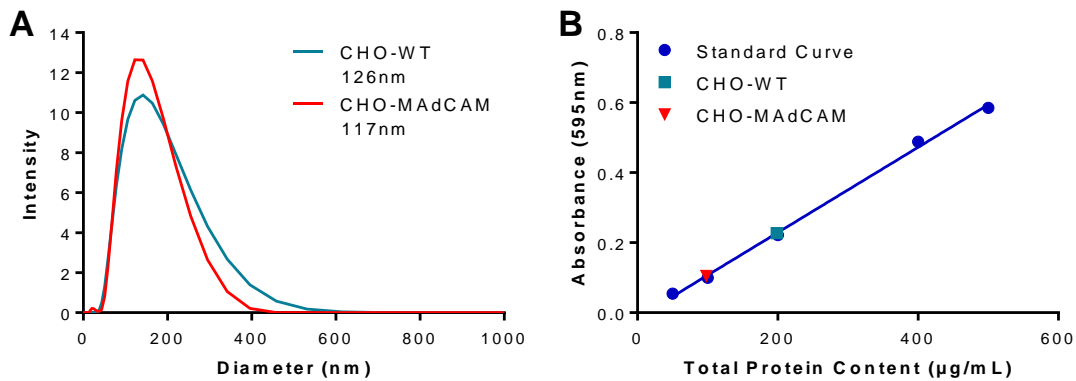


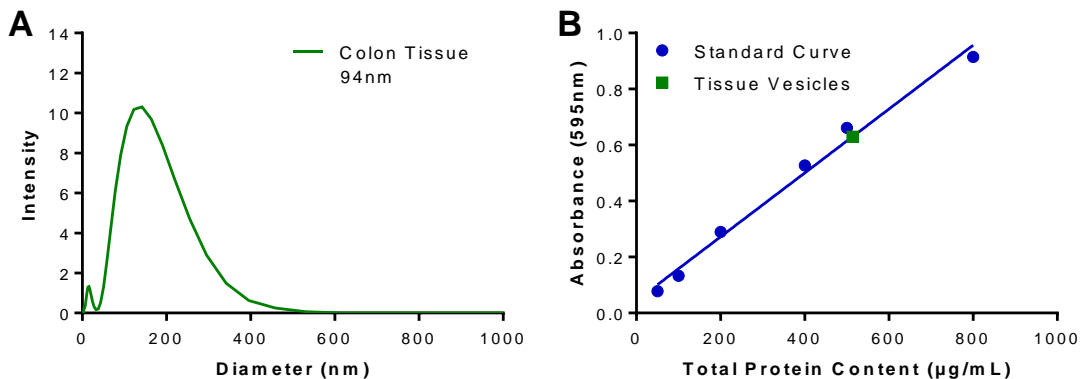
## Supporting information

Additional Supporting Information may be found in the online version of this article at the publishers web-site:

**Figure S1.** Characterization of CHO cell vesicles. Representative results for DLS (A) and Bradford Assay (B) of vesicle solutions made from CHO cells.



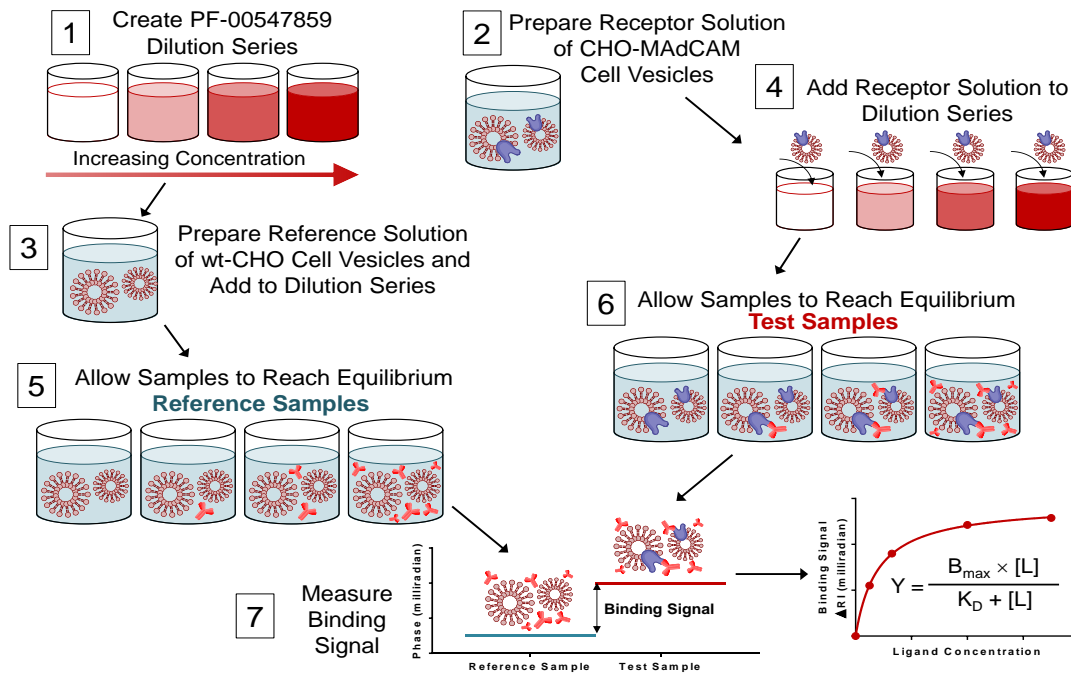
**Figure S2.** Characterization of human colon tissue VRH. Representative results for DLS (A) and Bradford Assay (B) of VRH made from colon tissue. Polydispersity index was measured and if greater than 0.28 by DLS, the VRH sample was probe sonicated again on ice for 90sec in a pulsed manner (5sec on, 1sec off), followed by centrifugation and characterization of the sample by DLS and Bradford as described.



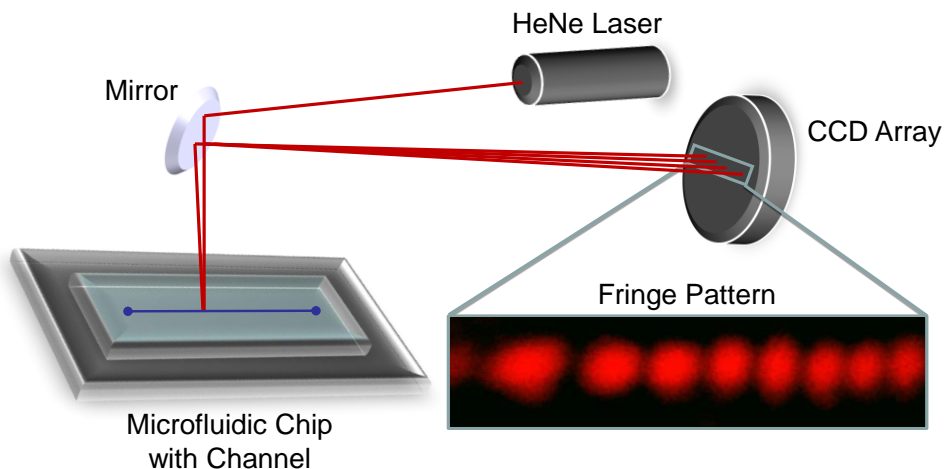
**Table S1.** Components of the reference and test samples. A pictorial representation of the reference and test samples used in the work-flow.

Matrix Tested	Binding Samples		Control Samples	
	Reference	Test	Reference	Test
Soluble F <sub>C</sub> Recombinant MAdCAM				
Recombinant Membrane MAdCAM (CHO cells)				
Native Soluble MAdCAM in Serum			Legend	
				Dilutions done with PBS
Native Membrane MAdCAM in Tissue				Dilutions done with serum
				Dilutions done with homogenate
Soluble and Membrane MAdCAM in Tissue				PF-00547659
				Isotype Control mAb
Soluble MAdCAM in Tissue				CHO-WT Cell Vesicles
				CHO-MAdCAM Cell Vesicles
				Tissue Vesicles
				MAdCAM Protein

**Figure S3.** Cell based assay protocol diagram. Illustration of the experimental procedure to measure the binding affinity in CHO cell vesicles



**Figure S4.** Block diagram of a Backscattering Interferometer.



*Measurement of hMAdCAM Concentration in Biological Samples by LC-MS/MS*

Briefly, for MAdCAM concentrations the LC system was configured with a WPS-3000 auto-injector and an FLM-3300 flow manager consisting of a temperature controlled column compartment set at 15°C and using 3 valves and 3 pumps: a Loading Pump connected to the anti-peptide antibody column, an NCS module supplying the analytical column with nanoflow rates, and Micro Pump washing and equilibrating the reversed-phase trap cartridge. There were three chromatographic separation stages employed: an anti-peptide antibody column, a reverse-phase trap cartridge and a reverse phase column. The loading and elution flow rates on the anti-peptide antibody column typically ranged between 0.3 and 0.6 mL/min, while the flow rate of the analytical column was 600 nL/min. The detection of MAdCAM-derived peptide was performed on an API5500Q mass spectrometer in multiple reaction monitoring (MRM) using a Nanospray III interface (AB Sciex, Toronto, Canada). AB Sciex Analyst software (Version 1.5.2) was used to acquire data and determine peak areas. Peak area ratios (PAR) of the MAdCAM to the internal standard peptide peak area were calculated. Calibration curves were obtained by plotting PAR of the calibration samples versus sample concentration. The curve was fitted with a nonlinear regression applying a 1/concentration weighted model using Labstats (Excel add-in). All sample concentrations were then calculated from their PARs against the calibration curve; a representative calibration curve is shown in Figure S5. The quality of the curve fit was evaluated by back-calculating the MAdCAM concentration of the calibrants. Each assay run was calibrated using an eight or ten point (tissue or serum assay, respectively) standard curve analyzed in duplicates across a MAdCAM quantification range from 2.29 to 5000 pM (tissue assay) and from 2.62 to 10000 pM (serum assay). Additionally, for quality control purposes, human serum and colon tissue homogenate, including their dilutions and spikes with recombinant human MAdCAM.Fc (R & D Systems, MN) were analyzed on multiple testing occasions in replicates (n=3 serum; n=2 tissue). A summary of all the values calculated are shown in Table S2. Coefficients of variation and relative error of the standards must be equal to or less than 20% for a run to be considered acceptable.

**Table S2.** MAdCAM sample concentrations. Tabulation of sample concentrations measured by LC-MS/MS and used in BSI

Sample	Concentration by LC-MS/MS (pM)	Dilution to BSI	Concentration in BSI (pM)
Human serum 100%	999 ± 32	various	Various
CHO-rhMAdCAM VRH	1719.5 <sup>a</sup>	50	34
Colon tissue VRH	15 <sup>a</sup>	349	0.043
Colon tissue ILH	44 <sup>a</sup>	4	11

<sup>a</sup> no SD was determined due to the rare sample type and availability

**Figure S4.** Quantification of MAdCAM using LC/MS. Representative results for LC/MS quantification of MAdCAM protein in vesicle solutions made from colon tissue.

