How carrier size and valency modulate receptor-mediated signaling: understanding the link between binding and endocytosis of ICAM-1-targeted carriers

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Supporting Information

Condition	Submicrometer Carrier[b] (Size, μm)	Micrometer Carrier[b] (Size, μm)
Freshly Prepared	0.260 ± 0.003	1.15 ± 0.02
30 min in Saline	0.262 ± 0.002	1.21 ± 0.06
30 min in Serum	0.245 ± 0.004	1.02 ± 0.17

[b]carriers with only anti-ICAM (no IgG on the coat).

Figure S1. Stability of anti-ICAM carriers. The size of submicrometer (250 nm) and micrometer (1 μm) anti-ICAM carriers was measured by dynamic light scattering after being freshly prepared, or after a 30 min incubation at 37°C in saline or 20% serum. Mean ± SEM.

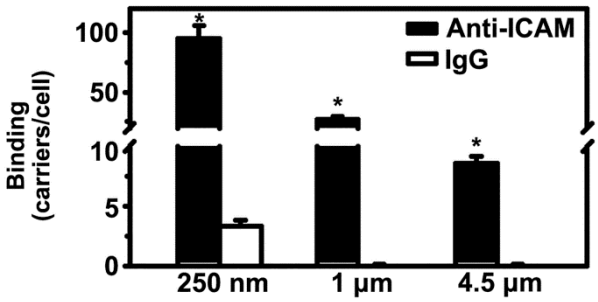

Figure S2. Specific binding of anti-ICAM carriers to endothelial cells. Binding of 250 nm, 1 μm or 4.5 μm anti-ICAM carriers *vs.* IgG carriers to fixed activated HUVECs after 30 min incubation at room temperature, analyzed by microscopy as in Figure 2. Mean ± SEM. *Compares IgG carriers to anti-ICAM carriers for each carrier size ($p<0.05$ by Student's *t*-test).

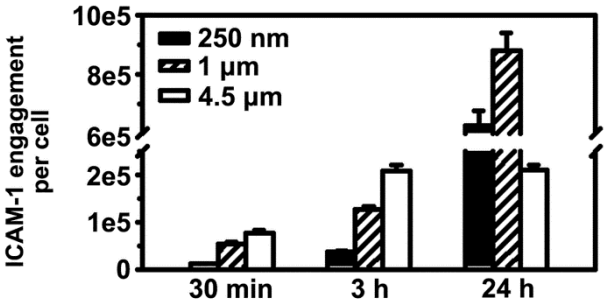

Figure S3. Estimated engagement of ICAM-1 by anti-ICAM carriers bound on endothelial cells. Number of ICAM-1 molecules theoretically engaged by 250 nm, 1 µm or 4.5 µm anti-ICAM carriers after 30 min, 3 h or 24 h incubation with fixed activated HUVECs at room temperature. ICAM-1 engagement is calculated by multiplying the total number of carriers bound per cell by the number of antibodies on the effective binding surface of each carrier (see Materials and Methods). Mean ± SEM.

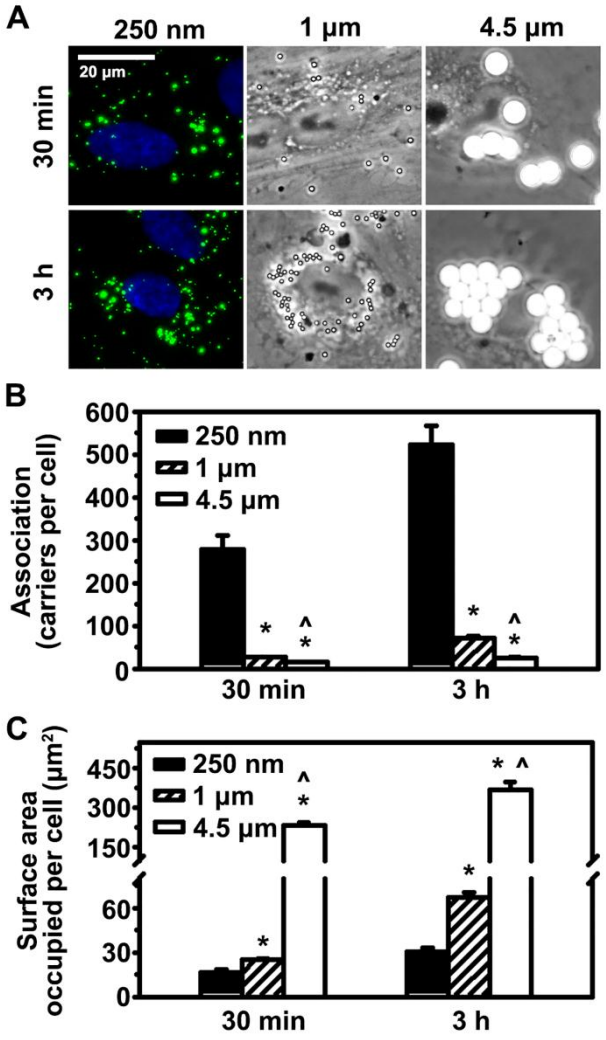

Figure S4. Association of anti-ICAM carriers to live endothelial cells. (A) Fluorescence (left) or phase-contrast (middle and right) microscopy showing association of 250 nm, 1 µm or 4.5 µm anti-ICAM carriers (^{b} in Table 1) after 30 min or 3 h incubation at 37˚C with live activated HUVECs. Scale bar = 20 µm. (B) Quantification of the total number of carriers associated per cell. (C) Estimation of the cell-surface area occupied by all carriers bound on a cell. Mean ± SEM. *Compares 1 µm or 4.5 µm to 250 nm; ^compares 4.5 µm to 1 µm (p<0.05 by Tukey's test after One-way ANOVA).

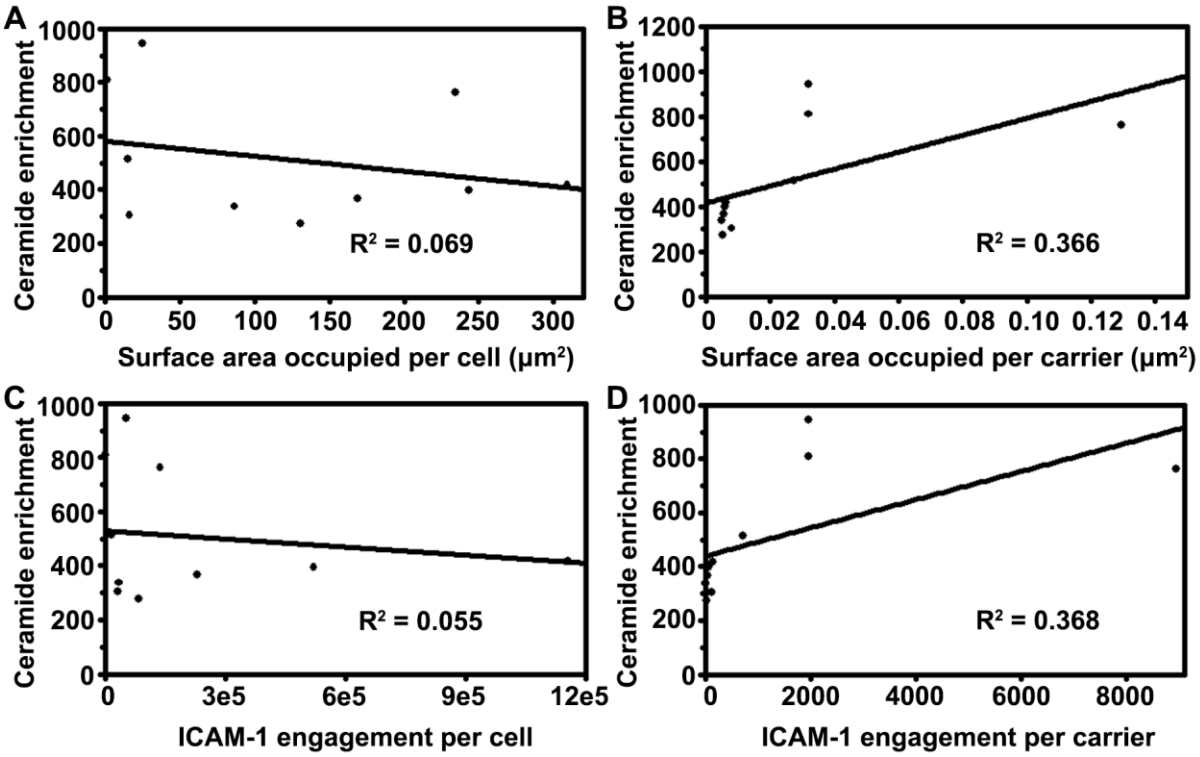

Figure S5. Analysis of factors influencing ceramide enrichment at sites of binding of anti-ICAM carriers to endothelial cells. Relationship between ceramide enrichment *vs.* the: (A) total surface area occupied by all carriers bound on a cell, (B) surface area occupied by each individual carrier bound on a cell, (C) total number of ICAM-1 molecules engaged by all carriers bound on a cell, or (D) the total number of ICAM-1 molecules engaged per individual carrier bound on a cell. All incubations were conducted for 15 or 30 min at 37˚C with live activated HUVECs, followed by ceramide staining and quantification, as in Figures 4 and 5. For details on the calculations see Materials and Methods. The line represents a linear regression, for which R^2 = regression coefficient.