

MASTER DEGREE IN BIOINFOMATICS AND SYSTEMS BIOLOGY

# SUPPLEMENTARY INFORMATION

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**Understanding the biology of Morpholinos in zebrafish  
through integrated gene expression analysis**

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# ***1) INTRODUCTION***

The current study analyses a pool of microarray datasets which were collected from GEO public repository through supervised machine learning. The supplementary information is divided in three chapters. The first chapter gives a deeper insight into the criteria used for the microarray selection and annotation. The second part describes the normalization strategies for the integration of the two microarray platforms; the final part provides further results which give more detailed information about the main results shown in the paper.

## **2) MATERIAL AND METHODS**

## 2.1 GEO repository details and experiment information from literature

**Table S.2.1** – Microarray data curation: the attributes below are assigned to each microarray dataset. GPL, GSE, GSM, Title and Pubmed attributes are used as identification for retrieving the microarray in the GEO repository. MO target, MOseq, COseq, MOtarget, source, Hpf, Treatment, MO type, MO dose and phenotype are directly extracted from the annexed scientific article. Dev. Stage and MO dose are created additionally from the provided information.

| Attribute  | Type        | Levels   | Description   |
|------------|-------------|--|---|
| GPL        | Descriptive | -  | Type of technology                                    |
| GSE        | Descriptive | -  | Study ID (coupled to unique publication)              |
| GSM        | Descriptive | -  | Data set ID   |
| Title      | Descriptive | -  | Data set description                                  |
| Pubmed     | Descriptive | -  | Associated publication ID                             |
| MO target  | Descriptive | -  | Gene targeted by morpholino                           |
| MOseq      | Descriptive | -  | Morpholino sequence                                   |
| COseq      | Descriptive | -  | Sequence of the control morpholino (if available)     |
| MO target  | Descriptive | -  | Gene targeted by morpholino                           |
| Source     | Categorical | kidney, endothelial cells, hearts,<br>whole embryo, embryo trunk | Tissue type   |
| Hpf        | Continuous  | -  | Age (hours post fertilization) of the sampled embryos |
| Treatment  | Categorical | Morpholino, Control  | Type of treatment                                     |
| MO type    | Categorical | Translation, splicing  | Mechanism of gene product inhibition                  |
| MO dose    | Continuous  | -  | Nanograms of morpholino injected                      |
| Phenotype  | Categorical | Normal, various  | Phenotype induced by the injection                    |
| Dev. Stage | Categorical | Blastula, Gastrula, Segmentation,<br>Pharyngula, Hatching        | Developmental stage of the sampled embryos*           |
| MO dose PM | Continuous  | -  | PicoMolar dose of the injected morpholino**           |

\* From: Zebrafish developmental staging series: [http://zfin.org/zf\\_info/zfbook/stages/](http://zfin.org/zf_info/zfbook/stages/) - ZFin data base  
Detailed description of the zebrafish developmental stages on page in section 2.2

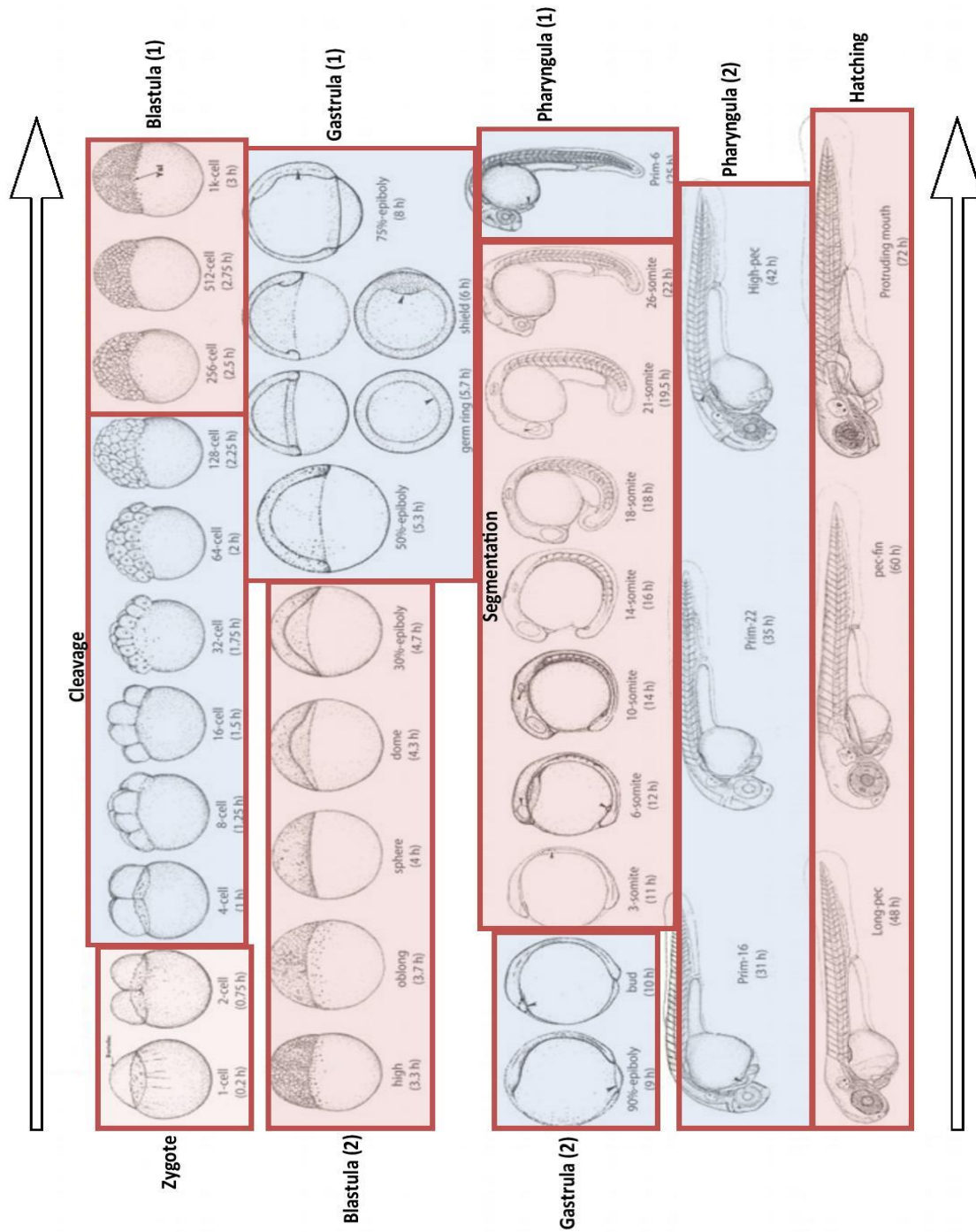
\*\* Calculated from the morpholino sequence molecular weight and the injected quantity (MO dose).

Molecular weights:

Morpholino ring: 87.12 g/mol, A subunit residue: 339.3 g/mole, C subunit residue: 315.3 g/mole, G subunit residue: 355.3 g/mole, T subunit residue: 330.3 g/mole, Additional weight of the ends of the chain: 101 g/mole

## 2.2 Zebrafish developmental stages

**Figure S.2.2** – The “Hours post fertilization” attributes extracted from the literature information is assigned to one of the developmental stages below. The stage in this case is used as a categorical variable



## 2.3 Affymetrix microarray profiles used for integrated data analysis

**Table S.2.3** – Relevant information used for the integrated microarray study- Affymetrix

| GSE      | GSM       | Description   | Treatment * | Replicates | Source      | MO dose pM | Dev stage  |
|----------|-----------|---|-------------|------------|-------------|------------|------------|
| GSE12012 | GSM303576 | zebrafish_48hpf_control_replicate1                                | WT          | 1          | endothelial | 0          | Pharyngula |
| GSE12012 | GSM303577 | zebrafish_48hpf_miR-126 MO-1_replicate1                           | miR-126     | 1          | endothelial | 0.455      | Pharyngula |
| GSE12012 | GSM303578 | zebrafish_48hpf_miR-126 MO-2_replicate1                           | miR-126     | 1          | endothelial | 0.563      | Pharyngula |
| GSE12012 | GSM303579 | zebrafish_48hpf_control_replicate2                                | WT          | 2          | endothelial | 0          | Pharyngula |
| GSE12012 | GSM303580 | zebrafish_48hpf_miR-126 MO-1_replicate2                           | miR-126     | 2          | endothelial | 0.455      | Pharyngula |
| GSE12012 | GSM303581 | zebrafish_48hpf_miR-126 MO-2_replicate2                           | miR-126     | 2          | endothelial | 0.563      | Pharyngula |
| GSE12012 | GSM303582 | zebrafish_48hpf_control_replicate3                                | WT          | 3          | endothelial | 0          | Pharyngula |
| GSE12012 | GSM303583 | zebrafish_48hpf_miR-126 MO-1_replicate3                           | miR-126     | 3          | endothelial | 0.455      | Pharyngula |
| GSE12012 | GSM303584 | zebrafish_48hpf_miR-126 MO-2_replicate3                           | miR-126     | 3          | endothelial | 0.563      | Pharyngula |
| GSE12012 | GSM303585 | zebrafish_48hpf_control_replicate4                                | WT          | 4          | endothelial | 0          | Pharyngula |
| GSE12012 | GSM303586 | zebrafish_48hpf_miR-126 MO-1_replicate4                           | miR-126     | 4          | endothelial | 0.455      | Pharyngula |
| GSE12012 | GSM303587 | zebrafish_48hpf_miR-126 MO-2_replicate4                           | miR-126     | 4          | endothelial | 0.563      | Pharyngula |
| GSE13157 | GSM329381 | Embryos injected with 15uM ERbeta2 MO biological repl. 1          | esr2a       | 1          | embryos     | 0.015      | Hatching   |
| GSE13157 | GSM329382 | Embryos injected with 15uM ERbeta2 MO biological repl. 2          | esr2a       | 2          | embryos     | 0.015      | Hatching   |
| GSE13157 | GSM329383 | Embryos injected with 15uM coMO biological repl. 1                | CO          | 1          | embryos     | 0.015      | Hatching   |
| GSE13157 | GSM329384 | Embryos injected with 15uM coMO biological repl. 2                | CO          | 2          | embryos     | 0.015      | Hatching   |
| GSE13157 | GSM329385 | Embryos uninjected biological repl. 1 (15uM)                      | WT          | 1          | embryos     | 0          | Hatching   |
| GSE13157 | GSM329386 | Embryos uninjected biological repl. 2 (15uM)                      | WT          | 2          | embryos     | 0          | Hatching   |
| GSE13158 | GSM329399 | Embryos injected with 50uM ERbeta2 MO biological repl. 1          | esr2a       | 1          | embryos     | 0.05       | Hatching   |
| GSE13158 | GSM329400 | Embryos injected with 50uM ERbeta2 MO biological repl. 2          | esr2a       | 2          | embryos     | 0.05       | Hatching   |
| GSE13158 | GSM329401 | Embryos injected with 50uM coMO biological repl. 1                | CO          | 1          | embryos     | 0.05       | Hatching   |
| GSE13158 | GSM329402 | Embryos injected with 50uM coMO biological repl. 2                | CO          | 2          | embryos     | 0.05       | Hatching   |
| GSE13158 | GSM329403 | Embryos uninjected biological repl. 1 (50uM)                      | WT          | 1          | embryos     | 0          | Hatching   |
| GSE13158 | GSM329404 | Embryos uninjected biological repl. 2 (50uM)                      | WT          | 2          | embryos     | 0          | Hatching   |
| GSE16740 | GSM419445 | RNA from embryos of 3 groups of Wildtype AB zebrafish at 36 hpf_1 | CO          | 1          | embryos     | 0.38       | Pharyngula |
| GSE16740 | GSM419446 | RNA from embryos of 3 groups of Wildtype AB zebrafish at 36 hpf_2 | CO          | 2          | embryos     | 0.38       | Pharyngula |
| GSE16740 | GSM419447 | RNA from embryos of 3 groups of Wildtype AB zebrafish at 36 hpf_3 | CO          | 3          | embryos     | 0.38       | Pharyngula |
| GSE16740 | GSM419448 | RNA from embryos of 3 groups of tnnt2 morpholino fish at 36 hpf_1 | tnnt2       | 1          | embryos     | 0.377      | Pharyngula |
| GSE16740 | GSM419449 | RNA from embryos of 3 groups of tnnt2 morpholino fish at 36 hpf_2 | tnnt2       | 2          | embryos     | 0.377      | Pharyngula |
| GSE16740 | GSM419450 | RNA from embryos of 3 groups of tnnt2 morpholino fish at 36 hpf_3 | tnnt2       | 3          | embryos     | 0.377      | Pharyngula |
| GSE16740 | GSM419451 | RNA from embryos of 3 groups of Wildtype AB zebrafish at 48 hpf_1 | CO          | 1          | embryos     | 0.38       | Pharyngula |
| GSE16740 | GSM419452 | RNA from embryos of 3 groups of Wildtype AB zebrafish at 48 hpf_2 | CO          | 2          | embryos     | 0.38       | Pharyngula |
| GSE16740 | GSM419453 | RNA from embryos of 3 groups of Wildtype AB zebrafish at 48 hpf_3 | CO          | 3          | embryos     | 0.38       | Pharyngula |
| GSE16740 | GSM419454 | RNA from embryos of 3 groups of tnnt2 morpholino fish at 48 hpf_1 | tnnt2       | 1          | embryos     | 0.377      | Pharyngula |
| GSE16740 | GSM419455 | RNA from embryos of 3 groups of tnnt2 morpholino fish at 48 hpf_2 | tnnt2       | 2          | embryos     | 0.377      | Pharyngula |
| GSE16740 | GSM419456 | RNA from embryos of 3 groups of tnnt2 morpholino fish at 48 hpf_3 | tnnt2       | 3          | embryos     | 0.377      | Pharyngula |
| GSE16740 | GSM419457 | RNA from embryos of 3 groups of Wildtype AB zebrafish at 60 hpf_1 | CO          | 1          | embryos     | 0.38       | Hatching   |

|          |            |   |                |   |         |       |              |
|----------|------------|---|----------------|---|---------|-------|--------------|
| GSE16740 | GSM419458  | RNA from embryos of 3 groups of Wildtype AB zebrafish at 60 hpf_2 | CO             | 2 | embryos | 0.38  | Hatching     |
| GSE16740 | GSM419459  | RNA from embryos of 3 groups of Wildtype AB zebrafish at 60 hpf_3 | CO             | 3 | embryos | 0.38  | Hatching     |
| GSE16740 | GSM419460  | RNA from embryos of 3 groups of tnnt2 morpholino fish at 60 hpf_1 | tnnt2          | 1 | embryos | 0.377 | Hatching     |
| GSE16740 | GSM419461  | RNA from embryos of 3 groups of tnnt2 morpholino fish at 60 hpf_2 | tnnt2          | 2 | embryos | 0.377 | Hatching     |
| GSE16740 | GSM419462  | RNA from embryos of 3 groups of tnnt2 morpholino fish at 60 hpf_3 | tnnt2          | 3 | embryos | 0.377 | Hatching     |
| GSE18830 | GSM466790  | Zebrafish wt embryo_ 30%E Biological rep1                         | WT             | 1 | embryos | 0     | Blastula     |
| GSE18830 | GSM466791  | Zebrafish wt embryo_ 30%E Biological rep2                         | WT             | 2 | embryos | 0     | Blastula     |
| GSE18830 | GSM466792  | Zebrafish wt embryo_ 75%E Biological rep1                         | WT             | 1 | embryos | 0     | Gastrula     |
| GSE18830 | GSM466793  | Zebrafish wt embryo_ 75%E Biological rep2                         | WT             | 2 | embryos | 0     | Gastrula     |
| GSE18830 | GSM466794  | Zebrafish wt embryo_ TB Biological rep1                           | WT             | 1 | embryos | 0     | Gastrula     |
| GSE18830 | GSM466795  | Zebrafish wt embryo_ TB Biological rep2                           | WT             | 2 | embryos | 0     | Gastrula     |
| GSE18830 | GSM466796  | Zebrafish QKD embryo_ 30%E Biological rep1                        | sox2/3/19a/19b | 1 | embryos | 0.068 | Blastula     |
| GSE18830 | GSM466797  | Zebrafish QKD embryo_ 30%E Biological rep2                        | sox2/3/19a/19b | 2 | embryos | 0.068 | Blastula     |
| GSE18830 | GSM466798  | Zebrafish QKD embryo_ 75%E Biological rep1                        | sox2/3/19a/19b | 1 | embryos | 0.068 | Gastrula     |
| GSE18830 | GSM466799  | Zebrafish QKD embryo_ 75%E Biological rep2                        | sox2/3/19a/19b | 2 | embryos | 0.068 | Gastrula     |
| GSE18830 | GSM466800  | Zebrafish QKD embryo_ TB Biological rep1                          | sox2/3/19a/19b | 1 | embryos | 0.068 | Gastrula     |
| GSE18830 | GSM466801  | Zebrafish QKD embryo_ TB Biological rep2                          | sox2/3/19a/19b | 2 | embryos | 0.068 | Gastrula     |
| GSE21539 | GSM537962  | zebrafish 12hpf control-1   | WT             | 1 | embryos | 0     | Segmentation |
| GSE21539 | GSM537963  | zebrafish 12hpf control-2   | WT             | 2 | embryos | 0     | Segmentation |
| GSE21539 | GSM537964  | zebrafish 12hpf control-3   | WT             | 3 | embryos | 0     | Segmentation |
| GSE21539 | GSM537965  | zebrafish 12hpf Ovo1 morphant-1                                   | Ovo1           | 1 | embryos | 0.286 | Segmentation |
| GSE21539 | GSM537966  | zebrafish 12hpf Ovo1 morphant-2                                   | Ovo1           | 2 | embryos | 0.286 | Segmentation |
| GSE21539 | GSM537967  | zebrafish 12hpf Ovo1 morphant-3                                   | Ovo1           | 3 | embryos | 0.286 | Segmentation |
| GSE27569 | GSM683599  | control embryos at 24hpf, biological rep 1                        | WT             | 1 | embryos | 0     | Segmentation |
| GSE27569 | GSM683600  | control embryos at 24hpf, biological rep 2                        | WT             | 2 | embryos | 0     | Segmentation |
| GSE27569 | GSM683601  | control embryos at 24hpf, biological rep 3                        | WT             | 3 | embryos | 0     | Segmentation |
| GSE27569 | GSM683602  | control embryos at 24hpf, biological rep 4                        | WT             | 4 | embryos | 0     | Segmentation |
| GSE27569 | GSM683603  | esco2 MO injected embryos at 24hpf, biological rep 1              | esco2          | 1 | embryos | 0.189 | Segmentation |
| GSE27569 | GSM683604  | esco2 MO injected embryos at 24hpf, biological rep 2              | esco2          | 2 | embryos | 0.189 | Segmentation |
| GSE27569 | GSM683605  | esco2 MO injected embryos at 24hpf, biological rep 3              | esco2          | 3 | embryos | 0.189 | Segmentation |
| GSE27569 | GSM683606  | esco2 MO injected embryos at 24hpf, biological rep 4              | esco2          | 4 | embryos | 0.189 | Segmentation |
| GSE27569 | GSM683607  | control embryos at 48hpf, biological rep 1                        | WT             | 1 | embryos | 0     | Pharyngula   |
| GSE27569 | GSM683608  | control embryos at 48hpf, biological rep 3                        | WT             | 2 | embryos | 0     | Pharyngula   |
| GSE27569 | GSM683609  | control embryos at 48hpf, biological rep 4                        | WT             | 3 | embryos | 0     | Pharyngula   |
| GSE27569 | GSM683610  | esco2 MO injected embryos at 48hpf, biological rep 1              | esco2          | 1 | embryos | 0.189 | Pharyngula   |
| GSE27569 | GSM683611  | esco2 MO injected embryos at 48hpf, biological rep 2              | esco2          | 2 | embryos | 0.189 | Pharyngula   |
| GSE27569 | GSM683612  | esco2 MO injected embryos at 48hpf, biological rep 3              | esco2          | 3 | embryos | 0.189 | Pharyngula   |
| GSE27569 | GSM683613  | esco2 MO injected embryos at 48hpf, biological rep 4              | esco2          | 4 | embryos | 0.189 | Pharyngula   |
| GSE32914 | GSM814795  | zebrafish_SB_4.3h_1   | Sox31          | 1 | embryos | 1.2   | Blastula     |
| GSE32914 | GSM814796  | zebrafish_SB_4.3h_2   | Sox31          | 2 | embryos | 1.2   | Blastula     |
| GSE32914 | GSM814797  | zebrafish_SB_4.3h_3   | Sox31          | 3 | embryos | 1.2   | Blastula     |
| GSE32914 | GSM814798  | zebrafish_WT_4.3h_1   | WT             | 1 | embryos | 0     | Blastula     |
| GSE32914 | GSM814799  | zebrafish_WT_4.3h_2   | WT             | 2 | embryos | 0     | Blastula     |
| GSE32914 | GSM814800  | zebrafish_WT_4.3h_3   | WT             | 3 | embryos | 0     | Blastula     |
| GSE32914 | GSM814801  | zebrafish_WT_2.5h_1   | WT             | 1 | embryos | 0     | Blastula     |
| GSE32914 | GSM814802  | zebrafish_WT_2.5h_2   | WT             | 2 | embryos | 0     | Blastula     |
| GSE32914 | GSM814803  | zebrafish_WT_4h_1   | WT             | 1 | embryos | 0     | Blastula     |
| GSE32914 | GSM814804  | zebrafish_WT_4h_2   | WT             | 2 | embryos | 0     | Blastula     |
| GSE46844 | GSM1139092 | Multiciliated cell_control_rep1                                   | CO             | 1 | kidney  | 1     | Hatching     |



|          |            |   |          |   |         |       |              |
|----------|------------|---|----------|---|---------|-------|--------------|
| GSE46844 | GSM1139093 | Multiciliated cell_control_rep2           | CO       | 2 | kidney  | 1     | Hatching     |
| GSE46844 | GSM1139094 | Multiciliated cell_miR-34B morphants_rep1 | mir-34B  | 1 | kidney  | 1     | Hatching     |
| GSE46844 | GSM1139095 | Multiciliated cell_miR-34B morphants_rep2 | mir-34B  | 2 | kidney  | 1     | Hatching     |
| GSE51541 | GSM1247632 | Uninjected control biological replicate 1 | WT       | 1 | heart   | 0     | Hatching     |
| GSE51541 | GSM1247633 | Uninjected control biological replicate 2 | WT       | 2 | heart   | 0     | Hatching     |
| GSE51541 | GSM1247634 | Uninjected control biological replicate 3 | WT       | 3 | heart   | 0     | Hatching     |
| GSE51541 | GSM1247635 | Control Morpholino biological replicate 2 | CO       | 1 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247636 | Control Morpholino biological replicate 3 | CO       | 2 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247637 | atg5 Morpholino biological replicate 1    | atg5     | 1 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247638 | atg5 Morpholino biological replicate 2    | atg5     | 2 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247639 | atg5 Morpholino biological replicate 3    | atg5     | 3 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247640 | becn1 Morpholino biological replicate 1   | becn1    | 1 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247641 | becn1 Morpholino biological replicate 2   | becn1    | 2 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247642 | becn1 Morpholino biological replicate 3   | becn1    | 3 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247643 | atg7 Morpholino biological replicate 1    | atg7     | 1 | heart   | 0.65  | Hatching     |
| GSE51541 | GSM1247644 | atg7 Morpholino biological replicate 2    | atg7     | 2 | heart   | 0.65  | Hatching     |
| GSE8800  | GSM218665  | MO  | C1q-like | 1 | embryos | 0.473 | Segmentation |
| GSE8800  | GSM218666  | Cont MO                                   | CO       | 2 | embryos | 0.474 | Segmentation |

\* Treatment

Control: **WT** – uninjected, **CO** – injected morpholino sequence

Morpholino treatment: **microRNA-** mir34B, mir126, **genes:** C1q-like, atg7, becn1, atg5, Sox31, esco2, Ovo1, sox2/3/19a/19b, tnnt2, esr2a

**Table S.2.3.1** – Number of microarrays per attribute factor. The table is a summary of Table S.2.3

|                            |   |
|----------------------------|---|
| <b>Developmental stage</b> | Blastula: 14<br>Gastrula: 8<br>Segmentation: 16<br>Pharyngula: 31<br>Hatching: 35 |
| <b>Treatment</b>           | Morpholino: 52<br>Control: 52 (WT:34 -CO:18)                                      |
| <b>Source</b>              | Embryos: 75<br>Kidney: 4<br>Hearts: 13<br>Endothelial cells: 12                   |
| <b>Dosage:</b>             | 0-0.189: 56<br>0.289-0.65: 41<br>1-1.2: 7   |

## 2.4 Agilent microarray profiles used for integrated data analysis

**Table S.2.4** – Relevant information used for the integrated microarray study- Agilent

| gpl      | gse      | gsm        | Description  | Treatment*  | Replicates | Source  | MO.dose pM | Dev.stage    |
|----------|----------|------------|--|-------------|------------|---------|------------|--------------|
| GPL14664 | GSE32594 | GSM807955  | Injection of control MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.         | CO          | 1          | embryos | 0.05       | Hatching     |
| GPL14664 | GSE32594 | GSM807956  | Injection of control MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.         | CO          | 2          | embryos | 0.05       | Hatching     |
| GPL14664 | GSE32594 | GSM807957  | Injection of control MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.         | CO          | 3          | embryos | 0.05       | Hatching     |
| GPL14664 | GSE32594 | GSM807961  | Injection of Nrf2a MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.           | Nrf2a       | 1          | embryos | 0.025      | Hatching     |
| GPL14664 | GSE32594 | GSM807962  | Injection of Nrf2a MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.           | Nrf2a       | 2          | embryos | 0.025      | Hatching     |
| GPL14664 | GSE32594 | GSM807963  | Injection of Nrf2a MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.           | Nrf2a       | 3          | embryos | 0.025      | Hatching     |
| GPL14664 | GSE32594 | GSM807967  | Injection of Nrf2a and Nrf2b MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf. | Nrf2a/Nrf2b | 1          | embryos | 0.05       | Hatching     |
| GPL14664 | GSE32594 | GSM807968  | Injection of Nrf2a and Nrf2b MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf. | Nrf2a/Nrf2b | 2          | embryos | 0.05       | Hatching     |
| GPL14664 | GSE32594 | GSM807969  | Injection of Nrf2a and Nrf2b MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf. | Nrf2a/Nrf2b | 3          | embryos | 0.05       | Hatching     |
| GPL14664 | GSE32594 | GSM807973  | Injection of Nrf2b MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.           | Nrf2b       | 1          | embryos | 0.025      | Hatching     |
| GPL14664 | GSE32594 | GSM807974  | Injection of Nrf2b MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.           | Nrf2b       | 2          | embryos | 0.025      | Hatching     |
| GPL14664 | GSE32594 | GSM807975  | Injection of Nrf2b MO at 1-4 cell stage. DMSO exposure at 48 hpf. RNA sampled at 52 hpf.           | Nrf2b       | 3          | embryos | 0.025      | Hatching     |
| GPL14664 | GSE42070 | GSM1031965 | whole fish, 6h, control, replicate 1   | CO_R        | 1          | embryos | 0.5        | Gastrula     |
| GPL14664 | GSE42070 | GSM1031966 | whole fish, 6h, control, replicate 2   | CO_R        | 2          | embryos | 0.5        | Gastrula     |
| GPL14664 | GSE42070 | GSM1031967 | whole fish, 6h, control, replicate 3   | CO_R        | 3          | embryos | 0.5        | Gastrula     |
| GPL14664 | GSE42070 | GSM1031968 | whole fish, 24h, control, replicate 1  | CO_R        | 1          | embryos | 0.5        | Segmentation |
| GPL14664 | GSE42070 | GSM1031969 | whole fish, 24h, control, replicate 2  | CO_R        | 2          | embryos | 0.5        | Segmentation |
| GPL14664 | GSE42070 | GSM1031970 | whole fish, 24h, control, replicate 3  | CO_R        | 3          | embryos | 0.5        | Segmentation |
| GPL14664 | GSE42070 | GSM1031971 | whole fish, 6h, PCSK7+P53 morpholinos, replicate 1   | PCSK7/P53   | 1          | embryos | 1.25       | Gastrula     |
| GPL14664 | GSE42070 | GSM1031972 | whole fish, 6h, PCSK7+P53 morpholinos, replicate 2   | PCSK7/P53   | 2          | embryos | 1.25       | Gastrula     |
| GPL14664 | GSE42070 | GSM1031973 | whole fish, 6h, PCSK7+P53 morpholinos, replicate 3   | PCSK7/P53   | 3          | embryos | 1.25       | Gastrula     |
| GPL14664 | GSE42070 | GSM1031974 | whole fish, 24h, PCSK7+P53 morpholinos, replicate 1  | PCSK7/P53   | 1          | embryos | 1.25       | Segmentation |
| GPL14664 | GSE42070 | GSM1031975 | whole fish, 24h, PCSK7+P53 morpholinos, replicate 2  | PCSK7/P53   | 2          | embryos | 1.25       | Segmentation |

|          |          |            |  |           |   |         |       |              |
|----------|----------|------------|--|-----------|---|---------|-------|--------------|
| GPL14664 | GSE42070 | GSM1031976 | whole fish, 24h, PCSK7+P53 morpholinos, replicate 3                          | PCSK7/P53 | 3 | embryos | 1.25  | Segmentation |
| GPL14664 | GSE45012 | GSM1095807 | FoxD5 Morpholino injection   | FoxD5     | 1 | embryos | 0.38  | Blastula     |
| GPL14664 | GSE45012 | GSM1095808 | FoxD5 Morpholino injection   | FoxD5     | 2 | embryos | 0.38  | Blastula     |
| GPL14664 | GSE45012 | GSM1095813 | FoxD5 Morpholino injection   | FoxD5     | 3 | embryos | 0.38  | Blastula     |
| GPL6457  | GSE20179 | GSM506241  | Whole embryo TNNT2sp morphants   | tnnt2     | 1 | embryos | 0.471 | Hatching     |
| GPL6457  | GSE20179 | GSM506241  | Whole embryo TNNT2sp morphants   | CO        | 1 | embryos | 0.471 | Hatching     |
| GPL6457  | GSE20179 | GSM506242  | Whole embryo TNNT2sp morphants   | tnnt2     | 2 | embryos | 0.471 | Hatching     |
| GPL6457  | GSE20179 | GSM506242  | Whole embryo TNNT2sp morphants   | CO        | 2 | embryos | 0.471 | Hatching     |
| GPL6457  | GSE20179 | GSM506243  | Whole embryo TNNT2sp morphants   | tnnt2     | 3 | embryos | 0.471 | Hatching     |
| GPL6457  | GSE20179 | GSM506243  | Whole embryo TNNT2sp morphants   | CO        | 3 | embryos | 0.471 | Hatching     |
| GPL6457  | GSE20179 | GSM506244  | Whole embryo TNNT2sp morphants   | tnnt2     | 4 | embryos | 0.471 | Hatching     |
| GPL6457  | GSE20179 | GSM506244  | Whole embryo TNNT2sp morphants   | CO        | 4 | embryos | 0.471 | Hatching     |
| GPL6457  | GSE24934 | GSM612962  | MO2-ers2a embryos at 8 hours post fertilization                              | CO        | 1 | embryos | 0.974 | Gastrula     |
| GPL6457  | GSE24934 | GSM612962  | MO2-ers2a embryos at 8 hours post fertilization                              | Ers2a     | 1 | embryos | 0.974 | Gastrula     |
| GPL6457  | GSE24934 | GSM612963  | MO2-ers2a embryos at 48 hours post fertilization                             | CO        | 2 | embryos | 0.974 | Pharyngula   |
| GPL6457  | GSE24934 | GSM612963  | MO2-ers2a embryos at 48 hours post fertilization                             | Ers2a     | 2 | embryos | 0.974 | Pharyngula   |
| GPL6457  | GSE25517 | GSM627464  | MO2-nr3c1-5m, 5hpf   | CO        | 1 | embryos | 0.783 | Blastula     |
| GPL6457  | GSE25517 | GSM627464  | MO2-nr3c1-5m, 5hpf   | MO        | 1 | embryos | 0.783 | Blastula     |
| GPL6457  | GSE25517 | GSM627465  | WT, 5hpf   | WT        | 1 | embryos | 0.783 | Blastula     |
| GPL6457  | GSE25517 | GSM627465  | WT, 5hpf   | MO        | 1 | embryos | 0.783 | Blastula     |
| GPL6457  | GSE25517 | GSM627466  | MO2-nr3c1-5m, 10hpf  | CO        | 2 | embryos | 0.783 | Gastrula     |
| GPL6457  | GSE25517 | GSM627466  | MO2-nr3c1-5m, 10hpf  | MO        | 2 | embryos | 0.783 | Gastrula     |
| GPL6457  | GSE25517 | GSM627467  | WT, 10hpf  | WT        | 2 | embryos | 0.783 | Gastrula     |
| GPL6457  | GSE25517 | GSM627467  | WT, 10hpf  | MO        | 2 | embryos | 0.783 | Gastrula     |
| GPL6457  | GSE38441 | GSM942059  | 16 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 1 | WT        | 1 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942059  | 16 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 1 | MO        | 1 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942060  | 16 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 2 | WT        | 2 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942060  | 16 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 2 | MO        | 2 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942061  | 16 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 3 | WT        | 3 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942061  | 16 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 3 | MO        | 3 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942062  | 24 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 1 | WT        | 1 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942062  | 24 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 1 | MO        | 1 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942063  | 24 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected Replicate 2 | WT        | 2 | trunk   | 0.023 | Segmentation |

|          |          |            |   |             |   |         |       |              |
|----------|----------|------------|---|-------------|---|---------|-------|--------------|
| GPL6457  | GSE38441 | GSM942063  | 24 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 2 | MO          | 2 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942064  | 24 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 3 | WT          | 3 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942064  | 24 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 3 | MO          | 3 | trunk   | 0.023 | Segmentation |
| GPL6457  | GSE38441 | GSM942065  | 48 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 1 | WT          | 1 | trunk   | 0.023 | Pharyngula   |
| GPL6457  | GSE38441 | GSM942065  | 48 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 1 | MO          | 1 | trunk   | 0.023 | Pharyngula   |
| GPL6457  | GSE38441 | GSM942066  | 48 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 2 | WT          | 2 | trunk   | 0.023 | Pharyngula   |
| GPL6457  | GSE38441 | GSM942066  | 48 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 2 | MO          | 2 | trunk   | 0.023 | Pharyngula   |
| GPL6457  | GSE38441 | GSM942067  | 48 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 3 | WT          | 3 | trunk   | 0.023 | Pharyngula   |
| GPL6457  | GSE38441 | GSM942067  | 48 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 3 | MO          | 3 | trunk   | 0.023 | Pharyngula   |
| GPL6457  | GSE38441 | GSM942068  | 72 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 1 | WT          | 1 | trunk   | 0.023 | Hatching     |
| GPL6457  | GSE38441 | GSM942068  | 72 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 1 | MO          | 1 | trunk   | 0.023 | Hatching     |
| GPL6457  | GSE38441 | GSM942069  | 72 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 2 | WT          | 2 | trunk   | 0.023 | Hatching     |
| GPL6457  | GSE38441 | GSM942069  | 72 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 2 | MO          | 2 | trunk   | 0.023 | Hatching     |
| GPL6457  | GSE38441 | GSM942070  | 72 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 3 | WT          | 3 | trunk   | 0.023 | Hatching     |
| GPL6457  | GSE38441 | GSM942070  | 72 hpf trunk region of zebrafish embryos: WT vs MO-grnA injected<br>Replicate 3 | MO          | 3 | trunk   | 0.023 | Hatching     |
| GPL14664 | GSE45011 | GSM1095795 | Klf2a Morpholino injection  | Klf2a       | 1 | embryos | 0.38  | Blastula     |
| GPL14664 | GSE45011 | GSM1095799 | Klf2a+Klf2b Morpholino injection  | Klf2a/Klf2b | 1 | embryos | 0.19  | Blastula     |
| GPL14664 | GSE45011 | GSM1095800 | Klf2a+Klf2b Morpholino injection  | Klf2a/Klf2b | 2 | embryos | 0.19  | Blastula     |
| GPL14664 | GSE45011 | GSM1095796 | Klf2a Morpholino injection  | Klf2a       | 2 | embryos | 0.38  | Blastula     |
| GPL14664 | GSE45011 | GSM1095797 | Klf2b Morpholino injection  | Klf2b       | 1 | embryos | 0.375 | Blastula     |
| GPL14664 | GSE45011 | GSM1095793 | Control Morpholino injection  | CO          | 1 | embryos | 0.38  | Blastula     |
| GPL14664 | GSE45011 | GSM1095794 | Control Morpholino injection  | CO          | 2 | embryos | 0.38  | Blastula     |
| GPL14664 | GSE45011 | GSM1095798 | Klf2b Morpholino injection  | Klf2b       | 2 | embryos | 0.375 | Blastula     |
| GPL14664 | GSE45012 | GSM1095801 | FoxD3 Morpholino injection  | FoxD3       | 1 | embryos | 0.38  | Blastula     |
| GPL14664 | GSE45012 | GSM1095802 | FoxD3+FoxD5 Morpholino injection  | FoxD3/FoxD5 | 1 | embryos | 0.19  | Blastula     |
| GPL14664 | GSE45012 | GSM1095803 | FoxD3+FoxD5 Morpholino injection  | FoxD3/FoxD5 | 2 | embryos | 0.19  | Blastula     |
| GPL14664 | GSE45012 | GSM1095804 | FoxD3 Morpholino injection  | FoxD3       | 2 | embryos | 0.38  | Blastula     |
| GPL14664 | GSE45012 | GSM1095805 | FoxD3 Morpholino injection  | FoxD3       | 3 | embryos | 0.38  | Blastula     |

|          |          |            |                                  |             |   |         |      |          |
|----------|----------|------------|----------------------------------|-------------|---|---------|------|----------|
| GPL14664 | GSE45012 | GSM1095806 | FoxD3+FoxD5 Morpholino injection | FoxD3/FoxD5 | 3 | embryos | 0.19 | Blastula |
| GPL14664 | GSE45012 | GSM1095809 | Klf4 Morpholino injection        | Klf4        | 1 | embryos | 0.38 | Blastula |
| GPL14664 | GSE45012 | GSM1095810 | Control Morpholino injection     | CO          | 1 | embryos | 0.38 | Blastula |
| GPL14664 | GSE45012 | GSM1095811 | Klf4 Morpholino injection        | Klf4        | 2 | embryos | 0.38 | Blastula |
| GPL14664 | GSE45012 | GSM1095812 | Control Morpholino injection     | CO          | 2 | embryos | 0.38 | Blastula |

\* Treatment

Control: **WT** – uninjected, **CO** – injected morpholino sequence

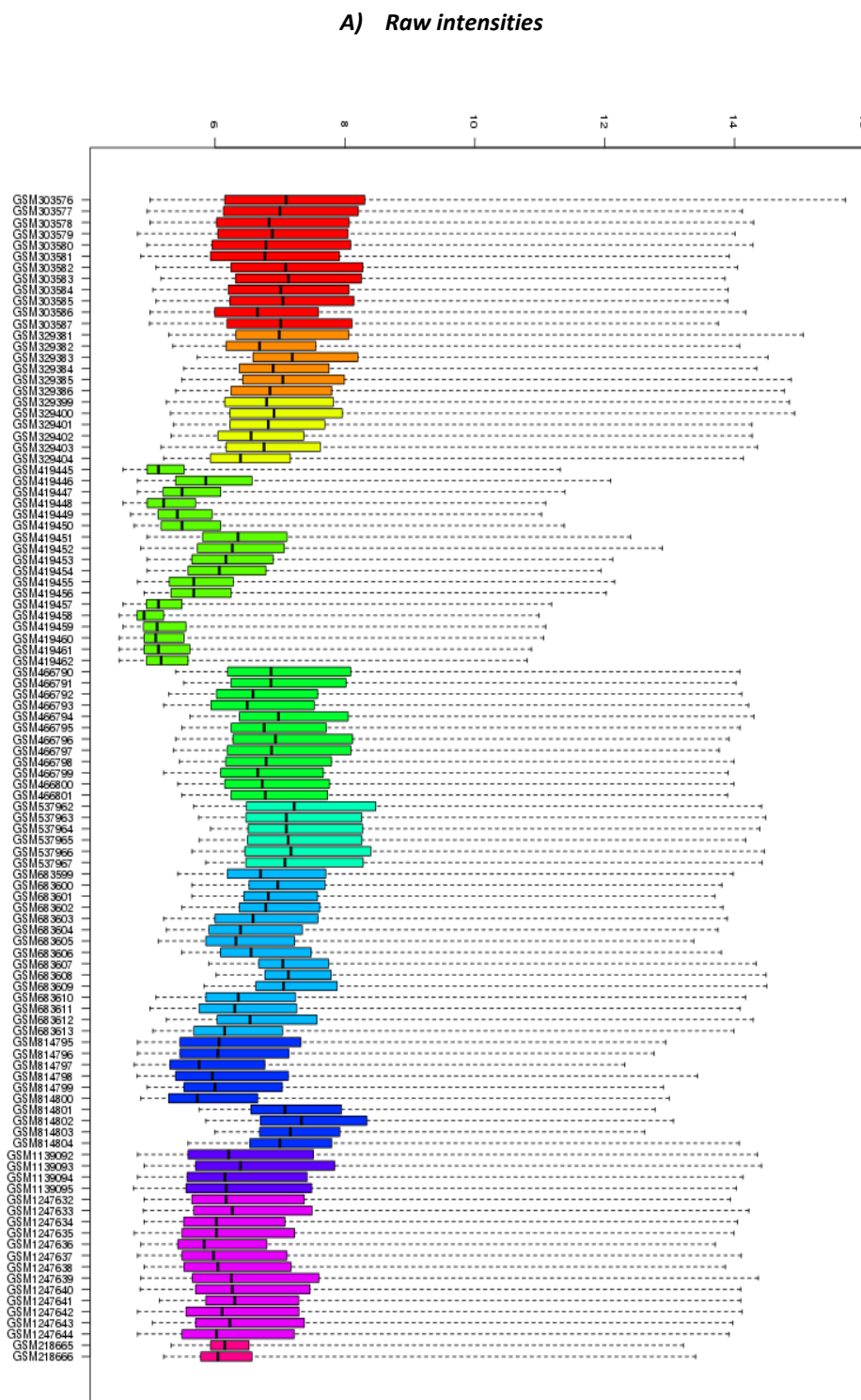
Morpholino treatment: **genes:** Klf4, FoxD3/FoxD5, FoxD3, Klf2b, Klf2a/Klf2b, tnnt2, FoxD5, PCSK7/P53, Nrf2b, Nrf2a/Nrf2b, Nrf2a

**Table S.2.4.1** – Number of microarrays per attribute factor. The table is a summary of Table S.2.4

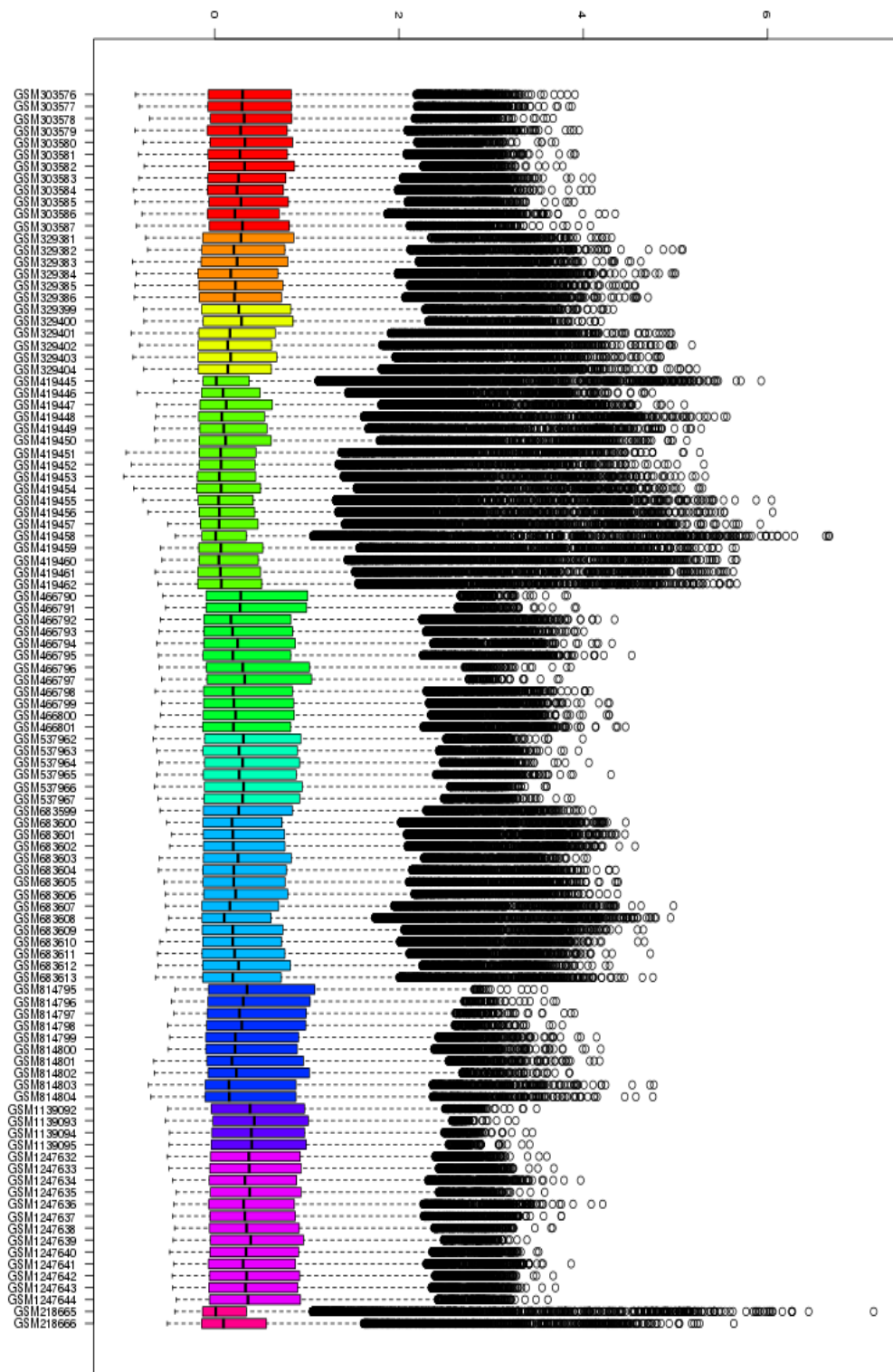
|                            |   |
|----------------------------|---|
| <b>Developmental stage</b> | Blastula: 25<br>Gastrula: 12<br>Segmentation: 18<br>Pharyngula: 8<br>Hatching: 26 |
| <b>Treatment</b>           | Morpholino: 54<br>Control: 32 (WT:14, CO:18)                                      |
| <b>Source</b>              | Embryos: 65<br>Trunk: 24  |
| <b>Dosage:</b>             | 0-0.19: 43<br>0.38-0.783: 36<br>0.974-1.25: 10                                    |

## 2.5 Data normalization and integration – Affymetrix GPL1319

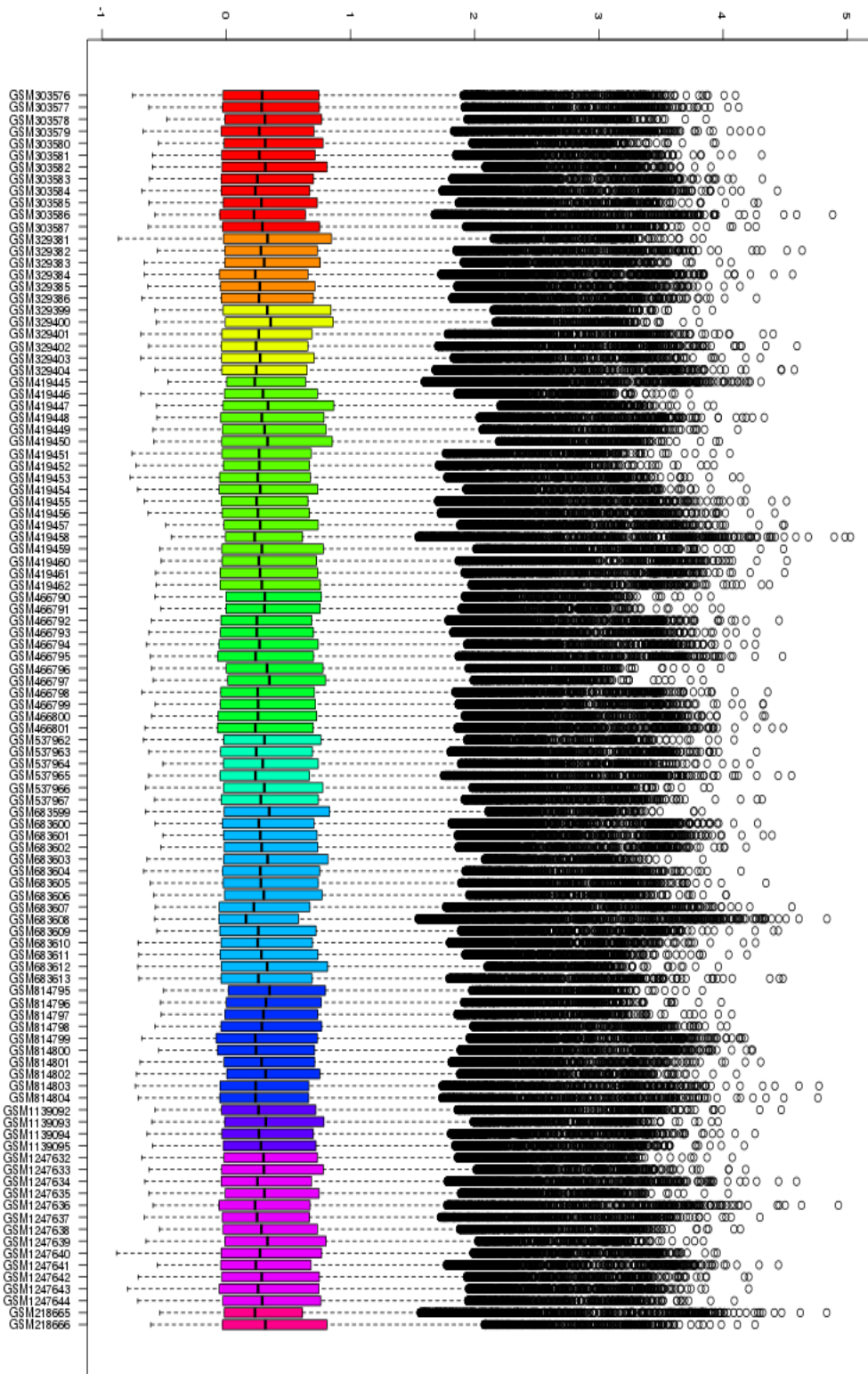
**Figure S.2.5** – Affymetrix: Boxplots of the microarray intensities. Each study is presented with different color. From the top: GSE12012, GSE13157, GSE13158, GSE16740, GSE18830, GSE21539, GSE27569, GSE32914, GSE46844, GSE51541, GSE8800



**B) Intensities normalized through Single Channel Array Normalization (SCAN)**



c) Intensities after Empirical Bayes “batch” standardization. The levels of the *Batch* covariate are the different studies and no *model matrix* is included in the correction

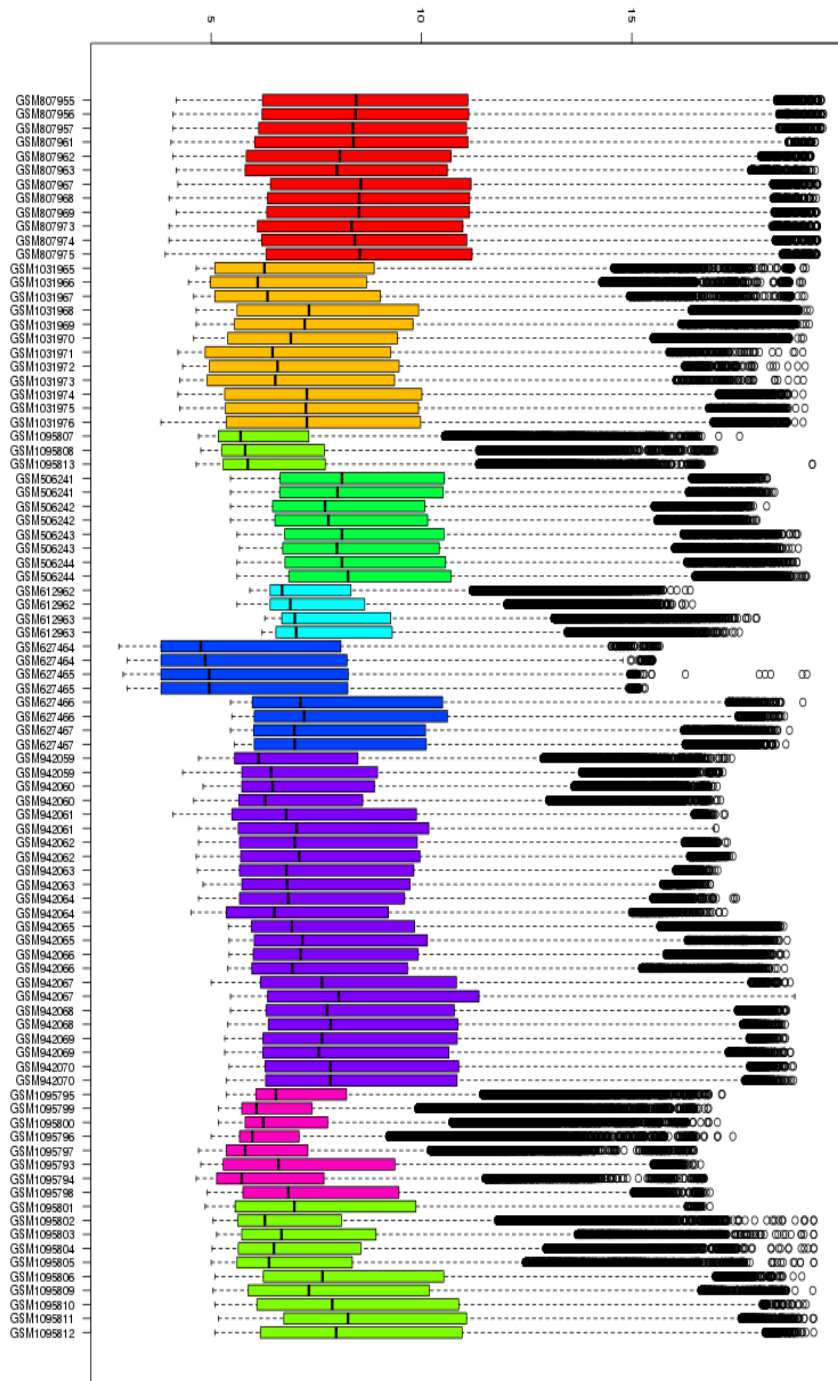




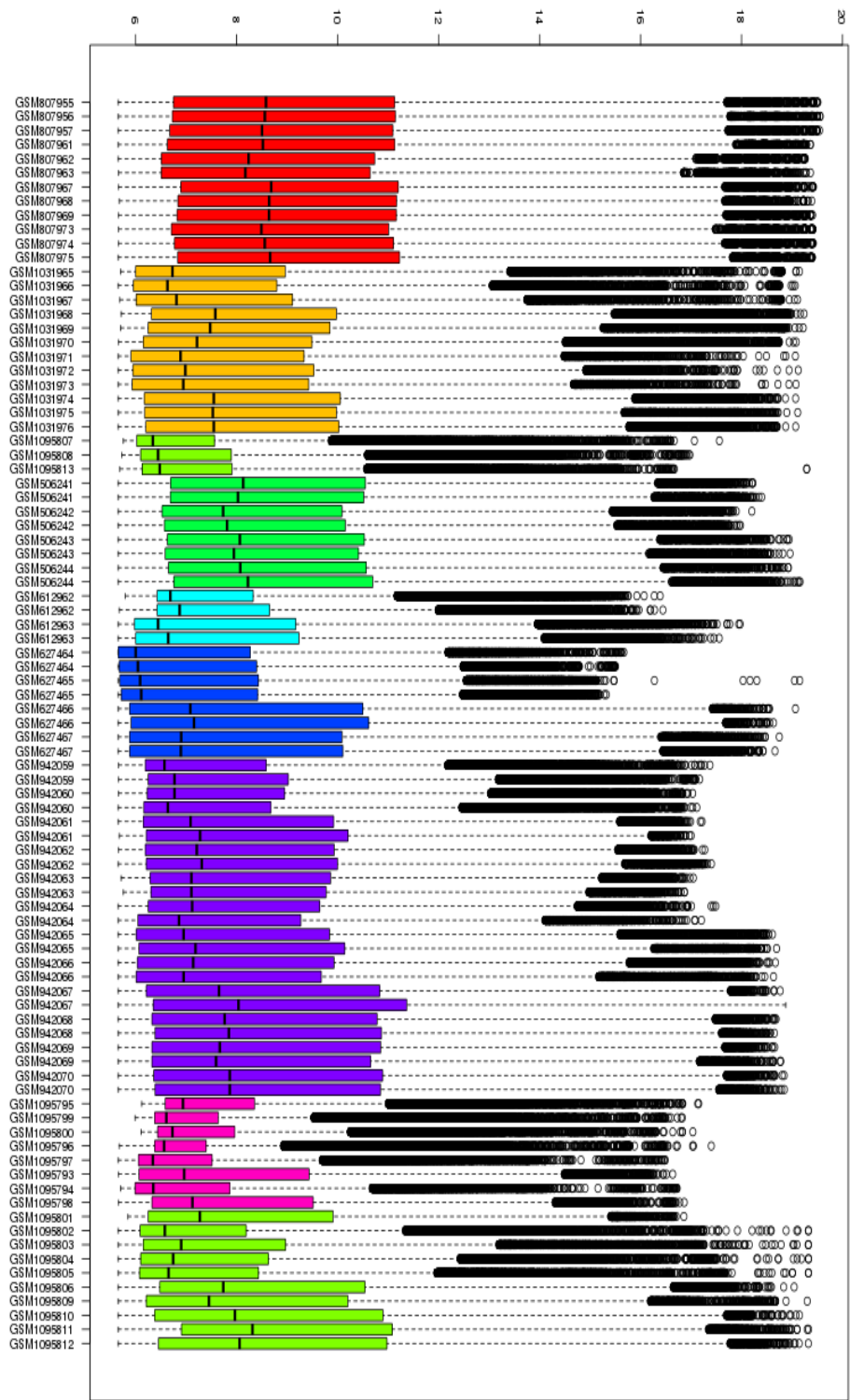
## 2.6 Data normalization and integration – Agilent GPL14664 – GPL6457

**Figure S.2.6** – Agilent: Boxplots of the microarray intensities. Each study is presented with different color. From the top: Single channel: GSE32594, GSE42070, GSE45012, GSE45011, Double channel: GSE20179, GSE24934, GSE25517, GSE38441, GSE45011

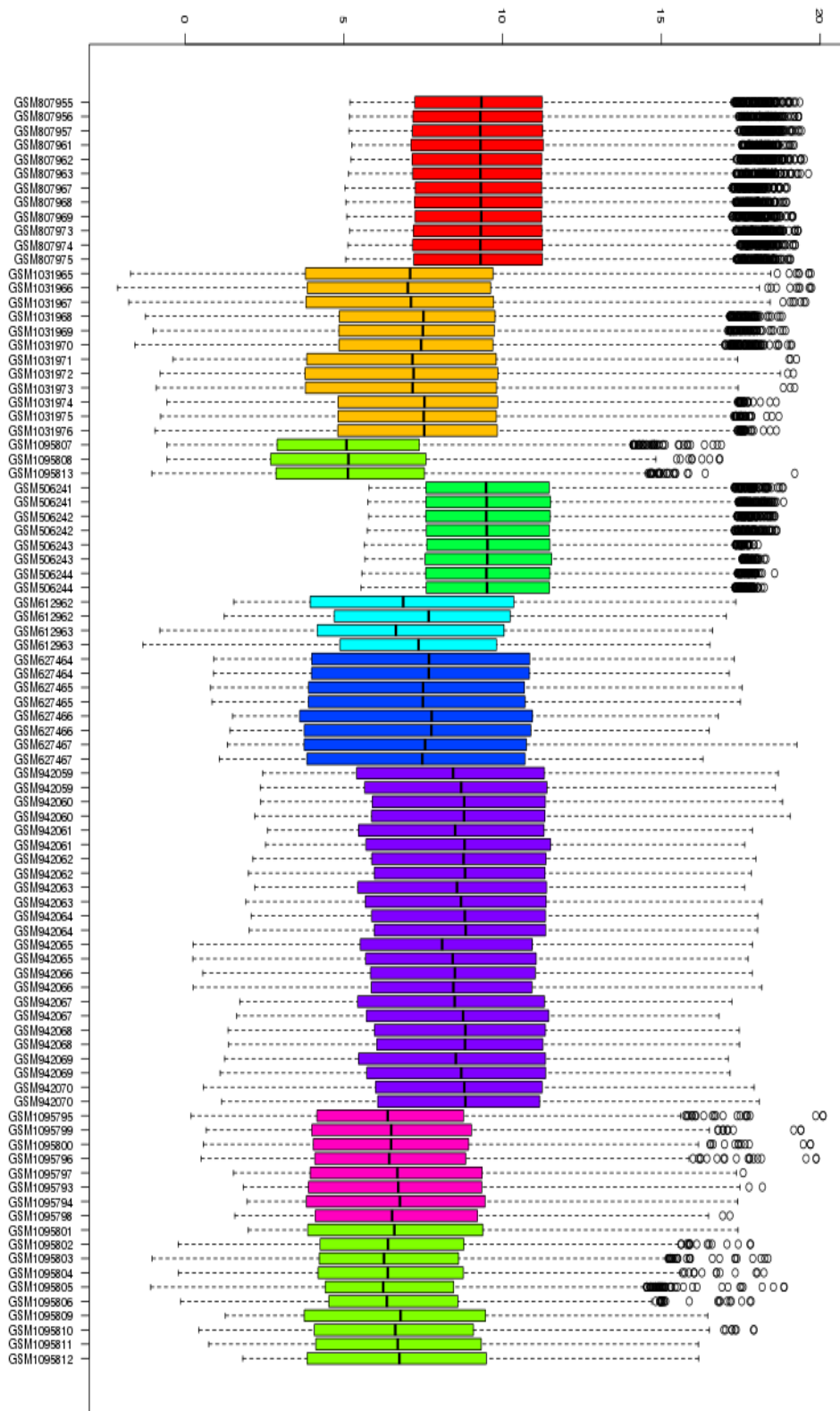
### A) Row intensities



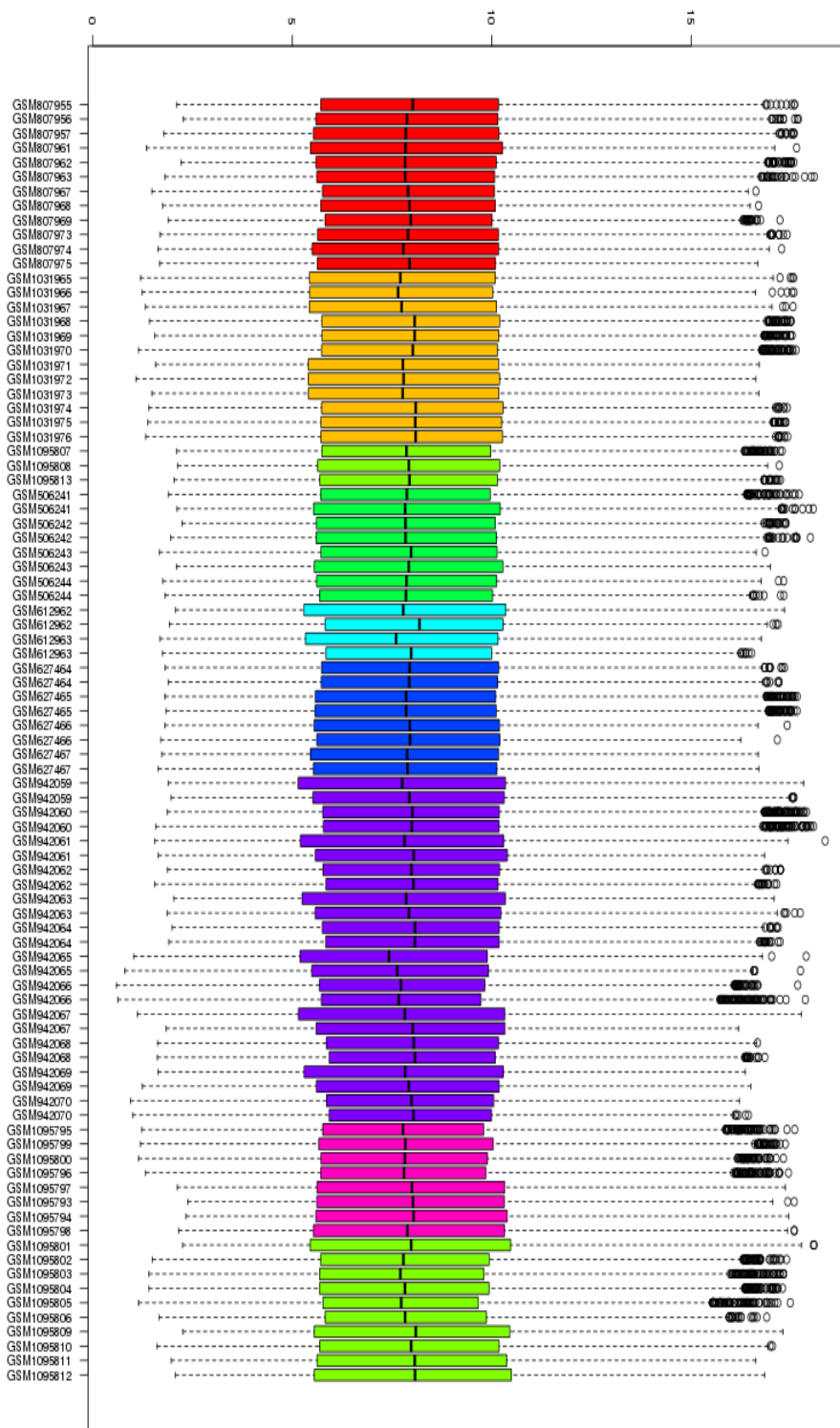
**B) Background corrected intensities with “minimum” method and offset score of 50. The correction aimed to the removal of the negative intensity values. The offset score is selected to shrink the log2 transformed intensities toward zero**



C) Intensities values after Variance Stabilization Normalization (VSN)



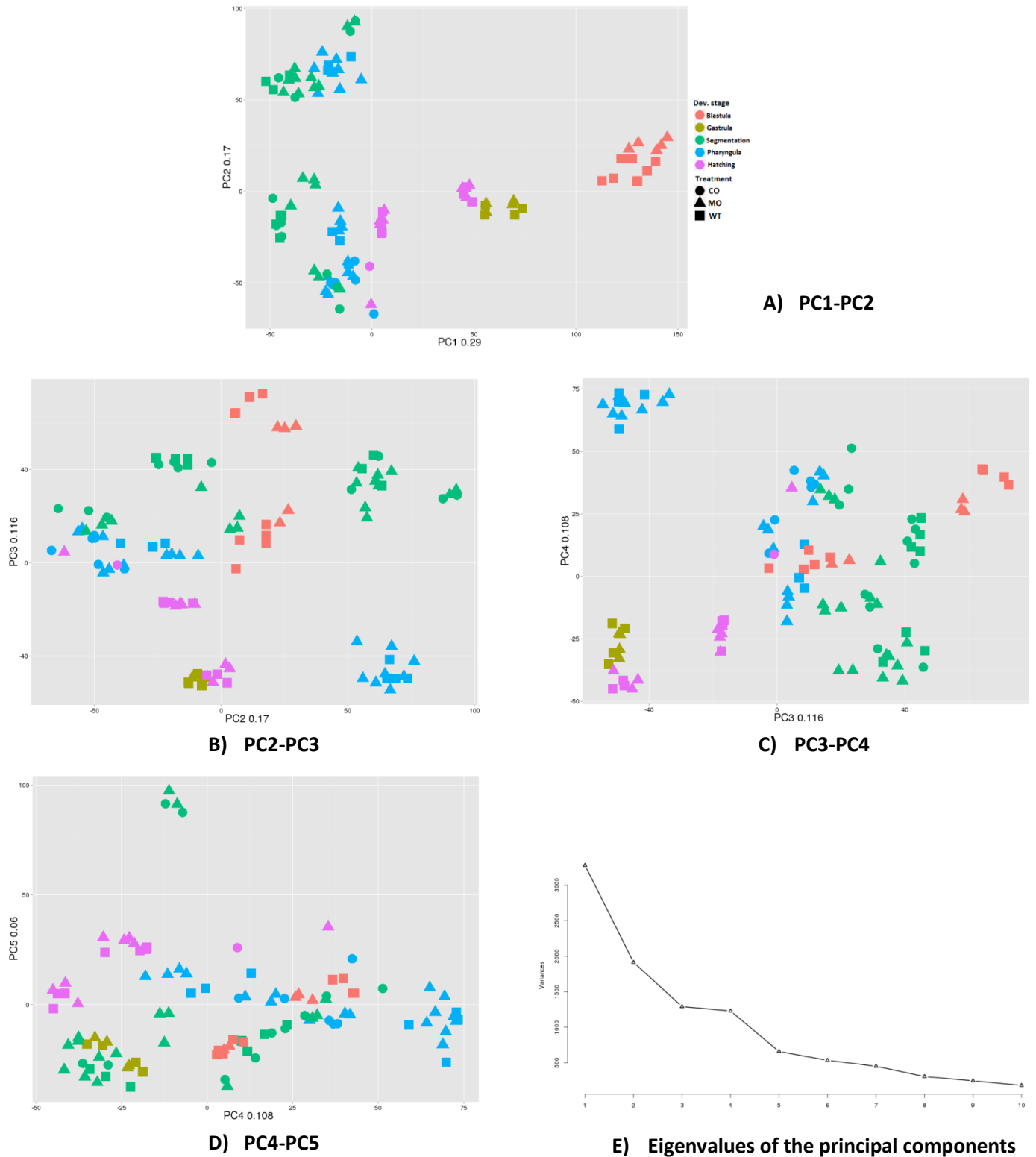
**D) Intensities after Empirical Bayes “batch” correction. The levels of the Batch covariate are the different studies. Studies with both single and double channel intensities are treated as two different levels of batch covariate. No model matrix is included in the “batch” correction**



## 3) RESULTS

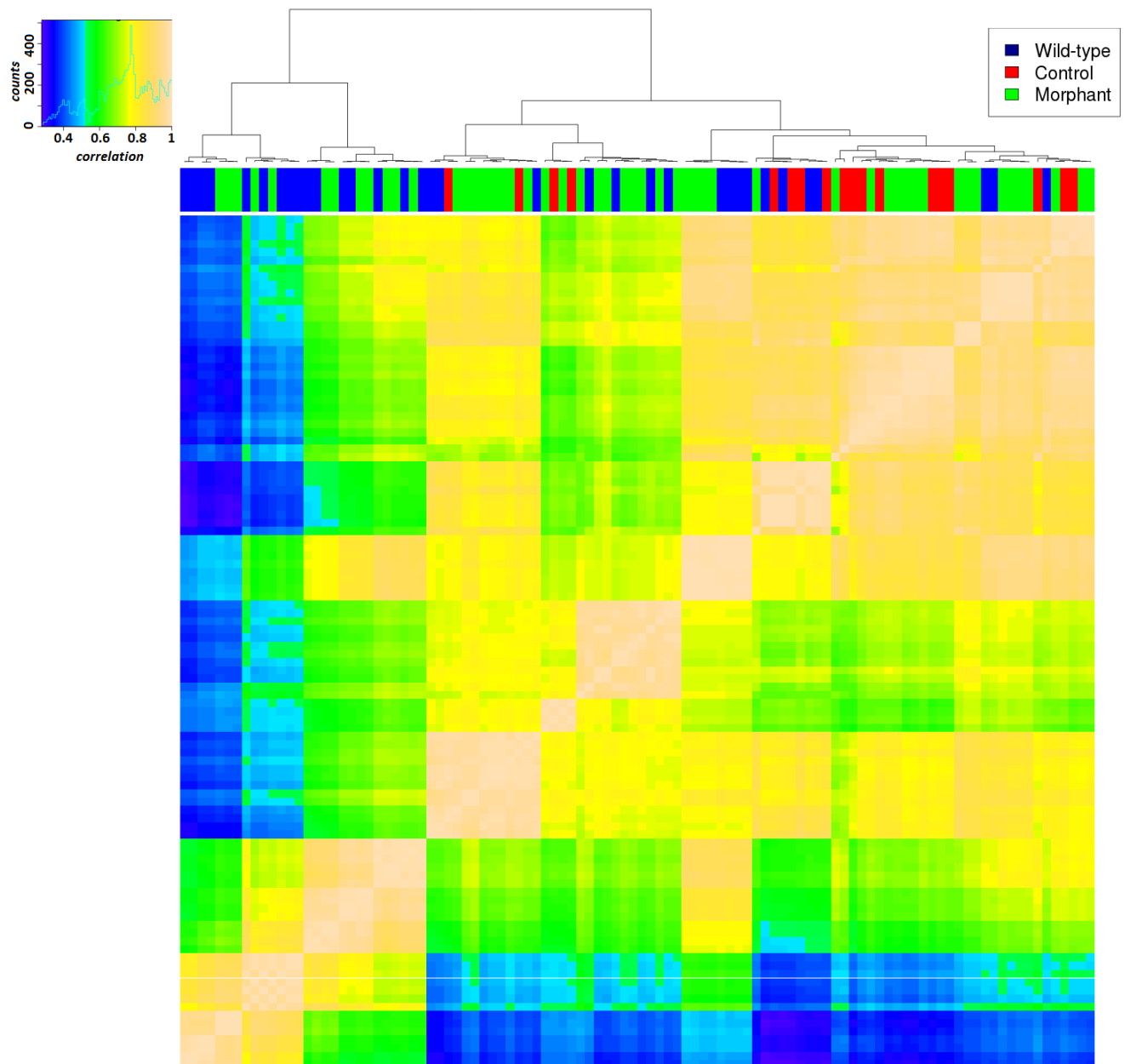
### 3.1 Principal component analysis -Affymetrix

**Figure S.3.1** – PCA labeled by developmental stage and treatment (The number of samples is shown in brackets): Developmental stages (see more details in Table S.2.3.1): Blastula (14), Gastrula (8), Segmentation (13), Pharyngula (31), Hatching (35), Treatment: WT (32): uninjected, CO (18): injected with control, MO (52): injected with target morpholino



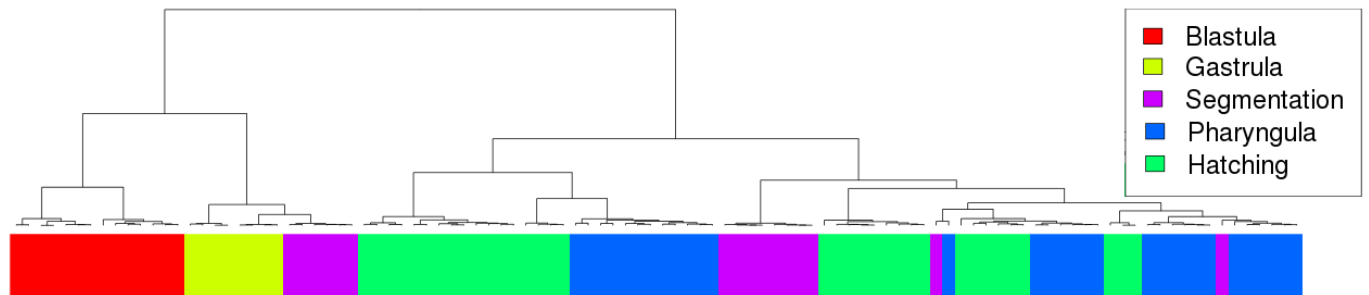
## 3.2 Hierarchical clustering - Affymetrix

**Figure S.3.2A** – Hierarchical clustering with Euclidian distance, Pearson correlation score, Ward’s minimum variance method (Murtagh and Legendre, 2014). The samples are labeled by treatment: Wild type, Control and Morpholino

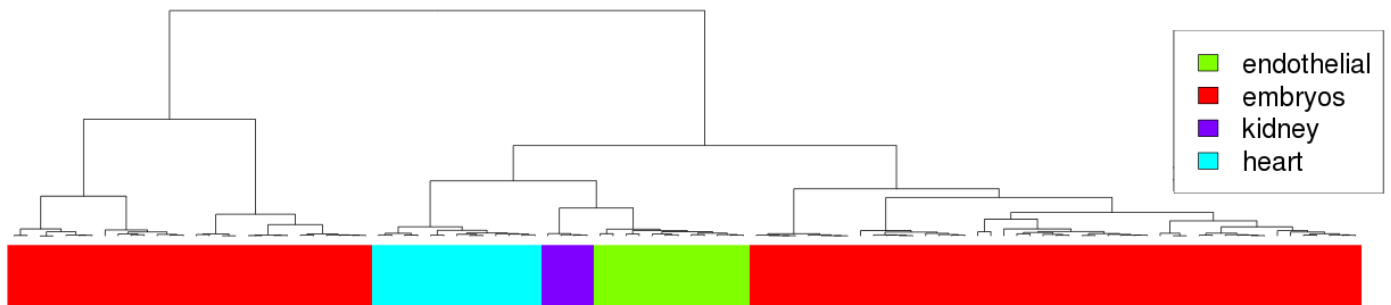


**Figure S.3.2B, C** – Hierarchical clustering with Euclidian distance, Pearson correlation score, Ward’s minimum variance method (Murtagh and Legendre, 2014) as the previous Figure S.3.2. The samples are labeled by developmental stage (B) or tissue type (C)

**B)**



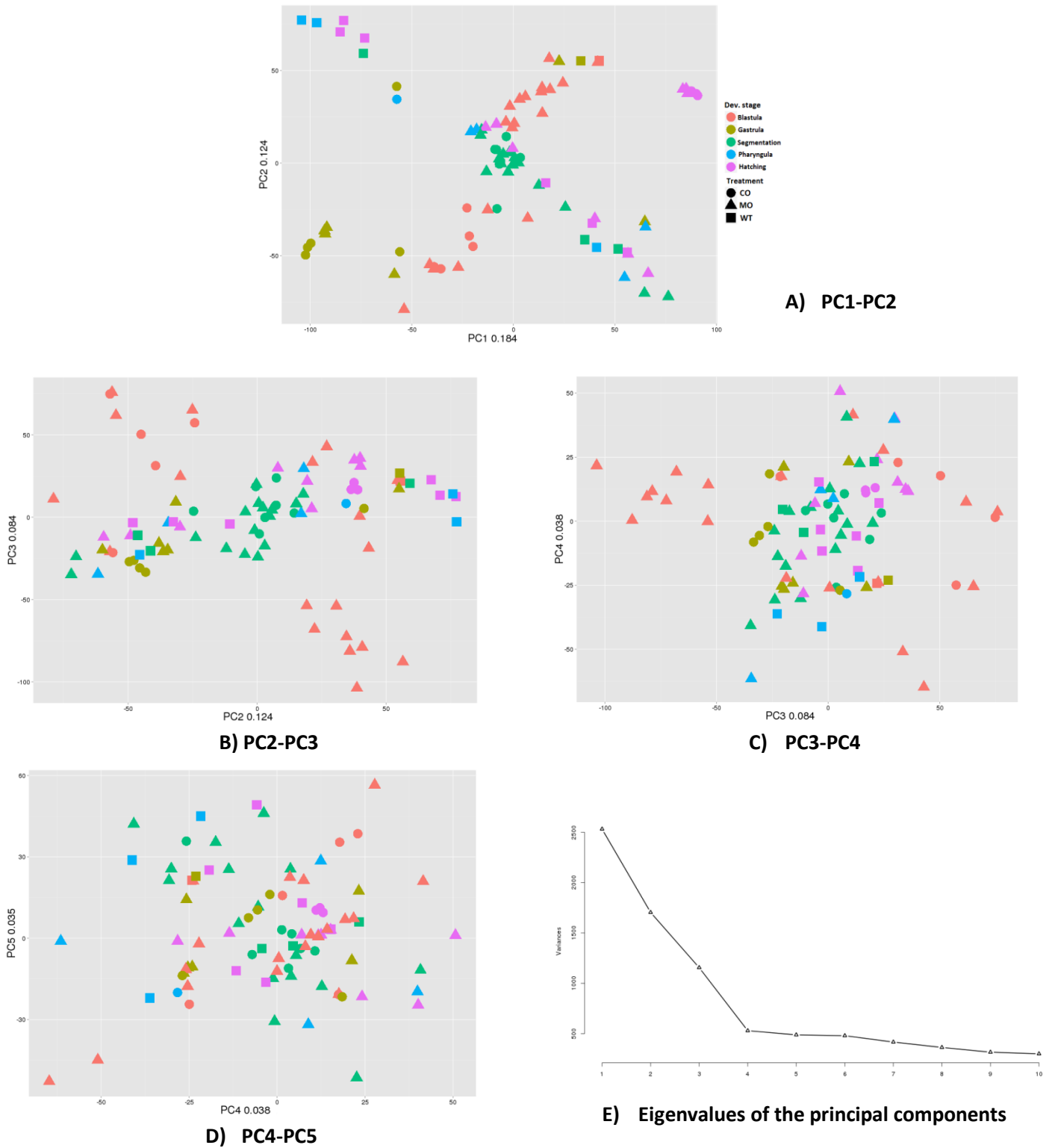
**C)**





### 3.3 Principal Component Analysis - Agilent

**Figure S.3.3** – PCA labeled by developmental stage and treatment (The number of samples is shown in brackets): Developmental stages (see more details in Table S.2.4.1): Blastula (25), Gastrula (12), Segmentation (18), Pharyngula (8), Hatching (26), Treatment: WT (14): uninjected, CO (18): injected with control, MO (54): injected with target morpholino



### 3.4 Machine learning performance - Agilent

**Table S.3.4A** – LOOCV accuracy performance of Embedded feature selector methods

| Feature selector        | LOOCV | LOOCV scrambled |
|-------------------------|-------|-----------------|
| Glmnet                  | 0.764 | 0.584 ± 0.042   |
| Gaussian Linear process | 0.652 | 0.494 ± 0.045   |
| SVM Linear kernel       | 0.652 | 0.494 ± 0.045   |
| Random Forest           | 0.706 | 0.539 ± 0.086   |
| J48                     | 0.741 | 0.526 ± 0.037   |
| Naive Bayes             | 0.685 | 0.557 ± 0.032   |
| SVM Radial kernel       | 0.674 | 0.517 ± 0.054   |
| SVM Polynomial kernel   | 0.741 | 0.55 ± 0.053    |
| PAM                     | 0.674 | 0.52 ± 0.045    |
| KNN                     | 0.607 | 0.559 ± 0.072   |

**Table S.3.4A** – LOOCV accuracy performance of filter feature selector methods tested with K-nearest neighbour

| Feature selector | Max LOOCV         | Max LOOCV scrambled | Max distance to scramble |
|------------------|-------------------|---------------------|--------------------------|
| t-test           | 0.805 (54 feat.)  | 0.635 (1 feat.)     | 0.803 (83 feat.)         |
| Relief           | 0.783 (189 feat.) | 0.643 (121 feat.)   | 0.78 (232 feat.)         |
| mRMR             | 0.673 (25 feat.)  | 0.69 (63 feat.)     | 0.657 (278 feat.)        |

### 3.5 Optimized parameters of embedded feature selectors

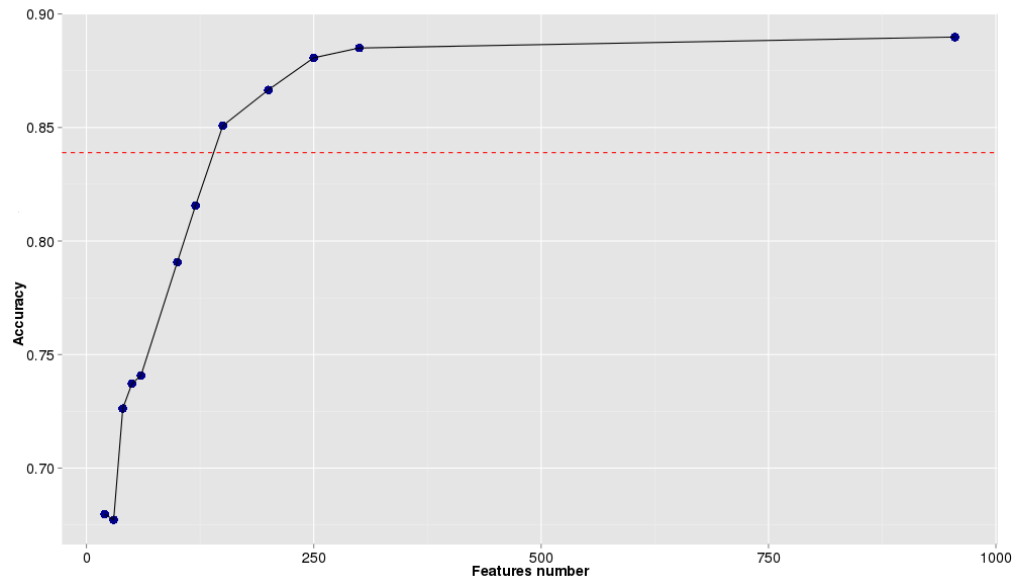
**Table S.3.5** – Algorithms optimal parameters for the Affymetrix and Agilent datasets

| Method                       | Parameters                      |
|------------------------------|---------------------------------|
| <b>Affymetrix</b>            |                                 |
| <b>GlmNet</b>                | Alpha: 0.1, Lambda: 0.1         |
| <b>Gaussian Linear</b>       | No parameters                   |
| <b>SVM Linear</b>            | C: 1                            |
| <b>Random Forest</b>         | Mtry: 3763 (max)                |
| <b>J48</b>                   | C: 0.25                         |
| <b>Naïve Bayes</b>           | fL: 0, usekerel: FALSE          |
| <b>SVM Radial kernel</b>     | Sigma: 0.00019, C: 0.5          |
| <b>SVM Polynomial kernel</b> | Degree: 1 , Scale: 0.01 , C: 1  |
| <b>PAM</b>                   | Threshold: 1.435                |
| <b>KNN</b>                   | K: 5                            |
| <b>Agilent</b>               |                                 |
| <b>GlmNet</b>                | Alpha: 1 , Lambda: 0.0986       |
| <b>Gaussian Linear</b>       | No parameters                   |
| <b>SVM Linear</b>            | C: 1                            |
| <b>Random Forest</b>         | Mtry: 103                       |
| <b>J48</b>                   | C: 0.25                         |
| <b>Naïve Bayes</b>           | fL: 0, usekerel: TRUE           |
| <b>SVM Radial kernel</b>     | Sigma: 0.00011, C: 1            |
| <b>SVM Polynomial kernel</b> | Degree: 2, Scale: 0.01, C: 0.25 |
| <b>PAM</b>                   | Threshold: 0.849                |
| <b>KNN</b>                   | K: 9                            |

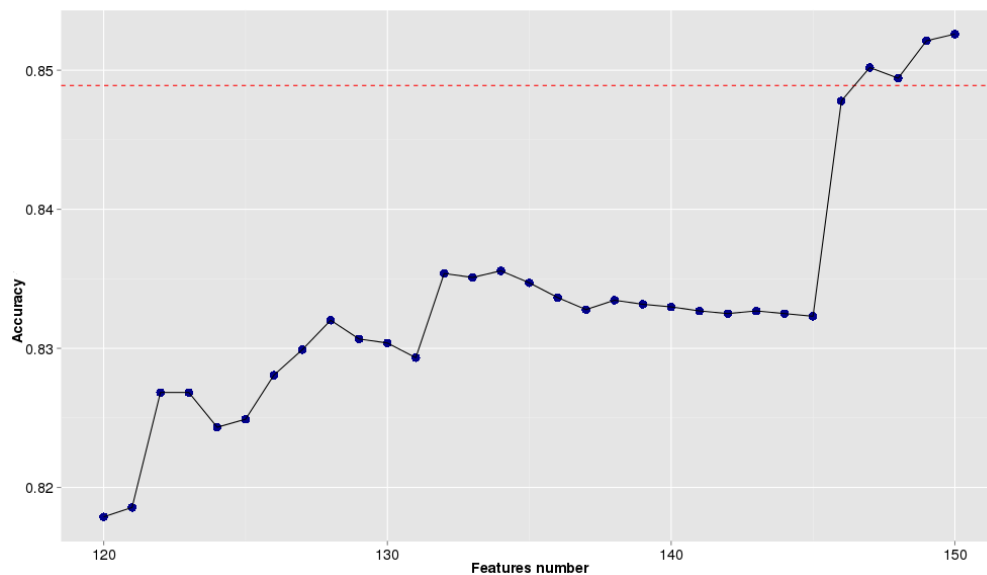
### 3.6 Recursive feature elimination – Affymetrix

**Figure S.3.6– A)** Model's accuracy for features number equal to 20, 30, 40, 50, 60, 100, 120, 150, 200, 250, 300 and 955. The red line shows the 5% tolerance interval **B)** Model's accuracy for features number from 120 to 150. The red line shows the 5% tolerance interval

A)



B)



### 3.7 Complete list of selected genes

**Table S.3.7** – Feature selection: list of the genes selected with Gaussian Linear model. The regulation is the observed gene expression in Morphants (+ Upregulated, -Downregulated). The score is the importance value assigned by the model for separating the two classes.

| Entrez gene ID | Gene name        | Regulation in MO | Score   |
|----------------|------------------|------------------|---------|
| 100534720      | isg20            | +                | 100.000 |
| 368779         | phlda3           | +                | 97.530  |
| 406311         | pik3r3a          | +                | 89.021  |
| 30590          | tp53             | +                | 88.747  |
| 393939         | atf3             | +                | 85.087  |
| 445220         | riok3            | +                | 83.806  |
| 792924         | wu:fb55g09       | +                | 82.708  |
| 555812         | ucp2             | -                | 78.957  |
| 559540         | bmb              | +                | 76.578  |
| 406397         | mmp9             | +                | 76.304  |
| 336967         |                  | +                | 75.206  |
| 100004299      | arhgap35a        | +                | 74.748  |
| 394198         | fos              | +                | 73.193  |
| 58081          | baxa             | +                | 72.736  |
| 677743         | rps27.2          | +                | 72.187  |
| 80963          | rnd3a            | +                | 71.272  |
| 100334953      | nck1b            | +                | 71.089  |
| 567253         | atf5b            | +                | 71.089  |
| 553721         | rpp14            | +                | 70.906  |
| 447811         | rassf1           | +                | 70.723  |
| 30637          | mdm2             | +                | 70.357  |
| 568593         | sdc4             | +                | 69.625  |
| 503589         | gna12a           | +                | 69.076  |
| 386815         |                  | +                | 68.893  |
| 30682          | vegfaa           | +                | 68.618  |
| 323503         |                  | +                | 68.253  |
| 336462         |                  | +                | 68.253  |
| 553212         | st8sia6          | +                | 68.253  |
| 368359         | txnipa           | +                | 67.887  |
| 100322182      |                  | +                | 67.521  |
| 393548         | gadd45aa         | +                | 67.521  |
| 100536496      |                  | +                | 67.429  |
| 30248          | calr             | -                | 67.246  |
| 556363         | GPR108           | +                | 67.063  |
| 561938         | thumpd3          | +                | 66.972  |
| 100003476      | FAM117B (1 of 2) | +                | 66.880  |

|           |                  |   |        |
|-----------|------------------|---|--------|
| 114424    | cdh1             | + | 66.514 |
| 323607    | tmx1             | + | 66.514 |
| 559260    | ENDOD1 (8 of 13) | - | 66.514 |
| 406172    | fat1a            | + | 66.423 |
| 678623    | slc11a2          | + | 66.331 |
| 140744    | ckbb             | - | 66.240 |
| 321708    |                  | + | 66.240 |
| 445149    |                  | + | 66.240 |
| 555328    | serpinh1a        | + | 66.148 |
| 564994    | rbms1a           | + | 66.148 |
| 335963    |                  | + | 65.874 |
| 57965     |                  | + | 65.874 |
| 323226    | wsb1             | + | 65.691 |
| 246093    | id3              | + | 65.599 |
| 566151    | gas2l3           | + | 65.508 |
| 65231     | mdkb             | - | 65.142 |
| 100004977 | mthfd2           | + | 64.684 |
| 324215    | igf2b            | + | 64.593 |
| 337513    |                  | + | 64.593 |
| 325181    | gpd1b            | - | 64.501 |
| 335839    | cnbpb            | + | 64.501 |
| 793663    | timmm8b          | + | 64.227 |
| 334086    | hspa4b           | + | 63.952 |
| 402843    | sfrp1a           | - | 63.952 |
| 321197    |                  | + | 63.769 |
| 335542    |                  | + | 63.769 |
| 116991    | ugdh             | - | 63.586 |
| 336335    | si:ch211-51e12.7 | - | 63.586 |
| 321314    | lmbr1l           | - | 63.403 |
| 386590    | hsp90b1          | - | 63.312 |
| 58094     | dap1b            | - | 63.312 |
| 100537724 |                  | + | 63.038 |
| 406528    | tdh              | + | 63.038 |
| 554119    | mlx              | + | 62.946 |
| 555997    | C2H1orf123       | + | 62.946 |
| 140614    | xbp1             | - | 62.855 |
| 334393    | iars             | + | 62.855 |
| 30719     | gbp              | - | 62.397 |
| 406562    | anp32e           | - | 62.397 |
| 322292    |                  | + | 62.306 |
| 393109    | rasl11b          | + | 62.031 |

|           |               |   |        |
|-----------|---------------|---|--------|
| 567241    | lepr          | + | 62.031 |
| 30081     | ptpn1         | + | 61.940 |
| 325029    |               | + | 61.940 |
| 337130    |               | + | 61.665 |
| 393421    | etnppl        | - | 61.665 |
| 572231    | pfdn4         | + | 61.299 |
| 335072    |               | + | 61.208 |
| 563060    | s100u         | + | 61.208 |
| 64603     | eomesa        | - | 61.208 |
| 100148022 | APOD (3 of 3) | - | 61.116 |
| 564413    | hipk3a        | + | 61.116 |
| 280647    | kars          | + | 60.750 |
| 322841    |               | + | 60.750 |
| 352925    |               | - | 60.750 |
| 406787    | kctd10        | + | 60.659 |
| 553622    | phlda2        | + | 60.659 |
| 393834    | arl4aa        | + | 60.567 |
| 407084    | riok2         | + | 60.567 |
| 553715    |               | + | 60.476 |
| 30295     | rho           | - | 60.293 |
| 799566    | eif4ebp1      | + | 60.201 |
| 321177    | sb:cb252      | + | 60.110 |
| 321986    |               | + | 60.110 |
| 336470    | pak1ip1       | + | 60.110 |
| 337658    |               | + | 60.110 |
| 335838    | lrrfip2       | + | 60.018 |
| 436766    | jdp2          | + | 60.018 |
| 449555    | zgc:101858    | + | 60.018 |
| 30596     | hbbe3         | + | 59.927 |
| 325586    | cyth1b        | + | 59.927 |
| 100007768 | MRPL52        | + | 59.835 |
| 325557    | anxa2a        | + | 59.744 |
| 117508    | klf2a         | - | 59.652 |
| 436988    | zgc:92360     | + | 59.652 |
| 335732    | elovl4b       | - | 59.561 |
| 100331225 | atad2b        | + | 59.561 |
| 751707    | acsf2         | - | 59.469 |
| 553799    | C9H2orf15     | + | 59.378 |
| 797137    |               | + | 59.286 |
| 326758    |               | + | 59.195 |
| 323343    |               | + | 59.103 |

|           |           |   |        |
|-----------|-----------|---|--------|
| 570360    | ppp6r2b   | + | 59.103 |
| 100536814 | dnali1    | + | 59.012 |
| 100003675 | p4ha1b    | + | 59.012 |
| 336227    | cdr2l     | - | 59.012 |
| 337391    |           | + | 59.012 |
| 100334857 | uqcfs1    | - | 58.920 |
| 337093    |           | + | 58.920 |
| 322404    | gltsr2    | + | 58.829 |
| 436826    | polr3k    | + | 58.737 |
| 140620    | prps1a    | + | 58.646 |
| 325655    |           | + | 58.646 |
| 445229    | naa50     | + | 58.646 |
| 566223    | atf5a     | + | 58.554 |
| 321298    | col14a1a  | + | 58.463 |
| 322065    |           | + | 58.463 |
| 561159    |           | + | 58.463 |
| 317638    | igfbp1a   | + | 58.371 |
| 564805    | arhgap29a | + | 58.371 |
| 79380     | EIF4E     | + | 58.371 |
| 325269    |           | + | 58.280 |
| 386850    | EIF6      | + | 58.280 |
| 325637    | NR2F6     | + | 58.280 |
| 171473    | CCNG1     | + | 58.188 |
| 30698     | NKX2-3    | + | 58.188 |
| 140820    | PTPRA     | + | 58.097 |
| 325009    |           | + | 58.097 |
| 677757    | MYCBP     | + | 58.097 |
| 323672    | NAA15A    | + | 58.005 |

**Table S.3.7.1** Genes filtered during the data processing with a strong correlation to the selected features (>0.95 Pearson correlation score)

|        |                  |   |        |           |   |
|--------|------------------|---|--------|-----------|---|
| 387293 | mmp13a           | + | 327494 | zgc:66472 | + |
| 793785 | rab6a            | + | 558677 | zp3a.2    | + |
| 567949 | piezo1           | + | 559966 | kbtbd8    | + |
| 1E+08  | zgc:173837       | + | 664700 | zp2.5     | + |
| 1E+08  | wu:fi42e03       | + | 327506 | ckmt2a    | + |
| 1E+08  | si:zf05-1505d6.3 | + | 559073 | galnt1    | + |
| 114438 | zp2.2            | + | 336236 |           | + |
| 114439 | zp2.3            | + | 336000 |           | + |
| 114440 | si:zf05-1505d6.3 | + | 567632 | dnali1    | + |
| 30593  | zp2.1            | + | 406359 | uqcfs1    | + |
| 327453 | pfkfb1           | + |        |           |   |



### 3.8 Detailed results from GSEA

**Table S.3.8 A)** Significant biological process GO terms and annexed genes

| Description   | P-value | Genes  |
|---|---------|--|
| intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator | 5.25E-6 | phlda3 - pleckstrin homology-like domain, family a, member 3<br>tp53 - tumor protein p53   |
| stress fiber assembly   | 1.01E-4 | sdc4 - syndecan 4<br>gna12a - guanine nucleotide binding protein (g protein) alpha 12a   |
| response to external stimulus   | 1.26E-4 | wipi2 - wd repeat domain, phosphoinositide interacting 2<br>ap1b1 - adaptor-related protein complex 1, beta 1 subunit<br>ctsd - cathepsin d<br>pcna - proliferating cell nuclear antigen<br>defbl1 - defensin, beta-like 1<br>atf3 - activating transcription factor 3<br>junba - jun b proto-oncogene a<br>cebpb - ccaat/enhancer binding protein (c/ebp), beta<br>mcm4 - mcm4 minichromosome maintenance deficient 4, mitotin (s. cerevisiae)<br>jun - jun proto-oncogene<br>atg4c - autophagy-related 4c (yeast)<br>angptl4 - angiopoietin-like 4<br>mmp9 - matrix metalloproteinase 9<br>nuak2 - nuak family, snf1-like kinase, 2<br>ins - preproinsulin<br>hadhaa - hydroxyacyl-coenzyme a dehydrogenase/3-ketoacyl-coenzyme a thiolase/enoyl-coenzyme a hydratase, alpha subunit a<br>hspa5 - heat shock protein 5<br>pik3r4 - phosphoinositide-3-kinase, regulatory subunit 4<br>cyp26a1 - cytochrome p450, subfamily xxvia, polypeptide 1<br>lamc1 - laminin, gamma 1<br>rho - rhodopsin<br>nbr1 - neighbor of brca1 gene 1<br>lamb1a - laminin, beta 1a |
| positive regulation of cell death   | 2.9E-4  | phlda3 - pleckstrin homology-like domain, family a, member 3<br>tp53 - tumor protein p53<br>baxa - bcl2-associated x protein, a  |
| actin filament bundle assembly  | 8.13E-4 | sdc4 - syndecan 4<br>gna12a - guanine nucleotide binding protein (g protein) alpha 12a   |
| positive regulation of peptidase activity   | 8.29E-4 | tp53 - tumor protein p53<br>baxa - bcl2-associated x protein, a  |
| response to stimulus  | 8.44E-4 | sdc4 - syndecan 4<br>mmp9 - matrix metalloproteinase 9<br>phlda3 - pleckstrin homology-like domain, family a, member 3<br>atf3 - activating transcription factor 3<br>tmx1 - thioredoxin-related transmembrane protein 1<br>tp53 - tumor protein p53<br>gadd45aa - growth arrest and dna-damage-inducible, alpha, a<br>mdm2 - transformed 3t3 cell double minute 2 homolog (mouse)<br>baxa - bcl2-associated x protein, a  |
| cell migration involved in heart development  | 8.95E-4 | vegfaa - vascular endothelial growth factor aa<br>gna12a - guanine nucleotide binding protein (g protein) alpha 12a  |

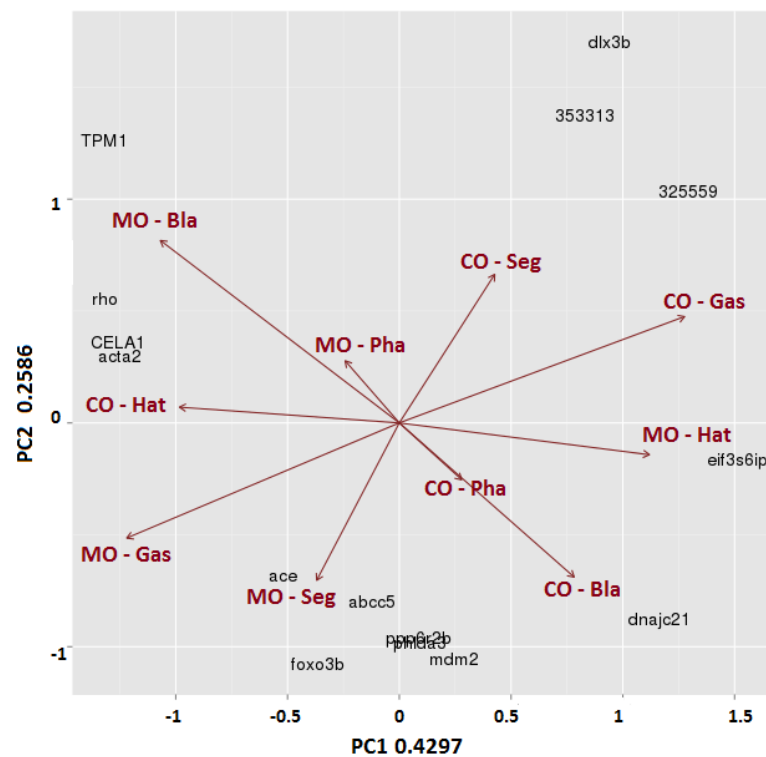
**Table S.3.8 B)** Significant molecular function GO terms and annexed genes

| <b>Description</b>                               | <b>P-value</b> | <b>Genes</b>   |
|--|----------------|--|
| phosphatidylinositol-4,5-bisphosphate binding    | 9.01E-5        | sdc4 - syndecan 4  |
| phosphatidylinositol-5-phosphate binding         | 3.84E-4        | phlda3 - pleckstrin homology-like domain, family a, member 3       |
| phosphatidylinositol-3,4-bisphosphate binding    |                | phlda3 - pleckstrin homology-like domain, family a, member 3       |
| phosphatidylinositol-3,4,5-trisphosphate binding |                |  |
| phosphatidylinositol 3-kinase regulator activity | 7.67E-4        | pik3r3a - phosphoinositide-3-kinase, regulatory subunit 3a (gamma) |

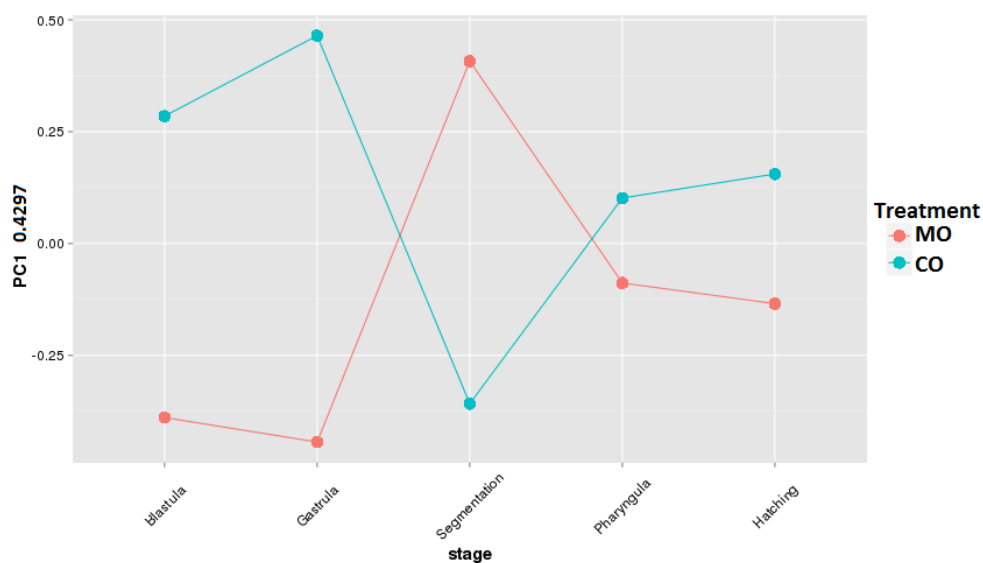
### 3.9 Interaction effect between developmental stage and treatment

**Figure S.3.9— A)** Biplot of the interaction coefficients: Developmental stage - Treatment **B)** Loadings of Biplot's principal component 1 (PC1)

**A)**



**B)**



### 3.10 Significant genes for developmental stage and treatment interaction in 2-way ANOVA and linear model

**Table S.3.10** List of significant genes after ANOVA and linear model

| Entrez gene ID | Gene name      | Significant | Entrez gene ID | Gene name      | Significant |
|----------------|----------------|-------------|----------------|----------------|-------------|
| 30295          | rho            | 0.001       | 335166         |                | 0.05        |
| 30296          | foxo3b         | 0.001       | 335716         | nme2a          | 0.05        |
| 30585          | dlx3b          | 0.001       | 368359         | txnipa         | 0.05        |
| 30637          | mdm2           | 0.001       | 369193         | aldocb         | 0.05        |
| 322509         | acta2          | 0.001       | 373080         | tuba1b         | 0.05        |
| 325559         |                | 0.001       | 393117         | ccdc94         | 0.05        |
| 336147         | abcc5          | 0.001       | 394087         | arl6ip1        | 0.05        |
| 336984         | dnajc21        | 0.001       | 402863         | gtpbp1l        | 0.05        |
| 353313         |                | 0.001       | 403023         | matn1          | 0.05        |
| 368779         | phlda3         | 0.001       | 406460         | ptp4a3         | 0.05        |
| 393908         | TPM1 (2 of 2)  | 0.001       | 406840         | sesn3          | 0.05        |
| 406402         | elf3s6ip       | 0.001       | 407696         | ddx52          | 0.05        |
| 445282         | CELA1 (1 of 7) | 0.001       | 415236         | ppil3          | 0.05        |
| 565980         | ace            | 0.001       | 445030         | pomp           | 0.05        |
| 570360         | ppp6r2b        | 0.001       | 445133         | kctd15a        | 0.05        |
| 100003142      | rpz5           | 0.05        | 556995         | lig1           | 0.05        |
| 100148022      | apoda.2        | 0.05        | 558540         | mybl1          | 0.05        |
| 100534720      | isg20          | 0.05        | 558911         | nol7           | 0.05        |
| 100535672      | prss59.2       | 0.05        | 559540         | bmb            | 0.05        |
| 171481         | fabp10a        | 0.05        | 561947         |                | 0.05        |
| 259189         | hgd            | 0.05        | 563328         | lamp1          | 0.05        |
| 30289          | her3           | 0.05        | 564608         | tmem198b       | 0.05        |
| 30590          | tp53           | 0.05        | 565144         | cenpk          | 0.05        |
| 30710          | igf2a          | 0.05        | 566445         | KRT78 (2 of 2) | 0.05        |
| 321114         | nhp2l1b        | 0.05        | 568021         | tp53inp1       | 0.05        |
| 323401         |                | 0.05        | 568155         | bnip4          | 0.05        |
| 325853         |                | 0.05        | 58216          | atoh7          | 0.05        |
| 326088         | ccnl1b         | 0.05        | 64812          | sox19b         | 0.05        |
| 326098         | med7           | 0.05        | 677743         | rps27.2        | 0.05        |