

Supplementary material

Quantification algorithm parameters

All quantification conducted using QuPath software, algorithms in Apache groovy.

p62 positive pixel count

```
setImageType('BRIGHTFIELD_H_DAB');
setColorDeconvolutionStains('{ "Name" : "H-DAB default", "Stain 1" : "Hematoxylin",
"Values 1" : "0.65111 0.70119 0.29049 ", "Stain 2" : "DAB", "Values 2" : "0.26917
0.56824 0.77759 ", "Background" : " 255 255 255 "});
runPlugin('qupath.imagej.detect.tissue.PositivePixelCounterIJ', '{"downsampleFactor": 3,
"gaussianSigmaMicrons": 2.0, "thresholdStain1": 0.3, "thresholdStain2": 0.2,
"addSummaryMeasurements": true}');
```

pTDP-43 positive pixel count

```
setImageType('BRIGHTFIELD_H_DAB');
setColorDeconvolutionStains('{ "Name" : "H-DAB default", "Stain 1" : "Hematoxylin",
"Values 1" : "0.65111 0.70119 0.29049 ", "Stain 2" : "DAB", "Values 2" : "0.26917
0.56824 0.77759 ", "Background" : " 255 255 255 "});
runPlugin('qupath.imagej.detect.tissue.PositivePixelCounterIJ', '{"downsampleFactor": 2,
"gaussianSigmaMicrons": 2.0, "thresholdStain1": 0.2, "thresholdStain2": 0.3,
"addSummaryMeasurements": true}');
```

CD68 cell count

```
setImageType('BRIGHTFIELD_H_DAB');
setColorDeconvolutionStains('{ "Name" : "H-DAB default", "Stain 1" : "Hematoxylin",
"Values 1" : "0.65111 0.70119 0.29049 ", "Stain 2" : "DAB", "Values 2" : "0.26917
0.56824 0.77759 ", "Background" : " 255 255 255 "});
runPlugin('qupath.imagej.detect.nuclei.PositiveCellDetection',
'{"detectionImageBrightfield": "Optical density sum", "requestedPixelSizeMicrons": 1.0,
"backgroundRadiusMicrons": 8.0, "medianRadiusMicrons": 0.0, "sigmaMicrons": 3.0,
"minAreaMicrons": 10.0, "maxAreaMicrons": 400.0, "threshold": 0.2, "maxBackground":
2.0, "watershedPostProcess": true, "excludeDAB": false, "cellExpansionMicrons": 5.0,
"includeNuclei": true, "smoothBoundaries": true, "makeMeasurements": true,
"thresholdCompartment": "Cell: DAB OD mean", "thresholdPositive1": 0.15,
"thresholdPositive2": 0.4, "thresholdPositive3": 0.6, "singleThreshold": true}');
```

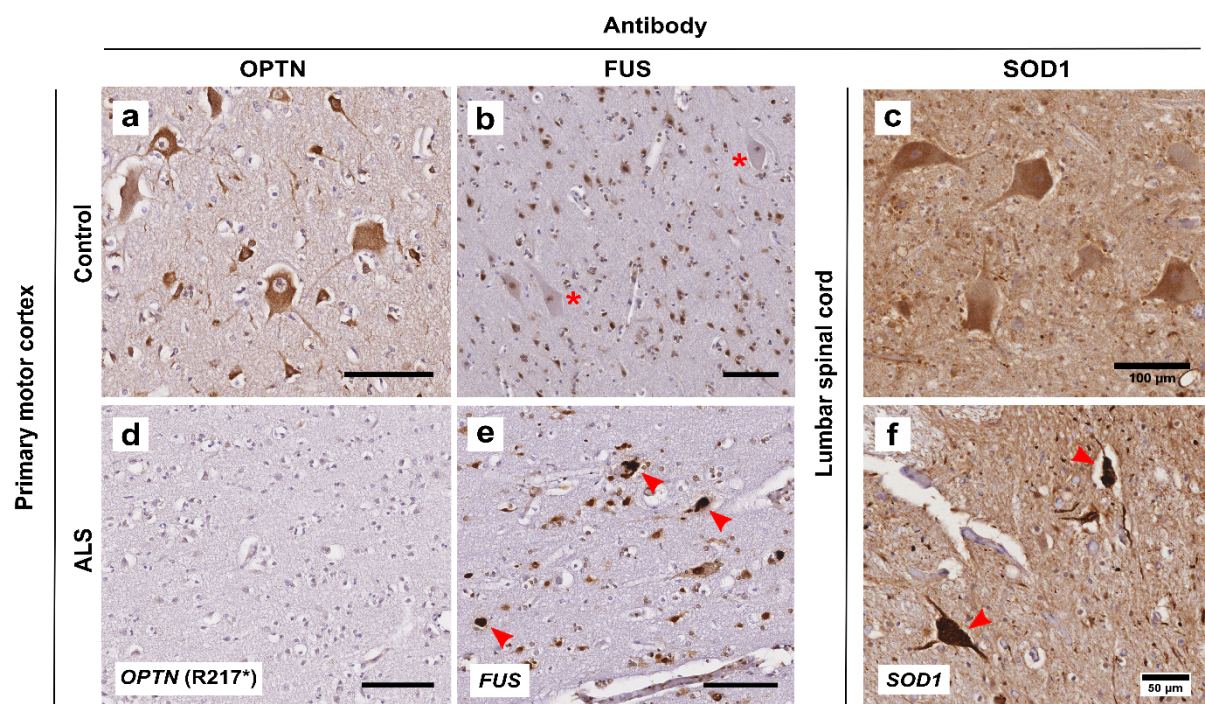
OLIG2 cell count

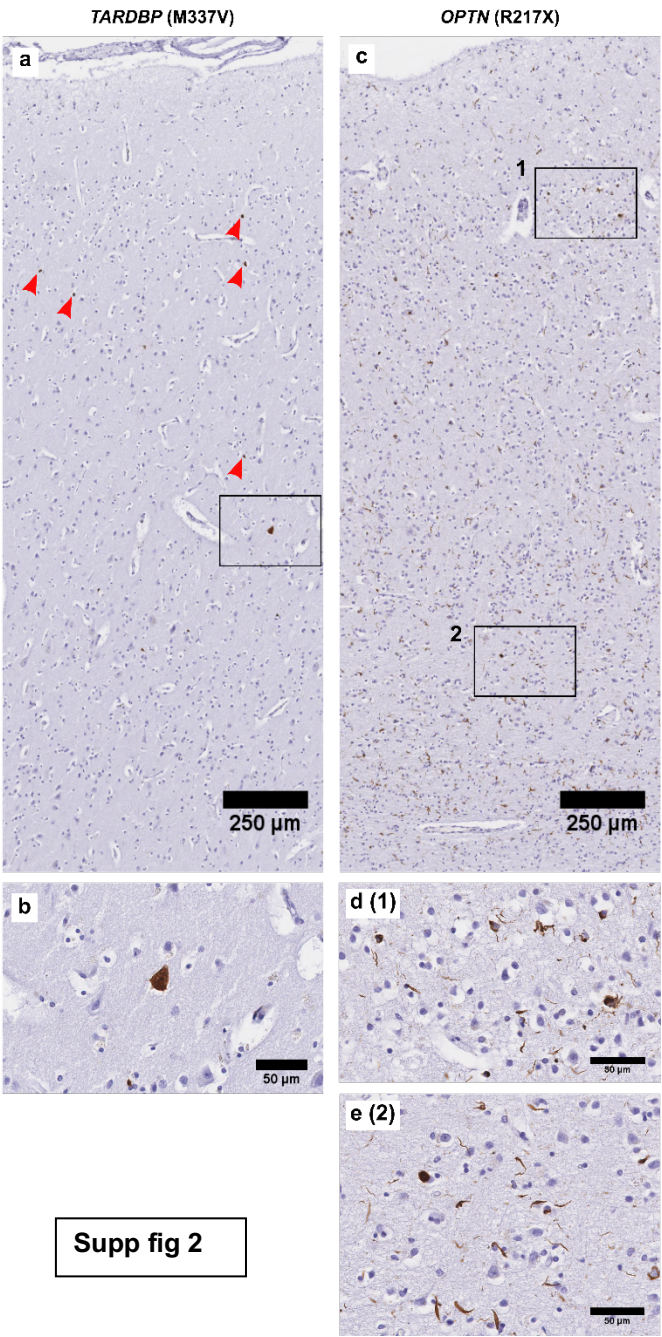
```
setImageType('BRIGHTFIELD_H_DAB');
setColorDeconvolutionStains('{ "Name" : "H-DAB default", "Stain 1" : "Hematoxylin",
"Values 1" : "0.65111 0.70119 0.29049 ", "Stain 2" : "DAB", "Values 2" : "0.26917
0.56824 0.77759 ", "Background" : " 255 255 255 "});
runPlugin('qupath.imagej.detect.nuclei.PositiveCellDetection',
'{"detectionImageBrightfield": "Optical density sum", "requestedPixelSizeMicrons": 0.5,
"backgroundRadiusMicrons": 8.0, "medianRadiusMicrons": 0.0, "sigmaMicrons": 1.5,
"minAreaMicrons": 10.0, "maxAreaMicrons": 400.0, "threshold": 0.5, "maxBackground":
2.0, "watershedPostProcess": true, "excludeDAB": false, "cellExpansionMicrons": 5.0,
"includeNuclei": true, "smoothBoundaries": true, "makeMeasurements": true,
"thresholdCompartment": "Nucleus: DAB OD mean", "thresholdPositive1": 0.2,
"thresholdPositive2": 0.4, "thresholdPositive3": 0.6, "singleThreshold": true}');
```

TPPP/p25 cell count

```
setImageType('BRIGHTFIELD_H_DAB');
setColorDeconvolutionStains('{ "Name" : "H-DAB default", "Stain 1" : "Hematoxylin",
"Values 1" : "0.65111 0.70119 0.29049 ", "Stain 2" : "DAB", "Values 2" : "0.26917
0.56824 0.77759 ", "Background" : " 255 255 255 "});
runPlugin('qupath.imagej.detect.nuclei.PositiveCellDetection',
'{"detectionImageBrightfield": "Optical density sum", "requestedPixelSizeMicrons": 0.5,
"backgroundRadiusMicrons": 8.0, "medianRadiusMicrons": 0.0, "sigmaMicrons": 2.0,
"minAreaMicrons": 10.0, "maxAreaMicrons": 400.0, "threshold": 0.55, "maxBackground":
2.0, "watershedPostProcess": true, "excludeDAB": false, "cellExpansionMicrons": 5.0,
"includeNuclei": true, "smoothBoundaries": true, "makeMeasurements": true,
"thresholdCompartment": "Nucleus: DAB OD mean", "thresholdPositive1": 0.2,
"thresholdPositive2": 0.4, "thresholdPositive3": 0.6, "singleThreshold": true}');
```

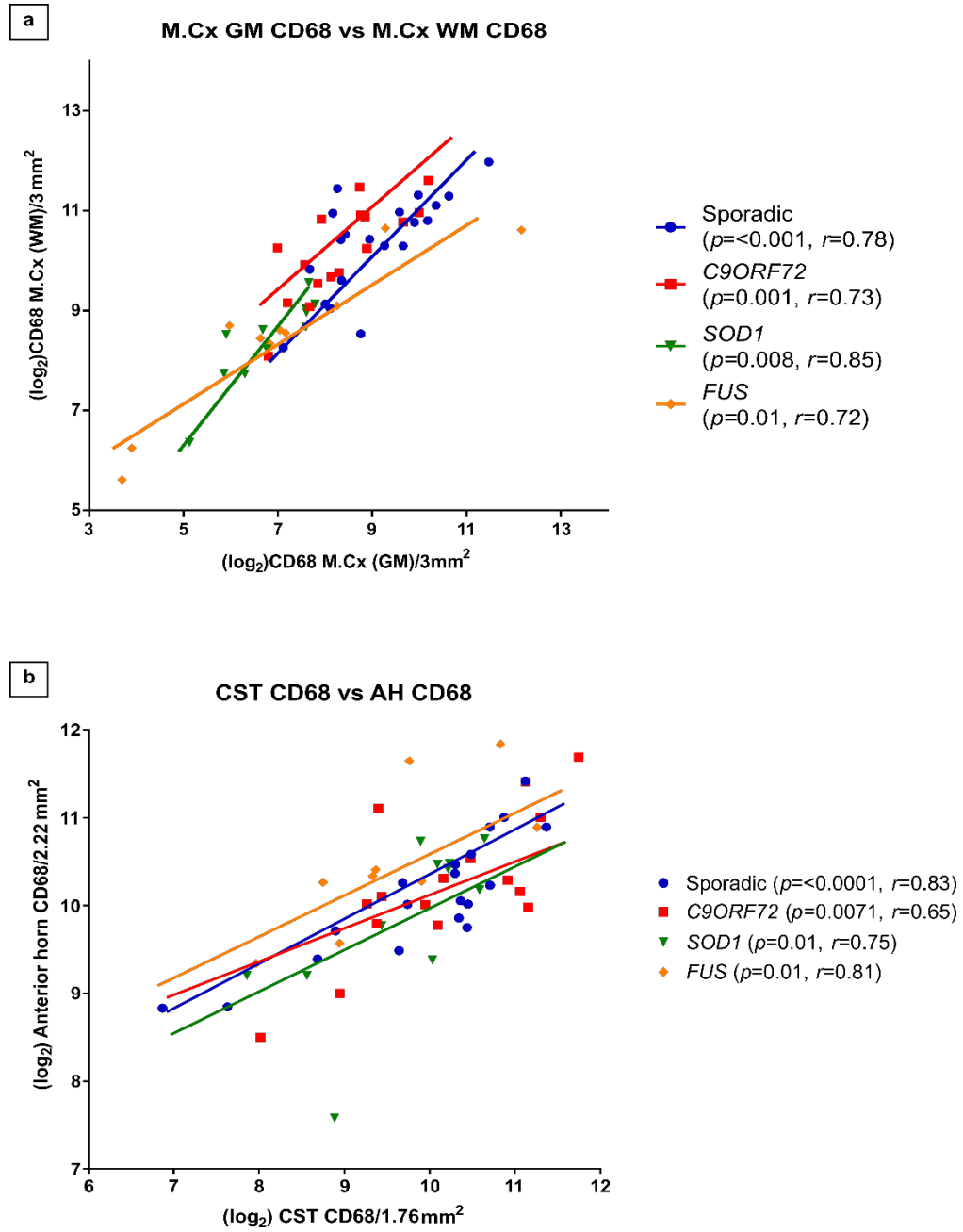
Supp fig 1





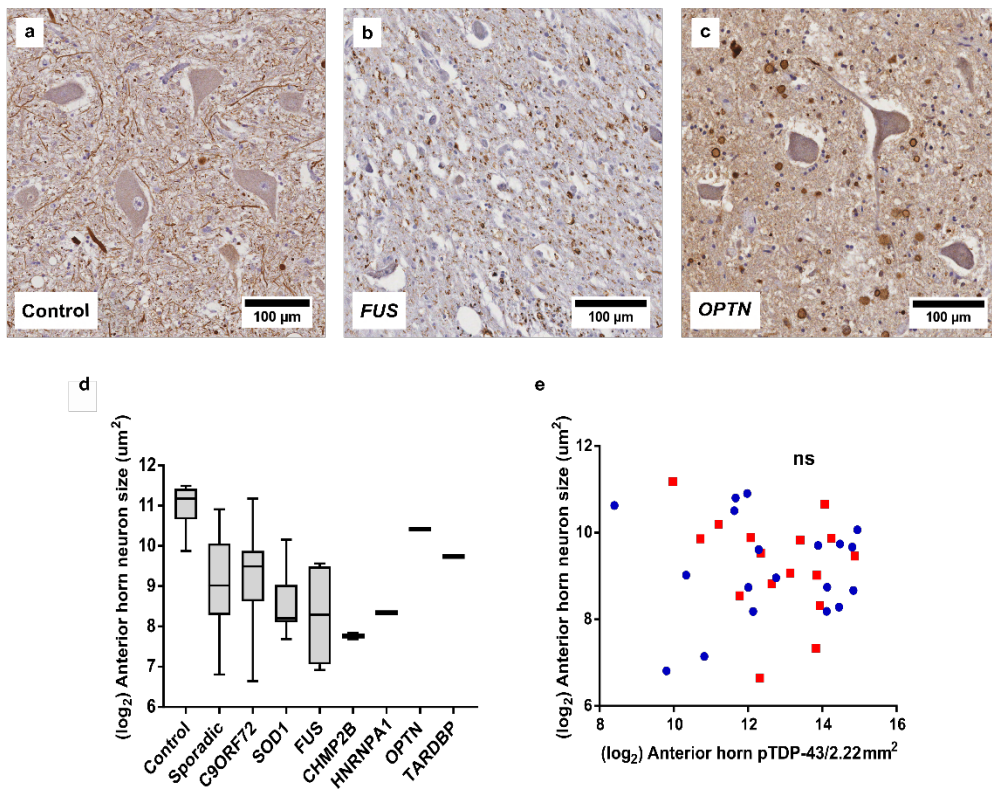
Supp fig 2

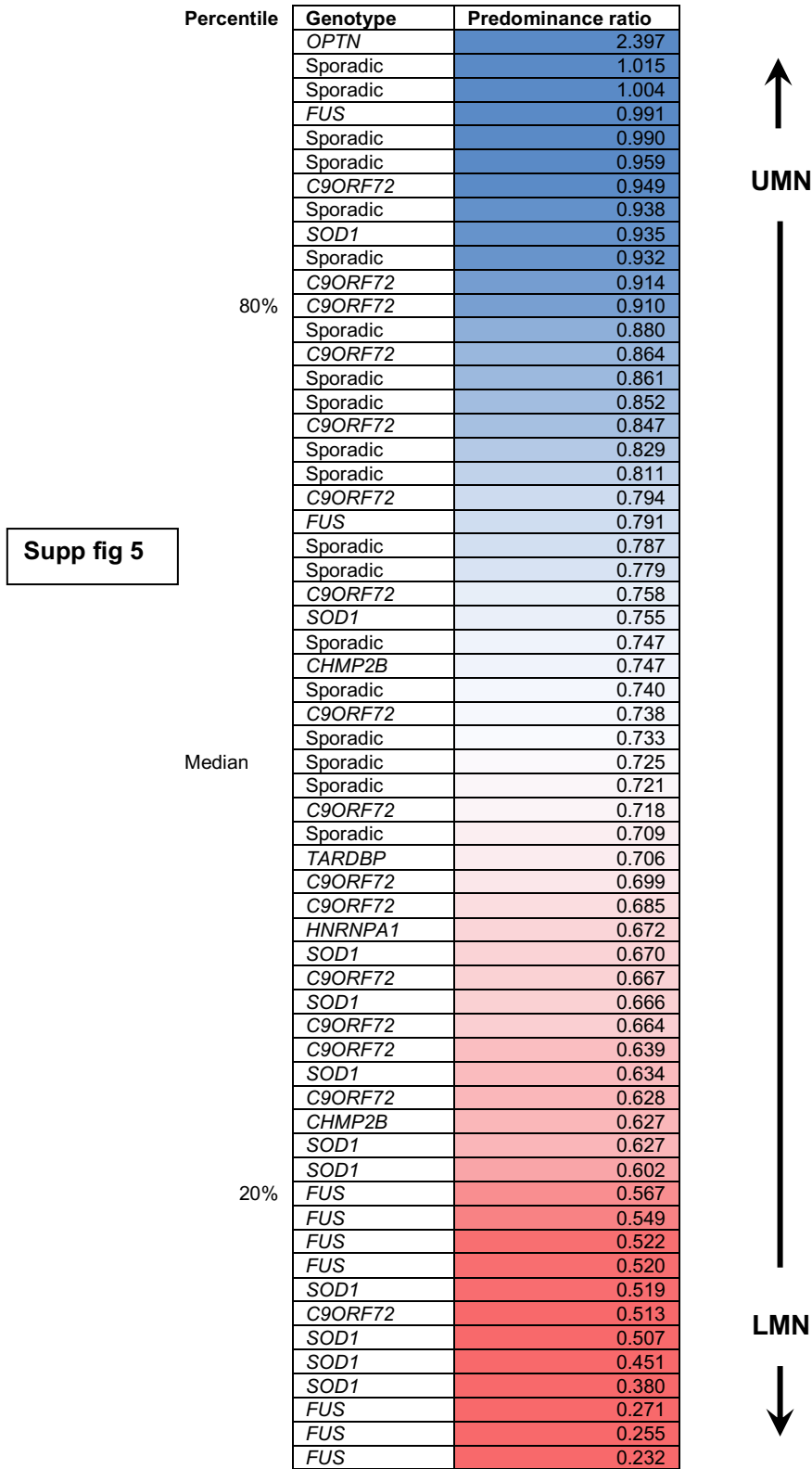
Supp fig 3



Supp fig 4

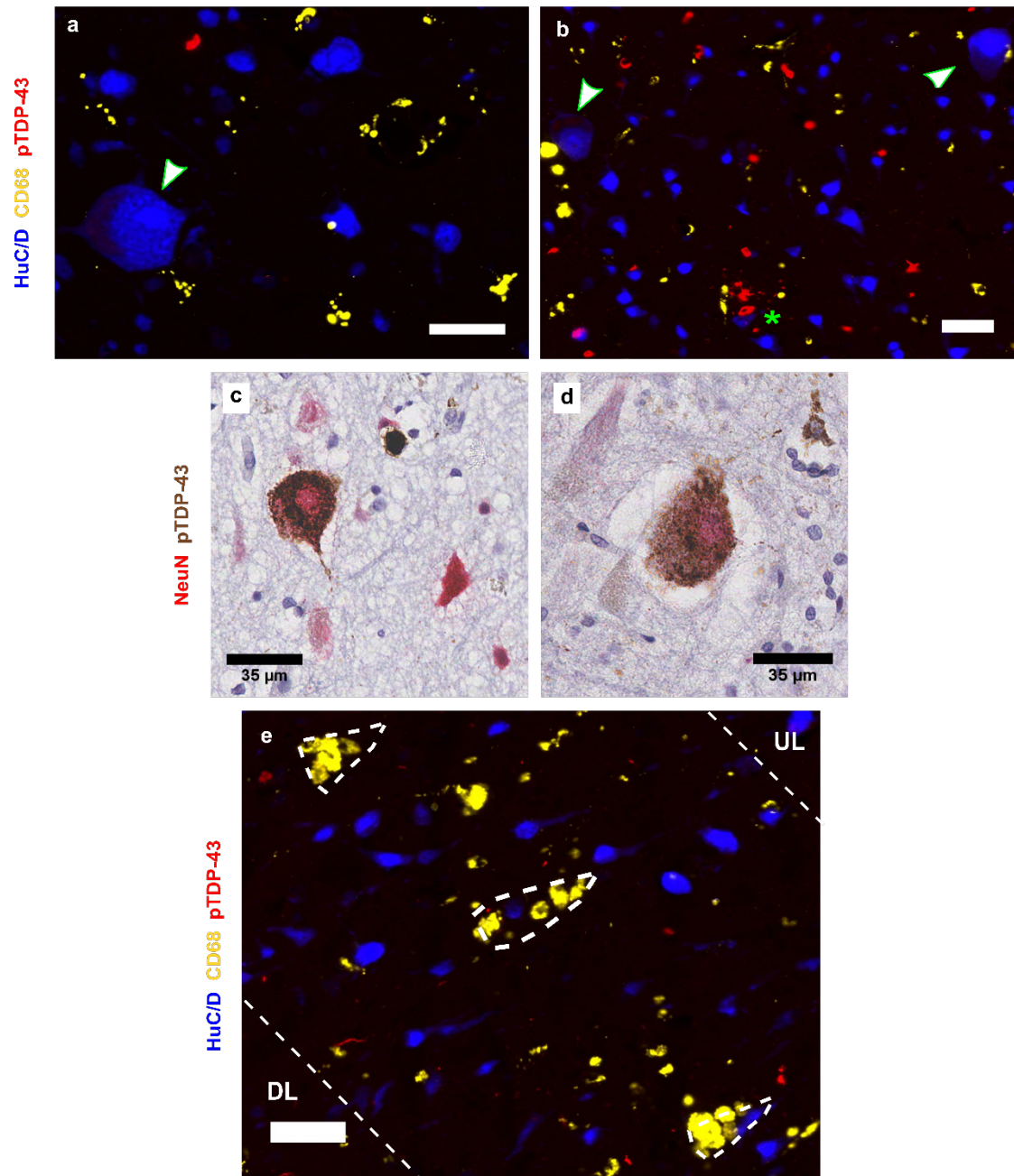
SMI-312





Supp fig 6

Primary motor cortex



Genotype	Mean OLIG2+ oligodendrocytes/3mm²	Mean Tppp/p25+ oligodendrocytes/3mm²
Control	1229	741
Control	1303	626
Control	903	766
sALS	1766	1135
sALS	1329	627
sALS	1326	666
sALS	1036	626
sALS	835	513
sALS	1237	773
C9-ALS	1064	Not available

Supp table 3: Numerical results for Olig and TPPP/p25 quantification.