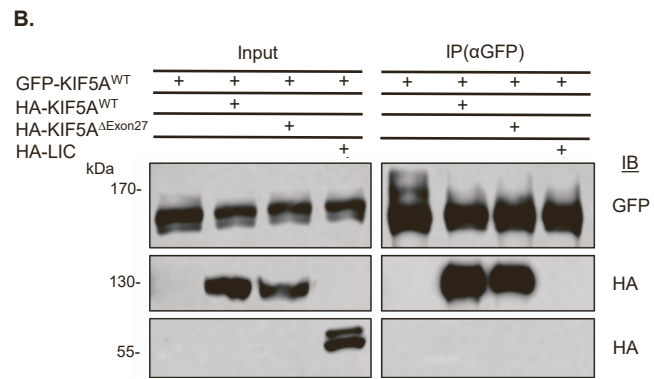
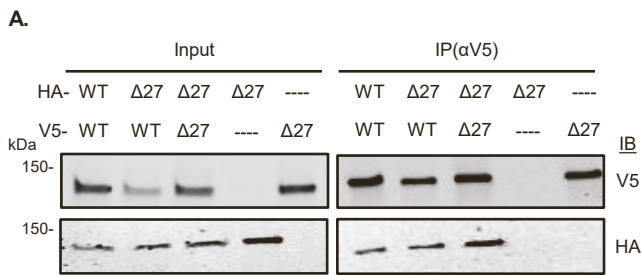


**Supplemental information**

**ALS-associated KIF5A mutations abolish autoinhibition  
resulting in a toxic gain of function**

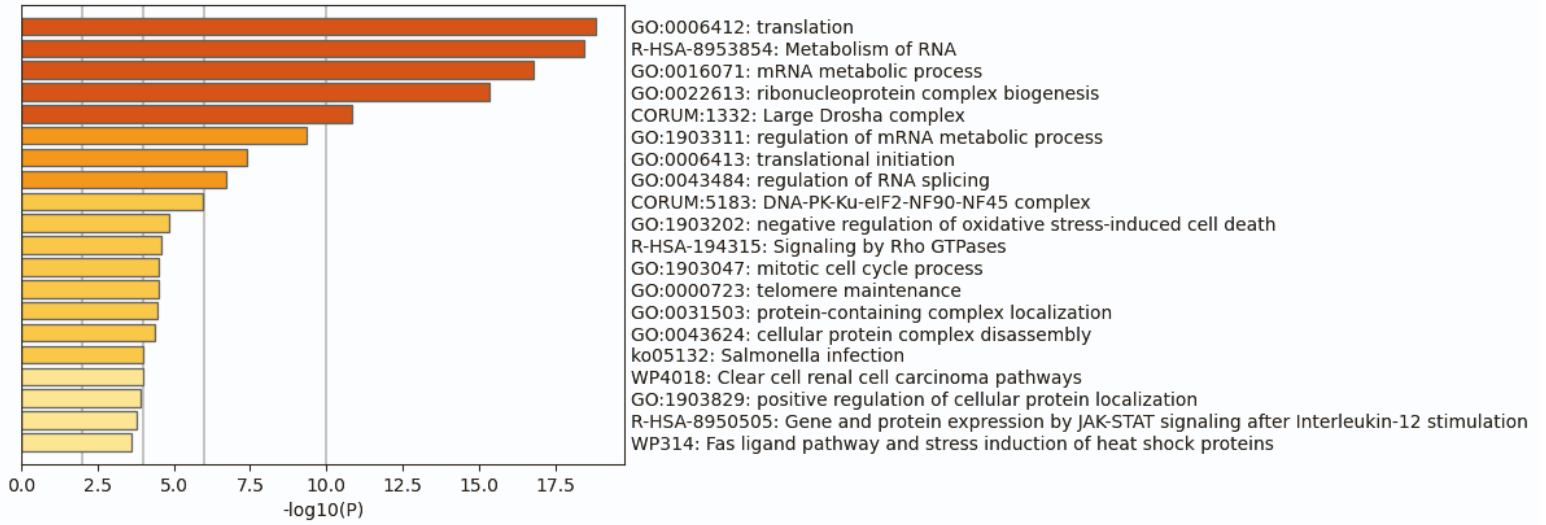
**Desiree M. Baron, Adam R. Fenton, Sara Saez-Atienzar, Anthony Giampetruzzi, Aparna Sreeram, Shankaracharya, Pamela J. Keagle, Victoria R. Doocy, Nathan J. Smith, Eric W. Danielson, Megan Andresano, Mary C. McCormack, Jaqueline Garcia, Valérie Bercier, Ludo Van Den Bosch, Jonathan R. Brent, Claudia Fallini, Bryan J. Traynor, Erika L.F. Holzbaur, and John E. Landers**



**Figure S1: Mutant KIF5A can homo/hetero dimerize with other forms of KIF5A suggesting a gain of function toxicity, Related to Figure 1 and Figure 2** (A) SKNAS were transfected with either V5-KIF5A and HA-KIF5A wild-type or  $\Delta$ Exon27 mutant, then the V5-KIF5A was immunoprecipitated and the lysate probed for V5- and HA-tagged protein. The blot is representative of N=2 biological replicates. (B) HEK293FT cells were transfected with GFP-KIF5A WT alone or co-transfected with HA-KIF5A Wild-Type,  $\Delta$ Exon27, or Dynein Light Intermediate Chain (LIC, negative control). The GFP-

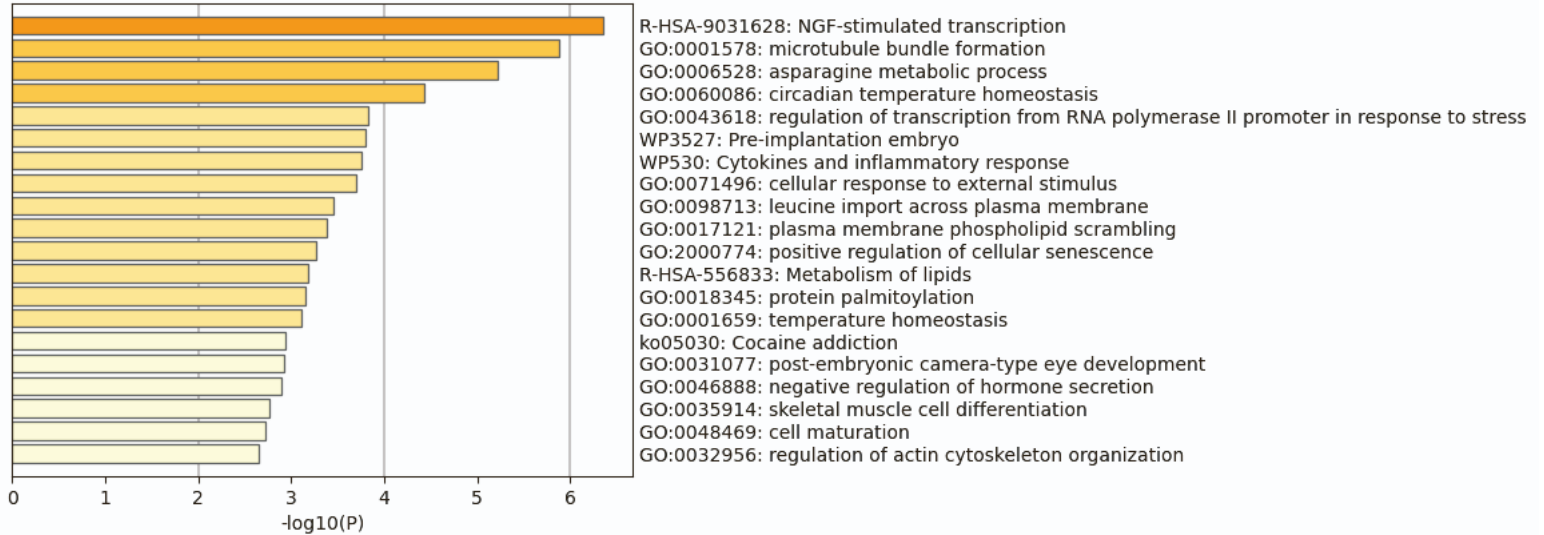
A.

Extended list of enriched GO terms for  $\geq 4x$  increased + unique  $\Delta$ Exon27 protein interactions

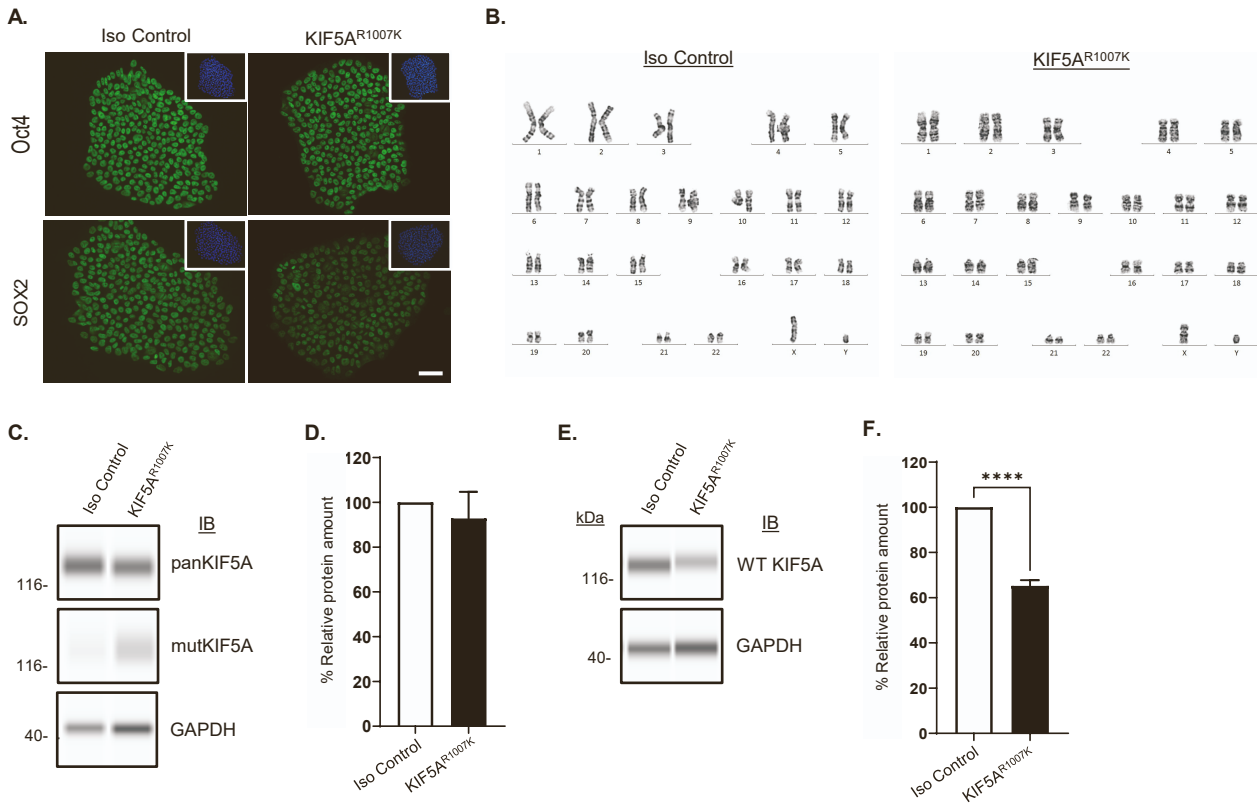


B.

Extended list of enriched GO terms for  $\geq 4x$  increased + unique  $\Delta$ Exon27 RNA interactions



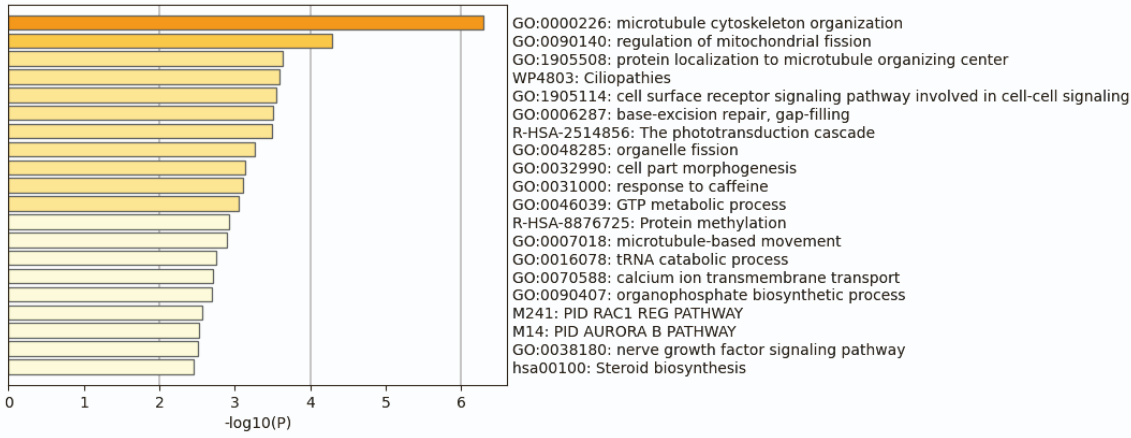
**Figure S2: Extended pathway analysis information from KIF5A $\Delta$ Exon27 interaction experiments, Related to Figure 4.** (A) Extended list of enriched GO terms for  $\geq 4x$  increased + unique KIF5A $\Delta$ Exon27 protein interactions. (B) Extended list of enriched GO terms for  $\geq 4x$  increased + unique KIF5A $\Delta$ Exon27 RNA interactions.



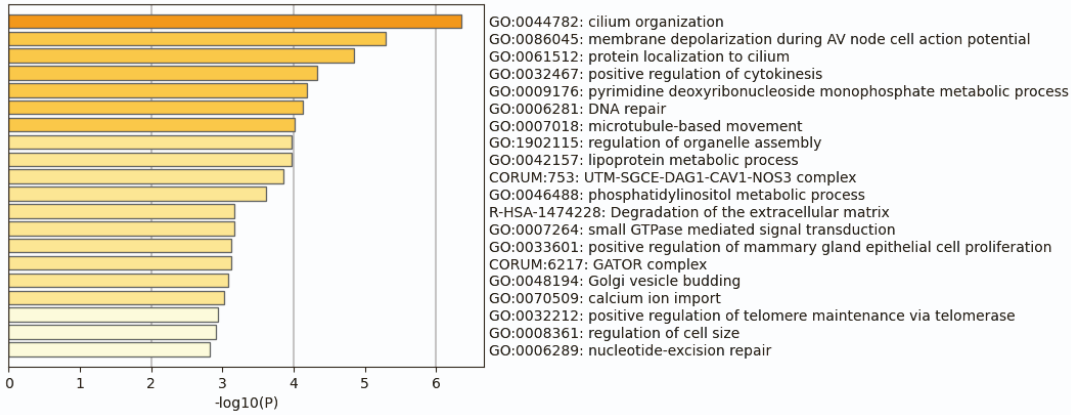
**Figure S3: KIF5A NIL iPSC characterization, Related to Figure 5.** (A) KIF5A<sup>R1007K</sup> mutant and isogenic control iPSC lines stained for the pluripotent markers Oct4 and Sox2. Inset: DAPI staining of iPSC lines. Scale bar = 50 $\mu$ m. (B) Karyotyping analysis of the lines shown in (a). (C-F) Expression of wild-type and ALS mutant forms of KIF5A in DIV15 KIF5A<sup>R1007K</sup> mutant and isogenic control iMNs. Using a panKIF5A antibody that recognizes both forms of the protein, capillary western blotting (c) shows a non-significant difference in total KIF5A protein levels (d). Blotting the same samples in (c) with a lab generated, mutant specific antibody (not shown), raised against the mutant C-terminus of KIF5A, confirms that the mutant protein is present in the KIF5A<sup>R1007K</sup> cells. Blotting KIF5A<sup>R1007K</sup>



**A.** Pathway Analysis of Exons displaying decreased skipping in KIF5A<sup>R1007K</sup> iMNs (1000)



**B.** Pathway Analysis of Exons displaying increased skipping in KIF5A<sup>R1007K</sup> iMNs (919)



**Figure S4: Extended pathway analysis information from KIF5A<sup>R1007K</sup> splicing analysis from RNAseq experiments, Related to Figure 6.** (A) Extended list of enriched GO terms for genes with decreased exon skipping in KIF5A<sup>R1007K</sup> iMNs. (B) Extended list of enriched GO terms for genes with increased exon skipping in KIF5A<sup>R1007K</sup> iMNs.