Supporting Information

Rengstl et al. 10.1073/pnas.1312509110

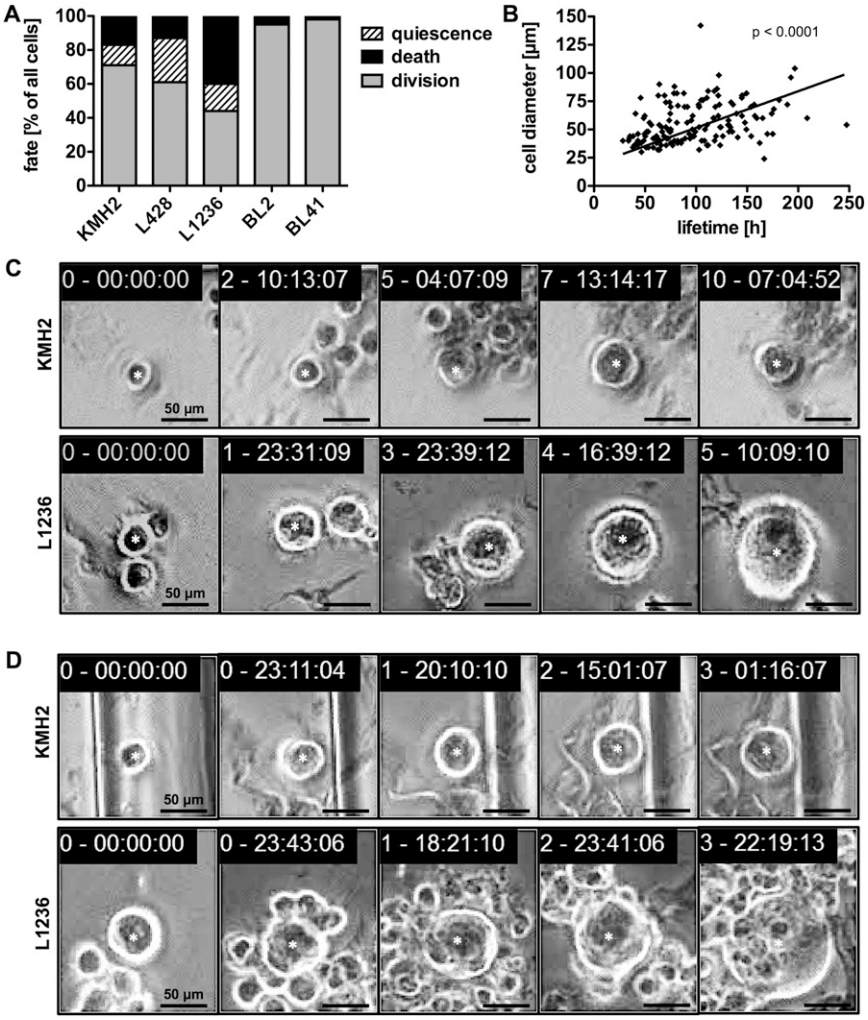


Fig. S1. Rare giant and long-lived Hodgkin and Reed–Sternberg (HRS) cells are present in Hodgkin lymphoma (HL) cell lines. HL cell lines KMH2, L428, and L1236 and Burkitt lymphoma (BL) cell lines BL2 and BL41 were tracked for 10–12 d using long-term time-lapse microscopy. (*A*) In addition to analysis of the seeded cells (n = 107–146, generation 0; Fig. 1*B*), the fate of the progeny (n = 166–490; generation 1–2) within the first 50 h was also classified as division, death, or quiescence. Fate values for the particular HL cell lines are compared with the BL cell lines that served as controls. (*B*) Correlation between lifetime and cell diameter was calculated for quiescent HRS cells of all HL cell lines through a combined analysis. (*C* and *D*) Compared with L428 HRS cells (Fig. 1 *E* and *F*), the lifespan of long-lived cells (*C*) and the growth of normal-sized cells forming giant HRS cells (*D*) are shown for HL cell lines KMH2 and L1236. Stars in *C* and *D* denote the cells tracked over time.

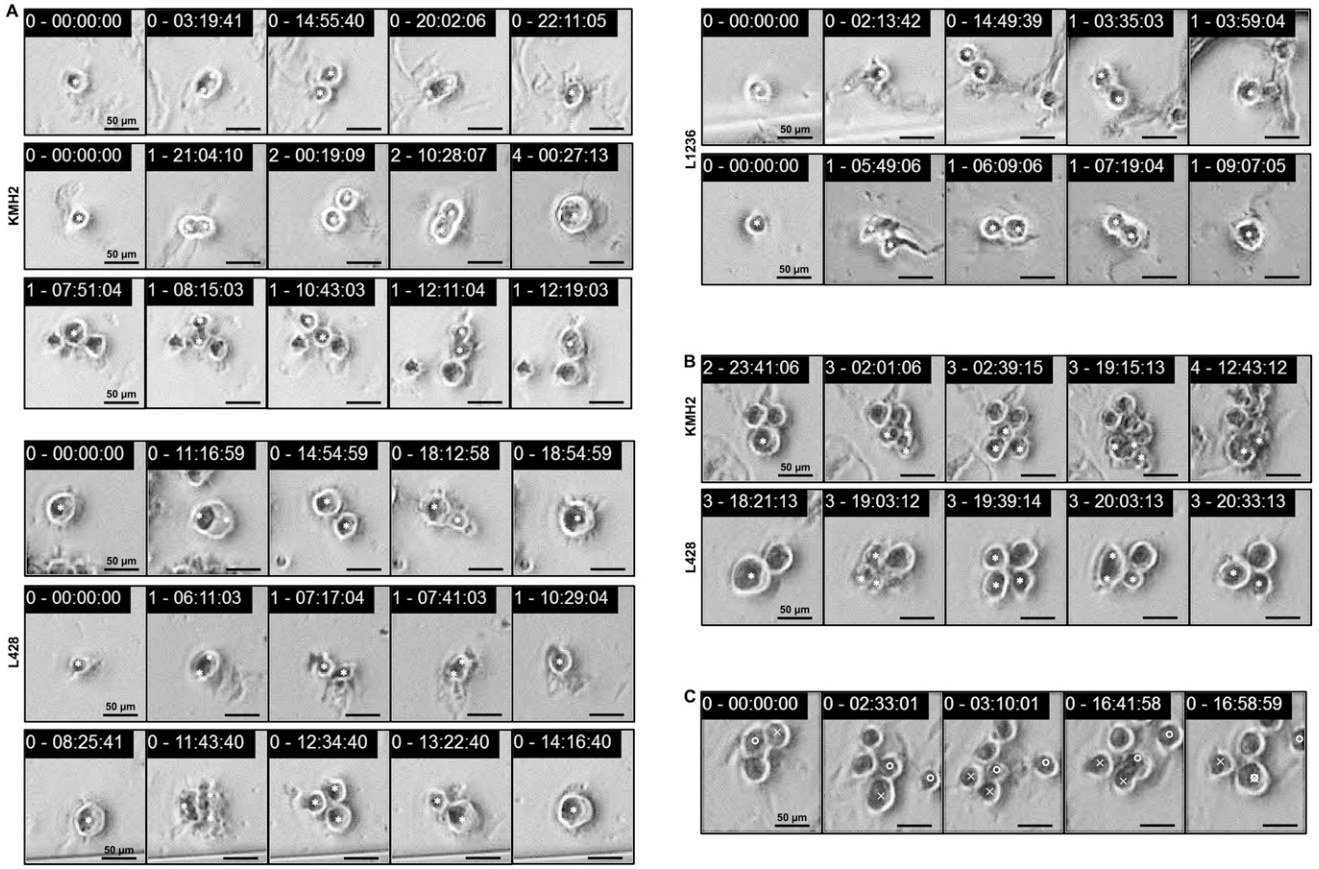


Fig. S2. Giant HRS cells originate mostly from re-fusion. The development of giant HRS cells was corroborated by long-term time-lapse microscopy of HL cell lines KMH2, L428, and L1236. (*A*) Examples of individual KMH2 ($n = 3$), L428 ($n = 3$), and L1236 ($n = 2$) HRS cells undergoing division and subsequent re-fusion are shown in selected images of the continuous time-lapse acquisition. (*B*) In addition to the highlighted trichotomy event of a L1236 HRS cell (Fig. 2*D*), other examples of trichotomy for the HL cell lines KMH2 and L428 are shown to demonstrate the alternative cell fusion route generating RS cells. First, a cell divides into three cells, and then two cells re-fuse to generate a fused giant HRS (fHRS) cell. (*C*) Selected images of the only cell fusion event observed between cells originating from different but related progenitors (HL cell line L428; Movie S4). Stars in *A* and *B* and circles and crosses in *C* indicate the tracked cells.

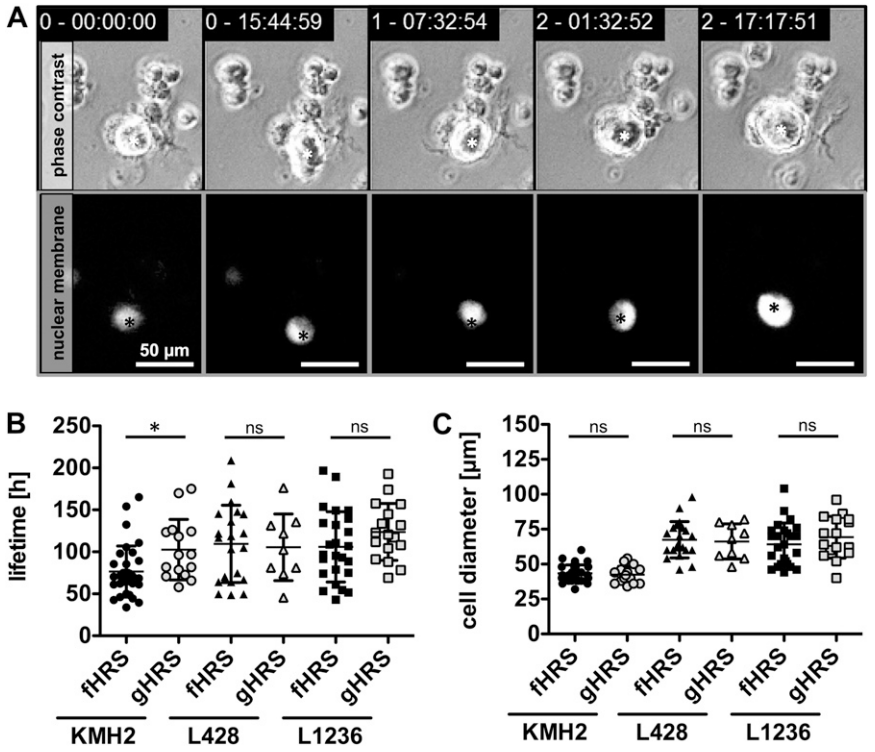


Fig. S3. Increase of nuclear mass in growth-induced giant HRS cells. HL cell lines KMH2, L428, and L1236 were transduced with a lentiviral vector encoding for tdTOMATO-hIMPORTIN. Subsequently, nuclear fluorescence-labeled HRS cells were tracked, focusing on cell fusion events, and gHRS cells missing a cell fusion event during their development were assessed as well. (*A*) Phase contrast (*Upper*) and fluorescence imaging (*Lower*) of an exemplary L1236 gHRS cell illustrates the increase of nuclear mass by time (Movie S6) in contrast to fHRS cells, which show multinuclearity after cell fusion, characteristic of RS cells (Fig. 2*F*). (*B* and *C*) Comparative analysis of lifetime (*B*) and cell diameter (*C*) of fHRS and gHRS cells from the particular HL cell lines.

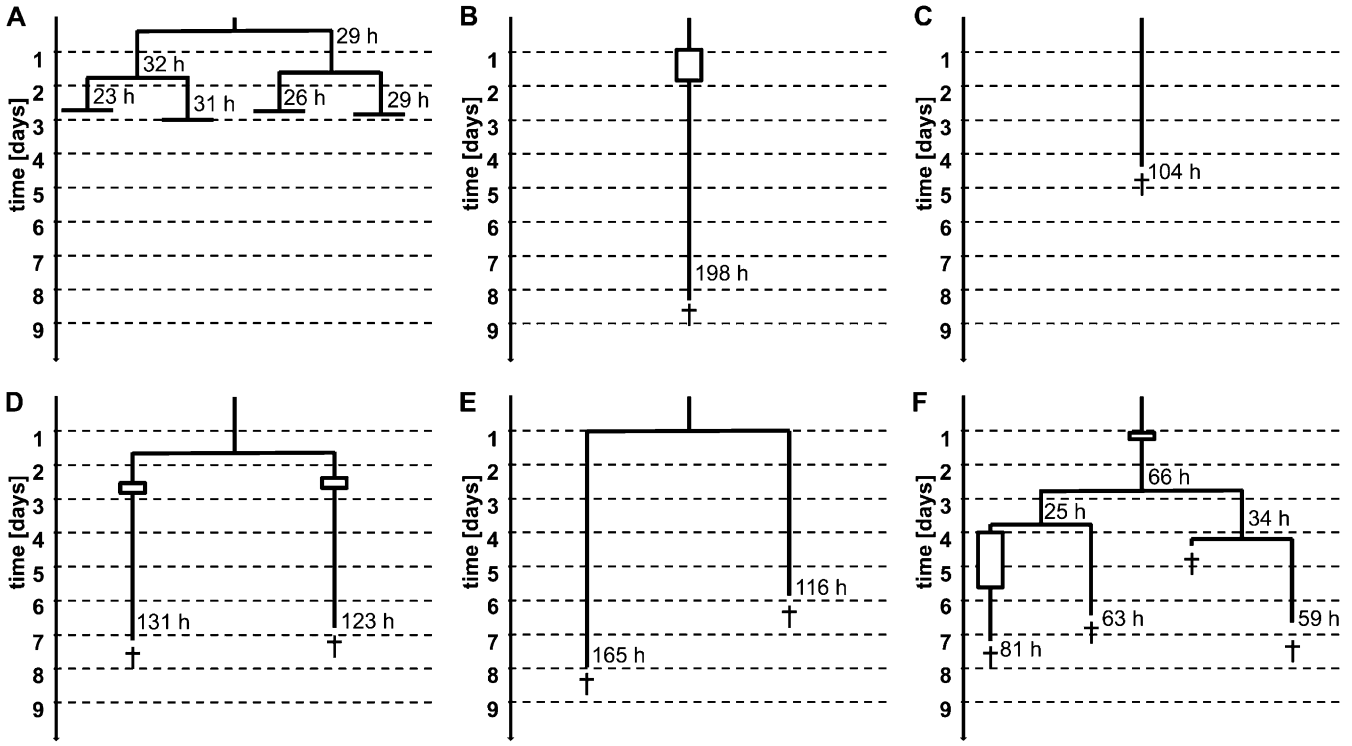
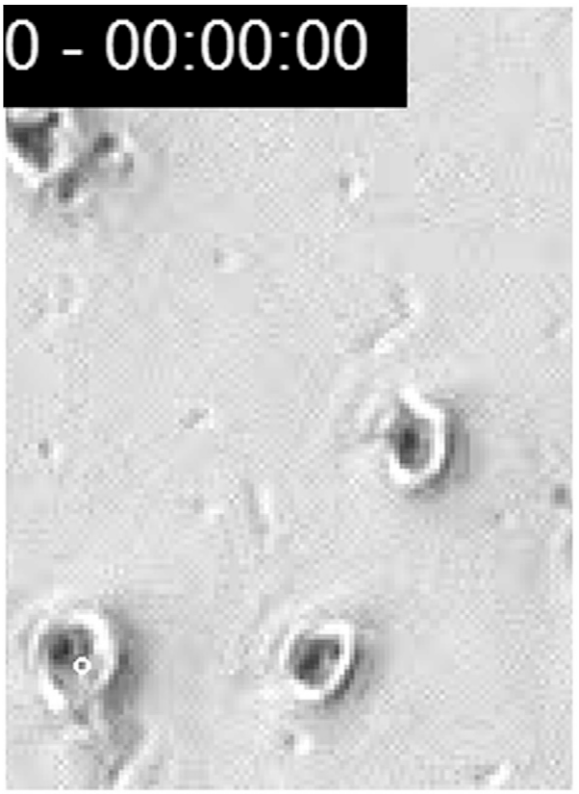
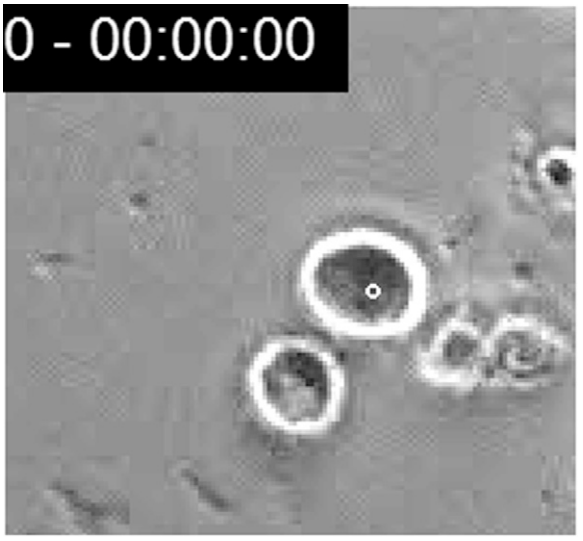


Fig. S4. Behavior of giant HRS cells in contrast to the bulk population of small mononucleated HRS cells. Examples of tracked pedigrees of HRS cells illustrate conventional proliferating cells (*A*), formation of a fHRS (*B*), formation of a gHRS cell (*C*), an fHRS sister cell pair (*D*), a gHRS sister cell pair (*E*), and a dividing fHRS together with its progeny (*F*). White boxes indicate cell division and re-fusion at a true time scale. Individual cell lifetimes are indicated.



Movie S1. Longevity of giant HRS cells. As an example of the longevity of giant HRS cells, an individual L428 cell (white circle track) is followed by time-lapse–based cell tracking for more than 8 d at 1-min resolution, demonstrating a lifetime of more than 192 h. An increase in size of the HRS cell over time is visible. Every seventh image was used for the movie assembly.

Movie S1

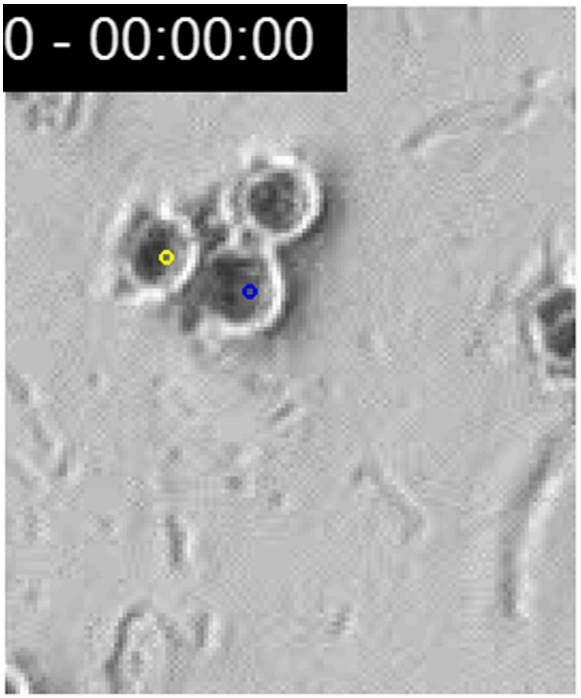


Movie S2. Fusion-based giant HRS cell development. Time-lapse microscopy of HL cell lines was performed to analyze giant cell formation. Cell fusion events were identified as the dominant route of giant HRS cell development. Here an individual L1236 cell (white circle track) is shown undergoing division with re-fusion of daughter cells after 21 h of separation. All images were used for the movie assembly.

Movie S2

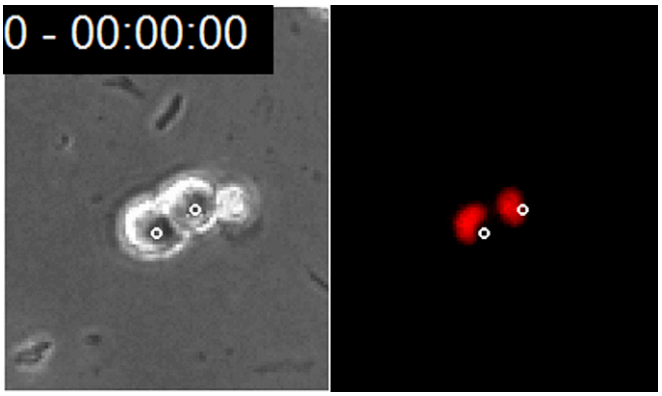
Movie S3. Trichotomy. Time-lapse microscopy of HL cell lines was performed to analyze giant cell formation. Trichotomy, division into three separate cells followed by re-fusion of two cells, exhibited a subgroup of cell fusion events. Here an L1236 cell is shown undergoing trichotomy with subsequent re-fusion of two cells. The third cell died 21 h later. All images were used for the movie assembly.

Movie S3



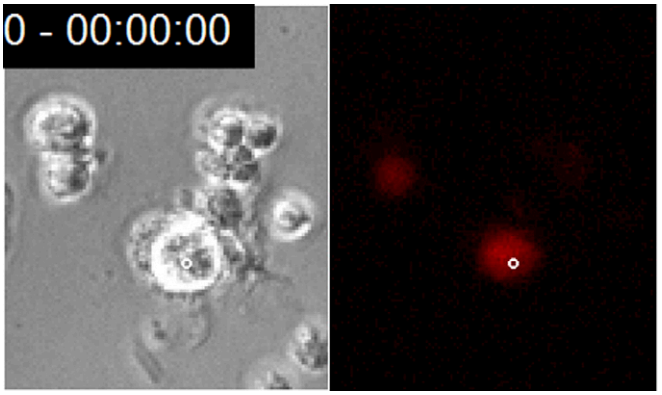
Movie S4. Cell fusion between HRS cells originating from different ancestors. Time-lapse microscopy of HL cell lines identified cell fusion as the dominant route of giant cell development. In most cases, cell fusion occurred between cells originating from the same progenitor; however, in one case, fusion of L428 cousin cells from different ancestors (in blue and yellow) was detected. All images were used for the movie assembly.

Movie S4



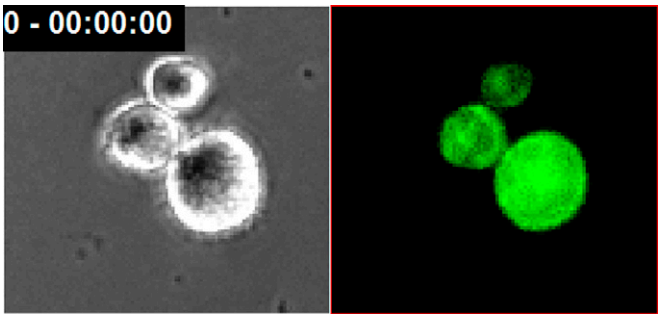
Movie S5. Development of multinucleated RS cells. Time-lapse microscopy of nuclear fluorescent-labeled HL cell lines enabled the analysis of mitotic events synchronous to fusion-based giant cell development. The video shows an L428 cell (white circle tracks), which divided into two cells, each containing a single nucleus. Shortly after division, the cells fused to give rise to a multinuclear RS cell with two separate nuclei. Every 67th image (corresponding phase-contrast and fluorescent images) was used for the movie assembly.

Movie S5



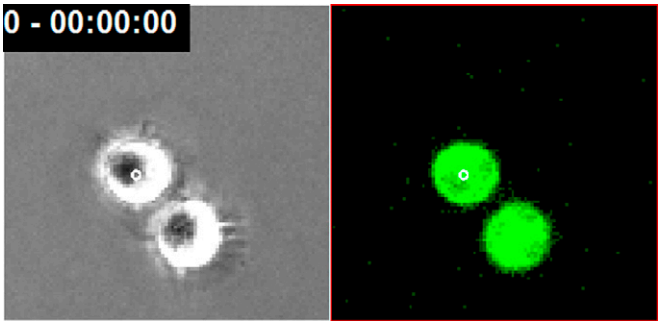
Movie S6. Development of giant gHRS cells. Time-lapse microscopy of nuclear fluorescent-labeled HL cell lines enabled the analysis of mitotic events synchronous to growth-induced giant cell development. The video shows a L428 cell (white circle tracks) containing a single nucleus that increased in size over time. The nuclear mass increased simultaneously with the cell diameter. Every 67th image (corresponding phase-contrast and fluorescent images) was used for the movie assembly.

Movie S6



Movie S7. Incomplete cytokinesis before re-fusion. Time-lapse microscopy of microtubule fluorescent-labeled HL cell lines was performed to clarify whether cytokinesis is complete or incomplete before re-fusion. The video shows an L428 cell (white circle tracks), which divided into two cells that remained connected through the midbody until re-fusion. Phase-contrast (*Left*) and RFP-tubulin (*Right*) are shown. Every 44th image (corresponding phase-contrast and fluorescent images) was used for the movie assembly.

Movie S7



Movie S8. Complete separation before re-fusion. Time-lapse microscopy of microtubule fluorescent-labeled HL cell lines was performed to clarify whether cytokinesis is complete or incomplete before re-fusion. The video shows a L428 cell (white circle tracks), which divided into two completely separated cells before re-fusion, with no visible microtubule bond (midbody connection). Phase-contrast (*Left*) and RFP-tubulin (*Right*) are shown. Every 44th image (corresponding phase-contrast and fluorescent images) was used for the movie assembly.

Movie S8