Supplementary Materials

YY1 silencing induces 5-Fluorouracil-resistance and BCL2L15 downregulation in colorectal cancer cells: diagnostic and prognostic relevance

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Table S1. Molecular and pathological features of the CRC cell line panel

Feature/Cell Line	HT29	SW620	HCT116	Caco2
Organ	Colon	Colon	Colon ascendens	Colon
Disease	Colorectal adenocarcinoma	Colorectal adenocarcinoma	Colorectal carcinoma	Colorectal adenocarcinoma
Patient	44-Year-old female	51-Year-old male	48-Year-old male	48-Year-old male
Stage	Dukes' C	Dukes' C	Dukes' D	Dukes' B
Derived from	Primary tumor	Lymph node metastasis	Primary tumor	nd
MSI status	MSS	MSS	MSI	MSS
CIMP panel 1	+	+	+	+
CIMP panel 2	+	−	+	−
CIN	+	46	−	48
APC mut 1	p.E853* (substitution - nonsense)	p.Q1338* (substitution - nonsense)	p.?	p.Q1367* (substitution - nonsense)
APC mut 2	p.T1556Nfs*3 (insertion - frameshift)	p.?	p.?	p.?
CTNNB1 mut 1	wt	wt	p.S45del (deletion - in frame)	p.G245A
CTNNB1 mut 2	wt	wt	p.?	p.?
TP53 mut 1	p.R273H (substitution - missense) Gain of function	p.R273H (substitution - missense) Gain of function	wt	p.Glu204Ter (substitution - nonsense - KO)
TP53 mut 2	p.?	p.P309S	wt	p.Glu204Ter (substitution - nonsense - KO)
KRAS mut 1	wt	p.G12V (substitution - missense)	p.G13D (substitution - missense)	wt
KRAS mut 2	wt	p.?	p.?	wt
BRAF mut 1	p.Val600Glu (substitution - missense)	wt	p.Ser45del	wt
BRAF mut 2	p.?	wt	p.?	wt
PIK3CA mut 1	p.Pro449Thr (substitution - missense)	wt	p.His1047Arg	wt
PIK3CA mut 2	p.?	wt	p.?	wt
SMAD4 mut 1	p.Q311* (substitution - nonsense)	p.?	nd	p.D351H (substituion - missense)
SMAD4 mut 2	p.?	p.?	nd	p.?
CDKN2A mut 1	nd	nd	p.Arg24fs*20	nd
CDKN2A mut 2	nd	nd	p.Glu74fs*15	nd

Table S2. Whole Transcription Factors ChIP-seq binding-clusters within BCL2L15 promoter/enhancer (all experiments)

Experiment Name	Tissue	Binding Score	co-IP TF ID
ATF2 IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	92	ATF2
ATF3 IP	epithelium, HCT116 cells	134	ATF3
ATF3 IP | CAMPTO	epithelium, HCT116 cells	311	ATF3
BATF IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	182	BATF
BCL11A IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	151	BCL11A
BHLHE40 IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	117	BHLHE40
CDX2 IP | 125DVD3	epithelium, LS180 cells	94	CDX2
CDX2 IP	epithelium, LS180 cells	126	CDX2
CEBPB IP - K562 cells	myeloid lineage, myeloblasts, CML, K562 cells	141	CEBPB
CEBPB IP	multipotent, MSCs	166	CEBPB
CEBPB IP	myeloid lineage, macrophages, MV-4-11 cells	180	CEBPB
CEBPB IP - HCT116 cells	epithelium, HCT116 cells	186	CEBPB
CEBPB IP - MCF7 cells	epithelium, MCF-7 cells	209	CEBPB
CEBPB ChIP-Seq - K562	myeloid lineage, myeloblasts, CML, K562 cells	361	CEBPB
CEBPB IP	multipotent, MSCs	419	CEBPB
CEBPB IP	multipotent, MSCs	486	CEBPB
E2F3 IP	epithelium, LoVo cells	155	E2F3
EBF1 IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	103	EBF1
ELF3 IP | KLF5 KO	duct, epithelium, CFPAC-1 cells	273	ELF3
ELF3 IP	duct, epithelium, CFPAC-1 cells	586	ELF3
ELF3 IP	duct, epithelium, CFPAC-1 cells	731	ELF3
EOMES IP - Rep 3	pluripotent, ESCs, HUES64 cells	266	EOMES
ETS1 IP	lymphoid lineage, lymphoblasts, ALL, CUTLL1 cells	183	ETS1
ETV5 IP	epithelium, LoVo cells	146	ETV5
FOSL1 IP - HCT116 cells	epithelium, HCT116 cells	524	FOSL1
FOXA1 ChIP-Seq - Rep 1	epithelium, ZR-75-1 cells	105	FOXA1
DHT | FOXA1 ChIP-Seq	epithelium, LNCaP cells	110	FOXA1
FOXA1 ChIP-Seq	epithelium, LNCaP cells	111	FOXA1
FOXA1 IP - ES D7	pluripotent, ESCs, Cyt49 cells	136	FOXA1
FOXA1 ChIP-Seq	epithelium, LNCaP-abl cells	156	FOXA1
FOXA1 IP - Rep 1	pluripotent, ESCs, HUES64 cells	164	FOXA1
CREB1 siRNA | FOXA1 ChIP-Seq - Rep 1	epithelium, LNCaP-abl cells	193	FOXA1
FOXA1 ChIP-Seq - LNCaP-abl	epithelium, LNCaP-abl cells	194	FOXA1
FOXA1 IP - T47D cells	epithelium, T47D cells	201	FOXA1
DEX | FOXA1 ChIP-Seq - Rep 2	epithelium, ZR-75-1 cells	221	FOXA1
FOXA1 ChIP-Seq - Rep 2	epithelium, LNCaP-abl cells	224	FOXA1
CREB1 siRNA | FOXA1 ChIP-Seq - Rep 2	epithelium, LNCaP-abl cells	244	FOXA1
FOXA1 IP	duct, epithelium, CFPAC-1 cells	295	FOXA1
FOXA1 IP	duct, epithelium, CFPAC-1 cells	308	FOXA1
FOXA1 ChIP-Seq	epithelium, LNCaP cells	359	FOXA1

FOXA1 IP - Rep 5	pluripotent, ESCs, HUES64 cells	540	FOXA1
FOXA2 IP - Rep 5	pluripotent, ESCs, HUES64 cells	193	FOXA2
FOXA2 IP - Rep 1	pluripotent, ESCs, HUES64 cells	289	FOXA2
FOXA2 IP - Rep 1	endodermal cells	398	FOXA2
FOXM1 IP - ECC1 cells	endometrium, epithelium, ECC-1 cells	139	FOXM1
FOXP1 IP	epithelium, LoVo cells	115	FOXP1
GABPA IP - Jurkat, Rep 1	lymphoid lineage, T lymphocytes, Jurkat	161	GABPA
FOXA1 KD | GATA2 ChIP-Seq	epithelium, LNCaP cells	179	GATA2
17BE2 | GATA3 ChIP-Seq	epithelium, MCF-7 cells	90	GATA3
GATA3 IP - T47D cells	epithelium, T47D cells	158	GATA3
GATA3 IP - SK-N-SH cells	other, neuroblastoma cells, SK-N-SH cells	198	GATA3
GATA4 IP - Rep 11	pluripotent, ESCs, HUES64 cells	118	GATA4
GATA4 IP - Rep 8	pluripotent, ESCs, HUES64 cells	151	GATA4
GATA4 IP - Rep 10	pluripotent, ESCs, HUES64 cells	164	GATA4
GATA4 IP - AGS	epithelium, AGS cells	186	GATA4
GATA4 IP - Rep 9	pluripotent, ESCs, HUES64 cells	187	GATA4
HNF1B IP	duct, epithelium, CFPAC-1 cells	416	HNF1B
HOXA4 IP	epithelium, LoVo cells	133	HOXA4
IRF1 IP	duct, epithelium, CFPAC-1 cells	180	IRF1
IRF1 IP	epithelium, LoVo cells	323	IRF1
IRF1 IP - Rep 3	lymphoid lineage, T lymphocytes, CD4+	373	IRF1
JUN IP	epithelium, LoVo cells	134	JUN
JUN IP - Rep 2	epithelium, MDA-MB-231 cells	184	JUN
JUN IP - Rep 1	epithelium, MDA-MB-231 cells	329	JUN
JUND IP	smooth muscle, whole	189	JUND
JUND IP - HCT116 cells	epithelium, HCT116 cells	766	JUND
KLF5 IP - KATOIII	epithelium, KATO III cells	125	KLF5
KLF5 IP	duct, epithelium, CFPAC-1 cells	383	KLF5
MAX IP - HCT116 cells	epithelium, HCT116 cells	172	MAX
MYB IP - Jurkat, Rep 3	lymphoid lineage, T lymphocytes, Jurkat	135	MYB
MYB IP - Jurkat, Rep 1	lymphoid lineage, T lymphocytes, Jurkat	194	MYB
MYB IP - Jurkat, Rep 5	lymphoid lineage, T lymphocytes, Jurkat	217	MYB
MYB IP - Jurkat, Rep 4	lymphoid lineage, T lymphocytes, Jurkat	529	MYB
NFYB IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	214	NFYB
OTX2 IP - Rep 8	pluripotent, ESCs, HUES64 cells	165	OTX2
OTX2 IP - Rep 6	pluripotent, ESCs, HUES64 cells	197	OTX2
SP1 IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	79	SP1
SP1 IP - HCT116 cells	epithelium, HCT116 cells	545	SP1
SPI1 IP - HL-60 cells, Rep 4	myeloid lineage, promyelocytes, APL, HL-60 cells	109	SPI1
SPI1 IP - U-937 cells, Rep 1	myeloid lineage, monocytes, U-937 cells	180	SPI1
SPI1 IP | RUNX1 KD	myeloid lineage, myeloblasts, AML, kasumi-1 cells	204	SPI1
SPI1 IP - U-937 cells, Rep 4	myeloid lineage, monocytes, U-937 cells	235	SPI1
SPI1 IP - GM12891 cells	lymphoid lineage, B lymphocytes, GM12891 cells	259	SPI1
SPI1 IP	myeloid lineage, macrophages, unspecified	264	SPI1
SPI1 IP	myeloid lineage, myeloblasts, AML, kasumi-1 cells	310	SPI1

SPI1 IP	myeloid lineage, macrophages, unspecified	339	SPI1
SPI1 IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	342	SPI1
SPI1 IP - HL cells	myeloid lineage, promyelocytes, APL, HL-60 cells	379	SPI1
SPI1 IP	myeloid lineage, macrophages, unspecified	643	SPI1
SPI1 IP	myeloid lineage, macrophages, unspecified	849	SPI1
SPI1 IP	myeloid lineage, macrophages, unspecified	1046	SPI1
SPI1 IP	myeloid lineage, macrophages, unspecified	1133	SPI1
SPI1 IP	myeloid lineage, macrophages, unspecified	1197	SPI1
SRF IP - HCT116 cells	epithelium, HCT116 cells	99	SRF
TAL1 IP	epithelium, LoVo cells	127	TAL1
TCF12 IP - ECC1 cells	endometrium, epithelium, ECC-1 cells	142	TCF12
TCF12 IP - SK-N-SH cells	other, neuroblastoma cells, SK-N-SH cells	207	TCF12
TEAD4 IP - HCT116 cells	epithelium, HCT116 cells	279	TEAD4
TEAD4 IP - ECC1 cells	endometrium, epithelium, ECC-1 cells	390	TEAD4
ZNF384 IP - GM12878 cells	lymphoid lineage, B lymphocytes, GM12878 cells	81	ZNF384

Table S3. GEO DataSets features, normality and correlation analyses

GEO Dataset Name	GSE ID	Year	Patients	YY1 Normality test	BCL2L15 Normality test	Pearson R	*Pearson P*	Spearman R	*Spearman P*
EXPO	GSE2109	2005	315	0.4169 (Y)	<0.0001 (N)	0.0439	*0.4368*	0.0408	*0.4710*
Watanabe	GSE4554	2006	84	<0.0001 (N)	<0.0001 (N)	0.1820	*0.0976*	0.1949	*0.0756*
Marra	GSE8671	2007	32	0.6751 (Y)	0.1307 (Y)	-0.1260	*0.4847*	-0.0955	*0.6029*
Jorissen	GSE13294	2009	155	0.0747 (Y)	<0.0001 (N)	0.0052	*0.9490*	0.0211	*0.7936*
Smith	GSE17538	2009	232	<0.0001 (N)	0.3461 (Y)	0.0577	*0.3815*	0.0723	*0.2722*
Sieber	GSE14333	2010	289	0.0025 (N)	<0.0001 (N)	0.0082	*0.8890*	0.0206	*0.7270*
Sugihara	GSE21510	2011	148	0.8457 (Y)	<0.0001 (N)	-0.0104	*0.8997*	0.0157	*0.8497*
Yagi	GSE28702	2011	83	0.7817 (Y)	0.339 (Y)	0.0096	*0.9312*	0.0905	*0.4159*
Olschwang	GSE37892	2012	130	0.0122 (N)	0.1917 (Y)	0.1169	*0.1853*	0.1923	*0.0284*
Wessels	GSE35896	2013	62	0.2448 (Y)	<0.0001 (N)	-0.1213	*0.3475*	-0.0368	*0.7766*
Clary	GSE41568	2016	133	0.6228 (Y)	0.0052 (N)	0.1024	*0.2409*	0.1232	*0.1578*
Sieber	GSE75316	2016	58	0.6715 (Y)	<0.0001 (N)	0.0641	*0.6329*	-0.0305	*0.8200*

Table S4. Primers used in the RT-PCR experiments

GENE ID	Primer Forward	Primer Reverse	bp
BCL2	TGAACTGGGGGAGGATTGTG	CGTACAGTTCCACAAAGGCA	183
BCL2L1 XS	AGCTTTGAACAGGATACTTTTGTGG	GGTGGGAGGGTAGAGTGGAT	183
BCL2L1 XL	CTGTGCGTGGAAAGCGTAGA	GCTGCTGCATTGTTCCCATAG	155
BCL2L2	CACCCAGGTCTCCGATGAAC	GCTGTGAACTCCGCCCAG	210
BCL2L3	TTTTCAGCGACGGCGTAACA	CAAACCCATCCCAGCCTCTTT	189
BCL2L4	CCCCGAGAGGTCTTTTTCCG	TGGTTCTGATCAGTTCCGGC	145
BCL2L7	GATCCCGGCAGGCTGATCC	GTAGCTGCGGAAAACCTCCT	156
BCL2L8	CTTTAAGAAGGGACTTCCTCGC	GTGGAGTTTCGGGATGTGGA	163
BCL2L15	ACCTGGTGTGCTCAGGATTC	TCCAGATTTTCCCAACCTCCC	194
BIRC3	TCTGGGCAGCAGGTTTACAA	GCATTCTTTGGATAGTAAAACACCA	191
BIRC4	TGTCCTGGCGCGAAAAGGT	CGTGCCAGTGTTGATGCTGA	190
BIRC5	CAAGGACCACCGCATCTCTA	TGTTCCTCTATGGGGTCGTCA	189
BIK	CCGCCAGAGGAGAAATGTCTGA	TCCTCCATAGGGTCCAGGTC	145
TP53	CCCCTCCTCAGCATCTTATCC	GTACAGTCAGAGCCAACCTCAG	124
YY1	GAGAGAACTCACCTCCTGAT	GGCTTCTCTCCAGTATGAAC	325
GAPDH	AGAAGGCTGGGGCTCATTTG	AGGGGCCATCCACAGTCTTC	258

Table S5. Antibodies used for immunoblot analyses

Antibody	Dilution in blocking buffer	Brand	ID
anti-YY1	1:1 000 dilution	Cell Signaling Technology, Danvers, MA, USA	CST-2185
anti-Bfk	1:1 000 dilution	Abcam, Cambridge, UK	Ab-18493
anti-β-Actin	1:10 000 dilution	Sigma-Aldrich, St. Louis, MO, USA	A1978
anti-GAPDH	1:1 000 dilution	Santa Cruz Biotech,Dallas, TX, USA	sc-137179
anti-cleaved-Caspase-3	1:1 000 dilution	Cell Signaling Technology, Danvers, MA, USA	CST-9664
anti-Flag-M2	1:1 000 dilution	Sigma-Aldrich, St. Louis, MO, USA	F1804
Goat Anti-Rabbit IgG Antibody, Fc, HRP conjugate	1:3 000 dilution	Chemicon International, Fisher Scientific, Waltham, MA, USA	AP156P
Goat Anti-Mouse IgG Antibody, Fc, HRP conjugate	1:3 000 dilution	Chemicon International, Fisher Scientific, Waltham, MA, USA	AP127P

Figure S1. Sq-RT-PCR densitometry analysis of YY1 putative transcriptional targets in the apoptotic pathway in CRC cells panel (HCT-116, Caco-2, HT-29 SW620). Signals are normalized with GAPDH, used as housekeeping. Values are presented as Mean ± SD.
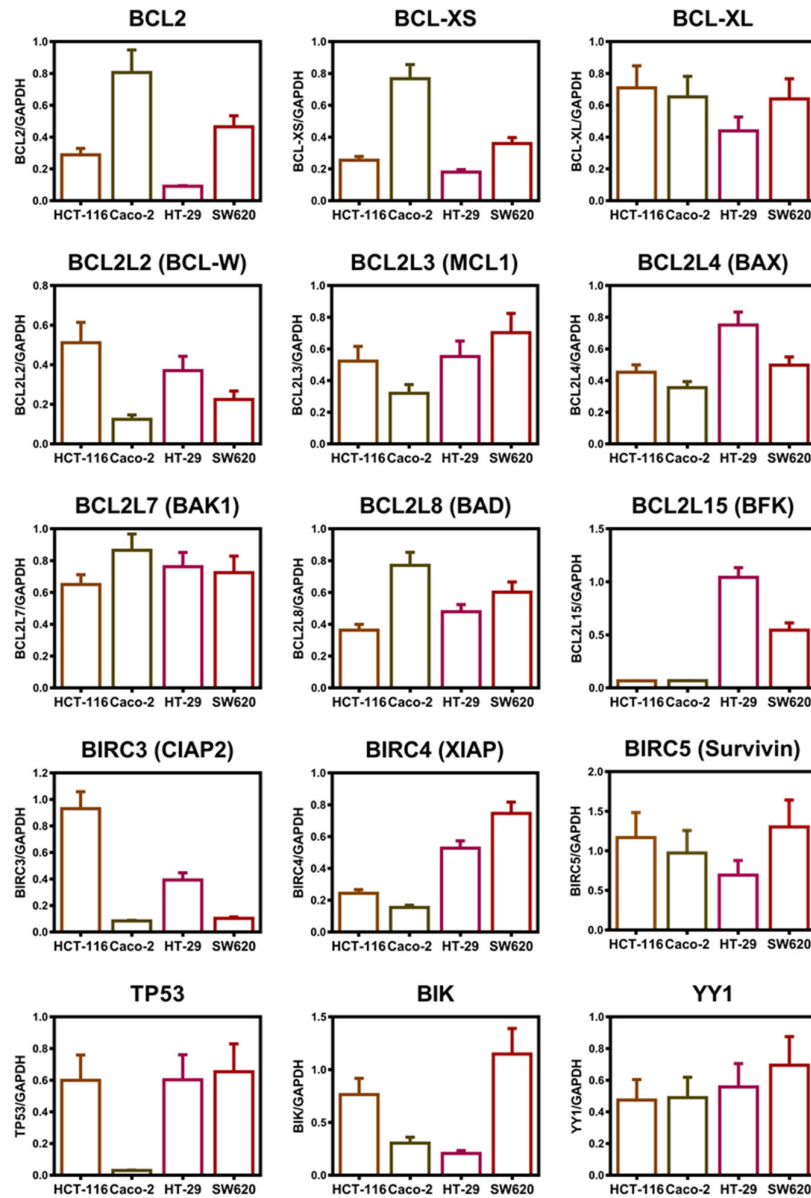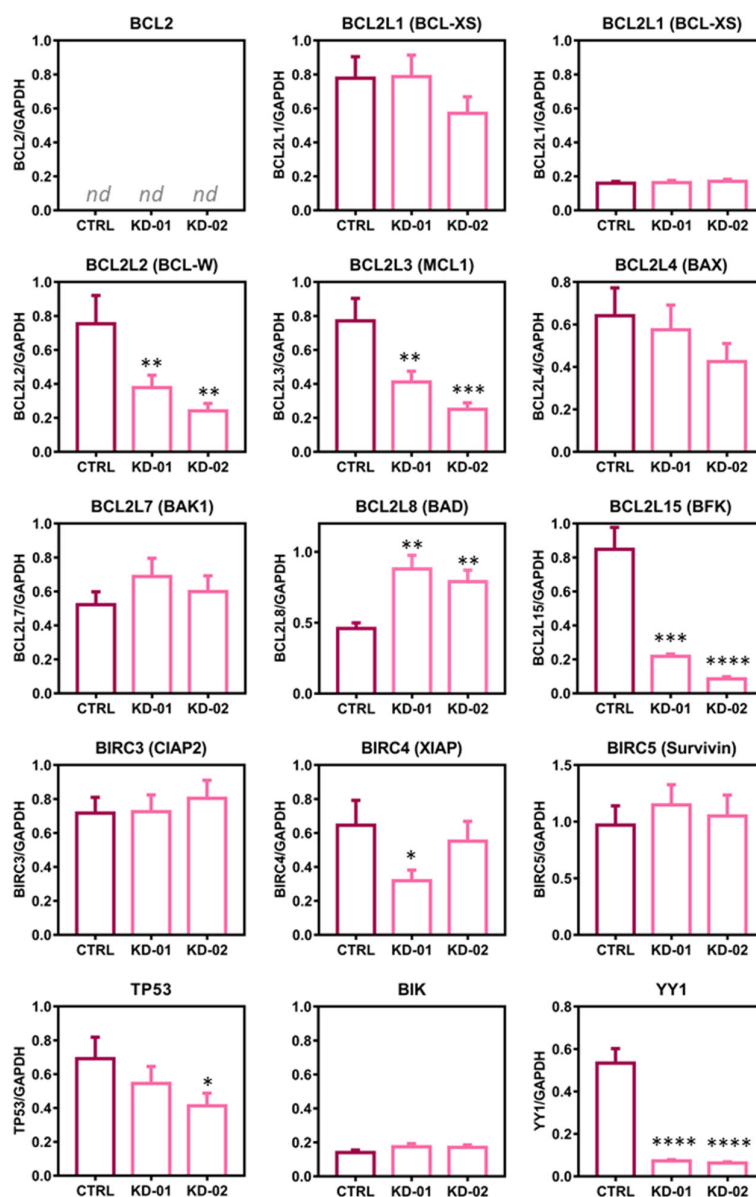
Figure S2. Sq-RT-PCR densitometry analysis of YY1 putative transcriptional targets in the apoptotic pathway in HT-29 cells (CTRL, KD-01, KD-02). Signals are normalized with GAPDH, used as housekeeping. Values are presented as Mean ± SD. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001*.

Figure S3. Sq-RT-PCR densitometry analysis of YY1 putative transcriptional targets in the apoptotic pathway in SW620 cells (CTRL, KD-01, KD-02). Signals are normalized with GAPDH, used as housekeeping. Values are presented as Mean ± SD. *P<0.05; **P<0.01; ***P<0.001.
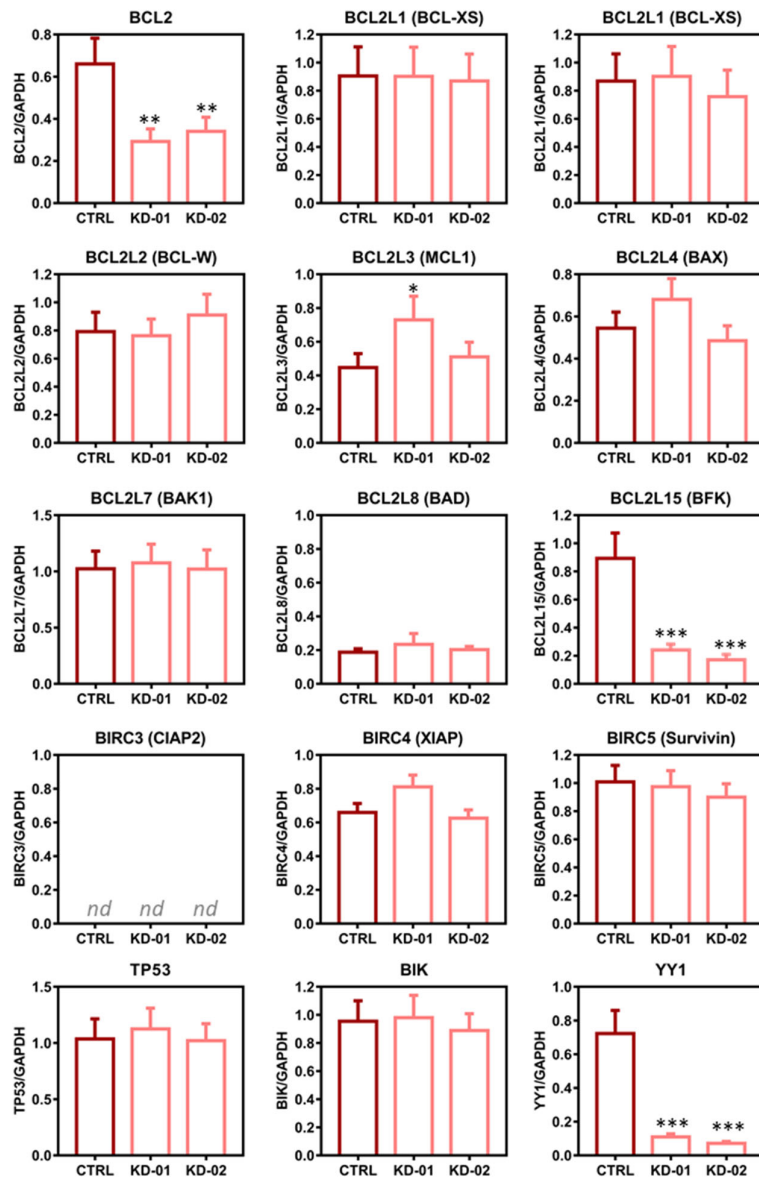
Figure S4. Whole Transcription Factors ChIP-seq binding-clusters within BCL2L15 regulatory region (±10Kb from Transcription Starting Site, TSS). Binding score arbitrary units (from 0 to 1200). Highlighted blue: Transcription factor class, Black: Transcription factor subclass, Light grey: Transcription factor gene name. Each red dot is an independent ChIP-seq experiment and indicates the intersection between the specific transcription factor gene name and its relative binding score to the BCL2L15 regulatory region.
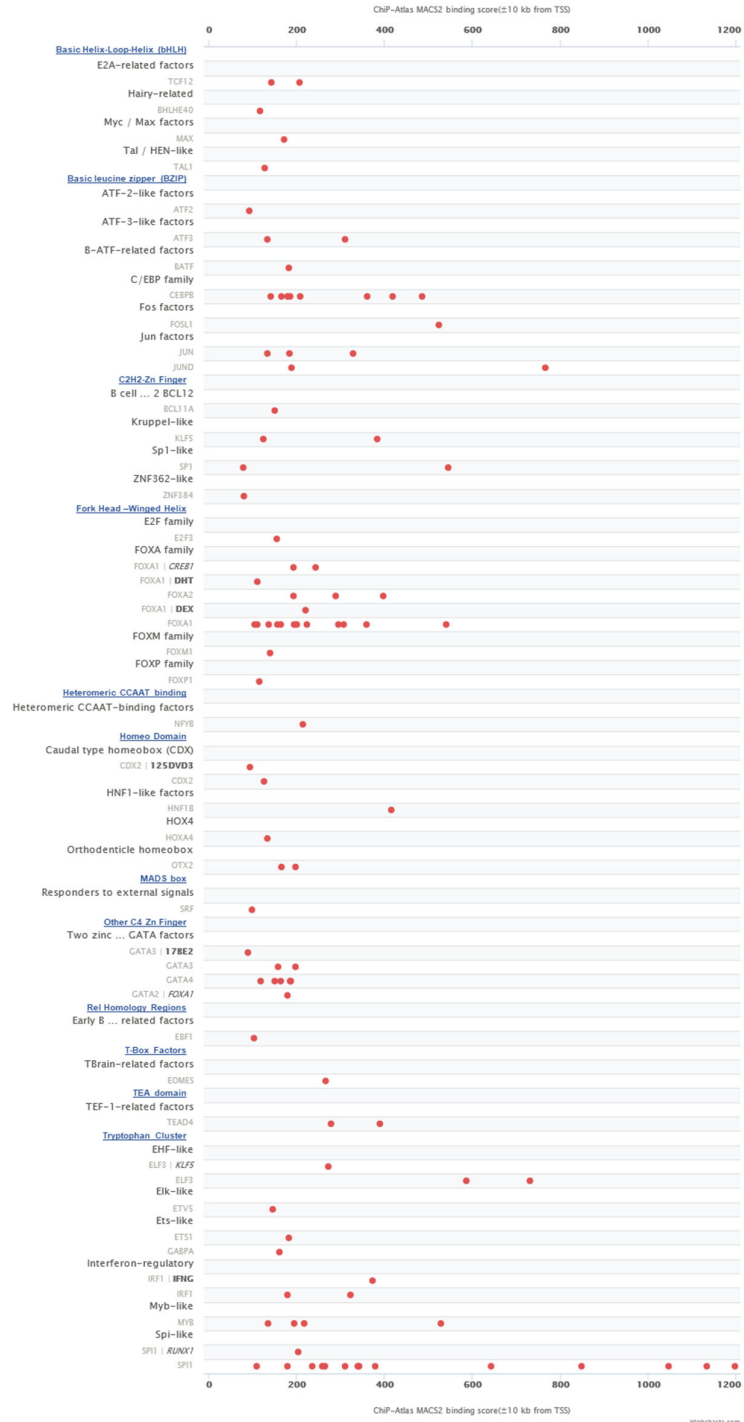
Figure S5. Whole Transcription Factors ChIP-seq binding-clusters, in gastro-intestinal cancer cells, within BCL2L15 regulatory region (±10Kb from Transcription Starting Site, TSS). Binding score arbitrary units (from 0 to 1200). Highlighted blue: Transcription factor class, Black: Transcription factor subclass, Light grey: Transcription factor gene name. Each red dot is an independent ChIP-seq experiment and indicates the intersection between the specific transcription factor gene name and its relative binding score to the BCL2L15 regulatory region.

Figure S6. Correlation plots between YY1 and BCL2L15 expression in twelve CRC GEO datasets. YY1 probe: YY1 200047_s_at; BCL2L15 probe: BCL2L15 236979_at.
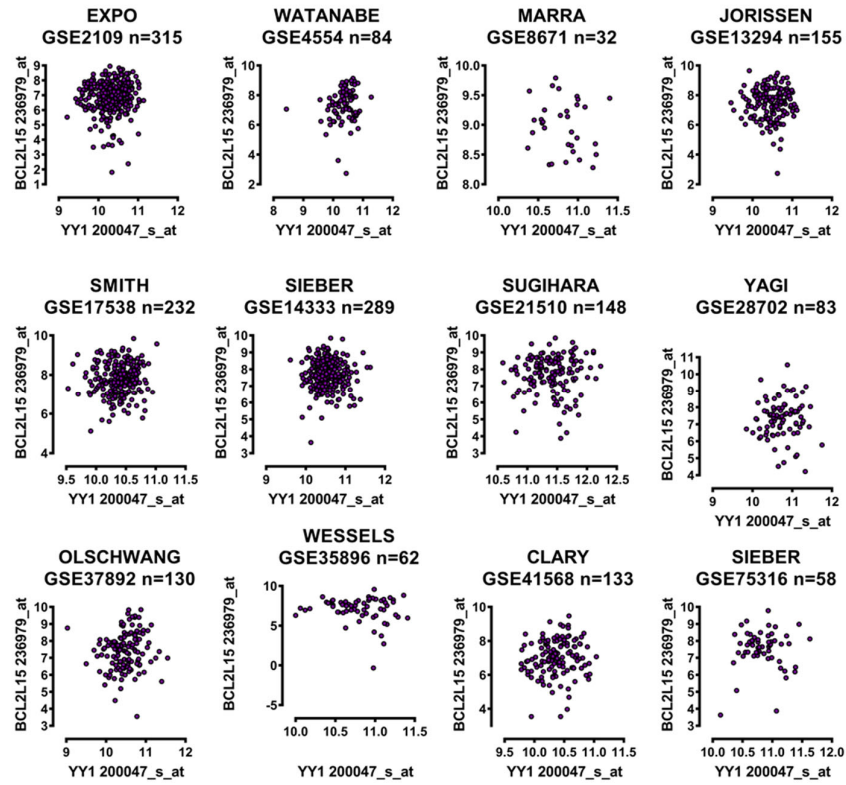
Figure S7. Correlation between YY1 expression and stratification of samples according to genetic mutational status in GSE35896. Dot plots with median of YY1 2log expression in CRC samples of: **A.** APC gene (WT vs. MUT). **B.** BRAF gene (WT vs. MUT). **C.** KRAS gene (WT vs. MUT). **D.** PIK3CA gene (WT vs. MUT). **E.** PTEN gene (WT vs. MUT). **F.** TP53 gene (WT vs. MUT). n.s.= not significant.
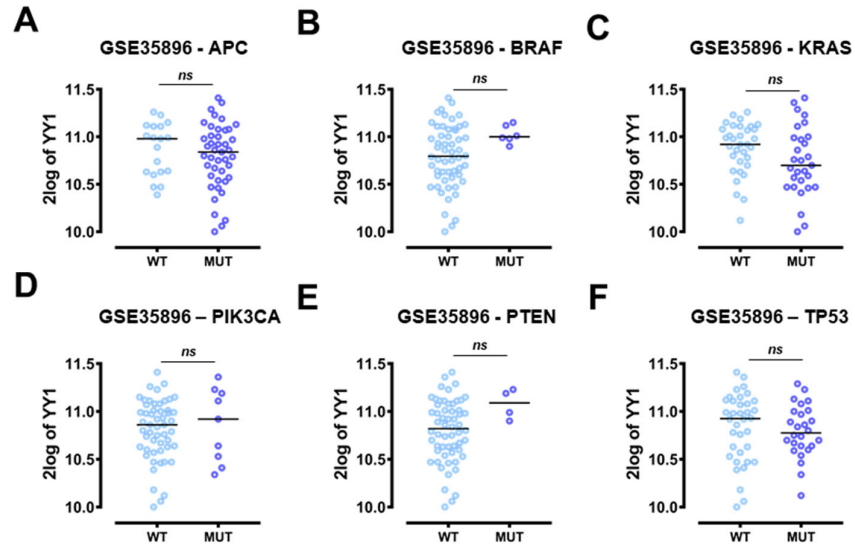
Figure S8. T-SNE plots of non-tumoral cells: YY1 and BCL2L15 expression. **A.** t-SNE plot of stromal cells (clusters). **B.** t-SNE plot of stromal cells (YY1 expression). **C.** t-SNE plot of stromal cells (BCL2L15 expression). **D.** t-SNE plot of myeloid cells (clusters). **E.** t-SNE plot of myeloid cells (YY1 expression). **F.** t-SNE plot of myeloid cells (BCL2L15 expression). **G.** t-SNE plot of T cells (clusters). **H.** t-SNE plot of T cells (YY1 expression). **I.** t-SNE plot of T cells (BCL2L15 expression). **J.** t-SNE plot of B cells (clusters). **K.** t-SNE plot of B cells (YY1 expression). **L.** t-SNE plot of B cells (BCL2L15 expression).
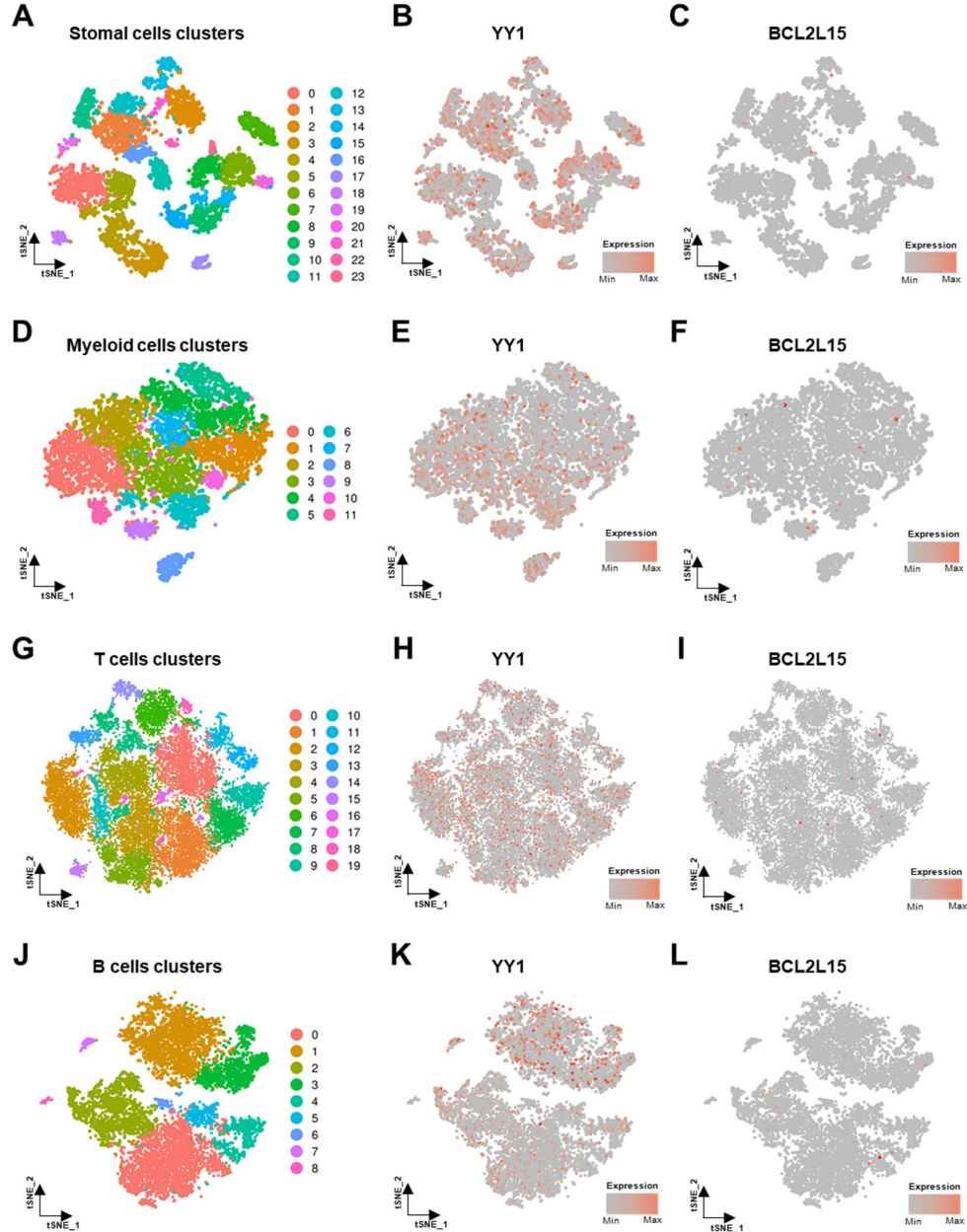
Figure S9. YY1 antibody mono-specificity. The YY1-mAb used for WB analyses selectively recognizes YY1 and not the homologous YY2. Flag-tagged human recombinant YY2 (hr-YY2) purified protein was loaded as control. Anti-FLAG antibody was used to detect hr-YY2.