

Neuroinflammatory Signatures of Complement Component 4 in the Subventricular Zone of Autism Spectrum Disorder and Schizophrenia

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ABSTRACT

In addition to an early inflammatory insult, genetic variants that impinge upon immune function are among the most recognized risk factors associated with neurodevelopmental psychiatric disorders. Among these, complement component 4 (C4), a molecule involved in inflammatory responses, has been strongly associated with schizophrenia (SZ). However, whether C4 is involved in other neurodevelopmental disorders, such as autism (ASD), is a matter of ongoing investigation. Moreover, while C4 in SZ has been implicated in the context of synaptic pruning, little is known about its neuroinflammatory role. The subventricular zone (SVZ) is a region heavily involved in neurodevelopment and neuroimmune interactions through the lifespan; thus, it is a region wherein C4 may play a vital role in disease pathology. Using *in situ* hybridization with radioactive riboprobes and RNAscope, we identified robust astrocytic expression of C4 in the SVZ and in the septum pellucidum. C4 was also expressed in ependyma, neurons, and Ki67⁺ progenitor cells. Examination of mRNA levels showed elevated C4 in both ASD and SZ, with higher expression in SZ compared to controls. Targeted transcriptomic analysis of inflammatory pathways revealed a strong association of complement system genes with SZ, and to a lesser extent, ASD, as well as generalized immune dysregulation without a strong association with known infectious pathways. Analysis of differentially expressed genes (DEGs) showed that ASD DEGs were enriched in adaptive immune system functions such as Th cell differentiation, while SZ DEGs were enriched in innate immune system functions, including NF- κ B and toll like receptor signaling. Moreover, the number of Ki67⁺ cells was significantly higher in ASD compared to SZ and controls. Taken together, these results support a role for C4 into inflammatory-neuroimmune dysregulation observed in SZ and ASD pathology.

TABLE 1

Characteristics	Group (no. of tissue sample donors)		
	TD control (n=16)	ASD (n=16)	SZ (n=16)
Age, mean \pm SD, yr	21.00 \pm 3.58	21.94 \pm 9.28	37.81 \pm 8.11
Sex, F:M	3:13	4:12	6:10
RIN, mean \pm SD	7.89 \pm 0.79	7.93 \pm 0.81	6.72 \pm 1.42
PMI, mean \pm SD, h	17.63 \pm 5.30	25.25 \pm 9.03	19 \pm 6.16
Race			
White	9	14	7
Black	7	2	9

TD= typical development; ASD= autistic spectrum disorder; SZ= schizophrenia; SD= standard deviation; RIN= RNA integrity number; PMI= post-mortem interval

Anatomical distribution of C4 mRNA in the SVZ. Photomicrographs of C4 *in situ* hybridization in the SVZ and surrounding areas. (A, B) Reference atlas showing human brain anatomy in the coronal plane: cingulate cortex (Cg), corpus callosum (cc), subventricular zone (SVZ), lateral ventricle (lv), caudate nucleus (Ca), and internal capsule (ic). (C): Representative Nissl staining of a coronal section at the level of (A, B); septum pellucidum (SP). Scale bar = 1 mm. Autoradiographic images of the mRNA signal for C4 (D) and control sense probe (E). (F-K) Representative images of C4 emulsion autoradiography (400X magnification) from regions indicated in (D); scale bar = 10 μ m. (F) SVZ; ependyma (e), hypocellular gap (h), astrocytic ribbon (ar); (G-H) ventricular zone; (I) SP; (J) cc and (K) caudate. * blood vessel

RESULTS

FIGURE 1

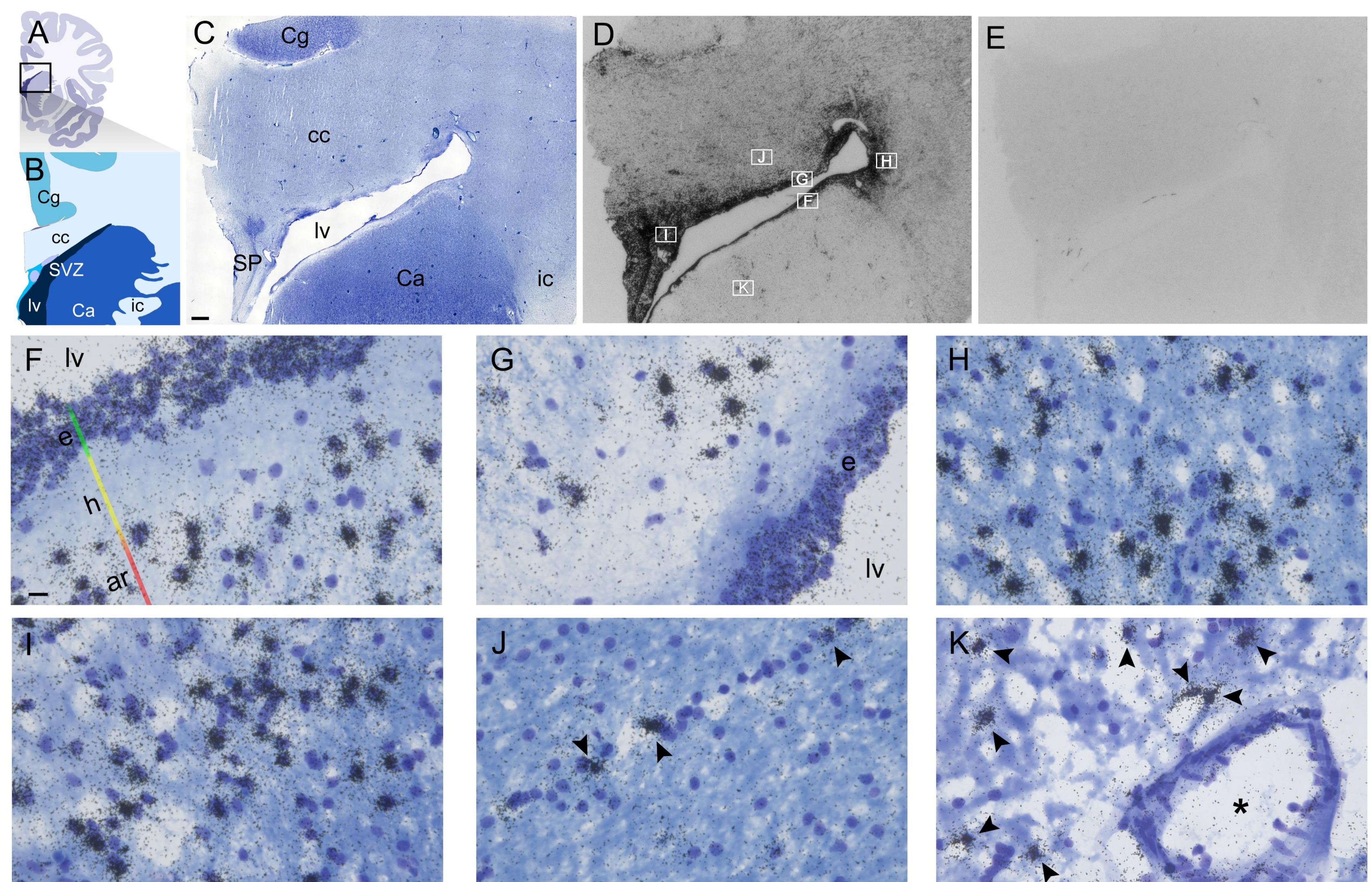
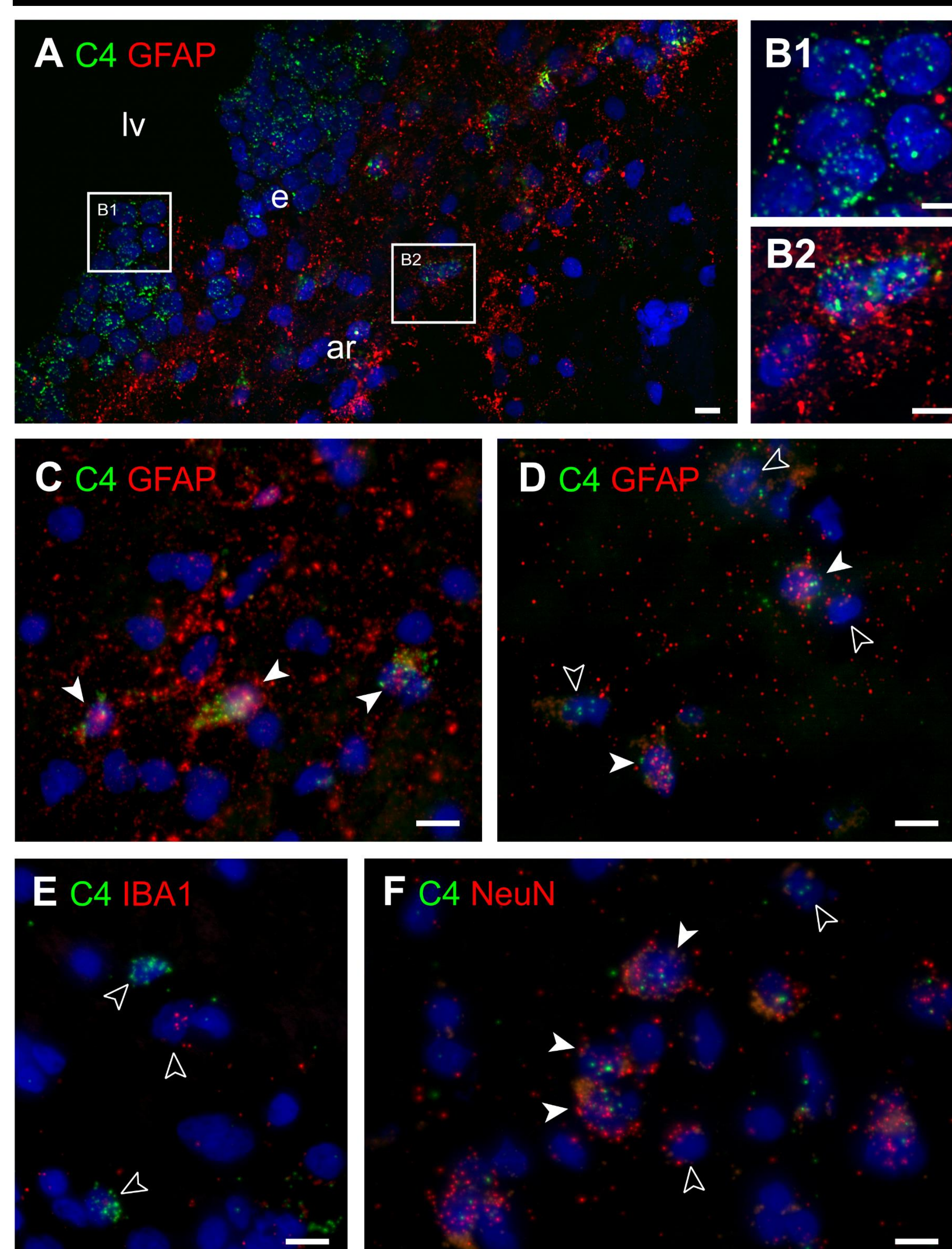
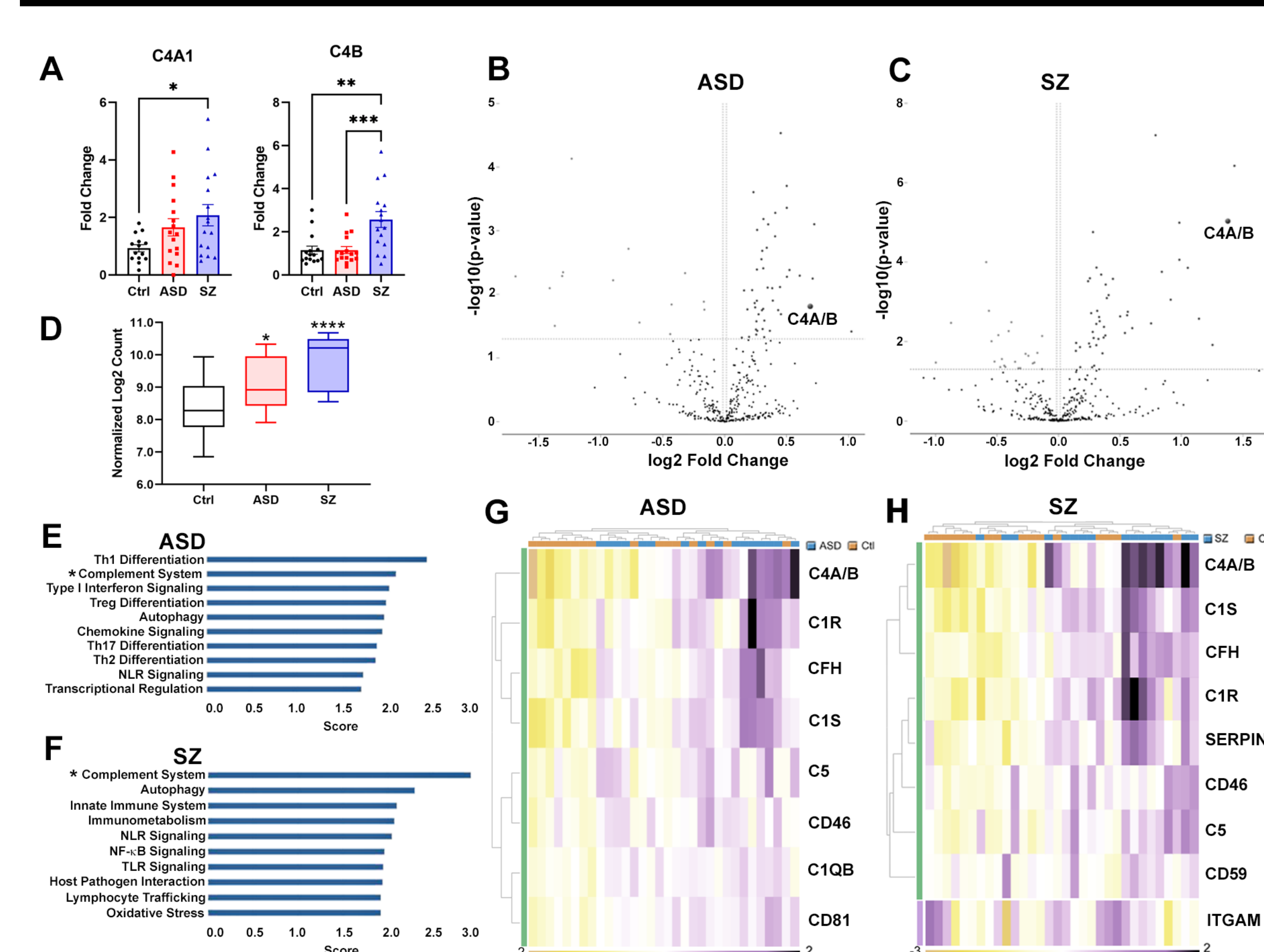


FIGURE 2



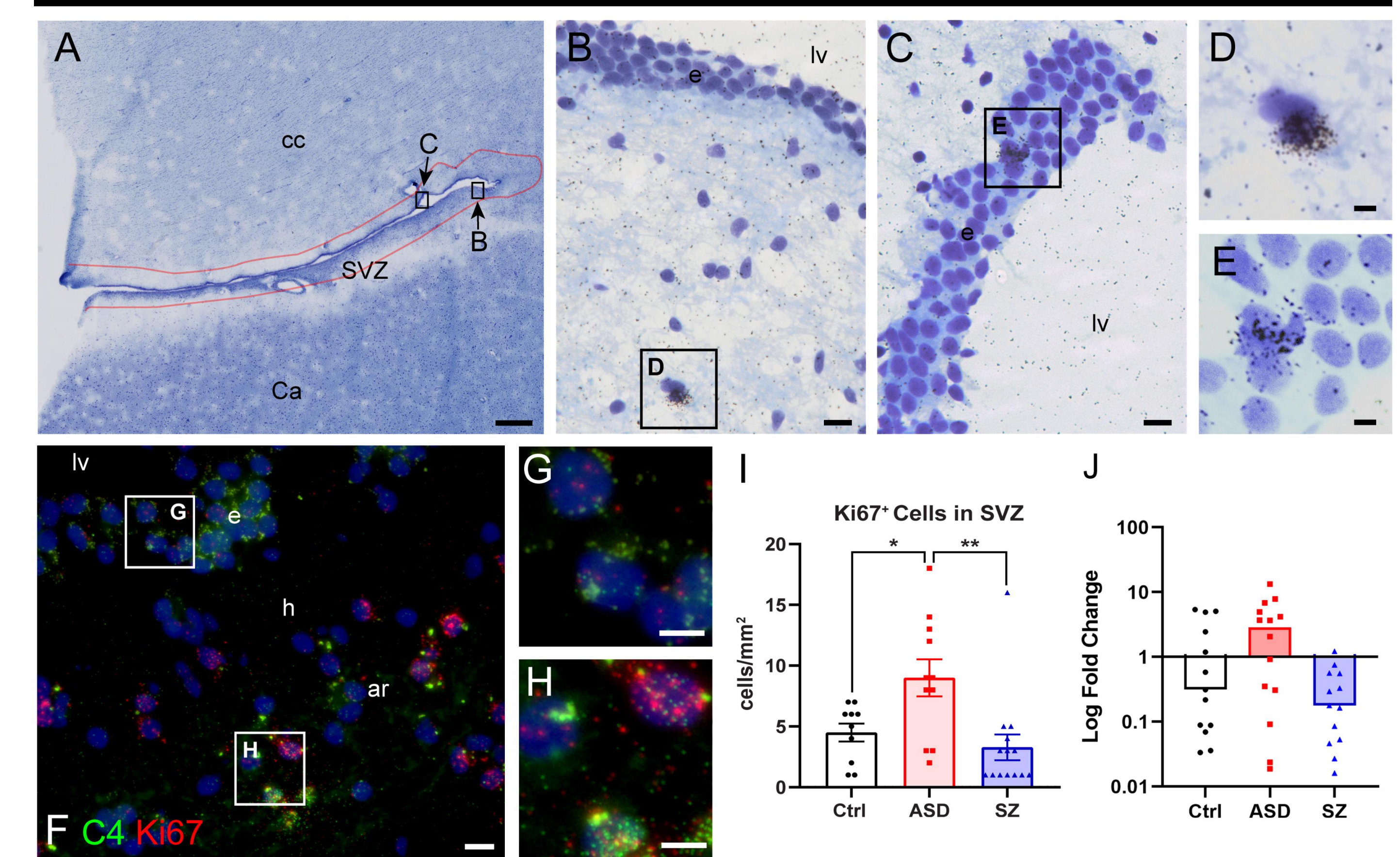
Cell-type specific expression of C4. (A) C4 (green) and GFAP (red) mRNA expression in the SVZ: lateral ventricle (lv), ependyma (e), and astrocytic ribbon (ar). (B1-2) Magnification of inserts shown in (A): ependymal cells expressing C4 (B1) and astrocytes co-expressing C4 and GFAP (B2). (C) C4⁺/GFAP⁺ astrocytes in the corpus callosum (cc), immediately dorsal to the SVZ. (D) C4⁺/GFAP⁺ astrocytes in the caudate nucleus, immediately ventral to the SVZ. (E) Iba1⁺ microglia (red) do not express C4 (green) in cc. (F) C4 (green) and NeuN (red) expression in the caudate nucleus, same area as in (D). Scale bar = 10 μ m (A, C, D, E, and F); = 5 μ m (B1-2). Solid arrows indicate co-expression; hollow arrows indicate single expression.

FIGURE 3



Inflammatory transcriptome analysis. (A) Quantification of mRNA expression for C4A and C4B by RT-PCR. (B, C) Volcano plots of differentially expressed genes (DEGs) ($p \geq 0.05$) in (B) autism spectrum disorder (ASD) and (C) schizophrenia (SZ) showing that C4A/B is upregulated. (D) Log₂ normalized counts for C4A/B in ASD and SZ compared to controls. (E, F) Gene set analyses for (E) ASD and (F) SZ showing the top ten gene sets including DEGs. (G, H) Heatmaps of DEGs in the Complement System gene sets for (G) ASD and (H) SZ. n = 16/group; ANOVA with Tukey posthoc test: * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

FIGURE 4



Ki67 expression in the SVZ. (A) Representative Nissl staining of a coronal section containing the SVZ. Red outline indicates the border within which Ki67⁺ cell counting was performed. (B, C) Representative images of Ki67 emulsion autoradiography from regions indicated in (A); (B) SVZ; (C) ependyma on the dorsal border of the lateral ventricle. (D-E) Magnification of inserts shown in (B-C), showing a Ki67⁺ cell in the SVZ. (D) and Ki67⁺ ependymal cells (E). (F) RNAscope images of C4 (green) and Ki67 (red) mRNA expression in the SVZ: lateral ventricle (lv), ependyma (e), hypocellular gap (h), astrocytic ribbon (ar). (G-H) Magnification of inserts shown in (F), confirming the co-expression of C4 and Ki67 in ependymal cells (G) and cells in the ar (H). (I) Quantification of Ki67⁺ cells by manual counting from coronal sections shown in (A) in control (Ctrl; n = 10), autism spectrum disorder (ASD; n = 11) and schizophrenia (SZ; n = 14). (J) RT-PCR quantification of Ki67 mRNA ($p = 0.056$). One way ANOVA: * $p \leq 0.05$; ** $p \leq 0.01$. Scale bar = 0.5 mm (A); 15 μ m (B, C); 5 μ m (D, E, G, and H); 10 μ m (F).

Conclusion

- C4 is expressed robustly in the SVZ and septum pellucidum.
- C4 is expressed by diverse cell types, including ependyma, astrocytes, putative progenitor cells, and neurons throughout the SVZ, surrounding corpus callosum, and caudate nucleus.
- C4 expression is significantly elevated in SZ, and to a lesser degree ASD, compared to control.
- In addition to the complement system, many other immune pathways are differentially regulated in ASD and SZ.
- Density of Ki67⁺ cell is increased in the ASD SVZ compared to ctrl and SZ.

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