nature immunology

Article

https://doi.org/10.1038/s41590-022-01385-x

SHP-2 and PD-1-SHP-2 signaling regulate myeloid cell differentiation and antitumor responses

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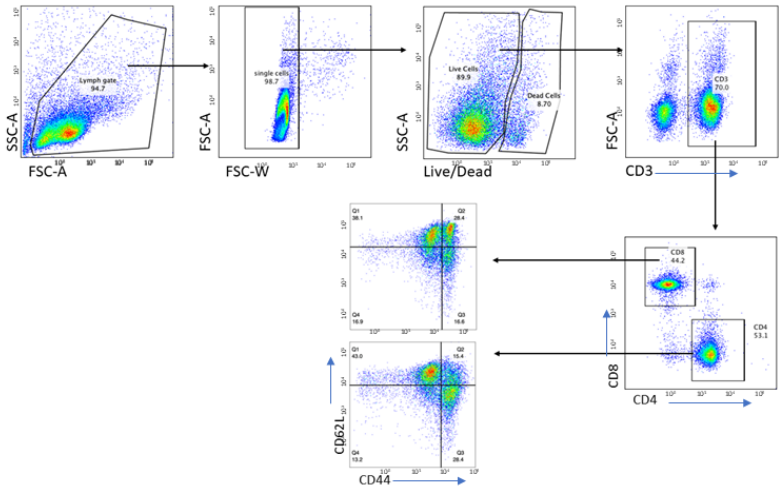
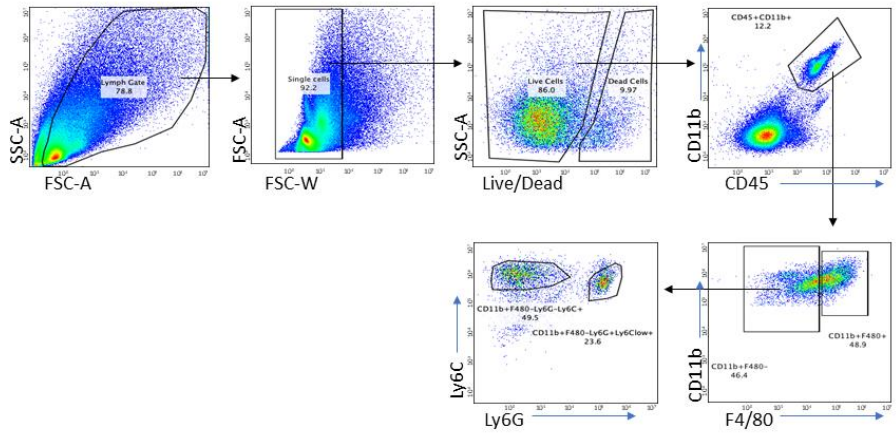
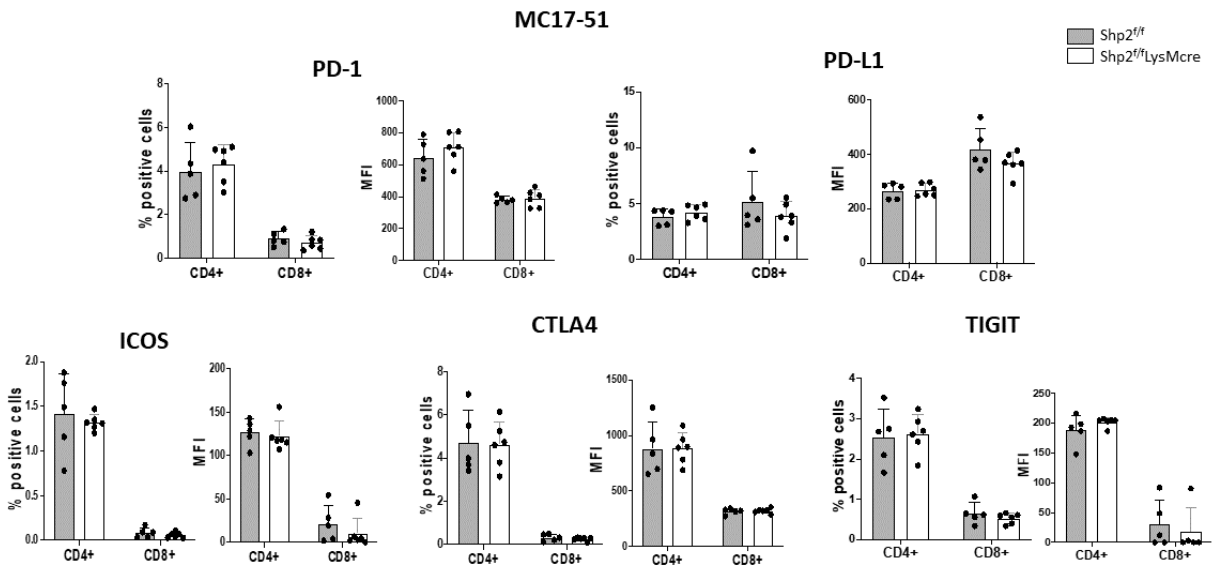


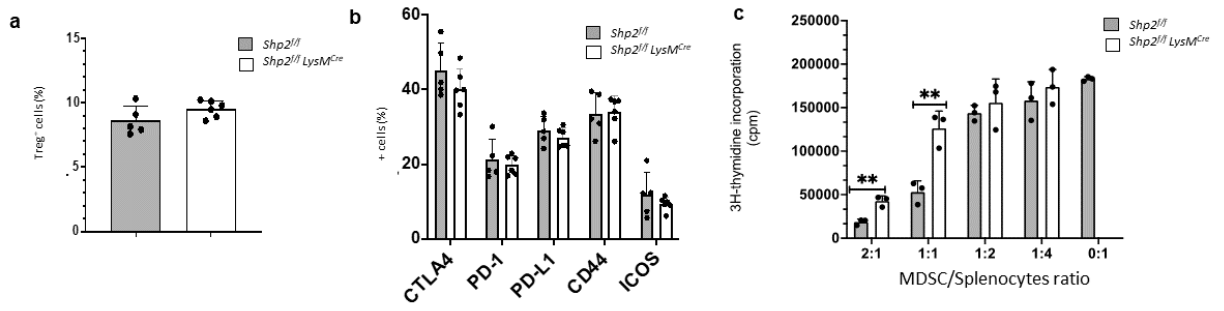
Supplementary Fig. 1. Gating strategy of activated CD4$^+$ and CD8$^+$ T cells in spleen and tumor draining lymph nodes. After excluding doublets and dead cells, CD3$^+$ T cells were divided into CD4$^+$ and CD8$^+$ T cells. Within the CD4$^+$ and CD8$^+$ gates, expression of CD44 and CD62L was assessed. CD4$^+$ and CD8$^+$ in the CD44^{hi}CD62L^{low} gate were defined as T effector (T_{EF}), whereas cells in the CD44$^+$CD62L^{hi} gate were defined as central memory (T_{CM})-like cells.



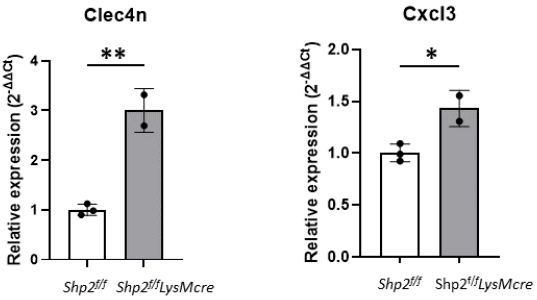
Supplementary Fig. 2: Gating strategy of myeloid subsets in the tumor site. After excluding doublets and dead cells, myeloid cells were identified as $CD45^{+}CD11b^{+}$. Within the myeloid cell population, macrophages were identified as $CD11b^{+}F4/80^{+}$, M-MDSCs as $CD11b^{+}F4/80^{-}Ly6C^{hi}Ly6G^{-}$ and PMN-MDSCs as $CD11b^{+}F4/80^{-}Ly6C^{lo}Ly6G^{+}$. The same gating strategy was used for identification of myeloid subsets in the spleen.



Supplementary Fig. 3. Myeloid-specific SHP-2 deletion does not affect the expression of co-inhibitory receptors in CD4$^+$ or CD8$^+$ T cells. Expression of the indicated markers was assessed by flow cytometry in T cells isolated from TDLN of *Shp2$^{f/f}$* and *Shp2$^{f/f}$LysM^{Cre}* mice bearing MC17-51 tumors. Representative plots and mean percentages ± SD in *Shp2$^{f/f}$* mice (grey bar) and *Shp2$^{f/f}$LysM^{Cre}* (black bars) are shown. Results are from one representative of four independent experiments with n=5 mice per group.



Supplementary Fig. 4. (a, b) Myeloid-specific SHP-2 deletion does not affect the expansion or activation of Treg cells. Tregs were identified by flow cytometry in TDLN of Shp2$^{f/f}$ and Shp2$^{f/f}$LysMCre mice bearing MC17-51 tumors **(a)**. The expansion of the indicated markers on Treg cells was examined **(b)**. Representative results from one of five independent experiments with n=4 to 6 mice per group are shown. **(c) Diminished suppressive activity of M-MDSC isolated from Shp2$^{f/f}$LysMCre tumor-bearing mice**. CD11b^{+}Ly6C^{hi}Ly6G^{-} monocytic (M-MDSC) cells were isolated from *Shp2$^{f/f}$ and Shp2$^{f/f}$LysM^{Cre}* MC17-51 tumor-bearing mice and cultured at various ratios with OTI splenocytes (2x10^{5}cells/well) stimulated with OVA$_{257-264}$ as described in Methods. Mean ± SEM of cpm values are shown. Results are representative of two separate experiments using MDSC from n=3 mice per group. (**p< 0.01).



Supplementary Fig. 5. RNAseq validation example. TAMs were isolated from *Shp2$^{f/f}$* and *Shp2$^{f/f}$LysM^{Cre}* tumor-bearing mice on day 15 and expression of mRNA for the indicated genes was examined by qPCR. Results shown are from one of four separate experiments with n=2-3 mice per group. (*p< 0.05, **p< 0.01).