1. **Data Preparation**

Table 1. In this study, 22 patients are enrolled from which no disease, diabetes with complication, and diabetes without complication are 7, 8, and 7, respectively. Each subject has at least three replicates.

|  |  |  |  |
| --- | --- | --- | --- |
|  | No disease  (N=7) | Diabet with complication (N=8) | Diabet without complication (N=7) |
| No treatment | 21 | 26 | 25 |
| Treated (with 30mM insulin) | 21 | 26 | 25 |

Design matrix for differential expression analysis

* DwC: Disease with complication
* DwoC: Disease without complication
* NoD: No disease

--------------------------------------------------------------------------------------------

Case\_Number Subject Condition Treatment

--------------------------------------------------------------------------------------------

DwC\_1026\_30mM.1 1 disease\_comp insulin

DwC\_1026\_30mM.2 1 disease\_comp insulin

DwC\_1026\_30mM.3 1 disease\_comp insulin

DwC\_1026\_30mM.4 1 disease\_comp insulin

DwC\_1026\_30mM.5 1 disease\_comp insulin

DwC\_1026\_norm.1 2 disease\_comp control

DwC\_1026\_norm.2 2 disease\_comp control

DwC\_1026\_norm.3 2 disease\_comp control

DwC\_1026\_norm.4 2 disease\_comp control

DwC\_1026\_norm.5 2 disease\_comp control

DwC\_6009\_30mM.1 3 disease\_comp insulin

DwC\_6009\_30mM.2 3 disease\_comp insulin

DwC\_6009\_30mM.3 3 disease\_comp insulin

DwC\_6009\_norm.1 4 disease\_comp control

DwC\_6009\_norm.2 4 disease\_comp control

DwC\_6009\_norm.3 4 disease\_comp control

DwC\_11167\_30mM.1 5 disease\_comp insulin

DwC\_11167\_30mM.2 5 disease\_comp insulin

DwC\_11167\_30mM.3 5 disease\_comp insulin

DwC\_11167\_norm.1 6 disease\_comp control

DwC\_11167\_norm.2 6 disease\_comp control

DwC\_11167\_norm.3 6 disease\_comp control

DwC\_13206\_30mM.1 7 disease\_comp insulin

DwC\_13206\_30mM.2 7 disease\_comp insulin

DwC\_13206\_30mM.3 7 disease\_comp insulin

DwC\_13206\_norm.1 8 disease\_comp control

DwC\_13206\_norm.2 8 disease\_comp control

DwC\_13206\_norm.3 8 disease\_comp control

DwC\_17075\_30mM.1 9 disease\_comp insulin

DwC\_17075\_30mM.2 9 disease\_comp insulin

DwC\_17075\_30mM.3 9 disease\_comp insulin

DwC\_17075\_norm.1 10 disease\_comp control

DwC\_17075\_norm.2 10 disease\_comp control

DwC\_17075\_norm.3 10 disease\_comp control

DwC\_21127\_30mM.1 11 disease\_comp insulin

DwC\_21127\_30mM.2 11 disease\_comp insulin

DwC\_21127\_30mM.3 11 disease\_comp insulin

DwC\_21127\_norm.1 12 disease\_comp control

DwC\_21127\_norm.2 12 disease\_comp control

DwC\_21127\_norm.3 12 disease\_comp control

DwC\_26008\_30mM.1 13 disease\_comp insulin

DwC\_26008\_30mM.2 13 disease\_comp insulin

DwC\_26008\_30mM.3 13 disease\_comp insulin

DwC\_26008\_norm.1 14 disease\_comp control

DwC\_26008\_norm.2 14 disease\_comp control

DwC\_26008\_norm.3 14 disease\_comp control

DwC\_27141\_30mM.1 15 disease\_comp insulin

DwC\_27141\_30mM.2 15 disease\_comp insulin

DwC\_27141\_30mM.3 15 disease\_comp insulin

DwC\_27141\_norm.1 16 disease\_comp control

DwC\_27141\_norm.2 16 disease\_comp control

DwC\_27141\_norm.3 16 disease\_comp control

DwoC\_2318\_30mM.1 17 disease insulin

DwoC\_2318\_30mM.2 17 disease insulin

DwoC\_2318\_30mM.3 17 disease insulin

DwoC\_2318\_30mM.4 17 disease insulin

DwoC\_2318\_30mM.5 17 disease insulin

DwoC\_2318\_norm.1 18 disease control

DwoC\_2318\_norm.2 18 disease control

DwoC\_2318\_norm.3 18 disease control

DwoC\_2318\_norm.4 18 disease control

DwoC\_2318\_norm.5 18 disease control

DwoC\_3395\_30mM.1 19 disease insulin

DwoC\_3395\_30mM.2 19 disease insulin

DwoC\_3395\_30mM.3 19 disease insulin

DwoC\_3395\_norm.1 20 disease control

DwoC\_3395\_norm.2 20 disease control

DwoC\_3395\_norm.3 20 disease control

DwoC\_6154\_30mM.1 21 disease insulin

DwoC\_6154\_30mM.2 21 disease insulin

DwoC\_6154\_30mM.3 21 disease insulin

DwoC\_6154\_norm.1 22 disease control

DwoC\_6154\_norm.2 22 disease control

DwoC\_6154\_norm.3 22 disease control

DwoC\_16362\_30mM.1 23 disease insulin

DwoC\_16362\_30mM.2 23 disease insulin

DwoC\_16362\_30mM.3 23 disease insulin

DwoC\_16362\_norm.1 24 disease control

DwoC\_16362\_norm.2 24 disease control

DwoC\_16362\_norm.3 24 disease control

DwoC\_18296\_30mM.1 25 disease insulin

DwoC\_18296\_30mM.2 25 disease insulin

DwoC\_18296\_30mM.3 25 disease insulin

DwoC\_18296\_norm.1 26 disease control

DwoC\_18296\_norm.2 26 disease control

DwoC\_18296\_norm.3 26 disease control

DwoC\_21183\_30mM.1 27 disease insulin

DwoC\_21183\_30mM.2 27 disease insulin

DwoC\_21183\_30mM.3 27 disease insulin

DwoC\_21183\_norm.1 28 disease control

DwoC\_21183\_norm.2 28 disease control

DwoC\_21183\_norm.3 28 disease control

DwoC\_25224\_30mM.1 29 disease insulin

DwoC\_25224\_30mM.2 29 disease insulin

DwoC\_25224\_30mM.3 29 disease insulin

DwoC\_25224\_30mM.4 29 disease insulin

DwoC\_25224\_30mM.5 29 disease insulin

DwoC\_25224\_norm.1 30 disease control

DwoC\_25224\_norm.2 30 disease control

DwoC\_25224\_norm.3 30 disease control

DwoC\_25224\_norm.4 30 disease control

DwoC\_25224\_norm.5 30 disease control

NoD\_7012\_30mM.1 31 normal insulin

NoD\_7012\_30mM.2 31 normal insulin

NoD\_7012\_30mM.3 31 normal insulin

NoD\_7012\_norm.1 32 normal control

NoD\_7012\_norm.2 32 normal control

NoD\_7012\_norm.3 32 normal control

NoD\_7344\_30mM.1 33 normal insulin

NoD\_7344\_30mM.2 33 normal insulin

NoD\_7344\_30mM.3 33 normal insulin

NoD\_7344\_norm.1 34 normal control

NoD\_7344\_norm.2 34 normal control

NoD\_7344\_norm.3 34 normal control

NoD\_11985\_30mM.1 35 normal insulin

NoD\_11985\_30mM.2 35 normal insulin

NoD\_11985\_30mM.3 35 normal insulin

NoD\_11985\_norm.1 36 normal control

NoD\_11985\_norm.2 36 normal control

NoD\_11985\_norm.3 36 normal control

NoD\_14381\_30mM.1 37 normal insulin

NoD\_14381\_30mM.2 37 normal insulin

NoD\_14381\_30mM.3 37 normal insulin

NoD\_14381\_norm.1 38 normal control

NoD\_14381\_norm.2 38 normal control

NoD\_14381\_norm.3 38 normal control

NoD\_14520\_30mM.1 39 normal insulin

NoD\_14520\_30mM.2 39 normal insulin

NoD\_14520\_30mM.3 39 normal insulin

NoD\_14520\_norm.1 40 normal control

NoD\_14520\_norm.2 40 normal control

NoD\_14520\_norm.3 40 normal control

NoD\_14569\_30mM.1 41 normal insulin

NoD\_14569\_30mM.2 41 normal insulin

NoD\_14569\_30mM.3 41 normal insulin

NoD\_14569\_norm.1 42 normal control

NoD\_14569\_norm.2 42 normal control

NoD\_14569\_norm.3 42 normal control

NoD\_14581\_30mM.1 43 normal insulin

NoD\_14581\_30mM.2 43 normal insulin

NoD\_14581\_30mM.3 43 normal insulin

NoD\_14581\_norm.1 44 normal control

NoD\_14581\_norm.2 44 normal control

NoD\_14581\_norm.3 44 normal control

------------------------------------------------------------------------------------------------------

1. **Data Preprocessing**

Data matrix: 47282 (probes) x 144 (samples).

Note that 47282 is the common probes among the four different batches.

1. **Raw data (box plot)**
2. 1-144 samples all together



1. 1-46 samples only



1. 47-92 samples only



1. 93-144 samples only



1. **Normalized data (box plot)**
   1. 1-144 samples all together



* 1. 1-46 samples only



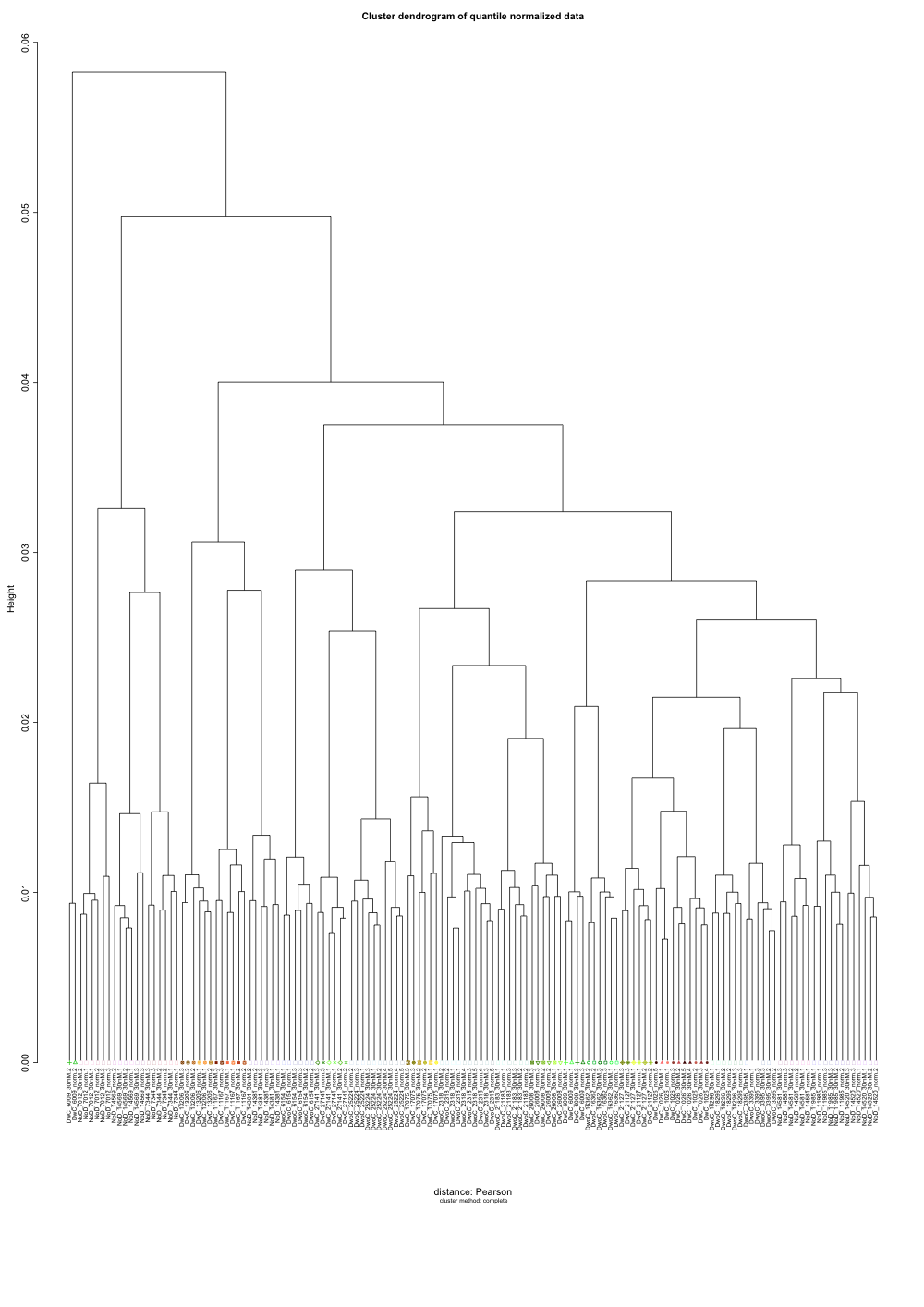
* 1. 47-92 samples only



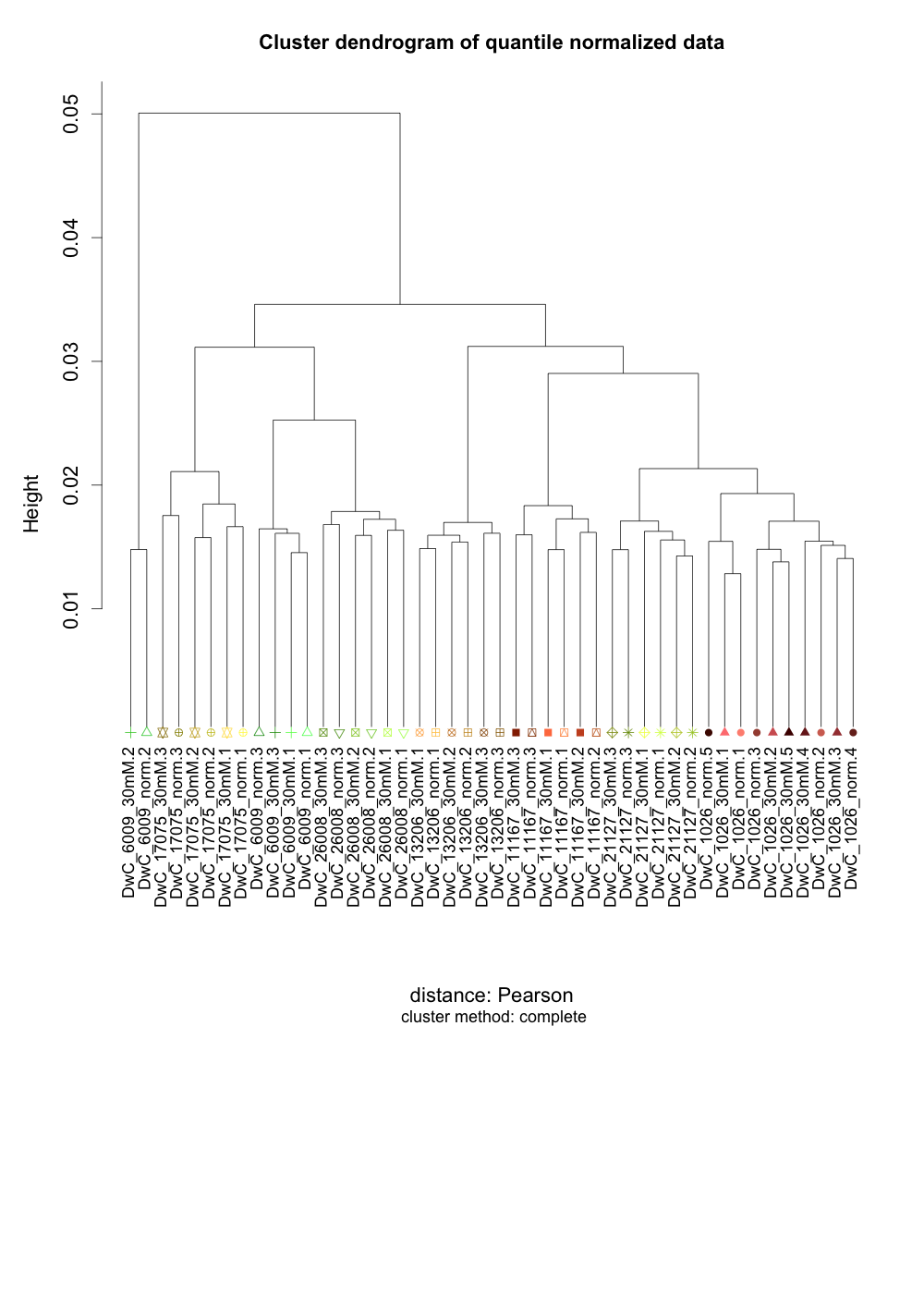
* 1. 93-144 samples only



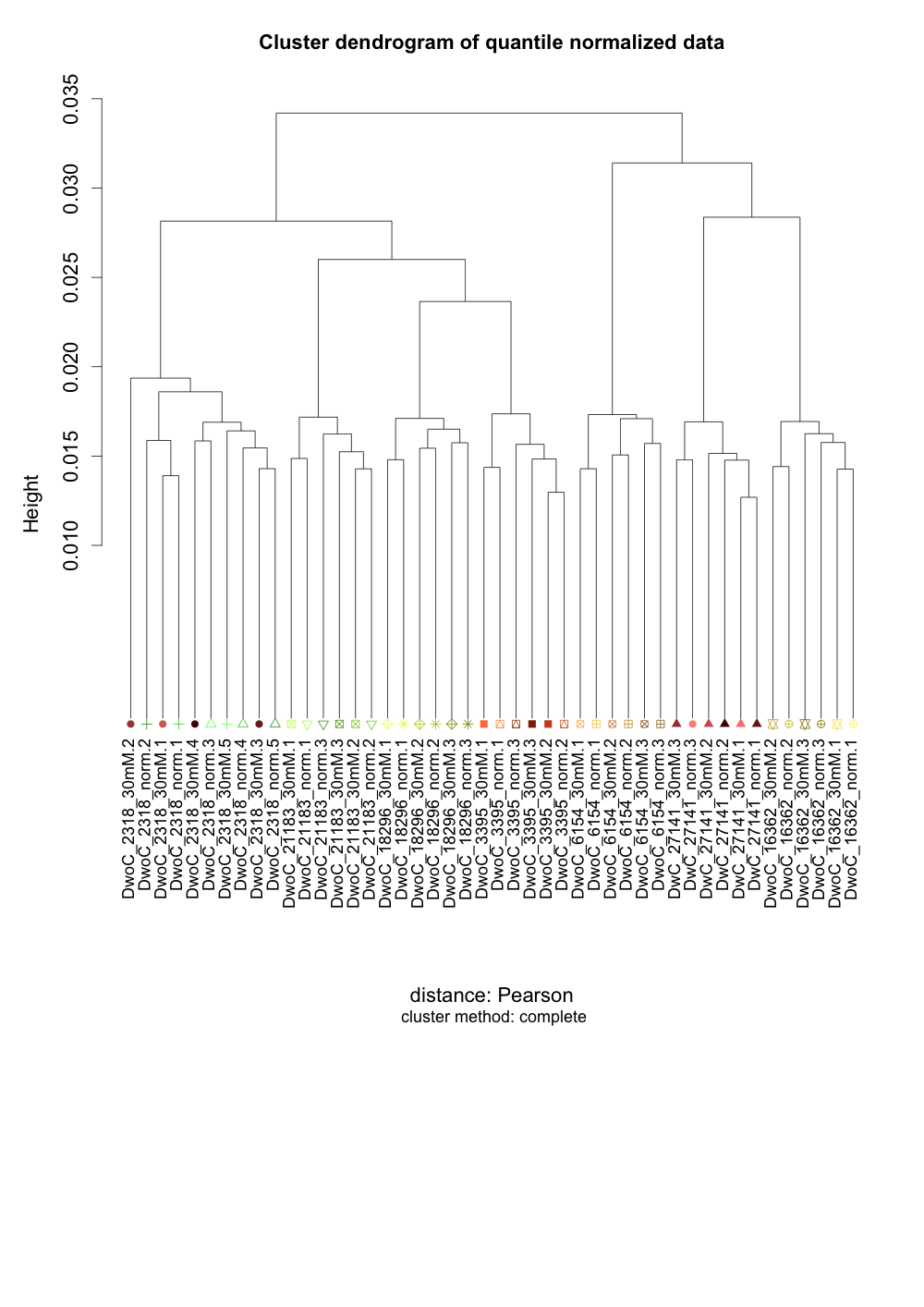
**Data clustering (normalized data only: 144 samples all together)**



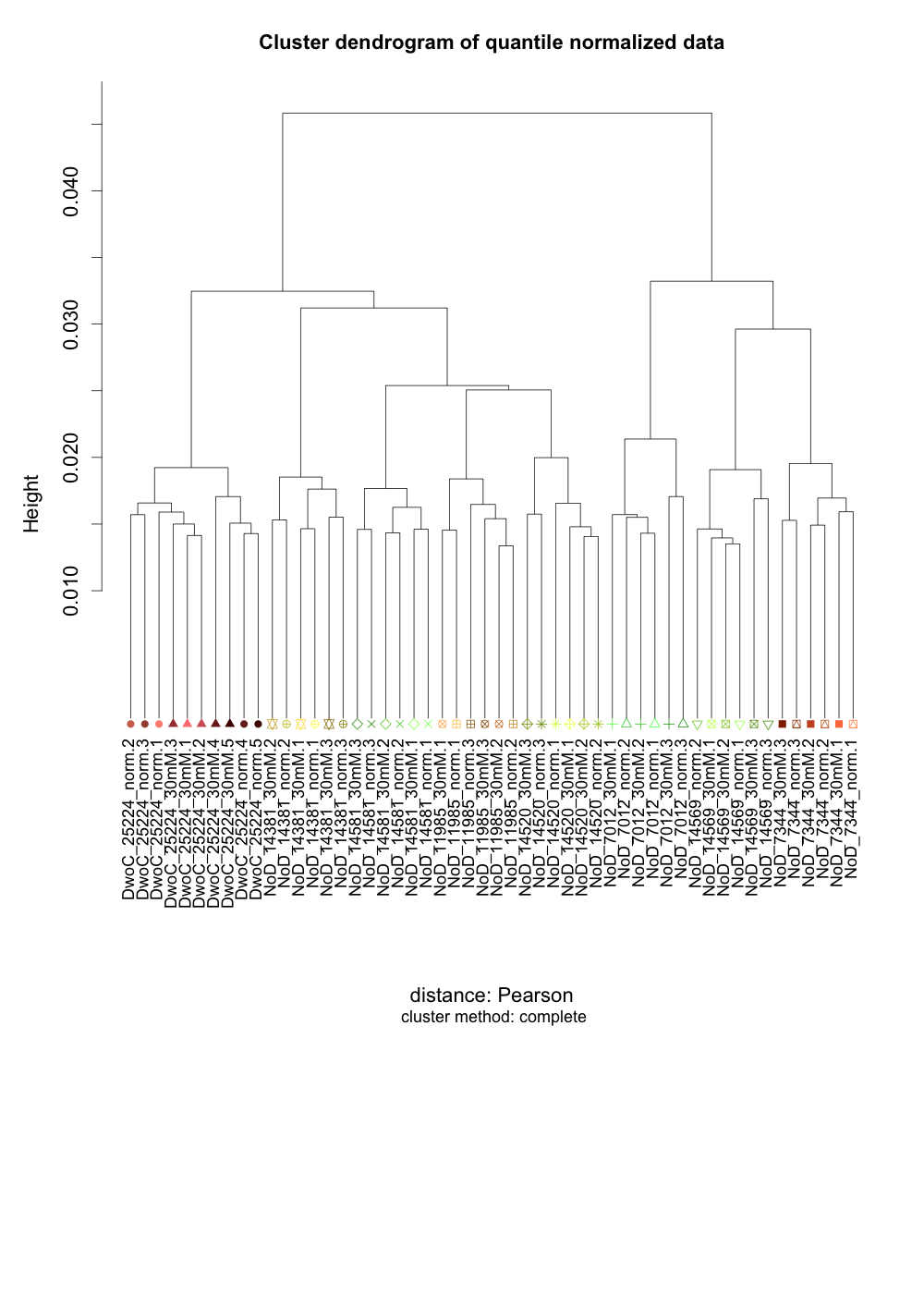
1. 1-46 samples only



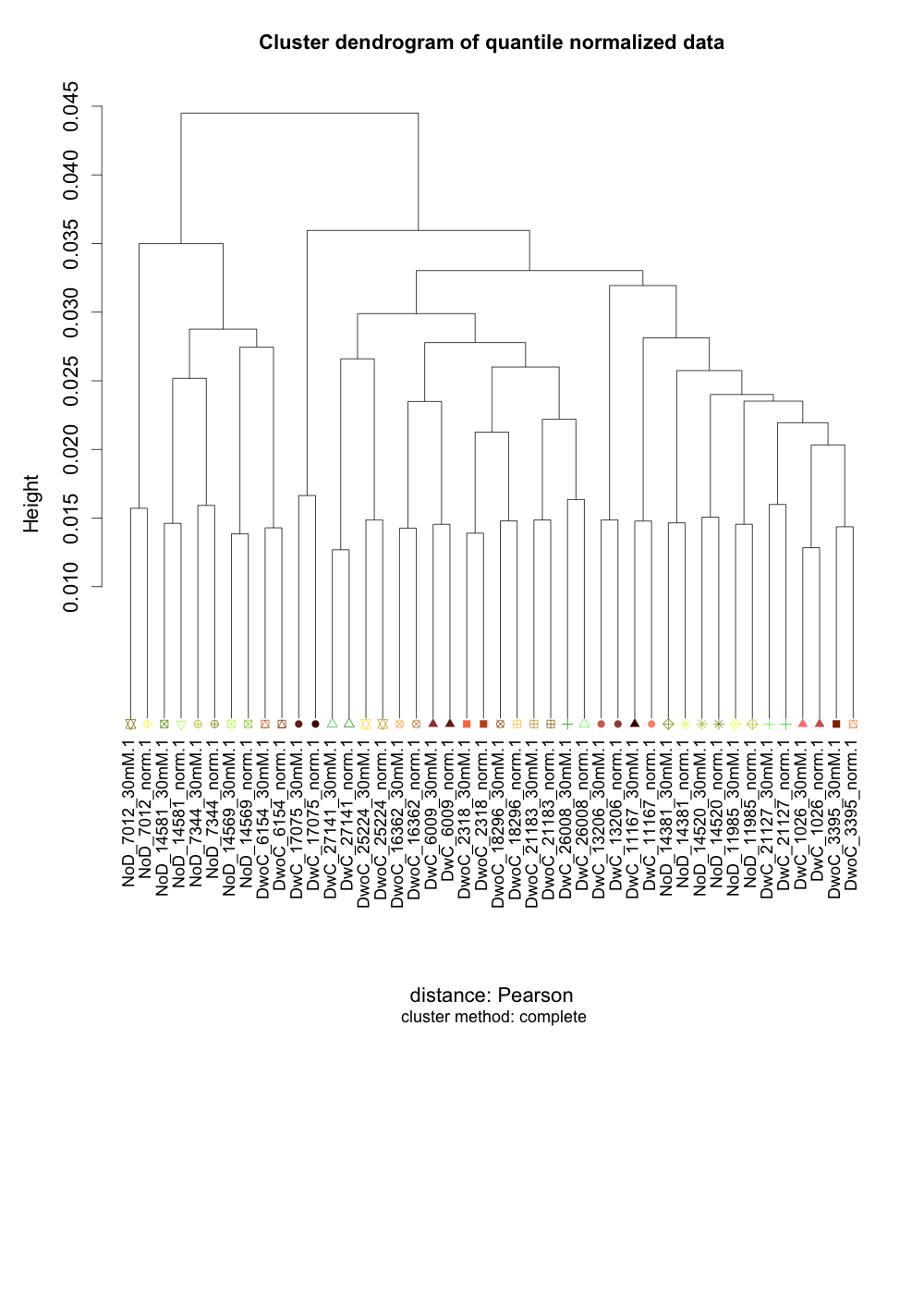
1. 47-92 samples only



1. 93-144 samples only



1. **44 samples without replicates**

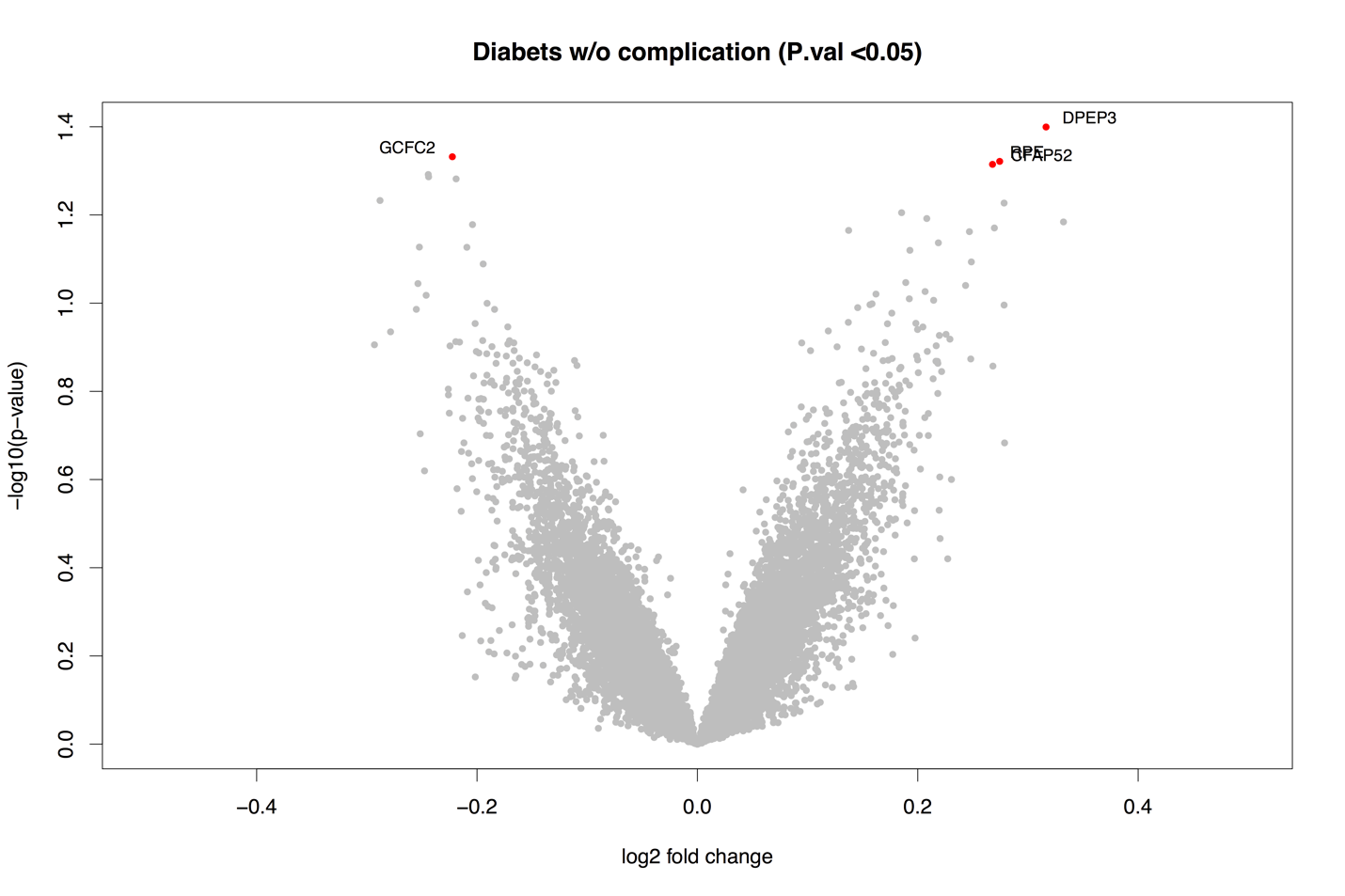


1. **Data filtering**
   1. 16436 unexpressed probes are removed that will reduce false positives (matrix size: 30846 x 144)
   2. 8871 probes that do not have annotated genes are removed (matrix size: 21975 x 144)
2. **Differential expression analysis (using limma)**

Input data matrix: 21975 probes x 144 samples

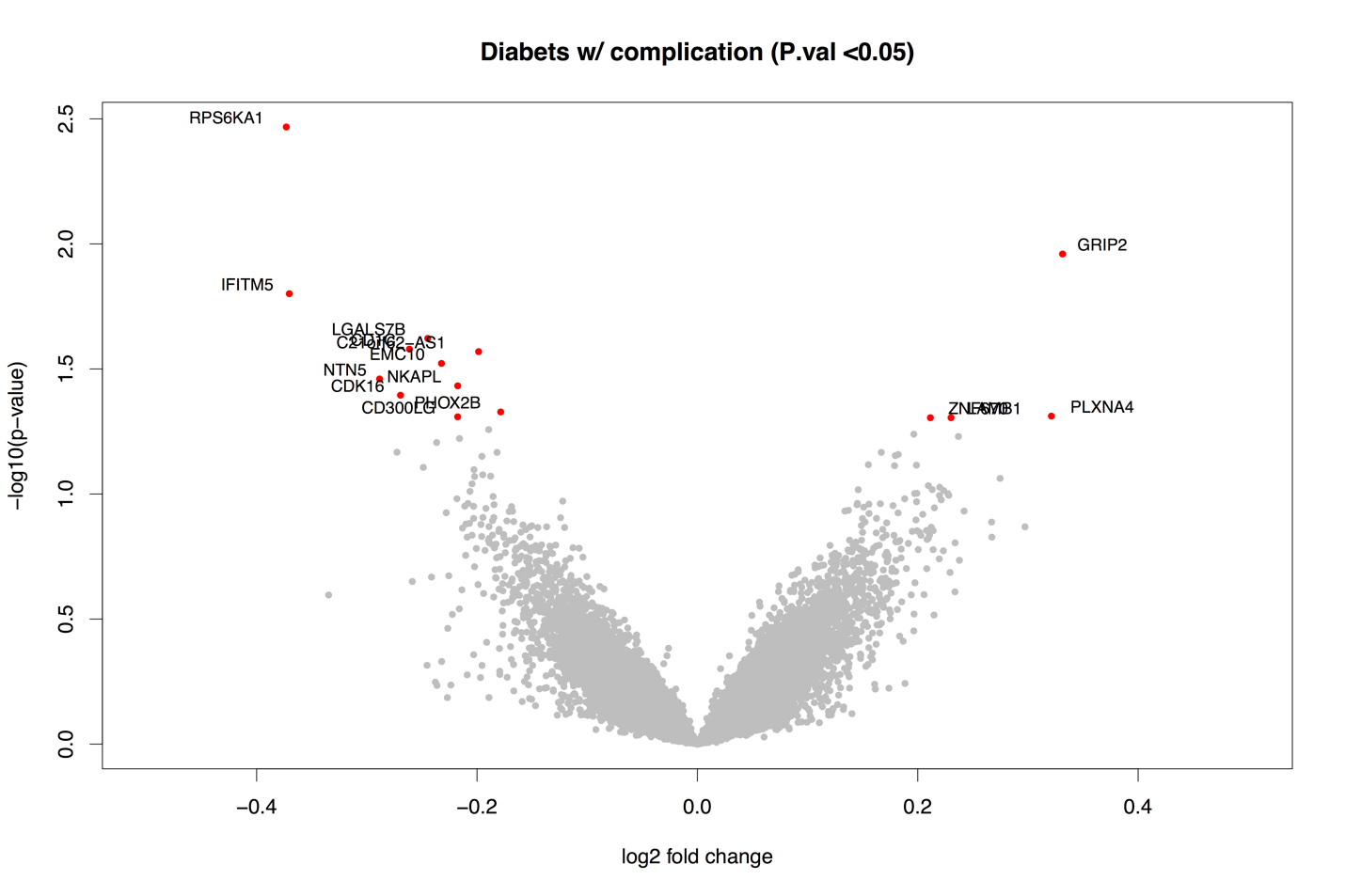
**Comparison design (cutoff: p.val <0.05) – note that no significant q-value is achieved**

1. disease without complication insulin vs control



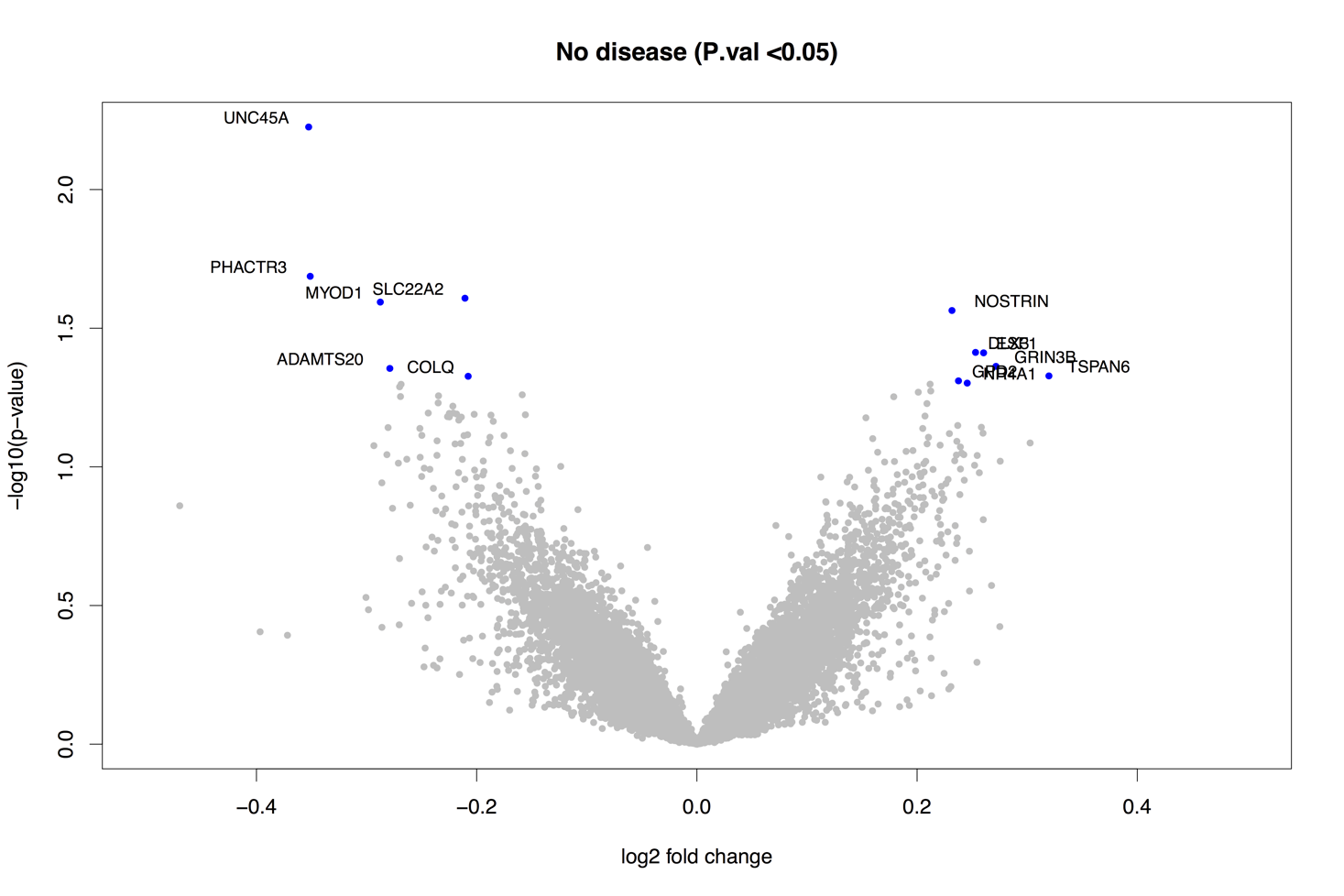
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene Symbol** | **Gene Name** | **log2 FC** | **Ave Expr** | **P.Value** |
| **DPEP3** | **dipeptidase 3** | **0.316** | **3.767** | **0.040** |
| **GCFC2** | **GC-rich sequence DNA-binding factor 2** | **-0.222** | **4.325** | **0.047** |
| **RPE** | **ribulose-5-phosphate-3-epimerase** | **0.274** | **3.754** | **0.048** |
| **CFAP52** | **cilia and flagella associated protein 52** | **0.268** | **3.864** | **0.048** |

1. disease with complication insulin vs control



|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene Symbol** | **geneName** | **Log2 FC** | **Ave Expr** | **P.Value** |
| **RPS6KA1** | **ribosomal protein S6 kinase, 90kDa, polypeptide 1** | **-0.373** | **4.194** | **0.003** |
| **GRIP2** | **glutamate receptor interacting protein 2** | **0.331** | **4.230** | **0.011** |
| **IFITM5** | **interferon induced transmembrane protein 5** | **-0.370** | **3.617** | **0.016** |
| **LGALS7B** | **lectin, galactoside-binding, soluble, 7B** | **-0.245** | **3.897** | **0.024** |
| **CD1C** | **CD1c molecule** | **-0.261** | **3.892** | **0.026** |
| **C21orf62-AS1** | **C21orf62 antisense RNA 1** | **-0.199** | **4.477** | **0.027** |
| **EMC10** | **ER membrane protein complex subunit 10** | **-0.232** | **4.140** | **0.030** |
| **NTN5** | **netrin 5** | **-0.288** | **3.932** | **0.035** |
| **NKAPL** | **NFKB activating protein-like** | **-0.218** | **4.108** | **0.037** |
| **CDK16** | **cyclin-dependent kinase 16** | **-0.269** | **3.937** | **0.040** |
| **PHOX2B** | **paired-like homeobox 2b** | **-0.179** | **4.186** | **0.047** |
| **PLXNA4** | **plexin A4** | **0.321** | **3.747** | **0.049** |
| **CD300LG** | **CD300 molecule-like family member g** | **-0.218** | **4.103** | **0.049** |
| **LAMB1** | **laminin, beta 1** | **0.230** | **4.260** | **0.050** |
| **ZNF670** | **zinc finger protein 670** | **0.211** | **4.453** | **0.050** |

1. no disease insulin vs control



|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene Symbol** | **Gene Name** | **log2 FC** | **Ave Expr** | **P.Value** |
| **UNC45A** | **unc-45 myosin chaperone A** | **-0.353** | **4.097** | **0.006** |
| **PHACTR3** | **phosphatase and actin regulator 3** | **-0.351** | **3.956** | **0.021** |
| **SLC22A2** | **solute carrier family 22 (organic cation transporter), member 2** | **-0.211** | **4.329** | **0.025** |
| **MYOD1** | **myogenic differentiation 1** | **-0.288** | **4.002** | **0.025** |
| **NOSTRIN** | **nitric oxide synthase trafficking** | **0.232** | **4.121** | **0.027** |
| **DLX3** | **distal-less homeobox 3** | **0.253** | **4.198** | **0.039** |
| **ESF1** | **ESF1 nucleolar pre-rRNA processing protein homolog** | **0.260** | **3.925** | **0.039** |
| **GRIN3B** | **glutamate receptor, ionotropic, N-methyl-D-aspartate 3B** | **0.272** | **3.980** | **0.043** |
| **ADAMTS20** | **ADAM metallopeptidase with thrombospondin type 1 motif, 20** | **-0.279** | **3.858** | **0.044** |
| **TSPAN6** | **tetraspanin 6** | **0.320** | **3.878** | **0.047** |
| **COLQ** | **collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase** | **-0.208** | **4.408** | **0.047** |
| **GPD2** | **glycerol-3-phosphate dehydrogenase 2 (mitochondrial)** | **0.238** | **4.114** | **0.049** |
| **NR4A1** | **nuclear receptor subfamily 4, group A, member 1** | **0.246** | **4.000** | **0.050** |