Line	ICOSL expression
A684	High
WM35	High
WM35P1N1	High
WM35P2N1	High
888	High
A682	High
A687	Low
938	Low
2084	Low
Memo	Low
WM793	Negative
WM793 P1N1	Negative
WM793 P2N1	Negative
624	Negative
A681	Negative
526	Negative
2088	Negative
2089	Negative
1007	Negative
A375	Negative
A375-S2	Negative
Skmel	Negative

Supplementary Table 1. Melanoma cell lines expression of ICOSL. Melanoma cell lines were harvested with protease free dissociation buffer and label with antibodies against ICOSL or isotype label IgG. Cells were analyzed by flowcytometry. Expression is evaluated as the shift of the cell population above the isotype control. **High** >50% cells shift, **Low**< 50% cells shift and **Negative**: no shift

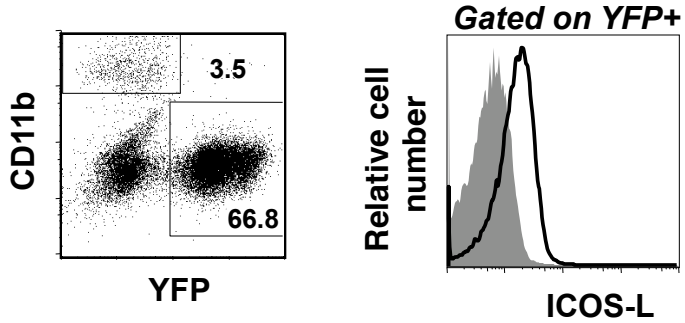

Figure S1. Mouse B16/F10 melanoma cells express ICOS-L. C57BL/6 mice were injected s.c with $2x10^5$ B16/F10-YFP melanoma cells which express yellow fluorescent protein (1) . Tumors were removed by excision after reaching 2 cm^2 and cell suspensions from the total tumors were stained with anti-CD11b and anti-ICOSL or isotype antibody. For the flow cytometry analysis, melanoma cells were identified by size and YFP expression and were gated independently of CD11b+ cells (left dot plot). Expression of ICOS-L (white histogram) and isotype control (grey histogram) is shown.

1. Overwijk WW, de Visser KE, Tirion FH, et al. Immunological and antitumor effects of IL-23 as a cancer vaccine adjuvant. J Immunol. 2006;176:5213-5222.

Supplementary Figure 1

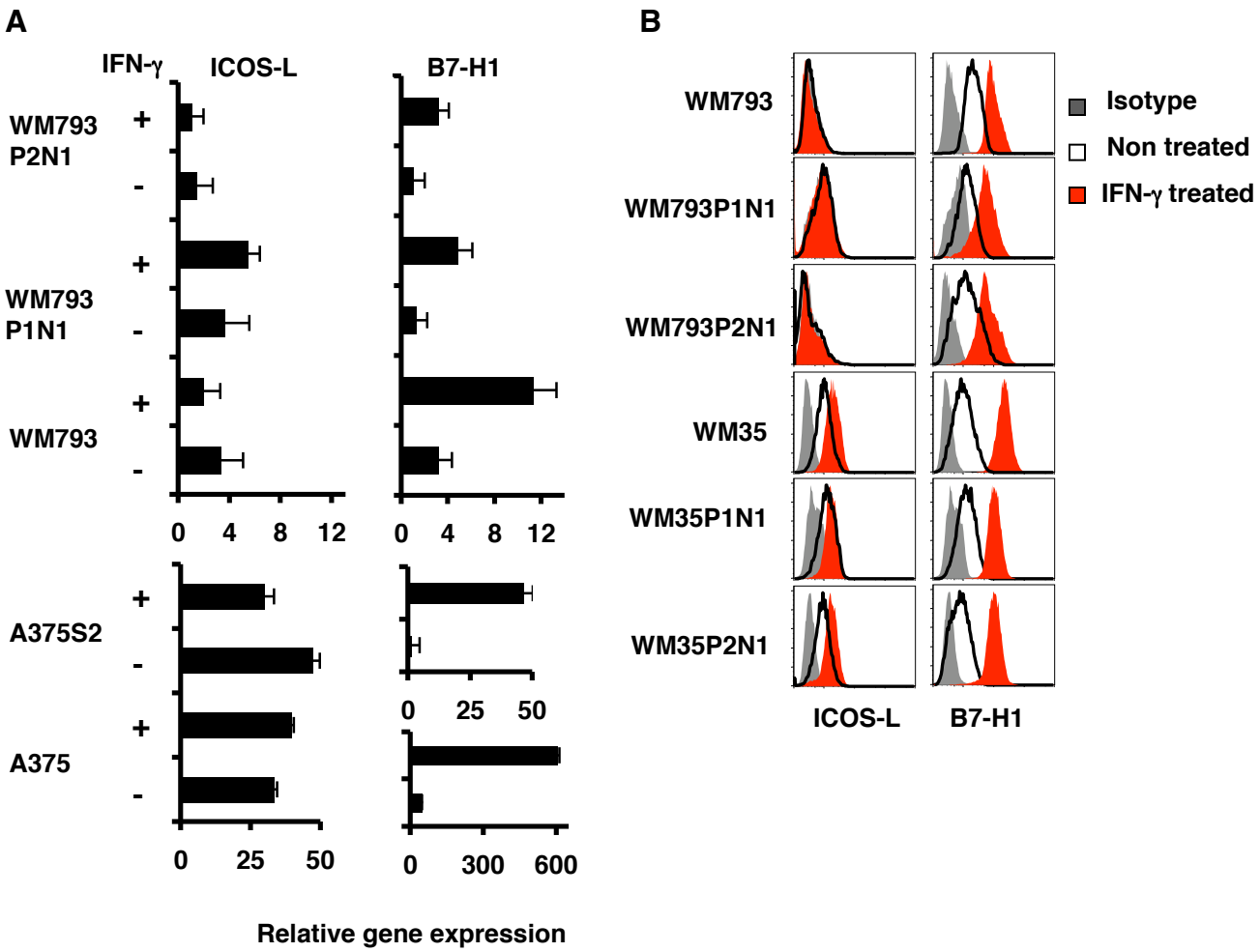

Figure S2. IFN-γ does not influence ICOS-L expression but induces B7-H1 expression in melanoma cell lines. **A-B.** Cells were grown with 100 U/ml of IFN-γ for 18 hours and cells were divided for mRNA extraction and flow cytometry analysis of ICOS-L or B7-H1 expression. **A.** RT- PCR was performed for the ICOS-L, B7-H1, and β-actin genes. Data was normalized to the β-actin gene (Mean ± SD). **B.** ICOS-L and B7-H1 cell surface protein expression in melanoma lines treated with IFN-γ. Red histograms indicate IFN-γ treated cells, white histograms indicate non-treated cell lines, and shaded histograms indicate isotype control Ab staining (rat IgG1).

Supplementary figure 2

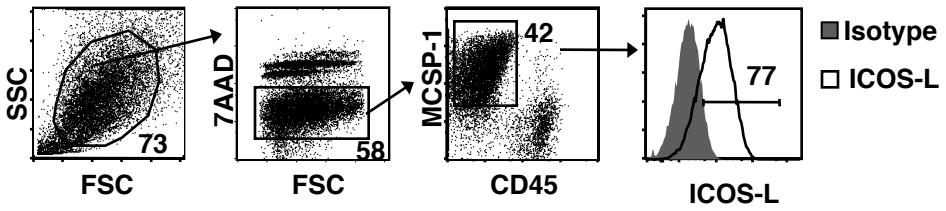

Figure S3. ICOS-L protein expression in fresh melanoma tumors. Strategy to detect ICOS-L expression on melanoma cells in tumor cell suspensions from fresh surgical samples. Cell suspensions from fresh tumor biopsies, were stained with mAbs against MCSP-1, ICOS-L, B7-H1 and HLA class II. 7-AAD was added before flow cytometry analysis. Melanoma cells were distinguished by MCSP-1-positive staining. First tumor cells were gated by size, then dead cells were excluded with 7-AAD. Finally we selected the MCSP-1$^+$ cells negative for CD45 (leukocytes) and determined the levels of ICOS-L, B7-H1, and HLA class II expression. Positive staining is indicated in the white histograms and shaded histograms indicate staining with the isotype control Ab.

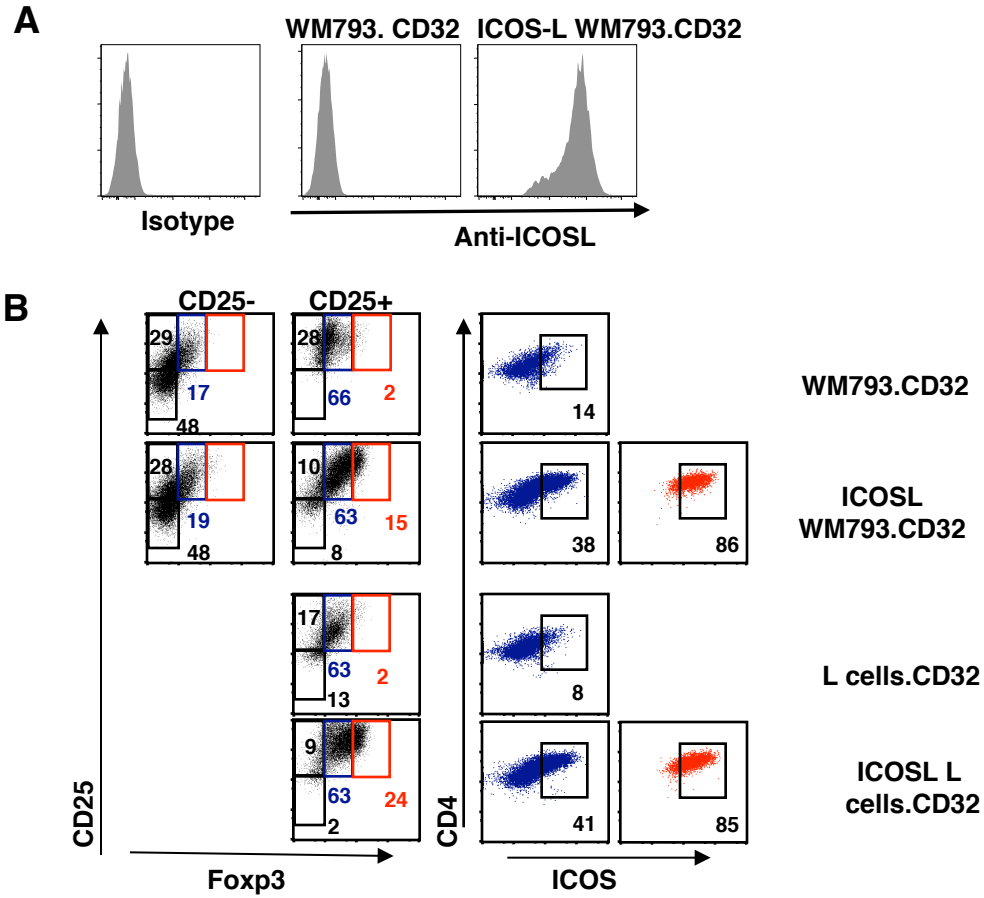

Figure S4.A. Transfection of ICOSL into WM793 melanoma line. WM793 melanoma cell line expressing human ICOS-L was generated by transfection with pcDNA3.1-ICOS-L in FuGENE (Invitrogen, Calrsbad, CA) and growth in culture medium containing G418. WM793-ICOS-L-transduced cells were sorted and the ICOS-L^{+} cells maintained by routine subculturing in G418-containing medium. Shown is the FACS analysis of ICOS-L on WM793 before (WM793.CD32) and after transfection (ICOSL WM793.CD32).**B. ICOS-L costimulation promotes high Foxp3 and CD25 expression on Tregs.** Sorted CD4^{+}CD25^{-} (CD25-) and CD4^{+}CD25^{-} (CD25+) cells were cultured with irradiated melanoma cell lines or L cells and anti-CD3 (OKT3). On day 5 the CD4^{+} cells were analyzed for the expression of Foxp3, CD25 and ICOS. Foxp3 expression on sorted CD25^{-} and CD25^{+} cells are shown in the left panels. ICOS expression on Foxp3^{+} (blue gate) and Foxp3^{hi} cells (red gate) on CD25^{+} cells are shown in right panel. The cell lines used in these co-cultures all expressed CD32.

Supplementary figure 4

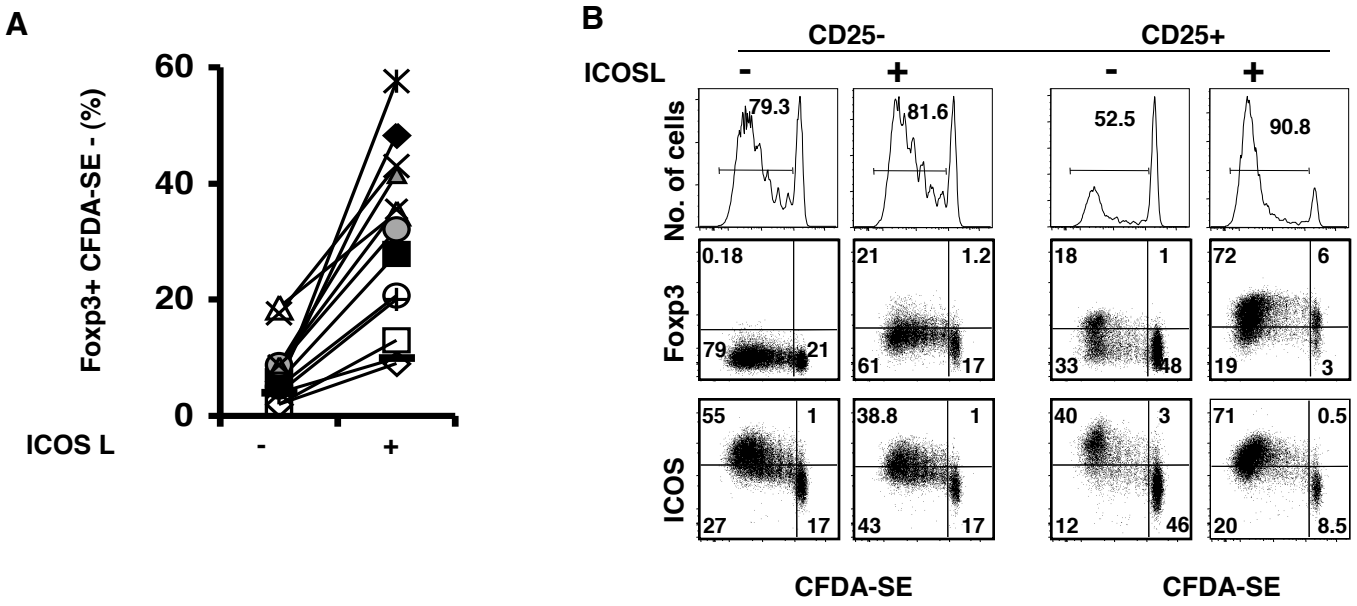

Figure S5. ICOS costimulation promotes the proliferation of Foxp3^{hi+}CD25^{+} ICOS^{+} T-cells. **A-B**. Sorted CD4^{+}CD25^{-} and CD4^{+}CD25^{-} cells were labeled with CFDA-SE and activated with anti-CD3 over a monolayer of irradiated melanoma lines, or over control L cells or ICOS-L- L cells. After 5 days, the expression of Foxp3, CD25, ICOS and CFSE dilution was analyzed. **A.** Percentage of CD25^{+} cells expressing high levels of Foxp3 and CFDA-SE ^{low} from different donors (n=12) cultured with ICOS^{+} or ICOSL^{-}melanomas. **C.** Co-cultures with L cells or ICOSL-L cells. Histograms show CFDA-SE dilution. Plots of Foxp3 and ICOS are shown.