

Additional File 1.pdf includes:

Table S1: Frequency of KMT2/MLL Mutations in Colon Cancer Cell Lines

Table S2: Sequences of individual siRNA and shRNA duplexes

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Figure S1: Validation of four oligos used for RNAi-mediated WDR5 depletion

Figure S2: Drug dose response curve for OICR-9429 in HCT116 cells

Figure S3: Representative propidium iodide cell cycle analysis following WDR5 depletion or OICR-9429 treatment in three colon cancer cell lines

Figure S4: Evaluation of apoptosis via Annexin V/PI staining following WDR5 depletion or OICR-9429 treatment in three colon cancer cell lines

Figure S5: RBBP5 depletion does not affect cell viability in a panel of colon cancer cell lines

Cell Line	KMT2A	KMT2B	KMT2C	KMT2D	KMT2F	KMT2G	WDR5
HCT116		D550fs <sup>2</sup> G1881fs <sup>1</sup> L2136fs <sup>2</sup>	Q419R <sup>1</sup> I1344fs <sup>1,2</sup>	V160M <sup>2</sup> R2173fs <sup>1</sup> R2443fs <sup>1,2</sup>		G1578fs <sup>2</sup>	Y260H <sup>1,2</sup>
HCT15	T771fs <sup>2</sup> S1062Y <sup>1,2</sup> V2178A <sup>1</sup>	R226W <sup>2</sup> R1125H <sup>2</sup> A1989T <sup>2</sup>	G3438D <sup>1,2</sup> S3543N <sup>1,2</sup>	P1152H <sup>2</sup> E1244D <sup>1</sup> E1517D <sup>1,2</sup> P1931H <sup>2</sup>		D159N <sup>2</sup> Q780H <sup>2</sup>	
SW480				D1633N <sup>2</sup>			
SW620		G273S <sup>2</sup> G292V <sup>1,2</sup>					
RKO	T771fs <sup>2</sup> S873fs <sup>2</sup> D877fs <sup>1</sup>	A905T <sup>2</sup> R1579H <sup>2</sup> D1819fs <sup>2</sup> P1823fs <sup>1</sup>	G2636C <sup>1</sup> R3853W <sup>2</sup>	R916H <sup>1</sup> R1189H <sup>1,2</sup> L1327P <sup>1</sup> L1600P <sup>1,2</sup> G1960fs <sup>2</sup> P2550fs <sup>2</sup> G3465* <sup>1,2</sup> L4516P <sup>1</sup> R4964fs <sup>2</sup> L5056P <sup>1,2</sup>	A1325T <sup>2</sup>	T1657A <sup>2</sup>	V217Ins <sup>1</sup>
LoVo		R525W <sup>1,2</sup> P2255L <sup>2</sup>	C1013R <sup>1</sup> E1313K <sup>1</sup>	H77fs <sup>1,2</sup> P647fs <sup>1,2</sup> P648fs <sup>1,2</sup> T3548I <sup>2</sup>		V1404I <sup>2</sup>	G277D <sup>1,2</sup>
T84				None <sup>1,2</sup>			

**Table S1: Frequency of KMT2/MLL Mutations in Colon Cancer Cell Lines**

<sup>1</sup>=COSMIC

<sup>2</sup>=Mouradov *et al*

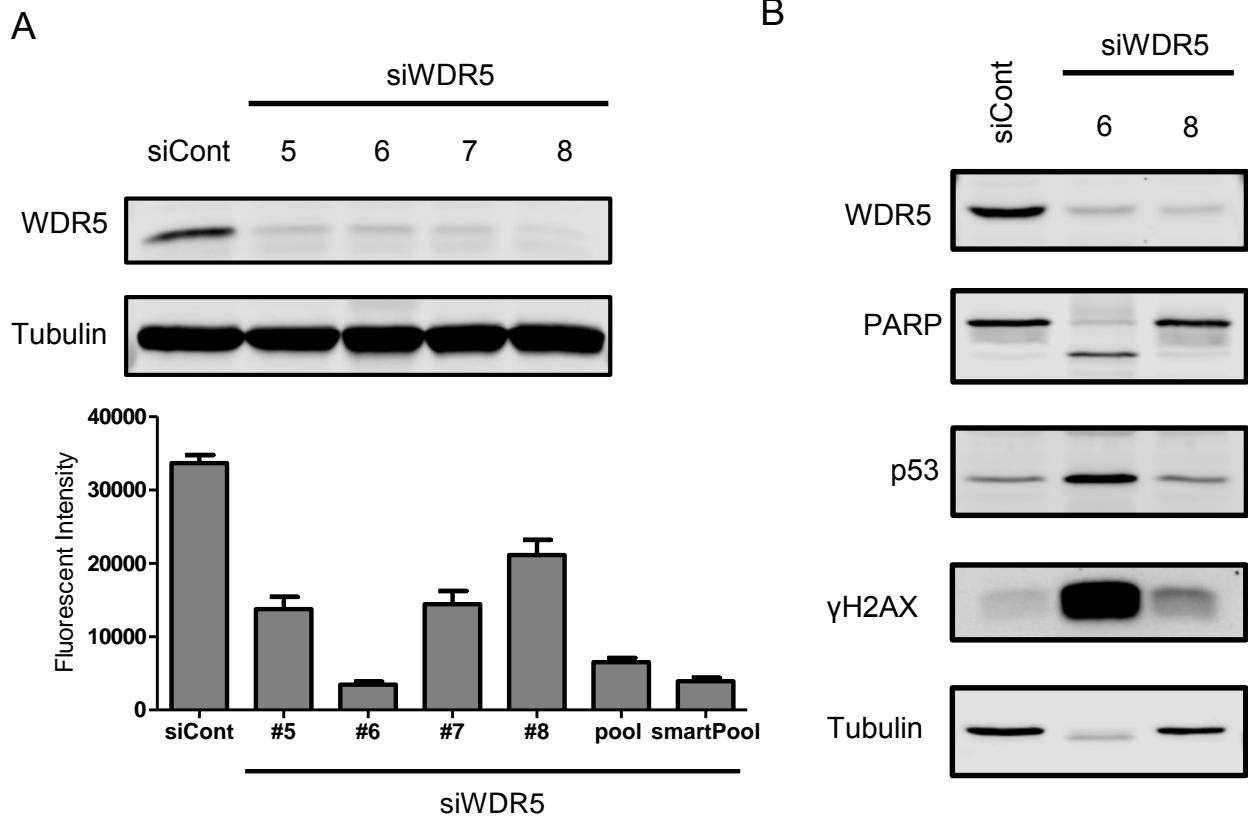
Note: SW480 were not evaluated in COSMIC

Target	Item #	siRNA	Target Sequence
Human WDR5	L-013383	5	GACGAAAGCGUGAGGAUUAU
		6	GUGGAAGAGUGACUGCUAA
		7	GACGUGAGCUCGGGCAAGU
		8	GAUGGAUCCUUGAUAGUUU
Human RBBP5	LU-012008	5	UAACACGGCAGAUCGAAUA
		6	UAUAGAACUUCAAGGAGUA
		7	GCAAUACCACAGCCAUUAA
		8	GAUGGAACUUUGGAUUGUA
Human Non-Targeting	D-001810	01	UGGUUUACAUGUCGACUAA

**Table S2: Sequences of individual siRNA and shRNA duplexes**

Target	Accession	Amplicon Length	Exons	Tm	F Primers 5' -> 3'	R primer 5' -> 3'
Human WDR5	NM_052821	135 bp	8-10	58°C	ATGCGACAGAGACCATCATAG	CGTGAGGATATGGGATGTGAA

**Table S3: Sequences of qPCR primers**

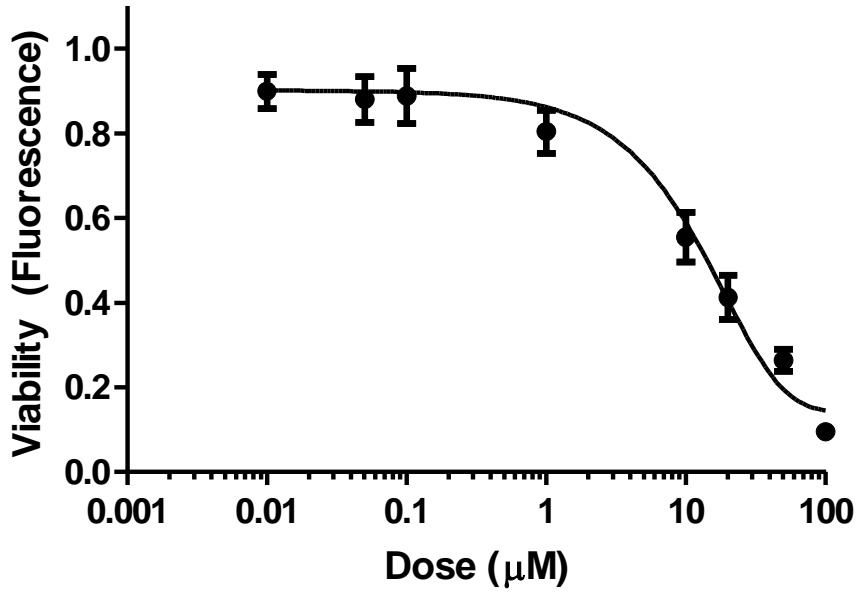


**C**

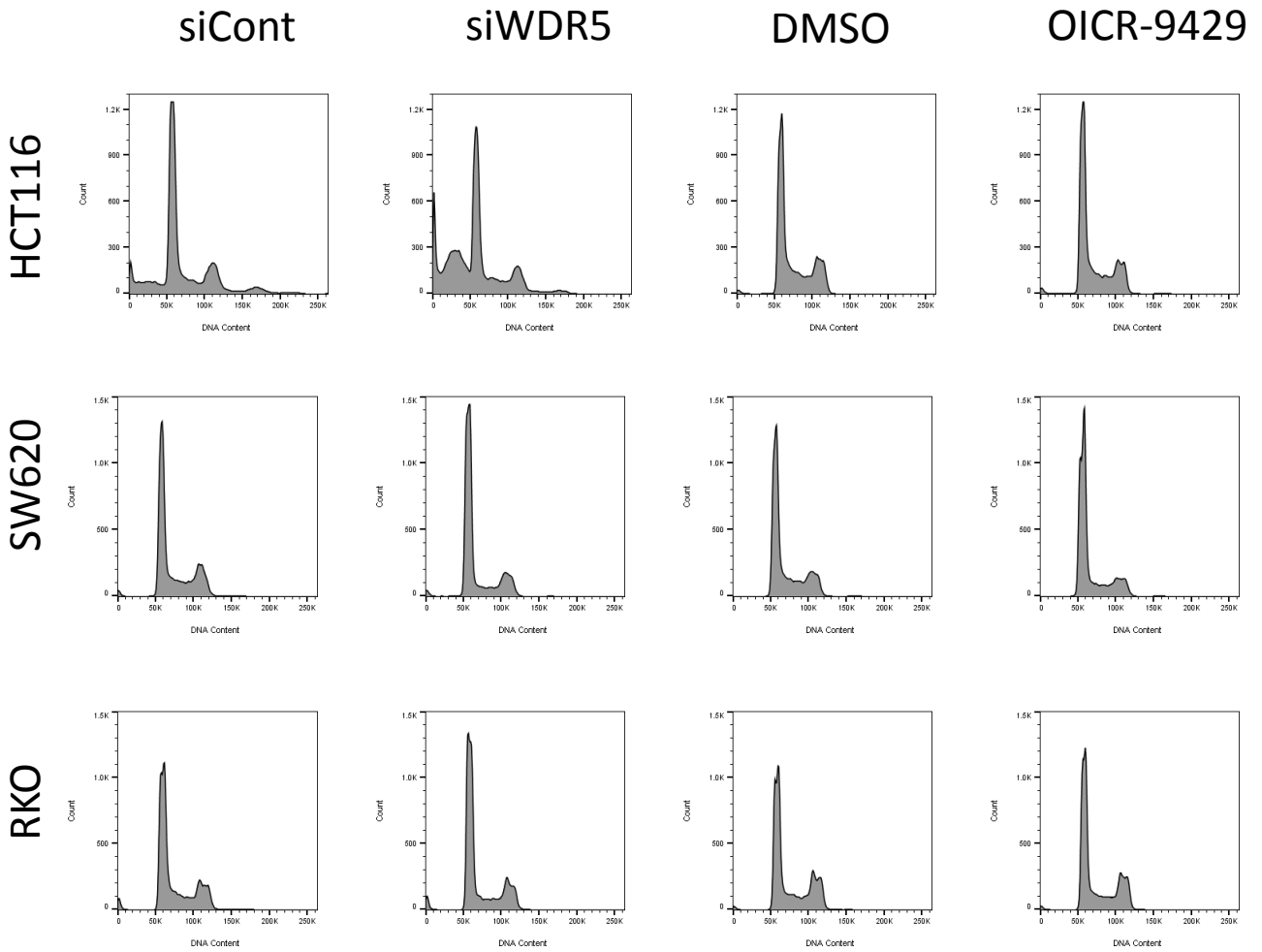
Oligo #6: GUGGAAGAGUGACUGCUAA

**Figure S1: Evaluation of individual WDR5 siRNA.** (A) Immunoblot of WDR5 and viability as measured by alamarBlue® following RNAi-mediated knockdown with individual siRNA oligos in HCT116 colon cancer cells. (B) Immunoblot of WDR5, PARP, p53, and γH2AX following RNAi-mediated WDR5 depletion using either oligo #6 or #8. (C) WDR5 #6 oligo sequence with nucleotides that match the ME1 sequence shown in red.

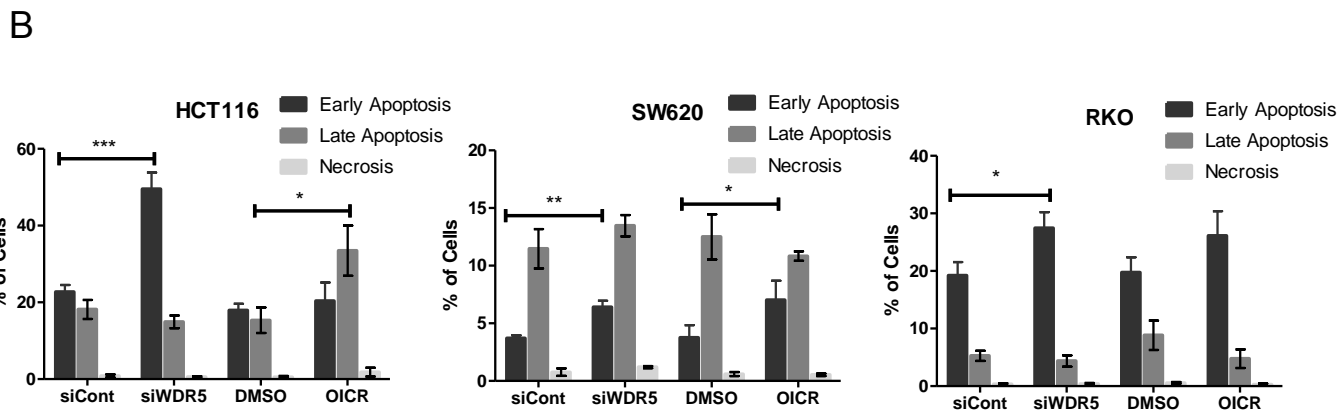
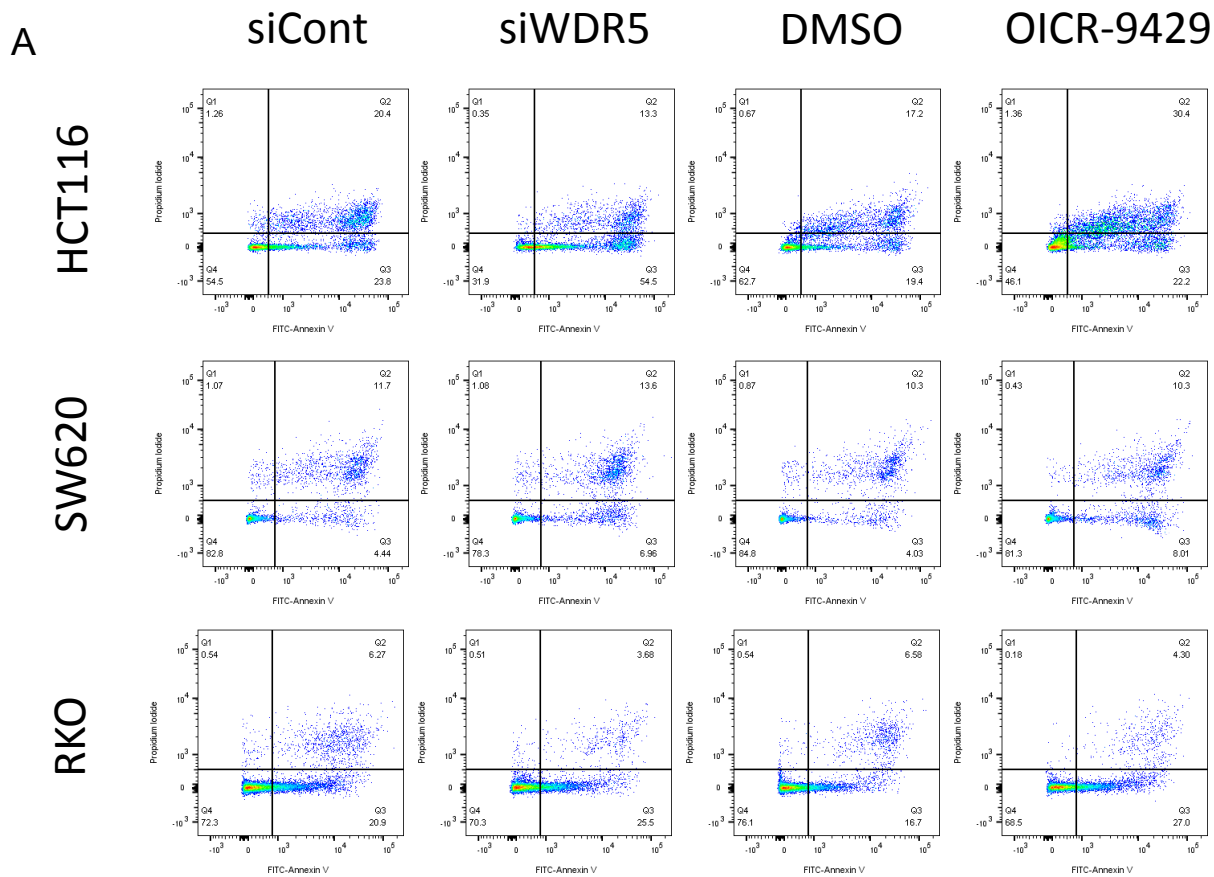
## HCT116



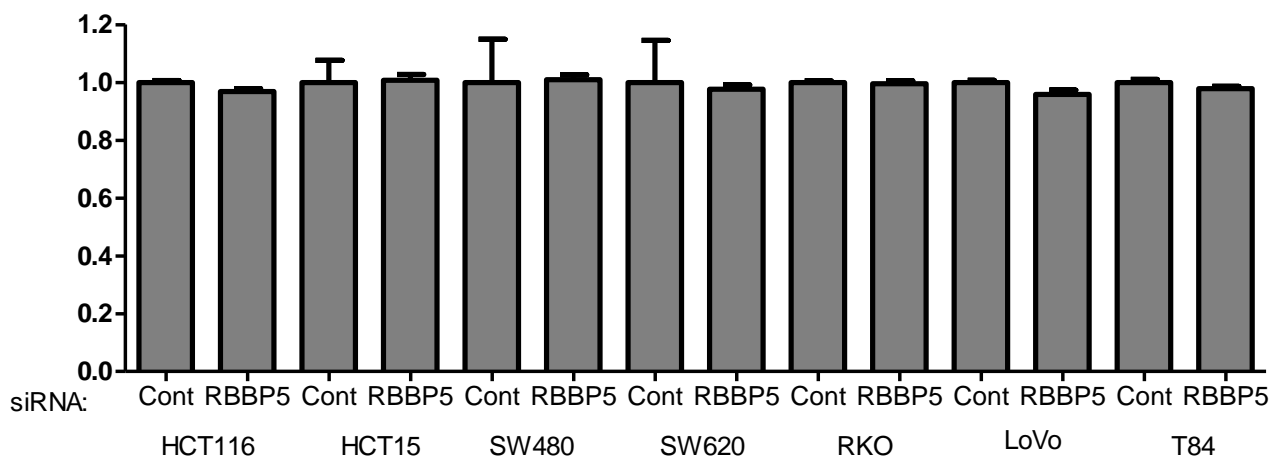
**Figure S2: Drug dose response curve for OICR-9429 in HCT116 cells.** Viability as measured by alamarBlue® following treatment with OICR-9429 at the specified doses for 48 hours in HCT116 colon cancer cells.



**Figure S3: Representative flow cytometry results from propidium iodide cell cycle analysis in three colon cancer cell lines following RNAi-mediated WDR5 depletion for 72 hours (left) or 10 μM OICR-9429 treatment (right) for 48 hours.**



**Figure S4: Evaluation of apoptosis following WDR5 depletion for 72 hours or 10  $\mu$ M OICR-9429 treatment for 48 hours in three colon cancer cell lines. (A) Representative flow cytometry results with (B) quantification from three biological replicates in three colon cancer cell lines.**



**Figure S5: RBBP5 depletion does not affect cell viability in a panel of colon cancer cell lines.** Cell viability in a panel of colon cancer cells as compared to HCECs following RNAi-mediated depletion of RBBP5. Viability was measured by alamarBlue® assays 72 hours after transfection.