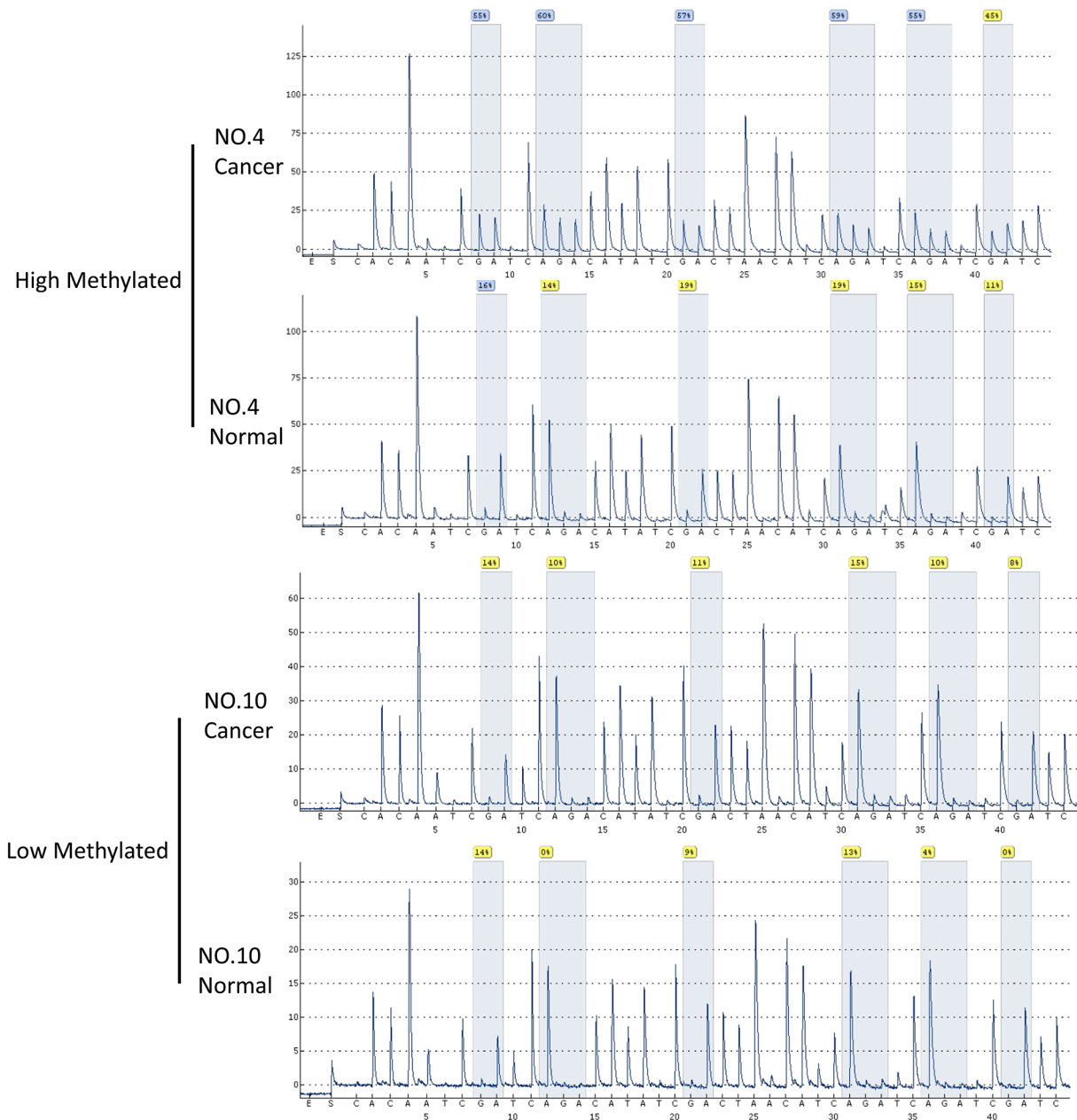


# Low FAT4 expression is associated with a poor prognosis in gastric cancer patients

## SUPPLEMENTARY MATERIALS

Methylation Rate (%)							Sample No.	Meth. Satus
Pos. 1	Pos. 2	Pos. 3	Pos. 4	Pos. 5	Pos. 6	Ave.		
Pos. 1 Meth. (%)	Pos. 2 Meth. (%)	Pos. 3 Meth. (%)	Pos. 4 Meth. (%)	Pos. 5 Meth. (%)	Pos. 6 Meth. (%)	Mean Meth. (%)		
						39.89	No.4	High Methylated (>5%)
						34.52	No.12	
						20.1	No.18	
						19.54	No.14	
						18.13	No.15	
						14.2	No.11	
						13.96	No.13	
						13.19	No.16	
						11.07	No.17	
						8.745	No.2	
						6.525	No.37	
						6.315	No.20	
						4.783	No.10	Low Methylated (<5%)
						3.493	No.32	
						2.462	No.9	
						0.258	No.6	
						-1.81	No.21	Un-Methylated
						-2.69	No.31	
						-4.29	No.5	
						-4.79	No.19	
						-5.42	No.22	
						-5.83	No.24	
						-7.78	No.25	
						-8.66	No.26	
						-10.8	No.1	
						-11	No.33	
						-11.5	No.35	
						-11.9	No.8	
						-12.2	No.34	
						-12.5	No.3	
						-13.3	No.30	
						-13.3	No.28	

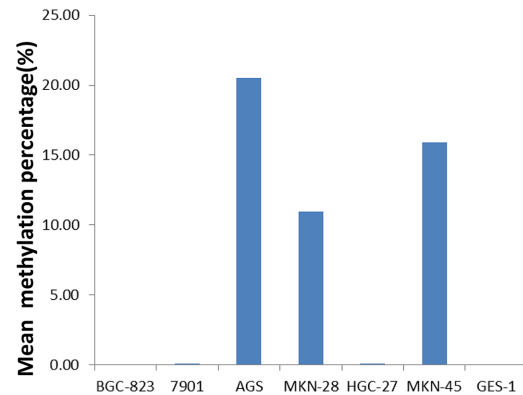
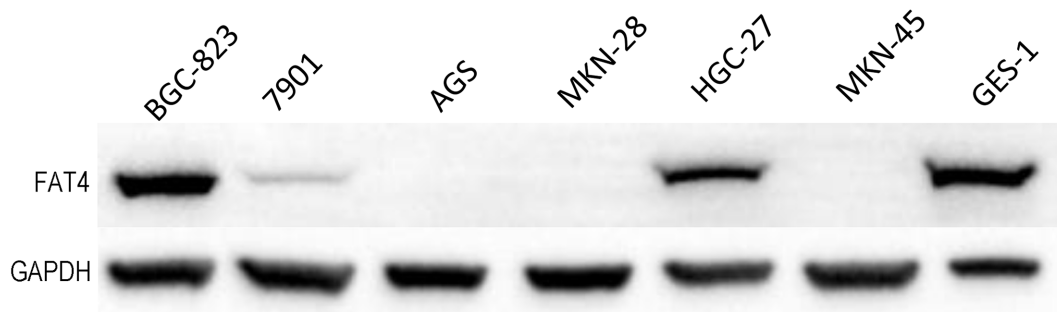
**Supplementary Figure 1: Methylation pyrosequencing analysis of FAT4 promoter in GC tissue samples.** Histogram shows mean methylation percentage based on pyrosequencing of 6 CpG sites in the FAT4 promoter region of GC tumor tissues relative to adjacent noncancerous tissues (n=32). Note: if mean methylation percentage was lower than 0%, the samples are referred to as unmethylated; if mean methylation percentage was between 0-5% or >5%, they are referred to as low and high methylated. Both low- and high- methylated groups were included in methylated group.



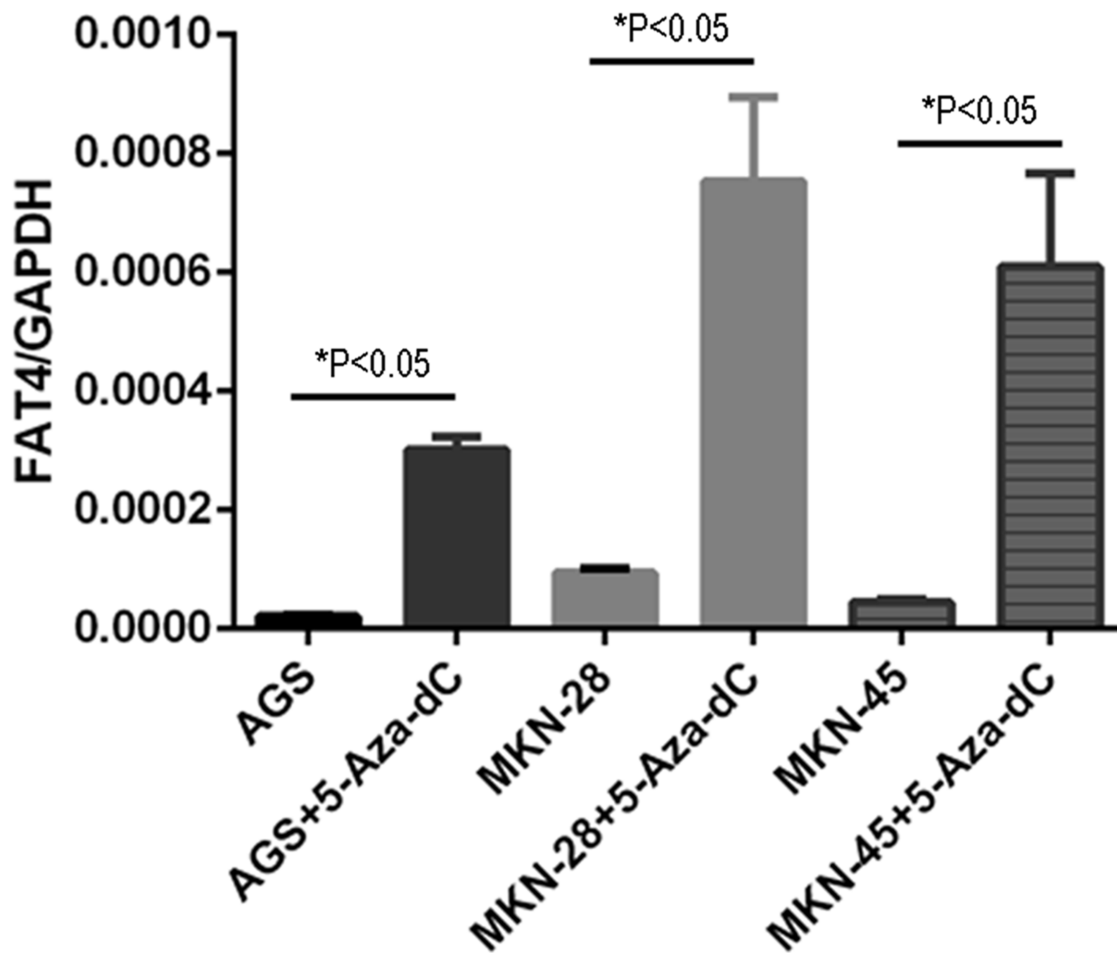
Supplementary Figure 2: Representative pyrosequencing results showing methylation patterns in high and low methylated GC patient samples.

**A**

Methylation Percentage (%)							Mean Meth. (%)	Samples
Pos. 1 Meth. (%)	Pos. 2 Meth. (%)	Pos. 3 Meth. (%)	Pos. 4 Meth. (%)	Pos. 5 Meth. (%)	Pos. 6 Meth. (%)	Mean Meth. (%)		
0.05	0.02	0.03	0.05	0.03	0.10	<b>0.05</b>	BGC-823	
0.08	0.06	0.09	0.09	0.06	0.05	<b>0.07</b>	7901	
20.36	26.53	21.71	23.08	16.41	14.93	<b>20.50</b>	AGS	
10.69	10.11	11.16	10.40	12.51	11.00	<b>10.98</b>	MKN-28	
0.08	0.06	0.09	0.09	0.06	0.05	<b>0.07</b>	HGC-27	
15.99	12.51	15.22	20.01	21.37	10.44	<b>15.92</b>	MKN-45	
0.06	0.05	0.10	0.04	0.05	0.05	<b>0.06</b>	GES-1	

**B**

**Supplementary Figure 3: FAT4 promoter methylation pyrosequencing of GC cell lines and FAT4 expression in GC cell lines.** (A) Mean methylation percentages of the *FAT4* promoter in various GC cell lines. The AGS, MKN-28 and MKN-45 cells show *FAT4* promoter methylation, the mean methylation percentage of between 10% and 20%. However, the GES-1, HGC-27, 7901 and BGC-823 cells shown no *FAT4* promoter methylation. (B) Representative western blots demonstrating FAT4 expression in various GC cell lines. The GC cells BGC-823, GES-1, 7901 and HGC-27 shown FAT4 expression, whereas AGS, MKN-28 and MKN-45 shown no FAT4 expression. GAPDH was used as loading control.



Supplementary Figure 4: 5-aza deoxycytidine increases FAT4 expression in BGC-823 cells. qRT-PCR analysis showing FAT4 levels in untreated and 5-Aza-Dc (1µM) treated BGC-823 GC cells. As shown, FAT4 levels increased upon 5-Aza-dC treatment (\*P<0.05).