Jungas et al., http://www.jcb.org/cgi/content/full/jcb.201602057/DC1

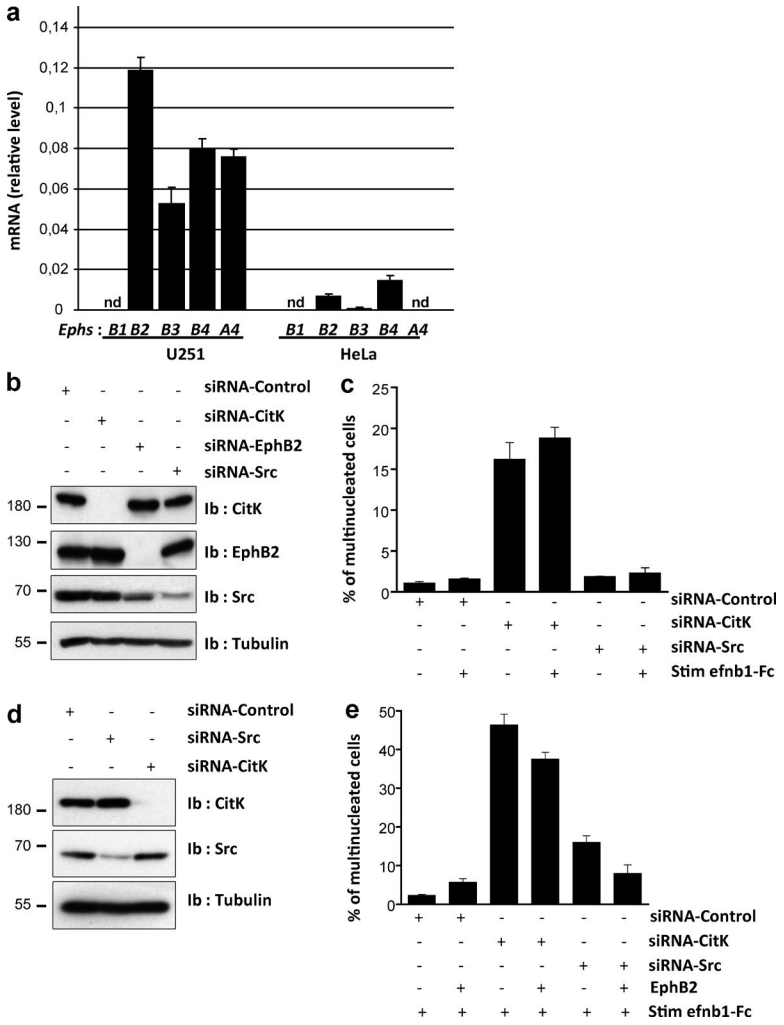


Figure S1. **Expression of Eph receptors and validation of siRNAs in U251 and HeLa cells.** (a) Total RNA extracted from U251 and HeLa cells was used for qRT-PCR as described in Materials and methods. *EphB1*, *EphB2*, *EphB3*, *EphB4*, and *EphA4* mRNA relative levels were compared with a standard (S16 ribosomal gene). nd, not detected. (b and d) Protein lysates from U251 (b) or HeLa (d) cells treated with different siRNAs were immunoblotted to detect EphB2, Src, and CitK. Tubulin was used as a loading control. (c and e) Quantification of the fraction of multinucleated U251 (c) or HeLa (e) cells after siRNA treatments from three independent experiments. Error bars correspond to SEM. Statistical p-value is indicated when significant.

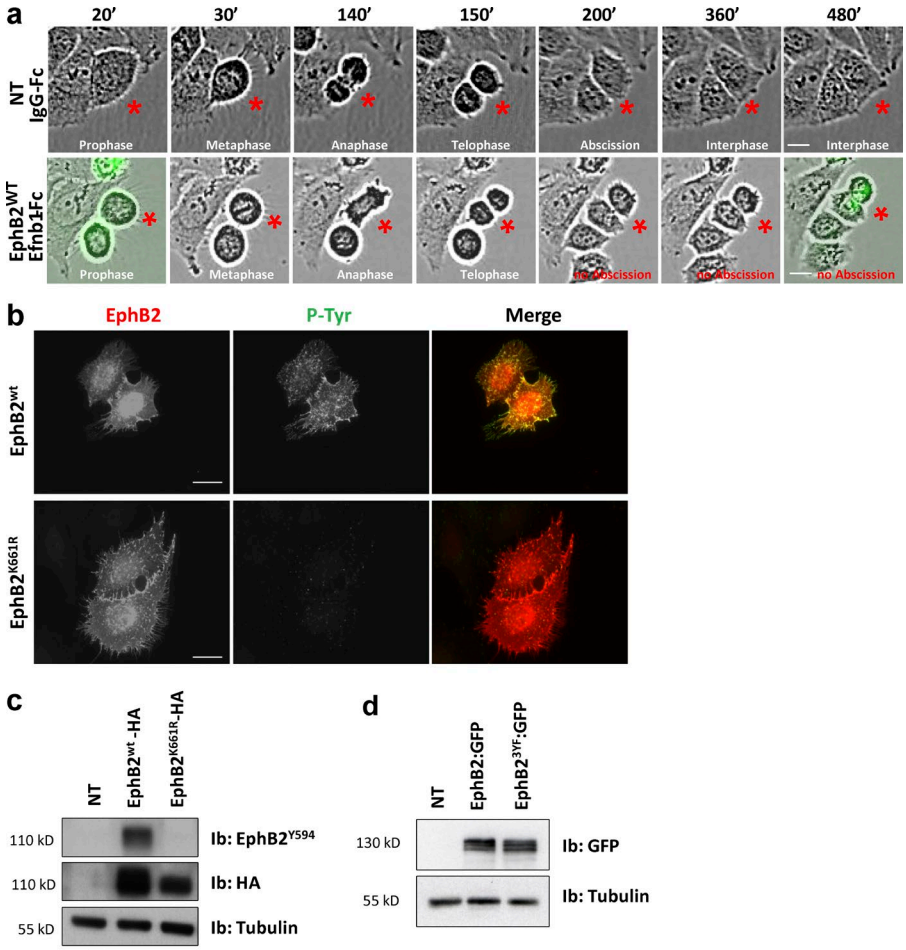


Figure S2. **Live imaging with Incucyte and characterization of EphB2 kinase-dead mutants.** (a) Example of frame-by-frame analysis with Incucyte for NT (top) and transfected + stimulated (bottom) HeLa cells undergoing cell division (asterisks). The ICB is visible as a refringent structure between daughter cells. Bars, 10 µm. (b) HeLa cells were transiently transfected with either wild-type EphB2 ($EphB2^{WT}$; top) or a kinase-dead version of EphB2 ($EphB2^{K661R}$; bottom). Cells were stimulated with Efnb1-Fc for 20 min and immunostained to detect EphB2 (red) and phosphorylated tyrosines (green, P-Tyr). Left panels are merged images. Bars, 10 µm. (c) HeLa cells either untransfected (NT) or transfected with HA-tagged $EphB2^{WT}$ or an HA-tagged $EphB2^{K661R}$ were stimulated with Efnb1-Fc for 20 min. Protein lysates were analyzed by immunoblotting using an antibody specific to phospho-EphB2 ($EphB2^{Y594}$) or an antibody to HA. Tubulin was used as a loading control. (d) HeLa cells either untransfected (NT) or transfected with GFP-tagged WT EphB2 or a GFP-tagged kinase mutated EphB2. Protein lysates were analyzed by immunoblotting using an antibody specific to GFP. Tubulin was used as a loading control.

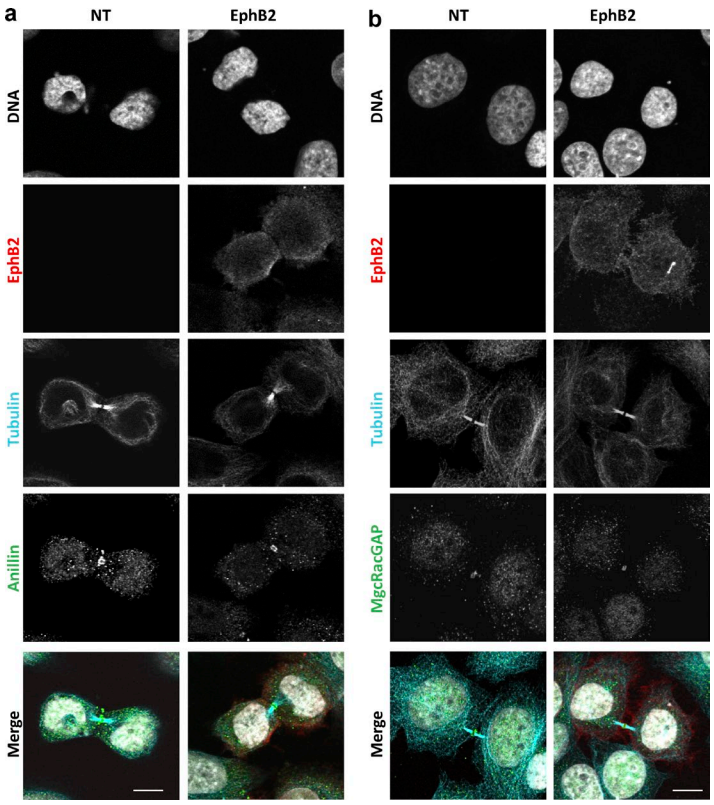


Figure S3. **Localization of Anillin and MgcRacGAP at the midbody is not modified after EphB2 activation.** (a and b) HeLa cells were either left untransfected (NT) or transiently transfected with EphB2, stimulated with Efnb1-Fc for 48 h, and immunostained as indicated. Anillin and MgcRacGAP properly localized at the midbody during cytokinesis of EphB2$^+$ stimulated cells. Images are representative of n = 10 cells. Bars, 10 µm.

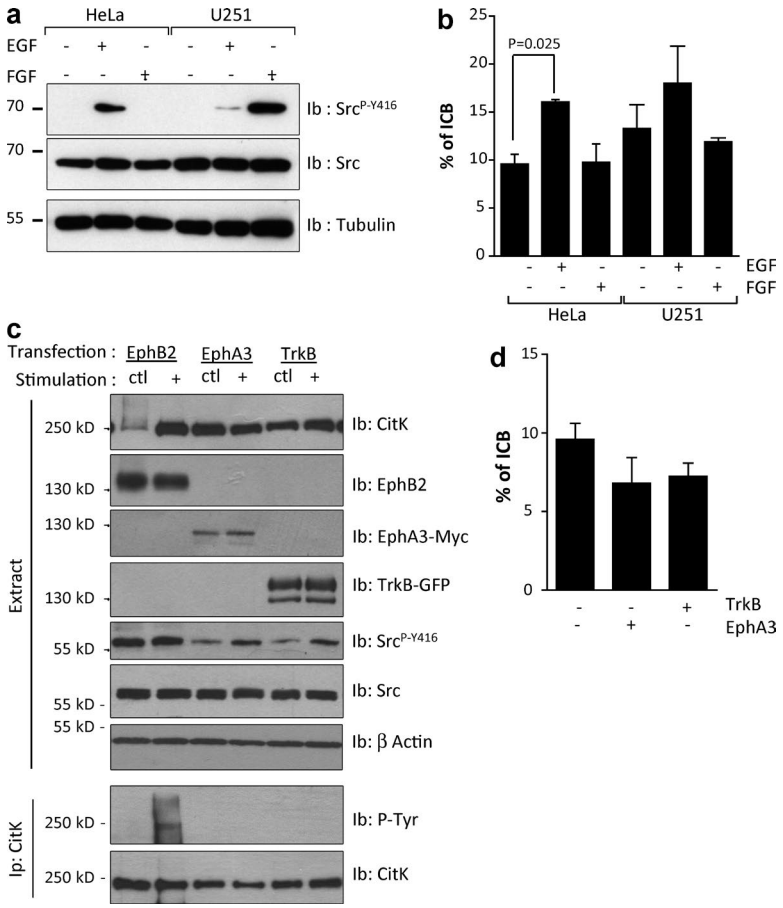


Figure S4. **Persistence of ICB is not a generic response to RTK and Src activation.** (a) U251 and HeLa cells were treated with EGF or FGF for 20 min to activate EGFR and FGFR, respectively. The status of Src activation was monitored by immunoblotting with $Src^{P\text{-}Y416}$ antibody. Tubulin was used as loading control. (b) Similar treatments were applied for 48 h, and the fraction of cells connected by ICBs was quantified by immunostaining on fixed cells. Tubulin was used to visualize ICB, and DAPI was used to visualize nuclei. *n* = 3 experiments. (c) HeLa cells were transfected with EphB2, EphA3, or TrkB and either mock stimulated or stimulated with corresponding ligands (Efnb1-Fc, EfnA5-Fc, and BDNF, respectively) for 20 min. The status of Src activation was monitored by immunoblotting with $Src^{P\text{-}Y416}$ antibody (Extract). The status of CitK phosphorylation was monitored by IP/Western blot (Ip). Actin was used as loading control. (d) HeLa cells were transfected with EphA3 or TrkB and stimulated with corresponding ligands (EfnA5-Fc and BDNF, respectively) for 48 h, and the fraction of cells connected by an ICB was quantified by immunostaining on fixed cells as described earlier. *n* = 3 experiments. Error bars correspond to SEM. Statistical p-value is indicated when significant.

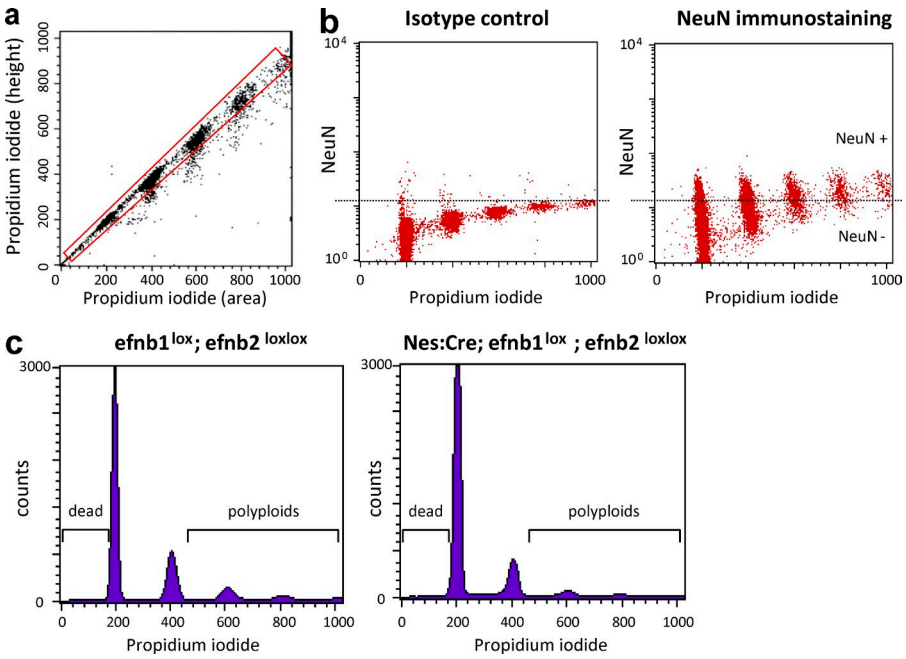
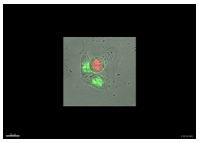


Figure S5. **FACS analyses.** (a) Dots plots used to exclude cell doublets from the analyses based on propidium iodide emission intensity (height) versus emission period (area). Only cells inside the drawing zone were considered as singlets and analyzed further. (b) Dot plots used to discriminate between NeuN-positive and NeuN-negative cells. (left) Cells incubated with secondary antibody only. (right) Cells immunostained with NeuN and secondary antibody. Cells above the dashed line were considered NeuN positive and analyzed for DNA content. (c) Representative histograms of DNA content analysis of NeuN-positive cells in a control (*Efnb1*^{loxlox}*; Efnb2*^{loxlox}) and a compound mutant (Nestin-Cre; *Efnb1*^{loxlox}*; Efnb2*^{loxlox}) embryo. Brackets show cells that were considered dead or polyploid based on their DNA content compared with cells in G1/G0 (first peak on the left).



Video 1. **Stimulated EphB2$^+$ HeLa cell undergoing cell death after entering telophase.** The video shows a dividing stimulated EphB2$^+$ HeLa cell undergoing cell death after entering telophase. The cell expresses EphB2-GFP (green) and Tubulin-mCherry (red) and was stimulated with Efnb1-Fc. Live imaging was performed on a 710 confocal microscope (ZEISS) using Zen software (ZEISS) for acquisition. Calibration was as follows: 7 fields/well, 1 frame/10 min for 12 h, and 15 Z-stacks of 2 µm. Related images are presented in Fig. 2.

Table S1. **Primer sequences**

Name	Sequence (5′–3′)
S16 sense	AGGAGCGATTTGCTGGTGTGG
S16 antisense	GCTACCAGGGCCTTTGAGATGG
Cre sense	GTCCGGGCTGCCACGACCAA
Cre antisense	ACGGAAATCCATCGCTCGACCAGTT
EphB1 sense	AGAGGAGGGAAAAGGACCAGG
EphB1 antisense	GGTTTCCCACGGCATCTC
EphB2 sense	AAAAGGGCTTGGGAGATTCAT
EphB2 antisense	GTCCATCTGTCCCGTCCTC
EphB3 sense	GCTGGGCTTGTCTTCGTGGTG
EphB3 antisense	CCTTGGCAAACTCCCGAACA
EphB4 sense	GGCTGCTCGCAACATCCTAGT
EphB4 antiense	CCACATCACAATCCCGTAAC
EphA4 sense	CGTTATGTGGGAAGTGATGTCATA
EphA4 antisense	TTCCTCAATGGCTTTAATCACATCT

Provided online is Table S2, showing the mass spectrometry dataset.