Figure S1. Microvilli and their components are dynamic. (A, top) Time points from JEG-3 cells expressing GFP-EBP50 to observe the microvillar lifecycle. Red arrows show a microvillus form and grow to its mature length. Bar, 2 µm. (bottom) Length measurements of individual microvilli over time show similar initial growth rates but variable lifetimes. (B) Photobleaching recovery curves of GFP-tagged EBP50 (n = 13), ezrin (n = 19), and TROP2 (n = 16). Error bars show SD, and black lines represent fitted curves with corresponding rates shown in the table.

Figure S2. EBP50 associates with ezrin stably in vitro. (A) Limiting amounts of PAGFP-EBP50 prebound to ezrin FERM beads were competed off with untagged-EBP50 for the times indicated. Gel was stained for total protein with IRDye. (B) Normalized amount of the remaining bound PAGFP-EBP50 from the representative experiment in A was fit to a single exponential decay curve shown as solid line. Dotted lines indicate the 95% confidence interval. Rate and half-life are indicated (±SD).
Figure S3. **Lysate effects on EBP50 dissociation from ezrin.** (A) Increasing concentrations of JEG-3 cell lysate were added to the in vitro photoactivation assay. Graphs represent the photoactivation decay curves for PAGFP-tagged EBP50 wild type (left) and PDZ1&2 mutant (mut; right) with increasing amounts of lysate. $n \geq 6$ for all curves. (B) Graph with addition of bacterial lysate to the in vitro photoactivation assay using either PAGFP-tagged wild type ($n = 9$) or PDZ1&2 mutant ($n = 8$). (C) Graph with addition of 2.4 mg/ml JEG-3 cell lysate depleted of ATP (ATP−; $n = 18$) or with ATP regeneration system (ATP+; $n = 17$) using PAGFP-tagged wild-type EBP50. All error bars show SD.