**This PDF file includes:**

Figure S1. Work flow for this study.

Figure S2. Detection of ERG-derived peptides in NCI-H660 vs. VCaP cell lines.

Figure S3. Detection of ERG-derived peptides in seven TMPRSS2-ERG gene fusion positive prostate tumor tissues.

Figure S4. Mapping of the peptides that have been selected for SRM analysis to various potential TMPRSS2- ERG fusion protein products.

Figure S5. Calibration curves of six ERG-derived peptides.

Figure S6. Abundance of all 7 detected ERG peptides in TMPRSS2-ERG fusion positive cell lines and tissues.

Figure S7. ERG peptides detected in TMPRSS2-ERG fusion positive tissues.

Table S1. Prostate cancer tissue specimens used for PRISM-SRM analysis.

Table S2. Selected transitions and optimal collision energy of each transition for SRM analysis of ERG protein.

Table S3. Summary of peak area ratios and CV values for the detected peptides in this study.
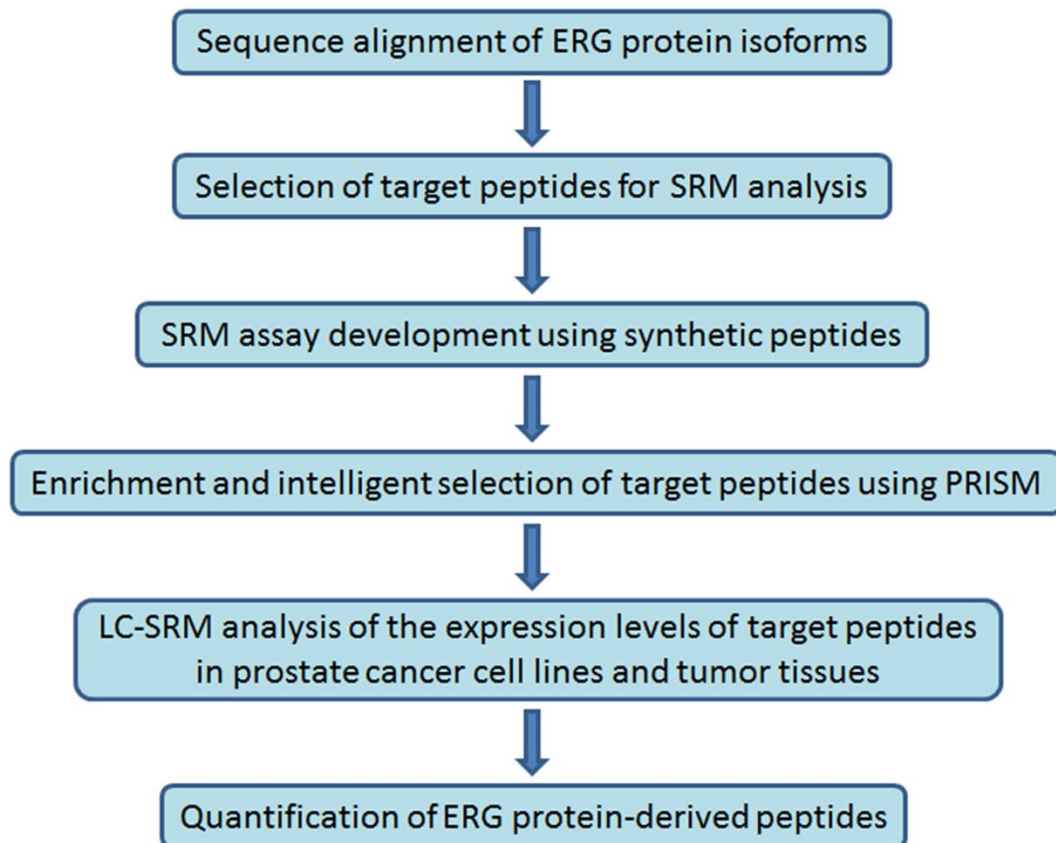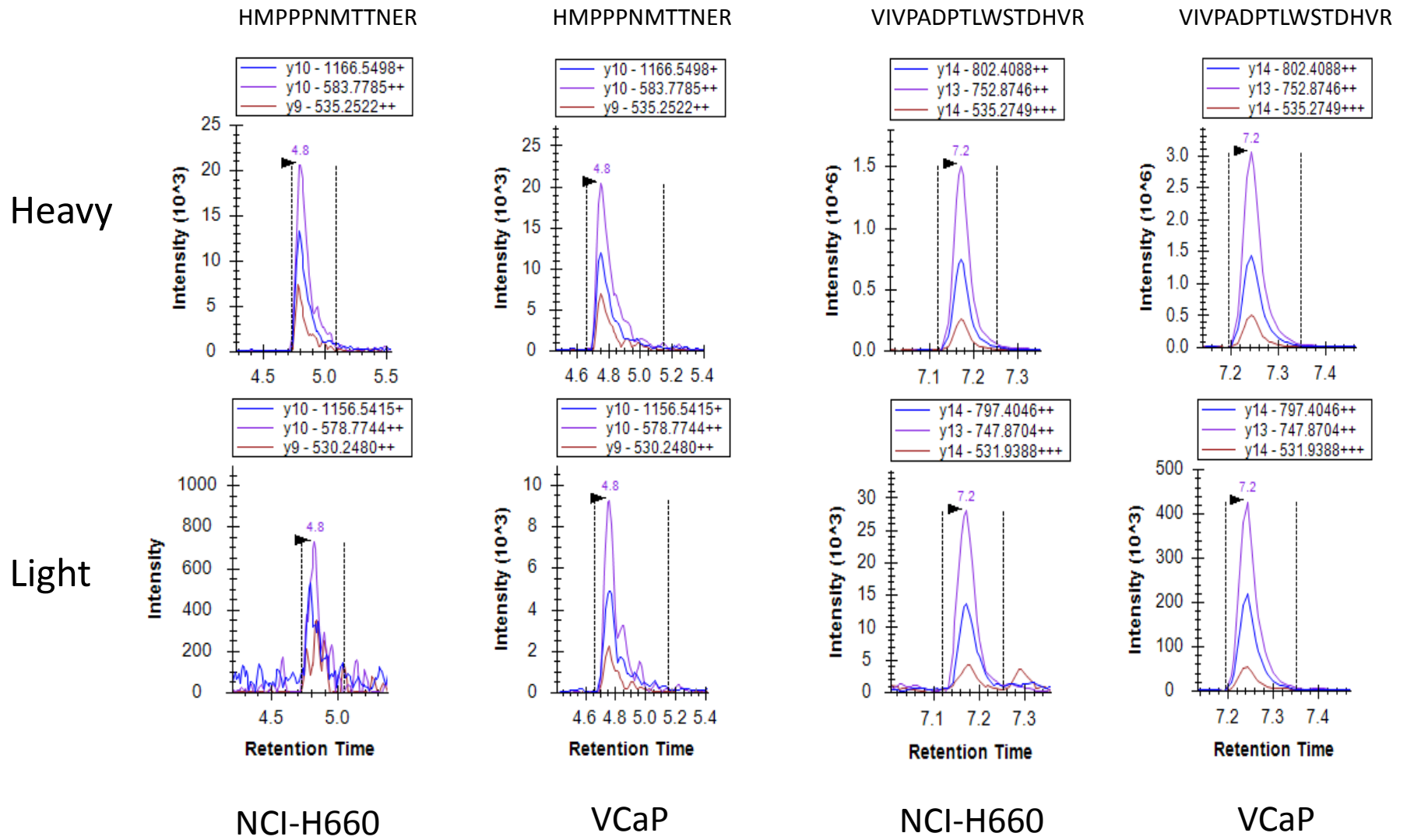
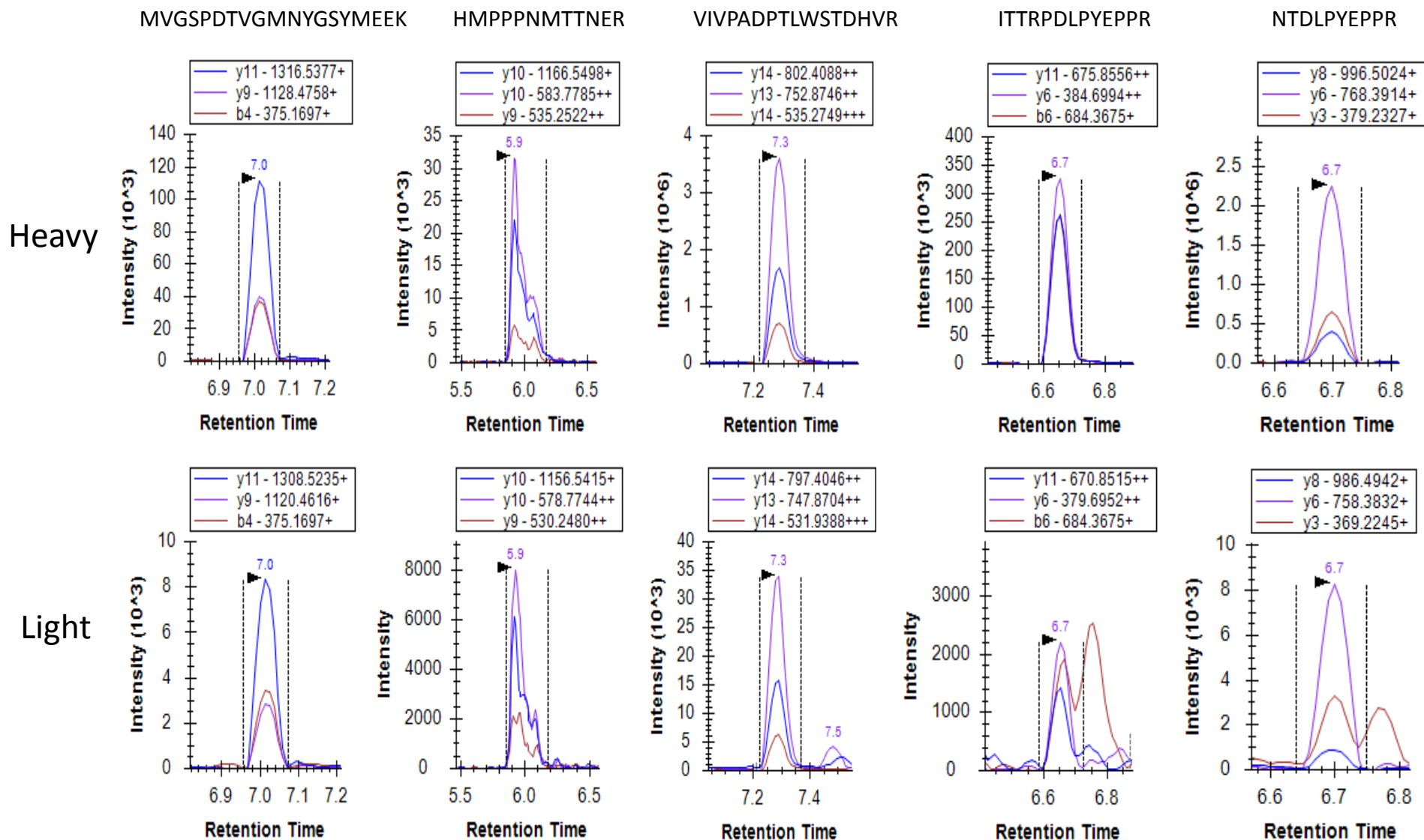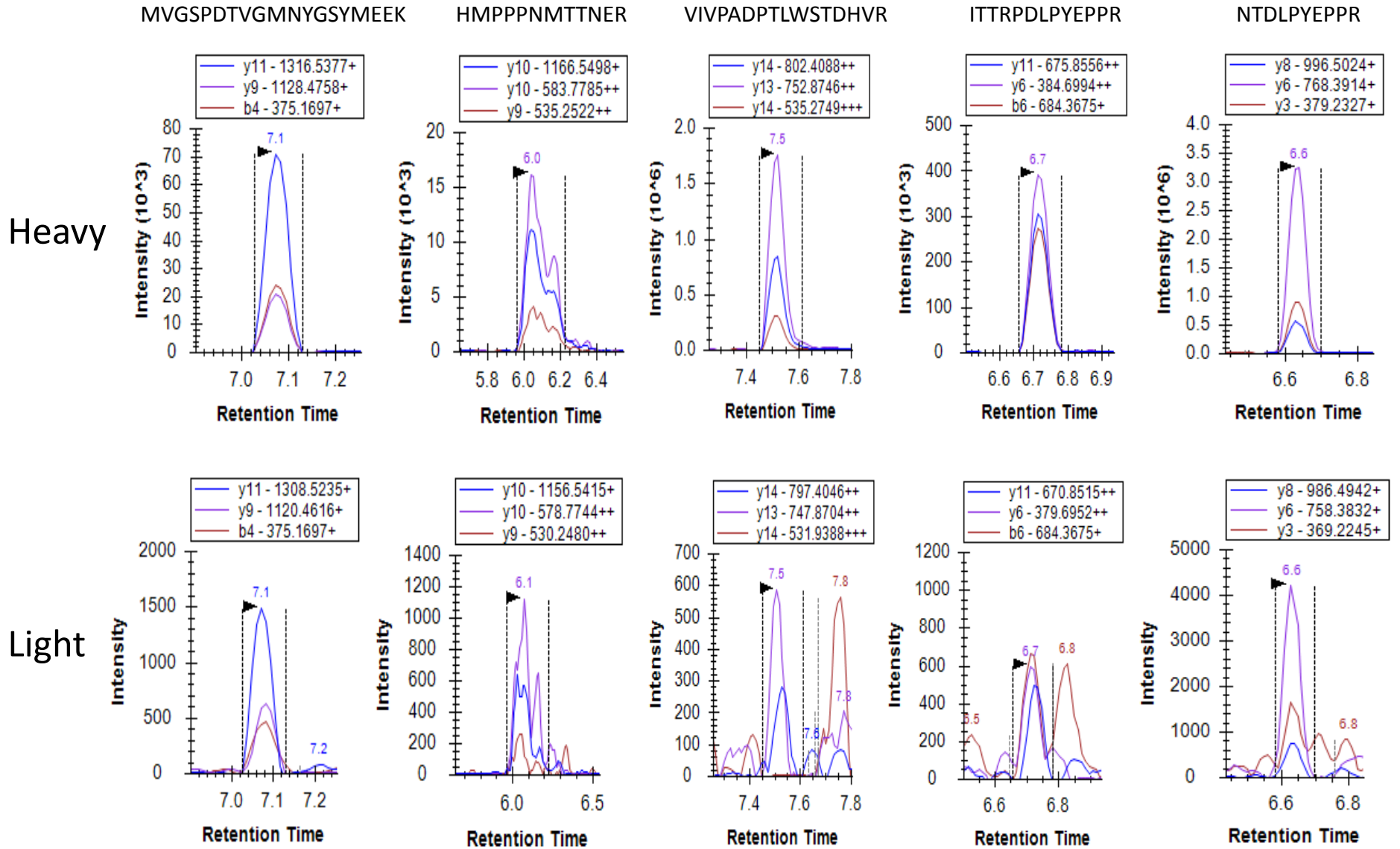**Figure S1. Work flow for this study**

**Figure S2. Detection of ERG-derived peptides in NCI-H660 vs. VCaP cell lines.**
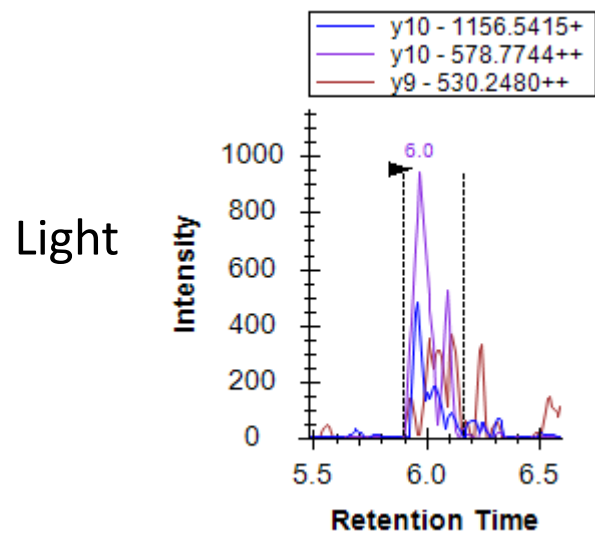
# Figure S3

## PT1



|  | MVGSPDTVGMNYGSYMEEK | HMPPPNMTTNER | VIVPADPTLWSTDHVR | ITTRPDLPYEPPR | NTDLPYEPPR |

# PT2



Heavy

| MVGSPDTVGMNYGSYMEEK | HMPPPNMTTNER | VIVPADPTLWSTDHVR | ITTRPDLPYEPPR | NTDLPYEPPR |

# PT3

HMPPPNMTTNER



Heavy

Legend:
- y10 - 1166.5498+
- y10 - 583.7785++
- y9 - 535.2522++

Light

Legend:
- y10 - 1156.5415+
- y10 - 578.7744++
- y9 - 530.2480++
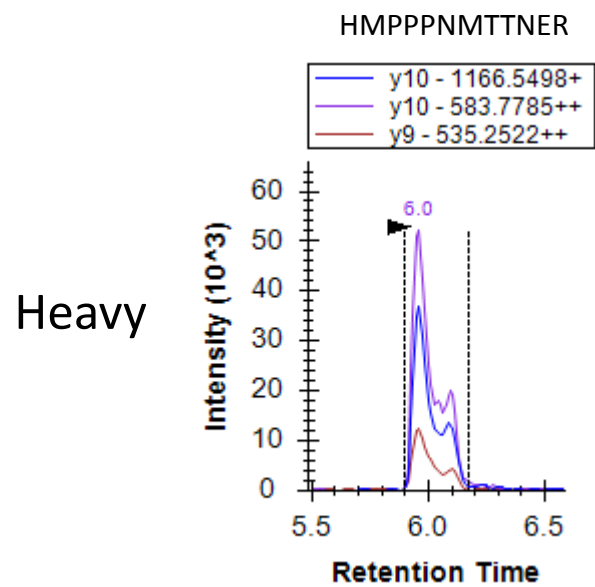
# PT4

# PT5

MVGSPDTVGMNYGSYMEEK  HMPPPNMTTNER  VIVPADPTLWSTDHVR
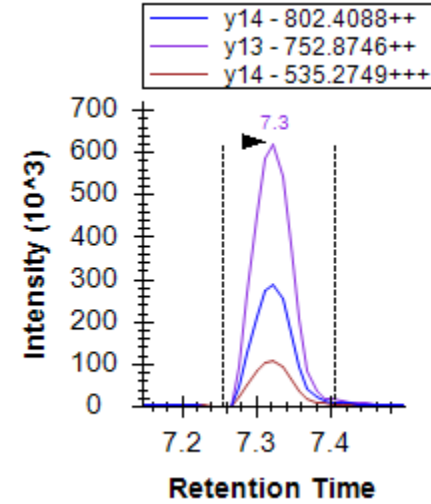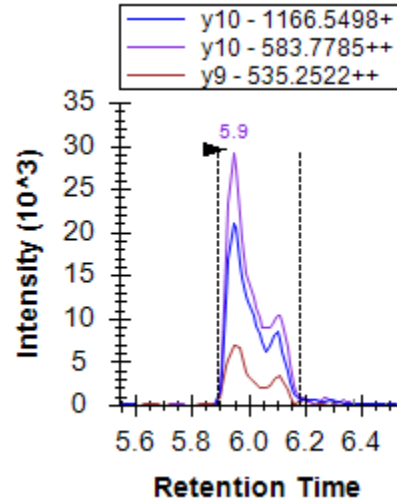
# PT6



MVGSPDTVGMNYGSYMEEK      HMPPPNMTTNER      VIVPADPTLWSTDHVR

Heavy
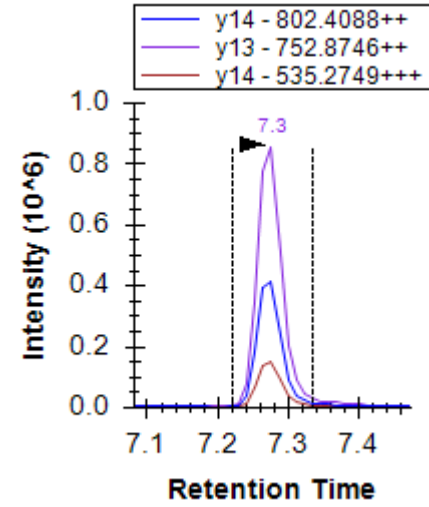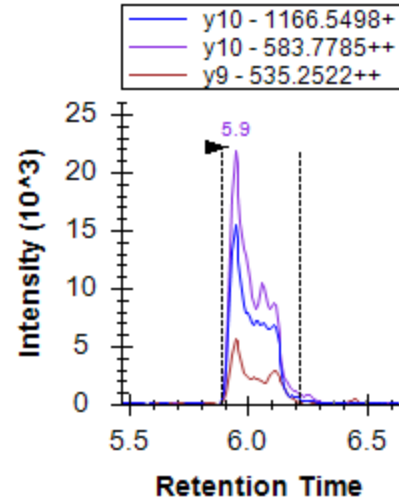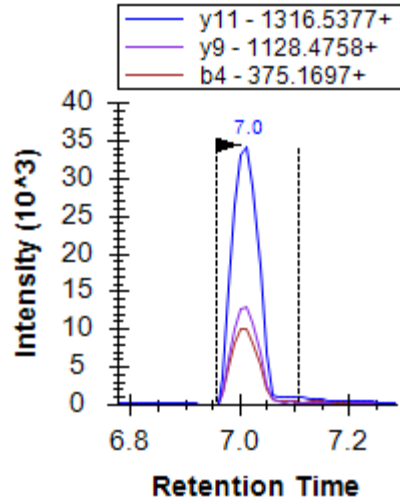
Light
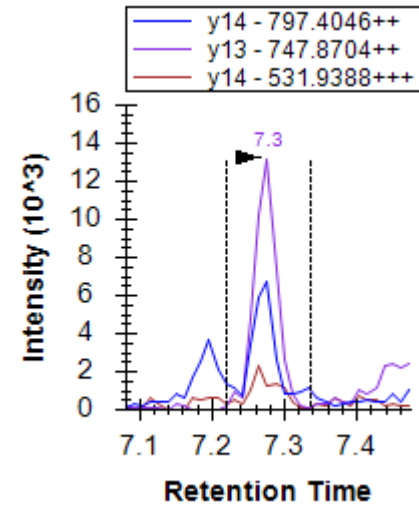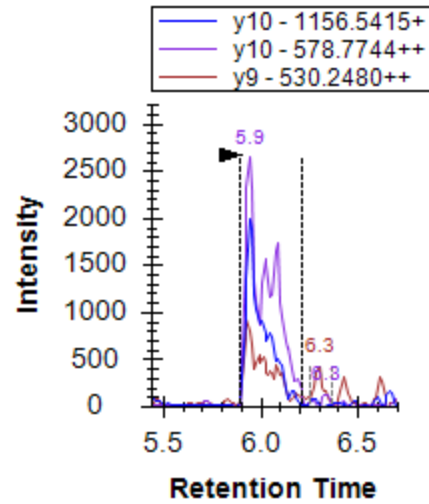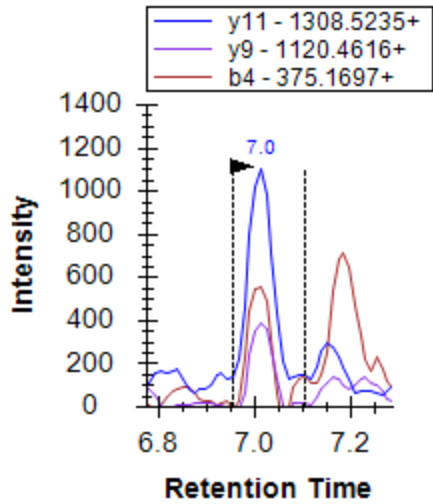
# PT7
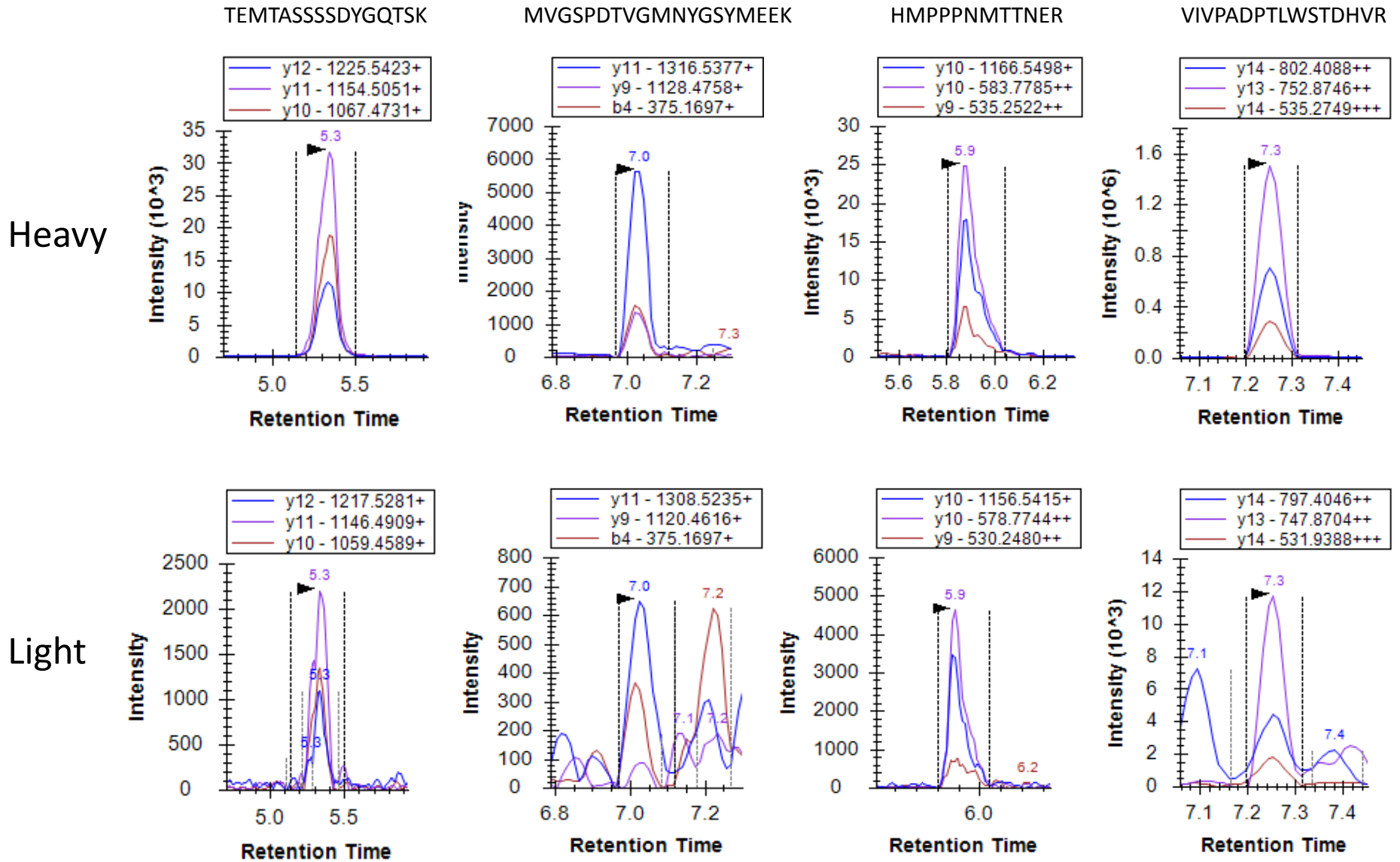


Figure S3. Detection of ERG-derived peptides in seven TMPRSS2-ERG fusion positive prostate tumor tissues
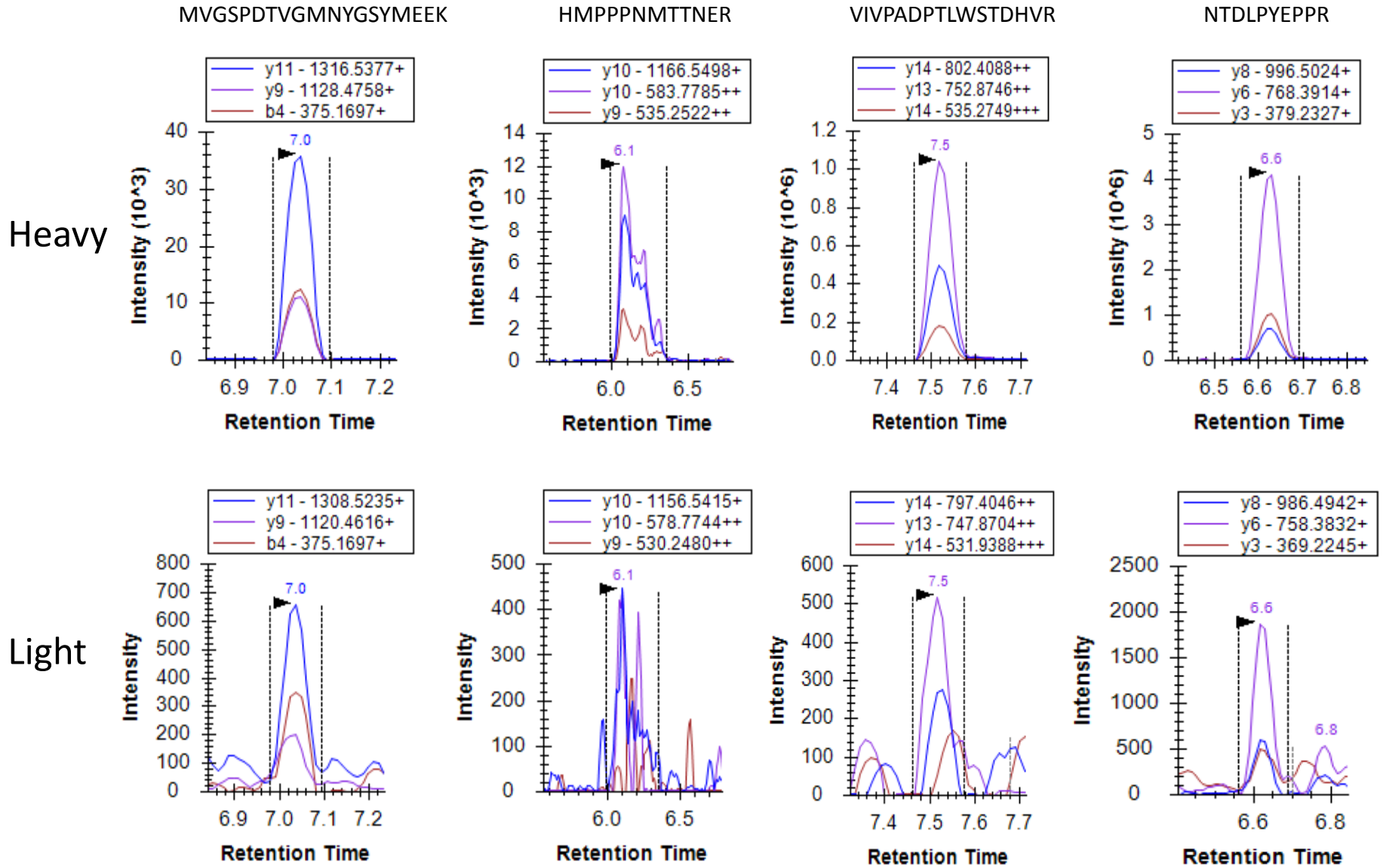
Figure S4

Figure S4. Mapping of the peptides that have been selected for SRM analysis to various potential TMPRSS2-ERG fusion protein products. Each peptide included in the final multiplexed PRISM-SRM assay (Ta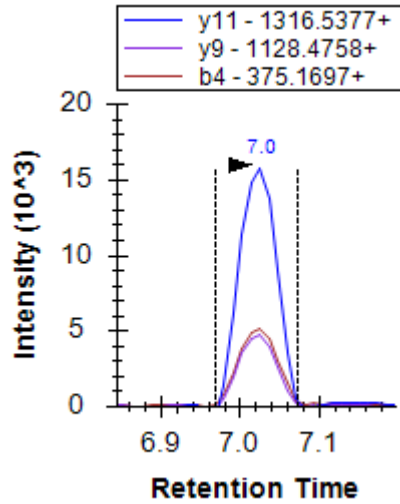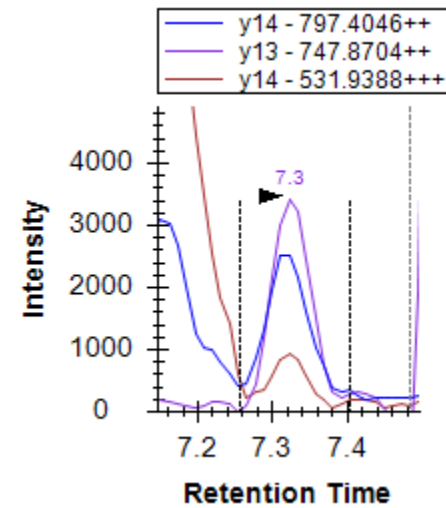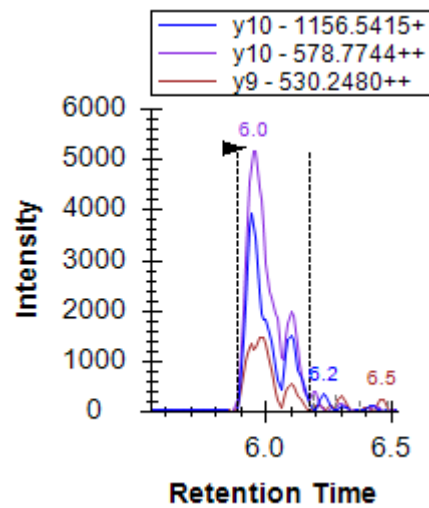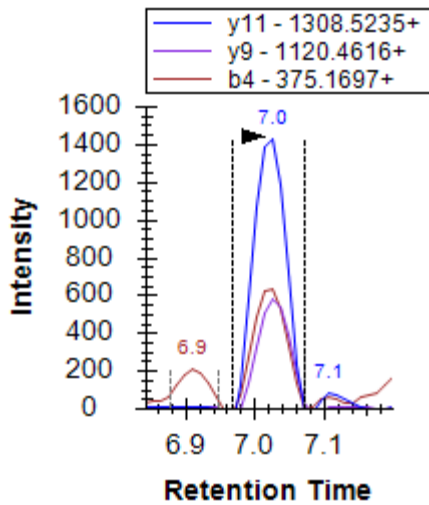ble 1) is represented as a bar on top of the aligned sequences of various putative protein products of the reported TMPRSS2-ERG gene fusion events. Peptides with sequences that skipped at least one exon are shown in bars that are connected using a solid line. The bars representing peptides detected in prostate cancer cell lines or tumor tissues in this study are grey shadowed. The exons are color coded. Dotted lines indicate sequence gap. Take the two mutually exclusive peptides as an example, ITTRPDLPYEPPR has sequences from both exons 10 (red) and 11 (purple), while NTDLPYEPPR has sequences from both exons 9 (blue) and 11 (purple).
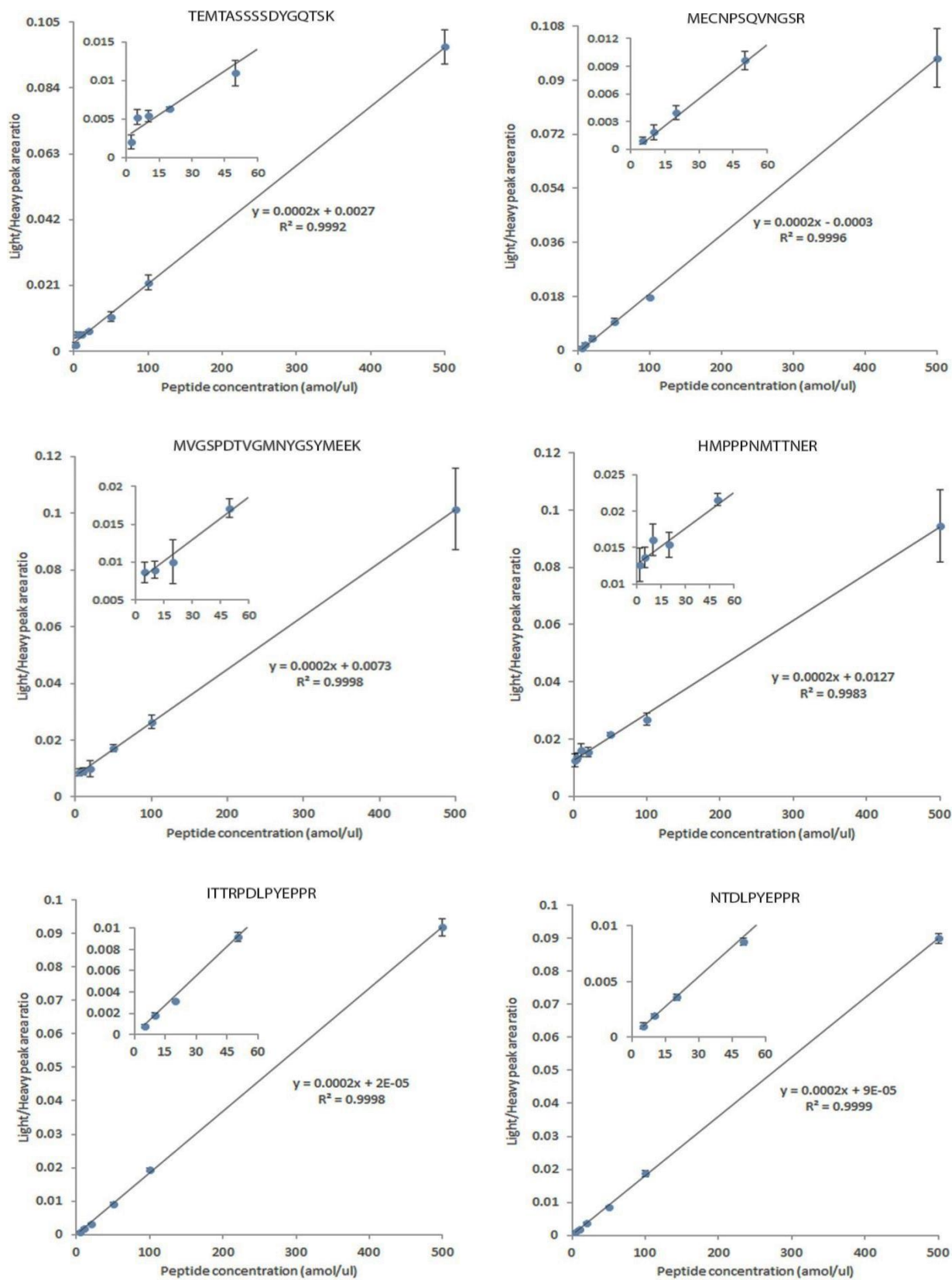
**Figure S5. Calibration curves of six ERG-derived peptides.**
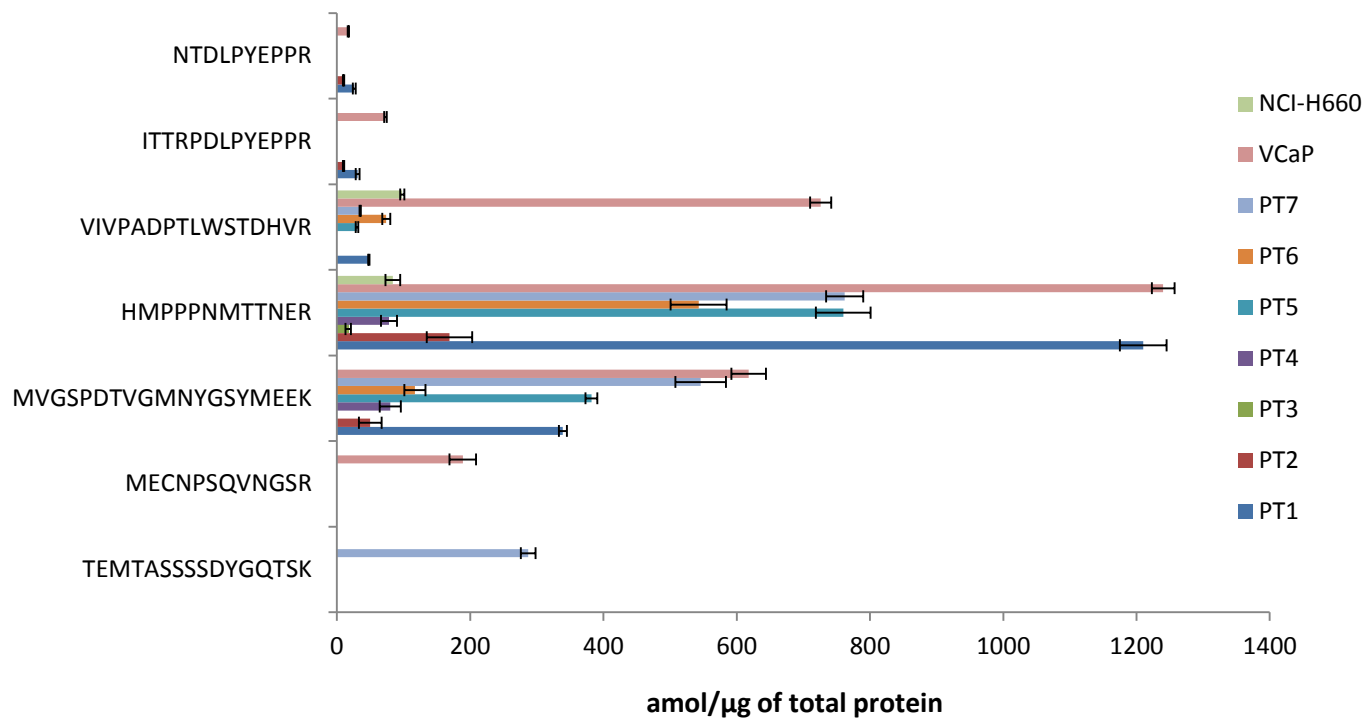
**Figure S6. Abundance of all 7 detected ERG peptides in TMPRSS2-ERG fusion positive cell lines and tissues.** The abundance of these peptides was represented by amol/µg of total protein. Each value represents the mean and SD of three replicate measurements.
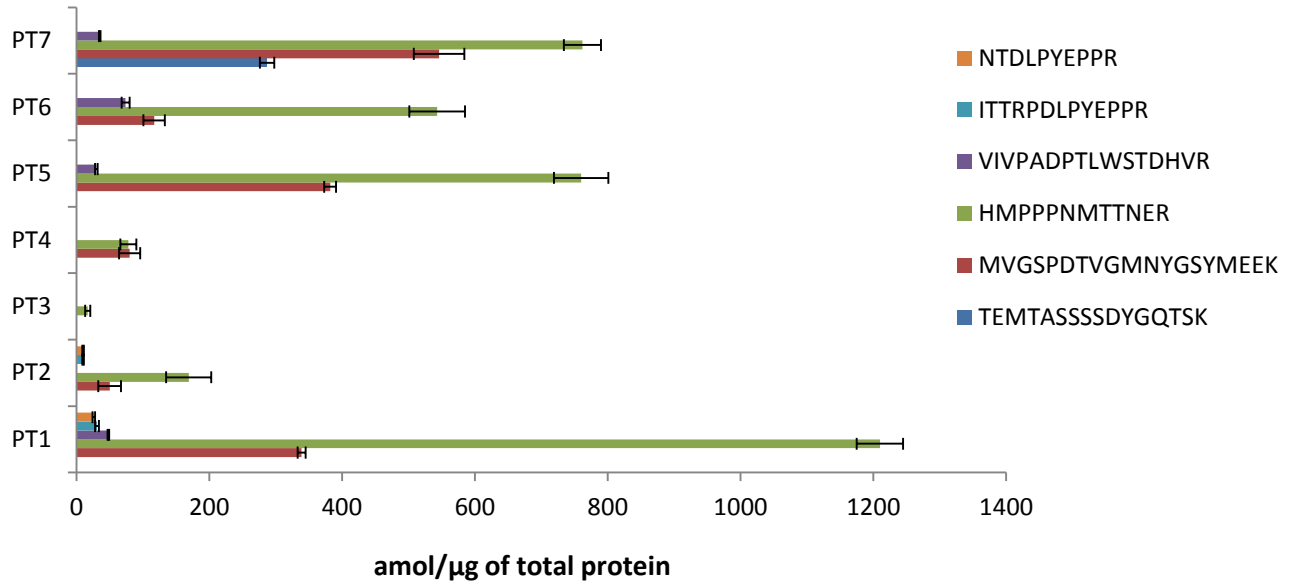
**Figure S7. ERG peptides detected in TMPRSS2-ERG fusion positive tissues.** The abundance of these peptides was represented by amol/μg of total protein. Each value represents the mean and SD of three replicate measurements.

**Table S1: Prostate cancer tissue specimens used for PRISM-SRM analysis.**

| Sample ID in the manuscript | Original Sample Name | Disease State | Tumor location[2] | ETS status[3] |
|---|---|---|---|---|
| PT1 | WA13 | CRPC[1] | Lung | ERG$^+$ |
| PT2 | WA39 | CRPC | Lymph node | ERG$^+$ |
| PT3 | WA40 | CRPC | Liver | ERG$^+$ |
| PT4 | WA49 | CRPC | Lower periaortic lymph node | ERG$^+$ |
| PT5 | WA53 | CRPC | Liver | ERG$^+$ |
| PT6 | STID 10162 B55A | Localized PC | Prostate | ERG$^+$ |
| PT7 | STID 10060 B53A | Localized PC | Prostate | ERG$^+$ |
| NT1 | WA32 | CRPC | Spleen | No ETS |
| NT2 | WA33 | CRPC | Periaortic lymph node | No ETS |
| NT3 | WA35 | CRPC | Mesentery | No ETS |
| NT4 | WA42 | CRPC | Left adrenal | No ETS |
| NT5 | WA51 | CRPC | Bone marrow | No ETS |

[1]CRPC: Castrate resistant metastatic prostate cancer. [2]Anatomic location of tissue. [3]Rearrangement in ETS.

**Table S2. Selected transitions and optimal collision energy of each transition for SRM analysis of ERG protein.** Bold red amino acids represent isotopically labeled residues. Cysteines are carbamidomethylated. CE, collision energy.

| Peptide Sequence | Parent Ion | Transition 1 | | Transition 2 | | Transition 3 | |
|---|---|---|---|---|---|---|---|
| | | Product ion 1 | CE1 | Product ion 2 | CE2 | Product ion 3 | CE3 |
| MIQTVPDPAAHIK | 474.26+++ | 424.73++ | 14 | 636.38+ | 17 | 848.46+ | 15 |
| MIQTVPDPAAHI**K** | 476.93+++ | 428.74++ | 14 | 644.40+ | 17 | 856.48+ | 15 |
| MASTIK | 325.68++ | 237.63++ | 21 | 448.28+ | 9 | 519.31+ | 15 |
| MASTI**K** | 329.69++ | 241.64++ | 21 | 456.29+ | 9 | 527.33+ | 15 |
| TEMTASSSSDYGQTSK | 840.36++ | 1059.46+ | 23 | 1146.49+ | 28 | 1217.53+ | 28 |
| TEMTASSSSDYGQTS**K** | 844.36++ | 1067.47+ | 23 | 1154.51+ | 28 | 1225.54+ | 28 |
| MTASSSSDYGQTSK | 725.31++ | 972.43+ | 26 | 1146.49+ | 27 | 1217.53+ | 27 |
| MTASSSSDYGQTS**K** | 729.32++ | 980.44+ | 26 | 1154.51+ | 27 | 1225.54+ | 27 |
| MECNPSQVNGSR | 689.80++ | 532.28+ | 25 | 844.43+ | 22 | 958.47+ | 25 |
| MECNPSQVNGS**R** | 694.80++ | 542.29+ | 25 | 854.44+ | 22 | 968.48+ | 25 |
| MVGSPDTVGMNYGSYMEEK | 1047.94++ | 375.17+ | 33 | 1120.46+ | 30 | 1308.52+ | 33 |
| MVGSPDTVGMNYGSYMEE**K** | 1051.95++ | 375.17+ | 33 | 1128.48+ | 30 | 1316.54+ | 33 |
| MNYGSYMEEK | 626.25++ | 843.36+ | 21 | 1006.42+ | 21 | 1120.46+ | 21 |
| MNYGSYMEE**K** | 630.26++ | 851.37+ | 21 | 1014.43+ | 21 | 1128.48+ | 21 |
| HMPPPNMTTNER | 712.82++ | 530.25++ | 29 | 578.77+ | 23 | 1156.54+ | 23 |
| HMPPPNMTTNE**R** | 717.83++ | 535.25++ | 29 | 583.78+ | 23 | 1166.55+ | 23 |
| VIVPADPTLWSTDHVR | 602.66+++ | 531.94+++ | 15 | 747.87++ | 15 | 797.40++ | 15 |
| VIVPADPTLWSTDHV**R** | 605.99+++ | 535.27+++ | 15 | 752.88++ | 15 | 802.41++ | 15 |
| VIVPADLPYEPPR | 733.41++ | 577.80++ | 21 | 758.38+ | 29 | 1154.58+ | 23 |
| VIVPADLPYEPP**R** | 738.41++ | 582.80++ | 21 | 768.39+ | 29 | 1164.59+ | 23 |
| ITTRPDLPYEPPR | 518.95++ | 379.70++ | 14 | 670.85++ | 16 | 684.37+ | 20 |
| ITTRPDLPYEPP**R** | 522.28+++ | 384.70++ | 14 | 675.86++ | 16 | 684.37+ | 20 |
| ITTRPAAQPSPSTVPK | 550.98+++ | 406.73++ | 15 | 812.45+ | 15 | 839.47+ | 17 |
| ITTRPAAQPSPSTVP**K** | 553.65+++ | 410.74++ | 15 | 820.47+ | 15 | 839.47+ | 17 |
| NTDLPYEPPR | 601.30++ | 369.22+ | 21 | 758.38+ | 16 | 986.49+ | 16 |
| NTDLPYEPP**R** | 606.30++ | 379.23+ | 21 | 768.39+ | 16 | 996.50+ | 16 |
| ITTRPGTK | 437.26++ | 380.72++ | 17 | 659.38+ | 20 | 760.43+ | 19 |
| ITTRPGT**K** | 441.27++ | 384.73++ | 17 | 667.40+ | 20 | 768.45+ | 19 |
| ITTRPVSYR | 364.88+++ | 439.75++ | 12 | 490.27++ | 13 | 621.34+ | 18 |
| ITTRPVSY**R** | 368.21+++ | 444.75++ | 12 | 495.27++ | 13 | 631.34+ | 18 |
| ALSHVIQR | 308.52+++ | 326.70++ | 12 | 370.21++ | 10 | 515.33+ | 14 |
| ALSHVIQ**R** | 311.85+++ | 331.70++ | 13 | 375.22++ | 10 | 525.34+ | 14 |

**Table S3. Summary of peak area ratios and CV values for the detected peptides in this study.** Ratio represents light peptide to heavy peptide peak area ratio. CV, or coefficient of variation, is calculated by dividing the standard deviation by the mean.

| Peptide | VCaP | | NCI-H660 | | PT1 | | PT2 | | PT3 | | PT4 | | PT5 | | PT6 | | PT7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ratio | CV | Ratio | CV | Ratio | CV | Ratio | CV | Ratio | CV | Ratio | CV | Ratio | CV | Ratio | CV | Ratio | CV |
| TEMTASSSSDYGQTSK | | | | | | | | | | | | | | | | | 0.0601 | 8.3% |
| MECNPSQVNGSR | 0.0376 | 9.6% | | | | | | | | | | | | | | | | |
| MVGSPDTVGMNYGSYMEEK | 0.131 | 9.5% | | | 0.0752 | 1.5% | 0.0159 | 22.7% | | | 0.0233 | 14.4% | 0.0838 | 10.8% | 0.0307 | 9.3% | 0.116 | 12.8% |
| HMPPPNMTTNER | 0.260 | 6.1% | 0.0294 | 12.3% | 0.255 | 7.7% | 0.0464 | 13.3% | 0.0162 | 8.2% | 0.0281 | 12.9% | 0.165 | 12.5% | 0.121 | 9.7% | 0.165 | 11.0% |
| VIVPADPTLWSTDHVR | 0.145 | 2.3% | 0.0197 | 3.9% | 0.0098 | 2.7% | 0.00027 | 11.2% | | | 0.0038 | 39.8% | 0.0062 | 9.1% | 0.0150 | 9.3% | 0.0071 | 4.1% |
| ITTRPDLPYEPPR | 0.0147 | 3.5% | | | 0.0062 | 10.4% | 0.0011 | 13.6% | | | | | | | | | | |
| NTDLPYEPPR | 0.0035 | 10.2% | | | 0.0052 | 8.5% | 0.0014 | 3.3% | | | 0.00049 | 5.7% | | | | | | |