β-Catenin Serves as a Clutch between Low and High Intercellular E-Cadherin Bond Strengths

Saumendra Bajpai,[†‡△] Yunfeng Feng,[‡§△] Denis Wirtz,[†‡*] and Gregory D. Longmore[‡§*]

[†]Department of Chemical and Biomolecular Engineering and [‡]Johns Hopkins Physical Sciences - Oncology Center, The Johns Hopkins University, Baltimore, Maryland; and [§]Departments of Medicine and Cell Biology and Physiology and BRIGHT Institute, Washington University School of Medicine, St. Louis, Missouri

Captions of supplemental figures

Supplemental 1. Frequency of Ecad/Ecad binding determined by calcium-depletion as control.

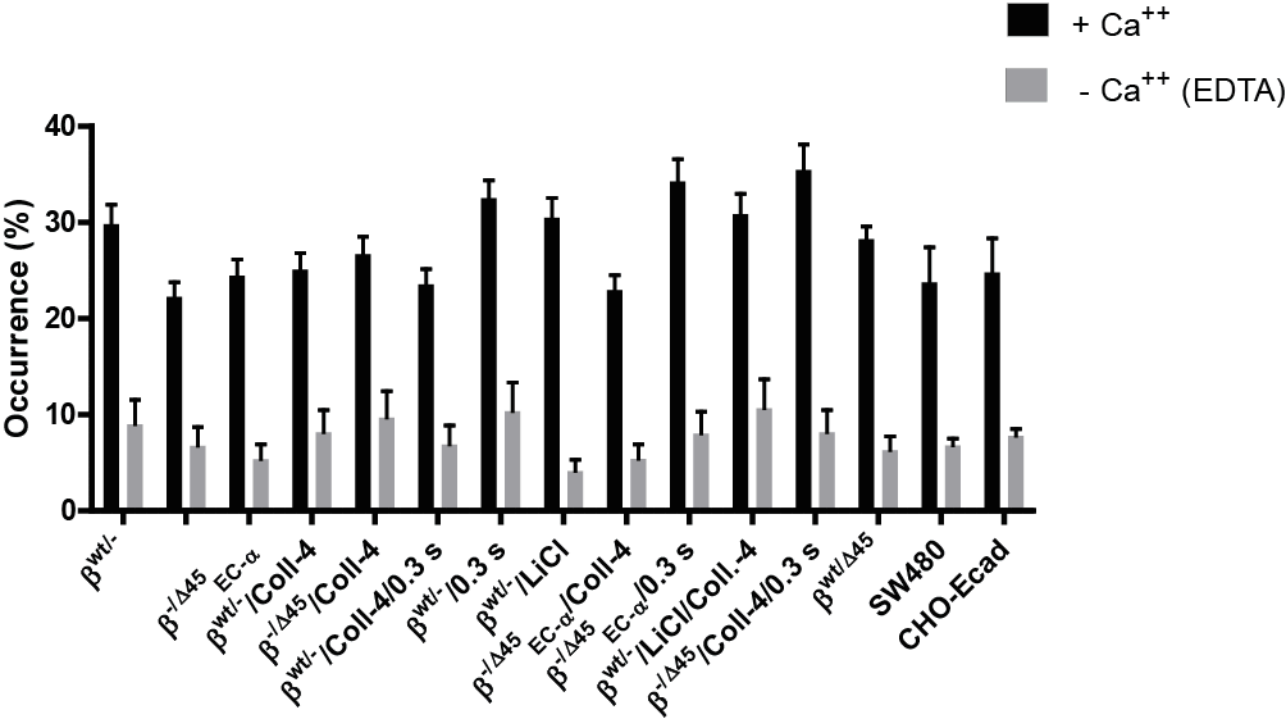
1 mM EGTA was used a chelating agent, to deplete Ca^{+2} from culture-media. EGTA was directly added to the cell-culture dish after changing to serum-free media and incubated for 30 min, before recording bond-rupture events. All retractions were measured at 10 μm/sec. Up to 5 independent experiments were conducted and n ~ 140 bond rupture events were recorded.

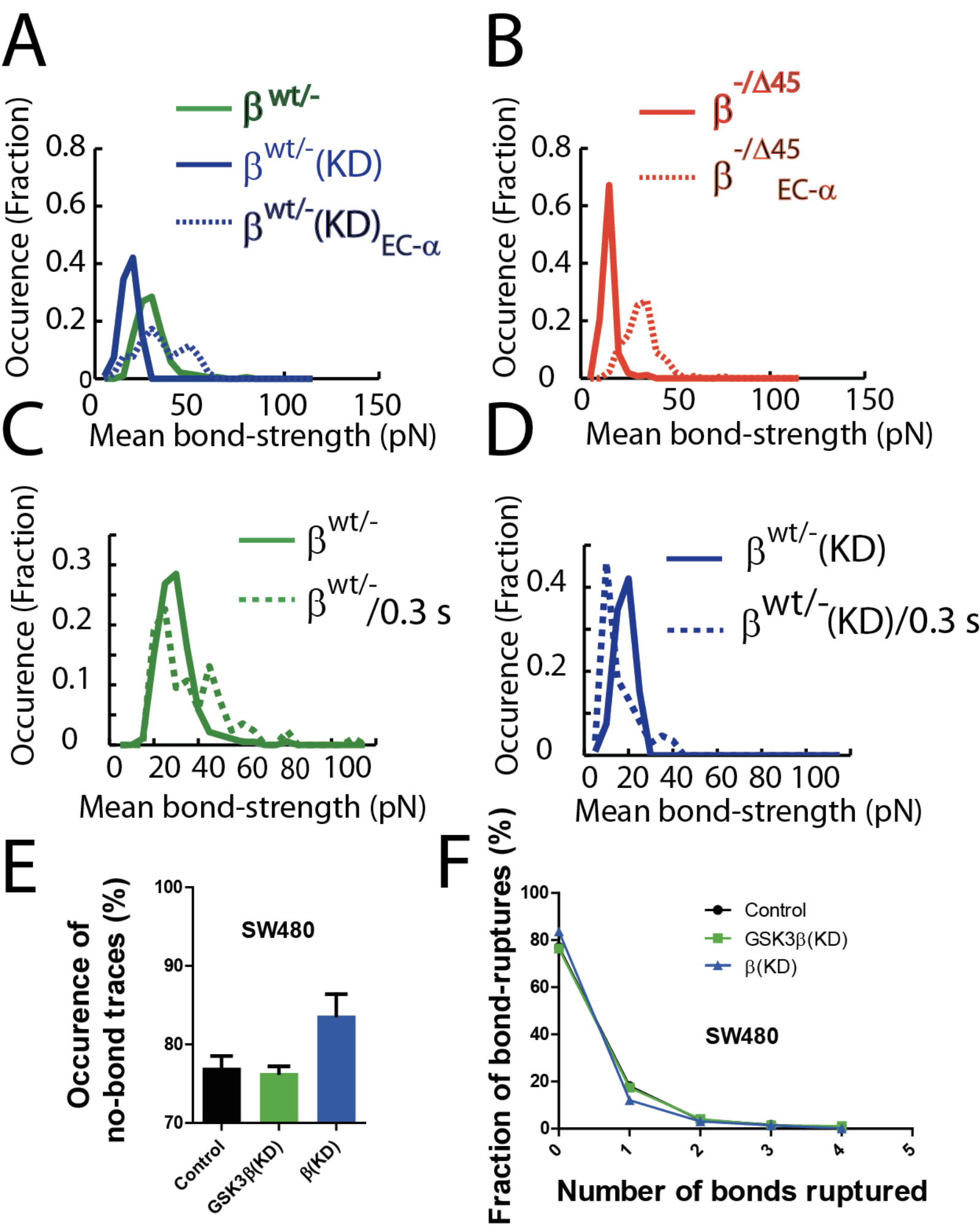
Supplemental 2. Occurrence of bond-ruptures of various strengths.

(A) Distribution of mean cell-cell bond strengths for cells expressing wt β-catenin, β-catenin knocked-down, and cells expressing a fusion protein. (B) Distribution of mean cell-cell bond strengths for cells expressing mut β-catenin and cells expressing the fusion protein. (C and D) Distributions of mean cell-cell bond strengths for short contact time (1 ms) and long contact time (300 ms) between cells expression wt β-catenin or with wt β-catenin knocked down.

Supplemental 3: Construction of HCT116 derived cell populations

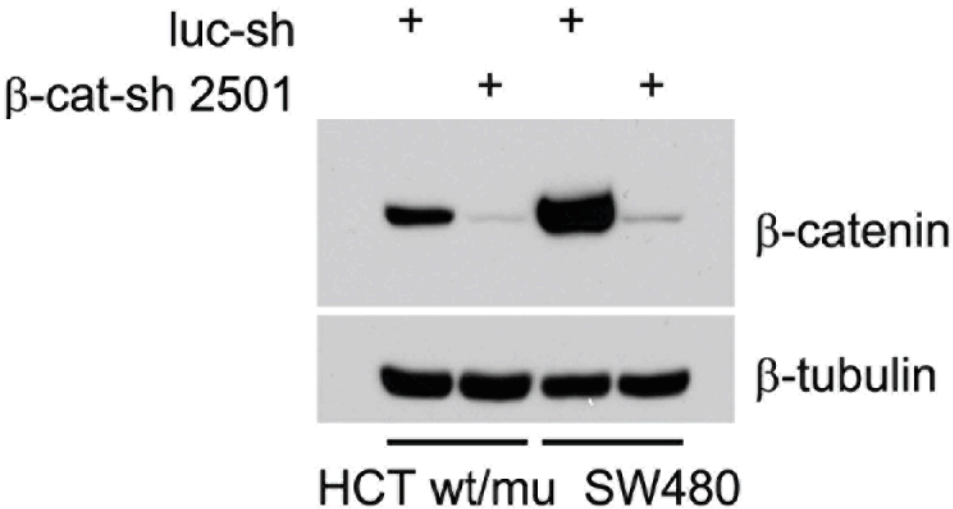
(A) Expression of E-cadherin-α-catenin fusion protein. HCT-/mu or wt/- were transduced with lentivirus that contains E-cadherin-α-catenin fusion expression cassette, cells were selected by puromycin. (B) Depletion of β-catenin in HCT wt/- that expressed E-cadherin-α-catenin fusion protein. Cells were transduced sequentially with the lentiviruses with different drug selection marker as indicated, cells were selected with puromycin and blasticidine. (C) Depletion of GSK3β in HCT116 derived cells. HCT wt/- and -/mu cells were transduced with lentiviruses that carried GSK3β shRNA expression cassette as indicated, selected with puromycin. (D) Depletion of α-catenin in HCT wt/- and -/mu that expressed E-cadherin-α-catenin fusion protein. Cells were transduced sequentially with the lentiviruses with different drug selection marker as indicated, cells were selected with puromycin and blasticidine. Western-blots were performed using antibodies, as indicated.


+ Ca++
- Ca++ (EDTA)
Occurrence (%)
40
30
20
10
0
βwt/-
β-/Δ45
EC-α
βwt/-/ColII-4
β-/Δ45/ColII-4
βwt/-/ColII-4/0.3 s
βwt/-/0.3 s
βwt/-/LiCl
β-/Δ45
EC-α/ColII-4
β-/Δ45
EC-α/0.3 s
βwt/-/LiCl/ColII.-4
β-/Δ45/ColII-4/0.3 s
βwt/Δ45
SW480
CHO-Ecad

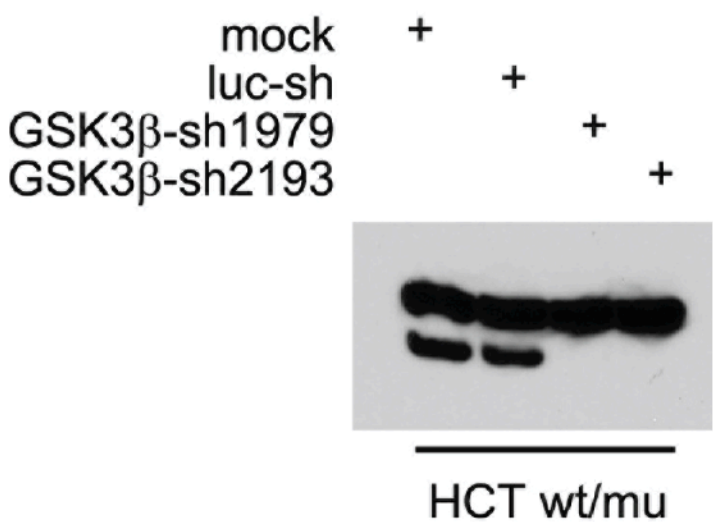


Supplemental Figure S2

A


luc-sh
β-cat-sh 2501
+
+
+
+
β-catenin
β-tubulin
HCT wt/mu
SW480

B


mock
luc-sh
GSK3β-sh1979
GSK3β-sh2193
+
+
+
+
HCT wt/mu