A Dual-Acting Nitric Oxide Donor and Phosphodiesterase 5 Inhibitor Activates Autophagy in Primary Skin Fibroblasts

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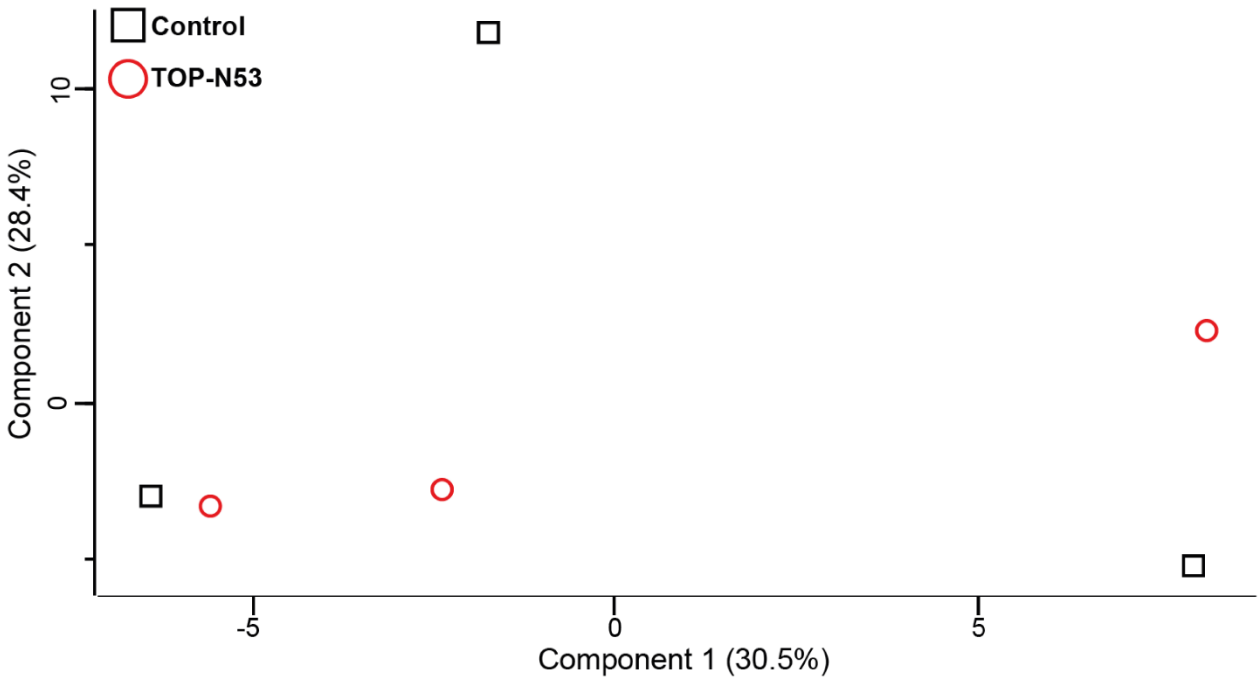
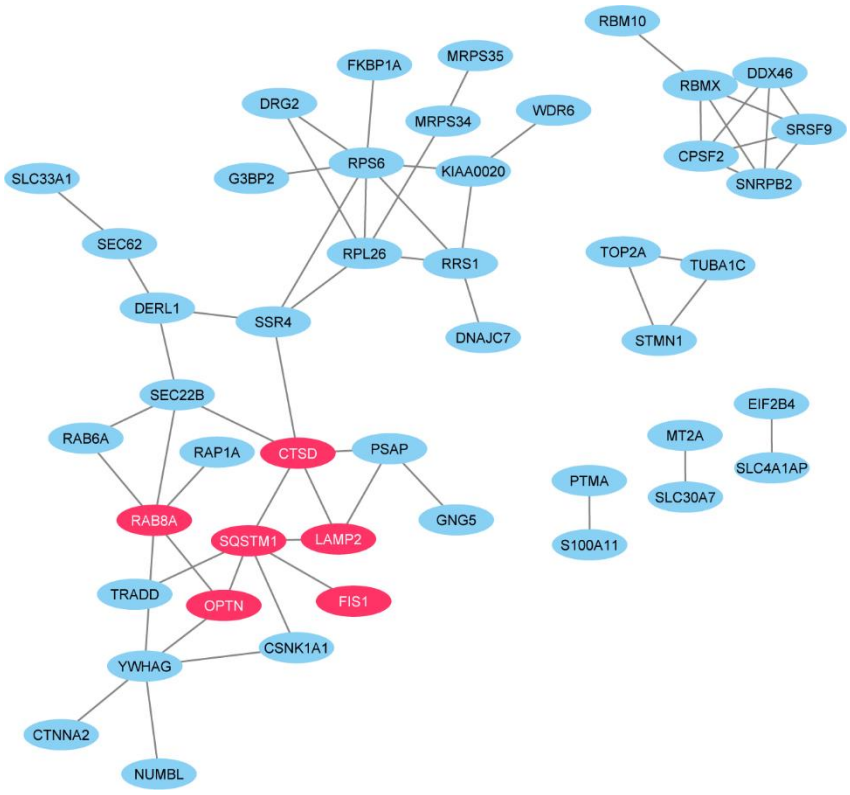
Supplementary Figures

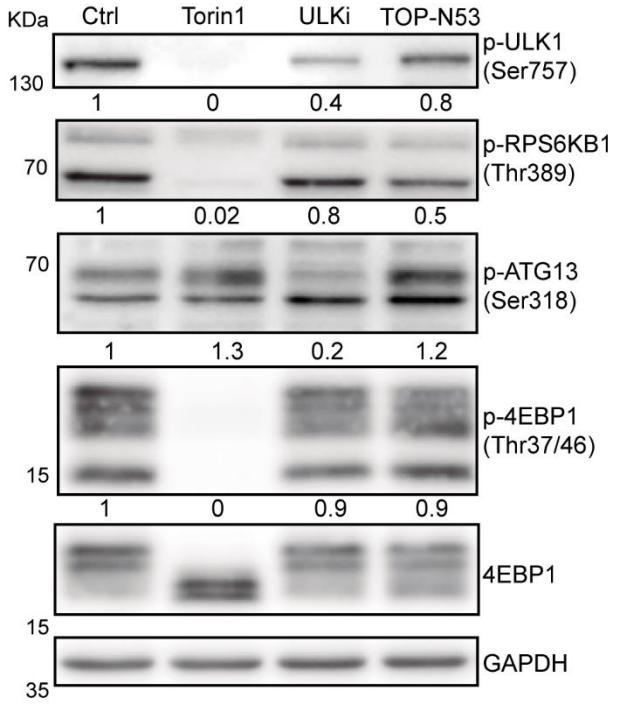
- **Supplementary Figure S1.** PCA of NHK.
- **Supplementary Figure S2.** Complete network analysis of significantly enriched proteins in TOP-N53 treated NHFs.
- **Supplementary Figure S3.** Analysis of mTORC1 signaling pathway and autophagy-related markers.



Supplementary Figure S1. PCA of NHK. Log_2-transformed LFQ intensities of identified proteins were used. The two experimental groups (Control and TOP-N53 treatment) do not separate, indicating no global effect of TOP-N53 on the proteome of NHK.



Supplementary Figure S2. Complete network analysis of significantly enriched proteins in TOP-N53 treated NHFs. Protein-protein interactions of 63 significantly enriched proteins in TOP-N53 treated NHF as analyzed by STRING DB (FDR < 0.05). Red labeled proteins carry terms "autophagy" (GOBP). Proteins that were not part of a node of at least 2 proteins were not included (n = 48).



Supplementary Figure S3. Analysis of mTORC1 signaling pathway and autophagy-related markers. Analysis of different markers related to mTORC1 signaling and autophagy upon TOP-N53 treatment. NHFs were treated with TOP-N53 (1 □M, 24 h), the specific ULK1 inhibitor MRT68921 (1 □M, 2 h) and the mTORC1 inhibitor Torin-1 (1 □M, 2 h) for comparison of triggered signaling. Representative western blot (n = 2) and its quantification below each protein panel (normalized to GAPDH).