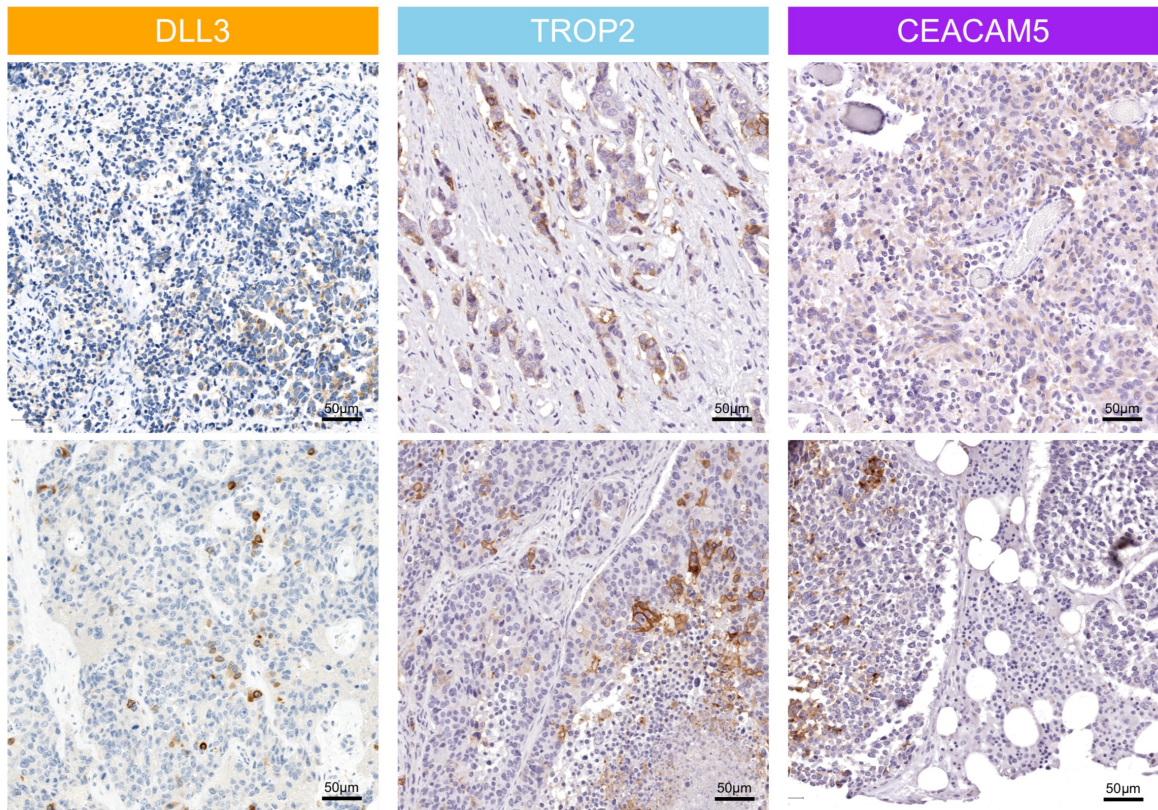


## **SUPPLEMENTARY DATA**

# **ASSESSMENT OF TROP2, CEACAM5 AND DLL3 IN METASTATIC PROSTATE CANCER: EXPRESSION LANDSCAPE AND MOLECULAR CORRELATES**

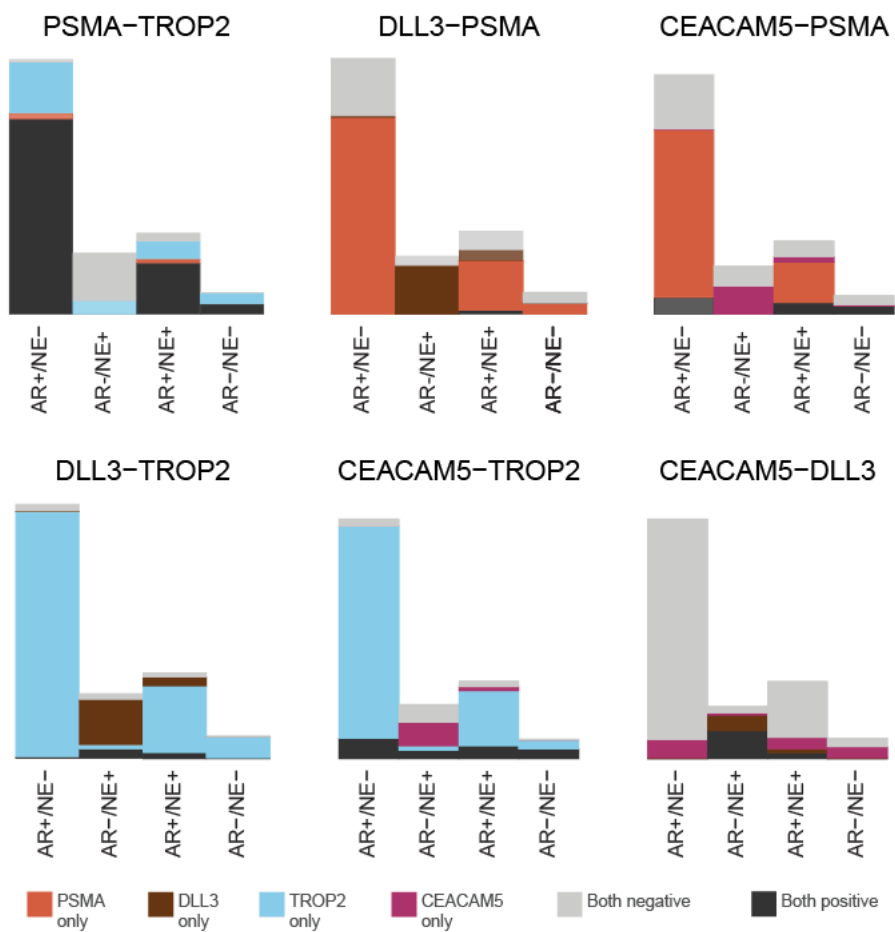
Azra Ajkunic, Erolcan Sayar, Martine P. Roudier, Radhika A. Patel, Ilsa M. Coleman, Navonil De Sarkar, Brian Hanratty, Mohamed Adil, Jimmy Zhao, Samir Zaidi, Lawrence D. True, Jamie M. Sperger, Heather H. Cheng, Evan Y. Yu, Robert B. Montgomery, Jessica E. Hawley, Gavin Ha, Thomas Persse, Patricia Galipeau, John K. Lee, Stephanie A. Harmon, Eva Corey, Joshua M. Lang, Charles Sawyers, Colm Morrissey, Michael T. Schweizer, Roman Gulati, Peter S. Nelson, Michael C. Haffner

**Supplementary Figure 1.** *Ajkunic, Sayar et al.*



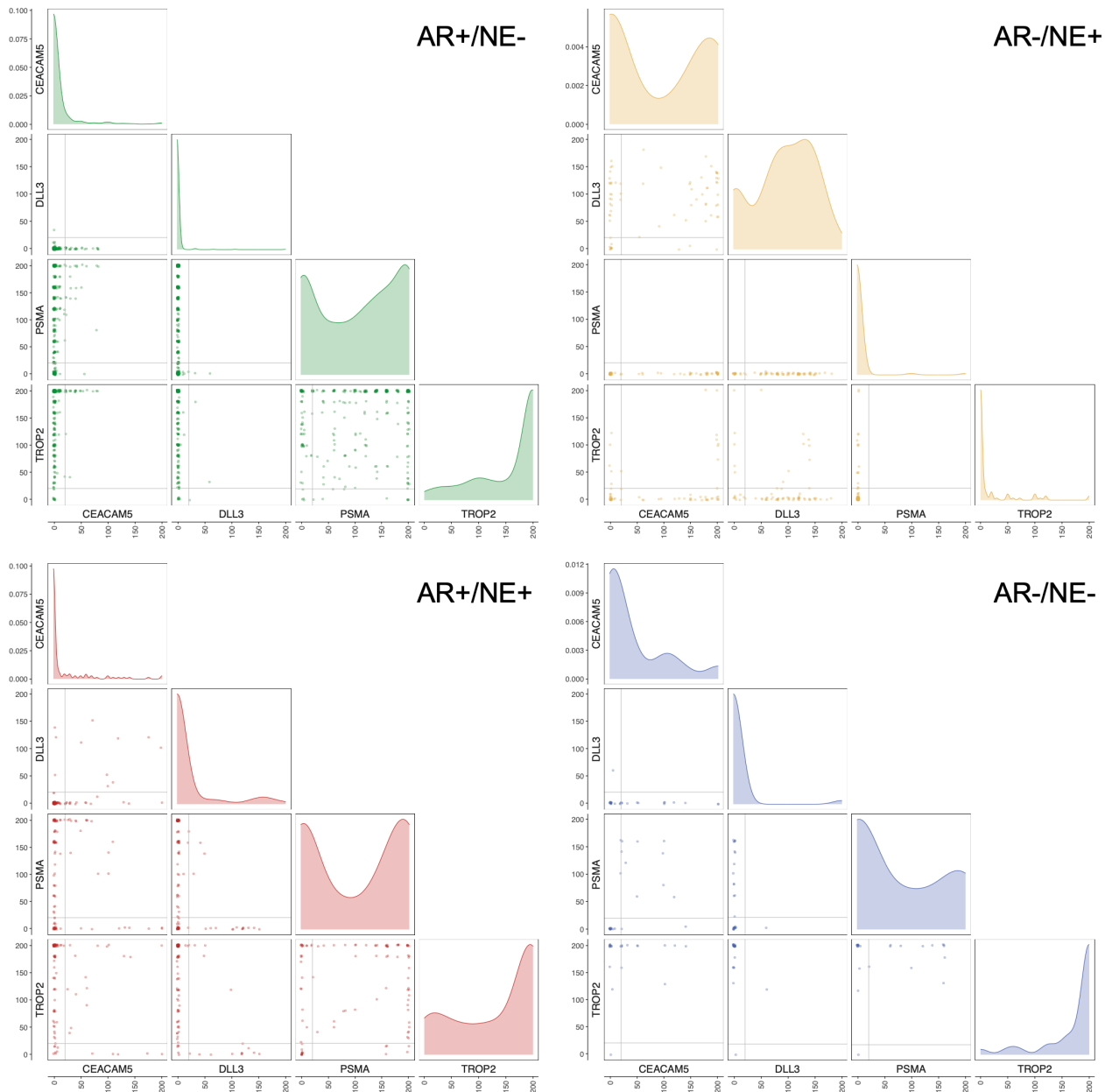
**Supplementary Figure 1.** Representative micrographs of mCRPC tumors with DLL3, TROP2 and CEACAM5 expression H-scores of 20.

**Supplementary Figure 2.** *Ajkunic, Sayar et al.*



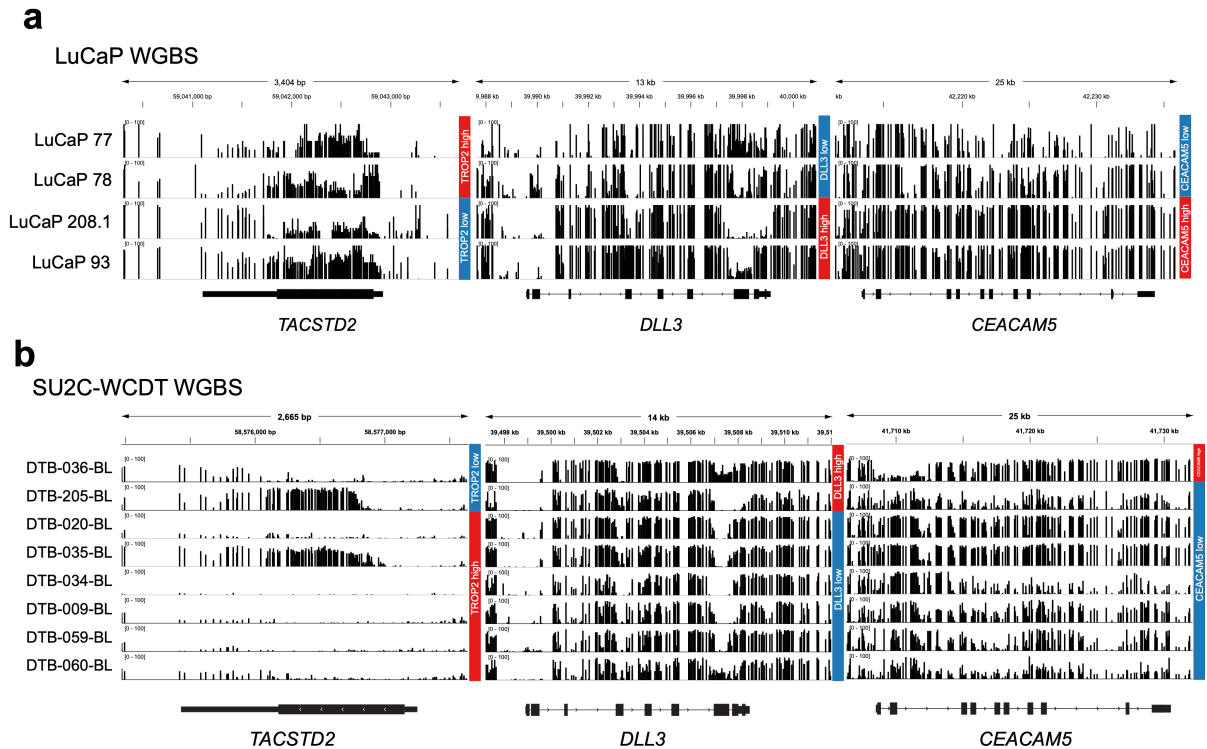
**Supplementary Figure 2.** Distribution of PSMA, TROP2, DLL3 and CEACAM5 co-expression pattern.

# Supplementary Figure 3. *Ajkunic, Sayar et al.*



**Supplementary Figure 3.** Scatter and density plots show the distribution of co-expression patterns of CEACAM5, DLL3, PSMA and TROP2 across different molecular subtypes.

## Supplementary Figure 4. *Ajkunic, Sayar et al.*



**Supplementary Figure 4.** Whole genome bisulfite sequencing studies show no consistent difference in CpG methylation pattern at the transcriptional start site of TACSTD2, DLL3 and CEACAM5 between high- and low-expressing tumors in **A.** LuCaP PDX lines and **B.** cases of the SU2C-WCDT cohort.

**Supplementary Table 1.** Clinical characteristics of patients from the UW-TAN rapid autopsy study with IHC expression data.

Total n = 52

| <b>Demographics data</b>                | <b>Median</b> | <b>Inter quartile range</b> |
|-----------------------------------------|---------------|-----------------------------|
| Age at initial diagnosis (median [IQR]) | 60.6          | [53.92, 66.33]              |
| PSA at diagnosis (median [IQR])         | 19.5          | [7.55, 111.25]              |
| Age at death (median [IQR])             | 68.6          | [61.91, 74.43]              |
| PSA at death (median [IQR])             | 90.6          | [8.69, 1007.51]             |

| <b>Treatment history</b>           | <b>Number of patients</b> | <b>Percent</b> |
|------------------------------------|---------------------------|----------------|
| Androgen deprivation therapy (ADT) | 51                        | 98.1           |
| Abiraterone acetate                | 33                        | 63.5           |
| Enzalutamide                       | 28                        | 53.8           |
| Taxane therapy                     | 36                        | 69.2           |
| Carboplatin                        | 27                        | 51.9           |
| Radiation therapy                  | 36                        | 69.2           |
| Alpharadin                         | 9                         | 17.3           |

| <b>Metastatic sites</b> | <b>Number of patients</b> | <b>Percent</b> |
|-------------------------|---------------------------|----------------|
| Bone                    | 48                        | 92.3           |
| Liver                   | 36                        | 69.2           |
| Lymph nodes             | 36                        | 69.2           |
| Lung                    | 31                        | 59.6           |
| Adrenal                 | 13                        | 25.0           |
| Spleen                  | 7                         | 13.5           |
| Other soft tissue sites | 18                        | 34.6           |

**Supplementary Table 2.** Co-expression pattern of cell surface antigens across different molecular subtypes of CRPC.**PSMA-TROP2**

| subtype | expressed  | percent |
|---------|------------|---------|
| AR+/NE- | Both       | 76.6    |
| AR+/NE- | PSMA only  | 2.3     |
| AR+/NE- | TROP2 only | 20.1    |
| AR+/NE- | Neither    | 1.0     |
| AR-/NE+ | Both       | 0.0     |
| AR-/NE+ | PSMA only  | 0.0     |
| AR-/NE+ | TROP2 only | 21.9    |
| AR-/NE+ | Neither    | 78.1    |
| AR+/NE+ | Both       | 62.9    |
| AR+/NE+ | PSMA only  | 5.2     |
| AR+/NE+ | TROP2 only | 21.6    |
| AR+/NE+ | Neither    | 10.3    |
| AR-/NE- | Both       | 46.2    |
| AR-/NE- | PSMA only  | 0.0     |
| AR-/NE- | TROP2 only | 50.0    |
| AR-/NE- | Neither    | 3.8     |

**TROP2-DLL3**

| subtype | expressed  | percent |
|---------|------------|---------|
| AR+/NE- | Both       | 0.7     |
| AR+/NE- | TROP2 only | 96.2    |
| AR+/NE- | DLL3 only  | 0.3     |
| AR+/NE- | Neither    | 2.7     |
| AR-/NE+ | Both       | 3.8     |
| AR-/NE+ | TROP2 only | 1.7     |
| AR-/NE+ | DLL3 only  | 17.7    |
| AR-/NE+ | Neither    | 2.4     |
| AR+/NE+ | Both       | 2.4     |
| AR+/NE+ | TROP2 only | 26.3    |
| AR+/NE+ | DLL3 only  | 3.4     |
| AR+/NE+ | Neither    | 1.7     |
| AR-/NE- | Both       | 0.3     |
| AR-/NE- | TROP2 only | 8.2     |
| AR-/NE- | DLL3 only  | 0.0     |
| AR-/NE- | Neither    | 0.3     |

**DLL3-CEACAM5**

| subtype | expressed    | percent |
|---------|--------------|---------|
| AR+/NE- | Both         | 0.0     |
| AR+/NE- | DLL3 only    | 0.3     |
| AR+/NE- | CEACAM5 only | 7.7     |
| AR+/NE- | Neither      | 92.0    |
| AR-/NE+ | Both         | 53.5    |
| AR-/NE+ | DLL3 only    | 29.6    |
| AR-/NE+ | CEACAM5 only | 4.2     |
| AR-/NE+ | Neither      | 12.7    |
| AR+/NE+ | Both         | 7.6     |
| AR+/NE+ | DLL3 only    | 4.8     |
| AR+/NE+ | CEACAM5 only | 15.2    |
| AR+/NE+ | Neither      | 72.4    |
| AR-/NE- | Both         | 0.0     |
| AR-/NE- | DLL3 only    | 3.6     |
| AR-/NE- | CEACAM5 only | 53.6    |
| AR-/NE- | Neither      | 42.9    |

**PSMA-CEACAM5**

| subtype | expressed    | percent |
|---------|--------------|---------|
| AR+/NE- | Both         | 6.8     |
| AR+/NE- | PSMA only    | 70.0    |
| AR+/NE- | CEACAM5 only | 0.3     |
| AR+/NE- | Neither      | 22.9    |
| AR-/NE+ | Both         | 0.0     |
| AR-/NE+ | PSMA only    | 0.0     |
| AR-/NE+ | CEACAM5 only | 57.4    |
| AR-/NE+ | Neither      | 42.7    |
| AR+/NE+ | Both         | 15.5    |
| AR+/NE+ | PSMA only    | 55.3    |
| AR+/NE+ | CEACAM5 only | 7.8     |
| AR+/NE+ | Neither      | 21.4    |
| AR-/NE- | Both         | 42.3    |
| AR-/NE- | PSMA only    | 0.0     |
| AR-/NE- | CEACAM5 only | 7.7     |
| AR-/NE- | Neither      | 50.0    |

**TROP2-CEACAM5**

| subtype | expressed    | percent |
|---------|--------------|---------|
| AR+/NE- | Both         | 8.4     |
| AR+/NE- | TROP2 only   | 88.6    |
| AR+/NE- | CEACAM5 only | 0.0     |
| AR+/NE- | Neither      | 3.0     |
| AR-/NE+ | Both         | 14.9    |
| AR-/NE+ | TROP2 only   | 9.0     |
| AR-/NE+ | CEACAM5 only | 43.3    |
| AR-/NE+ | Neither      | 32.8    |
| AR+/NE+ | Both         | 16.7    |
| AR+/NE+ | TROP2 only   | 70.8    |
| AR+/NE+ | CEACAM5 only | 5.2     |
| AR+/NE+ | Neither      | 7.3     |
| AR-/NE- | Both         | 50.0    |
| AR-/NE- | TROP2 only   | 45.8    |
| AR-/NE- | CEACAM5 only | 0.0     |
| AR-/NE- | Neither      | 4.2     |

**PSMA-DLL3**

| subtype | expressed | percent |
|---------|-----------|---------|
| AR+/NE- | Both      | 0.0     |
| AR+/NE- | PSMA only | 76.9    |
| AR+/NE- | DLL3 only | 0.9     |
| AR+/NE- | Neither   | 22.2    |
| AR-/NE+ | Both      | 0.0     |
| AR-/NE+ | PSMA only | 0.0     |
| AR-/NE+ | DLL3 only | 83.1    |
| AR-/NE+ | Neither   | 16.9    |
| AR+/NE+ | Both      | 4.5     |
| AR+/NE+ | PSMA only | 60.0    |
| AR+/NE+ | DLL3 only | 12.7    |
| AR+/NE+ | Neither   | 22.7    |
| AR-/NE- | Both      | 0.0     |
| AR-/NE- | PSMA only | 48.3    |
| AR-/NE- | DLL3 only | 3.4     |
| AR-/NE- | Neither   | 48.3    |

**Supplementary Table 3.** Associations between genomic alterations and TROP2, PSMA, DLL3 and CEACAM5 protein expression in the UW-TAN cohort. Estimated associations between biomarker immunohistochemistry H-score  $\geq 20$  and genomic calls from generalized linear mixed effects models.

| Characteristic       | OR*  | 95% CI*      | p-value |
|----------------------|------|--------------|---------|
| TROP2~gAR            | 59.3 | 1.46; 2,405  | 0.031   |
| TROP2~gBRCA2#        | #    | #            | #       |
| TROP2~gCHD1#         | #    | #            | #       |
| TROP2~gPTEN          | 1.09 | 0.03; 39.9   | >0.9    |
| TROP2~gRB1 BAL       | 0    | 0.00; 0.08   | 0.001   |
| TROP2~gRB1 BAL_MAL   | 0.03 | 0.00; 13.8   | 0.3     |
| TROP2~gTP53          | 1.46 | 0.04; 54.5   | 0.8     |
| PSMA~gAR             | 37.2 | 4.41; 314    | <0.001  |
| PSMA~gBRCA2          | 92.7 | 0.94; 9,154  | 0.053   |
| PSMA~gCHD1           | 83.2 | 0.27; 25,886 | 0.13    |
| PSMA~gPTEN           | 0.46 | 0.05; 4.07   | 0.5     |
| PSMA~gRB1 BAL        | 0.01 | 0.00; 0.09   | <0.001  |
| PSMA~gRB1 BAL_MAL    | 0.11 | 0.01; 1.90   | 0.13    |
| PSMA~gTP53#          | #    | #            | #       |
| DLL3~gAR             | 0.09 | 0.00; 10.8   | 0.3     |
| DLL3~gBRCA2#         | #    | #            | #       |
| DLL3~gCHD1#          | #    | #            | #       |
| DLL3~gPTEN           | 2.66 | 0.03; 218    | 0.7     |
| DLL3~gRB1 BAL        | 34   | 0.19; 5,993  | 0.2     |
| DLL3~gRB1 BAL_MAL    | ^    | ^            | ^       |
| DLL3~gTP53#          | #    | #            | #       |
| CEACAM5~gAR          | 0.14 | 0.01; 1.39   | 0.094   |
| CEACAM5~gBRCA2       | #    | #            | #       |
| CEACAM5~gCHD1        | 1.59 | 0.01; 276    | 0.9     |
| CEACAM5~gPTEN        | 1.77 | 0.17; 18.6   | 0.6     |
| CEACAM5~gRB1 BAL     | 4.15 | 0.38; 45.5   | 0.2     |
| CEACAM5~gRB1 BAL_MAL | 1.31 | 0.08; 21.1   | 0.9     |
| CEACAM5~gTP53        | 0.45 | 0.04; 4.75   | 0.5     |

\*OR = Odds Ratio, CI = Confidence Interval

# Regression model did not converge

^Regression model could not be estimated owing to complete separation

BAL denotes biallelic loss only

BAL\_MAL includes biallelic and monoallelic loss

**Supplementary Table 4.** Associations between genomic alterations and *TACSTD2*, *FOLH1*, *DLL3* and *CEACAM5* expression in the SU2C-WCDT. Estimated associations between biomarker expression (based on RNA-seq) and genomic calls from generalized linear mixed effects models.

| Comparison    | OR <sup>1</sup> | 95% CI <sup>1</sup> | p-value |
|---------------|-----------------|---------------------|---------|
| TACSTD2~AR    | 13.1            | 1.82; 263           | 0.025   |
| TACSTD2~BRCA2 | #               | #                   | #       |
| TACSTD2~CHD1  | #               | #                   | #       |
| TACSTD2~PTEN  | 1.53            | 0.21; 30.7          | 0.7     |
| TACSTD2~RB1   | #               | #                   | #       |
| TACSTD2~SPOP  | #               | #                   | #       |
| TACSTD2~TP53  | 1.11            | 0.18; 8.73          | >0.9    |
| FOLH1~AR      | 3.35            | 0.95; 11.9          | 0.055   |
| FOLH1~BRCA2   | #               | #                   | #       |
| FOLH1~CHD1    | #               | #                   | #       |
| FOLH1~PTEN    | 0.72            | 0.21; 2.90          | 0.6     |
| FOLH1~RB1     | #               | #                   | #       |
| FOLH1~SPOP    | #               | #                   | #       |
| FOLH1~TP53    | 1.04            | 0.31; 3.74          | >0.9    |
| DLL3~AR       | 0.06            | 0.00; 0.39          | 0.011   |
| DLL3~BRCA2    | 2.12            | 0.10; 15.5          | 0.5     |
| DLL3~CHD1     | #               | #                   | #       |
| DLL3~PTEN     | 0.52            | 0.03; 3.40          | 0.6     |
| DLL3~RB1      | #               | #                   | #       |
| DLL3~SPOP     | #               | #                   | #       |
| DLL3~TP53     | 2.89            | 0.54; 21.6          | 0.2     |
| CEACAM5~AR    | 0.19            | 0.05; 0.74          | 0.018   |
| CEACAM5~BRCA2 | 1.12            | 0.06; 7.24          | >0.9    |
| CEACAM5~CHD1  | #               | #                   | #       |
| CEACAM5~PTEN  | 4.86            | 1.27; 20.6          | 0.022   |
| CEACAM5~RB1   | 1.87            | 0.09; 13.4          | 0.6     |
| CEACAM5~SPOP  | #               | #                   | #       |
| CEACAM5~TP53  | 0.89            | 0.22; 3.35          | 0.9     |

<sup>1</sup> OR = Odds Ratio, CI = Confidence Interval

# Regression model did not converge