nature research

Corresponding author(s): Eduard Batlle

☐ 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.

Experimental group sizes were practically associated to cage sizes (5 mice/cage) and treatment experiments were designed to have n >= 5 per group (1 or more cages) .

2. Data exclusions

Describe any data exclusions.

In the DSS/tam induction of the genetic model, 13% of mice died in the first 2 weeks from DSS treatment and were excluded from analysis.
One mouse was excluded from the Fig. 2f because it showed no recombination upon tamoxifen treatment.
In the KM survival curve in Fig 2e, some cohorts of mice (Con and Gal) were sacrificed at endpoint of Con (metastasis-associated severe morbidity) for tumour nodule count. Fully cured Gal mice that were culled this way were censored from the survival plot (ticks).

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experiments were reproducible.

4. Randomization

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

Because daily drug treatments were given as one type per cage (to avoid confusion and errors), we found the best way to randomize was to inject mice with tumour organoids in semi-random order: balanced among the predetermined treatment arms so as to not have any difference in cell viability but otherwise unpredictable to the person doing the injection.

5. Blinding

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

T (tumour) status scoring from H&E stained sections was performed by two blinded observers.
As stated above, the investigator doing the injection was blinded to treatment arm. In addition, the technicians doing the daily drug administrations were blinded to the experiment design including groups. Data collection and analysis was not blinded.

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.	We used R (v3.4.2), RStudio (v1.1.383) and FIJI/ImageJ (v1.51d), and have custom codes that are available upon request. In addition, we used QuPath (v0.1.2), GraphPad Prism (v7.03), netWHCpan (v2.8), bwa (v bwa-0.7.4), Sambamba (v0.5.9), Picard (v1.128), GATK (v3.5), snpEff (v4.1), deconstructSigs (c1.8.0), Star (v2.3.0e), GeneAtlas, FlowJo (v10.4),

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.	The MTO biobank established in this paper is available to the community upon request, subject to restrictions imposed by the original mouse strain MTAs. All other materials are readily available from indicated standard commercial sources.

9. Antibodies

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

Only commercial antibodies have been used:
Rat αCD8α (YTS 169.4; BioXCell BE0117) or Rat αCD4α (GK1.5; BioXCell BE0003-1) or Rat IgG2b (LTF-2, BioXCell BE0090) isotype control antibodies were used for in vivo T cell depletion. All routinely validated and confirmed by flow cytometry analysis on peripheral blood (by us).
Rat αPD-L1 (10F.9G2; BioXCell BE0101) or Rat IgG2b (LTF-2, BioXCell BE0090) isotype control antibodies were used for PD-1/PD-L1 therapy. Validation reported on supplier's website.

For IHC, we used: Antibodies against CALD1 (Rabbit, Sigma, ref HPA008066; 1:250), IGFBP7 (Rabbit, Sigma, HPA002196; 1:200), phopsho-SMAD2 (rabbit, Cell Signaling, ref 3108; 1:50), CD4 (Rabbit, Sino Biological, ref 50134-R001; 1:1000), CD8 (Rabbit, Biorbyt, ref orb10325-200; 1:200), FoxP3 (Rabbit, Abcam, ref ab54501, 1:1000), T-bet (Santa Cruz, ref sc-21003; 1:500), phospho-SMAD3 (Rabbit, Abcam, ref ab52903; 1:500) and PD-L1 (Cell Signalling, ref 16764988S; 1:25); staining o/n at 4ºC. We used anti-GFP (Rabbit, Life Technologies, ref A11122; 1:500), anti-CD3 (Rabbit, DAKO, ref. IS50330; 1:30), staining for 2h at room temperature. Antibodies were validated extensively by the provider (including westernblot analysis) and confirmed by the authors for specific localization and--in case of TGF-beta targets--on modulation upon pathway inhibition in vivo.

The following (routinely used and community-validated) antibodies were used for flow cytometry staining: anti-CD45 (clone 30-F11), anti-CD4 (clone GK1.5), anti-aCD8 (clone 53-6.7), anti-CD69 (clone H1.2F3), anti-CD104b (PDGFRb, clone APB5), and anti-MHCII (clone M5/114.15.2) were obtained from eBioscience; Epcam (clone EBA-1), anti-CD3e (clone 145-2C11), anti-CD44 (clone G44-26), CD62L (clone MEL-14), anti-IFNγ (clone XMG1.2), anti-CD274 (PD-L1, clone MIH-5), and anti-Ly6C (clone AL-32) were obtained from BD Pharmingen; anti-CD31 (clone 390) was obtained from Abcam; and anti-CD11b (clone M1/70), anti-CD11c (clone H418), anti-GZMB (GB11), anti-CD279 (PD-1, clone 29F.1A12), anti-CD8a (clone 53-6.7), anti-T-bet (clone 4B10), anti-Ly6G (clone 1A8) were obtained from BioLegend. Extra validation was performed by comparing expression in internal controls (known negative populations).

For Westernblot, we used antibodies against SMAD4 (B-8, Santa Cruz, ref: sc7966) and actin (Abcam, ref: ab20272), validated on protein size and by knockout (SMAD4).

10. Eukaryotic cell lines

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

Mouse tumour organoids were derived from genetic models in our lab.
MC38 and CT26 murine cell lines as well as L cells producing Wnt3a (L-Wnt3a) were obtained from the ATCC.

b. Describe the method of cell line authentication used.

Cancer cell lines used were exome sequenced. Wnt3a production was tested in a cellular reporter assay, sensitive to Wnt ligands.

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

Mycoplasma contamination was tested for (bi-monthly) and always negative.

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.

None 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 details on animals and/or animal-derived materials used in the study.

Genetic models: We used inbred (backcrossed) C57BL/6J mice in which we induced recombination at age 12-15 weeks, observing up to 1 year after induction or sacrificing upon severe morbidity.
From tumours/polyps in this model we derived tumour organoids (MTOs) to study metastasis vastly more efficiently than otherwise possible with purely genetic (spontaneous) mouse models. Injection and transplantation of these MTOs was performed again in C57BL/6J hosts or in BALB/c Nude mice, aged 7-8 weeks. (This age limit was made more flexible (7-10 weeks) when injecting in cohorts of genetic mice such as the UBC-creERT2;Tgfbr2-fl/fl or Lgr5-creERT2 hosts, to reduce total numbers of litters necessary.) Experimental mice were typically observed for 3-5 weeks, but treatment survivors are being kept for over 1 year after injection.
All mice were closely monitored by authors, facility technicians (during treatments) and by an external veterinary scientist responsible for animal welfare.

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 subjects.

nature research

Corresponding author(s): Eduard Batlle

☐ Initial submission ☐ Revised version ☒ Final submission

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.	Livers with tumours were removed, lobules (and in case of micro-dissection: individual liver metastases) were carefully dissected and finely minced with scalpels. The tissue was enzymatically digested in 10 ml of DMEM supplemented with 10% FBS, 1% HEPES, sodium pyruvate, glutamine, streptomycin and penicillin and 0.1% β -mercaptoethanol (Gibco) and containing 1 mg/ml Collagenase A (Roche), 0.2 mg/ml Dispase II (Sigma) and 0.2 mg/ml DNAse I (Roche), during 25 min at 37ºC with rotation. The enzymatic reaction was quenched by the addition of 30 ml of ice-cold DMEM (10% FBS, supplement). Cell suspension was filtered through a 70 µm cell strainer (BD). The filter was washed with 10 ml of ice-cold 10% FBS DMEM and the cells were pelleted at 280 g for 5 min at 4ºC. Lysis of erythrocytes was performed in Red Cell Lysis Buffer (RCLB, 155 mM NH4Cl, 12 mM NaHCO3, 0.1 mM EDTA) during 4 min at room temperature and immediately washed with ice-cold 10% FBS DMEM, followed by filtration through a 70 µm cell strainer and centrifugation. Sequentially, cells were purified by centrifugation 30 min at 2,400 rpm in 40/80 Percoll (Sigma) gradient. Cells were resuspended in DMEM (10% FBS, supplement; FACS buffer).
6. Identify the instrument used for data collection.	Flow cytometry analysis and cell separation were performed in a FACSAriaFusion flow cytometer (Beckton Dickinson).
7. Describe the software used to collect and analyze the flow cytometry data.	Data were analyzed using FlowJo software v10.4.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Purity was not assessed
9. Describe the gating strategy used.	Figure 1g (MTO biobank generation): To sort Lgr5-GFP+ tumour cells, we gated: Cells/Single cells/Living cells/GFP+. Figures 2g, ED Figs 7b and 8a: To assess genetic recombination in the UbC-CreERT2; Tgfbr2fl/fl; R26mTmG model, or to sort tumour cell populations, we gated: Living cells/Cells/ Single cells/PDGFRb+ (CAFs). From the negative population, we followed: Endothelial cells CD31+/CD45- and Leukocytes CD31-/CD45+, further refined to small size and good viability. Figure 3c-d: T-cell activation markers were assessed inside: Living cells/Cells/Single cells, than CD45+/ CD3+, then CD4+/CD8- or CD4-/CD8+. Figure 3e and 4g: T-cell cytokine levels were assessed inside: Living cells/Cells/Single cells/CD45+/CD3+/

NK1.1-, then CD4+/CD8- or CD8+/CD4-. Figure 3f: CD8+ T cells were sorted using gates: Living cells/Cells/Single cells/CD45+/CD19-/MHCII-/CD3+/CD4-/CD8+. Figure 4b: PD-1 expression was assessed inside: Living cells/Cells/Single cells/CD45+/CD3+, then CD4+/CD8- or CD8+/CD4-. Figure 4d and ED Fig 10e: PD-L1 populations were determined using: Living cells/Cells/Single cells/PD-L1+. Within these we measured for CAFs (PDGFRb+/Epcam-) or Epithelial cells (PDGFRb-/Epcam+). From the double-negative population, we followed: Endothelial cells (CD31+/CD45-) and Leukocytes (CD45+/CD31-). Fig. 4d: PD-L1 was measured inside Living cells/Cells/Single cells/CD45+. Figure 4f: For T-cell activation markers we gated: Living cells/Cells/Single cells/CD45+/CD3+/NK1.1-, then CD4+/CD8- or CD8+/CD4-. ED Fig. 10f: Myeloid populations were assessed inside: Living cells/Cells/Single cells/CD45+/PD-L1+/Ly6G-/Ly6C+/CD11b+.

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