Thioflavin Staining and Amyloid Formation are Unique to Mixed Tauopathies

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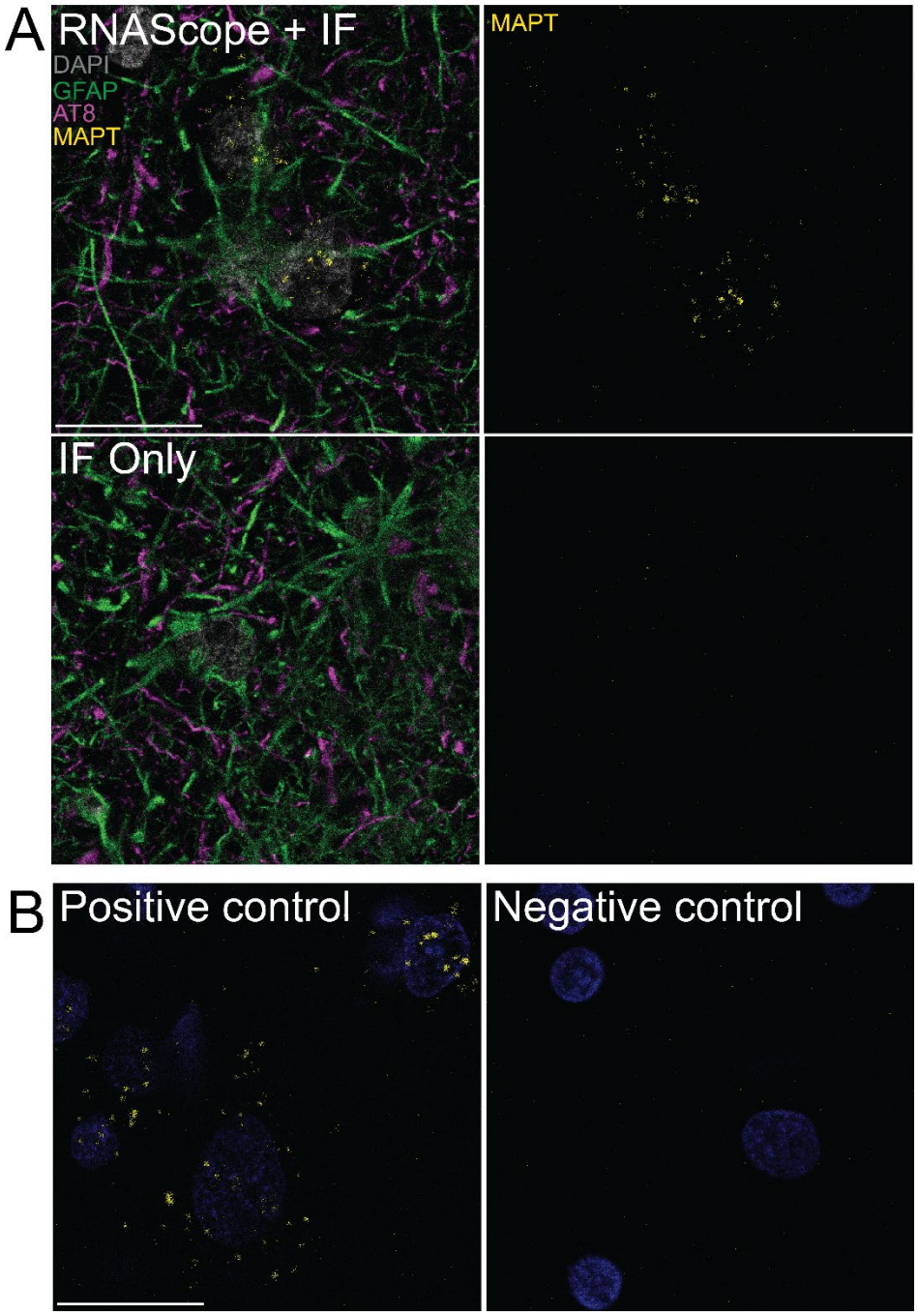
Supplementary Figure 1.



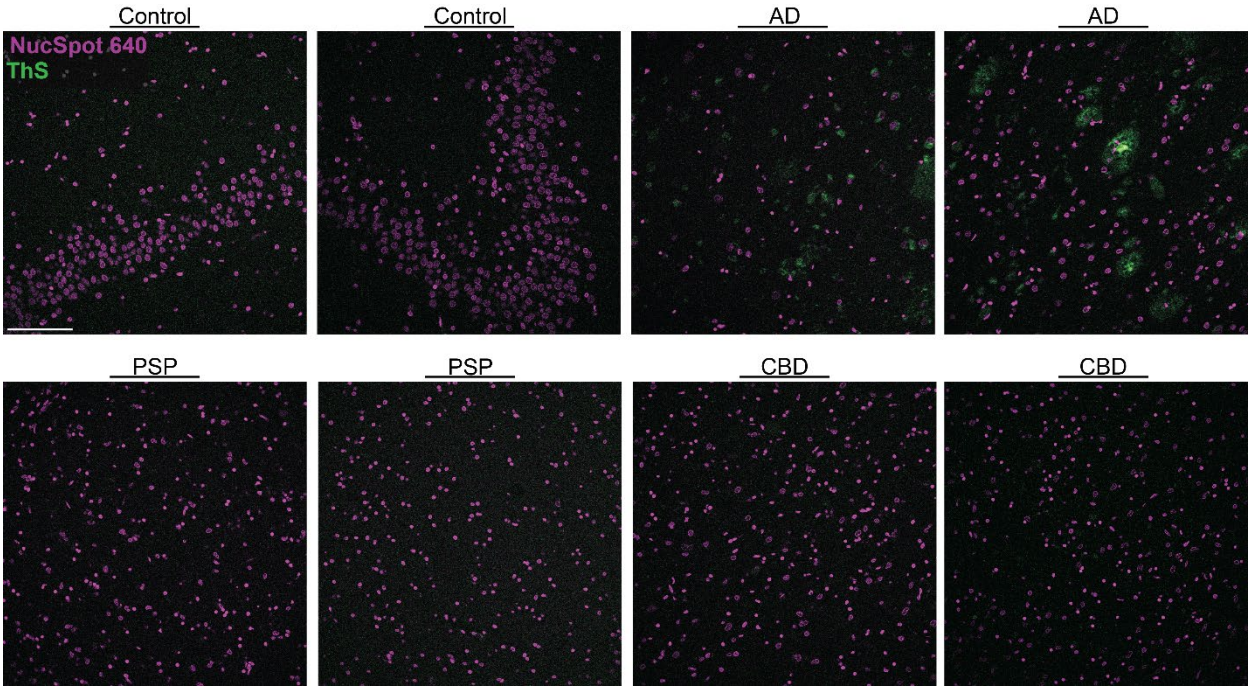
Supplementary Figure 1.
Validation of RNAscope and immunofluorescence staining techniques. (*A*) Immunofluorescence staining without *MAPT* probe (yellow) shows no *in situ* hybridization signal compared to adjacent region on the same slide of human brain tissue stained with probe. (*B*) Further validation using manufacturer provided positive (yellow) and negative control probes on adjacent regions on the same slide of human brain tissue. DAPI (blue) was used to highlight nuclei. Scale bar= 20μm for both (*A*) and (*B*).

Supplementary Figure 2. Quantification code

```
ds=imageDatastore('Binarized Images\');                    %create an image store of Cy5 images
file_names=ds.Files;                                       %read file names
[a,b]=size(file_names);                                    %determine how many files there are
Cy5=zeros([a,1]);                                          %initialize the vector (saves processing
time)
for i=1:a
   I=readimage(ds,i);                                      %read image i
   x=nnz(I);                                               %calculate the number of non-zero pixels
                                                           in the %thresholded image
   Cy5(i,1)=x;                                             %add to vector
end


%create a vector for the diagnoses, transpose it to a vertical, and turn it into a categorical
Dx=["AD" "PSP" "CBD" "Control" "Control" "AD" "Control" "AD" "CBD" "CBD" "PSP" "PSP"];
Dx=Dx';
Dx=categorical(Dx);

%%create indices for each diagnosis
idx_AD=Dx=="AD";
idx_CBD=Dx=="CBD";
idx_PSP=Dx=="PSP";
idx_Con=Dx=="Control";

%%run ANOVA to check  if any comparison is significant.
[~,~,stats]=anova1(Cy5,Dx);

%%run multcompare to figure out which
[c,~,~,gnames]=multcompare(stats);
tbl = array2table(c,"VariableNames", ...
   ["Group A","Group B","Lower Limit","A-B","Upper Limit","P-value"]);
tbl.("Group A") = gnames(tbl.("Group A"));
tbl.("Group B") = gnames(tbl.("Group B"))


%generate the plot
scatter(1,Cy5(idx_Con),"black","filled")
hold on
scatter(2,Cy5(idx_AD),"black","filled")
scatter(3,Cy5(idx_CBD),"black","filled")
scatter(4,Cy5(idx_PSP),"black","filled")
ylabel("Number of Positive Pixels")
```

```
xlabel("")
xticks([1 2 3 4])
xticklabels({'Control' 'AD' 'CBD' 'PSP'})
xlim([0.5 4]);
line([1 2],[29000 29000])
text(1.5,30000,"p="+round(tbl{2,6},4,"significant"),'HorizontalAlignment', 'center')
line([2 3],[28000 28000])
text(2.5,29000,"p="+round(tbl{3,6},4,"significant"),'HorizontalAlignment', 'center')
line([2 4],[30000 30000])
text(3.5,3.1e4,"p="+round(tbl{1,6},4,"significant"),'HorizontalAlignment', 'center')
hold off
```

Supplementary Figure 3.



Supplementary Figure 3.
Validation of thioflavin S staining pattern using alternative source of thioflavin S (see methods). Thioflavin staining (green) is present in AD but is not found in control, PSP, or CBD. NucSpot 640 (magenta) was used to highlight nuclei. Two cases are shown per disease (total n=8). Scale bar= 50μm. Abbreviations: *AD* Alzheimer disease; *PSP* progressive supranuclear palsy; *CBD* corticobasal degeneration; *ThS* thioflavin S

Supplementary Table 1. Quantification of colocalization using FIJI BIOP JACOP to calculate Mander's M1/M2 and Pearson's correlation coefficients for images shown in main text Fig. 2.

Case ID	Pathologic Diagnosis	Area A	Area B	Area Overlap	Pearson's Coefficient
#3	Control	0.012	0.856	0	-3.15E-04
#8	AD	81.496	18.834	9.876	0.288
#13	PSP	5.346	2.067	0.047	0.016
#20	CBD	6.455	8.827	0.163	0.02
#22	PiD	10.668	0.093	3.86E-04	-8.35E-04
#24	ALS and PART	9.992	8.778	1.684	0.245

Case ID	M1	M2	Threshold A	Threshold B	Thresholded M1	Thresholded M1
#3	1.11E-04	1.53E-04	92	37	0	0
#8	0.331	0.641	116	97	0.126	0.531
#13	0.049	0.039	98	37	0.01	0.023
#20	0.128	0.032	111	37	0.027	0.019
#22	4.47E-04	0.029	116	31	3.23E-05	0.003
#24	0.272	0.203	139	37	0.176	0.302

AD Alzheimer's disease; *PSP* Progressive supranuclear palsy; *CBD* Corticobasal degeneration; *PiD* Pick's disease; *ALS* Amyotrophic lateral sclerosis; *PART* Primary age-related tauopathy