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### **Acute kidney injury promotes development of papillary renal cell adenoma and carcinoma from renal progenitor cells.**

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1  
2 **Title: Acute kidney injury promotes development of papillary renal cell adenoma**  
3 **and carcinoma from renal progenitor cells**  
4

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6

## 1 **Abstract**

2 Acute tissue injury causes DNA damage and repair processes involving increased cell mitosis and  
3 polyploidization, leading to cell function alterations that may potentially drive cancer development. Here  
4 we show that acute kidney injury (AKI) increased the risk for papillary renal cell carcinoma (pRCC)  
5 development and tumor relapse in humans as confirmed by data collected from several single center and  
6 multicentric studies. Lineage tracing of tubular epithelial cells (TECs) after AKI induction and long-term  
7 follow-up in mice showed time-dependent onset of clonal papillary tumors in an adenoma-carcinoma  
8 sequence. Among AKI-related pathways, NOTCH1 overexpression in human pRCC associated with  
9 worse outcome and was specific for type 2 pRCC. Mice overexpressing NOTCH1 in TECs developed  
10 papillary adenomas and type 2 pRCCs, and AKI accelerated this process. Lineage tracing in mice  
11 identified single renal progenitors as the cell of origin of papillary tumors. Single-cell RNA sequencing  
12 showed that human renal progenitor transcriptome showed similarities to PT1, the putative cell of origin  
13 of human pRCC. Furthermore, NOTCH1 overexpression in cultured human renal progenitor cells  
14 induced tumor-like 3D growth. Thus, AKI can drive tumorigenesis from local tissue progenitor cells. In  
15 particular, we find that AKI promotes the development of pRCC from single progenitors through a  
16 classical adenoma-carcinoma sequence.

## 1 **Introduction**

2 Chronic tissue injury related to toxin exposure or viral infections are proven factors in carcinogenesis  
3 (1). Whether acute tissue injury and remodeling may promote cancer is purely speculative. Acute tissue  
4 injury causes DNA damage and repair involving extensive proliferation of resident stem cell populations  
5 (2) and/or polyploid genome alterations of differentiated cells (3). Both conditions may facilitate cancer  
6 development. In addition, repair processes trigger signaling pathways that control cell cycle activation,  
7 cell survival, and proliferation, stimulating pathways that drive cancer development (3, 4). Here, we  
8 examine a putative link between acute kidney injury (AKI) and subsequent cancer development. The  
9 kidney provides a unique opportunity to study this possibility, since patients with AKI survive for a long  
10 time even when developing end stage renal disease (ESRD) with supportive or renal replacement  
11 therapies, such as dialysis and kidney transplantation. In both situations, the native kidneys remain *in*  
12 *situ* and allow assessing tumor rates (5).

13 AKI is a global public health concern resulting in 1.7 million deaths per year (6). If not lethal in the acute  
14 phase, AKI is largely considered reversible as indicated by resumed urine production or biomarkers  
15 assessing injury resolution (7). However, even mild AKI episodes give a substantial risk for subsequent  
16 chronic kidney disease (CKD) due to irreversible nephron loss (8). In this study, we hypothesized that  
17 AKI, and particularly ischemia reperfusion injury (IRI), may be a risk factor for one or more types of  
18 renal cell carcinoma (RCC), by inducing tissue repair signaling pathways that may drive neoplastic  
19 growth.

20

## 21 **Results**

22 *AKI is associated with RCC in two different human cohorts*

1 To assess the potential role of AKI insults as a risk factor for RCC development, we reviewed the hospital  
2 database of the Careggi University Hospital, Florence, Italy for all the patients with incident RCC  
3 (n=1618) who underwent surgery between 2012 and 2018 (fig. S1). We retrieved laboratory information  
4 for 468 patients with a minimum follow-up of one year before tumor diagnosis. AKI episodes were  
5 identified in the patients' clinical history in 76/468 (16.2%), corresponding to an incidence rate estimate  
6 of 420 AKI episodes per 10,000 person-years, while the expected rate in the general population is 50 to  
7 150 per 10,000 person-years (9, 10). In detail, comparing our population with the one described by  
8 Sawhney et al. (9) yielded an AKI incidence ratio of 3.2 (95%CI: 2.6-4.1).

9 To confirm the observed association between AKI episodes and RCC, we analyzed a nationwide cohort  
10 study in Denmark (population 5.4 million) using population-based medical databases (11, 12). We  
11 identified all patients with a hospital diagnosis of AKI between 1977 and 2006 (fig. S2). We obtained  
12 data on subsequent incident RCCs and quantified the risk of RCC after AKI. Among 12,480 patients  
13 diagnosed with AKI, 7 (0.06%) were diagnosed with RCC within the first year and 14 (0.1%) were  
14 diagnosed with RCC more than one year after the first AKI episode. Comparing the observed number of  
15 RCC with the expected number computed based on age- and sex-specific cancer incidence rates in the  
16 general Danish population (1.6 in the first year and 6.7 cancer cases in the subsequent years) led to  
17 calculation of standardized incidence ratios for developing RCC of 4.4 (95%CI: 1.7-9.0) one year after  
18 the AKI episode and of 2.1 afterwards (95%CI: 1.1-3.5). These results suggest that AKI episodes  
19 associate with RCC.

20

21 *AKI episodes are a risk factor for pRCC development and recurrence in a single center study*

22 We evaluated whether AKI episodes associated with clear cell RCC (ccRCC) and/or pRCC, the two most  
23 frequent tumor histotypes accounting for approximately 75% and 15% of RCCs, respectively (13). We

1 retrieved clinical and laboratory information for 56 pRCC and 75 ccRCC stage T1 or T2 patients with a  
2 minimum follow-up of one year before tumor diagnosis (Table 1, fig. S1). The frequency of the exposure  
3 (that is previous AKI episodes) was significantly higher in pRCC patients compared to a cohort of 101  
4 in-house non-tumor controls (21.4% vs. 6.9%,  $p = 0.008$ , Table 1). For ccRCC, there was no significant  
5 difference (12.0% vs. 6.9%,  $p = 0.247$ , Table 1). To further investigate the association with AKI episodes,  
6 a binary logistic regression was performed including known risk factors for RCC such as male gender,  
7 age, presence of diabetes mellitus (DM), and CKD as covariates (14). With multivariate analysis, a  
8 previous AKI episode was significantly associated with pRCC development (OR 3.48, 95%CI: 1.14-  
9 10.67) compared to controls (Table 1), but there was no significant correlation in ccRCC patients (Table  
10 1). pRCC can be classified into type 1 and type 2 based on histology (56 pRCC patients: 21 type 1, 28  
11 type 2, and 7 undefined). Among the 28 patients affected by type 2 pRCC, 7 had a previous AKI episode  
12 (25% vs. 6.9% of controls  $p=0.012$ ), while out of the 21 type 1 patients, 3 had AKI (14.3% vs. 6.9%  
13  $p=0.37$ ). The remaining 2 patients with AKI had unclassified pRCC histotype. These findings suggest  
14 that AKI could be a risk factor for pRCC development and particularly for the type 2 subtype.

15 Resection of localized pRCC is frequently associated with postoperative AKI, because of renal artery  
16 clamping, parenchymal mass reduction, intraoperative hypotension, and decreased renal perfusion  
17 pressure (15). We investigated whether AKI upon pRCC surgery could be a risk factor for tumor  
18 recurrence. In 83 patients, a follow-up of at least one year after nephrectomy for pRCC (mean follow-up  
19  $3.31 \text{ years} \pm 1.84$ ) was available (fig. S1). In this cohort, postoperative AKI occurred in 18/83 (21.7%)  
20 patients, similar to previous reports (16). While the cumulative recurrence frequency was 14/83 (16.9%),  
21 it was significantly more frequent among patients who experienced postoperative AKI compared to non-  
22 AKI patients (7/18, 38.9% vs 7/65, 10.8%,  $p=0.005$ ) (table S1). Five-year recurrence-free survival was  
23 significantly ( $p=0.008$ ) lower in postoperative AKI patients (Fig. 1A). A binary logistic regression was  
24 performed to evaluate the association between postoperative AKI and recurrence, including age at

1 surgery, clinical T stage, and R.E.N.A.L. (radius, exophytic/endophytic properties, nearness of deepest  
2 tumor portion to collecting system or sinus, anterior/posterior, and location relative to polar line)  
3 nephrometry score, a surgical indicator of tumor complexity previously associated with recurrence (17),  
4 as covariates. By multivariate analysis, postoperative AKI was significantly associated with recurrence  
5 (OR 7.24, 95%CI: 1.65-31.86) (table S1). Taken together, these results suggest that an AKI episode could  
6 be a previously unrecognized major risk factor for pRCC recurrence.

7

### 8 *A multicentric study confirms that AKI episodes are a risk factor for pRCC recurrence*

9 To validate the association between AKI and pRCC, we analysed The Italian Registry of Conservative  
10 Renal Surgery (RECORD1 Project), a 4-year prospective observational multicentric study promoted by  
11 the Leading Urological Non-Profit Foundation for Advanced Research (LUNA) of the Italian Society of  
12 Urology (18). Overall, 769 consecutive patients underwent partial nephrectomy for a renal tumor at 19  
13 Italian urological institutions from 2009 to 2012 (fig. S3). For the current study, we extracted data on  
14 594 patients with a histologic diagnosis of RCC and with a minimum of 1-year follow-up after surgery.  
15 Renal function was evaluated at baseline and at 1<sup>st</sup> postoperative day. Table S2 summarizes the baseline  
16 features of the entire cohort.

17 In 89 patients with pRCC histotype, 18 (20.2%) patients experienced local recurrence, while no systemic  
18 recurrences were recorded. Forty-six (51.7%) patients experienced postoperative AKI. In these patients,  
19 a significantly higher rate of local recurrence was found (30.4% vs 9.3% in non-AKI patients; p=0.01)  
20 (table S3). Survival analysis s showed a significantly (p=0.021) lower 5-year recurrence-free survival in  
21 patients experiencing a postoperative AKI episode compared to those without (Fig. 1B). By multivariate  
22 analysis, the association between postoperative AKI and tumor recurrence remained significant

1 (p=0.023) after adjustment for age at surgery and clinical T stage (table S3). Taken together, these results  
2 confirm that AKI at surgery is associated with pRCC recurrence.

3  
4 *Long-term follow-up reveals that AKI induces papillary tumors in mice*

5 To experimentally test the hypothesis that AKI promotes tumor development, we induced unilateral  
6 ischemia-reperfusion injury (IRI) in wild-type mice and examined their kidneys in the long term at  
7 different time points (fig. S4A). Development of type 1 as well as type 2 papillary tumors occurred in a  
8 time-dependent manner (Fig. 2, A-H). Over the course of 36 weeks, the lesions were detectable by  
9 ultrasound imaging (Fig. 2, A and B). Papillary tumors ranged from small adenomas with low nuclear  
10 grade to pRCC appearing as larger papillary tumors with high nuclear grade (Fig. 2, C-F). Type 1  
11 papillary tumors appeared as papillae and tubular structures covered with monolayers of small cells  
12 containing basophilic cytoplasm and small, uniform oval nuclei, often forming cysts (Fig. 2C). Type 2  
13 papillary tumors appeared as multilayered papillae covered by large cells with eosinophilic cytoplasm  
14 and large spherical nuclei with prominent nucleoli (Fig. 2D) and frequent high nuclear grade (Fig. 2E)  
15 as described in the most recent World Health Organization (WHO) pRCC classification (19). In addition,  
16 focal accumulation of foamy macrophages in the stroma of the papillary stalks (a highly characteristic  
17 pRCC finding) was also seen in papillary mouse tumors (Fig. 2F). No tumors were found 4 weeks after  
18 AKI, but 55.6% of mice had papillary adenomas at 12 and at 36 weeks (Fig. 2G). At 36 weeks, 22.2%  
19 of mice showed pRCC (Fig. 2G). Overall, 68.75% of the tumors were of type 1 and 31.25% of type 2  
20 (Fig. 2H). In contrast, mice that did not undergo IRI did not develop any tumors (fig. S4B). These results  
21 suggest that AKI is a risk factor for type 1 and type 2 mouse papillary tumors, similar to findings in  
22 humans.

23

1 *AKI-driven papillary tumors originate from proliferation of single mouse tubular epithelial cells*

2 To study the origin of papillary tumors, we evaluated their clonality using a lineage tracing approach  
3 based on the Confetti reporter stochastic labeling of tubular epithelial cells (TECs) at single cell level  
4 with different fluorochromes, that is *Pax8.rtTA;TetO.Cre;R26.Confetti* (Pax8/Confetti) mice (fig. S4, C  
5 and D). Both type 1 (Fig. 2, I and J) and type 2 (Fig. 2, K and L) papillary tumors tagged at 36 weeks  
6 after IRI were mostly of single colors. Indeed, clonal analysis revealed that >95% of type 1 and type 2  
7 papillary tumors were monoclonal (Fig. 2M). Of note, type 1 tumors stained positively for cytokeratin 7  
8 (CK7) (Fig. 2J) while type 2 did not (Fig. 2K), as reported in pRCC patients (13). Additionally, type 2  
9 pRCCs presented extensive nuclear atypia, as shown in Fig. 2L. 3D analysis confirmed that clonal  
10 populations from the primary tumors invaded the kidney parenchyma (Fig. 2N) and that papillary tumors  
11 showed characteristic aberrant vascularization, confirming invasive growth typical of carcinomas (Fig.  
12 2O). Thus, papillary tumors developing after post-ischemic AKI originate from clonal amplification of  
13 single TECs.

14

15 *Human pRCCs associate with altered AKI-activated pathways*

16 To understand how AKI could drive pRCC formation, we speculated that AKI-activated signaling  
17 pathways could promote pRCC pathogenesis. To this aim, we analyzed AKI-activated pathways included  
18 in the protein array deposited in The Cancer Genome Atlas (TCGA),  
19 (<https://cancergenome.nih.gov/cancersselected/kidneypapillary>) from 161 patients with pRCC (20).  
20 Among the pathways involved in post-AKI repair (table S4), a decreased expression of mTOR,  $\beta$ -catenin,  
21 VHL, and an increased expression of NOTCH1 were associated with advanced pRCC stage (T3-T4) at  
22 diagnosis (Fig. 3A). A survival analysis indicated that among them, only increased NOTCH1 associated  
23 with worse prognosis (Fig. 3B and table S4). Low amounts of PTEN and SMAD4 also associated with

1 worse prognosis (Fig. 3, C and D; table S4). Type 1 and type 2 pRCC associated with differences in  
2 expression of distinct AKI-related signaling pathways (Fig. 3E). In particular, high amounts of mTOR,  
3  $\beta$ -catenin, and VHL associated with type 1, while high amounts of NOTCH1 strongly associated with  
4 type 2 (Fig. 3E). These results suggest that pRCC is associated with altered AKI-activated pathways.

5

#### 6 *NOTCH1 overexpression is specific for human type 2 pRCC and predicts poor prognosis*

7 NOTCH1 strongly associates with disease severity and patient survival in pRCC, is highly specific for  
8 type 2 subtype, and has critical importance in AKI repair (21). These specificities prompted us to examine  
9 by the Cancer Genome Atlas (TCGA) Research Network data, focusing our attention on NOTCH1 (20).  
10 The results presented by TCGA Research Network indicate that type 2 pRCC consists of at least three  
11 subtypes based on molecular features (clusters C2a, C2b, and C2c-CIMP) (20). NOTCH1 protein was  
12 increased in all these subgroups, but not in type 1 pRCCs (cluster C1) (Fig. 3F). Type 1 and type 2 pRCCs  
13 are both associated with several gene mutations (20). Germline or somatic mutations of the tricarboxylic  
14 acid cycle enzyme gene fumarate hydratase (*FH*), or the NRF2/antioxidant response element (ARE)  
15 pathway, including *CUL3*, *KEAP1*, *NF2*, and *NFE2L2* (NRF2) (22), can cause sporadic pRCC that is  
16 usually type 2. In contrast, activating germline *MET* mutations cause hereditary type 1 pRCCs (20). Type  
17 1 pRCCs with *MET* mutations did not show increased NOTCH1 (Fig. 3G). In contrast, type 2 pRCCs  
18 characterized by *CDKN2A* silencing, or by *SETD2/BAP1/PBRM1* mutations or by mutations in *FH* or  
19 NRF2–ARE pathway (20), showed increased NOTCH1 protein in comparison to non-mutated tumors  
20 (Fig. 3G). Lifetime survival analysis revealed that NOTCH1 protein amounts correlated with survival in  
21 patients with type 2 pRCC (Fig. 3H). Finally, multivariate analysis showed that NOTCH1 expression in  
22 tumors correlated with prognosis even when compared to known risk factors for pRCC (table S5). These

1 results indicate that the AKI-associated pathway Notch1 is a critical prognostic factor for all subgroups  
2 of type 2 but not for type 1 pRCC.

3

#### 4 *NICD1 overexpression in mouse tubular cells drives clonal type 2 papillary tumors*

5 To verify whether NOTCH1 overexpression could trigger type 2 pRCC development, we generated  
6 *Pax8.rtTA;TetO.Cre;R26.NICD1.GFP* (Pax8/NICD1) transgenic mice with overexpression of the  
7 intracellular portion of the *Notch1* gene (NICD1) in Pax8+ TECs after induction by administration of  
8 doxycycline in drinking water (fig. S5A). In order to verify the effective activation of the transgenes and  
9 subsequent expression of the NICD1 and GFP proteins, we stained Pax8/NICD1 kidney sections with an  
10 anti-GFP antibody. We observed that TECs expressed high amounts of GFP, while interstitial cells  
11 remained negative (fig. S5B). We analyzed the animals at weeks 4, 12, and 36 after transgene induction  
12 (fig. S5C). Blood urea nitrogen (BUN) measurements revealed a progressive decline of renal excretory  
13 function in induced versus uninduced Pax8/NICD1 mice (fig. S5D). Kidney weights and volumes were  
14 higher and increased over time in induced Pax8/NICD1 mice (fig. S5, E and F). Histological analysis  
15 revealed cystic changes (fig. S5G), with or without epithelial hyperplasia or tumors (fig. S5, H and I). In  
16 particular, kidneys of Pax8/NICD1 mice developed numerous papillary tumors in the cortex and outer  
17 stripe of the outer medulla at 12 weeks that increased in size, number, and complexity over time (fig. S5,  
18 H and I). The uninduced Pax8/NICD1 mice did not develop CKD, renomegaly, or other lesions (fig. S5J).  
19 Notably, animals that were homozygous for the *R26.NICD1.GFP* effector transgene (Pax8/NICD1<sup>+/+</sup>)  
20 had significantly more papillary adenomas (pAdenomas)/pRCCs than heterozygotes (Pax8/NICD1<sup>+/-</sup>) at  
21 24-36 weeks (p=0.03 and p=0.005, respectively), suggesting that high NICD1 amounts are critical for  
22 generation of papillary tumors (fig. S5K).

1 All Pax8/NICD1 mice had CKD (fig. S5D) and 93.75% developed papillary tumors at 36 weeks (Fig.  
2 4A), reaching up to 1.6 millimeters in size (Fig. 4B) and detectable by Power Doppler imaging as  
3 vascularized masses (Fig. 4C). Interestingly, papillary tumors consisted of both adenomas (Fig. 4D) as  
4 well as pRCC (Fig. 4, E and F), but were only type 2 (100% of the tumors). Papillary tumors were  
5 negative for carbonic anhydrase IX (CAIX) (Fig. 4G), as reported in human pRCC (13). Strong BCL-2  
6 expression was detected, suggesting resistance to cell death (Fig. 4H).

7 We then evaluated the clonal composition of NOTCH1 overexpression-induced type 2 papillary tumors  
8 by generating *Pax8.rtTA;TetO.Cre;R26.NICD1.GFP;R26.Confetti* (Pax8/NICD1/Confetti) mice (fig.  
9 S5L). After induction, clonal analysis revealed that over 80% of the papillary tumors in  
10 Pax8/NICD1/Confetti mice at 36 weeks were either mono- or biclonal (Fig. 4, I-K, and fig. S5, M-O).  
11 3D analysis confirmed that multiple papillary tumors were interconnected and generated by a clonal  
12 population spreading from the primary pRCC and invading the surrounding kidney tissue (Fig. 4L, video  
13 S1). Primary tumor masses also showed vascularization (Fig. 4M).

14 Tumors invading the surrounding kidney tissue or the kidney capsule and/or metastasizing at distant sites  
15 were observed in all (100%) mice. Interestingly, metastasis was associated with areas that showed  
16 rhabdoid-like morphology, characteristic of high-grade pRCC (23), within the dominant pRCC mass (Fig.  
17 4, N and O), as well as in the metastasis (Fig. 4P). Metastases were also positive for PAX2, confirming  
18 their renal origin (Fig. 4Q). Cytokeratins (PanCK), vimentin, and epithelial membrane antigen (Mucin 1,  
19 cell surface associated (MUC1)) were also expressed, as reported for rhabdoid variants of human RCC  
20 metastases (Fig. 4, R-T) (23). These results suggest that NOTCH1 overexpression promotes development  
21 of papillary adenomas and highly aggressive type 2 pRCCs derived from clonal amplification of single  
22 TECs.

23

1 *AKI accelerates clonal type 2 papillary tumor development in NOTCH1-overexpressing mice*

2 To investigate whether AKI could also act as a tumor-accelerating factor in Pax8/NICD1 mice, we  
3 performed unilateral IRI in induced and uninduced Pax8/NICD1 mice (fig. S6A). While activated  
4 NOTCH1 expression was virtually absent in uninduced healthy mice (Fig. 5A), IRI alone induced strong  
5 NOTCH1 activation in uninduced mice 1 day after injury (Fig. 5B); it remained higher compared to  
6 controls thereafter (Fig. 5C). Strong nuclear NOTCH1 immunostaining was seen, indicating diffuse  
7 NOTCH1 activation in TECs of Pax8/NICD1 mice (Fig. 5D). AKI increased NOTCH1 in Pax8/NICD1  
8 mice (Fig. 5, E and F). We then checked whether AKI could accelerate pRCC formation when acting  
9 together with NICD1 overexpression. Not surprisingly, the combination of IRI and NICD1  
10 overexpression in induced Pax8/NICD1 and Pax8/NICD1/Confetti mice accelerated the formation of  
11 cysts, papillary adenomas, CKD (fig. S6B), and pRCCs in just 4 weeks (Fig. 5, G-I, and fig S6, C and  
12 D). Papillary RCC included foamy macrophages (Fig. 5G), stained positive for PAX2 (Fig. 5H), similar  
13 to human pRCCs, and were mostly of type 2 (Fig. 5J). By contrast, no tumors were observed in uninduced  
14 Pax8/NICD1 or Pax8/Confetti mice with AKI (fig. S6, E and F) or in Pax8/NICD1 and  
15 Pax8/NICD1/Confetti mice without AKI at 4 weeks (Fig. 5I and fig. S6, G and H). Notably, papillary  
16 tumors induced by AKI were clonal (Fig. 5, K and L). 3D analysis confirmed that papillary tumors  
17 showed vascularization and clonal population spread from the primary tumors into the surrounding  
18 parenchyma (Fig. 5, M and N). These results establish AKI as an accelerating factor for papillary tumors.

19  
20 *Human renal progenitors are transformed by NOTCH1 overexpression*

21 The monoclonality of papillary tumors as well as their association with AKI suggested a possible origin  
22 from renal progenitors. We previously reported that renal progenitors consist of a multipotent progenitor  
23 population (24) characterized by co-expression of CD133 and VCAM1, and a committed tubular

1 progenitor that is CD133+VCAM1- (24). CD133+VCAM1+ cells are localized at the urinary pole of the  
2 Bowman's capsule and represent a rare subset of the scattered CD133+ cells localized mostly in proximal  
3 tubule (Fig. 6, A and B). To evaluate the contribution of tubular cell subsets to pRCC in humans, we  
4 performed immunomagnetic sorting of CD133+ cells, and stained them with the fluorescent label CFDA-  
5 SE. We then seeded labeled CD133+ cells in coculture with unlabeled CD133- cells in a ratio of 1:1.  
6 After 5 days of culture, the labeled cell population had undergone many cell divisions, becoming more  
7 than 98% of cultured cells at day 10 (Fig. 6C). This enrichment of CD133+ cells related to their ~50 fold  
8 higher proliferative capacity compared to CD133- cells (Fig. 6D, p=0.00003), as also demonstrated by  
9 the high number of cell divisions observed with CFDA-SE labelling (Fig. 6E). Accordingly, overgrown  
10 traced cells were virtually all CD133+VCAM1+ (Fig. 6F), in agreement with previous studies (24). We  
11 then overexpressed NICD1 in primary cultures of human renal progenitors by ZsGreen1-NICD1  
12 lentivirus infection (25). In comparison to mock-infected cells (Fig. 6, G and H), NICD1-infected renal  
13 progenitors acquired an enlarged, eosinophilic cytoplasm with prominent nucleoli and nuclear grooves  
14 (Fig. 6I), typical of pRCC histology (26), and a more developed cytoskeleton (Fig. 6J). More importantly,  
15 when mock-infected renal progenitors were cultured in a microfluidic device under fluid shear stress,  
16 they reconstituted a 3D tubule with a lumen (Fig. 6K), whereas NICD1 overexpression changed the  
17 orientation of renal progenitor cell divisions leading to generation of papilla-like structures within the  
18 lumen (Fig. 6L) that elongated, filling the lumen and generating tumor-like masses (Fig. 6M).

19

#### 20 *NOTCH1 overexpression induces renal progenitor proliferation and aberrant mitosis*

21 We next analysed how NOTCH1 overexpression could promote renal progenitor transformation. We  
22 previously reported that Notch pathway is active in renal progenitors in culture and controls their  
23 proliferation (25). Indeed, NICD1-infection further enhanced renal progenitor proliferation (fig. S7A).

1 Mitoses in mock-infected cultures were normal (fig. S7, B and C), whereas NICD1-infected progenitors  
2 frequently showed abnormal mitotic spindle distribution (fig. S7, D and E). These results suggest that  
3 NOTCH1 activation induced normal renal progenitor mitosis, whereas NOTCH1 hyperactivation  
4 disrupted cell polarity signaling leading to a striking number of aberrant mitoses (fig. S7, B-F).  
5 Transcriptome analysis identified numerous genes differentially expressed between NICD1-infected  
6 renal and mock-infected progenitor cells (fig. S7G). Gene ontology analysis demonstrated  
7 downregulation in NICD1-infected progenitors of several pathways involved in check-points and/or  
8 mitotic spindle control, including E2F targets (27), G2M checkpoint, and MYC targets V1 and V2 (28)  
9 (fig. S7H). In particular, several genes that control mitotic spindle polarity, such as *INSC*, *DYNC2H1*,  
10 *PRKAA2*, *DLG1*, *PARD3*, *PARD6G*, *STK33*, *HNF1B*, and *CCNE1* were deregulated (fig. S7I) (29, 30).  
11 Of note, survival analysis of 285 patients with pRCC from the Human Protein Atlas (31)  
12 (<http://www.proteinatlas.org>) showed that the modulation of transcripts of some of these mitotic spindle  
13 genes in tumor tissue of patients with pRCCs correlated with reduced survival (fig. S7, J-M). Taken  
14 together, NOTCH1 overactivation deregulates the expression of genes controlling mitotic spindle polarity  
15 and promotes renal progenitor proliferation as well as aberrant mitosis.

16

### 17 *Renal progenitors express NOTCH1 after AKI and represent the cell of origin of pRCC in humans*

18 To evaluate NOTCH1 expression in humans after AKI, we analysed biopsies of four patients. Patients  
19 with AKI showed nuclear expression of NOTCH1 in  $77\pm 4.7\%$  of CD133+ vs.  $51.3\pm 6.9\%$  of CD133-  
20 tubular cells (Fig. 6, N-O;  $p=0.03$ ).

21 Recently, Young et al. (32) identified the putative cell of origin of pRCCs using single-cell RNA  
22 sequencing (scRNAseq) as a rare VCAM1+ SLC17A3+ SLC7A13- proximal tubular cell. This cell  
23 cluster signature was defined as PT1. We then analyzed their pRCC scRNAseq dataset together with

1 scRNAseq data obtained from wild-type human renal progenitor cell cultures from our laboratory to  
2 evaluate if they represented the same population. After correcting for batch effects by matching mutual  
3 nearest neighbors (33), we reduced dimensionality by running a principal-component analysis (PCA) on  
4 the most highly variable genes, performed graph-based clustering on the significant ( $p < 0.05$ ) principal  
5 components (PCs), and finally visualized distinct cell subgroups using Uniform Manifold Approximation  
6 and Projection (UMAP) algorithm. Unsupervised clustering of the entire pooled dataset identified 7  
7 transcriptionally distinct populations (Fig. 6P), 4 deriving from Young's (1, 3, 4, 5) and 3 from our data  
8 (0, 2, 6). The pRCC cell cluster 5 characterized by PT1 signature markers clustered together with the  
9 renal progenitor cell clusters 0 and 6, which showed a similar pattern of mRNA expression (Fig. 6, Q  
10 and R). Importantly, all three clusters expressed *CD133* (*PROM1*) and *PAX2*, as well as the epithelial  
11 cell adhesion molecule *EPCAM*, further suggesting that the cell of origin of pRCC corresponded to renal  
12 progenitor cells. Taken together, these results suggest that NOTCH1 overexpression may induce pRCC  
13 and papillary tumor-like growth from human renal progenitors.

14

#### 15 *AKI induces papillary tumors by promoting clonal expansion of renal progenitors in mice*

16 To evaluate the role of renal progenitors in pRCCs in vivo, we used *Pax2.rtTA;TetO.Cre* transgenic mice,  
17 which specifically track renal progenitors in the mouse kidney, where CD133 cannot be used because it  
18 is species-specific (34, 35). For this purpose we generated conditional  
19 *Pax2.rtTA;TetO.Cre;R26.NICD1.GFP* (*Pax2/NICD1*) and *Pax2.rtTA;TetO.Cre;R26.NICD1.GFP;*  
20 *R26.Confetti* (*Pax2/NICD1/Confetti*) mice (fig. S8A), allowing NICD1 overexpression in the Pax2+  
21 subpopulation of TECs (renal progenitors) and their clonal tracing upon doxycycline induction (fig. S8B),  
22 as previously described (34, 35). In order to verify the effective activation of the transgenes, we stained  
23 *Pax2/NICD1* kidney sections with an anti-GFP antibody (fig. S8C). We observed positive cells in the

1 Bowman's capsule and scattered in the tubules, consistent with Pax2+ cell distribution (34, 35). Four  
2 weeks after IRI, more than 85% of induced Pax2/NICD1 mice presented with type 2 papillary tumors  
3 that stained positive for BCL-2 (Fig. 7A-E and fig. S8, D and E), similar to those observed in  
4 Pax8/NICD1 mice at 4 weeks after IRI (Fig. 5). Most of the papillary lesions were of type 2 (Fig. 7, A-  
5 D and F). Histological analysis of uninduced Pax2/NICD1 mice 4 weeks after IRI and Pax2/NICD1 mice  
6 without AKI did not show neoplastic lesions (fig. S8, F and G). 3D analysis confirmed papillary tumor  
7 vascularization and clonal invasive tumor growth in the surrounding parenchyma (Fig. 7, C and D).  
8 Clonal analysis of papillary tumors in Pax2/NICD1/Confetti mice 4 weeks after IRI (Fig. 7, B-D, fig.  
9 S8H) revealed that 88% of pAdenomas/pRCCs were monoclonal, the rest being biclonal and triclonal  
10 (Fig. 7G). A direct quantitative comparison of type 2 papillary tumors in Pax8/NICD1 and Pax2/NICD1  
11 4 weeks after IRI showed similar numbers of these lesions in both mice (Fig. 7H). In addition, no CKD  
12 was seen in Pax2/NICD1 mice after 4 weeks (Fig. 7E and fig. S8I), whereas a large proportion of  
13 Pax8/NICD1 mice showed reduced kidney function (Fig. 7E and fig. S6B). To further verify whether the  
14 role of AKI in papillary tumor induction relates to endogenous NOTCH1 activation, we induced IRI in  
15 Pax2/NICD1/Confetti mice and treated them for one week with N-[N-(3,5-Difluorophenacetyl)-L-  
16 alanyl]-S-phenylglycine t-butyl ester (DAPT). In this setting, DAPT blocks only the endogenous AKI-  
17 induced Notch1 activation, but not the transgene-derived NICD1. DAPT-treated Pax2/NICD1/Confetti  
18 mice developed significantly ( $p=0.008$ ) fewer papillary tumors, compared to vehicle-treated mice 4  
19 weeks after IRI (Fig. 7I). These results suggest that single renal progenitors are the source of papillary  
20 tumors induced by NOTCH1 overexpression.

21 To address whether clonal tumors developing spontaneously in mice after AKI also originated from renal  
22 progenitors, we first confirmed that in Pax2/Confetti mice NOTCH1 was expressed by renal progenitors  
23 at a percentage significantly higher than that of Pax2- cells after AKI ( $p=0.03$ ) and of renal progenitors  
24 in healthy mice ( $p=0.03$ ) (fig. S9A). A representative staining is shown in Fig. 7J. We then performed

1 lineage tracing for 36 weeks after IRI induction using Pax2/Confetti mice (Fig. 7K-R). Papillary tumors  
2 spontaneously developed in Pax2/Confetti mice after IRI (Fig. 7K). As previously observed in  
3 Pax8/Confetti mice (Fig. 2M), Pax2/Confetti mice showed a clonal origin (Fig. 7L). Type 1 papillary  
4 tumors were prevalent (Fig. 7M). DAPT-treated Pax2/Confetti mice developed significantly ( $p=0.03$ )  
5 fewer papillary tumors, compared to vehicle-treated mice 4 weeks after IRI (Fig. 7N). This reduction  
6 was related to a decrease of type 2 papillary tumors (fig. S9, B and C). 3D analysis confirmed that  
7 papillary tumors showed vascularization and that clonal populations spread from the primary tumors into  
8 the surrounding parenchyma, promoting invasive clonal tumor growth (Fig. 7, Q and R). These results  
9 suggest that papillary tumors that develop after AKI derive from clonal proliferation of single renal  
10 progenitors in a Notch-dependent manner.

11

## 12 **Discussion**

13 We hypothesized that AKI may drive the development of one or more subtypes of RCC. Our results  
14 reveal that: 1. AKI is a risk factor for pRCC development and for pRCC recurrence in humans in different  
15 cohorts. 2. AKI promotes long-term development of papillary tumors in mice by activating tumor  
16 growth-promoting pathways in a type 1- or 2-specific manner. 3. Overexpression of the AKI-associated  
17 pathway Notch1 is a specific feature of type 2 pRCC, which correlates with outcome in humans, and  
18 drives type 2 papillary tumors in an inducible mouse model of pRCC. 4. pRCC originates from clonal  
19 proliferation of renal progenitors - the cellular source of tubule regeneration upon AKI - in a classical  
20 adenoma-carcinoma sequence leading to invasive pRCC growth and even remote metastasis.

21 AKI, and particularly IRI, is common and often overlooked. In addition, patients with AKI have a  
22 reduced life expectancy (36), which may explain why the link between AKI and pRCC has remained  
23 unrecognized until now (6). Autopsy studies suggest that papillary adenomas are common, with a

1 prevalence ranging from 5 to 10% before the age of 40 and rising to almost 40% above the age of 70  
2 (37). In contrast, pRCCs are considerably rarer, with the exception of patients on dialysis or with a kidney  
3 transplant, two patient categories at high risk of AKI (38, 39), where prevalence increases up to 100  
4 times, reaching 2-3% (40). These epidemiologic data are in line with our findings in mice, where  
5 papillary adenomas were observed in over half of the animals 12 weeks after an AKI episode, whereas  
6 pRCCs appeared only 36 weeks after AKI and in only 16% of the animals. These results suggest that the  
7 adenoma-carcinoma transformation is a stochastic event that develops only in a minority of patients with  
8 papillary adenomas and requires long periods of time and likely additional factors to develop. As further  
9 evidence that AKI is an important risk factor for pRCC, postoperative AKI episodes also directly  
10 increased the risk for pRCC relapse. These results suggest that all patients at high risk for pRCC, that is  
11 patients carrying germinal mutations in pRCC-related genes or patients undergoing pRCC surgery,  
12 require rigorous measures to prevent AKI episodes. Once AKI has occurred, careful follow-up for pRCC  
13 relapse would be required. These findings may be particularly relevant for dialysis patients or patients  
14 with kidney transplant in whom pRCC represents an important cause of death (41). Our results suggest  
15 that this risk may relate to AKI episodes that are inherent to advanced CKD and kidney transplantation  
16 (5).

17 How could AKI promote development of pRCC? Our analysis of human tumor samples reported by  
18 TCGA study (20) showed that some AKI-associated signaling pathways that critically control healing  
19 responses to kidney injury are overexpressed in pRCCs and predict tumor severity and patient survival.  
20 Notably, type 1 and type 2 pRCCs reveal associations with different pathways, suggesting that these  
21 subtypes are promoted by AKI through activation of distinct signaling pathways involved in cell growth  
22 control. To verify this hypothesis, we tried to reproduce papillary tumors by overexpressing one of these  
23 AKI-associated pathways in mice. We chose to overexpress the Notch1 pathway as a proof of concept  
24 because our analysis of patients from TCGA study (20) found NOTCH1 overexpression to predict

1 disease severity and outcome in pRCC and to be a specific feature of all three molecular subgroups of  
2 type 2 pRCC independently of causative germline or somatic mutations. In agreement with our  
3 hypothesis, NOTCH1 overexpression in TECs induced papillary adenomas and type 2 pRCC  
4 development, and blocking NOTCH hyperactivation after AKI prevented their development in mice.  
5 Conversely, a previous study showed that knocking out *Notch1* in the kidney epithelium during  
6 development can lead to generation of type 1 papillary adenomas at birth (42). Consistent with this result,  
7 we also found that type 1 papillary tumors had low amounts of NOTCH1 and were not caused by NOTCH  
8 overexpression. However, both NOTCH under- and overexpression determine malfunctioning of the  
9 mitotic spindle and hyperproliferation (43), as also observed in this study. Taken altogether, these results  
10 suggest that environmental or genetic factors that promote hypo or hyperactivation of signaling pathways  
11 that control tubular cell proliferation, such as the Notch pathway, can drive papillary tumors. Previous  
12 studies reported that NOTCH overexpression in TECs promotes CKD, increased proliferation, and even  
13 epithelial dysplasia, but not RCC (44-46). However, in these studies: 1. mice were studied in the absence  
14 of AKI and only 4-8 weeks after transgene activation (44, 45), a time that is not sufficient for tumor  
15 development, in agreement with our observations; 2. *Notch* transgene was overexpressed under the  
16 control of a promoter (46) that is not expressed in renal progenitors as previously reported (47). Indeed,  
17 our results demonstrate that NOTCH1 activation induces RCC only after overexpression specifically in  
18 renal progenitors.

19 Until now, the cell of origin for pRCC has been unknown. Because the renal tubule contains 10 to 20  
20 different epithelial cell subtypes (48), not every TEC may be amenable to cancer transformation, making  
21 identification of the cell type of pAdenoma/pRCC origin a challenging task. Immunolabeling and mRNA  
22 profiling support a possible proximal tubular origin (49, 50). Our lineage tracing studies indicate that  
23 pAdenoma/pRCC sequentially develop from an aberrant clonal expansion of renal progenitors occurring  
24 after AKI or after genetically-induced changes of an AKI-activated pathway specifically in renal

1 progenitors. These results demonstrate a crucial role of the cell of origin in determining the renal papillary  
2 tumor type. Our results also provide a possible explanation for recent results obtained from scRNAseq  
3 suggesting the similarity of pRCCs with a scattered, uncharacterized population of tubular cells  
4 expressing VCAM1, defined as PT1 (32). We previously reported that a subset of CD133+ renal  
5 progenitors in humans has self-renewal and clonogenic capacity and is VCAM1+ (3, 24). Our scRNAseq  
6 analysis indicated that the PT1 signature closely resembled CD133+ human renal progenitor cells, further  
7 confirming that this is the cell of origin of pRCC. In this setting, AKI-induced hypoxia may also be  
8 critical. Indeed, hypoxia promotes the undifferentiated cell state in various stem and precursor  
9 populations by activating Notch-responsive promoters and increasing expression of genes directly  
10 downstream of Notch (51). In turn, NOTCH also drives a HIF1a-to-HIF2a switch that favors  
11 transformation into cancer stem cells and supports tumor growth (52).

12 Our study also has some limitations. In mouse models we tested only IRI, the most frequent type of AKI  
13 in clinical settings (6). Other studies are needed to determine whether other types of AKI, such as toxic  
14 or obstructive, also promote papillary tumor development in animal models. In addition, we did not assess  
15 whether the risk of pRCC was related to the severity of AKI. Other studies are needed to address this  
16 important point.

17 In summary, the results of this study provide evidence that acute organ injury can drive tumorigenesis  
18 and, in particular, that AKI promotes the development of pRCC from single renal progenitors through a  
19 classical adenoma-carcinoma sequence. The process is initiated by overactivation of AKI-associated  
20 pathways that promote clonal expansion of renal progenitors as an overshooting healing response. We  
21 identify NOTCH1 overexpression as a common pathomechanism of type 2 pRCC formation, suggesting  
22 that this pathway could be targeted in this RCC type. Finally, the discovery of AKI as a risk factor for

1 pRCC development and relapse suggests that pRCCs unrelated to known risk factors may actually relate  
2 to previous AKI episodes, requiring AKI prevention and risk stratification programs.

3

## 4 **Materials and methods**

5

### 6 **Study Design**

7 The purpose of this study was to examine the effect of AKI on RCC development and to identify the  
8 mechanisms involved in this process. These objectives were addressed by (i) analyzing AKI insults as a  
9 risk factor for RCC development in humans, (ii) analyzing the effect of postoperative AKI related to  
10 tumor resection in promoting pRCC recurrence in patients, (iii) confirming AKI as a risk factor for pRCC  
11 development in mice, and evaluating pRCC clonality, (iv) analyzing the AKI-related pathways that are  
12 involved in tumor progression in human pRCC tissues, (v) evaluating the effect of the AKI-related  
13 pathway NICD1 on pRCC formation in a transgenic mouse model of pRCC, (vi) evaluating the  
14 transforming effect of NICD1 overexpression on human renal progenitors, the critical cell for the  
15 response to AKI, (vii) evaluating renal progenitors as the cell of origin of pRCC in NICD1  
16 overexpressing transgenic mouse and/or in response to AKI.

17 For all clinical studies, all eligible patients from the available observation period were included without  
18 previous sample size calculation and were assigned to predefined clinical groups (control/pRCC/ccRCC,  
19 AKI/non-AKI) after retrospective clinical adjudication, as per study design.

20 *Single center study* We analyzed the incidence of previous AKI episodes in the RCC population  
21 irrespectively of the histological diagnosis. All patients who underwent surgery for RCC at the Careggi  
22 University Hospital, Florence, Italy, from 2012 to 2018 (fig. S1) with available clinical and laboratory

1 information and a follow-up of at least one year before tumor diagnosis were included in the analysis.  
2 Patients were excluded if no detailed information about the tumor clinical stage was available and in case  
3 of TNM stage 3 or 4. Historical clinical records for laboratory, surgical, and histological data of RCC  
4 patients were retrospectively and independently collected by two blinded observers. We evaluated the  
5 exposure to previous AKI episodes as an independent risk factor for the development of pRCC or ccRCC.  
6 AKI that occurred in the 6 months before surgery were excluded from the analysis. When at least one  
7 year of follow-up after surgery was available (fig. S1), we evaluated the association between  
8 postoperative AKI and tumor recurrence both in a survival analysis and in a multivariate model. This  
9 study was approved by the Ethical Committee on Human Experimentation of the Azienda Ospedaliero-  
10 Universitaria Careggi, Florence, Italy.

11 ***Danish National Registry of Patients*** A Danish nationwide cohort study using population-based medical  
12 databases (11, 12) between 1977 and 2006 was screened for the association between AKI and RCC (fig.  
13 S2). All patients diagnosed with AKI were included in the analysis. Data were extracted by an  
14 investigator blinded to the purpose of the analysis. The study was approved by the Danish Data Protection  
15 Agency (record No 2005-41-5281).

16 ***Multicentric study*** We analyzed the Italian Registry of Conservative Renal Surgery (RECORD1 Project)  
17 of the Italian Society of Urology (fig. S3) (18) to confirm the association between postoperative AKI and  
18 tumor recurrence both in a survival analysis and in a multivariate model. The RECORD project includes  
19 all patients who underwent conservative surgical treatment for radiologically diagnosed renal cell  
20 carcinoma (RCC) between January 2009 and December 2012 at 19 urological Italian Centres. We  
21 included all patients with a histologic diagnosis of RCC and with a minimum 1-year oncologic follow-  
22 up (fig. S3). Patients were excluded if no detailed information about the tumor clinical stage was available  
23 and in case of TNM stage 3 or 4. Data were extracted by an investigator blinded to the purpose of the

1 analysis. The study protocol was approved by the local ethical committee and patients' acceptance of the  
2 written informed consent .

3 ***Cancer Genome Atlas Research Network*** We analyzed gender, age, smoking exposure, and mutations  
4 on genes previously described as specific for pRCC in relation to expression of NOTCH1 and other  
5 pathways associated with AKI (20) in all available pRCC patients.

6 ***Patient dataset from Human Protein Atlas*** We analysed the survival probability of all pRCC patients  
7 from The Human Protein Atlas (31), (<http://www.proteinatlas.org>) based on PRKAA2, PARD6G,  
8 HNF1B, and CCNE1 expression.

9 ***Patient biopsies*** A total of 5 normal-appearing kidneys (3 men and 2 woman, mean age  $64.8 \pm 12.4$  years)  
10 and 4 kidney biopsies from patients with AKI (2 men and 2 women, mean age  $58.8 \pm 5.1$  years, time  
11 post-AKI: 2, 2, 14, and 15 days) were analyzed in agreement with the Ethical Committee on Human  
12 Experimentation of the Azienda Ospedaliero-Universitaria Careggi, Florence, Italy. Normal-appearing  
13 kidney fragments were obtained from the pole opposite to the tumor of patients who underwent  
14 nephrectomy for localized renal tumors.

15 ***Animal studies*** Mice were randomly assigned to experimental and control groups, but investigators were  
16 not blinded. Sample size was calculated based on the primary endpoint rate of tumor formation and other  
17 assumptions based on preliminary experiments. No data were excluded from studies in this manuscript.  
18 Pathology analysis was performed in a blinded fashion. All procedures were performed in accordance  
19 with institutional protocols approved by the Institutional Animal Care and Use Committee of the  
20 University of Florence, Italy.

21

22 **Statistical analysis**

1 **Human studies** Results are reported as mean±SD unless otherwise stated. Between-group comparisons  
2 for continuous variables were performed by Student's t test for unpaired data and Mann–Whitney *U* test  
3 according to a normal or non-parametric distribution. Frequencies among groups were compared by chi-  
4 squared ( $\chi^2$ ) test for categorical variables, applying Fisher's exact test when appropriate (Table 1 and  
5 tables S1-S3). A two-sided p-value of <0.05 was considered statistically significant.

6 To test the association between previous AKI episodes and postoperative AKI with pRCC development  
7 and recurrence, respectively, binary logistic regression models were constructed. pRCC or ccRCC  
8 development and pRCC recurrence served as outcome or dependent variables. Covariates or explanatory  
9 variables were treated as categorical (AKI, gender, CKD, DM, clinical T) or continuous (age, R.E.N.A.L.  
10 score) as appropriate. The results are presented as odds ratio and 95% confidence interval. A Wald test  
11 statistics' p-value of <0.05 was considered statistically significant. For survival analyses, Kaplan-Meier  
12 estimates for postoperative AKI and non-AKI patients were compared by log-rank test with statistical  
13 significance set for a p-value lower than 0.05. Cumulative 4-year recurrence-free survival and standard  
14 error are reported.

15 Kaplan-Meier estimates were used to generate overall survival curves for:

- 16 1. NOTCH1, PTEN, and SMAD4 RPPA amounts in patients affected by pRCC, or NOTCH1 (using  
17 50<sup>th</sup> percentile as a cutoff value) in patients affected by type 1 or 2 pRCC obtained from RPPA  
18 data from the Cancer Genome Atlas Research Network (20).
- 19 2. PRKAA2, PARD6G, HNF1B, and CCNE1 expression (using best expression cut off) in patients  
20 affected by pRCC from Human Protein Atlas (31).

21 Differences between groups were assessed by log-rank test. For each Kaplan-Meier plot, a Cox regression  
22 model was constructed to provide hazard ratio (HR) and 95% confidence interval.

1 To test the independent effects of different variables on the prognosis of pRCC, a multiple logistic  
2 regression model was constructed by entering TNM stages >2 as dependent variable while all parameters  
3 previously described as risk factors for pRCC served as covariates.

4 ***Animal and in vitro studies*** The number of mice used, numbers of replicates, and statistical values (where  
5 applicable) are provided in the figure legends. Results were expressed as mean±SEM. Comparison  
6 between groups was performed by the Mann-Whitney test, or through the analysis of variance for  
7 multiple comparisons (ANOVA for repeated measures) with Bonferroni post hoc analysis, with the  
8 Fisher's exact test (two-tailed p-value) or with t-test (two-tailed p-value). A p-value < 0.05 was  
9 considered statistically significant.

10 Statistical analysis was performed using SPSS (RRID:SCR\_002865), OriginPro (RRID:SCR\_015636),  
11 and Stata (RRID:SCR\_012763) statistical softwares.

12

### 13 **Supplementary Materials**

#### 14 Supplementary Materials and Methods

15 Fig. S1. The flow chart illustrates the selection criteria for the single center study population.

16 Fig. S2. The flow chart illustrates the selection criteria for the study population from the Danish National  
17 Registry of Patients.

18 Fig. S3. The flow chart illustrates the selection criteria for the multicentric study population.

19 Fig. S4. AKI-driven papillary tumors originate from clonal proliferation of single tubular epithelial cells  
20 in mice.

21 Fig. S5. NICD1 overexpression in mouse tubular cells induces papillary tumors and CKD.

- 1 Fig. S6. AKI accelerates clonal type 2 papillary tumor development in mice.
- 2 Fig. S7. NOTCH1 overexpression enhances cell division and promotes aberrant mitosis.
- 3 Fig. S8. AKI induces papillary tumors by promoting clonal expansion of renal progenitors in mice.
- 4 Fig. S9. AKI induces papillary tumors from renal progenitors in a NOTCH1-dependent manner.
- 5 Table S1. Postoperative AKI is a risk factor for the development of pRCC recurrence in humans in a  
6 single center study.
- 7 Table S2. The preoperative characteristics of 594 patients treated with partial nephrectomy for kidney  
8 tumors and clinical details of 89 pRCC patients (the RECORD1 project).
- 9 Table S3. Postoperative AKI is a risk factor for the development of pRCC recurrence in humans in a  
10 multicentric study (RECORD1 project).
- 11 Table S4. AKI-activated pathways are overexpressed in human pRCC in a subtype-specific manner.
- 12 Table S5. Multivariate analysis indicates NOTCH1 as a risk factor for pRCC prognosis.
- 13 Video S1. 3D reconstruction of a papillary tumor in Pax8/NICD1/Confetti kidney at 36 weeks
- 14

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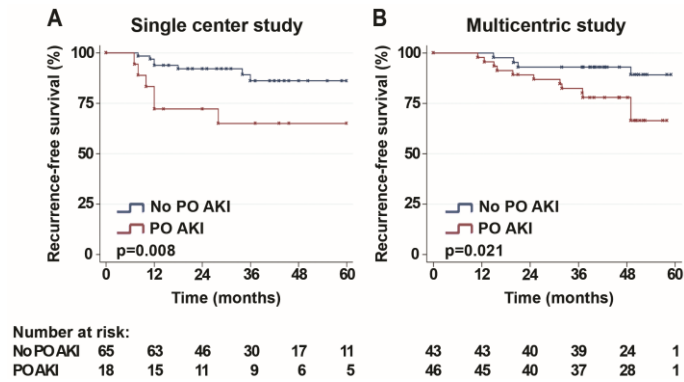
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10 Minervini, S.S., M.C., A. Mari, F.D.M, A.A., F.P., R.S., V.F., C.F.C. and R.M.R collected patient clinical  
11 data, M.A., F.G., C.F.C and A. Mari analysed them. A.J.P., G.A., M.L.A., A.S. and L.D.C. performed  
12 immunohistochemistry and confocal microscopy. A.J.P., G.A., A.S., C.C., B.M., S.N. and M.E.M.  
13 designed and carried out experiments in the mouse system. G.J.N. and M.R.R. performed the  
14 histopathological tissue analysis of the mouse H&E samples. A.J.P., G.A., M.L.A., A.S. and P.R.  
15 analyzed the data. T.L. performed ultrasound imaging. S.L. and S.G. performed single cell RNA  
16 sequencing, R.S. and A. Magi. analysed the datasets. M.R., A.Mari, M.A. and F.G. performed statistical  
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22 NCBI's Gene Expression Omnibus repository as GSE137704 and GSE137620, respectively. All data

- 1 associated with this study are present in the paper or the Supplementary Materials, or are available from
- 2 The Human Protein Atlas (<https://www.proteinatlas.org/>).

1 **Figures:**

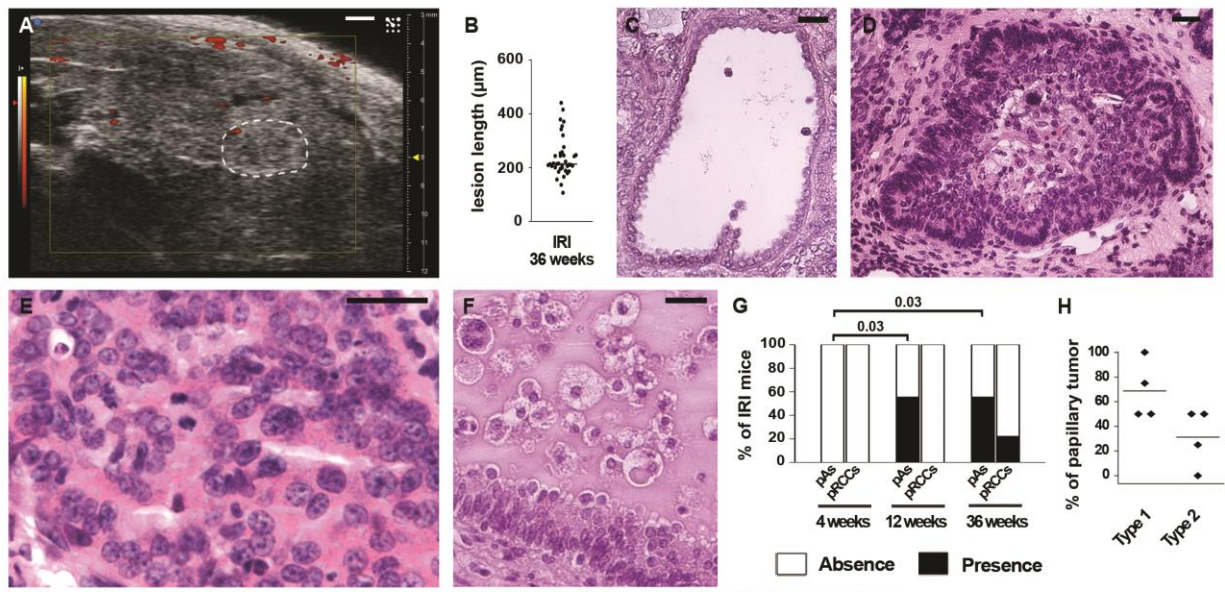


2

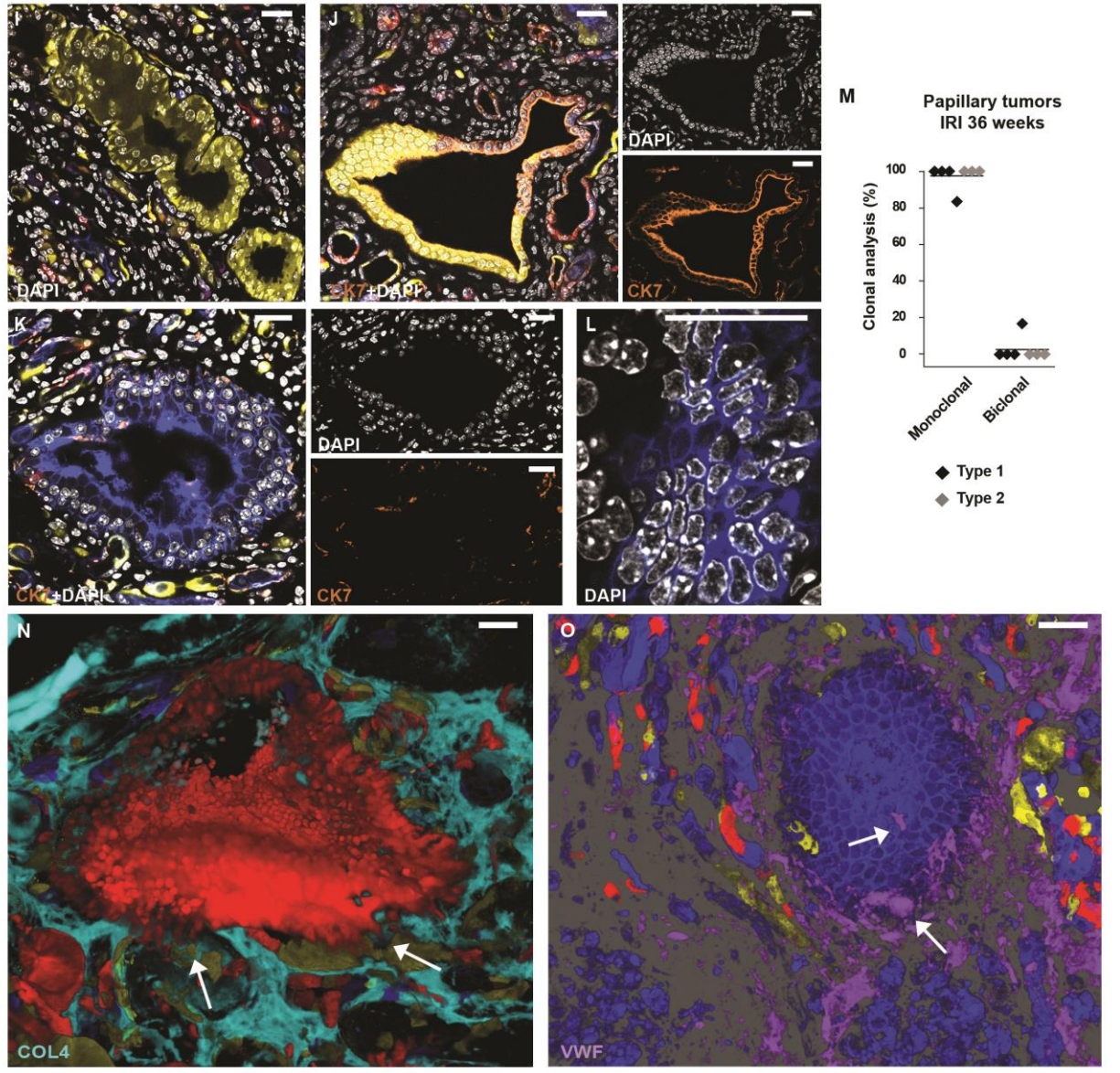
3 **Fig. 1. Recurrence-free survival in patients with pRCC according to the presence or absence of a**  
 4 **postoperative AKI episode. (A, B) pRCC patients' recurrence-free survival based on the presence or**  
 5 **absence of a postoperative AKI episode in (A) a single center study (n=65 no postoperative AKI, n=18**  
 6 **postoperative AKI) and (B) a multicentric study (n=43 no postoperative AKI, n=46 postoperative AKI).**  
 7 Censored patients and patients at risk are shown. Kaplan-Meier curves are compared by log-rank test  
 8 (7.143 and 5.36, respectively) and Cox regression ( $HR_{(A)}=3.6$  [1.3-10.2];  $HR_{(B)}=3.4$  [1.1-10.3]).  
 9 Statistical significance was set for a p-value <0.05. PO AKI: Postoperative AKI.

10

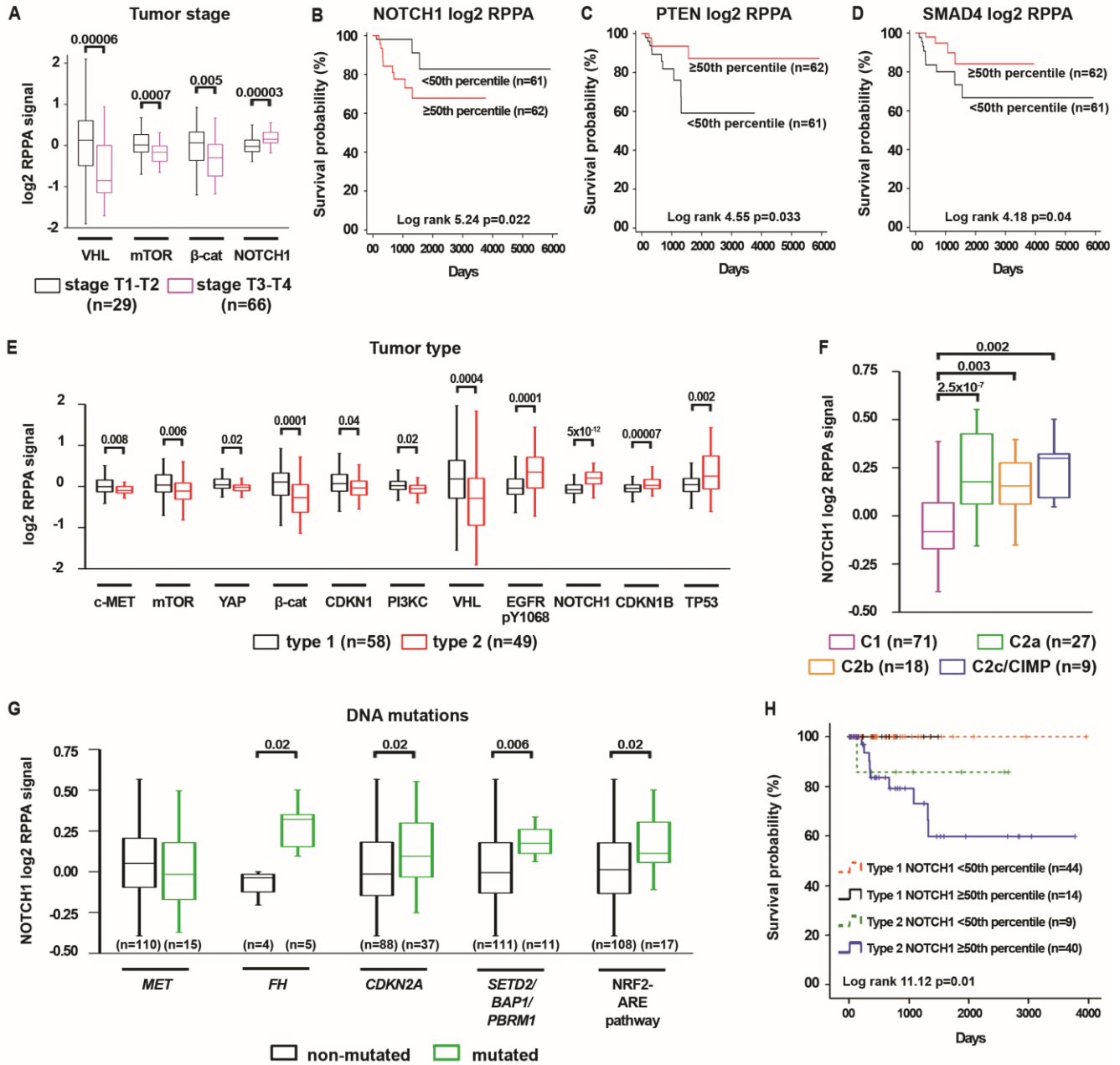
Wild-type AKI



Pax8/Confetti AKI



1 **Fig. 2. Long-term follow-up reveals that AKI induces papillary tumors in mice.** (A) Ultrasound  
2 image with Power Doppler Mode (red) of a wild-type mouse kidney (displayed in transverse plane) at 36  
3 weeks after ischemia-reperfusion injury (IRI) (representative of n=3 mice). The region of interest (ROI,  
4 dashed line) indicates tumoral tissue. Bar=1 mm. (B) Length ( $\mu\text{m}$ ) of the lesions in wild-type mouse  
5 kidneys at 36 weeks after IRI (n=5 mice). Bar indicates median value. (C-F) Representative histological  
6 images with H&E staining of papillary tumors from wild-type mouse kidneys after IRI. In (C), a type 1  
7 papillary tumor. In (D), a type 2 papillary tumor. In (E), nuclear atypias. In (F), foamy macrophages  
8 within a papillary lesion. Bars=25  $\mu\text{m}$ . (G) Percentages of mice at 4 weeks (n=8), 12 weeks (n=9), and  
9 36 weeks (n=9) after IRI presenting with papillary adenomas (pAs) and pRCCs. Numbers above graph  
10 represent p-values. (H) Percentage of type 1 vs. type 2 papillary tumors in wild-type mice at 36 weeks  
11 after IRI (n=4 mice). Bars indicate mean values. No significant difference between the groups. (I)  
12 Representative confocal image of a type 1 papillary tumor from Pax8/Confetti mouse kidneys at 36 weeks  
13 after IRI. Bar=25  $\mu\text{m}$ . (J) Split confocal images of CK7 staining (orange) of a type 1 papillary tumor  
14 from Pax8/Confetti mouse kidneys at 36 weeks after IRI. Bar=25  $\mu\text{m}$ . (K) Split confocal images of CK7  
15 staining (orange) of a type 2 papillary tumor from Pax8/Confetti mouse kidneys at 36 weeks after IRI.  
16 Bar=25  $\mu\text{m}$ . (L) Detail of nuclear atypias in a type 2 papillary tumor from Pax8/Confetti mouse kidneys  
17 at 36 weeks after IRI. Bar=25  $\mu\text{m}$ . (M) Clonal analysis in type 1 and 2 papillary tumors from  
18 Pax8/Confetti mouse kidneys at 36 weeks after IRI (n=7 mice). Bars indicates mean value. (N, O) 3D  
19 reconstruction of representative pRCC in Pax8/Confetti mouse kidneys at 36 weeks after IRI. In (N),  
20 collagen IV (COL4) staining (cyan). Arrows indicate invasion. In (O), Von Willebrand Factor (VWF)  
21 staining (purple). Arrows indicate tumor vascularization. Bars= 25  $\mu\text{m}$ . DAPI (white) counterstains  
22 nuclei. Signals for fluorescent Confetti proteins are as follow: GFP, green; RFP, red; YFP, yellow, and  
23 CFP, blue. Statistical power was assessed using Fisher's exact test (G) or Mann-Whitney test (H). N.S  
24 (p>0.05).



1

2 **Fig. 3. NOTCH1 overexpression is specific for human type 2 pRCC and predicts poor prognosis.**

3 (A) Log<sub>2</sub> RPPA signal for proteins differently expressed in tumor stage T1-T2 (black boxes, n=29

4 patients) vs T3-T4 (pink boxes, n=66) pRCC. (B-D) Overall survival of pRCC patients based on high

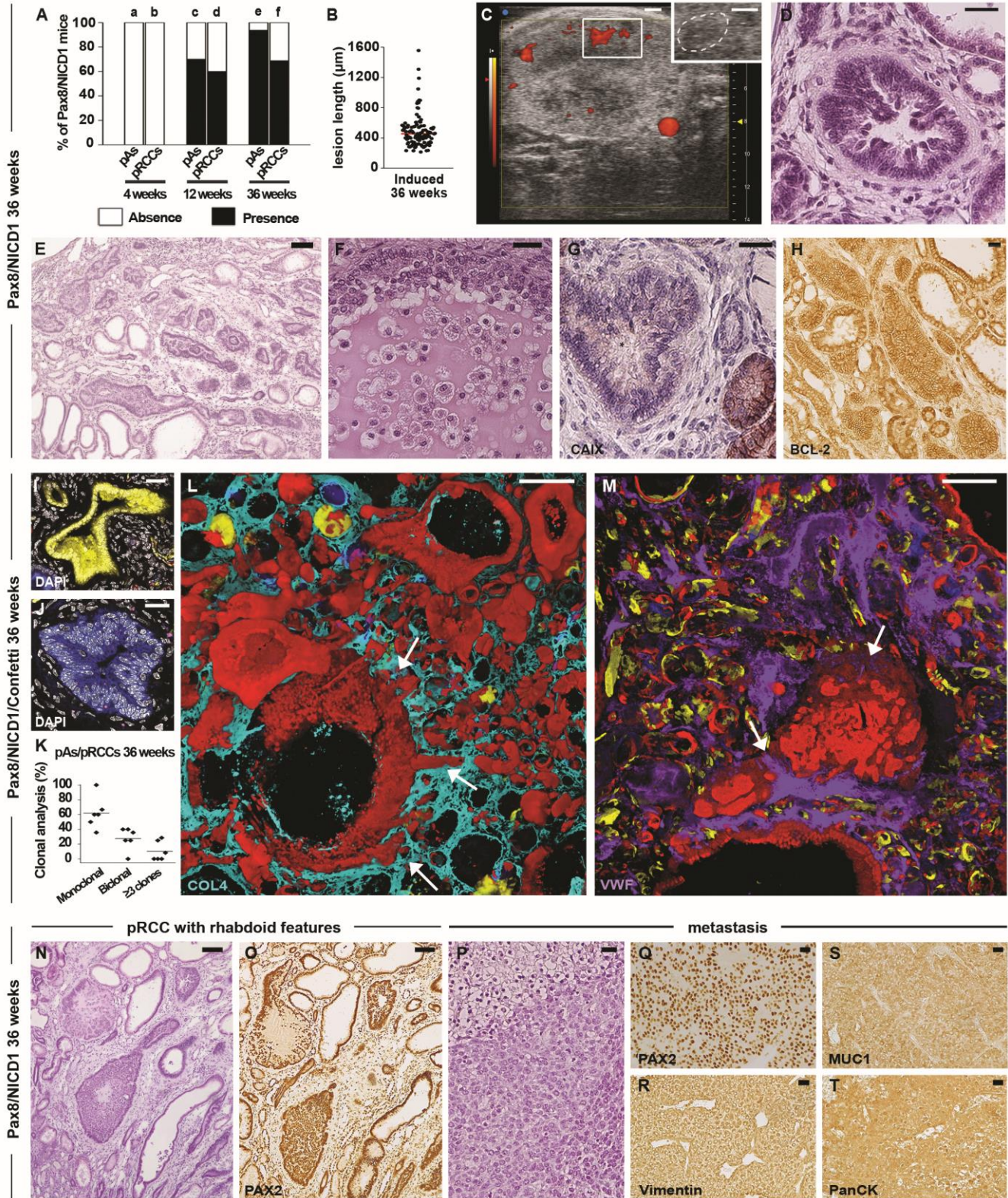
5 ( $\geq 50^{\text{th}}$  percentile, n=62) or low ( $< 50^{\text{th}}$  percentile, n=61) expression of different proteins: NOTCH1 (B),

6 PTEN (C), or SMAD4 (D). (E) Log<sub>2</sub> RPPA signal for proteins different in tumor type 1 (black box,

7 n=58) vs. type 2 (red box, n=49) pRCC. (F) NOTCH1 log<sub>2</sub> RPPA signal in patients with pRCC of

1 clusters C1, C2a, C2b, and C2c/CIMP (n=71, 27, 18, and 9, respectively). (G) NOTCH1 log2 RPPA  
2 signal in patients with non-mutated (black boxes) or mutated (green boxes) *MET* (non-mutated n=110,  
3 mutated n=15), *FH* (non-mutated n=4, mutated n=5), *CDKN2A* (non-mutated n=88, mutated n=37),  
4 *SETD2/BAP1/PBRM1* (non-mutated n=111, mutated n=11), or NRF2–antioxidant response element  
5 (ARE) pathway (non-mutated n=108, mutated n=17). (H) Differences in overall survival of patients with  
6 type 1 or type 2 pRCC based on high ( $\geq 50^{\text{th}}$  percentile, type 1 n=14 and type 2 n=40) or low ( $< 50^{\text{th}}$   
7 percentile, type 1 n=44 and type 2 n=9) NOTCH1 protein expression.  $\beta$ -cat:  $\beta$ -catenin. RPPA: reverse  
8 phase protein array. Box-and-whisker plots: line = median, box = 25-75%, whiskers = min-max.  
9 Statistical significance was calculated by Mann-Whitney test. Kaplan-Meier curves are compared by log-  
10 rank test and Cox regression ( $\text{HR}_{(B)}=4.0$  [1.1-14.3];  $\text{HR}_{(C)}=3.3$  [1.0-10.8];  $\text{HR}_{(D)}=3.2$  [1.0-10.1];  
11  $\text{HR}_{(F)}=4.6$  [1.0-20.5]). Numbers above graphs represent p-values.

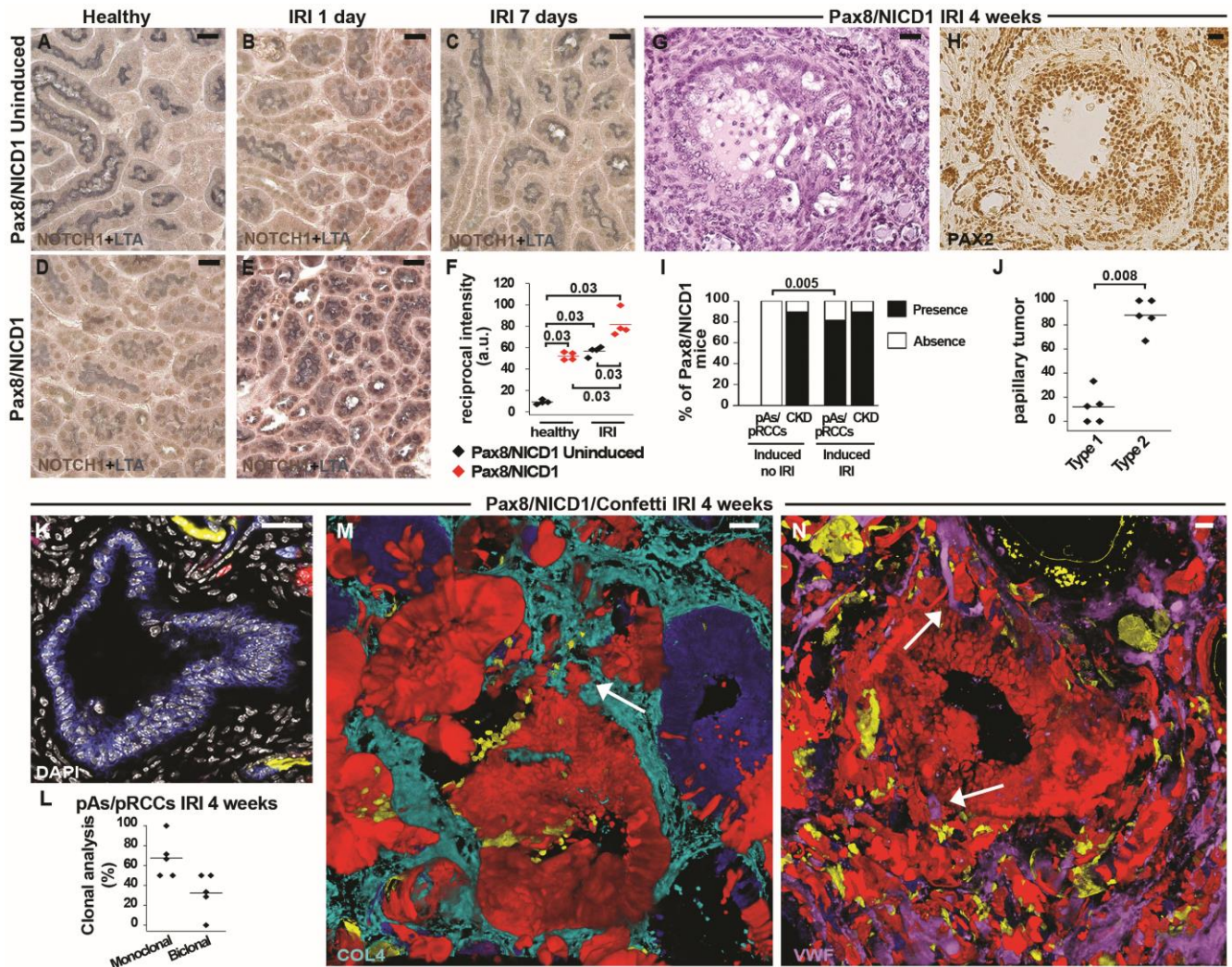
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1

2 **Fig. 4. NICD1 overexpression in mouse tubular cells induces clonal type 2 papillary tumors. (A)**  
 3 Percentages of Pax8/NICD1 mice at 4 weeks (n=8), 12 weeks (n=10), and 36 weeks (n=16) presenting

1 with papillary adenomas (pAs) and pRCCs. Statistical power was assessed using Fisher's exact test: a vs.  
2 c  $p=0.004$ , a vs. e  $p=0.0001$ , c vs. e  $p=0.2642$  and b vs. d  $p=0.0128$ , b vs. f  $p=0.002$ , d vs. f  $p=0.6924$ .  
3 **(B)** Length ( $\mu\text{m}$ ) of the lesions in Pax8/NICD1 mouse kidneys at 36 weeks (n=5 mice). Bar (red) indicates  
4 median value. **(C)** Ultrasound image with Power doppler Mode (red) of a wild-type mouse kidney  
5 (displayed in transverse plane) at 36 weeks (representative of n=4 mice). Inset: high magnification of the  
6 ultrasound appearance of a tumor area (dashed line). Bar=1 mm. **(D)** Representative image of H&E  
7 staining of papillary adenoma from Pax8/NICD1 mouse kidney at 36 weeks. Bar=25  $\mu\text{m}$ . **(E)** Type 2  
8 papillary carcinoma from Pax8/NICD1 mouse kidney at 36 weeks. Bar=100  $\mu\text{m}$ . **(F)** H&E staining of  
9 foamy macrophages within a papillary lesion from Pax8/NICD1 mouse kidney at 36 weeks. Bar=25  $\mu\text{m}$ .  
10 **(G)** Representative image of a carbonic anhydrase IX (CAIX) negative papillary tumor (brown) from  
11 Pax8/NICD1 mouse kidney at 36 weeks. Bar=25  $\mu\text{m}$ . **(H)** BCL-2 positive papillary tumor (brown) from  
12 Pax8/NICD1 mouse kidney at 36 weeks. Bar=25  $\mu\text{m}$ . **(I)** Representative type 2 papillary tumor from  
13 Pax8/NICD1/Confetti mouse kidney at 36 weeks. Bar=25  $\mu\text{m}$ . **(J)** Representative type 2 papillary tumor  
14 from Pax8/NICD1/Confetti mouse kidney at 36 weeks. Bar=25  $\mu\text{m}$ . **(K)** Clonal analysis of papillary  
15 tumors in Pax8/NICD1/Confetti mice at 36 weeks (n=6 mice). Bars indicate mean values. **(L, M)** 3D  
16 reconstruction of representative lesions in Pax8/NICD1/Confetti kidney. In **(L)**, COL4 staining (cyan).  
17 Arrows indicate invasion. In **(M)**, VWF staining (purple). Arrows indicate vascularization of the tumor.  
18 Bars=100  $\mu\text{m}$ . **(N, O)** Low-power images of a papillary tumor with rhabdoid features. In **(N)**, H&E  
19 staining. In **(O)**, PAX2 staining (brown). Bars=100  $\mu\text{m}$ . **(P-T)** Histologic images of a subcutaneous  
20 metastasis. In **(P)**, H&E staining. In the upper part of the image, subcutaneous adipose tissue; in the lower  
21 part, metastatic tissue with rhabdoid features. In **(Q)**, PAX2 staining (brown). In **(R)**, vimentin staining  
22 (brown). In **(S)**, MUC1 staining (brown). In **(T)**, PanCK staining (brown). Bars=25  $\mu\text{m}$ . DAPI (white)  
23 counterstains nuclei. Signals for fluorescent Confetti proteins are as follow: GFP, green; RFP, red; YFP,  
24 yellow; and CFP, blue.

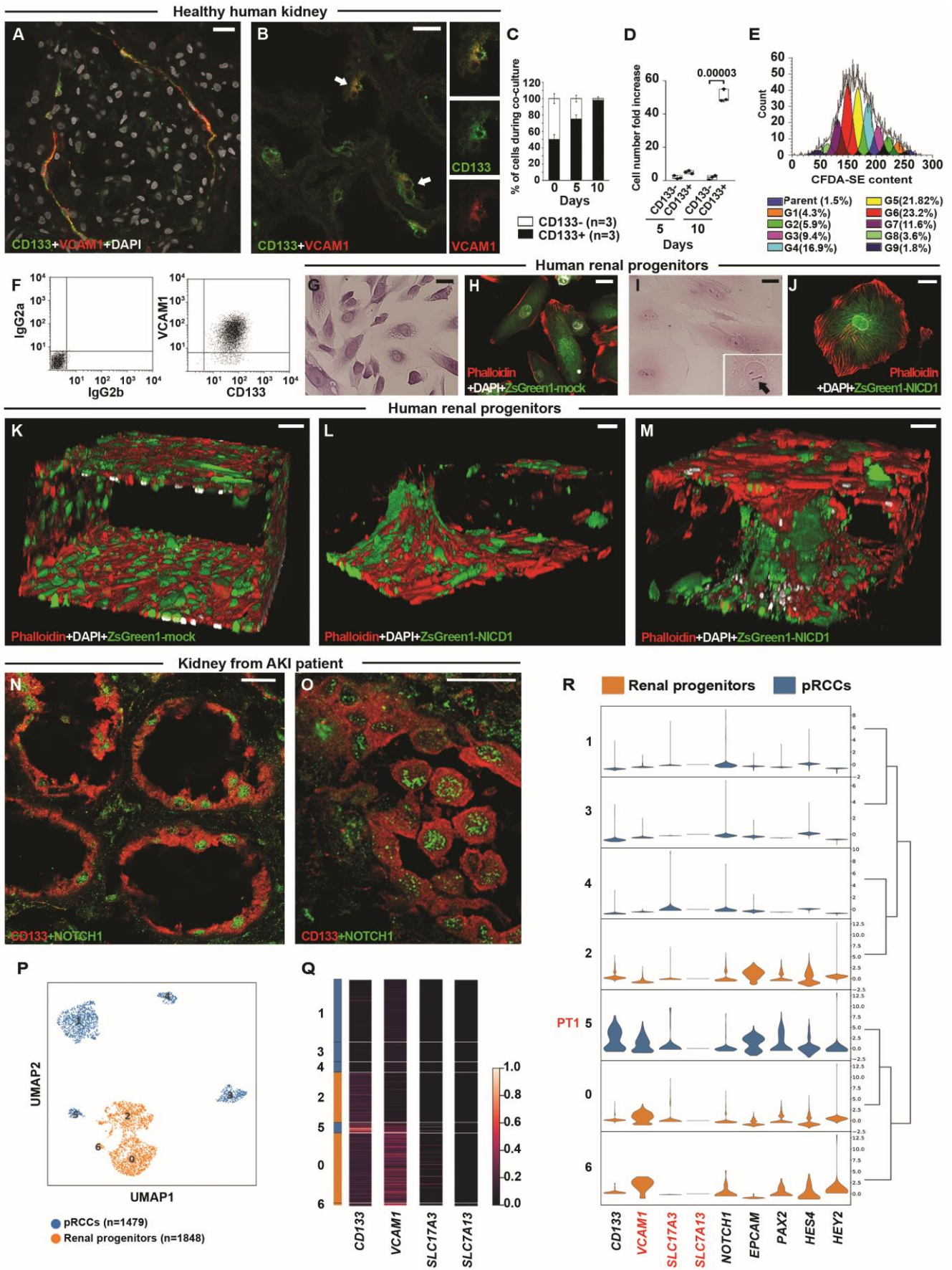


1

2 **Fig. 5. AKI accelerates clonal type 2 papillary tumor development in mice.** (A) Representative image  
 3 of activated NOTCH1 staining (brown) and Lotus TetraglonoLobus Lectin (LTA, blue) in healthy  
 4 uninduced Pax8/NICD1 mouse kidney at 1 day. Bar=25  $\mu$ m. (B, C) Representative images of activated  
 5 NOTCH1 staining (brown) and LTA (blue) in uninduced Pax8/NICD1 mouse kidney 1 day (B) and 7  
 6 days (C) after IRI. Bars=25  $\mu$ m. (D) Representative image of activated NOTCH1 staining (brown) and  
 7 LTA (blue) in healthy induced Pax8/NICD1 mouse kidney at 1 day. Bar=25  $\mu$ m. (E) Representative  
 8 image of activated NOTCH1 staining (brown) and LTA (blue) in induced Pax8/NICD1 mouse kidney 1  
 9 day after IRI. Bar=25  $\mu$ m. (F) Graph of the reciprocal intensity of activated NOTCH1 per nucleus in  
 10 uninduced and induced Pax8/NICD1 mice 1 day after IRI (n=4 mice for each group). a. u.: arbitrary units.

1 Bars indicate mean values. **(G)** Papillary tumor with foamy macrophages in the lumen, present in  
2 Pax8/NICD1 mice 4 weeks after IRI. Bar=25  $\mu$ m. **(H)** PAX2 positive papillary tumor (brown) from  
3 Pax8/NICD1 mouse kidneys 4 weeks after IRI. Bar=25  $\mu$ m. **(I)** Percentages of induced Pax8/NICD1  
4 mice with or without IRI presenting with pAdenomas and pRCCs (pAs/pRCCs, n=5 uninduced and n=11  
5 induced mice) and CKD (n=7 uninduced and n=5 induced mice) after 4 weeks. **(J)** Percentages of type  
6 1 vs. type 2 papillary tumors in Pax8/NICD1 mice 4 weeks after IRI (n=5 mice). Bars indicate mean  
7 values. **(K)** Representative papillary tumor from Pax8/NICD1/Confetti mouse kidney 4 weeks after IRI.  
8 Bar=25  $\mu$ m. DAPI (white) counterstains nuclei. **(L)** Clonal analysis of papillary tumors from  
9 Pax8/NICD1/Confetti mice 4 weeks after IRI (n=5 mice). Bars indicate mean values. **(M, N)** 3D  
10 reconstruction of representative lesions in Pax8/NICD1/Confetti kidneys after IRI. In **(M)**, COL4  
11 staining (cyan). Arrow indicates invasion. In **(N)**, VWF staining (purple). Arrows indicate  
12 vascularization. DAPI (white) counterstains nuclei. Signals for fluorescent Confetti proteins are as  
13 follow: GFP, green; RFP, red; YFP, yellow; and CFP, blue. Bars 25  $\mu$ m. Statistical power was assessed  
14 using Fisher's exact test **(I)** or Mann-Whitney test **(F, J)**; numbers above graphs represent p-values.

15



1 **Fig. 6. Human renal progenitors are transformed by NOTCH1 overexpression.** (A, B)  
2 Representative confocal images of healthy human kidney labelled with CD133 (green) and VCAM1 (red)  
3 (n=5 patients). In (A), glomerulus. In (B), tubules (arrows indicate CD133+VCAM1+ cells). Bars=25  
4  $\mu\text{m}$ . (C) Graph representing the evolution over time of the percentages of CD133+ and CD133-  
5 populations (n=3 biological replicates). (D) Graph representing the number of CD133- and CD133+ cells  
6 over time (n=3 biological replicates). Bars indicate median value. Statistical power was assessed using t  
7 test; number above graph represents p-value. (E) Representative cell division analysis of CFDA-SE-  
8 labeled CD133+ cells at day 10, out of 3 biological replicates. In the graph, the far right peak indicates  
9 undivided cells and each peak toward the left-hand side represents one cell division or generation. The  
10 percentages of cells in each division are shown in the graphs. (F) Representative FACS analysis for the  
11 contemporaneous expression of CD133 and VCAM1 in CD133+ fraction (right) and corresponding  
12 isotype controls (left) (n=3 biological replicates). (G, H) Representative H&E (G) and confocal (H)  
13 images of human renal progenitors infected with ZsGreen1-mock (n=3). In red, phalloidin staining.  
14 Bars=25  $\mu\text{m}$ . (I, J) Representative H&E (I) and confocal (J) images of human renal progenitors infected  
15 with ZsGreen1-NICD1 (n=3) (black arrow indicates nuclear groove). In red, phalloidin staining. Bars=25  
16  $\mu\text{m}$ . (K-M) 3D reconstruction of a confocal z-stack of human renal progenitors infected with either  
17 ZsGreen1-mock (K) or ZsGreen1-NICD1 (L, M) and showing tubular morphology with a lumen (K) or  
18 growth towards inside the tubule lumen (L), generating a tumor-like mass (M) (1 representative image  
19 of n=3 biological replicates each). In red, phalloidin staining. Bars=50  $\mu\text{m}$ . DAPI counterstains nuclei.  
20 (N) Representative confocal images of biopsies from patients with AKI (n=4). In red, CD133, in green  
21 activated NOTCH1. Bars= 25  $\mu\text{m}$ . (O) Higher magnification of a representative confocal images of a  
22 biopsy from a patient with AKI (n=4). In red, CD133, in green activated NOTCH1. Bars= 25  $\mu\text{m}$ . (P)  
23 UMAP representation of 3,327 cells, with 1479 pRCC (blue) and 1848 human renal progenitor (orange)  
24 cells. (Q) Expression of canonical PT1 signature and renal progenitor (*PROM1*) genes. (R) Violin plots

1 of single cell RNA sequencing data from pRCC (blue) (32) and from human renal progenitor cell cultures  
2 (orange), showing the expression of selected marker genes. In red, genes of the PT1 signature. *VCAM1*:  
3 vascular cell adhesion molecule 1, *EPCAM*: epithelial cell adhesion molecule, *SLC17A3*: Solute carrier  
4 family 17 member 3 and *SLC7A13*: Solute carrier family 7 member 13, CFDA-SE: Carboxyfluorescein  
5 diacetate succinimidyl ester.

6



1 **Fig. 7. AKI induces papillary tumors by promoting clonal expansion of renal progenitors in mice.**  
2 (A) Representative histological image with H&E staining of a type 2 papillary carcinoma present in  
3 Pax2/NICD1 mice 4 weeks after IRI. Bar=25  $\mu$ m. (B) Representative confocal image of type 2 papillary  
4 tumor of Pax2/NICD1/Confetti mice 4 weeks after IRI. Bar=25  $\mu$ m. (C, D) 3D reconstruction of  
5 representative lesions in Pax2/NICD1/Confetti kidneys 4 weeks after IRI. In (C), COL4 staining (cyan).  
6 Arrows indicate invasion. In (D), VWF staining (purple). Arrows indicate vascularization of the tumor.  
7 Bars=25  $\mu$ m. (E) Percentages of Pax2/NICD1 and Pax8/NICD1 mice presenting with pAdenomas and  
8 pRCCs (pAs/pRCCs) (n=11 for Pax2/NICD1 and n=11 for Pax8/NICD1) and CKD (n=6 for  
9 Pax2/NICD1 and n=5 for Pax8/NICD1) 4 weeks after IRI. (F) Percentages of type 1 vs. type 2 papillary  
10 tumors in Pax2/NICD1 mice 4 weeks after IRI (n=6 mice). Bars indicate mean values. (G) Clonal  
11 analysis of papillary tumors in each Pax2/NICD1/Confetti mouse 4 weeks after IRI (n=7 mice). Bars  
12 indicate mean value. (H) Graph of the number of papillary tumors per kidney section in Pax2/NICD1  
13 (n=6) and Pax8/NICD1 (n=6) mice 4 weeks after IRI. Bars indicate mean values. No significant  
14 difference between the groups. (I) Graph of the number of papillary tumors per kidney section in  
15 Pax2/NICD1/Confetti mice treated with vehicle (n=5 mice) or DAPT (n=5 mice) and sacrificed 4 weeks  
16 after IRI. Bars indicate mean values. (J) Representative confocal image of activated NOTCH1 (cyan) in  
17 Pax2/Confetti mice 2 days after IRI (n=4). Arrows indicate Pax2+Notch1+ cells. Bar=25  $\mu$ m. (K)  
18 Percentages of Pax2/Confetti mice at 4 weeks (n=8), 12 weeks (n=3), and 36 weeks (n=9) after IRI  
19 presenting with papillary adenomas (pAs) and pRCCs. (L) Clone frequency analysis in papillary tumors  
20 of Pax2/Confetti mouse kidneys at 36 weeks (n=7 mice). Bars indicate mean values. (M) Percentages of  
21 type 1 vs. type 2 papillary tumors in Pax2/Confetti mice at 36 weeks (n=5 mice). Bars indicate mean  
22 values. (N) Graph of the numbers of papillary tumors per kidney section in Pax2/Confetti mice treated  
23 with vehicle (n=7) or DAPT (n=5) and sacrificed 36 weeks after IRI. Bars indicate mean values. (O, P)  
24 Representative confocal images of type 1 (O) and type 2 (P) papillary tumors of Pax2/Confetti mice 36

1 weeks after IRI. Bars=25  $\mu$ m. (**Q**, **R**) 3D reconstruction of representative lesions in Pax2/Confetti kidney  
2 4 weeks after IRI. In (**Q**), COL4 staining (cyan). Arrows indicate invasion. In (**R**), VWF staining (purple).  
3 Arrows indicate vascularization of the tumor. Bars=25  $\mu$ m. DAPI (white) counterstains nuclei. Signals  
4 for fluorescent Confetti proteins are as follow: GFP, green; RFP, red; YFP, yellow; and CFP, blue. Data  
5 are mean $\pm$ SEM. Statistical power was assessed using Fisher's exact test (**E**, **K**) or Mann-Whitney test (**F**,  
6 **H**, **I**, **M**, **N**); numbers above graphs represent p-values.

7

	<b>pRCC (n=56)</b>	<b>Controls (n=101)</b>	<b>P<sup>(a)</sup></b>
<b>Previous AKI episodes</b>	<b>12/56 (21.4%)</b>	<b>7/101 (6.9%)</b>	<b>0.008</b>
Age at surgery (years)	65.3 ± 11.7	63.7 ± 9.9	0.184
<b>Gender (male)</b>	<b>47/56 (83.9%)</b>	<b>65/101 (64.4%)</b>	<b>0.009</b>
Preoperative CKD (eGFR < 60)	10/56 (17.9%)	11/101 (10.9%)	0.219
DM	8/56 (14.3%)	14/101 (13.9%)	0.942
Length of follow-up (years)	4.6 ± 2.8	4.3 ± 2.9	0.571
<b>Variable</b>	<b>OR</b>	<b>(95%CI)</b>	<b>P<sup>(b)</sup></b>
<b>AKI (y/n)</b>	<b>3.479</b>	<b>(1.135 - 10.667)</b>	<b>0.029</b>
Age (years)	0.979	(0.947 - 1.012)	0.213
Gender (M/F)	2.907	(1.250 - 6.761)	0.013
CKD (y/n)	1.093	(0.377 - 3.163)	0.870
DM (y/n)	0.715	(0.258 - 1.978)	0.518
	<b>ccRCC (n=75)</b>	<b>Controls (n=101)</b>	<b>P<sup>(a)</sup></b>
Previous AKI episodes	9/75 (12.0%)	7/101 (6.9%)	0.247
<b>Age at surgery (years)</b>	<b>66.6 ± 9.3</b>	<b>63.7 ± 9.9</b>	<b>0.047</b>
Gender (male)	48/75 (64.0%)	65/101 (64.4%)	0.961
Preoperative CKD (eGFR < 60)	12/75 (16.0%)	11/101 (10.9%)	0.320
DM	11/75 (14.7%)	14/101 (13.9%)	0.880
Length of follow-up (years)	4.2 ± 2.2	4.3 ± 2.9	0.916
<b>Variable</b>	<b>OR</b>	<b>(95%CI)</b>	<b>P<sup>(b)</sup></b>
AKI (y/n)	1.549	(0.514 - 4.664)	0.437
Age (years)	0.971	(0.939 - 1.004)	0.085
Gender (M/F)	0.945	(0.516 - 1.852)	0.945
CKD (y/n)	1.202	(0.463 - 3.115)	0.706
DM (y/n)	0.888	(0.366 - 2.155)	0.793

1

2 **Table 1. AKI episodes are a risk factor for the development of pRCC but not for ccRCC in humans.**

3 Clinical characteristics of pRCC, ccRCC and controls are described. Between-group comparisons were  
4 performed using the Mann–Whitney U test for continuous variables or the chi-squared test for categorical  
5 variables. Binary logistic regression analysis was used for the association between previous acute kidney  
6 injury (AKI) episodes (yes/no) and pRCC development adjusted for age, gender, preoperative chronic  
7 kidney disease (CKD), and diabetes mellitus (DM). eGFR, estimated glomerular filtration rate; OR, odds

- 1 ratio; RCC, renal cell carcinoma; pRCC: papillary RCC; ccRCC: clear cell RCC; SD, standard deviation;
- 2 CI, confidence interval. Continuous variables are presented as mean $\pm$ SD.