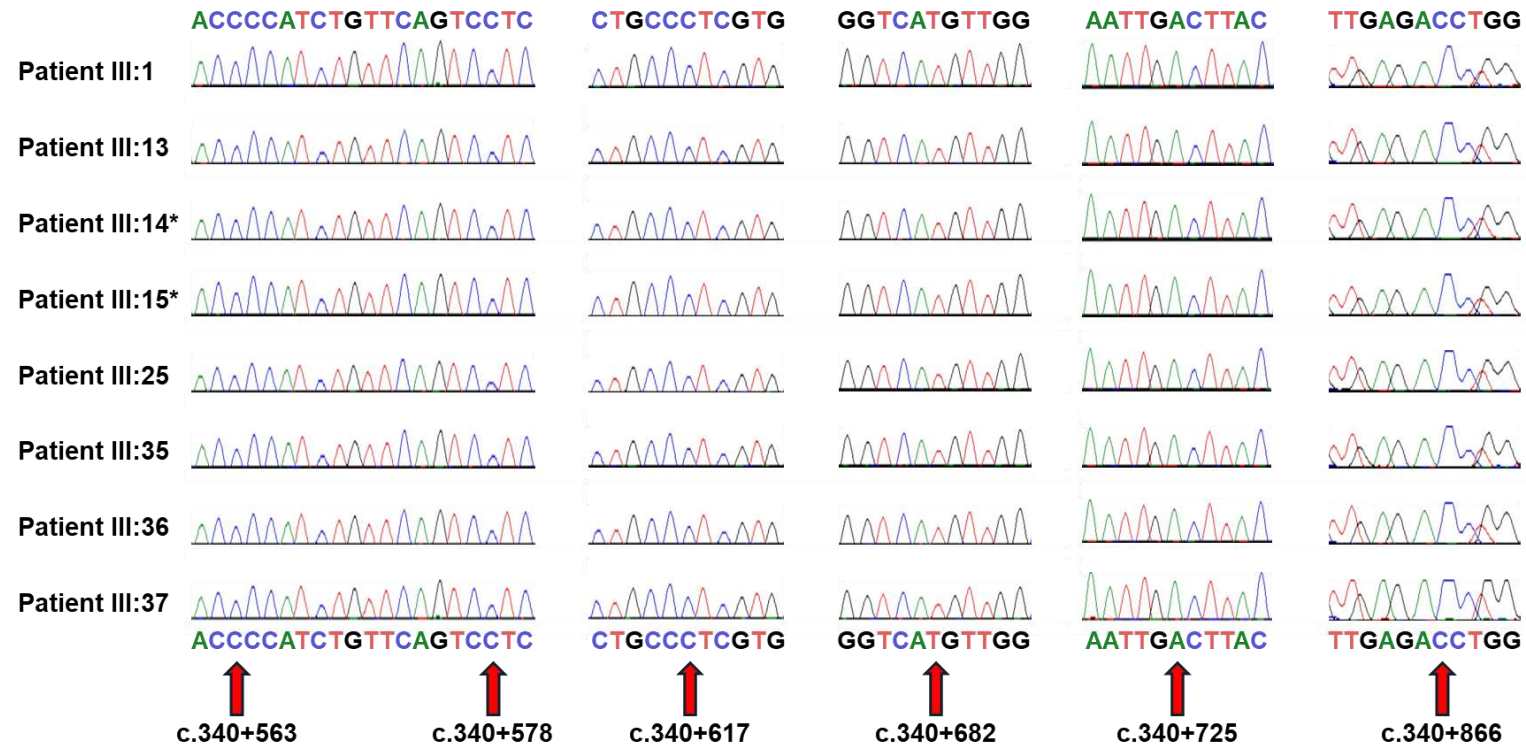
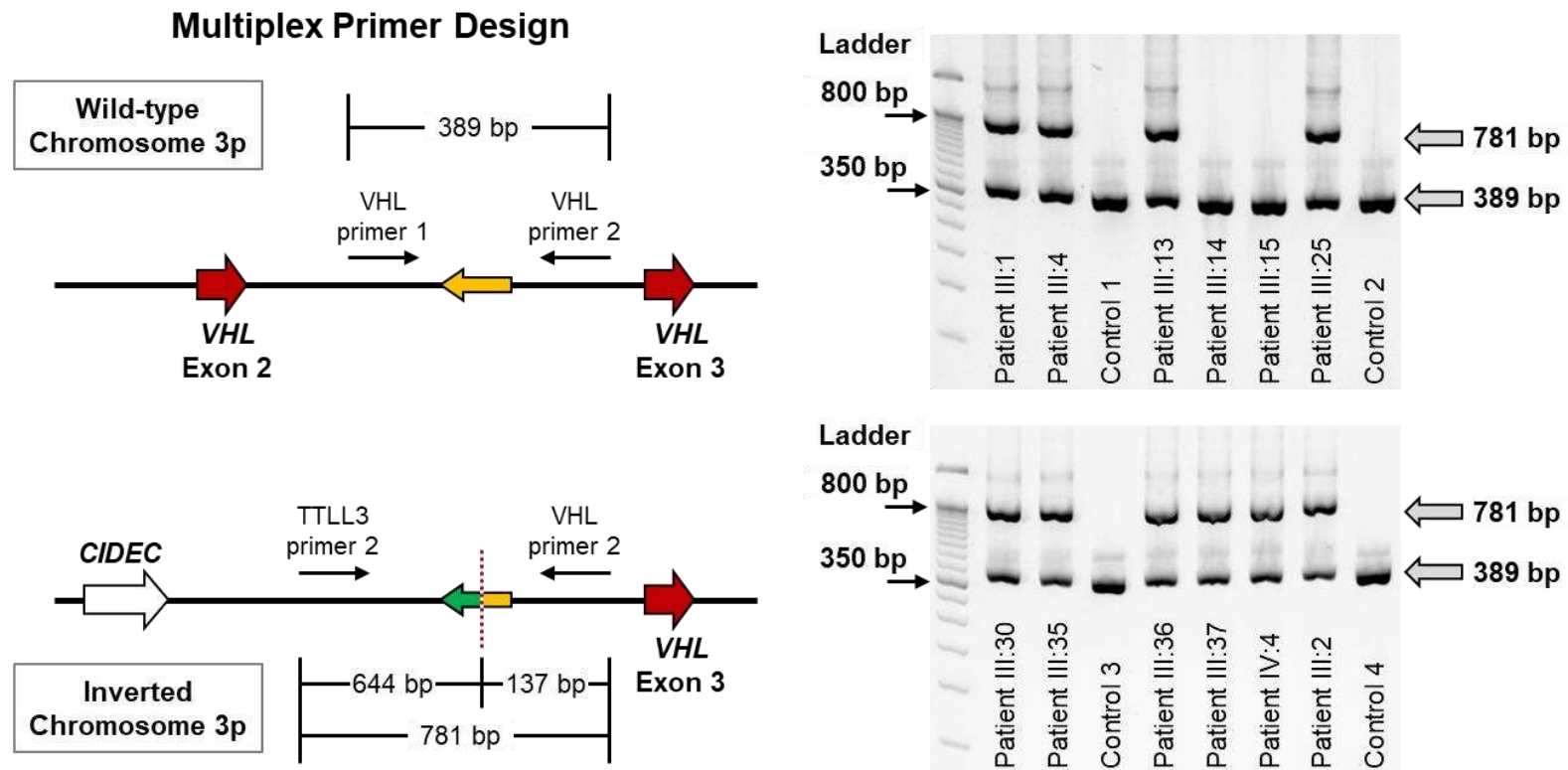


Supplementary Data

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.

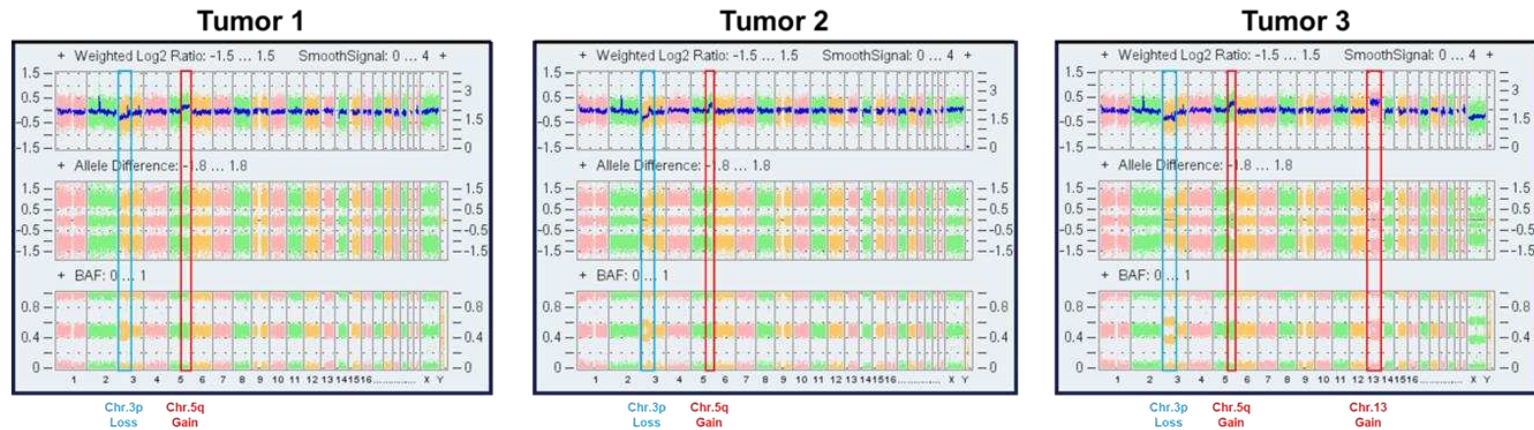


Supplementary Figure S2: Multiplex PCR-based analysis of germline DNA

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, indicating 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.

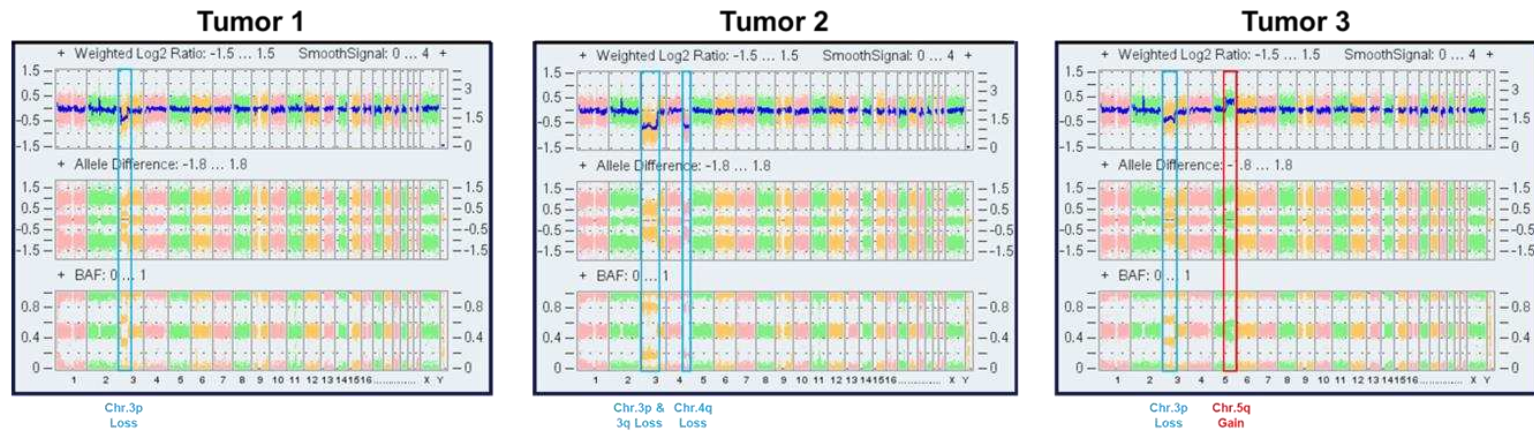
A

Patient III:1



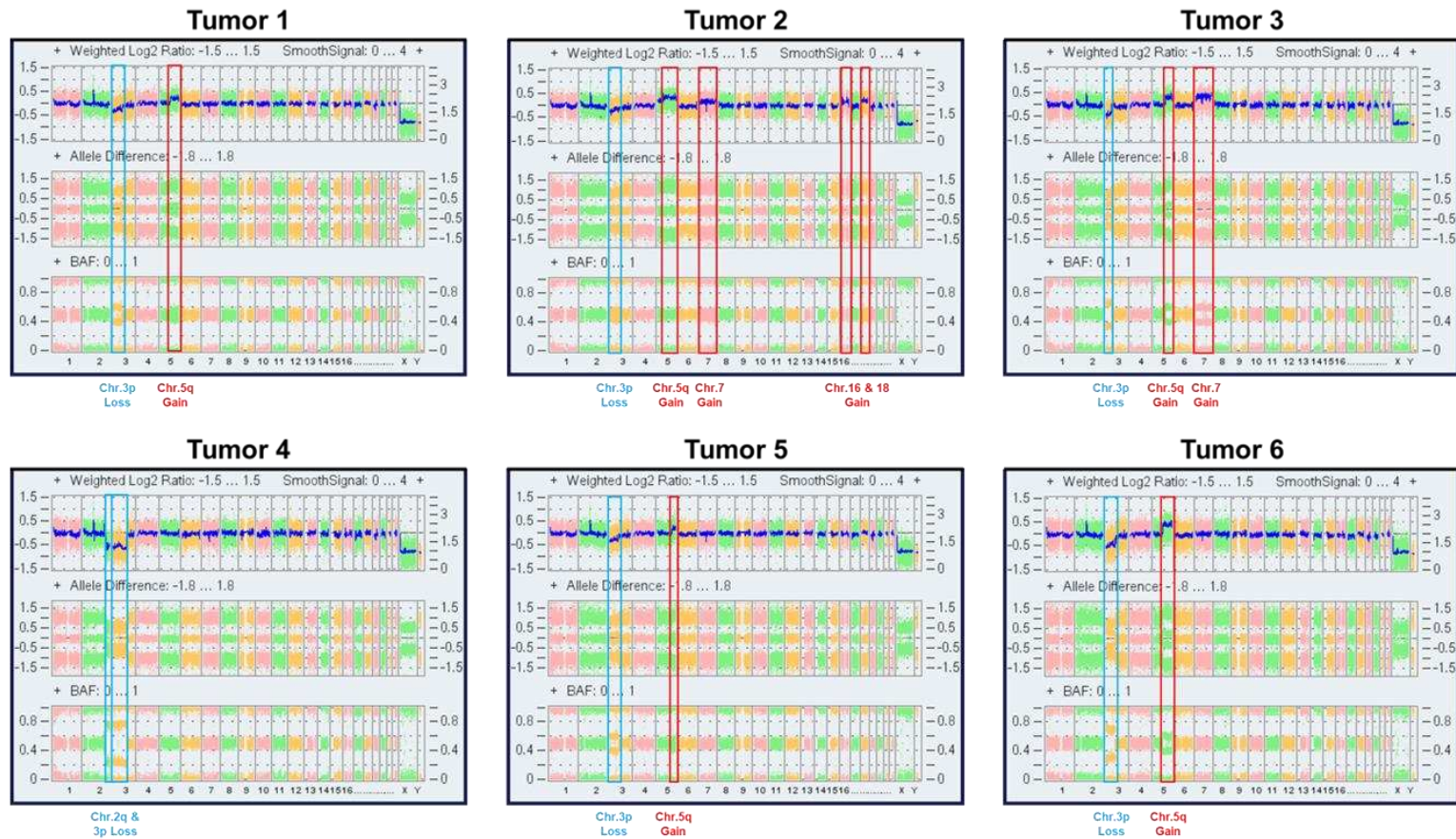
B

Patient III:25

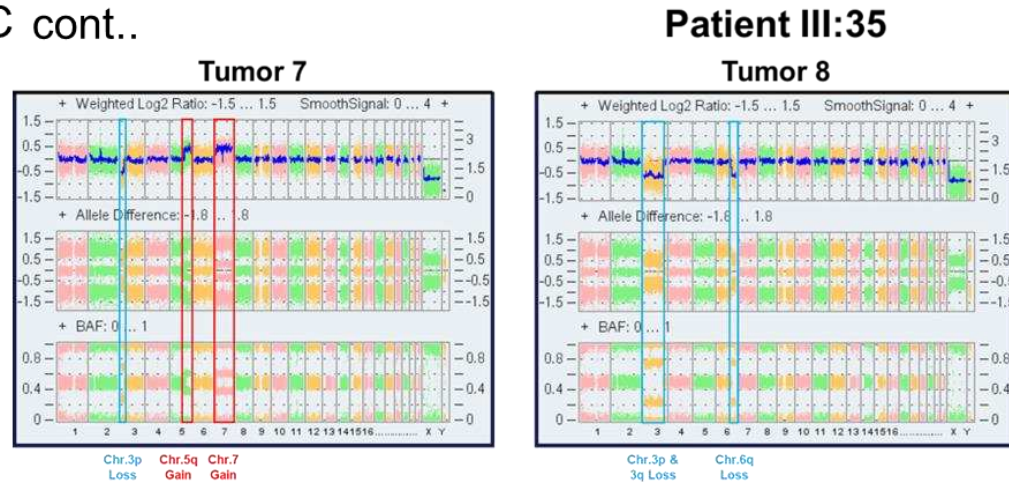


C

Patient III:35

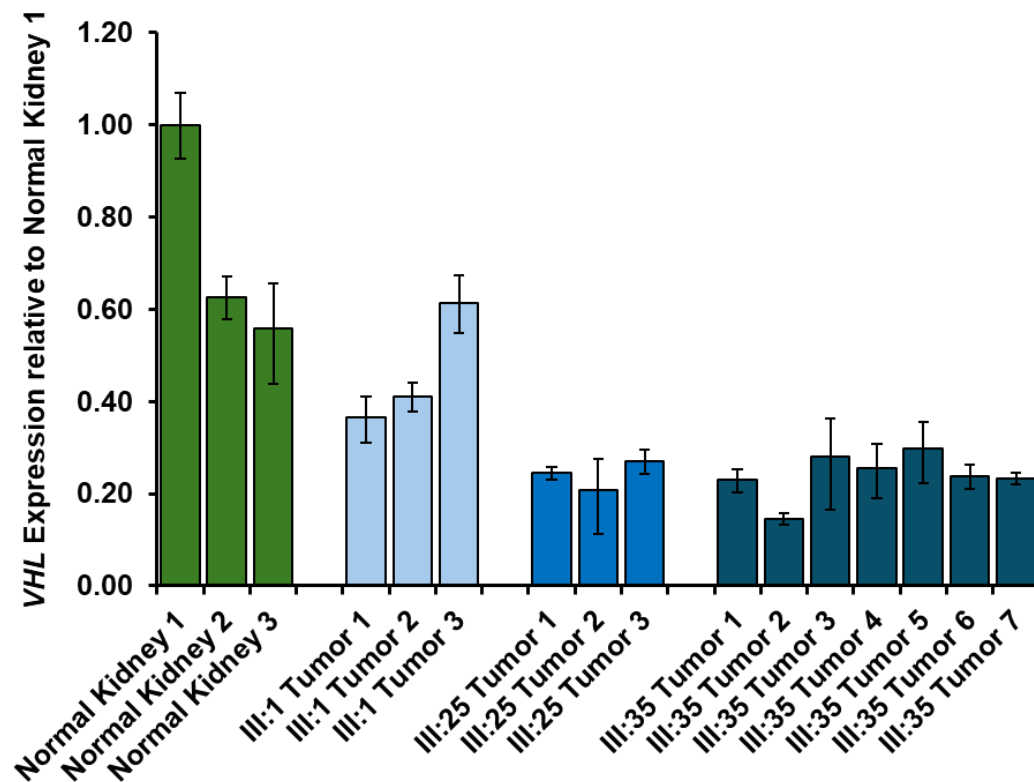


C cont..



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	<i>MTOR, TET2</i>
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