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Supporting Material

A Molecular Dynamics Investigation of Vinculin Activation

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Figure S1. Conformational changes in Vinculin.
During simulation of vinculin activation with external force electrostatic interactions between Vt and D1 are sequentially broken. As the salt bridges break vinculin advances in its conformational changes. (A) VMD (44) rendered image of Vt interacting with D1 via the three sets of salt bridges. Vt is shown in orange, linker region in yellow, Vh in green, and VBS in black. (B) The first salt bridge to break during simulation is bridge 2, the interaction between E14 (blue-green) and K996 (dark purple). (C) Salt bridge 2 is the second bridge to break. The interaction between E14 (blue-green) and K996 (dark purple) is broken at around 520 ps into the simulation. (D) The interaction between E29 (blue) and R1008 (green) is the last interaction to break; afterwards the D1 domain is pulled apart from Vt rapidly until it is in an activated conformation.

Figure S2. Other possible mechanisms of vinculin activation explored.
Using molecular dynamics two other possible mechanisms of vinculin activation can be explored. (A) It could be possible that vinculin activation involves the complete separation of Vt from Vh. To simulate such a separation using molecular dynamics for comparison to simulation of vinculin stretching a constant force was applied to the four residues nearest the center of mass of Vt away from the four residues nearest to the center of mass of Vh. Direction of force is shown in black arrow. Simulation with 100 pN over 1 ns was unable to induce significant conformational changes in this direction. (B) Another direction in which Vt could move if vinculin activation involved complete separation of Vt from Vh would be in a direction away from domain 3. The direction force was applied in molecular dynamics simulation for simulating this movement is indicated by a black arrow on a 90° rotated view of vinculin. Simulation in this direction also failed to achieve activation.

Figure S3. Normal mode analysis of vinculin.
Using WEBnm@ (61) and ElNemo (37) normal mode analysis was carried out on vinculin. Normal mode analysis shows regions of vinculin that are flexible due to the general structure of vinculin. (A) The results show the lowest frequency and therefore the most likely vibrational movement in vinculin involves the movement of its flexible linker region (movement shown with black arrows). This proline-rich linker region is so flexible its crystal structure has yet to be fully defined. (B) Other movement in the lowest frequency normal modes all involve movement of linker regions not near Vt. Shown here is the movement of the linker region in D4in normal mode 8, which is representative of the movements of linker regions not near Vt.

Video S1. Electrostatic interactions between Vt and D1 govern vinculin activation.

During simulation of vinculin activation with external force electrostatic interactions between Vt and D1 are sequentially broken. Vt interacts with D1 via the three sets of salt bridges. Salt bridge 1: E29-E31, R945-R1008, Salt bridge 2: E14-K996, Bridge 3, R7-E986. The first salt bridge to break during simulation is bridge 3. Results shown are from Vinculin-VBS being simulated under a constant external force of 125pN. Salt bridge 2 is the second bridge to break at around 260ps. Salt-bridge 1 is the last interaction to break. Each breakage corresponds to an increase in distance between the actin binding region, Vt, and the VBS binding region.

Figure S1

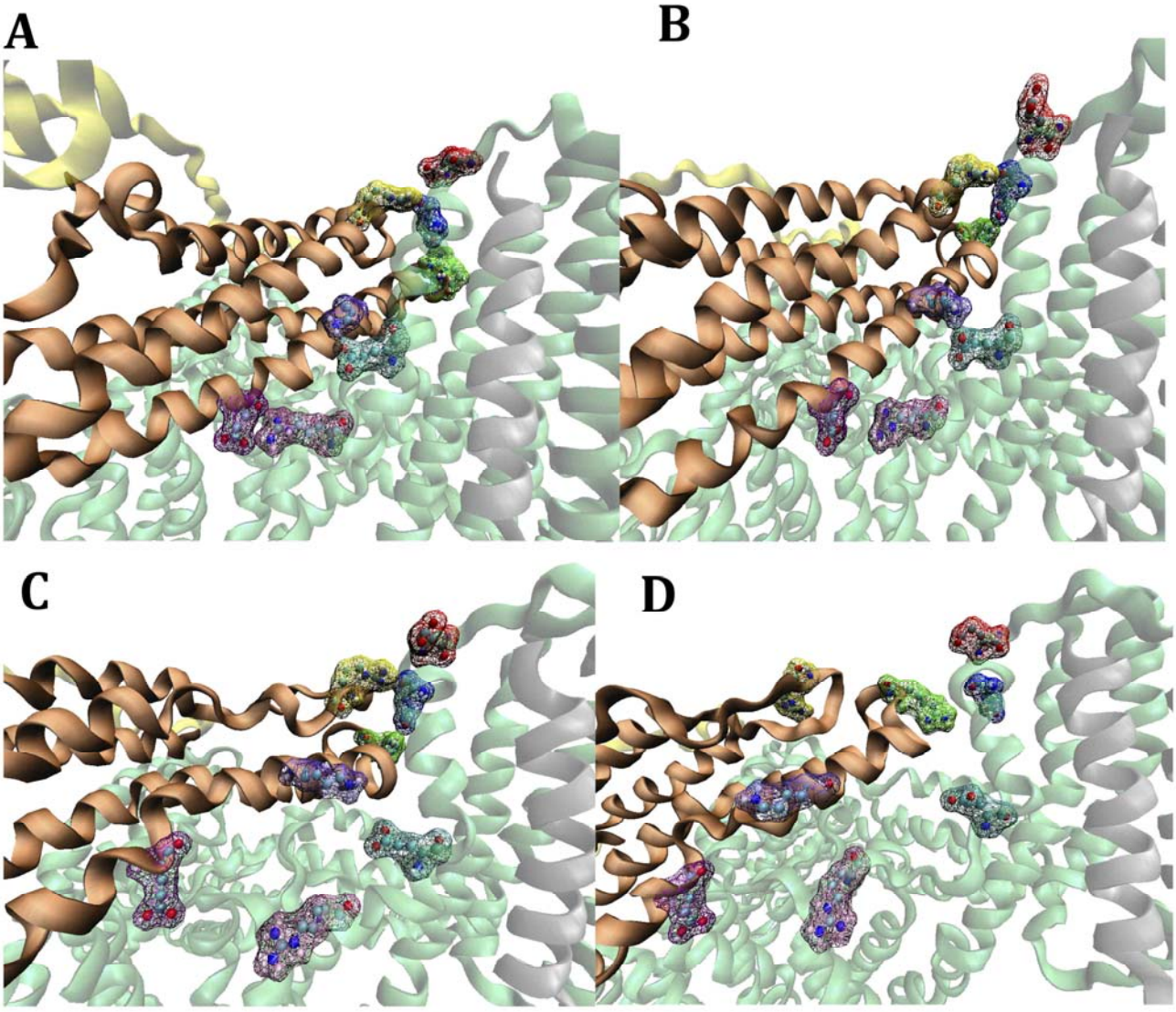

Figure S2

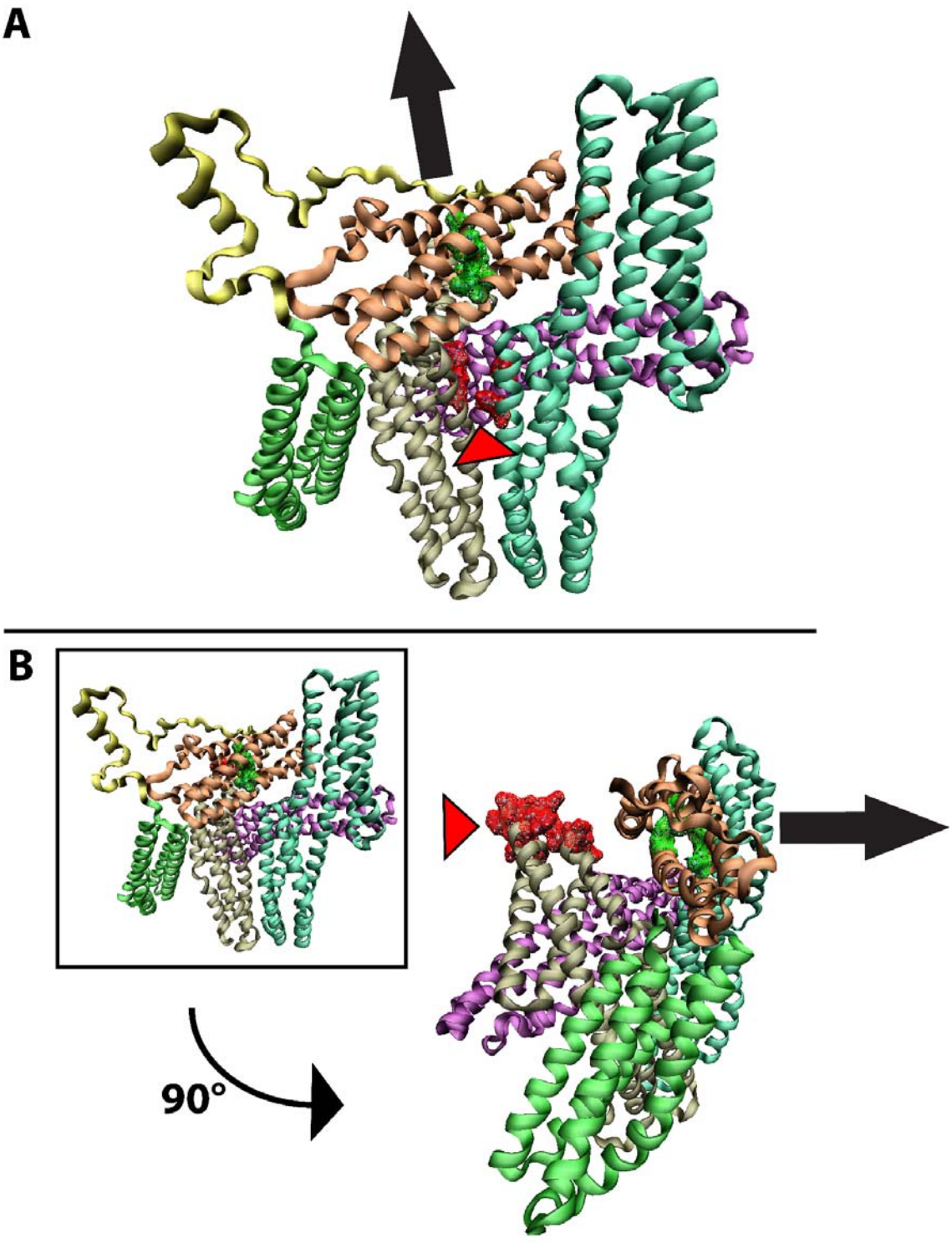

Figure S3