ONLINE METHODS

Modeling of T2-TrpRS/VE-cadherin complex. First we aligned the VE-cadherin sequence with the sequences of five other homologous type II cadherins (with known crystal structures) using ClustalW[37]. The five type II cadherin crystal structures included human cadherin-8 (EC1-3, PDB 2A62), cadherin-11 (EC1-2, PDB 2A4E **[AU: Code correct?]**), MN-cadherin (EC1, PDB 1ZVN), cadherin-8 (EC1, PDB 1ZXK) and mouse cadherin-11 (EC1, PDB 2A4C)[31]. We then used the alignment and the five structures to model the structure of the EC1 domain of VE-cadherin using Modeller 8v2 (http://salilab.org/modeller/).

For modeling of the complex, we first determined the initial orientation of VE-cadherin EC1 for docking to T2-TrpRS (PDB 1O5T)[35] according to the respective global surface electrostatic properties, calculated by APBS package (http://apbs.sourceforge.net/) as a plug-in in program PyMol (http://www.pymol.org/). We then manually adjusted the position of the EC1 domain relative to T2-TrpRS to allow its N-terminal end to enter into the Trp-AMP pocket, with the least steric clash for the rest of EC1 domain. The N-terminal D-W2-I-W4 was further adjusted to fit into the active site. This initial model was then subject to molecular dynamic refinement in CNS (http://cns-online.org/v1.21/). After refinement, the interaction energy converged at 17186.937 kcal mol^{-1} indicative of a favorable binding state without any steric clash. The final model of the complex was validated by PROCHECK[38] with no residues in the disallowed regions of the Ramachandran plot. The coordinates of the complex model and the result of the PROCHECK analysis are included in Supplementary Data.

Endothelial cell tube formation assay. The tube formation assay was performed with murine endothelial cells 3B11 using a detailed protocol described previously [36].

Preparation of recombinant human T2-TrpRS. T2-TrpRS mutants were constructed with QuikChange Site-Directed Mutagenesis Kit (Stratagene). Wild-type and mutant T2-TrpRS proteins were expressed in *E. coli* and purified as described earlier [14].

Transfection of 293T cells with VE-cadherin constructs. Wild type VE-cadherin-Fc cDNA-pCEP4 (a gift from Prof. D. Michael Shasby [39]) was used to make VE-cadherin mutants. 293T cells were transfected with either wild-type or mutant VE-cadherin-Fc constructs, using the Lipofectamine LTX (Invitrogen) kit. Two days after transfection, the cells were selected for 3 weeks with 10 μg ml^{-1} blasticidine S (Invitrogen). The stably transfected cells were used for study of the interaction between VE-cadherin and T2-TrpRS.

Immunoprecipitation and Western blotting. The stably transfected (with various genes for VE-cadherin and its mutants) 293T cells were washed with PBS and extracted in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, and 1x protease inhibitors). The supernatants (0.4 ml) of cell lysates were mixed with equal volumes of either purified T2-TrpRS (10 μg ml^{-1}) or of lysates of *E. coli* cells that had been transformed with a gene expressing T2-TrpRS or its mutants. Incubation for 1 h at 4˚C in the presence 5 mM $CaCl_2$ was followed by incubation with 30 μl protein G

sepharose for 1 h at 4˚C (with continuous mixing) to allow protein G to capture the VE-cadherin-Fc and T2-TrpRS complex.

To test the effects of agents on the binding of T2-TrpRS to VE-cadherin, purified human VE-cadherin-Fc (R&D System) was incubated with T2-TrpRS and various testing agents, and then incubated with protein G sepharose.

The protein G bound proteins, after elution with 60 µl of reduced slab gel sample buffer (final 5% SDS, 10 mM DTT), were analyzed by Western blotting using rabbit anti-C-terminal human TrpRS and goat anti-human IgG-Fc antibody (Jackson Immuno Research) as primary antibodies to detect T2-TrpRS and VE-cadherin, respectively.

Isothermal titration calorimetry. ITC was performed at 25 ^{O}C using an Omega titration calorimeter (Microcal) as described previously by Wiseman *et al* [40]. Proteins were dialyzed overnight in 20 mM sodium phosphate buffer (pH 7.5) containing 150 mM NaCl and the final dialysate was used to prepare ligand solutions. Typically, 700 µM of ligand solution was added to 70 µM protein (present in the sample cell (1.34 ml)) as a 2 µl injection followed by thirty 6 µl injections using a syringe rotating at 400 rpm. Between successive injections, an interval of 3 min was given for the baseline to stabilize.

One of the requirements for determining binding constants in an Omega calorimeter is that the C-values should lie between 1 and 1000, where C = (K_A) X ([M]), with K_A as the association constant and [M] as the macromolecule concentration. The experimental conditions ensured that the C-value was always in this range, and heats of dilution of ligand were subtracted from the titration data. The data so obtained were fitted

via the nonlinear least squares minimization method to determine binding constants using Origin software (Microcal).

Equilibrium dialysis assay. Because of the low affinity of T2-TrpRS for AMP or ATP [33], an indirect method was used to evaluate the adenosine pocket of T2-TrpRS mutants (A310W and G172M). Tryptophan binding to T2-TrpRSs was measured in the presence the Trp-AMP analog, Trp-SA, as a binding inhibitor. A disrupted adenosine pocket would attenuate the inhibition by Trp-SA. Equilibrium dialysis tryptophan binding assay was performed using a modification of the method described earlier[41]. Prior to the binding experiment, protein stocks were dialyzed in phosphate buffered saline (pH 7.4). Experiment was performed by placing T2-TrpRS or mutants (20 μM) and tryptophan ([^{14}C]-labeled) suspended in PBS in the two chambers on either side of a semipermeable membrane in the absence or the presence of increasing concentrations of Trp-SA. After overnight dialysis, the radioactivity in both the chambers was measured by scintillation counting and free and bound tryptophan calculated at each Trp-SA concentration.

In the absence of Trp-SA, WT, A310W and G172M T2-TrpRS have similar affinity for tryptophan (Kd is consistent with the number obtained by ITC), and the binding of tryptophan in the absence of Trp-SA was normalized to 100%. As expected, in the presence Trp-SA, tryptophan binding was inhibited (Supplementary Fig. 2). The inhibition effect with increased Trp-SA concentration was fit with a single site binding equation $y = 100 \times (1-1/(1+K_I/x))$. The inhibition was attenuated for both T2-TrpRS mutants, with stronger attenuation by A310W than by G172M mutation. The result suggested that the adenosine pocket of T2-TrpRS was disrupted in either mutant, and the

degree of disruption was consistent with their disrupted ability in VE-cadherin binding (Fig. 4B).