Optogenetic construction of de novo integrin-adhesion complexes reveals role for biocondensation in adhesion nucleation

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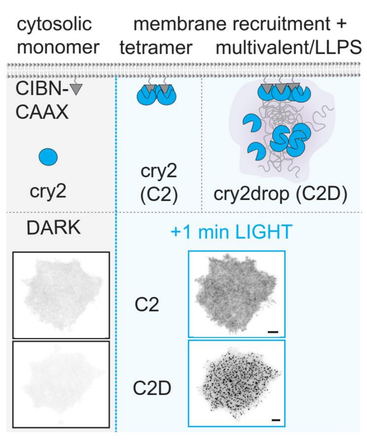
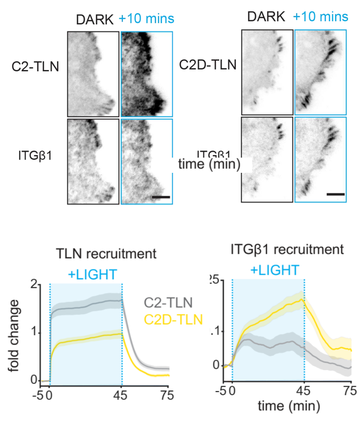
Optogenetic membrane droplets

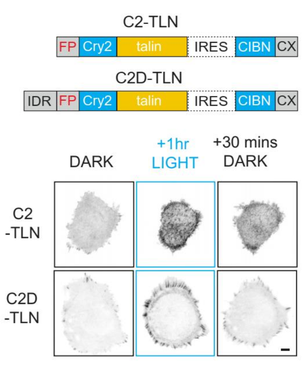


- Cry2 simultaneously undergoes tetramerization and binding to CIBN in blue light.
- Fusion of an IDR to Cry2 causes it to instead form liquid-liquid phase separated (LLPS) membrane droplets
- These will revert to the dark state within minutes

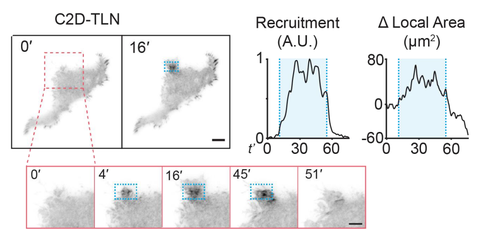
IDRs promote integrin clustering and recruitment



Light-mediated talin self association drives focal adhesion formation

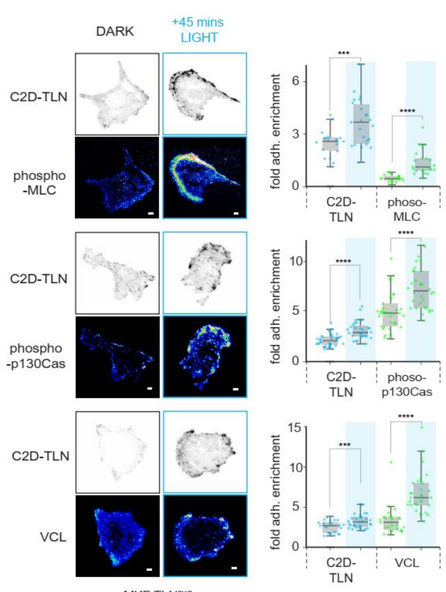


Local clustering of talin induces IAC formation

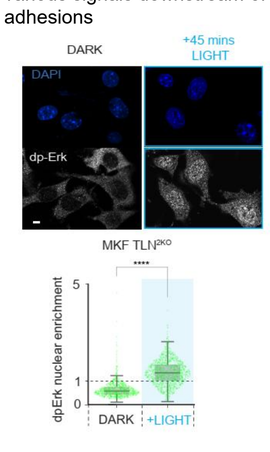


- Increased talin self-association leads to integrin-adhesion complex (IAC) formation and integrin clustering
- IACs are only formed on the edge of cells

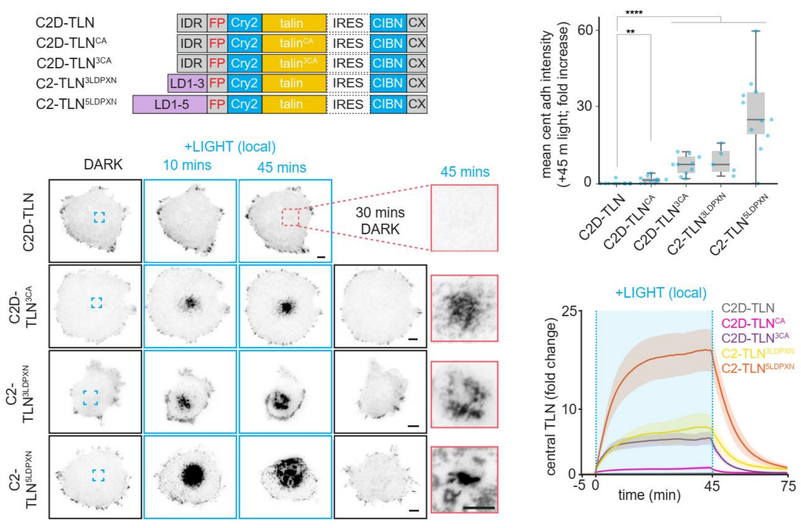
Optogenetic talin enable cell signaling



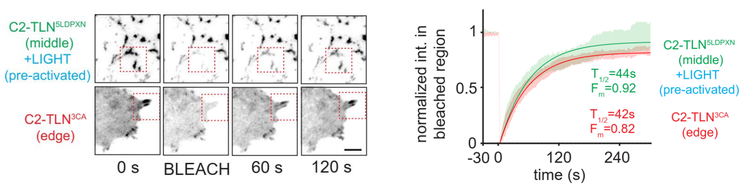
- C2D-Talin rescues TLN1/2 KO MEFs
- C2D-Talin activation increases various signals downstream of adhesions



Talin phase separation enables IACs

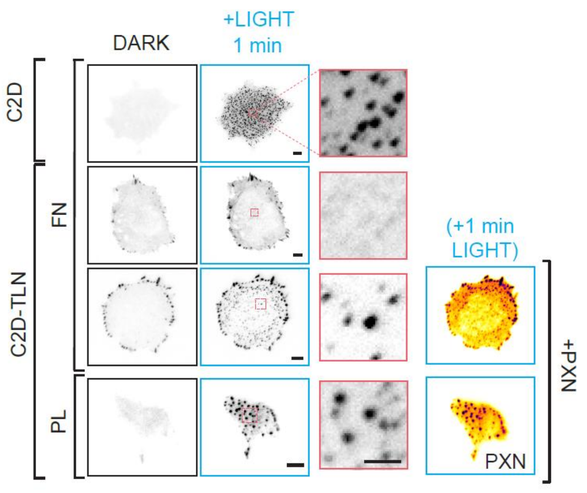


- Mutations which break talin autoinhibition and unfolding (CA [M319A] / 3CA [M319A, T1767L, & E1770K] allow for central IAC formation in the light. Stabilization of unfolded talin through expression of a deregulated vinculin yields similar results.
- Fusion of paxillin LD domains to talin also allows it to form central IACs, with the 5LD fusion leading to dramatic phase separation



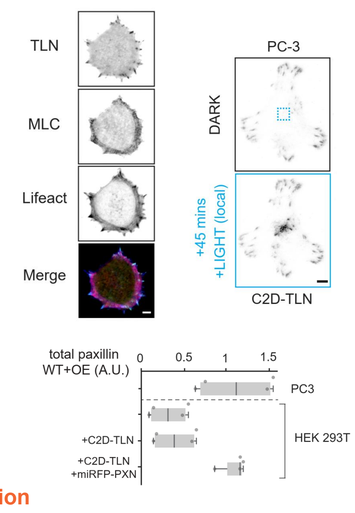
Paxillin expression enables biocondensation of talin and central IAC formation

Paxillin expression enables central talin recruitment and IAC formation

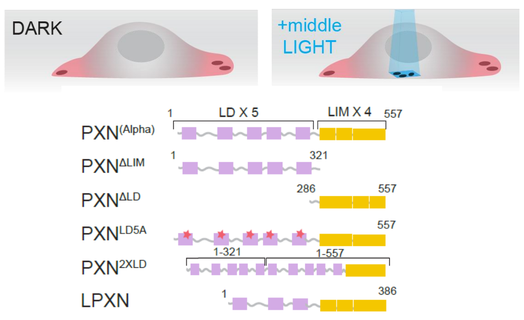


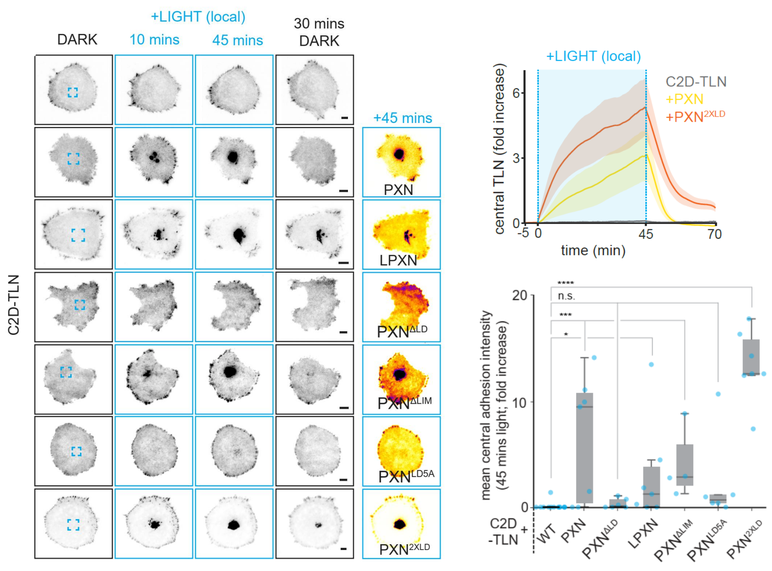
- 293Ts lack IACs and actomyosin in the center of cells
- 293Ts have minimal expression of paxillin and its isoforms
- Paxillin expression enables central IAC formation in response to light activation
- Paxillin is recruited with talin immediately, and even without ECM engagement
- Talin droplets show rounded morphology and fusion in the absence of ECM

293T cells lack central actomyosin and significant paxillin expression



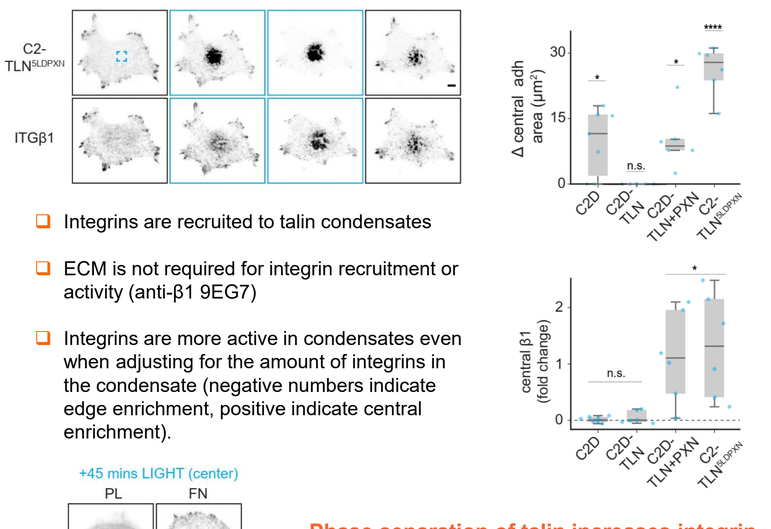
LD Domains of paxillin are sufficient for talin LLPS/central FA nucleation



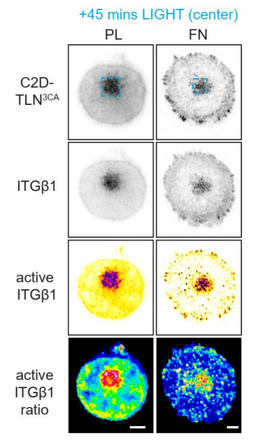


- Paxillin LD domains have been shown to allow for paxillin phase separation
- Paxillin LD domains are co-recruited with talin producing phase separated aggerates
- Intact FAK binding sites seem to be required

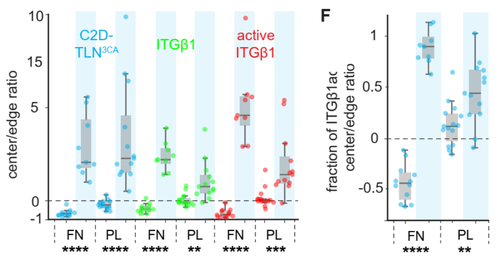
Talin LLPS activates and partitions integrins

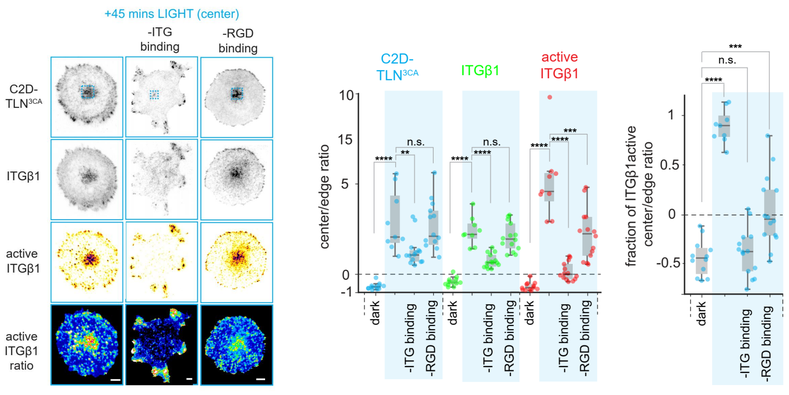


- Integrins are recruited to talin condensates
- ECM is not required for integrin recruitment or activity (anti-β1 9EG7)
- Integrins are more active in condensates even when adjusting for the amount of integrins in the condensate (negative numbers indicate edge enrichment, positive indicate central enrichment).

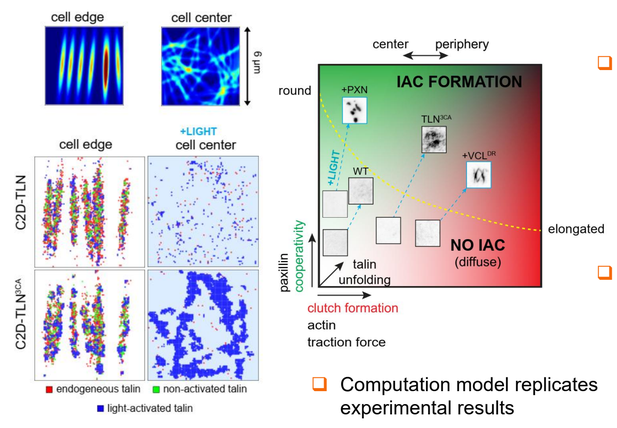


Phase separation of talin increases integrin activation independently of ECM



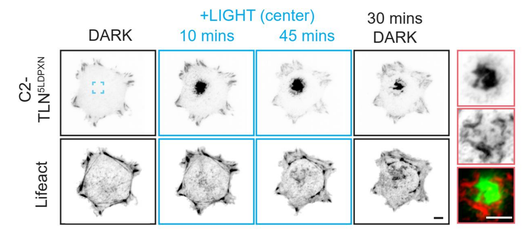


Model of IAC nucleation and stabilization

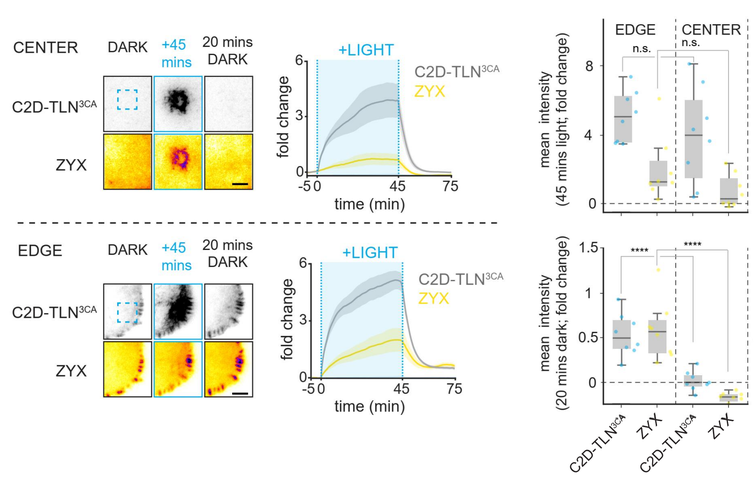


- Edge traction force enhances clutch formation, talin unfolding, and thus stabilizes and enables IAC formation
- Clutch formation and cooperativity work together to set the phase separation threshold
- Computation model replicates experimental results

Actomyosin engagement stabilizes IACs



- Central IACs can recruit actin slowly
- A fraction talin and zyxin recruited to the edge, but not the center of cells remains stable after dark recovery



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