Targeting USP22 reprograms the tumor microenvironment and sensitizes KRAS/p53-driven lung tumor to anti-PD1 immunotherapy

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Supplementary Figure 1

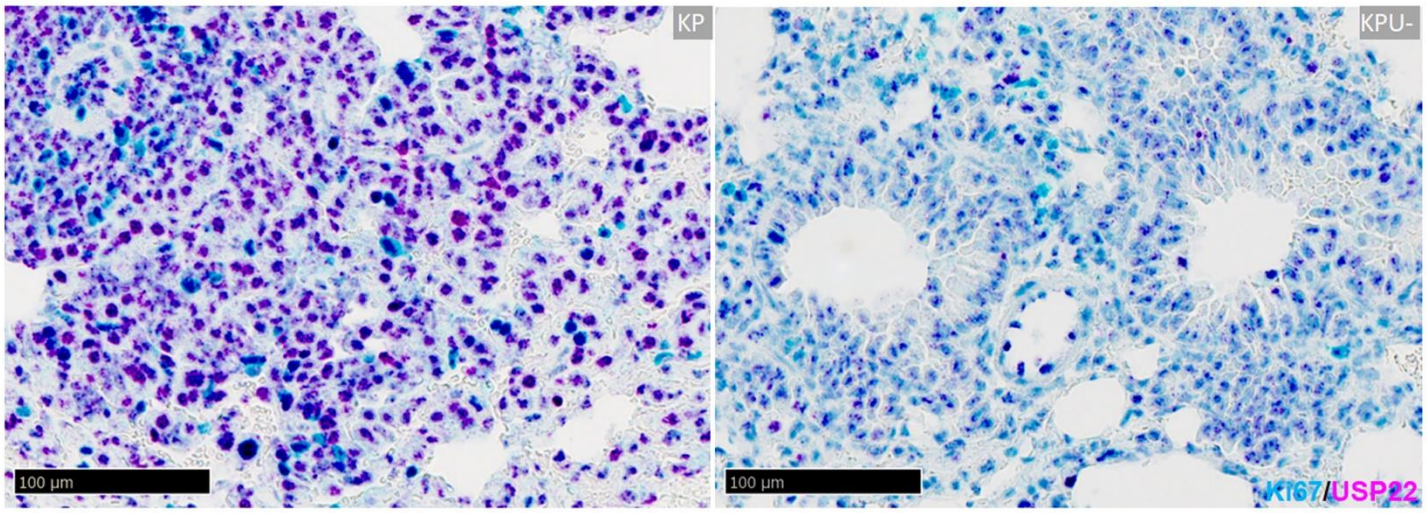

Figure S1. IHC staining images (scale bar: 100 μm, 200×x Magnification) of USP22 (purple) and Ki67 (cyan) in KP and KPU- lung tumors at 8 weeks after administration of Ad-Cre.

Supplementary Figure 2

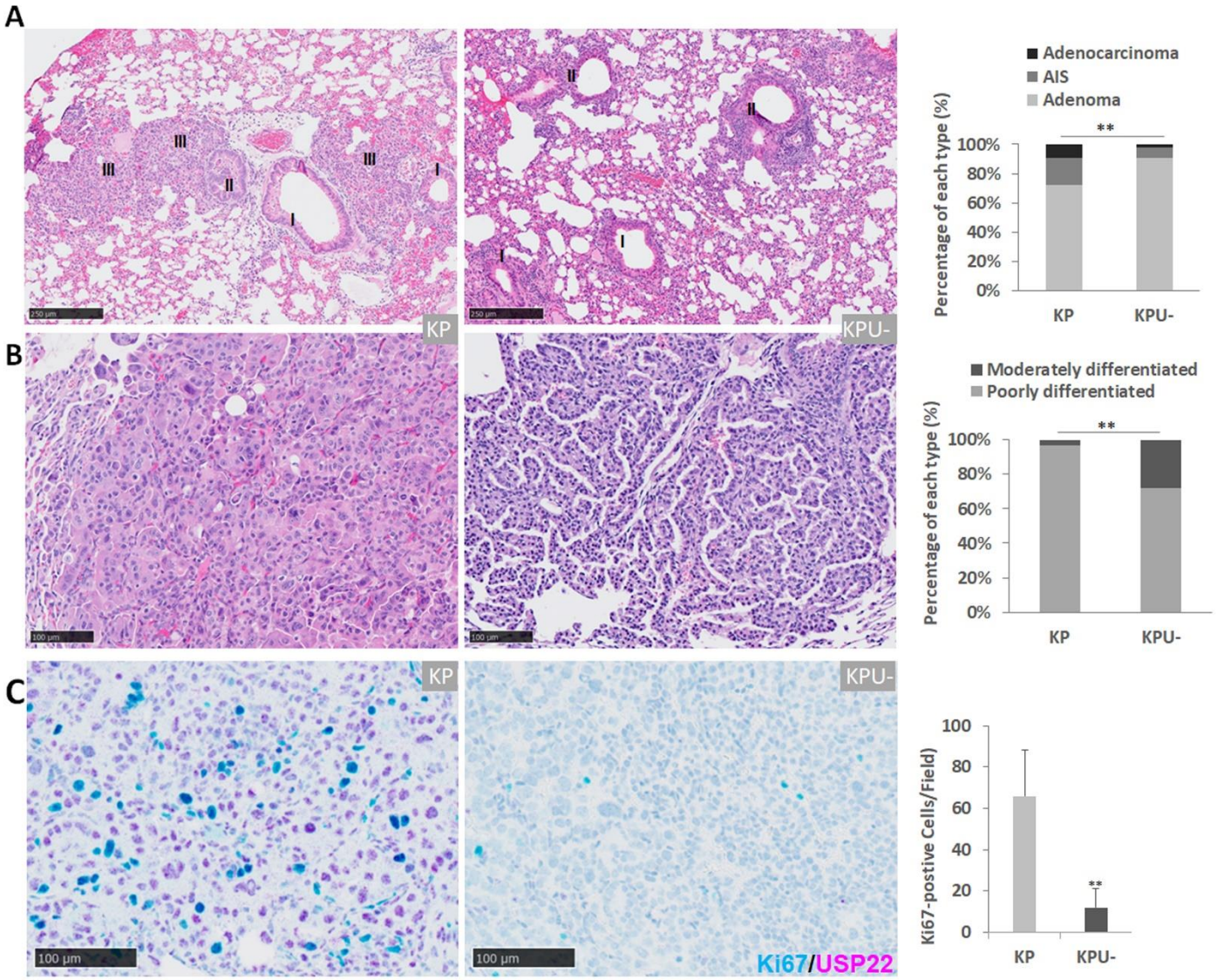

Figure S2. Usp22-KO suppressed carcinogenesis and differentiation of KRAS-G12D/p53-null mouse lung tumor. (**A**) H&E staining images (scale bar: 100 μm, 100× magnification) of KP and KPU- tumor tissues at 8 weeks of past-administration of Ad-Cre (left panel), areas labeled I as representative lesions of adenoma, II as lesions of adenocarcinoma in situ (AIS), and III as lesions of adenocarcinoma. Right panel: quantitative analysis of adenoma, AIS, and adenocarcinoma in KP and KPU- lung tumors (n = 7, $^{**}P < 0.01$, KPU- vs. KP). (**B**) H&E images (scale bar: 100 μm, 100× magnification): representative poorly differentiated adenocarcinomas in KP and moderately differentiated adenocarcinomas in KPU- tissue; right panel: quantitative analysis of each type of adenocarcinoma in KP and KPU- samples (n = 7, $^{**}P < 0.01$, KPU- vs. KP). (**C**) IHC staining images (scale bar: 100 μm, 200× magnification) of USP22 (purple) and Ki67 (cyan) in KP and KPU- lung tissues at 16 weeks after administration of Ad-Cre (left panel) and semiquantitative analysis of Ki67-positive tumor cells (n = 7, $^{**}P < 0.01$, KPU- vs. KP).

Supplementary Figure 3

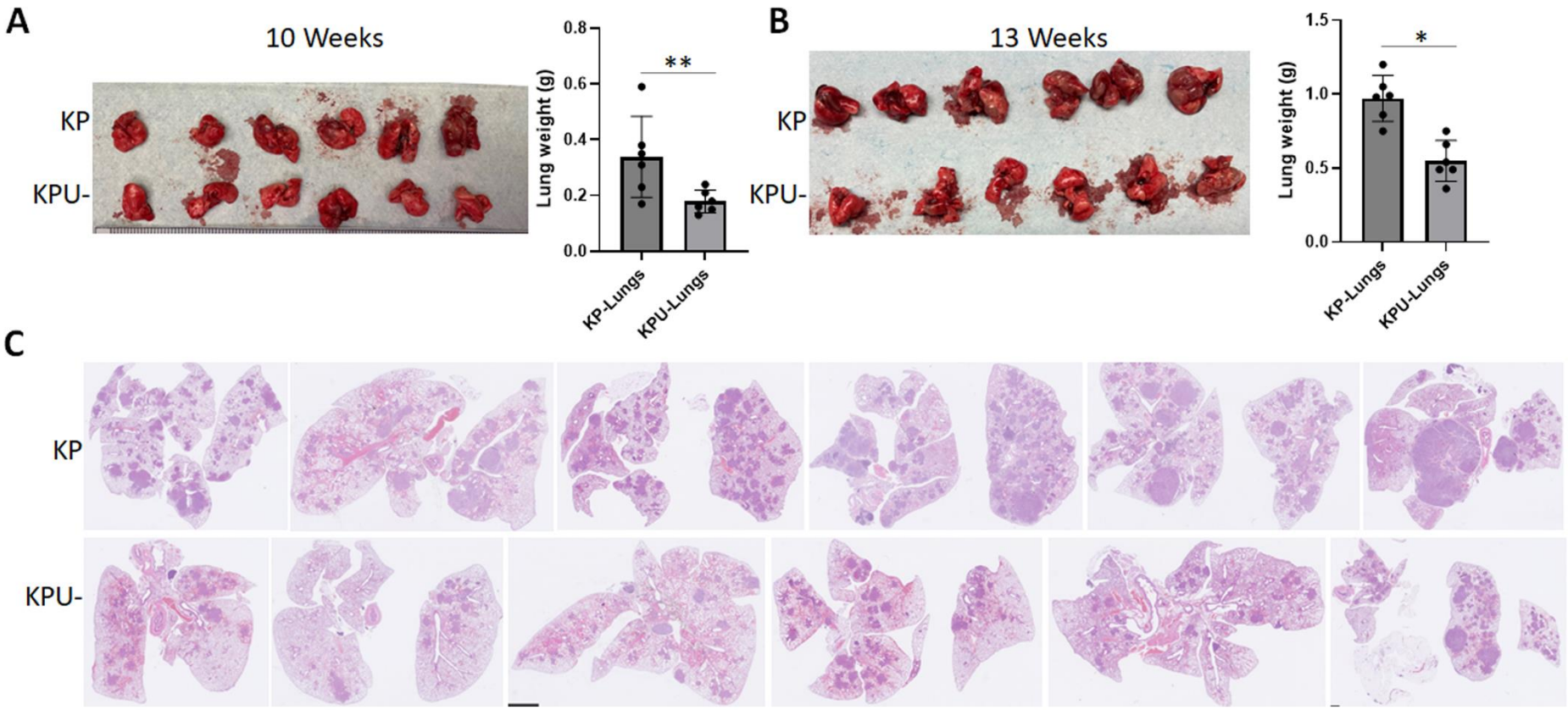

Figure S3. Gross lungs and H&E staining images of KP and KPU- lung tumors. Images of representative gross lungs and the wet weights of whole lungs at (**A**)10 and (**B**)13 weeks after tumor initiation, n = 6, *$P < 0.05$, **$P < 0.01$, KPU- versus KP. (**C**) H&E staining images of tumor areas in KP and KPU- mouse lungs (12 weeks post-initiation), which were stained deep blue.

Supplementary Figure 4

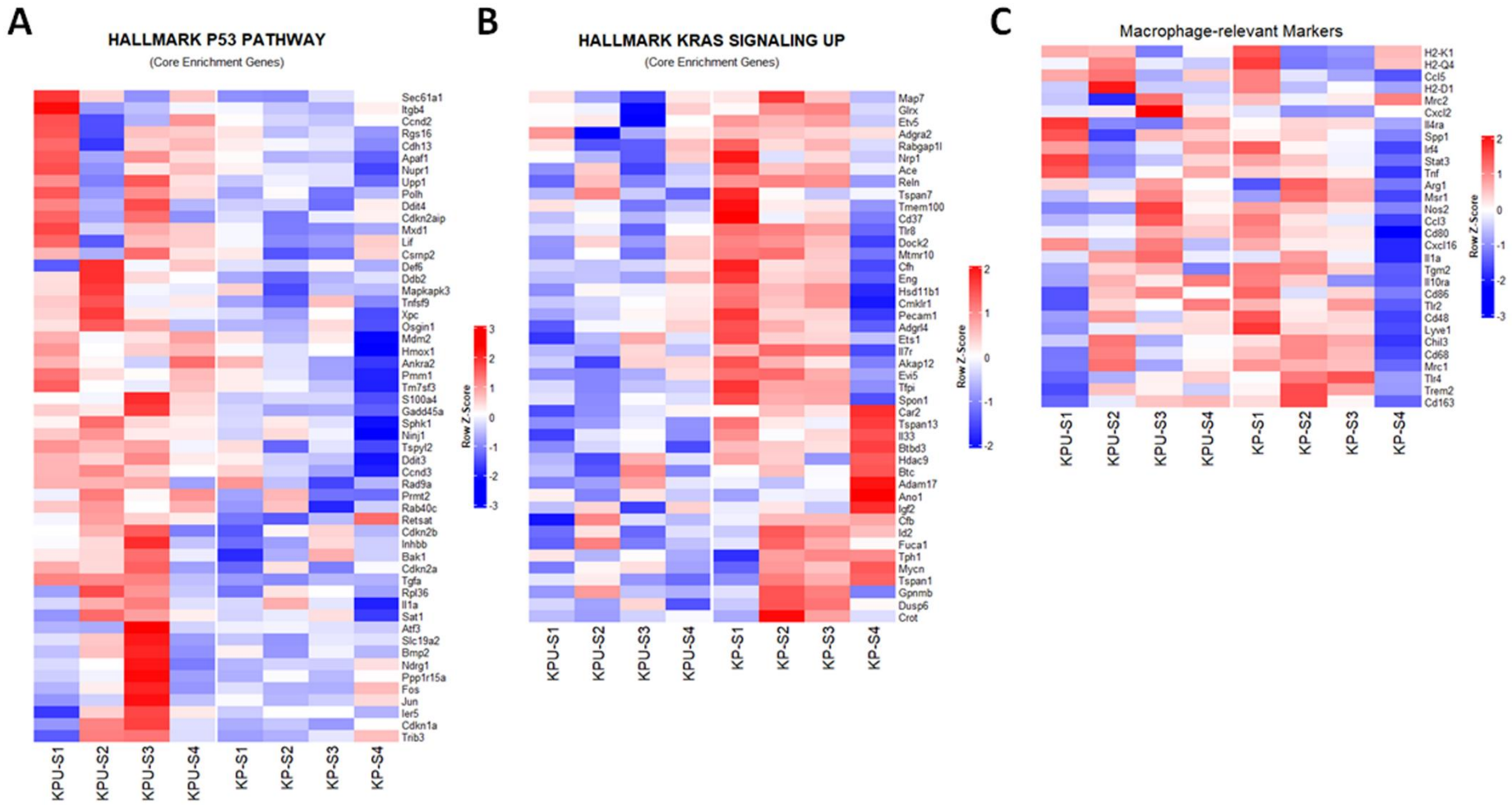

Figure S4. Unsupervised heatmaps of the expression of genes in KP and KPU- lung tumor samples (n = 4)**.** (**A**) p53 signaling pathway, (**B**) KRAS signaling pathway, and (**C**) Macrophage-relevant gene markers.

Supplementary Figure 5

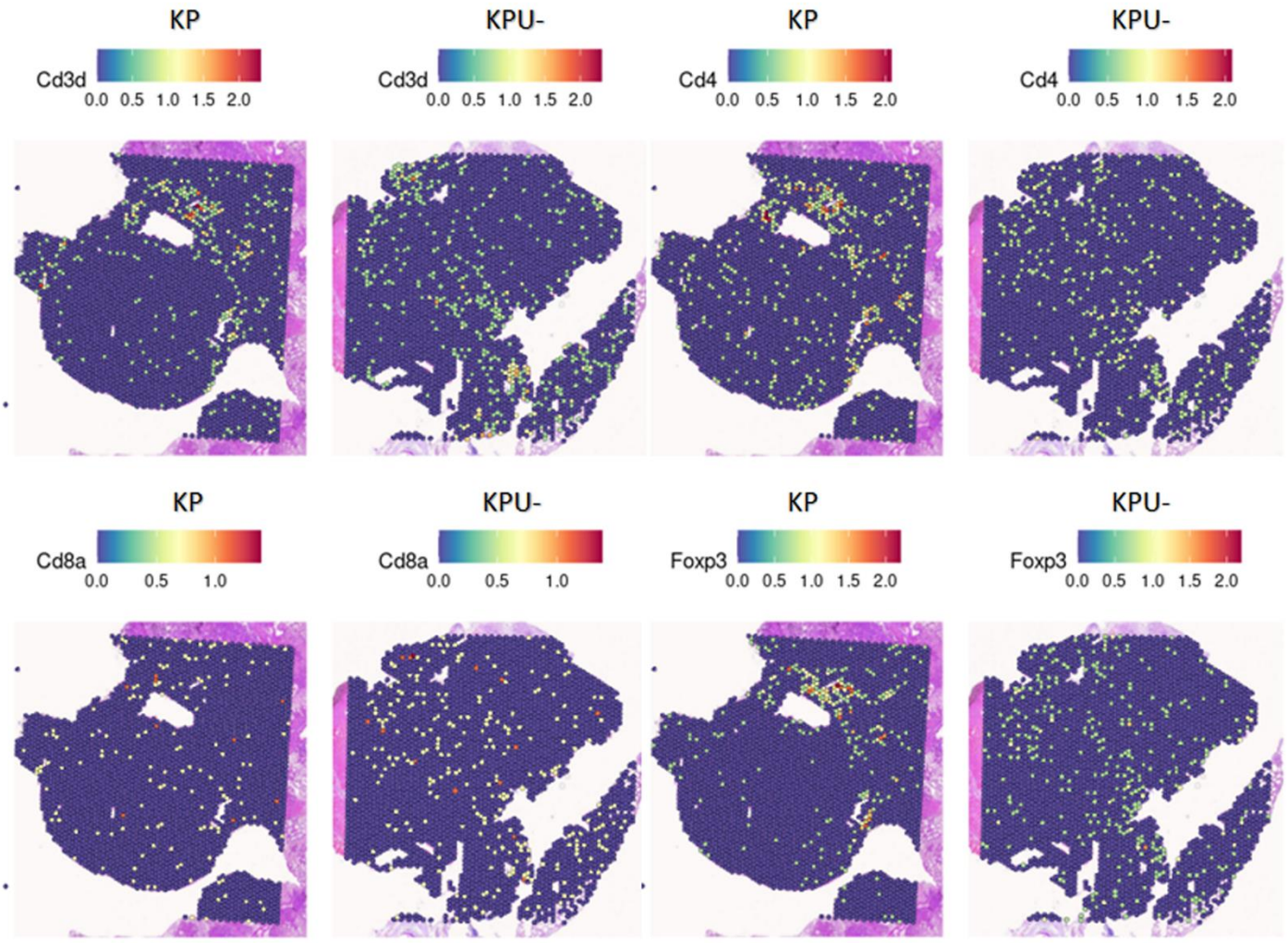

Figure S5. Spatial transcriptomic plots of Cd3d (CD3), CD4, Cd8a (CD8), and FOXP3 in a representative KP and KPU- mouse lung tumor tissues.

Supplementary Figure 6

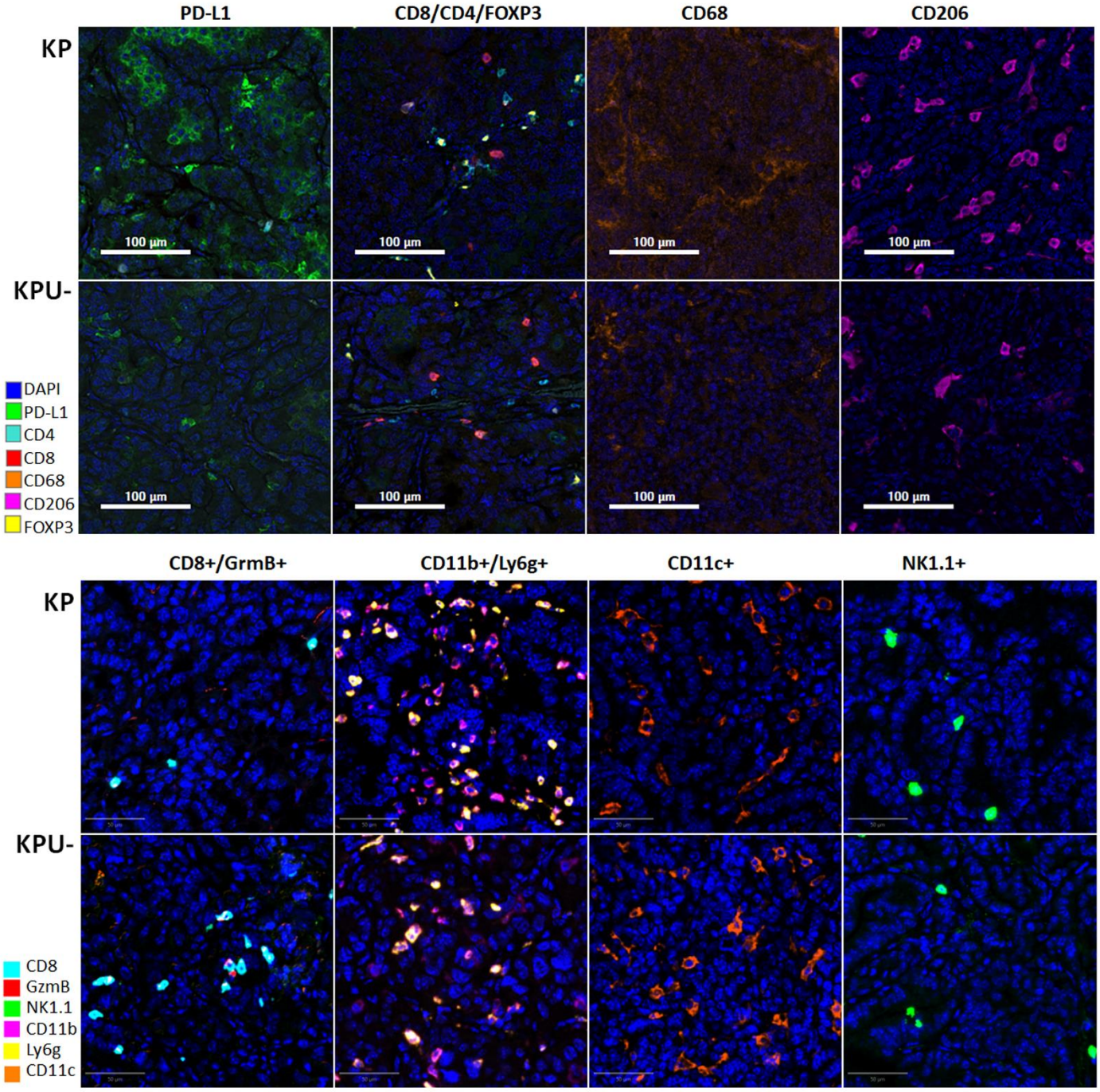

Figure S6. Representative mIF images of **PD-L1** (Green), **CD8/CD4/FOXP3** (Red/Cyan/Yellow), **CD68** (Brown), and **CD206** (purple) in upper panel); **Granzyme B** (GzmB), **NK1.1**, **CD11b**, **CD11c**, and **Ly6g** in lower panel, Scale bar: 50 µm; 200× magnification) in KP and KPU- lung tumor tissues.

Supplementary Figure 7

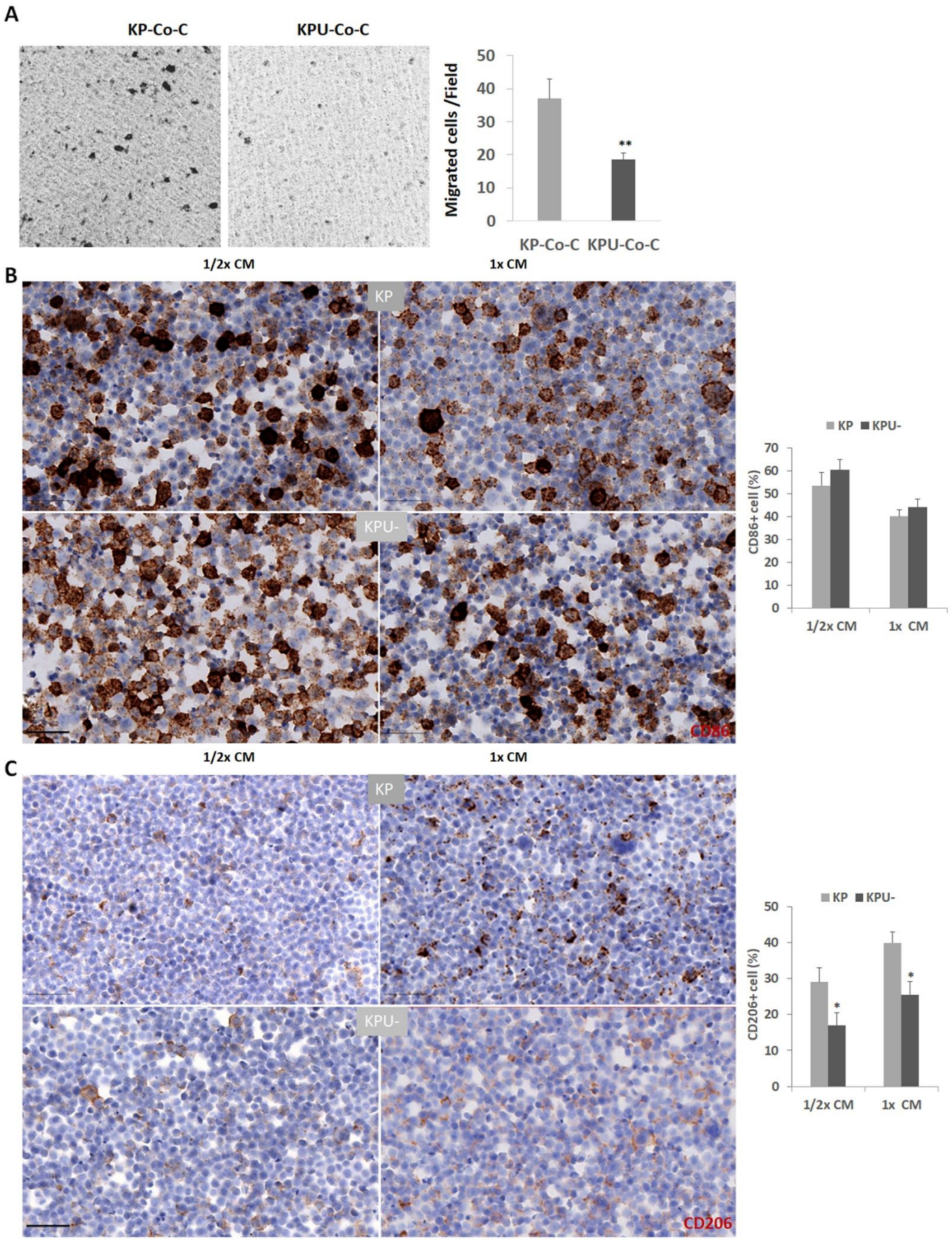

Figure S7. **Effects of KP and KPU- tumor cells on RAW264.7 macrophage migration and polarization.** (**A**) Left panel: representative images of chemotactic migration of RAW264.7 cells co-cultured with KP (KP-Co-C) or KPU- (KPU-Co-C) lung tumor cells in a Transwell assay; right panel: semiquantitative analysis of migrated cells (***P* < 0.01, KPU^- vs. KP). Dose-dependent suppression or enhancement of LPS/IFN-γ-induced M1 polarization (**B**) or TGF-β1-induced M2 polarization (**C**) by KP-CM and KPU-CM. Left panel: IHC staining of CD86 or CD206 (M1/M2 macrophage markers; scale bar: 50 μm; 200× magnification) in RAW264.7 cells cultured with medium containing 50% CM (1/2 CM) or 100% CM (1× CM); right panel: semiquantitative analysis of CD86- or CD206-positive cells based on data from triplicate samples from two independent experiments (**P* < 0.05, KPU vs. KP).

Supplementary Figure 8

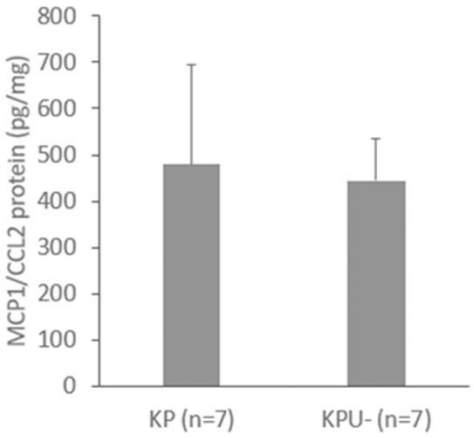

Figure S8. MCP1/CCL2 protein levels in KP and KPU- mouse lung tumors (n = 7) measured by ELISA.