

Morales-Betanzos et al.

## Supplemental Dataset 2

PRM traces for detection of target peptides in all samples

Part I. PD-1, PD-L1, PD-L2 (PRM with stable isotope dilution)

Part II. IDO1, LAG3, HAVCR2 (TIM-3), C10orf54 (VISTA), CD40 (PRM with labeled reference peptide normalization)

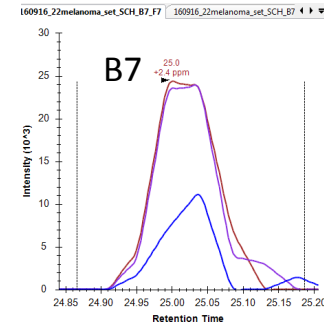
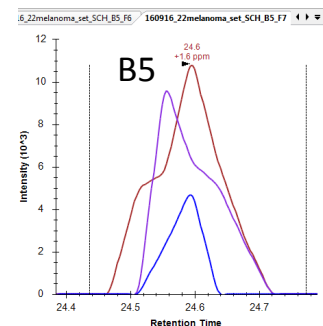
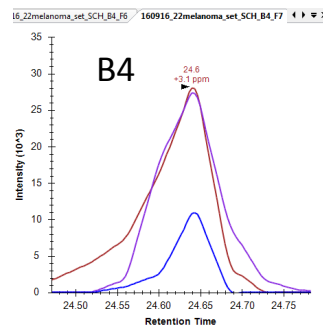
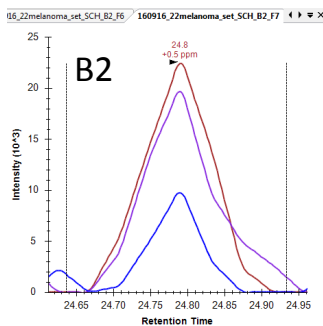
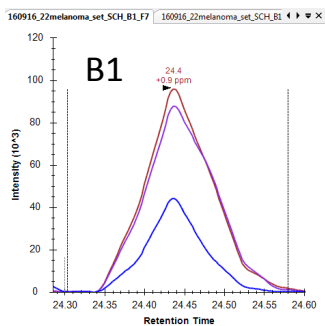
**Part I. Quantitation of PD-1, PD-L1, and PD-L2 by PRM with stable isotope dilution**

# PD1

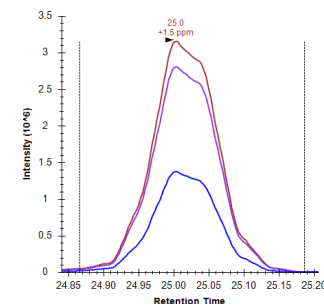
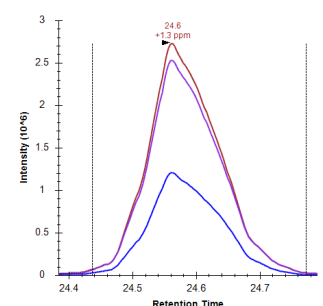
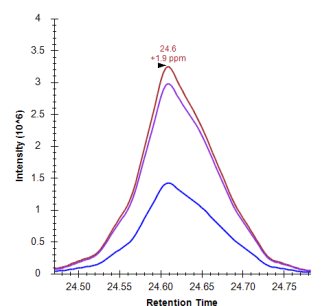
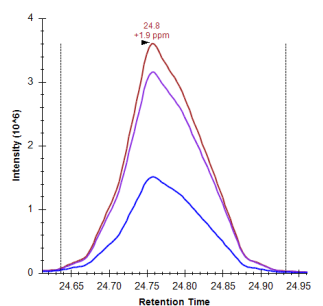
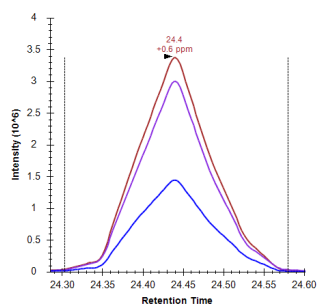
PD-1 was quantified via the proteotypic peptide LAAFPEDR. PRM transitions traces for the endogenous LAAFPEDR peptide and the ( $^{13}\text{C}^{15}\text{N}$ ) standard for each sample are shown in the following 3 pages.

# PD1 endogenous= LAAFPEDR standard = LAAFPEDR (C<sup>13</sup> N<sup>15</sup>)

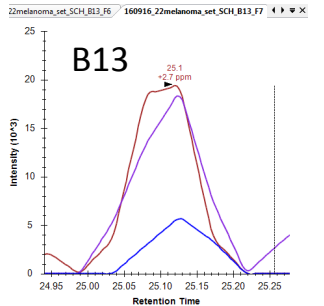
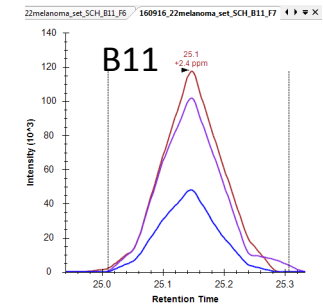
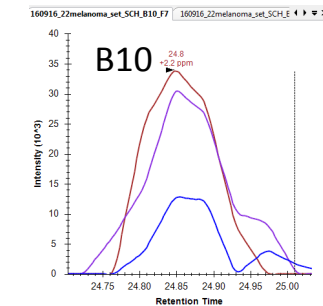
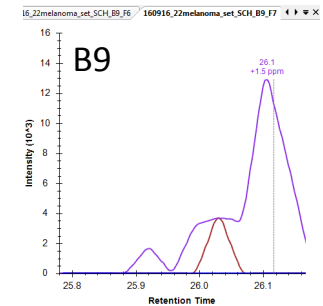
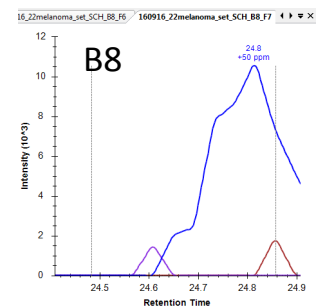
Endogenous



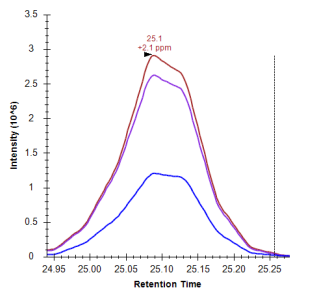
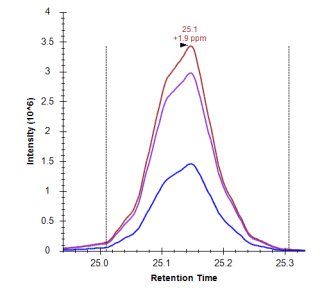
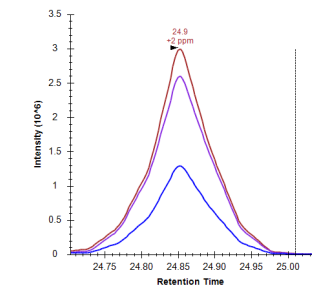
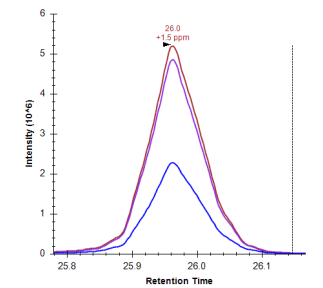
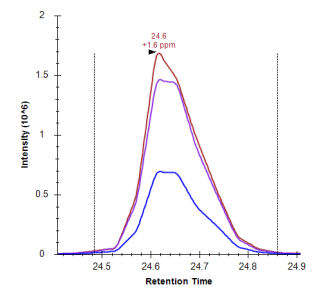
Standard



Endogenous

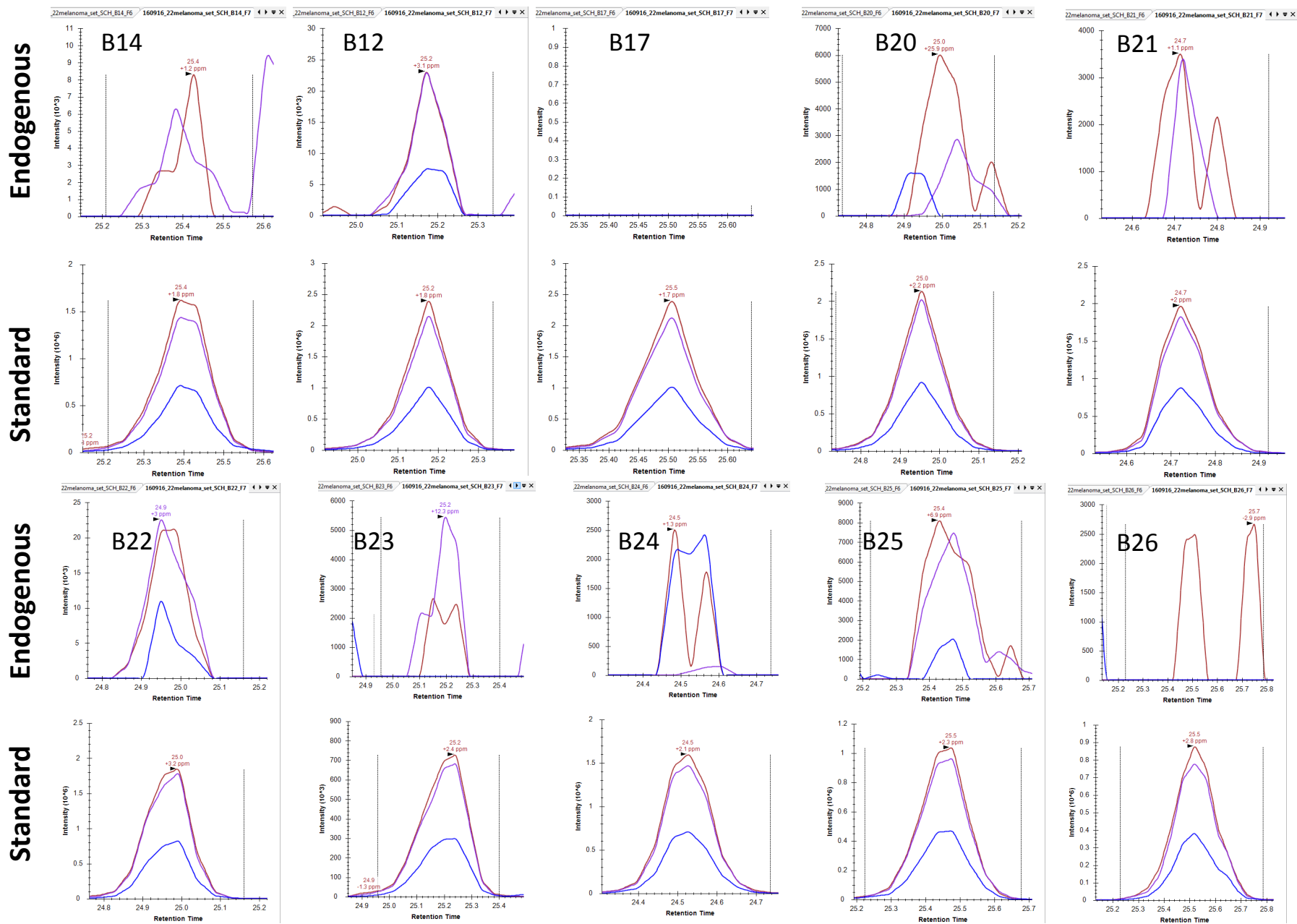


Standard



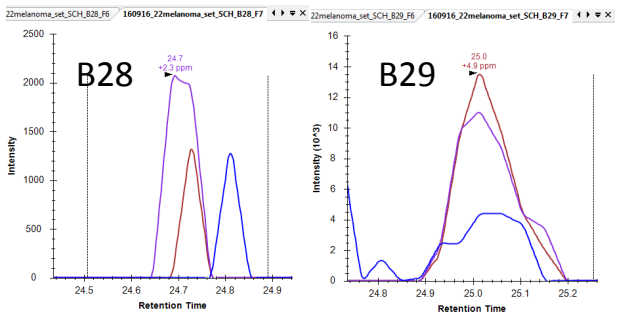


# PD1 endogenous= LAAFPEDR standard = LAAFPEDR (C<sup>13</sup> N<sup>15</sup>)

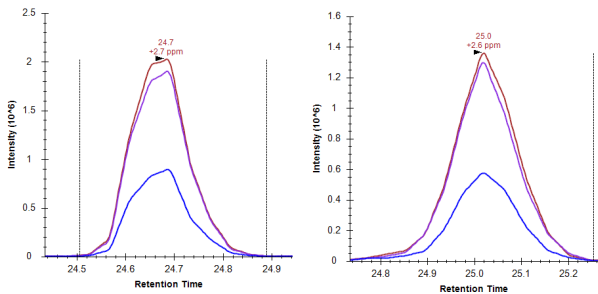


# PD1 endogenous = LAAFPEDR standard = LAAFPEDR (C<sup>13</sup> N<sup>15</sup>)

Endogenous



Standard



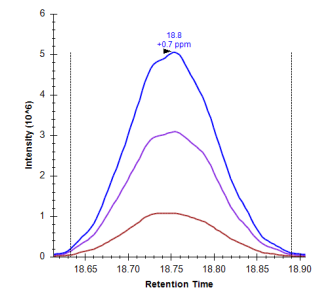
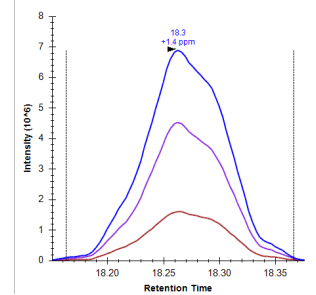
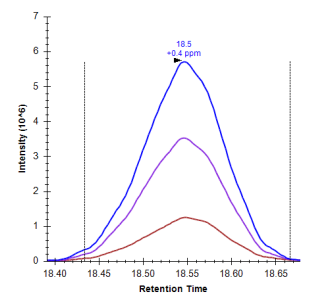
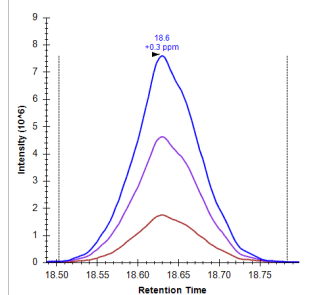
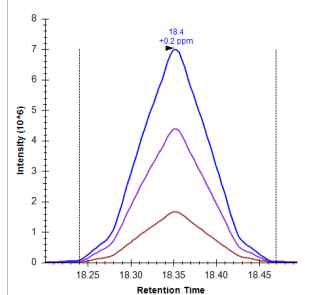
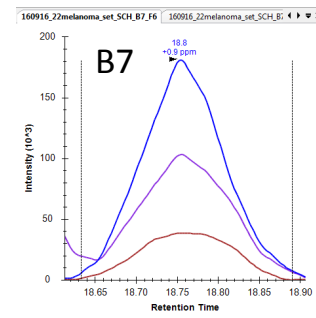
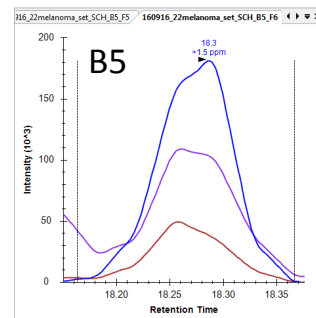
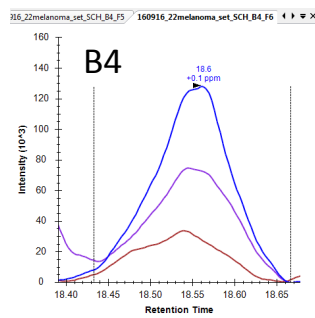
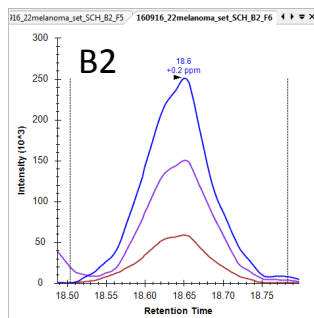
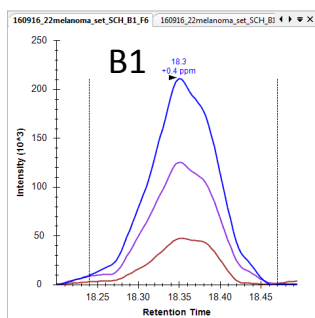
# PD-L1

PD-L1 was quantified via the proteotypic peptides LQDAGVYR and VNAPYNK. PRM transitions traces for the endogenous LQDAGVYR and VNAPYNK peptides and the  $^{13}\text{C}^{15}\text{N}$  standard for each sample are shown in the following 3 pages.

# PDL1 endogenous= LQDAGVYR standard = LQDAGVYR (C<sup>13</sup> N<sup>15</sup>)

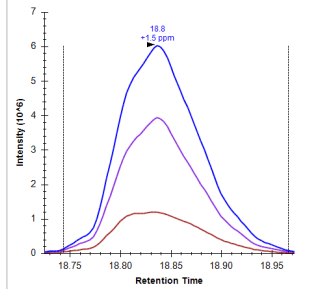
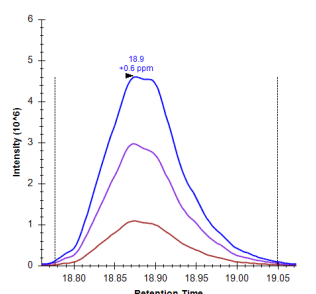
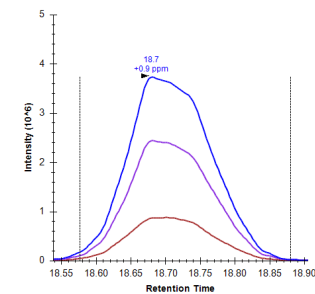
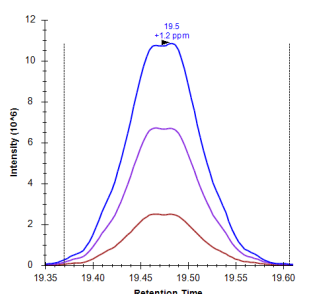
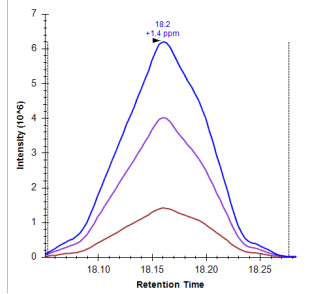
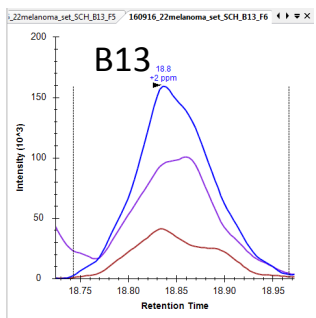
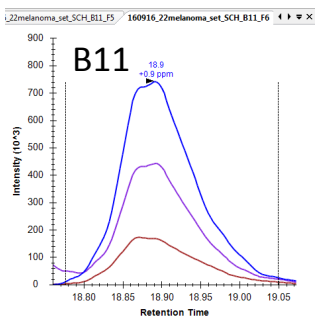
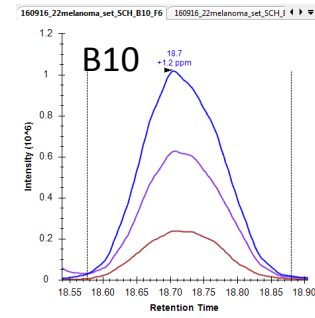
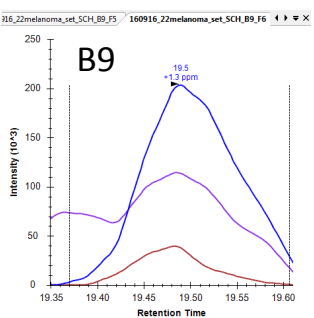
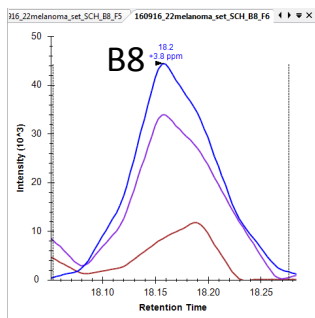
Endogenous

Standard

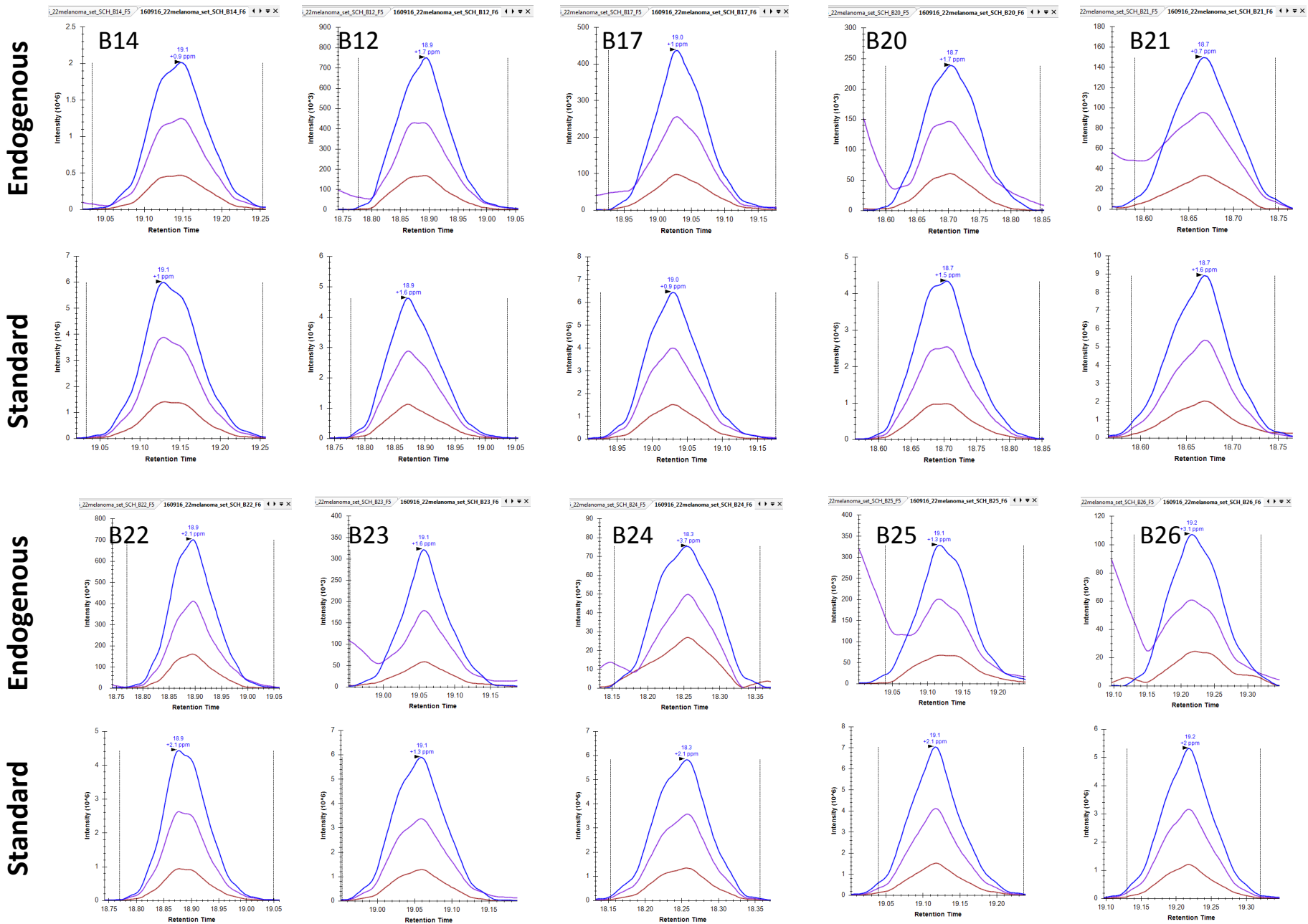


Endogenous

Standard

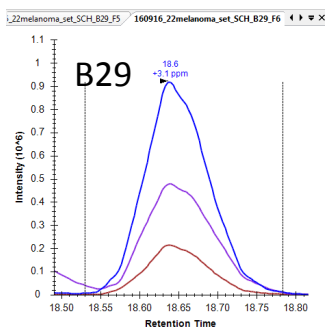
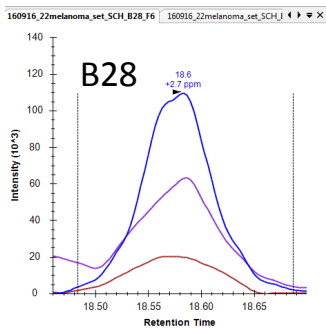


# PDL1 endogenous= LQDAGVYR standard = LQDAGVYR (C<sup>13</sup> N<sup>15</sup>)

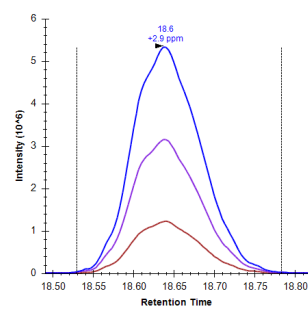
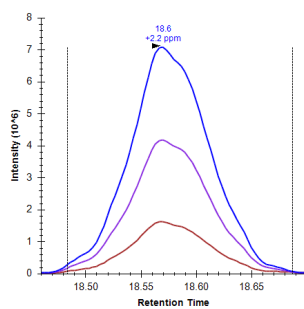


# PDL1 endogenous= LQDAGVYR standard = LQDAGVYR (C<sup>13</sup> N<sup>15</sup>)

Endogenous

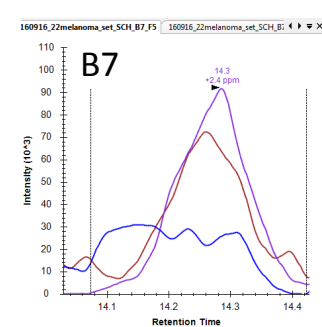
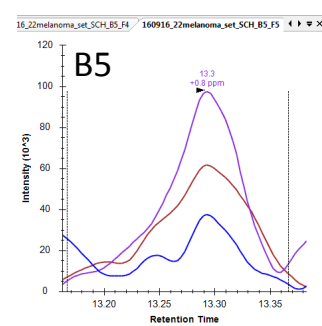
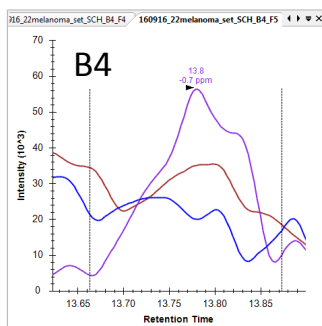
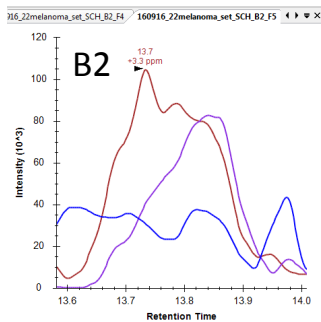
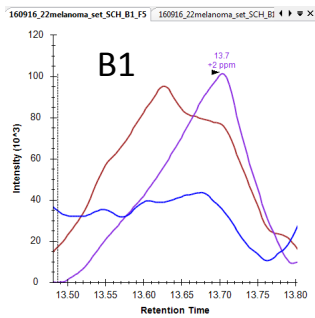


Standard

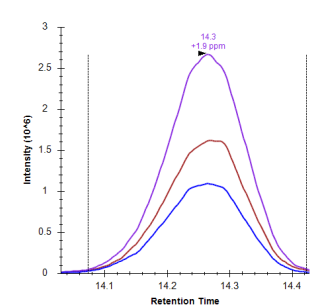
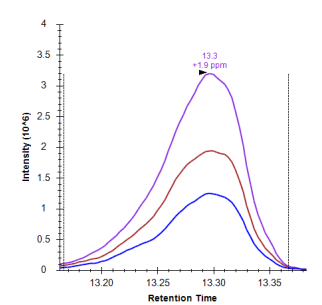
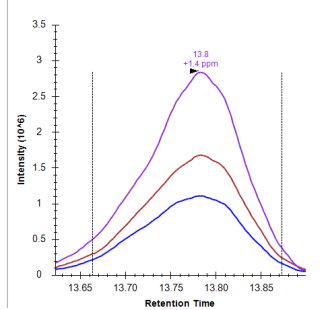
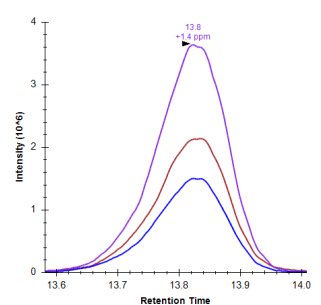
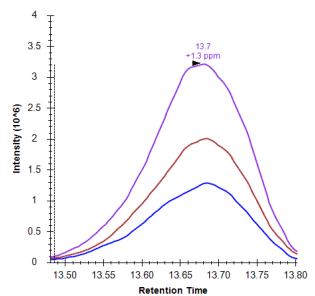


# PDL1 endogenous= VNAPYNK standard = VNAPYNK(C<sup>13</sup> N<sup>15</sup>)

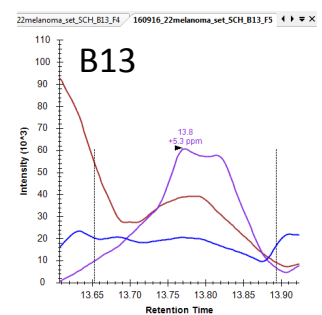
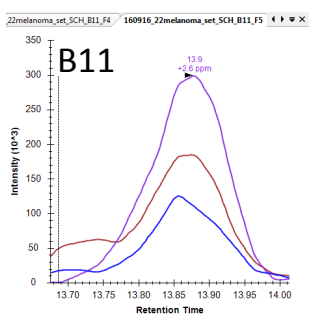
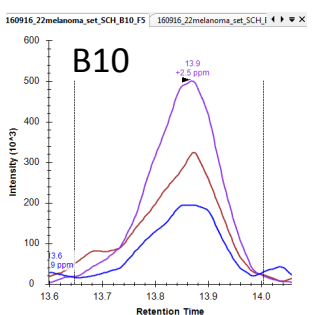
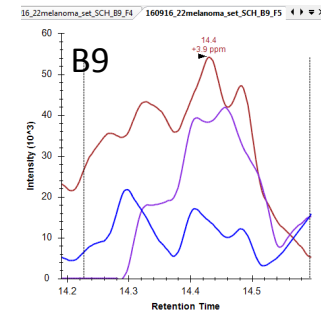
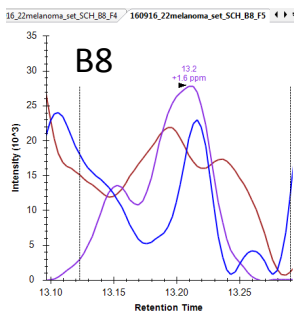
Endogenous



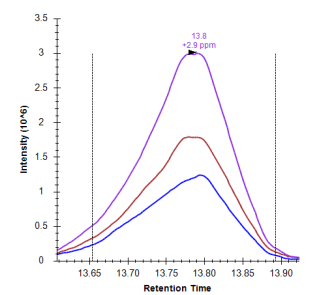
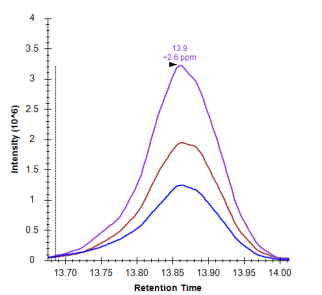
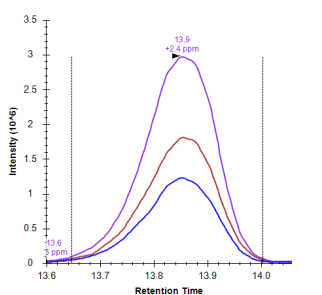
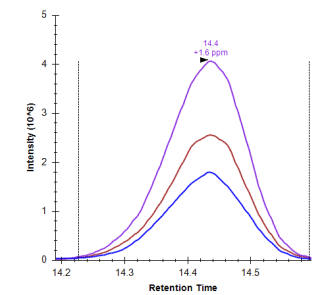
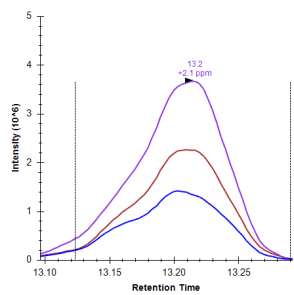
Standard



Endogenous

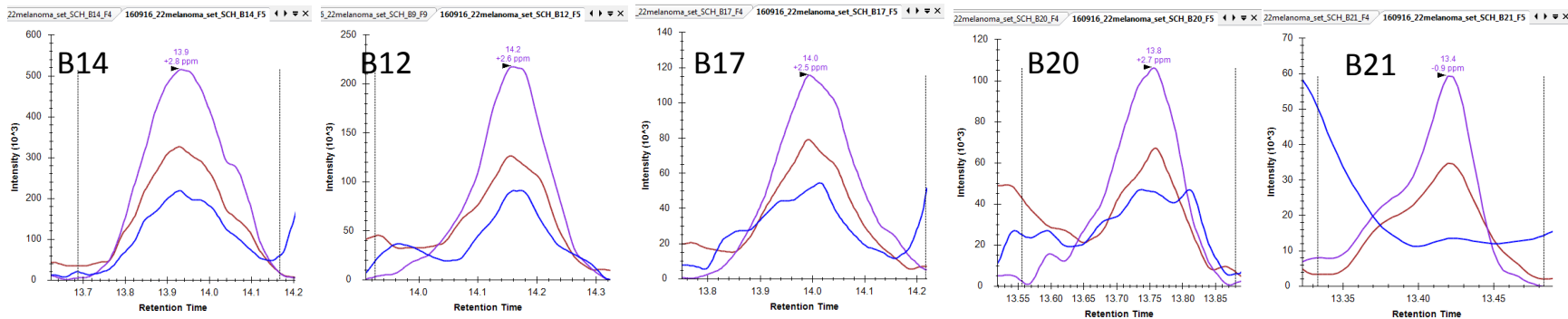


Standard

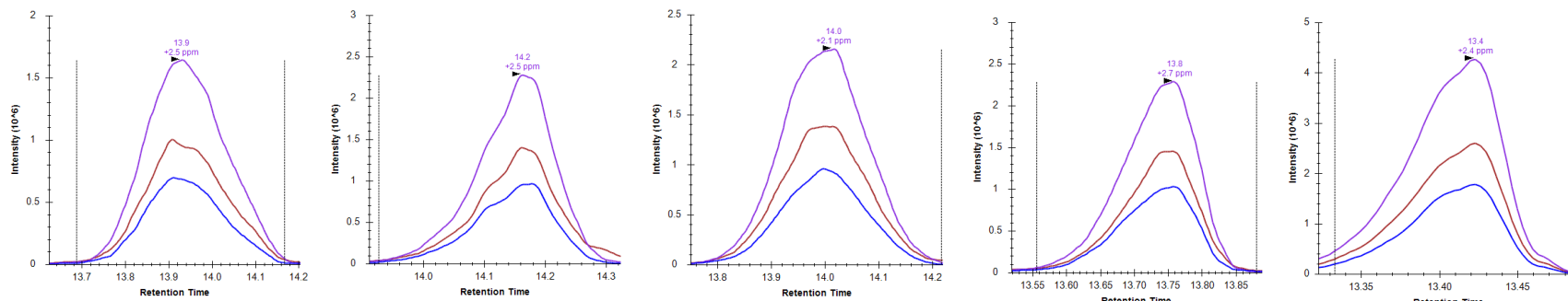


# PDL1 endogenous= VNAPYNK standard = VNAPYNK(C<sup>13</sup> N<sup>15</sup>)

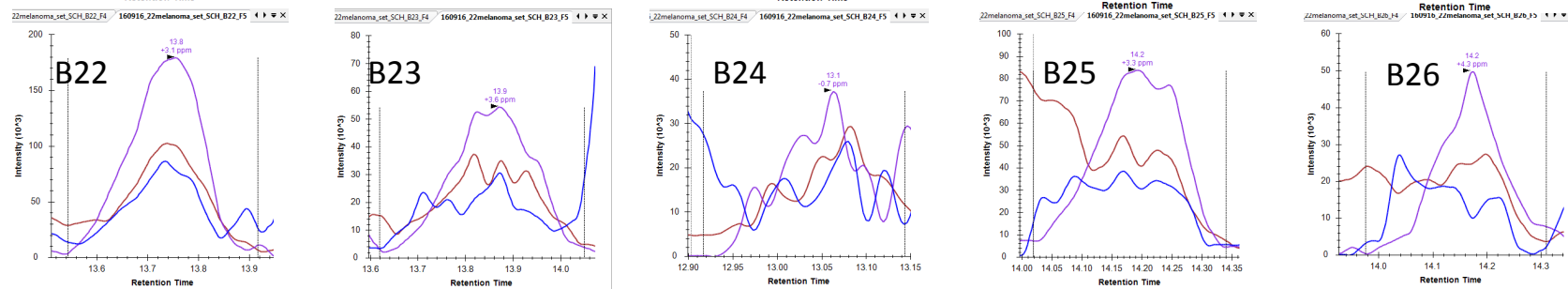
Endogenous



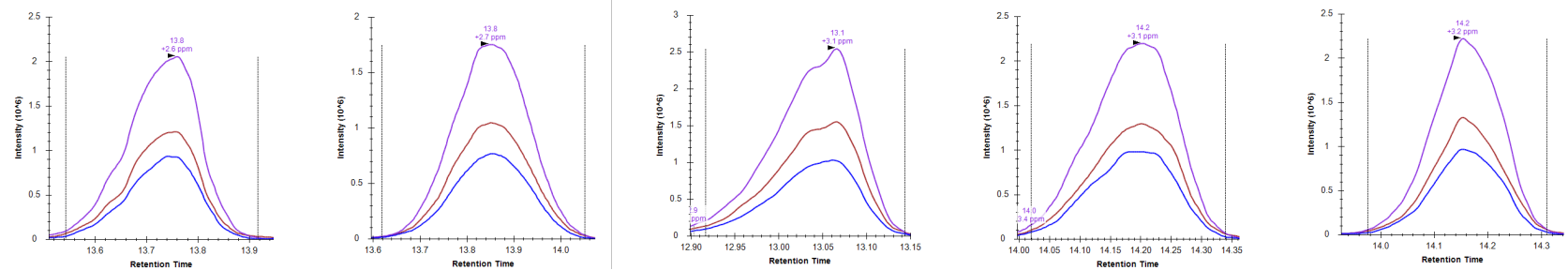
Standard



Endogenous



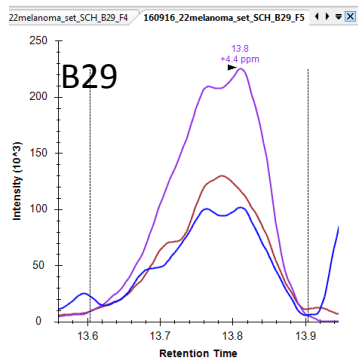
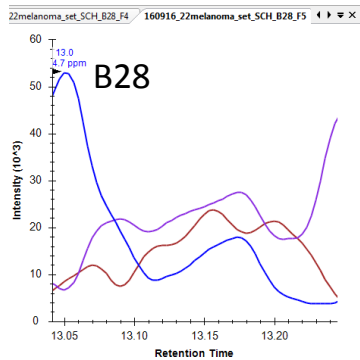
Standard



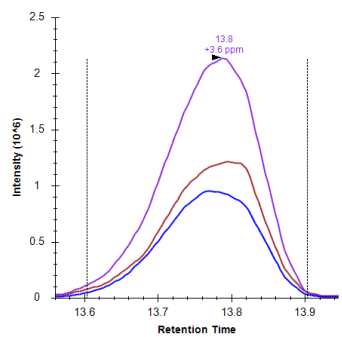
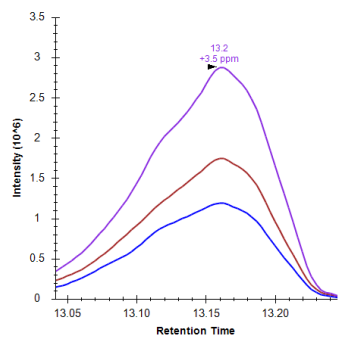


# PDL1 endogenous= VNAPYNK standard = VNAPYNK(C<sup>13</sup> N<sup>15</sup>)

Endogenous



Standard

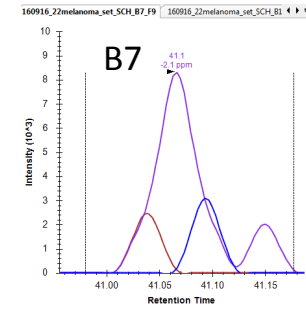
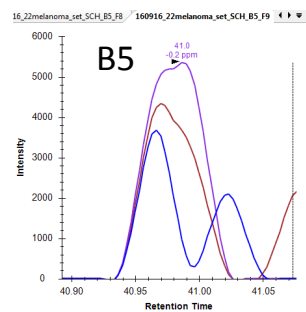
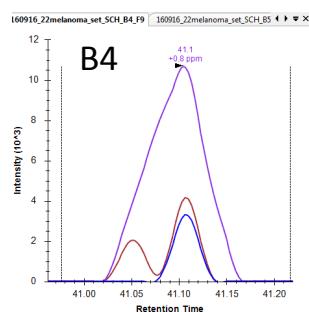
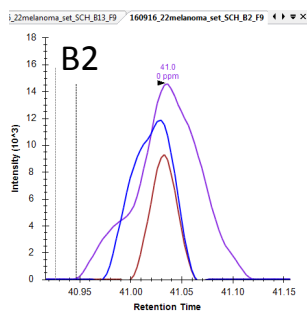
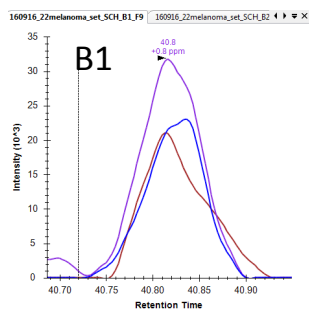


# PD-L2

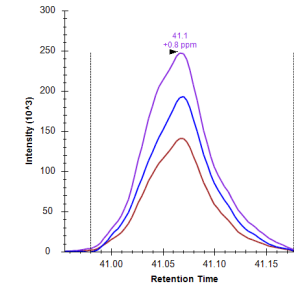
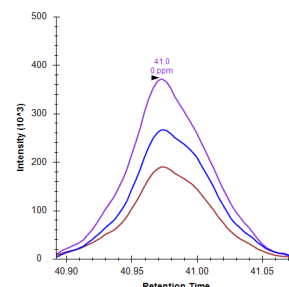
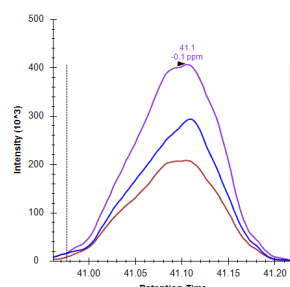
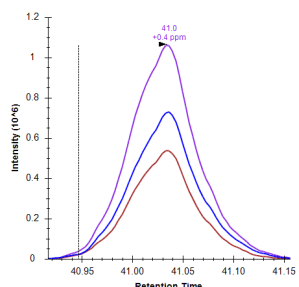
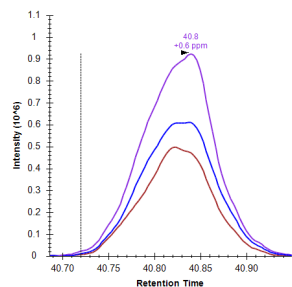
PD-L2 was quantified via the proteotypic peptide TPEGLYQVTSVLR. PRM transitions traces for the endogenous TPEGLYQVTSVLR peptide and the  $(^{13}\text{C}^{15}\text{N})$  standard for each sample are shown in the following 3 pages.

# PDL2 endogenous= TPEGLYQVTSVLR standard = TPEGLYQVTSVLR (C<sup>13</sup> N<sup>15</sup>)

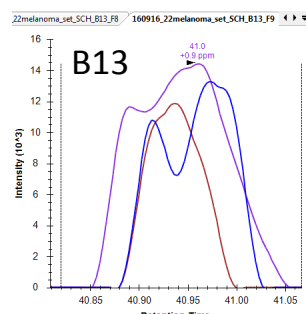
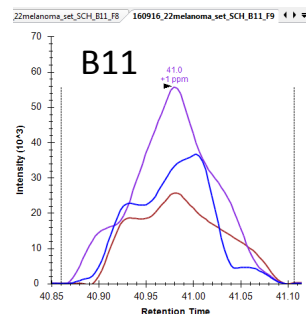
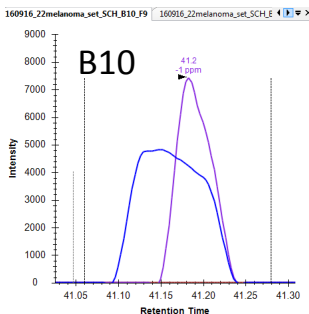
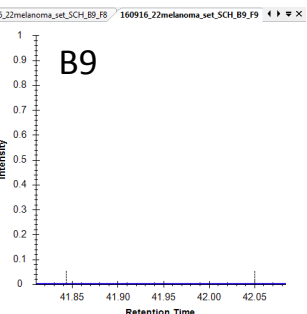
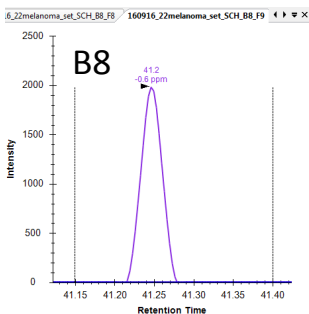
Endogenous



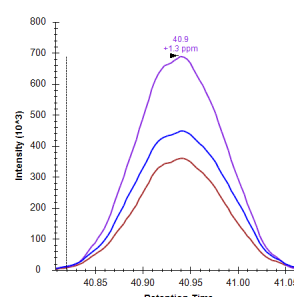
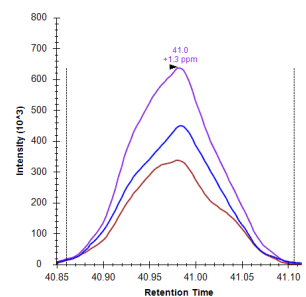
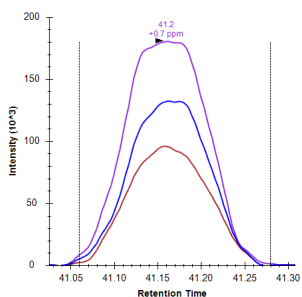
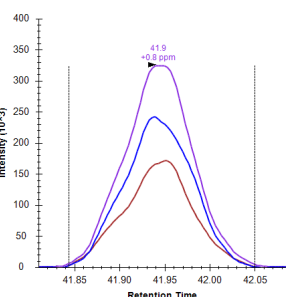
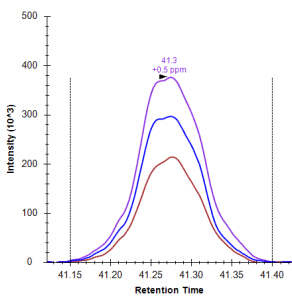
Standard



Endogenous

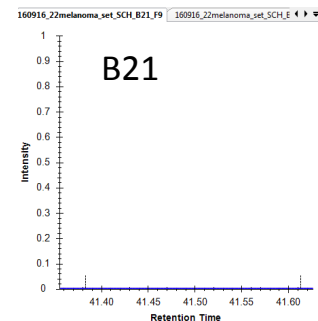
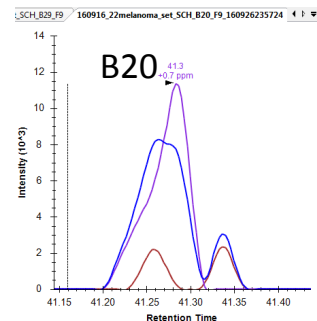
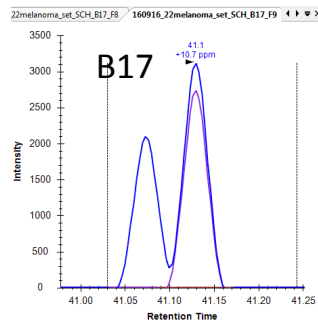
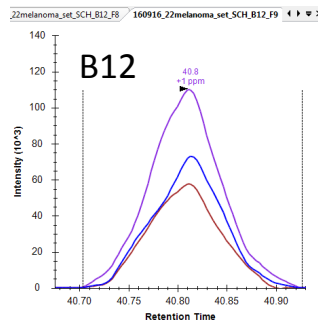
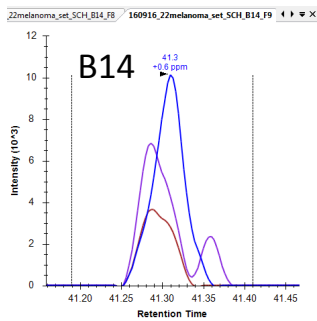


Standard

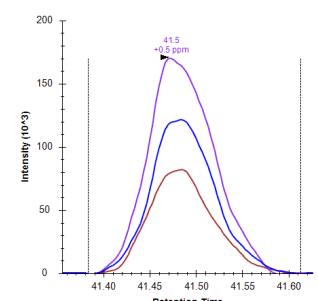
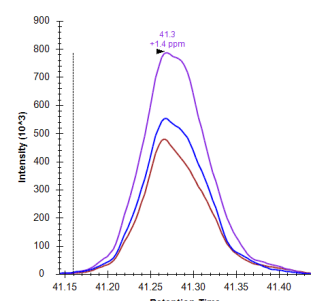
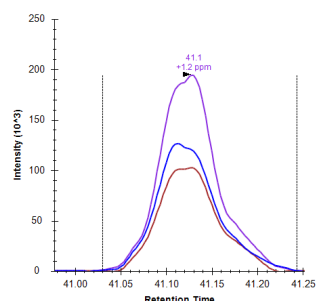
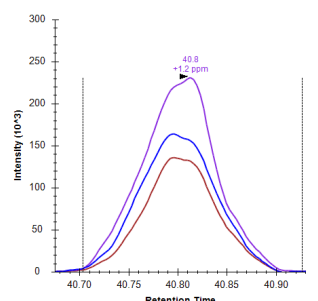
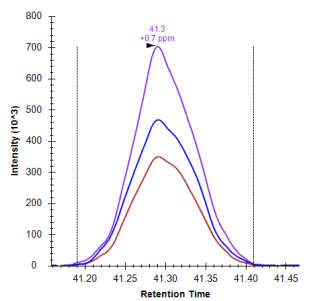


# PDL2 endogenous= TPEGLYQVTSVLR standard = TPEGLYQVTSVLR (C<sup>13</sup> N<sup>15</sup>)

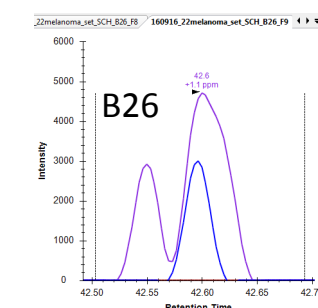
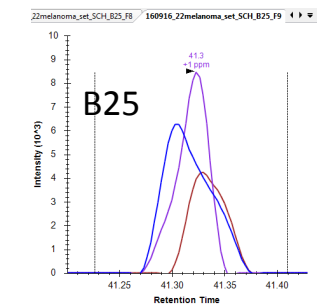
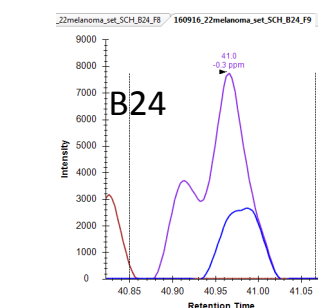
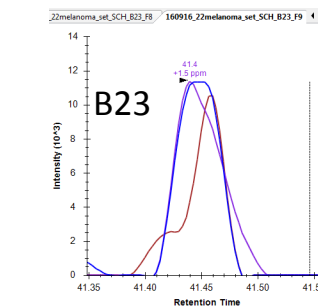
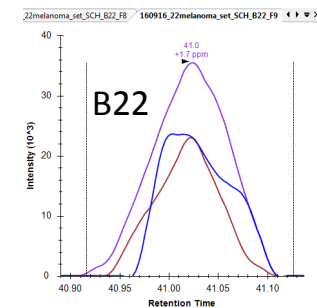
Endogenous



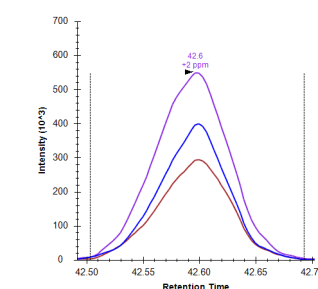
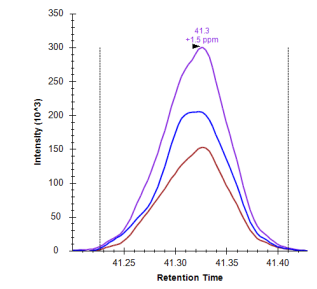
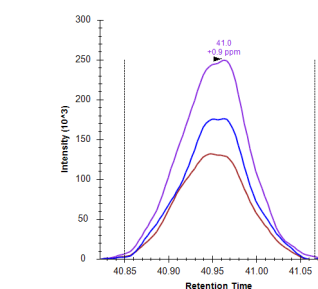
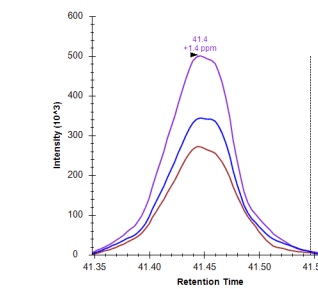
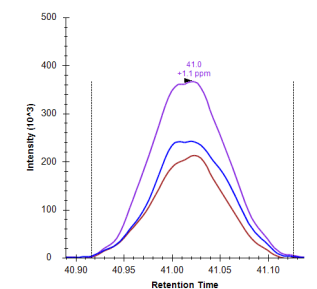
Standard



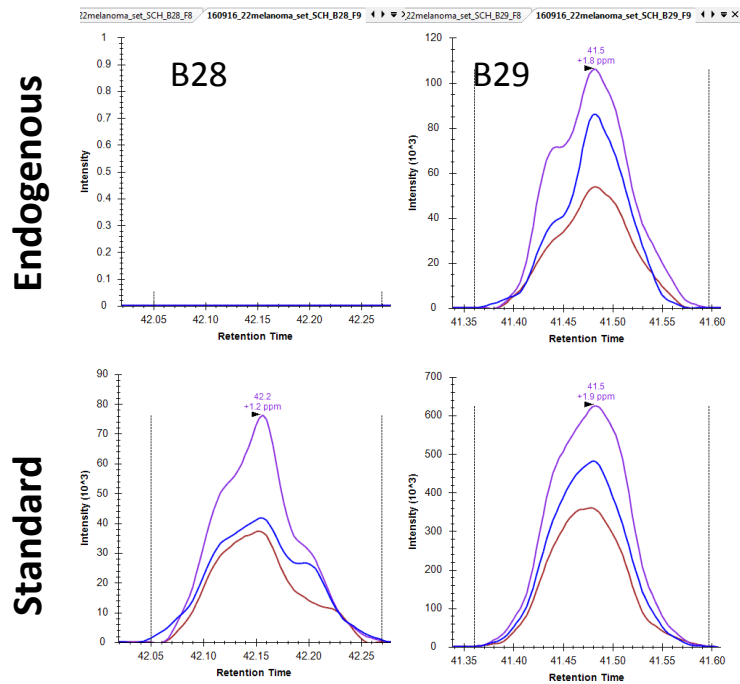
Endogenous



Standard

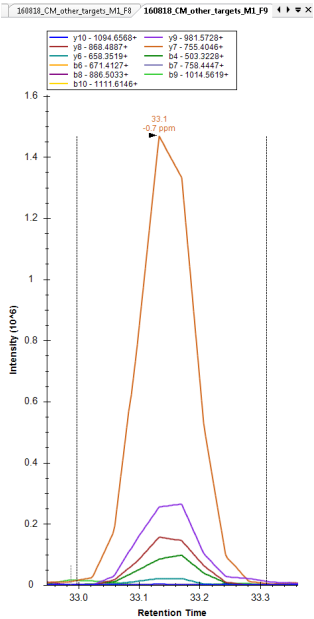


# PDL2 endogenous = TPEGLYQVTSVLR standard = TPEGLYQVTSVLR (C<sup>13</sup> N<sup>15</sup>)

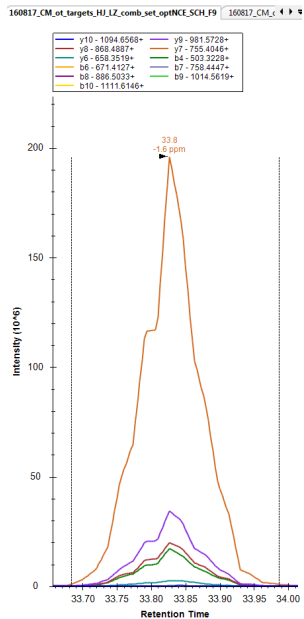


# **Part II. Relative quantitation of IDO1, LAG3, HAVCR2 (TIM-3), C10orf54 (VISTA) and CD40 by the labeled reference peptide (LRP) method**

- 1. IDO1 YILIPASQQPK; reference standard CD4-ILGNQGSFLTK (C<sup>13</sup> N<sup>15</sup>)**
- 2. LAG3 LPAGVGTR; reference standard PDL1-LQDAGVYR (C<sup>13</sup> N<sup>15</sup>)**
- 3. HAVCR2 (TIM-3) LANDLR; reference standard PDL1-VNAPYNK (C<sup>13</sup> N<sup>15</sup>)**
- 4. C10orf54 (VISTA) HPLSYVAQR; reference standard CD3G- WNLGSNAK (C<sup>13</sup> N<sup>15</sup>)**
- 5. CD40 YCDPNLGLR; reference standard PD1-LAAFPEDR (C<sup>13</sup> N<sup>15</sup>)**



**melanoma  
endogenous**  
YILIPASQQPK



**synthetic  
standard**  
YILIPASQQPK

# IDO1

## YILIPASQQPK

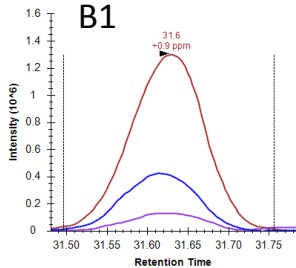
A representative PRM transitions trace for the peptide YILIPASQQPK is shown at left, along with that for a synthetic standard (unlabeled) YILIPASQQPK peptide.

For quantitation by the LRP method the summed transitions for YILIPASQQPK were normalized to the summed transitions for the CD4 peptide ILGNQGSFLTK (13C15N), which eluted in the same bRPLC fraction. See traces for each sample on the following 3 pages.

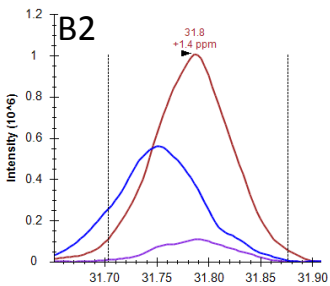
# IDO1 endogenous = YILIPASQQPK reference standard = CD4-ILGNQGSFLTK (C<sup>13</sup> N<sup>15</sup>)

Endogenous

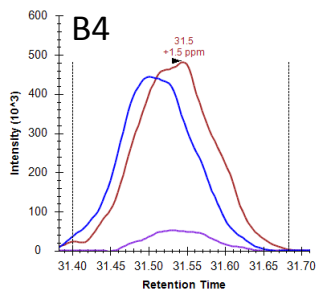
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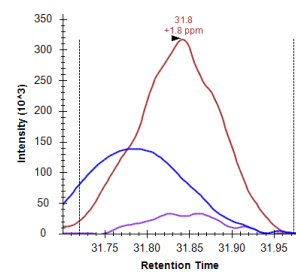
ma\_set\_SCH\_B13\_F9 | 160916\_22melanoma\_set\_SCH\_B2\_F9



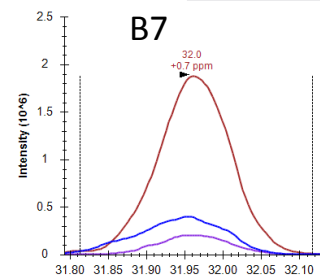
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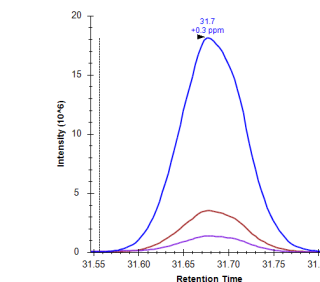
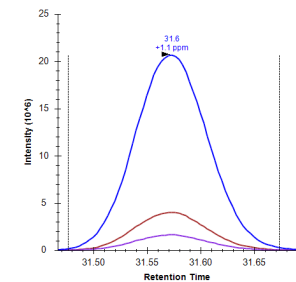
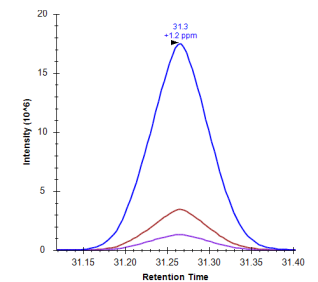
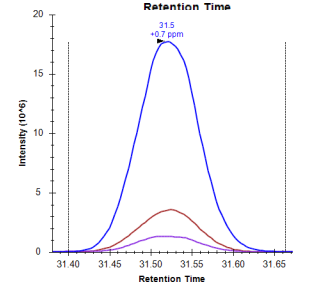
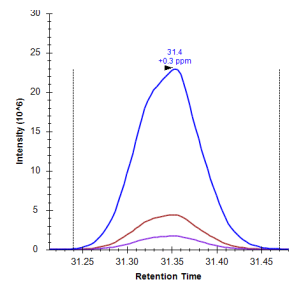
oma\_set\_SCH\_B5\_F8 | 160916\_22melanoma\_set\_SCH\_B5\_F9



160916\_22melanoma\_set\_SCH\_B7\_F9 | 160916\_22melanoma\_

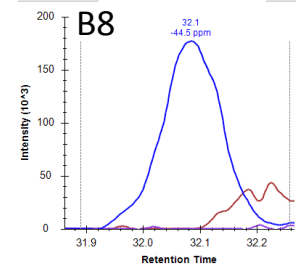


Standard Ref

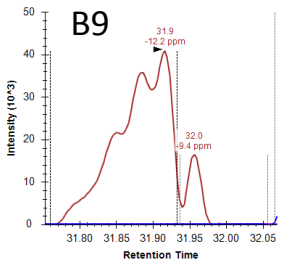


Endogenous

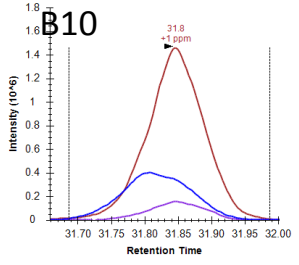
oma\_set\_SCH\_B8\_F8 | 160916\_22melanoma\_set\_SCH\_B8\_F9



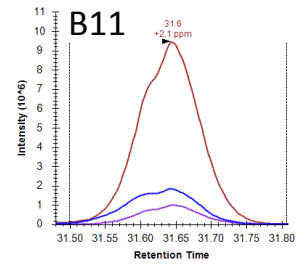
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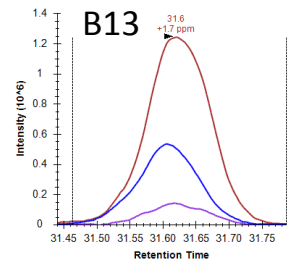
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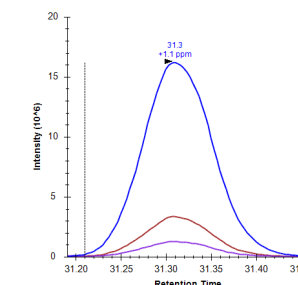
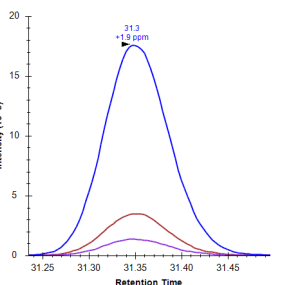
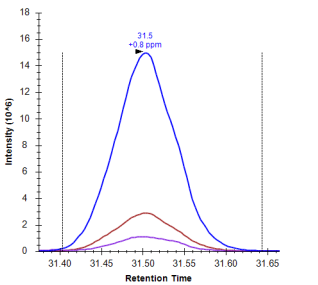
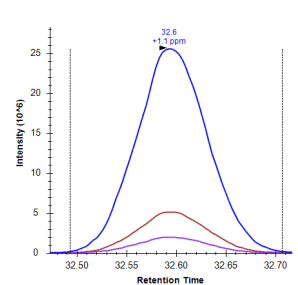
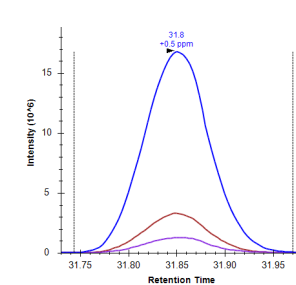
ma\_set\_SCH\_B11\_F8 | 160916\_22melanoma\_set\_SCH\_B11\_F9



ma\_set\_SCH\_B13\_F8 | 160916\_22melanoma\_set\_SCH\_B13\_F9

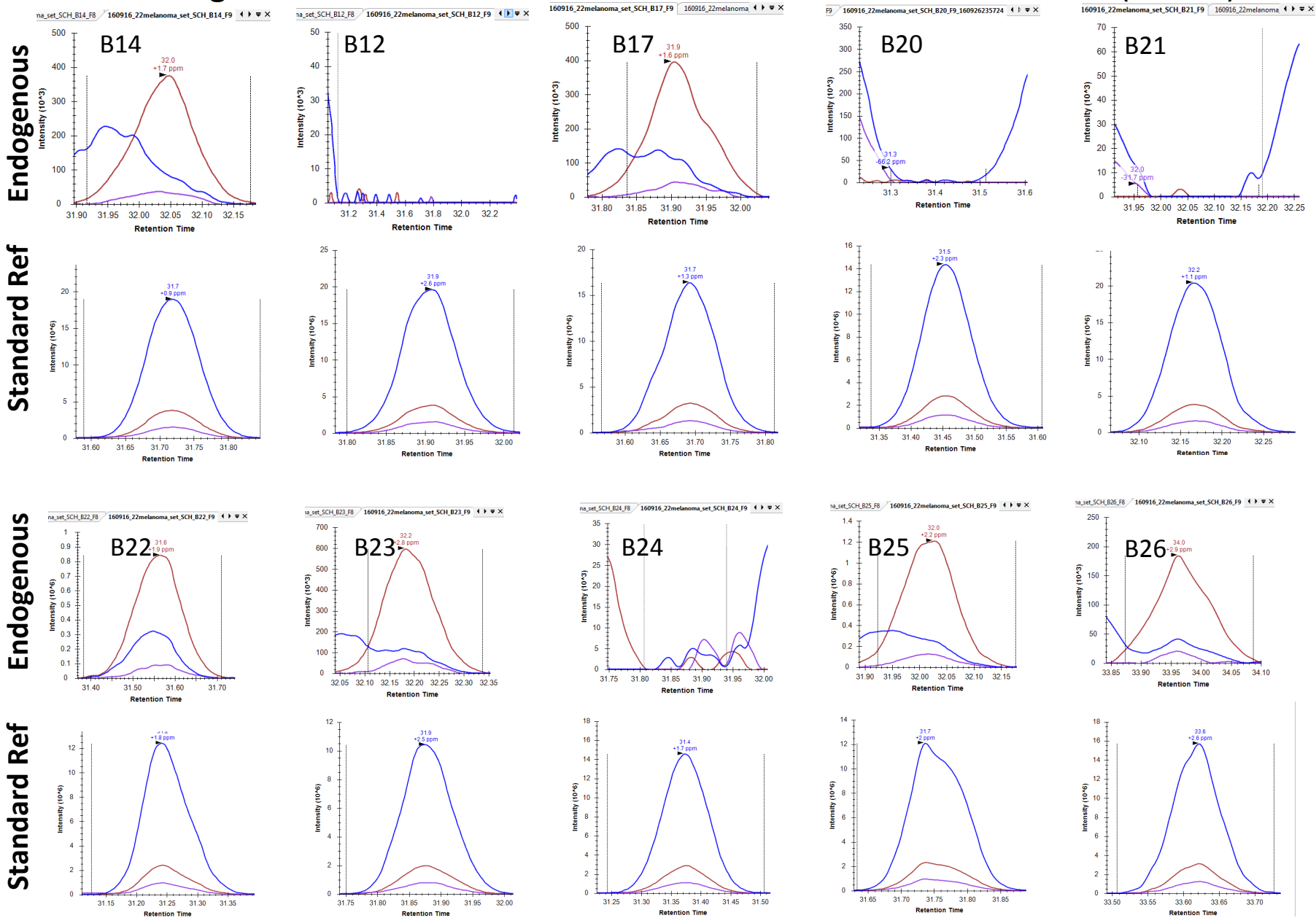


Standard Ref



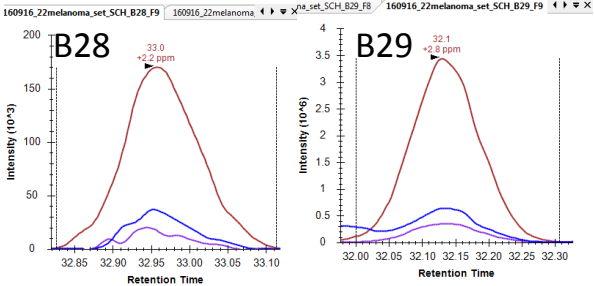


# IDO1 endogenous= YILIPASQQPK reference standard = CD4-ILGNQGSFLTK (C<sup>13</sup> N<sup>15</sup>)

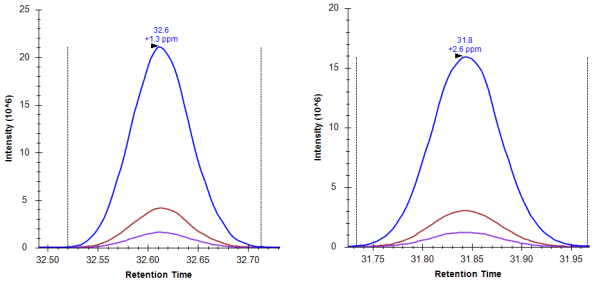


IDO1 endogenous= YILIPASQQPK reference standard = CD4-ILGNQGSFLTK (C<sup>13</sup> N<sup>15</sup>)

Endogenous

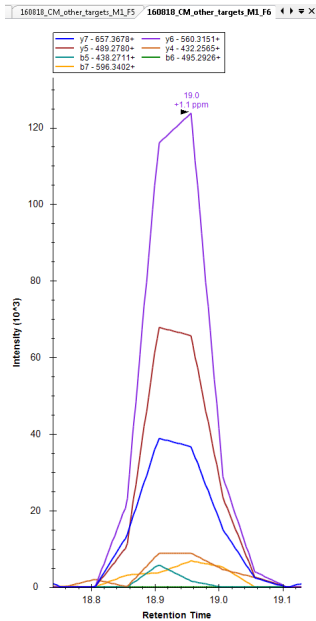


Standard Ref

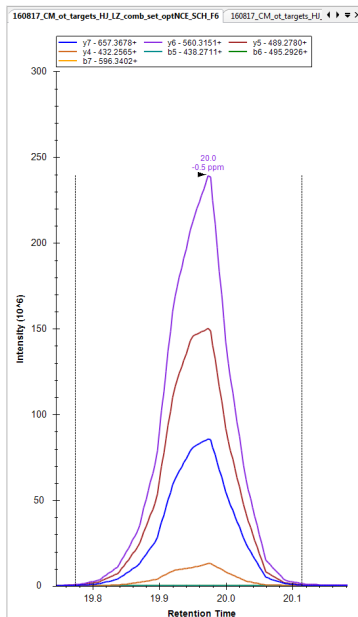


# LAG3

## LPAGVGTR



**melanoma  
endogenous  
LPAGVGTR**



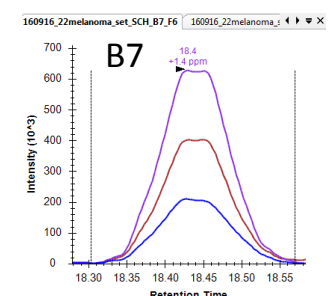
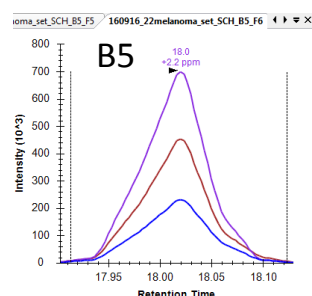
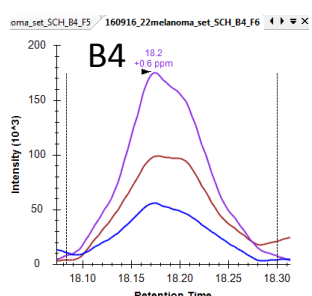
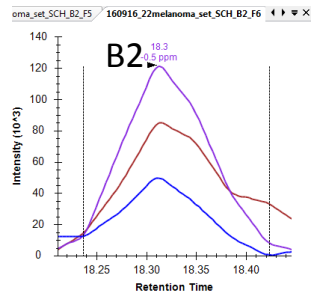
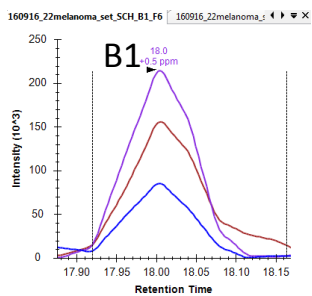
**synthetic  
standard  
LPAGVGTR**

A representative PRM transitions trace for the peptide LPAGVGTR is shown at left, along with that for a synthetic standard (unlabeled) LPAGVGTR peptide.

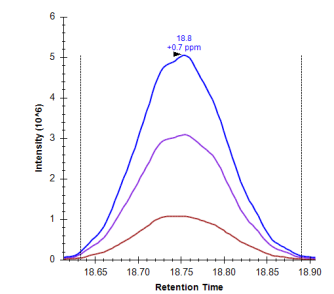
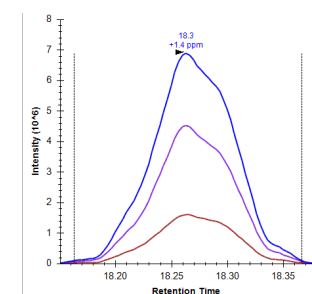
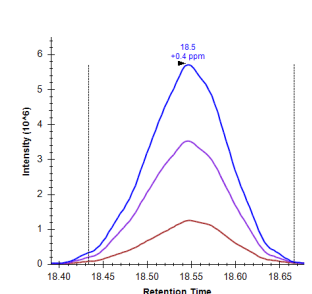
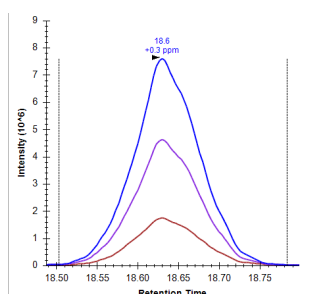
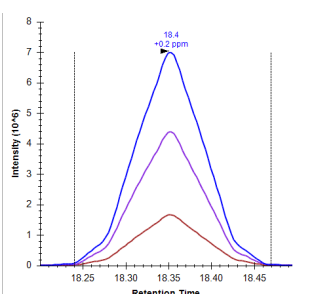
For quantitation by the LRP method the summed transitions for LPAGVGTR were normalized to the summed transitions for the PD-L1 peptide LQDAGVYR ( $^{13}\text{C}^{15}\text{N}$ ), which eluted in the same bRPLC fraction. See traces for each sample on the following 3 pages.

# LAG3 endogenous= LPAGVGTR reference standard = PDL1-LQDAGVYR (C<sup>13</sup> N<sup>15</sup>)

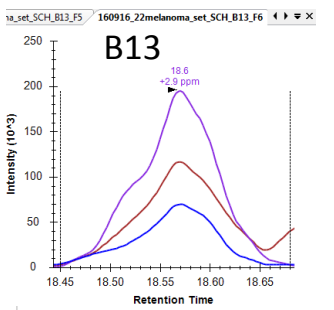
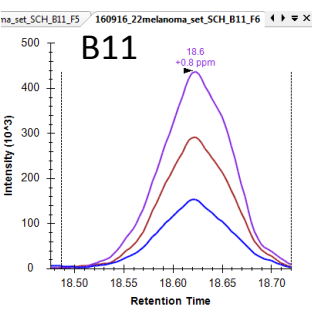
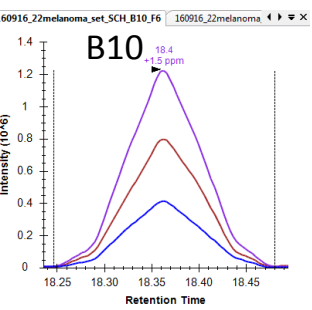
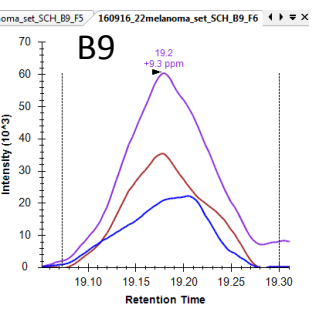
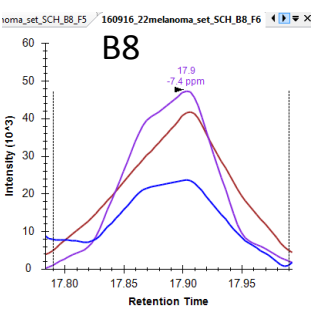
Endogenous



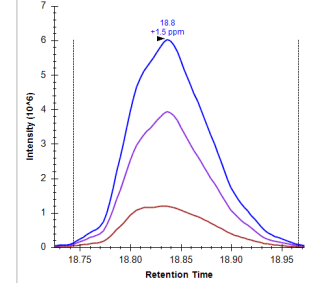
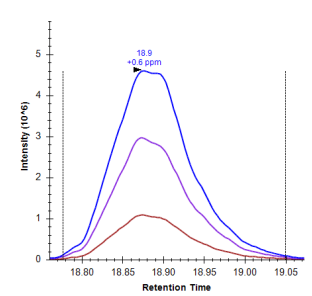
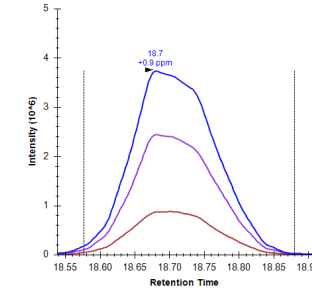
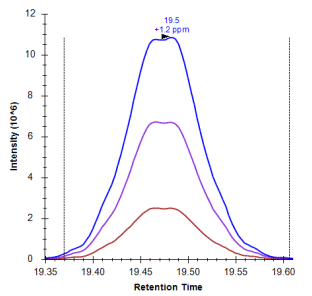
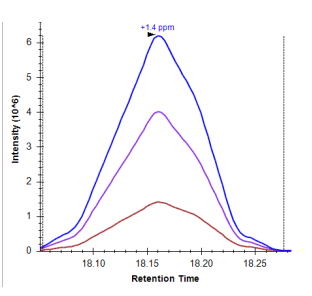
Standard Ref



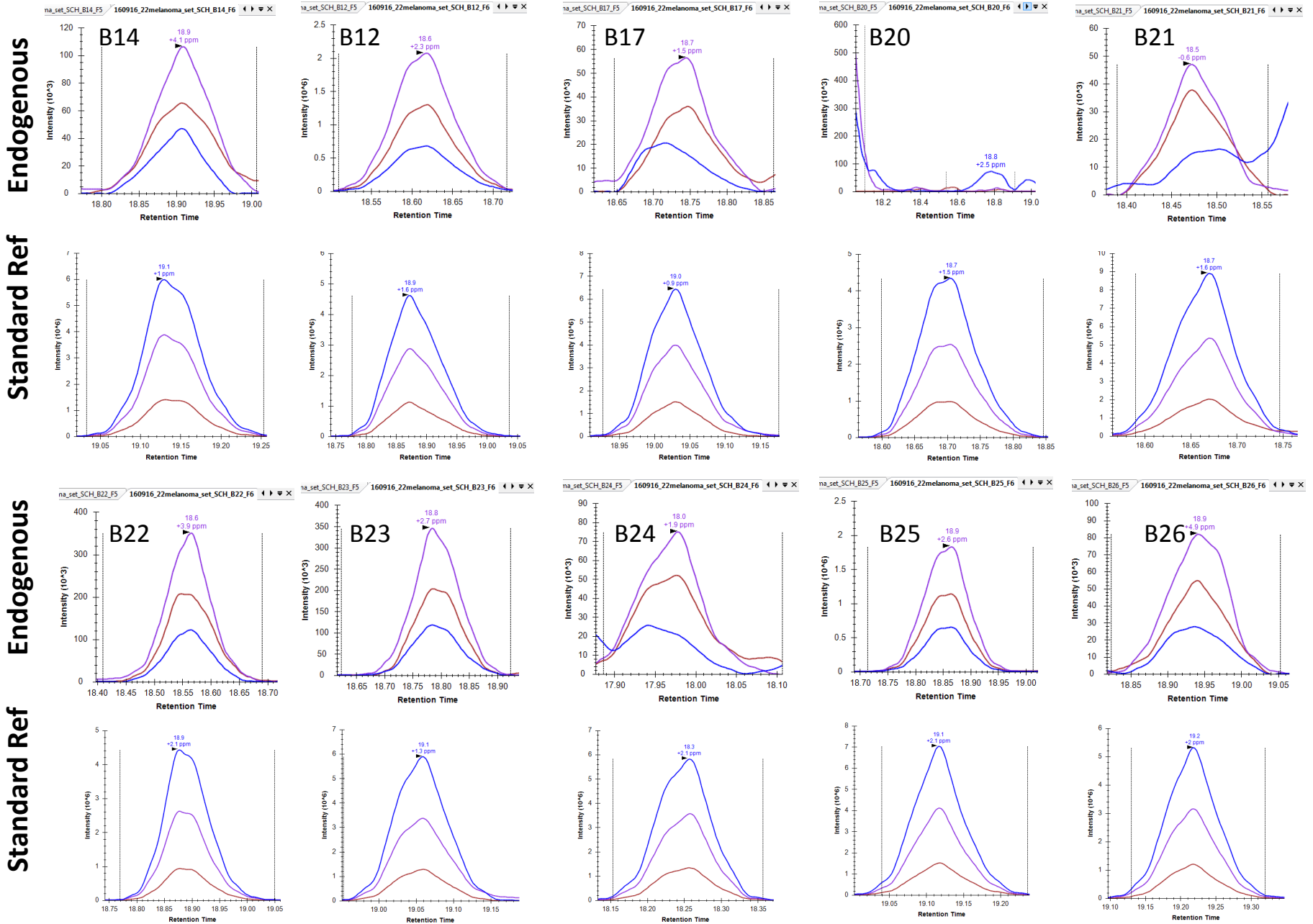
Endogenous



Standard Ref

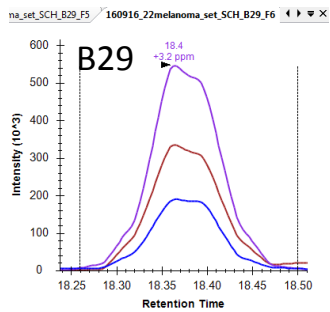
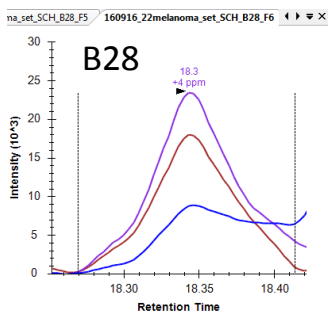


# LAG3 endogenous= LPAGVGTR reference standard = PDL1-LQDAGVYR (C<sup>13</sup> N<sup>15</sup>)

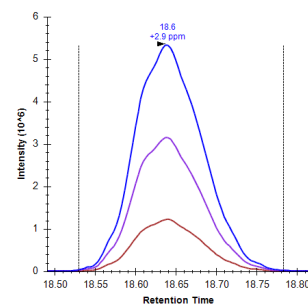
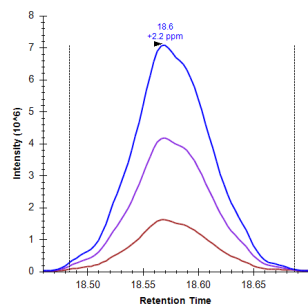


# LAG3 endogenous= LPAGVGTR reference standard = PDL1-LQDAGVYR (C<sup>13</sup> N<sup>15</sup>)

Endogenous



Standard Ref



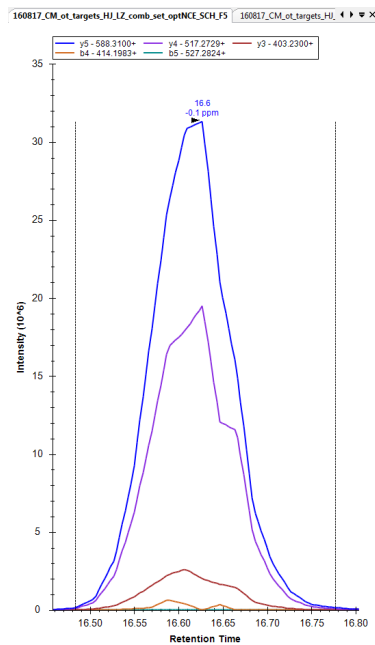
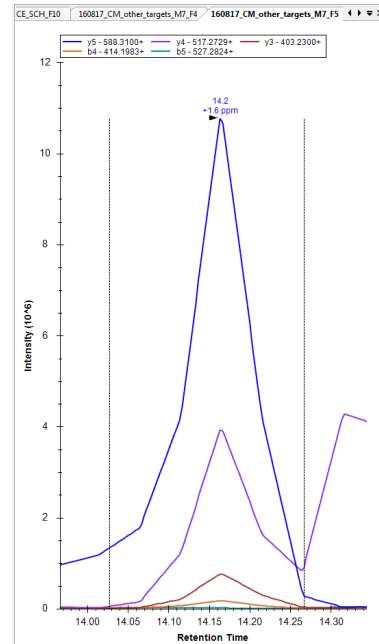
# HAVCR2 (TIM-3)

## LANDLR

melanoma  
endogenous

LANDLR

A representative PRM transitions trace for the peptide LANDLR is shown at left, along with that for a synthetic standard (unlabeled) LANDLR peptide.

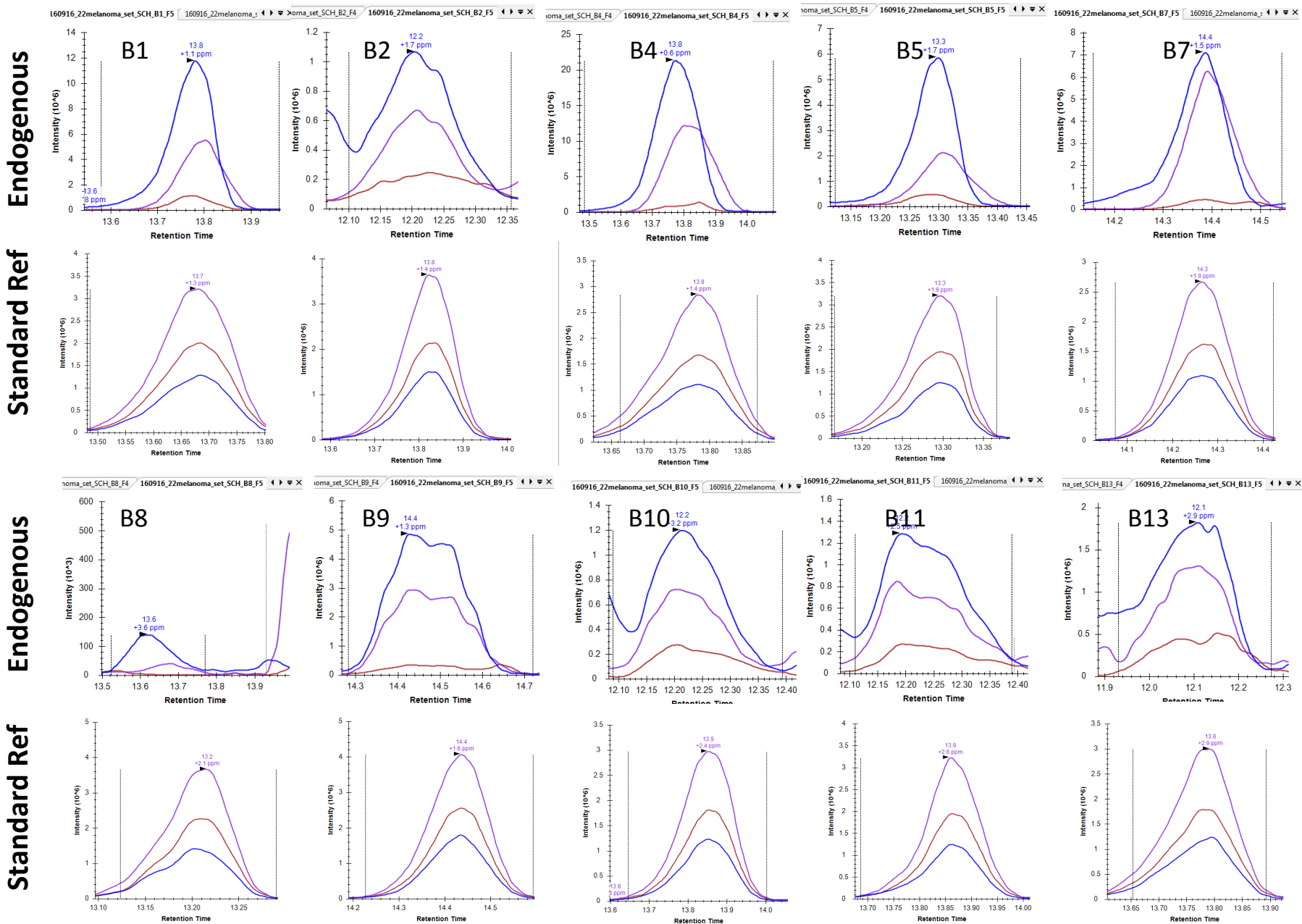


synthetic  
standard

LANDLR

For quantitation by the LRP method the summed transitions for LANDLR were normalized to the summed transitions for the PD-L1 peptide VNAPYNK (13C15N), which eluted in the same bRPLC fraction. See traces for each sample on the following 3 pages.

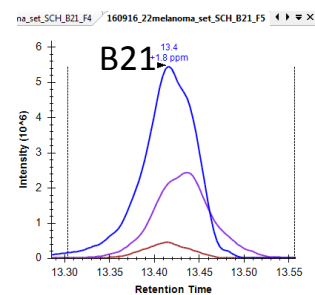
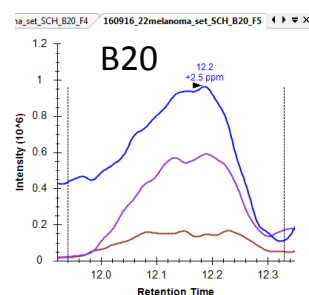
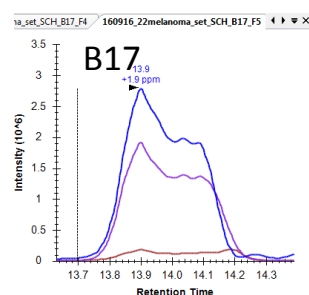
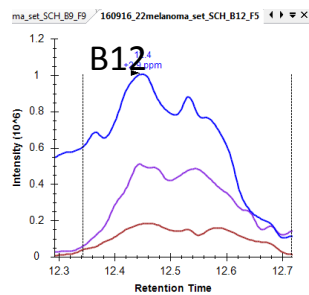
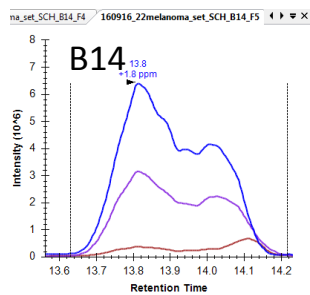
# HAVCR2 (TIM-3) endogenous = LANDLR reference standard = PDL1-VNAPYNK (C<sup>13</sup> N<sup>15</sup>)



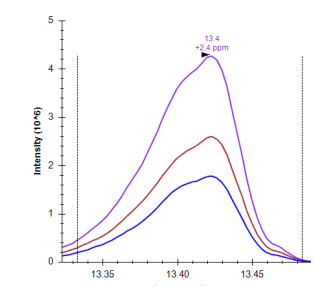
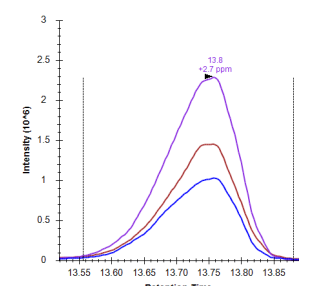
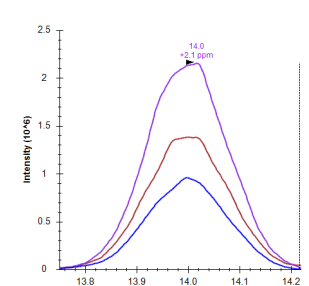
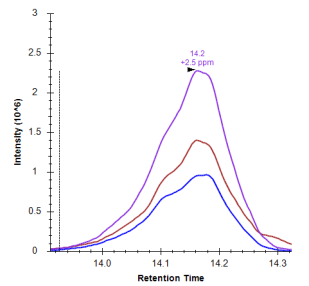
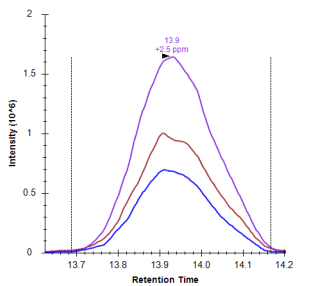


# HAVCR2 (TIM-3) endogenous= LANDLR reference standard = PDL1-VNAPYNK (C<sup>13</sup> N<sup>15</sup>)

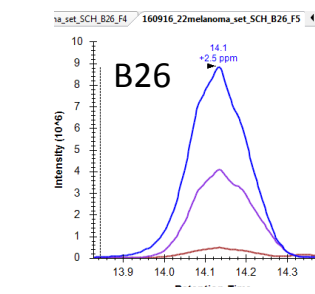
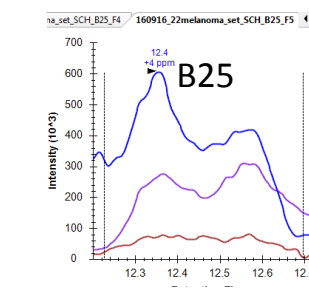
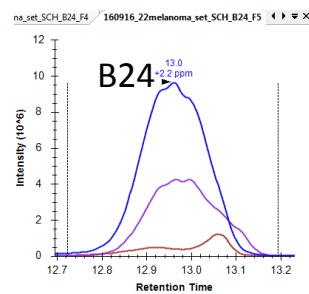
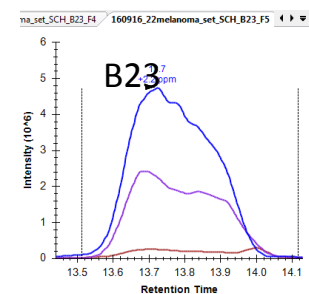
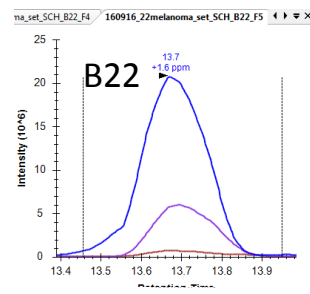
Endogenous



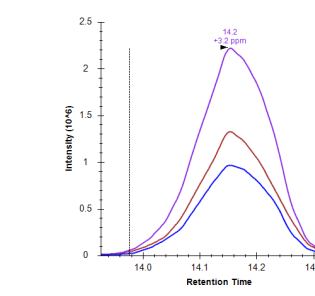
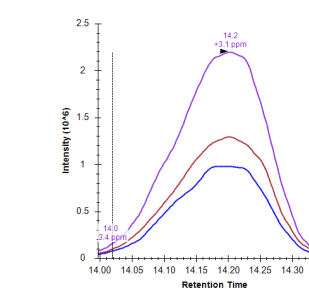
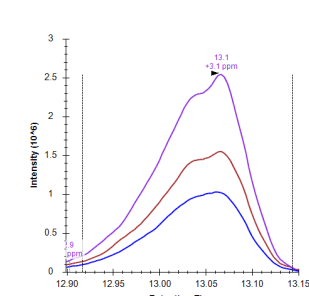
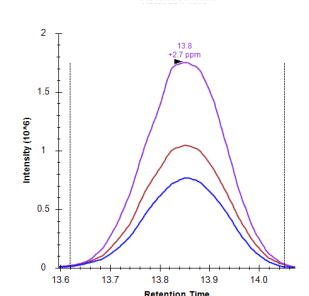
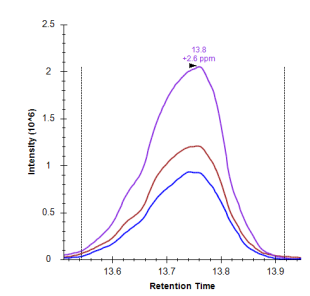
Standard Ref



Endogenous

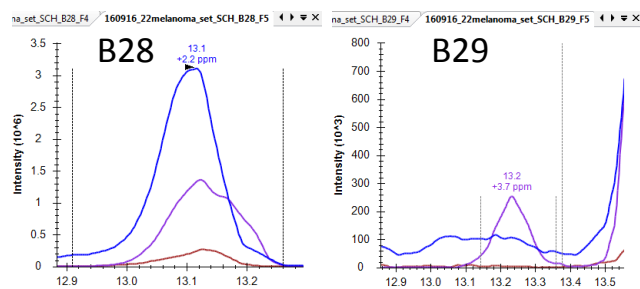


Standard Ref

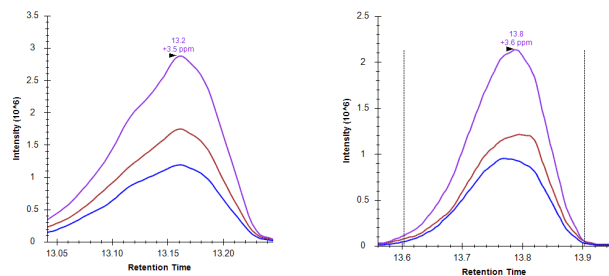


# HAVCR2 (TIM-3) endogenous = LANDLR reference standard = PDL1-VNAPYNK (C<sup>13</sup> N<sup>15</sup>)

Endogenous



Standard Ref

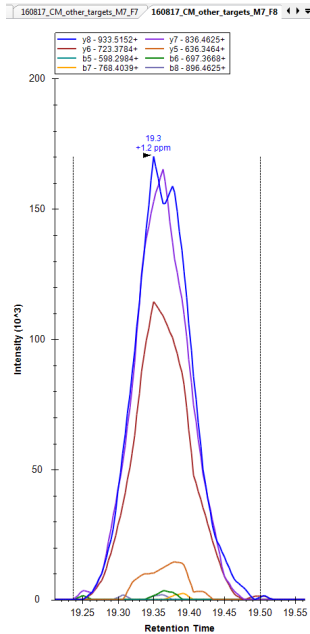


# C10orf54 (VISTA)

## HPLSYVAQR

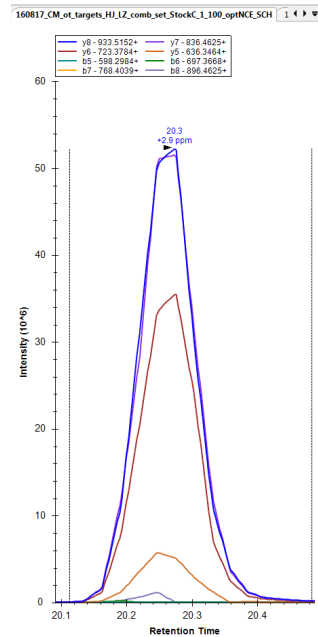
A representative PRM transitions trace for the peptide HPLSYVAQR is shown at left, along with that for a synthetic standard (unlabeled) HPLSYVAQR peptide.

For quantitation by the LRP method the summed transitions for HPLSYVAQR were normalized to the summed transitions for the CD3G peptide WNLGSNAK ( $^{13}\text{C}^{15}\text{N}$ ), which eluted in the same bRPLC fraction. See traces for each sample on the following 3 pages.



melanoma  
endogenous

HPLSYVAQR

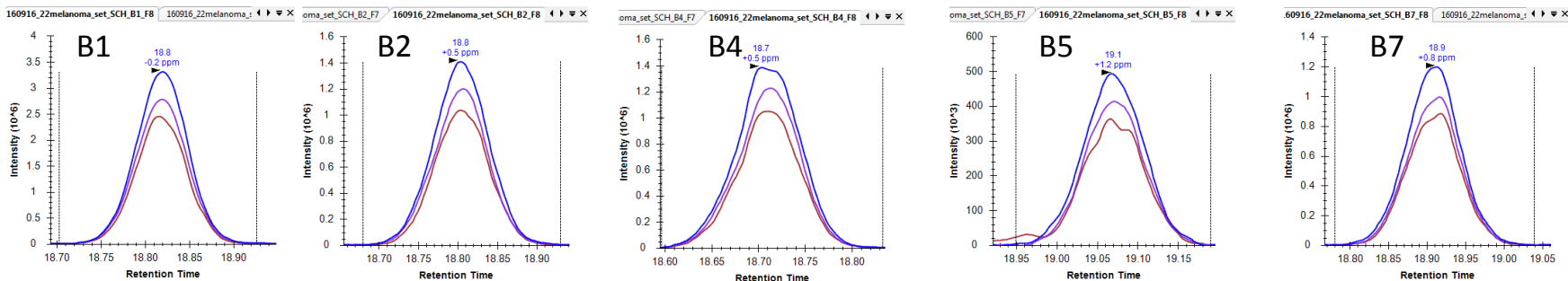


synthetic  
standard

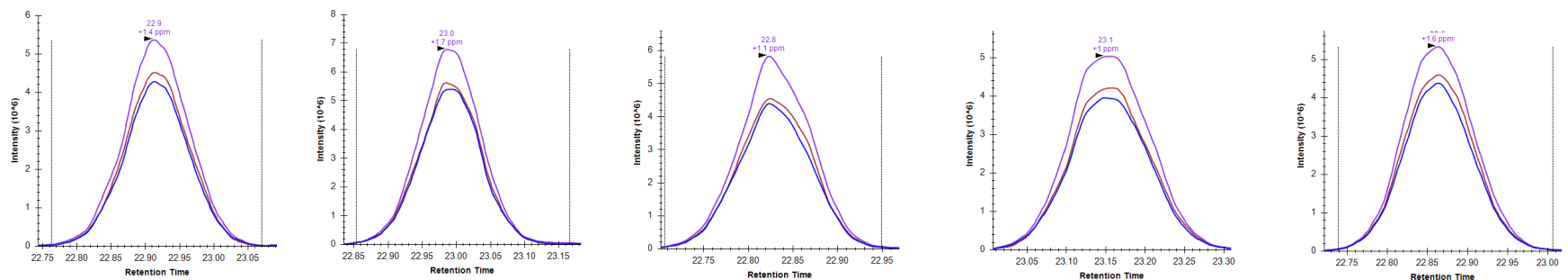
HPLSYVAQR

# C10orf54 (VISTA) = HPLSYVAQR reference standard = CD3G- WNLGSNAK (C<sup>13</sup> N<sup>15</sup>)

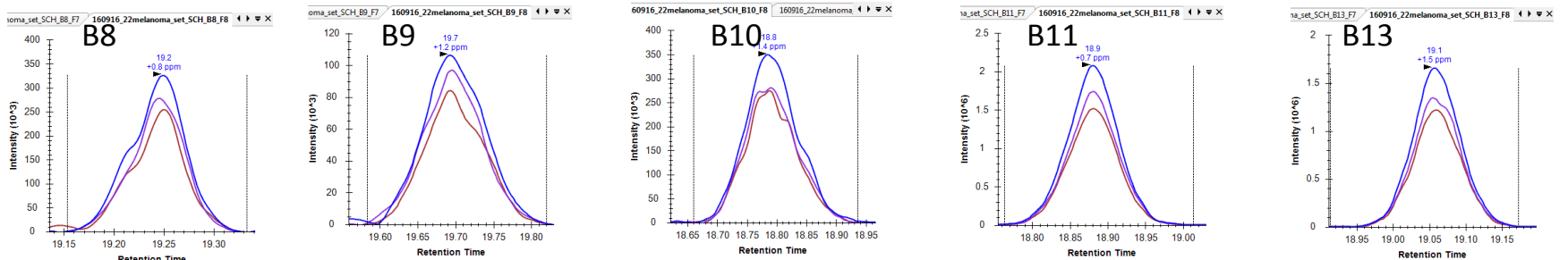
Endogenous



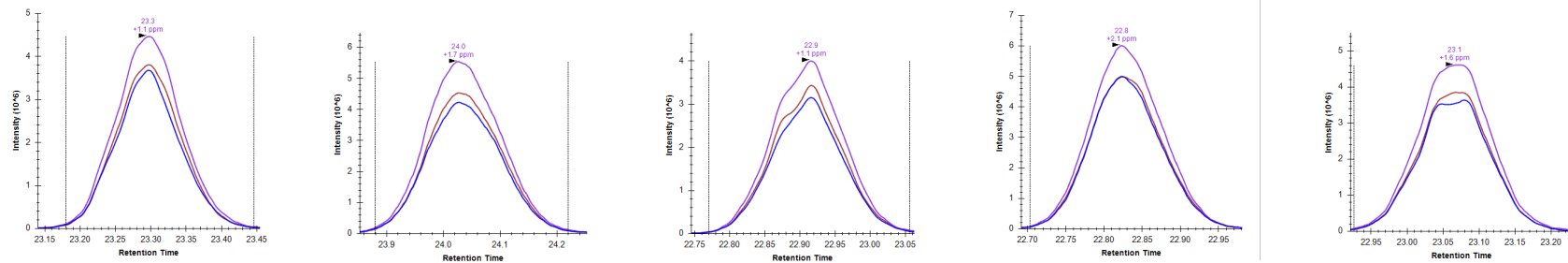
Standard Ref



Endogenous

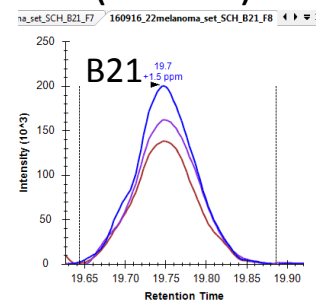
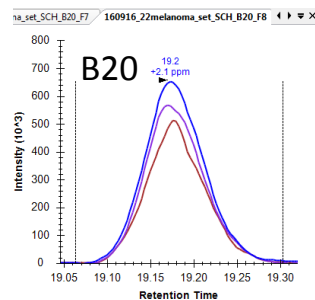
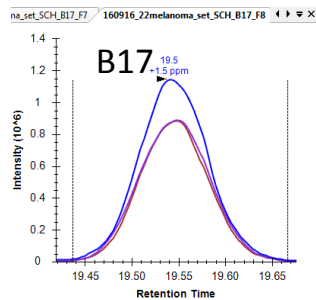
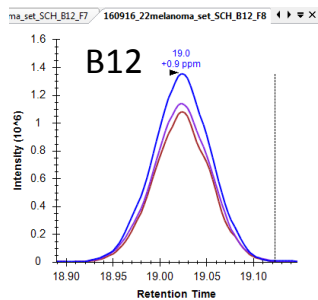
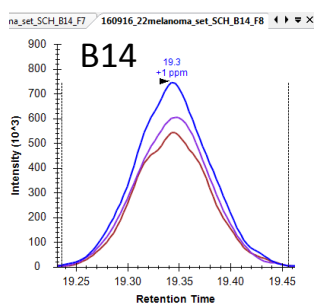


Standard Ref

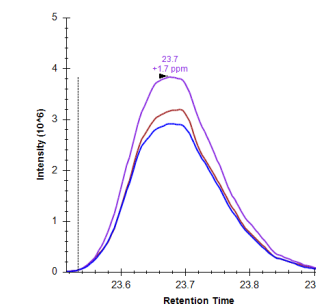
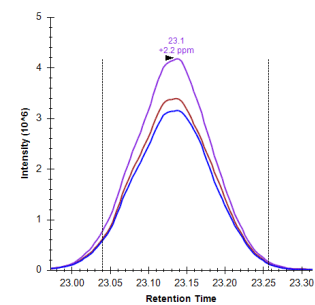
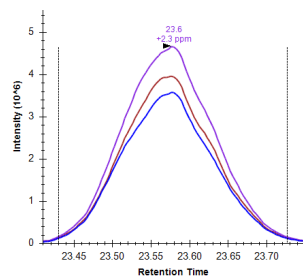
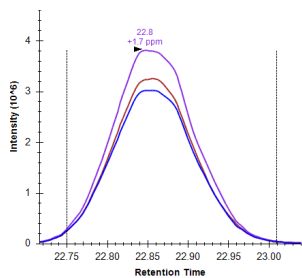
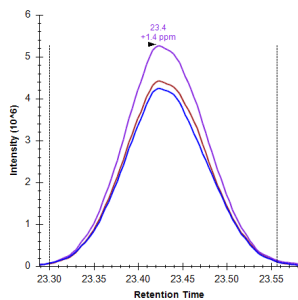


# C10orf54 (VISTA) = HPLSYVAQR reference standard = CD3G- WNLGSNAK (C<sup>13</sup> N<sup>15</sup>)

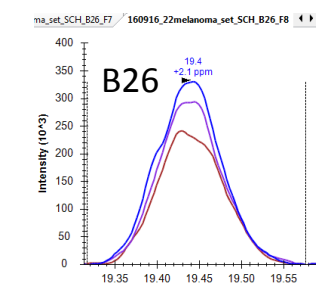
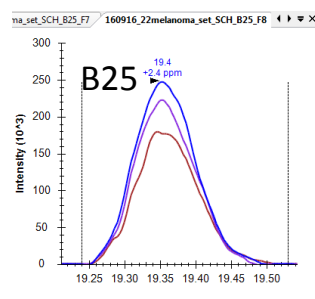
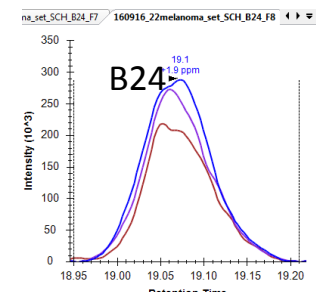
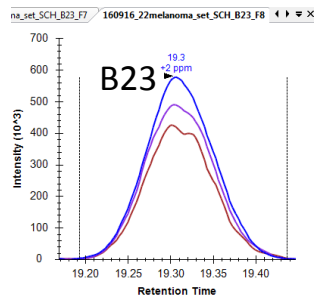
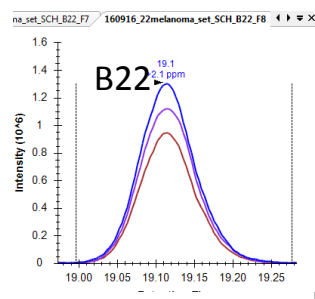
Endogenous



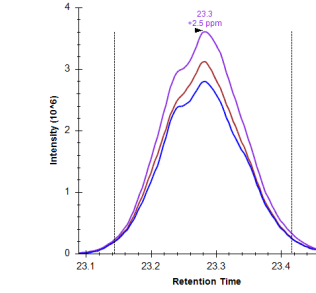
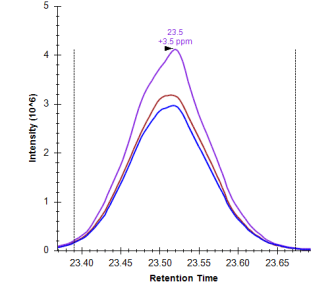
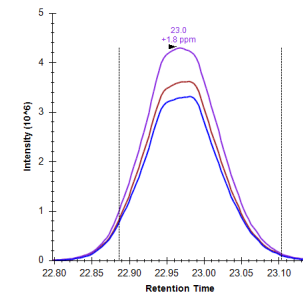
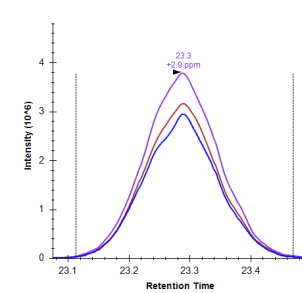
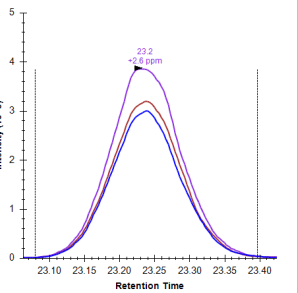
Standard Ref



Endogenous

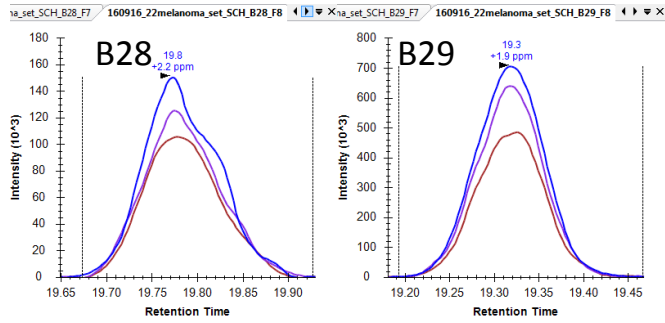


Standard Ref

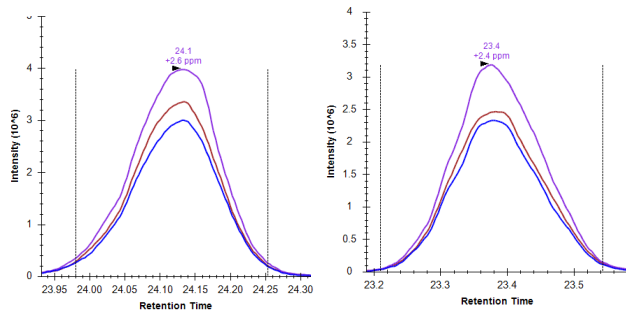


# C10orf54 (VISTA) = HPLSYVAQR reference standard = CD3G- WNLGSNAK (C<sup>13</sup> N<sup>15</sup>)

Endogenous



Standard Ref

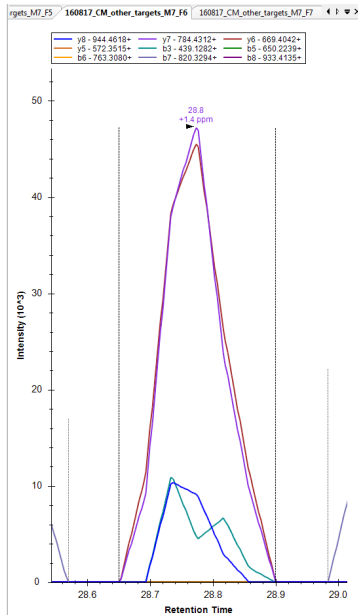


# CD40

## YC[+57]DPNLGLR

**melanoma  
endogenous**

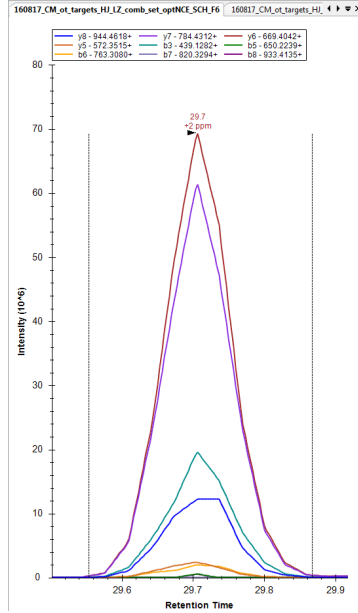
YC[+57]DPNLGLR



A representative PRM transitions trace for the peptide YC[+57]DPNLGLR is shown at left, along with that for a synthetic standard (unlabeled) YC[+57]DPNLGLR peptide.

**synthetic  
standard**

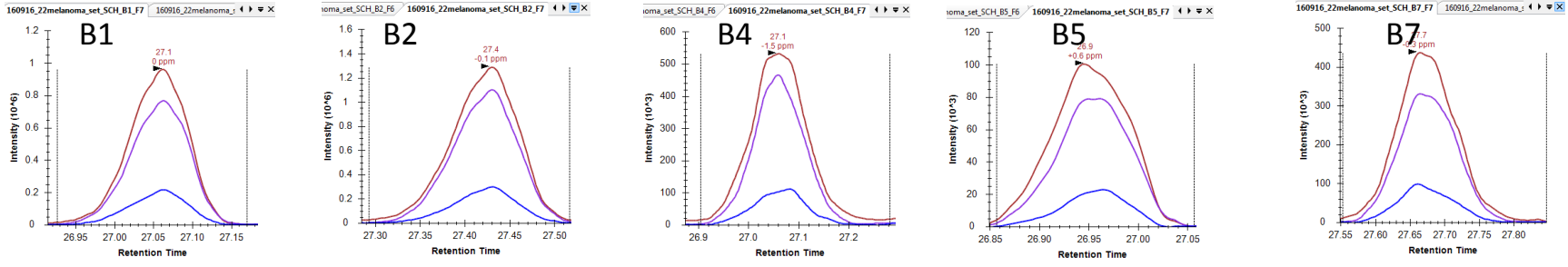
YC[+57]DPNLGLR



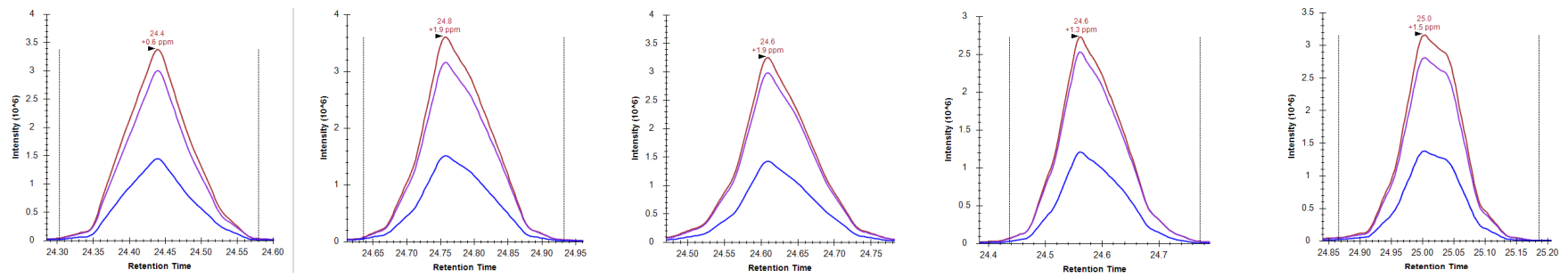
For quantitation by the LRP method the summed transitions for YC[+57]DPNLGLR were normalized to the summed transitions for the PD-1 peptide LAAFPEDR (13C15N), which eluted in the same bRPLC fraction. See traces for each sample on the following 3 pages.

# CD40 endogenous= YC[+57]DPNLGLR reference standard = PD1-LAAFPEDR (C<sup>13</sup> N<sup>15</sup>)

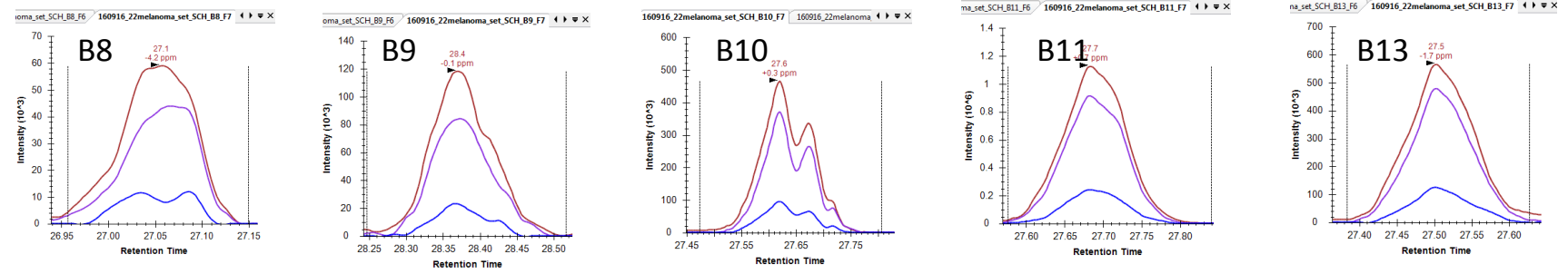
Endogenous



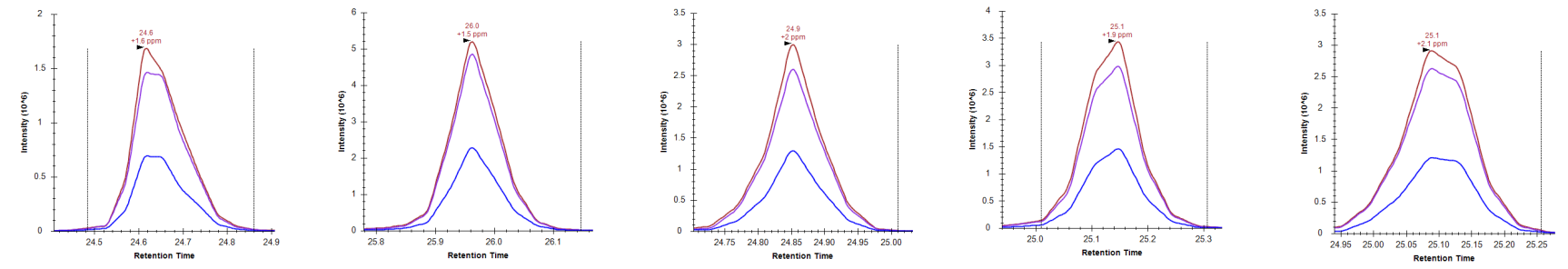
Standard Ref



Endogenous



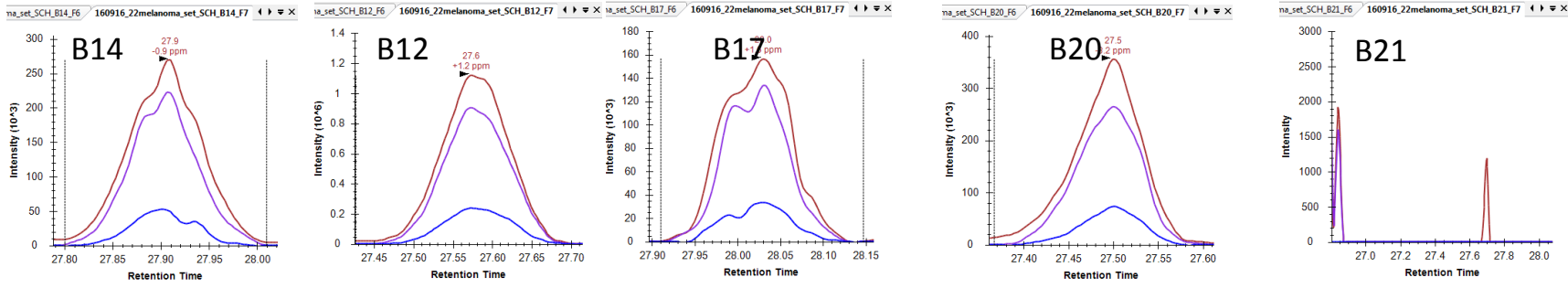
Standard Ref



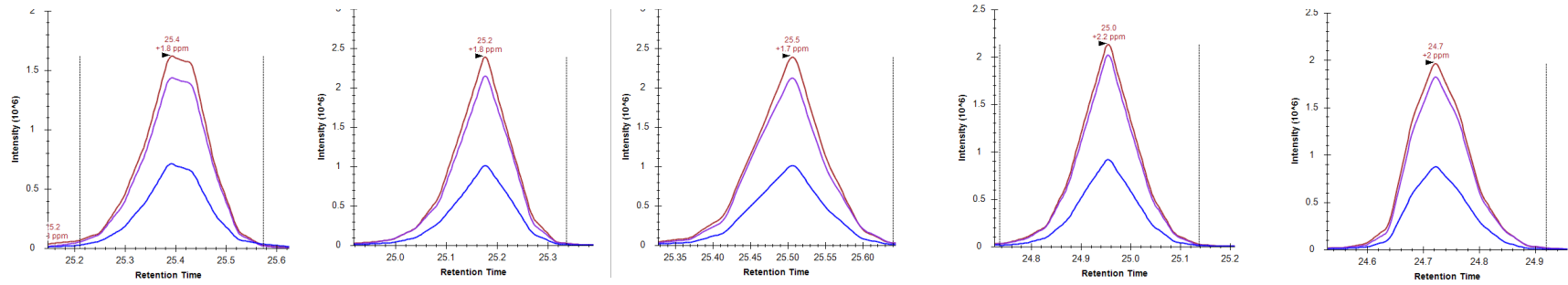


# CD40 endogenous= YC[+57]DPNLGLR reference standard = PD1-LAAFPEDR (C<sup>13</sup> N<sup>15</sup>)

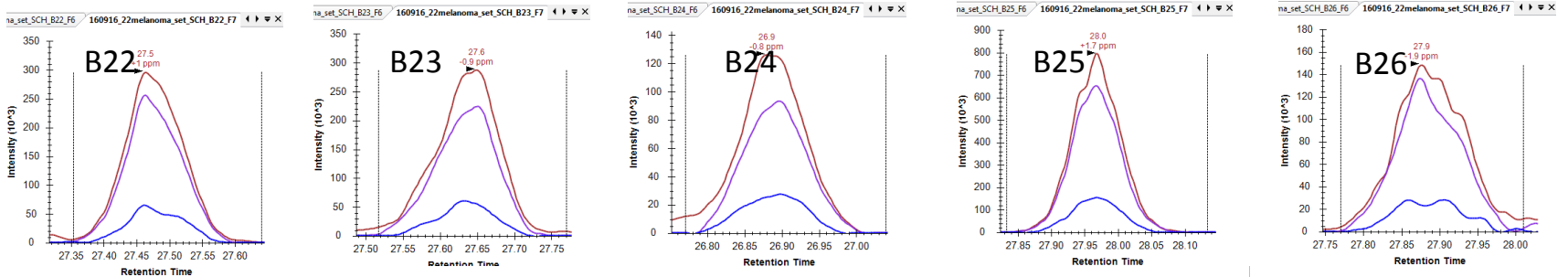
Endogenous



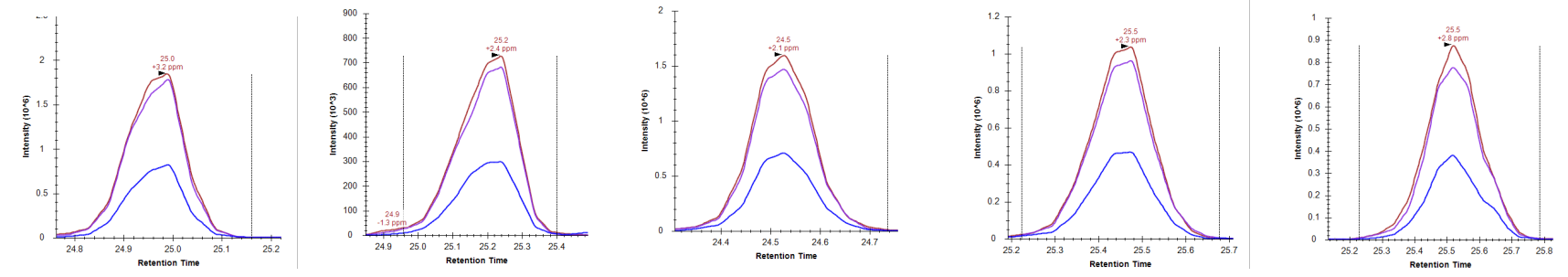
Standard Ref



Endogenous

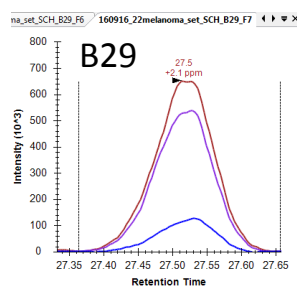
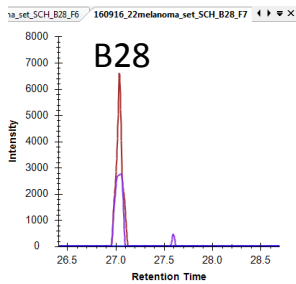


Standard Ref



# CD40 endogenous= YC[+57]DPNLGLR reference standard = PD1-LAAFPEDR (C<sup>13</sup> N<sup>15</sup>)

Endogenous



Standard Ref

