SUPPLEMENTAL INFORMATION

Collaborative Enhancement of Endothelial Targeting of Nanocarriers by Modulating Platelet Endothelial Cell Adhesion Molecule-1/CD31 Epitope Engagement

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Table S1.[a] Binding Epitopes and Parameters of Radioiodinated anti-PECAM Ab to Live Endothelial Cells (EC)

Ab	Species Reactivity	clone	Amino Acid (AA) Epitope Mapping, Ig Domain (IgD)	PECAM		Effect on PECAM Homophilic Adhesion
				K_d (nM)	B_{max} (10^5 Ab/cell)	
Ab_{1h}	Human	62	AA26-AA30, IgD1	4.32 ± 0.30[b]	2.62 ± 0.26[b]	Inhibit
Ab_{2h}	Human	37	AA42-AA45, IgD1	0.24 ± 0.02[b]	1.51 ± 0.12[b]	No effect
Ab_{1m}	Mouse	MEC13	AA164-AA177, IgD2	2.81 ± 0.13[c]	5.81 ± 0.2[c]	No effect
Ab_{2m}	Mouse	390	AA194-AA205, IgD2	0.25 ± 0.01[c]	2.60 ± 0.06[c]	Inhibit

[a]Ref.[1]
[b]Ab binding to native huPECAM on HUVEC
[c]Ab binding to native muPECAM on murine MS1 cells

Table S2.[a] Lung:Blood Tissue Selectivity Summary

Formulation	Localization Ratio		
	IgG	Paired Ab	Specificity Index
^{125}I-Ab_{1m}	2.98	10.22	3.4
^{125}I-Ab_{2m}	2.40	7.75	3.2
Ab_{1m}/^{125}I-NC	17.55	183.58	10.5
Ab_{2m}/^{125}I-NC	62.42	238.75	3.8

[a]Mice were injected intravenously with IgG or paired Ab (at t=-30 min), followed by radiolabeled probe (anti-PECAM ^{125}I-Ab or ^{125}I-NC) (at t=0 min). Tissues were harvested at t=30 min. Specificity Index denotes the relative lung:blood localization ratio of paired Ab group to IgG control group.

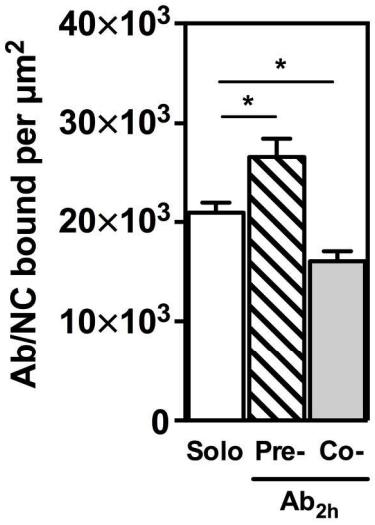

Figure S1. Free paired Ab_{2h} pre-incubation specifically enhances EC binding of Ab_{1h}/NCs. EC binding of Ab_{1h}/NC at high Ab density over particle surface (200 Ab/NC) was enhanced by paired free A_{2h} when pre-incubated with cells (hashed bar) than when cells were co-incubated with paired Ab and Ab_{1h}/NC (grey bar) when compared to solo binding (open bar). The number of total EC bound fluorescent particles in each image field as quantified by fluorescence microscopy. Data are mean ± SE (n=8).

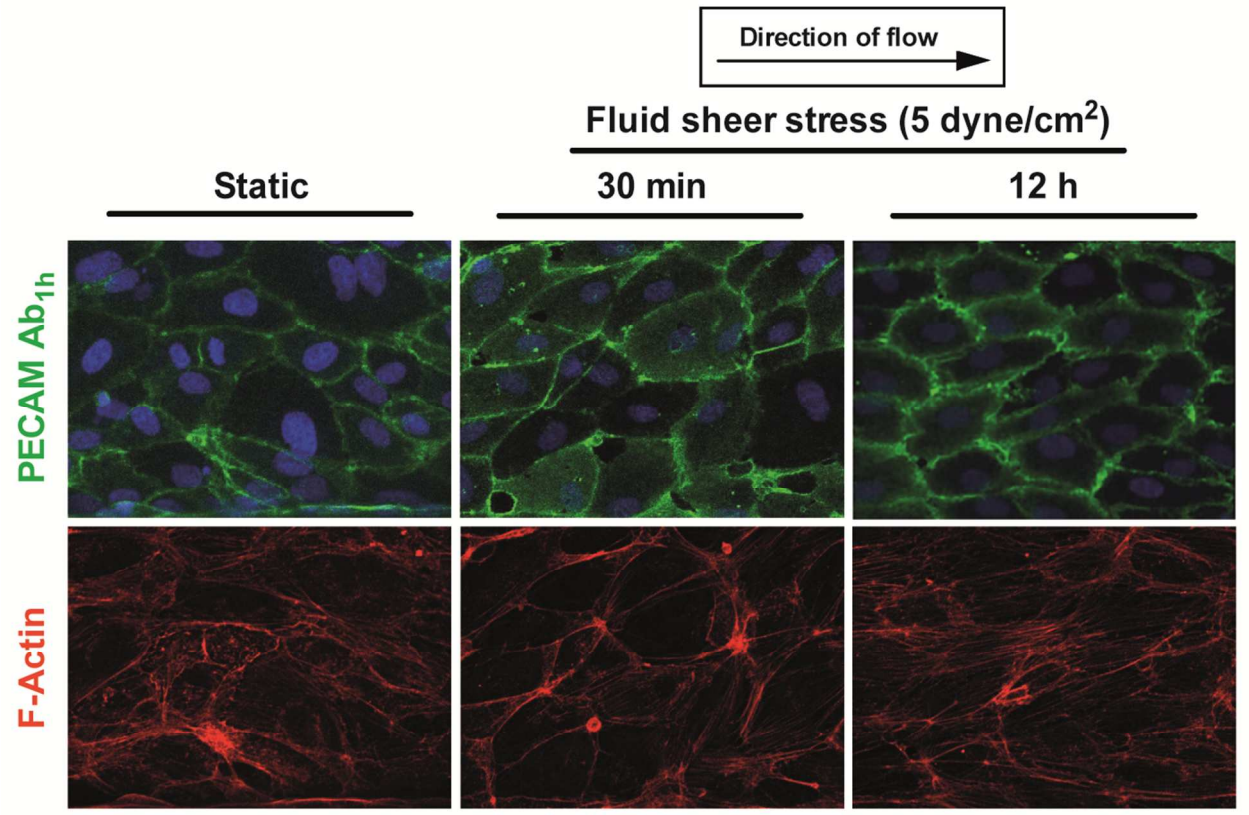

Figure S2. Distribution of PECAM and F-actin with and without exposure to flow. Confluent monolayers of HUVEC were exposed to static conditions, short-term (30 min) acute flow, or overnight (12 h) flow adaption and subsequently fixed, permeabilized, and stained for PECAM (green) and F-actin (red). In contrast to F-actin, PECAM staining demonstrates neither a change in intensity nor distribution, with PEMCAM remaining predominantly at the cell-cell junctions.

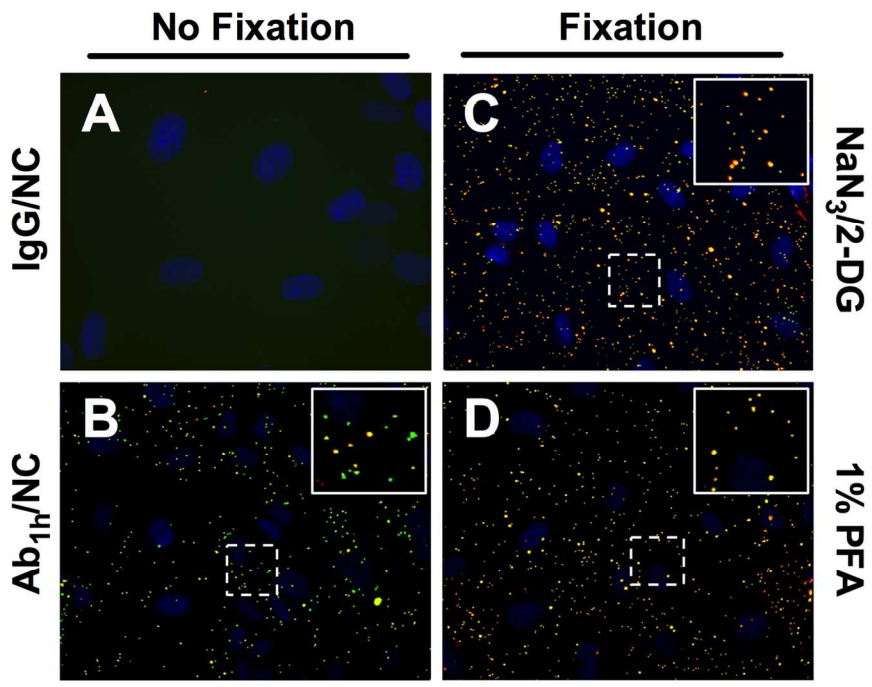

Figure S3. Microscopy studies reveal no internalization of fully coated Ab_{1h}/NC in fixed ECs *versus* live ATP-depleted ECs under fluid shear stress (4 dyne/cm^2) following counterstain with secondary red anti-IgG. Merged fluorescence images show internalized (green) and surface-bound (yellow) Ab/NC, with DAPI-stained nuclei (blue). (A) Control IgG/NC have negligible surface binding, whereas Ab_{1h}/NC undergo endocytosis under flow (B). (C) Endocytosis of Ab_{1h}/NC was blocked in ATP-depleted EC treated with NaN_3/2-DG (5 mM, 30 min) as well as with (D) EC pre-fixed with PFA (1%, 10 min). Inset in each panel is close up of selected field.

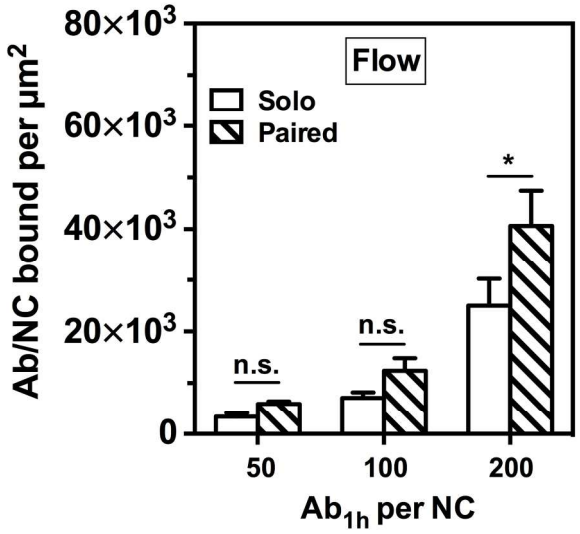

Figure S4. Under flow conditions (4 dyne/cm^2), pre-incubation of paired Ab_{2h} (20 nM) stimulated endothelial binding of Ab_{1h}/NCs with different targeted antibody density on particle surface (*i.e.*, # Ab per particle) as compared to pre-incubation with solo control IgG. The number of total EC bound fluorescent particles in each image field as quantified by fluorescence microscopy. Data are shown as mean ± SE (n=8). *, P<0.05; ***, P<0.001; n.s., P>0.05. Open bars show NC binding with solo treatment; hashed bars show NC binding with paired free Ab treatment.

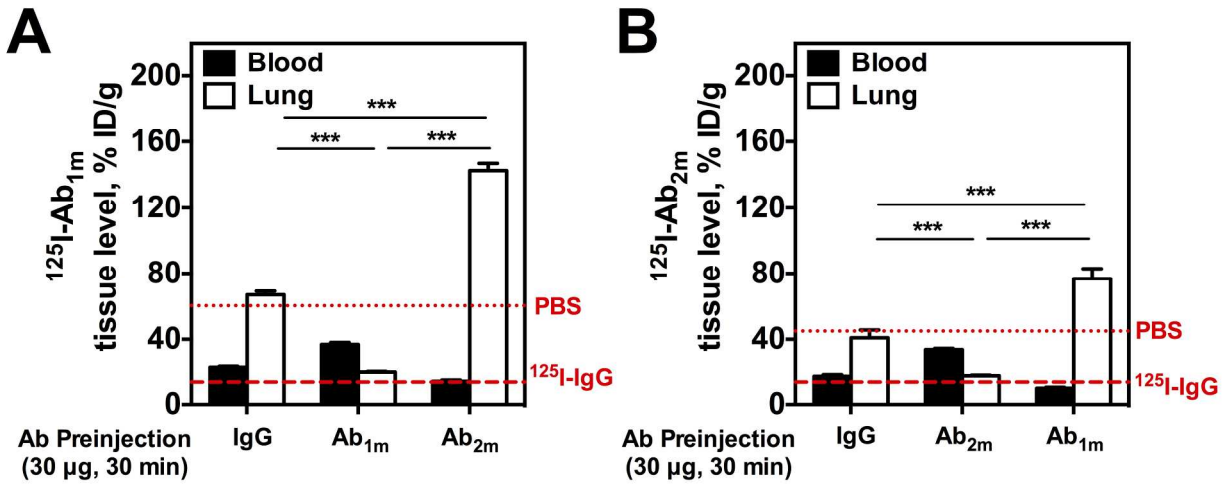

Figure S5. *In vivo* endothelial targeting of Ab/^{125}I-NC to muPECAM is enhanced by paired muPECAM Ab. (A) Lung tissue radioactivity levels at 30 min p.i. of anti-muPECAM ^{125}I-Ab$_{1m}$ is enhanced when mice are preinjected with paired Ab$_{2m}$ and uptake blocked with self-paired mAb$_{1m}$. Paired enhancement of lung activity levels results in significantly decreased blood levels. The dashed line indicates non-specific lung activity levels of control ^{125}I-IgG and dotted line indicates lung activity levels of probe with vehicle control PBS pre-injection. (B) Lung tissue activity of Ab$_{1m}$/^{125}I-NC (200 µg NC per mouse) at 30 min p.i. is enhanced significantly with paired Ab$_{2m}$ (30 µg for 30 minutes) as compared to IgG pre-injection. Blood levels of Ab$_{1m}$/^{125}I-NC following paired Ab treatment is lower than blood in IgG pre-treated mice. Dashed line indicates lung uptake of non-specific IgG/^{125}I-NC and dotted line is Ab$_{1m}$/^{125}I-NC lung uptake blocked with Ab$_{1m}$ pretreatment. Data represented as mean ± SE (n = 5 mice per group). *, *P*<0.05, ***, *P*<0.001.

REFERENCES

1. Chacko, A. M.; Nayak, M.; Greineder, C. F.; Delisser, H. M.; Muzykantov, V. R. Collaborative enhancement of antibody binding to distinct PECAM-1 epitopes modulates endothelial targeting. *PLoS One* **2012**, 7, e34958.