Modeling FUS-ALS hallmark neuropathology using patient-specific iPSCs and iPSC-derived cortical neurons

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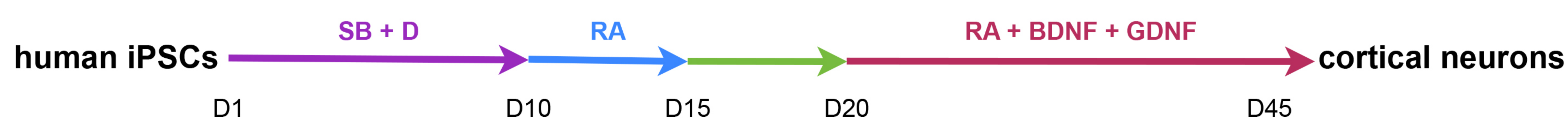
Abstract:

Amyotrophic lateral sclerosis (ALS) is an adult onset disorder in which about 5% of familial cases are caused by autosomal-dominant mutations within the *FUS* (fused in sarcoma) gene. ALS is considered as aggregate prone disease with spreading of disease pathology during disease progression, affecting spinal and cortical motor neurons. We use patient-specific and isogenic induced pluripotent stem cell (iPSC) lines to model FUS-ALS in human *in vitro* cultures. That allows the (patho-)physiological investigation of FUS in iPSCs and iPSC-derived cortical neurons carrying endogenous mutation. We analyze iPSC-derived cortical neurons during different steps of cortical layer differentiation and maturation and compare these to hindbrain/spinal neuronal phenotypes. We found typical hallmarks of neuropathology including aggregate formation, cytoplasmic mislocalization and neurodegeneration. We found that the amount of cytoplasmic FUS depends on the severity of the underlying mutation. Cytoplasmic FUS inclusions formed spontaneously in mutated iPSC-derived cortical neurons but not iPSCs depending both on the severity of *FUS* mutation and neural aging. Our study thereby highlights the value and usefulness of patient-derived cell models in FUS-ALS and the importance to study pathophysiology in cell types specifically affected in disease.

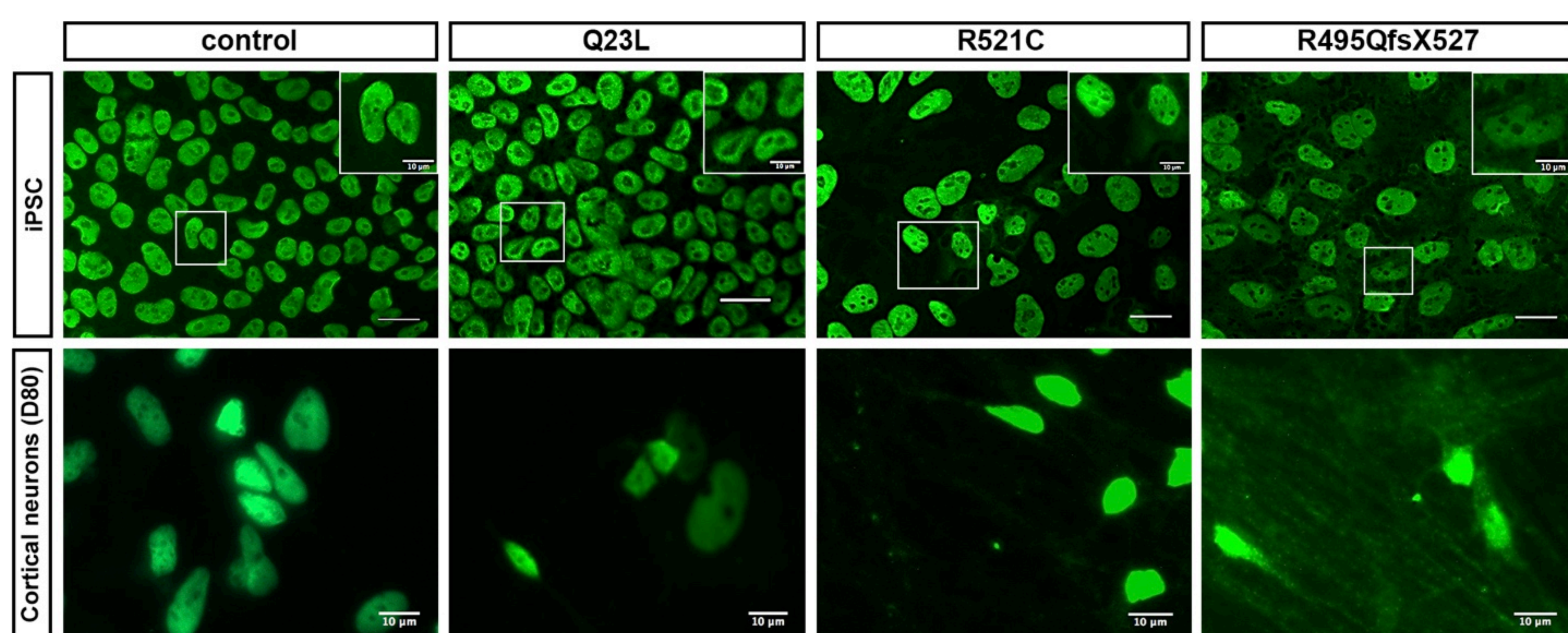
Methods: Cortical differentiation protocol



Neural differentiation of high-density monolayer h-iPSCs was induced by supplementation with SB431542 and Dorsomorphin. On day 20 cells were re-plated and neural differentation medium was supplemented with RA, BDNF and GDNF for neural maturation. On day 45, cells were re-plated again for further maturation and aging up to 120 days in total.

Results:

Figure 1: Cytoplasmic mislocalization of FUS protein in FUS-ALS patient-derived cell lines depends on both severity of mutation and aging of cells



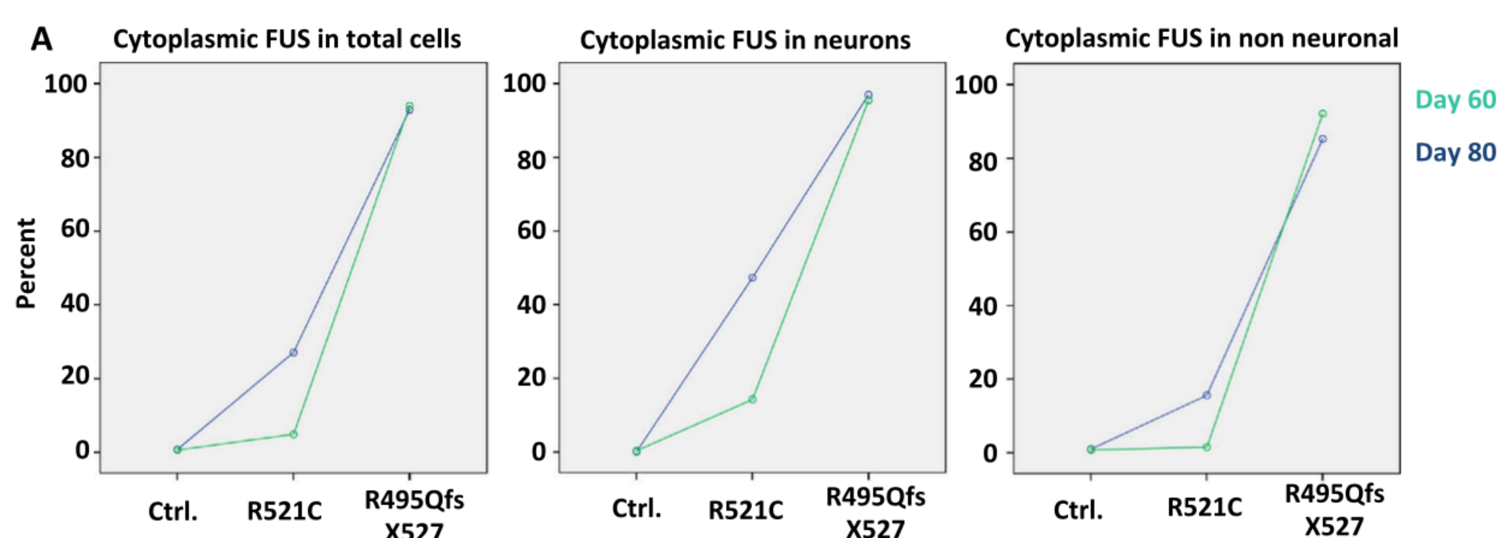


Figure 2: Isogenic iPSC cell lines with EGFP-tagged FUS. NLS mutation of FUS influences FUS localization in iPSCs, spinal motor neurons and cortical neurons

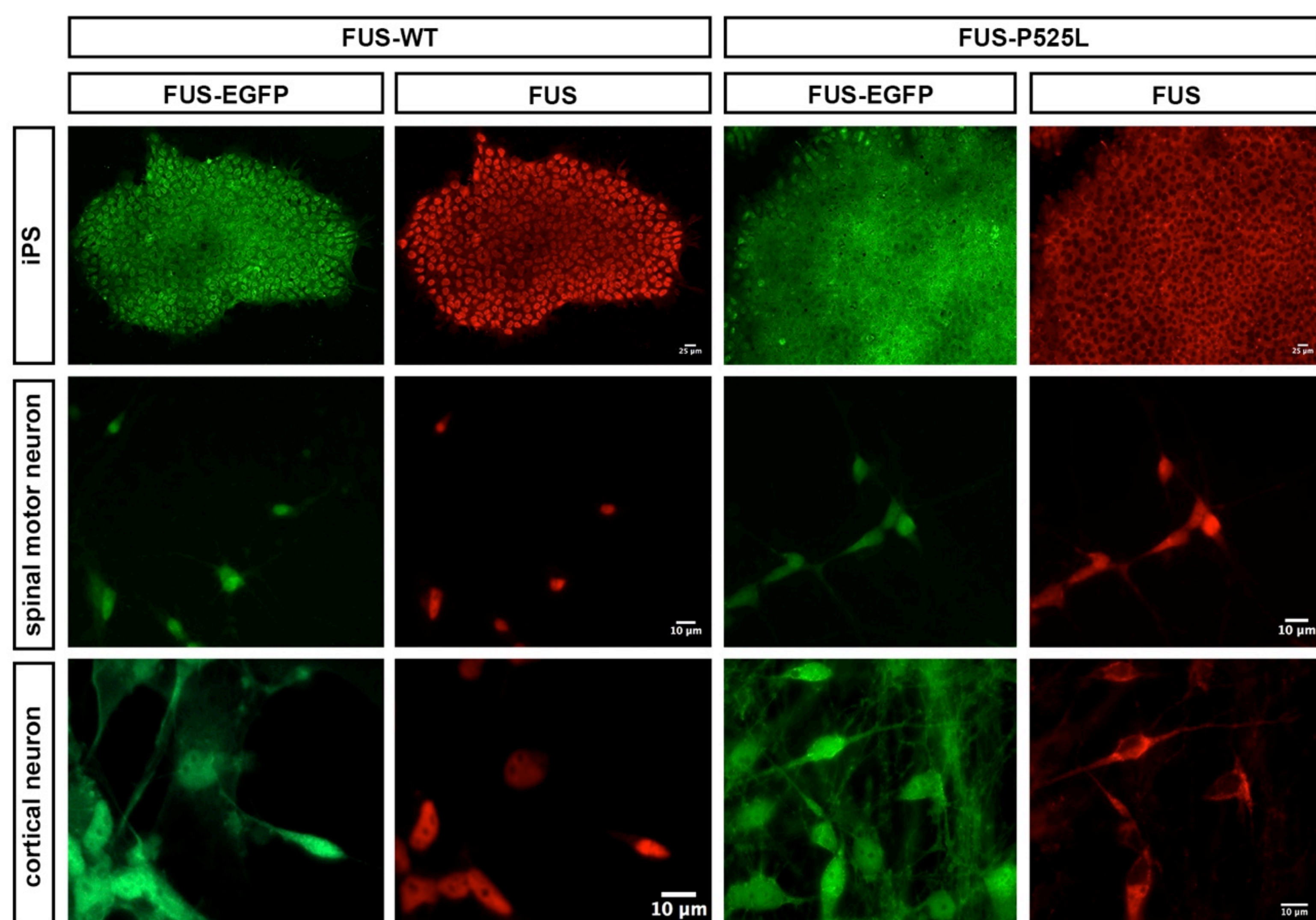


Figure 3: FUS+ cytoplasmic inclusions occur spontaneously in patient-derived FUS-ALS mutant cortical neurons and increase in size in aging neurons

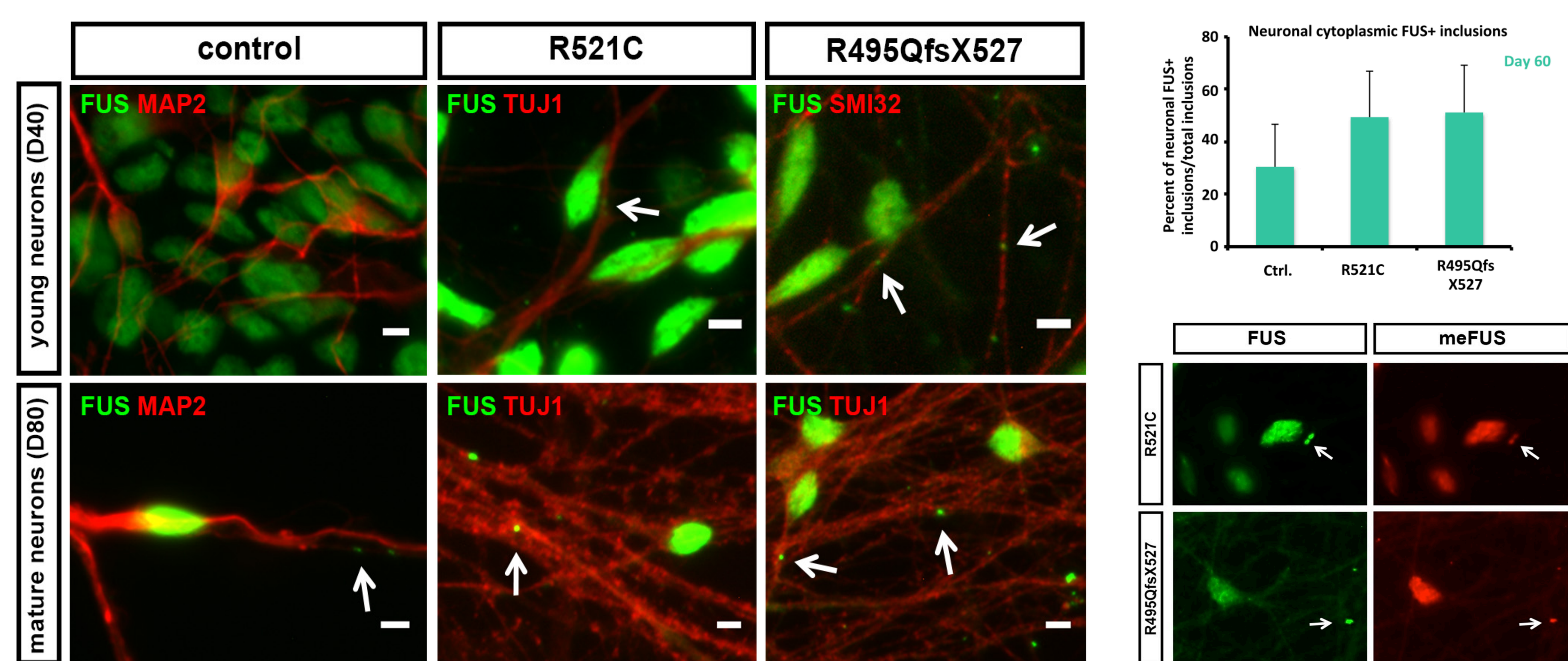


Figure 4: Patient-derived FUS-ALS mutant cell lines show a higher vulnerability to sodium arsenite compared to control

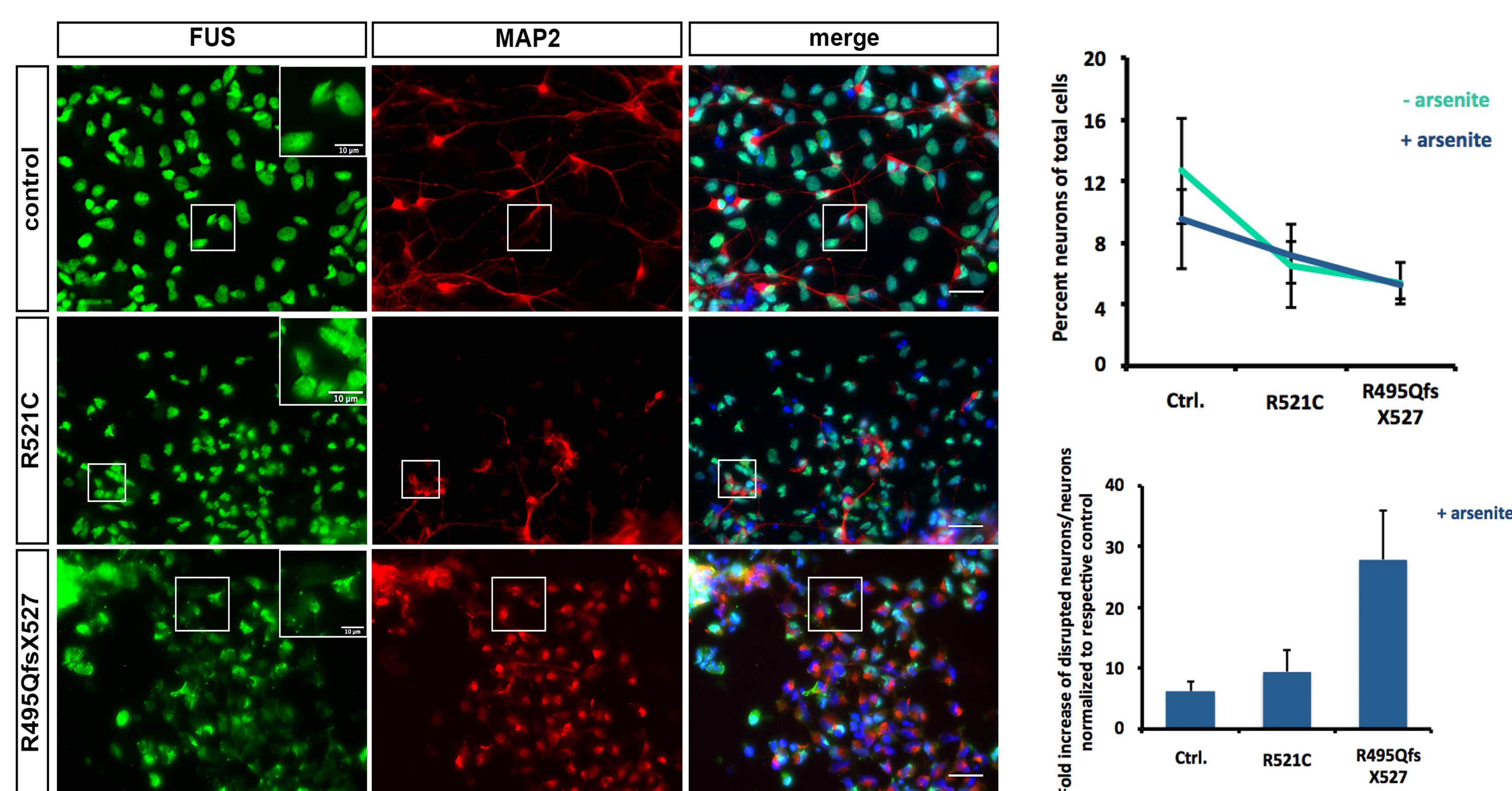
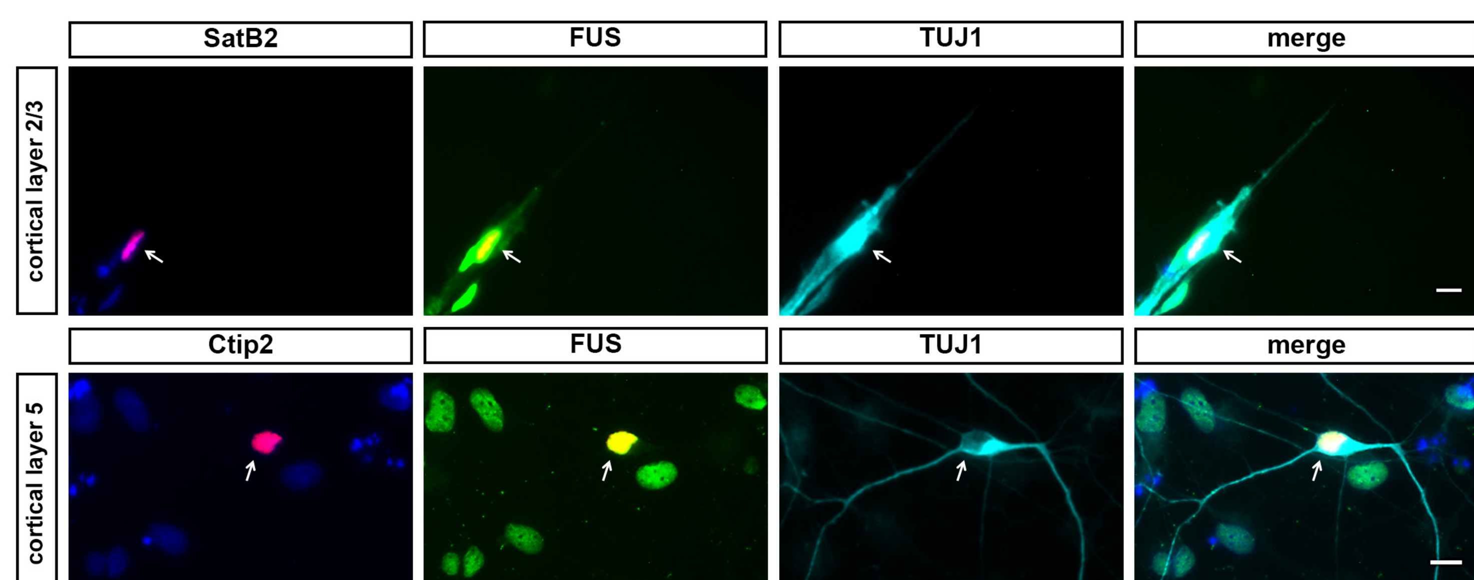


Figure 5: FUS pathology in FUS-ALS patient-derived cortical neurons is not specific to layer V corticospinal motor neurons (Ctip2) but also seen in Layer II/III cells (SatB2)



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