Optogenetic control of vinculin tail interactions to probe durotaxis

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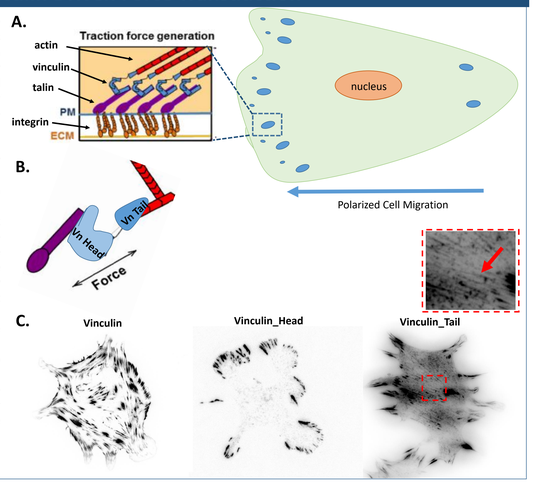
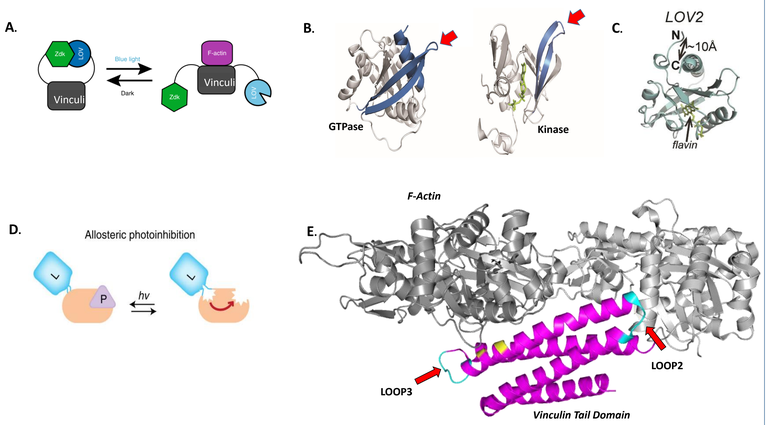
Vinculin Driven Force Transduction

Vinculin provides a mechanical link to the actin cytoskeleton at focal adhesions. **A**. Cancer cells migrate towards increasing stiffness through a process known as durotaxis[1]. Cells sense their environment through focal adhesions. Focal adhesions consists of clusters of transmembrane integrin proteins and a wide range of intracellular proteins, including talin and vinculin. Talin links integrin to actin filaments, and this linkage is stabilized by vinculin. **B**. Vinculin consists of an N-terminal globular head domain that binds to talin and a C-term helical tail domain that binds to actin. These domains are linked by a proline rich neck region[2]. **We predict that by inserting a photo-responsive LOV2 domain in the vinculin tail we will be able to control vinculin-driven force transduction in a migrating cell, thereby probing vinculin's role in durotaxis**. **C**. Transient expression of NeonGreen vinculin reveals focal adhesions. Expressing the head domain alone shows strong localization at focal adhesions. Expressing the vinculin tail shows localization to the focal adhesions and to the actin cytoskeleton (red arrow in closeup). Here, we test light-dependent localization of our optogenetic vinculin tail analogs to gauge the photosensitivity of vinculin binding to actin and focal adhesions

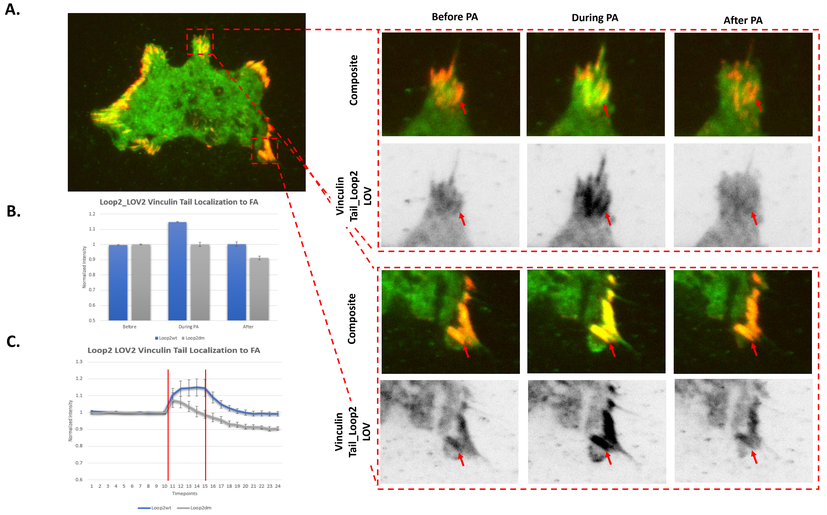


Optogenetic Control with Loopology and ZLock

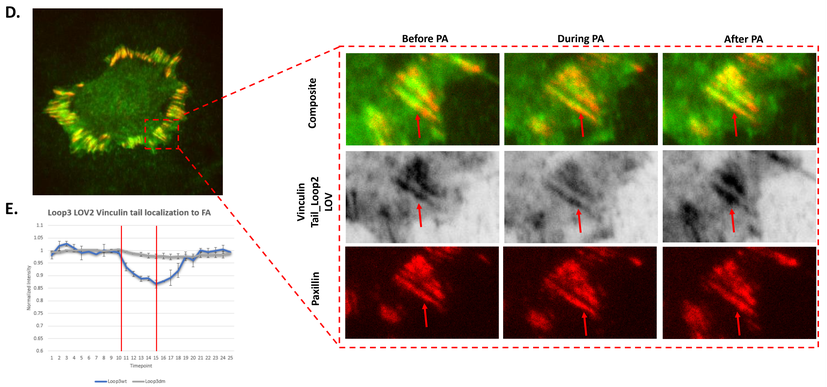


A. Design of Z-lock vinculin tail. Zdk2, an engineered protein that binds only to the dark conformation of LOV2, is inserted at the N-term of the vinculin linker region. LOV2 is inserted in the Loop2 region of the vinculin tail. Only in the dark, LOV2 binding to Zdk blocks vinculin binding to F-actin. **B**. In "Loopology", another application of the LOV domain[3], we insert LOV2 in surface loops of well folded secondary structures (here a GTPase and Kinase). Altering the conformation of the inserted LOV with light allosterically affects distant portions of the protein (eg the active site). **C**. LOV2 domain crystal structure. The close proximity of the N and C termini allows for successful insertion into surface loops. **D**. LOV2 is inserted into vinculin to allosterically perturb specific binding interactions when illuminated. **E**. Crystal structure of Vinculin-tail domain bound to F-actin. Red arrows indicate insertion sites tested for allosteric control of vinculin binding. I996 and V1001 (yellow residues) are known point mutations that inhibit F-actin binding.

Insertion of LOV2 in Loop2 results in Photoactivatable Vinculin Tail

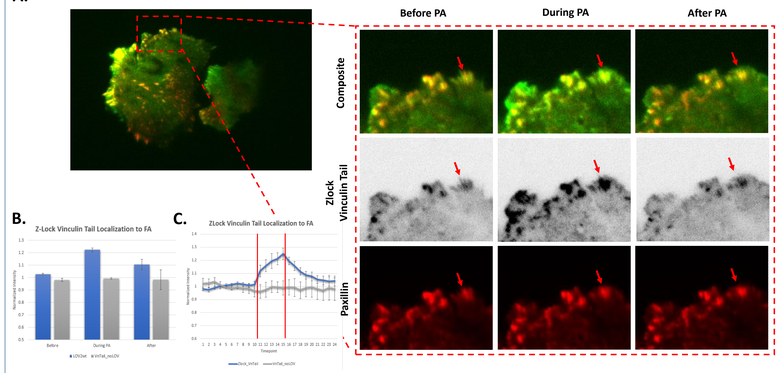


Insertion of LOV2 in Loop3 results in a Photoinhibitable Vinculin Tail

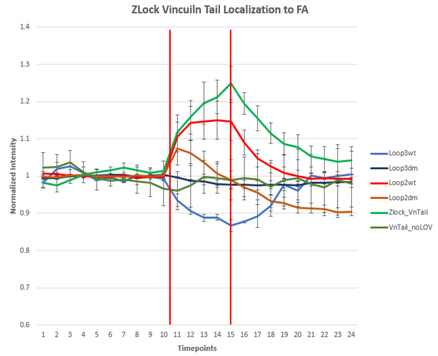


A. mCherry_Vinculin tail with LOV2 in Loop2 was transiently expressed in Mouse Embryonic Fibroblasts (MEFs) along with Halo_Paxillin labeled with Janelia 646 ligand and imaged using TIRF microscopy. Images were collected every 15 seconds for 25 timepoints. At timepoint 11, a 488nm laser was turned on for 5 time points (5 second duration each) and turned off at timepoint 16. Arrows indicate focal adhesions that were affected by photoactivation. **B**. Quantification of vinculin tail construct intensity at paxillin focal adhesions. A focal adhesion mask was created using fliuorescent paxillin as an adhesion marker, and vinculin intensity colocalizaing with paxillin was monitored. Bar graphs show average vinculin intensity before, during, and after irradiation. **C**. Vinculin tail intensity over time. Red lines indicate when light is on. (Data for **B** and **C:** 7 cells wt, and 4 cells LOV dark mutant). **D**. mCherry_Vincuiln tail with LOV2 in Loop3 expressed in MEFs and imaged as above. **E**. Quantification of mean vinculin intensity. Data represents n=3 cells for Loop3_LOVwt and n=4 for Loop3_LOV dark mutant control.

Zlock vinculin tail localizes to FA during photoactivation



Zlock vinculin tail increases localization to focal adhesions upon photoactivation. **A**. Snap_vinculin_Zlock tail construct was transiently expressed in MEFs along with Halo_paxillin. These were labeled with Janelia646 Snap ligand and Janelia549 Halo ligand respectively. Cells were imaged as described in the previous figures. **B** and **C**. Quantification of mean vinculin_tail_Zlock intensity at paxillin focal adhesions before, during and after photoactivation. Data from n=5 and n=3 cells for LOVwt and vinculin tail control respectively. **D**. Overlay of all three experiments. Data between red lines represents photoactivation timepoints.



Future Directions

- Optimizing photo-reactivity by testing linker lengths and Zdk mutants of varying affinity.
- Generating a full length vinculin with light-sensitive actin binding.
- Using traction force microscopy to study effects of vinculin modulation on extracellular force generation.
- Elucidate the role that vinculin-driven tension at focal adhesion plays in cell polarity and durotaxis.

References

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2. Bays, J. L. & DeMali, K. A. Vinculin in cell–cell and cell–matrix adhesions. *Cellular and Molecular Life Sciences* **74**, 2999–3009 (2017).
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