

N-acetylaspartate rescues DUSP6 deficiency-induced neurogenesis blockage and synaptic loss in Alzheimer's disease

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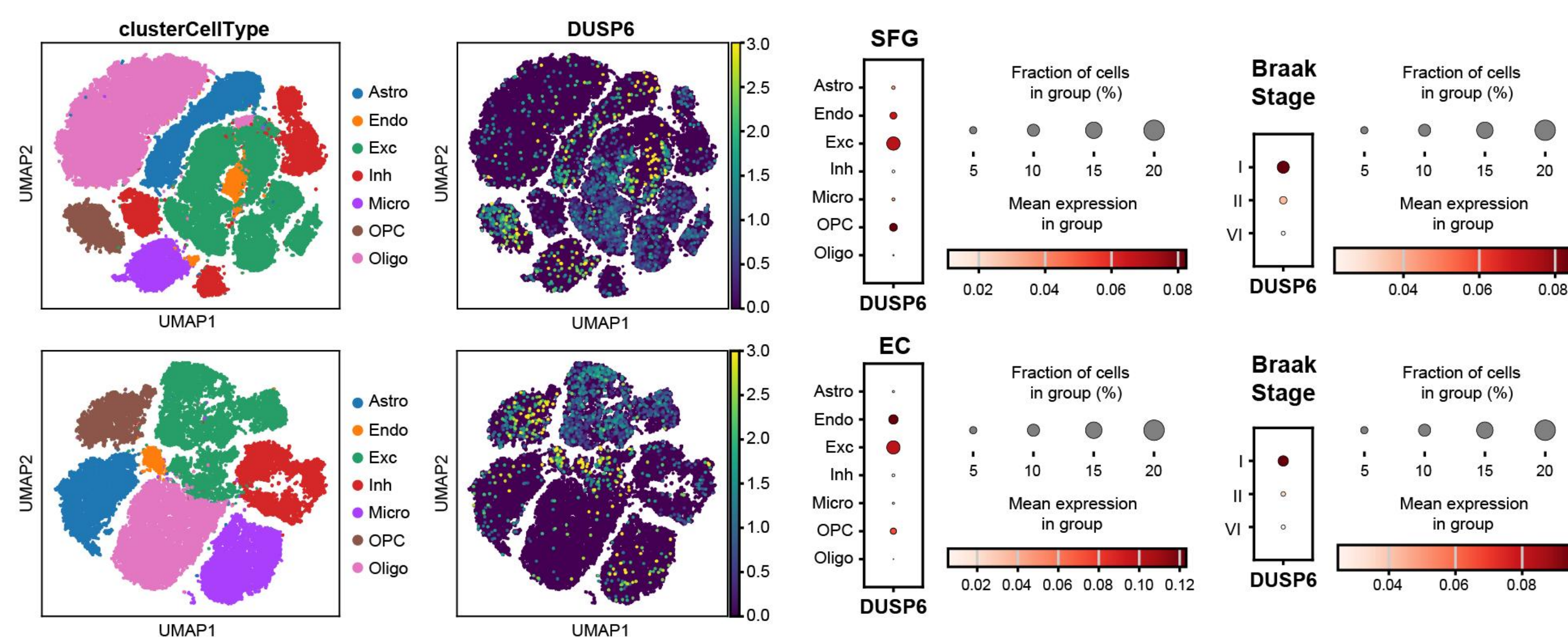
Introduction

Excessive activation of extracellular signal-regulated kinase (ERK) signaling underlies Alzheimer's disease (AD). Dual-specificity phosphatase 6 (DUSP6) is the most abundant enzyme for ERK dephosphorylation in brain that guards against over-activation. Importantly, DUSP6 is suppressed in AD patients, yet its consequences remain understudied in vivo.

Methods

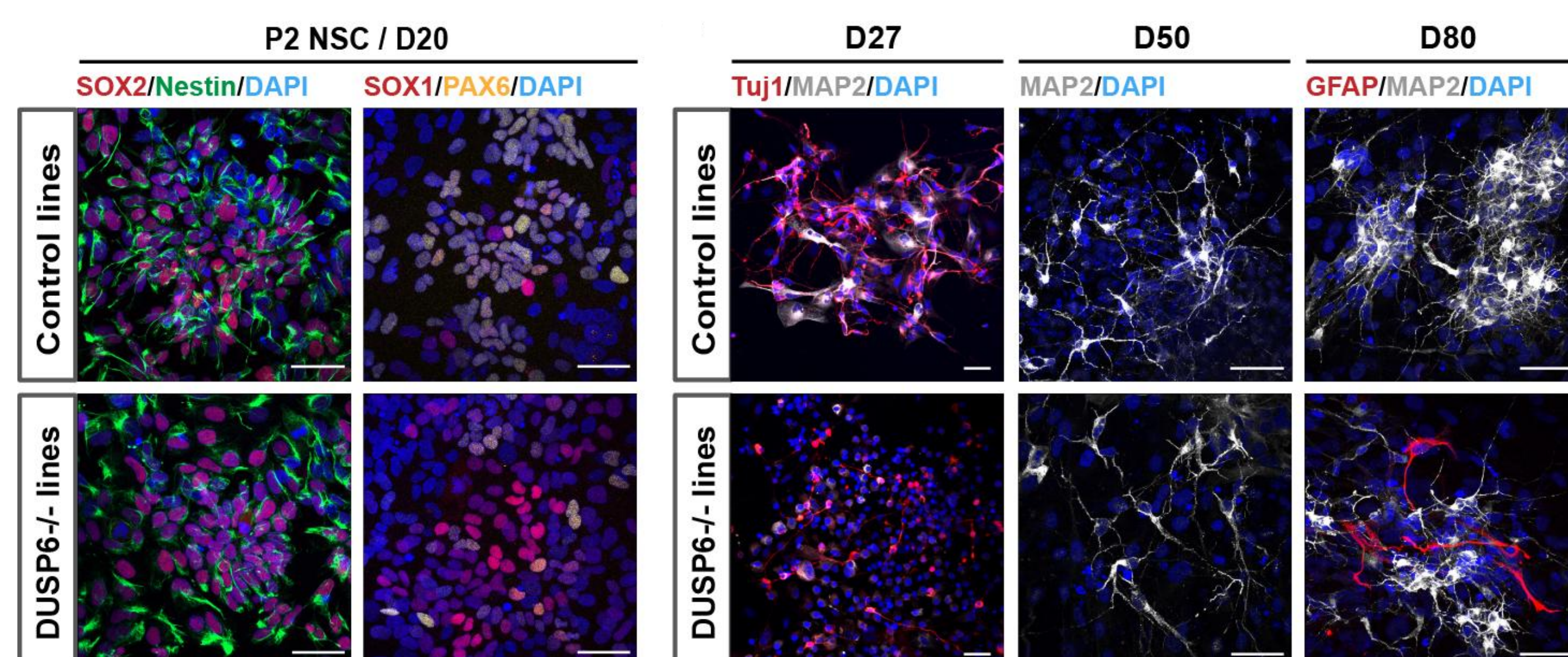
Transcriptome analysis of DUSP6 from 20 post-mortem patients with AD revealed preferential neuronal expression in prefrontal and entorhinal cortex, with more pronounced reduction in cases with later BRAAK stages. To model DUSP6 reduction, we generated knockout models in human embryonic stem cell lines (H9-ESCs) and in mice. To test the therapeutic effect of supplementing DUSP6, we injected adeno-associated virus in mice's dentate gyrus for over expression. We examined neuronal differentiation and maturation through morphological evaluation and immunostaining of specific markers. We measured the effect on the cognitive function with Morris water maze (MWM), novel object recognition (NOR) and fear conditioning (CFC). The brain glucose metabolism was measured by positron emission tomography (PET).

1. DUSP6 is downregulated in AD



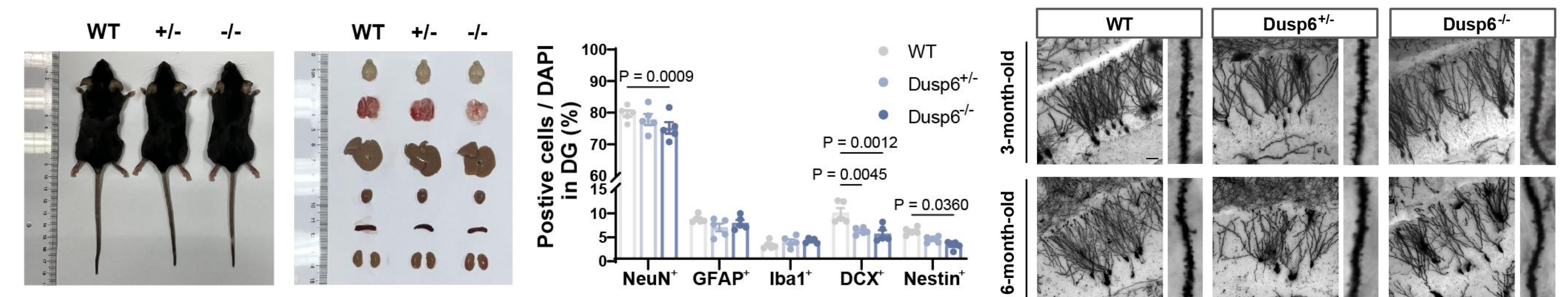
We first examined single-cell sequencing datasets of prefrontal cortex and entorhinal cortex from postmortem brains of 20 AD patients downloaded from public databases, and found that DUSP6 was expressed predominantly in excitatory neurons, and that the levels of its expression declined with increasing Braak staging.

2. DUSP6 controls neuronal differentiation in human embryonic stem cells and mice,



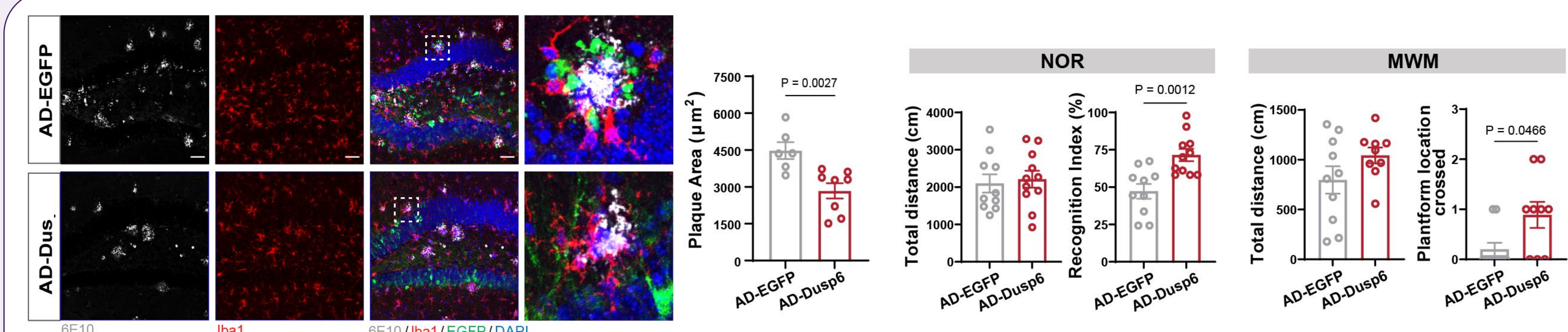
To determine the role of DUSP6 in NSC differentiation, we first generated DUSP6-deficient ESCs via CRISPR/Cas9. Loss of DUSP6 significantly impaired neuronal differentiation and enhanced glial cell proliferation.

3. DUSP6 deficiency impairs synaptic maturation, energy utilization, and cognition.



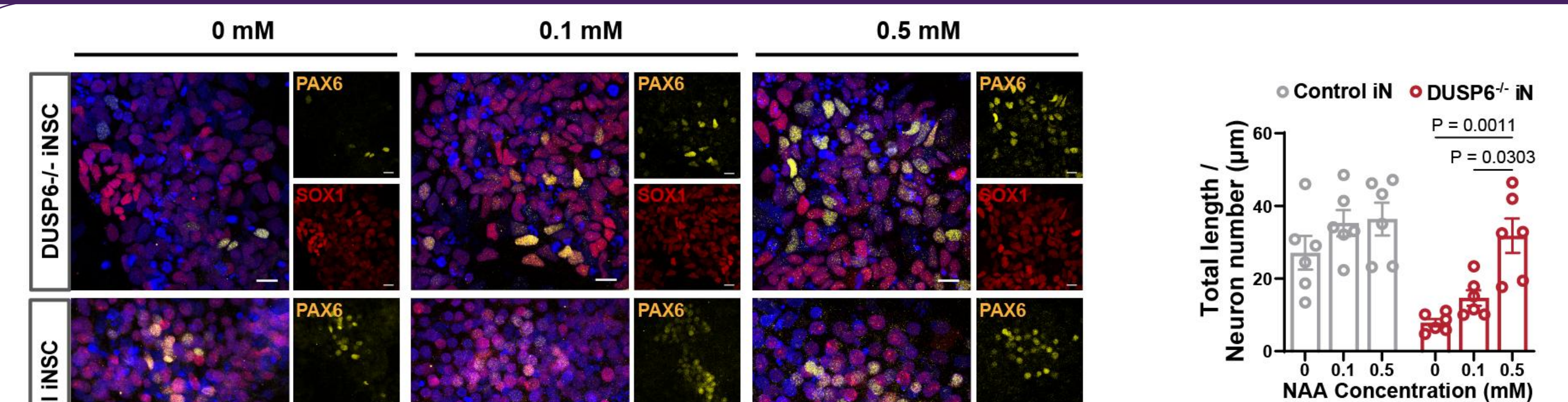
In adult mice, Dusp6 deficiency reduced hippocampal neurogenesis, simplified dendritic arbors, and decreased synaptic protein. These abnormalities were accompanied by defective cerebral glucose metabolism and impaired cognitive functions.

4. DUSP6 overexpression ameliorates AD-related pathology and cognitive deficits in mice



DUSP6 overexpression in 5xFAD mice showed protective effects including elevated neurogenesis, attenuated amyloid plaques deposition, and boosted metabolic and cognitive functions.

5. DUSP6 promotes neuronal differentiation by controlling N-acetylaspartate synthesis



We further found that DUSP6 regulates the expression of the mitochondrial gene NAT8L, which encodes the only known NAA synthase. Consistent with this regulatory link, exogenous NAA supplementation rescued the neuronal differentiation defects caused by DUSP6 deficiency in a dose-dependent manner.