Supplemental material

Xu et al., **https://doi.org/10.1084/jem.20171285**

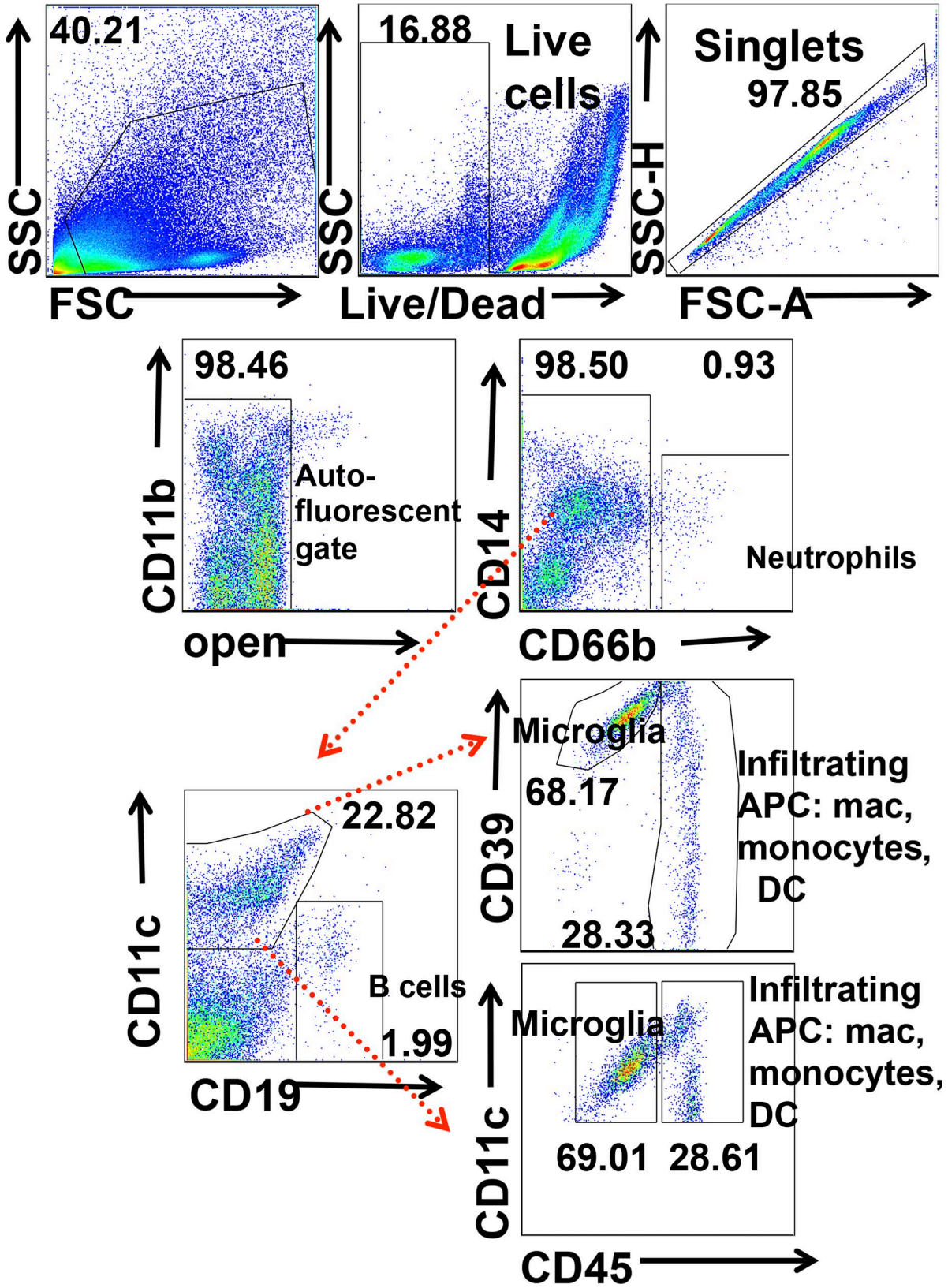

Figure S1. **Gating strategy for microglia and brain-infiltrating APCs.** Patient FCD20 is shown as a representative to demonstrate the sequential gating strategy used to discriminate neutrophils (CD66b$^+$), B cells (CD66b$^-$/CD19$^+$CD11c$^-$), microglia (CD66b$^-$/CD19$^-$/CD11c$^+$/CD39^{hi}/CD45^{int}), and infiltrating APCs (CD66b$^-$/CD19$^-$CD11c$^+$/CD39^{int}/CD45^{hi}), including macrophages, monocytes, and DCs. FSC, forward scatter; SSC, side scatter. Numbers in the flow plots indicate the frequencies of positive cell populations.

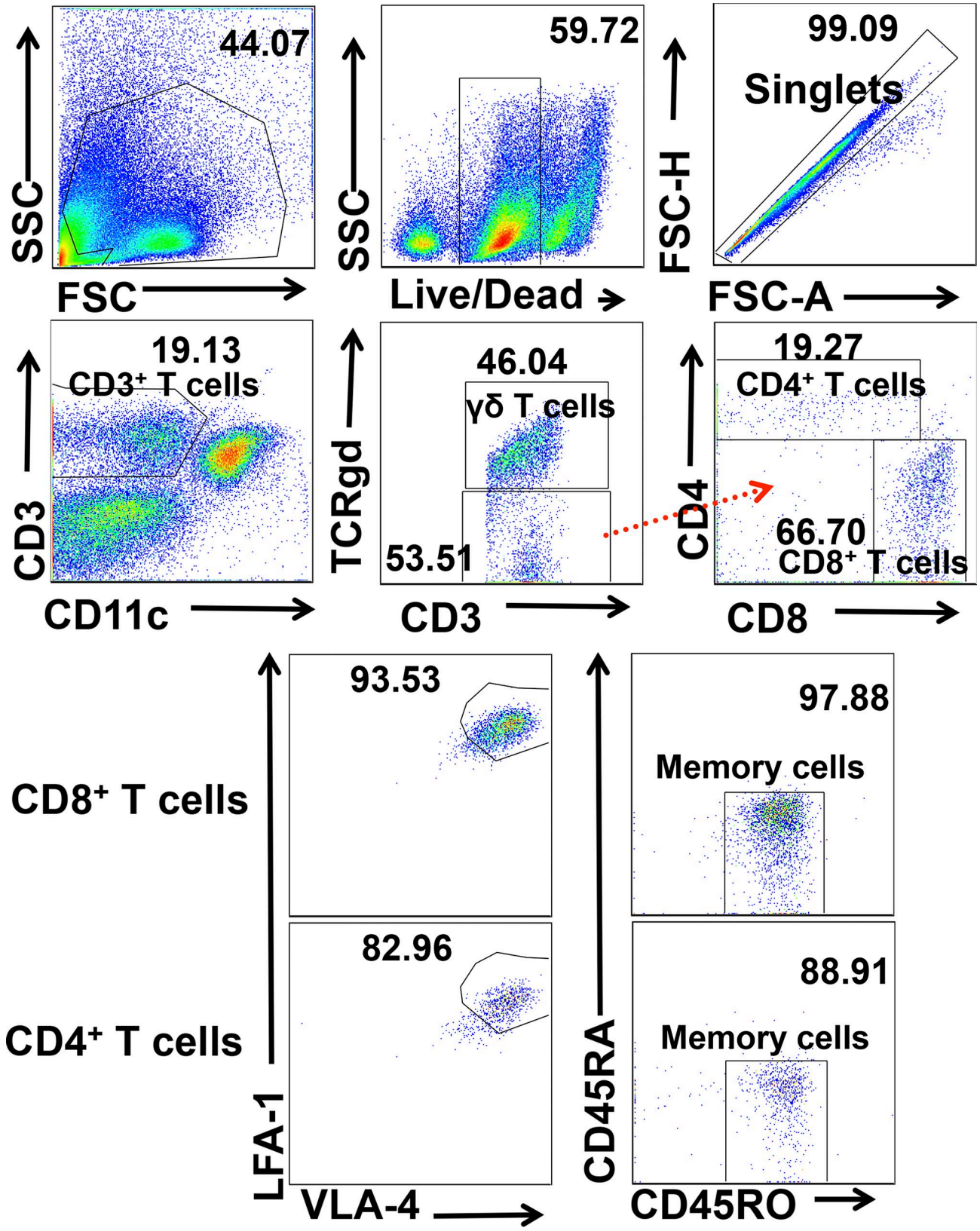

Figure S2. **Gating strategy for T cells.** Patient EM5 is shown as a representative to demonstrate the sequential gating strategy used to discriminate γδ T cells (CD11c/CD3$^+$/TCRγδ$^+$), CD4$^+$ T cells (CD11c$^-$/CD3$^+$/TCRγδ$^-$/CD8$^-$/CD4$^+$), CD8$^+$ T cells (CD11c$^-$/CD3$^+$/TCRγδ$^-$/CD4-/CD8$^+$), and memory T cells (CD45RO$^+$/CD45RA$^-$). FSC, forward scatter; SSC, side scatter. Numbers in the flow plots indicate the frequencies of positive cell populations.

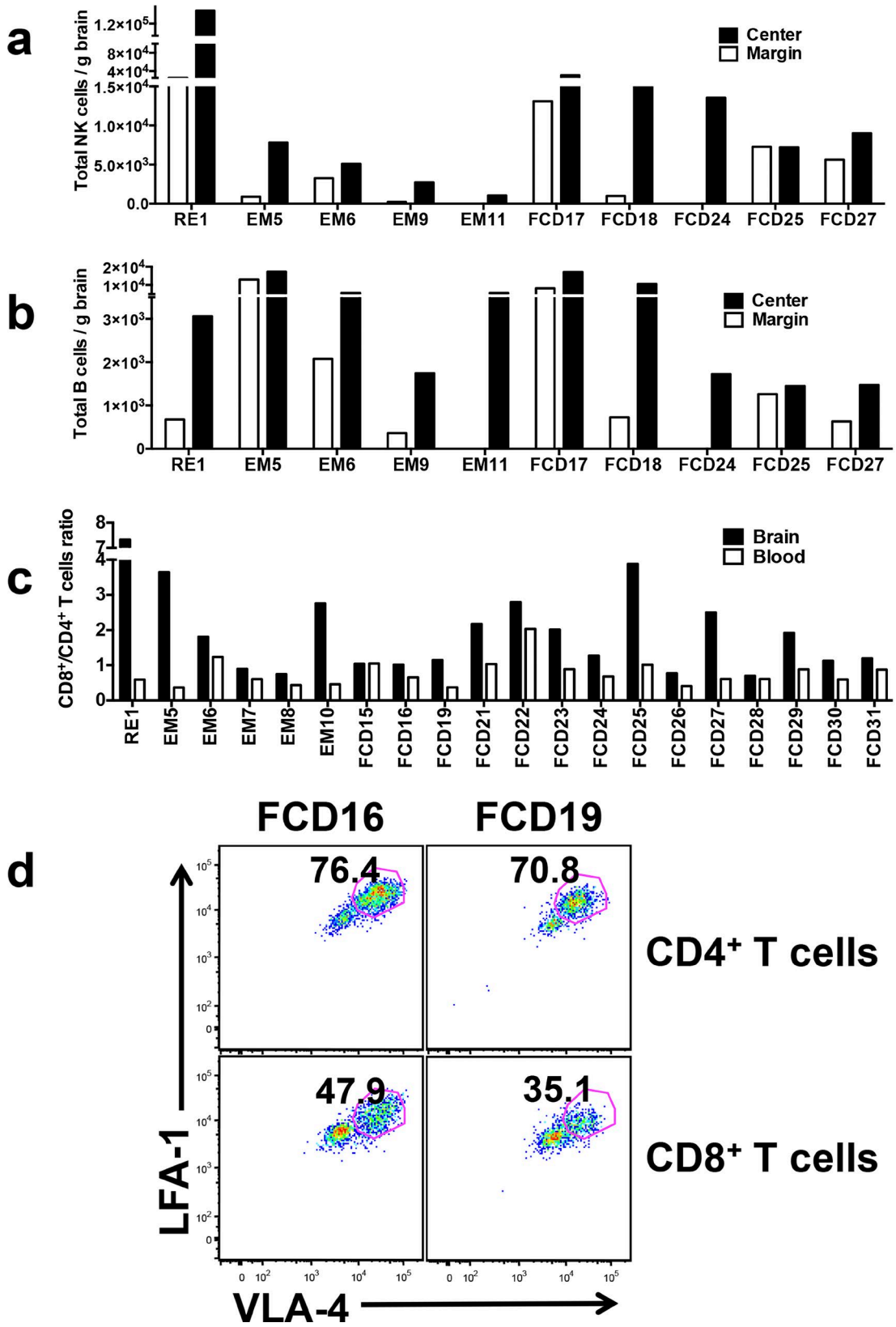

Figure S3. **Numbers of NK cells and B cells in epileptogenic lesions. (a and b)** Absolute count of NK (a) and B cells (b) in the margin and epileptogenic center from representative patients in each diagnosis category as assessed by flow cytometric analysis. **(c)** Ratio of $CD8^+/CD4^+$ T cells in the epileptogenic center and peripheral blood. **(d)** Visualization of integrin molecule expression by T cells in the epileptogenic center. VLA-4 and LFA-1 expression on $CD4^+$ and $CD8^+$ T cells were determined by flow cytometric analysis in representative patients. Numbers in the flow plots indicate the frequencies of positive cell populations.

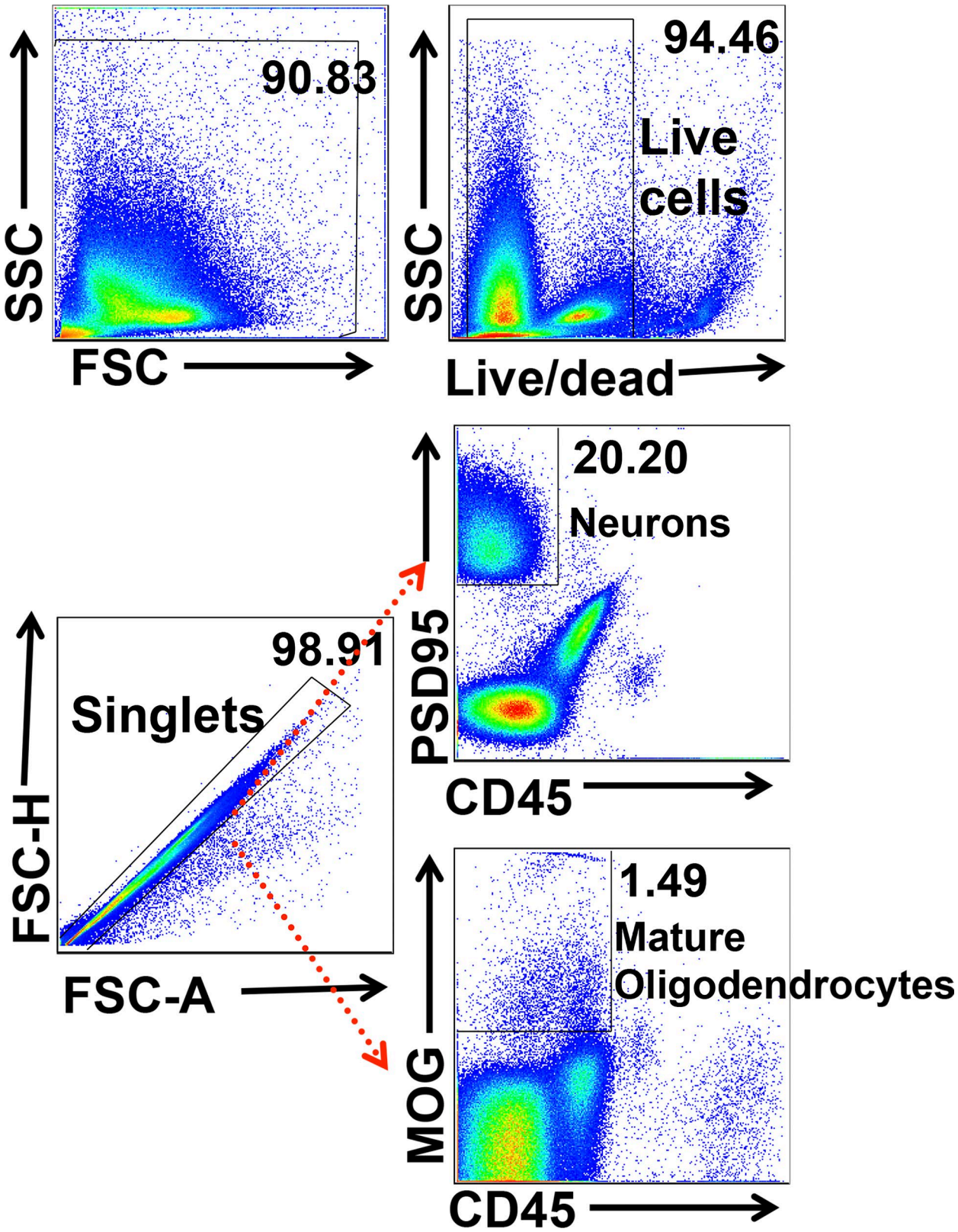

Figure S4. **Gating strategy for brain resident cells.** PatientFCD26 is shown as a representative to demonstrate the sequential gating strategy used to discriminate neurons ($CD45^-/PSD95^+$) and mature oligodendrocytes ($CD45^-/MOG^+$). FSC, forward scatter. Numbers in the flow plots indicate the frequencies of positive cell populations.

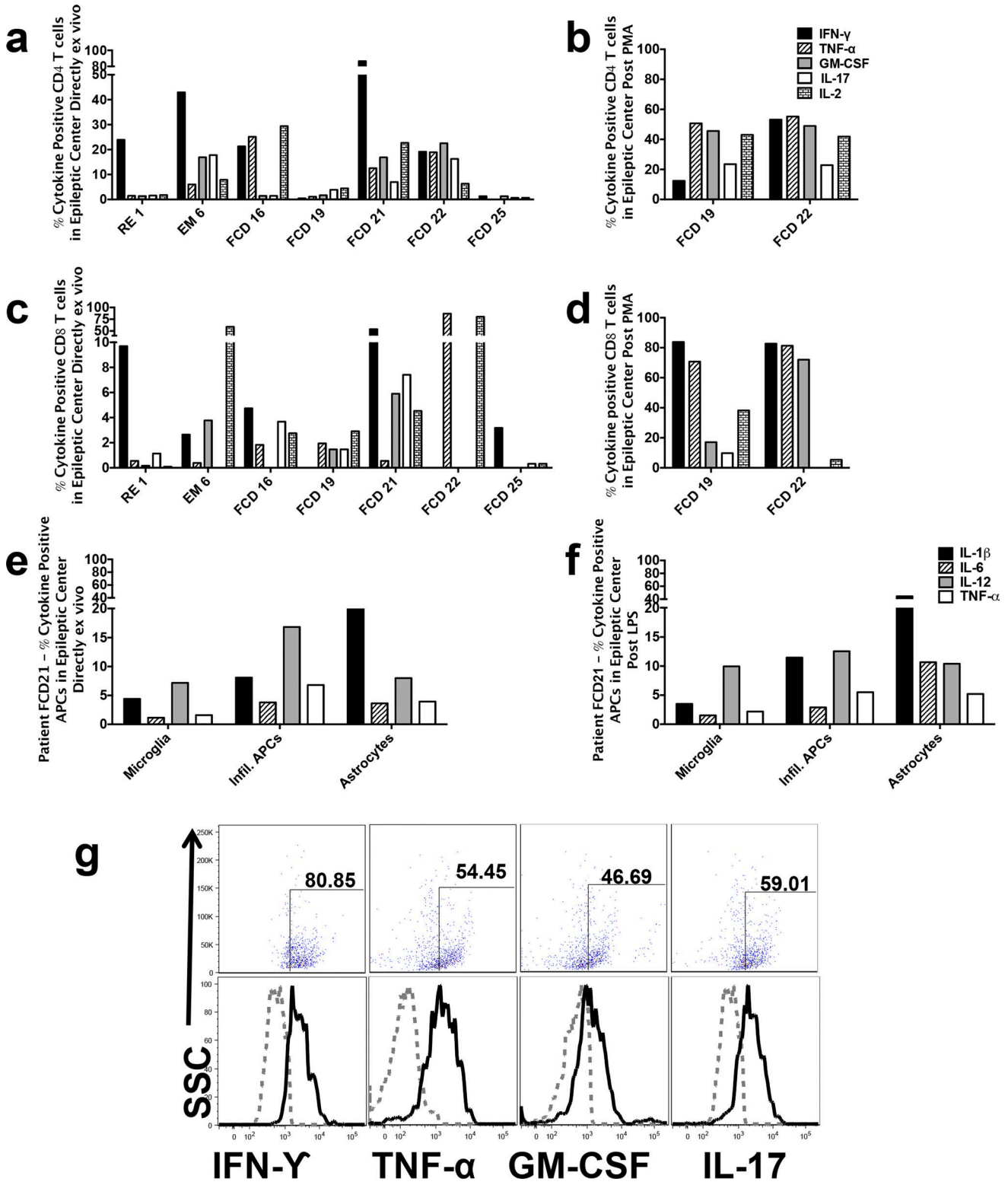

Figure S5. **Cytokine profiles of brain-infiltrating T cells. (a–d)** Percentages of cytokine producing CD4⁺ (a and b) and CD8⁺ (c and d) T cells from the epileptogenic center of patients without (a and c) and with (b and d) stimulation by T cells mitogens determined by intracellular cytokine staining. **(e and f)** Percentage of cytokine-producing brain-resident and brain-infiltrating APCs without (e) or after stimulation with lipopolysaccharide (LPS; f). Intracellular cytokine staining profiles of brain-infiltrating γδ T cells. Patient FCD22 is shown as a representative to demonstrate gating strategy used to discriminate cytokine positive versus negative γδ T cells. **(g)** Number shown is the percentages of cytokine producing γδ T cells from the epileptogenic center without stimulation by T cells. SSC, side scatter. Numbers in the flow plots indicate the frequencies of positive cell populations.

Table S1. **Characteristics of clinical findings in patients used in this study.**

Patient		Dx	Age (yr) at		Seizure			IQ	AED		Post-op f/u		MRI lesion
No.	S		Op	Onset	Dur (yr)	Freq/ month	Feb sz		Past	Current	Dur (yr)	Sz	
1	F	RE	15	6	9	>100	N	74	OXC PHT TPM LVT LTG LCM CLB IVIG LZP	CLB LZP DZP	1	free	L hem
2	F	RE	6	4	2	>300	N	NA	LVT LZP IVIG	PB LVT LZP	1	sz	R temp
3	F	MTLE	7	1.3	6	12-20	Y	99	PB LVT	OXC	2	free	L temp
4	M	MTLE	12	3	9	10	Y	NA	CBZ LVT TPM	CBZ CLB	4.5	free	L temp
5	M	EM	15	0.4	3	>450	Y	NA	ACTH LZP DZP VPA LTG PB PHT TPM ZNS LTM CBZ VGB	LTG DZP	1	free	R hem
6	M	EM	8	1	7	30-60	Y	MR	TPM PB CLB steroid	VGB RUF CZP LTG CLB DZP	1	sz	L frontal cyst
7	M	EM	2	0.1	2	>600	Y	NA	LVT ACTH VGB	LVT CLB LCM	<1	free	L hem
8	M	EM	2	0.5	1.5	8-16	Y	NA	ACTH VGB LVT	VGB	1	free	L PTO
9	M	EM	5	0.1	5	8-16	Y	NA	VPA CBZ CZP	LTG DZP	2	free	L hem
10	M	EM	17	0.1	17	2-8	Y	42	CBZ TPM VPA LVT PB DZP CZP GBP	RUF CLB DZP	1	free	L temp
11	M	EM	17	0.4	7	1-30	Y	92	CBZ	LVT CBZ	2	sz	L frontal
12	F	EM	8	6	2	>30	N	69	CBZ VPA LTM ZNS LTG DZP OXC	VPA DZP	3	free	R temp
13	M	EM	13	9.8	3	4-8	N	78	OXC TPM CBZ MAD	LVT LTG ZNS	3	free	R temp
14	F	EM	9	7	2	5-15	N	55	OXC LVT ZNS TPM LCM	LVT ZNS DZP	4	free	L hem
15	F	FCD 2B	14	7	7	60-150	N	NA	OXC TPM LCM CLB	LTG LVT	< 1	sz	L temp
16	F	FCD 3	18	12	6	2-60	N	97	OXC LVT	OXC LVT DZP	1	free	R temp
17	M	FCD 2A	4	1.8	2	15-375	N	75	LVT OXC VPA CZP FBM ACTH	RUF	3	free	L frontal
18	M	FCD 2B	1	0.6	0.4	>100	Y	NA	LVT VPA CZP ZNS ACTH VGB	VPA LVT	3	free	L frontal
19	M	FCD 2A	2.5	0.1	2	>450	Y	NA	PB LVT TPM LCM	LVT	2	free	L PTO
20	M	FCD 2A	9	3	6	>450	N	NA	CLZ VPA steroid LVT OXC TPM CLB AZ PHT	VPA DZP	3	free	L frontal
21	M	FCD 2A	2	0.5	1.5	30-60	N	NA	LVT PB DZP ZNS steroid	OXC ZNS LVT	1	free	R hem
22	M	FCD 2A	19	16	3	1-2	N	81	OXC LTG LVT LCM	OXC LCM	1	free	R frontal
23	F	FCD 2A	10	4	6	< 1	N	NA	LVT OXC TPM	LVT steroid	1	aura	L frontal
24	M	FCD 2A	4	3.5	0.5	>100	N	NA	LVT OXC VPA TPM	CLB CBZ ZNS CZP	1.5	sz	L temp
25	F	FCD 2A	11	0.1	11	30	Y	43	PB PHT OXC ZNS LVT steroid IVIG LTG CLB CZP VPA	VPA LTG OXC LCM GBP DZP	21	sz	L PTO
26	F	FCD 2A	1	0.2	1	150-300	Y	NA	ACTH VGB steroid	PB ZNS	< 1	free	R hem
27	M	FCD 2A	18	10	8	30-120	N	71	CBZ LVT LTG	CBZ LTG MDL	1	free	R hem
28	F	FCD 2B	2.4	1.4	1	30-60	Y	NA	OXC VPA ACTH	CLB	2	free	L frontal
29	M	FCD 2A	12	7	5	2-3	N	NA	OXC LTG ZNS	DZP LTG ZNS MDL	< 1	free	L temp
30	F	FCD 2A	2	0.3	1.5	30-60	N	NA	PB TPM VGB	LVT CLB	< 1	sz	R frontal
31	F	FDC 2A	1	8.25	2	8	N	NA	LVT	CBZ ZNS	< 1	free	R hem

Table S1. **Characteristics of clinical findings in patients used in this study.** *(Continued)*

Patient		Dx	Age (yr) at		Seizure			IQ	AED		Post-op f/u		MRI lesion
No.	S		Op	Onset	Dur (yr)	Freq/ month	Feb sz		Past	Current	Dur (yr)	Sz	
32	M	FCD 2B	9	4	5	30-60	N	77	LVT PB PHT VPA OXC ZNS	OXC	3	free	L frontal
33	M	FCD 1	11	5	6	4-8	N	83	DZP LTG	LTG	2	free	R temp

Mean values ± SD. Age at operation was 8.7 ± 5.8 yr. Age at seizure onset was 3.8 ± 4.1 yr. Epilepsy duration was 4.6 ± 3.6 yr. Steroid designates prednisone or dexamethasone 10-30 day course. Number of AEDs used: past AEDs, 4.7 ± 3.1; current AEDs, 2.6 ± 1.3. Abbreviations used: ACTH, adrenocorticotrophin hormone; AED, antiepileptic drug treatment; CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; Dur, duration; Dx, diagnosis; DZP, diazepam; F = female; f/u, follow-up; Feb sz, febrile seizure; freq, frequency; Hem, Hemisphere; Inf, inferior; IQ, full-scale intelligence quotient; IVIG, i.v. Ig monthly infusion; L, left; LCM, lacosamide; LTG, lamotrigine; LVT, levetiracetam; LZP, lorazepam; M, male; MR, mental retardation; NA, not available; Op, operation; OXC, Oxcarbazepine; PB, pyridostigmine bromide; PHT, phenytoin; PTO, parietal temporal occipital; R, right; S, sex; steroid, prednisone or dexamethasone 10–30 d course; Sz, seizure; Temp, temporal lobectomy; TPM, topiramate; VGB, vigabatrin; VPA, Valproic acid; ZNS, Zonisamide.