

Supplementary Information for

Antibody-mediated blockade of the IL-23 receptor destabilizes intratumoral regulatory T cells and enhances immunotherapy.

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Supplementary Figures and Legends to S1 to S5

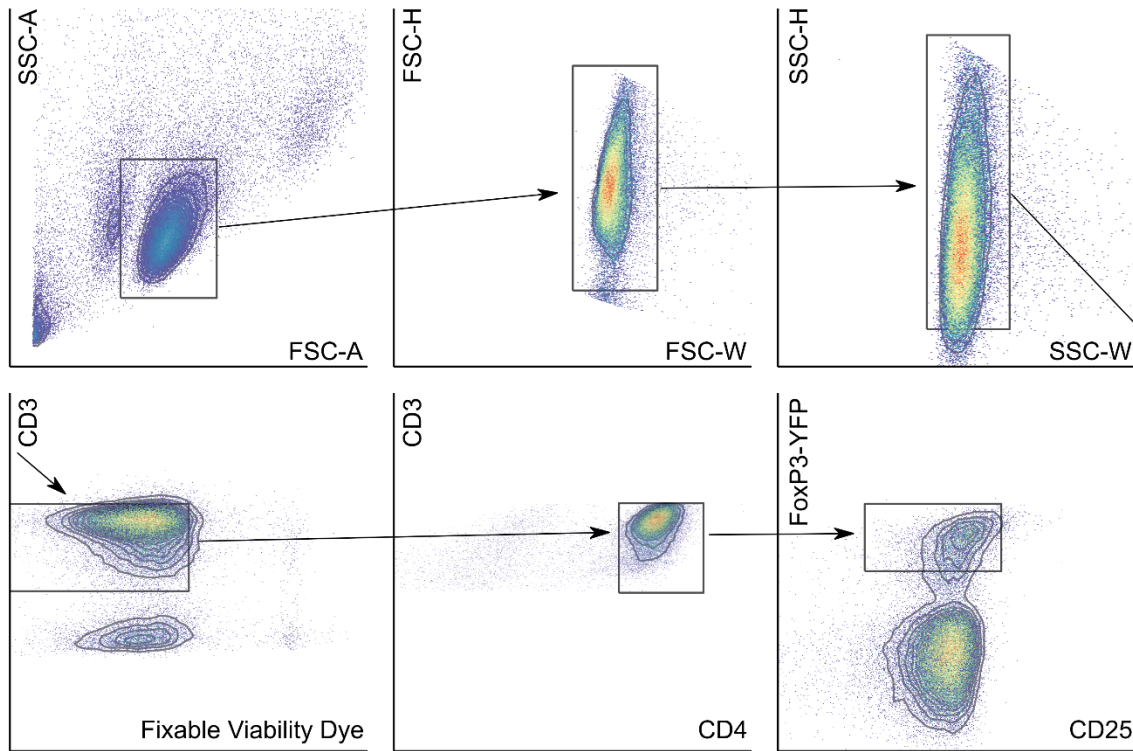


Fig. S1. Treg sorting strategy (related to Figs. 1, 2, and 3).

After magnetic depletion of Ter119⁺ cells (RBCs), B220⁺ cells (B cells & pDCs), and CD8a⁺ cells (CD8 T cells & CD8a⁺ DCs), cells from FoxP3-YFP reporter mice were sorted on a FACS AriaIII. Lymphocytes were identified and passed through two doublet-discrimination filters, then sorted on live T cells by fixable viability stain and CD3, CD4⁺ T cells by CD4 expression, and finally Treg were isolated by YFP expression. Example plots are taken from a tumor-infiltrating lymphocyte sample.

Gene	logFC	PValue	Annotation	Reference	Notes
Tnfrsf11a	7.71	0.0022	Proinflammatory/Th1	1	Induced in destabilized Treg
Tnfrsf21	5.92	0.0015	Proinflammatory/Th1	2	Suppresses Th2, improves CD4 migration to inflammatory sites
Fgr	5.64	0.0061	Proinflammatory/Th1	3	Src kinase required for generation of inflammatory environments
Socs5	4.67	0.0056	Proinflammatory/Th1	4	Favours Th1 program over Th2
Socs4	3.9	0.018	Proinflammatory/Th1	5	Required for antiviral CD8 T cell recruitment
Il18rap	3.8	0.0233	Proinflammatory/Th1	6	Involved in inflammatory IL18 signaling
Cx3cr1	3.66	0.015	Other	NA	NA
Taok2	3.34	0.0003	Other	NA	NA
Itga5	3.03	0.0221	Other	NA	NA
Bcl2	3.01	0.0274	Other	NA	NA
Tcf7l2	2.91	0.0251	Other	NA	NA
Il15	2.88	0.0462	Proinflammatory/Th1	NA	Promotes NK cells and CD8 T cells
Ikbke	2.86	0.0159	Other	NA	NA
Ccr4	2.75	0.009	Other	NA	NA
Il12rb2	2.49	0.0317	Proinflammatory/Th1	7	Induces STAT4 signaling and IFNg production
Traf3	2.4	0.0031	Proinflammatory/Th1	8	Required for TCR signaling
Tollip	2.34	0.0099	Other	NA	NA
Ifngr2	2.24	0.0396	Proinflammatory/Th1	9	Required for IFNg-mediated immune responses
Tnfsf10	2.17	0.0072	Proinflammatory/Th1	10	Promotes cancer apoptosis
Taok3	2.1	0.0065	Other	NA	NA
Syk	1.99	0.0341	Other	NA	NA
Irak4	1.68	0.0074	Proinflammatory/Th1	11	Essential for IL18-mediated anti-tumor responses
Nfkb2	1.65	0.0154	Other	NA	NA
Lck	1.42	0.0158	Other	NA	NA
Nfkb1	-1.67	0.0045	Other	NA	NA
Lta	-1.72	0.0314	Other	NA	NA
Mapk14	-1.89	0.0444	Regulatory/Th2	12	Expressed as cells acquire Treg phenotype
Socs2	-1.9	0.0236	Regulatory/Th2	13	Promotes Treg stability
Itga4	-2.06	0.0141	Other	NA	NA
Ep300	-2.37	0.0435	Regulatory/Th2	14	Stabilizes Treg phenotype
Map3k3	-2.57	0.0234	Other	NA	NA
Mapk3	-3.07	0.028	Regulatory/Th2	14	Favours Treg over Th17 development
Il1r1	-3.2	0.0453	Regulatory/Th2	15	Enhances Th2 and Treg functions
Smad2	-3.29	0.0194	Regulatory/Th2	16	Mediates TGFb signaling, promotes Treg, suppresses IFNg
Tec	-3.49	0.0191	Regulatory/Th2	17	Suppresses IL23R expression and Th17 phenotype
Bid	-3.62	0.0376	Other	NA	NA
Il17rb	-3.79	0.0476	Other	NA	NA
Traf6	-3.79	0.0035	Regulatory/Th2	18	Supports Treg stability
Tnfrsf14	-4.51	0.041	Other	NA	NA
Il11	-7.62	0.0005	Regulatory/Th2	19	Suppresses anti-tumor CD4 responses

Fig. S2. Table of significant DEG and their inflammatory/regulatory annotation (related to Figure 2). See supplemental references for citations supporting functional annotation.

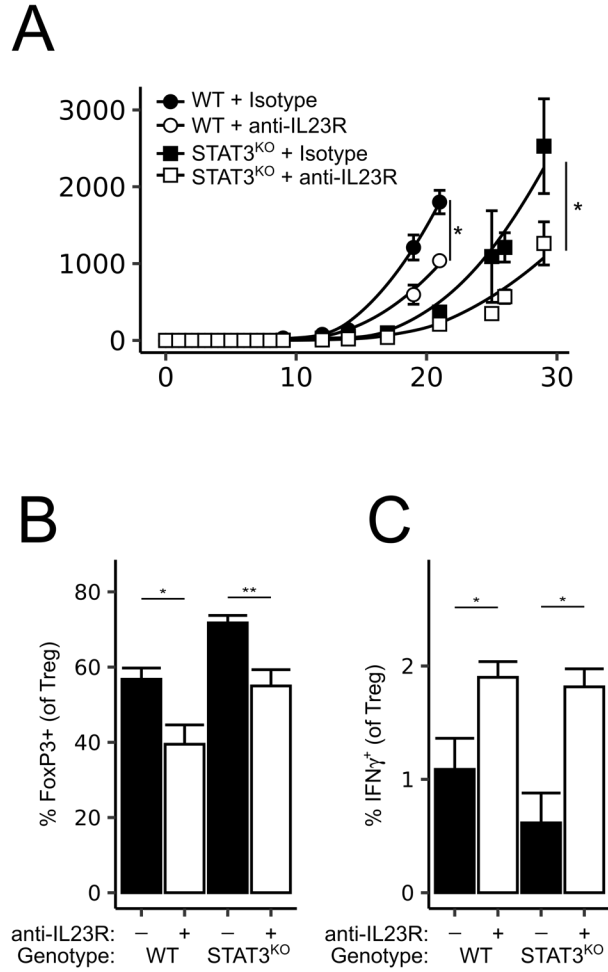


Fig. S3. IL23R interference operates independent from STAT3 signaling (related to Figure 2).

(A) Tumor growth curves in WT or Treg-specific STAT3^{KO} mice treated with anti-IL23R antibody or isotype control as in Figure 3. **(B)** FoxP3 expression and **(C)** IFN γ expression on tumor-infiltrating Treg at tumor endpoint (D21 for WT, D29 for STAT3KO).

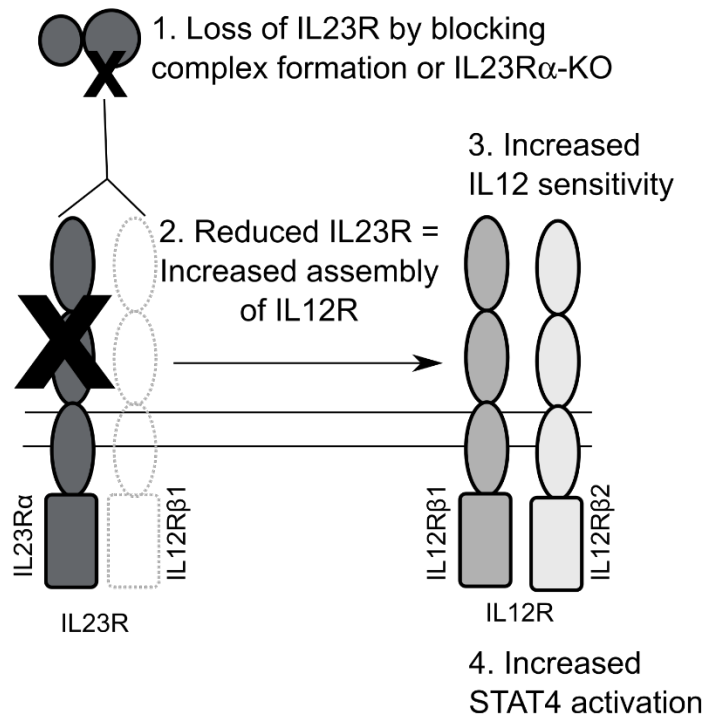


Fig. S4. : Schematic showing the functional relationship between IL23R and IL12R.

IL23R and IL12R compete for a shared signaling chain, IL12R β 1. Therefore, (1) interfering with IL23R α (2) liberates IL12R β 1 to assemble more IL12R complexes. This increased surface assembly of high-affinity IL12 receptors (3) increases IL12 sensitivity in the cell, (4) leading to increased STAT4 activation and expression of STAT4-regulated genes like IFN γ .

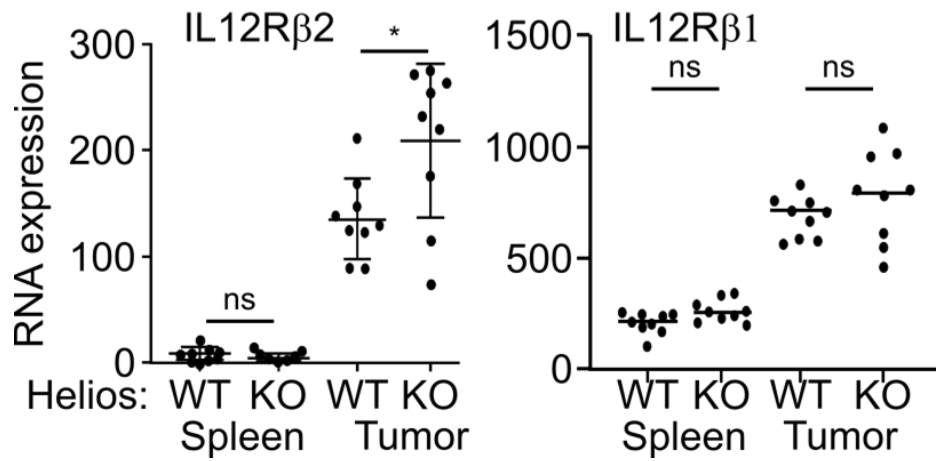


Fig. S5. Helios deficiency induces *Il12rb2* but not *Il12rb1* mRNA.

Re-analysis of RNA-Seq data generated in Yates et al. (ref. 4 in the ms). Normalized read-counts shown for each indicated gene. Mean \pm SEM is shown, with each point representing a single mouse. Statistical comparisons done by Wilcoxon test.

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