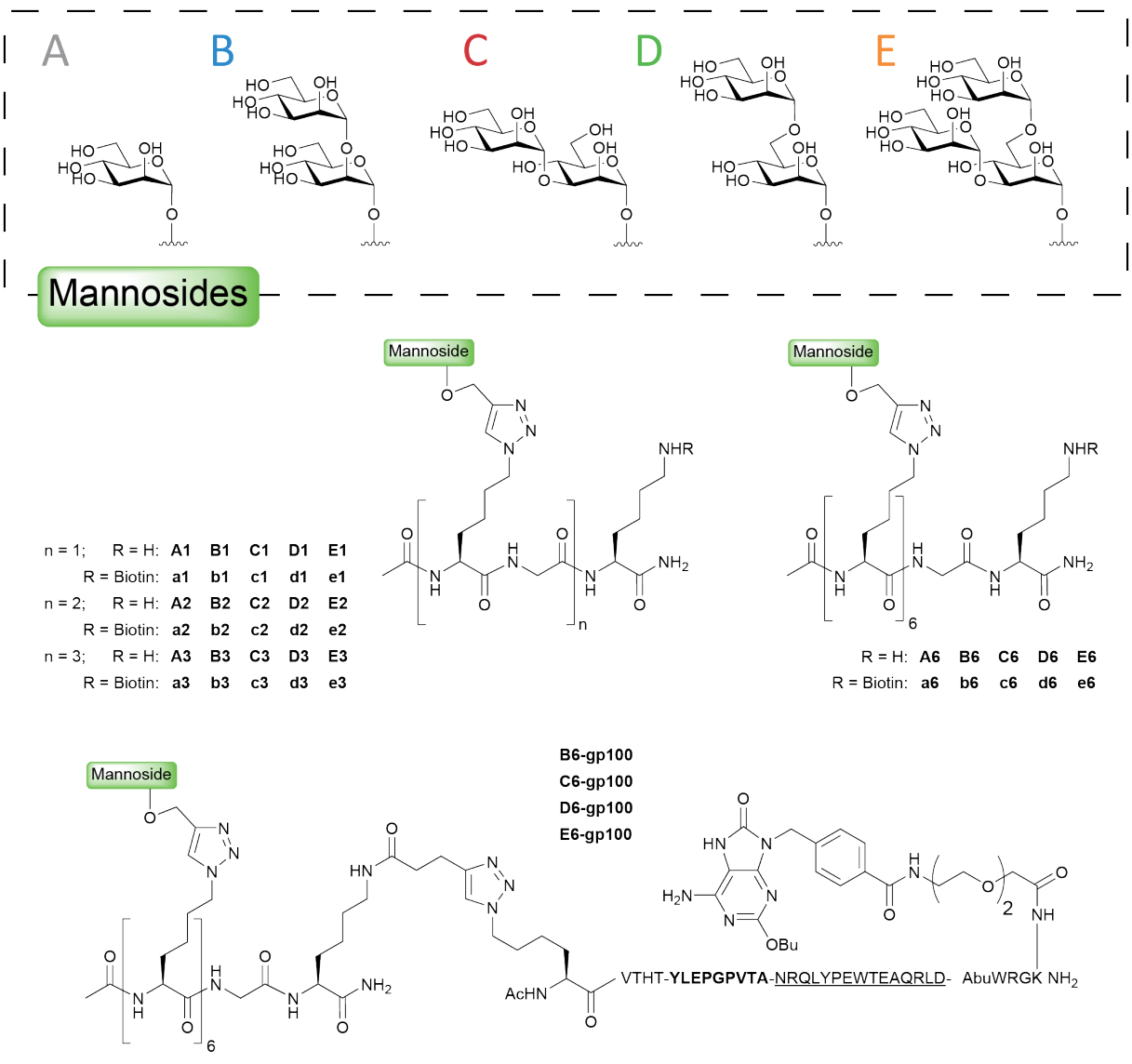
Targeting of the C-Type Lectin Receptor Langerin Using Bifunctional Mannosylated Antigens

Supplementary Material

Supplementary figures

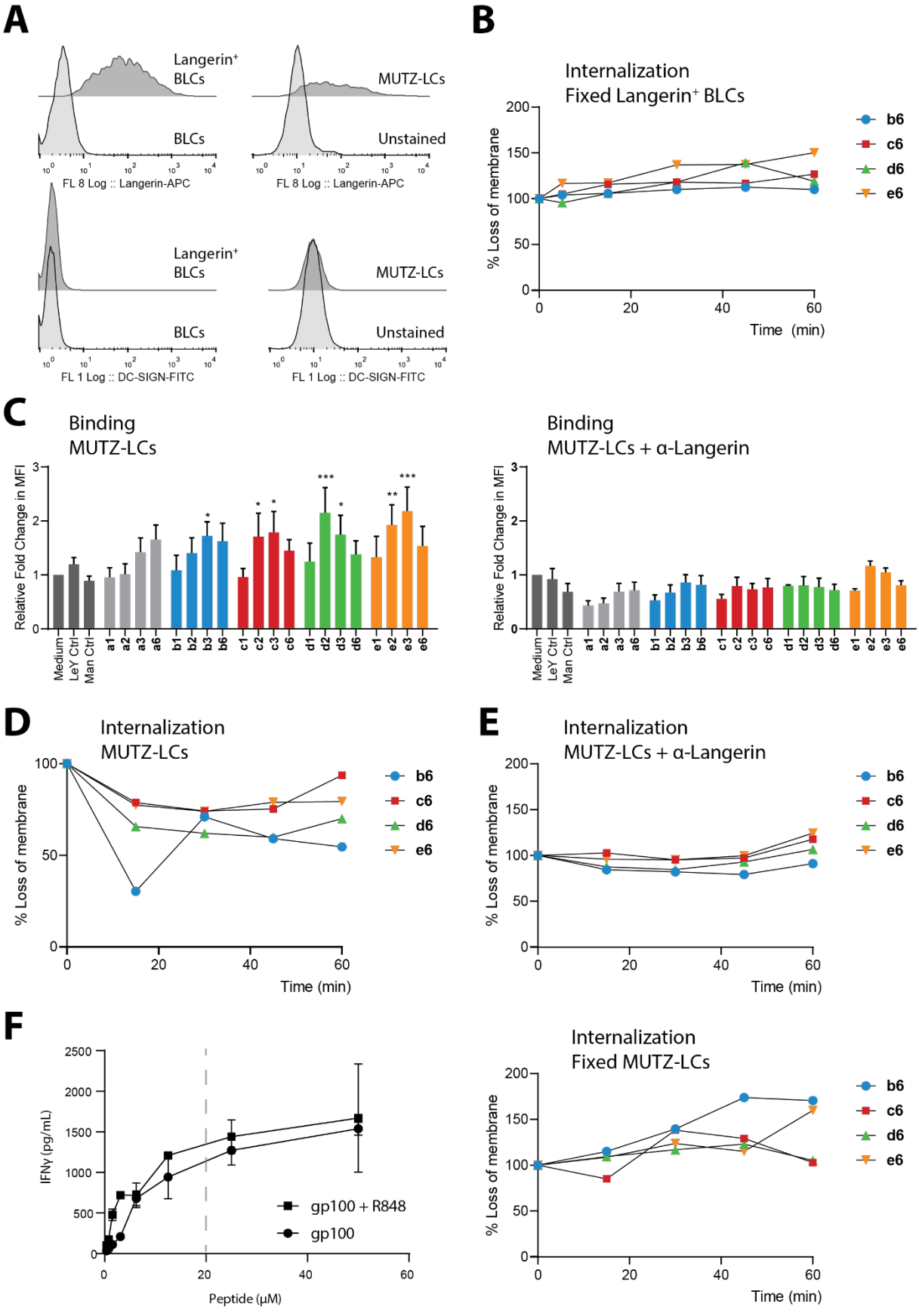
11 Supplementary Material

Supplementary Figure 1 | Schematic overview of the mannosylated compounds Five mannosides (upper panel) with 1, 2, 3, or 6 copy numbers were used to synthesize the library of 20 constructs (middle panel). The alkyne handle could also be functionalized with a biotin, resulting in a biotynilated cluster (coded with a lower case). In the lower panel, the clusters were functionalized with a gp100$_{280\text{-}288+44\text{-}59}$ antigen.



Supplementary Figure 2 | Langerin-, binding-, and internalization profiles of Langerin$^+$ BLCs and MUTZ-LCs
(A) Flowcytometric quantification of Langerin and DC-SIGN expression on the BLCs, Langerin transfected BLCs and MUTZ-LCs demonstrate only expression of Langerin in the Langerin$^+$ BLCs and MUTZ-LCs, and no expression of DC-SIGN. (B) The internalization of the hexavalent mannoside clusters by gently fixed Langerin$^+$ BLCs was measured by flow cytometry, and demonstrates no internalization. (C) Binding of biotin-functionalized clusters to MUTZ-LCs was measured by flow cytometry and normalized to medium (upper panel). The binding is inhibited by blocking with a Langerin blocking antibody (lower panel). (D) Internalization by MUTZ-LCs was measured by flow cytometry, and demonstrate a decrease of 20-45% hexavalent mannoside cluster at the cell membrane. (E) Blocking the Langerin receptor or gently fixing the MUTZ-LCs prevents internalization. One experiments is shown as a representative of 3. (F) The antigenic peptide gp100 was used for antigen presentation with and without a TLR stimulus. 20 μM was chosen because this concentration

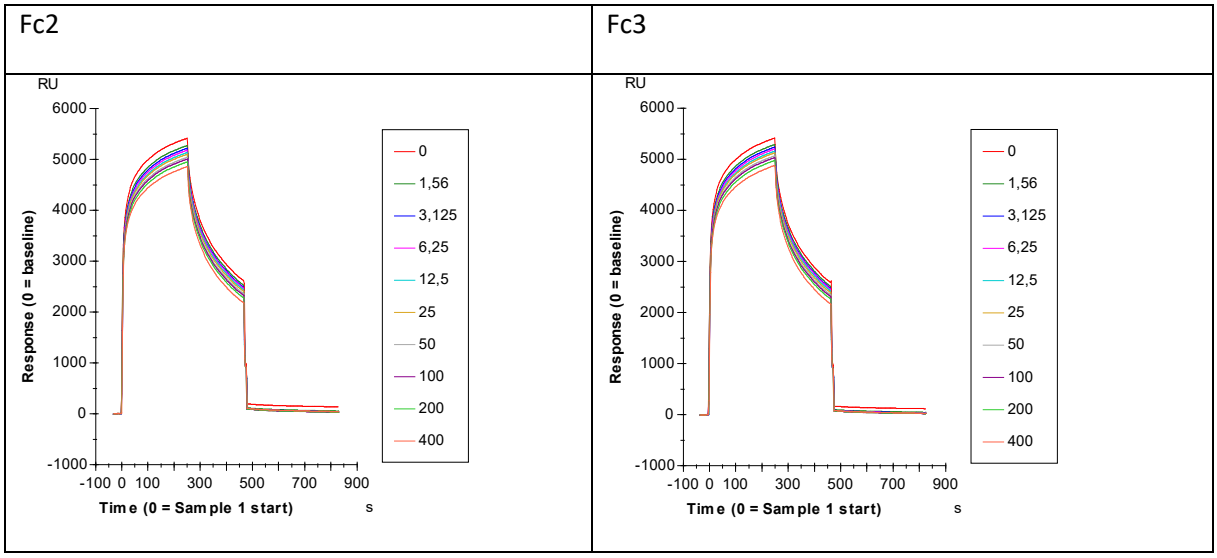
provides the largest experimental window.



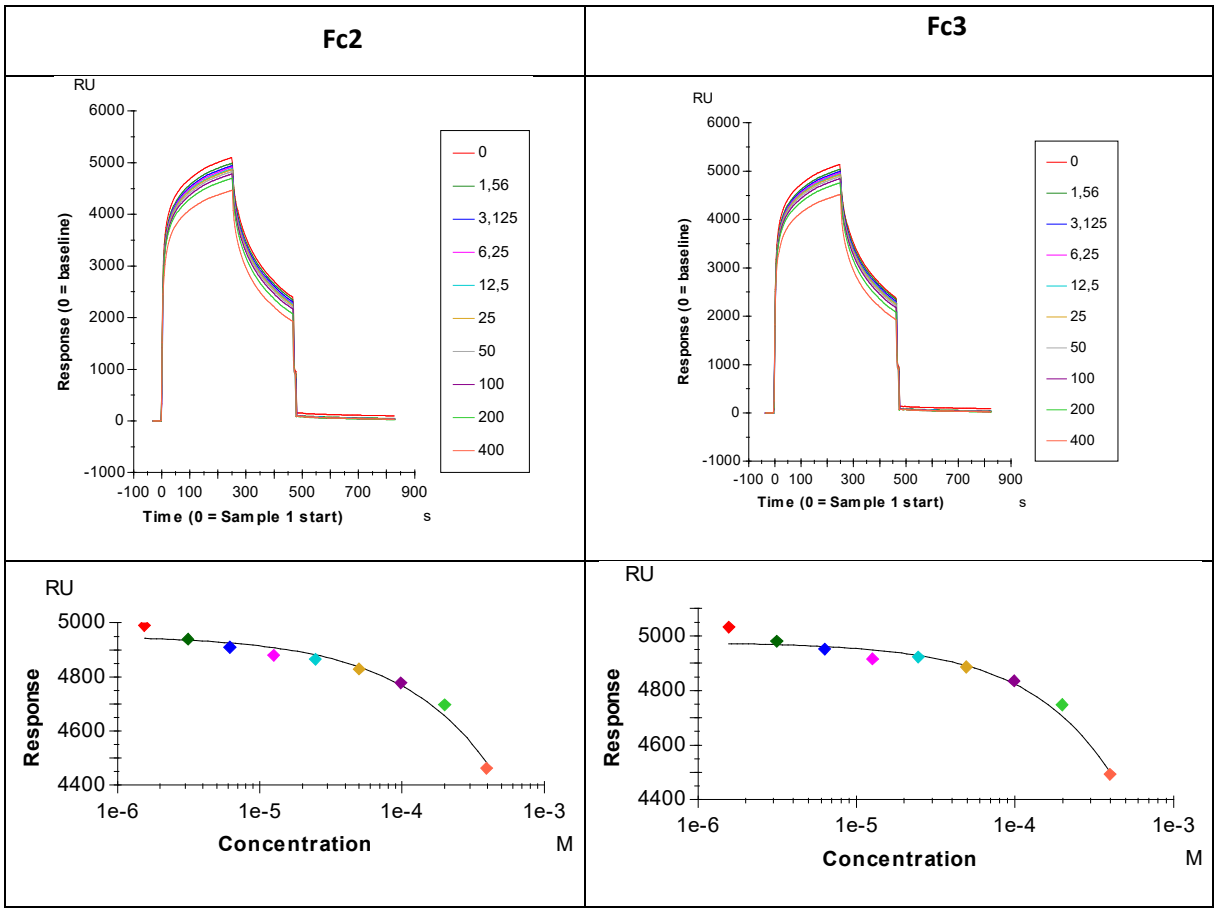
Supplementary Figure 3 | Competition assays (SPR)

It should be taken into consideration that Langerin interacts with the CM-dextran surface and, therefore, no reference subtraction will be done prior to the extraction of binding response.

A1 Langerin



B1 Langerin



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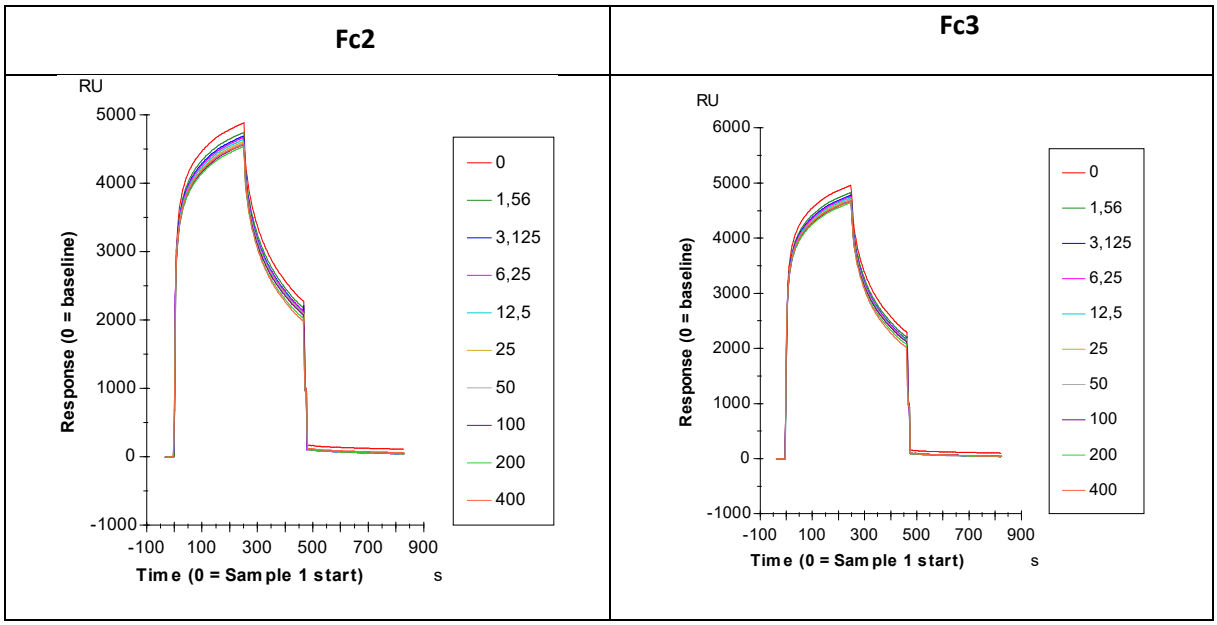
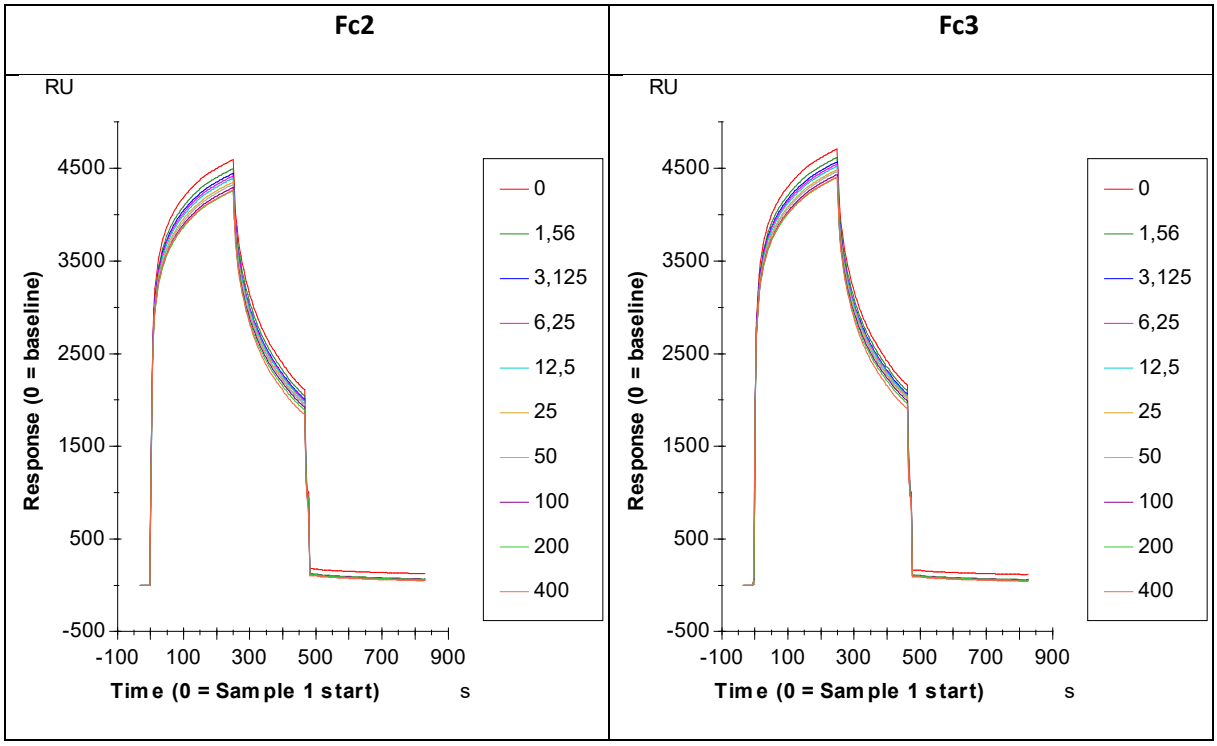
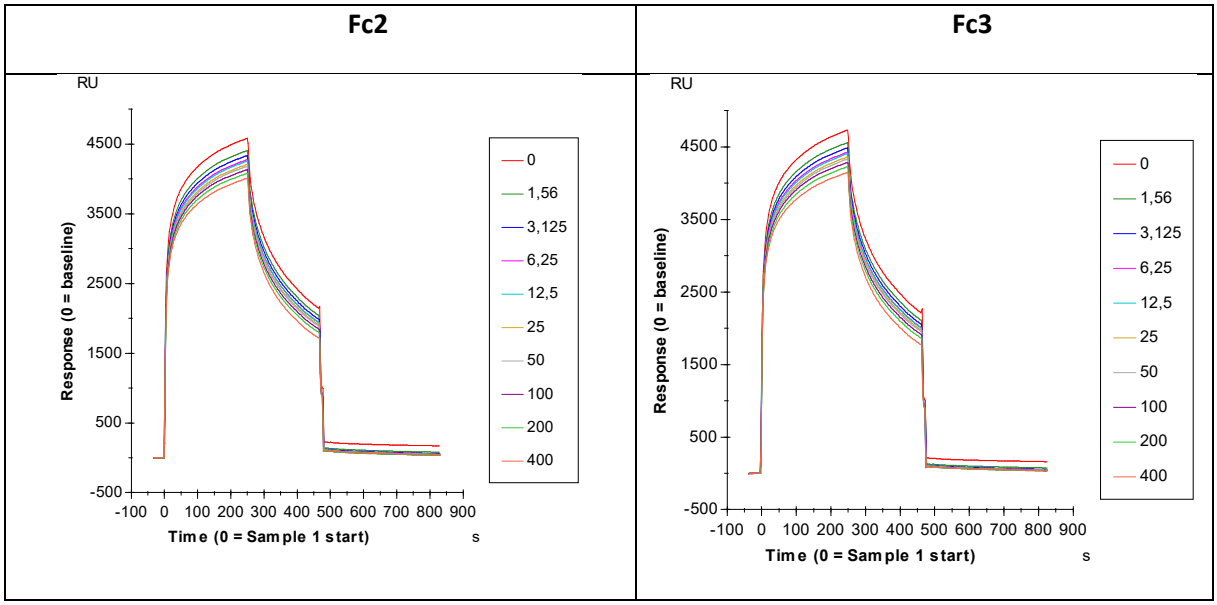

Fc2
Fc3
RU
Response (0 = baseline)
Time (0 = Sample 1 start)
s
0
1,56
3,125
6,25
12,5
25
50
100
200
400

D1 Langerin


Fc2
Fc3
RU
Response (0 = baseline)
Time (0 = Sample 1 start)
s
0
1,56
3,125
6,25
12,5
25
50
100
200
400

E1 Langerin



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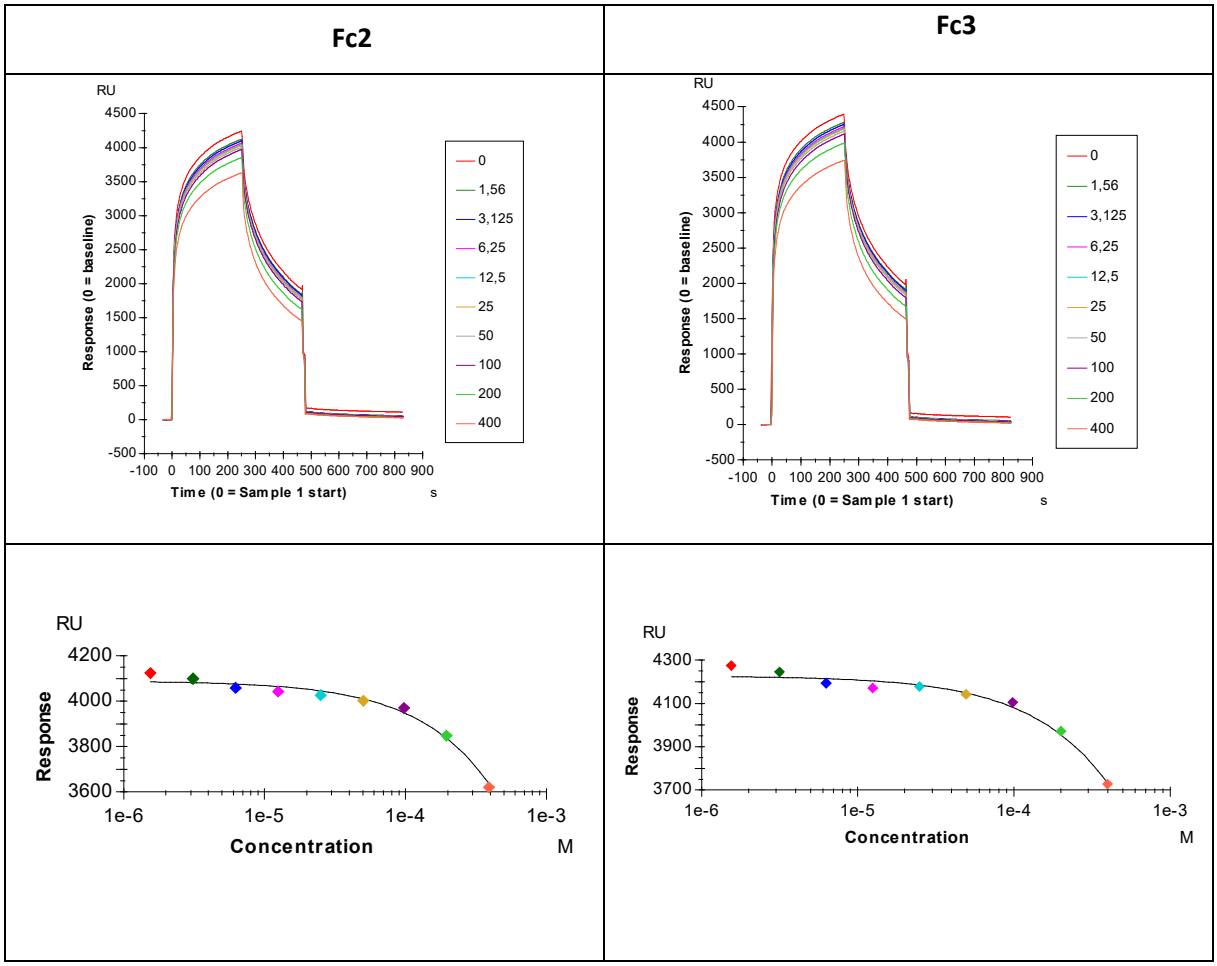
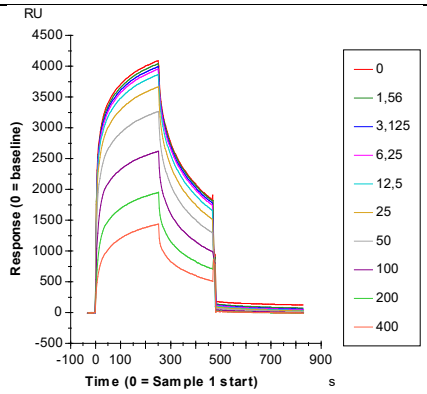
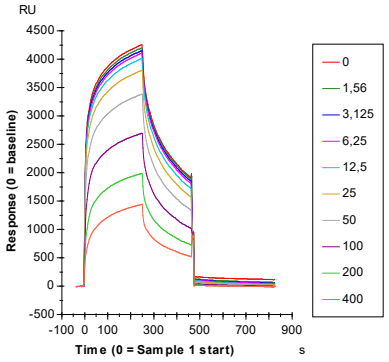
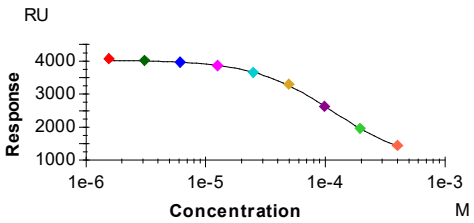
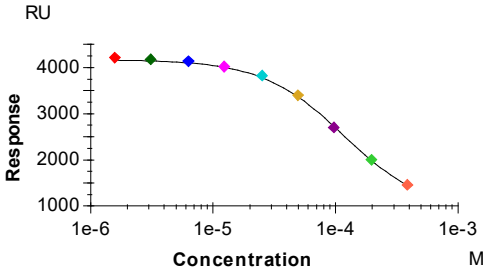
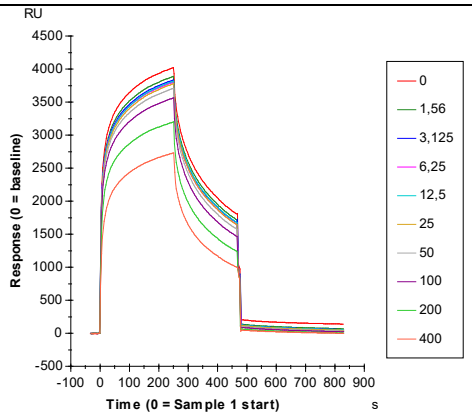
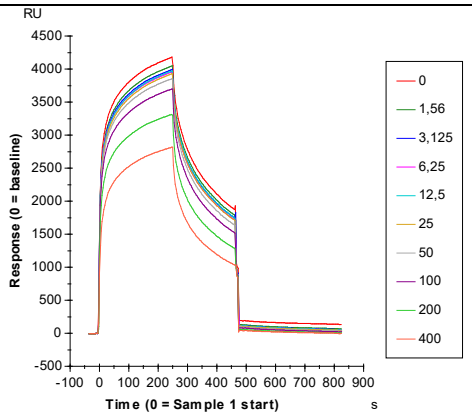
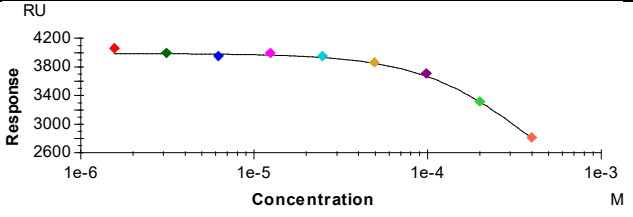
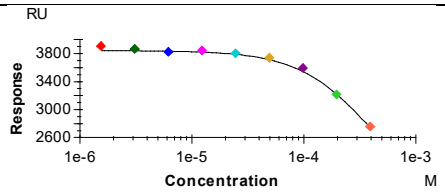


B2 Langerin

Fc2	Fc3
	
	

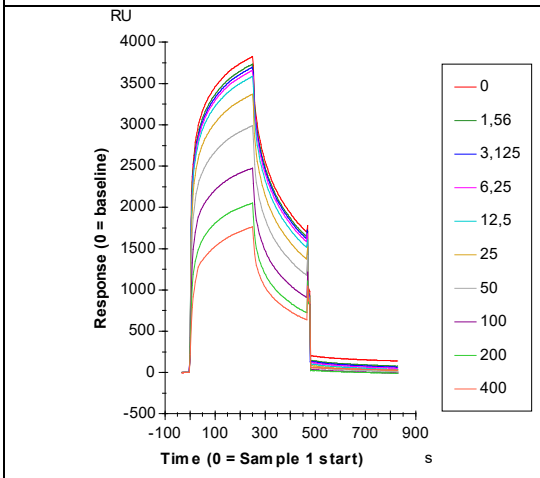
C2 Langerin

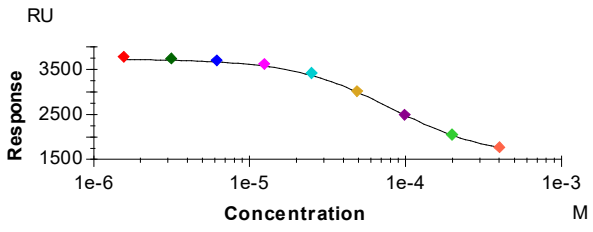
Fc2	Fc3
	
	



C3 Langerin

Fc2





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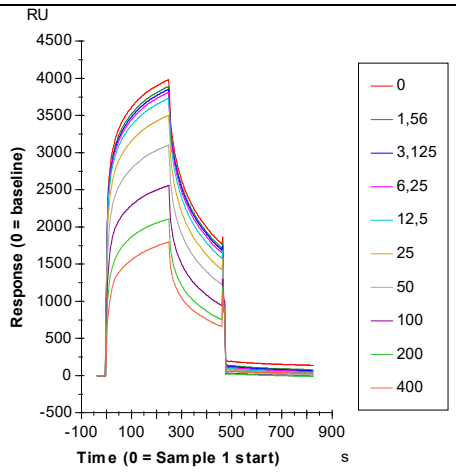

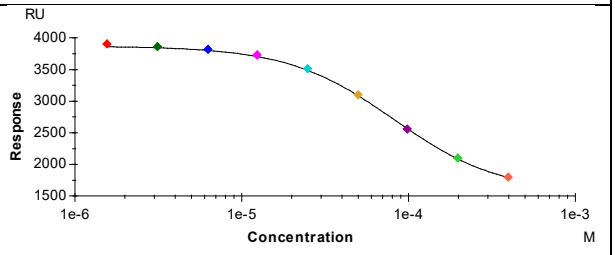

Supplementary Figure 4 | Direct interaction assays (SPR)

A6

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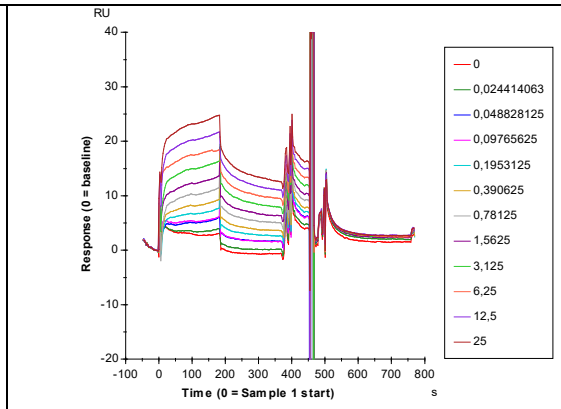

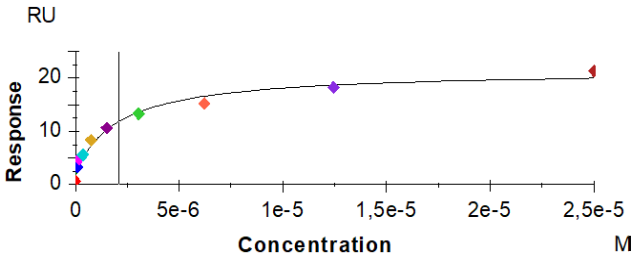

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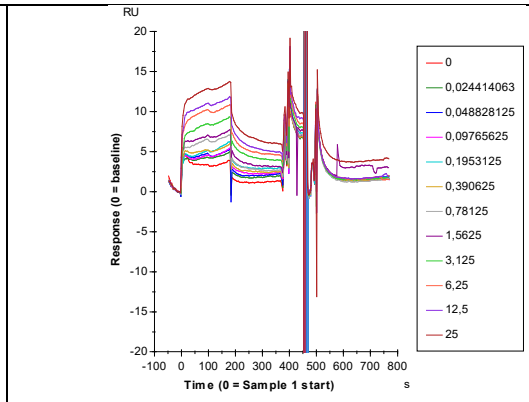

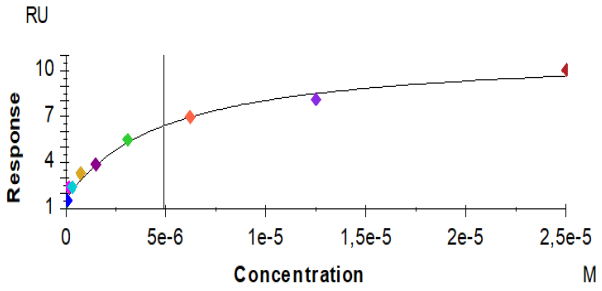

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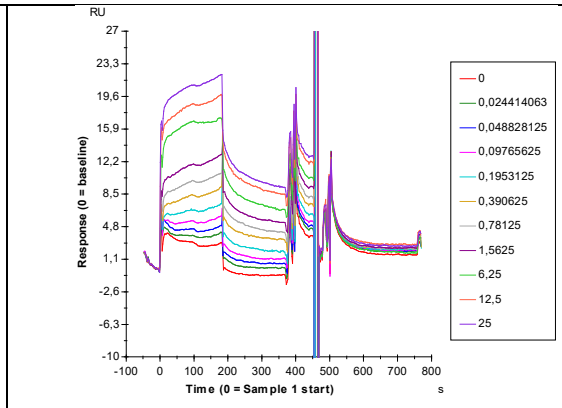

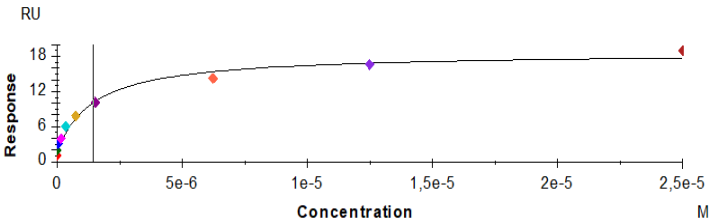

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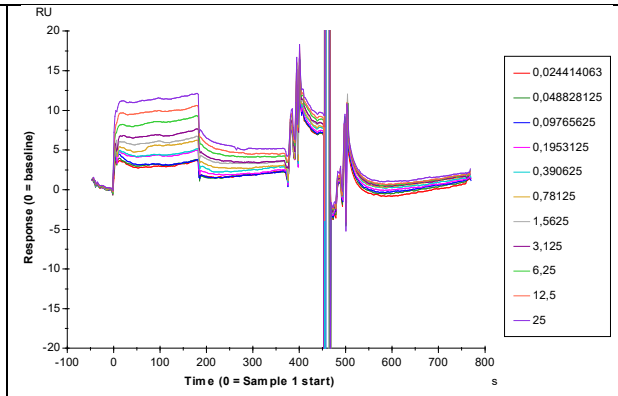

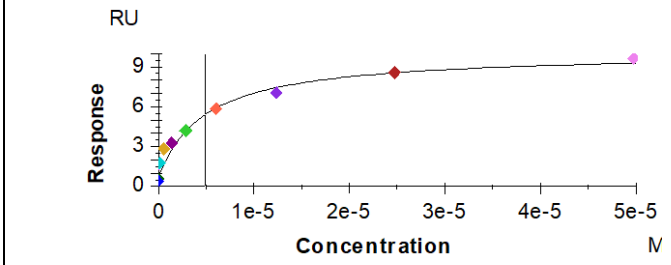

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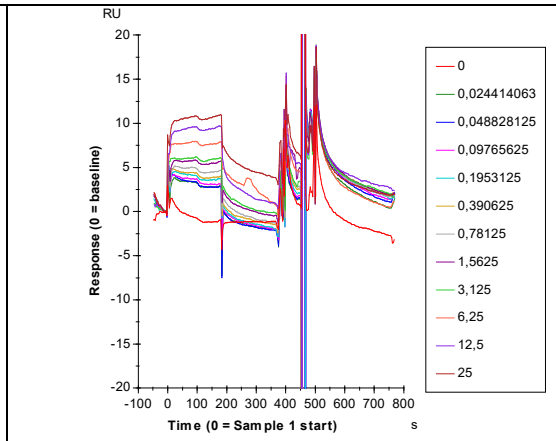

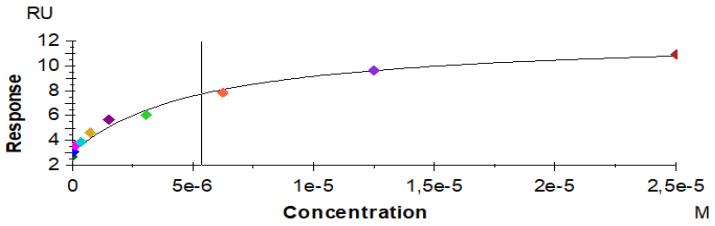

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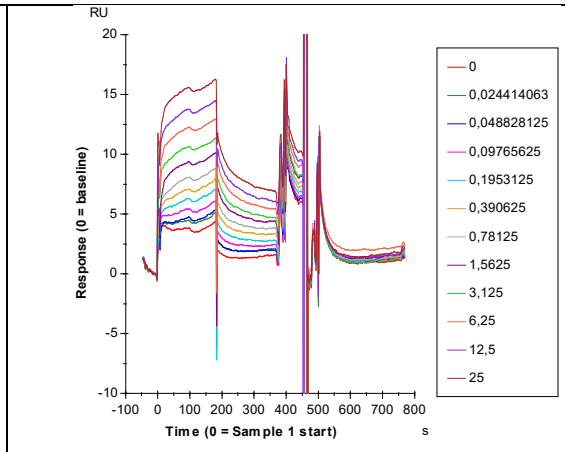

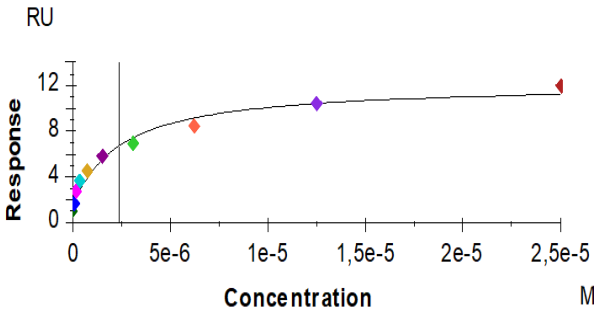

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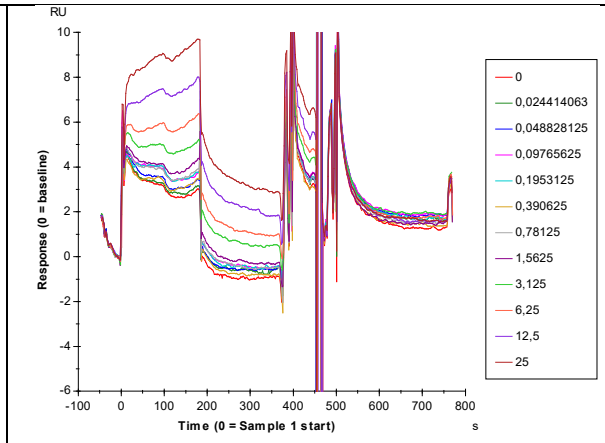

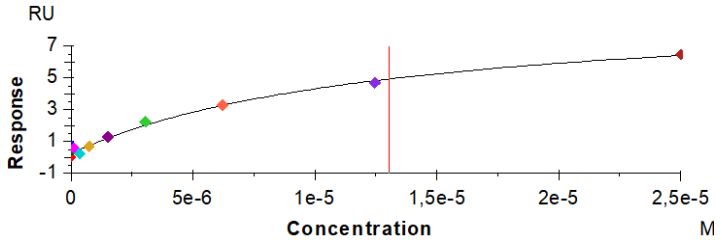

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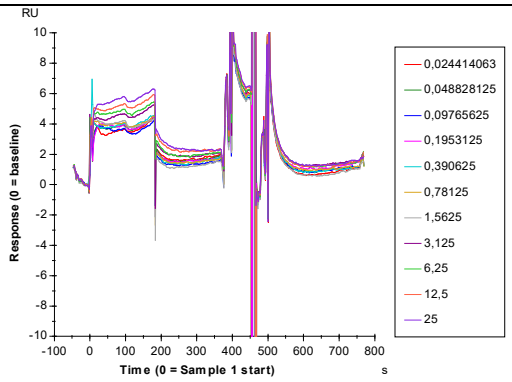

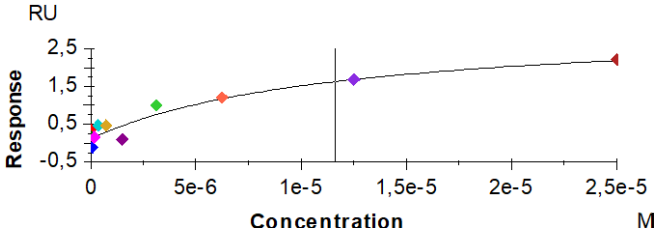

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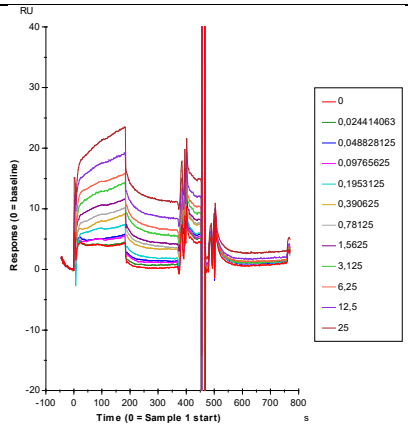

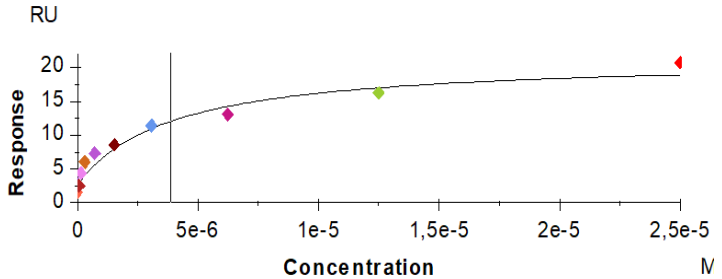

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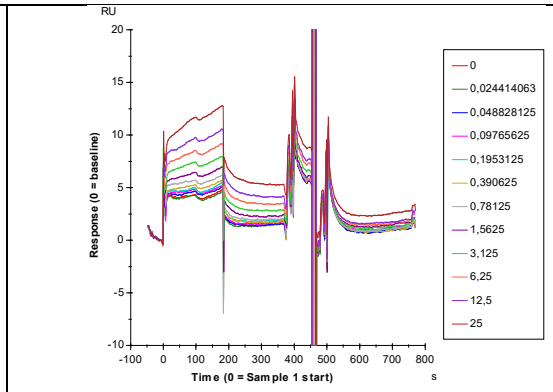

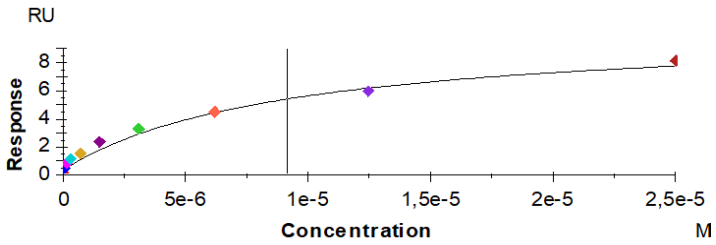

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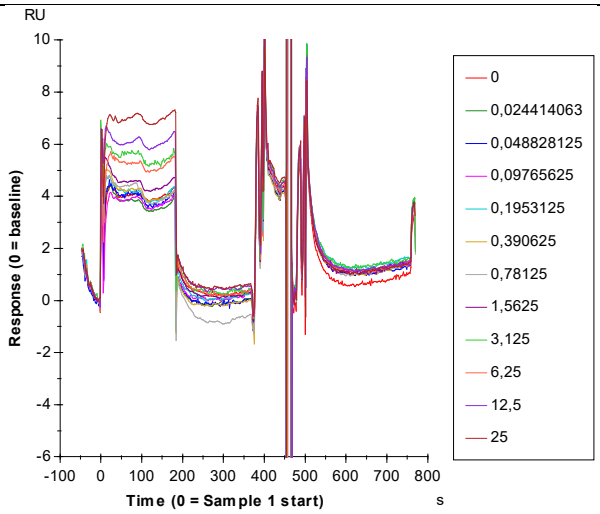

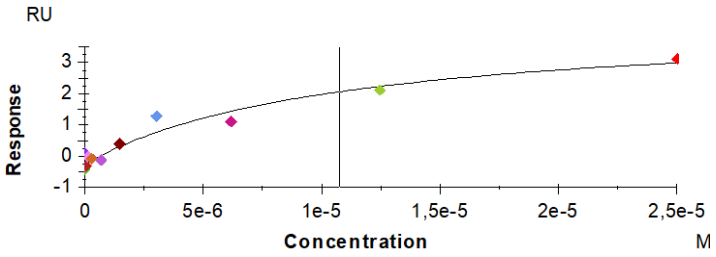

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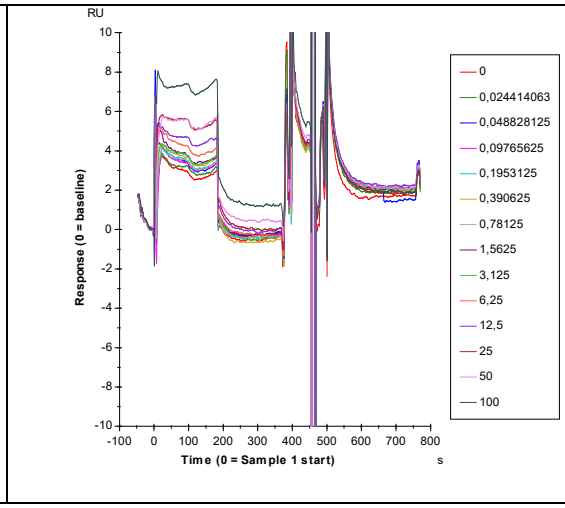

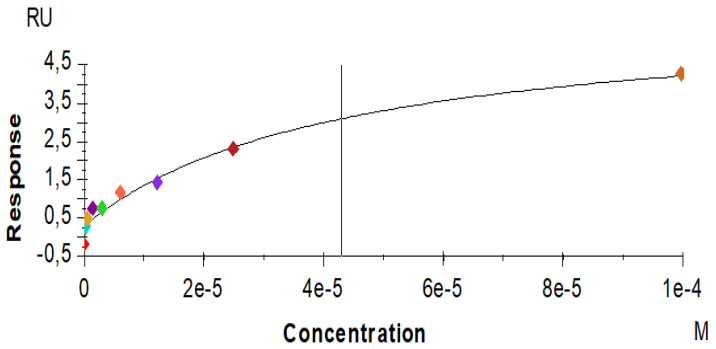

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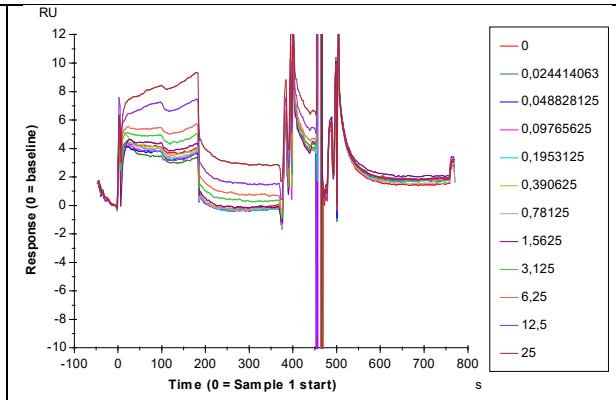

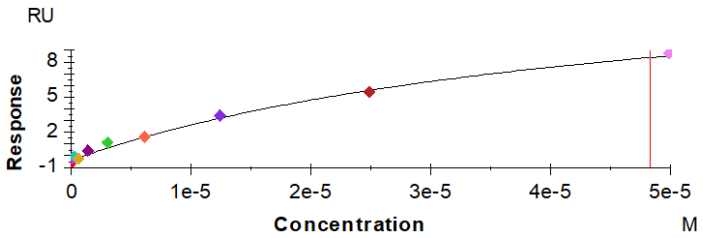

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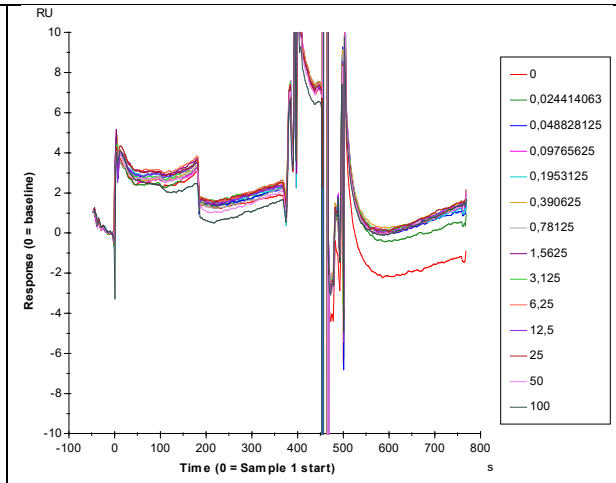

Not possible to determine

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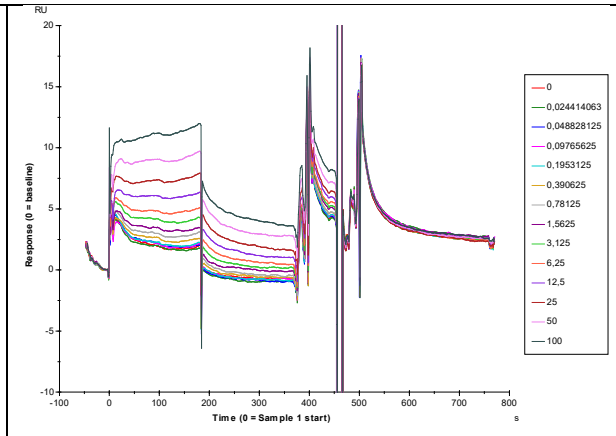

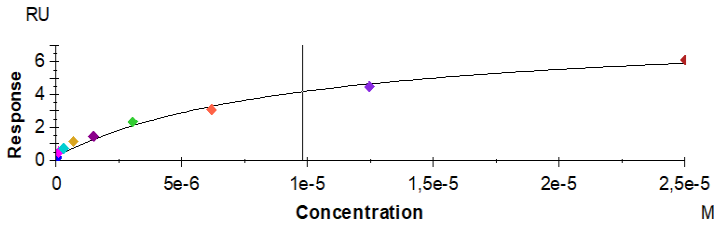

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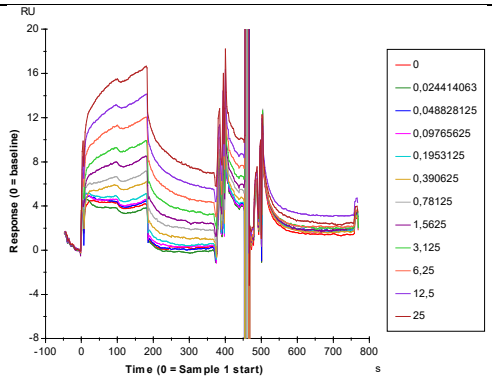
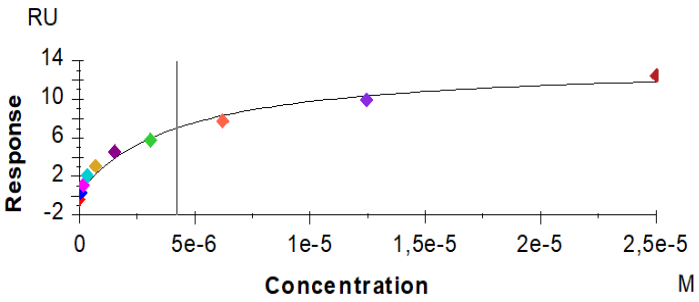
Fc4-1 Langerin		

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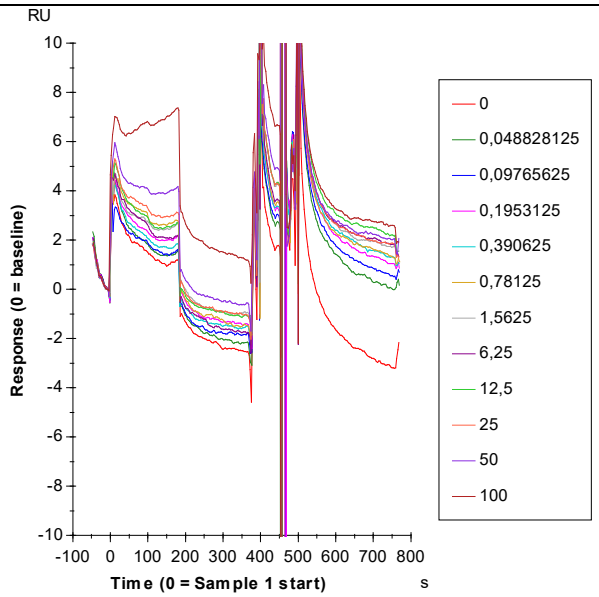
Fc4-1 Langerin		*Not possible to determine*

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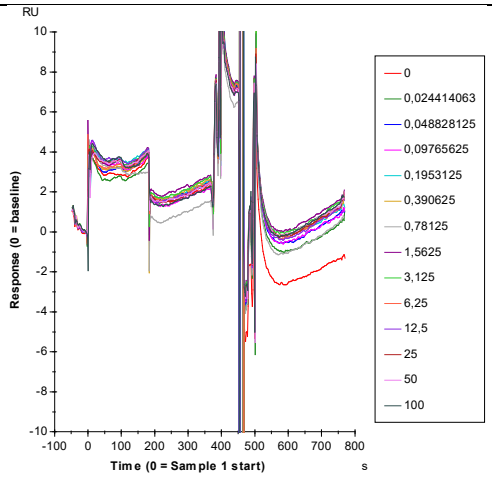
Fc4-1 Langerin		*Not possible to determine*

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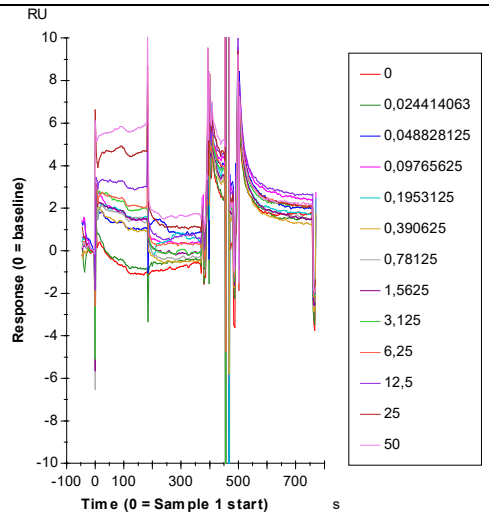
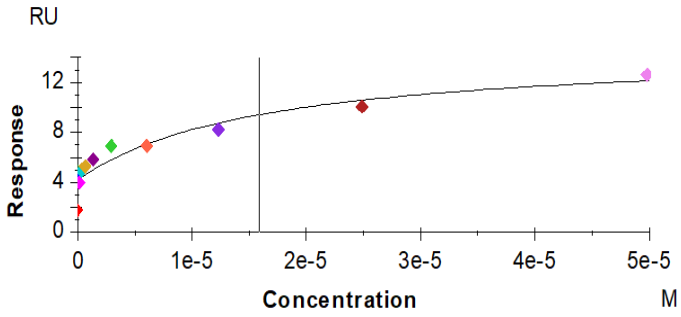
Fc4-1 Langerin		

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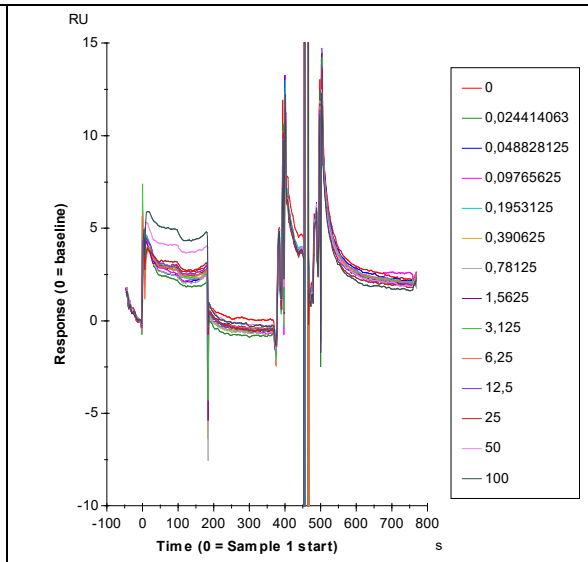
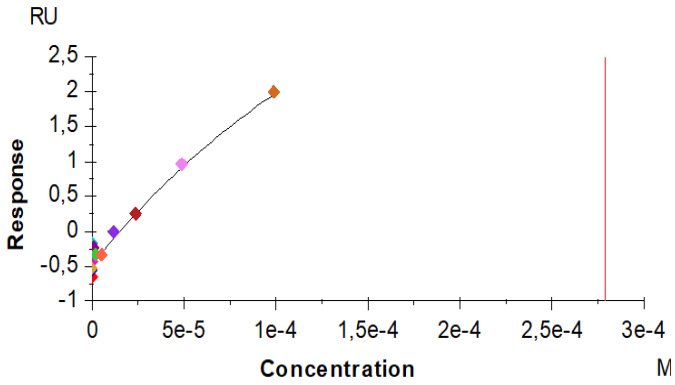
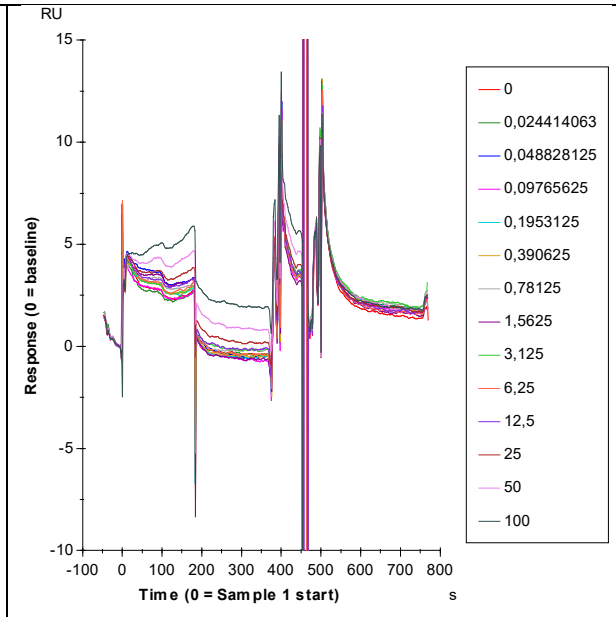
Fc4-1 Langerin

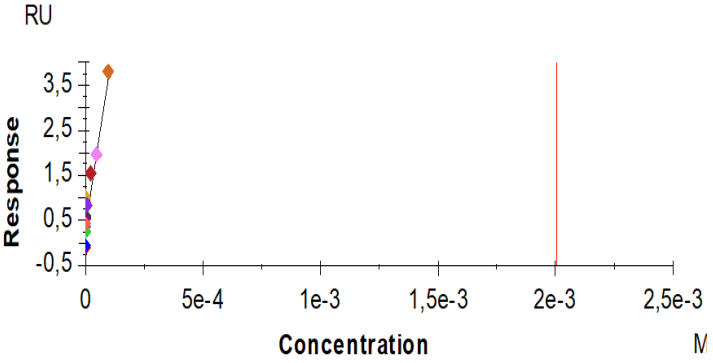




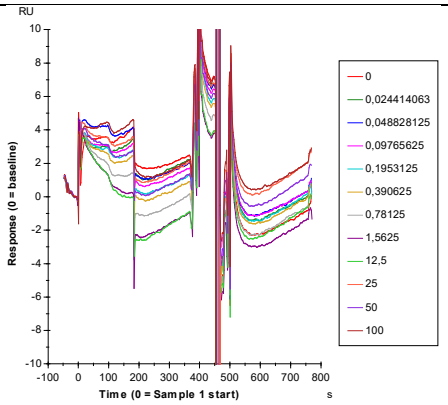
B1

Fc4-1 Langerin

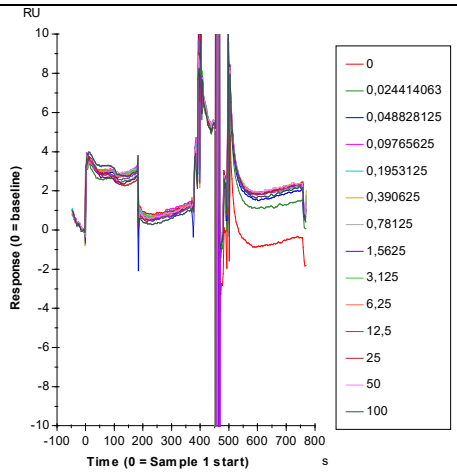




E1

Fc4-1 Langerin		*Not possible to determine*

D1

Fc4-1 Langerin		*Not possible to determine*