**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure 1.** (A) Western blot for Kras following transduction with Kras shRNA (sh.Kras) or scrambled control shRNA (sh.Scr) into Tcon3077 and Tcon3944 cells. -actin, loading control. (B) Proliferation assay for Tcon3077 and Tcon3944 cells following transduction with sh.Kras or sh.Scr. (C) Photos and graphs of migration and invasion assays for Tcon3077 and Tcon3944 cells after transduction with sh.Kras or sh.Scr. \*p<0.05 compared to control. (D) Photos of spheroid formation assay for Tcon3077 and Tcon3944 cells after transduction with sh.Kras or sh.Scr. Graphs quantifying CD44 (E), phospho-MEK1/2 (F), and combined CD44/phospho-MEK1/2 immunofluorescence staining (G) in primary gastric tumors, microscopic lung metastases, and macroscopic lung metastases from the GA GEMM. \*p<0.05 compared to other groups.

**Supplementary Figure 2.** (A). Western blot analysis demonstrating levels of active and total Kras, MEK1/2, and ERK1/2 in HFE-145 cells transduced with oncogenic *KRAS* (*KRASG12V*), wild-type *KRAS* (*KRASWT*) or control (Vector). (B) Graph showing cell growth over 3 days of HFE-145 cells transduced with *KRASG12D, KRASWT,* or Vector. (C-D) Representative data and graph of fluorescence-activated cell sorting (FACS) analysis demonstrating proportion of CD44(+) cells in monolayer cells and in spheroids transduced with *KRASG12D, KRASWT,* or Vector. β-actin, loading control. Bars represent standard deviation. \*p<0.05 compared to control.

**Supplementary Figure 3.** (A) Western blot showing expression of CD44, Sox2, Oct-4, Nanog, and c-Myc in gastric cancer cell lines grown as monolayer cells versus as spheroids. β-actin was used as housekeeping gene. (B) Immunofluorescence photos of gastric cancer cells in spheroids transduced with sh.KRAS or sh.Scr. Scale bar, 100 m. (C) Photos and graphs of single cell assay in AGS and KATOIII spheroid cells showing diameter of spheroids at selected time points following transduction sh.KRAS ras or sh.Scr and grown in spheroid formation conditions. Bars represent standard deviation. \*p<0.05 compared to control.

**Supplementary Figure 4.** (A) Western blot analysis of AGS and KATOIII gastric cancer spheroid cells treated with PD0325901 or carrier (DMSO) in spheroid formation condition for levels of phospho-ERK1/2, ERK, CD44, Sox2, Oct-4, Nanog, and c-Myc. (B) Immunofluorescence photos of gastric cancer cells in spheroids treated with PD0325901 or DMSO. Scale bar, 100 m. (C) Immunofluorescence photos and graph of AGS- and KATOIII spheroids with GFP treated with PD0325901 or DMSO in spheroid formation condition. (D) Photos and graphs of single-cell assay in AGS and KATOIII gastric cancer spheroid cells showing diameter of spheroids at selected time points treated with PD0325901 or DMSO and grown in spheroid formation conditions. β-actin, loading control. Bars represent standard deviation. \*p<0.05 compared to control.

**Supplementary Figure 5.** (A)Western blot analysis for total and phosphorylated ERK1/2 and stemness factors in AGS and KATO spheroid cells that have been FACS sorted for CD44 expression and treated with the MEK inhibitor PD0325901 or carrier (DMSO). (B) Photos and graphs of spheroid formation assay for AGS spheroid cells stably transduced with GFP which were FACS-sorted for CD44(+) and CD44(-) following treated with PD0325901 or DMSO. (C) Western blot analysis for total and phosphorylated ERK1/2 and EMT-related factors in AGS spheroid cells that have been FACS sorted for CD44 expression and treated with PD0325901 or carrier (DMSO). (D) Graphs of migration and invasion assays for AGS and KATO spheroid cells that have been FACS sorted for CD44 expression and treated with the MEK inhibitor PD0325901 or carrier (DMSO).

**Supplementary Figure 6.** (A) Proliferation assays for monolayers and spheroids of gastric cancer cells following treatment with 5-fluorouracil (5-FU) or cisplatin chemotherapy. (B) Proliferation assays for AGS and KATOIII gastric cancer spheroid cells treatment with sh.KRAS or sh.Scr, 5-fluorouracil (5-FU) or cisplatin chemotherapy. (C) Tumor growth curves for AGS xenografts treatment with sh.Scr or sh.KRAS and PBS or cisplatin. (D) Immunofluorescence photos and graphs following immunohistochemical analysis of tumors for proliferation using Ki-67 (green), apoptosis using cleaved caspase-3 (red), and CD44 (green). Scale bar, 50 m. Bars represent standard deviation. \*p<0.05 compared to control.

**Supplemental Figure 7.** (A) Photos and graphs of migration and invasion assays (B) and soft agar assay for spheroids of AGS and KATOIII transduced with sh.KRAS or sh.Scr. (C) Western blot analysis for MAPK proteins and EMT-related proteins in AGS and KATOIII spheroid cells treated with the MEK inhibitor PD0325901 or carrier (DMSO). (D) Western blot analysis for KRAS protein in AGS spheroid cells with sh.Scr or sh.KRAS. Bars represent standard deviation. \*p<0.05 compared to control.