Supplementary Materials for

CLEC-1 is a death sensor that limits antigen cross-presentation by dendritic cells and represents a target for cancer immunotherapy

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Other Supplementary Material for this manuscript includes the following:

Data S1
Supplementary Text

**Generation of Fc CLEC-1 proteins**

Human and mouse CLEC-1 IgG Fc chimera proteins (Fc CLEC-1) were generated by cloning into pcDNA3.1, the extracellular domain of mouse (Q73-Q269) or human CLEC-1 (Q74-D280) fused in N-terminal to mouse IgG1e2 Fc (Invivogen) or human IgG1e3 Fc (Invivogen) sequences respectively. Synthesis of Fc CLEC-1 sequences was ordered at Genscript with restriction sites XhoI in 5’ and XbaI in 3’ for cloning in pcDNA3.1. Kozak sequence (GCCACC – SEQ ID No.10) and IgK leader signal peptide (METDTLLLWVLLWVPGSTGD–SEQ ID No.9) were added in the beginning of the sequences for expression in mammalian cells. Midiprep of plasmid pcDNA3.1 containing Fc CLEC-1 was produced with Nucleobond Xtra Midi EF kit (Macherey Nagel). Recombinant Fc CLEC-1 proteins were produced in 60.10⁶ FreeStyle 293-F (HEK) (Thermofisher) by transient transfection with 60µg of plasmid pcDNA3.1-Fc CLEC-1 using 120µl of 293 Fectine transfection reagent (Thermofisher) in 60ml of FreeStyle 293 medium (Thermofisher). After 5 days, supernatant was clarified by centrifugation 30min at 3000g and filtered on 0.22µm Stericup (Merck Millipore) and proteins were purified on HiTrap protein A column (GE) and eluted with 0.1M citric acid pH3. Eluate was concentrated on Microsep Advance10kDa (Pall) and filtered on 0.22µm filter.

**Generation of anti-human CLEC-1 mAbs**

Hybridomas against human CLEC-1 were generated by Diaclone SAS (Besançon, France) by immunization of mice with human His CLEC-1 (Biotechne) and were selected by ELISA according to conventional protocols. Briefly, 1µg of protein was administered in the foot pad of naive mice, one day per week for three weeks and then one day per two weeks for the last two injections. Draining lymph nodes were collected and hybridomas were obtained by fusing ganglion cells with the mouse myeloma X63/AG.8653 cell line. Hybridomas were selected by their abilities to recognize by flow cytometry cell lines expressing or not CLEC-1. Variable sequence of heavy chain (VH) and light chain (VL) were sequenced and cloned respectively in pcDNA3.4 human IgG4m expression plasmid containing CH1-hinge-CH2-CH3 domains of hIgG4, mutated at S228P to stabilize hinge region and in pcDNA3.4 CLig-hkappa expression plasmid containing human CLkappa (plasmids from OseImmunotherapeutics, France).
Plasmids were co-transfected in CHO cells and Abs from supernatant (at d7) were purified by Protein A chromatography and quantified by UV (280nm).

**Enrichment of CLEC-1 ligand(s) and mass spectrometry**

UV-treated Raji and HPB-ALL were lysed in 5 volumes of ice cold mild RIPA-IP lysis buffer (PBS (Corning) containing 1% NP-40, 0.25% Na deoxycholate, 1X Protease Inhibitor cocktail (PIC) (Sigma Aldrich)) followed by 2 cycles of thermal lysis and centrifugation at 14000g (15 min, 4°C) and concentration of whole cell protein extracts (WCE) was determined by BCA assay. For co-IP, protein G Surebeads magnetic beads (Biorad) (resuspended in PBS+0.1% Tween-20 (Sigma-Aldrich)) were coated with Fc-fusion proteins (hFc alone, hFc CLEC-1, or hFc CLEC7A (Invivogen) (1h, 4°C on a rotating wheel) and were added after washing (3 times in PBS-tween and then in PBS) to WCE in cold PBS+1X PIC (3h, 4°C on a rotating wheel). After washing in PBS+500mM NaCl and PBS, eluated proteins (elution buffer (1X Laemmli buffer (Biorad), 250mM DTT (Sigma-Aldrich)), were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis on stain free gels and imaged on a Chemidoc Imaging System (Biorad). Band of interest was cut out in a gel stacking for nanoscale liquid chromatography coupled to tandem mass spectrometry analysis (LSMBO,Strasbourg) performed on a nanoACQUITY Ultra-Performance-LC-system (Waters, Milford, MA) coupled to a TripleTOF 5600 mass spectrometer (ABSciex). Data were analyzed by MASCOT 2.6.2 algorithm (Matrix Science) successively filtered by applying a false discovery rate (FDR) of 1%, and removing the classical contaminants and proteins purified in Fc control and beads only conditions. Eluate was revealed by WB with anti-human TRIM21 mAbs (AB01/1G5, Biorad). Final shortlist was filtered for candidates displaying over 20% sequence coverage and at least 7 specific peptides.

**Animals**

OT-II.Ly5.1 homozygous mice were obtained by intercrossing OVA-specific TCR-transgenic OT-II mice (C57BL/6-Tg(TcraTcrb)425Cbn/Crl) (Charles River) with Ly5.1 mice (B6.SJL-PtprcaPepcb/BoyCrl) (Charles River). RIPmOVA (C57BL/6-Tg (Ins2-TFRC/OVA)296Wehi/WehiJ) transgenic mice (50) (6–8 weeks of age) were purchased from The Jackson Laboratory (Bar Harbor, ME), bred heterozygously and screened by PCR for OVA expression according to standard procedure.

**Cell lines**
Human non-small-cell lung carcinoma (NSCLC) (A549), colorectal cancer (CRC) (DLD-1, HT-29), Ovarian (HeLa), embryonic kidney (HEK293T), hepatocarcinoma (HCC) (HepG2, Huh7), osteosarcoma (U2OS), glioblastoma (U373), T-ALL (Hpb-all, DND41, Jurkat), lymphoma (T2), B-ALL (Raji, Ramos, RPMI8866), myeloma (U266, RPMI8226) and C57Bl/6 mouse mesothelioma (AK-7), hepatocarcinoma (Hepa1.6) and colon adenocarcinoma (MC38) cells were purchased from ATCC or Leibniz Institute DSMZ and cultured according to the manufacturers’ instructions in DMEM or RPMI medium (Life Technologies) supplemented with (10% endotoxin-free fetal calf serum (Thermo Fisher), 2 mM L-glutamine (Sigma-Aldrich), 100U/ml Penicillicine; 100µg/ml Streptamycine (Life Tecnologies)) at 37°C and 5% CO₂.

**Generation of RIPmOVA bone marrow chimeric mice and induction of autoimmune diabetes**

For generation of RIPmOVA chimeric mice, 5.10⁶ of bone-marrow cells collected from femurs and tibiae of WT and Clec1a-deficient mice and subjected to red blood cell (RBC) lysis (ACK buffer (0.15 M NH₄Cl, 1 mM KHCO₃, and 0.1 mM Na₂EDTA (pH 7.4))) were injected i.v. into lethally whole-body X-irradiated (Faxitron CP 160 (Faxitron X-Ray Corp., Wheeling) (day−1, 11 Gy) RIPmOVA recipient mice. Reconstituted mice were injected sc. with Terramycin (200mg/kg) (Sigma-Aldrich) and drinking water was supplemented with neomycin trisulfate (2mg/ml) (Coopahavet) for 2 weeks. Eight weeks after reconstitution, chimeras were i.v. injected with 5.10⁶ of purified CD8⁺ T cells (CD8a⁺ T cell Isolation Kit II, Miltenyi Biotec) from spleens and lymph nodes of OT-I TCR-transgenic mice ± 0.5 mg of anti-OVA polyclonal antibody (IgG from serum of OVA-hyperimmunized rabbits purified by protein A affinity chromatography; Covalab, Villeurbanne, France). Blood glucose levels were assessed with a StatStrip Xpress Glucose/Ketone Meter monitoring system (Nova Biomedical). Mice were considered diabetic after two consecutive measurements >250mg/dl.

**In vivo and in vitro T cell assays**

For in vivo T cell assays, OVA-specific CD4⁺ T conventional cells from OT-II.Ly5.1 mice were isolated using CD4⁺ T cell isolation Kit II (according to the manufacturer’s instructions (Miltenyi Biotec)), labeled with Cell Proliferation Dye (CPD) eFluor670 (eBioscience) and i.v. injected (total of 1–2.10⁶ cells/mouse) in WT and Clec1a KO mice. One day later, recipient mice were i.p. injected with 100µg of EndoFit OVAprotein (InvivoGen) or with 5.10⁷ of UV-C light treated (150mJ/cm²) MCA101-FcROVA cells (expressing membrane-bound OVA and
with no class I MHC expression). After 3 days, spleen was harvested and proliferation (CPD dilution) of injected cells (CD45.1+CD4+) was assessed by flow cytometry.

For *in vitro* T cells assays, bone marrow cells were cultured (5.10^5 cells/ml) in complete RPMI medium in the presence of 20ng/ml of mouse GM-CSF (Miltenyi Biotec) for 8 days. BMDCs were harvested and incubated in a 96-well plate (1.25.10^4 cells per well) with 500µg/ml of EndoFit OVAprotein (InvivoGen), 10µg/ml of OVA_{323-339} (ISQAVHAAHAINEAGR) peptides (GenScript) or 1.25.10^4 of UV-C light treated (150mJ/cm²) MCA101-FcROVA cells for 5h. Then, BMDCs were washed 3 times and added (1.25.10^4 cells/well) to CPD-labeled purified CD4+ T cells from OT-II-Ly5.1 mice (5.10^4 cells per well) and proliferation (CPD dilution) was assessed by flow cytometry 3 days later.

FACS data were acquired on a BD FACSCanto™ (BD Biosciences) and analyzed with FlowJo software (Tree Star).

**Flow cytometry analysis**

Cells were killed by treatment with UV-C light (CL-1000 UV Crosslinker, Analytik Jena) (150mJ/cm²), X-ray (Faxitron CP 160 (Faxitron X-Ray Corp., Wheeling)) (10 Gy for PBMC, 2 for mouse splenocytes) or chemotherapies (staurosporine (1µM Sigma-Aldrich), cisplatin (20µM Merck)) and were subsequently incubated in culture conditions for 18h at 37°C. Mouse splenocytes and human PBMCs were stained with recombinant mouse or human recombinant proteins (5µg/ml) respectively together with fluorescently labeled monoclonal antibodies (mAbs) (anti-CD19 for B, anti-CD3 for T, anti-CD11b for myeloid, anti-NK1.1 and anti-CD56 for NK cells) (**Table S4**). For Annexin V/PI staining, mouse splenocytes or human PBMCs were stained with mouse or human Fc CLEC-1-AF488 protein (5µg/ml) respectively and with Annexin V (BD Biosciences) and propidium iodide (Sigma-Aldrich) according to manufacturer’s instructions. FACS data were acquired on a BD FACScelesta (BD Biosciences) and analyzed with FlowJo software (Tree Star).

FACS analysis of splenocytes from controls or hepatocarcinoma-burdened livers of WT and Clec1a KO mice was conducted using a BD FACSymphony™ flow cytometer and FlowJo v.10 software (Tree Star).

**Murine BMDCs generation and stimulation**

For *in vitro* BMDCs stimulation, bone marrow cells from WT and Clec1a KO mice were cultured (5.10^5 cells/ml) in complete RPMI medium in the presence of 20ng/ml of mouse GM-CSF (Miltenyi Biotec) for 8 days at 37°C and 5% CO₂. Then, BMDCs were stimulated with
UV-C light treated (150mJ/cm²) dead splenocytes at ratio 1:10 respectively ± Poly I:C (20µg/ml) (Invivogen). Cells were harvested, extensively washed and subjected for RNA extraction (at 6h) for Q-PCR analysis (Table S5) or for phenotypic analysis (at 24h).

**In vitro uptake of dead cells**

Splenocytes treated by UV-C light (150 mJ/cm²) were labeled with 25nM of Cell Proliferation Dye (CPD) eFluor670 (Invitrogen) or with 5µM of pH Rodo Red (Molecular Probes) according to the manufacturer’s instruction. Bone marrow derived dendritic cells (BMDCs) generated from WT and Cleca KO mice were labeled with 250nM of Cell Proliferation Dye (CPD) eFluor450 (Invitrogen). For uptake of dead cells, BMDCs and dead splenocytes were co-incubated in 96-well culture plate at ratio 1:1 (2.5.10⁵ cells per well) at various time points (30 to 240 min) at 37°C and 5% CO2. BMDCs were stained with anti-CD11c (PE) and CD11b (PE-Cy7) mAbs and engulfment of dead cells was assessed by flow cytometry.

**Gene ontology and pathway enrichment analysis**

Pathway enrichment analysis was performed using the Enrichr (51) tool based on MSigDB Hallmark 2020 database, using differential expression genes (DEG). Enrichment analyses for gene ontology (GO) were performed with WebGestalt (52) tool using. The GO enrichment was performed for biological processes and Benjamini–Hochberg false-discovery rate (FDR) was adopted for screening statistically significance and was set at FDR < 0.05. For nSolver™ generated raw counts, DESeq2 R package was used for differential expression analysis between samples (53).

**Selection of anti-human CLEC-1 antagonist mAbs**

Specificity of anti-hCLEC-1 mAbs (dose-response) was evaluated according to their binding by ELISA on coated recombinant human Fc CLEC-1 protein (Biotechne, 10nM) revealed by anti-mouse IgG-HRP) (0.5µg/ml) and according to their binding by flow cytometry to CLEC-1 expressing cell lines revealed by anti-mouse IgG-PE (5µg/ml).

Antagonist activity of anti-human CLEC1 mAbs was evaluated by flow cytometry by their ability (dose-response) to block the interaction of human Fc CLEC-1-AF488 protein (10µg/ml) to UV-C light treated (150mJ/cm²) necrotic Raji cells.
Fig. S1: Mouse CLEC-1 recognizes intracellular ligands exposed upon programmed necrosis.
(A) Flow cytometry analysis of mouse splenocytes either fresh, permeabilized, freeze thawing or previously (18h earlier followed by incubation in culture conditions) treated by UV or X-ray (RX), and stained with DAPI and indicated Fc-AF488 fusion proteins or Fc-AF488 alone as control. (a) Representative dot plots and (b) histograms indicate % of CLEC-1 ligands positive cells in DAPI+ cells (N=5-8, Mean ± SEM, paired t-test **p<0.01, ***p<0.01). (B) Flow cytometry analysis of UV-treated (a) human PBMCs and (b) mouse splenocytes incubated in culture conditions for 6h or 18h, and stained with Annexin V, propidium iodide (PI) and human or mouse Fc CLEC-1 fusion protein respectively.
Fig. S2. CLEC-1 ligands are expressed in all cell types upon programmed necrosis and are conserved between species.

(A) (a) Human PBMCs and (b) mouse splenocytes and previously UV-treated (18h earlier followed by incubation in culture conditions) were stained with cell-type specific mAbs to differentiate T (CD3ε), NK (NK1.1/CD56), B (CD19) and myeloid cells (CD11b). (B) (a) mouse splenocytes and (b) human PBMCs previously UV-treated (18h earlier followed by incubation in culture conditions) were stained with indicated Fc fusion proteins. Dot plots are representative of at least 3 independent experiments (m:mice, h:human). (C) Flow cytometry analysis of human tumor cell lines previously treated with UV or X-ray radiation or with the cytotoxic chemotherapies cisplatin (20µM) or staurosporin (1µM) for 18h in culture conditions.
and stained with DAPI and human Fc-CLEC-1 fusion protein or Fc alone as control. Histograms indicate % of CLEC-1 ligands positive cells in DAPI+ cells (n=21, Mean ± SEM, paired t-test ****p<0.001). (D) Flow cytometry analysis of fresh or previously UV-treated mouse splenocytes incubated with RNase A (10µg/ml), DNase I (50U/ml), heat-denatured (20min-65°C) or treated with trypsin (100µg/ml) (30min-37°C) or cycloheximide (100µg/ml) and stained with viability dye and mouse Fc-CLEC-1 fusion protein or Fc alone as control. Histograms indicate MFI staining of CLEC-1 ligand positive cells in dead cells (with MFI of Fc alone subtracted) (N=5-11, Mean ± SEM, paired t-test *p<0.05, **p<0.01). (E) Flow cytometry analysis of human tumor cell lines previously treated with UV or X-ray radiation or with the cytotoxic chemotherapies cisplatin (20µM) or staurosporin (1µM) for 18h in culture conditions and stained with anti-human TRIM21 Abs or isotype as control. Histograms indicate % of positive cells (n=21, Mean ± SEM, paired t-test ****p<0.001).
Fig.S3: Absence of Clec1a increases anti-tumor response in spleen in mouse model of Hepa1.6 hepatocarcinoma.

(A) Flow cytometry analysis of different immune cell subsets representing PMNs/MDSCs (CD11b+Ly6G+ MHC-II+), monocytes (CD11b+Ly6C+ MHC-II+), total DCs (CD11c+ MHC-II+), Bmem (CD19+CD45R+CD24+CD38+), TemL (CD44+CD62L−CD27+) and Tcm (CD44+CD62L+) CD3+ CD4/8+ T cells from the spleen of controls (Ctrl) or WT and Clec1a KO mice at day 13 after Hepa1.6 inoculation (Data are expressed in % of CD45+ or in particular cell subset) (N=5 for controls, N=15 for Hepa1.6 treated, Mean ± SEM of 3 independent experiments, unpaired t-test *p<0.05, **p<0.01, ***p<0.01).
Fig.S4: Single-Cell RNA Sequencing reveals that absence of Clec1a modifies the transcriptomic profile of the myeloid TME.

(A) Uniform Manifold Approximation and Projection (UMAP) clustering of the integrated datasets representing the identified cellular clusters (12) (colored by cell type) from tumor-
burdened livers of WT and Clecl KO mice at day 13 post HeLa1.6 tumor inoculation. (B) Cell proportion of different cell type distribution by genotype. (C-D) Bar chart of overall results of pathways enrichment analysis using Enrichr tool from macrophage and MDSCs from tumor-burdened livers of WT and Clecl KO mice. The blue bars indicate up-regulated pathways, and the red bar indicate down-regulated pathways in Clecl KO mice compared to WT ones. (E-F) Bar chart of the GO enrichment analyzed by WebGestalt of macrophages and MDSCs clusters from tumor-burdened livers of WT versus Clecl KO mice.
Fig.S5: Loss of CLEC-1 does not influence antigen presentation to CD4+ T cells by DCs nor their maturation but enhances their cytokine and chemokine expression. (A) Clec1a and CLEC1A mRNA expression in mouse and human myeloid cell subtypes from ImmGen public RNAseq database (http://www.immgen.org/) (OpenSource Mononuclear
Phagocytes Project for mice, GSE122108) (Bld: Blood, Lv: Liver, Sp: spleen, Mo: monocytes, MF: macrophages, p:plasmacytoid). (B) Representative profile and histograms evaluated by flow cytometry of the proliferation (CPD dilution) of splenic OVA-specific CD4\(^+\) T (OT-II) cells following in vivo inoculation of WT and Clec1a KO mice with UV-treated necrotic MCA cells expressing membrane-bound form of OVA (FcROVA) (OVA dead cells) or with soluble OVA protein (OVAs). Control (Ctrl) represents naïve mice without OVA injection (N=5-7, Mean ± SEM of two independent experiments). (C) Clec1a mRNA expression assessed by qPCR in mouse BMDCs stimulated (6h) with coated anti-CD40 (10\(\mu\)g/ml), LPS (0.1mg/ml), IFN\(\gamma\) (1\(\mu\)g/ml), TNF\(\alpha\) (1000U/ml) and TGF\(\beta\) (20ng/ml) (data are expressed in arbitrary unit (AU) relative to Hprt expression) (N=5-13, Mean ± SEM of 3 independent experiments, paired t-test, **p<0.01, ***p<0.001, ****p<0.0001). (D) Representative flow cytometry profile and histograms of the proliferation (reflected by CPD dilution) of OVA-specific CD4\(^+\) T (OT-II) cells in response to WT and Clec1a KO BMDCs either unstimulated (US) or loaded with UV-treated MCA cells expressing membrane-bound OVA (OVA dead cells), OVA peptide SIINFEKL (OVAp) or soluble OVA (OVAs) (N=5-7, Mean ± SEM of 3 independent experiments). (E) CPD eFluor450-labeled BMDCs and CPD eFluor670/pH rodo labeled-dead cells were co-incubated (ratio 1:1) for 30-300 min in culture conditions. Percentage of BMDCs that engulfed dead cells was calculated by the percentage of double positive CPDe450\(^+/\)CPDe670\(^+\) BMDCs assessed by flow cytometry. Dead cell uptake was quantified by the MFI of pHrodo-labeled dead cells in phagocytic double positive CPDe450\(^+/\)CPDe670\(^+\) BMDCs assessed by flow cytometry (N=5, Mean ± SEM of 3 independent experiments). (F) Flow cytometry analysis of BMDC phenotype (MHC-I, MHC-II, CD86, CD40) from WT and Clec1a KO mice stimulated with UV-treated necrotic splenic cells and/or TLR3-L for 24h. Data are expressed in histograms (N=5, Mean ± SEM of 3 independent experiments). (G) Cxcl2, Ccl2, Isg15, and Il1b mRNA expressions assessed by qPCR in BMDCs from WT and Clec1a KO mice stimulated with UV-treated necrotic splenic cells and/or TLR3-L for 6h. Results are expressed in histograms (N=9-21, Mean ± SEM of 6 independent experiments in AU of specific cytokine/Hprt ratio, paired t-test *p<0.05, **p<0.01).
Fig. S6. Loss of CLEC-1 signaling does not break cross-tolerance following homeostatic cell death.

Chimeric RIP-mOVA mice reconstituted with bone-marrow from WT or Clecl1a KO mice were injected with $5.10^6$ of OT-I cells alone (A) or with rabbit anti-OVA polyclonal IgG (i.p., 0.5mg) (B). Diabetes incidence was assessed by monitoring blood glycemia every other day during at least 12 days (N=8-20, Mean ± SEM).
Fig.S7.

**Fig.S7: Specificity of anti-human CLEC-1 mAbs and expression of CLEC-1 in CLEC-1 humanized mice.**

Specificity of the anti-human CLEC-1 mAbs (#1 and #2) evaluated by (A) ELISA on human Fc CLEC-1 and His-CLEC-1 recombinant proteins and by (B) flow cytometry on CLEC-1 expressing cell line. (C) Antagonism property of mAbs was evaluated by flow cytometry by their dose-dependent potential to block the interaction of human recombinant Fc CLEC-1 protein to UV-treated necrotic PBMCs. (D) CLEC-1 expression in T cells (CD45⁺ CD3⁺), neutrophils (CD45⁺ CD11b⁺ Ly6G⁺), cDC1 (CD45⁺ CD11c⁺ MHC-II⁺ XCR1⁺), cDC2 (CD45⁺ CD11c⁺ MHC-II⁺ CD11b⁺), alveolar macrophages (AP) (CD45⁺ CD11b⁺ CD11c⁺ CD64⁺) and endothelial cells (ECs) (CD31⁺ CD45⁻) from lung and spleen of CLEC-1 humanized mice evaluated by flow cytometry with anti-human CLEC-1 mAb#1. Data are expressed in histograms (delta MFI: substraction of the MFI obtained with isotype control to the specific antibody, N=4-9, Mean ± SEM of 4 independent experiments).
Table S1: Steady state frequency of most common cell populations in Cleclα KO mice.

| Cell Type       | Markers         | WT               | Cleclα-KO         |
|-----------------|-----------------|------------------|-------------------|
| Blood           |                 |                  |                   |
| T cells         | CD3+            | 18.90 ± 3.46     | 28.00 ± 14.50     |
| CD4+ T cells    | CD3+CD4+        | 8.33 ± 1.40      | 15.30 ± 6.71      |
| CD8+ T cells    | CD3+CD8+        | 9.16 ± 1.74      | 13.36 ± 5.33      |
| Treg            | CD3+CD4+FoxP3+  | 0.54 ± 0.07      | 0.81 ± 0.17       |
| NK Cells        | CD3 NK1.1+      | 3.69 ± 0.59      | 2.14 ± 1.22       |
| B Cells         | CD3 CD19+       | 54.27 ± 0.80     | 49.73 ± 10.12     |
| Monocytes       | CD11bLy6C+      | 2.74 ± 0.40      | 2.42 ± 0.50       |
| Neutrophiles    | CD11bLy6g+Ly6cint | 8.47 ± 4.46   | 7.00 ± 3.48       |
| cDCs            | CD11cMHC-II+    | 0.27 ± 0.03      | 0.26 ± 0.25       |
| Spleen          |                 |                  |                   |
| T cells         | CD3+            | 32.67 ± 4.24     | 29.03 ± 2.70      |
| CD4+ T cells    | CD3+CD4+        | 20.87 ± 4.73     | 18.58 ± 3.96      |
| CD8+ T cells    | CD3+CD8+        | 11.15 ± 1.54     | 10.78 ± 1.74      |
| Treg            | CD3+CD4+FoxP3+  | 2.27 ± 0.76      | 2.03 ± 0.51       |
| NK Cells        | CD3 NK1.1+      | 4.43 ± 1         | 4.42 ± 0.11       |
| B Cells         | CD3 CD19+       | 45.60 ± 9.59     | 50.86 ± 6.62      |
| Monocytes       | CD11bLy6C+      | 1.53 ± 0.42      | 1.46 ± 0.42       |
| Neutrophiles    | CD11bLy6g+Ly6cint | 2.96 ± 0.26   | 2.86 ± 2.25       |
| cDCs            | CD11cMHC-II+    | 1.38 ± 0.33      | 1.53 ± 0.66       |
| cDC1            | CD11cMHC-II+CD11bLy6cXCR1+ | 0.48 ± 0.17   | 0.65 ± 0.27       |
| cDC2            | CD11cMHC-II+CD11bXCR1+ | 0.83 ± 0.13   | 0.73 ± 0.19       |
| Lymph nodes     |                 |                  |                   |
| T cells         | CD3+            | 51.34 ± 9.89     | 53.60 ± 5.86      |
| CD4+ T cells    | CD3+CD4+        | 25.27 ± 8.88     | 28.17 ± 4.54      |
| CD8+ T cells    | CD3+CD8+        | 21.46 ± 5.59     | 22.56 ± 4.38      |
| Treg            | CD3+CD4+FoxP3+  | 3.46 ± 2.02      | 4.29 ± 1.19       |
| NK Cells        | CD3 NK1.1+      | 0.79 ± 0.30      | 0.58 ± 0.13       |
| B Cells         | CD3 CD19+       | 26.90 ± 10.42    | 38.07 ± 4.19      |
| Monocytes       | CD11bLy6C+      | 0.13 ± 0.08      | 0.14 ± 0.08       |
| Neutrophiles    | CD11bLy6g+Ly6cint | 0.16 ± 0.11   | 0.06 ± 0.04       |
| cDCs            | CD11cMHC-II+    | 1.30 ± 0.56      | 1.15 ± 0.20       |
| cDC1            | CD11cMHC-II+CD11bLy6cXCR1+ | 0.54 ± 0.27   | 0.44 ± 0.08       |
| cDC2            | CD11cMHC-II+CD11bXCR1+ | 0.41 ± 0.36   | 0.43 ± 0.13       |
Table S2: Steady state frequency of most common cell populations in CLEC-1 humanized mice.

| Blood          |            |            |            |            |
|----------------|------------|------------|------------|------------|
| Cell Type      | Markers    | WT         | CLEC1A-KI  |
| T cells        | CD3^+      | 16.24 ± 5.31| 15.20 ± 6.29|
| CD4^+ T cells  | CD3^+CD4^+ | 6.73 ± 1.90 | 5.65 ± 0.89 |
| CD8^+ T cells  | CD3^+CD8^+ | 6.47 ± 1.72 | 5.87 ± 1.35 |
| Treg           | CD3^+CD4^+ | 0.43 ± 0.1  | 0.35 ± 0.06 |
| NK Cells       | CD3 Nk1.1^+| 1.83 ± 0.72 | 1.63 ± 0.81 |
| B Cells        | CD3 CD19^+ | 56.29 ± 13.00 | 54.52 ± 13.31 |
| Monocytes      | CD11b^ly6c^+| 5.18 ± 2.02 | 5.16 ± 2.40 |
| Neurophiles    | CD11b^ly6g^+Ly6c^int | 7.95 ± 3.73 | 9.47 ± 6.40 |
| cDCs           | CD11c^MHC-II^+ | 0.85 ± 0.18 | 0.71 ± 0.42 |

| Spleen         |            |            |            |            |
|----------------|------------|------------|------------|------------|
| Cell Type      | Markers    | WT         | CLEC1A-KI  |
| T cells        | CD3^+      | 31.50 ± 3.67 | 33.70 ± 6.64 |
| CD4^+ T cells  | CD3^+CD4^+ | 16.07 ± 1.70 | 17.72 ± 3.24 |
| CD8^+ T cells  | CD3^+CD8^+ | 12.10 ± 1.71 | 12.37 ± 2.23 |
| Treg           | CD3^+CD4^+ | 2.49 ± 0.34 | 2.94 ± 0.71 |
| NK Cells       | CD3 Nk1.1^+| 3.61 ± 0.43 | 2.26 ± 0.80 |
| B Cells        | CD3 CD19^+ | 52.40 ± 3.16 | 53.35 ± 5.95 |
| Monocytes      | CD11b^ly6c^+| 1.38 ± 0.12 | 1.21 ± 0.22 |
| Neurophiles    | CD11b^ly6g^+Ly6c^int | 1.66 ± 0.71 | 1.22 ± 0.40 |
| cDCs           | CD11c^MHC-II^+ | 1.33 ± 0.34 | 0.88 ± 0.26 |
| cDC1           | CD11c^MHC-II^+CD11b^ly6c^+XCR1^- | 0.30 ± 0.08 | 0.19 ± 0.04 |
| cDC2           | CD11c^MHC-II^+CD11b^XCR1^- | 0.91 ± 0.21 | 0.60 ± 0.19 |

| Lymph nodes    |            |            |            |            |
|----------------|------------|------------|------------|------------|
| Cell Type      | Markers    | WT         | CLEC1A-KI  |
| T cells        | CD3^+      | 69.65 ± 4.46 | 61.03 ± 4.51 |
| CD4^+ T cells  | CD3^+CD4^+ | 35.70 ± 4.10 | 31.78 ± 1.96 |
| CD8^+ T cells  | CD3^+CD8^+ | 30.20 ± 0.57 | 26.28 ± 3.22 |
| Treg           | CD3^+CD4^+ | 5.74 ± 0.34 | 5.90 ± 0.40 |
| NK Cells       | CD3 Nk1.1^+| 0.82 ± 0.04 | 0.50 ± 0.15 |
| B Cells        | CD3 CD19^+ | 25.00 ± 4.67 | 34.00 ± 4.17 |
| Monocytes      | CD11b^ly6c^+| 0.11 ± 0.04 | 0.08 ± 0.04 |
| Neurophiles    | CD11b^ly6g^+Ly6c^int | 0.03 ± 0.02 | 0.02 ± 0.01 |
| cDCs           | CD11c^MHC-II^+ | 0.92 ± 0.14 | 0.55 ± 0.35 |
| cDC1           | CD11c^MHC-II^+CD11b^ly6c^+XCR1^- | 0.44 ± 0.12 | 0.25 ± 0.19 |
| cDC2           | CD11c^MHC-II^+CD11b^XCR1^- | 0.41 ± 0.36 | 0.43 ± 0.13 |
Table S3.

| Primers     | Length (pb) | Sequences 5’-3’                        | Genotype      | Tm (°C) |
|-------------|-------------|----------------------------------------|---------------|---------|
| Clec1a Fw   | 20          | TGCCCTAGTGTTGTTGCCTTG                  | Clec1a WT/KO  | 62      |
| Clec1a R    | 20          | GATGTGAGTGTTTCTAGGG                    |               |         |
| Cre Fw      | 22          | ACCAGGTTCGTTCACTCATGGA                 | Clec1a WT/KO  | 62      |
| Cre R       | 23          | GGAACCGAGATGTAGTACCCAGC                |               |         |
| Clec1a Fw   | 18          | AGTCAGAGGCTCCCTTG                      | Clec1a WT/KI  | 58      |
| Clec1a R    | 18          | CGATTTTCTCCAGAGCTC                     |               |         |
| CLEC1A_F    | 19          | ATGAACAAAGGTGGCTAT                     | Clec1a WT/KI  | 58      |
| CLEC1A_R    | 18          | CGATTTTCTCCAGAGCTC                     |               |         |

Table S3: Primers used for PCR genotyping.
Table S4: Panels of mAbs used for FACS analysis.

| Antibody     | Colour | Ref.       | Furnisher | Clone | Reactivity |
|--------------|--------|------------|-----------|-------|------------|
| BDC1A3       | PE     | 559781     | BD        | 1A4   | HS         |
| CD11b        | PB     | 558123     | BD        | ICRF44| HS         |
| CD11c        | PE-Cy7 | 337216     | Biolegend | Bu15  | HS         |
| CD19         | BV786  | 563325     | BD        | SJ25C1| HS         |
| CD1c         | PerCp-eFluor710 | 46-0015-42 | eBioscience | L161  | HS         |
| CD3          | BV605  | 317322     | Biolegend | OKT3  | HS         |
| CD56         | PE     | 555516     | BD        | B159  | HS         |
| HLA-DR       | APC-Cy7| 335831     | BD        | L243  | HS         |
| LIN-1        | FITC   | 340546     | BD        |       |            |
| CD11b        | BUVE395| 563553     | BD        | M1/70 | MM         |
| CD11b        | PE-Cy7 | 552850     | BD        | M1/70 | MM         |
| CD11c        | BV711  | 563048     | BD        | H13   | MM         |
| CD19         | PE     | 553786     | BD        | 103   | MM         |
| CD19         | BV711  | 563157     | BD        | 103   | MM         |
| CD24         | APC    | 562349     | BD        | M1/69 | MM         |
| CD27         | PE     | 558754     | BD        | LG.3A10 | MM    |
| CD27         | BV605  | 558754     | BD        | LG.3A10 | MM    |
| CD279 (PD-1) | BV711  | 744547     | BD        | J43   | MM         |
| CD38         | PE     | 553764     | BD        | 90    | MM         |
| CD3c         | PerCP-Cy5.5 | 551163 | BD        | 145-2C11 | MM    |
| CD4          | PE-Cy7 | 552775     | BD        | RM4-5 | MM         |
| CD44         | BV711  | 563971     | BD        | IM7   | MM         |
| CD45         | PerCP-Cy5.5 | 550994 | BD        | 3D11  | MM         |
| CD45.1       | FITC   | 553775     | BD        | A20   | MM         |
| CD45.2       | BV395  | 564616     | BD        | 104   | MM         |
| CD45.2       | APC-Cy7| 560694     | BD        | 104   | MM         |
| CD45.2       | PE     | 560695     | BD        | 104   | MM         |
| CD45R (B220) | APC-Cy7| 561102     | BD        | RA3-6B2 | MM   |
| CD62L        | APC    | 553152     | BD        | MEL-14| MM         |
| CD69         | FITC   | 553236     | BD        | H1.2F3| MM         |
| CD8a         | BV737  | 564297     | BD        | 53.6-7| MM         |
| CD8a         | V450   | 560469     | BD        | 53.6-7| MM         |
| CD8a         | PE-Cy7 | 552877     | BD        | 53.6-7| MM         |
| IFN-γ        | PE     | 554412     | BD        | XMG1.2| MM         |
| Fc Block     | Purified| 553141     | BD        | 2.4G2 | MM         |
| Ly6C         | V450   | 560594     | BD        | AL-21 | MM         |
| Ly6G         | BV605  | 563005     | BD        | 1A8   | MM         |
| Ly6G         | FITC   | 551460     | BD        | 1A8   | MM         |
| Ly6G         | BV605  | 563005     | BD        | 1A8   | MM         |
| MHCI (I-A/I-E)| FITC | 11-5321-82 | eBioscience | M5/114.15.2 | MM |
| MHCI (I-A/I-E)| BV421 | 562928     | BD        | AF-6.120.1 | MM |
| MHCI (H2Kb)  | FITC   | 553569     | BD        | AF-6.88.5 | MM |
| CD80         | PerCp-Cy5.5 | 560526 | BD        | 16-10A1 | MM |
| CD86         | PE     | 553692     | BD        | GL1   | MM         |
| NK-1.1       | PerCp-Cy5.5 | 551114 | BD        | PK136 | MM         |
| NK-1.1       | BV395  | 564144     | BD        | PK136 | MM         |
| XCR1         | APC    | 148205     | Biolegend | ZET   | MM         |
| XCR1         | APC-Cy7| 148223     | Biolegend | ZET   | MM         |
| VL eFlour506  | AmCyan | 65-0866    | eBioscience |       | MM         |
Table S5:

| Primers  | Length (pb) | Sequences 5’-3’                | RNA Source | Tm-3 (°C) |
|----------|-------------|--------------------------------|------------|-----------|
| Hpri_Fw  | 20          | AAATGTCAGTTTATTCGCTGG         | Mm         | 73        |
| Hpri_R   | 20          | GCGACATCTATCCAGAGGGT          | Mm         | 77        |
| Cxcl2_Fw | 20          | ACTCTCAAGGGCGGTCAAA           | Mm         | 81        |
| Cxcl2_R  | 20          | CATCAGGTAGTGGATCCAGGGCT       | Mm         | 77        |
| Cxcl2_Fw | 20          | AGGTGTCACAAAGGACTGT           | Mm         | 81        |
| Cxcl2_R  | 20          | ACAGAGGTGCTGGAGTG            | Mm         | 77        |
| Il1b_Fw  | 20          | TCAGGCAGGGTCATACGCT           | Mm         | 77        |
| Il1b_R   | 20          | AGCTCATATGGGTCGACAG          | Mm         | 77        |
| Clec1a_Fw| 23          | GAGAGGCCCTTGCCAAATAAGAGTA      | Mm         | 73        |
| Clec1a_R | 21          | AGGAAGAGCTGGATTTGCCA         | Mm         | 79        |
| Igf15_Fw | 20          | TGGTACAGAACCTGCAGGAG        | Mm         | 79        |
| Igf15_R  | 20          | CTCGAAGCTCAGGACAACT          | Mm         | 79        |
**Data S1:** List of DEG genes in macrophages, MDSCs and DCs between tumor burdened livers from WT and *Clec1a* KO mice.
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