



**Figure S3** - Replenishment of TECPR2 alleviates basal accumulation of autophagosomes in SPG49 patient fibroblasts in a LIR-dependent manner. **(A)** Primary fibroblasts of SPG49 Ex.8 mutant patient (and healthy control) were transfected for expression of TECPR2-Flag, TECPR2[ΔLIR]-Flag or empty Flag construct control using JetPrime transfection reagent for 48 h. Cells were then fixed with methanol, immunostained for LC3B and Flag and analyzed by confocal microscopy. Scale bar: 20 μm. **(B)** Endogenous TECPR2 was immunoprecipitated from *TECPR2* KO or parental HeLa cells. Enrichment of selected HOPS was probed. **(C)** Endogenous TECPR2 was immunoprecipitated from HeLa cells that were transfected with siNT or siVAMP8 using Darmophact transfection reagent for 72 h. Coimmunoprecipitation of VPS41 (on background of siVAMP8) was probed. **(D)** Cells lysates from **(C)** were subjected to the western blot analysis and the efficiency of siVAMP8 was detected by anti-VAMP8 antibody.