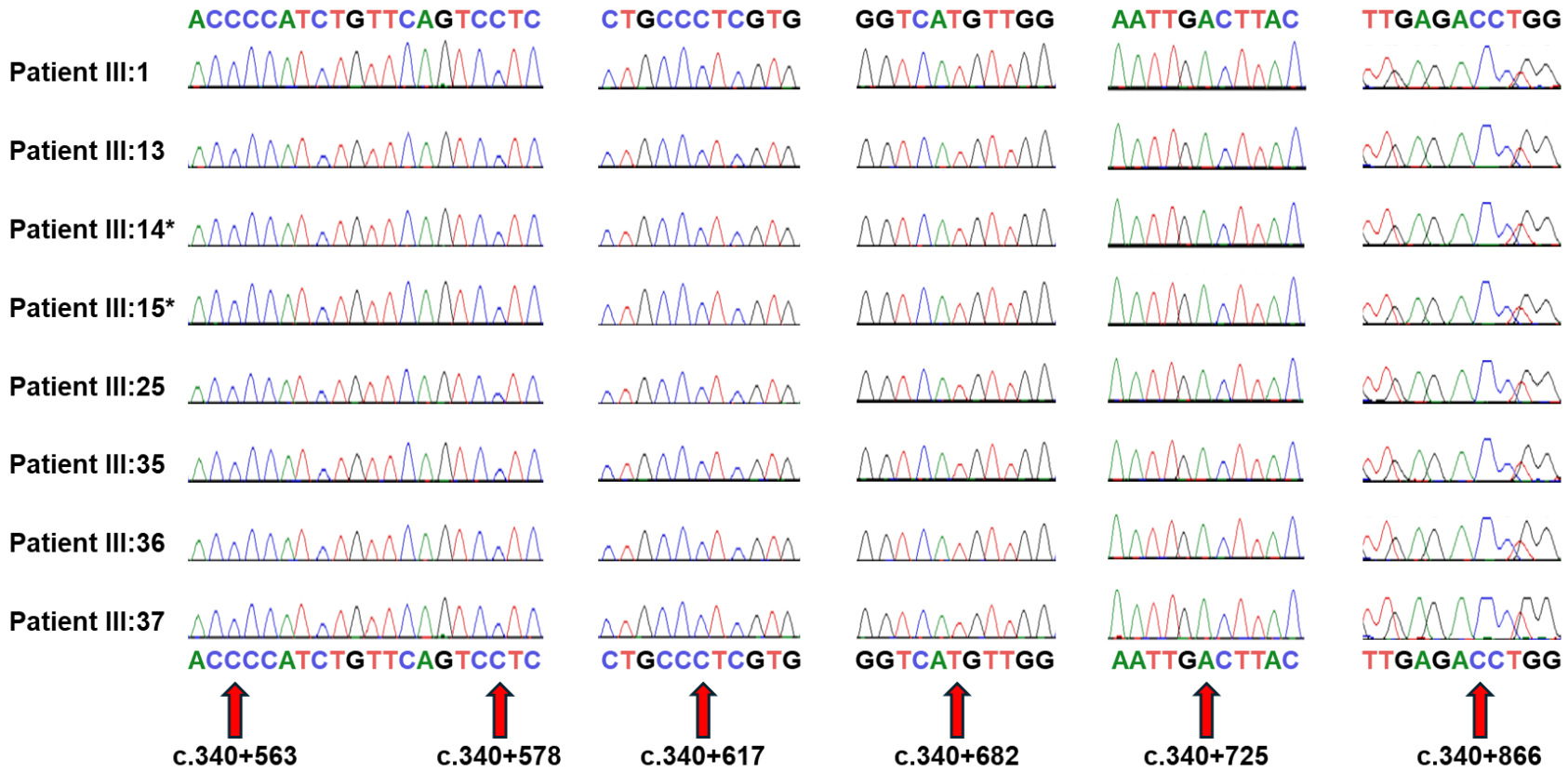
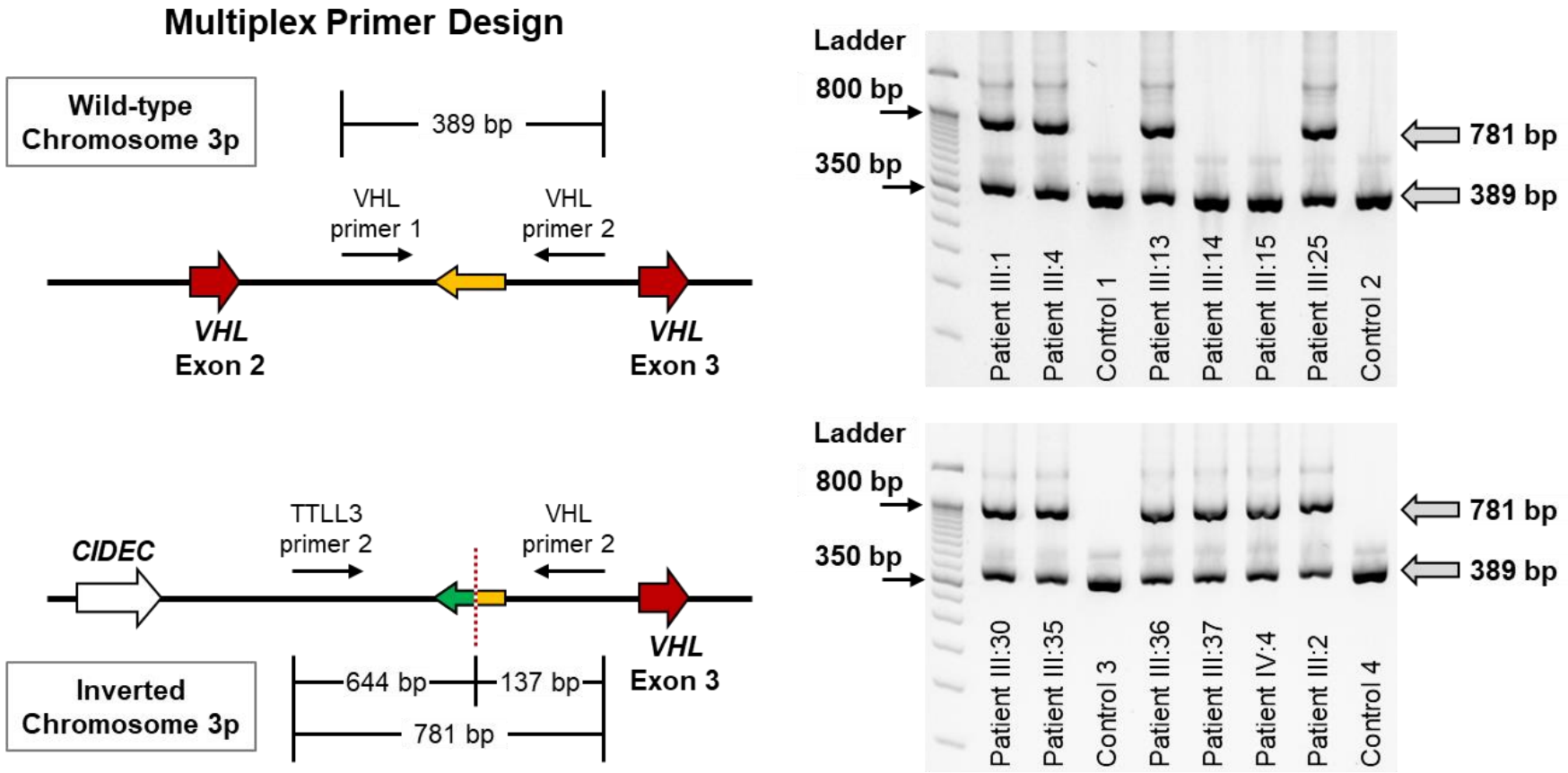
# Supplementary Data




**Supplementary Figure 1: Sanger sequencing of the *VHL* E1' cryptic exon**

DNA sequencing of the E1' cryptic exon was performed on eight family members, all of whom exhibited wild type sequences, including at c.340+617 and c.340+682, which have been reported as pathogenic variants, and additional loci that have been reported as benign or VUS [12]. Primer sequences were as follows: forward GAATAGTTTGCATTTGAAGGTG, reverse TCACCTTTTAAGCATCCTCTC. * denotes family members (III:14 and III:15) who have no clinical manifestations of VHL disease, while the other six patients have at least one manifestation.



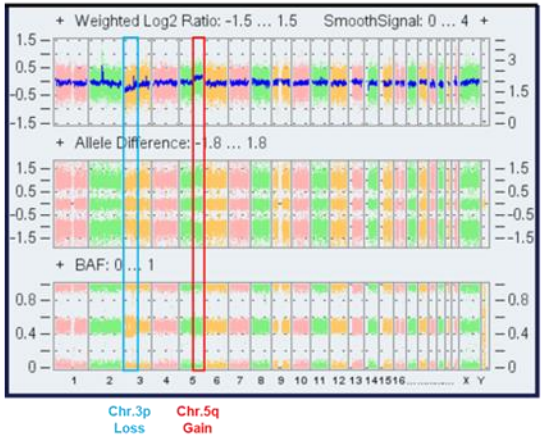

**Supplementary Figure S2: Multiplex PCR-based analysis of germline DNA**

Multiplex PCR primer sets were designed to identify both the wild-type sequence between the second and third exons of the *VHL* gene and across the breakpoint created by the chromosome 3p inversion, creating 389 bp and 781 bp fragments respectively. Twelve individuals from the family were evaluated with ten demonstrating evidence of both the wild-type and inversion induced fragments, indicting the germline presence of the inversion. Four control DNAs from unrelated, unaffected individuals and two family members (III:14 and III:15) only demonstrated the wild-type fragment.

A

## Patient III:1

### Tumor 1




### Tumor 2

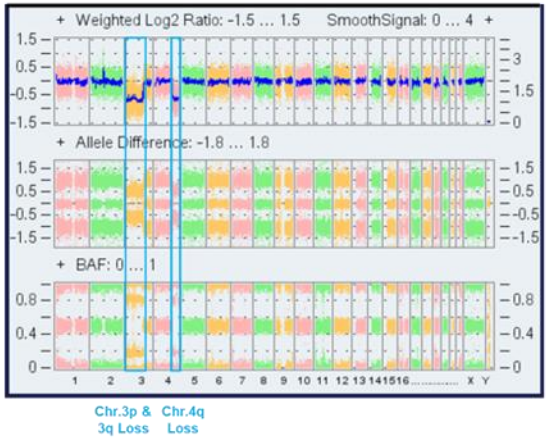

### Tumor 3


B

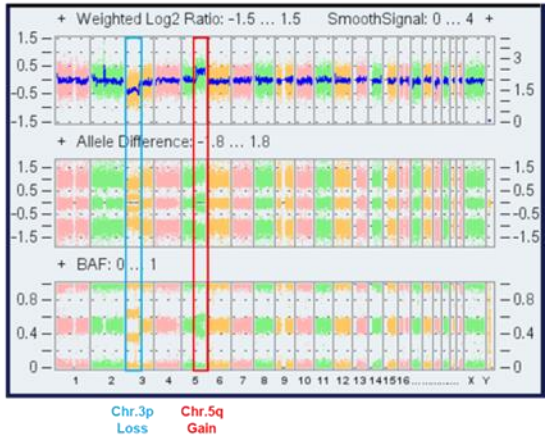
## Patient III:25

### Tumor 1


### Tumor 2



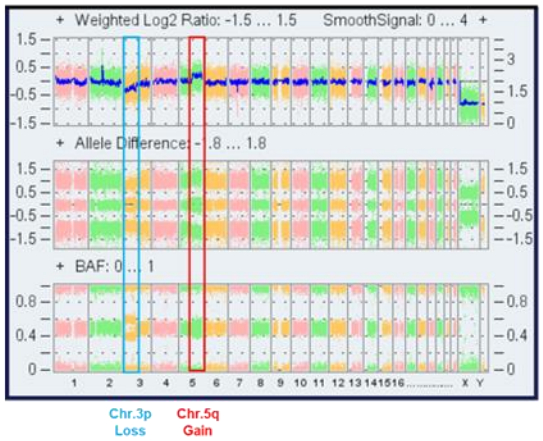

### Tumor 3



C

## Patient III:35

### Tumor 1



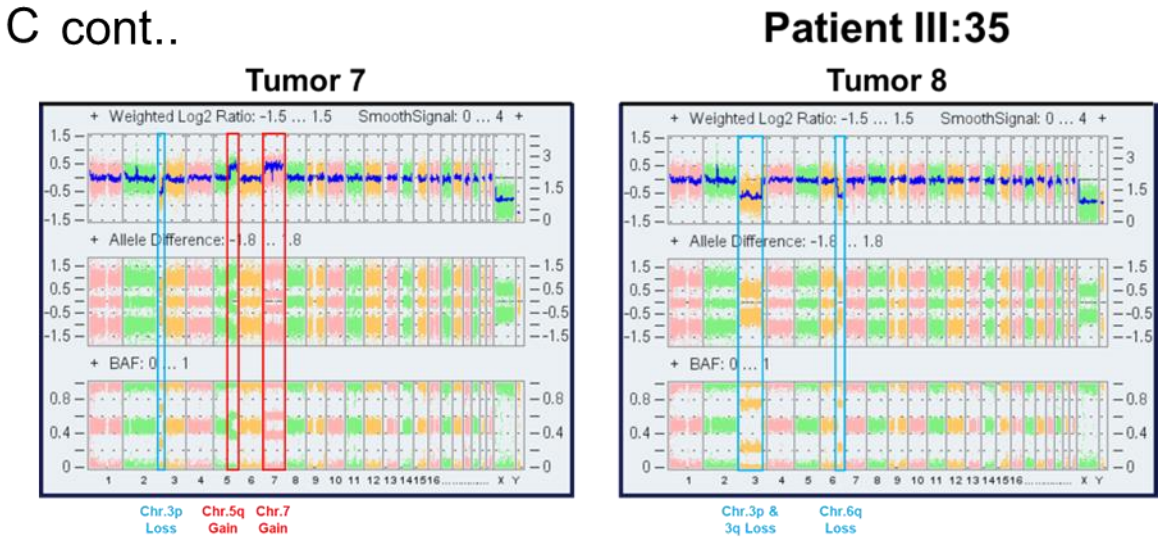

### Tumor 2


### Tumor 3


### Tumor 4

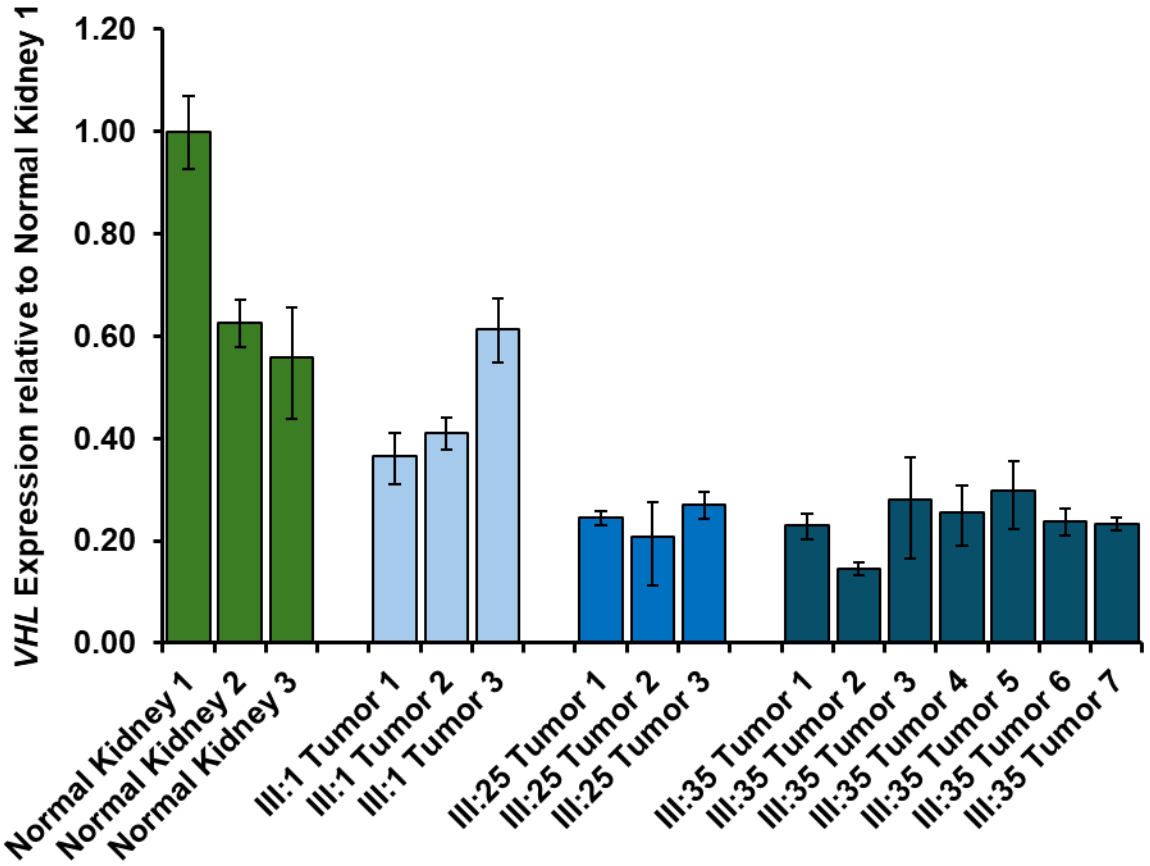

### Tumor 5


### Tumor 6




**Supplementary Figure S3: OncoScan analysis of chromosomal copy number alterations in tumor DNA**

Fourteen tumor DNAs were evaluated using the OncoScan CNV assay and significant copy number events were highlighted. Three tumors were available for both patient III:1 (A) and patient III:25 (B). Eight tumors were assessed from patient III:35 (C).




**Supplementary Figure S4: Expression analysis of *VHL* mRNA in ccRCCs**

Expression analysis of *VHL* was performed on 13 ccRCCs from patients III:1 (n=3), III:25 (n=3), and III:35 (n=7) and compared to normal kidney samples derived from three independent, unrelated VHL patients. All values are presented as the relative expression of VHL in comparison to the normal kidney 1 sample. Expression was normalized by 18S expression.

**Supplementary Table S1: PCR primer sequences**

| Primer | Sequence |
|---|---|
| TTLL3 1 | TGGAAGTCTGTCTTGTGCTAC |
| TTLL3 2 | GACTAGGTGTTGGTTTGGTG |
| VHL1 | GGATACCCAATTCAGTTGTAC |
| VHL2 | TAGTTTTGGGGTGGTAGTCAC |

**Supplementary Table S2: Clinical Manifestations**

| Patient | Clinical Manifestations of VHL Syndrome | | | | | | Germline status | Mortality Status |
|---|---|---|---|---|---|---|---|---|
| | Kidney | Pancreas | CNS | Pheo | ELST | Retinal | | |
| II:2 | Yes | Yes | | | | | n.d. | d.50s |
| II:4 | Yes | | Yes | | | | n.d. | d.70s |
| II:6 | | | | | | | n.d. (obligate carrier) | Alive – 50s |
| II:7 | Yes | | Yes | | | | n.d. | d.50s |
| II:8 | | | Yes | | | | n.d. | d.40s |
| II:10 | Yes | Yes | Yes | Yes | | | n.d. | Alive – 50s |
| II:11 | Yes | | Yes | | | | n.d. | d.40s |
| II:12 | | Yes | | | | | n.d. | d.20s |
| III:1 | Yes | Yes | Yes | | | | Positive | Alive – 20s |
| III:2 | Yes | Cysts | Yes | | | | Positive | Alive – 30s |
| III:3 | | | | | | Yes | n.d. | d.20s |
| III:4 | | | Yes | | | | Positive | Alive – 30s |
| III:5 | | | Yes | | | | n.d. | Alive – 30s |
| III:13 | Yes | Cysts | Yes | | | | Positive | Alive – 30s |
| III:21 | | | Yes | | | | n.d. | Alive – 30s |
| III:23 | | | Yes | | | | n.d. | Alive – 20s |
| III:24 | Yes | | | | | | n.d. | d.20s |
| III:25 | Yes | Yes | Yes | Yes | Yes | Yes | Positive | Alive – 30s |
| III:30 | Yes | Yes | Yes | | Yes | | Positive | Alive – 20s |
| III:35 | Yes | | Yes | | | | Positive | Alive – 30s |
| III:36 | Cysts | Cysts | | | | | Positive | Alive – 20s |
| III:37 | | Cysts | | | | | Positive | Alive – teens |
| IV:4 | | | | | | | Positive | Alive – teens |

**Supplementary Table S3: Renal Tumor Characteristics**

| Patient Tumors | Age at Surgery | Laterality | Size (cm) WHO/ISUP Grade | Chr. 3p Loss | Chr. 5q Gain | Additional Genetic Alterations |
|---|---|---|---|---|---|---|
| III:1 – 1 | 20s | Right | 1.3 – FNG2 | Yes | Yes | |
| III:1 – 2 | 20s | Right | 2.0 – FNG2 | Yes | No | |
| III:1 – 3 | 20s | Right | 0.8 – FNG2 | Yes | Yes | Chr. 13 Gain |
| III:25 – 1 | 30s | Left | 4.0 – FNG2 | Yes | No | *MTOR*, *TET2* |
| III:25 – 2 | 30s | Left | 1.1 – FNG2 | Yes | No | Chr. 3q & 4q Loss |
| III:25 – 3 | 30s | Left | 0.8 – FNG2 | Yes | Yes | |
| III:35 – 1 | 20s | Right | 1.9 – FNG2 | Yes | Yes | |
| III:35 – 2 | 20s | Right | 0.9 – FNG2 | Yes | Yes | Chr. 7, 16 & 18 Gain |
| III:35 – 3 | 20s | Right | 2.9 – FNG2 | Yes | Yes | Chr. 7 Gain |
| III:35 – 4 | 20s | Right | 1.2 – FNG2 | Yes | No | Chr. 2q & 3q Loss |
| III:35 – 5 | 20s | Right | 0.6 – FNG2 | Yes | Yes | |
| III:35 – 6 | 20s | Right | 0.8 – FNG2 | Yes | Yes | |
| III:35 – 7 | 20s | Right | 1.1 – FNG2 | Focal | Yes | Chr. 7 Gain |
| III:35 – 8 | 20s | Right | 0.5 – FNG2 | Yes | No | Chr. 3q & 6q Loss |