Ubiquitin-proteasome-rich cytoplasmic structures in neutrophils of patients with Shwachman-Diamond syndrome

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SUPPLEMENTARY APPENDIX

Supplementary Figure S1. Representative western blot of lysed blood granulocytes from a healthy control subject and an SDS patient with SBDS gene mutation. Lysates were resolved by SDS-PAGE (4-20%) and immunoblotted for FK1 (polyubiquitinated proteins), 20S proteasome (Prt) and p62 protein. β-Actin was used as a loading control. The graph on the right, obtained by densitometry and normalized to β-actin, shows the increment, expressed as a percentage, of FK1, Prt and p62 in SDS patients compared to that in control granulocytes.
Online Supplementary Figure S2. Aldehyde-osmium-fixed blood neutrophils from a healthy control, either untreated (A1, 1,000x) or treated with the xanthine/xanthine oxidase system (A2, 1,000x; B, 4,000x; b1, 20,000x). In toluidine-blue-stained semithin resin sections a single cell (black arrow) in A1, and several cells in A2, show round, pyknotic nuclei. The apoptotic nature of these cells was confirmed by electron microscopy of an adjacent thin section (B, enlarged in b1) showing a round dense, chromatin aggregate, leaving a thin crescentic remnant of karyoplasm in direct contact with a mostly homogeneous cytoplasm, at a thin borderline (white arrowheads) devoid of a nuclear membrane envelope. A few secretory granules, dense bodies and several membrane-delimited vesicles, but no PaCS, can still be recognized in the cytoplasm.