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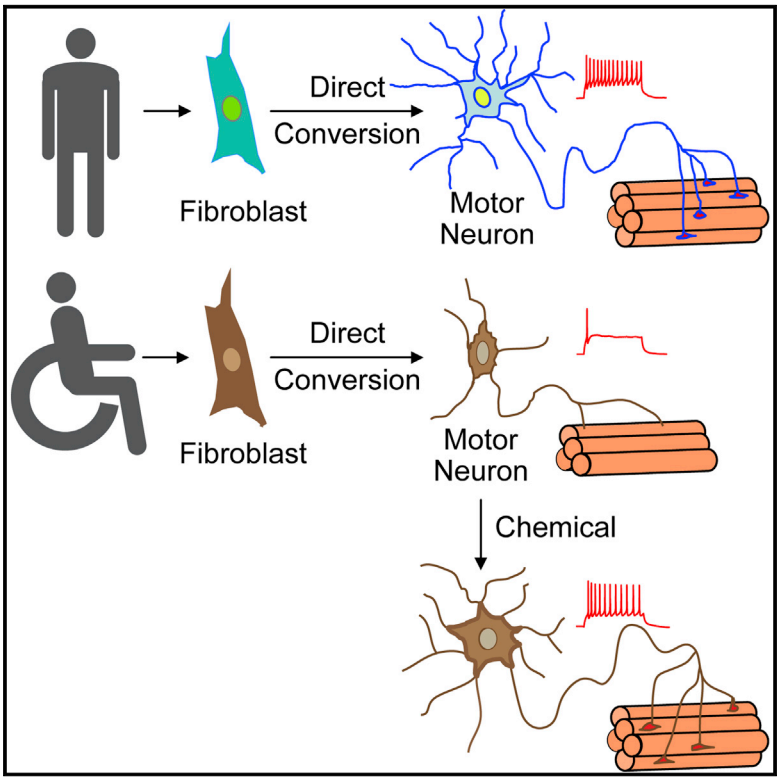
# Direct Lineage Reprogramming Reveals Disease-Specific Phenotypes of Motor Neurons from Human ALS Patients

## Graphical Abstract

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## In Brief

Liu et al. demonstrate that adult human fibroblasts can be efficiently and directly reprogrammed into highly pure spinal motor neurons (hiMNs). These neurons exhibit mature electrophysiology, form neuromuscular junctions, and control muscle activity. Interestingly, hiMNs from ALS fibroblasts show physiological deficits, which can be ameliorated by a small chemical compound.

## Highlights

- Directly convert adult human fibroblasts to highly pure hiMNs
- hiMNs are physiologically mature controlling muscle activity
- hiMNs derived from ALS patient fibroblasts show pathophysiology
- Pathophysiology of ALS hiMNs can be rescued by a small molecule

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# Direct Lineage Reprogramming Reveals Disease-Specific Phenotypes of Motor Neurons from Human ALS Patients

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## SUMMARY

**Subtype-specific neurons obtained from adult humans will be critical to modeling neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). Here, we show that adult human skin fibroblasts can be directly and efficiently converted into highly pure motor neurons without passing through an induced pluripotent stem cell stage. These adult human induced motor neurons (hiMNs) exhibit the cytological and electrophysiological features of spinal motor neurons and form functional neuromuscular junctions (NMJs) with skeletal muscles. Importantly, hiMNs converted from ALS patient fibroblasts show disease-specific degeneration manifested through poor survival, soma shrinkage, hypoactivity, and an inability to form NMJs. A chemical screen revealed that the degenerative features of ALS hiMNs can be remarkably rescued by the small molecule kenpaullone. Taken together, our results define a direct and efficient strategy to obtain disease-relevant neuronal subtypes from adult human patients and reveal their promising value in disease modeling and drug identification.**

## INTRODUCTION

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a devastating adult-onset neurological disorder prevalent worldwide with no effective cure (Arbab et al., 2014; Gladman et al., 2012). ALS is characterized by progressive motor neuron (MN) dysfunction and death; however, the mechanisms leading to selective MN loss around the age of onset remain poorly understood (Arbab et al., 2014; Robberecht and Philips, 2013). This is largely due to the lack of available patient-specific MNs during disease progression (Arbab et al., 2014). Studies of post-mortem tissues, as well as transgenic cellular and animal models, have provided valuable insights into pathogenic ALS phenotypes (Bruijn et al., 1998; Gurney et al., 1994; Hadzipasic et al., 2014; Haidet-Phillips et al., 2011; Kiernan and Hudson, 1991; Qiu et al., 2014; Re et al., 2014; Spalloni et al., 2011; Wada et al., 2012). Nonetheless, major discrepancies and controversies exist in these models owing to genetic, anatomical, and experimental variations (Bories et al., 2007; Delestrée et al., 2014; Haidet-Phillips et al., 2011; Kuo et al., 2004; Leroy et al., 2014; Re et al., 2014; Saxena et al., 2013). Unsurprisingly, no therapeutic has succeeded in translation to the clinic (Gladman et al., 2012; Musarò, 2013).

Induced pluripotent stem cells (iPSCs) derived from human skin fibroblasts and differentiated into spinal MNs are emerging as a cellular model for investigating ALS (Chen et al., 2014; Dimos et al., 2008; Kiskinis et al., 2014). This model utilizes genetic mutation(s) naturally occurring in human patients and thus avoids potential pitfalls associated with ectopic overexpression of mutant genes. New insights into the pathology of ALS have been gained with this model (Chen et al., 2014; Kiskinis et al., 2014); however, the generation of iPSCs and their stepwise differentiation into MNs are lengthy and complex processes accompanied by technical limitations due to iPSC line variation and the heterogeneity of differentiated neurons (Arbab et al., 2014). Furthermore, because iPSCs are reset to an embryonic stage during reprogramming (Lapasset et al., 2011; Miller et al., 2013; Rando and Chang, 2012), a major difficulty with modeling ALS is the induction of an adult-onset pathology using iPSC-derived fetal stage neurons (Arbab et al., 2014).

Direct lineage reprogramming bypasses pluripotency and converts fully differentiated somatic cells into functional neurons (Pang et al., 2011; Vierbuchen et al., 2010; Yoo et al., 2011). This technology is also capable of creating subtype-specific neurons, such as dopaminergic neurons (Caiazzo et al., 2011), striatal medium spiny neurons (Victor et al., 2014), nociceptive neurons (Wainger et al., 2015), and cholinergic neurons (Liu et al., 2013; Son et al., 2011). Despite limitations with reprogramming efficiency and neuronal purity, directly converted subtype-specific neurons are potentially more valuable to disease modeling and drug identification for late-onset human neurological disorders (Arbab et al., 2014). As a proof-of-concept study, we provide a protocol for direct and highly efficient conversion of adult human fibroblasts to functionally mature MNs with high purity. We further reveal pathology of MNs derived from ALS patient fibroblasts with FUS mutations. Most importantly, a pilot drug screen