SUPPLEMENTARY TABLES

Table S1. Lasers and filter sets used for sorts.

Antibody to be detected	Laser	Filter Set
DAPI	355 nm	460/50 nm
O1/BV421	405 nm	460/50 nm
Reference channel (no antibody)	488 nm	528/38 nm
TUBB3-555	561 nm	593/40 nm
GFAP-647	640 nm	670/30 nm

Table S2. Post-sort event, cell, and protein yield for neural cell types isolated from non-symptomatic and Alzheimer's disease brain. Counts, calculated using a hemocytometer, were based on single cells (i.e., no clumps were included). AD-severe diagnosis indicates Braak staging of VI/VI. Sorting runs used all cells isolated from a given donor sample (see Table S2).

Donor #	Diagnosis	Cell Population	Event Count	Cell Count	Protein Yield (µg)
1	NS	Unsorted	$1x10^6$	$1.3x10^6$	13.9
1	NS	O1+	$2.4x10^4$	--	--
1	NS	TUBB3+	$9.4x10^5$	$1.4x10^6$	8.7
1	NS	GFAP+	$7.1x10^5$	$5.8x10^5$	10.3
1	NS	Myelin debris	--	--	145
2	NS	Unsorted	$5.5x10^5$	$4.6x10^5$	5.0
2	NS	O1+	$1x10^5$	--	--
2	NS	TUBB3+	$3x10^5$	$4.4x10^5$	6.8
2	NS	GFAP+	$6.2x10^5$	$1.1x10^6$	4.5
2	NS	Myelin debris	--	--	170
3	NS	Unsorted	$5x10^5$	$5.7x10^5$	9.8
3	NS	O1+	$8.3x10^4$	--	--
3	NS	TUBB3+	$2.5x10^5$	$2.9x10^5$	5.5
3	NS	GFAP+	$3.2x10^6$	$2.7x10^6$	24.8
3	NS	Myelin debris	--	--	178
4	AD-severe	Unsorted	$1x10^6$	$1.3x10^6$	14.3
4	AD-severe	O1+	$5.7x10^4$	--	--
4	AD-severe	TUBB3+	$1x10^6$	$1.1x10^6$	11.8
4	AD-severe	GFAP+	$8x10^5$	$2.7x10^5$	5.3
4	AD-severe	Myelin debris	--	--	127
5	AD-severe	Unsorted	$1x10^6$	$1.6x10^6$	13.7
5	AD-severe	O1+	$1.7x10^4$	--	--
5	AD-severe	TUBB3+	$1.3x10^6$	$1.5x10^6$	11.6
5	AD-severe	GFAP+	$4.8x10^5$	$6.3x10^5$	7.8
5	AD-severe	Myelin debris	--	--	119
6	AD-severe	Unsorted	$5x10^5$	$1.1x10^6$	8.0
6	AD-severe	O1+	$1.4x10^5$	--	--
6	AD-severe	TUBB3+	$1.1x10^6$	$7.4x10^5$	8.6
6	AD-severe	GFAP+	$1.2x10^6$	$1.6x10^6$	11.8
6	AD-severe	Myelin debris	--	--	168

Table S3. Lasers and filter sets used for confocal imaging.

Laser	Nikon AIR Multiphoton microscope settings	Zeiss LSM800 with Airyscan confocal microscope settings
405 nm	425-475 nm PMT; HV 100, Offset -15, Power 4.50	Pinhole 37 μm, Offset 0, Gain 725V
488 nm	N/A	Pinhole 44 μm, Offset 0, Gain 725V
561 nm	570-620 nm GaAsP; HV 80, Offset -15, Power 0.25	Pinhole 51 μm, Offset 0, Gain 700V
640 nm	N/A	Pinhole 56 μm, Offset 0, Gain 750V
655 nm	663-738 nm PMT; HV 120, Offset -15, Power 3.50	N/A

Table S4. Top 50 proteins identified from fresh and PFA-fixed rat brain cells. Listed proteins are ranked by abundance for fresh samples (left columns) and PFA samples (right columns). Units for quantified proteins are averaged spectrum counts.

Top 50 Proteins for Fresh Condition			*Top 50 Proteins for PFA Condition*		
Protein Name	**Fresh Condition Averaged Spectrum Counts**	**PFA Condition Averaged Spectrum Counts**	**Protein Name**	**PFA Condition Averaged Spectrum Counts**	**Fresh Condition Averaged Spectrum Counts**
SPTA1	68.80	46.47	PRPF8	82.07	57.80
SPTB	66.67	37.67	SNRNP200	67.40	49.13
PRPF8	57.80	82.07	NUMA1	62.73	31.80
SNRNP200	49.13	67.40	TPR	56.73	28.73
LMNA	46.73	50.53	DHX9	52.60	42.80
DHX9	42.80	52.60	LMNA	50.53	46.73
HNRNPM	42.13	50.27	HNRNPM	50.27	42.13
LMNB1	40.67	45.33	SF3B1	46.67	30.20
MATR3	34.60	42.20	SPTA1	46.47	68.80
LMNB2	33.13	36.47	LMNB1	45.33	40.67
NUMA1	31.80	62.73	RANBP2	44.67	27.87
SF3B1	30.20	46.67	MATR3	42.20	34.60
HBB	29.00	20.93	TUBB4A	40.73	26.73
TPR	28.73	56.73	SUGP2	39.00	26.27
SLC4A1	28.53	25.73	SPTB	37.67	66.67
ANK1	28.00	21.67	ATP2A2	37.07	22.33
HNRNPL	27.93	33.27	EFTUD2	36.87	25.87
RANBP2	27.87	44.67	LMNB2	36.47	33.13
HSPA8	27.87	32.20	SF3B3	34.53	25.80
HNRNPU	26.73	34.33	NUP205	34.47	21.73
TUBB4A	26.73	40.73	HNRNPU	34.33	26.73
HNRNPUL2	26.47	34.27	HNRNPUL2	34.27	26.47
YWHAE	26.40	26.20	DDX5	34.00	25.13
SUGP2	26.27	39.00	HNRNPL	33.27	27.93
EFTUD2	25.87	36.87	HSPA8	32.20	27.87
SF3B3	25.80	34.53	ILF3	30.93	25.27
MYEF2	25.60	30.13	NUP210	30.40	17.93
HNRNPA2B1	25.40	27.87	MYEF2	30.13	25.60
ILF3	25.27	30.93	TRIM28	30.00	22.33
DDX5	25.13	34.00	ZFR	29.60	20.60
ACTB	24.47	23.87	HSPA5	29.40	24.07
HSPA5	24.07	29.40	TP53BP1	29.33	13.93
HNRNPA1	22.60	24.93	NONO	29.00	21.60
HNRNPA3	22.33	24.13	NUP98	28.80	18.20
TRIM28	22.33	30.00	DDX17	28.60	22.33
ATP2A2	22.33	37.07	KRT1	28.53	16.07
DDX17	22.33	28.60	SRRM2	28.40	14.73
HP1BP3	22.07	26.53	NUP93	28.27	18.07
NUP205	21.73	34.47	ATP1A1	28.07	10.20
NONO	21.60	29.00	SMARCA5	28.00	13.07
MECP2	21.33	27.20	HNRNPA2B1	27.87	25.40
SFPQ	21.00	24.40	SMARCA2	27.87	11.40
ANXA3	20.87	20.40	SMARCC2	27.47	11.60
NOP56	20.67	23.80	RBM14	27.27	18.40
ZFR	20.60	29.60	MECP2	27.20	21.33
HBA1	20.47	11.07	ATP5F1B	26.60	17.27
CAMK2A	19.67	20.67	HP1BP3	26.53	22.07
RBMXRTL	19.20	22.73	CDC5L	26.27	14.93
HNRNPR	19.00	23.67	YWHAE	26.20	26.40
HNRNPK	18.73	22.80	NUP133	26.00	14.00

SUPPLEMENTARY FIGURES

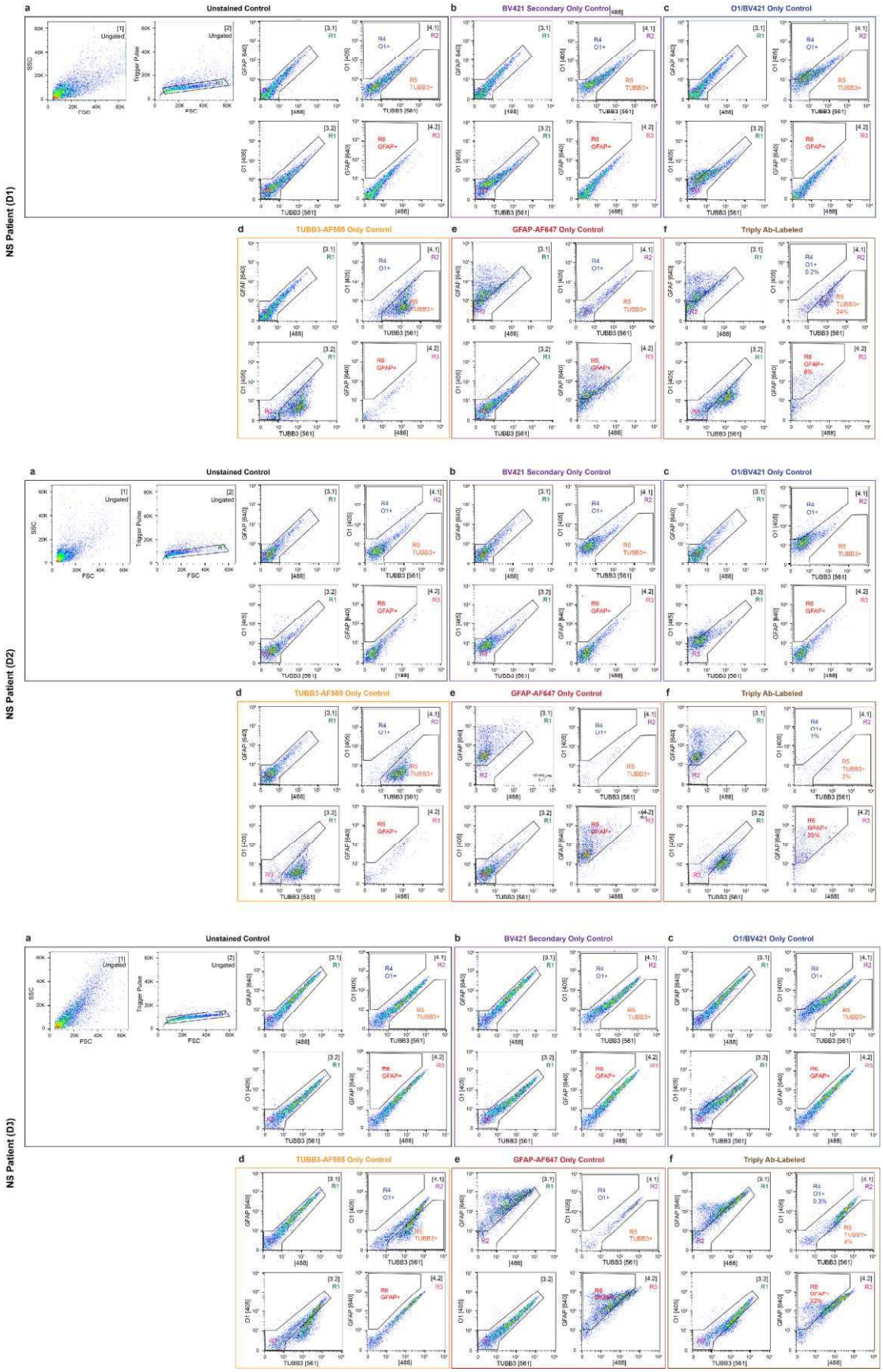
NS Patient (D1)
NS Patient (D2)
NS Patient (D3)
a
Unstained Control
b
BV421 Secondary Only Control
c
O1/BV421 Only Control
d
TUBB3-AF555 Only Control
e
GFAP-AF647 Only Control
f
Triply Ab-Labeled
[1] Ungated
[2] Ungated
[3.1] R1
[4.1] R2
[3.2] R1
[4.2] R3
R4 O1+
R5 TUBB3+
R6 GFAP+
SSC
FSC
Trigger Pulse
GFAP [640]
O1 [405]
TUBB3 [561]
[488]

Figure S1. Multi-color fluorescence-activated cell sorting of heterogeneous brain cell populations from non-symptomatic patients. All gating was established based on (a) unstained controls, was validated with (b) secondary only and (c-e) single stain controls, and was finally applied to (f) triply antibody-labeled experimental conditions. The gating strategy is as follows: [1] Distribution of events based on forward (FSC) and side (SSC) scatter properties. [2] Trigger pulse width assessment to exclude doublet/triplet events. "R1" gate represented singlet detection, which was then applied to initial bivariate gating schemes. [3] Bivariate assessment of fluorescence channels. Gating encompassed baseline autofluorescence ("R2" and "R3") and was subsequently applied to secondary bivariate gating schemes. Channel 488 was used as a placeholder for bivariate delineation. [4] Final detection of singly positive O1+ (blue), TUBB3+ (orange) populations [4.1], or GFAP+ (red) population [4.2]. Abbreviations: Ab, antibody; BV, Brilliant Violet; D, donor; FSC, forward scatter; R#, region; SSC, side scatter.

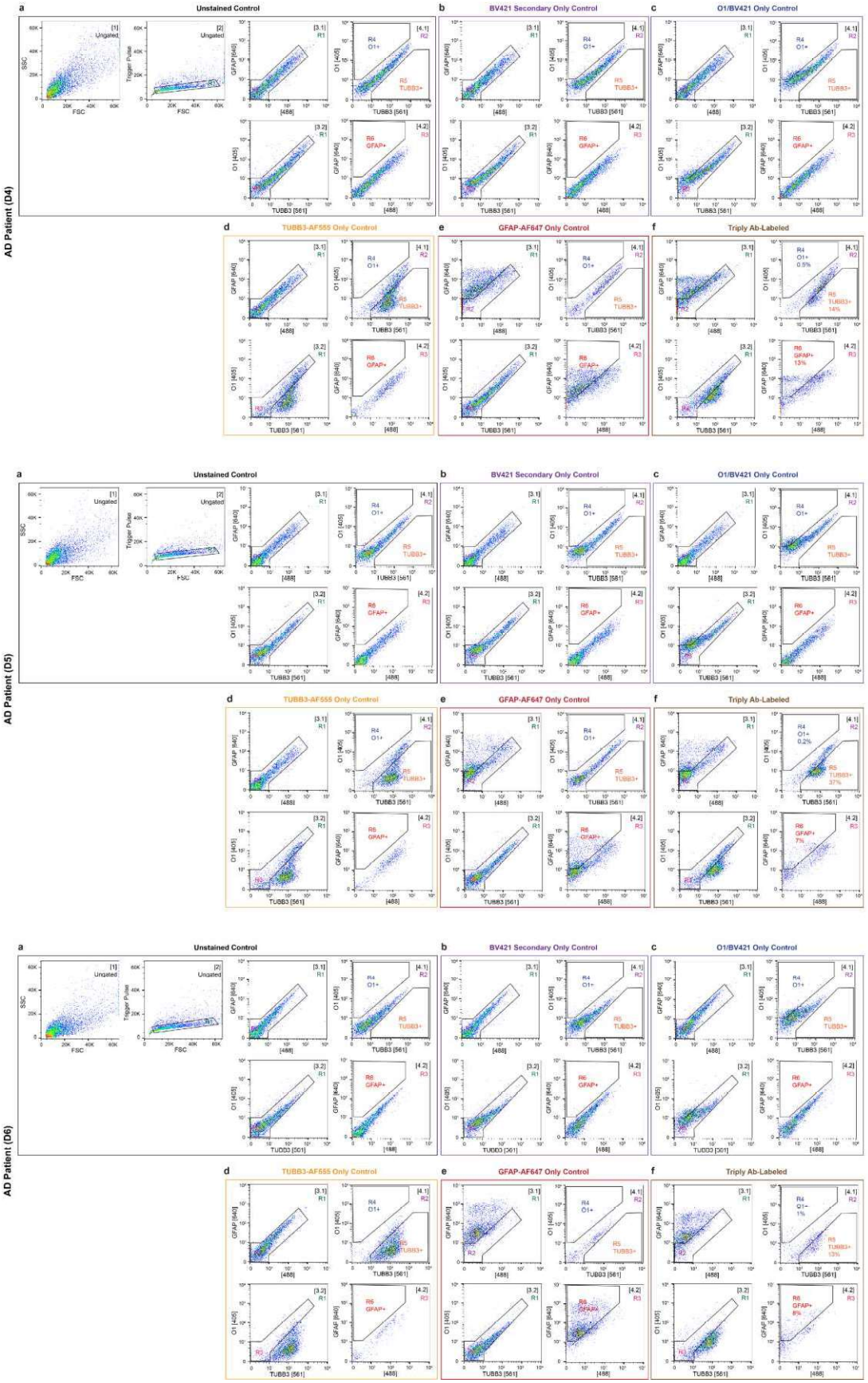
AD Patient (D4)
AD Patient (D5)
AD Patient (D6)
a Unstained Control
b BV421 Secondary Only Control
c O1/BV421 Only Control
d TUBB3-AF555 Only Control
e GFAP-AF647 Only Control
f Triply Ab-Labeled
[1] Ungated
[2] Ungated
SSC
FSC
Trigger Pulse
GFAP [640]
O1 [405]
TUBB3 [561]
[488]
[3.1] R1
[4.1] R2
[3.2] R1
[4.2] R3
R4 O1+
R5 TUBB3+
R6 GFAP+
R5 TUBB3+ 14%
R6 GFAP+ 13%
R4 O1+ 0.5%
R4 O1+ 0.2%
R5 TUBB3+ 37%
R6 GFAP+ 7%
R4 O1+ 1%
R5 TUBB3+ 13%
R6 GFAP+ 8%

Figure S2. Multi-color fluorescence-activated cell sorting of heterogeneous brain cell populations from Alzheimer's disease patients. All gating was established based on (a) unstained controls, was validated with (b) secondary only and (c-e) single stain controls, and was finally applied to (f) triply antibody-labeled experimental conditions. The gating strategy is as follows: [1] Distribution of events based on forward (FSC) and side (SSC) scatter properties. [2] Trigger pulse width assessment to exclude doublet/triplet events. "R1" gate represented singlet detection, which was then applied to initial bivariate gating schemes. [3] Bivariate assessment of fluorescence channels. Gating encompassed baseline autofluorescence ("R2" and "R3") and was subsequently applied to secondary bivariate gating schemes. Channel 488 was used as a placeholder for bivariate delineation. [4] Final detection of singly positive O1+ (blue), TUBB3+ (orange) populations [4.1], or GFAP+ (red) population [4.2]. Abbreviations: Ab, antibody; BV, Brilliant Violet; D, donor; FSC, forward scatter; R#, region; SSC, side scatter.

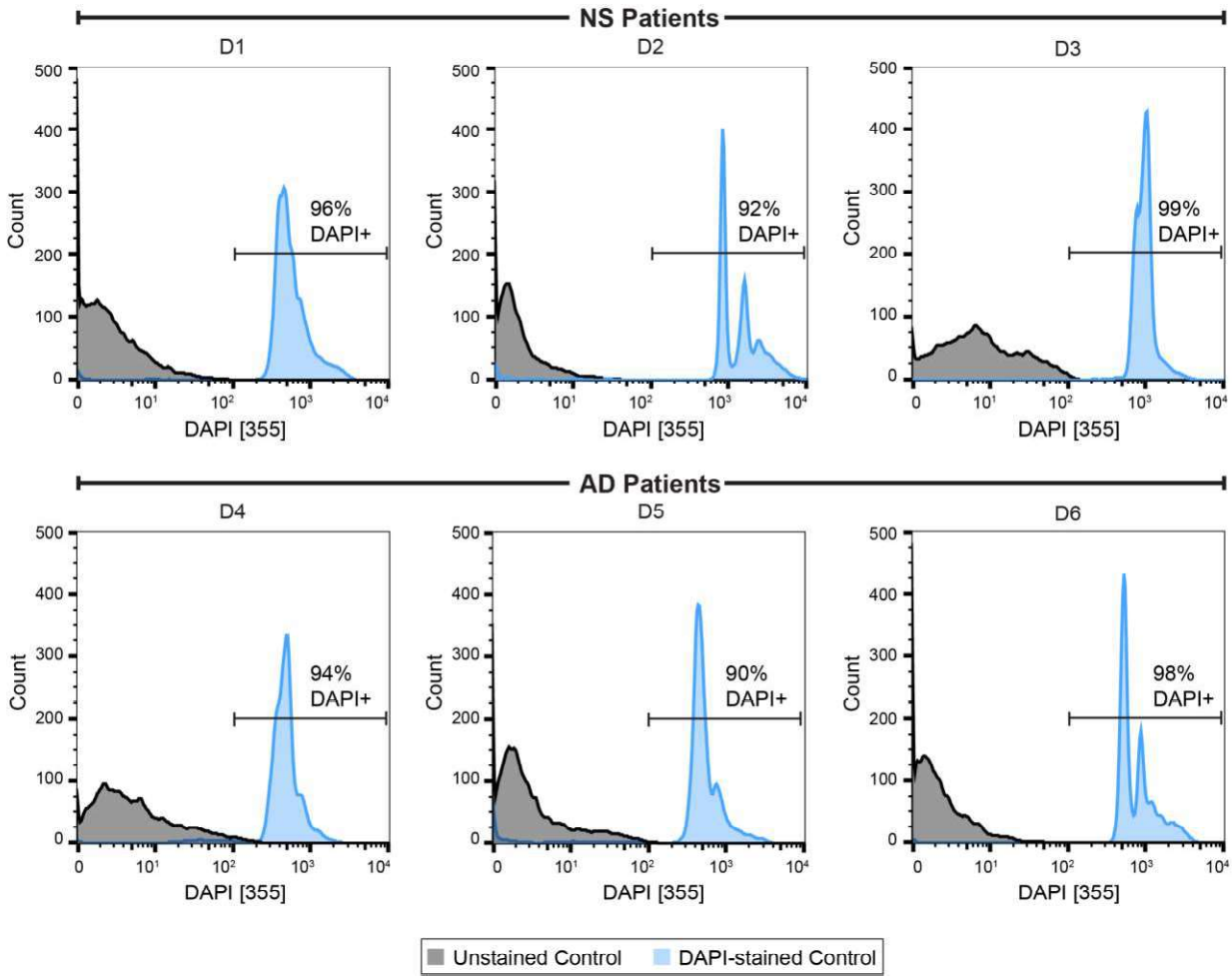

Figure S3. Flow cytometry analysis of DAPI-stained donor samples. Each histogram depicts an unstained control (grey) and a DAPI-stained sample (blue). Percentages of DAPI+ events are listed for each donor.

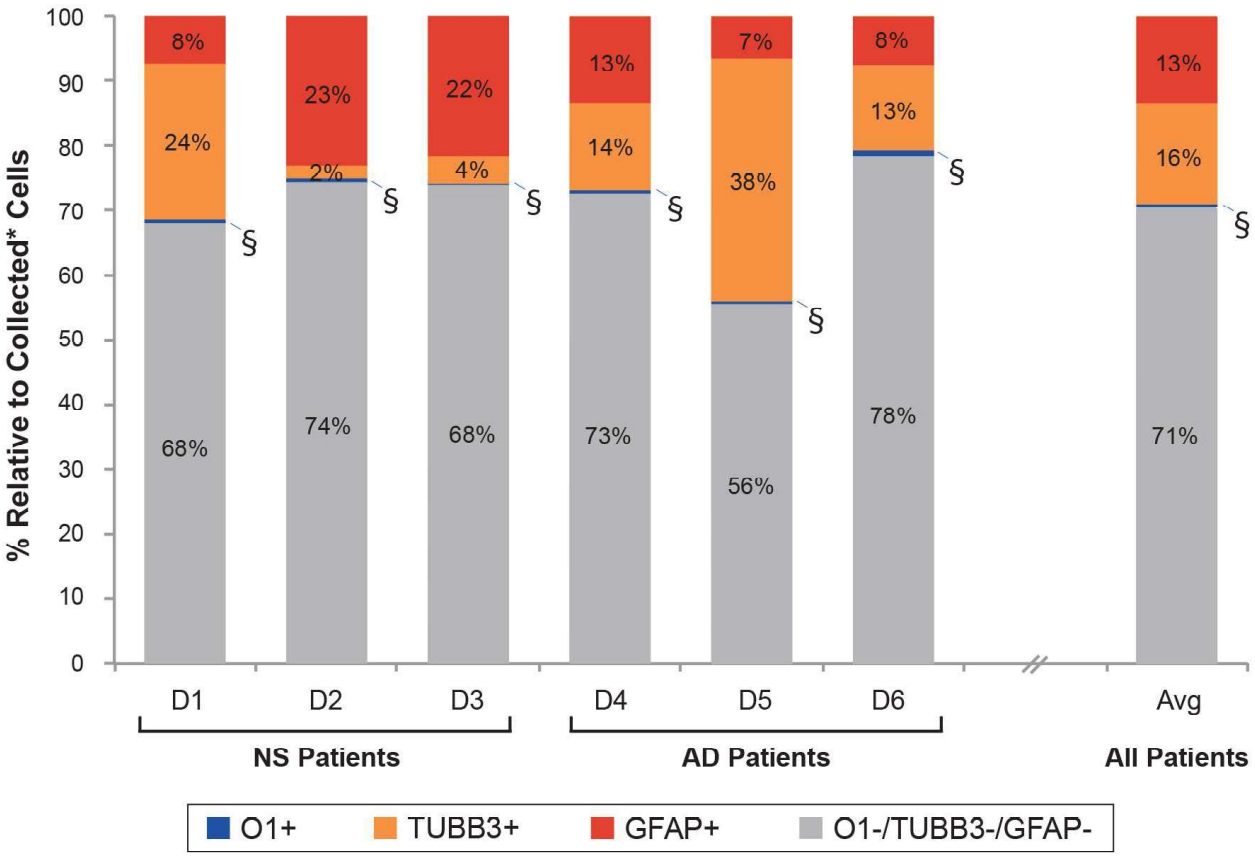

Figure S4. Proportions of individual cell types collected from non-symptomatic and Alzheimer's disease donors by fluorescence-activated cell sorting. Raw data are presented for each donor sample, and mean data are presented for all patients combined. O1 cell populations (§) made up <1% of cell total in all donors assessed. A 2-way ANOVA was performed to determine significance among disease states ($P = 1$), cell types ($P < 0.001$), and interactions between factors ($P = 0.082$). D, donor; Avg, average. Abbreviations: R#, region #; y.o., years old.

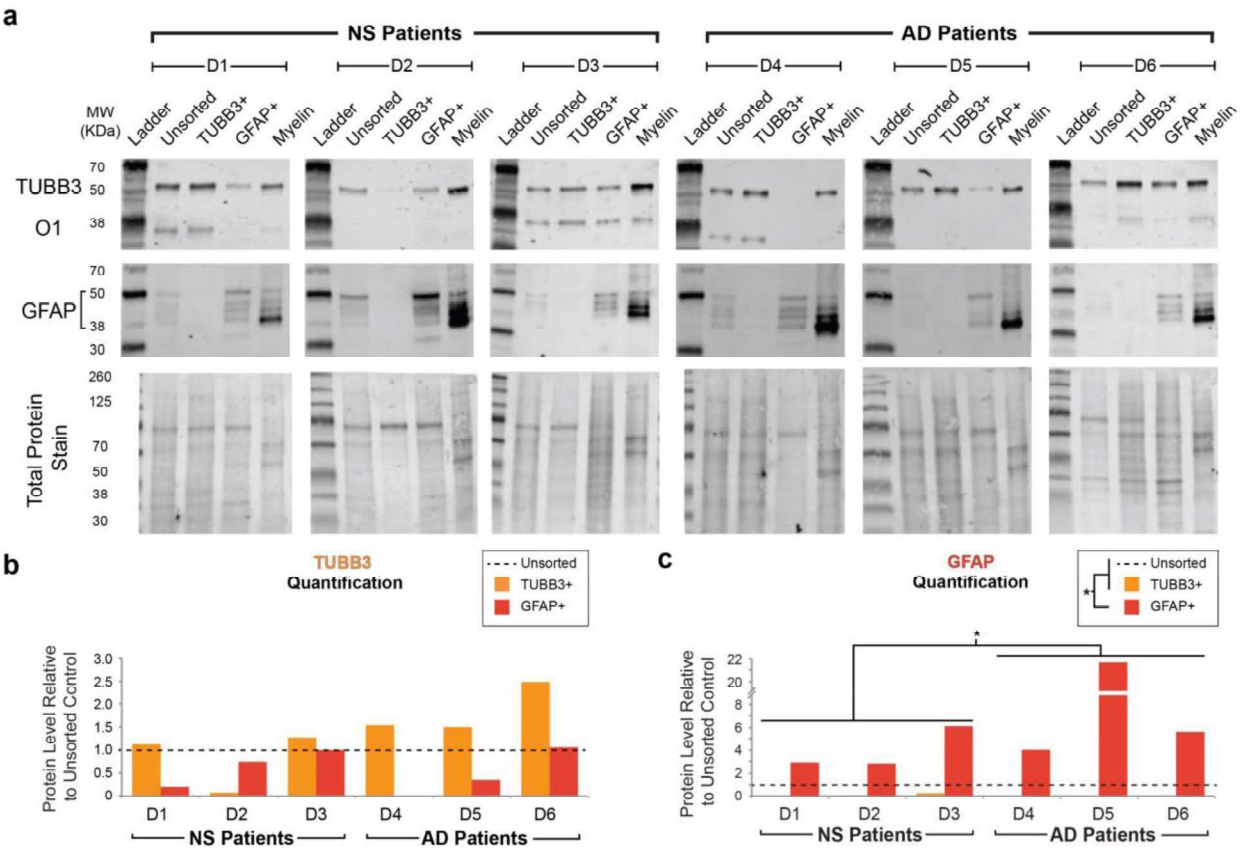

Figure S5. Western blots supported validity of successful neural cell enrichment for neurons (TUBB3+) and astrocytes (GFAP+) isolated from non-symptomatic and Alzheimer's disease brain. Donors 1 and 4 were shown in main Figure 3e. (a) Western blots of post-FACS, collected cell populations and myelin debris protein lysates for non-symptomatic (NS) and Alzheimer's disease (AD) donors 2 and 3, assessing TUBB3 (55 kDa), O1 (35 kDa), GFAP (38-50 kDa), and Total Protein Stain. Densitometry-based quantification of (b) TUBB3 and (c) GFAP (50 kDa isoform). Dashed line indicates the normalized unsorted control protein level. 2-way ANOVA were performed to determine significance among disease states, cell types, and interactions between factors (* denotes a statistically significant difference for indicated comparison, $P < 0.005$).

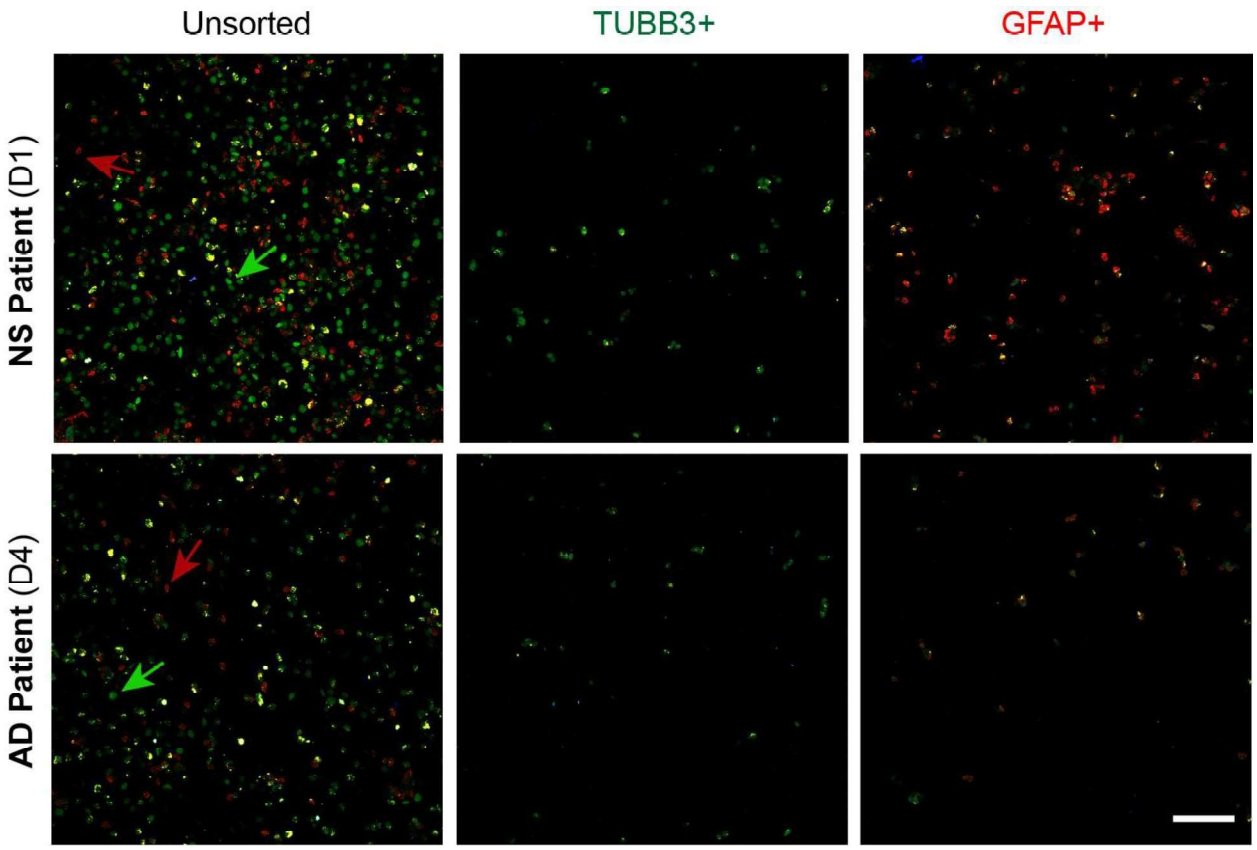

Figure S6. Low magnification confocal images of unsorted, neurons (TUBB3+), and astrocytes (GFAP+) from non-symptomatic and Alzheimer's disease brain. Green, TUBB3 protein; Red, GFAP protein; Yellow, overlap between TUBB3+ and GFAP+ protein. Arrows highlight cells types of interest. Scale bar, 100 µm.

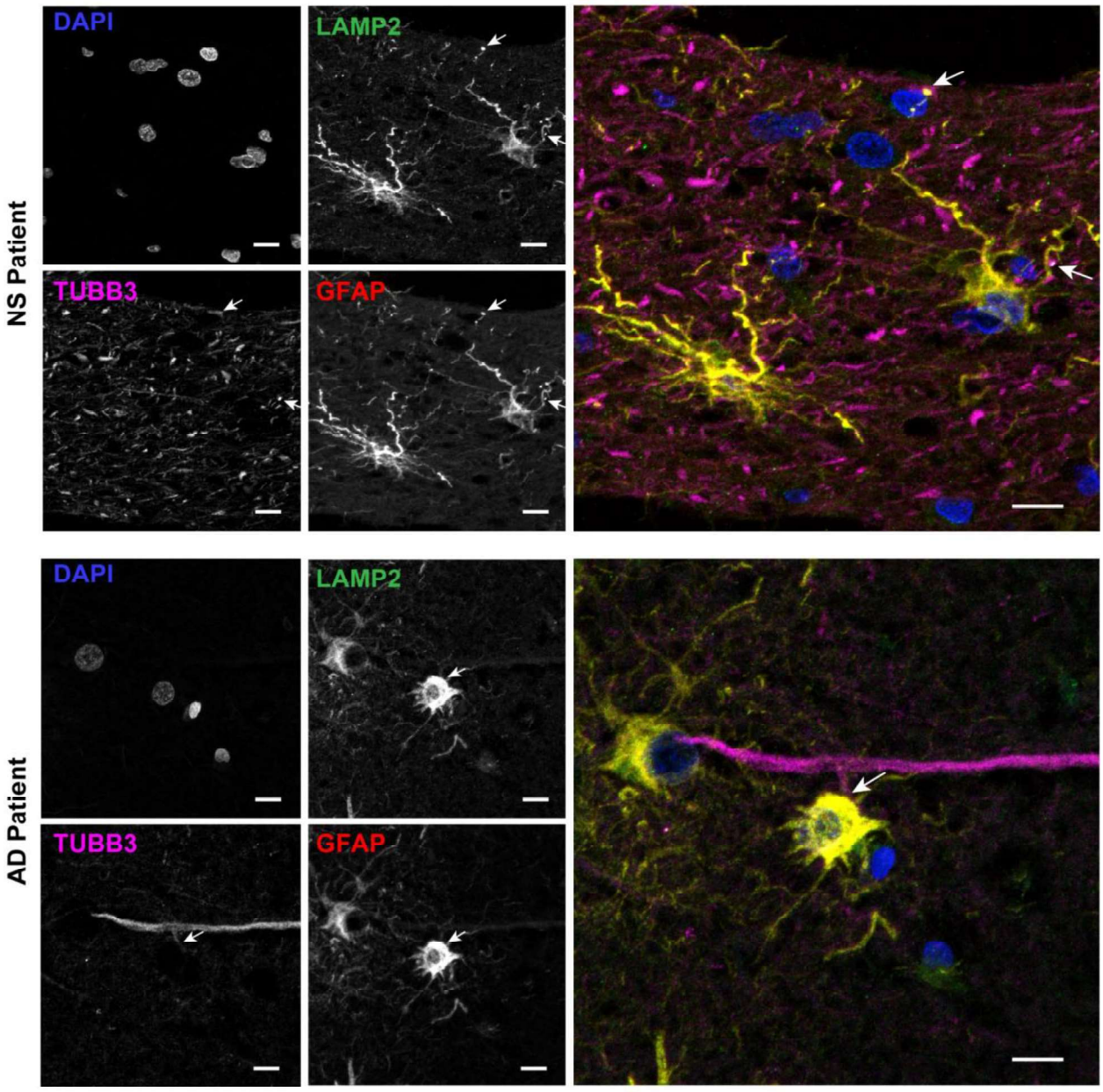

Figure S7. High magnification confocal images of non-symptomatic and Alzheimer's disease brain tissue sections. Blue, DAPI; Green, LAMP2 protein; Magenta, TUBB3 protein; Red, GFAP protein. Arrows highlight TUBB3 protein localization to LAMP2+ lysosomes in GFAP+ astrocytes. Scale bars, 10 μm.