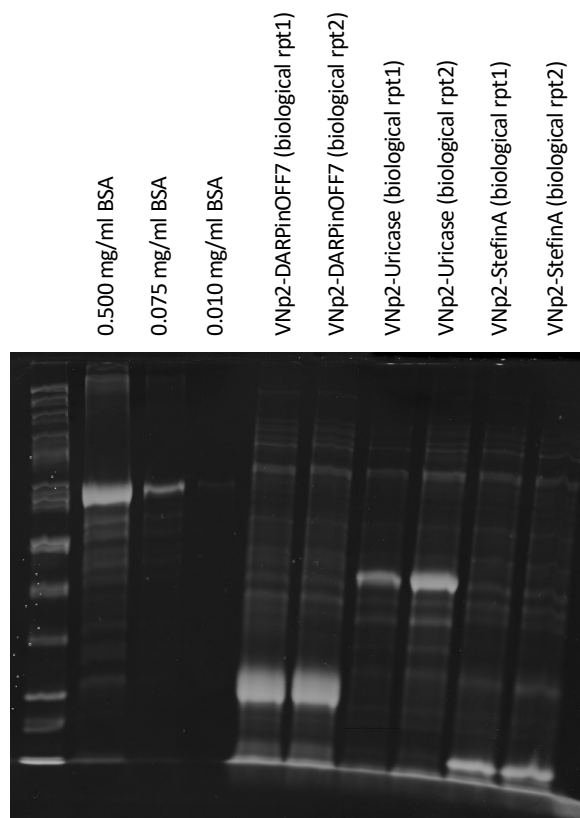
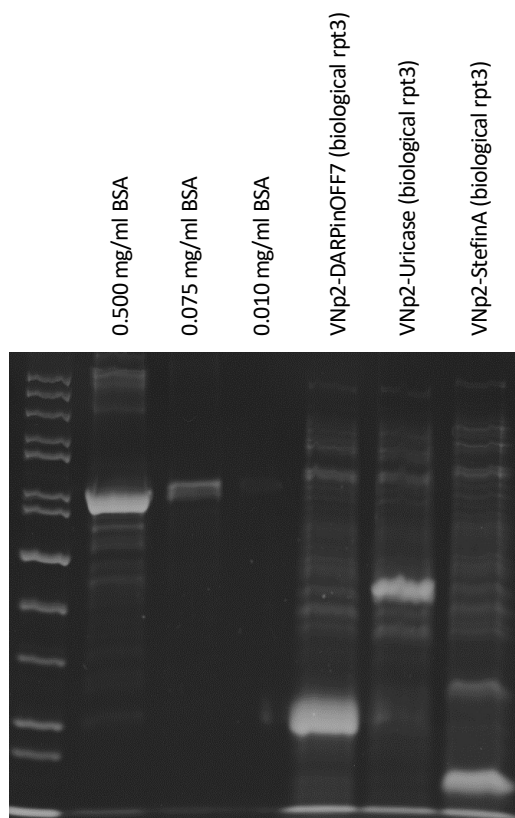


Determination of protein yield from filtered culture media:

Vesicles were isolated directly from overnight bacterial cell cultures by passing the culture through a sterile and detergent-free 0.45 µm polyethersulfone (PES) filter. 10 µl of filtered culture media was loaded onto a 10% acrylamide gel for SDS-PAGE analysis. 10 µl of BSA loading standards also were loaded for calibration of each gel by densitometry analysis. The coomassie stained gels were scanned and analysed using ImageJ software.



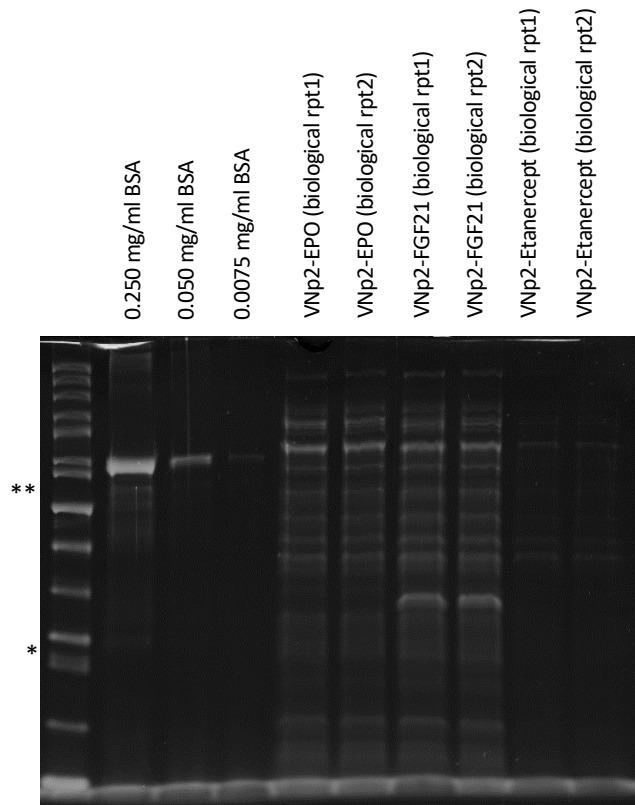
Filtered culture media samples.



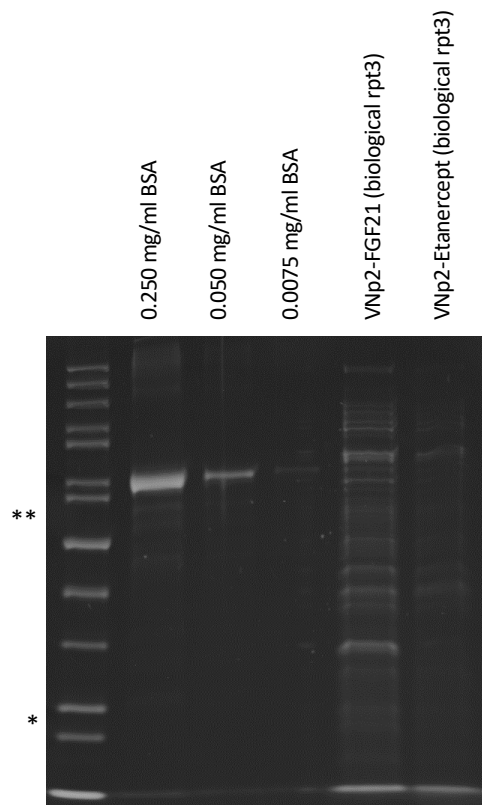
Filtered culture media samples.

Calculated yields – mg/L of culture:

<u>VNp2-DARPinOFF7:</u>	
Biological rpt1	865.7 mg/L
Biological rpt2	853.3 mg/L
Biological rpt3	876.7 mg/L
<u>VNp2-Uricase:</u>	
Biological rpt1	183.9 mg/L
Biological rpt2	416.5 mg/L
Biological rpt3	367.8 mg/L
<u>VNp2-StefinA:</u>	
Biological rpt1	397.1 mg/L
Biological rpt2	402.1 mg/L
Biological rpt3	419.7 mg/L



Filtered culture media samples.
* VNP2-EPO – 23.7 kDa
** VNP2-Etanercept – 56.6 kDa



Filtered culture media samples.

Calculated yields – mg/L of culture:

VNP2-EPO:

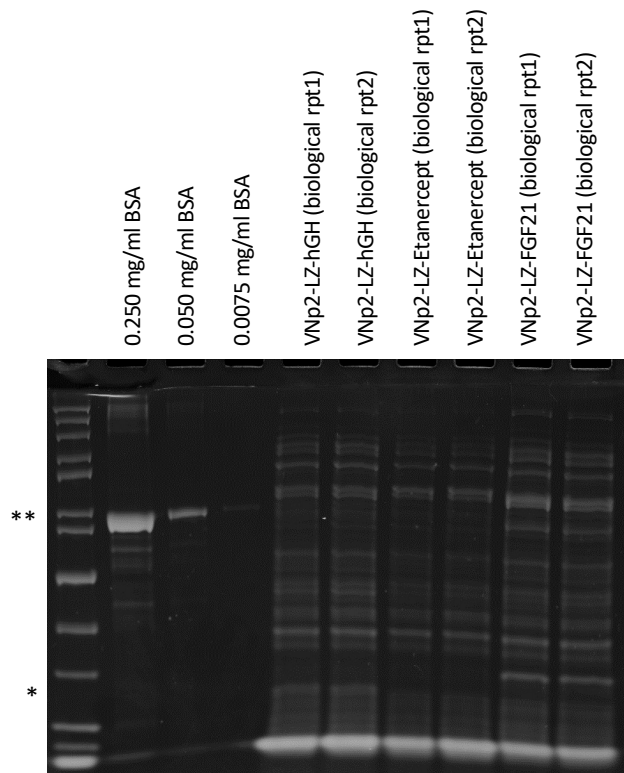
Biological rpt1	ND
Biological rpt2	ND

VNP2-FGF21:

Biological rpt1	50.3 mg/L
Biological rpt2	58.0 mg/L
Biological rpt3	46.8 mg/L

VNP2-Etanercept:

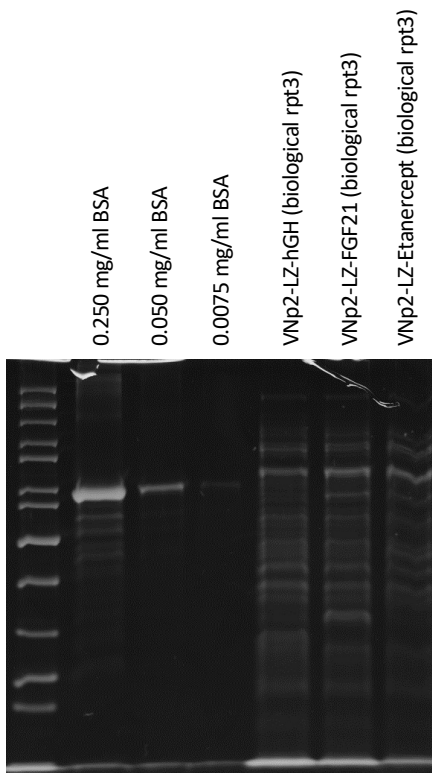
Biological rpt1	ND
Biological rpt2	ND
Biological rpt3	ND



Filtered culture media samples.

* VNp2-LZ-hGH – 30.1 kDa

** VNp2-LZ-Etanercept – 60.5 kDa



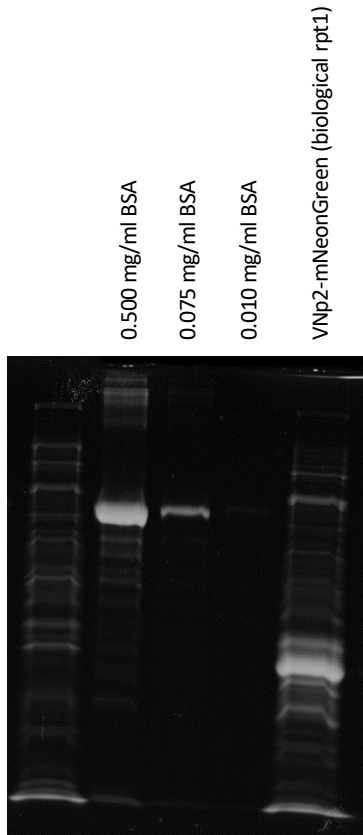
Filtered culture media samples.

Calculated yields – mg/L of culture:

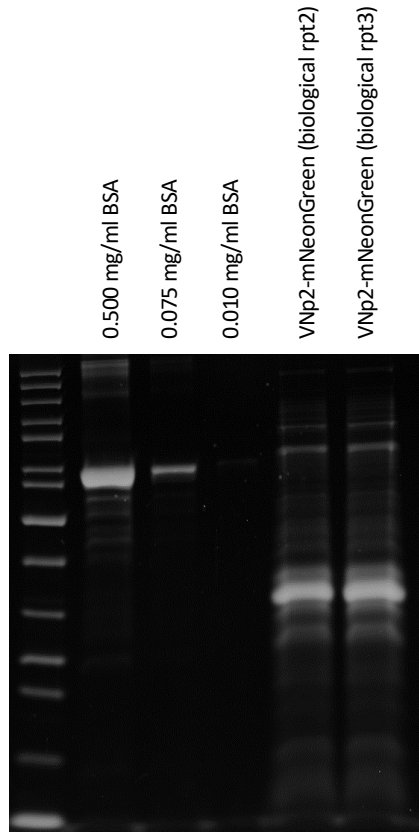
<u>VNp2-LZ-hGH:</u>	
Biological rpt1	9.0 mg/L
Biological rpt2	11.3 mg/L
Biological rpt3	10.1 mg/L

<u>VNp2-LZ-Etanercept:</u>	
Biological rpt1	ND
Biological rpt2	ND
Biological rpt3	ND

<u>VNp2-LZ-FGF21:</u>	
Biological rpt1	37.6 mg/L
Biological rpt2	36.7 mg/L
Biological rpt3	32.4 mg/L



Filtered culture media samples.

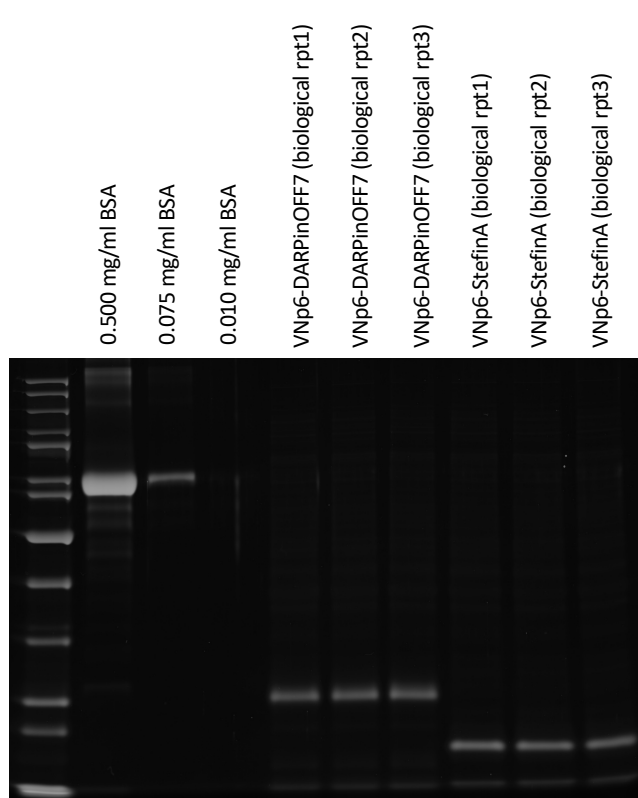


Filtered culture media samples.

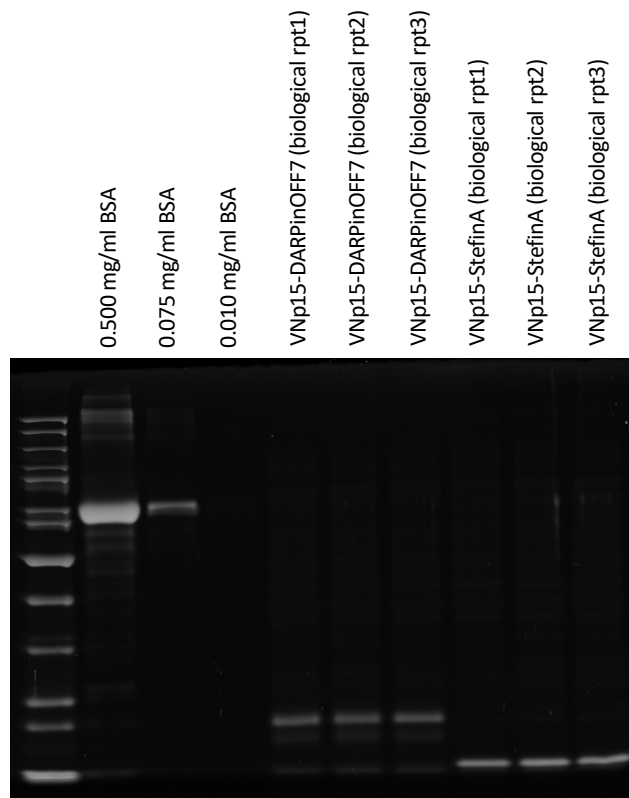
Calculated yields – mg/L of culture:

<u>VNp2-mNeonGreen:</u>	
Biological rpt1	525.4 mg/L
Biological rpt2	552.9 mg/L
Biological rpt3	685.5 mg/L

Filtered culture media was diluted 10-fold and 10 µl loaded to a 10% Acrylamide gel for SDS-PAGE analysis.



1:10 Filtered culture media samples.



1:10 Filtered culture media samples.

Calculated yields – mg/L of culture:

VNp6-DARPinOFF7:

Biological rpt1	2009.1 mg/L
Biological rpt2	2111.6 mg/L
Biological rpt3	2312.6 mg/L

VNp6-StefinA:

Biological rpt1	2021.4 mg/L
Biological rpt2	1792.8 mg/L
Biological rpt3	1708.9 mg/L

VNp15-DARPinOFF7:

Biological rpt1	1605.8 mg/L
Biological rpt2	1500.2 mg/L
Biological rpt3	1479.0 mg/L

VNp15-StefinA:

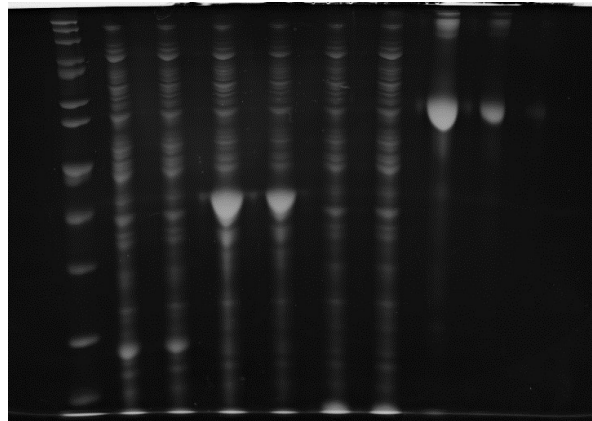
Biological rpt1	2047.6 mg/L
Biological rpt2	2385.3 mg/L
Biological rpt3	2358.6 mg/L

Determination of soluble protein yield from cell pellets:

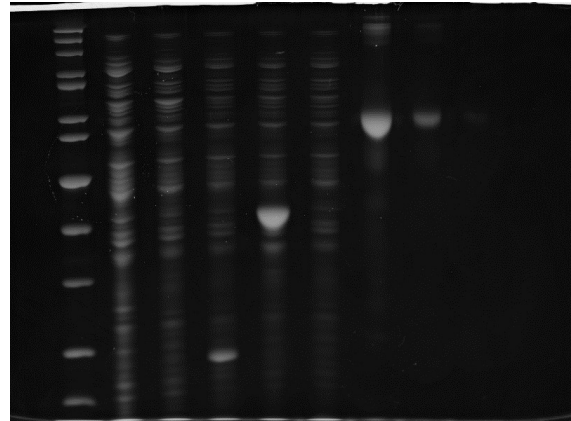
Cell pellets from 50 ml of culture were harvested by centrifugation and resuspended in 5 ml of soluble extract buffer (20 mM TRIS, 500 mM NaCl, pH 8.0). Cells were lysed by sonication for a total of 2 min (6 x 20 sec pulses), and cell debris removed by centrifugation at 39,000 xg (4 °C) for 30 min. Cell lysates were diluted 10-fold and 10 µl of diluted lysate was loaded onto a 10% acrylamide gel for SDS-PAGE analysis. 10 µl of BSA loading standards also were loaded for calibration of each gel by densitometry analysis. The coomassie stained gels were scanned and analysed using ImageJ software.

VNp2-DARPinOFF7 (biological rpt1)
 VNp2-DARPinOFF7 (biological rpt2)
 VNp2-Uricase (biological rpt1)
 VNp2-Uricase (biological rpt2)
 VNp2-StefinA (biological rpt1)
 VNp2-StefinA (biological rpt2)
 0.500 mg/ml BSA
 0.075 mg/ml BSA
 0.010 mg/ml BSA

 **VNp2-FGF21 (biological rpt3)
 ***VNp2-Etanercept (biological rpt3)
 VNp2-DARPinOFF7 (biological rpt3)
 VNp2-Uricase (biological rpt3)
 VNp2-StefinA (biological rpt3)
 0.250 mg/ml BSA
 0.050 mg/ml BSA
 0.0075 mg/ml BSA



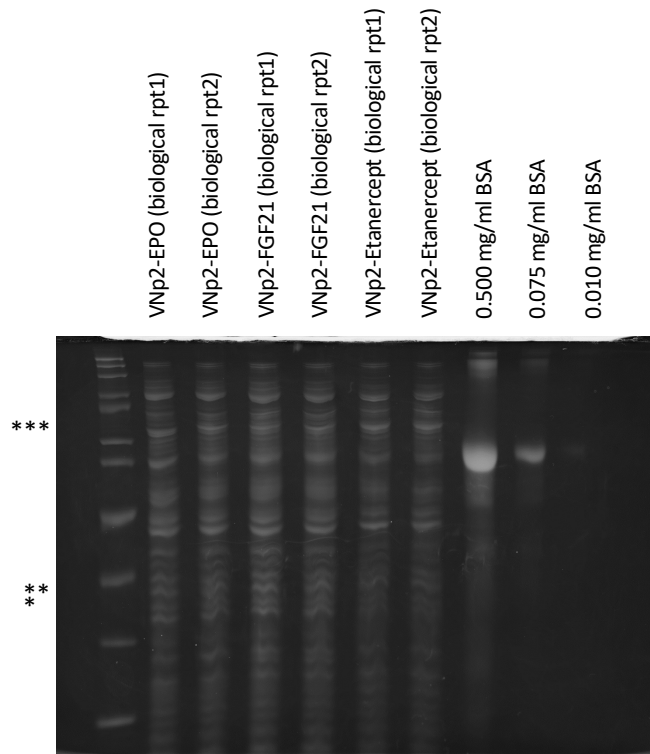
1:10 Soluble extract samples.



1:10 Soluble extract samples.

Calculated yields – mg/L of culture:

<u>VNp2-DARPinOFF7:</u>	
Biological rpt1	76.2 mg/L
Biological rpt2	32.0 mg/L
Biological rpt3	119.7 mg/L
<u>VNp2-Uricase:</u>	
Biological rpt1	742.4 mg/L
Biological rpt2	523.8 mg/L
Biological rpt3	465.4 mg/L
<u>VNp2-StefinA:</u>	
Biological rpt1	94.2 mg/L
Biological rpt2	103.2 mg/L



VNp2-EPO (biological rpt1)

VNp2-EPO (biological rpt2)

VNp2-FGF21 (biological rpt1)

VNp2-FGF21 (biological rpt2)

VNp2-Etanercept (biological rpt1)

VNp2-Etanercept (biological rpt2)

0.500 mg/ml BSA

0.075 mg/ml BSA

0.010 mg/ml BSA

**

1:10 Soluble extract samples.

* VNp2-EPO – 23.7 kDa

** VNp2-FGF21 – 24.7 kDa

*** VNp2-Etanercept – 56.6 kDa

Calculated yields – mg/L of culture:

VNp2-EPO:

Biological rpt1 ND

Biological rpt2 ND

VNp2-FGF21:

Biological rpt1 ND

Biological rpt2 ND

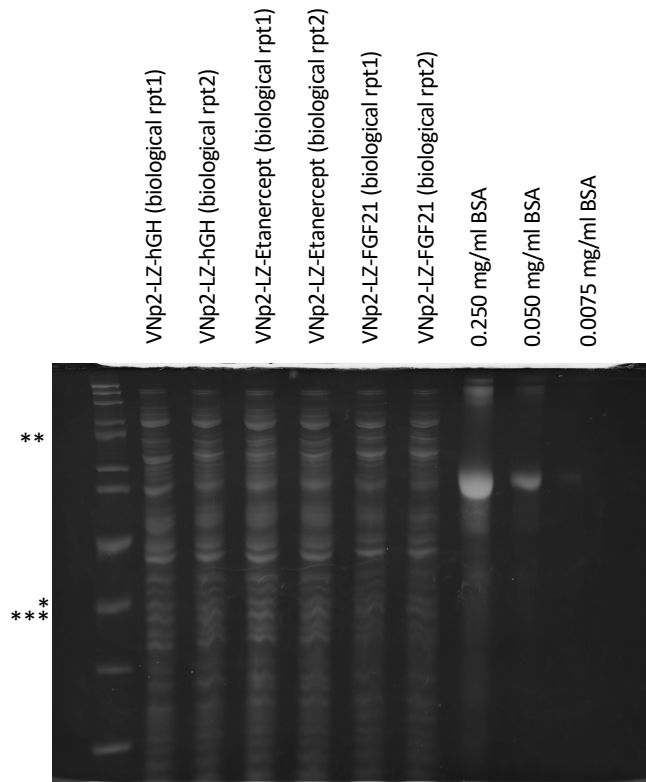
Biological rpt3 ND

VNp2-Etanercept:

Biological rpt1 ND

Biological rpt2 ND

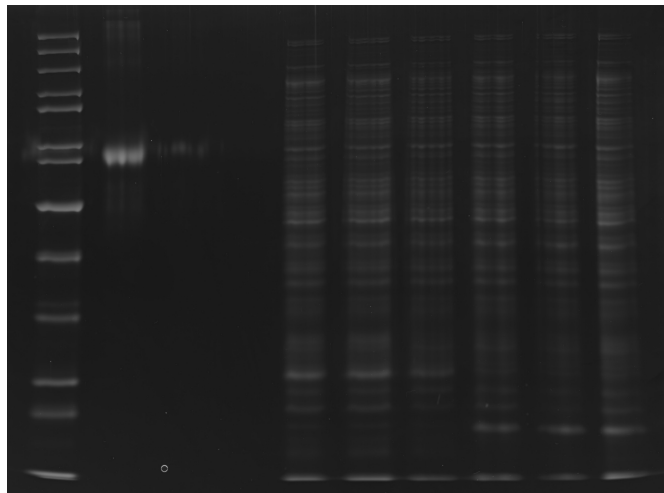
Biological rpt3 ND



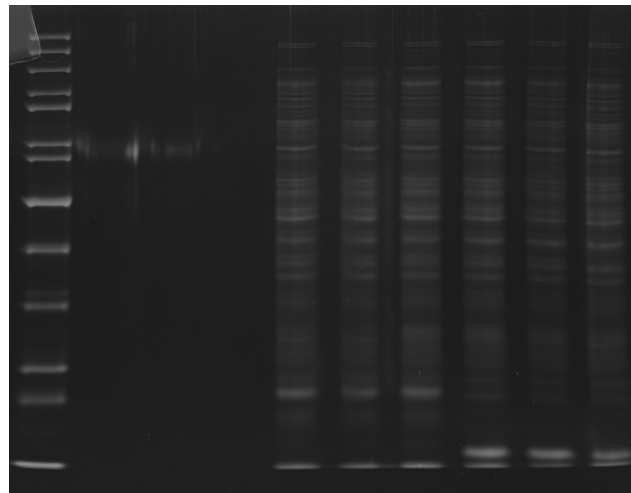
1:10 Soluble extract samples.
 * VNp2-LZ-hGH – 30.1 kDa
 ** VNp2LZ--Etanercept – 60.5 kDa
 *** VNp2-LZ-FGF21 – 28.7 kDa

Calculated yields – mg/L of culture:

<u>VNp2-LZ-hGH:</u>	
Biological rpt1	ND
Biological rpt2	ND
<u>VNp2-LZ-Etanercept:</u>	
Biological rpt1	ND
Biological rpt2	ND
<u>VNp2-LZ-FGF21:</u>	
Biological rpt1	ND
Biological rpt2	ND



1:10 Soluble extract samples.



1:10 Soluble extract samples.

Calculated yields – mg/L of culture:

VNp6-DARPinOFF7:

Biological rpt1	73.6 mg/L
Biological rpt2	74.2 mg/L
Biological rpt3	2.5 mg/L

VNp6-StefinA:

Biological rpt1	37.3 mg/L
Biological rpt2	30.0 mg/L
Biological rpt3	62.6 mg/L

VNp15-DARPinOFF7:

Biological rpt1	124.0 mg/L
Biological rpt2	65.2 mg/L
Biological rpt3	123.0 mg/L

VNp15-StefinA:

Biological rpt1	365.7 mg/L
Biological rpt2	310.5 mg/L
Biological rpt3	284.6 mg/L