

Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: A movie of constant-force SMD simulations of α Ncat-ABD-WT. Cartoon representation shows that the α 1-helix (blue) starts to unfold at \sim 60 ns

File Name: Supplementary Movie 2

Description: A close-up view of constant-force SMD simulations of α Ncat-ABD-WT. V796_N flips out from the interior pocket (red and blue surface) shortly before the α 1-helix (blue surface and ribbon) unfolds and pulls away from the pocket at \sim 60 ns.

File Name: Supplementary Movie 3

Description: Wide-field fluorescence spinning-disk live-cell microscopy of α EcatFL-mGFP (WT) or α Ecat-H1-mGFP (H1) expressing R2/7 cells. Cells stably expressing α cat-mGFP lines were infected with LifeAct Ruby, resulting in some cells not displaying the Ruby signal. Cells were plated on glass-bottomed dish coverslips \sim 48hr before imaging, and scratch-wounds were generated 18 hours before imaging. Time-lapse sequences were acquired at 30 s intervals for both GFP and Life-Act-Ruby channels. Cell line, time-stamp and scale bars (21 μ m) are burned-into the movie sequence. A six-panel composite of grayscale inverted and color overlay movies are shown.

File Name: Supplementary Movie 4

Description: Phase contrast live imaging of scratch wounded R2/7 cell monolayers. A four-panel composite of image sequences is shown for α EcatFL (WT), α Ecat-H1 (H1), α EcatFL- $\Delta\beta$ H (WT $\Delta\beta$) and α Ecat-H1 $\Delta\beta$ H (H1 $\Delta\beta$)-expressing cells. Time-lapse sequences were acquired at 10-min intervals for 15 hours.