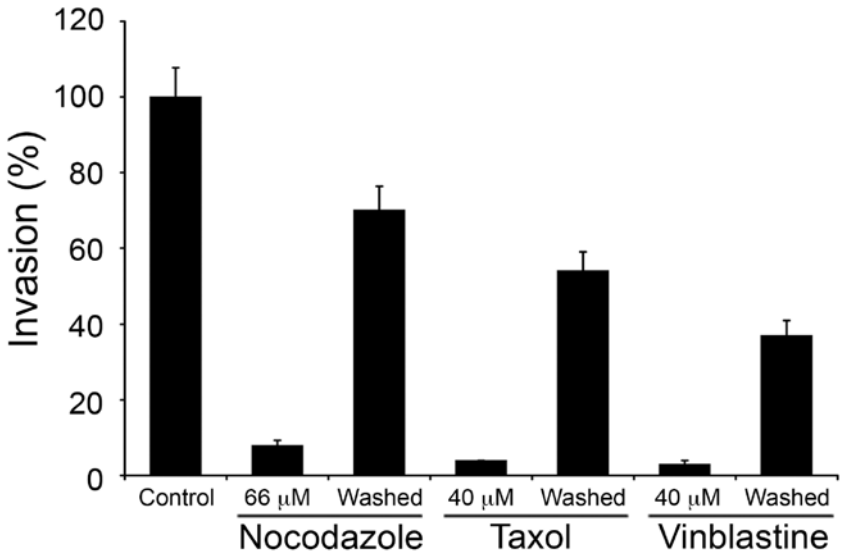
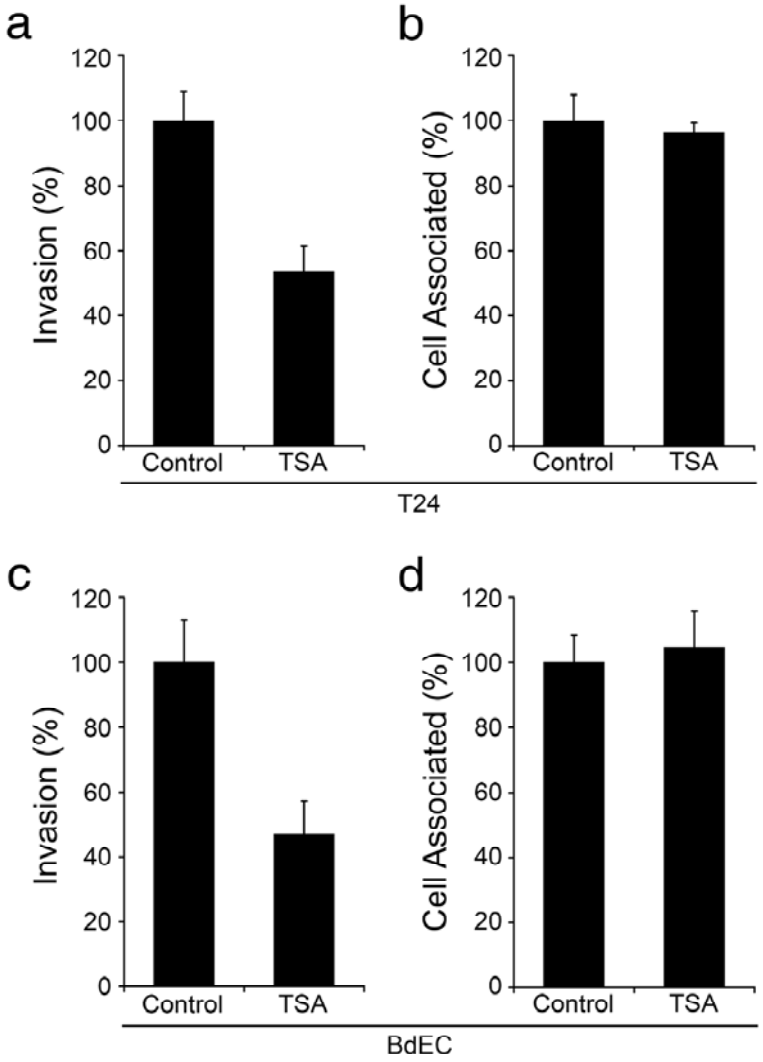
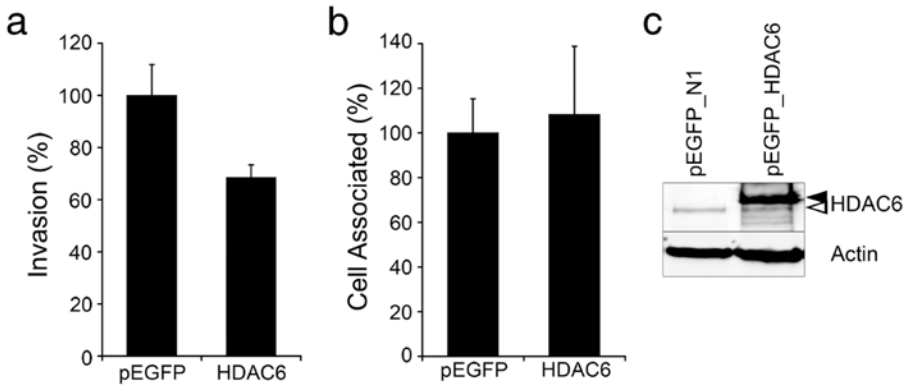


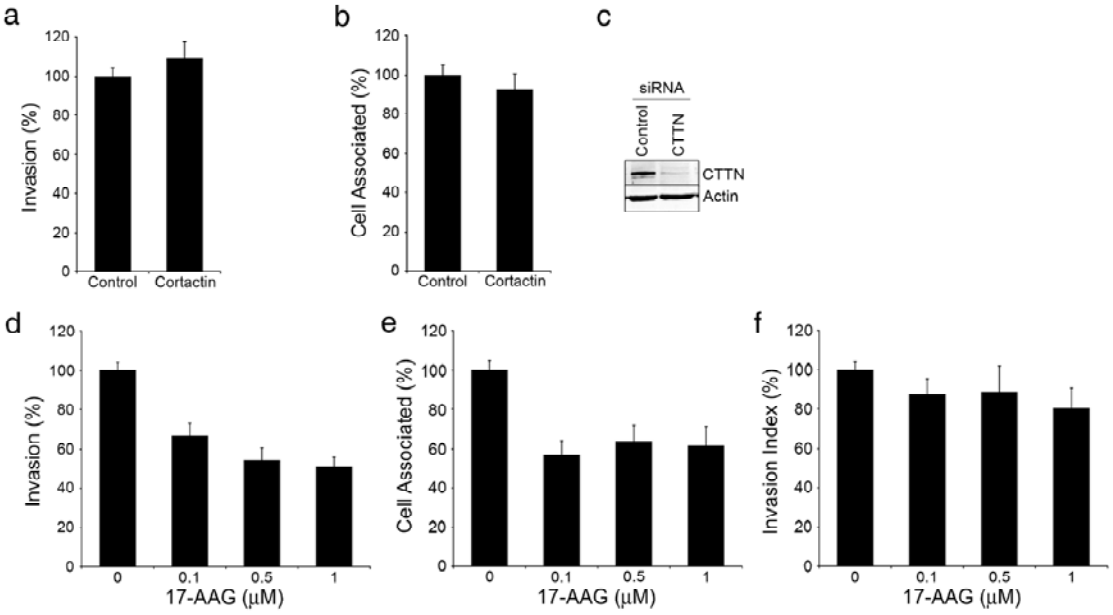
Supplemental Fig. 1. Effects of microtubule-disrupting drugs on UPEC invasion are reversible. Following a 1 h pre-treatment with the indicated drugs, bladder epithelial cells were infected with UTI89 in the continued presence or absence (washed samples) of drugs. Intracellular (gentamicin-protected) bacterial titers were determined after an additional 2 h incubation. Data are expressed relative to untreated controls as the means ± s.e.m. of at least three independent experiments carried out in triplicate.



Supplemental Fig 2. HDAC6 modulates UPEC invasion of T24 and normal primary human bladder epithelial cells. (a and b) T24 or (c and d) primary normal human bladder epithelial (BdEC) cells were treated with 300 nM TSA for 3 h prior to infection with UTI89. Two h later (a and c) intracellular and (b and d) total cell-associated bacterial titers were determined. Data are expressed relative to DMSO-treated controls as the means ± s.e.m. of at least three independent experiments carried out in triplicate.



Supplemental Fig 3. Over expression of HDAC6 inhibits UPEC invasion. 5637 bladder cells were transfected with either control pEGFP-N1 or pEGFP_HDAC6 and GFP-positive cells expressing either GFP alone or GFP-tagged HDAC6 were sorted using a BD FACSVantage Cell Sorter. These were plated, grown to confluency and infected with UTI89. Two h later, numbers of (a) intracellular and (b) total cell-associated bacteria were determined. Data are expressed relative to pEGFP-N1-transfected controls as the means ± s.e.m. of two independent experiments performed in triplicate. (c) The Western blot shows levels of native (white arrowhead) and GFP-tagged (black arrowhead) HDAC6, as well as actin, in the two transfected bladder cell populations.



Supplemental Fig. 4. The HDAC6 substrates Hsp90 and cortactin are not required for host cell invasion by UPEC. (a and b) 5637 bladder cells were transfected with non-specific control siRNA or siRNA specific for cortactin 72 h prior to infection with UTI89. (c) The efficiency of siRNA-mediated knockdown of cortactin (CTTN) protein levels was assessed by Western blot, using anti-actin antibody to verify equal protein loading. Alternately, bladder cells were treated with the indicated concentrations of 17-AAG for 3 h before infecting with UTI89 for an additional 2 h. (d and e) Intracellular and total cell-associated bacterial titers are expressed relative to controls as the means ± s.e.m. of at least three independent experiments carried out in triplicate. Data obtained using 17-AAG is also presented in (f) as relative invasion indices (number of intracellular bacteria/number of cell-associated bacteria) to better show that the drug has no significant inhibitory effect on bacterial entry. 17-AAG did not alter the growth or viability of UTI89 (data not shown), though it did affect bacteria-host cell interactions, possibly by disturbing the maturation and/or stability of host receptor proteins that are recognized by UPEC.