SUPPLEMENTAL DATA

Fig. S1. (A) Confluent MDCK cells were incubated in LCM for 16h then switched back to HCM supplemented with DMSO or with okadaic acid (OA, 1mM dissolved in DMSO) for the indicated times. Before Ca^{2+} switch, cells were exposed to DMSO or OA (1mM) in LCM for 2 hours. Cell lysates were separated on SDS-PAGE and probed with indicated antibodies. (B) Confluent MDCK cells were incubated in LCM for 16 h followed by pretreatment with DMSO, SB216763, NaCl or LiCl for 1 h before they were switched back to HCM, with the aforementioned chemicals present in the HCM. Cells were fixed at the indicated times after the switch from LCM to HCM and immunostained for ZO-1. Bar=30 μm. (C) Quantification of ZO-1 relocalization to junction regions during the Ca^{2+} switch in (A). Error bars represent standard deviations of the mean ZO-1 length per cell within each of the four randomly picked view fields. The asterisks (*) denote significant differences in the indicated pairs ($P < 0.05$) by Student's *t*-test. (D) Confluent MDCK cells were incubated in LCM for 16 h followed by replacement of the media with fresh LCM supplemented with DMSO, 10 μM SB216763, 20 mM NaCl or 20 mM LiCl. Cells were fixed at the indicated time points after the replacement of medium and immunostained for PATJ and PAR-3. Bar=30 μm. Arrowheads highlight strands of protein present in the sites of cell-cell contacts. (E) MDCK cells were incubated in LCM for 16 h. Medium was replaced by fresh LCM supplemented with the indicated reagents. Cells were lysed 2 h after the medium change and the Triton soluble (s) and insoluble (i) fractions were probed with the indicated antibodies in a Western blot analysis. (F) Confluent MDCK cells were incubated in LCM for 16 h followed by replacement of the media with fresh LCM supplemented with the indicated concentrations of different test reagents. Cells were fixed 2 h and 10 h after the medium change and immunostained for occludin. Bar=30 μm. (G) Quantification of occludin relocalization to cell membrane in (E). Error bars represent standard deviations of the mean occludin length per cell within each of the four randomly picked view fields. The asterisks (*) denote significant differences in the indicated pairs ($P < 0.05$) by Student's *t*-test.

Fig. S2. (A) Confluent MDCK cells were incubated in HCM with 1 mM AICAR, DMSO, 10 μM SB216763, 20 mM NaCl or 20 mM LiCl for 1 h before they were switched to LCM in the presence of the aforementioned test reagents. Cells were fixed 4 h and 10 h after the medium change and immunostained for occludin. Bar=30 μm. (B) Quantification of occludin on the cell membrane. Error bars represent standard deviations of the mean occludin length per cell within each of the four randomly picked view fields. The asterisks (*) denote significant differences in the indicated pairs ($P < 0.05$) by Student's *t*-test.

Fig. S3. (A) Confluent MDCK cells were incubated in LCM for 16 h followed by the replacement of the media with fresh LCM supplemented with the indicated concentrations of AICAR. Cells were fixed 2 h after the medium change and immunostained for ZO-1. Bar=30 μm. (B) Quantification of ZO-1 relocalization to the cell membrane in (A). Error bars represent standard deviations of the mean ZO-1 length per cell within each of the four randomly picked view fields.

(C) Cell lysates from two lines of stable AMPK knock-down cells and two lines of control cells were probed with indicated antibodies in a Western blot analysis. The bands in each of the three blots were scanned from the same film, with extra lanes between the depicted samples removed via image processing. (D) Confluent stable AMPK knock-down MDCK cells and control cells were incubated in LCM for 16 h followed by the replacement of the media with fresh LCM supplemented with DMSO, SB216763, NaCl, LiCl or AICAR. Cells were fixed 2 h after the medium change and immunostained for occludin. Bar=30 μm. (E) Quantification of occludin relocalization to cell membrane in (D). Error bars represent standard deviations of the mean occludin length per cell within each of the four randomly picked view fields. The asterisk (*) denotes significant differences in the indicated pairs ($P < 0.05$) by Student's *t*-test.

Fig. S4. (A) shRNA constructs targeting E-cadherin plus cadherin-6 (E-cadherin) or an unrelated protein (Luc-ctrl) were transiently transfected into MDCK cells. Cells were cultured for 48 h in HCM. Cell lysates were probed with the indicated antibodies in a Western blot analysis. (B) MDCK cells transfected with control or E-cadherin shRNAs were incubated in LCM for 16 h followed by replacement of the media with fresh LCM supplemented with 1 mM AICAR, DMSO, 10 μM SB216763, 20 mM NaCl or 20 mM LiCl. Cells were fixed 2 h after the medium change and immunostained for occludin. Bar=30 μm. (C) Quantification of occludin relocalization to cell membrane in (B). Error bars represent standard deviations of the mean occludin length per cell within each of the four randomly picked view fields.

Fig. S5. (A) shRNA constructs targeting two different sequences on afadin or an unrelated protein (Luc-ctrl) were transiently transfected into MDCK cells. Cells were cultured for 48 h in HCM. Cell lysates were probed with the indicated antibodies in a Western blot analysis. Bands in each blot corresponding to different antibodies were scanned from the same film, with extra lanes between the depicted samples removed by image processing. (B) MDCK cells transfected with control or afadin shRNAs were incubated in LCM for 16 h followed by replacement of the media with fresh LCM supplemented with 1 mM AICAR, DMSO, 10 μM SB216763, 20 mM NaCl or 20 mM LiCl. Cells were fixed 2 h after the medium change and immunostained for occludin. Bar=30 μm. (C) Quantification of occludin relocalization to cell membrane in (B). Error bars represent standard deviations of the mean occludin length per cell within each of the four randomly picked view fields. The asterisk (*) denotes significant differences in the indicated pairs ($P < 0.05$) by Student's *t*-test.

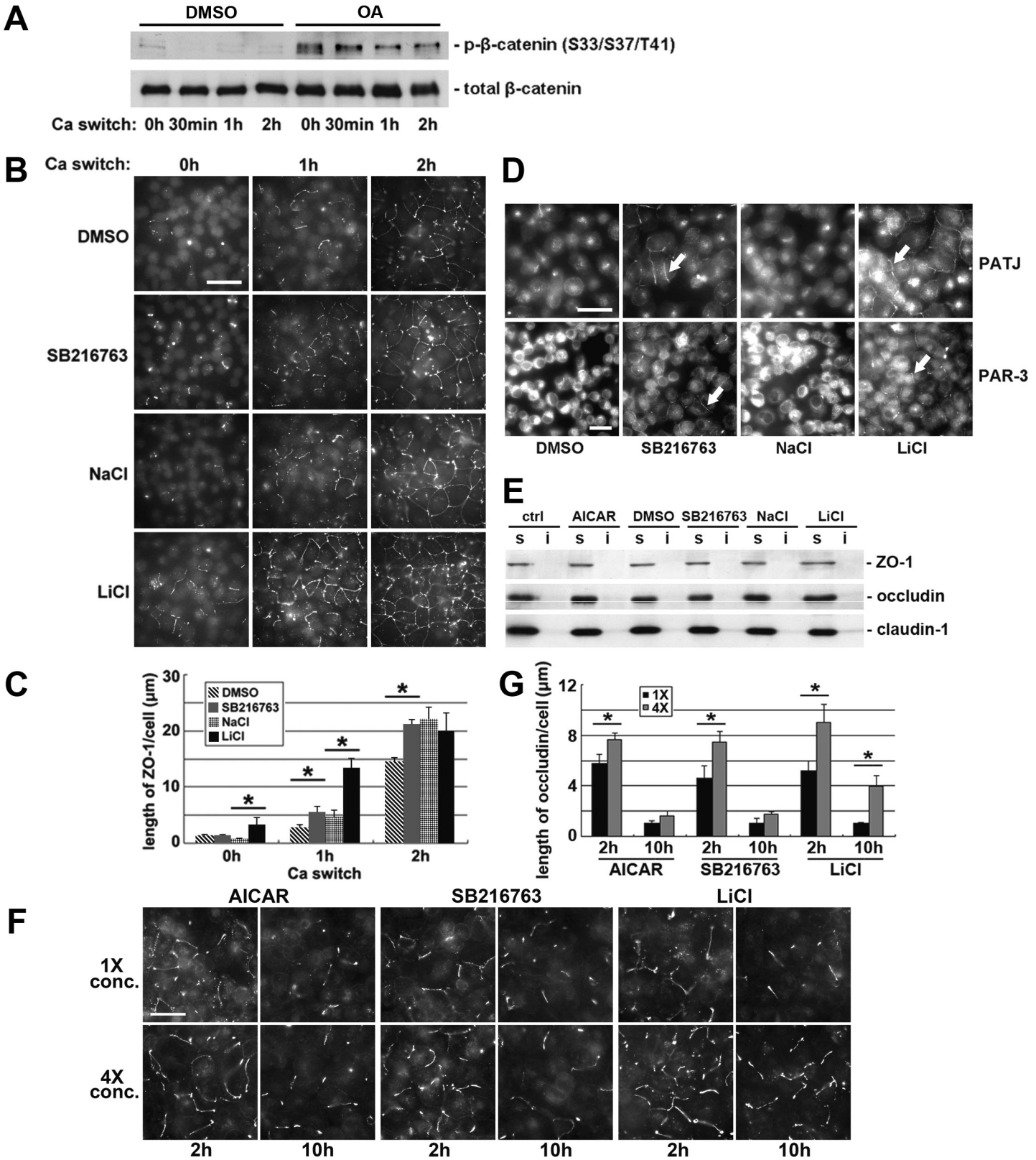
Fig. S6. (A) A schematic diagram of the phosphoproteomic study that made use of SILAC labeling to compare levels of phosphorylation of specific afadin peptides in response to AICAR treatment. Equal amounts of total protein from the control and the SILAC labeled treated samples were mixed and the afadin peptide compositions in the mixture were analyzed by LC-MS/MS. The changes in the phosphorylation levels of the peptides were detected as changes in these peptides' SILAC ratios. (B) SILAC labeled MDCK cells were incubated in LCM for 16 h before being incubated with 1 mM AICAR for 2 h in LCM (AICAR), or in LCM with no added drug for

2 h (control). Cell lysates from these treatments (AICAR and control) were mixed with the cell lysate from unlabeled MDCK cells incubated in LCM for 18 h. Peptide compositions of afadin immunoprecipitated from each condition were then analyzed by LC-MS/MS. Selected isolated peptides from MDCK cells are listed on the x-axis. The corrected ratio of light to heavy (SILAC ratio) of each peptide is plotted on the y-axis. (C) The SILAC phosphoproteomic study identified one afadin peptide whose phosphorylation increases in response to afadin. The sequence of this peptide (top line) is consistent with a consensus AMPK phospohorylation site (bottom line).

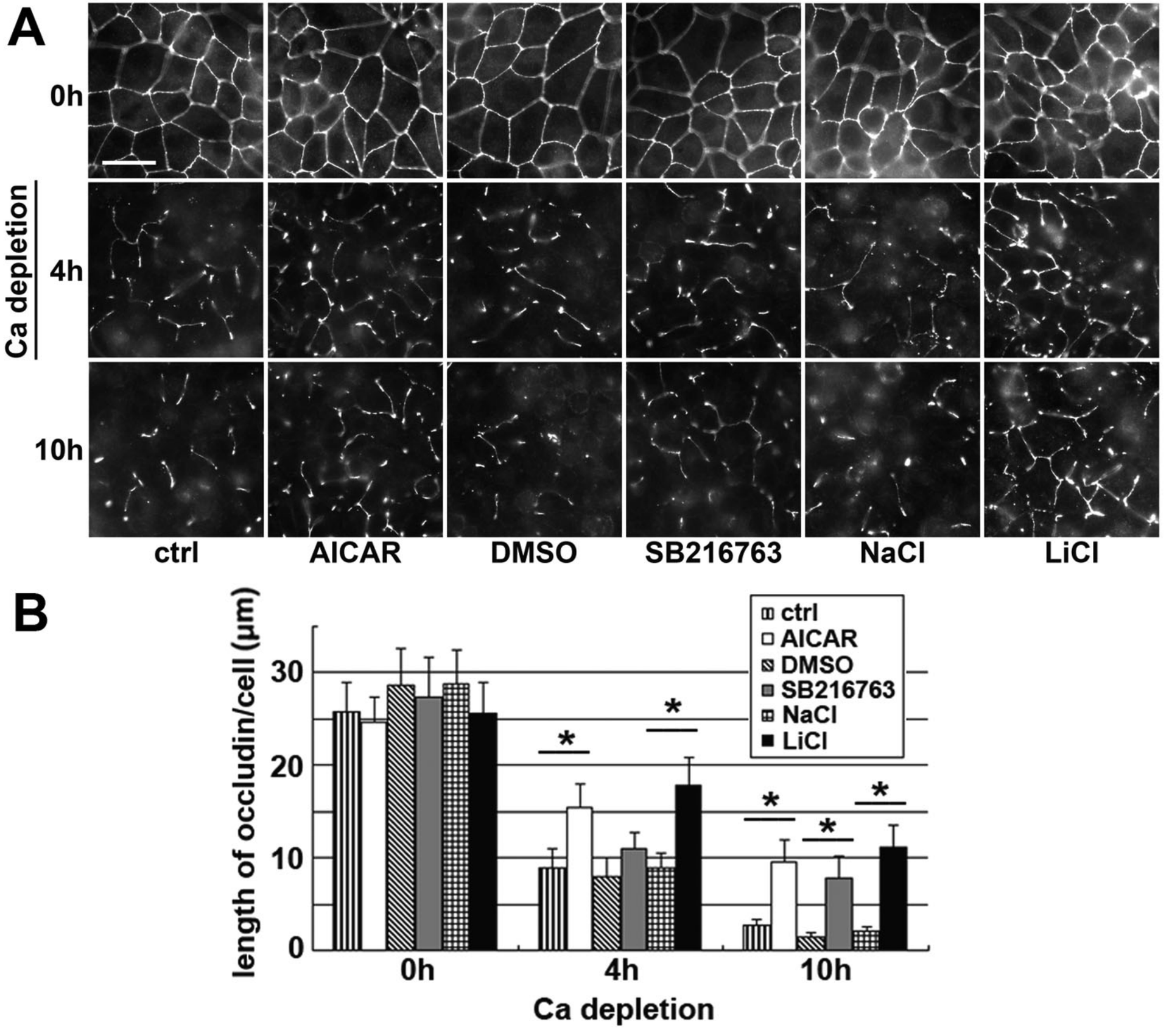
Table S1. Select peptides from native afadin (gi|73946249) identified by LC-MS/MS

Modification	**Sequence**	**TiO_2 fraction**	**m/z**	**'heavy' m/z**
	572- VLSASSTAER -581	Flow through	510.77	515.77 1xR
	590- VEQQQDYR -597	Flow through	533.25	538.26 1xR
Phosphorylation (S1102)	1100- TSSVVTLEVAK -1110	Enriched	607.31	610.32 1xK
Phosphorylation (S1201)	1191- SSPNVANQPPSPGGK -1205	Enriched	758.85	761.86 1xK
	1729- TASSLQAQALSPDSLYTAR -1747	Flow through	990.51	995.51 1xR

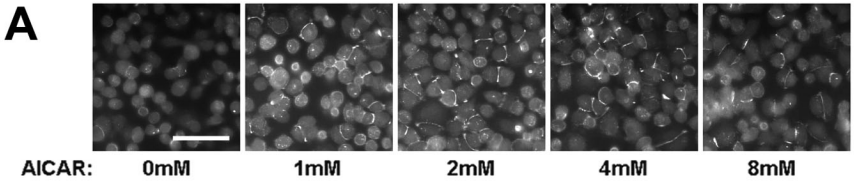
Phosphopeptides identified in LC-MS/MS analysis of afadin immunoprecipiated from MDCK cells. Afadin peptides were subjected to titanium dioxide (TiO_2) enrichment to separate phospho (enriched) and non-phosphopeptide (flow through) fraction for LC-MS/MS analysis.

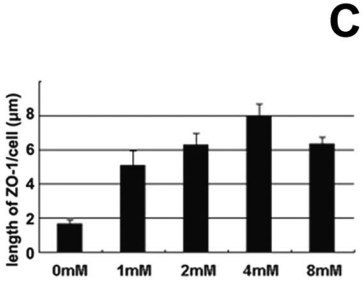


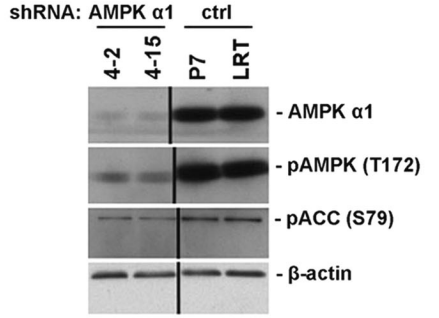
Zhang *et al*, Figure S1

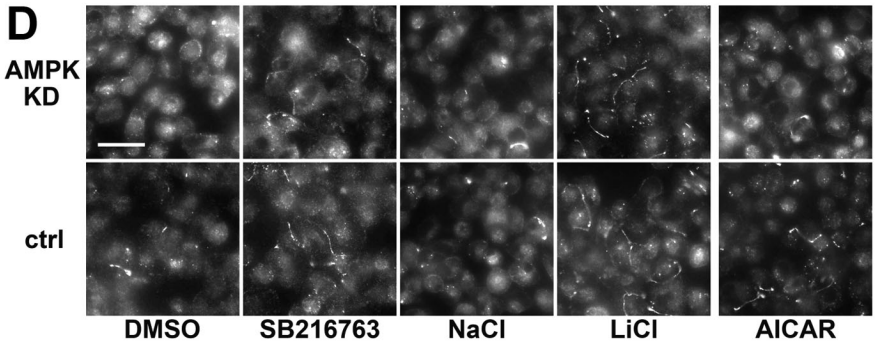


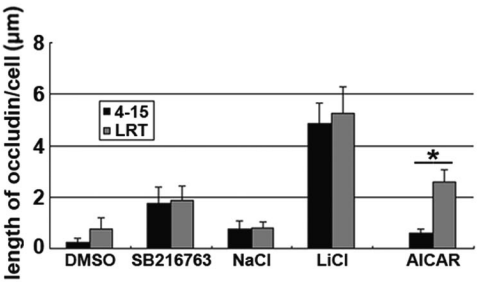
Zhang *et al*, Figure S2



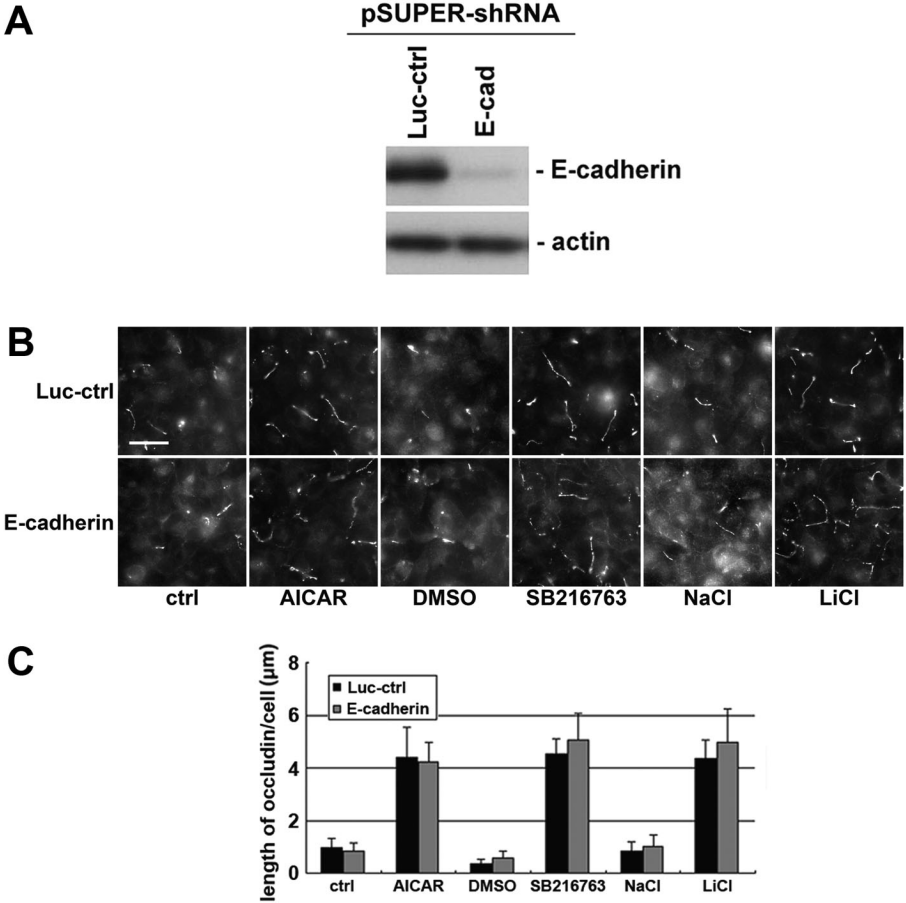




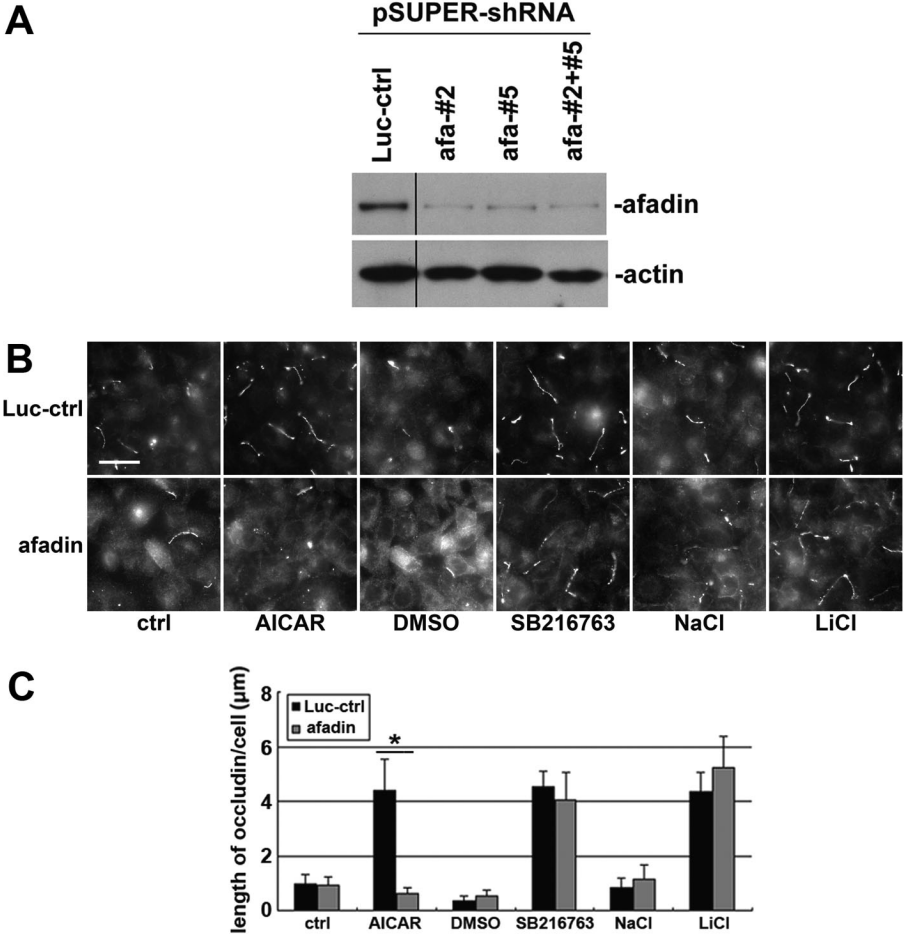




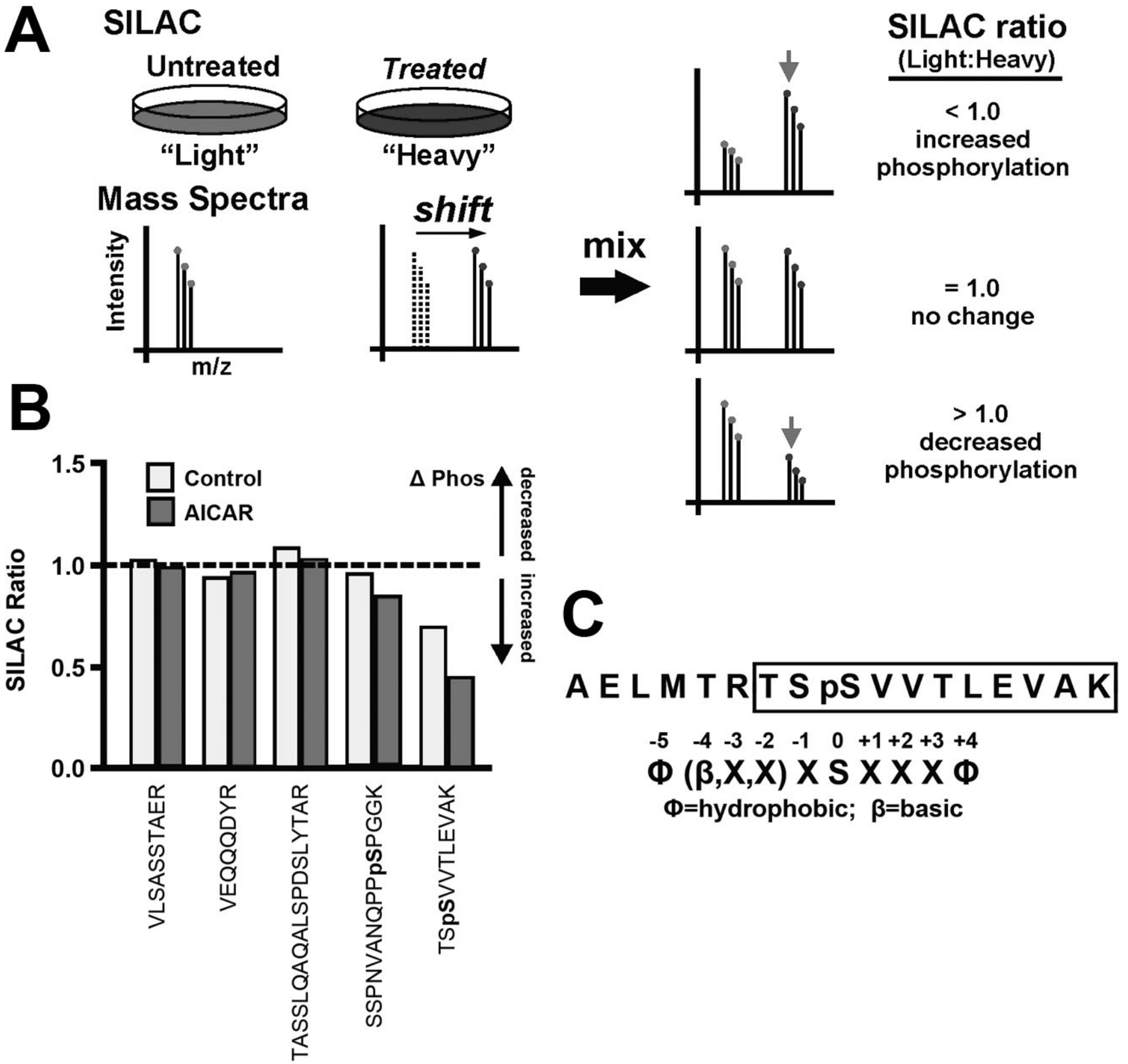
Zhang *et al*, Figure S3



Zhang *et al*, Figure S4



Zhang *et al*, Figure S5



Zhang *et al*, Figure S6