Fig. S1. A) Validation of siRNA silencing of HD-PTP. Left: lysates from HeLa cells were harvested and proteins detected by Western blotting. Graph shows quantification of total protein expression relative to tubulin, values represent mean ± SD. Unpaired two-tailed student’s t-test, **p<0.01, n=4. Right: HeLa cells were immunostained for HD-PTP (red) and with FK2 anti-ubiquitin conjugate (green). Scale bar = 10 µm. B) Validation of siRNA silencing of Alix. C) Validation of siRNA silencing of Rabaptin-5. Unpaired two-tailed student’s t-test, ***p<0.001, n=4. D) Validation of siRNA silencing of GAPVD1. Unpaired two-tailed student’s t-test, ***p<0.0001, n=6.
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A

GFP-RBPT5

HA-HD-PTP

HA-HD-PTP

FL-WT

FL-LI/DD

B

HA

EEA1

Rab5

MERGE

C

HA-HD-PTP

FL-LI/DD

HA-HD-PTP

FL-WT

EEA1

Rab5

Graph

Cells with WT EEA1 staining (%)
Fig. S2. A) HeLa cells were transfected with HA-HD-PTP and GFP-Rabaptin-5 constructs as indicated (FL = full-length), and labelled for endogenous Rab5. Scale bar represents 10 μm. Insets magnified x 3.
B) HeLa cells were transfected with HA-HD-PTP constructs and labelled as indicated. Arrowheads indicate non-endosomal HA-HD-PTP clusters. Scale bar represents 20 μm. Insets magnified x 3.
C) Left: HeLa cells were depleted by siRNA and rescued with RNAi-resistant HA-HDPTP as indicated and immunostained for EEA1. HA-HD-PTP transfected cells are asterisked. Scale bar represents 10 μm. Insets magnified x4. Right: Histogram showing the percentage of cells that displayed a normal distribution of EEA1 (mean ± SD from 3 independent experiments, 100 cells counted per experiment). One-way ANOVA with Bonferroni’s test for multiple comparisons, ***p=0.0004.
Table S1. siRNA oligonucleotides used in this study.

| Protein | Company        | Commercial name | Sequence                        | Conditions |
|---------|----------------|-----------------|---------------------------------|------------|
| HD-PTP  | Thermo Scientific | J-009417-06     | GCAAACAGCGGAUGAGCAA              | 5nM, 48hr  |
| Rabaptin-5 | Qiagen        | Custom          | GUAGUAUGCUGUAUGAAUA              | 10nM, 48hr |
| ALIX    | Thermo Scientific | J-004233-12     | GUACCUCAGUCUAUAUGAUU             | 20nM, 48hr |
|         |                |                 | followed by 20nM, 48hr          |            |
| GAPVD1  | Thermo Fisher Scientific | s25136 | | 5nM, 48hr |
| UBAP1   | Qiagen         | Custom          | CCCAAUGGCUUUUAUAACCUUA          | 20nM, 72hr |
| Hrs     | Thermo Scientific | J-016835-06     | GCACGUCUUUCCAGAAUUC             | 20nM, 72hr |
| VPS4A   | Thermo Scientific | L-013092-05     | CCACAAACAUCCCCAUGGGU            | 5nM, 72hr  |
| VPS4B   | Thermo Scientific | L-013119-05     | GGGCAAGUGUACAGAAUA              | 5nM, 72hr  |