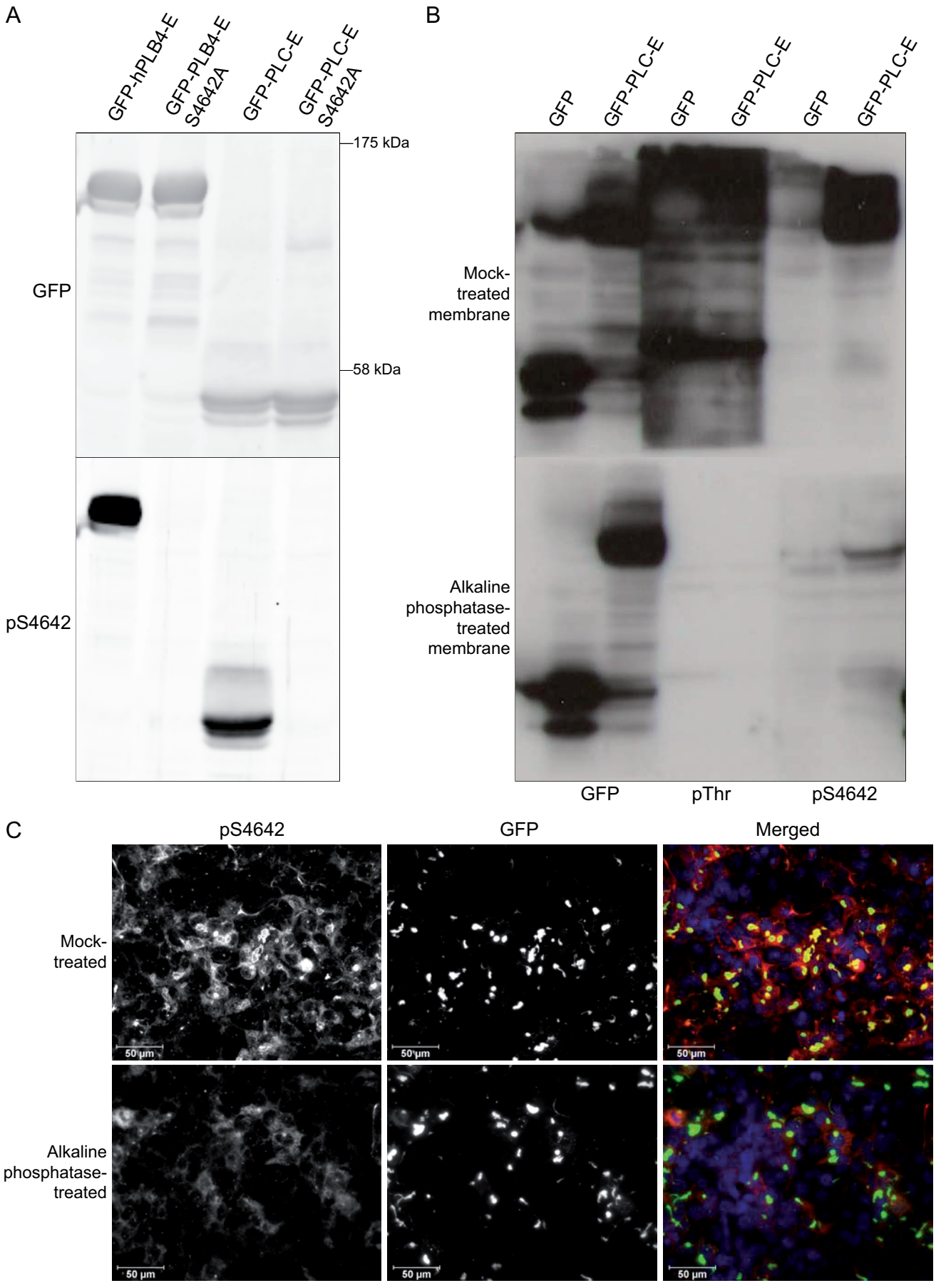

Figure S1. Specificity of the anti-plectin pS4642 Abs. A) WB analysis of whole extracts prepared from transiently transfected HEK 293T cells expressing various recombinant plectin proteins as indicated (8 % SDS-PAGE) with anti-GFP or –pS4642 Abs . Anti-pS4642 Abs recognized the GFP-tagged plectin proteins PL-B4-E and -PL-C-E but did not bind to the corresponding phospho-deficient S4642A mutants. WB development: upper panel: fluorescence, lower panel: ECL. B) Alkaline phosphatase treatment of WBs, prepared with whole extracts from transfected HEK 293T cells expressing GFP or GFP-PL-C-E (10 % SDS-PAGE), strongly decreased the immuno-signals (ECL) obtained with anti-phospho-threonine and anti-pS4642 Abs. C) Alkaline phosphatase treatment of transiently transfected HEK 293T cells expressing GFP-PL-B4-E (green) and fixed with methanol significantly decreased the immuno-reactivity of the anti-pS4642 Abs (red) toward the recombinant protein. The same laser intensities and exposure times were used for all pictures. Cells expressing S4642 mutants (A, G, D or E) were not recognized by the anti-pS4642 Abs (data not shown). Bar, 50 µm.

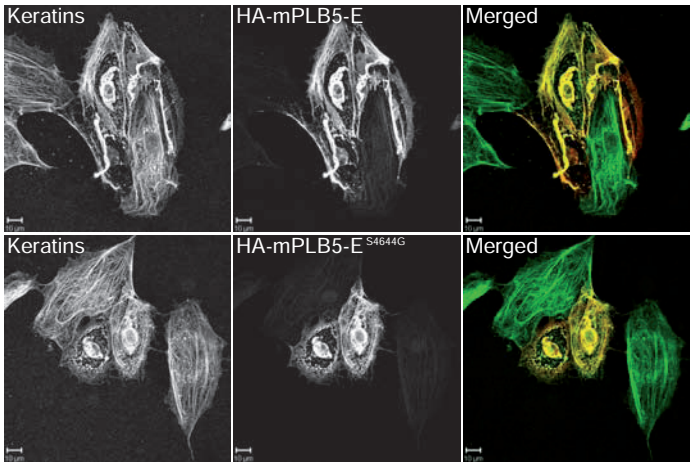

Figure S2. HA-mPL-B5-E and HA-mPL-B5-E-S4644G colocalize with and bundle IFs in transfected PtK2 cells. Immunofluorescence analysis of the localization of HA-tagged plectin proteins (red) with the keratins 8/18 network (green). No significant difference in localization pattern or IFs bundling was observed between the two recombinant proteins. Bar, 10 µm.

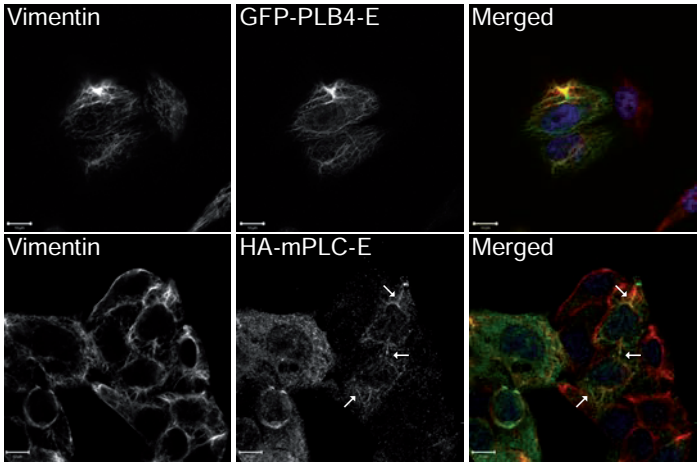

Figure S3. GFP-PL-B4-E and HA-mPL-C-E colocalize with vimentin IFs in transfected HeLa cells. Tagged recombinant plectin proteins (green), IFs (red), arrows: colocalized signals. Bar, 10 µm.

A

GAL4-BD	GAL4-AD: Vimentin	Desmin	K5/K14	K8/K14	NFL/NFH
mPLC-E	-	-	-	-	-
mPLC-E^{S4644G}	+	+	+	+	+

B

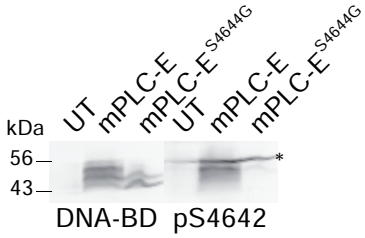

Figure S4. Serine 4644 in mPL-C-E prevents its interaction with various IF proteins in Y3H assays. A) +, - means growth or no growth of cotransformed PJ694 yeast strain on selection media. mPL-C-E, in contrast to mPL-C-ES4644G, did not interact with all tested IF proteins: vimentin, desmin, heterodimers of keratins 5/14, 8/18 and of NFL/NFH. B) Whole extracts from untransformed (UT) PJ694A yeasts or transformed with plasmids coding for GAL4-BD-mPL-C-E or GAL4-BD-mPL-C-ES4644G were analyzed by WB with anti-GAL4-BD and anti-pS4642/4 Abs. GAL4-BD-mPL-C-E was recognized by the anti-pS4642 Abs, indicating that it was phosphorylated (calculated size of GAL4-DNA-BD-PL-C-E: 48.6 kDa; *: unspecific band).

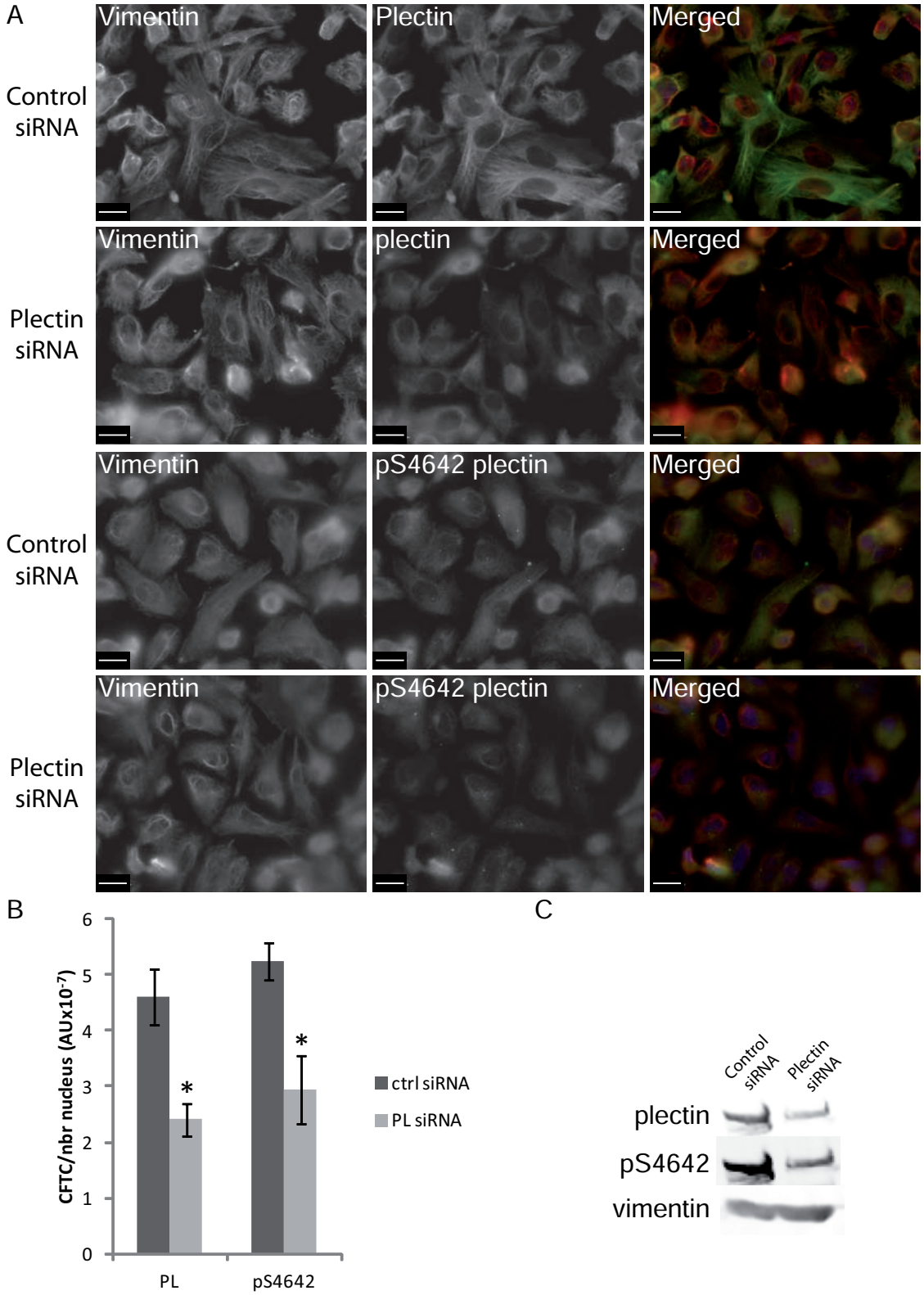

Figure S5. Anti-pS4642 and GP21 anti-plectin Abs have a similar specificity to plectin. A) SK-MEL-2 cells were cultured and subjected to control and plectin knock-down. Cells were fixed and immunofluorescently-labeled for vimentin, plectin and pS4642. After knock-down of plectin, signals given by both the anti-plectin and pS4642 Abs decreased. Bar, 10 µm. B) Quantification of the fluorescent signals in A with the Image J software showed an equivalent decrease of plectin and pS4642 signals. The CFTC represents the "corrected total fluorescence" of a picture which was divided by the number of nucleus. *P<0.005 (n>3). C) The efficacy of plectin knock down was checked by WB. Anti-vimentin Abs were used as loading control.

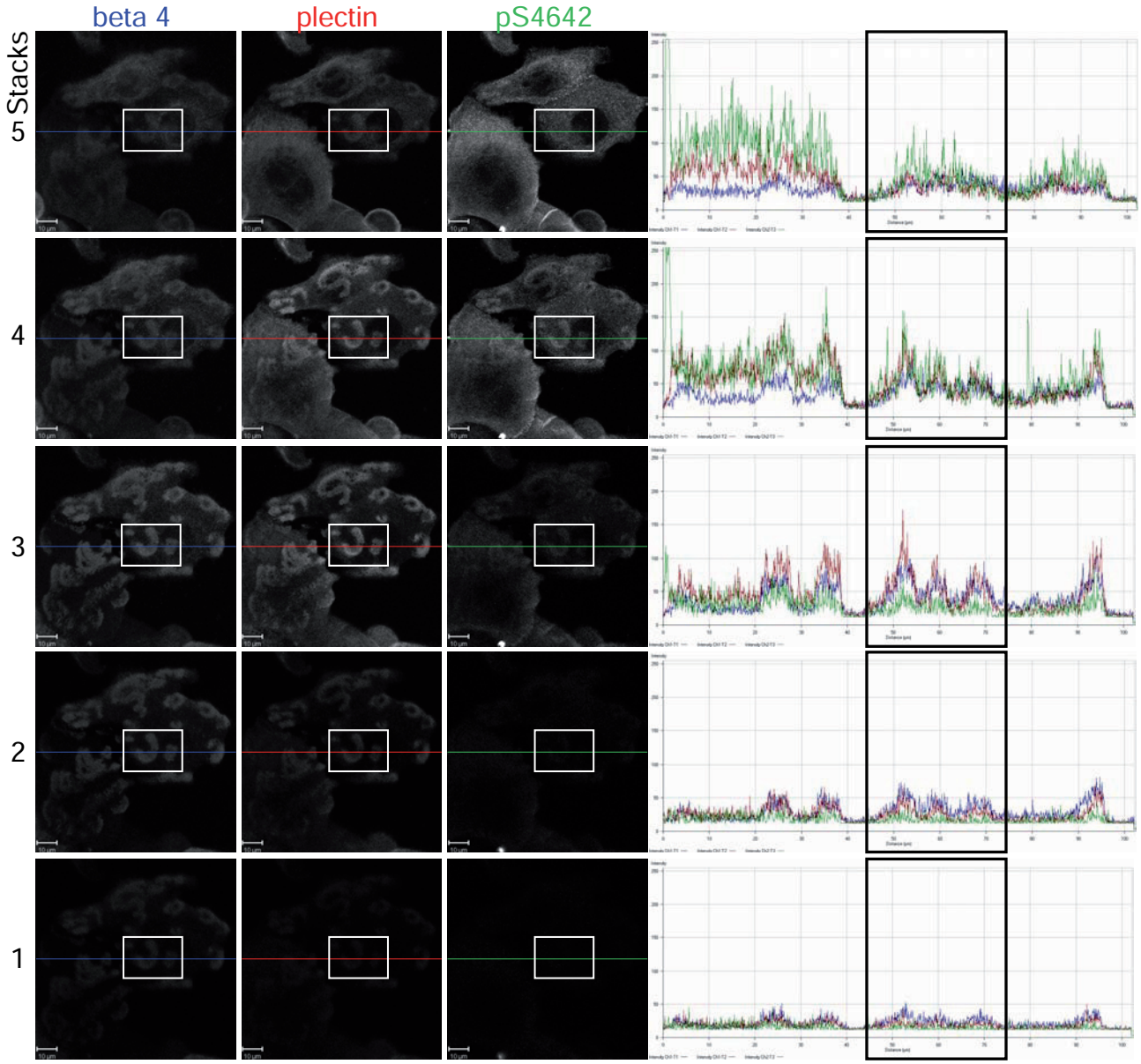

Figure S6. In the core of PAJEB-β4 cell-substrate contacts plectin is weakly phosphorylated at S4642. Cells cultured in high calcium for 24 h were fixed with formaldehyde and co-immunostained for integrin β4 subunit (blue), plectin (GP21; red) and pS4642 (green) as indicated. The three immunostaining intensity profiles along the Z stacks (measured along the colored horizontal lines) were different. In hemidesmosome-like structures (β4), the intensity ratio between pS4642 and plectin staining was not constant, as it strongly increased from stack 3 to 4. The white boxes in the pictures correspond to the black ones in the intensity profile graphs. Bar, 10 µm.

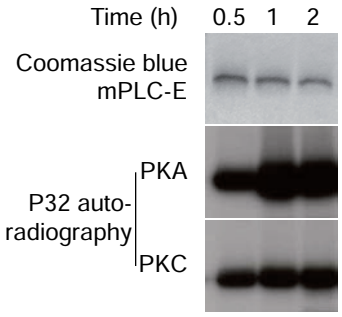

Figure S7. PL-C-E is phosphorylated in vitro by PKA and PKC. Purified recombinant H6-mPL-C-E was incubated with either PKA or PKC in the presence of γ-P32-ATP, separated on 10 % SDS-PAGE, stained with Coomassie blue and autoradiographed. Both PKs phosphorylated mPL-C-E. PKA phosphorylated a second serine residue located in the thrombin cleavage site encoded by the pET15b vector, explaining the stronger signals than with PKC.

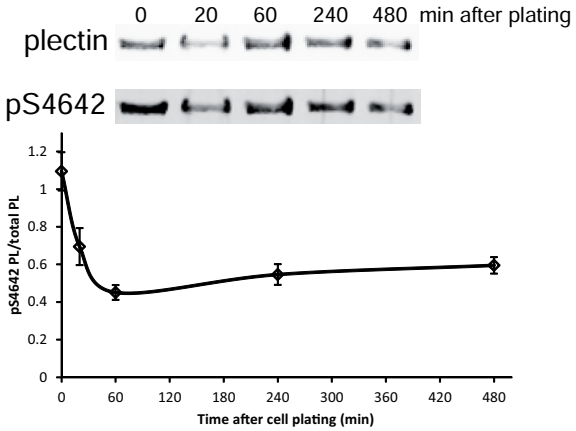

Figure S8. Replating of trypsinized HeLa cells is accompanied by a decrease in plectin S4642 levels. HeLa cells were detached by trypsin treatment and replated. Levels of plectin and pS4642 plectin were analyzed by WBs for different time points after replating.

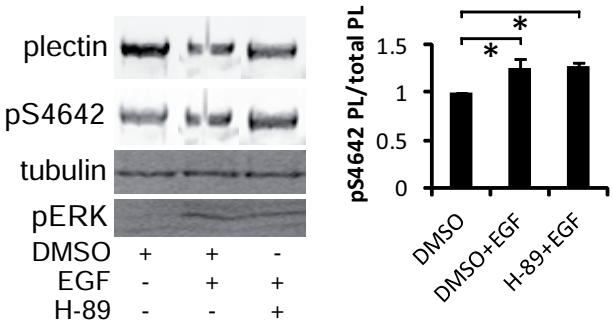

Figure S9. H-89 PK inhibitor does not block the EGF-induced increase in plectin pS4642 levels. Starved HeLa cells were pretreated 1 h with 10 μM H-89 or DMSO (control) prior to stimulation with 100 ng/mL EGF for 15 min. Levels of plectin, pS4642, tubulin and pERK 1/2 were analyzed by WB. *P<0.05 (n=3).

Antibodies	Targets	Raised in	Dilutions		Companies	References
			Microscopy	Western blot		
10F6	plectin	mouse		1:1000	Santa Cruz Biotechnology	sc-33649
4.3E1	β4-integrin	mouse	1:800			Hessle et al., 1984
DP I & II	desmoplakin	mouse	1:200		Boehringer	
Flag (M2)	flag-tag	mouse		1:1000	Sigma	F1804
GAL4 (DBD)	DNA BD	mouse		1:1000	Santa Cruz Biotechnology	sc-510
GAPDH	GAPDH	mouse		1:3000	Santa Cruz Biotechnology	sc-47724
GFP (B-2)	GFP	mouse		1:1000	Santa Cruz Biotechnology	sc-9996
GFP (FL)	GFP	rabbit		1:4000	Santa Cruz Biotechnology	sc-8334
GP21	plectin	guinea pig	1:500	1:10,000	Progen	GP21
HA	HA-tag	rabbit	1:100	1:1000	NeoMarkers	RB-1438-P1
HA	HA-tag	mouse		1:1000	Thermo scientific	sc-805
NW161	desmoplakin	rabbit		1:1000		Bornslaeger et al., 1996
PanKer	K5, 6, 8. 17	mouse	1:200	1:1000	DakoCytomation	M 0821 (MNF116)
pERK	p-ERK 1/2 (Thr 202)	rabbit		1:1000	Santa Cruz Biotechnology	sc-101760
pP38	phospho-p38 MAPK (Thr180/Tyr182)	rabbit		1:1000	Cell Signalling	9211S
pS4642	pS4642 plectin	rabbit	1:200	1:4000		
pS4642 Anti serum	pS4642 plectin	rabbit		1:1000		
pThr	p-threonine	rabbit		1:1000	Zymed	71-8200
RCK 106 (ascites fluid)	K18	mouse		1:10		J.H. Vost et al., 1993
TBN06	β-tubulin	mouse		1:1000	Thermo scientific	MA5-11732
Tubulin	α-tubulin	rabbit		1:10,000	Epitomics	2871-1
V9	vimentin	mouse	1:200	1:1000	Santa Cruz Biotechnology	sc-6260
Anti guinea pig-Alexa 568	guinea pig IgG (H+L)	goat	1:400		Life technologies	A11075
Anti guinea pig-Alexa 488	guinea pig IgG (H+L)	goat		1:15,000	GE Healthcare	A-11073
Anti mouse-Alexa 488	mouse IgG (H+L)	goat	1:400	1:2000	Life technologies	A-11029
Anti mouse-Alexa 568	mouse IgG (H+L)	goat	1:400		Life technologies	A11031
Anti mouse-Alexa 633	mouse IgG (H+L)	goat	1:400		Life technologies	A21052
Anti mouse-HRP	mouse IgG (H+L)	sheep		1:15,000	GE Healthcare	NA931
Anti mouse-IRDye 680	mouse Ig G (H+L)	goat		1:30,000	LI-COR	926-32220
Anti mouse-IRDye 800 CW	mouse Ig G (H+L)	donkey		1:15,000	LI-COR	926-32212
Anti rabbit-Alexa 488	rabbit Ig G (H+L)	goat	1:400		Life technologies	A-11008
Anti rabbit-Alexa 568	rabbit Ig G (H+L)	goat	1:400		Life technologies	A11011
Anti rabbit-HRP	rabbit Ig G (H+L)	goat		1:15,000	GE Healthcare	NA934
Anti rabbit-IRDye 800 CW	rabbit Ig G (H+L)	goat		1:15,000	LI-COR	926-32211