

Supplementary information

Examination of oestrus-dependent alterations of bovine cervico-vaginal mucus glycosylation for potential as optimum fertilisation indicators

Marie Le Berre ^{a,b}, Jared Q. Gerlach ^{a,b}, Catherine Loughrey ^{a,b}, Aileen Creavin ^{a,b}, Katarzyna Pluta ^c, Mary Gallagher ^c, Stephen D. Carrington ^c, Lokesh Joshi ^{a,b,*}, Michelle Kilcoyne ^{b,d,*}

^a Glycoscience Group, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

^b Advanced Glycoscience Research Cluster, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

^c Veterinary Sciences Centre, UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland

^d Carbohydrate Signalling Group, Discipline of Microbiology, National University of Ireland Galway, Galway, Ireland

* Joint corresponding authors

Table of contents

Supplementary Table 1. Bovine cervical mucus, print information and concentrations used. Print buffer was PBS, pH 7.4, supplemented with varying percentages of Tween-20 indicated by % T.

Supplementary Figure 1. Representative image of a subarray from a mucus microarray slide incubated with SNA-I.

Supplementary Figure 2. Glycosylation profiles of bovine cervico-vaginal mucus at different time points around oestrus detected following oestrus induction in cows (A) 664, (B) 673 and (C) 685, zoom-in on lectin binding intensity below 8,000 RFU. Bars represents the average binding intensity of fluorescently labelled lectins and MECA-79 antibody to printed mucus from three replicates experiments (in duplicate for MECA79, SNA-I, Con A and MAA) and error bars represent +/- 1 standard deviation.

Supplementary Figure 3. Glycosylation profiles of bovine cervico-vaginal mucus at different time points following oestrus induction in cow 685 and relationship to sampling time points. (A) Bars represents the average binding intensity of fluorescently labelled lectins and MECA-79 antibody to printed mucus from three replicates experiments (in duplicate for MECA79, SNA, Con A and MAA) and error bars represent +/- 1 standard deviation. (B) Zoom-in on lectin binding intensity below 8,000 RFU (C) Sampling time line describing relationship between sampling number, sampling day and oestrus observed.

Supplementary Figure 4. Glycosylation profiles of bovine cervico-vaginal mucus at different time points following oestrus induction in cow 673 and relationship to sampling time points. (A) Bars represents the average binding intensity of fluorescently labelled lectins and MECA-79 antibody to printed mucus from three replicates experiments (in duplicate for MECA79, SNA, Con A and MAA) and error bars represent +/- 1 standard deviation. (B) Zoom-in on lectin binding intensity below 8,000 RFU (C) Sampling time line describing relationship between sampling number, sampling day and oestrus observed.

Supplementary Figure 5. Glycosylation profiles of bovine cervico-vaginal mucus at different time points following oestrus induction in cow 664 and relationship to sampling time points. (A) Bars represents the average binding intensity of fluorescently labelled lectins and MECA-79 antibody to printed mucus from three replicates experiments (in duplicate for MECA79,

SNA, Con A and MAA) and error bars represent +/- 1 standard deviation. (B) Zoom-in on lectin binding intensity below 8,000 RFU (C) Sampling time line describing relationship between sampling number, sampling day and oestrus observed.

Supplementary Figure 6. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

Supplementary Figure 7. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

Supplementary Figure 8. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

Supplementary Figure 9. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

Supplementary Figure 10. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

Supplementary Figure 11. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

Supplementary Figure 12. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

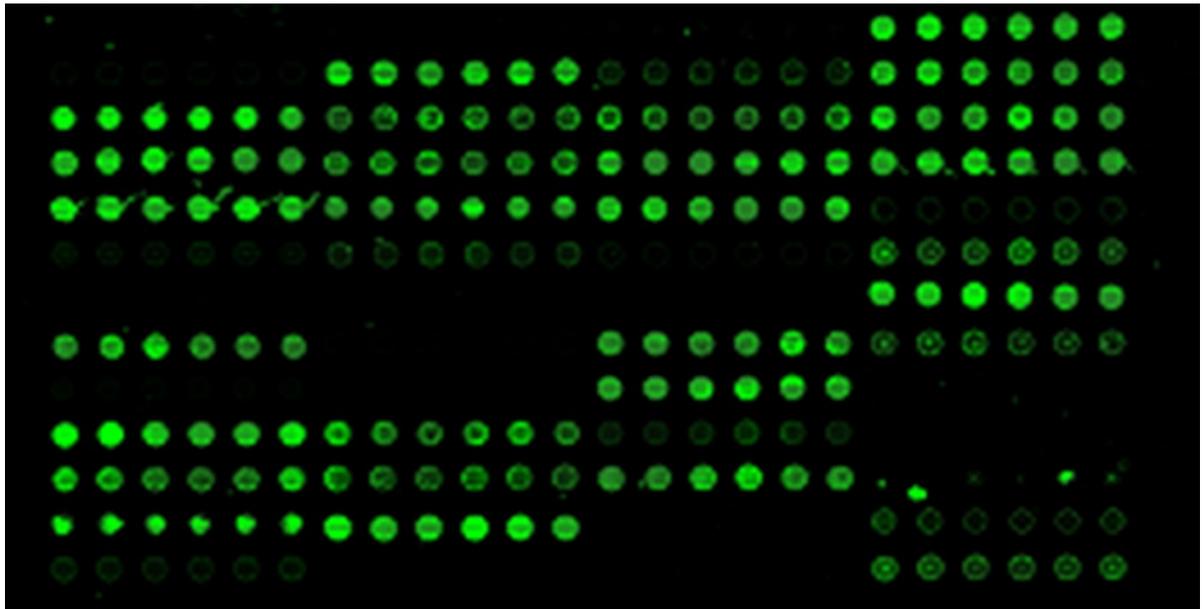
Supplementary Figure 13. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

Supplementary Figure 14. Matrix plot analysis of lectins binding onto mucus microarray showing grouped time points at oestrus observed (left column) and potentially oestrus detected (right column).

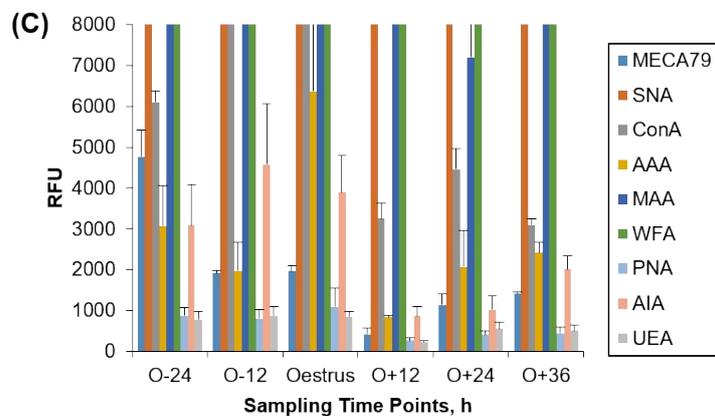
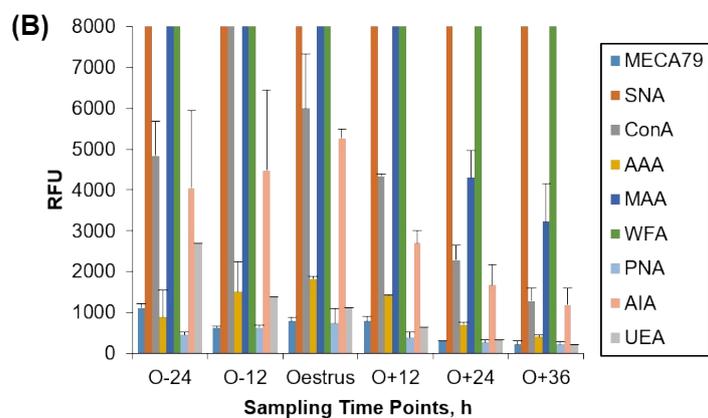
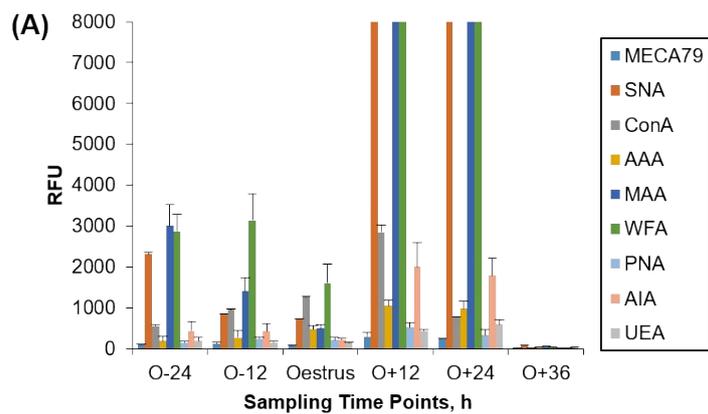
Supplementary Table 1. Bovine cervical mucus, print information and concentrations used.

Print buffer was PBS, pH 7.4, supplemented with varying percentages of Tween-20 indicated by % T.

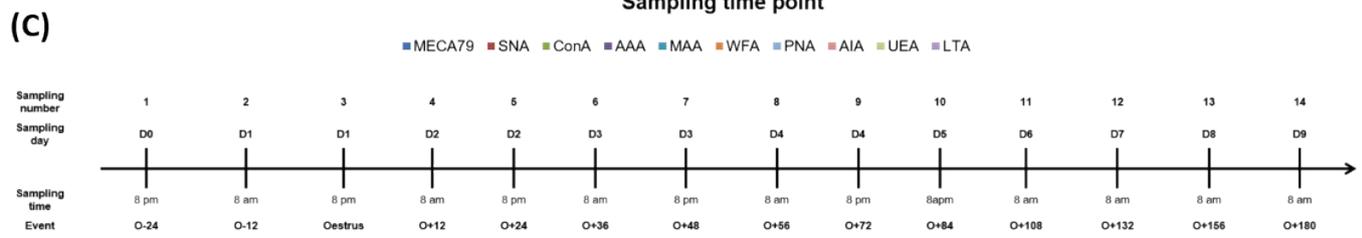
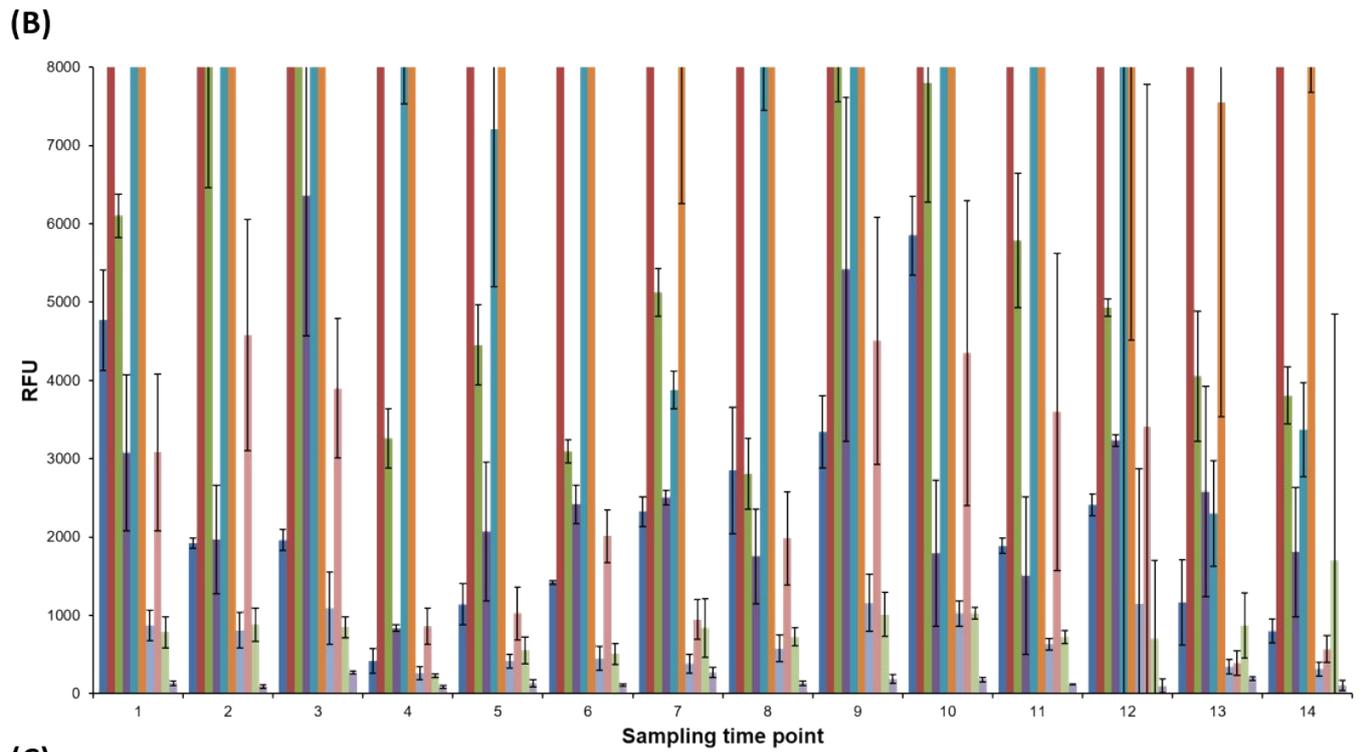
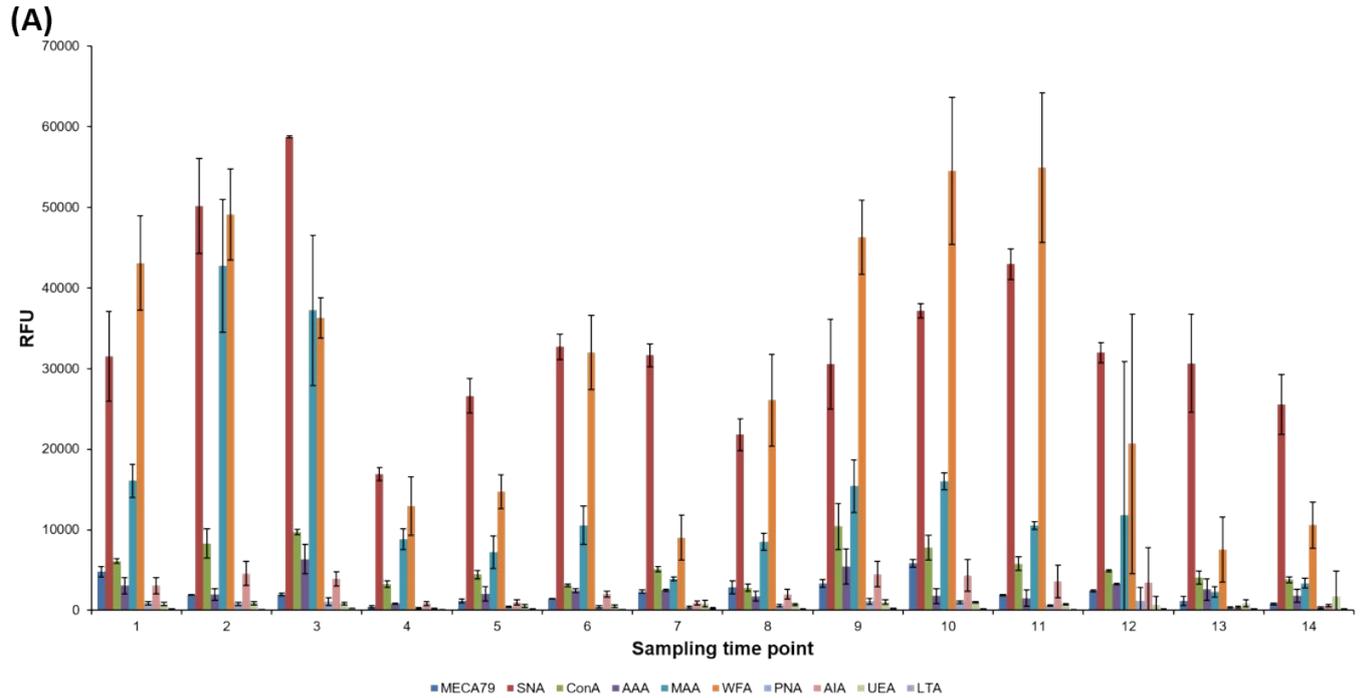
Cow	Sampling number	Print buffer % T	Mucus conc (mg/mL)
664	1	PBS 0.025% T	0.9
664	2	PBS 0.025% T	0.5
664	3	PBS 0.025% T	0.9
664	4	PBS 0.025% T	0.9
664	5	PBS 0.025% T	0.9
664	6	PBS 0.025% T	0.9
664	7	PBS 0.025% T	0.9
664	8	PBS 0.025% T	0.9
664	9	PBS 0.05% T	0.5
664	10	PBS 0.05% T	0.9
664	11	PBS 0.025% T	0.9
664	12	PBS 0.025% T	0.9
664	13	PBS 0.025% T	0.5
664	14	PBS 0.025% T	0.25
685	1	PBS 0.025% T	0.25
685	2	PBS 0.025% T	0.75
685	3	PBS 0.025% T	0.5
685	4	PBS 0.05% T	1
685	5	PBS 0.05% T	0.25
685	6	PBS 0.05% T	0.5
685	7	PBS 0.025% T	1
685	8	PBS 0.05% T	0.5
685	9	PBS 0.025% T	1
685	10	PBS 0.025% T	0.5
685	11	PBS 0.025% T	1
685	12	PBS 0.025% T	0.5
685	13	PBS 0.025% T	0.5
685	14	PBS 0.05% T	1
673	1	PBS 0.025% T	0.5
673	2	PBS 0.05% T	0.75
673	3	PBS 0.05% T	0.75
673	4	PBS 0.05% T	0.75
673	5	PBS 0.025% T	0.9
673	6	PBS 0.05% T	0.9
673	7	PBS 0.025% T	0.9
673	8	PBS 0.025% T	0.9
673	9	PBS 0.025% T	0.5
673	10	PBS 0.05% T	0.9
673	11	PBS 0.05% T	0.9
673	12	PBS 0.025% T	0.25
673	13	PBS 0.05% T	0.9
673	14	PBS 0.05% T	0.9



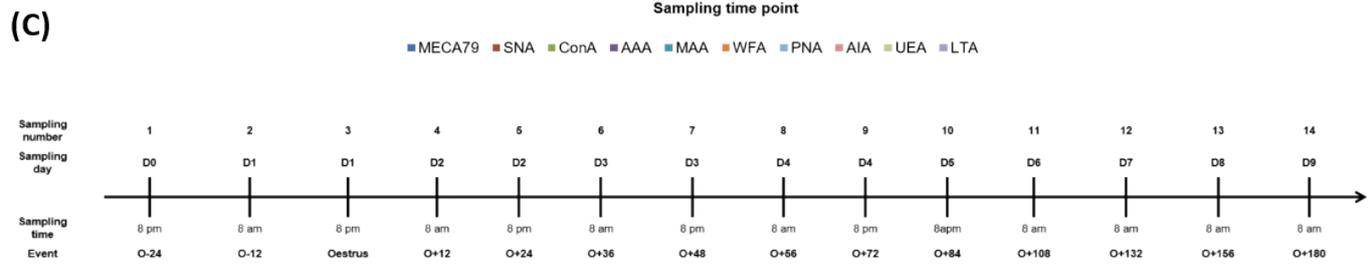
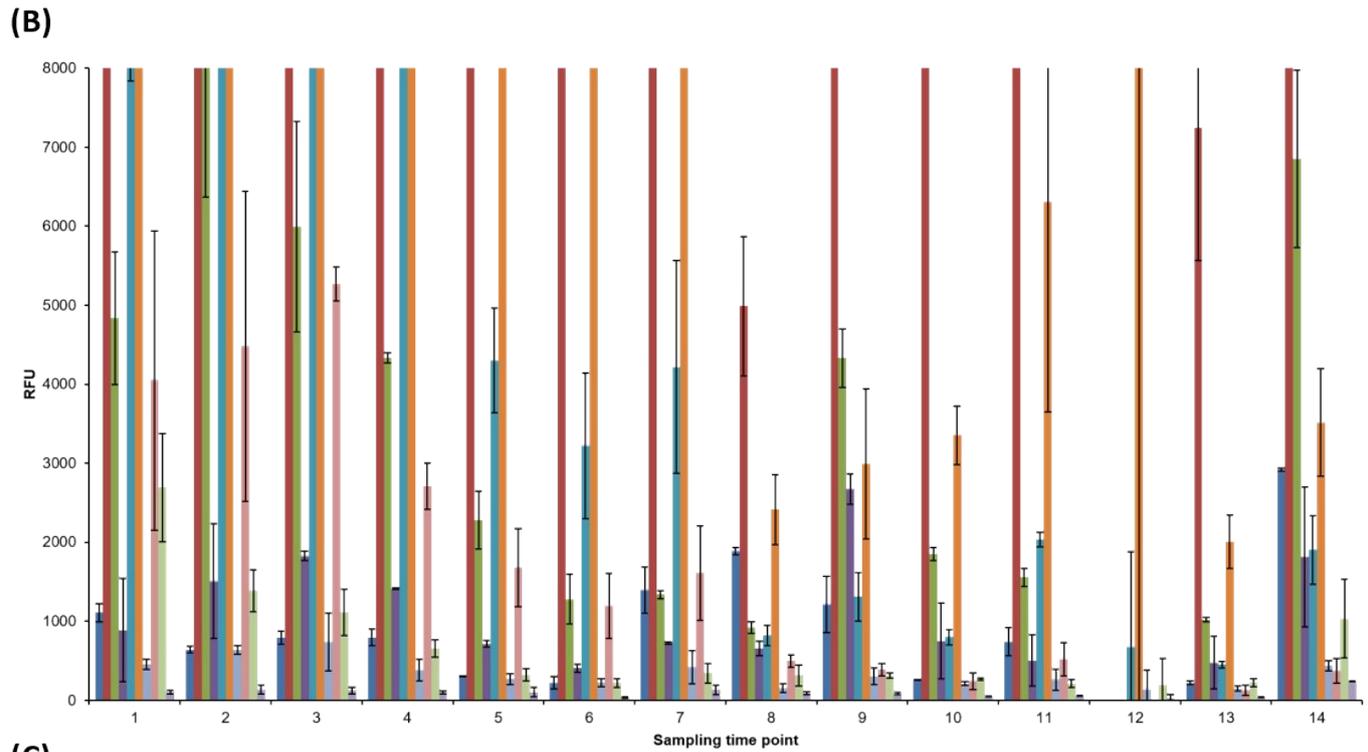
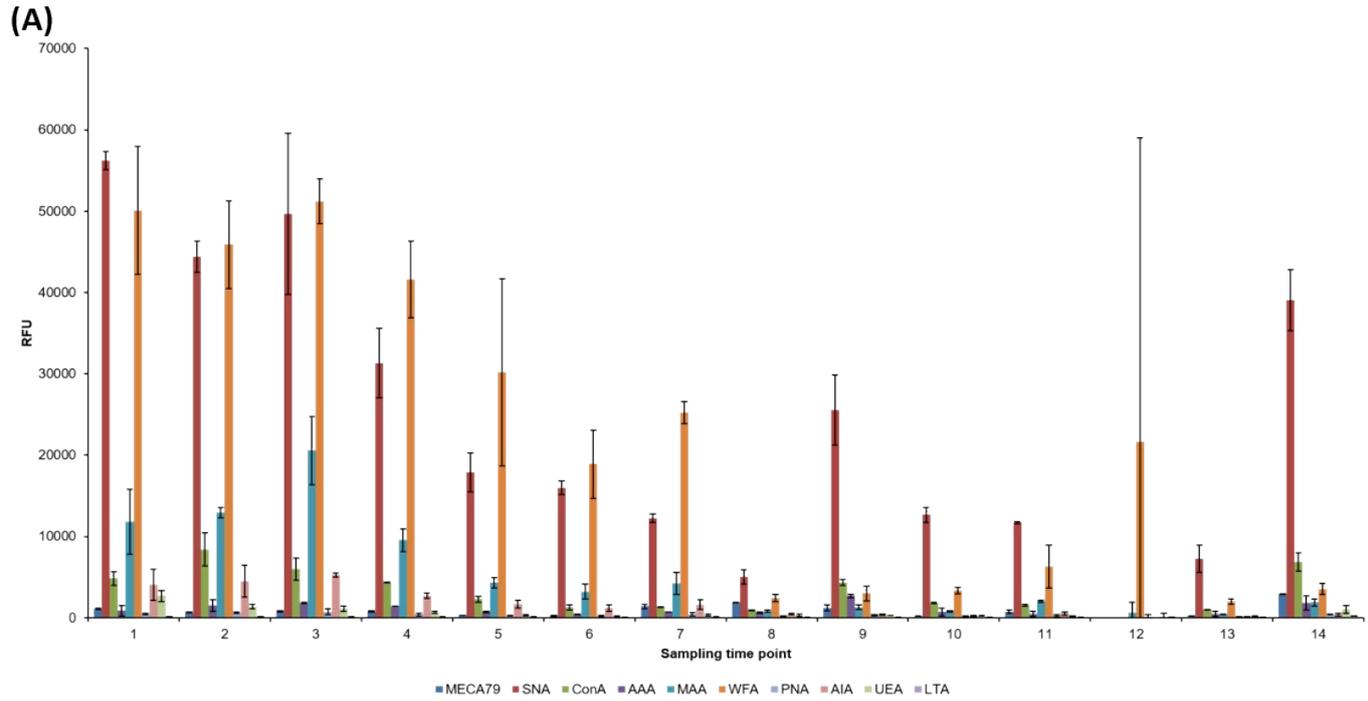
Supplementary Figure 1.



Supplementary Figure 2.

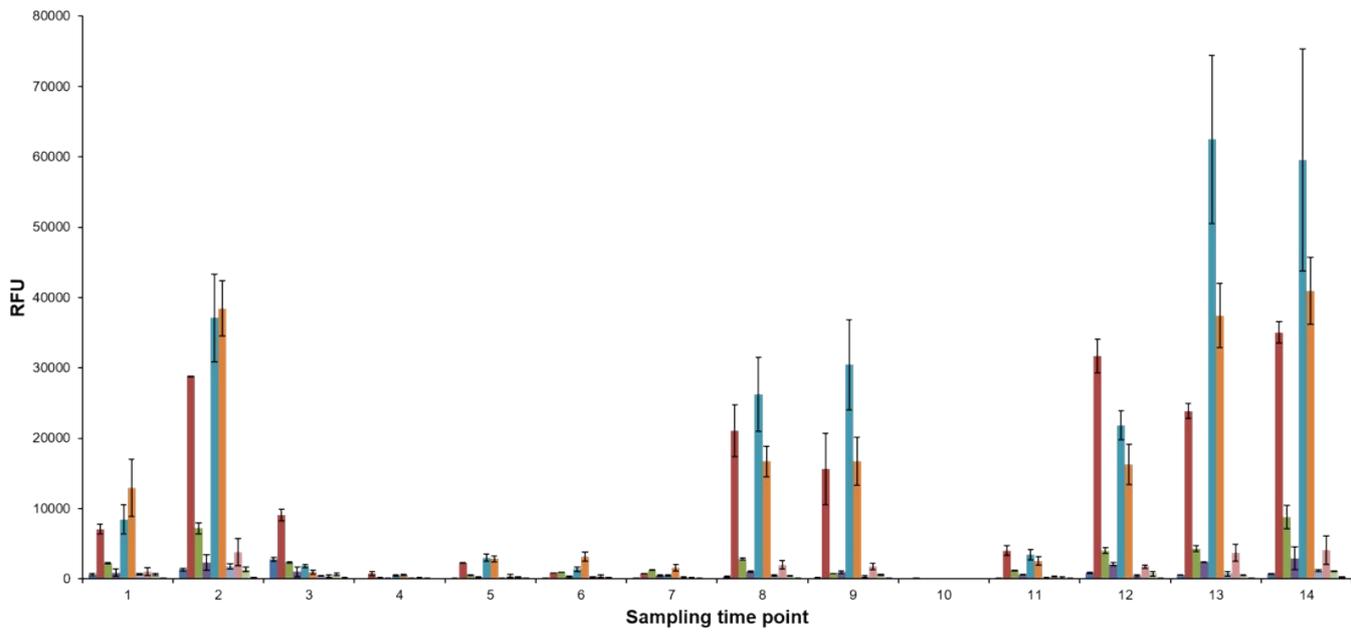


Supplementary Figure 3.

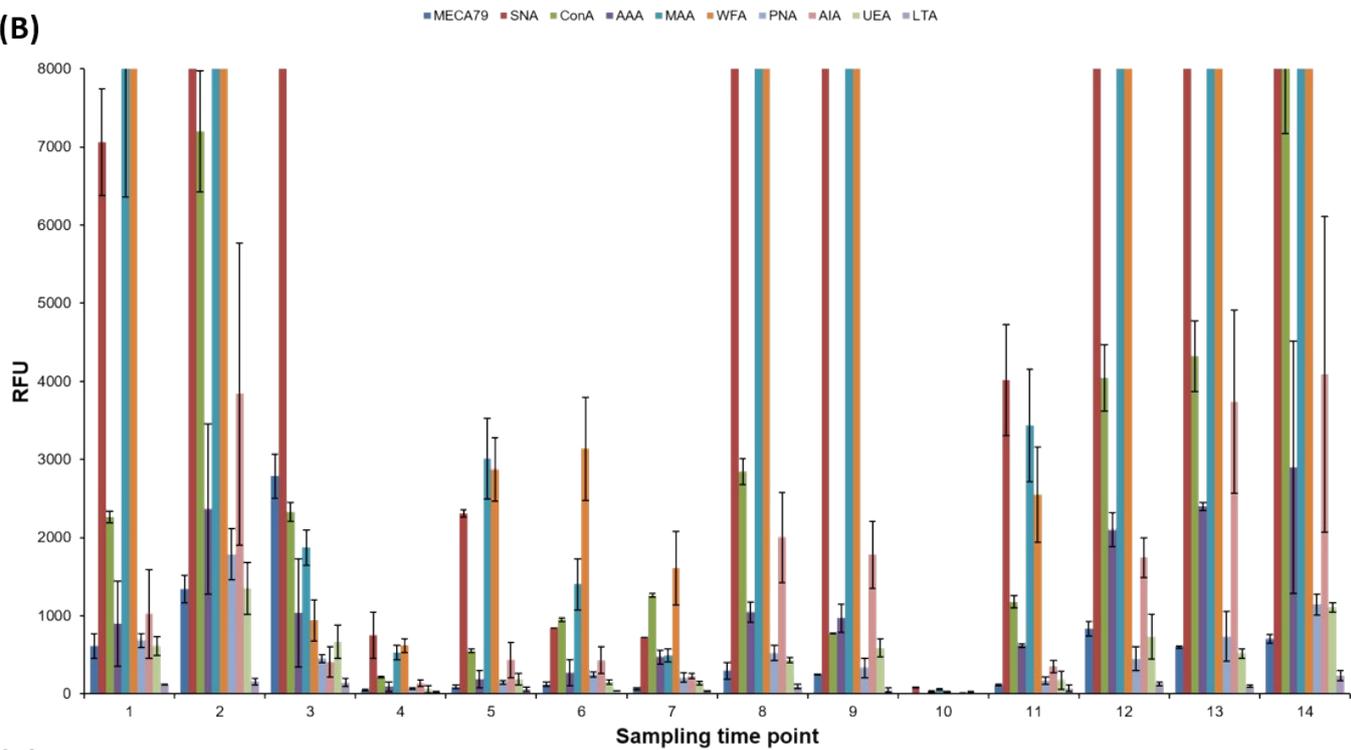


Supplementary Figure 4.

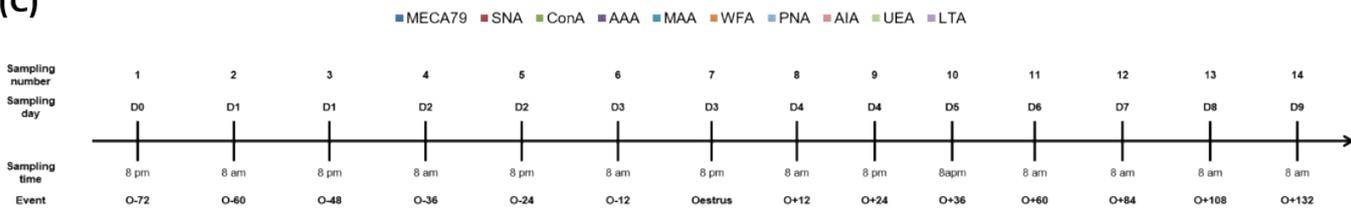
(A)



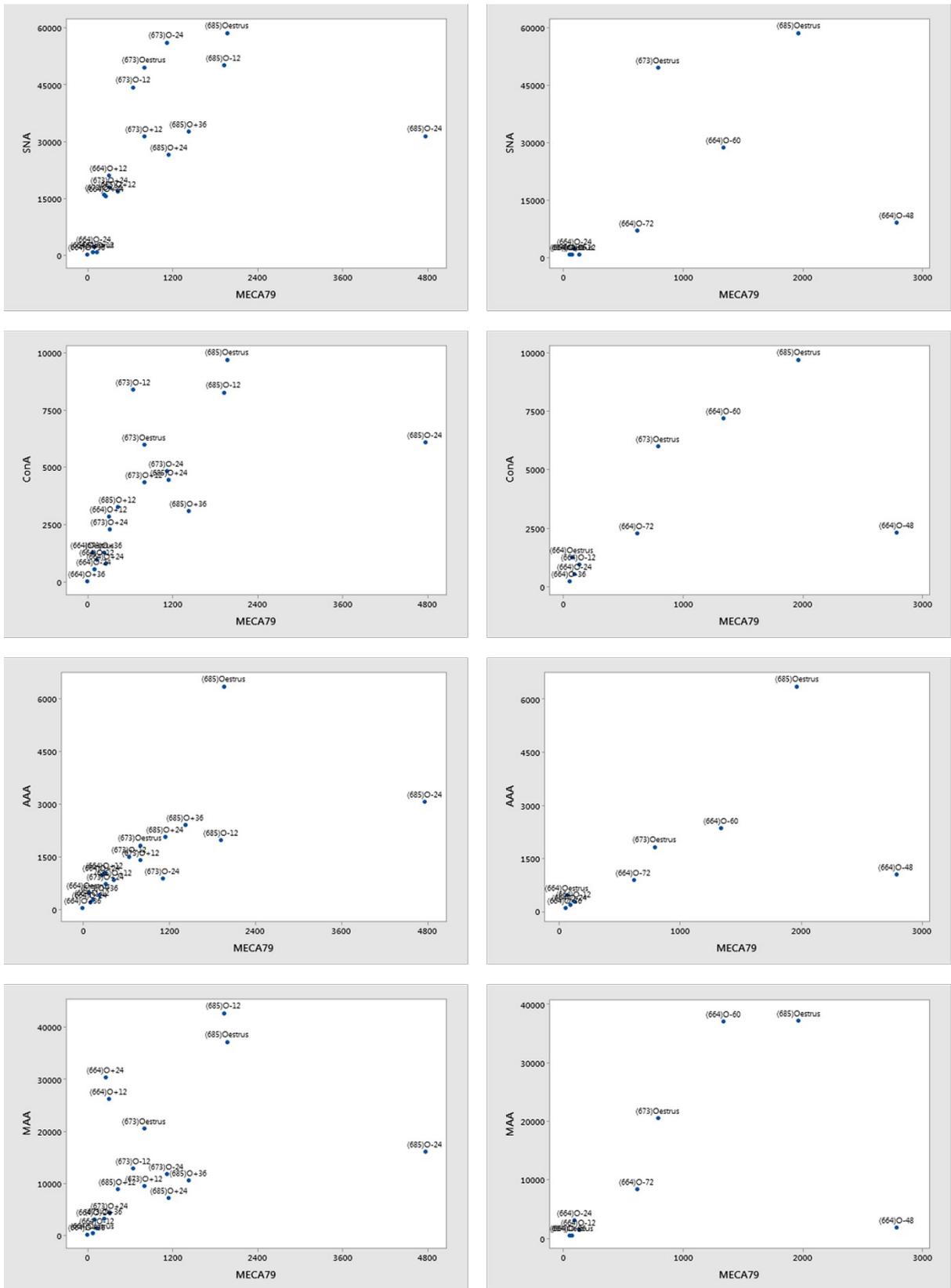
(B)



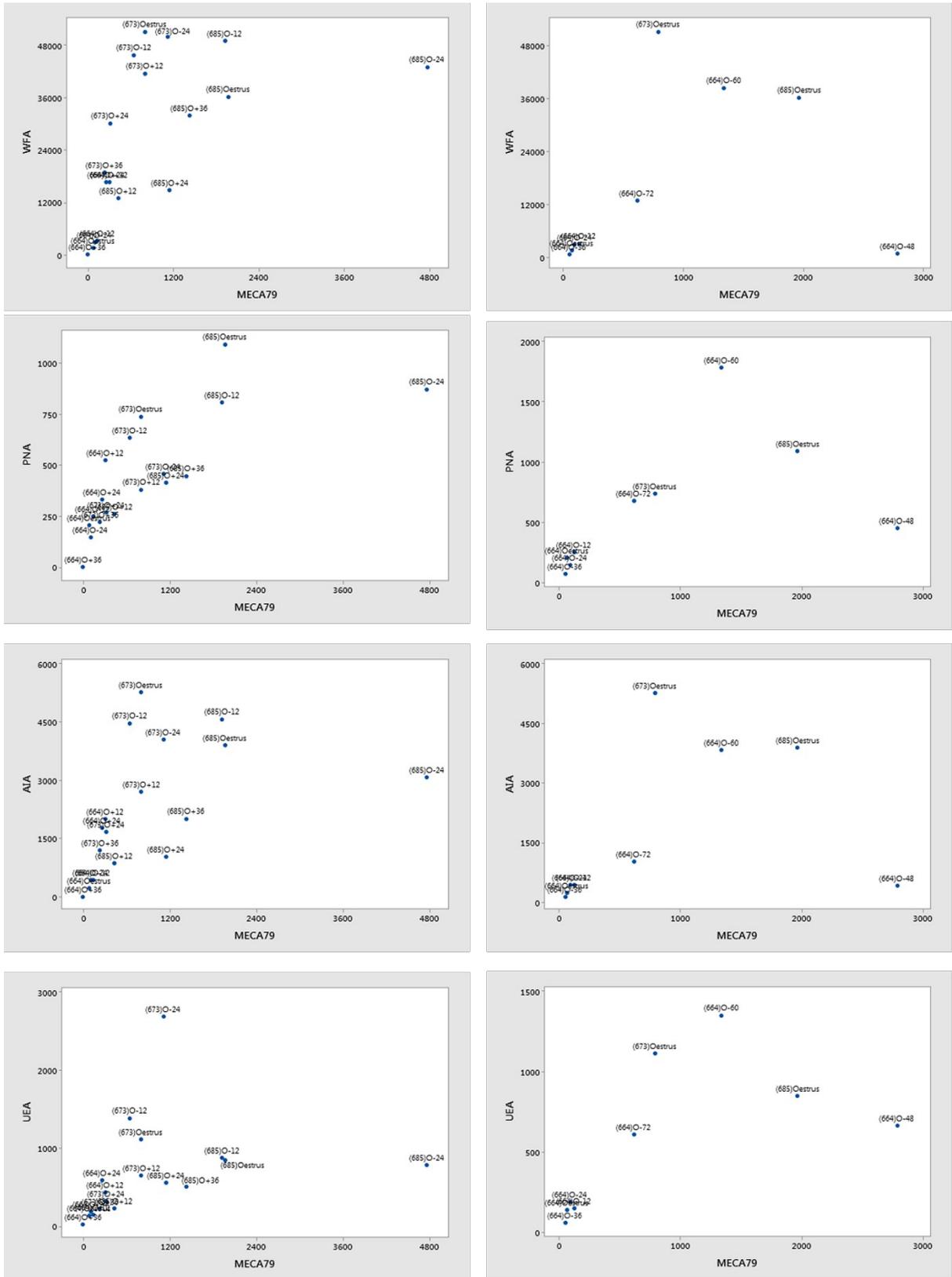
(C)



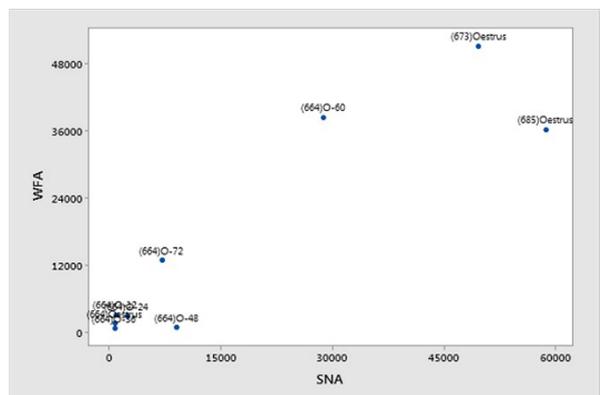
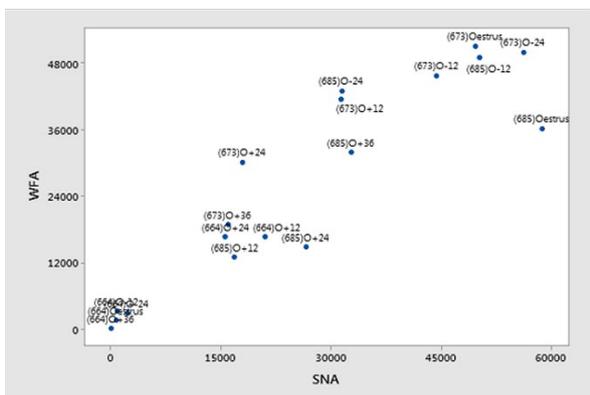
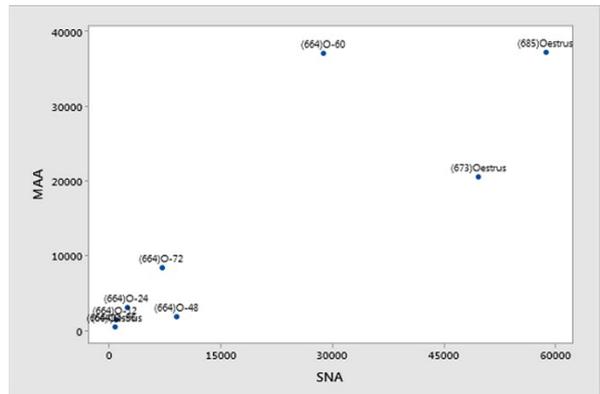
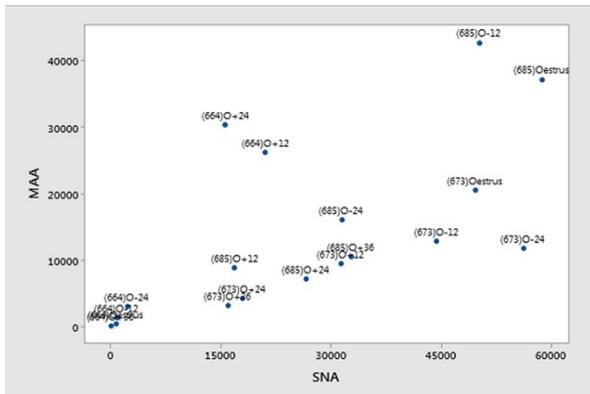
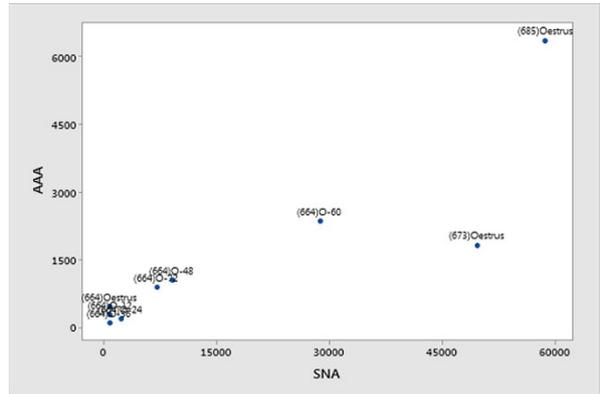
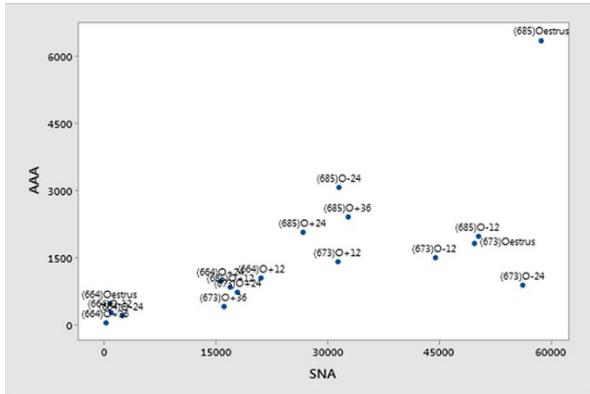
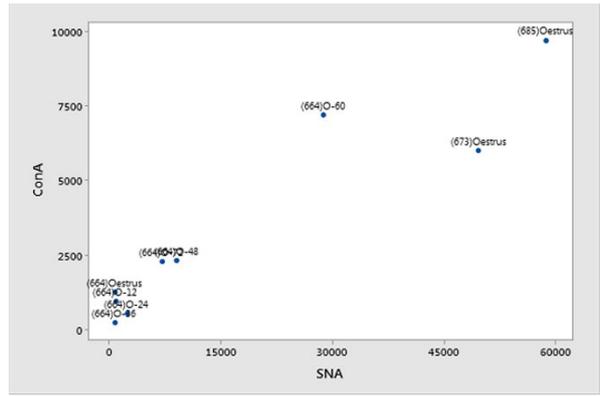
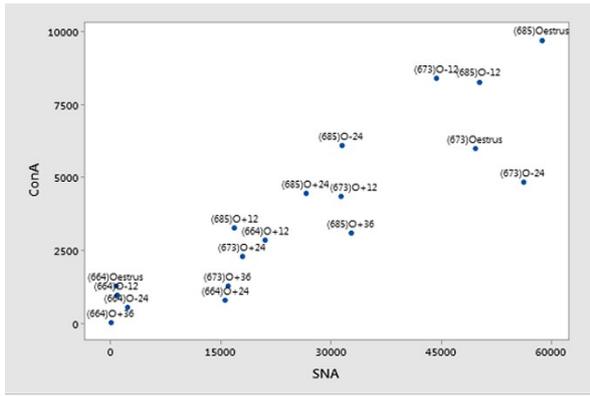
Supplementary Figure 5.



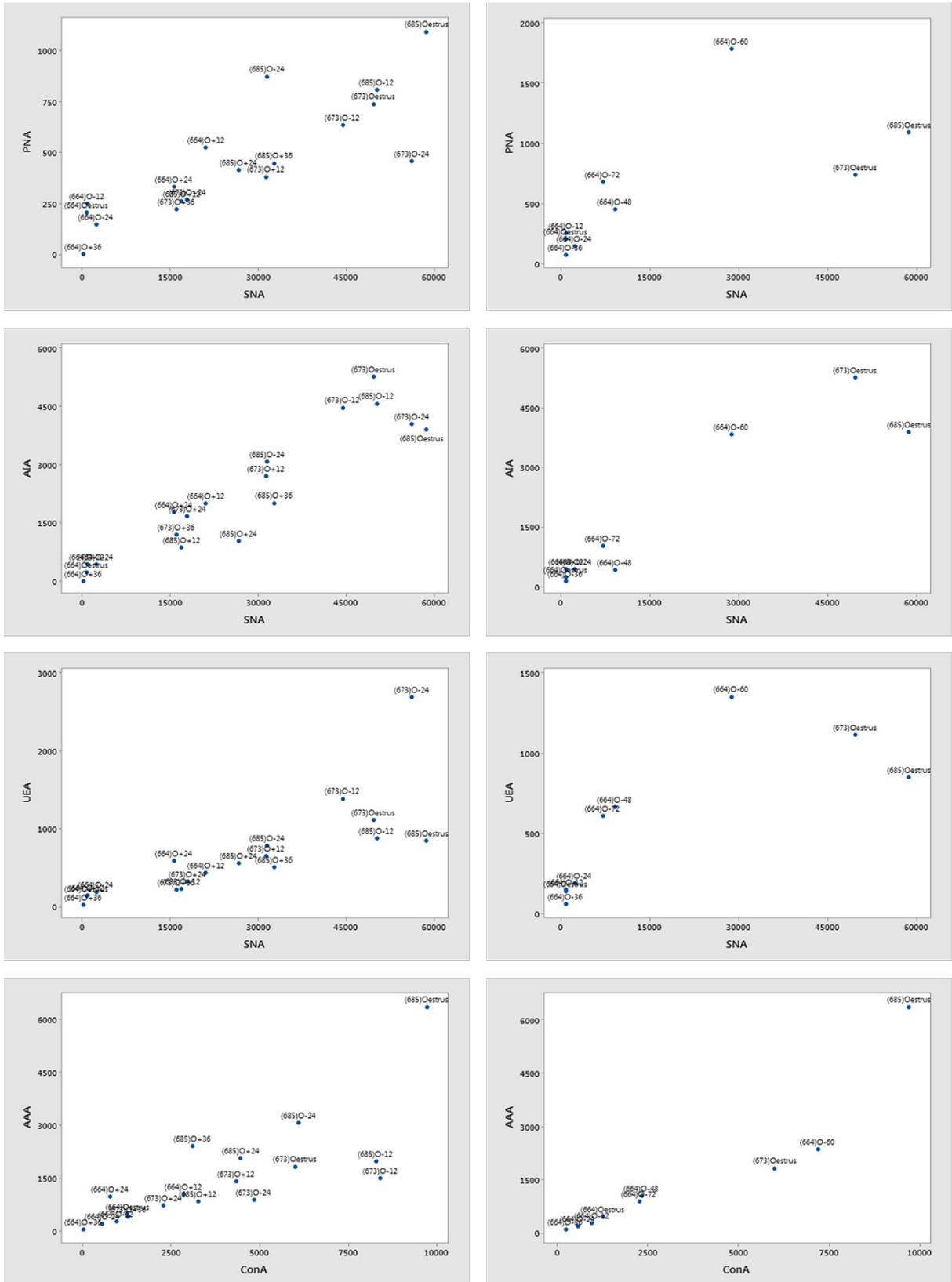
Supplementary Figure 6.



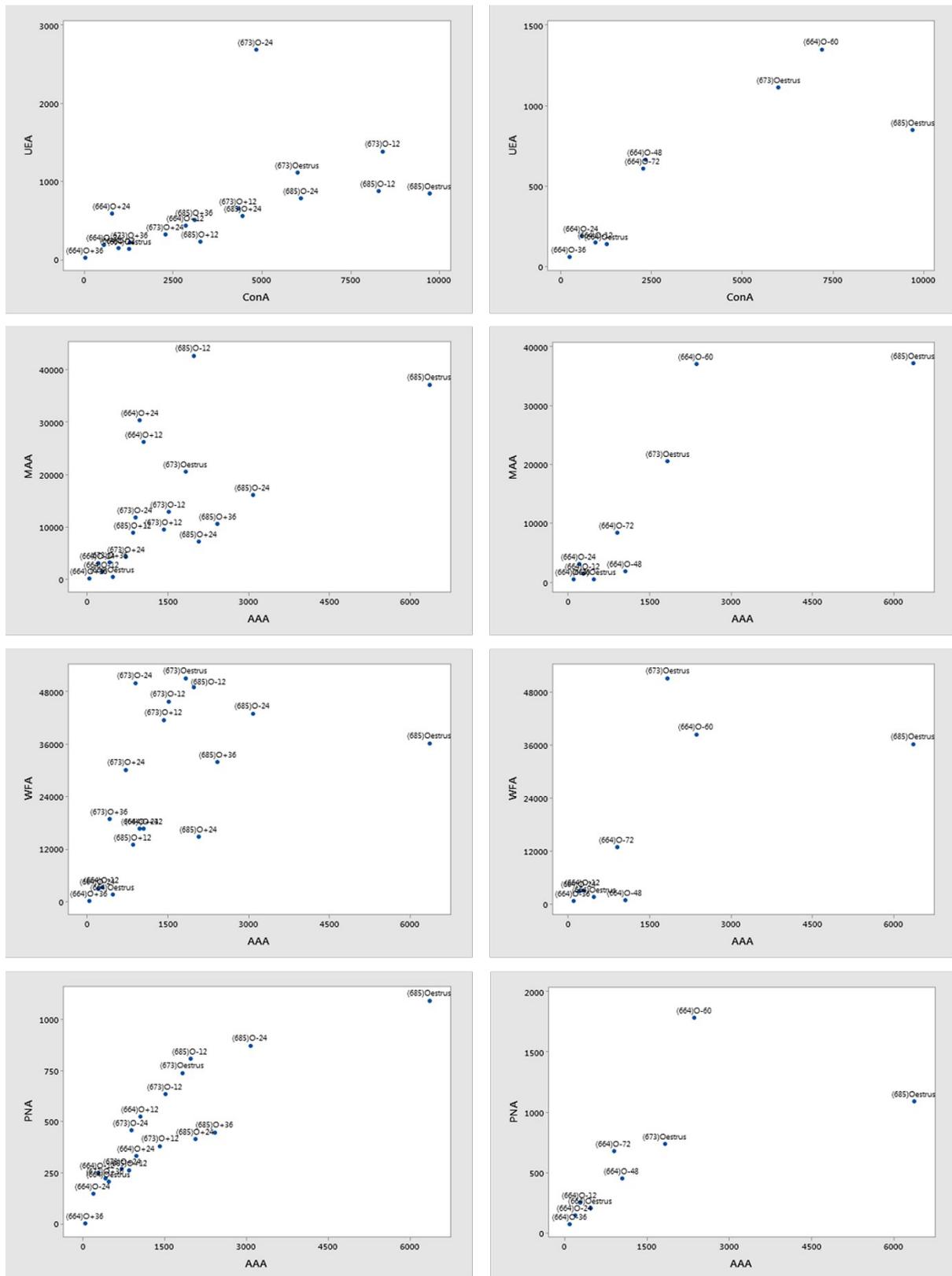
Supplementary Figure 7.



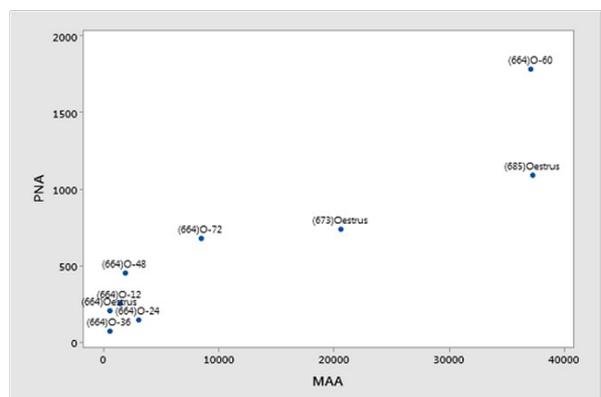
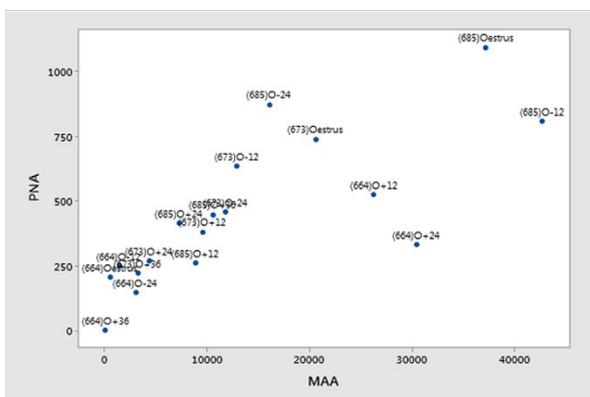
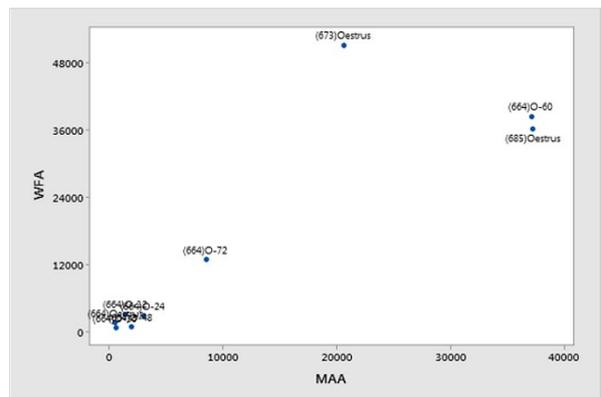
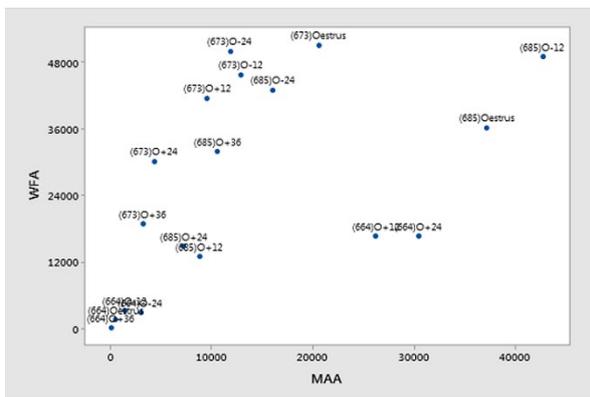
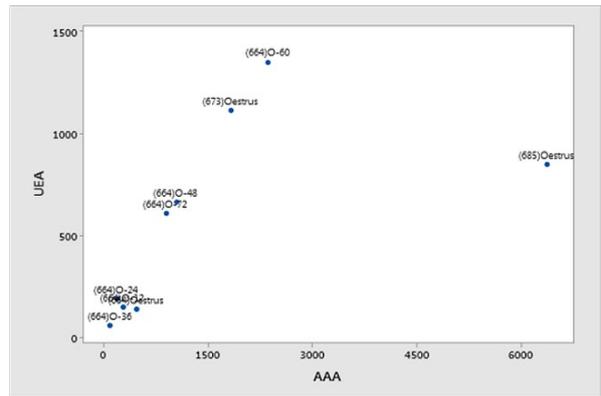
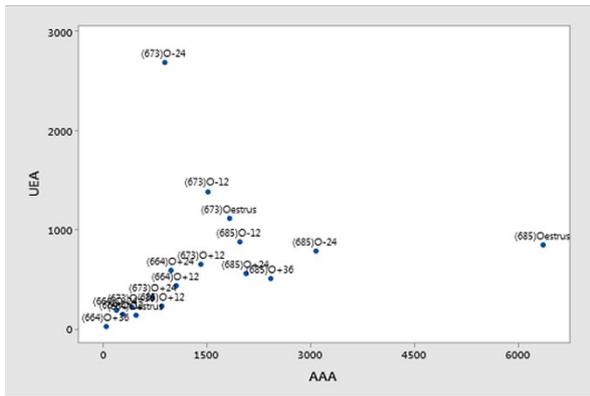
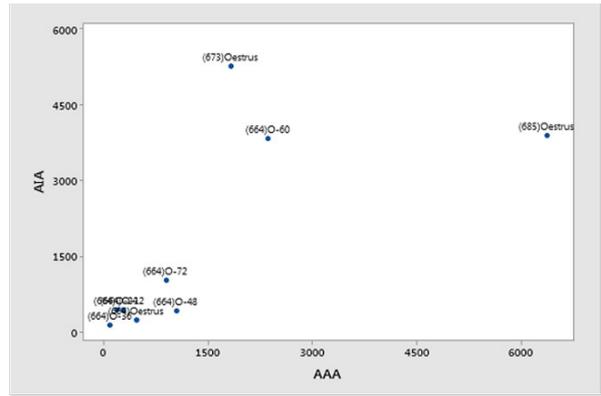
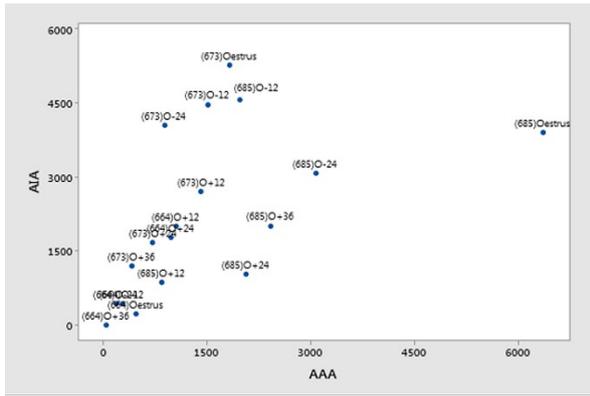
Supplementary Figure 8.



Supplementary Figure 9.



Supplementary Figure 11.



Supplementary Figure 12.

