

Oncogenic KRAS and BRAF drive metabolic reprogramming in colorectal cancer

Supplementary Figures

Josiah E. Hutton¹, Xiaojing Wang², Lisa J. Zimmerman^{1,3}, Robbert J. C. Slebos^{1,3}, Irina A. Trenary⁴, Jamey D. Young^{4,5}, Ming Li⁶, and Daniel C. Liebler^{1,3}

¹Department of Biochemistry, ²Department of Biomedical Informatics, ³Jim Ayers Institute for Precancer Detection and Diagnosis, ⁴Chemical & Biomolecular Engineering, ⁵Molecular Physiology & Biophysics, ⁶Department of Biostatistics, Vanderbilt University, Nashville, Tennessee 37232

Address correspondence to:

Daniel C. Liebler, Ph. D.

Vanderbilt University School of Medicine

465 21st Avenue South

Nashville, TN 37232-6350

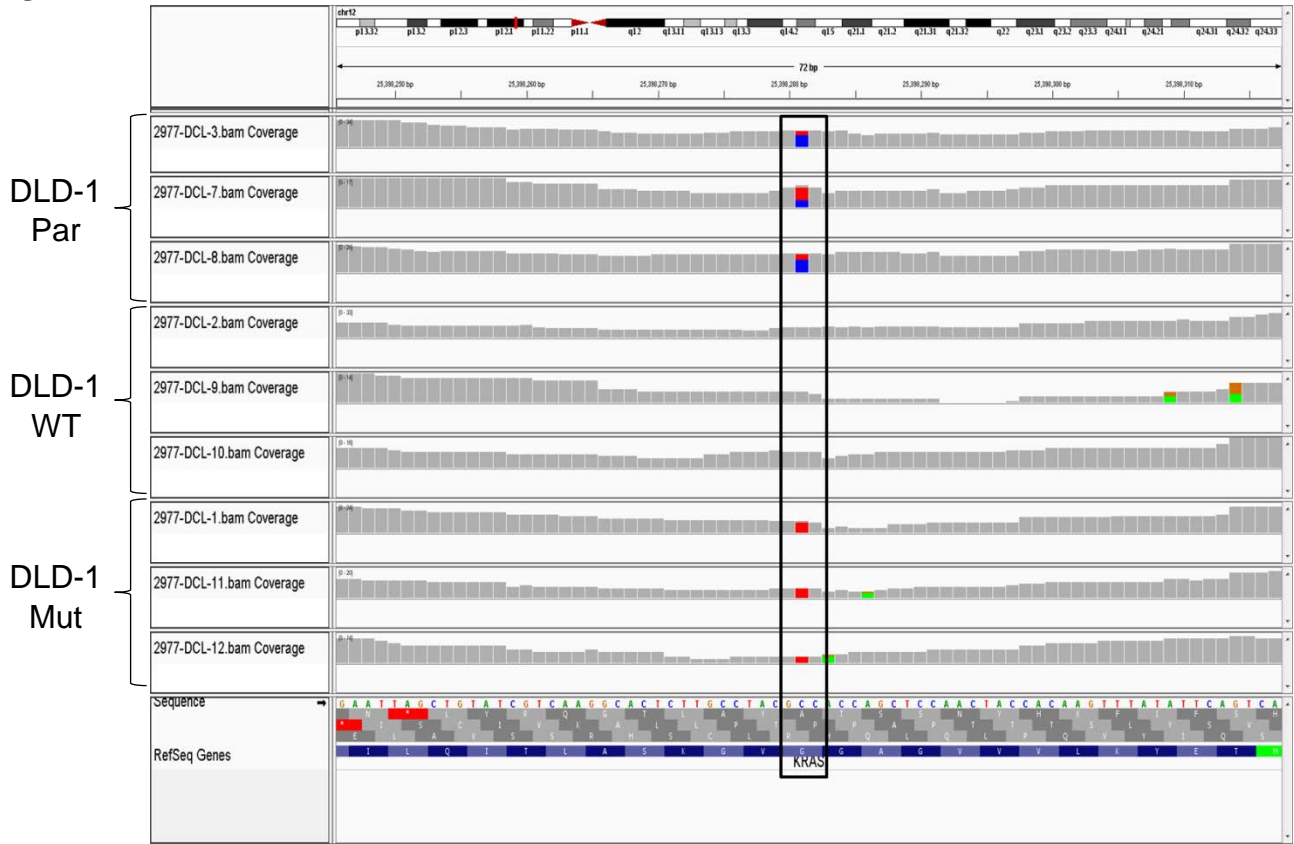
Email: daniel.liebler@vanderbilt.edu

Running Title: Metabolic reprogramming in colon cancer

Keywords: colon cancer, metabolism, parallel reaction monitoring, KRAS, BRAF, oncogene

Figure S1. Integrated Genomve Viewer of KRAS and BRAF RNA-Seq reads in DLD-1 and RKO cells. RNA-Seq was performed on DLD-1 and RKO cells to verify that cell lines contained the expected mutant and/or wild type KRAS or BRAF mRNA sequences. A) IGV for KRAS in DLD-1 cells. DCL-3, DCL-7, and DCL-8 are DLD-1 Par cells; and DCL-1, DCL-11, and DCL-12 are DLD-1 Mut cells. The blue or grey bar at nucleotide position 38 corresponds to the wild type reference genome sequence (38 G), while a red bar at position 38 indicates the mutation of 38G→A for the KRAS G13D mutation. B) IGV for BRAF in RKO cells. DCL-13, DLC-14, and DCL-15 are RKO WT cells; DCL-16, DCL-17, and DCL-18 are RKO Par cells; and DCL-19, DCL-20, and DCL-21 are RKO Mut cells. A green bar at nucleotide position 1799 indicate that the wild type nucleotide (1799 T) is at that position, while a red bar indicates that the mutant 1799T→A nucleotide is present coding for BRAF V600E.

S1A



S1B

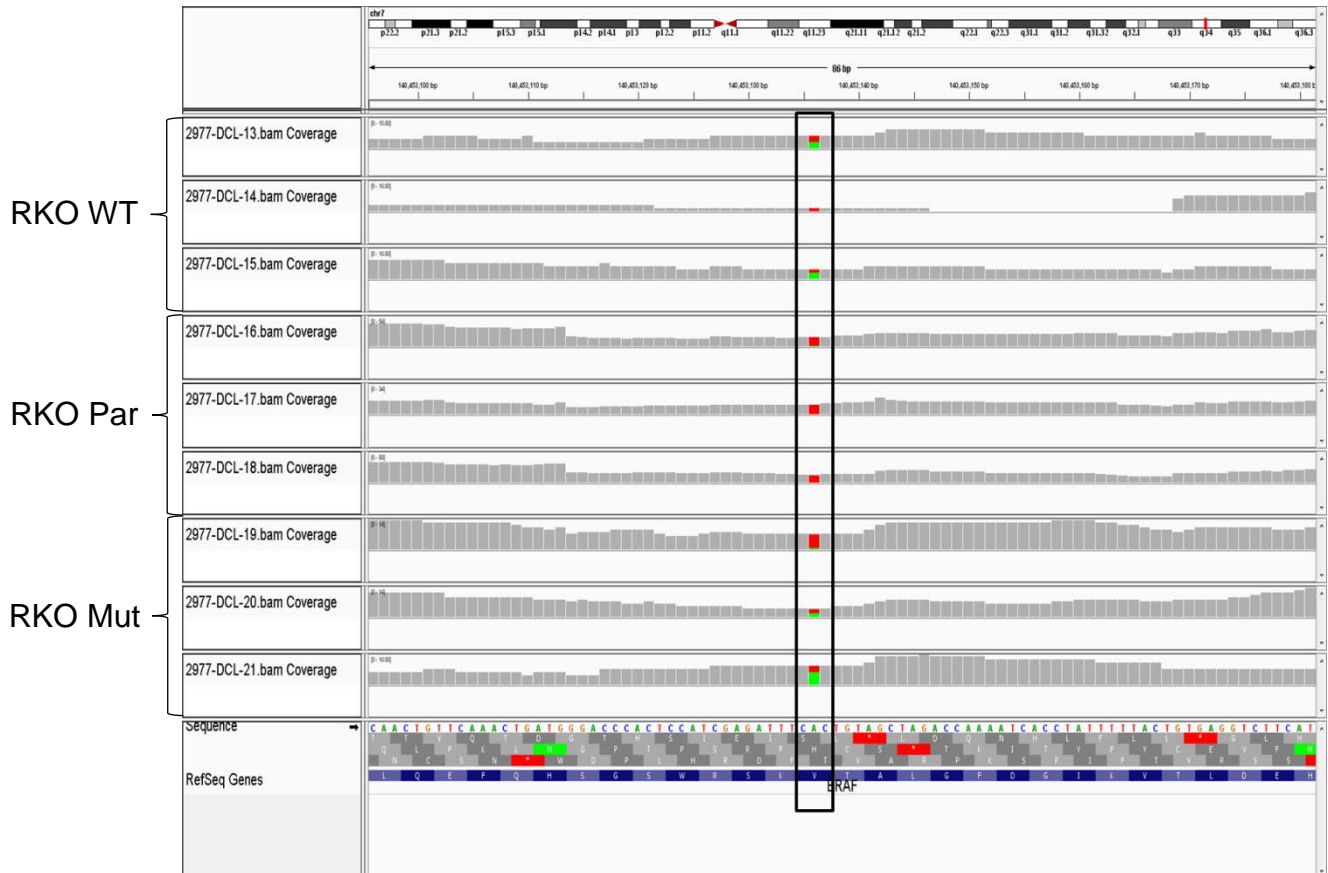
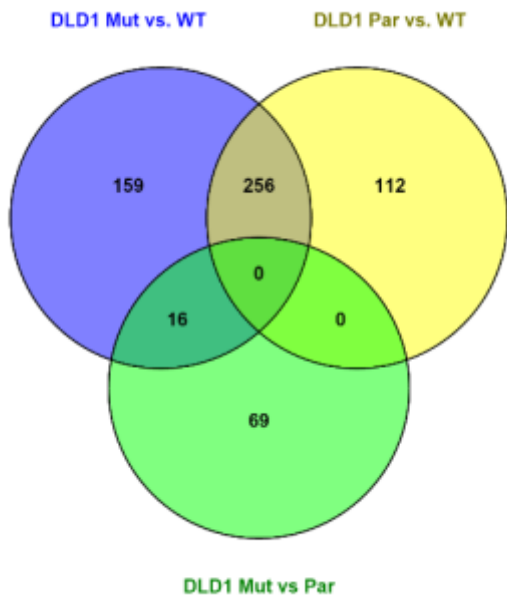
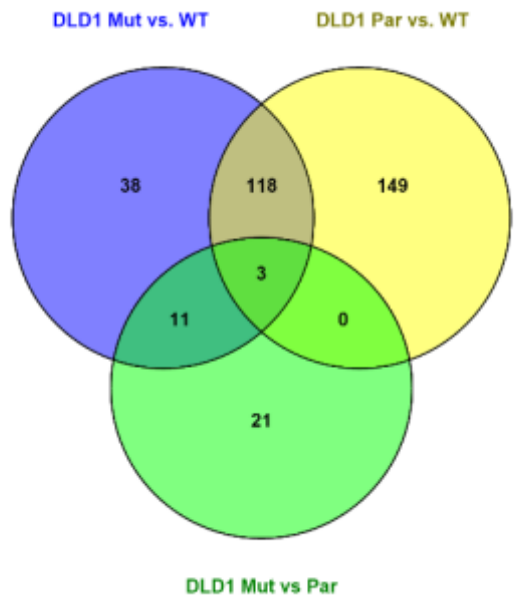


Figure S2. Venn diagram comparisons of 2-fold differentially expressed proteins in shotgun analyses of cell lines. Pairwise comparisons identified proteins with 2-fold differential expression between cell lines. A) Comparisons of 2-fold increased proteins in DLD-1 cell lines. B) Comparisons of 2-fold decreased proteins in DLD-1 cell lines. C) Comparisons of 2-fold increased proteins in RKO cell lines. D) Comparisons of 2-fold decreased proteins in RKO cell lines.

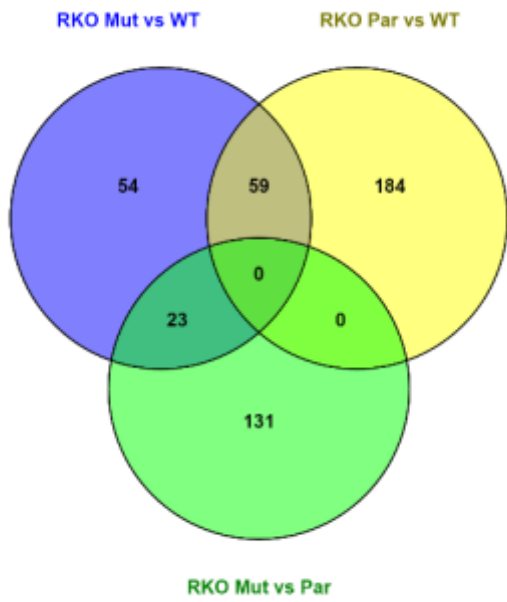
S2A



S2B



S2C



S2D

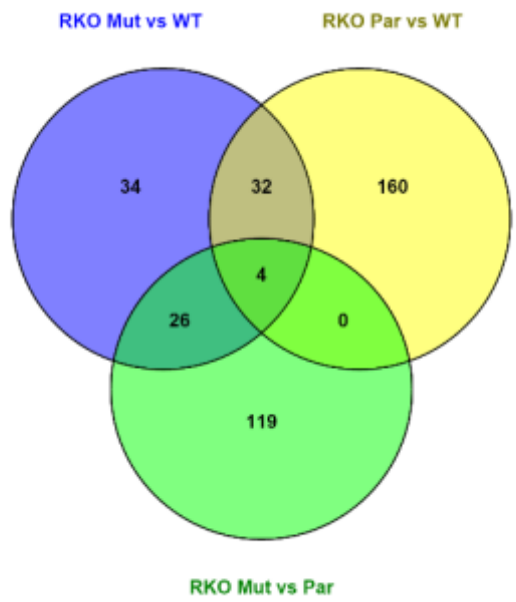
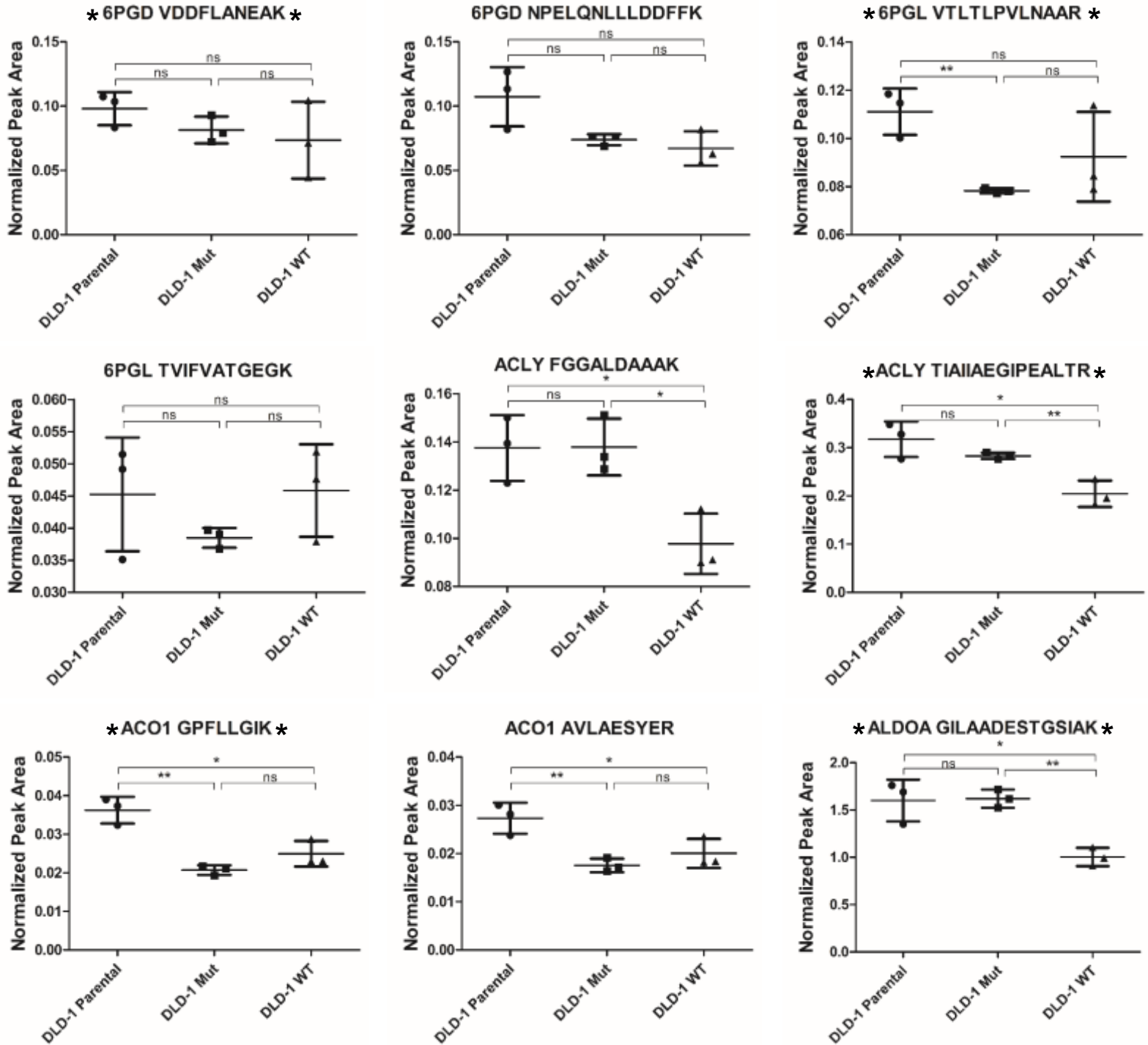
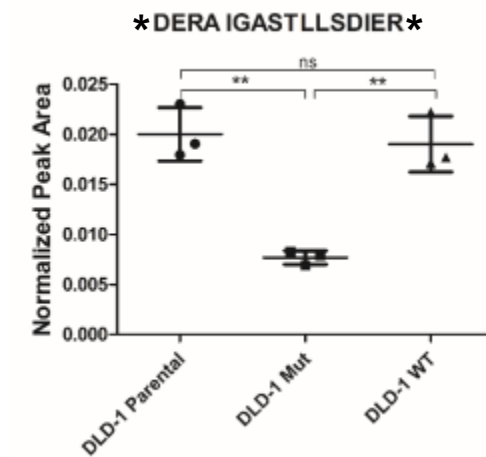
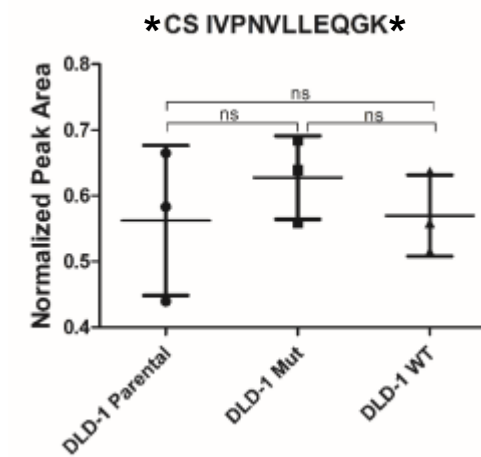
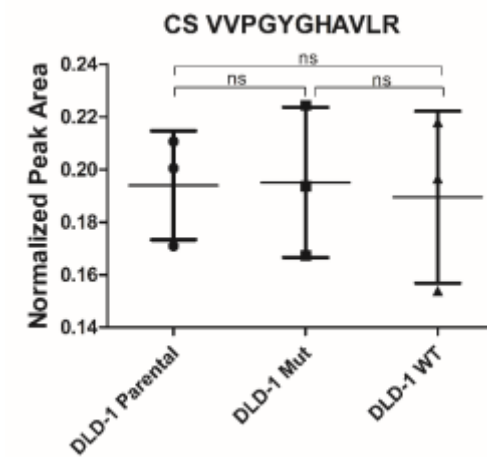
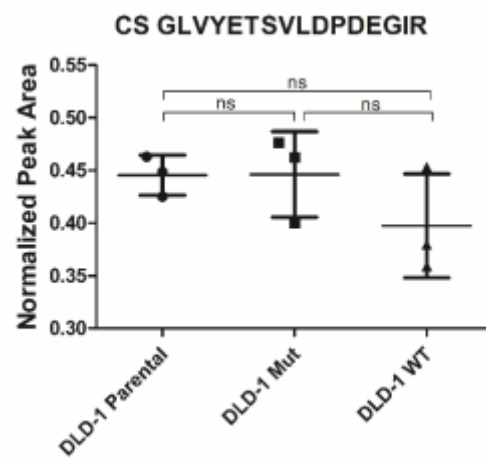
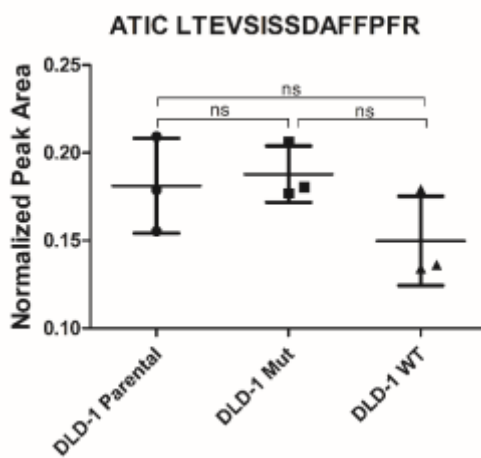
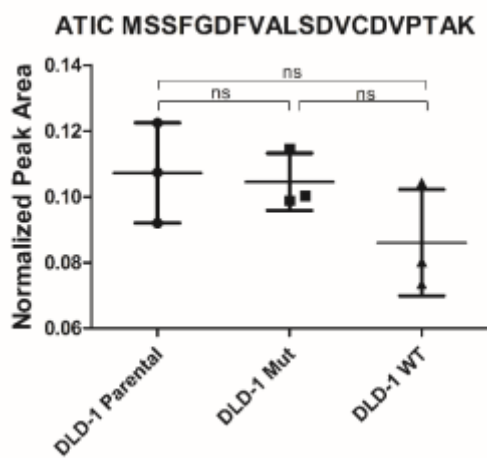
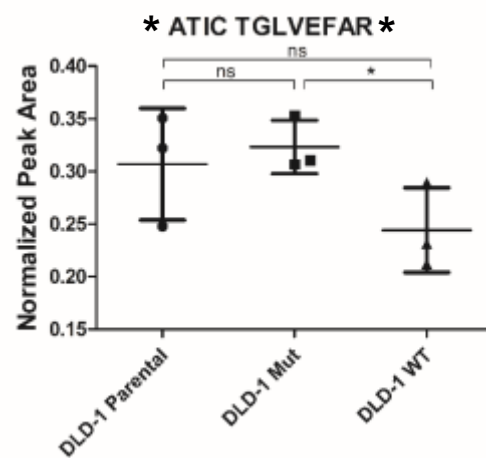
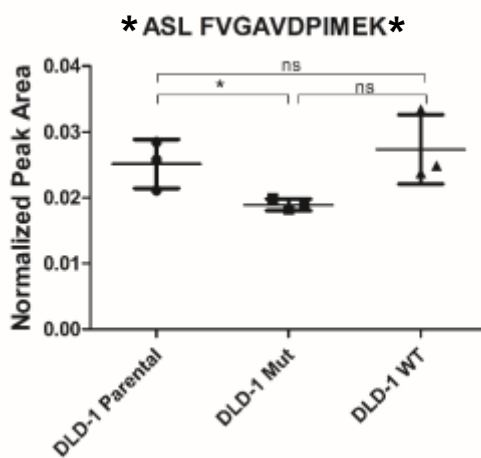
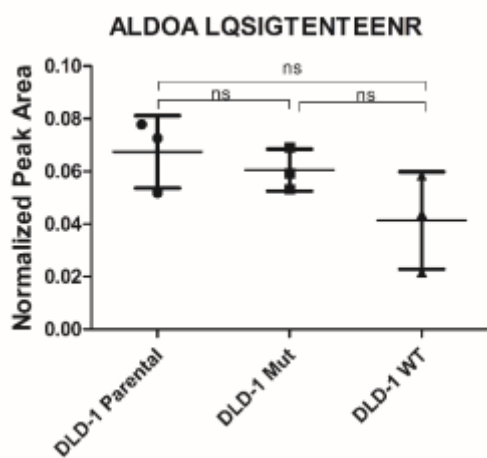


Figure S3. Quantitative comparisons of peptides from metabolic proteins by PRM in DLD-1 cell lines. Each peptide peak area was normalized to that of the LRP standard. Pairwise comparisons are shown with bars above the plots. Significant differences were determined using Student's t-test, where the asterisks denote p-values: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; ns indicates no significant difference. Peptides selected for quantitative comparisons and for Figure 4 are listed with an asterisk (*) before and after the protein name and peptide sequence.

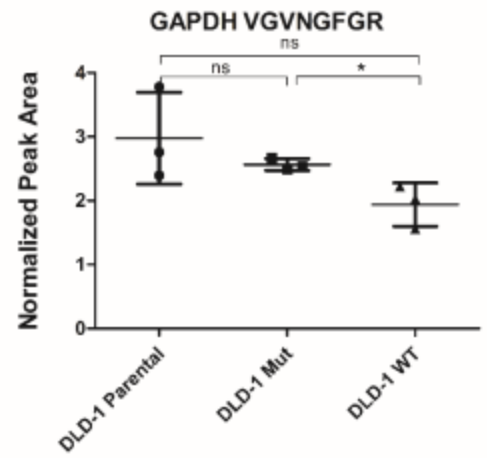
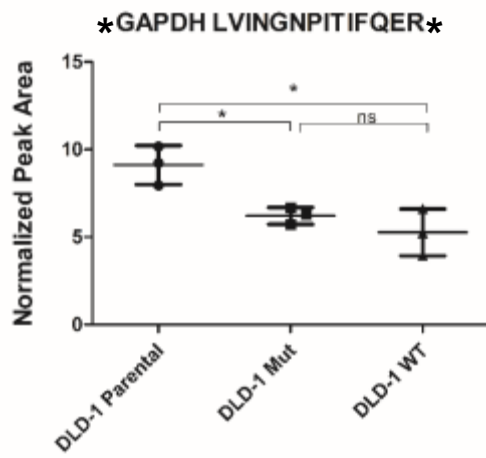
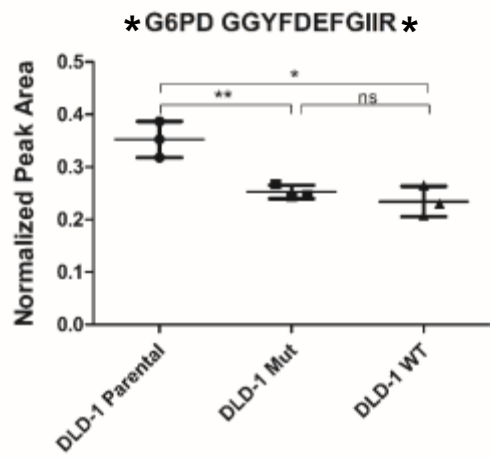
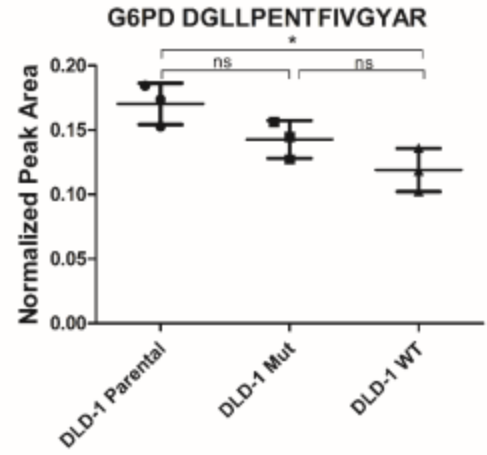
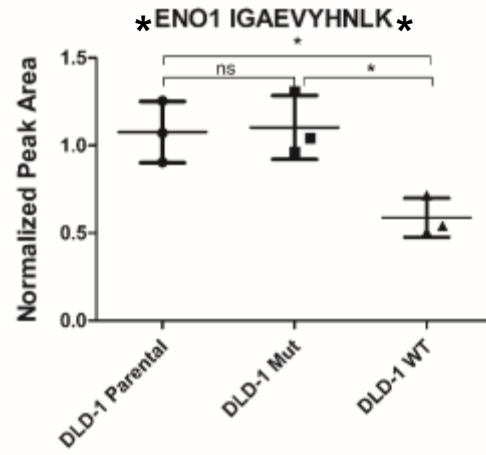
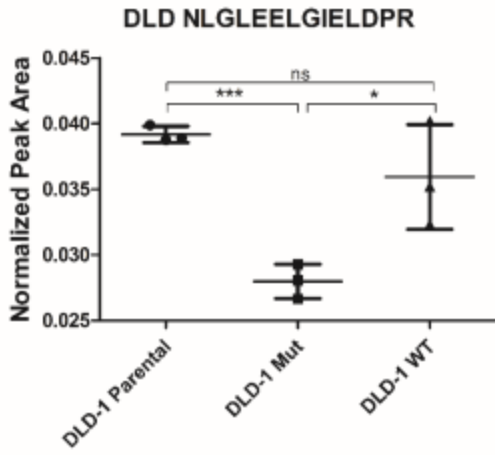
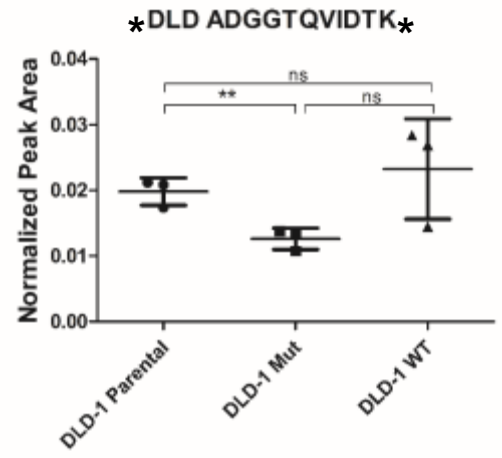
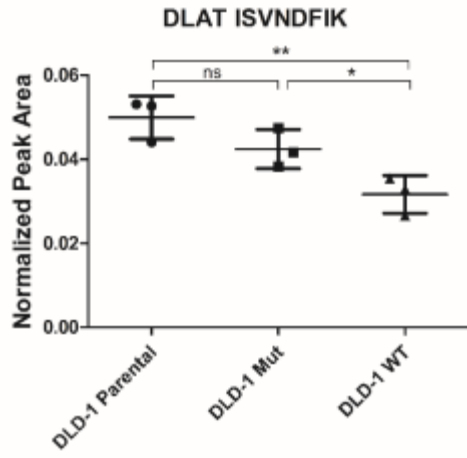
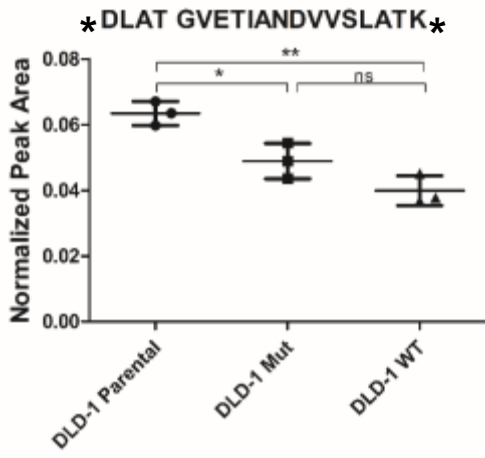
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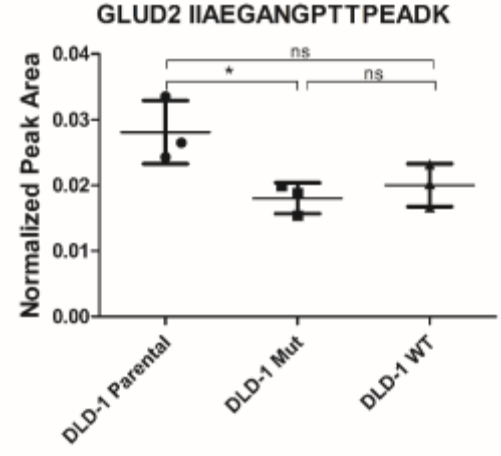
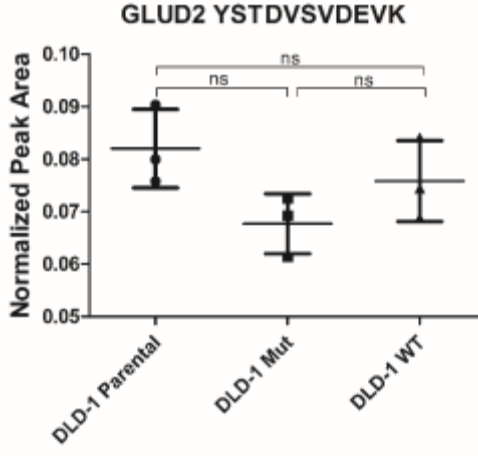
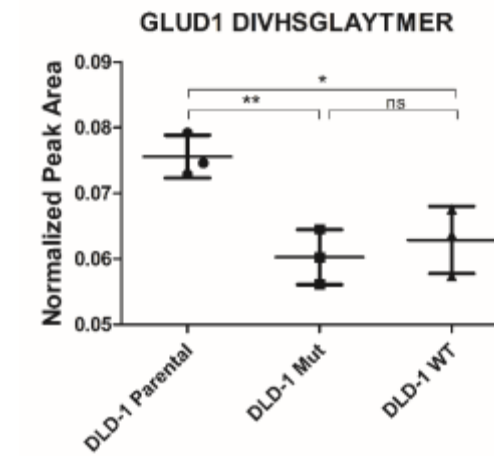
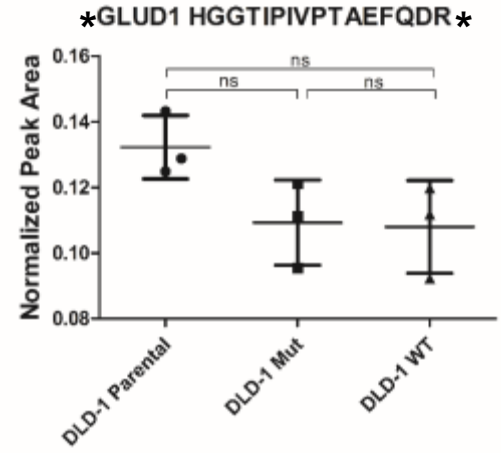
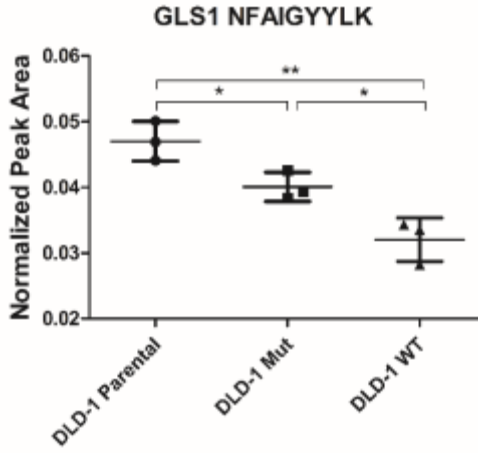
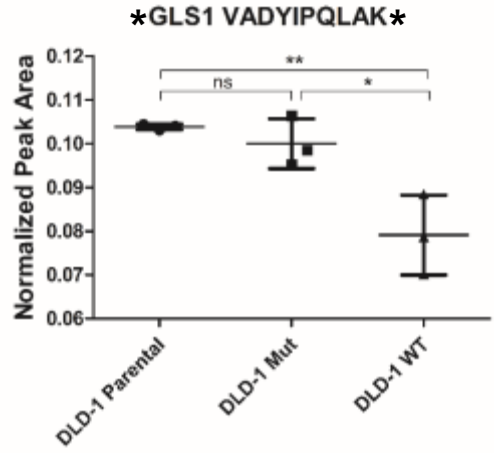
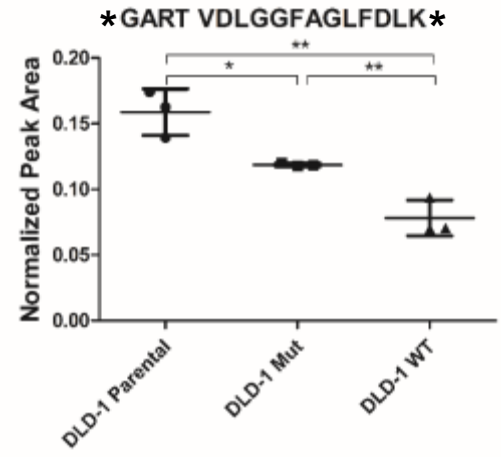
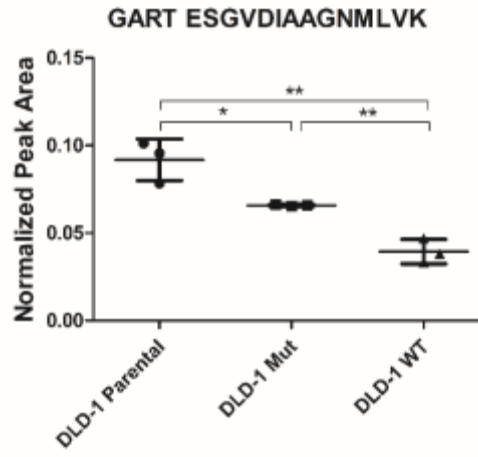
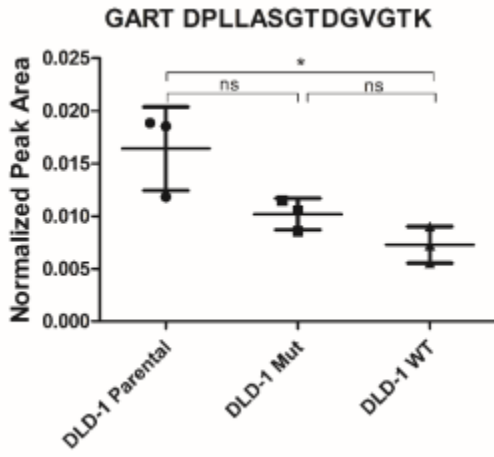
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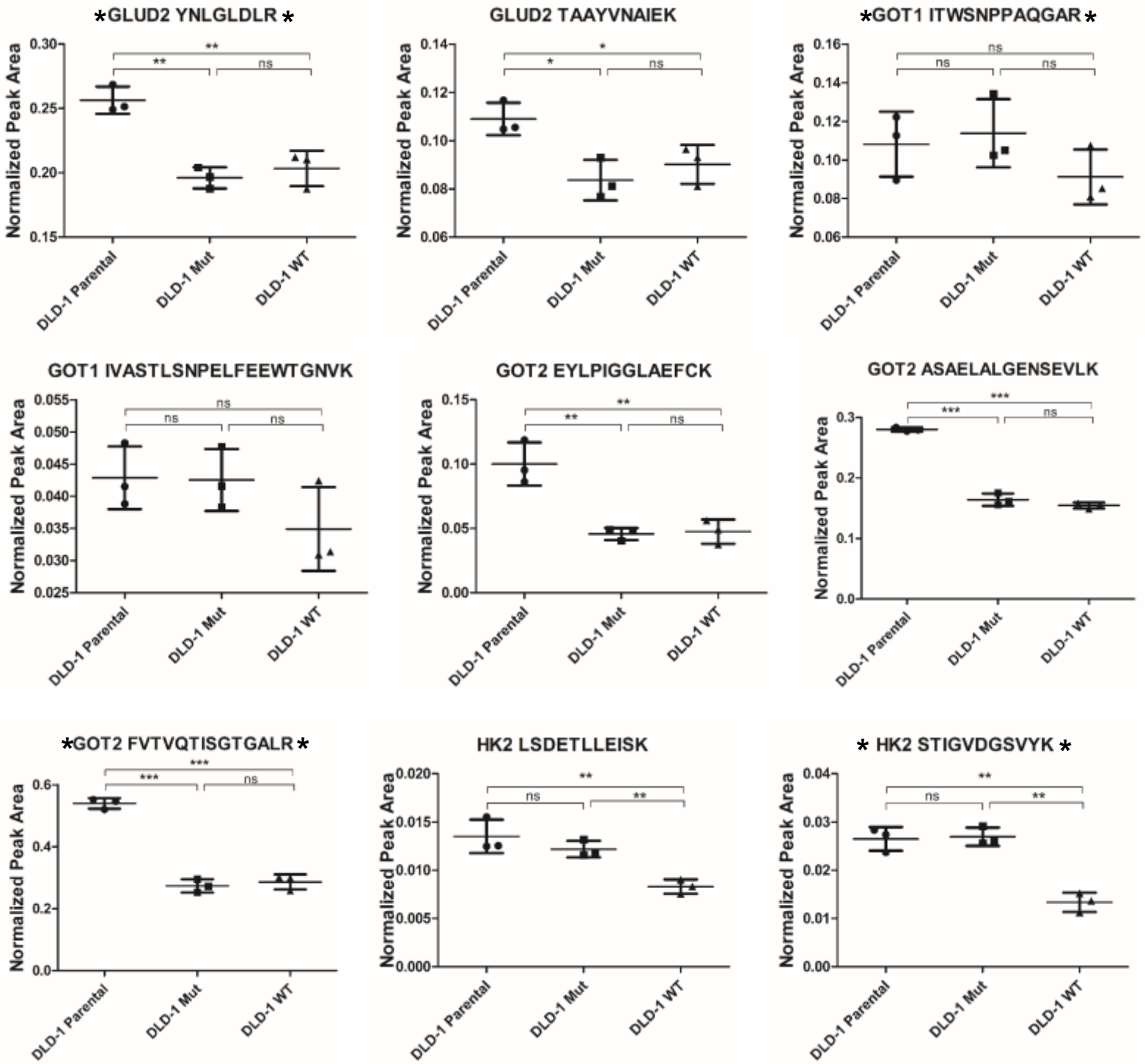
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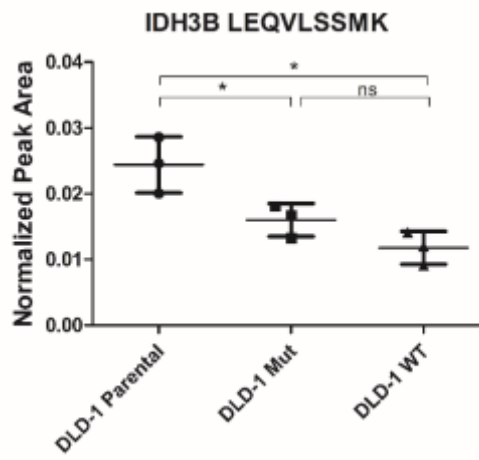
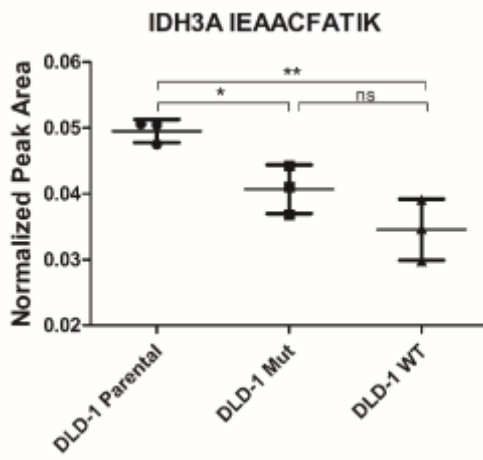
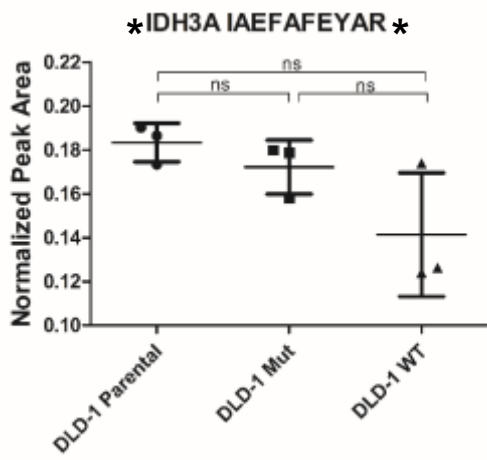
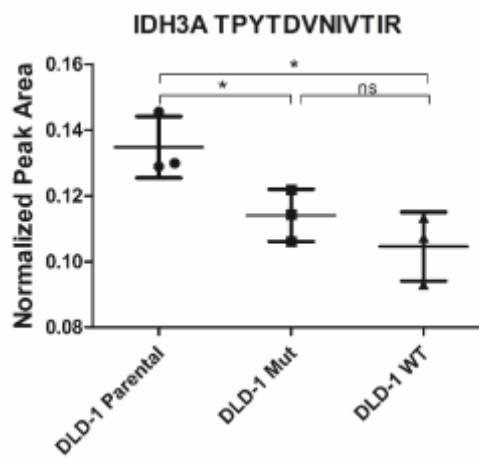
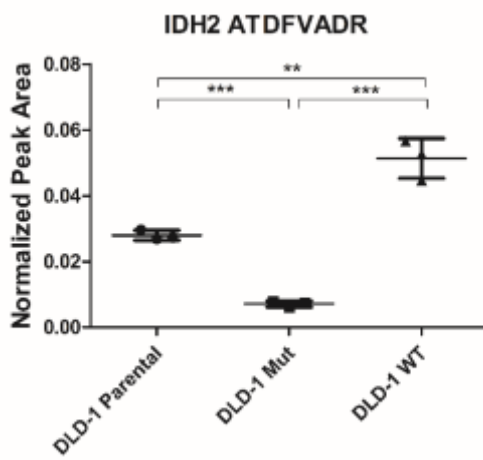
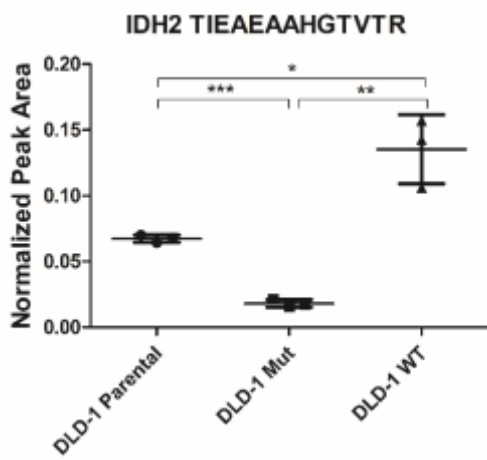
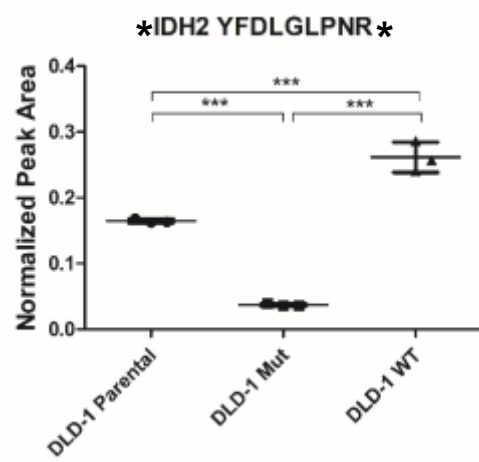
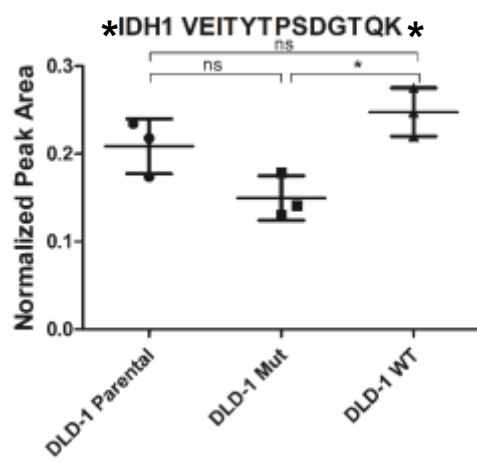
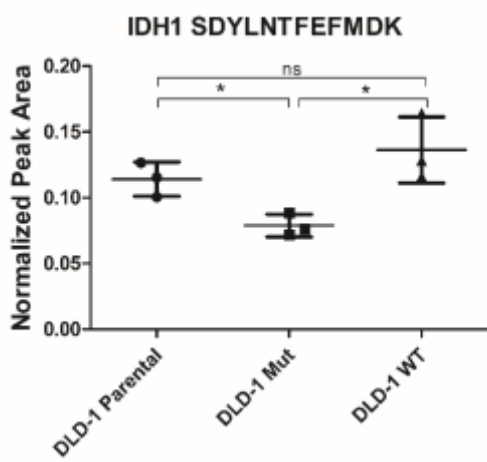
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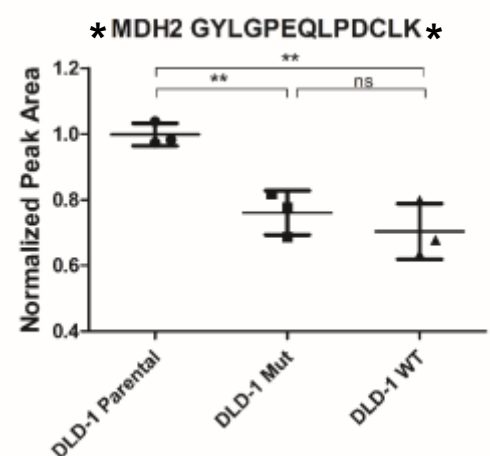
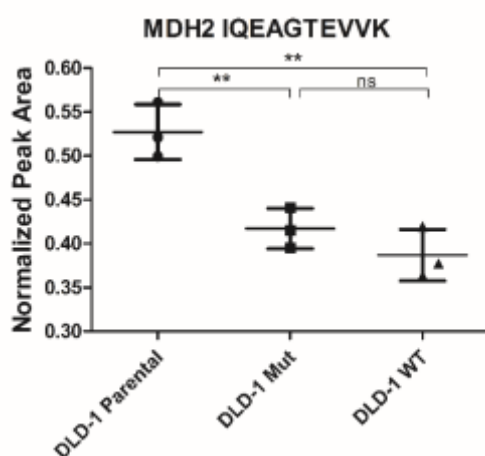
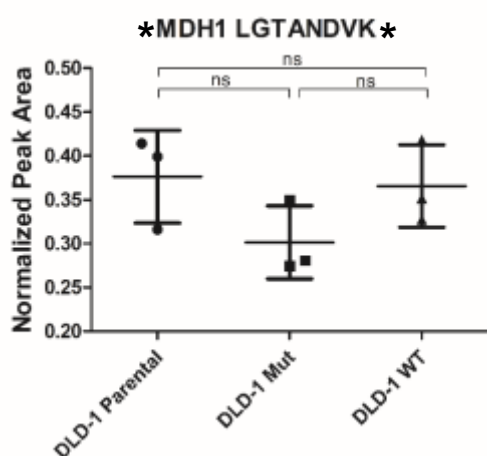
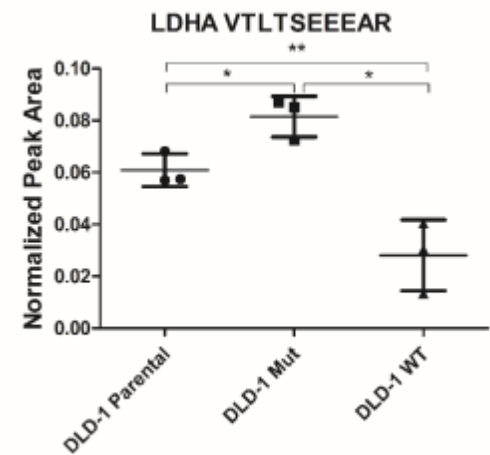
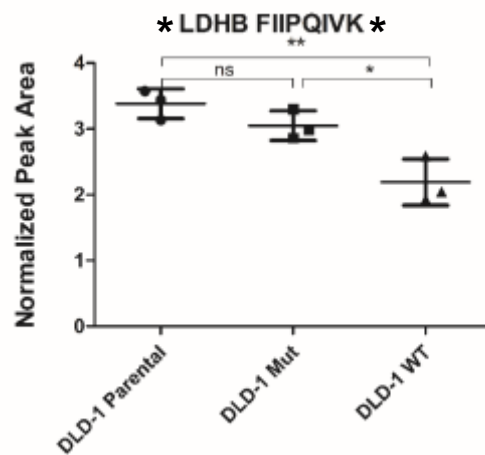
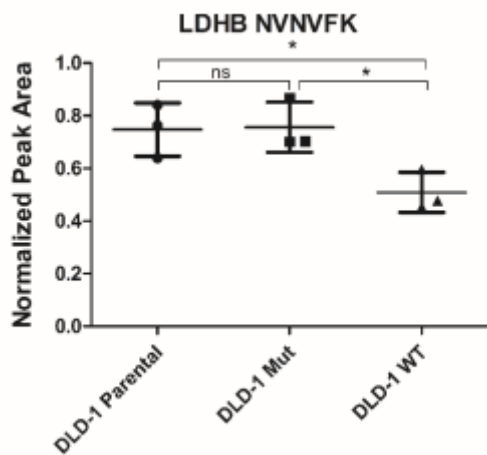
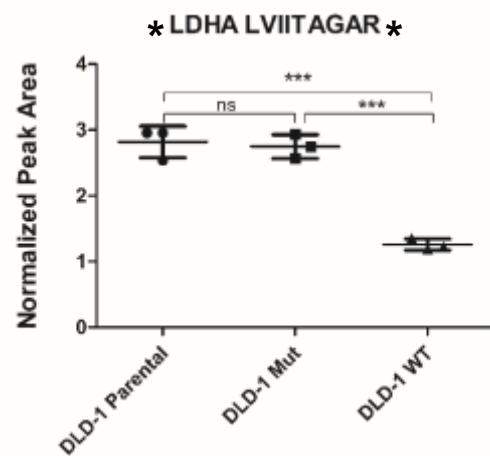
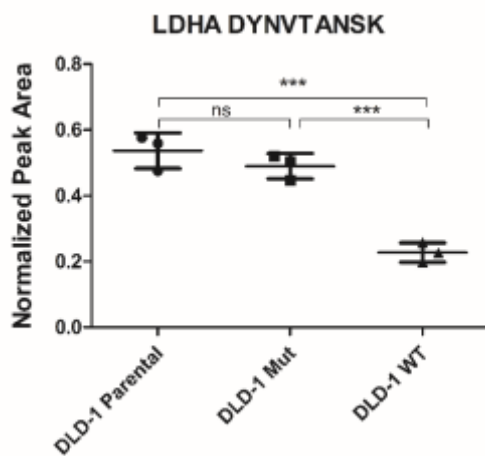
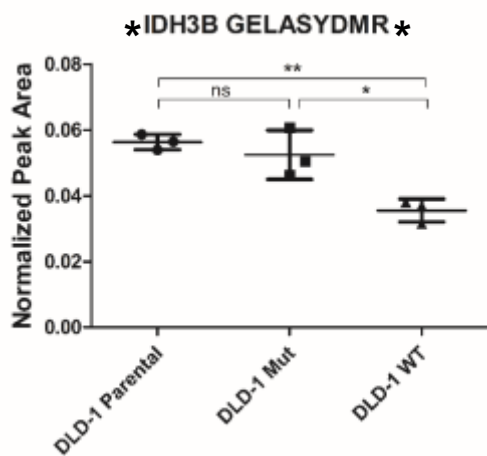
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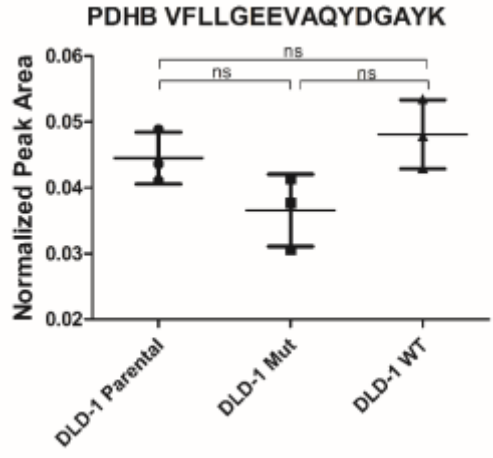
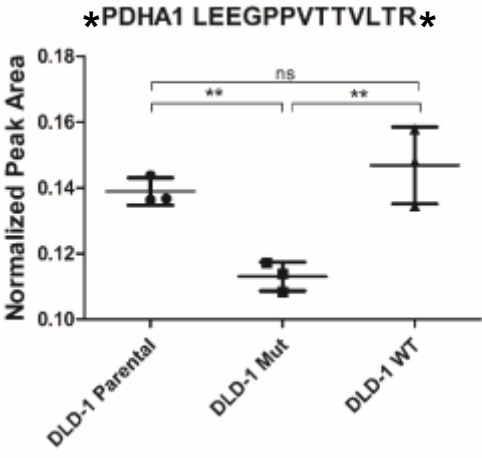
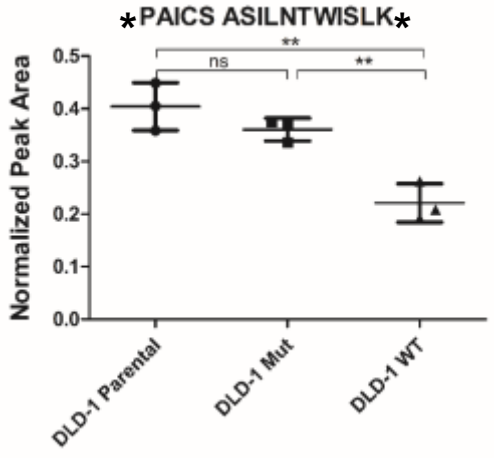
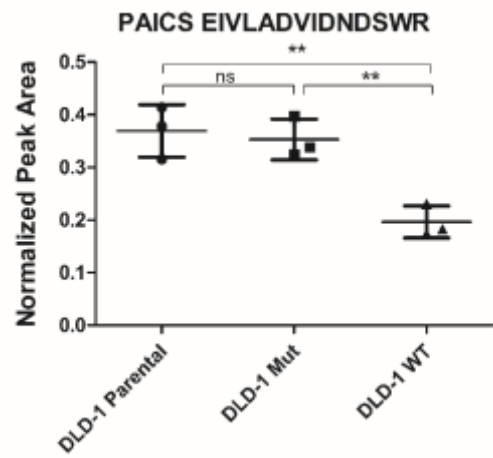
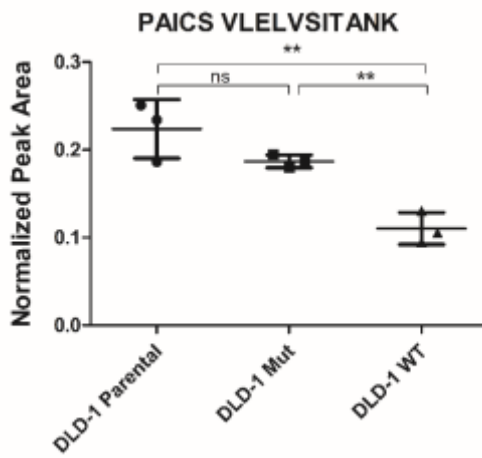
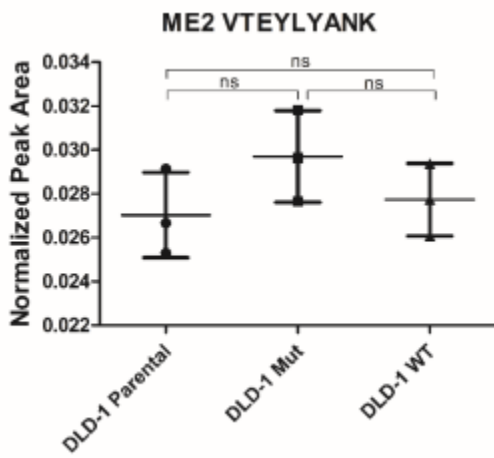
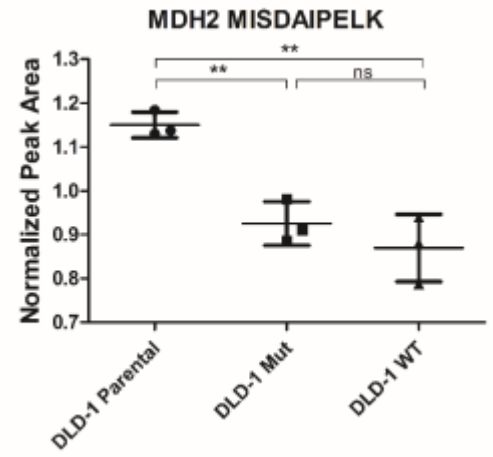
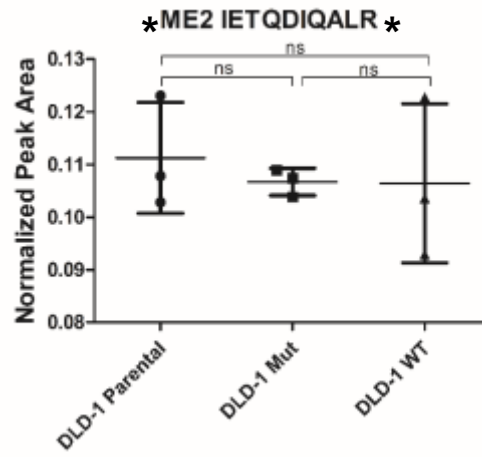
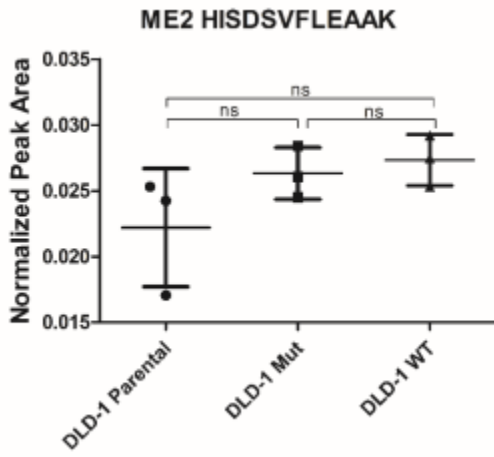
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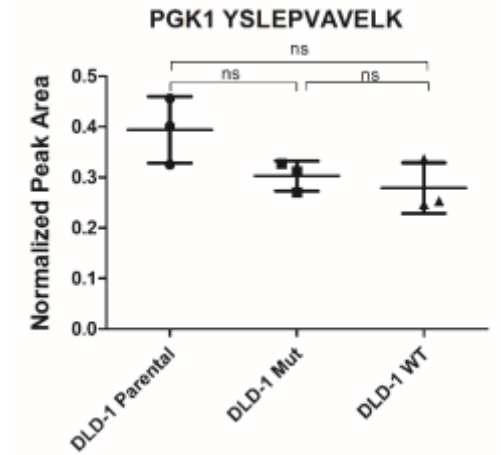
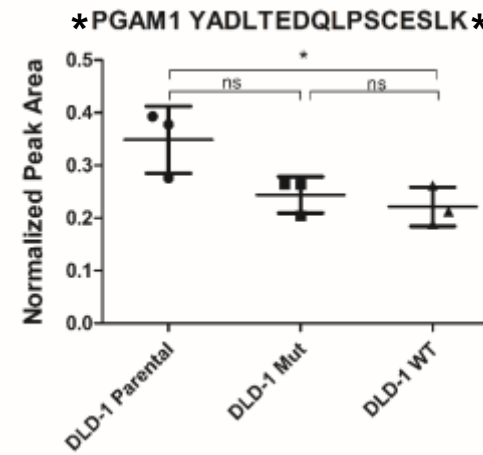
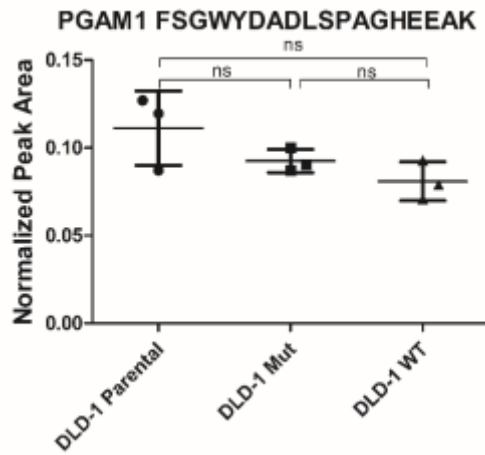
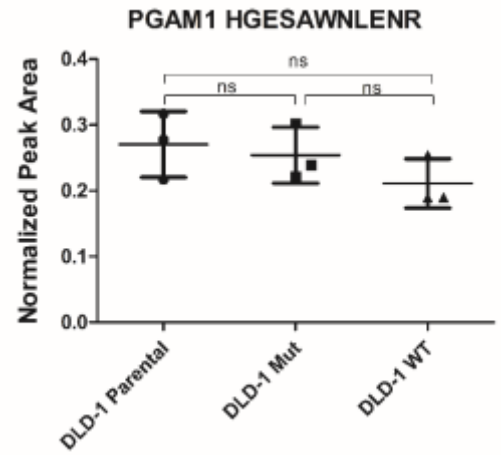
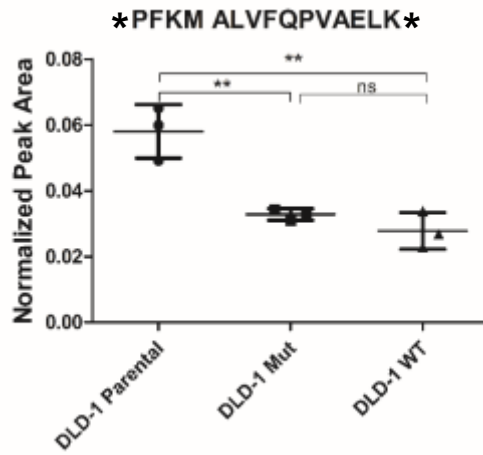
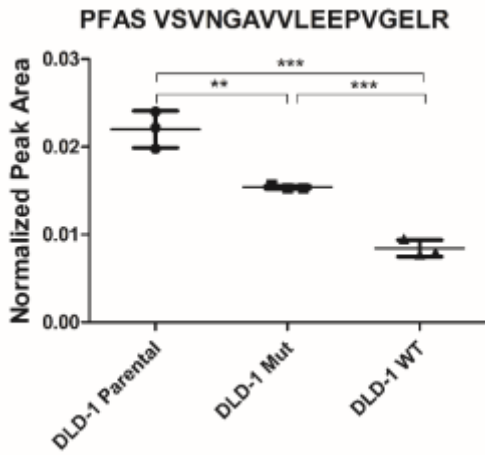
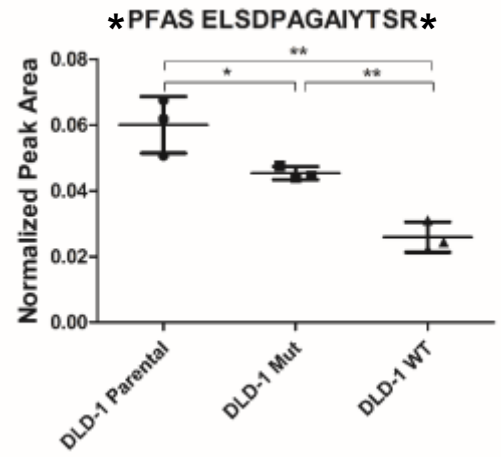
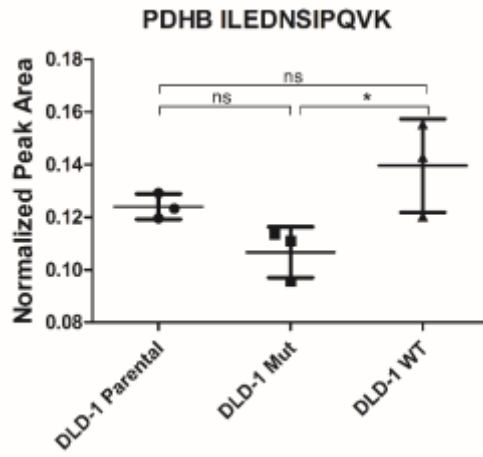
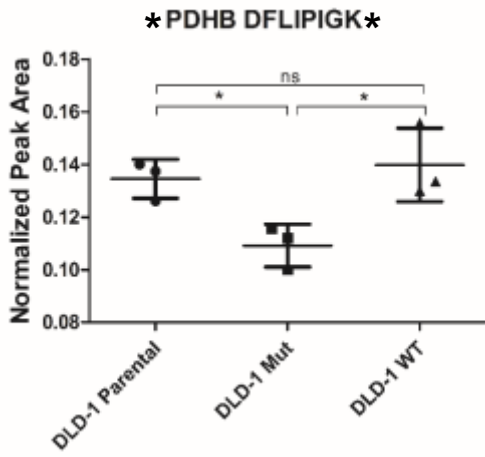


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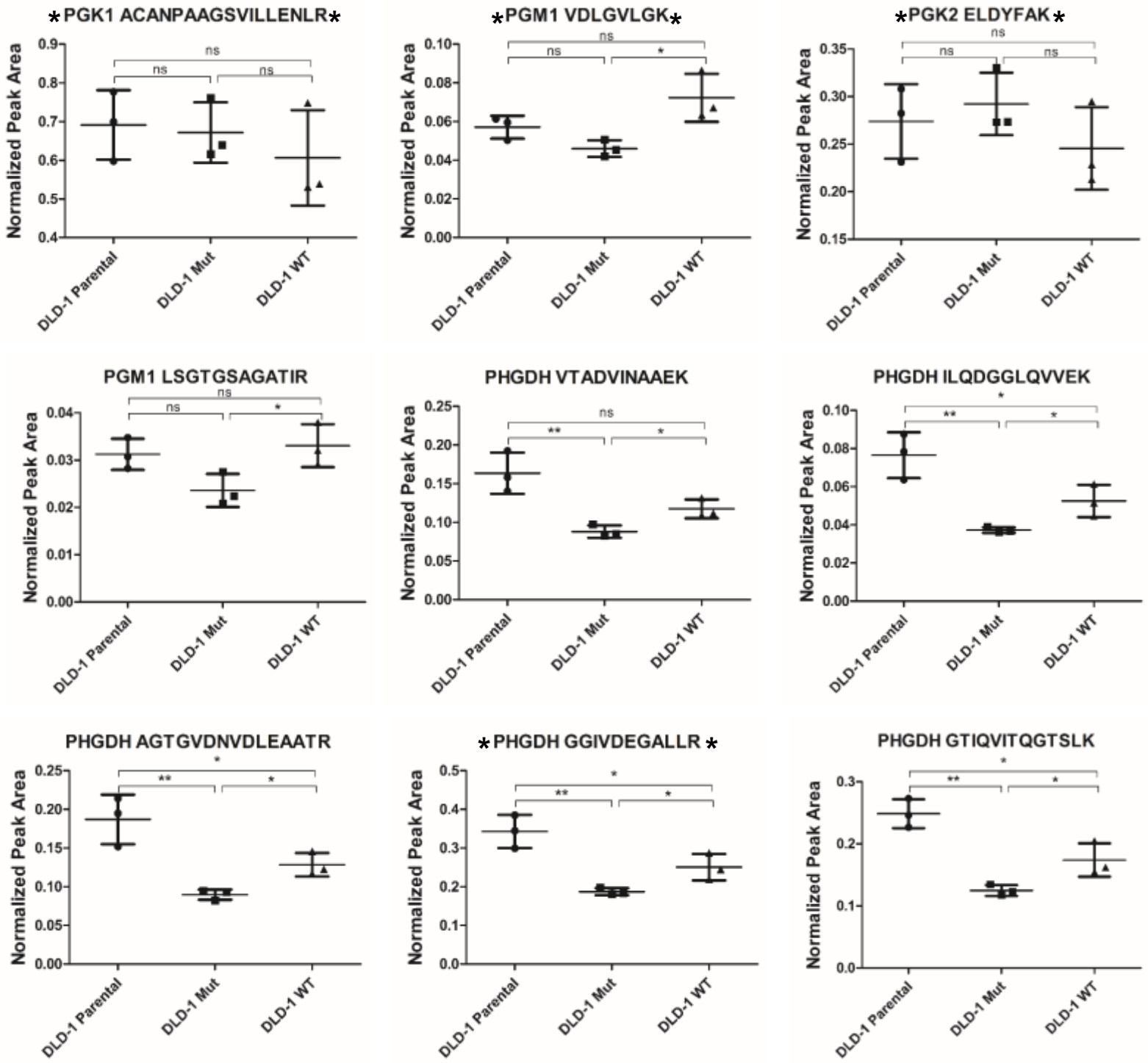


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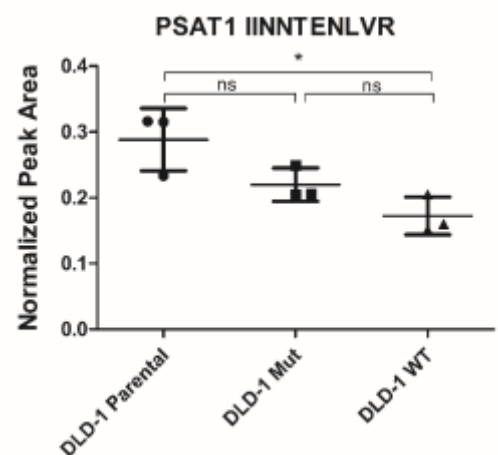
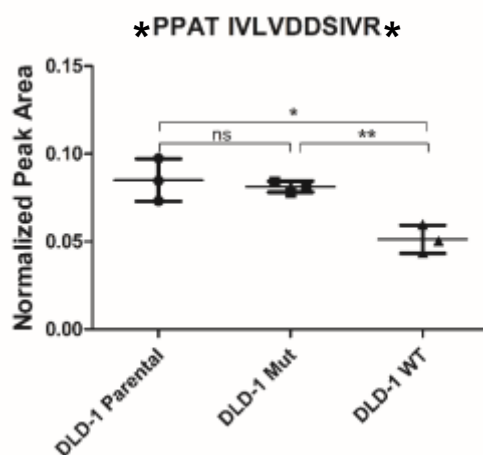
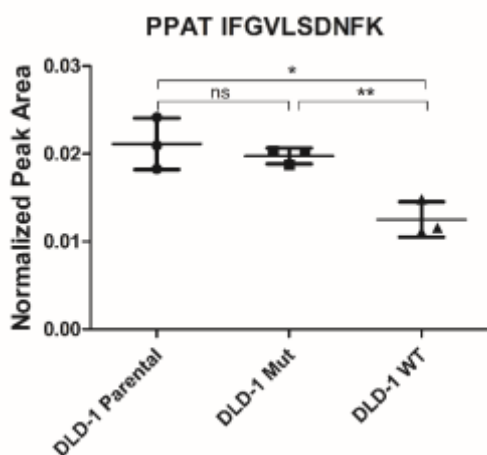
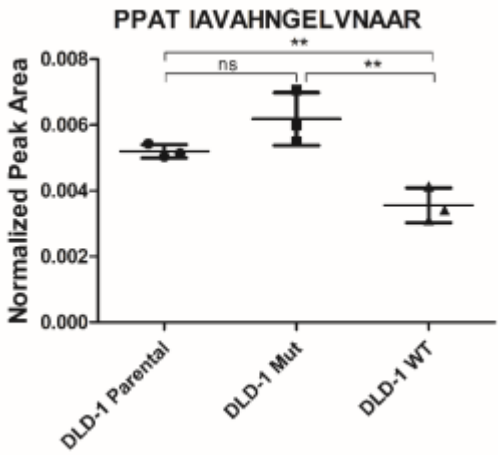
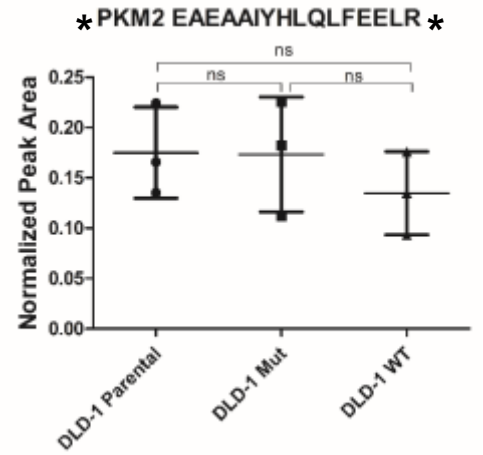
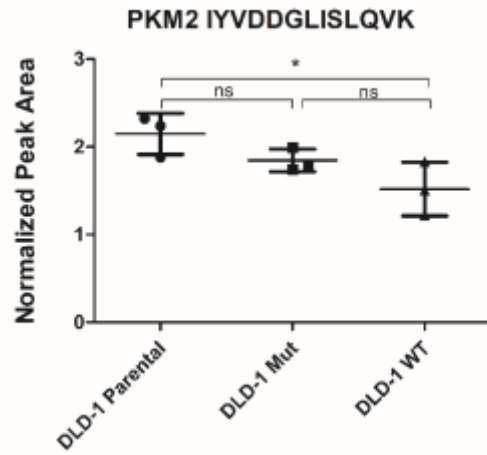
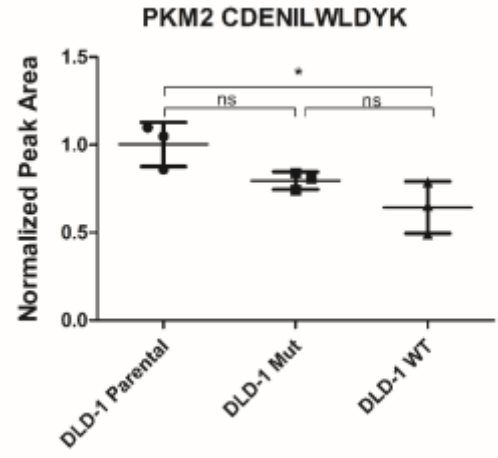
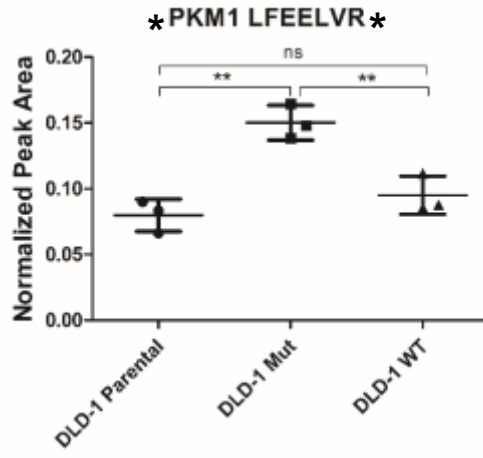
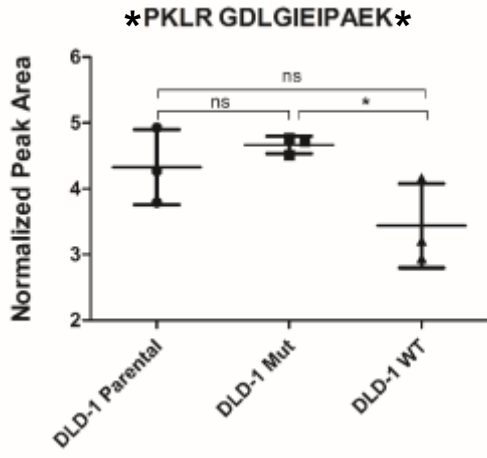




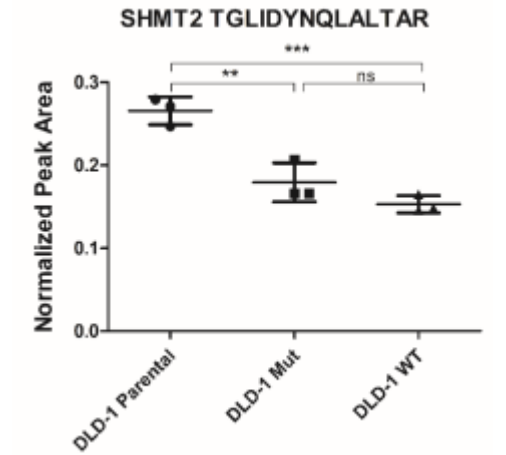
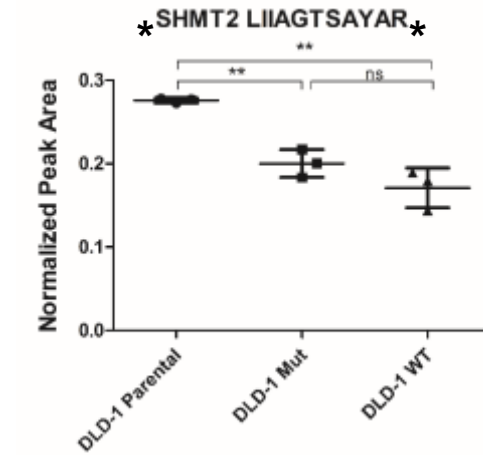
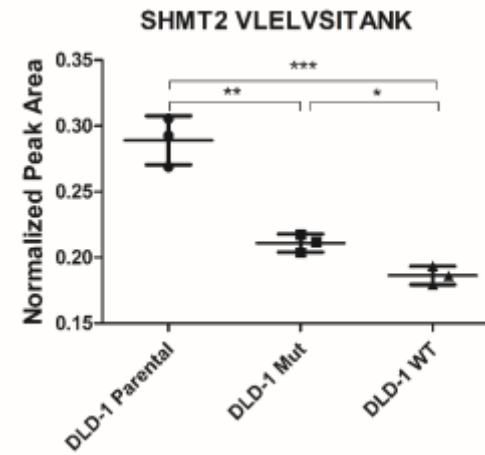
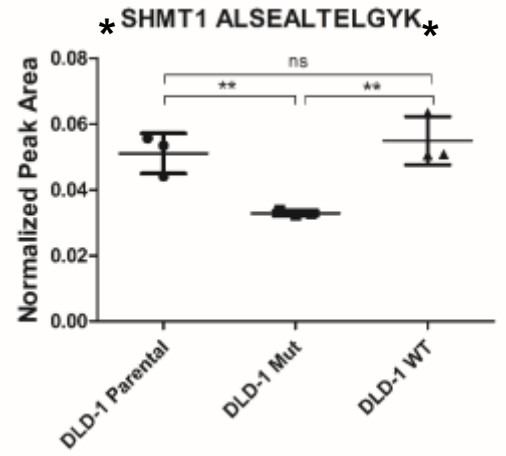
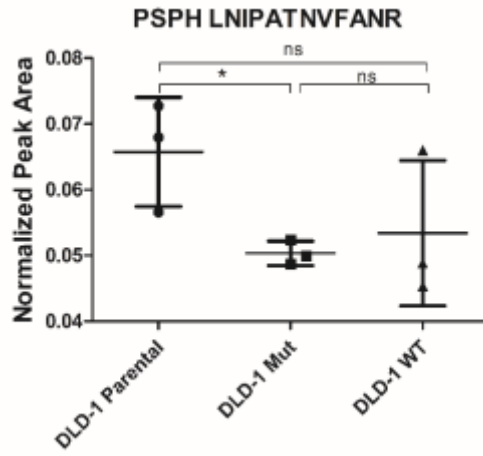
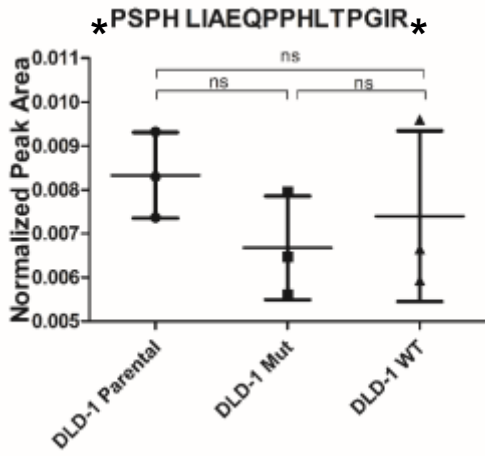
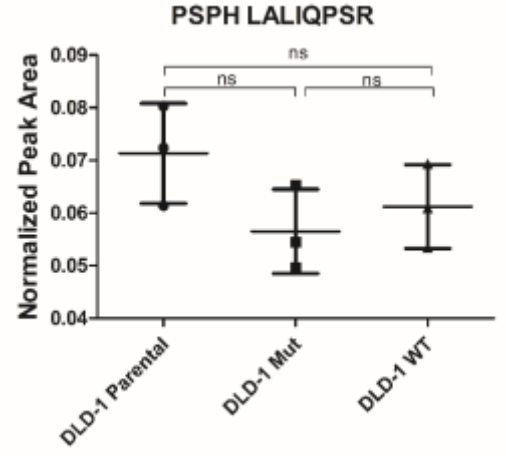
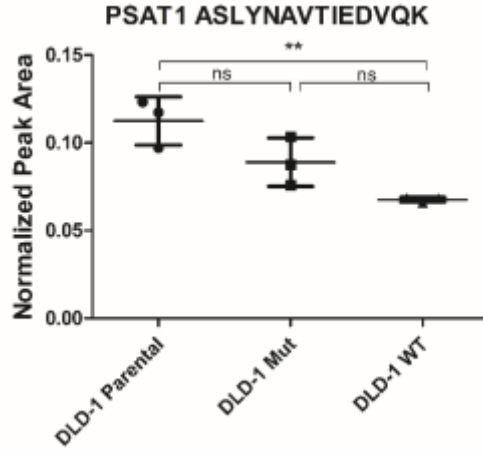
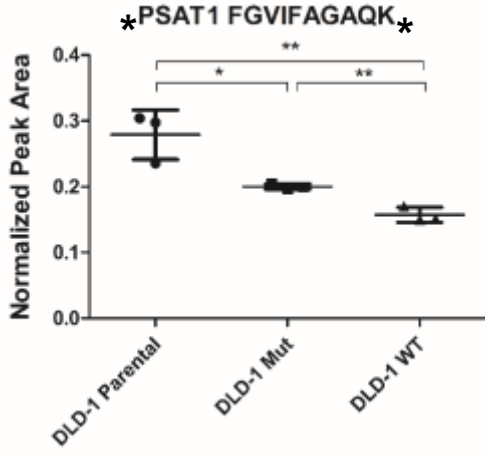
S3J



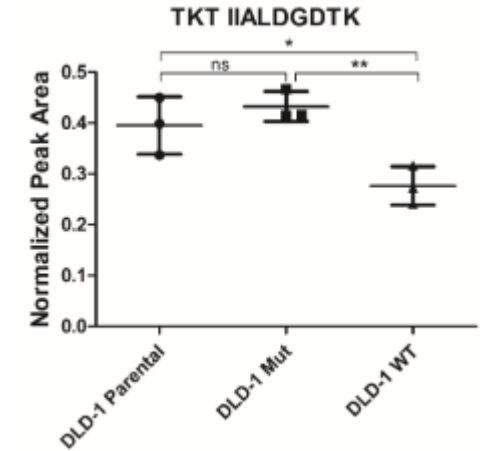
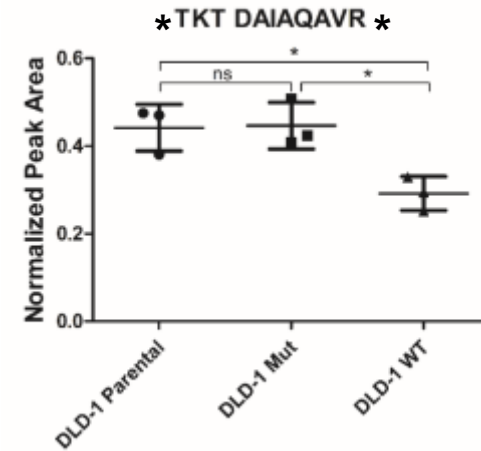
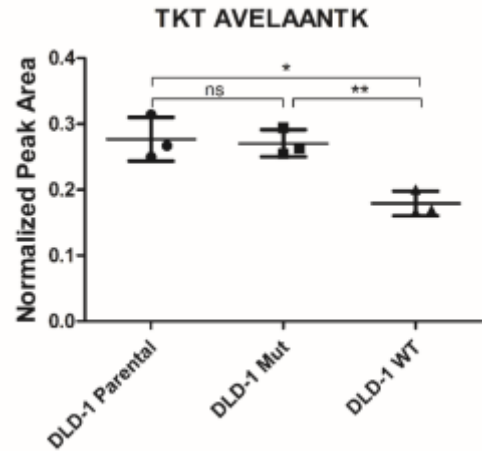
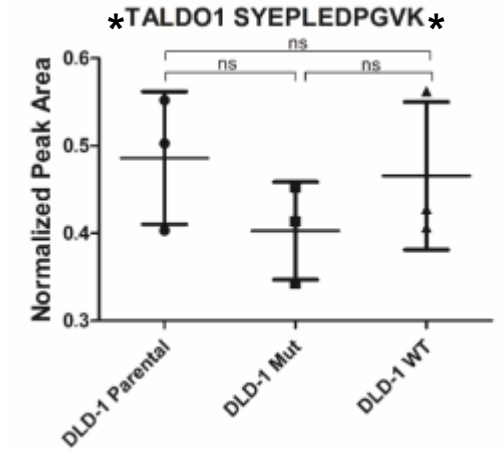
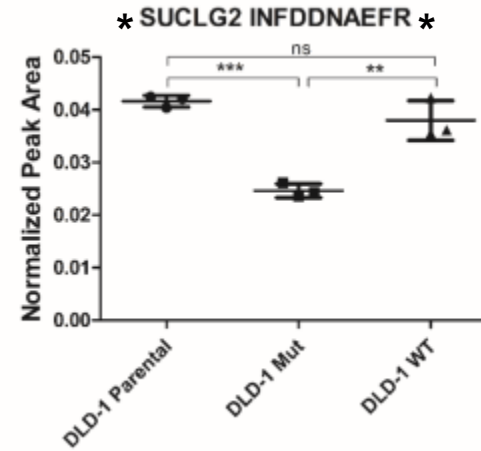
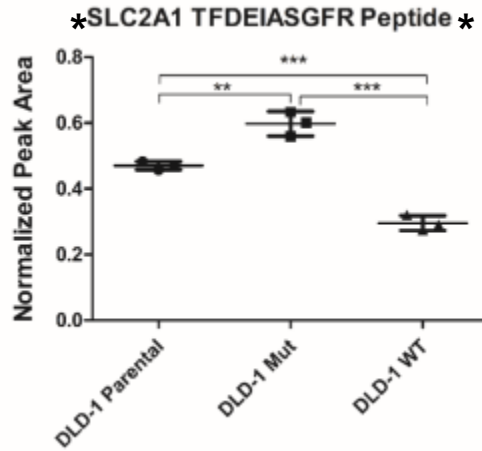
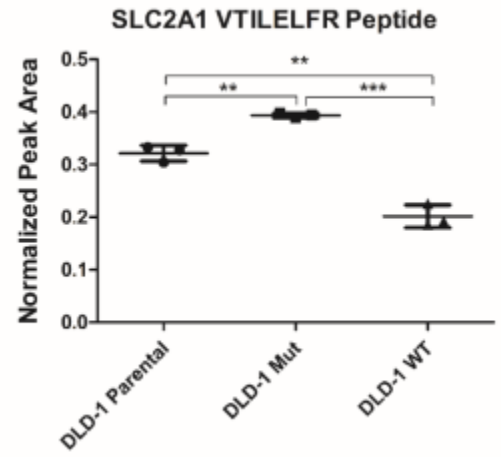
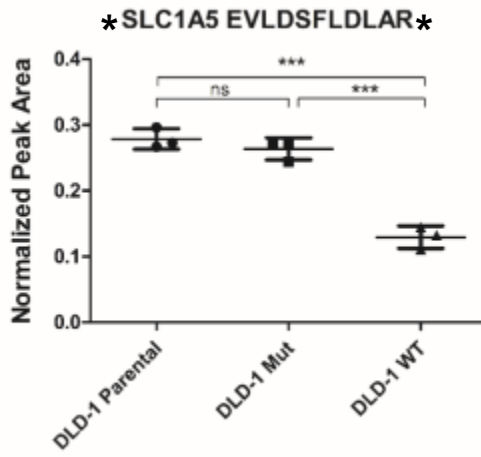
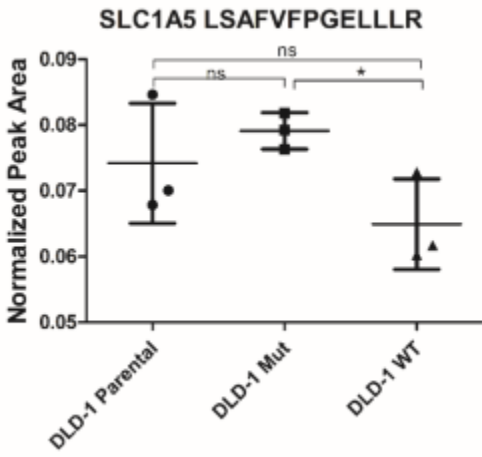
S3K



S3L



S3M



S3N

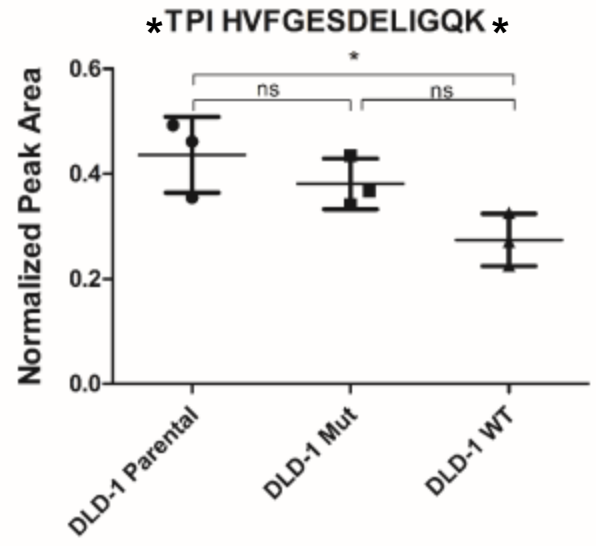
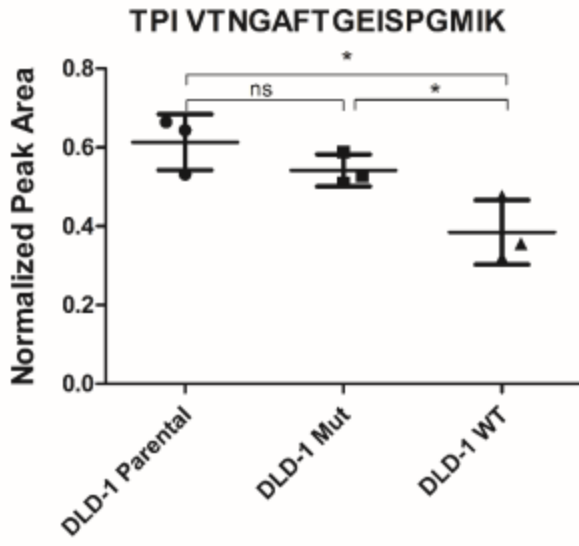
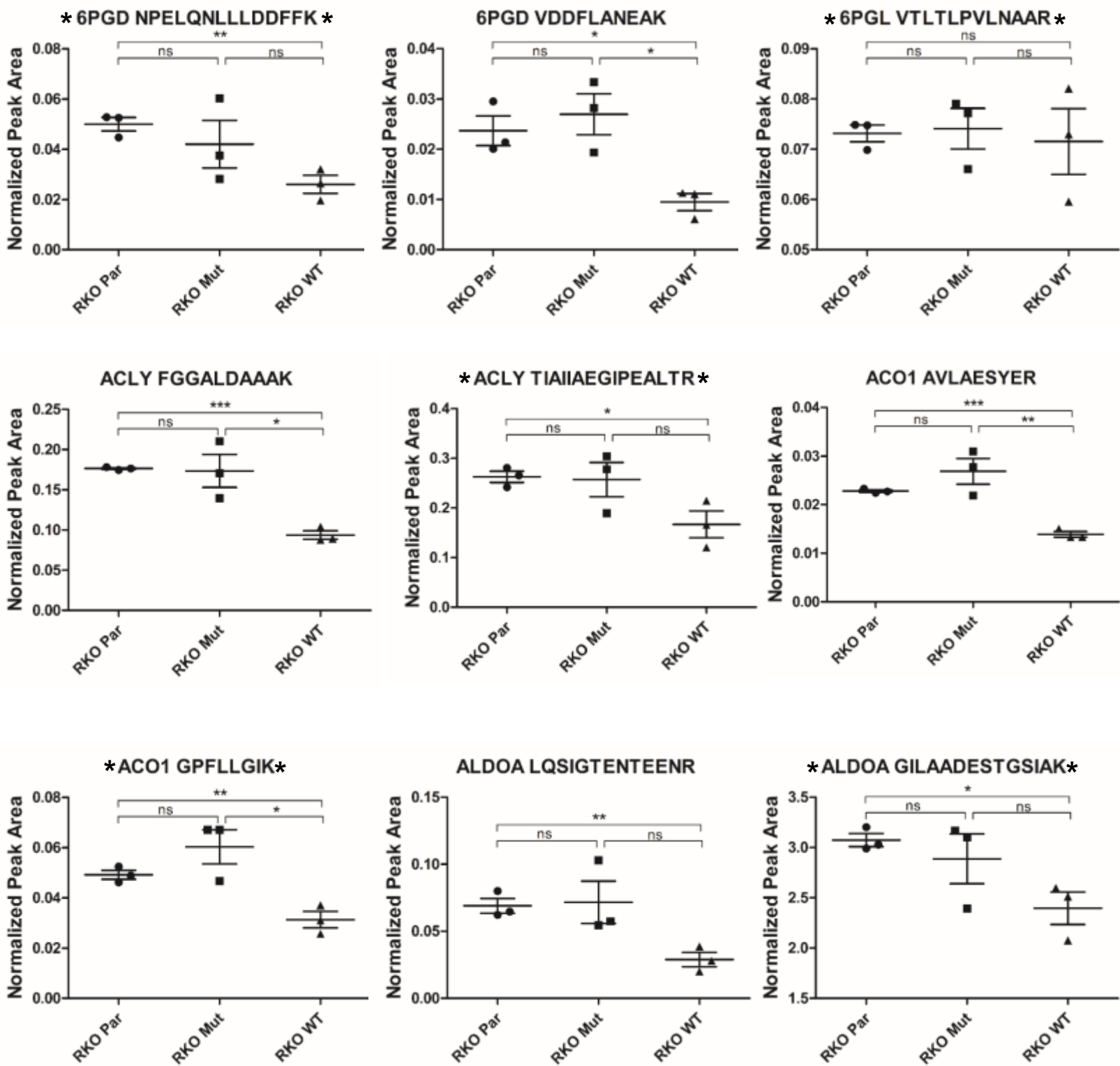
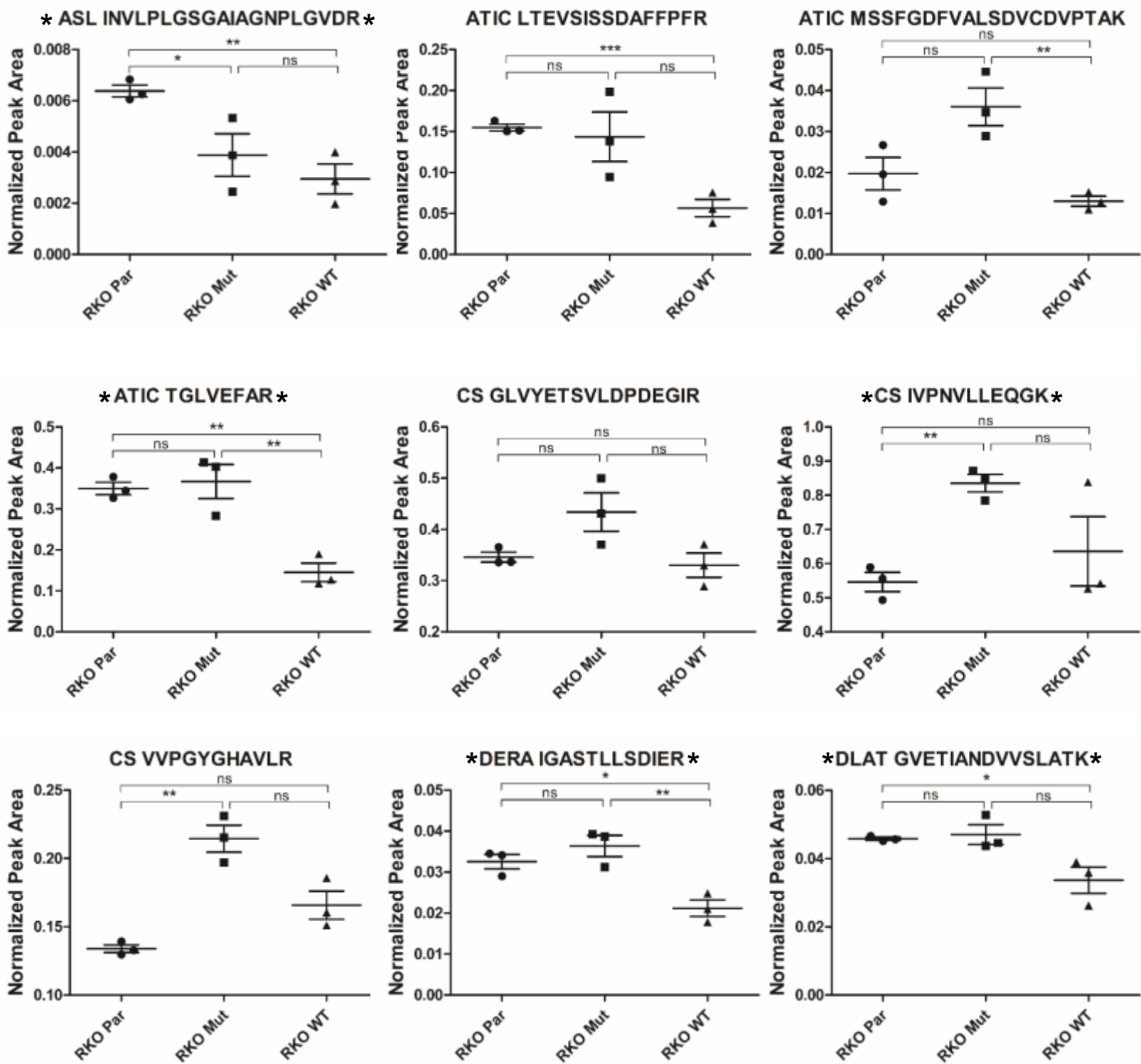


Figure S4. Quantitative comparisons of peptides from metabolic proteins by PRM in RKO cell lines. Each peptide peak area was normalized to that of the LRP standard. Pairwise comparisons are shown with bars above the plots. Significant differences were determined using Student's t-test, where the asterisks denote p-values: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; ns indicates no significant difference. Peptides selected for quantitative comparisons and for Figure 5 are listed with an asterisk (*) before and after the protein name and peptide sequence.

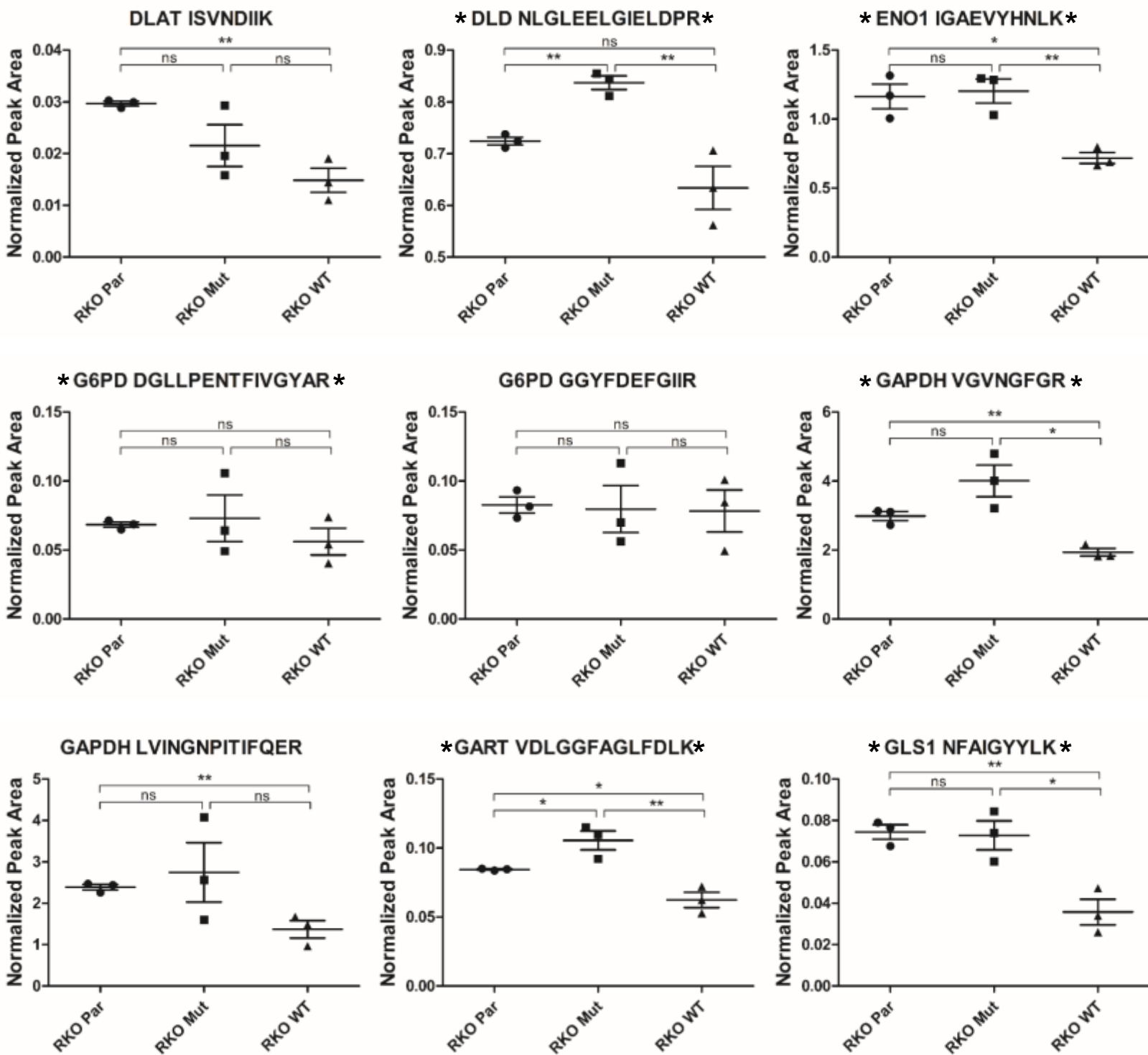
S4A



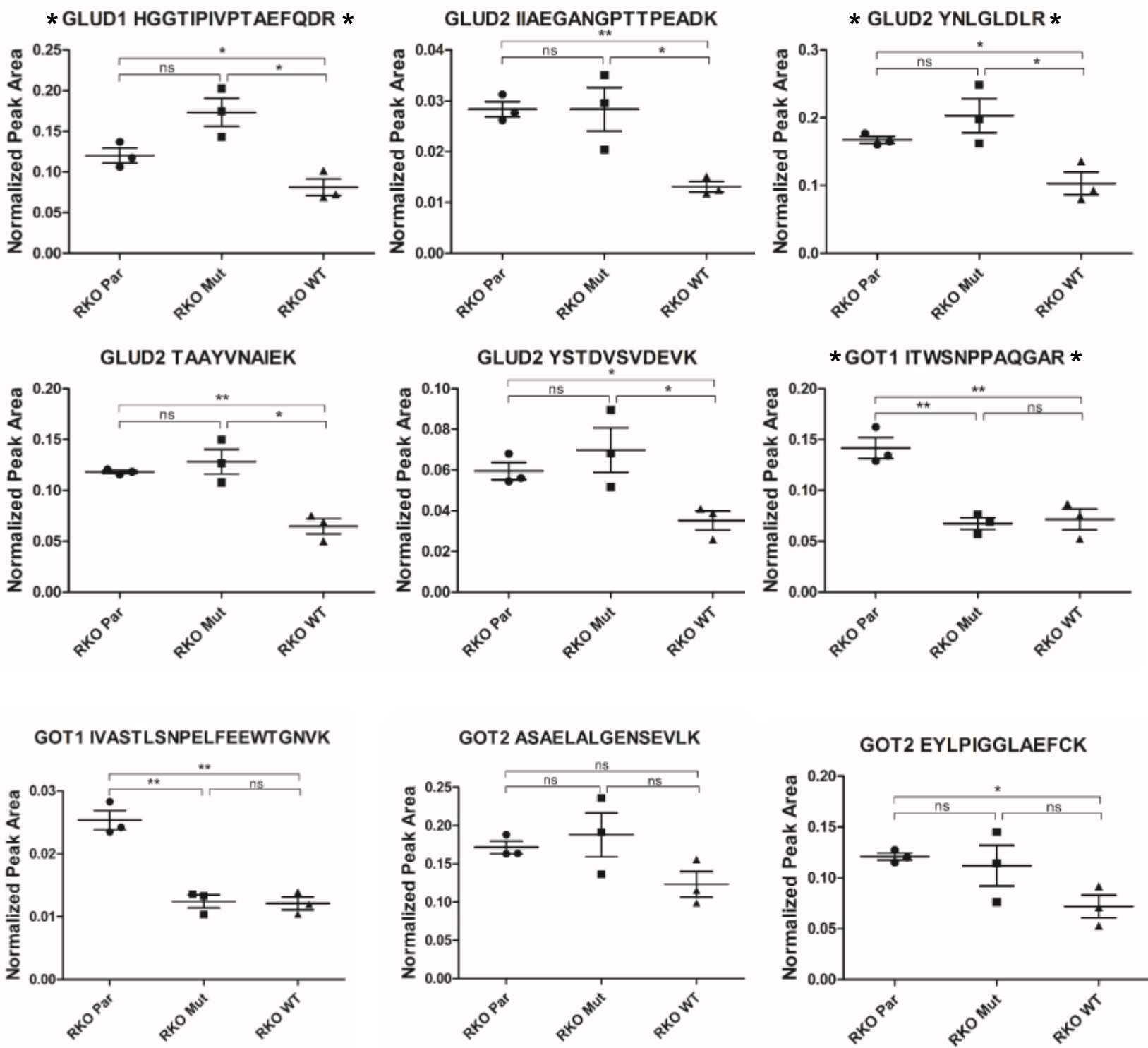
S4B



S4C

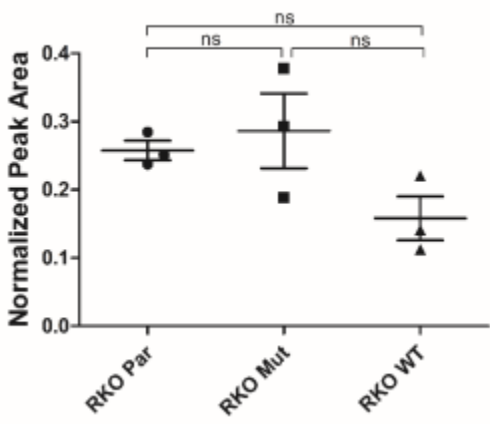


S4D

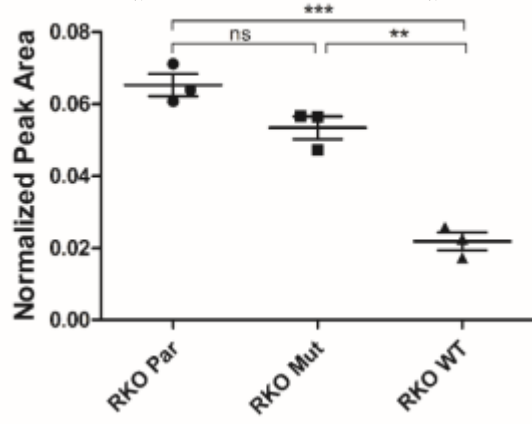


S4E

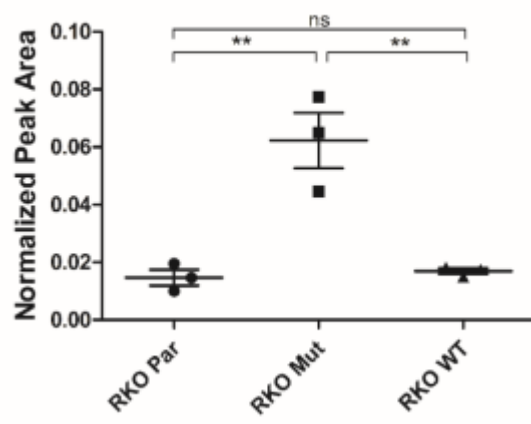
***GOT2 FVTVQTISGTGALR ***



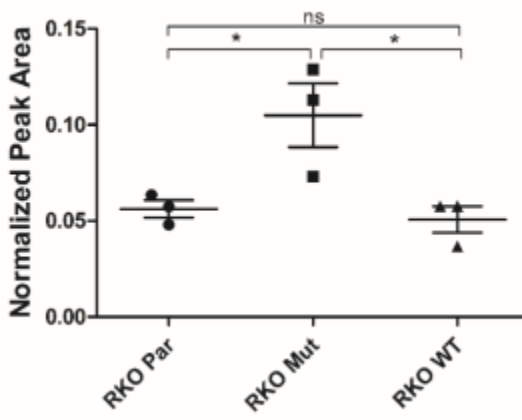
***HK2 STIGVDGGSVYK ***



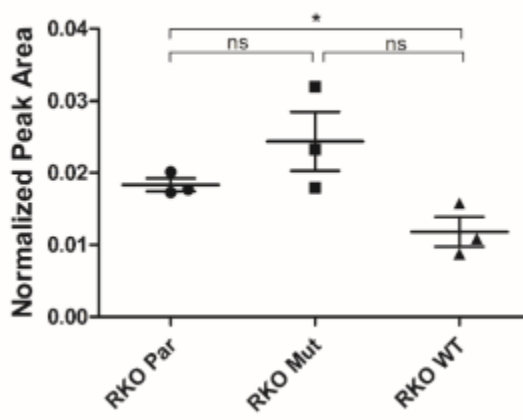
IDH1 SDYLNTFEFMDK



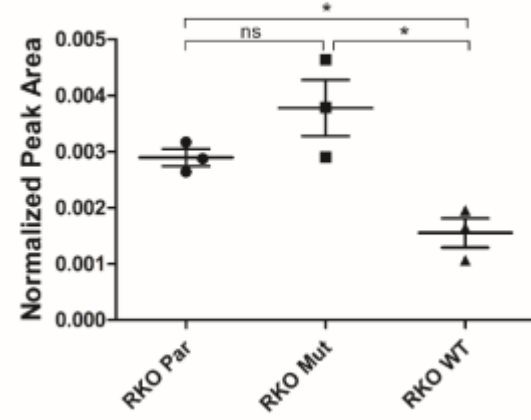
***IDH1 VEITYTPSDGTQK ***



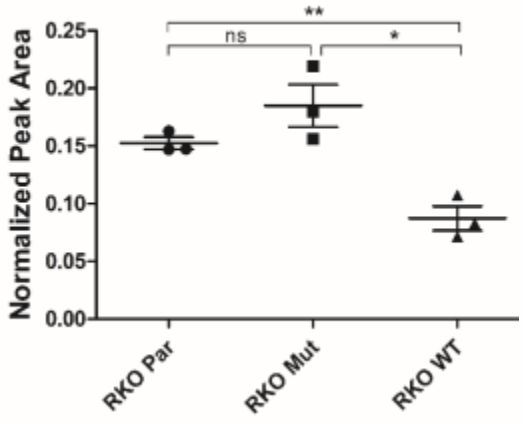
***IDH2 YFDLGLPNR ***



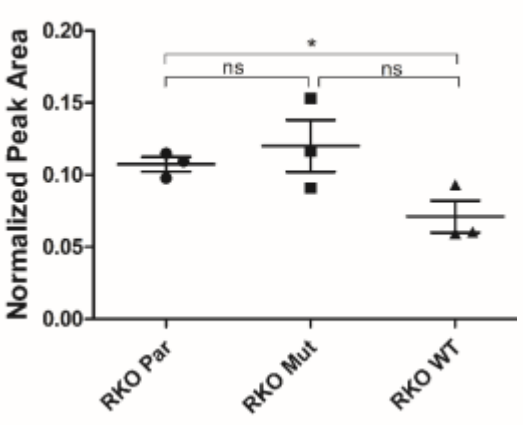
IDH2 TIEAEEAAGTVTR



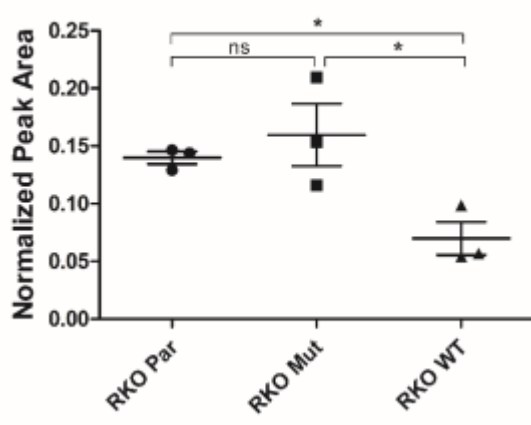
IDH3A IAEFAFEYAR



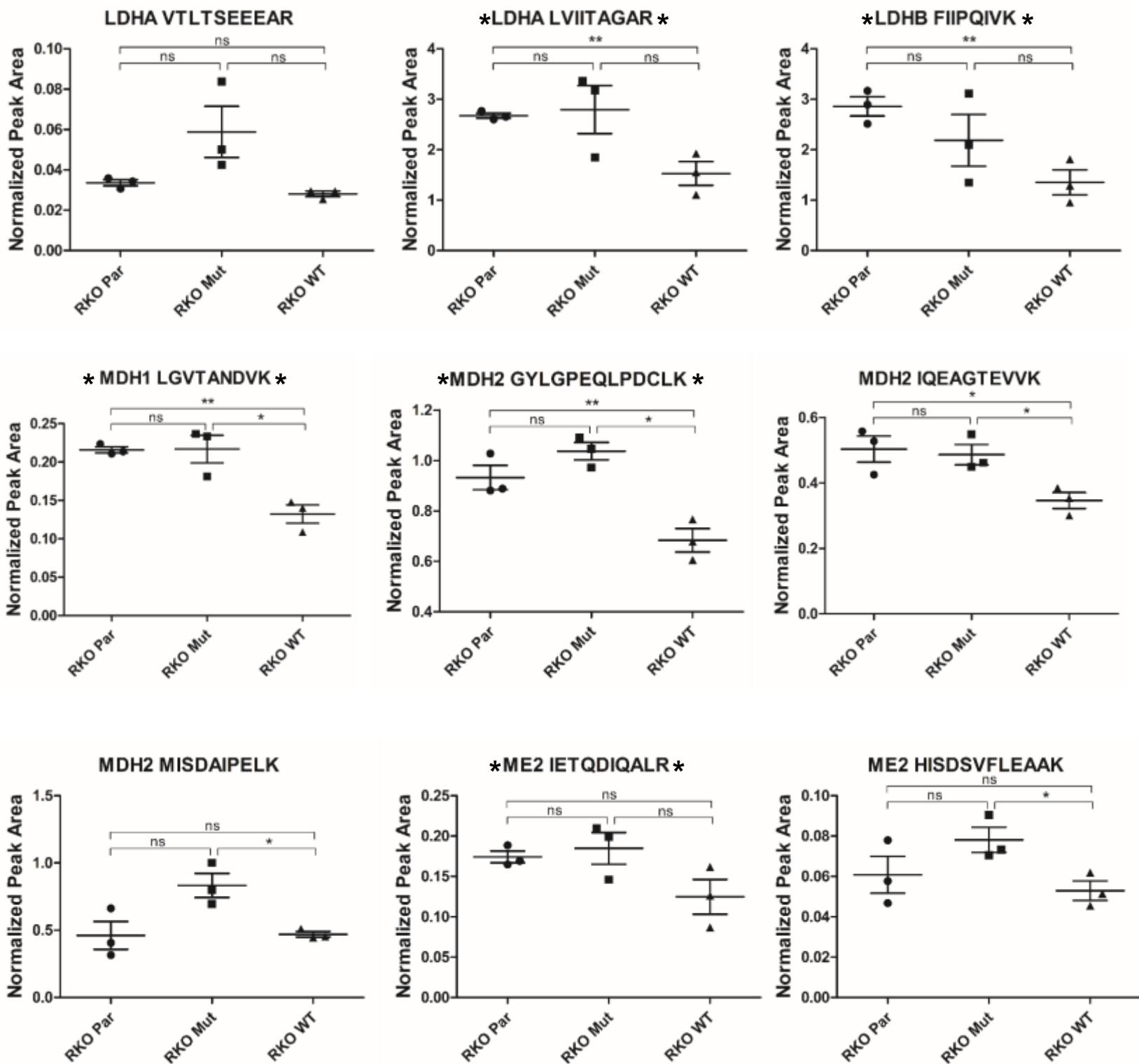
IDH3A IEAACFATIK



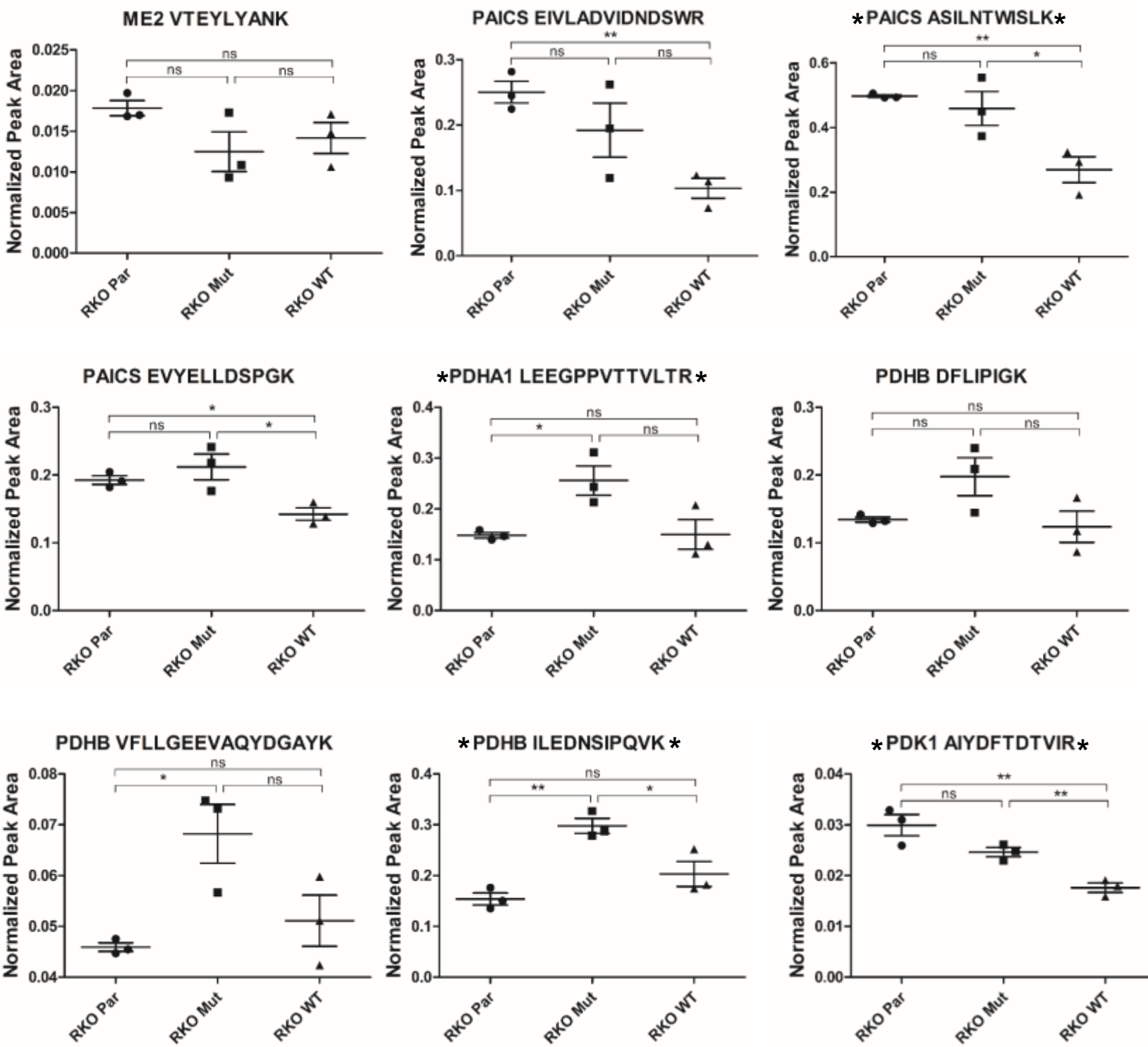
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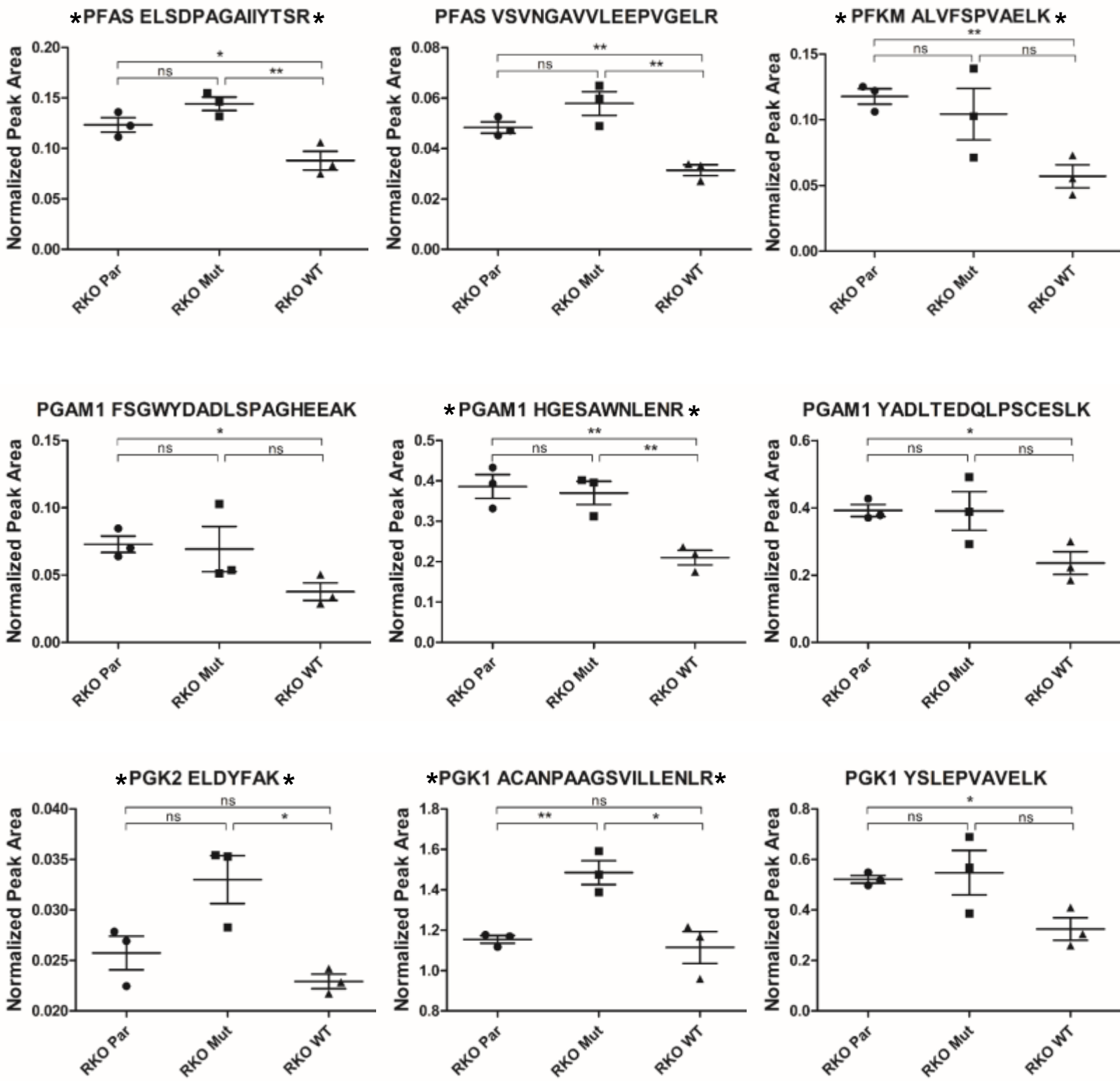
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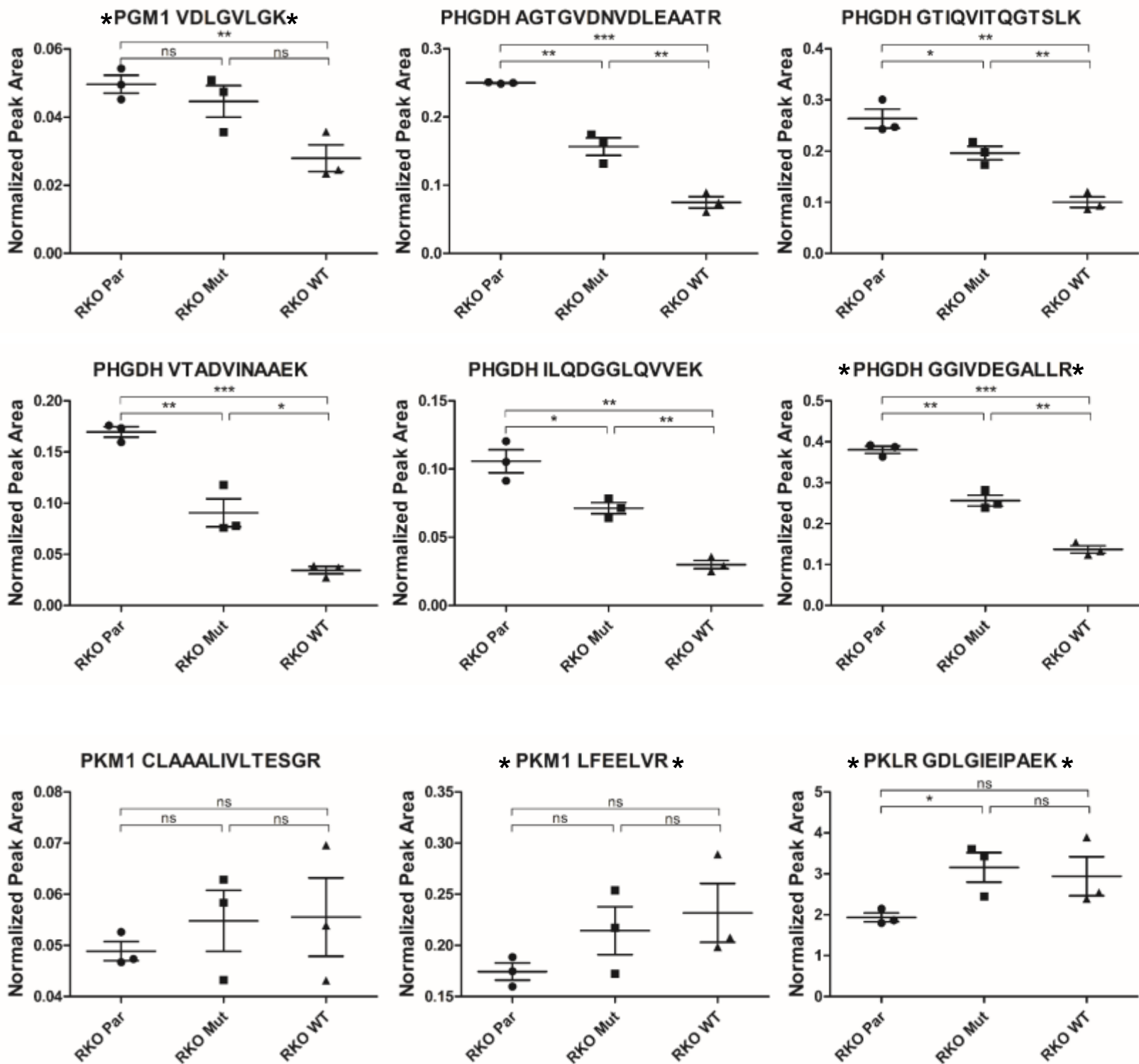
S4G



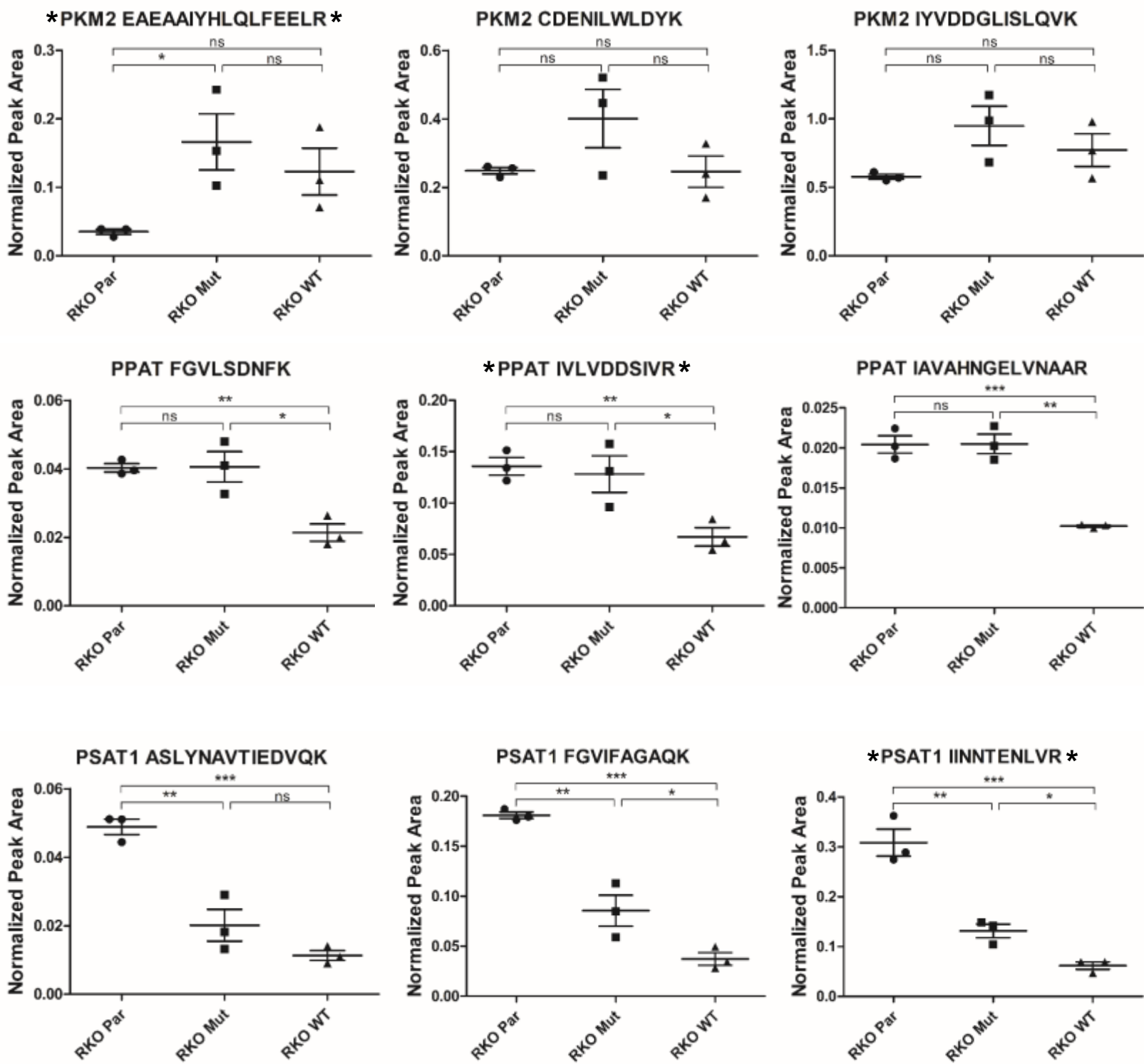
S4H



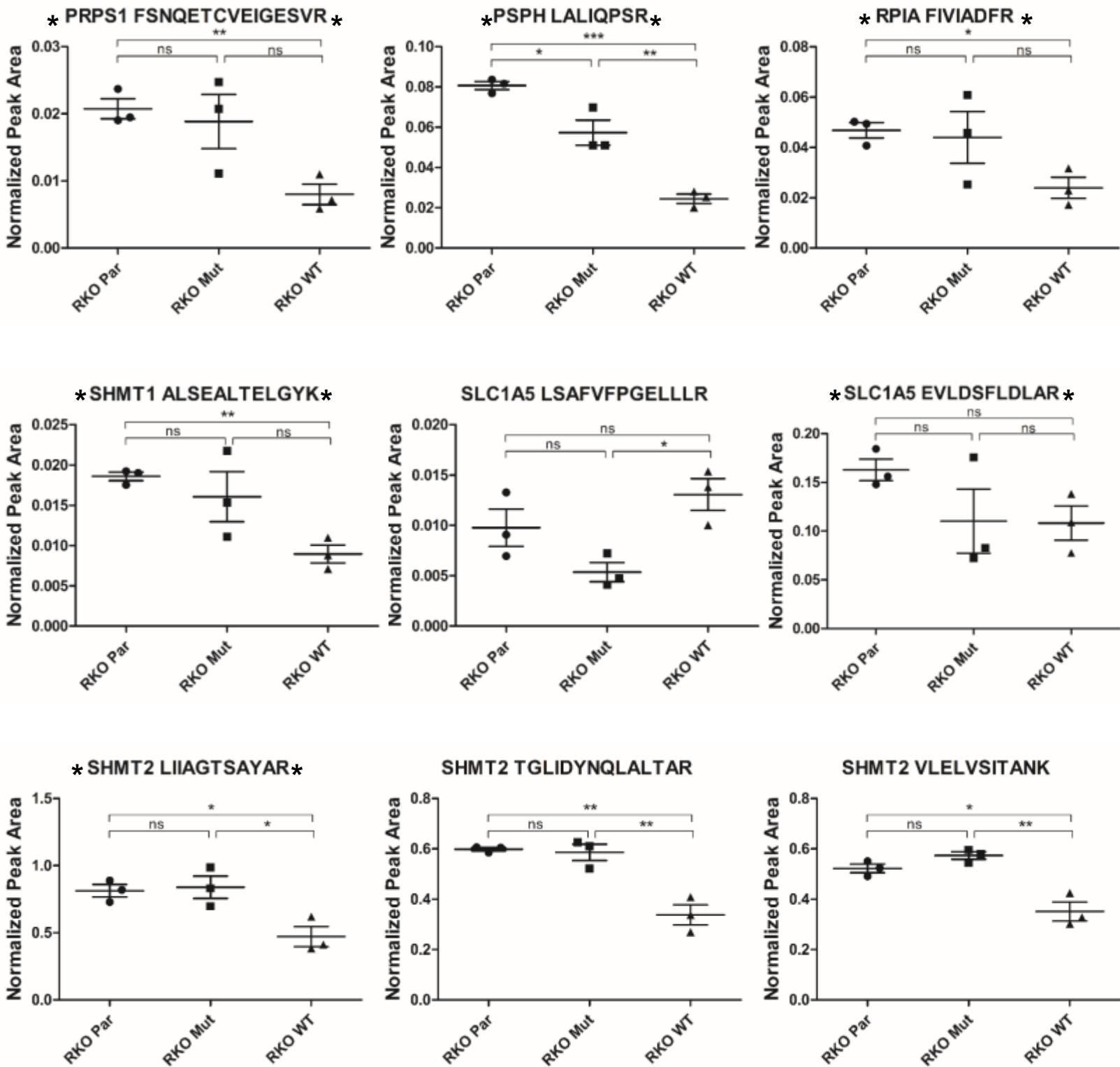
S4I



S4J



S4K



S4K

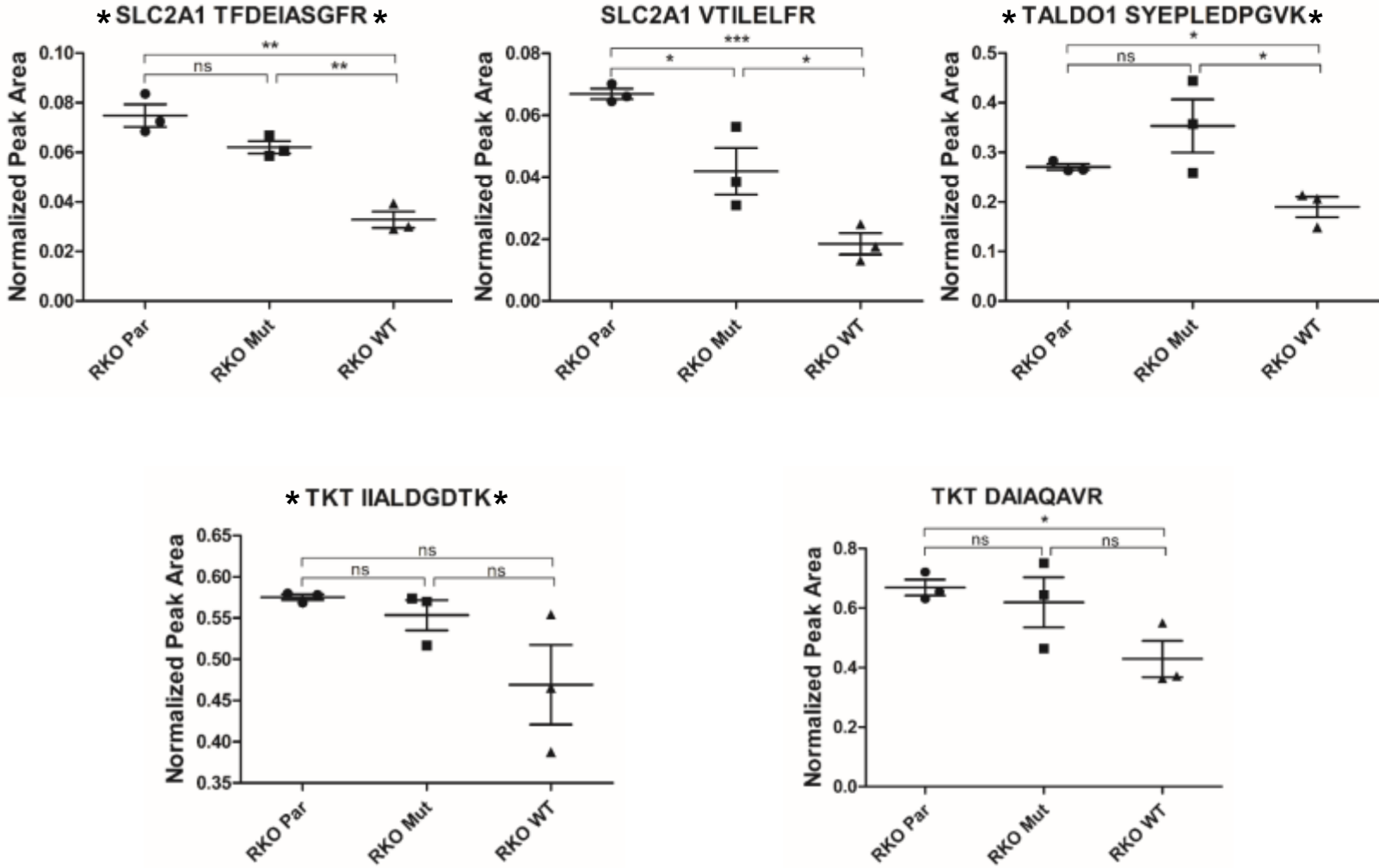


Figure S5. Summary of PRM measurements from Figures 4 and 5. The PRM comparisons depicted in Figures 4 and 5 were consolidated into a single figure. The legend for each pairwise comparison shows fold changes relative to the cell line listed first in each comparison. Proteins with a CV < 0.25, an ICC > 0.6, and a $p < 0.05$ and that are higher in the first cell line are shown in red (at least a 2-fold difference) or light red (between 1.2- and 1.9-fold difference). Proteins with a CV < 0.25, an ICC > 0.6, and a $p < 0.05$ and that are lower in the first cell line are shown in green (at least a 2-fold difference) or light green (between 1.2- and 1.9-fold difference). Proteins with a CV < 0.25, an ICC > 0.6, but a $p > 0.05$ or with a CV < 0.25 but an ICC < 0.6 are listed in grey (no difference). Proteins with a CV > 0.25 or with no detectable peak area are shown in white.

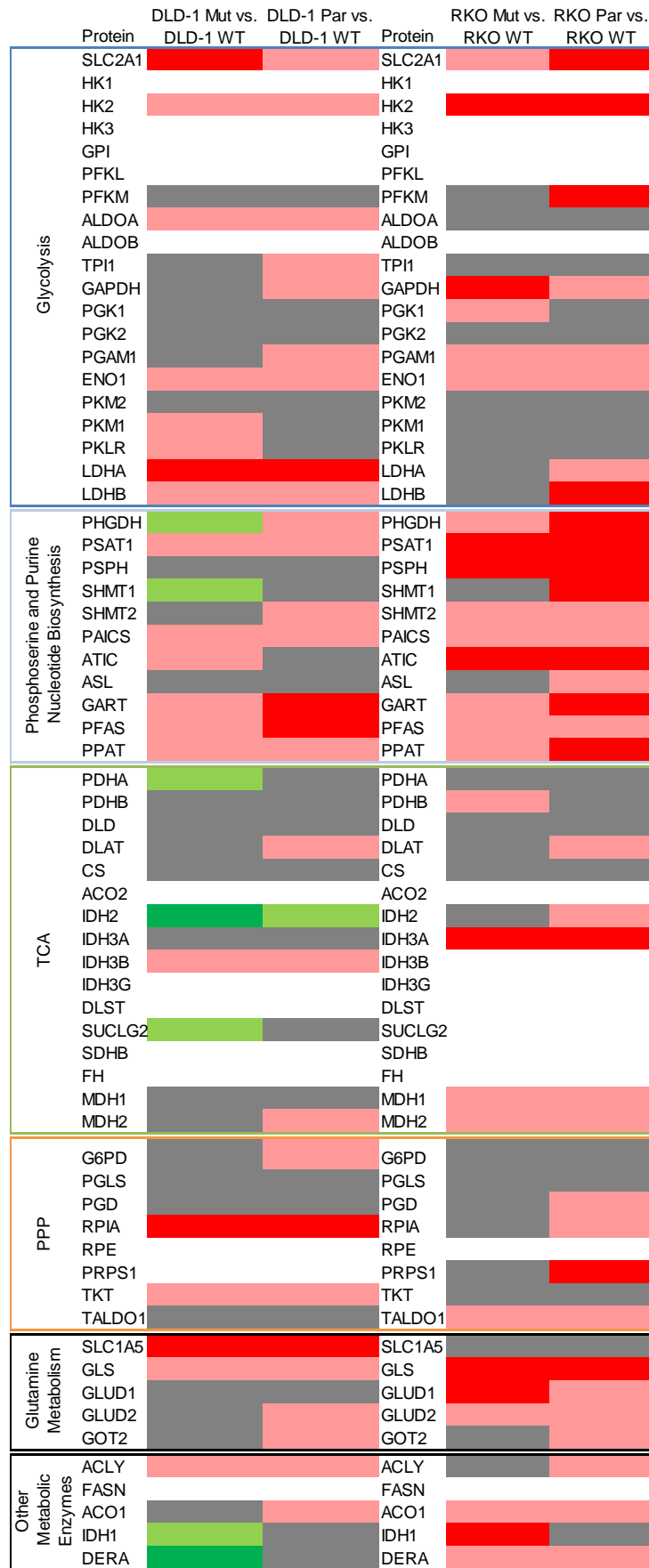
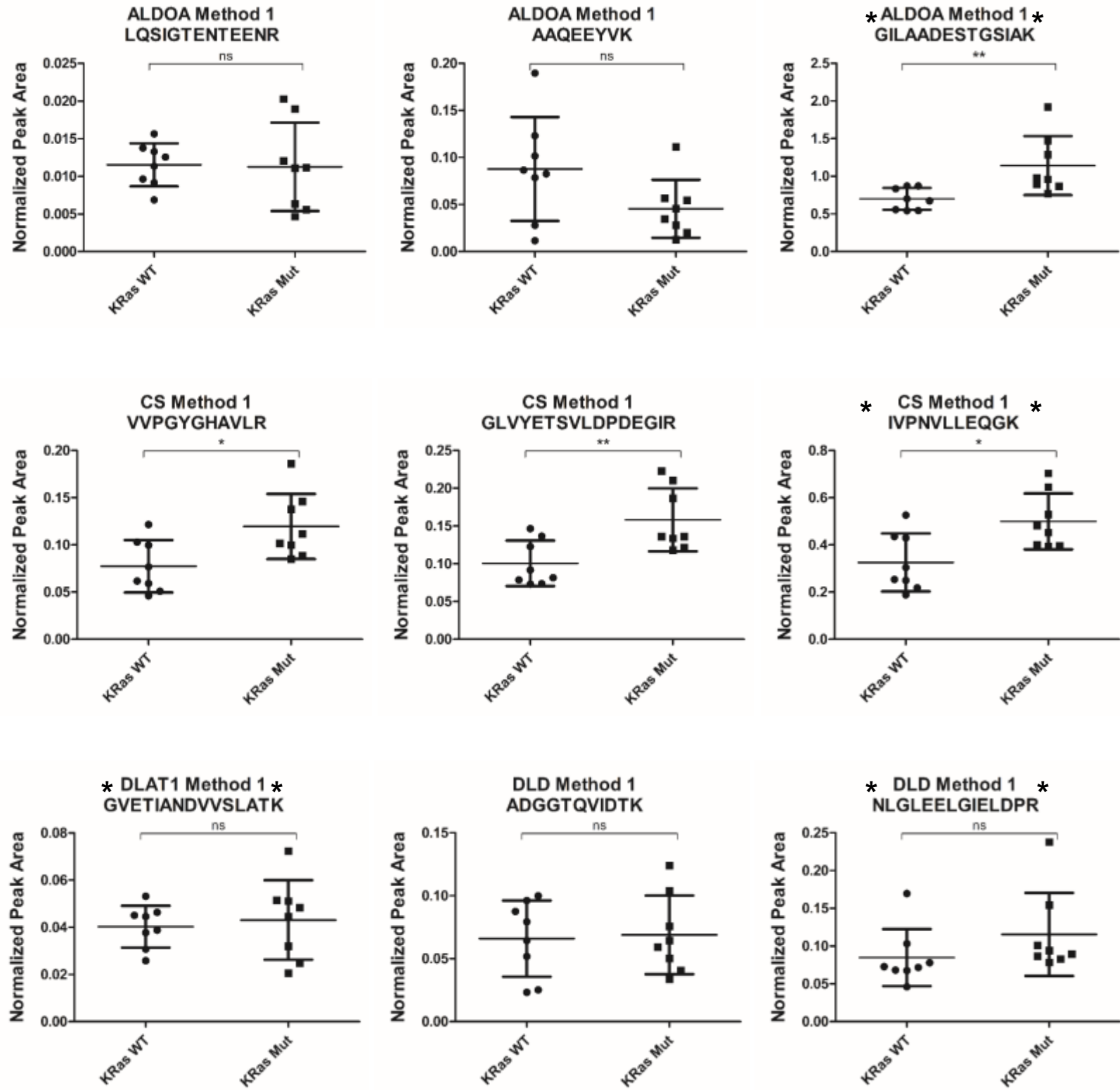
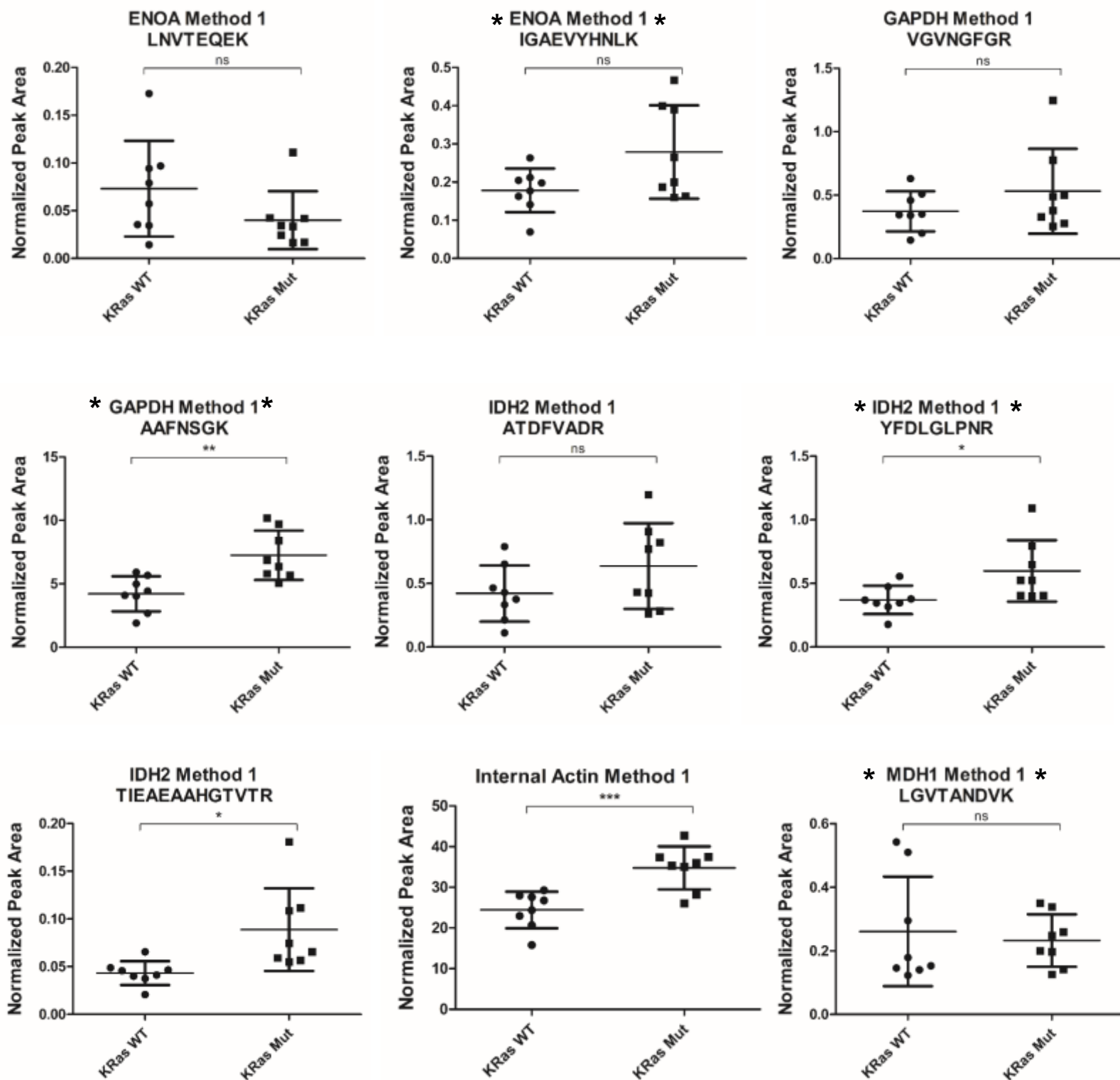


Figure S6. Quantitative comparisons of peptides from metabolic proteins by MRM in primary human colorectal tumors. Each peptide peak area was normalized to that of the LRP standard. Pairwise comparisons are shown with bars above the plots. Each point represents the average of three technical replicate analyses of a single tumor. Significant differences were determined using Student's t-test, where the asterisks denote p-values: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; ns indicates no significant difference. Peptides used for quantitation and for Figure 8 are listed with an asterisk (*) before and after the protein name and peptide sequence.

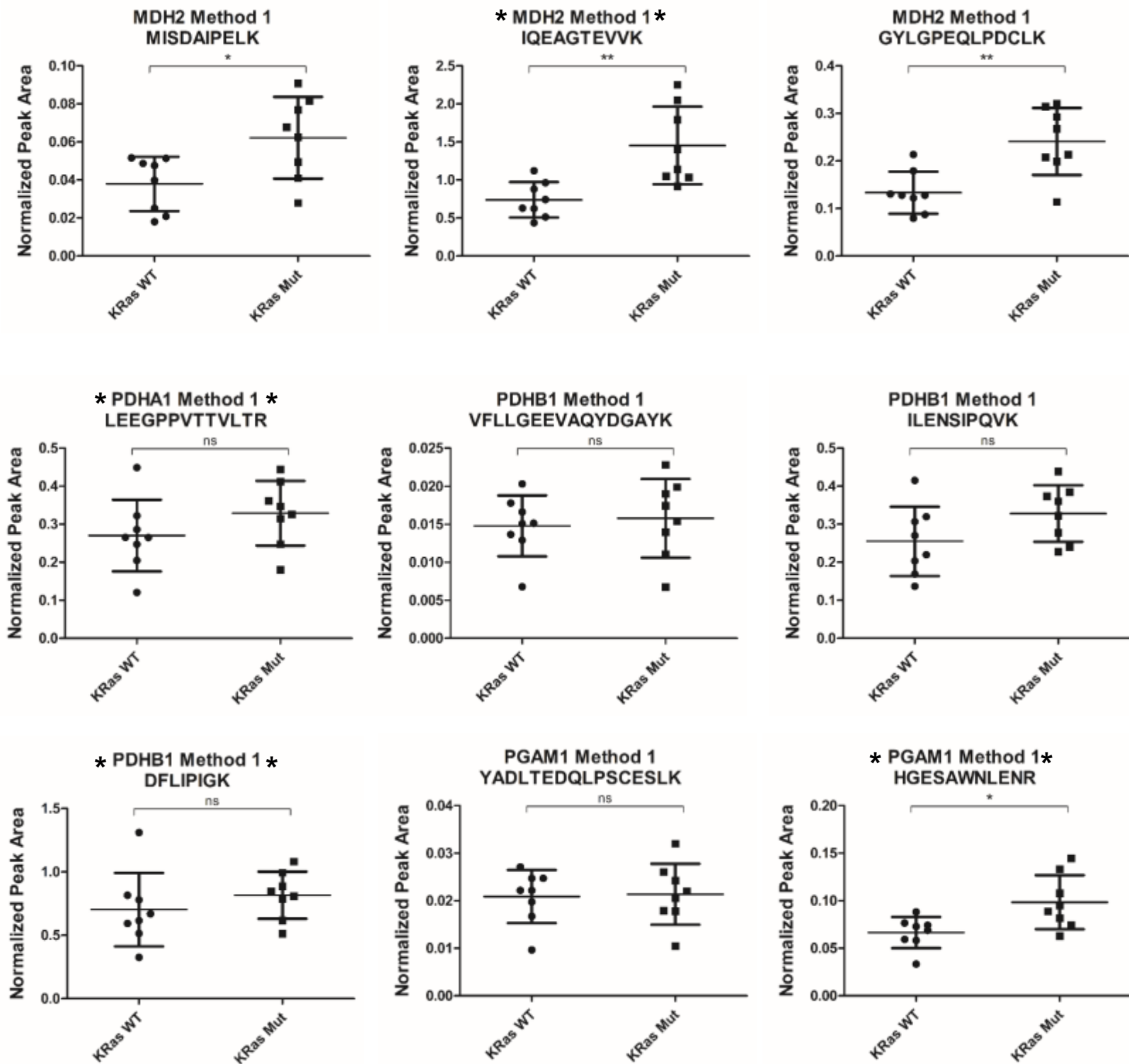
S6A



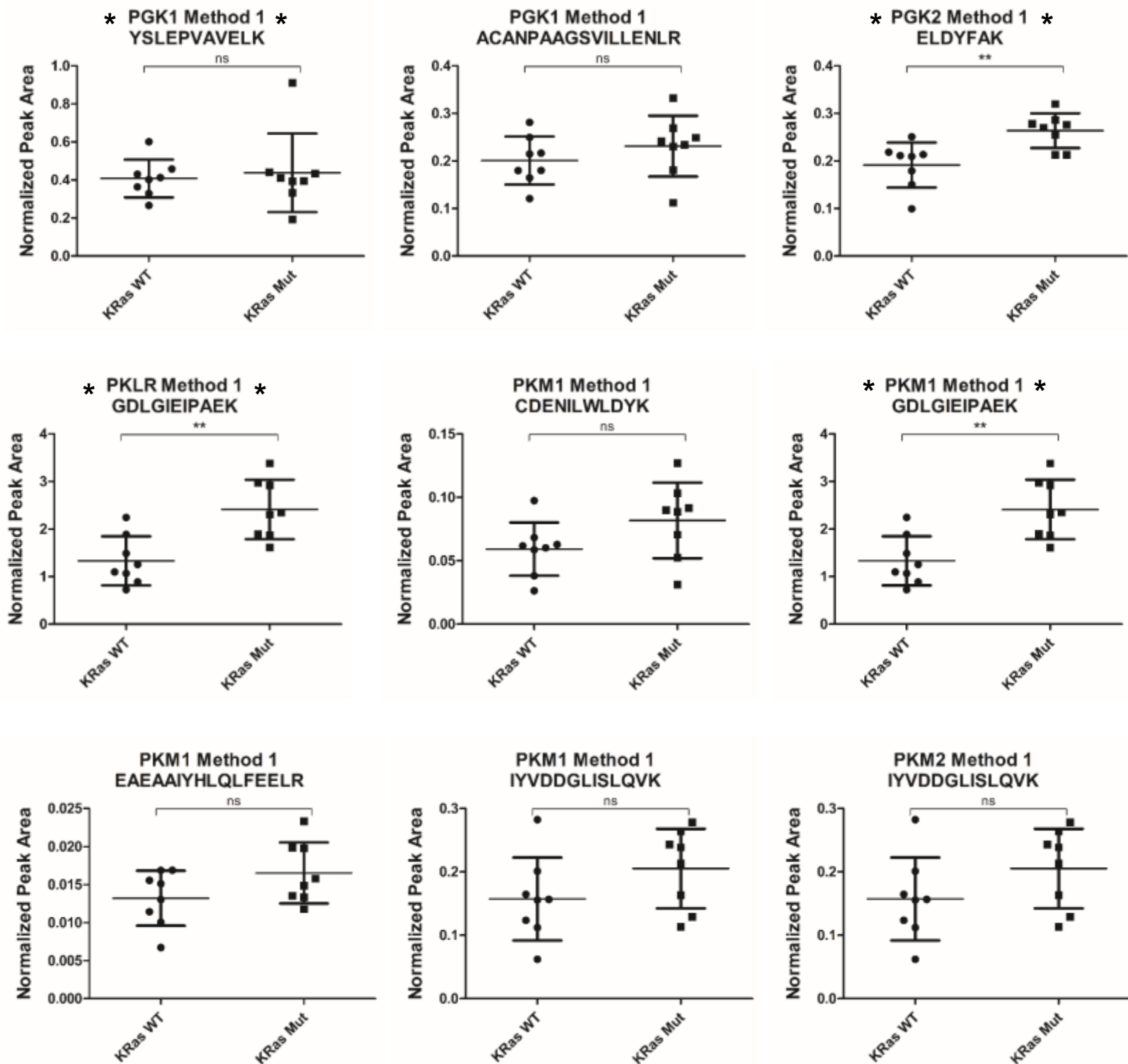
S6B



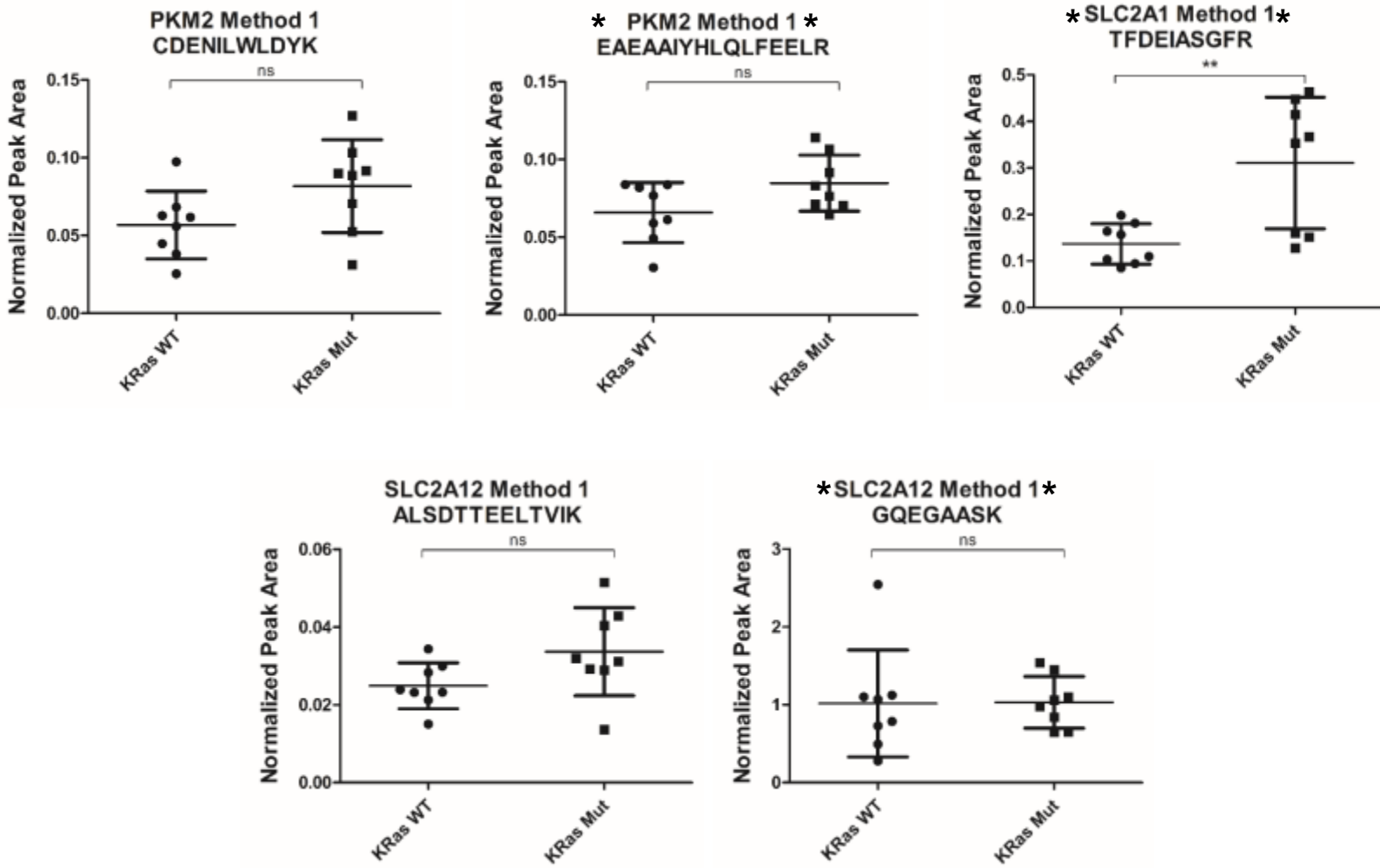
S6C



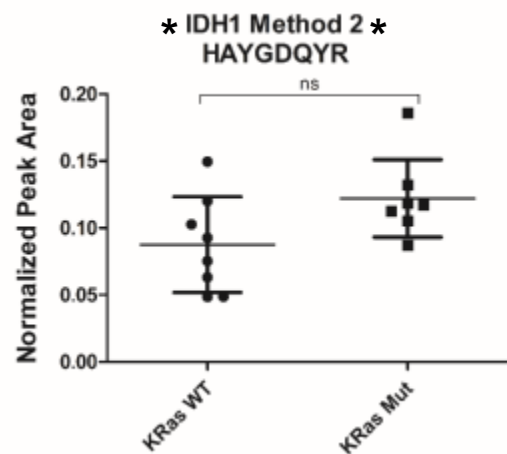
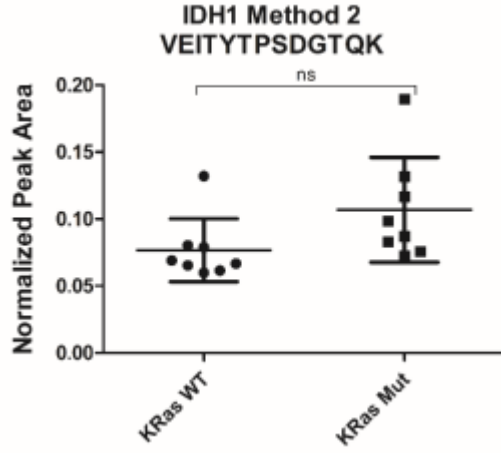
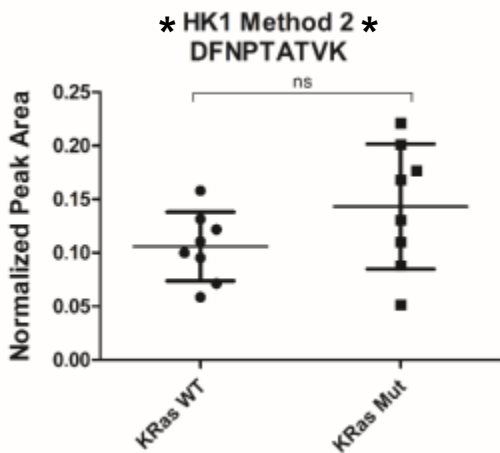
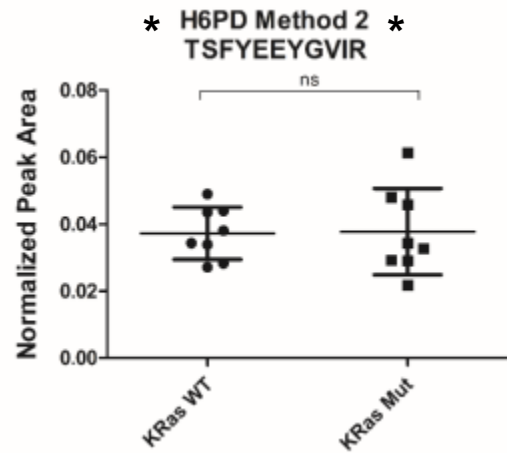
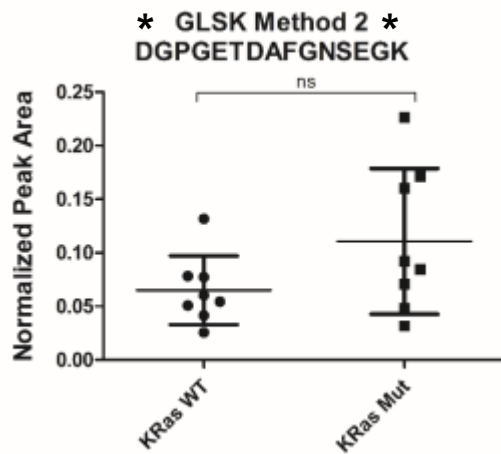
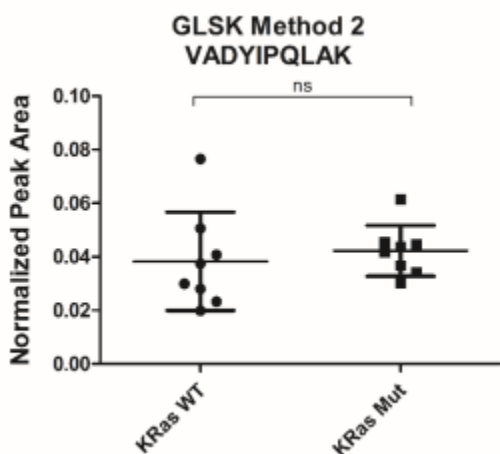
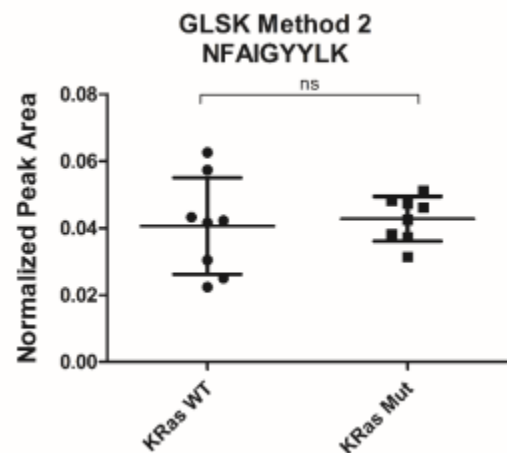
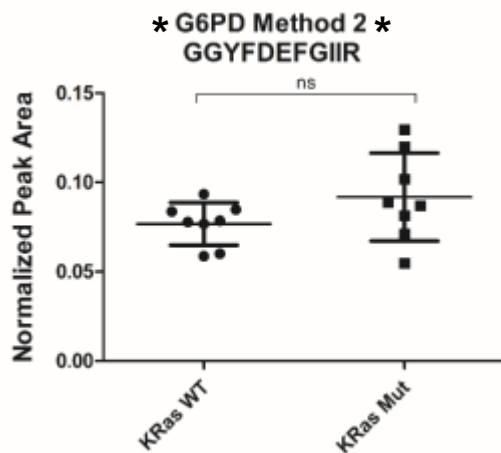
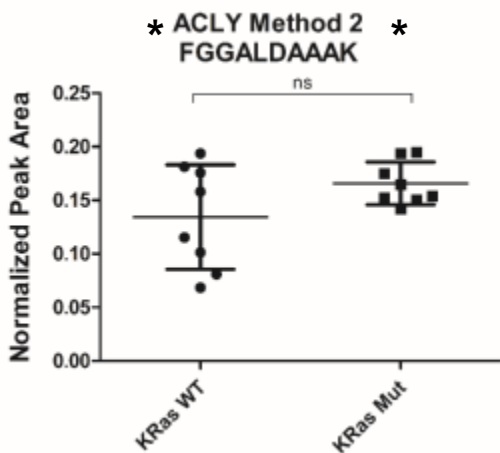
S6D



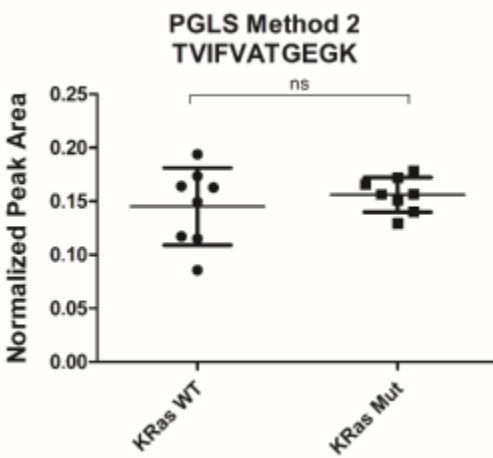
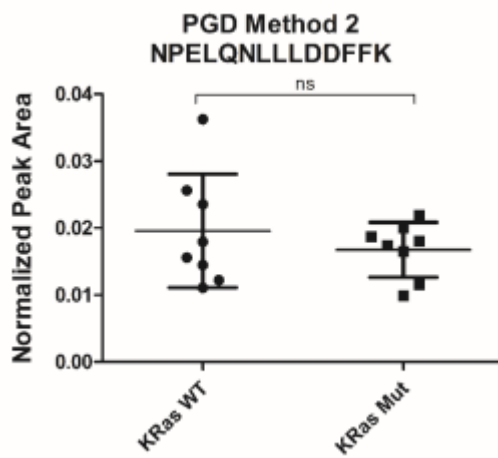
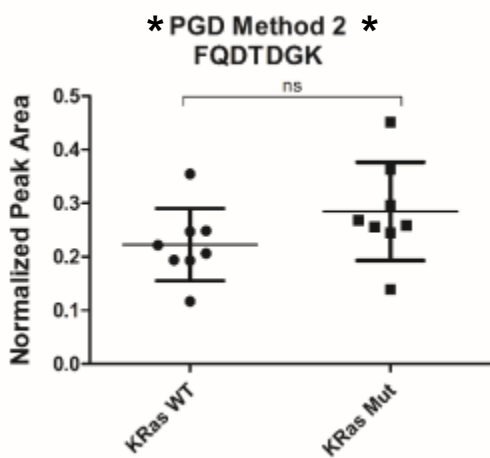
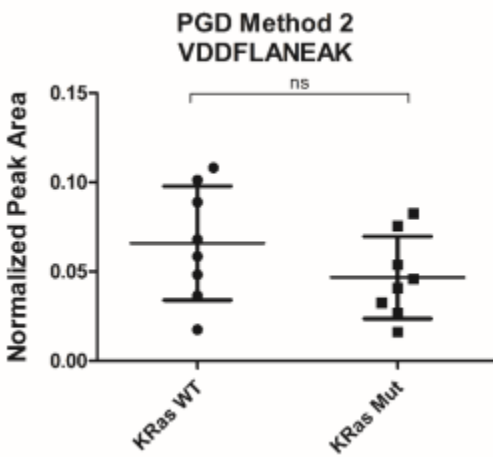
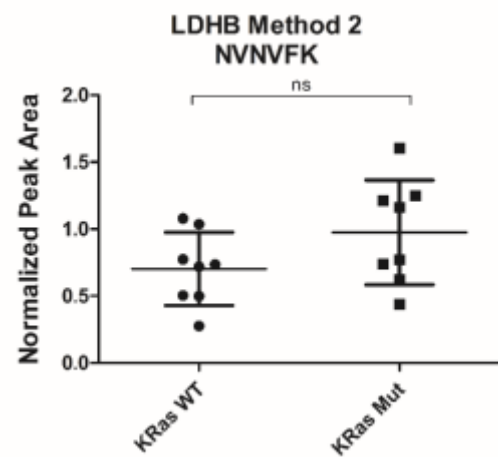
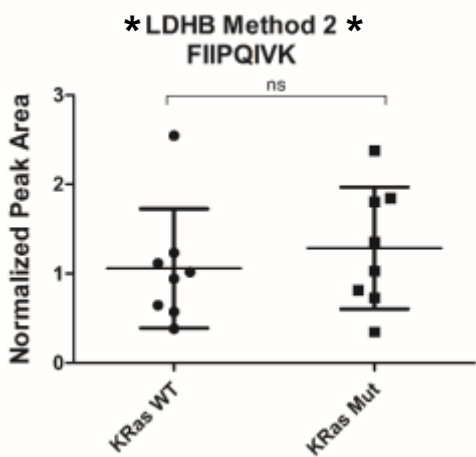
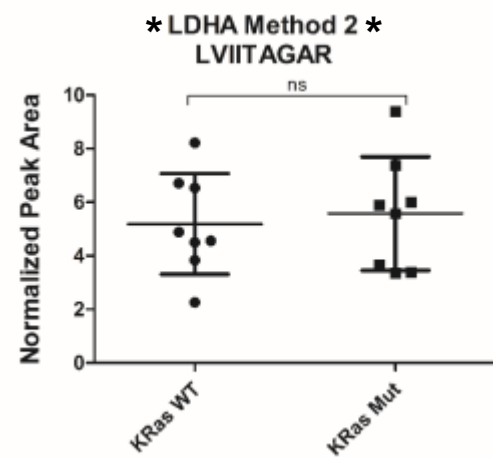
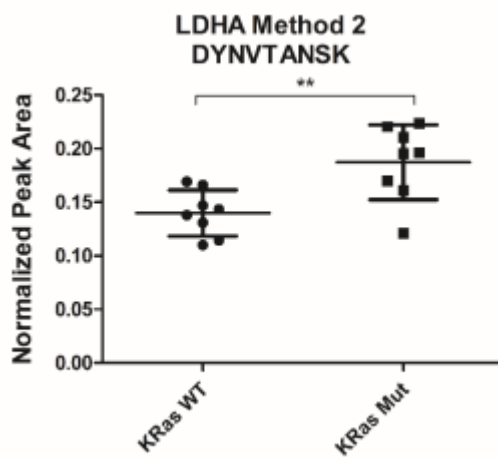
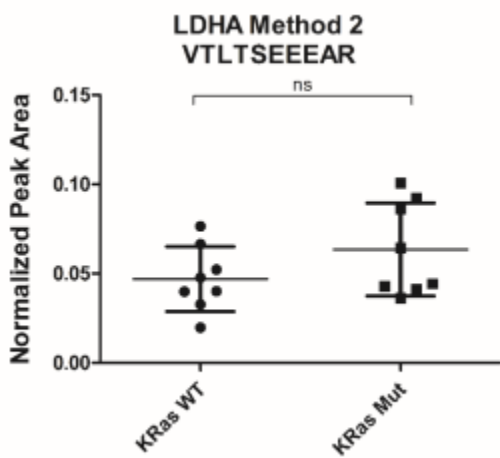
S6E



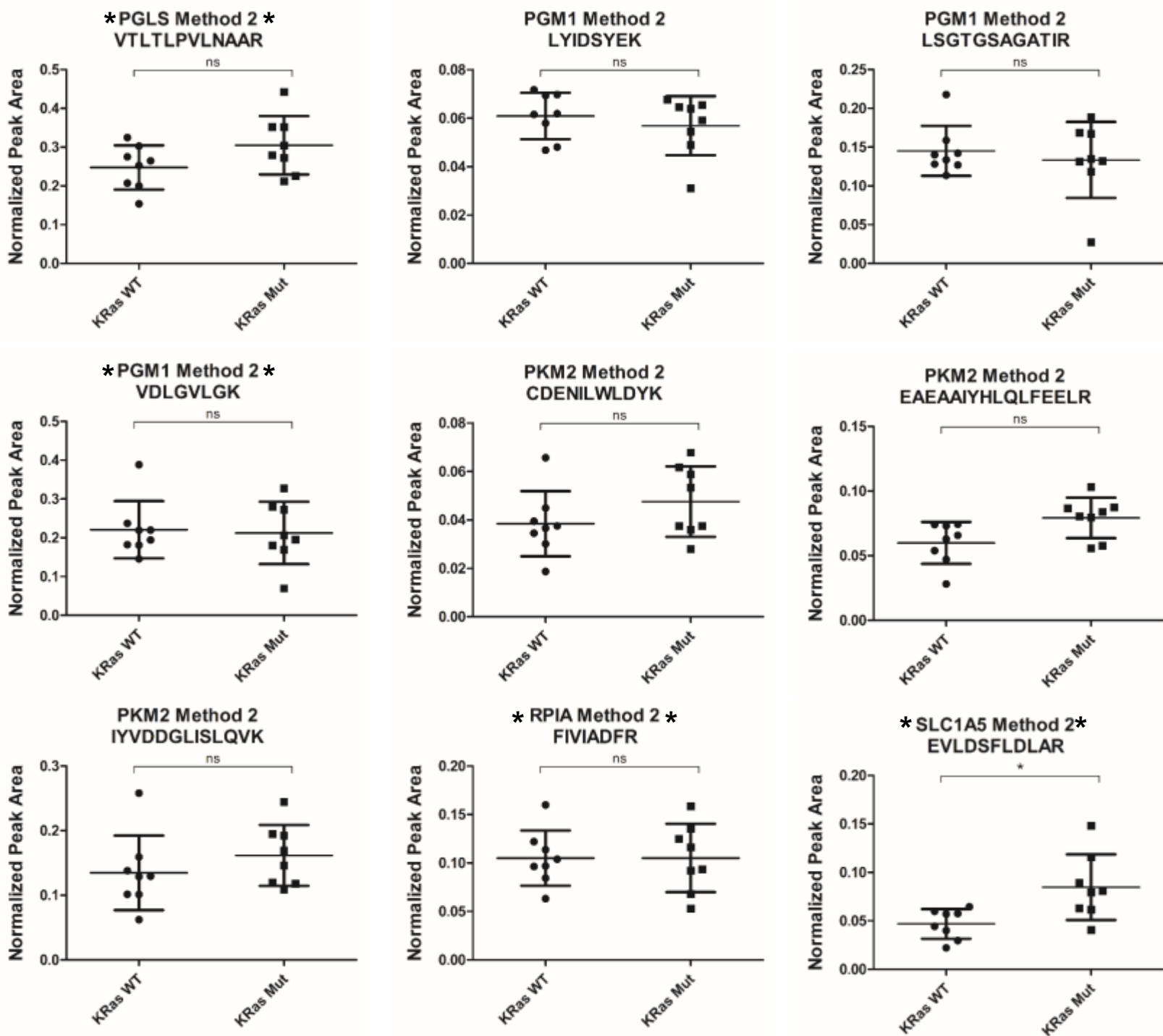
S6F



S6G



S6H



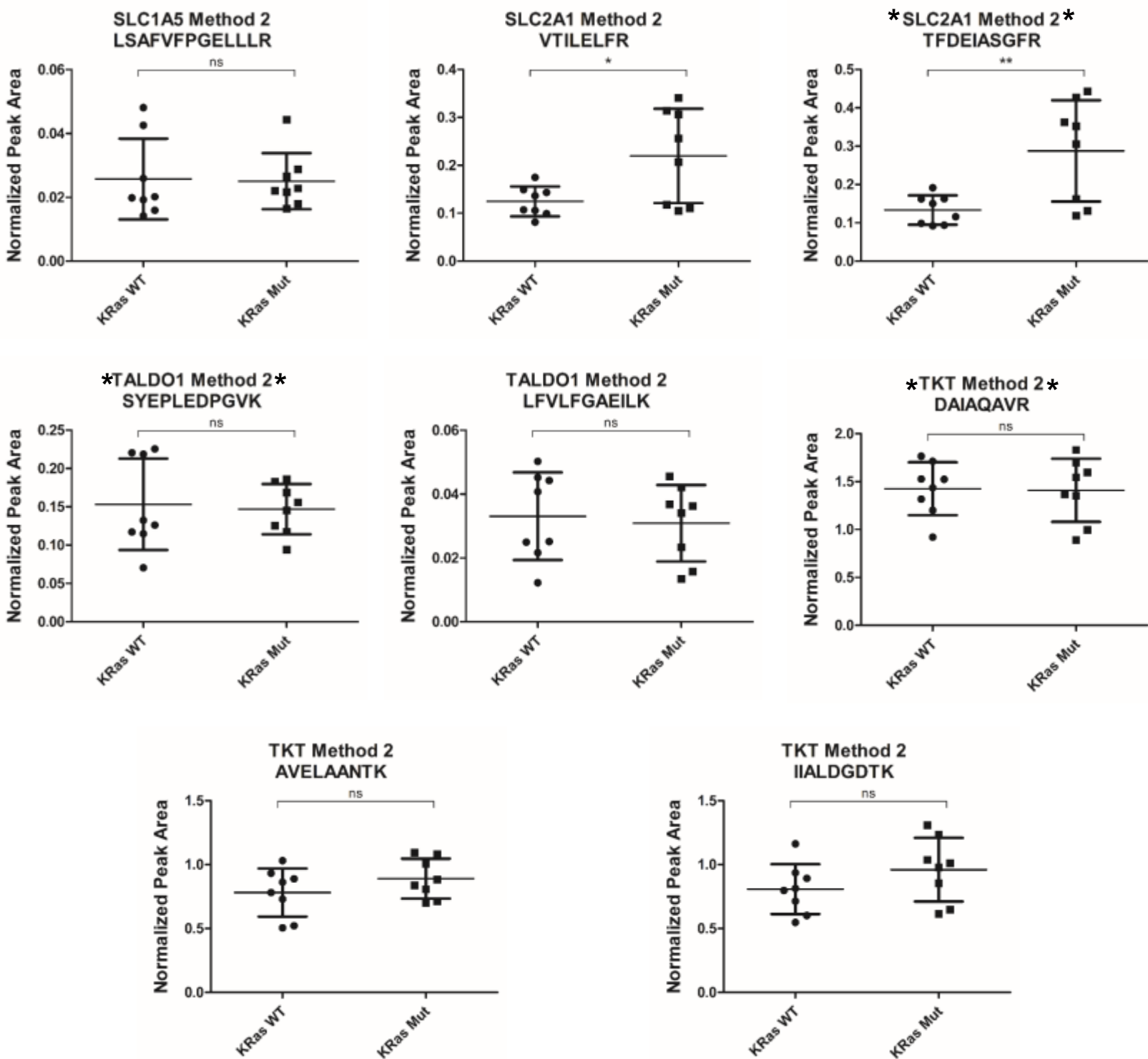


Figure S7. Pathway map of quantitative comparisons of metabolic proteins in stage II human FFPE colorectal tumor samples. The LRP-normalized values for each protein were averaged within the KRAS mutant and KRAS wild type groups and fold-change differences between the groups depicted are from comparisons of the averages. A Student's t-test analysis of the individual LRP-normalized values was used to determine significance of expression differences between KRAS mutant and wild type tumors. Proteins with $CV < 0.25$, $ICC > 0.7$, and a $p < 0.05$ are shown in either red or green. Proteins with higher average values in the KRAS mutant tumors compared to the KRAS wild type tumors are highlighted in red (fold change greater than 2-fold) or light red (fold change between 1.9- and 1.2-fold). Proteins with lower average values in the KRAS mutant tumors compared to KRAS wild type tumors are highlighted in green (fold change greater than 2-fold) or light green (fold change between 1.9- and 1.2-fold). Proteins with a $CV < 0.25$, $ICC < 0.7$, and a $p > 0.05$ or with a $CV < 0.25$ but an $ICC > 0.7$ are shown in grey. Proteins with $CV > 0.25$ or with no detectable peak area are shown in white.

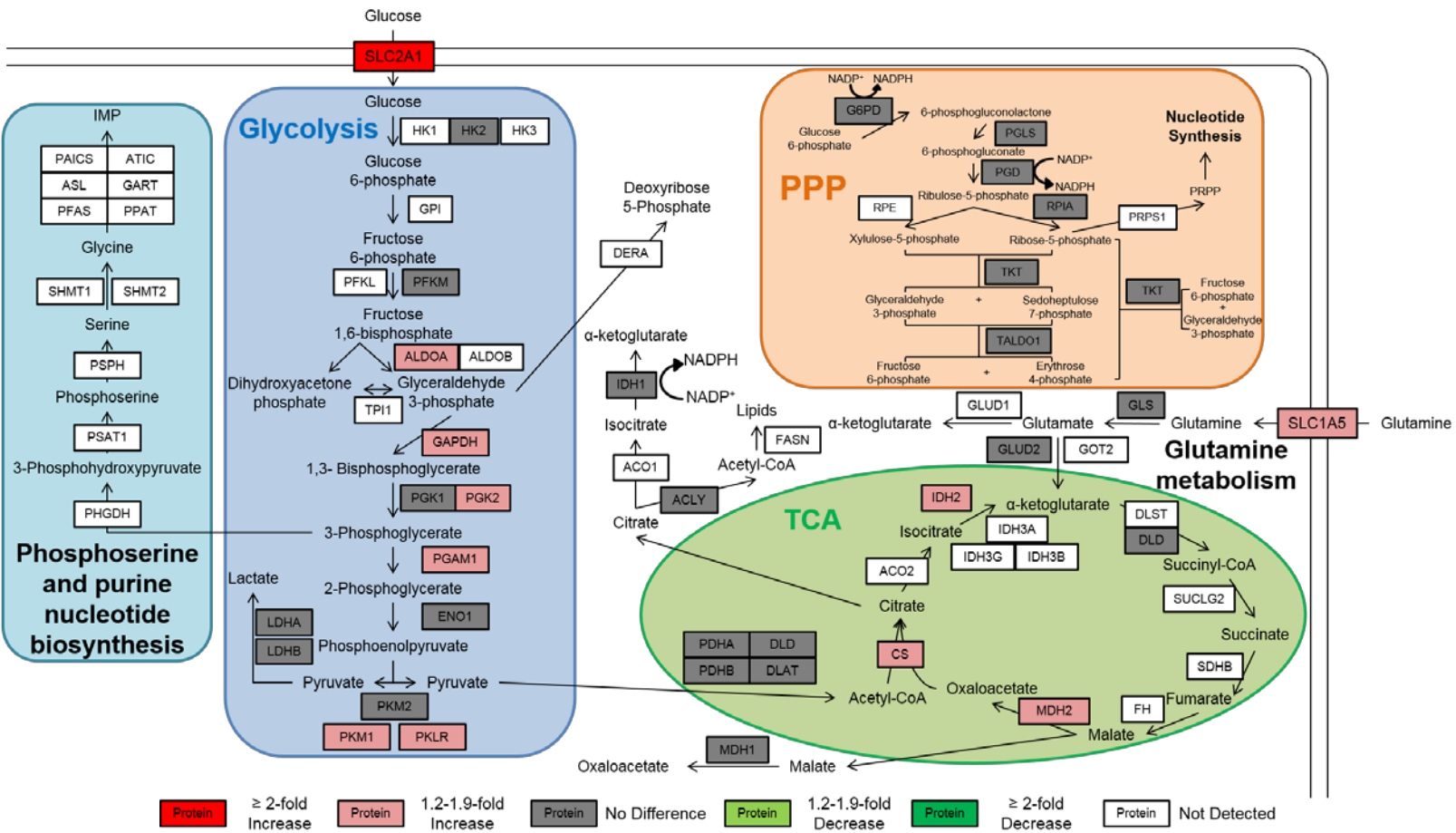
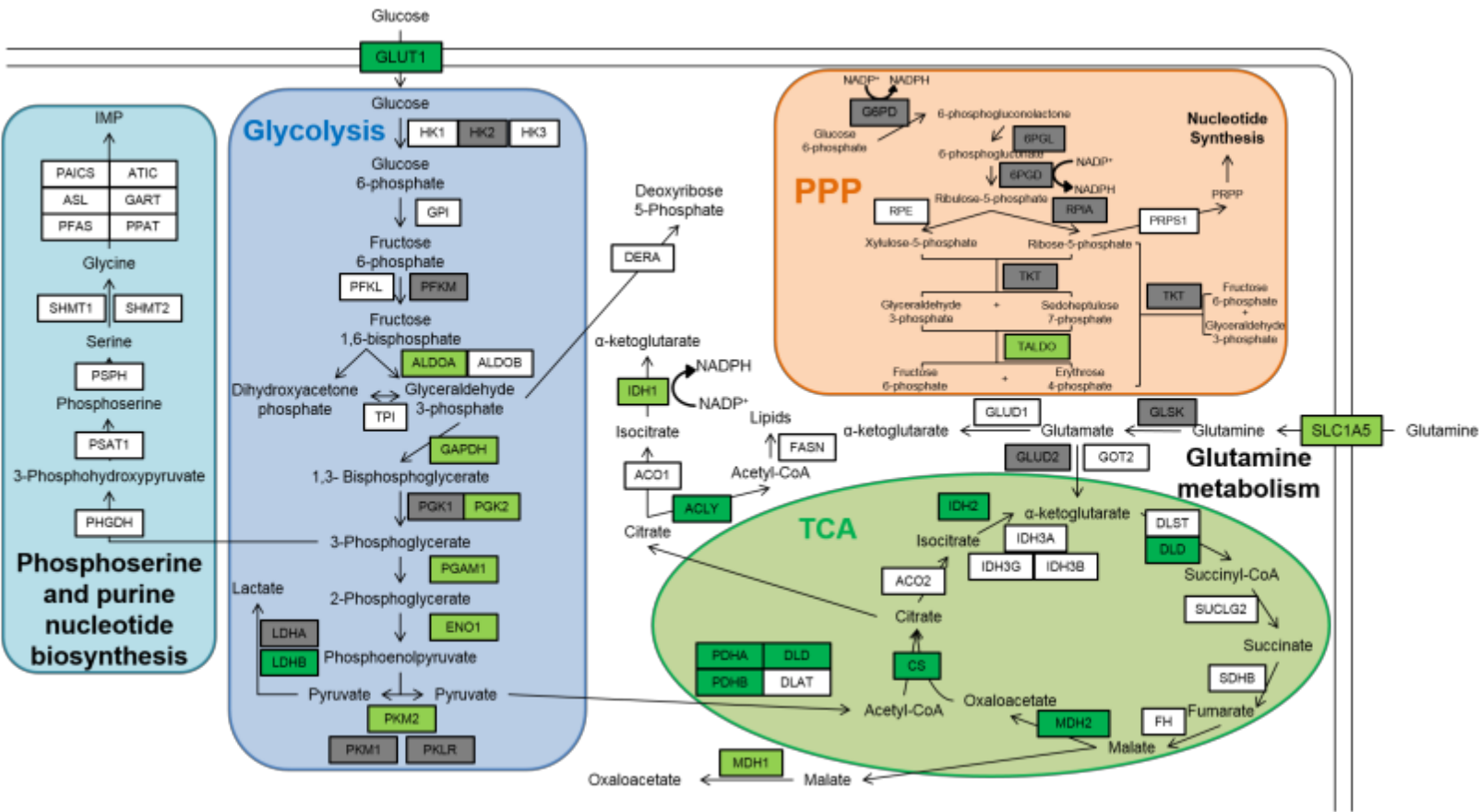


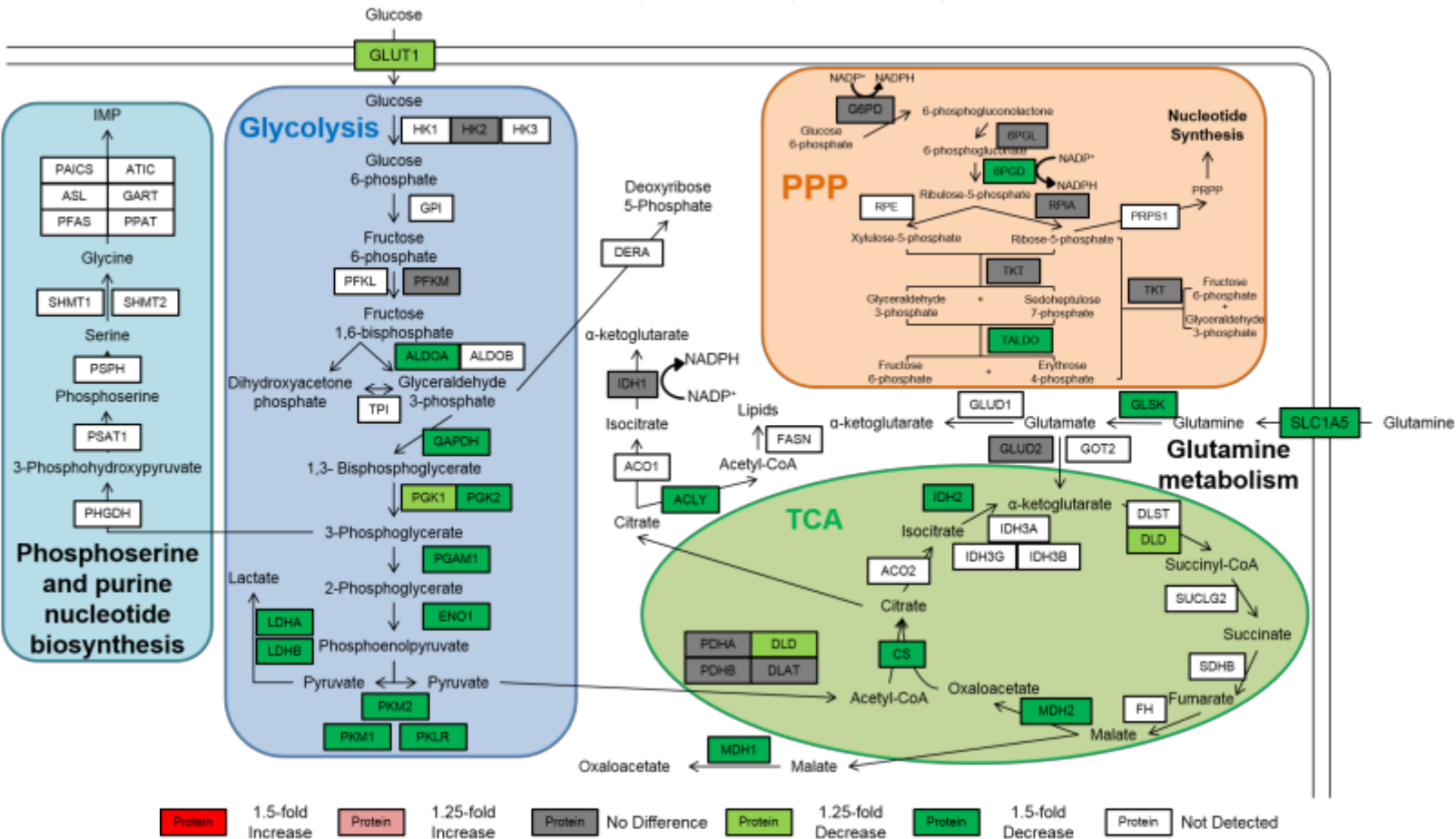
Figure S8. Pathway maps of metabolic protein abundance differences in individual human Stage II colon tumors. Protein measurements for each tumor were compared to the average measurement for all samples analyzed in the dataset. Mutational status of KRAS, NRAS, BRAF, and PI3K for each tumor are shown next to each tumor and are also listed in Supplemental Table S20. Panels A-H depict KRAS WT tumors; panels I-P depict KRAS mutant tumors. Proteins shown with a white box were not detected. Proteins shown with grey boxes were less than 1.25 fold different than the average measurement for that protein. Proteins shown with light green or light red boxes were at least a 1.25-fold higher or lower, respectively, than the average protein measurement. Proteins shown with a dark green or a dark red box were more than 1.5-fold lower or higher, respectively, than the average measurement for that protein.

Tumor C KRAS WT, NRAS WT, BRAF V600E, PI3K WT

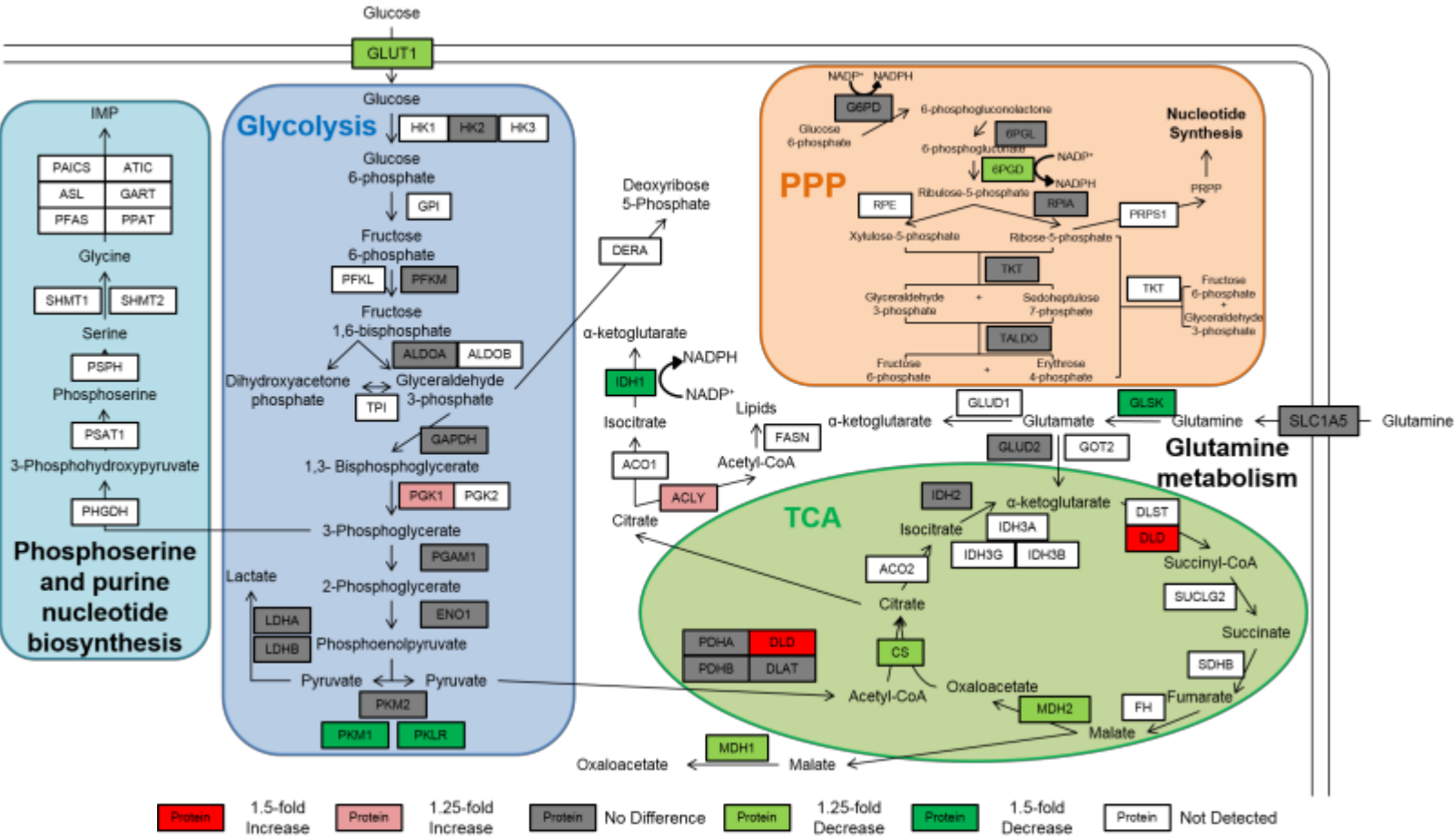


Protein 1.5-fold Increase
 Protein 1.25-fold Increase
 Protein No Difference
 Protein 1.25-fold Decrease
 Protein 1.5-fold Decrease
 Protein Not Detected

Tumor S KRAS WT, NRAS WT, BRAF WT, PI3K WT

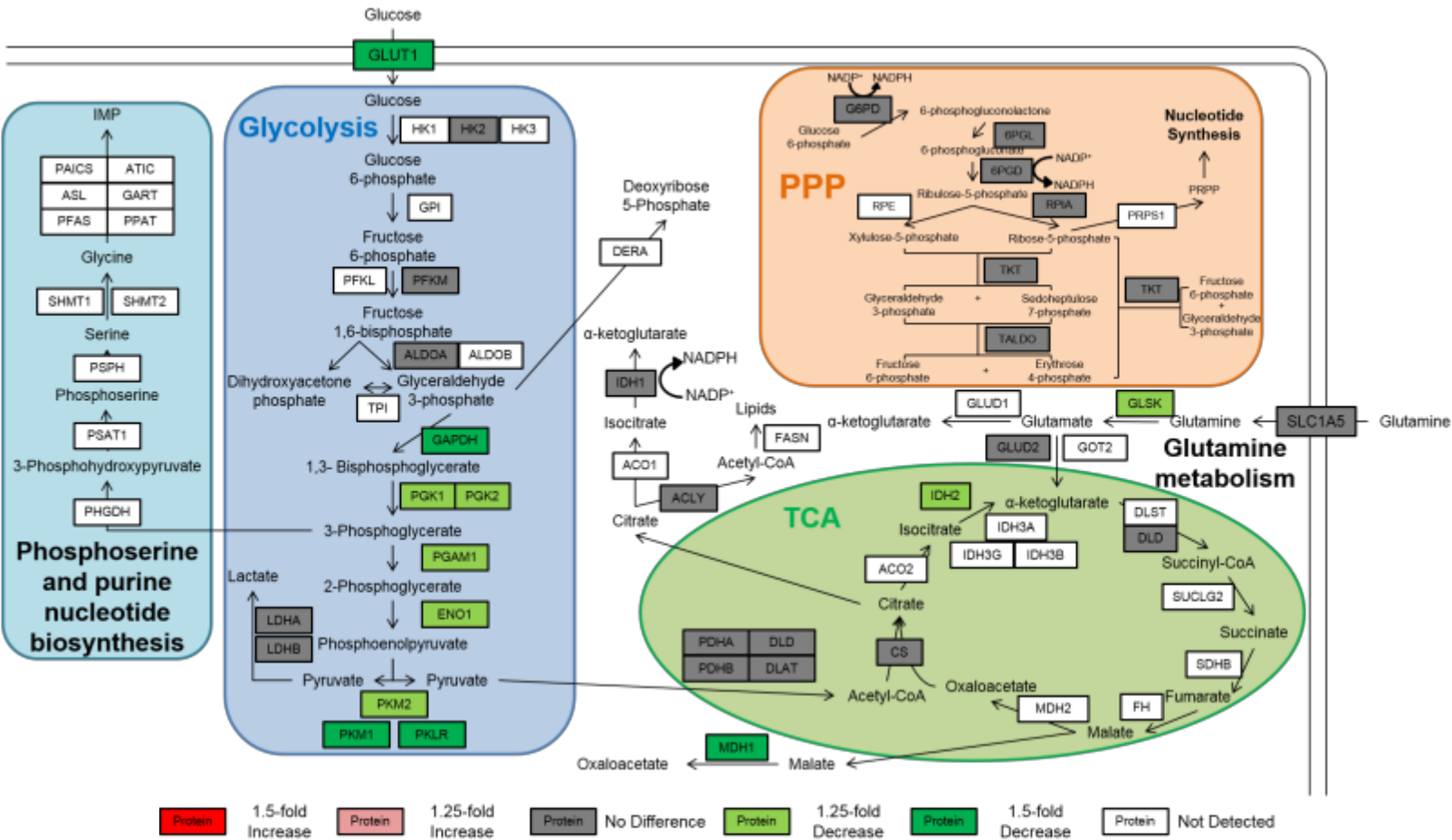


Tumor N KRAS WT, NRAS WT, BRAF WT, PI3K WT

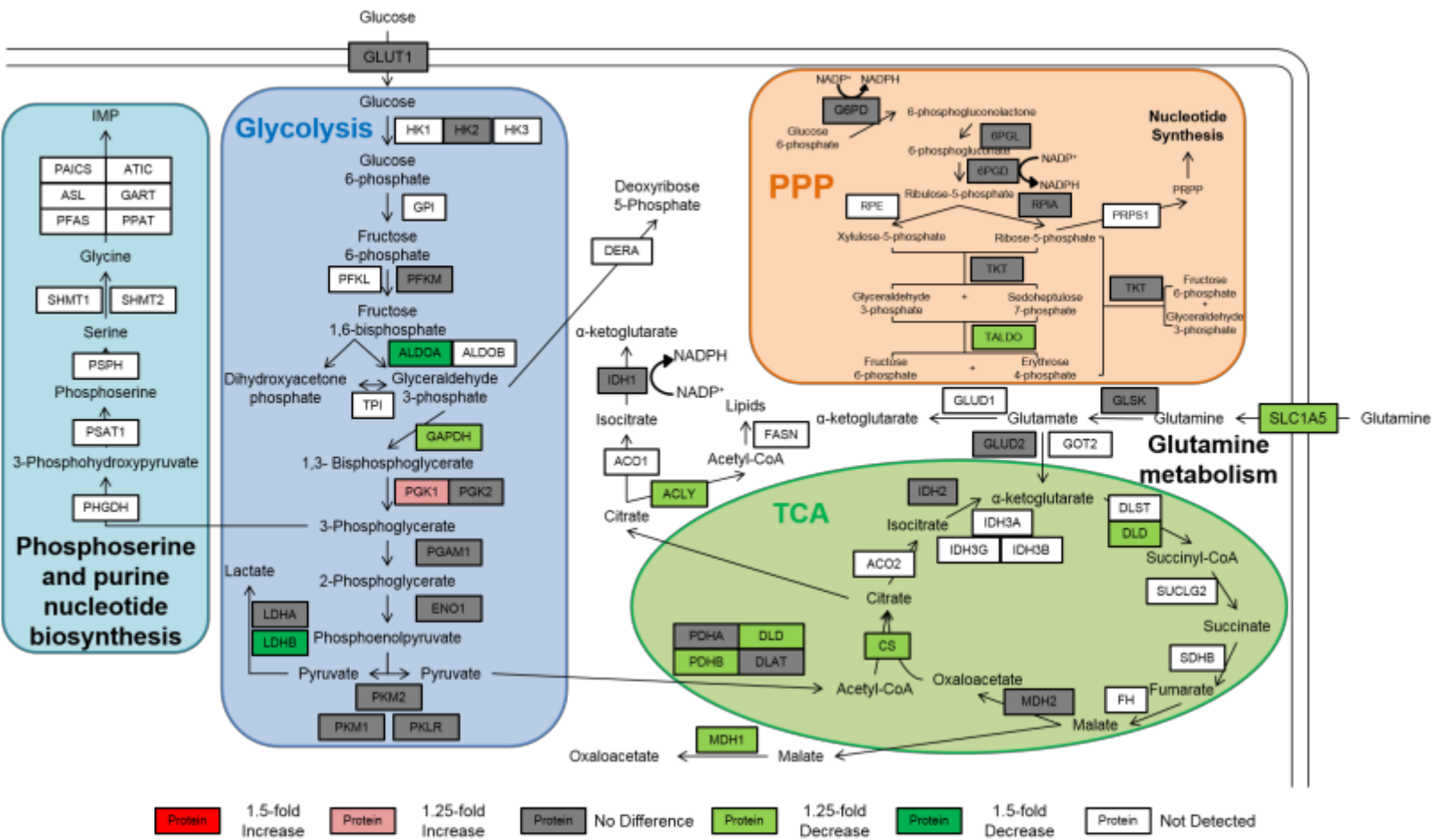


S8D

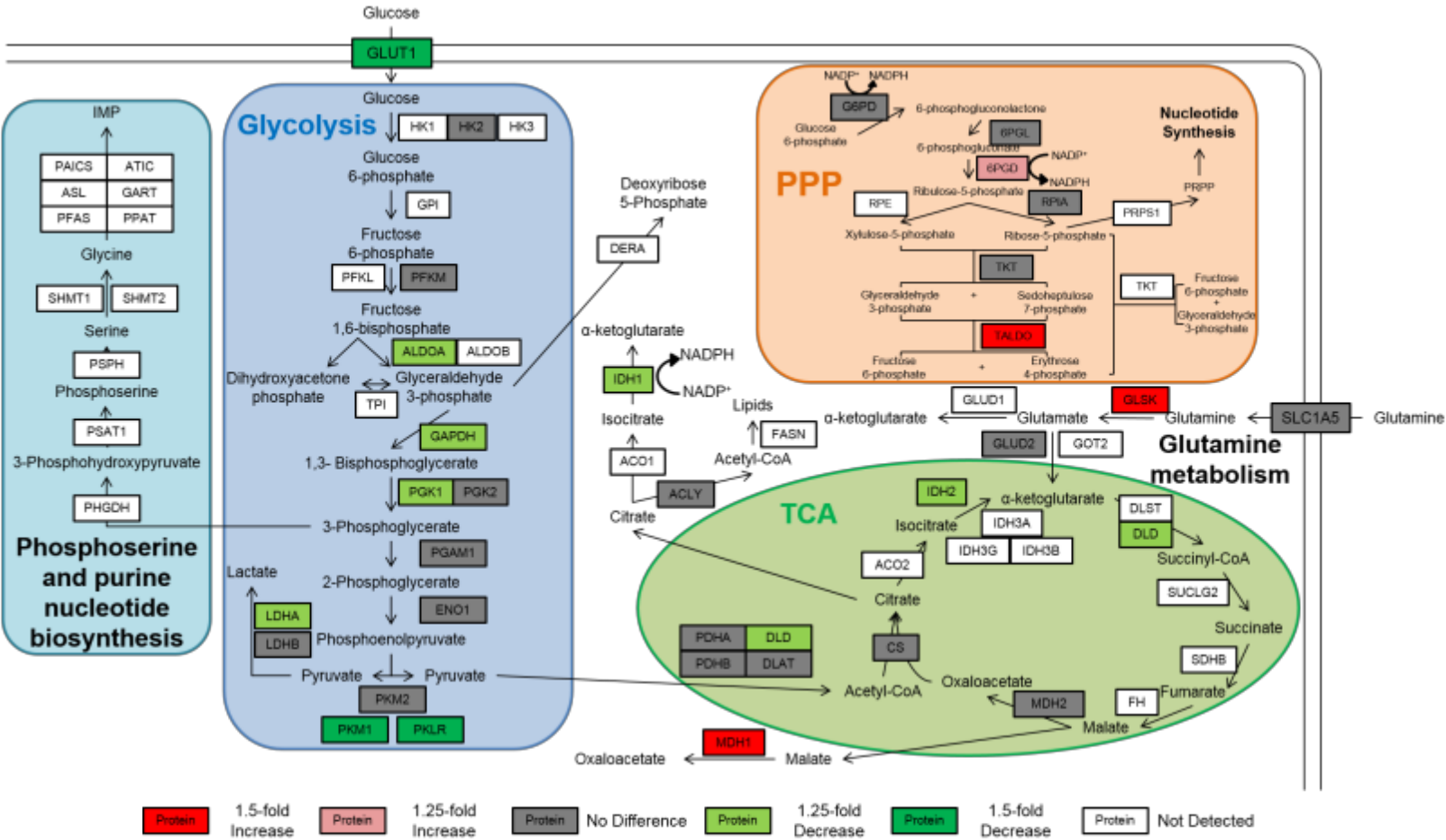
Tumor O KRAAS WT, NRAS WT, BRAF WT, PI3K WT



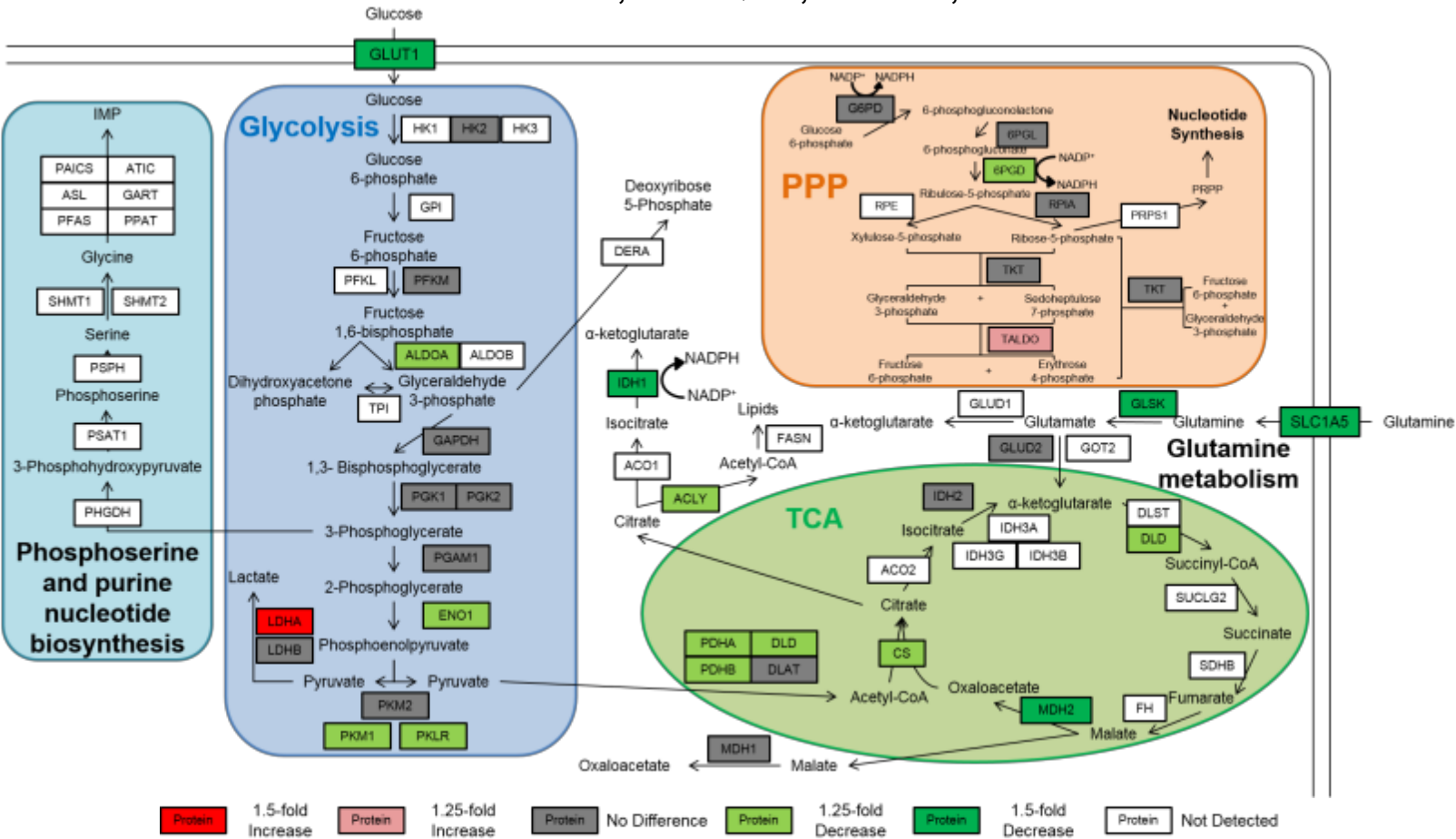
Tumor E KRAS WT, NRAS WT, BRAF WT, PI3K WT



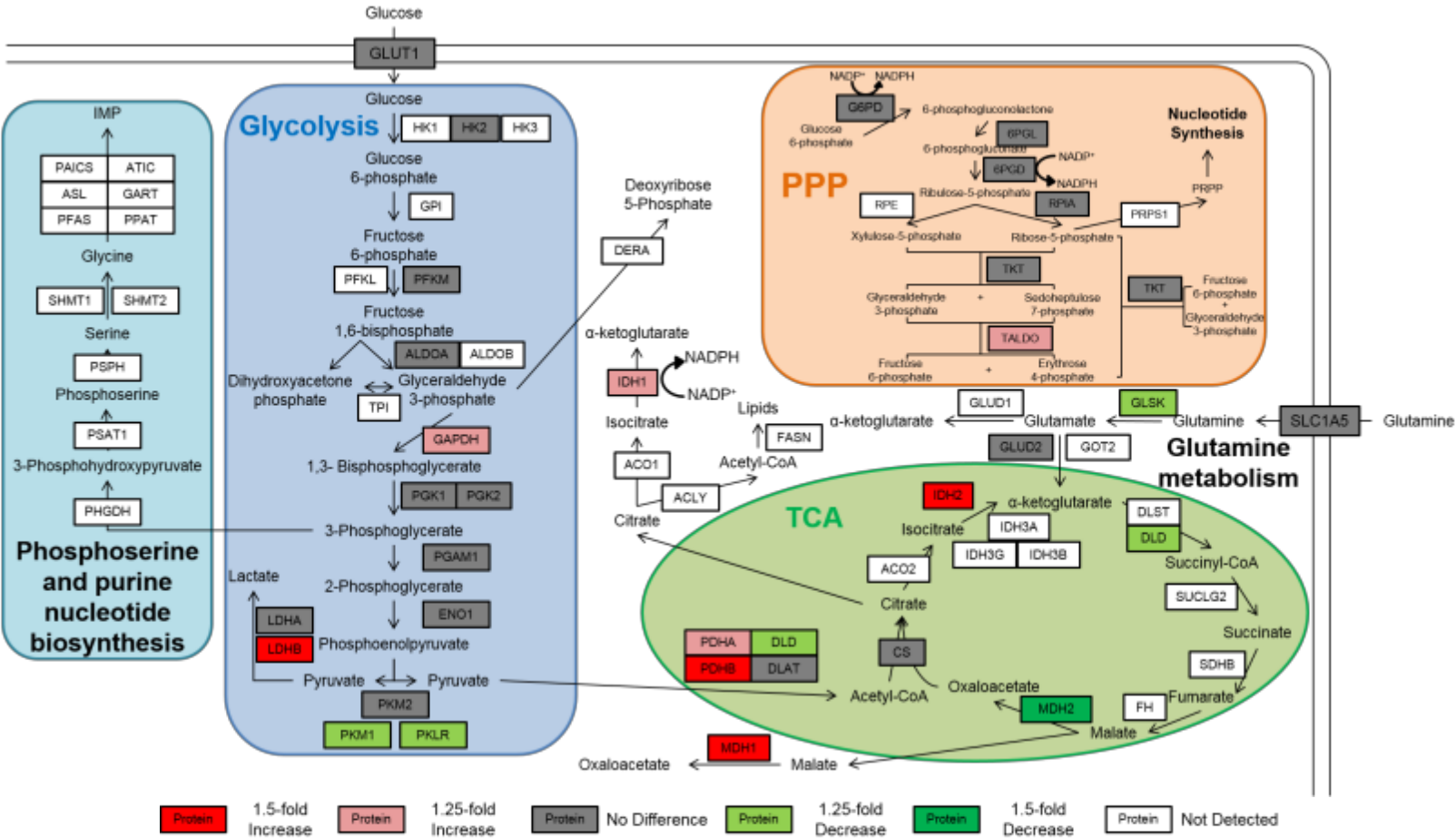
Tumor G KRAS WT, NRAS WT, BRAF WT, PI3K WT



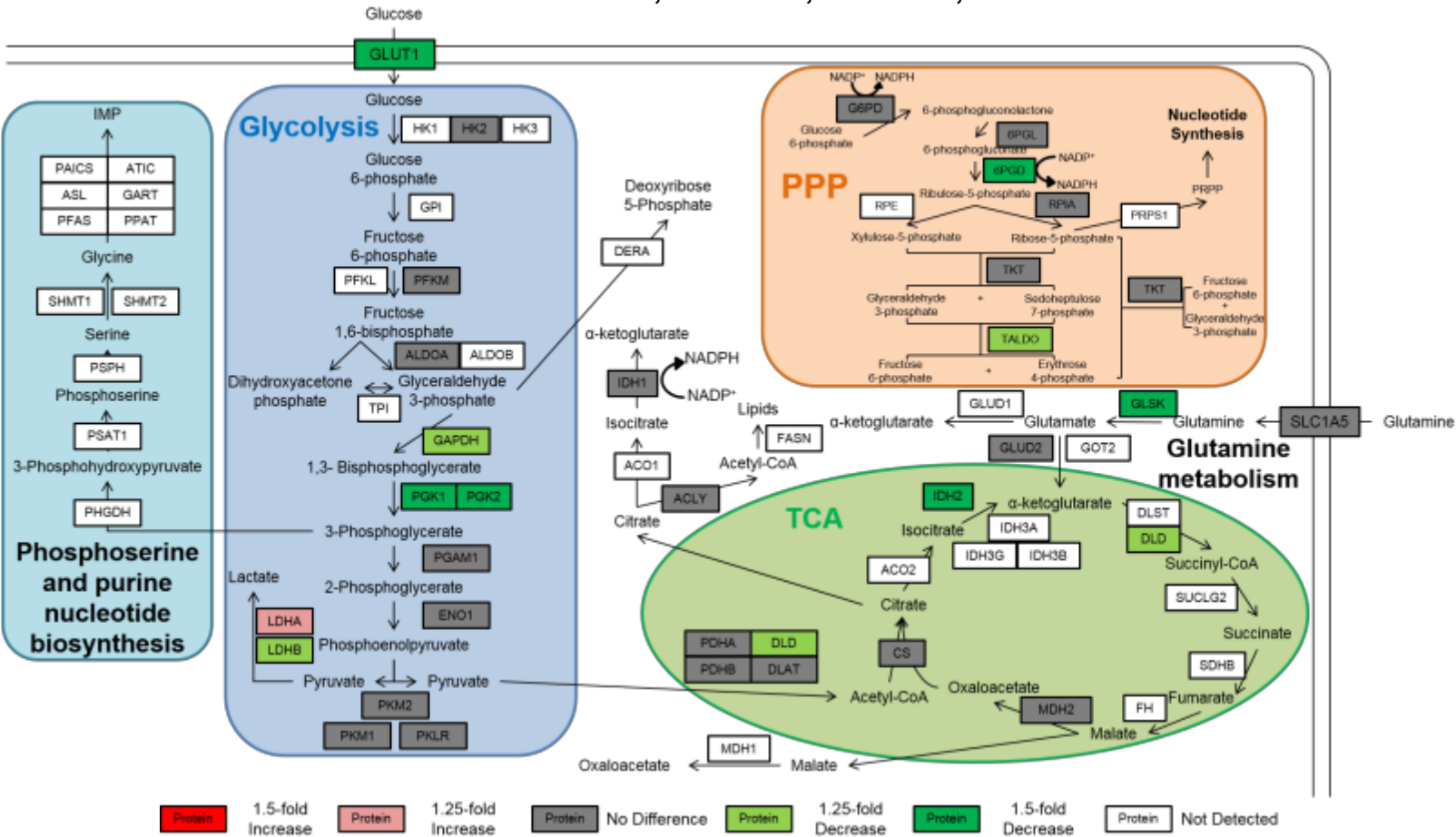
Tumor I KRAS WT, NRAS Q61K, BRAF WT, PI3K WT



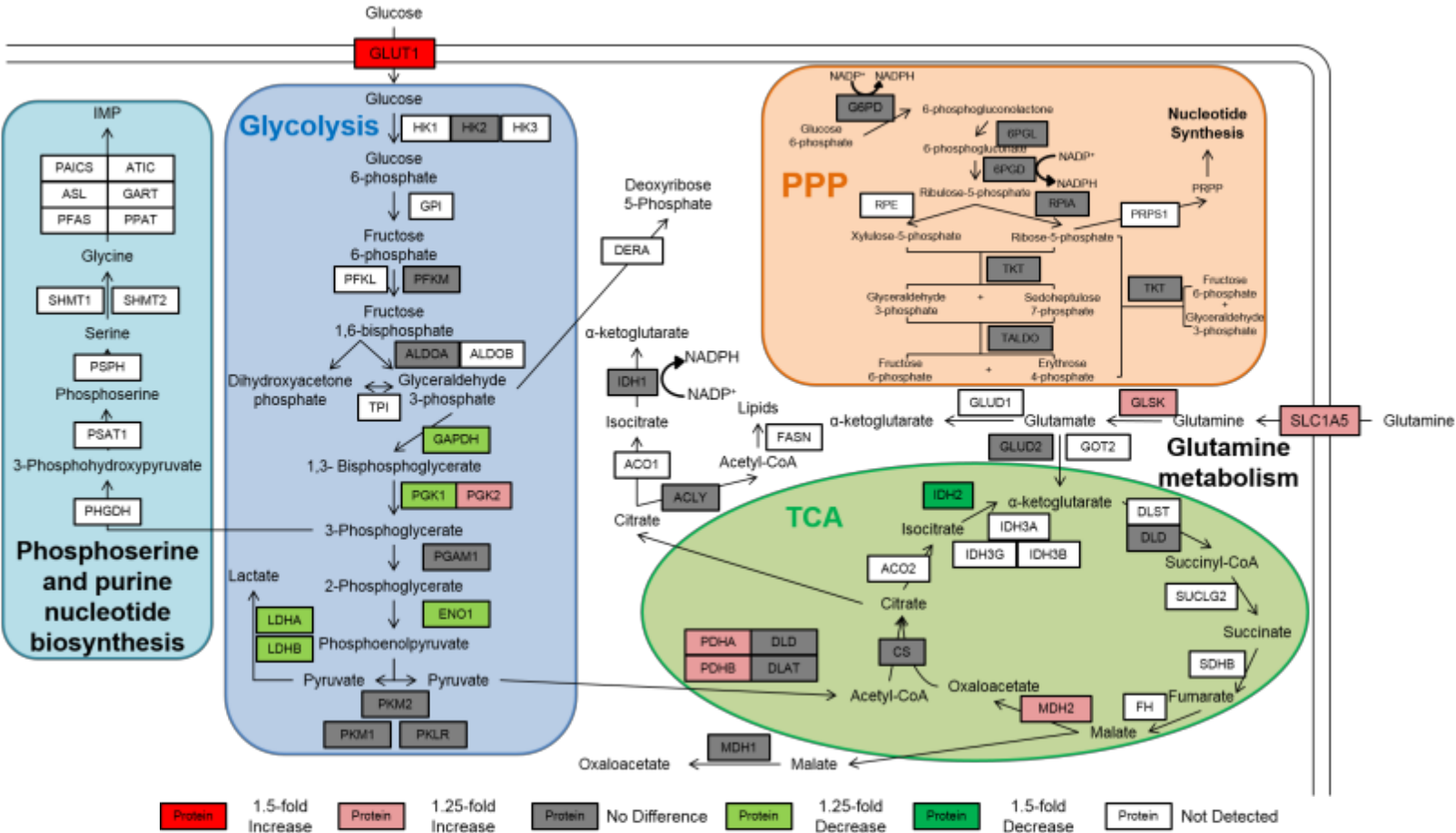
Tumor U KRAS WT, NRAS WT, BRAF WT, PI3K WT



Tumor X KRAS G12V, NRAS WT, BRAF WT, PI3K WT

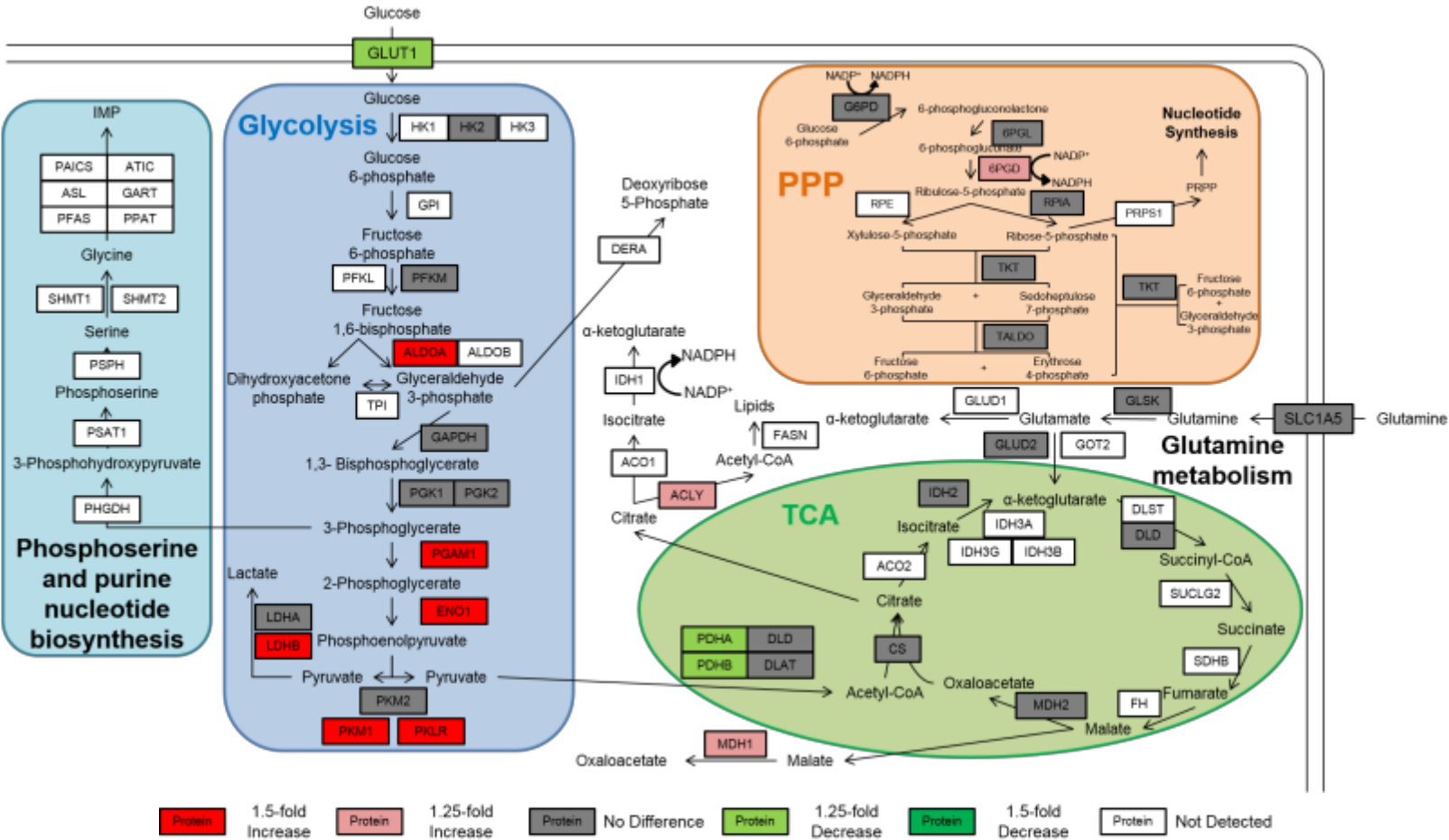


Tumor Q KRAS G12V, NRAS WT, BRAF WT, PI3K WT

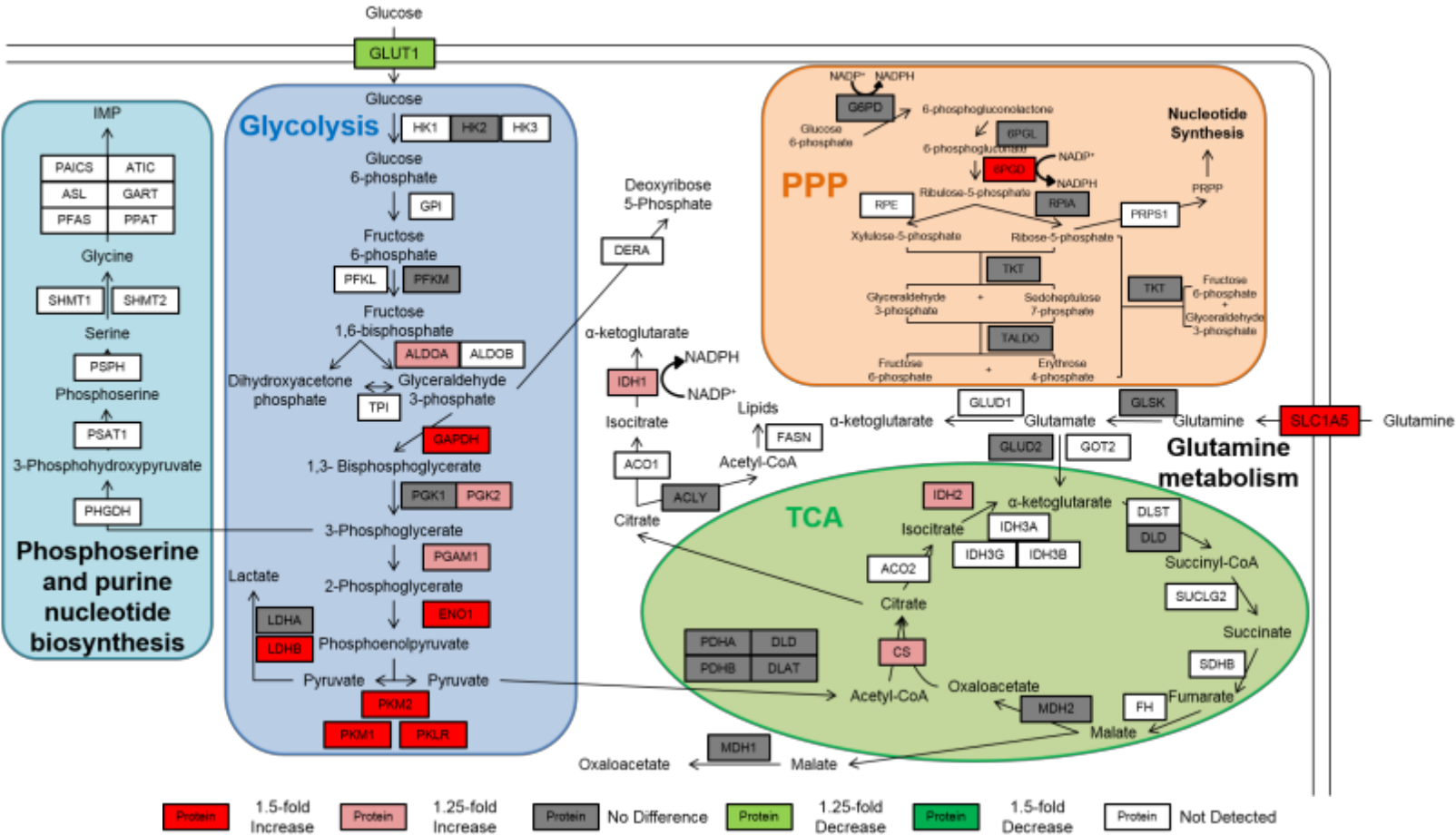


S8K

Tumor T KRAS G12D, NRAS WT, BRAF WT, PI3K WT

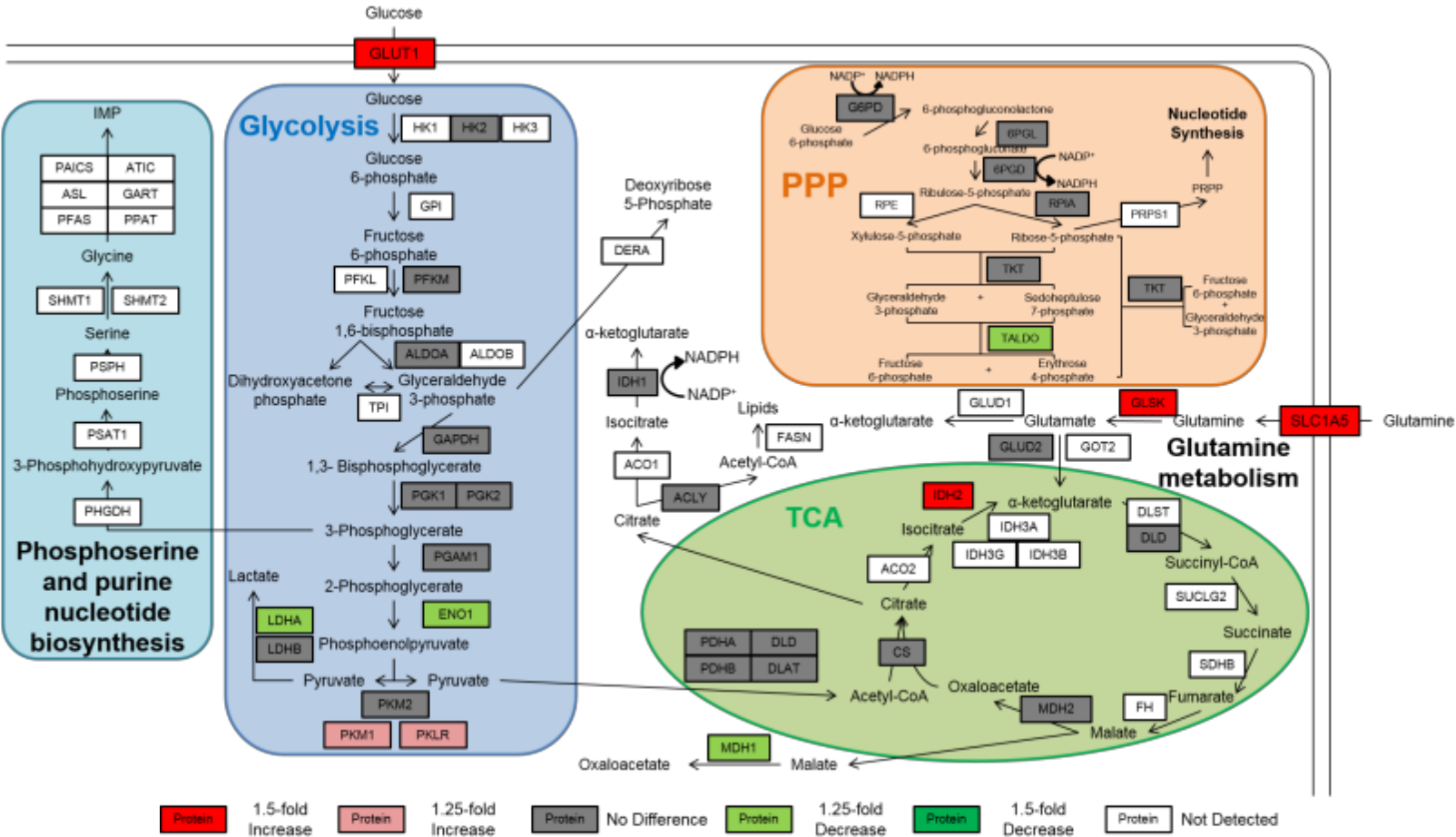


Tumor V KRAS G12V, NRAS WT, BRAF WT, PI3K WT

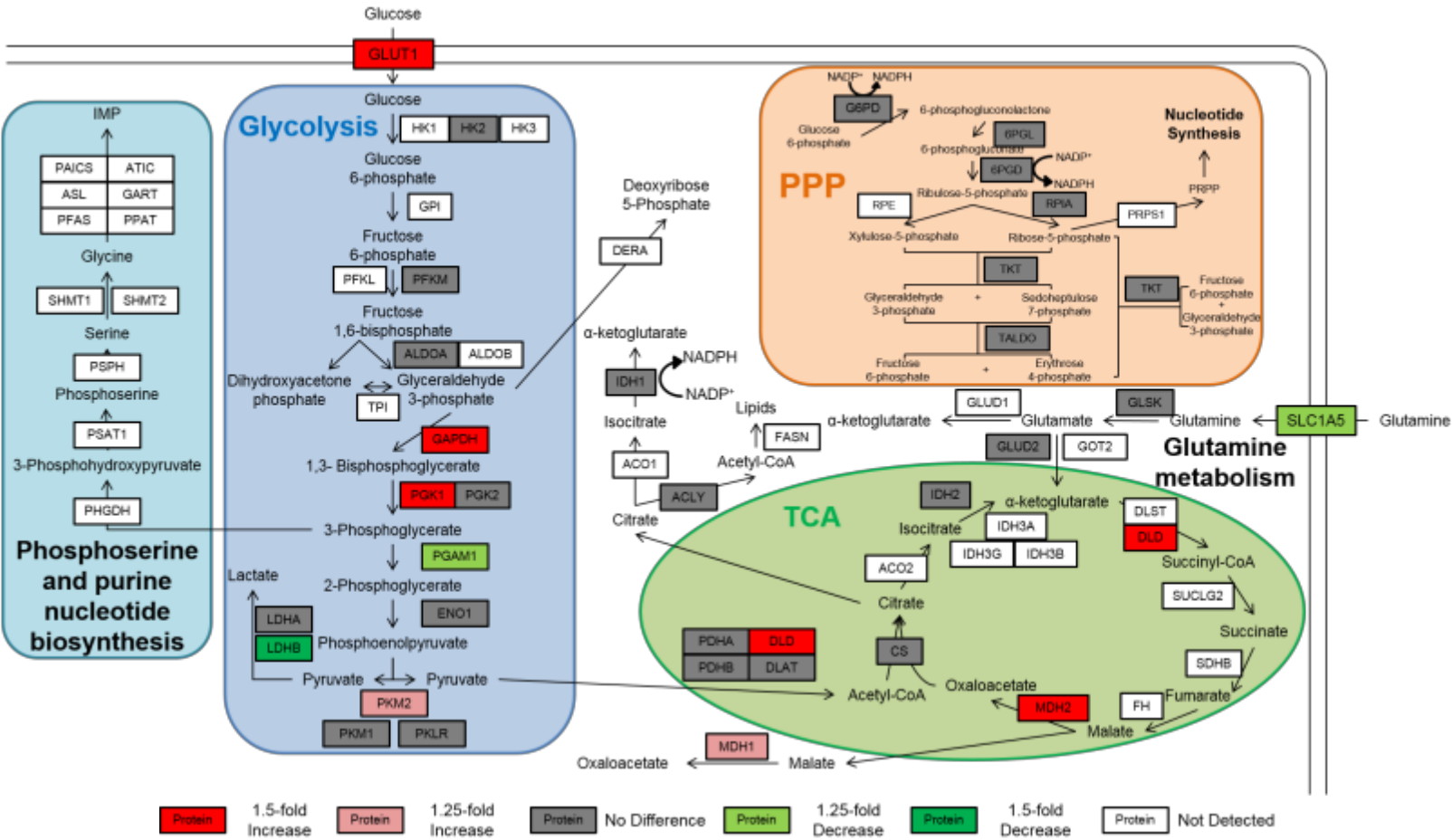


S8M

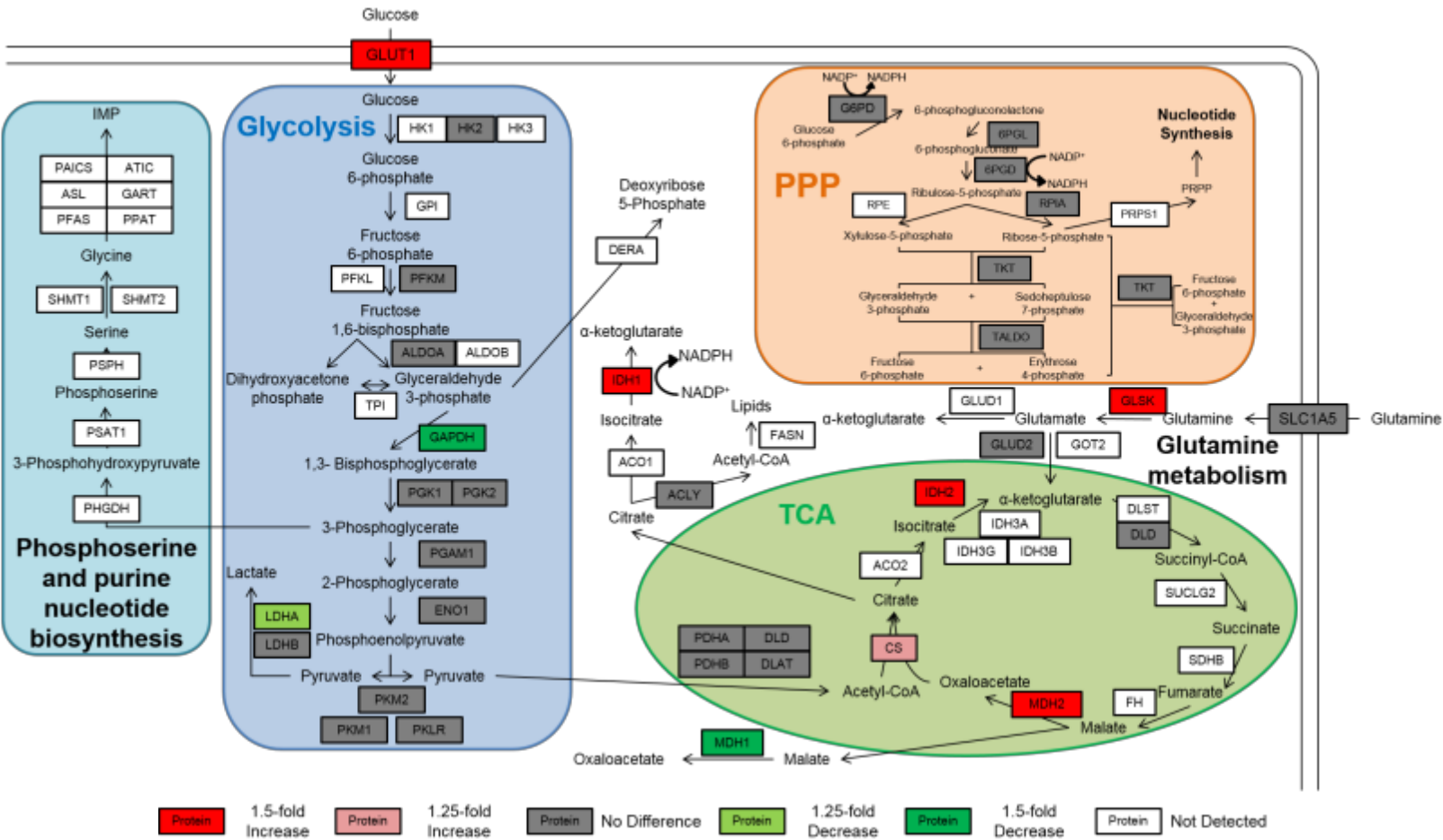
Tumor P KRAS G12V, NRAS WT, BRAF WT, PI3K WT



Tumor H KRAS G12V, NRAS WT, BRAF WT, PI3K WT



Tumo L KRAS G12V, NRAS WT, BRAF WT, PI3K E545K



Tumor A KRAS G12V, NRAS WT, BRAF WT, PI3K E545K

