Supplemental Materials

Molecular Biology of the Cell

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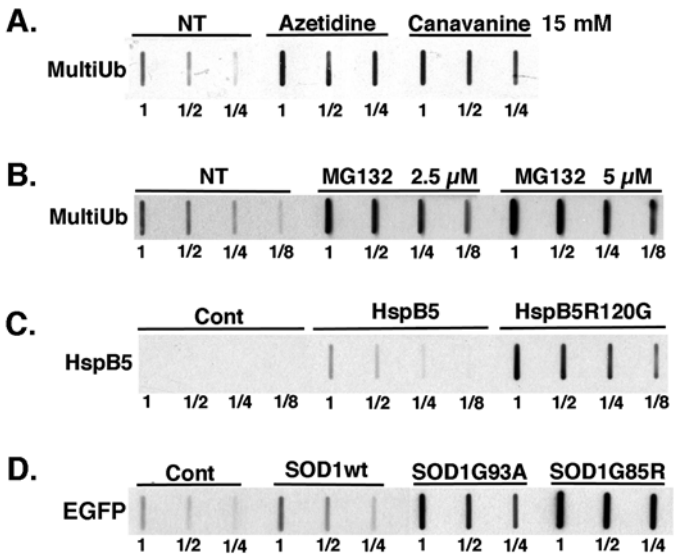

Figure S1: Amino acid analog, MG132 treatments and overexpression of mutated forms of HspB5 and SOD1 induce the formation of protein aggregates.

HeLa cells were either untreated (NT) or submitted to 15 mM azetidine or canavanine (A) treatments or to 2.5 and 5 μM MG132 treatments (B) during 6 hours and allowed to recover in fresh culture medium for 16h. HeLa cells were transiently transfected with control (Cont) plasmids (see materials and methods) or plasmids expressing wild type HspB5 or its mutated form HspB5R120G (C) or wild type SOD1-EGFP or its mutated forms SOD1G93A-EGFP and SOD1G85R-EGFP (D) and were analyzed 48h after transfection. Total protein extracts were prepared for filter-trap assays. Samples were slot-blotted at different dilutions (1 to 1/8) onto a nitrocellulose membrane and aggregates were probed with antibodies against Multi-Ubiquitin for azetidine, canavanine (A) or MG132 (B) treatments or with HspB5 (C) or EGFP (D) antibodies.

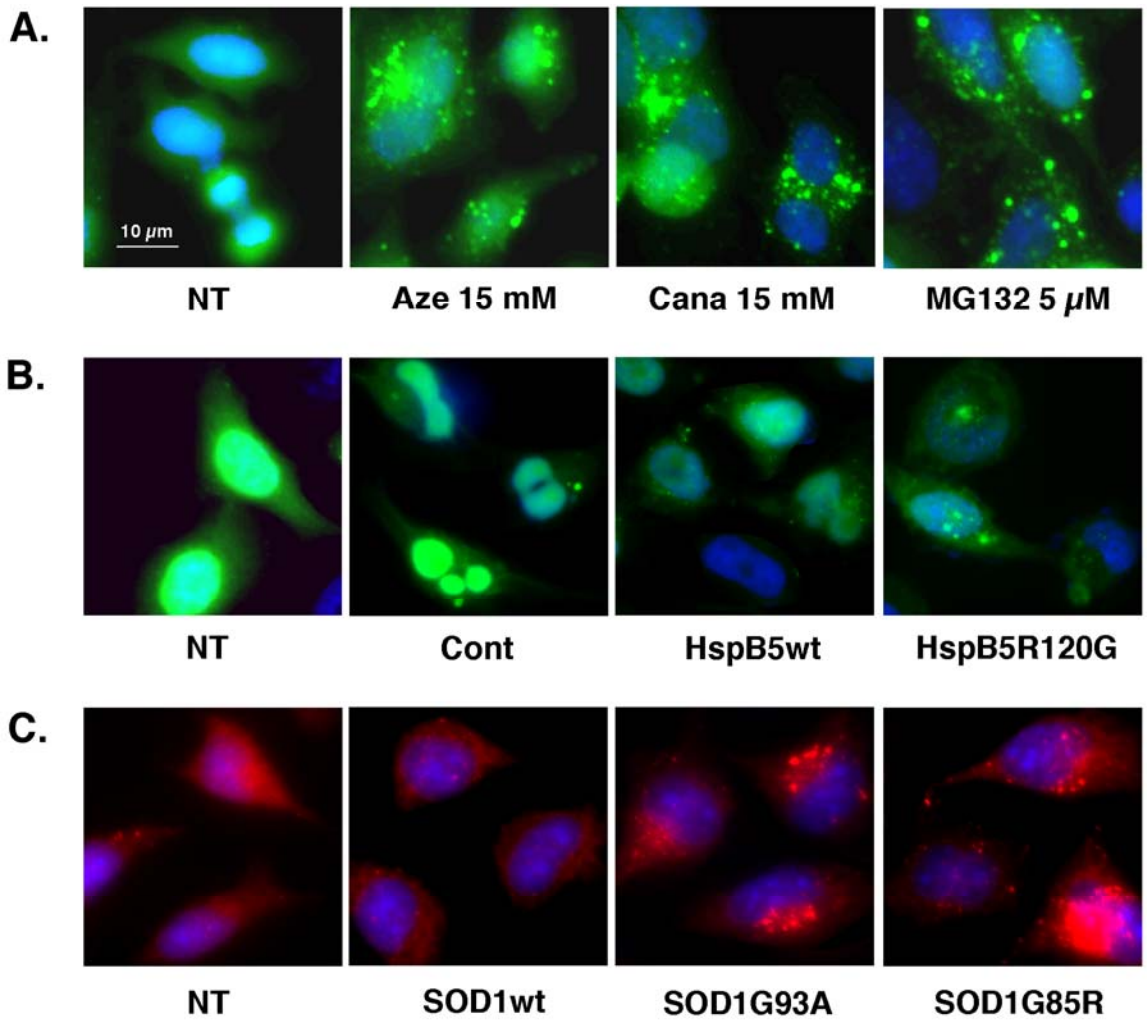

Figure S2: Amino acid analogs MG132 treatments and overexpression of mutated forms of HspB5 and SOD1 activate autophagy

(A) Stable HeLa transfectants expressing GFP-LC3 were either non treated (NT) or treated by azetidine 15 mM (Aze), canavanine 15 mM (Cana) or MG132 5 μM during 6 hours. (B) Stable HeLa transfectants expressing GFP-LC3 were transiently transfected or not (NT) with control plasmid (Cont) or plasmids expressing wilt type HspB5 or its mutated form HspB5R120G. (C) HeLa cells were non transfected (NT) or transfected with wilt type SOD1-EGFP or its mutated forms SOD1G93A-EGFP and SOD1G85R-EGFP. 16 hours after treatments or 24 hours after transfection, cells were fixed, permeabilized and stained with Hoechst (A, B and C) and with anti-LC3 antibody (C). Cells were analyzed with a fluorescence microscope: nuclei, blue staining; GFP-LC3, green staining; LC3, red staining. n=3.