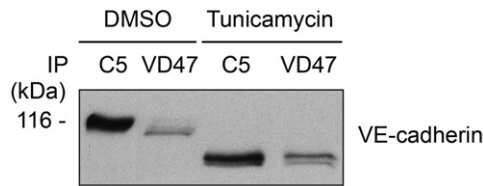# Expanded View Figures

Figure EV1. Reduced apparent molecular weight of VE-cadherin proform is due to immature glycosylation.

bEnd.5 cells were treated with tunicamycin or DMSO for 48 h prior to cell lysis. VE-cadherin was precipitated from cell lysates with the antibodies C5 (mature VE-cadherin) or VD47 (proform of VE-cadherin). Precipitates were analyzed by Western blotting with antibodies against VE-cadherin. Molecular weight marker is indicated in kDa.
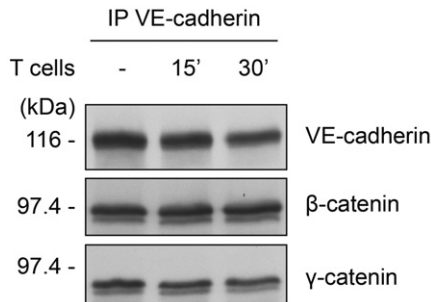

Figure EV2. Leukocytes do not trigger the dissociation of β-catenin or plakoglobin from VE-cadherin.

bEnd.5 cells were stimulated with TNF-α for 18 h prior to incubation with antigen-stimulated mouse T cells for 0–30 min. VE-cadherin was immunoprecipitated from cell lysates, and precipitates were analyzed by immunoblots for indicated antigens.
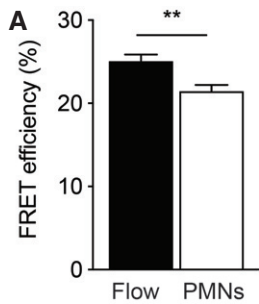

Figure EV3. Leukocyte-induced tension across VE-cadherin is independent of SHP2, and tension is not required for SHP2 dissociation from PECAM-1.

A   Quantification of FRET efficiency (percentage) at junctions of HUVEC treated with siRNAs for SHP2 and VE-cadherin prior to transducing VE-cadherin-FL. Cells were exposed either to flow alone or to flow in the presence of PMNs and then fixed with 4% PFA followed by FLIM measurements at sites of PMN transmigration (PMN) or at junctions without PMNs (Flow).

B   Representative immunoblot for SHP2 and actin expression of HUVEC used for the experiments in (A).

C   HUVEC were grown on collagen-coated polyacrylamide gels of varying physiologic stiffness of 0.2 kPa (left) and 20 kPa (right) and stimulated with TNF-α for 17 h prior to adding HL60-derived neutrophils for 20 min. PECAM-1 immunoprecipitates and cell lysates were immunoblotted for the indicated antigens. Note that leukocytes triggered SHP2 dissociation from PECAM-1 independent of substrate stiffness.

Data information: Graph in (A) represents $n = 21$ and 20 measurements in a total of four independent experiments. Bars and error bars indicate mean ± SEM. Statistical significance was tested with unpaired t-test, **$P < 0.01$. Blots are representative for 3 experiments (C).