

Journal of Hepatology

CTAT methods

Tables for a “Complete, Transparent, Accurate and Timely account” (CTAT) are now mandatory for all revised submissions. The aim is to enhance the reproducibility of methods.

- Only include the parts relevant to your study
- Refer to the CTAT in the main text as ‘Supplementary CTAT Table’
- Do not add subheadings
- Add as many rows as needed to include all information
- Only include one item per row

1.1 Antibodies

| Name | Citation | Supplier | Cat no. | Clone no. |
|---|----------|-----------|----------|-----------|
| anti-mouse PD-1 in vivo blocking antibody | | Bioxcell | BE0273 | 29F.1A12 |
| Rat IgG2a isotype control in vivo | | Bioxcell | BP0089 | 2A3 |
| anti-mouse CD4 in vivo depletion antibody | | Bioxcell | BE0003-1 | GK1.5 |
| anti-mouse PD-L1 in vivo blocking antibody | | Bioxcell | BP0101 | 10F.9G2 |
| Rat IgG2b isotype control in vivo | | Bioxcell | BP0090 | LTF-2 |
| anti-mouse VEGFR2 in vivo blocking antibody | | Bioxcell | BP0060 | DC101 |
| Rat IgG1 isotype control in vivo | | Bioxcell | BP0088 | HRPN |
| anti-mouse CD8 in vivo depletion antibody | | Bioxcell | BP0061 | 2.43 |
| anti-mouse CD31 monoclonal antibody, Immune fluorescence staining | | Biolegend | 102516 | MEC13.3 |
| anti-mouse CD8 monoclonal antibody, Immune fluorescence staining | | Biolegend | 100702 | 53-6.7 |
| BV605 anti-mouse CD3 | | Biolegend | 100237 | 17A2 |
| Alexa Fluor700 anti-mouse CD8a | | Biolegend | 100729 | 53-6.7 |
| PE/Cyanine7 anti-mouse CD44 | | Biolegend | 103029 | IM7 |

| | | | | |
|-------------------------------------|--|-----------------------|------------|----------|
| PerCP/Cyanine5.5 anti-mouse CD62L | | Biologend | 104431 | MEL-14 |
| FITC anti-mouse PD-1 | | Invitrogen | 11-9985-82 | J43 |
| PE anti-mouse PD-1 | | Invitrogen | 12-9981-82 | RMP1-30 |
| PB anti-mouse Tim3 | | Biologend | 119723 | RMT3-23 |
| Alexa Fluor700 anti-mouse CD4 | | Biologend | 100429 | GK1.5 |
| Brilliant Violet421 anti-mouse CD8a | | Biologend | 100737 | 53-6.7 |
| APC anti-mouse IFN-g | | Biologend | 505809 | XMG1.2 |
| PE anti-mouse TNF α | | Biologend | 506306 | MP6-XT22 |
| FITC anti-mouse Granzyme B | | Biologend | 515403 | GB11 |
| PE/Cyanine7 anti-mouse CXCR6 | | Biologend | 151103 | SA051D1 |
| APC/Fire 570 anti-mouse TCR β | | Biologend | 109246 | H57-597 |
| APC anti-mouse CD1d Tetramer | | NIH Tetramer Facility | 40239 | N/A |
| AF594 anti-mouse CD8 | | Biologend | 100758 | 53-6.7 |
| AF647 anti-mouse CD31 | | Biologend | 102516 | MEC13.3 |

1.2 Cell lines

| Name | Citation | Supplier | Cat no. | Passage no. | Authentication test method |
|---------|---------------------------------|-------------------------------------|----------|--|---|
| CT26 | Cancer Res. 1980; 40, 2142-2146 | ATCC | CRL-2638 | Within 5 passages after thawing, tumor cells were used for in vivo assay | Authentication performed by supplier |
| RIL-175 | J Hepatol. 2019; 70, 449-457 | Scott Lowe / Lars Zender in Germany | N/A | Within 5 passages after thawing, tumor cells were used for in vivo assay | Authentication was performed by i) pathology analysis of RIL-175 tumor confirms HCC morphology and ii) by luciferase expression |

1.3 Organisms

| Name | Citation | Supplier | Strain | Sex | Age | Overall n number |
|-----------------------|---------------------------------|--------------------|-------------------------|-----|---------------------------------------|------------------|
| Wild type mice | | Charles River | C57BL/6 | F | 8-10 week old when received treatment | 443 |
| Wild type mice | | Charles River | BALB/c | F | 8-10 week old when received treatment | 94 |
| Albino C57BL/6 | | Jackson Laboratory | B6(Cg)-Tyr<c-2J>/J | F | 8-10 week old when received treatment | 80 |
| B6.CXCR6-GFP knock-in | J Immunol. 2013; 190, 5226-5236 | Jackson Laboratory | B6.129P2-Cxcr6tm1Litt/J | F | 8-10 week old when received treatment | 16 |

1.4 Sequence based reagents

| Name | Sequence | Supplier |
|----------------------------------|---|---|
| RNeasy Mini Kit | N/A | Miltenyi Biotec (Ref. 74104) |
| nCounter Metabolic Pathway Panel | N/A | Nanostring technology (Ref. XT-CSO-MMP1-12) |
| iScript™ cDNA synthesis kit | N/A | BIO-RAD (Ref. 170-8891) |
| iQ SYBR Green Supermix | N/A | BIO-RAD (Ref. 1708882) |
| Fbp1 | Forward 5'-GCATCGCACAGCTCTATGGT-3', Reverse 5'-ACAGGTAGCGTAGGACGACT-3' | Eurofins Genomics |
| Pck1 | Forward 5'-AATATGACAACCTGTTGGCTG-3', Reverse 5'-AATAGCTTTCTCAAAGTCCTC-3' | Eurofins Genomics |
| Mpc1 | Forward 5'-GGTACAACCTCGAACTGG-3', Reverse: 5'-TCAAGAGCTGGTCCTTGTACC-3' | Eurofins Genomics |
| Adh1 | Forward 5'-GCTATGGCTCTGCCGTCAAAGT-3', Reverse 5'-TGTCCACAGCAATGATCCTGGC-3' | Eurofins Genomics |
| Aldh2 | Forward 5'-GAGGACTGTGTTGGGAGGTC-3', | Eurofins Genomics |

| | | |
|-------|--|-------------------|
| | Reverse 5' GTAGGTCCGGTCCCGTTC-3' | |
| Adh4 | Forward 5'- GCTATGGCTCTGCCGTCAAAGT- 3', Reverse 5'- TGTCCACAGCAATGATCCTGGC-3' | Eurofins Genomics |
| GAPDH | Forward 5'-CCT GCA CCA CCA ACT GCT TA-3', Reverse 5'-TCA TGA GCC CTT CCA CAA TG-3' | Eurofins Genomics |

1.5 Biological samples

N/A

1.6 Deposited data

| Name of repository | Identifier | Link |
|---------------------------------------|------------|---|
| NCBI Gene Expression Omnibus database | GSE184231 | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE184231 |

1.7 Software

| Software name | Manufacturer | Version |
|----------------------------|----------------------------|---------|
| FlowJo | Becton Dickinson & Company | 10.6.2 |
| Prism | GraphPad Software, LLC. | 8.4 |
| Advanced Analyses software | Nanostring Technologies | 2.0.115 |
| Imaris software | Bitplane AG | 9.5.1 |

1.8 Other (e.g. drugs, proteins, vectors etc.)

| Name | Manufacturer | Purpose |
|---|---|------------------------------|
| Metformin 1,1-Dimethylbiguanide hydrochloride | Sigma-Aldrich (Ref. D150959-5G) | For invivo treatment |
| Western diet | Envigo (Ref. TD.120528) | For invivo treatment |
| MCD | Research diets (Ref. A02082002BR) | For invivo treatment |
| CDA | Dyets inc (Ref. 518753) | For invivo treatment |
| Leukocyte activation cocktail (Phorbol 12-Myristate 13-Acetate [PMA], ionomycin and GolgiPlug™ [Brefeldin A]) | Bioscience (Ref. 51-2042 ^E) | For invitro T cell treatment |
| 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose | Invitrogen (Ref. N1395) | For Glucose uptake staining |

| | | |
|---|--------------------------------------|----------------------------------|
| MitoTracker Deep Red | Invitrogen (Ref. M22426) | For mitochondrial staining |
| MitoTracker Deep Green | Invitrogen (Ref. C2925) | For mitochondrial staining |
| MTT Cell Proliferation Kit | Abcam (Ref. ab211091) | For invitro experiment |
| DAPI | Abcam (Ref. ab138903) | For immune fluorescence staining |
| CellTracker Deep Red | Invitrogen (Ref. C34565 A) | For invitro experiment |
| CellTracker Green | Invitrogen (Ref. C2925) | For invitro experiment |
| Bovine PureCol | Advanced Biomatrix (Ref. 5005-100ml) | For invitro experiment |
| Microscopy Dish | MatTek (Ref. P35G-1.5-14.C) | For invitro experiment |
| Mouse CD8 ⁺ AutoMACS pro sorting kit | Miltenyi Biotec (Ref. 130-104-075) | For invitro experiment |

1.9 Please provide the details of the corresponding methods author for the manuscript:

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2.0 Please confirm for randomised controlled trials all versions of the clinical protocol are included in the submission. These will be published online as supplementary information.

N/A