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## Early blood transcriptomic signature predicts patients' outcome after out-of-hospital cardiac arrest

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1                    **Early blood transcriptomic signature predicts patients' outcome**  
2                    **after out-of-hospital cardiac arrest**

3  
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59

60 **Abstract**

61 **Background:** Early prognostication is a major challenge after out-of-hospital cardiac arrest  
62 (OHCA).

63 **Aims:** We hypothesized that a genome-wide analysis of blood gene expression could offer  
64 new prognostic tools and lines of research.

65 **Methods:** Sixty-nine patients were enrolled from an ancillary study of the clinical trial  
66 NCT00999583 that tested the effect of erythropoietin (EPO) after OHCA. Blood samples  
67 were collected in comatose survivors of OHCA at hospital admission and 1 and 3 days after  
68 resuscitation. Gene expression profiles were analyzed (Illumina HumanHT-12 V4 BeadChip;  
69 >34,000 genes). Patients were classified into two categories representing neurological  
70 favorable outcome (Cerebral Performance Category [CPC] = 1-2) vs unfavorable outcome  
71 (CPC>2) at Day 60 after OHCA. Differential and functional enrichment analyses were  
72 performed to compare transcriptomic profiles between these two categories.

73 **Results:** Among the 69 enrolled patients, 33 and 36 patients were treated or not by EPO,  
74 respectively. Among them, 42% had a favorable neurological outcome in both groups. EPO  
75 did not affect the transcriptomic response at Day-0 and 1 after OHCA. In contrast, 76  
76 transcripts differed at Day-0 between patients with unfavorable vs favorable neurological  
77 outcome. This signature persisted at Day-1 after OHCA. Functional enrichment analysis  
78 revealed a down-regulation of adaptive immunity with concomitant up-regulation of innate  
79 immunity and inflammation in patients with unfavorable vs favorable neurological outcome.  
80 The transcription of many genes of the HLA family was decreased in patients with  
81 unfavorable vs favorable neurological outcome. Concomitantly, neutrophil activation and  
82 inflammation were observed. Up-stream regulators analysis showed the implication of  
83 numerous factors involved in cell cycle and damages. A logistic regression including a set of  
84 genes allowed a reliable prediction of the clinical outcomes (Specificity=88%; Hit Rate=83%).

85 **Conclusions:** A transcriptomic signature involving a counterbalance between adaptive and  
86 innate immune responses is able to predict neurological outcome very early after hospital  
87 admission after OHCA. This deserves confirmation in a larger population.

88 **Keywords:** Cardiac arrest; Cardiopulmonary resuscitation; Prognostication; Transcriptomics;  
89 Innate immunity; Inflammation.

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91

92 **Introduction**

93 Out-of-hospital cardiac arrest (OHCA) is a major cause of mortality in western countries.  
94 After resuscitation and return of spontaneous circulation, neurological, cardiovascular, and  
95 multi-organ failures contribute to a “post-cardiac arrest syndrome” that compromises the  
96 outcome. Regarding several aspects, this syndrome is considered as a “sepsis-like  
97 response”<sup>1</sup>.

98 Using current clinical and biological methods, it is extremely difficult to establish an early  
99 and accurate prognosis in this situation. In resuscitated patients with an uncertain outcome,  
100 the most recent guidelines specify that clinicians should consider prolonged observation<sup>2,3</sup>.  
101 Use of molecular biomarkers is now also proposed to improve prognostication, using micro  
102 RNA analyses<sup>4-7</sup>. For instance, higher levels of miR-124-3p were significantly associated  
103 with lower survival.<sup>8</sup> However, the use of a single biomarker does not provide a sufficient  
104 predictive value and still needs to be combined with other clinical and biological markers<sup>9</sup>.

105 Our goal was thus to identify a specific signature using a systemic screening evaluation of  
106 the whole transcriptome, allowing a deep evaluation of the sepsis-like, inflammatory and  
107 blood signaling responses very early after OHCA, *i.e.*, as soon as possible after hospital  
108 admission. An exploratory study in 22 patients previously demonstrated that transcriptome  
109 analysis could be relevant after OHCA but this was only evaluated after 2 days following  
110 resuscitation<sup>10</sup>. Here, we had the opportunity to address the transcriptome since hospital  
111 admission in an ancillary study from the large-scale EPO-ACR 02 trial, that failed to  
112 demonstrate a clinical effect of erythropoietin (EPO) on survival and neurological outcomes  
113 after out-of-hospital cardiac arrest<sup>11</sup>. We compared transcriptomic profiles according to the  
114 Cerebral Performance Category (CPC) of the patients at the end of the study. Differential  
115 analysis and functional enrichment analysis were performed between patients with  
116 unfavorable versus favorable outcome, with the purpose to determine the differentially  
117 expressed genes, over-represented canonical pathways, and upstream regulators after  
118 cardiac arrest in patients. We then identified a gene signature that could predict the

119 neurological outcome from whole blood very early after OHCA. However, this study should  
120 be considered as hypothesis generating as we included a limited number of patients.

121

## 122 **Methods**

### 123 *General design of the study*

124 The present study was an ancillary study from the EPO-ACR-02 trial (High Dose of  
125 Erythropoietin Analogue After Cardiac Arrest), which was a multicenter, phase 3,  
126 randomized, single-blind, controlled trial that evaluated the safety and efficacy of a high dose  
127 of EPO in patients resuscitated from a cardiac arrest. The designs and results of this  
128 principal study were recently published <sup>11</sup>. Briefly, in the intervention group, patients received  
129 the first dose of EPO (40,000 units i.v.) as soon as possible after resuscitation. It was  
130 followed by 4 injections every 12 h during the first 48 h. In the Control group, patients  
131 received standard care without any EPO treatment. Neurological performance was assessed  
132 at Day-60, according to the Cerebral Performance Category (CPC) scale. <sup>11</sup> We considered  
133 favorable and unfavorable neurological outcomes as CPC values  $\leq 2$  and  $> 2$ , respectively.

### 134 *Gene expression profiling*

135 Blood samples were drawn in patients as soon as possible after hospital admission (Day-  
136 0) as well as 24 hours (Day-1) and 72 hours (Day-3) after cardiac arrest. Samples were  
137 stored at - 80°C before transcriptomic analyses using Illumina Human HT-12V4 BeadChips  
138 that targets 47,323 probes corresponding to 34,694 genes, as described in supplemental  
139 material.

### 140 *Statistical analyses*

141 Epidemiological and clinical variables were expressed as mean $\pm$ SD or numbers and  
142 percentages for continuous and categorical parameters, respectively. Continuous  
143 parameters were compared between patients treated by EPO vs Control and between  
144 patients with CPC1-2 vs CPC $>$ 2 at Day 60 using a two-way analysis of variance. Categorical  
145 variables were compared using a Chi-square test. Significant differences were determined by  
146 p-values  $< 0.05$ .

147 The differential analysis of the transcriptome consisted in identifying genes differentially  
148 expressed between patients treated or not by EPO and/or with CPC1-2 vs CPC $>$ 2 at Day 60.

149 We also compared transcriptome within different categories of patients according to  
150 epidemiological or resuscitation parameters using t-test analyses. Genes with a fold-  
151 change>1.5 and corrected p-value<0.05 were considered as differentially expressed as  
152 compared to control conditions. Such a high fold-change threshold provides very robust  
153 results, despite the rather low number of patients. Functional enrichment analysis was then  
154 made using the QIAGEN Ingenuity® Pathways Analysis™ software  
155 ([www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity)) using a fold-change>1.3 and a p-value<10<sup>-3</sup>. Indeed, functional  
156 enrichment generates hypotheses using wider gene profiling, requiring the use of lower fold-  
157 change. Analyses were performed using R (<http://www.r-project.org>) and Bioconductor  
158 (<http://www.bioconductor.org>). The logistic regression analyses were also performed using R.

#### 159 *Predictive models and logistic regression*

160 A signature of genes that were commonly up- or down-regulated in patients with favorable  
161 vs unfavorable outcome was derived. The ability of this gene signature to predict the  
162 neurological outcome was evaluated by logistic regression. Several regressions were tested  
163 in order to determine the best combination regarding specificity, hit rate, and area under the  
164 curve (AUC) of the receiving operating curves (ROC).

165 To compare the prognostic value of the “transcriptomics-based” model to usual clinical  
166 parameters, we performed a regression analysis with clinical end-points, including age, low-  
167 flow time, no-flow time, initial rhythm during cardiac arrest and epinephrine dosages.

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171 **Results**

172 *Patients and samples characteristics*

173 As shown in **Table 1**, we enrolled 69 patients, *i.e.*, 33 and 36 patients treated by EPO or  
174 not, respectively. No difference was observed regarding epidemiological characteristics and  
175 resuscitation management between these two groups, except for a higher rate of post-  
176 cardiac arrest coronary intervention in patients treated by EPO vs Control. Nevertheless, the  
177 neurological outcome was not different since 42% of the patients achieved CPC1-2 at Day  
178 60 in both groups (*i.e.*, 14/33 and 15/36 patients, respectively). The majority of patients with  
179 CPC1-2 belonged to the CPC1 category and only 2 patients belonged to the CPC2 category  
180 (*i.e.*, 1 in each EPO and control group). Conversely, a large majority of patients with poor  
181 neurological (CPC>2) outcome belonged to the CPC5 category (death) and only 2 patients  
182 belonged to the CPC3 category (*i.e.*, 1 in each EPO and control group). No patient was in the  
183 CPC4 category. As compared to patients with CPC>2, patients with favorable outcome  
184 (CPC1-2), were younger, with a shorter low-flow time and less epinephrine used during  
185 resuscitation.

186 Among these 69 patients, 181 blood samples were collected from the 207 expected  
187 samples (3 samples planned for each patient). Missing samples were related to early death  
188 (n=14) or lack of sampling in surviving patients (n=12). Among the 181 available samples, 7  
189 samples were excluded for technical reasons after quality control. We ultimately analyzed  
190 transcriptomic data from 174 blood samples (64, 62 and 52 at Day-0, 1 and 3, respectively).  
191 Sample repartition among the different conditions and time of collection after cardiac arrest  
192 are shown in **Supplemental Table 1**.

193 *Effect of EPO on the transcriptome after cardiac arrest*

194 As shown in **Figure 1A**, only 1 and 4 genes were differentially expressed at Day-0 and  
195 Day-1 between patients treated with EPO or not, respectively. This number significantly  
196 increased at Day-3 (141 genes), with a homogeneous modification of the transcriptome  
197 among most patients (**Figure 1B**). As expected and illustrated by **Figure 1C**, functional

198 enrichment revealed an activation of heme biosynthesis pathways at Day-3 in patients  
199 treated by EPO.

200 *Effect of epidemiological and clinical parameters on the transcriptome after cardiac arrest*

201 As illustrated in **Figure 2A**, we then assessed the effect of epidemiological and  
202 resuscitation parameters on gene transcriptome, as compared to EPO proper effect. Gene  
203 expressions mostly differ regarding gender (*e.g.*, 48 genes at Day-0), low-flow duration (142  
204 genes at Day-0), ultimate CPC category (CPC>2 vs CPC1-2; 76 genes at Day-0) and  
205 epinephrine dose (>1mg vs ≤1 mg; 235 genes at Day-0). Among the modified transcripts, no  
206 gene was commonly modified between age category or gender and CPC (**Figure 2B**).  
207 Similarly, no gene was commonly modified between CPC and EPO categories. This  
208 demonstrates that the transcriptomic signature marking CPC was not biased by age, gender  
209 or EPO treatment. Conversely, many genes were commonly modified among CPC  
210 categories and low-flow duration or epinephrine dose. This was expected as the latter  
211 parameters are well-known predictors of neurological outcome. For instance, 54 and 12  
212 genes were commonly modified by CPC categories, low-flow duration or epinephrine dose at  
213 Day-0 and Day-1 after cardiac arrest. **Supplemental Figure 1** lists these common genes.

214 Low-flow duration and epinephrine dose modified many other genes beyond the CPC  
215 signature, demonstrating a transcriptomic response non-specific of the ultimate outcome. For  
216 instance, only 43 and 26% of the genes modified by low-flow duration and epinephrine dose  
217 at Day-0 were common with those modified in CPC>2 vs CPC1-2 patients, respectively.  
218 Therefore, further analyses were focused on the proper transcriptomic signature of CPC  
219 category at Day-0 and Day-1, which was the most relevant for a prognostication purpose. We  
220 also focused on Day-0 and Day-1 as 15 samples were missing at Day-3, due to the death of  
221 patients or technical considerations, which probably flawed the results at this time point and  
222 explains the decreased number of differentially expressed genes.

223

## 224 *Transcriptomic differences in patients with favourable vs unfavourable outcome*

225 As illustrated in **Figure 3A** (Day-0) and **3B** (Day-1), we then examined the top up- and  
226 down-differentially expressed genes in patients with CPC>2 vs CPC1-2. Among them, we  
227 found a majority of genes involved in inflammation, innate immunity and cell migration. We  
228 also found several genes involved in cell cycle regulation and heat shock proteins.

229 In order to assess the most relevant and time-independent genes differing in patients  
230 with CPC>2 vs CPC1-2, we determined the common ones at Day-0 and Day-1 (**Figure 3C**).  
231 As illustrated in **Figure 3D**, we identified 17 genes with, again, a majority is known to be at  
232 least in part linked to immunity and inflammatory response. We also found genes related to  
233 cell cycle or response to DNA damages.

## 234 *Functional enrichment analyses of transcriptomic data*

235 We pursued our analysis by a functional enrichment of the gene list generated at Day-0  
236 and Day-1. **Figure 4A** illustrates the canonical pathways found to be over-represented  
237 between patients with unfavorable vs favorable outcome. Briefly, antigen presentation  
238 pathways, OX40, or Cdc42 signaling were significantly down-regulated in patients with  
239 CPC>2 compared to CPC1-2. In contrast, interleukin-6 signaling and toll-like receptor (TLR)  
240 signaling were significantly up-regulated and enriched in patients with CPC>2 vs CPC1-2.  
241 Communication between innate and adaptive immune cells and crosstalk between dendritic  
242 cells and natural killer cells pathways followed a biphasic pattern of alterations with a  
243 significant up-regulation at Day-0 and subsequent a down-regulation at Day-1 in patients with  
244 CPC>2 vs CPC1-2.

245 As illustrated in **Figure 4B**, upstream regulators analysis further predicted the implication  
246 of many factors that could be linked to inflammation (e.g., CCAAT/enhancer-binding protein  
247 alpha [CEBPA], signal transducer and activator of transcription 3 [STAT3], high mobility  
248 group box 1 [HMGB1]) or cell cycle regulation and nuclear damage signaling.

249 In order to further explore the putative pathways linked to the ultimate outcome, we  
250 conducted a further analysis using only the genes that were commonly modified at Day-0

251 and Day-1 in patients with CPC>2 vs CPC1-2. This includes 94 genes using a 1.3 fold  
252 change (vs 17 genes in the previous analysis illustrated by Figure 3 with a 1.5 fold change).  
253 As illustrated in **Figure 4C**, the canonical pathways linked to the ultimate patient's outcome  
254 were then all related to adaptive immune responses including antigen presentation, OX40  
255 signaling pathways, Th1 / Th2 activation pathway or Cdc42 signaling.

#### 256 *Prognostication value of the transcriptomic signature*

257 Using the wide list of 94 genes commonly modified at Day-0 and Day-1, we determined  
258 whether a logistic regression using a combination of genes could better predict the ultimate  
259 outcome at Day 60 (CPC>2 vs CPC1-2). In order to be more discriminant for a  
260 prognostication purpose, this model was tested on the entire bank of blood samples, *i.e.*, in  
261 blood samples obtained at Day-0, Day-1 but also Day-3. The general linear model with the  
262 best prognostication value was obtained with a combination of 38 genes (**Figure 5A**). Using  
263 this combination of genes, we generated a general linear model providing a high specificity of  
264 88%, a hit rate of 83%, and a ROC AUC of 0.94 (**Figure 5B**).

265 As a reference approach, we then tested a logistic regression using conventional clinical  
266 and epidemiological parameters, *i.e.*, age, epinephrine dose, low-flow time, no-flow time and  
267 initial rhythm before resumption of spontaneous circulation (**Table 1**). As illustrated in **Figure**  
268 **5C**, the combination of these clinical and epidemiological parameters predicts outcome with  
269 a specificity, hit rate, and AUC of ROC lower than the transcriptomics model.

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274 **Discussion**

275 Using a screening analysis of the whole transcriptome, we deciphered the systemic  
276 response following OHCA, generating a new hypothesis for patient prognostication after out-  
277 of-hospital cardiac arrest. We used a population treated or not by EPO, which did not affect  
278 outcome. Furthermore EPO did not modify the early transcriptomic response to OHCA but  
279 only at Day-3 after cardiac arrest. Accordingly, we focused our analyses on the  
280 transcriptomic response of the patients at Day-0 and Day-1. Since the first blood sample  
281 drawn after hospital admission, more than 300 genes were differentially expressed between  
282 patients with unfavorable (CPC>2) vs favorable neurological outcome (CPC≤2), i.e., only few  
283 hours after resuscitation. Using a combination of genes, we were able to predict the outcome  
284 with a good specificity, hit rate and AUC of the ROC curves. This is one the first biomarker  
285 approach providing promising results for prognostication since hospital admission. However,  
286 results should be interpreted with caution according to the small number of patients.

287 To our knowledge, this is the first wide genome-wide analysis of the very early and global  
288 response following resuscitation in OHCA patients. A transcriptomic signature was observed  
289 in patients with unfavorable outcome involving both adaptative immunity, through antigen  
290 presentation, and innate immunity and inflammation transcripts. Even if several clinical and  
291 non-clinical prognostic markers are already usable, there is still a need for very early  
292 prognostication tools. Here we observed that the transcriptomic signature may provide  
293 information on hospital admission.

294 In patients with an unfavorable outcome, a complex balance occurred in the immune  
295 response through up-regulation of innate immunity and down-regulation of antigen  
296 presentation processes. The latter event was striking with a significant alteration at both Day-  
297 0 and Day-1 in patients with CPC>2 vs CPC≤2. This was supported by the functional  
298 enrichment analysis as well as many top up- and top down- transcripts related to the HLA  
299 family (HLA-DMB, HLA-DRB3, HLA-DRB4, HLA-DRB6). This could suggest a deficiency of  
300 antigen-presentation in patients with unfavorable outcome.

301 Beyond the down-regulation of adaptative immunity in patients with an unfavorable  
302 outcome, we observed an activation of innate immunity and inflammation in this category of  
303 patients. For example, neutrophil activation is shown by metalloproteinase-8 (MMP-8) and  
304 CD177 upregulation in patients with the unfavorable vs favorable outcome, suggesting a  
305 deleterious effect of neutrophil activation. Proteinase 3 (PRTN3) and MMP8 are well-known  
306 protease genes markers of sepsis, showing a clear engagement of neutrophils response <sup>12</sup>.  
307 We also observed a significant activation of several interleukins and TLRs, as well as an up-  
308 regulation of CD83, Regulator of G-protein signaling 1 (RGS1) and Proteinase 3 (PRTN3),  
309 which play a key role in inflammation in patients with poor outcome. Among these genes,  
310 regulator of G-protein signaling 1 (RGS1) has an important role in the regulation of B and T  
311 lymphocytes and macrophages trafficking and functions. RGS1 has been involved in  
312 macrophage-mediated vascular inflammation in atherosclerotic plaques <sup>13</sup>. Genome-wide  
313 association studies (GWAS) also identified a link between polymorphic variants of RGS1 and  
314 chronic inflammatory diseases in humans, including celiac disease, multiple sclerosis, and  
315 type I diabetes <sup>14-16</sup>.

316 Importantly, the apparent contradiction between the down-regulation of antigen  
317 presentation pathways and the activation of innate immunity could be explained by the  
318 biphasic pattern of evolution of the crosstalk between dendritic cells and natural killer cells  
319 and the communication between innate and adaptive immune cells. This supports that a  
320 dysfunctional immune response occurs in patients with an unfavorable outcome, with an  
321 inappropriate balance between innate and adaptative responses through antigen  
322 presentation. In other words, a strong innate immunity and inflammatory response may occur  
323 in these patients, with no ability to activate a subsequent and beneficial adaptative response.  
324 Such an hypothesis could represent a paradigm shift as compared to the concept of sepsis-  
325 like response after OHCA, which should be revisited in the light of a more specific activation  
326 of some immune pathways. Obviously, these results are only hypothesis-generating.

327 The activation of innate immunity and inflammation is also consistent with up-stream  
328 regulators analysis which identified a role of the HMGB1 pathway. This is a well established

329 damage-associated molecular pattern (DAMPs) that could activate immunity and have  
330 multiple functions in the regulation of immunity and inflammation. This is in agreement with  
331 the danger-model of immunity which was deciphered during stroke <sup>17</sup> or sepsis <sup>18</sup>, as well as  
332 the differential expression of several genes related to DNA damages. Beyond the immunity  
333 alteration provoked by danger signaling, the up-stream regulators analysis also evidenced  
334 the implication of many other factors. For instance, carcinoembryonic antigen-related cell  
335 adhesion molecule 1 (CEBPA) is known to increase macrophage inflammatory protein (MIP)-  
336 2 production from mast cells upon bacterial stimulation <sup>19</sup>. Likewise, Forkhead box O1  
337 (FOXO1) affects several critical aspects of neutrophil functions including mobilization of  
338 neutrophils from the bone marrow to the vasculature, recruitment of neutrophils and  
339 clearance of bacteria. Furthermore, bacteria-induced nuclear localization of FOXO1 is  
340 dependent upon TLR-2 and/or TLR4 <sup>20</sup>, that could be related to the sepsis-like syndrome  
341 observed in OHCA. The involvement of estrogen receptor 1 (ESR1), as a direct regulator of  
342 the innate and adaptive immune system was also observed and could participate in gender  
343 differences <sup>21</sup>. These findings allow generating hypothesis regarding the putative damage  
344 (DNA damage, apoptosis) or repairing process (cell lineage, maturation and hematopoiesis)  
345 occurring after cardiac arrest.

346 This study presents several limitations. For instance, we might further improve the  
347 specificity of the transcriptomic signature through a deeper analysis taking into account the  
348 cause of death, *e.g.*, hemodynamic or cerebral failure. A replication of the results will also be  
349 required prior to a wide implementation of the transcriptomic signature as prognostic tool in  
350 the clinical arena. We should also keep in mind that the results were only obtained using a  
351 limited number of patients.

352

353

354 **Conclusion**

355 In conclusion, this wide analysis of the transcriptome demonstrates a counterbalance  
356 between adaptive and innate immune response after cardiac arrest. A clear transcriptomic  
357 signature was observed and allows to predict favorable outcome very early after cardiac  
358 arrest since hospital admission. This generates new hypotheses and provides promising  
359 perspectives for the prognostication after OHCA, and more widely in cardiovascular  
360 diseases. A combinatory approach including transcriptomic signatures could be the key for  
361 the future management of OHCA <sup>22-25</sup>. In the future general practice, one would speculate  
362 that dedicated micro-arrays or next generation sequencing (NGS) approaches could provide  
363 rapid prognostic tools at admission, as also expected for trauma brain injury or stroke <sup>26-27</sup>.  
364 However, even if providing important information on outcome, further prospective validation  
365 studies on larger samples are required that may improve the predictive performance of the  
366 transcriptomic model and its use for individual prognostication.

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368

369 **Conflict of interest**

370 The authors do not disclose conflict of interest.

371

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450 **Legends to figures**

451

452 **Figure 1: Effect of erythropoietin (EPO) on the transcriptome after cardiac arrest.**

453 (A) Bar chart representation showing the number of probes differentially expressed between  
454 EPO and control samples at Days 0, 1 and 3. Up-regulated probes are indicated in red while  
455 down-regulated probes are indicated in green.

456 (B) Heatmap showing the relative expressions for genes differentially expressed in at least  
457 one timepoint. Probes were organized based on their expression profiles using a hierarchical  
458 clustering.

459 (C) Bar chart representation showing the p-values, displayed as  $-\log_{10}$ , of pathways found to  
460 be significantly over-represented at Day-3 in patients treated by EPO vs controls. Functional  
461 enrichment revealed an activation of heme biosynthesis pathways at Day-3 in patients  
462 treated by EPO (*e.g.*,  $p=10^{-7.9}$ ,  $10^{-6.5}$ , and  $10^{-2.3}$  for heme biosynthesis II, heme biosynthesis  
463 from uroporphyrinogen III-1, and Tetrapyrrole Biosynthesis II pathways, respectively). We  
464 also observed an activation of other pathways linked to EPO signaling such as phagosome  
465 maturation, NRF2-mediated Oxidative Stress Response or hypoxia signalling in the  
466 cardiovascular system ( $p=10^{-3.4}$ ,  $10^{-2.5}$ , and  $10^{-2.5}$ , respectively).

467

468

469

470 **Figure 2: Effect of epidemiological, resuscitation and outcome parameters on the**  
471 **blood transcriptome evaluated at Day-0, Day-1 and Day-3 after cardiac arrest.**

472 **(A)** Bar chart representation showing the number of probes differentially expressed between  
473 patients depending upon the age, gender, initial rhythm of cardiac arrest, epinephrine (Epi)  
474 dose before resuscitation, low-flow (LF) duration, no-flow-duration, cerebral performance  
475 category (CPC) at day 60 after cardiac arrest, and putative treatment (EPO). Up-regulated  
476 probes are indicated in red, while down-regulated probes are indicated in green.

477 **(B)** Venn diagram showing the number of differentially expressed genes in patients with  
478 CPC>2 vs CPC1-2 and other above-listed investigated parameters.

479

480

481 **Figure 3: Differentially expressed genes in patients with an ultimate cerebral**  
482 **performance category (CPC) > 2 vs CPC 1-2.**

483 *Down-regulated genes are indicated in green, while up-regulated genes are indicated in red.*

484 **(A and B)** List of the top up- and down-regulated genes with their associated fold-changes at  
485 Day-0 and Day-1 after cardiac arrest in patients with CPC>2 vs CPC1-2, respectively. A  
486 majority of genes are involved in inflammation, innate immunity and cell migration, such as  
487 matrix metalloproteinase-8 (MMP-8, an enzyme stored in secondary granules within  
488 neutrophils), several genes encoding for major histocompatibility complex (HLA-DMB, HLA-  
489 DRB4, HLA-DRB6), CD177 (cell surface glycoprotein playing a role in neutrophil activation),  
490 CD83 (member of the immunoglobulin superfamily receptors, involved in immune response),  
491 IL1R2 (Interleukin [IL]-1 receptor, type II), IL18R1 (Interleukin [IL]-18 receptor 1), PRTN3  
492 (Proteinase 3, that could play a role in in neutrophil migration), RGS1 (regulator of G-protein  
493 signaling 1), PRG2 (Proteoglycan 2, Pro Eosinophil Major Basic Protein) or CX3CR1 (CX3C  
494 chemokine receptor 1, a transmembrane protein and chemokine involved in the adhesion  
495 and migration of leukocytes). We also observe several genes involved in cell cycle regulation  
496 and heat shock proteins, such as DNAJ (DnaJ [Hsp40] homolog subfamily, members 1 and  
497 4), HSPB1 (Heat shock 27 kDa protein 1) or G0S2 – G0/G1 switch 2. Finally, we observed  
498 an up-regulation of cathepsin L1 (CTSL1), a lysosomal cysteine protease that plays a major  
499 role in intracellular protein catabolism, as well as other genes with still questionable putative  
500 function in the setting of cardiac arrest (*e.g.*, albumin).

501 **(C)** Venn diagram showing the number of differentially expressed genes found at Day-0 and  
502 Day-1 in patients with CPC>2 vs CPC1-2.

503 **(D)** List of the top up- and down-regulated genes commonly modified at Day-0 and Day-1 in  
504 patients with an ultimate cerebral performance category (CPC) > 2 vs CPC 1-2 with their  
505 associated fold-changes. A majority of genes are known to be at least in part linked to  
506 immunity and inflammatory response, *e.g.*, MMP8, CD177, CLEC1B (C-type lectin-like  
507 receptor, which is expressed in myeloid cells and natural killer cells), GPR84 (G Protein-  
508 Coupled Receptor 84, also known as Inflammation-Related G Protein-Coupled Receptor

509 EX33), RETN (adipose tissue-specific secretory factor, which promotes chemotaxis in  
510 myeloid cells), SAMSN1 (SAM domain-containing protein, a negative regulator of B-cell  
511 activation), CEACAM1 (Carcinoembryonic antigen-related cell adhesion molecule 1, which  
512 belongs to the immunoglobulin superfamily), TNFAIP6 (Tumor necrosis factor alpha-induced  
513 protein 6), HLA-DMB, or CPVL (carboxypeptidase vitellogenic-like protein, which may  
514 participate to the inflammatory protease cascade). Some genes were also related to cell  
515 cycle or response to DNA damages such as DDTI4 (DNA-damage-inducible transcript 4),  
516 CDC20 (cell-division-cycle protein 20, which interacts with several other proteins at multiple  
517 points in the cell cycle). The putative function of the 5 last identified genes is still more  
518 challenging to identify in the cardiac arrest setting, *i.e.*, CTSL1, SMIM1 1 (Small Integral  
519 Membrane Protein 1), ANKRD22 (Ankyrin Repeat Domain 22), STOM (Stomatin, a highly  
520 conserved family of integral membrane proteins), LGALS2 (lectin galactoside-binding  
521 soluble, also known as Galectin 2).

522

523

524 **Figure 4: Functional enrichment of the transcriptomic signature predicting patient**  
525 **outcomes after cardiac arrest in patients with cerebral performance category (CPC) >**  
526 **2 vs CPC 1-2.**

527 (A) Functional enrichment analysis showing the canonical pathways found to be over-  
528 represented at Day-0 and Day-1 in patients with CPC>2 vs CPC1-2. Some pathways were  
529 significantly down-regulated at Day-0 and/or Day-1, such as antigen presentation pathways,  
530 OX40, Cdc42 signaling and Nur77 Signaling (e.g.,  $p=10^{-7.5}$ ,  $10^{-5.3}$ ,  $10^{-4.2}$ ,  $10^{-4.2}$  at Day-0 and  
531  $p=10^{-4.4}$  at Day-1 respectively). Other pathways were significantly up-regulated, such as  
532 interleukin-6 signaling ( $p=10^{-4.1}$  at Day-0), toll-like receptor (TLR) signaling ( $p=10^{-4.2}$  at Day-  
533 1), and pattern recognition receptors in recognition of bacteria and viruses ( $p=10^{-4.0}$  at Day-  
534 1). Communication between innate and adaptive immune cells and crosstalk between  
535 dendritic cells and natural killer cells pathways followed a biphasic pattern of alterations with  
536 a significant up-regulation at Day-0 and subsequent a down-regulation at Day-1.

537 (B) Functional enrichment analyses of upstream regulators found to be over-represented in  
538 gene signatures at Day-0 and Day-1 in patients with CPC>2 vs CPC1-2. The p-value,  
539 associated with each pathway, is represented  $-\log_{10}(p\text{-value})$  using a gradient color scale. For  
540 instance, CCAAT/enhancer-binding protein alpha (CEBPA) and Sp1 transcription factor  
541 (SP1) were significantly activated at both Day-0 and Day-1 (e.g.,  $p=10^{-10.1}$  and  $p=10^{-8.3}$  at  
542 Day-0). Other regulators were significantly modified at only Day-0, with either down-  
543 regulation (TP53 – tumor protein 53, ESR1 – Estrogen Receptor 1, and HSF1 – Heat Shock  
544 Transcription Factor 1) or up-regulation (SATB1 – Special AT-rich sequence binding protein  
545 1, STAT3 – signal transducer and activator of transcription 3, FOXO1 – Forkhead box O1,  
546 HMGB1 – high mobility group box 1). Many factors could be linked to inflammation (CEBPA,  
547 STAT3, HMGB1) or cell cycle regulation and nuclear damage signaling (TP53, FOXO1,  
548 SATB1, HMGB1, SP1).

549 (C) Functional enrichment analysis showing the pathways found to be over-represented  
550 among the common genes at Day-0 and Day-1 in patients with CPC>2 vs CPC1-2. The p-  
551 value is represented using a color-gradient scale.

552 **Figure 5: Logistic model based on the transcriptomic signature (Panels A and B) or**  
553 **clinical and epidemiological findings (Panel C) to predict the outcome of the patients**  
554 **with cerebral performance category (CPC) > 2 vs CPC 1-2.**

555 **(A)** Coefficients associated with each gene in the logistic regression analysis. P-values  
556 associated with each coefficient are indicated using a color gradient scale. The regression  
557 analysis was based on 38 genes, including a majority of genes already presented in previous  
558 analyses, such as CDC20, DDIT4, HLA-DMB, STOM, RETN, CLEC1B, TNFAIP6,  
559 ANKRD22, HLA-DRB6, PRTN3, LGALS2, MMP8, HLA-DRB4, SAMSN1, GPR84, CD177  
560 and CPVL (see Legend of Figures 3 and 4). Additional genes were also listed here, *e.g.*,  
561 SLC2111 (Solute Carrier Family 2 Member 11), COL17A1 (collagen type XVII alpha 1 chain),  
562 PHGDH (phosphoglycerate dehydrogenase), OST-ALPHA (organic solute transporter  
563 subunit alpha) or TCN1 (transcobalamin 1).

564 **(B)** Summary of the ability of the transcriptomic logistic model to predict the outcome of the  
565 patients, including the truth/decision table, specificity, Hit Rate, AUC, and the ROC curve.

566 **(C)** Summary of the ability of the logistic model based on clinical and epidemiological  
567 parameters to predict the outcome of the patients, including the same parameters.

568

569

570 **Table 1:** Epidemiological and clinical findings.

Mean±SD, N or %	Control		EPO		Pooled CPC1-2	Pooled CPC>2
	CPC1-2	CPC>2	CPC1-2	CPC>2		
Number of patients and % among group	15 (42%)	21 (58%)	14 (42%)	19 (58%)	29 (42%)	40 (58%)
Age (years)	53±16	62±12*	55±16	60±11*	54±16	61±12*
Gender (Male/Female)	12 / 3	16 / 5	11 / 3	17 / 2	23 / 6	33 / 7
Initial shockable rhythm (N [%])	9 (60%)	10 <sup>§</sup> (50%)	11 (79%)	9 (47%)	20 (69%)	20 <sup>§</sup> (51%)
Epinephrine dose (mg)	0.33±0.90	4.00±4.05*	1.57±2.93	3.00±2.93*	0.63±2.19	3.53±3.55*
No- flow time(min)	2.9±2.9	5.8±5.8	2.9±5.8	4.3±4.3	2.9±4.3	4.3±4.3
Low-flow time (min)	14.4±8.6	27.4±15.8*	14.4±11.5	25.9±14.4*	14.4±10.1	27.4±14.4*
Post-cardiac arrest PCI (N [%])	4 (27%)	7 (33%)	9 (64%) †	12 (63%) †	13 (45%)	19 (48%)
Thrombotic events (N [%])	1 (7%)	2 (10%)	2 (14%)	3 (16%)	3 (10%)	5 (13%)
Cardiogenic shock status (N [%])	3 (20%)	7 (33%)	5 (36%)	10 (53%)	8 (28%)	17 (42%)
Body temperature at H24 (°C)	34.0±1.6	33.9±1.7	33.0±0.8	33.8±1.3	33.3±1.3	33.9±1.5
Body temperature at H48 (°C)	37.5±0.9	37.1±1.7	37.5±1.0	37.3±1.2	37.5±0.9	37.3±1.4

571

572 *Data are expressed as number, percentage or mean±SD. Percentages were calculated*

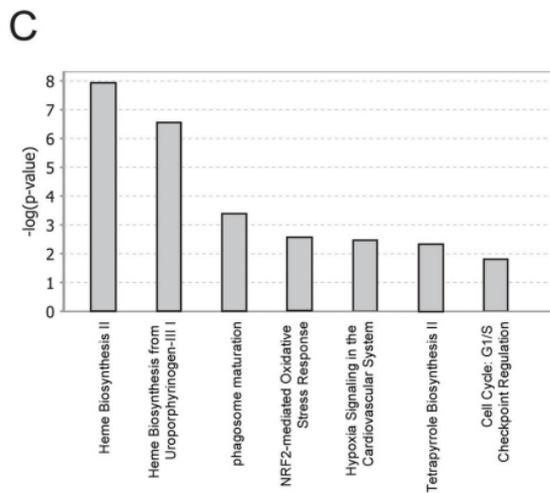
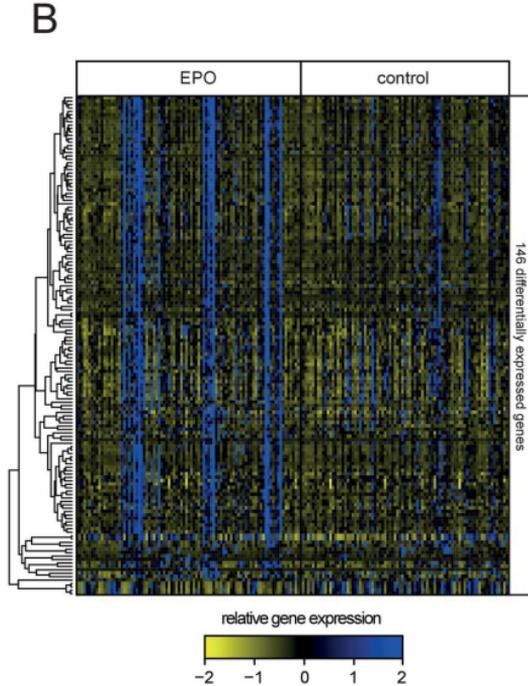
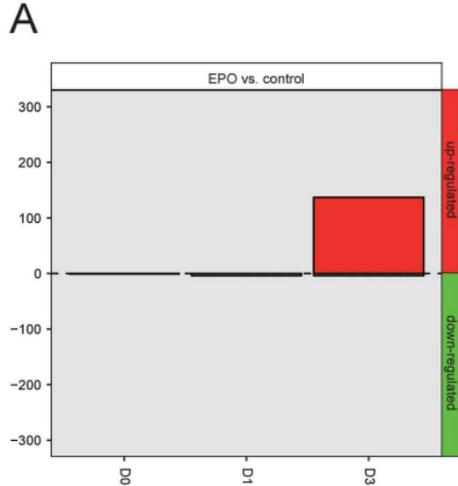
573 *within each sub-group (i.e., column), except for the total number of patients with CPC 1-2 or*

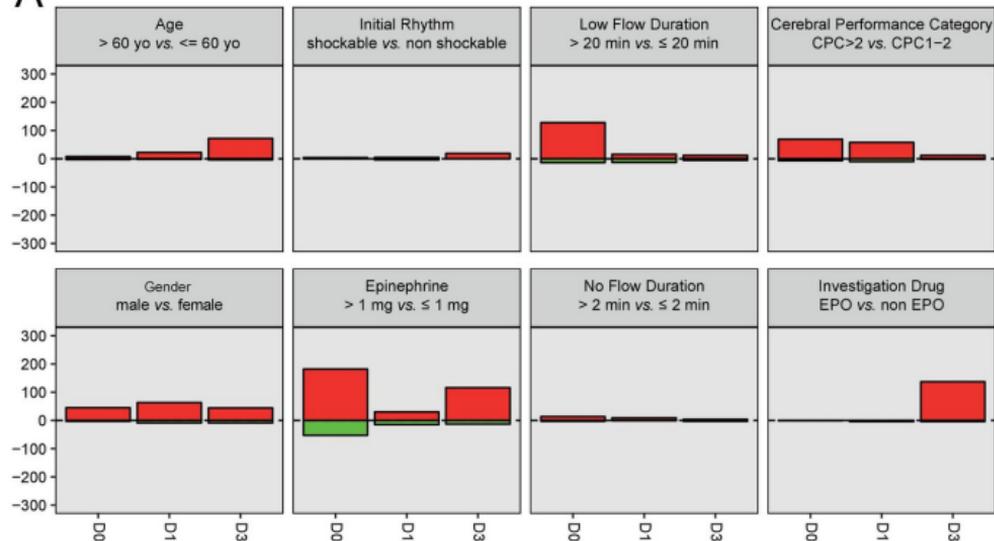
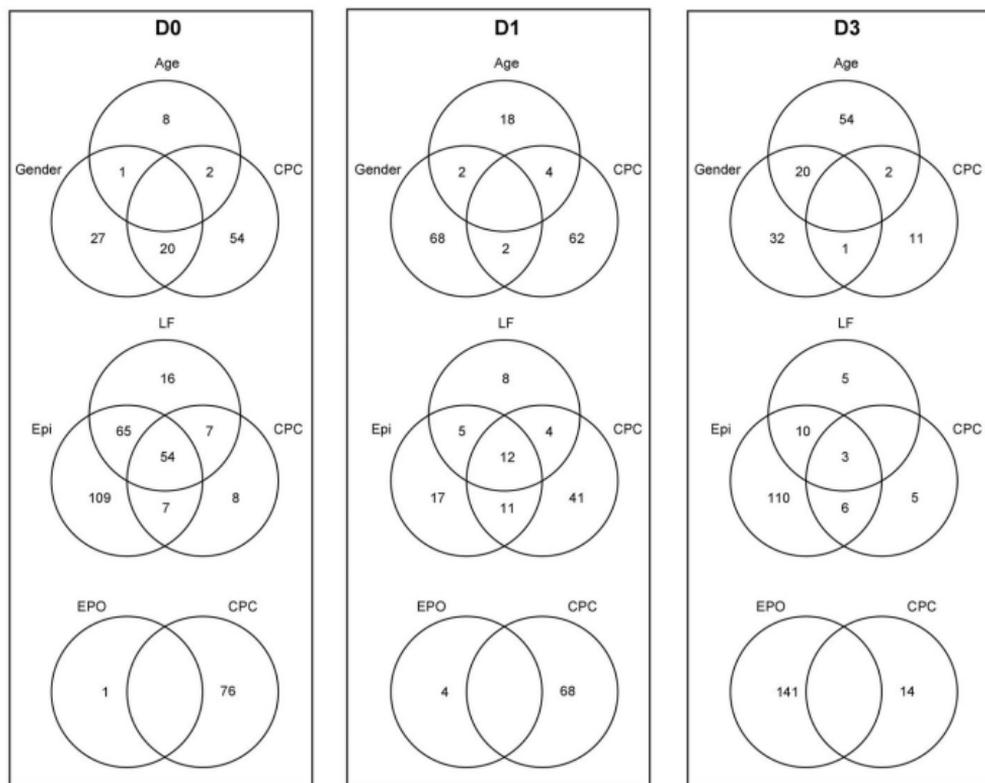
574 *CPC>2 which includes the percentage of patients among the whole Control and EPO groups.*

575 *PCI, percutaneous intervention; <sup>§</sup>, unknown for 1 patient; \*, p<0.05 vs corresponding CPC1-*

576 *2; †, p<0.05 vs corresponding Control*

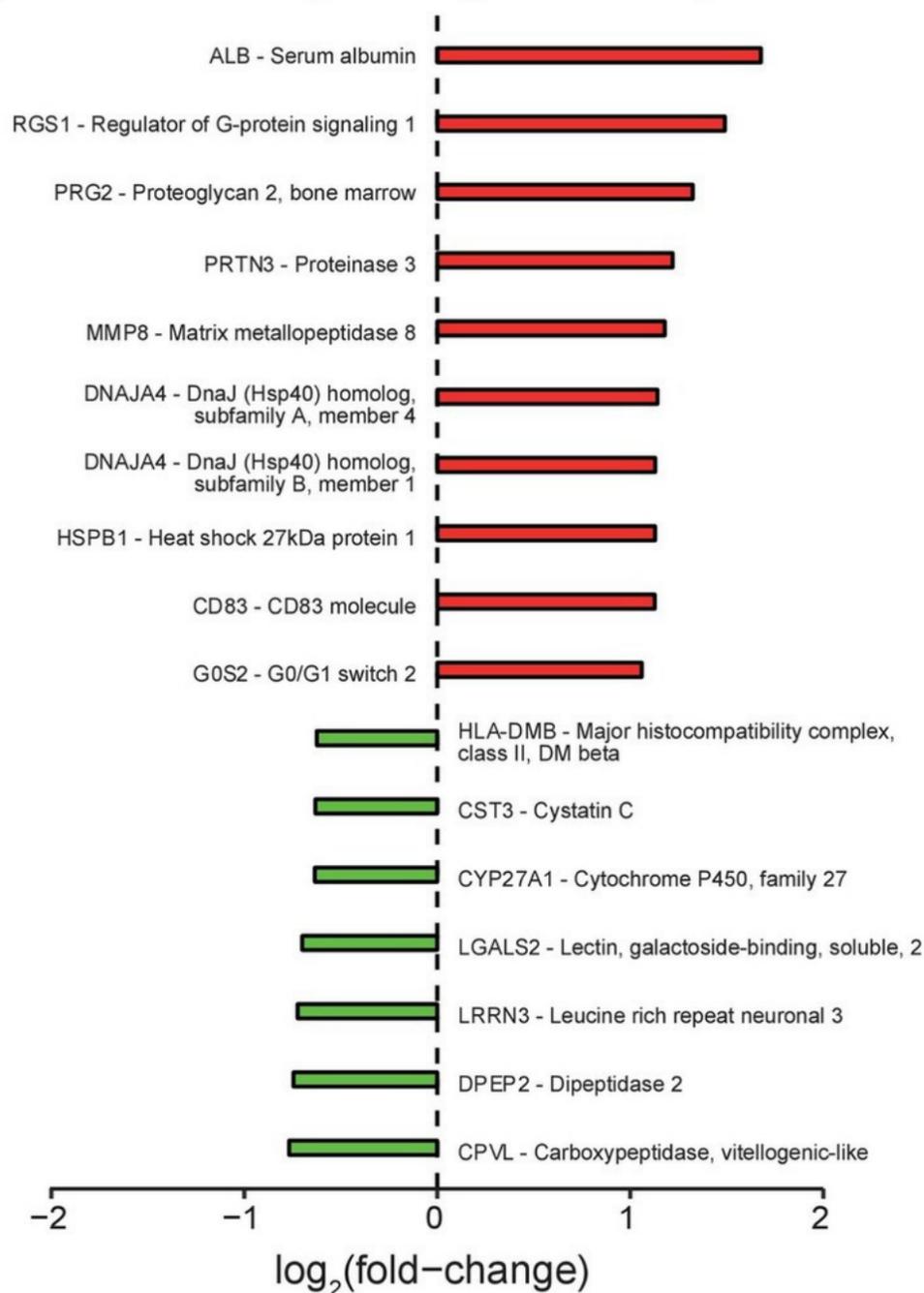
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**A****B**

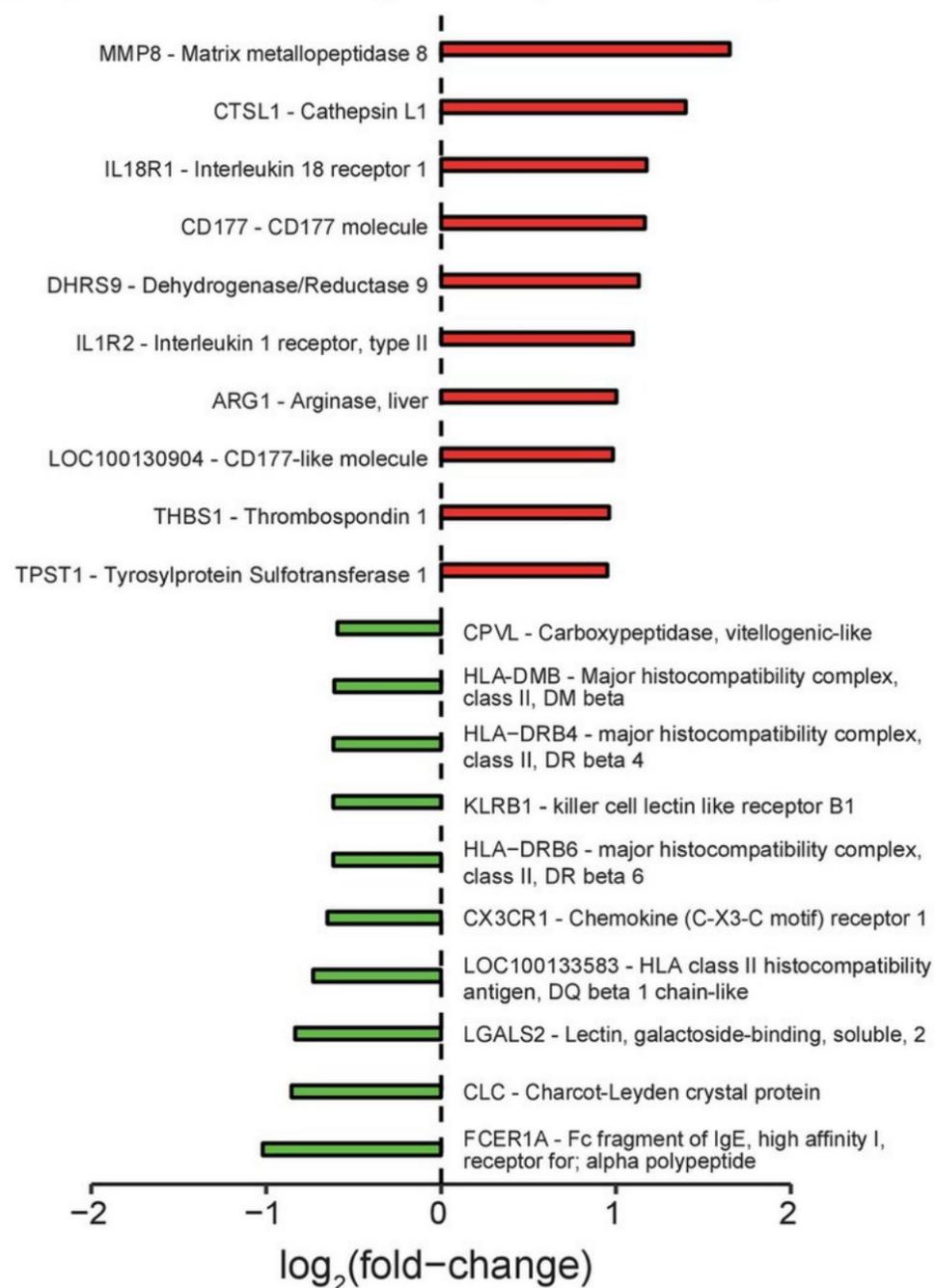
A

top up- and down- regulated genes at day 0

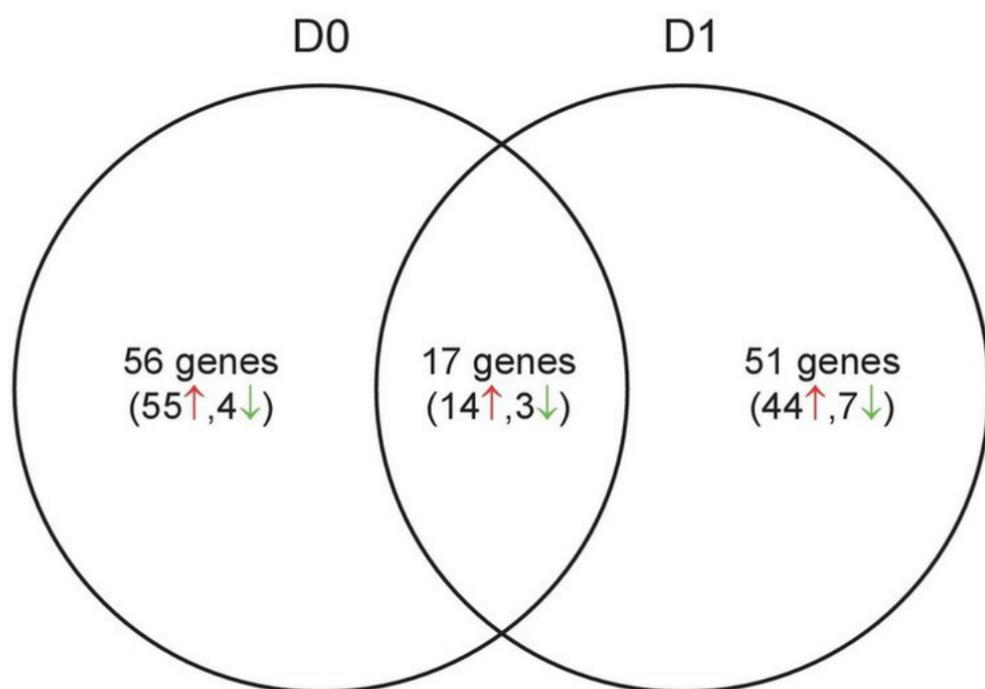


B

top up- and down- regulated genes at day 1



C

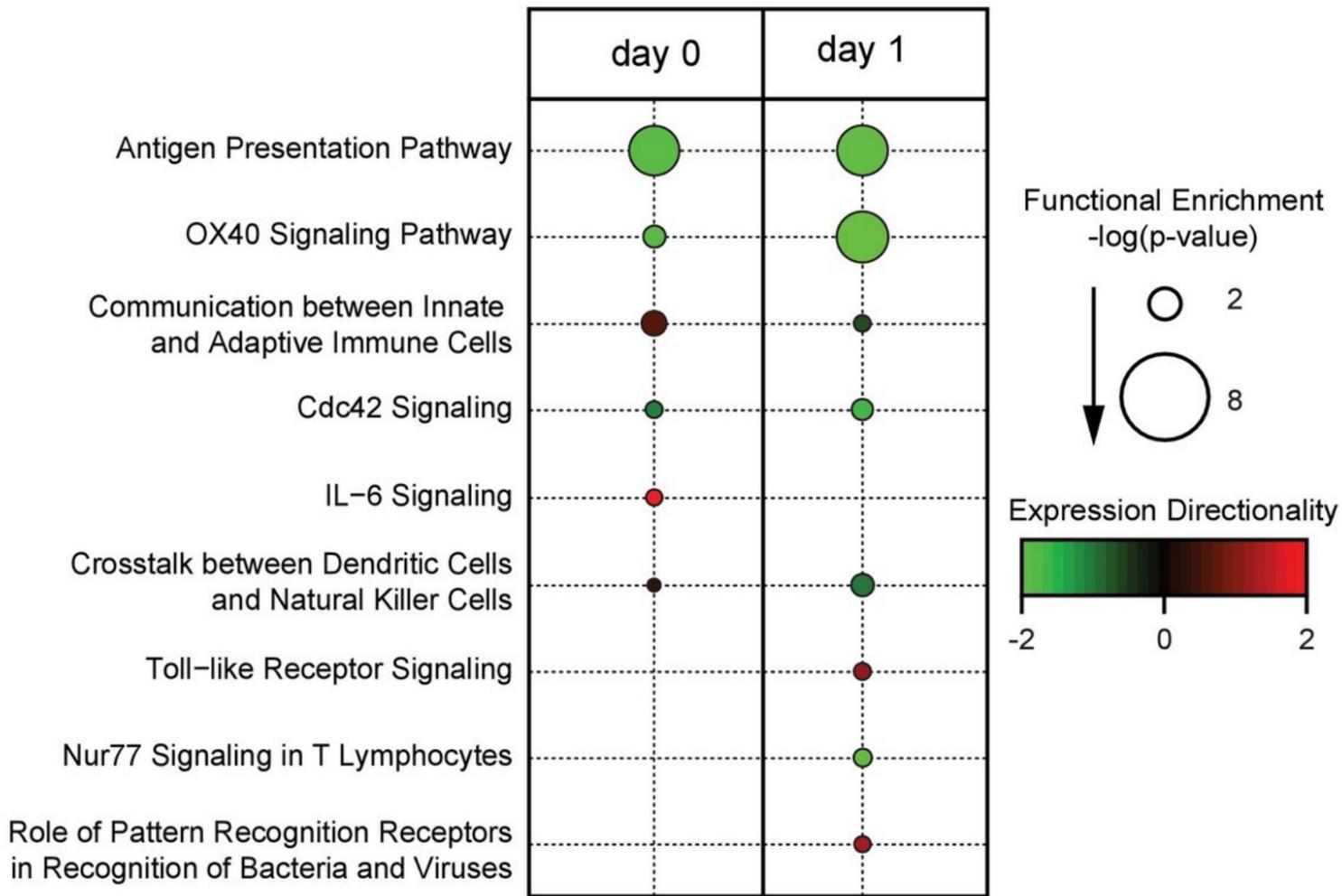


D

Gene Symbol	D0	D1	Gene Name
MMP8	2.26	3.14	Matrix metalloproteinase 8
CTSL1	1.85	2.64	Cathepsin L1
CD177	1.93	2.24	CD177 molecule
DDIT4	1.80	1.84	DNA-damage-inducible transcript 4
SMIM1	1.78	1.85	Small integral membrane protein 1
CLEC1B	1.68	1.80	C-Type Lectin Domain Family 1 Member B
GPR84	1.90	1.57	G protein-coupled receptor 84
RETN	1.83	1.61	adipose tissue-specific secretory factor
CDC20	1.68	1.71	cell-division cycle protein 20
ANKRD22	1.58	1.71	Ankyrin Repeat Domain 22
SAMSN1	1.53	1.74	SAM domain-containing protein SAMSN-1
CEACAM1	1.52	1.75	Carcinoembryonic antigen-related cell adhesion molecule 1
STOM	1.51	1.55	Stomatin
TNFAIP6	1.50	1.54	TNF Alpha Induced Protein 6
HLA-DMB	-1.54	-1.53	Major histocompatibility complex, class II, DM beta
CPVL	-1.70	-1.51	carboxypeptidase, vitellogenic-like
LGALS2	-1.62	-1.78	lectin, galactoside-binding, soluble, 2

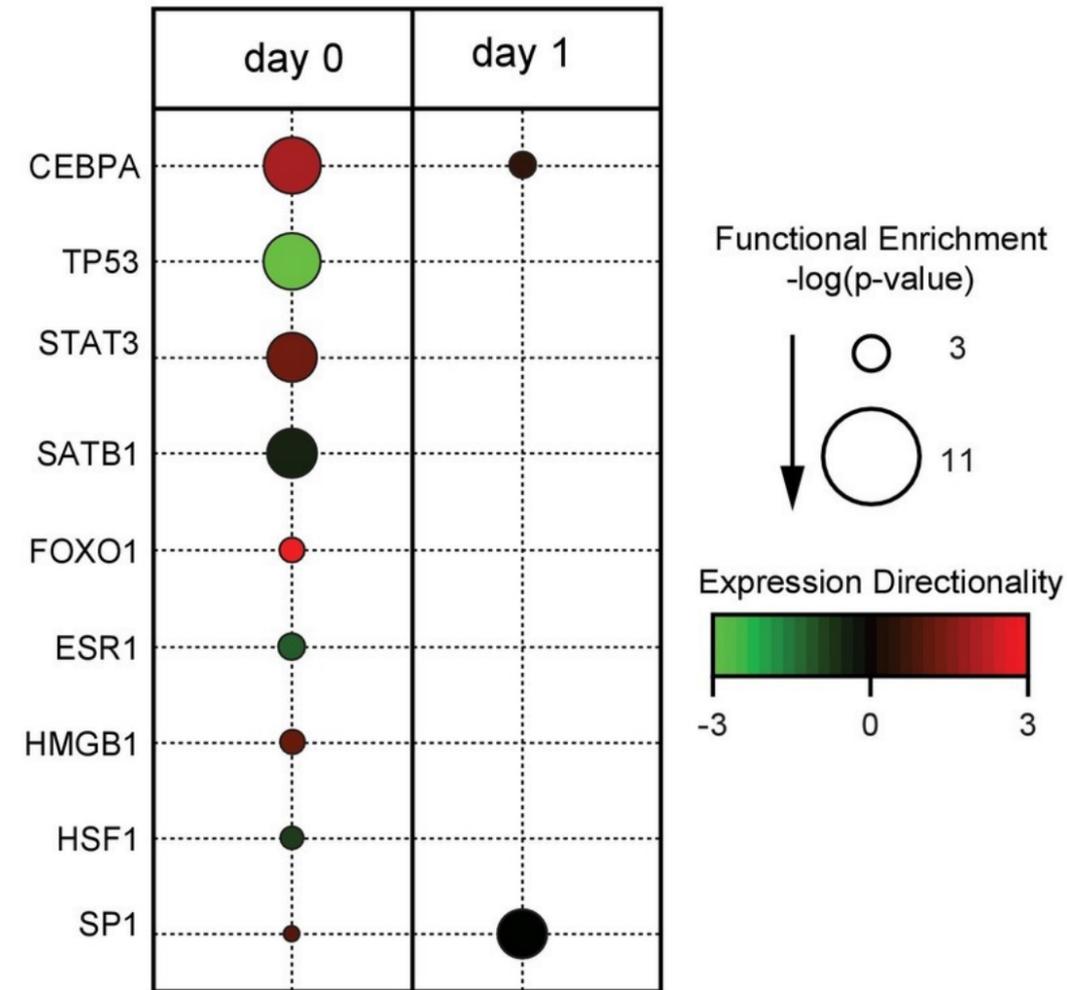
A

## Canonical Pathways



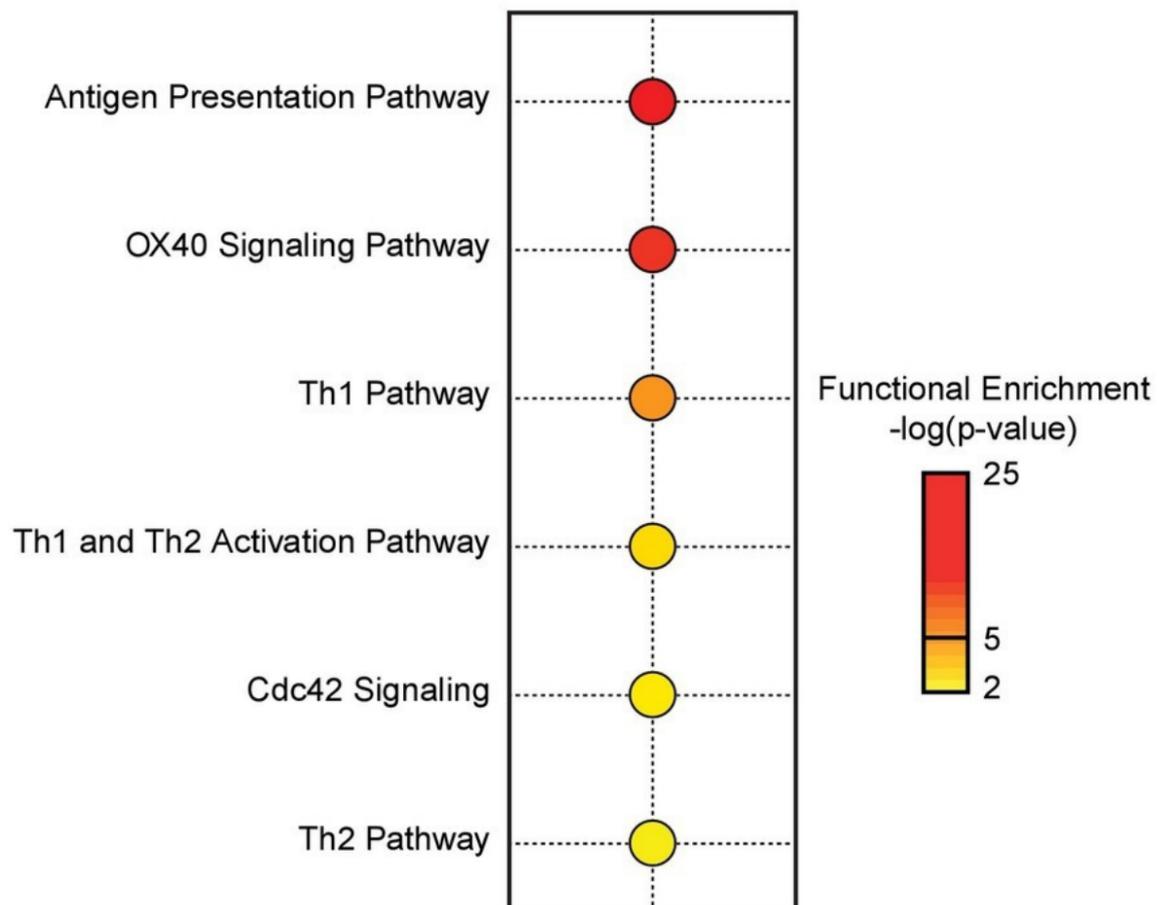
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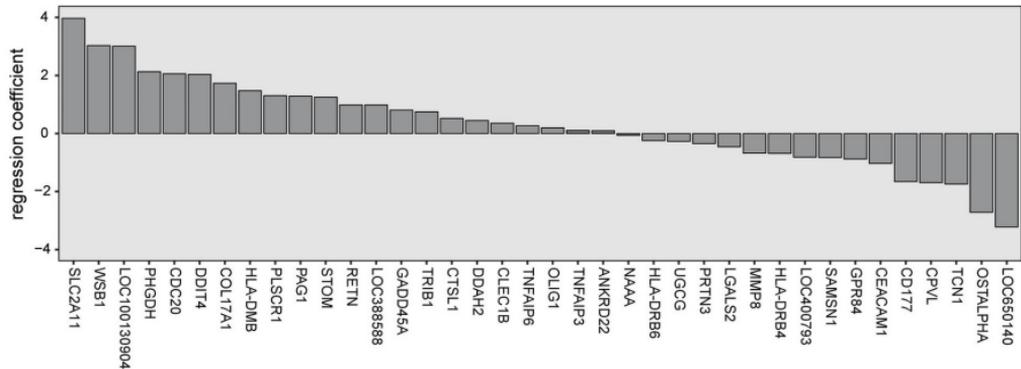
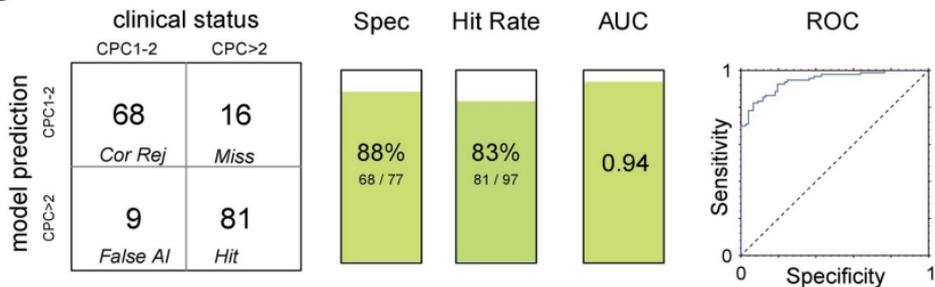
## Upstream Regulators



C

## Canonical Pathways



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