

Expanded View Figures

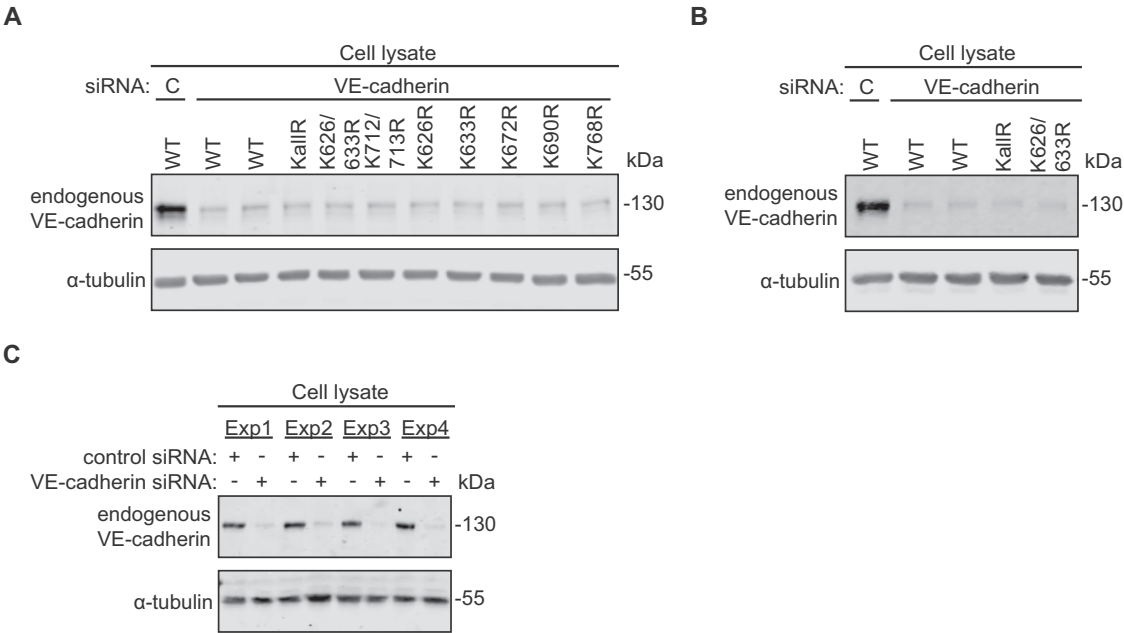


Figure EV1. Endogenous VE-cadherin knockdown efficiency for experiments in Fig. 1.

(A–C) Endogenous VE-cadherin was silenced in HUVEC via VE-cadherin siRNA. Whole cell lysates were immunoblotted for endogenous VE-cadherin and α-tubulin. Molecular sizes are indicated in kilodaltons (kDa). (A) Experiment shown in Fig. 1B. (B) Experiment shown in Fig. 1D. (C) Experiment shown in Fig. 1F.

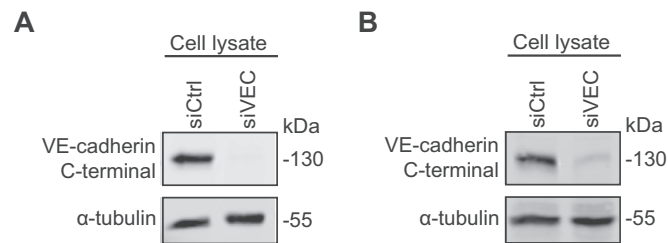


Figure EV2. Endogenous VE-cadherin knockdown efficiency for experiments in Fig. 2.

(A, B) Endogenous VE-cadherin was silenced in HUVEC via VE-cadherin siRNA. Whole cell lysates were immunoblotted for endogenous VE-cadherin and α -tubulin. Molecular sizes are indicated in kilodaltons (kDa). (A) Experiment shown in Fig. 2A. (B) Experiment shown in Fig. 2C.

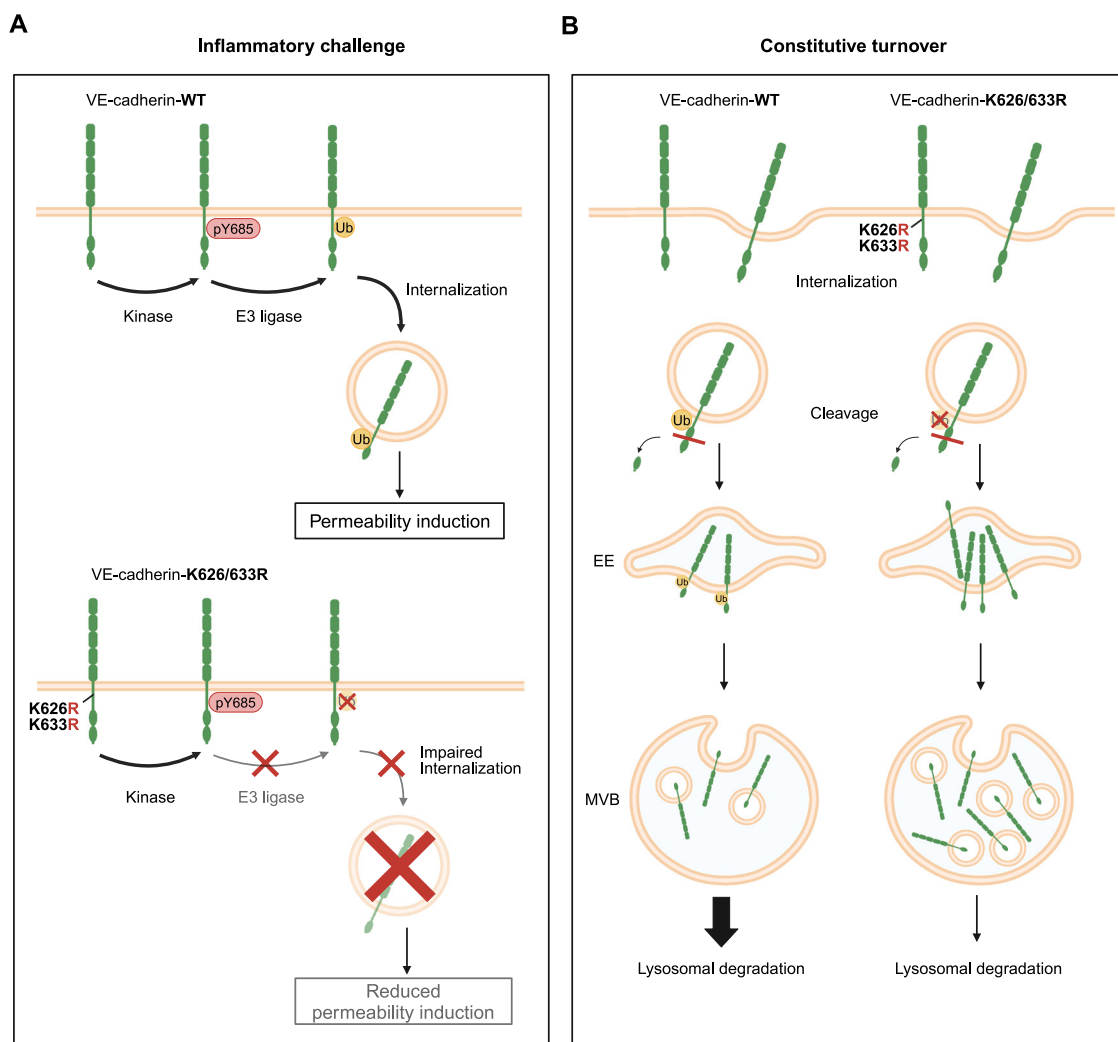


Figure EV3. Dual function of VE-cadherin ubiquitination in inflammation-induced endocytosis and lysosomal targeting.

(A) Upon inflammatory challenge, Y685 of VE-cadherin gets phosphorylated followed by ubiquitination of VE-cadherin lysine residues K626 and K633, leading to VE-cadherin endocytosis and an increase in vascular permeability. This is prevented when K626 and K633 are mutated to arginine leading to impaired induction of vascular permeability. (B) Besides inflammation-induced endocytosis, ubiquitination of VE-cadherin controls a second process, the targeting of VE-cadherin to the lysosomal degradation pathway. Mutation of K626 and K633 to arginine slows down lysosomal targeting of mutated VE-cadherin leading to its accumulation in early endosomes (EE) and multivesicular bodies (MVB). Our results imply that K/R mutated VE-cadherin can be constitutively endocytosed in resting cells by lysine independent mechanisms, and therefore accumulates inside the cell, due to impaired degradation.