

ChIP-seq Analysis of TGF-beta Treated SMAD2 and SMAD3 Peaks

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Methods and Code

Note: the following code takes a long time to run. Hence, it shall just be displayed here for transparency but will not be run to compile the document.

Instead, the objects from the image saved in “overall_analysis.RData” after running this code will be loaded to present and discuss the results.

Libraries

The following libraries were loaded during this analysis:

Annotating Peaks

Peaks were annotated using the **ChIPseeker** package. The annotated peaks for each of the conditions were further filtered into three subsets: 1. All peaks 2. Peaks with only promoter annotations. 3. Peaks lying within genic regions, i.e., peaks without the annotations “Distal Intergenic” and “Downstream”

```
## Takes a filename as string input, and if it has suffix '.narrowPeak',
## converts it to a GRanges object containing a q_value metadata column.
## Requires tidyverse and GRanges packages.

narrow_to_granges <- function(file) {
  stopifnot(file.exists(file), endsWith(x = file, suffix = ".narrowPeak")) ## checking for existence

  bed <- as_tibble(read_tsv(file = file, col_names = c("chrom", "start", "end",
    "name", "score", "strand", "signal_value", "p_value", "q_value", "peak")))

  gr <- GRanges(seqnames = bed$chrom, ranges = IRanges(start = bed$start,
    end = bed$end), strand = if_else(condition = bed$strand == "+" | bed$strand ==
    "-", true = bed$strand, false = "*"), q_value = bed$q_value)

  return(gr)
}

### To read in multiple .narrowPeak files and create a GRanges list of GRanges
### objects with the input of respective files.

files <- dir(path = ".", pattern = "\\..narrowPeak$") ## specify files in character vector

grl <- lapply(files, narrow_to_granges)

TxDb <- TxDb.Hsapiens.UCSC.hg38.knownGene

v <- str_remove_all(string = files, pattern = "\\..narrowPeak$") ## vector of variable names for granges

### Assign individual granges within grl to variable names in v.
```

```

for (i in seq_along(grl)) {
  assign(x = v[i], value = grl[[i]])
}

ap_v <- paste0("AP_", v)  ## variable names for annotated peak objects

### Create annotated peak objects and assign them to names in ap_v

for (i in seq_along(grl)) {
  assign(x = ap_v[i], value = annotatePeak(peak = grl[[i]], TxDb = TxDb, annoDb = "org.Hs.eg.db"))
}

ap_v_df <- paste0("df_", ap_v)  ## variable names for tibbles derived from annotated peaks

### Create tibbles from annotated peak objects listed in ap_v and assign them
### to names in ap_v_df

for (i in seq_along(ap_v)) {
  assign(x = ap_v_df[i], value = AnnotationDbi::as.data.frame(eval(parse(text = ap_v[i]))) %>%
    as_tibble)
}

ap_v_prom_df <- paste0("prom_df_", ap_v)  ## variable names for tibbles derived from annotated peaks li

### Create subset of tibbles from variable names listed in ap_v_df and assign
### them to variable names in ap_v_prom_df
for (i in seq_along(ap_v_df)) {
  assign(x = ap_v_prom_df[i], value = parse(text = ap_v_df[i]) %>% eval() %>%
    filter(str_detect(annotation, "Promoter")))
}

ap_v_gen_df <- paste0("gen_df_", ap_v)  ## variable names for tibbles derived from annotated peaks limi

### Create tibbles from variable names listed in ap_v_df and assign them to
### names in ap_v_gen_df

for (i in seq_along(ap_v_df)) {
  assign(x = ap_v_gen_df[i], value = parse(text = ap_v_df[i]) %>% eval() %>%
    filter(!str_detect(annotation, "Distal Intergenic|Downstream")))
}

```

GO Term Enrichment Analysis of Annotated Peaks

GO term enrichment analysis of the annotated peaks was carried out using the **ClusterProfiler** package. Analysis was carried out for all four conditions and each subset of annotated peaks within each condition

(all peaks, promoter-limited peaks and genic region-limited peaks).

Note: The following code takes *very* long to run.

```
library(clusterProfiler)

ego_v <- paste0("ego_", v) ### Vector of variable names for go term enrichment objects

### Performing go term analysis using the clusterProfiler package and
### assigning the results to the variables in ego_v

for (i in seq_along(ego_v)) {
  ego <- enrichGO(gene = eval(parse(text = (ap_v_df[i])))[["geneId"]], OrgDb = org.Hs.eg.db,
    ont = "BP", pAdjustMethod = "BH", readable = TRUE)
  if (!nrow(as.data.frame(ego)) == 0)
    assign(x = ego_v[i], value = ego)
}

###

prom_ego_v <- ego_v <- paste0("prom_ego_", v) ### Vector of variable names for go term enrichment objects

for (i in seq_along(prom_ego_v)) {
  ego <- enrichGO(gene = eval(parse(text = (ap_v_prom_df[i])))[["geneId"]],
    OrgDb = org.Hs.eg.db, ont = "BP", pAdjustMethod = "BH", readable = TRUE)
  if (!nrow(as.data.frame(ego)) == 0)
    assign(x = prom_ego_v[i], value = ego)
}

gen_ego_v <- paste0("gen_ego_", v) ### Vector of variable names for go term enrichment objects limited

for (i in seq_along(gen_ego_v)) {
  ego <- enrichGO(gene = eval(parse(text = (ap_v_gen_df[i])))[["geneId"]],
    OrgDb = org.Hs.eg.db, ont = "BP", pAdjustMethod = "BH", readable = TRUE)
  if (!nrow(as.data.frame(ego)) == 0)
    assign(x = gen_ego_v[i], value = ego)
}

save(list = ls(), file = "overall_analysis.RData")
```

Results

The following file, which contained the results of the previous analyses was loaded.

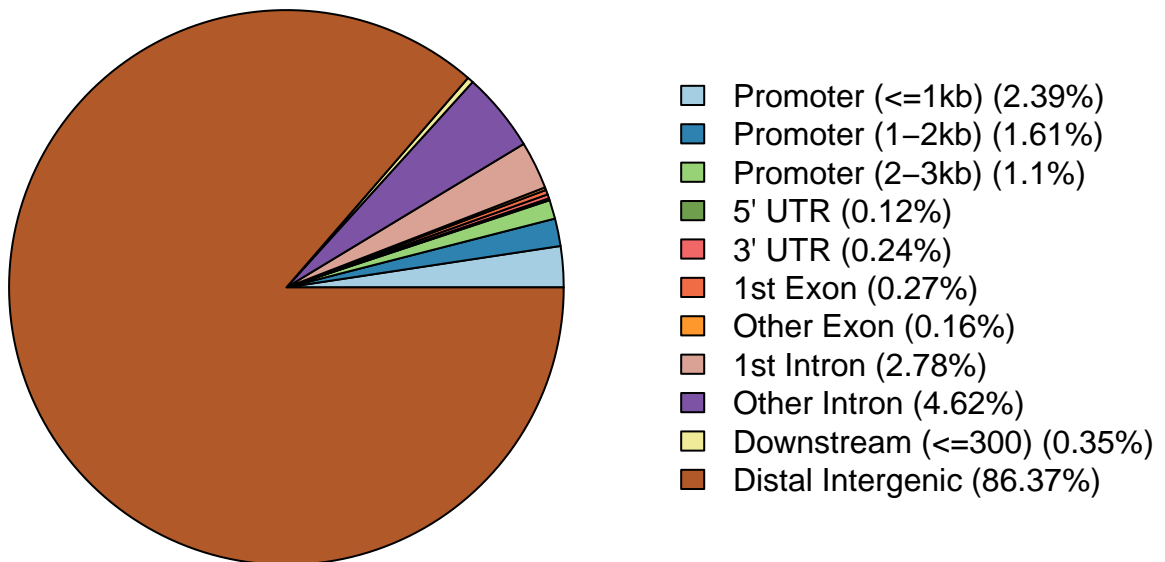
```
load("overall_analysis.RData")
```

Peak Annotation Results

SMAD2 Peaks - Untreated

Most of the untreated SMAD2 peaks were in the distal intergenic regions (about 86%).

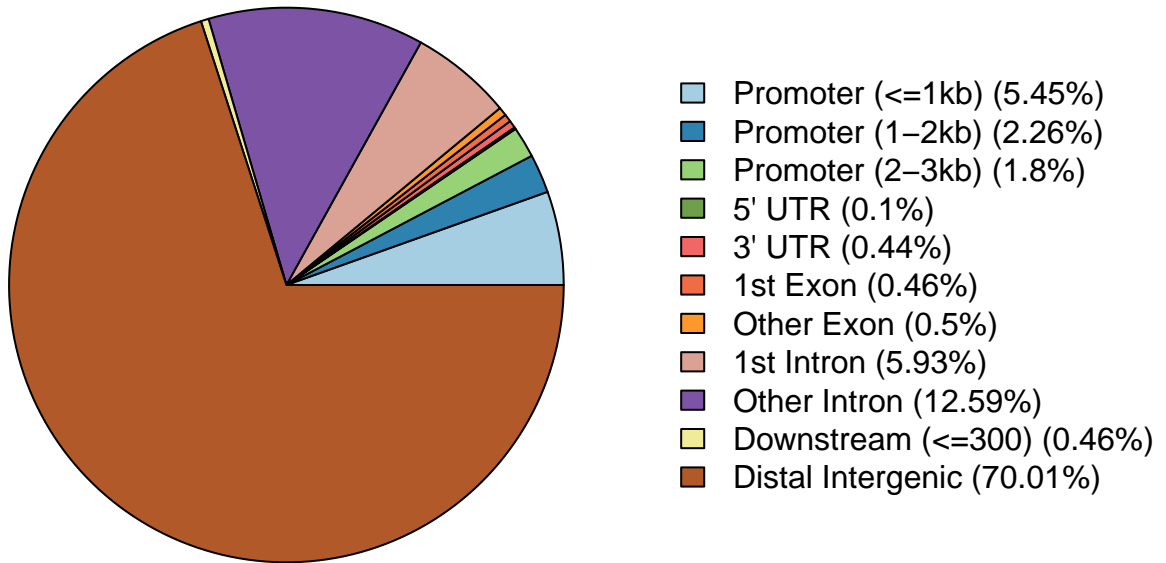
SMAD2 Peaks: Untreated



SMAD2 Peaks - Treated

Similar to the untreated SMAD2 peaks, most of the treated SMAD2 peaks also lay within the intergenic regions (about 71%), but in a lower proportion as compared to the untreated peaks (about 86%). On the other hand, the proportion of SMAD2 peaks in the genic regions rose upon treatment from 13.28% to 29.53%.

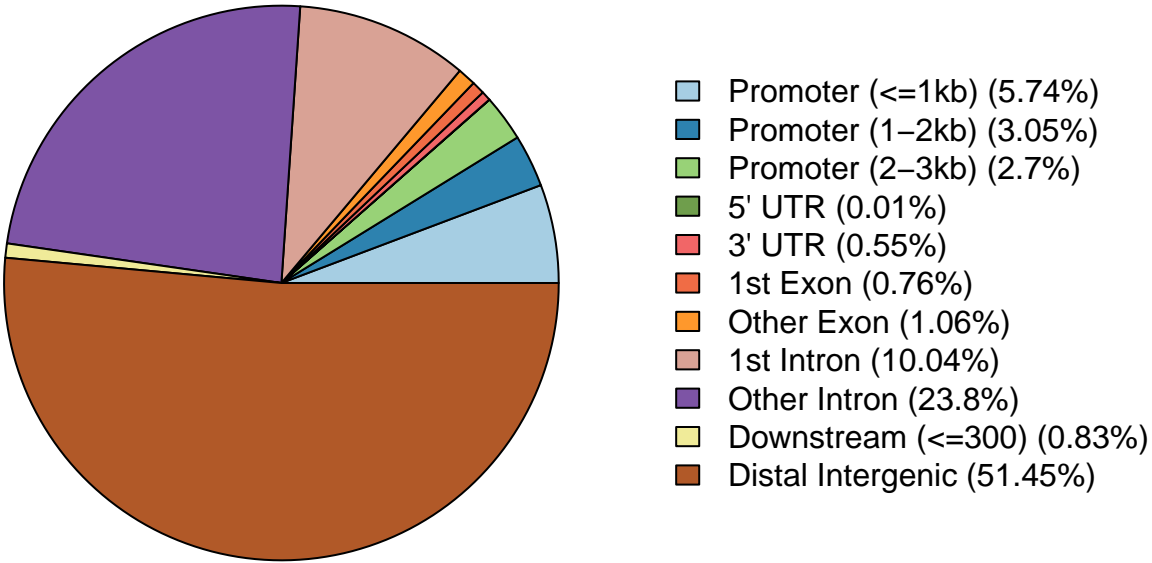
SMAD2 Peaks: Treated



SMAD3 Peaks - Untreated

Around half of the untreated SMAD3 peaks were in the intergenic regions, while a majority of the peaks in the genic regions (33.84 %) overlapped with intronic sequences. Compared to the SMAD2 peaks (both treated and untreated), the SMAD3 untreated peaks had a far lower proportion of peaks within the intergenic regions. On the other hand, the proportion of SMAD3 peaks in intronic regions was noticeably elevated as compared to the SMAD2 peaks.

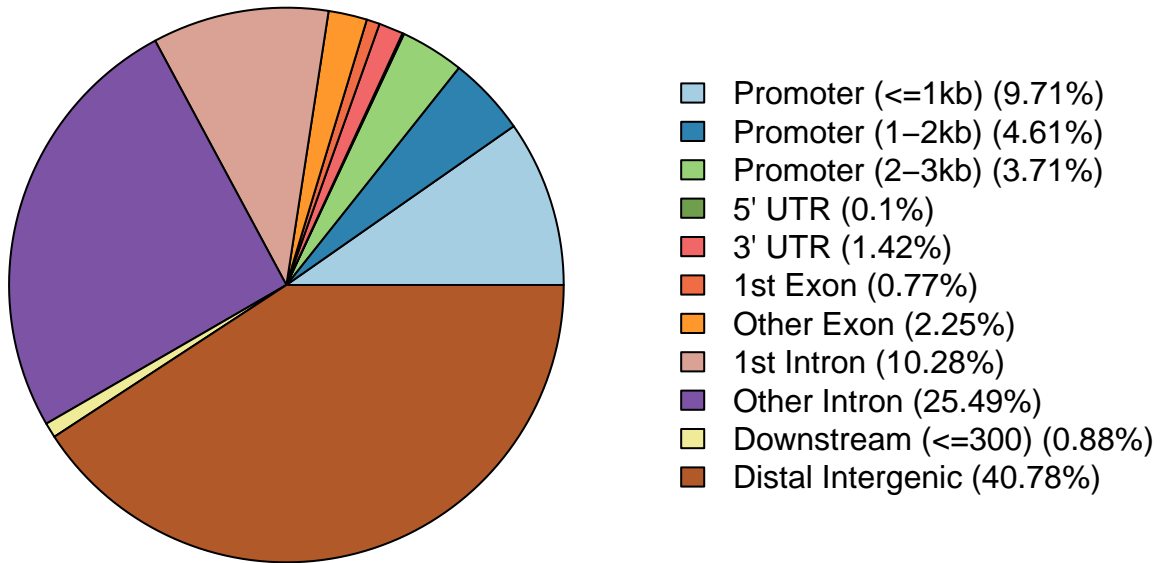
SMAD3 Peaks: Untreated



SMAD3 Peaks - Treated

TGF- β ligand treatment reduced the proportion of SMAD3 peaks in the intergenic regions (52.28% without treatment vs. 41.66% with treatment). For the most part, those peaks seem to have been displaced to the promoter regions, which accounted for a total of 11.49% before treatment but rose to 18.03% after treatment.

SMAD3 Peaks: Treated



GO Term Analysis Results

GO term analysis was limited to biological process (BP) terms; GO Terms relating to Cellular Component and Molecular Function were not analysed.

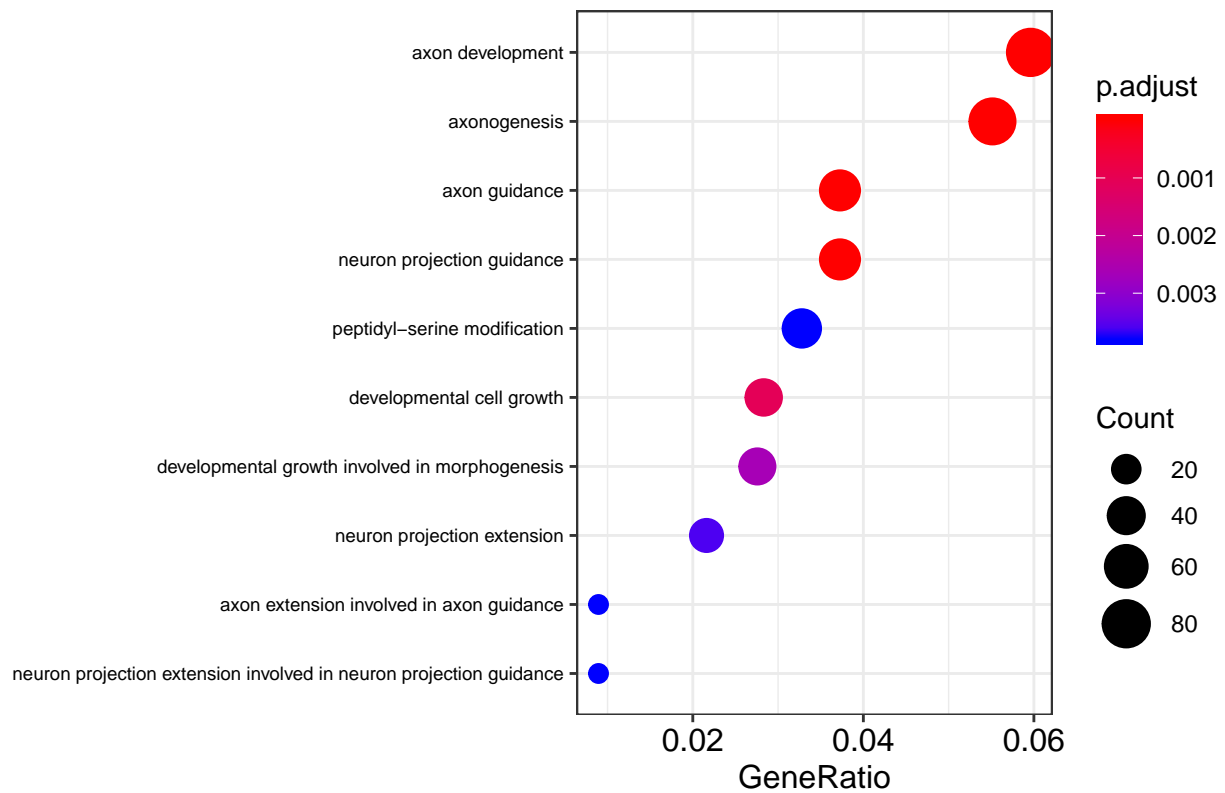
GO Term Analysis for All Peaks

No enriched BP GO terms were found within annotations for untreated SMAD2 peaks. This may have been due to the majority of these peaks being located in the intergenic regions. For treated SMAD2 peaks, GO terms related to processes in neuronal development were over-represented.

Note: Only the first 20 enriched GO terms for each peak subset and condition are shown. Terms were arranged in ascending order by adjusted p value, then in descending order by gene ratio and absolute count.

Enriched GO Terms in Treated SMAD2 Peaks	p.adjust	GeneRatio	BgRatio
axon development	0.0000000	80/1342	493/18493
axonogenesis	0.0000001	74/1342	449/18493
axon guidance	0.0000002	50/1342	259/18493
neuron projection guidance	0.0000002	50/1342	260/18493
developmental cell growth	0.0010681	38/1342	226/18493
developmental growth involved in morphogenesis	0.0026364	37/1342	227/18493
neuron projection extension	0.0035945	29/1342	161/18493
peptidyl-serine modification	0.0037980	44/1342	301/18493
axon extension involved in axon guidance	0.0037980	12/1342	37/18493
neuron projection extension involved in neuron projection guidance	0.0037980	12/1342	37/18493
regulation of developmental growth	0.0043011	47/1342	334/18493
cell junction assembly	0.0043011	37/1342	238/18493
regulation of axonogenesis	0.0066075	30/1342	180/18493
axon extension	0.0108430	22/1342	116/18493
cell growth	0.0121295	60/1342	485/18493
peptidyl-serine phosphorylation	0.0121295	40/1342	282/18493
regulation of cell morphogenesis involved in differentiation	0.0125971	41/1342	293/18493
cell junction organization	0.0161102	40/1342	287/18493
smooth muscle contraction	0.0165590	20/1342	105/18493
mesenchymal cell development	0.0165590	17/1342	82/18493

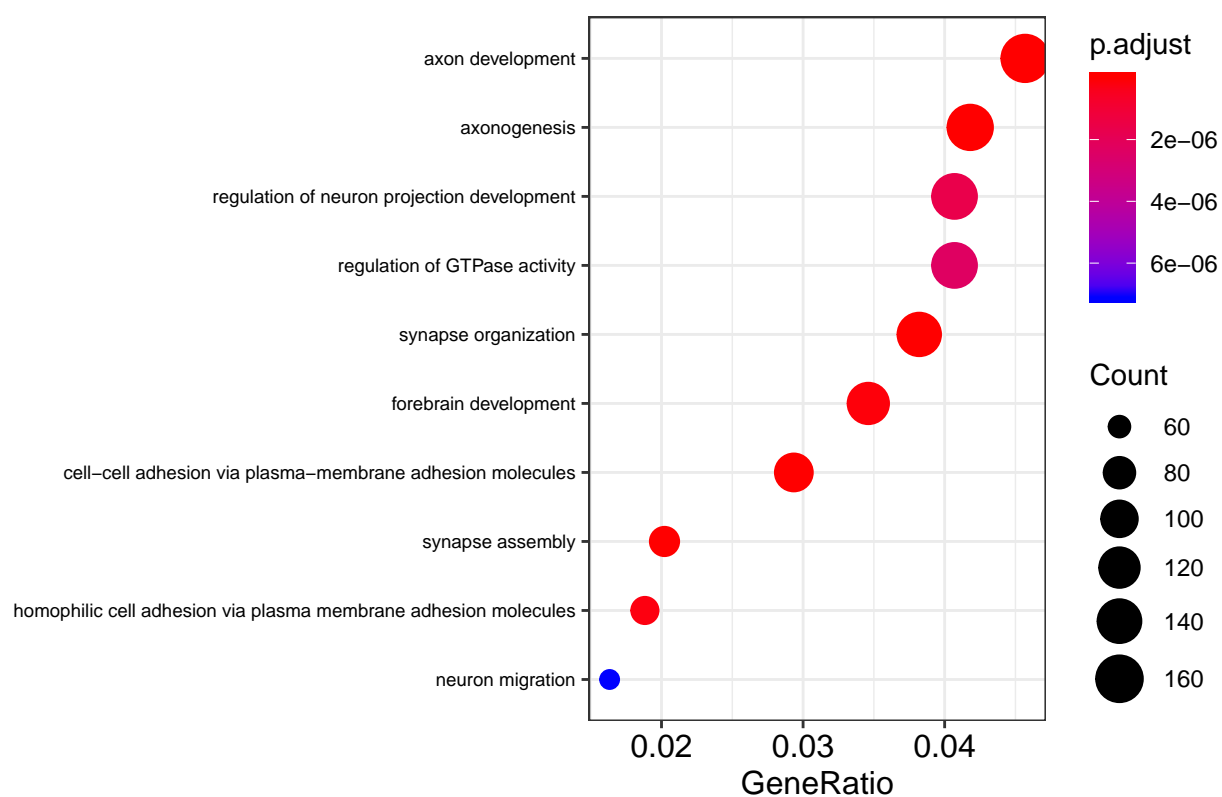
Enriched BP GO Terms in Treated SMAD2 Peaks



Untreated SMAD3 peaks also showed an enrichment in neuronal development-related terms. In addition, peaks in regions related to GTPase activity and cell-cell adhesion were also enriched.

Enriched GO Terms in Untreated SMAD3 Peaks	p.adjust	GeneRatio	BgRatio
synapse organization	0.00e+00	138/3612	384/18493
cell-cell adhesion via plasma-membrane adhesion molecules	0.00e+00	106/3612	270/18493
axon development	0.00e+00	165/3612	493/18493
synapse assembly	0.00e+00	73/3612	165/18493
axonogenesis	0.00e+00	151/3612	449/18493
forebrain development	1.00e-07	125/3612	374/18493
homophilic cell adhesion via plasma membrane adhesion molecules	2.00e-07	68/3612	167/18493
regulation of neuron projection development	1.60e-06	147/3612	478/18493
regulation of GTPase activity	2.30e-06	147/3612	481/18493
neuron migration	7.10e-06	59/3612	149/18493
positive regulation of GTPase activity	1.50e-05	125/3612	405/18493
modulation of chemical synaptic transmission	1.56e-05	128/3612	418/18493
regulation of trans-synaptic signaling	1.69e-05	128/3612	419/18493
neuron projection guidance	2.69e-05	87/3612	260/18493
regulation of synapse organization	2.69e-05	75/3612	214/18493
postsynapse organization	2.69e-05	60/3612	159/18493
positive regulation of neurogenesis	2.84e-05	135/3612	453/18493
regulation of synapse structure or activity	3.65e-05	76/3612	220/18493
axon guidance	3.95e-05	86/3612	259/18493
regulation of small GTPase mediated signal transduction	6.12e-05	101/3612	321/18493

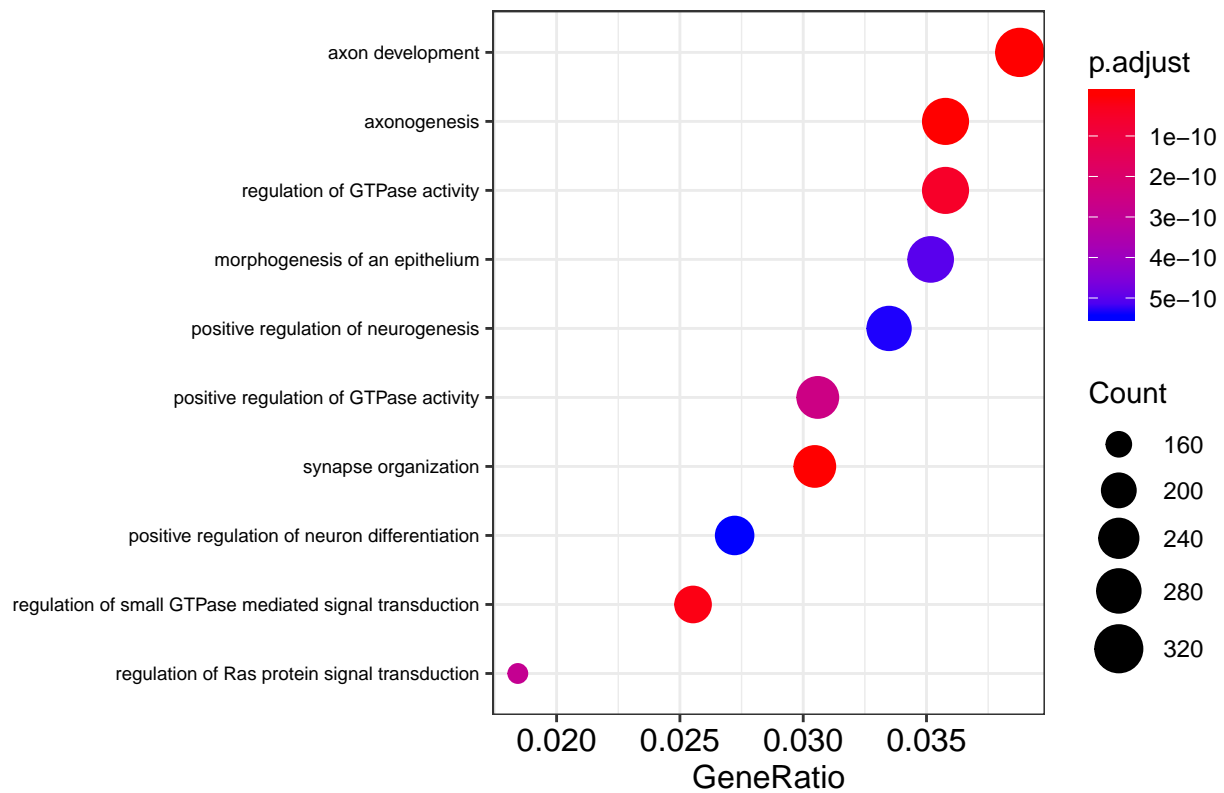
Enriched BP GO Terms in Untreated SMAD3 Peaks



Similar terms related to neuronal development also appeared in treated SMAD3 peaks. However, additional enriched terms such as “regulation of small GTPase-mediated signal transduction”, as well as “regulation of Ras protein signal transduction” were also found to be enriched.

Enriched GO Terms in Treated SMAD3 Peaks	p.adjust	GeneRatio	BgRatio
axon development	0	322/8303	493/18493
axonogenesis	0	297/8303	449/18493
synapse organization	0	253/8303	384/18493
regulation of small GTPase mediated signal transduction	0	212/8303	321/18493
regulation of GTPase activity	0	297/8303	481/18493
positive regulation of GTPase activity	0	254/8303	405/18493
regulation of Ras protein signal transduction	0	153/8303	222/18493
morphogenesis of an epithelium	0	292/8303	479/18493
positive regulation of neurogenesis	0	278/8303	453/18493
positive regulation of neuron differentiation	0	226/8303	356/18493
synapse assembly	0	119/8303	165/18493
neuron projection guidance	0	172/8303	260/18493
epithelial tube morphogenesis	0	201/8303	313/18493
axon guidance	0	171/8303	259/18493
developmental growth involved in morphogenesis	0	153/8303	227/18493
regulation of trans-synaptic signaling	0	257/8303	419/18493
modulation of chemical synaptic transmission	0	256/8303	418/18493
regulation of neuron projection development	0	284/8303	478/18493
Ras protein signal transduction	0	259/8303	430/18493
positive regulation of cell projection organization	0	227/8303	371/18493

Enriched BP GO Terms in Treated SMAD3 Peaks

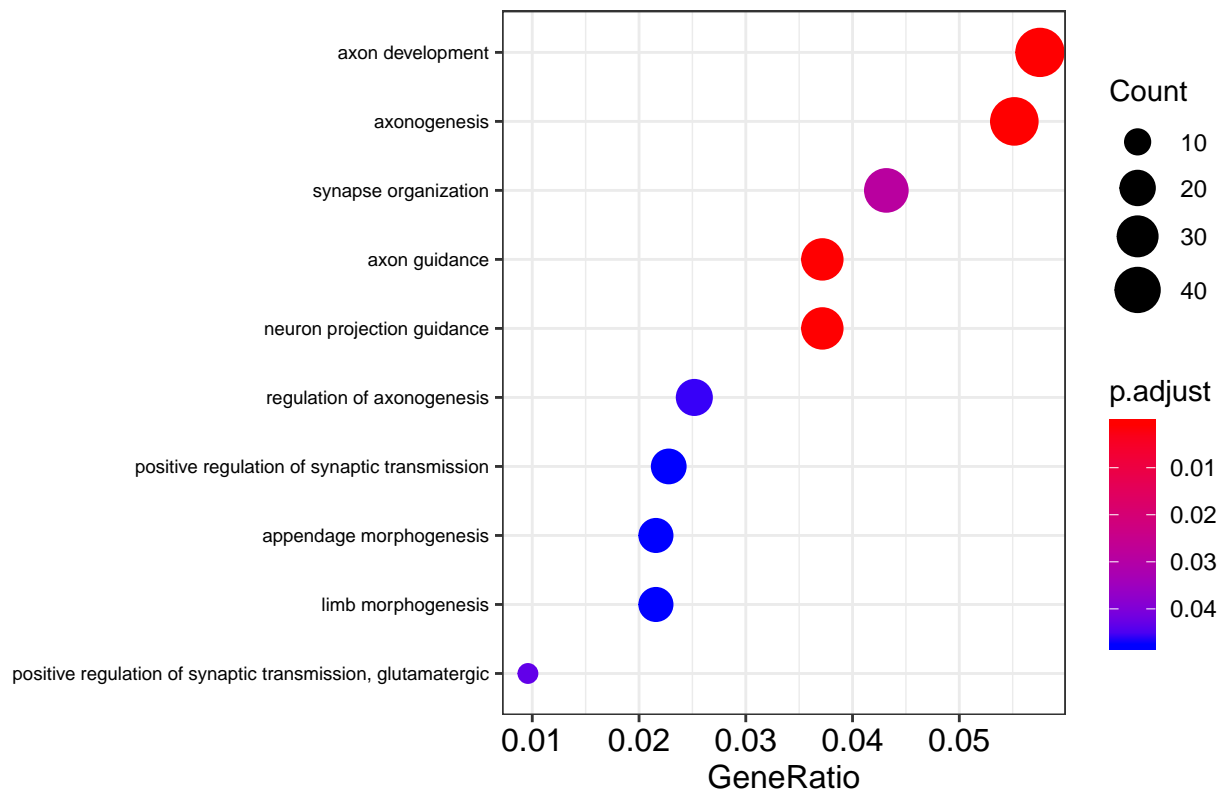


GO Term Analysis for Peaks Restricted to Gene Regions

The enriched GO Terms for SMAD2 peaks limited to gene regions did not differ much from their counterparts where all the peaks were considered. The pattern remains the same, with terms associated with neuronal development being predominantly featured, although terms for limb and appendage morphogenesis also turn up.

Enriched GO Terms in Treated SMAD2 Peaks Restricted to Gene Regions	p.adjust	GeneRatio	BgRatio
axonogenesis	0.0009522	46/834	449/18493
axon development	0.0010088	48/834	493/18493
axon guidance	0.0010088	31/834	259/18493
neuron projection guidance	0.0010088	31/834	260/18493
synapse organization	0.0287847	36/834	384/18493
positive regulation of synaptic transmission, glutamatergic	0.0433635	8/834	31/18493
regulation of axonogenesis	0.0461952	21/834	180/18493
peptidyl-serine modification	0.0474595	29/834	301/18493
positive regulation of synaptic transmission	0.0474595	19/834	159/18493
appendage morphogenesis	0.0474595	18/834	146/18493
limb morphogenesis	0.0474595	18/834	146/18493

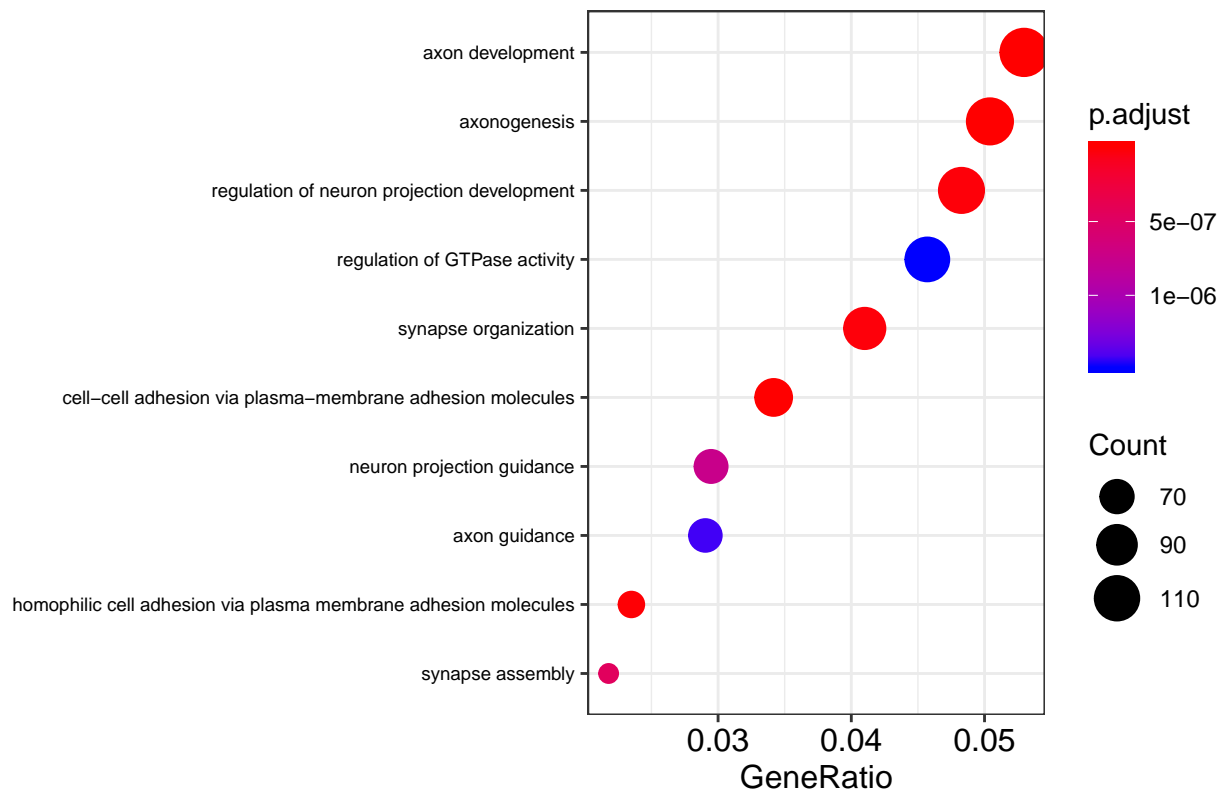
Enriched BP GO Terms in Treated SMAD2 Genic Peaks



Untreated SMAD3 peaks in gene regions showed a similar neuronal development theme for the most significantly enriched genes.

Enriched GO Terms in Untreated SMAD3 Peaks Restricted to Gene Regions	p.adjust	GeneRatio	BgRatio
axonogenesis	0.00e+00	118/2341	449/18493
axon development	0.00e+00	124/2341	493/18493
cell-cell adhesion via plasma-membrane adhesion molecules	0.00e+00	80/2341	270/18493
homophilic cell adhesion via plasma membrane adhesion molecules	0.00e+00	55/2341	167/18493
regulation of neuron projection development	0.00e+00	113/2341	478/18493
synapse organization	0.00e+00	96/2341	384/18493
synapse assembly	5.00e-07	51/2341	165/18493
neuron projection guidance	7.00e-07	69/2341	260/18493
axon guidance	1.40e-06	68/2341	259/18493
regulation of GTPase activity	1.50e-06	107/2341	481/18493
modulation of chemical synaptic transmission	1.60e-06	96/2341	418/18493
regulation of trans-synaptic signaling	1.70e-06	96/2341	419/18493
neuron migration	1.80e-06	46/2341	149/18493
positive regulation of GTPase activity	4.80e-06	92/2341	405/18493
positive regulation of neurogenesis	5.00e-06	100/2341	453/18493
forebrain development	1.39e-05	85/2341	374/18493
sensory system development	1.39e-05	82/2341	357/18493
eye development	1.39e-05	81/2341	351/18493
visual system development	1.46e-05	81/2341	352/18493
positive regulation of neuron differentiation	2.32e-05	81/2341	356/18493

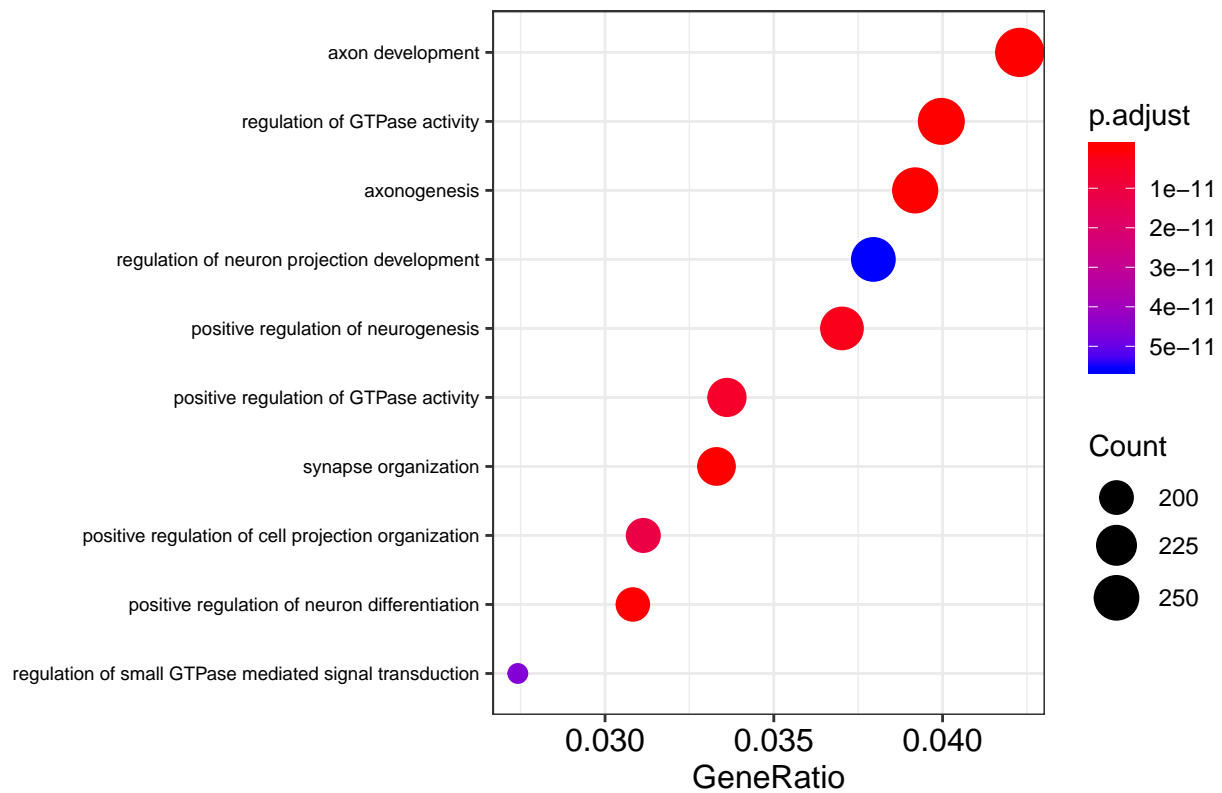
Enriched BP GO Terms in Untreated SMAD3 Genic Peaks



Treated SMAD3 peaks in gene regions did not show too many changes either, as compared to the the set of all treated SMAD3 peaks.

Enriched GO Terms in Treated SMAD3 Peaks Restricted to Gene Regions	p.adjust	GeneRatio	BgRatio
axon development	0	273/6456	493/18493
axonogenesis	0	253/6456	449/18493
regulation of GTPase activity	0	258/6456	481/18493
synapse organization	0	215/6456	384/18493
positive regulation of neuron differentiation	0	199/6456	356/18493
positive regulation of neurogenesis	0	239/6456	453/18493
positive regulation of GTPase activity	0	217/6456	405/18493
positive regulation of cell projection organization	0	201/6456	371/18493
regulation of small GTPase mediated signal transduction	0	177/6456	321/18493
regulation of neuron projection development	0	245/6456	478/18493
regulation of Ras protein signal transduction	0	130/6456	222/18493
regulation of axonogenesis	0	110/6456	180/18493
neuron projection guidance	0	147/6456	260/18493
axon guidance	0	146/6456	259/18493
modulation of chemical synaptic transmission	0	213/6456	418/18493
regulation of trans-synaptic signaling	0	213/6456	419/18493
positive regulation of neuron projection development	0	147/6456	269/18493
developmental growth involved in morphogenesis	0	128/6456	227/18493
regulation of cell morphogenesis involved in differentiation	0	157/6456	293/18493
synapse assembly	0	99/6456	165/18493

Enriched BP GO Terms in Treated SMAD3 Genic Peaks

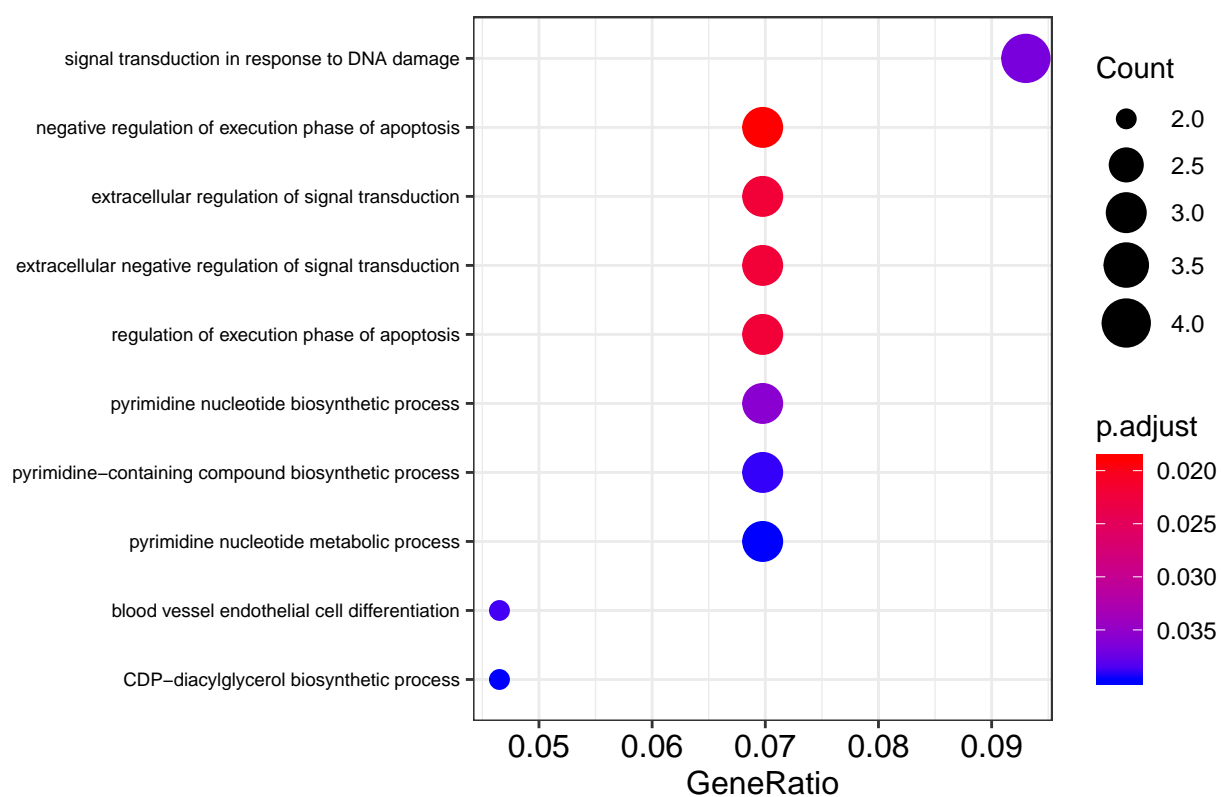


GO Term Analysis for Peaks Restricted to Promoter Regions

When peaks were limited to promoter regions, the enriched GO term sets showed noticeable differences from sets derived from all peaks as well as sets derived from peaks limited to gene regions. No enriched GO terms were found for treated promoter-proximal SMAD2 peaks.

Enriched GO Terms in Untreated SMAD2 Peaks Restricted to Promoters	p.adjust	GeneRatio	BgRatio
negative regulation of execution phase of apoptosis	0.0190118	3/43	23/18493
extracellular regulation of signal transduction	0.0220964	3/43	36/18493
extracellular negative regulation of signal transduction	0.0220964	3/43	36/18493
regulation of execution phase of apoptosis	0.0220964	3/43	38/18493
pyrimidine nucleotide biosynthetic process	0.0356605	3/43	48/18493
signal transduction in response to DNA damage	0.0367235	4/43	130/18493
blood vessel endothelial cell differentiation	0.0387759	2/43	11/18493
pyrimidine-containing compound biosynthetic process	0.0391230	3/43	58/18493
pyrimidine nucleotide metabolic process	0.0396258	3/43	64/18493
CDP-diacylglycerol biosynthetic process	0.0396258	2/43	13/18493
CDP-diacylglycerol metabolic process	0.0396258	2/43	14/18493
morphogenesis of an endothelium	0.0396258	2/43	15/18493
endothelial tube morphogenesis	0.0396258	2/43	15/18493

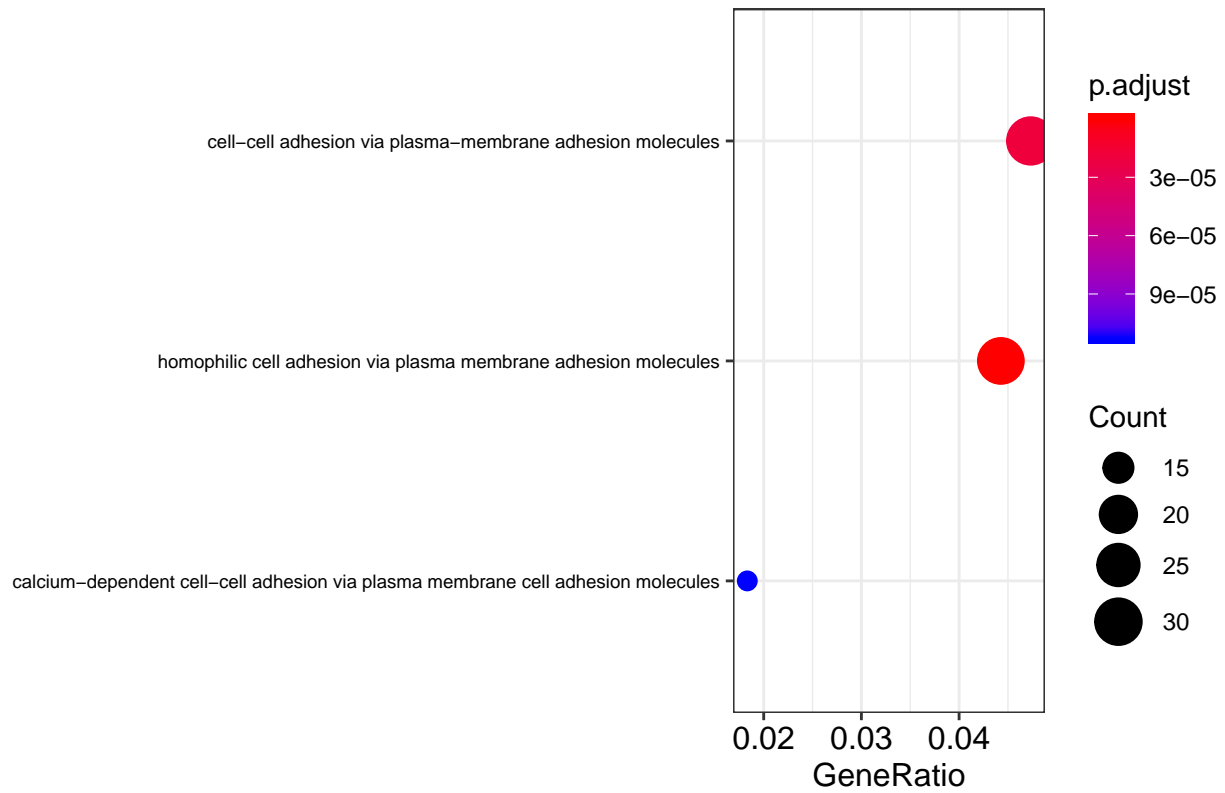
Enriched BP GO Terms in Untreated SMAD2 Promoter Peaks



Untreated SMAD3 peaks restricted to promoters showed the least number of enriched terms, all of which were related to cell-cell adhesion.

Enriched GO Terms in Untreated SMAD3 Peaks Restricted to Promoters	p.adjust	GeneRatio	BgRatio
homophilic cell adhesion via plasma membrane adhesion molecules	0.0000000	29/655	167/18493
cell-cell adhesion via plasma-membrane adhesion molecules	0.0000194	31/655	270/18493
calcium-dependent cell-cell adhesion via plasma membrane cell adhesion molecules	0.0001125	12/655	48/18493

Enriched BP GO Terms in Untreated SMAD3 Promoter Peaks



Treatment with ligand seemed to show many more enriched GO terms in promoter-restricted SMAD3 peaks, as compared to untreated promoter-restricted SMAD3 peaks.

Enriched GO Terms in Treated SMAD3 Peaks Restricted to Promoters	p.adjust	GeneRatio	BgRatio
regulation of small GTPase mediated signal transduction	0.0000001	99/2990	321/18493
regulation of Ras protein signal transduction	0.0000001	76/2990	222/18493
regulation of GTPase activity	0.0000017	129/2990	481/18493
positive regulation of GTPase activity	0.0000017	113/2990	405/18493
regulation of Rho protein signal transduction	0.0000017	51/2990	136/18493
Rho protein signal transduction	0.0002438	61/2990	199/18493
activation of GTPase activity	0.0007166	34/2990	91/18493
Ras protein signal transduction	0.0013685	107/2990	430/18493
epithelial tube morphogenesis	0.0045268	81/2990	313/18493
extracellular matrix organization	0.0230640	85/2990	348/18493
pinocytosis	0.0230640	11/2990	19/18493
morphogenesis of an epithelium	0.0276288	110/2990	479/18493
extracellular structure organization	0.0276288	95/2990	402/18493
regulation of phosphoprotein phosphatase activity	0.0349439	35/2990	114/18493
endosomal transport	0.0392397	55/2990	208/18493
neural tube closure	0.0392397	29/2990	89/18493
tube closure	0.0401784	29/2990	90/18493
histone H3-K36 methylation	0.0401784	8/2990	12/18493
distal tubule development	0.0401784	8/2990	12/18493
tube formation	0.0492196	41/2990	145/18493

Enriched BP GO Term in Treated SMAD3 Promoter Peaks

