Cancer stem cell-associated miRNAs serve as prognostic biomarkers in colorectal cancer

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Supplementary Information

Supplementary Fig 1: Chemoresistant cells express high levels of CD44v6 A, Schematics of chemoresistant cell generation. **B,** Images of 5-fluorouracil resistant (5FUR) cells compared to their respective parental cells. **C,** Cytotoxicity of 5FU on 5FUR cells in comparison to their parental cells (n = 6 per time point Mann-Whitney's U test). **D,** The expression of CD44 variants between 5FUR and parental cells (n=3, Mann-Whitney's U test). **E,** CD44v6 was consistently overexpressed between 5FUR and CDCSCs among all CD44 variants. **F,** Immunofluorescence images of CD44v6 (GFP, right) and DAPI nuclear staining (middle) and merged image (right) **G,** western blot of CD44v6 expression in HCT116/SW480 **H,** The expression of stemness-suppressive miRNAs between 5FUR cells and parental cells. * $p < 0.05$, ** $p < 0.01$

Supplementary Fig 2: Pathways associated with CD44v6 positive and negative cancer stem cells. Pathway analysis of downstream target genes of differentially expressed miRNAs identified between CD44v6 positive and negative colorectal cancer stem cells.

Supplementary Fig 3: Pathways associated with miRNAs downregulated in CD44v6 positive cancer stem cells. A, Representation of pathways associated with downstream target genes of identified overexpressed miRNAs. Colored square represents pathways and colored circles are genes associated with the same colored square. **B**, Representation of pathways associated with downstream target genes of identified downregulated miRNAs. Colored square represents pathways and colored circles are genes associated with the same colored square.

Supplementary Table 1. Cohort characteristics

		Cohort 1	Cohort 2
Variable		No. (%)	No. (%)
Age	Median (range)	68 (32-92)	65 (35-90)
Sex	Female	47 (42)	41 (39)
	Male	65 (58)	65 (61)
Tumor location	Colon	76 (68)	64 (60)
	Rectum	36 (32)	42 (40)
	I	21 (19)	22 (21)
	II	32 (29)	39 (37)
	III	29 (26)	18 (17)
	IV	30 (27)	27 (25)
	I	13 (12)	7 (7)
	II	17 (15)	18 (17)
	III	68 (61)	42 (40)
	IV	14 (12)	39 (37)
Histology	Well and mod	70 (62)	99 (93)
	Poor and muc	42 (38)	7 (7)
Venous invasion	Presence	Cohort 2	90 (85)
	Absence	No. (%)	16 (150)
Lymphatic invasion	Presence	68 (32-92)	60 (57)
	Absence	47 (42)	46 (43)

Supplementary Table 2: Xenograft transplantation assay: HCT116 parental v. SDCSC comparison

Xenotransplantation assay*			
Cell No.	**1 x 10^5**	**1 x 10^4**	**1 x 10^3**
HCT116 parental	**6/6**	**4/6**	**1/6**
HCT116 SDCSC	**6/6**	**6/6**	**6/6**

*Immunocompromised mice were injected with various numbers of SDCSCs or their parental cells and the number of mice that developed tumors counted

Supplementary Table 3: Xenograft transplantation assay: HCT116 SDCSC CD44v6$^-$ v. CD44v6$^+$ comparison

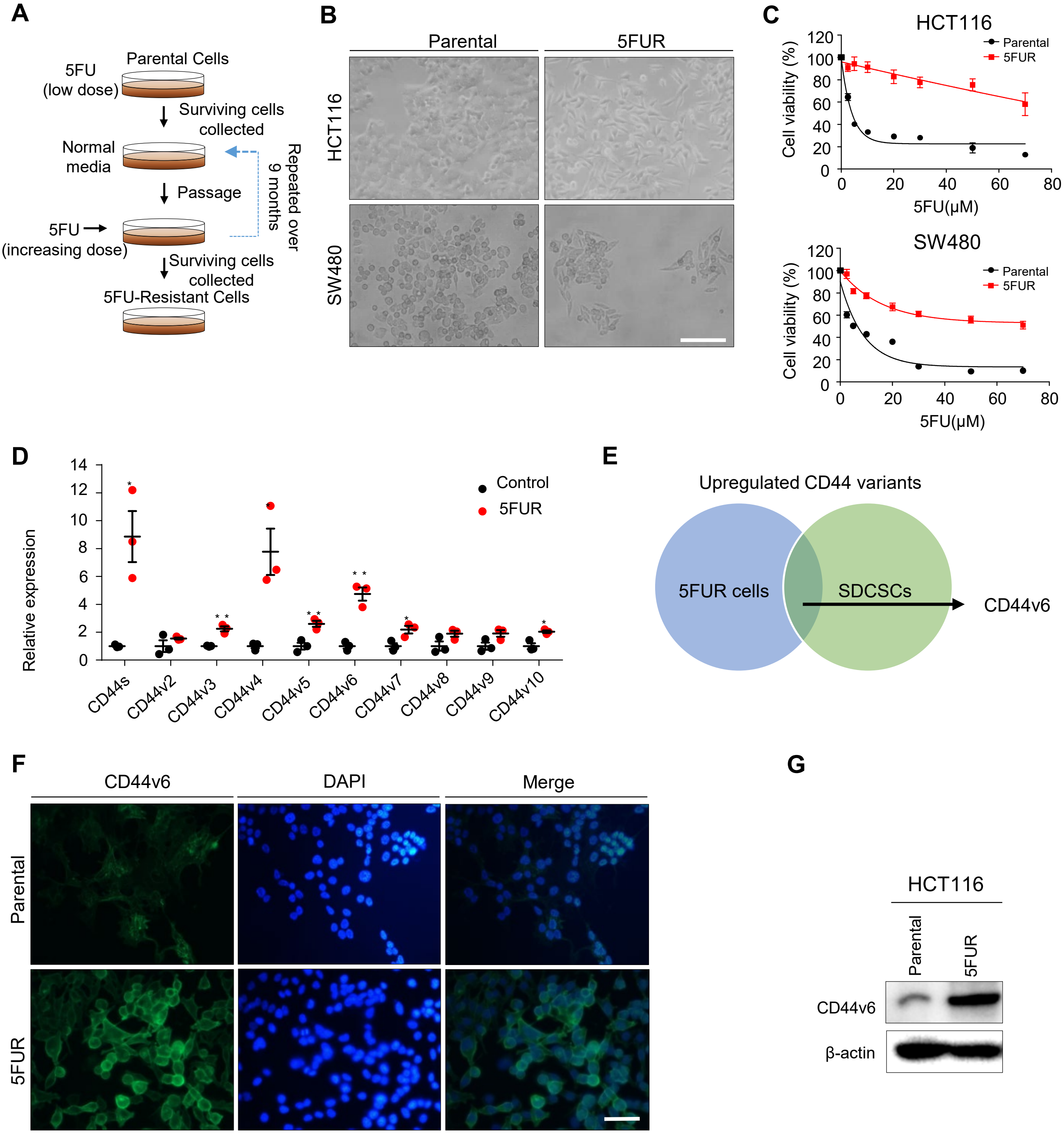
Xenotransplantion assay*		
Cell No	**1 x 10^3**	**1 x 10^2**
SDCSC CD44v6$^-$	**0/4**	**0/4**
SDCSC CD44v6$^+$	**4/4**	**2/4**

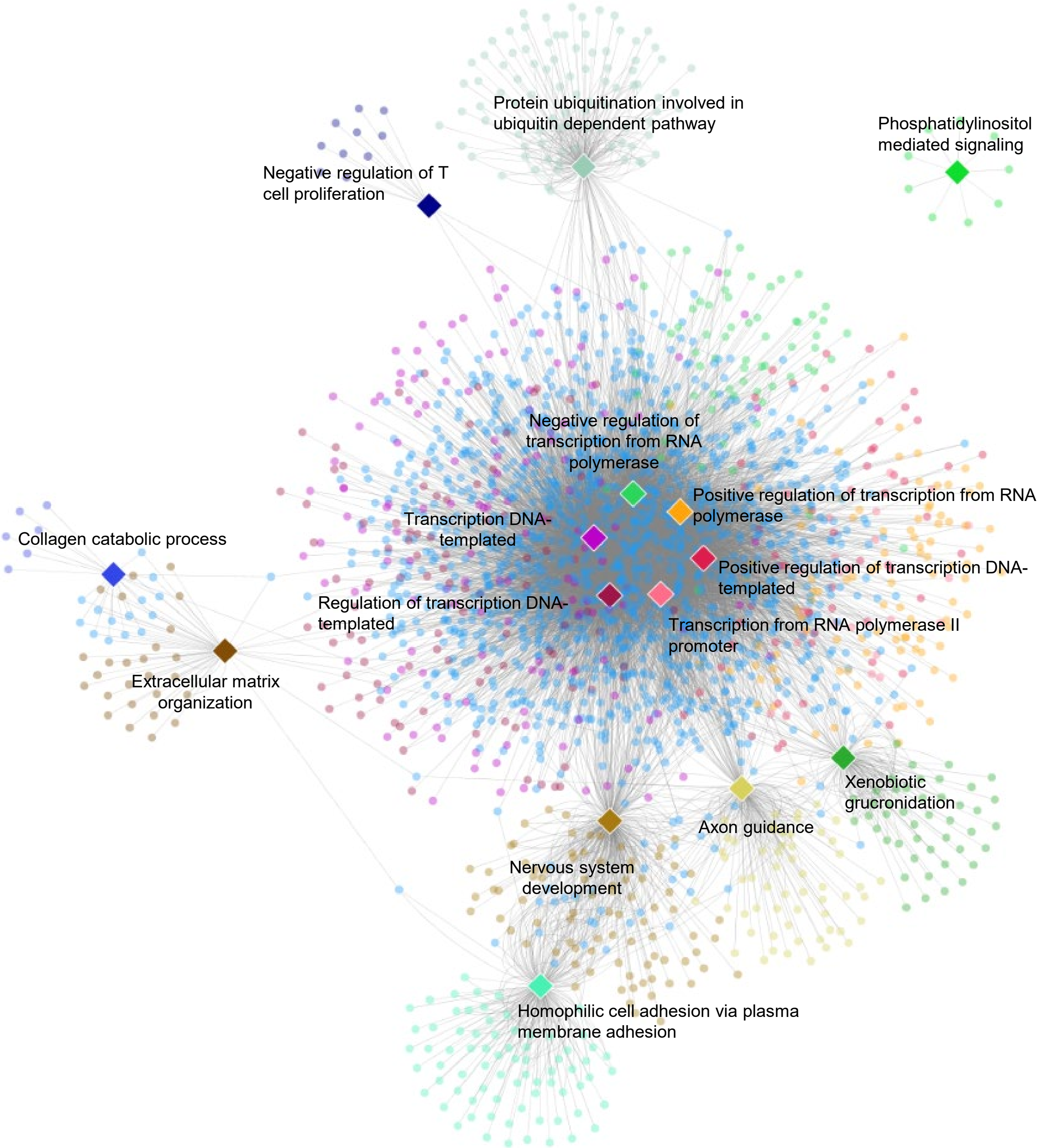
*****Immunocompromised mice were injected with various numbers of CD44v6$^+$ SDCSCs and CD44v6$^-$ SDCSCs and the number of mice that developed tumors counted.

Supplementary Table 4: List of differentially expressed miRNAs between CD44v6 positive and negative spheroid derived cancer stem cells

microRNA	log2FoldChange	p-value
Upregulated		
hsa-miR-1246	4.284343	2.07E-08
hsa-miR-122-5p	5.142072	1.80E-07
hsa-miR-3605-3p	3.699013	4.29E-06
hsa-miR-423-5p	3.173	0.000179
hsa-miR-3182	3.600355	0.000192
hsa-miR-4284	4.153804	0.000231
hsa-miR-338-3p	2.773659	0.020168
hsa-miR-2116-3p	2.288411	0.03215
hsa-miR-3609	2.449211	0.038835
hsa-miR-320b	1.932736	0.039924
Downregulated		
hsa-miR-31-3p	-3.91411	1.70E-05
hsa-miR-19a-3p	-3.29285	5.60E-05
hsa-miR-29b-3p	-3.43576	0.000144
hsa-miR-19b-3p	-3.18754	0.000231
hsa-miR-21-3p	-2.81419	0.000257
hsa-miR-374a-5p	-3.10058	0.000257
hsa-miR-340-5p	-3.03188	0.00027
hsa-miR-18a-5p	-2.80731	0.000795
hsa-miR-374a-3p	-2.69516	0.001777
hsa-miR-101-3p	-2.61788	0.002071
hsa-miR-561-5p	-2.61604	0.002427
hsa-miR-106b-5p	-2.44869	0.002903
hsa-miR-100-3p	-2.39362	0.008258
hsa-miR-20a-5p	-2.87515	0.008258
hsa-miR-590-3p	-2.63081	0.008301
hsa-miR-141-3p	-2.36889	0.013367
hsa-miR-153-3p	-2.57055	0.013721
hsa-miR-30b-5p	-2.33372	0.013721
hsa-miR-30e-5p	-2.10295	0.015321
hsa-miR-660-5p	-2.3817	0.016878
hsa-miR-429	-2.05656	0.018854
hsa-miR-1307-5p	-2.34819	0.019584
hsa-miR-141-5p	-2.06997	0.020168
hsa-miR-15b-3p	-2.56947	0.021944
hsa-miR-140-5p	-2.51024	0.026942
hsa-miR-34a-3p	-3.158	0.028502
hsa-miR-7-5p	-1.96159	0.034788
hsa-miR-577	-1.99848	0.034948
hsa-let-7f-1-3p	-2.23188	0.039924
hsa-miR-17-5p	-2.16379	0.04355
hsa-miR-7-1-3p	-1.98415	0.04355
hsa-miR-200a-3p	-1.94489	0.048989

Supplementary Fig 1




Protein ubiquitination involved in ubiquitin dependent pathway
Phosphatidylinositol mediated signaling
Negative regulation of T cell proliferation
Negative regulation of transcription from RNA polymerase
Positive regulation of transcription from RNA polymerase
Transcription DNA-templated
Collagen catabolic process
Positive regulation of transcription DNA-templated
Regulation of transcription DNA-templated
Transcription from RNA polymerase II promoter
Extracellular matrix organization
Xenobiotic grucronidation
Axon guidance
Nervous system development
Homophilic cell adhesion via plasma membrane adhesion

Supplementary Fig. 3