PROTEOMICS PLATFORM
CHU DE QUEBEC RESEARCH CENTER
http://proteomique.ulaval.ca

Analyse LF97

Label Free relative protein quantification

Rapport de recherche présenté à Amita Singh and Hugo Germain

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1.0 SAMPLE PREPARATION AND MASS SPECTROMETRY

Protein digestion and mass spectrometry analyses were performed by the Proteomics Platform of the CHU de Québec Research Center (Quebec, Qc, Canada),

ÉCHANTILLONS ET DOSAGE PAR LE CLIENT – reçus le 15 Août 2019

Échantillon	Tampon	Volume (µL)	Conc. (µg/µL)	Quantité (µg)
1	50mM ABC + 1% DOC	25	2,4	48.2
2	50mM ABC + 1% DOC	25	1,8	36.5
3	50mM ABC + 1% DOC	25	1,7	34.37
4	50mM ABC + 1% DOC	25	3	60
5	50mM ABC + 1% DOC	25	2,8	56
6	50mM ABC + 1% DOC	25	1,9	39.5
7	50mM ABC + 1% DOC	25	2	40
8	50mM ABC + 1% DOC	25	1,7	35.45
9	50mM ABC + 1% DOC	25	1,1	22.48
10	50mM ABC + 1% DOC	25	2,8	57.5
11	50mM ABC + 1% DOC	25	1,3	26.59
12	50mM ABC + 1% DOC	25	1,5	30

Dosage par la plateforme – 04 Septembre 2019 – VF

Rien n'a pu être dosé à deux reprises : Le dosage au niveau des peptides sera effectué.

- DIGESTION –09 Septembre 2019 – VF
- Digérer tout (culot insoluble malgrès bioruptor + surnageant à pooler ensemble)
- Ajuster le volume à 30µL avec du 1% DOC 50mM ABC
- Chauffer à 95°C pendant 5 mins.
- Ajouter 2µL de DTT 0.5mg/mL (équivaut à 3,2mM, conc. finale = 0.2mM)
- Incuber à 37°C pendant 30 mins.
- Ajouter 2,74µL d'iodoacétamide 2.5mg/mL. (conc. finale = 0.8mM)
- Incuber à 37°C pendant 30 mins.
- Ajouter 0.2µg de trypsine (ratio TRP:protéine = 1:50)
- Incuber O/N à 37°C.
- Ajouter 45µL de Sample Buffer pour acidifier l'échantillon (vérifier le pH).
- Centrifuger 5 mins à 13000rpm.
- Procéder à la purification sur stagetip.
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PURIFICATION SUR STAGETIP – 10 Septembre 2019 – VF

- Préparer le stagetip avec 30µL de MeOH (centrifuger 3 mins à 3000rpm).
- Laver le stagetip avec 30µL de Sample buffer (centrifuger 3 mins à 3000rpm).
- Récupérer le surnageant de l'échantillon et le mettre dans le stagetip (centrifuger 3 mins à 3000rpm).
- Laver avec 30µL de Sample buffer (centrifuger 3 mins à 3000rpm).
- Changer de tube avant l'élution.
- Éluer avec 45µL de ACN 80%/Acide acétique 0,5% (centrifuger 3 mins à 3000rpm).
- Sécher les échantillons au speedvac.

DOSAGE NANODROP

	1	2	3	4	5	6	7	8	9	10	11	12
Abs 205nm	2.542	2.776	0.331	4.189	3.504	0.249	1.063	2.125	1.02	0.794	0.981	0.834
		2.822	0.339	4.055	3.549	0.25	1.072	2.094	1.054	0.864	0.963	0.849
		2.848	0.348	3.972	3.586	0.258	1.068	2.092	1.059	0.822	0.946	0.842
Abs moyenne	2.542	2.815	0.339	4.072	3.546	0.252	1.068	2.104	1.044	0.827	0.963	0.842
Concentration (ng/µL)	1015.391	1119.834	173.728	1600.020	1399.157	140.485	452.032	847.899	443.116	359.944	412.165	365.675
Concentration (µg/µL)	**1.015**	**1.120**	**0.174**	**1.600**	**1.399**	**0.140**	**0.452**	**0.848**	**0.443**	**0.360**	**0.412**	**0.366**
Volume	10	10	10	10	10	10	10	10	10	10	10	10
Quantité totale (µg)	10.15	11.20	1.74	16.00	13.99	1.40	4.52	8.48	4.43	3.60	4.12	3.66

MASS SPECTROMETRY

Samples (1.4 ug) were analysed by nanoLC/MSMS using a Dionex UltiMate 3000 nanoRSLC chromatography system (Thermo Fisher Scientific) connected to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA,USA) equipped with a nanoelectrospray ion source. Peptides were trapped at 20 µl/min in loading solvent (2% acetonitrile, 0.05% TFA) on a 5mm x 300 µm C18 pepmap cartridge pre-column (Thermo Fisher Scientific) during 5 minutes. Then, the pre-column was switch online with Pepmap Acclaim column (ThermoFisher) 50 cm x 75µm internal diameter separation column and the peptides were eluted with a linear gradient from 5-40% solvent B (A: 0,1% formic acid, B: 80% acetonitrile, 0.1% formic acid) in 90 minutes, at 300 nL/min. Mass spectra were acquired using a data dependent acquisition mode using Thermo XCalibur software version 4.1.50. Full scan mass spectra (350 to 1800m/z) were acquired in the orbitrap using an AGC target of 4e5,

a maximum injection time of 50 ms and a resolution of 120 000. Internal calibration using lock mass on the m/z 445.12003 siloxane ion was used. Each MS scan was followed by acquisition of fragmentation MSMS spectra of the most intense ions for a total cycle time of 3 seconds (top speed mode). The selected ions were isolated using the quadrupole analyzer in a window of 1.6 m/z and fragmented by Higher energy Collision-induced Dissociation (HCD) with 35% of collision energy. The resulting fragments were detected by the linear ion trap in rapid scan rate with an AGC target of 1e4 and a maximum injection time of 50ms. Dynamic exclusion of previously fragmented peptides was set for a period of 30 sec and a tolerance of 10 ppm.

DATA ANALYSIS

Spectra acquired were processed using ProteomeDiscoverer 2.3 (Thermo) with the Minora feature detector and Mascot for the search engine. Files were search against Uniprot REF homo sapiens protein database (74485 entries). Trypsin was set as enzyme and 2 missed cleavages were allowed. Deamidation (N, Q), oxidation (M), were set as dynamic modifications and carbamidomethylation (C), was set as static modifications. Mass search tolerance were 10ppm and 0.6Da for MS and MS/MS respectively. For protein validation, a maximum False Discovery Rate of 1% at peptide and protein level was used based on a target/decoy search. Unique and razor peptides are considered for protein quantification and normalisation was performed based on summed abundance of peptides. Peptides and protein result tabs were exported from proteome Discoverer into Excel. An adjusted p-value (Benjamini Hochberg) is calculated by proteome discoverer and a z-score was calculated. A protein was considered as differentially expressed if the adjusted p-value was lower than 0.01 and that its zscore was higher than 1.96 or lower than -1.96.

2.0 RESULTS

Result File: LF97 GermainH_201901017_ Results_proteins

LF97 GermainH _peptides

Main results in protein results files:

6033 identified proteins (Protein ID tab of the protein results file)

4849 quantified proteins (Quant proteins tab of the protein results file)

121 statistically variant proteins for the ratio CTRL/IFN

99 statistically variant proteins for the ratio CTRL/Virus

145 statiscally variant protein for the ratio CTRL/IFN virus

129 statiscally variant protein for the ratio IFN/ virus

151 statiscally variant protein for the ratio IFN/IFN virus

130 statiscally variant protein for the ratio virus/IFN virus

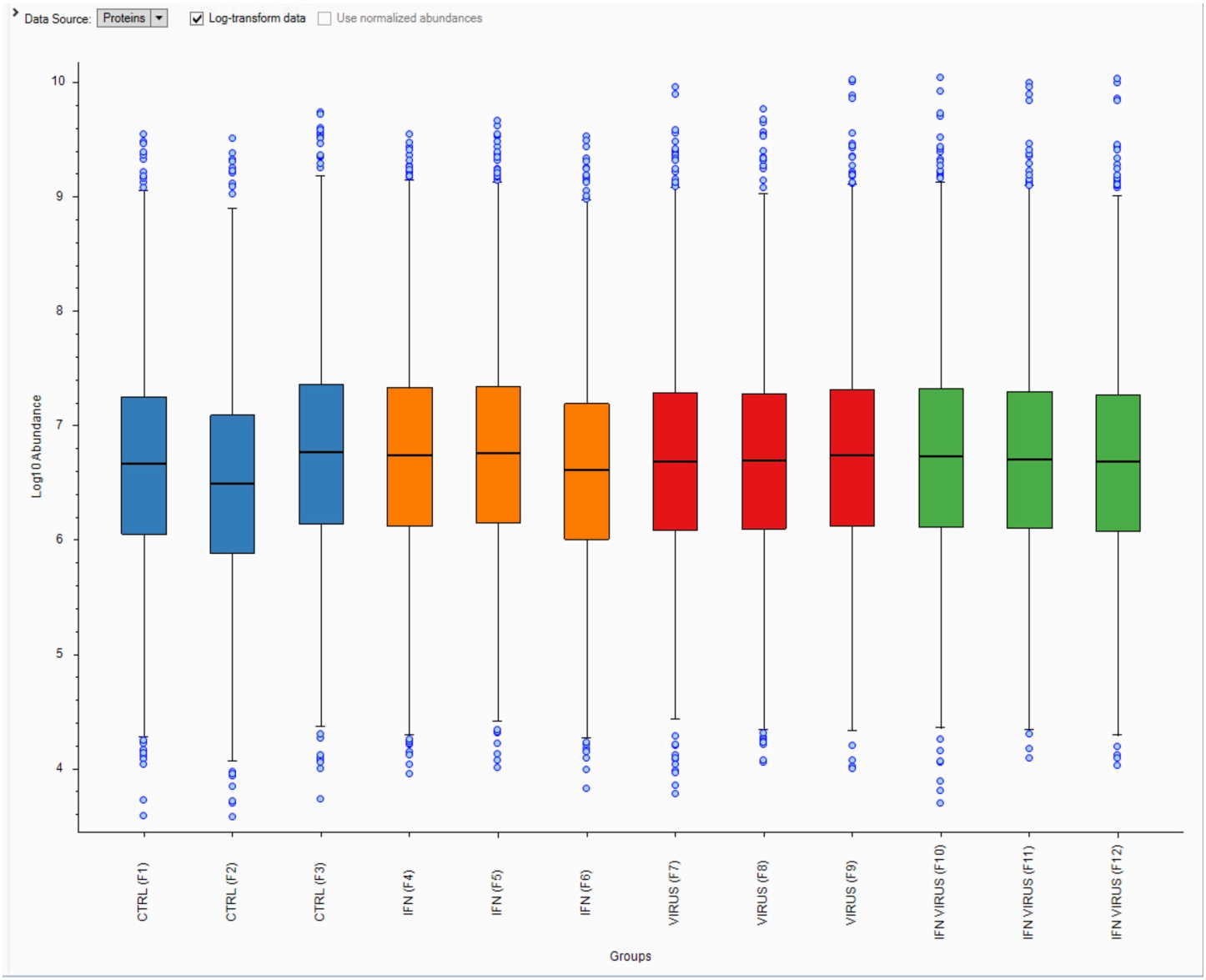
At least two unique quantifed peptides per protein were needed in order to obtain quantification for the protein.

A p-value, an adjusted p-value and a z-score were calculated.

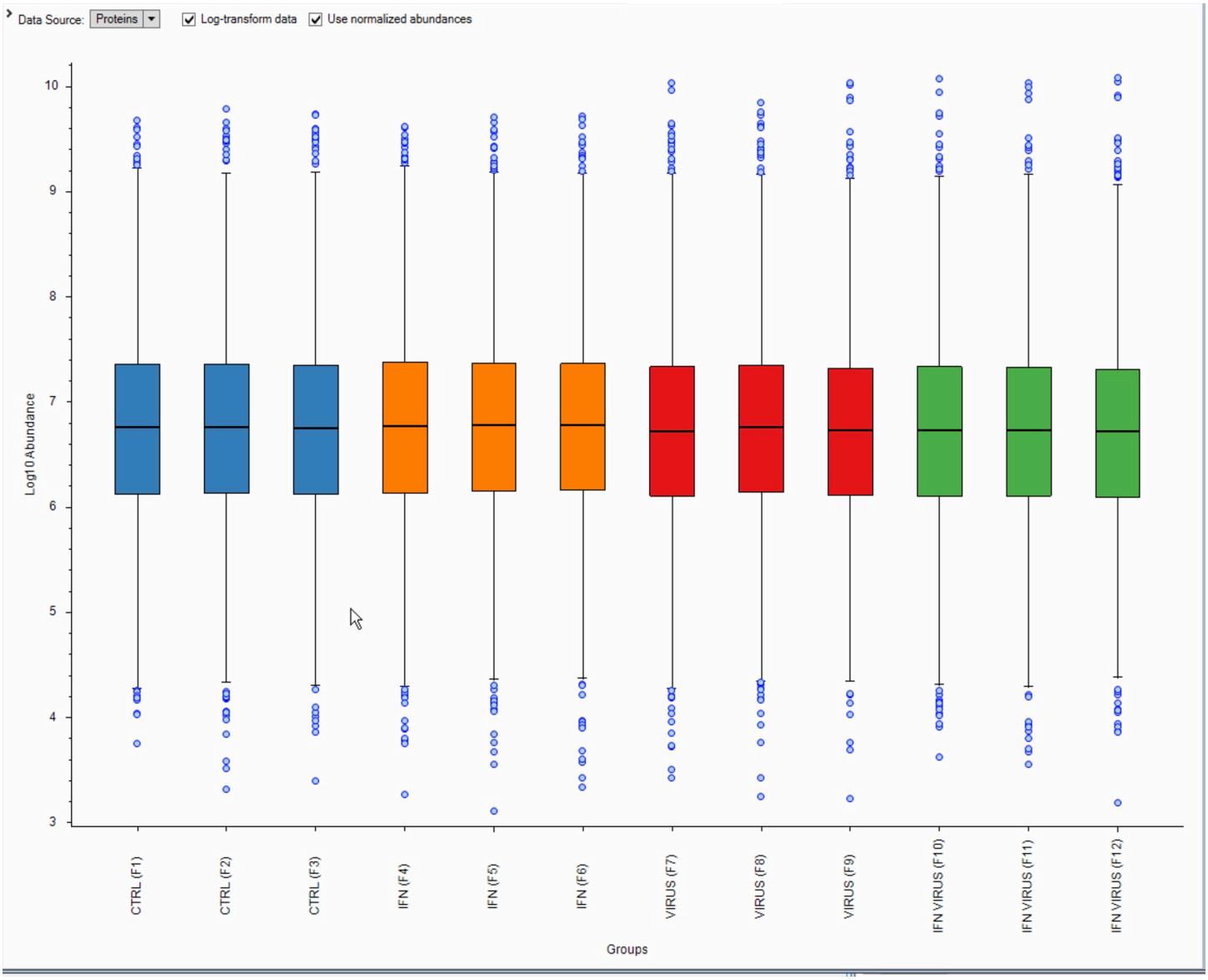
To be significanly differentially expressed, a protein have to have an adjusted p-value less than 0.01 and a z-score lower than -1.96 or higher than 1.96.

Graphical results:

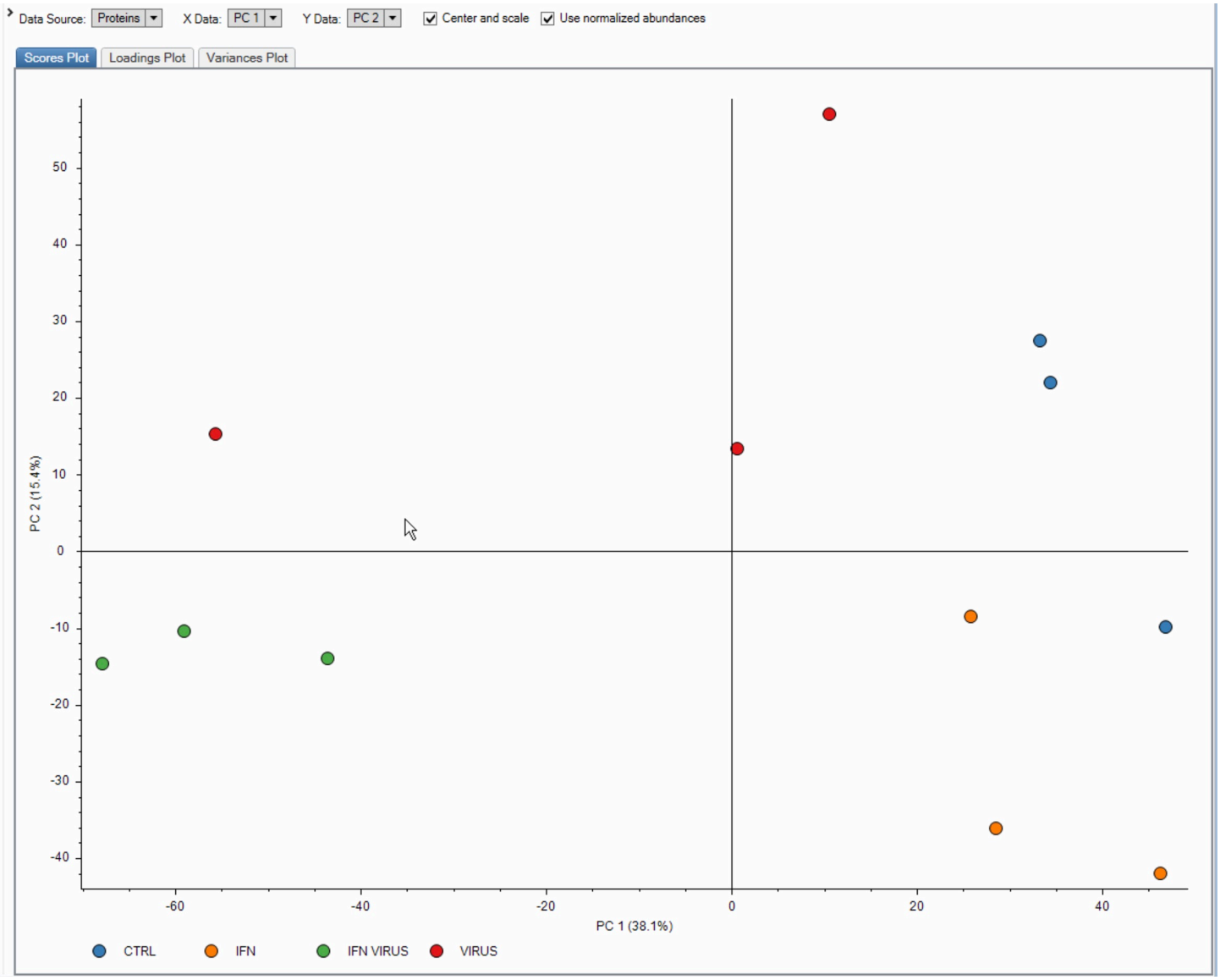
LF97 Boxplot before normalisation



LF97 Boxplot after normalisation

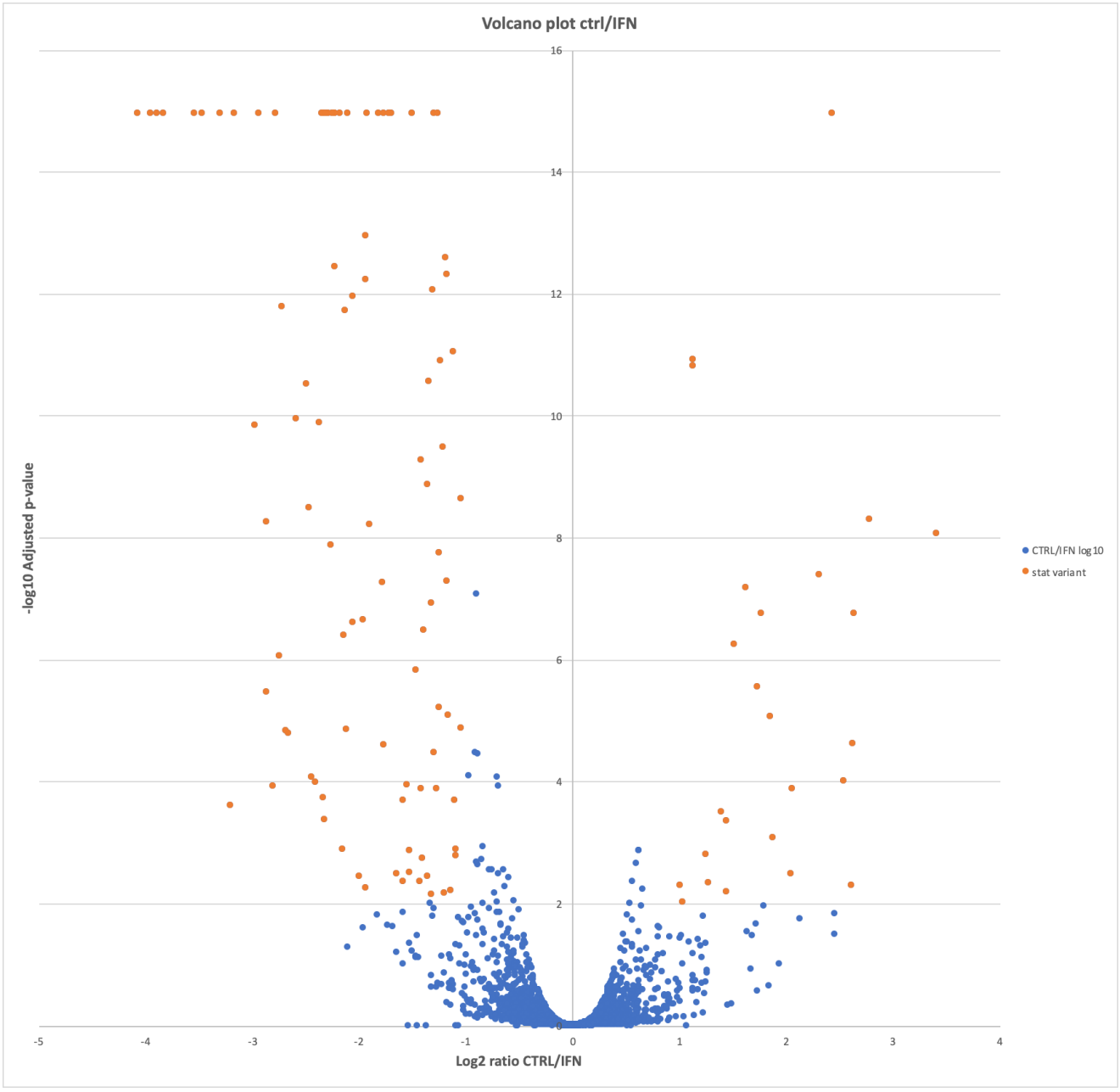


LF97 Principal Component Analysis

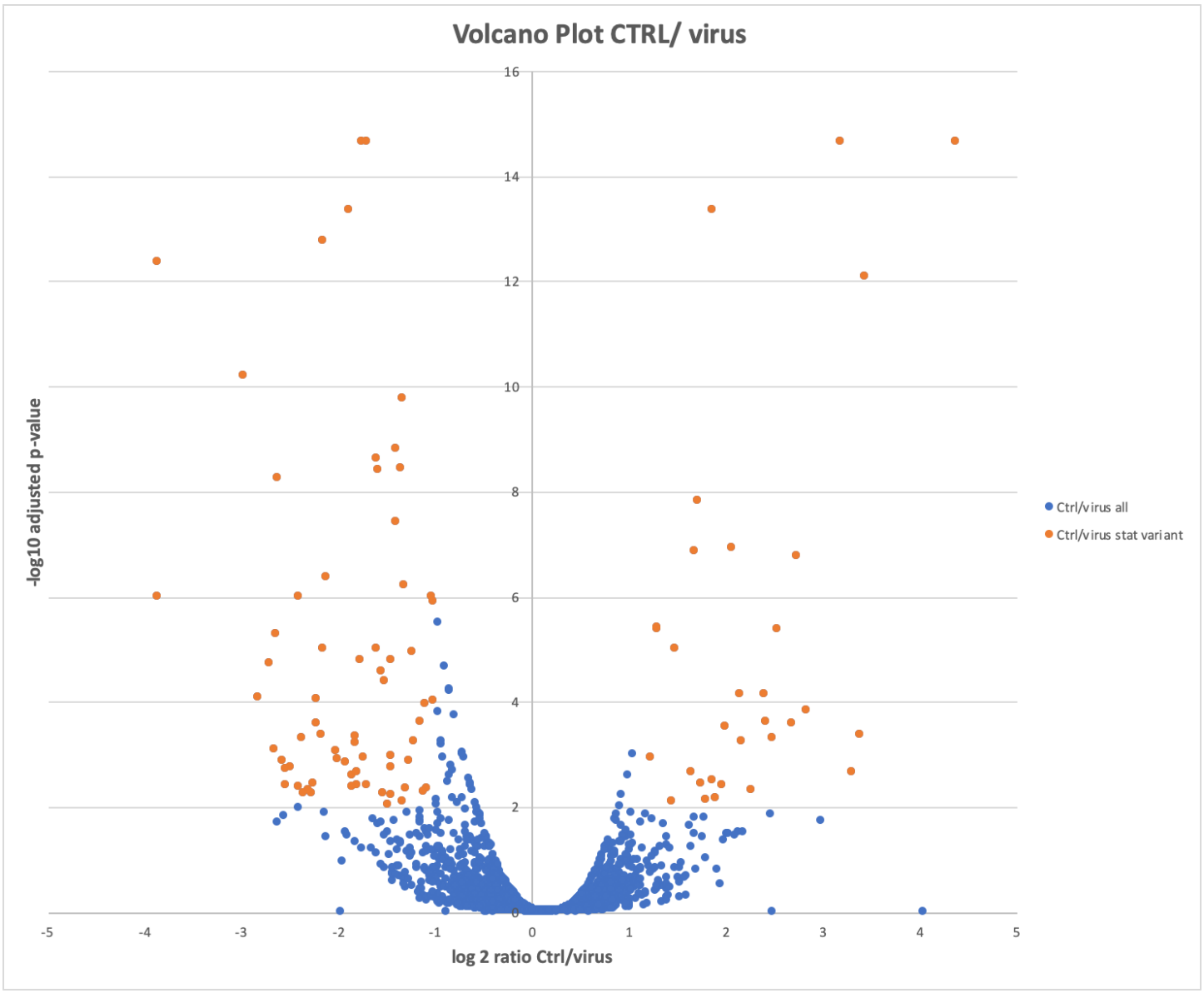


LF97 Volcano plot

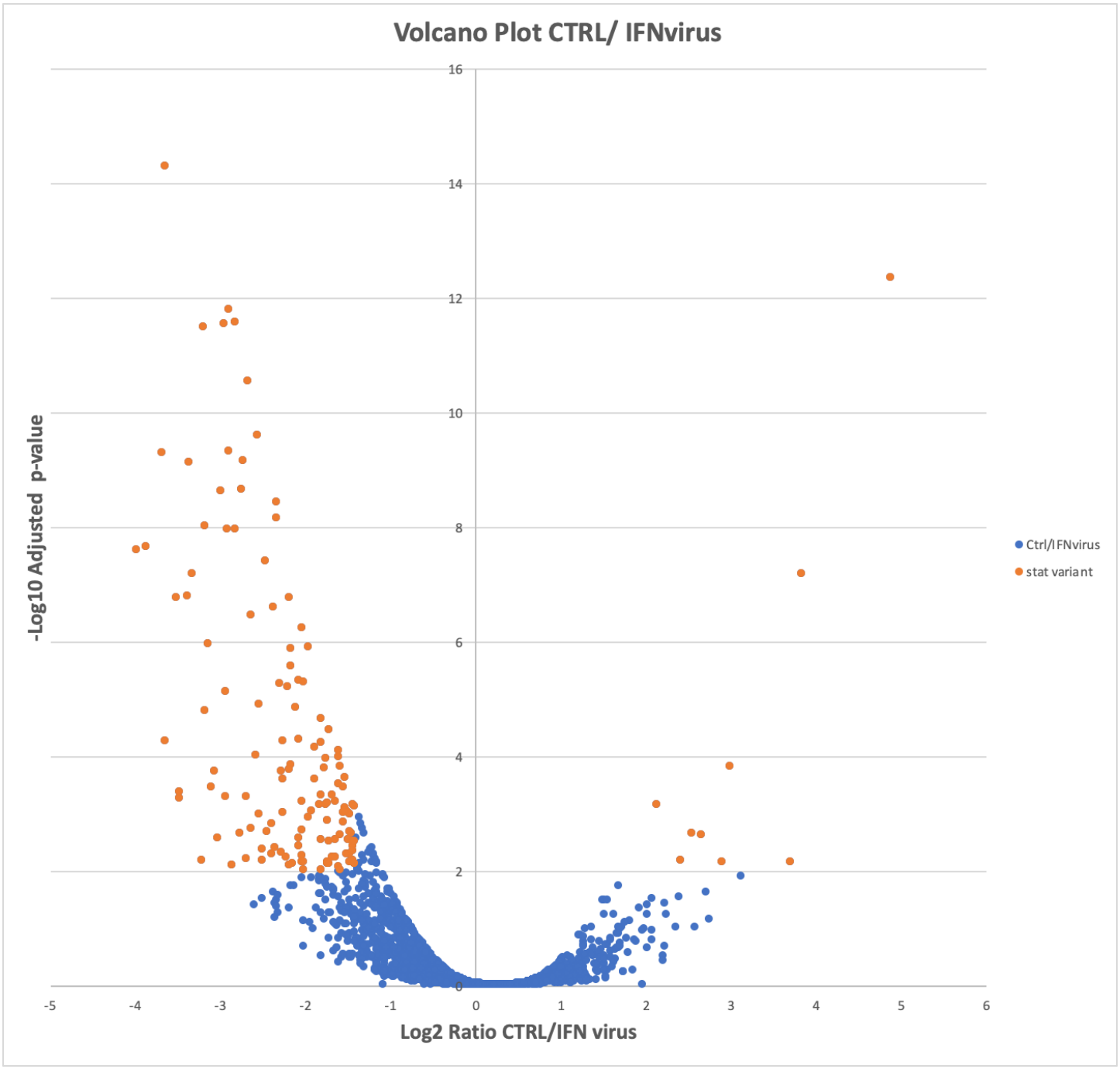
CTRL/IFN



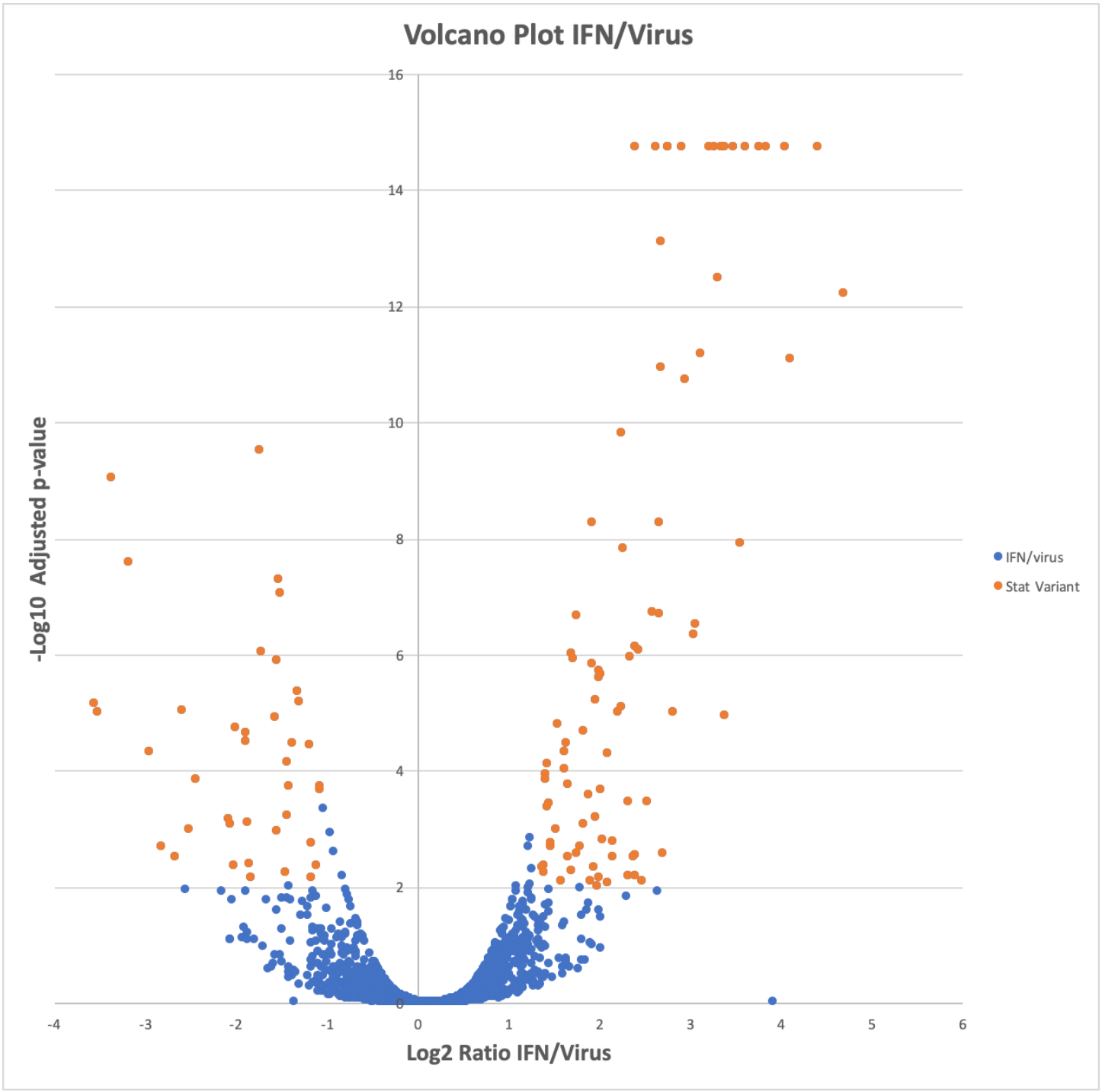
CTRL/Virus


Volcano Plot CTRL/ virus
-log10 adjusted p-value
log 2 ratio Ctrl/virus
16
14
12
10
8
6
4
2
0
-5
-4
-3
-2
-1
0
1
2
3
4
5
Ctrl/virus all
Ctrl/virus stat variant

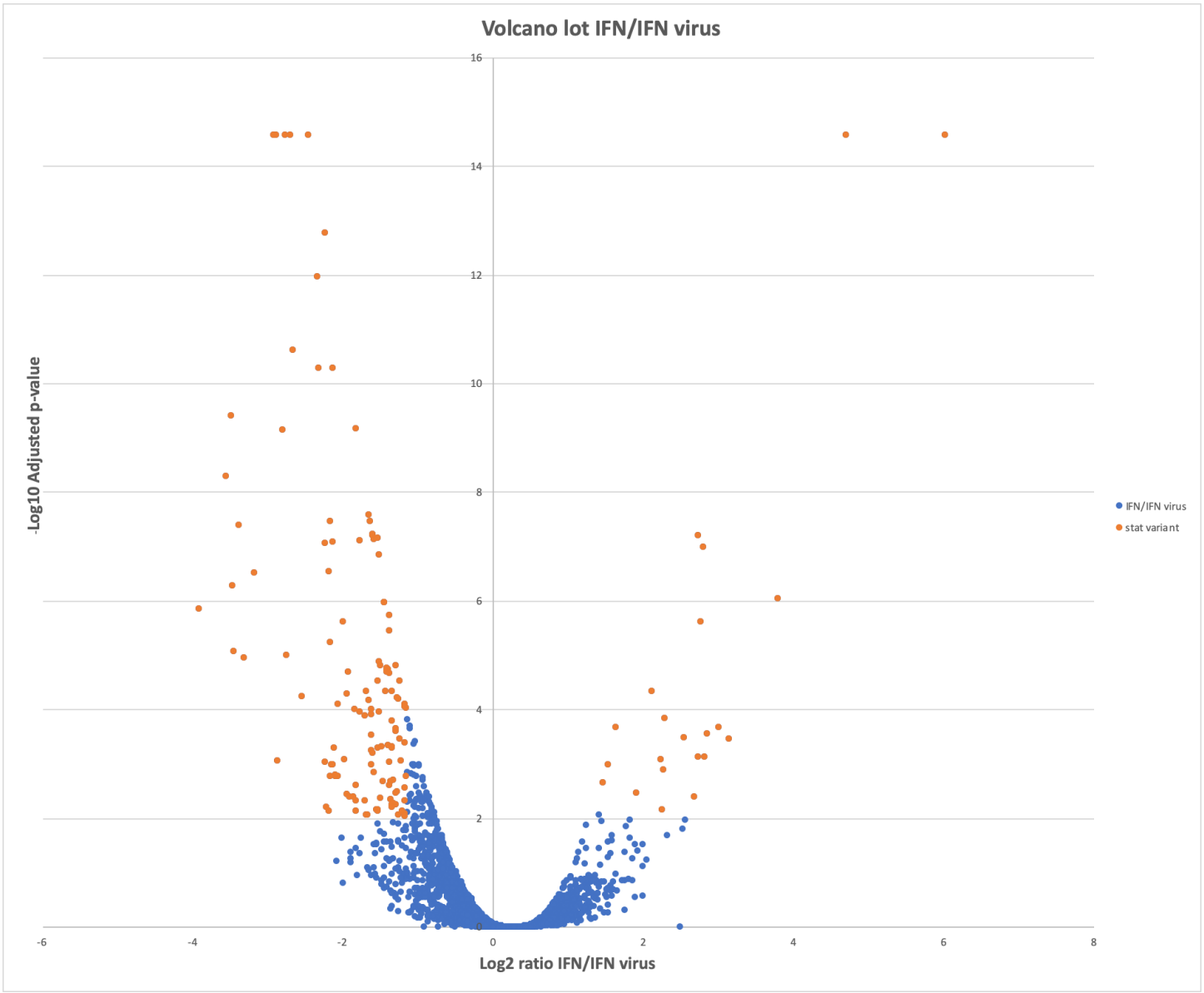
CTRL/IFN Virus



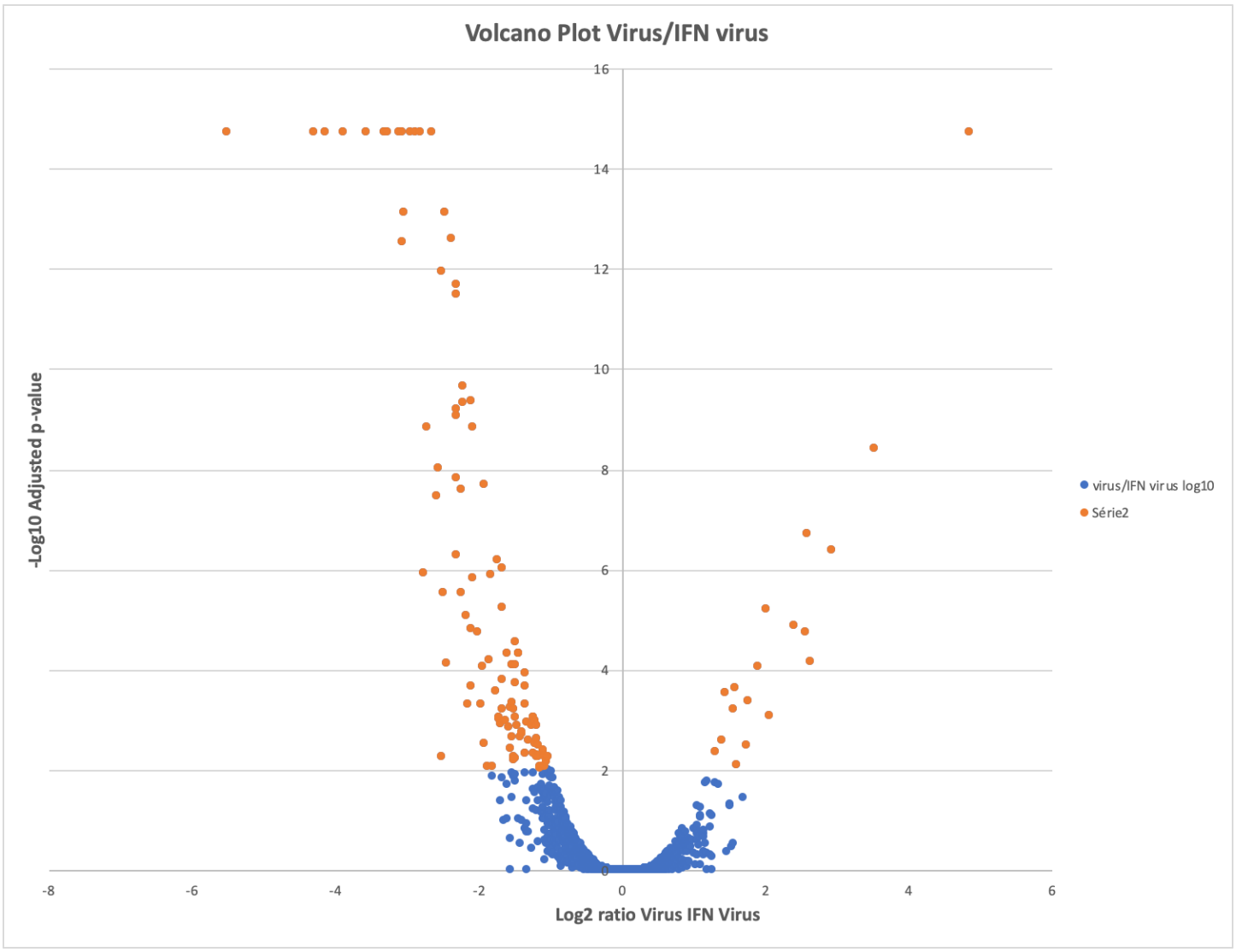
IFN/Virus


Volcano Plot IFN/Virus
-Log10 Adjusted p-value
Log2 Ratio IFN/Virus
16
14
12
10
8
6
4
2
0
-4
-3
-2
-1
0
1
2
3
4
5
6
IFN/virus
Stat Variant

IFN/IFNvirus



Virus/IFN virus



LF97 Heatmap