WILEY-VCH

Supporting Information

for *Adv. Sci.*, DOI: 10.1002/advs.202003712

Steroids Enable Mesenchymal Stromal Cells to Promote $CD8^+$ T Cell Proliferation via VEGF-C

Yurun Gan, Tao Zhang, Xiaodong Chen, Wei Cao, Liangyu Lin, Liming Du, Yu Wang, Fei Zhou, Xuefeng He, Yulong He, Jianhe Gan, Huiming Sheng, Lydia Sorokin, Yufang Shi[*]*, and Ying Wang*[*]

Supporting Information

Steroids Enable Mesenchymal Stromal Cells to Promote CD8$^+$ T cell Proliferation via VEGF-C

Yurun Gan, Tao Zhang, Xiaodong Chen, Wei Cao, Liangyu Lin, Liming Du, Yu Wang, Fei Zhou, Xuefeng He, Yulong He, Jianhe Gan, Huiming Sheng, Lydia Sorokin, Yufang Shi[*]*, and Ying Wang*[*]

Supplementary Figures:

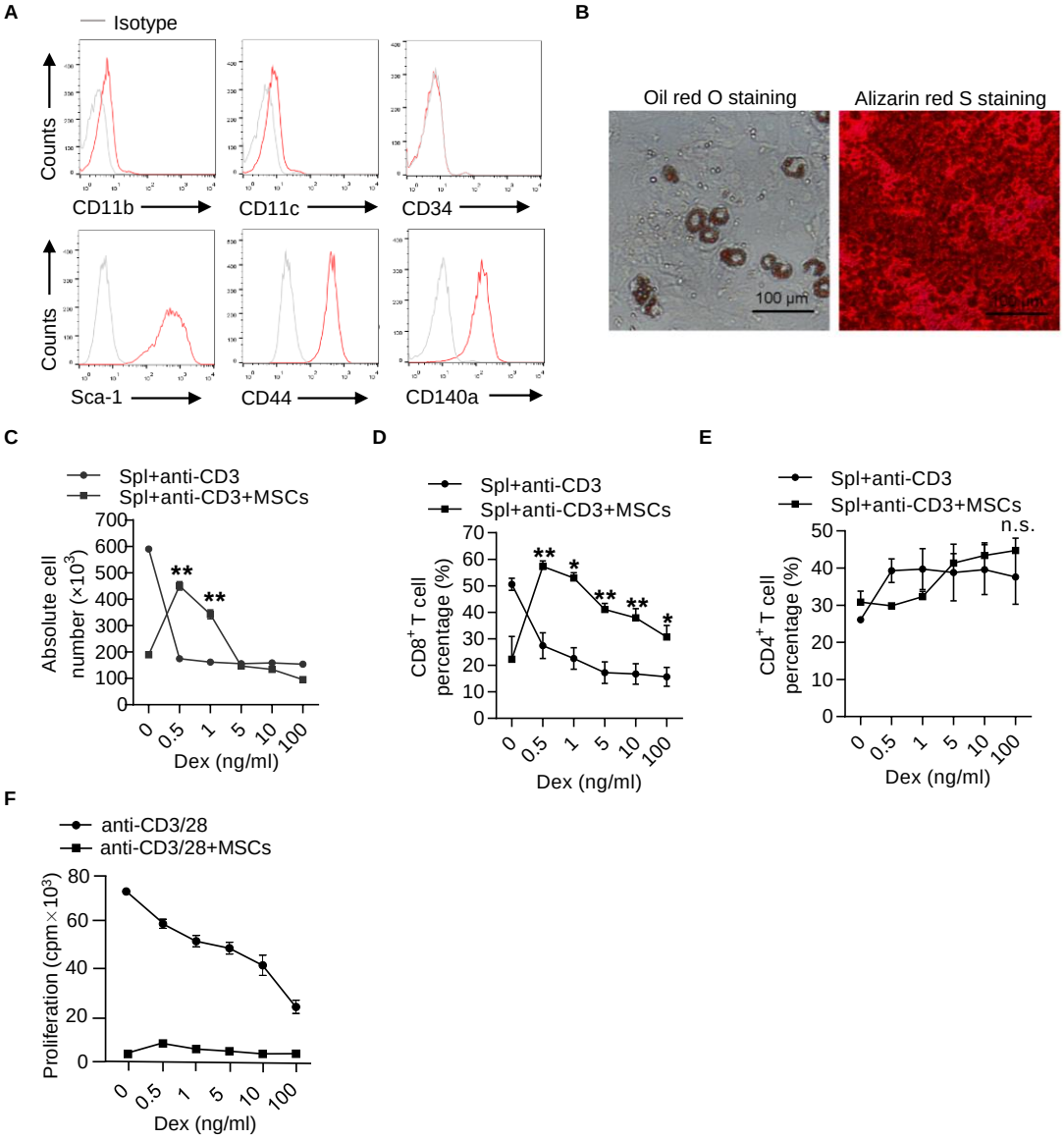
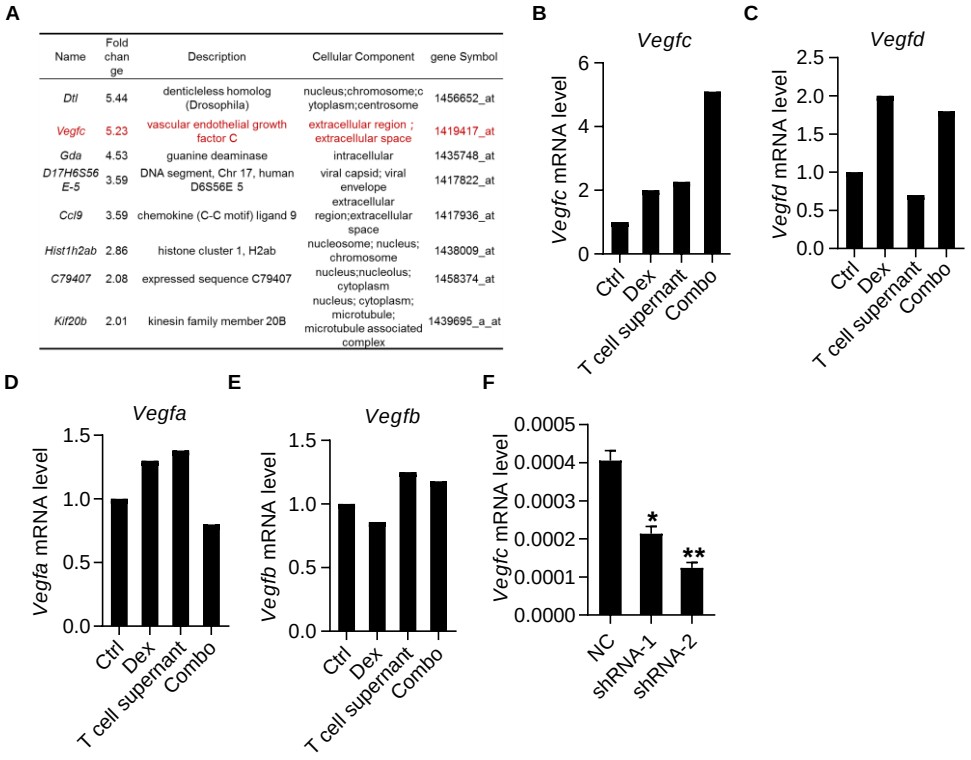


Figure S1. $CD4^+$ T cells are not involved in immune promotion by Dex-treated MSCs. A) Flow cytometric analysis of the expressions of CD11b, CD11c, CD34, Sca-1, CD44 and CD140a in MSCs. B) Oil red O staining and Alizarin red S staining of MSCs cultured in adipogenic and osteogenic differentiation medium for 7 and 21 days, respectively. C) Splenocytes were activated by anti-CD3 and co-cultured with or without MSCs in the presence or absence of Dex at indicated concentrations, and the total cell numbers were counted. D-E) Flow cytometric analysis of the percentages of $CD4^+$ T cells and $CD8^+$ T cells among splenocytes. F) The proliferation of $CD4^+$ T cells. $CD4^+$ T cells isolated from the mouse spleen were stimulated with anti-CD3 and anti-CD28 with or without MSCs, in the presence or absence of Dex at indicated concentrations. Cell proliferation was assessed by ^3H-

thymidine incorporation. Data are presented as mean ± SEM. n.s., no significance, *p < 0.05 and **p < 0.01.



Name	Fold change	Description	Cellular Component	gene Symbol
Dtl	5.44	denticleless homolog (Drosophila)	nucleus;chromosome;cytoplasm;centrosome	1456652_at
Vegfc	5.23	vascular endothelial growth factor C	extracellular region ; extracellular space	1419417_at
Gda	4.53	guanine deaminase	intracellular	1435748_at
D17H6S56E-5	3.59	DNA segment, Chr 17, human D6S56E 5	viral capsid; viral envelope	1417822_at
Ccl9	3.59	chemokine (C-C motif) ligand 9	extracellular region;extracellular space	1417936_at
Hist1h2ab	2.86	histone cluster 1, H2ab	nucleosome; nucleus; chromosome	1438009_at
C79407	2.08	expressed sequence C79407	nucleus;nucleolus; cytoplasm	1458374_at
Kif20b	2.01	kinesin family member 20B	nucleus; cytoplasm; microtubule; microtubule associated complex	1439695_a_at

Figure S2. Expressions of VEGF family members in MSCs upon Dex treatment. A) Top 9 up-regulated genes in MSCs by Dex in the presence of activated T cells. Four groups were presented, including MSCs (G1), MSCs with Dex treatment (G2), MSCs co-cultured with $CD8^+$ T cells (G3) and MSCs co-cultured with $CD8^+$ T cells and with Dex treatment (G4). B-E) mRNA expressions of *Vegfc*, *Vegfd*, *Vegfa* and *Vegfb* in MSCs stimulated with activated T cell supernatant, with or without Dex addition. F) Efficiency of short hairpin RNA (shRNA)-mediated *Vegfc* knockdown in MSCs. NC, scramble shRNA control. Data are presented as mean ± SEM. *p < 0.05 and **p < 0.01.

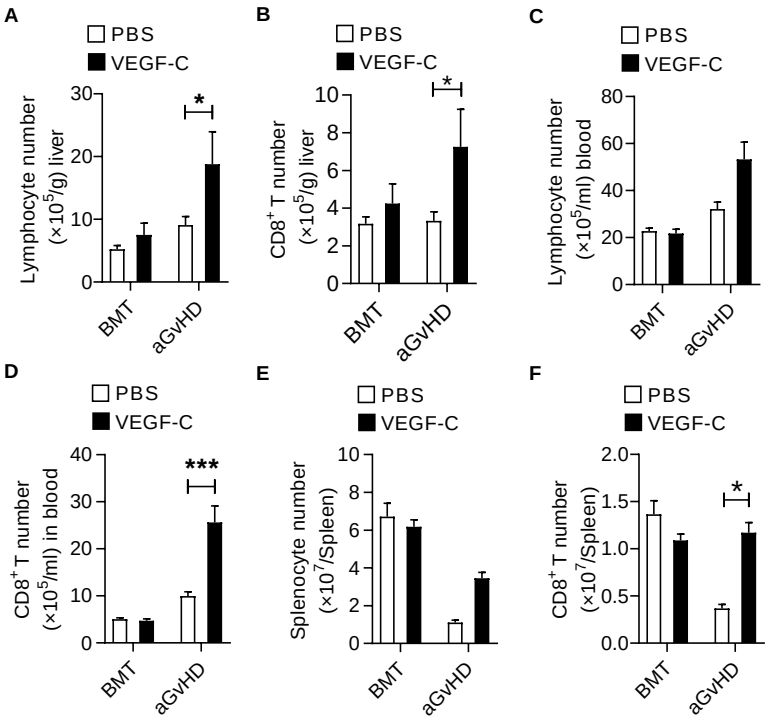


Figure S3. The influence of VEGF-C on the numbers of immune cells in the liver, blood and spleen of aGvHD mice. A-B) The numbers of lymphocytes (A) and $CD8^+$ T cells (B) in the liver of aGvHD mice treated with PBS or VEGF-C. Lethally irradiated C3H × C57 BL/6 F1 mice were adoptively transferred with bone marrow cells (5×10^6) and splenocytes (5×10^7) from C57BL/6 mice to induce aGvHD. On day 7 post aGvHD induction, mice were *i.p.* injected with VEGF-C at 10 μg per mouse daily. For the BMT group, recipient mice were adoptively transferred with bone marrow cells (5×10^6), with or without VEGF-C administration. C-D) The numbers of lymphocytes (C) and $CD8^+$ T cells (D) in peripheral blood of aGvHD mice treated with PBS or VEGF-C. E-F) Numbers of splenocytes (E) and $CD8^+$ T cells (F) in the spleen of aGvHD mice treated with PBS or VEGF-C. Data are presented as mean ± SEM. *$p < 0.05$ and ***$p < 0.001$.

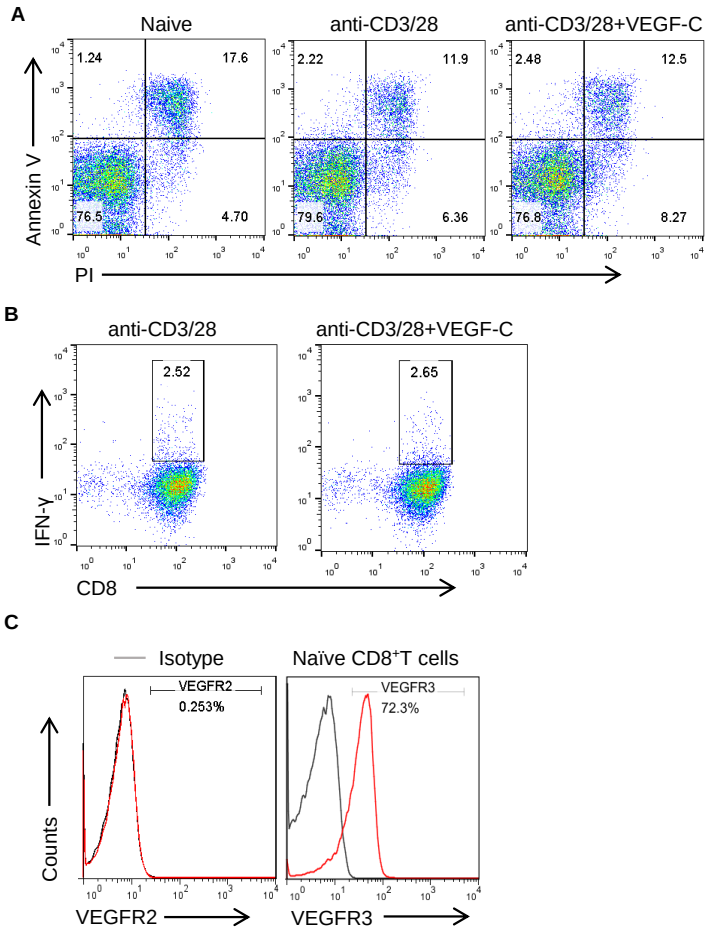


Figure S4. The effects of VEGF-C on $CD8^+$ T cell apoptosis and IFN-γ expression. A) Flow cytometric analysis of apoptotic cells among $CD8^+$ T cells activated with anti-CD3 and anti-CD28 for 48 hours, in the presence or absence of VEGF-C (800 ng/ml). B) Flow cytometric analysis of IFN-γ expression in $CD8^+$ T cells stimulated with anti-CD3 and anti-CD28 in the presence of VEGF-C. C) Flow cytometric analysis of the expressions of VEGFR2 and VEGFR3 on naïve $CD8^+$ T cells.

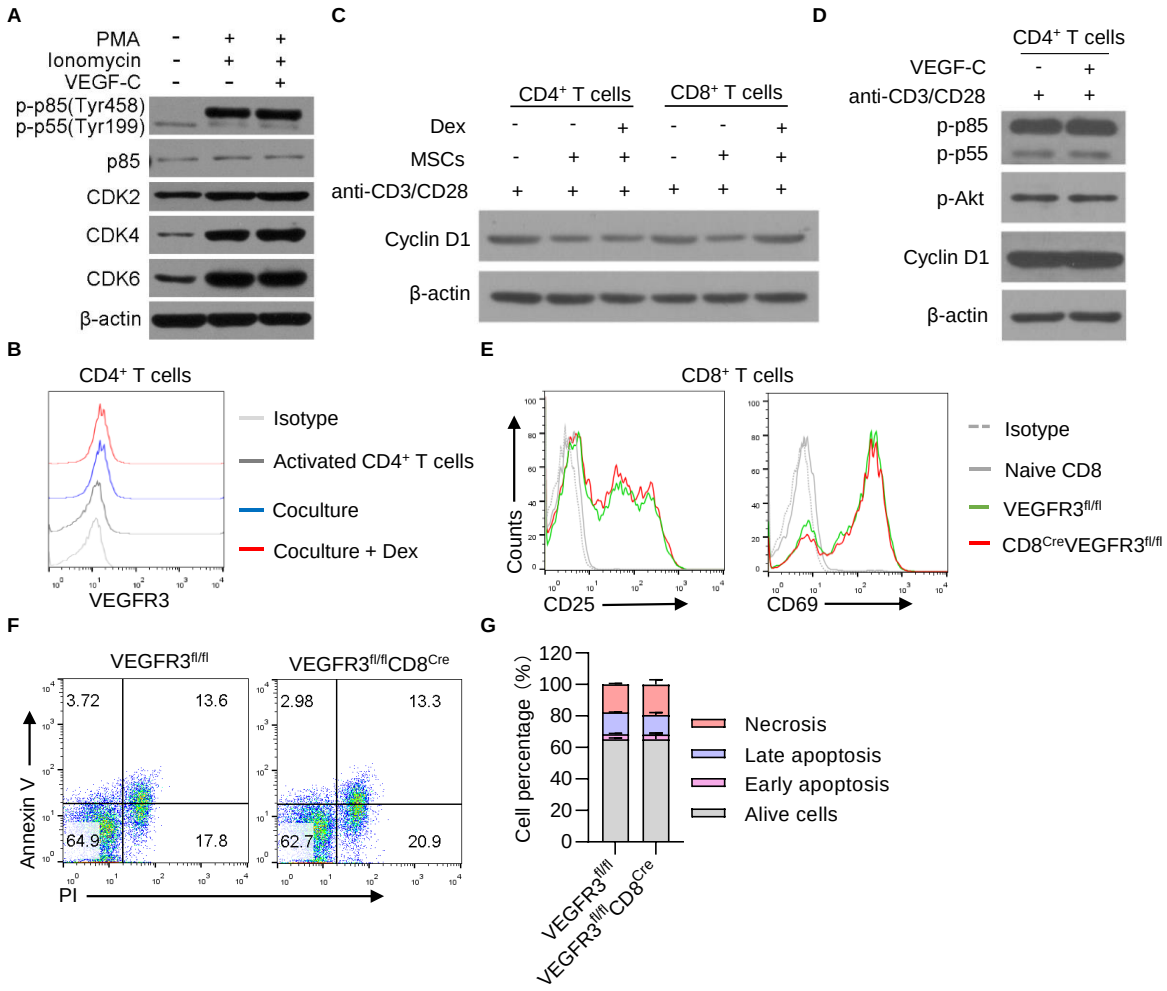


Figure S5. Activation and apoptosis in CD8$^+$ T cells isolated from VEGFR3$^{fl/fl}$ and VEGFR3$^{fl/fl}$CD8^{Cre} mice. A) Immunoblotting analysis of total p85, phosphorylated p85/p55, CDK2, CDK4, and CDK6 in CD8$^+$ T cells stimulated with PMA and ionomycin. β-actin was used as a loading control. B) Flow cytometric analysis of the expression of VEGFR3 on CD4$^+$ T cells co-cultured with MSCs, with or without Dex (10 ng/ml) additon. C) Immunoblotting analysis of Cyclin D1 expression in CD4$^+$ T cells and CD8$^+$ T cells co-cultured with MSCs, with or without Dex (10 ng/ml) addition. D) Immunoblotting analysis of the expressions of p-p85/p-p55, p-Akt and Cyclin D1 in CD4$^+$ T cells stimulated with anti-CD3 and anti-CD28 and in the presence or absence of VEGF-C (800 ng/ml). E) Flow cytometric analysis of the expressions of CD25 and CD69 on CD8$^+$ T cells activated by anti-CD3 and anti-CD28. F-G) Flow cytometric analysis of apoptotic cells in anti-CD3 and anti-CD28 activated CD8$^+$ T cells isolated from VEGFR3$^{fl/fl}$ and VEGFR3$^{fl/fl}$CD8^{Cre} mice.

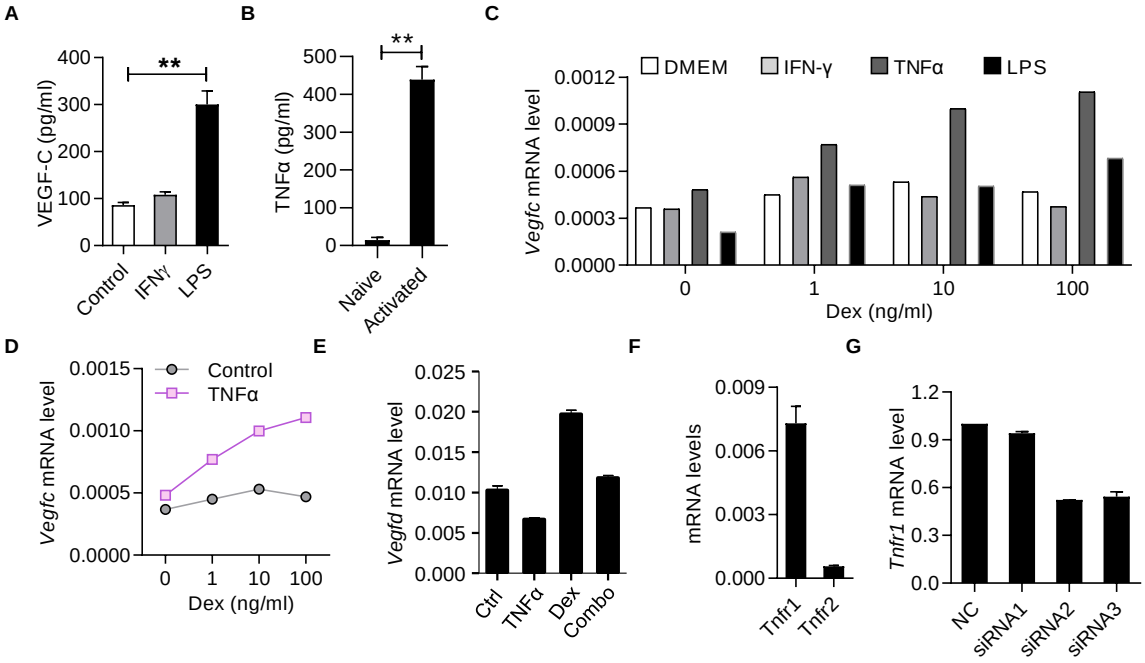


Figure S6. TNFα and Dex synergistically enhance VEGF-C expression in MSCs. A) The protein levels of VEGF-C in the supernatant of MSCs treated with PBS, LPS (100 ng/ml) or IFN-γ (10 ng/ml). B) The protein level of TNFα in the supernatant of $CD8^+$ T cells stimulated with anti-CD3 and anti-CD28. C) *Vegfc* mRNA expressions in MSCs stimulated with LPS (20 ng/ml), IFN-γ (5 ng/ml) or TNFα (5 ng/ml) and treated with Dex at indicated concentrations. D) mRNA expressions of *Vegfc* in MSCs treated with TNFα (5 ng/ml) and Dex at indicated concentrations. E) mRNA expressions of *Vegfd* in MSCs treated with TNFα (10 ng/ml), Dex (10 ng/ml) or their combination. F) mRNA expressions of *Tnfr1* and *Tnfr2* in MSCs. G) Efficiency of siRNA-mediated *Tnfr1* knockdown in MSCs. NC: scramble siRNA control. Data are presented as mean ± SEM. **p < 0.01.