Supplementary information, Data S1 Materials and Methods

Quantification of cell-in-cell structures and "winner" and "loser" cell identity. To quantify cell-in-cell structures, 1×10^5 cells were cultured in 6-well Ultra-low Attachment plates (Corning) for 6h followed by cytospin (Wescor) of cells onto glass slides. Samples were fixed with 10% TCA at 4^oC for 10 min and then stained with E-cadherin and β-catenin for analysis by confocal microscopy. Cell structures with more than half of one cell body internalized inside of another cell were counted. For identity analysis of winners and losers, equal numbers of two different cell lines, stained with red and green CellTrackers as described[3], were mixed together and cultured in suspension for 3-12 h as needed, and then plated into soft agar or cytospun onto glass slides for analysis. To assess the effects of gene expression on winner or loser cell identity, MCF-10A-mCherry-CAAX cells were nucleofected with constructs expressing GFP tagged-genes and plated for overnight culture. Cells were then suspended for 3-4h, and then plated into 0.4% agar-containing medium for confocal microscopic analysis of live cells. Structures formed between GFP expressing and non-expressing cell were analyzed. All analyses were performed using the Ultraview Vox spinning disc confocal system (Perkin Elmer) equipped with a Yokogawa CSU-X1 spinning disc head, and EMCCD camera (Hamamatsu C9100-13), and coupled with a Nikon Ti-E microscope. All imaging with live cells was performed within incubation chambers at 37°C and 5% CO_2. Confocal image acquisition and analysis was performed with Volocity software (Perkin Elmer).

Cell competition assay. MCF7 cells expressing GFP and VmCUB3 cells expressing mCherry were mixed in suspension for 7 h with or without Y27632, and then plated onto adherent cultures overnight in the absence of Y27632, followed by quantification of relative cell numbers microscopy the next morning. This procedure was repeated for the indicated number of cycles. To examine the effects of Y27632 on cell death in suspension co-culture, DAPI was added into suspension cultures 10 min prior to cytospinning for analysis of DAPI-positive red and green cells

in the co-culture. To examine the effects of Y27632 on cell proliferation in suspension co-culture, BrdU was incubated with mixed cultures in suspension for 2 h before cytospinning for staining and analysis. More than 10 different fields were imaged using a 10X objective lens (Nikon Ti-E) for analysis.

Cell fate analysis. Quantification of entotic cell fate was performed as described [2]. Briefly, to quantify engulfed cell fate in structures formed between MCF7 and VmCUB3 cells, cells were first labeled with red and green CellTrackers and then co-cultured in suspension for 6h followed by plating on 35mm glass-bottomed dishes (MatTek) overnight. Cell-in-cell structures in adherent cultures were identified by cell tracker fluorescence and then imaged by time-lapse DIC microscopy over the next day. To quantify cell fate in soft agar, MDA-MB-453/E-cadherin-GFP cells were cultured in suspension for 4h, and then put into polyhema-coated glass-bottom dishes (MatTek) for analysis. Cell-in-cell structures were identified by DIC and E-cadherin-GFP; only completely engulfed cells were chosen for analysis. Images were acquired every 15 min for 20 h using a Nikon Ti-E inverted microscope.

Micropipette aspiration. Micropipette aspiration (MPA) was performed as described [3], on intermediate entotic structures of MCF-10A cells or single cells as indicated in suspension. To analyze the MPA data, the length of the deformation of cortex pulled into the micropipette (L_p) was normalized by the pipette radius (R_p), and L_p/R_p values were plotted as a function of applied pressure. Slopes (m) derived from linear fits of the plots of the inner and outer cell data were determined and analyzed. These data were converted into apparent elastic moduli (Table 1) [4].

Physical model of entosis. The two cells undergoing entosis may adopt different geometrical configurations, depending, in part, on the concentrations and activities of myosin II and cadherin proteins. From a physics point of view, the energy change of the system consisting of these two cells, ΔE_{total} , has a simple form of $\Delta E_{total} = \Delta E_{bending} + \Delta E_{dilation} - \Delta E_{adhesion} - T_{myo} A_{myo}$, where

$\Delta E_{adhesion}$, $\Delta E_{bending}$, and $\Delta E_{dilation}$ are the change of adhesion energy, bending energy associated with curvature change, and dilation energy of the actin cortex, respectively. T_{myo} is the tension per unit length due to elevated actomyosin contractility, and A_{myo} is the corresponding effective area in the inner cell. Thus, the term $T_{myo}A_{myo}$ represents the mechanical work performed by actomyosin contractility. If $\Delta E_{total} \geq 0$, the system remains stationary. If $\Delta E_{total} < 0$, entosis proceeds spontaneously, *i.e.* strong cell-cell adhesion and a relative increase in myosin contractility in the inner cell are required for entosis to complete.

The change of the system energy for different configurations can be calculated by varying the major geometrical parameters once the concentrations and activities of myosin II and cadherin are quantitatively defined. How the geometry of these two cells changes can be numerically identified by finding the minimum energy path of ΔE_{total} in the geometrical space using the nudged elastic band method [5]. The geometrical parameters include r_1, r_2, and r_3 with the assumptions that local cell outlines are more or less spherical and that the volumes are conserved through the process (Figure S4). The surface areas of the corresponding spherical caps are designated as A_1, A_2, and A_3, respectively. The change of bending energy is

$$\Delta E_{bending} = \frac{1}{2}k_2\left(\frac{1}{r_3}-\frac{1}{\bar{r}_3}\right)^2 A_3 + \frac{1}{2}k_2\left(-\frac{1}{r_2}-\frac{1}{\bar{r}_2}\right)^2 A_2 + \frac{1}{2}k_1\left(\frac{1}{r_2}-\frac{1}{\bar{r}_2}\right)^2 A_2 + \frac{1}{2}k_1\left(\frac{1}{r_1}-\frac{1}{\bar{r}_1}\right)^2 A_1.$$

The terms k_1 and k_2 (k_1= k_2 =400 k_BT [6]) are the bending moduli for inner and outer cells, respectively. $\bar{r}_i$ (i=1, 2, 3) are the initial values of r_i before symmetry breaking. The change of dilation energy is

$$\Delta E_{dilation} = \frac{1}{2}\frac{D_2}{{A_0}^2}\left(A_3 + A_2 - A_0\right)^2 + \frac{1}{2}\frac{D_1}{{A_0}^2}\left(A_1 + A_2 - A_0\right)^2.$$

The term D_1 is the area dilation modulus (D_1=2×10^4 k_BT) for the inner cell cortex and was determined by converting the measured inner cell slope (m=1.5 μm^2/nN; Figure 2D) into a dilation modulus, assuming the cortex is ~0.5 μm thick. D_2 is the area dilation modulus (D_2=1×10^4 k_BT) for the outer cell cortex and is approximately half of D_1 based on the measured slopes (Figure 3A). A_0 is the initial surface area before symmetry breaking. The change of adhesion energy is

$$\Delta E_{adhesion} = (\varepsilon\rho - 2\gamma)A_2,$$

where ρ (ρ =100 molecules/μm^2 [7]) is the cadherin bond density at the cell-cell interface, ε (ε = 20 k_BT [8, 9]) is the bond energy of cadherin-cadherin complexes, and γ ($\gamma = 0$) is the interface energy between the cell surface and the culture media. The work associated with myosin contractile force is

$$T_{myo}A_{myo} = 2N\chi f / r_1 \cdot \pi r_1^{\,2} = 2\pi N\chi f r_1,$$

where N (N=1000 molecules/μm^2 [10]) is the difference in the number of active myosin II molecules (in bipolar thick filament form) per unit area in the actin cortex of the inner cell vs. the outer cell, χ is the duty ratio of mammalian myosin II (0.8 for myosin IIB [11, 12]), and f (4 pN [13]) is the force generated by one myosin head. The simulated energy landscapes for the progression of entosis with and without a myosin II contractility differential between the inner and outer cells and from different starting geometries are shown in Figure 4.

References

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