

Supplemental Materials

Molecular Biology of the Cell

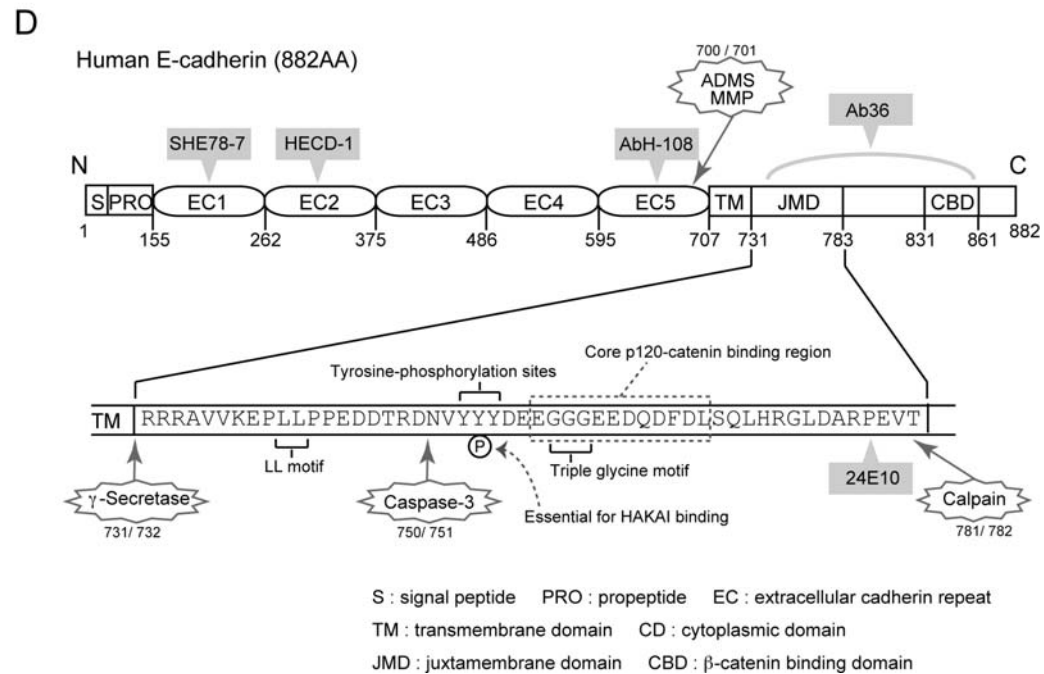
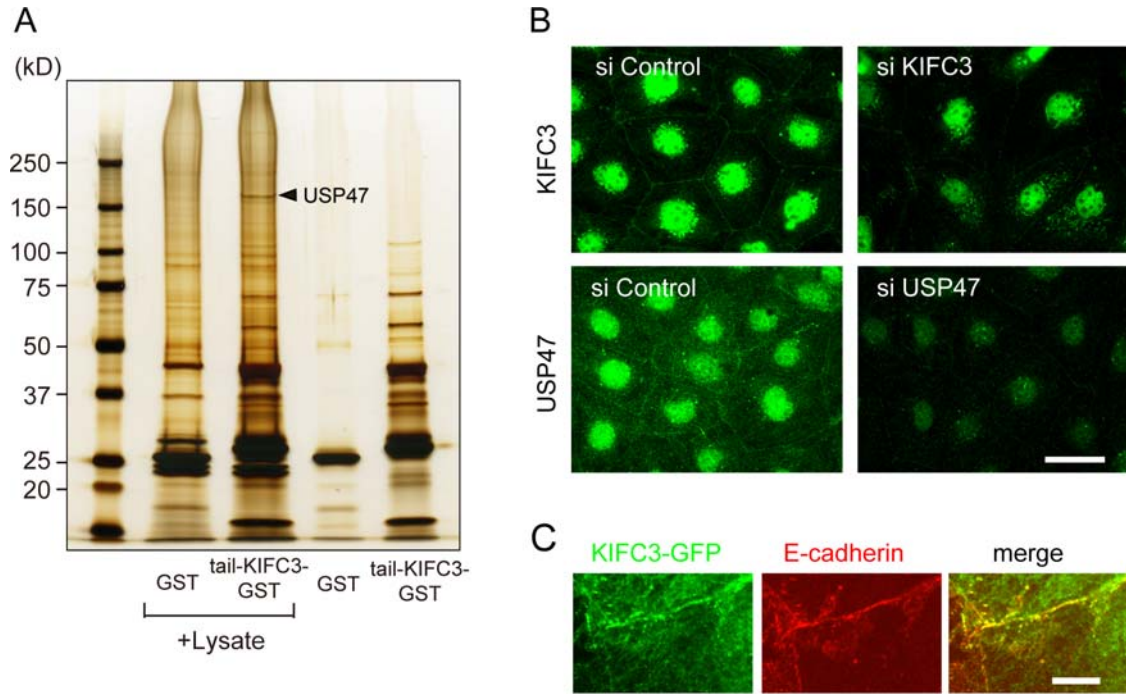
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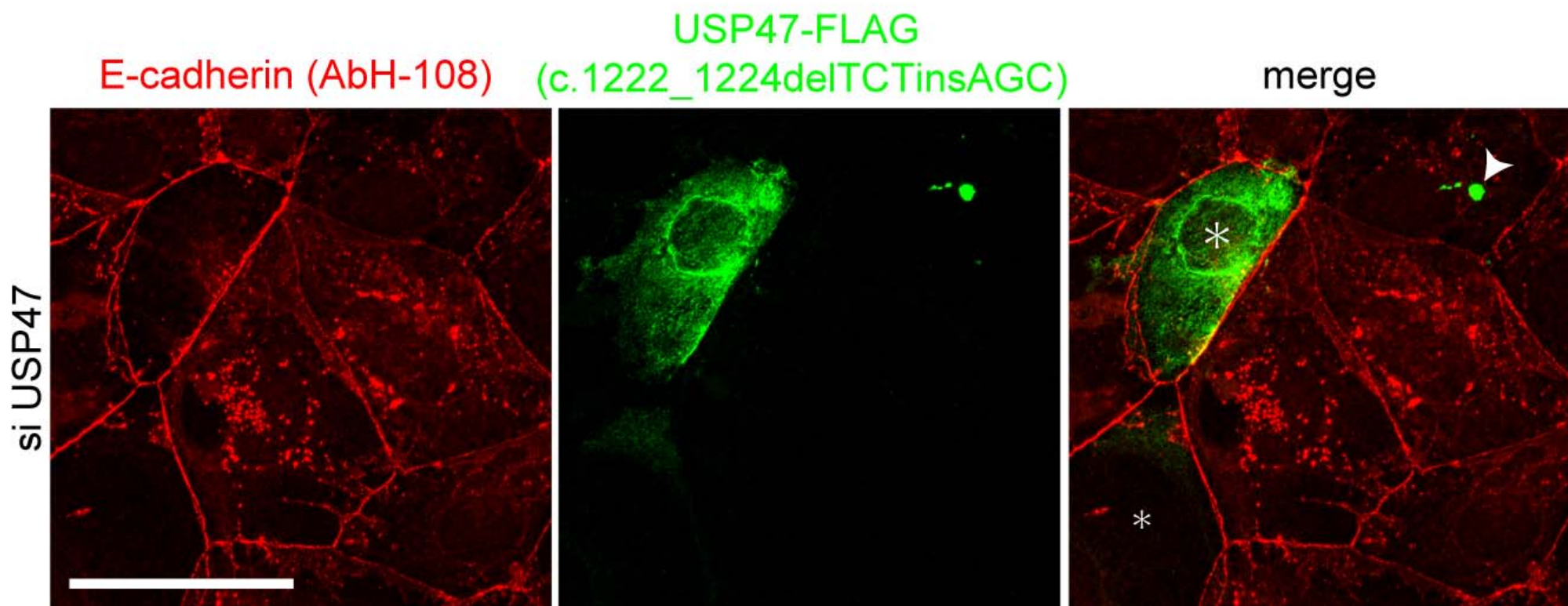
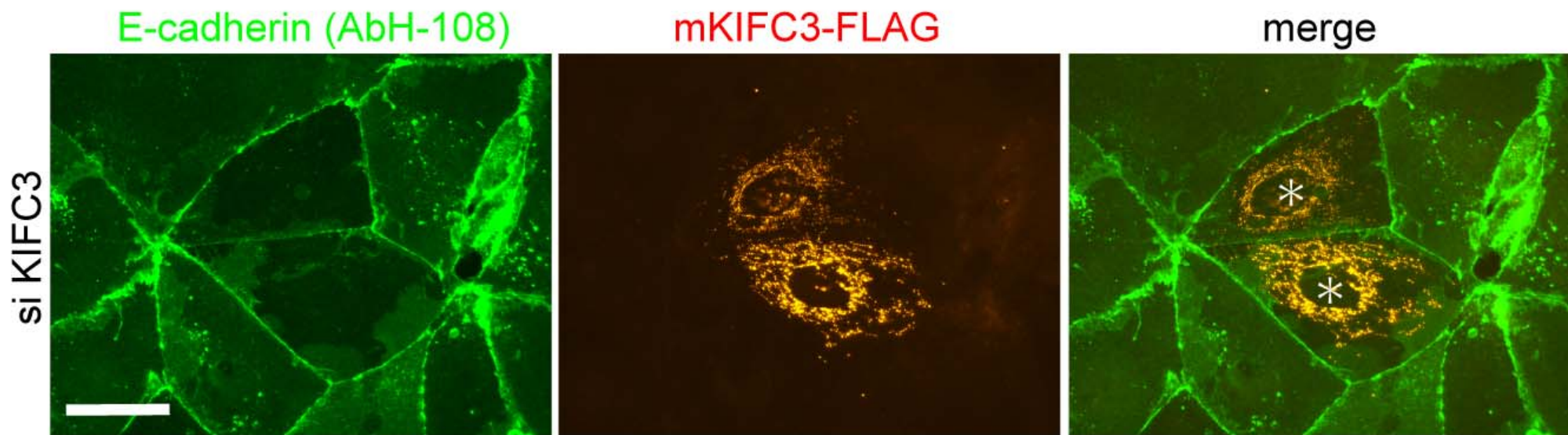
SUPPLEMENTAL FIGURE LEGENDS

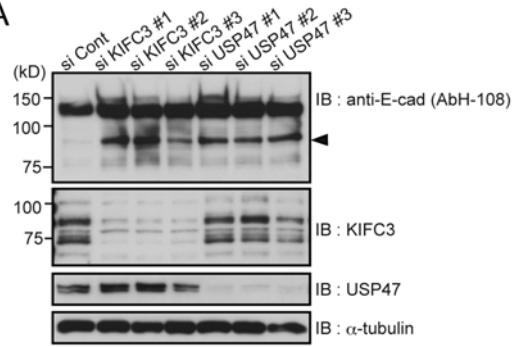
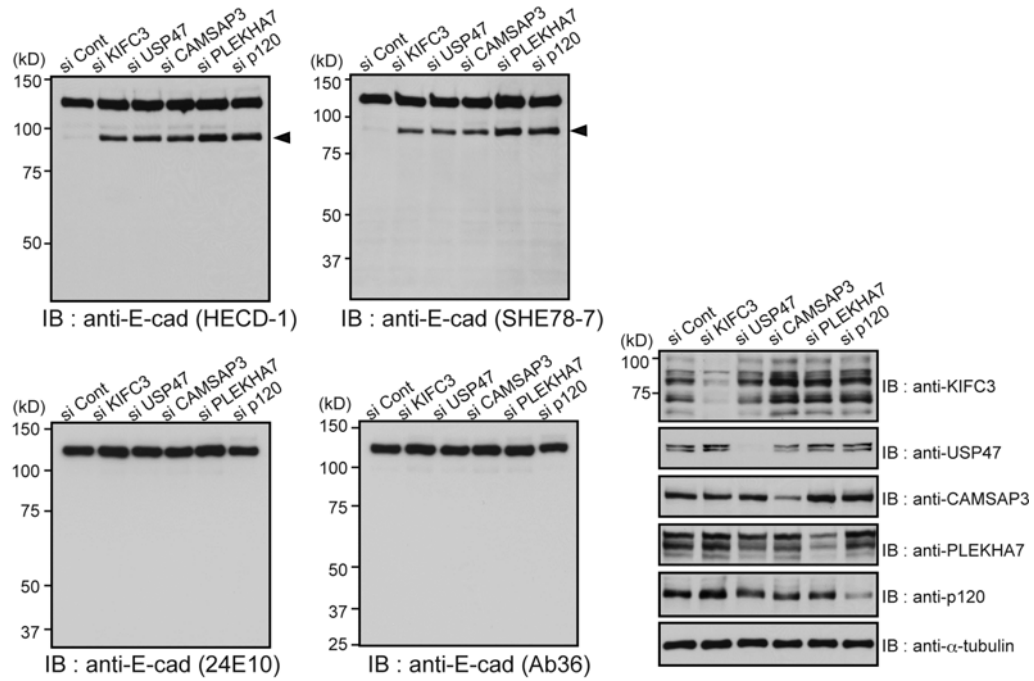
SUPPLEMENTAL FIGURE S1: (A) Mass spectrometry analysis to identify KIFC3-binding proteins. GST pull-down assay was performed with tail-KIFC3-GST protein using Caco-2 cell lysates. Proteins were separated by gel electrophoresis, and silver-stained. Among proteins specifically pulled down with tail-KIFC3-GST, a band was identified as USP47. (B) Treatments of Caco-2 cells with siRNAs specific for KIFC3 or USP47 reduce their immunostaining signals along cell junctions. Immunostaining signals were also reduced in the cytoplasm and nuclei, particularly in USP47-knocked down cells. All photographs were taken under an identical exposure condition. Scale bar, 50 μm . (C) Double-immunostaining for endogenous E-cadherin and exogenous KIFC3-EGFP in Caco-2 cells. Scale bar, 10 μm . (D) Mapping of various sites in human E-cadherin. Immunogenic regions for the antibodies SHE78-7, HECD-1, AbH-108, 24E10 and Ab36 are indicated by grey boxes. Cleavage sites for peptidases, ADMS, MPP, γ -Secretase, Caspase-3 and Calpain, are indicated by starbursts/arrows. LL motif, tyrosine phosphorylation sites, and triple glycine motif are involved in p120-catenin binding. Phosphorylation of the middle tyrosine in the tyrosine phosphorylation site is essential for HAKAI binding.

SUPPLEMENTAL FIGURE S2: Rescue experiments to confirm the specificity of siRNA effects. At 6 hr after transfection of Caco-2 cells with a human KIFC3-specific siRNA, a murine KIFC3-FLAG expression vector was introduced into the same cells (*top*). Similarly, a siRNA-resistant mutant of USP47-FLAG, c.1222_1224delTCTinsAGC, was introduced into cells transfected with a USP47-specific siRNA (*bottom*). At 24 hr after the first transfection, cells were double-immunostained with AbH-108 and anti-FLAG antibody. Cytoplasmic E-cadherin signals were quenched in FLAG-positive cells (large asterisks). The cell marked with small asterisk also expresses the mutant USP47-FLAG but in a lower level. Arrowhead indicates a non-specific stain. Scale bars, 50 μm .

SUPPLEMENTAL FIGURE S3: (A) Effects of various siRNAs on 90kD E-cadherin fragment production. (B) Detection of 90kD E-cadherin fragments with various antibodies. Antibodies reacting with the extracellular region of E-cadherin, HECD-1 and SHE78-7, detect the 90kD band, whereas those recognizing the intracellular region, 24E10 and Ab36, do not. (C) Effects of protein degradation inhibitors on 90-kD fragment production. Caco-2 cells were transfected with control or KIFC3-specific siRNA. At 5 hr after transfection, 0.5 μM L685,458, 2.5 μM GM6001 or 20 μM DEVD-CHO was added to culture medium. Calpain inhibitors, MDL-28170 and calpeptin, in varying concentrations were also added to culture medium. These reagents show no effects on 90kD E-cadherin fragment production.





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