Supplementary Table 1 Legend

Excel file listing proteomic results from EcadBL screens. Description refers to *Canis familiaris* Ref Seq database; N-PSM, normalized peptide spectral match (PSM for each protein/total PSM for that mass spectrometry analysis; inclusion of any protein required presence in 2/3 or 3/3 separate analyses and N-PSM values were averaged for each protein to generate final values, # ob peptides, peptides in size range detectable in mass spectrometry (see methods), N-PSM/ob peptides was used to estimate relative abundance of recovered proteins. Proteins are listed in order of estimated abundance; only proteins present at twice the level of proteins recovered from cells expressing biotin ligase alone (see (Van Itallie et al., 2013)) are included; list 2 are ribosomal proteins and list 3 includes all proteins identified in MDCK cells (in at least 2/3 isolations) expressing biotin ligase alone.

Supplementary Figure Legends

Supplementary Fig. 1. ZO-1, ZO-2 and E-cadherin are dispensable for targeting LPP to cell contacts. Top panels. Mixed cultures of ZO-1/ZO-2 double knockdown cells (Fanning et al., 2012) were stained for ZO-1 (left) and LPP (middle); merged confocal image (right) revealed normal LPP localization in double knockdown cells. Bottom panels. Similarly, mixed cultures of control and E-cadherin knockdown cells (Capaldo and Macara, 2007) were stained for E-cadherin (left) and LPP (middle); merged image (right) revealed normal LPP localization in double knockdown cells.

Supplementary Fig. 2. Immunofluorescent localization of cortical actin and non-muscle myosin 2A and 2B are similar in control and LPP knockdown cells. Top panels. MDCK and LPP knockdown cells were stained for myosin 2A (left panels) and for actin using Rhodamine phalloidin (middle panels); confocal imaging revealed similar myosin 2A and actin localization in control and knockdown cells. Bottom panels. MDCK and LPP knockdown cells were stained for myosin 2B (left panels) and actin (middle panels) and merge (right panels); similar to myosin

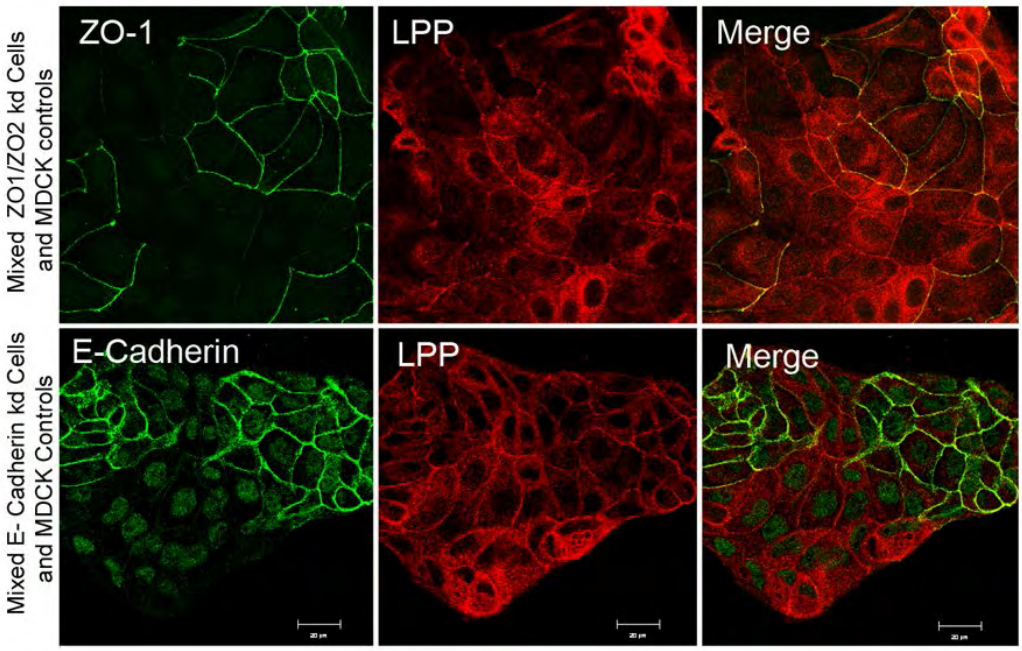
2A, there was no clear difference in myosin 2B or actin staining in control and LPP knockdown cells.

Supplementary Fig. 3. Immunofluorescent localization of VASP , α-actinin and VASP reveals no clear differences between control MDCK and LPP knockdown cells. Top Panels. Rhodamine phalloidin localization of actin (left panels) and VASP (middle panels; merge right panels) reveals similar cell contact staining in control and LPP knockdown cells. The increase in nuclear staining in the LPP knockdown cells was reproducible but presently without explanation. Bottom panels. Left panels: α-actinin localization was similar in control and knockdown cells (although LPP knockdown cells were flatter) and zyxin staining (middle panels) was more evident at focal adhesions in LPP knockdown cells than in control cells. Cell contact staining was faint and discontinuous in both control and knockdown cells. Zyxin nuclear staining appears here brighter in the LPP knockdown cells than in control cells; this was not reproducible.

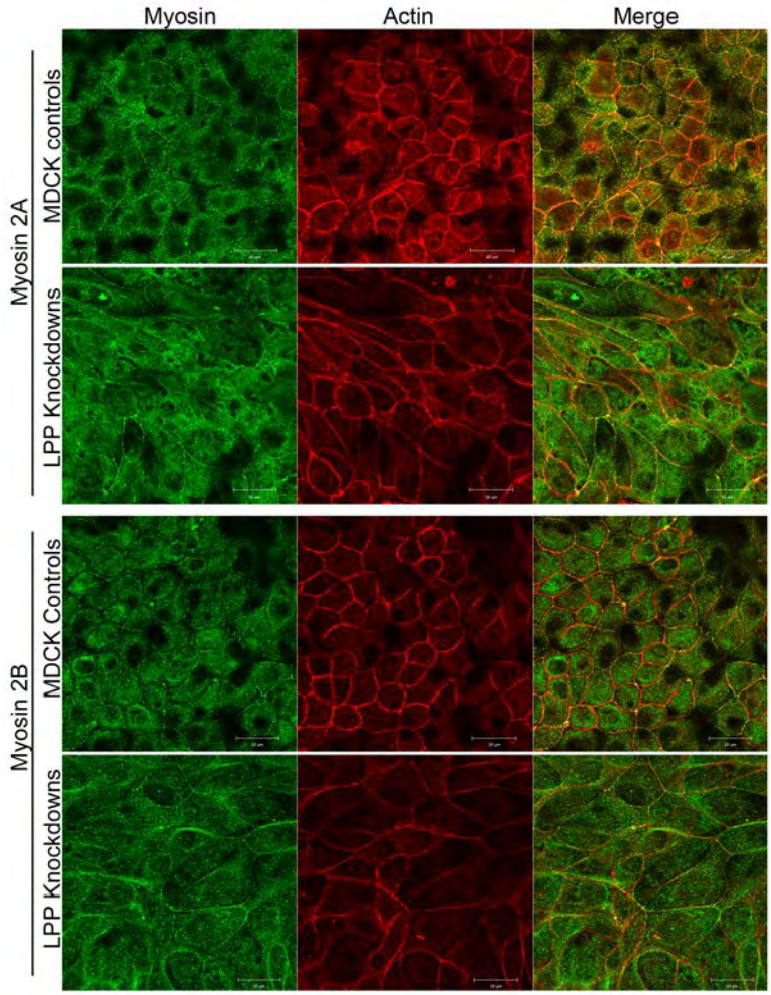
Capaldo, C. T. and Macara, I. G. (2007). Depletion of E-cadherin disrupts establishment but not maintenance of cell junctions in Madin-Darby canine kidney epithelial cells. *Mol Biol Cell* **18**, 189-200.

Fanning, A. S., Van Itallie, C. M. and Anderson, J. M. (2012). Zonula occludens-1 and -2 regulate apical cell structure and the zonula adherens cytoskeleton in polarized epithelia. *Mol Biol Cell* **23**, 577-590.

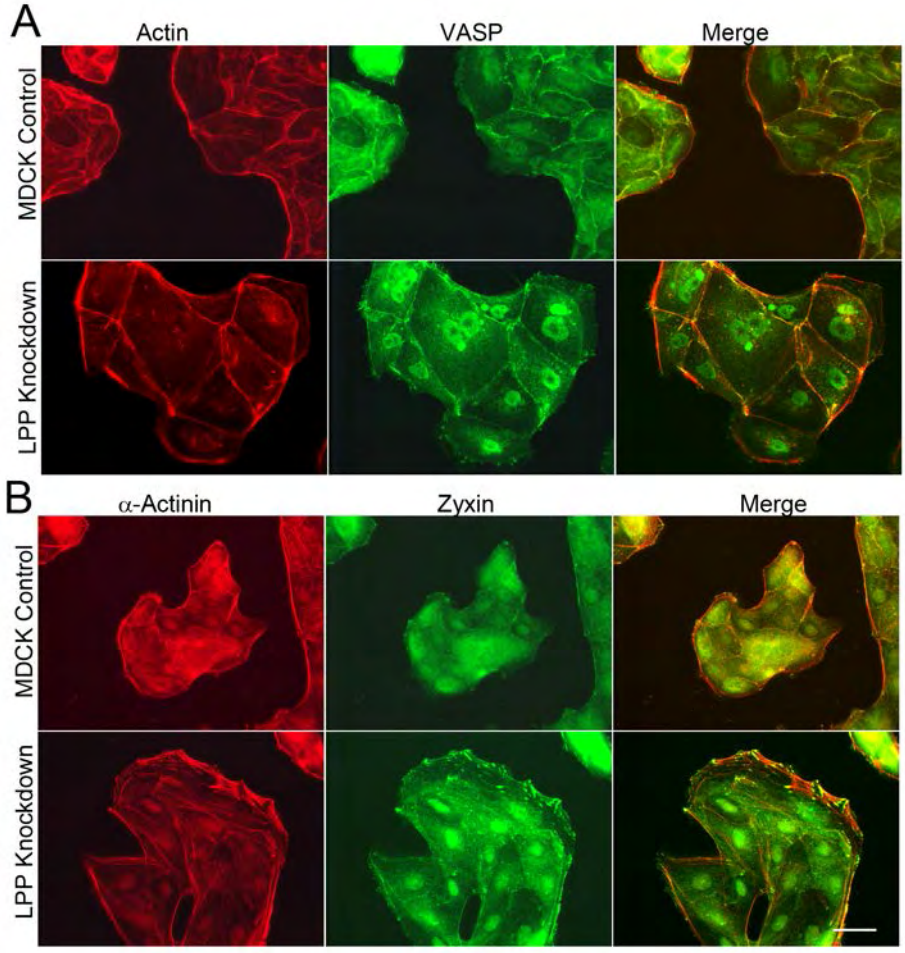
Van Itallie, C. M., Aponte, A., Tietgens, A. J., Gucek, M., Fredriksson, K. and Anderson, J. M. (2013). The N and C termini of ZO-1 are surrounded by distinct proteins and functional protein networks. *J Biol Chem* **288**, 13775-13788.



Supplementary Fig. 1



Supplementary Fig. 2



Supplementary Fig. 3

Table S1. Proteins recovered from cells expressing ECadBL (2-fold above biotin ligase alone)

Download Table S1