# Supplementary Methods

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## mRNA synthesis and LNP formulation

mRNA was synthesised in vitro by T7 RNA polymerase-mediated transcription where the UTP was substituted with N1-methylpseudoUTP, from a linearized DNA template, which incorporates the 5′ and 3′ untranslated regions (UTRs) and a poly-A tail as described (24). The final mRNA utilizes Cap1 to increase mRNA translation efficiency.

LNP formulations were prepared using a modified method previously described (26) with the structure and composition of the LNP as described (25). Briefly, the ionizable lipid heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8- oxooctyl)amino)octanoate and other lipid components were dissolved in ethanol at molar ratios of 50:10:38.5:1.5 (ionizable:DSPC:Cholesterol:DMG-PEG2k). The lipid mixture was combined with an acidification buffer of 50 mM citrate buffer (pH 4.0) containing mRNA at a ratio of 2:1 (aqueous: ethanol) using synchronized syringe pumps (Harvard Apparatus). Formulations were diafiltered and concentrated against 20 mM Tris (pH 7.4) with 8% sucrose using Pellicon XL 100 kDa tangential flow membranes (EMD Millipore) then passed through a 0.22 µm filter. The formulated mRNA is then diluted in buffer to the desired concentration and stored at –20˚C until use. Formulations were tested for particle size, RNA encapsulation, and endotoxin. All were found to be between 80 to 120 nm in size by dynamic light scattering and with ≥80% encapsulation and <10 EU/ml endotoxin.

## Mouse and human tumor cells

HeLa, A20, A431, BT-20, BT-549, HCC1806, Hs 578&, SH-4, SK-MEL-3, A375, CAL 27, HCC38 and RPMI-7951 cells were all from the American Type Culture Collection. PE/CA-PJ34, PE/CA-PJ41 and PE/CA-PJ49 cells were from the European Collection of Authenticated Cell Cultures. KYSE-30 cells were from the German Collection of Microorganisms and Cell Cultures. Mouse colon adenocarcinoma model MC38 materials were obtained under MTA from the Biological Testing Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis [National Cancer Institute (NCI)]. MDA-MB-231 cells were from the National Cancer Institute. The two variants of the MC38 cell line MC38-S and MC38-R used in this study have been described previously (23). B16F10-AP3 cells are a B16F10 subline derived by *in vivo* passage, and were from AstraZeneca (36). For in vitro culture, tumor cell lines were maintained in their individual culture media (Supplementary Table 5).

## In vitro IL-12p70 expression assays

HeLa cells or human primary hepatocytes (Female Human Plateable Cryopreserved Hepatocytes, Bioreclamation IVT) in 6 well plates were transfected with 2.97 μg of mIL-12 mRNA ± miR122 binding site using Lipofectamine 2000 (L2K, Thermo Fisher) for 24h prior to supernatant collection. HeLa cells were transfected with 3 µg of secreted IL-12 or tethered IL-12 mRNA per well of a 6-well plate with L2000 (Thermo Fisher), or were mock transfected with L2000 alone. Supernatants were collected 24 hours after transfection and cells were lysed in RIPA Buffer with EDTA (Boston Bioproducts) with 1X Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher). Human monocyte-derived macrophages (MoDM) were prepared from healthy donor blood leukocyte cones (NHSBT). PBMC were isolated by Ficoll gradient centrifugation. CD14+ monocytes were enriched from PBMC by negative selection (StemCell Technologies) and differentiated for 6 days in RPMI with 10% FBS, 1% Penicillin / Streptomycin and 100ng/ml human M-CSF (Peprotech). MoDM were harvested, seeded in 96-well plates, rested overnight, and treated with MEDI1191 for 24h prior to supernatant collection. Human tumor cell lines were seeded in 96 well plates, rested overnight, incubated for 4h with LNP-formulated MEDI1191 mRNA, and then incubated for another 18h in fresh media prior to supernatant collection and analysis.

**In vitro mRNA-derived IL-12p70 bioactivity**

mIL-12 mRNA derived mIL-12p70 was generated by transfecting HeLa cells as described. Mouse CD8+ T cells were enriched from C57Bl/6 splenocytes by immunomagnetic negative selection (EasySep™ Mouse CD8+ T Cell Isolation Kit, STEMCELL Technologies). T cells were then stimulated for 48h with mouse T-Activator CD3/CD28 DynabeadsTM (ThermoFisher, 1:3 bead to cell ratio) in the presence of HeLa supernatant containing mIL-12 mRNA-derived mIL-12p70 or recombinant mIL-12p70 (R&D Systems). IFN-γ was measured in CD8+ T cellculture supernatants by DuoSet ELISA (R&D Systems).

HeLa cells transfected with tethered mIL-12 mRNA were growth arrested by 50 µg/mL Mitomycin C (Abcam) for 20 minutes at 37ºC, washed 4 times with PBS, and harvested. Transfected HeLa cells were seeded in 96 well plates at a range of cell densities (0.5 – 10,000 cells / well) and co-cultured with 50,000 mouse CD8+ T cells / well in the presence of mouse T-Activator CD3/CD28 DynabeadsTM (1:2 bead to cell ratio). Following 72 hours of co-culture, IFNγ levels were measured by DuoSet ELISA (R&D Systems).

MEDI1191-derived hIL-12p70 was generated by transfecting HeLa cells with 3.3 μg hIL-12 mRNA per well of a 6-well plate using FuGENE HD transfection reagent (Promega) for 72 hours. Human CD8+ T cells were enriched from human PBMCs by negative selection (CD8+ T Cell Isolation Kit, Miltenyi Biotec). T cells were then stimulated for 72 hours with Dynabeads™ Human T-Activator CD3/CD28 cocktail (Thermofisher, 0.3-fold manufacturer recommended amount) in the presence of HeLa supernatant containing MEDI1191-derived hIL-12p70 or recombinant hIL-12p70 (R&D Systems). IFN-γ was measured in CD8+ T cellculture supernatants by DuoSet ELISA (R&D Systems).

## Animal studies

All *in vivo* experiments carried out at AstraZeneca (UK) were performed in accordance with the UK Animal (Scientific Procedures) Act 1986 and the EU Directive 2010/63/EU, under a UK Home Office Project License and approved by the Babraham Institute Animal Welfare and Ethical Review Body. All *in vivo* experiments carried out by AstraZeneca (US), an AAALAC-accredited facility, were performed in compliance with the laws, regulations, and guidelines of the National Institutes of Health (NIH), Office of Laboratory Animal Welfare (OLAW), and were approved by the AstraZeneca (US) Institutional Animal Care and Use Committee (IACUC). All animal procedures and experiments carried out at Moderna Inc. were conducted in compliance with all the laws, regulations, and guidelines of the National Institutes of Health (NIH) and with the approval of the Moderna Therapeutics Institutional Animal Care and Use Committee (IACUC). All animal procedures carried out at Molecular Imaging (MI) Bioresearch were conducted in compliance with all the laws, regulations and guidelines of the National Institutes of Health (NIH) and with the approval of MI Bioresearch’s Animal Care and Use Committee. MI Bioresearch is an AAALAC accredited facility. All PDX studies at Crown Bioscience San Diego were performed in compliance with all the laws, regulations, and guidelines of the National Institutes of Health (NIH) as applicable and in accordance with IACUC-approved Animal Protocol No. EB17-030. All animals were 6–10 weeks of age and > 18g and housed under specific pathogen-free conditions with a 12-hour light/12-hour dark cycle.

Animals for Moderna studies were provided water (Innovive® Aquavive® prefilled, purified, laboratory-grade acidified water) and food (irradiated ProLab® Isopro® RMH 3000 Diet, consisting of 22.0% crude protein, 5.0% crude fat, and 5.0% crude fiber) ad libitum. The mice were housed on irradiated Enrich-o’cobs™ enrichment bedding in Innovive® Disposable IVC Innocage®caging system. Dose groups were housed in separate cages. Animal rooms were set to 20°C–22°C and 40%-60% humidity, with a programmed 15 complete room air exchanges per hour.

Animals for AstraZeneca studies were housed in Tecniplast IVC cages holding a maximum of 6 animals with irradiated aspen chip bedding, Nestlets nesting material, a cardboard tunnel and wooden chew blocks,with *ad libitum* UV-treated or filtered water and RM1 rodent diet.

Animals for PDX studies carried out at Crown Bioscience were housed in individual HEPA-ventilated cages (Innovive® IVC Innocage®caging system [Innovive (San Diego, CA)]); dose groups were housed in separate cages. Temperature and humidity were monitored and recorded daily and maintained to the maximum extent possible between 20°C–23°C and 30%–70% humidity, respectively. Animals were provided 18% soy irradiated rodent feed (Teklad, 2920X.10) and autoclaved acidified water (pH 2.5–3) ad libitum.

Animals for studies carried out at MI Bioresearch were fed irradiated Teklad 2918.15 Rodent Diet and water ad libitum. Animals were housed in Innovive® disposable ventilated caging with corn cob bedding inside Biobubble® Clean Rooms that provide H.E.P.A filtered air into the bubble environment at 100 complete air changes per hour. All treatments, body weight determinations, and tumor measurements were carried out in the bubble environment. The environment was controlled to a temperature range of 70°±2°F and a humidity range of 30-70%.

For single flank subcutaneous tumor models, 5 x 105 MC38-S, MC38-R and A20 or 5 x 103 for B16F10-AP3 cells in 100-200 μl were injected in the right flank. For rechallenge of animals with a complete response to therapy, the same number of tumor cells was implanted subcutaneously in the contralateral flank of the animal. For the bilateral MC38-S tumor model, 5 x 105 MC38S cells were implanted in both flanks on the same day.

Body weight and tumor size were measured two or three times weekly. Mice were randomized on the basis of tumor volume prior to treatment. mRNA was administered IT using insulin syringes with Ultra-Fine™ 6mm x 31G needles (BD Biosciences) as a bolus injection (25 μl fixed volume). For the bilateral tumor model, only right flank tumors received IT injection.

**Tumor and plasma mIL-12p70 and mIFNγ quantification**

Plasma was collected from in-life and terminal bleeds and tumors were collected and snap frozen at various protocol-specified endpoint times after mIL-12 mRNA administration. Snap frozen tumors were pulverized, extracted at 100 mg/ml in RIPA buffer with protease inhibitors and clarified by centrifugation. mIL-12p70 and mIFNγ levels in syngeneic mouse tumor lysates and plasma were quantified with the ProcartaPlex Mix&Match Mouse 5-plex, the Cytokine and Chemokine 36-Plex Mouse ProcartaPlex Panel 1A (Thermo Fisher), or for NK PD by electrochemiluminescent V-plex (MesoScaleDiscovery), according to manufacturer’s instructions with minor modifications. MEDI1191-derived hIL-12p70 was quantified in tumor lysates and plasma by DuoSet ELISA (R&D Systems) according to the manufacturer’s instructions. Protein was normalized to either tumor weight (ng protein per gram of tumor tissue) or to plasma volume (ng protein per ml of plasma).

**Sample processing for flow cytometry**

Tumors were weighed, dissociated with a GentleMacs mouse Tumor Dissociation Kit (Miltenyi) and cells resuspended in staining buffer (PBS/2% FBS). Spleens were processed by passing tissues through a 70 µm cell strainer and resuspending cells in staining buffer. Peripheral blood was lysed with ACK lysis buffer (Thermo Fisher). Intracellular staining was carried out with Foxp3 / Transcription Factor Staining Buffer Set (Thermo Fisher). Samples were otherwise fixed with Intracellular Fixation buffer (Thermo Fisher).

## Flow cytometry gating

All gating strategies included doublet cell exclusion gates (FSC-W/FSC-H and SSC-W/SSC-H) and a dead cell exclusion gate (eFluor 506 low or negative with Fixable Viability Dye).

Gating strategy for lymphocytes:
CD8+ T cells: CD45+, SSClow, CD3e+, CD8a+
CD4+ T cells: CD45+, SSClow, CD3e+, CD4+, FoxP3–
Tregs: CD45+, SSClow, CD3e+, CD4+, FoxP3+
NK cells: CD45+, SSClow, CD3e–, CD49b+

NK cells (blood and spleen, in NK PD): live CD45+, CD19–, CD11b–, CD3– NKp46+

NK cells (tumor, in NK PD): live CD45+, CD19–, CD3–, NKp46+

Gating strategy for monocytic cell types:
CD11b+ myeloid cells: CD45+, CD11b+
Monocytes: CD45+, CD11b+, Ly6G–, Ly6Chigh

Gating strategy for dendritic cell populations:
CD103 cDCs: CD45+, CD11c+, MHCII+, B220–, CD103+
CD8 cDCs: CD45+, CD11c+, MHCII+, B220–, CD8a+
CD11b+ cDCs: CD45+, CD11c+, MHCII+, B220–, CD11b+, SiRPa+

Gating strategy for MC38-R and B16F10 tumor cells:
Tumor: CD45–, FSC hi

Gating strategy for peripheral blood samples:
CD8+ T cells: CD45+, SSClow, CD3e+, Ly6G–, CD8a+
CD4+ T cells: CD45+, SSClow, CD3e+, Ly6G–, CD4+
Monocytes: CD45+, CD3e–, CD11b+, CD19–, Ly6G–, Ly6Chigh

## Syngeneic tumor and patient tumor slice immunohistochemistry

Tumor samples were fixed in 10 % neutral buffered formalin for 24h before paraffin embedding. 4 µm sections were cut, dewaxed, rehydrated and antigens were retrieved using BioCare Medical decloaking chamber with high pH buffer (Dako). Following endogenous peroxidase block with H2O2 in methanol, slides were Immunostained on a Dako Link48 autostainer with rabbit monoclonal anti-mouse CD8 (Calico) or rabbit monoclonal anti-mouse PDL-1 (Cell Signaling), followed by detection with goat anti rabbit EnVision polymer HRP (Dako) with Dako DAB+ substrate (MC38-R) or magenta substrate (B16F10-AP3) as the chromogen, and hematoxylin as a counterstain. Slides were digitally scanned using the Aperio AT2 system. Representative tumor regions were annotated by the Pathologist (P.L.M) and analysed with Definiens Tissue Studio 4.4.2 software for automated quantification of CD8+ cell densities. HALO 2.1 software was used for automated quantification of percent tumor area positive for PDL-1 expression.

One representative patient tumor slice was fixed immediately after vibratome sectioning (10% neutral buffered formalin for 24 – 72h) and embedded in paraffin for immunohistochemistry. Blocks were sectioned at 4 µm, mounted on slides, deparaffinized, rehydrated and then stained for CD3 (antibody clone 2GV6 (Roche Diagnostics) with OmniMap detection on a Ventana Discovery autostainer (Roche Diagnostics), or for NKp46 (antibody clone MOG1-M-H46-2/3-8E5B-5G2 (Innate Pharma) with Polymer Refine detection on a Leica Bond Rx autostainer (Leica Biosystems), both using DAB as chromogen and hematoxylin as a counterstain. Negative control slides substituting a non-specific antibody of the same isotype as the primary were included in each staining run. After staining, the slides were digitally scanned at 20x magnification using an Aperio AT2 scanner (Leica Biosystems), and positive cells were counted using Halo image analysis software (Indica Labs).

**Table S1. Transcriptomics full DEG lists and pathway analysis**

See additional data file

**Table S2. Tumor volumes at time of treatment**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Model**  | **Treatment**  | **Type of study (pharmacodynamic response (PD) or efficacy)** | **Main manuscript or supplementary (S) figure panel(s)**  | **First IT dose (day post-implantation)** | **Randomization day (days post-implantation)** | **Tumor volume at randomization (mm3)** |
| MC38-R | mIL-12 mRNA | PD response, cytokines | 1C, 1D, 3B, 3C | 15 | 14 | 108 - 666 |
| A20 | mIL-12 mRNA | PD response, cytokines | S1C, S1D, S5H, S5I | 20 | 19 | 144 - 405 |
| A20 | mIL-12 mRNA | Efficacy | 1E, 1H, S1E-H, S3A | 18 | 18 | 88 – 256 |
| MC38-S | mIL-12 mRNA | Efficacy | 1F, 1H, S1E-H, S3B | 12 | 12 | 75 – 144  |
| MC38-R | mIL-12 mRNA | Efficacy. PD response (cytokines) | 1G, 1H, S1E-H, S2, S3C | 11 | 10 | 65 - 251 |
| MC38-R | mIL-12 mRNA | PD response (flow cytometry) | 2A–D, 3A, 3H, S5A-F, S7A, S7B, 4B, S8A, S8B | 13 | 12 | 126 – 399  |
| MC38-R | mIL-12 mRNA | PD response (transcriptomics) | 2E, 3G, S5G, S7C-G | 11 | 11 | 128 – 306 |
| MC38-R | mIL-12 mRNA, cell depleting antibodies | Efficacy | S4B | 11 | 9 | 14 – 75 |
| MC38-R | mIL-12 mRNA, IFNγ-blocking antibody | Efficacy and PD | 2F, S4C, 3D, 3E, S8C | 13 | 10 | 62 – 144 |
| MC38-R | mIL-12 mRNA + αPD-L1 | PD (tumor IHC, splenocyte restimulation) | 4A, D-G | 7 | 7 | 79 - 175 |
| MC38-R | mIL-12 mRNA + αPD-L1 | Efficacy | 4C, S8E | 11 | 10 | 67 - 216 |
| MC38-R | mIL-12 mRNA + αPD-L1 | Efficacy | S8D, S8F | 11 | 10 | 69 - 234 |
| B16F10-AP3 | mIL-12 mRNA | PD (flow cytometry, cytokines) | S9A, S9B, S9D-G | 11 | 10 | 90 - 330 |
| B16F10-AP3 | mIL-12 mRNA + αPD-L1 | PD (tumor IHC)  | S9C, S9H | 7 | 7 | 111 - 155 |
| B16F10-AP3 | mIL-12 mRNA + αPD-L1 | PD (splenocyte restimulation) | S9K | 7 | 7 | 40 - 128 |
| B16F10-AP3 | mIL-12 mRNA + αPD-L1 | Efficacy | S9I, S9J | 10 | 9 | 80 – 160 |
| Dual flank MC38-S | mIL-12 mRNA + αPD-L1 | Efficacy | 5A, B | 13 | 12 | 71 – 195 (treated tumor) 60 – 298 (un-treated distal tumor) |
| Dual flank MC38-S | Tethered mIL-12 mRNA | Efficacy and PD (cytokines) | 5F–H | 13 | 12 | 61 – 219 |
| ME12057  | MEDI1191 | PD (cytokines) | S10D, S10E | 34 | 33 | 136 – 257 |
| ME12058 | MEDI1191 | PD (cytokines) | S10E | 36 | 35 | 151 – 199 |
| HN5111 | MEDI1191 | PD (cytokines) | S10D, S10E | 48 | 47 | 130 – 249 |
| HN5116 | MEDI1191 | PD (cytokines) | S10E | 71 | 70 | 142 – 265 |
| MC38-R | mIL-12 mRNA +/- αNK1.1 or IgG2a isotype | Efficacy, PD, and cytokines | 3F, S6 | 11  | 11 | 52 - 257 |

**Table S3. Antibodies and controls for in vivo dosing**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Protein / antibody** | **Clone** | **Grade** | **Supplier** | **Catalog #** |
| Anti–CD4 | GK1.5 | InVivoPlus  | Bio X Cell | BP0003-1 |
| Anti–CD8α | 2.43 | InVivoPlus  | Bio X Cell | BP0061 |
| Anti–IFNγ | XMG1.2 | InVivoPlus  | Bio X Cell | BP0055 |
| Rat IgG2b Isotype | LTF-2 | InVivoPlus  | Bio X Cell | BP0090 |
| Rat IgG1 Isotype | HRPN | InVivoPlus  | Bio X Cell | BP0088 |
| Mouse Anti–mPD-L1 | Clone 80, D265A | – | MedImmune | – |
| Mouse IgG1 Isotype | NIP-228 D265A | – | MedImmune | – |
| Mouse anti-NK1.1 | PK136 | InVivoPlus | Bio X Cell | BE0036 |
| Mouse IgG2a Isotype | NIP-228 | – | MedImmune | – |

Table S4. Flow Cytometry Staining Antibodies

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Antibody** | **Fluor** | **Clone** | **Isotype** | **Vendor** | **Catalog No.** | **Study** |
| BV421-A | CD3 | BV421 | 145-2C11 | Arm Ham IgG1 | BioLegend | 100341 | Depletion/Block |
| BV421-A | FoxP3 | eFluor450 | FJk-16s | Rat IgG2a, κ | eBioscience | 48-5773-82 | Tumor PD |
| BV421-A | Iso Control | eFluor450 | eBR2a | Rat IgG2a, κ | eBioscience | 48-4321-82 | Tumor PD |
| BV421-A | CD86 | BV421 | GL1 | Rat IgG2a κ | BD | 564198 | Tumor PD |
| BV421-A | Iso Control | BV421 | R35-95 | Rat IgG2a κ | BD | 562602 | Tumor PD |
| BV421-A | CD335 | BV421 | 29A1.4 | Rat IgG2a κ | BioLegend | 137612 | NK PD |
| BV605-A | CD45 | BV605 | 30-F11 | Rat IgG2b, κ | BioLegend | 103140 | Tumor PD |
| BV605-A | CD4 | BV605 | RM4-4 | Rat IgG2b, κ | BD | 740336 | Depletion/Block |
| BV605-A | Ly6C | BV605 | HK1.4 | Rat IgG2c, κ | Biolegend | 128036 | NK PD |
| BV510-A | FVD506 | eFluor 506 | NA | NA | Thermo Fisher | 65-0866-14 | Tumor PD |
| BV510-A | Ly6C | BV510 | HK1.4 | Rat IgG2c, κ | BioLegend | 128033 | Depletion/Block |
| BV650-A | CD11b | BV650 | M1/70 | Rat IgG2a  | BD | 563402 | Tumor PD |
| FITC-A | PD-L1 | FITC | 104.9G2 | Rat IgG2b | Elab Sciences | E-AB-2156 | Depletion/Block  |
| FITC-A | CD3 | FITC | 145-2C11 | Arm Ham IgG1  | Biolegend | 100306 | Tumor PD |
| FITC-A | CD45 | FITC | 30-F11 | Mouse IgG1 κ | BioLegend | 103108 | NK PD |
| FITC-A | PDCA1 | FITC | eBio927 | Rat IgG2b κ | eBioscience | 11-3172-82 | Tumor PD |
| PerCP-Cy5.5-A | CD45 | PerCP-Cy5.5 | 30-F11 | Rat IgG2b κ | Biolegend | 103132 | Depletion/Block |
| PerCP-Cy5.5-A | I-A/I-E | PerCP-Cy5.5 | M5/114.15-2 | Rat IgG2b κ | BD | 562363 | Tumor PD |
| PerCP-Cy5.5-A | CD3 | PerCP-eFluor710 | 17A2 | Rat IgG2b κ | eBioscience | 46-0032-82 | NK PD |
| PE-A | Ly6C | PE | AL-21 | Rat IgM κ | BD | 560592 | Tumor PD |
| PE-A | CD8a | PE | 53-6.7 | Rat IgG2a  | BioLegend | 100707 | Depletion/Block |
| PE-A | CD274 | PE | MIH7 | Rat IgG2a  | BioLegend | 155404 | NK PD |
| PE-CF594-A | CD4 | PE-CF594 | RM4-5 | Rat IgG2a κ | BD | 562285 | Tumor PD |
| PE-CF594-A | CD19 | PE-eFluor 610 | eBio1D3 | Rat IgG2a κ | eBioscience | 61-0193-82 | Depletion/Block  |
| PE-CF594-A | CD8a | PE-CF594 | 53-6.7 | Rat IgG2a κ | BD | 562283 | Tumor PD |
| PE-CF594-A | Foxp3 | PE-eFluor610 | FJK-16S | Rat IgG2a κ | eBioscience | 61-5773-82 | NK PD |
| PE-Cy7-A | CD11c | PE-Cy7 | HL3 | Arm Ham IgG1 λ2 | BD | 558079 | Tumor PD |
| PE-Cy7-A | Ly6G | PE-Cy7 | 1A8 | Rat IgG2a κ | BD | 560601 | Depletion/Block |
| Alexa Fluor 700-A | CD8a | APC-R700 | 53-6.7 | Rat IgG2a κ | BD | 564983 | Tumor PD |
| Alexa Fluor 700-A | CD11b | APC-R700 | M1/70 | Rat IgG2b κ | BD | 564985 | Tumor PD |
| Alexa Fluor 700-A | CD103 | APC-R700 | M290 | Rat IgG2a κ | BD | 565529 | Tumor PD |
| APC-A | CD19 | eFluor660 | 1D3 | Rat IgG2a κ | eBioscience | 50-0193-82 | NK PD |
| APC-Cy7-A | Zombie NIR | Zombie NIR | NA | NA | Biolegend | 423105 | Depletion/Block |
| APC-Cy7-A | CD69 | APC-e780 | H1.2F3 | Arm Ham IgG | eBioscience | 47-0691-82 | Tumor PD |
| APC-Cy7-A | I-A/I-E | APC-Cy7 | M5/114.15.2 | Rat IgG2b κ | Biolegend | 107628 | Tumor PD |
| APC-Cy7-A | B220 | APC-Cy7 | RA3-6B2 | Rat IgG2a κ | BioLegend | 103224 | Tumor PD |
| APC-Cy7-A | CD11b | APC-Cy7 | M1/70 | Rat IgG2b κ | BioLegend | 101226 | NK PD |
| BV711-A | CD11b | BV711 | M1/70 | Rat IgG2b κ | BioLegend | 101242 | Depletion/Block |
| BV711-A | PD-L1 | BV711 | 10F.9G2 | Rat IgG2b κ | Biolegend | 124319 | Tumor PD |
| BV711-A | SiRPa | BV711 | P84 | Rat IgG1 κ | BD | 740766 | Tumor PD |
| BV786-A | CD49b | BV786 | HMα2 | Arm Ham IgG1 κ | BD | 740895 | Tumor PD |
| BV786-A | Ly6G | BV786 | 1A8 | Rat IgG2a κ | Biolegend | 127645 | Tumor PD |
| BV786-A | F4/80 | BV786 | BM8 | Rat IgG2a κ | Biolegend | 123141 | Tumor PD |
| BV786-A | Ly6G | BV785 | 1A8 | Rat IgG2a κ | BioLegend | 127645 | NK PD |
| BUV496-A | Live/Dead  | Fixable Aqua | - | - | eBioscience | L34957 | NK PD |
| BUV661-A | CD4 | BUV661 | GK1.5 | Rat IgG2b κ | BD | 612974 | NK PD |
| BUV737-A | CD8a | BUV737 | 53-6.7 | Rat IgG2a κ | BD | 564297 | NK PD |

**Table S5. Cell line culture media**

|  |  |
| --- | --- |
| **Cell line** | **Growth media** |
| A20 | RPMI 1640, 1mM NaPy, 10mM HEPES, 2.5g/L glucose, 10% FBS |
| A375 | DMEM, 10 % FBS |
| A431 | DMEM, 5 % FBS; 1 % NEAA |
| B16F10-AP3 | DMEM, 4.5g/L glucose, L glutamine, 110mg/L sodium pyruvate, 10% FBS |
| BT-20 | MEM, 10 % FBS, 1 % NEAA, 1 % sodium pyruvate |
| BT-549 | RPMI, 10 % FBS, 0.023 IU/ml bovine insulin |
| CAL 27 | DMEM, 10 % FBS |
| HCC1806 | RPMI, 10 % FBS |
| HCC38 | RPMI, 10 % FBS |
| HeLa | RPMI, 10 % FBS |
| Hepatocytes | nVitroGRO CP medium with Torpedo antibiotic mix |
| Hs 578T | DMEM, 10 % FBS, 100 µg/ml bovine insulin |
| KYSE-30 | RPMI/Hams F12 (1:1), 10 % FBS, 1 % Glutamax-I |
| MC38-SMC38-R | DMEM, 10% FBS, 2mM glutamine, Penicillin / Streptomycin, 25µg/mL GentamicinDMEM, 10% FBS, GlutaMAX |
| MDA-MB-231 | RPMI, 10 % FBS |
| PE/CA-PJ34 | IMDM, 10 % FBS |
| PE/CA-PJ41 | IMDM, 10 % FBS |
| PE/CA-PJ49 | IMDM, 10 % FBS |
| RPMI-7951 | MEM, 10 % FBS, 1 % NEAA, 1 % NaPy |
| SH-4 | DMEM, 10 % FBS |
| SK-MEL-3 | McCoys 5A, 15 % FBS |