

## **Supplementary Figures.**

### **Characterisation of IL-23 receptor antagonists and disease relevant mutants using fluorescent probes**

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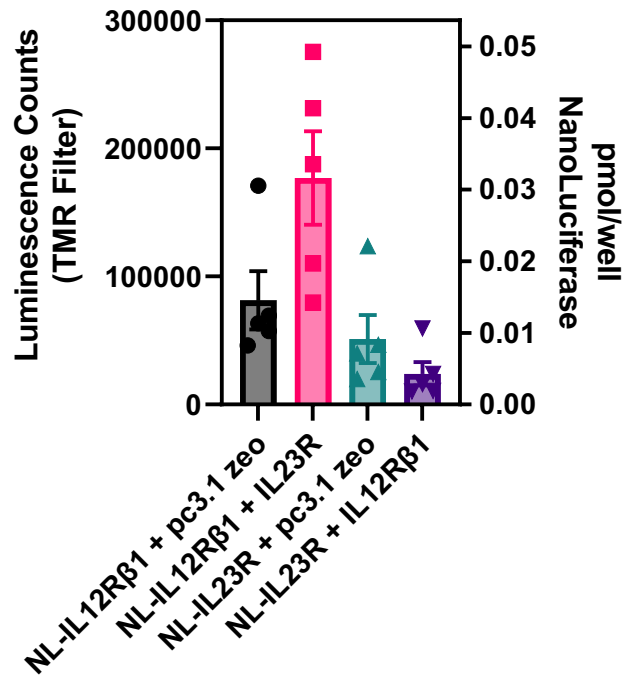
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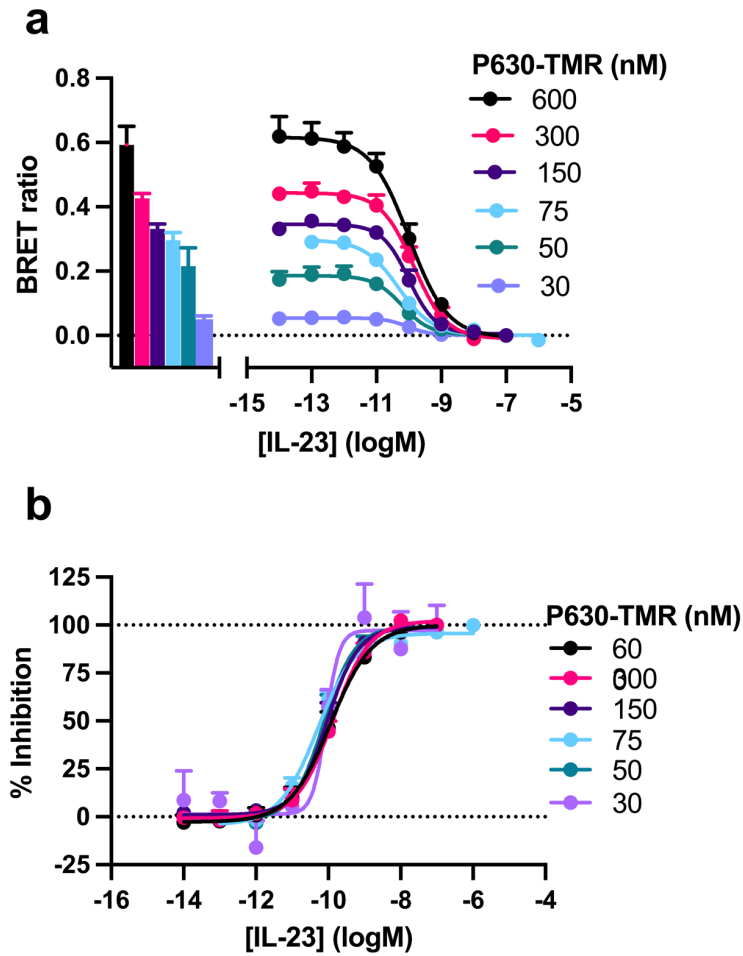
<sup>4</sup> Division of Bimolecular Science and Medicinal Chemistry, School of Pharmacy, Biodiscovery Institute, University of Nottingham, NG7 2RD, UK

<sup>5</sup> Crick-GSK Biomedical Linklabs, Medicine Design, GlaxoSmithKline, Stevenage SG1 2NY, UK

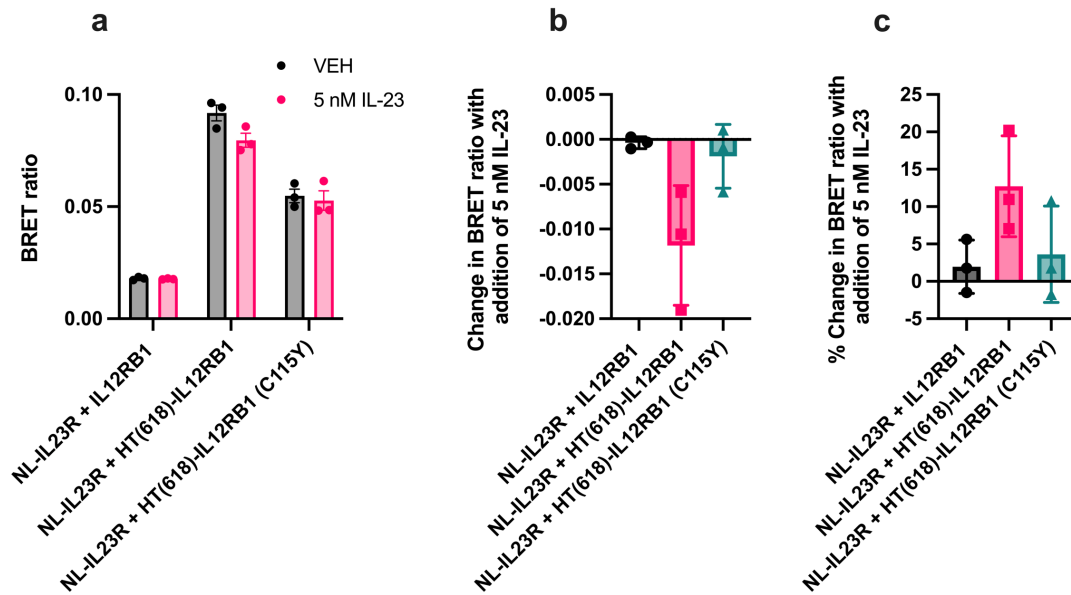


**Supplementary Fig. 1: Prediction of receptor expression label using a standard curve.**

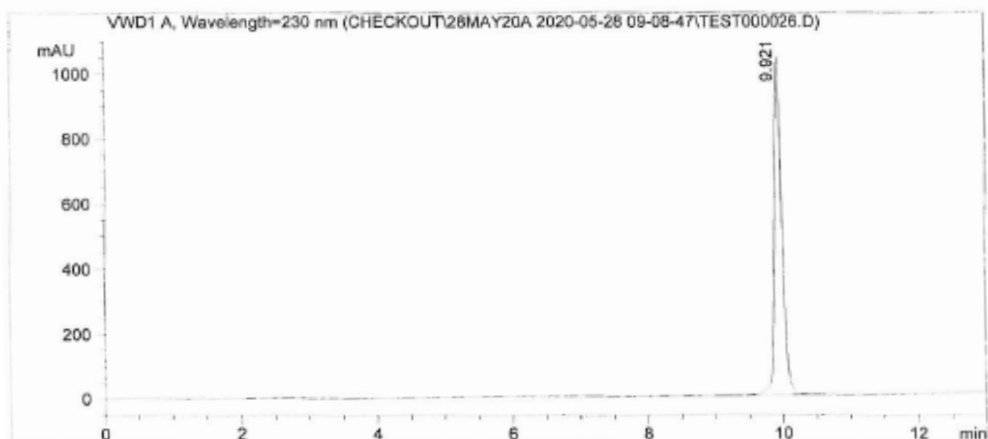
A previously generated standard curve of purified NanoLuc<sup>24</sup> was used to predict the expression level of IL-23 receptor components from luminescence values (in the absence of ligand) measured in the binding experiments shown in Figure 2. Data are mean  $\pm$  SEM from five independent experiments performed in triplicate.



**Supplementary Fig. 2: Non-competitive interaction between IL-23 and P630-TMR.** (a) Competitive displacement of several concentrations of P630-TMR by increasing concentrations of IL-23. (b) The data from (a) normalised to % of the maximum inhibition of the BRET signal. Data are mean  $\pm$  SEM from five independent experiments performed in triplicate.



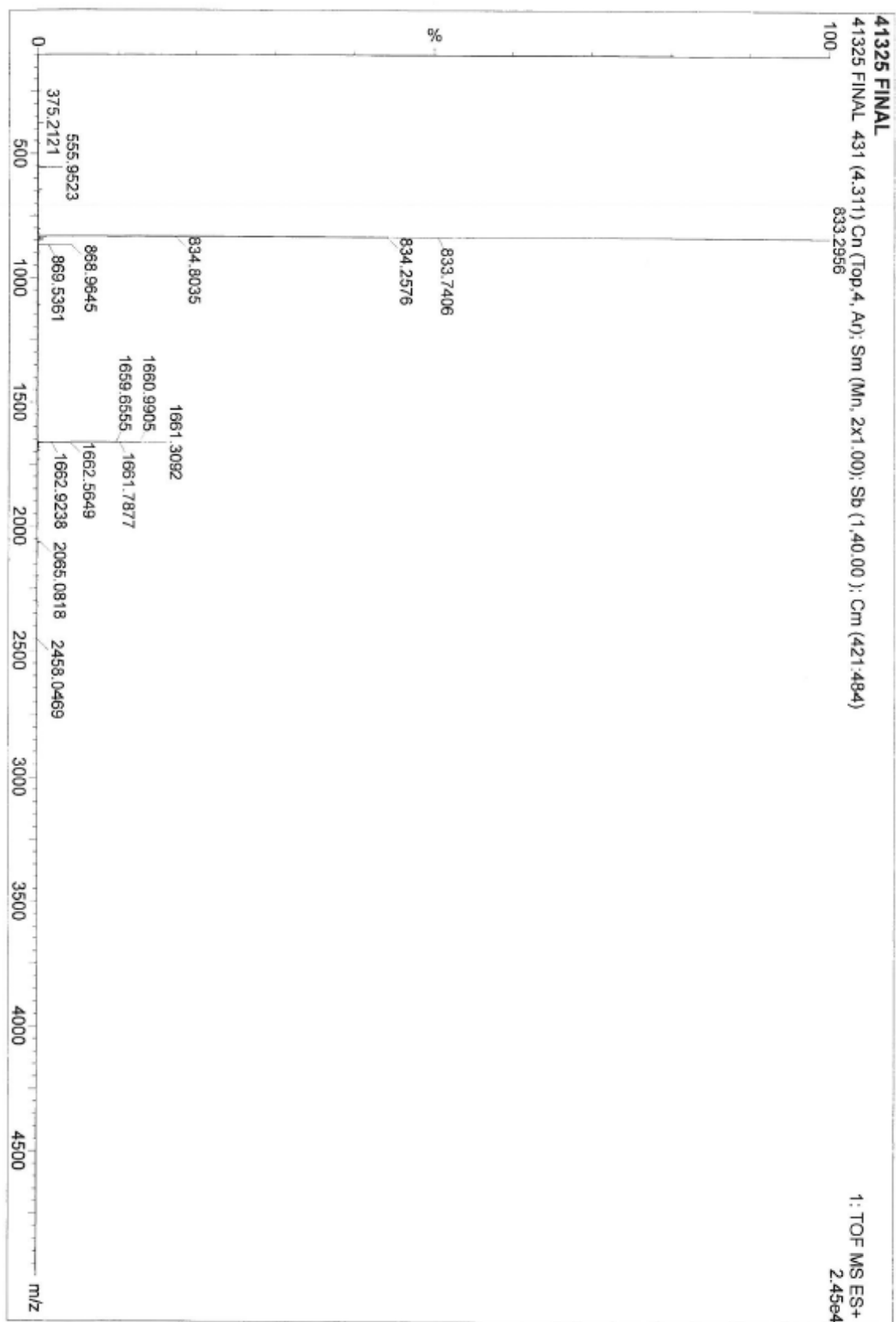
**Supplementary Fig. 3: The C115Y mutant of IL23R associated with IL12R $\beta$ 1 but does not undergo ligand induced conformational change.** (a) The BRET ratio generated by transiently transfected cells labelled with HT618 ligand in the presence and absence of IL-23. (b) the change in BRET ratio when the cells from (a) were treated with IL-23. (c) the data from (b) transformed to % change in BRET signal. Data are mean  $\pm$  SEM from three independent experiments conducted in pentuplicate.



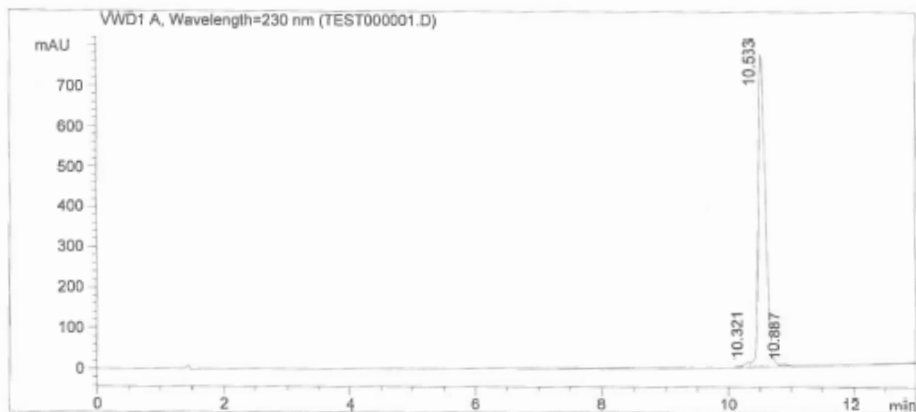
Column: ACE 3 C18-300 300A 150x2.1mm  
Buffer A: 0.1 % TFA in H<sub>2</sub>O  
Buffer B: 0.1 % TFA in MeCN  
Flow rate: 0.35mL/min  
Gradient: 2 to 70% B over 13 min

| # | RT (Min) | Area     | Height   | Area % |
|---|----------|----------|----------|--------|
| 1 | 9.921    | 8081.531 | 1040.918 | 100.0  |

**Supplementary Fig. 4: High Performance Liquid chromatography analysis of P630.** These data were provided by Cambridge Research Biochemicals.



**Supplementary Fig. 5: Mass spectroscopy analysis of P630.** These data were provided by Cambridge Research Biochemicals.



Column: ACE 3 C18-300 300A 150x2.1mm  
Buffer A: 0.1 % TFA in H<sub>2</sub>O  
Buffer B: 0.1 % TFA in MeCN  
Flow rate: 0.35mL/min  
Gradient: 2 to 70% B over 13 min

| # | RT (Min) | Area     | Height  | Area % |
|---|----------|----------|---------|--------|
| 1 | 10.321   | 63.384   | 9.180   | 1.0    |
| 2 | 10.533   | 6116.216 | 772.829 | 98.4   |
| 3 | 10.887   | 35.843   | 4.312   | 0.5    |

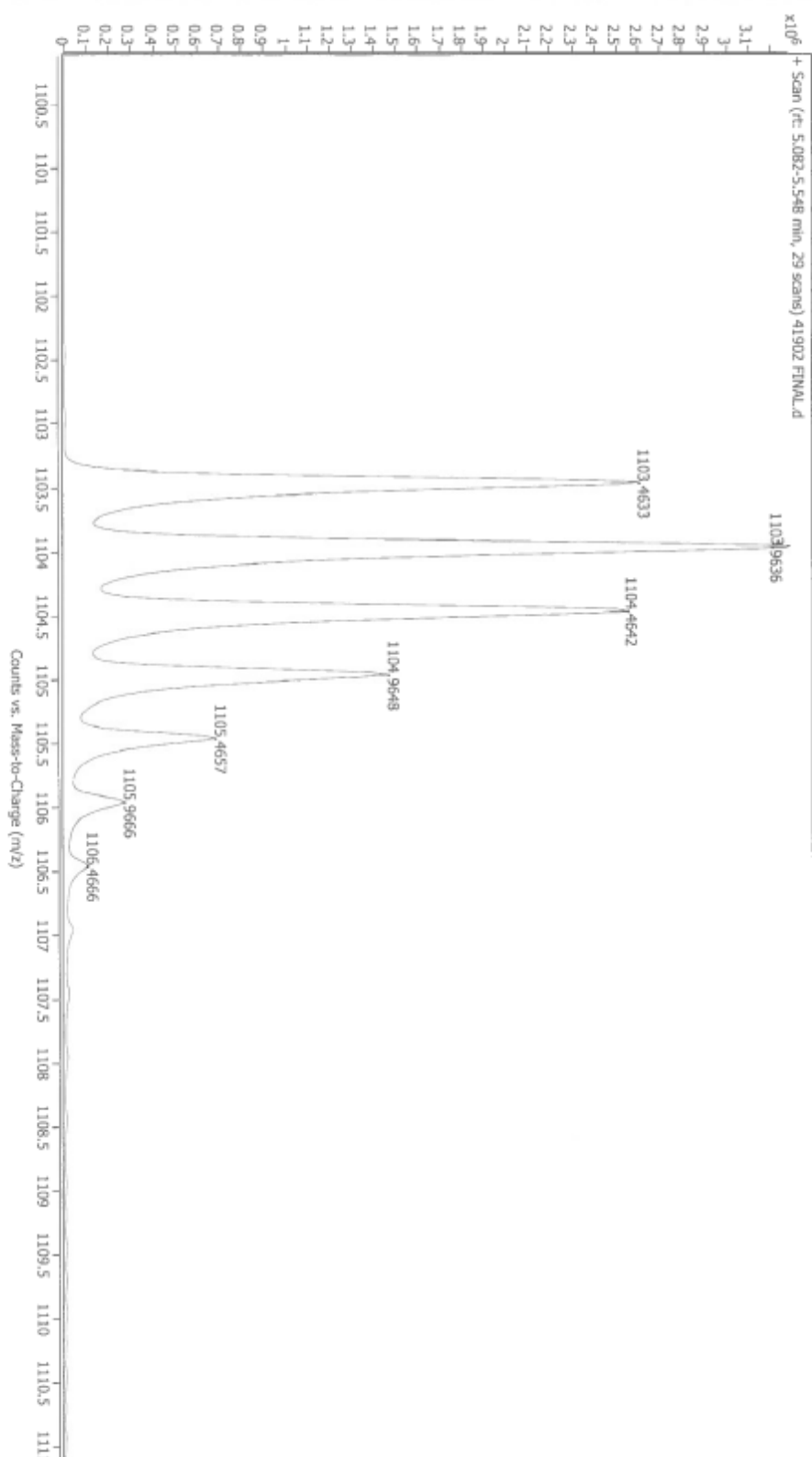
**Supplementary Fig. 6: High Performance Liquid chromatography analysis of P630-TMR.**

These data were provided by Cambridge Research Biochemicals.



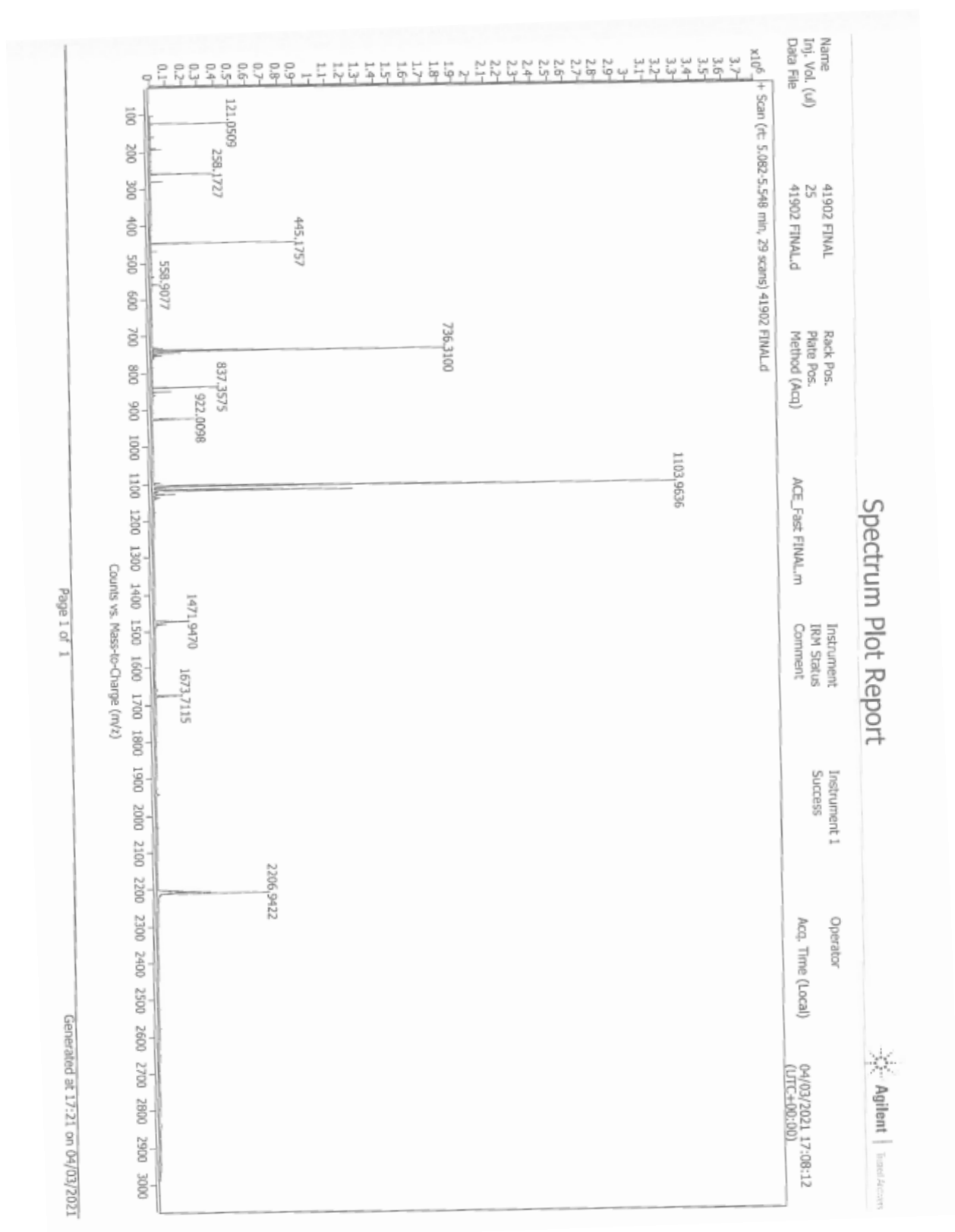
Agilent

|                |               |              |                  |                   |                                 |
|----------------|---------------|--------------|------------------|-------------------|---------------------------------|
| Name           | 41902.FINAL   | Rack Pos.    | Instrument       | Operator          |                                 |
| Inj. Vol. (ul) | 25            | Plate Pos.   | IRM Status       | Success           |                                 |
| Data File      | 41902.FINAL.d | Method (Acq) | ACE_Fast.FINAL.m | Acq. Time (local) | 04/03/2021 17:08:12 (UTC+00:00) |



**Supplementary Fig. 7: Mass spectroscopy analysis of P630-TMR.** These data were provided by Cambridge Research Biochemicals.





**Supplementary Fig. 8: Mass spectroscopy analysis of P630-TMR.** These data were provided by Cambridge Research Biochemicals.