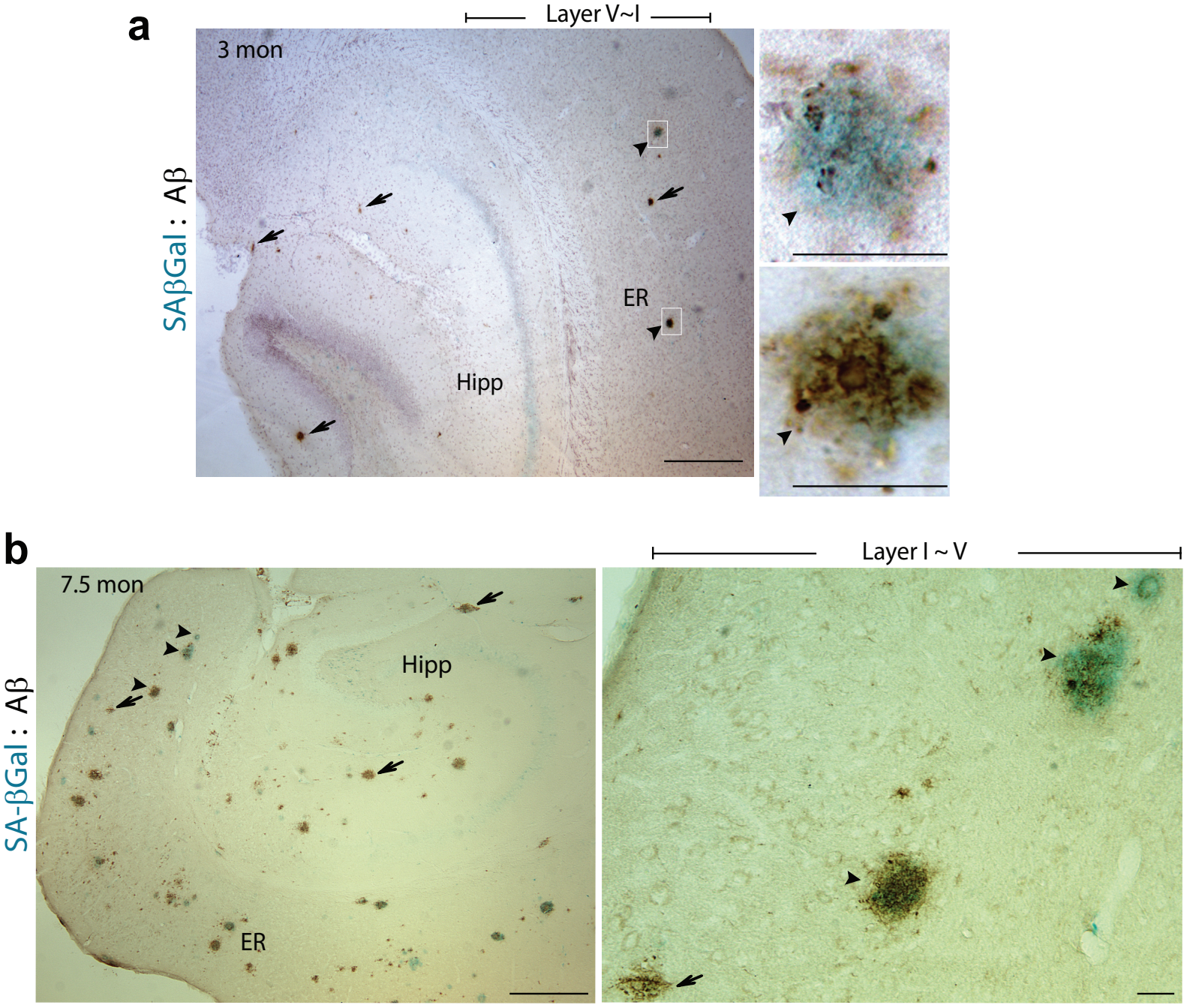


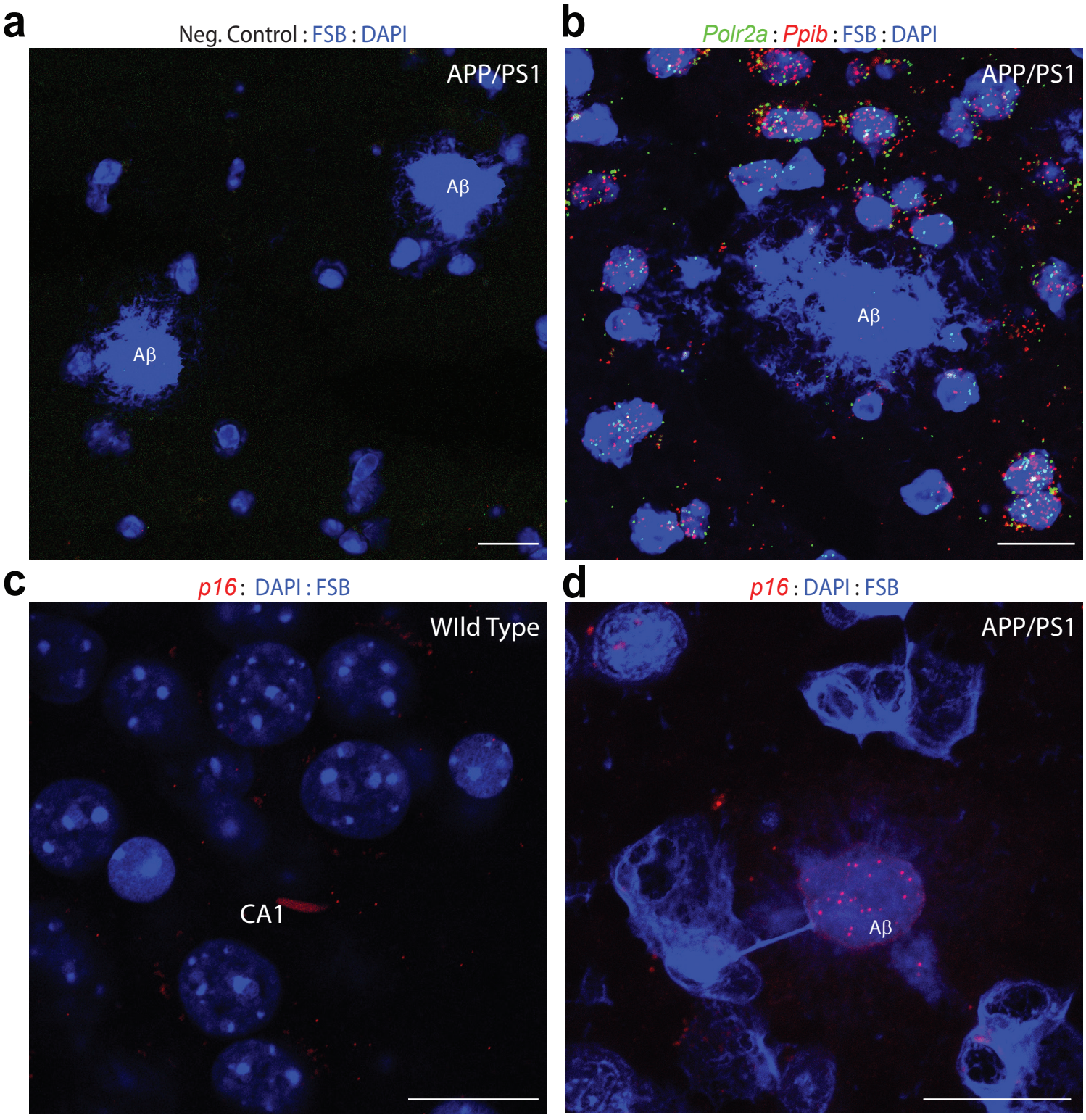
Supplementary Figure 1. Confocal images showing little or no autofluorescent signals present in a human AD brain as well as in a NDC control brain. Brain sections were immunostained with anti-Aβ monoclonal antibody 6E10 and visualized using 3,3' diaminobenzidine (DAB) staining. The images on the left show Aβ-immunoreactive plaques (arrows) visualized by differential interference contrast optics. The images on the right were acquired with excitation at the indicated wavelengths (405, 488 and 568 nm. The results represent 5 images per brain in each group. Scale bar = 20μm.



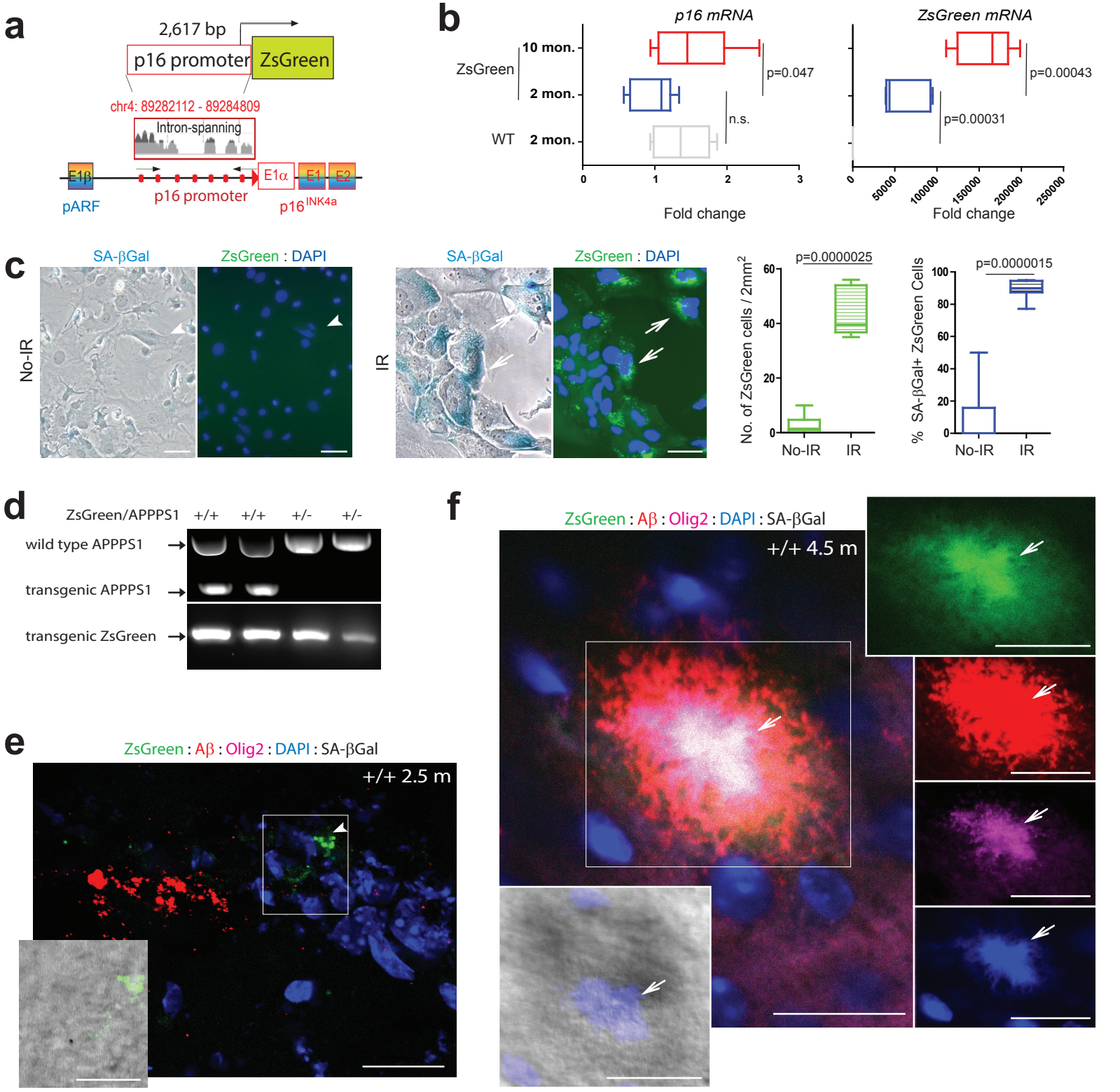
Supplementary Figure 2. Immunofluorescence images showing the locations of senescent cells, microglia and astrocytes in the Aβ plaque environment in the inferior parietal cortex of AD patients. **(a)** Confocal images showing fibrillar Aβ (blue; FSB staining), and immunostaining for the microglial marker Iba1 (red) and the cell senescence marker p21 (green). Microglia do not exhibit p21 immunoreactivity (arrows). **(b)** Confocal images showing fibrillar Aβ (blue; FSB staining), and immunostaining for the astrocyte marker GFAP (green) and the senescent cell marker p16 (red). Arrows point to GFAP-negative cells exhibiting a senescence phenotype (p16 immunoreactivity). Scale bar = 20 μm. The results in a and b represent more than 10 images from two human AD brains per group.



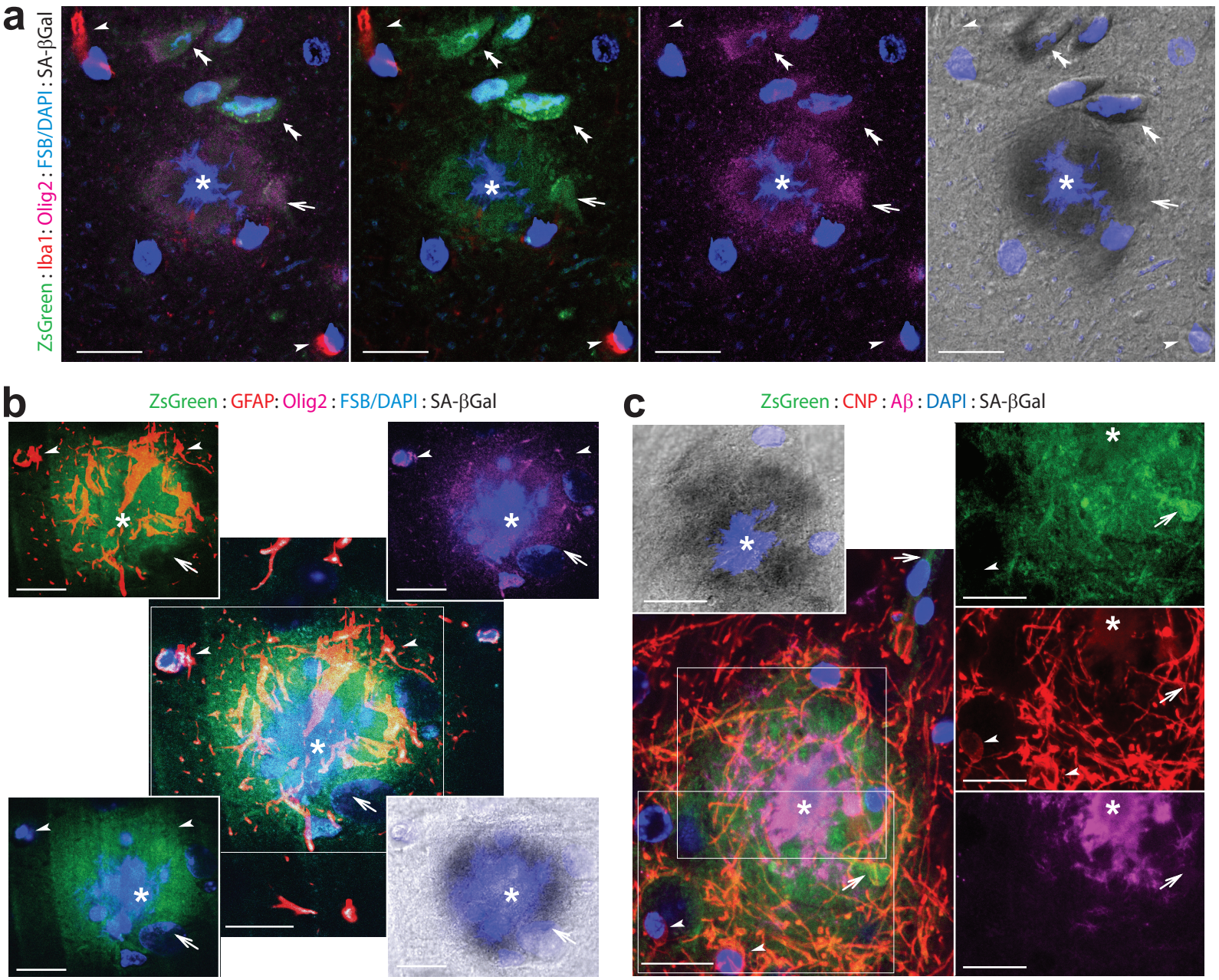
Supplementary Figure 3. Association of cellular senescence with Aβ plaques in the brains of APP/PS1 double mutant transgenic mice. Brain sections from a 3-month-old (a) and 7.5-month-old (b) APP/PS1 mutant transgenic mouse were stained for Aβ (brown) and SA-βGal (blue), with (a) and without (b) cresyl violet counterstaining. Arrows and arrowheads point to the variation in sizes of Aβ deposits between different ages and their uneven distributions over SA-βGal foci. Note the paucity of Aβ deposits in the 3-month-old mouse. Right panels are high magnifications of boxed areas in left panels. Hipp, hippocampus; EC, entorhinal cortex. Scale bar: left panels = 100μm ; right panels =20μm. Images represent three sections per mice, and three mice for each group. The statistic results are shown in Figure 4d.



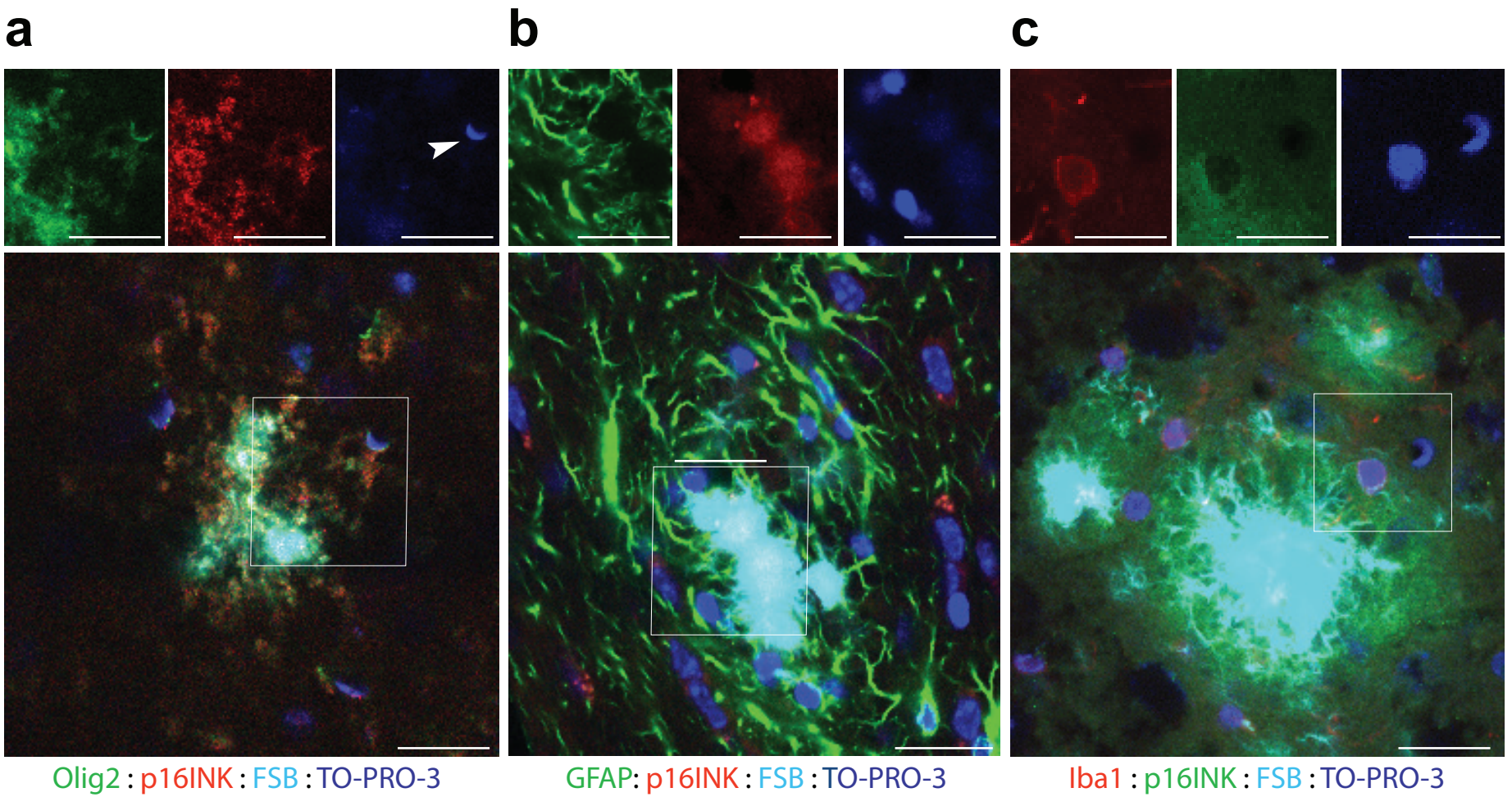
Supplementary Figure 4. RNAscope fluorescence in situ hybridization (ISH) using brain sections from 8-month-old APP/PS1 transgenic and wild type mice. **(a)** Confocal image showing no RNA signal was detected in the negative control using a probe` against bacterial gene *DapB*. The 10 µm section from an APP/PS1 mouse brain was counterstained with FSB and DAPI to label Aβ plaques and nuclei, respectively. **(b)** An adjacent section was hybridized with the RNAscope probes against the murine houseeeping gene` *Polr2a* (green) and *Ppib* (red) as positive controls. The RNA puncta` were readily detectable in the regions devoid of Aβ plaques. **(c)** A section of age-matched wild type brain was hybridized with the target-specific probe against *p16* transcript as a control. The image showing a sparse distribution of` *p16* RNA`near the nuclei of CA1 neurons. **(d)** Image showing numerous copies of *p16* transcripts concentrated in cells associated with a FSB-labeled Aβ plaque, but distributed sparsely in the regions devoid of Aβ plaques Scale bar = 20 µm in each panel. The results in a, b and c represent 5-8 images per group in two independent replicates per group. The statistic analysis and replica in d are shown in Figure 2e.



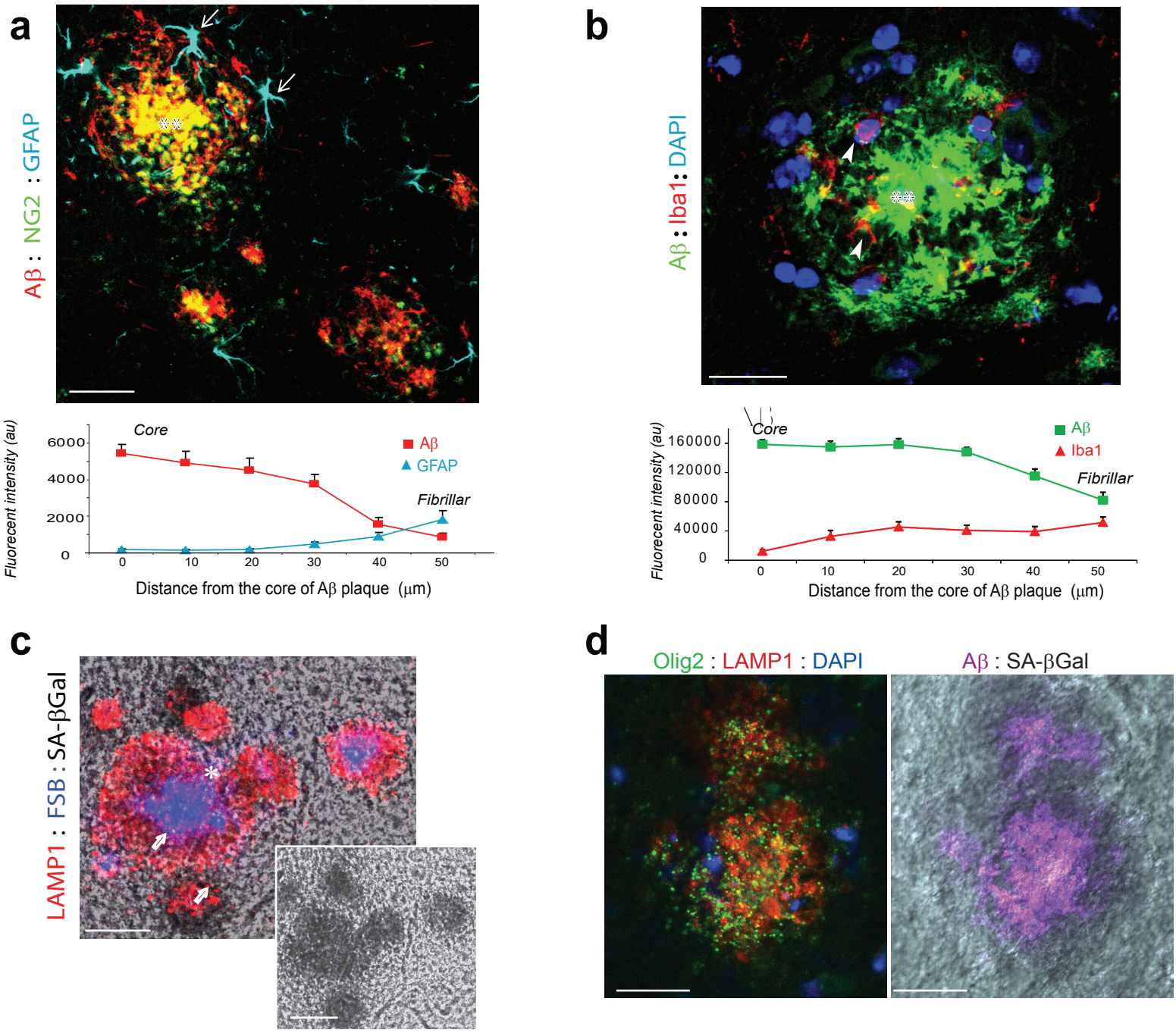
Supplementary Figure 5. Generating the ZsGreen senescence reporter mouse. **(a)** Schematics: a murine p16 promoter (see details in ref. 7) was cloned by PCR to drive the expression of the ZsGreen fluorescent protein. **(b)** The kidney from ZsGreen transgenic mice and wild type littermates at 2- and 10-month of ages were analyzed by RT-qPCR for the expression of *p16* and *ZsGreen* against *Gapdh*. n=3~4 mice per group. Two replicas per mice. **(c)** Four adult ZsGreen mouse brains were pooled and extracted for primary glial cultures for 3 d, and then subjected to 10 Gy irradiation (IR) and no-IR controls for 9 d. An arrowhead and arrows in the image panel point to the correlated expression of ZsGreen proteins with SA-βGal activities in glial cells upon IR exposure (right) and no-IR control (left). n=150~200 cells from 10 images in each group were analyzed. Scale bar = 10μm. The statistics in b~c: two tailed Student's t-test; the state of box plots are described in Method. **(d)** Genotyping ZsGreen/APPPS1 mutant and wild type mice. **(e)** Image showing that a cell harboring few ZsGreen puncta (arrowhead, SA-βGal negative) was apart from a newly generated small Aβ aggregate (red) in 2.5-month old ZsGreen/APPPS1 mouse brains. **(f)** Image showing the robust expression of ZsGreen in the SA-βGal+ and Olig2+ cell around a large-sized (>30 μm) Aβ plaques in 4.5-month-old ZsGreen/APPPS1 mouse brains. Arrows point to the colocalization of ZsGreen (green) with Aβ (red) and Olig2 (purple). Lower left insert showing the SA-βGal activity (black) in the ZsGreen cell. Scale bar = 20 μm. The results in d, e and f represent 8-20 images. n =3 mice per group.



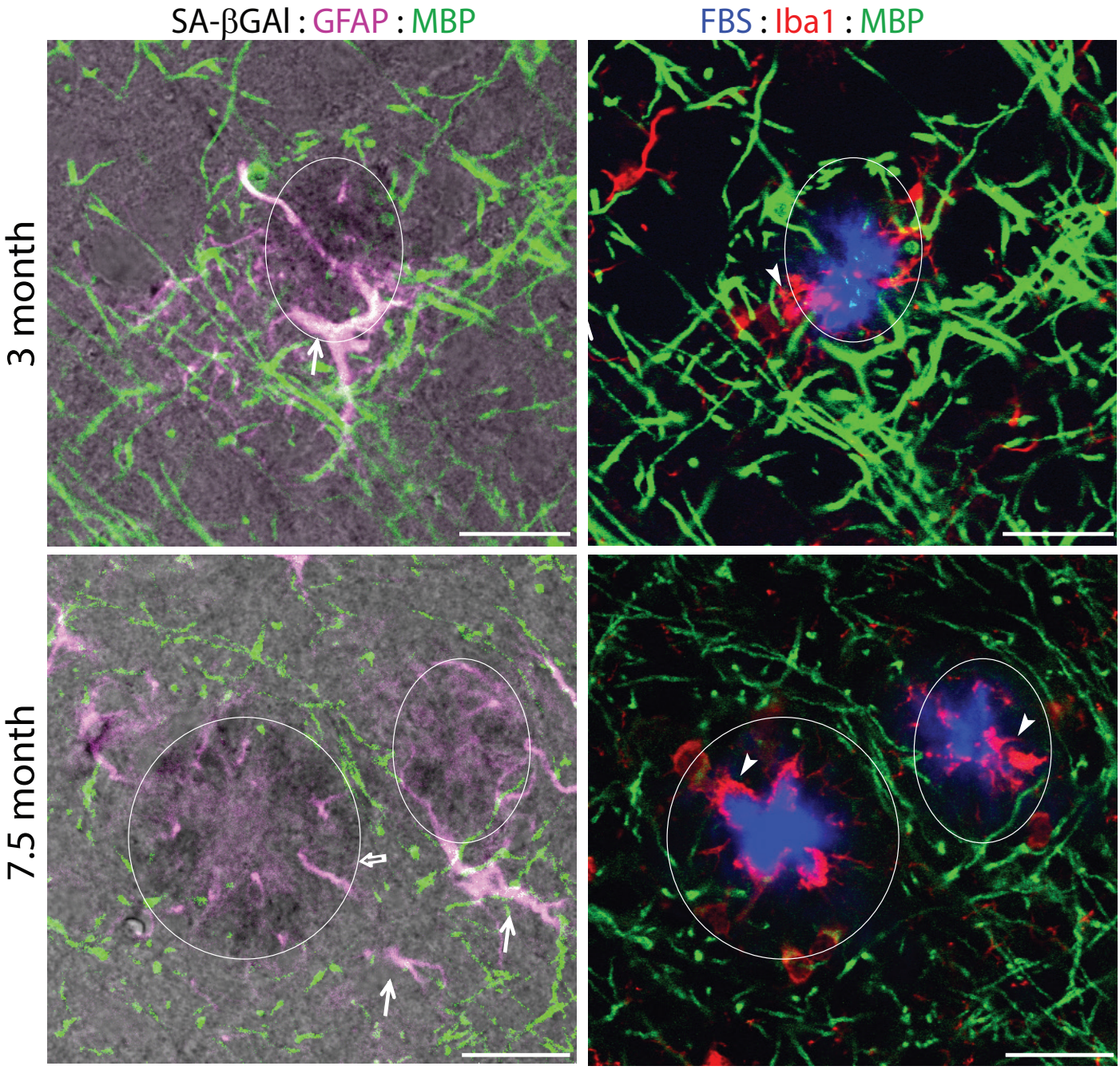
Supplementary Figure 6. Evidence that Aβ-associated ZsGreen senescent cells are OPCs, but not astrocytes or microglia in ZsGreen/APPPS1 AD mouse brains. **(a)** The representative image showing that the Aβ (blue FSB staining; asterisk)-associated ZsGreen cell exhibited Olig2 immunoreactivity (purple), but not Iba1 immunoreactivity (red). Arrowheads point to two Iba1+ cells around the Aβ plaque. A arrow point to a flattened Olig2+ / SA-βGal+ ZsGreen cell surrounding the Aβ plaque. Double arrows point to five of Olig2+ and SA-βGal+ ZsGreen cells with polarized soma were either next or merged with the Aβ plaque. **(b)** The representative image showing the GFAP immunoreactivity (red) were undetectable in the Aβ-associated ZsGreen cells. Arrowheads point to Aβ-associated GFAP+ astrocytes and their processes. **(c)** The representative image showing that the CNP immunoreactivity (red) of oligodendrocytes (OLs) were barely detectable in an Aβ (purple)-associated ZsGreen cell. Arrowheads point to CNP+ cells around the Aβ plaque. An insert on up-left is the top boxed area in the merged image. The insets on right are the bottom boxed area in the merged image. Scale bar in each panel = 20 μm. The images in a, b, and c represent 34, 32, 15 and 19 cells for each analysis. n=3 mouse brains.



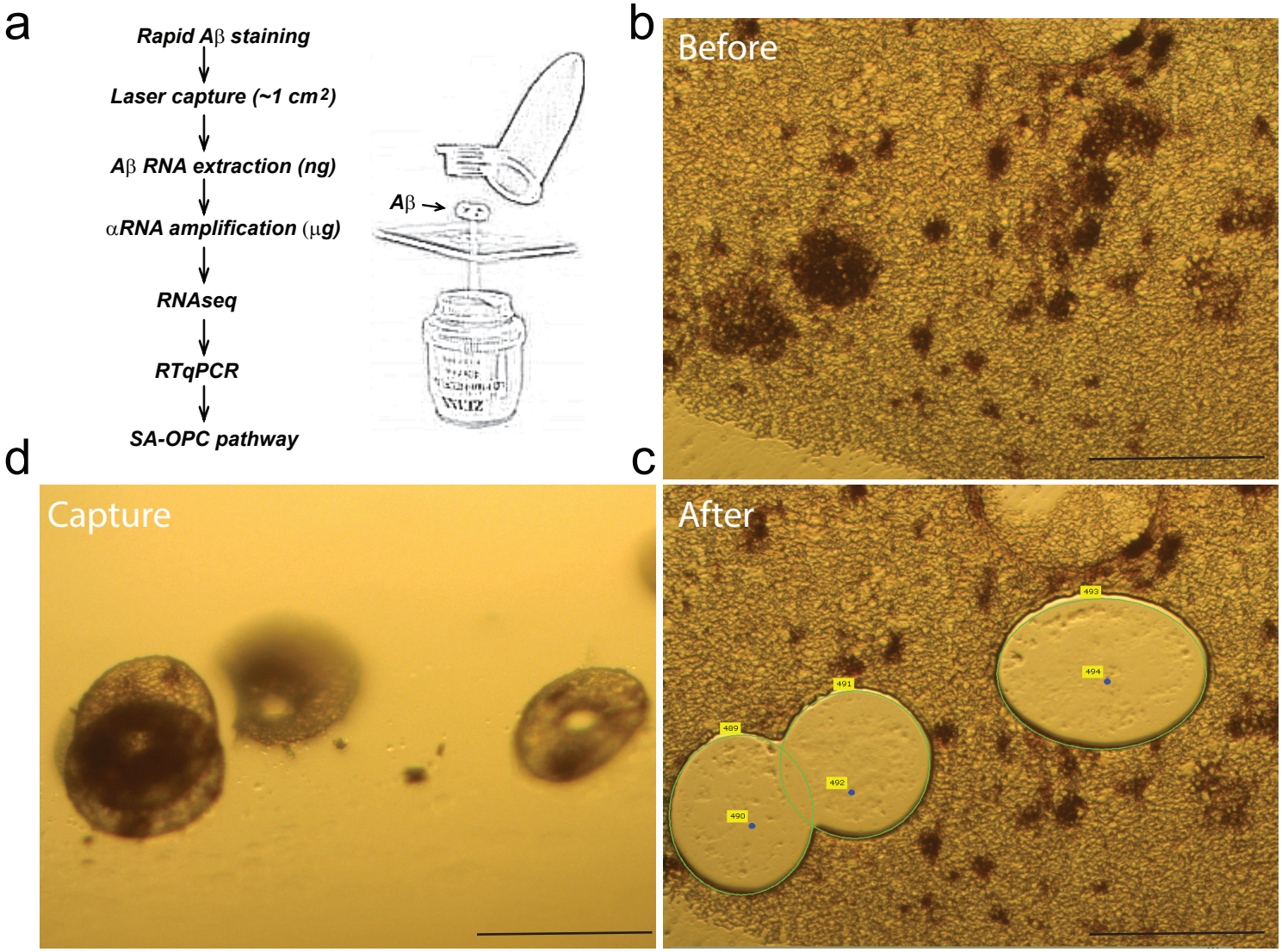
Supplementary Figure 7. Evidence that Aβ plaque-associated senescent cells are OPCs, but not astrocytes or microglia in APP/PS1 AD mouse brains. **(a-c)** Spatial relationships between p16 immunoreactivity, Olig2+ OPCs (a), **G**FAP+ astrocytes (b) and **I**ba1+ microglia (c) in the Aβ plaque (cyan) environment. Blue, TO-PRO3 nuclear staining. Top panels are single-color images of the boxed areas in the merged images shown in the lower panels. Scale bar in each panel = **2**0 μm. The images in a, b, and c represent 8-10 images per analysis. n = 3 mouse brains.



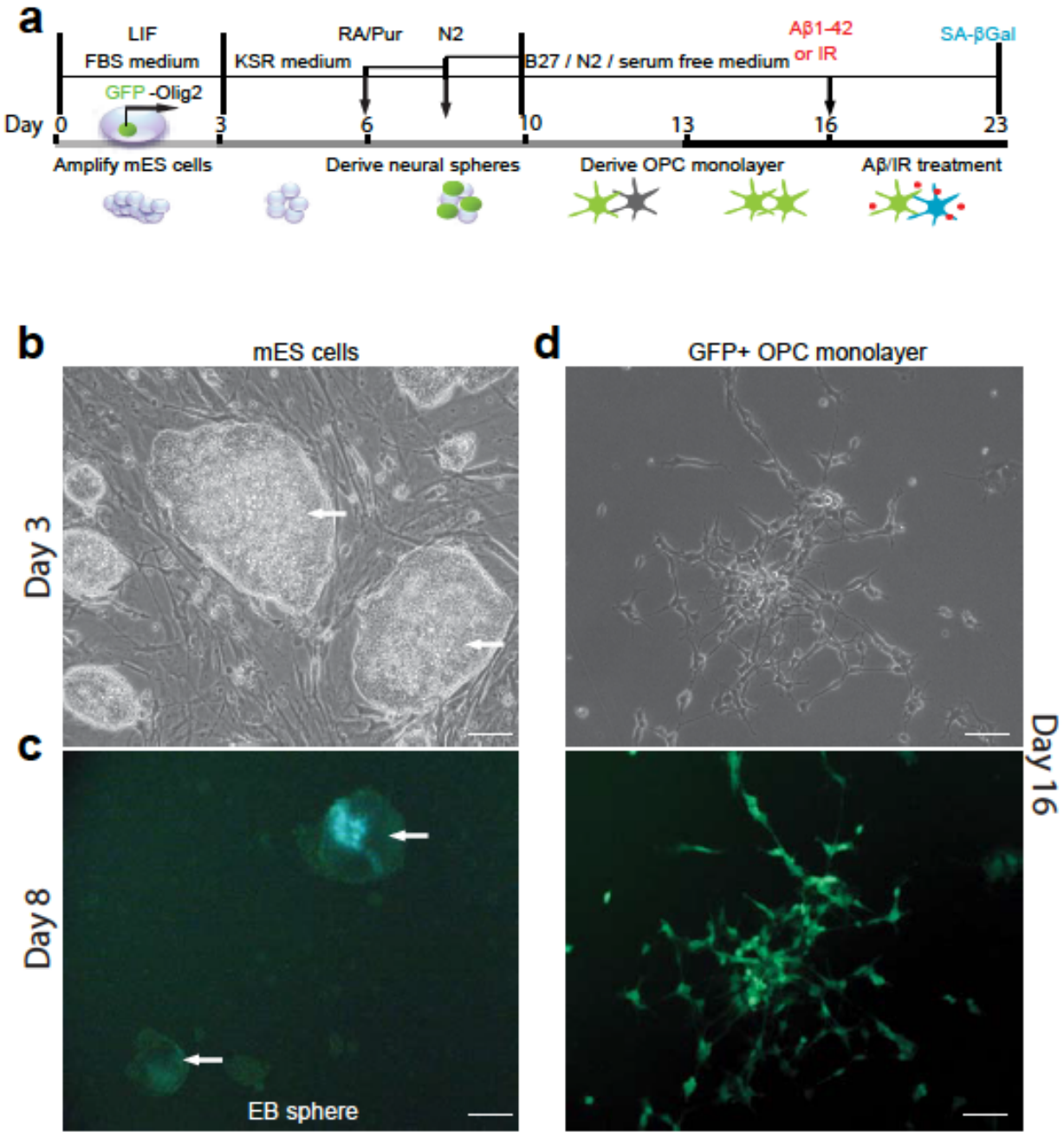
Supplementary Figure 8. Cell type identity of senescent cells. **(a)** Representative images showing that GFAP positive astrocytes (cyan blue, arrows) were located at the periphery of Aβ plaques (red), which were distinct from NG2+ OPCs (green). The graph shows the relative fluorescence intensities of the indicated protein markers from the plaque core to the peripheral edge of the plaque (values are the mean and SEM of measurements made on 23 plaques). **(b)** Representative images of plaque-associated microglia identified by microglial marker Iba1 (red) at the periphery of Aβ plaque. The graph shows the relative fluorescence intensities of the indicated proteins from the core to the peripheral edge of the plaque (values are the mean and SEM of measurements made on 22 plaques). **(c-d)** Confocal images showing robust co-localization of SA-βGal staining (black), LAMP1(red) and Olig2 (green in d) immunoreactivity around the core of Aβ plaques (blue in c, purple in d). Arrows in c point to colocalization of SA-βGal and LAMP1 in lysosomal compartments. Scale bars are 20 μm in each panel. The images in a, b, c and d represent 22- 35 plaques per analysis. n = 3 mouse brains.



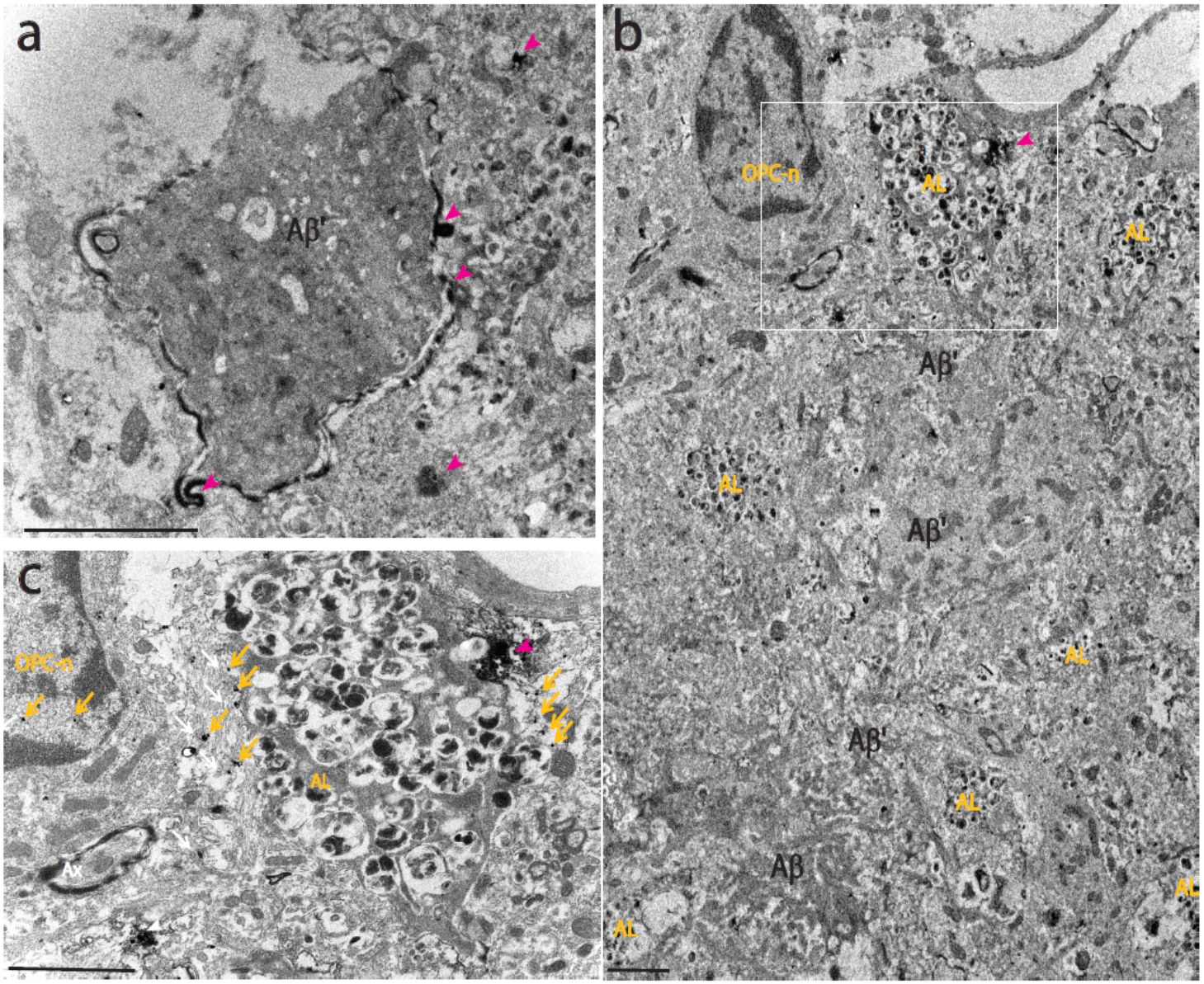
Supplementary Figure 9. Spatial relationships between cells exhibiting SA-βGal activity, astrocytes, microglia, and myelinated axons in regions of Aβ plaques in the brains of 3- and 7.5-month-old APP/PS1 mutant mice. Brain sections were stained for SA-βGal (black), and immunostained with antibodies against MBP (green; myelinated axons) and GFAP (pink; astrocytes) or Iba1 (red; microglia). Sections were also stained with FSB to label fibrillar Aβ (blue). The circles demarcate regions exhibiting SA-βGal activity and extensive demyelination. Arrowheads point to microglia. Scale bar = 20 µm. The images represent 15- 25 plaques per analysis. n = 3 mouse brains.



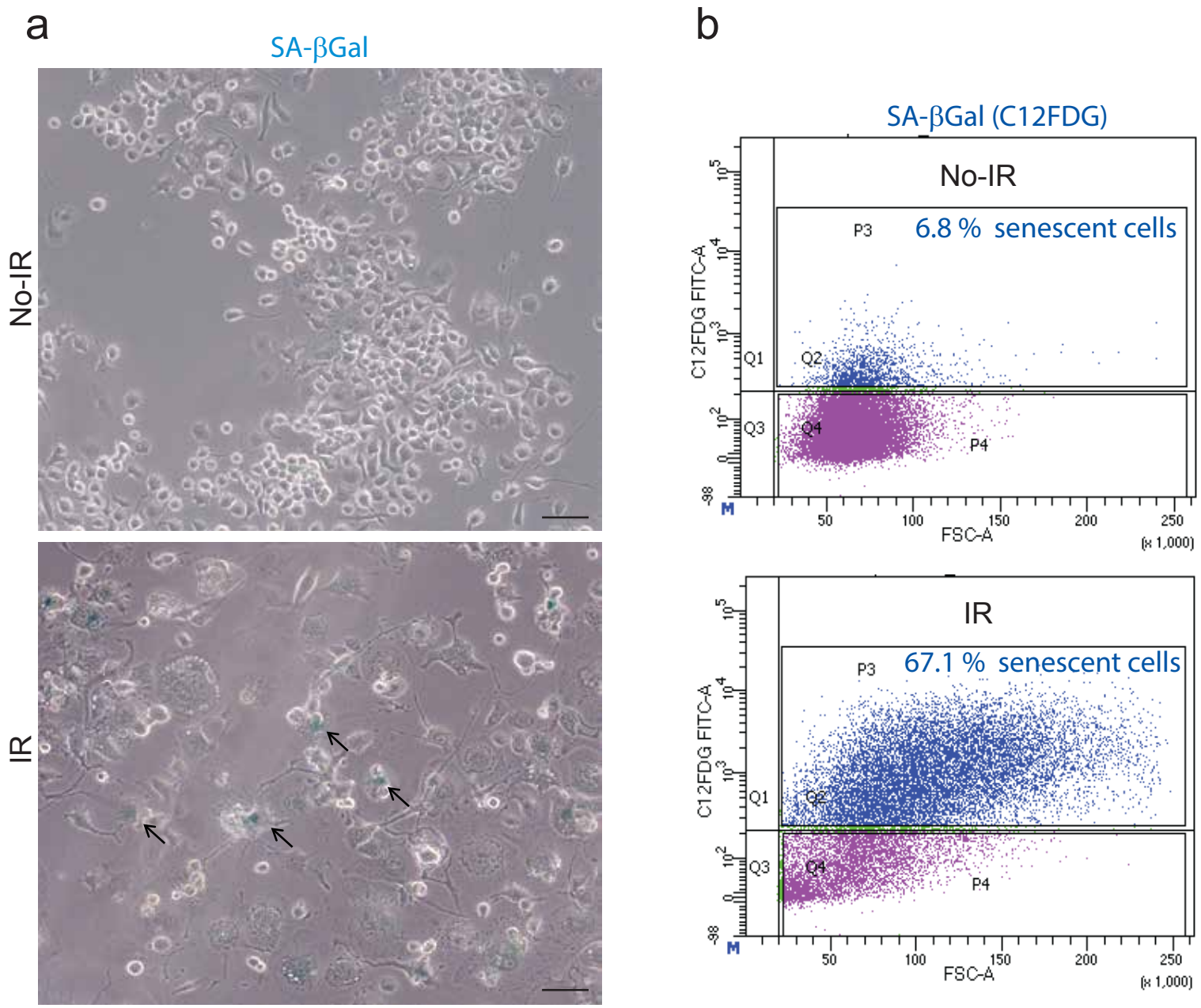
Supplementary Figure 10. Laser capture microdissection (LCM) and RNA analysis of Aβ plaques in brain tissue samples. **(a)** Schematic illustration of LCM workflow **(b - c)** Images of an Aβ plaque-laden brain section from a 9-month-old APP/PS1 mutant mouse before (b) and after (c) LCM. **(d)** Representative image showing the pieces of tissue removed from the same brain section. The brain section was immunostained with anti-Aβ antibody using the DAB detection method for plaque visualization prior to LCM. Scale bar in each panel =100 μm. The images in a, b, c and d represent 160- 220 times of laser dissections from 5-6 sections per brain. n = 3 mouse brains.



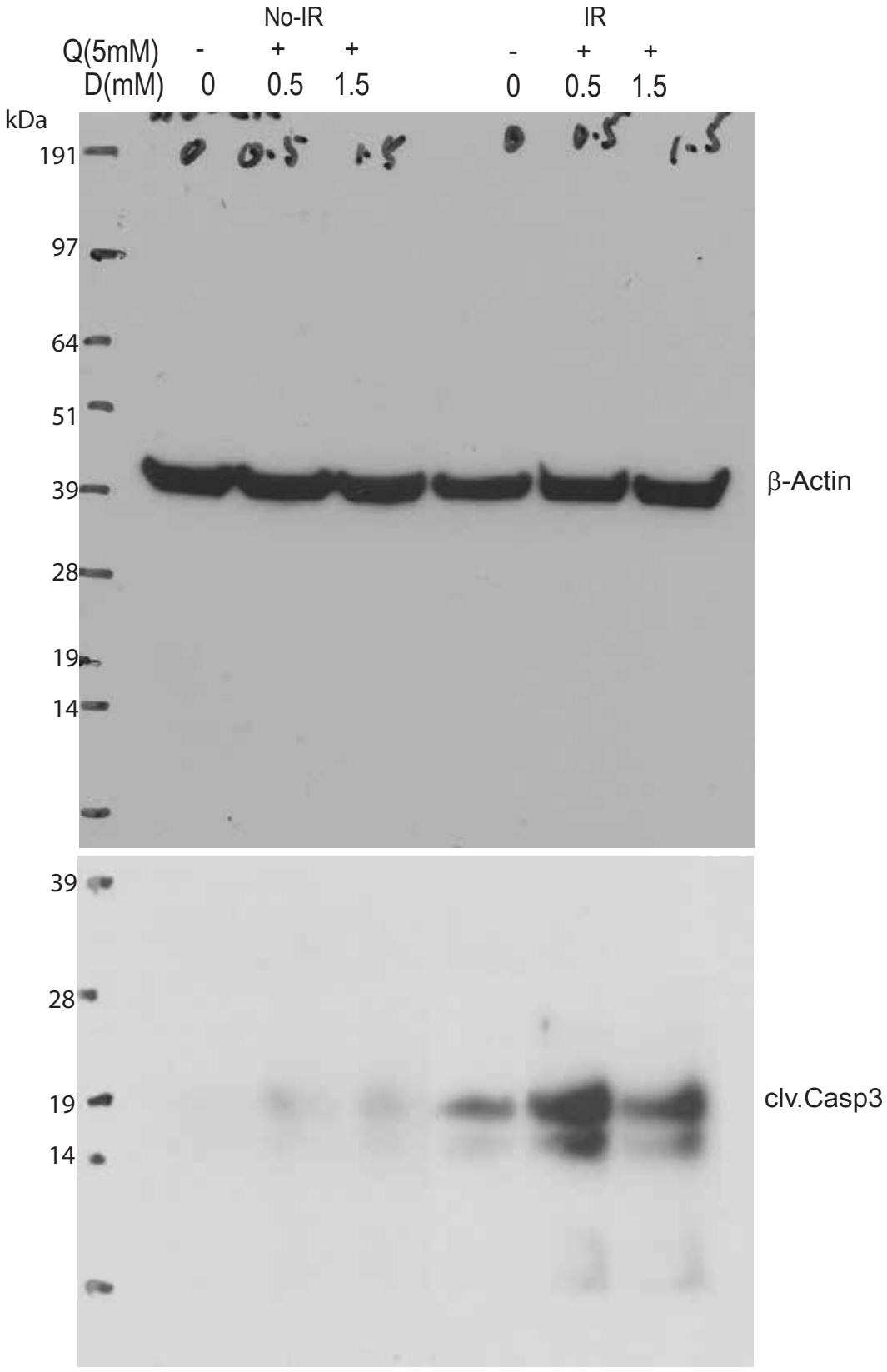
Supplementary Figure 11. Differentiation of GFP-Olig2 mouse embryonic stem cell (mESC)-derived OPCs. **(a)** Schematic illustration showing the protocol for differentiating mESCs. **(b)** Phase-contrast image showing undifferentiated mESC colonies not expressing GFP-Olig2 fusion protein (arrows) growing on a feeder layer of mouse embryonic fibroblasts. **(c)** The fluorescent image showing GFP-Olig2 expressing OPCs in embryoid bodies (EB, arrows) on culture day 8. **(d)** Highly enriched GFP-expressing OPC populations at culture day 16. Scale bar = 50 μm. The images in b, c and d represent the results from 3 different experiments.



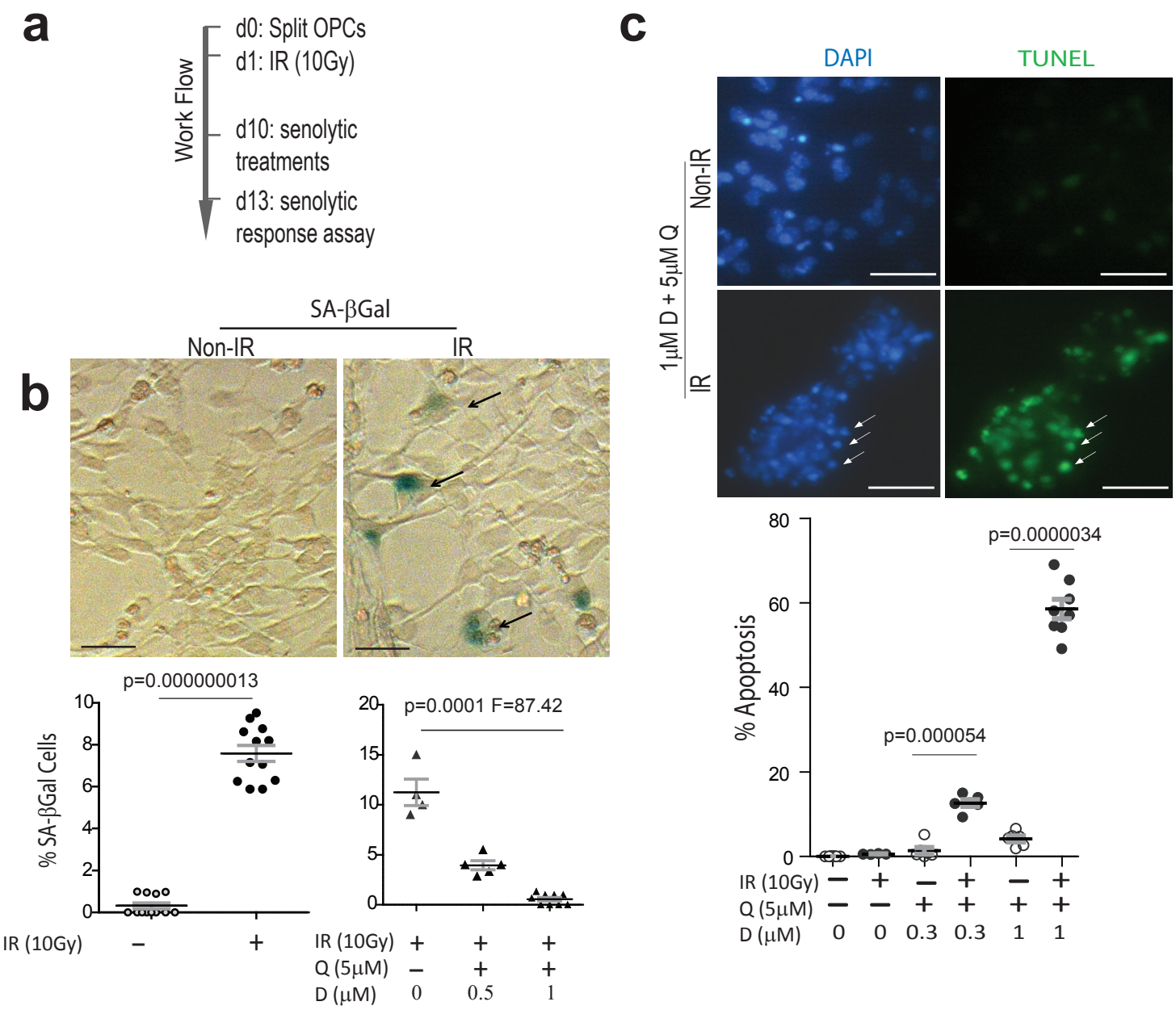
Supplementary Figure 12. Electron micrographs showing the Aβ plaque environment in brain tissue sections from APP/PS1 AD mice. **(a)** Electron micrograph showing Aβ signals seen as dark scattered material (pink arrowheads) around Aβ aggregates (Aβ'). **(b)** Spatial relationships of an OP C with the Olig2-positive autolysosomes (AL) and the Aβ aggregates in the periphery of a plaque (Aβ'). OPCn , OPC nucleus; Aβ, plaque core. Arrowhead points to autolysosome-mediated up take of Aβ in the OPC. **(c)** The enlarged image of the boxed area in panel b shows Olig2+ gold particles (yellow arrows) located adjacent to the Aβ deposit and the AL as well as in the cytoplasm and nucleus of OPC. The pink arrowhead points to an Aβ seen as dark scattered materials in contact with the AL. Scale bar = 2 μm. The images represent the results from seven EM blocks (n =7).



Supplementary Figure 13. Validation of C12FDG-FACS method for detecting senescence activity. **(a)** Upon exposure of neuro2a (N2a) cells to 10 Gy irradiation (IR) for 7 d, less than 15% senescent cells (arrows) were visible via the conventional method of SA-βGal staining. The images represent 10-15 image per group. **(b)** 5 d after IR, more then 60% cells manifested senescence activity by fluorescence-activated cell sorting (FACS) analysis using the fluorogenic substrate C12FDG. The results represent the date from three different experiments.

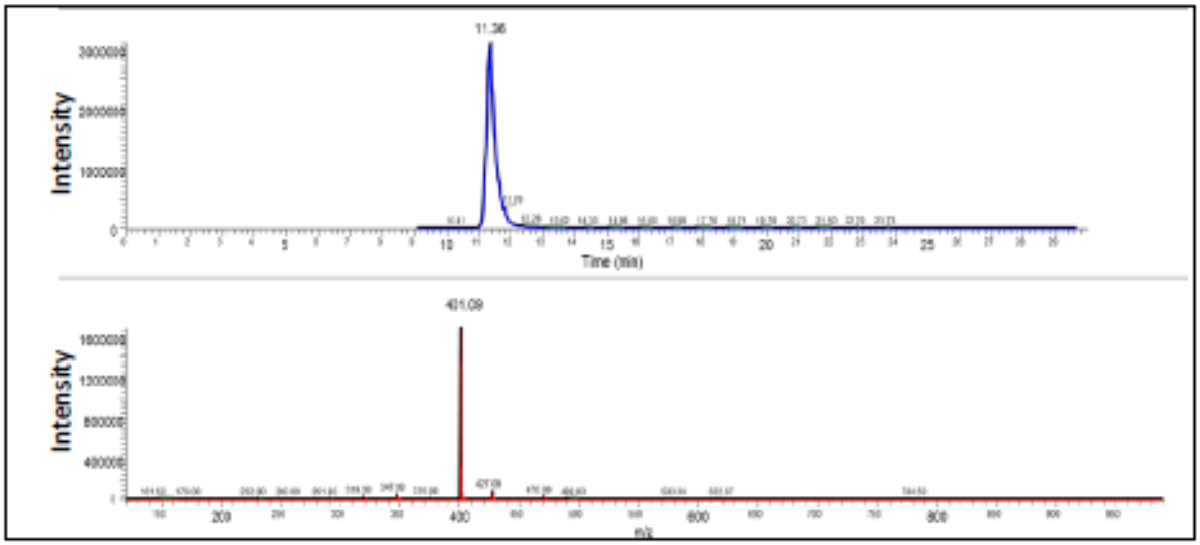


Supplementary Figure 14. Full scan of western blots for the insert in Figure 4a. n= 3 bioloigal replicates.



Supplementary Figure 15. The dasatinib and quercetin cocktail (D+Q) has senolytic activity in vitro. **(a)** Schematic of experimental design for sequential exposure of OPC cultures to 10 Gy ionizing radiation (IR) and D+Q. **(b)** 10 d after IR, irradiated OPCs and non-IR counterparts were treated with 5uM of Q plus varied does of D for 3 d, and then were subjected to SA-βGal staining Arrows in upper panel point to SA-βGal positive cells. The graphs show the percentage of senescent cells with or without D+Q treatments. Two tailed Student's t-test (left graph) and one-way ANOVA with Dunnett's post hoc (right graph) were made in 3 separate cultures (n = 3) with 4 - 12 images (200 - 240 cells) per condition. Values are the means and SEM. Scale bar = 30 μm. **(c)** D+Q mediated apoptosis were examined by TUNEL assay. 10 d after IR, irradiated OPCs and non-IR controls were treated with D+Q for 3 d, and then were subjected to DAPI (blue) and TUNEL (green) fluorescence assay. Arrows in lower panel point to apoptotic nuclei. Scale bar = 25 μm. The graph shows the percentage of apoptotic OPCs in response to various doses of D+Q. Values are the means and SEM of measurements made in 3 separate cultures (n = 3) of 4 - 8 images with total 100 - 200 cells, and significance was determined with two-tailed Student's t test.

a



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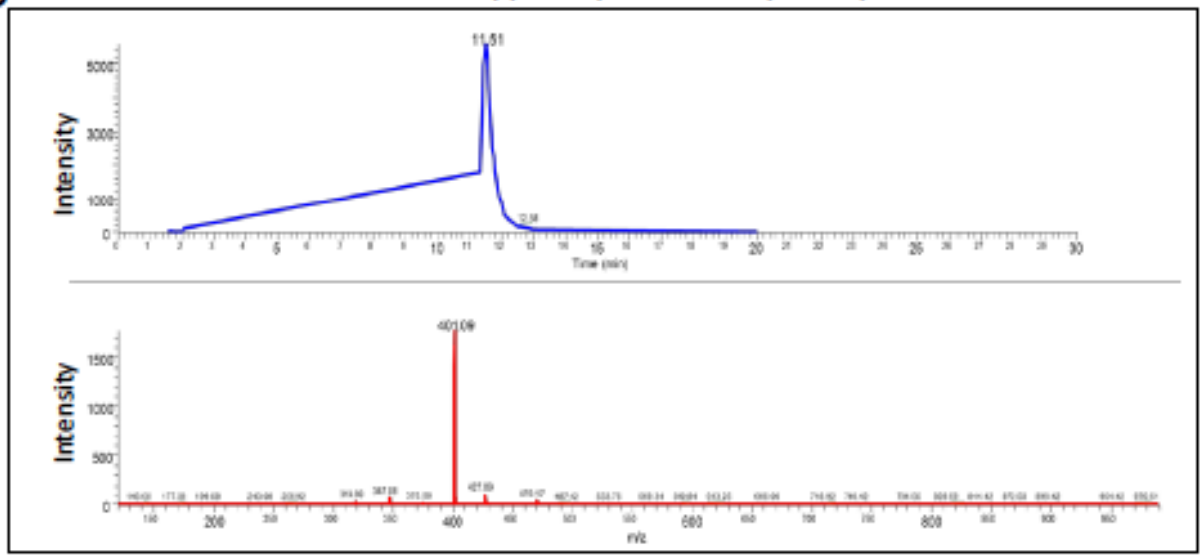
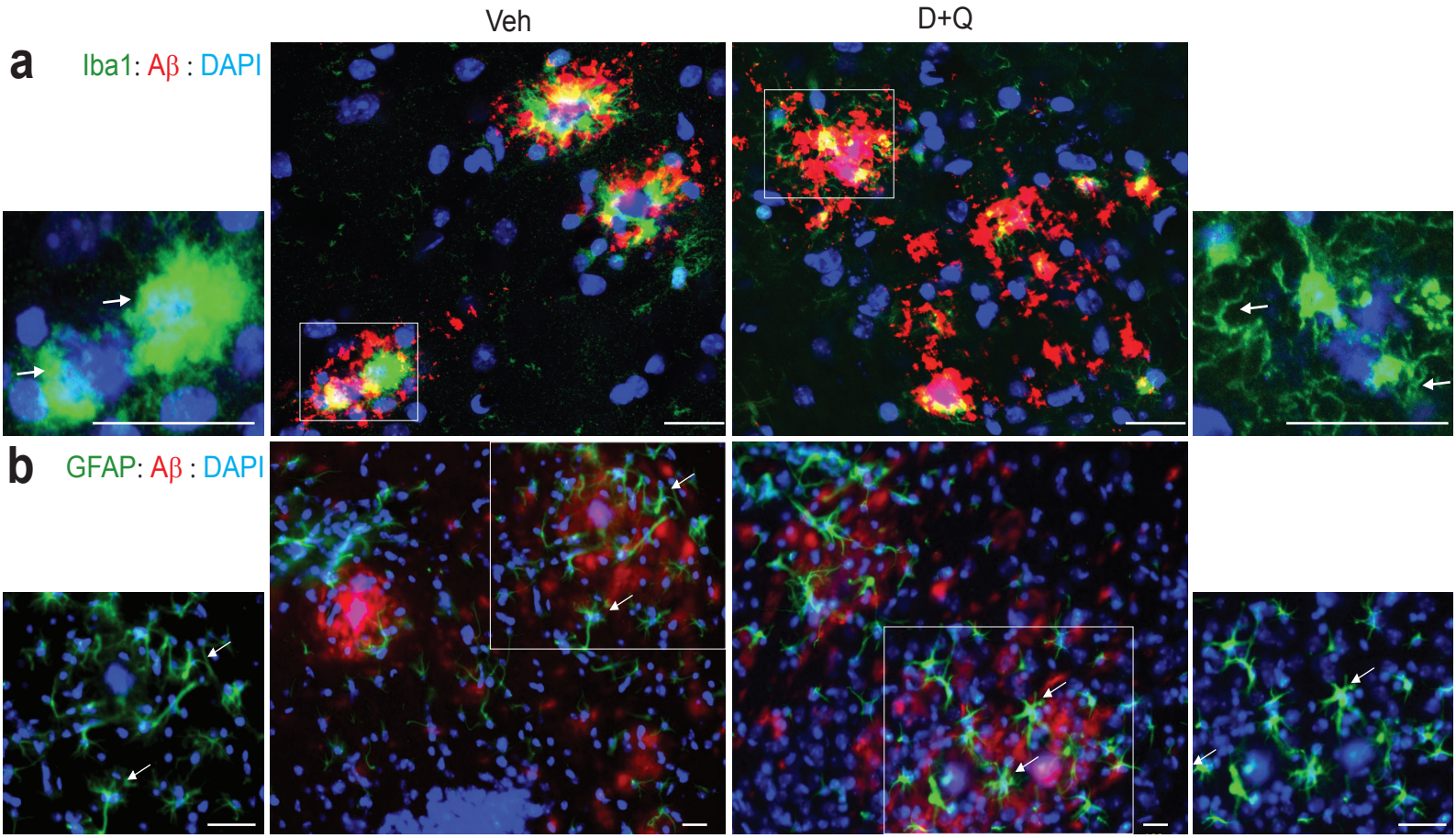


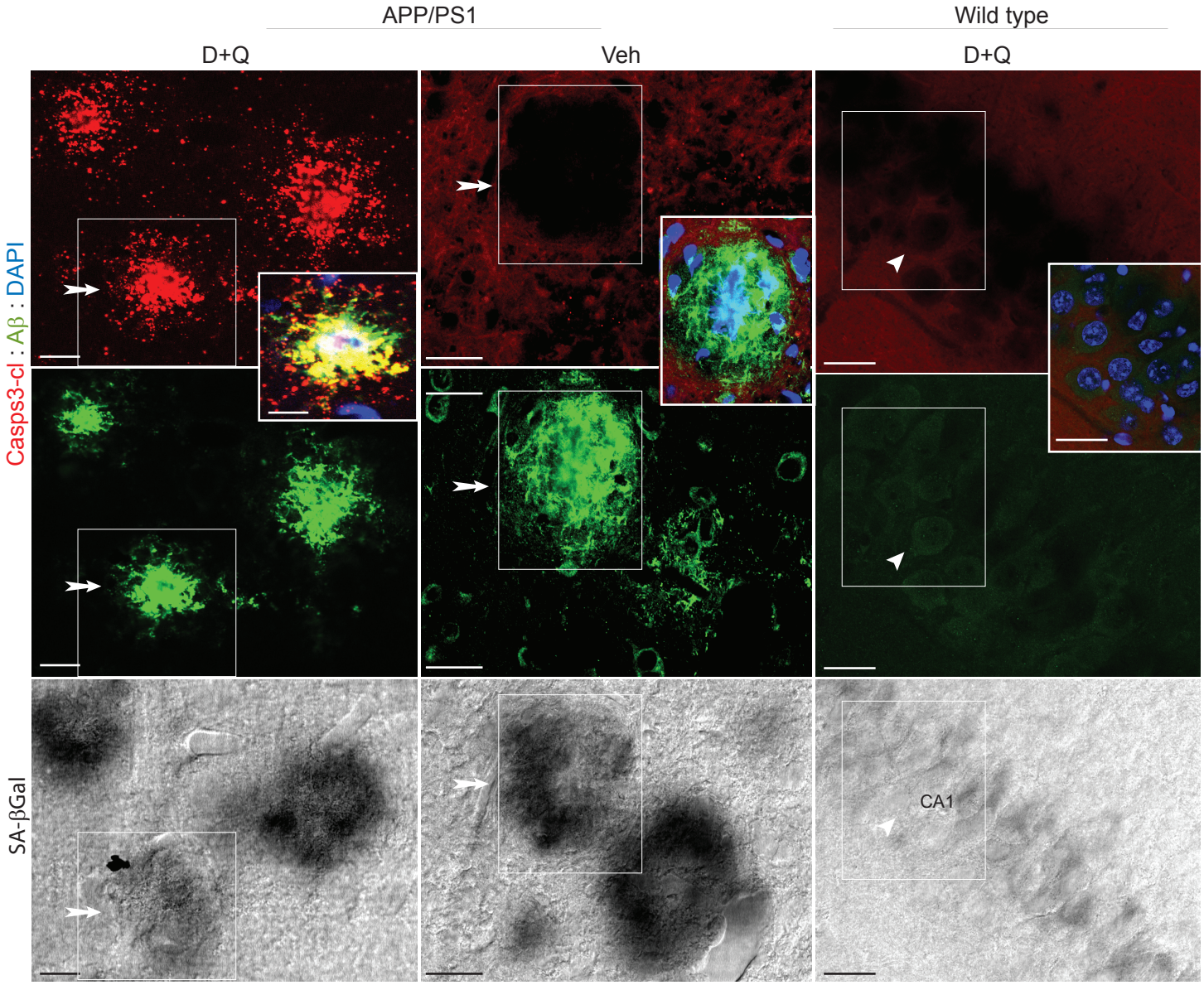
c

Substance measured	Mice	Plasma	Brain tissue	
			Hippocampus	Cerebellum
Dasatinib (ng/ml)	APP/PS1-a	22.34	57.17	13.98
	APP/PS1-b	8.05	10.90	14.33

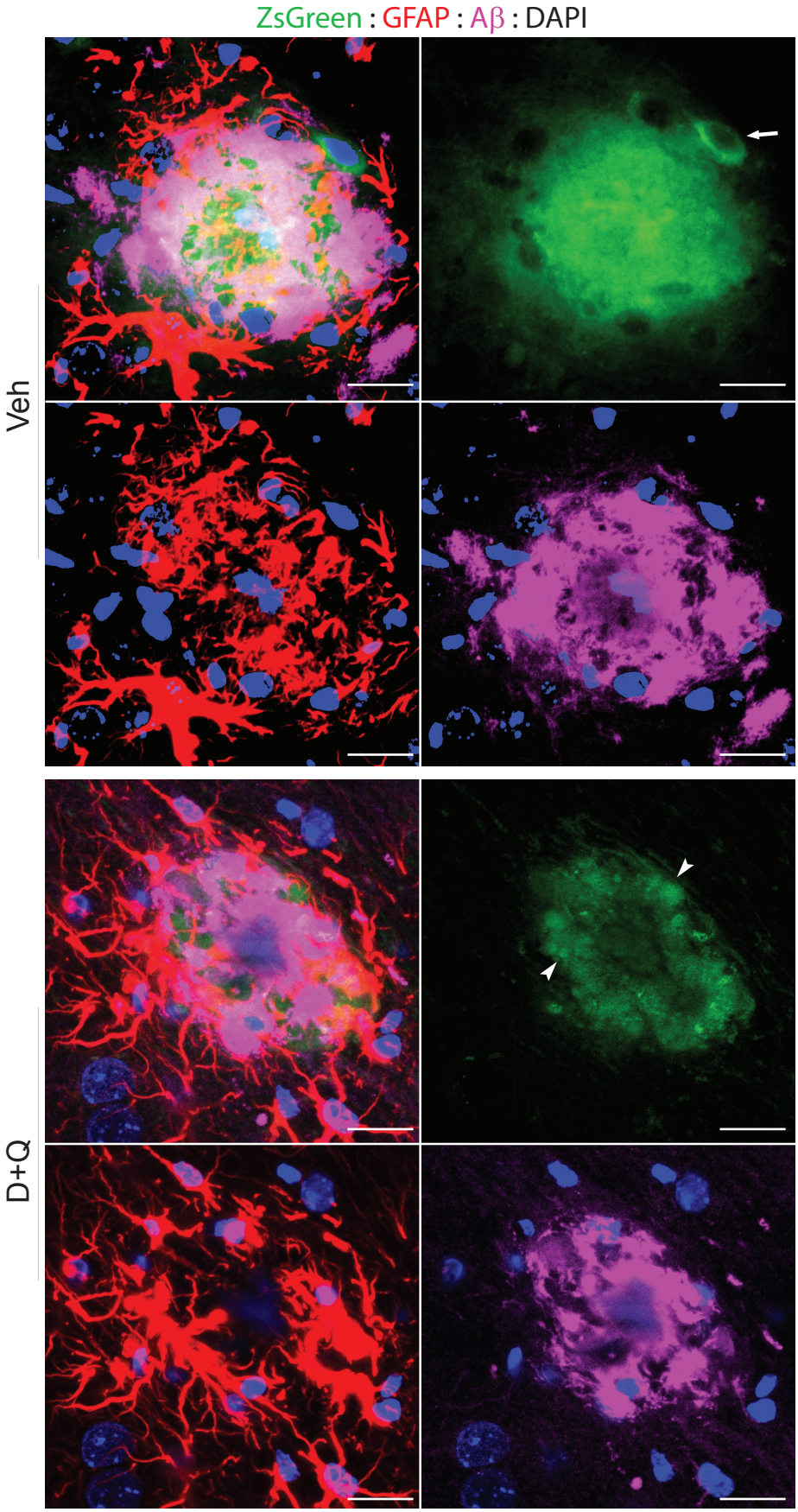
Supplementary Figure 16. Mass spectrometric characterization of dasatinib (D) in mouse hippocampus and cerebellum. **(a, b)** Chromatograms of D standard (a), and its level in hippocampus (b) 2 hours after oral treatment with 12 mg/kg of D. **(c)** Average concentrations of D in the plasma and brain tissues of two APP/PS1 mice at 2 hours after oral administration of D (12 mg/kg). The results in a, b represent two separated experiments from two mice tissues as indicated in c.



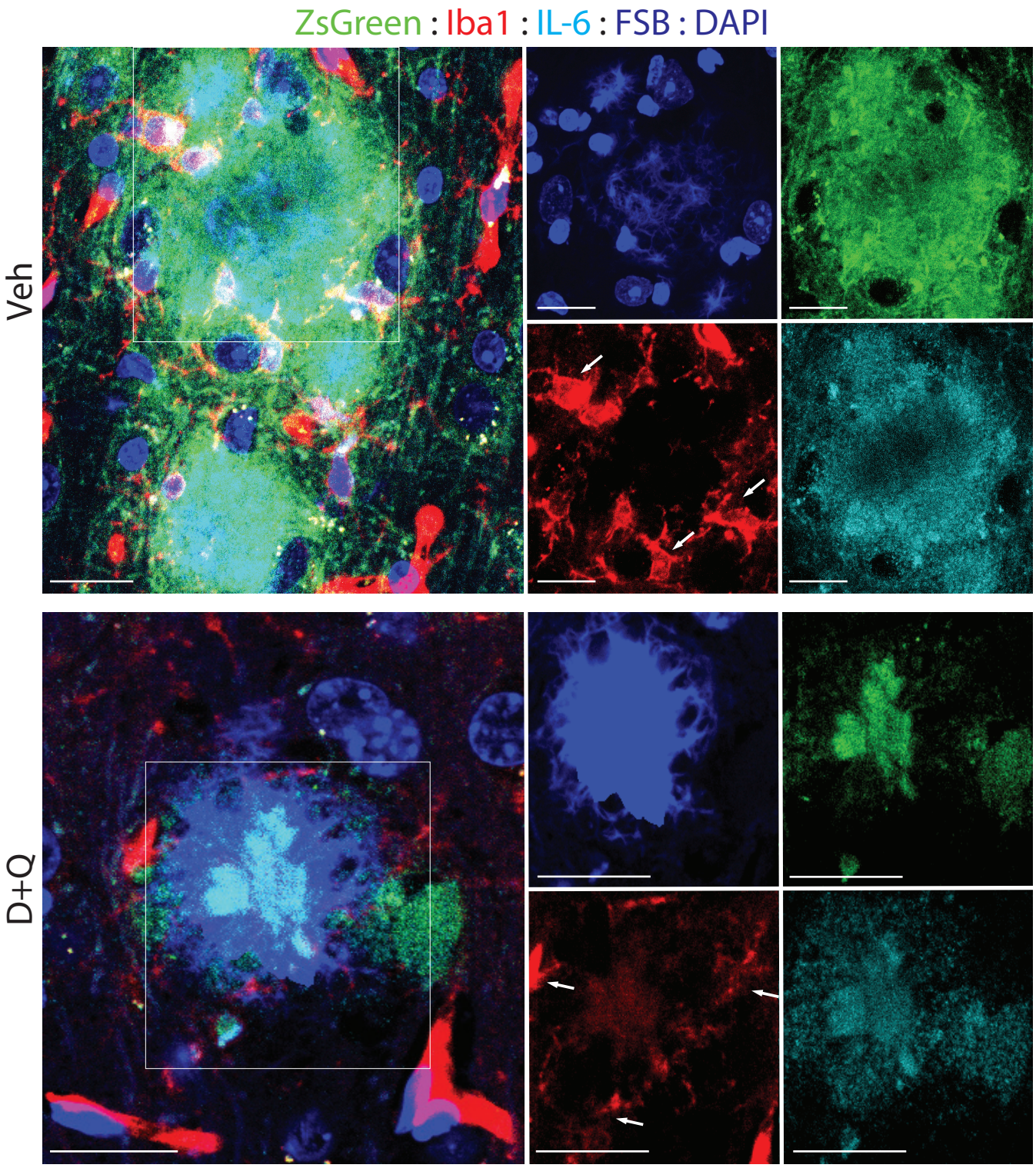
Supplementary Figure 17. Plaque-associated cellular responses of 9-day short-term D+Q treatment in APP/PS1 mice. The sections from same brains were immunostained using antibodies against Iba1 (green in a), GFAP (green in b) and Aβ (red). Arrows in a point to microglia with retracted processes and an enlarged soma in vehicle control (left most insert) or with ramified fine processes in the D+Q group (right most insert). Arrows in b point to astrocytes with similar morphologies in D+Q and vehicle control groups. Scale bar = 20 μm. The images in a and b represent 15 images for each analysis. n = 3 mouse brains in each group.



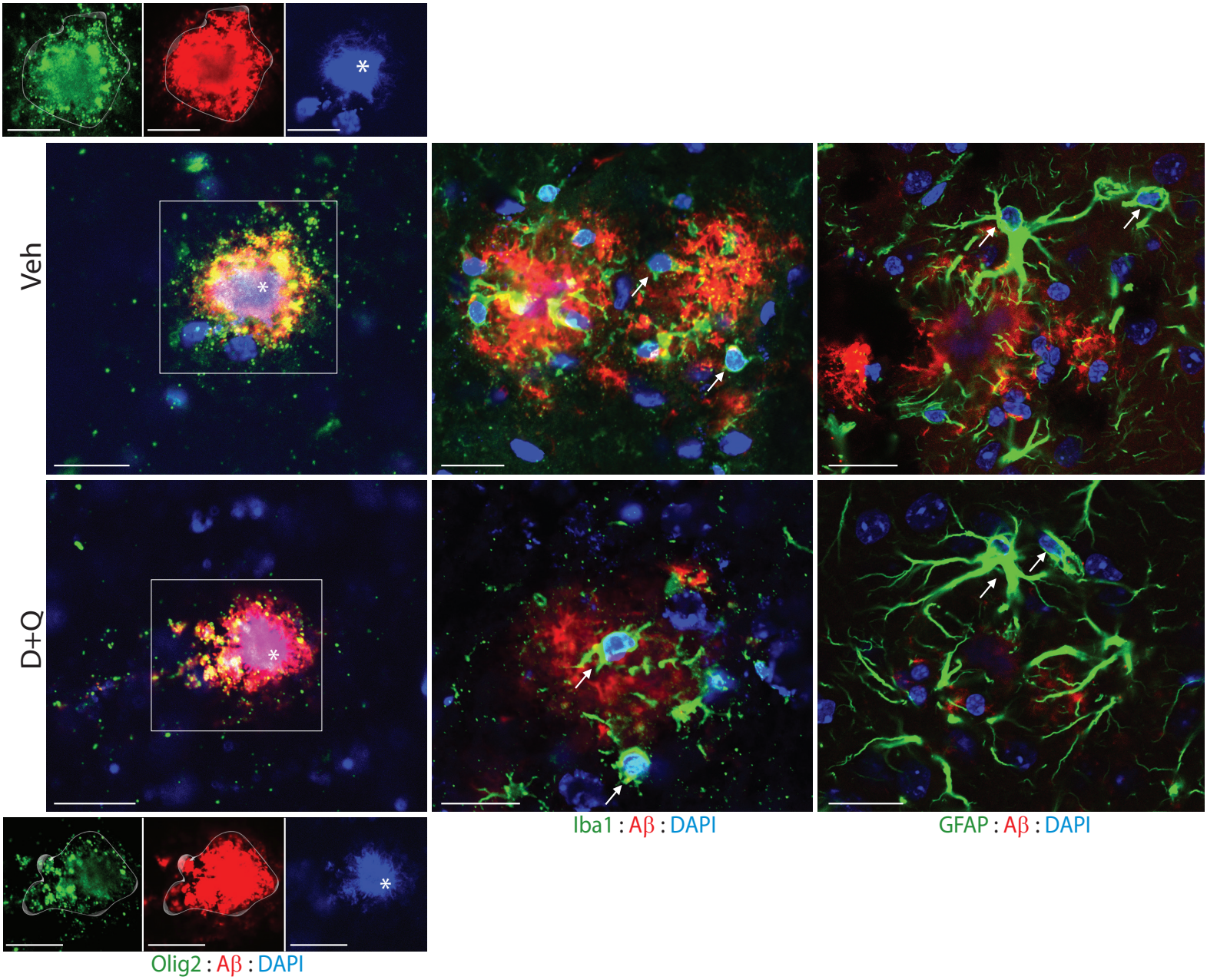
Supplementary Figure 18. Short-term D+Q treatment elicits a senolytic response in the brains of APP/PS1 mutant mice. The 8-month-old APP/PS1 mice and wild type littermates were administrated either D+Q or vehicle daily for 9 d. Left, representative confocal images showing that the activation and cleavage of Caspase 3 (clv. Caps 3) indicative of apoptosis were observed in the brains of D+Q treated APP/PS1 mice. Arrows point to the immunoreactivity of clv. Casp 3 (red) were colocalized with Aβ plaques (green) and SA-βGal foci (black). n=4. brains. Middle, clv. Casp 3 immunoreactivity was absent in Aβ plaques and SA-βGal foci in the vehicle-treated APP/PS1 mouse brains. n=4 brains. Right, little or no caspase 3 activation was detected in the D+Q treated wild type mouse brains, where CA1 pyramidal neurons (arrowhead) exhibited defuse SA-βGal activity. n=2 brains. The inserts at the right of each upper panel are merged fluorescence images of the boxed areas. Scale bar = 20 μm. The images represent 12-15 images collected from 5 section of two brains in each group.



Supplementary Figure 19. Reduction of plaque-associated ZsGreen p16 reporter levels in response to D+Q treatment has little or no impact on Aβ-associated astrocyte activation. ZsGreen/APPPS1 mice (5 months-old) were treated for 9 days with either vehicle (upper panel) or D+Q (lower panel). The images show ZsGreen (green) and astrocytes (red) in the Aβ (purple) environment. Scale bar = 20 μm. The images represent 12-15 images per group. n = 3 mouse brains in each group.



Supplementary Figure 20. Evidence that short-term senolytic treatment reduces Aβ plaque-associated inflammation in APP/PS1 AD mice. ZsGreen/APPPS1 mice (5 months old) were treated for 9 days with either vehicle (upper panel) or with D+Q (lower panel). Images show ZsGreen p16 reporter fluorescence (green), Iba1-labeled microglia (red) and IL-6 immunoreactivity (cyan) in FSB labeled Aβ plaques. Inserts in right panels are unmerged images in the boxed areas. Arrows point to reactive (upper panel) and dereactive microglia (lower panel) in response to vehicle and (D+Q)S treatments respectively. Scale bar =20 μm. The images represent 12-15 plaques per mouse and n=3 biological replicates per group.



Supplementary Figure 21. Examples of Aβ plaque-associated cells in APP/PS1 AD mice treated for 11 weeks with vehicle or D+Q. Brain sections were immunostained with antibodies against Olig2 (green in left panel), Iba1 (green in middle panel) and GFAP (green in right panel). The Aβ plaque (red)-associated fluorescence Olig2 flourescence intensity (lasso enclosure in unmerged insert), and the number of GFAP-, Ib a1-postive cells (arrows) were quantified and those data are shown in Fig. 5b . Asterisks in leftmost panels mark DAPI-stained nuclei in the plaque environment. Scale bar = 20 μm. The images represent 10-18 image per mouse brain. n = 6 and 8 mice for Veh control and D+Q treatment, respectively.

Supplemental table 1: Demographics and characteristics of postmortem examined individuals

Reference (no)	*Clinical Diagnosis*	*Age (years)*	*Gender (M/F)*	*Braak Stage*	*PMD*	*Aβ plaques*[a]		*p21&Olig2/Aβ*[b]	
						Total	*AVG*	*Total*	*AVG*
1244	NDC	90	F	2	2.25	2*	0.4	1	0.2
1245	NDC	88	M	2	2.17	45	2.3	18	0.9
1280	NDC	86	M	2	4	2*	0.4	1	0.2
1161	NDC	84	F	0	2.5	0*	0.0	0	0.0
1221	NDC	81	M	2	2.83	40	1.9	23	1.1
1260	NDC	80	F	1	4.42	6*	0.6	0	0.2
1271	NDC	71	M	0	2.6	18*	1.8	3	0.3
1268	NDC	68	F	1	4	5*	0.5	3	0.3
1289	MCI	92	F	3	2.33	93	3.1	45	1.5
1164	MCI	88	F	3	3	84	4.0	53	2.5
1122	MCI	87	M	4	2.75	0*	0.0	0	0.0
1065	MCI	87	M	4	3.5	68	3.6	42	2.2
1152	MCI	84	M	4	3.5	61	3.1	23	1.2
1225	MCI	82	F	5	5	75	3.6	47	2.2
1087	MCI	82	F	3	3	29	2.9	11	1.1
1277	MCI	78	F	3	3.5	80	4.0	16	0.8
1086	AD	90	F	6	2.75	64	3.0	54	2.6
1285	AD	88	M	5	1.5	111	5.6	100	5.0
1084	AD	86	F	6	3.25	108	5.4	104	5.2
1144	AD	85	M	6	2.75	79	4.9	64	4.0
1050	AD	81	M	6	3.75	67	3.5	53	2.8
1202	AD	80	F	6	3.33	83	4.2	48	2.4
1160	AD	73	M	6	2	96	4.4	75	3.4
1181	AD	67	F	6	2.25	161	8.1	153	7.7

NDC: non-demented controls
MCI: mild cognitive impairment
Braak stage: scored by the existence of neurofibrillary tangles
PMD: post modem delay
a: scored by the gray matter of cortex (inferior parietal lobule) with Aβ plaque size ≥50μm
b: scored by one or more of p21+/ olig2+ positive granule(s) per Aβ plaque
* All cases were scored from more than 20 of 0.5mm^2 fields except NDC* and MCI subject 1122 that were scored from 5 to 10 fields due leaking Aβ plaque

Supplementary table 2

	Symb	Forward	Reverse	Genbank #	Gene name
1	hAPP	CCCCAGATTGCCATGTTCTG	TGGATGGTCACTGGTTGGTT	NM_000484.3	*human amyloid beta A4 protein precursor
2	mApp	TGCAGCAGAACGGATATGAG	ACACCGATGGGTAGTGAAGC	NM_001198823.1	amyloid beta precursor protein
3	mPsen1	TGGTTGGTGAATATGGCTGA	CTCCCACTCCTCACTGAAGC	NM_008943.2	presenilin 1
4	mMapt	GCCCTCTGGCACTTCTGTAG	AGACGTGGGCATGATAGGAC	NM_001038609.2	microtubule associated protein tau
5	mNotch1	ACCCACTCTGTCTCCCACAC	GCTTCCTTGCTACCACAAGC	NM_008714.3	Notch1
6	mBace2	TCTTTGTGCGGATGTCTCAG	ACATTCCTGGATGGCTCTTG	NM_019517.4	beta-site APP-cleaving enzyme 2
7	mCspg4	ATTCAGCACGCTCTGTTCCT	GACCAACAAGGCCAAGGTAA	NM_139001.2	chondroitin sulfate proteoglycan 4 (NG2)
8	mOlig2	AGCAATGGGAGCATTTGAAG	CAGGAAGTTCCAGGGATGAA	NM_016967.2	oligodendrocyte transcription factor 2
9	mOlig1	GGTTTCCGAGCTGGATGTTA	GCGAGCCTGAAAAACAGAAC	NM_016968.4	oligodendrocyte transcription factor 1
10	mPdgfra	ACCACAATGGTGCTGTTGAA	AATCTCTGGGGCAAAGGTCT	NM_001083316.1	platelet derived growth factor receptor alpha
11	Mbp	CCTCTGCCCTCTCATGCC	CCATAATGGGTAGTTCTCGTGT	NM_001025251.2	myelin basic protein
12	mCdkn1a	CACAGCTCAGTGGACTGGAA	ACCCTAGACCCACAATGCAG	NM_001111099.2	cyclin-dependent kinase inhibitor 1A , p21CIP1
13	mCdkn2a	ATCTGGAGCAGCATGGAGTC	GGGGTACGACCGAAAGAGTT	NM_001040654.1	cyclin-dependent kinase inhibitor 2AP16INK4A
14	mCdkn1c	AGAGAACTGCGCAGGAGAAC	TCTGGCCGTTAGCCTCTAAA	NM_001161624.1	cyclin-dependent kinaseinhibitor,p57KIP2
15	mTgfb1	GGACTCTCCACCTGCAAGAC	GACTGGCGAGCCTTAGTTTG	NM_011577.2	transforming growth factor beta 1
16	IL-1beta	ACTCATTGTGGCTGTGGAGA	TTGTTCATCTCGGAGCCTGT	NM_008361.4	interleukin 1 beta
17	Tnfalpha	CCACCACGCTCTTCTGTCTA	CTGATGAGAGGGAGGCCATT	NM_013693.3	tumor necrosis factor isoform1
18	Rn18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG	NR_003278	18S ribosomal RNA
19	Actb	TGTTACCAACTGGGACGACA	GGGGTGTTGAAGGTCTCAAA	NM_007393.5	Beta Actin
20	Galc	GAGGCTATGAGTGGTGGCTA	CTTTGCCCAGCCATCCAG	NM_008079.4	galactocerebrosidase

*human transgene