Supplemental figure legends

Supplemental Figure 1. Reproducibility of sample preparation.

The tissue sample, Cm-8 (Supplemental Table 1), was crushed and separated into two pieces (Cm-8a and Cm-8b). Membrane fraction proteins prepared from these pieces were alkylated and then digested with trypsin after adding BSA as an internal standard. Cm-8a and -8b were labeled with iTRAQ reagent 114 or 116, respectively. The labeled samples were mixed and analyzed by LC-MS/MS. The raw data were analyzed using Proteome Discoverer (Experimental Procedures). The unique peptides of BSA were searched by Proteome Discoverer ver.1.1 (Thermo Fisher Scientific) against MSIPI-human version 3.67 containing the sequences of BSA and used to obtain the iTRAQ ratio of added BSA. iTRAQ ratios of identified proteins were normalized with the iTRAQ ratio of BSA.

Supplemental Figure 2. Example of quantitation using SRM/MRM.

The result of ITGA5 is shown as representative of SRM/MRM analysis. Peak area ratios of endogenous and SI peptide of each transition are shown. The coefficients of variation (CV) of peak area ratios of four transitions were below 15.8%. Assays were constructed to measure two distinct peptides per-protein listed in Supplemental table 5 and that the

individual assays for each of the two peptides are labeled SRM-1 and SRM-2. Endogenous, endogenous peptide. SI, stable isotope-labeled peptide.

Supplemental Figure 3. Results of iTRAQ and SRM/MRM analysis for confirmation.

The iTRAQ data and SRM/MRM data are presented in a dot plot. Horizontal bar through each set of data shows the mean. P, polyp. C, cancer without metastasis. Cm, cancer with metastasis. Area ratio, the ratio of peak area of endogenous peptide to that of SI peptide. A, Proteins increased between polyps and cancer without metastasis. B, Proteins increased between cancer with and without metastasis. C, Proteins decreased between polyps and cancer without metastasis. D, Proteins decreased between cancer with and without metastasis. E, Proteins increased between polyps and cancer with metastasis. Assays were constructed to measure two distinct peptides per-protein listed in Supplemental table 5 and that the individual assays for each of the two peptides are labeled SRM-1 and SRM-2.

Supplemental Figure 4. Correlation of iTRAQ and SRM/MRM quantitation results.

The iTRAQ and SRM ratios of ITGA5, GPRC5A, PDGFRB and TFRC were plotted on each graph. Each data point represents a given peptide ratio in the same samples,

which were quantified by either iTRAQ or SRM assay. Correlation coefficients are shown in plots. Assays were constructed to measure two distinct peptides per-protein listed in Supplemental table 5 and that the individual assays for each of the two peptides are labeled SRM-1 and SRM-2.

Supplemental Figure 5. Verification of candidate proteins by SRM/MRM.

The SRM/MRM data for verification are presented in a dot plot. Horizontal bar through each set of data shows the mean. P, polyp. C, cancer without metastasis. Cm, cancer with metastasis. Area ratio, the ratio of peak area of endogenous peptide to that of SI peptide.

Supplemental Figure 6. Tissue microarray of C8orf55.

Multiple cancer tissue microarray (TMA1150) (A) and normal tissue array (B) were stained with anti-C8orf55 antibody. TMA1150 has 1150 cores from 14 common cancer types ((a) lung (squamous cell carcinoma), (b) breast, (c) biliary tract, (d) liver (hepatocellular carcinoma), (e) stomach, (f) lung (adenocarcinoma), (g) kidney (renal cell carcinoma), (h) thyroid, (i) colon, (j) prostate, (k) pancreas, (l) urothelial, (m) ovary, (n) uterine corpus). The normal tissue array has 20 cores from 13 tissues ((a) lung, (b)

breast, (c) bile tract, (d) liver, (e) stomach, (f) lung, (g) kidney, (h) thyroid, (i) colon, (j) prostate, (k) pancreas, (l) urine, (m) uterine tube/surface epithelium, (n) uterus).