**Supplemental Material**

# CKAP4 regulates cell migration via the interaction with and recycling of integrin

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Running title: CKAP4 regulates integrin recycling

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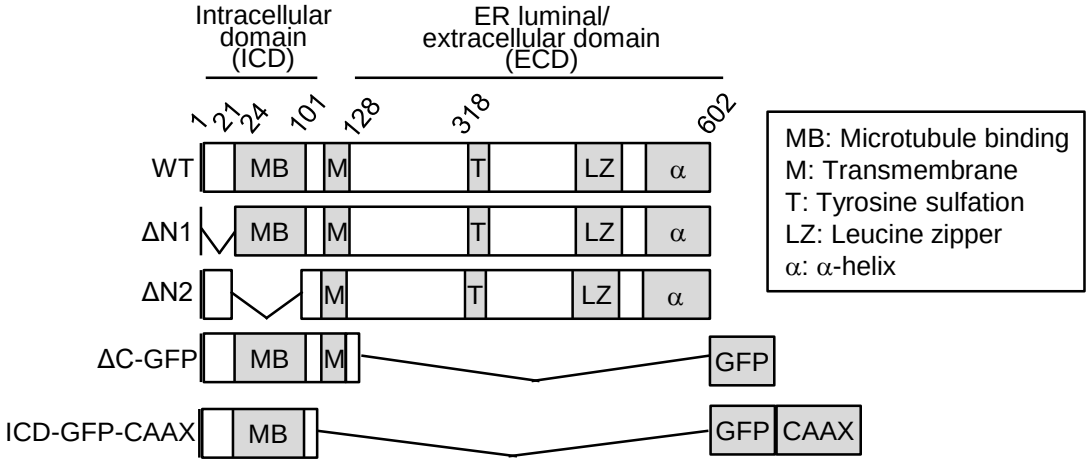
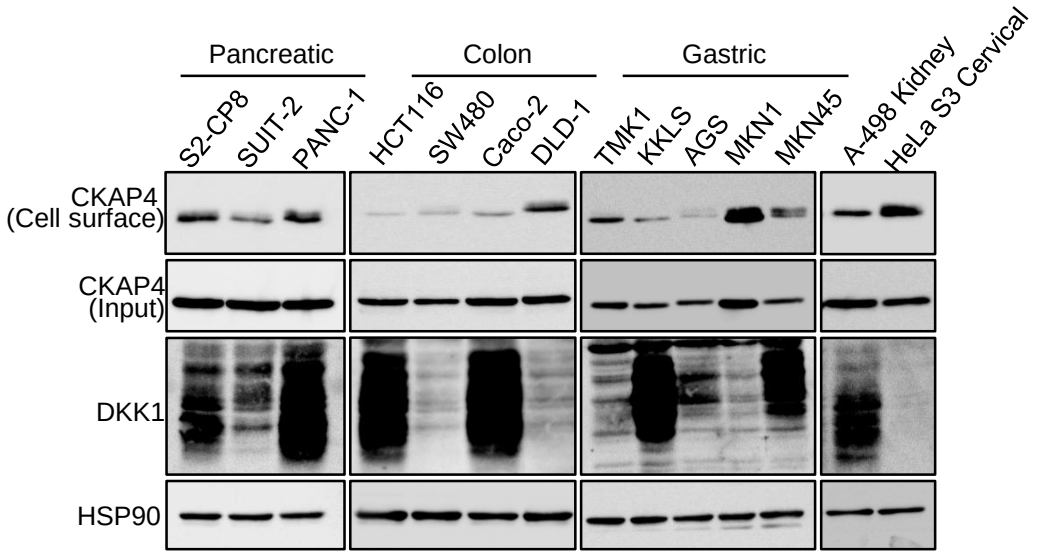
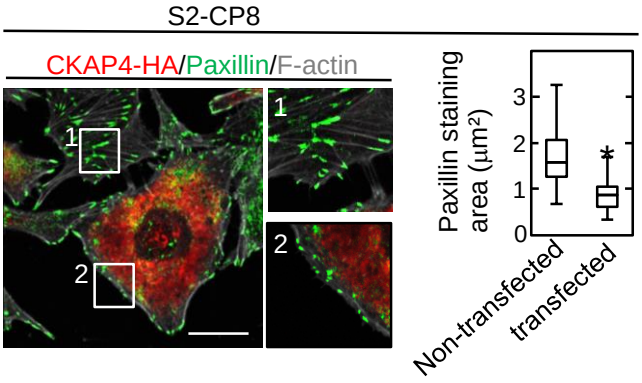
All authors have declared no conflicts of interest.




**Supplemental Figure S1. CKAP4-HA deletion mutant constructs.** Schematic representation of CKAP4-HA deletion mutants used in this study. WT, wild type.

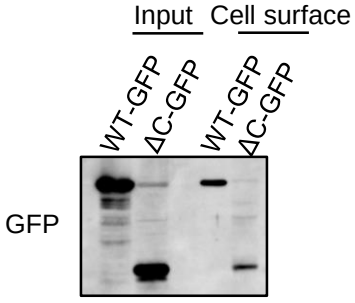



**Supplemental Figure S2. The levels of CKAP4 on the cell surface or DKK1.**
In various cancer cell lines, cell surface proteins were biotinylated and precipitated with NeutrAvidin agarose beads. The precipitates were probed with anti-CKAP4 antibody and total cell lysates (input) were probed with indicated antibodies. HSP90 was used as a loading control.

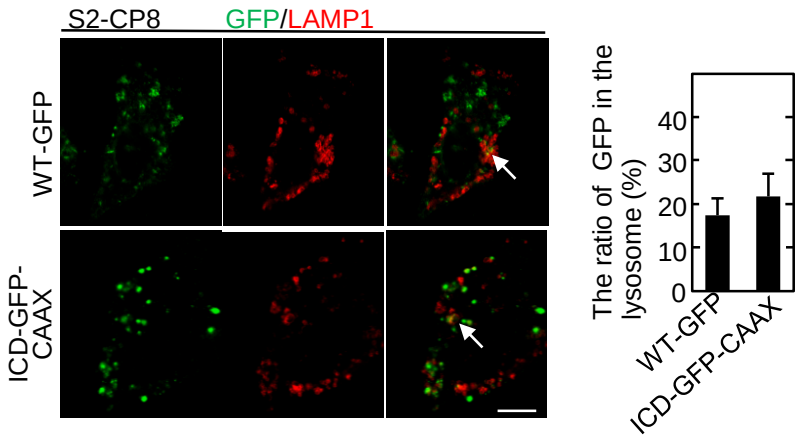



**Supplemental Figure S3. CKAP4 overexpression in S2-CP8 cells reduces cell adhesion sites.**

Left panels, CKAP4-HA was transiently overexpressed in S2-CP8 cells and the cells were stained with anti-HA and anti-paxillin antibodies and phalloidin. Boxed area 1 and 2 indicate paxillin-staining areas in a non-transfected and CKAP4-HA-transfected cells, respectively. The paxillin-staining area (green) in cells with (red) or without CAKP4 expression were measured. Right panel, box and whisker plot for the measured areas of individual paxillin positive regions. Scale bar, 20 μm. *, $p < 0.0001$.

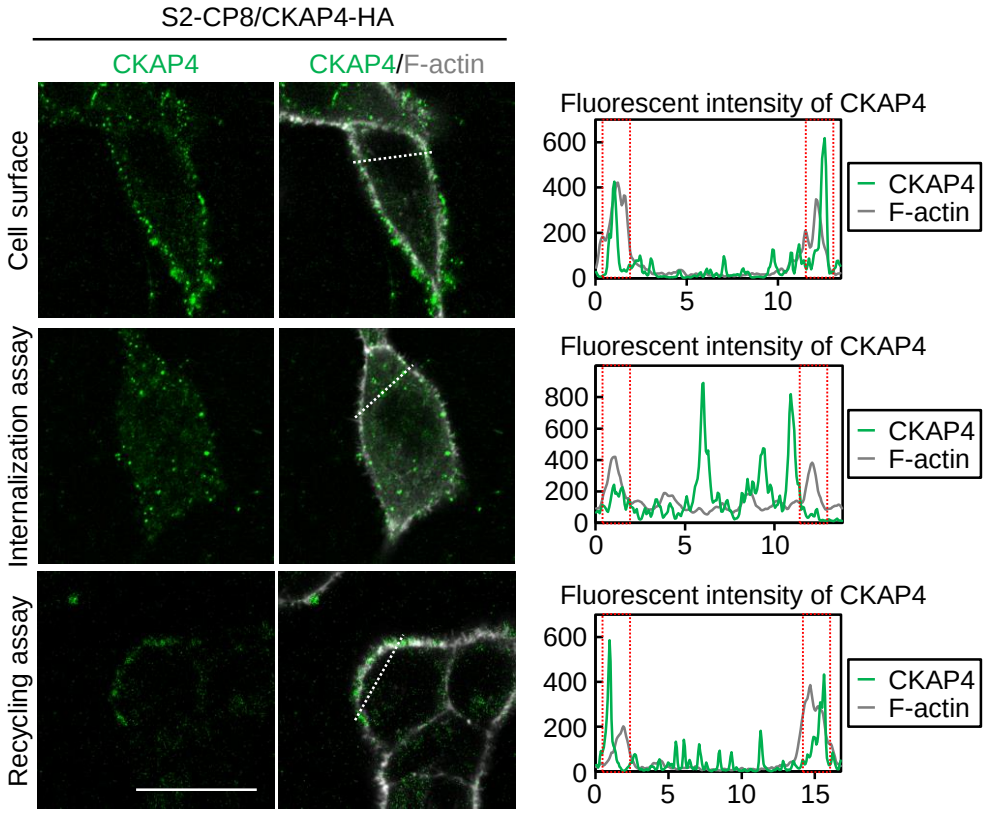



**Supplemental Figure S4. The levels of CKAP4 on the cell surface.**
In CKAP4 (WT-GFP or ΔC-GFP)-expressing HeLa S3 cells, cell surface proteins were biotinylated and precipitated with NeutrAvidin agarose beads. The precipitates were probed with anti-GFP antibody.



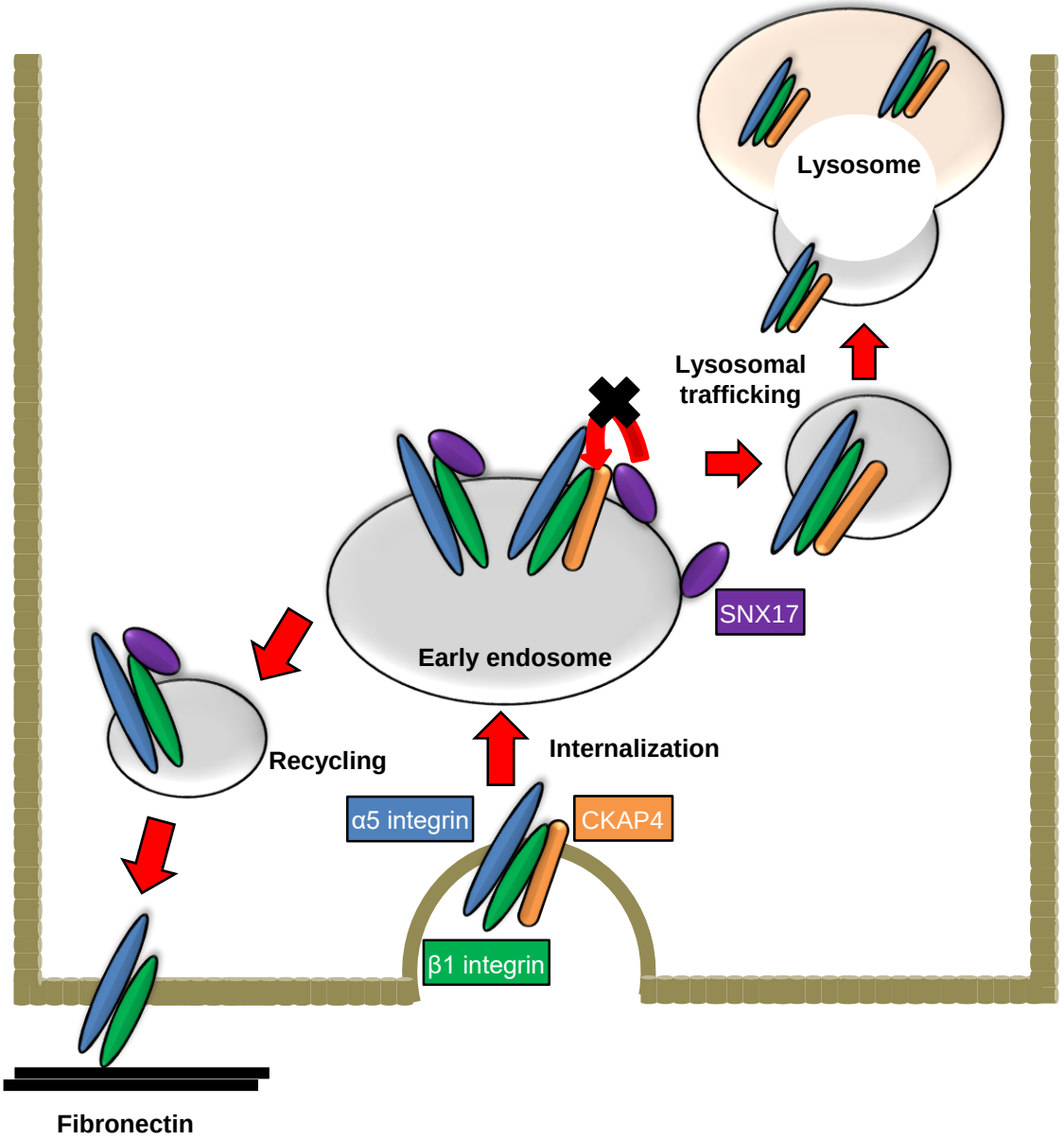

**Supplemental Figure S5. CKAP4 (ICD-GFP-CAAX) construct behaves as WT CKAP4 does.**

Left panels, CKAP4 construct (WT-GFP or ICD-GFP-CAAX)-expressing S2-CP8 cells were treated with bafilomycin. The cells were stained with anti-LAMP1 (red) and anti-GFP (green) antibodies. White arrows indicate CKAP4 colocalized with the LAMP1-positive lysosomes. Right panel, the ratio of GFP in the lysosome was quantified. Scale bar, 10 μm.




**Supplemental Figure S6. Cell surface localized CKAP4 is internalized and recycled.**

Upper panels, cell surface CKAP4 in S2-CP8/CKAP4-HA cells was labelled with anti-CKAP4 antibody in a non-permeabilized condition. Subsequently, the cells were subjected to the internalization (middle panels) and recycling (lower panels) assays. Cells were stained with a secondary antibody and phalloidin. Scale bar, 20 μm. Right panels, CKAP4 (green) and F-actin (gray) fluorescence intensities aligned with white dotted lines shown in left panels were measured and shown as arbitrary units. Red dashed line boxed areas indicate cell cortex. Scale bar, 20 μm.




**Supplemental Figure S7. Schematic illustration demonstrating how the cell surface CKAP4 regulates α5β1 integrin.**

CKAP4 in the cell surface membrane is associated with α5β1 integrin and internalized to the early endosome where SNX17 is localized and plays a role in the sorting of vesicles containing α5β1 integrin to the recycling endosome through the binding to β1 integrin. CKAP4 competes with SNX17 for the binding to β1 integrin; the vesicles containing α5β1 integrin complexed with CKAP4 are sorted to the lysosome, in contrast, those without CKAP4 are recycled to the plasma membrane. Thus, CKAP4 coordinates the balance of degradation and recycling of α5β1 integrin.

**Supplemental Table S1. List of antibodies used in this study.**

| Antibody | Distributor | Application (dilution ratio) |
|---|---|---|
| CKAP4 | Kimura et al, 2016[1]<br>Kimura et al, 2019[2] | IB (1:10000),<br>IF (1:600) |
| DKK1 | R&D Systems (Minneapolis, MN) | IB (1:1000) |
| Paxillin | BD Bioscience (San Jose CA) | IF (1:300) |
| HSP90 | BD Bioscience (San Jose CA) | IB (1:2000) |
| β-actin | Sigma-Aldrich (St Louis, MO) | IB (1:5000) |
| HA | Roche (Basel, Switzerland) | IF (1:600) |
| HA | MBL Life Science (Nagoya, Japan) | IP (1:50) |
| HA | Santa Cruz Biotechnology (Santa Cruz, CA) | IB (1:2000) |
| HA | Cell signaling Technology (Danvers, CA) | IF (1:400) |
| GFP | Aves Labs (Tigard, OR) | IF (1:300) |
| FLAG | Novus Biologicals (Centennial, CO) | IF (1:300) |
| LC3 | MBL Life Science (Nagoya, Japan) | IB (1:1000) |
| Cyclin A | BD Bioscience (San Jose CA) | IB (1:1000) |
| LAMP-1 | Santa Cruz Biotechnology (Santa Cruz, CA) | IF (1:300) |
| LAMP-1 | Sigma-Aldrich (St Louis, MO) | IF (1:300) |
| SNX17 | Santa Cruz Biotechnology (Santa Cruz, CA) | IB (1:1000) |
| β1 integrin (18/CD29) | BD Bioscience (San Jose CA) | IB (1:1000) |
| β1 integrin (EP1041Y) | Abcam (Cambridge, United Kingdom) | IB (1:1000) |
| β1 integrin (6S6) | Millipore (Burlington, MA) | IF (1:300) |
| β1 integrin (TS2/16) | Santa Cruz Biotechnology (Santa Cruz, CA) | IP (1:500) |
| β4 integrin | BD Bioscience (San Jose CA) | IB (1:1000) |
| α2 integrin | BD Bioscience (San Jose CA) | IB (1:1000) |
| αv integrin | BD Bioscience (San Jose CA) | IB (1:1000) |
| α6 integrin | Cell signaling Technology (Danvers, CA) | IB (1:1000) |
| α5 integrin | BD Bioscience (San Jose CA) | IB (1:1000) |
| FAK | BD Bioscience (San Jose CA) | IB (1:1000) |
| AKT | Cell signaling Technology (Danvers, CA) | IB (1:1000) |
| pAKT (T308) | Cell signaling Technology (Danvers, CA) | IB (1:1000) |

IB, Immunoblotting; IP, Immunoprecipitation; IF, Immunofluorescence

[1] Kimura H, Fumoto K, Shojima K, Nojima S, Osugi Y, Tomihara H, Eguchi H, Shintani Y, Endo H, Inoue M, Doki Y, Okumura M, Morii E, Kikuchi A. 2016. CKAP4 is a Dickkopf1 receptor and is involved in tumor progression. J Clin Invest 126:2689-2705

[2]Kimura H, Yamamoto H, Harada T, Fumoto K, Osugi Y, Sada R, Maehara N, Hikita H, Mori S, Egu H, Ikawa M, Takehara T, Kikuchi A. 2019. CKAP4, a DKK1 Receptor, Is a Biomarker in Exosomes Derived from Pancreatic Cancer and a Molecular Target for Therapy. Clin Cancer Res 25(6):1936-1947

**Supplemental Table S2. Target sequences for siRNAs used in this study**

| Gene | Sequence |
|---|---|
| Human *DKK1-1* | 5' - GGAATAAGTACCAGACCAT -3’ |
| Human *DKK1-2* | 5' - CCTGGAGTGTAAGAGCTTT -3’ |
| Human *CKAP4-1* | 5' - GCTTCTGCATTTGGTTGGT -3’ |
| Human *CKAP4-2* | 5' - GCAGATTAACCTCAGAAAT -3’ |
| Human *α5 integrin* | 5' - GCTACCTCTCCACAGATGA -3’ |
| Human *FN1* | 5' - GGACCTGCAAGCCCATAGC -3’ |
| Human *SNX17* | 5' - TGGTCAAACTCTCAAGTGA -3’ |
| Human *β1 integrin-1* | 5' - GCACCAGCCCATTTAGCTA -3 |
| Human *β1 integrin -2* | 5' - CCCACAACACTGAATGCGA -3 |