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Supplementary information

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Ligand Functionalization of Titanium Nanopattern Enables the Analysis of Cell-Ligand Interactions by Super-Resolution Microscopy

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Supplementary Method 1.

TIRF Angle adjustment

TIRF angle was adjusted according to the manufacturer's instructions (https://www.research.uky.edu/uploads/nikon-storm-manual, https://manualzz.com/doc/33848772/overview-nikon-a1r-storm-microscope-). Briefly, achieving total internal reflection requires a collimated laser beam and an incident angle greater than the critical angle. For many recent commercially available TIRF microscopes, this likely can be performed using the standard automated TIRF adjustment functions. For manual adjustment (such as for TIRF illuminator with single automated axis), the first step is to focus the objective at the sample z-plane and the laser angle to epi-illumination. Alignment of the X-axis is performed by changing the angle on the software (or manually), while the alignment of the Y-axis is performed by turning the actuator knob on the TIRF illumination arm. The purpose of XY-beam position alignment is to ensure the beam is directly above the objective. The laser is then focused on a far-off point (usually a target point directly co-axial with the objective lens previously marked on the ceiling) by adjusting the collimator lens (usually via a side slider) on the TIRF illuminator until the most tightly focused spot is observed on the target. Once the beam is collimated and aligned, to achieve the appropriate incident angle, the laser angle is increased with a coarse setting, starting from the epi-illumination angle until the laser is no longer visible by naked eye on the objective at low power (i.e., TIRF condition). Further fine increase in laser angle can be performed using a fluorescent sample (e.g., MEF cells expressing Paxillin-EGFP spread on fibronectin coated glass-bottom dish) until no cytoplasmic signal is visible and the Paxillin signal at the adhesions has a high signal-to-noise ratio. This can be determined visually by observing cell nucleus boundary in a non-TIRF angle and a drastic increase in the Paxillin signal at the adhesions (**Extended Data Fig. 1**)[1–3].

References

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