Supporting Information

Photoinduced reconfiguration to control the protein-binding affinity of azobenzene-cyclized peptides

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S1. Druggability analysis of the surface of VCAM1

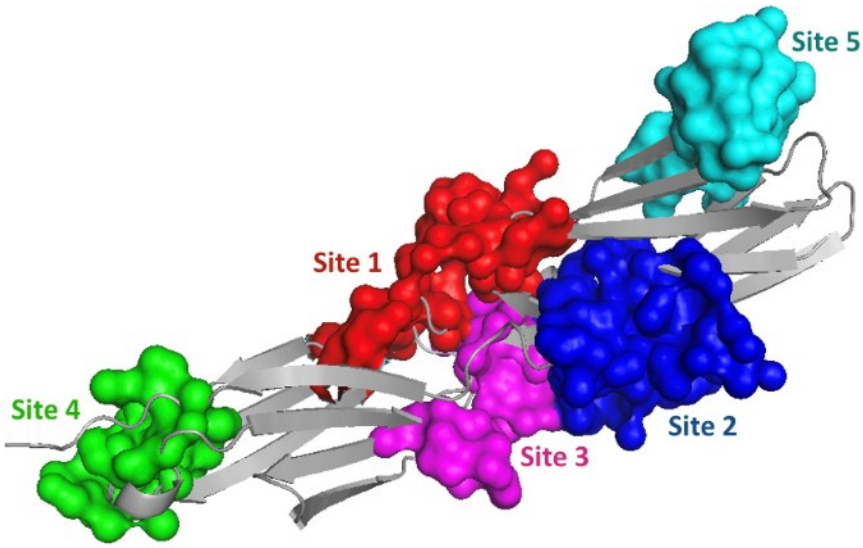

Fig. S1 Putative peptide binding sites (S1 – S5) on VCAM1 identified by performing a druggability analysis of the crystal structure of VCAM1 (PDB ID: 1VCA) using SiteMap.

Table S1 SiteMap parameters for the top five scored potential VCAM1 binding pockets. All scores are unitless unless otherwise noted.

Property	**S1**	**S2**	**S3**	**S4**	**S5**
S-score	0.89	0.86	0.89	0.87	0.73
Size (points)	323.00	142.00	137.00	122.00	66.00
D-score	1.01	0.99	1.01	1.00	0.82
Volume ($Å^3$)	192.42	77.86	84.38	73.06	35.67
Exposure	0.82	0.88	0.81	0.87	0.91
Enclosure	0.36	0.31	0.37	0.32	0.31
Contact	0.31	0.27	0.33	0.29	0.23
Phobic	0.08	0.11	0.12	0.11	0.03
Philic	0.46	0.41	0.47	0.42	0.39
Balance	0.18	0.26	0.25	0.27	0.07
Don/Acc	1.72	1.15	1.26	0.89	0.84

S2. Photophysical characterization of cyclo$_{AZOB}$[VHGKQHRP-K*]

While our analysis focused on the π-π^* peak, the n-π^* peak changes were also detected, as shown in Fig. S2. Changes in both peaks are consistent with azobenzene isomerization. The first peak was selected for analysis due to high signal strength. Where signal strength was high enough, the rate constants for π-π* and n-π* peaks were similar. In addition, *cis-trans* and *trans-cis* isomerization of cyclo$_{AZOB}$[VHGKQHRP-K*] was investigated at different concentration (0.15, 0.38, and 1.5 mM) and light intensities. The resulting data are collated in Fig. S3.

The photoisomerization of azobenzene-cyclic peptides studied in this work was induced using a Dymax BlueWave 200 lamp, whose spectral output is reported in Fig. S4. A Dymax Accu-cal 50 radiometer was used to measure the incident intensity across a narrow spectral window (320-390 nm). To calculate the incident intensity on the sample, the unfiltered UV lamp intensity was initially measured using Accu-cal 50 at the sample plane. Filtered lamp output was calculated by multiplying the filter's spectral response with the Dymax lamp output. By using the ratio of the total unfiltered output of the lamp between 320-390 nm and the total filtered output of the lamp, incident power of the filtered lamp was calculated from the Dymax Accu-cal 50 readings.

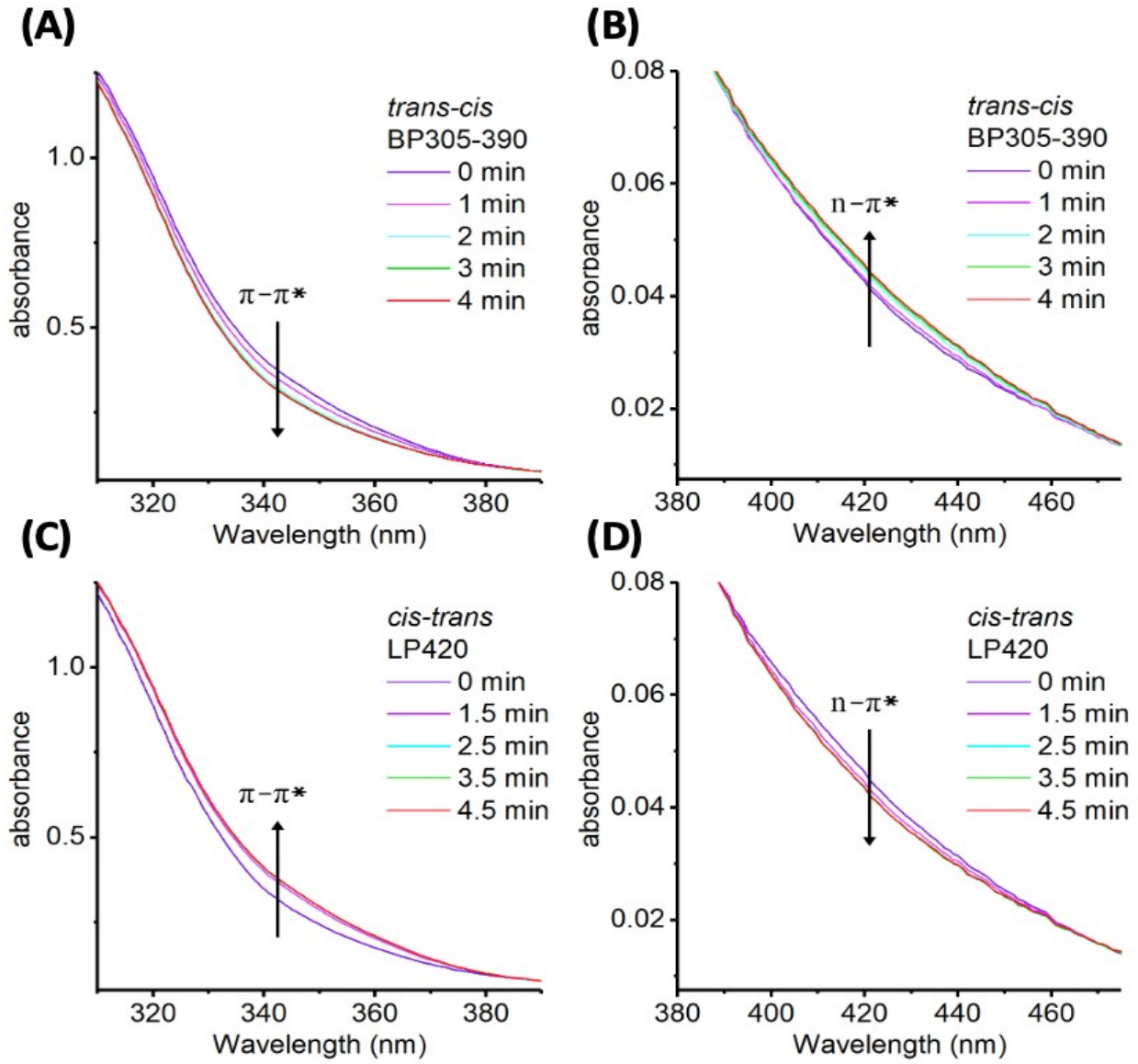

Fig. S2 Spectral absorbance changes of a 0.38 mM solution of cyclo$_{AZOB}$[VHGKQHRP-K*] in MilliQ water upon exposure to UV bandpass filtered light (A and B) and subsequent LP420 filtered light (C and D). Spectra are scanned from 500 nm to 250 nm at 10 nm/s and time indicates light exposure at the start of each scan.

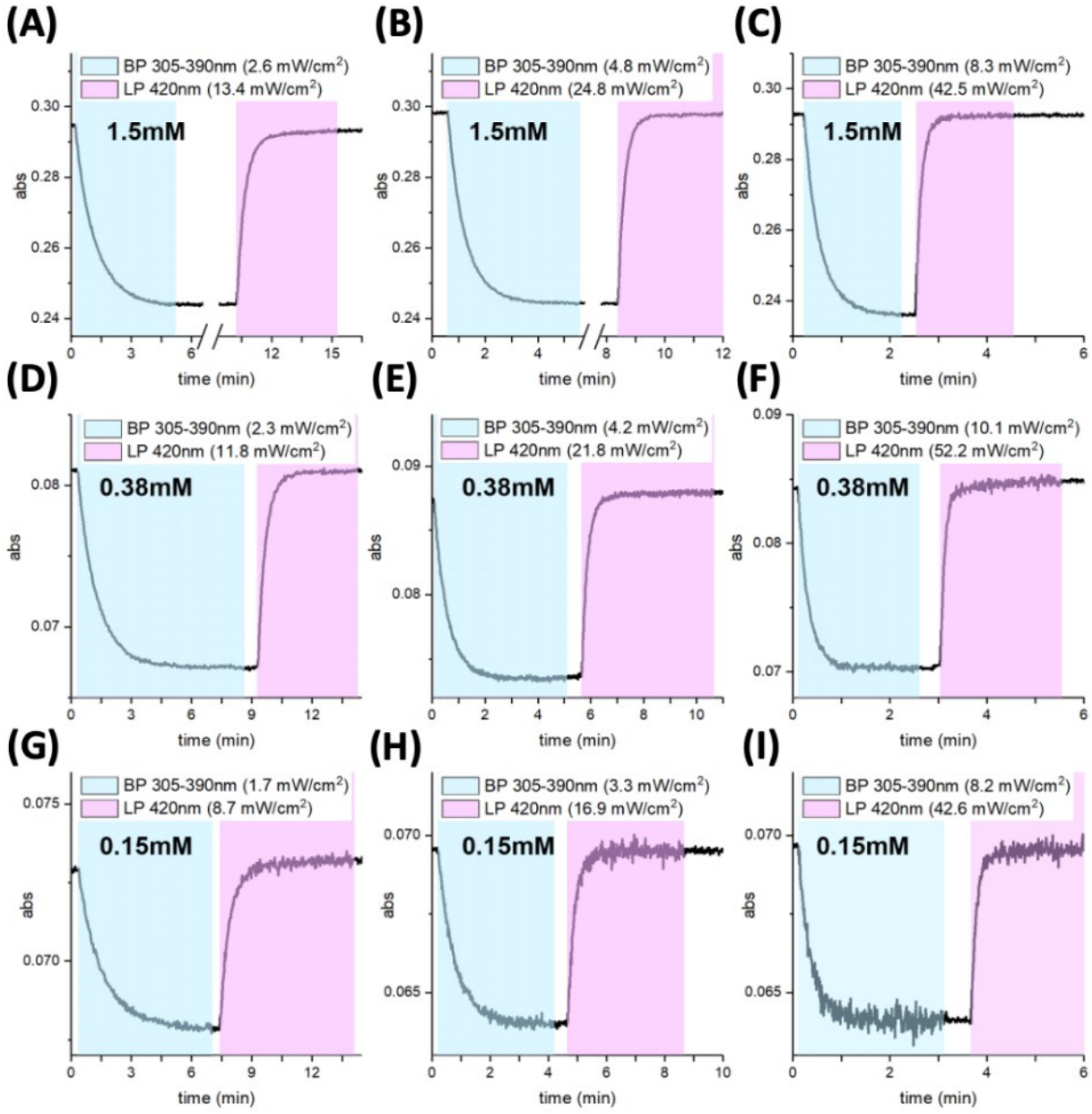

Fig. S3 Representative changes of absorbance at 350 nm for 1.5 mM (A, B, C), 0.38 mM (D, E, F), and 0.15 mM (G, H, I) solutions of cyclo$_{AZOB}$[VHGKQHRP-K*] upon exposure to light filtered with UV bandpass (BP 305-390nm) and LP420 filters at different values of light intensity.

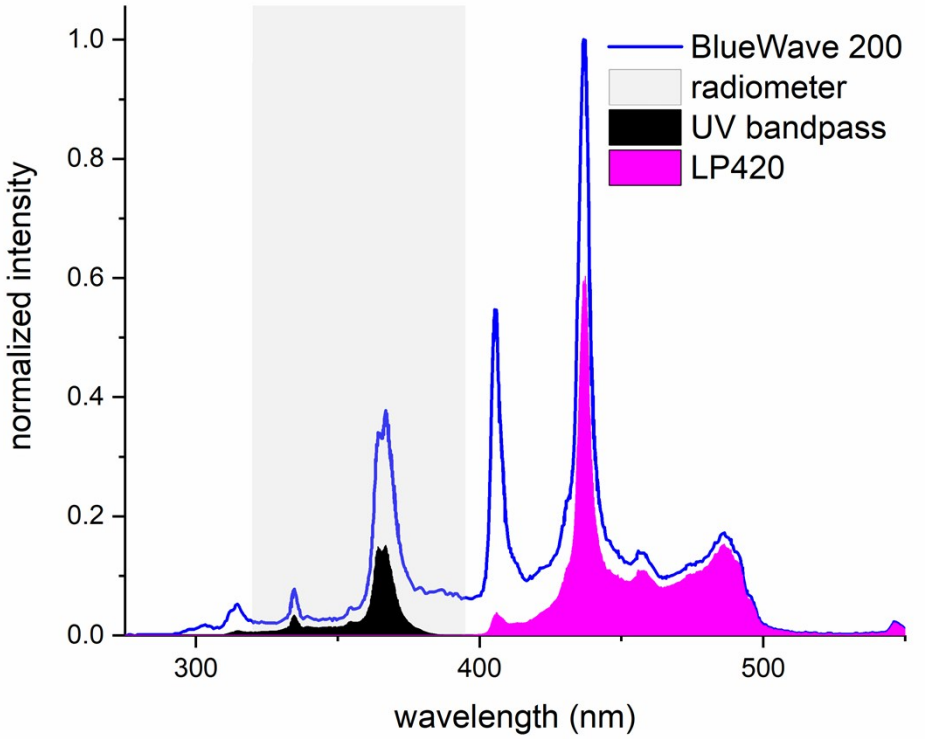

Fig. S4 Spectra of Dymax BlueWave 200 curing lamp and corresponding filtered outputs using UV bandpass (BP305-390) and LP420 filters.

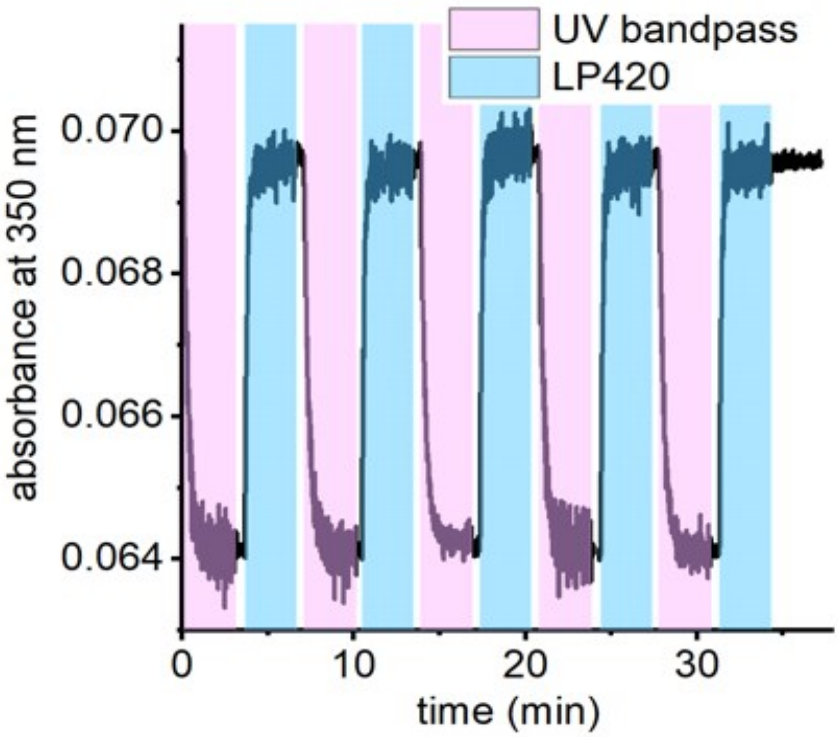

Fig. S5 Reversible and repeatable *trans-cis* and *cis-trans* isomerization of cyclo$_{AZOB}$[VHGKQHRP-K*] dissolved in MilliQ water at the concentration of 0.15 mM.

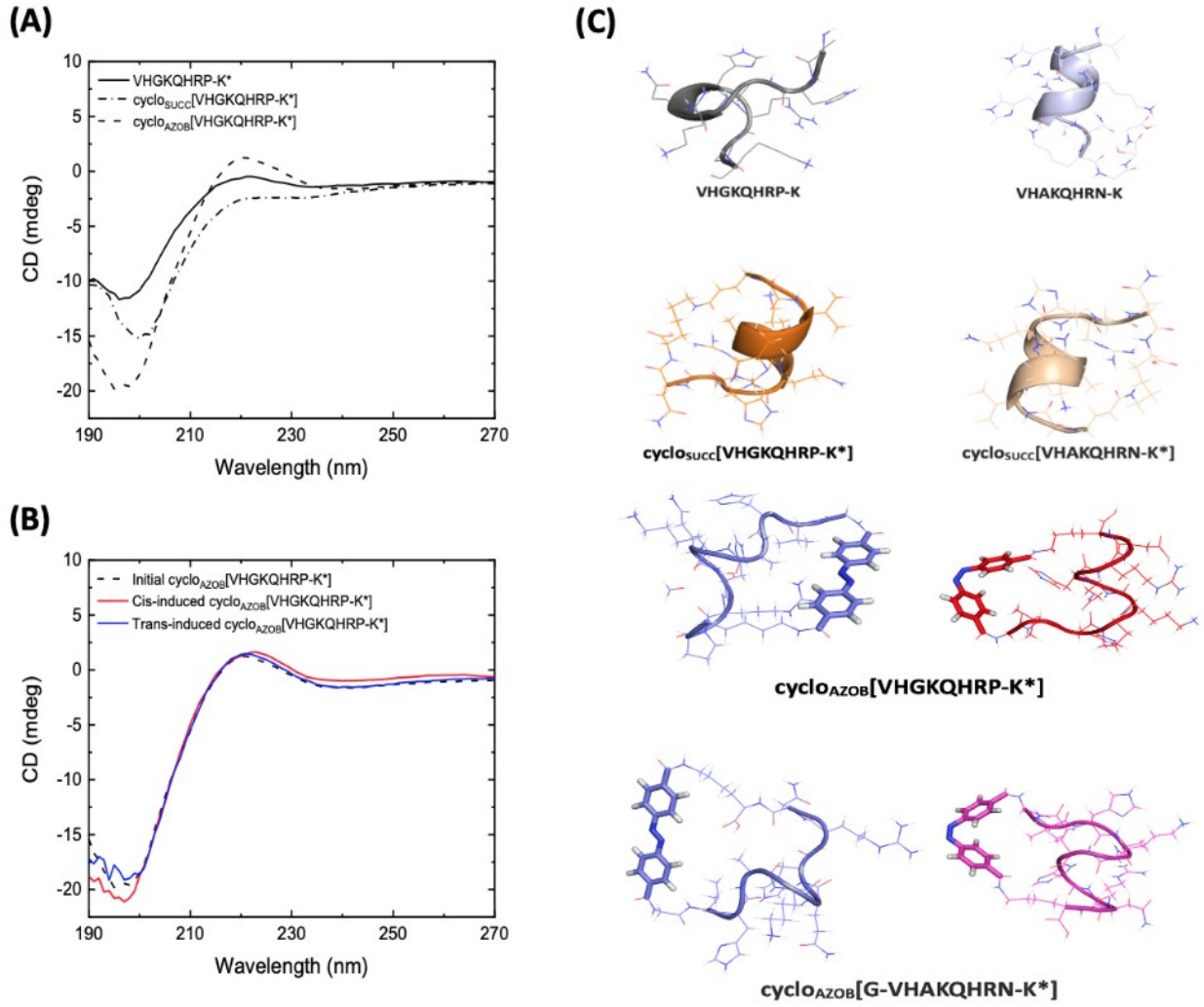

Fig. S6 (A) Circular dichroism (CD) spectra of cyclo$_{AZOB}$[VHGKQHRP-K*] peptide upon photo-induced isomerization, indicating a random coil confirmation, (B) CD spectra of peptide variants spectra, indicating a random coil conformation for all peptides. (C) Structure of VHPKQHR-GSG and cyclo$_{SUCC}$[G-VHAKQHRN-K*] peptides with an α-helix segment, and cyclo$_{AZOB}$[G-VHAKQHRN-K*] with random coil in both *trans* and *cis* conformation.

S3. Binding affinity by surface plasmon resonance (SPR)

Table S2 Average thickness of the SAM and SAM-peptide monolayers, and corresponding peptide density determined by ellipsometry.

Sample	Thickness (Å)	Peptide density (molecules nm^{-2})
15:85 N3-PEG : HO-PEG SAM (N_3 SAM)	20.1 ± 3.0	- - -
15:85 NH_2-PEG : HO-PEG SAM (amino SAM)	22.3 ± 1.5	- - -
$cyclo_{AZOB}$[G-VHAKQHRN-K] on N_3SAM	35.0 ± 2.3	0.85 ± 0.22
$cyclo_{AZOB}$[G-VHPKQHRS-K] on N_3SAM	33.7 ±	0.79 ± 0.27
$cyclo_{AZOB}$[G-VHAKQHRD-K] on N_3SAM	33.7 ±	0.93 ± 0.21
VHPKQHR-GSG on N_3 SAM	30.5 ±	1.01 ± 0.21
Anti-VCAM1 antibody on amino SAM	68.2 ± 11.6	- - -

S4. Cell-labelling with cyclo$_{AZOB}$[G-VHAKQHRN-K*]

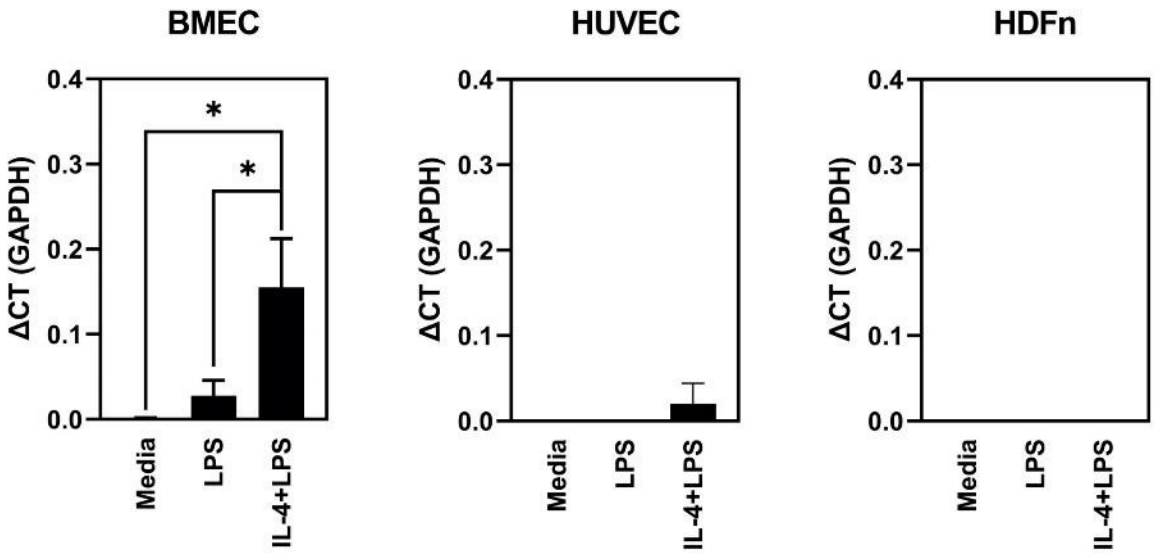

Fig. S7 Confirmation of VCAM1 induction by LPS exposure via immunohistochemical analysis and qRT-PCR characterization. BMEC was demonstrated to express VCAM-1 when treated with IL-4, followed by LPS. LPS treatment alone showed no significant change in VCAM-1 expression. HUVEC and HDFn controls showed no increase in VCAM-1 expression with either treatment condition. Statistical analysis was performed using a 2way ANOVA with Tukey's post hoc comparison test with an alpha value of 0.05 (*) considered statistically significant.

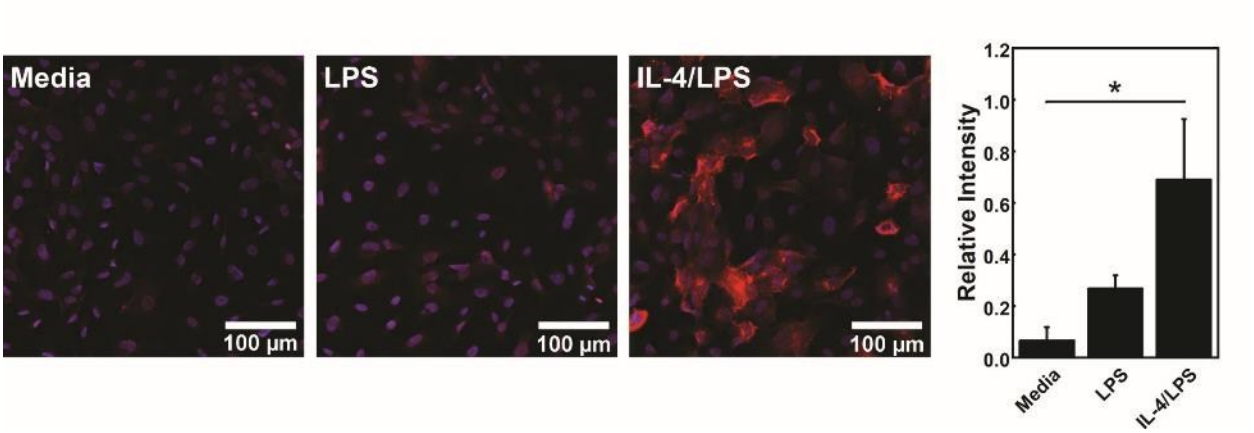

Fig. S8 Confirmation of VCAM1 induction in BMECs by synergistic treatment with IL-4 and LPS via immunohistochemical analysis quantification. Relative intensity of tagged VCAM-1 antibody compared across different cell treatment condition aggress with RT-qPCR measurements, showing the most induction of VCAM-1 in BMECs treated with IL-4 followed by LPS. Error bars represent standard deviation. Statistical analysis was performed using a paired t-test with an alpha value of 0.05 (*) considered statistically significant.