

AP/NTS Gfral TRAP-seq

Alan Rupp

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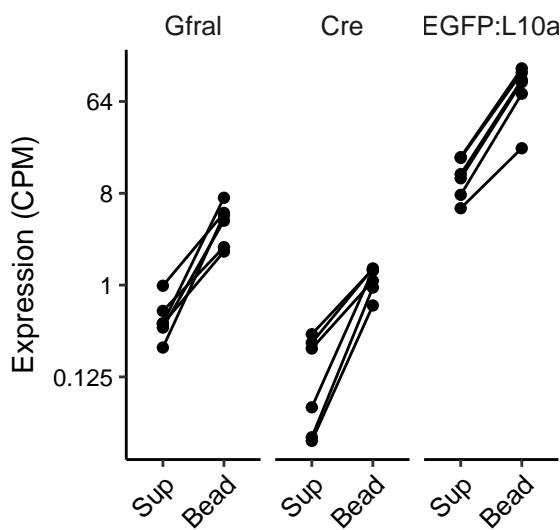
Analyzing TRAP-seq data from *GfralCre::L10* mice dissected from the AP/NTS. Data is combined from 2 runs, one of which had mice that were treated with either GDF15 or Vehicle. I'm looking for genes enriched in *Gfral* neurons and genes that are regulated in *Gfral* neurons in response to GDF15 treatment.

Samples

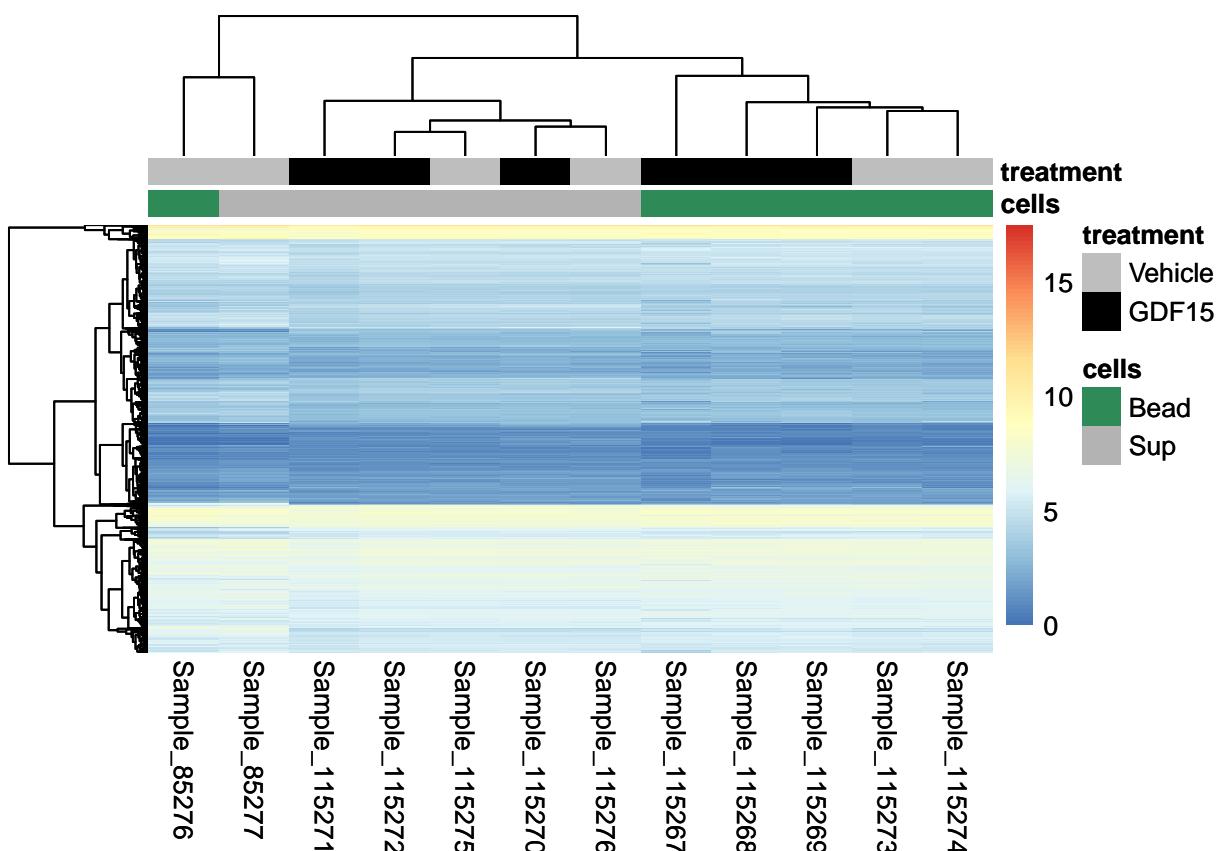
Sample_ID	cells	run	treatment
Sample_85276	Bead	Run_1905	Vehicle
Sample_85277	Sup	Run_1905	Vehicle
Sample_115267	Bead	Run_2487	GDF15
Sample_115268	Bead	Run_2487	GDF15
Sample_115269	Bead	Run_2487	GDF15
Sample_115270	Sup	Run_2487	GDF15
Sample_115271	Sup	Run_2487	GDF15
Sample_115272	Sup	Run_2487	GDF15
Sample_115273	Bead	Run_2487	Vehicle
Sample_115274	Bead	Run_2487	Vehicle
Sample_115275	Sup	Run_2487	Vehicle
Sample_115276	Sup	Run_2487	Vehicle

Quality control

Positive controls



Heatmap clustering



Samples Sample_85276 and Sample_85277 appear to be outliers.

tSNE Clustering

```
## [1] "Running PCA ..."
```

```
## [1] "Choosing PCs by statistical significance ..."  
## [1] "Running tSNE with perplexity = 3.7 ..."
```

● Sup ● Bead ● Vehicle ▲ GDF15

▲ Sample_115271

Sample_115276

● Sample_115270

▲ Sample_115272

● Sample_115275

Sample_85277

● Sample_85276

● Sample_115269

● Sample_115268

● Sample_115274

▲ Sample_115267

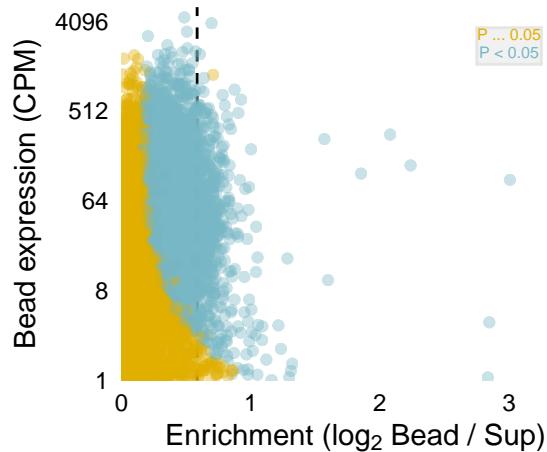
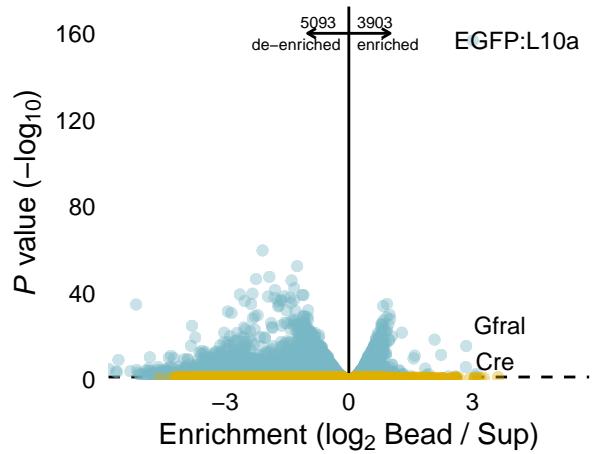
Sample_85277 appears to be an outlier using tSNE clustering as well. I'm going to remove it and its pair from all analysis.

Differential gene expression

I'll use DESeq2 1.26.0 to find differentially expressed genes.

Enrichment

```
## [1] "Creating DESeq2 object ..."  
## [1] "Running DESeq ..."  
## [1] "Finding enriched genes ..."  
## [1] "Calculating CPM for each sample ..."
```

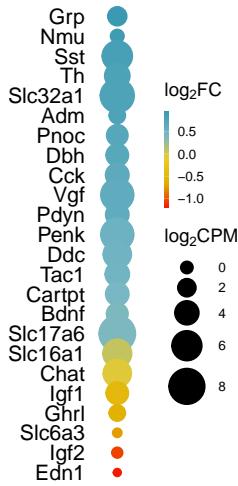


3903 genes significantly enriched. 530 genes significantly enriched > 1.5 -fold change.

Table 1: Top 10 enriched protein-coding genes (excluding Gfral, Cre, and GFP)

gene_name	gene_id	CPM	Enrichment	P
Taar1	ENSMUSG00000056379	0.5	3.09	1.7e-03
Cdc20b	ENSMUSG00000078926	0.3	2.59	2.8e-02
Pbk	ENSMUSG00000022033	0.3	2.41	2.7e-02
Bahcc1	ENSMUSG00000039741	144.5	2.24	2.8e-12
Afp	ENSMUSG00000054932	0.7	1.93	2.1e-03
Yeats2	ENSMUSG00000041215	119.8	1.85	6.6e-06
Casr	ENSMUSG00000051980	10.2	1.60	6.7e-12
Tnrc18	ENSMUSG00000039477	266.3	1.57	1.3e-10
Insm2	ENSMUSG00000045440	1.5	1.32	2.6e-03
Btn2a2	ENSMUSG00000053216	1.3	1.31	2.4e-03

Enrichment of known neuronal signaling genes



GDF15 effects

Now using DESeq2 to identify the effects of GDF15.

```
## [1] "Setting up DESeq2 object ..."  
## [1] "Running DESeq ..."  
## [1] "Finding regulated genes ..."
```

1 genes are significant regulated by GDF15 treatment in the Bead.

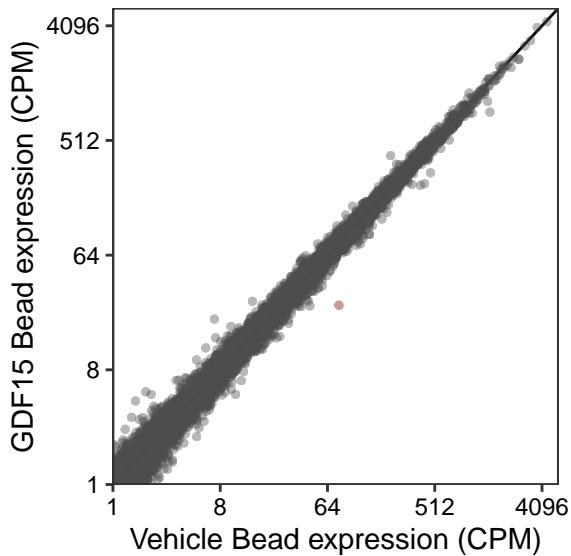
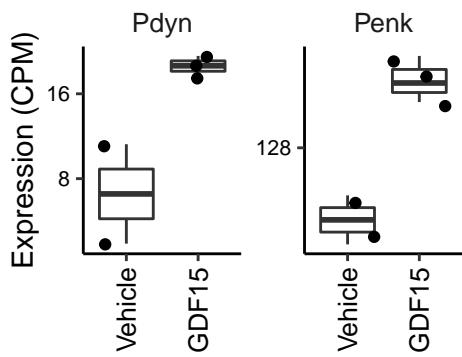


Table 2: Top regulated genes in Bead

Gene	Vehicle	GDF15	Regulation (log2)	P
Rn7sk	6.34	4.74	-1.68	5.6e-04
Pdyn	3.02	4.40	1.41	2.5e-01
Penk	6.72	7.29	0.59	7.5e-01
Ide	6.82	6.28	-0.54	8.6e-01



Files

I'm saving the R image as `analysis.Rdata` for future easy loading.

Session info

```
## R version 3.6.3 (2020-02-29)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 18.04.5 LTS
##
## Matrix products: default
## BLAS:    /usr/lib/x86_64-linux-gnublas/libblas.so.3.7.1
## LAPACK:  /usr/lib/x86_64-linux-gnulapack/liblapack.so.3.7.1
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
## [3] LC_TIME=en_US.UTF-8      LC_COLLATE=en_US.UTF-8
## [5] LC_MONETARY=en_US.UTF-8   LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
## [9] LC_ADDRESS=C              LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics   grDevices utils      datasets  methods   base
##
## other attached packages:
## [1] ggdendro_0.1.21  wesanderson_0.3.6 reticulate_1.16  ggrepel_0.8.2
## [5]forcats_0.5.0    stringr_1.4.0    dplyr_1.0.2     purrr_0.3.4
## [9]readr_1.3.1     tidyverse_1.2.1  kableExtra_1.2.1 knitr_1.30
## [13]tidyverse_1.2.1
##
## loaded via a namespace (and not attached):
## [1] Rtsne_0.15          colorspace_1.4-1
## [3] ellipsis_0.3.1      htmlTable_1.12
## [5] XVector_0.26.0      GenomicRanges_1.38.0
## [7]base64enc_0.1-3      rstudioapi_0.11
## [9]farver_2.0.3        bit64_4.0.5
## [11]AnnotationDbi_1.48.0 fansi_0.4.1
## [13]lubridate_1.7.9     xml2_1.3.2
## [15]splines_3.6.3       geneplotter_1.64.0
## [17]Formula_1.2-3       jsonlite_1.7.1
## [19]broom_0.7.0         annotate_1.64.0
## [21]cluster_2.1.0       pheatmap_1.0.12
## [23]compiler_3.6.3      httr_1.4.2
## [25]backports_1.1.10    assertthat_0.2.1
## [27]Matrix_1.2-18       cli_2.0.2
## [29]htmltools_0.5.0     tools_3.6.3
## [31]gtable_0.3.0        glue_1.4.2
## [33]GenomeInfoDbData_1.0.0 Rcpp_1.0.5
## [35]Biobase_2.46.0      cellranger_1.1.0
## [37]vctrs_0.3.4         xfun_0.18
## [39]rvest_0.3.6         lifecycle_0.2.0
## [41]XML_3.99-0.3        zlibbioc_1.32.0
## [43]MASS_7.3-53         scales_1.1.1
## [45]hms_0.5.3           parallel_3.6.3
## [47]SummarizedExperiment_1.16.1 RColorBrewer_1.1-2
## [49]yaml_2.2.1          memoise_1.1.0
## [51]gridExtra_2.3        rpart_4.1-15
## [53]latticeExtra_0.6-28  stringi_1.5.3
## [55]RSQLite_2.2.0        genefilter_1.68.0
## [57]S4Vectors_0.24.4     checkmate_2.0.0
## [59]BiocGenerics_0.32.0  BiocParallel_1.20.1
## [61]GenomeInfoDb_1.22.1  rlang_0.4.8
## [63]pkgconfig_2.0.3      matrixStats_0.56.0
```

```
## [65] bitops_1.0-6           evaluate_0.14
## [67] lattice_0.20-41        htmlwidgets_1.5.2
## [69] labeling_0.3            bit_4.0.4
## [71] tidyselect_1.1.0         magrittr_1.5
## [73] DESeq2_1.26.0           R6_2.4.1
## [75] IRanges_2.20.2          generics_0.0.2
## [77] Hmisc_4.4-1             DelayedArray_0.12.3
## [79] DBI_1.1.0                pillar_1.4.6
## [81] haven_2.3.1              foreign_0.8-76
## [83] withr_2.3.0              survival_3.2-7
## [85] RCurl_1.98-1.2           nnet_7.3-14
## [87] modelr_0.1.2             crayon_1.3.4
## [89] rmarkdown_2.4              locfit_1.5-9.4
## [91] grid_3.6.3                readxl_1.3.1
## [93] data.table_1.13.0         blob_1.2.1
## [95] digest_0.6.25            webshot_0.5.2
## [97] xtable_1.8-4              stats4_3.6.3
## [99] munsell_0.5.0             viridisLite_0.3.0
```