Supplementary Figure 1. A monoclonal antibody specific for hSPE-39. Lysates of HeLa cells either transfected or not transfected with the hSPE-39-EGFP construct were resolved by PAGE, blotted and probed with the anti-hSPE-39 monoclonal antibody. Endogenous hSPE-39 is indicated with a hollow arrow. Full-length hSPE-39-EGFP fusion protein is indicated with a filled arrow. hSPE-39-EGFP is partially degraded when over expressed in HeLa cells and degraded products of various sizes were also detected. Sizes of protein standards are shown in kilodaltons.

Supplementary Figure 2. An antiserum that recognizes mammalian VPS33B. Human Embryonic Kidney (HEK) 293 cells were either transfected or not transfected with the *hVPS33B-HA* construct before they were analyzed by immunoblotting. The anti-VPS33B antiserum detected a single major endogenous band. Migration of this band (top arrow) is indistinguishable from the band detected by the anti-HA antibody in HEK cells expressing hVPS33B-HA (bottom arrow). This indicates that the endogenous hVPS33B band was the correct size. Sizes of protein standards are shown in kilodaltons.

Supplementary Figure 3. hSPE-39 interacts with hVPS33B in HEK 293T cells. HEK cells transfected (lanes 3, 4, 7, 8, 11 and 12) and not transfected (lanes 1, 2, 5, 6, 9 and 10) with hSPE-39-EGFP were incubated with (even lanes) or without (odd lanes) DSP, lysed and immunoprecipitated with antibodies against the γ subunit of adaptor complex AP-1 (lanes 1-4) or hSPE-39 (lanes 5-8). Anti-hSPE-39 antibodies precipitated both (arrows) endogenous hSPE-39 (lanes 5, 6, 7 and 8) and transfected hSPE-39-EGFP (lanes 7 and 8) together with the HOPS subunit hVPS33B. hSPE-39 or hVPS33B was not detected in beads coated with anti-γantibody (lanes 1-4). Bands marked with an asterisk

in lanes 1-4 are the heavy chain of the anti-γ antibody. Each input was loaded at 2% of the total amount of cell lysate used for corresponding immunoprecipitation experiments.

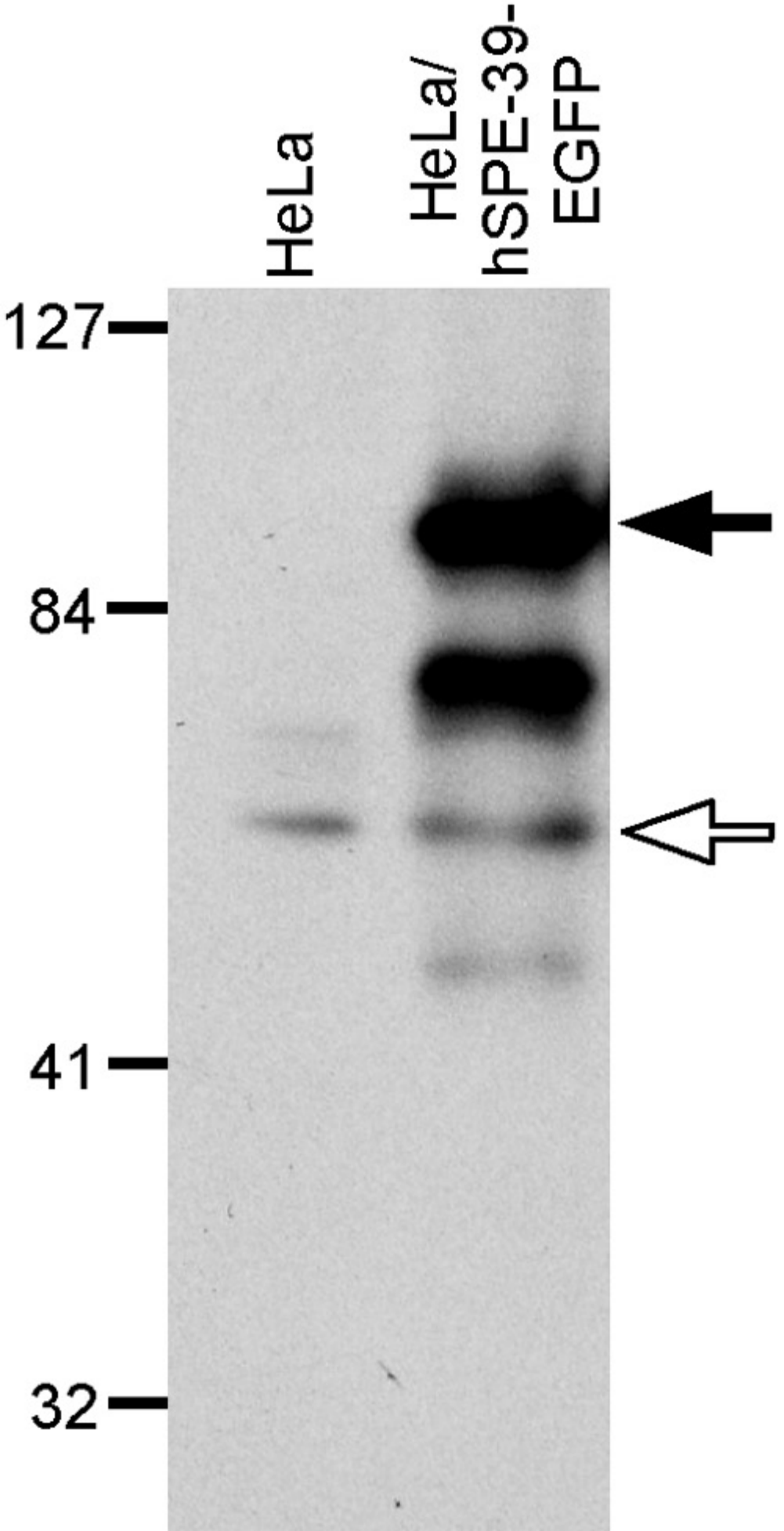
Supplementary Figure 4. Co-immunoprecipitation of hSPE-39-HA with recombinant HOPS subunits. (A) HEK cell lines expressing tagged HOPS subunits were transiently transfected with hSPE39-HA and incubated with (even lanes) or without (odd lanes) DSP. Cell lysates were immunoprecipitated with anti-hSPE-39 antibodies. As we observed before with the hSPE39-EGFP fusion protein, the HA tagged form of hSPE-39 co-precipitates VPS11-HA (lanes 1 and 2), VPS16-HA (lanes 3 and 4), VPS18-MYC (lanes 5 and 6) and VPS41-MYC (lanes 7 and 8). (B) Lysates of cells expressing hSPE-39-HA/VPS18-MYC (lanes 1 and 2) or hSPE-39-HA/VPS41-MYC (lanes 3 and 4) were immunoprecipitated with anti-MYC antibodies and the presence of hSPE-39-HA in the precipitated protein complexes was detected by Western analysis. These results indicate that, like hSPE-39-EGFP, hSPE-39-HA also interacts with the HOPS subunits. Each input was loaded at 1.7% of the total amount of cell lysate used for corresponding immunoprecipitation experiments.

Supplementary Figure 5. Golgi, endosomal and lysosomal markers in cells overexpressing hSPE-39-EGFP. HeLa cells transfected with the hSPE-39-EGFP construct were stained for EEA1 (A), transferrin receptor (D), CD63 (G), LAMP1 (J), GM130 (M), and cathepsin D (P). Each row consists of corresponding images that show immunostaining of a compartment marker (in red, left panels), hSPE-39-EGFP signals (in green, middle panels) and a merge (right panels). Bars, 20 μm.

Supplementary Figure 6. hSPE-39 knockdown phenotypes can be rescued by siRNA-resistant (SR) hSPE-39-EGFP expression. (A) HeLa cells treated with control siRNAs

(lanes 1, 3 and 4) or *hSPE-39* siRNA (lanes 2, 5 and 6) were transfected with either control vector plasmid pEGFP-N1 (lanes 1 and 2), wild-type (lanes 3 and 5) or siRNA-resistant (lanes 4 and 6) *hSPE-39-EGFP* construct. Western results indicate that the *hSPE-39* siRNA duplex abolished the expression of both endogenous hSPE-39 (lanes 2, 5 and 6) and wild-type hSPE-39-EGFP (lane 5) expression but did not interfere with the expression of hSPE-39-EGFP(SR) (lane 6). (B and C) HeLa cells transfected with both *hSPE-39* siRNA and *hSPE-39-EGFP(SR)* construct were stained for cathepsin D (B) and M6PR (C). Cathepsin D levels are low in cells treated with hSPE-39 knockdown, but its level is increased with hSPE-39-EGFP expression (B). In *hSPE-39* siRNA-treated cells, M6PR signals are highly clustered, which can be corrected by an appropriate level of hSPE-39-EGFP (C). These results indicate that the observed RNAi phenotypes in cultured cells are not off-target effects and that low, physiological level of hSPE-39-EGFP expression can functionally compensate for the lack of endogenous hSPE-39. Bars, 10 ⌈m.

Supplementary Figure S7. hSPE-39 co-localizes with RAB11 and RAB7 compartments. HEK293 cells stably expressing VPS16-HA either alone (A-C and G-L) or transiently transfected with a plasmid encoding a GFP-tagged RAB5 GTPase mutant Q79L (D-F) were co-stained with antibodies against endogenous hSPE-39, RAB5 and the HA epitope (A-C); hSPE-39 and the HA epitope (D-F), hSPE-39, RAB7B and the HA epitope (G-I); and hSPE-39, RAB11 and the HA epitope (J-L). M shows a representative HEK293 cell transiently transfected with Hrs-MYC and stained with antibodies against hSPE-39 and the MYC epitope. All images were acquired by wide field deconvolution microscopy and pseudo-colored green and red. Bars, 5 μm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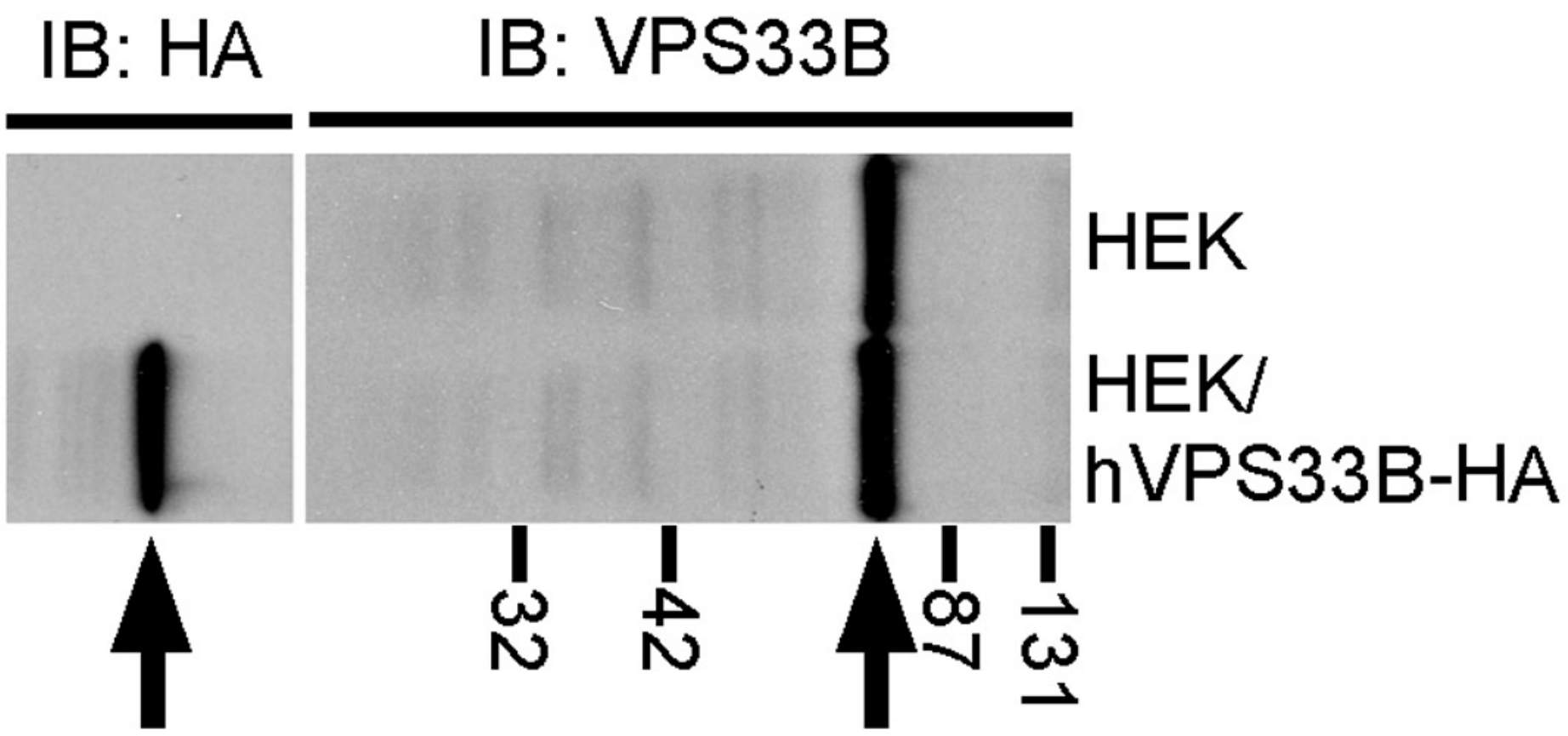
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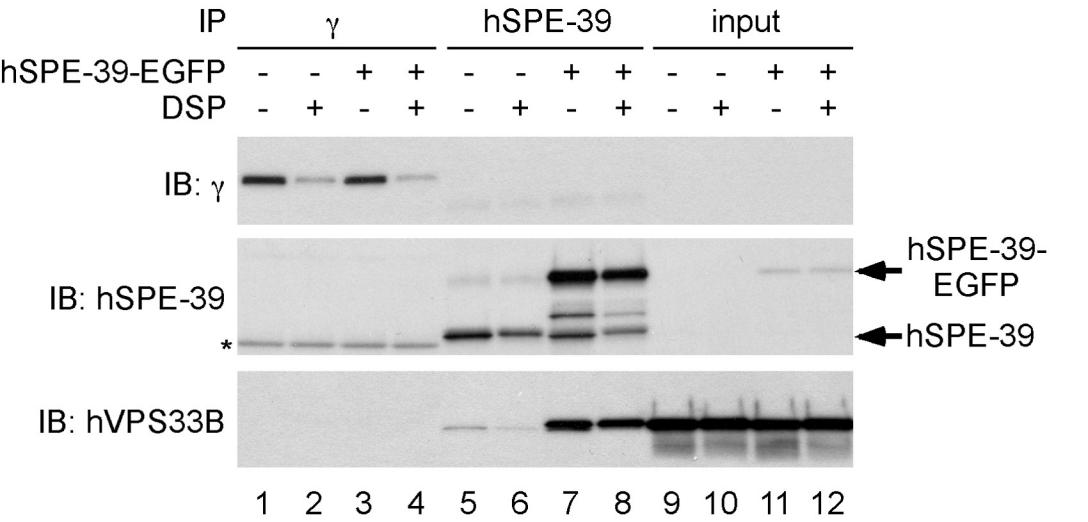
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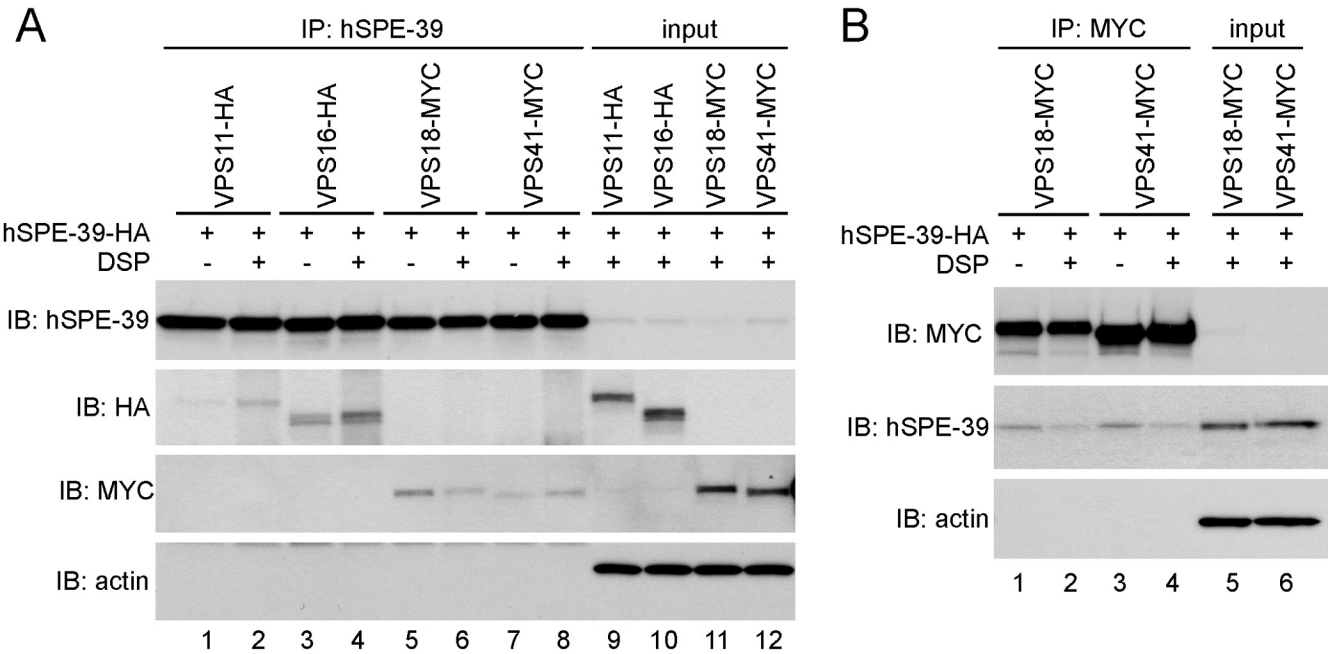
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DSP - + - + - + - + + + + +
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IB: HA
IB: MYC
IB: actin
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B
IP: MYC
input
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VPS41-MYC
VPS18-MYC
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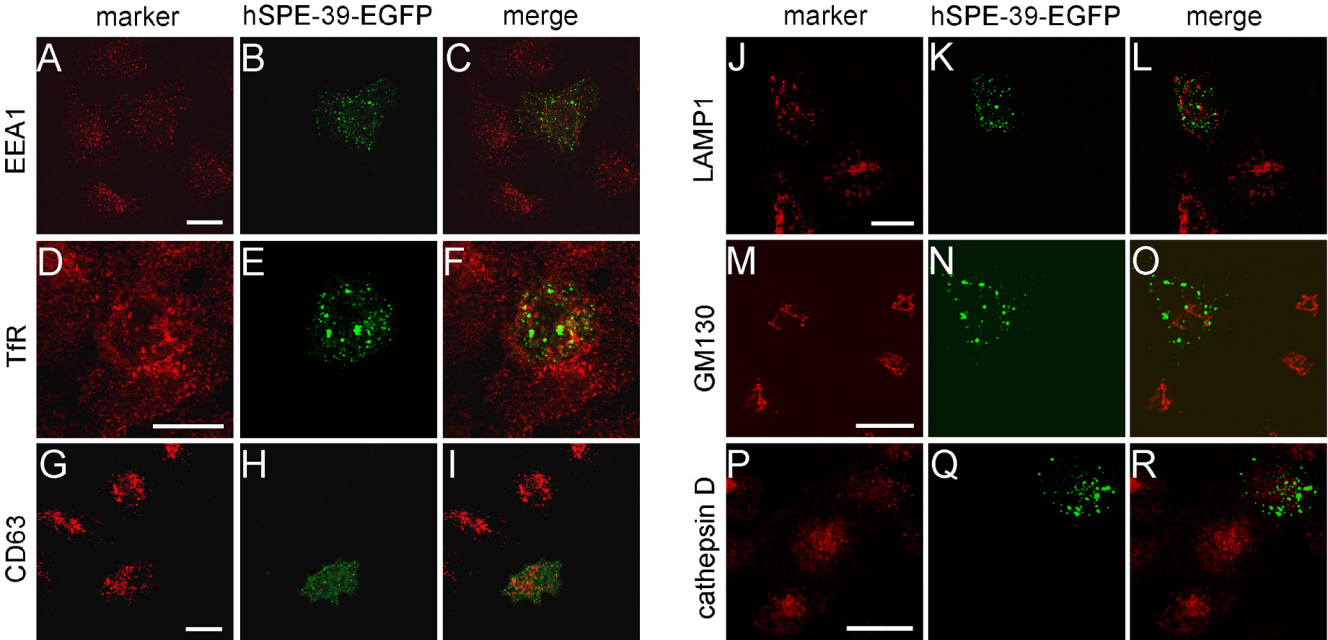
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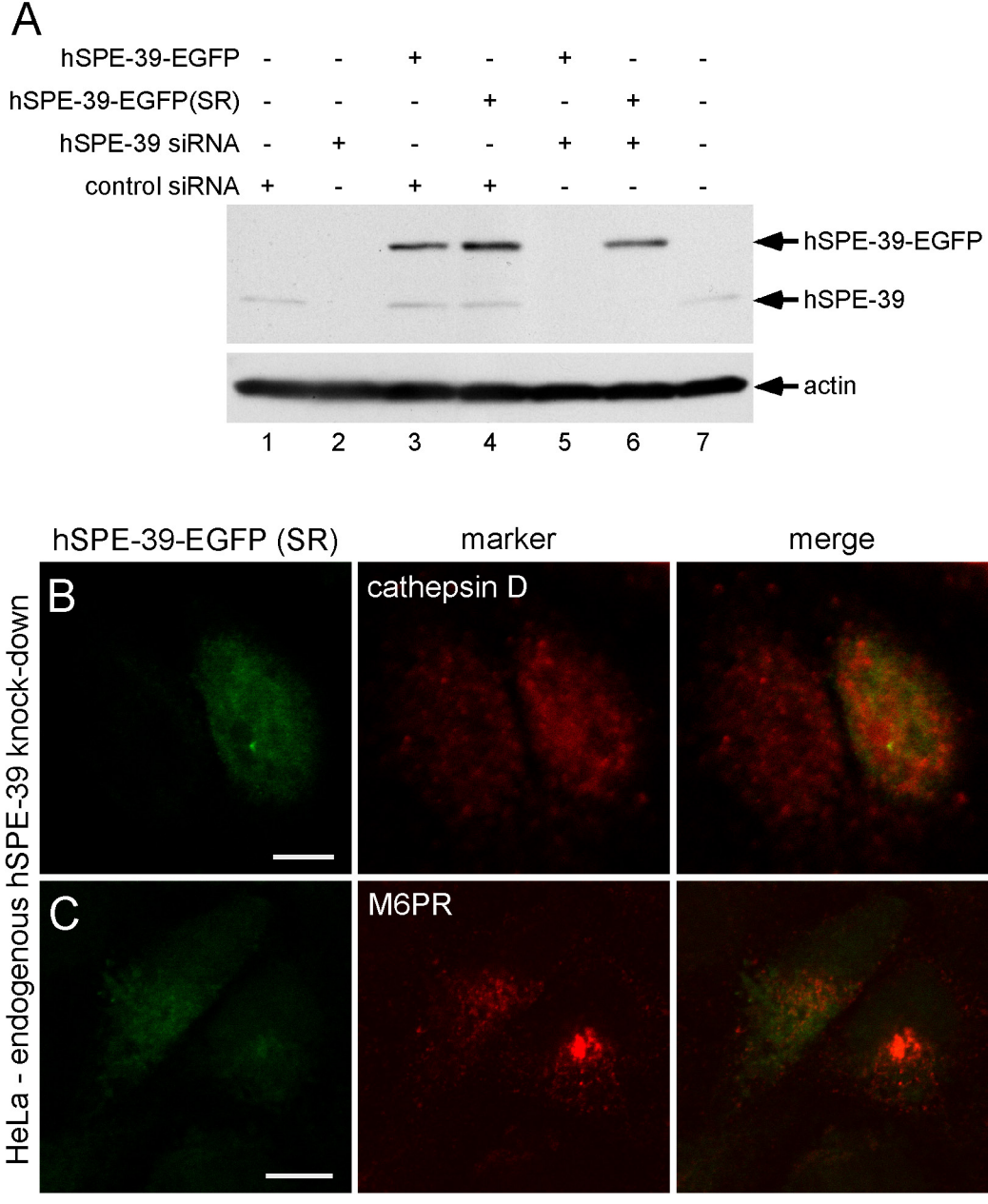
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control siRNA + - + + - - -
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hSPE-39
actin
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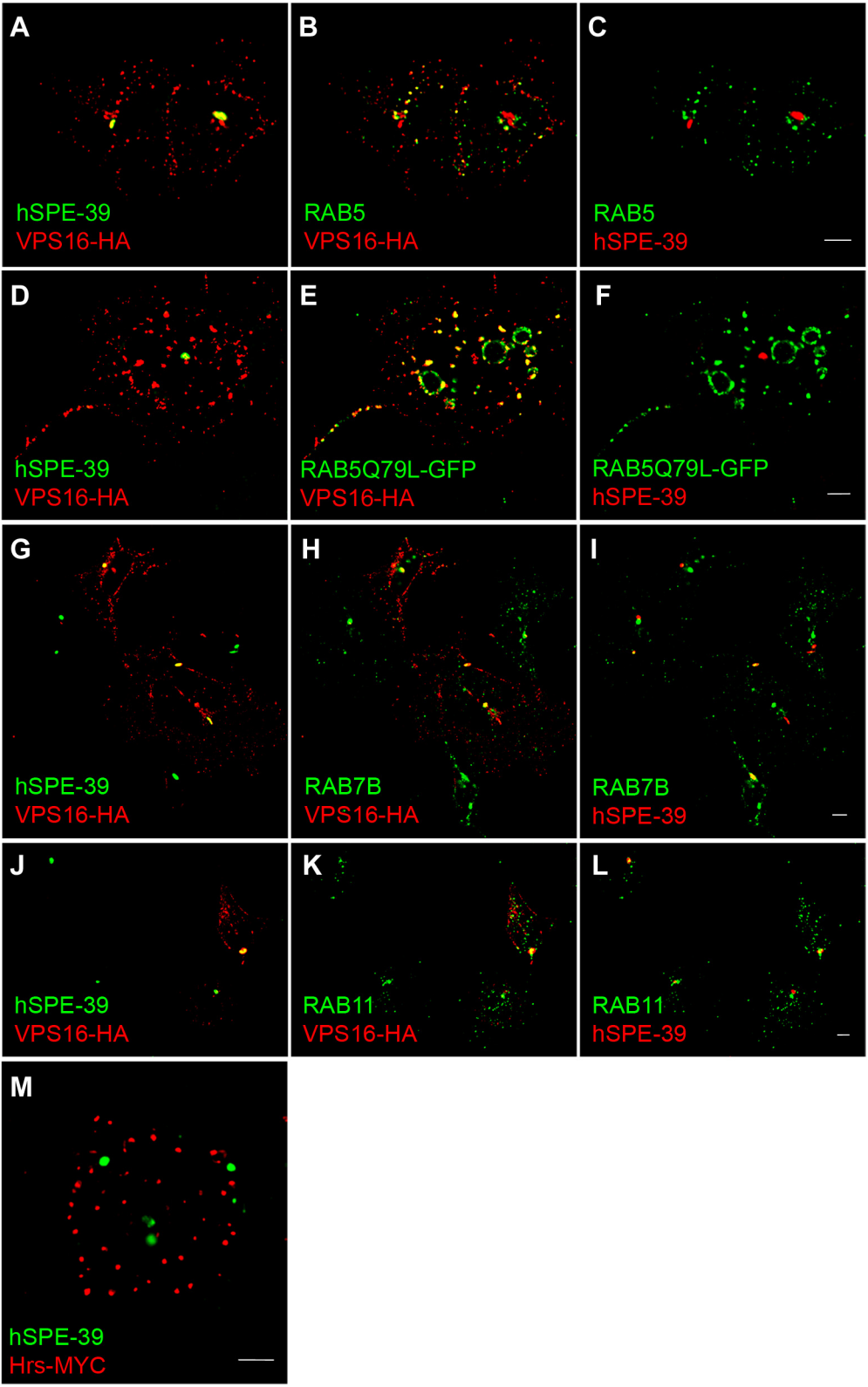
merge
B
cathepsin D
C
M6PR
HeLa - endogenous hSPE-39 knock-down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