



Figure S11

Figure S11. Evaluation of efficiencies RNAi mediated knockdown of VASH1, VASH2 and TTL and their impact on cellular detyrosination. **a and b** Immunoblot analysis of cells expressing SVBP-Myc or VASH1-GFP transfected with control or siRNAs specific for SVBP or VASH1. The knockdown efficiency of SVBP and VASH1 specific siRNAs were observed using anti-Myc or anti-GFP respectively with GAPDH as loading control. **c** Assessment of VASH1/2 knockdown by RNAi. Efficiency of multiple siRNA combinations assessed by immunoblot of protein lysates from cells transfected with combinations of VASH1-A&B and VASH2-A&B siRNAs and probing with detyrosinated tubulin antibody with GAPDH as loading control. **d** Representative immunofluorescence images of U2OS cells transfected with siRNAs against VASH1/2 or TTL. Detyrosinated and total α -tubulin pools were detected using the respective antibodies and DNA was counterstained with DAPI. In merged image, Detyrosinated tubulin in red, total α -tubulin in green, and DNA in blue. Scale bar: 20 μ m. **e** Detyrosinated tubulin levels were quantified from sum projections of the immunofluorescence images of interphase cells and normalized to respective levels of total α -tubulin. N(number of cells, number of independent experiments): siCTRL (48, 2); siVASH1/2 (48, 2); siTTL (47, 2). P-values were calculated using Mann-Whitney U test. ** $p < 0.01$, *** $p < 0.001$. **f** Immunoblot demonstrating the effect of knockdown of TTL on α -tubulin detyrosination levels with GAPDH serving as a loading control.