Figure S1.  **Silencing and reexpression of CEACAM1 in NBT-II cells.** (A) Western blot of CEACAM1, α3-integrin, α6-integrin, and E-cadherin in wild-type (wt) cells, a clone of stably down-regulated cells (C2Dc3), and mock-transfected cells are shown. (B) Western blot of reexpressed CEACAM1-YFP isoforms in C2Dc3 cells detected by anti-CEACAM1 [αCC16] and anti-GFP antibodies. (C) Confocal microscopy of reexpressed CEACAM1-YFP isoforms in C2Dc3 cells (CC1-L, CEACAM1-4L–YFP [a]; CC1-S, CEACAM1-4S–YFP [b]; CC1-ΔCyto, CEACAM1-4ΔCyto–YFP [c]; ΔN-CC1-L, ΔN-CEACAM1-3L–YFP [d]). CEACAM1 in wild-type (e) cells was detected by indirect immunofluorescence with mAb Be9.2. (D) Direct confocal immunofluorescence of CEACAM1-4L–YFP and CEACAM1-4S–CFP demonstrating colocalization on the cell surface of C2Dc3 cells. (E) Wild-type NBT-II, silenced C2Dc3, or C2Dc3 cells expressing CEACAM1-YFP isoforms were cross-linked with BS3 and lysed, and CEACAM1 was detected by Western blotting [αCC16]. The various CEACAM1 isoforms appeared as monomers (M), dimers (D), and oligomers (O). Black lines indicate that intervening lanes have been spliced out. MW, molecular weight. Bars, 10 µm.
Figure S2. **Adhesion and cell surface expression of CEACAM1-expressing NBT-II cells.** Attached cells were detected by crystal violet staining. (A) Silenced C2Dc3 cells and C2Dc3 cells stably transfected with CEACAM1-YFP isoforms were seeded in the presence of 10% FBS in uncoated dishes or dishes coated with rat CEACAM1-Fc, rat ΔN–CEACAM1-Fc, human CEACAM1-Fc, or BSA. The data are presented as relative number of attached cells compared with uncoated dishes. (B and C) Wild-type NBT-II cells (WT CC1) and C2Dc3 cells with reexpressed CEACAM1-YFP isoforms were seeded in dishes coated with rat CEACAM1-Fc (B) or collagen I (C). w/o FBS, no FBS added; FBS, 10% FBS added; c, no inhibitor; PP2, Src family kinase inhibitor; NSC, SHP-1/SHP-2–specific phosphatase inhibitor NSC-87877. Cell attachment is presented as relative adhesion compared with serum-free conditions. (A–C) Mean ± SD of representative experiments performed in triplicate are presented. *, P ≤ 0.01 (Student’s t test). (D) Cell surface expression of CEACAM1-L–YFP after drug treatment or antibody ligation. Trypsinized cells were suspended in BSA-coated dishes in the absence (w/o) or presence (+) of 10% FBS together with the indicated antibodies (20 µg/ml) or pharmacological inhibitors for 2 h on ice or 2 h at 37°C. Surface-associated CEACAM1 was analyzed by flow cytometry, detecting the primary antibodies with FITC-conjugated secondary antibodies or staining the cells with 20 µg/ml mAb 5.4 and secondary antibodies. Note the total disappearance of surface-associated CEACAM1 when macroclustering was induced with polyclonal αCC16 at 37°C in contrast to all other cases in which the reduction of surface expression accounted for only 8–20%. The data represent mean ± SD of three independent experiments.
Figure S3. **Recognition of monomeric and dimeric CEACAM1 by various anti-CEACAM1 antibodies.** Adherent or suspended C2Dc3 cells expressing CEACAM1-L–YFP were chemically cross-linked with BS3 and lysed. CEACAM1-L–YFP was immunoprecipitated with mAb Be9.2, pAb αCC16, or pAb αcyto-L antibodies. Precipitated CEACAM1 monomers (M) or dimers (D) were detected by immunoblotting with Be9.2. IP, immunoprecipitation; MW, molecular weight; WB, Western blot.

Figure S4. **Comparison of binding affinities of mAbs Be9.2 and 5.4 to cell surface–exposed CEACAM1.** C2Dc3 cells expressing CEACAM1-4S–YFP were trypsinized and incubated on ice with primary antibodies (0.01, 0.04, 0.125, 0.625, 2.5, 10, and 40 µg/ml) and FITC-conjugated secondary antibodies. Mean fluorescence was plotted against the concentration of primary antibody (Ab conc).