Figure 7

This notebook reproduces the main analyses from figure 7 of the manuscript. First we import the necessary packages.

```r
library(Seurat)
library(tidyverse)
library(patchwork)
library(voxhunt)
```

Now we set up some colors and load the data. The loaded seurat object contains the bulk RNA-seq data from the patterning screen. Each sample represents one organoid. We also do some feature selection with VoxHunt.

```r
data("voxel_meta")
load_aba_data('voxhunt_data/')
patscreen_data <- read_rds('patscreen_srt.rds')

ps_names <- c(
    'Ctrl_no_dose',
    'SHH_high', 'SHH_low',
    'CHIR_high', 'CHIR_low',
    'RSPO2_high', 'RSPO2_low',
    'RSPO3_high', 'RSPO3_low',
    'SB_DM_high', 'SB_DM_low'
)
ps_colors <- c(
    'gray',
    '#f57f17', '#ffc107',
    '#186a3b', '#28b463',
    '#c2185b', '#f48fb1',
    '#512da8', '#9575cd',
    '#2874a6', '#5dade2'
)
names(ps_colors) <- ps_names

struct_markers <- structure_markers('E13', 'custom_2')
genes_use <- struct_markers %>%
    group_by(group) %>%
    top_n(10, auc) %>%
    pull(gene) %>% unique()

print(patscreen_data)
```

```
## An object of class Seurat
## 98973 features across 61 samples within 3 assays
## Active assay: RNA (32991 features, 0 variable features)
##  2 other assays present: counts, TPM
```

1

Now we perform PCA to get an impression how the samples are distributed.

```
patscreen_data <- patscreen_data %>% FindVariableFeatures(nfeatures=2000) %>% ScaleData() %>% RunPCA(np
```

```
## Warning in FindVariableFeatures.Assay(object = assay.data, selection.method =
## selection.method, : selection.method set to 'vst' but count slot is empty; will
## use data slot instead
```

```
## Centering and scaling data matrix
```

```
## PC_ 1
## Positive:  PTRF, LGALS3, S100A14, GSTA2, KYNU, KDELR3, TMEM37, GSTA1, APOE, SOAT2
##      LCP1, MIR4435-2HG, VIL1, NPNT, ANXA4, GOS2, AKR1D1, HNF4A, IGF2, LGALS2
##      ARSE, F2, AMBP, TMEM92, TF, FABP1, PLA2G12B, AGT, GGT1, SLCO2B1
## Negative:  TCEAL7, TMSB15A, H2AFZ, HMGN2, SMC4, PLEKHO1, FABP7, BTBD17, CENPV, GINS2
##      FAM64A, TCEAL5, PCNA, BEX2, IMMP1L, HMGB2, PIK3R3, CCDC144NL-AS1, HIST1H4C, TUBA1B
##      CENPF, H2AFV, HMGB1, MARCKSL1, C16orf45, RTN1, HN1, ACOT7, TMSB15B, NCALD
## PC_ 2
## Positive:  FAM212A, FABP5, TNNC2, SIX1, SCRG1, RGS10, NPFFR2, CD163L1, ROPN1, S100A6
##      RP11-45A12.1, ACTC1, S100A5, TFAP2B, MIA, GPHA2, FAM110A, PLAC9, GAS2, SOX10
##      ISL1, PGF, SLC9A9, NRP2, LRRC53, ROPN1B, ERVMER61-1, DLX1, S100B, EGFL8
## Negative:  CXADR, VAT1L, NUAK2, POR, SDC2, COL4A6, PROM1, DDR1, ELP4, GDPD2
##      GGA2, KLHDC8B, CLU, C5orf22, GJA1, ODC1, FGFR2, PALLD, ALPL, PAM
##      PLAGL1, UBE3D, EPHA7, TUBB4A, CD82, MID1, TRPM3, TMEM2, TSC22D1, EFHD1
## PC_ 3
## Positive:  CTNNA1, MTHFD2, PLS3, ACLY, HDAC2, PPP2R1B, VCP, DDX5, EFTUD2, CTPS1
##      CCNC, ZNF385C, EYA1, UNC5C, CCT8, HNRNPK, HEYL, XPOT, API5, SRSF1
##      SF3B3, C5orf30, CAPRIN1, XRCC6, TRIM9, SRSF6, DLD, MAPRE1, FAR2, CSE1L
## Negative:  RP4-616B8.4, RP6-65G23.5, RP11-1035H13.3, RP11-461A8.1, BARHL2, RP11-371E8.4, RP11-256P1.
##      C1orf194, RP11-2B6.3, C2orf80, PRLH, GRIK1, FETUB, RP11-316M1.12, ORM1, HLA-B, CORIN
##      AP001615.9, HELT, MUC12, ENKUR, AC079742.4, CIB1, RP11-849I19.1, RP11-109P14.10, RP11-493L12.4, 
## PC_ 4
## Positive:  VIT, TUBB2A, ALCAM, RSPO2, WNT1, UNC5C, TNFRSF19, CDK6, CHGB, ASCL1
##      PCDH9, C8orf4, PCSK1N, CTHRC1, ZIC4, RP11-77H9.2, ZIC1, GAP43, PAM, NHLH2
##      SLC17A6, STMN2, WNT10B, RP11-363J20.2, SYT4, NDRG2, WFIKKN1, WNT4, RSPO3, RASD1
## Negative:  RAX, SIX3, SHISA2, SIX6, RP11-89K21.1, LINC01551, FZD5, FAM69C, LHX2, DLK1
##      F2RL2, DLX6, PPP2R2B, TNNT1, RP11-665C14.2, GPR160, ATP6V1B1, CAPN6, ADAM28, FEZF1-AS1
##      RP11-114G22.1, EDN3, FEZF1, FGF8, SCUBE1, DANT1, CDA, BST2, PAMR1, CLDN10
## PC_ 5
## Positive:  NOS2, NTN5, PAX3, LINC01198, ZNF436-AS1, COL11A2, MUSTN1, PAX6, LMX1A, GREM1
##      ARHGAP15, CRABP2, RP11-96L14.7, NRIP3, TPPP3, RP11-314M24.1, AGR3, NEUROG1, SLC39A8, CST4
##      ZIC2, ID3, LHX5-AS1, FOXD3-AS1, EGFL8, DKK1, WNT1, LGI1, CDH9, TAL2
## Negative:  TECRL, RP11-162P23.2, RP11-568A7.4, PCAT14, GDF3, RP11-267L5.1, CH507-513H4.4, PLVAP, SLP
##      PCOLCE, RGS1, CH507-513H4.6, TDGF1, PRRX1, RP11-85G21.3, SPINK4, HOXB6, LBH, CD300E
##      HOXB-AS3, NANOG, CH507-513H4.3, ECSCR, SLC7A3, CH507-513H4.5, ICAM2, MYH10, POU5F1, ALDH2
```
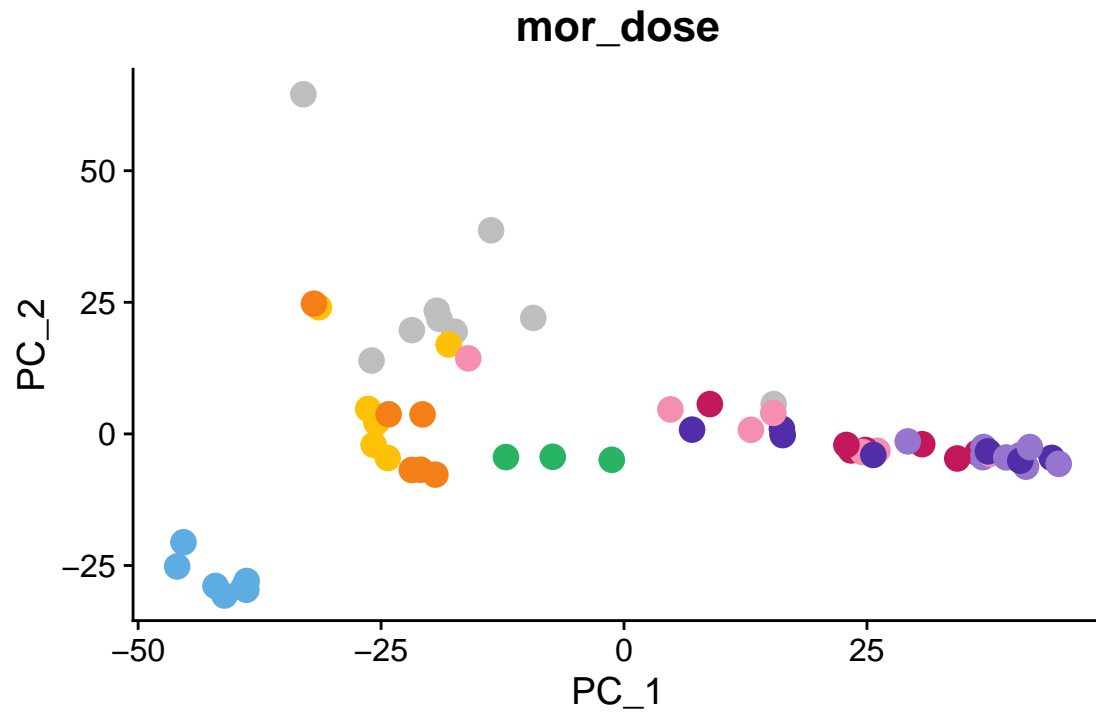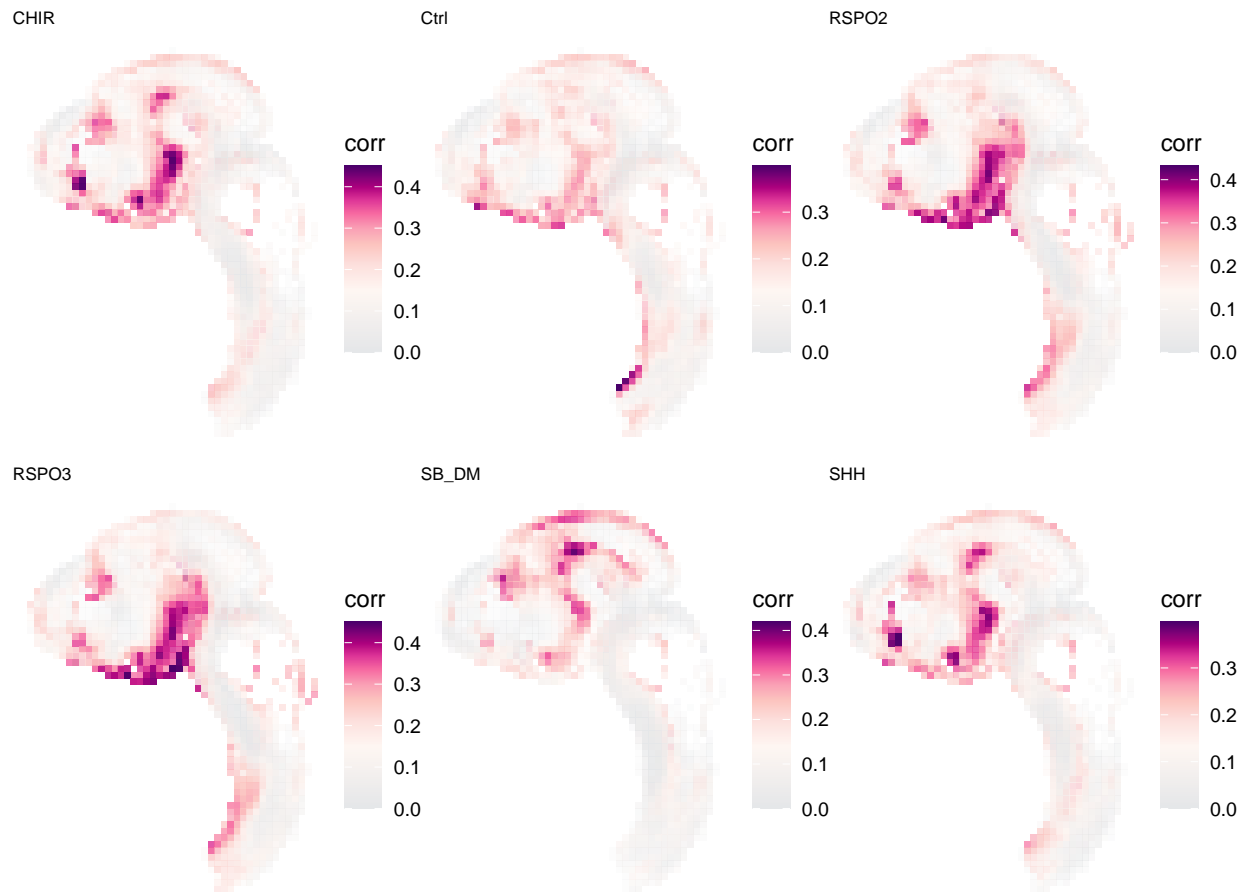
```
patscreen_data@reductions$pca@stdev
```

```
## [1] 29.44264 16.70063 12.20770  8.94130  7.89276
```

```
DimPlot(patscreen_data, group.by='mor_dose', pt.size=4) +
    scale_color_manual(values=ps_colors) +
    no_legend()
```

mor_dose

Now we map the data with VoxHunt.

```
ps_map <- voxel_map(patscreen_data, stage='E11', genes_use = genes_use, group_name = 'morphogen')
plot_map(ps_map)
```

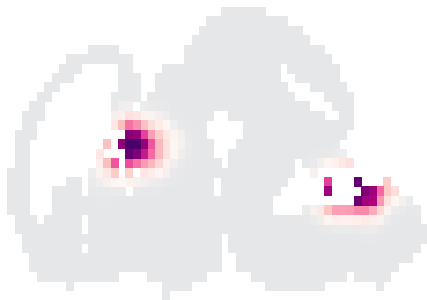Further, we'll have a closer look at the correlation patterns of RSPO2/3.

```
rspo_data <- subset(patscreen_data, morphogen%in%c('RSPO2', 'RSPO3'))
ps_map <- voxel_map(rspo_data, stage='E13', genes_use = genes_use, group_name = 'morphogen')
plot_map(ps_map, slices = 6)
```
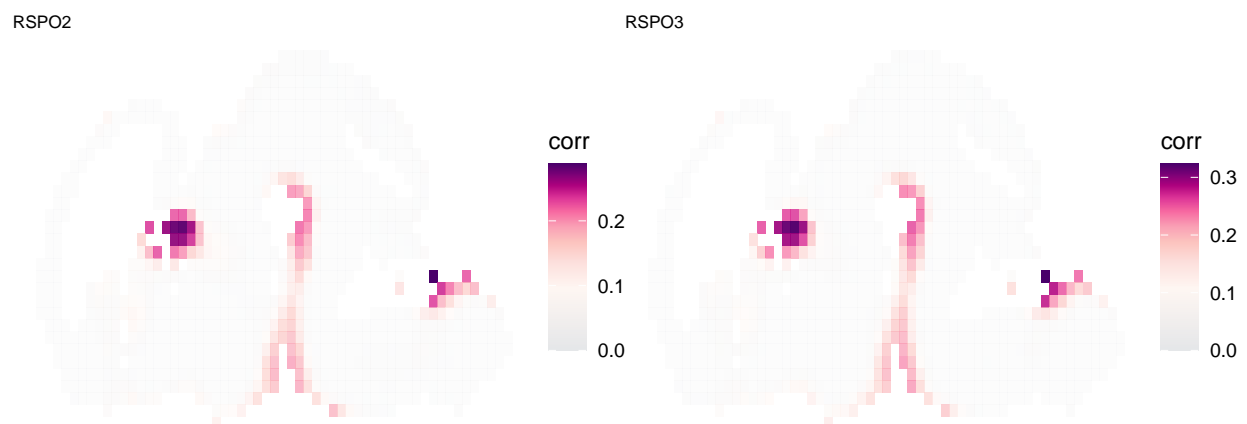

Lastly, we can look at some markers on the same slice to see that RSPO2 & 3 are expressed adjacent to the highlighted position.

```
plot_expression('E13', slices = 6, genes = c('RSPO2', 'RSPO3', 'TTR', 'SPINT2'))
```

TTR

RSPO2

SPINT2

RSPO3