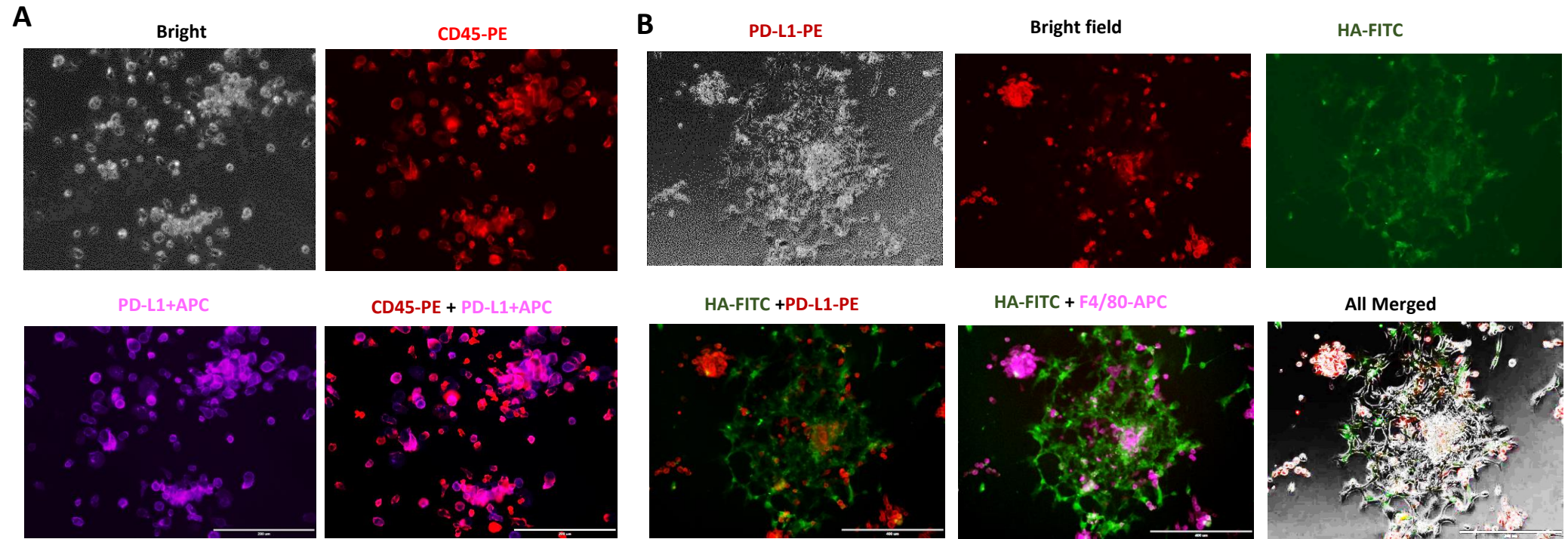
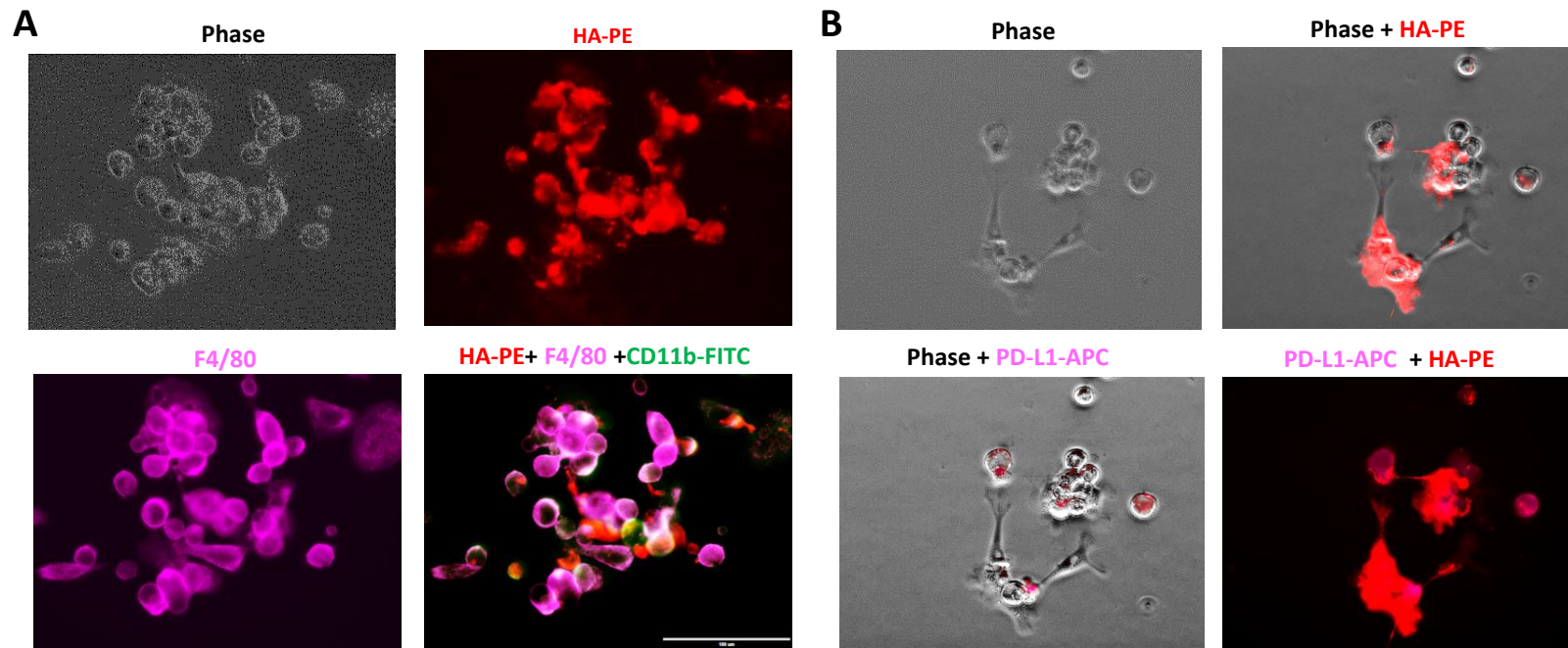


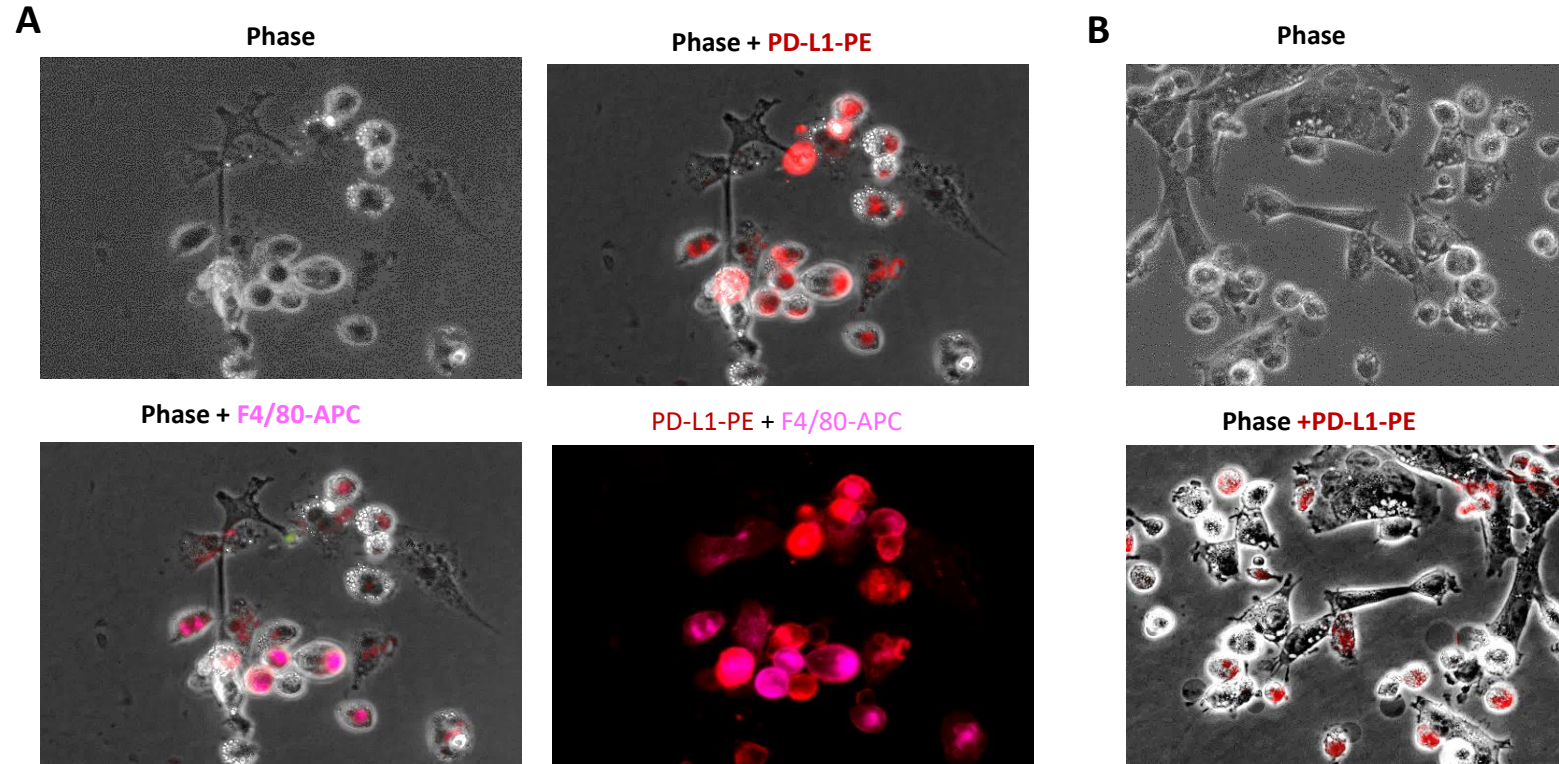
Supporting Fig. S1. The stroma-associated HA supports development of PD-L1⁺ cells in tumor tissue and tumor-draining lymph nodes. MBT2 tumor cells were injected into C3/He mice. Two weeks later mice were sacrificed, and tumors were surgically excised. Tissue slices prepared using tumor tissue pieces (**A**, **B**, **C**). Forty eight hours later plates were fixed with 4% formaldehyde and stained for the expression of PD-L1 (magenta) and HA (red). Representative images of tumor stroma are shown.



Supporting Fig. S2. HA-enriched stroma supports development of PD-L1⁺F4/80⁺ macrophages. MBT2 tumor cells were injected into C3/He mice. MBT2 tumor tissue slices were cultured in 24-well plates, allowing develop the stromal clusters and produce hyaluronan (HA). Plates were fixed with 4% formaldehyde and stained for (A): CD45-PE (red) and PD-L1-APC (magenta) ; and (B): HA (green), PD-L1 (red) and F4/80 (magenta). Representative images are shown.

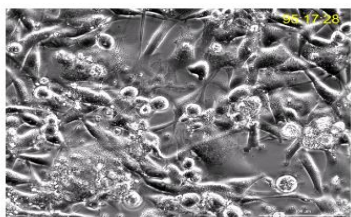
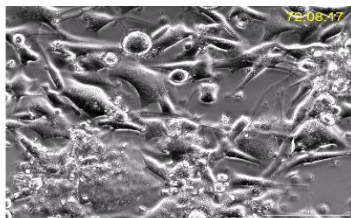
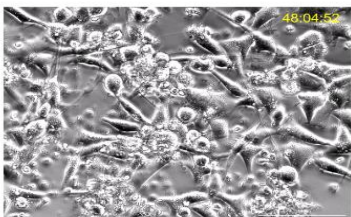
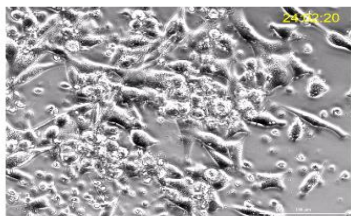
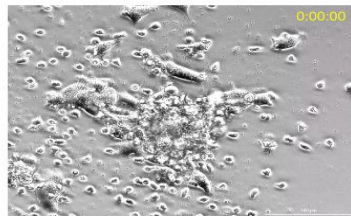


Supporting Figure S3. PD-L1-expressing cells detected in human HA-enriched tumor stroma. **A:** Murine MBT2 tumor tissue slices were cultured in 24-well plates. Plates were fixed formaldehyde and stained for HA-PE (red), F4/80 (magenta); and CD11b (green). Representative images are shown. **B:** Representative images showing the presence of HA-enriched stroma interacting with PD-L1+ cells in human bladder cancer tissue. Tumor tissue slices prepared cultured for 7 days. After removing culture medium and tissue slices, plates were fixed, stained for HA (red), PD-L1-APC (magenta) and evaluated by IF microscopy.

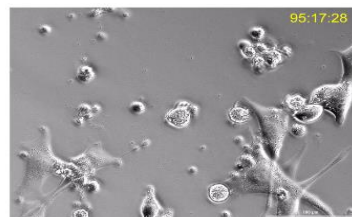
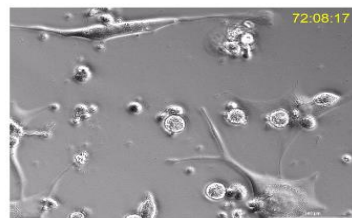
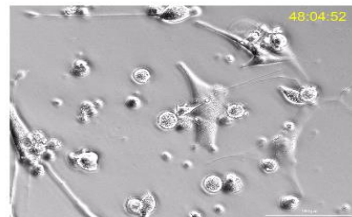
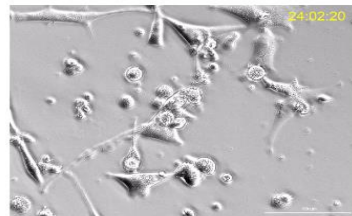
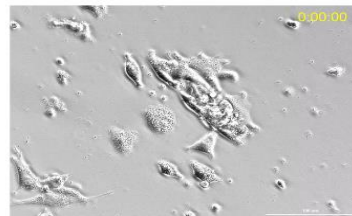


Supporting S4. Fibroblast-like cells support development of PD-L1⁺ cells in both TDLNs and tumor tissue. MBT2 cells were injected into C3/He mice. Two weeks later, mice were sacrificed, and tumor-draining LNs (A) were surgically excised. Prepared tissue slices were cultured for 5 days to produce HA and attach to the plates. Plates were fixed and stained for the presence of PD-L1-PE (red) and F4/80-APC (magenta). Tumor-tissue slices (B) were analyzed by live imaging (not fixed). Anti-PD-L1-PE antibody (red) to the tissue slice cultures and pictures taken 10 minutes later. Representative images are shown.

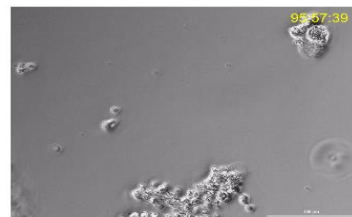
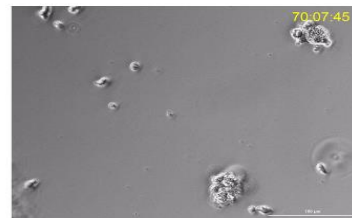
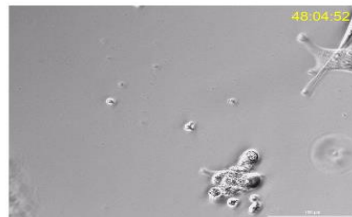
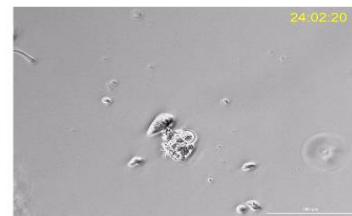
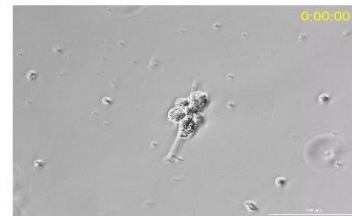
Large Cluster TCM (+)



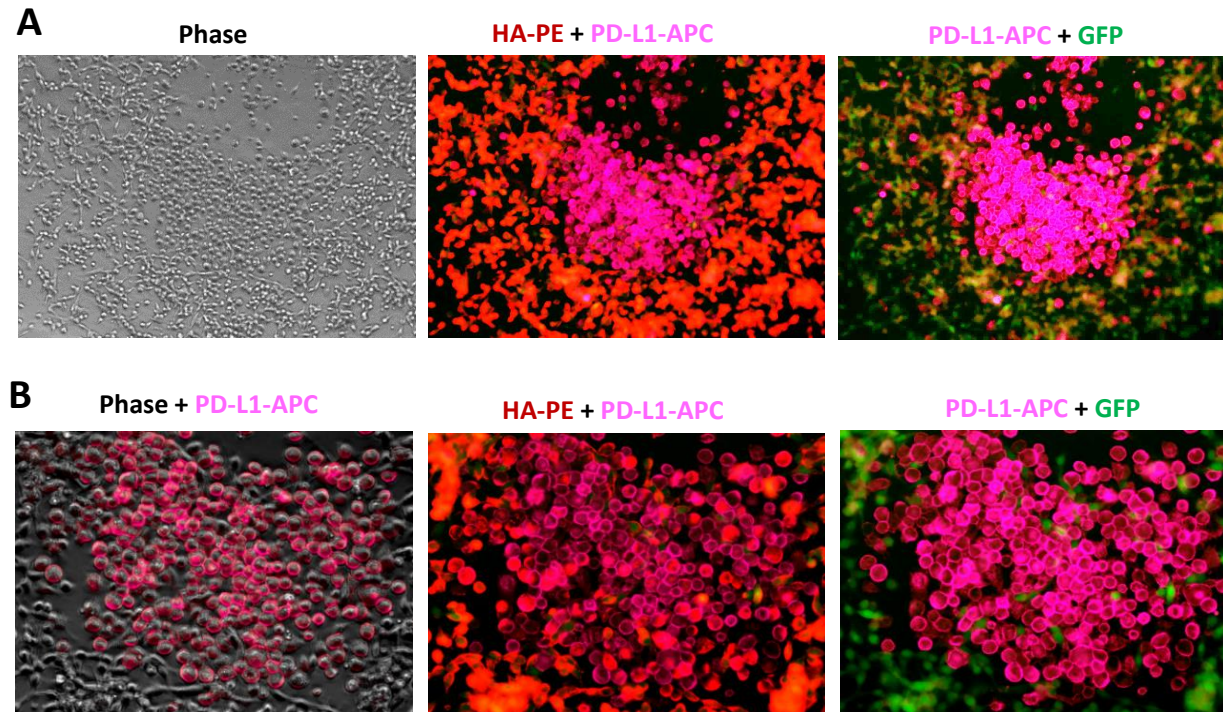
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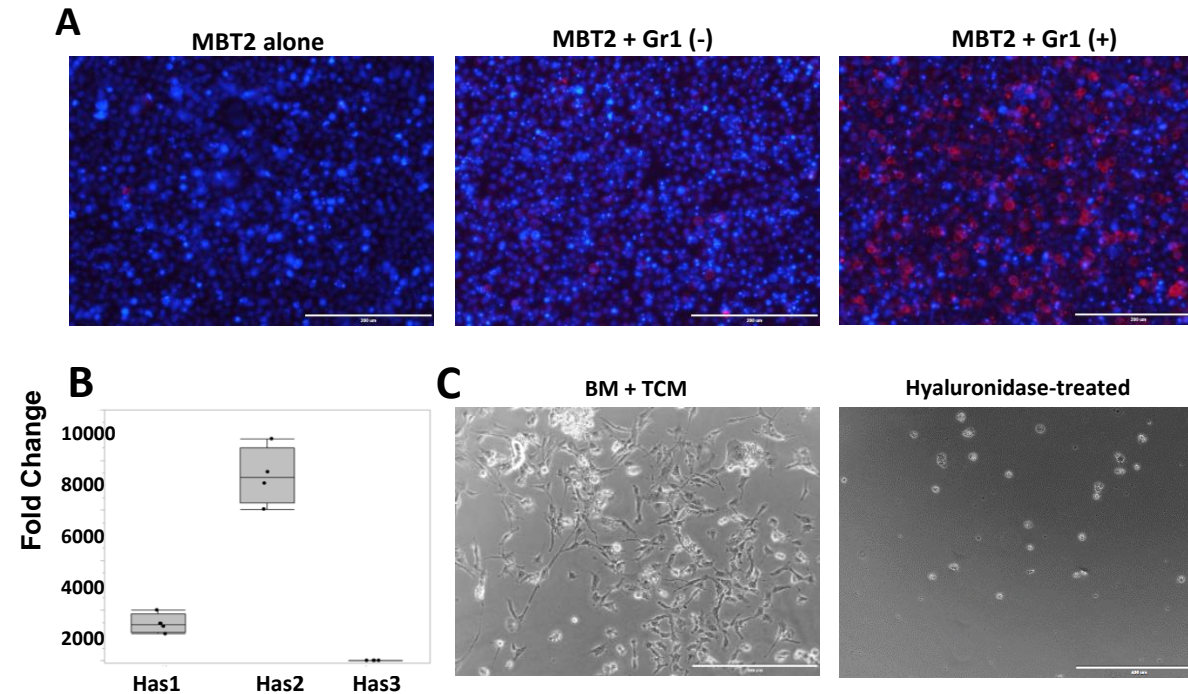
Small Cluster TCM (-)



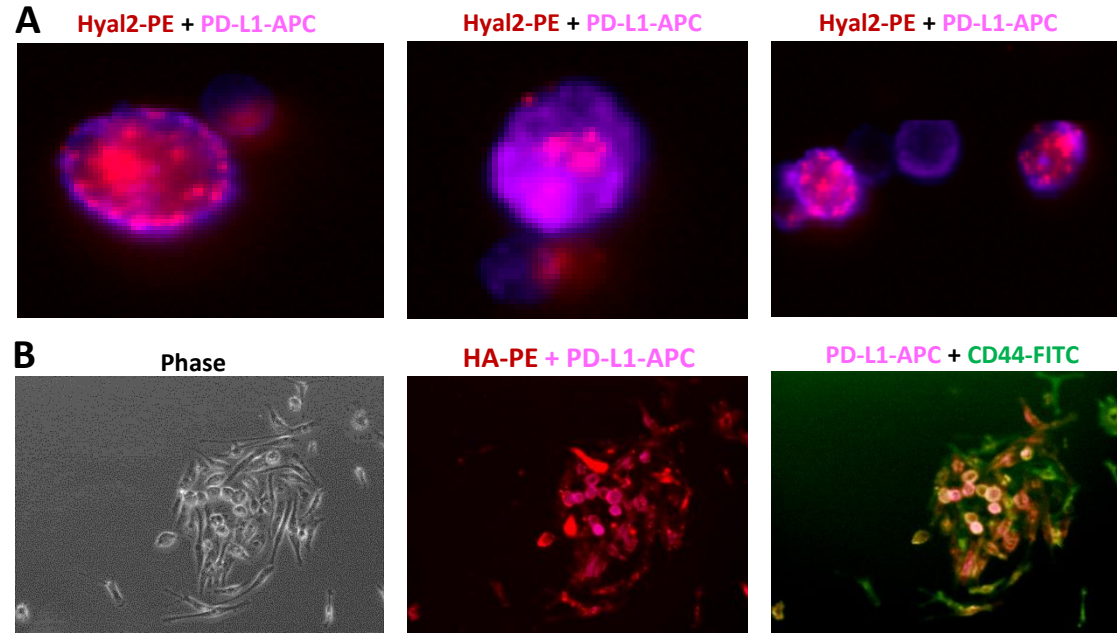
Supporting Figure S5. Formation of stromal clusters using time-lapse. Infiltrating cells from bladder cancer slice culture were imaged for 96h the presence of TCM (+) or without TCM(-). Both Large and Small Clusters TCM (+) displayed dynamic motility, proliferation, and viability through the experiment. The TCM (-) only developed small clusters that displayed some motility and proliferation; however they began to display decreased viability 72h. TCM provides factors that enhance infiltrate survival, proliferation, and motility.



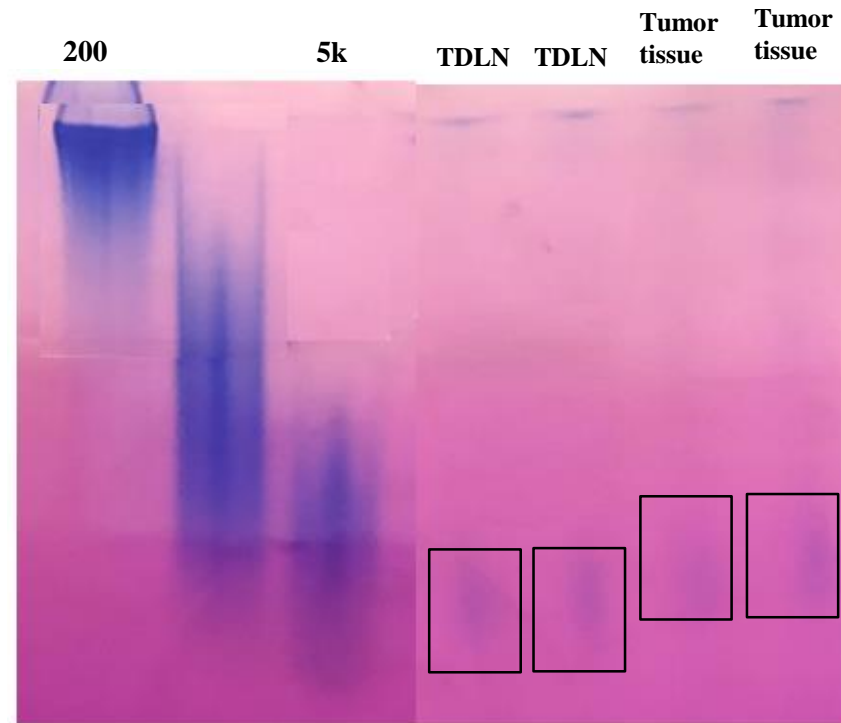
Supporting Fig S6. A: Clusters of PD-L1⁺ cells are developing in close contact with stroma that enriched with HA produced by both MBT2 tumor cells and CAFs. GFP-expressing MBT2 (A) and CT26 (B) murine tumor cells were injected in C3/He and BALB/c mice, respectively. Two weeks later the harvested tumor was used for the preparation of tumor tissue slice culture. Tumor tissue slices were cultured for 7 days, then fixed and stained with PD-L1-APC and HA-PE Abs.



Supporting Figure \$7: A: Gr-1⁺ cells were enriched using magnetic beads from the spleen of MBT2 bearing mice. Isolated Gr-1-positive (right panel) and Gr-1-negative (middle panel) splenic cells were mixed with MBT-2 tumor cells and added to the 24-well plate in a complete culture medium. On Day 7 cells were collected and stained with Dapi (blue) and anti-PD-L1-PE Abs (red). B: Expression of HAS1, HAS2 and HAS3 in MBT-2 murine bladder tumor cells. Frozen MBT2 cells were thawed and cultured for 48 hours. Collected cells were subjected to RNA isolation. Expression of murine hyaluronan synthases 1, 2 and 3 (HAS1, 2, 3) was measured using Quantitative Real-Time PCR. C: MBT2 tumor-conditioned medium added to the 24-well plate overnight with or without hyaluronidase I (Sigma-Aldrich). Myeloid cells isolated from murine bone marrow cells were added to the TCM-treated cell. Microphotographs were taken on day 7 after initiation of cell culture. Representative pictures are shown.



Supporting Fig. S8. A: CD11b myeloid cells isolated from murine naïve BM were cultured in the presence or absence of TCM. On Day 4 later cultured cells were collected. Collected cells were stained for Hyal2 (red) and PD-L1 (magenta) using immunofluorescent microscopy. **B:** Renca tumor cells were injected into BALB/c mice. Two weeks later mice were sacrificed, and tumors were surgically excised. Prepared tissue slices were cultured in the complete culture medium, fixed and stained for the HA (red), PD-L1 (magenta), and CD44 (green).



Supporting Figure S9. Electrophoretic analysis of HA produced by TDLNs and MBT2 tumor tissue slices. Cell-free supernatants collected from TDLN and tumor tissue slice cultures were treated with ethanol, proteinase K, and benzonase before applying samples to polyacrylamide electrophoresis. Commercial HA with MW 200, 10, and 5kDa were used as control. Hyaluronan was visualized by staining with “Stains All” dye.