Megat et al.

Integrative genetic analysis illuminates ALS heritability and identifies novel risk genes

SUPPLEMENTARY INFORMATION

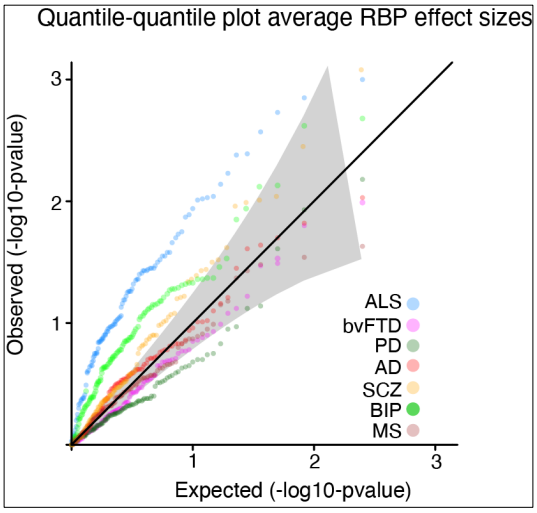

Fig. S1:Quantile-quantile plot average RBP effects binding sizes
Related to Figure 1C. Shaded gray area represent 95% confidence interval for the observed p-values.

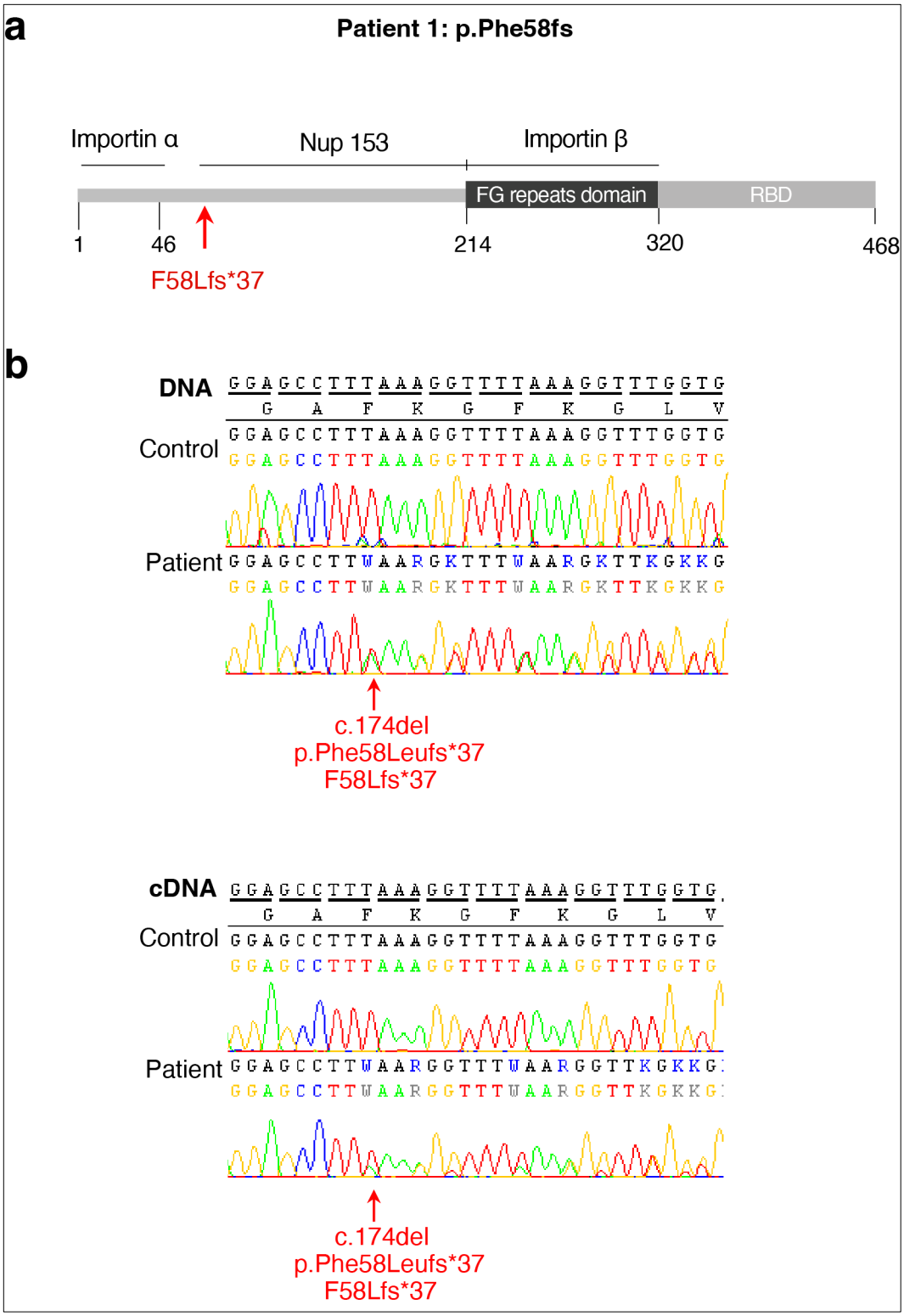

Fig. S2: *NUP50* Phe58Leufs*37 mutation

a- Representation of NUP50 protein domains with the position of the identified mutation in red. Regions of the protein interacting with importin α (Imp. α), Nup153 and importin β (Imp. β) are indicated.

b- Part of the chromatograms showing the position of the c.174del (p.F58Lfs*37) in the patient and the corresponding normal sequence (healthy control) and cDNA amplified from mRNA extracted from lymphoblasts.

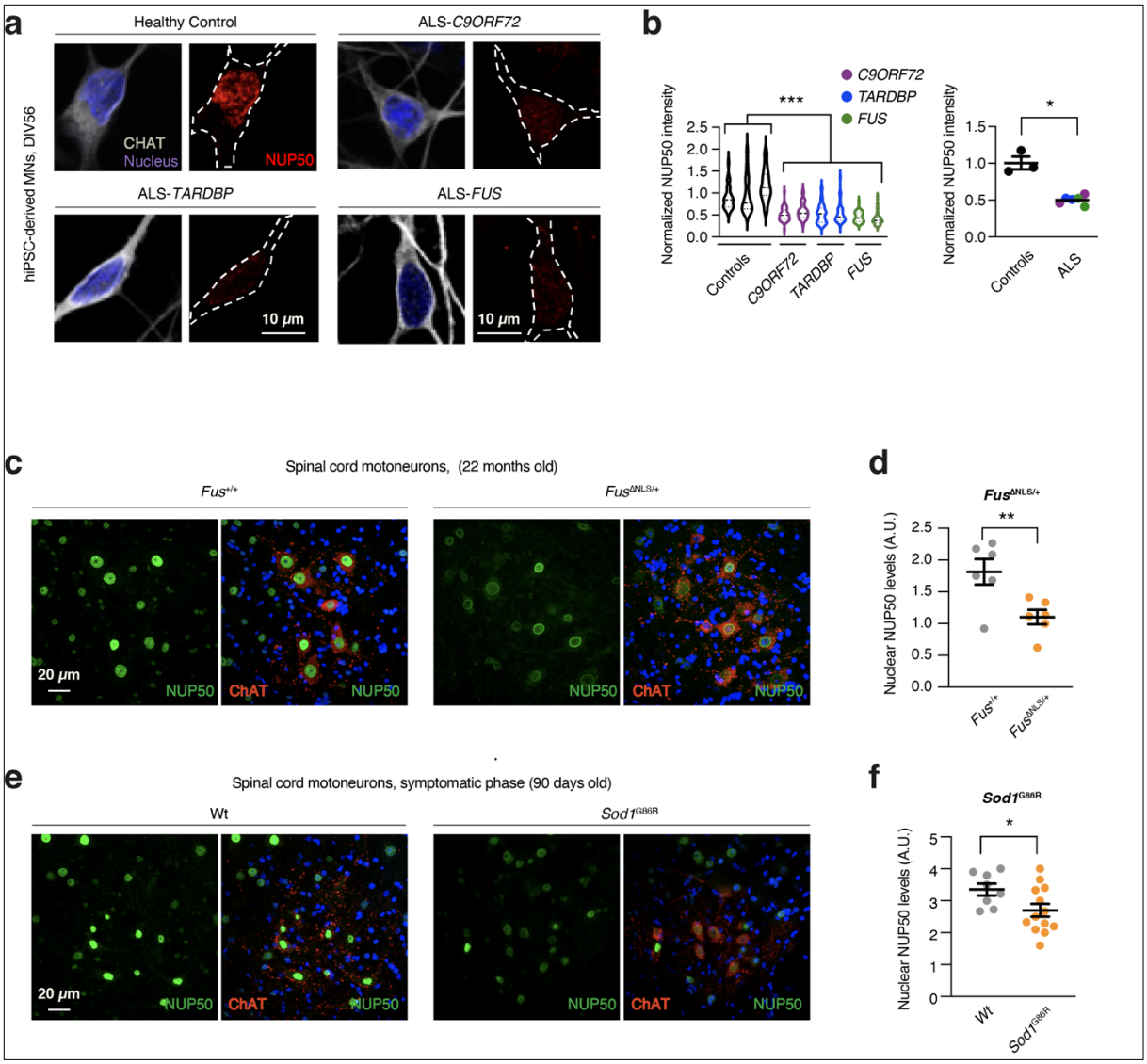

Fig. S3: NUP50 nuclear loss is a common feature in cell and animal models of ALS

a-b: Representative image of iPSCs-derived motoneurons from fALS (FUS, TDP-43 and C9ORF72 HRE carriers) immunostained for CHAT (white), nucleus (DAPI, blue) and NUP50 (red). Control 1: n= 56 cells, Control 2: n=39 cells, Control 3: n=52 cells, *C9ORF72* 1: n= 54 cells, *C9ORF72* 2: n= 53 cells, *TARDBP* 1: n=54 cells, *TARDBP* 2: n=57 cells, *FUS* 1: n= 63 cells, *FUS* 2: 65 cells, examined over 3 independent experiments. The scatter plot on the left shows the mean per subject (either control or ALS). Panel b shows quantification of NUP50 levels : Left: Kruskal Wallis, ***, p<0.001 for all comparisons between control and fALS cell lines. Right: Unpaired two tailed t-test : t=5,552, df=2,320, p = 0.0220. Data are presented as mean values +/- SEM

c-f- Representative image of immunohistochemistry for NUP50 and CHAT in spinal cord sections showing a decrease in nuclear NUP50 levels in motor neurons of (c-d) $Fus^{\Delta NLS/+}$ (Two-tailed Nested-t-test : t=3,293, df=10, p = **0,0081) and (e-f) $Sod1^{G86R}$ (Two-tailed Nested-t-test : t=2,212, df=19, p = *0.038) mice. A.U.: arbitrary units. Data are presented as mean values +/- SEM

For panel d, n=113 motor neurons examined in n=6 independent 22 months old male Wt animals, and n=132 motor neurons examined in n=6 independent $Fus^{\Delta NLS/+}$ littermates. Each dot in the scatter plot indicates the mean of an individual animal.

For panel f, n=60 motor neurons examined in n=8 independent 90 days old Wt animals, and n=106 motor neurons examined in n=13 independent $Sod1^{G86R}$ littermate animals. Each dot in the scatter plot indicates the mean of an individual animal.

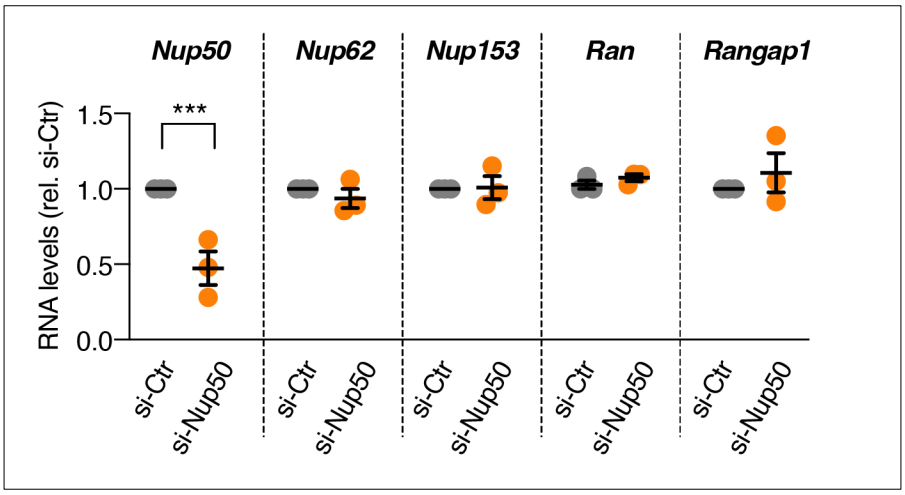

Fig. S4: mRNA levels after *Nup50* knockdown

Dot-plots showing mRNA levels of different nucleoporins after *Nup50* knock-down. Significant decrease of Nup50 expression (Two-tailed Nested t-test t=8,124, df=14, ****p < 0.0001) in HT22 cell lines. n=3 independent experiments performed at least in duplicate. Data are presented as mean values +/- SEM

Each dot in the scatter plot indicates the mean of an individual experiment.

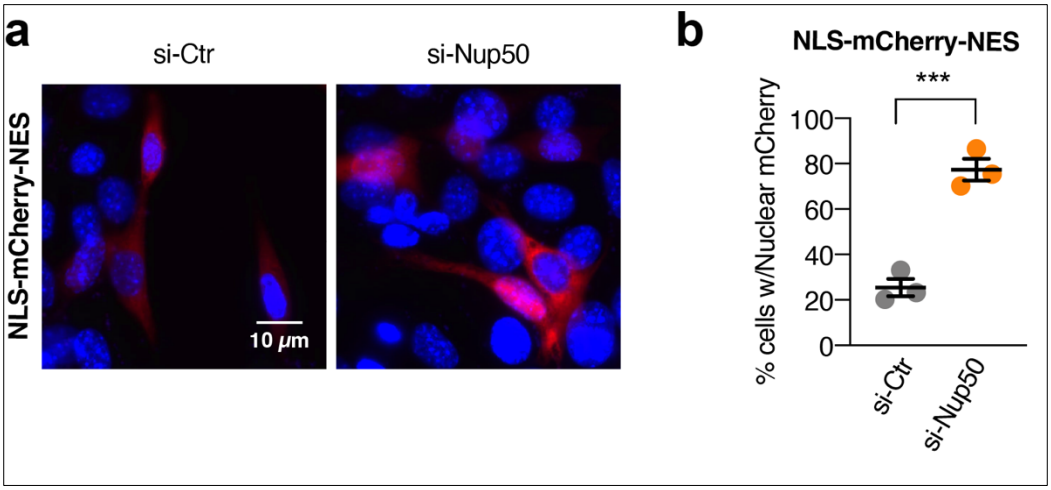

Fig. S5: *Nup50* knockdown consequences on nuclear export activity in HT22 neuronal cells.

a-b: Representative images of NLS-mCherry-NES reporter of nuclear export activity in HT22 cells transfected with si-Ctr or si-Nup50 (a), and (b) quantification (Two-tailed Nested t-test t=12,76, df=38, ****p < 0.0001) in HT22 cell lines. n=3 independent experiments performed at least in duplicate. Data are presented as mean values +/- SEM. Each dot in the scatter plot indicates the mean of an individual experiment.

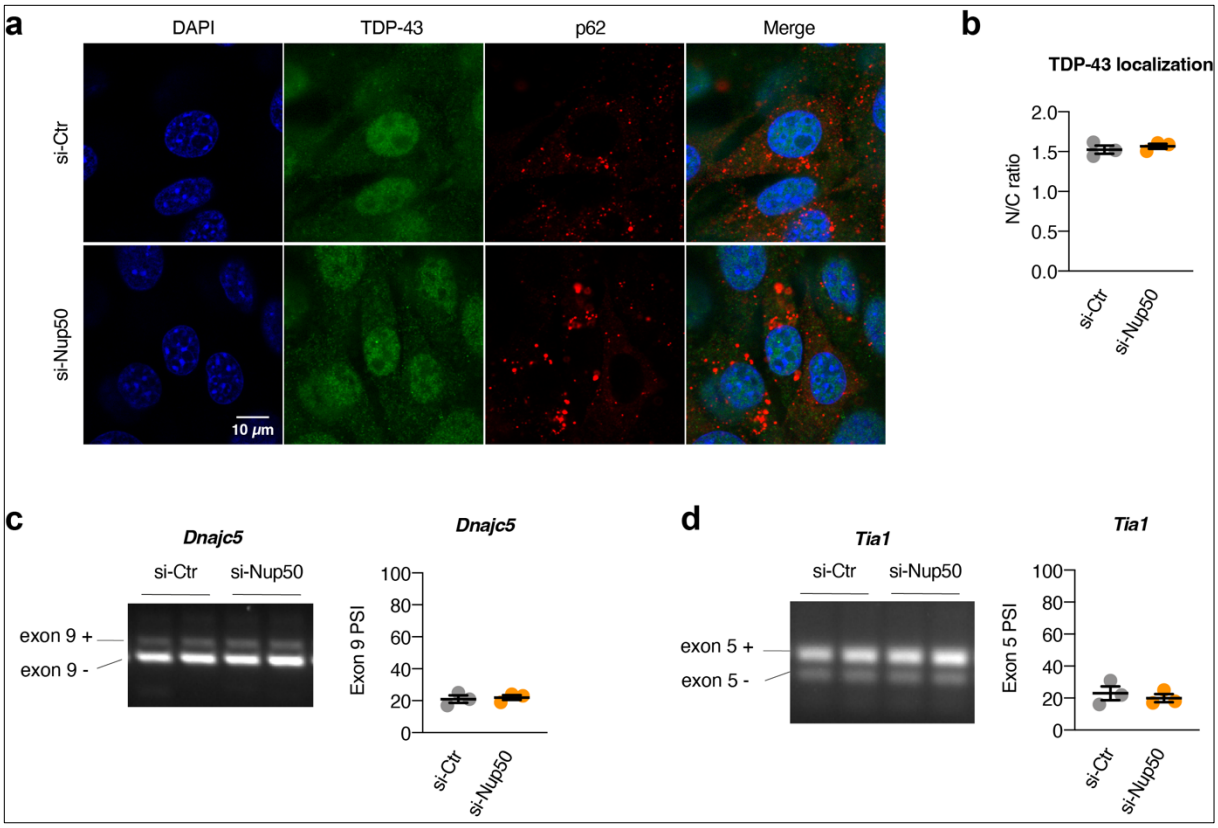

Fig. S6: TDP-43 localization and function upon *Nup50* knock-down in HT22 cells

a- representative immunofluorescence images of HT22 cells transfected either with a control siRNA (si-Ctr) or *Nup50* targeting siRNA (si-Nup50). Cells were stained 24 hours after transfection with DAPI (for nuclear staining, blue), TDP-43 (green) and p62 (red). While knockdown of *Nup50* led to p62 aggregates, we did not observe mislocalization of TDP-43. n=3 independent experiments performed at least in duplicate.
b- quantification of nuclear/cytoplasmic ratio as presented in panel a. There was no difference in TDP-43 nuclear enrichment according to *Nup50* knockdown. Data are presented as mean values +/- SEM. Data are presented as mean values +/- SEM. n=3 independent experiments performed at least in duplicate. Each dot in the scatter plot indicates the mean of an individual experiment.
c-d- splicing assays for *Dnajc5* exon 9 inclusion (C) and *Tia1* exon 5 inclusions (D), two splicing events known to be TDP-43 dependent in mouse neurons. There was no alteration in inclusion of either exon upon *Nup50* knock down. Data are presented as mean values +/- SEM. n=3 independent experiments performed at least in duplicate. Each dot in the scatter plot indicates the mean of an individual experiment.

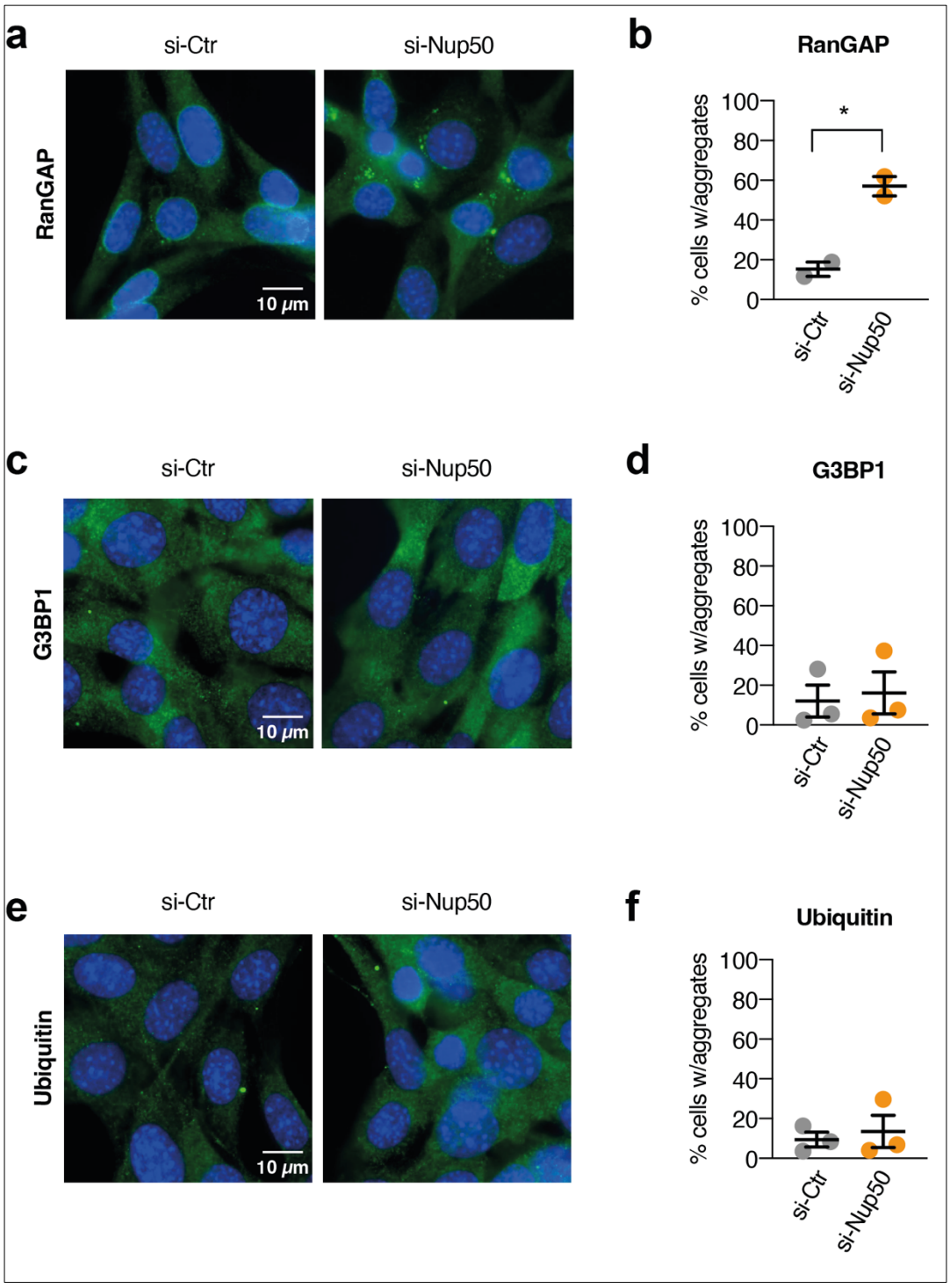

Fig. S7: additional characterization of *Nup50* knockdown in HT22 neuronal cells.
a-f- Representative images of RanGAP (a) G3BP1 (c),and Ubiquitin (e) immunofluorescence in HT22 cells transfected with si-Ctr or si-Nup50, and respective quantifications (b, d, f). Note the lack of increase in G3BP1 (Two tailed Nested t-test, t=0,6486, df=18, p=0.52) or Ubiquitin inclusions (Nested t-test , t=11.095, df=17, p= 0.28). Conversely, Nup50 knock-down increases the number of RanGAP1 inclusion (Two-tailed Nested t-test ,t=10,78, df=10, *p<0.0001). Data are presented as mean values +/- SEM. For panel a-b : n=2 independent experiments performed in triplicate. For panels c-f: n=3 independent experiments performed at least in triplicate. Each dot in the scatter plot indicates the mean of an individual experiment.

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