Lysyl-tRNA synthetase-expressing colon spheroids induce M2 macrophage polarization to promote metastasis

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Supplementary figures.

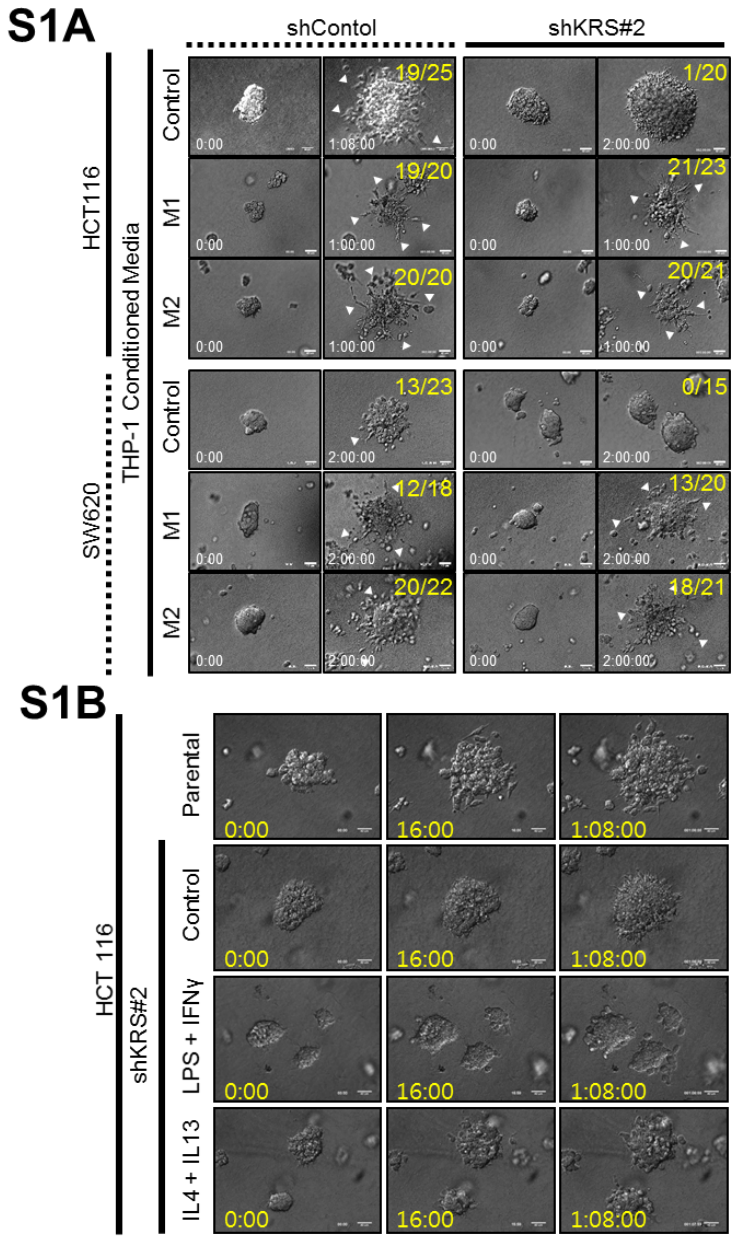


Figure S1. Conditioned media of macrophages affect invasive migration of HCT116 spheroids in 3D collagen I gels. (A) HCT116 or SW620 spheroids in 3D collagen I gels were treated with conditioned-media (CM) of THP-1 or its differentiated M1 or M2 macrophages (MΦ) and then time-lapse imaged for 1 day or 2 days (0^{day}:00^{h}:00^{min}). (B) HCT116-shKRS#2 spheroids in 3D collagen I gels were treated with vehicle (Control), LPS (100 ng/ml) + IFNγ (20 ng/ml), or IL4 (20 ng/ml) + IL13 (20 ng/ml). The spheroids were then time-lapse imaged for 1 day and 8 h (1:08:00), together with HCT116-parental spheroids, and the representative snap images of each spheroid were presented. Data represent three independent experiments. See also Figure 1.

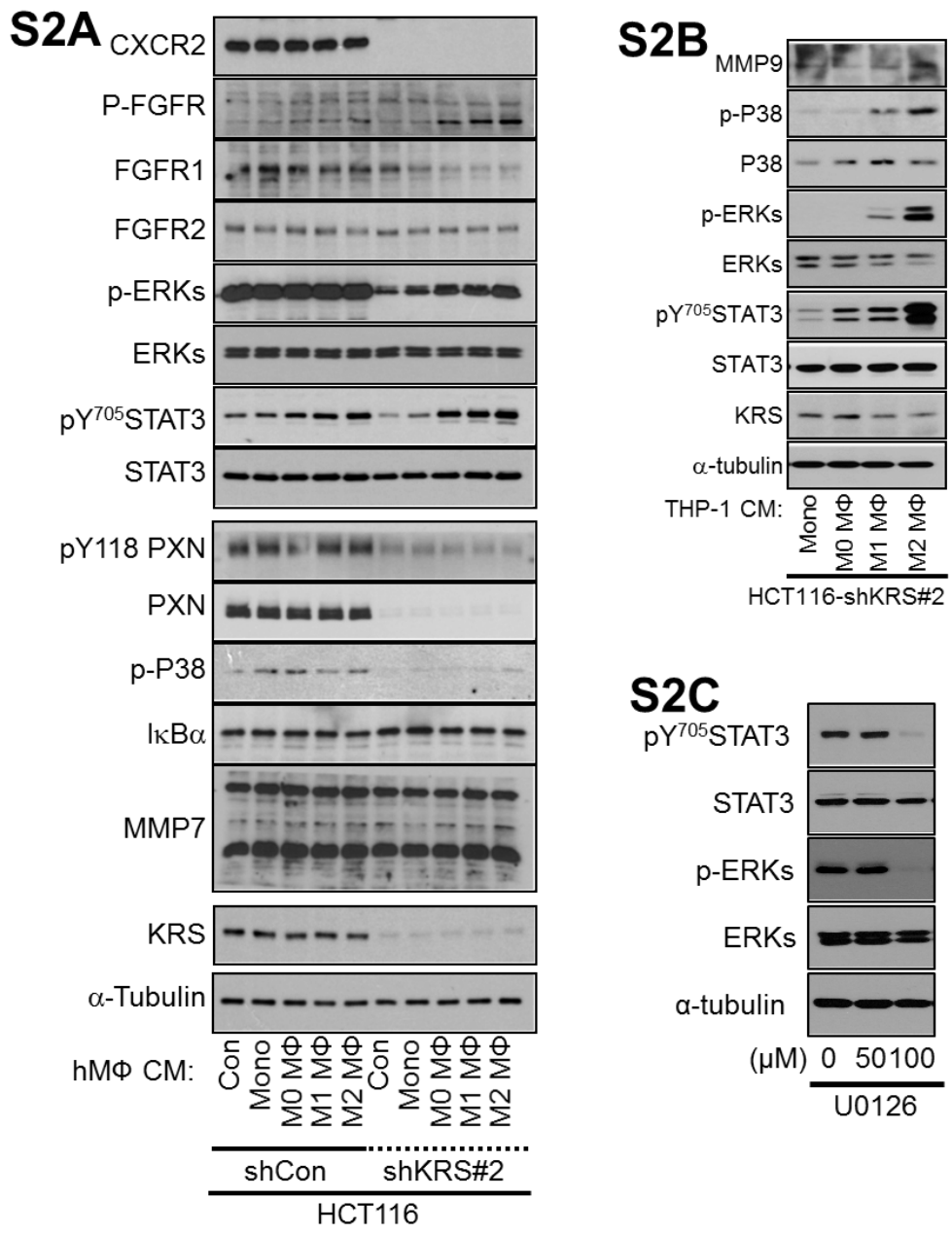


Figure S2. KRS-mediated intracellular signaling activity. (A and B) HCT116 spheroids embedded in 3D collagen I gels with various KRS expression levels (parental KRS-positive shControl spheroids and KRS-suppressed spheroids, shKRS#2) in the absence or presence of the conditioned media (CM) from human monocytes (Mono) or macrophages (MΦ) for 24 h were processed to immunoblotting. (C) Parental HCT116 spheroids in 3D collagen I gels treated with vehicle (0) or U0126 ERKs inhibitor for 24 h were analyzed by immunoblotting. Data represent three independent experiments. See also Figure 1.

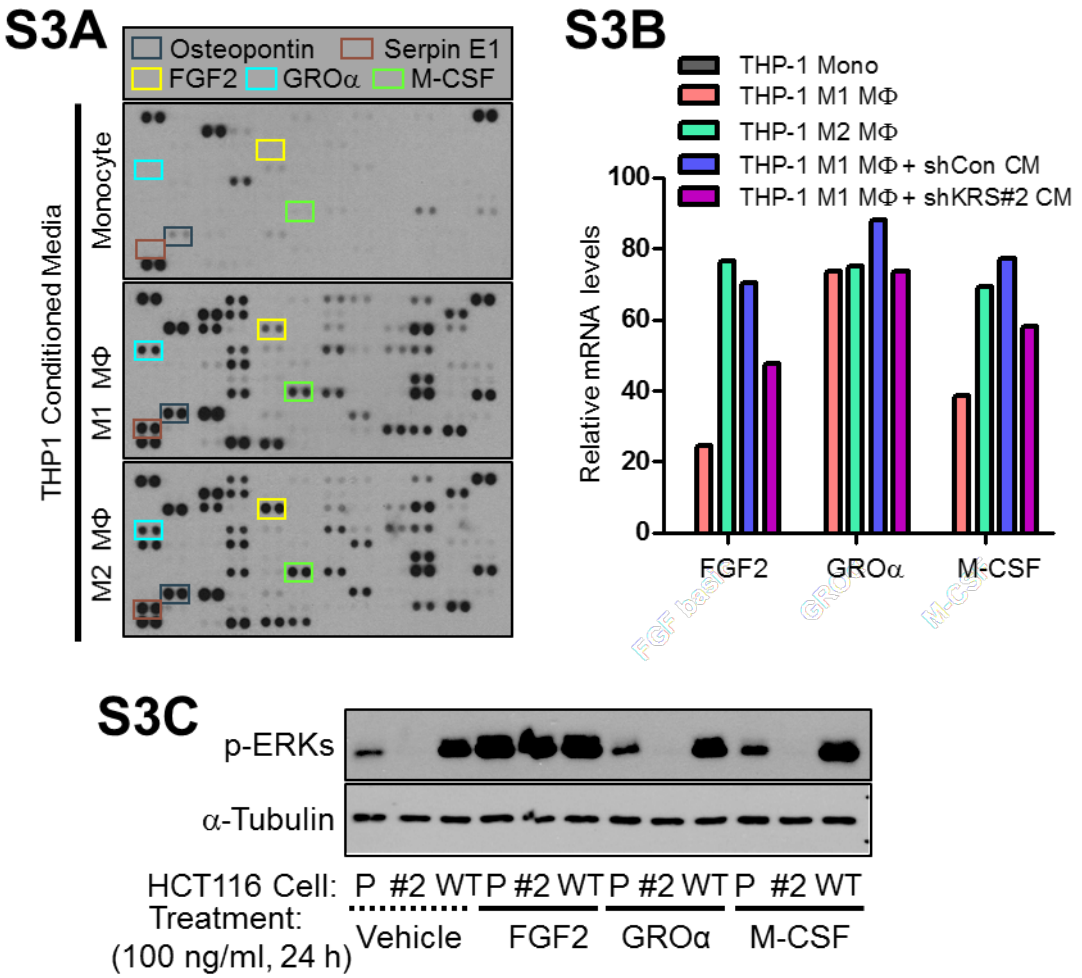
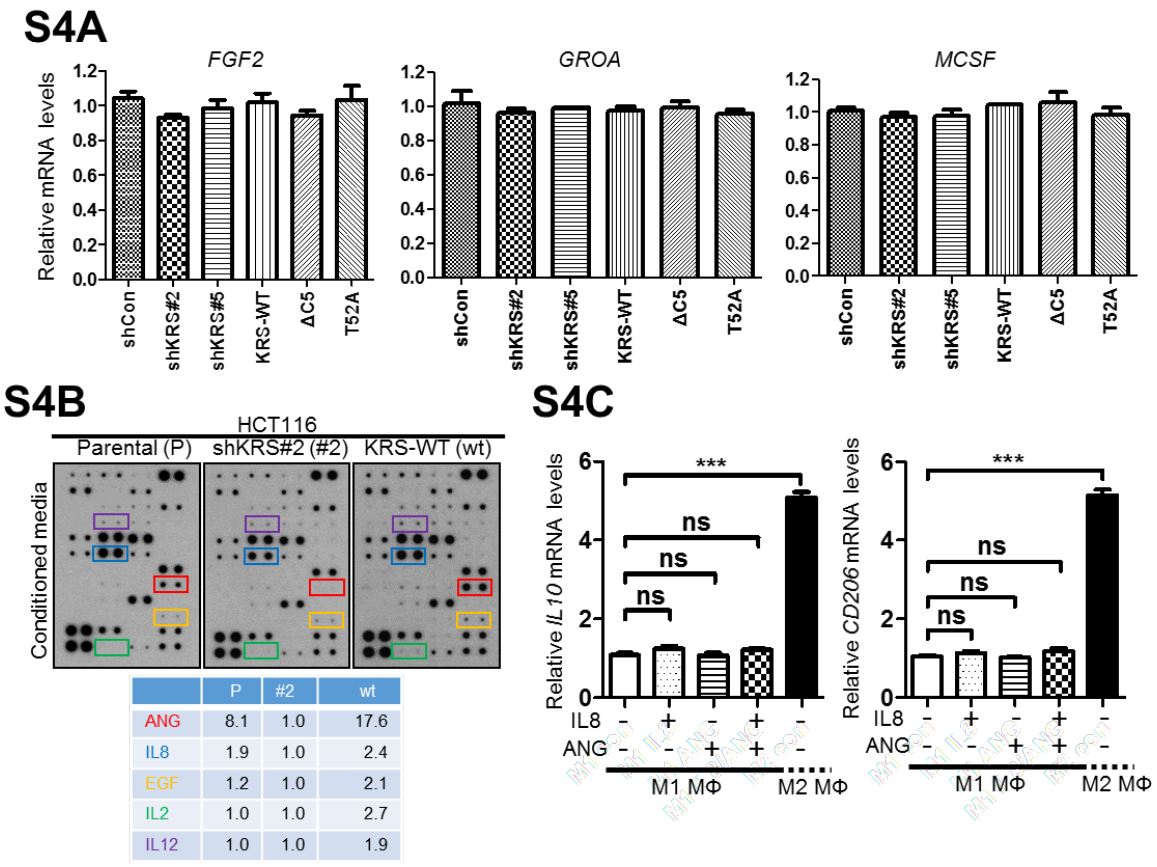


Figure S3. Macrophages produce cytokines to affect KRS-dependent signaling activity. (A) Antibody array for human cytokines and soluble factors was performed using the conditioned media (CM) from cultures of THP-1 monocytes, M1, and M2 macrophages. (B) mRNA levels of the soluble factors that were expressed in macrophages more than in monocytes and in macrophages treated with CM of the HCT116 spheroids were analyzed by q-PCR. (C) FGF2 that was expressed in M2 macrophages more than in THP-1 monocytes and M1 macrophages affected KRS-dependent ERKs activity. Data represent three independent experiments. See also Figure 2.



	P	#2	wt
ANG	8.1	1.0	17.6
IL8	1.9	1.0	2.4
EGF	1.2	1.0	2.1
IL2	1.0	1.0	2.7
IL12	1.0	1.0	1.9

Figure S4. Certain soluble factors induced by macrophages or KRS-positive cancer spheroids. (A) mRNA levels of the *FGF2*, *GROA*, and *MCSF* that are secreted by M2 macrophages more than by M1 macrophages or monocytes were analyzed for their expression levels in HCT116 spheroids. One-way ANOVA with Dunnett tests showed no significant differences between experimental conditions. (B) Cytokine antibody array was performed using conditioned media from HCT116 spheroids [of parental (P), KRS-suppressed (shKRS#2), or KRS WT-overexpressing (KRS-WT) cells] in 3D collagen I gels. (C) IL8 and ANG that were highly expressed in HCT116 spheroids depending on KRS expression were treated to M1 macrophages, before analysis of *IL10* or *CD206* mRNA levels. The data are presented as the mean ± standard deviation (SD). *** represents significance levels of $p < 0.001$ by one-way ANOVA with Dunnett tests. NS indicates non-significance. Data represent three independent experiments. See also Figures 3 and 4.

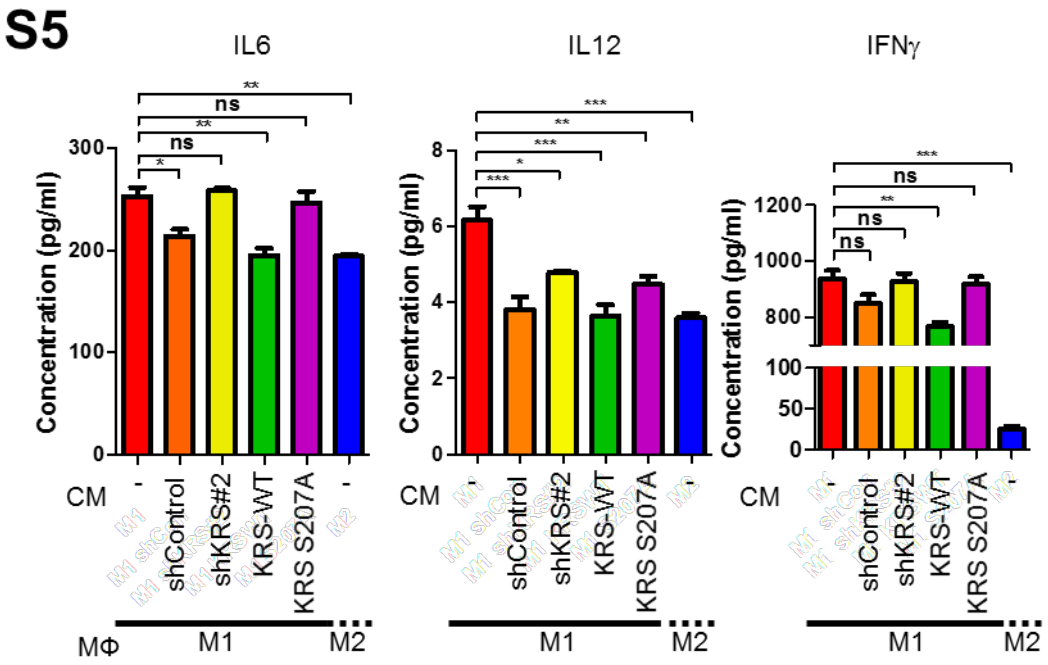


Figure S5. Macrophage (MΦ) markers induced by conditioned media from KRS-positive cancer spheroids. Conditioned media (CM) of HCT116 spheroids in 3D collagen I gels with various KRS expression levels (at shControl, KRS-suppressed (shKRS#2), KRS WT-overexpressing (KRS-WT), or KRS-S207A mutant-overexpressing levels) were treated to THP-1 M1 MΦ for 24 h, before determination of protein levels for IL-6, IL-12, and IFNγ via ELISA analysis. As M1 MΦ markers, IL6, IL12, and IFNγ showed a tendency to be decreased by CM from the KRS-positive spheroids, but not by CM from KRS-suppressed or KRS-S207A mutant spheroids, indicating an M2 polarization. The data are presented as the mean ± standard deviation (SD). *, **, and *** represent significance levels of $p < 0.05$, 0.01, and 0.001 by one-way ANOVA with Dunnett tests, respectively. NS indicates non-significance. Data represent three independent experiments. See also Figure 4.

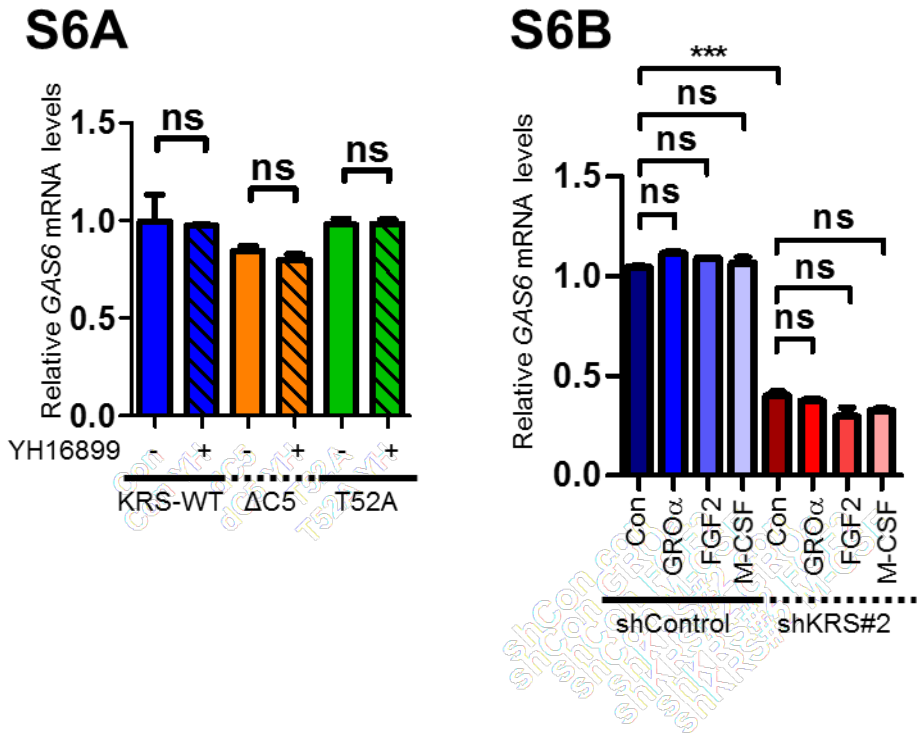


Figure S6. *GAS6* mRNA levels in cancer spheroids are not affected by a specific inhibitor of membranous KRS or the treatment of the soluble factors that were induced from M2 cytokines. (A) HCT116 spheroids expressing wildtype or mutants (ΔC5 unable to be secreted or T52A unable to be dissociated from cytosolic MSC enough to be localized to plasma membranes) were treated with YH16899, a specific inhibitor against KRS (12), prior to analysis of *GAS6* mRNA levels. (B) Vehicle (Con) or cytokines that were highly expressed from M2 macrophages were treated to KRS-positive shControl or KRS-suppressed shKRS#2 spheroids, prior to analysis of *GAS6* mRNA levels. The data are presented as the mean ± standard deviation (SD). *** represents significance levels of $p < 0.001$ by one-way ANOVA with Dunnett tests. NS indicates non-significance. Data represent three independent experiments. See also Figure 7.

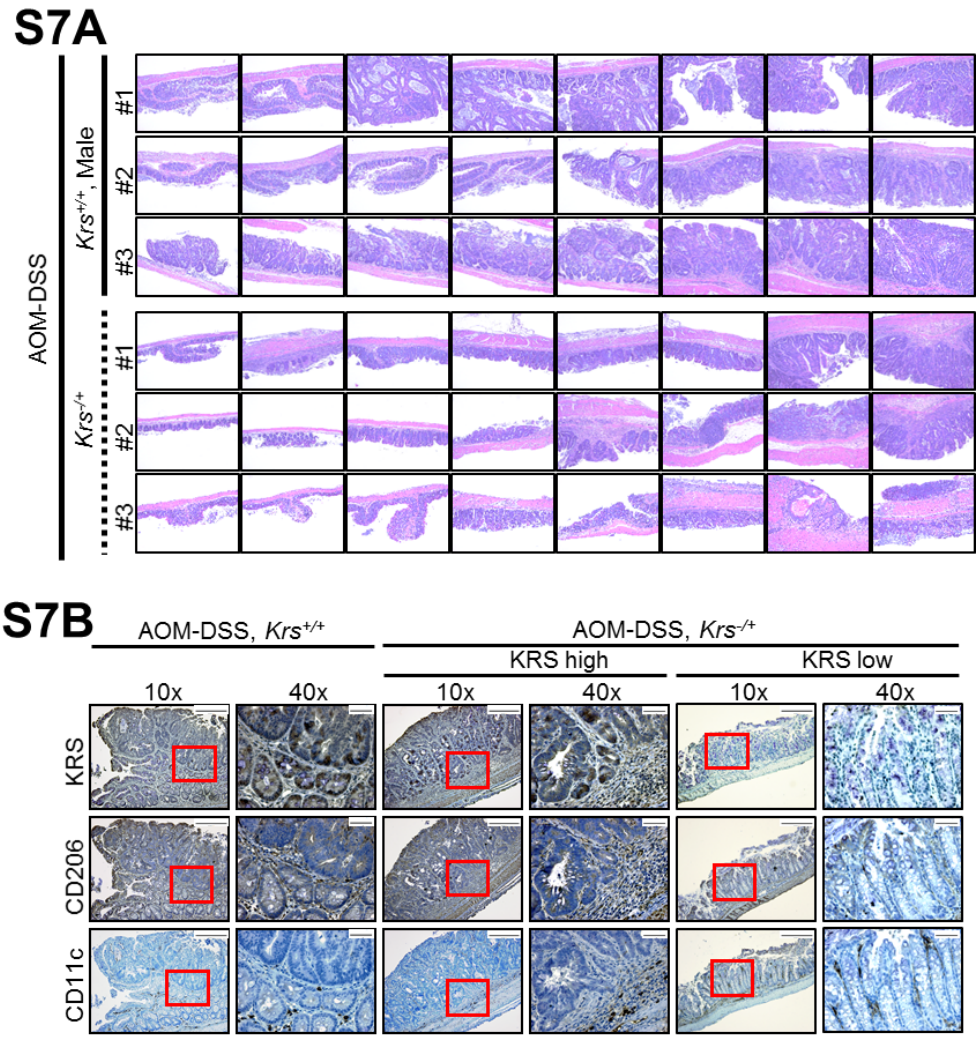


Figure S7. KRS-dependent colon carcinogenesis and metastasis. Ten-week-old C57BL/6 male normal mice (n=4) or *Krs*$^{-/+}$ knock-out mice (n=4) were treated with AOM-DSS, as explained in the Materials and Methods. (A) AOM-DSS-mediated colon carcinogenesis were severe in KRS-positive mice rather than *Krs*$^{-/+}$ knock-out mice. (B) Normal mice or *Krs*$^{-/+}$ knock-out mice treated with AOM-DSS, as above, were processed to immunostaining analysis of colon tissues to show that the KRS-positive colon tumor regions were near CD206- but not CD11c- positive immune cells. See also Figure 9.

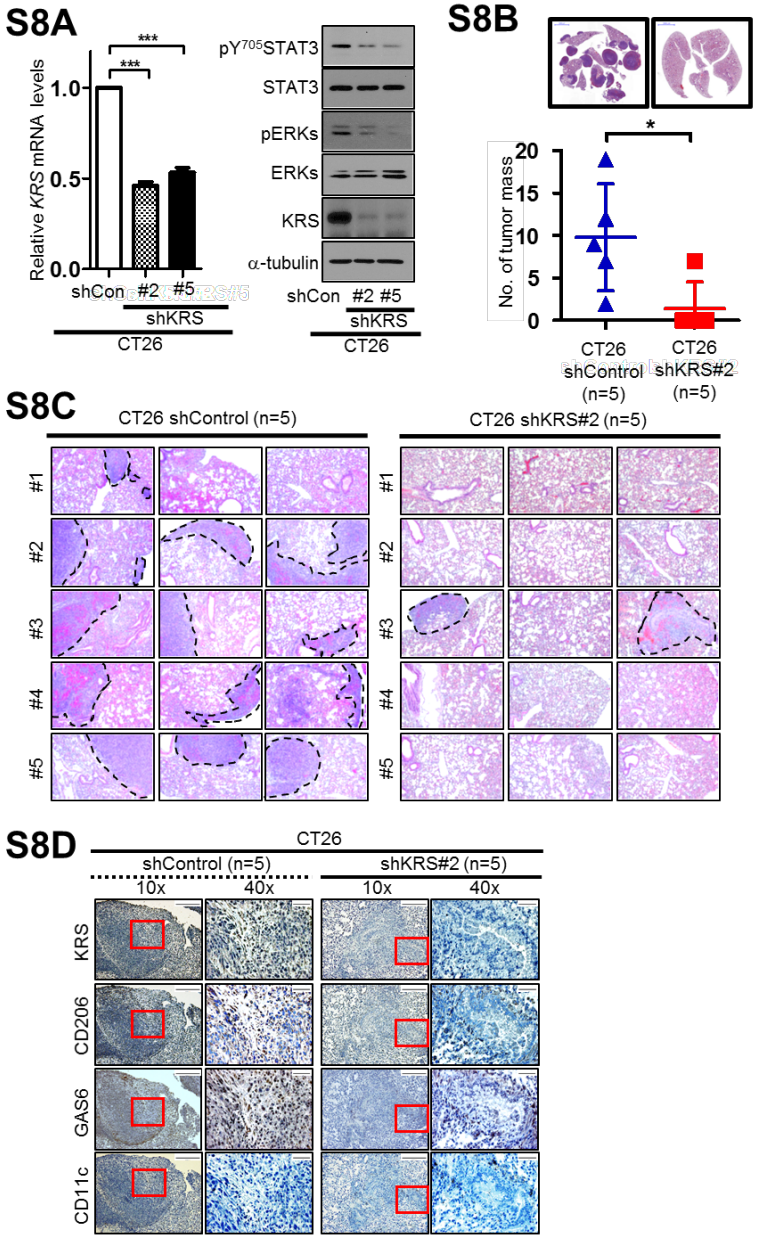


Figure S8. Tail vein injection of murine colon cancer CT26 cells shows KRS-dependent lung metastasis. Tail vein injection of murine colon cancer CT26 cells with control shRNA or shRNA against KRS [targeting sequence #2 or #5] was done to 8-week-old female Balb/c mice (each n=5) and 3 weeks later the lung tissues were evaluated for the signaling activities via immunoblottings, compared with the cells with KRS suppression (A). The data are presented as the mean ± standard deviation (SD). *** represents significance levels of $p < 0.001$ by one-way ANOVA with Dunnett tests. NS indicates non-significance. The lung tissues were also immunostained to see that control KRS-positive CT26 cells led to more severe cancer cell colonization in animal lungs. Data values were presented at mean ± standard deviation (B and C). * represents significance levels of $p < 0.05$ by Student's t test. The lung tissues were also immunostained to show KRS expression-dependent overlapping of KRS, CD206 and GAS6 but not CD11c. See also Figure 9.