

## SUPPLEMENTARY MATERIALS

Sex	
Female	35 (51%)
Male	33 (49%)
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Age (median + range)	73 years (40-87)
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Clinical response	
CR	38 (56%)
PR	14 (21%)
SD	4 (6%)
PD	8 (12%)
N/A	4 (6%)
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N stage	
N0	1 (1%)
N1	1 (1%)
N2	1 (1%)
N2c	37 (54%)
N3	28 (41%)
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M stage	
M0	62 (91%)
N/A	6 (9%)
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Perfusion vessel	
Brachial	9 (13%)
Femoral	59 (87%)
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Perfusion chemo	
Melphalan	59 (87%)
Melphalan + TNF- $\alpha$	9 (13%)
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**Table S1.** Patient and tumor characteristics.

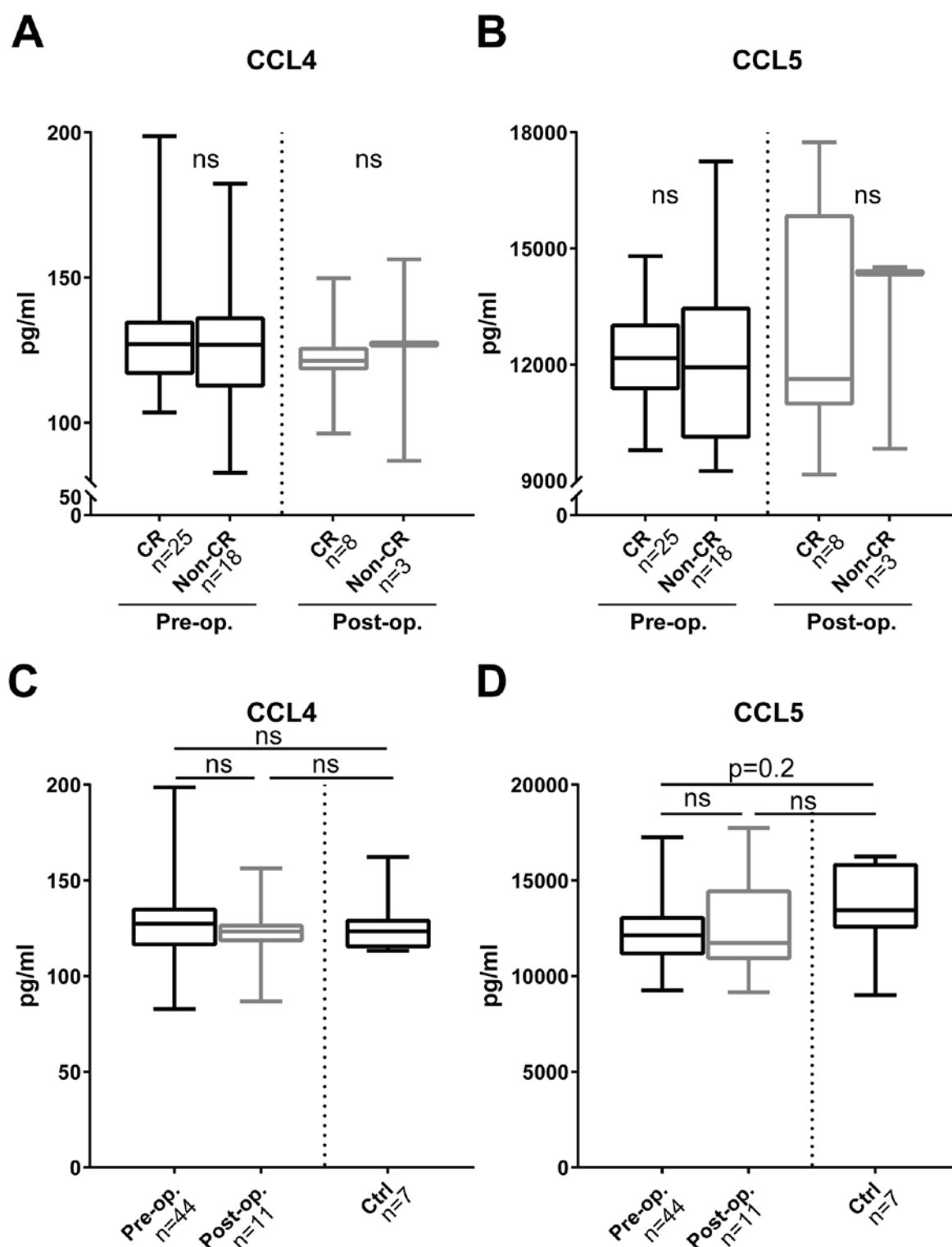
<b>Antigen</b>	<b>Fluorophore</b>	<b>Clone</b>	<b>Vendor</b>	<b>Article number</b>
CCR4	BV421	1G1	BD Biosciences	562579
CCR5	FITC	2D7	BD Biosciences	561747
CD127	Alexa Fluor® 647	HIL-7R-M21	BD Biosciences	558598
CD25	PE	M-A251	BD Biosciences	555432
CD3	BV711	UCHT1	BD Biosciences	563725
CD4	APC-H7	RPA-T4	BD Biosciences	560158
CD8	PerCP-Cy5.5	RPA-T8	BD Biosciences	560662
CD56	BUV737	NCAM16.2	BD Biosciences	564447
CXCR3	BUV395	1C6	BD Biosciences	565223
PD-1	BV421	EH12.1	BD Biosciences	562516

**Table S2.** List of all conjugated antibodies for flow cytometry utilized in the study.

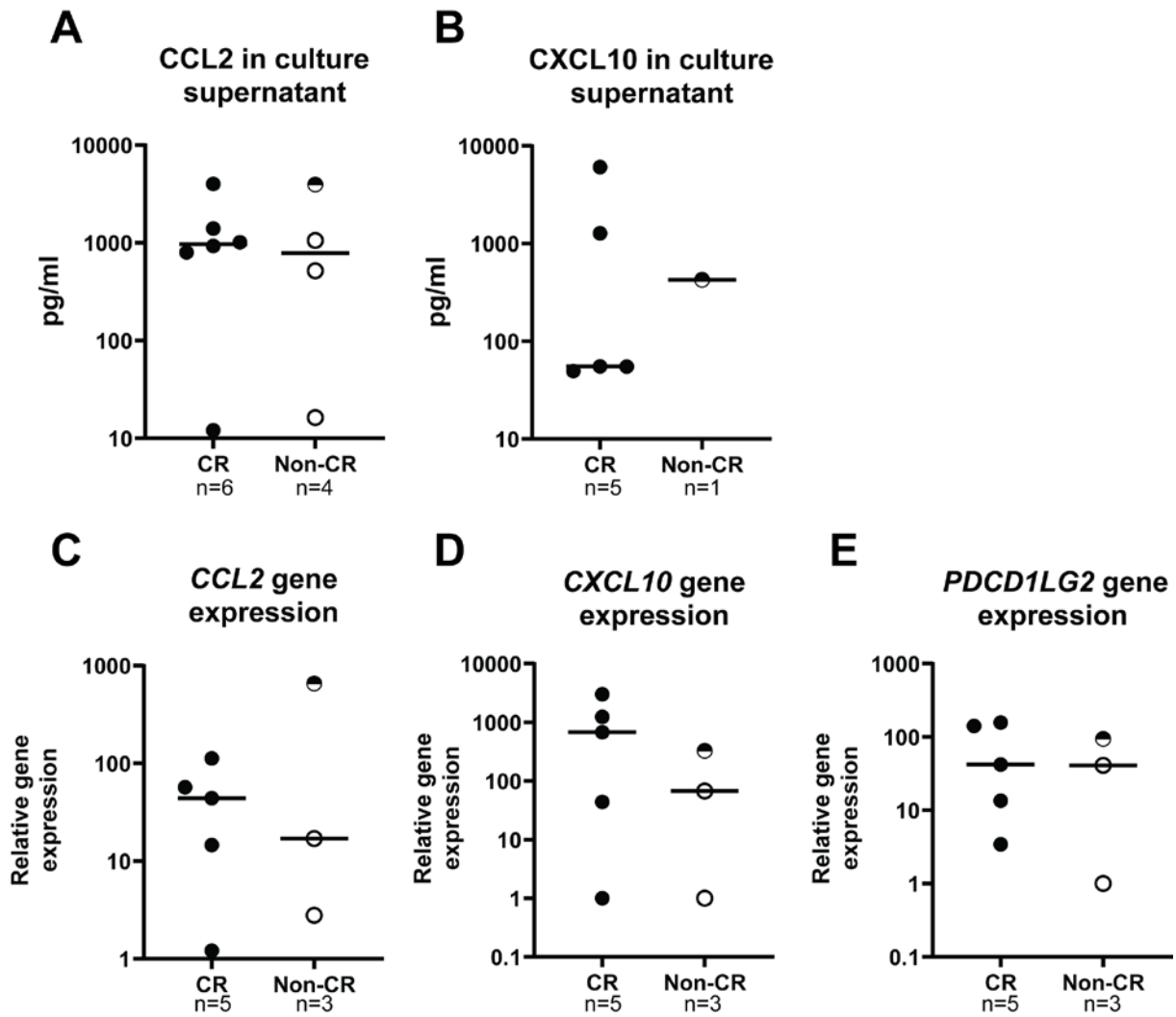
<b>Gene</b>	<b>Forward primer</b>	<b>Reverse primer</b>
<i>CCL2</i>	TCAAAGTGAAGCTCGCACTCT	GGCATTGATTGCATCTGGC
<i>CXCL10</i>	AGCAGTTAGCAAGGAAAGGTCTA	GGGAAGTGATGGGAGAGGCA
<i>GAPDH</i>	CCCACTCCTCCACCTTTGAC	GCCAAATTCGTTGTCATACCAGG
<i>PDCD1LG2</i>	CGTGAGGGAACTTACTTTGGC	GCAGAAGGGGATGAAAATGTGA

**Table S3.** Primers for RT-qPCR.

SUPPLEMENTARY FIGURES

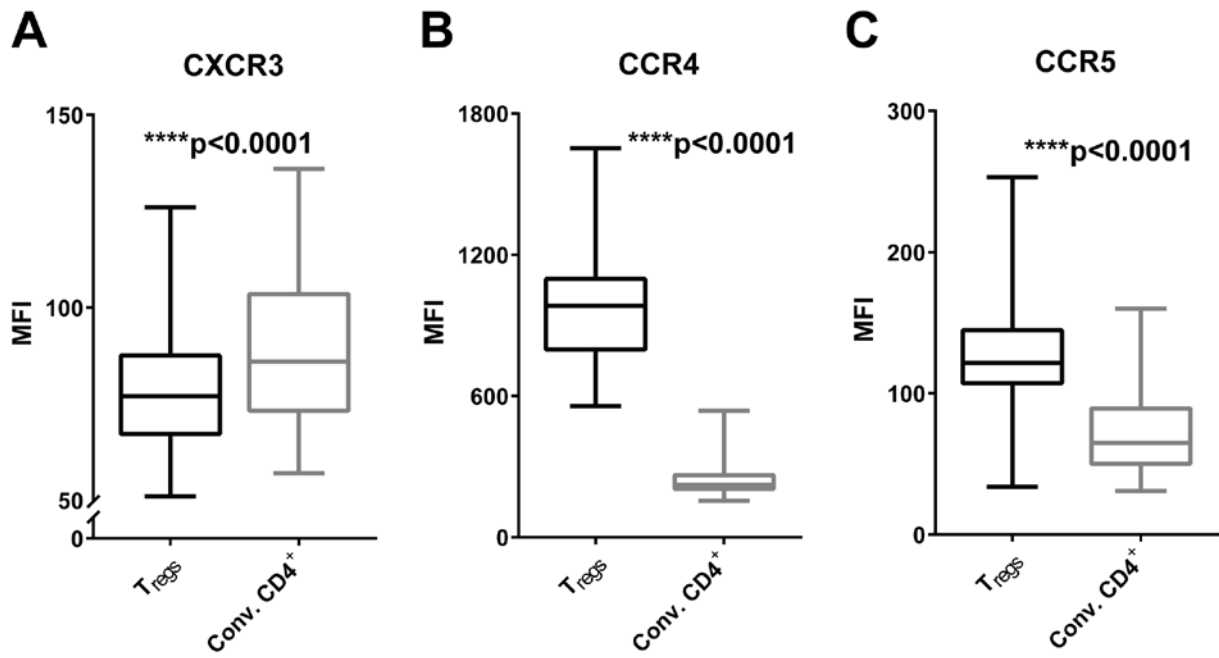


**Figure S1. Serum levels of CCL4 and CCL5 in melanoma patients and healthy controls.** Serum levels of **A, C**) CCL4 and **B, D**) CCL5 in **A-B**) melanoma patients who achieved and did not achieve CR (non-paired Mann-Whitney test), and **C-D**) between melanoma patients and healthy controls (paired Wilcoxon test between pre-op. and post-op., non-paired Kruskal-Wallis test followed by Dunn's multiple comparison test for ctrl vs. pre-op. and vs. post-op). Pre-op. indicates blood sample obtained before ILP, post-op. indicates samples obtained one month after ILP. Data are presented in box-and-whiskers plots with min. and max.

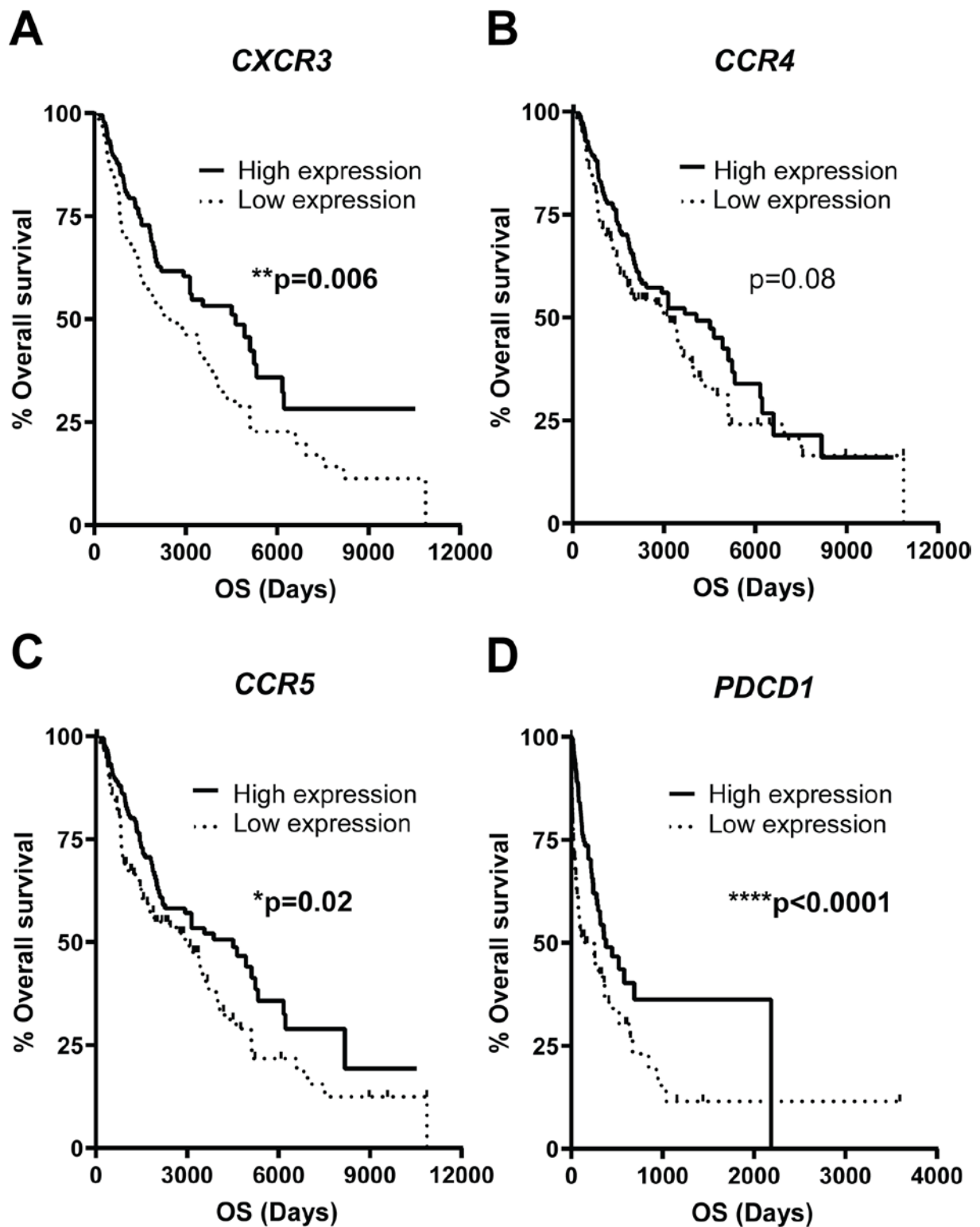


**Figure S2. Correlation between treatment response and intratumoral levels of ISGs and ISG products.**

Levels of **A) CCL2** and **B) CXCL10** in tumor biopsy culture supernatants from ILP patients stratified into CR and Non-CR. Biopsies were obtained during the operational procedure. mRNA was extracted from the same biopsy cultures, and subsequent RT-qPCR analyzes were made with primers for **C) CCL2**, **D) CXCL10** and **E) PDCD1LG2**. RT-qPCR data were normalized to the reference gene *GAPDH*, converted to relative units and stratified into CR or Non-CR. Among the Non-CR patients one patient was a partial responder (PR) and is represented by a half-filled circle.

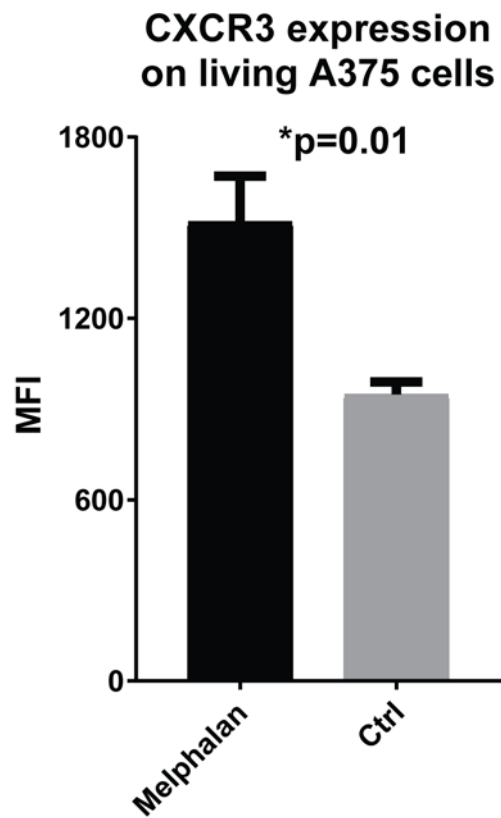


**Figure S3.  $T_{reg}s$  and conventional  $CD4^+$  T cells express different levels of chemokine receptors.** The expression of A) CXCR3, B) CCR4 and C) CCR5 on  $T_{reg}s$  and conventional  $CD4^+$  T cells in peripheral blood from melanoma patients before ILP (n=54; paired Wilcoxon test). MFI = Median Fluorescence Intensity. Data are presented in box-and-whiskers plots with min. and max.



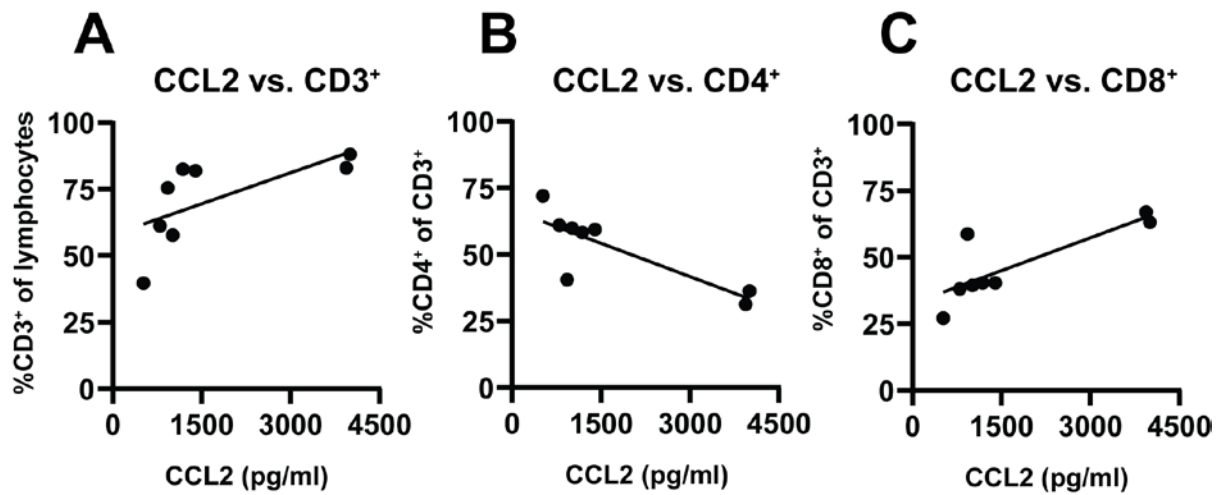
**Figure S4. High mRNA expression of receptors to ISG products correlates with prolonged overall survival in advanced melanoma.**

Melanoma patients in the TCGA database were dichotomized into two groups based on above or below median gene expression of the receptors **A) *CXCR3***, **B) *CCR4***, **C) *CCR5*** and **D) *PDCD1*** (n=470; Log-rank test).



**Figure S5. Melanoma cells exposed to melphalan upregulates CXCR3.**

The expression of CXCR3 on melphalan-exposed and non-exposed melanoma cells (n=5; paired t-test). MFI = Median Fluorescence Intensity. Data are presented as mean with SEM.



**D**

**Intratumoral levels of CCL2 and percent TILs**

		%CD3 <sup>+</sup> of lymphocytes	%CD4 <sup>+</sup> of CD3 <sup>+</sup>	%CD8 <sup>+</sup> of CD3 <sup>+</sup>
<b>CCL2 vs.</b>	Spearman r	0.91	-0.81	0.83
	P value	**0.0046	*0.022	*0.016

**Figure S6. High levels of CCL2 in tumors correlate with infiltration of T cells.**

Tumor biopsies were obtained during ILP and processed into single cell suspensions. TILs were obtained and analyzed with FACS, and supernatants were collected after 48 hours of culture of the cell suspensions and analyzed for the presence of CCL2. The level of CCL2 is depicted against the percentage of **D**) CD3<sup>+</sup> T cells among lymphocytes, **E**) CD4<sup>+</sup> and **F**) CD8<sup>+</sup> T cells among CD3<sup>+</sup> T cells, with the lines adapted from linear regression. **G**) The level of CCL2 was correlated against the T cell populations utilizing Spearman correlation. (n=8)