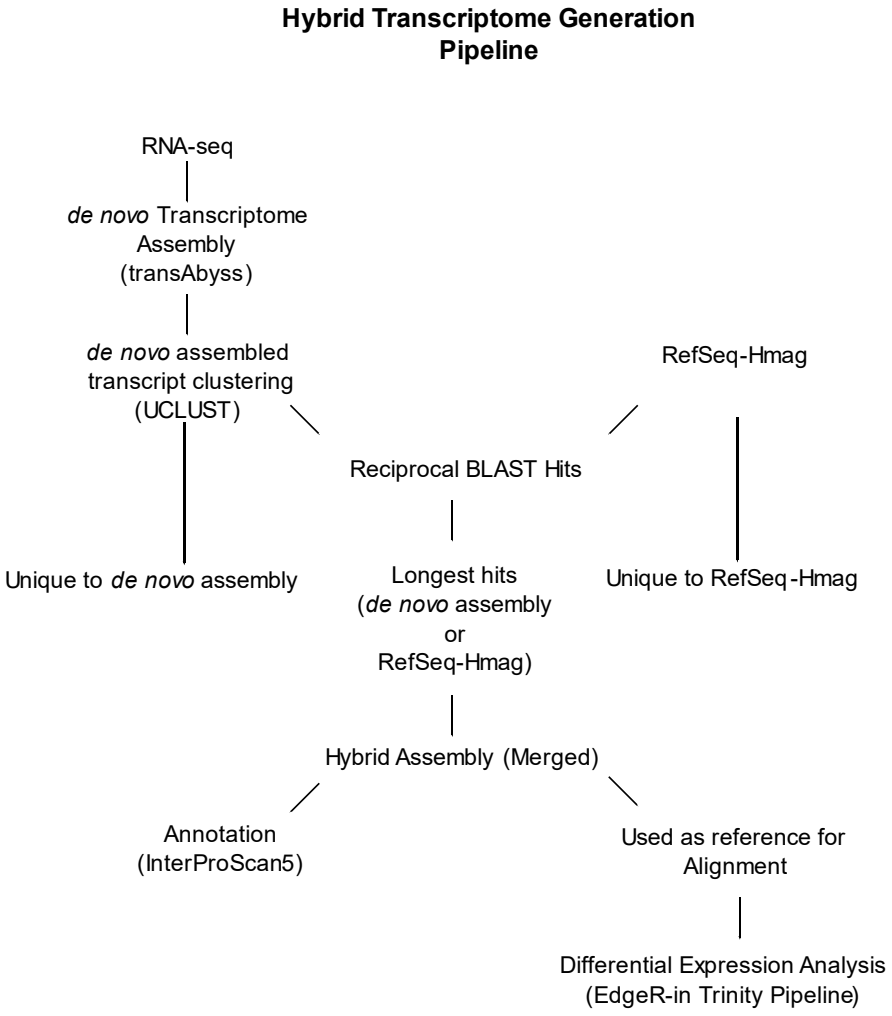
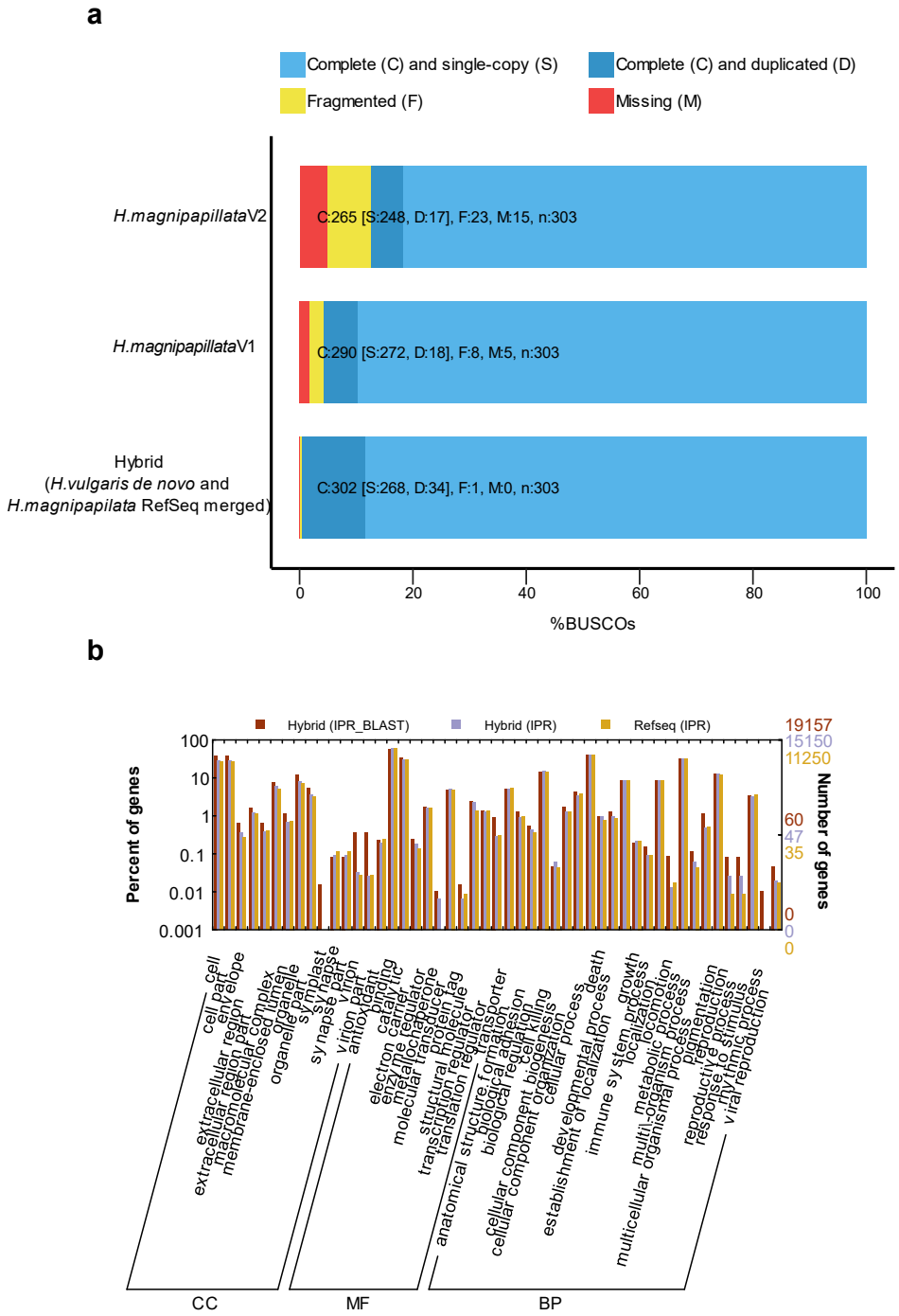
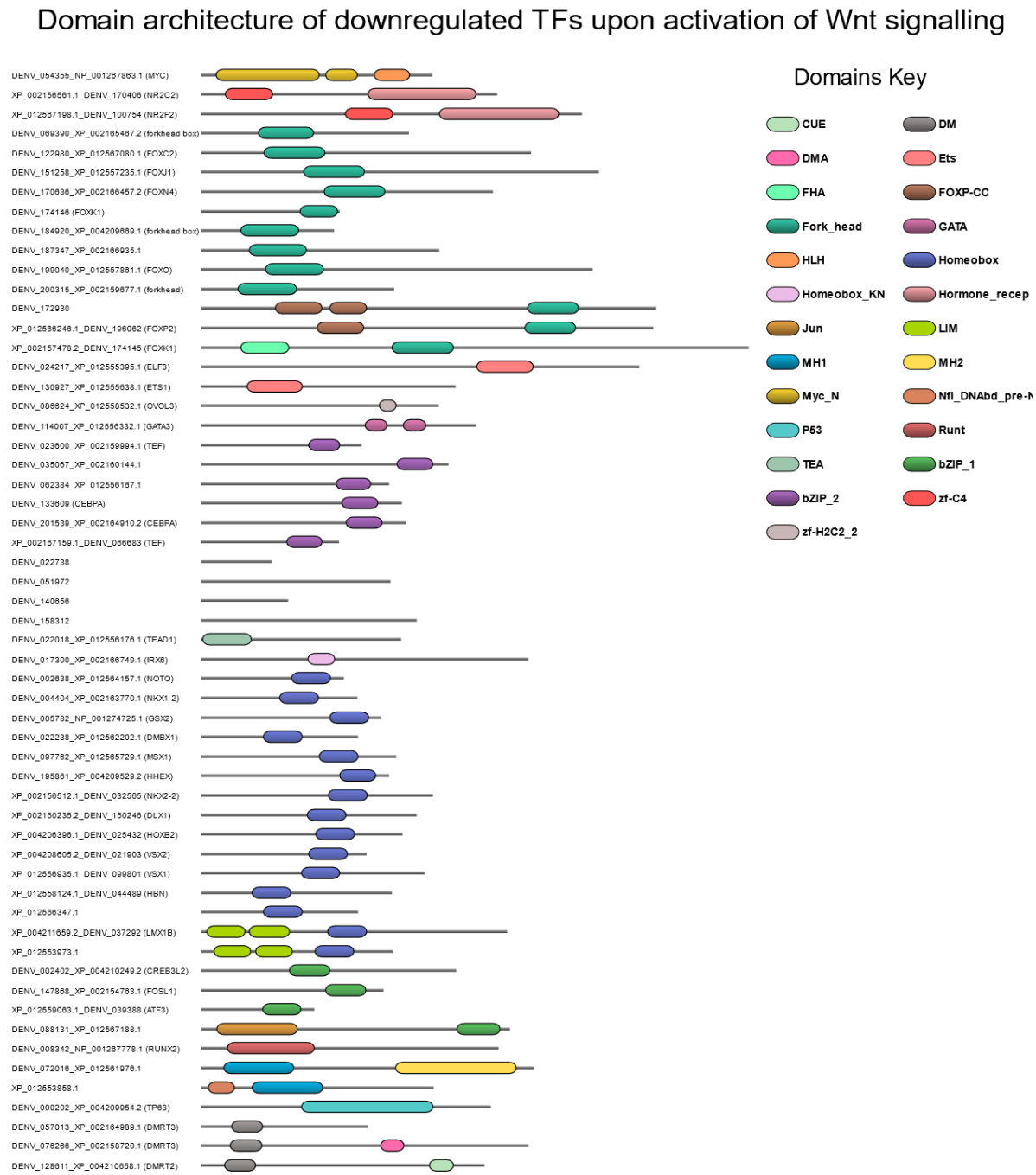


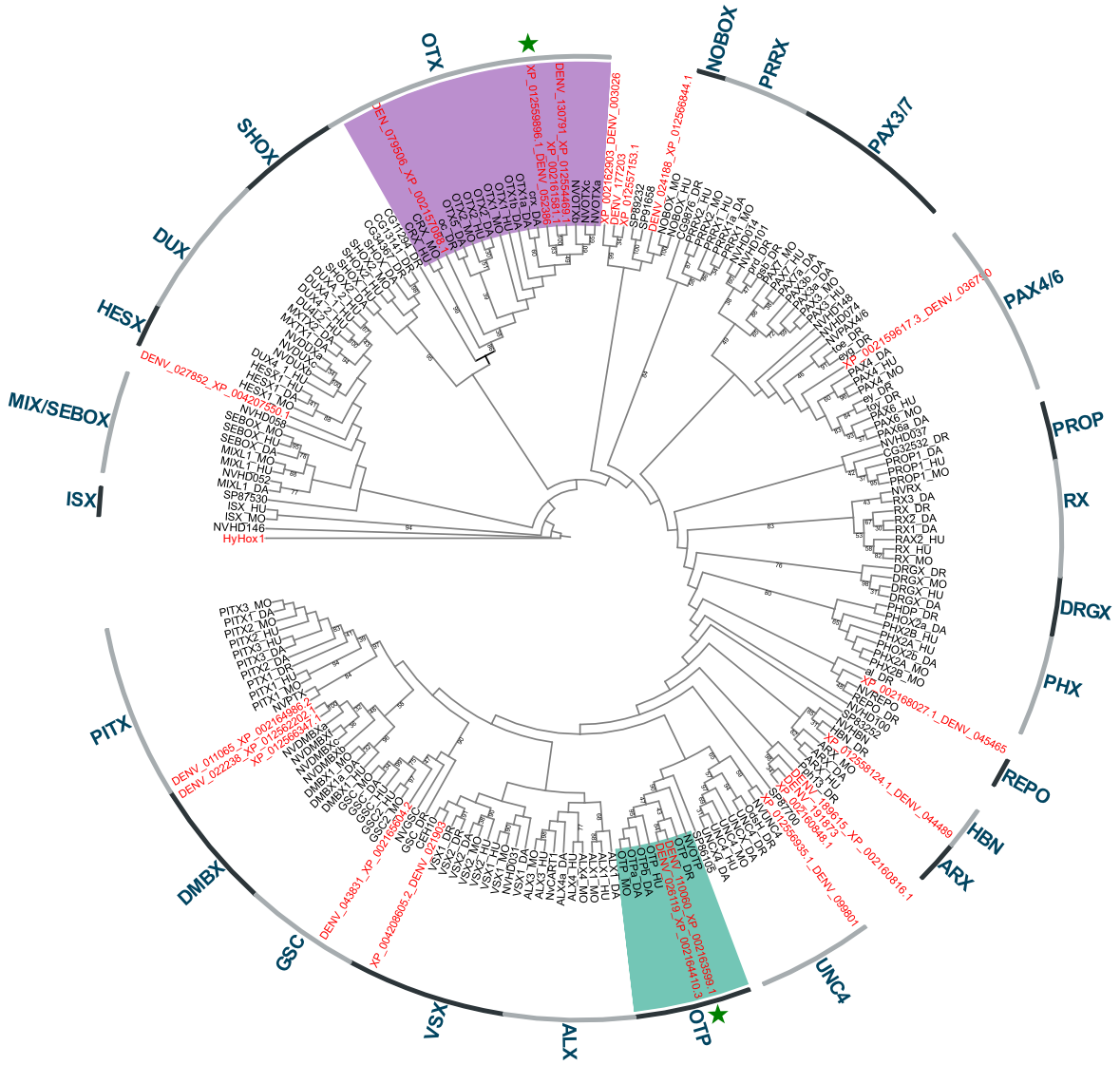
Supplementary Figure 1: Generation of a hybrid transcriptome assembly. After RNA sequencing raw reads from all experimental conditions were used to build the *de novo* assembly. A hybrid assembly was generated by merging missing sequences or longer transcripts from *Hydra magnipapillata* RefSeq.



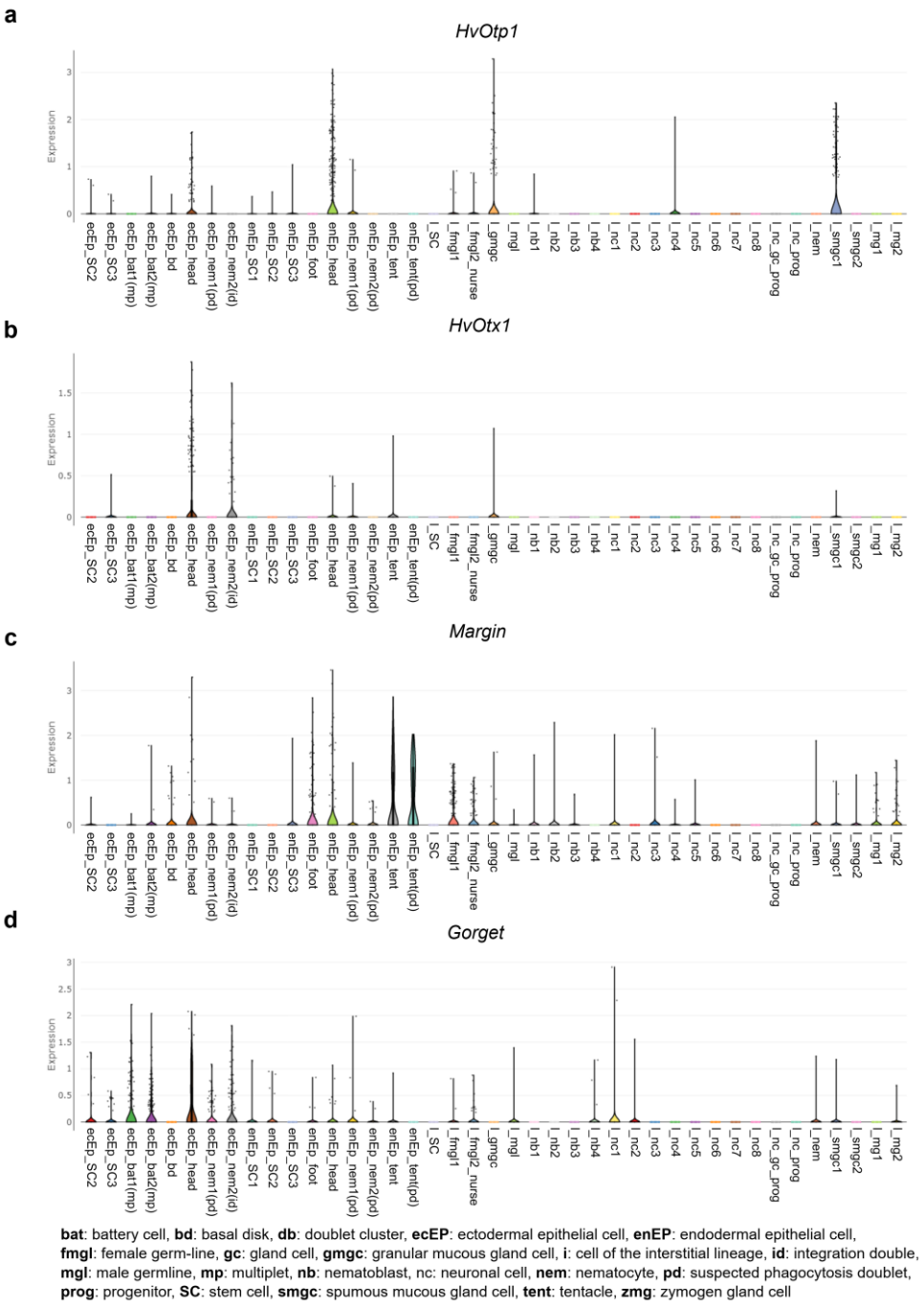
Supplementary Figure 2: Quality assessment and annotation of hybrid assembly. a, Evaluation of the quality of hybrid assembly and comparison with RefSeq data of *H. magnipapillata* and Hydra2.0 genome (*H. magnipapillata*). **b,** Comparison of different functional annotation methods on hybrid and RefSeq data. Barplot shows the coverage of gene ontology (GO) terms coverage. Hybrid assembly with InterProScan and BLAST based method gave best coverage.



Supplementary Figure 3: Domain organization of downregulated TFs upon activation of Wnt signalling by inhibition of GSK3-β. Colour coded key labels indicate the corresponding Pfam domain.

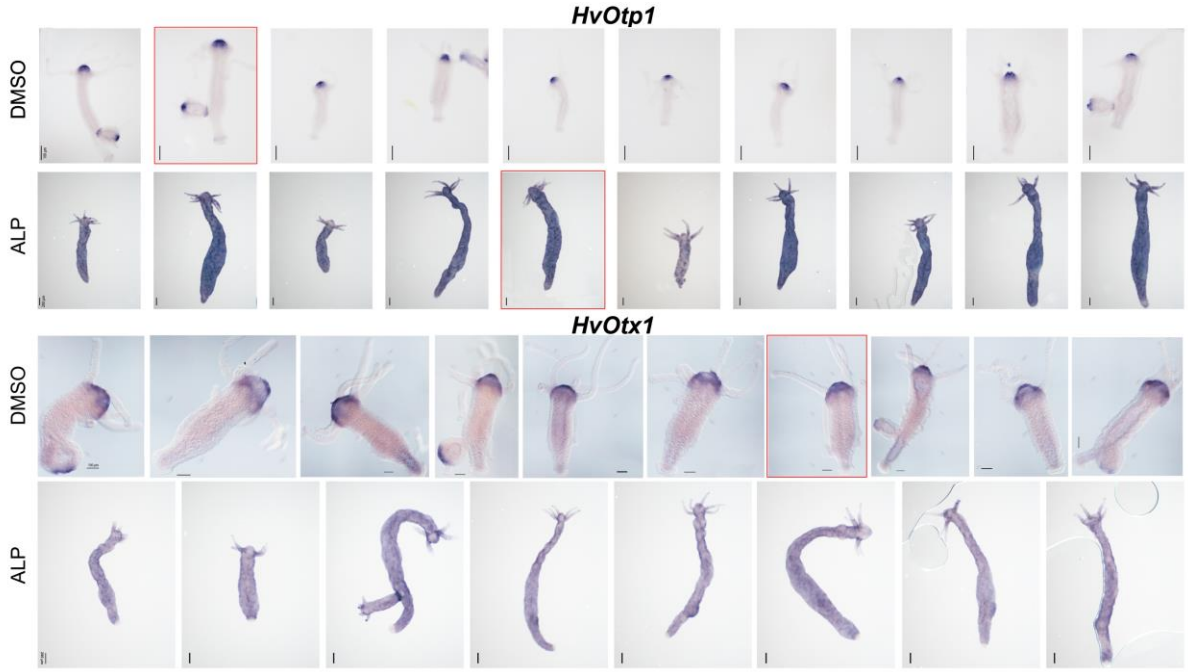


Supplementary Figure 4: Phylogenetic analysis of PRD class of homeodomain proteins. Phylogenetic analysis of homeodomains from PRD members of selected animal phyla along with *Hydra* components was carried out by RAxML method. Here, the sequence alignments were performed using MUSCLE default parameters and after manually trimming they were used in analysis. Values on branches indicate the percentage confidence in branching point after 1000 bootstrap iterations. The sequence details are given in Supplementary Information. Sequences denoted with green star were used for further study in current work.

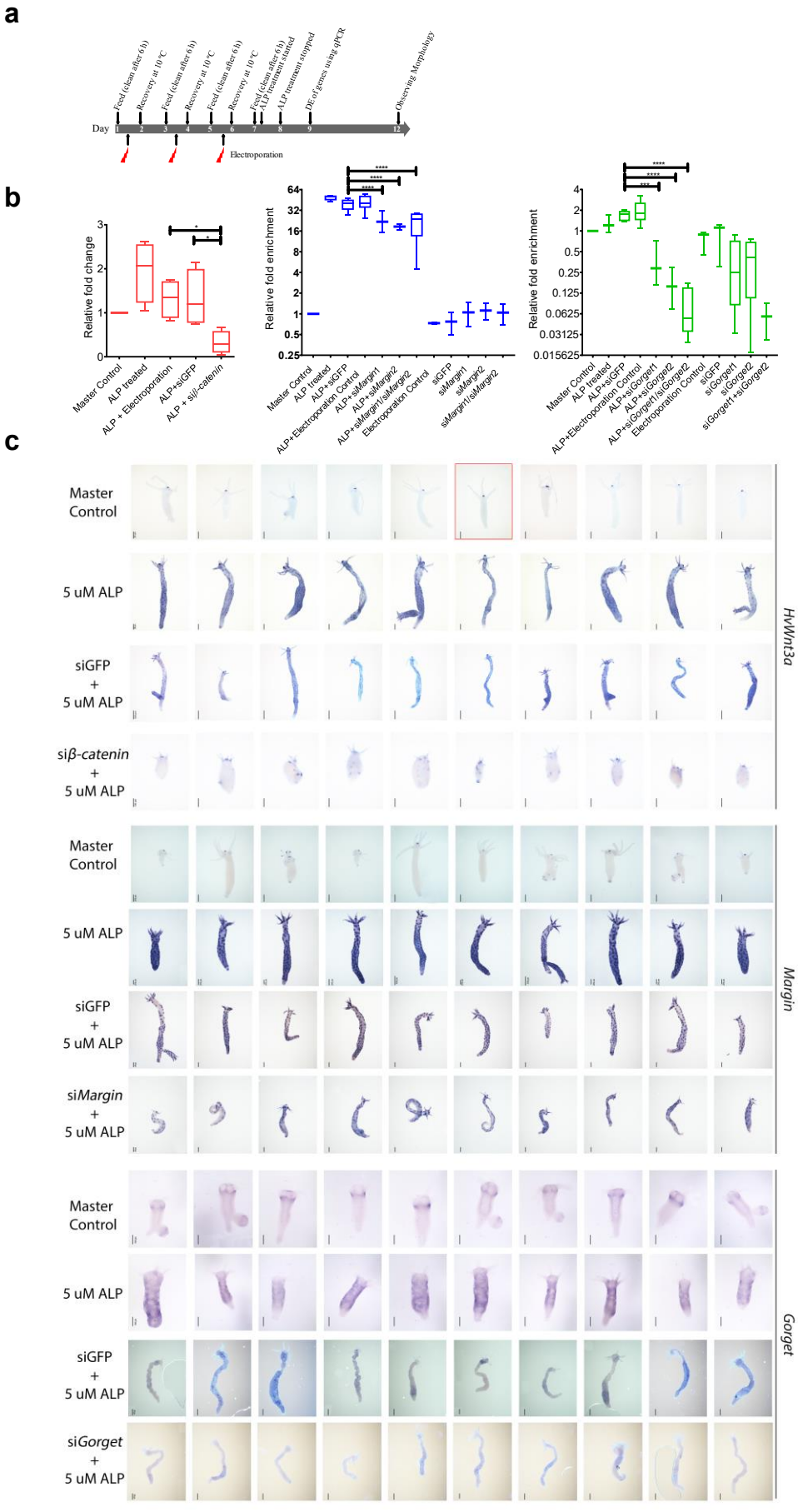


bat: battery cell, **bd**: basal disk, **db**: doublet cluster, **ecEP**: ectodermal epithelial cell, **enEP**: endodermal epithelial cell, **fmgl**: female germ-line, **gc**: gland cell, **gmgc**: granular mucous gland cell, **i**: cell of the interstitial lineage, **id**: integration double, **mgl**: male germline, **mp**: multiplet, **nb**: nematoblast, nc: neuronal cell, **nem**: nematocyte, **pd**: suspected phagocytosis doublet, **prog**: progenitor, **SC**: stem cell, **smgc**: spumous mucous gland cell, **tent**: tentacle, **zmg**: zymogen gland cell

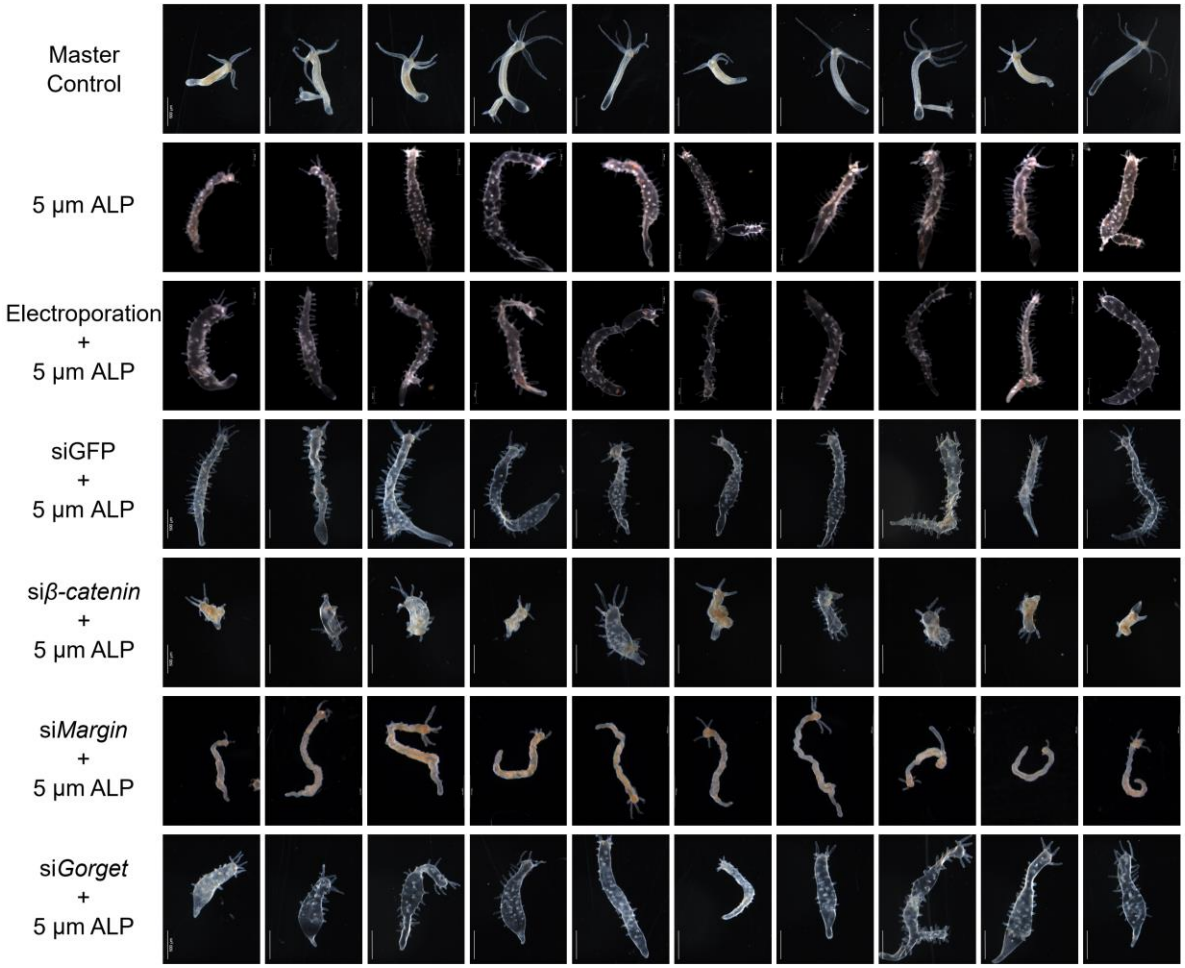
Supplementary Figure 5: Expression of *HvOtp1*, *HvOtx1*, *Margin* and *Gorget* in different cell type cluster based on single cell transcriptome analysis. The expression plots were used from the data available online (https://portals.broadinstitute.org/single_cell/study/SCP260/stem-cell-differentiation-trajectories-in-hydra-resolved-at-single-cell-resolution). This data was generated by Siebert et. al., (2019) [1] for tracing the stem cell lineages in *Hydra* using single cell RNA-seq. **a**, Expression of *HvOtp1* in different cell-type clusters. **b**, Expression of *HvOtx1* in different cell-type clusters. **c**, Expression of *Margin* in different cell-type clusters. **d**, Expression of *Gorget* in different cell-type clusters. x-axis, names of the different cell-type clusters; y-axis, expression in the cells of different cell-type clusters.



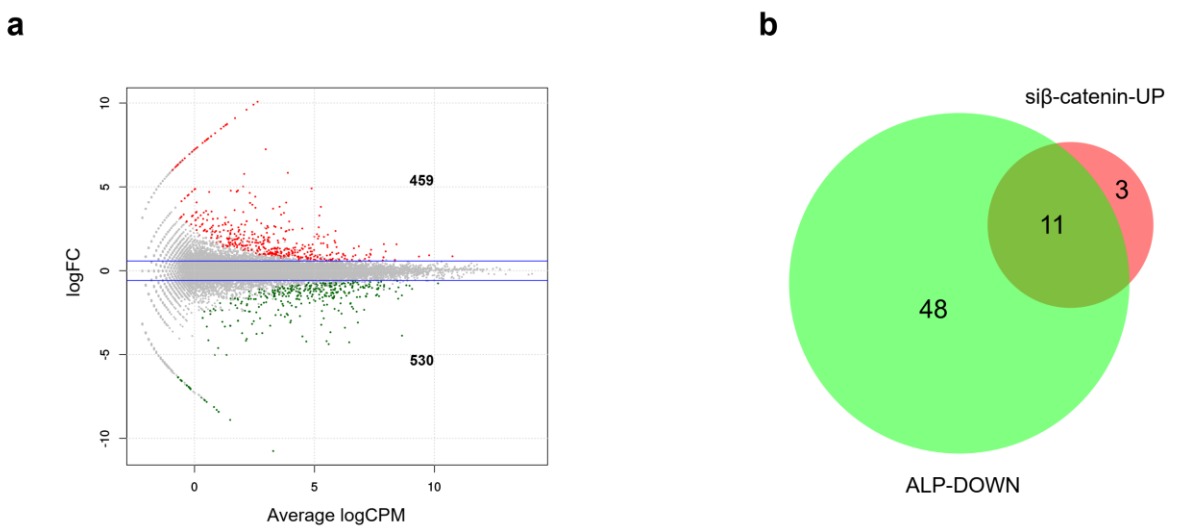
Supplementary Figure 6: Expression pattern of *HvOtp1* and *HvOtx1* upon activation of Wnt signalling. Family pictures of expression pattern of *HvOtp1* and *HvOtx1* by *in situ* hybridization after treatment with Alsterpaullone with respective controls. DMSO, vehicle control; ALP, 5 µM Alsterpaullone. The images with Red border line were used as representative images in Figure 2d.


a
Feed (clean after 6 h)
Recovery at 10 ℃
Feed (clean after 6 h)
Recovery at 10 ℃
Feed (clean after 6 h)
Recovery at 10 ℃
Feed (clean after 6 h)
ALP treatment started
ALP treatment stopped
DE of genes using qPCR
Observing Morphology
Day
Electroporation
b
Relative fold change
Master Control
ALP treated
ALP + Electroporation
ALP+siGFP
ALP + siβ-catenin
Relative fold enrichment
Master Control
ALP treated
ALP+siGFP
ALP+Electroporation Control
ALP+siMargin1
ALP+siMargin2
ALP+siMargin1/siMargin2
Electroporation Control
siGFP
siMargin1
siMargin2
siMargin1/siMargin2
Relative fold enrichment
Master Control
ALP treated
ALP+siGFP
ALP+Electroporation Control
ALP+siGorget1
ALP+siGorget2
ALP+siGorget1/siGorget2
Electroporation Control
siGFP
siGorget1
siGorget2
siGorget1+siGorget2
c
Master Control
5 uM ALP
siGFP + 5 uM ALP
siβ-catenin + 5 uM ALP
HvWnt3a
Master Control
5 uM ALP
siGFP + 5 uM ALP
siMargin + 5 uM ALP
Margin
Master Control
5 uM ALP
siGFP + 5 uM ALP
siGorget + 5 uM ALP
Gorget

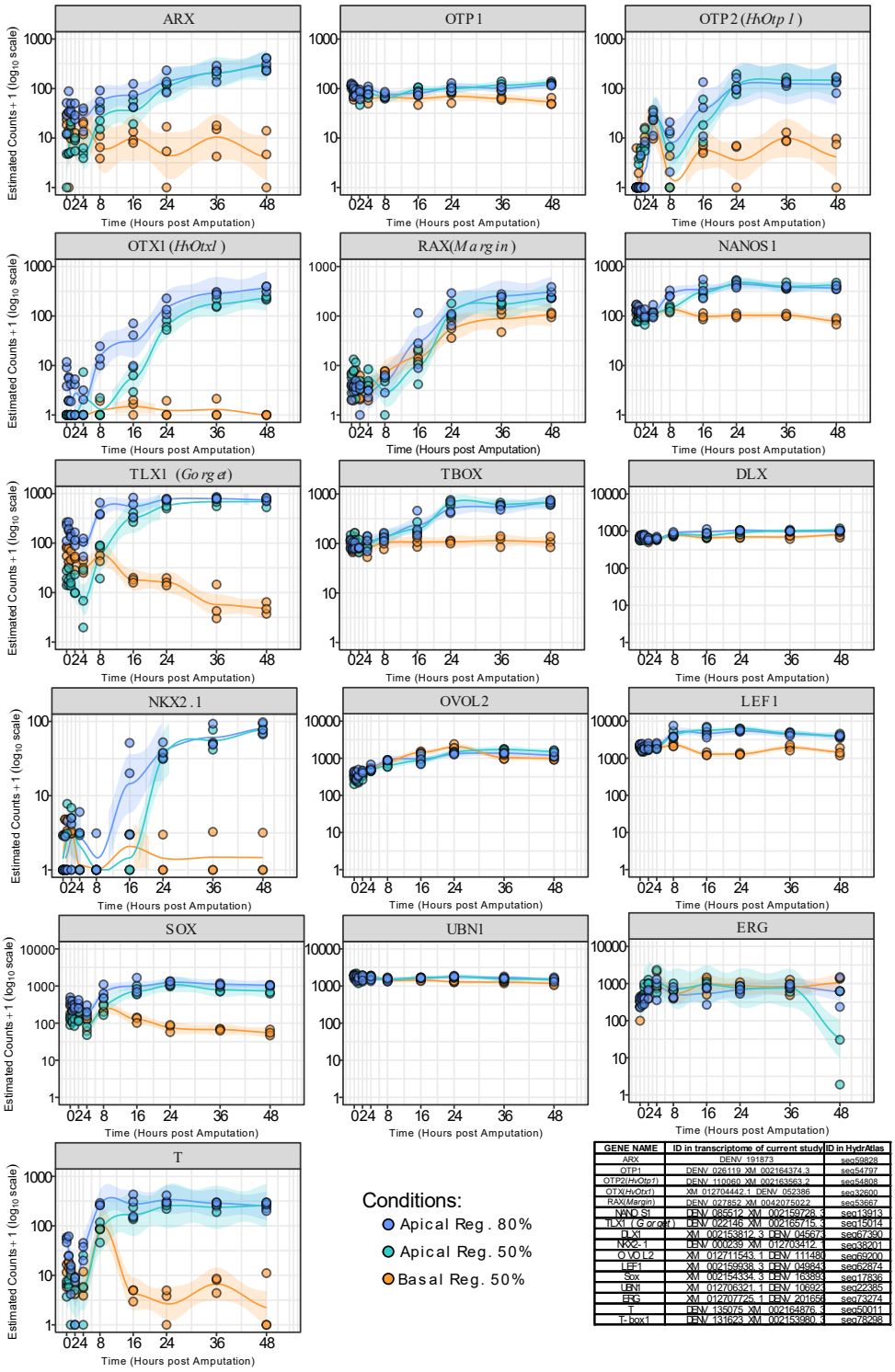
Supplementary Figure 7: Knockdown of *β-catenin*, *Margin* and *Gorget*. Experimental design of siRNA mediated knockdown of *β-catenin*, *HvRx1* and *Gorget* followed by the activation of Wnt signalling. **b,** Validation of *β-catenin*, *HvRx1* and *Gorget* knockdown by qRT-PCR; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. **c**, Family pictures of expression scored by *in situ* hybridization upon knockdown of *β-catenin, Margin* and *Gorget* with controls. Image with red line border was used as the representative image in Figure 1c.



Supplementary Figure 8: Morphology of *Hydra* polyps following Knockdown of *β-catenin*, *Margin* and *Gorget*. Pictures of morphological changes followed by knockdown of *β-catenin*, *Margin* and *Gorget* in the background of Alsterpaullone treatment.



Supplementary Figure 9: Differential gene expression analysis upon *β-catenin* knockdown. a, Smear plot showing the differentially expressed genes upon knockdown of *β-catenin* followed by activation of Wnt signalling. Red and Green colour dots denote significantly up and down regulated genes respectively. **b,** Venn diagram showing the overlap of TFs downregulated by activation of Wnt signalling and TFs upregulated by *β-catenin* knockdown. ALP-DOWN, downregulated TFs upon Alsterpaullone treatment; siβ-catenin-UP, upregulated TFs after *β-catenin* knockdown.



GENE NAME	ID in transcriptome of current study	ID in HydrAtlas
ARX	DENV_191873	seq59828
OTP1	DENV_026119 XM_002164374.3	seq54797
OTP2(HvOtp1)	DENV_110960 XM_002163563.2	seq54808
OTX(HvOtx1)	XM_012704442.1 DENV_052386	seq32600
RAX(Margin)	DENV_027852 XM_004207502.2	seq53667
NANOS1	DENV_085512 XM_002159728.3	seq13913
TLX1 (Gorget)	DENV_022146 XM_002165715.3	seq15014
DLX1	XM_002153812.3 DENV_045672	seq67380
NKX2-1	DENV_000239 XM_012703412.1	seq38201
OVOL2	XM_012711543.1 DENV_111480	seq69200
LEF1	XM_002159938.3 DENV_049843	seq62874
Sox	XM_002154334.3 DENV_163893	seq17836
UBN1	XM_012706321.1 DENV_106923	seq22385
ERG	XM_012707725.1 DENV_201658	seq73274
T	DENV_135075 XM_002164876.3	seq50011
T-box1	DENV_131623 XM_002163980.3	seq78298

Supplementary Figure 10: Expression dynamics of Wnt/β-catenin regulated TFs identified in this study during regeneration of Hydra. Expression plots for selected genes were extracted from HydrATLAS server (https://hydratlas.unige.ch/)[2,3]. Plots depict the estimated counts (y-axis) for transcripts during different time points of regeneration (y-axis). The table in the bottom right corner includes details of gene names, identification numbers (IDs) of our hybrid transcriptome assembly and corresponding IDs in HydrATLAS.

Information regarding the versions of programs and command lines used in the *de novo* transcriptome assembly, functional annotation and differential expression analysis.

1) Data processing by FASTX toolkit (version 36.06)

I) Trimming first 10 and last 2 bases using fastx_trimmer

```
$fastx_trimmer -Q 33 -f 10 -l 99 -i $file -o $file.trimmed
```

II) Read correction using SEECER

```
$seecer Read1.fastq Read2.fastq
```

2) Transcriptome assembly by abyss-pe (version 3.81) and transAbyss (version 1.4.8)

I) Assembly at different k-mers (35 to 88 in the interval of 3)

```
#!/bin/bash
for k in {35..88..3}
do
echo "Processing k-mer ******** : $k"
mkdir k$k
echo "Running k-mer: $k" >> kmer.log
abyss-pe -C k$k np=80 name=illumina k=$k lib='VC_rep1 VC_rep2 AP_rep1
AP_rep2' \
VC_rep1='/data/transcriptome/SO_2135/VC_control/rep1/SO_2135_Set_I_B_VC_R
1.fastq.trimmed_corrected.fa
/data/transcriptome/SO_2135/VC_control/rep1/SO_2135_Set_I_B_VC_R2.fastq.trim
med_corrected.fa' \
VC_rep2='/data/transcriptome/SO_2135/VC_control/rep2/SO_2135_Set_II_A_VC_R
1.fastq.trimmed_corrected.fa
/data/transcriptome/SO_2135/VC_control/rep2/SO_2135_Set_II_A_VC_R2.fastq.tri
mmed_corrected.fa' \
AP_rep1='/data/transcriptome/SO_2135/AP_treated/rep1/SO_2135_Set_I_B_AP_R
1.fastq.trimmed_corrected.fa
/data/transcriptome/SO_2135/AP_treated/rep1/SO_2135_Set_I_B_AP_R2.fastq.trim
med_corrected.fa' \
AP_rep2='/data/transcriptome/SO_2135/AP_treated/rep2/SO_2135_Set_II_B_AP_R
1.fastq.trimmed_corrected.fa
/data/transcriptome/SO_2135/AP_treated/rep2/SO_2135_Set_II_B_AP_R2.fastq.tri
mmed_corrected.fa'
done
```

II) Filtering of the assemblies (FEM)

Transcriptome libraries:

• junction contigs and indel bubbles are extended

• short contigs and short islands are removed
• overlapping/redundant sequences are merged
• sequences shorter than read length are removed

```
for k in 35 38 41 44 47 50 53 56 59 62 65 68 73 78 83 88
do
        bash ../wrappers/abyss-ta-filter -n illumina -i illumina/trans-abyss-
v1.4.8/assembly/k$k -o illumina/trans-abyss-v1.4.8/filter/k$k -k $k
done
```

```
bash ../wrappers/abyss-rmdups-iterative -n illumina -i illumina/trans-abyss-
v1.4.8/filter/ -o illumina/trans-abyss-v1.4.8/merge/ -p 80
```

3) Prediction of ORFs from the transcripts

```
$perl /home/amol/trinityrnaseq_r2012-10-05/trinity-
plugins/transdecoder/transcripts_to_best_scoring_ORFs.pl -t traabyss_transcripts.fa
-m 50 --CPU 30
```

4) *De novo* transcript clustering

The UCLUST algorithm divides a set of sequences into clusters. The cluster_fast and cluster_smallmem commands are based on UCLUST. A cluster is defined by one sequence, known as the centroid or representative sequence. Every sequence in the cluster must have similarity above a given identity threshold with the centroid, as shown in the figure below.

```
$./usearch7.0.1001_i86linux32 -cluster_fast
best_candidates.eclipsed_orfs_removed.pep -id 0.9 -centroids centroid.fasta -uc
clusters.uc -fastapairs fastapairs.txt -userout hits.m8 -userfields
query+target+id+alnlen+mism+opens+qlo+qhi+tlo+thi+evalue+bits
```

5) Prediction of ORFs from the reference based transcripts

```
$perl /home/amol/trinityrnaseq_r2012-10-05/trinity-
plugins/transdecoder/transcripts_to_best_scoring_ORFs.pl -t
reference_transcripts.fa -m 50 --CPU 30
```

6) Merging *de novo* (*Hydra vulgaris*** Ind-Pune**) and RefSeq (***Hydra magnipapillata***) assemblies**

#refseq source

ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/invertebrate/Hydra_vulgaris/latest_assembly_versions/GCF_000004095.1_Hydra_RP_1.0/

#make blast database

$makeblastdb -in ./blastdb/refseq_protein.fasta -dbtype prot -out ./blastdb/refseq_protein

$makeblastdb -in ./blastdb/denovo_pep.fasta -dbtype prot -out ./blastdb/denovo_pep

$makeblastdb -in ./blastdb/uniref90.fasta -dbtype prot -out ./blastdb/uniref90

#run BLASTP reciprocally

$blastp -num_threads 12 -query ./blastdb/denovo_pep.fasta -db ./blastdb/refseq_protein -out ./RBH/denovo_vs_refseq -evalue 0.01 -outfmt 7 -max_target_seqs 1;

$blastp -num_threads 12 -query ./blastdb/refseq_protein.fasta -db ./blastdb/denovo_pep -out ./RBH/refseq_vs_denovo -evalue 0.01 -outfmt 7 -max_target_seqs 1

#select top hits

$awk '/^DENV*/' ./RBH/denovo_vs_refseq|awk '{if($3>=70) print$0}'|awk '{print$1,$2}'|sort -u > ./RBH/denovo_refseq_table

$awk '/^NP|^XP/' ./RBH/refseq_vs_denovo|awk '{if($3>=70) print$0}'|awk '{print$1,$2}'|sort -u > ./RBH/refseq_denovo_table

select same pair

$awk 'NR==FNR{a[$1,$2];next} ($2,$1) in a' ./RBH/denovo_refseq_table ./RBH/refseq_denovo_table > ./RBH/selected_matches

calculate the length of the protein sequences of denovo assembly and refseq

$awk '/^>/ {if (seqlen)print seqlen;print;seqlen=0;next} { seqlen+= length($0)}END{print seqlen}' ./blastdb/denovo_pep.fasta | awk 'NR%2{s=$0; next} {print s,$0}' OFS="\t" > ./RBH/denovo_pep_length

$awk '/^>/ {if (seqlen)print seqlen;print;seqlen=0;next} { seqlen+= length($0)}END{print seqlen}' ./blastdb/refseq_protein.fasta| awk 'NR%2{s=$0; next} {print s,$0}' OFS="\t" > ./RBH/refseq_protein_length

calculate the RBH ratio and select better covered refseq

$join -j 1 -o 1.2,1.1,2.2 <(sort -k1 ./RBH/selected_matches) <(sort -k1 ./RBH/refseq_protein_length_mod) > ./RBH/selected_matches_refseq_length

$join -j 1 -o 1.1,1.2,2.2,1.3 <(sort -k1 ./RBH/selected_matches_refseq_length) <(sort -k1 ./RBH/denovo_pep_length)|awk '{ratio=$4/$3; if(ratio>1) print $0, ratio}' > ./RBH/selected_matches_sigRBHratio_refseq

get the protein fasta sequences from the selected refseqIDs [RBH ratio based]

$awk '{print $2}' ./RBH/selected_matches_sigRBHratio_refseq > ./RBH/finla_assembly/selected_matches_sigRBHratio_refseq_IDs

#fetch FASTA sequences with better RBH ratio from refseq data

$awk 'BEGIN{while((getline<"./RBH/finla_assembly/selected_matches_sigRBHratio_refseq_IDs")>0)l[">"$1]=1}/^>/{f=l[$1]?1:0}f' ./blastdb/refseq_protein.fasta > ./RBH/finla_assembly/sigRBHratio_refseq_protein.fasta

#fetch FASTA sequences unique to refseq data by romoving matching IDs (orthologs from RBH)

$awk 'BEGIN{while((getline<"./RBH/selected_matches")>0)l[">"$1]=1}/^>/{f=!l[$1]?1:0}f' ./blastdb/refseq_protein.fasta> ./RBH/finla_assembly/unique_refseq.fasta

#fetch FASTA sequences common to *de novo* and refseq data [from denovo assembly] (orthologs from RBH)

$awk 'BEGIN{while((getline<"./RBH/selected_matches")>0)l[">"$2]=1}/^>/{f=l[$1]?1:0}f' ./blastdb/denovo_pep.fasta > ./RBH/finla_assembly/common_denovo.fasta

#fetch FASTA sequences common to denovo and refseq data after filtering significant RBH hits from refseq [from *de novo* assembly] (orthologs from RBH)

$awk 'BEGIN{while((getline<"./RBH/selected_matches_sigRBHratio_refseq")>0)l[">"$1]=1}/^>/{f=!l[$1]?1:0}f' ./RBH/finla_assembly/common_denovo.fasta > ./RBH/finla_assembly/common_denovo_denovo.fasta

#fetch FASTA sequences unique to *de novo* data by romoving matching IDs (orthologs from RBH)

$awk 'BEGIN{while((getline<"./RBH/selected_matches")>0)l[">"$2]=1}/^>/{f=!l[$1]?1:0}f' ./blastdb/denovo_pep.fasta > ./RBH/finla_assembly/unique_denovo.fasta

get the CDS fasta sequences from the selected refseqIDs [RBH ratio based]

$join -1 3 -2 1 -o 1.2 <(sort -k3 ~/mouli/projects/hydra_regeneration/hmag_genome/ID_maps/IDs_map_mod) <(sort

-k1 ./RBH/finla_assembly/selected_matches_sigRBHratio_refseq_IDs) |sort -u > ./RBH/finla_assembly/selected_matches_sigRBHratio_refseq_XM_NM_IDs

#fetch FASTA sequences with better RBH ratio from refseq data

$awk 'BEGIN{while((getline<"./RBH/finla_assembly/selected_matches_sigRBHratio_refseq_XM_NM_IDs")>0)l[">"$1]=1}/^>/{f=l[$1]?1:0}f' ./blastdb/refseq_rna.fasta > ./RBH/finla_assembly/sigRBHratio_refseq_cds.fasta

$join -1 3 -2 1 -o 1.2 <(sort -k3 ~/mouli/projects/hydra_regeneration/hmag_genome/ID_maps/IDs_map_mod) <(sort -k1 ./RBH/selected_matches) |sort -u > ./RBH/selected_matches_XM_NM_IDs

#fetch FASTA sequences unique to refseq data by romoving matching IDs (orthologs from RBH)

$awk 'BEGIN{while((getline<"./RBH/selected_matches_XM_NM_IDs")>0)l[">"$1]=1}/^>/{f=!l[$1]?1:0}f' ./blastdb/refseq_rna.fasta > ./RBH/finla_assembly/unique_refseq_cds.fasta

#fetch FASTA sequences common to *de novo* and refseq data [from denovo assembly] (orthologs from RBH)

$grep ">" ./RBH/finla_assembly/common_denovo.fasta| awk '{split($1,a,">"); print a[2]}' > ./RBH/finla_assembly/common_denovo_IDs

$awk 'BEGIN{while((getline<"./RBH/finla_assembly/common_denovo_IDs")>0)l[">"$1]=1}/^>/{f=l[$1]?1:0}f' ./blastdb/denovo_cds.fasta > ./RBH/finla_assembly/common_denovo_cds.fasta

#fetch FASTA sequences common to *de novo* and refseq data after filtering significant RBH hits from refseq [from *de novo* assembly] (orthologs from RBH)

$join -1 3 -2 2 -o 2.1 <(sort -k3 ~/mouli/projects/hydra_regeneration/hmag_genome/ID_maps/IDs_map_mod) <(sort -k2 ./RBH/selected_matches_sigRBHratio_refseq) |sort -u > ./RBH/finla_assembly/selected_matches_sigRBHratio_denovo_IDs

$awk 'BEGIN{while((getline<"./RBH/finla_assembly/selected_matches_sigRBHratio_denovo_IDs")>0)l[">"$1]=1}/^>/{f=!l[$1]?1:0}f' ./RBH/finla_assembly/common_denovo_cds.fasta > ./RBH/finla_assembly/common_denovo_denovo_cds.fasta

#fetch FASTA sequences unique to denovo data by romoving matching IDs (orthologs from RBH)

```
$awk
'BEGIN{while((getline<"./RBH/selected_matches")>0)l[">"$2]=1}/^>/{f=!l[$1]?1:0}f'
./blastdb/denovo_cds.fasta > ./RBH/finla_assembly/unique_denovo_cds.fasta

# remove line breaks to protein fasta files


$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/sigRBHratio_refseq_protein.fasta >
./RBH/finla_assembly/sigRBHratio_refseq_protein_nolinebreaks.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/unique_refseq.fasta>./RBH/finla_assembly/unique_refseq_nili
nebreak.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/common_denovo.fasta >
./RBH/finla_assembly/common_denovo_nolinebreak.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/common_denovo_denovo.fasta >
./RBH/finla_assembly/common_denovo_denovo_nolinebreaks.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/unique_denovo.fasta >
./RBH/finla_assembly/unique_denovo_nolinebreak.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./blastdb/denovo_pep.fasta > ./RBH/finla_assembly/denovo_pep_nolinebreaks.fasta

# remove line breaks to CDS fasta files

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/sigRBHratio_refseq_cds.fasta >
./RBH/finla_assembly/sigRBHratio_refseq_cds_nolinebreaks.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/unique_refseq_cds.fasta >
./RBH/finla_assembly/unique_refseq_cds_nolinebreaks.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/common_denovo_cds.fasta >
./RBH/finla_assembly/common_denovo_cds_nolinebreaks.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/common_denovo_denovo_cds.fasta >
./RBH/finla_assembly/common_denovo_denovo_cds_nolinebreaks.fasta

$awk '!/^>/ { printf "%s", $0; n = "\n" }  /^>/ { print n $0; n = "" } END { printf "%s", n }'
./RBH/finla_assembly/unique_denovo_cds.fasta >
./RBH/finla_assembly/unique_denovo_cds_nolinebreaks.fasta
```

modify protein fasta headers of refseq [remove description and ">" and keep IDs]

$sed 's/\ .*$//g'
./RBH/finla_assembly/sigRBHratio_refseq_protein_nolinebreaks.fasta|sed 's/>//g' >
./RBH/finla_assembly/sigRBHratio_refseq_protein_nolinebreaks_modIDs.fasta

modify cds fasta headers of refseq [remove description and ">" and keep IDs]

$sed 's/\ .*$//g' ./RBH/finla_assembly/sigRBHratio_refseq_cds_nolinebreaks.fasta|
sed 's/>//g' >
./RBH/finla_assembly/sigRBHratio_refseq_cds_nolinebreaks_modIDs.fasta

$sed 's/\ .*$//g' ./RBH/finla_assembly/unique_refseq_cds_nolinebreaks.fasta|sed
's/>//g' > ./RBH/finla_assembly/unique_refseq_cds_nolinebreaks_modIDs.fasta

convert no line break protein fasta to tab limited

$awk 'NR%2{s=$0; next} {print s,$0}' OFS="\t"
./RBH/finla_assembly/sigRBHratio_refseq_protein_nolinebreaks_modIDs.fasta >
./RBH/finla_assembly/sigRBHratio_refseq_protein_nolinebreaks_modIDs_tablimited.
fasta

$sed 's/>//g' ./RBH/finla_assembly/common_denovo_denovo_nolinebreaks.fasta
|awk 'NR%2{s=$0; next} {print s,$0}' OFS="\t"
>./RBH/finla_assembly/common_denovo_denovo_nolinebreaks_tablimited.fasta

$awk'NR%2{s=$0; next} {print s,$0}' OFS="\t"
./RBH/finla_assembly/denovo_pep_nolinebreaks.fasta >
./RBH/finla_assembly/denovo_pep_nolinebreaks_tablimited.fasta

convert no line break cds fasta to tab limited

$awk 'NR%2{s=$0; next} {print s,$0}' OFS="\t"
./RBH/finla_assembly/sigRBHratio_refseq_cds_nolinebreaks_modIDs.fasta>
./RBH/finla_assembly/sigRBHratio_refseq_cds_nolinebreaks_modIDs_tablimited.fast
a

$sed 's/>//g'
./RBH/finla_assembly/common_denovo_denovo_cds_nolinebreaks.fasta| awk
'NR%2{s=$0; next} {print s,$0}' OFS="\t" >
./RBH/finla_assembly/common_denovo_denovo_cds_nolinebreaks_tablimited.fasta

add *de novo* IDs to RBH best hit IDs (protein)

$join -j 1 -o 1.1,1.2,2.2 <(sort -k1 ./RBH/selected_matches) <(sort -k1
./RBH/finla_assembly/sigRBHratio_refseq_protein_nolinebreaks_modIDs_tablimited.
fasta) | awk '{print">"$1"_"$2,$3}' OFS="\n" >
./RBH/finla_assembly/sigRBHratio_refseq_proteinID_denovoID.fasta

add *de novo* IDs to RBH best hit IDs (cds)

$join -1 3 -2 1 -o 1.2 2.2 <(sort -k3 ~/mouli/projects/hydra_regeneration/hmag_genome/ID_maps/IDs_map_mod) <(sort -k1 ./RBH/selected_matches) |sort -u > ./RBH/selected_matches_NM_XM_denv_IDs

$join -j 1 -o 1.1,1.2,2.2 <(sort -k1 ./RBH/selected_matches_NM_XM_denv_IDs) <(sort -k1 ./RBH/finla_assembly/sigRBHratio_refseq_cds_nolinebreaks_modIDs_tablimited.fasta) | awk '{print">"$1"_"$2,$3}' OFS="\n" > ./RBH/finla_assembly/sigRBHratio_refseqID_denovoID.fasta

add refseqIDs to *de novo* matching sequences (protein)

$join -1 2 -2 1 -o 1.2,1.1,2.2 <(sort -k2 ./RBH/selected_matches) <(sort -k1 ./RBH/finla_assembly/common_denovo_denovo_nolinebreaks_tablimited.fasta) | awk '{print">"$1"_"$2,$3}' OFS="\n" > ./RBH/finla_assembly/common_denovo_refseq.fasta

add refseqIDs to *de novo* matching sequences (cds)

$join -1 2 -2 1 -o 1.2,1.1,2.2 <(sort -k2 ./RBH/selected_matches_NM_XM_denv_IDs) <(sort -k1 ./RBH/finla_assembly/common_denovo_denovo_cds_nolinebreaks_tablimited.fasta) | awk '{print">"$1"_"$2,$3}' OFS="\n" > ./RBH/finla_assembly/common_denovoID_refseqID.fasta

#merge all sequences

$cat ./RBH/finla_assembly/protein_sequences/*.* > ./RBH/finla_assembly/protein_sequences/hybrid_protein.fasta

$cat ./RBH/finla_assembly/CDS_sequences/*.* > ./RBH/finla_assembly/CDS_sequences/hybrid_cds.fasta

7) Calculation of completeness of different transcriptome assemblies by BUSCO (version 3.0.2)

a) training augustus

Initially gene prediction algorithm was trained to get custom specific models for running AGUSTUS. This was achieved through BUSCO wrapper. Here, I have used filtered gene sets from RefSeq genome annotation containing multi exon and single copy genes after removing UTRs.

split test and training set (~1:5)

$./scripts/randomSplit.pl ./gene_set_filter4_mod_withoutUTR.gb 200
grep -c LOCUS ./gene_filter4_mod_withoutUTR.gb*

create new file for hydra vulgaris Ind-Pune

$./scripts/new_species.pl --species=HvulIP

#etrain

$./bin/etraining --species=HvulIP ./ gene_set_filter4_mod_withoutUTR.gb.train

#test
$./bin/augustus --species=HvulIP ./ gene_set_filter4_mod_withoutUTR.gb.test | tee firsttest.out

#optimize
$./scripts/optimize_augustus.pl --cpus=10 --species=HvulIP ./MAKER_filter4_mod_withoutUTR.gb.train --metapars=./config/species/HvulIP/HvulIP_metapars.cfg

b) Run BUSCO for each transcriptome assembly or refseq data

#busco
$./scripts/run_BUSCO.py -c 10 --long -sp HvulIP -o Hmag1 -i /home/hydra/mouli/projects/Hydra_Genome/BUSCO/busco/data/hma.fasta -l /home/hydra/mouli/projects/Hydra_Genome/BUSCO/busco/data/eukaryota_odb9 -m genome -o out_hmag1

$python scripts/generate_plot.py -wd ./BUSCO_summaries_hydra_assemblies

8) Functional annotation by InterProScan (version 5.14-53.0) and BLAST (version 2.2.28+)

functional annotation by InterProScan

$./interproscan-5.14-53.0/interproscan.sh -i /home/hydra/mouli/projects/wnt/new_analysis/denovo_refseq_merge/RBH/finla_assembly/protein_sequences/hybrid_protein.fasta -appl ProDom,ProSiteProfiles,PRINTS,Pfam,TIGRFAM --goterms -o /home/hydra/mouli/projects/wnt/new_analysis/denovo_refseq_merge/annotation/hybrid_protein_interproscan -f TSV

$awk 'BEGIN {FS="\t"};{print$1"\t"$14}' ./hybrid_protein_interproscan | awk '/GO:/{print$0}'| sort -u > ./hybrid_protein_interproscan_go_raw

$awk '{FS="\t"} {for(j=2;j<=NF;j++) a[$1]=a[$1]"\t"$j}END{for(i in a) {print i""a[i]}}' ./annotation/hybrid_protein_interproscan_go_raw > ./annotation/hybrid_protein_interproscan_go_WEGO_native_format

#remove repeted GO IDs per record

$awk '{ while(++i<=NF) printf (!a[$i]++) ? $i FS:""; i=split("",a); print ""}' ./annotation/hybrid_protein_interproscan_go_WEGO_native_format > ./annotation/hybrid_protein_interproscan_go_WEGO_native_format_mod

annotation by performing BLAST against ubniref90

$blastp -query ./RBH/finla_assembly/protein_sequences/hybrid_protein.fasta -db ./blastdb/uniref90 -num_threads 11 -max_target_seqs 1 -outfmt 6 > ./annotation/hybrid_protein_blastp_uniref90.outfmt6

$awk '{print $2}' ./annotation/hybrid_protein_blastp_uniref90.outfmt6 > ./annotation/hybrid_protein_blastp_hits_uniref90_IDs

#go to uniprot retrieve/ID mapping and paste the list of UniRef90 hits and converts IDs (UniRef) to UniProtKB, download as tab limited file and change UNIREF90 to UniRef90.

$join -1 2 -2 1 -o 1.1 2.4 -t $'\t' <(sort -k2 ./annotation/hybrid_protein_blastp_uniref90.outfmt6) <(sort -k1 ./annotation/uniref90_go_IDs.tab)| awk '/GO:/{print $0}' |awk '$1=$1' FS=";" OFS="\t" |sort -u >./annotation/hybrid_protein_uniref90blastP_go_raw

$awk '{FS="\t"} {for(j=2;j<=NF;j++) a[$1]=a[$1]"\t"$j}END{for(i in a) {print i""a[i]}}' ./annotation/hybrid_protein_uniref90blastP_go_raw > ./annotation/hybrid_protein_uniref90blastP_go_raw_WEGO_native_format

#remove repeated GO IDs per record

$awk '{ while(++i<=NF) printf (!a[$i]++) ? $i FS:""; i=split("",a); print ""}' ./annotation/hybrid_protein_uniref90blastP_go_raw_WEGO_native_format > ./annotation/hybrid_protein_uniref90blastP_go_raw_WEGO_native_format_mod

$cat ./annotation/hybrid_protein_uniref90blastP_go_raw_WEGO_native_format_mod ./annotation/hybrid_protein_interproscan_go_WEGO_native_format_mod > ./annotation/merged_GO_IDs

$awk '{FS=" "} {for(j=2;j<=NF;j++) a[$1]=a[$1]"\t"$j}END{for(i in a) {print i""a[i]}}' ./annotation/merged_GO_IDs > ./annotation/merged_GO_IDs_unique

#remove repeated GO IDs per record

$awk '{ while(++i<=NF) printf (!a[$i]++) ? $i FS:""; i=split("",a); print ""}' ./annotation/merged_GO_IDs_unique > ./annotation/merged_GO_IDs_mod

9) **Read alignment and quantitation by Trinity pipeline (version 2.1.1)**

#!/bin/bash

for i in $(ls /home/hydra/mouli/projects/wnt/new_analysis/refseqbased_DE/tophat/*.fastq|awk -F"/" '{split($NF,a,"."); print a[1]}'|cut -c -11| sort -u)

do ./util/align_and_estimate_abundance.pl --thread_count 10 --transcripts /home/hydra/mouli/projects/wnt/new_analysis/trinity_DE/hybrid_cds_mod.fasta --seqType fq --left /home/hydra/mouli/projects/wnt/new_analysis/refseqbased_DE/tophat/${i}_R1.fastq --right

/home/hydra/mouli/projects/wnt/new_analysis/refseqbased_DE/tophat/${i}_R2.fastq --est_method RSEM --aln_method bowtie --prep_reference --output_dir /home/hydra/mouli/projects/wnt/new_analysis/trinity_DE/alignment_out/${i}

done;

Note: similar command line was used for analysis using different experimental samples. The final count matrix was used in edgeR for differential expression analysis. All the commands listed above are generic and require additional details (directory structure and file names) in order to re-execute them.

Phylogenetic analysis of PRD class of homeobox genes.

Phylogenetic analysis was carried out using the homeobox genes collected from HomeoDB for human, mouse, zebrafish, fruitfly and nematode [4](http://homeodb.zoo.ox.ac.uk). Sequences from *Nematostella* and sponges were included for comparative analysis based on previous classification [5]. Here, initial broad classification was carried out using CLANS to segregate PRD class members based on BLAST similarity scores using bioinformatic toolkit available at htpps://toolkit.tubingen.mpg.de [6]. Homeobox domain regions were collected from these sequences after performing hmmscan using HMMER v3.1. All the fetched sequences from these group were aligned using ETC3 tool kit [7]. These alignments were cured manually in Jalview v2 and used for further analysis (Waterhouse et. al., 2009). Phylogentic tree was computed using randomized accelerated maximum likelihood (RAxML-HPC v.8 on XSEDE) implemented in CIPRES Science Gateway [8]. Here, protein CAT model with LG substitution matrix was used with 1000 iterations of rapid bootstrap analysis and HyHox1 is used as an outgroup. The phylogenetic tree was visualized and annotated in iTOL and branch support values above 30 were displayed on branches [9].

1) PRD class protein sequence files

```
>al_DR

MGISEEIKLEELPQEAKLAHPDAVVLVDRAPGSSAASAGAALTVSMSVSGGAPSGASGASGGTNSPVSDGNSDCE
ADEYAPKRKQRRYRTTFTSFQLEELEKAFSRTHYPDVFTREELAMKIGLTEARIQVWFQNRRAKWRKQEKVGPQS
HPYNPYLPGGAATMQTVVGAALPPNPFTHLGFQLRKPFDAQHAANLAAFRYPHLSAAPMIPSGYFNQFQRAPPHM
LPHGMAGMYSPSSSFQSLLANMTAVPRGTPLGKPPALLVGSPDLHSPNHMLASPPTSPASGHASQHQQHPTAHPP
PPQAPPQMPVGVQPAQLSPQHLVGIALTQQASSLSPTQTSPVALTLSHSPQRQLPPPSHQAPPPPPRAATPPEDR
RTSSIAALRLKAREHELKLELLRQNGHGNDVVS

>ALX1_DA

MEYLSDKFSLKSPAIKGSDYYMDQVMDTLDNVQYYNKASPKCVQAFPMQSNDQHSSMDRSSPCDNQSSVTYCAPK
SEESSLHAMENCCSLRVSPATSGPDKTDLDELGEKCDSNVSSSKKRRHRTTFTSAQLEELEKVFQKTHYPDVYVR
EQLAMRTELTEARVQVWFQNRRAKWRKRERYGQIQQAKSHFAATYDISMLPRTDSYSQISNNLWTGPSAGSSVVS
SCMIPRGSPPCVTSPYPHSPRAAEHGYVGFPNHQQNQFGVNHVSLNNFFADSLLASSANSHAAFETKPEFERRSS
SIAVLRMKAKEHTANISWAM
```

>ALX1_HU

MEFLSEKFALKSPPSKNSDFYMGAGGPLEHVMETLDNESFYSKASAGKCVQAFGPLPRAEHHVRLERTSPCQDSS
VNYGITKVEGQPLHTELNRAMDNCNSLRMSPVKGMQEKGELDELGDKCDSNVSSSKKRRHRTTFTSLQLEELEKV
FQKTHYPDVYVREQLALRTELTEARVQVWFQNRRAKWRKRERYGQIQQAKSHFAATYDISVLPRTDSYPQIQNNL
WAGNASGGSVVTSCMLPRDTSSCMTPYSHSPRTDSSYTGFSNHQNQFSHVPLNNFFTDSLLTGATNGHAFETKPE
FERRSSSIAVLRMKAKEHTANISWAM

>ALX1_MO

MEFLSEKFALKSPPSKNSDFYMGTGGALEHVMETLDNESFYGKATAGKCVQAFGPLPRAEHHVRLDRTSPCQDSS
VNYGITKVEGQPLHTELNRAMDNCNNLRMSPVKGMPEKSELDELGDKCDSNVSSSKKRRHRTTFTSLQLEELEKV
FQKTHYPDVYVREQLALRTELTEARVQVWFQNRRAKWRKRERYGQIQQAKSHFAATYDISVLPRTDSYPQIQNNL
WAGNASGGSVVTSCMLPRDASSCMTPYSHSPRTDSSYTGFSNHQNQFSHVPLNNFFTDSLLTGATNGHAFETKPE
FERRSSSIAVLRMKAKEHTANISWAM

>ALX3_HU

MDPEHCAPFRVGPAPGPYVASGDEPPGPQGTPAAAPHLHPAPPRGPRLTRFPACGPLEPYLPEPAKPPAKYLQDL
GPGPALNGGHFYEGPAEAEEKTSKAASFPQLPLDCRGGPRDGPSNLQGSPGPCLASLHLPLSPGLPDSMELAKNK
SKKRRNRTTFSTFQLEELEKVFQKTHYPDVYAREQLALRTDLTEARVQVWFQNRRAKWRKRERYGKIQEGRNPFT
AAYDISVLPRTDSHPQLQNSLWASPGSGSPGGPCLVSPEGIPSPCMSPYSHPHGSVAGFMGVPAPSAAHPGIYSI
HGFPPTLGGHSFEPSSDGDYKSPSLVSLRVKPKEPPGLLNWTT

>ALX3_MO

MDPERCAPFSVGPAAGPYAAAGDEAPGPQGTPDAAPHLHPAPPRGPRLSRFPACGPLEPYLPEPAKPPAKYLQDL
GPGPVLNGGHFYEGSAEAEEKASKAASFPQLPVDCRGGPRDGPSNVQASPGPCLASLSVPLSPGLPDSMELAKTK
SKKRRNRTTFSTFQLEELEKVFQKTHYPDVYAREQLALRTDLTEARVQVWFQNRRAKWRKRERYGKMQEGRNPFT
TAYDISVLPRTDSHPQLQNSLWPSPGSGSPGGPCLMSPEGIPSPCMSPYSHSHGNVAGFMGVPASPAAHPGIYSI
HGFPPALGGHSFEPSPDGDYKSPSLVSLRMKPKEPPGLLNWTT

>ALX4_HU

MNAETCVSYCESPAAAMDAYYSPVSQSREGSSPFRAFPGGDKFGTTFLSAAAKAQGFGDAKSRARYGAGQQDLAT
PLESGAGARGSFNKFQPQPSTPQPQPPPQPQPQQQQPQPQPPAQPHLYLQRGACKTPPDGSLKLQEGSSGHSAAL
QVPCYAKESSLGEPELPPDSDTVGMDSSYLSVKEAGVKGPQDRASSDLPSPLEKADSESNKGKKRRNRTTFTSYQ
LEELEKVFQKTHYPDVYAREQLAMRTDLTEARVQVWFQNRRAKWRKRERFGQMQQVRTHFSTAYELPLLTRAENY
AQIQNPSWLGNNGAASPVPACVVPCDPVPACMSPHAHPPGSGASSVTDFLSVSGAGSHVGQTHMGSLFGAASLSP
GLNGYELNGEPDRKTSSIAALRMKAKEHSAAISWAT

>ALX4_MO

MNAETCVSYCESPAAAMDAYYSPVSQSREGSSPFRGFPGGDKFGTTFLSAGAKGQGFGDAKSRARYGAGQQDLAA
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EPELPPDSEPVGMDNSYLSVKETGAKGPQDRASAEIPSPLEKTDSESNKGKKRRNRTTFTSYQLEELEKVFQKTH
YPDVYAREQLAMRTDLTEARVQVWFQNRRAKWRKRERFGQMQQVRTHFSTAYELPLLTRAENYAQIQNPSWIGNN
GAASPVPACVVPCDPVPACMSPHAHPPGSGASSVSDFLSVSGAGSHVGQTHMGSLFGAAGISPGLNGYEMNGEPD
RKTSSIAALRMKAKEHSAAISWAT

>ALX4a_DA

MNAETCVSYCEMTSMDSYYSPSAPQGRDHQANPFRTFQASDTKYSPAFLTNKGQGYGEKSGSPFQQECQSLDATA
GEGTFNKYHLFMQRSSCKTPPDSSKLQQENSGHNGGLIACYGKDSTGLTDSELPQNSDPAGMDGSYLSVKDSGVK
SPQQATSELASPLDKTEGESNKGKKRRNRTTFTSYQLEELEKVFQKTHYPDVYAREQLALRTDLTEARVQVWFQN
RRAKWRKRERFGQMQQVRTHFSTAYELPLLTRPENYAQIQNPSWIGGSSAASPVPGCVVPCDSVTSCMPPHPHAA
SGVSDFLGVPSPGSHMGQTHMGSLFGSPGMGTGINGYDLNMDPDRKSSSIAALRMKAKEHSAAISWAT

>ARX_DA

MSSQYDDDSRDRSECKSKSPTVLSSYCIDSILGRRSPCKVRQLGAQSLPAPVRPDHEMTTEVTSKENSFDSDMHL
PPKLRRLYGPGGKYLDSGRGFHEHLEKGERERLLDQACESLKISQAPQVSISRSKSYRENAPFSQSDEGQSPEHM

AQELVELSTLKFEEDVVKEEACGDNSLSPKDEESLHNDGDVKDGEDSVCLSAGSDSEEGMLKRKQRRYRTTFTSY
QLEELERAFQKTHYPDVFTREELAMRLDLTEARVQVWFQNRRAKWRKREKAGVQAHPTGLPFPGPLAAAHPLSHY
LEGGPFPPHPHPALESAWTAAAAAAAAFPGLAPPPNSSALPPATPLGLGTFLGTAMFRHPAFIGPTFGRLFSSMG
PLTSASTAAALLRQTAPPVESPVQPSAALPEPPSSSSSTAADRRASSIAALRLKAKEHSAQLTQLNILPSGTAGK
EVC

>ARX_HU

MSNQYQEEGCSERPECKSKSPTLLSSYCIDSILGRRSPCKMRLLGAAQSLPAPLTSRADPEKAVQGSPKSSSAPF
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AAAAAWDTLKISQAPQVSISRSKSYRENGAPFVPPPPALDELGGPGGVTHPEERLGVAGGPGSAPAAGGGTGTED
DEEELLEDEEDEDEEEELLEDDEELLEDDARALLKEPRRCPVAATGAVAAAAAAAVATEGGELSPKEELLLHPE
DAEGKDGEDSVCLSAGSDSEEGLLKRKQRRYRTTFTSYQLEELERAFQKTHYPDVFTREELAMRLDLTEARVQVW
FQNRRAKWRKREKAGAQTHPPGLPFPGPLSATHPLSPYLDASPFPPHHPALDSAWTAAAAAAAAAFPSLPPPPGS
ASLPPSGAPLGLSTFLGAAVFRHPAFISPAFGRLFSTMAPLTSASTAAALLRQPTPAVEGAVASGALADPATAAA
DRRASSIAALRLKAKEHAAQLTQLNILPGTSTGKEVC

>ARX_MO

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PEDAEGKDGEDSVCLSAGSDSEEGLLKRKQRRYRTTFTSYQLEELERAFQKTHYPDVFTREELAMRLDLTEARVQ
VWFQNRRAKWRKREKAGAQTHPPGLPFPGPLSATHPLSPYLDASPFPPHHPALDSAWTAAAAAAAAAFPSLPPPP
GSASLPPSGAPLGLSTFLGAAVFRHPAFISPAFGRLFSTMAPLTSASTAAALLRQPTPAVEGAVASGALADPATA
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>CG11294_DR

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FTMMHPAFQQQHQQQQHQGHQQATDQDKLSKTYTELKLYKAPSHGMELGGMAALSGHSDEGSDGSDSEEIDLTSG
ACIDFSQSSKLQQQQQQQQQQGTQGAAGSAEANGGL

>CG13141_DR

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FTLDQLNELERLFEETHYPDAFMREELSQRLGLSEARVQVWFQNRRAKCRKHENQMHKGFLVGSRSPPIATPLEP
CRVAPYVSLAALRSSSVPSHPAAATSSNPQAPGVSGTSSSGKVVTVDSPHNHNPAISRSAIKQFSSTVAAAAAFS
AFDPAIISVAAHQYAAAITNGTVPAGLFSVPQYSINLAAFAAAHSKSSSIADLRMKAKKHSESLGLQADMVL

>CG32532_DR

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ASHAHSSHAHGSHAHALAIAHGHHGLLPSVVPQQQQQQQQQQQQQQQQQQAQQLPPGVGVASPLQQQQQQQQQQQ
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MGIGGSHENRLLSLASSVQDTRSPITTLEKSSSSSLNHQRKCSSTPEDFSALYSGLPTPGMDSSSHHHTPAHTPP
SRLSDHTISAEGAFKKLKPEPNSGLSTVSAGITSPGSGLSSLSQHAGHTPTTASCPTPARRRHRTTFTQEQLAEL
EAAFAKSHYPDIYCREELARTTKLNEARIQVWFQNRRAKYRKQEKQLQKALAPSVIPSCNGMMRNIQGYSVSRGY
QPYPHHNTMNRYPQDLFQMGASSYPGMTQPFSMAHSTNMGSVGVRQDSMGEFHGMSPEDEWYNKSLSALRMNSSH
HPNLSAPMLQYQT

>CG34367_DR

MEQLAQFVTKSFEFNLTNNSVHALFNSMTGARLKKPTEVKNSIFINSISSGGSYADTCASGEESVDIDITDLSVS
ESNTILAIDPEPTEESHSQREEHVETYSLSPTLHKAAVEPKPKNWLISEDLDVDSQPEDPKMSGLPTASITECTE
DSNTPKLAPDKPLLPEECVSPEPSRNREHCDPLDTSLVNTKQRRSRTNFTLDQLNELERLFEETHYPDAFMREEL
SQRLGLSEARVQVWFQNRRAKCRKHENQMHKGFLVGSRSPPIATPLEPCRVAPYVSLAALRSSSVPSHPAAATSS

NPQAPGVSGTSSSGKVVTVDSPHNHNPAISRSAIKQFSSTVAAAAAFSAFDPAIISVAAHQYAAAITNGTVPAGL
FSVPQYSINLAAFAAAHSKSSSIADLRMKAKKHSESLGLQADMVL

>CG9876_DR

MLNYQQQLHSLPVGNPGNFYYGPTVSGEIYSSHQSHNLESEDKLEDREESGRNLDKIHRFSVDNIMEMKHDAYSK
GKMAMELSSNFGPTGAGCGGADRPAPCSGNLPAGGGHHSRKPRRNRTTFSSAQLTALEKVFERTHYPDAFVREEL
ATKVHLSEARVQVWFQNRRAKFRRNERSVGSRTLLDTAPQLVPAPISNNMHKYANIPHPHPQPPPPPGAYALNFG
PLELRSCQNYTNCYGGFGSSGASGSGVCSFFGATNYCVAANYAKNAYPPL

>crx_DA

MMSYIKQPHYAVNGLTLSASGMDLLHTAVGYPATPRKQRRERTTFTRTQLDILEALFTKTRYPDIFMREEVALKI
NLPESRVQVWFKNRRAKCRQQQQQTSGQPKPRPPKKKSSPPPDLTSDPGTSSSVVAAPTPTVPPSVSAGTAPVSV
WSPTSLSPLPDPLCGSGTPCVQRPAPYPMSYGQPSSYSQGYGSSPYFSGLDCSPYLSPMTTQLSASGGALSPLTV
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>CRX_HU

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>PAX7_MO

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>PAX7a_DA

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>PHX2A_HU

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>PHX2A_MO

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>PHX2B_HU

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>PITX1_DA

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>PTX1_DR

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>RX_DR

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2) Homeodomain alignments of PRD class used for phylogenetic analysis

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PKRTRTAYSNSQLDQLELIFATTHYPDVFTREDLSRRLGIREDRIQVWFQNRRARFRKQE
>toe_DR
FRRNRTTFSPEQLDELEKEFDKSHYPCVNTREKLAARTALSEARVQ------TSEMAAPP
>toy_DR
LQRNRTSFSNEQIDSLEKEFERTHYPDVFARERLADKIGLPE----VWFSNRRAKWRREE
>UNC4_DR
RRRSRTNFNSWQLEELERAFSASHYPDIFMREALAMRLDLKESRVAVWFQNRRAKVRKRE
>UNC4_HU
RRRTRTNFTGWQLEELEKAFNESHYPDVFMREALALRLDLVESRVQVWFQNRRAKWRKKE
>UNC4_MO
RRRTRTNFTGWQLEELEKAFNESHYPDVFMREALALRLDLVESRVQVWFQNRRAKWRKKE
>UNCX_DA
RRRTRTNFTGWQLEELEKAFNESHYPDVFMREALALRLDLIESRVQVWFQNRRAKWRKKE
>UNCX4_DA
RRRTRTNFTGWQLEELEKAFNESHYPDVFMREALALRLDLVESRVQVWFQNRRAKWRKKE
>VSX1_DA
KRRHRTVFTSHQLEELEKAFNEAHYPDVYAREMLAMKTELPEDRIQVWFQNRRAKWRKRE
>VSX1_DR
RRHGRTIFTSSQLEELEKAFKEAHYPDVSARELLSMKTGLAEDRIQVWYQNRRAKWRKTE
>VSX1_HU
KRRHRTVFTAHQLEELEKAFSEAHYPDVYAREMLAVKTELPEDRIQVWFQNRRAKWRKRE
>VSX1_MO
KRRHRTVFTAHQLEELEKAFGEAHYPDVYAREMLAAKTELPEDRIQVWFQNRRAKWRKRE
>VSX2_DA
KRRHRTIFTSYQLEELEKAFNEAHYPDVYAREMLAMKTELPEDRIQVWFQNRRAKWRKRE
>VSX2_DR
RRHSRTIFTSYQLEKLEEAFKEAHYPDVYAREMLSLKTELPEDRIQVWFQNRRAKWRKTE
>VSX2_HU
KRRHRTIFTSYQLEELEKAFNEAHYPDVYAREMLAMKTELPEDRIQVWFQNRRAKWRKRE
>VSX2_MO
KRRHRTIFTSYQLEELEKAFNEAHYPDVYAREMLAMKTELPEDRIQVWFQNRRAKWRKRE
```

Finding orthologs using RBH (reciprocal BAST hits) in planaria and Xenopus

1) Command lines used for generating the RBH data

$makeblastdb -in ./blast_db/hydra_hybrid_protein.fasta -dbtype prot -out ./blast_db/hydra_hybrid_protein

$makeblastdb -in ./blast_db/planaria_longest_orfs_mod.pep -dbtype prot -out ./blast_db/planaria_longest_orfs_mod

$makeblastdb -in ./blast_db/XL9_1_v20161019_primaryTranscripts_pep.fa -dbtype prot -out ./blast_db/XL9_1_v20161019_primaryTranscripts_pep

#./reverse_blast.sh

$blastp -num_threads 12 -query ./blast_db/hydra_hybrid_protein.fasta -db ./blast_db/planaria_longest_orfs_mod -out ./RBH/hydra_vs_planaria -evalue 0.01 -outfmt 7 -max_target_seqs 1

$blastp -num_threads 12 -query ./blast_db/planaria_longest_orfs_mod.pep -db ./blast_db/hydra_hybrid_protein -out ./RBH/planaria_vs_hydra -evalue 0.01 -outfmt 7 -max_target_seqs 1

$blastp -num_threads 12 -query ./blast_db/hydra_hybrid_protein.fasta -db ./blast_db/XL9_1_v20161019_primaryTranscripts_pep -out ./RBH/hydra_vs_xenopus -evalue 0.01 -outfmt 7 -max_target_seqs 1

```
$blastp -num_threads 12 -query
./blast_db/XL9_1_v20161019_primaryTranscripts_pep.fa -db
./blast_db/hydra_hybrid_protein -out ./RBH/xenopus_vs_hydra -evalue 0.01 -outfmt
7 -max_target_seqs 1

$awk '/^DENV|^XP/' ./RBH/hydra_vs_planaria|awk '{if($3>=30) print$0}'|awk
'{print$1,$2}'|sort -u  > ./RBH/hydra_planaria_table
$awk '/^tr5/' ./RBH/planaria_vs_hydra |awk '{if($3>=30) print$0}'|awk
'{print$1,$2}'|sort -u > ./RBH/planaria_hydra_table
$awk '/^DENV|^XP/' ./RBH/hydra_vs_xenopus|awk '{if($3>=30) print$0}'|awk
'{print$1,$2}'|sort -u > ./RBH/hydra_xenopus_table
$awk '/^gnl/' ./RBH/xenopus_vs_hydra|awk '{if($3>=30) print$0}'|awk
'{print$1,$2}'|sort -u > ./RBH/xenopus_hydra_table
$awk 'NR==FNR{a[$1,$2];next} ($2,$1) in a' ./RBH/planaria_hydra_table
./RBH/hydra_planaria_table > ./RBH/hydra_planaria_rbh
$awk 'NR==FNR{a[$1,$2];next} ($2,$1) in a' ./RBH/xenopus_hydra_table
./RBH/hydra_xenopus_table > ./RBH/hydra_xenopus_rbh
```

2) **Protein IDs of genes upregulated upon knockdown of β-catenin with their orthologs (based on RBH-reciprocal BLAST hits) in planaria**

```
DENV_002883_XP_002166177.1    tr5_comp8157_c0_seq1_m_4534
DENV_003721 tr5_comp1472_c0_seq1_m_2764
DENV_008342_NP_001267778.1    tr5_comp2989_c0_seq1_m_10262
DENV_017300_XP_002166749.1    tr5_comp18327_c0_seq1_m_12529
DENV_031968_XP_012554474.1    tr5_comp11238_c0_seq1_m_12911
DENV_055772_XP_002157026.1    tr5_comp17606_c0_seq1_m_12097
DENV_057854 tr5_comp21026_c0_seq1_m_17528
DENV_066788_XP_002163988.1    tr5_comp2749_c0_seq1_m_8218
DENV_076867_XP_012560246.1    tr5_comp316_c0_seq1_m_8874
DENV_097762_XP_012565729.1    tr5_comp24701_c0_seq1_m_17136
DENV_097978_XP_012563379.1    tr5_comp7445_c0_seq1_m_1572
DENV_098962_XP_004208490.1    tr5_comp3340_c0_seq1_m_5018
DENV_127367_XP_002163146.3    tr5_comp7567_c0_seq1_m_4070
DENV_134105 tr5_comp150_c0_seq1_m_450
DENV_163354_XP_012555860.1    tr5_comp2868_c0_seq1_m_10311
XP_002166590.1_DENV_075186    tr5_comp19681_c0_seq1_m_12643
XP_002168605.3_DENV_067560    tr5_comp7514_c0_seq1_m_4569
XP_004208605.2_DENV_021903    tr5_comp39772_c0_seq1_m_18769
XP_012553532.1_DENV_100559    tr5_comp4330_c0_seq1_m_551
XP_012554603.1_DENV_178355    tr5_comp6149_c0_seq1_m_7319
XP_012555020.1_DENV_049455    tr5_comp1616_c0_seq1_m_8832
XP_012555758.1_DENV_013271    tr5_comp20054_c0_seq1_m_17081
XP_012555832.1_DENV_170349    tr5_comp7416_c0_seq1_m_6801
XP_012556963.1_DENV_068869    tr5_comp11764_c0_seq1_m_12451
XP_012558898.1    tr5_comp5422_c0_seq1_m_2396
XP_012559357.1_DENV_023644    tr5_comp14634_c0_seq1_m_14972
XP_012561605.1_DENV_008711    tr5_comp3700_c0_seq1_m_5956
XP_012564494.1_DENV_176993    tr5_comp4647_c0_seq1_m_5548
XP_012565081.1    tr5_comp803_c0_seq1_m_10400
XP_012565777.1    tr5_comp16411_c0_seq1_m_10830
XP_012566719.1_DENV_174502    tr5_comp8109_c0_seq1_m_47
```

3) **Protein IDs of genes downregulated upon knockdown of β-catenin with their orthologs (based on RBH-reciprocal BLAST hits) in planaria**

```
DENV_012348_XP_012553992.1    tr5_comp5362_c0_seq1_m_7568
DENV_029380_XP_012555637.1    tr5_comp13078_c0_seq1_m_14058
```

DENV_051689 tr5_comp4080_c0_seq1_m_396
DENV_056127_XP_004207355.2 tr5_comp12334_c0_seq1_m_13170
DENV_056573_XP_012566931.1 tr5_comp14231_c0_seq1_m_14133
DENV_060128_XP_012553600.1 tr5_comp9437_c0_seq1_m_8970
DENV_073439 tr5_comp12477_c0_seq1_m_13438
DENV_078983_XP_004206814.1 tr5_comp3625_c0_seq1_m_4072
DENV_085512_XP_002159764.2 tr5_comp13566_c0_seq1_m_10678
DENV_089748_NP_001267780.1 tr5_comp2508_c0_seq1_m_7772
DENV_091231_XP_004206634.1 tr5_comp8204_c0_seq1_m_4187
DENV_102767_XP_012555741.1 tr5_comp9535_c0_seq1_m_8607
DENV_103840_XP_012555807.1 tr5_comp19981_c0_seq1_m_18049
DENV_110060_XP_002163599.1 tr5_comp27318_c0_seq1_m_17771
DENV_139448_XP_002160774.1 tr5_comp4102_c0_seq1_m_1317
DENV_144578 tr5_comp7938_c0_seq1_m_8690
DENV_164073_XP_002162811.3 tr5_comp20320_c0_seq1_m_18500
DENV_173944_XP_002155943.3 tr5_comp6293_c0_seq1_m_6003
DENV_191448_XP_002164795.3 tr5_comp125_c0_seq1_m_10183
DENV_198081_XP_002155049.1 tr5_comp4681_c0_seq1_m_7022
DENV_198433 tr5_comp637_c0_seq1_m_5451
XP_002153848.3_DENV_045673 tr5_comp24446_c0_seq1_m_17218
XP_002159974.1_DENV_049843 tr5_comp6898_c0_seq1_m_10014
XP_002163296.3_DENV_106178 tr5_comp11071_c0_seq1_m_10577
XP_002163691.2_DENV_170250 tr5_comp36486_c0_seq1_m_19493
XP_002164046.1 tr5_comp1487_c0_seq1_m_9540
XP_002165472.2 tr5_comp4801_c0_seq1_m_3100
XP_002165493.1_DENV_051715 tr5_comp10336_c0_seq1_m_13562
XP_002166171.3_DENV_197934 tr5_comp11025_c0_seq1_m_15317
XP_002168125.2_DENV_176500 tr5_comp5964_c0_seq1_m_8909
XP_004208612.2_DENV_171524 tr5_comp9177_c0_seq1_m_10244
XP_012554604.1_DENV_101910 tr5_comp32538_c0_seq1_m_19111
XP_012555618.1_DENV_004709 tr5_comp14941_c0_seq1_m_14112
XP_012556297.1_DENV_058222 tr5_comp3789_c0_seq1_m_2565
XP_012556498.1_DENV_200779 tr5_comp4929_c0_seq1_m_8401
XP_012558698.1_DENV_051053 tr5_comp347_c0_seq1_m_457
XP_012559634.1_DENV_168885 tr5_comp3801_c0_seq1_m_2460
XP_012561026.1_DENV_084438 tr5_comp1908_c0_seq1_m_9940
XP_012561260.1 tr5_comp2726_c0_seq1_m_2598
XP_012561295.1_DENV_008382 tr5_comp9858_c0_seq1_m_11192
XP_012562708.1_DENV_062145 tr5_comp9081_c0_seq1_m_2737
XP_012566382.1_DENV_178167 tr5_comp14597_c0_seq1_m_11204

4) Protein IDs of genes upregulated upon knockdown of β-catenin with their orthologs (based on RBH-reciprocal BLAST hits) in Xenopus

DENV_002883_XP_002166177.1 Xelaev18033356m
DENV_008342_NP_001267778.1 Xelaev18011464m
DENV_008531 Xelaev18026968m
DENV_009915_XP_012556947.1 Xelaev18033977m
DENV_010691_XP_012557009.1 Xelaev18041017m
DENV_017300_XP_002166749.1 Xelaev18000174m
DENV_022783_XP_012557245.1 Xelaev18036422m
DENV_023080_XP_002164291.1 Xelaev18025040m
DENV_029253_NP_001274286.1 Xelaev18027357m
DENV_031402_XP_002165888.1 Xelaev18046281m
DENV_031968_XP_012554474.1 Xelaev18018810m
DENV_055772_XP_002157026.1 Xelaev18039153m
DENV_057854 Xelaev18002488m
DENV_066788_XP_002163988.1 Xelaev18030893m
DENV_067234_XP_012566767.1 Xelaev18011720m
DENV_070660_XP_002157611.3 Xelaev18022709m
DENV_097762_XP_012565729.1 Xelaev18017244m
DENV_097978_XP_012563379.1 Xelaev18037770m

DENV_098962_XP_004208490.1 Xelaev18043355m
DENV_127367_XP_002163146.3 Xelaev18011004m
DENV_146956 Xelaev18028129m
DENV_148889_XP_002164528.2 Xelaev18026705m
DENV_157487 Xelaev18038520m
DENV_163354_XP_012555860.1 Xelaev18024895m
DENV_188436_XP_002154928.1 Xelaev18035982m
XP_002154008.1 Xelaev18037744m
XP_002163087.2_DENV_169466 Xelaev18026971m
XP_002166590.1_DENV_075186 Xelaev18033038m
XP_002168605.3_DENV_067560 Xelaev18013914m
XP_004208605.2_DENV_021903 Xelaev18026576m
XP_004211659.2_DENV_037292 Xelaev18018977m
XP_012553451.1 Xelaev18029006m
XP_012553532.1_DENV_100559 Xelaev18044849m
XP_012554388.1_DENV_061537 Xelaev18006883m
XP_012555020.1_DENV_049455 Xelaev18021210m
XP_012555758.1_DENV_013271 Xelaev18021235m
XP_012556963.1_DENV_068869 Xelaev18026122m
XP_012557068.1 Xelaev18003904m
XP_012557269.1 Xelaev18003719m
XP_012558523.1_DENV_090796 Xelaev18009322m
XP_012559357.1_DENV_023644 Xelaev18023669m
XP_012560419.1_DENV_152770 Xelaev18014156m
XP_012561605.1_DENV_008711 Xelaev18045680m
XP_012563378.1_DENV_206654 Xelaev18003717m
XP_012564439.1_DENV_006543 Xelaev18037126m
XP_012564494.1_DENV_176993 Xelaev18015103m
XP_012566923.1_DENV_170123 Xelaev18012891m

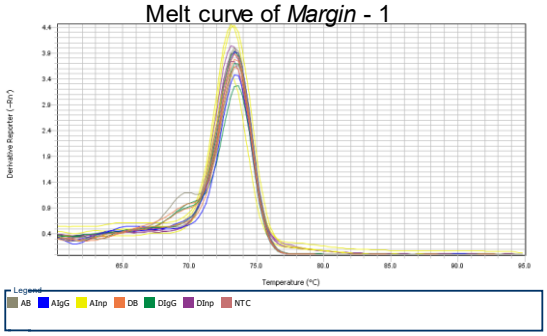
5) Protein IDs of genes downregulated upon knockdown of β-catenin with their orthologs (based on RBH-reciprocal BLAST hits) in Xenopus

DENV_008833_XP_012554670.1 Xelaev18026018m
DENV_012348_XP_012553992.1 Xelaev18020051m
DENV_022146_XP_002165751.1 Xelaev18034616m
DENV_027001_XP_002158990.1 Xelaev18018204m
DENV_046948 Xelaev18033688m
DENV_048507_XP_012562827.1 Xelaev18012794m
DENV_056127_XP_004207355.2 Xelaev18020972m
DENV_056573_XP_012566931.1 Xelaev18013013m
DENV_060128_XP_012553600.1 Xelaev18022960m
DENV_069649 Xelaev18012288m
DENV_076720_XP_002158844.1 Xelaev18020482m
DENV_078983_XP_004206814.1 Xelaev18005453m
DENV_085512_XP_002159764.2 Xelaev18034293m
DENV_089748_NP_001267780.1 Xelaev18031149m
DENV_093426_XP_004210083.1 Xelaev18043203m
DENV_098364_XP_012555674.1 Xelaev18039397m
DENV_101803_XP_012565167.1 Xelaev18045463m
DENV_102767_XP_012555741.1 Xelaev18040863m
DENV_109387_XP_012562538.1 Xelaev18011397m
DENV_113734_XP_012556903.1 Xelaev18006583m
DENV_118389_NP_001274292.1 Xelaev18043371m
DENV_128081_XP_012559396.1 Xelaev18005839m
DENV_129246_XP_012560559.1 Xelaev18011685m
DENV_135075_XP_002164912.3 Xelaev18029079m
DENV_139448_XP_002160774.1 Xelaev18002478m
DENV_141689_XP_002158070.2 Xelaev18030633m
DENV_164073_XP_002162811.3 Xelaev18028982m

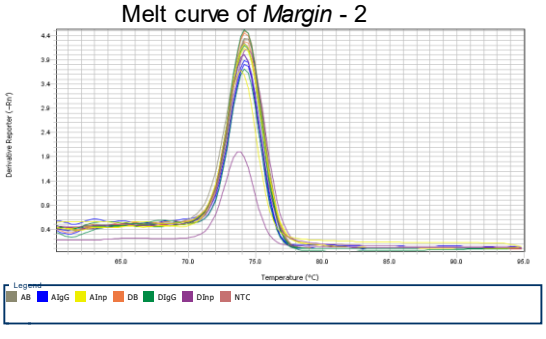
DENV_173944_XP_002155943.3 Xelaev18045581m
DENV_179050_XP_012555495.1 Xelaev18022865m
DENV_183673_XP_002154419.2 Xelaev18005899m
DENV_184349_XP_002157228.2 Xelaev18009169m
DENV_191448_XP_002164795.3 Xelaev18011935m
DENV_192159_XP_012558303.1 Xelaev18040932m
DENV_198081_XP_002155049.1 Xelaev18029658m
DENV_198433 Xelaev18026677m
DENV_203339_XP_012561918.1 Xelaev18041536m
XP_002153848.3_DENV_045673 Xelaev18032986m
XP_002156487.1_DENV_099471 Xelaev18032949m
XP_002159974.1_DENV_049843 Xelaev18019997m
XP_002162975.1_DENV_038595 Xelaev18015825m
XP_002165472.2 Xelaev18029500m
XP_002165493.1_DENV_051715 Xelaev18016633m
XP_002166171.3_DENV_197934 Xelaev18031534m
XP_002167951.2_DENV_065194 Xelaev18025043m
XP_002168125.2_DENV_176500 Xelaev18017736m
XP_004208612.2_DENV_171524 Xelaev18025326m
XP_004211105.1_DENV_034494 Xelaev18017003m
XP_012553964.1 Xelaev18025341m
XP_012554405.1_DENV_105277 Xelaev18021564m
XP_012554629.1_DENV_039347 Xelaev18021990m
XP_012554760.1 Xelaev18013127m
XP_012556498.1_DENV_200779 Xelaev18025960m
XP_012557119.1_DENV_141373 Xelaev18038940m
XP_012558698.1_DENV_051053 Xelaev18032838m
XP_012559409.1_DENV_179707 Xelaev18041001m
XP_012559634.1_DENV_168885 Xelaev18027250m
XP_012560374.1_DENV_179864 Xelaev18005545m
XP_012561260.1 Xelaev18031383m
XP_012561295.1_DENV_008382 Xelaev18031124m
XP_012562678.1_DENV_130362 Xelaev18013693m
XP_012562708.1_DENV_062145 Xelaev18022141m
XP_012564205.1_DENV_168699 Xelaev18034771m
XP_012566382.1_DENV_178167 Xelaev18038200ma

Melt curve plots for ChIP qRT-PCR

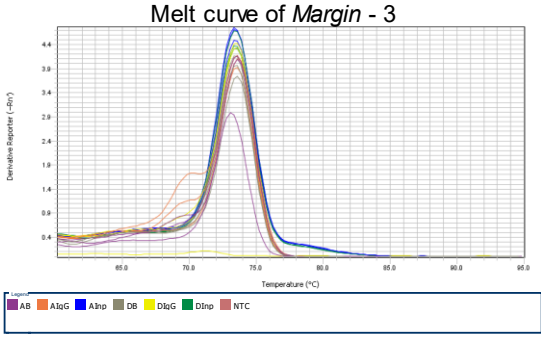
Melt curve of *Margin* - 1



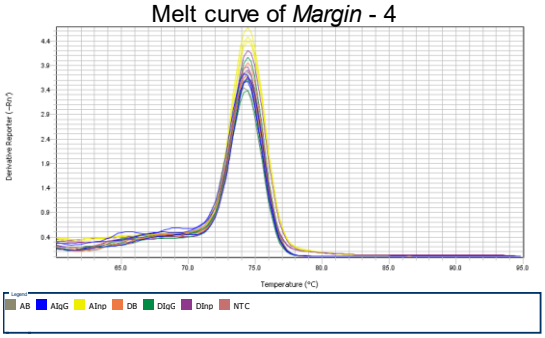
Melt curve of *Margin* - 2



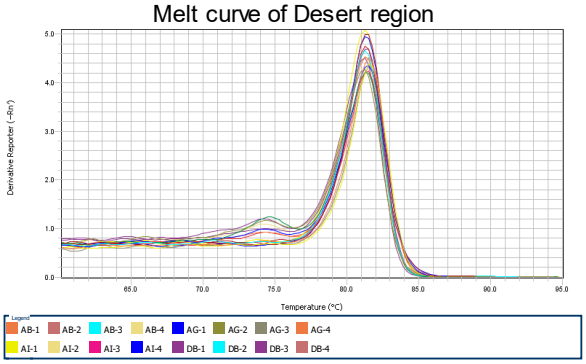
Melt curve of *Margin* - 3



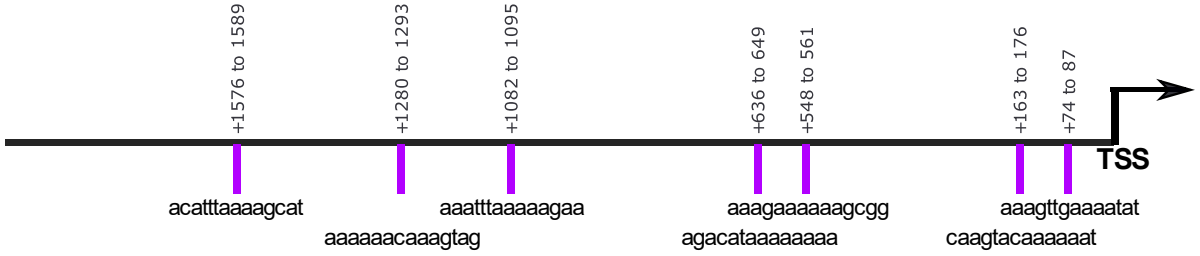
Melt curve of *Margin* - 4



Melt curve of Desert region

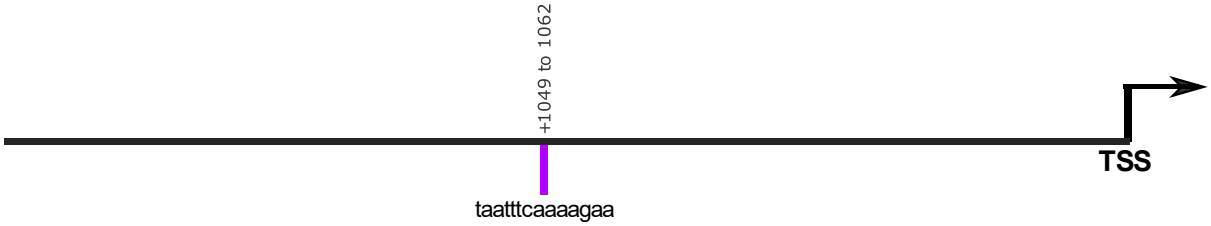


TCF7L2 binding motifs on promoter regions of *HvOtp1*, *HvOtx1* and *Gorget*



HvOtp1 promoter - NW_004167801.1: 137146-139146

HvOtx1 promoter - NW_004169543.1: 87178-89178



Gorget promoter - NW 004172274.1: 24425-26425

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