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Supplemental Material to:

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Cardiotrophin-1 determines liver engraftment of a syngenic colon carcinoma cell line through an immune-mediated mechanism

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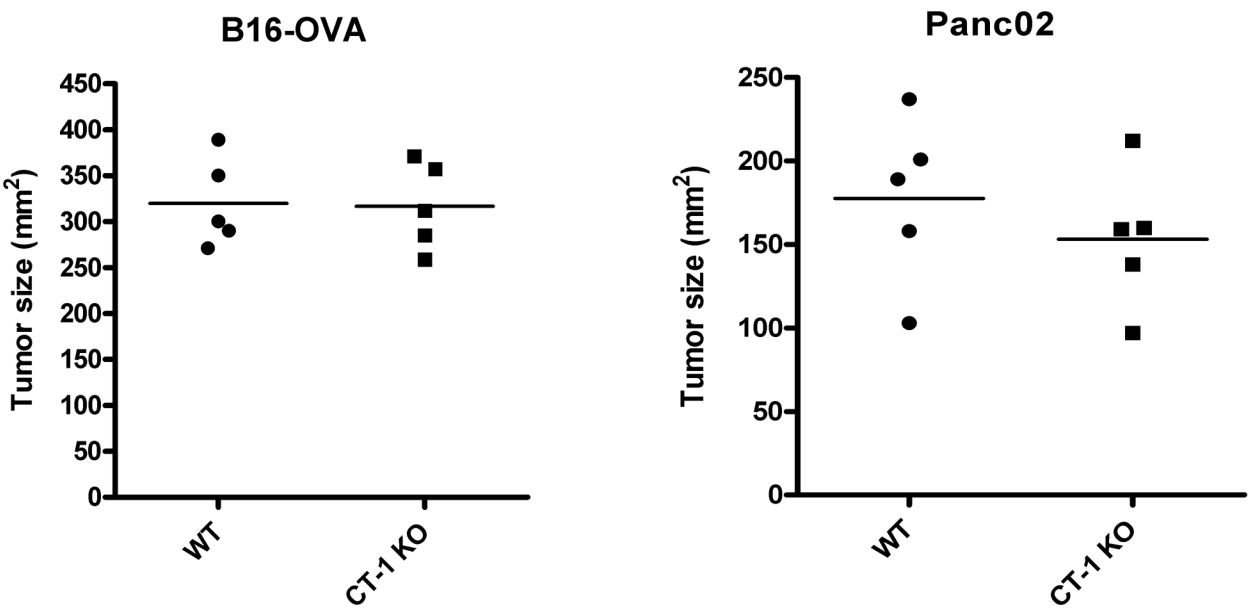
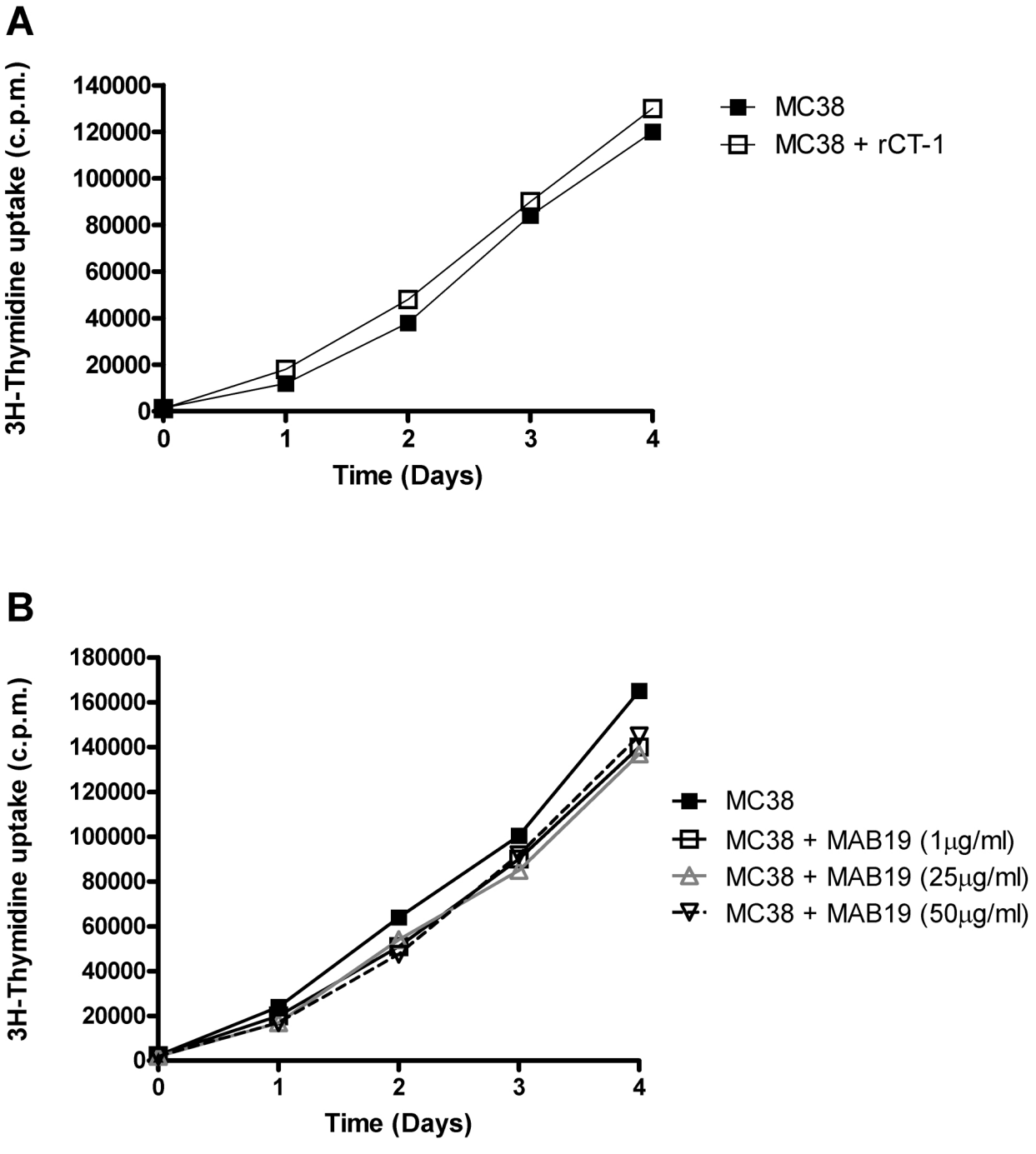
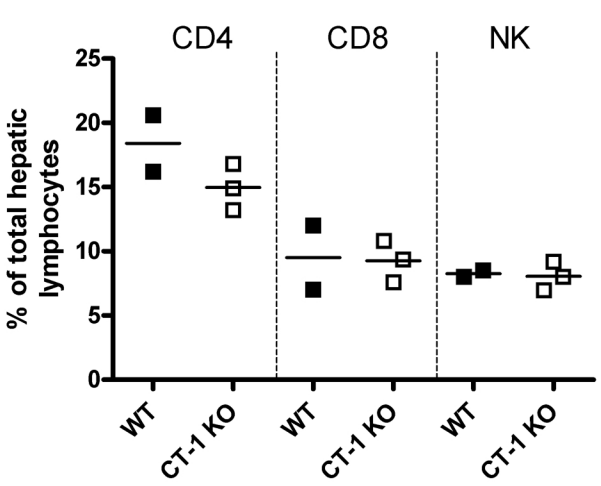
Supplementary figure 1

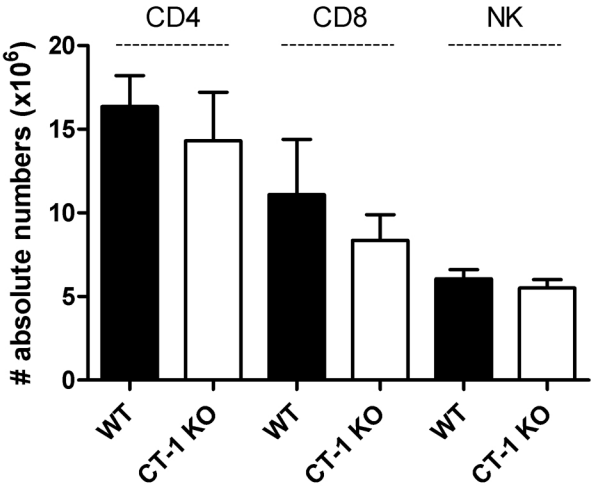


Supplementary figure 2

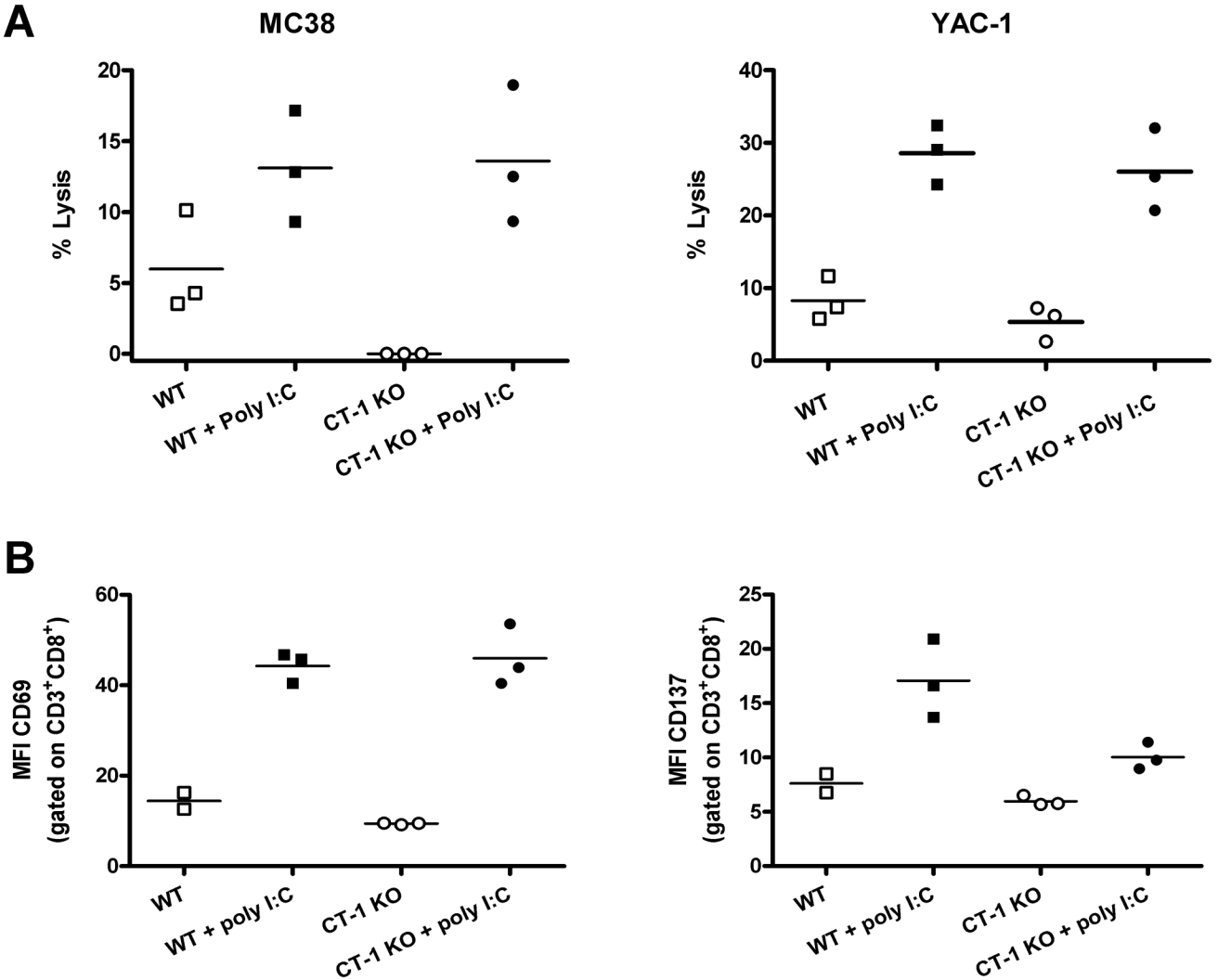


Supplementary figure 3





Supplementary figure 4



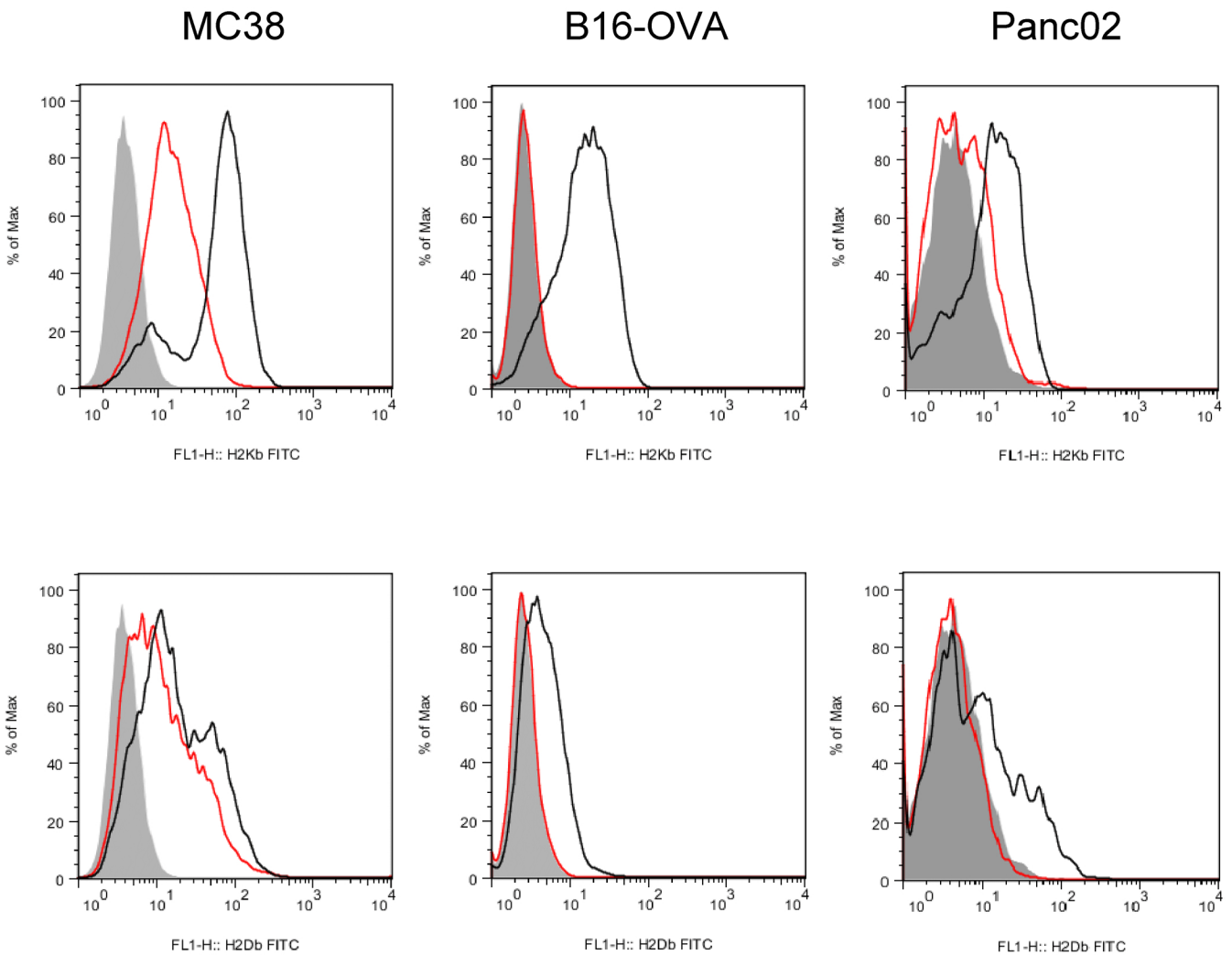
Supplementary figure 5

H-2K^b

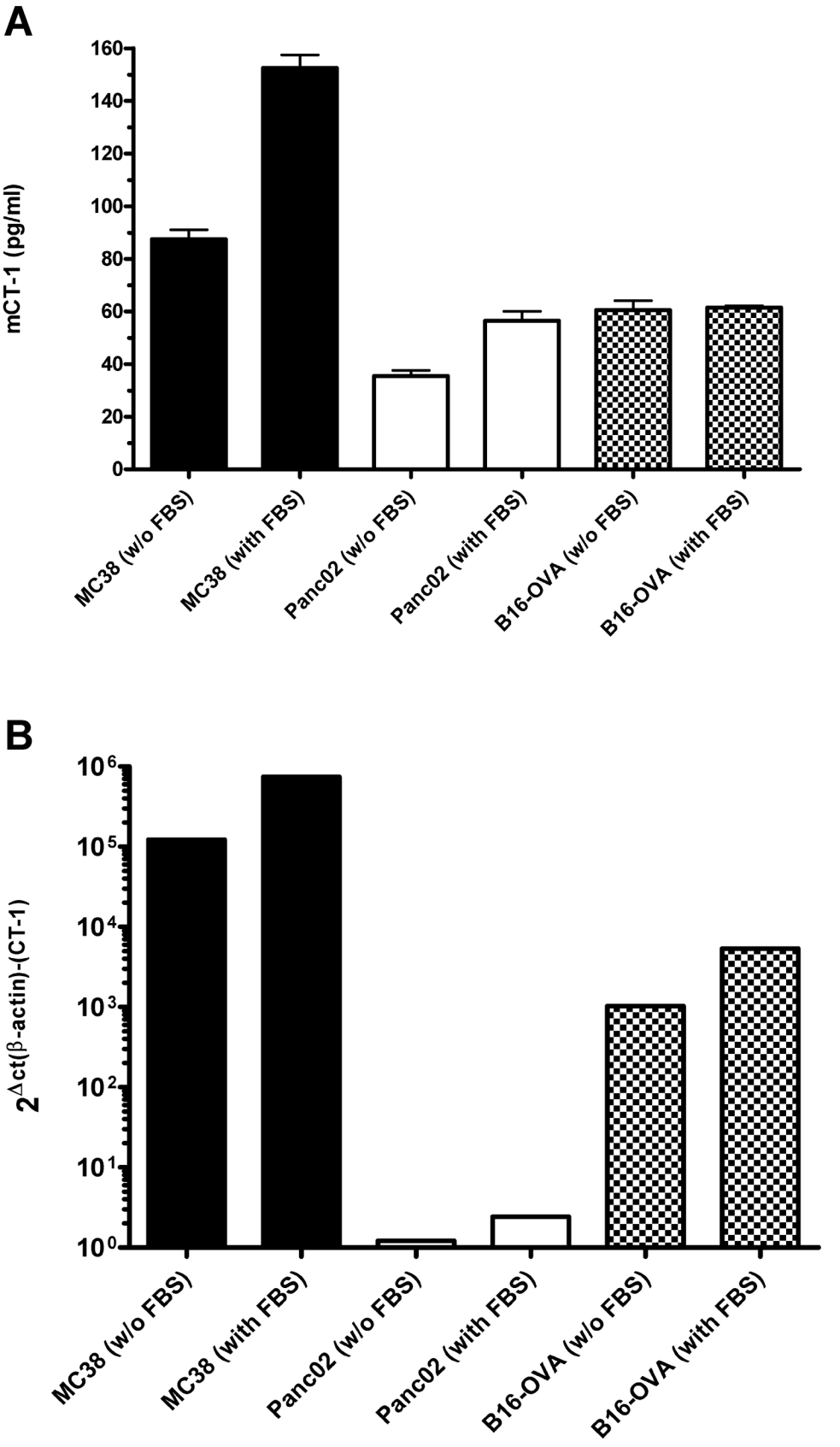
		MFI		
		MC38	B16-OVA	Panc02
	Isotype control	9,03	4,37	5,12
-	H2Kb (Basal)	18,5	2,89	5,39
-	H2Kb (+ IFNγ)	70,4	18,9	20,25

H-2D^b

		MFI		
		MC38	B16-OVA	Panc02
	Isotype control	8,04	3,38	5,33
-	H2Db (Basal)	29,3	3,91	5,05
-	H2Db (+ IFNγ)	36,6	9,37	20,4



Supplementary figure 6



Legends to Supplementary Figures

Supplementary Figure 1. **The resistance to the hepatic engraftment of tumor cells of *CT-1*$^{-/-}$ mice is not shared by other syngenic tumors.** Graphs show wild type (WT) and *CT-1*$^{-/-}$ mice inoculated with 5x10^6 Panc02 and 10^6 B16-OVA carcinoma cells in the left lobe of the liver by laparotomy under anaesthesia and euthanized on day 14 to monitor the diameter of individual lesions on the surface of the liver.

Supplementary Figure 2. **CT-1 does not affect the growth of MC38 cells in culture.** **A.** Experiments of *in vitro* of ^{3}H-thymidine incorporation with MC38 cells treated or not with 100 μg CT-1. **B.** Proliferation of MC38 cells cultured in RPMI 1640 with 10% FCS upon the addition of 1-50 μg/mL of the anti.CT-1 neutralizing MAB19 antibody, when indicated.

Supplementary Figure 3. **The abundance of CD4$^+$ (CD3$^+$ CD4$^+$), CD8$^+$ (CD3$^+$ CD8$^+$), and NK cells (CD3$^-$ NK1.1$^+$) in the liver of wild type (WT) and *CT-1*$^{-/-}$ mice is comparable.** The livers of WT and *CT-1*$^{-/-}$ mice were excised and, upon collagenase D/DNAse I digestion, a Percoll gradient was carried out to isolate the leukocyte population. Graphs show the percentage of total hepatic lymphocytes (left) and absolute numbers (right) of CD4$^+$, CD8$^+$ and NK cells.

Supplementary Figure 4. **Hepatic lymphocytes from *CT-1*$^{-/-}$ and wild type (WT) mice exert comparable spontaneous tumor cytotoxicity. A.** Total hepatic leukocytes were isolated as described in **Supplementary Figure 3** to measure cytolytic activity in five-hour ^{51}Cr release assays. Where indicated, wild type or *CT-1*$^{-/-}$ were mice injected or not with 100 μg polyI:C *i.v.* to enhance the activity of NK cells. Lymphocytes were co-cultured with ^{51}Cr-labeled MC38 (left panel) or YAC-1 (right panel) cells at 5:1

effector:target ratio. **B.** Levels of CD69 (left panel) and CD137 (right panel) activation markers on the surface of the $CD3^+$ $CD8^+$ population from WT and *CT-1*$^{-/-}$ mice pre-treated or not with polyI:C.

Supplementary Figure 5. **Analysis of MHC class I expression on MC38, Panc02 and B16-OVA cells.** Surface expression of H-$2K^b$ and H-$2D^b$ molecules on the indicated cell lines was examined by flow cytometry. Figure shows the FACS histograms and MFI of baseline (red line) MHC class I expression or the levels detected upon the addition of 1,000 U/mL interferon γ (IFNγ) during the last 48h of culture (black line) The corresponding isotype-matched background staining is provided.

Supplementary Figure 6. **Production of CT-1 by tumor cell lines. A.** ELISA determination of the concentration of mouse CT-1 in the supernatants of MC38, Panc02 and B16-OVA cultured cells. All cells were cultured either with or without fetal bovine serum (FBS), as indicated. **B.** *CT-1* mRNA was quantified by RT-PCR in tumor cells cultured as in **A**.