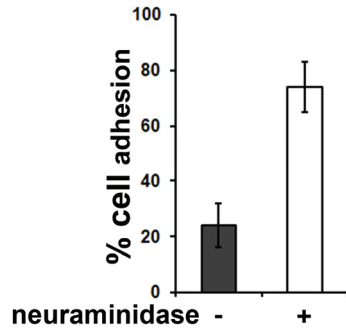
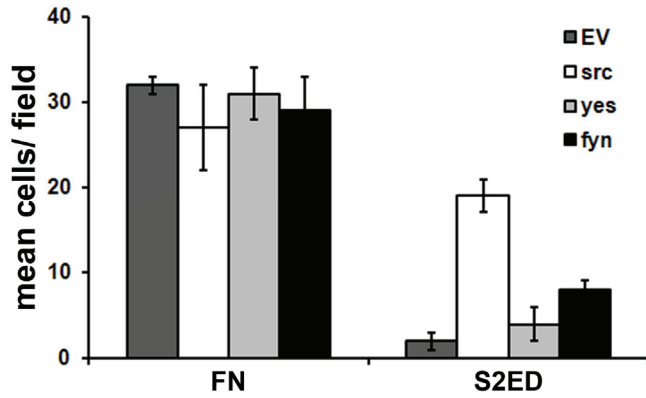


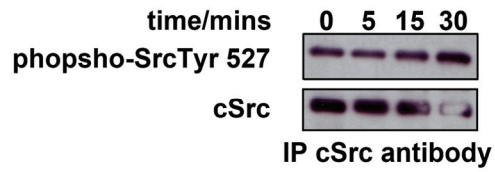
Supplemental Figure 1. Phorbol ester stimulated leukocyte adhesion to S2ED is mediated by P¹²⁴-F1⁴¹ of murine syndecan-2. (A). U937 cells were seeded in serum-free media in the presence or absence of 10 ng/ml of PMA for 40 min prior to fixation. Values obtained for cell adhesion to S2ED in the presence of PMA were set at 100% and error bars represent the standard deviation of 4 replicates. (B). U937 cells express CD148. FACS was performed on cells stained with CD148 (black solid line) or IgG control antibodies (grey dotted line).



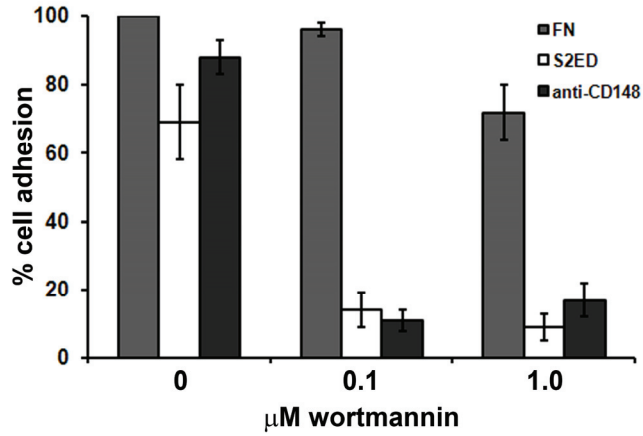
Supplemental Figure 2. ID8 Mouse ovarian surface epithelial cell adhesion to S2ED is stimulated by neuraminidase treatment. ID8 cells were in the presence or absence of neuraminidase (500U). Cells were incubated for 15 mins at 37 °C prior to seeding on wells coated with S2ED (light grey bars). Adhesion is shown relative to cell adhesion of untreated cells seeded on FN (data not shown). Error bars represent the standard deviation of data from 4 replicates.



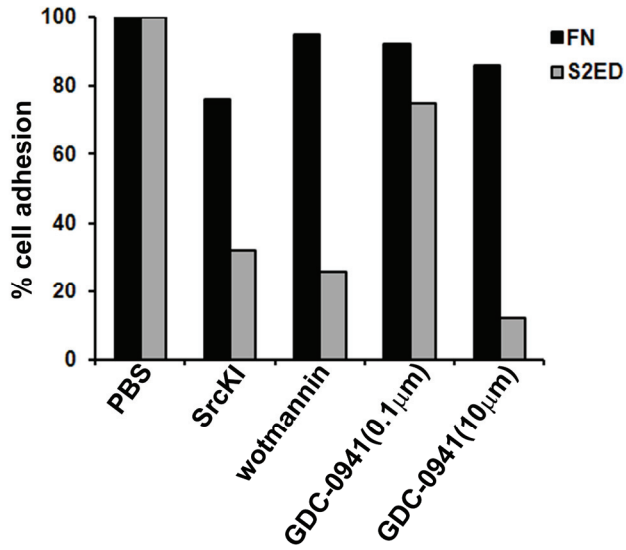
Supplemental Figure 3. Src kinase and not Yes or Fyn kinases are required for adhesion to S2ED. Syf cells were transfected with plasmids to express either Src (white bars), Yes (light grey bars) or Fyn (black bars) or empty vector (EV; dark grey bars). Transfected cells were seeded in serum free medium into wells coated with either FN or S2ED. After 1 hour cells were washed twice in PBS and adherent cells in 5 separate fields were counted using a 10x objective on an inverted microscope. Mean values were calculated and error bars represent standard deviation. Only expression of murine src restored adhesion by Syf cells S2ED.



Supplemental Figure 4. Phosphorylation of Src at Tyr⁵²⁷ remains unchanged during adhesion to S2ED. Syf+Src cells were seeded on S2ED coated plates for the time intervals indicated. Src was immunoprecipitated from cell lysates and analysed by western blot using antibodies specific for phospho-Src Tyr⁵²⁷.



Supplemental Figure 5. Wortmannin inhibits WI38 fibroblast adhesion to S2ED. WI38 fibroblasts were seeded in the presence of the PI3K inhibitor wortmannin at the concentrations indicated on FN (grey bars), S2ED (white bars) or anti-CD148 coated wells. Adhesion in serum-free conditions for 60 min was quantified. Error bars represent the standard deviation from values obtained from separate wells per condition.



Supplemental Figure 6. Adhesion to S2ED by primary murine lung fibroblasts requires both Src kinase and PI3 kinase activity. Primary murine lung fibroblasts were seeded on the substrates indicated in serum free media treated with either SrcKI, wortmannin or GDC-0941. Percentage cell adhesion was calculated based on adhesion to FN or S2ED with no inhibitors.

Supplemental table 1; Inhibition of Class I, II and III PI3K isoforms by Wortmannin and GDC-0941 (previously reported IC₅₀ values for lipid kinase activity, μ M).

Enzyme	Wortmannin (Domin et al., 1997; Linassier et al., 1997; Arcaro et al., 1993; Chaussade et al., 2007)	GDC-0941 (Folkes et al., 2008)
p110α (class IA)	0.0005	0.003
p110β (class IA)	0.0023	0.0033
PI3K-C2α (class II)	<i>Insensitive</i> (0.420, max at 10 mM)	<i>Not published</i>
PI3K-C2β (class II)	0.0016	0.670
Vps34 (class III)	0.01	<i>Insensitive</i> (> 10)

Supplemental table 2; Primer sequences

Primer	Sequence	Construct
Ms2-gagfor	agtccagttctgacaacatc	S2ED-GAG
Ms2-gagrev	gtcgtcgtcatcaccgga	S2ED-GAG
Ms2mut1for	tagcccaaagtggaaacctgac	S2ED-mut1
Ms2mut1rev	ggaagcagcactagtgagtg	S2ED-mut1
Ms2mut2for	tagcctgaagaaactgacaagga	S2ED-mut2
Ms2mut2rev	tgactcagtctgagcaggtgt	S2ED-mut2
Ms2mut3for	tagcctgctataaaaagcacagat	S2ED-mut3
Ms2mut3rev	gccagcttctcttctgcct	S2ED-mut3
Ms2mut4for	cctgctataaaaagcacagatg	S2ED-mut4
Ms2mut4for	ggatgtgtcagaactggac	S2ED-mut4
CD148sffor	tatataggatccgcaggtggcaccctagt	pCD148sf
CD148sfrev	tataaaagctttccagttctattgcaaactgtc	pCD148sf