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Supplementary information to:

Original article:

HYPERMETHYLATION OF *RAD9A* INTRON 2 IN CHILDHOOD CANCER PATIENTS, LEUKEMIA AND TUMOR CELL LINES SUGGEST A ROLE FOR ONCOGENIC TRANSFORMATION

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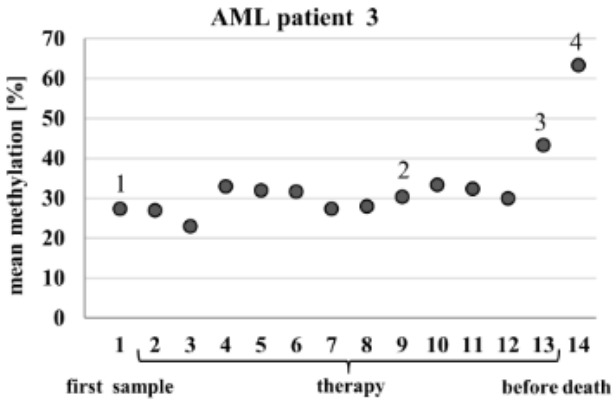
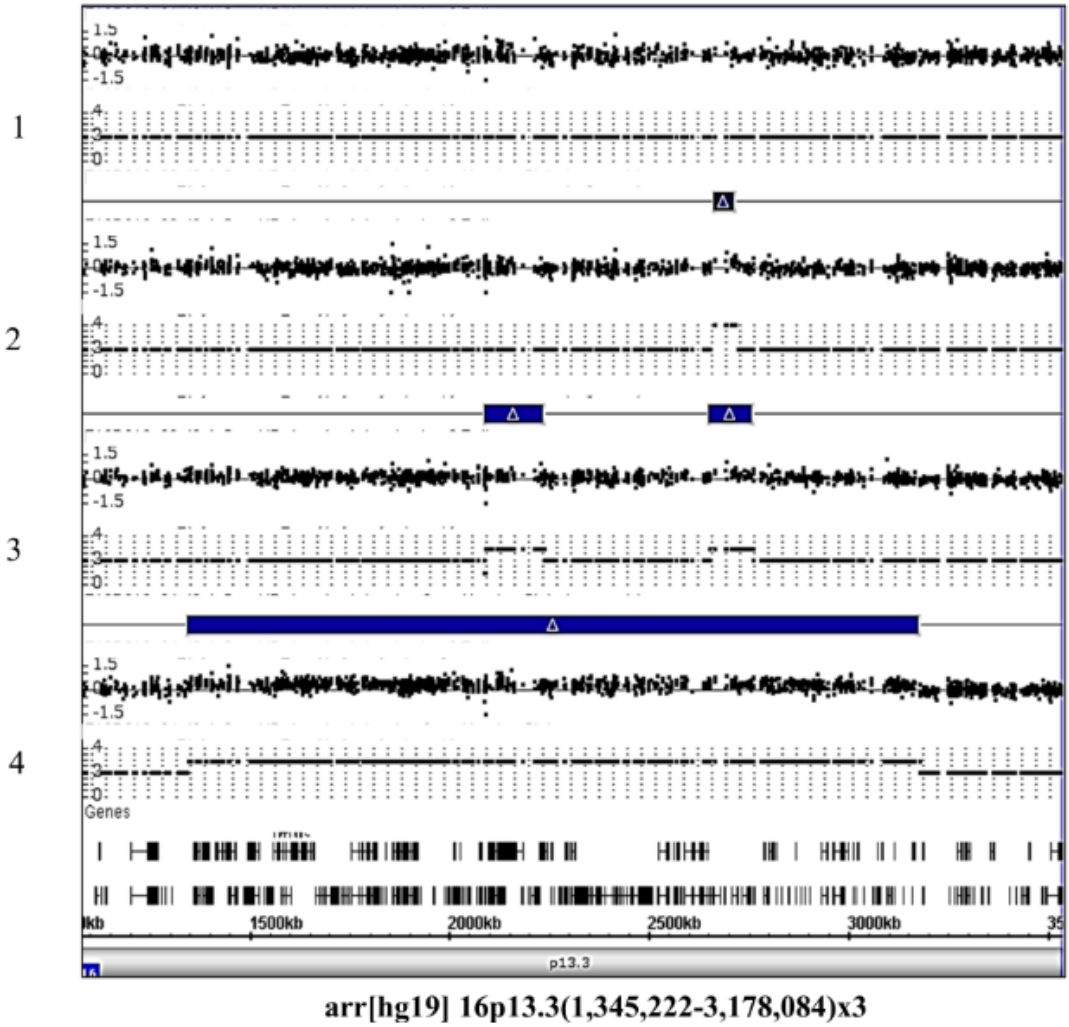
E



F



Supplementary Figure 2E, F

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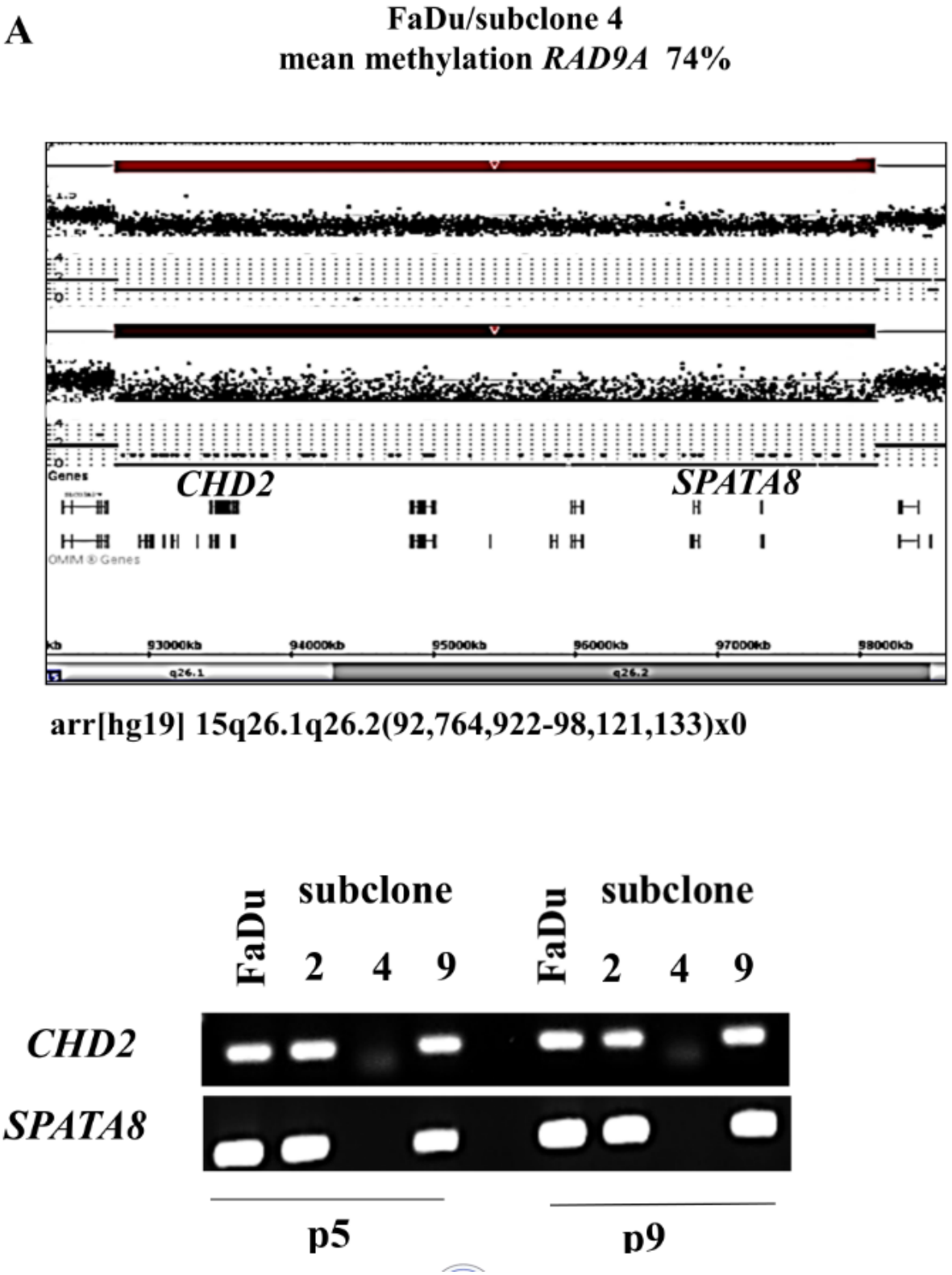

Supplementary Figure 5A: Molecular karyotyping of FaDu subclones 4, 2, 6, 9 and 10. (A) *CHD2* and *SPATA8* are homozygously deleted in subclone 4. The upper bar mark represents the parental FaDu cell line. PCR analysis of *CDH2* and *SPATA8* genes confirmed the SNP Array result.

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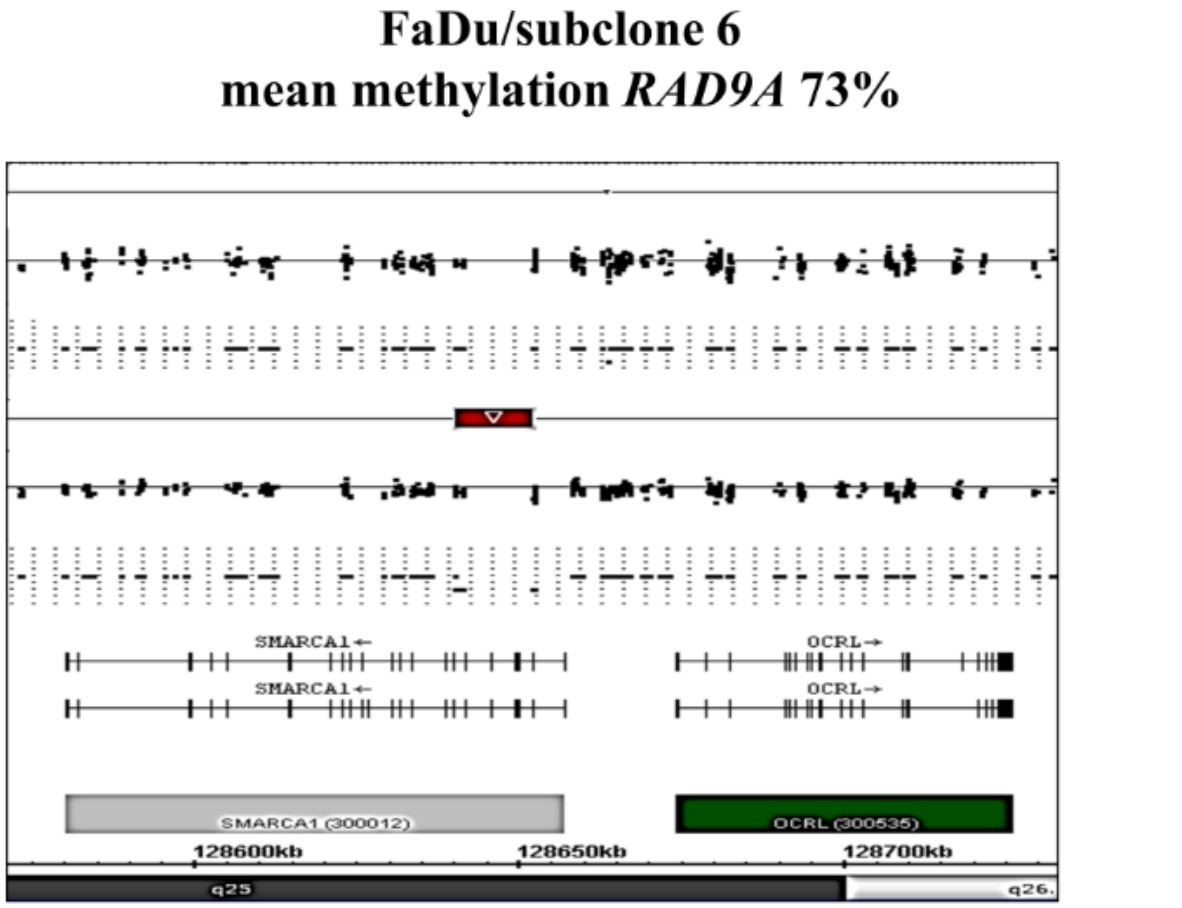

Supplementary Figure 5B: Homozygous mutation (deletion using SNP-Array and stop mutation using Sanger sequencing) is shown for *SMARCA1* in subclone 6. The analysis of **A** and **B** was conducted in two different passages (p5 and p9).

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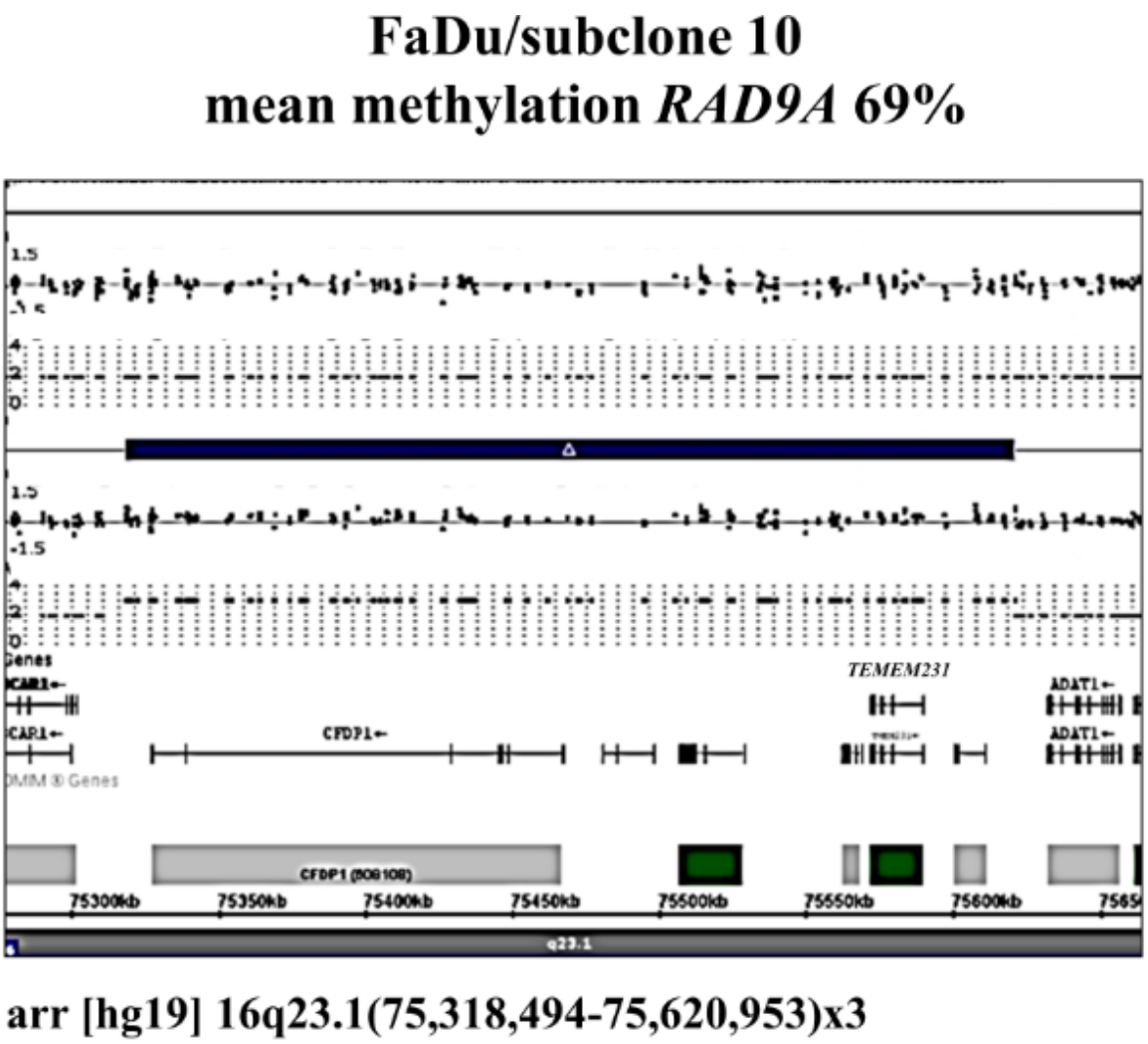

Supplementary Figure 5C: The subclone 10 displayed a 302 kb duplication (indicated as a blue bar) in 16q23.1(75,318,494-75,620,953).

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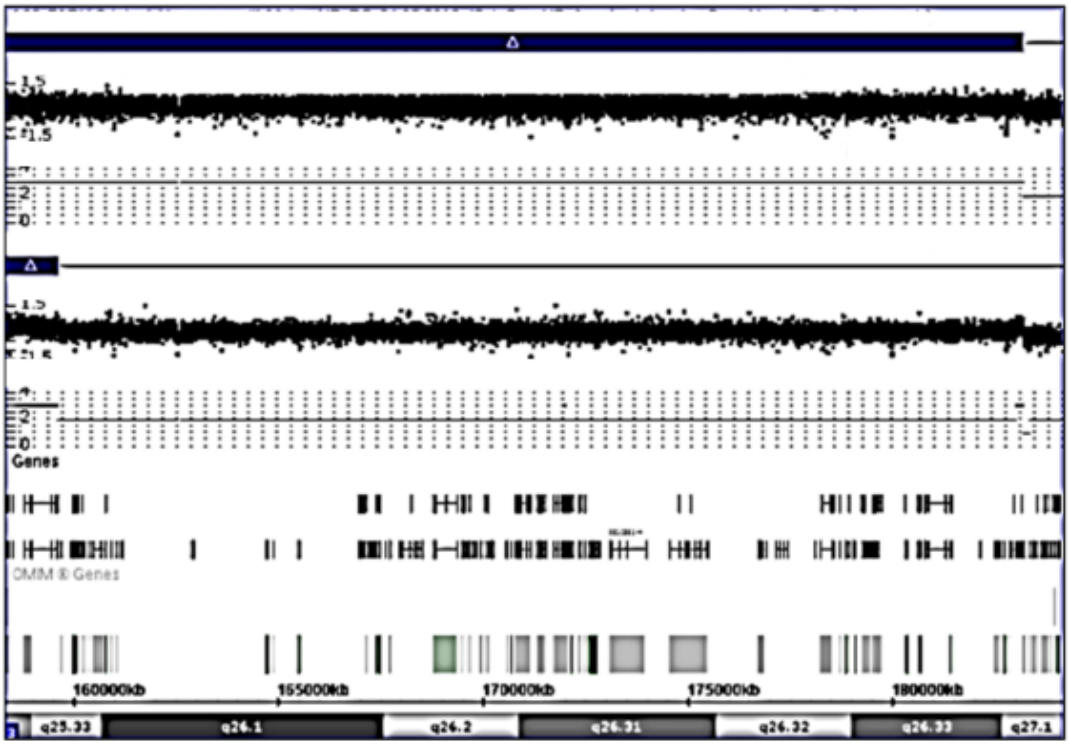
FaDu/subclone 2
mean methylation *RAD9A* 42%



arr [hg19] 3q25.33q26.33(159,599,190-183,199,315)x2

Supplementary Figure 5D-F show the restoration of duplicated areas in subclones 2 and 9. The upper blue bar represents the duplicated chromosome section in the parental cell line FaDu.

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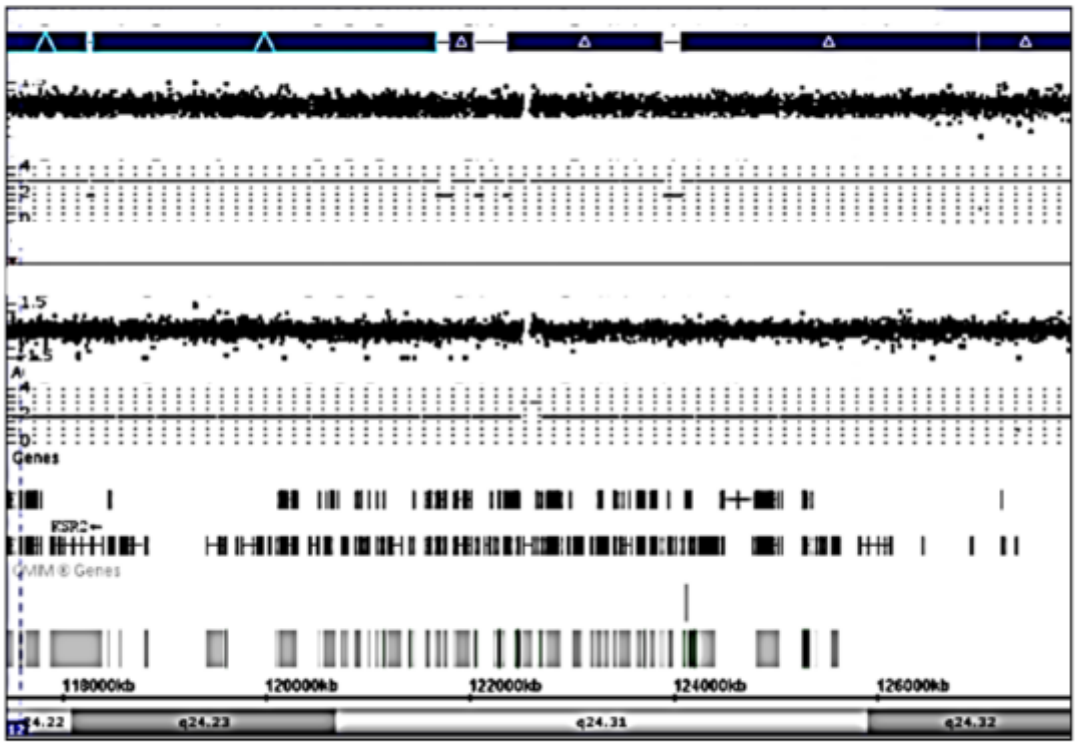
FaDu/subclone 2
mean methylation *RAD9A* 42%



arr[hg19] 12q24.22q24.32(117,452,580-133,584,910)x2

Supplementary Figure 5E

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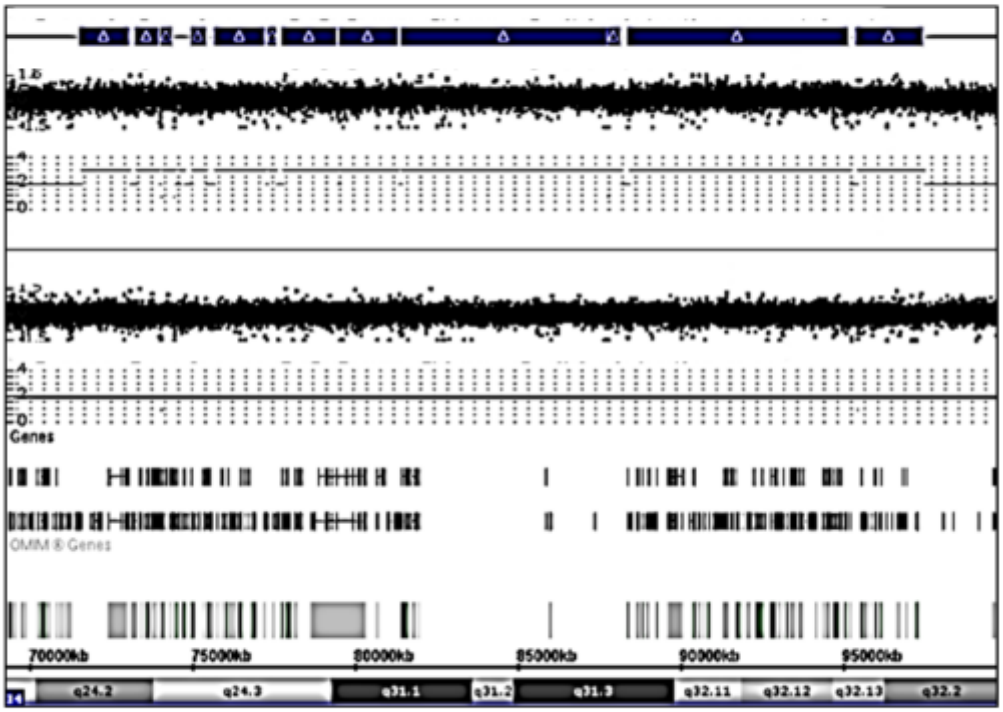
FaDu/subclone 9
mean methylation *RAD9A* 40%



arr[hg19] 14q24.2q32.13(71,543,696-97,490,985)x2

Supplementary Figure 5F

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Supplementary Table 1: List of PCR- and sequencing primer (5´-3´orientation) for bisulfite pyrosequencing

Gene	Chromosomal localization[a] (bp)	Amplicon size (bp)	Forward primer	Reverse primer	Sequencing primer	No. of CpG sites	Reference
BRCA1	Chr17: 43,125,274-43,125,506	232	ATTTAGAGTAG-AGGGTGAAGG	*TCTATCCCTCCCATCCTCTAATT	TGGGTGGTTAATTTAGAGT	5	Galetzka et al., 2012
CDKN2A	Chr9: 21,974,960-21,975,129	169	GGTTGTTTTYGGTT-GGTGTTTT	*ACCCTATCCCTCA-AATCCTCTAAAA	TTTTTGTTTG-GAAAGAT	2	Feng et al., 2007
TP53	Chr17: 7,674,136 - 7,674,298	162	*TTTTTTAGGTTGGTTT-TGATTGTA	AAAACACAACAAACCAA-TATACA	TAATAATAAAAA TAAACCTC	2	Designed for this study (exon 6)
APC (part a) Promoter 1A	Chr5: 112,737,678-112,737,871	193	*GGTTAGGGTTAGG-TAGGTTGT	ACTACACCACTACAACCA-CATATC	CCACACCCAAC-CAA	7	Modified after Schatz et al., 2006
APC (part b) Promoter 1A	Chr5: 112,737,677-112,737,779	102	GGGTTAGGGTTAGGTAGGT	*TCCAAC-CAATTACACAAC-TACTTCTCTCT	AG-GGTTAGGTAGGTT	6	Modified after Schatz et al., 2006
RAD9A	Chr11: 67,392,508-67,392,610	102	GGTTTTTATGGG-GAAAGGAGG	*CCACAAACCCAAC-CCTCTAAC	TTTTATGGG-GAAAGGA	3	Modified after Cheng et al., 2005
EFNA5	Chr5: 107,670,853-107,670,957	104	GAGGGTTTAGGAG-GAAAAAGGAATTA	*CCCCCCAAACACAACTTAAC	AATTATAAGATG-GAGAGAAG	5	Kuang et al., 2008
FBN1	Chr15: 48,417,049-48,417,285	236	GTAGTAGGGTAG-AAATTTATAGT-TAGGTTT	*CCACTTTTATCCAC-CTATTTTCTAAT	ATTATAGTGTTT-TTTAAGAG	1	Flanagan et al., 2006

[a] according to Ensemble NCBI human assembly GRCh37 (Ensembl release 92).
* biotinylated

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Supplementary Table 2: Primers (5´-3´orientation) for deep bisulfite sequencing

Gene	Primer	Sequence (5′ to 3′)	CpG No.	Chromosomal localization (bp)	Amplicon length
APC	Forward	ACACTCTTTCCCTACACGACGCTCTT-CCGATCTGGTTAGGGTTAGGTAGGTTGT	16	Chr5:112,737,678-112,737,871	193bp
	Reverse	GTGACTGGAGTTCAGACGTGTGCTCTT-CCGATCTACTACACCACTACAACCACATATC			
CDKN2A	Forward	ACACTCTTTCCCTACACGACGCTCTT-CCGATCTGGTTGTTTT**Y**GGTTGGTGTTTT	10	Chr9:21,974,960-21,975,129	169bp
	Reverse	GTGACTGGAGTTCAGACGTGTGCTCTT-CCGATCTACCCTATCCCTCAAATCCTCTAAAA			
RAD9A	Forward	ACACTCTTTCCCTACACGACGCTCTT-CCGATCTGGTTTTTATGGGGAAAGGAGG	3	Chr11:67,392,508-67,392,610	102bp
	Reverse	GTGACTGGAGTTCAGACGTGTGCTCTT-CCGATCTCCACAAACCCAACCCTCTAAC			
TP53	Forward	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTT-TTTTAGGTTGGTTTTGATTGTA	2	Chr17:7,674,136-7,674,298	162bp
	Reverse	GTGACTGGAGTTCAGACGTGTGCTCTT-CCGATCTAAAACACAACAAACCAATATACA			

[a] according to Ensemble NCBI human assembly GRCh38 (Ensembl release 104).