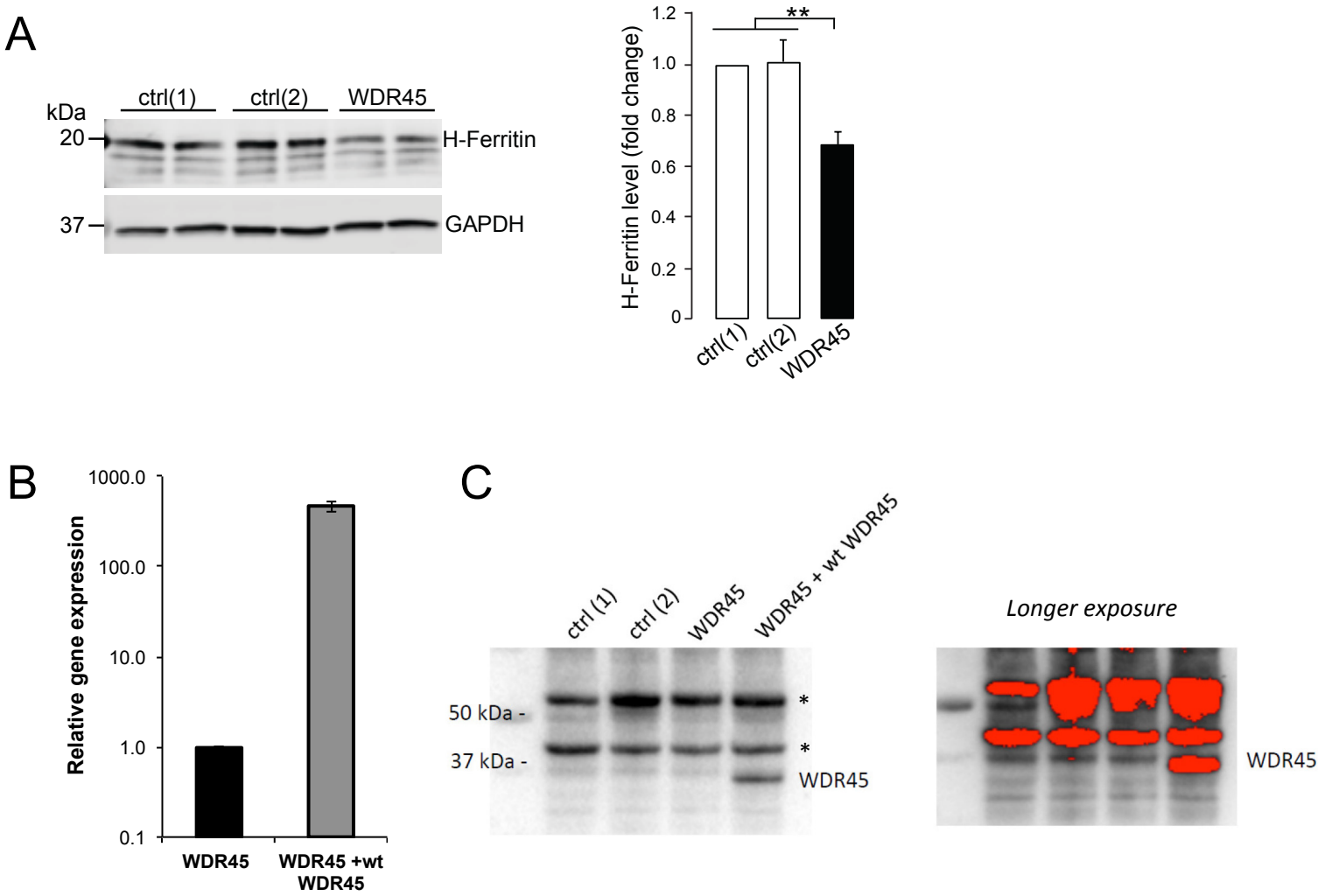
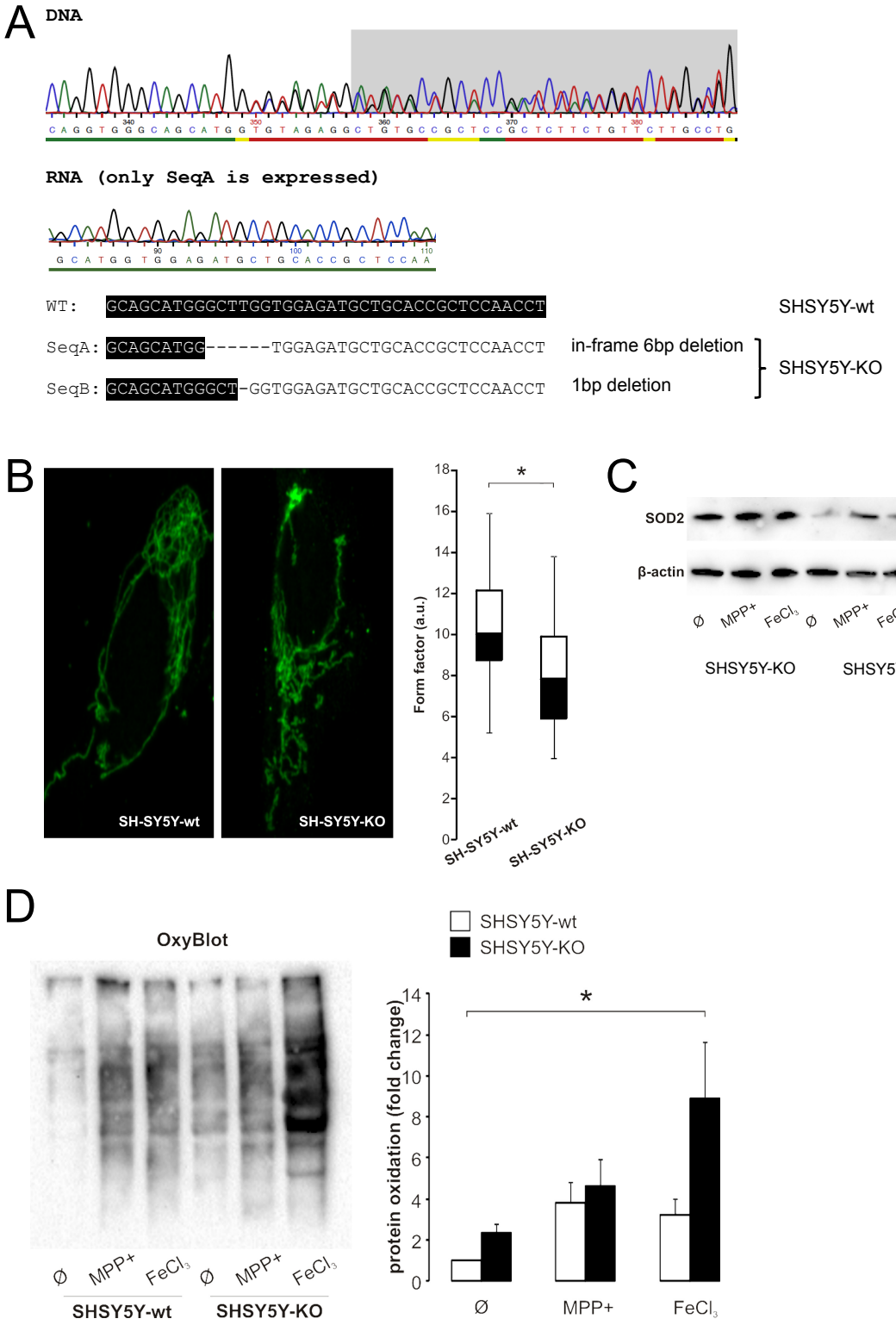
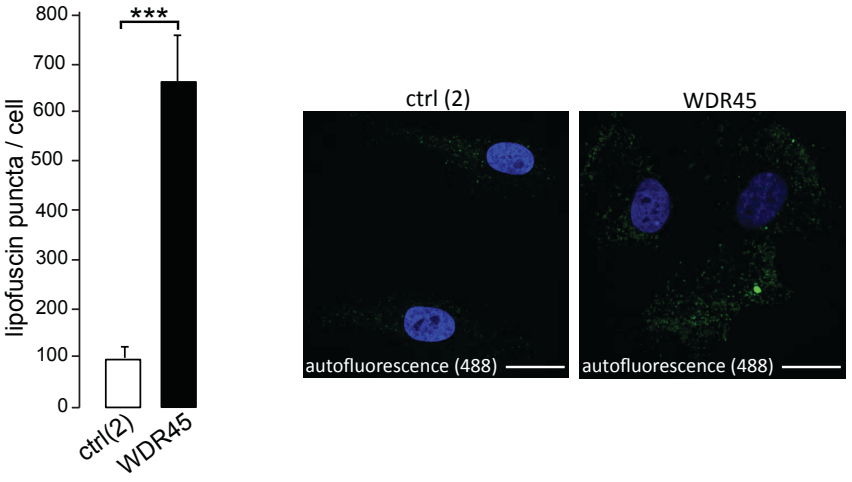


Supplementary Figure 1. Reduced levels of H-Ferritin in *WDR45* mutant cells and overexpression of wildtype WDR45. **(A)** Western blot analysis shows H-Ferritin protein levels in the *WDR45* mutant and control fibroblasts with GAPDH as loading control. **(B)** Relative gene expression levels of *WDR45* were assessed by RT-PCR. Patient fibroblast cells were infected with lentivirus to overexpress wildtype (wt) WDR45. RNA was extracted from patient fibroblast cells infected and not-infected with the lentivirus. Samples from not-infected cells were set to 1. **(C)** Western blot analysis was performed with two control (ctrl) fibroblast lines, the patient *WDR45* mutant line, and the patient line overexpressing wt WDR45. The blot was probed with anti-WDR45 antibody, which only detected overexpressed protein. Asterisk indicates unspecific band.



Supplementary Figure 2. SH-SY5Y cells with a heterozygous knockout in *WDR45*. **(A)** A double-strand DNA break was induced by CRISPR/Cas9 into exon 4 of *WDR45* in SH-SY5Y cells. Non-homologous end-joining repair mechanism caused a 6bp in-frame deletion in the first allele and a 1bp deletion in the second allele of one cell leading to a heterozygous knockout (KO) of *WDR45*. **(B)** Mitochondrial morphology was investigated under basal conditions by fluorescence microscopy in fixed cells immunostained with anti-GRP75 (green). The form factor as a measure for mitochondrial interconnectivity was calculated for wildtype (wt) and heterozygous KO cells. **(C)** Western blot analysis performed on samples under basal conditions and upon treatment with 1-methyl-4-phenylpyridinium (MPP+) or ferric chloride ($FeCl_3$). The western blot was probed with antibodies against superoxide dismutase 2 (SOD2) and β-actin (loading control). **(D)** Oxidation of proteins in wt and KO cells was determined by OxyBlot using an antibody against DNP.

A



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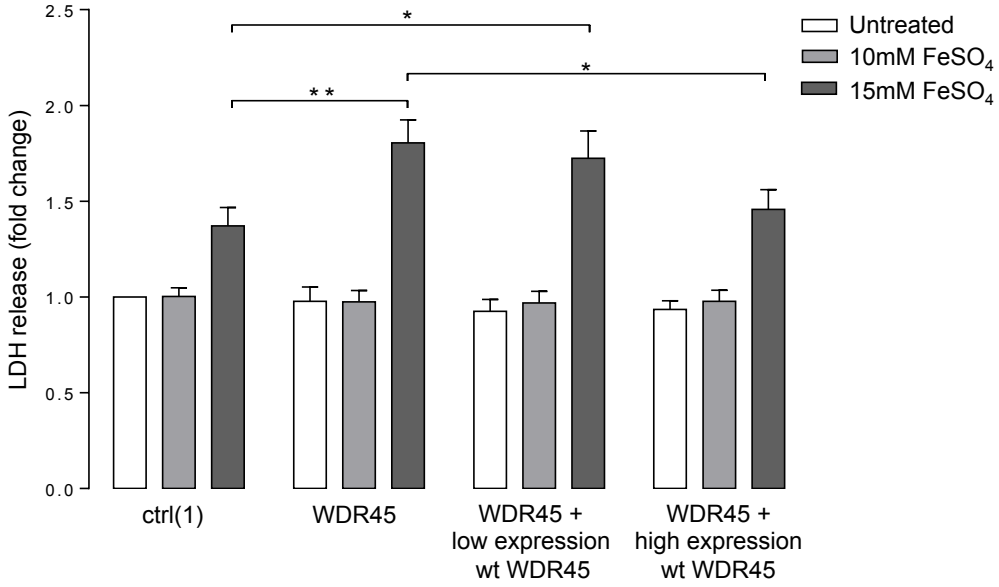
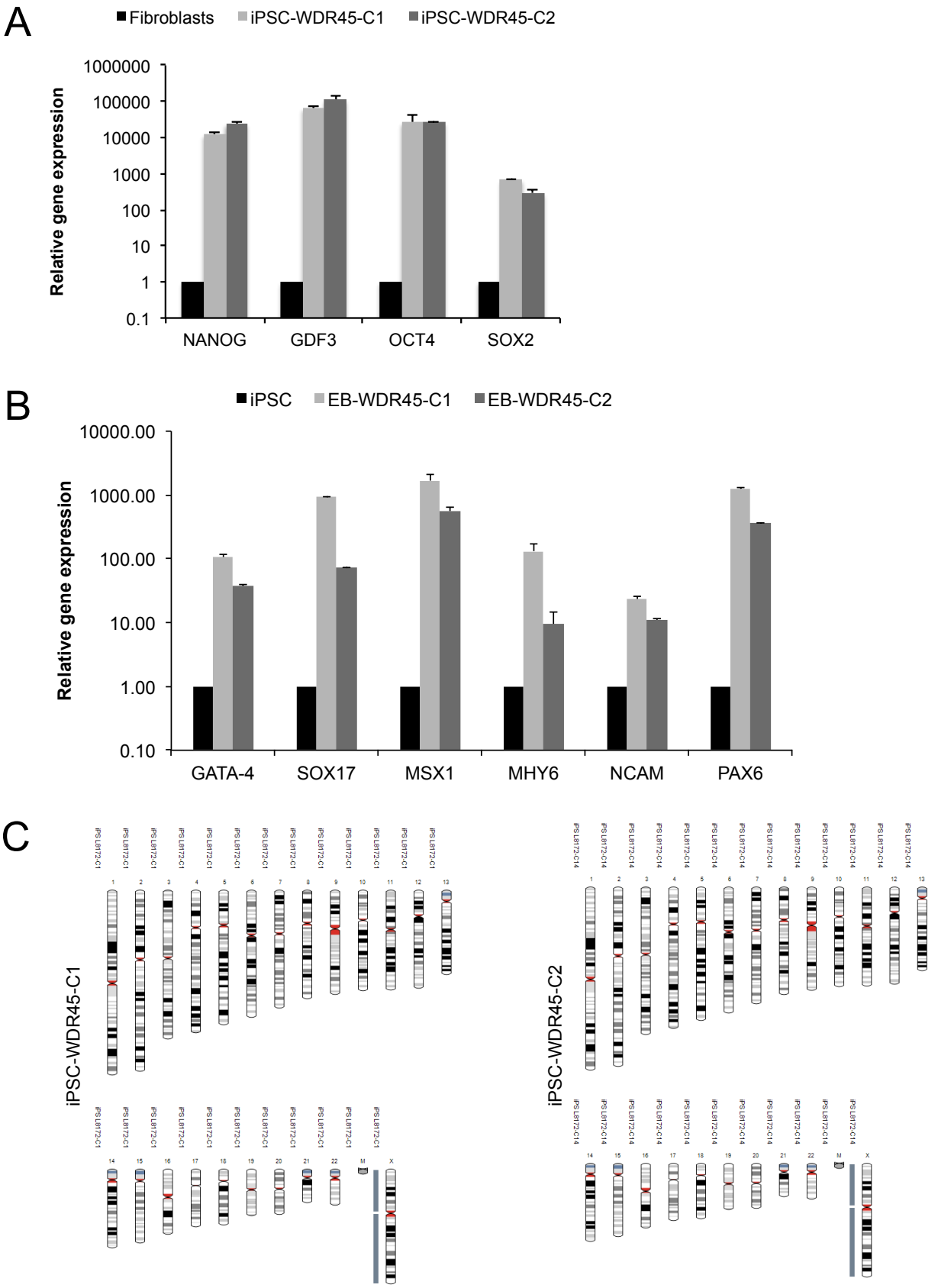
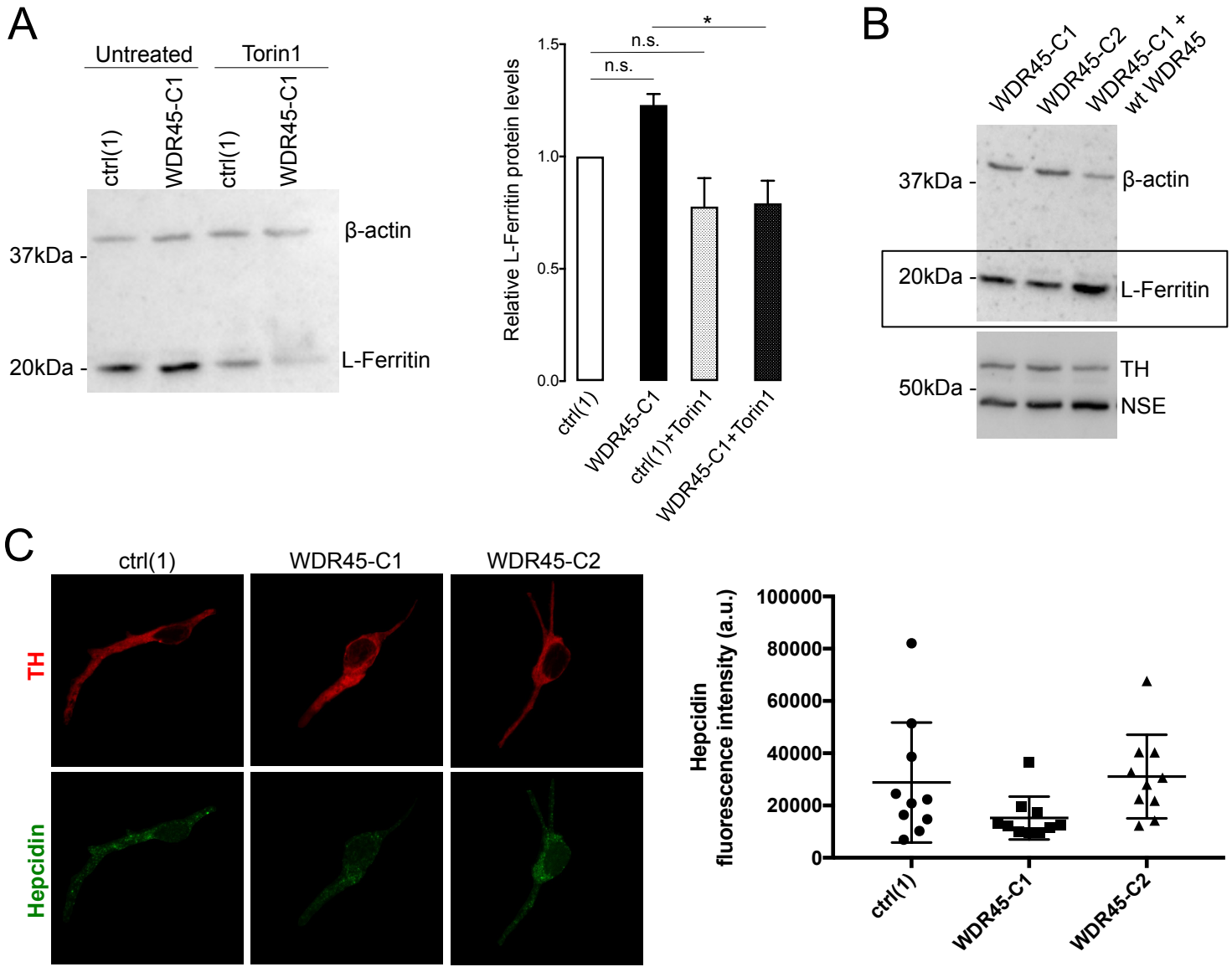


Supplementary Figure 3. Lipofuscin accumulation and increased susceptibility to iron stress in *WDR45* mutant fibroblasts. **(A)** Accumulation of lipofuscin, an autofluorescent electron-dense aggregate of undigested lysosomal material, was examined by taking images of autofluorescence signal using the 488 channel (n=37 cells; scale bar 25µm). **(B)** Viability of cells was analyzed by measuring the release of lactate dehydrogenase (LDH) in the supernatant of untreated cells and upon treatment with $FeSO_4$. Values were normalized to the untreated control ctrl(1). Lentiviral overexpression of wt WDR45 was performed at a multiplicity of infection of 0.75 virus particles per cell ("low expression") and 15 virus particles per cell ("high expression"), respectively.



Supplementary Figure 4. Characterization of iPSC lines derived from *WDR45* mutant fibroblasts. **(A)** Expression levels of pluripotency markers NANOG, GDF3, OCT4, and SOX2 in fibroblasts and iPSC lines relative to β-actin (a loading control) as assessed by quantitative RT-PCR. The values from parental fibroblasts were set to 1. **(B)** RT-PCR analyses of various differentiation markers for the three germ layers (endoderm: GATA-4, SOX17; mesoderm: MSX1, MHY6; ectoderm: NCAM, PAX6) in iPSCs that were undifferentiated (iPSC) and after 4d in suspension culture followed by 7d in adherent culture to form embryoid bodies (EB). **(C)** Karyograms produced from SNP array show no gross abnormalities in the previously unpublished iPSC lines used in this study. Red indicates loss or single copy, green indicates gain of copy, grey indicates loss of heterozygosity on autosomes, or two copies of X chromosome (i.e. female lines). The clones iPSC-WDR45-C1 and iPSC-WDR45-C2 were named internally iPSL8172-C1 and iPSL8172-C14, respectively.



Supplementary Figure 5. Cellular dysfunction in iPSC-derived dopaminergic neurons harboring mutant *WDR45*. **(A-B)** Western blot analysis from control (ctrl) and *WDR45* mutant neurons. Samples were probed against L-Ferritin, tyrosine hydroxylase (TH), neuron-specific enolase (NSE), and β-actin (loading control). Upon treatment with Torin1, L-Ferritin levels decreased significantly in mutant neurons (A). Overexpression of WDR45 led to an increase in L-Ferritin protein levels in *WDR45* mutant neurons (B). **(C)** Immunofluorescence staining shows expression of TH and hepcidin in control and mutant neurons. Fluorescence intensity was quantified from z-stack images (n=10).