

Supplementary Figure legends

Supplementary Figure 1 - (A) Schematic of the main pathway of DNA demethylation. TET enzymes (TET1-3) progressively oxidize 5mC to 5hmC, 5fC, and 5caC. TDG excises 5fC and 5caC, and downstream BER enzymes yield cytosine. DNMTs convert cytosine into 5mC. **(B)** Schematic of the mating strategy employed to generate mice with the four genotypes of interest.

Supplementary Figure 2 - *Tet1* and *Tdg* inactivation modifies tumor multiplicity or size in the *Apc*^{Min} background. Graphical representation of the total number **(A)** and size distribution **(B)** of small intestinal (left panels) and colonic (right panels) adenomas in *Tet1*^{-/-}*Tdg*^{+/+} (*Tet1* knockout) *Apc*^{Min/+} mice; *Tet1*^{+/-}*Tdg*^{N151A/+} (double heterozygotes) *Apc*^{Min/+} mice; *Tet1*^{+/+}*Tdg*^{N151A/+} (*Tdg* heterozygotes) *Apc*^{Min/+} mice, and control *Tet1*^{+/+}*Tdg*^{+/+} (“wild type”) *Apc*^{Min/+} mice. * *P*-value < .05, ** *P*-value < .01.

Supplementary Figure 3 - Immunohistochemistry of adenomas of different genotypes stained with anti-F4/80 antibody **(A) or anti-CD3 antibody **(B)**;** the scale bar is 100 microns. **(C)** Deconvolution of differential gene expression between control *Apc*^{Min/+} adenomas and *Tet1* and *Tdg* mutant adenomas (see Fig. 4) using GEDIT (<https://webtools.mcdb.ucla.edu/>) confirms the prevalent involvement of myelomonocytic and T-cell lineages.

Supplementary Figure 4 - (A) Pie chart showing the percentage of hypermethylation and hypomethylation at CpG sites by genomic feature/location (promoter, exonic, intronic and intergenic) for comparison between *Tet1*^{-/-}*Tdg*^{+/+} (*Tet1* knockout) *Apc*^{Min/+} adenomas, *Tet1*^{+/-}*Tdg*^{N151A/+} (double heterozygous) *Apc*^{Min/+} adenomas, *Tet1*^{+/+}*Tdg*^{N151A/+} (*Tdg* heterozygous) *Apc*^{Min/+} adenomas vs. control *Tet1*^{+/+}*Tdg*^{+/+} (“wild type”) *Apc*^{Min/+} adenomas. **(B)** Examples of DNA methylation level validation by bisulfite sequencing of a CGI (*Semaf5b*) and a NCGI (*Calb2*) locus differentially methylated between *Tet1*^{-/-}*Tdg*^{+/+} (*Tet1* knockout) *Apc*^{Min/+} colonic adenomas and *Tet1*^{+/+}*Tdg*^{+/+} (“wild type”) *Apc*^{Min/+}

colonic adenomas. (C) Representative electropherograms of analysis of microsatellite instability in adenomas of the indicated genotypes, conducted for murine microsatellites A27, A33, D14MIT15, D17M123, D1MIT36. Normal colonic mucosae from C57BL/6J mice and Apc-Min mice were the reference.

Supplementary Figure 5 - (A) cBioPortal Oncoprint showing alterations of *TET1-3* and *TDG* in various CRC data sets. Each column denotes an individual tumor and each row represents a gene. The total frequency of alterations per gene is noted on the left. Colors and symbols refer to type of genomic alteration and study of origin, as indicated. **(B)** cBioPortal Oncoprint showing alterations and mRNA expression changes of *TET1-3* and *TDG* levels in TCGA COAD and READ data sets. Each column denotes an individual tumor and each row represents a gene. The total frequency of alterations and changes in expression levels is noted on the left. Colors and symbols refer to type of genomic alteration or change in expression and study of origin, as indicated.

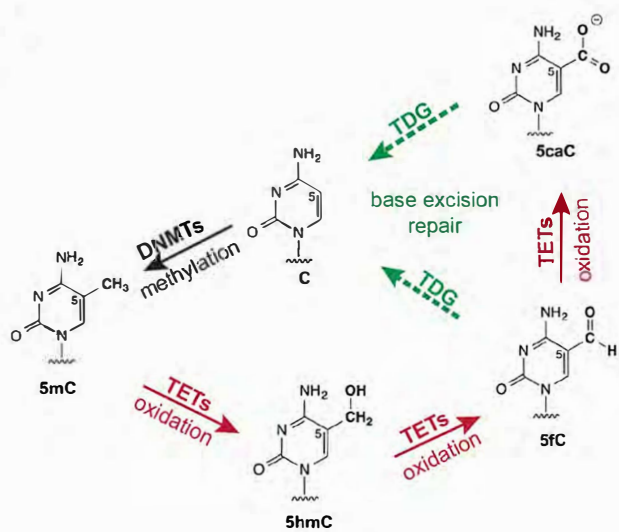
Supplementary Figure 6 - (A) Expression levels of *TET1-3* and *TDG* mRNA (z-score relative to all samples, log RNA Seq V2 RSEM) in CRC samples in TCGA COAD (n=439) and READ (n=155) data sets. **(B)** *TET1* expression by lower/upper quartile of *TDG* expression, and **(C)** *TDG* expression by lower/upper quartile of *TET1* expression in TCGA COAD and READ data sets, showing that lower *TET1* expression corresponds to lower *TDG* levels and vice versa, as indicated.

Supplementary Figure 7 - Cluster analysis of TCGA COAD (colon adenocarcinoma) cases with the 127-gene human *TET1*/*TDG*-related inflammatory signature, as in Fig. 6A, to mark clusters C1-C4 **(A)**, with the signature unique to Te1-Te1-Am adenomas which identified the MSI/CIMP cases (yellow) **(B)**, with the signature unique to Te1-Td-Am (double het) adenomas **(C)**, and with the signature unique to Td-Am adenomas **(D)**.

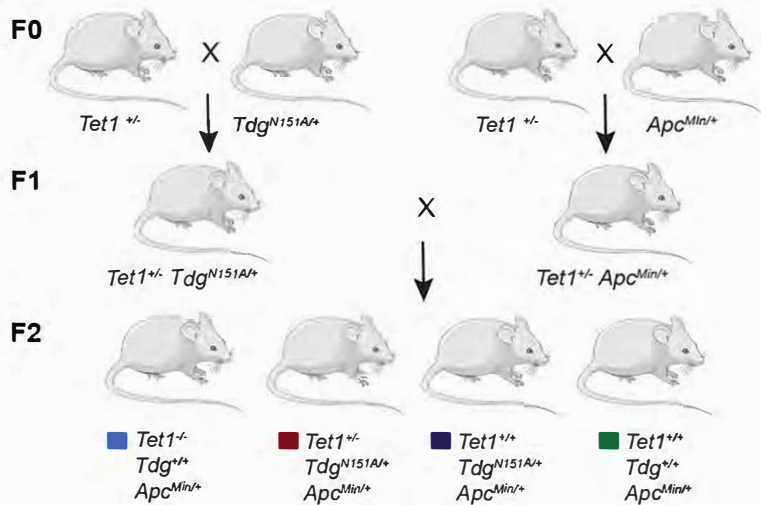
Supplementary Figure 8 - Cluster analysis of TCGA COAD (colon adenocarcinoma) cases with a 44-gene signature derived from the 127-gene inflammation set by linear discriminant analysis (LDA) followed by a leave-one-out cross validation; clusters C1-C4 are identified with a success rate of approximately 75%.

Supplementary Figure 9 - (A) Hypoxia score and (B) Female/Male ratio in TCGA COAD (colon adenocarcinoma) cases in clusters CIN-*cold* (C2) and CIN-*hot* (C4).

A

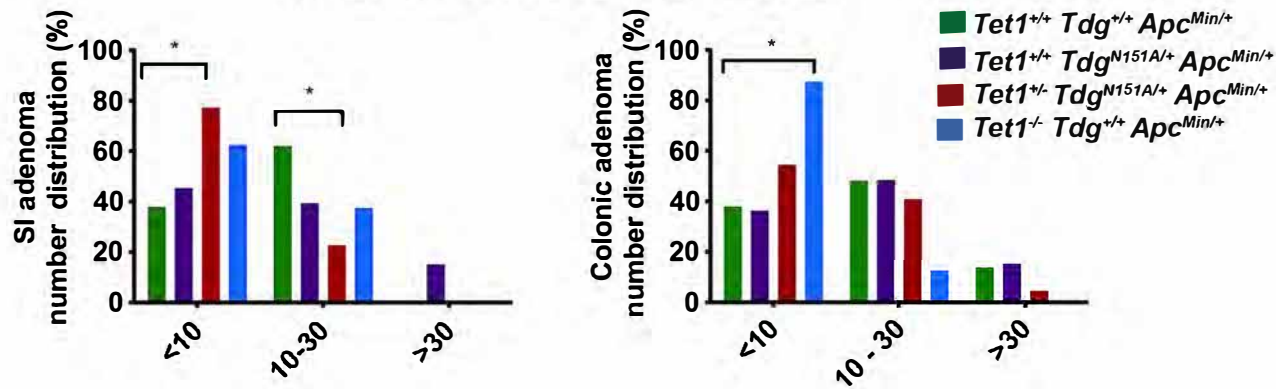


B

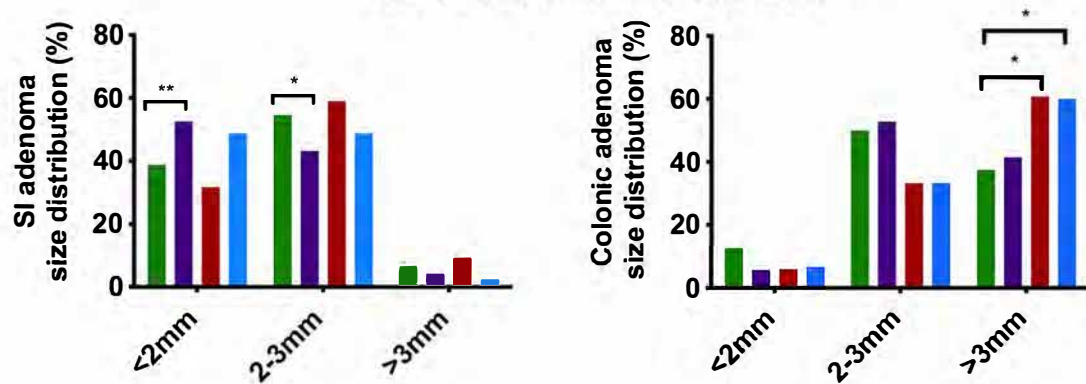


Suppl. Figure 1

A Adenoma number distribution

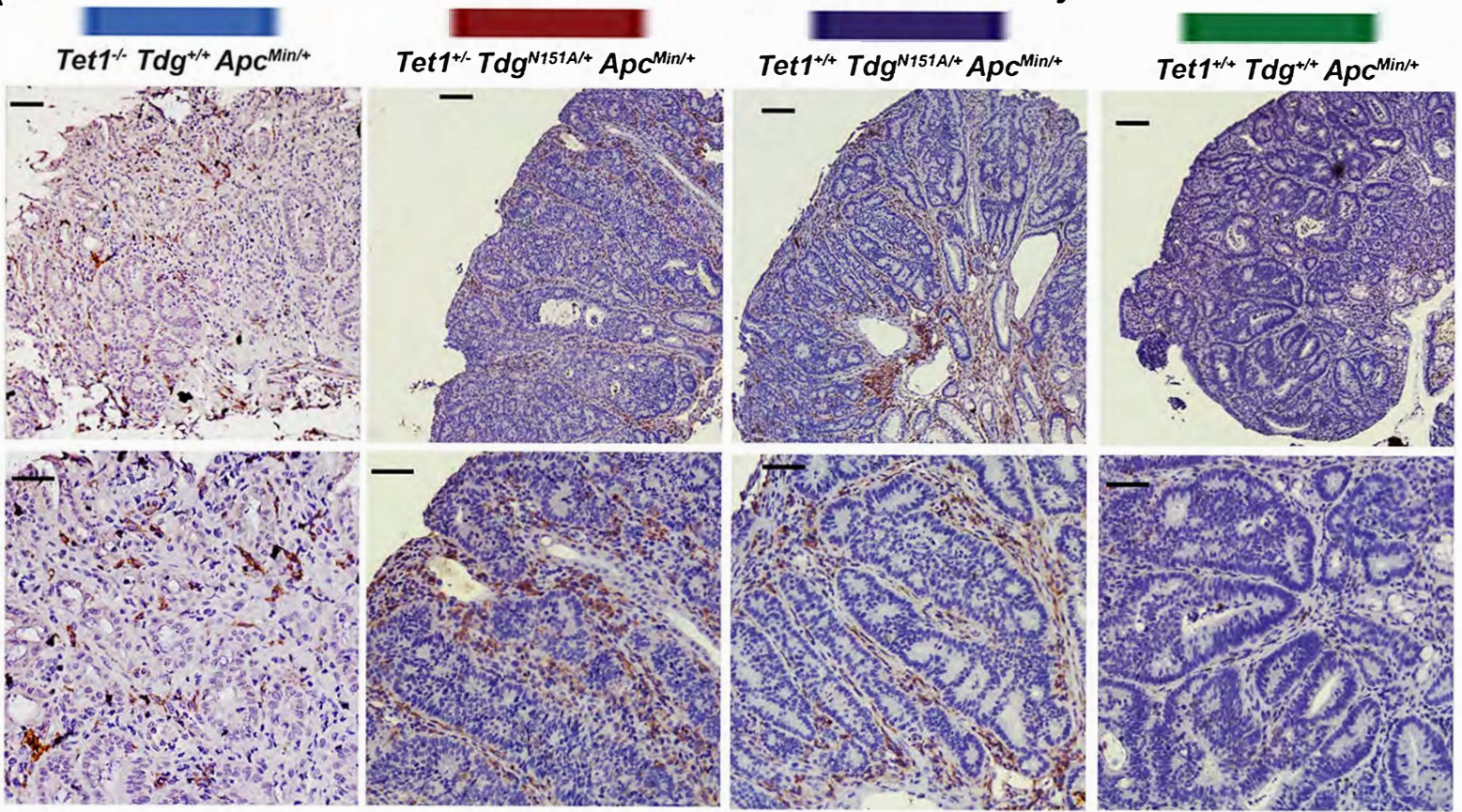


B Adenoma size distribution



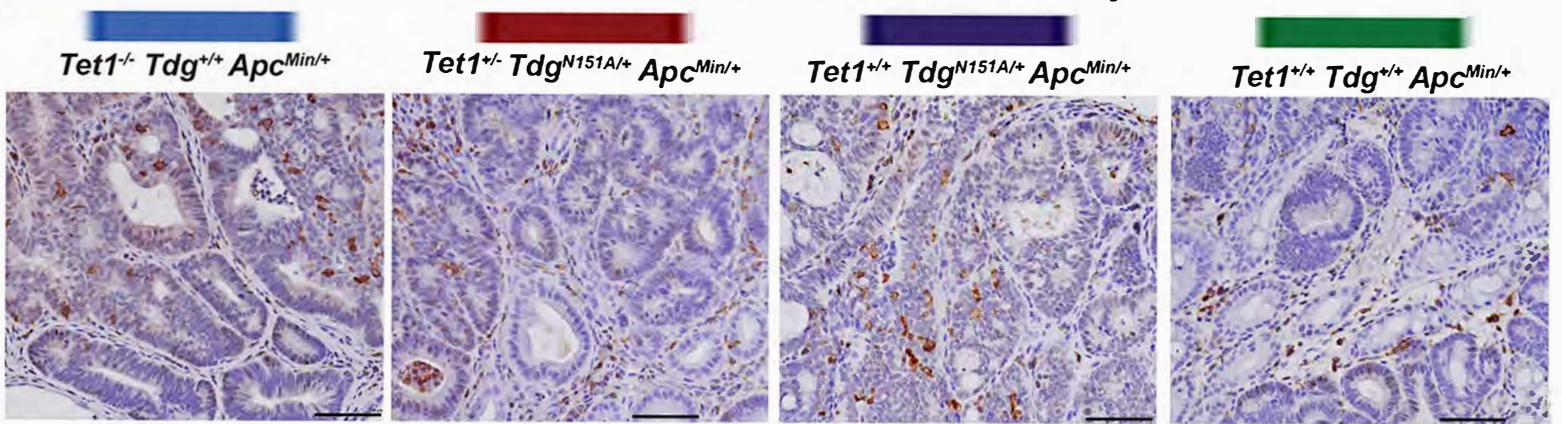
A

Anti-F4/80 immunohistochemistry



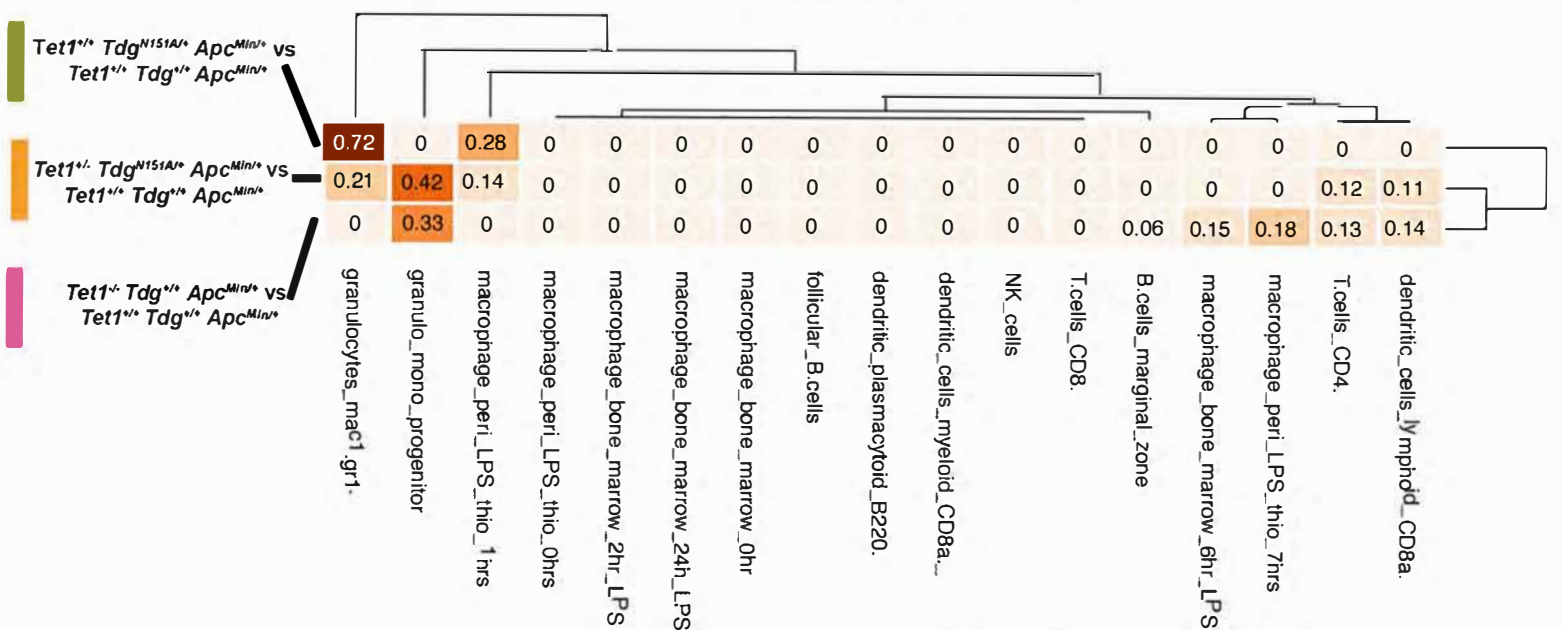
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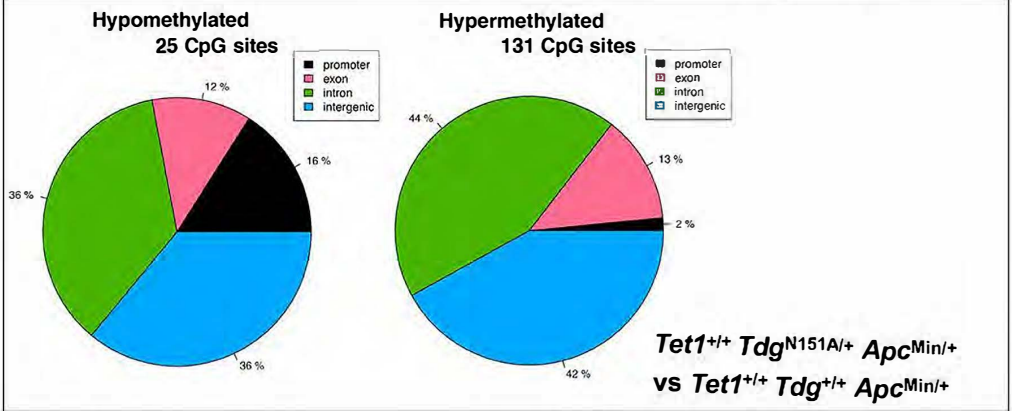
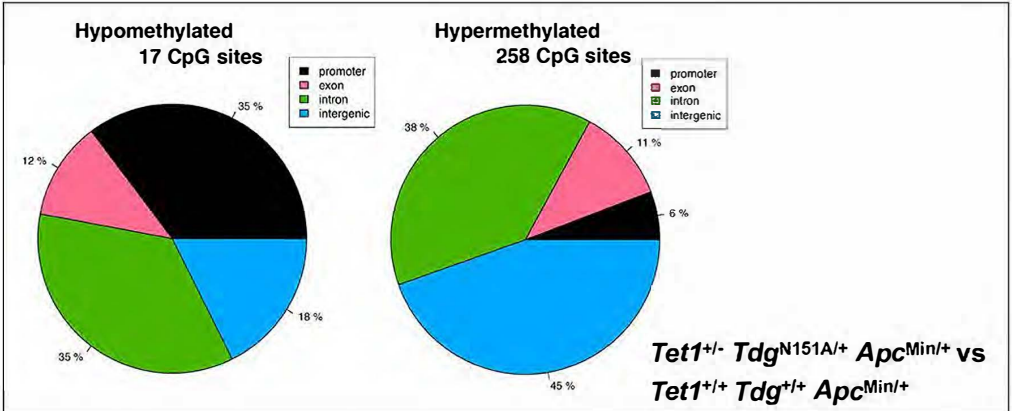
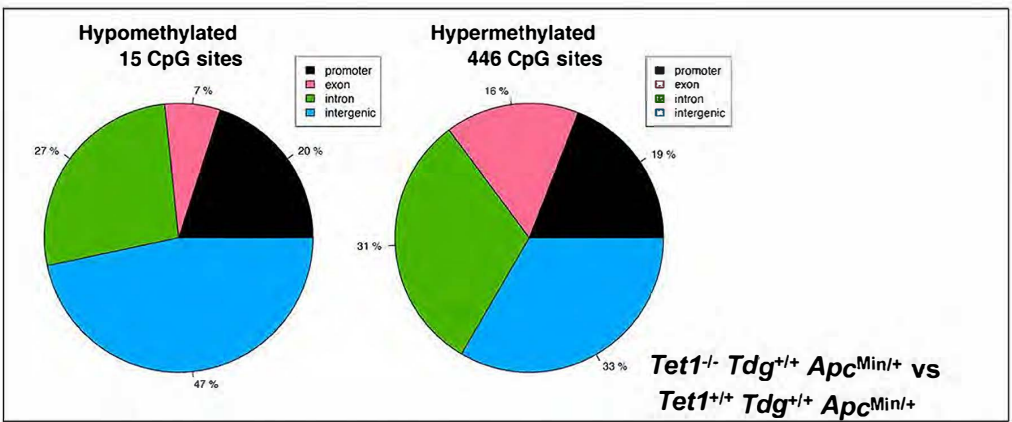
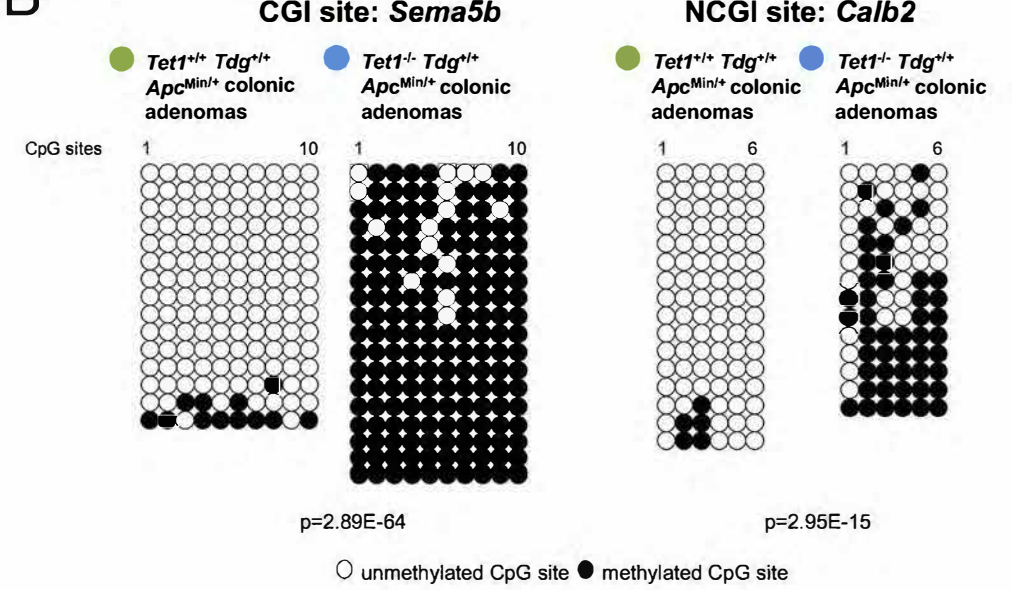
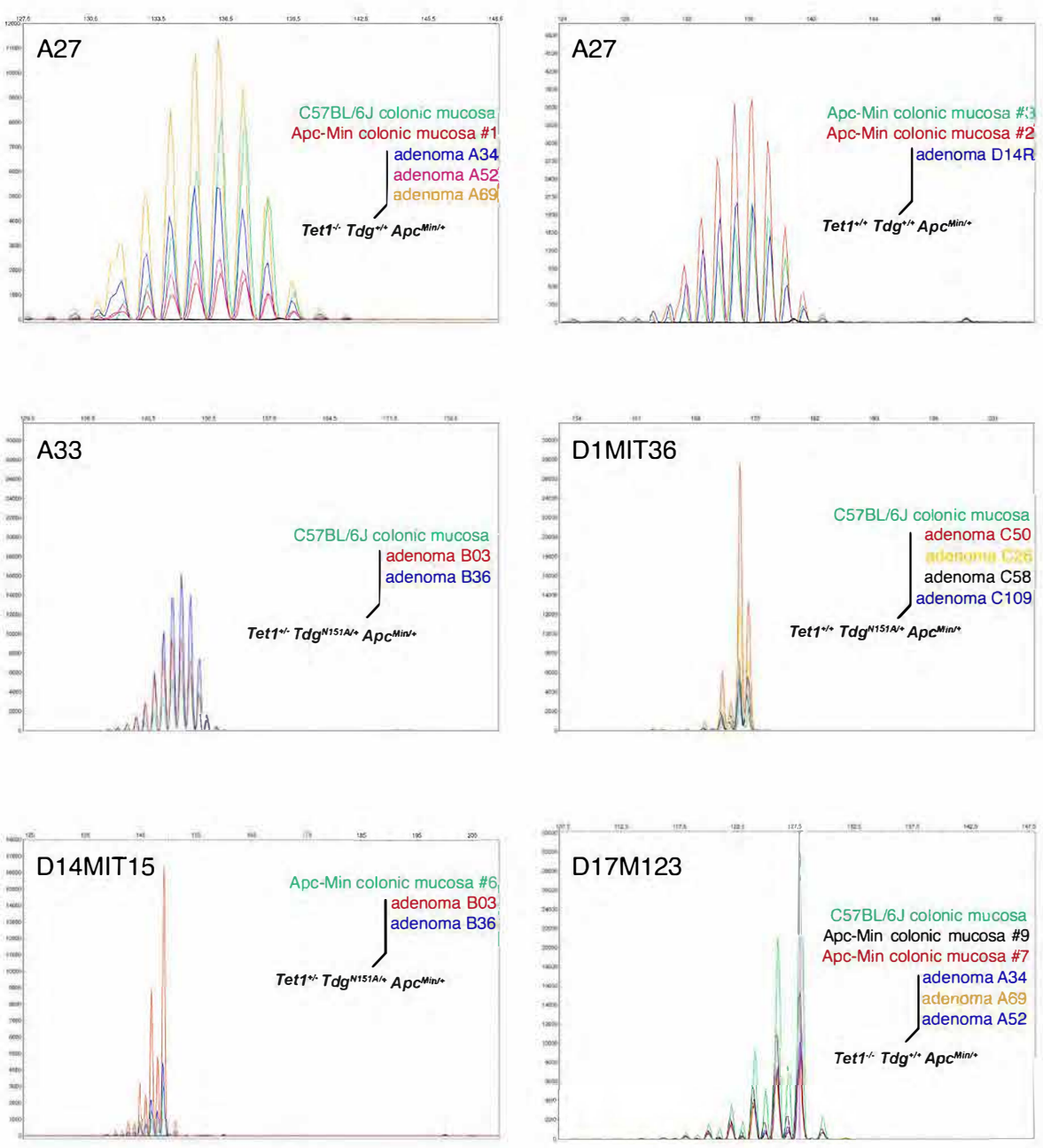
Anti-CD3 immunohistochemistry



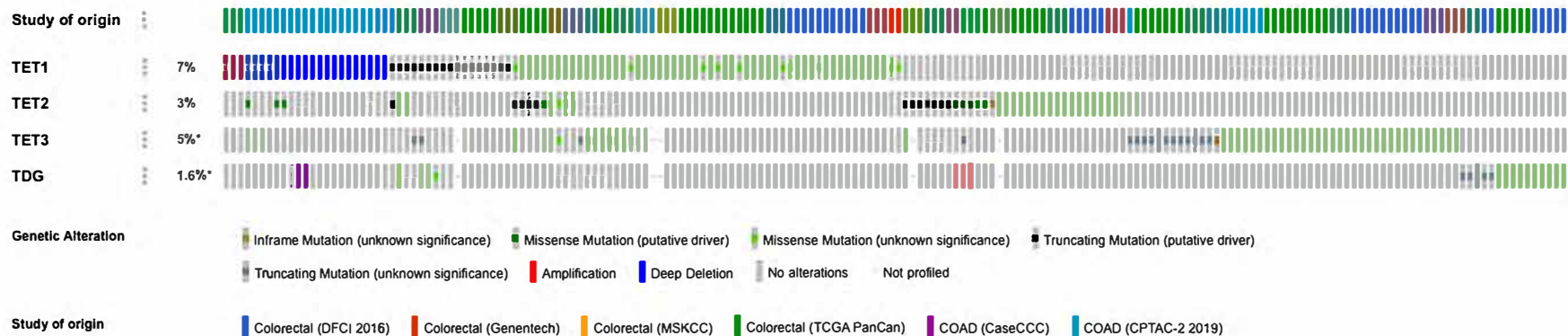
C

Deconvolution of immune cells

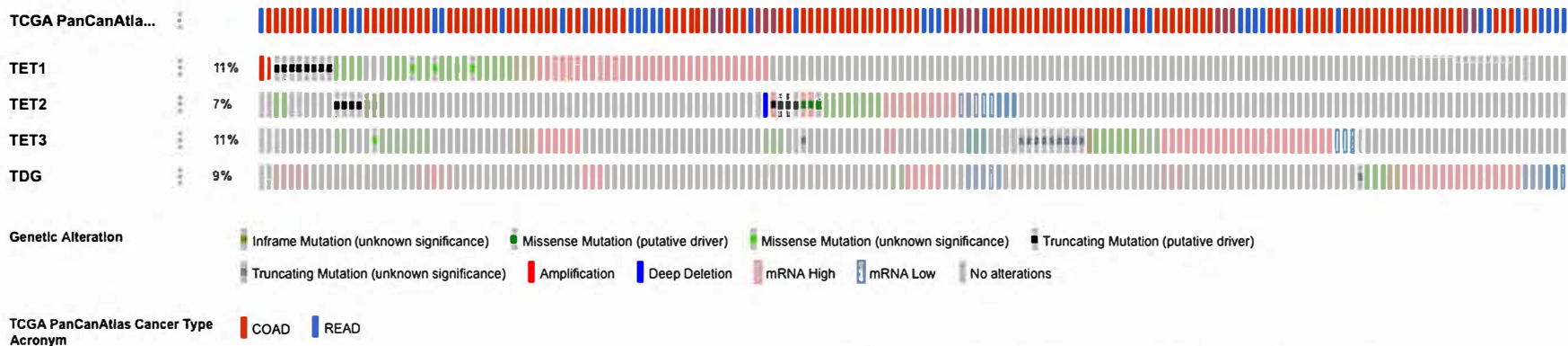


A**B****C**

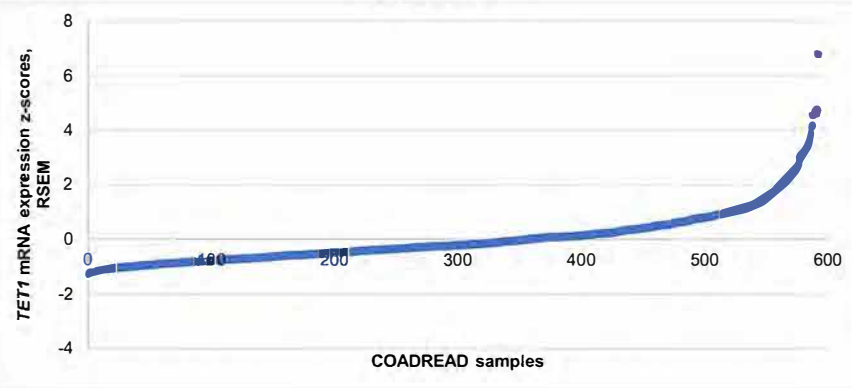
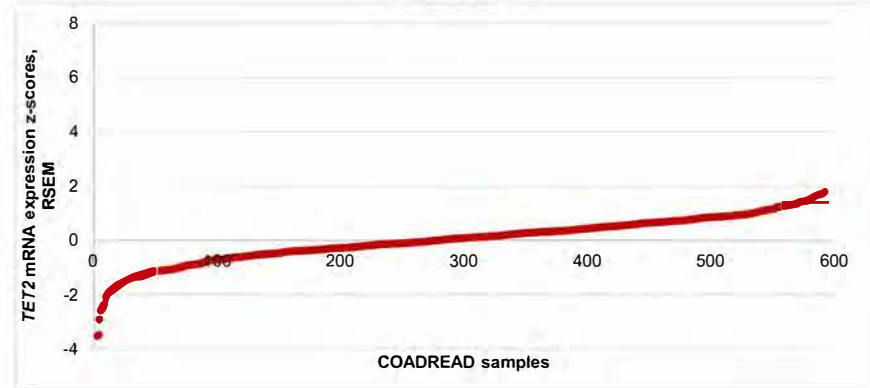
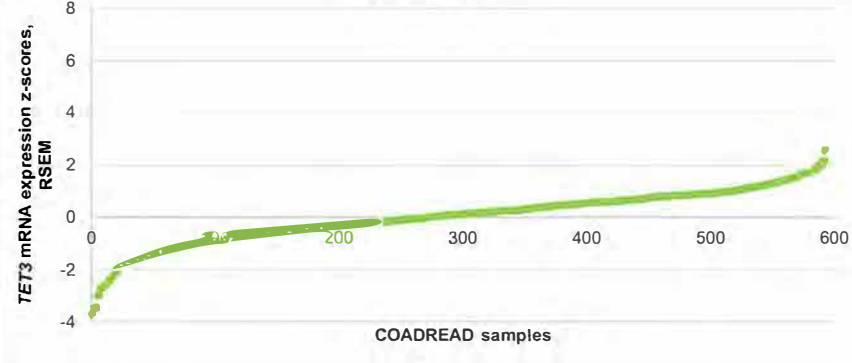
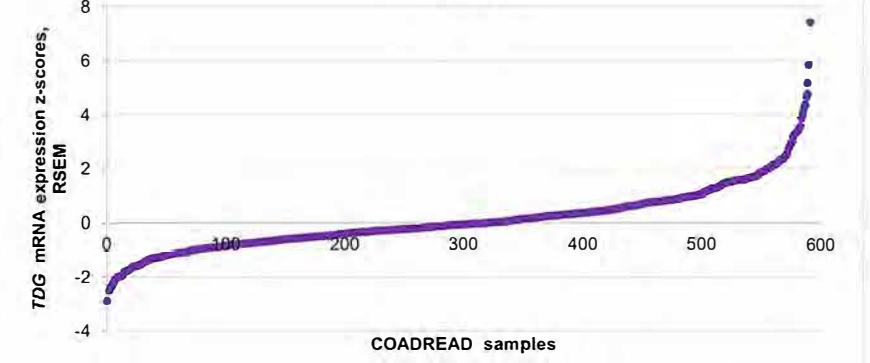
A



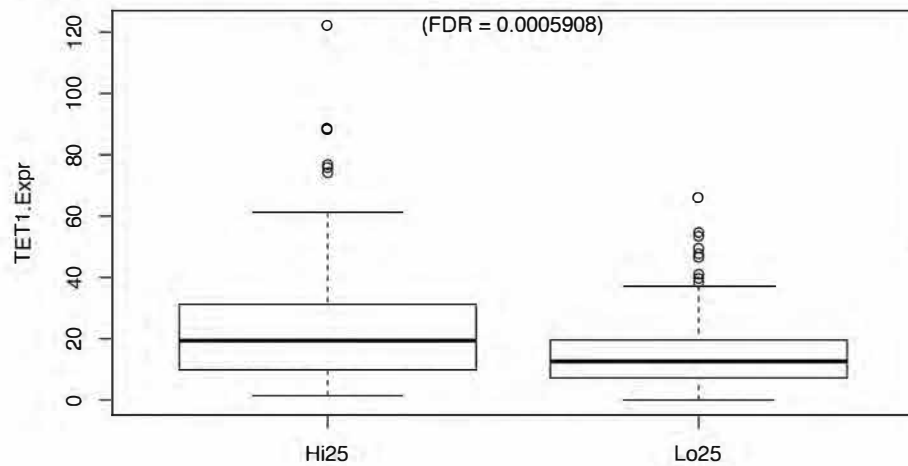
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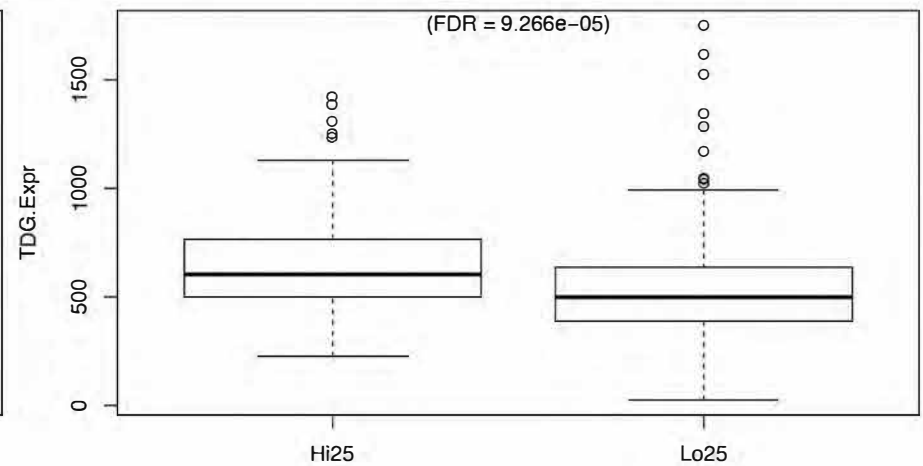
Suppl. Figure 5

A*TET1**TET2**TET3**TDG***B**

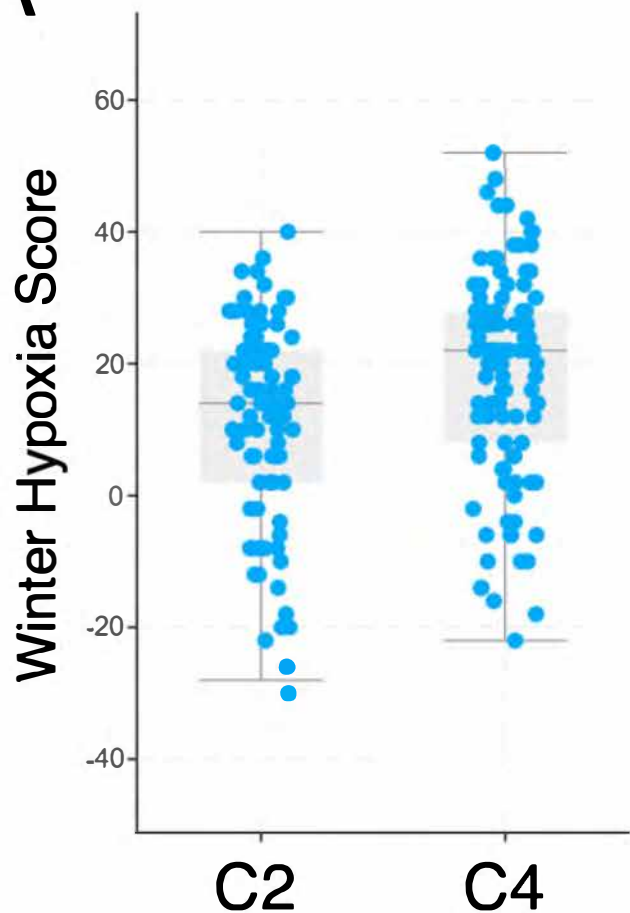
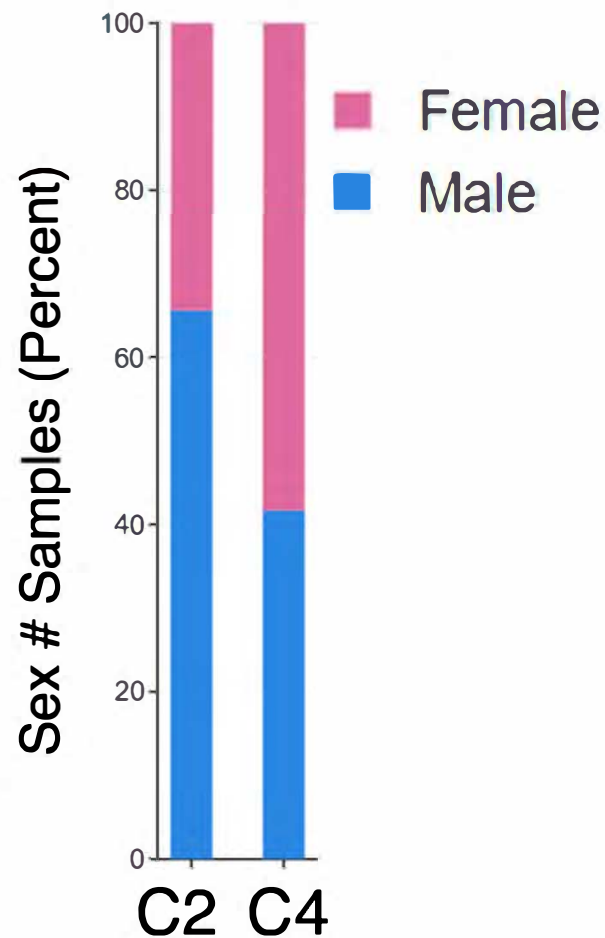
TET1.Expr by upper/lower quartile of TDG expression

**C**

TDG.Expr by upper/lower quartile of TET1 expression



Suppl. Figure 6

A**B**

	Statistical Test	p-Value	q-Value
Winter Hypoxia Score	Wilcoxon Test	9.615e-4	0.0345
Sex	Chi-squared Test	1.522e-3	0.0345

Supplemental Table 1 – WGEM-seq sequencing parameters

Colonic adenoma genotype	Total number of reads	Depth (-fold)	Percent CpG sites in mm10 covered	5x CpG coverage threshold	10x CpG coverage threshold
<i>Tet1^{-/-}Tdg^{+/+}Apc^{Min/+}</i>	187,065,022	8x	80.63%	11,826,719	1,331,939
<i>Tet1^{+/-}Tdg^{N151A/+}Apc^{Min/+}</i>	251,431,684	10x	81.70%	16,757,681	5,830,675
<i>Tet1^{+/+}Tdg^{N151A/+}Apc^{Min/+}</i>	335,808,976	14x	81.54%	17,864,331	8,105,411
<i>Tet1^{+/+}Tdg^{+/+}Apc^{Min/+}</i>	287,077,894	12x	81.58%	17,929,046	7,773,202

Supplementary Table 2 - 127-Genes Signature

Gene	Entrez
MCEMP1	199675
ACSBG1	23205
ADAMTSL4	54507
ADM	133
ALDH1A3	220
AQP8	343
ATP12A	479
CA1	759
CBR3	874
CCL3L3	414062
CCL4	6351
CES1	1066
CLCA2	9635
CLEC4D	338339
CLEC4E	26253
COL17A1	1308
CSF3	1440
CSF3R	1441
CSRNP1	64651
CWH43	80157
CXCL3	2921
CXCR2	3579
CYP2C18	1562
CYP4F2	8529
DES	1674
DHRS9	10170
DUSP1	1843
EPHX2	2053
EREG	2069
FOSB	2354
FPR1	2357
FPR2	2358
G0S2	50486
GJB4	127534
CSTA	1475
GSDMC	56169
HOGA1	112817
HP	3240
IL11	3589
IL1A	3552
IL1R2	7850
IL5RA	3568
ITGA2	3673

ITIH5	80760
KRT13	3860
LIX1	167410
MMP3	4314
MMP8	4317
MSLN	10232
NAT8L	339983
NLRP3	114548
NQO1	1728
OR51E2	81285
OLR1	4973
OSM	5008
PCDH17	27253
PLEK	5341
PRDM16	63976
PRSS22	64063
PRSS27	83886
PTGS2	5743
RDH12	145226
RET	5979
RETNLB	84666
S100A8	6279
S100A9	6280
SAMSN1	64092
SERPINA3	12
SERPINE1	5054
SLC4A11	83959
SLPI	6590
SPRR1A	6698
SPRR2D	6703
SRGN	5552
SYNM	23336
SYT8	90019
TNIP3	79931
TPPP3	51673
TREM1	54210
TRIM10	10107
TRIM15	89870
B2M	567
BST2	684
C3	718
CPLX2	10814
CTNND2	1501
CXCL9	4283
DDX60	55601

DHX58	79132
GABRP	2568
GBP2	2634
GBP4	115361
GBP6	163351
HLA-DRB5	3127
HLA-A	3105
HDC	3067
HSPA1A	3303
HSPA1B	3304
IFI44	10561
IFIT3	3437
IRF7	3665
ISG15	9636
LARS2	23395
LTF	4057
CPT1B	1375
NLRC5	84166
NOS2	4843
OAS1	4938
OAS2	4939
OAS3	4940
OASL	8638
PHF11	51131
PRAP1	118471
PSMB8	5696
RDH16	8608
RSAD2	91543
RTP4	64108
SLC38A4	55089
SP100	6672
SPP1	6696
STAT1	6772
TAP1	6890
THBS2	7058
USP18	11274
XAF1	54739
XIST	7503
ZBP1	81030

Supplementary Table 3 - Clusters C1 through C4

Patient	Cluster
TCGA-A6-5664	C1
TCGA-A6-6138	C1
TCGA-A6-6781	C1
TCGA-A6-6782	C1
TCGA-AA-3489	C1
TCGA-AD-6901	C1
TCGA-AD-6963	C1
TCGA-AD-6964	C1
TCGA-AY-6196	C1
TCGA-AZ-6607	C1
TCGA-CA-6718	C1
TCGA-CK-5914	C1
TCGA-D5-5540	C1
TCGA-D5-5541	C1
TCGA-D5-6536	C1
TCGA-D5-6540	C1
TCGA-D5-6923	C1
TCGA-D5-6924	C1
TCGA-D5-6926	C1
TCGA-D5-6927	C1
TCGA-D5-6928	C1
TCGA-D5-6932	C1
TCGA-F4-6703	C1
TCGA-G4-6628	C1
TCGA-NH-A50V	C1
TCGA-WS-AB45	C1
TCGA-3L-AA1B	C2
TCGA-4N-A93T	C2
TCGA-4T-AA8H	C2
TCGA-A6-5656	C2
TCGA-A6-5659	C2
TCGA-A6-5660	C2
TCGA-A6-5662	C2
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TCGA-A6-A5ZU	C2
TCGA-AA-3502	C2
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TCGA-AA-A02K	C2
TCGA-AD-6965	C2
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TCGA-AM-5820	C2
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TCGA-AZ-4313	C2
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TCGA-F4-6569	C4
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TCGA-NH-A50T	C4
TCGA-NH-A5IV	C4
TCGA-NH-A6GA	C4
TCGA-NH-A6GB	C4
TCGA-NH-A6GC	C4
TCGA-QG-A5YV	C4
TCGA-QG-A5YW	C4
TCGA-QG-A5Z2	C4
TCGA-QL-A97D	C4

Supplementary Table 4 - Gene Overlaps among Signatures

	Inflammatory 127-gene	Chen/Scurrah Metaplasia	Chen/Scurrah Wnt.StemCell	Leach BA.down	Leach BA	Leach BA.up	Leach WA
Inflammatory 127	127						
Chen/Scurrah Metaplasia	1	18					
Chen/Scurrah Wnt.StemCell	0	0	11				
Leach.BA.down	0	0	0	22			
Leach.BA	2	1	0	22	56		
Leach.BA.up	2	1	0	0	34	34	
Leach.WA	0	0	2	0	1	1	8

Supplementary Table 5 - Number of focal CNVs (gains or losses <10Mb)

	C2	C4	ratio	95%CI	P-val
global	4.19	3.65	1.15	(1.11 - 1.18)	0.0000
chr					
1	6.31	4.98	1.27	(1.13 - 1.42)	0.0000
2	5.63	4.34	1.30	(1.15 - 1.47)	0.0000
3	3.97	3.96	1.00	(0.87 - 1.15)	0.9833
4	5.63	5.28	1.07	(0.95 - 1.20)	0.2720
5	4.30	4.77	0.90	(0.79 - 1.02)	0.1079
6	4.39	5.99	0.73	(0.65 - 0.83)	0.0000
7	3.51	3.53	0.99	(0.86 - 1.15)	0.9378
8	7.77	6.62	1.17	(1.06 - 1.30)	0.0017
9	2.88	2.41	1.19	(1.01 - 1.41)	0.0370
10	5.10	4.77	1.07	(0.95 - 1.21)	0.2840
11	3.53	3.19	1.11	(0.96 - 1.28)	0.1685
12	3.83	3.51	1.09	(0.95 - 1.26)	0.2152
13	4.78	2.86	1.67	(1.45 - 1.92)	0.0000
14	2.69	2.38	1.13	(0.96 - 1.34)	0.1496
15	3.12	1.97	1.59	(1.34 - 1.88)	0.0000
16	3.59	3.94	0.91	(0.79 - 1.05)	0.1934
17	3.98	3.63	1.10	(0.96 - 1.26)	0.1868
18	4.61	2.85	1.62	(1.40 - 1.86)	0.0000
19	1.98	1.58	1.25	(1.02 - 1.53)	0.0281
20	6.91	4.83	1.43	(1.28 - 1.60)	0.0000
21	1.84	2.03	0.91	(0.75 - 1.10)	0.3315
22	2.37	1.20	1.97	(1.60 - 2.42)	0.0000
23	3.59	3.41	1.05	(0.91 - 1.21)	0.4961

Number of focal gains (<10Mb)

	C2	C4	ratio	95%CI	P-val
global	1.47	1.06	1.38	(1.31 - 1.45)	0.0000
chr					
1	1.89	1.13	1.67	(1.34 - 2.08)	0.0000
2	1.38	0.81	1.70	(1.31 - 2.20)	0.0001
3	0.92	0.81	1.14	(0.85 - 1.51)	0.3894
4	1.60	1.14	1.40	(1.11 - 1.77)	0.0040
5	1.00	1.10	0.91	(0.70 - 1.18)	0.4734
6	1.32	1.70	0.78	(0.62 - 0.97)	0.0263
7	1.57	1.19	1.32	(1.05 - 1.66)	0.0178
8	3.11	3.08	1.01	(0.87 - 1.18)	0.8915
9	1.04	0.45	2.30	(1.66 - 3.20)	0.0000

10	1.50	1.24	1.21	(0.96 - 1.52)	0.1071
11	1.38	0.84	1.63	(1.26 - 2.11)	0.0002
12	1.46	1.08	1.35	(1.06 - 1.71)	0.0139
13	2.98	1.17	2.54	(2.08 - 3.10)	0.0000
14	0.39	0.48	0.80	(0.53 - 1.22)	0.2990
15	0.63	0.32	1.98	(1.32 - 2.95)	0.0009
16	1.27	1.16	1.10	(0.86 - 1.40)	0.4642
17	1.68	1.23	1.36	(1.09 - 1.70)	0.0070
18	1.01	0.35	2.88	(2.01 - 4.11)	0.0000
19	0.81	0.52	1.57	(1.13 - 2.19)	0.0076
20	4.44	2.99	1.49	(1.29 - 1.71)	0.0000
21	0.50	0.39	1.28	(0.86 - 1.91)	0.2296
22	0.73	0.16	4.69	(2.85 - 7.74)	0.0000
23	1.12	1.13	1.00	(0.77 - 1.29)	0.9848

Number of focal losses (<10Mb)

	C2	C4	ratio	95%CI	P-val
global	2.72	2.59	1.05	(1.02 - 1.09)	0.0047
chr					
1	4.42	3.85	1.15	(1.01 - 1.31)	0.0403
2	4.26	3.52	1.21	(1.05 - 1.38)	0.0066
3	3.04	3.15	0.97	(0.83 - 1.13)	0.6680
4	4.03	4.14	0.97	(0.85 - 1.11)	0.7000
5	3.30	3.67	0.90	(0.78 - 1.04)	0.1497
6	3.07	4.29	0.71	(0.62 - 0.83)	0.0000
7	1.94	2.34	0.83	(0.69 - 1.00)	0.0496
8	4.66	3.54	1.32	(1.15 - 1.50)	0.0001
9	1.83	1.96	0.93	(0.77 - 1.14)	0.5020
10	3.60	3.53	1.02	(0.88 - 1.18)	0.7911
11	2.16	2.34	0.92	(0.77 - 1.10)	0.3636
12	2.38	2.43	0.98	(0.82 - 1.16)	0.8079
13	1.80	1.69	1.07	(0.87 - 1.31)	0.5346
14	2.30	1.89	1.22	(1.01 - 1.46)	0.0384
15	2.49	1.65	1.51	(1.25 - 1.82)	0.0000
16	2.32	2.78	0.83	(0.70 - 0.99)	0.0385
17	2.30	2.39	0.96	(0.81 - 1.15)	0.6676
18	3.60	2.50	1.44	(1.23 - 1.68)	0.0000
19	1.17	1.06	1.10	(0.85 - 1.42)	0.4716
20	2.47	1.84	1.34	(1.12 - 1.61)	0.0016
21	1.34	1.64	0.82	(0.66 - 1.02)	0.0811
22	1.63	1.05	1.56	(1.23 - 1.97)	0.0002
23	2.47	2.29	1.08	(0.91 - 1.28)	0.4010