**Supplemental Materials**

Table S1. Antibodies used for IHC staining

| Primary antibody | Clone | Vendor | Catalog No. | Antigen retrieval | Primary antibody dilution | Primary antibody concentration |
|---|---|---|---|---|---|---|
| SPT16 | polyclonal | Santa Cruz | sc-28734 | Citrate buffer, 40 minutes | 1/100 | 2 µg/ml |
| SSRP1 | 10D1 | Biolegend | 609702 | EDTA buffer, 60 minutes | 1/200 | 2.5 µg/ml |

Table S2. Primers and conditions of PCR

| Target gene | Sequence of forward primer | Sequence of reverse primer | Tm | Cycles # |
|---|---|---|---|---|
| SPT16 | ctagggtttgggatgggaat | gattgtggcaatgtgaaacg | 50ºC | 23 |
| SSRP1 | cgtggtcgttatgacattcg | ttcatgccctctttcaatcc | 50ºC | 22 |
| GAPDH | gcttccgtgtccccactgc | ggctggtggtccaggggtct | 58ºC | 19 |

## Table S3 – Adjusted Survival Analysis

| FACT | Outcome | Adjusted Hazard Ratio (95% CI) | P-value |
|---|---|---|---|
| SSRP1 | Overall Survival | 1.14 (0.84, 1.54) | 0.41 |
| | Disease Specific | 1.02 (0.67, 1.55) | 0.93 |
| | Recurrence Free | 1.19 (0.82, 1.74) | 0.36 |
| | Progression Free | 1.06 (0.77, 1.47) | 0.72 |
| | | | |
| SPT16 | Overall Survival | 0.78 (0.51, 1.18) | 0.24 |
| | Disease Specific | 0.76 (0.42, 1.34) | 0.34 |
| | Recurrence Free | 0.94 (0.53, 1.65) | 0.81 |
| | Progression Free | 1.00 (0.61, 1.62) | 0.98 |

95% CI = 95% Confidence Interval

Hazard Ratio for *high* versus *low* expression

## Table S4 – Associations between SSRP1Expression and Survival Outcomes in patient Sub-groups

| | OS | | DS | | RFS | | PFS | |
|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Overall Sample | 1.20 (0.91, 1.57) | 0.20 | 1.09 (0.75, 1.58) | 0.67 | 1.29 (0.92, 1.80) | 0.15 | 1.19 (0.89, 1.60) | 0.23 |
| ER/PR + | 0.78 (0.48, 1.28) | 0.33 | 0.53 (0.24, 1.19) | 0.12 | 0.85 (0.46, 1.57) | 0.61 | 0.68 (0.39, 1.20) | 0.19 |
| ER/PR - | 1.25 (0.82, 1.90) | 0.30 | 0.98 (0.58, 1.65) | 0.93 | 1.21 (0.71, 2.07) | 0.49 | 1.09 (0.70, 1.69) | 0.71 |
| Triple + | 0.59 (0.26, 1.33) | 0.20 | 0.34 (0.08, 1.41) | 0.14 | 0.63 (0.24, 1.63) | 0.34 | 0.50 (0.22, 1.12) | 0.09 |
| Triple - | 1.12 (0.67, 1.89) | 0.67 | 0.86 (0.43, 1.74) | 0.68 | 1.12 (0.56, 2.23) | 0.75 | 1.11 (0.61, 2.00) | 0.73 |
| Grade 1/2 | 1.12 (0.61, 2.06) | 0.70 | 0.83 (0.31, 2.23) | 0.71 | 1.56 (0.78, 3.12) | 0.21 | 1.49 (0.82, 2.71) | 0.19 |
| Grade 3 | 1.09 (0.80, 1.49) | 0.60 | 1.07 (0.71, 1.61) | 0.75 | 1.06 (0.72, 1.57) | 0.77 | 0.96 (0.69, 1.35) | 0.83 |
| Clin Stage 0/1 | 0.93 (0.36, 2.40) | 0.88 | 1.28 (0.23, 6.96) | 0.78 | 2.68 (0.80, 8.99) | 0.11 | 1.81 (0.60, 5.51) | 0.29 |
| Clin Stage 2 | 0.97 (0.54, 1.72) | 0.91 | 0.61 (0.29, 1.27) | 0.19 | 0.94 (0.49, 1.82) | 0.86 | 0.90 (0.49, 1.63) | 0.72 |
| Clin Stage 3/4 | 1.95 (0.96, 3.98) | 0.07 | 1.64 (0.77, 3.50) | 0.20 | 2.11 (0.73, 6.12) | 0.17 | 1.40 (0.71, 2.78) | 0.33 |
| Path Stage 0/1 | 1.09 (0.56, 2.09) | 0.81 | 0.72 (0.20, 2.53) | 0.61 | 2.65 (1.20, 5.85) | 0.02 | 1.89 (0.93, 3.84) | 0.08 |
| Path Stage 2 | 0.93 (0.60, 1.45) | 0.75 | 0.69 (0.36, 1.33) | 0.27 | 0.73 (0.44, 1.24) | 0.25 | 0.69 (0.42, 1.13) | 0.14 |
| Path Stage 3/4 | 1.30 (0.75, 2.25) | 0.36 | 1.31 (0.70, 2.48) | 0.40 | 1.21 (0.56, 2.62) | 0.62 | 1.02 (0.59, 1.78) | 0.93 |

HRs are for *h*SSRP1 versus *l*SSRP1
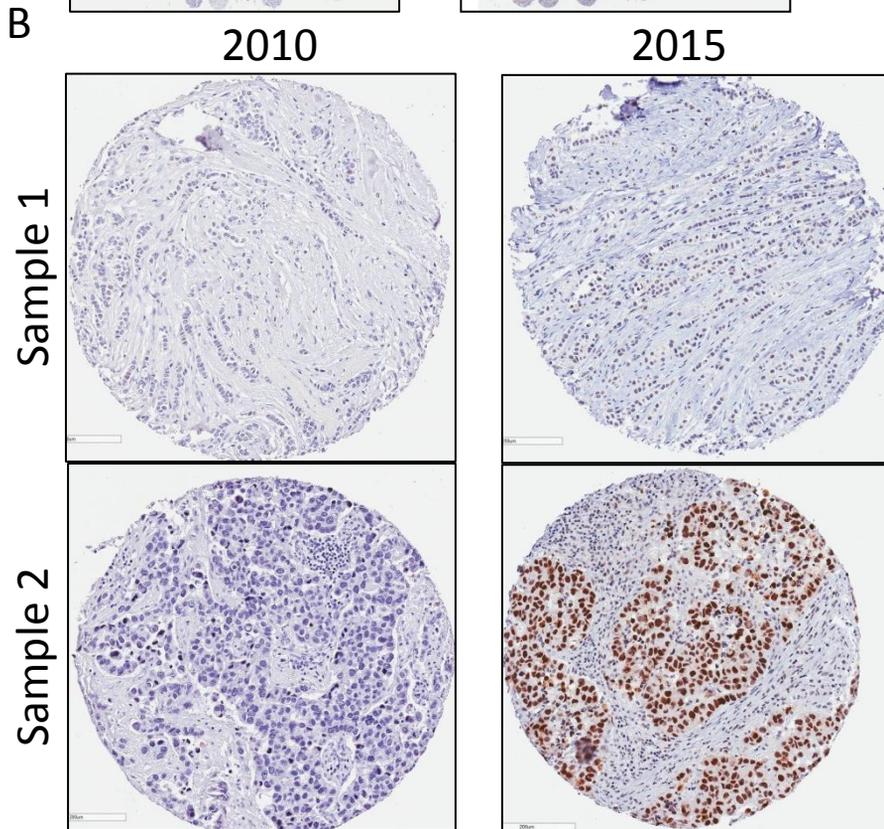
Supplemental Figure S1. Comparison of SSRP1 IHC staining done in 2010 (reported in Garcia et al., Cell Reports, 2013) and in 2015 (this study). A. 4x magnification view of scanned TMA slides stained with antibody to SSRP1 oriented the same way. 10x magnification view of two random samples from the scanned image of the same TMA, stained in 2010 and 2015.
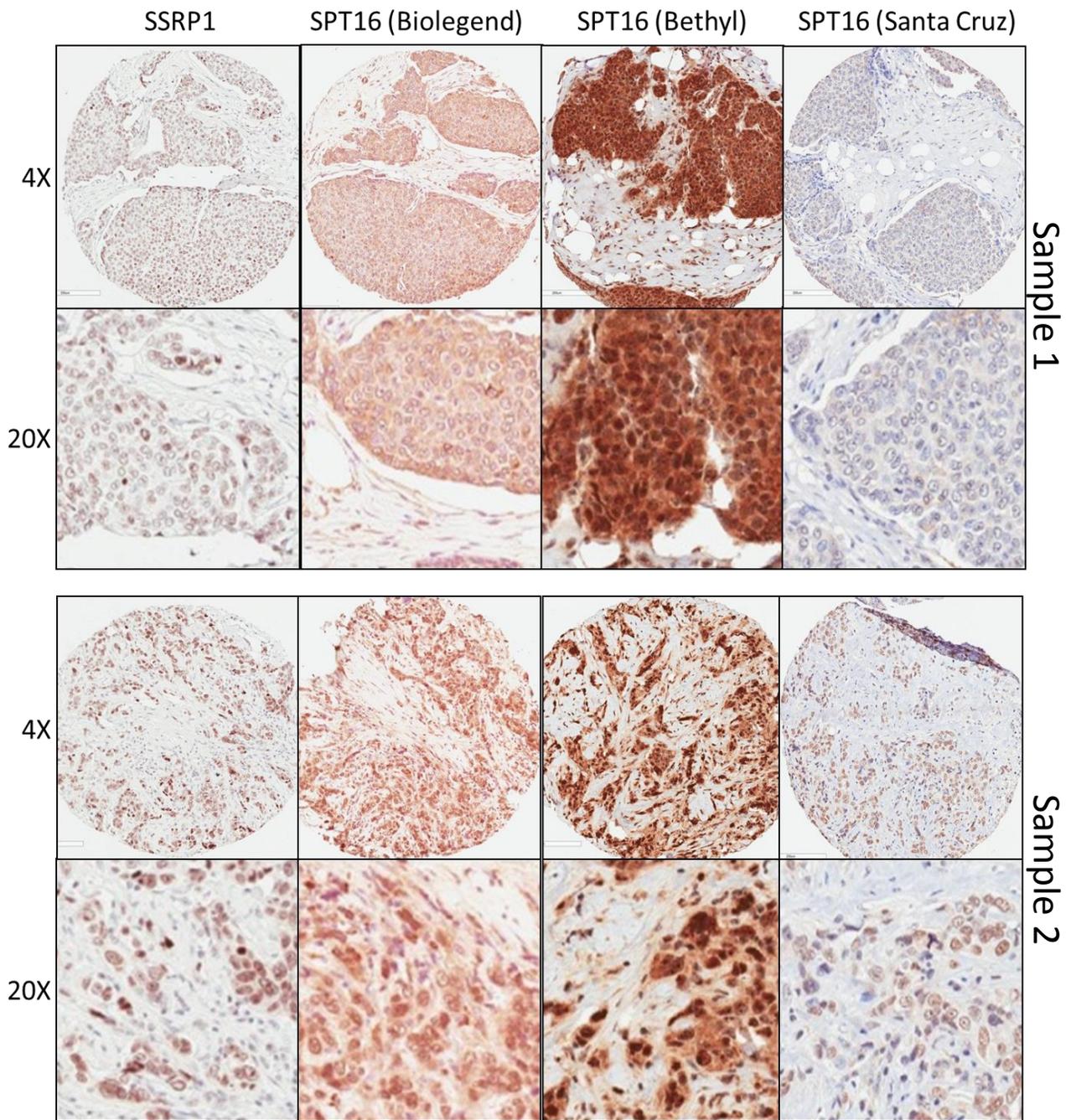
Supplemental Figure S2. Examples of staining of two random samples with antibodies to SSRP1 and SPT16 available from different commercial vendors. Santa-Crus antibody was selected for the study due to the most selective nuclear staining of tumor cells similarly to SSRP1 antibody.
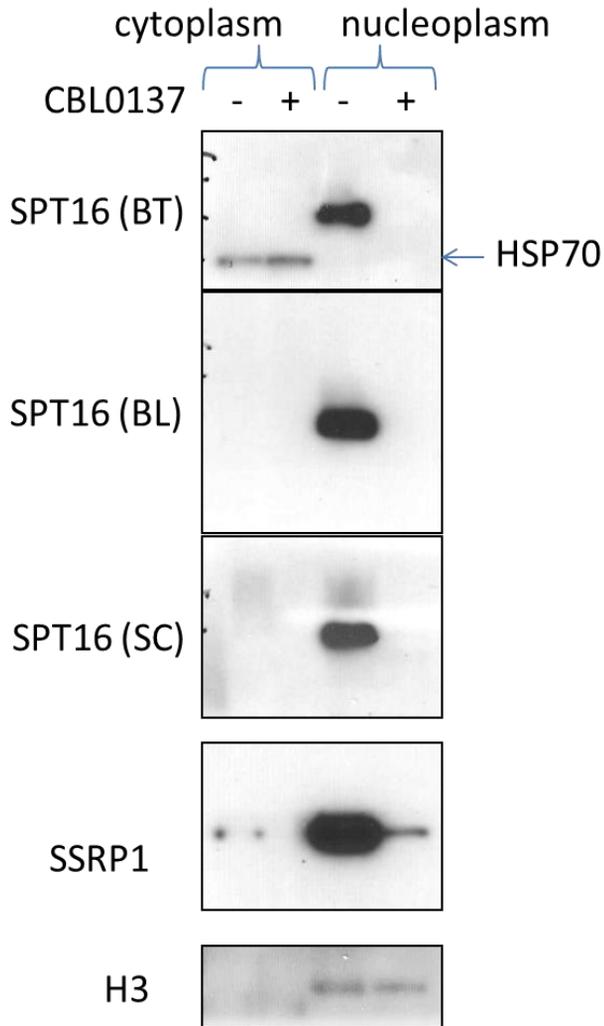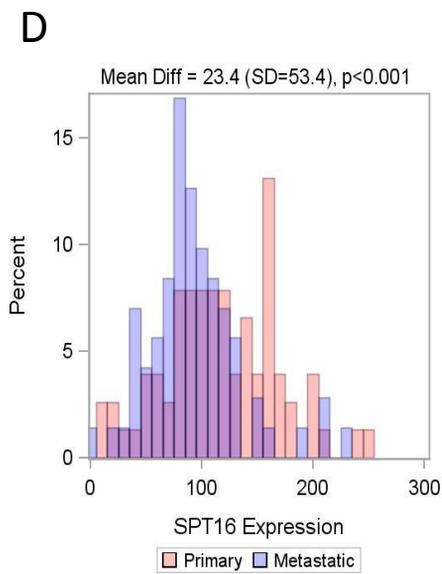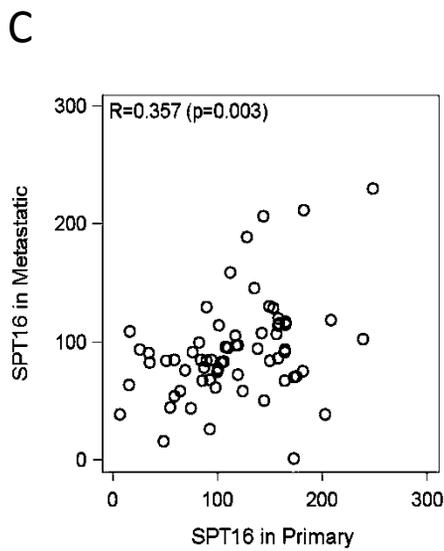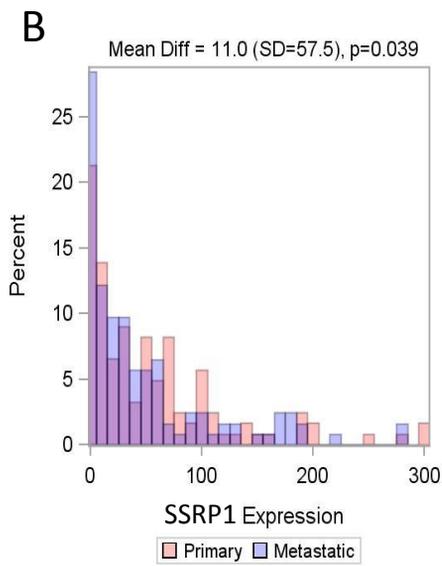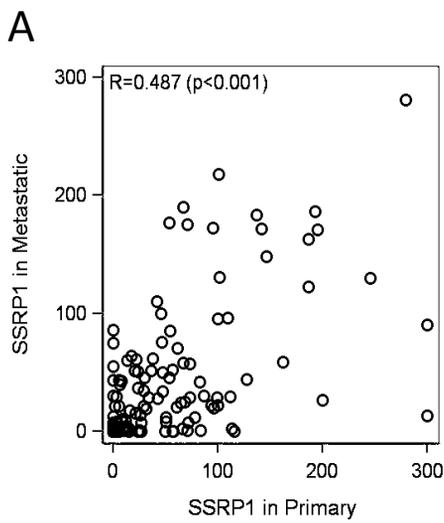
Supplemental Figure S3. Testing of specificity of cytoplasmic signal observed with SPT16 antibody upon IHC staining. Hela cells were treated with 3μM of CBL0137 for 30 minutes, what led to the disappearance of FACT from soluble fraction of cell extract (FACT is precipitated in chromatin pellet (Gasparian et al., Sci Transl Med, 2011). Cytoplamic and nuclear fractions of samples were leaded on gel several times to be independently stained with different antibodies: SPT16 from different vendors, BT from Bethyl Laboratories, BL – from Biolegend, SC – from Santa Cruz Biotehnology. Antibodies to Hsp70 and histone H3 were used for the control of cytoplasmic and nuclear extractions respectively.
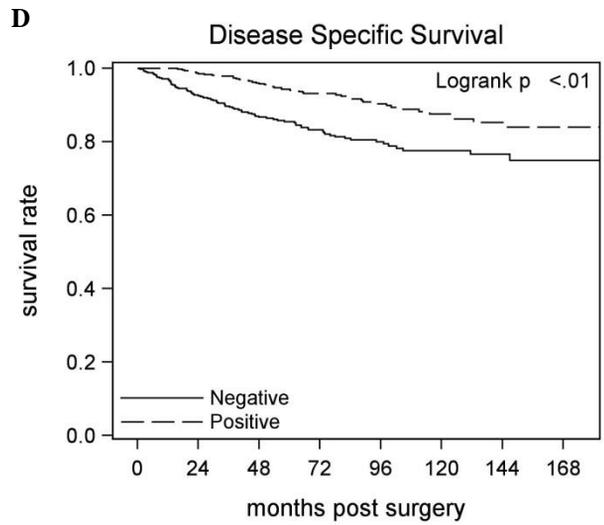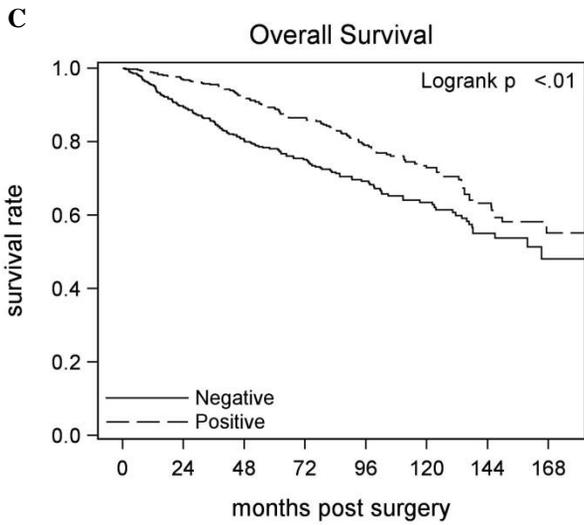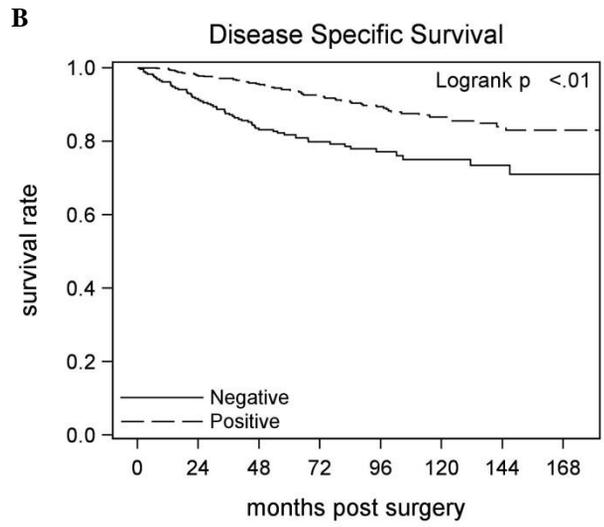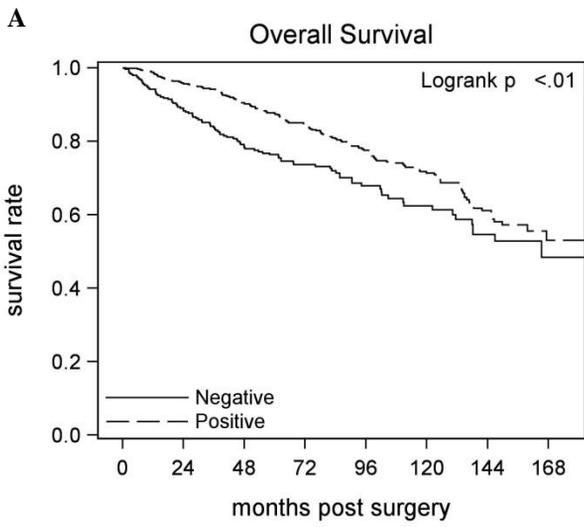
Supplemental Figure S4: Kaplan-Meier curves of overall and disease specific survival by ER (A,B) and PR (C,D) status. Comparisons were made using the log-rank test.
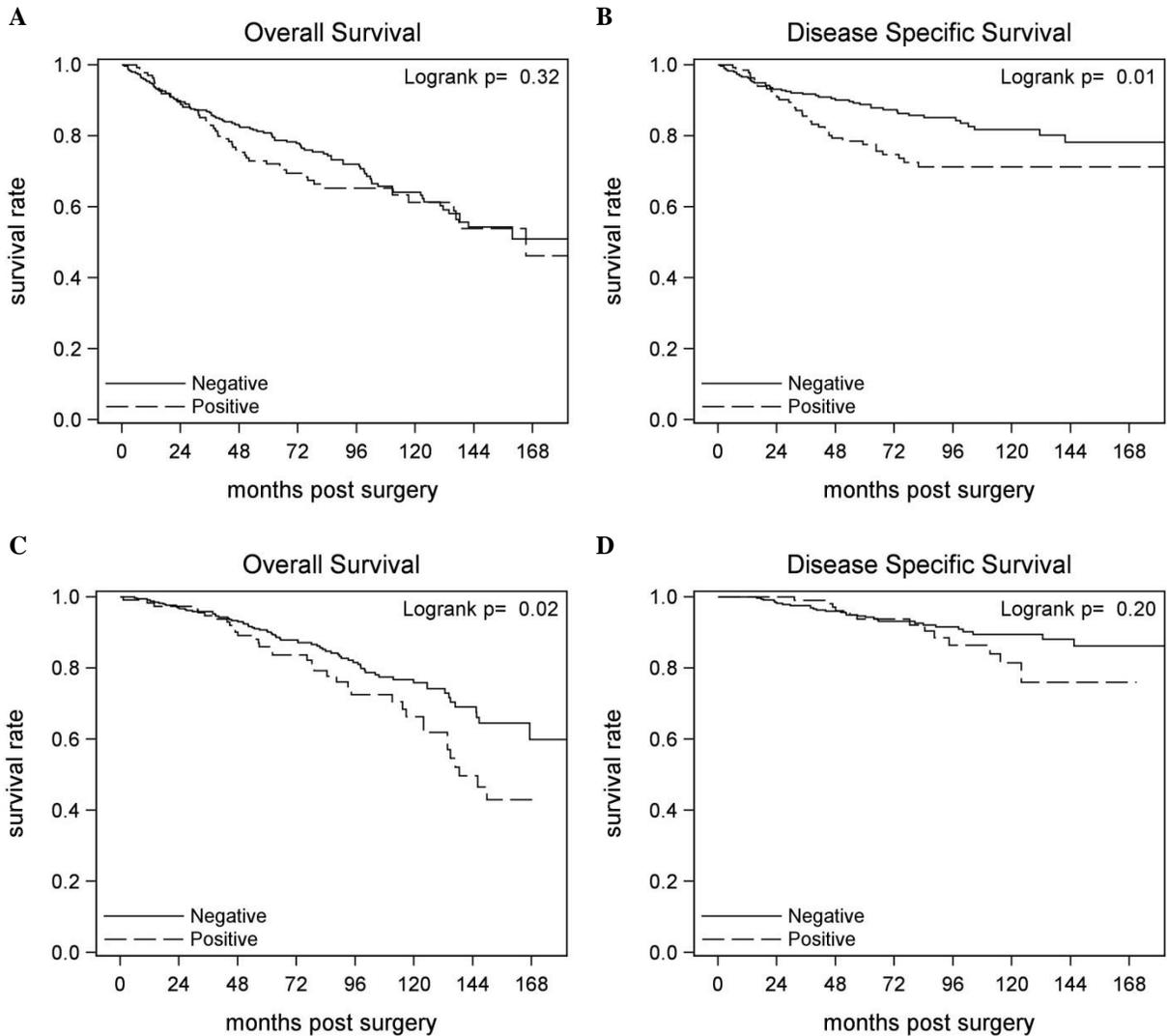
Supplemental Figure S5: Kaplan-Meier curves of overall and disease specific survival by HER-2 status in ER/PR double negative (A,B) and ER/PR non-double negative (C,D) patients. Comparisons were made using the log-rank test.

**A** R=0.487 (p<0.001)

**B** Mean Diff = 11.0 (SD=57.5), p=0.039

**C** R=0.357 (p=0.003)

**D** Mean Diff = 23.4 (SD=53.4), p<0.001

Supplemental Figure S6: Comparison of SSRP1 and SPT16 scores in primary and metastatic samples from the same patients. A, C. Scatter plot of SSRP1 (A) and SPT16 (D) scores from metastatic versus primary samples. B.E. Histograms of distribution of SSRP1 (B) and SPR16 (E) scores in primary and metastatic samples.

A
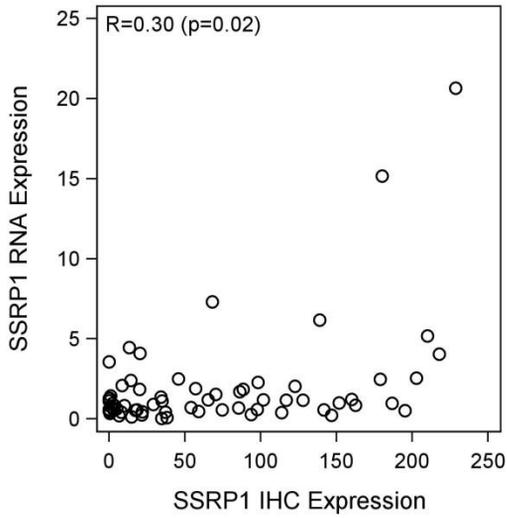
B
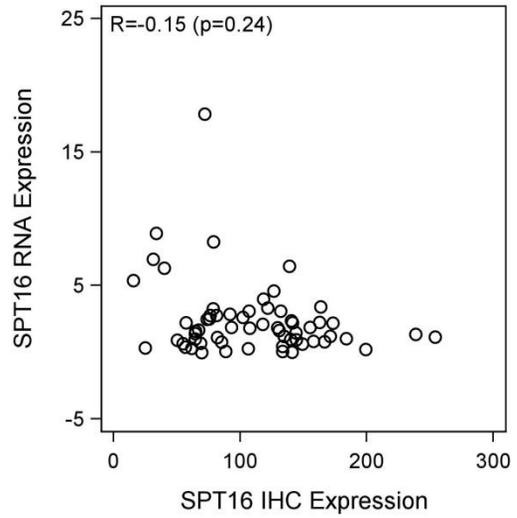


Supplemental Figure S7: Scatter plot of IHC and RNA expression for SSRP1 (A) and SPT16 (B). The potential relationships were assessed using the Spearman correlation coefficient (R).
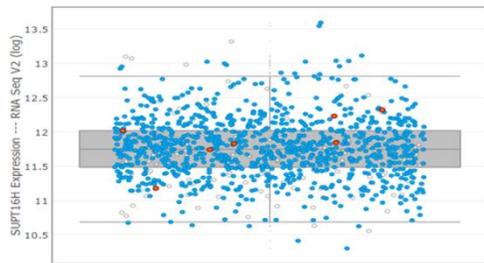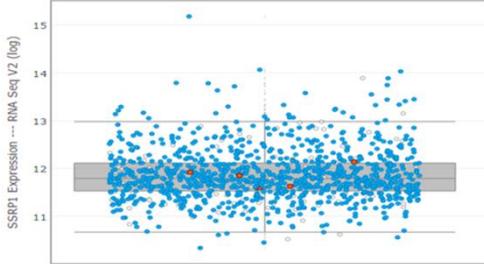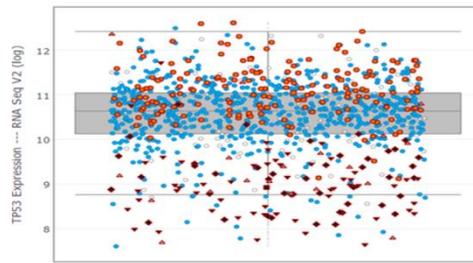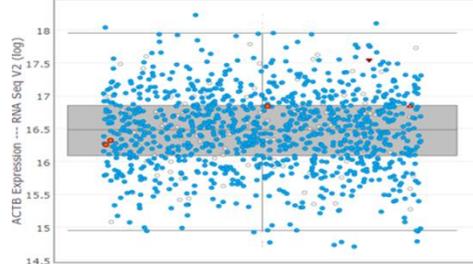
Supplemental Figure S8: Comparison of mutations found in different cancers in
SUPT16H (A), SSRP1 (B), TP53 (C) and ACTB (D). Two latter genes were used for
comparison. TCGA data analyzed using cBioPortal.