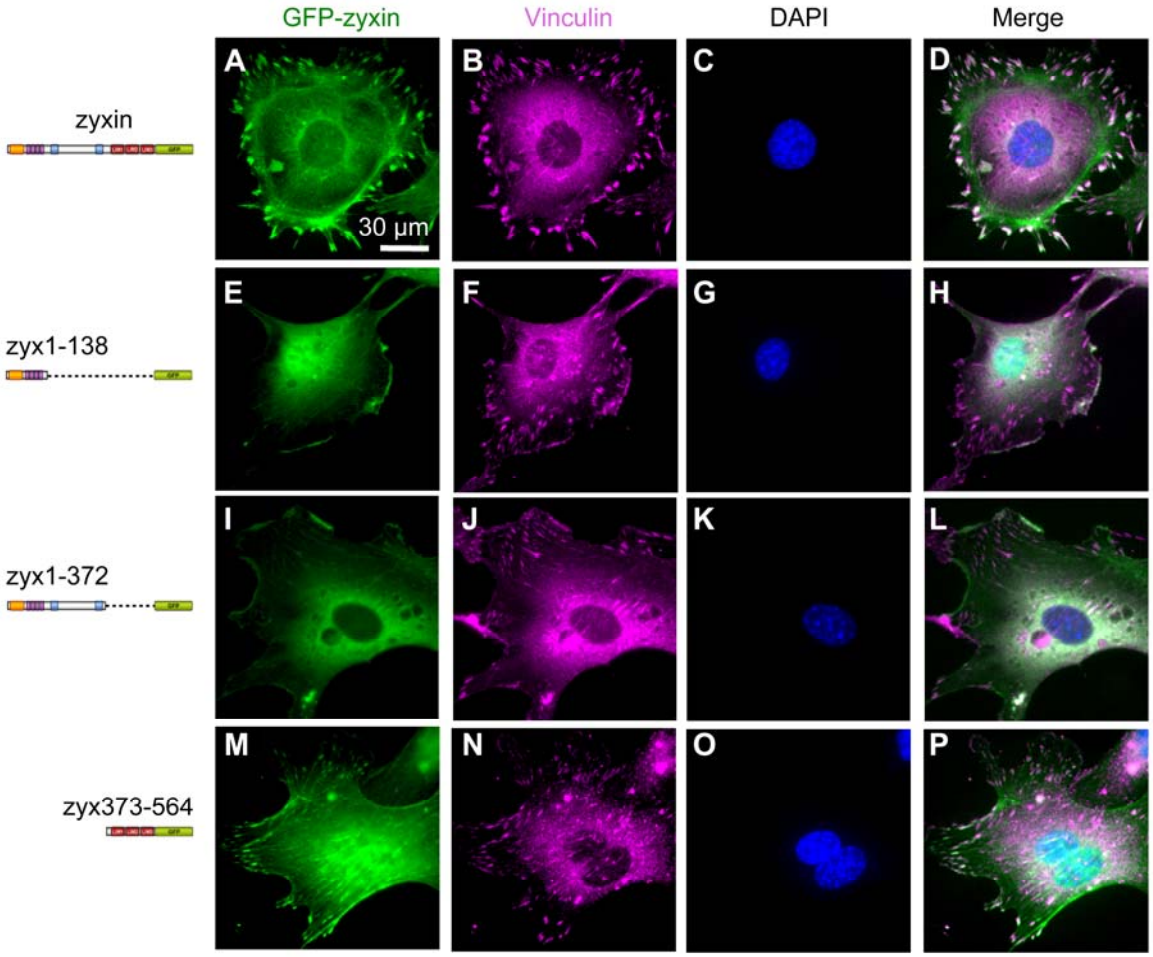
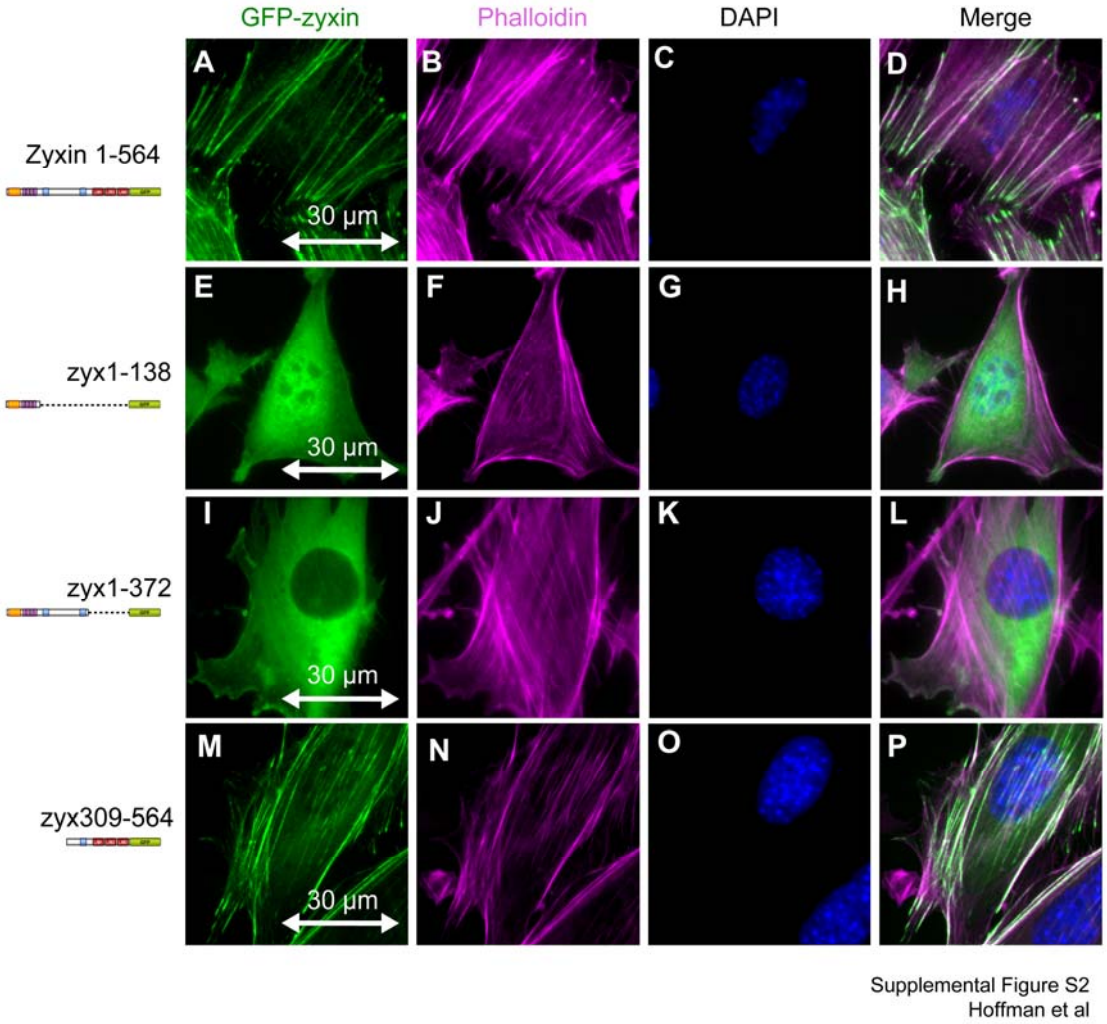


Supplemental Figure S1. Zyxin constructs lacking the LIM domains do not accumulate at focal adhesions. Zyxin-null fibroblasts expressing eGFP-zyxin variants (A,E,I,M) were fixed and stained for vinculin at focal adhesions (B,F,J,N) and DAPI at nuclei (C,G,K,O). Full length zyxin1-564 (A) and LIM domain construct zyx373-564

(M) both co-localized with vinculin at focal adhesions. Zyxin N-terminus zyx1-138 exhibited strong nuclear localization and failed to accumulate at the vinculin-rich focal adhesions within the cell (compare E to F). Zyx1-372 lacks only the LIM domains and it also failed to accumulate at the vinculin-rich focal adhesions (I,J). Interestingly, since zyx1-372 retains the nuclear export sequences, it did not accumulate in the nucleus, but some ruffled edge accumulation was observed. To better show the focal adhesion colocalization, merged images (D,H,L,P) of

eGFP-zyxin variants (green) and vinculin (magenta) are shown which are white with overlap (D,P). Scale bar is 30 μm.



Supplemental Figure S2. Zyxin constructs lacking the LIM domains do not accumulate along stretch-induced actin stress fibers. Zyxin-null fibroblasts expressing eGFP-zyxin variants (A,E,I,M) were grown on Collagen and Fibronectin coated silicone membranes, then subjected to uniaxial cyclic stretch (1hr 15% 0.5Hz), fixed and stained for F-actin (phalloidin;B,F,J,N) and nuclei (DAPI;C,G,K,O). Full length zyxin1-564 (A) and LIM domain construct zyx309-564 (M) both accumulated along actin stress fibers (B,N). Although phalloidin-stained actin stress fibers (F,J) were detectable in cells expressing zyx1-138 and zyx1-372, these constructs did not accumulate on stretch-induced SFs (E,I). (D,H,L,P) images of stretched cells expressing eGFP-zyxin variants (green) and phalloidin-stained SFs (magenta) were merged to show colocalization of the two signals (white) with LIM domain containing constructs. Stretch direction is in the horizontal plane (double-headed arrow, 30 µm scale).

Supplemental Video 1. Disruption of GFP-zyxin-containing stress fibers by Jasplakinolide (video1.avi). Zyxin-null fibroblasts expressing GFP-zyxin were treated for 2h with the actin stabilizer Jasplakinolide (200 nM) and images collected at 3 min intervals. Arrow indicates site of actin rupture corresponding to Figure 7J.