



Vitamin B12 Reduces TDP-43 Toxicity by Alleviating Oxidative Stress and Mitochondrial Dysfunction

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Supplementary Methods

Supplemental Table S1. Primers used in qPCR experiments.

Gene	Accession number	Direction	Primer sequence	Species
ATF4	NM_001675	Forward	5'-GAGGTGGCCAAGCACTTCAA	<i>H. sapiens</i>
		Reverse	5'-GCCCCGCTTAGCCTTGTC	
CHOP	NM_001675	Forward	5'-GGGAGCTGGAAGCCTGGTAT	
		Reverse	5'-CCCCATTTTCATCTGAAGACA	
GADD34	U83981	Forward	5'-CCCAGAAACCCCTACTCATGATC	
		Reverse	5'-TCGGAGAAGCGCACCTTT	
ND1	LC178901	Forward	5'-CCCTTCGCTGACGCCATA	
		Reverse	5'-TGGTAGATGTGGCGGGTTTT	
ND2	AF014899	Forward	5'-GCAAGCAACCGCATCCATA	
		Reverse	5'-CATGTCCGGAGAGTATATTGTTGA	
ND3	JN034128	Forward	5'-CCTACCATGAGCCCTACAAACAAC	
		Reverse	5'-GGTGATGATTAATAAGAGGGATGACA	
ND4	JN034131	Forward	5'-AGCTCCATCTGCCTACGACAA	
		Reverse	5'-TATGTGGCTGATTGAAGAGTATGCA	
ND5	DQ926980	Forward	5'-CACAGCAGCCATTCAAGCAA	
		Reverse	5'-AGGCGAGGATGAAACCGATA	
POLG	NM_002693	Forward	5'-AAGCAGGGCAAACACAAGGT	
		Reverse	5'-GGCTTTCCTCTGGGACTTCTG	
cGAS	KC294566	Forward	5'-CGGGCGGTTTTGGAGAA	
		Reverse	5'-GCCGCCGTGGAGATATCA	
STING	NM_198282	Forward	5'-CTGGCCCCAGCTGAGATCT	
		Reverse	5'-CATGGGCCACGTTGAAATTC	
TBK1	XM_005268809	Forward	5'-TGGAAGGCTGGCACAACAT	
		Reverse	5'-GAGGTTCCCGGCTTACTACAAA	
IRF3	NM_001571	Forward	5'-TAAGCCAGACCTGCCAACCT	
		Reverse	5'-CTGCTAAACGCAACCCTTCTTT	
ATF5	NM_012068	Forward	5'-GGACCGCAAGCAAAAGAAGA	
		Reverse	5'-CGCTGGCGGTACCTCAGA	
LonP1	XM_011528441	Forward	5'-CCAACGTCACGGACACCAT	
		Reverse	5'-CGACACGTTGATCATCTCCATAC	
HSP60	AF143723	Forward	5'-GGCGGCCATCGGAGTT	
		Reverse	5'-ATCCTTATAGACGGCCTCACATG	
HSPA9	NM_004134	Forward	5'-TTCTTGTGGGTGGCATGACTAG	
		Reverse	5'-TGCCAAAAAGATCCTGTACAGTCT	
18S rRNA	M10098	Forward	5'-CGGCGACGACCCATTC	
		Reverse	5'-CACGGCGACTACCATCGAA	
GAPDH	NM_002046	Forward	5'-GTCAGTGGTGGACCTGACCT	
		Reverse	5'-TGCTGTAGCCAAATTCGT TG	

Supplementary Figure

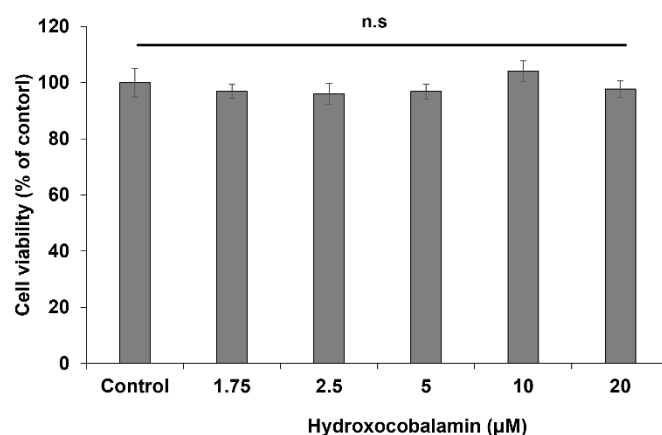
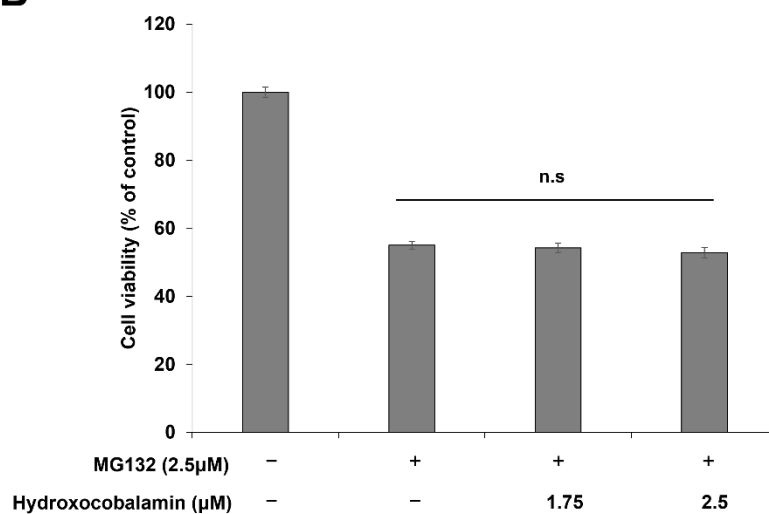
A**B**

Figure S1. MG132-induced toxicity was not affected by hydroxycobalamin. (A) SH-SY5Y cells were treated with various concentrations of hydroxycobalamin for 24 h, cell viability was determined by CCK-8 assay, and the percentage of cell viability was expressed as the mean \pm SD of three independent experiments (n.s., not significant, one-way ANOVA using Tukey's multiple comparison test). (B) A CCK-8 assay was performed to evaluate the effect of Hb on MG132 (2.5 μM)-induced toxicity in SH-SY5Y cells. Hb had no effect on MG132-induced toxicity. Data are presented as the mean \pm SD of three independent experiments. The significance was determined by one-way ANOVA with Tukey's multiple comparison test (n.s., not significant).

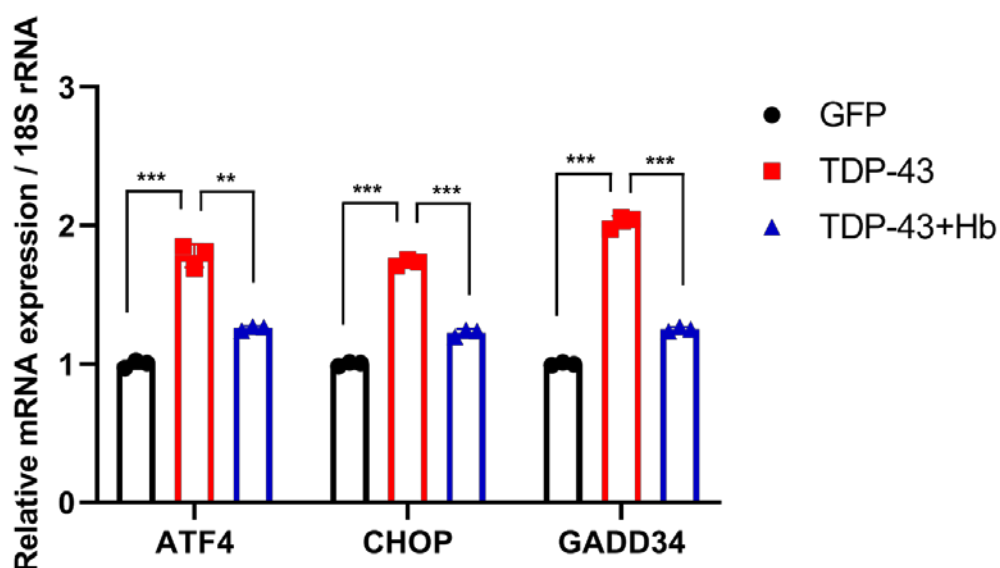


Figure S2. Hydroxocobalamin mitigates the TDP-43-induced unfolded protein response in neuronal cells. Cells were infected with the GFP- or GFP-tagged human WT TDP-43 containing lentivirus and grown for 2 days. The day before harvest, the cells were treated with Hb (10 μ M) for 24 h. The transcription levels of ER stress-related genes, including *ATF4*, *CHOP*, and *GADD34*, were detected using real-time PCR. 18S rRNA was used as an internal control. Error bars represent the mean values \pm SD of three independent experiments. The significance of differences was determined using one-way ANOVA with Tukey's multiple comparison test (** $p < 0.01$ and *** $p < 0.001$).