

Supplemental Table 1. Significant differentially expressed genes per cluster. The number of differentially expressed genes per (super-)cluster with adjusted p-value <0.05, fold change >1.5 or <1/1.5, and mean expression >200

Cluster	C1 (N=15)	C2 (N=11)	C3 (N=18)	C4 (N=38)	C1-2 vs C3-4
Transcript	6024	782	266	1111	3935
unique genes	2404	409	107	593	1738
up-regulated	1044	34	29	509	533
down-regulated	1360	375	78	84	1205

Supplemental Table 2. Clinical variables per cluster. Clinical variables among clusters were compared using the chi-square or Kruskal-Wallis test. Variables with a *P* value <0.10 are included in Figure 4.

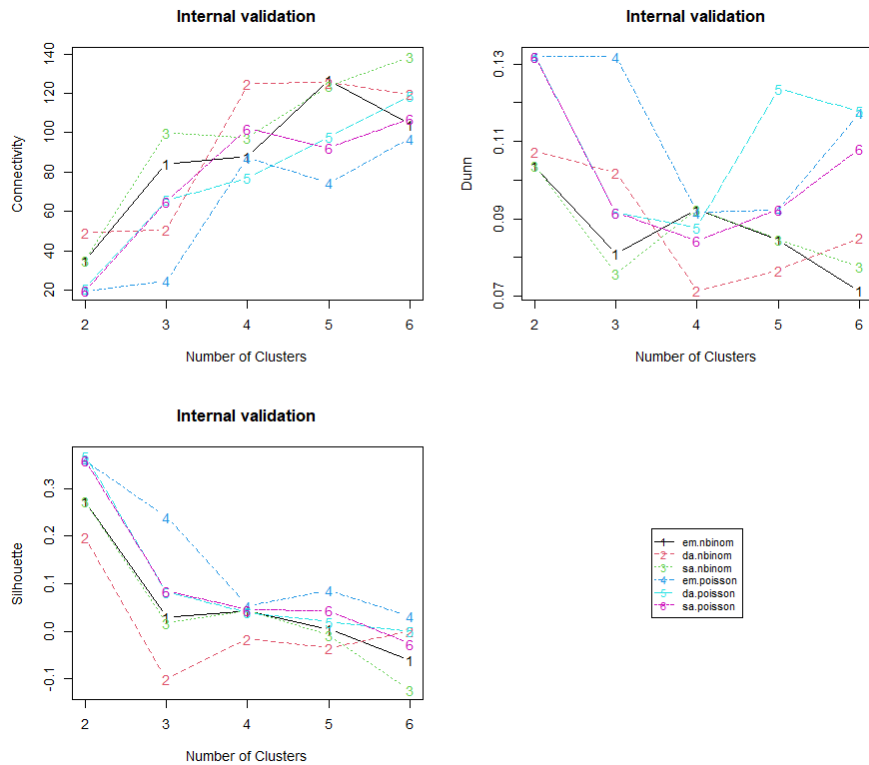
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	p-value
<i>Age, year</i>	56.13 ± 13.15	57.45 ± 11.67	58.50 ± 8.12	53.08 ± 12.78	0.41
<i>Time from diagnosis, month</i>	1.9 [1.2-5.3]	4.2 [1.5-5.4]	2 [0.5-7]	2.6 [1.1-7.3]	0.98
<i>Female, n (%)</i>	3 (20.00)	1 (9.09)	4 (22.22)	14 (36.84)	0.24
<i>Diabetes mellitus, n (%)</i>	0 (0.00)	1 (9.09)	2 (11.11)	6 (15.79)	0.43
<i>Heart rate, b.p.m.</i>	70.00 [65.50-83.00]	76.00 [66.00-81.50]	73.00 [66.00-78.75]	72.50 [62.25-82.00]	0.97
<i>Systolic blood pressure, mm Hg</i>	124.00 [113.50-139.00]	146.00 [129.50-155.00]	135.50 [130.00-139.50]	126.00 [120.00-140.00]	0.13
<i>Diastolic blood pressure, mm Hg</i>	78.20 ± 12.14	85.00 ± 14.83	83.00 ± 9.06	77.37 ± 11.66	0.14
<i>NYHA functional class ≥III, n (%)</i>	10 (66.67)	0 (0.00)	7 (38.89)	14 (36.84)	0.007
<i>Hypertension, n (%)</i>	5 (33.33)	3 (27.27)	8 (44.44)	14 (36.84)	0.81
<i>Body mass index, kg/m²</i>	26.73 ± 3.82	27.44 ± 4.32	26.55 ± 4.19	27.64 ± 5.08	0.34
<i>Disease modifiers, n (%)</i>					
<i>Genetic mutation</i>	12 (80.00)	5 (45.45)	9 (50.00)	15 (39.47)	0.067
<i>Genetic group</i>		\]]	0.003
<i>LMNA</i>	1 (6.67)	1 (9.09)	0 (0.00)	5 (13.16)	
<i>TTNtv</i>	10 (66.67)	4 (36.36)	5 (27.78)	6 (15.79)	
<i>Other genetic</i>	1 (6.67)	0 (0.00)	4 (22.22)	4 (10.53)	
<i>Gene-elusive</i>	3 (20.00)	6 (54.54)	9 (50.00)	23 (60.52)	
<i>Familial disease</i>	9 (60.00)	3 (27.27)	5 (27.78)	9 (23.68)	0.075
<i>Toxic trigger</i>	2 (13.33)	1 (9.09)	1 (5.56)	4 (10.53)	0.89
<i>Auto-immune disease</i>	1 (6.67)	1 (9.09)	1 (5.56)	2 (5.26)	0.97
<i>Cardiac inflammation</i>	4 (26.67)	2 (18.18)	4 (22.22)	13 (34.21)	0.67
<i>Significant viral load</i>	2 (13.33)	1 (9.09)	4 (22.22)	6 (15.79)	0.80
<i>Electrical trigger</i>	3 (20.00)	4 (36.36)	10 (55.56)	16 (42.11)	0.22
<i>Echocardiography</i>					
<i>LV ejection fraction, %</i>	24.00 [47.50-65.50]	26.00 [21.00-36.50]	30.00 [21.00-31.75]	26.00 [22.25-35.75]	0.91
<i>LV EDD index, mm/m²</i>	31.91 [30.83-33.51]	30.41 [28.38-33.50]	31.04 [27.80-34.25]	30.59 [27.77-33.47]	0.68
<i>LV ESD, mm</i>	53.20 ± 7.36	54.74 ± 8.17	52.06 ± 9.13	52.40 ± 9.73	0.79
<i>LA volume index, mL/m²</i>	55.08 [43.95-63.75]	52.00 [41.47-57.91]	41.19 [37.68-44.20]	44.01 [36.45-54.77]	0.10
<i>LV mass index, g/m²</i>	111.87 [99.90-120.89]	118.18 [102.71-133.56]	117.49 [102.91-125.23]	112.79 [94.69-133.38]	0.94
<i>E/e' ratio</i>	11.10 [9.75-13.41]	10.60 [8.32-11.50]	9.36 [8.47-10.89]	10.50 [9.01-12.23]	0.17
<i>Posterior WT, mm</i>	9.00 [8.00-9.50]	9.00 [8.50-10.50]	9.00 [8.22-9.95]	9.00 [8.00-10.00]	0.90
<i>Intraventricular ST, mm</i>	8.40 [8.00-9.00]	9.00 [8.00-10.03]	9.00 [8.00-9.22]	9.05 [8.02-10.00]	0.18
<i>Laboratory data</i>					
<i>NT-proBNP, pmol/L</i>	142.00 [26.65-389.00]	90.10 [25.57-185.00]	11.05 [7.22-61.02]	108.00 [17.57-335.00]	0.055

<i>Hs-Troponin T, ng/L</i>	16.00 [11.50-23.00]	12.75 [9.21-18.00]	13.18 [9.25-22.00]	13.21 [8.00-27.74]	0.84
<i>Creatinine, μmol/L</i>	102.00 [90.00-116.50]	99.00 [80.50-111.00]	104.00 [84.25-113.25]	99.50 [86.75-134.25]	0.96
<i>sIL-2R elevation, n (%)</i>	3 (20.00)	2 (18.18)	6 (33.33)	6 (15.79)	0.50
<i>Neopterin elevation, n (%)</i>	8 (53.33)	2 (18.18)	4 (22.22)	14 (36.84)	0.17
<i>Cardiac auto-antibodies, n (%)</i>	1 (6.67)	0 (0.00)	0 (0.00)	1 (2.63)	0.60
<i>C-reactive protein, mg/L</i>	2.00 [1.71-7.50]	3.00 [1.00-7.50]	2.13 [1.77-4.60]	3.00 [1.50-5.51]	0.98
<i>ASAT, U/L</i>	28.00 [25.50-37.50]	24.00 [20.00-27.50]	23.00 [20.25-36.25]	25.50 [20.72-29.50]	0.19
<i>ALAT, U/L</i>	32.00 [23.50-39.00]	29.00 [22.50-36.14]	30.50 [20.25-45.00]	24.80 [19.25-31.57]	0.54
<i>ECG/Holter, n (%)</i>					
<i>Atrial fibrillation</i>	7 (46.67)	3 (27.27)	5 (27.78)	11 (28.95)	0.59
<i>Non-sustained VT</i>	6 (40.00)	3 (27.27)	8 (44.44)	15 (39.47)	0.83
<i>Atrioventricular block</i>	3 (20.00)	0 (0.00)	2 (11.11)	5 (13.16)	0.49
<i>Left bundle branch block</i>	3 (20.00)	3 (27.27)	10 (55.56)	14 (36.84)	0.17
<i>Out of hospital cardiac arrest</i>	1 (6.67)	0 (0.00)	3 (16.67)	3 (7.89)	0.45
<i>Cardiovascular MR</i>					
<i>LV mass index, g/m²</i>	57.45 [48.83-71.80]	77.06 [66.00-87.70]	72.05 [56.17-84.54]	72.65 [60.46-88.06]	0.041
<i>LV EDV index, mL/m²</i>	142.39 [120.26-156.12]	117.77 [107.77-134.51]	122.83 [115.71-156.72]	122.88 [106.18-175.57]	0.79
<i>LV ESV index, mL/m²</i>	101.58 [79.75-133.04]	83.81 [66.28-100.80]	92.70 [72.63-111.95]	79.10 [55.52-132.63]	0.78
<i>LV stroke volume index, mL/m²</i>	34.26 [28.34-41.19]	41.26 [32.84-45.29]	42.12 [32.97-45.33]	41.05 [35.09-47.36]	0.09
<i>LV ejection fraction, %</i>	29.00 [20.08-35.50]	28.00 [23.00-42.08]	29.08 [24.46-39.25]	35.58 [24.00-44.50]	0.33
<i>Late gadolinium enhancement, n (%)</i>	11 (73.33)	3 (27.27)	8 (44.44)	19 (50.00)	0.12
<i>Endomyocardial biopsy</i>					
<i>Collagen volume fraction, %/area</i>	4.69 [2.47-12.80]	3.37 [2.25-9.18]	6.10 [3.68-8.61]	6.81 [4.82-11.21]	0.44
<i>Medication, n (%)</i>					
<i>Beta blocker</i>	15 (100.00)	11 (100.00)	17 (94.44)	36 (94.74)	0.69
<i>ACE inhibitor</i>	12 (80.00)	9 (81.81)	11 (61.11)	27 (71.05)	0.56
<i>Angiotensin II receptor blocker</i>	2 (13.33)	2 (18.18)	7 (38.89)	9 (23.68)	0.35
<i>Mineralocorticoid receptor antagonist</i>	12 (80.00)	8 (72.72)	13 (72.22)	23 (60.53)	0.53
<i>Diuretics</i>	11 (73.33)	7 (63.63)	11 (61.11)	24 (63.16)	0.89
<i>Angiotensin receptor/neprilysin inhibitor</i>	1 (6.67)	0 (0)	0 (0.00)	2 (5.26)	0.62
FOOTNOTE: Variables are presented using the mean ± standard deviation (SD) or median with 25th-75th percentiles [Q1-Q3] for continuous variables and count (percentage) for categorical variables.					

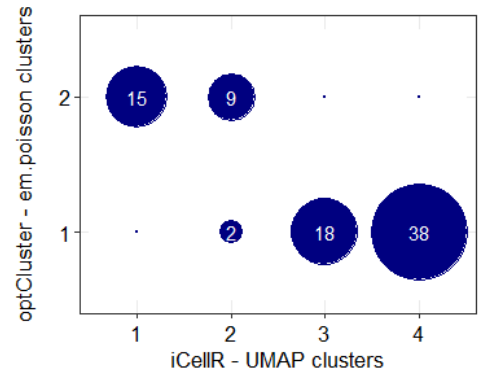
Supplemental Table 3. Comparison between the early-stage and end-stage DCM cohort. Seven clinical variables were measured in both cohorts of DCM patients.

	Early-stage DCM patients (MUMC)	End-stage DCM patients (MAGNet)	p-value
Age, year	55.4 ± 11.9	54.8 ± 7.1	0.31
Female, n (%)	22 (27)	30 (34)	0.33
Diabetes mellitus, n (%)	9 (11)	20 (23)	0.065
Hypertension, n (%)	30 (37)	39 (44)	0.35
Body mass index, kg/m ²	27.2 ± 4.5	26.5 ± 4.9	0.18
LV ejection fraction, %	26 [21-35]	15 [10-20]	<0.001
Atrial fibrillation	26 (32)	44 (50)	0.019
FOOTNOTE: Variables are presented using the mean ± standard deviation (SD) or median with 25th-75th percentiles [Q1-Q3] for continuous variables and count (percentage) for categorical variables.			

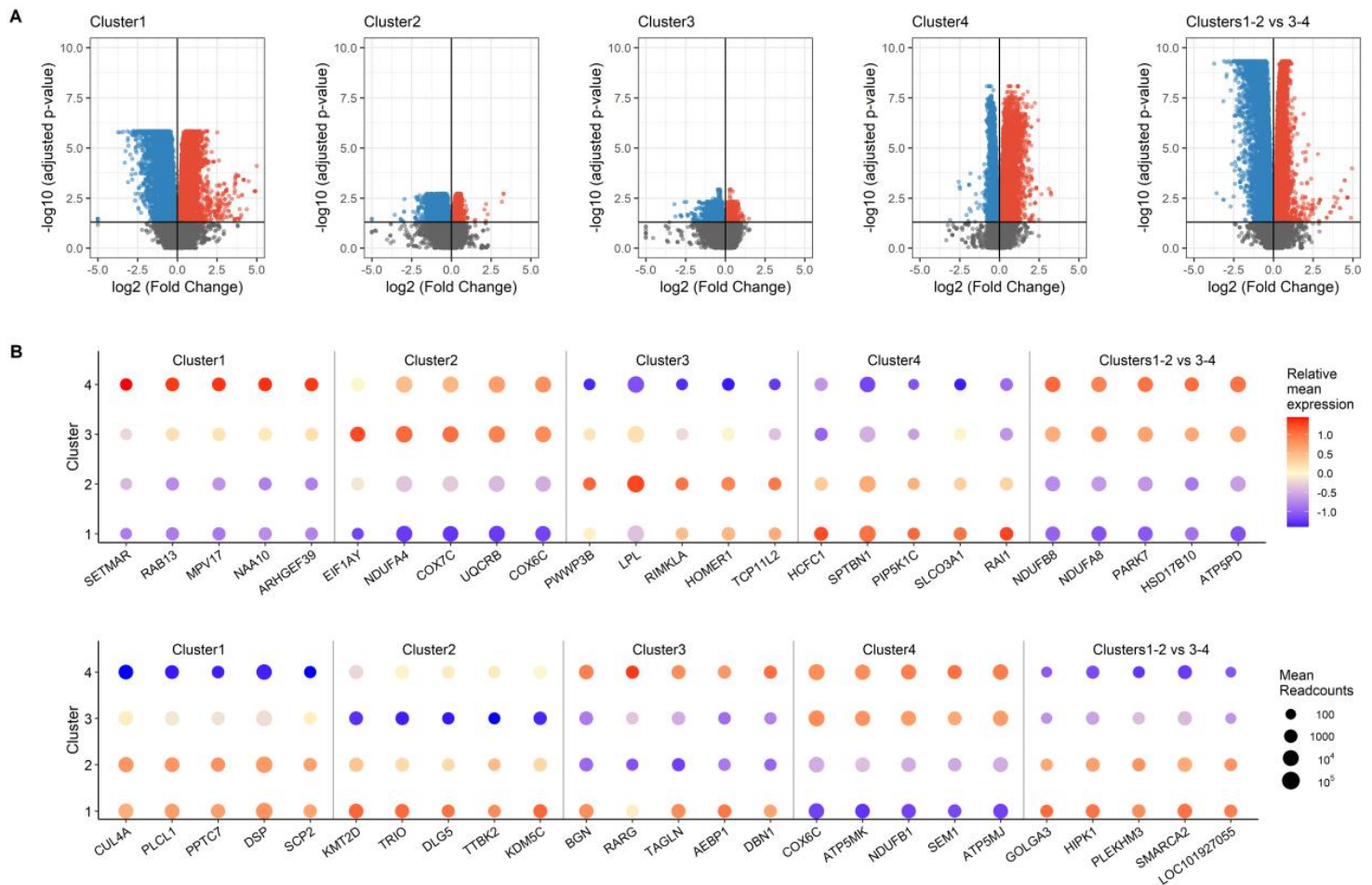
A.



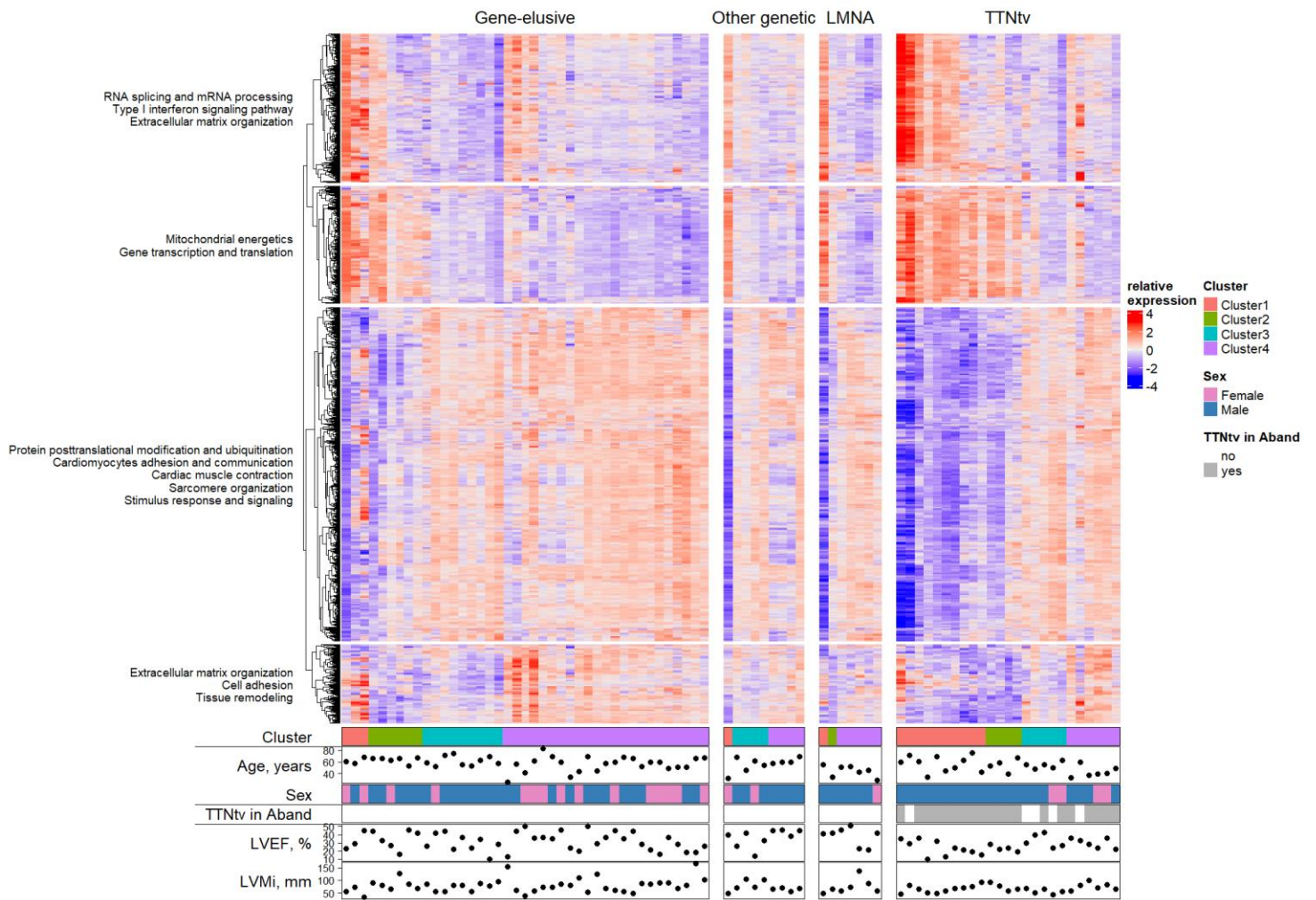
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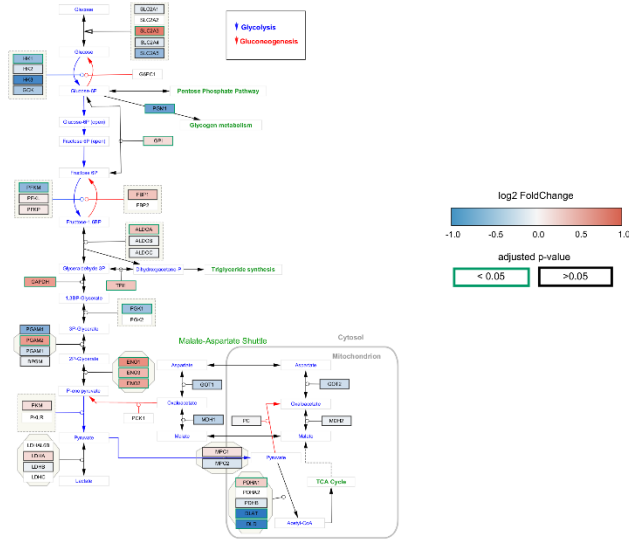
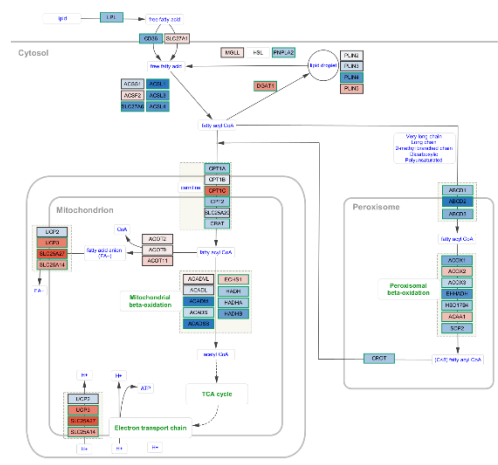
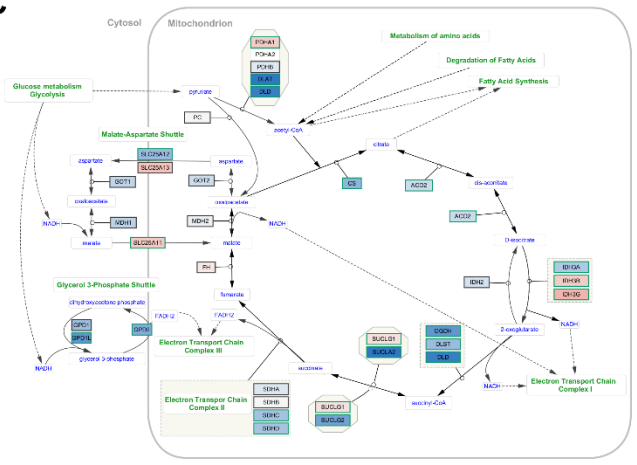
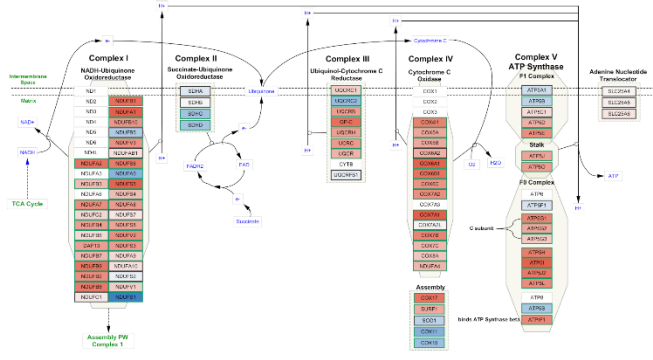
Supplemental Figure 1. RNA-seq Clustering with optCluster methods. (A) Internal validation metrics show poisson distribution with the expectation maximization algorithm (em.poisson, blue line marked --4--) for 2 clusters is the best among all 6 model-based clustering methods for RNA-seq data. **(B)** The partitioning of samples in clusters defined by dimensionality reduction combined with the graphic-based clustering based on UMAP (iCellR - UMAP) and clusters defined by best model-based clustering (optCluster - em.poisson). The super-cluster 1-2 and 3-4 are highly assembled with a Jaccard similarity coefficient =0.92.



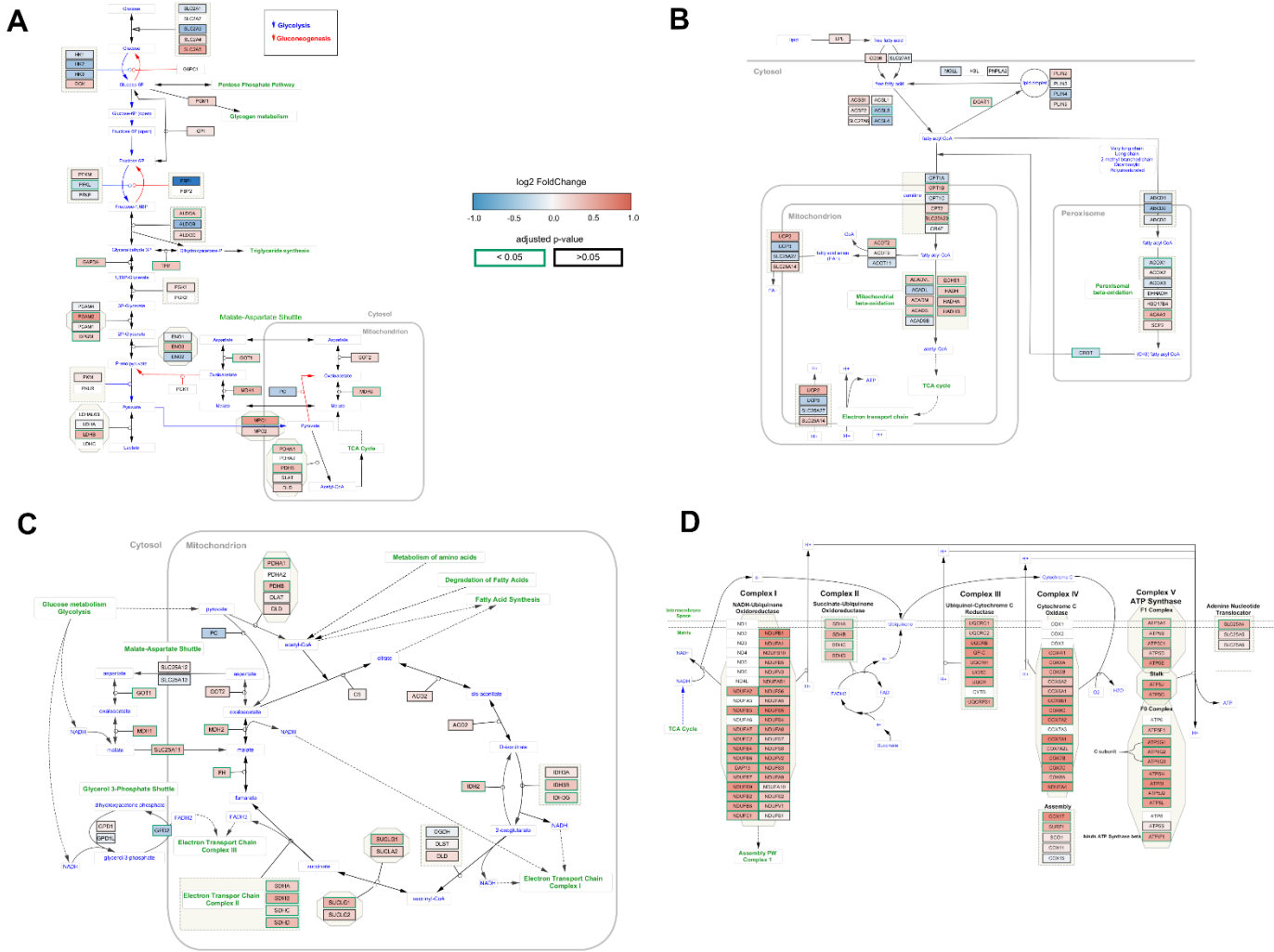
Supplemental Figure 2. Differential expression analysis. (A) Volcano plots of transcripts per cluster compared to the other three clusters, or inter-superclusters. Adjusted p-value < 0.05 is plotted as a cutoff to distinguish transcripts. **(B)** The top5 most significant differentially expressed up- and down-regulated cluster marker genes, among genes with adjusted p-value < 0.05 , $|FC| > 1.5$ and mean reads > 400 in the higher expressed part.



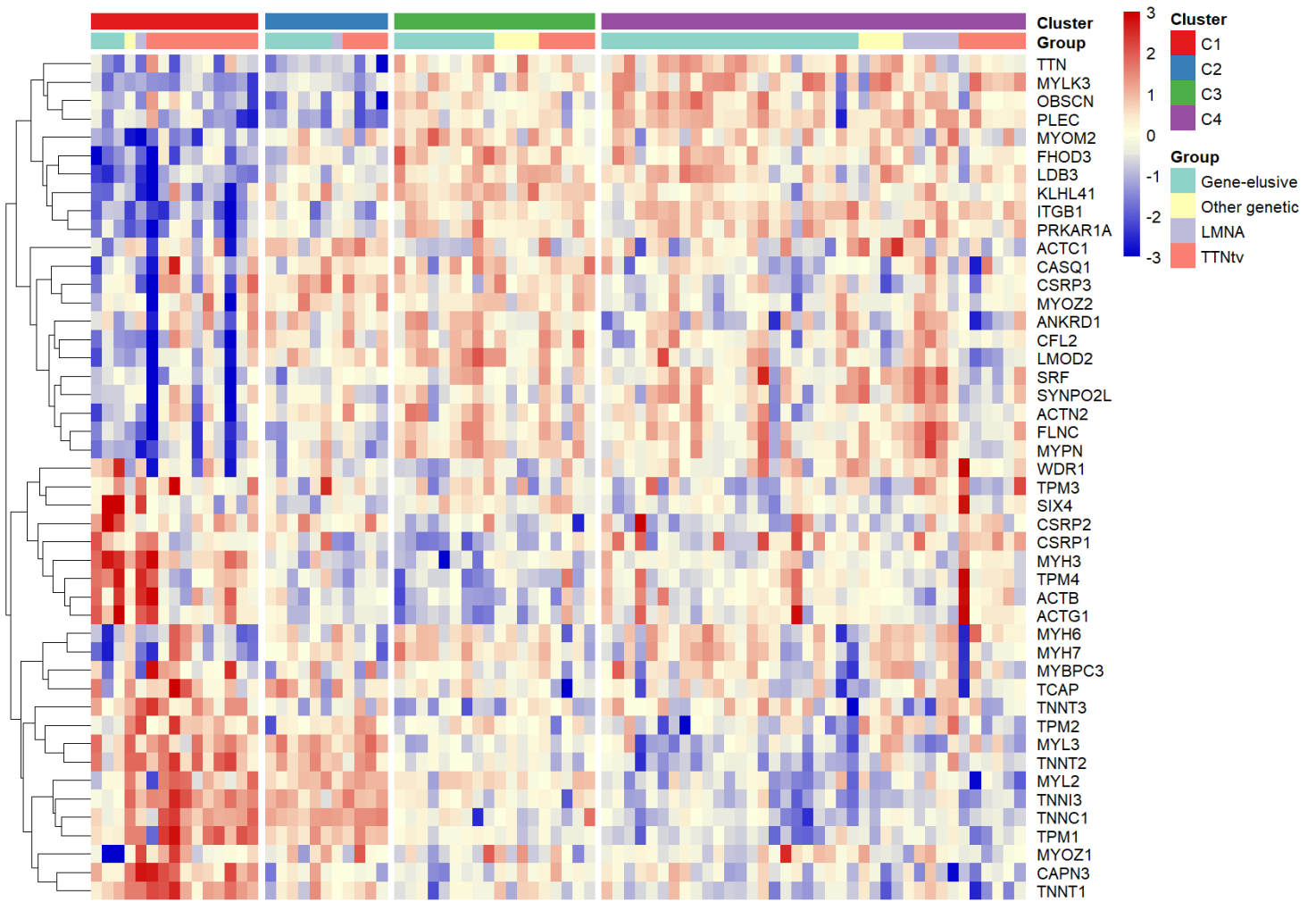
Supplemental Figure 3. Heatmap of significant differentially expressed genes of clustering stratified per genotype. Heatmap combining genetic, clinical and transcriptomic information.

A**B****C****D**

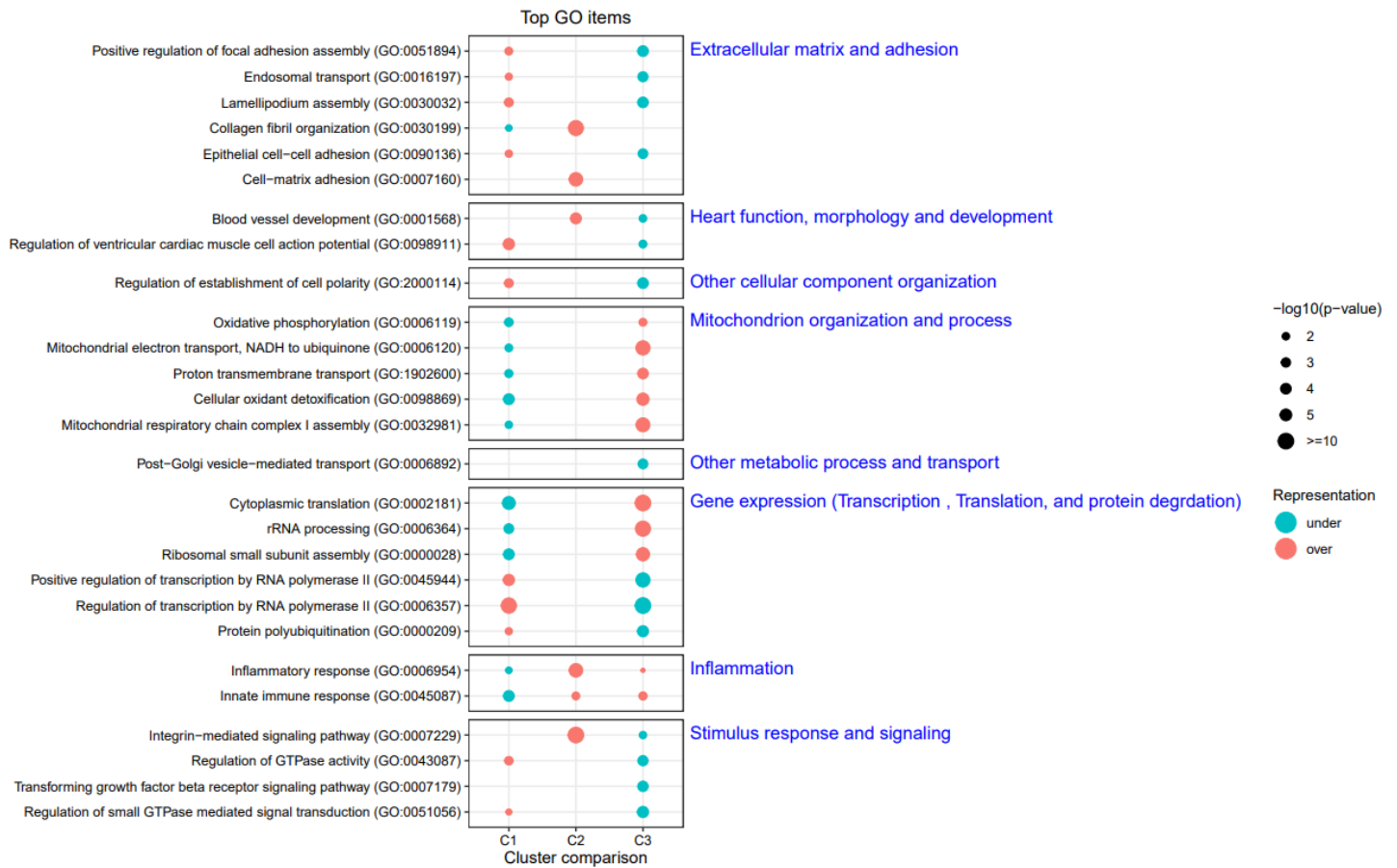
Supplemental Figure 4. Transcriptomic expression of the substrate metabolism pathways in patients from cluster 1. (A) glucose uptake and glycolysis pathway. **(B)** Fat uptake, storage and beta-oxidation. **(C)** Tricarboxylic cycle (TCA). **(D)** Electron transport chain, the oxidative phosphorylation system. Blue indicates a down-regulation of a gene, red indicates upregulation of a gene. The border of the Gene name, green: adjusted p-value <math>< 0.05</math>; black: adjusted p-value >math>> 0.05</math>; light grey: not expressed.



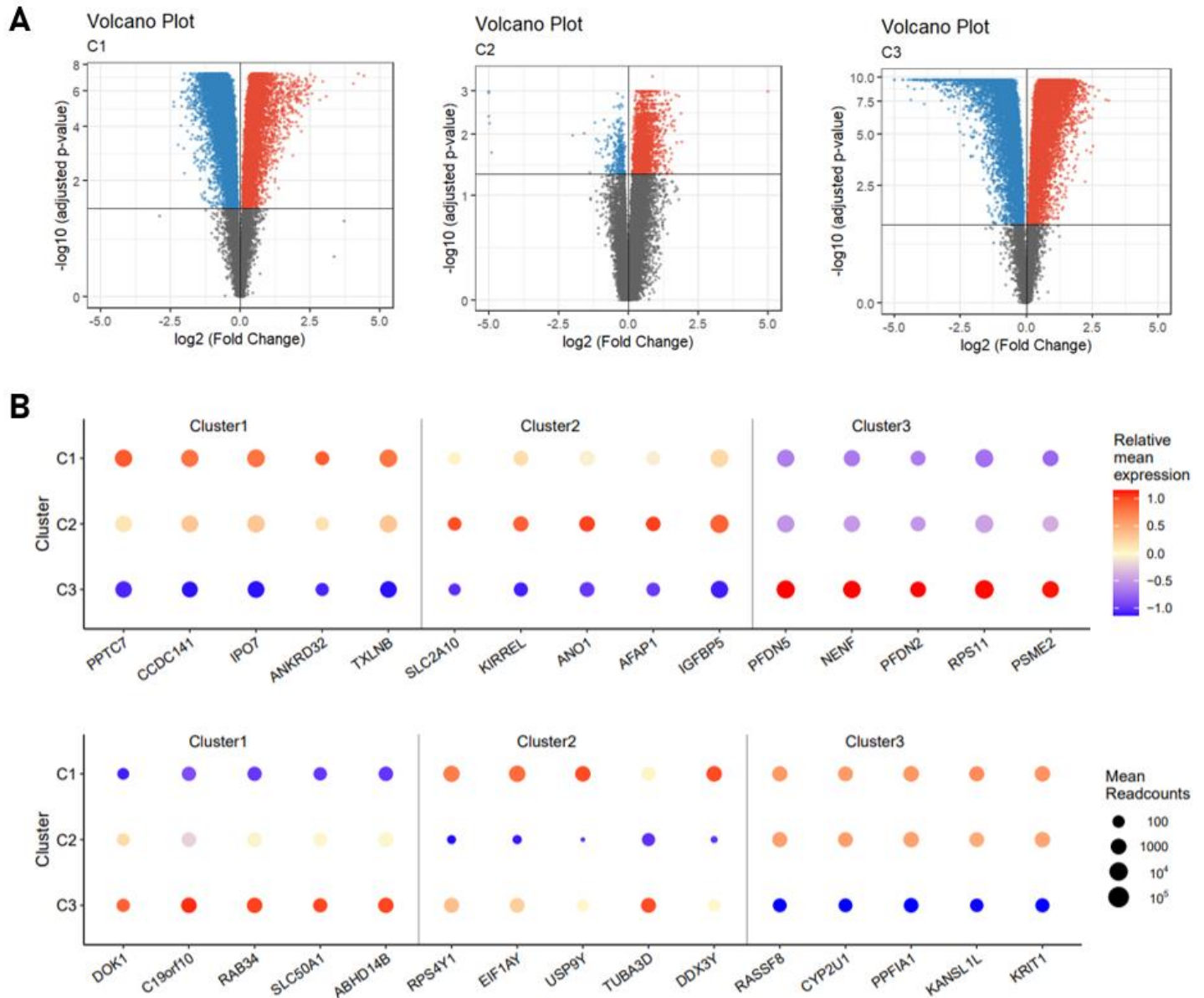
Supplemental Figure 5. Transcriptomic expression of the substrate metabolism pathways in patients from cluster 2. (A) glucose uptake and glycolysis pathway. (B) Fat uptake, storage and beta-oxidation. (C) Tricarboxylic cycle (TCA). (D) Electron transport chain, the oxidative phosphorylation system. Blue indicates a down-regulation of a gene, red indicates upregulation of a gene. The border of the Gene name, green: adjusted p-value <0.05; black: adjusted p-value >0.05; light grey: not expressed.



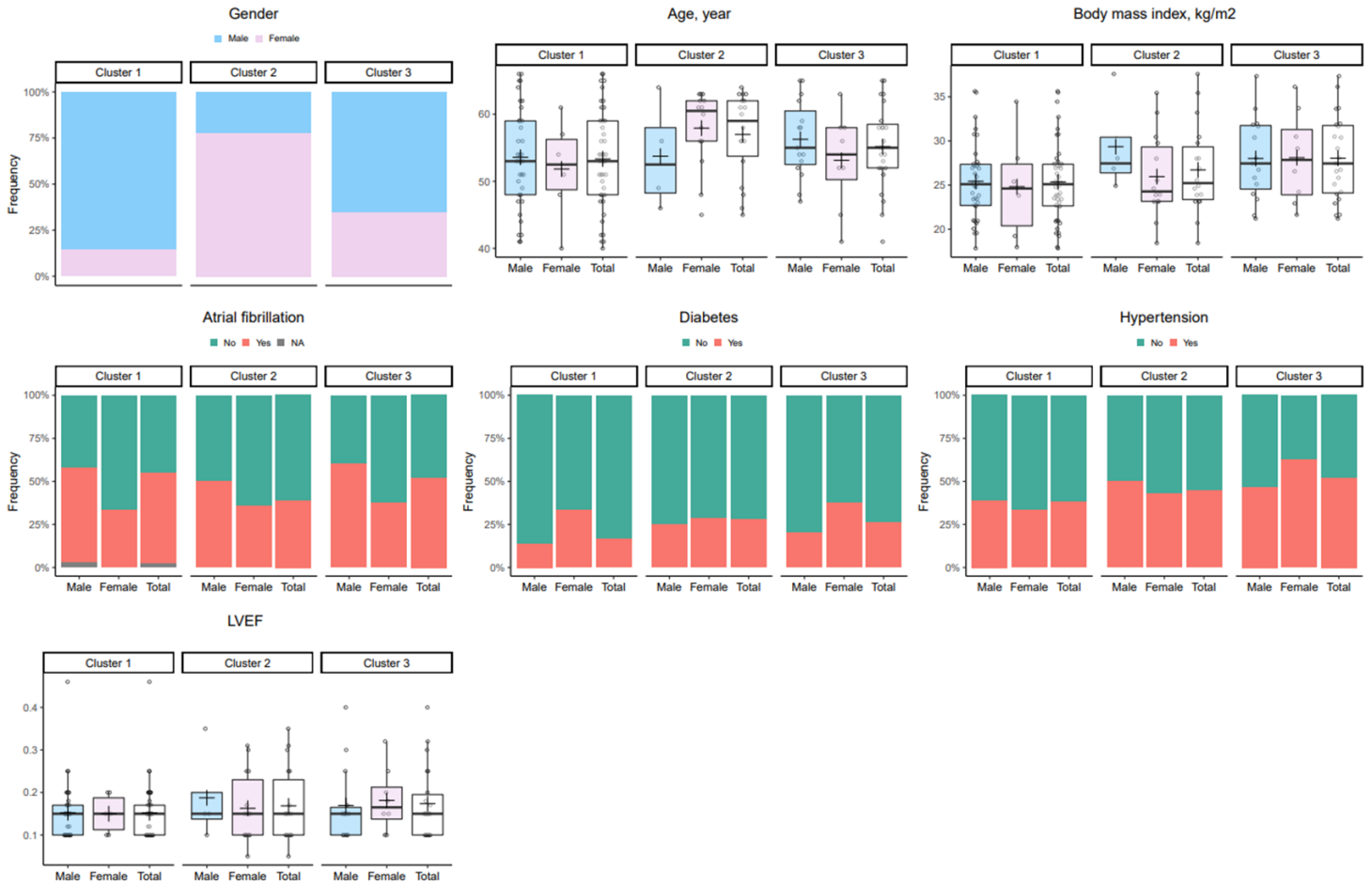
Supplemental Figure 6. Heatmap of the genes involved in sarcomere pathways divided per cluster and genotype.



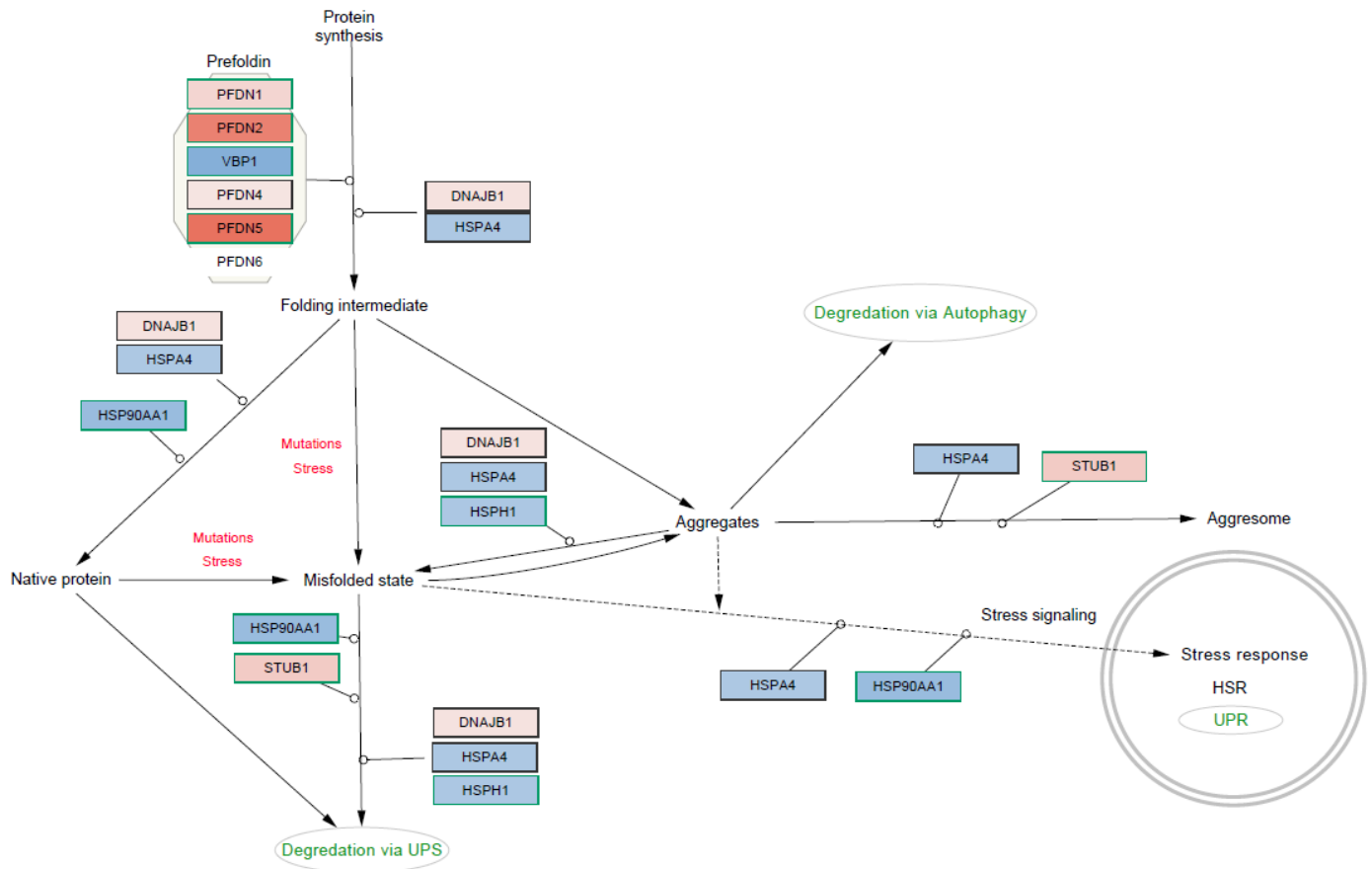
Supplemental Figure 7. Top GO items of each cluster identified in the end-stage DCM patients grouped to biological functions. Results are displayed per cluster in comparison to the other clusters.



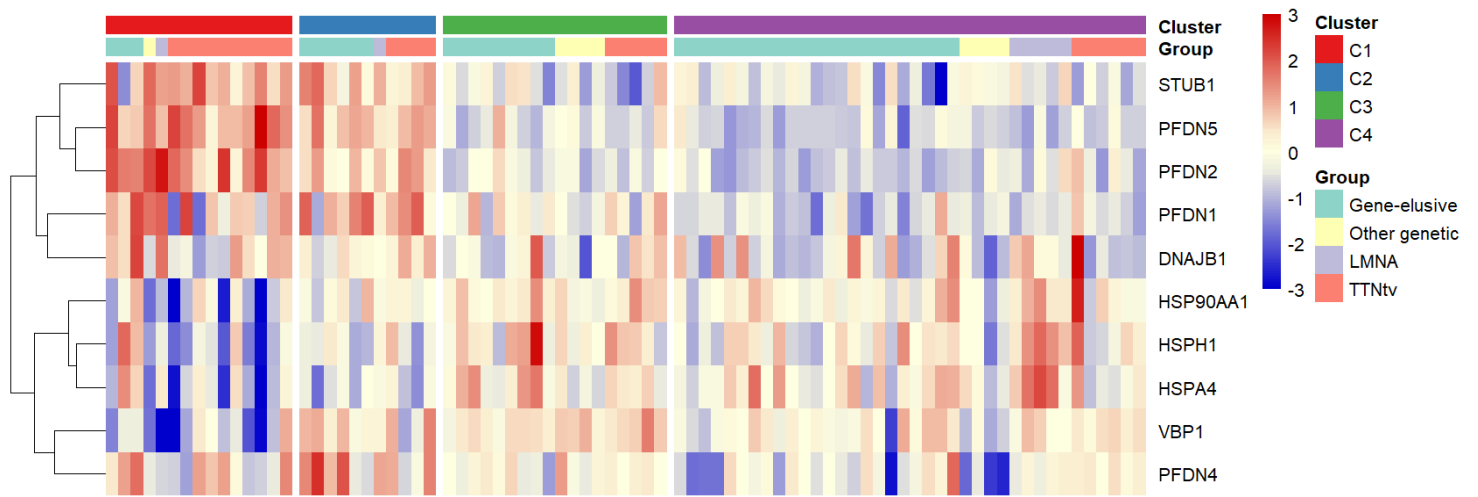
Supplemental Figure 8. Differential expression analysis. (A) Volcano plots of transcripts per cluster compared to the other two clusters in the end-stage DCM patients. Adjusted p-value <0.05 is plotted as a cutoff to distinguish transcripts. **(B)** The top5 most significant differentially expressed up- and down-regulated cluster marker genes, among genes with adjusted p-value < 0.05 , |FC| >1.5 and mean reads > 400 in the higher expressed part.



Supplemental Figure 9. Association between phenotype and clusters based on cardiac transcriptome in end-stage DCM patients. Details of all available variables by grouped bar plot, or violin plot + box plot for categorical variables and quantitative variables, respectively.



Supplemental Figure 10. Transcriptomic expression of the pathways involved in proteostasis in patients from cluster 1. Blue indicates a down-regulation of a gene, red indicates upregulation of a gene. The border of the Gene name, green: adjusted p-value <0.05; black: adjusted p-value >0.05; light grey: not expressed.



Supplemental Figure 11. Heatmap genes involved in proteostasis pathways divided per cluster and genotype.