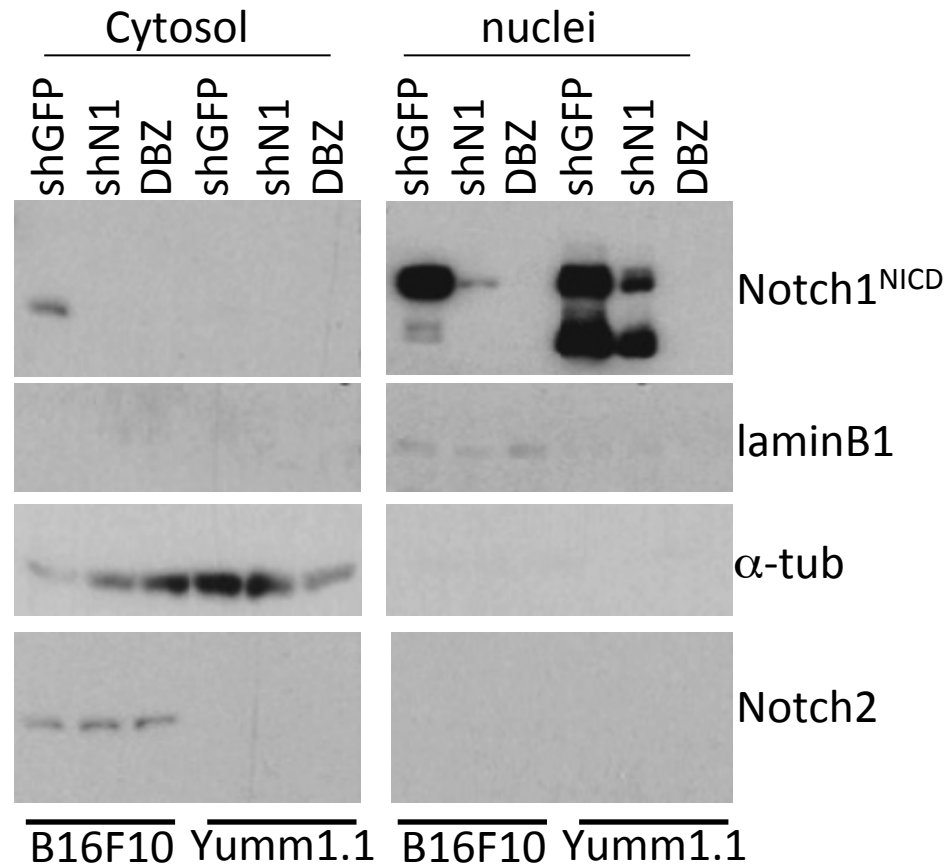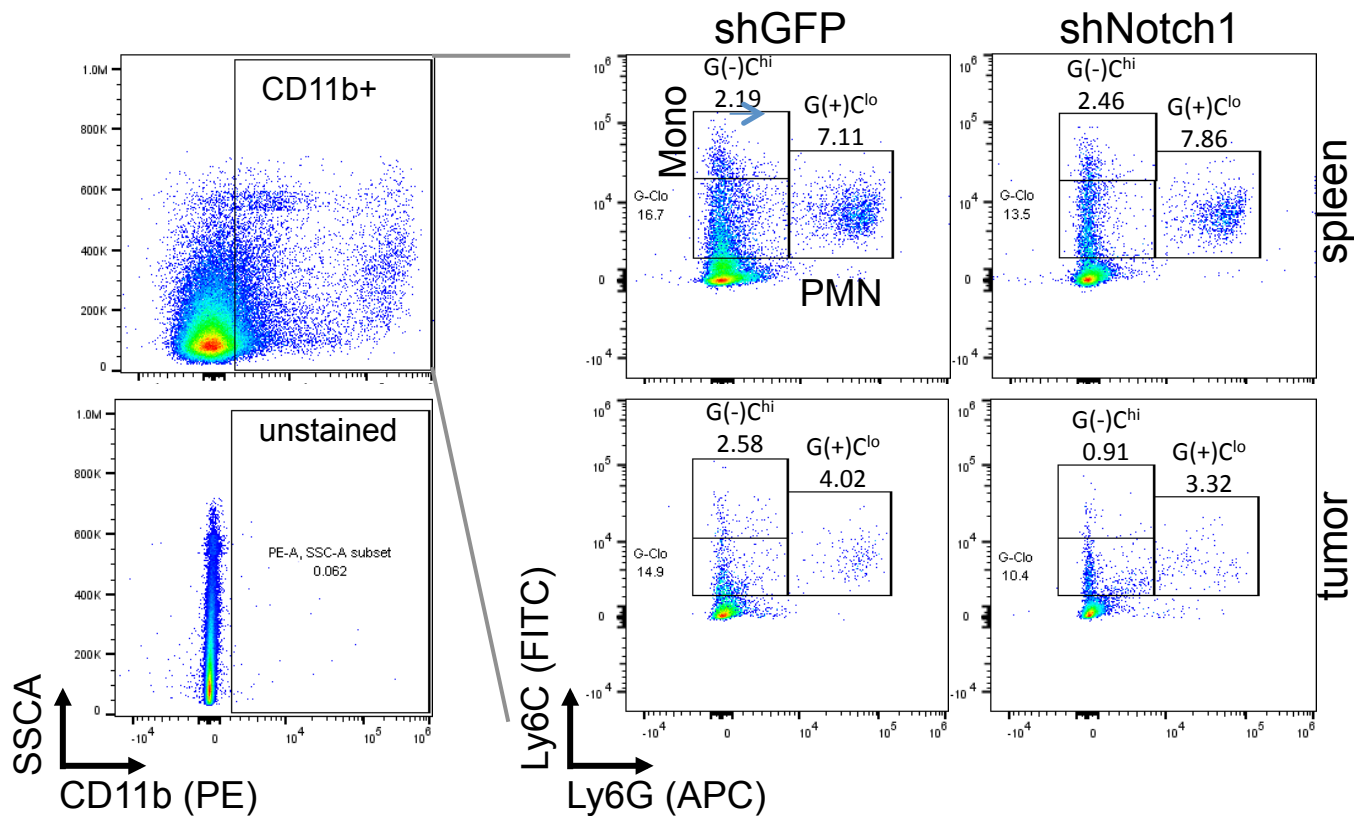| Suppl. Table 1: primer sequences.			
Human		**mouse**	
VAMP3F	GCAGCCAAGTTGAAGAGGAA	il6f	GACAACTTTGGCATTGTGG
VAMP3R	CAGTTTTGAGTTCCGCTGGT	il6r	ATGCAGGGATGATGTTCTG
VAMP2F	GCACCTCCTCCAAATCTTAC	β-actinf	ATGGGTCAGAAGGACTCCTATG
VAMP2R	GAGGATGATGGCGCAAATCAC	β-actinr	ATCTCCTGCTCGAAGTCTAGAG
VAMP8F	TGATCGTGTGCGGAACCTGCA	Arg1f	TTGGGTGGATGCTCACACTG
VAMP8R	GCTCAGATGTGGCTTCCAGATCCT	Arg1r	TTGCCCATGCAGATTCCC
VTI1BF	CATCCTGGTCGGTCTGGTGT	Foxp3f	CCCAGGAAAGACAGCAACCTT
VTI1BR	AGCCCACAGCAGCAACACAT	Foxp3r	TTCTCACAACCAGGCCACTTG
STX4F	GTCTGACGAGGAGCTGGAAC	TGF-b1f	TGACGTCACTGGAGTTGTACGG
STX4R	CCGAGCTCAGGATGTTCTTC	TGF-b1r	GGTTCATGTCATGGATGGTGC
SNAP23F	ATGAGTCTCTGGAAAGTACGAGG	IL-10f	ATTTGAATTCCCTGGGTGAGAAG
SNAP23R	CCACAGCATTTGTTGAGTTCTG	IL-10r	CACAGGGGAGAAATCGATGACA
IL6F2	GGTACATCCTCGACGGCATCT	ccl4f	CCATGAAGCTCTGCGTGTCTG
IL6R2	GTGCCTCTTTGCTGCTTTCAC	ccl4r	GGCTTGGAGCAAAGACTGCTG
IL8F	CTTGGCAGCCTTCCTGATTT	PD1f	CGTCCCTCAGTCAAGAGGAG
IL8R	GGGTGGAAAGGTTTGGAGTATG	PD1r	GTCCCTAGAAGTGCCCAACA
CCL5-F	GCTGTCATCCTCATTGCTACTG	ccl5f	ATATGGCTCGGACACCACTC
CCL5-R	TGGTGTAGAAATACTCCTTGATGTG	ccl5r	TCCTTCGAGTGACAAACACG
		mNotch1f	TCAATGTTCGAGGACCAGATG
	Hong Qiu	mNotch1r	TCACTGTTGCCTGTCTCAAG

Suppl. Figure 1. FACS gating of CD11b+/Gr1+ cells indicating 90% purity. These were the cells sorted for the migration assay.
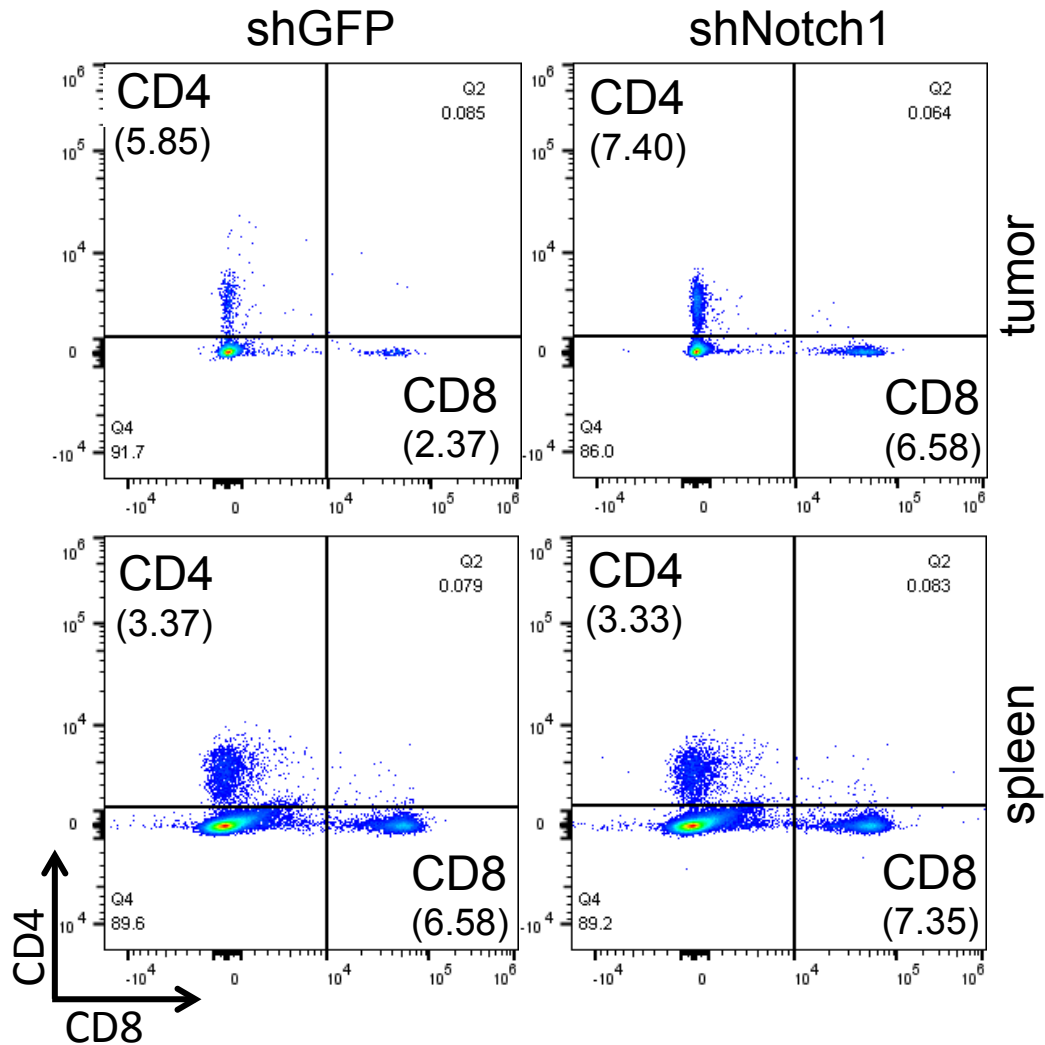
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Suppl. Figure 2. Notch1-NICD expression in B16F10 and Yumm1.1 cells expressing shGFP or shMT1-MMP. DBZ (dibenzazepine, 10uM), a γ-secretase inhibitor, was added as control. LaminB1 and α-tubulin serve as loading control of nuclear and cytosolic fractions, respectively. Notch2 is also shown.
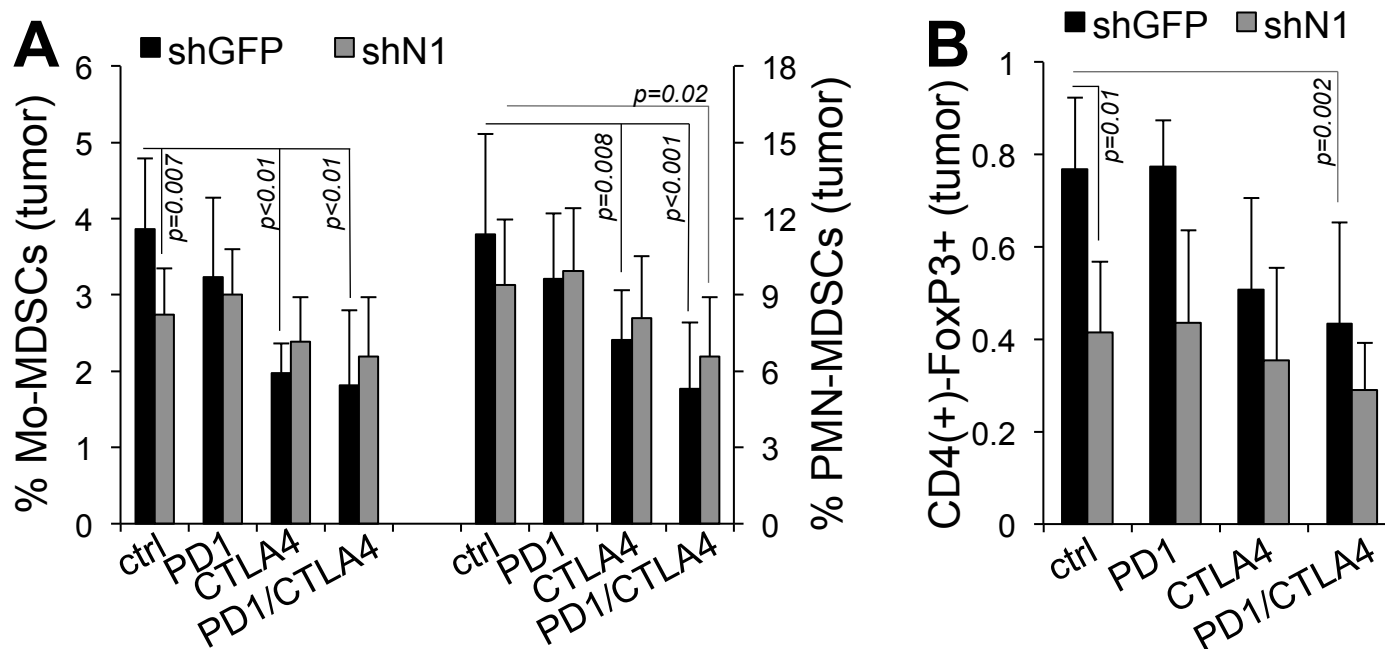
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Suppl. Figure 3. FACS gating of CD11b+ cells further analyzed for the % of Ly6C^{hi}Ly6G- (Monocytic MDSCs) and Ly6C^{lo}Ly6G+ (polymorphonuclear MDSCs).
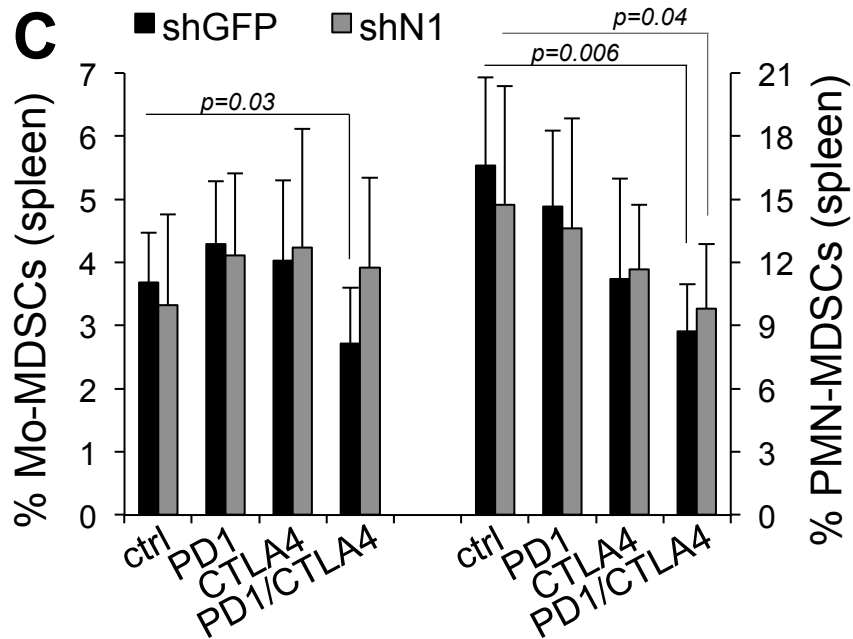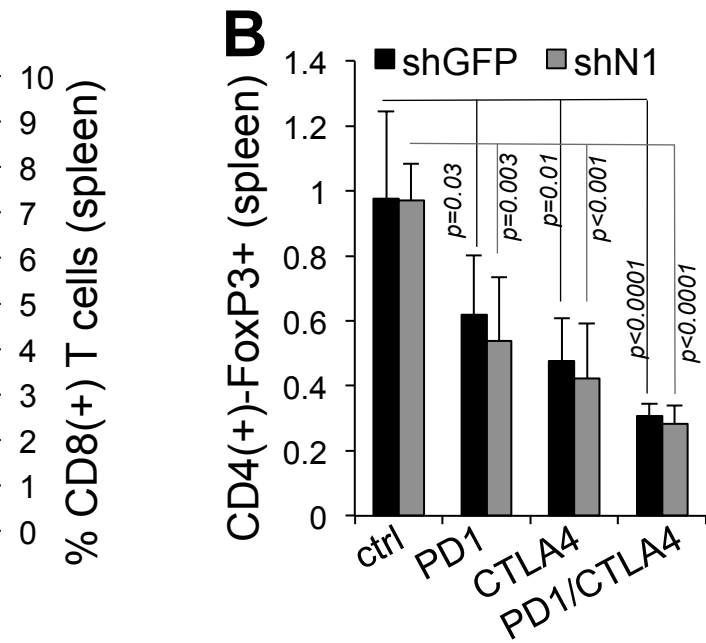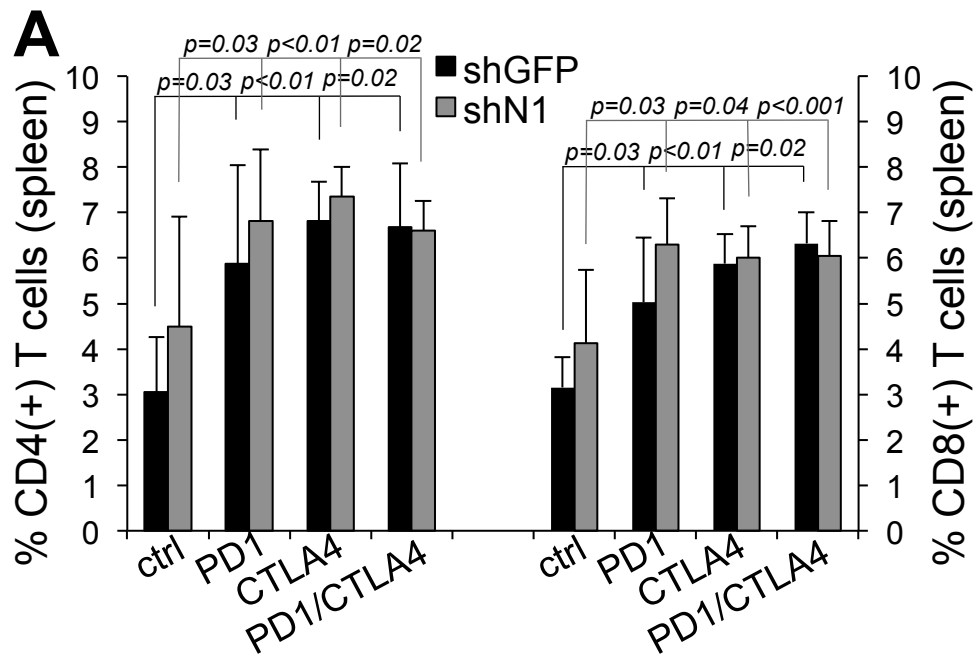
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Suppl. Figure 4: Representative FACS gating for CD4 and CD8 positive cells in tumors and spleens.
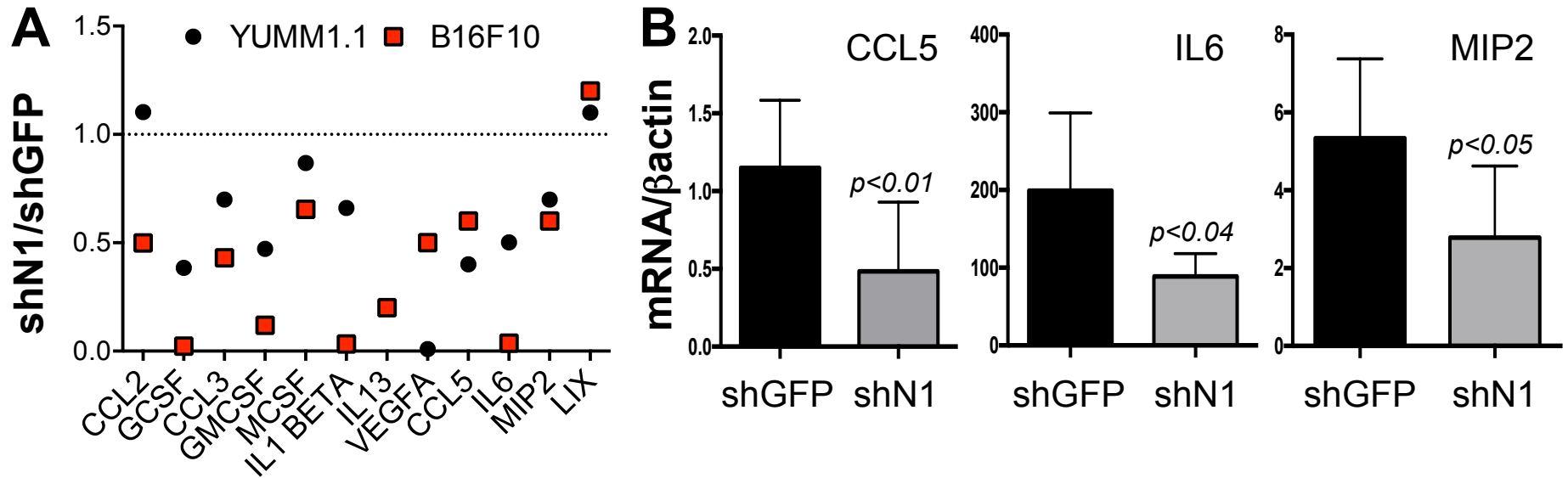
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Suppl. Figure 5: A-B) MDSCs and Tregs in tumors. A) % Monocytic-MDSCs (CD11b+; Ly6C^{hi}; ly6G$^-$); and polymorphonuclear (CD11b+; Ly6C^{lo}; ly6G$^+$) in B16F10 tumors treated with ICIs (n=10). **B)** % Tregs (CD4+/FoxP3+) cells in B16F10 tumors treated as in A. n=10. P values were calculated by the Student's T test.

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Suppl. Fig. 6. Immune cells in spleen after ICI treatment. A) % CD4(+) and CD8(+) T cells in the spleens of mice bearing B16F10 tumors and treated with ICIs. N=10). **B)** % Tregs (CD4+/FoxP3+) cells in the spleens of mice bearing B16F10 tumors treated as in A. n=10. **C)** % Monocytic-MDSCs (CD11b+; Ly6C^{hi}; ly6G$^-$); and polymorphonuclear (CD11b+; Ly6C^{lo}; ly6G$^+$) in the spleens of mice bearing B16F10 tumors and treated with ICIs (n=10). P values were calculated by the Student's T test.

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Suppl. Figure 7: A) Chemokine array (mouse): Protein levels of chemokines associated with MDSCs function, secreted in media of mouse cell lines B16F10 and Yumm1.1, expressing either shGFP or shNotch1. The values are the ratio between shNotch1 and shGFP (set at 1 – dotted line). Serum free media was collected after three days incubation and volumes normalized to adjust for cell number differences, evaluated as the total protein content of cell lysates. **B)** Levels of CCL5, IL6 and MIP2 detected in tumor lysates (n=20). Values are the average of two repeats, each done using 10 tumors per group. P values were calculated by the Student's T test.

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