Supplemental data

Developmentally-regulated RNA-binding protein 1 (Drb1)/RNA-binding motif protein 45 (RBM45), a nuclear-cytoplasmic trafficking protein, forms TAR DNA-binding protein 43 (TDP-43)-mediated cytoplasmic aggregates

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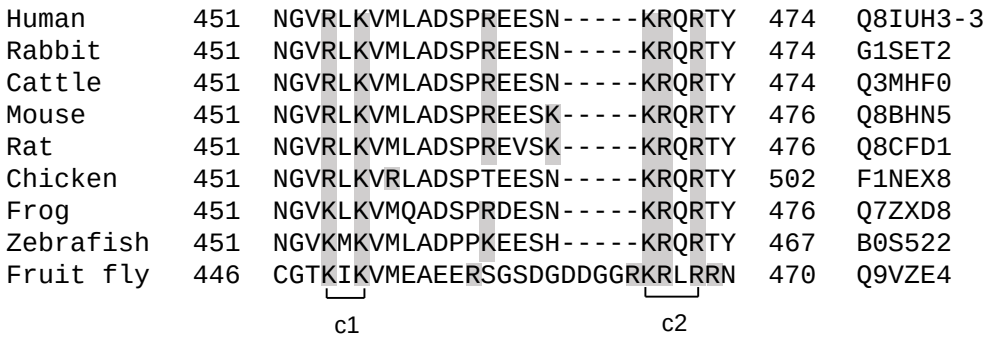
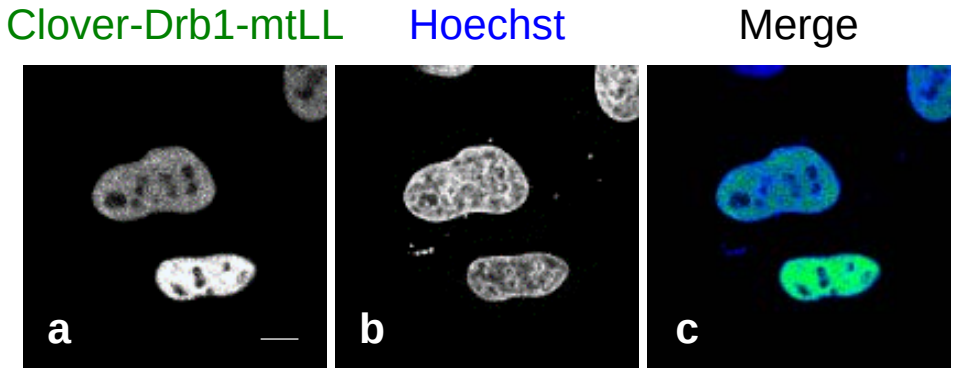
Supplemental Fig. S1



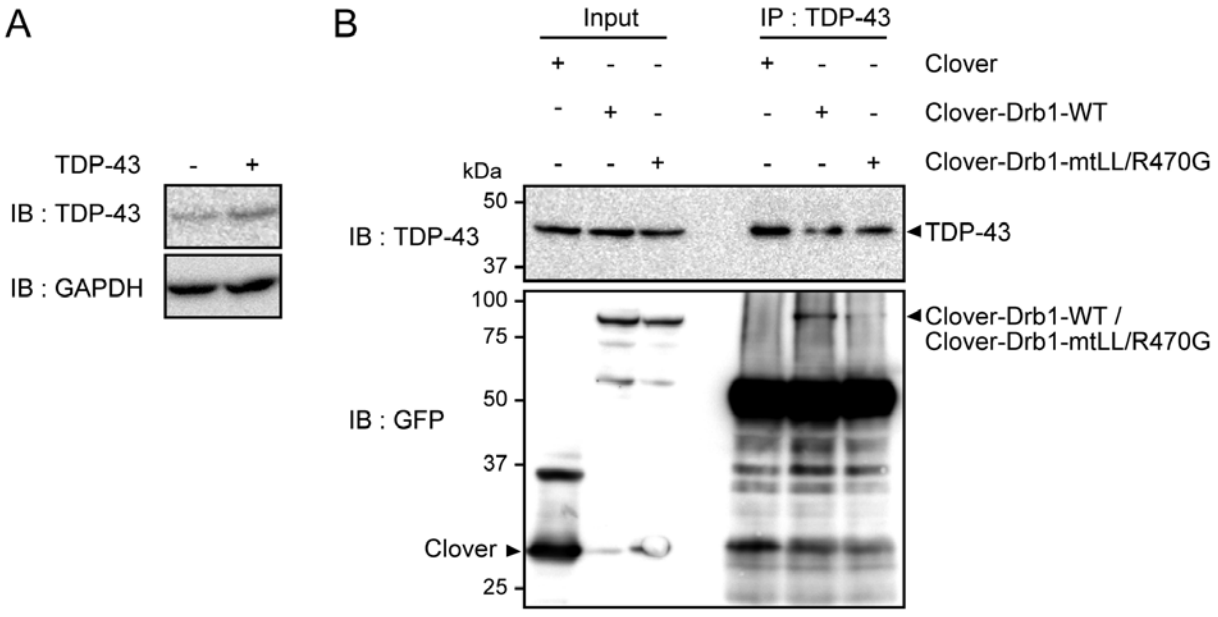
Supplemental Fig. S1. Two clusters of basic amino acids are highly conserved at the carboxyl-terminus of Drb1. Alignment of Drb1 proteins in 9 species. Shadowed letters indicate positively charged amino acids. Each protein sequence was cited from UniProt knowledgebase and each accession number is described on the right. The terms “c1” and “c2” indicate the cluster 1 and cluster 2 of positively charged amino acids of the putative bipartite NLS, respectively.

Supplemental Fig. S2



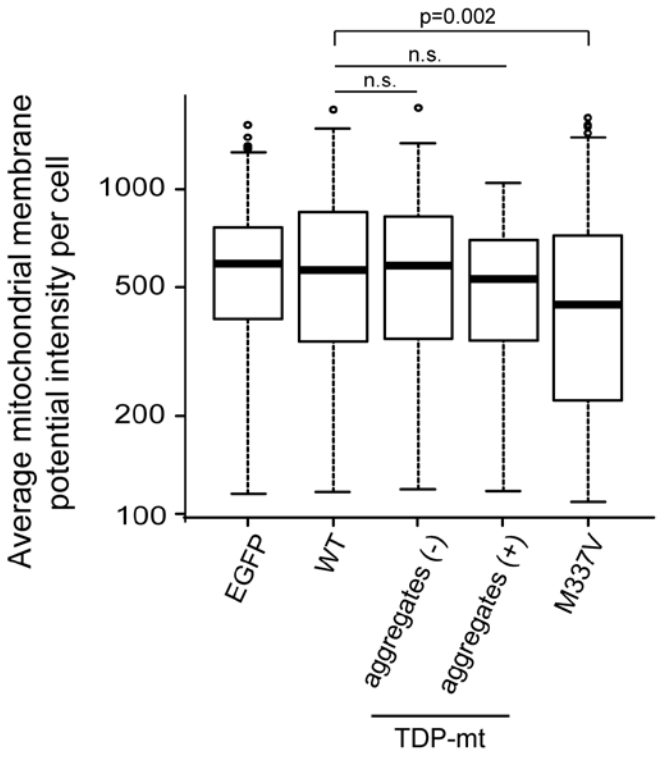
Supplemental Fig. S2. (A) Subcellular localization of mtLL Drb1 fused with Clover-T7 in HeLa cells. At 24 h post-transfection, cells were fixed and permeabilized. The nuclei were stained with Hoechst 33258. Scale bar indicates 10 μm.

Supplemental Fig. S3



Supplemental Fig. S3. Endogenous TDP-43 interacts with wild-type and mutant Drb1 through protein-protein interaction. (A) Cell lysates from control or TDP-43-expressing HeLa cells were treated with Benzonase nuclease and subjected to immunoblot analysis with antibodies against TDP-43 (1:2000, Abnova, upper) or GAPDH (1:3000, MAB374, Millipore, bottom). (B) Co-immunoprecipitation (Co-IP) of wild-type (WT) or mtLL/R470G Drb1 with endogenous TDP-43. The immunoprecipitation was performed as described in the legend of Fig. 3E except to use a rabbit anti-TDP-43 antibody (12892-1-AP, raised for 216–414 amino acids of human TDP-43, Proteintech, Rosemont, IL, USA) for IP. TDP-43 was detected by a mouse anti-TDP-43 antibody (1:2000, Abnova) and Clover-tagged proteins were detected by a rabbit anti-GFP antibody (1:3,000, Torrey Pines Biolabs). Clover-Drb1-WT and -mtLL/R470G, but not Clover alone, were co-immunoprecipitated with endogenous TDP-43 in HeLa cells. Probably due to a difference in subcellular distribution (endogenous TDP-43 in the nucleus in a majority of cells, whereas Clover-Drb1-mtLL/R470G in cytoplasm, see also Fig. 5D), the precipitated amount of Clover-Drb1-mtLL/R470G markedly reduced, compared with Clover-Drb1-WT.

Supplemental Fig. S4



Supplemental Fig. S4. The effect of overexpression of mutant TDP-43 on mitochondrial membrane potential. Box plot of average mitochondrial membrane potential ($\Delta\psi m$) in HeLa cells transiently expressing EGFP, EGFP-TDP-43-WT (WT), EGFP-TDP-43-mt (TDP-mt), or ALS-associated mutant EGFP-TDP-43 M337V (M337V). Mitochondrial membrane potential was evaluated as described in Fig. 6. EGFP-TDP-43-mt-expressing cells were divided into two classes by the presence of cytoplasmic aggregates [aggregates (-) and aggregates (+)]. No significant difference in mitochondrial membrane potential was found in both aggregates (-) and aggregates (+) of EGFP-TDP-43-mt-expressing cells compared with WT expressing cells. EGFP-TDP-43-M337V has a single amino acid change from methionine to valine at TDP-43 codon 337. EGFP-TDP-43-M337V displayed predominantly nucleoplasmic localization and no cytoplasmic aggregates in HeLa cells (data not shown). EGFP-TDP-43-M337V-expressing cells showed a significant reduction of mitochondrial membrane potential, compared with TDP-43-WT. Expression construct of pEGFP-TDP-43-M337V was generated from pEGFP-TDP-43-WT by the overlap extension PCR method using the following two sets of primers, forward, 5´-GCAAGCTTTCTGAATATATTCGGGTAACCG-3´ and reverse, 5´-CTGGCTAACATGCCCACCATACCCCAACTGCTCTG-3´, and forward, 5´-GAGCAGTTGGGGTATGGTGGGCATGTTAGCCAGCC-3´ and reverse, 5´-AGGGATCCTACATTCCCCAGCCAGAAGAC-3´.