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The biosynthetic routes leading to de novo Nicotinamide Adenine Dinucleotide (NAD⁺) production are involved in acute kidney injury (AKI) with a critical role for Quinolinate Phosphoribosyl Transferase (QPRT), a bottleneck enzyme of de novo NAD⁺ biosynthesis. However, the molecular mechanisms determining reduced QPRT in AKI, and the role of impaired NAD⁺ biosynthesis in the progression to chronic kidney disease (CKD) are unknown. We demonstrate that a high urinary quinolinate to tryptophan ratio, an indirect indicator of impaired QPRT activity and reduced de novo NAD⁺ biosynthesis in the kidney, is a clinically applicable early marker of AKI after cardiopulmonary bypass, and is predictive of progression to chronic kidney disease (CKD) in kidney transplant recipients. We also provide evidence that the Endoplasmic Reticulum (ER) stress response impairs de novo NAD⁺ biosynthesis by repressing QPRT transcription. In conclusion, NAD⁺ biosynthesis impairment is an early event in AKI embedded with the ER stress response, and persistent reduction of QPRT expression is associated with AKI to CKD progression. This defines non-invasive metabolic biomarkers of kidney injury with prognostic and therapeutic implications.

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1 Cell stress response impairs de novo NAD⁺ biosynthesis in the kidney

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54

55 **Abstract**

56 The biosynthetic routes leading to de novo Nicotinamide Adenine Dinucleotide (NAD⁺)
57 production are involved in acute kidney injury (AKI) with a critical role for Quinolinate
58 Phosphoribosyl Transferase (QPRT), a bottleneck enzyme of de novo NAD⁺ biosynthesis. The
59 molecular mechanisms determining reduced QPRT in AKI, and the role of impaired NAD⁺
60 biosynthesis in the progression to chronic kidney disease (CKD) are unknown.

61 We demonstrate that a high urinary quinolinate to tryptophan ratio, an indirect indicator of
62 impaired QPRT activity and reduced de novo NAD⁺ biosynthesis in the kidney, is a clinically
63 applicable early marker of AKI after cardiac surgery and is predictive of progression to chronic
64 kidney disease (CKD) in kidney transplant recipients. We also provide evidence that the
65 Endoplasmic Reticulum (ER) stress response may impair de novo NAD⁺ biosynthesis by
66 repressing QPRT transcription.

67 In conclusion, NAD⁺ biosynthesis impairment is an early event in AKI embedded with the ER
68 stress response, and persistent reduction of QPRT expression is associated with AKI to CKD
69 progression. This defines non-invasive metabolic biomarkers of kidney injury with prognostic
70 and therapeutic implications.

71

72

73 **Introduction**

74 Nicotinamide adenine dinucleotide (NAD⁺) is a cofactor involved in oxido-reduction reactions
75 and serves as an energy transfer intermediate in multiple metabolic pathways (1). NAD⁺ is an
76 important cosubstrate for histones deacetylases (sirtuins) and poly(ADP-ribose) polymerases
77 (PARPs), which regulate several aspects of cellular homeostasis (1). Cellular NAD⁺ reduction
78 in aging and in association with several diseases contributes to overall fitness decline and to
79 a lower resistance to cell stress. Conversely, increasing NAD⁺ content can prolong health and
80 life span in experimental organisms (2)(3). Recent experimental and clinical data
81 demonstrated a critical role for NAD⁺ homeostasis in acute kidney injury (AKI) (4)(5)(6)(7).
82 Proximal tubular cells (PTCs) are highly metabolically active and their survival and function
83 depend on the possibility to couple their energetic needs to the regulation of energy generation,
84 antioxidant responses, and mitochondrial biogenesis and quality control, all of which rely on
85 cytosolic and mitochondrial NAD⁺ (5)(8). NAD⁺ is continuously degraded, and stable NAD⁺
86 cellular concentrations are maintained by constant new supply through the nicotinamide
87 salvage pathway (nicotinamide is generated as a by-product of enzymatic activities) and
88 biosynthetic pathways (**Suppl. Figure 1**). NAD⁺ biosynthesis involves diverse dietary sources,
89 including nicotinic acid, nicotinamide, nicotinamide riboside, and tryptophan. The de novo
90 pathway converts dietary tryptophan to NAD⁺ in eight steps with the last catalyzed by
91 quinolinate phosphoribosyltransferase (QPRT). Reduction in QPRT activity during AKI is
92 considered critical in NAD⁺ biosynthetic impairment (6), but the molecular mechanisms
93 determining reduced QPRT upon kidney injury and its role in long-term outcomes after AKI
94 remain unclear. The clinical relevance a non-invasive monitoring of QPRT activity in patients
95 with a kidney disease is not established.

96

97 **Results**

98 **Impaired NAD⁺ biosynthesis is a very early event in AKI**

99 A previous study showed that quinolinate accumulates as a consequence of reduced QPRT
100 activity and that urinary quinolate/tryptophan ratio (uQ/T) is elevated in AKI (6). Metabolic
101 profiling of urine samples collected at baseline and the day after cardio pulmonary bypass
102 (CPB) in 41 cardiac surgery patients (**Supp. Figure 3** for metabolomic data and **Suppl. Table**
103 **2** for clinical data), including 11 patients (26%) developing AKI in the 7 days following CPB
104 according to KDIGO criteria, confirmed that uQ/T was higher at day 1 after surgery in patients
105 developing AKI, whereas at baseline, the levels were the same (**Figure 1A**), and severity of
106 AKI correlated with uQ/T levels at day 1 (**Figure 1B**). uQ/T was one of few urinary metabolic
107 parameters significantly discriminating AKI at day 1 after surgery (but not at baseline) in this
108 cohort (**Figure 1C**). uQ/T on day 1 after cardiac surgery predicted AKI with a higher accuracy
109 than other AKI biomarkers, including kidney injury molecule 1 (KIM-1) or neutrophil gelatinase
110 associated lipocalin (NGAL) (**Figure 1D**). In a multivariate analysis, uQ/T measured the day
111 after CPB was an independent predictor of the occurrence of AKI (**Suppl. Table 3** and **4** for
112 uni and multivariate analyses). Urinary QPRT transcripts (urinary gene expression patterns
113 reflect the dynamics of biopsy genes signatures (9)(10)(11)) were negatively correlated with
114 uQ/T levels, in line with the fact that elevated uQ/T is an indirect marker of reduced QPRT
115 activity (**Figure 1E**). QPRT mRNA levels were significantly reduced in the first -after ischemia-
116 reperfusion injury (IRI) in protocol biopsies obtained in 42 kidney transplant recipients (KTR)
117 before and after reperfusion (12) (**Figure 1F**). QPRT transcripts levels were highly
118 anticorrelated with HAVCR1 (the gene encoding KIM-1) transcripts (**Figure 1G**), suggesting
119 that patients with more severe AKI in the post transplantation biopsy had more pronounced
120 QPRT reduction after reperfusion. Finally, in a mouse model of bilateral IRI (13), Qprt achieved
121 the lowest level within 24 h after IRI (**Figure 1H**). Thus, reduced QPRT transcription and
122 activity is an early event associated with AKI. Indirectly determined by uQ/T in a clinical context,
123 this is the first metabolic change predictive of AKI detectable by urinary metabolic profiling.

124 **QPRT reduction is associated with progression to chronic kidney disease**

125 To investigate the long-term impact of the early reduction of de novo NAD⁺ biosynthesis
126 associated with IRI, we performed a targeted urinary metabolic profiling in 237 KTR 10 days
127 after transplantation (**Suppl. Table 5** for clinical data). Hierarchical clustering according to
128 metabolic profiling identified 3 clusters (**Figure 2A**). Donor related parameters, such as age,
129 history of hypertension, renal function, or death from stroke (ie. Extended Criteria Donor
130 kidneys), were balanced among the clusters (**Suppl. Figure 4A and B**), but cluster 2 was
131 enriched with patients who undergone more severe ischemic injury, as indicated by longer cold
132 ischemia time, higher serum creatinine the day of urine sampling, and higher frequency of
133 delayed graft function episodes (need for at least on dialysis session during the 7 days after
134 transplantation) and a higher number of dialysis sessions (**Figure 2B to D and Suppl. Figure**
135 **4C**). Among the urinary metabolites that best characterized cluster 2, quinolinate and uQ/T
136 had the largest and significant size effect (**Figure 2E and Suppl. Table 6** for the list of
137 metabolites). Critically, multivariate models integrating all variables associated with graft
138 outcomes showed that high uQ/T 10 days after transplantation was an independent predictor
139 of allograft function 3 and 12 months after transplantation (**Tables 1 and 2**).

140 To investigate the role of QPRT along the transition from AKI to CKD, we took advantage of
141 the previously reported cohort of 42 KTR followed by bulk RNAseq in protocol biopsies
142 obtained 3 and 12 months after transplantation (12). A computational model to characterize
143 the transcriptional transition from AKI to CKD has been developed, based on the principle that
144 the progression to fibrosis is a transitional process from acute injury to fibrosis (12) (see
145 **Methods**). According to this computational model, QPRT was progressively reduced along the
146 transition to CKD (**Figure 2F**), a finding confirmed by immunohistochemistry in kidney biopsies
147 obtained from patients with CKD (**Figure 2G**), by the correlation between renal function and
148 QPRT transcripts levels in urinary cell pellets in a cohort of 55 CKD patients (**Figure 2H and**
149 **Suppl. Table 7** for clinical data), and by a lower expression of QPRT transcripts in tubular

150 human diabetic tissues (14) (**Figure 2I**). Thus, persistent QPRT reduction and impaired de
151 novo NAD⁺ biosynthesis after AKI is a feature of the transition to chronic renal damage.

152 **ER stress reduces de novo NAD⁺ biosynthesis in the kidney**

153 We next investigated the potential mechanisms determining the reduction of NAD⁺ biosynthetic
154 activity upon AKI. Among the several factors involved in the early transcriptional response to
155 AKI, we focused on the endoplasmic reticulum (ER) stress response, which is known to be
156 activated in the early kidney injury response in mouse (15)(16) and human (17). We modeled
157 the interaction between ER stress and NAD⁺ biosynthesis in PTCs by performing *in vitro*
158 studies in the Human Kidney 2 (HK2) cell line, a proximal tubule-derived cell line expressing
159 QPRT (**Suppl. Figure 5**). In line with this, analysis of mouse single nucleus RNA sequencing
160 datasets indicated that Qprt is almost exclusively expressed in PTCs (**Figure 3A**), which is in
161 line with transcriptomic and proteomic data in micro dissected tubules in rats (18)(19) (**Suppl.**
162 **Figure 6A and B**), and supported by an immunohistochemistry study on normal human kidney
163 (**Figure 3B**).

164 In an unbiased approach, we induced ER stress with tunicamycin (Tun), an inhibitor of GlcNAc
165 phosphotransferase that promotes ER stress and activates the Unfolded Protein response
166 (UPR) (**Suppl. Figure 7**), and we performed metabolomic analysis in the cell lysate. ER stress
167 profoundly reshaped the intracellular metabolic profile of HK2 cells (**Figure 3C**). Among the
168 metabolites strongly enriched in response to ER stress we found asparagine (ER stress
169 induces asparagine synthase (20)(21)), and quinolinate (**Figure 3D** and **Suppl. Table 8A** for
170 the list of metabolites). Similar results were obtained after ER stress induction with Brefeldin A
171 (BFA) (**Suppl. Figure 7**), a lactone that inhibits protein transport from the ER to the Golgi
172 complex (**Suppl. Figure 8** and **Suppl. Table 8B** for the list of metabolites). ER stress induced
173 by Tun or BFA reduced NAD⁺ intracellular contents (**Figure 3E**). This process was inhibited by
174 TES1025 (**Figure 3F**), an inhibitor of the α -amino- β -carboxymuconate- ϵ -semialdehyde
175 decarboxylase (ACMSD), indicating ER stress represses de novo NAD⁺ synthesis since
176 ACMSD decreases the proportion of substrates able to undergo spontaneous cyclization into

177 quinolinic acid (**Suppl. Figure 1**). Importantly, the decrease in NAD⁺ intracellular content was
178 unlikely a consequence of accelerated consumption of NAD⁺ by PARPs, because ER stress
179 was associated with PARP cleavage and a defective PARylation activity (**Figure 3G to I**). Note
180 that Etoposide, used as a positive control to promote apoptosis, does not induces ER stress
181 and does not affect QPRT expression (**Figure 3G and H**). We observed a slight increase in
182 expression of the transcripts of the ectonuclease CD38 upon ER stress in HK2 (**Figure 3J**).
183 CD38, an ecto-enzyme capable of reducing extracellular NAD⁺ precursors availability (22),
184 could theoretically participate to the decrease in NAD⁺ contents observed in ER stressed cells,
185 albeit the culture medium used (Dulbecco's Modified Eagle Medium) contain high and likely
186 saturating concentrations of nicotinamide (4 mg/L). These results suggest that ER stress may
187 be involved in NAD⁺ biosynthetic impairment, and that quinolinate accumulation is consistent
188 with a reduction in the de novo biosynthetic pathway activity.

189 **QPRT expression is repressed in response to kidney injuries associated with ER stress**

190 A diminished expression of Qprt was found in several models of kidney injuries associated with
191 ER stress (using DNA Damage Inducible Transcript 3 (DDIT3), also known as C/EBP
192 homologous protein (CHOP)), as a surrogate marker (23), for instance injection of Tun; a
193 transgenic mouse model expressing mutant uromodulin (UmodC147W/+) that accumulates in
194 the ER lumen; and streptozotocin-induced diabetic nephropathy (**Figure 4A to C**). In addition,
195 in the first hours after IRI in mice, single nucleus RNA sequencing indicated that PTCs
196 displayed a fundamental change of their transcriptional profile with the expression of
197 established injury markers (e.g. Havcr1), and the parallel loss of classical differentiation
198 markers (eg. Lrp2, encoding megalin), leading to the characterization of novel PTCs clusters
199 (called PT1 to 3), which were referred to as “injured proximal tubule” (**Figure 4D and E**) and
200 **Suppl. Figure 6C to E**) (8). PTCs in the early damaged state displayed low levels of Qprt
201 (**Figure 4F**) associated with high levels of the ER stress marker Ddit3 (**Figure 4G**), indicating
202 that the ER stress response is engaged in PTCs following IRI, and that in this condition, Qprt
203 expression is repressed.

204 ER stress induced by Tun, BFA, thapsigargin (Tg, an inhibitor of the sarco/endoplasmic
205 reticulum Ca(2+)-ATPase (SERCA)), dithiothreitol (DTT, a reducing agent) and glucose
206 starvation activated the UPR and repressed QPRT levels in HK2 cells (**Figure 5A to C**),
207 whereas other key enzymes of the NAD⁺ biosynthesis and salvage pathways, including
208 indoleamine 2,3-dioxygenase 1 (IDO1), ACMSD, nicotinic acid phosphoribosyl transferase
209 (NAPRT) and nicotinamide phosphoribosyl transferase (NAMPT), were not affected by ER
210 stress (**Suppl. Figure 9**). Time-course analysis of QPRT transcripts levels in HK2 cells after
211 ER stress induction showed QPRT down regulation starting 4h after stimulation (**Figure 5D**
212 and **E**). The transcription factor DDIT3 has a repressive activity on numerous target genes
213 (24)(25)(26)(23), leaving the possibility that it could repress QPRT transcription. siRNA-
214 mediated RNA interference against DDIT3 attenuated QPRT reduction upon ER stress (**Figure**
215 **5F**). The over-expression of DDIT3 in non-stressed cells had no impact on QPRT expression
216 (**Figure 5G**), suggesting that additional factors embedded in the ER stress response are
217 required for DDIT3 to repress QPRT. These findings were replicated in primary cultured PTCs
218 (**Suppl. Figure 10**). Together, these results indicate that ER stress impairs QPRT expression
219 and that DDIT3 may participate in QPRT repression.

220 **Discussion**

221 A better understanding of the cellular and molecular processes activated in response to kidney
222 injury, a better characterization of the structural determinants that foster progression from AKI
223 toward CKD, and the identification of biomarkers of ongoing tissue injury in individuals during
224 the early stages of the disease are required to improve patient care in nephrology. Our findings
225 link the ER stress response to fundamental changes in PTCs biology associated with AKI and
226 AKI to CKD progression. Furthermore, this study provides a comprehensive characterization
227 of non-invasive metabolic biomarkers of kidney disease that ultimately could be used to
228 optimize therapies to slow disease progression.

229 We provide evidence for the first time that the ER stress response has a repressive role in
230 NAD⁺ biosynthesis. The relationship between ER stress and cellular energetic metabolism is

231 complex, and multiple pathways appear to be at work (27). Our results suggest a novel
232 metabolic role for the UPR in modulating NAD⁺ biosynthesis, possibly mediated by DDIT3. The
233 consequences of these findings are multiple and raise several avenues for further
234 investigation. The landscape of the biological impact of de novo NAD⁺ down regulation appears
235 endless given the hundreds of redox reactions involving NAD⁺/NADH, and dozens of reactions
236 involving NAD⁺ consumption throughout the cell (28). Considering that NAD⁺ is a key
237 modulator of mitochondrial homeostasis, a prime issue to be addressed is the way the UPR
238 affects mitochondrial function and biogenesis. Indeed, the kidney is second only to the heart
239 in the abundance of mitochondria, and the elevated energetic burden may render the kidney
240 especially vulnerable to ischemia. This also implies to better understand the heterogeneity of
241 individual cellular stress responses and cell fate in face of IRI. A subset of PTCs may fail to
242 recover from acute stress and face an unresolved ER stress that may ultimately become
243 deleterious and may prompt progression toward irreversible lesions. One could envision that
244 early events that affect NAD⁺ contents in a proportion of PTCs imprint a metabolic memory in
245 these cells, possibly through altered sirtuins activity and epigenetic reprogramming, and which
246 would endow them with a maladaptive phenotype, fuelling fibrogenesis, inflammation and
247 progression to CKD if a critical threshold of PTCs number is reached.

248 Our results suggest that DDIT3 could participate in the attenuation of QPRT transcription,
249 which contributes to the expanding body of data indicating that DDIT3 is not only an apoptotic
250 regulator but carries out non-apoptotic functions as well. It is clear that DDIT3 alone is not
251 sufficient to inhibit metabolic gene expression because overexpressed DDIT3 was not
252 associated with QPRT repression in the absence of ER stress. This observation suggests that
253 ER stress signals are necessary to potentiate its repressive action. Our observations are
254 reminiscent of what have been described for the suppression of genes encoding the
255 transcriptional regulators of lipid metabolism such as PPAR α , or SREBF1 by DDIT3 (25),
256 raising the possibility that a regulatory program is engaged upon ER stress, and which could
257 affect metabolic genes expression in a similar manner. In the case of NAD⁺ biosynthesis

258 impairment upon ER stress, the precise mechanisms by which DDIT3 repress QPRT upon ER
259 stress remain to be examined in detail. This transcriptional regulation of QPRT is not exclusive,
260 and ER stress may affect QPRT enzyme activity through other mechanisms.

261 Our highlight the fact that a molecular reprogramming process occurring within a stressed cell
262 in an injured tissue can be non-invasively detected and quantified and provide information
263 regarding the magnitude of the damage. Implementing uQ/T as a non-invasive biomarker to
264 improve prediction performance will provide a validated tool to increase the earliness and
265 specificity of the diagnosis of kidney injury, which is a major prerequisite for successful
266 intervention. It is anticipated that the earlier that therapy can be started, the greater the
267 chances are to stop the pathological process. In agreement, the detection of increased uQ/T
268 could lead to a better stratification of the risk of kidney disease progression. uQ/T is a new
269 monitoring tool that could be routinely used in the follow-up of individuals with kidney disease.
270 Individuals at high risk of disease progression will benefit from early nephroprotective
271 strategies whose purpose is to increase intracellular NAD⁺ contents, and that would slow
272 disease progression.

273 In conclusion, ER stress impairs de novo NAD⁺ biosynthesis and the transcription factor DDIT3
274 may be involved in QPRT repression. Unresolved ER stress and QPRT down regulation
275 constitute a signature of the transition from AKI to CKD. Finally, Elevated uQ/T levels reflect
276 the severity of tissue damage upon acute kidney allograft injury and are predictive of kidney
277 outcomes and chronic kidney damage.

278

279 **Methods**

280 **Study populations**

281 *HEGP Cardiopulmonary bypass cohort for urinary metabolome*: From 17 February 2017 to 26
282 April 2017, 42 patients undergoing scheduled cardiac surgery with CPB were enrolled.
283 Detailed information on the cohort is available in the Supplementary file. The exclusion criteria
284 were: an eGFR <30 ml/min/1.73 m², infusion of a radio contrast agent within the 24 h before
285 surgery, a preoperative left ventricular ejection fraction <40%, age <18 years, pregnancy, and
286 the inability to provide consent. AKI was diagnosed according to the KDIGO Clinical Practice
287 Guideline for AKI criteria (<http://kdigo.org/>) in measuring serum creatinine concentrations and
288 urine output after the surgery. All medications acting on the renin-angiotensin system and all
289 diuretics were stopped on the day before surgery. Lost blood was recovered using Cell Saver®
290 Elite® (Haemonetics™, France) and re-transfused when possible. Vasotropic or inotropic
291 agents, fluids and transfusion products were administered at the discretion of the
292 anesthesiologist based on clinical, echocardiographic and biological findings. After surgery, all
293 patients were transferred to the cardiovascular intensive care unit. Hemodynamic data were
294 extracted from the Philips system using IXTREND® software version 2.1.0 FW14 (Ixellence
295 GmbH, Germany). MAP values were recorded every 1.25 sec during the CPB procedure.
296 Clinical data were prospectively extracted from the hospital's electronic medical records. Urine
297 samples were collected in Corning 50-ml conical tubes and centrifuged at 2,000 g for 20 min
298 within 4 h of collection. Cell pellets were conserved in 300 µL of RLT® buffer (Qiagen™,
299 France) and stored until mRNA extraction. Supernatants and cell pellets were stored at -80°C
300 until analysis. All clinical data and samples were de-identified. Urine samples of 41 patients
301 were technically adequate for further analysis. These urine samples used in this study have
302 been part of a previously published analysis (29).

303 *Leuven Kidney transplant recipients cohort for kidney allograft RNA-seq*: 41 KTR were enrolled
304 at the University Hospitals of Leuven (12). In each case, protocol biopsy was performed at four
305 different time points: before implantation (PRE, kidney flushed and stored in ice), after
306 reperfusion (POST, at the end of the surgical procedure) and 3 and 12 months after
307 transplantation. Genome-wide gene expression profiling using RNA-seq was performed in
308 kidney allograft recipients as previously described (12). Based on RNAseq profiling of protocol
309 biopsies, a computational model to characterize the transition from AKI to CKD has been
310 developed, as previously described (12). Briefly, a pseudotime analysis including all 3-months
311 and 12 months samples was performed to cover this transition. The pseudotime line separated
312 into 2 branches: a branch with transcriptomes depicting the progression to fibrosis over time

313 (called "transition" and "progression"), and an opposite branch with transcriptomes moving
314 toward recovery (called "recovery").

315 *Necker kidney transplant recipients cohort for urinary metabolome:* All of the consecutive
316 patients (405) who received a kidney transplant at our center from January 2010 to June 2012
317 were considered for this prospective, longitudinal, single-center cohort study. The reasons for
318 exclusion were: non-inclusion criteria (n=48), primary non-function/early graft loss (n=12),
319 other study with urine monitoring (n=16), patients death within the first 6 months (n=7), and
320 early loss of follow-up (n=22). Post-transplantation, urine was collected on day 10 for 237 out
321 of 300 individuals initially included in the study. Glomerular filtration rate was measured using
322 Iohexol clearance calculation. Urine samples were centrifuged at 1,000g for 10 min within 4 h
323 of collection. The supernatant was collected after centrifugation and stored with protease
324 inhibitors at -80°C. These urine samples used in this study have been part of a previously
325 published analysis (30).

326 *HEGP Chronic Kidney Disease cohort for urinary transcripts monitoring:* Between November
327 2017 and February 2018, 55 consecutive individuals who were referred to the Nephrology
328 Department at the Georges Pompidou European Hospital (Paris, France) for a kidney biopsy
329 were evaluated for potential inclusion in the study. Indications for biopsy were estimated GFR
330 < 60ml/min and/or proteinuria > 0.5 g/l. Kidney biopsies were only for patient care. At the time
331 of biopsy, urine samples were collected for routine clinical chemistry analyses and stored at -
332 80°C. Detailed information regarding the clinical, medical, demographic, biological, and
333 histological status of the patients was collected using an information-based data warehouse.

334 **Study approvals**

335 *HEGP Cardiopulmonary bypass cohort:* this single-center, prospective, pilot study was
336 approved by the French ethical committee on 7 February 2017 (CPP Sud Est III n° 2016-072
337 B) and registered under the EudraCT n° 2016-A01871-50. All patients provided written consent
338 for study participation and for the biological analysis before inclusion.

339 *Leuven Kidney transplant recipients cohort for kidney allograft RNA-seq:* patients were
340 enrolled at the University Hospitals of Leuven (12). Participants provided written consents, and
341 the local ethic committee approved this consent procedure and studies of human.

342 *Necker kidney transplant recipients cohort:* This study was approved by the Ethics Committee
343 of Ile-de-France XI (#13016), and all of the participating patients provided written informed
344 consent.

345 *HEGP Chronic Kidney Disease cohort.* Analyses were performed anonymously. The patients
346 were informed of the study and did not object to the use of their clinical and biological data
347 collected within the framework of the care exclusively. Data management complies with French
348 reference methodologies.

349 *Animal experiments.* Experiments were conducted according to French veterinary guidelines
350 and those formulated by the European Commission for experimental animal use (L358–
351 86/609EEC) and/or to the Guide for the Care and Use of Laboratory Animals as published by
352 the US National Institutes of Health. Animals were fed ad libitum, had free access to water,
353 and housed at constant ambient temperature in a 12-hour light cycle.

354 **Urine analyses**

355 *Enzyme-linked immunosorbent assays.* Urinary KIM-1 levels were quantified using the KIM-1
356 ELISA immunoassay (R&D Systems), NGAL using the Lipocalin-2/NGAL Quantikine® ELISA
357 Immunoassays (R&D Systems), tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-
358 like growth factor-binding protein 7 (IGFBP7) using TIMP2 ELISA immunoassay (R&D
359 Systems) and IGFBP7 ELISA immunoassay (MyBiosource) respectively, according to the
360 manufacturer's protocol. Multiplication of the two markers ([TIMP-2]*[IGFBP7]) was performed
361 as previously described (31). Because the distribution of the values of urinary KIM1, NGAL,
362 RBP and TIMP2*IGFBP7 concentrations was skewed, we performed analyses using log-
363 transformed values to obtain a Gaussian distribution, but this did not change the results of the
364 comparative study.

365 *Clinical chemistry analyses.* The urine protein measurements were performed at the Clinical
366 Chemistry Department of the Hôpital Européen Georges Pompidou. The urinary levels of
367 retinol binding protein (RBP) were measured using a Siemens BN II nephelometer Analyzer II
368 and kits from Siemens. Values considered normal were < 0.5 mg/L.

369 *Urine mRNA processing.* Urine samples from the CKD HEGP cohort were collected in Corning
370 50-ml conical tubes and centrifuged at 2,000 g for 20 mins within 4 h of collection. Cell pellets
371 were conserved in 300 µL of RLT® buffer (Qiagen™, France) and stored until mRNA
372 extraction. Supernatants and cell pellets were stored at -80°C until analysis. RNA was
373 extracted from the pellets using the RNeasy mini kit® (Qiagen™) and reverse-transcribed into
374 cDNAs using TaqMan® Reverse Transcription Reagents (Applied Biosystems™).

375 **Targeted metabolomics**

376 HK2 cells were washed twice with ice-cold PBS, drained, snapped-frozen in liquid nitrogen
377 and stored at -80°C until analyses. After addition of an extraction solution made of 50%

378 methanol, 30% acetonitrile, and 20% water (32) (1 mL/1.10⁶ cells or 500ul for 20 µl urine), the
379 samples were vortexed for 5 min at 4°C, and then centrifuged at 16,000 g for 15 min at 4°C.
380 The supernatants were collected and separated by liquid chromatography–mass spectrometry
381 using SeQuant ZIC-pHilic column (Millipore). The aqueous mobile-phase solvent was 20-mM
382 ammonium carbonate plus 0.1% ammonium hydroxide solution and the organic mobile phase
383 was acetonitrile. The metabolites were separated over a linear gradient from 80% organic to
384 80% aqueous for 15 min. The column temperature was 50°C and the flow rate was 200 µl/min.
385 The metabolites were detected across a mass range of 75-1,000 m/z using the Q-Exactive
386 Plus mass spectrometer at a resolution of 35,000 (at 200 m/z) with electrospray ionization and
387 polarity switching mode. Lock masses were used to insure mass accuracy below 5 ppm. The
388 peak areas of different metabolites were determined using Thermo TraceFinder software using
389 the exact mass of the singly charged ion and known retention time on the HPLC column. Data
390 analysis was performed in the MetaboAnalyst 4.0 software (33).

391 **Human kidney immunohistochemistry**

392 Kidney biopsies were fixed in alcohol-formalin-acetic acid, dehydrated with ethanol and xylene,
393 embedded in paraffin, and cut into 3µm sections. Samples were then deparaffinized,
394 rehydrated and heated for 20 min at 97°C in citrate buffer. Endogenous peroxidase was
395 inactivated by incubation for 10 min at room temperature in 0.3% H₂O₂. Sections were
396 incubated with PBS containing 1:100 anti QPRT (orb317756, Biorbyt). Next, sections were
397 incubated with anti-mouse antibody conjugated with peroxydase labeled polymer (Dako), then
398 visualized with a peroxydase kit (Dako). Finally, the tissue sections were counterstained with
399 hematoxylin.

400 **Animal experiments**

401 *Tunicamycin injection*: 12 weeks-old C57/BL6 background male mice (Charles River
402 laboratories) were intraperitoneally injected with Tunicamycin (Sigma-Aldrich, T7765) (1
403 mg/kg) or vehicle (DMSO) at day 0, and mice were sacrificed 2 days post-injection (n=4-5 per
404 condition). Total RNA was extracted from kidneys using the RNeasy Mini Kit® (Qiagen)
405 according to the manufacturer's protocol.

406 *Induction of Diabetes Mellitus*: Twelve-week-old males of the Sv129J genetic background
407 (Charles River laboratories) were rendered diabetic by Streptozotocin (Sigma-Aldrich, S-0130)
408 (100 mg/kg in sodium citrate buffer pH = 4.5) intraperitoneal injection on 2 consecutive days.
409 Control mice received citrate buffer alone. Mice with fasting glycemia above 300 mg/dL were
410 considered diabetic. Mice were euthanized 10 weeks after the induction of diabetes. (n=3-4
411 per condition). Total RNA was extracted from kidneys using the RNeasy Mini Kit® (Qiagen)
412 according to the manufacturer's protocol.

413 *UmodC147W/+ mouse*: A detailed description of the methods for the of the UmodC147W/+
414 mouse line of the C57BL/6J genetic background was previously reported (34). Public
415 repositories pertaining to the transcriptome of mRNA isolated from whole-kidney tissue from
416 mutant mice and littermate controls at multiple time points, including 12 and 24 weeks (Gene
417 Expression Omnibus [GEO] database accession no. GSE102566) were analyzed for QPRT
418 and DDIT3 expression.

419 *Bilateral Ischemia reperfusion injury*: this procedure has been detailed in (13). A 21-minutes
420 warm IRI was performed, and cohorts of injured mice were examined at: 2 and 4 h; 1, and 2,
421 and 3 days after IRI (n=3-4). Warm renal ischemia-reperfusion injury was performed on 10- to
422 12-week-old (25–28 g) C57BL/6CN male mice.

423 **Bulk RNA-sequencing of kidneys after bilateral IRI.**

424 This procedure has been detailed in (13).

425 **Single-nucleus RNA-seq**

426 snRNAseq data were obtained from an experimental model previously described (8). The
427 experimental protocol is shown in **Suppl. Figure 2**. Seurat v3.2.0 in R v4 was used for
428 analyses, including normalization, scaling, and clustering of nuclei. First, we analyzed each
429 dataset separately and excluded nuclei with less than 150 or more than 8,000 genes detected.
430 We also excluded nuclei with a relatively high percentage of UMIs mapped to mitochondrial
431 genes (>1) and ribosomal genes (>1, for normal kidney sample, and > 2, all other samples).
432 We performed curated doublet removal based on known lineage-specific markers. The
433 samples from different datasets were integrated to avoid batch effect using Seurat standard
434 workflow splitting by dataset. Following ScaleData, RunPCA, FindNeighbours and FindCluster
435 at a resolution of 0.5 were performed. We focused our analysis on control and early time points
436 post IRI (4 and 12 h) resulting in total 34,755 renal nuclei including 19,926 PTCs from 15
437 samples (8 controls and 7 post IRI). Cluster reassignment was performed based on manual
438 review of lineage-specific marker expression. For data visualization we used RunUMAP,
439 FeaturePlot, Dotplot from Seurat and dittoHeatmap from dittoSeq package.

440 **Cells**

441 *HK2 cell line*: Normal human renal epithelial cells of proximal origin (HK-2) were purchased
442 from ATCC/LGC Standards (lot number 60352186), and cultured according to previously
443 published method (35). HK-2 is a cell line derived from primary proximal tubule cells. HK-2
444 cells are cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 5 µg/mL insulin,
445 10 µg/mL human apotransferrin, 500 ng/mL hydrocortisone, 10 ng/mL epithelial growth factor,
446 6.5 ng/mL triiodothyronin, 5 ng/mL sodium selenite, 1% fetal calf serum, 25 IU/mL penicillin,

447 25 µg/mL streptomycin and 10 mM HEPES buffer. These cells lines are Mycoplasma free
448 (Mycoalert Mycoplasma Detection Kit, Lonza). Tunicamycin, thapsigargin, dithiotreitol,
449 brefeldin A, and and etoposide were from Sigma Aldrich.

450 *Primary culture:* human renal epithelial cells were harvested from human nephrectomy
451 specimens removed for renal cell carcinoma, and isolated according to previously published
452 methods, with minor modifications (36). Fragments of non-malignant renal cortex were minced
453 and digested with collagenase IV (250 IU/mL) for three hours at 37°C. Cells were centrifuged
454 and the pellets washed three times with phosphate-buffered saline. Cells were then cultured
455 in Dulbecco's Modified Eagle Medium (DMEM) containing 5 µg/mL insulin, 10 µg/mL human
456 apotransferrin, 500 ng/mL hydrocortisone, 10 ng/mL EGF, 6.5 ng/mL triiodothyronin, 5 ng/mL
457 sodium selenite, 1% FCS, 25 IU/mL penicillin, 25 µg/mL streptomycin and 10 mM HEPES
458 buffer. Cells were incubated at 37°C in 5% CO₂ and 95% air. The characterization of our cellular
459 model has been published previously (37), confirming the proximal descent of the vast majority
460 of the cultured tubular epithelial cells. Experiments were not performed with cells beyond the
461 third passage.

462 **RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)**

463 Total RNA was extracted using the RNeasy Mini Kit® (Qiagen) according to the manufacturer's
464 protocol. Transcript expression levels were quantified through SYBR green RT-qPCR using
465 an ABI PRISM 7900 sequence detector system (Applied Biosystems). Vehicle-treated samples
466 were used as controls, and the fold-changes for each tested gene were normalized to the
467 Ribosomal Protein L13A (RPL13A) housekeeping gene. The relative expression levels were
468 calculated using the $2^{-\Delta\Delta CT}$ method (38). By definition, the expression level of a given gene in
469 control sample, using the $2^{-\Delta\Delta CT}$ method to calculate relative expression levels, is 1. Primers
470 sequences are listed in the **Suppl. Table 1**

471 **Protein extraction and immunoblotting**

472 Cells were washed in PBS and incubted for 30 min at 4°C and in mPER lysis buffer (Thermo
473 Fisher Scientific) with protease (Halt™ Protease Inhibitor Cocktail 100X, Thermo Fisher
474 Scientific) and phosphatase inhibitors (Halt™ Phosphatase Inhibitor Cocktail 100X, Thermo
475 Fisher Scientific). Extracts were centrifuged at 14,000 xg for 15 min. Protein concentrations in
476 the supernatant were measured by using a Pierce BCA Protein Assay Kit (Thermo Fisher
477 Scientific) and Tecan Safire® plate reader. Protein extracts (25 µg) were resolved by 4-12%
478 SDS-PAGE (Invitrogen) and transferred to nitrocellulose membranes (iBlot, Invitrogen).
479 Membranes were blocked with SEABLOCK blocking buffer (Thermo-Scientific) for 1 h at room
480 temperature and then incubated overnight at 4°C with primary antibody diluted in blocking

481 buffer. Primary antibodies were anti QPRT (HPA011887, Sigma-Aldrich), anti DDIT3 (L63F7,
482 Cell Signaling Technology), anti PARP (ref 95424, Cell Signaling Technology), anti BiP
483 (GRP78) (sc-1050, Santa Cruz Biotechnology), anti-PERK (C33E10, Cell Signalling
484 technology), anti eiF2 α (#9722, Cell Signaling Technology), anti phospho-eiF2 α (#9721, Cell
485 Signaling Technology), anti-Tubulin (T9026, Sigma-Aldrich). After washings in PBS-Tween
486 buffer, membranes were incubated with secondary antibody coupled to IRDye fluorophores.
487 Infrared signal of membranes was revealed using an Odyssey detection system (Li-Cor
488 biosciences, Lincoln, NE, USA).

489 **siRNA transfections**

490 The transient inactivation of DDIT3 was achieved using small interfering synthetic RNAs
491 (siRNAs) designed and obtained from Qiagen and transfected using HiPerFect® (Qiagen)
492 according to the manufacturer's protocol. Two different siRNA directed against the same target
493 were transfected: Hs_DDIT3_1 FlexiTube siRNA (ref SI00059528) and
494 Hs_DDIT3_3 FlexiTube siRNA (ref SI00059542). AllStars Negative Control siRNA (5'-
495 AACGAUGACACGAACACACTT-3') has no homology to any known mammalian gene, and
496 validation has been performed using Affymetrix GeneChip arrays and a variety of cell-based
497 assays to ensure minimal nonspecific effects on gene expression and phenotype. Cells were
498 incubated with siRNA for 24 h before conducting the experiments.

499 **Expression vectors**

500 Cells were cultured at 37°C in 5% CO₂ and were studied while subconfluent. Transient
501 transfection of the gene DDIT3_OHu16873C_pcDNA3.1(+) cloned by HindIII/BamHI in a
502 pcDNA3.1 vector (Cat No: OHu16873C, Gene script) was performed using Lipofectamine 2000
503 (Invitrogen) according to manufacturer instructions. pcDNA3.1 vector was used as a control.
504 After, 36 h of transfection, cells were harvested for mRNA and proteins preparation.

505 **NAD measurement**

506 The levels of NAD⁺ and intracellular NADH were measured by a colorimetric enzymatic test
507 (BioVision®, # K337-100) according to the manufacturer's protocol. The cells of a determined
508 number of wells containing the 2.10⁵ cells required for each test were washed with ice-cold
509 PBS, lysed with 400 μ L of extraction buffer by two cycles of freeze-thaw (freezing 10 min at -
510 80°C, thaw 10 min on dry ice), harvested in tubes, vortexed for 10 s and centrifuged at 19,000
511 g for 5 min at 4°C. Concentrations of NAD⁺ or NADH in cell lysates were measured at 450 nm
512 against a calibration range with an Infinite 200 Plate Reader (TECAN). The levels of the
513 dinucleotides were expressed per million cells.

514 **PARP activity**

515 The levels of PARP activity were measured by ELISA (PARP/Apoptosis Colorimetric Assay Kit
516 Catalog Number: 4684-096-K, RD Systems) according to the manufacturer's protocol. $5 \cdot 10^4$
517 cells/200 μ L fresh medium/well in a 96 well flat-bottom plate were incubated 24 h for ER
518 stressors or 10 mM Etoposide. 25 μ L Samples containing 200 μ g proteins were evaluated in
519 triplicate. PARP activity in samples was evaluated in semi-quantitatively detecting PAR
520 deposited onto immobilized histone proteins in a 96-well format. An anti-PAR monoclonal
521 antibody, goat anti-mouse IgG-HRP conjugate, and HRP substrate are used to generate a
522 colorimetric signal (450 nm). Absorbance correlates with PARP activity.

523 **Statistical analysis**

524 Graphs were generated using GraphPad Prism 7 Software (GraphPad Software, Inc.).
525 Statistical analyses were performed with JMP.10 software (SAS Institute Inc). Student's t tests
526 were 2 tailed and one-way ANOVA was performed when indicated. A p value less than 0.05
527 was considered significant.

528 **Data availability**

529 RNA-seq data for human kidney transplant biopsies are available at GEO (GSE126805).
530 Transcriptomic data for human diabetic kidneys are available at GEO (GSE30122).
531 RNA-seq data for mouse IRI are available at GEO (GSE52004).
532 RNAseq data for UmodC147W/+ kidneys and UMOD-expressing epithelium are available at
533 GEO (GSE1102566).
534 RNAseq and proteomic data for rat microdissected tubules are available at GEO (GSE56743
535 and PXD16958).
536 RNAseq data for renal cell lines are available at GEO (GSE135441, GSE 111837 and
537 GSE99701).
538 snRNA-seq data are available at GEO (GSE151167, GSE139107, GSE163863).
539 Raw data of metabolomics analyses and clinical data are available from the corresponding
540 authors upon reasonable request.

541 **Author contributions**

542 N.P. conceived and designed the project. N.P., Y.B., A.R. Z.N., and V.P., performed
543 experiments. N.P. analyzed CBP datasets. I.N. performed metabolomic analyses. A.H. and
544 P.G. validated data interpretation. O.L. provided diabetic mice kidneys. B.F and P.WR.
545 generated the CPB database urine biocollection. A.K. generated HEGP CKD datasets. M.N.
546 and P.E.C. provided RNA-seq data from kidney allograft recipients and P.E.C. performed
547 analyses. D.A. generated Necker's KTR datasets and urine biocollection. P.E.C. and A.R.

548 conducted snRNA-seq experiments and analysis. N.P and P.E.C. wrote the manuscript. First
549 authorship order was assigned according to the relative amount of data generated in the
550 project in agreement with the 3 co-authors (Y.B, A.R. and Z.N.).

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554 Biomédecine.

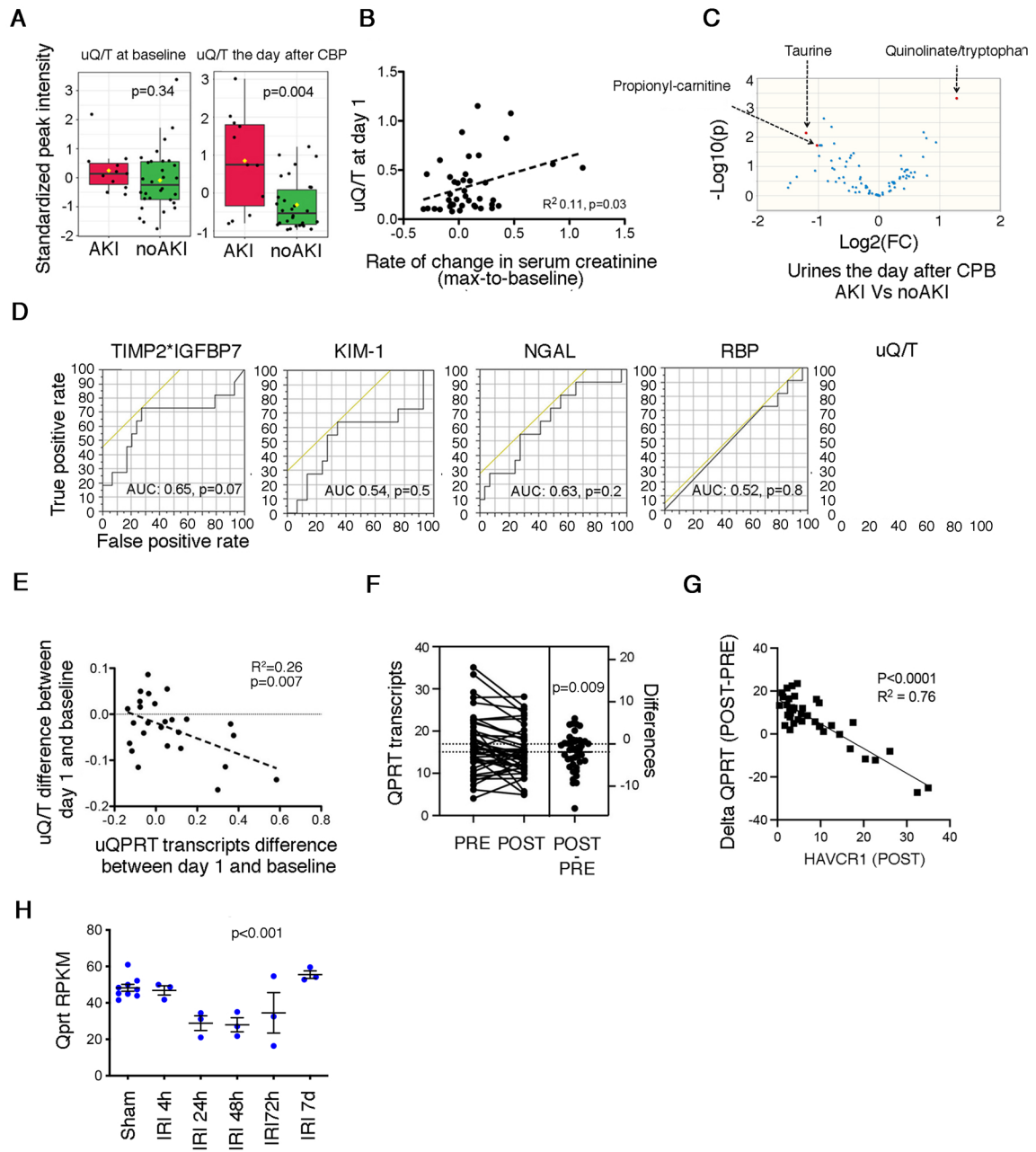
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- 641
- 642

FIGURE 1



644 **Figure 1. Impaired NAD⁺ biosynthesis is a very early event in AKI**

645 **A.** Distribution of quinolinate/tryptophan (uQ/T) levels measured in urine at baseline, and the
646 day after cardiopulmonary bypass (CPB), according the occurrence of AKI during the week
647 after CPB.

648 **B.** Correlation between quinolinate/tryptophan (uQ/T) levels measured in urine the day after
649 CPB and the rate of variation in serum creatinine between the baseline and the maximum
650 value in the week after CPB. P value was computed with a Student's t-test.

651 **C.** Volcano plot comparing urinary metabolites of 41 patients collected 24 h after CPB and who
652 eventually developed acute kidney injury the week after surgery.

653 **D.** Receiver operating characteristic curves for the association between the concentrations of
654 urinary TIMP-2*IGFBP7, KIM-1, NGAL, RBP, and uQT collected the day after CPB and the
655 occurrence of AKI the week after surgery. p values were computed with a Chi2 test, n=41.

656 **E.** Increase of uQ/T levels between the day after CPB and baseline as a function of increase
657 of urinary QPRT transcripts levels between the day after CPB and baseline. P value was
658 computed with a Student's t-test.

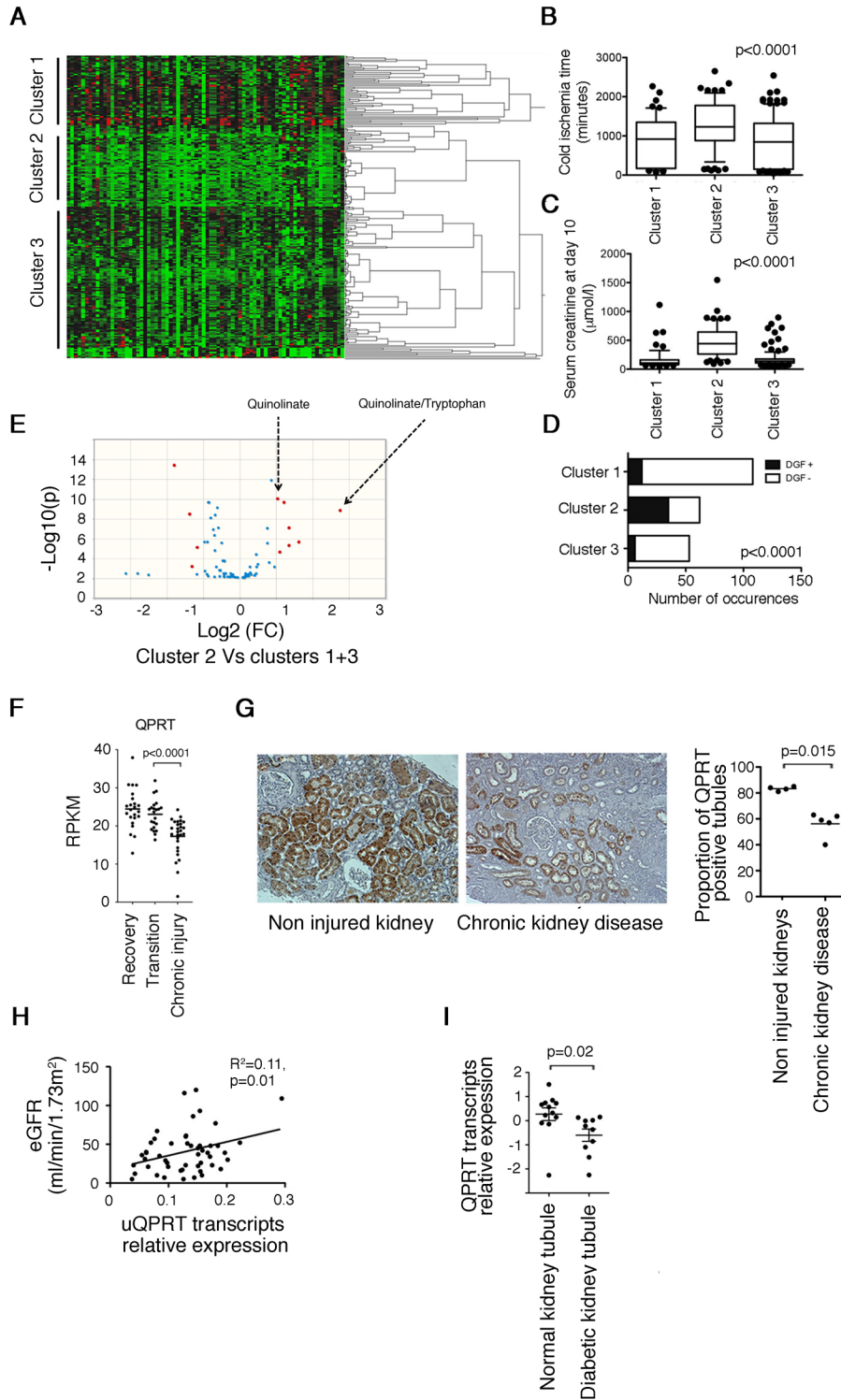
659 **F.** Expression of QPRT measured by RNA-sequencing (RNA-seq) of mRNA isolated from
660 whole-kidney biopsies in a cohort of 42 KTR before implantation (PRE) and shortly after the
661 restoration of the blood flow (POST). p value was computed with a Student's t-test.

662 **G.** Decrease of QPRT transcripts levels as a function of the increase in HAVCR1 transcripts
663 levels measured by RNA-sequencing (RNA-seq) of mRNA isolated from whole-kidney biopsies
664 in a cohort of 42 KTR shortly after the restoration of the blood flow (POST). P value was
665 computed with a Student's t-test.

666 **H.** Expression of Qprt transcripts measured by RNA-seq of mRNA isolated from whole-mice
667 kidneys examined at different time points following bilateral ischemia-reperfusion injury (IRI):
668 (3 to 4 mice per condition). p value was computed with Oneway ANOVA.

669

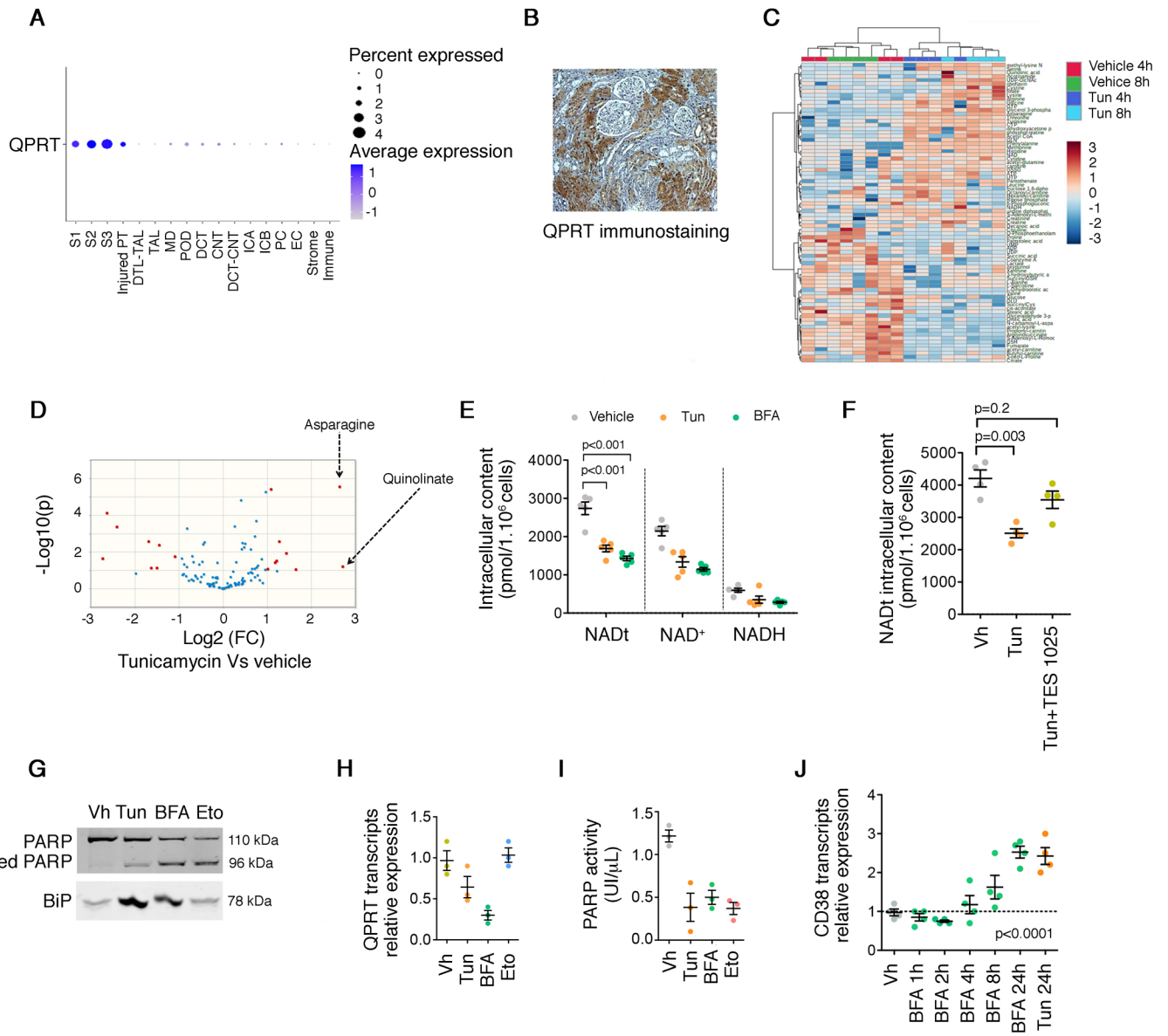
FIGURE 2



670 **Figure 2. Persistent QPRT reduction is associated with progression to chronic kidney**
671 **disease**

- 672 **A.** Hierarchical clustering of 237 urine samples collected 10 days after kidney transplantation.
673 **B.** Distribution of cold ischemia times, according to the clusters identified by hierarchical
674 clustering in (A). p value was computed using one-way ANOVA.
675 **C.** Distribution of serum creatinine 10 days after transplantation, according to the clusters
676 identified by hierarchical clustering in A. p value was computed using one-way ANOVA.
677 **D.** Proportion of delayed graft function (DGF, need for a dialysis session during the 7 days
678 after transplantation) events according to the clusters identified by hierarchical clustering in A.
679 p value was computed using one-way ANOVA.
680 **E.** Volcano plot comparing urinary metabolites of 273 patients collected 10 days after kidney
681 transplantation in cluster 2 or clusters 1+3. Urinary quinolinate/tryptophan (uQ/T) remained
682 significantly increased when using FDR adjusted p values.
683 **F.** Expression of QPRT measured by RNA-sequencing (RNA-seq) of mRNA isolated from
684 whole-kidneys in the group of 42 kidney transplant recipients who recovered or progressed to
685 fibrosis according to the computational model described in (12) which identified 2 main
686 transcriptional trajectories leading to kidney recovery or to sustained injury with associated
687 fibrosis and renal dysfunction. p values were computed using Student's t-test.
688 **G.** Representative photomicrograph of human QPRT expression evaluated by
689 immunohistochemistry in kidney from an individual without chronic kidney disease (left) and
690 advanced chronic kidney disease (right). Original magnification x40. The distributions of QPRT
691 positive tubules sections corresponding to each condition (n=4) were compared with a
692 Student's T test.
693 **H.** Correlation between urinary QPRT transcripts levels and eGFR in 55 patients explored for
694 a chronic kidney disease. P value was computed with a Student's t-test.
695 **I.** Relative expression of QPRT transcripts in diabetic kidney disease tubulointerstitium
696 samples (n=10) compared with control samples (n=12). p value was computed using Student's
697 t-test. Data are from public repositories (NCBI accession GSE126805).
698

FIGURE 3



699 **Figure 3. ER stress reduces de novo NAD⁺ biosynthesis in the kidney**

700 **A.** Qprt gene expression in the renal cell types identified by snRNAseq of 8 control kidney
701 samples (25,896 cells).

702 **B.** Representative photomicrograph of human QPRT expression evaluated by
703 immunohistochemistry in kidney. Original magnification x40.

704 **C.** Hierarchical clustering of HK2 cells incubated with 2.5 µg/mL of Tun or DMS or vehicle for
705 4 h and 8 h (4 replicates).

706 **D.** Volcano plot comparing urinary metabolites of HK2 cells incubated with or without 2.5 µg/mL
707 Tun for 8 h.

708 **E.** Concentrations of total NAD concentrations, NAD⁺ and NADH in HK2 cells incubated with
709 2.5 µg/mL Tun or 5 µg/ml BFA or DMSO for 24 h (4-5 replicates). Bars represent mean±sem.
710 p value was computed with a Dunnett's multiple comparison test.

711 **F.** Concentrations of total NAD concentrations, NAD⁺ and NADH in HK2 cells incubated with
712 2.5 µg/mL Tun alone with or without 100 µmol/L of TES 1025 for 24 h (3-4 replicates). Bars
713 represent mean±sem. p value was computed with a Dunnett's multiple comparison test.

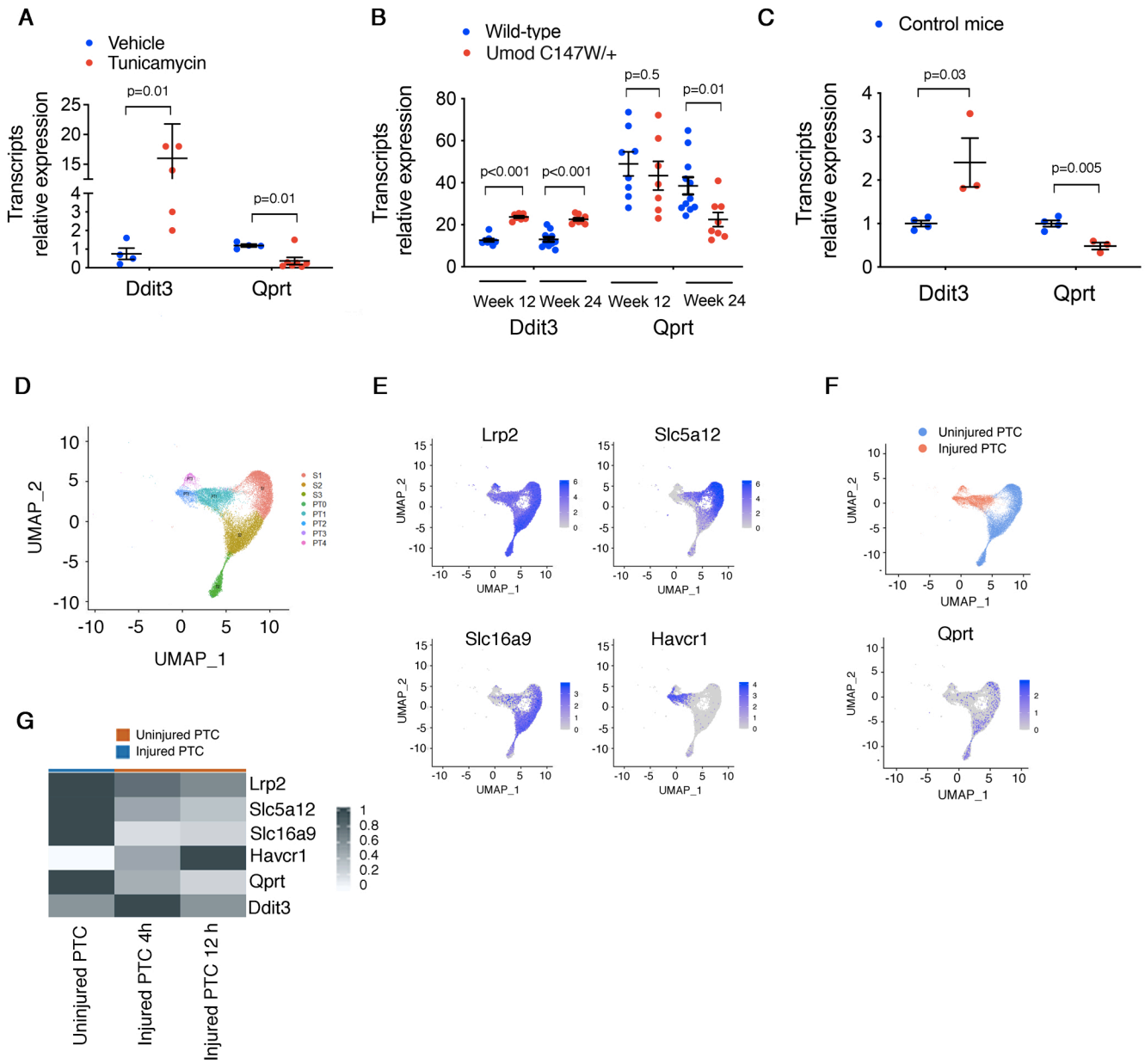
714 **G.** Immunoblot representing PARP, its cleaved fragment and Bip expression in HK2 24 h after
715 incubation with DMSO, 2.5 µg/mL Tun, 5 µg/ml BFA, or 100 µM Eto. The immunoblot shown
716 is representative of 3 independent experiments.

717 **H.** Relative expression of QPRT measured by RT-qPCR in HK2 cells incubated with 2.5 µg/mL
718 Tun, 5 µg/ml BFA, 100 µM Eto or DMSO for 24 h (3 replicates per condition). Bars represent
719 mean±sem.

720 **I.** the PARylation activity of PARP in HK2 cells incubated with DMSO, 2.5 µg/mL Tun, 5 µg/ml
721 BFA, or 100 µM Eto (3-4 replicates). Bars represent mean±sem.

722 **J.** Relative expression of CD38 measured by RT-qPCR in HK2 cells incubated with 2.5 µg/mL
723 Tun or 5 µg/ml of BFA, or DMSO for 24 h (3-4 replicates per condition). Bars represent
724 mean±sem. p value was computed with Oneway ANOVA.
725

FIGURE 4



726 **Figure 4. QPRT expression is repressed in response to kidney injuries associated with**
727 **ER stress**

728 **A.** Expression of *Ddit3* and *Qprt* transcripts by q-PCR in kidneys cortex of mice 48h after
729 intraperitoneal injection of 1 mg/kg Tun or DMSO (4 to 5 mice per condition). Bars represent
730 mean±sem. p values were computed with a Student's t-test.

731 **B.** Expression of *Ddit3* and *Qprt* transcripts by RNA-seq in whole-kidneys of 12 and 24 weeks
732 old *UmodC147W/+* mice and wild type mice (5 to 10 mice per condition). Data are from public
733 repositories (NCBI accession GSE102566). Bars represent mean±sem. p values were
734 computed with a Student's t-test.

735 **C.** Expression of *Ddit3* and *Qprt* transcripts by q-PCR in kidneys cortex of diabetic mice. Bars
736 represent mean±sem. p values were computed with a Student's t-test.

737 **D.** UMAPs of 7 mouse IRI and 8 control kidney samples analyzed by snRNAseq (n=19,926
738 cells) identify segments of the proximal tubule (S1, S2, S3) and new (injured) proximal tubule
739 clusters (PT1, PT2, PT3).

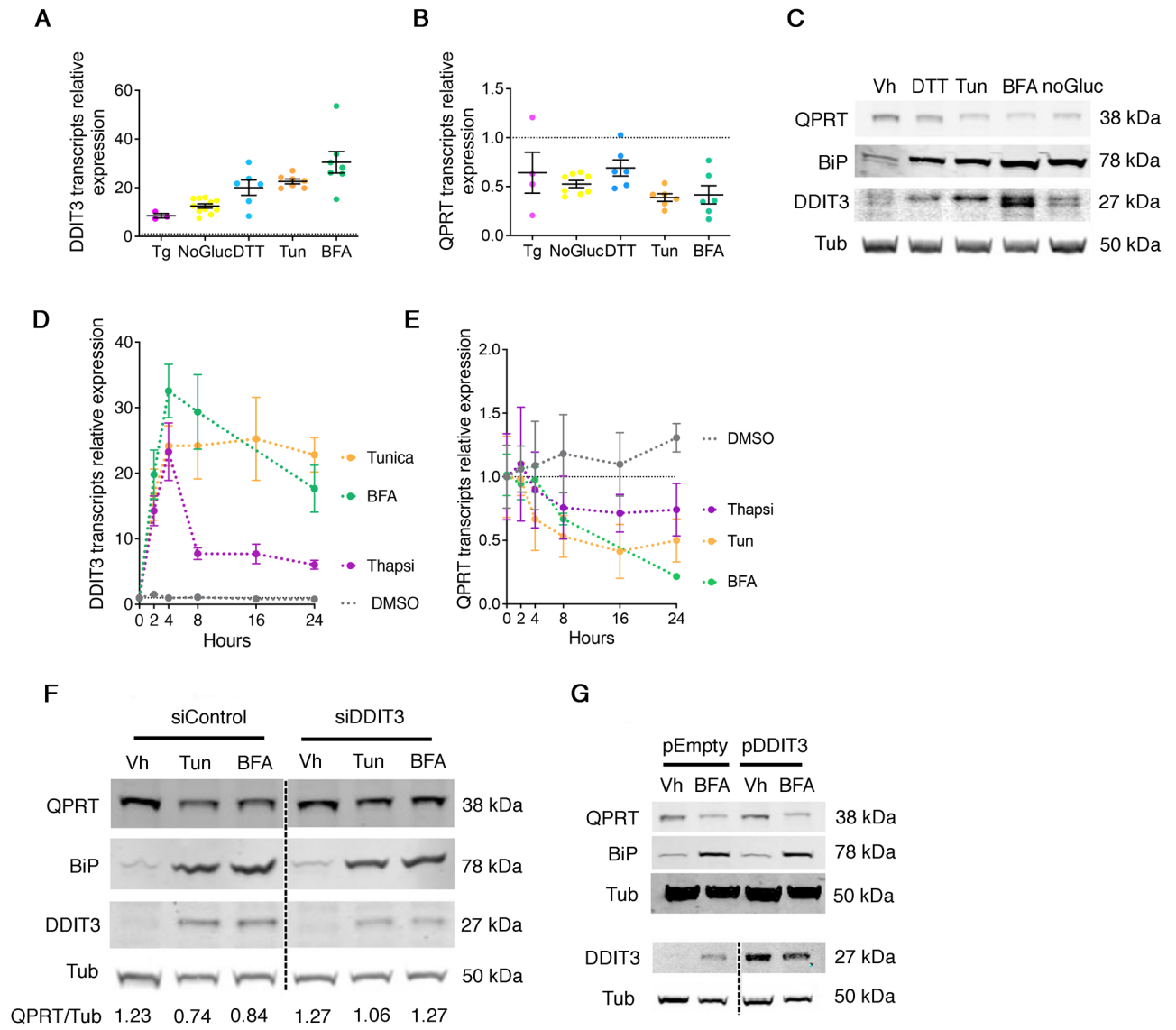
740 **E.** UMAPs of 7 mouse IRI and 8 control kidney samples analyzed by snRNAseq (n=19,926
741 cells) identify the expression of differentiation markers (*Lrp2*, *Slc5a12* and *Slc16a9*) and injury
742 marker (*Havcr1*) in segments of the proximal tubule (S1, S2, S3) and new proximal tubule
743 clusters (PT1, PT2, PT3)

744 **F.** (Left). UMAP of 7 mouse IRI and 8 control kidney samples analyzed by snRNAseq
745 (n=19,926 cells) highlighting uninjured (blue) and injured proximal tubular cells (PTCs) cells
746 (red) populations. (Right) UMAP of 7 mouse IRI and 8 control kidney samples analyzed by
747 snRNAseq (n=19,926 cells) identify the differential expression of *Qprt* in injured and non-
748 injured PTCs.

749 **G.** Expression of classical differentiation and injury markers over time after IRI in 7 mouse IRI
750 and 8 control kidney samples analyzed by snRNAseq. Each column represents the average
751 expression per cell state in control PTCs, injured PTCs at 4h and 12h). (n=15 samples,
752 n=19,164 cells).

753

FIGURE 5



754 **Figure 5. ER stress reduces QPRT expression in HK2 cells**

755 **A. B.** Scatter dot plots representing the relative expression of DDIT3 (A) and QPRT (B)
756 transcripts measured by RT-qPCR in HK2 cells incubated 8 h with either with 0.25 μ M
757 thapsigargin (Tg), with 1 μ M dithiotreitol (DTT), with 2.5 μ g/ml tunicamycin (Tun), with 5 μ g/mL
758 brefeldin A (BFA), or 48 h in a glucose-deprived culture medium (No Gluc.), and compared
759 with vehicle (DMSO)-treated cells (6-8 replicates). The dashed line represents 1, the reference
760 value of vehicle-treated cells. Bars represent mean \pm sem.

761 **C.** Immunoblot representing QPRT, BiP, DDIT3 and tubulin protein expression in HK2 24 h
762 after incubation with DMSO (vehicle), 1 μ M DTT, 2.5 μ g/ml tunicamycin (Tun), with 5 μ g/mL
763 brefeldin A (BFA), or 48 h in a glucose-deprived culture medium (No Gluc.). The immunoblot
764 shown is representative of 3 independent experiments.

765 **D. E.** Time course analysis of the relative expression of DDIT3 (D) and QPRT (E) transcripts
766 measured by RT-qPCR in HK2 cells incubated with either with vehicle (DMSO), 0.25 μ M
767 thapsigargin (Tg), 5 μ g/mL brefeldin A (BFA), or with 2.5 μ g/ml tunicamycin (Tun) for up to 24
768 h (4 replicates). Bars represent mean \pm sem.

769 **F.** Immunoblot representing the expression of QPRT, BiP, DDIT3 and Tubulin proteins in HK2
770 cells transfected with DDIT3 siRNA (siDDIT3) or with control siRNA (siScramble) and
771 incubated with 2.5 μ g/ml tunicamycin (Tun), 5 μ g/mL brefeldin A (BFA), or with DMSO (vehicle)
772 for 24h. The immunoblot shown is representative of two independent experiments.

773 **G.** Immunoblot representing the expression of QPRT, BiP, DDIT3 and tubulin proteins in HK2
774 cells transfected with a pcDNA3.1 vector expressing DDIT3 or an empty vector, and incubated
775 with 5 μ g/mL brefeldin A (BFA) or with DMSO (vehicle) for 24h. The immunoblot shown is
776 representative of two independent experiments. Bip/QPRT
777

778

779 **Table 1. Determinants of measured glomerular filtration rate 3 months after kidney**
 780 **transplantation (146 KTR, living donors excluded).**

781 Model F ratio 11.8, p<0.0001

Characteristic	Estimate	95% CI	P value
Extended criteria donor ¶¶ (yes)	-7.32	-10 to -4.6	<0.0001
Cold ischemia time (minutes)	-0.0001	-0.006 to 0.006	0.95
Delayed graft function ¶¶¶ (yes)	0.46	-3.3 to 4.3	0.81
Serum creatinine at day 10 (µmol/L)	-0.01	-0.03 to 0	0.048
Urinary Quinolate-to-Tryptophan at day 10	-4.5	-8 to -1	0.01

782

783 ¶¶ Extended criteria donors are normally aged 60 years or older, or over 50 years with at least two of the
 784 following conditions: hypertension history, serum creatinine > 133 µmol/L or cause of death from
 785 cerebrovascular accident.

786 ¶¶¶ Delayed graft function (DGF) is defined as failure of the renal transplant to function immediately, with
 787 the need for dialysis in the first post-transplantation week.

788 Glomerular filtration rate was determined in measuring Iohexol clearance.

789

790 **Table 2. Determinants of measured glomerular filtration rate 12 months after kidney**
 791 **transplantation (104 KTR, living donors excluded).**

Characteristic	Estimate	95% CI	P value
Extended Criteria donor ¶¶ (yes)	-7.82	-10 to -5.2	<0.0001
Cold ischemia time (minutes)	-0.0009	-0.005 to 0.007	0.76
Delayed graft function ¶¶¶ (yes)	-1.71	-5.22 to 1.75	0.33
Serum creatinine at day 10 (µmol/L)	-0.01	-0.03 to -0.003	0.01
Urinary Quinolate-to-tryptophan at day 10	-3.7	-6.9 to -0.61	0.02

792

793 Model F ratio 14.1, p<0.0001

794

795 ¶¶ Extended criteria donors are normally aged 60 years or older, or over 50 years with at least two of the
 796 following conditions: hypertension history, serum creatinine > 133 µmol/L or cause of death from
 797 cerebrovascular accident.

798 ¶¶¶ Delayed graft function (DGF) is defined as failure of the renal transplant to function immediately, with
 799 the need for dialysis in the first post-transplantation week.

800 Glomerular filtration rate was determined in measuring Iohexol clearance.

801

802