Supplementary Data

MPO promoter polymorphism (rs2333227) programs malignant phenotypes of colorectal cancer by altering the binding affinity of AP-2α

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Running title: *MPO* polymorphism and colorectal cancer development

Key words: colorectal cancer; *MPO*; survival; AP-2α; polymorphism

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Fig.S1 Creation of the genomic MPO -463 C>T mutation in CRC cells with CRISPR/cas9

(A) Upper panel, schematic of the CRISPR/Cas9-mediated DNA cleavage. The wild type rs2333227 bases are shown in red. The sgRNA sequence is shown in green. The sequence for the single-stranded DNA donor is described in the bottom part. (B) Sanger sequencing for wild-type and mutation in CRC cells (SW620, andSW480) with CRISPR/Cas9-mediated.

Figure S1

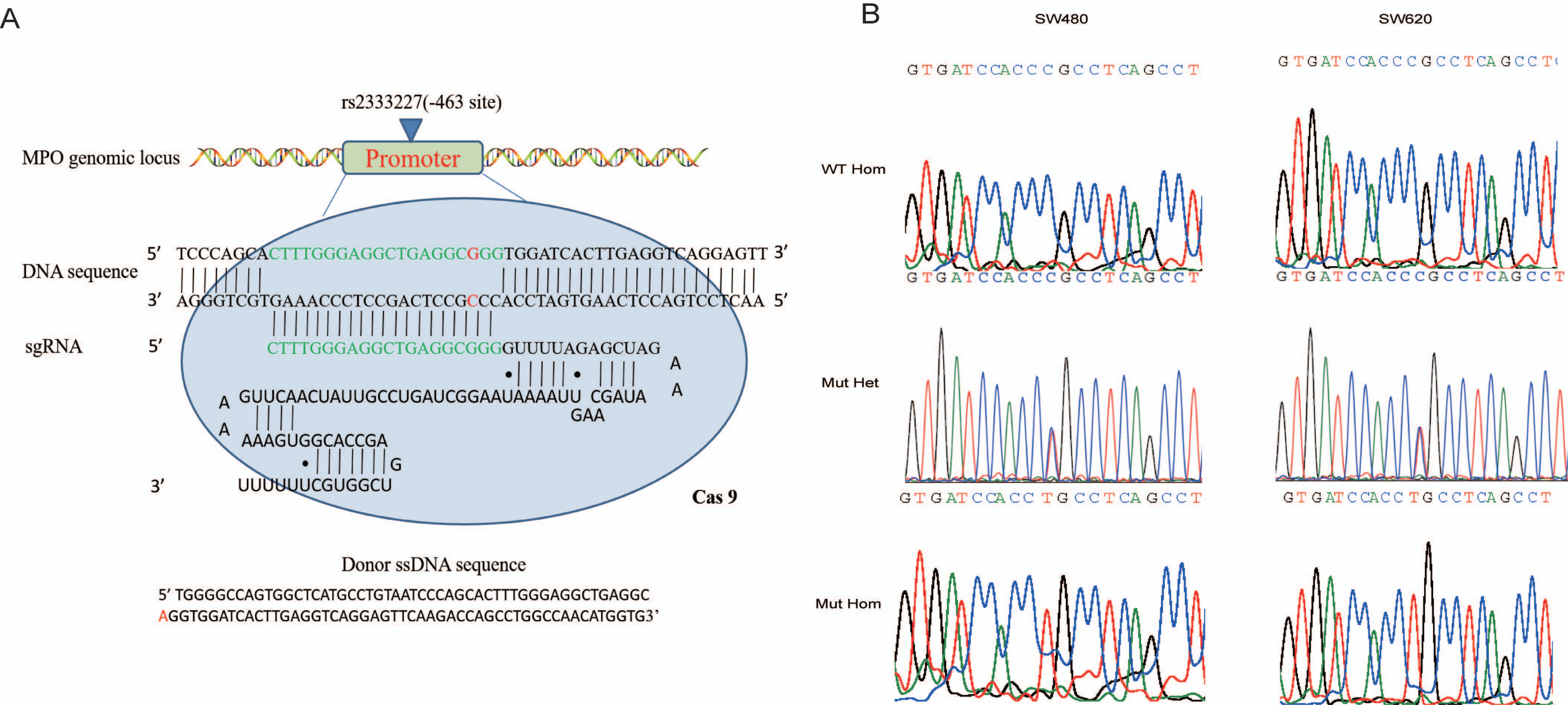
A
rs2333227(-463 site)
MPO genomic locus
Promoter
DNA sequence
5' TCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATCACTTGAGGTCAGGAGTT 3'
3' AGGGTCGTGAAACCCTCCGACTCCGCCCACCTAGTGAACTCCAGTCCTCAA 5'
sgRNA
5' CTTTGGGAGGCTGAGGCGGGUUUUAGAGCUAG
A
A
GUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAUA
GAA
A
A
AAAGUGGCACCGA
G
3' UUUUUUCGUGGCU
Cas 9
Donor ssDNA sequence
5' TGGGGCCAGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGC
AGGTGGATCACTTGAGGTCAGGAGTTCAAGACCAGCCTGGCCAACATGGTG3'
B
SW480
SW620
WT Hom
Mut Het
Mut Hom
GTGATCCACCCGCCTCAGCCT
GTGATCCACCTGCCTCAGCCT

Fig.S2 Protein interaction network for genes regulated by MPO

(A-I) Correlation analysis between mRNA expression levels of MPO and 9 genes predicted to interact with MPO.

Figure S2

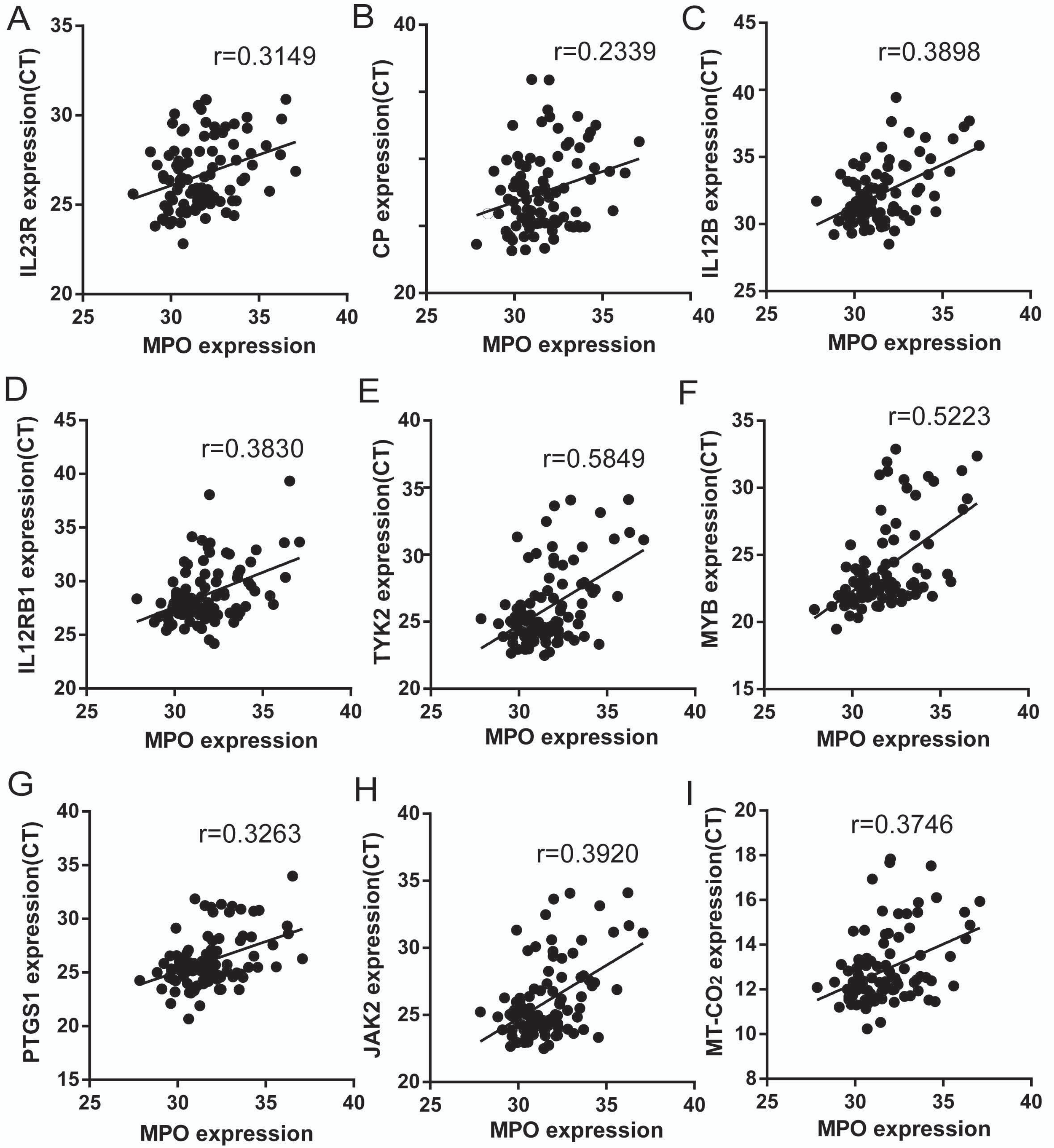
A
IL23R expression(CT)
r=0.3149
35
30
25
20
25
30
35
40
MPO expression
B
CP expression(CT)
r=0.2339
40
20
25
30
35
40
MPO expression
C
IL12B expression(CT)
r=0.3898
45
40
35
30
25
25
30
35
40
MPO expression
D
IL12RB1 expression(CT)
r=0.3830
45
40
35
30
25
20
25
30
35
40
MPO expression
E
TYK2 expression(CT)
r=0.5849
40
35
30
25
20
25
30
35
40
MPO expression
F
MYB expression(CT)
r=0.5223
35
30
25
20
15
25
30
35
40
MPO expression
G
PTGS1 expression(CT)
r=0.3263
40
35
30
25
20
15
25
30
35
40
MPO expression
H
JAK2 expression(CT)
r=0.3920
40
35
30
25
20
25
30
35
40
MPO expression
I
MT-CO2 expression(CT)
r=0.3746
20
18
16
14
12
10
8
25
30
35
40
MPO expression

Fig.S3 MPO, IL-23A and MMP9 expression in wild type and CRISPR/Cas9-mediated CRC cell

(A-F) MPO, IL-23A and MMP9 mRNA expression levels were detected in CRC cells by qRT-PCR (A, C, E: SW620, and B, D, F: SW480). (G, H) The MPO levels of CRC cell culture supernatant were detected by ELISA (G: SW620, and H: SW480). (I, J) IL-23A levels of CRC cell culture supernatant were detected by ELISA (G: SW620, and H: SW480). *** $P < 0.001$, ** $P < 0.01$,*$P < 0.05$.

Figure S3

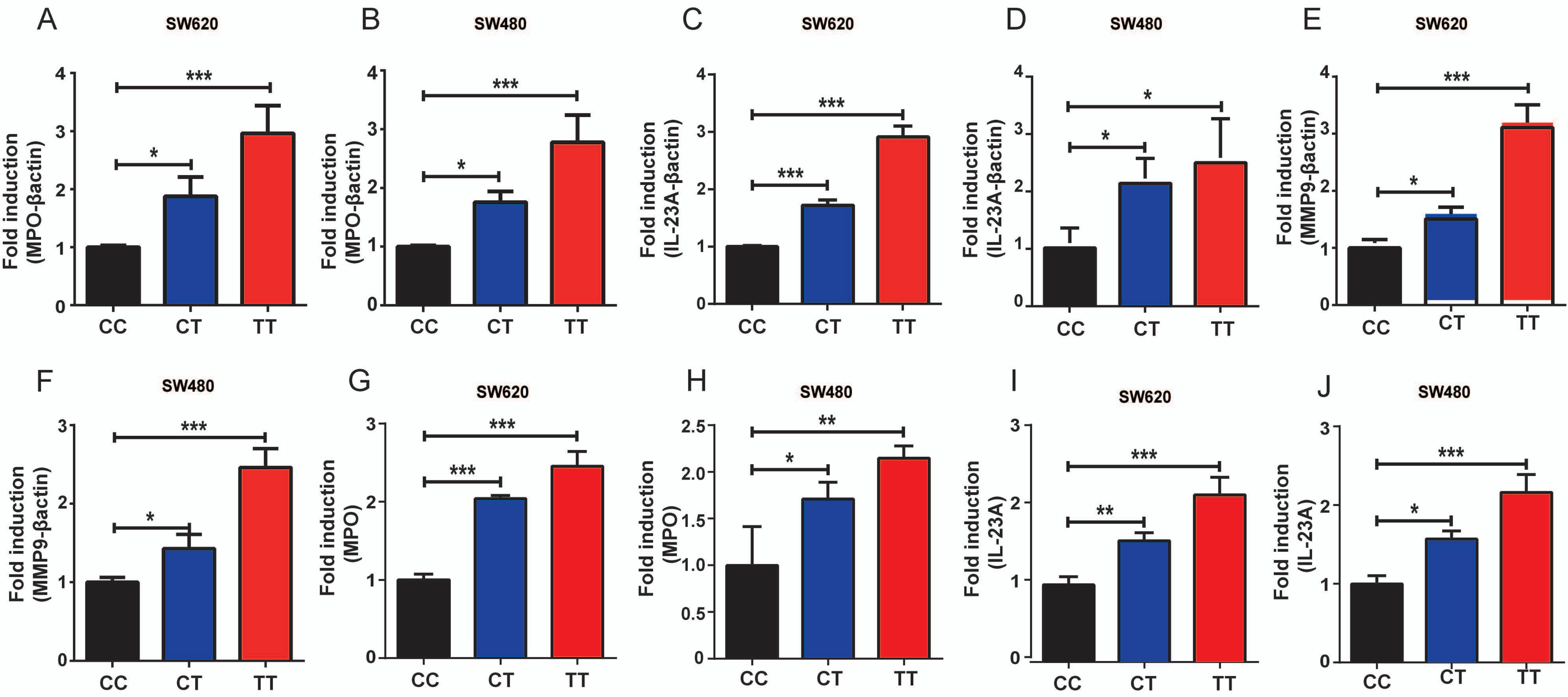
A
SW620
Fold induction (MPO-βactin)
0
1
2
3
4
***
*
CC
CT
TT
B
SW480
Fold induction (MPO-βactin)
0
1
2
3
4
***
*
CC
CT
TT
C
SW620
Fold induction (IL-23A-βactin)
0
1
2
3
4
***
***
CC
CT
TT
D
SW480
Fold induction (IL-23A-βactin)
0
1
2
3
4
*
*
CC
CT
TT
E
SW620
Fold induction (MMP9-βactin)
0
1
2
3
4
***
*
CC
CT
TT
F
SW480
Fold induction (MMP9-βactin)
0
1
2
3
***
*
CC
CT
TT
G
SW620
Fold induction (MPO)
0
1
2
3
***
***
CC
CT
TT
H
SW480
Fold induction (MPO)
0
0.5
1.0
1.5
2.0
2.5
**
*
CC
CT
TT
I
SW620
Fold induction (IL-23A)
0
1
2
3
***
**
CC
CT
TT
J
SW480
Fold induction (IL-23A)
0
1
2
3
***
*
CC
CT
TT

Table S1. Stratification analysis of the *MPO* rs2333227 genotypes associated with CRC risk by demographic variables.

Variables	Genotypes（cases/controls）		P	Adjusted OR (95% CI) [a]
	GG	GT/TT		
Age				
≤56	405/454	162/151	0.1624	1.20(0.92-1.56)
>56	341/421	170/149	0.0103	1.42(1.09-1.85)
Gender				
Male	476/517	183/169	0.1912	1.18(0.92-1.51)
Female	270/358	149/131	0.0043	1.53(1.15-2.04)
Drinking status				
Never	510/630	226/220	0.0331	1.21(0.97-1.51)
Ever	236/245	106/80	0.0663	1.45(0.96-2.21)
Smoking status				
Never	457/581	212/208	0.0252	1.24(0.98-1.57)
Ever	289/294	120/92	0.0794	1.21(0.84-1.73)
Location				
Colon	308/875	167/300	<.0001	1.64(1.30-2.08)
Rectum	438/875	165/300	0.4055	1.10(0.88-1.37)
Grade				
Low	246/875	110/300	0.0451	1.31(1.01-1.71)
Intermediate/High	500/875	222/300	0.0135	1.31(1.07-1.61)
Depth of invasion				
T1	28/875	7/300	0.4586	1.14(0.89-1.47)
T2	178/875	66/300	0.622	1.09(0.87-1.37)
T3	139/875	65/300	0.0585	1.25(0.99-1.57)
T4	401/875	194/300	0.0017	1.44(1.09-1.91)
Lymph node metastasis				
N0	453/875	168/300	0.4848	1.08(0.87-1.35)
N1	293/875	164/300	<.0001	1.67(1.32-2.11)
Distant metastasis				
M0	681/875	277/300	0.0803	1.20(0.99-1.46)
M1	65/875	55/300	<.0001	2.49(1.70-3.66)
TNM				
I	151/875	45/300	0.4423	0.86(0.60-1.23)
II	286/875	115/300	0.2168	1.18(0.92-1.53)
III	244/875	117/300	0.0102	1.44(1.11-1.87)
IV	65/875	55/300	<.0001	2.49(1.70-3.66)

[a] Adjusted for age, Gender, smoking and drinking status.

Table S2. Stratification analysis of the rs2333227 genotypes associated with colorectal cancer patients' survival

Variables	Genotypes(Patients/Deaths)		P	Adjusted HR (95% CI) [a]
	CC	CT/TT		
Age				
≤56	405/226	162/100	0.0413	1.10(0.87-1.40)
>56	341/201	170/117	0.007	1.33(1.05-1.67)
Gender				
Male	476/290	183/120	0.096	0.97(0.78-1.21)
Female	270/137	149/97	0.0003	1.61(1.24-2.10)
Drinking status				
Never	457/246	212/136	0.0011	1.24(1.01-1.53)
Ever	289/181	120/81	0.1393	1.17(0.88-1.55)
Smoking status				
Never	510/275	226/140	0.0105	1.25(1.01-1.54)
Ever	236/152	106/77	0.0126	1.13(0.87-1.48)
Location				
Colon	308/191	167/114	0.0503	1.20(0.95-1.52)
Rectum	438/236	165/103	0.0083	1.19(0.94-1.51)
Grade				
Low	246/157	110/79	0.0208	1.14(0.87-1.51)
Intermediate/High	500/270	222/138	0.0089	1.25(1.02-1.54)
Depth of invasion				
T1	28/8	7/5	0.0513	5.70(1.32-24.66)
T2	178/75	66/33	0.1144	1.37(0.91-2.07)
T3	139/75	65/35	0.6826	1.15(0.77-1.73)
T4	401/269	194/144	0.0123	1.33(1.09-1.64)
Lymph node metastasis				
N0	453/193	168/68	0.9411	0.93(0.70-1.23)
N1	293/234	164/149	0.0005	1.45(1.17-1.78)
Distant metastasis				
M0	681/371	277/164	0.0635	1.20(1.00-1.45)
M1	65/56	55/53	0.0309	1.40(0.95-2.08)
TNM				
I	151/44	45/12	0.9236	0.89(0.46-1.70)
II	286/134	115/49	0.6212	0.89(0.64-1.25)
III	244/193	117/103	0.0425	1.29(1.01-1.65)
IV	65/56	55/53	0.0309	1.40(0.95-2.08)

[a] Adjusted for age, Gender , smoking and drinking status, location, grade, TNM stage.

Table S3. Stepwise Cox regression analysis of colorectal cancer survival

Entered variables	b	SE	HR (95% CI)	P
Age (> 56 *vs.* ≤56)	0.4514	0.08083	1.57(1.34-1.84)	<.0001
Drinking status (Ever *vs.* Never)	0.32549	0.08337	1.39(1.18-1.63)	<.0001
TNM (III/IV *vs.* I/II)	1.75775	0.08697	5.80(4.89-6.88)	<.0001
rs2333227 (CT/TT *vs.* CC)	0.20785	0.08371	1.23(1.05-1.45)	0.013

β, regression coefficient

Table S4. The serum MPO levels of CRC patients with rs2333227 genotypes

	CC	CT/TT
N	40	40
mean	463.3	535.5
SD	30.2	43.0
Range (ng/ml)	389-543	474-631