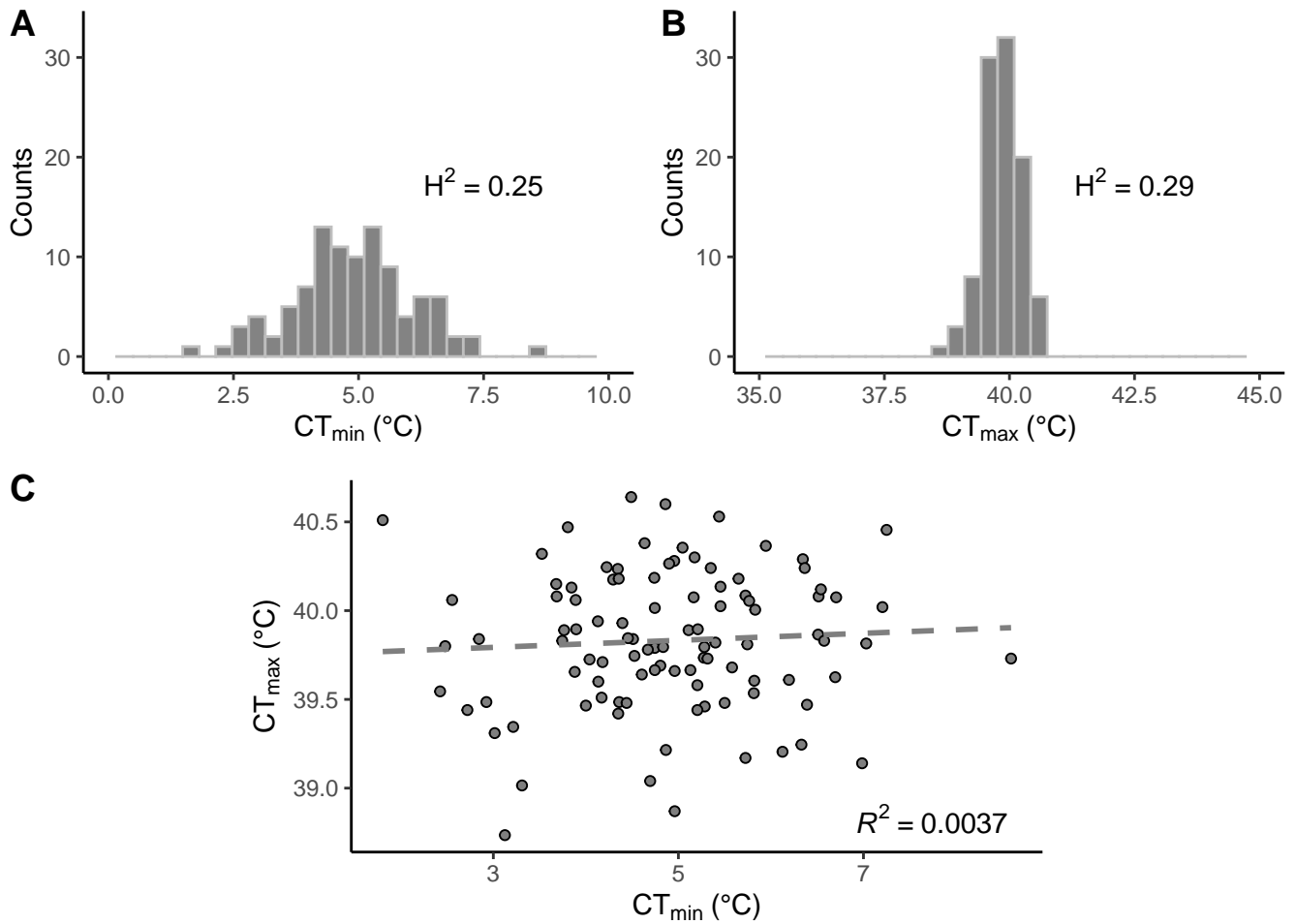


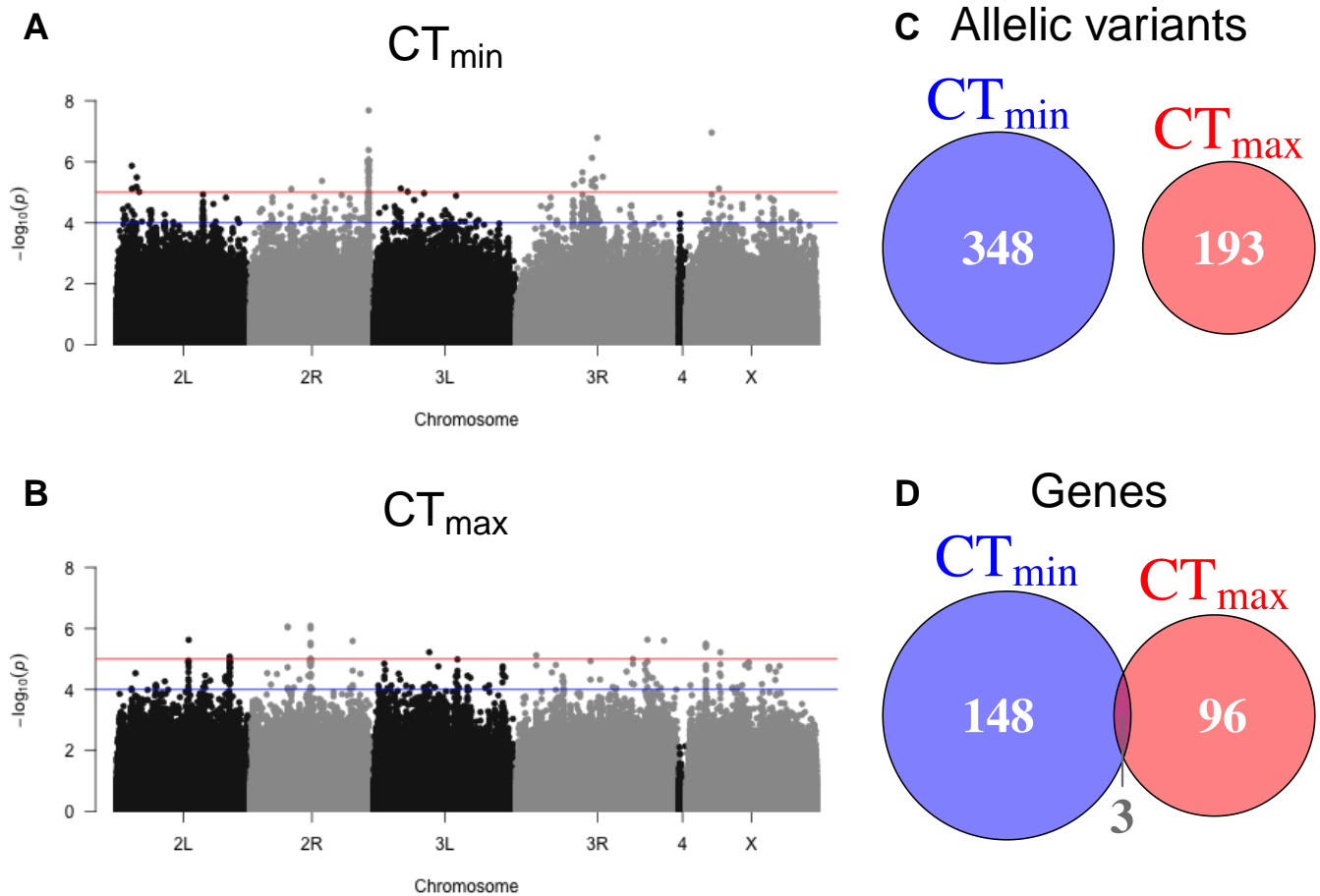
## Sketch of the final figures

**Figure 1: Phenotypes**



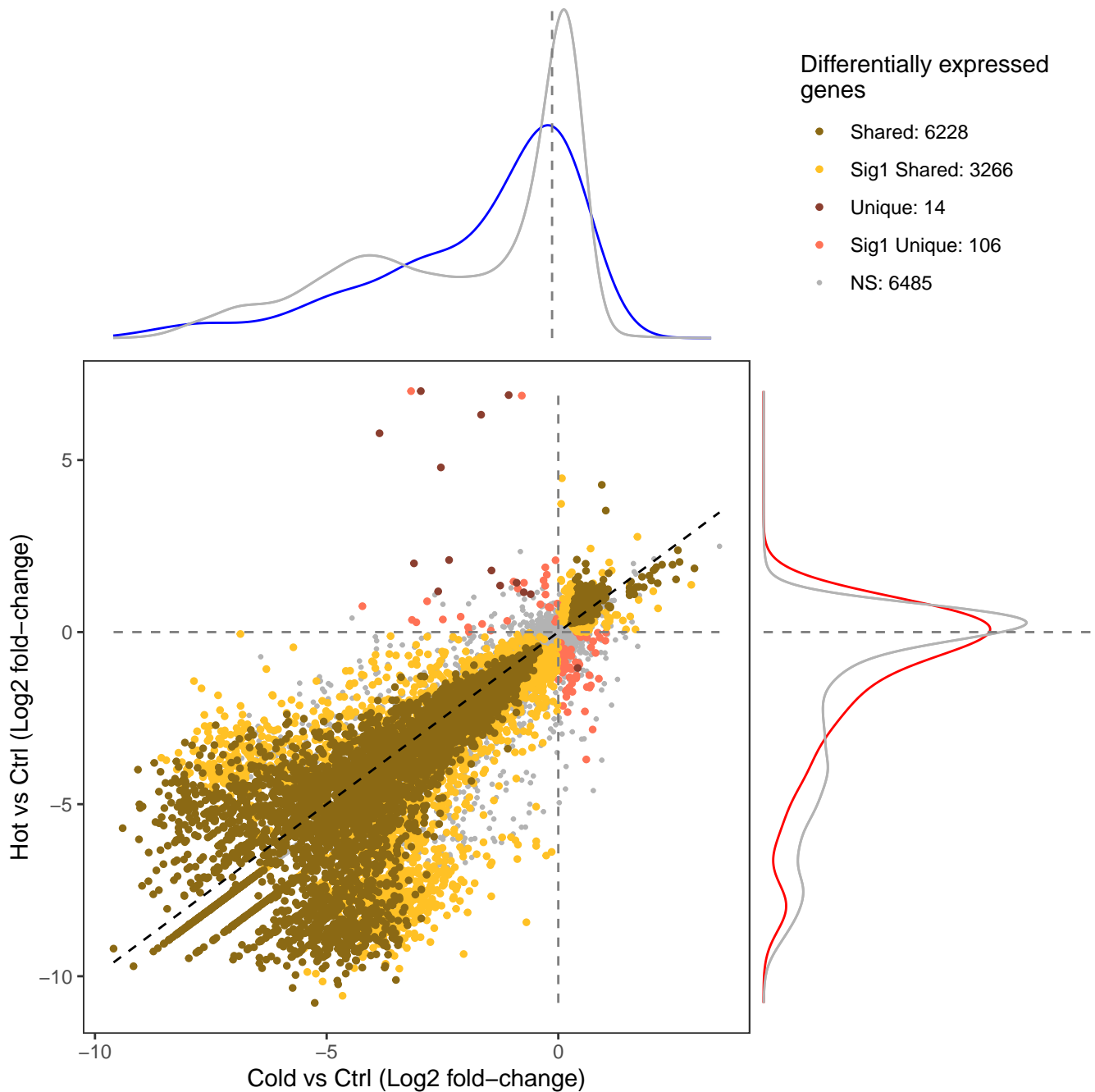
**Figure 1: Distribution of thermal limits in the DGRP.** Histograms of mean phenotypes across 100 DGRP lines for (A)  $CT_{min}$  and (B)  $CT_{max}$ . (C) Correlation between line-means of  $CT_{min}$  and  $CT_{max}$ .

**Figure 2: Manhattan plots**



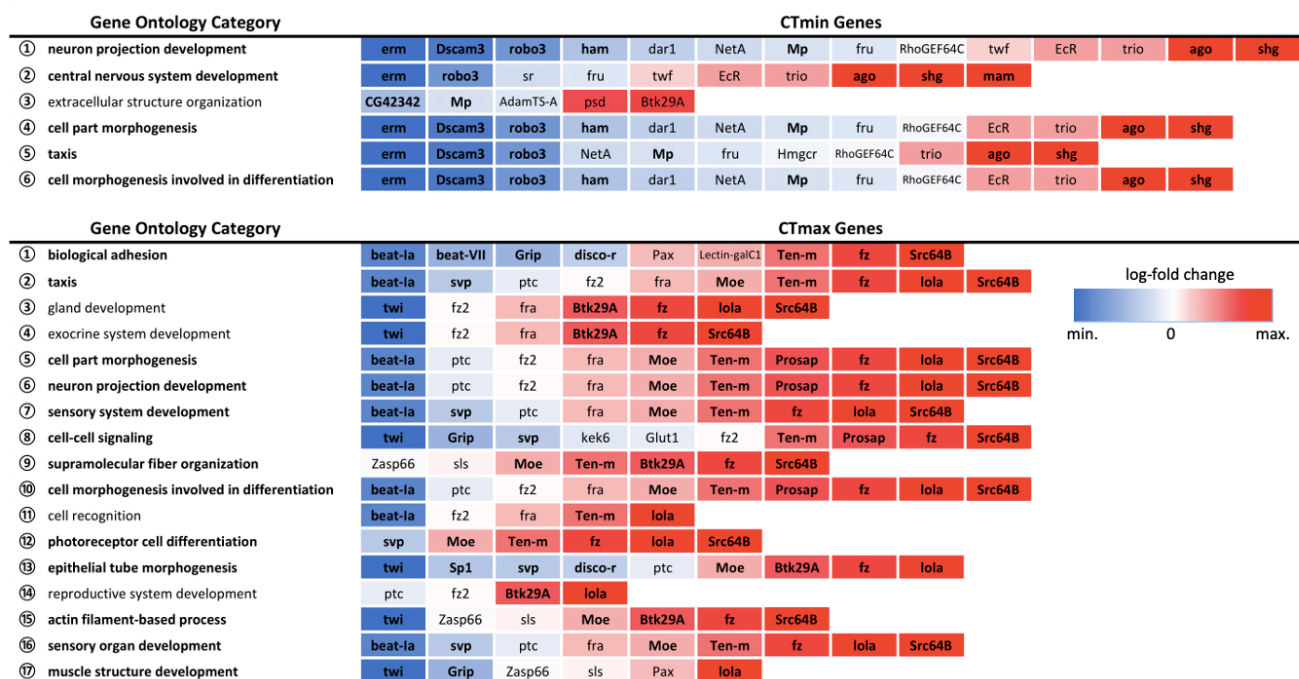
**Figure 2: Results of GWAS to identify polymorphisms associated with thermal tolerance.** Manhattan plots of the results from the GWAS for **(A)**  $CT_{min}$  and **(B)**  $CT_{max}$ . The blue line corresponds to  $p < 1E-4$  and the red line corresponds to  $p < 1E-5$ . The overlap of **(C)** allelic variants and **(D)** unique genes between  $CT_{min}$  and  $CT_{max}$  is also shown.

**Figure 3: DEG & GWAS integration**



**Figure 3: Scatter plot of the gene expression log-fold change of heat-shock (37°C) and cold-shock (4°C) relative to the 25°C control.** The density plots indicate the density of the genes that are associated with  $CT_m^{in}$  (blue) or  $CT_m^{ax}$  (red) relative to the log fold change expression of all other genes (gray).

**Figure 4: ORA for GWAS**



**Figure 4: Expression patterns of GWAS genes within significantly over-represented GO biological process categories.** All expression patterns are expressed relative to the 25°C control. Bolded categories indicate those also over-represented in the full DEG dataset for the corresponding temperature extreme. Bolded genes indicate significant differential gene expression at an  $\text{fdr} < 0.01$ .

**Table 1: Gene counts**

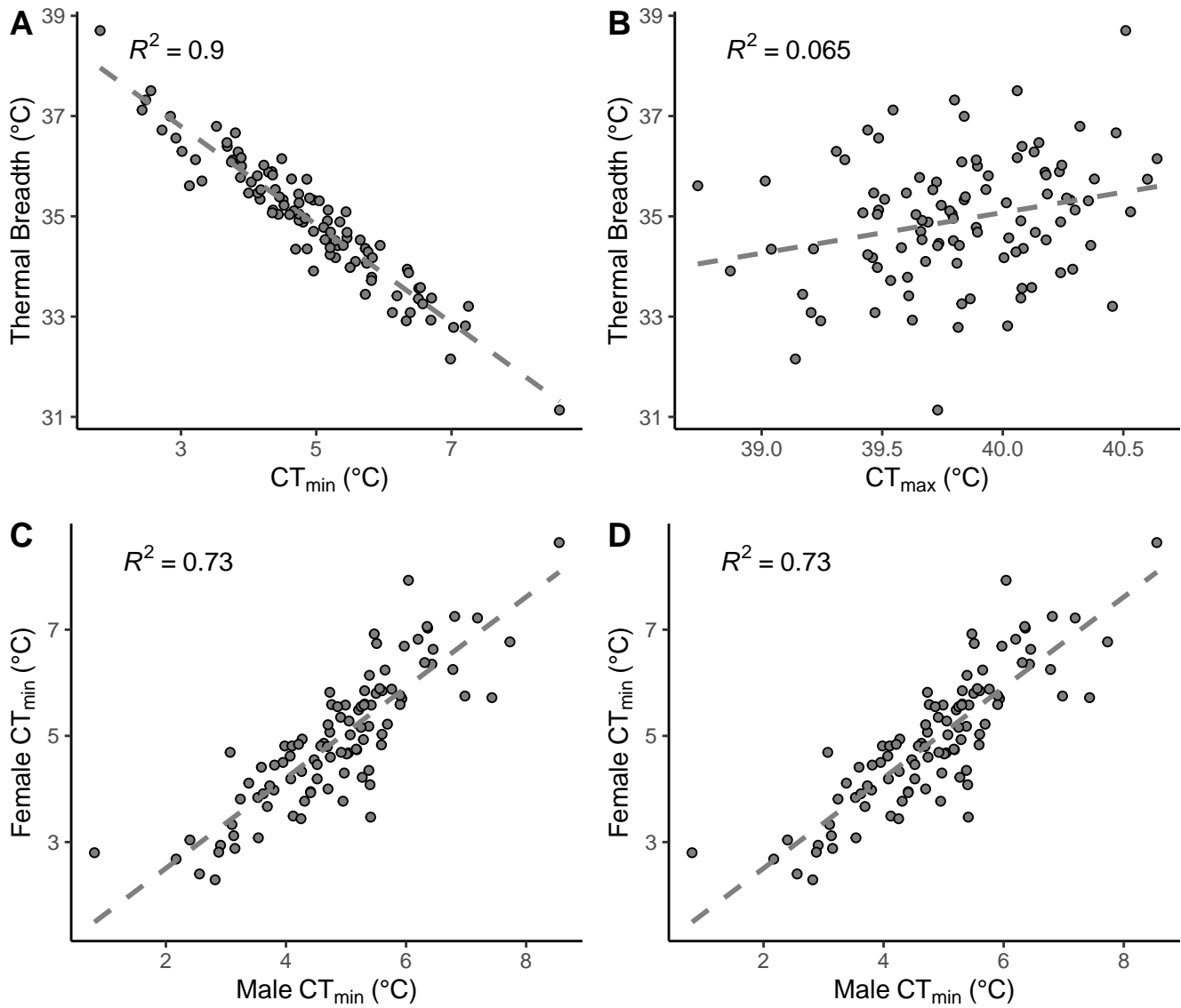
<b>GWAS</b>	<b>Relaxed SNPs</b>	<b>Strict SNPs</b>	<b>Unique genes</b>	<b>DEGs</b>	<b>TFs</b>	<b>DE TFs</b>
CTmax	193	21	99	59	9	9
CTmin	348	53	151	72	12	3

**Table 2: Feature counts**

Feature	CTmin	CTmax
Intron	171	152
Downstream	40	22
Upstream	35	16
Synonymous coding	31	13
3' UTR	11	3
Non-synonymous coding	10	7
5' UTR	8	2
Exon	4	0
Start gained	1	0

Supplementary Information

Thermal Breadth Correlations



## CTmin ORA results

GO category	FDR	Genes
neuron projection development	0.0297	robo3, ham, erm, shg, EcR, dar1, ago, RhoGEF64C, Mp, trio, Dscam3, fru, twf, NetA
central nervous system development	0.0297	robo3, erm, shg, EcR, mam, ago, trio, sr, fru, twf
cell part morphogenesis	0.0392	robo3, ham, erm, shg, EcR, dar1, ago, RhoGEF64C, Mp, trio, Dscam3, fru, NetA
taxis	0.0392	robo3, erm, shg, ago, RhoGEF64C, Mp, trio, Dscam3, fru, Hmgcr, NetA
extracellular structure organization	0.0392	psd, Btk29A, Mp, AdamTS-A, CG42342
cell morphogenesis involved in differentiation	0.0392	robo3, ham, erm, shg, EcR, dar1, ago, RhoGEF64C, Mp, trio, Dscam3, fru, NetA

## CTmax ORA results

GO category	FDR	Genes
biological adhesion	0.00134	beat-la, Pax, Lectin-galC1, fz, Ten-m, Src64B, beat-VII, disco-r, Grip
taxis	0.00789	beat-la, ptc, lola, fra, fz, fz2, Ten-m, Src64B, svp, Moe
exocrine system development	0.01030	Btk29A, twi, fra, fz, fz2, Src64B
gland development	0.01030	Btk29A, twi, lola, fra, fz, fz2, Src64B
cell part morphogenesis	0.01840	beat-la, ptc, lola, fra, Prosap, fz, fz2, Ten-m, Src64B, Moe
neuron projection development	0.01840	beat-la, ptc, lola, fra, Prosap, fz, fz2, Ten-m, Src64B, Moe
sensory system development	0.01840	beat-la, ptc, lola, fra, fz, Ten-m, Src64B, svp, Moe
supramolecular fiber organization	0.01840	Btk29A, fz, sls, Ten-m, Src64B, Zasp66, Moe
cell-cell signaling	0.01840	twi, Prosap, fz, fz2, Ten-m, Src64B, Glut1, kek6, svp, Grip
cell morphogenesis involved in differentiation	0.01940	beat-la, ptc, lola, fra, Prosap, fz, fz2, Ten-m, Src64B, Moe
cell recognition	0.02080	beat-la, lola, fra, fz2, Ten-m
photoreceptor cell differentiation	0.03670	lola, fz, Ten-m, Src64B, svp, Moe
epithelial tube morphogenesis	0.03670	Btk29A, twi, ptc, lola, fz, svp, disco-r, Moe, Sp1
reproductive system development	0.03670	Btk29A, ptc, lola, fz2
actin filament-based process	0.04230	Btk29A, twi, fz, sls, Src64B, Zasp66, Moe
sensory organ development	0.04260	beat-la, ptc, lola, fra, fz, Ten-m, Src64B, svp, Moe
muscle structure development	0.04910	Pax, twi, lola, sls, Zasp66, Grip



Cold DEG ORA results

Hot DEG ORA results