

<b>URINE</b>
# ID
volume in mL
date of collection
date of processing x CFU
# vials
<b>COLLECTION:</b>
pretreatment (Prostate massage/ catheter) NONE
Ethical Y/N
collection method (Mid-stream)/catheter
collection time
collection date
volume collected
collection device
<b>STORAGE</b>
after collection store at 4°C
Transfer to the lab in ice Y/N
arrived at the lab date
hours passed after collection <4h/ <6h
light protection Y/N
Exclusion macrohematuria Y/N
quality -pH Y/N
quality protein level Y/N
<b>PROCESSING</b>
1) homogenize sample before processing Y/N
2) urine centrifugation 3000rpm x 15 min=704 G force
3) centrifugation at 4°C Y/N
4) supernatant: loose pellet pipetting without disturbs Y/N
5) cell pellet stored Y/N
6) register # aliquots CFU
7) Storage at -80°C/ location
8) method freeze: quick -80°C
9) Defrost: in Room temp water bath until defrost

**Supplementary Table S1:** Data collected with the urine samples. Urine collection, processing, and storage.

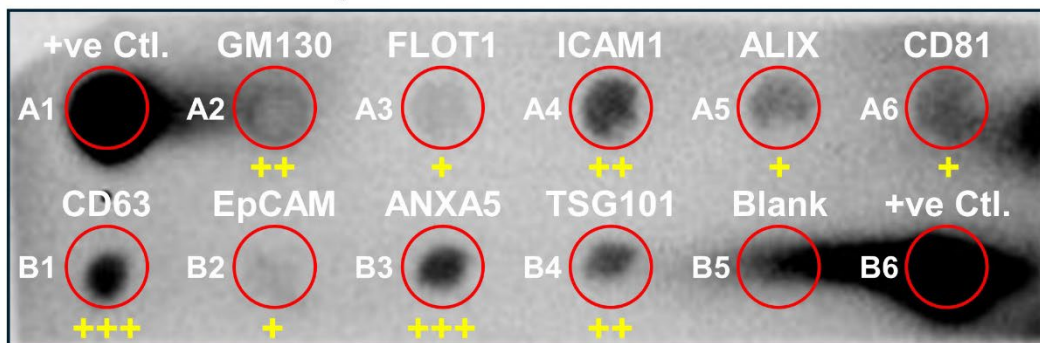
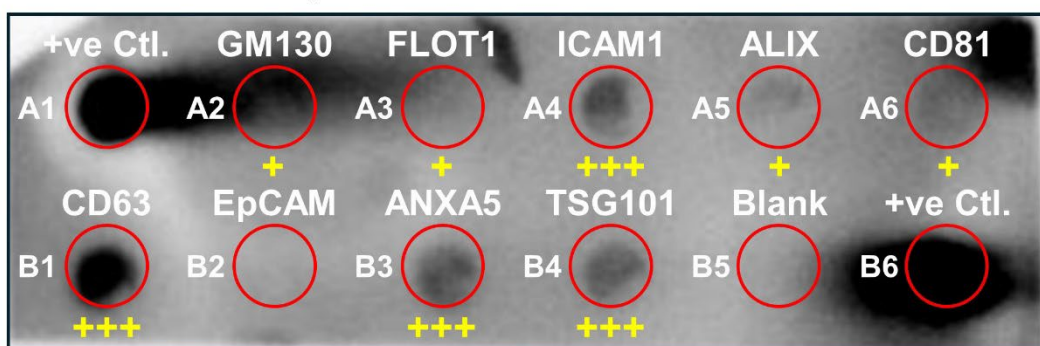
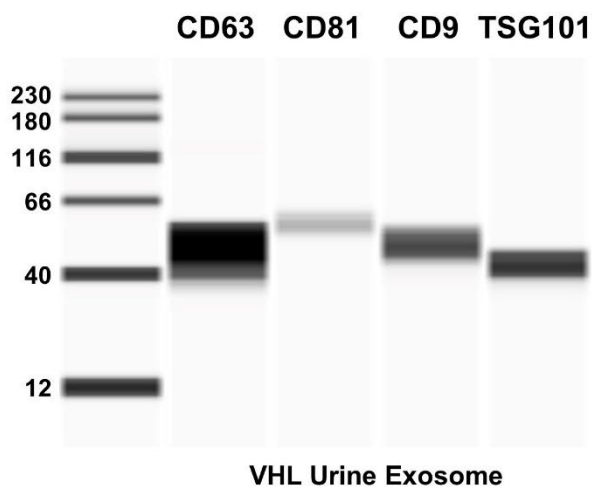
Position	ID	Description	VHL Urine					Control Urine		
A1	Positive Ctl.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
A2	GM130	Cis-golgi Matrix Protein	++	+	++	+	N/A	+	N/A	+
A3	FLOT1	Flotillin-1	+	+	+	-	-	+	-	+
A4	ICAM1	Intercellular Adhesion Molecule 1	+++	+++	++	++	++	+++	-	+++
A5	ALIX	Programmed Cell Death 6 Interacting Protein	++	+	+	+	+	++	-	+
A6	CD81	Tetraspanin	+	+	+	+	+	+	+	+
B1	CD63	Tetraspanin	+++	+++	+++	+++	+++	+++	+	+++
B2	EpCAM	Epithelial Cell Adhesion Molecule	+	-	+	-	-	+	-	-
B3	ANXA5	Annexin A5	+++	+++	+++	+++	++	+++	-	+++
B4	TSG101	Tumor Susceptibility Gene 101	+++	+++	++	+++	++	+++	-	+++
B5	Blank	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
B6	Positive Ctl.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

**Supplementary Table S2:** Collated results for Exo-Check® exosome array.

CD81 and CD63 were expressed in all the samples.

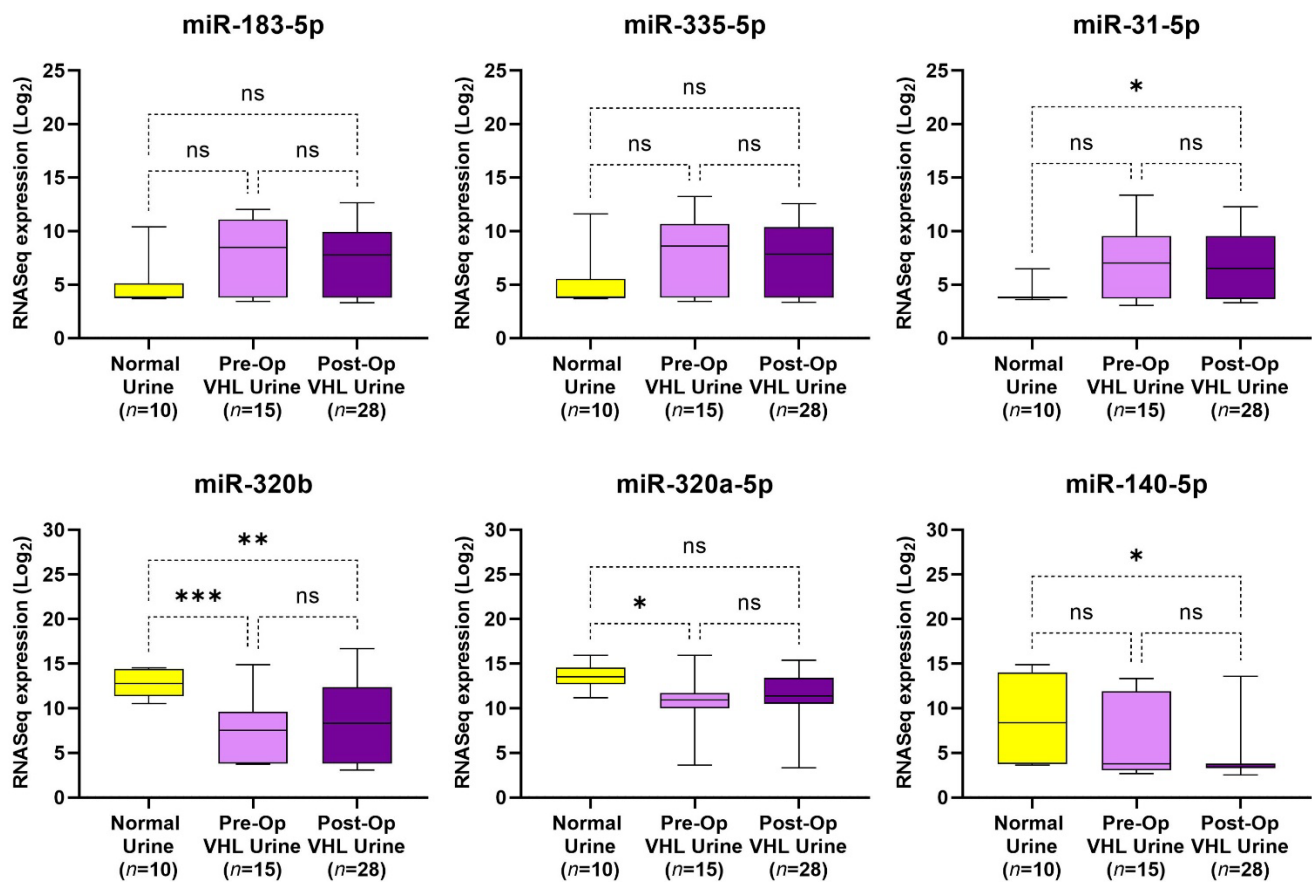
miRNA	Comparison	Fold change	Fold change (log2)	p-value	Adjusted p-value
hsa-miR-25-5p	All VHL Urine vs. Normal Urine	33.579	5.069	0.00366812	0.011658735
hsa-miR-183-5p	All VHL Urine vs. Normal Urine	28.702	4.843	1.27945E-15	4.04307E-13
hsa-miR-182-5p	All VHL Urine vs. Normal Urine	23.972	4.583	0.00108442	0.010659708
hsa-miR-335-5p	All VHL Urine vs. Normal Urine	22.809	4.512	9.9528E-15	1.57254E-12
hsa-miR-429	All VHL Urine vs. Normal Urine	13.549	3.760	0.000113908	0.005999152
hsa-miR-241-5p	All VHL Urine vs. Normal Urine	11.866	3.569	0.001793835	0.010659708
hsa-miR-31-5p	All VHL Urine vs. Normal Urine	9.679	3.275	5.60748E-09	5.90655E-07
hsa-miR-1281-5p	All VHL Urine vs. Normal Urine	8.960	3.164	0.009559349	0.024558977
hsa-miR-148b-5p	All VHL Urine vs. Normal Urine	6.620	2.727	0.005461802	0.015273712
hsa-miR-361-5p	All VHL Urine vs. Normal Urine	5.505	2.461	0.007342814	0.019498564
hsa-miR-99a-5p	All VHL Urine vs. Normal Urine	5.164	2.368	0.000800252	0.010659708
has-let-7g-5p	All VHL Urine vs. Normal Urine	4.345	2.119	0.001113487	0.010659708
has-let-7e-5p	All VHL Urine vs. Normal Urine	3.953	1.983	0.000562593	0.010659708
hsa-miR-200a-5p	All VHL Urine vs. Normal Urine	3.374	1.755	0.000512254	0.010659708
hsa-miR-146a-5p	All VHL Urine vs. Normal Urine	3.305	1.725	0.00554204	0.015362145
hsa-miR-1269a	All VHL Urine vs. Normal Urine	2.760	1.465	0.003138054	0.011141855
has-let-7f-5p	All VHL Urine vs. Normal Urine	2.263	1.178	0.006737131	0.018512464
hsa-miR-144-5p	All VHL Urine vs. Normal Urine	2.211	1.144	0.0069259	0.018867106
hsa-miR-3065-5p	All VHL Urine vs. Normal Urine	2.181	1.125	0.000805411	0.010659708
hsa-miR-139-5p	All VHL Urine vs. Normal Urine	-2.308	-1.207	0.00448479	0.013244801
hsa-miR-140-5p	All VHL Urine vs. Normal Urine	-2.804	-1.488	0.000450757	0.010659708
hsa-miR-423-5p	All VHL Urine vs. Normal Urine	-3.097	-1.631	0.001827433	0.010659708
hsa-miR-320a-5p	All VHL Urine vs. Normal Urine	-8.055	-3.010	7.99371E-06	0.000505202
hsa-miR-486-5p	All VHL Urine vs. Normal Urine	-18.390	-4.201	0.007812706	0.020573458
hsa-miR-23a-5p	All VHL Urine vs. Normal Urine	-20.598	-4.364	0.007145916	0.019300081
hsa-miR-320b	All VHL Urine vs. Normal Urine	-24.592	-4.620	1.73401E-07	1.36987E-05
hsa-miR-320c	All VHL Urine vs. Normal Urine	-30.663	-4.938	0.002451932	0.010659708
hsa-miR-186-5p	PreOp VHL Urine vs. PostOp VHL Urine	4.995	2.320	0.006942929	0.068379087
hsa-miR-142-5p	PreOp VHL Urine vs. PostOp VHL Urine	4.965	2.312	0.002707284	0.068379087
hsa-miR-542-5p	PreOp VHL Urine vs. PostOp VHL Urine	4.116	2.041	0.002120531	0.068379087
hsa-miR-339-5p	PreOp VHL Urine vs. PostOp VHL Urine	3.528	1.819	0.007420709	0.068379087
hsa-miR-30c-5p	PreOp VHL Urine vs. PostOp VHL Urine	2.990	1.580	0.008631719	0.068379087
hsa-miR-424-5p	PreOp VHL Urine vs. PostOp VHL Urine	-3.034	-1.601	0.007158388	0.068379087
hsa-miR-660-5p	PreOp VHL Urine vs. PostOp VHL Urine	-3.353	-1.745	0.00681669	0.068379087
hsa-miR-1307-5p	PreOp VHL Urine vs. PostOp VHL Urine	-4.152	-2.054	0.001871645	0.068379087
hsa-miR-205-5p	PreOp VHL Urine vs. PostOp VHL Urine	-4.605	-2.203	0.008428092	0.068379087
hsa-miR-152-5p	PreOp VHL Urine vs. PostOp VHL Urine	-5.281	-2.401	0.001539034	0.068379087
hsa-miR-483-5p	PreOp VHL Urine vs. PostOp VHL Urine	-8.650	-3.113	0.00074045	0.04679644
hsa-miR-1281-5p	PreOp VHL Urine vs. PostOp VHL Urine	-11.290	-3.497	1.60436E-05	0.001267448
hsa-miR-107	PreOp VHL Urine vs. PostOp VHL Urine	-11.898	-3.573	1.79421E-08	2.83484E-06
hsa-miR-196b-5p	PreOp VHL Urine vs. PostOp VHL Urine	-19.890	-4.314	8.02039E-09	2.53444E-06
hsa-miR-22-5p	PreOp VHL Urine vs. PostOp VHL Urine	-21.897	-4.453	4.15856E-08	4.38035E-06
hsa-miR-148b-5p	VHL ccRCC tumor vs. Normal Kidney	214.497	7.745	1.36061E-06	0.000429952
hsa-miR-181a-5p	VHL ccRCC tumor vs. Normal Kidney	213.319	7.737	2.26995E-05	0.001024722
hsa-miR-19b1-5p	VHL ccRCC tumor vs. Normal Kidney	103.881	6.699	4.08949E-06	0.000448001
hsa-miR-19b2-5p	VHL ccRCC tumor vs. Normal Kidney	102.896	6.685	4.25318E-06	0.000448001
hsa-miR-15a-5p	VHL ccRCC tumor vs. Normal Kidney	97.931	6.614	7.75185E-06	0.000612396
hsa-miR-107	VHL ccRCC tumor vs. Normal Kidney	77.192	6.270	1.58782E-05	0.001003501
hsa-miR-15b-5p	VHL ccRCC tumor vs. Normal Kidney	68.018	6.088	2.23287E-05	0.001024722
hsa-miR-186-5p	VHL ccRCC tumor vs. Normal Kidney	57.945	5.857	3.42475E-05	0.001352774
hsa-miR-7-5p	VHL ccRCC tumor vs. Normal Kidney	53.111	5.731	0.000128474	0.004510866
hsa-miR-497-5p	VHL ccRCC tumor vs. Normal Kidney	43.863	5.455	0.0038488	0.079281287
hsa-miR-185-5p	VHL ccRCC tumor vs. Normal Kidney	36.374	5.185	0.001186854	0.037504587
hsa-miR-455-5p	VHL ccRCC tumor vs. Normal Kidney	25.307	4.661	0.006334346	0.111202963
hsa-miR-542-5p	VHL ccRCC tumor vs. Normal Kidney	18.784	4.231	0.003764689	0.079281287
hsa-miR-320b	VHL ccRCC tumor vs. Normal Kidney	14.563	3.864	0.003901437	0.079281287
hsa-miR-615-5p	VHL ccRCC tumor vs. Normal Kidney	14.117	3.819	0.003414275	0.079281287
hsa-miR-629-5p	VHL ccRCC tumor vs. Normal Kidney	10.610	3.407	0.003845643	0.079281287
hsa-miR-126-5p	VHL ccRCC tumor vs. Normal Kidney	10.395	3.378	0.009019775	0.137601969
hsa-miR-365a-5p	VHL ccRCC tumor vs. Normal Kidney	8.835	3.143	0.008617076	0.137601969
hsa-miR-338-5p	VHL ccRCC tumor vs. Normal Kidney	8.457	3.080	0.009381057	0.137601969
hsa-miR-891a-5p	VHL ccRCC tumor vs. Normal Kidney	-54.119	-5.758	0.004014242	0.079281287
hsa-miR-9-5p	VHL ccRCC tumor vs. Normal Kidney	-88.233	-6.463	0.005606667	0.10362988
hsa-miR-206	VHL ccRCC tumor vs. Normal Kidney	-122.508	-6.937	0.009579884	0.137601969

**Supplementary Table S3:** Selected miRNAs from comparative analyses with FC>2.0 and p-value <0.01.

**A****Representative VHL urine****Representative Control urine****B****Supplementary Figure S1: Characterization of Exosome Isolates.**

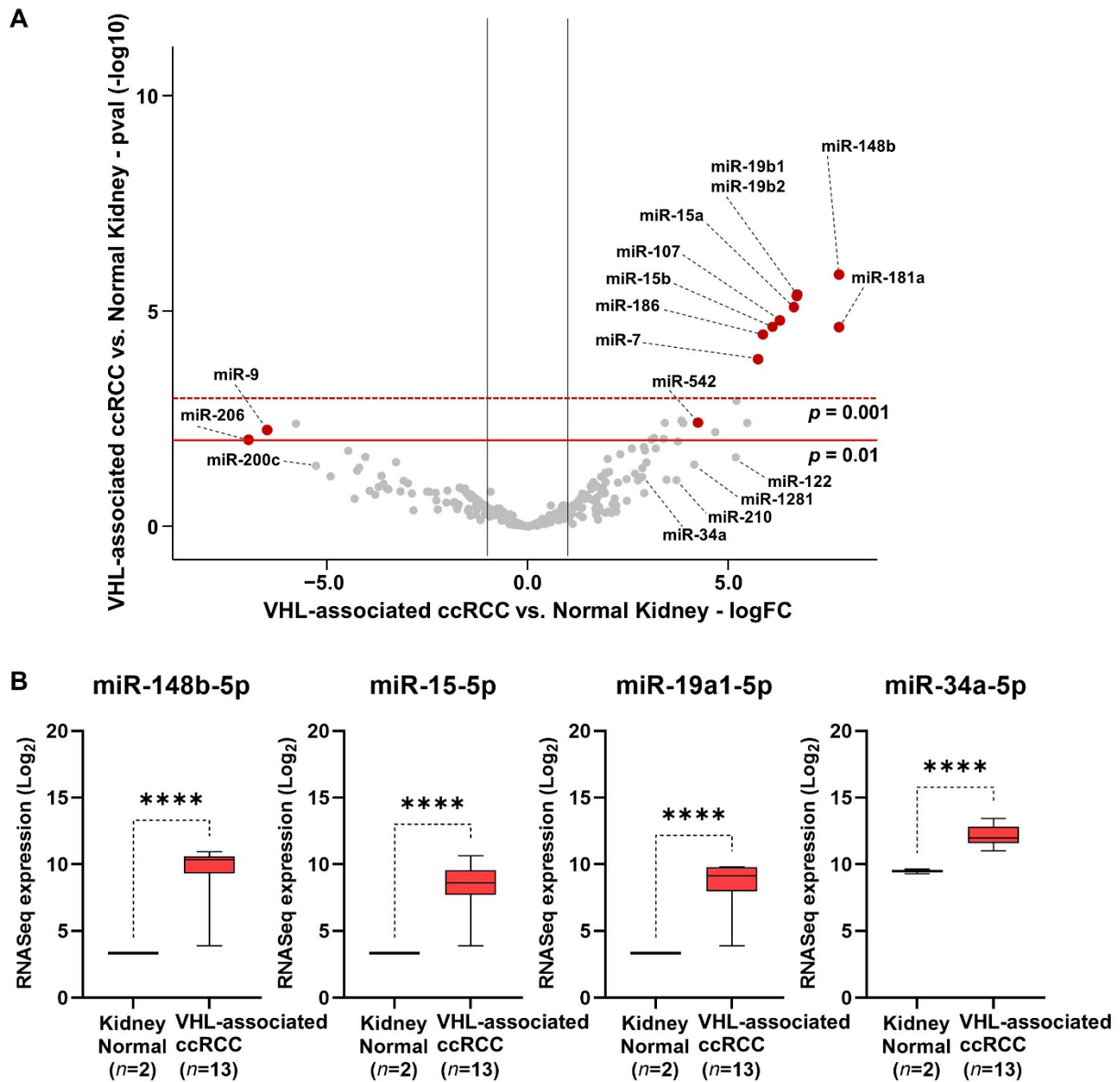
A) Representative VHL-urine and control urine samples for Exosome characterization by Exo-Check® exosome array.

B) Western blot analysis of CD63, CD81, CD9, and TSG101 antibodies for an example of a VHL urine exosome sample.



**Supplementary Figure S2:** Pre- and Postoperative VHL Urine analysis for marker miRNAs.

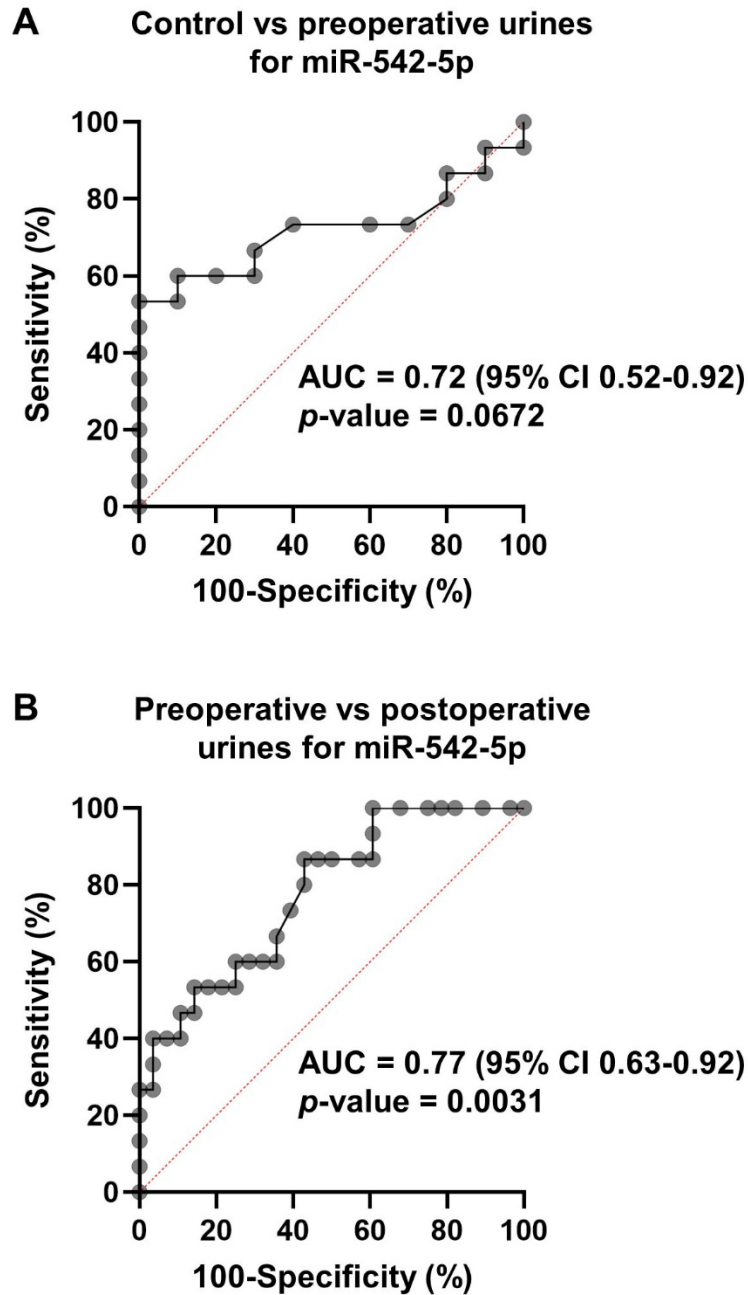
Confirmatory box plots for six selected up- and downregulated miRNAs comparing normal urine exosomes to VHL urine exosomes, with the VHL urines separated into pre- and postoperative urines, one-way ANOVA. \*  $p$ -value <0.05, \*\*  $p$ -value <0.01, \*\*\*  $p$ -value <0.001, ns – not significant



**Supplementary Figure S3: Differential miRNA expression in VHL-associated ccRCCs**

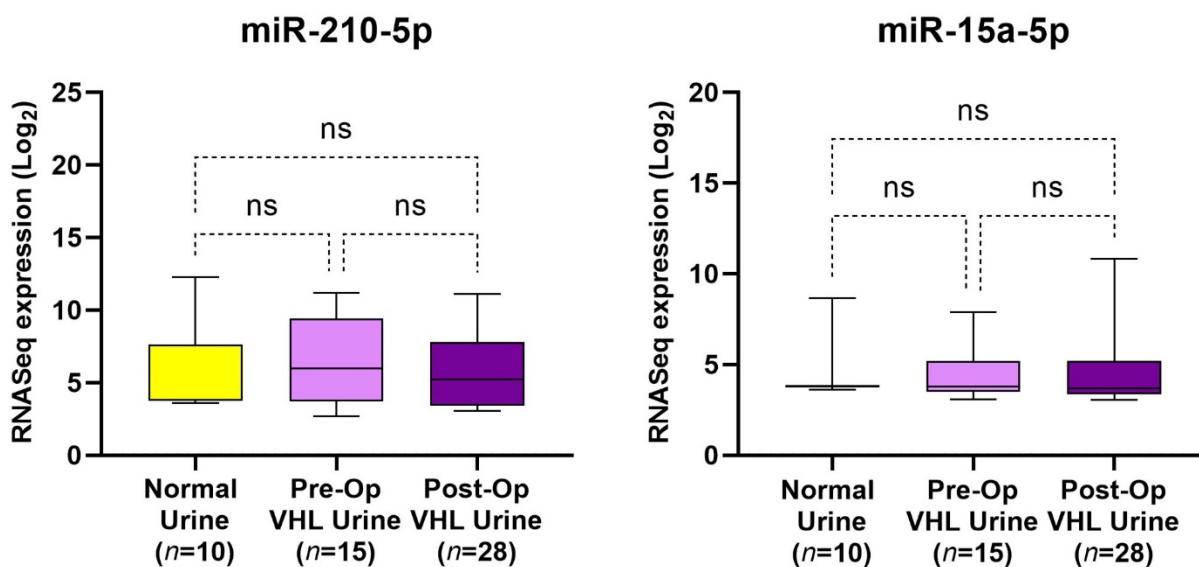
A) Volcano plot analysis comparing 13 VHL-associated ccRCC samples excised from 13 VHL patients within this study compared to normal kidney, consisting of an in-house pool of microdissected FFPE normal kidney. Red line represents  $p=0.01$  and dashed dark red line represents  $p=0.001$ . MiRNAs with  $p$ -values  $> 0.001$  and fold changes  $> 2$  are labeled in red, all other miRNAs are labeled in gray. The gray lines represent log2 fold-changes of  $-1.0$  and  $1.0$ . B) Selected miRNAs, including miR-15a-5p, with significantly increased expression in VHL-associated ccRCC in comparison to normal kidney, Welch's t-test.

\*\*\*\*  $p$ -value  $< 0.0001$



**Supplementary Figure S4. ROC curves for miR-542-5p.**

Receiver operating characteristic (ROC) curves for miR-542-5p demonstrating the comparison between preoperative urines and control urines (A) and between preoperative urines and postoperative urines (B).



**Supplementary Figure S5. Pre- and postoperative VHL urine analysis for known marker miRNAs associated with ccRCC.**

Box plots comparing the expression of known miRNA markers of sporadic ccRCC within the normal urine exosomes and the VHL urine exosomal miRNA, with the VHL urines separated into pre- and postoperative urines, one-way ANOVA. ns – not significant