Supplementary Online Content

Song N, Pogue-Geile KL, Gavin PG, et al. Clinical outcome from oxaliplatin treatment in stage II/III colon cancer according to intrinsic subtypes: secondary analysis of NSABP C-07/NRG Oncology randomized clinical trial. *JAMA Oncol*. Published online June 6, 2016. doi:10.1001/jamaoncol.2016.2314

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eTable 1. **Clinical and Pathological Features of eligible patients and patients in study cohort**

	Eligible patients N=2370		Patients in study cohort N=1729		P Value [a]
Characteristic	n	%	n	%	
Gender					
Female	1028	43.4	744	43.0	0.85
Male	1342	56.6	985	57.0	
Stage					
II	680	28.7	497	28.7	1
III	1689	71.3	1232	71.3	
NA	1	0	0	0	
Node positive					
0	680	28.7	497	28.7	0.97
1-3	1085	45.8	797	46.1	
4+	604	25.5	435	25.2	
NA	1	0	0	0	
Grade					
Differentiated	1905	80.4	1385	80.1	0.86
Undifferentiated	465	19.6	344	19.9	
Tumor stage					
T1	66	2.8	34	2	0.39
T2	222	9.4	157	9.1	
T3	1916	80.8	1420	82.1	
T4	160	6.8	118	6.8	
NA	6	0.3	0	0	
Perforation					
No	2305	97.3	1687	97.6	0.66
Yes	64	2.7	42	2.4	
Obstruction					
No	1917	81.3	1389	80.8	0.72
Yes	441	18.7	330	19.2	
NA	0	0	4	0.4	
Recurrence-free interval status					
Censored	1704	71.9	1231	71.2	0.65
Event	666	28.1	498	28.8	

[a]P Value: Associations of clinical and pathological features variable with discovery and validation cohort were analyzed by the chi-square test without correcting for missing values.

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eMethods 1. Selection of the discovery cohort in NSABP C-07

In an unpublished study, we tried to develop a predictive algorithm for oxaliplatin benefit through gene expression profiling of entire NSABP C-07 cases with WG-DASL array, which was designed to be optimized for FFPET samples. However, after we had assayed about half of the cohort in a chronological order of block submission, Illumina stopped manufacturing WG-DASL arrays. Because we did not have a good alternative technology for microarray profiling of FFPET at that time and with budget considerations, we decided to use the WG-DASL assayed cases to discover candidate prognostic and predictive genes and designed a custom nCounter probe set, which was then used to profile the entire C-07 cohort. During the entire process, those cases that were not profiled with WG-DASL remained un-linked to clinical data.

For the testing of the hypothesis that molecular subtypes interact with oxaliplatin, we considered several issues. First, although molecular subtypes were described after the design of our custom nCounter probe set and we can justify testing the hypothesis using the entire cohort, we decided not to do so because we did not know the direction of interaction. We determined that first we had to discover the direction of interaction and then validate in an independent cohort. However, access to tumor tissue bank from other oxaliplatin trials was not certain. Therefore, we decided to split NSABP C-07 into discovery and validation cohorts. Second, we thought it would be prudent to keep the original cohort separation intact because the nCounter probe set was designed based on WG-DASL data, and therefore decided to divide the C-07 cohort into discovery and validation cohorts based on whether the cases were assayed with WG-DASL or not.

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eTable 2. Clinical and Pathological Features of Discovery and Validation Cohorts: NSABP C-07

	Discovery N=848		Validation N=881		P Value [a]
Characteristic	n	%	n	%	
Gender					
Female	357	42.1	387	43.9	0.47
Male	491	57.9	494	56.1	
Stage					
II	260	30.7	237	26.9	0.09
III	588	69.3	644	73.1	
Node positive					
0	260	30.7	237	26.9	0.04
1-3	396	46.7	401	45.5	
4+	192	22.6	243	27.6	
Grade					
Differentiated	680	80.2	705	80.0	0.98
Undifferentiated	168	19.8	176	20.0	
Tumor stage					
T1	13	1.5	21	2.4	0.36
T2	71	8.4	86	9.8	
T3	709	83.6	711	80.7	
T4	55	6.5	63	7.2	
Perforation					
No	827	97.5	860	97.6	1.00
Yes	21	2.5	21	2.4	
Obstruction					
No	701	83.3	688	78.1	0.01
Yes	141	16.7	189	21.5	
NA	0	0	4	0.4	
MMR status					
pMMR	665	88.8	554	62.9	0.68
dMMR	84	11.2	76	8.6	
NA	0	0	251	28.5	
BRAF					
Wild type	716	84.4	682	77.4	0.67
Mutant	126	14.9	112	12.7	
NA	6	0.7	87	9.9	
KRAS					
Wild type	506	59.7	426	48.4	0.98
Mutant	317	37.4	269	30.5	
NA	25	2.9	186	21.1	

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NRAS					
Wild type	799	94.2	657	74.6	0.91
Mutant	20	2.4	18	2.0	
NA	29	3.4	206	23.4	
PIK3CA					
Wild type	644	75.9	569	64.6	0.16
Mutant	179	21.1	131	14.9	
NA	25	2.9	181	20.5	
Recurrence-free interval status					
Censored	601	70.9	630	71.5	0.81
Event	247	29.1	251	28.5	

[a]P Value: Associations of clinical and pathological features variable with discovery and validation cohort were analyzed by the chi-square test without correcting for missing values.
Abbreviations: MMR, mismatch repair status; pMMR, proficient MMR; dMMR, deficient MMR.

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Gene Expression Profiling Using nCounter Assay

eMethods 2. Selection of genes for nCounter code set

The 295 genes were selected for the following reasons: the genes were prognostic or predictive for oxaliplatin benefit in the C-07 discovery cohort or were part of significant pathways using C-07 WG-DASL data. We also included prognostic genes from a discovery set of NSABP C-08 WG-DASL data and C-08 nCounter data. It should be noted however, that the analytical measurement of the same gene targets by WG-DASL and nCounter are only moderately correlated, and therefore we cannot build a predictive algorithm using WG-DASL data and hope that the same algorithm will work in nCounter dataset despite the fact that same gene targets were measured. Therefore, it is meaningless to discuss WG-DASL assay results or detailed design of the nCounter probe set. Suffice it to state that WG-DASL provided a starting point to pick potential candidate predictive genes when we designed our nCounter assay.

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eTable 3. Genes Included in the Custom-Designed nCounter Assay

GENE
ABCA3
ABCC1
ABCC2
ABCC5
ABCC9
ABCD1
ABCG2
ACOT7
ACTA2
ADAM28
ADAMTS12
ADAMTS5
ADRA1B
AGPAT5
AKAP12
AKR1E2
ALDH3B2
ANKRD44
ANKRD6
ARHGEF10L
ASPRV1
ASPSCR1
ATF1
ATG9A
ATP5E
ATP7B
BAX
BCL2
BGN
BHLHE41
BMP7
BST1
BTBD11
BTG1
C13orf16
C16orf45
C17orf79
C19orf18
C19orf48
C19orf59
C1QTNF3

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C20orf103
C20orf195
C5orf4
C6orf15
C7orf44
C8orf84
CAB39L
CALB2
CASP3
CASP8
CCDC74A
CCDC85A
CCL25
CCR7
CD160
CD27
CD28
CD3D
CD4
CD8A
CDCA2
CDH23
CDK1
CDK14
CDKN2B
CGB1
CKMT2
CLEC4E
CNOT7
COL11A1
COL17A1
COL8A1
COMP
COX17
CPE
CRYAB
CSGALNACT1
CTLA4
CXCL10
CXCL11
CXCL13
CXCL2
CXCL9

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CXCR6
CXCR7
CYP1B1
CYP2C18
CYP4F2
DAPK1
DCBLD2
DENND3
DFFB
DLX5
DNER
DPEP1
DUSP10
EPB41
EPB41L4B
EPHB6
ERAP2
ERCC1
ERCC4
ERCC8
EXO1
F5
FAM178B
FAP
FCRL4
FGF19
FGL2
FN1
FNDC1
FOXC2
FOXN3
FOXP3
FREM1
FSTL5
GABRR1
GADD45B
GBP1
GBP4
GLDN
GNG12
GPX1
GPX3
GRB2

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GRM8
GSTM5
GSTP1
GUSB
GZMB
HCG9
HDAC9
HEATR8
HELB
HEYL
HGD
HIST1H3B
HIST1H3I
HJURP
HNF1B
HOXA13
HPRT1
HSD17B2
HTRA2
HYAL1
ID4
IDO1
IGFBP3
IKZF3
IL23R
IL2RA
IL2RB
IL8
INHBA
INMT
ISM2
ITGB1BP1
KCNAB1
KIAA1539
KIAA1919
KIR2DL1
KLF5
KLK4
KTN1
LEF1
LGR6
LMO3
LRRC17

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LRRC26
LRRC32
MADD
MAP6
MEG3
MFAP5
MGC50722
MGP
MKI67
MLH3
MMP11
MMP28
MRPL12
MYBL2
MYC
MYOM3
NDP
NFIB
NFYC
NKX3-2
NLRC5
NOMO2
NPIPL1
NPR3
NR6A1
NTSR1
NUP155
OAS2
OGFOD1
OR10H1
OR2T27
P2RY2
PAK4
PAPPA
PAPPAS
PCDHB12
PCDHB13
PDCD10
PDZD3
PGK1
PHF7
PIGR
PLA2G12B

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PLA2G4C
PLAG1
PLOD2
POU2AF1
PPAPDC2
PPIA
PRAP1
PRND
PROM2
PSMB9
PTGER3
PTPRC
QRICH1
RAB1A
RCC1
RECQL
REV3L
RNASEH1
RNF180
RNF39
ROBO1
RRM2
RUNX1T1
SALL4
SAP18
SDC2
SELL
SERP2
SERPINE1
SETBP1
SFRP2
SFXN5
SGK2
SH3PXD2A
SHARPIN
SLC2A12
SLC4A4
SLIT2
SMC4
SMOX
SPARC
SPP1
SRPK1

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SSPN
STC1
STMN2
STX3
SYN1
TACSTD2
TCEAL3
TCF21
TCN2
TFR2
TGFBR3
THSD1P1
TK1
TM4SF1
TMTC1
TNFRSF10D
TSHZ2
TSPYL4
TYMS
UBB
UBD
UPK1A
USP10
VCAN
VDAC2
VEPH1
VNN1
WDR27
WNT11
XRCC1
ZBTB39
ZNF135
ZNF174
ZNF205
ZNF449
ZNF451
ZNF471
ZNF576
ZNFN1A3

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eMethods 3. Analytical performance of mRNA expression profile by nCounter

Dynamic range and limit of detection for the nCounter assay were estimated from synthetic spike-in controls included with every sample. Positive controls are spiked into the reaction at concentrations from 0.125 – 128 fM, representing a fold-change of 1024. Measurements of these controls are highly linear (median $r^2 = 0.99$ across all discovery samples) in this range. Eight negative control probes, representing sequences not found in the human transcriptome, are also included in each reaction. The lowest positive control, 0.125fm, represents approximately 0.2 copies per cell, and is detected at least 2 standard deviations above the mean of the negative controls. If r^2 for the positive spikes drops below 0.95 or if the 0.125fm spike is not detected 2 standard deviations above the mean of the negative controls, the reaction is considered failed and that sample is repeated or removed from further analysis.

We also tested 11 pairs of samples as duplicate. For these duplicate samples, we calculated the correlation coefficients for the gene expression; the minimum of correlation coefficient was 0.9912, and mean was 0.9925, standard deviation is 0.0021.

To address assay reproducibility and required amount of input RNA, we first performed several samples using 100, 150, 200, and 250ng of total RNA as input. Results are shown for one representative sample in the following figure. The raw data demonstrates an almost linear increase in signal with increased input material. However, normalized data were nearly identical, demonstrating that we can use 100ng of total RNA as starting material for the nCounter assay.

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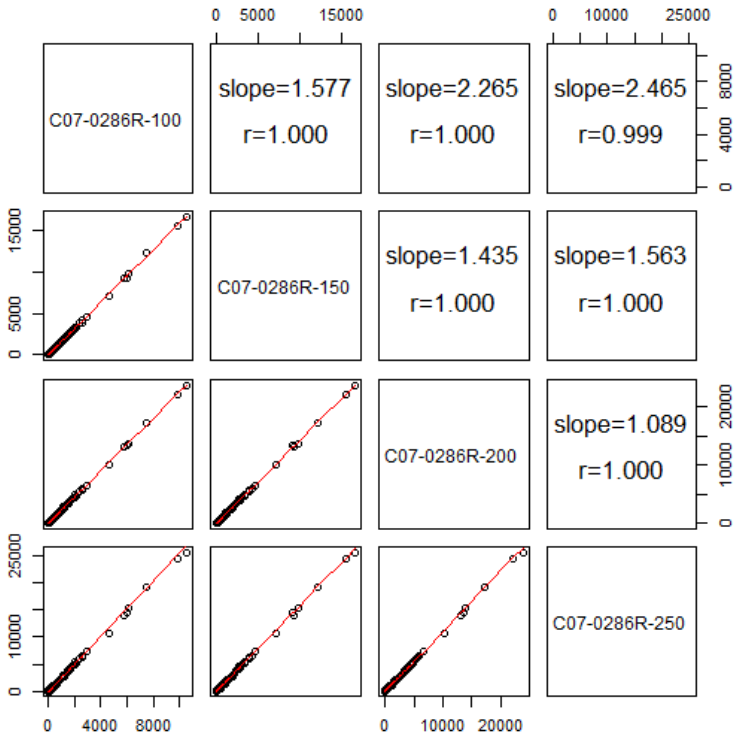
eFigure 1. Reproducibility of C-07 Sample (C-07-0286R) Measured by nCounter



This sample was tested using 100, 150, 200, and 250ng of total RNA as input.

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eMethods 4. Quality control of the nCounter data

Quality control of the data was performed using default flags in the NSolver software that is provided by the manufacturer:

1) Imaging Flag – sample removed if less than ¾ of the expected fields of view are captured by the camera (0.75 * 600 = 450 minimum FOVs)

2) Binding Density – sample removed if the binding density falls outside the range 0.05 – 2.25

3) Positive Control Flag – sample removed if the positive spikes do not follow the expected linear trend ($r^2 < 0.95$)

4) 0.5fm Detection Flag – sample removed if the 0.5fm positive control is within two standard deviations of the negative controls

5) Tech Normalization Flag – for the raw data, median(sum(pos(controls))/sum(positive control); sample removed if the technological normalization factor > 3 or < 0.3

6) Biological Normalization Flag – after adjusted by technical normalization, median (geomean(pos(house-keeping controls))/geomean(house-keeping control); sample removed if the biological normalization factor <0.1 or >10. In this data, *KIAA1539, MADD, RAB1A, C17orf79, PDCD10, NFYC* were selected as house-keeping genes.

7) If repeated measurements for an individual both pass above criteria, the lane with the lesser total counts were be removed.

There were 778 out of 848 samples (91.75%) in the discovery cohort that passed QC, and 825 out of 881 (93.64%) in the validation cohort passed QC. After preprocessing data, we normalized each tumor for technical variability with the sum of the positive controls inherent to the nCounter assay and within sample reference normalized with the geometric mean of 6 internal reference genes (*KIAA1539, MADD, RAB1A, C17orf79, PDCD10, NFYC).*

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Development of 72-gene Colorectal Cancer Assigner (CRCA) Classifier

eTable 4. Centroids for Redeveloped 72-gene Colorectal Cancer Assigner (CRCA) Classifier

	Enterocyte	Goblet-like	Inflammatory	Stem-like	TA
SLC4A4	1.099	0.1349	0.0933	-0.461	-0.4826
SFRP2	-0.2103	-0.5022	0.0497	1.0716	-0.3926
MGP	0.0509	-0.4134	-0.1745	0.8961	-0.3097
CYP1B1	-0.3509	-0.242	0.0875	0.8449	-0.3248
FNDC1	-0.2566	-0.3827	-0.0213	0.8409	-0.2125
CXCL13	0.11	-0.2356	0.8126	-0.1015	-0.4541
MFAP5	1.00E-04	-0.3641	-0.1964	0.8067	-0.2264
IDO1	-0.2064	-0.1664	0.7754	-0.2062	-0.1969
COMP	-0.4057	-0.202	-0.1175	0.7747	-0.1101
COL11A1	-0.4436	-0.3797	0.189	0.7476	-0.1898
CXCL9	-0.2303	-0.2078	0.7334	-0.006	-0.2731
CXCL10	-0.1897	-0.1776	0.7137	-0.0052	-0.302
VCAN	-0.1637	-0.3038	-0.0031	0.7104	-0.2344
GBP4	-0.1999	-0.1174	0.7101	-0.1899	-0.1937
FAP	-0.2881	-0.2525	0.1586	0.6969	-0.301
GBP1	-0.1684	-0.2325	0.6939	0.0308	-0.2952
BGN	-0.2625	-0.3363	0.1203	0.684	-0.2285
CXCL11	-0.203	-0.115	0.6691	-0.0649	-0.2545
HSD17B2	0.6687	-0.0054	0.1407	-0.3991	-0.2153
SPARC	-0.1227	-0.3431	0.0146	0.6662	-0.218
AKAP12	-0.1564	-0.3258	-1.00E-04	0.6652	-0.1946
INHBA	-0.3224	-0.2377	0.1021	0.6646	-0.2233
CDKN2B	0.6162	-0.4035	-0.0118	0.222	-0.2867
C8orf84	-0.4073	0.5791	-0.0724	-0.1541	0.0682
PTPRC	0.0416	-0.252	0.5649	0.1694	-0.4187
FN1	-0.2859	-0.4114	0.1302	0.5476	-0.0764
SDC2	-0.0982	-0.2924	-0.0399	0.5358	-0.1257
SSPN	-0.0575	-0.0801	-0.1318	0.5308	-0.206
NFIB	0.0535	-0.0317	-0.5168	0.0032	0.3672
GRM8	-0.0273	-0.1092	-0.3808	-0.2021	0.5153
PLA2G12B	-0.0591	-0.2634	-0.3123	-0.1001	0.5015
PSMB9	-0.1726	-0.0019	0.4983	-0.2105	-0.1072
VNN1	0.0753	0.2341	0.4957	-0.2096	-0.4016
DPEP1	-0.1993	0.0196	-0.4644	-0.0446	0.4881
SGK2	0.2445	-0.2138	-0.4084	-0.2707	0.4818
CAB39L	-0.0904	-0.2837	-0.4717	0.1316	0.4798
CPE	0.091	0.1071	-0.4766	0.2741	0.0311
TYMS	-0.1895	0.1904	0.4728	-0.3805	-0.0701

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MMP11	-0.1743	-0.2439	-0.1581	0.462	0.0318
PRAP1	-0.0074	-0.2188	-0.4551	0.0767	0.4181
PIGR	0.2984	0.2771	0.2527	-0.4536	-0.2038
GPX3	0.0253	-0.1816	-0.0432	0.4493	-0.203
OAS2	-0.1136	0.0199	0.4392	-0.0611	-0.2234
DAPK1	-0.2495	0.3342	0.3441	0.1757	-0.4336
SPP1	-0.3134	-0.1681	0.4032	0.4321	-0.3238
GZMB	-0.2513	-0.4275	0.3314	-0.167	0.2865
MMP28	0.4253	0.2425	0.1082	-0.1931	-0.3441
ID4	-0.1789	0.0325	-0.2977	0.4248	0
ERAP2	-0.0712	0.0416	0.4002	-0.1821	-0.1445
SERPINE1	-0.1613	-0.116	0.0757	0.3678	-0.1574
EPB41L4B	0.1808	-0.0063	-0.2888	-0.3419	0.3594
PAPPA	-0.0641	-0.2033	0.0437	0.3593	-0.1342
POU2AF1	0.3543	0.2138	0.147	-0.1889	-0.3155
CKMT2	0.0206	-0.2473	-0.2254	-0.0649	0.3515
HGD	0.2535	0.3355	-0.0656	-0.3365	-0.0593
DCBLD2	-0.2662	-0.1791	0.1036	0.335	-0.0522
IL8	-0.166	-0.0459	0.3226	0.0624	-0.156
FGL2	0.1732	-0.1883	0.3211	0.1058	-0.3093
STC1	-0.3185	-0.1163	0.2305	0.2822	-0.1135
BHLHE41	0.1079	0.075	0.0015	0.2663	-0.3092
ROBO1	-0.1221	-0.2991	0.0818	0.2661	-0.0014
PROM2	0.2046	0.1593	-0.0655	-0.2981	0.0462
CSGALNACT1	-0.2585	-0.0478	-0.0254	0.2363	0.0324
SELL	0.0788	-0.1222	0.2456	0.0016	-0.1589
BMP7	-0.2046	-0.0645	-0.143	0.04	0.2408
ANKRD44	0.0701	-0.1685	0.2335	0.0874	-0.1798
CXCL2	-0.1416	0.0466	0.1917	-0.2284	0.0826
HOXA13	-0.0301	0.1091	-0.0917	-0.2152	0.1795
TACSTD2	-0.0954	0.2113	-0.0379	0.109	-0.1196
F5	-0.2002	0.0195	0.2094	-0.0196	-0.03
TM4SF1	-0.1527	-0.1754	-0.0883	0.0963	0.1938
TGFBR3	-0.1721	-0.0829	-0.016	0.04	0.1378

Abbreviations: TA, transit amplifying.

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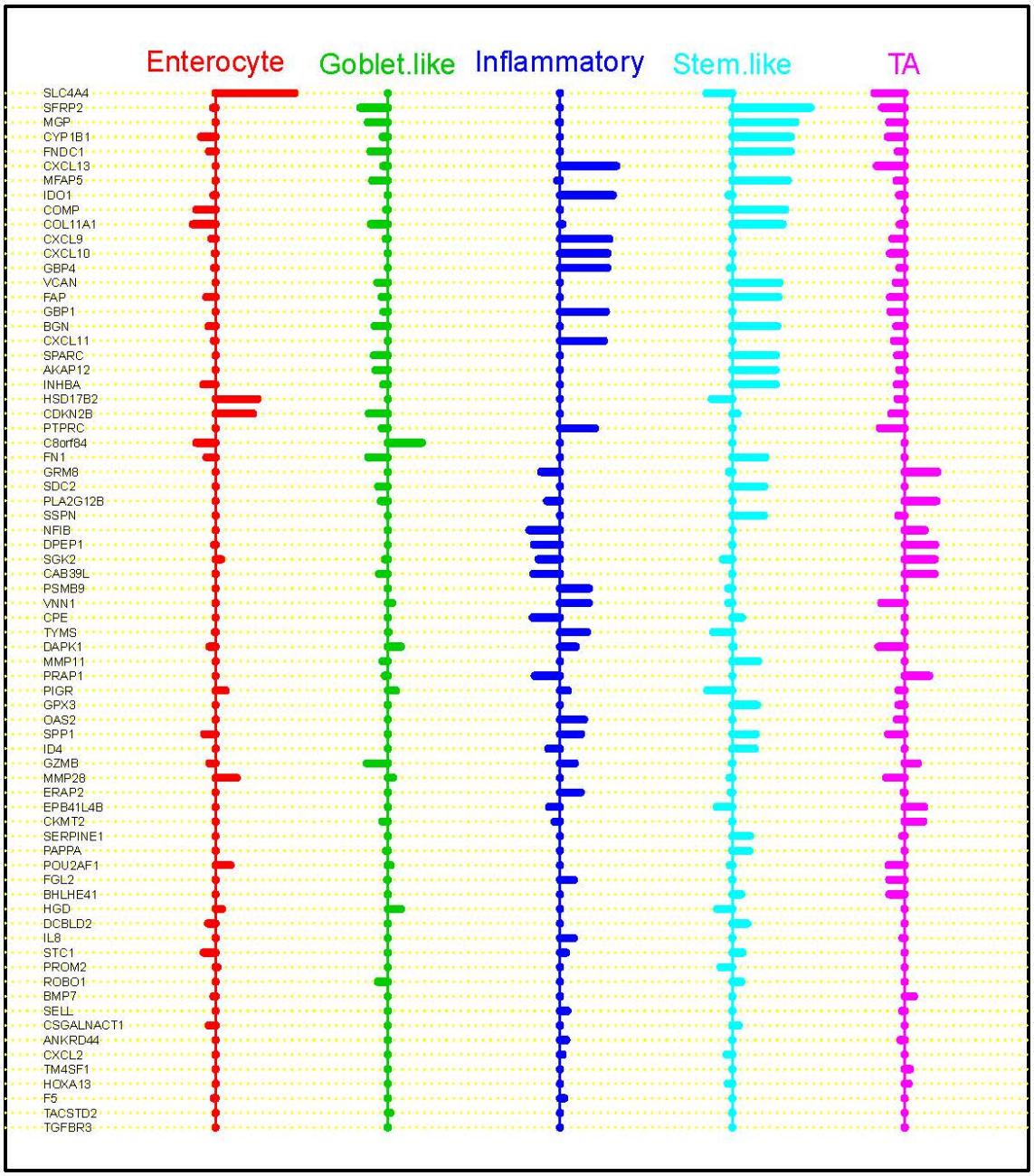
eFigure 2. Graphical Presentation of Centroids for Redeveloped 72-gene Colorectal Cancer Assigner (CRCA) Classifier



Abbreviations: TA, transit amplifying.
Enterocyte and goblet-like subtype classifier genes are under-represented among 72 genes.

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eMethods 5. Cross-validation error of redeveloped colorectal cancer assigner classifier

To evaluate the performance of re-developed colorectal cancer assigner classifier, we used 10-fold cross-validation. Specifically, the core training dataset (N=387), which was used by Sadanandam to develop the original CRCA classifier and used in this study to redevelop the centroid, were randomly divided into 10 approximately equally-sized parts. For each part in turn, the classifier is built on the other 9 parts then tested on the remaining part. Cross-validation error was evaluated by comparing prediction with the original subtype assignment by Sadanandam. Analysis was carried out using the pamr packages implemented in R.

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eTable 5. Cross-Validation Error Rate of Redeveloped 72-gene Colorectal Cancer Assigner (CRCA) Classifier Compared to the Original Subtype Assignment by Sadanandam et al[1]

	Enterocyte	Goblet-like	Inflammatory	Stem-like	TA	Classification Error Rate
Enterocyte	46	8	6	2	2	0.285
Goblet-like	1	44	6	6	6	0.302
Inflammatory	4	1	68	3	2	0.128
Stem-like	0	0	3	73	1	0.052
TA	1	2	3	4	95	0.095
Overall error rate = 0.157						

Abbreviations: TA, transit amplifying.

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Development of a 166-gene Colon Cancer Subtypes (CCS) classifier

eMethods 6. Colon Cancer Subtypes (CCS) assignment for C-07 samples

We redeveloped a classifier using the core training dataset in which the original Colon Cancer Subtypes (CCS) classifier was originally discovered (N=90) because only 10 out of 146 genes in the CCS classifier were included in our nCounter code set (De Sousa, Wang, et al. 2013).[2] We generated the centroid for each subtype using Prediction Analysis of Microarray (PAM) method (Tibshirani, Hastie, et al. 2002).[3] PAM ranks genes using a penalized t-statistic, and identifies a set of genes (n=166) for classification; the number of genes is selected by 10-fold cross validation. The redeveloped centroids of the CCS dataset are shown in **eTable 5.** The analysis was done using pamr packages implemented in R. To predict subtype for a single sample, we calculated the spearman rank correlation between each sample and the value of centroids for each subtype and assigned the sample to the most correlated subtypes.

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eTable 6. Centroid for Redeveloped Colon Cancer Subtypes (CCS) Classifier

	CCS1	CCS2	CCS3
TYMS	-0.0984	0.5169	-0.0909
KCNAB1	-0.0714	-0.188	0.4959
SLIT2	-0.1298	-0.0624	0.4879
NPR3	-0.1243	-0.0646	0.4797
SSPN	-0.0799	-0.1464	0.4734
CRYAB	-0.1322	-0.0404	0.472
CYP1B1	-0.251	0	0.4669
BHLHE41	-0.203	0	0.4667
INHBA	-0.2638	0	0.4647
ABCC9	-0.1994	0	0.4485
MFAP5	-0.1205	-0.0341	0.4448
CDK14	-0.23	0	0.4327
COMP	-0.0998	-0.0544	0.4256
PTGER3	-0.0253	-0.2035	0.4255
RUNX1T1	-0.0126	-0.2273	0.4241
FAP	-0.2584	0	0.4224
AKAP12	-0.1276	0	0.4198
ANKRD6	-0.0836	-0.0787	0.418
PRND	-0.1053	-0.0347	0.4176
SPARC	-0.1138	0	0.3982
ACTA2	-0.0629	-0.0976	0.3974
VCAN	-0.155	0	0.3933
ADAMTS5	-0.1511	0	0.3877
CALB2	-0.2368	0	0.3866
SDC2	-0.0698	-0.0699	0.3846
C1QTNF3	-0.0397	-0.1246	0.3796
EXO1	-0.0728	0.3747	-0.0074
C16orf45	-0.0489	-0.0985	0.3727
TFR2	-0.1148	0.3714	0
MGP	-0.0619	-0.0663	0.367
CPE	0	-0.3537	0.3598
MRPL12	0.0504	0.077	-0.3557
GADD45B	-0.1886	0	0.3519
MYC	0.234	0	-0.3508
COL8A1	-0.1035	0	0.3501
EPB41L4B	0.3301	-0.2997	-0.1834
BGN	-0.1726	0	0.3287
SGK2	0.2881	-0.3267	-0.0816
STMN2	0	-0.3233	0.1973
COL11A1	-0.1614	0	0.3216
BST1	-0.0916	0	0.3151

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MEG3	-0.0476	-0.0338	0.311
ID4	0	-0.306	0.2529
CAB39L	0.1237	-0.3047	0
GRM8	0.2437	-0.3031	-0.0218
SRPK1	0.0883	0	-0.3026
SPP1	-0.2998	0.1769	0.2403
NFYC	0	0.2921	-0.1759
TK1	0	0.2907	-0.2069
DAPK1	-0.2119	0	0.2866
SETBP1	-0.0212	-0.0584	0.285
PAK4	0.071	0	-0.2837
GPX3	-0.1314	0	0.281
SERPINE1	-0.2553	0.0445	0.2802
RCC1	0	0.2792	-0.0595
STX3	0.1651	0	-0.2716
AGPAT5	0.0384	0.0074	-0.2699
HJURP	0	0.2697	-0.1731
LRRC17	0	-0.0785	0.2598
FN1	-0.0473	0	0.2578
RRM2	0	0.2487	-0.2178
ABCC5	0.0534	-0.2445	0
CASP3	0	0.2392	-0.1595
PPIA	0.1088	0	-0.2344
DCBLD2	-0.0757	0	0.2328
LRRC32	0	-0.2257	0.2317
ZBTB39	0.0693	0	-0.23
MKI67	0	0.2275	-0.1438
PIGR	0	0.0591	-0.2207
GBP1	-0.1401	0.22	0
TNFRSF10D	0	0.2131	-0.033
WNT11	0.1074	-0.212	0
IL8	-0.2046	0.2104	0.0351
TACSTD2	-0.0463	0	0.2098
LMO3	0	-0.0811	0.2046
DPEP1	0.1811	-0.2044	0
CSGALNACT1	-0.0774	0	0.2039
GNG12	0	-0.2032	0.0443
HDAC9	0	-0.0164	0.2022
HTRA2	0	0.1967	-0.0077
DUSP10	-0.1443	0.1899	0
F5	-0.1898	0.0799	0.1276
EPB41	0	0.1185	-0.1878
SH3PXD2A	0	-0.0673	0.1847

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SELL	-0.1843	0.1418	0.0608
P2RY2	0.1843	-0.0564	-0.139
STC1	-0.1833	0.0299	0.1615
CDK1	0	0.1804	-0.11
QRICH1	-0.0024	0.1802	0
CXCL10	-0.1099	0.179	0
HNF1B	0.0826	0	-0.1784
PSMB9	0	0.1749	0
CNOT7	0	0.0056	-0.1727
VDAC2	0	0.1709	-0.1112
ABCA3	-0.0737	0.1641	0
CXCL9	-0.0618	0.146	0
VNN1	-0.1405	0	0.1231
PDZD3	0.1367	-0.0991	-0.0127
TCF21	0	-0.1366	0.0047
RNASEH1	0.0097	-0.1348	0
OAS2	-0.0955	0.1347	0
CXCL11	-0.0518	0.1337	0
PHF7	0	0	-0.1331
CD28	-0.0939	0	0.1313
IL2RB	-0.055	0.1269	0
MYBL2	0.0898	0	-0.1264
CKMT2	0.1231	-0.0215	-0.0589
HYAL1	-0.1213	0.0994	0
PTPRC	-0.1166	0	0.0799
DFFB	0	0.1147	-0.0626
SMC4	0	0.1131	-0.0067
SAP18	0.0124	-0.1084	0
REV3L	-0.1066	0.0446	0.0074
ZNF576	0.1065	-0.0033	-0.0451
COL17A1	0	-0.1035	0
CXCL13	-0.1022	0.0923	0
ATP5E	0.1018	-0.0374	-0.0053
UBB	-0.0614	0.0973	0
IGFBP3	-0.0467	0	0.0947
ABCC1	0.0393	0	-0.0939
CLEC4E	-0.0928	0	0.0806
TSPYL4	0	-0.091	0.0505
IDO1	-0.004	0.0803	0
DLX5	0	0	0.0774
GRB2	-0.0773	0.0452	0
ADAM28	-0.021	0	0.0754
ABCC2	0.0445	-0.0725	0

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BTG1	-0.0706	0	0.0409
SLC4A4	0	0.0694	0
PAPPA	-0.0665	0.0275	0
MLH3	-0.0657	0	0.0143
ASPSCR1	9.00E-04	0	-0.0649
PLA2G4C	0	0	0.0646
ATP7B	0.0636	0	0
CXCL2	0	0.0596	-0.062
CD8A	-0.0608	0.0203	0
ABCG2	0	0	0.0562
CASP8	0.0562	0	-0.0448
BAX	0.001	0	-0.0514
IL2RA	-0.0454	0.0478	0
ACOT7	0	0.0477	-0.0061
NTSR1	-0.0453	0	0.0399
TGFBR3	-0.0444	0	0.0107
HCG9	0	0	0.0418
RECQL	0	0	0.0392
TCN2	-0.0379	0	0
BMP7	0	-0.0371	0.0127
MMP28	-0.0361	0	0
ERAP2	-0.0014	0.0329	0
LEF1	0	0	0.0318
GABRR1	0.0289	0	0
CTLA4	0	0.0246	0
MMP11	0	-0.0241	0
CCR7	-0.0209	0	0
PLAG1	0	-0.0141	0.0204
ZNF205	0.0173	0	0
NFIB	0	0.0168	0
FGL2	0	0	0.0166
ROBO1	-0.0151	0	0
PLOD2	0	0	0.0127
NUP155	0	0	-0.011
DENND3	-0.0077	0	0
CD4	-0.0064	0	0
GZMB	0	0	-0.0032
EPHB6	0	0.0017	0
HPRT1	0	9.00E-04	0

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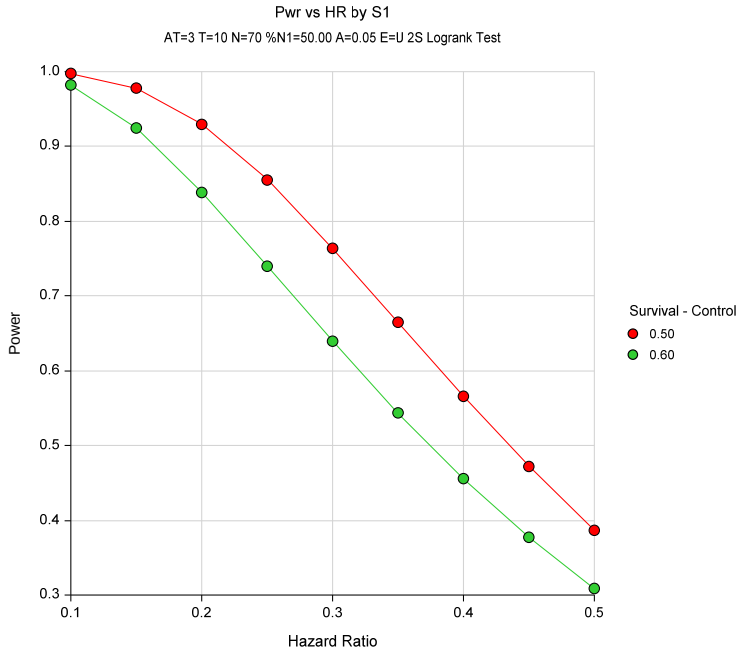
Statistical Power Analyses

eMethods 7. Power calculation of oxaliplatin benefit in enterocyte subtype

Logrank test was used to test the null hypothesis and assumes that there is no difference between the clinical outcome for enterocyte patients treated by FULV or FLOX. The test was performed at a 0.05 significance level. The accrual time was 2.8 years and the follow-up time is 10 years. The sample size is 70. We calculated power by varying the Hazard Ratio (HR) (0.2, 0.5), and a control survival proportion at 10 years of (0.5, 0.6). The following figure graphs power versus HR.

Power of Oxaliplatin Benefit in Patients with the Enterocyte Subtype



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eMethods 8. Power calculation of enterocyte subtype as predictive biomarker

For the primary aim, enterocyte was classified as a benefit group, and other subtypes were classified as a non-benefit group. We tested the null hypothesis that there is no interaction between treatment and the genomic predictor using a two-arm survival interaction test with the method developed by Peterson and George[4] using the SWOG webtool (http://www.swogstat.org/stat/public/int_survival.htm).

With the assumption of exponential distribution, the formula for power calculation is as follows:

$$Z_{1-\beta} = \sqrt{N(log\Delta)^2 / \sum \frac{1}{(e_{ij}f_{ij})}} - Z_{1-\propto/2}$$

in which N is the sample size; f_{ij} are the frequencies in treatment *i* (FLOX or FULV) in stratum *j* (*e.g.* benefit group or non-benefit group); e_{ij} are the event probabilities in treatment *i* and stratum *j*, with the exponential failure assumptions, e_{ij} is calculated using the accrual, minimum follow-up, competing risk, and the hazard rate λ_{ij} where λ_{ij} represent hazard rate in treatment *i* and stratum *j*, λ_{ij} can be inferred from hazard rate λ_i and frequency of genomic predictor (f_j) $\lambda_i = \sum \lambda_{ij} * f_j$, and Δ is defined as $\Delta = \frac{\Delta_1}{\Delta_2} = \frac{\lambda_{11}/\lambda_{21}}{\lambda_{12}/\lambda_{22}}$ (the ratio of hazard ratios). In this analysis, treatment refers to FLOX or FULV, and stratum refers to patients stratified by the predictor. For power consideration, we need to specify variables α, N, f_{ij}, Δ, accrual, minimum follow-up, competing risk, and λ_{ij}.

In C-07, accrual time was 2.8 years, minimum follow-up is 10 years, and competing risk hazard of death is 0.0069 deaths per patient-year, the hazard rate for FLOX is 0.0245 and the hazard rate for FULV is 0.0291. In the C-07 validation cohort, 606 stage III patients have been successfully profiled and assigned to subtypes, among them, 70 (12%) are enterocyte, and 536 (88%) belong to other subtypes. We also calculated power by varying λ_{11} and $\lambda_{21}$to have the values of (0.020, 0.022, 0.024, 0.026, 0.028, 0.03, 0.032, and 0.034). The following figure shows how the power changes with the differing assumptions with regard to the degree of oxaliplatin benefit in the different groups or specifically in differing values for Δ (the ratio of hazard ratio) including HRs for the non-benefit group ranging from 0.9 to 1.1 and HRs for the benefit group ranging from 0.25 to 0.7. In the current validation cohort, the Δ was approximately 0.55, resulting in a power of less than 0.4. If the Δ had been 0.4, then the power would still have been less than 0.7.

Power of Detecting a Difference in Benefit from Oxaliplatin in Patients with an Enterocyte Subtype versus Other Subtypes

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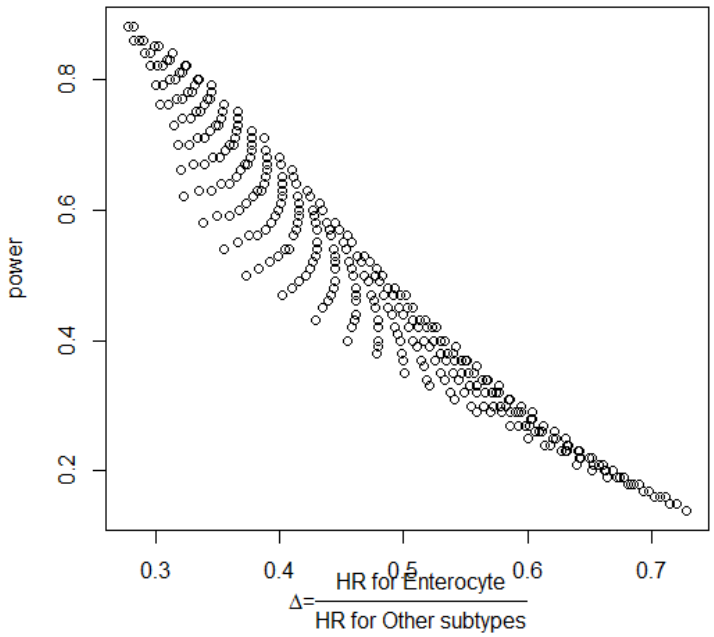

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Prognostic and Predictive Values of Clinical Variables in the Entire C-07 Cohort

eTable 7. Univariate Analysis of Prognostic Value for Clinical Variables in C-07 Entire Dataset

		HR	Lower (95%)	Upper (95%)	P Value
Age	≥70 vs <70	0.96	0.75	1.23	0.741
Gender	Male vs Female	0.91	0.76	1.09	0.294
Stage	III vs II	3.19	2.42	4.20	0.000
Grade	High vs low	1.08	0.86	1.35	0.514
Tumor stage	T3 vs T1&T2	2.17	1.47	3.20	0.000
	T4 vs T1&T2	3.92	2.46	6.25	0.000
Perforation	Yes vs No	1.67	1.04	2.67	0.033
Obstruction	Yes vs No	1.38	1.11	1.71	0.003

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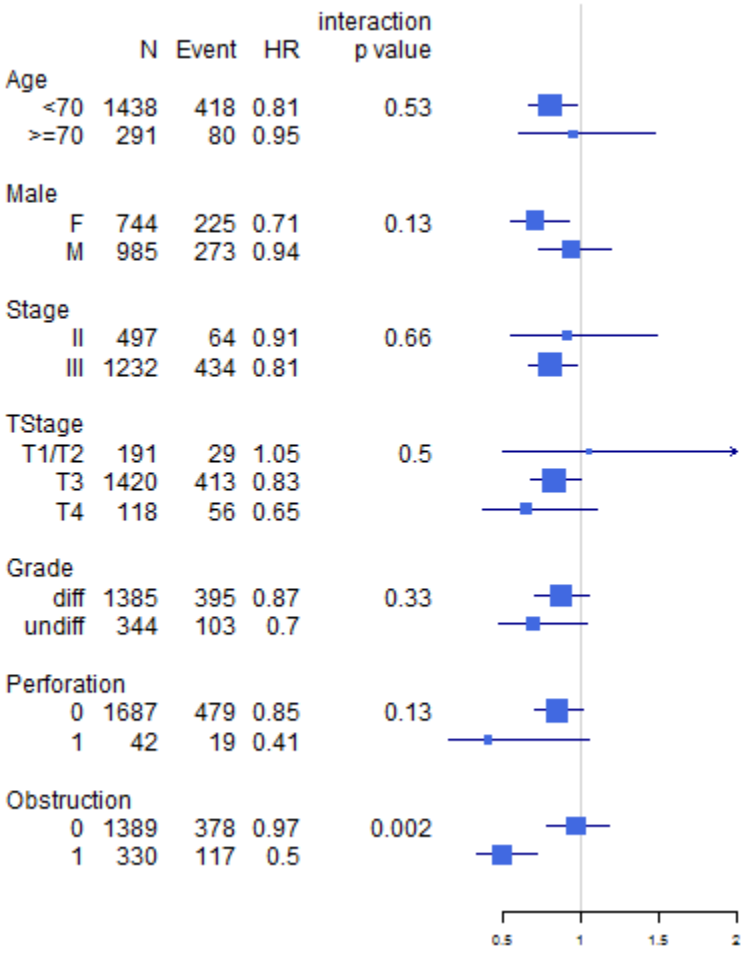
eFigure 3. Forest Plot of Clinical Variables



HR <1 indicated that patients receive benefit from oxaliplatin.

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eResults 1. The NCCN and oxaliplatin benefit in C-07

NCCN recommended all stage III and high-risk stage II patients with lymphovascular invasion, perforation, obstruction, T4 lesions, less than 12 lymph nodes examined, grade 3-4 lesions, and perineural invasion to be treated with oxaliplatin. However, analysis in the entire C-07 data (including discovery and validation cohort) indicated that the NCCN biomarker is not predictive (interaction HR=1.13, interaction p=0.79), and the gain in high-risk patients are small (HR=0.821, 95% CI: 0.686-0.982) (**eFigure 4**). Furthermore, the NCCN guideline classified 89.6% of the cohort as high risk, and most likely subjected many patients to unnecessary toxic therapy.

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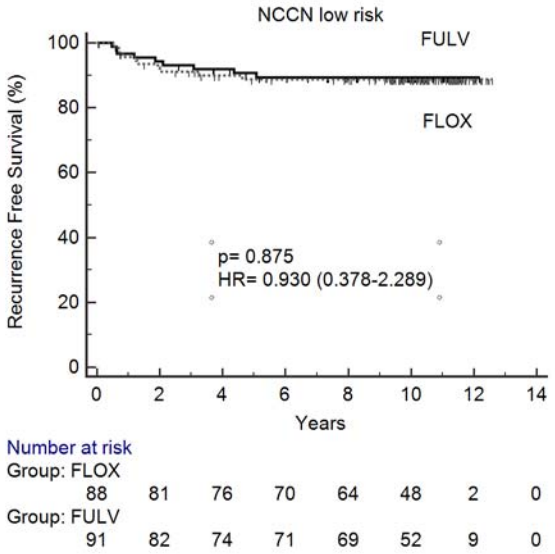
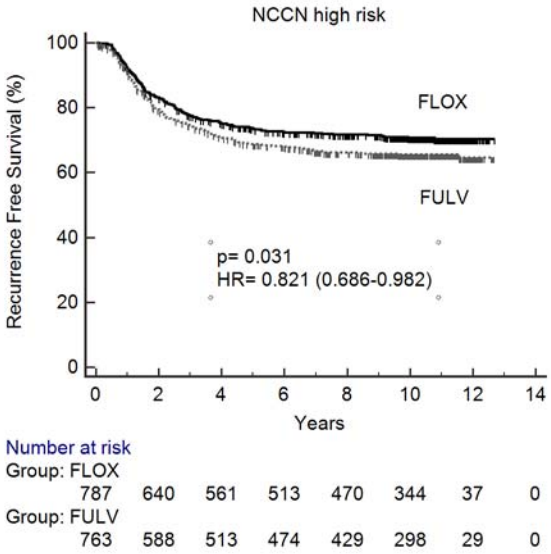
eFigure 4. Recurrence-free survival for NCCN Low-risk (A) and NCCN High-risk (B) Patients in NSABP C-07 (including discovery and validation cohorts) treated with FULV or FLOX (interaction p=0.125)

A.



B.



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Prognostic and Predictive Values of Subtypes

eTable 8. Correspondences between Colorectal Cancer Assigner (CRCA) and Colon Cancer Subtypes (CCS) Classifiers for Samples in Discovery, Validation, and Entire Datasets

	Colorectal cancer	CCS1	CCS2	CCS3
Discovery				
	Enterocyte	54	4	33
	Goblet-like	27	25	11
	Inflammatory	14	153	35
	Stem-like	11	10	218
	TA	152	8	23
Validation				
	Enterocyte	51	4	38
	Goblet-like	38	25	16
	Inflammatory	12	165	26
	Stem-like	13	12	214
	TA	177	11	23
Entire dataset				
	Enterocyte	105	8	71
	Goblet-like	65	50	27
	Inflammatory	26	318	61
	Stem-like	24	22	432
	TA	329	19	46

Abbreviations: TA, transit amplifying.

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eTable 9. Distribution of Colorectal Cancer Assigner (CRCA) Subtypes with Clinical and Pathological Variables in Discovery, Validation, and Entire Datasets

	Enterocyte	Goblet-like	Inflammatory	Stem-like	Transit-Amplifying	P Value [a]
Discovery Cohort						
Gender						
Female	43 (47.3%)	22 (34.9%)	89 (44.1%)	95 (39.7%)	79 (43.2%)	0.51
Male	48 (52.7%)	41 (65.1%)	113 (55.9%)	144 (60.3%)	104 (56.8%)	
Stage						
II	26 (28.6%)	20 (31.7%)	88 (43.6%)	57 (23.8%)	42 (23%)	0
III	65 (71.4%)	43 (68.3%)	114 (56.4%)	182 (76.2%)	141 (77%)	
Grade						
Differentiated	80 (87.9%)	51 (81%)	126 (62.4%)	194 (81.2%)	168 (91.8%)	0
Undifferentiated	11 (12.1%)	12 (19%)	76 (37.6%)	45 (18.8%)	15 (8.2%)	
Tumor stage						
T1	3 (3.3%)	3 (4.8%)	2 (1%)	1 (0.4%)	3 (1.6%)	0
T2	12 (13.2%)	8 (12.7%)	15 (7.4%)	6 (2.5%)	25 (13.7%)	
T3	71 (78%)	46 (73%)	176 (87.1%)	213 (89.1%)	144 (78.7%)	
T4	5 (5.5%)	6 (9.5%)	9 (4.5%)	19 (7.9%)	11 (6%)	
Perforation						
No	89 (97.8%)	62 (98.4%)	196 (97%)	231 (96.7%)	179 (97.8%)	0.91
Yes	2 (2.2%)	1 (1.6%)	6 (3%)	8 (3.3%)	4 (2.2%)	
Obstruction						
No	77 (85.6%)	54 (85.7%)	177 (88.1%)	174 (74%)	158 (86.3%)	0
Yes	13 (14.4%)	9 (14.3%)	24 (11.9%)	61 (26%)	25 (13.7%)	
Nodes positive						
0	26 (28.6%)	20 (31.7%)	88 (43.6%)	57 (23.8%)	42 (23%)	0
1-3	43 (47.3%)	32 (50.8%)	78 (38.6%)	107 (44.8%)	108 (59%)	
4+	22 (24.2%)	11 (17.5%)	36 (17.8%)	75 (31.4%)	33 (18%)	
dMMR						
pMMR	77 (100%)	50 (87.7%)	131 (72.4%)	193 (94.1%)	162 (97.6%)	0
dMMR	0 (0%)	7 (12.3%)	50 (27.6%)	12 (5.9%)	4 (2.4%)	
BRAF						
Wild type	83 (91.2%)	48 (78.7%)	145 (72.9%)	205 (86.1%)	177 (96.7%)	0

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Mutant	8 (8.8%)	13 (21.3%)	54 (27.1%)	33 (13.9%)	6 (3.3%)	
KRAS						
Wild type	64 (71.9%)	26 (44.1%)	129 (66.8%)	126 (54.1%)	121 (66.9%)	0
Mutant	25 (28.1%)	33 (55.9%)	64 (33.2%)	107 (45.9%)	60 (33.1%)	
MET						
Wild type	85 (96.6%)	56 (94.9%)	184 (95.3%)	227 (97.4%)	174 (96.1%)	0.8
Mutant	3 (3.4%)	3 (5.1%)	9 (4.7%)	6 (2.6%)	7 (3.9%)	
NRAS						
Wild type	85 (96.6%)	56 (94.9%)	190 (99%)	226 (97%)	176 (97.2%)	0.46
Mutant	3 (3.4%)	3 (5.1%)	2 (1%)	7 (3%)	5 (2.8%)	
PIK3CA						
Wild type	74 (84.1%)	46 (76.7%)	142 (73.2%)	175 (74.8%)	158 (87.3%)	0
Mutant	14 (15.9%)	14 (23.3%)	52 (26.8%)	59 (25.2%)	23 (12.7%)	
RFS						
Censored	59 (64.8%)	47 (74.6%)	173 (85.6%)	141 (59%)	132 (72.1%)	0
Event	32 (35.2%)	16 (25.4%)	29 (14.4%)	98 (41%)	51 (27.9%)	
Validation Cohort						
Gender						
Female	42 (45.2%)	35 (44.3%)	93 (45.8%)	104 (43.5%)	90 (42.7%)	0.97
Male	51 (54.8%)	44 (55.7%)	110 (54.2%)	135 (56.5%)	121 (57.3%)	
Stage						
II	23 (24.7%)	19 (24.1%)	78 (38.4%)	54 (22.6%)	45 (21.3%)	0
III	70 (75.3%)	60 (75.9%)	125 (61.6%)	185 (77.4%)	166 (78.7%)	
Grade						
Differentiated	76 (81.7%)	67 (84.8%)	137 (67.5%)	188 (78.7%)	190 (90%)	0
Undifferentiated	17 (18.3%)	12 (15.2%)	66 (32.5%)	51 (21.3%)	21 (10%)	
Tumor stage						
T1	7 (7.5%)	5 (6.3%)	2 (1%)	0 (0%)	4 (1.9%)	0
T2	9 (9.7%)	17 (21.5%)	18 (8.9%)	10 (4.2%)	26 (12.3%)	
T3	73 (78.5%)	50 (63.3%)	171 (84.2%)	203 (84.9%)	169 (80.1%)	
T4	4 (4.3%)	7 (8.9%)	12 (5.9%)	26 (10.9%)	12 (5.7%)	
Perforation						
No	90 (96.8%)	76 (96.2%)	201 (99%)	233	205 (97.2%)	0.59

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				(97.5%)		
Yes	3 (3.2%)	3 (3.8%)	2 (1%)	6 (2.5%)	6 (2.8%)	
Obstruction						
No	78 (83.9%)	66 (83.5%)	172 (85.1%)	158 (66.1%)	171 (82.2%)	0
Yes	15 (16.1%)	13 (16.5%)	30 (14.9%)	81 (33.9%)	37 (17.8%)	
Nodes positive						
0	23 (24.7%)	19 (24.1%)	78 (38.4%)	54 (22.6%)	45 (21.3	0
1-3	47 (50.5%)	34 (43%)	78 (38.4%)	104 (43.5%)	113 (53.6%)	
4+	23 (24.7%)	26 (32.9%)	47 (23.2%)	81 (33.9%)	53 (25.1%)	
dMMR						
pMMR	61 (96.8%)	48 (85.7%)	93 (61.6%)	157 (95.2%)	161 (98.2%)	0
dMMR	2 (3.2%)	7 (12.5%)	58 (38.4%)	8 (4.8%)	3 (1.8%)	
	0 (0%)	1 (1.8%)	0 (0%)	0 (0%)	0 (0%)	
BRAF						
Wild type	78 (90.7%)	54 (79.4%)	131 (70.8%)	192 (87.7%)	180 (96.8%)	0
Mutant	8 (9.3%)	14 (20.6%)	54 (29.2%)	27 (12.3%)	6 (3.2%)	
KRAS						
Wild type	46 (63%)	32 (54.2%)	111 (70.3%)	110 (56.7%)	104 (63.8%)	0.07
Mutant	27 (37%)	27 (45.8%)	47 (29.7%)	84 (43.3%)	59 (36.2%)	
MET						
Wild type	75 (100%)	59 (100%)	155 (95.7%)	182 (95.3%)	161 (97%)	0.17
Mutant	0 (0%)	0 (0%)	7 (4.3%)	9 (4.7%)	5 (3%)	
NRAS						
Wild type	69 (97.2%)	52 (98.1%)	153 (98.7%)	183 (96.3%)	154 (96.9%)	0.71
Mutant	2 (2.8%)	1 (1.9%)	2 (1.3%)	7 (3.7%)	5 (3.1%)	
PIK3CA						
Wild type	67 (93.1%)	38 (64.4%)	123 (75.9%)	160 (82.9%)	146 (88%)	0
Mutant	5 (6.9%)	21 (35.6%)	39 (24.1%)	33 (17.1%)	20 (12%)	
RFS						
Censored	68 (73.1%)	51 (64.6%)	171 (84.2%)	142 (59.4%)	153 (72.5%)	0
Event	25 (26.9%)	28 (35.4%)	32 (15.8%)	97 (40.6%)	58 (27.5%)	
All Patients						
Gender						
Female	85	57 (40.1%)	182 (44.9%)	199	169 (42.9%)	0.7

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	(46.2%)			(41.6%)		
Male	99 (53.8%)	85 (59.9%)	223 (55.1%)	279 (58.4%)	225 (57.1%)	
Stage						
II	49 (26.6%)	39 (27.5%)	166 (41%)	111 (23.2%)	87 (22.1%)	0
III	135 (73.4%)	103 (72.5%)	239 (59%)	367 (76.8%)	307 (77.9%)	
Grade						
Differentiated	156 (84.8%)	118 (83.1%)	263 (64.9%)	382 (79.9%)	358 (90.9%)	0
Undifferentiated	28 (15.2%)	24 (16.9%)	142 (35.1%)	96 (20.1%)	36 (9.1%)	
Tumor Stage						
T1	10 (5.4%)	8 (5.6%)	4 (1%)	1 (0.2%)	7 (1.8%)	0
T2	21 (11.4%)	25 (17.6%)	33 (8.1%)	16 (3.3%)	51 (12.9%)	
T3	144 (78.3%)	96 (67.6%)	347 (85.7%)	416 (87%)	313 (79.4%)	
T4	9 (4.9%)	13 (9.2%)	21 (5.2%)	45 (9.4%)	23 (5.8%)	
Perforation						
No	179 (97.3%)	138 (97.2%)	397 (98%)	464 (97.1%)	384 (97.5%)	0.93
Yes	5 (2.7%)	4 (2.8%)	8 (2%)	14 (2.9%)	10 (2.5%)	
Obstruction						
No	155 (84.7%)	120 (84.5%)	349 (86.6%)	332 (70%)	329 (84.1%)	0
Yes	28 (15.3%)	22 (15.5%)	54 (13.4%)	142 (30%)	62 (15.9%)	
Nodes positive						
0	49 (26.6%)	39 (27.5%)	166 (41%)	111 (23.2%)	87 (22.1%)	0
1-3	90 (48.9%)	66 (46.5%)	156 (38.5%)	211 (44.1%)	221 (56.1%)	
4+	45 (24.5%)	37 (26.1%)	83 (20.5%)	156 (32.6%)	86 (21.8%)	
dMMR						
pMMR	138 (98.6%)	98 (86.7%)	224 (67.5%)	350 (94.6%)	323 (97.9%)	0
dMMR	2 (1.4%)	14 (12.4%)	108 (32.5%)	20 (5.4%)	7 (2.1%)	
NA	0 (0%)	1 (0.9%)	0 (0%)	0 (0%)	0 (0%)	
BRAF						
Wild type	161 (91%)	102 (79.1%)	276 (71.9%)	397 (86.9%)	357 (96.7%)	0

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Mutant	16 (9%)	27 (20.9%)	108 (28.1%)	60 (13.1%)	12 (3.3%)	
KRAS						
Wild type	110 (67.9%)	58 (49.2%)	240 (68.4%)	236 (55.3%)	225 (65.4%)	0
Mutant	52 (32.1%)	60 (50.8%)	111 (31.6%)	191 (44.7%)	119 (34.6%)	
MET						
Wild type	160 (98.2%)	115 (97.5%)	339 (95.5%)	409 (96.5%)	335 (96.5%)	0.6
Mutant	3 (1.8%)	3 (2.5%)	16 (4.5%)	15 (3.5%)	12 (3.5%)	
NRAS						
Wild type	154 (96.9%)	108 (96.4%)	343 (98.8%)	409 (96.7%)	330 (97.1%)	0.37
Mutant	5 (3.1%)	4 (3.6%)	4 (1.2%)	14 (3.3%)	10 (2.9%)	
PIK3CA						
Wild type	141 (88.1%)	84 (70.6%)	265 (74.4%)	335 (78.5%)	304 (87.6%)	0
Mutant	19 (11.9%)	35 (29.4%)	91 (25.6%)	92 (21.5%)	43 (12.4%)	
RFS						
Censored	127 (69%)	98 (69%)	344 (84.9%)	283 (59.2%)	285 (72.3%)	0
Event	57 (31%)	44 (31%)	61 (15.1%)	195 (40.8%)	109 (27.7%)	

[a]P Value: Associations of clinical and pathological features variable with subtypes were analyzed by the chi-square test without correcting for missing values.
Abbreviations: MMR, mismatch repair status; pMMR, proficient MMR; dMMR, deficient MMR.

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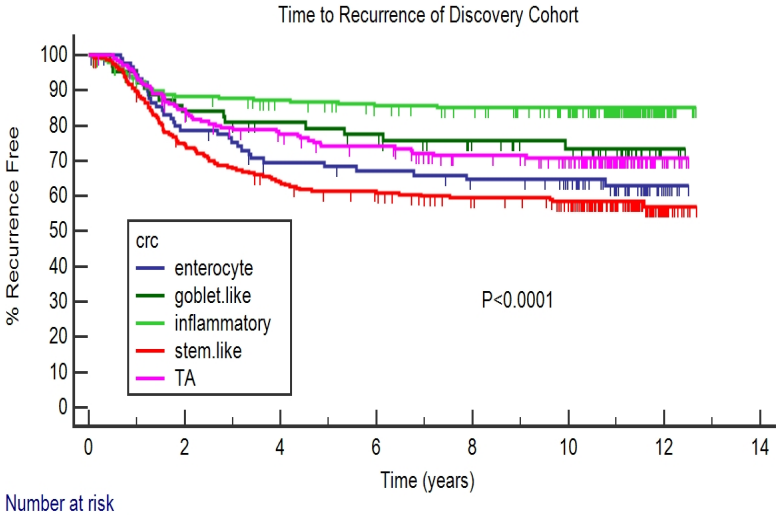
eFigure 5. Recurrence-free Survival for Colorectal Cancer Assigner subtypes (CRCA) and Colon Cancer Subtypes (CCS) in the Discovery Cohort

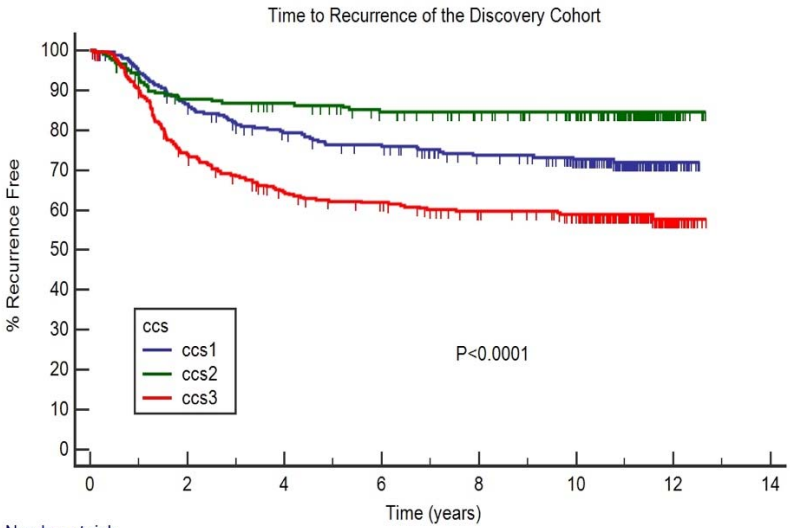
A.



Number at risk

Group: enterocyte							
91	70	59	56	52	46	7	0
Group: goblet.like							
63	53	47	44	36	31	3	0
Group: inflammatory							
202	170	161	151	144	128	16	0
Group: stem.like							
239	175	148	137	124	111	15	0
Group: TA							
183	150	132	122	106	93	10	0

B.



Number at risk

Group: ccs1							
258	217	191	179	159	139	18	0
Group: ccs2							
200	170	163	151	140	122	14	0
Group: ccs3							
320	231	193	180	163	148	19	0

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eTable 10. **Univariate and Multivariable Cox Model for Colorectal Cancer Assigner (CRCA) Subtype Prognostic Value in Discovery Cohort**

		Univariate Analysis				Multivariate Analysis			
		HR	**Lower (95%)**	**Upper (95%)**	**P Value**	**HR**	**Lower (95%)**	**Upper (95%)**	**P Value**
Age	>70 vs <70	1.00	0.71	1.41	0.986	1.05	0.74	1.48	0.781
Gender	Male vs Female	0.85	0.65	1.10	0.207	0.88	0.68	1.15	0.359
Stage	III vs II	3.39	2.29	5.00	0.000	3.36	2.26	4.99	0.000
Grade	High vs low	1.03	0.74	1.43	0.854	1.10	0.78	1.55	0.575
Tumor stage	T3 vs T1&T2	1.77	1.03	3.04	0.040	2.09	1.20	3.66	0.010
	T4 vs T1&T2	4.42	2.33	8.41	0.000	4.54	2.31	8.91	0.000
Perforation	Yes vs No	2.20	1.20	4.04	0.011	1.48	0.78	2.82	0.229
Obstruction	Yes vs No	1.41	1.02	1.94	0.037	1.24	0.89	1.72	0.214
CRCA subtypes	Enterocyte vs Stem-like	0.81	0.55	1.21	0.311	0.96	0.64	1.44	0.840
	Goblet-like vs Stem-like	0.56	0.33	0.95	0.031	0.69	0.40	1.18	0.172
	Inflammatory vs Stem-like	0.31	0.21	0.47	0.000	0.41	0.27	0.64	0.000
	TA vs Stem-like	0.63	0.45	0.88	0.007	0.71	0.50	1.01	0.056

Abbreviations: TA, transit amplifying.

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eTable 11. **Univariate and Multivariable Cox model for Colon Cancer Subtypes (CCS) subtype Prognostic Value in Discovery Cohort**

		Univariate Analysis				Multivariate Analysis			
		HR	**Lower (95%)**	**Upper (95%)**	**P Value**	**HR**	**Lower (95%)**	**Upper (95%)**	**P Value**
Age	>70 vs <70	1.00	0.71	1.41	0.986	1.03	0.73	1.45	0.882
Gender	Male vs Female	0.85	0.65	1.10	0.207	0.90	0.69	1.18	0.446
Stage	III vs II	3.39	2.29	5.00	0.000	3.30	2.22	4.90	0.000
Grade	High vs Low	1.03	0.74	1.43	0.854	1.08	0.77	1.52	0.654
Tumor stage	T3 vs T1&T2	1.77	1.03	3.04	0.040	2.11	1.21	3.69	0.009
	T4 vs T1&T2	4.42	2.33	8.41	0.000	4.86	2.47	9.58	0.000
Perforation	Yes vs No	2.20	1.20	4.04	0.011	1.48	0.78	2.80	0.230
Obstruction	Yes vs No	1.41	1.02	1.94	0.037	1.18	0.84	1.64	0.342
CCS	CCS1 vs CCS3	0.59	0.44	0.79	0.000	0.72	0.53	0.98	0.036
	CCS2 vs CC3	0.33	0.22	0.49	0.000	0.42	0.28	0.64	0.000

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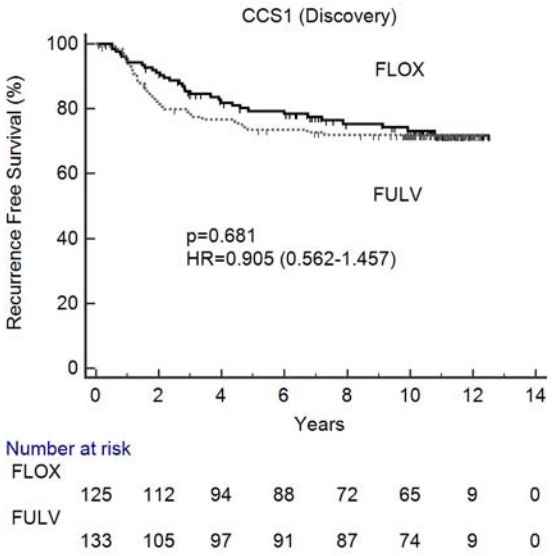
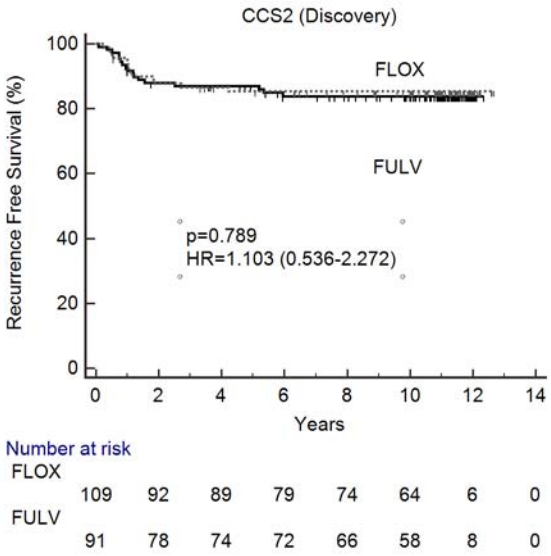
eFigure 6. Recurrence-free Survival for Colon Cancer Subtypes (CCS) and Colorectal Cancer Assigner (CRCA) Subtypes in the Discovery Cohort Treated with FULV and FLOX

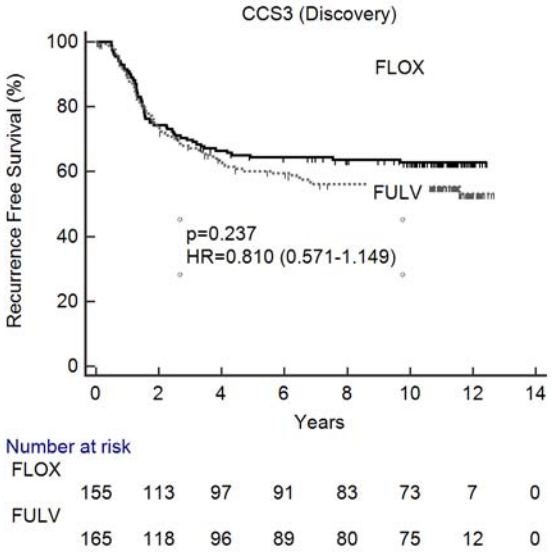
A.



B.



C.



D.

E.

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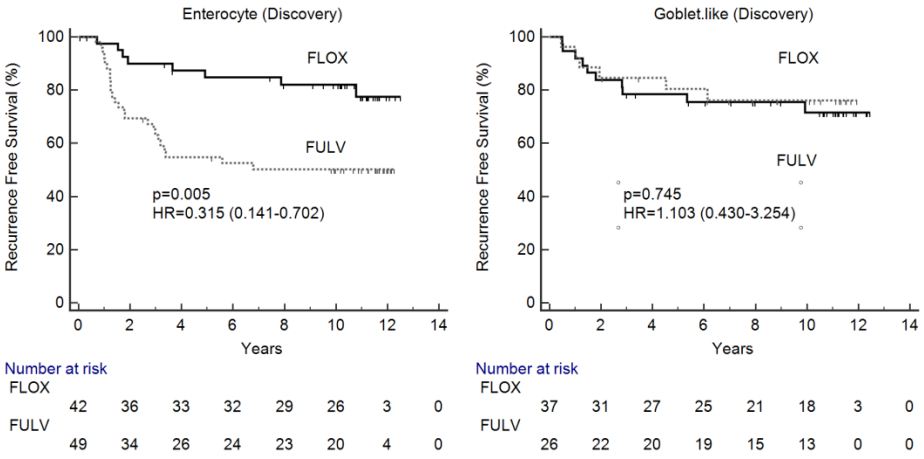
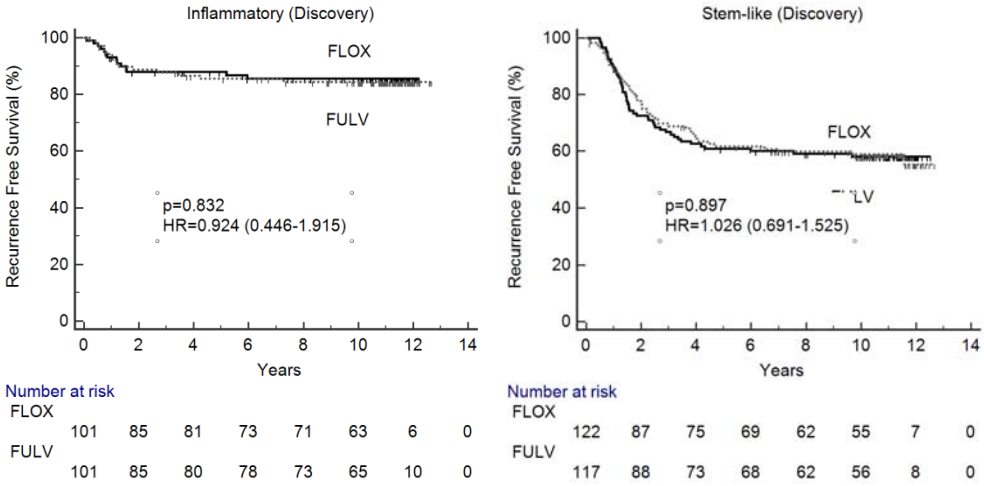
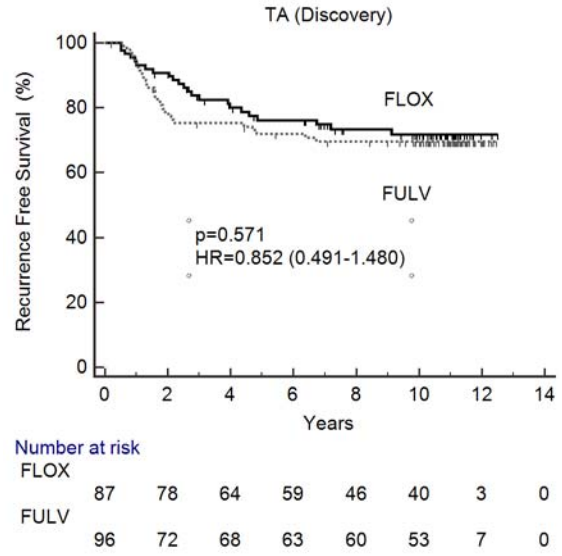


F.

G.



H



Abbreviations: TA, transit amplifying.

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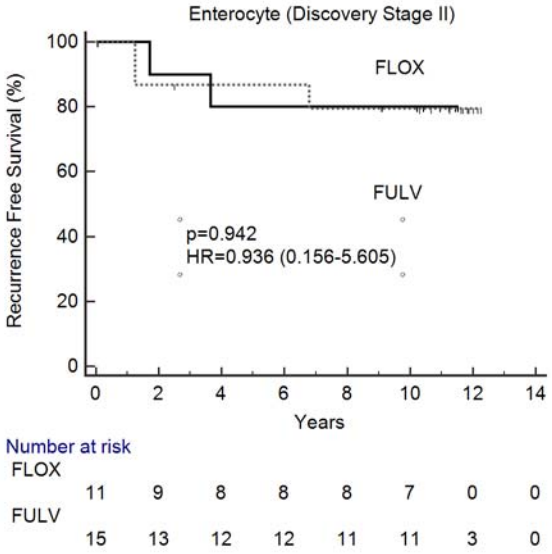
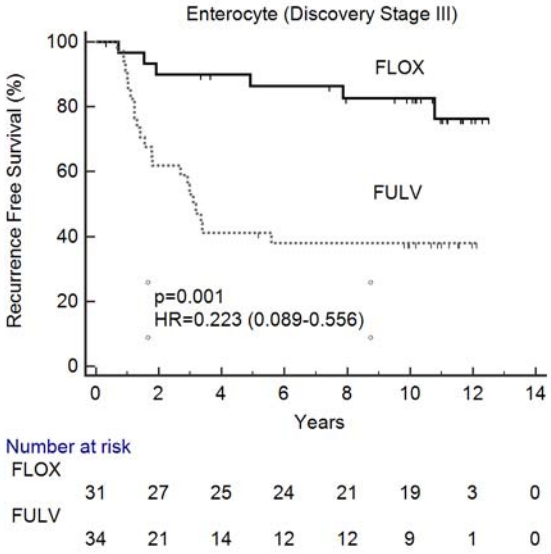
eFigure 7. Recurrence-free Survival for Colorectal Cancer Assigner (CRCA) Subtypes of Stage II and III Patients in the Discovery Cohort Treated with FULV and FLOX

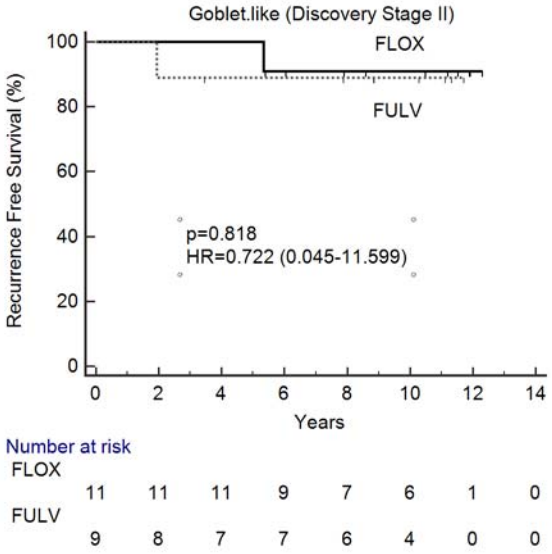
A.



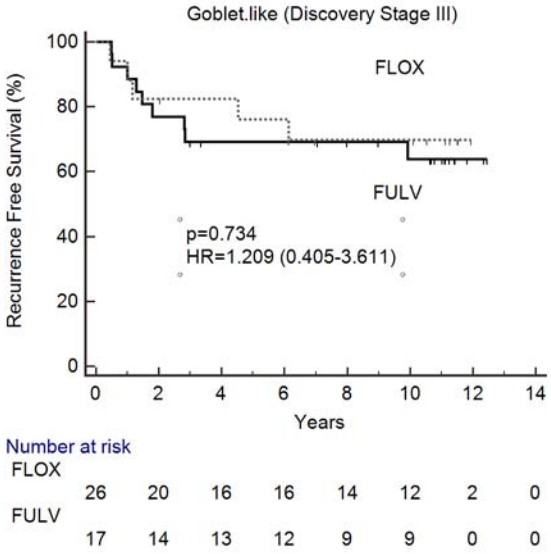
B.



C.



D.



E.

F.

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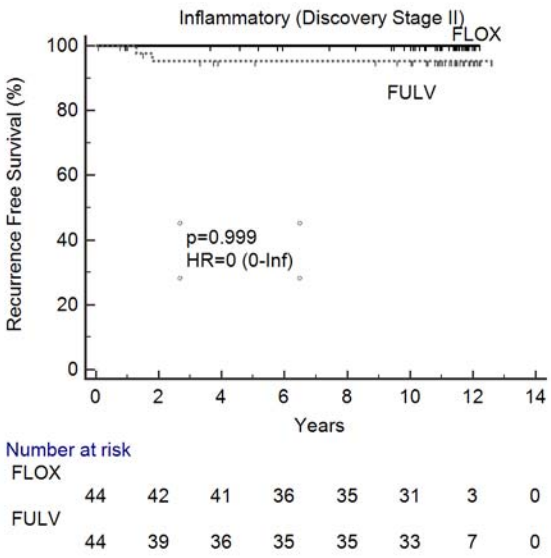
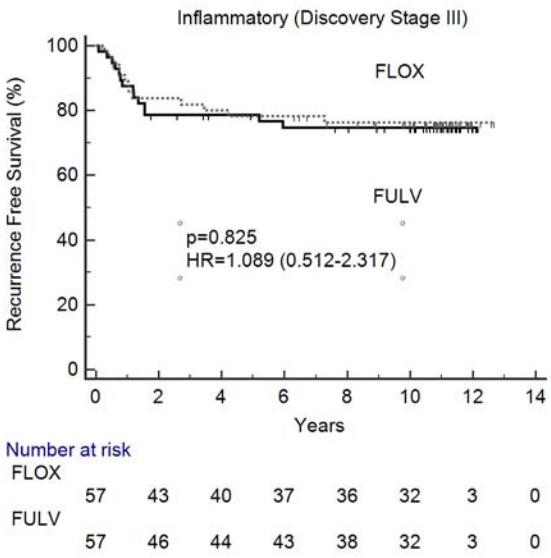
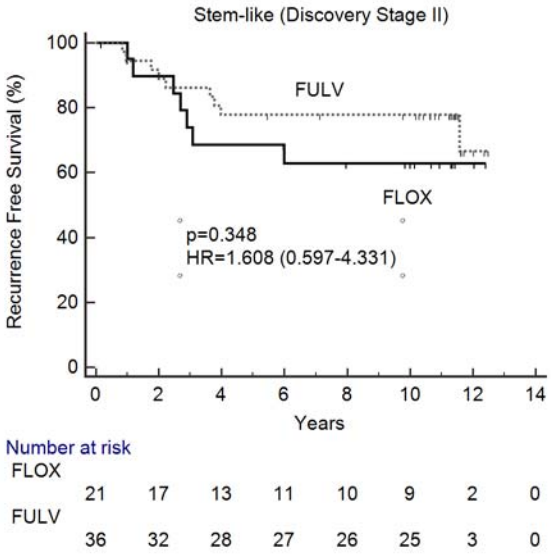
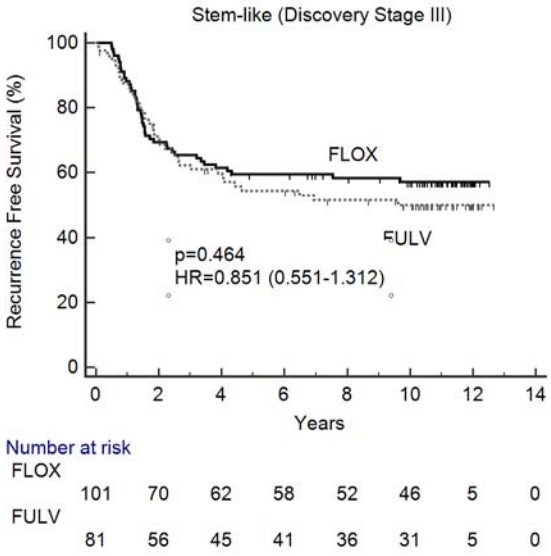




G.



H.



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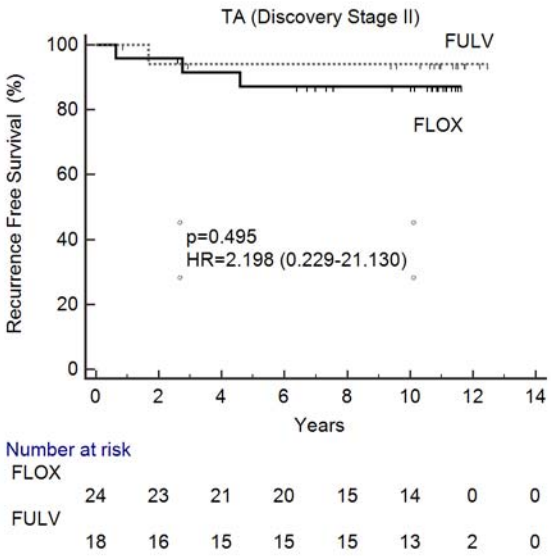
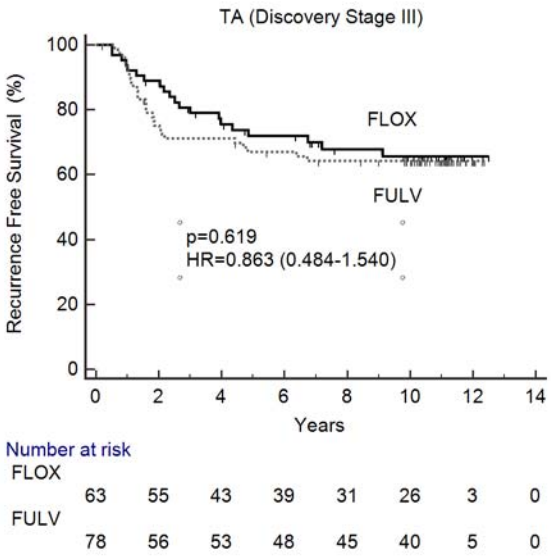
I.



J.



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Prognostic and Predictive Value of Colorectal Cancer Assigner (CRCA) Subtypes in Validation Cohort

eTable 12. Univariate and Multivariable Cox model for CRC Subtype Prognostic Value in Validation Cohort

		Univariate Analysis				Multivariate Analysis			
		HR	**Lower (95%)**	**Upper (95%)**	**P Value**	**HR**	**Lower (95%)**	**Upper (95%)**	**P Value**
Age	>70 vs <70	0.92	0.64	1.31	0.627	0.92	0.64	1.31	0.644
Gender	Male vs Female	0.97	0.75	1.25	0.797	0.95	0.74	1.24	0.718
Stage	III vs II	3.00	2.04	4.43	0.000	3.38	2.26	5.05	0.000
Grade	High vs low	1.12	0.82	1.53	0.471	1.15	0.83	1.58	0.395
Tumor stage	T3 vs T1&T2	2.61	1.49	4.58	0.001	3.38	1.91	6.00	0.000
	T4 vs T1&T2	3.48	1.77	6.88	0.000	4.14	2.06	8.35	0.000
Perforation	Yes vs No	1.22	0.57	2.58	0.610	1.31	0.60	2.87	0.499
Obstruction	Yes vs No	1.36	1.02	1.81	0.038	1.18	0.87	1.58	0.288
CRC	Enterocyte vs Stem-like	0.60	0.39	0.94	0.024	0.72	0.46	1.13	0.154
	Goblet-like vs Stem-like	0.84	0.55	1.28	0.413	1.10	0.71	1.68	0.679
	Inflammatory vs Stem-like	0.33	0.22	0.49	0.000	0.41	0.27	0.61	0.000
	TA vs Stem-like	0.61	0.44	0.85	0.003	0.70	0.50	0.98	0.036

Abbreviations: CRC, Colorectal cancer; TA, transit amplifying.

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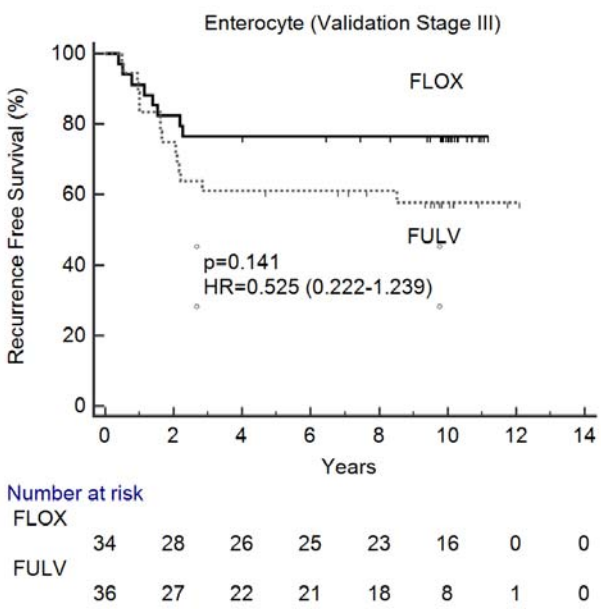
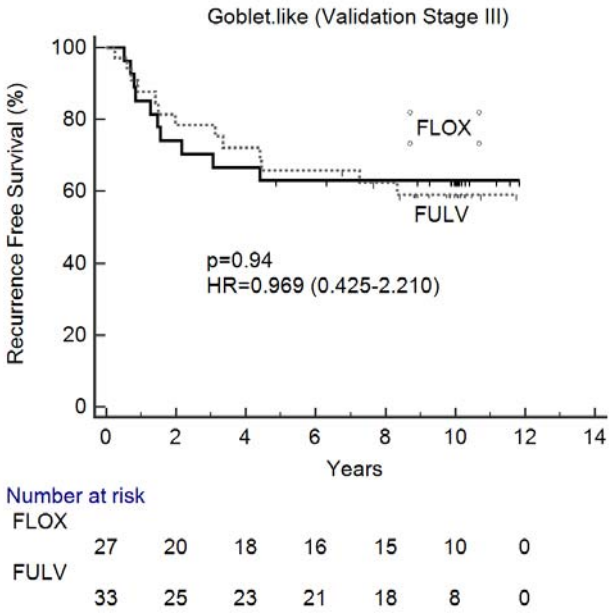
eFigure 8. Recurrence-free Survival for Colorectal Cancer Assigner (CRCA) and Subtype of Stage III Patients in the Validation Cohort Treated with FULV and FLOX

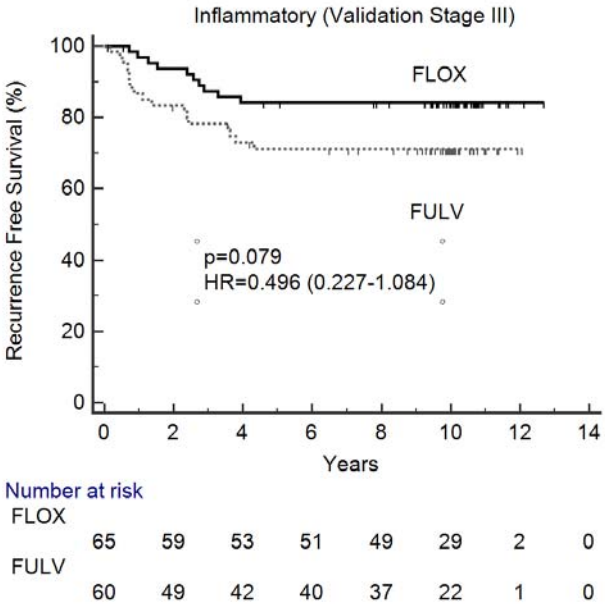
A.



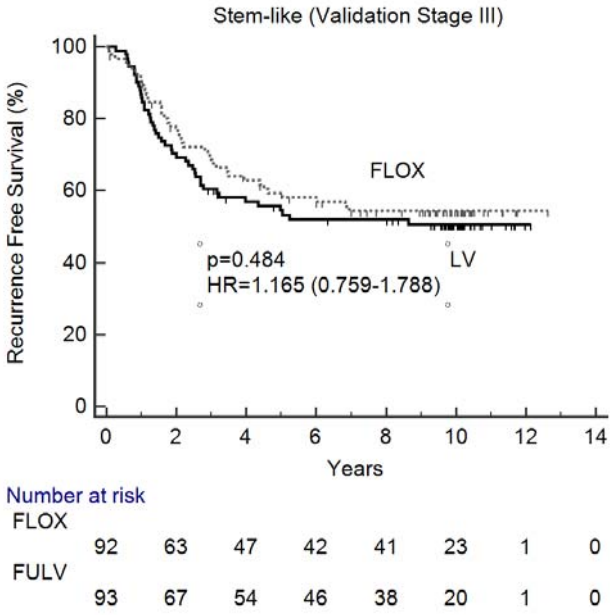
B.



C.



D.



E.

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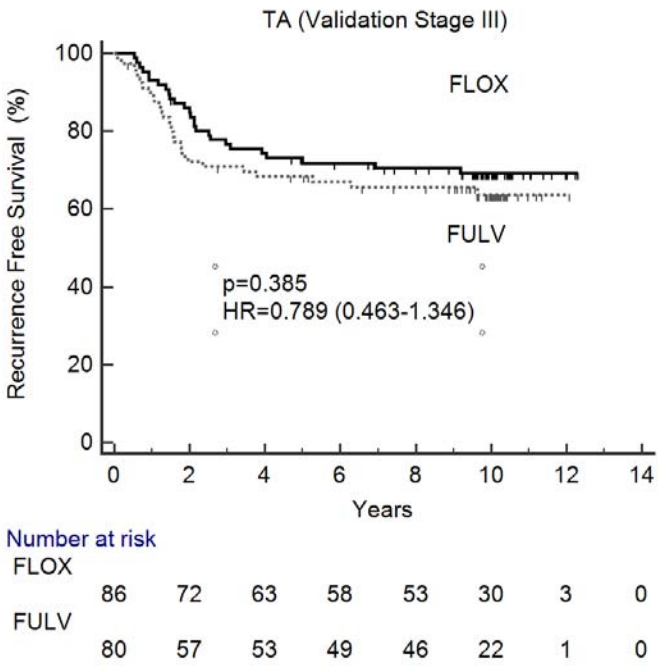

Abbreviations: TA, transit amplifying.

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Post-hoc Exploratory Analyses of the Entire C-07 Cohort with Colorectal Cancer Assigner (CRCA) and Consensus Molecular Subtypes (CMS)

eResults 2. Consensus subtype assignment for C-07 samples

Recently, Guinney et al.[5] studied the association among subtypes from six different subtyping classifiers, and clustered the 27 subtypes from six classifiers to 4 groups, named as Consensus Molecular Subtypes (CMS) 1-4 in the consensus subtype classifier. They further developed a 'single sample predictor' SSP method, which can be used to identify the CMS subtype for any new sample. The SSP method is based on a similarity-to-centroid approach, with the Pearson coefficient as a similarity measure. Note, the CMS classifier only classified samples with high confidence into consensus subtypes, resulting in some samples remaining unclassified.

To assign C-07 samples to the CMS subtype, we used the SSP method developed by Guinney et al. Note, the classifier for consensus subtype identification includes 693 genes, 37 of which were profiled by the nCounter code set used in C-07. We assigned C-07 samples to the consensus subtypes based on the centroid of the 37 genes. We used centroid information from the original consensus subtype classifier directly without redeveloping them. This may cause some bias of subtype identification.

In C-07, the association of CRCA and CMS in the discovery and validation cohorts is similar to the consensus paper and other datasets. Note, a greater portion of enterocyte samples fall into the unknown subtype than other CRCA subtypes.

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eTable 13. Correlation between Colorectal Cancer Assigner (CRCA) and Consensus Molecular Subtypes (CMS) Classifiers

	Enterocyte	Goblet-like	Inflammatory	Stem-like	TA
CMS1	4	22	**174**	28	3
CMS2	**57**	11	24	29	**261**
CMS3	19	**53**	6	2	6
CMS4	36	6	11	**271**	10
Unknown	19	11	24	37	27

Numbers in bold indicate the greatest number of tumors categorized by the two different classifiers.

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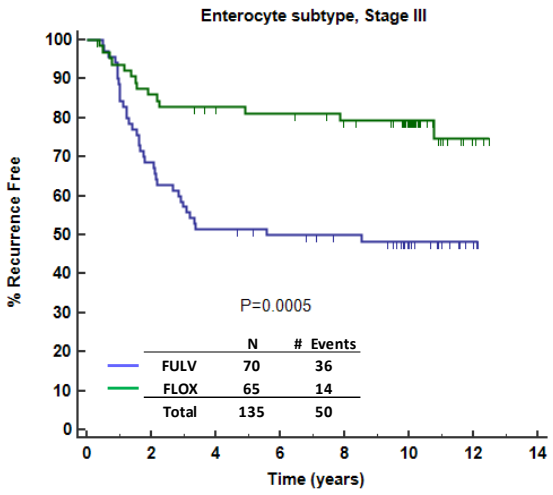
eFigure 9. Oxaliplatin Benefit in Enterocyte versus Other Subtypes in All Stage III patients

A.



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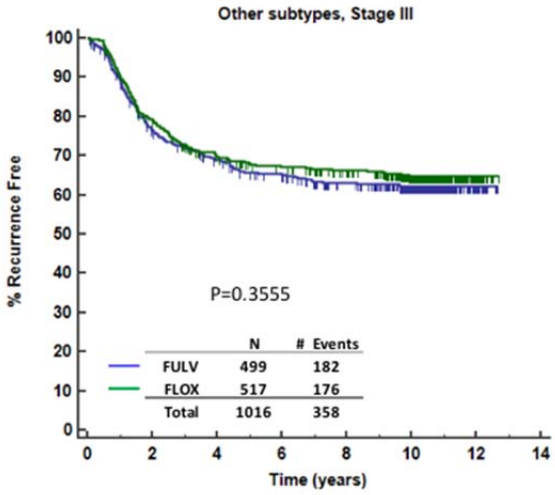

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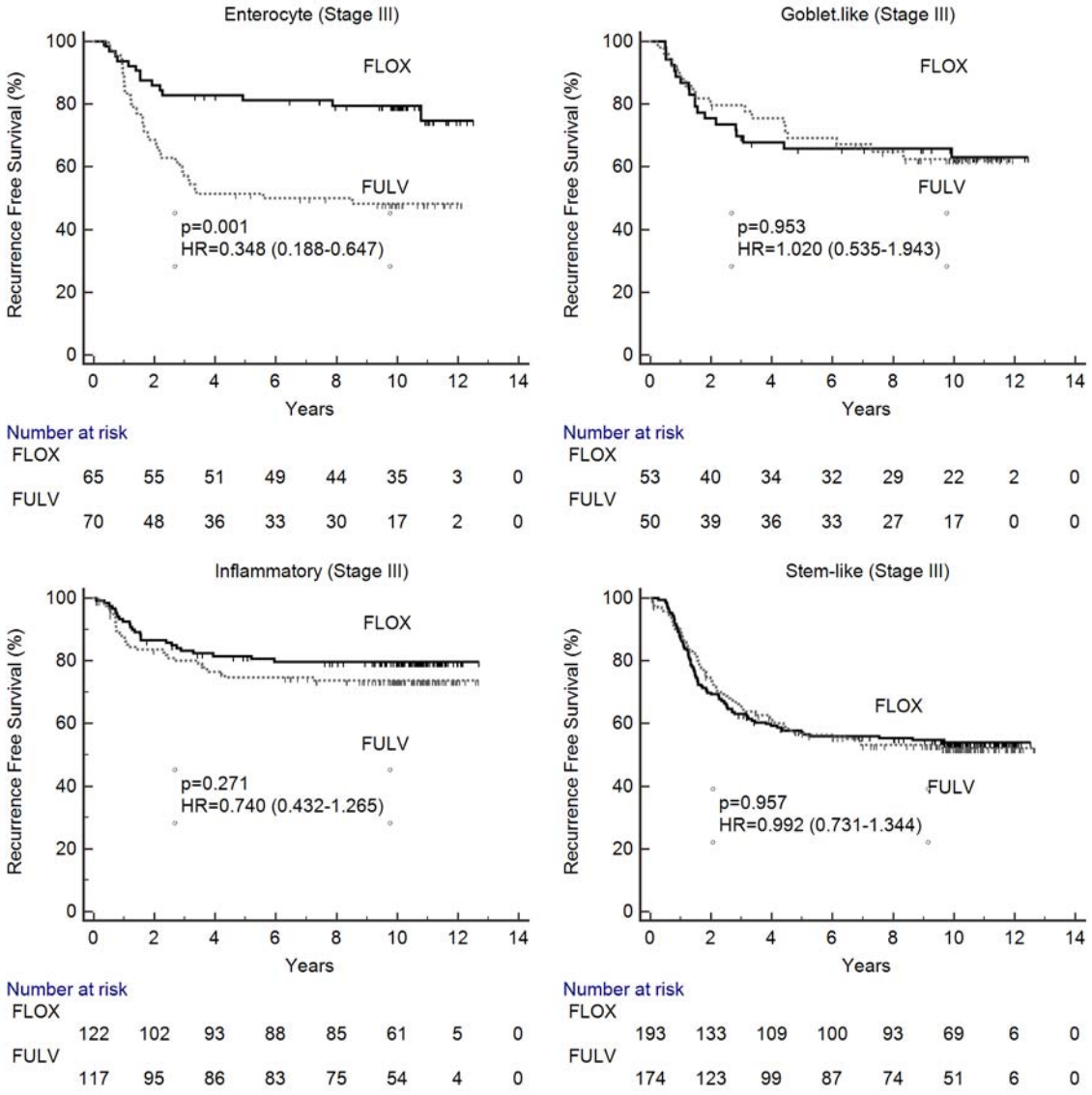
eFigure 10. Recurrence-free Survival for Colorectal Cancer Assigner (CRCA) and CCS Subtype of Stage III Patients in the entire C-07 dataset Treated with FULV and FLOX



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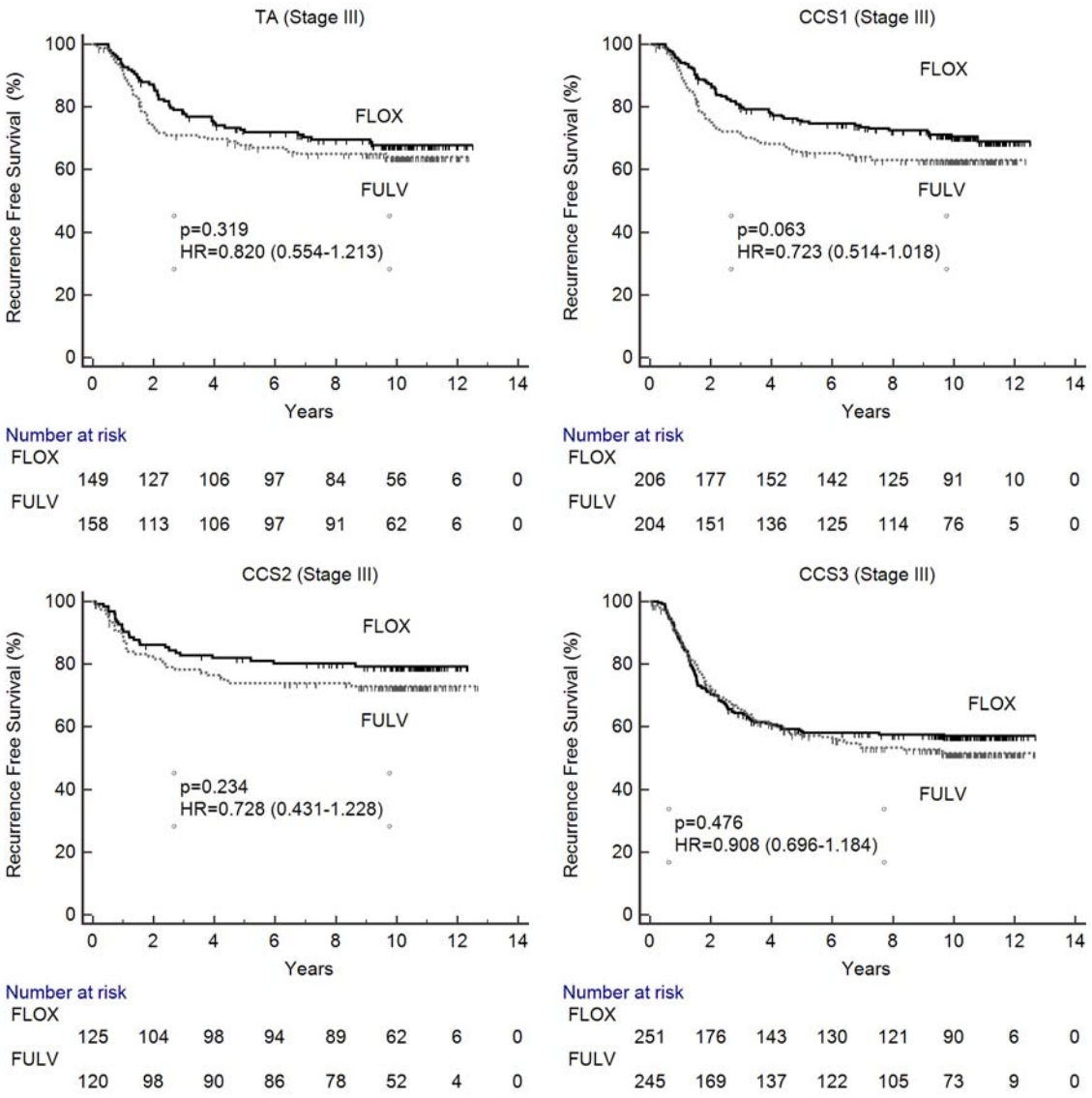

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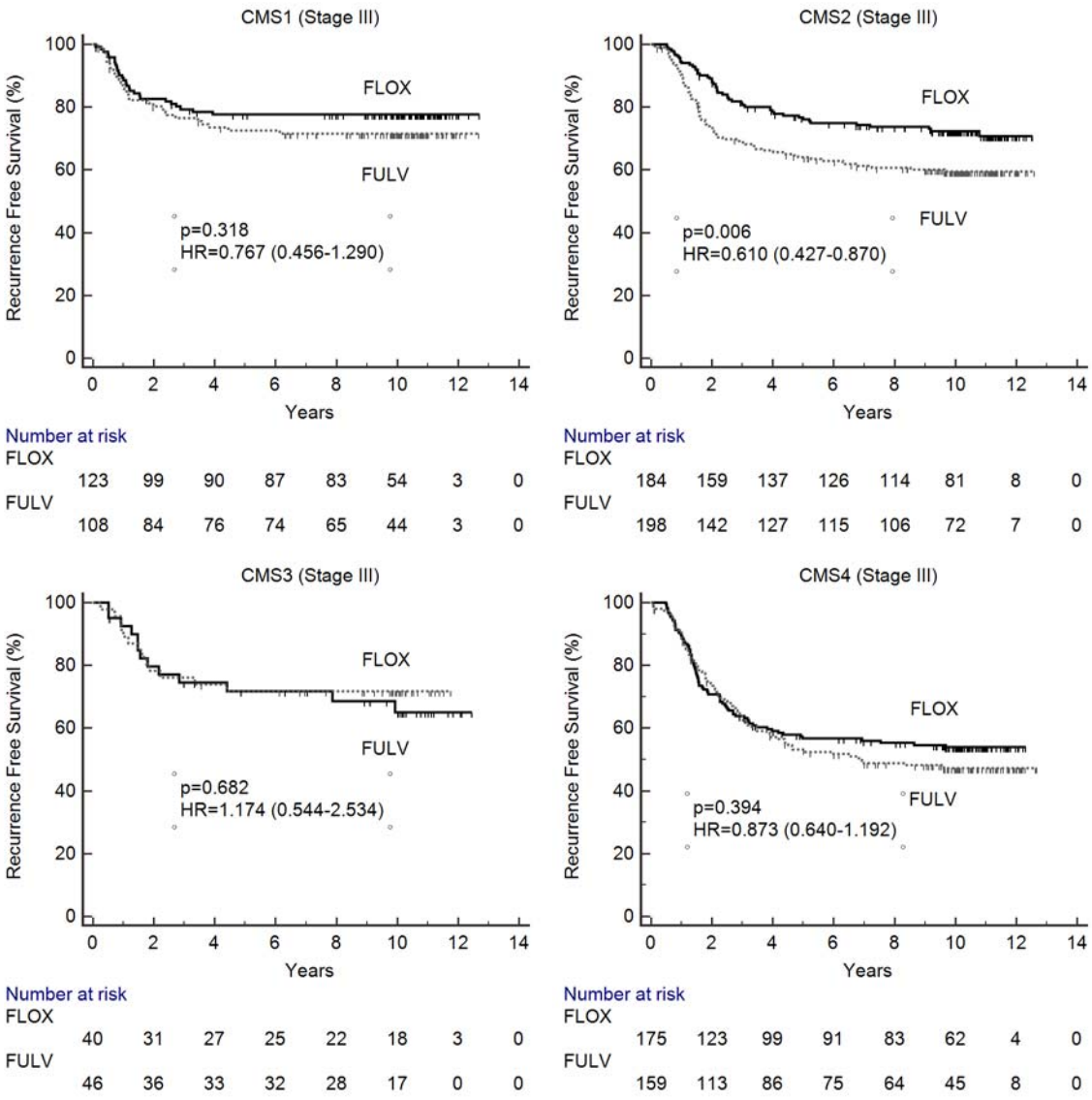

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