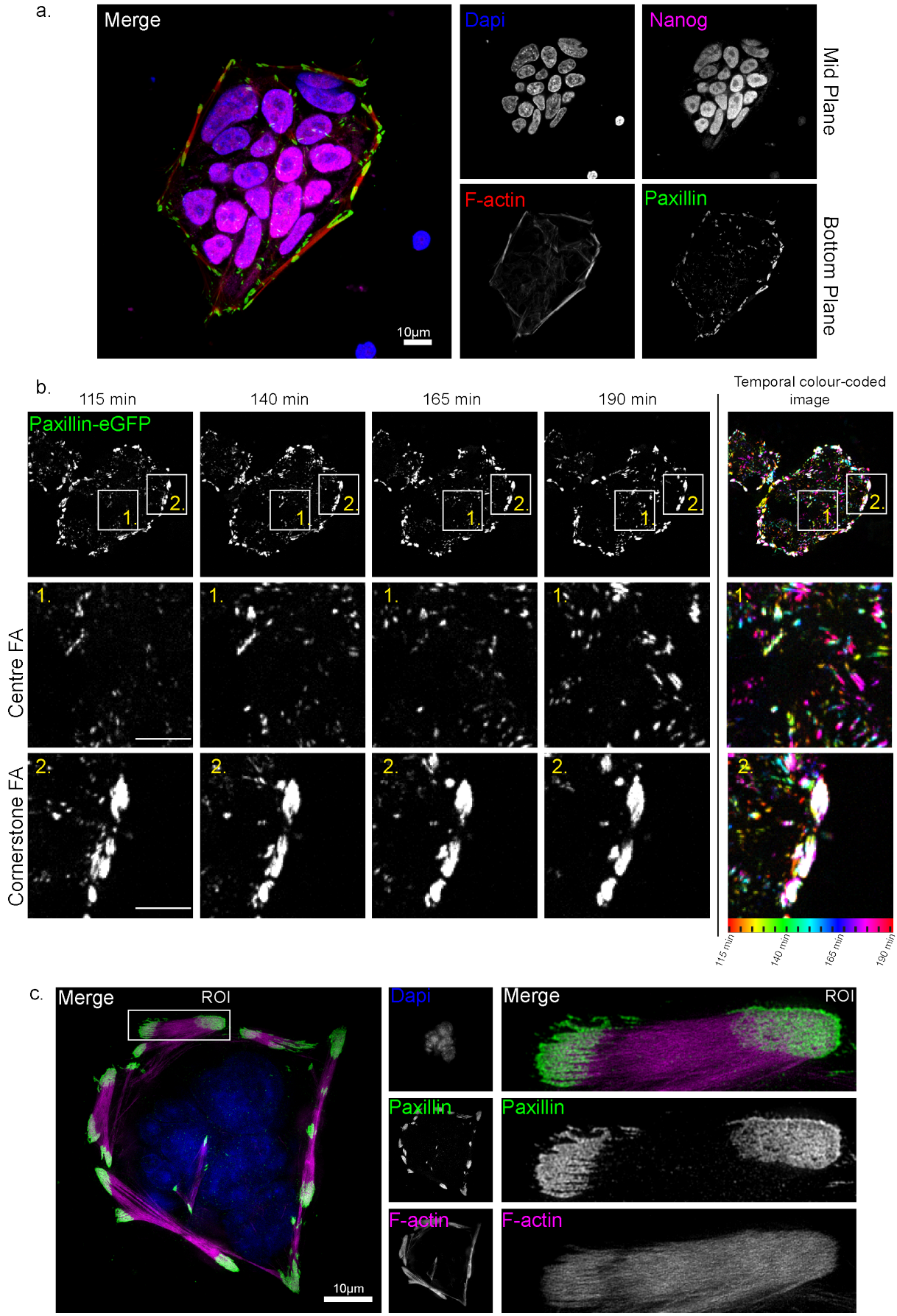
Supplemental material

Movie S1: Paxillin dynamics in an hPSC colony plated on vitronectin

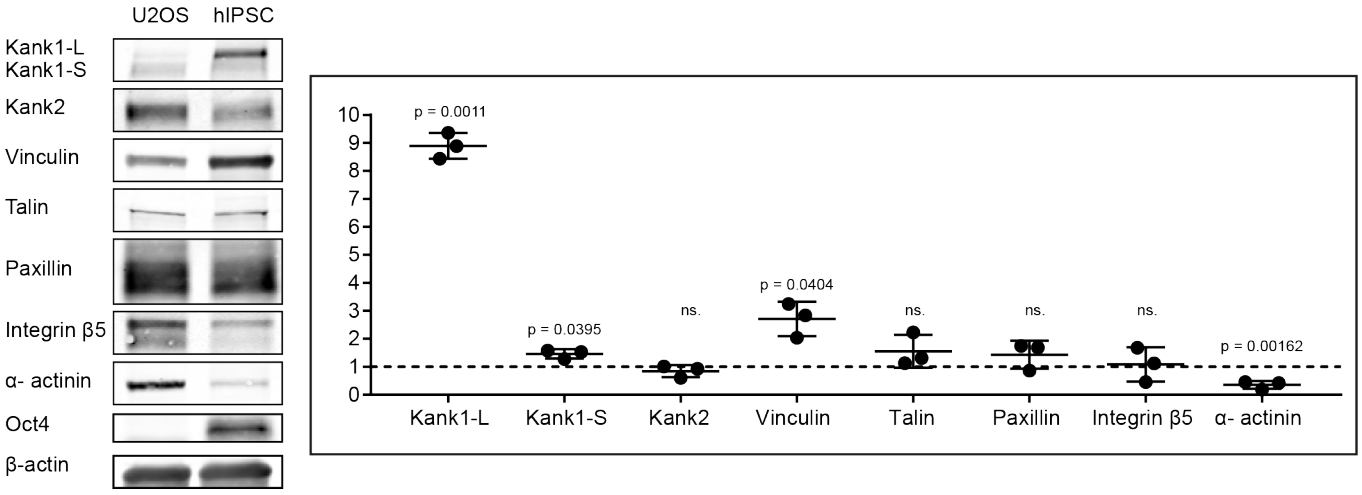
Live-cell imaging of endogenously tagged paxillin hPSC. Images were acquired using a spinning disk microscope.

Movie 2: Nanoscale kank1 localisation in cornerstone adhesions

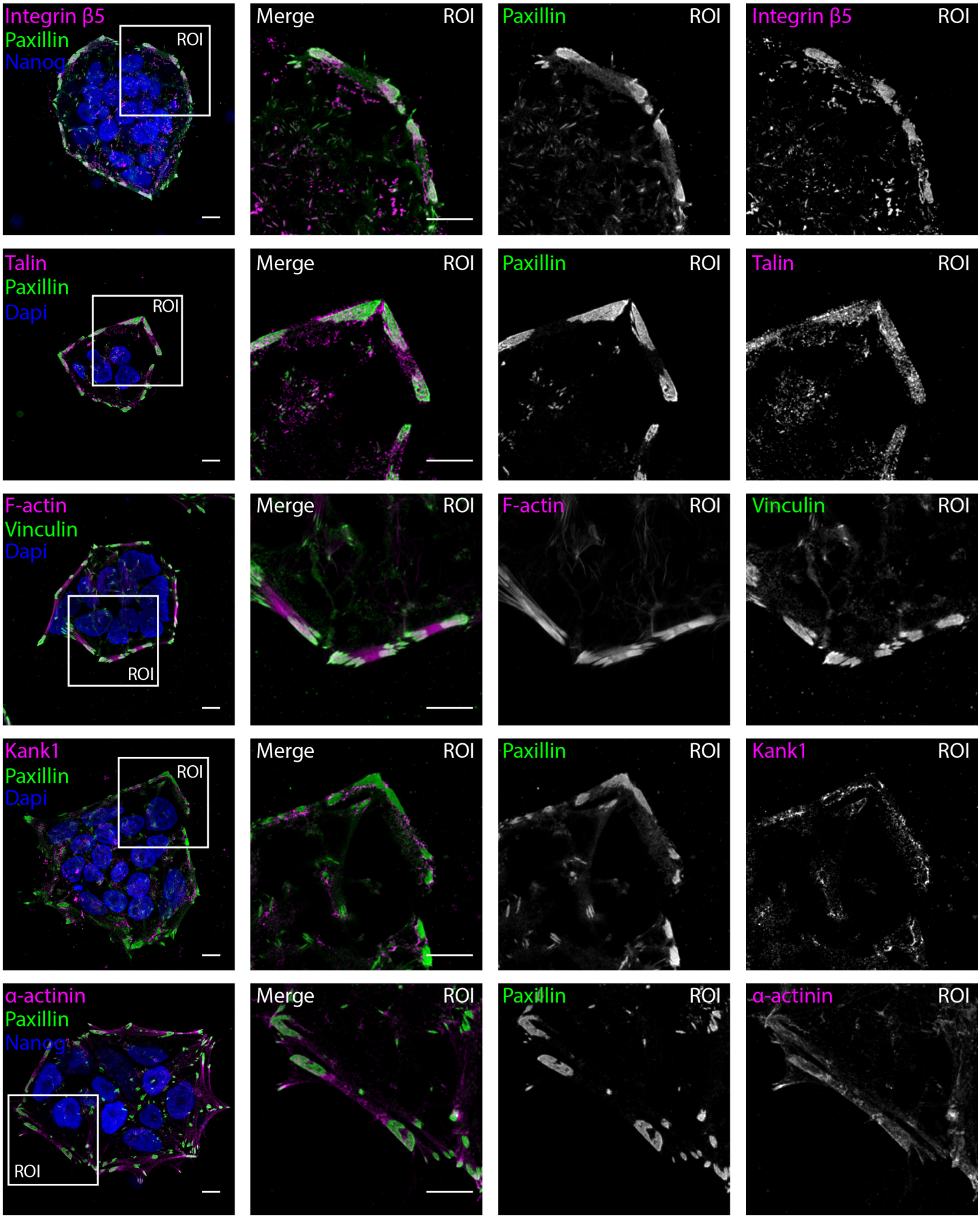
3D reconstruction of kank1 (green) and paxillin (red) iPALM data highlighting that kank1 assembles a wall surrounding paxillin-positive adhesions.



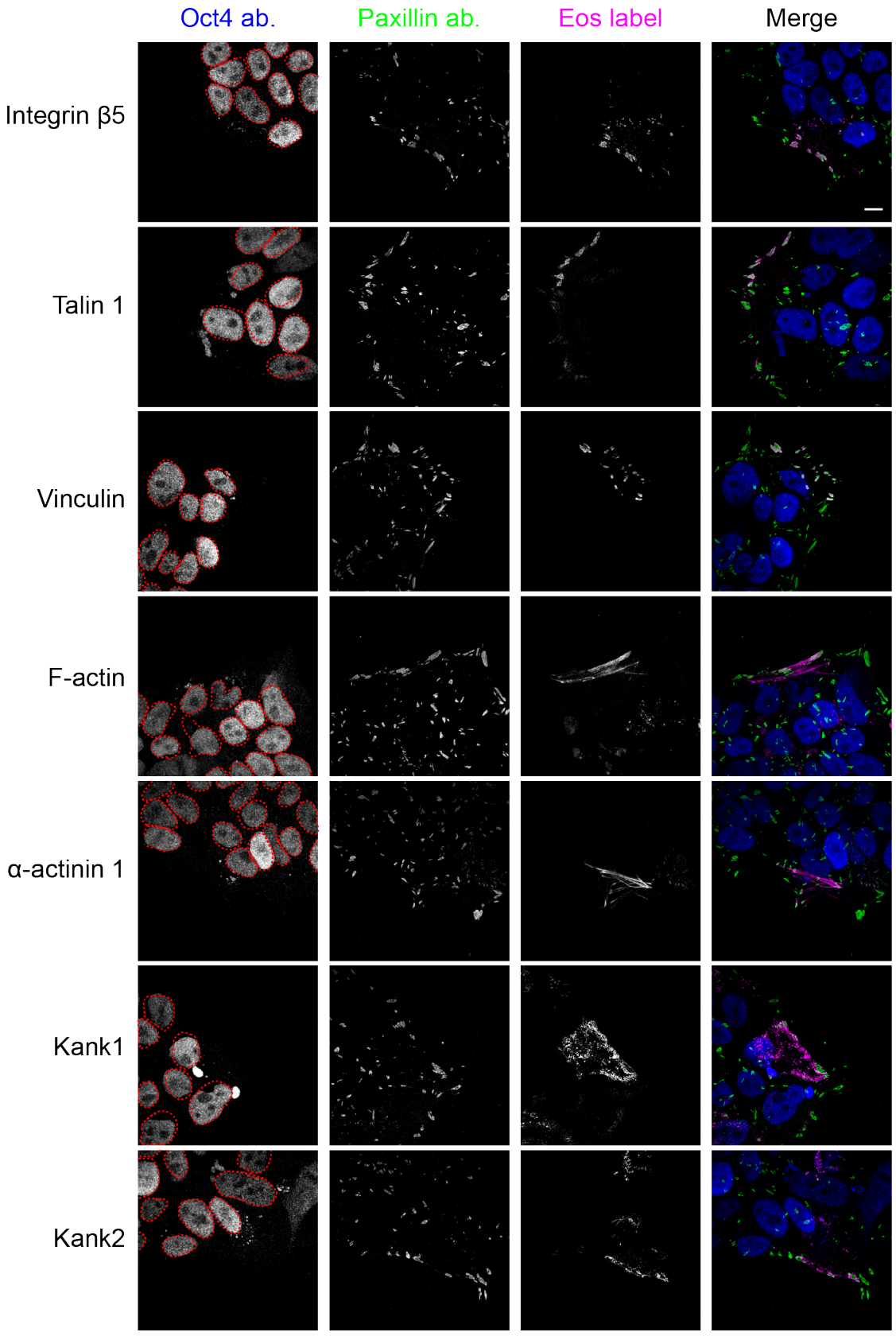
Supplementary Figure 1: hPSC cornerstone adhesions (a) Spinning disk images of hPSC plated on VTN and stained for F-actin, paxillin, DAPI and the pluripotency marker Nanog. Scale bar 10 µm. (b) Live-cell imaging of endogenously tagged paxillin in hPSC. Images were acquired using a spinning disk microscope. The full video is available as supplementary information (Video 1). White squares highlight regions of interests (ROI), which are magnified (ROI size 30 µm). Several time points of interest as well as temporal colour-coded images are displayed (white represents very stable adhesions). (c) Structured illumination microscopy images of hPSC plated on VTN and stained for filamentous actin (F-actin) and paxillin. Scale bar 10 µm. The white square highlights an ROI, which is magnified.



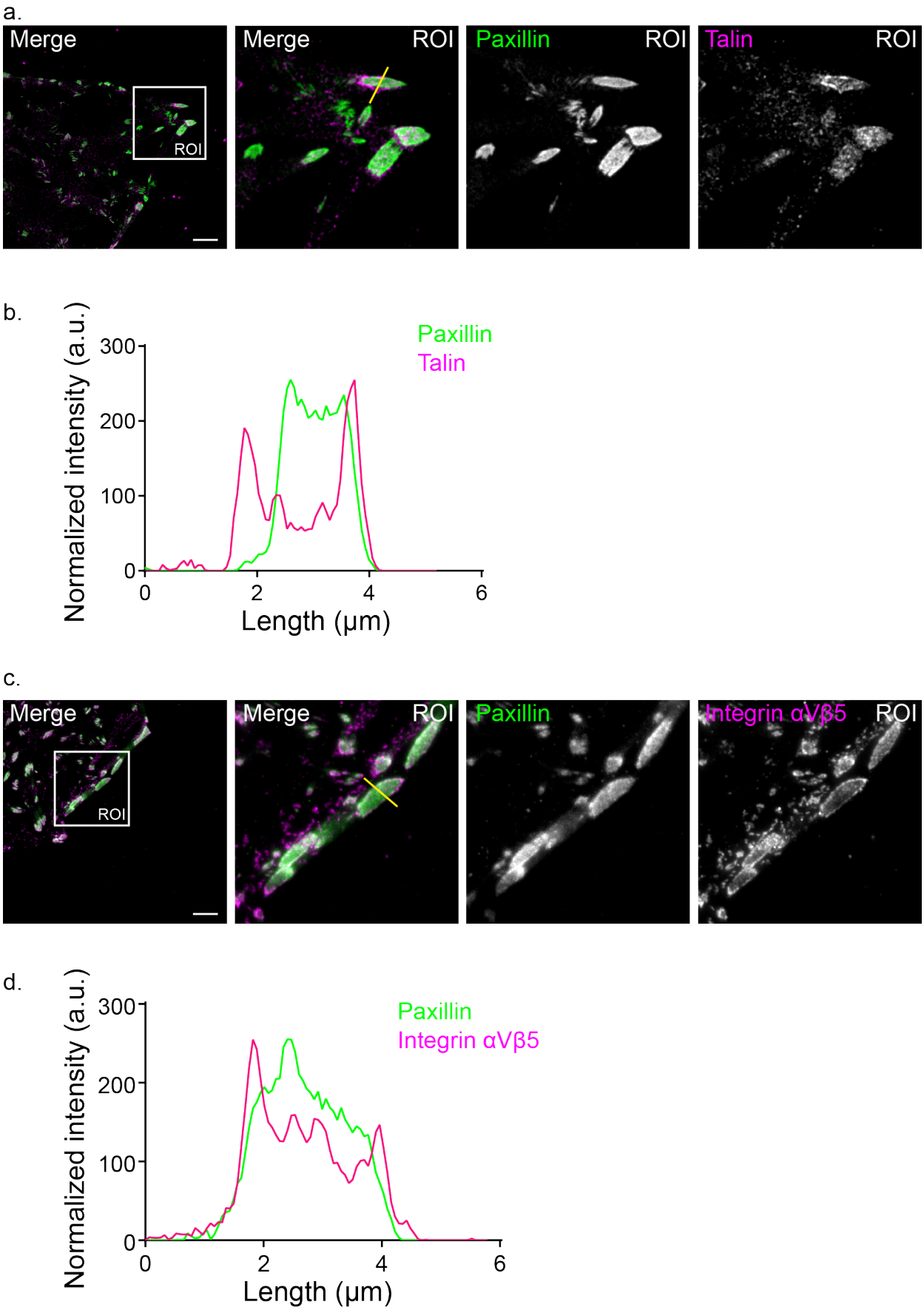
Supplementary Figure 2: Adhesion protein levels in hPSC cells. (a,b) Western blot analysis (a) and quantification (b) of endogenous FA components in U2OS cells and in hPSCs grown on VTN for 24h. Oct4 was used as a pluripotency marker and β-actin as a loading control. For quantification, integrated density values for each protein were first normalised to the loading control β-actin for all samples. Results from hPSC were then displayed normalised to U2OS cells (dotted line) (n = 3).



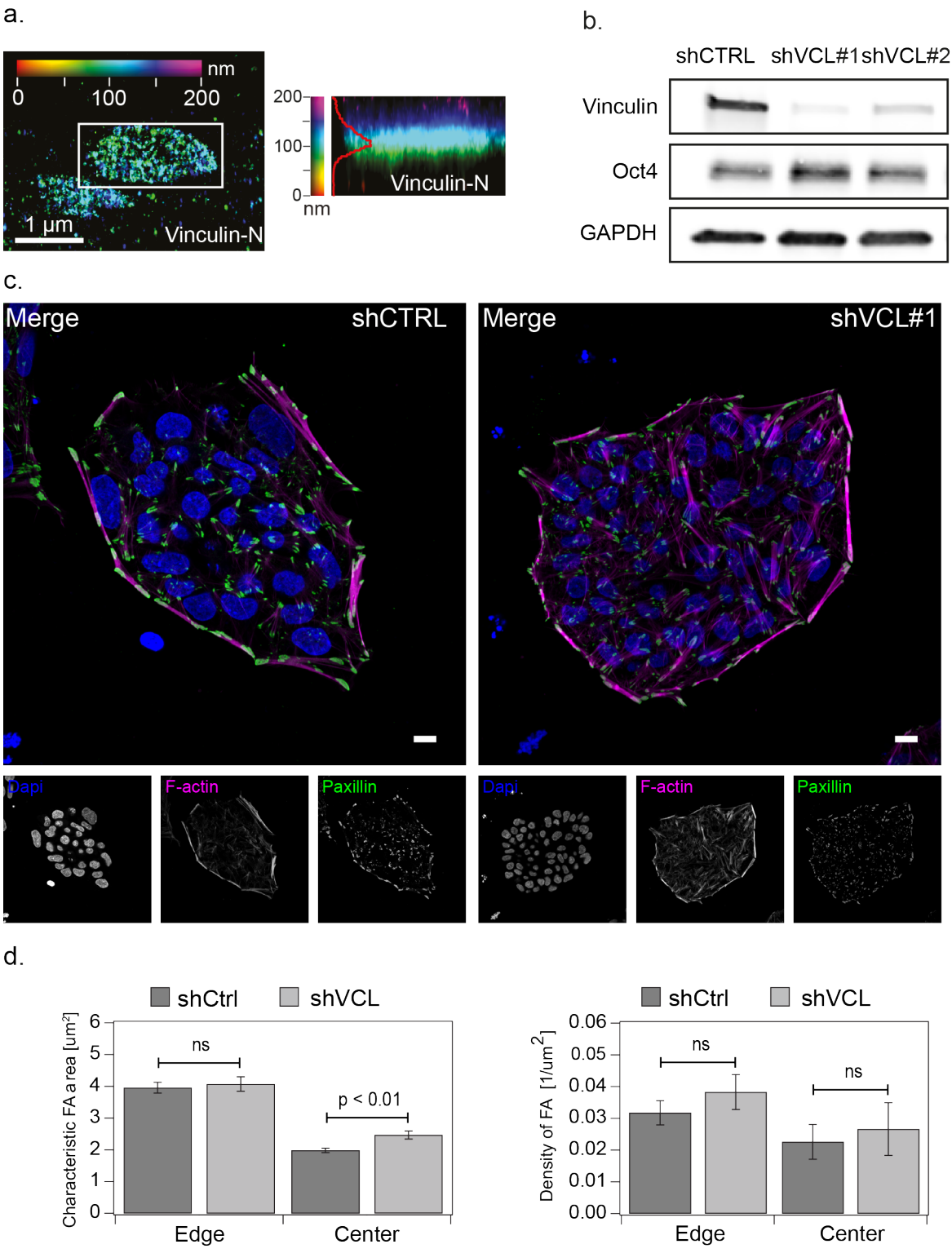
Supplementary Figure 3: Endogenous straining of multiple adhesion proteins in hPSC. Spinning disk images of hPSC plated on VTN and stained for paxillin, vinculin, β5 integrin, talin, kank1, α-actinin-1, Nanog and DAPI. White squares highlight regions of interests (ROI), which are magnified. Scale bar 10 µm.



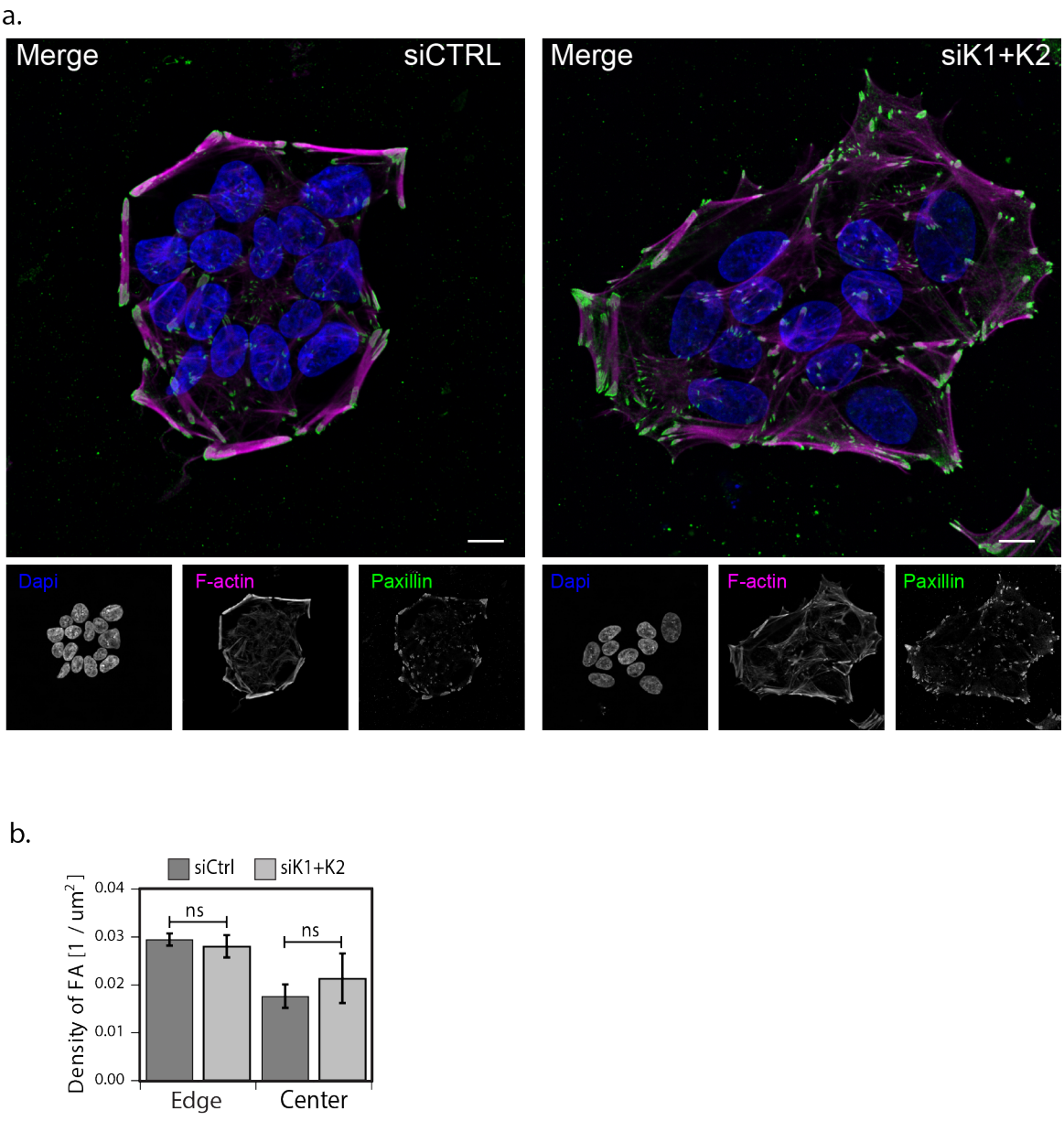
Supplementary Figure 4: Validation of the constructs used for iPALM. Spinning disk images of hPSC plated on VTN and transiently expressing various Eos-constructs used for iPALM in this study. Red dotted lines highlight the nucleus. Oct4 and paxillin were used as pluripotency and FA markers, respectively. Scale bar 10µm.



Supplementary Figure 5: lateral segregation of proteins within cornerstone adhesions. (a,b) Spinning disk images of hPSC plated on VTN and stained for endogenous talin and paxillin. Scale bar 10 μm. The yellow line in the magnified ROI indicates the area used to measure the intensity profiles displayed in (b). (c,d) TIRF images of hPSC plated on VTN and stained for endogenous αVβ5 integrin and paxillin. Scale bar 10 μm. The yellow line in the magnified ROI indicates the area used to measure the intensity profiles displayed in (d).



Supplementary Figure 6: Vinculin and cornerstone FA (a) iPALM images of N-terminally tagged (Eos) vinculin (vinculin-N) in cornerstone FA. Vinculin-N localisation is shown in top-view and side-view images that are colour-coded as a function of the z-position of Vinculin-N molecules. Scale bar 1 µm. (b) Vinculin, Oct4 and GAPDH protein levels in hPSC previously infected with lentivirus containing control shRNA (shCTRL) or two individual shRNA targeting vinculin (shVin#1 and shVin#2). (c) Spinning disk images of shCTRL or shVinculin hPSC plated on VTN and stained for paxillin, F-actin and DAPI. Scale bar 10 µm. (d) Quantification of the characteristic FA area and FA density at the edge and at the centre of shCTRL and shVinculin hPSC colonies (n = 3, see methods for details).



Supplementary Figure 7: kank1 and kank2 in cornerstone FA. (a) Spinning disk images of siCTRL or sikank1 and sikank2 (siK1 + siK2) hPSC plated on VTN and stained for paxillin, F-actin and DAPI. Scale bar 10 µm. (b) Quantification of FA density at the edge and at the centre of siCTRL or sikank1 and sikank2 hPSC colonies (n = 3, see methods for details).