Figure S1

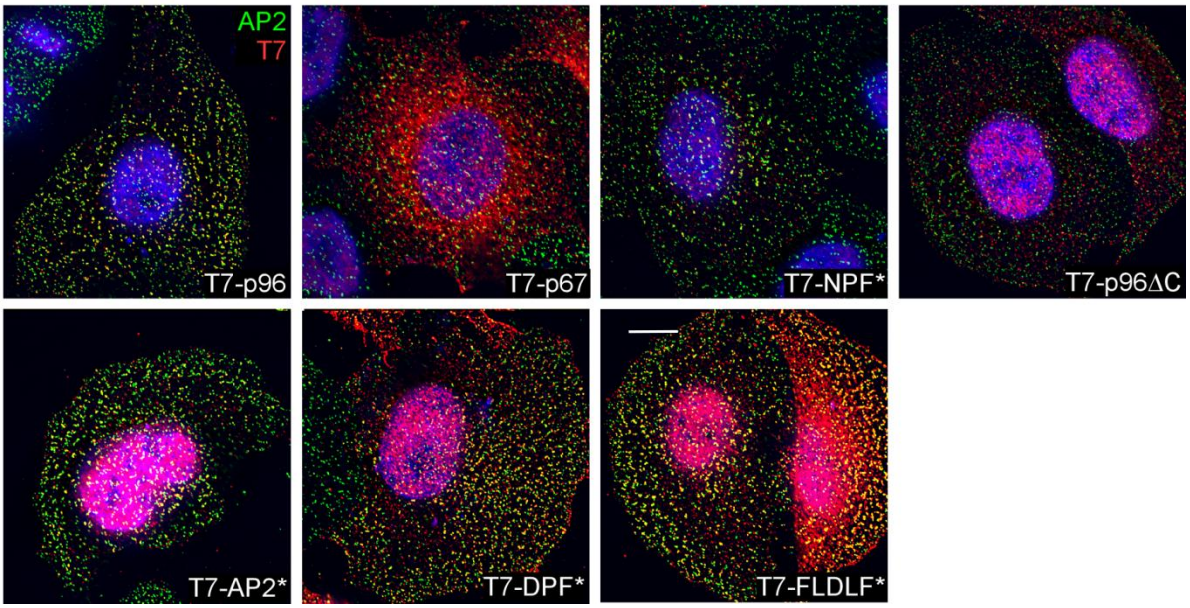


Figure S2

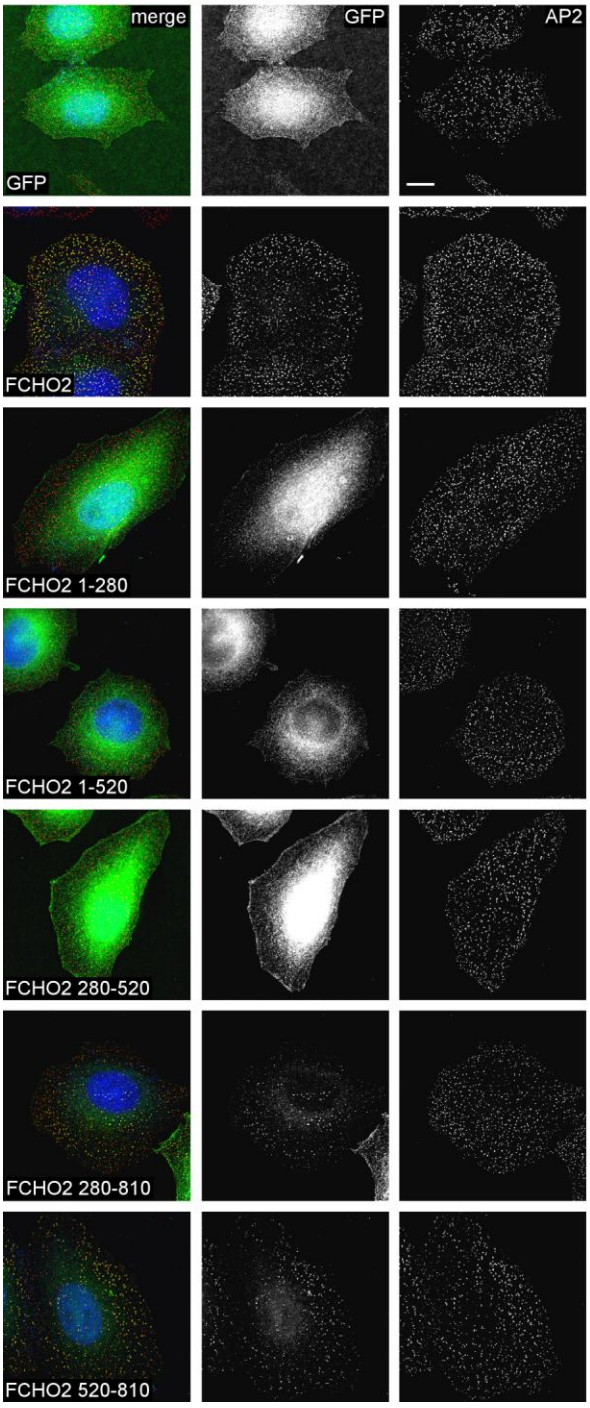


Figure S3

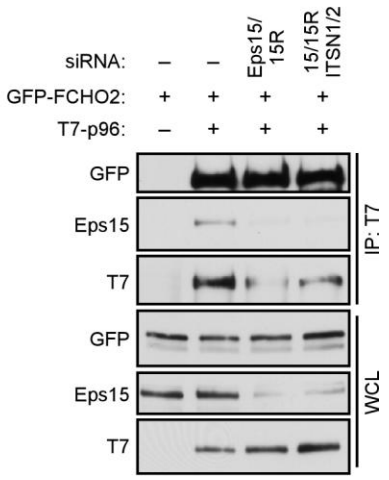


Figure S4

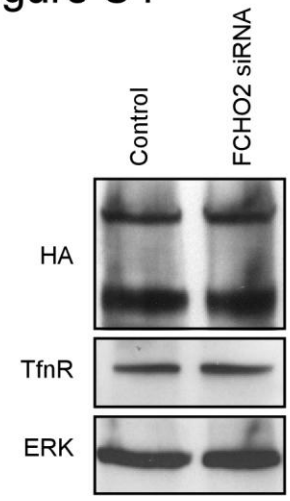
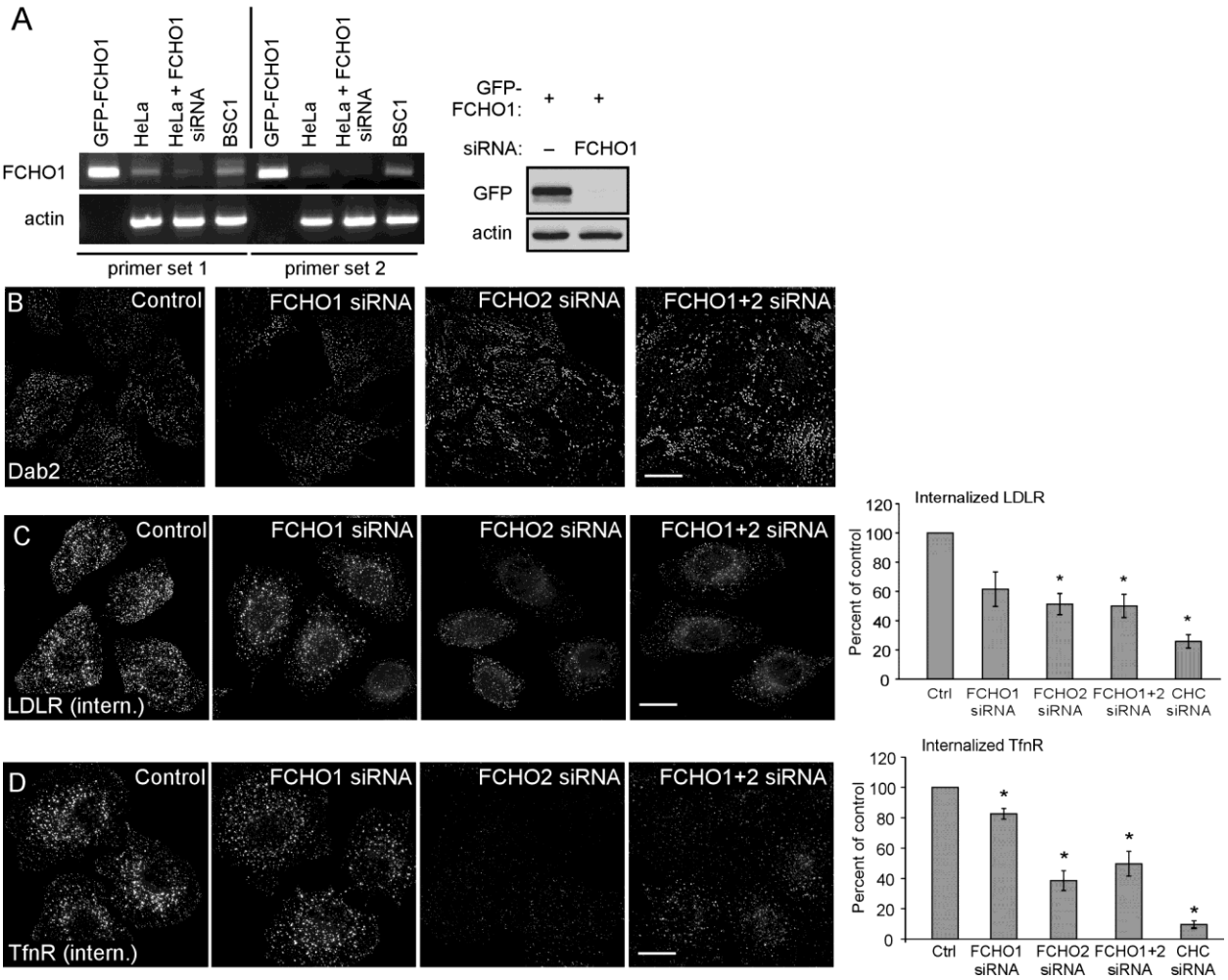


Figure S5


A
GFP-FCHO1
HeLa
HeLa + FCHO1 siRNA
BSC1
GFP-FCHO1
HeLa
HeLa + FCHO1 siRNA
BSC1
FCHO1
actin
primer set 1
primer set 2
GFP-FCHO1: + +
siRNA: – FCHO1
GFP
actin
B
Control
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
Dab2
C
Control
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
LDLR (intern.)
Internalized LDLR
Percent of control
120
100
80
60
40
20
0
Ctrl
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
CHC siRNA
D
Control
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
TfnR (intern.)
Internalized TfnR
Percent of control
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100
80
60
40
20
0
Ctrl
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
CHC siRNA

Figure S6

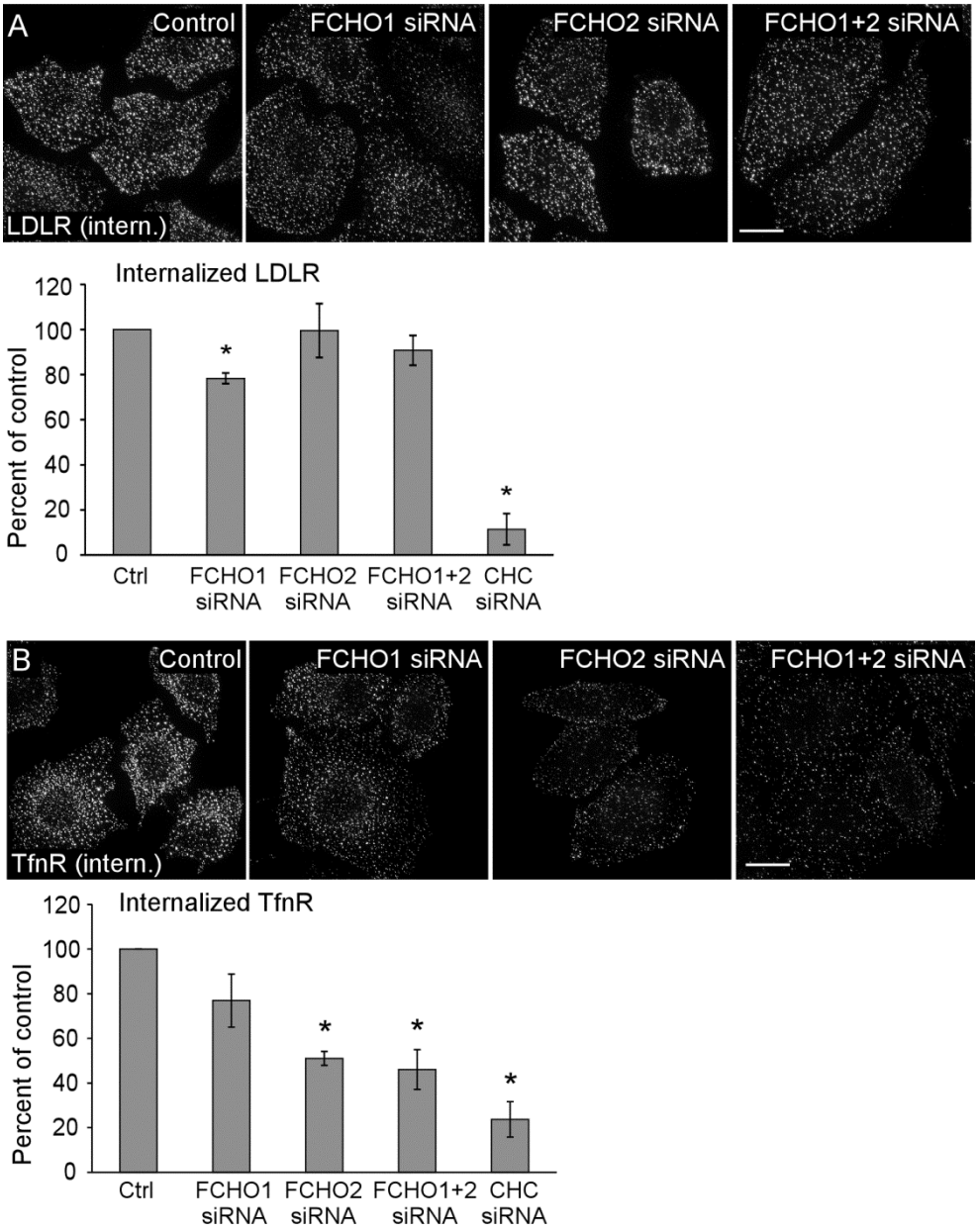

A
Control
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
LDLR (intern.)
Internalized LDLR
Percent of control
120
100
80
60
40
20
0
*
*
Ctrl
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
CHC siRNA
B
Control
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
TfnR (intern.)
Internalized TfnR
Percent of control
120
100
80
60
40
20
0
*
*
*
Ctrl
FCHO1 siRNA
FCHO2 siRNA
FCHO1+2 siRNA
CHC siRNA

Figure S7

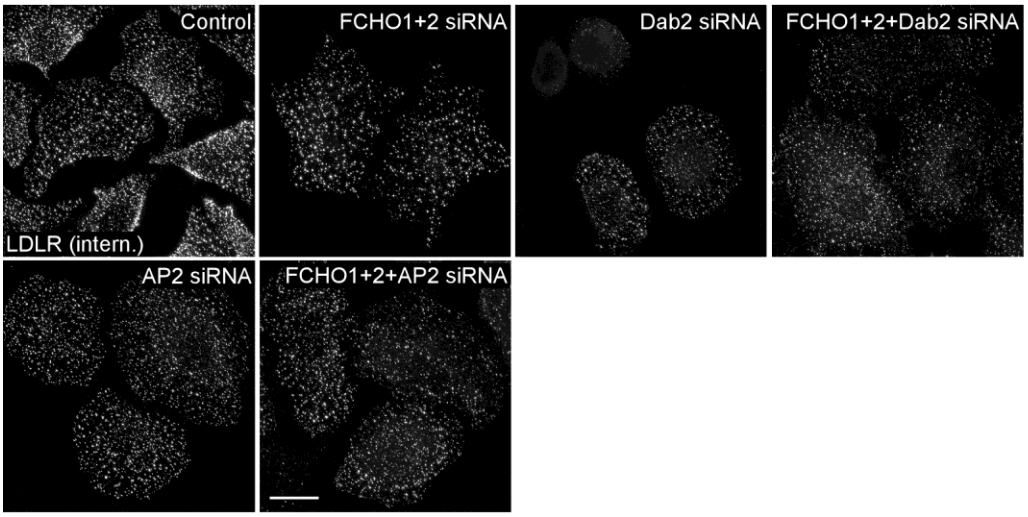
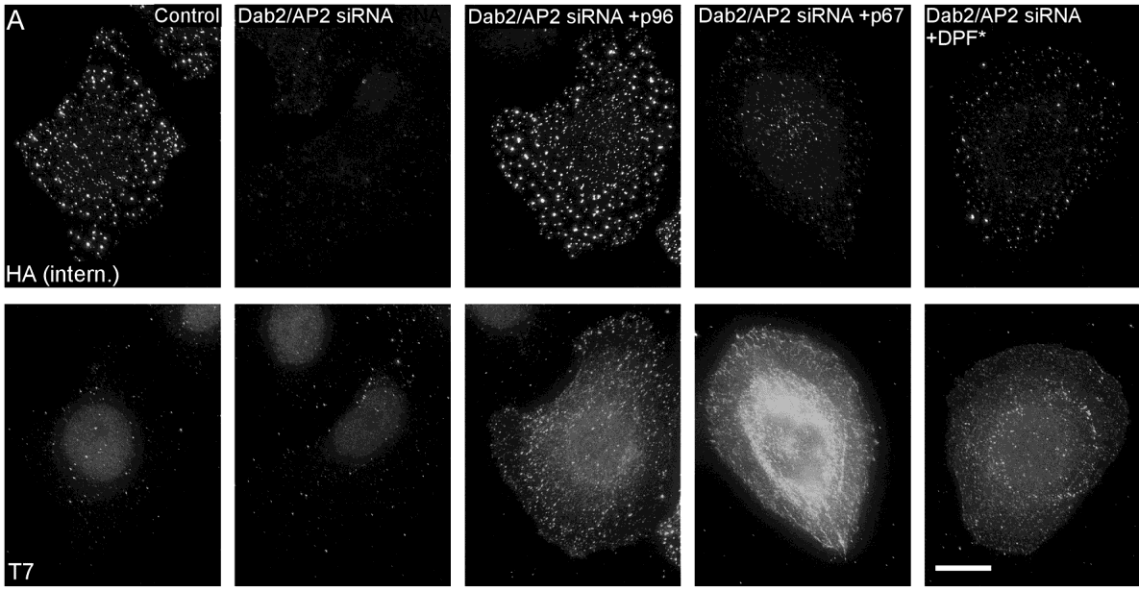
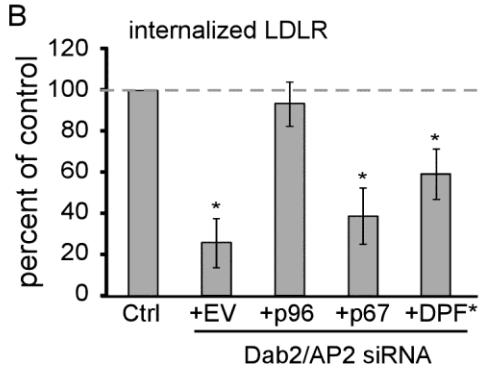


Figure S8





Supplemental Figure Legends

Figure S1
Localization of T7-Dab2 mutants
Fixed HeLa cells were permeabilized and stained with antibodies to T7 and α-adaptin. The adherent surface of the cell is shown. Nuclear T7 staining is non-specific background. Scale bar is 10μm.

Figure S2
The μHD of FCHO2 is required to direct it to clathrin-coated pits
HeLa cells were transfected with GFP-tagged FCHO2 fragments, permeabilized, and stained for AP2. Shown is the adherent surface of the cell. Scale bar is 10μm.

Figure S3
Depletion of EH domain proteins does not affect Dab2-FCHO2 binding
HeLa cells were transfected with two rounds of siRNA for Eps15/15R or Eps15/15R+ITSN1/2. Cells were also transiently transfected with DNA for T7-Dab2 and GFP-FCHO2. Cells were lysed and subjected to immunoprecipitation with antibodies to T7.

Figure S4
FCHO2 depletion does not affect total cellular levels of TfnR or HA-miniLDLR.
HeLa cells transfected with siRNA to FCHO2 were lysed and immunoblotted for either TfnR or HA-miniLDLR. For HA-miniLDLR, the upper band corresponds to the unprocessed, ER form of the protein and the lower is the processed form (Li et al., 2001). ERK was used as a loading control.

Figure S5
Effect of FCHO1+2 siRNA on LDLR and TfnR endocytosis at physiological conditions
(A) (Left) FCHO1 siRNA depletes both FCHO1 mRNA and GFP-FCHO1 protein. HeLa-miniLDLR cells were transfected with FCHO1 siRNA or buffer control on days 1 and 3 and lysed for RNA on day 5. Only low levels of FCHO1 were detected in HeLa cells. BSC1 cells were used as a positive control for FCHO1 expression (Henne *et al.*, 2010), and GFP-FCHO1 as a positive control for PCR. (Right) HeLa cells were transfected on days 1 and 3 with FCHO1 siRNA or buffer control and GFP-FCHO1 DNA on day 2, lysed, and subjected to Western blotting. (B) CCS size in cells transfected with siRNA for FCHO1, FCHO2, FCHO1+2, or buffer control. Cells were fixed and stained with anti-Dab2 antibody. Images are the adherent surface of a field of cells. Scale bar is 20 μm. (C, D) Cells were transfected with FCHO1, FCHO2, FCHO1+2, or CHC siRNA or buffer control on days 1 and 3, and endocytosis was measured on day 5. Cells were given antibody against HA (C) or TfnR (D) and allowed to internalize for 2 min at 37°C. Values shown are means +/- standard error for at least three experiments. Images are z-stack projections of the entire cell height. Scale bars are 20 μm. *, P-value < 0.05 by Student's t-test. In neither case were FCHO2 and FCHO1+2 cells significantly different from one another.

Figure S6
Effect of FCHO1+2 siRNA on LDLR and TfnR endocytosis with a 4°C pre-incubation

HeLa-miniLDLR cells were transfected with FCHO1, FCHO2, FCHO1+2, or CHC siRNA or buffer control on days 1 and 3, and endocytosis was measured on day 5. Cells were given antibody against HA (A) or TfnR (B) and incubated for 1 hr at 4°C. Cells were then placed in a 37°C waterbath and allowed to internalize for 2 min. Values shown are means +/- standard error for at least three experiments. Images are z-stack projections. Scale bars are 20 μm. *, P-value < 0.05 by Student's t-test. In neither case were FCHO2 and FCHO1+2 cells significantly different from one another.

Figure S7
Additional depletion of Dab2 or AP2 with FCHO1+2 does not further decrease LDLR endocytosis with a 4°C pre-incubation
HeLa-miniLDLR cells were transfected with combinations of FCHO1, FCHO2, Dab2, and AP2 siRNA or buffer control. Cells were given antibody against HA and incubated for 1 hr at 4°C. Cells were then placed in a 37°C waterbath and allowed to internalize for 2 min. Images are z-stack projections. Scale bar is 20 μm.

Figure S8
Dab2-DPF* is deficient for LDLR endocytosis even when cells are given a 4°C pre-incubation.
(A) HeLa-miniLDLR cells were transfected with siRNA to Dab2 and AP2 and DNA for T7-tagged Dab2 forms. Cells were incubated at 4°C for 1 hr with anti-HA, warmed to 37°C for 2 min, and then stained with antibodies to internalized HA and T7. Images are z-stack projections of the entire cell. Bar, 10μm. (B) Means and standard errors of fluorescence intensity of at least five cells from three separate experiments are shown. *, $P < 0.05$ by Student's t-test compared to control cells. Dashed line indicates control level, EV: empty vector.