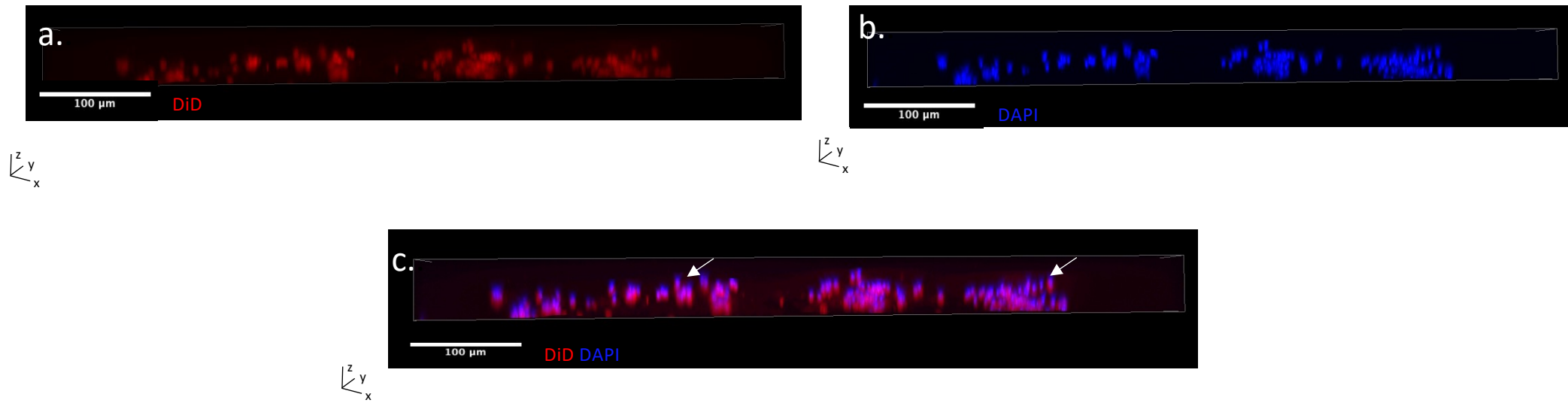


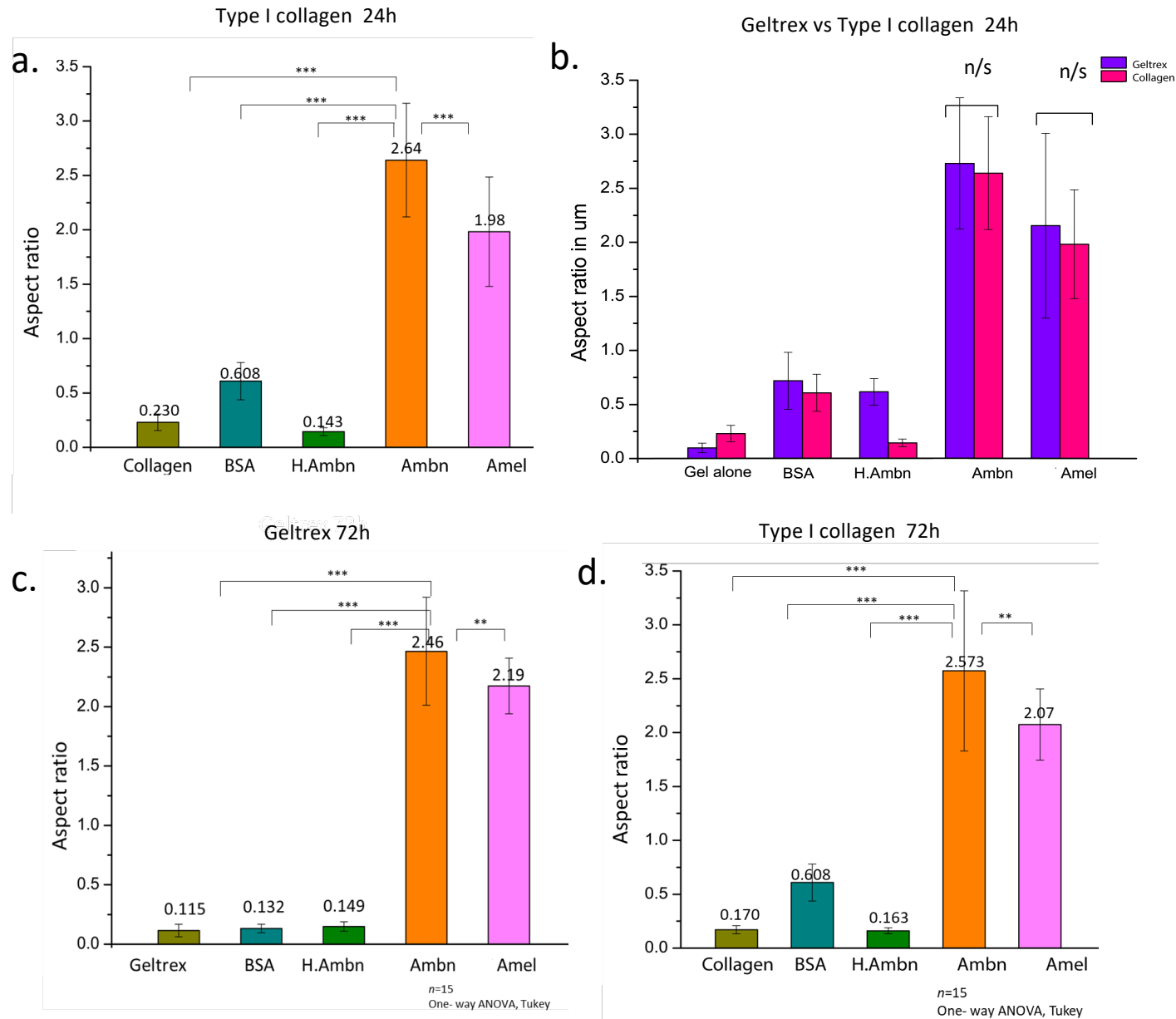
Matrix Biology manuscript figures

9 Supplementary images

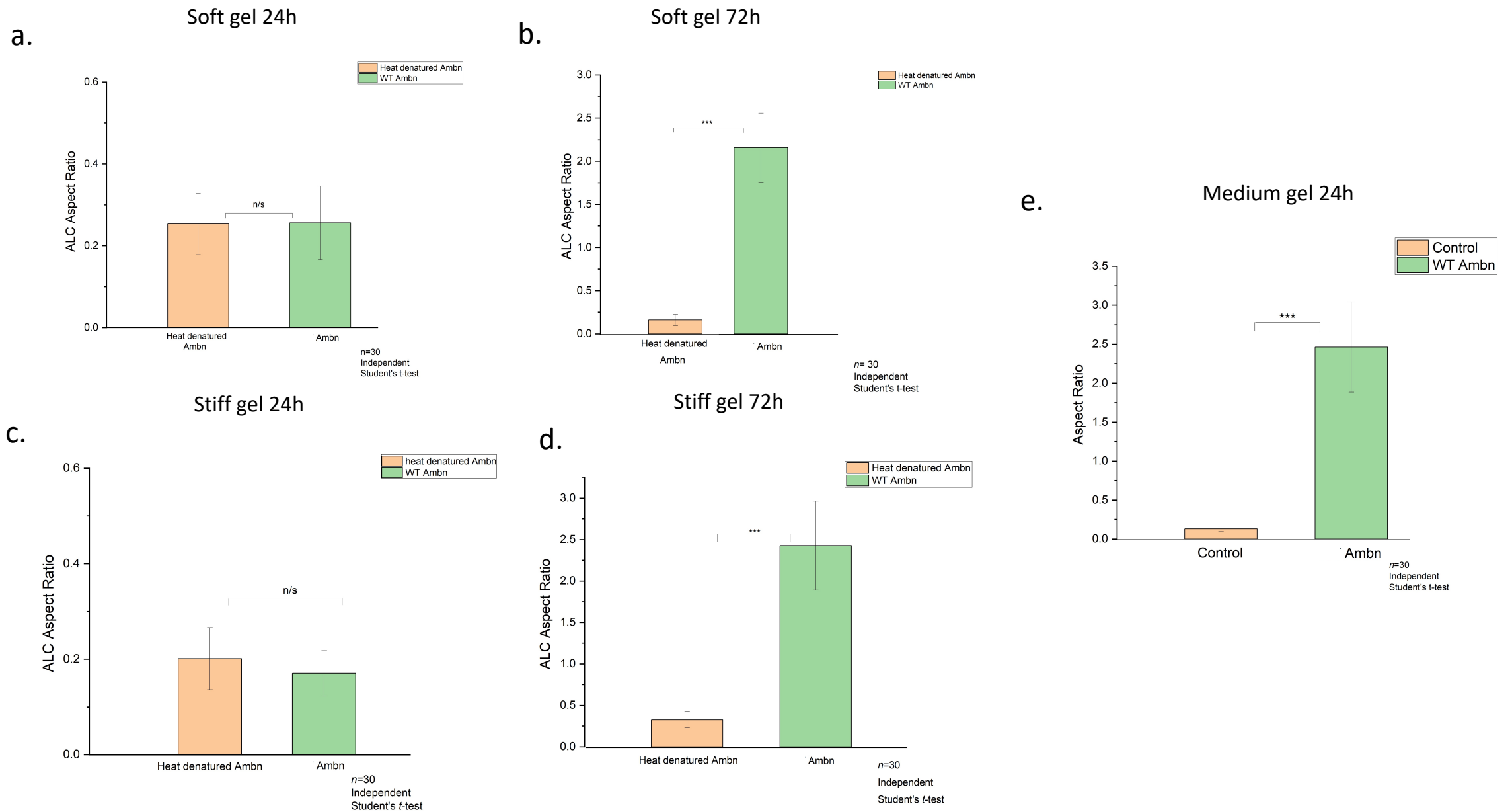
Cell clusters with nuclear localization to one pole of the cell



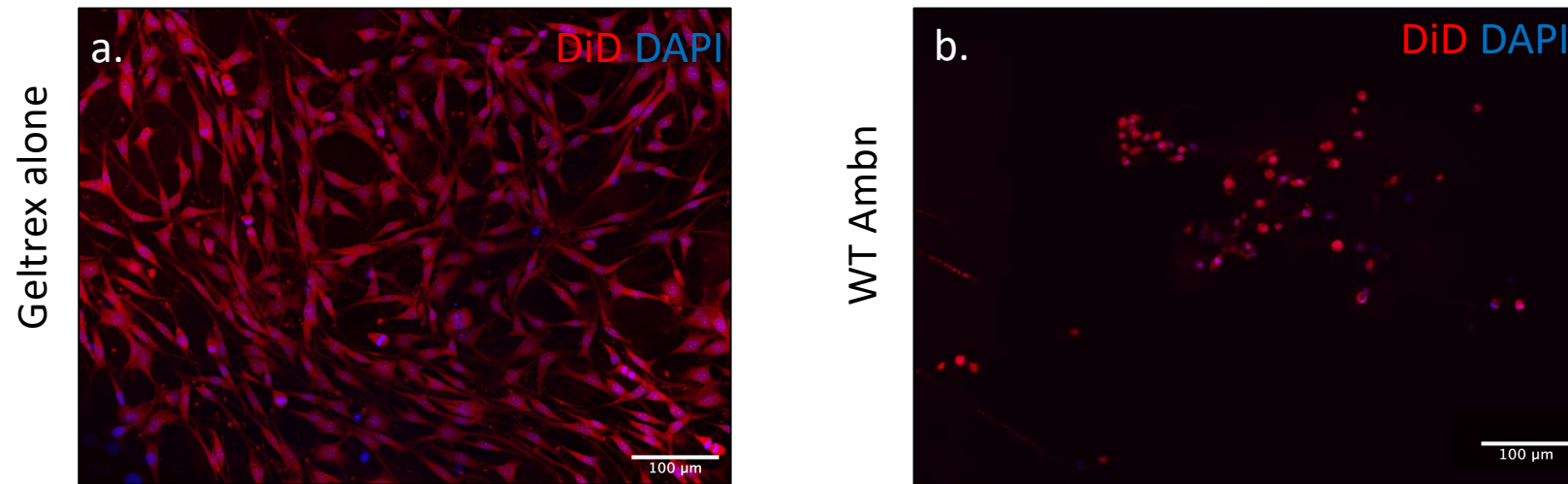
Supplementary Figure 1. 3D Z stack image of DiD and DAPI labeling showing nuclear polarization. Individual channels for membrane and nucleus shown in (a) and (b) respectively. Note that in the merged image, nucleus localization shown by DAPI is limited to one pole of the membrane shown in DiD (white arrows in c).



Supplementary Figure 2. ALC aspect ratio in Type I Collagen gel (a,c) and after 72h in Geltrex (c). Note that the difference in ALC aspect ratio between the controls and EMPs remains consistent at the end of 72h in Collagen and Geltrex gels. No significant difference in aspect ratios observed between Type I Collagen and Geltrex gels (b). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

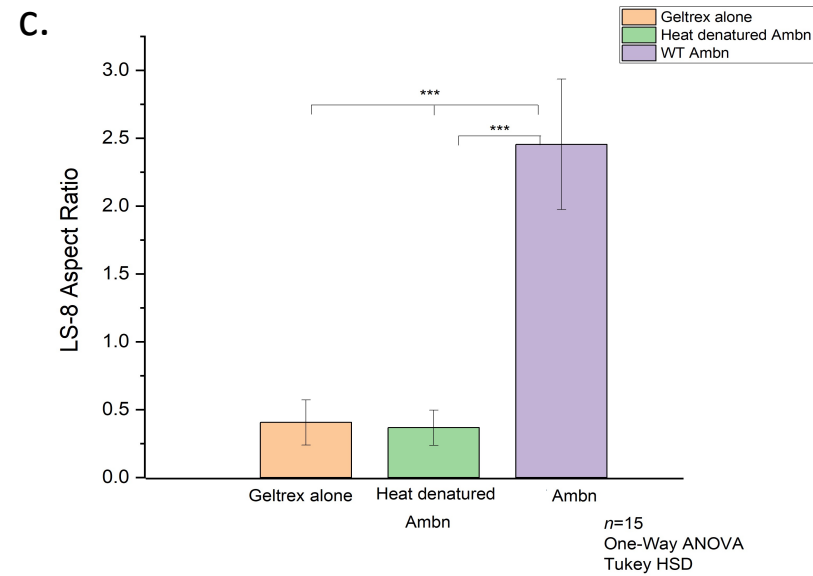


Supplementary Figure 3. Data from transglutaminase crosslinked gelatin gels. (a,b) Soft gel, (c,d) Stiff gel and (e) Medium gel. Note that with the Soft and Stiff gels, ALC aspect ratio does not increase at the end of 24h (a,c). Whereas with the Medium gel, aspect ratio is enhanced at the end of 24h (e). Independent Student's t-test * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



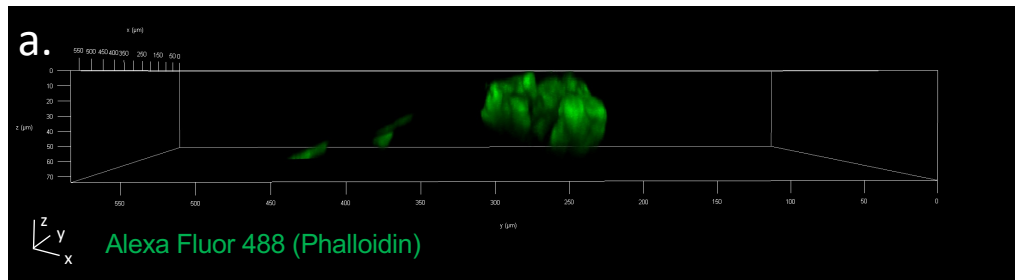
Geltrex alone

WT Ambn

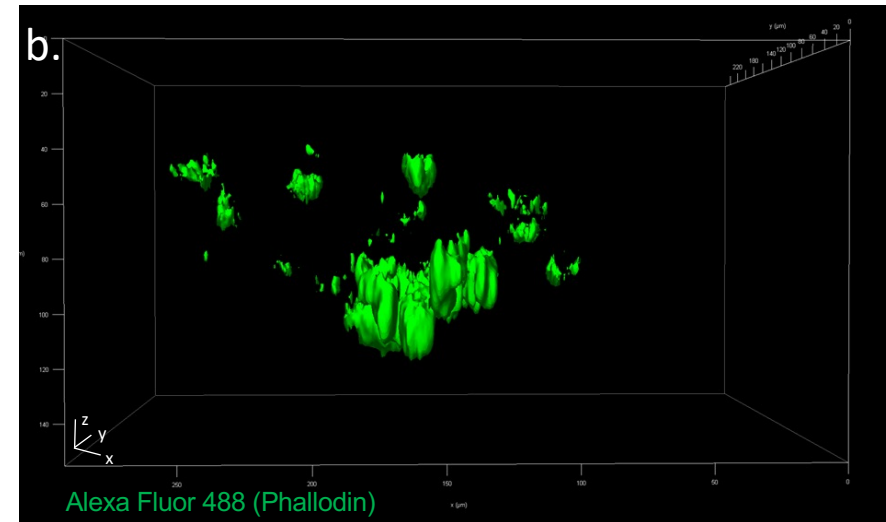


Supplementary Figure 4. LS-8 cells in Geltrex. (a,b) Fluorescent images showing the presence of discrete cells in the control (a) and the formation of cell clusters with WT Ambn (b). Measurement of cell aspect ratio reveals a significantly increased aspect ratio in the presence of WT Ambn (c). One way ANOVA, Tukey HSD * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

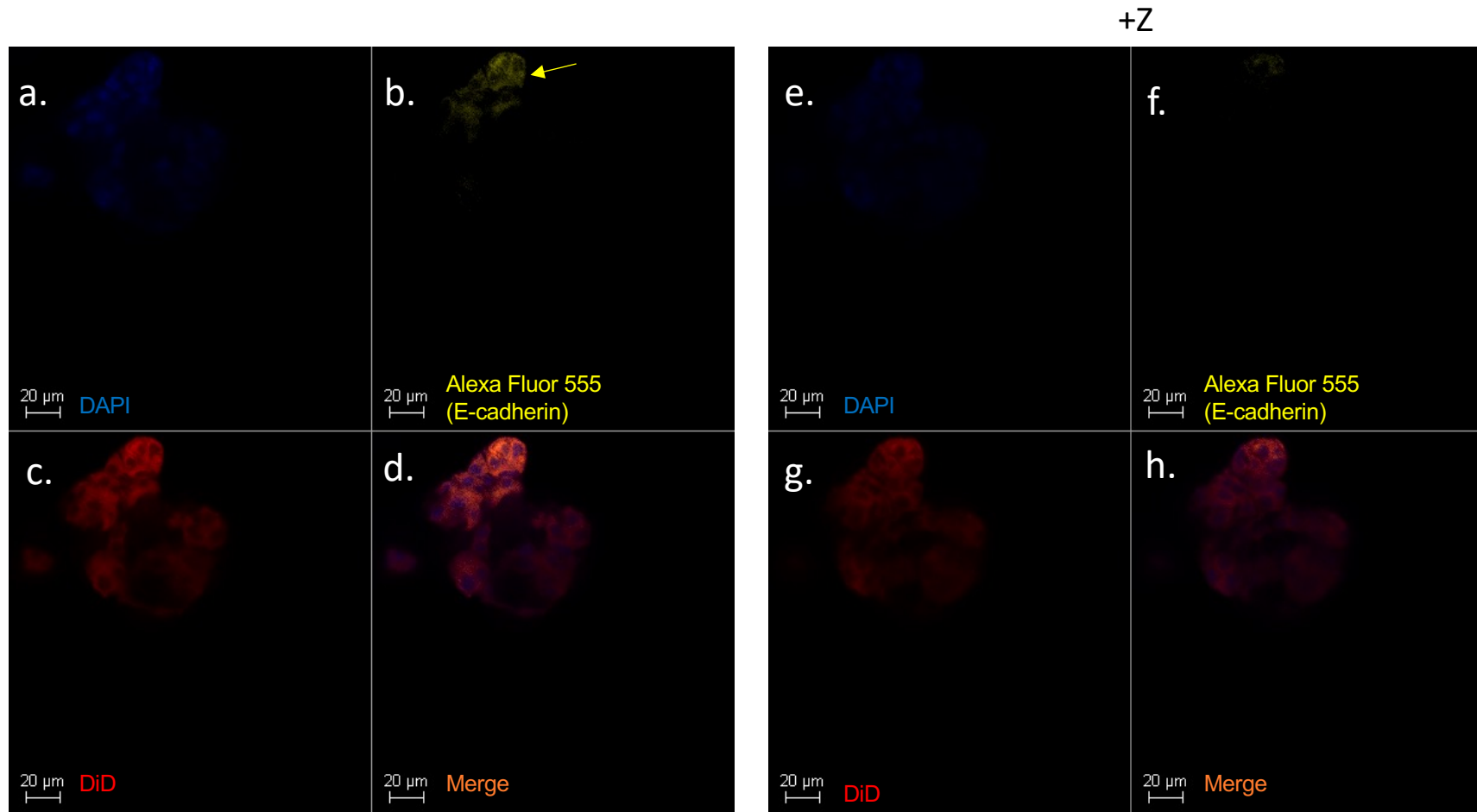
Phalloidin labeling axial view



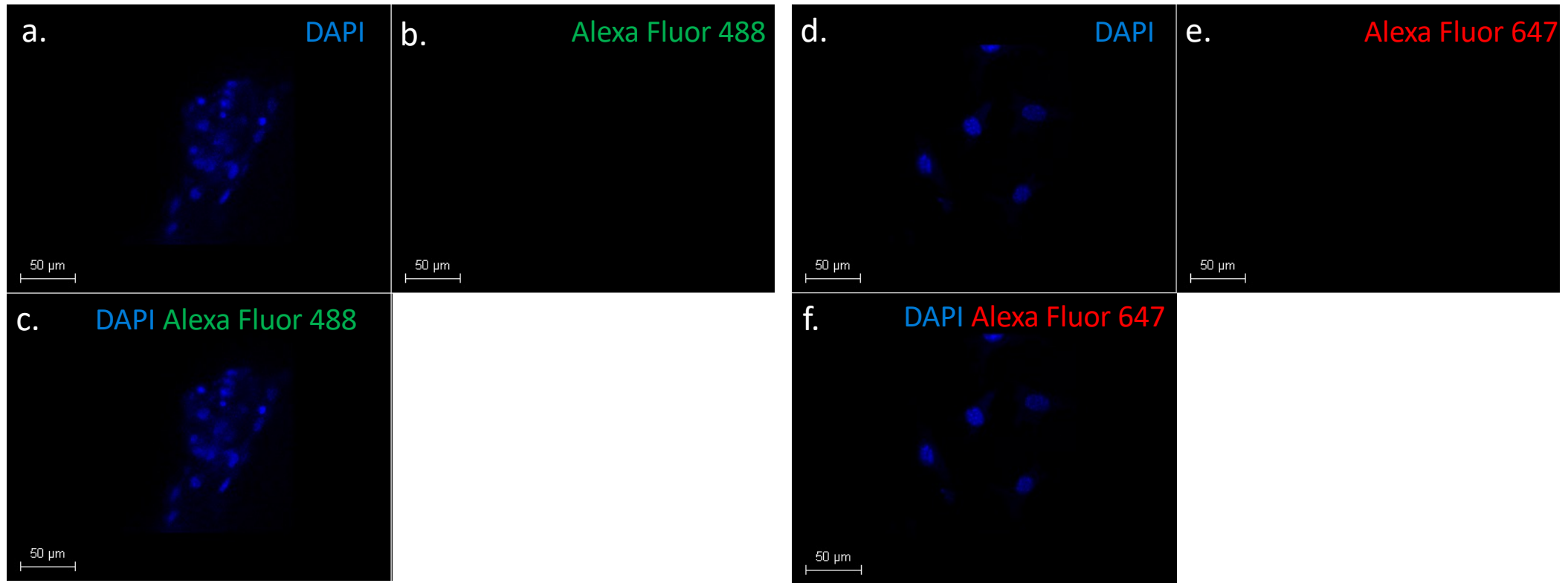
Phalloidin surface rendering



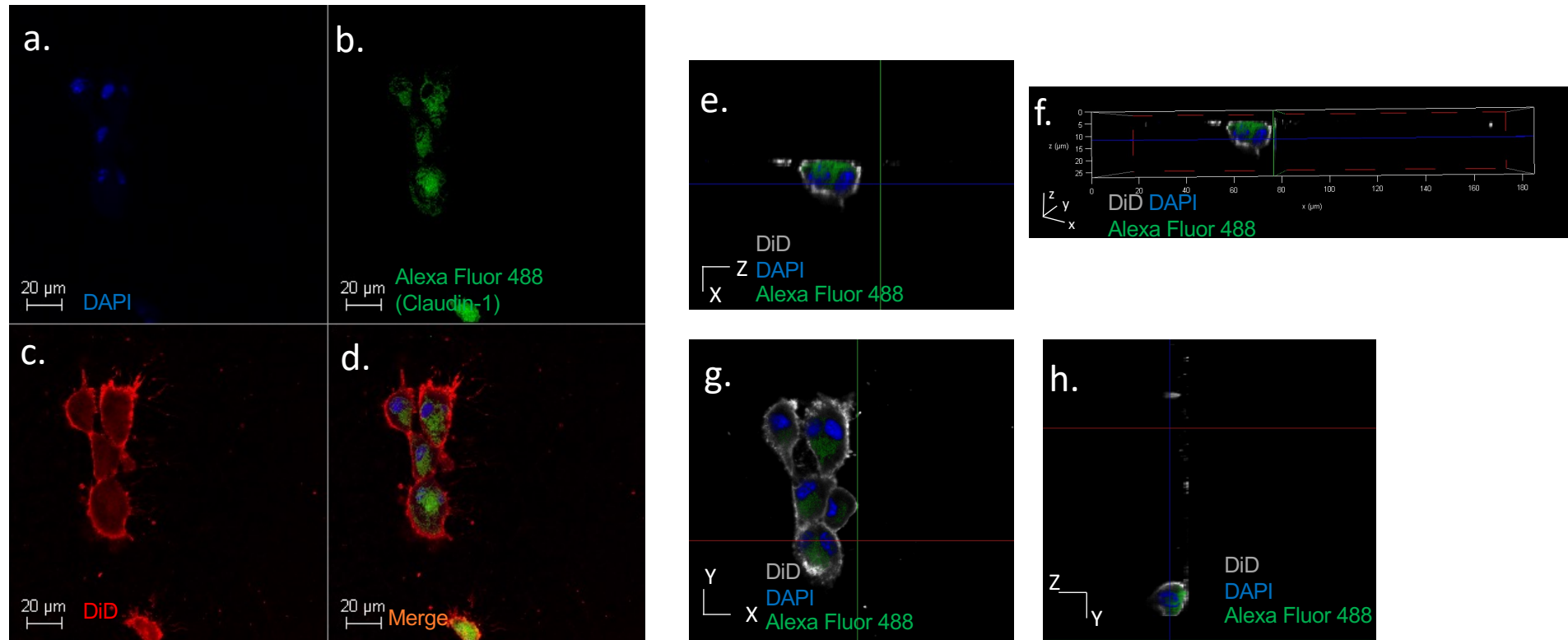
Supplementary Figure 5. Confocal laser scanning microscope axial image of a representative cell cluster labelled with Alexa Fluor 488 conjugated Phalloidin. 3D reconstruction and surface rendering reveals the presence of elongated cells within the clusters (b).



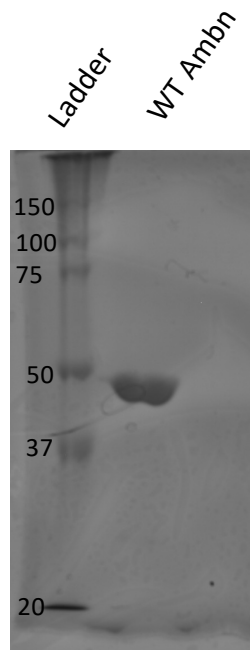
Supplementary Figure 6. 2D range imaging of E-cadherin membrane double labeling.(a-d) represent 2D images of a representative cell cluster at a certain Z depth. (e-h) represent the same cluster at a higher Z depth from the surface the of the gel. Note that the Alexa Fluor 555 signal (yellow arrow in b) representative of E-cadherin labeling disappears with increasing Z depth (f) , whereas the DiD signal is still present at both Z depths (c,g).



Supplementary Figure 7. Confocal secondary antibody controls for Alexa Fluor 488 (a-c) and Alexa Fluor 647 (d-f) secondary antibodies.



Supplementary Figure 8. Claudin-1 labeling. Alexa Fluor 488 labeled Cln-1 distribution is cytoplasmic in reference to the membrane labeled with DiD as seen in (b,d). (e-h) represent XZ(e), XY (g) and YZ(h) sections of the same cluster showing the cytoplasmic positioning of Cln-1 label within the cells. Blue (DAPI; nucleus), Green (Alexa Fluor 488; Cln-1); Red (DiD; membrane) Grey pseudo color (DiD; membrane).



Supplementary Figure 9. Recombinant full-length WT Ambn characterization. 12% SDS gel stained with Coomassie Blue.