

# Scanning of the Renin First Intron for Cis-Regulatory Relevance

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# Abstract

## Background

The introns have been of great interest to molecular biologists since their discovery in 1977. Their copying and splicing require large and complex molecular machines and put a high energetic cost on the cell. Does the benefit of their existence exceed the burden on the cell? Recent studies propose that introns may influence gene expression. Here, we focused on the renin gene - a key component of a hormone system that regulates blood pressure and electrolyte balance.

## Methods and Results

We scanned the first intron of renin (intron A) for the *cis*-regulatory relevance in the natural genomic context of a kidney-derived cell line by utilizing a powerful RNA-guided DNA targeting tool: clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) fused with a transcription activator complex. We recruited the transcription activator complex with the help of specific RNA guides to defined locations in intron A (+ 4107 to + 137 bp downstream of the transcription start site (TSS)). Guide-RNAs specific for the renin promoter were used as a reference (-445 to + 28 bp upstream TSS). Moreover, we performed the qRT-PCR with the custom-designed primers for the identification of the secreted and non-secreted renin isoforms.

## Conclusions

With our approach, we identified the five *cis*-regions in renin intron A, activation of which led to a non-secreted renin isoform expression.

## INTRODUCTION

Even though introns do not encode proteins and put an expensive energetic cost on a cell, they constitute approximately 25% of the human genome. The elongation rate of RNA polymerase II (RNAP II) is, on average of 60 bases per second; therefore, the transcription of some long introns may last for many hours. Nevertheless, the benefit of having introns that burden the cell exceeds the energy costs spent on their transcription [1, 2].

The ability of introns to influence gene expression has been identified in a wide range of organisms, including mammals, nematodes, insects, fungi, and plants [3]. Several authors have shown that the first introns are, on average, longer than later introns. Moreover, Bradnam and Korf analyzed all available GenBank data on intron lengths and found a significant trend of increased length in first introns throughout a wide range of species. The generally accepted interpretation of this finding is that longer introns might harbor *cis*-regulatory sequences, likely related to transcription initiation. Furthermore, when

the orthologous introns in different species were compared, it was found that the first introns have the highest level of conservation among all introns in a given gene [2, 4, 5]. Previous studies have indicated that conserved regions of first introns might play a role in transcription regulation. Park et al. analyzed the enrichment level for specific regulatory signals using chromatin marks and protein-binding data from the ENCODE project. They have analyzed the following data: DNase I hypersensitivity sites (DHS), transcription factor binding sites (TFBSs), active chromatin marks (e.g., H3K4me1, H3K4me3), a repressive chromatin mark (e.g., H3K9me3), and the insulator protein CTCF binding sites. The authors of this study reported that first introns are enriched with the mentioned above signals in comparison to other introns [5].

Gallegos and Rose reported that intron DNA sequences might play a more significant role in the determination of the transcription initiation site than a proximal promoter. Few studies were mentioned, where the promoter was inactive unless endogenous introns were present; or, in other cases, the introns changed the spatial expression patterns of tissue-specific genes. Their examples demonstrated that gene expression might be influenced by introns, even though the prior promoter activity was lacking [6]. Moreover, some introns host an alternative promoter that utilizes a different transcription start site [2].

In recent years, many important discoveries have been made, which have allowed new tools to study gene expression. The clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) system was discovered as an adaptive immune system in bacteria, and it became a powerful RNA-guided DNA targeting tool [7]. In 2015, Konermann and co-workers developed a novel technology, based on CRISPR-Cas9, which converted Cas9 nuclease into an RNA-guided transcription activator. The complex consists of three components: a nucleolytically inactive Cas9-VP64 fusion, a sgRNA incorporating two MS2 RNA aptamers, and an MS2-P65-HSF1 activation helper protein. VP64, P65, and HSF1 are transcription activators. The CRISPR-Cas9 Synergistic Activation Mediator (SAM) system was developed for transcriptional activation at any endogenous genomic loci [8].

Renin is the key enzyme for cardiovascular and renal homeostasis [9]. Authors of several studies have shown that alternative transcripts of renin have a transcriptional start site within the first intron, and they lack the first exon in several species, including humans. As exon 1 encodes for the signal sequence needed for the secretory renin pathway, those transcripts lead to the production of a truncated protein, so-called non-secreted renin [10, 11].

In this study, we investigated the relevance of the first intron of the human renin gene, also called intron A, for renin transcription regulation as well as its influence on the expression of the secretory and non-secreted isoforms. We created a modified human embryonic kidney 293 (HEK 293) cell line, which had stably incorporated the CRISPR-SAM system and the renin gene tagged with firefly luciferase. Every transcription activation of renin led to the generation of bioluminescence due to the tagging of renin with the firefly luciferase reporter gene. The generated cell line allowed the analysis of renin transcriptional activation in the natural genomic context of a kidney-derived cell line. Renin expression was activated

after transient transfection with the promoter- and intron-specific guides that recruited the transcription activator complex to defined sites in the renin gene.

## METHODS

### Cell culture

All cell lines were purchased from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). The modified HEK293 cell line was cultured in DMEM media without Phenol Red (Thermo Fisher Scientific), supplemented with 10% Fetal Bovine Serum (FBS) and 1% Pen/Strep (Merck). In addition, culture media contained Blasticidin (0,1 mg/ ml) and Puromycin (0,03 mg/ml), both from Invivogen and Hygromycin B (0,05 mg/ml, Thermo Fisher Scientific). Cells were grown in a humidified incubator with 5%CO<sub>2</sub> at 37°C. The media was changed every three days.

### Stable integration of CRISPR/gRNA-directed synergistic activation mediator (SAM) system

The HEK293 cell line was modified as described by Mrowka et al. [12]. Briefly, HEK-293T cells were transfected with lentiviral packaging plasmids: pVSV-G (12259), pRSV-REV (12253), pMDLg/pRRE (12251), and plasmids lenti dCAS-VP64\_Blast (61425) or lenti MS2-P65-HSF1\_Hygro (61426) (all from Addgene) to produce the lentiviral particles. Lentivirus-containing supernatants were harvested 48 h after transfection. Afterward, HEK-293 cells were infected with lentiviral supernatant from lenti dCAS-VP64\_Blast-transfected HEK293T. Next, cells were infected with lentiviral supernatant from lenti MS2-P65-HSF1\_Hygro-transfected HEK-293T. Finally, cells were selected with 10 µg/ml blasticidin and 100 µg/ml hygromycin B.

### Renin tagging

Renin was tagged in frame with firefly luciferase, G418, and puromycin resistance markers encoded by plasmids pGuideit-tdtomato (Takara Bio Europe) and a renin homology arm encoding a donor plasmid containing firefly luciferase, G418 resistance gene, and puromycin resistance gene (LA\_REN\_Firefly\_G418\_PuroTK\_RA-REN, Transposagen, SGK-005). Briefly, the guide GGCTTCGCCTTGGCCCGCTG (at renin exon 10) was integrated into pGuide-it-tdTomato vector according to the manufacturer's protocol. Flanking homology arms were amplified by polymerase chain reaction (PCR) from the gDNA of HEK293. Next, PCR products were integrated into MV-PGK-Puro-TK flanking P2A-firefly luciferase - T2A - G418 resistance- PGK promoter-puromycin resistance - T2A. P2A and T2A sequences enable the production of renin, firefly luciferase, and antibiotic resistance proteins from one mRNA. Therefore, if renin expression was activated, we could evaluate it with the help of a luciferase activity assay. Cells were selected with 2 µg/ml puromycin [12].

## Transient transfections and luciferase activity assays

Modified HEK293 cells were seeded in the cavities of a 96-well plate. We seeded 80000 cells per well in 140 µl of Luciferin medium (High Glucose DMEM without phenol red, 10% FBS, 1% Pen/Strep, 250 µM D-Luciferin (Promega). Cells were 80–90% confluent at the moment of transfection. Each well was transfected with 100 ng of plasmid DNA in 10 µl Opti-MEM™ containing 0,5 µl Lipofectamine ® 2000 Transfection Reagent (both Thermo Fisher Scientific) 24 h after the cell seeding. Finally, the plate was sealed and placed in the modified Packard TopCount NXT Microplate Scintillation and Luminescence Counter (Packard Instrument Company) with a consistently maintained temperature of 37°C for the luciferase activity assay. The luminometer acquired data every 5 to 20 minutes within 70 hours.

## Real-time quantitative PCR (RT-qPCR)

RNA was isolated from  $5 \times 10^5$  cells with Monarch Total RNA Miniprep Kit (NEB), corresponding to the manual. RNA concentration was determined with a NanoDrop Spectrophotometer. The cDNA synthesis was performed with High Capacity Reverse Transcription Kit (Applied Biosystems). The 2X RT master mix was prepared on ice as follows: 10 µl of RNA (aliquoted to a concentration of 200 ng/µl) was added to 10 µl of 2X master mix, which resulted in a reaction mix final concentration of 100 ng/µl. The RT-qPCR was performed with Luminaris HiGreen High ROX qPCR Master Mix (Thermo Fisher Scientific) in a StepOnePlus™ Real-Time PCR System (Applied Biosystems) according to the user's manual. Housekeeping genes EMC7, GAPDH, and TBP were used as an endogenous control. Samples were analyzed in triplicate. One primer pair did not include the first exon of renin, which is present only in the secreted isoform, allowing us to distinguish splice-specific renin isoforms. Primers are listed in the supplementary table S1.

## Statistical analysis

Statistical analysis was performed in GraphPad Prism. A one-way analysis of variance (ANOVA) test was used to analyze the data obtained in a luciferase activity assay. An unpaired T-test was used to analyze RT-qPCR data.

## Oligonucleotides sequences

All DNA oligonucleotide sequences are listed in the supplementary table S1.

## RESULTS

### HEK293 with stably expressed components of the CRISPR-SAM system and tagged renin

We created a cell line with stably expressed components of the CRISPR-SAM system: a modified Cas9 and the transcription activator complex [8, 12]. Furthermore, the renin encoding gene was tagged in frame by a firefly luciferase reporter gene to follow the transcriptional activation of renin by detection of generated luminescence (Fig. 1). The generated cell line did not express renin endogenously. After introducing the guide sequence by the transient transfection, the CRISPR-SAM complex was assembled. The guide sequence recruited the Cas9-based transcription activation complex to a defined position in the human renin gene. We designed 10 different RNA-guides for the promoter region (specific for certain positions within the region – 445 to + 28 bp upstream the transcription start site (TSS) and 20 renin intron A guides (distinct for + 4107 to + 137 bp downstream the TSS). Twenty renin intron A guides we tested one by one. Their target sequences were located within the first intron, starting from proximity to renin exon one and further towards exon two. Thus, renin intron A was “scanned” for the *cis*-regulatory significance of intron A for renin transcription regulation with the help of the CRISPR-SAM system.

The components of the dCas9-dependent Synergistic Activation Mediator system [8] were stably expressed in the modified HEK-293 cell line. Moreover, renin was tagged in frame with the luciferase reporter gene. The cell line did not express renin endogenously. After introducing the guide sequence by the transient transfection, the CRISPR-SAM complex was assembled. The guide sequence recruited the dCas9-based transcription activation complex to a defined position in the human renin gene. We designed 10 RNA-guides for the promoter region (specific for certain positions within the region – 445 to + 28 bp upstream the transcription start site (TSS) and 20 renin intron A guides (distinct for + 4107 to + 137 bp downstream the TSS). Twenty renin intron A guides we tested one by one. Their target sequences were located within the first intron, starting from proximity to renin exon one and further towards exon two.

## Renin expression activation by single intron A guides

To investigate the *cis*-regulatory significance of intron A for renin transcription regulation, we designed twenty intron-specific guide RNAs. Evolutionarily conserved regions and DNase I hypersensitivity sites were identified using the UCSC Genome Browser, and the guides were designed to target these regions. Renin intron A guides were tested one by one. Their target sequences were located within the first intron, starting from the proximity to the renin exon one and further towards exon two. As the measurement of light from a chemical reaction always has the background noise, we consider that guides that did not lead to the luciferase activity, higher than 100 cps, did not show a significant increase in renin expression. Fifteen guides, namely guides 1–7 as well as guides 9–16, did not lead to significant activation of renin expression. Five renin intron A guides, guide 8, as well as guides 17–20, led to a significant activation of renin expression. Renin intron A guide 8 resulted in 437 cps, the guides 17–20 showed 1002, 747, 1161, 308 cps correspondingly (see Fig. 2).

The Y-axis represents luciferase activity in counts per second (cps), the X-axis - groups. The error bars show SD. Modified HEK cells were seeded into the cavities of 96-well plates and transfected with 100 ng of plasmid DNA per well encoding RNA-guide oligos to recruit the SAM complex to defined positions in the human renin gene. A luciferase reporter gene assay was performed afterwards, following the renin

transcriptional activation. **Promoter Guide** encodes one RNA-guide specific for – 131 to -111 bp upstream the TSS; **Intron A guides** (analyzed one by one) encode for 20 RNA-guides specific for certain positions within the region + 4107 to + 137 bp downstream the TSS; the **empty vector** represents a control.

## Renin expression activation in the natural promoter vs in the renin intron A

To activate the renin promoter for the reference, guide RNA targeting the promoter region (encoded on plasmid) was transiently transfected into a modified HEK293 cell line (100 ng of plasmid DNA per well). By conducting the stably expressed modified CRISPR-SAM complex to a specific position in the renin promoter, renin transcription was activated and followed by luciferase enzyme activity measurement. The promoter guide we used encodes the RNA-guide specific for – 131 to -111 bp upstream from the renin gene TSS.

The intron A guide 17, which encodes the RNA-guide specific for + 3784 to + 3764 bp downstream from the renin TSS, was utilized to test whether there is a difference between the single promoter and intron A guide transcription activation mode. The guides, which carried transcription factors to the promoter region, led to luciferase production in a steady manner, whereas guides targeting DNase I hypersensitive regions within intron A, led to a quick increase at the beginning and then went to a plateau (see Fig. 3). The empty vector backbone was used as a control and did not lead to renin transcription activation. The emitted light was measured in a live cell assay as counts per second (cps).

The Y-axis represents luciferase activity in counts per second (cps), and the X-axis represents measurement time in hours. The number of replicates for each experiment varies from 4 to 9 and represents the mean of 1 to 3 independent experiments. The cells were seeded into the cavities of 96-well plates and were transfected with 100 ng of plasmid DNA per well encoding RNA-Guide oligos to recruit the Cas9-based transcription activation complex to a defined position in the human renin gene. The generated bioluminescence was followed by the luciferase reporter gene assay. The luminometer acquired data every 5 to 20 minutes within 70 hours. The **Promoter Guide** encodes the RNA-Guide specific for – 131 to -111 bp upstream from the TSS; **Intron A Guide 17** encodes RNA-Guide specific for + 3784 to + 3764 bp downstream from the TSS; the **Empty Vector Backbone** represents a control.

## The splice-specific renin expression

Not all of the designed sgRNA guides were able to activate the transcription of renin in the first intron. Only five of the twenty guides led to significant activation of renin expression, and we grouped them as a pool of renin intron A transcription activators (Intron A Pool). We also used a pool of the renin promoter guides to activate the transcription of the classical renin isoform. To identify the alternative isoforms of renin, we designed splice-specific primers, either including or skipping the first exon (Fig. 4). The modified HEK-293 cell line was transfected with the renin promoter and intron A pool of guides as well as with single guides. The mRNA was isolated at 70 hours post-transfection and analyzed with RT-qPCR.

Renin 1 represents the secreted renin isoform (primer pair binds to the first exon), while Renin 2 represents both secreted and non-secreted (cytosolic) renin isoforms, as the primers bind to the renin exons 3–5. After the transfection with the pool of renin promoter-specific guides, no significant difference in renin expression was found between Renin 1 and Renin 2 primer pairs utilization.

**A:** renin has secreted and non-secreted isoforms. Non-secreted renin lacks the first exon. **B:** splice-specific primers were designed for RT-qPCR to evaluate the level of both isoforms' expression after transfection of a modified HEK393 cell line with the promoter and intron A guides. Exons are shown with the gray boxes. Primers are indicated with the arrows. Dashes represent the spanning region in introns. **C:** splice-specific renin expression. Renin 1 represents the secreted (kidney-specific), Renin 2 represents both secreted and non-secreted (cytosolic) renin isoforms, as the primers bind to renin exons 3–5. Relative renin expression was normalized to the empty vector control. Unpaired T-test: stars indicate a statistical significance with  $** p < 0,01$ . The promoter pool, as well as the promoter pool mix with intron A pool of guides, did not result in a statistically significant difference between Renin 1 and Renin 2 primer pairs. Renin intron A pool as well as single intron A guides 8 and 17 led predominantly to non-secreted renin isoform expression ( $p < 0,01$ ). The cells transfected with an empty vector did not show a significant increase in renin expression, as the modified HEK293 cell line did not express renin endogenously.

Transfection with the intron A pool of guides led predominantly to an expression of the transcript encoding non-secreted renin ( $p < 0,01$ , Unpaired T-test), as the renin expression, while utilizing the Renin 1 primer pair with the binding in the first exon, was significantly lower as compared to the Renin 2 primers. Simultaneous transfection with promoter pool and intron A pool did not lead to a statistically significant difference. Transfection with the single intron A guides: guide 8 and guide 17 predominantly led to an expression of the transcript, encoding non-secreted renin ( $p < 0,01$ ). Cells transfected with an empty vector did not show a significant increase in renin expression, as the modified HEK293 cell line did not express renin endogenously.

## DISCUSSION

More and more evidence is emerging that introns are involved in transcription regulation. It has been found that first introns in particular cell types and genes are methylated as enhancers, and this feature is linked with gene expression. Anastasiadi and co-workers used different tissues from a modern fish to show a relationship between DNA methylation and gene expression. A study identified more differences in tissue-specific DNA methylation patterns in the first intron than in any other part of the gene. Moreover, they reported that differently methylated regions in the first intron have a positive or negative correlation with the gene expression [13]. The stunning idea that introns can enhance gene expression in the absence of the promoter arose after promoter deletion studies in *Caenorhabditis elegans* and *Arabidopsis* [14]. Also, there is evidence that renin has an alternative transcription start site in the first intron in rats, mice, and humans [15]. All mentioned above make the first intron attractive in terms of studying its effect on the level of gene expression.

We focused on the renin gene because renin expression is the rate-limiting step of the renin-angiotensin-aldosterone system (RAAS). RAAS is a hormone system that regulates blood pressure and electrolyte balance. Understanding of renin transcription regulation is significant as a malfunction of RAAS might lead to hypertension and cardiovascular diseases [16]. Also, we were interested in whether the first intron of renin may participate in the transcription regulation since it has specific characteristics. The first intron of renin occupies 35% of the REN-201 splice variant. In addition to transcription binding sites in the promoter region, it contains regulatory elements (Open Regulatory Annotation, OREG1685612); accessible chromatin, as it includes the DNaseI hypersensitivity cluster in 123 cell types; a transcription factor binding cluster, and evolutionarily conserved DNA sequences present in 100 vertebrates [17, 18].

The juxtaglomerular (JG) cells in the kidney are the primary source of circulating renin. The lack of an established human JG renin-expressing cell line complicates the study of renin transcription regulation [19]. Yet, few cell lines expressing renin were established: the human pulmonary carcinoma cell line CALU-6 (ACC 734) and the mouse kidney AS4.16 (ATCC® CRL2193) [12]. In order to study the *cis*-regulatory importance of the first intron of the renin gene, we modified the HEK293 cell line, which allowed us to analyze renin transcriptional activation in the natural genomic context of a kidney-derived cell line. The modified HEK-293 cell line had stably incorporated the CRISPR-SAM system.

The SAM system consists of a modified Cas9 protein used to recruit a transcription activation complex to defined positions in gene promoters, enabling the switch of the gene transcription [8]. The target position is determined by the guide-RNA-oligonucleotides encoded by transiently transfected vectors. The transcription activation of renin led to the generation of bioluminescence due to the tagging of renin with the firefly luciferase reporter gene. We used CRISPR-SAM-based “scanning” of the first intron of the renin gene to investigate its role in transcription regulation. By simply designing sgRNA for the target DNA region, we could guide the CRISPR-SAM system to any position genome-wide.

The SAM system always led to the initiation of renin transcription when promoter guides were introduced into the modified HEK-293 cells. We used twenty guides to “scan” the first intron of renin. Five guides led to the activation of renin expression, whereas fifteen did not differ significantly from the empty backbone control. As cells did not express renin endogenously, transcription of renin was activated approximately 10 hours post-transfection. The luciferase activity assay was followed in the luminometer for up to 70 hours.

Moreover, the existence of alternative renin isoforms, which lack the first exon and are incapable of being secreted, was reported by many authors. In the kidney, transcription is initiated at the first exon (so-called exon 1A). It utilizes a canonical renin promoter. Non-secreted isoforms have the initiation of transcription at exon 1B (brain-specific) and exon 1C (lung-specific). Although it is known that tissue-specific renin isoforms exist, the molecular mechanism responsible for the choice of transcriptional start site remains unclear [15].

Furthermore, the existence of an alternative promoter in the first intron of renin was reported in the rat [10]. Besides, Sinn and Sigmund stated that the lung-specific, non-secreted renin isoform has

transcription initiation 79 bp directly upstream of exon two and fuses to it without splicing. Our findings (Fig. 4) show that transcription activators brought by the SAM system to intron A led predominantly to non-secreted renin expression, whereas activation of the renin promoter equally led to both isoforms production. Therefore, intron A might be responsible for the non-secreted renin isoform expression.

Our approach focuses on the study of renin transcriptional expression in its genomic context. Modified CRISPR/Cas9 SAM system, developed by Konermann and co-workers, commanded transcription activators to bind DNA, and we could evaluate the transcription rate (if it was present) with the reporter gene and splice-specific qRT-PCR. Other approaches, e.g., cloning of the renin locus, would have disrupted the surrounding context and contact domain boundaries of renin. For example, a genome browser at NCBI (<https://www.ncbi.nlm.nih.gov/gene/5972>) identifies intron A as a silencer element based on reporter gene assay evidence, which was performed in 1996. Briefly, the authors cloned renin intron A into the basic vector downstream of the luciferase gene and then transfected the Calu-6 cell line. They evaluated the level of renin expression with a luciferase activity assay. We do not deny the importance of this work; we suppose that the assessment of intron A solely, without the surrounding context, could have been misleading.

## CONCLUSION

To sum up, our results, along with the other available data, show that the first intron of renin may actively participate in transcription regulation. The utilization of the SAM system, in combination with reporter tagging, allows quick and reliable estimation of the regulatory significance of DNA sequences within a given gene. The recruitment of the transcription activation complex to renin intron A led to a significant level of renin expression. Analyzing twenty RNA-guides specific for certain positions within the region + 4107 to + 137 bp downstream of the TSS of renin one by one, we could identify areas, targeting of which led to renin expression. These specific areas might play a role in the expression of alternative renin isoforms.

## Declarations

## SUPPLEMENTARY DATA

Supplementary data are available online.

## CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

## FUNDING

## Author Contribution

O.M., S.R., and R.M. made substantial contributions to the conception and design of the work. S.R. and A.W. carried out cell line creation and established the cloning and transfection procedures, and data evaluation. R.M. established the luciferase read-out assay; created R-Studio scripts for the data evaluation. S.K. and A.O. performed the design and cloning of the renin promoter guides. O.M. performed the design and cloning of the renin intron guides and primer design for the RT-qPCR; carried out the transfection procedures, luciferase activity assays, and qRT-PCR. O.M. drafted the manuscript. S.R., A.W. and R.M. revised the manuscript and advised on the improvement. All authors approved the final version to be published and agree to be accountable for all aspects of the work.

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## Figures

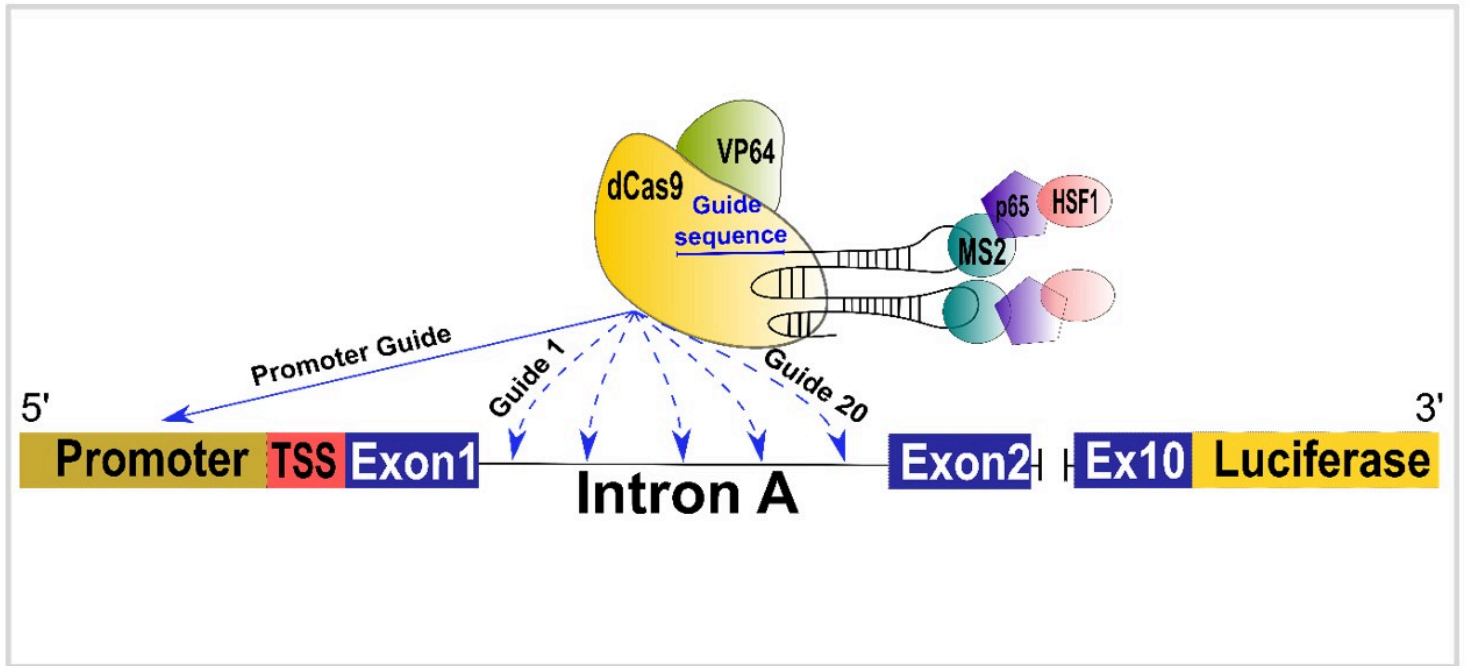


**Figure 1**

Assembled CRISPR-SAM complex “scanning” for the *cis*-regulatory relevance of the human renin promoter and intron A.

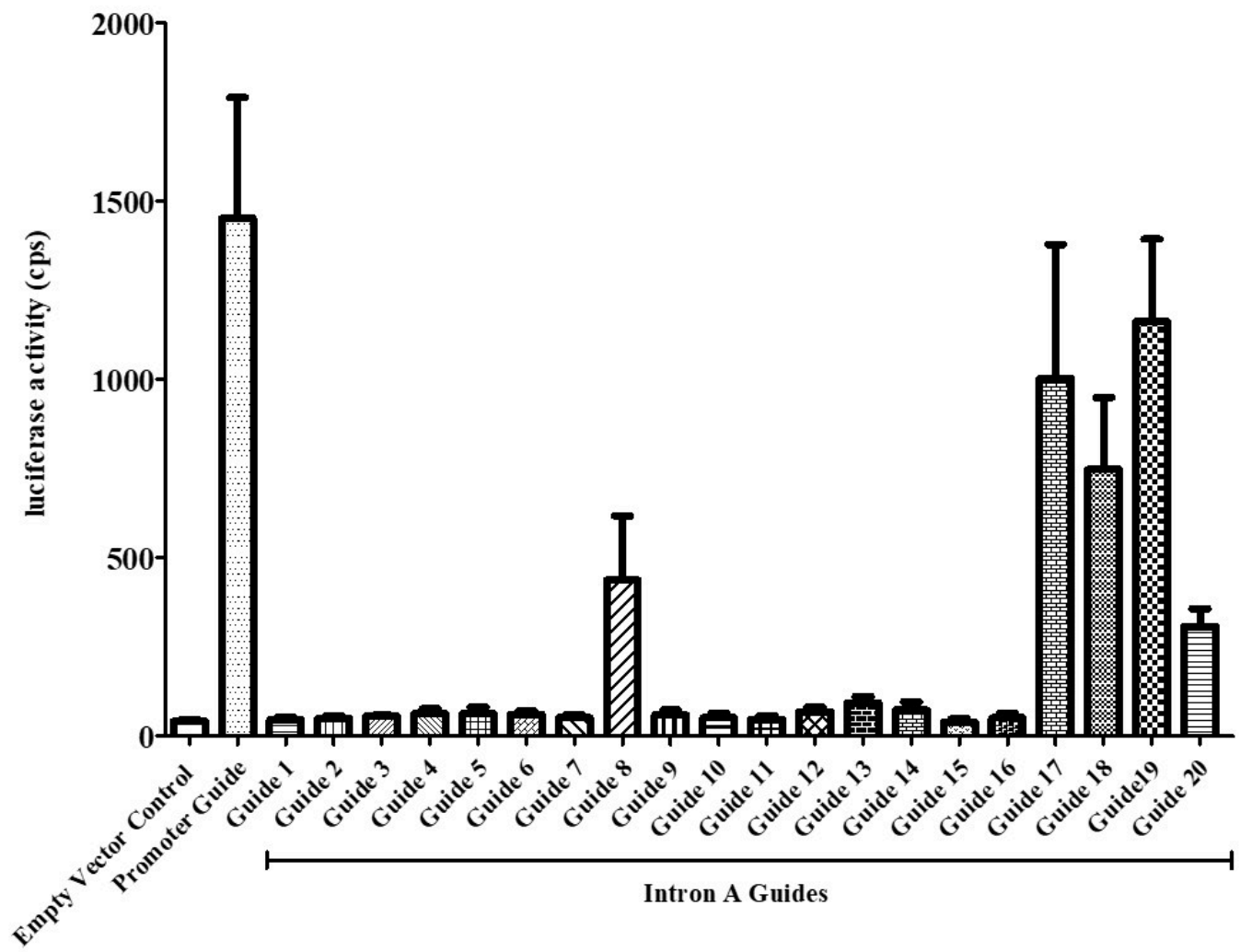


**Figure 2**

The luciferase activity assays after single renin promoter and intron A guides transfection, 70 h after transfection.

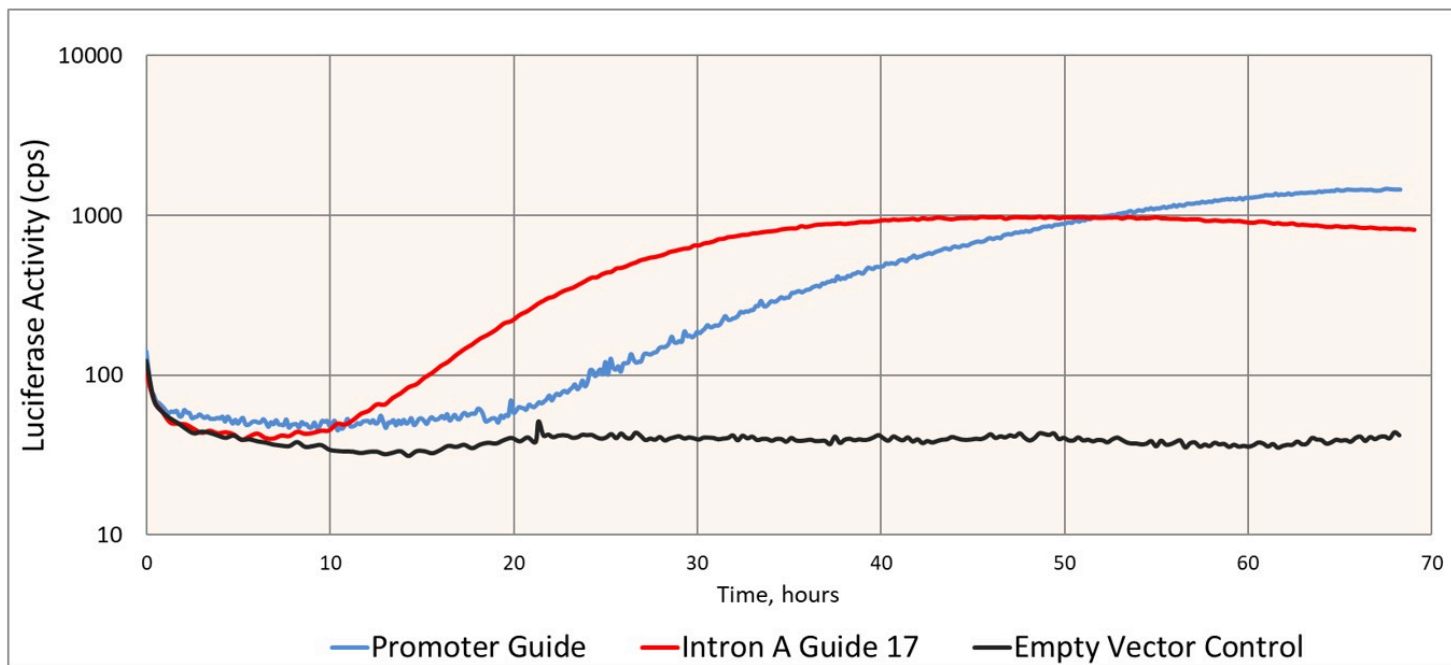


**Figure 3**

**Luciferase activity assay after renin promoter and intron A guide 17 transfection**

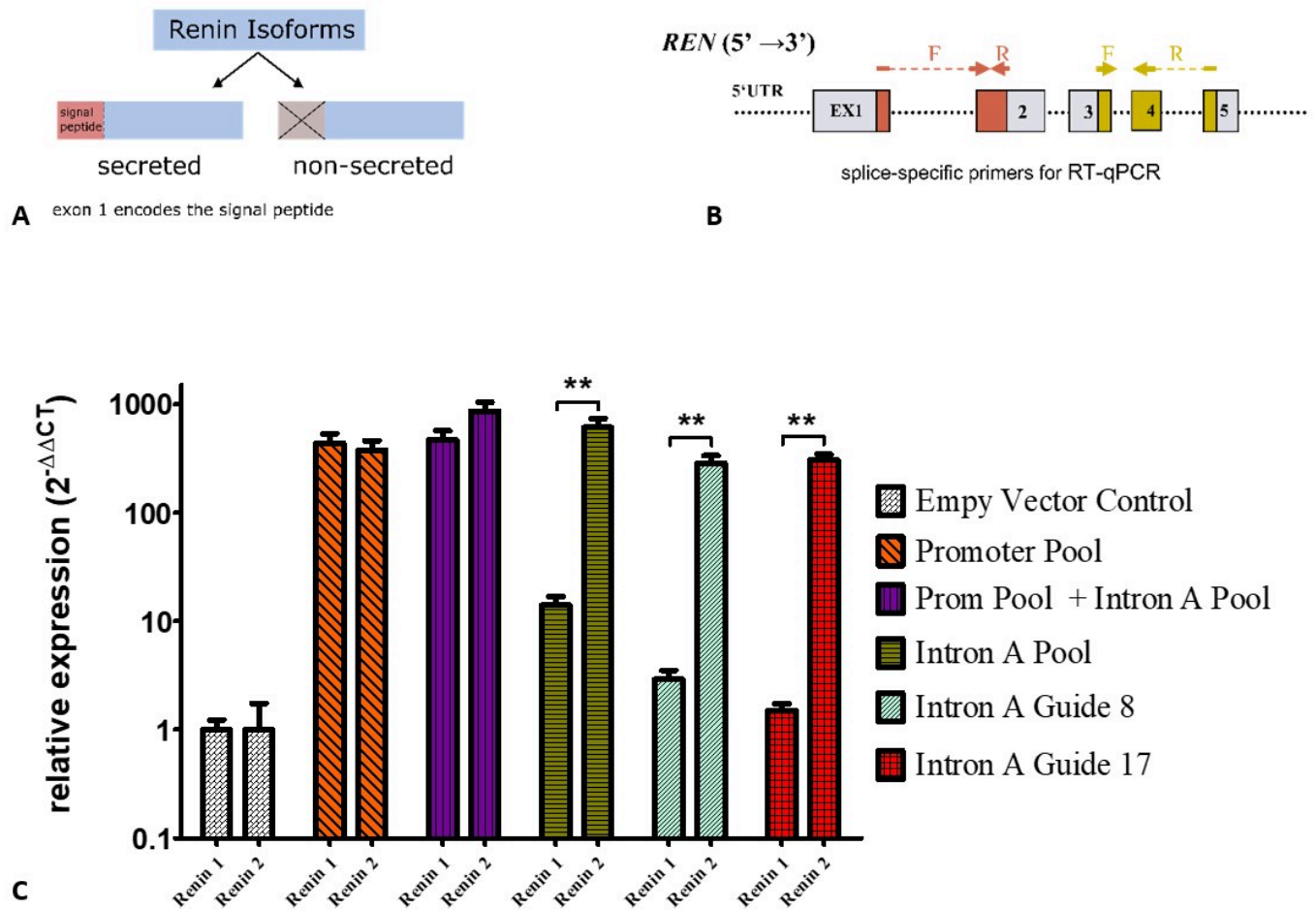


**Figure 4**

#### Renin isoforms and splice-specific renin expression

**A:** renin has secreted and non-secreted isoforms. Non-secreted renin lacks the first exon. **B:** splice-specific primers were designed for RT-qPCR to evaluate the level of both isoforms' expression after transfection of a modified HEK393 cell line with the promoter and intron A guides. Exons are shown with the gray boxes. Primers are indicated with the arrows. Dashes represent the spanning region in introns. **C:** splice-specific renin expression. Renin 1 represents the secreted (kidney-specific), Renin 2 represents both secreted and non-secreted (cytosolic) renin isoforms, as the primers bind to renin exons 3-5. Relative renin expression was normalized to the empty vector control. Unpaired T-test: stars indicate a statistical significance with \*\*  $p < 0,01$ . The promoter pool, as well as the promoter pool mix with intron A pool of guides, did not result in a statistically significant difference between Renin 1 and Renin 2 primer pairs. Renin intron A pool as well as single intron A guides 8 and 17 led predominantly to non-secreted renin isoform expression ( $p < 0,01$ ). The cells transfected with an empty vector did not show a significant increase in renin expression, as the modified HEK293 cell line did not express renin endogenously.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryDataS1.xlsx](#)