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Supplemental Information

**Unraveling the Receptor-Ligand Interactions between Bladder Cancer
Cells and the Endothelium Using AFM**

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Supporting Information

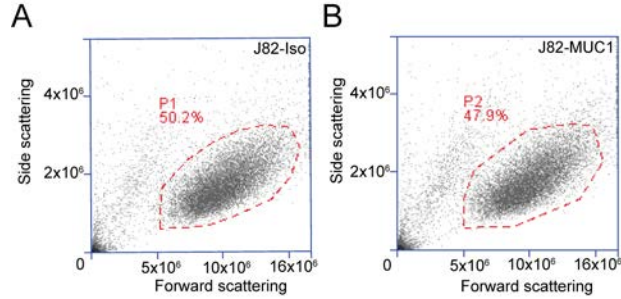


Figure S1: Scatter plots showing the gating of cells. J82 cells are gated for isotype control (A) and MUC1 (B) to analyze the fluorescence signals.

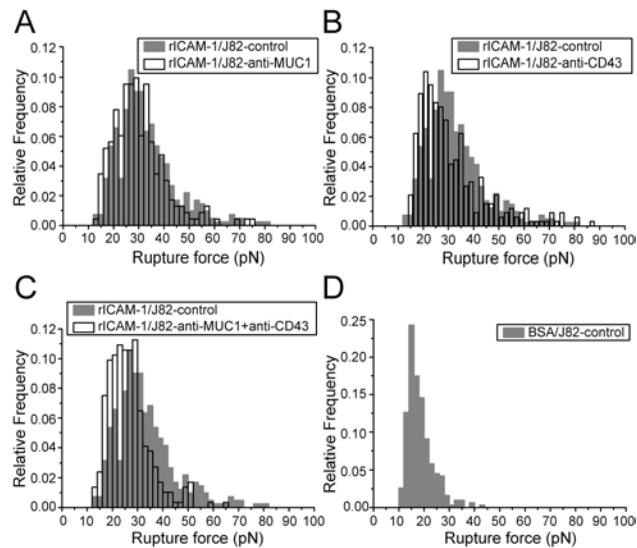


Figure S2: Quantification of bladder cancer-rICAM-1 adhesion using SCFS while blocking different receptors involved in the interaction. Force histograms showing the distribution of rupture events for the adhesion of rICAM-1 with J82 bladder cancer cells for different conditions were obtained from the force curves (applied force 500 pN, time of contact 10 s, velocity 5 $\mu\text{m/s}$). Histograms obtained while blocking MUC1 (A), blocking CD43 (B) and blocking MUC1+CD43 (C) on J82 cells were compared with the control. (D) Rupture force histogram for nonspecific interactions was obtained by using BSA-coated substrate.

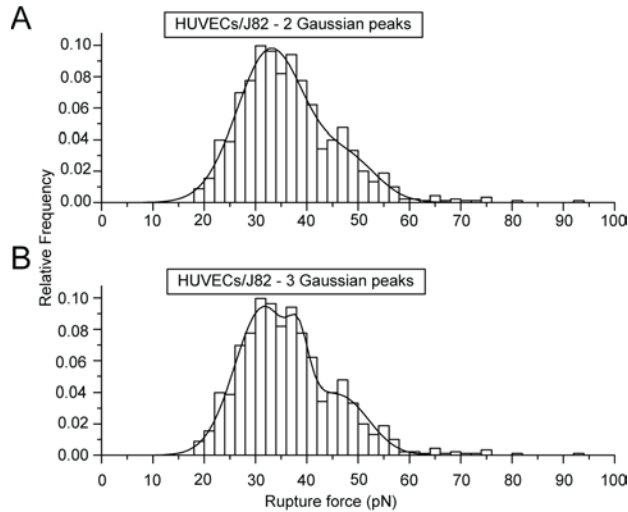


Figure S3: Qualitative analysis of Gaussian Mixture Model. Force histogram obtained from the adhesion of HUVEC monolayer with J82 bladder cancer cell was fitted with two (A) Gaussian models (adjusted R-square 0.974) or three (B) Gaussian models (adjusted R-square 0.981).

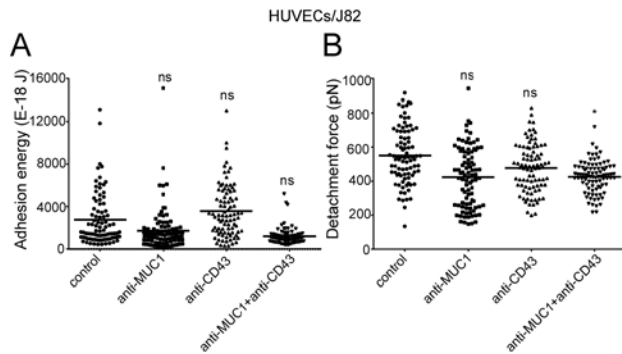


Figure S4: Adhesion energy and detachment force for different conditions. Adhesion energy (A) and detachment force (B) obtained for the interaction of HUVEC monolayers with J82 cancer cell while blocking MUC1, CD43 or both. Bars indicate mean values. GLMM (R software) was performed to check the significance with respect to the control, * $p < 0.05$ and ns $p > 0.05$.

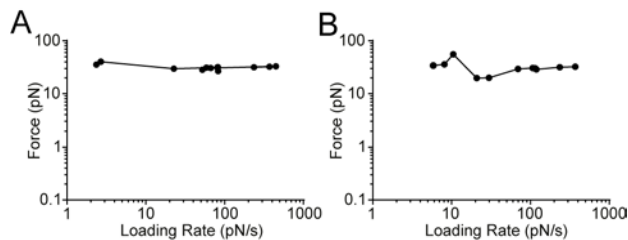


Figure S5: The local loading rate before the rupture events in a force curve was calculated from the slope before rupture. We checked the local loading rate for two curves, and the results are shown on the two graphs. In both cases, there were around 10 rupture events for each curve and the rupture force was plotted as a function of the loading rate. From the graphs, we can observe that there is hardly any effect of the local loading rate on the rupture forces, in the range observed in our experiments.

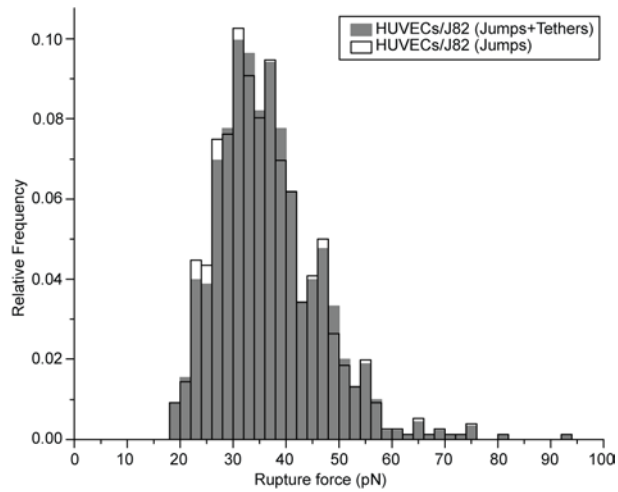


Figure S6: Including the tethers do not alter the shape of force distribution. A force histogram showing the distribution of rupture events for the adhesion of HUVEC monolayer with J82 bladder cancer cells considering both jumps and tethers was compared with one considering jumps alone.

Bin start – Bin end (pN)	control (events)	anti-MUC1 (events)	anti-CD43 (events)	anti-MUC1+anti-CD43 (events)
28-30	70	86	84	76
30-32	90	89	83	64
32-34	87	82	73	58
34-36	74	68	68	61
36-38	85	50	47	43
38-40	70	25	46	21
40-42	56	25	38	11
42-44	31	13	30	11
44-46	36	10	16	8
46-48	43	7	10	4
48-50	30	8	10	6
50-52	18	4	8	6
52-54	12	7	5	4
54-56	17	3	4	3
56-58	9	0	3	2
58-60	2	0	5	1

Table S1: Rupture events <36pN are unresponsive when blocking the receptors. The number of rupture events obtained for bins (28-60pN) is represented for different conditions (control, anti-MUC1, anti-CD43 and anti-MUC1+anti-CD43). No significant decrease in the number of rupture events was observed when blocking the receptors (MUC1 or CD43 or both) for forces < 36 pN as compared to the control. In contrast, when considering the data >36 pN, the number of rupture events significantly decreased when blocking the receptors (MUC1 or CD43 or both) as compared to the control (indicated in red in the Table). When performing similar analysis with rICAM-1 as the substrate we found a different limit of 30 pN (data not shown).

substrate	J82 cell condition	peak 1 mean±SEM	peak 2 mean±SEM	peak 3 mean±SEM
HUVECs	control	30.0±0.28	39.5±0.4	53.0±1.82
	anti-MUC1	29.1±0.23	43.4±1.53	
	anti-CD43	31.0±0.28		50.4±1.75

Table S2: Force range obtained from GMM analysis of SCFS data for the interaction of BCs with ECs considering jumps alone (velocity 5 μm/s).

GMM analysis revealed that the interaction of CD43 is mediated by a mean rupture force of ~43 pN and the interaction of MUC1 is mediated by a mean rupture force of ~50pN.