Supplementary Online Content

Van Allen EM, Lui VWY, Egloff AM, et al. Genomic correlate of exceptional erlotinib response in head and neck squamous cell carcinoma. *JAMA Oncol.* Published online March 5, 2015. doi:10.1001/jamaoncol.2015.34

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Patient enrollment: Patient data, including treatment history, smoking status, tumor histology, anatomical site, and stage were obtained through the University of Pittsburgh Head and Neck SPORE neoplasm virtual repository[1]. The patient samples and clinical data were collected using protocols approved by the Institutional Review Boards of the University of Pittsburgh and the Broad Institute of MIT and Harvard.

DNA extraction and exome sequencing: Hematoylin and eosin stained slides were cut from frozen tissue blocks and examined by a board-certified pathologist to select high-density cancer foci and ensure high purity of cancer DNA. Biopsy cores were taken from the corresponding frozen tissue block for DNA extraction. DNA was extracted and quantified using Picogreen dsDNA Quantitation Reagent (Invitrogen). DNA was stored at -20 degrees Celsius. Whole exome capture libraries were constructed from 100ng of DNA from tumor and normal tissue after sample shearing, end repair, and phosphorylation and ligation to barcoded sequencing adaptors. Ligated DNA was size selected for lengths between 200-350 bp and subjected to exonic hybrid capture using SureSelect v2 Exome bait (Agilent). The sample was multiplexed and sequenced using Illumina HiSeq technology for a mean target exome coverage of 85X in the tumor sample and 183X in the germline sample.

Sequence data processing: Exomes sequence data processing and analysis were performed using pipelines at the Broad Institute. A BAM file aligned to the hg19 human genome build was produced using Illumina sequencing reads for the tumor and normal sample and the Picard pipeline. The BAM was uploaded into the Firehose pipeline (http://www.broadinstitute.org/cancer/cga/Firehose) for managing intermediate analysis files executed by GenePattern.

Sequencing quality control: Sequencing data was incorporated into quality control modules in Firehose to compare the tumor and normal genotypes and ensure concordance between samples. Cross-contamination between samples from other individuals sequenced in the same flow cell was monitored with the ContEst algorithm[2].

Alteration calling, annotation, and prioritization: The MuTect algorithm was applied to identify somatic single-nucleotide variants in targeted exons[3]. Indelocator (http://www.broadinstitute.org/cancer/cga/indelocator) was applied to identify small insertions or deletions. Copy ratios were calculated for each hybrid capture bait by dividing the tumor coverage by the median coverage obtained in a set of reference normal samples[4]. The resulting copy ratios were segmented using the circular binary segmentation algorithm[5]. Alterations were annotated using Oncotator (http://www.broadinstitute.org/cancer/cga/oncotator), and heuristics were applied to nominate clinically and biologically relevant alterations for individual patient samples using PHIAL[6]. All alterations nominated by PHIAL were manually reviewed in the Integrated Genomics Viewer (IGV)[7]. In addition, all sequencing reads for *EGFR* were manually reviewed in IGV, including locations of known *EGFR* hotspot activating mutations[8].

Human Papilloma Virus (HPV) Detection: Using *in situ* hybridization - An HPV pan-specific DNA probe (Dako, Wide Spectrum HPV DNA Probe Cocktail, Biotinylated) was applied to formalin-fixed paraffin-embedded tissue blocks from the patient tumor. This probe detects HPV subtypes 6, 11, 16, 18, 31, 33, 35, 45, 51, and 51. A tumor with punctuate nuclear staining is scored as HPV-positive.

Cell lines: HNSCC cell line CAL-33 was obtained from Dr. Gerard Milano (University of Nice, France), and FaDu cells were obtained from ATCC (Manassas, VA). The HSC-6 cell line (harboring endogenous $MAPK1^{E322K}$ mutation) was a kind gift from Prof. Johji Inazawa (Tokyo Medical and Dental University, Japan). All cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA), penicillin (100 U/ml)/streptomycin (100 µg/ml) at 37^oC in a humidified atmosphere of 5% CO_2.

Retroviral Infection of HNSCC cells: Retroviruses were generated using the Platinum Retrovirus Expression Systems (Cell Biolabs, San Diego, CA) according to manufacturer's instructions. Briefly, Plat-A cells were transfected with 3 µg of retroviral vector carrying the gene of interest (pMXs-puro-EGFP as control, pMXs-puro-$MAPK1$ WT, pMXs-puro-$MAPK1^{E322K}$ mutant). Three days after transfection, fresh retroviruses (in the supernatant of the Plat-A cells) were collected by centrifugation at 1,500 rpm for 5 mins at 4°C. Cell debris was removed by filtering through a 0.45 µm syringe filter. Fresh retroviruses were used for infection of FaDu cells. FaDu cells were plated at 20% confluency in a T25 flask one day before infection. Infection of HNSCC cells was performed by adding 1.5 ml of retrovirus to the cells containing 2.5 ml of complete culture media. Then, 12 µl of polybrene (4 µg/µl, Sigma-Aldrich, St. Louis, MO) was added to the cells with gentle mixing. Cells were then incubated at 37°C and 5% CO_2 for an additional 48-72 hours, and the infection medium was replaced with fresh complete medium. Expression of the gene of interest and alteration of the signaling pathway was assessed by Western blotting.

MAPK1 siRNA Knockdown: HSC-6 cells were transfected with either MAPK1-targeting siRNA or control siRNA (catalog numbers SR303751C and SR30004, respectfully,

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Origene Technologies, Inc.) using siTran (Origene Technologies, Inc.) according to manufacturer's instructions. 48h after transfection, cells were transferred to 12-well plates and allowed to attach for 24h before erlotinib treatment and cell viability assessment.

Senescence Assay: Cells were treated with vehicle (DMSO) or erlotinib (0.5μM and 5μM) for 48h after which b-galactosidase activity at pH 6 was detected in senescent cells by light microscopy (100x) following staining using the senescence staining kit (Cell Signaling Technology, USA). Number of senescent cells and total number of cells per field were analyzed for ≥5 fields for each cell type and treatment condition. Average percent of senescent cells from a representative of 3 independent experiments is presented.

Trypan Blue Exclusion Assay: Cells were treated with vehicle (DMSO) or erlotinib (0.5μM and 5μM) for 48h then harvested by trypsinization for cell counting. Viable cells upon trypan blue staining (Invitrogen, Carlsbad, CA) were counted under light microscope. Triplicate wells were counted each time. Cumulative data from 2 independent experiments were analyzed for statistical significance.

Western blotting: Primary antibodies for phospho-p42/44-MAPK, p42/44-MAPK and phospho-EGFR(Y1068) were from Cell Signaling Technology, Inc. (Boston, MA), while EGFR antibody was obtained from BD biosciences (San Jose, CA). Anti-tubulin antibody was from Abcam (Cambridge, MA) and secondary antibodies from BioRad (Hercules, CA).

eFigure 1. MAPK1 and Erlotinib Sensitivity in HNSCC Cell Lines
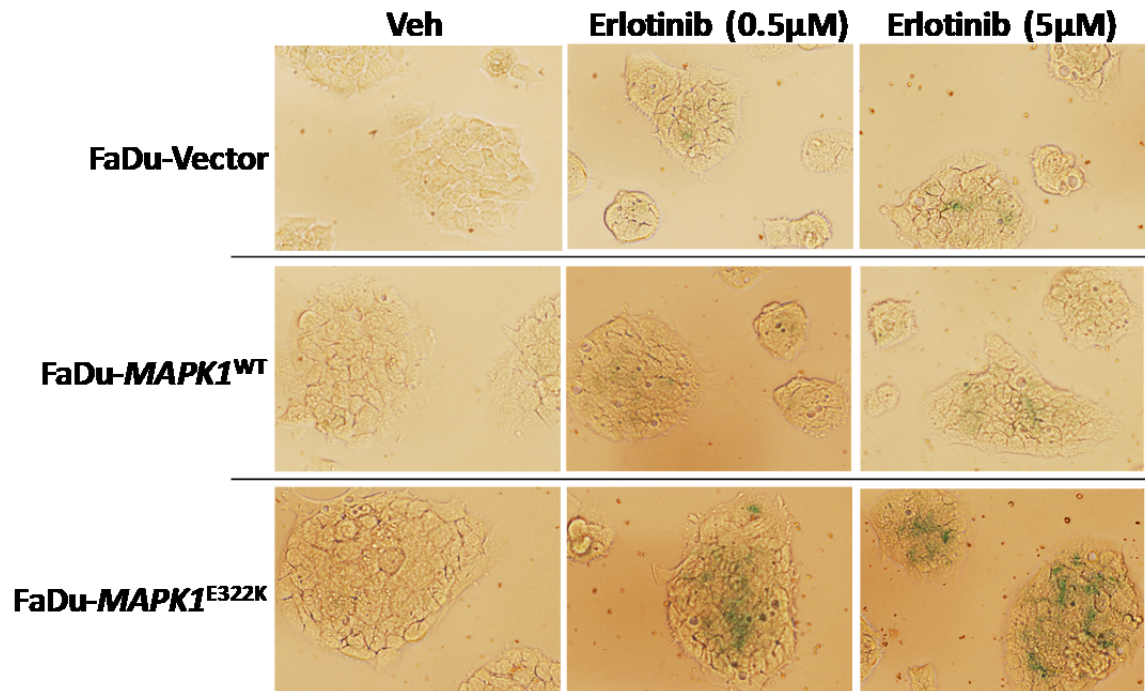
Representative pictures of senescence staining of stably transfected FaDu cells. FaDu-Vector, FaDu-*MAPK1*[WT] and FaDu-*MAPK1*[E322K] were stained for β-galactosidase activity at pH 6 following 48h erlotinib treatment. Staining was detected by light microscopy (pictured at 100x).

eFigure 2. Expression of *MAPK1*^{E322K} Increased Erlotinib Sensitivity in HNSCC Cells
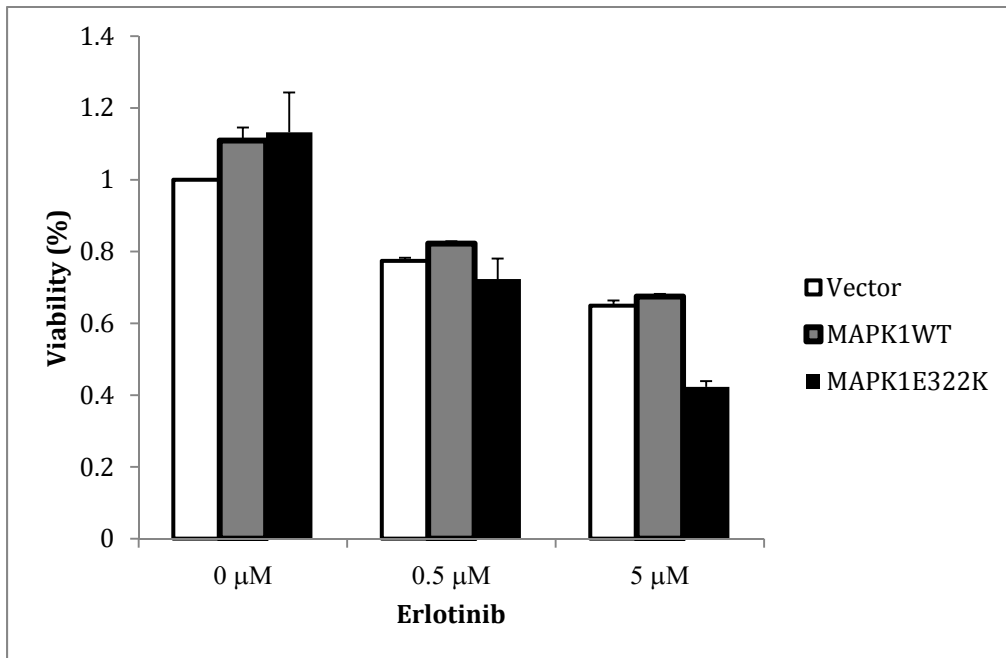
Stably transfected FaDu cells, FaDu-Vector, FaDu-*MAPK1*^{WT} and FaDu-*MAPK1*^{E322K} , were assessed for viability as measured by vital dye exclusion assay following 48h erlotinib treatment. Viability presented relative to vehicle treated vector transfected cells (** denotes p<0.005; t-test).

eFigure 3. Erlotinib Sensitivity Was Abrogated by ERK2 Knockdown in HSC-6 (*MAPK1*^{E332K}) Cells
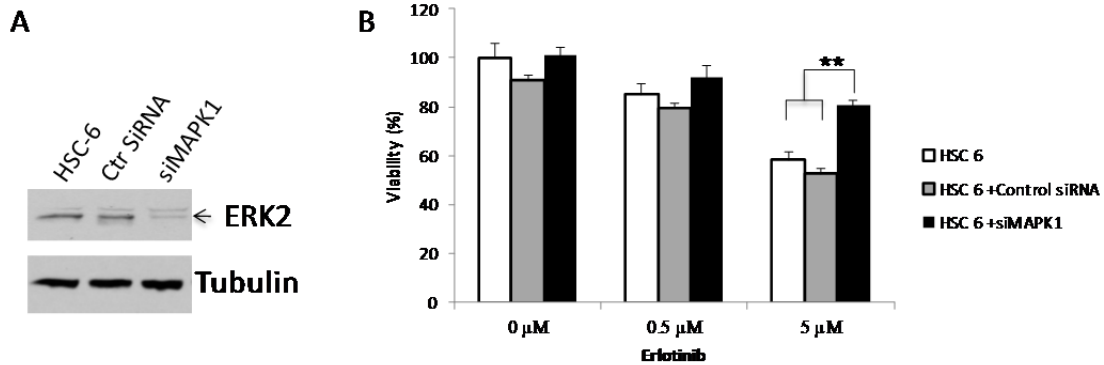
(A) siRNA targeting MAPK1 (ERK2) resulted in reduced ERK2 protein expression and

(B) significantly reduced HSC-6 growth inhibition in response to 48h treatment with 5 µM

erlotinib as measured by vital dye exclusion assay (** denotes $p<0.005$; t-test). Viability

data are presented relative to parental HSC-6 ells without erlotinib treatment.

eTable 1. Complete Listing of Somatic Alterations in the Exome

Gene	Variant_Classification	Alteration	Tumor_allele	Reference_allele	dbSNP_RS	Chromosome	Start_position	End_position	Coverage	Allelic_fraction	Number_of_Probes	Segment_Mean	In_Segment
TP53	Nonsense_ Mutation	p.Y236*	T	G		17	7577573	7577573	54	0.241	NA	NA	NA
ABL2	Missense_ Mutation	p.G1028S	T	C		1	179077320	179077320	80	0.125	NA	NA	NA
ARID1A	Missense_ Mutation	p.Y1279C	G	A		1	27099957	27099957	20	0.150	NA	NA	NA
MAPK1	Missense_ Mutation	p.E322K	T	C		22	22127164	22127164	88	0.125	NA	NA	NA
ABCC2	Splice_Site	p.M1139_splice	A	G		10	10159 5847	10159 5847	32	0.125	NA	NA	NA
C8B	Missense_ Mutation	p.P189Q	T	G		1	57417821	57417821	217	0.129	NA	NA	NA
CD209	Missense_ Mutation	p.R129W	A	G	rs146082308	19	7810767	7810767	115	0.043	NA	NA	NA
CHD4	Missense_ Mutation	p.R813H	T	C		12	6702658	6702658	123	0.065	NA	NA	NA
CHGB	Missense_ Mutation	p.N172H	C	A		20	5903304	5903304	36	0.167	NA	NA	NA
COL11A1	Missense_ Mutation	p.E286V	A	T		1	103491812	103491812	123	0.146	NA	NA	NA
DDX17	Missense_ Mutation	p.E416K	T	C		22	38890105	38890105	94	0.106	NA	NA	NA
EPS8L2	Copy Number	Deleted	NA	NA	NA	11	703152	710488	NA	NA	2	-1.1004	FALSE
GAPVD1	Missense_ Mutation	p.R963C	T	C		9	128103465	128103465	51	0.176	NA	NA	NA
GTF3C4	Missense_ Mutation	p.H478Q	A	C	rs147481161	9	135554440	135554440	63	0.127	NA	NA	NA
MUC16	Missense_ Mutation	p.L10956P	G	A		19	9048764	9048764	98	0.051	NA	NA	NA
MUC16	Missense_ Mutation	p.F10954L	G	A		19	9048771	9048771	98	0.051	NA	NA	NA
NDUFC1	Missense_ Mutation	p.E33K	T	C		4	140216271	140216271	73	0.055	NA	NA	NA

NID2	Missense_Mutation	p.P716Q	T	G	rs111540553	14	52505575	52505575	160	0.044	NA	NA	NA
PDE2A	Missense_Mutation	p.P40Q	T	G		11	72353323	72353323	4	0.500	NA	NA	NA
POLA2	Missense_Mutation	p.T356S	T	A		11	65055246	65055246	92	0.054	NA	NA	NA
RPL9	Missense_Mutation	p.L144I	T	A		4	39456526	39456526	69	0.058	NA	NA	NA
SGCE	Missense_Mutation	p.S325W	C	G		7	94230021	94230021	45	0.089	NA	NA	NA
SOX3	Missense_Mutation	p.K213R	C	T		X	139586588	139586588	20	0.150	NA	NA	NA
ST14	Missense_Mutation	p.P452T	A	C		11	130066595	130066595	88	0.057	NA	NA	NA
TTC14	Missense_Mutation	p.R593M	T	G		3	180327795	180327795	290	0.090	NA	NA	NA
ACER2	Missense_Mutation	p.R119W	T	C		9	19424829	19424829	53	0.245	NA	NA	NA
ATG7	Missense_Mutation	p.R481Q	A	G		3	11400049	11400049	43	0.070	NA	NA	NA
BCMO1	Missense_Mutation	p.A463V	T	C		16	81320985	81320985	53	0.113	NA	NA	NA
C17orf47	Missense_Mutation	p.K378N	G	T		17	56620414	56620414	29	0.138	NA	NA	NA
C6orf211	Missense_Mutation	p.V52M	A	G		6	151779469	151779469	204	0.137	NA	NA	NA
COL22A1	Missense_Mutation	p.G753R	T	C		8	139734323	139734323	4	0.500	NA	NA	NA
CYP8B1	Missense_Mutation	p.P398T	T	G		3	42916117	42916117	107	0.103	NA	NA	NA
DEAF1	Copy Number	Deleted	NA	NA	NA	11	703152	710488	NA	NA	2	-1.1004	FALSE
DHX37	Missense_Mutation	p.R327Q	T	C		12	125459965	125459965	57	0.140	NA	NA	NA
EPHA6	Missense_Mutation	p.H1119Y	T	C		3	97467507	97467507	51	0.137	NA	NA	NA
FAM151B	Nonsense_Mutation	p.Q197*	T	C		5	79817875	79817875	100	0.100	NA	NA	NA
FAM198B	Missense_Mutation	p.E300Q	G	C		4	159091630	159091630	77	0.091	NA	NA	NA
FAM71C	Missense_Mutation	p.P83T	A	C		12	100042199	100042199	111	0.099	NA	NA	NA

GPATCH8	Missense_Mutation	p.G767D	T	C		17	42477145	42477145	67	0.060	NA	NA	NA
NCOA6	Missense_Mutation	p.P749L	A	G		20	33337752	33337752	122	0.115	NA	NA	NA
NLRP13	Missense_Mutation	p.A883P	G	C		19	56413543	56413543	24	0.125	NA	NA	NA
PCDHA4	Missense_Mutation	p.A642T	A	G		5	140188696	140188696	99	0.131	NA	NA	NA
PDE6C	Missense_Mutation	p.V9M	A	G		10	95372507	95372507	91	0.066	NA	NA	NA
SSX7	Frame_Shift_Del	p.P127fs	-	T		X	52677396	52677396	122	0.172	NA	NA	NA
TBC1D10A	Missense_Mutation	p.R107Q	T	C		22	30695530	30695530	39	0.128	NA	NA	NA
TBCEL	Nonsense_Mutation	p.Q313*	T	C		11	120930775	120930775	65	0.077	NA	NA	NA
TGIF2LY	Translation_Start_Site		T	C		Y	3447268	3447268	14	0.357	NA	NA	NA
TMEM80	Copy Number	Deleted	NA	NA	NA	11	703152	710488	NA	NA	2	-1.1004	FALSE
ZNF518A	Frame_Shift_Del	p.E296fs	-	A		10	97916966	97916966	6	0.333	NA	NA	NA
ADAMTSL2	Intron		-	CA		9	136400505	136400506	6	0.333	NA	NA	NA
ANKS1B	Silent	p.P410P	T	C		12	100048887	100048887	41	0.122	NA	NA	NA
APOBEC3H	Intron		T	-		22	39497592	39497593	6	0.333	NA	NA	NA
C10orf79	Silent	p.D1284D	A	G		10	105906024	105906024	121	0.116	NA	NA	NA
CCDC18	3'UTR		-	A		1	93744059	93744059	159	0.101	NA	NA	NA
CCDC88C	Intron		-	TC		14	91773333	91773334	7	0.429	NA	NA	NA
CNOT2	Intron		T	-		12	70739864	70739865	6	0.333	NA	NA	NA
ERGIC2	Silent	p.S351S	A	G		12	29494685	29494685	204	0.093	NA	NA	NA
FLJ40330	Intron		-	AAG		2	89102192	89102194	6	0.333	NA	NA	NA
FOXP2	Intron		-	GGT		7	114036725	114036727	6	0.333	NA	NA	NA
GFOD1	Silent	p.I79I	T	G		6	13486886	13486886	61	0.066	NA	NA	NA

HLA-B	Intron		-	A		6	31245032	31245032	33	0.273	NA	NA	NA
HSPG2	Intron		-	A		1	22198881	22198881	6	0.333	NA	NA	NA
KLF8	Intron		-	AG		X	56296838	56296839	6	0.333	NA	NA	NA
KNG1	Intron		CCA	-		3	18643562 2	18643562 3	6	0.333	NA	NA	NA
LATS1	3'UTR		T	C		6	14998276 9	14998276 9	19	0.211	NA	NA	NA
LOC100 286793	RNA		C	G	rs140521978	1	14374441 1	14374441 1	6	0.500	NA	NA	NA
MARCO	Intron		CCC TGC	-		2	11973409 7	11973409 8	6	0.500	NA	NA	NA
NHLRC2	Silent	p.P144P	C	T		10	11563638 0	11563638 0	93	0.140	NA	NA	NA
NOL8	Intron		-	A		9	95062034	95062034	6	0.500	NA	NA	NA
NSMAF	Intron		CG	-	rs141516286;r s151015572	8	59535859	59535860	47	0.170	NA	NA	NA
PCDHB1 9P	3'UTR		T	C		5	14062122 0	14062122 0	79	0.063	NA	NA	NA
PDCL3	Intron		-	T		2	10118554 0	10118554 0	7	0.429	NA	NA	NA
PIGK	Intron		-	AAAT		1	77595400	77595403	11	0.364	NA	NA	NA
PION	Silent	p.L283L	A	G		7	77003411	77003411	132	0.076	NA	NA	NA
PLA1A	3'UTR		G	T	rs139478101	3	11934836 0	11934836 0	8	0.250	NA	NA	NA
PRPF40 A	Intron		-	A		2	15351588 1	15351588 1	6	0.333	NA	NA	NA
PXDNL	Intron		-	G	rs113739338	8	52526045	52526045	6	0.333	NA	NA	NA
REG3A	5'UTR		A	G	rs56413330	2	79386560	79386560	34	0.118	NA	NA	NA
RPF2	Intron		AAA C	-	rs147892015	6	11130604 4	11130604 5	6	0.500	NA	NA	NA
SF3B4	Silent	p.S277S	T	C		1	14989781 0	14989781 0	31	0.129	NA	NA	NA
SMAD9	3'UTR		T	C		13	37422731	37422731	53	0.113	NA	NA	NA
STK4	Intron		T	-		20	43623633	43623634	6	0.500	NA	NA	NA
SUMF1	Intron		G	-	rs144078985	3	4315430	4315431	7	0.429	NA	NA	NA
SYMPK	Intron		-	AGGG		19	46320240	46320243	6	0.333	NA	NA	NA
TAC1	Intron		-	G		7	97362	97362	6	0.3	N	NA	NA

							958	958		33	A		
TARS	Intron		-	TT	rs111754118	5	33453369	33453370	8	0.375	NA	NA	NA
TBL3	Intron		C	A		16	202680	202680	22	0.273	NA	NA	NA
TNKS	Intron		G	-	rs149114426	8	9566157	9566158	6	0.667	NA	NA	NA
Unknown	RNA		G	A	rs926027	14	22192106	22192106	186	0.032	NA	NA	NA
Unknown	5'Flank		T	G		15	102304722	102304722	21	0.143	NA	NA	NA
Unknown	IGR		-	AGG		10	29543272	29543274	6	0.333	NA	NA	NA
Unknown	IGR		-	CC		10	52467359	52467360	6	0.333	NA	NA	NA
Unknown	IGR		-	TG		16	32399771	32399772	10	0.400	NA	NA	NA
Unknown	IGR		-	A		18	49139477	49139477	9	0.333	NA	NA	NA
Unknown	IGR		-	C	rs60129451	2	232770987	232770987	6	0.333	NA	NA	NA
Unknown	IGR		-	GGA	rs71327417	20	58657085	58657087	6	0.333	NA	NA	NA
Unknown	IGR		-	TA	rs79867563;rs117711458	7	57659470	57659471	6	0.333	NA	NA	NA
Unknown	IGR		CT	-		8	20900645	20900646	6	0.333	NA	NA	NA
ZNF679	Silent	p.K78K	A	G		7	63721279	63721279	24	0.208	NA	NA	NA

This table provides information about the point mutations, short insertion/deletions, and copy number alterations observed in this patient's tumor. Additional data regarding allelic fraction, segment mean, and coverage is provided for these alterations.

eTable 2. Exome Somatic Alteration COSMIC Mutations and Pathways

Gene	Alteration	Pathways	COSMIC_overlapping_mutations	COSMIC_total_alterations_in_gene	UniProt_Region
TP53	p.Y236*		p.Y236C(46)|p.Y236N(12)|p.Y236H(9)|p.Y236*(9)|p.0?(7)|p.Y236D(6)|p.Y236del(4)|p.Y236S(3)|p.Y236Y(2)|p.Y236fs*4(2)|p.Y236_M237delYM(1)|p.Y236_M237insXX(1)|p.Y236fs*5(1)|p.H233fs*6(1)|p.N235_Y236delNY(1)|p.Y236_M243delYMCNSSCM(1)|p.V225fs*23(1)|p.I232_Y236delIHYNY(1)|p.Y236_M237>*L(1)|p.H233_C242del10(1)	22245	Required for interaction with FBXO42.||Interaction with HIPK1 (By similarity).|Interaction with AXIN1 (By similarity).
ABL2	p.G1028S			14	Pro-rich.|F-actin-binding (By similarity).
ARID1A	p.Y1279C			142	
MAPK1	p.E322K	BIOCARTA_CDMAC_PATHWAY | BIOCARTA_IGF1R_PATHWAY | BIOCARTA_41BB_PATHWAY | KEGG_THYROID_CANCER | BIOCARTA_HER2_PATHWAY | BIOCARTA_MET_PATHWAY | KEGG_BLADDER_CANCER | KEGG_NON_SMALL_CELL_LUNG_CANCER | KEGG_COLORECTAL_CANCER | KEGG_PANCREATIC_CANCER | KEGG_ENDOMETRIAL_CANCER | KEGG_RENAL_CELL_CARCINOMA | KEGG_PROSTATE_CANCER | KEGG_PATHWAYS_IN_CANCER |		3	
ABCC2	p.M1139_splice			1	
C8B	p.P189Q			4	MACPF.

Gene	Mutation		Count	Domain
CD209	p.R129W		1	Extracellular (Probable).|2.|7 X approximate tandem repeats.
CHD4	p.R813H		2	Helicase ATP-binding.
CHGB	p.N172H		6	
COL11A1	p.E286V		12	Nonhelical region.
DDX17	p.E416K		4	Helicase C-terminal.
EPS8L2	Deleted	NA	NA	NA
GAPVD1	p.R963C		4	
GTF3C4	p.H478Q		2	
MUC16	p.L10956P		57	Thr-rich.|Extracellular (Potential).
MUC16	p.F10954L		57	Thr-rich.|Extracellular (Potential).
NDUFC1	p.E33K		0	
NID2	p.P716Q		7	Nidogen G2 beta-barrel.
PDE2A	p.P40Q		4	
POLA2	p.T356S		0	
RPL9	p.L144I		1	
SGCE	p.S325W		1	Cys-rich.|Helical; (Potential).
SOX3	p.K213R		1	
ST14	p.P452T		5	Extracellular (Potential).|LDL-receptor class A 1.
TTC14	p.R593M		1	
ACER2	p.R119W		2	Cytoplasmic (Potential).
ATG7	p.R481Q		1	
BCMO1	p.A463V		0	
C17orf47	p.K378N		1	
C6orf211	p.V52M		0	
COL22A1	p.G753R		13	Collagen-like 5.|Pro-rich.|Gly-rich.
CYP8B1	p.P398T		2	
DEAF1	Deleted	NA	NA	NA
DHX37	p.R327Q		1	Helicase ATP-binding.

Gene	Mutation		Count	Domain
EPHA6	p.H1119Y		16	SAM.|Cytoplasmic (Potential).
FAM151B	p.Q197*		0	
FAM198B	p.E300Q		0	Extracellular (Potential).
FAM71C	p.P83T		0	
GPATCH8	p.G767D		4	
NCOA6	p.P749L		7	TBP/GTF2A-binding region.|NCOA1-binding region.|Gln-rich.|CREBBP-binding region.
NLRP13	p.A883P		9	
PCDHA4	p.A642T		6	Cadherin 6.|Extracellular (Potential).
PDE6C	p.V9M		4	
SSX7	p.P127fs		1	
TBC1D10A	p.R107Q		1	
TBCEL	p.Q313*		1	
TGIF2LY			0	
TMEM80	Deleted	NA	NA	NA
ZNF518A	p.E296fs		1	
ADAMTSL2		NA	1	NA
ANKS1B	p.P410P		0	
APOBEC3H		NA	2	NA
C10orf79	p.D1284D		0	
CCDC18		NA	5	NA
CCDC88C		NA	3	NA
CNOT2		NA	0	NA
ERGIC2	p.S351S		1	Cytoplasmic (Potential).
FLJ40330		NA	0	NA
FOXP2		NA	8	NA
GFOD1	p.I79I		2	
HLA-B		NA	0	NA
HSPG2		NA	9	NA
KLF8		NA	1	NA
KNG1		NA	1	NA
LATS1			6	
LOC100286793			0	
MARCO		NA	6	NA
NHLRC2	p.P144P		1	Thioredoxin.
NOL8		NA	1	NA
NSMAF		NA	1	NA
PCDHB19P			0	
PDCL3		NA	0	NA
PIGK		NA	3	NA
PION	p.L283L		1	

PLA1A			3	
PRPF40A		NA	0	NA
PXDNL		NA	2	NA
REG3A			1	
RPF2		NA	2	NA
SF3B4	p.S277S		1	
SMAD9			0	
STK4		NA	2	NA
SUMF1		NA	1	NA
SYMPK		NA	1	NA
TAC1		NA	0	NA
TARS		NA	2	NA
TBL3			0	
TNKS		NA	7	NA
ZNF679	p.K78K		1	KRAB.

This table provides contextual information about the patient exome somatic alterations including similar COSMIC mutations and implicated functional pathways.

eTable 3. Gene-Level Depth of Coverage

Gene	Total coverage	Average coverage (X)
ABL1	202739	57.21
ABL2	423709	114.45
ACVR1B	152952	81.23
AKT1	54075	36.44
AKT2	105321	58.51
AKT3	123321	85.64
ALK	395533	81.27
ANXA7	135702	92.5
APC	770706	89.83
AR	86018	30.6
ARAF	40989	22.46
ARHGAP26	301023	121.87
ARID1A	683181	99.62
ARID2	742492	134.7
ASCL1	11060	15.56
ASXL1	424665	91.66
ATM	1008052	109.92
ATR	962029	120.15
ATRX	753004	100.47
AURKA	132659	108.12
AURKB	44037	42.42
AXL	185059	68.92
BAP1	129756	57.44
BCL2	48711	64.69
BCL6	251371	118.52
BIVM	147619	108.3
BLM	403164	94.77
BMPR1A	218705	136.78
BRAF	292242	127.01
BRCA1	648038	114.53
BRCA2	883373	86.12
BRIP1	451606	120.43
BUB1B	368291	114.73
CAMK1D	90565	75.91
CARD11	227263	65.59
CBFB	63231	106.27
CBL	317931	116.84
CBLB	385539	129.16
CBLC	73335	51.46

CCND1	47900	53.94
CCND2	76309	87.71
CCND3	48422	49.26
CCNE1	97476	77.92
CD79A	22961	33.72
CD79B	28036	40.46
CDC73	187027	117.18
CDH1	261563	93.58
CDK12	369113	82.52
CDK4	119100	130.59
CDK6	76332	77.81
CDK8	214603	153.84
CDKN1A	22372	44.74
CDKN1B	48508	65.55
CDKN2A	74152	75.05
CDKN2B	11477	23.05
CDKN2C	43318	85.44
CDX1	21382	25.98
CEBPA	8705	8.08
CHEK1	168986	99.17
CHEK2	135231	76.4
CHUK	225259	100.65
CIC	203123	42.08
CREBBP	594665	78.94
CRKL	86275	94.6
CRLF2	63563	82.44
CSF1R	196939	65.69
CTNNA1	260776	95.77
CTNNB1	282292	120.33
CYLD	412737	143.61
DAXX	166850	75.06
DCBLD1	58278	353.2
DDB2	97935	76.27
DDR2	407545	148.52
DICER1	809066	138.97
DLC1	485281	103.96
DNMT1	352347	71.79
DNMT3A	152786	53.35
EGFR	364059	90.36
EP300	727860	100.46
EPHA3	441212	148.16

EPHA5	411249	131.77
EPHB6	244401	79.71
ERBB2	212383	56.31
ERBB2IP	432645	105.11
ERBB3	456657	106.55
ERBB4	514369	130.98
ERCC2	95455	40.86
ERCC3	229330	89.69
ERCC4	272163	98.9
ERCC5	325909	91.52
ERCC6	474578	148.21
ERG	132297	86.02
ESR1	138291	77.34
ETV1	115913	77.53
ETV4	102289	70.3
ETV5	182785	113.74
ETV6	199024	140.16
EWSR1	158219	74.56
EXT1	238359	106.36
EXT2	288974	123.02
EZH2	264342	117.17
FAM123B	95173	27.46
FANCA	239680	54.78
FANCB	166270	64.45
FANCC	101729	60.66
FANCD2	473383	104.68
FANCE	115854	71.91
FANCF	102037	90.7
FANCG	147981	79.18
FANCI	488448	121.5
FANCL	115939	104.73
FANCM	533855	86.74
FBXO31	76194	47.03
FBXW7	438647	173.24
FGFR1	181393	67.68
FGFR2	280110	103.36
FGFR3	81333	27.27
FGFR4	93259	29.37
FH	186407	121.6
FHIT	32547	73.3
FKBP9	199038	116.12

FLCN	138490	66.07
FLT1	435751	104.3
FLT3	302240	101.05
FLT4	175369	40.84
FOXA1	48262	34.01
FOXL2	18139	16.04
FUBP1	240296	123.99
GATA1	21500	15.97
GATA2	53105	36.8
GATA3	105562	78.95
GATA4	60242	45.23
GATA6	66490	37.19
GNA11	57689	53.42
GNAQ	130778	121.09
GNAS	220465	55.2
GOPC	118421	95.81
GPC3	190108	104.45
GPSM3	29332	41.08
GUCY1A2	206110	93.73
HGF	285669	128.39
HIF1A	303053	122
HNF1A	80617	30.65
HRAS	38929	61.5
HSP90AA1	194524	75.13
IDH1	125153	100.52
IDH2	93165	68.55
IGF1R	343014	83.58
IGF2	23129	32.12
IKBKE	135898	63.18
IKZF1	67041	42.98
IL6ST	403850	146.48
IL7R	161604	103.86
INPP4B	365787	131.82
JAK1	321626	92.82
JAK2	351217	103.33
JAK3	150262	42.6
KDM5C	125594	26.29
KDM6A	185237	44.04
KDR	492121	120.88
KEAP1	102631	54.74
KIAA1549	359538	61.04

KIT	333831	113.74
KLF6	102692	100.48
KRAS	49850	72.56
LMO1	35210	74.76
MAP2K1	114989	97.28
MAP2K2	20481	17.02
MAP2K4	160586	133.82
MAP3K1	411309	90.62
MAP3K5	452372	103.42
MAP3K7	198756	109.15
MAP3K8	130827	86.58
MAP3K9	211927	59.9
MAPK1	98692	91.13
MAPK3	77315	63.63
MCL1	87814	82.53
MDM2	209887	139.92
MDM4	174940	118.76
MED12	265864	40.29
MEN1	113108	61.21
MET	656493	152.11
MITF	124770	69.32
MLH1	277069	120.05
MLL	1328268	111.43
MLL2	698507	42.04
MLL3	2484144	168.34
MPL	158771	79.66
MRE11A	263308	123.79
MSH2	292535	104.29
MSH6	399527	97.02
MTOR	824357	105.21
MTUS2	200742	47.08
MUTYH	114110	67.56
MYC	80470	58.95
MYCL1	66921	51.08
MYCN	46413	33.27
MYD88	79073	82.89
MYST3	650857	107.99
NBN	296354	130.84
NF1	1164121	135.3
NF2	125402	66.67
NFE2L2	241265	127.65

NFKBIA	72359	75.85
NKX2-1	14634	11.88
NLRP2	191154	59.94
NOTCH1	240046	31.3
NOTCH2	1162047	147
NOTCH3	183498	26.32
NOTCH4	309992	58.51
NPM1	66279	74.14
NRAS	118605	208.08
NTRK1	101396	40.2
NTRK2	308333	119.32
NTRK3	233338	83.84
PALB2	313157	87.94
PARK2	148532	105.42
PAX5	99619	82.53
PBRM1	458893	93.56
PDGFRA	394491	119.15
PDGFRB	240006	71.54
PDPK1	259824	155.12
PHF6	75881	62.87
PHOX2B	34090	36.07
PIK3CA	442838	138
PIK3CG	375748	113.55
PIK3R1	324428	139.78
PMS1	278983	95.28
PMS2	305228	117.89
PPP2R1A	132134	69.91
PRDM1	287281	115.51
PREX2	556517	114.18
PRF1	72803	43.65
PRKAR1A	169019	147.49
PTCH1	327669	71.33
PTEN	194480	160.46
PTK2B	248386	76.97
PTPN11	163842	91.74
PTPRD	836277	143.57
RAD51L1	104794	89.41
RAF1	197628	101.5
RARA	100834	48.25
RB1	252249	90.51
REL	216332	116.31

RET	205569	60.87
RICTOR	936700	182.7
ROS1	877648	124.6
RPTOR	327196	80.91
RUNX1	142181	89.65
RUNX1T1	240525	122.22
RUNX3	65587	50.22
SBDS	77274	102.62
SDCCAG8	3289	74.75
SDHB	71808	85.18
SDHC	70549	114.34
SDHD	31244	90.3
SETD2	722130	93.84
SF3B1	464307	116.51
SGK1	218804	101.39
SGK3	184343	123.47
SMAD2	169638	120.82
SMAD3	123245	91.16
SMAD4	223665	134.82
SMARCA4	270868	53.65
SMARCB1	106944	88.24
SMO	128480	54.35
SOCS1	5496	8.64
SOX2	36281	38.03
SRC	70817	43.96
STK11	25835	19.84
SUFU	152110	94.42
SYK	179266	93.95
TARBP2	80631	67.7
TCF4	296015	144.12
TERT	118693	34.92
TET2	335159	54.36
TGFBR2	199284	112.02
TNFAIP3	263440	111.02
TNFRSF14	35738	41.95
TOP1	194108	84.47
TP53	79457	62.91
TRIM62	67656	45.84
TSC1	351042	98.41
TSC2	258974	46.42
TSHR	325410	133.8

UNKNOWN	211255	13.59
VHL	24860	38.72
VRK2	1709	47.47
WAS	28289	18.75
WRN	420997	97.93
WT1	95107	61.04
XPA	41305	50.25
XPC	185700	65.78
XPO1	301024	92.99
ZNF276	39133	46.15
ZNF668	88959	46.12

This table provides mean target coverage per gene. Data was generated using DepthOfCoverage[9].

eTable 4. Coverage by Intervals Across the Exome

Target	Total coverage	Average coverage (X)
1:2488104-2488172	529	7.67
1:2489165-2489273	1109	10.17
1:2489782-2489907	13060	103.65
1:2490320-2490438	0	0
1:2490475-2490573	0	0
1:2491262-2491417	1974	12.65
1:2492063-2492153	2814	30.92
1:2492933-2492963	0	0
1:2493112-2493254	7728	54.04
1:2494304-2494335	2720	85
1:2494587-2494712	5804	46.06
1:11166997-11167181	0	0
1:11167542-11167557	630	39.38
1:11168238-11168343	15020	141.7
1:11169347-11169427	11020	136.05
1:11169706-11169786	5298	65.41
1:11171185-11171188	0	0
1:11172909-11172974	8554	129.61
1:11174375-11174510	12563	92.38
1:11174870-11174944	7921	105.61
1:11175453-11175525	16604	227.45
1:11177061-11177143	13824	166.55
1:11181303-11181425	8859	72.02
1:11182036-11182183	6174	41.72
1:11184555-11184690	6358	46.75
1:11186679-11186853	20893	119.39
1:11187067-11187201	8205	60.78
1:11187681-11187863	33438	182.72
1:11188061-11188183	20680	168.13
1:11188511-11188609	9115	92.07
1:11188912-11189008	4281	44.13
1:11189795-11189895	6330	62.67
1:11190586-11190834	7620	30.6
1:11193137-11193254	15293	129.6
1:11194408-11194523	18324	157.97
1:11199361-11199492	19129	144.92
1:11199590-11199715	20118	159.67
1:11204705-11204812	13207	122.29
1:11205025-11205102	17595	225.58

1:11206733-11206848	10760	92.76
1:11210183-11210283	5433	53.79
1:11217209-11217348	14648	104.63
1:11227499-11227574	12269	161.43
1:11259315-11259460	11522	78.92
1:11259598-11259760	26277	161.21
1:11264618-11264760	9448	66.07
1:11269369-11269515	30127	204.95
1:11270871-11270963	11001	118.29
1:11272369-11272531	21448	131.58
1:11272853-11272965	15089	133.53
1:11273456-11273623	23229	138.27
1:11276205-11276291	7867	90.43
1:11288725-11288975	31513	125.55
1:11290982-11291111	19490	149.92
1:11291357-11291491	22829	169.1
1:11292493-11292585	12102	130.13
1:11293455-11293544	9337	103.74
1:11294200-11294322	13649	110.97
1:11297900-11298105	15158	73.58
1:11298459-11298674	29461	136.39
1:11300360-11300604	17059	69.63
1:11301610-11301738	10838	84.02
1:11303171-11303357	14042	75.09
1:11307682-11307790	19023	174.52
1:11307876-11308151	21713	78.67
1:11313896-11314030	20035	148.41
1:11316049-11316249	5890	29.3
1:11316990-11317222	9531	40.91
1:11318542-11318650	9507	87.22
1:11319305-11319466	17009	104.99
1:17345376-17345453	10376	133.03
1:17349103-17349225	9114	74.1
1:17350468-17350569	4263	41.79
1:17354244-17354360	14529	124.18
1:17355095-17355231	16043	117.1
1:17359555-17359640	6998	81.37
1:17371256-17371383	9547	74.59
1:17380443-17380514	938	13.03
1:25228613-25229157	20804	38.17
1:25233750-25233908	5587	35.14

1:25245731-25245835	6879	65.51
1:25254065-25254221	27626	175.96
1:25255531-25255533	0	0
1:25256078-25256359	3505	12.43
1:25291005-25291062	1186	20.45
1:27022895-27024031	4103	3.61
1:27056142-27056354	26778	125.72
1:27057643-27058095	166655	367.89
1:27059167-27059283	11015	94.15
1:27087347-27087587	26026	107.99
1:27087875-27087964	9884	109.82
1:27088643-27088810	15757	93.79
1:27089464-27089776	18254	58.32
1:27092712-27092857	12977	88.88
1:27092948-27093057	2914	26.49
1:27094281-27094490	22913	109.11
1:27097610-27097817	13062	62.8
1:27098991-27099123	11062	83.17
1:27099303-27099478	12876	73.16
1:27099837-27099987	8916	59.05
1:27100071-27100208	13676	99.1
1:27100293-27100389	31569	325.45
1:27100820-27101711	68011	76.25
1:27102068-27102198	9040	69.01
1:27102308-27102391	268	3.19
1:27105514-27107247	197693	114.01
1:33611460-33611507	0	0
1:33612778-33613328	39293	71.31
1:33623854-33623969	10245	88.32
1:33625289-33625545	12887	50.14
1:33631072-33631167	2196	22.88
1:33642009-33642053	0	0
1:33646626-33647033	3035	7.44
1:40363044-40363642	62807	104.85
1:40366486-40367115	4114	6.53
1:40367480-40367560	0	0
1:43803520-43803598	1188	15.04
1:43803770-43803902	9447	71.03
1:43804213-43804391	16652	93.03
1:43804942-43805240	20063	67.1
1:43805635-43805797	7297	44.77

1:43806058-43806184	28433	223.88
1:43812116-43812300	21878	118.26
1:43812463-43812605	13259	92.72
1:43814514-43814673	93	0.58
1:43814934-43815115	10519	57.8
1:43817887-43817974	10629	120.78
1:43818189-43818443	19313	75.74
1:45794978-45795109	21851	165.54
1:45795738-45795740	0	0
1:45796188-45796229	609	14.5
1:45796854-45797006	13196	86.25
1:45797092-45797228	4068	29.69
1:45797333-45797544	3432	16.19
1:45797695-45797758	5123	80.05
1:45797838-45797982	4773	32.92
1:45798063-45798160	4083	41.66
1:45798246-45798359	6542	57.39
1:45798435-45798506	7706	107.03
1:45798590-45798631	2432	57.9
1:45798769-45798842	14566	196.84
1:45798957-45798996	1397	34.93
1:45799085-45799275	10510	55.03
1:45800063-45800183	11692	96.63
1:45803858-45803869	0	0
1:45805875-45805926	2130	40.96
1:51436041-51436169	12722	98.62
1:51439565-51439942	30596	80.94
1:65300245-65300340	8051	83.86
1:65301079-65301189	9326	84.02
1:65301781-65301898	10292	87.22
1:65303615-65303787	14638	84.61
1:65304148-65304272	7297	58.38
1:65305286-65305478	14886	77.13
1:65306928-65307022	8730	91.89
1:65307134-65307284	17439	115.49
1:65309747-65309898	26546	174.64
1:65310437-65310572	9991	73.46
1:65311196-65311323	19690	153.83
1:65312332-65312419	4488	51
1:65313215-65313358	16428	114.08
1:65316487-65316593	5491	51.32

1:65321192-65321381	5685	29.92
1:65323339-65323462	9712	78.32
1:65325788-65325945	14597	92.39
1:65330470-65330655	8425	45.3
1:65332549-65332891	57691	168.2
1:65334994-65335157	18470	112.62
1:65339053-65339206	11689	75.9
1:65344708-65344831	10004	80.68
1:65348960-65349158	11715	58.87
1:65351942-65351947	345	57.5
1:78413199-78413237	0	0
1:78414451-78414459	610	67.78
1:78414558-78414599	112	2.67
1:78414840-78414985	25433	174.2
1:78420940-78421017	6446	82.64
1:78422257-78422385	16570	128.45
1:78425869-78425948	4268	53.35
1:78426029-78426180	2595	17.07
1:78428455-78428615	20056	124.57
1:78429259-78429400	8140	57.32
1:78429747-78429847	27014	267.47
1:78429939-78430041	7320	71.07
1:78430331-78430432	28048	274.98
1:78430555-78430653	18034	182.16
1:78430753-78430915	38542	236.45
1:78432378-78432435	4407	75.98
1:78432568-78432639	6356	88.28
1:78432733-78432785	3120	58.87
1:78433311-78433350	3973	99.33
1:78433849-78433887	1811	46.44
1:78434706-78434768	0	0
1:78435609-78435699	6780	74.51
1:78444569-78444688	10773	89.78
1:115251156-115251275	26422	220.18
1:115252190-115252349	15824	98.9
1:115256421-115256599	46723	261.02
1:115258671-115258781	29636	266.99
1:120457929-120459317	176623	127.16
1:120460288-120460385	18266	186.39
1:120461029-120461176	19997	135.11
1:120461935-120462236	18528	61.35

1:120462852-120463020	11701	69.24
1:120464336-120464432	10080	103.92
1:120464859-120465069	15812	74.94
1:120465259-120465401	31460	220
1:120466260-120466607	33265	95.59
1:120467928-120468433	76413	151.01
1:120469122-120469234	3087	27.32
1:120471599-120471835	19744	83.31
1:120478095-120478227	22014	165.52
1:120479905-120480089	35340	191.03
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X:133549046-133549252	10958	52.94
X:133551199-133551336	7097	51.43
X:133559231-133559360	8287	63.75

This table provides mean coverage for each coding interval covered by the exome bait design. Data was generated using DepthOfCoverage[9].

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