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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   We chose sufficient sample size to establish that the values compared were derived from normal distributions. (Mentioned in "Statistical analysis and reproducibility" section)

2. Data exclusions

   Describe any data exclusions.

   All the data were included.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   Yes, all the experimental findings were reliably reproduced.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   No aminal or human research participants were used, so there was no issue about randomization.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   No aminal or human research participants were used, so there was no big issue about blinding for group allocation in this study. For the imaging analysis in Fig.2d, we mentioned a criteria to pick up the cells to analyze in "online methods" section as follows. "For statistical analysis of CD43ex-45int-mCherry segregation, we picked up cells that were clearly in contact through scFv-antigen interaction. This was achieved by observing accumulation of CFP at the cell-cell interface with HEK-HER2. (In the case of contact with HEK-iRFP, CFP accumulation at the cell interface was never observed, so sensor cells that appeared to be in contact with HEK-iRFP were picked up). "

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	No special software was used in this study.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	Correspondence and requests for materials should be addressed to M.F. (mentioned in "additional information" section.)

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	For the use of ML39 (anti HER2 scFv), Ec4 (anti Epcam DARPin), and SP6 (irrelevant antibody), papers are cited in the text and figure legends. Source of them are described in "online methods" section and supplementary information as well. Confirmation of HER2 expression on SKBR3 cells were done by using a commecially available antibody. (Biolegend, #324405) (Fig.S4c)

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

The HEK-293 and HEK-293T cell lines were from DSMZ. hMSC-TERT were provided by the authors of ref #36. SKBR3 cells were gift from Dr. Nancy Hynes (FMI, Basel, Switzerland). These are mentioned in "online methods" section.

b. Describe the method of cell line authentication used.

The HEK-293 and HEK-293T cell lines were authenticated by DSMZ. For hMSC-TERT cells, the original paper reporting this cell line was cited (#36 in the revised manuscript). For SKBR3 cells (gift from Nancy Hynes), we confirmed HER2 expression by ourselves with FACS. (Fig S4c) These things are mentioned in "online methods" section.

c. Report whether the cell lines were tested for mycoplasma contamination.

For HEK-293T and HEK-293 cells which we used mainly in this paper, microplasma (-) is guaranteed by the supplier. (Online methods, cell culture and transfection section) (https://www.dsmz.de/catalogues/details/culture/ACC-305.html?tx_dsmzresources_pi5%5BreturnPid%5D=192) Also, we exclusively use cells we regularly check for bacterial contamination

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animal studies was conducted.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No human research participants were involved in this study.