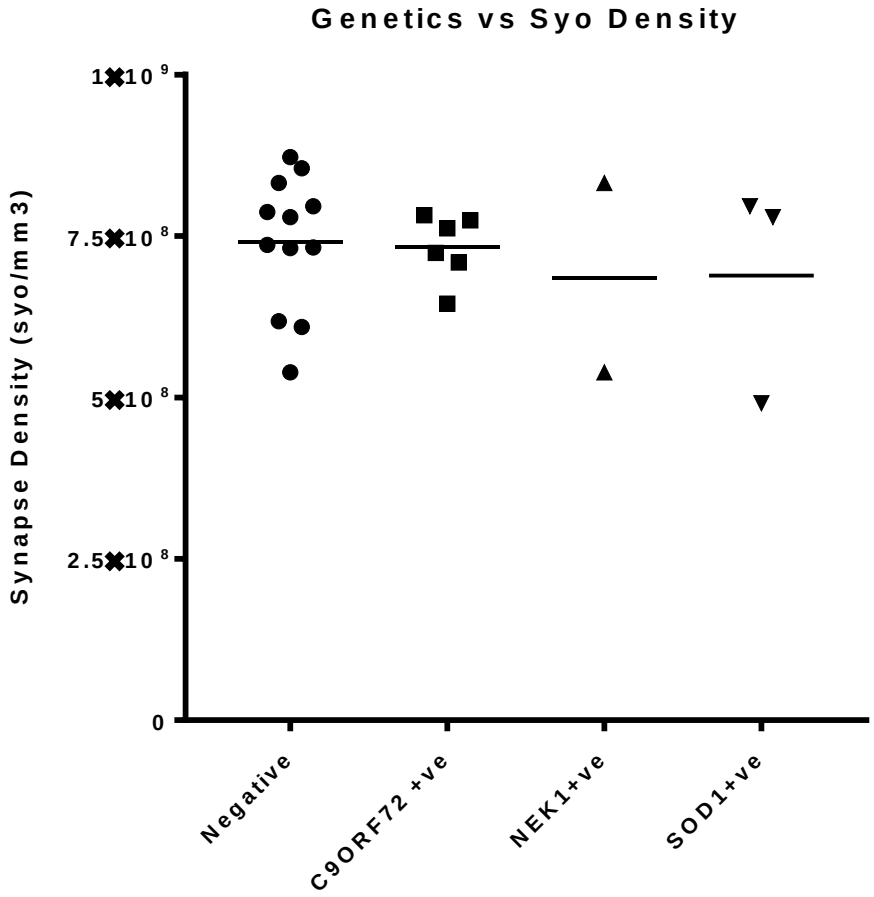
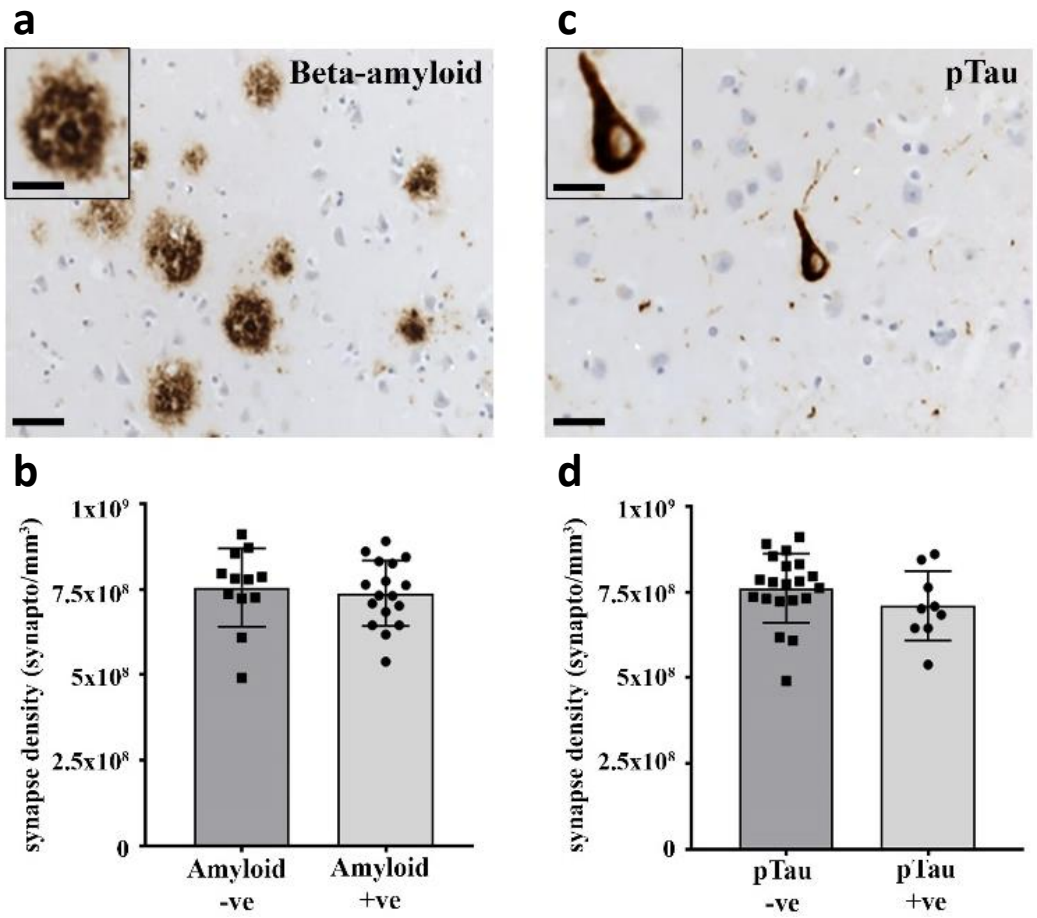
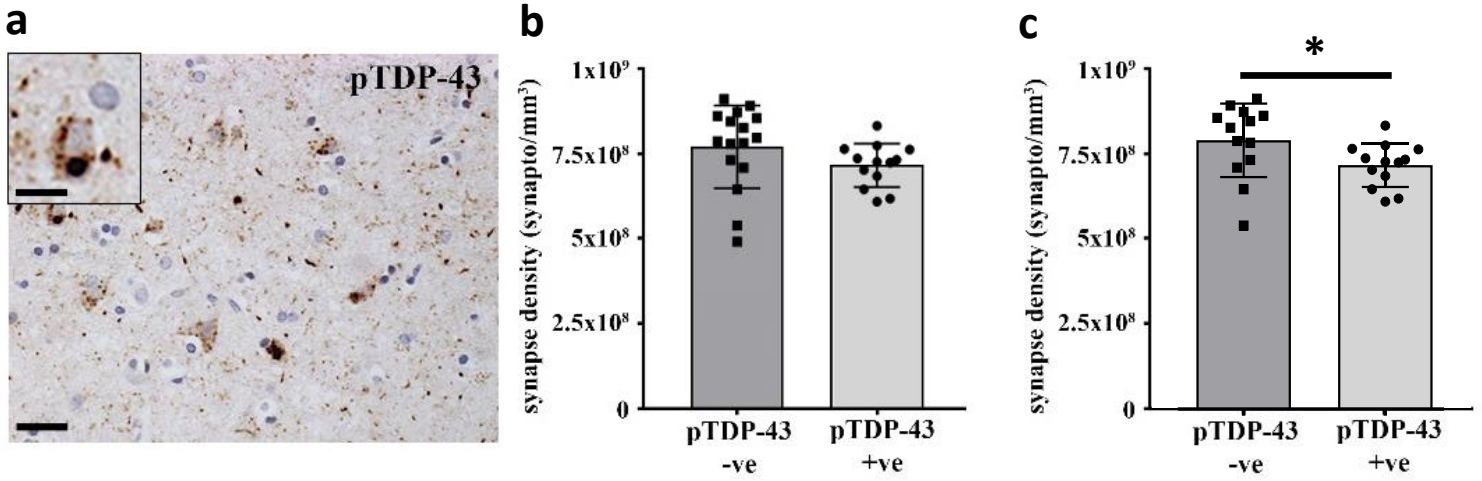


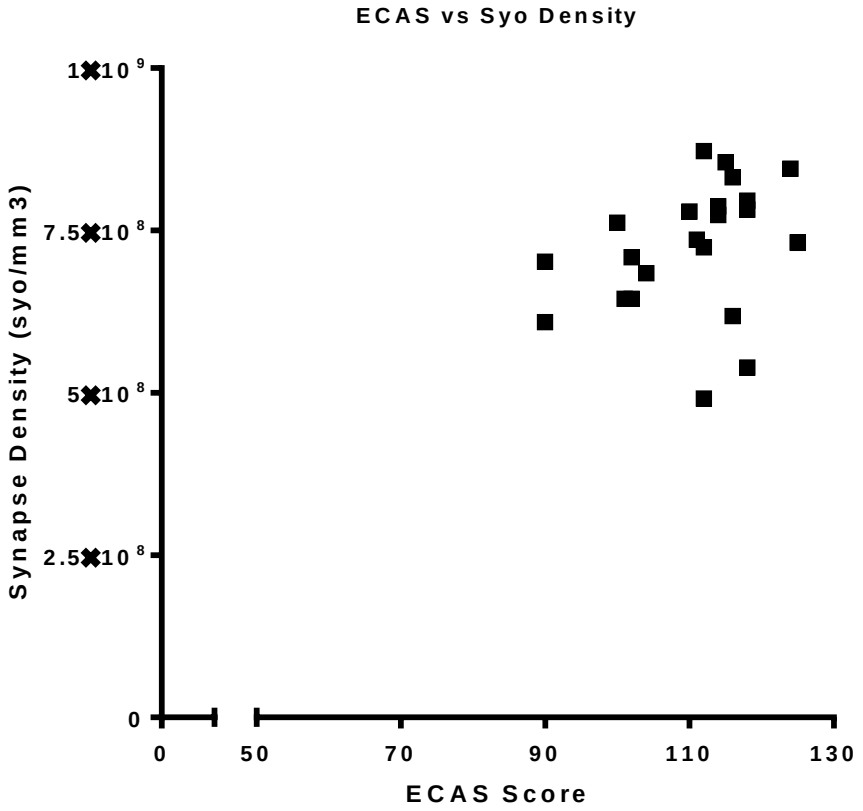
Supplementary Figure 1: Genetic status does not affect synapse density in frontal cortex. Histogram representing no difference in synapse density between ALS patients with no known ALS-associated variants (n=12), C9ORF72+ve (n=6), NEK1+ve (n=2) and SOD1+ve (n=3) ALS cases (One-way ANOVA; F=0.277; p=0.84). Each data point represents the mean synapse density of each case.



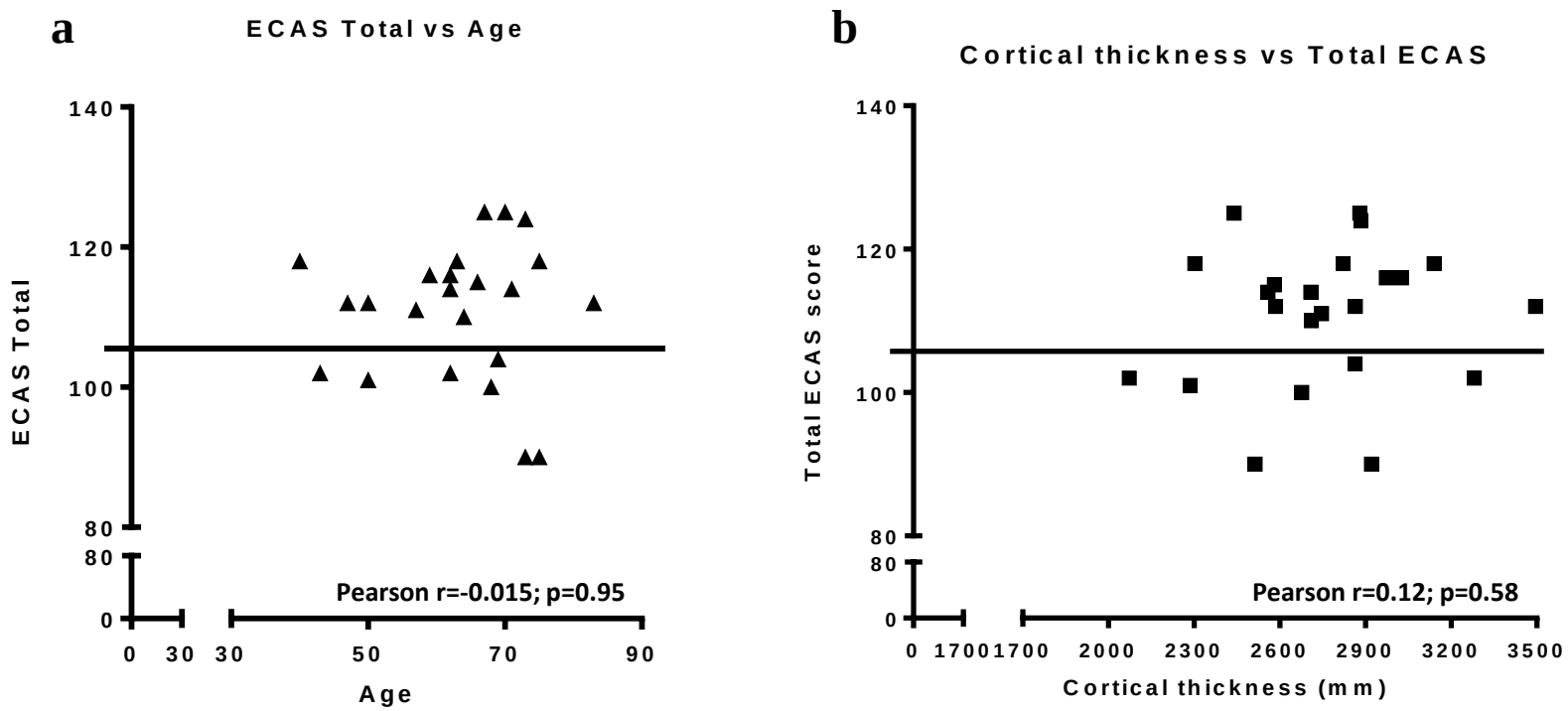
Supplementary Figure 2: Synapse loss is not associated with Beta-amyloid or pTau in BA9
a. Immunohistochemical stain for beta-amyloid shows the presence of amyloid-positive plaques in the frontal cortex of a number of ALS cases. Scale bar is 100µm. Inset shows a higher magnification of a dense-core plaque. Scale bar is 50µm. **b.** Synapse density in amyloid-negative (n=12) and amyloid-positive (n=17) cases reveals no difference (2-tailed unpaired t-test; p=0.67). **c.** Immunohistochemical stain for pTau shows the presence of pathology in the frontal cortex of a number of ALS cases. Scale bar is 50µm. Inset shows a higher magnification of a pTau-positive tangle. Scale bar is 20µm. **d**. Synapse density in pTau-negative (n=20) and pTau-positive (n=9) cases reveals no difference (2-tailed unpaired t-test; p=0.22).



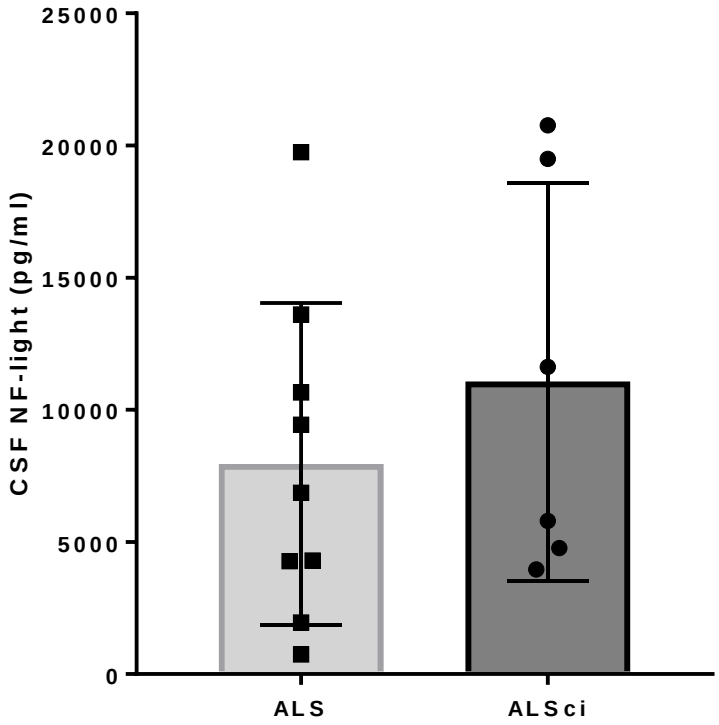
Supplementary Figure 3: pTDP-43 pathology in BA9 may associate with lower synapse density
a. Immunohistochemical stain for pTDP-43 shows the presence of pathology in the frontal cortex of almost half the ALS cases. Scale bar is 50µm. Inset shows a higher magnification of a pTDP-43-positive cell. Scale bar is 20µm. **b.** Synapse density in pTDP-43-negative (n=16) and pTDP-43-positive (n=13) cases reveals no difference (2-tailed unpaired t-test; p=0.11). **c.** However, if the 3 SOD1+ve cases are removed from the dataset, analysis reveals a lower synapse density in the pTDP-43-positive group (2-tailed unpaired t-test; p=0.047). Each data point represents the mean synapse density of each case.



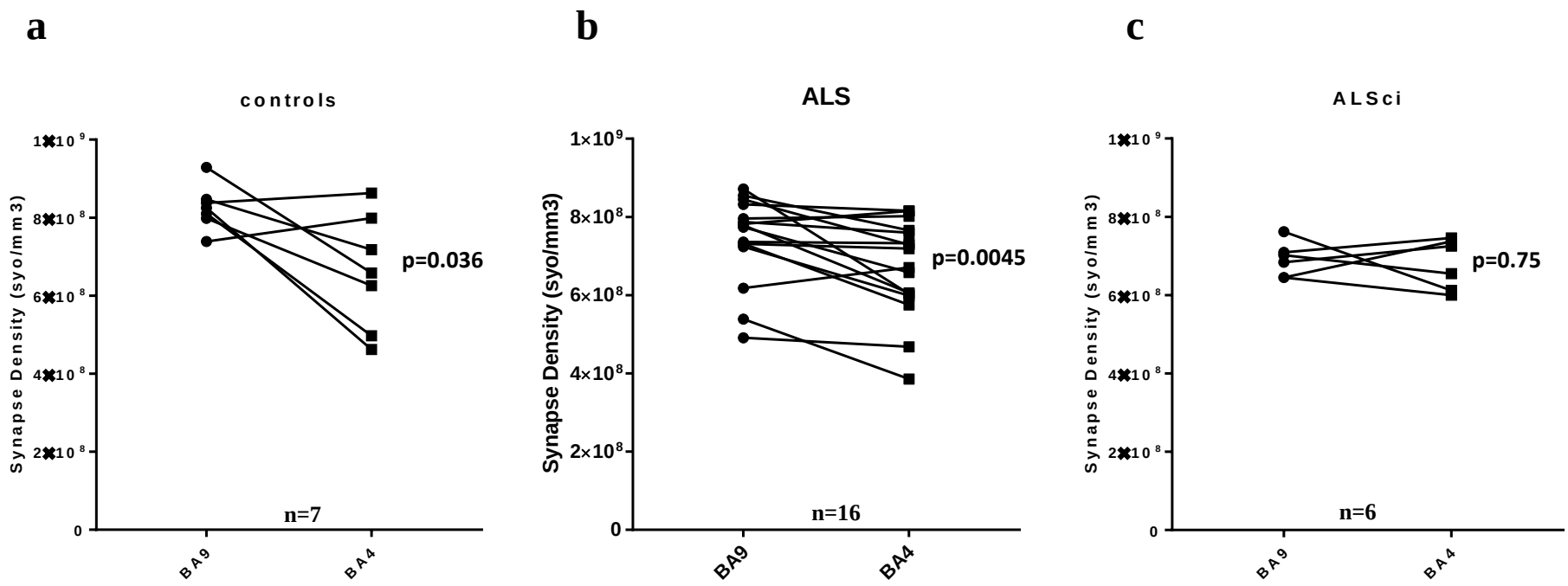
Supplementary Figure 4: Comparison of ECAS score and BA9 synapse density
Plotting ECAS score against BA9 synapse density for all 23 ECAS-tested ALS patients reveals a trend towards positive correlation (Pearson r=0.31, p=0.15).



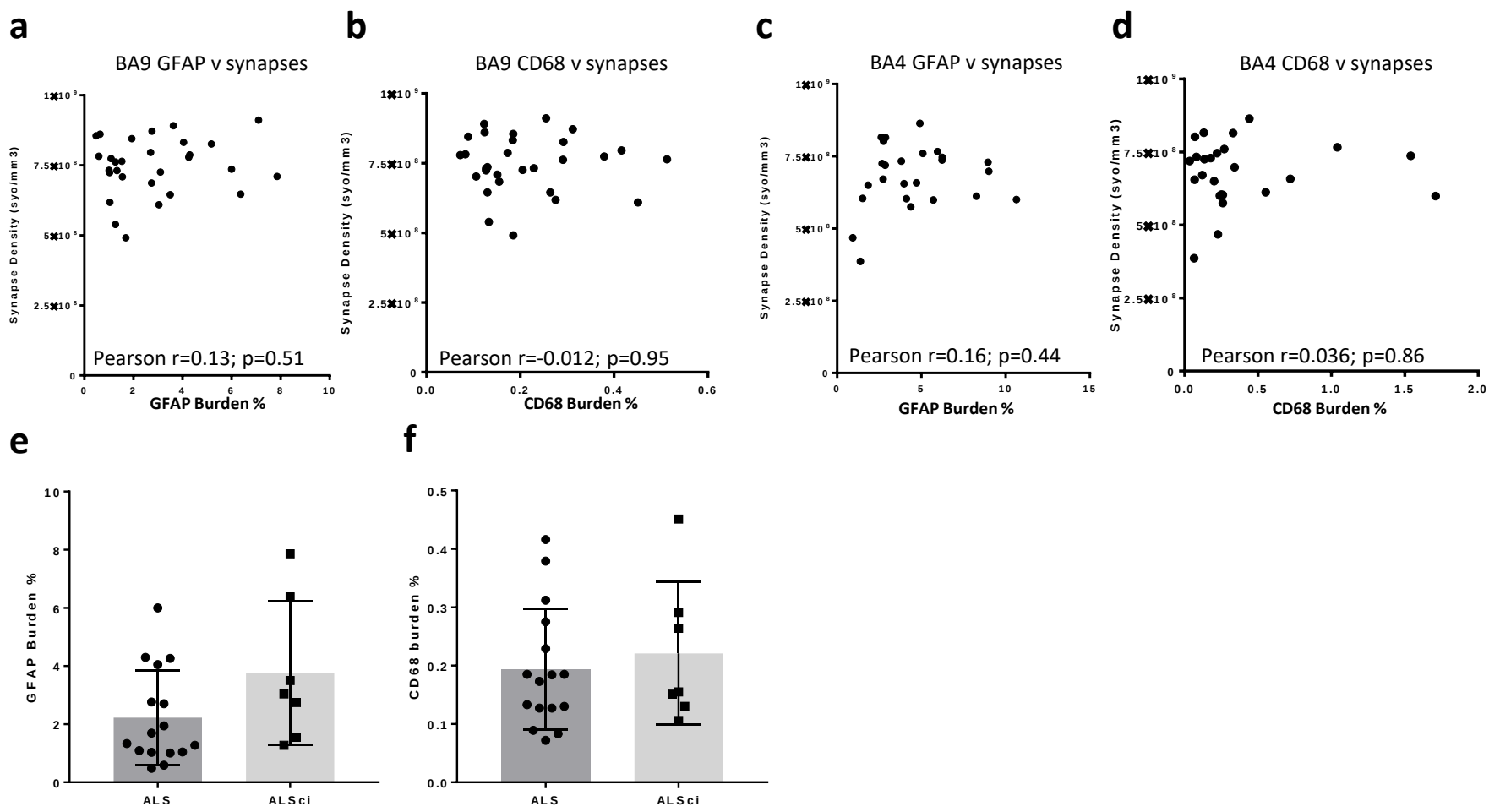
Supplementary Figure 5: ECAS score is not affected by age or cortical thickness.
No association was found between age at death (**a**) or frontal cortex cortical thickness (**b**) and ECAS total score. Each data point represents an individual ALS case. Black line represents impairment cut off.



Supplementary Figure 6: Neurofilament light-chain levels in CSF of ALS patients
Neurofilament light-chain levels in the CSF were measured using a commercial ELISA kit (Uman Diagnostics, Sweden). Each data point represents the CSF level of NF-light in an individual patient (ALS n=9, ALSci n=6). There is no difference in NF-light levels between ALS and ALSci patients (ALS = 7950 ± 2030 pg/ml, ALSci = 11068 ± 3073 pg/ml; 2-tailed unpaired t-test; p=0.39).



Supplementary Figure 7: Regional synapse density changes in ALSci brains.
Paired scatterplots representing BA9 and BA4 synapse densities within control (**a**), ALS unimpaired (**b**) and ALS cognitively impaired (**c**) brains. Each line links the BA9 and BA4 densities from the same brain. Paired t-tests reveal a lower density in BA4 compared to BA9 in control and ALS unimpaired cases (p=0.036 and p=0.0045 respectively), however no difference in the ALS cognitively impaired group (p=0.75).



Supplementary Figure 8: Synapse loss and cognitive decline are not associated with increased gliosis
Scatterplots of GFAP burden versus synapse density in BA9 (**a**) and BA4 (**c**) show now correlation between these parameters (BA9: n=29; BA4: n=25). Furthermore, scatterplots of CD68 burden versus synapse density in BA9 (**b**) and BA4 (**d**) also reveal no association(BA9: n=29; BA4: n=25). There is no difference in BA9 GFAP burden (**e**) between ALS (n=16) and ALSci (n=7) patients (2-tailed unpaired t-test; p=0.09) or BA9 CD68 burden (**f**) (2-tailed unpaired t-test; p=0.59).