Figures and figure supplements

Discrete spatial organization of TGFβ receptors couples receptor multimerization and signaling to cellular tension

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Figure 1. Spatial segregation of TβRII from TβRI. Spinning disc confocal imaging of endogenous TβRII (A, Figure 1—figure supplement 1) demonstrates punctate staining. Imaging of mEmerald-labeled TβRII (B) reveals TβRII-absent domains in ATDC5 (B,C) and NIH3T3 (D) cells expressing mEmerald-TβRII. Switching from widefield (E) to TIRF mode imaging (F) on the same cell unveils a specific spatial organization of TβRII, which is discrete from that of TβRI (Alk5 and Alk1) (G,H). ATDC5 cells co-expressing mEmerald-TβRII and mCherry-TβRI (Alk1) reveal that TβRII surrounds specific domains of TβRI (I-L). Quantitative profile plot of expression intensity demonstrates separate and distinct localization patterns of TβRI and TβRII (L).

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Figure 1—figure supplement 1. Endogenous staining of TβRII insufficient for spatial organization visualization. Wildtype ATDC5 cells (A-C) and ATDC5 cells expressing TβRII-mCherry were stained with two different anti-TβRII (A,D and B,E) antibodies and an IgG control (C,F). Staining of TβRII between wildtype and transfected cells differs only in intensity and not structurally, indicating that the observed spatial organization is not due to expression constructs alone. Although TβRII antibodies showed specificity relative to IgG staining, this bright punctate staining is insufficient for visualizing fine structural organization.

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Figure 2. Limited TβRI (Alk5) and TβRII mobility in areas of receptor spatial organization. All mEos2-tagged TβRI and TβRII sptPALM single molecule trajectories with durations of at least 5 frames (500 ms) are plotted for representative ATDC5 cells, in which each color represents a different track (A,B). Cellular domains outside the imaging plane appear black. The histogram represents the distribution within a single cell of trajectory durations for individual TβRI and TβRII molecules (C). Representative individual TβRI sptPALM single molecule trajectories exhibiting immobile (red), confined (green), and freely diffusive (blue) movement are plotted in (D), with calculated mean squared displacement (MSD) plots for each population of TβRI and TβRII shown in E (mean ± SEM). Comparison of diffusion coefficients for TβRI and TβRII (F, mean ± SEM) in whole cells relative to areas of segregated TβRI/TβRII identify a less mobile population of TGFβ receptors in these regions of interest (ROI). See Source code 1 and Figure 2—source data 1.

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The following source data is available for figure 2:

Source data 1. sptPALM single molecule trajectories
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Figure 3. Focal adhesions sequester TβRII from TβRI. TIRF mode imaging and a custom colocalization analysis were used to evaluate localization of TβRII (A), Alk5 (B), or Alk1 (C) with integrin α2 in ATDC5 cells. TβRII surrounds integrin α2 (A), whereas both subtypes of TβRI, Alk5 (B) and Alk1 (C), are included within integrin-rich focal adhesions, as reflected by profile plots and the slope values of the regression lines (A,B,C). Quantification of colocalization reveals that Alk5 and Alk1 are significantly more colocalized with integrin α2 relative to TβRII (**p < 0.001, mean ± SD, D, Figure 3—source data 1). This organization is also present in ATDC5 cells when the fluorescent labels for TβRII and integrin α2 have been switched (E), in osteosarcoma Saos-2 cells (F), or in epithelial MCF10A cells (G), when labeling focal adhesions with integrin αV (G), and when TβRII is expressed and imaged alone (H). TβRII spatial organization is unaffected by addition of TGFβ, indicated by red outlines in the same cellular region following 15 min of TGFβ treatment (I). See Source code 2.

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The following source data is available for figure 3:

**Source data 1.** Colocalization Index

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Figure 3—figure supplement 1. Focal adhesion formation and TßRII spatial organization are dependent on integrin activity. TßRII spatial organization (A,B,E,F) and focal adhesion formation (C,D,G,H) are absent on poly-l-lysine (B,D,F,H) coated glass substrates relative to collagen II (A,C,E,G). Spinning disc confocal microscopy at 40x (A-D) and TIRF microscopy at 100x (E–H) of ATDC5 cells expressing TßRII-mEmerald and integrin α2-mCherry demonstrate a loss of TßRII depleted regions (A,B), TßRII peripheral ring formations (E,F), and integrin α2 developed adhesions (C,D,G,H) on poly-l-lysine relative to collagen II-coated substrates. DOI: http://dx.doi.org/10.7554/eLife.09300.009
Figure 4. Dynamic interaction of TβRs with integrins facilitate spatial organization. Representative trajectories for TβRI (Alk5) overlaid with the tagged focal adhesion marker vinculin are consistent with TIRF results showing a colocalization and interaction between integrin-based adhesions and TβRI (A) but not TβRII (B). Quantification of these regions shows that TβRI is preferentially enriched inside adhesions relative to outside, and that TβRII is preferentially excluded at these same sites (*p < 0.01, mean ± SD, C). Representative single molecule trajectories show sequestration of TβRI in focal adhesions (D, i-ii) and free diffusion outside adhesions (D, iii-iv), whereas TβRII bounces around the edges of focal adhesions in a freely diffusive (E, i-ii) or confined (E, iii-iv) manner. Analyzing TβR trajectories at focal adhesions based on diffusion (Red: Immobile, Green: Confined, Blue: Freely Diffusive) shows a higher density of tracks inside adhesions for TβRI (F) compared to TβRII (G), and demonstrates a higher fraction of immobile TβRI trajectories inside relative to outside adhesions (H). The diffusion coefficient of TβRI trajectories decreases inside adhesions (mean ± SD, I). See Source code 1 and Figure 4—source data 1.

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The following source data is available for figure 4:

Source data 1. Enrichment Ratio and Diffusion Coefficient
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**Figure 5.** TβRs form complexes with integrin αV and coflin. High-energy collision dissociation–tandem mass spectra obtained from precursor ions with mass 549.7775+2 (A) and 669.3185+2 (B) found in tryptic digests of immunoaffinity pulldowns of TβRI/II, corresponding to peptides spanning residues Y153-K165 of human integrin αV (A) and Y82-K92 of human coflin (B). b- and y- type ion series are labeled in the figure. Insets show the sequences of the peptides as well as representative peptide counts for integrin αV (A) and coflin (B) for mock (M), TβRI (RI, Alk5), and TβRII (RII) pulldowns. Co-immunoprecipitation of Flag-tagged TβRI and TβRII demonstrate the presence of integrin αV and coflin in these complexes (C,D).

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Figure 6. Tension-sensitive regulation of TβR spatial organization. Within 15 min of disrupting cellular tension by adding the ROCK inhibitor Y27632 (A, B) or the myosin II inhibitor blebbistatin (C, D), the peripheral ring of TβRII-mEmerald around focal adhesions (A, C) completely collapses (B, D). Colocalization quantification (Ai, Bi, Ci) demonstrates that TβRII is significantly more colocalized with integrin α2 post-treatment (Y27632, blebbistatin) relative to pre-treatment (**p < 0.001, mean ± SD, E, Figure 6—source data 1). Disruption of tension with Y27632 enhances integrin αV association with TβRI but reduces its association with TβRII (F). See Source code 2.
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The following source data is available for figure 6:
Source data 1. Colocalization Index (vehicle and treatment)
DOI: http://dx.doi.org/10.7554/eLife.09300.014
Figure 7. Disruption of tension-sensitive TβR segregation increases TβRI/TβRII multimerization and phosphorylation of Smad3. ROCK inhibition releases the discrete spatial organization of TβRs at focal adhesions and drives the formation of heteromeric TβRI/TβRII complexes within 15 min of Y27632 exposure (A), as shown by Flag co-immunoprecipitation (IP) and immunoblotting (IB). Likewise, manipulation of cellular tension through culturing cells on collagen II-coated glass or 0.5 kPa PDMS substrates increases co-immunoprecipitation of TβRI with Flag-tagged TβRII (p < 0.05, B). In cells grown on collagen II-coated compliant (0.5 kPa, p < 0.05) or stiff (16 kPa) PDMS substrates, endogenous Smad3 phosphorylation is increased (C). The effect of TGFβ on Smad3 phosphorylation is substrate-dependent, such that maximal TGFβ-inducibility is observed on 0.5 kPa substrates (p < 0.05), consistent with a tension-sensitive calibration of TβR localization and activity (C). See Figure 7 – source data.

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The following source data is available for figure 7:

Source data 1. Western Quantitative Analysis
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