Supplementary Figure Legends

Supplementary Fig. 1. Inclusion formation by YFP-tagged mutant SOD1 in HeLa cells under proteasome-inhibiting conditions. (A) Sedimentation analysis of SOD1-YFPs. Cell lysates were fractionated by centrifugation and analyzed by western blotting using antibodies against the indicated proteins. (B and C) Filter-trap assay of SOD1-YFPs (WT or G85R) during formation (B) or disappearance (C) of inclusions. (D) Immunofluorescence staining of cells expressing SOD1-G85R-YFP in the presence of MG-132 for 16 hours. White arrows indicate inclusions. Bar=20 μm. (E) Number of cells containing SOD1-YFP inclusions during recovery following 17 hours treatment with 50 nM epoxomicin (mean ± S.D., n>600). (F) Disappearance of cells containing SOD1-YFP inclusions under inhibition of autophagy and lysosomal degradation (mean ± S.D., n>600). Cells were treated with MG-132 for 16 hours, and recovered in the absence of MG-132 and in the presence of 0.1 μM bafilomycin A1. Inset images show LC3-accumulated autophagosome formation upon addition of bafilomycin A1 or DMSO as a negative control.

Supplementary Fig. 2. Recovery of proteasome activity. After being treated with MG-132 for 16 hours, cells were transferred to recovery cultures lacking MG-132. Fluorescence intensities were determined for GFPu, a GFP variant rapidly degraded by proteasomes, and mGFP (non-degraded control) over a 10 hour period. Decreasing GFPu fluorescence intensity represents increasing proteasome activity.

Supplementary Fig. 3. Expression and solubility of SOD1 tagged with mPAGFP (A) or mGFP (B). SOD1-mPAGFP or SOD1-mGFP were expressed in HeLa cells. Cell lysates containing equal amounts of protein were fractionated by centrifugation, and then the supernatants and pellets were subjected to SDS-PAGE. Separated proteins were blotted onto PVDF membranes, and SOD1-mPAGFP or SOD1-mGFP proteins were detected using anti-GFP antibody. GAPDH was used as a loading control and detected using anti-GAPDH antibody.

Supplementary Fig. 4. Fluorescence correlation spectroscopy (FCS) analysis of soluble mutant SOD1 oligomers during treatment with MG-132 for the indicated periods (A), followed by transfer to recovery media for the indicated periods (B). Lysates were prepared from cells expressing YFP, SOD1-WT-YFP, or SOD1-G85R-YFP, and then treated with 100 mM DTT. Vales are means ± S.D. (n = 3)

Supplementary Fig. 5. Sucrose density-gradient fractionation of cell lysates containing SOD1-G85R-YFP in the presence or absence of 2 μM MG-132.

Supplementary Fig. 6. Poly-ubiquitination analysis of SOD1-WT-YFP and SOD1-G85R-YFP in the presence or absence of 2 μM MG-132.

Supplementary Fig. 7. Fluorescence lifetime of FRET donors in FRET-FLIM analysis of controls. (A) Fluorescence lifetime of mTFP1 in HeLa cells expressing SOD1-G85R-mTFP1 as the FRET donor and SOD1-G85R-mVenus as the acceptor. Experiments were performed in the presence of MG-132 or DMSO (as a negative control). (B) Fluorescence lifetime of mTFP1 in cells expressing SOD1-wt-mTFP1 and SOD1-wt-cp173mVenus. (C) Fluorescence lifetime of mTFP1 in cells expressing the mTFP1 monomer, with or without the cp173mVenus monomer. (D) Fluorescence lifetime of mTFP1 in cells expressing SOD1-wt-mTFP1 without other fluorophores as acceptors.

Fig. S1. Kitamura *et al.*

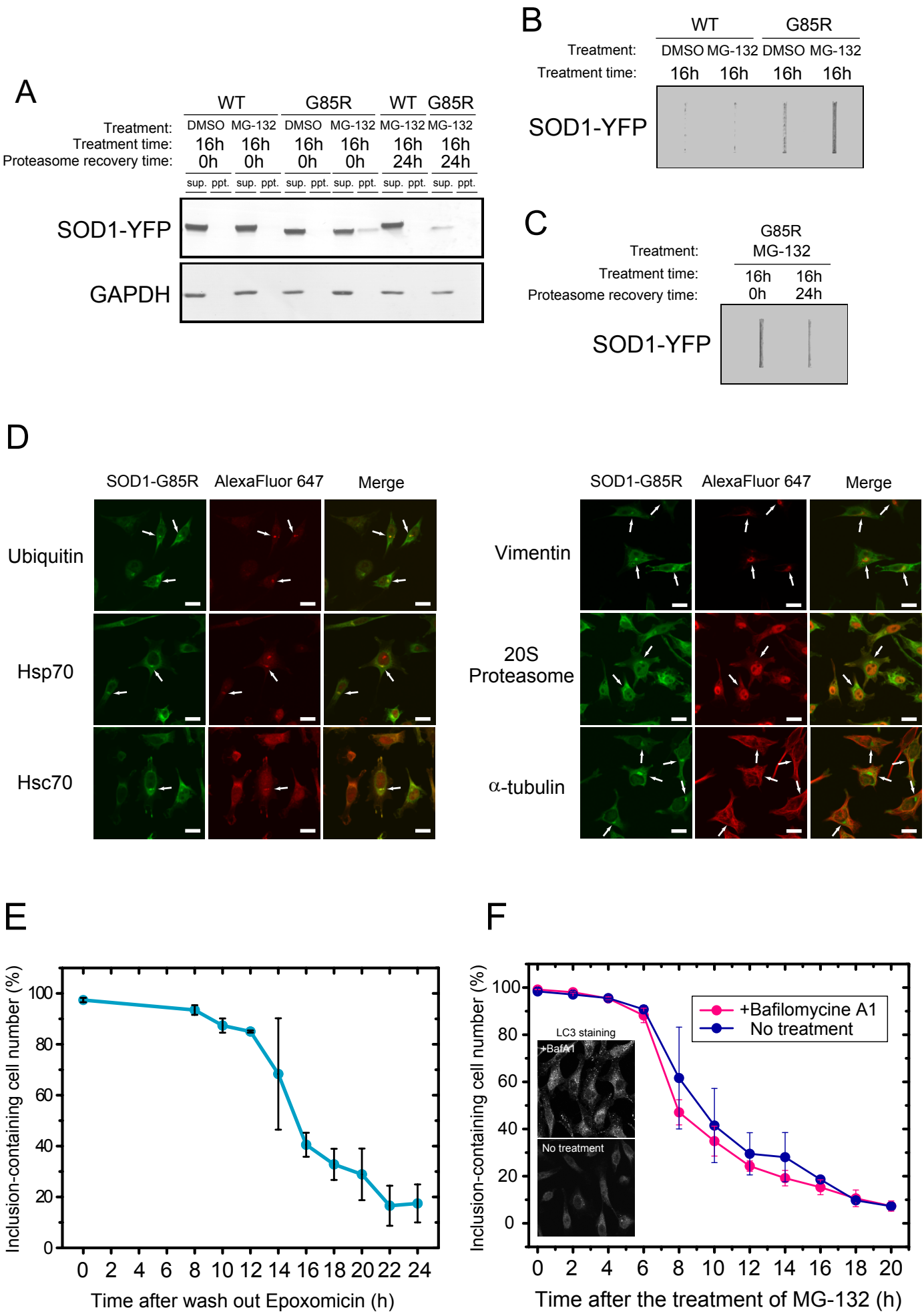


Fig. S2. Kitamura *et al.*

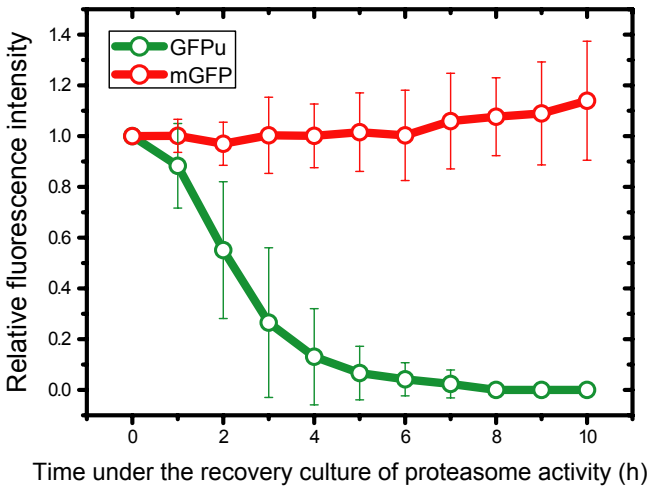
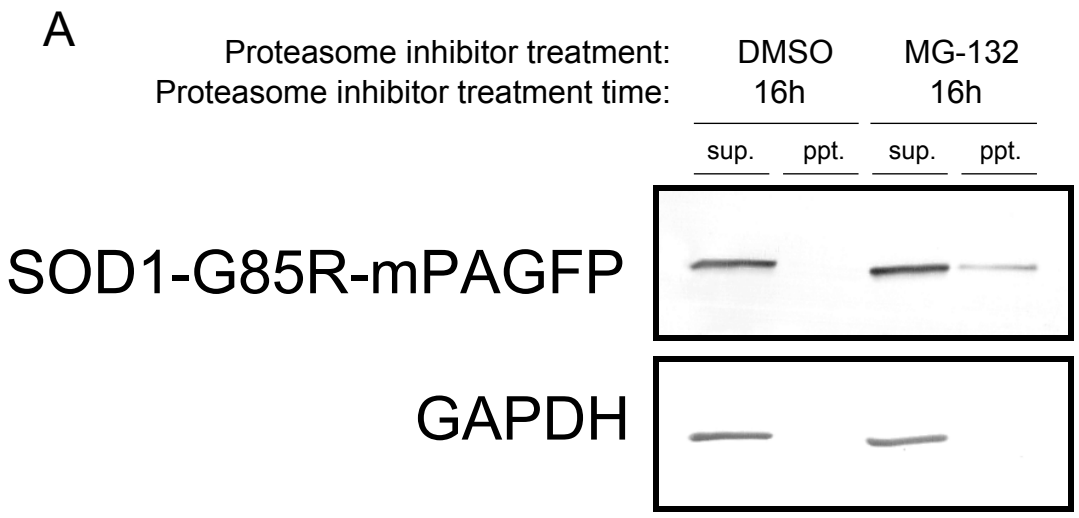


Fig. S3. Kitamura *et al.*



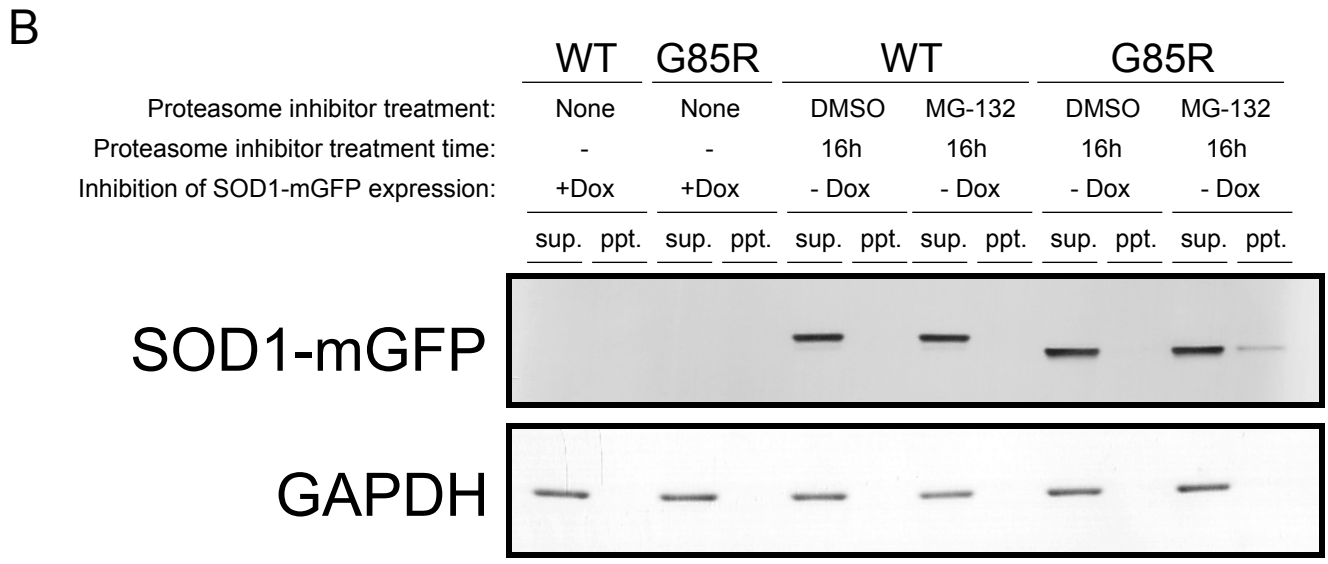


Figure S4. Kitamura *et al.*

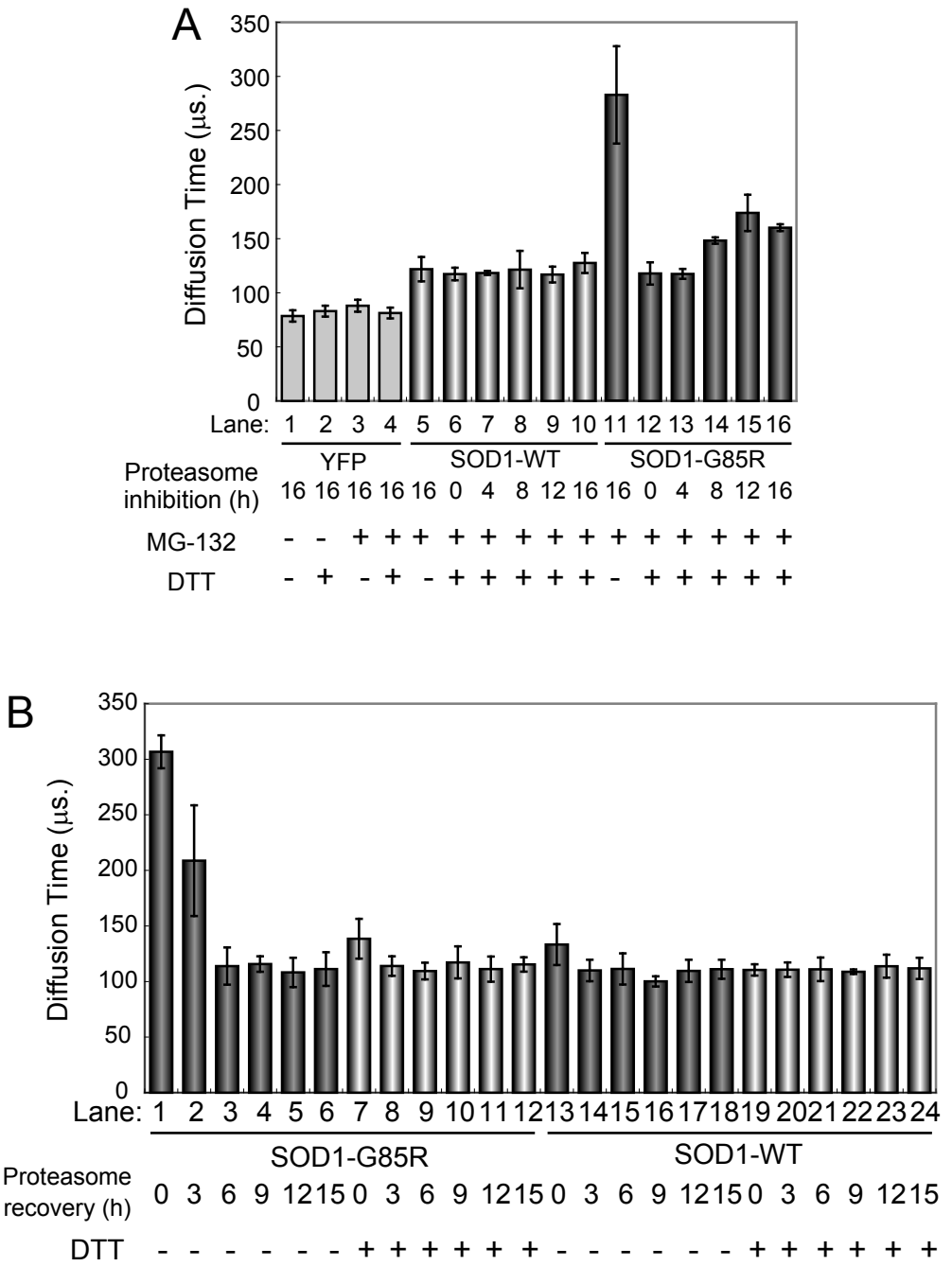


Fig. S5 Kitamura *et al.*

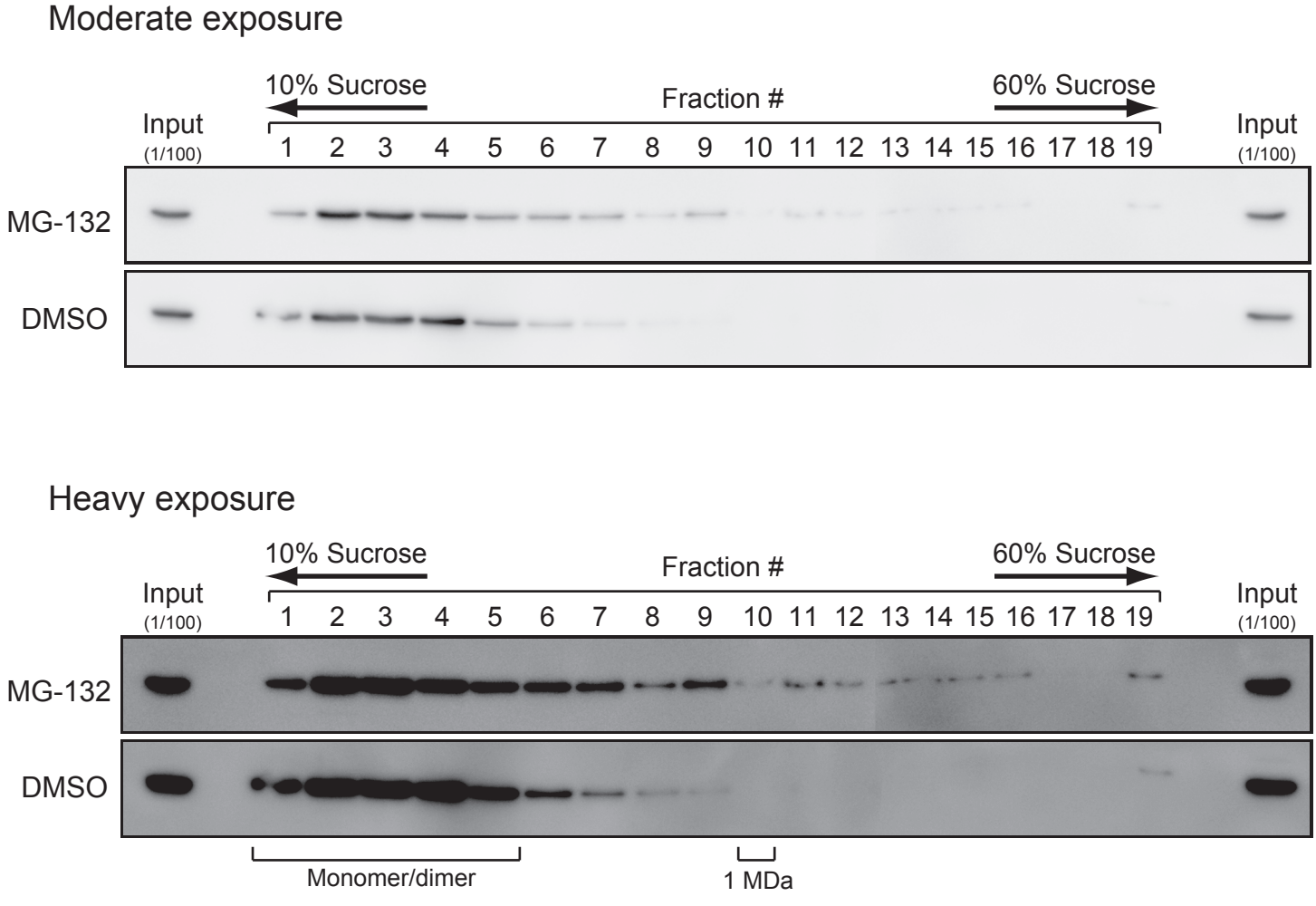


Fig. S6 Kitamura *et al.*

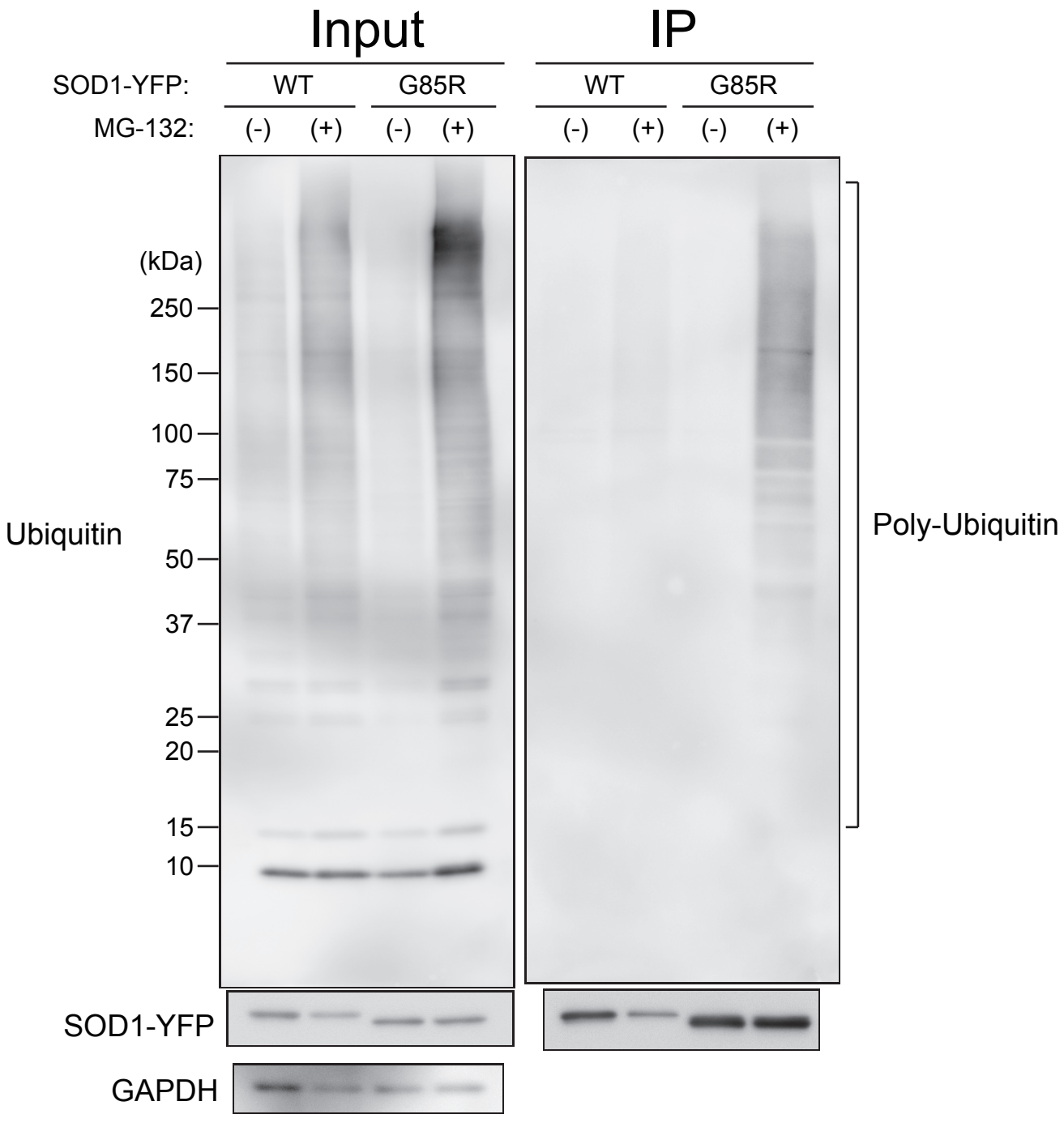


Fig. S7 Kitamura *et al.*