Figure S1

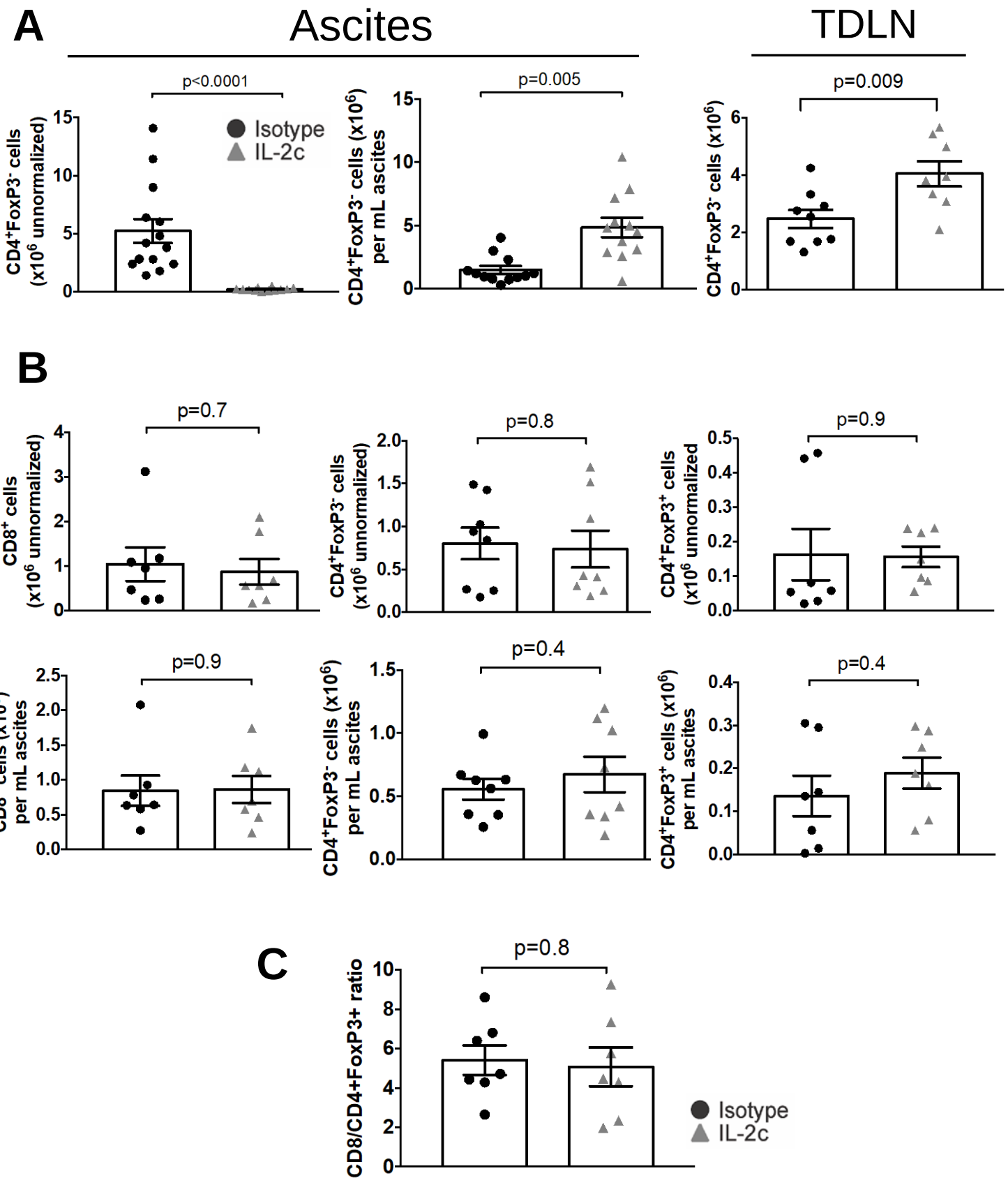
A
Ascites
TDLN
p<0.0001
p=0.005
p=0.009
Isotype
IL-2c
CD4+FoxP3- cells (x10^6 unnormalized)
CD4+FoxP3- cells (x10^6) per mL ascites
CD4+FoxP3- cells (x10^6)
B
p=0.7
p=0.8
p=0.9
p=0.9
p=0.4
p=0.4
CD8+ cells (x10^6 unnormalized)
CD4+FoxP3- cells (x10^6 unnormalized)
CD4+FoxP3+ cells (x10^6 unnormalized
CD8+ cells (x10^6) per mL ascites
CD4+FoxP3- cells (x10^6) per mL ascites
CD4+FoxP3+ cells (x10^6) per mL ascites
C
p=0.8
CD8/CD4+FoxP3+ ratio
Isotype
IL-2c

Figure S1. IL-2c increases conventional CD4 T cell in ascites three weeks after final IL-2c, but does not affect ascites Treg and effector T cell numbers at one week after final IL-2c administration. **A.** Absolute numbers of $CD4^+$ $FoxP3^+$ Tregs in ascites either raw (left) or normalized to ascites volume (middle) and in TDLN (right) isolated three weeks after final IL-2c dose**. B.** Numbers of $CD8^+$ (left), $CD4^+FoxP3^-$ (middle) and $CD4^+FOXP3^+$ (right) Tregs either raw (top) or normalized to ascites volume (bottom) isolated one weeks after final IL-2c dose. **C.** $CD8^+/CD4^+FoxP3^+$ ratios were calculated one weeks after final IL-2c dose.

Figure S2

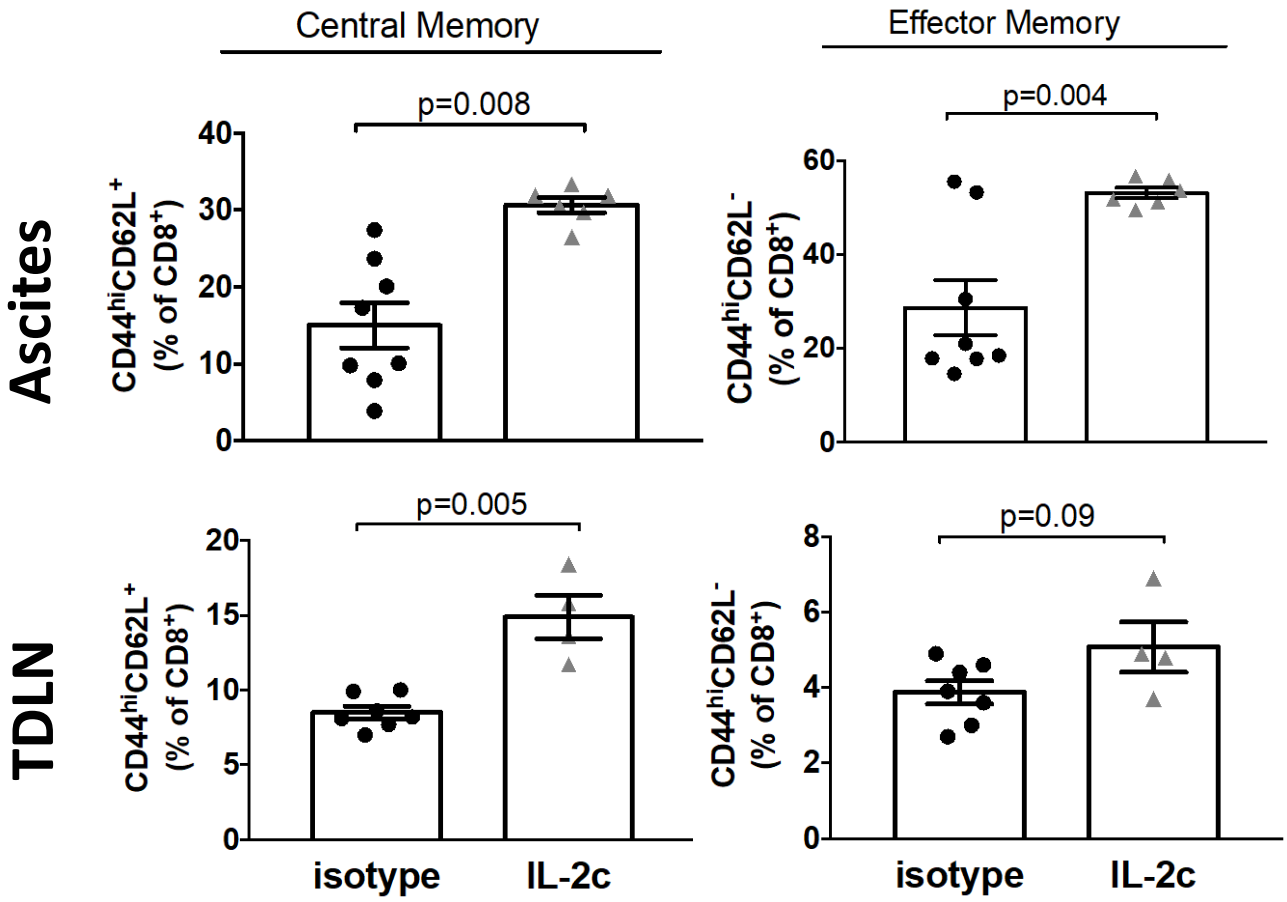

Figure S2. IL-2c treatment increases CD8⁺ central and effector memory T cells in the ascites, and central memory T cells in TDLN. Mice were challenged with ID8agg-Luc, ± IL-2c and sacrificed three weeks after final IL-2c dose. $CD44^{hi}CD62L^{+}$ (left) and $CD44^{hi}$ $CD62L^{-}$ (right) cells in ascites (top) and TDLN (bottom) were determined by flow cytometry.

Figure S3

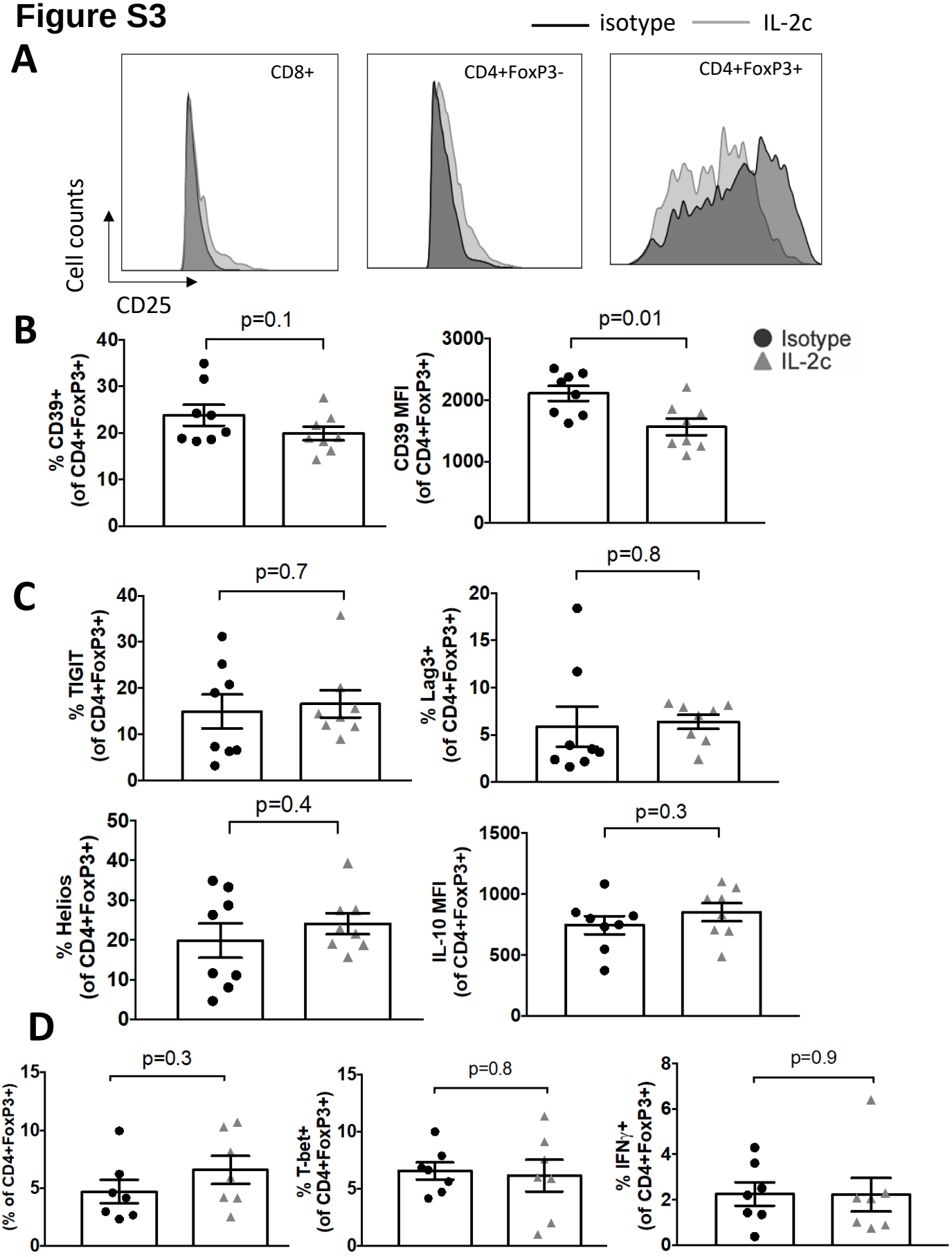
isotype
IL-2c
A
CD8+
CD4+FoxP3-
CD4+FoxP3+
Cell counts
CD25
B
p=0.1
% CD39+ (of CD4+FoxP3+)
p=0.01
CD39 MFI (of CD4+FoxP3+)
Isotype
IL-2c
C
p=0.7
% TIGIT (of CD4+FoxP3+)
p=0.8
% Lag3+ (of CD4+FoxP3+)
p=0.4
% Helios (of CD4+FoxP3+)
p=0.3
IL-10 MFI (of CD4+FoxP3+)
D
p=0.3
PD-1+ (% of CD4+FoxP3+)
p=0.8
% T-bet+ (of CD4+FoxP3+)
p=0.9
% IFNγ+ (of CD4+FoxP3+)

Fig S3. Additional Treg phenotype in ascites and TDLN after IL-2c treatment. A. Histogram overlay of CD25 expression in CD8+ (left), CD4+FoxP3- (middle) and CD4+FoxP3+ (right) in ascites plotted from Fig 3B. **B.** Summary graph of CD39 prevalence (left) and MFI (right) in ascites CD4+FoxP3+ cells. **C**. Summary graph of TIGIT, Lag3, Helios prevalence and IL-10 MFI in ascites CD4+FoxP3+ cells. **D**. Summary graph of PD-1, T-bet and IFN**γ** prevalence in TDLN CD4+FoxP3+ cells. P value determined by unpaired Student *t*-test.

Figure S4

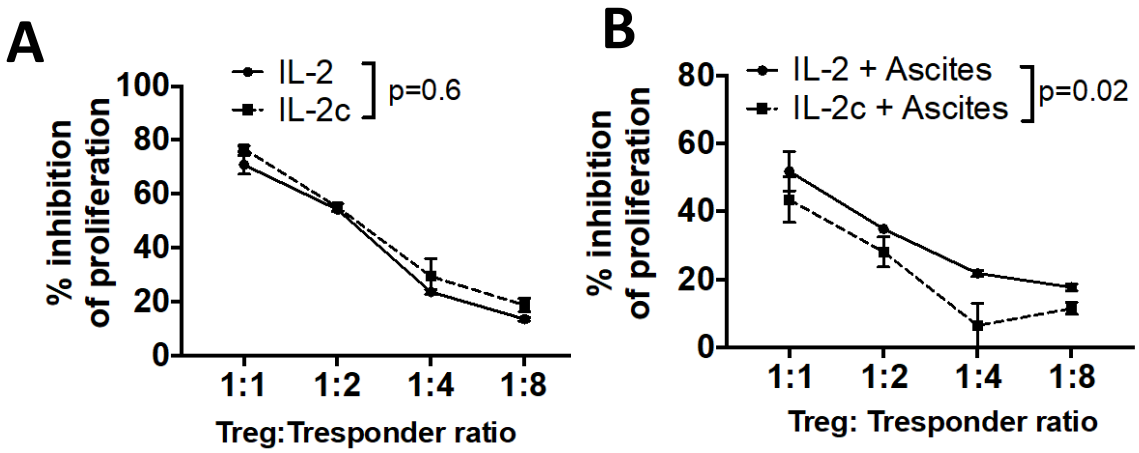

Fig S4. IL-2c directly inhibits Treg suppression specifically in the tumor microenvironment. $CD4^{+}FoxP3^{+}$ Tregs were sorted from naïve FIR mice and cultured *ex vivo* without (**A**) or with (**B**) 50% ascites in complete medium for 5 days followed by suppression assay. P value determined by two-way ANOVA.

Figure S5

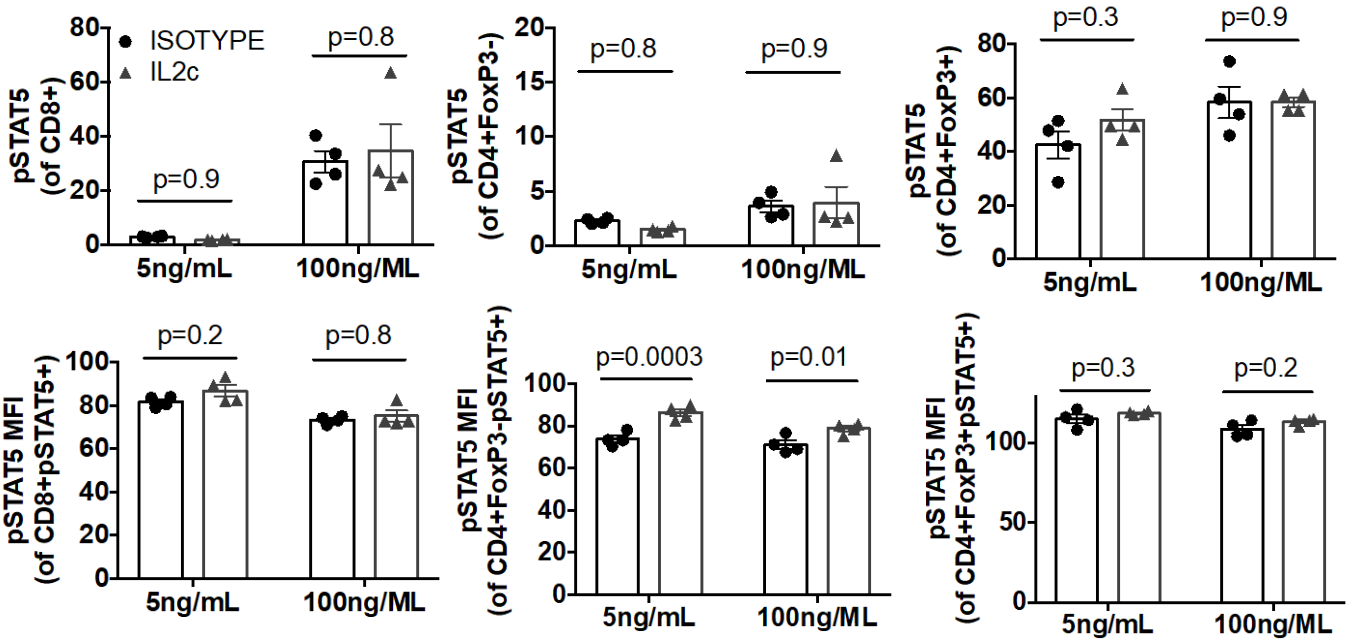

Fig S5. IL-2 signaling is not impaired in TDLN after IL-2c treatment. WT challenged with ID8agg were treated with isotype control or IL-2c. CD3+ T cells were sorted from TDLN 3 weeks after last IL-2c dose, stimulated with 5ng/mL or 100ng/mL mouse IL-2 for 30 minutes and assessed by flow cytometry.

Figure S6

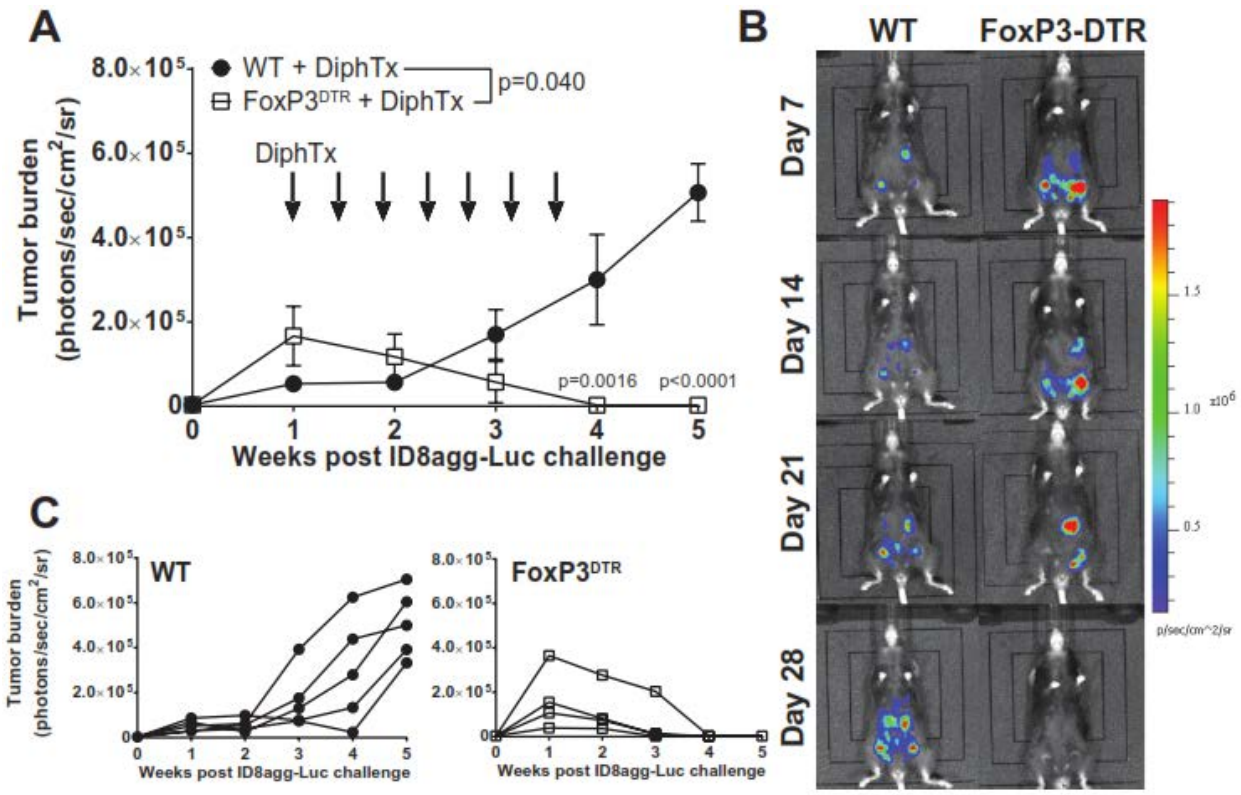

Figure S6. Specific Treg depletion is highly effective against ID8agg. A. ID8agg-Luc tumor burden after administration of diphtheria toxin (DiphTx) as indicated (arrows). **B.** Representative bioluminescent images from **A**. **C.** Individual tumor growth curves from **A**. P values determined by two-way ANOVA.

Figure S7

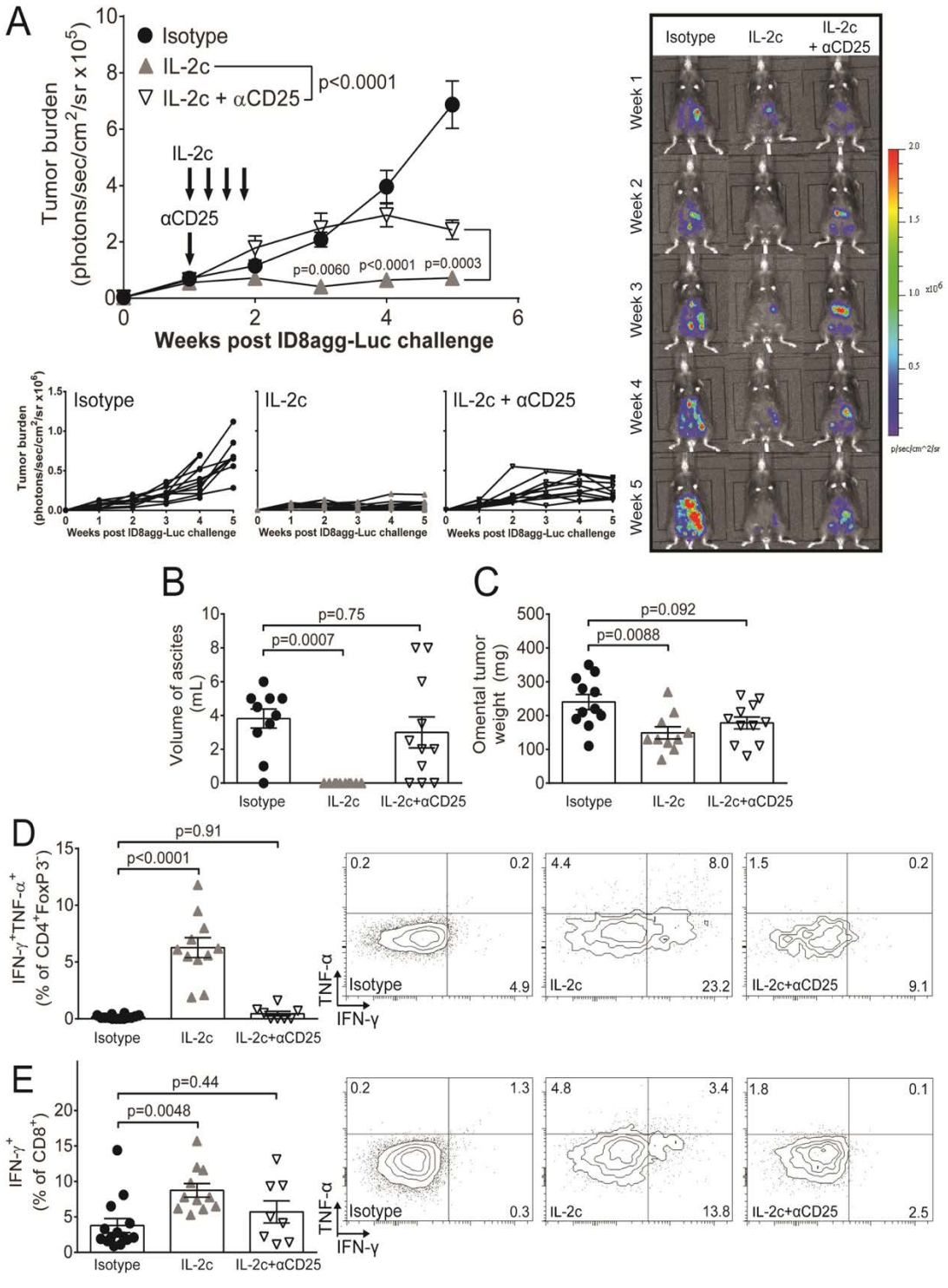
A
Isotype
IL-2c
IL-2c + αCD25
p<0.0001
Tumor burden (photons/sec/cm2/sr x 10^5)
IL-2c
αCD25
p=0.0060
p<0.0001
p=0.0003
Weeks post ID8agg-Luc challenge
Isotype
IL-2c
IL-2c + αCD25
Tumor burden (photons/sec/cm2/sr x10^6)
Weeks post ID8agg-Luc challenge
Week 1
Week 2
Week 3
Week 4
Week 5
B
Volume of ascites (mL)
p=0.75
p=0.0007
Isotype
IL-2c
IL-2c+αCD25
C
Omental tumor weight (mg)
p=0.092
p=0.0088
Isotype
IL-2c
IL-2c+αCD25
D
IFN-γ+TNF-α+ (% of CD4+FoxP3-)
p=0.91
p<0.0001
Isotype
IL-2c
IL-2c+αCD25
0.2
0.2
Isotype
4.9
4.4
8.0
IL-2c
23.2
1.5
0.2
IL-2c+αCD25
9.1
TNF-α
IFN-γ
E
IFN-γ+ (% of CD8+)
p=0.44
p=0.0048
Isotype
IL-2c
IL-2c+αCD25
0.2
1.3
Isotype
0.3
4.8
3.4
IL-2c
13.8
1.8
0.1
IL-2c+αCD25
2.5
TNF-α
IFN-γ

Figure S7. αCD25 attenuates IL-2c-induced tumor rejection and T cell function in ID8agg OC. A. Tumor burden by bioluminescence after challenge with ID8agg-Luc and IL-2c treatment as indicated (black arrows) (top left), with representative images of ID8agg-Luc-bearing mice ± IL-2c or αCD25 (right) and individual tumor growth curves (bottom). **B.** Omental tumor weight 3 weeks after the last IL-2c. **C.** Ascites volume 3 weeks after the last IL-2c. **D.** Summary graph of ascites $CD45^+CD3^+CD4^+FoxP3^-$ IFN-γ^+TNF-α^+ T cell frequency 3 weeks after IL-2c (left) with representative flow plots (right). **E.** Summary graph of ascites $CD45^+CD3^+CD8^+$ IFN-γ^+TNF-α^+ T cell frequency 3 weeks after the last IL-2c (left) with representative flow plots (right). All *p* values from one-way ANOVA with *post hoc* Sidak's test, except **A** (two-way repeated measures ANOVA (figure legend) with *post hoc* Sidak's test (graph points)). N=10 for isotype, 10 for IL-2c, 8-13 for IL-2c + αCD25 for all panels. All data are from two pooled independent experiments. The datasets for control and IL-2c-treated mice used here are also used in **Fig 1**.

Figure S8

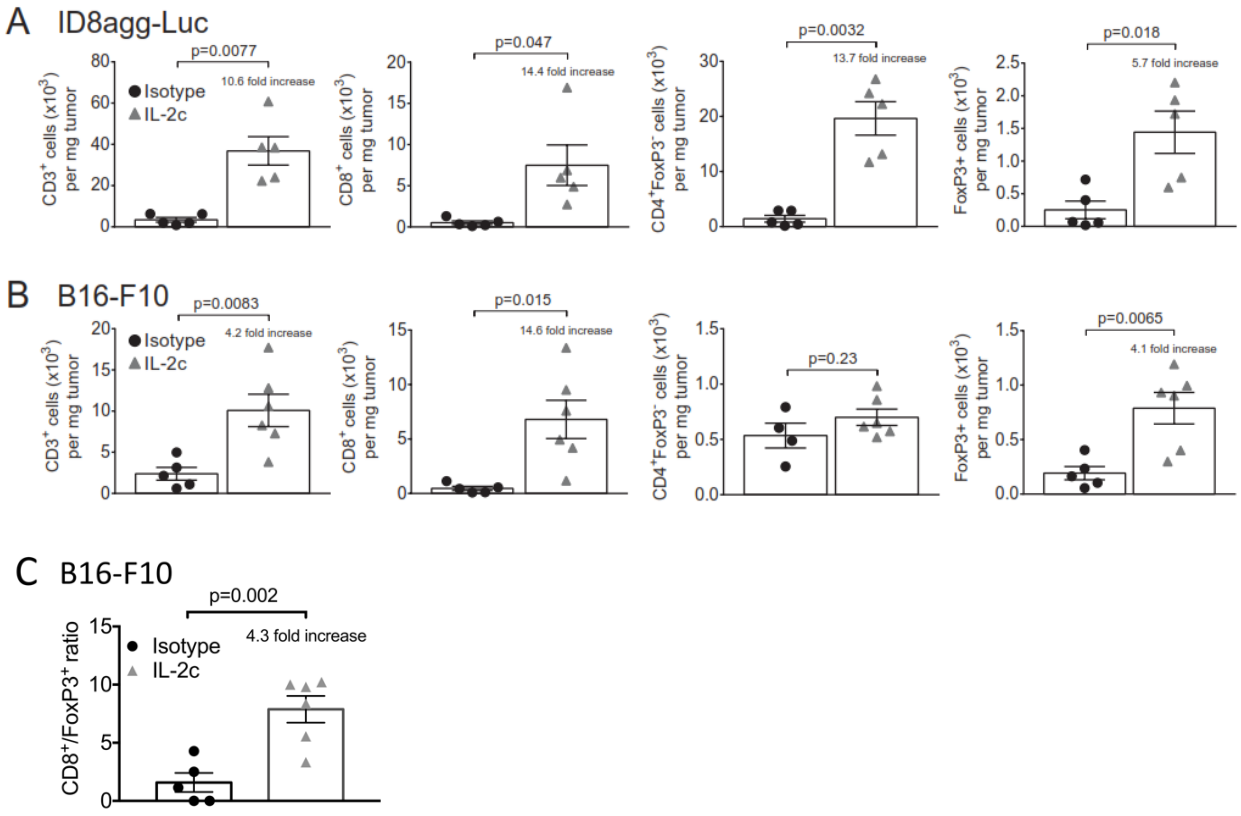

Figure S8. IL-2c promotes intratumoral T cell infiltration. A. Absolute numbers of ID8agg intratumoral T cell subsets normalized to tumor weight 12 days after the final IL-2c dose. **B.** Absolute numbers of B16-F10 intratumoral T cell subsets normalized to tumor 1 day after the final IL-2c dose. Data from one experiment representative of many with similar results. N=4-5/group. **C.** $CD8^+/Treg^+$ ratio of B16-F10 tumors 1 day after the final IL-2c dose.

Figure S9

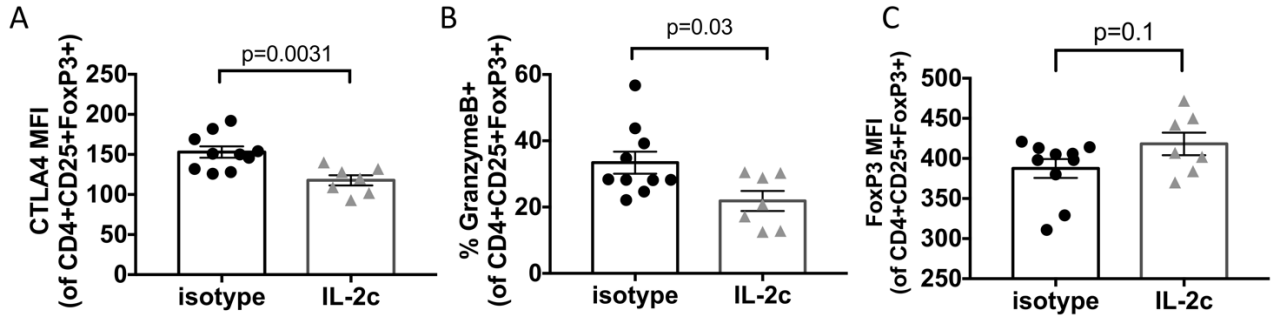

Figure S9. IL-2c reduces intratumoral Treg activation and functional molecules in B16 melanoma. CTLA-4 (**A**) and Granzyme B (**B)** and FoxP3 (**C**) expression in intratumoral $CD45^+CD3^+CD4^+CD25^+Foxp3^+$ Tregs in B16 tumors by flow cytometry after IL-2c treatment one day after final IL-2c treatment. N=7-10 per group.

Figure S10

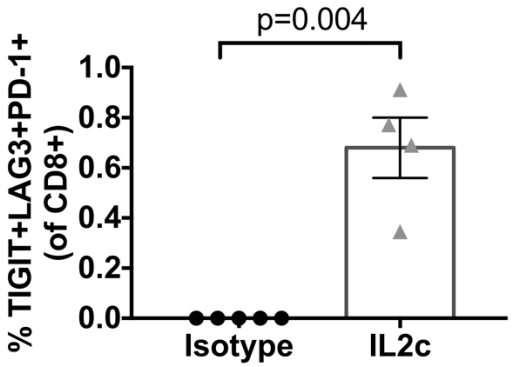

Figure S10. IL-2c increases TIGIT$^+$LAG-3$^+$PD-1$^+$ CD8$^+$ T cells in B16 tumors. TIGIT$^+$LAG-3$^+$PD-1$^+$ prevalence among CD45$^+$CD3$^+$CD8$^+$ T cells in B16 tumors one day after last IL-2c dose by flow cytometry. N=4-5 per group.