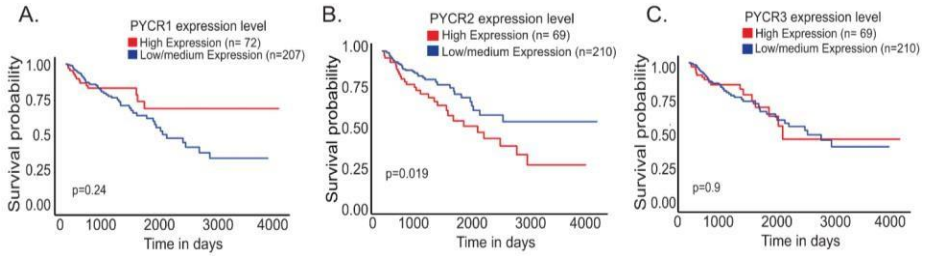

Figure S1. PYCR2 expression in CRC significantly associated with poor patient survival. (A) The Kaplan-Meier (Km) plot survival probability analysis for PYCR1 in CRC patients (*p*=0.24). **(B)** The Km plot analysis for survival probability of CRC patient with PYCR2 expression *(p*=0.019*).* **(C)** The Km plot analysis for survival probability of CRC patient with PYCR3 expression (*p*=0.9).

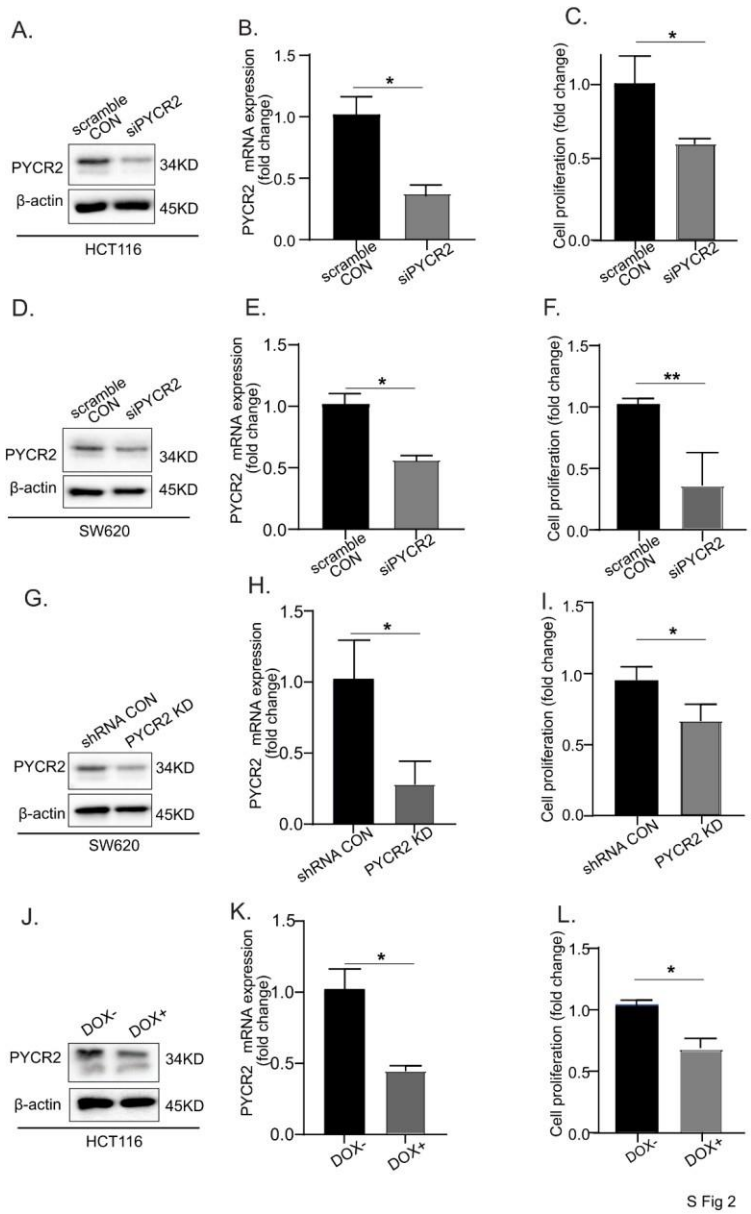

Figure S2. Effects of PYCR2 loss-of-expression in CRC cells using different genetic manipulation tools. (A-F) The loss of function of PYCR2 was performed by using the anti-Human PYCR2 siRNA in HCT116 and SW620 cells. Representatives

immunoblot, and RT-qPCR analysis for PYCR2 mRNA for HCT116, and SW620 respectively and cell proliferation assay for HCT116 and SW620 cells respectively. **(G-I)** PYCR2 knockdown using constitutive shRNA plasmid in SW620 cells. Representative immunoblots of PYCR2 mRNA expression and cell proliferation analysis. **(J-L)** PYCR2 knock down using doxycycline inducible shRNA plasmid in HCT116 cells. The representative images of immunoblots, PYCR2 mRNA expression analysis and cell proliferation assay. *p<0.05, **p<0.01.

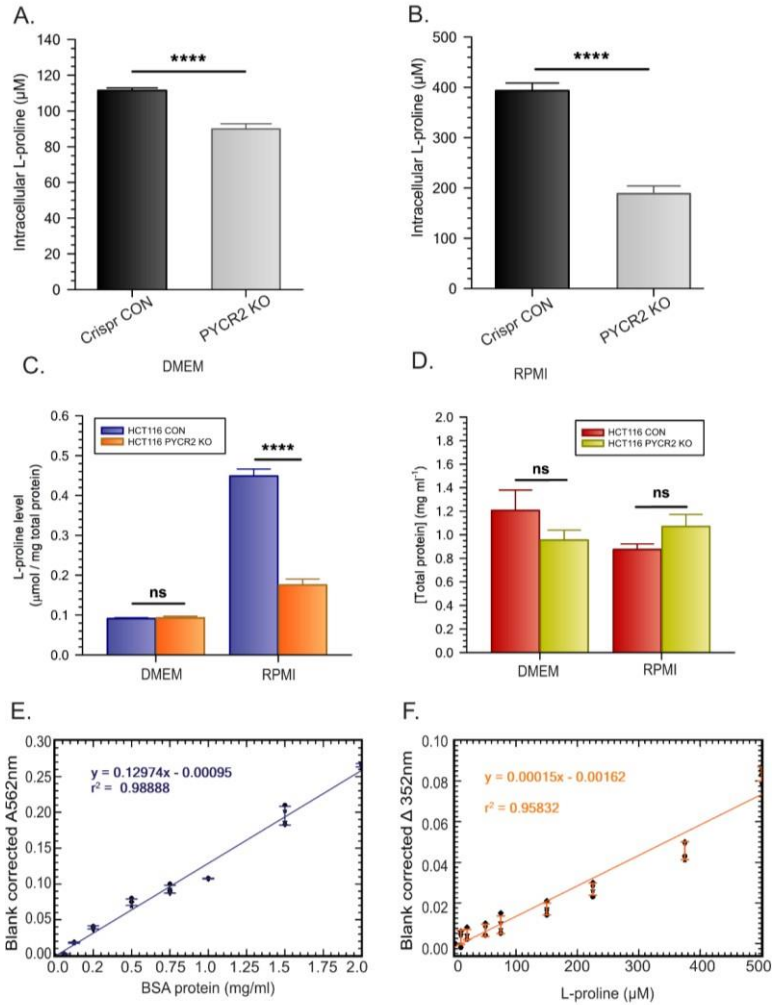

Figure S3. PYCR2 regulates the intracellular proline level. Intracellular L-proline concentrations in HCT116 control and PYCR2-KO (knock out) cells cultured in DMEM. **(A)** and in RPMI-1640. **(B)**. Quantification was performed using modified acid ninhydrin assay**. (C)** Normalized intracellular L-proline levels in HCT116 cells cultured in different growth media and collected at 10^5 cells. Data plotted as mean ± SEM of (n=3) biological replicates, each in triplicate. L-proline concentration values in (μM) units measured by using modified acid ninhydrin assay, were normalized to corresponding total protein concentration values in (mg ml^{-1}) units measured by using BCA Pierce protein assay, to express vertical axis as intracellular L-proline level in (μmol/mg total protein) units. **(D)** Total protein concentrations in HCT116 cells cultured in different growth media and quantified by using BCA Pierce protein assay. Data plotted as mean + SEM of (n=3) biological replicates, each in triplicate. **(E)** BSA known protein concentration standards' calibration plot using BCA Pierce protein assay. **(F)** L-proline known concentration standards' calibration plot using modified acid ninhydrin assay. Statistical significance was determined by Student's t-test, ****, $p < 0.0001$, ns, not statistically significant.

$$\mathrm{L-proline\ level\ (\mu mol/mg\ total\ protein)} = \frac{\mathrm{L-proline\ concentration\ (\mu mol\ L^{-1})}}{\mathrm{Total\ protein\ concentration\ (mg\ mL^{-1})}} \times \frac{1\ \mathrm{L}}{10^3\ \mathrm{mL}}$$

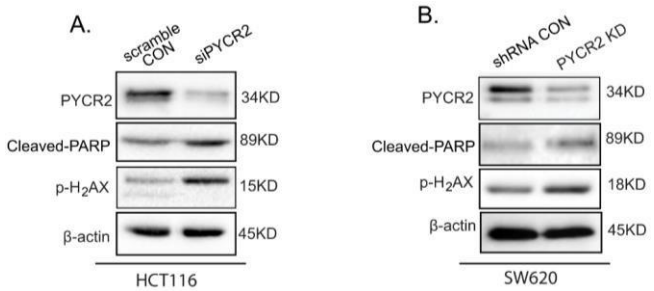

Figure S4. Effects of transient (siRNA-mediated) and constitutive (shRNA plasmid) PYCR2-KD upon markers of apoptosis/cell death in HCT116 and SW620 cells. (A-B) The immunoblot analysis for p-H_2AX and cleaved PARP expression after PYCR2 knock down.

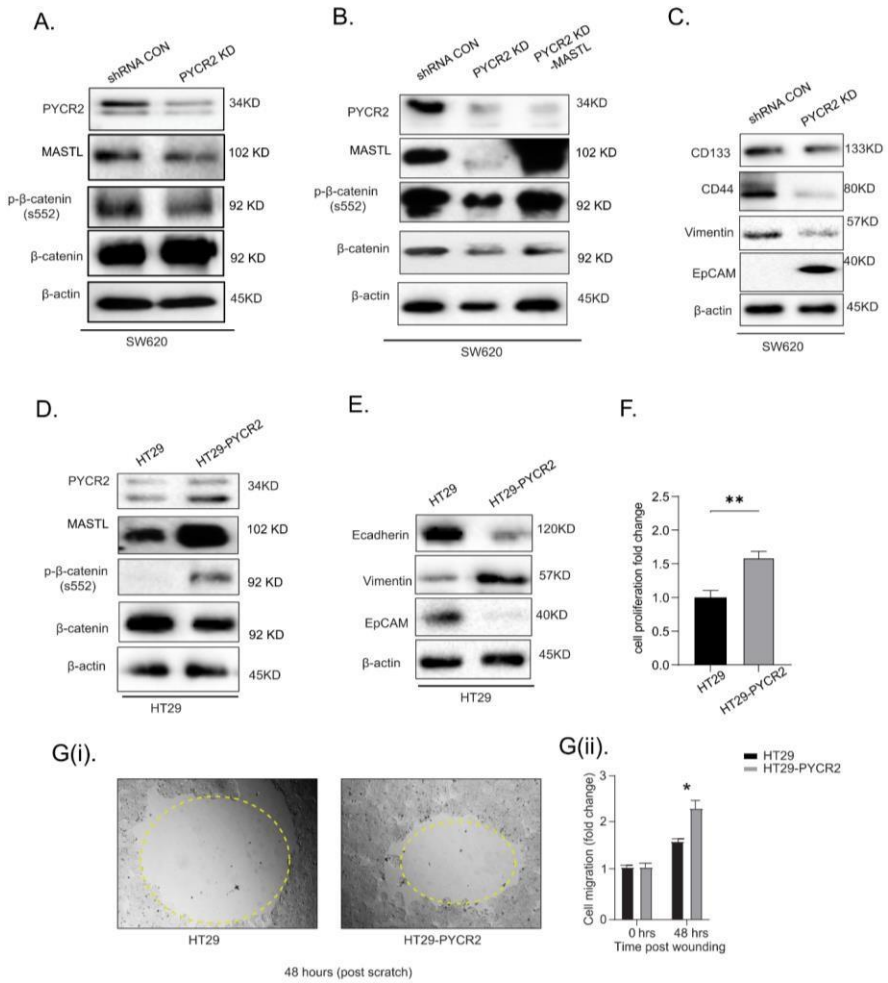

Figure S5. PYCR2 modulates the MASTL/Wnt/β-catenin-signaling. (A-B) Representative immunoblot analysis to investigate the expression of MASTL/Wnt/β-catenin-signaling in control, PYCR2 KD and MASTL overexpression in SW620 cells. **(C)** Immunoblot analysis for CSCs and EMT markers in PYCR2-KD SW620 cells. **(D-E)** Immunoblot analysis of MASTL/Wnt/β-catenin-signaling along with EMT markers in HT29-PYCR2 cells. **(F-Gi-ii)** Cell proliferation and cell migration assay in control and HT29-PYCR2 cells. *p<0.05, **p < 0.01

Table **S1. List of siRNA, shRNA, Crispr/Cas9, pCDNA3 PYCR2 and MASTL cDNA clone used in study**

Name	Company	Catalogue	Sequence
Human Anti-PYCR2 siRNA	Ambion™	AM16708	Sense: GGUCCUUGUGAUAAAACCUtt Antisense: AGGUUUUAUCACAAGGACCtg
Human Anti-PYCR2 shRNA	Sigma Millipore	TRCN0000046368	GCCCTTAAGAAGACCCTCTTA
SMARTvector™ Inducible Human PYCR2 shRNA	Dharmacon	V3SH11252-226257373	AGCAGCGTACGCAGGGTTG
3× sgRNA/Cas9 all-in-one expression clone targeting PYCR2	Genecopoeia	HCP262887-CG12-3-B	a) atccGTGAACCGCGGACCATGAGCGGTTTTAGAGCTAGAAA TAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCTTTTTT b) atccGAGCTGGCCTATGCTCTGGCGGTTTTAGAGCTAGAAA TAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA AGTGGCACCGAGTCGGTGCTTTTTT c) atccGAAGCCCCGCGCCAGAGCATGTTTTAGAGCTAGAAAT AGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCTTTTTT
MASTL PCS6 (pCMVSPORT6)	Transomic	BC009107	
pCDNA3 PYCR2			Prepared in lab

Table S2. List of antibodies and reagents with their dilution used in the study.

SN	Name of Antibodies/chemicals	Company	Catalog #	Dilution used
1	PYCR2	ProteinTech	17146-1-AP	1:1000 (WB) and 1:250 (IHC)
2	β-actin	ProteinTech	20536-1-AP	1:20000
3	p-H2AX	Cell Signaling	2577	1:1000 (WB) and 1:500 (IHC)
4	Cleaved PARP	Cell Signaling	5625S	1:1000 (WB)
5	p-AKT	Cell Signaling	4060	1:1000 (WB)
6	AKT	Santa Cruz	Sc-5298	1:1000 (WB)
7	Cyclin D1	ABclonal	A19038	1:1000 (WB)
8	Cleaved caspase 3	Cell Signaling	9664S	1:1000 (WB) and 1:1000 (IHC)
9	Mastl	Sigma Millipore	MABT372	1:1000 (WB)
10	p-β-catenin	Cell Signaling	5651S	1:1000 (WB)
11	β-catenin	BD Bioscience	610154	1:1000 (WB)
12	EpCam	Cell Signaling	3599S	1:500 (IF)/1:1000 (WB)
13	Ecadherin	BD Bioscience	610405	1:1000 (WB)
14	Vimentin	Cell Signaling	5741S	1:1000 (WB)
15	CD133	Cell Signaling	64326T	1:1000 (WB)
16	CD44	Cell Signaling	3578S	1:1000 (WB)
17	SOX2	Cell Signaling	2748S	1:1000 (WB)
18	Precision plus protein standard	Biorad	1610375	
19	GKI			

Spheroid media protocol (50ml)

- B-27 supplement (# 17504044 Thermofisher) -1X
- HEPES (# 15630106 Thermofisher) - 10mM
- DMEM/F-12, GlutaMAX (# 10565018 Thermofisher)- Remaining volume

Table S3. List of qPCR primers used in study.

PYCR1

1	Forward	GGACAAGGTGAAGCTGGACT
	Reverse	AGGACGTGTCAATCCTTGC
2	Forward	TTTCTGCTCTCAGGAAGATG
	Reverse	ACCACAATGTGTCTGTCCTC
3	Forward	AAGATGGCAGGCTTGTGGAGCA
	Reverse	CAGAGCATCCAGGGCTGTGAAA

PYCR2

1	Forward	TCCCTCGCTGAGGGGGTTCGT
	Reverse	CCATCTTCCTGAGCGCGGACACC
2	Forward	AGATGGGTGTGAACCTGACA
	Reverse	CTTCACAGCCAGAAACAGGA
3	Forward	CGGCTCACAAGATAATAGCC
	Reverse	TTCACCGTCTCCTTGTTGC

PYCR3

1	Forward	CCCAGACCCTGCTGGGGGACG
	Reverse	CTCCACGGCGCTCATGGTGG
2	Forward	GTGGAAGCTCAGCACATACTGG
	Reverse	CTTGGTGGCAAAGATGACGAGC

CD133

Forward	GGTGCTGTTCATGTTCTCGA
Reverse	ACCGACTGAGACCCAACATC

CD44

Forward	CCAGAAGGAACAGTGGTTTGGC
Reverse	ACTGTCCTCTGGGCTTGGTGTT

Sox2

Forward	GAGCTTTGCAGGAAGTTTGC
Reverse	GCAAGAAGCCTCTCCTTGAA

β-actin

Forward	CACCATTGGCAATGAGCGGTTC
Reverse	AGGTCTTTGCGGATGTCCACGT