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Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample size (mouse and implantable microenvironment) were chosen on the basis of previously published findings (Lee, J et al, PNAS 2012, Cancer Research 2014) and preliminary experiments so as to provide sufficient power (80%) for statistical comparison (5% significance).

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the study.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   The reproducibility of biomaterial fabrication, implanted-tissue-microenvironment formation with human bone marrow stromal cells, and recruitment of human tumor cells generated from an orthotopic xenograft tumors were confirmed by previously published results (Lee, J et al. Biomaterials 2009, PNAS 2012, Cancer Research 2014). The impact of hPBMC on implantable metastatic tumor microenvironment was repeated two times to ensure the reproducibility of the experimental finding. We observed the comparable pattern of metastatic tumor development in these experiments. Complete optical sectioning of implantable microenvironment was performed for at least three independent scaffolds. All other characterizations including immunohistostaining were performed in at least three independent scaffolds and tissue samples to ensure experimental reproducibility.

4. Randomization

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

   Experimental groups (hPBMC and control PBS-injection mice) were randomized.

5. Blinding

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

   Blinding was not relevant to our study. Implanted scaffolds in each mouse were retrieved and reimplanted to the same mouse. Surgeons knew which mice received hPBMCs and control PBS during the surgery.

Note: all in vivo studies must report how sample size was determined and 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes 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 in all relevant figure captions (with explicit mention of central tendency and variation)

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.

Image J was used for image analysis. Nikon Elements AR v4.5 was used for 3D rendering and creation of a video. The video was annotated in Adobe Premiere Pro CC 2014 and encoded in Adobe Media Encoder CC 2014.

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 third party.

The implantable hydrogel scaffolds are readily available from the authors. All other materials are available from standard commercial sources (Fisher scientific). Human fresh bone marrow aspirate is available from Lonza.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Anti-human vimentin (M072529-02) was purchased from DAKO. Anti-alpha smooth muscle actin (ab5694), anti-ki67 (ab16667), anti-human cytokeratin 8 (ab107115), and anti-mouse Ly6C were purchased from Abcam. Anti-mouse CD31 (550274), anti-human CD45 (555480), anti-human CD4 (555631), anti-human CD8 (555344), anti-mouse F4/80 (565409), anti-mouse Ly6G (557445), and anti-human CD44 (550392) were purchased from BD Pharmingen. Anti-human cytoeratin polyclonoal (LS-C193787) was purchased from Lifespan Boscience. Anti-MMP was purchased from ThermoFisher. Absence of cross-reactivity of human vimentin, CD45 and cytokertain antibodies was validated with mouse tissues. The other antibodies were not validated before use.

10. Eukaryotic cell lines

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

Luc-GFP PC3 cell line was obtained from the Center for Engineering in Medicine, Massachusetts General Hospital. The original PC3 cell line was acquired from American Type Culture Collection (Manassas, VA, USA)

b. Describe the method of cell line authentication used.

The cell line used has not been authenticated.

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

No testing was performed.

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.

No commonly misidentified cells line were used.

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

6–13 week-old male NSG mice were used. Initial breeding pairs were purchased from the Jackson Laboratory (Cat #: 005557)

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.

Three healthy male (age 20–38) donated peripheral blood.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Mouse tissue and implanted scaffolds were collected and digested in collagenase, type II. Tissue pieces were passed through a 40 µm cell strainer and spun down at 1500 RPM.
6. Identify the instrument used for data collection.	Becton Dickinson LSRFortessa
7. Describe the software used to collect and analyze the flow cytometry data.	BD FACSDiva was used for collection and analysis
8. Describe the abundance of the relevant cell populations within post-sort fractions.	N/A
9. Describe the gating strategy used.	Due to tissue heterogeneity positive cells percentages were calculated by dividing positive cell counts by total events run through cytometer. Gates were determined by analyzing fresh hCD45-PE stained hPBMCs from human donors.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒