

CLEC-1 is a novel myeloid immune checkpoint for cancer immunotherapy limiting tumor cells phagocytosis and tumor antigen cross-presentation

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Abstract

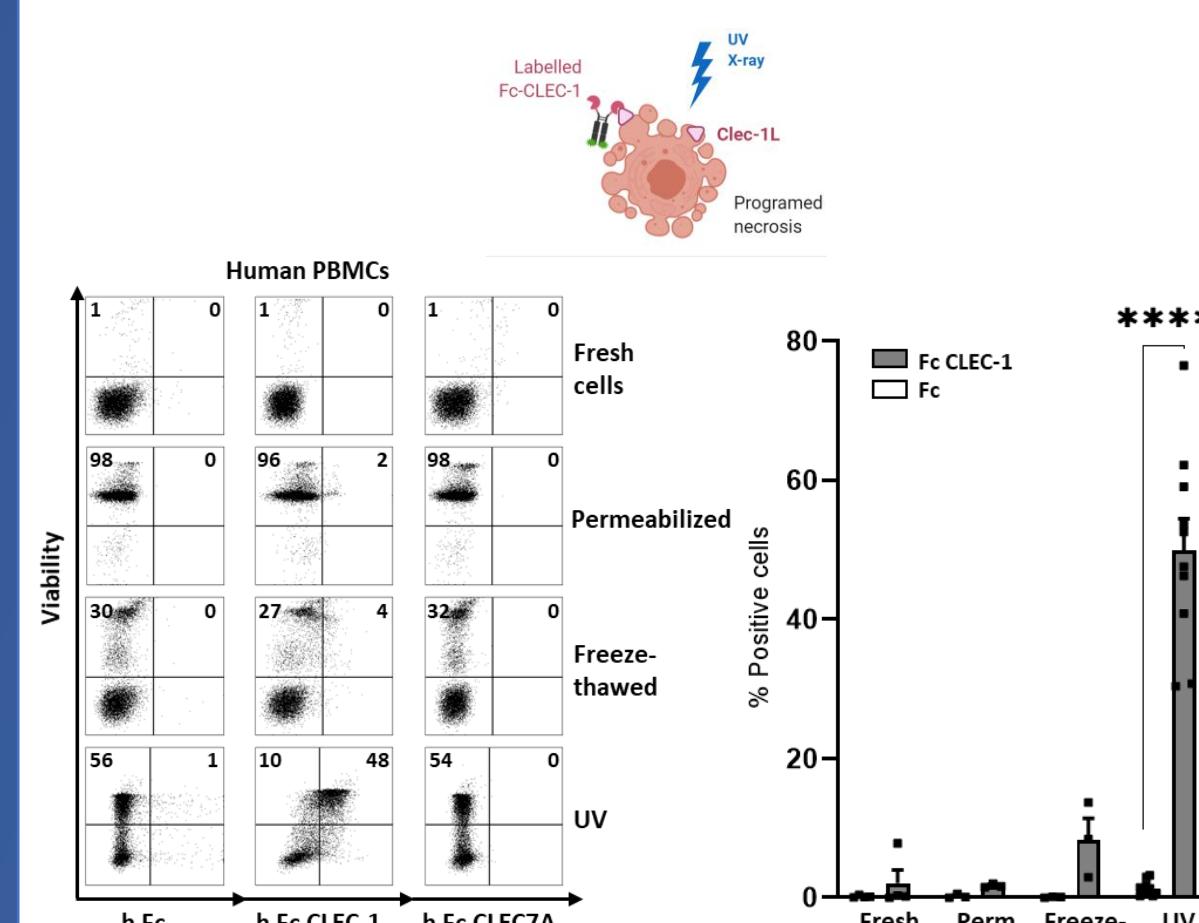
Myeloid cells are one of the most abundant immune cell types in solid tumors that impede myeloid phagocytosis by triggering “don’t eat me” and “don’t find me” signals. Recent literature demonstrates that C-type lectin receptors (CLRs) are powerful pattern recognition receptors shaping immune cell-mediated tissue damage by positively or negatively regulating myeloid cell functions and hence effecting tumor elimination or evasion. We previously reported that the orphan CLR CLEC-1 expressed by dendritic cells (DCs) and macrophages (MΦ) is enhanced by TGFβ and tempers downstream T cells responses. Furthermore, we observed that **CLEC-1 is highly expressed by myeloid cells** purified from **human tumor microenvironment**, in particular tumor-associated MPs. We evaluated whether CLEC-1 could also function as a receptor for damage associated molecular pattern and influences phagocytosis. We show that CLEC-1 specifically recognizes late apoptotic and secondary necrotic healthy cells or tumor cells induced by chemotherapy, radiation (UV, X-ray) or culture stress conditions, and acts as a damage-sensing **negative receptor on dendritic cell antigen cross-presentation and tumor cell phagocytosis**. Furthermore, CLEC-1 inhibition synergizes with immunogenic chemotherapy to favor efficient and robust anti-tumor immune responses. Finally, we report that anti-CLEC-1 mAbs release the break on tumor cell phagocytosis by macrophages and dendritic cells, thus highlighting CLEC-1 as a **novel myeloid checkpoint inhibitor and DON’T EAT ME signal**.

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CLEC-1 ligand (CLEC-1L) is inducible under cell stress conditions and exposed by dying cells

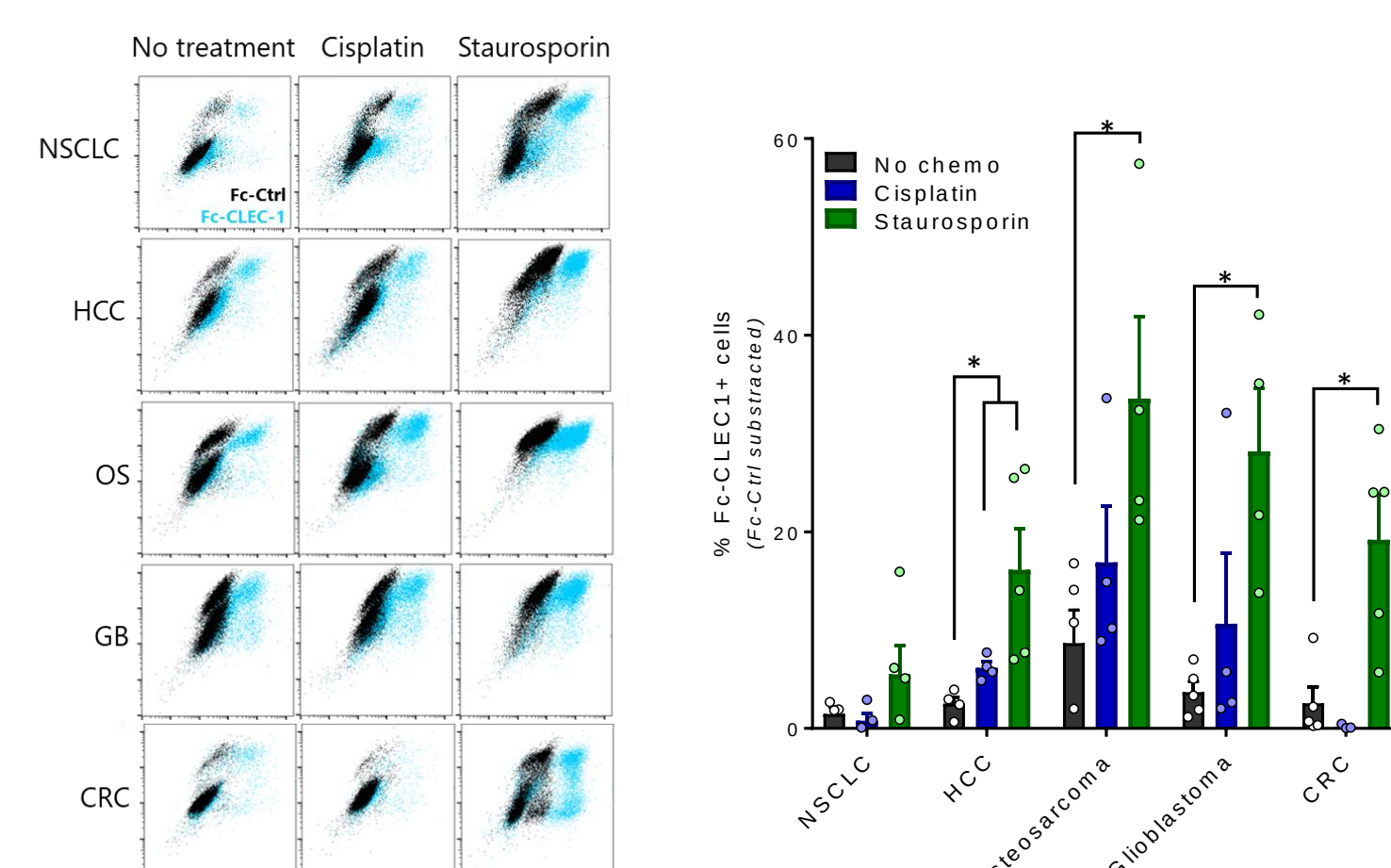
CLEC-1 recognizes DAMPs exposed by dying cells

Human PBMCs were subjected to UV rays, X rays, or freeze-thawed cycles and stained for flow cytometry analysis either by a Fc-Ctrl, an irrelevant CLR (Fc-Dectin1), or Fc-CLEC-1.



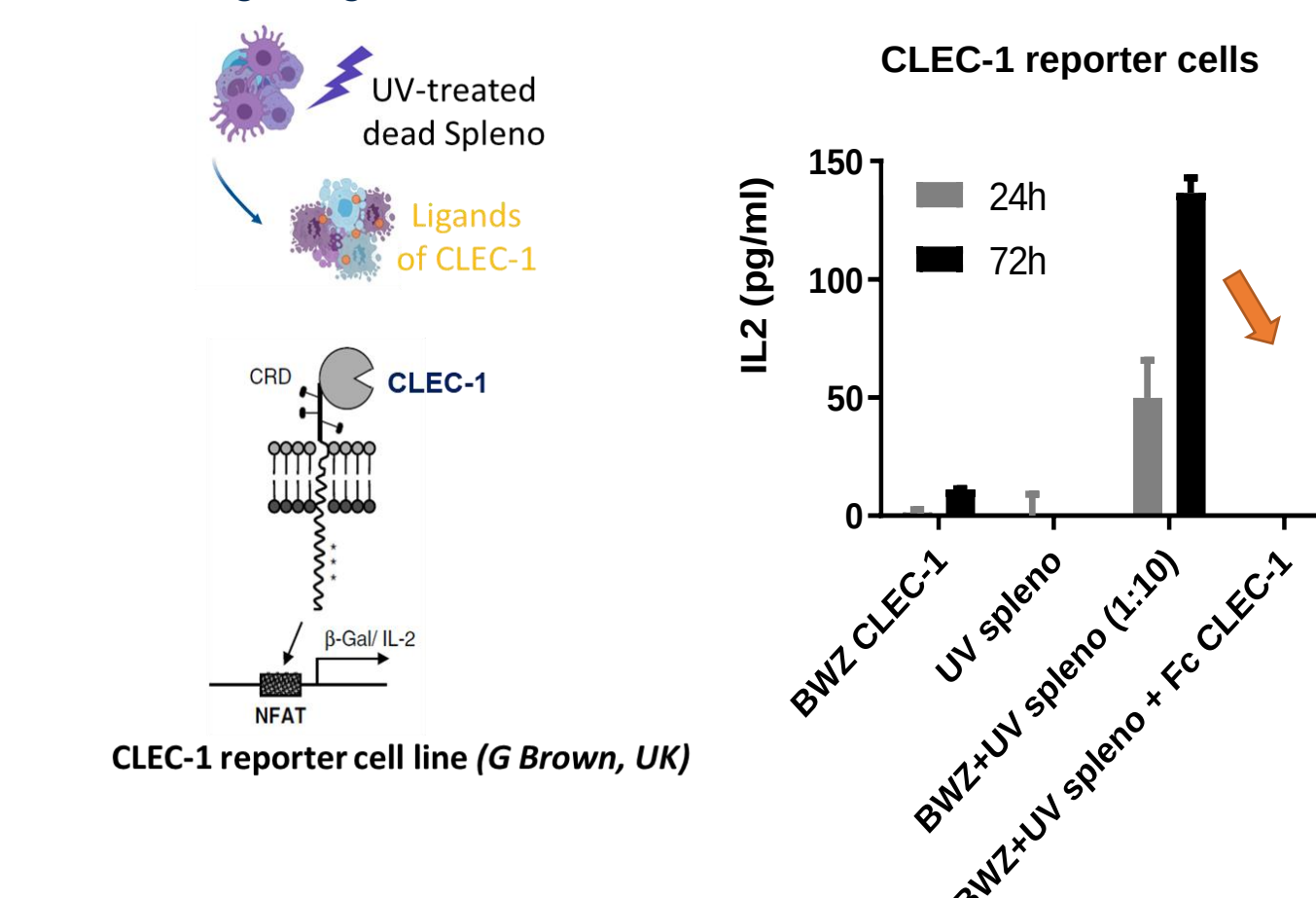
The ligand of CLEC-1 is detectable on tumor cells after chemotherapy

Human tumor cell lines were treated with 20μM of cisplatin or 1μM of staurosporin for 24h and stained for flow cytometry analysis either by a Fc-Ctrl, or Fc-CLEC-1. (NSCLC : non-small cells lung cancer A549; HCC : hepatocellular carcinoma Huh7; OS : osteosarcoma U2OS; GB : glioblastoma U373; CRC : colorectal cancer HT-29)



CLEC-1L triggers CLEC-1 signalling in reporter cell line

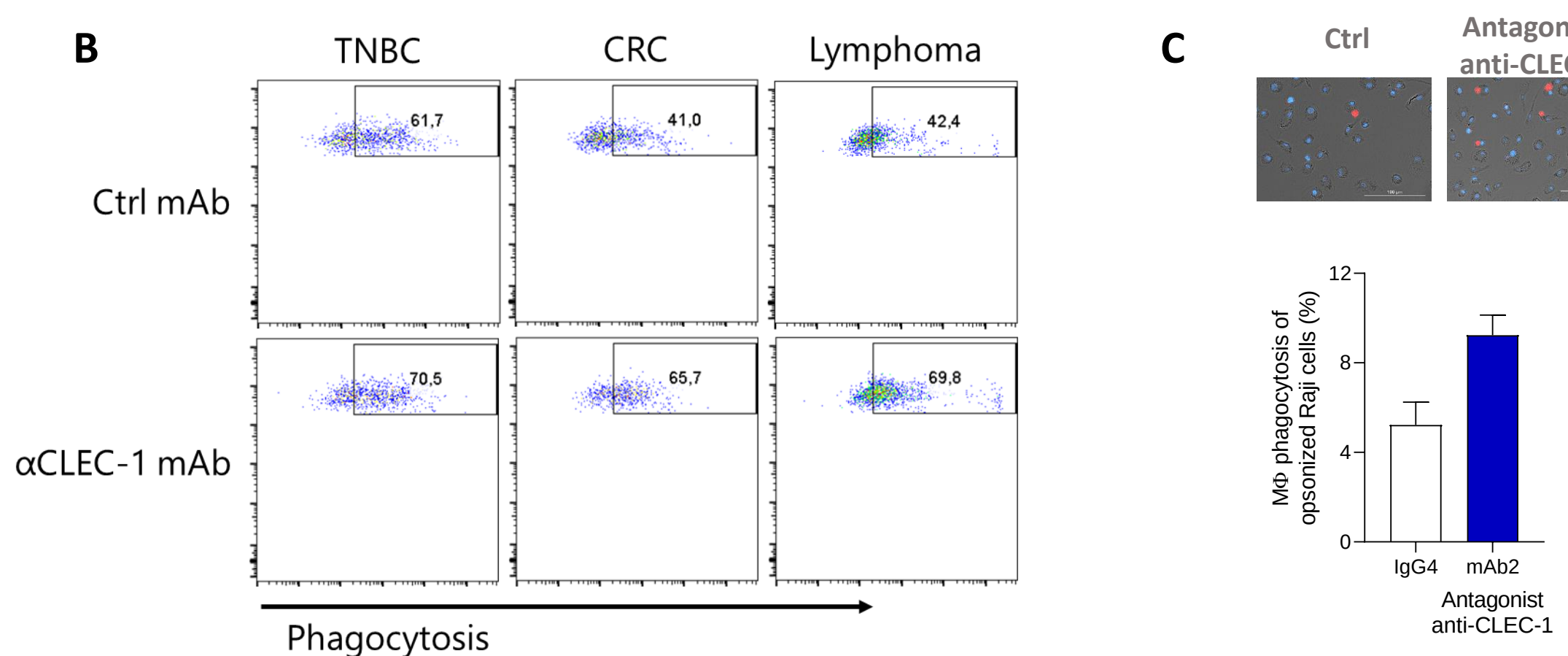
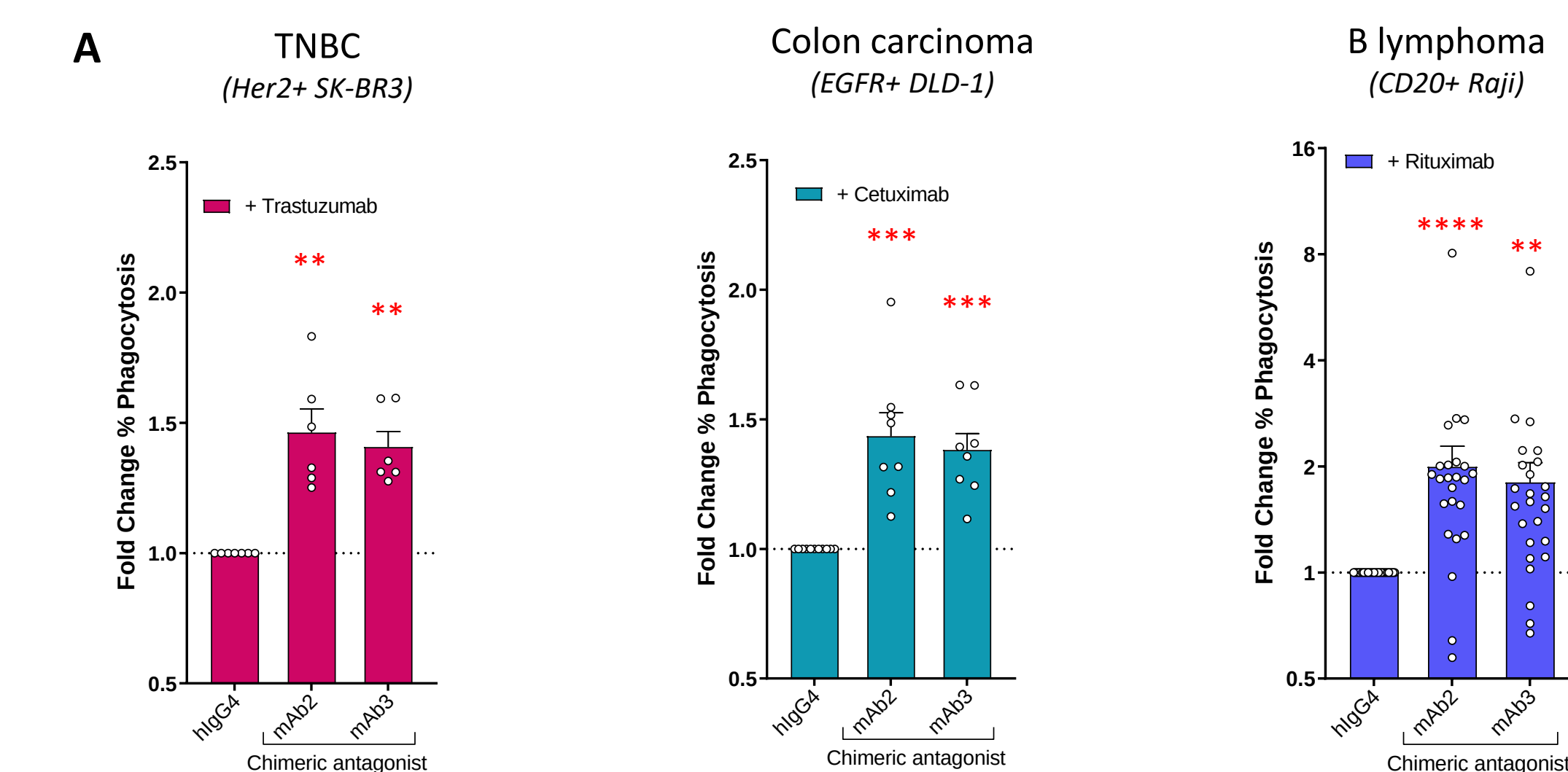
Splenocytes from naïve mice were subjected to UV rays and presented to the BWZ cell line that expresses the murine CLEC-1 fusion to the signalling domain of the CD3 zeta chain which upon activation leads to beta-galactosidase and IL-2 production upon activation. Recombinant Fc-CLEC-1 antagonist protein inhibits CLEC-1L-induced signalling.



5

CLEC-1 antagonist mAbs increase epithelial & hematopoietic tumor cell phagocytosis by **human MΦ**

Macrophages (MΦ) were generated from monocytes with M-CSF (100ng/mL) for 5 days, then polarized with IFNγ (70ng/mL) for two days to generate phagocytic MΦ. MΦ were cultured with either the triple negative breast cancer (SK-BR3), the osteosarcoma (U2OS), the colon carcinoma (DLD-1), the non-Hodgkin's lymphoma (Raji), at a 1:1 ratio +/- the anti-Her2 mAb (Trastuzumab), the anti-EGFR mAb (Cetuximab), or the anti-CD20 mAb (Rituximab) at 10ng/mL providing the “Eat-me” signal, for 2 hours for MΦ. Phagocytosis was analyzed by flow cytometry (A-B) or by microscopy (C) and normalized over the control antibody condition for each donor. * p<0.05; ** p<0.01, *** p<0.005 compared to isotype control



Conclusion

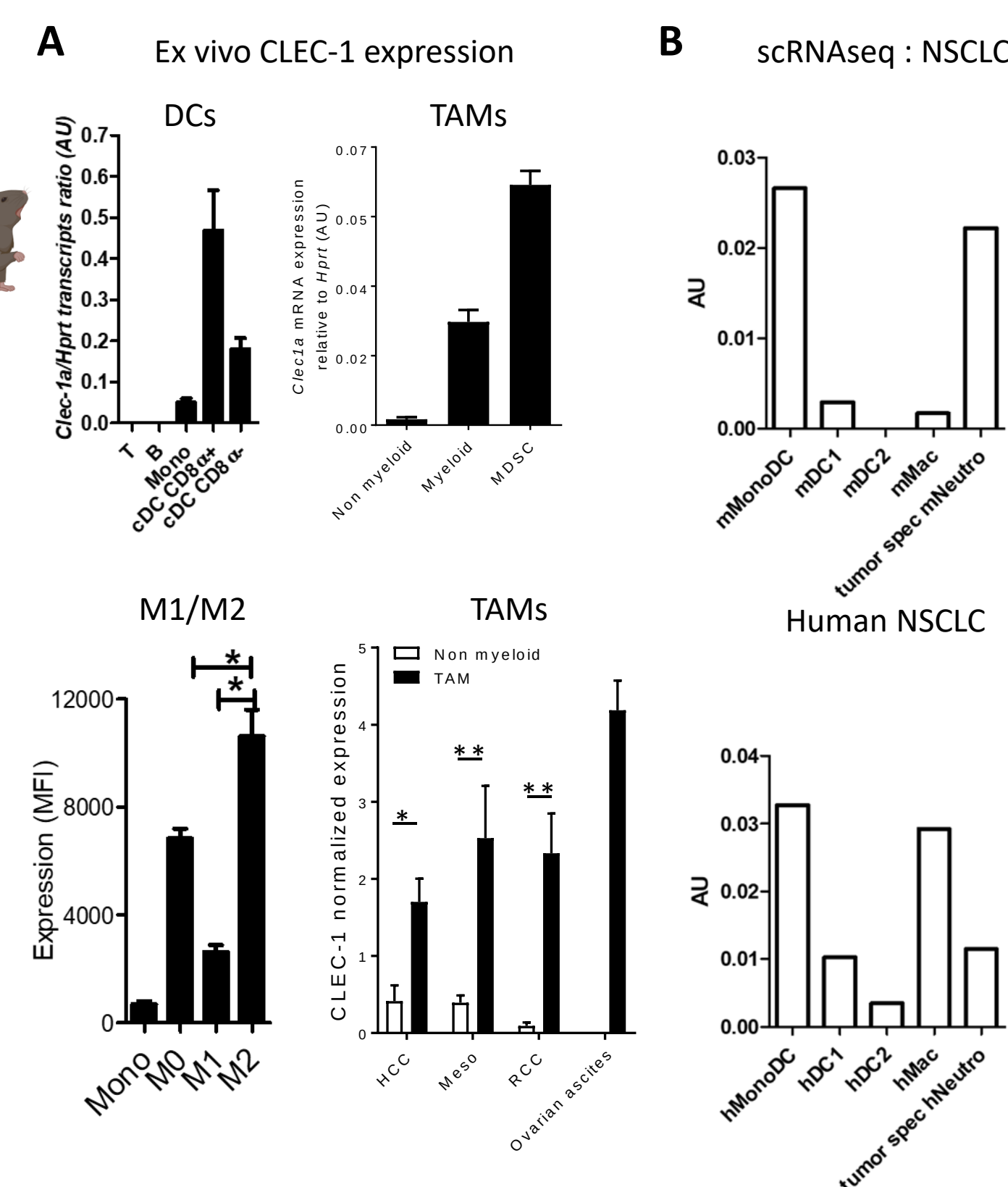
- CLEC-1/CLEC-1L interaction inhibits macrophages phagocytosis of tumor cells and DCs antigen cross-presentation.
- CLEC-1 inhibition represents a novel therapeutic target for immuno-oncology that modifies T cell immune responses and blunts *in vivo* chemotherapeutic anti-tumor immune response
- Antagonists of the CLEC-1 myeloid immune checkpoint release the break on tumor cell phagocytosis by preventing a novel DON’T EAT ME signal.

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CLEC-1 is expressed by macrophages and cross-presenting dendritic cells (cDC1)

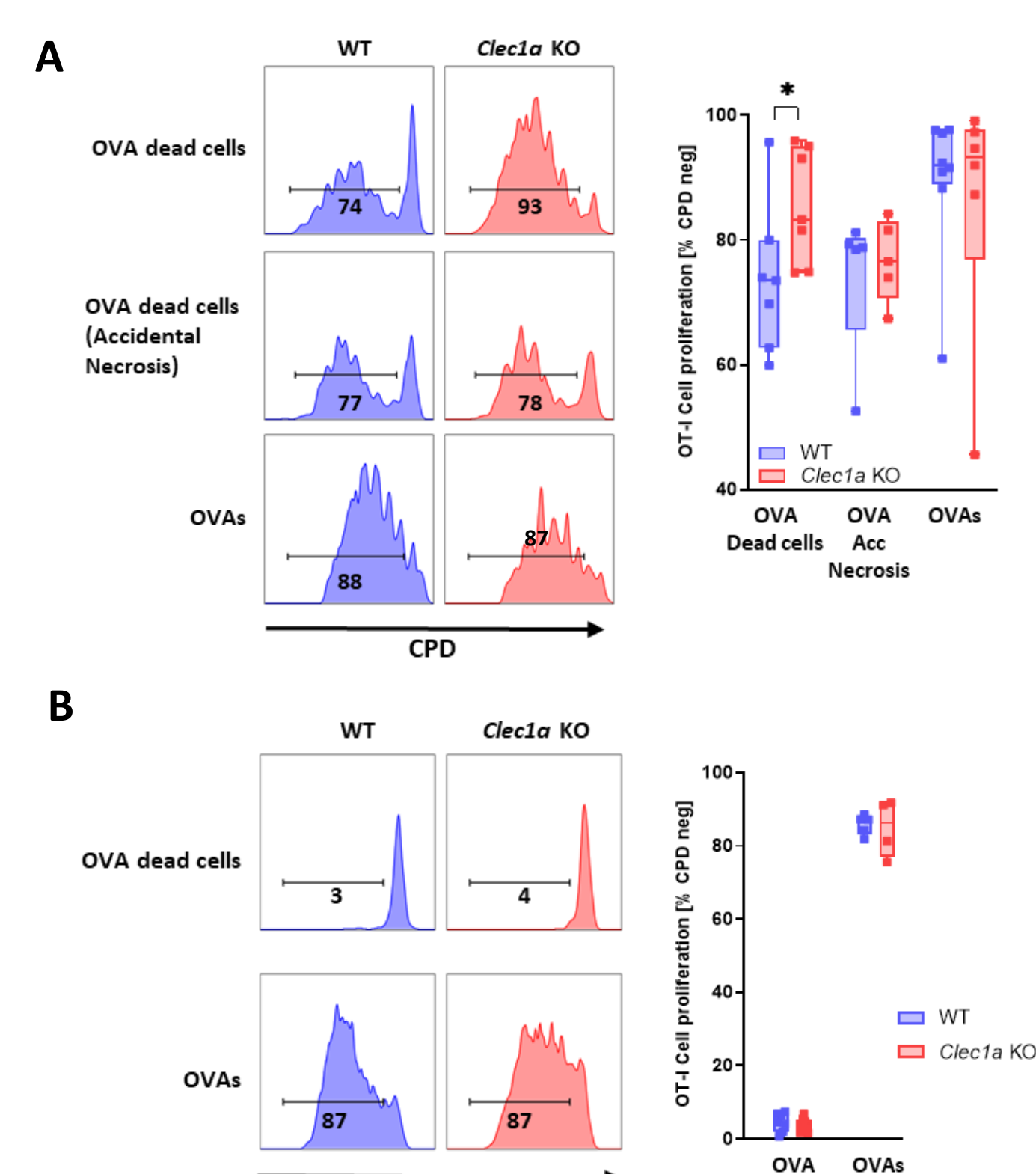
CLEC-1 expression in mouse and human immune cells and MΦ/DCs subsets from different solid tumor microenvironment context or models was analyzed by flow cytometry or RT-qPCR (A). CLEC1A expression was also characterized in mouse and human non-small cell Lung carcinoma (NSCLC) tumor using single cell RNA sequencing public datasets (Zilionis et al, Immunity 2019) (B).



3

CLEC-1 deficiency specifically enhances T cell antigen cross-priming under stress settings

MCA101-FcR OVA cells (expressing membranous OVA) treated either with UV (to induce CLEC-1L expression) or with freeze-thaw cycles (not expressing CLEC-1L) ; or soluble OVA protein were concomitantly injected into Clec1a KO or WT mice with OVA-specific CD8+ OT-1 cells (A) or OVA-specific CD4 OT-II cells (B). OT-1 and OT-II cell proliferation was analyzed by flow cytometry.



4

CLEC-1 inhibition impairs tumor growth and modifies TME in immunogenic HCC cancer and chemotherapy-sensitive CRC

Clec1a KO or WT mice received either 2.5x10⁶ Hepa 1.6 hepatocarcinoma cells in the portal vein (A) or subcutaneously 1x10⁶ MC38 colorectal cancer cells (B). Chemotherapy (100mg/kg cyclophosphamide) was administered intraperitoneally (i.p.) once at d12. Mouse Fc-CLEC-1 recombinant protein was intraperitoneally injected twice a week for two weeks from d4 at 3mg/kg. Tumor microenvironment (TME) analysis was conducted by flow cytometry on Percoll-sorted non parenchymal cells from tumor at d13-20 after tumor inoculation according to the tumor model.

