Surface plasmon resonance (SPR) assays. The assays were done using a Biacore X (Biacore AB). We used a CM5 sensor chip and Amine Coupling Kit for the protein immobilization (Biacore AB). The recombinant or affinity purified human VAP-1 was immobilized on the sensor chip according to the manufacturer's instructions. 0.19 mg/ml (1.1 μM) VAP-1 in 10 mM Na-acetate buffer, pH 4.0 was run on the activated surface. This resulted to about 14,000 resonance units (RU) of coupled VAP-1 on the surface. A non-coupled surface was left as a reference channel. Before the interaction assays the coupled surface was subjected to a regeneration procedure: 5 μL of 0.0125% SDS and 25mM NaOH (regeneration solution) was injected at a flow rate 20 μL/min.

First we tested the binding of Siglec-9 derived peptide (P1) to immobilized VAP-1. For the measurements the peptide was dissolved into 10 mM HEPES pH 7.4, 150 mM NaCl, 0.005% P-20 (running buffer) resulting 400 μM solution. This solution was used for the preparation of more diluted solutions (1-200 μM). The concentrations were verified by spectroscopy (A280). The measurements were done at +25°C. In the assay 20 μL of each concentration was injected over the VAP-1 coupled surface at the flow rate of 20 μL/min and the binding of the peptide was monitored versus time. Each measurement was done twice to ensure the reproducibility of the observation. When needed, 1-3 regeneration injections were performed before the next peptide injection. To test the specific binding of the peptide to VAP-1, the interaction of the peptide to the reference channel was subtracted from the VAP-1 curve.

To test qualitatively the role of two individual arginine residues of the

peptide in the VAP-1/Siglec-9 interaction we measured the binding of four different cyclic peptides to immobilized VAP-1: wild type peptide (P1), peptide without arginines (P1_noArg), peptide without Arg284 (P1_noArg284) and peptide without Arg290 (P1_noArg290). We measured the binding on the immobilized VAP-1 using 10, 100 and 200 μM solutions for P1, P1_noArg284 and P1_noArg290. P1_noArg could only be dissolved into 50 μM solution. Additional concentrations for P1 in dose response studies were 1, 50, 150, 300 and 400 μM.

Peptides used:

Abbreviation	Sequence
P1	Cys-Ala-Arg-Leu-Ser-Leu-Ser-Trp-Arg-Gly-Leu-Thr-Leu-Cys-Pro-Ser-NH_2 Cyclic Cys1, Cys14
P1_noArg	Cys-Ala-Ala-Leu-Ser-Leu-Ser-Trp-Ala-Gly-Leu-Thr-Leu-Cys-Pro-Ser-NH_2 Cyclic Cys1, Cys14
P1_noArg284	Cys-Ala-Ala-Leu-Ser-Leu-Ser-Trp-Arg-Gly-Leu-Thr-Leu-Cys-Pro-Ser-NH_2 Cyclic Cys1, Cys14
P1_noArg290	Cys-Ala-Arg-Leu-Ser-Leu-Ser-Trp-Ala-Gly-Leu-Thr-Leu-Cys-Pro-Ser-NH_2 Cyclic Cys1, Cys14

Table S1. Parameters of intravital microscopic experiments

Antibody treatment	**Vessel diameter (µm)***	**Centerline velocity (mm/s)***	**Wall shear rate (x 1000 s^{-1})***
Control mAb	33 ± 4	5.8 ± 0.6	2.1 ± 0.2
Siglec-9 mAb	28 ± 3	6.5 ± 0.8	2.7 ± 0.4

*Values are expressed as mean ± SEM

Table S2. *In vivo* biodistribution of ^{68}Ga-DOTA-peptide in VAP-1 KOTG and KO mice with DNCB induced inflammation

	KOTG	**KO**	***P* value**
Heart	0.86 ± 0.41	0.40 ± 0.07	0.04
Inflammation	0.66 ± 0.27	0.34 ± 0.06	0.03
Kidney	2.68 ± 1.38	1.51 ± 0.27	0.13
Liver	0.86 ± 0.29	0.73 ± 0.33	0.61
Lung	0.48 ± 0.22	0.21 ± 0.05	0.02
Muscle	0.32 ± 0.15	0.16 ± 0.04	0.04
Urinary bladder	9.52 ± 7.84	17.52 ± 2.53	0.06

KOTG, VAP-1 deficient mice expressing human VAP-1 as a transgene on endothelium under Tie-1 promoter; KO, VAP-1 negative knock-out mice; DNCB, 1-chloro-2,4-dinitrobenzene. Results are expressed as standardized uptake values (mean ± SEM; KOTG n = 4, KO n = 7). Note that higher signals in several organs of KOTG mice is based on the fact that VAP-1 is also expressed on other endothelia besides the inflamed ear.

Figure S1. Mouse Siglec-E does not bind to mouse VAP-1. Binding of CFSE labelled CHO-Siglec-E transfectants to CHO cells expressing wild type mouse VAP-1 or to mock transfected controls (CHO-mock). Binding is expressed as relative binding (mean ± SEM). Results are from two experiments, both having four parallel wells.

Figure S2. Siglec-9 peptide detects inflammation by PET also in mice

(A) Representative whole-body coronal PET images of VAP-1 KOTG and KO mice intravenously injected with ^{68}Ga-DOTA-peptide. Images are summations from 10-30 min after injection of the peptide, standardized with injected radioactivity and mouse body weight, and displayed in the same color scale. Radioactive signal in the inflamed ear of VAP-1 KOTG mouse is clearly higher compared to that of KO mouse. Excess of radioactivity is excreted through the kidneys to the urinary bladder, especially in case of KO mouse. (B) Time-activity curves of ^{68}Ga-DOTA-peptide in a inflammation focus of VAP-1 KOTG and KO mice obtained from dynamic PET imaging.

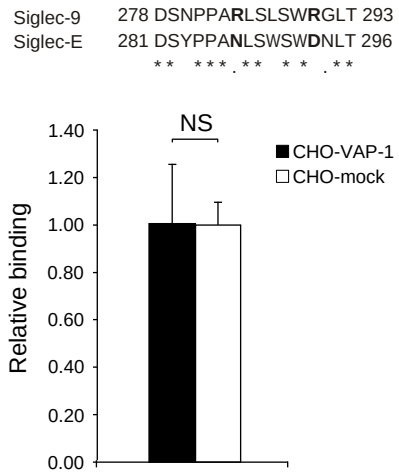
Siglec-9 278 DSNPPA**R**LSLSW**R**GLT 293
Siglec-E 281 DSYPPA**N**LSWSW**D**NLT 296
NS
CHO-VAP-1
CHO-mock
Relative binding
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00

Figure S1

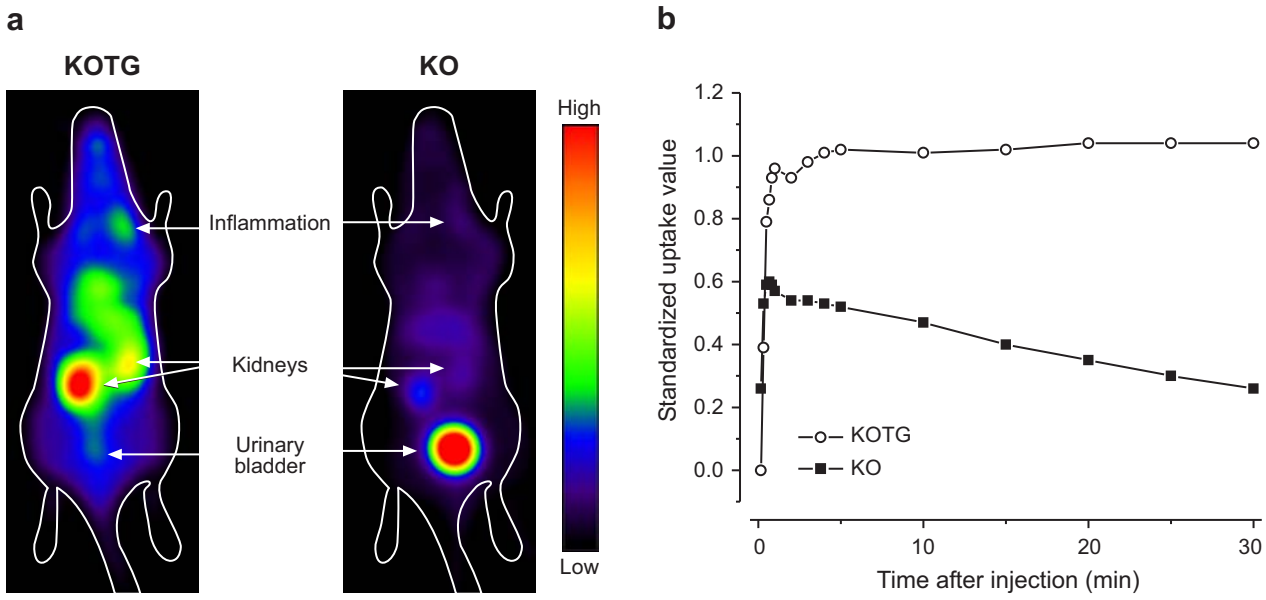
a
KOTG
KO
High
Low
Inflammation
Kidneys
Urinary
bladder
b
Standardized uptake value
1.2
1.0
0.8
0.6
0.4
0.2
0.0
KOTG
KO
0
10
20
30
Time after injection (min)

Figure S2