



## Supplementary Materials: Identification and Validation of an Aspergillus nidulans Secondary Metabolite Derivative as an Inhibitor of the Musashi-RNA interaction

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Figure S1. Structures of other azaphilones in the screening.



**Figure S2.** Plot of relative peak intensity for all non-overlapping MSI2-RRM1 resonances in the ligand bound form versus free state (I1:0.3/I1:0). Gray dashed line corresponds one standard deviation from the mean.



**Figure S3.** Aza-9 titration against <sup>13</sup>C ILV methyl labeled MSI2-RRM1. A) <sup>1</sup>H-<sup>13</sup>C HSQC spectra of 80  $\mu$ M <sup>15</sup>N and <sup>13</sup>C ILV methyl labeled MSI2-RRM with increasing molar ratios of AZA-9. B) Residues with peak intensity ratio lower than 0.40 are colored red. C) Plot of relative peak intensity for all non-overlapping MSI2-RRM1 ILV methyl resonances in the ligand bound form versus free state (I1:0.3/I1:0). Gray dashed line corresponds one standard deviation from the mean.

lop panel							
HCT-116 b/W numb-Bi pull-down	(0		HCT-116 b/W numb-Bi pull-down				
250KDa 150KDa 075KDa 37KDa 37KDa 200KDa 150KDa 150KDa 150KDa 100KDA 100KDA	500g Input (1/1	oll signaling		q Oswa numb-Bi	Control-Bi	Soug Input (1)	11-coll signaling
25KDa 20KDa	-		1000	65	432	2 1 ***	
		6	5	4	3	2	1
	Intensity	0.016	10.2	9.89	0.171	3.29	1.07
Bottom panel	ratio	0	1	0.97	0.017	0.32	0.105
50ug input (170) 37KDa	MSH		50ug input (1/10)		<b>-116 b/W</b>		<b>ASI1</b>
		-					
				INPUT	numb	DMSO	Aza-9
			Intensity	1.15	-0.01	6.91	5.1
			ratio	0.17	0	1	0.74

Figure S4. Figure 5B Supplemental.



Figure S5. Figure 8B Supplemental.





Figure S6. Figure 9B left panel Supplemental.



**Figure S7.** MSI direct targets APC and NUMB protein levels increased in RKO cells with Aza-9-liposome treatment.

	Red channel	Green channel	Intensity
116 24H Aza-9-lip Lip 50 100	116 24H Lip Aza-9-lip 50 100	116 24H Aza-9-lip Lip 50 100	116 24H Aza-9-lip Lip 50 100
250KDa	250KDa	250KDa	250KDa 0.387 0.368 0.501
100KDa 75KDa	100KDa 75KDa	100KDa	100KDa 75KDa
50KDa	50KDa	50KDa	50KDa
RKO <u>24H</u> Lip <u>A9L</u>	RKO <u>24H</u> Lip <u>A9L</u>	RKO <u>24H</u> Lip <u>A9L</u> 50	RKO <u>24H</u> Lip <u>A9L</u>
250KDa	250KDa	250KDa	250KDa
150KDa	150KDa	150KDa	150KDa
100KDa	100KDa	100KDa	100KDa
75KDa	75KDa	75KDa	75KDa 0.559 0.527
50KDa	50KDa	50KDa	50KDa 4.82 3.68

Figure S8. Figure 9B right panel and Figure S7 Supplemental.



**Figure S9.** Aza-9-lip inhibits Wnt/ $\beta$ -catenin reporter. HCT-116 cells were transfected with TOPflash or FOPflash reporter constructs. Cells were treated with Aza-9-lip or liposomes only for 24 h in the presence of 20 mM LiCl.



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