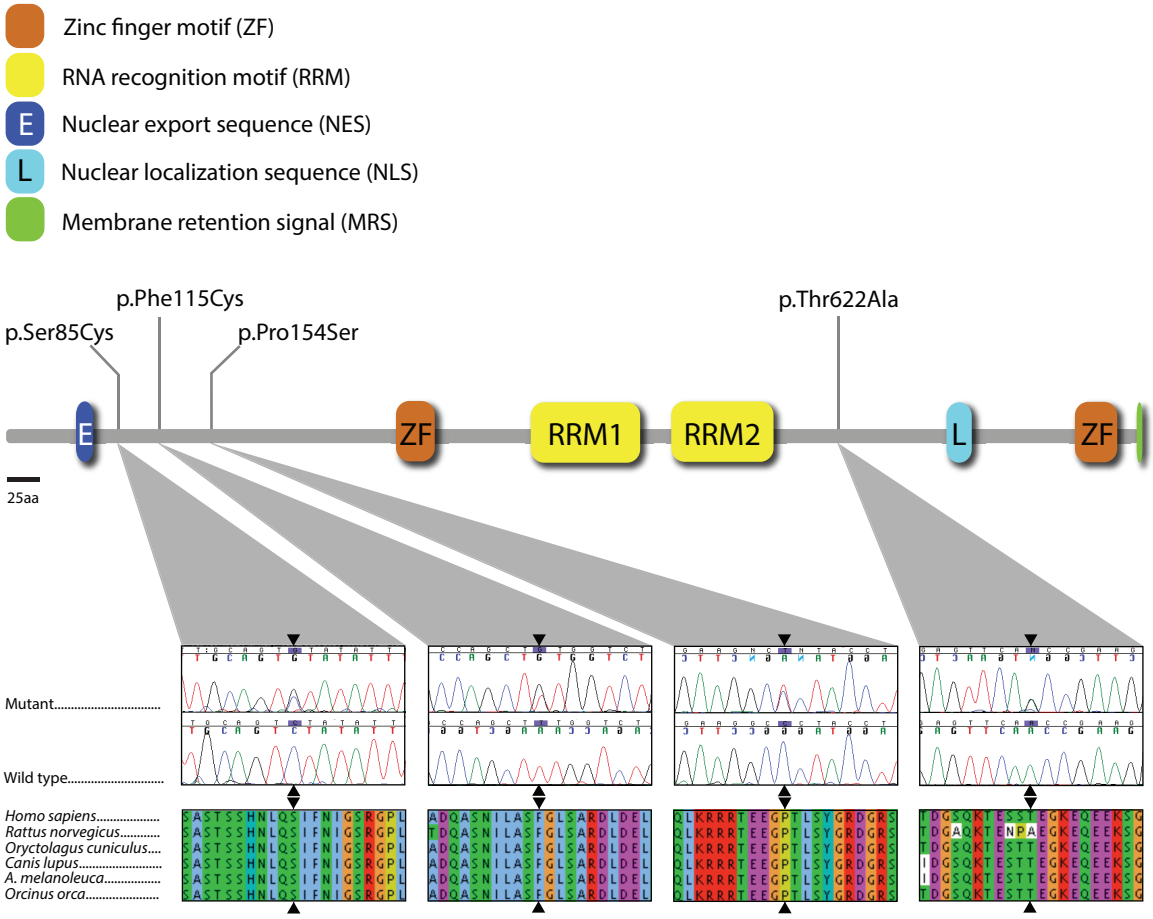
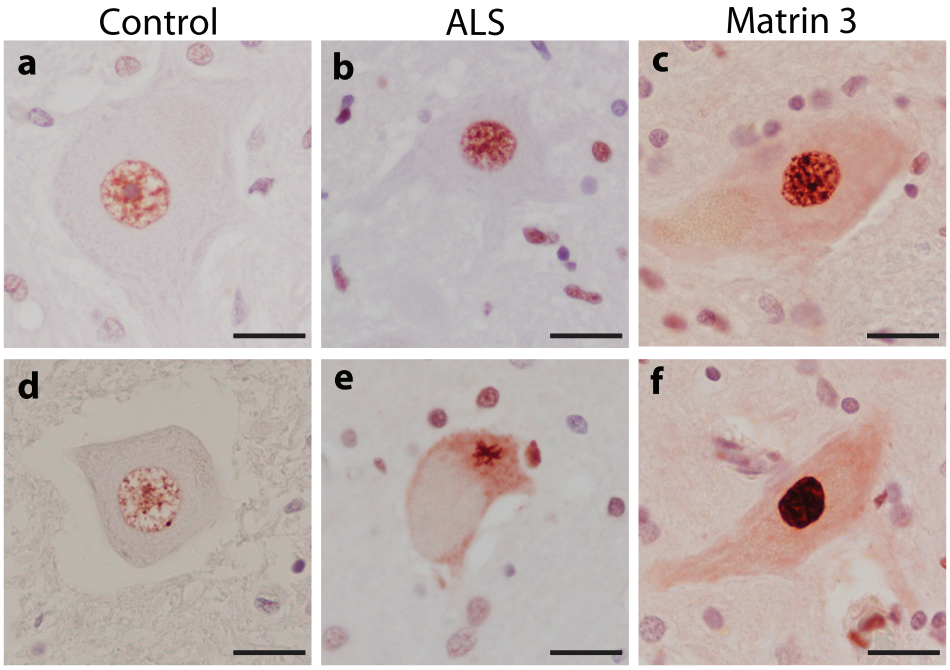
Supplementary Material

Mutations in the *Matrin 3* gene cause familial amyotrophic lateral sclerosis

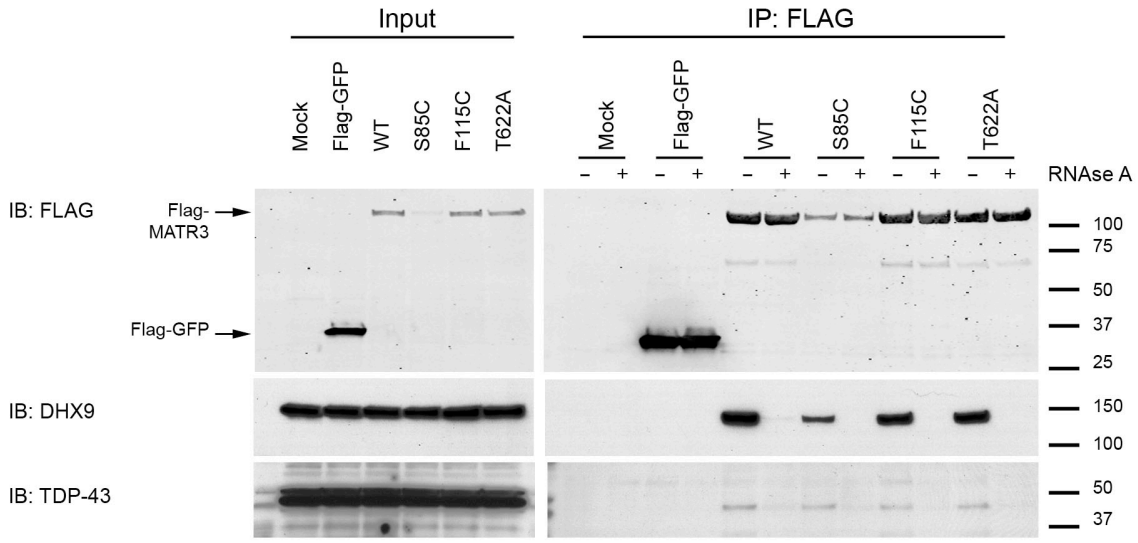
Janel O. Johnson, Erik P. Pioro, Ashley Boehringer, Ruth Chia, Howard Feit, Alan E. Renton, Hannah A. Pliner, Yevgeniya Abramzon, Giuseppe Marangi, Brett J. Winborn, J Raphael Gibbs, Michael A. Nalls, Sarah Morgan, Maryam Shoai, John Hardy, Alan Pittman, Richard W. Orrell, Andrea Malaspina, Katie C. Sidle, Pietro Fratta, Matthew B. Harms, Robert H. Baloh, Alan Pestronk, Conrad C. Weihl, Ekaterina Rogaeva, Lorne Zinman, Vivian E. Drory, Giuseppe Borghero, Gabriele Mora, Andrea Calvo, Jeffrey D. Rothstein, ITALSGEN, Carsten Drepper, Michael Sendtner, Andrew B. Singleton, J. Paul Taylor, Mark R. Cookson, Gabriella Restagno, Mario Sabatelli, Robert Bowser, Adriano Chiò, Bryan J. Traynor.



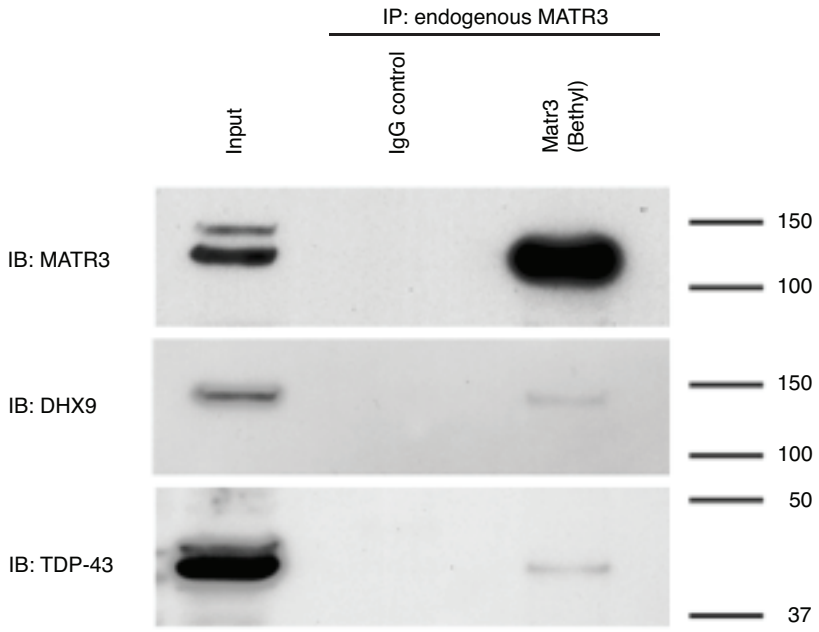
Supplementary Figure 1. Distribution of *MATR3* mutations detected in familial ALS patients.
The upper panel shows the location of detected mutations and of the domains of MATR3 as determined by Hibino, Y., et al. Biochim. Biophys. Acta 1759, 195–207 (2006). Corresponding chromatograms showing mutant and wild-type alleles are as indicated, and conservation of amino acid residue across species is highlighted at the bottom (generated using the Clustal Omega online tool, www.ebi.ac.uk/Tools/msa/clustalo/).



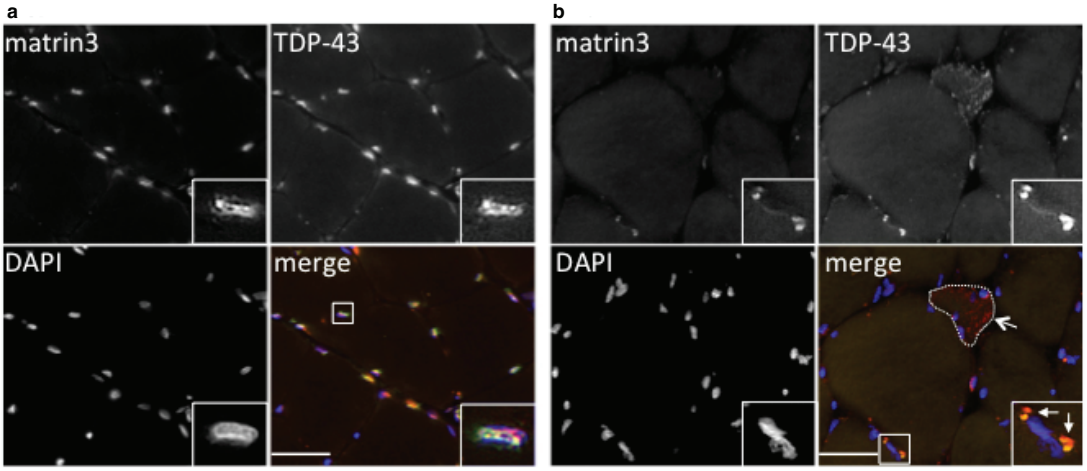
Supplementary Figure 2. MATR3-immunoreactive staining in spinal cord neurons of ALS patients.
(**a** & **d**) Control cases exhibit a nuclear staining pattern with staining not filling the entire nucleus. (**b** & **e**) ALS cases display stronger nuclear staining pattern with cytoplasmic staining present in some cells. Cytoplasmic staining is either diffuse across the entire cell or found in cytoplasmic puncta. E shows a MATR3-positive cytoplasmic inclusion, which are occasionally observed (this patient was known to carry a pathogenic *C9ORF72* repeat expansion). (**c** & **f**) Patient carrying the pPhe115Cys *MATR3* mutation shows strong nuclear staining and cytoplasmic staining in many cells. Immunohistochemistry was performed using the HPA036565 antibody (Sigma-Aldrich). Similar results were seen with a different anti-MATR3 antibody. MATR3 was not detected within cytoplasmic inclusions containing TDP-43 (data not shown). All images were taken at 40x magnification, and the scale bars represent 25μm.



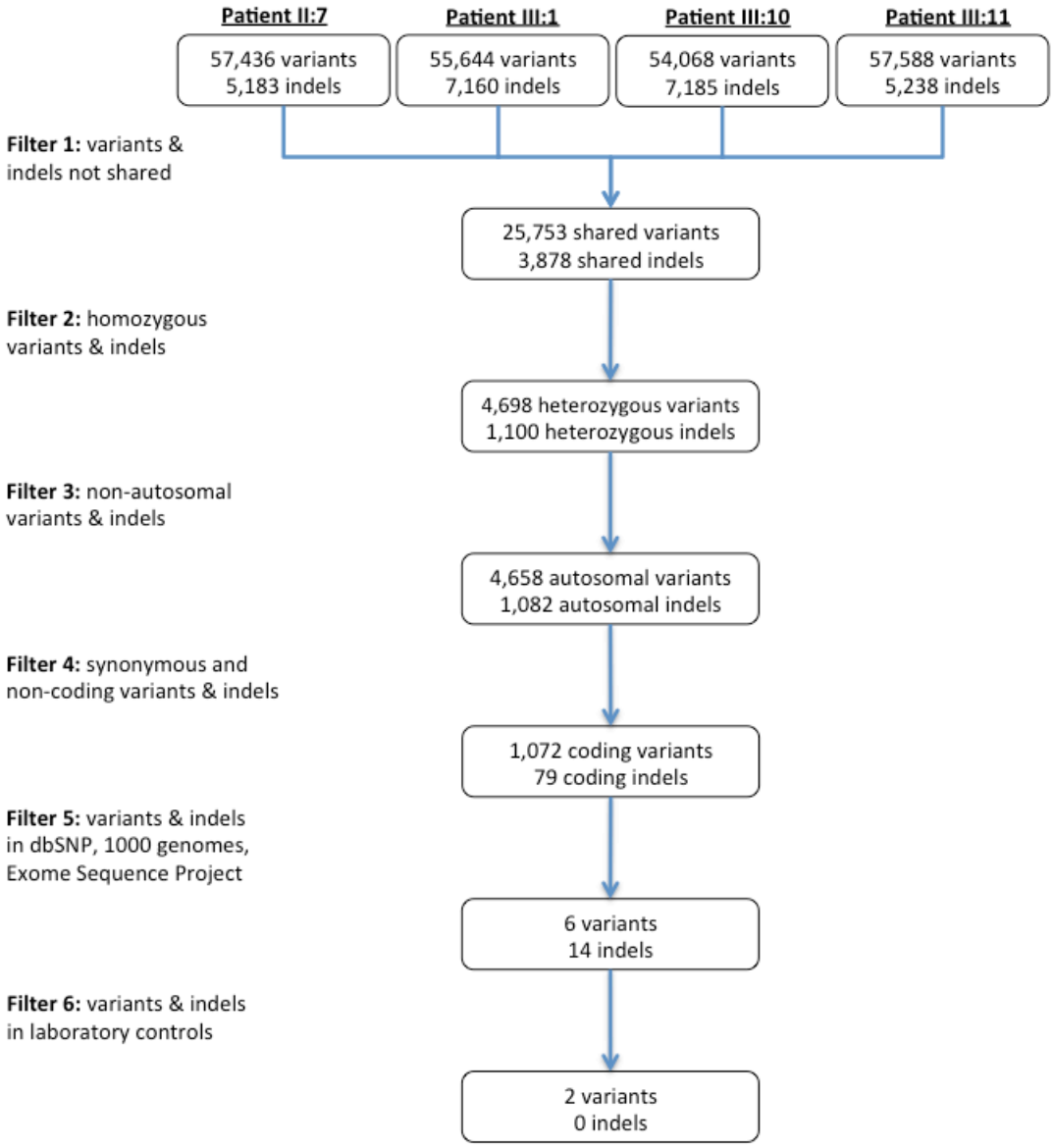
Supplementary Figure 3. MATR3 and TDP-43 interaction is RNA dependent. FLAG-MATR3 was expressed in 293FT cells, immunoprecipitated using anti-FLAG antibody followed by treatment with RNase A and probed with TDP-43 and DHX9 antibodies. Representative blots from two independent experiments are shown. Interaction of MATR3 and DHX9 is consistent with Salton, M. et al., PLoS One 6, e23882 (2011) showing that the interaction is RNA dependent, as is the interaction with TDP-43.



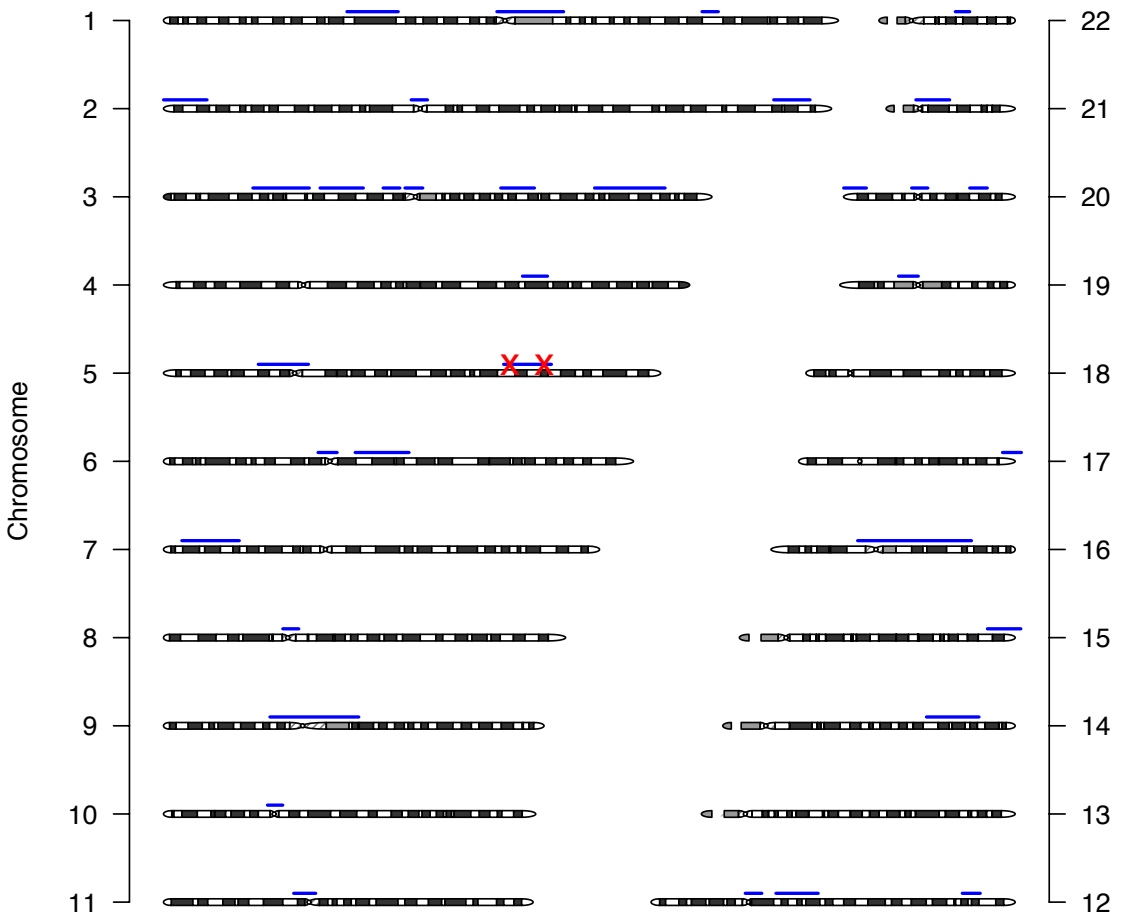
Supplementary Figure 4. Co-immunoprecipitation experiments using endogenous MATR3.
Endogenous MATR3 was immunoprecipitated from 293FT cells and probed with DHX9 and TDP-43 antibodies. Representative blots from two independent experiments are shown. These data show that MATR3, TDP-43 and DHX9 interact at the endogenous level.



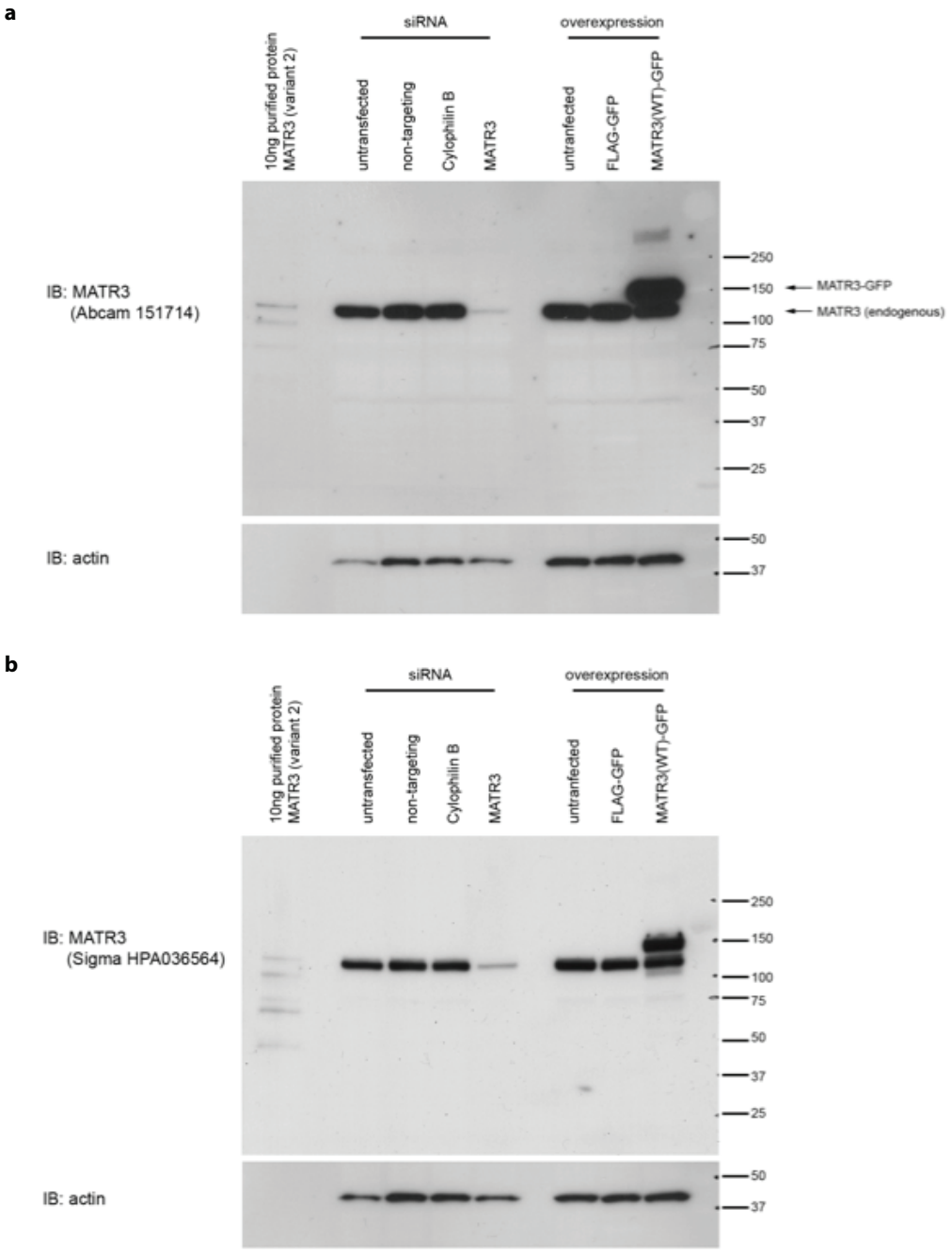
Supplementary Figure 5. Immunofluorescence of skeletal muscle biopsy from (a) a normal control, and (b) a patient carrying the p.Ser85Cys missense mutation in *MATR3* using anti-TDP-43 and anti-MATR3 antibodies.
In normal skeletal muscle, MATR3 and TDP-43 localize to nuclei including myonuclei. In the patient with the *MATR3* mutation, there is decreased nuclear MATR3 immunoreactivity, whereas TDP-43 accumulates in the sarcoplasm and is restricted from the nucleus. In addition, MATR3 and TDP-43 co-aggregate in the sarcoplasm adjacent to myonuclei. Open arrow highlights a TDP-43 positive fiber (outlined in white). Closed arrows demonstrate MATR3 and TDP-43 co-localized in perinuclear inclusions. Insets are enlarged myonuclei and the scale bar is 50uM.



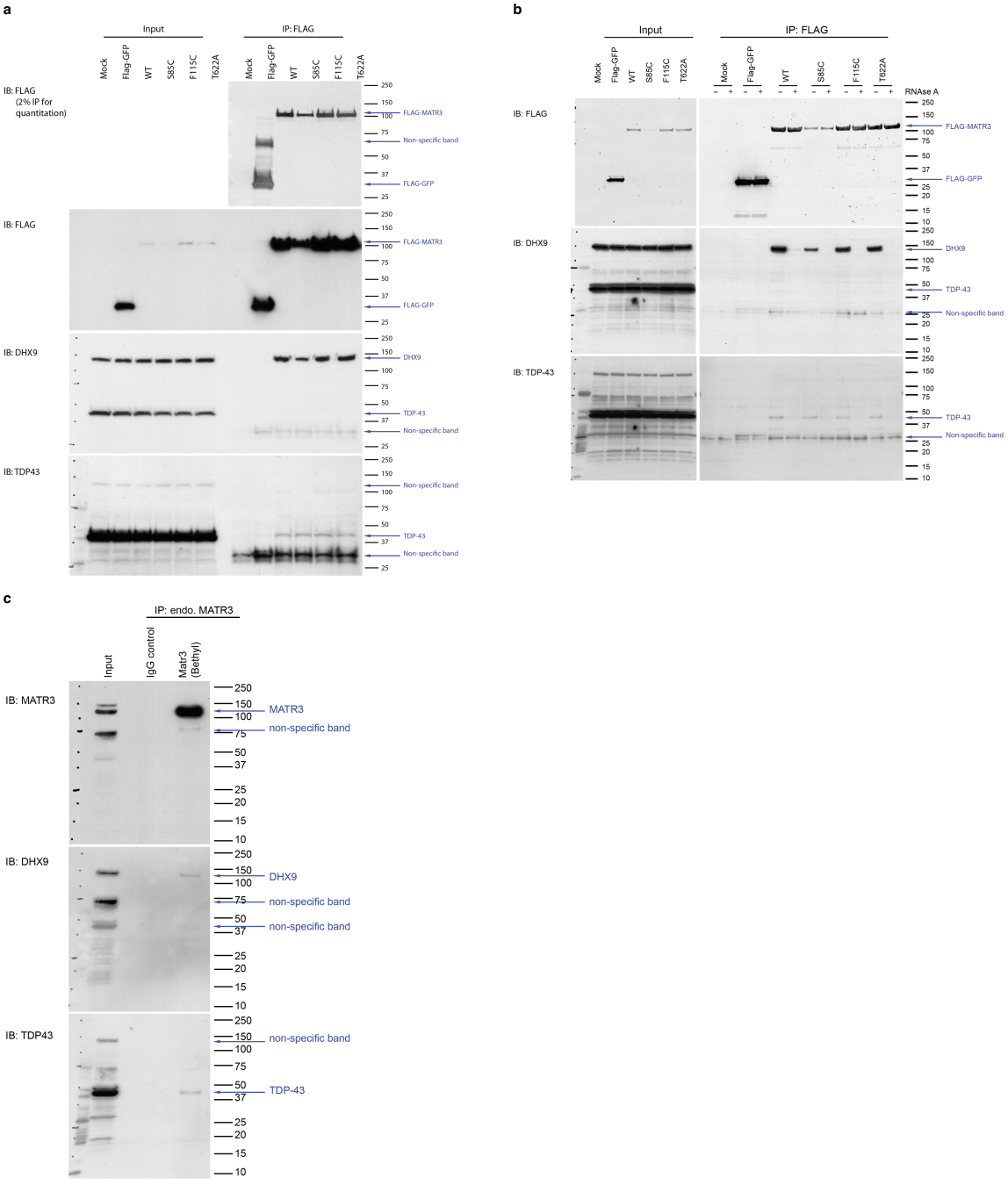
Supplementary Figure 6. Filters applied to variants and indels detected by exome sequencing in affected individuals of the USALS#3 pedigree.



Supplementary Figure 7. Novel coding variants identified in the USALS#3 kindred by exome sequencing.
Graphical representation of autosomes showing genomic regions shared by the four affected individuals of the USALS#3 pedigree (blue lines). Whole genome data was generated using Infinium OmniExpress genotyping arrays (Illumina Inc.). *LMNB1* and *MATR3* variants are shown as red crosses located within a 17.4 Mb shared segment on chromosome 5q. No other novel, coding variants were shared across affected individuals of the USALS#3 family.



Supplementary Figure 8. Characterization of MATR3 antibodies.
The specificity of MATR3 antibodies Abcam 151714 (**a**) and Sigma HPA0036564 (**b**) were tested against purified MATR3 protein (Origene TP323258), lysates from HEK293FT cells treated with siRNA (untransfected control, non-targeting control, Cyclophilin B and MATR3), and cells overexpressing MATR3(WT)-GFP. Both antibodies were specific to MATR3 as indicated by the MATR3 siRNA sample showing a reduction in MATR3 protein level compared to untransfected, non-targeting and Cyclophilin B controls.



Supplementary Figure 9. Full-length blots for (a) Figure 3, (b) Supplementary Figure 3 and (c) Supplementary Figure 4.

The ITALSGEN Consortium
Cristina Moglia[1], Stefania Cammarosano[1], Antonio Canosa[1], Sara Gallo[1], Maura Brunetti[2], Irene Ossola[2], Kalliopi Marinou[3], Laura Papetti[3], Fabrizio Pisano[3], Giuseppe Lauria Pinter[4], Amelia Conte[5], Marco Luigetti[5], Marcella Zollino[6], Serena Lattante[6], Giuseppe Marangi[6], Vincenzo la Bella[7], Rossella Spataro[7], Tiziana Colletti[7], Stefania Battistini[8], Claudia Ricci[8], Claudia Caponnetto[9], Gianluigi Mancardi[9], Paola Mandich[9], Fabrizio Salvi[10], Ilaria Bartolomei[10], Jessica Mandrioli[11], Patrizia Sola[11], Christian Lunetta[12], Silvana Penco[13], Maria Rosaria Monsurrò[14], Gioacchino Tedeschi[14], Francesca Luisa Conforti[15], Antonio Gambardella[15], Aldo Quattrone[15], Paolo Volanti[16], Gianluca Floris[17], Antonino Cannas[17], Valeria Piras[17], Francesco Marrosu[17], Maria Giovanna Marrosu[18], Maria Rita Murru[18], Maura Pugliatti[19], Leslie D. Parish[19], Alessandra Sotgiu[20], Giuliana Solinas[20], Lucia Ulgheri[20], Anna Ticca[21], Isabella Simone[22], Giancarlo Logroscino[22], and Angelo Pirisi[23].

Affiliations
[1] Department of Neuroscience, University of Turin, Turin, Italy;
[2] Laboratory of Molecular Genetics, ASO OIRM-Sant'Anna, Turin, Italy
[3] Salvatore Maugeri Foundation, IRCCS, Scientific Institute of Milan, Milan, Italy
[4] Department of Neurology, National Institute of Neurology 'Carlo Besta', Milan, Italy
[5] Neurological Institute, Catholic University and I.CO.M.M. Association for ALS Research, Rome, Italy
[6] Molecular Genetics Laboratory, Catholic University of Rome, Rome, Italy
[7] ALS Clinical Research Center, Bio. Ne. C., University of Palermo, Palermo, Italy
[8] Department of Neuroscience, Neurology Section, University of Siena, Siena, Italy
[9] Department of Neuroscience, Ophthalmology and Genetics, University of Genoa, Genoa Italy
[10] Center for Diagnosis and Cure of Rare Diseases, Department of Neurology, Bellaria Hospital, Bologna, Italy
[11] Department of Neuroscience, S. Agostino-Estense Hospital, and University of Modena, Modena, Italy
[12] NeuroMuscular Omnicenter, Serena Foundation, Milan
[13] Department of Laboratory Medicine, Medical Genetics, Niguarda Ca' Granda Hospital, Milan, Italy
[14] Department of Neurological Sciences, Second University of Naples, Naples, Italy
[15] Institute of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy
[16] Salvatore Maugeri Foundation, IRCCS, Scientific Institute of Mistretta, Mistretta, Italy
[17] Azienda Universitaria-Ospedaliera di Cagliari, and University of Cagliari, Cagliari, Italy
[18] Centro Sclerosi Multipla, Ospedale Binaghi, Università di Cagliari, Cagliari, Italy
[19] Department of Neuroscience, University of Sassari, Italy
[20] Department of Biomedical Science, University of Sassari, Sassari, Italy
[21] AO San Francesco, Nuoro, Italy
[22] Department of Neuroscience, University of Bari, Bari, Italy
[23] Department of Neurology, University of Sassari, Sassari, Italy