Immune checkpoint regulator PD-L1 expression on tumor cells by contacting CD11b positive bone marrow derived stromal cells

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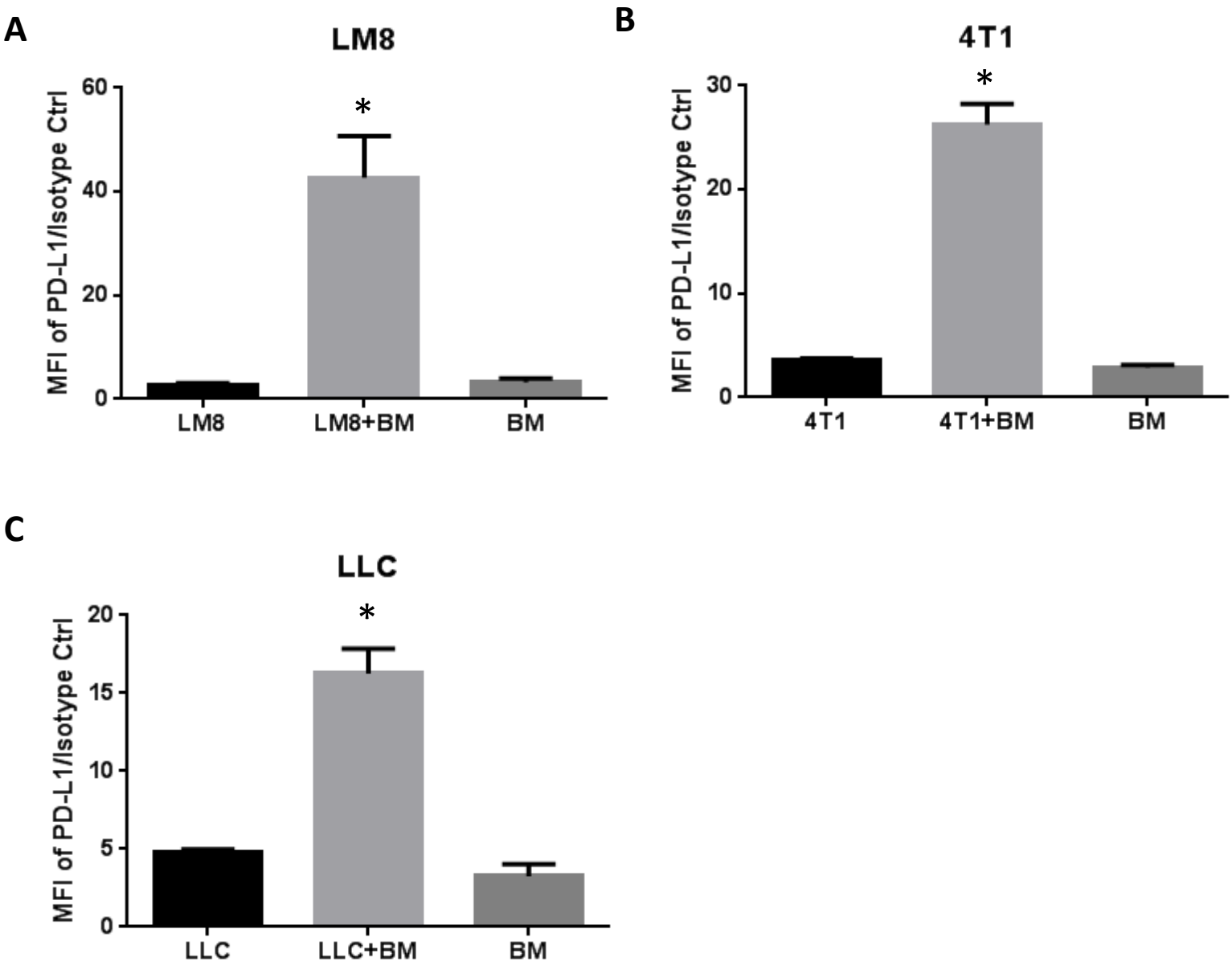
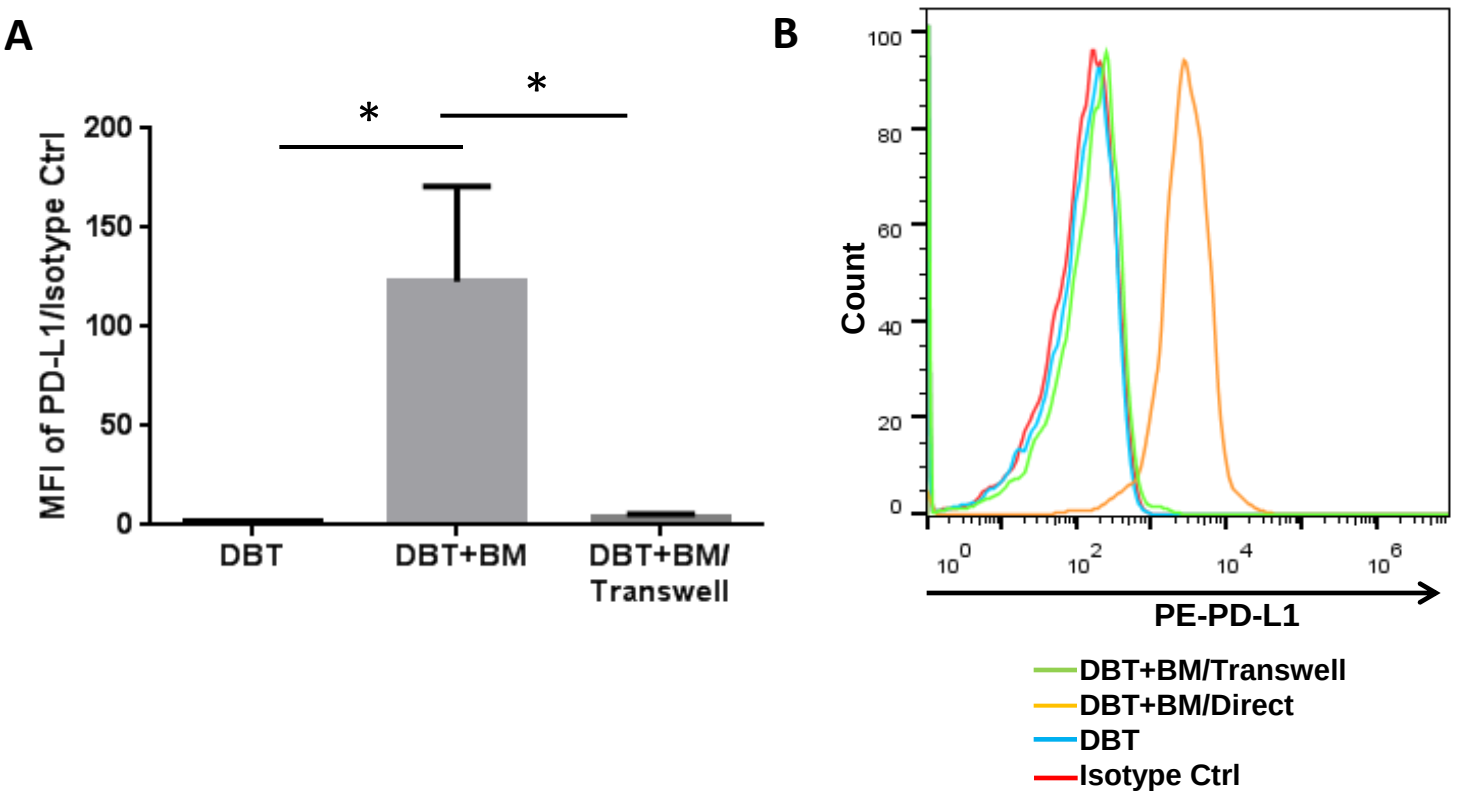
Supplemental S1



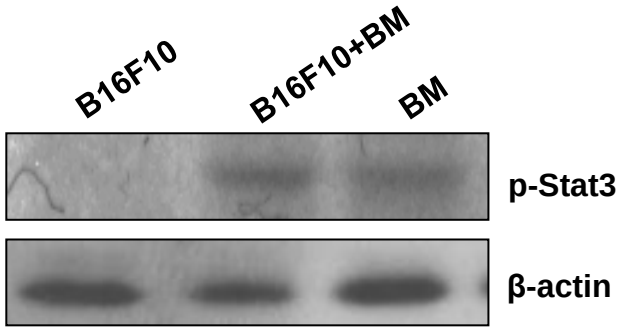
S1. Bone Marrow cells induce PD-L1 expression on tumor cells. (A) LM8, (B) 4T1, and (C) LLC tumor cell surface PD-L1 expression after co-culture with BM cells for 48hrs. Cells were stained with isotype control or PE-PD-L1 antibody. PD-L1 expression level was determined using flow cytometry. Data are presented as mean ± standard error (n=3). *, $P < 0.05$ versus B16F10 alone, student *t* test.

Supplemental S2



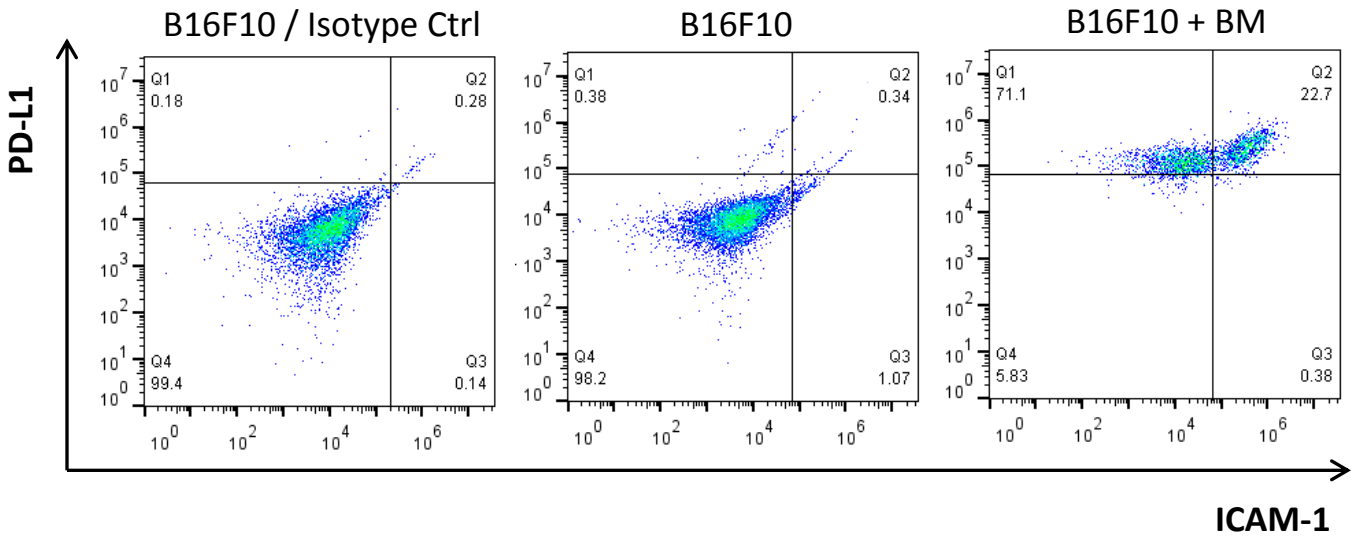
S2. Direct interaction between BM and tumor cells is required for PD-L1 expression. DBT brain tumor cells were co-cultured with BM cells together or separately using transwell membrane. Cells were stained with isotype control or PE-PD-L1 antibodies, followed flow cytometry analysis. (A) Bar graph, Data are presented as mean ± standard error (n=3). (B) Histogram *, $P < 0.05$ versus B16F10 alone, student *t* test.

Supplemental S3



S3. pStat3 was not activated by BM co-culture in B16F10 cells.
B16F10 cells co-cultured with BM cells were subjected to lysis, and cell lysates were subjected to immunoblotting to detect pStat3 levels. β-actin was used as a loading control.

Supplemental S4



S4. PD-L1 induction is not correlated with ICMA-1 on tumor cells. B16F10 tumor cells were co-cultured with BM cells for 2 days. Cells were stained with isotype control, PE/Cy7-PD-L1 or PE-ICAM-1 antibodies, followed flow cytometry analysis. Results are representative of three independent experiments.