

## **SUPPLEMENTAL INFORMATION**

<b>Table S1. Significantly expressed miRNAs in different CSC subpopulations*.</b>	
<u>LGR5/ALDH FACS Isolation</u>	<u>miRNAs</u>
LGR5+/ALDH- cells	310
LGR5-/ALDH+ cells	15
<u>CD166/ALDH FACS Isolation</u>	<u>miRNAs</u>
CD166+/ALDH- cells	1
CD166-/ALDH+ cells	2
<u>CD166/LRG5 FACS Isolation</u>	<u>miRNAs</u>
CD166+/LRG5- cells	1
CD166-/LRG5+ cells	102
<u>LRIG1/LGR5 FACS Isolation</u>	<u>miRNAs</u>
LRIG1+/LGR5- cells	4
LRIG1-/LGR5+ cells	673
<u>LRIG1/ALDH FACS Isolation</u>	<u>miRNAs</u>
LRIG1+/ALDH- cells	101
LRIG1-/ALDH+ cells	4
<u>CD166/LRIG1 FACS Isolation</u>	<u>miRNAs</u>
CD166+/LRIG1- cells	29
CD166-/LRIG1+ cells	4
*Based on $p$ -value < 0.05.	

<b>Table S2. The mRNAs predicted to be targeted by the miRNAs in each CSC subpopulation were found to have different functional classifications</b>				
LGR5 vs. ALDH	zinc finger motifs	vs.	phosphoproteins, and protein binding	Figure 2 vs. Figure 3
CD166 vs. ALDH	alternative splicing, phosphoproteins, and membrane	vs.	phosphoprotein, nucleus, cytoplasm, and nucleus functions	Figure S2 vs. Figure S3
LRIG1 vs. CD166	alternative splicing, transcriptional regulation, and nucleus	vs.	phosphoprotein functions	Figure S5 vs. Figure S6
LRIG1 vs. ALDH	alternative splicing and as protein kinase domains	vs.	phosphoproteins, protein binding	Figure S8 vs. Figure S9
LGR5 vs. LRIG1	cytoplasm and different protein domains	vs.	alternative splicing, protein binding, and phosphoprotein	Figure S11 vs. Figure S12
LGR5 vs. CD166	splice variant, phosphoprotein and zinc finger motifs	vs.	phosphoprotein, protein binding, cytoplasm, and nucleus	Figure S14 vs. Figure S15

### Tables S3 & S4. BIOINFORMATICS ANALYSIS OF LGR5 and ALDH DATA

To determine what might distinguish the different CSC subpopulations from each other, a detailed analysis of the LGR5 and ALDH data was done. Then a bioinformatics analysis was done to determine which genes in the Retinoic Acid (Table 1) and WNT (Table 2) signaling pathways were predicted to be targeted by these miRNAs.

<b>Table S3. Retinoic Acid Pathway Genes Analyzed</b>	
<i>ADH1A</i>	Alcohol Dehydrogenase 1A
<i>ADH1B</i>	Alcohol Dehydrogenase 1B
<i>ADH1C</i>	Alcohol Dehydrogenase 1C
<i>ADH4</i>	Alcohol Dehydrogenase 4
<i>ADH5</i>	Alcohol Dehydrogenase 5
<i>ADH6</i>	Alcohol Dehydrogenase 6
<i>ADH7</i>	Alcohol Dehydrogenase 7
<i>ALDH16A1</i>	Aldehyde Dehydrogenase 16A1
<i>ALDH18A1</i>	Aldehyde Dehydrogenase 18A1
<i>ALDH1A1</i>	Aldehyde Dehydrogenase 1A1
<i>ALDH1A2</i>	Aldehyde Dehydrogenase 1A2
<i>ALDH1A3</i>	Aldehyde Dehydrogenase 1A3
<i>ALDH1B1</i>	Aldehyde Dehydrogenase 1B1
<i>ALDH1L1</i>	Aldehyde Dehydrogenase 1L1
<i>ALDH1L2</i>	Aldehyde Dehydrogenase 1L2
<i>ALDH2</i>	Alcohol Dehydrogenase 2
<i>ALDH3A1</i>	Aldehyde Dehydrogenase 3A1
<i>ALDH3A2</i>	Aldehyde Dehydrogenase 3A2
<i>ALDH3B1</i>	Aldehyde Dehydrogenase 3B1
<i>ALDH3B2</i>	Aldehyde Dehydrogenase 3B2
<i>ALDH4A1</i>	Aldehyde Dehydrogenase 4A1
<i>ALDH5A1</i>	Aldehyde Dehydrogenase 5A1
<i>ALDH6A1</i>	Aldehyde Dehydrogenase 6A1
<i>ALDH7A1</i>	Aldehyde Dehydrogenase 7A1
<i>ALDH8A1</i>	Aldehyde Dehydrogenase 8A1
<i>ALDH9A1</i>	Aldehyde Dehydrogenase 9A1
<i>CRABP1</i>	Cellular Retinoic Acid Binding Protein 1
<i>CRABP2</i>	Cellular Retinoic Acid Binding Protein 2
<i>CRBP</i>	Cellular Retinol Binding Protein
<i>CYP26A1</i>	Cytochrome P450 26A1
<i>CYP26B1</i>	Cytochrome P450 26B1
<i>CYP26C1</i>	Cytochrome P450 26C1
<i>RARA</i>	Retinoic Acid Receptor Alpha
<i>RARB</i>	Retinoic Acid Receptor Beta

<i>RARG</i>	Retinoic Acid Receptor Gamma
<i>RBP1</i>	Retinol Binding Protein 1
<i>RBP2</i>	Retinol Binding Protein 2
<i>RBP3</i>	Retinol Binding Protein 3
<i>RBP4</i>	Retinol Binding Protein 4
<i>RBP5</i>	Retinol Binding Protein 5
<i>RBP7</i>	Retinol Binding Protein 7
<i>RDH10</i>	Retinol Dehydrogenase 10
<i>RDH11</i>	Retinol Dehydrogenase 11
<i>RDH12</i>	Retinol Dehydrogenase 12
<i>RDH13</i>	Retinol Dehydrogenase 13
<i>RDH14</i>	Retinol Dehydrogenase 14
<i>RDH16</i>	Retinol Dehydrogenase 16
<i>RDH5</i>	Retinol Dehydrogenase 5
<i>RDH8</i>	Retinol Dehydrogenase 8
<i>RXRA</i>	Retinoid X Receptor Alpha
<i>RXRB</i>	Retinoid X Receptor Beta
<i>RXRG</i>	STRA6
<i>STRA6</i>	Stimulated by retinoic acid 6
<i>TTR</i>	Transthyretin

<b><u>Table S4. WNT Pathway Genes Analyzed</u></b>	
<i>APC</i>	adenomatous polyposis coli
<i>AXIN1</i>	aka Axin 1 a protein coding gene
<i>AXIN2</i>	aka Axin 2 a protein coding gene
<i>BTRC</i>	Beta-transducin repeat containing E3 ubiquitin protein ligase
<i>CSNK1A1/CK1</i>	casein kinase 1 alpha 1
<i>CK1 beta</i>	casein kinase 1 beta 1
<i>CK1 gamma 1</i>	casein kinase 1 gamma 1
<i>CK1 gamma 2</i>	casein kinase 1 gamma 2
<i>CK1 gamma 3</i>	casein kinase 1 gamma 3
<i>CK1 delta</i>	casein kinase 1 delta
<i>CK1 epsilon</i>	casein kinase 1 epsilon
<i>CTNNB1</i>	Beta-catenin 1
<i>DVL1</i>	dishevelled (DSH) DVL in mammals 1
<i>DVL2</i>	dishevelled (DSH) DVL in mammals 2
<i>DVL3</i>	dishevelled (DSH) DVL in mammals 3
<i>FZD1</i>	Frizzled Class Receptor 1
<i>FZD2</i>	Frizzled Class Receptor 2
<i>FZD3</i>	Frizzled Class Receptor 3

<i>FZD4</i>	Frizzled Class Receptor 4
<i>FZD5</i>	Frizzled Class Receptor 5
<i>FZD6</i>	Frizzled Class Receptor 6
<i>FZD7</i>	Frizzled Class Receptor 7
<i>FZD8</i>	Frizzled Class Receptor 8
<i>FZD9</i>	Frizzled Class Receptor 9
<i>FZD10</i>	Frizzled Class Receptor 10
<i>GSK3B</i>	Glycogen Synthase Kinase 3 Beta
<i>LGR4</i>	Leucine Rich Repeat Containing G Protein-Coupled Receptor 4
<i>LGR5</i>	Leucine Rich Repeat Containing G Protein-Coupled Receptor 5
<i>LGR6</i>	Leucine Rich Repeat Containing G Protein-Coupled Receptor 6
<i>LRP5</i>	Low-density lipoprotein receptor-related protein 5
<i>LRP6</i>	Low-density lipoprotein receptor-related protein 6
<i>RNF43</i>	Ring finger protein 43
<i>ZNRF3</i>	Zinc and Ring Finger 3

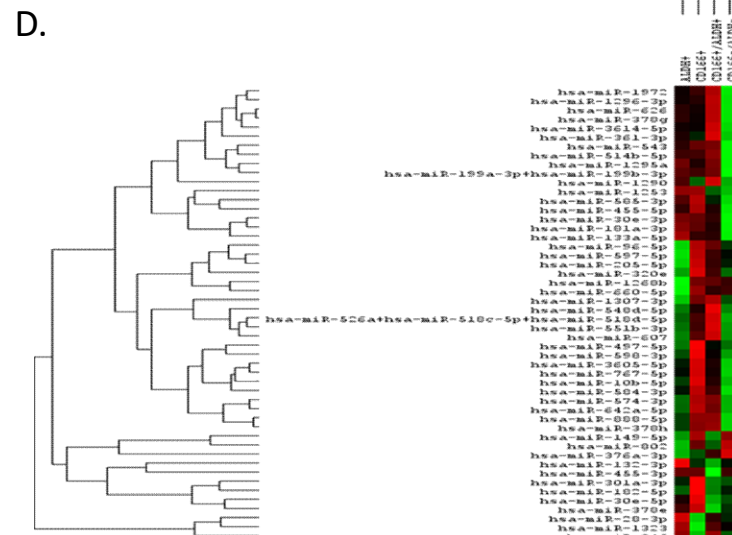
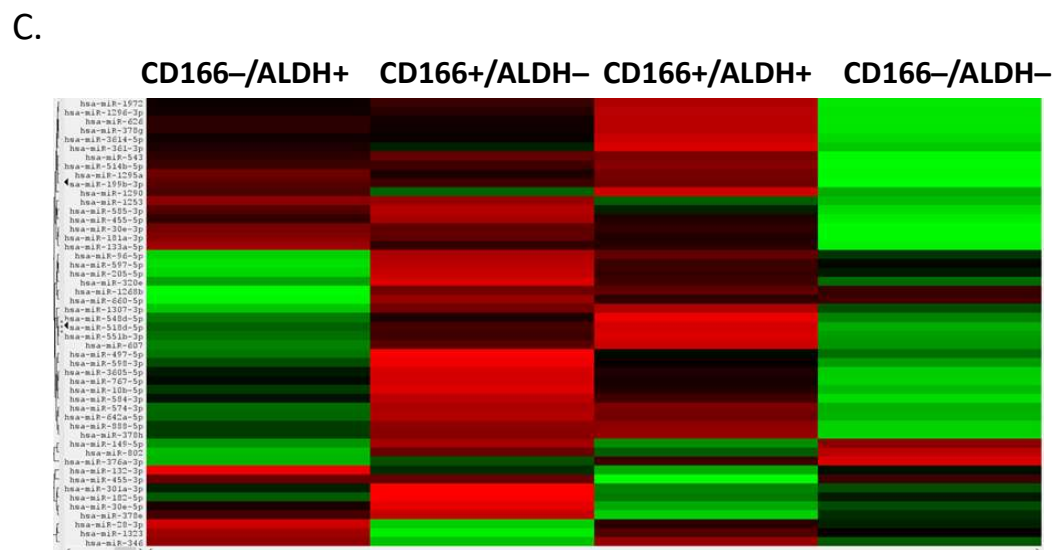
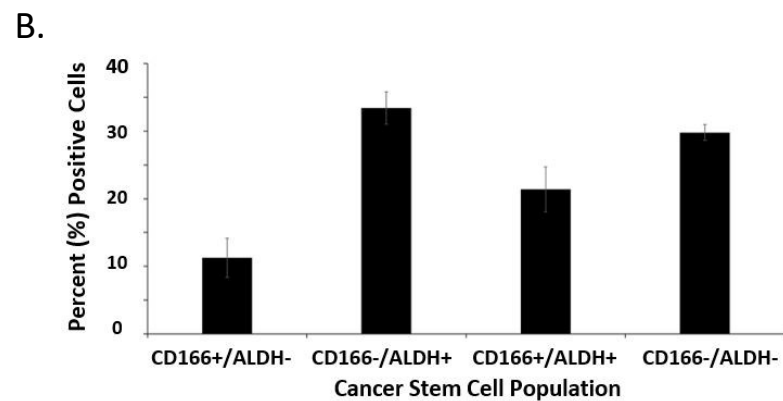
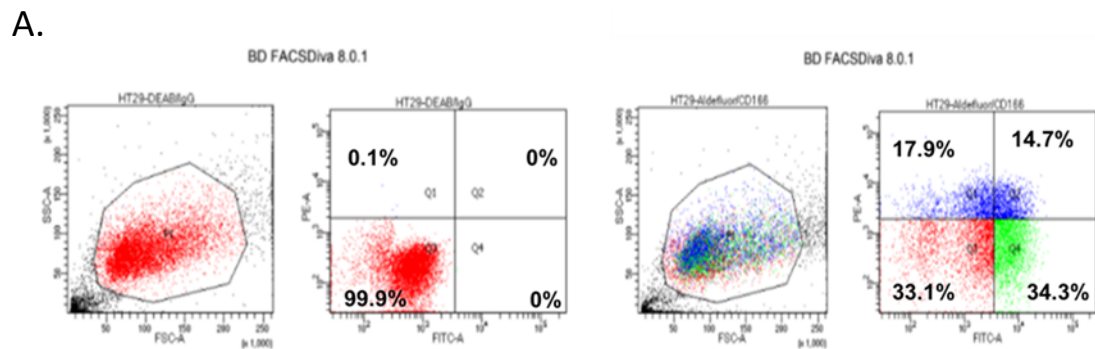
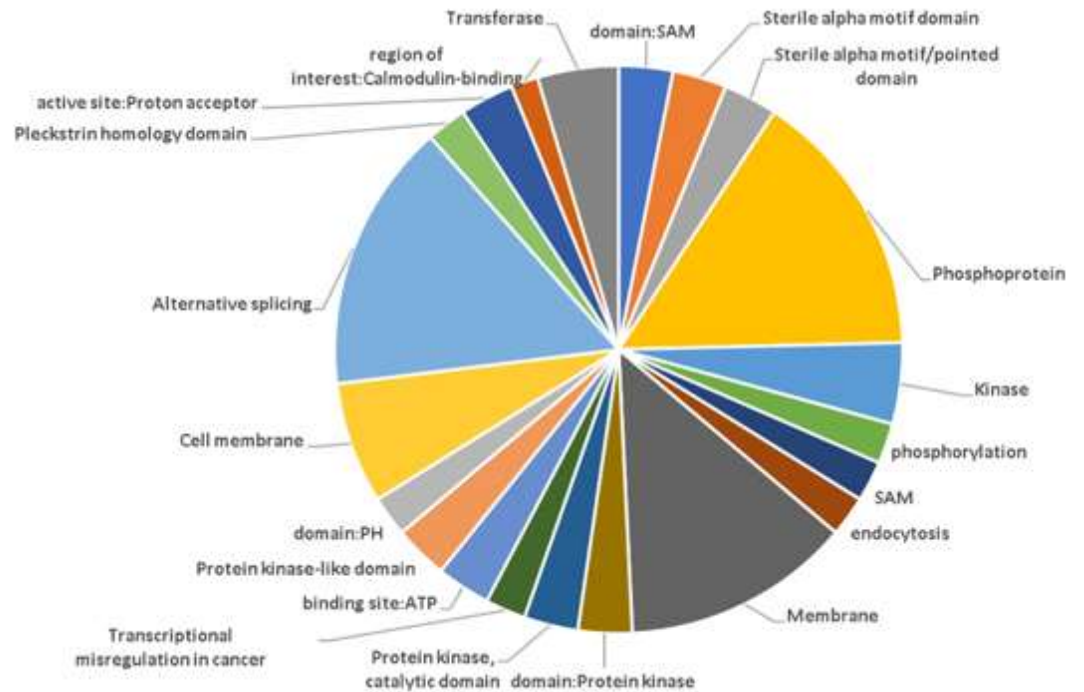


Figure S1

**E. CD166+/ALDH- Top miRNAs**

Name	p-Value	Ratio
hsa-miR-185-5p	0.005335	1.52
hsa-miR-34a-5p	0.079482	1.84
hsa-miR-28-5p	0.097872	1.80
hsa-miR-135b-5p	0.13191	1.42
hsa-miR-183-5p	0.148579	1.23
hsa-miR-1246	0.185359	1.91
hsa-miR-1323	0.341565	2.08
hsa-miR-26a-5p	0.377387	1.21
hsa-miR-196a-5p	0.413641	1.31
hsa-miR-1299	0.42265	1.22



**F. ALDH+/CD166- Top miRNAs**

Name	p-Value	Ratio
hsa-miR-301a-3p	0.024285	1.59
hsa-miR-96-5p	0.041435	1.61
hsa-miR-29b-3p	0.071412	1.18
hsa-miR-429	0.084041	1.54
hsa-miR-182-5p	0.085607	1.54
hsa-miR-660-5p	0.100104	1.92
hsa-miR-200a-3p	0.100196	1.47
hsa-miR-10a-5p	0.104863	1.16
hsa-miR-93-5p	0.106804	1.25
hsa-miR-194-5p	0.107797	1.32

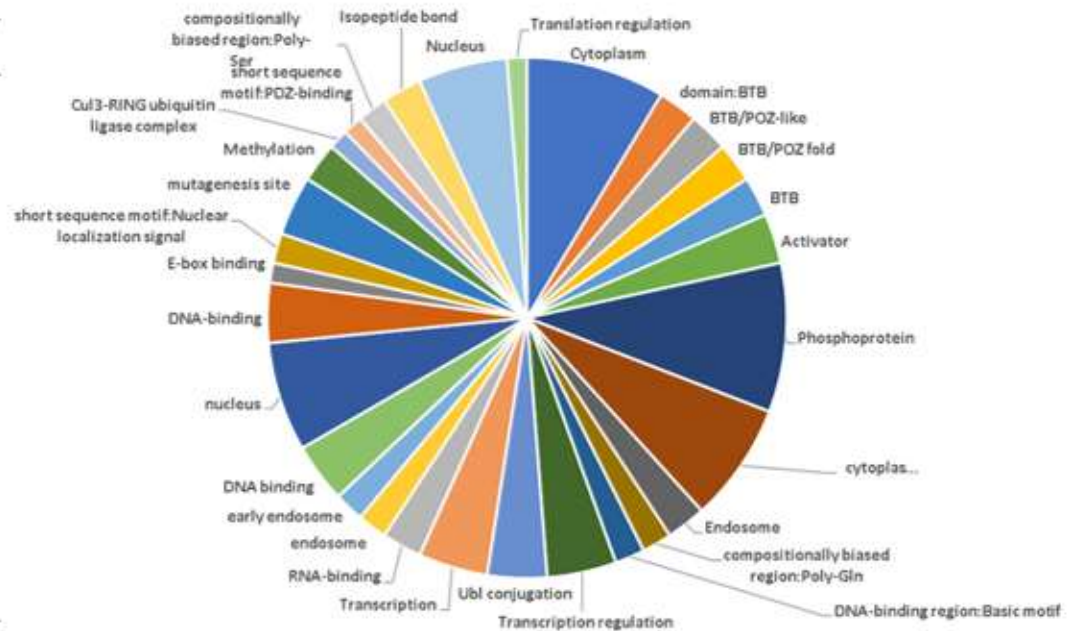
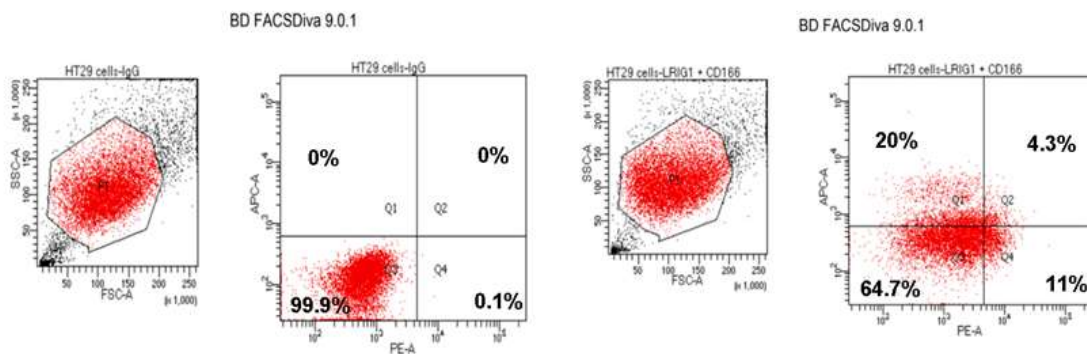
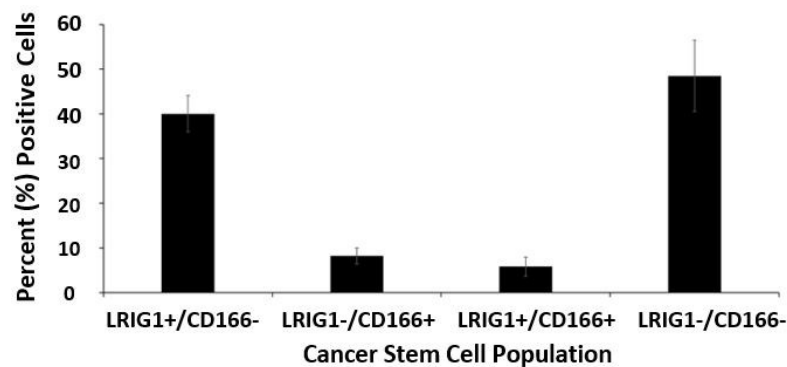


Figure S1

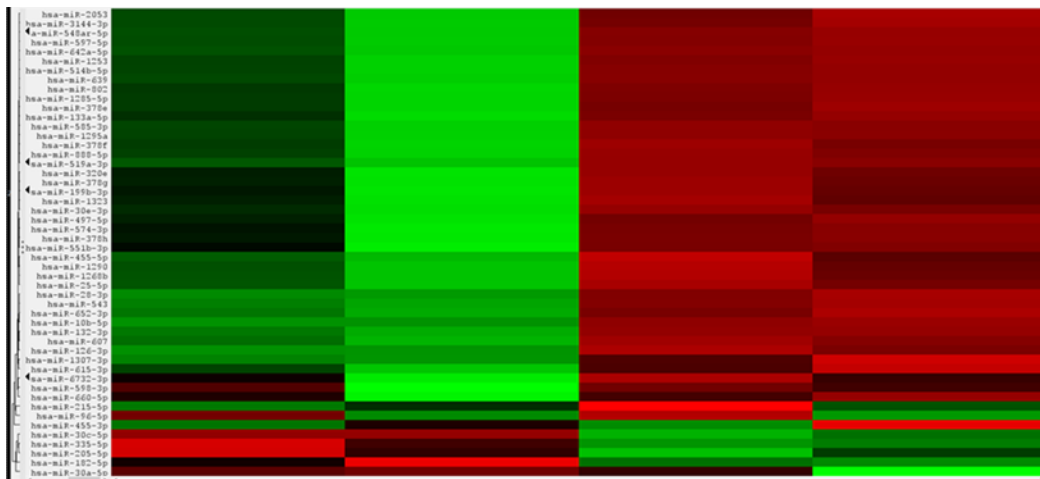
A.



B.



C. LRIG1-/CD166+ LRIG1+/CD166+ LRIG1+/CD166- LRIG1-/CD166-



D.

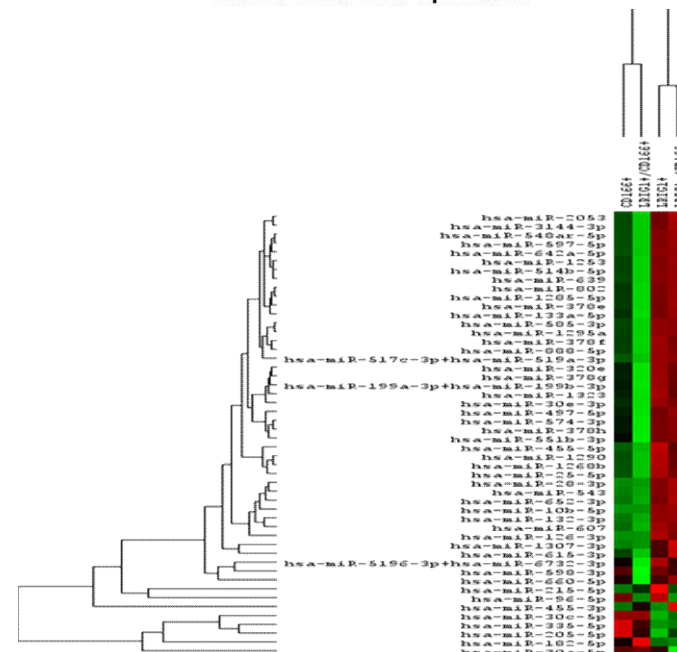
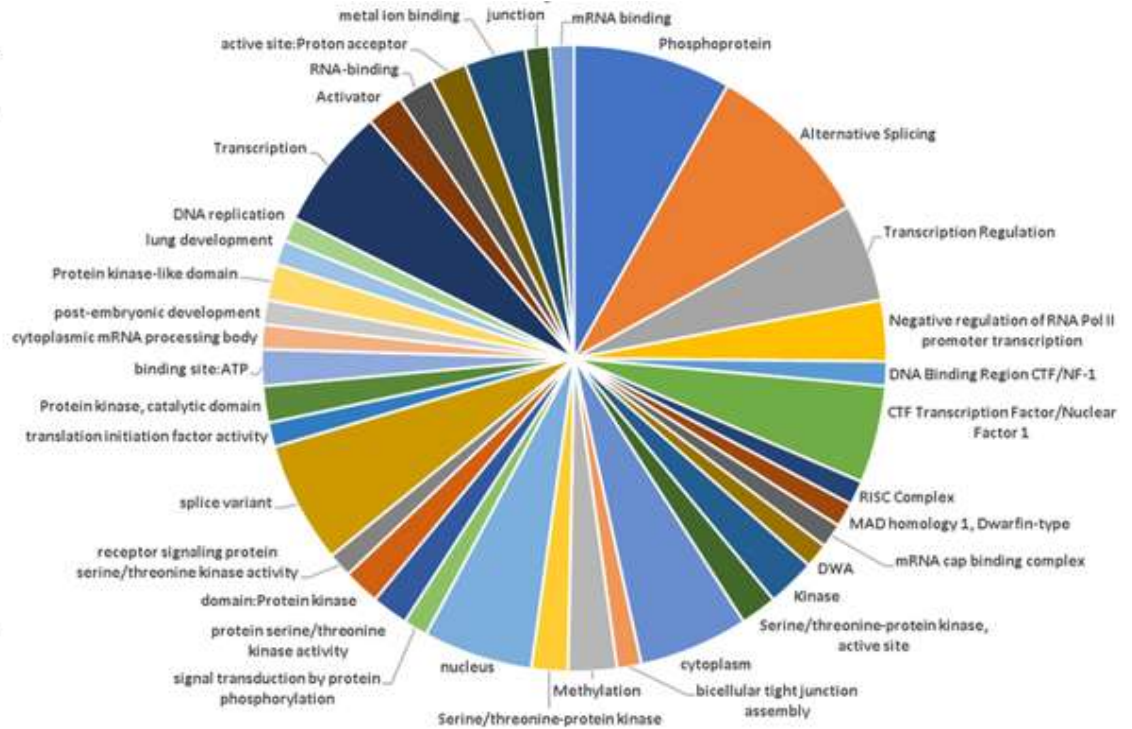


Figure S2



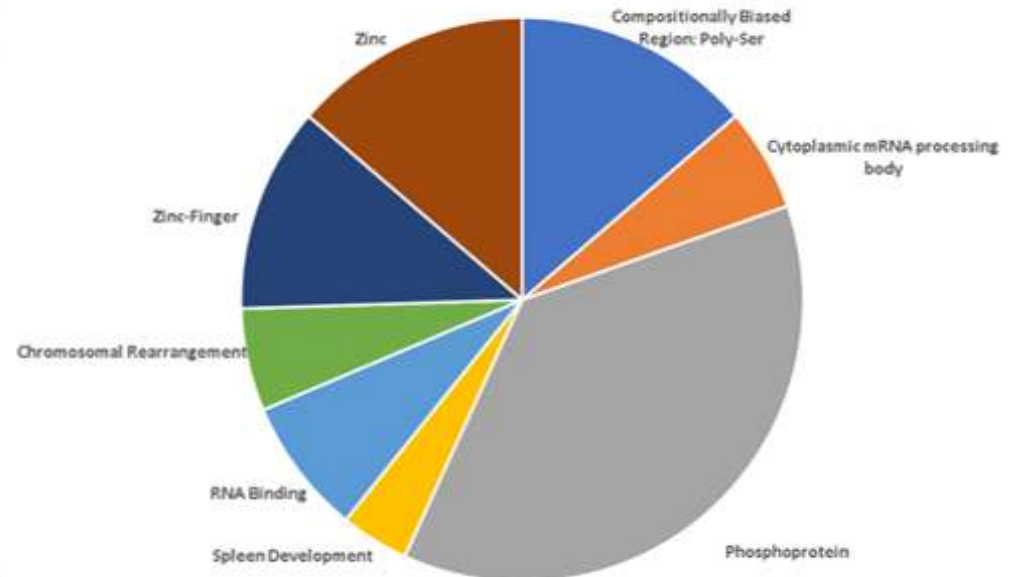
**E. LRIG1+/CD166- Top miRNAs**

Name	p-Value	Ratio
hsa-miR-374b-5p	0.009738	1.39
hsa-miR-205-5p	0.010734	4.21
hsa-let-7i-5p	0.044163	1.58
hsa-miR-192-5p	0.04518	1.62
hsa-miR-429	0.0745	1.64
hsa-miR-106b-5p	0.116775	1.43
hsa-miR-590-5p	0.126675	1.56
hsa-miR-361-5p	0.128048	1.18
hsa-miR-27b-3p	0.131583	1.4
hsa-miR-374a-5p	0.198531	1.58



**F. CD166+/LRIG1- Top miRNAs**

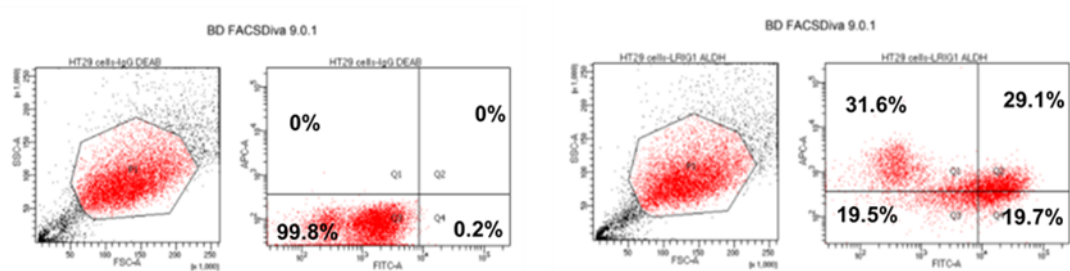
Name	p-Value	Ratio
hsa-miR-608	0.004408	1.61
hsa-miR-1200	0.006728	1.72
hsa-miR-582-3p	0.011376	2.00
hsa-miR-548y	0.012319	2.13
hsa-miR-30a-3p	0.014378	2.08
hsa-miR-28-3p	0.02381	1.64
hsa-miR-517c-3p*	0.024478	2.13
hsa-miR-1302	0.025088	2.08
hsa-miR-28-5p	0.02628	2.04
hsa-miR-628-3p	0.027493	1.75



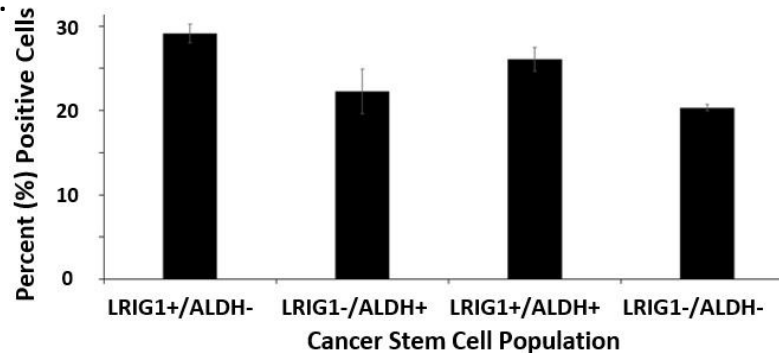
\*hsa-miR-517c-3p+hsa-miR-519a-3p

Figure S2

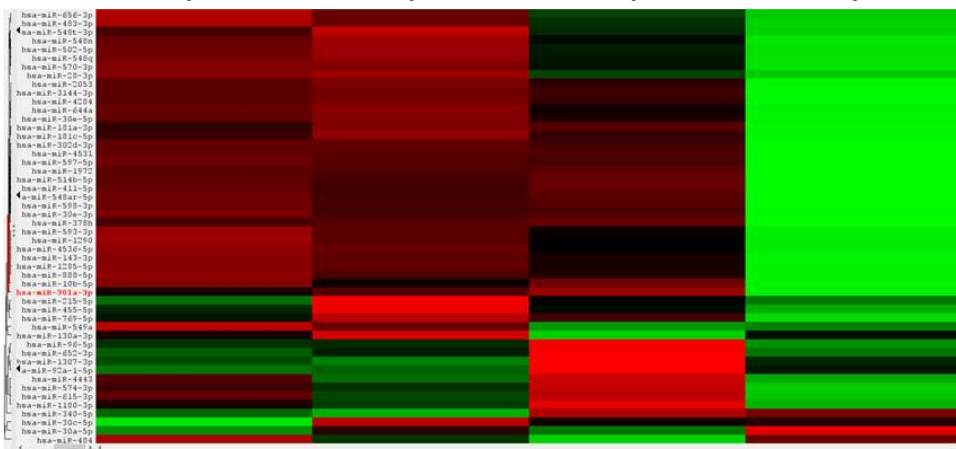
A.



B.



C. LRIG1-/ALDH+ LRIG1+/ALDH+ LRIG1-/ALDH- LRIG1+/ALDH-



D.

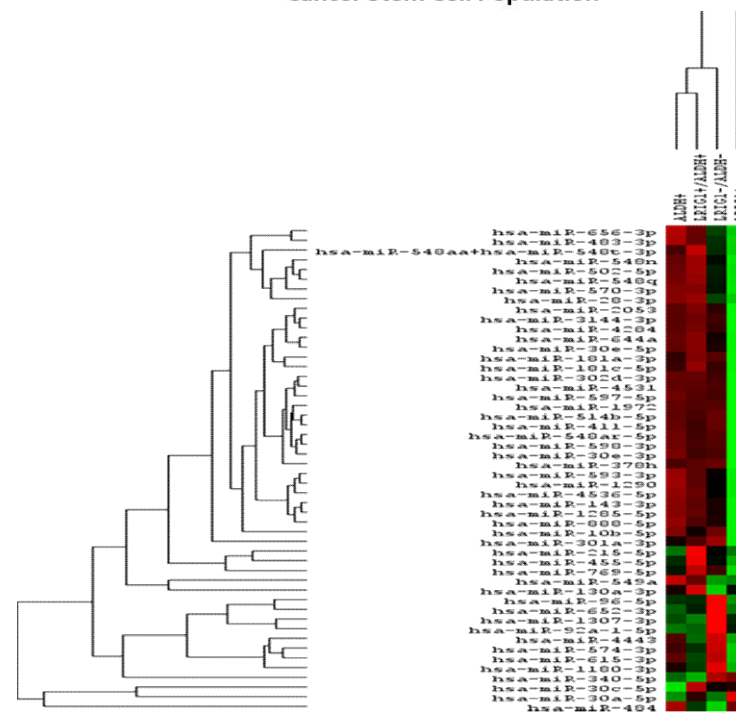
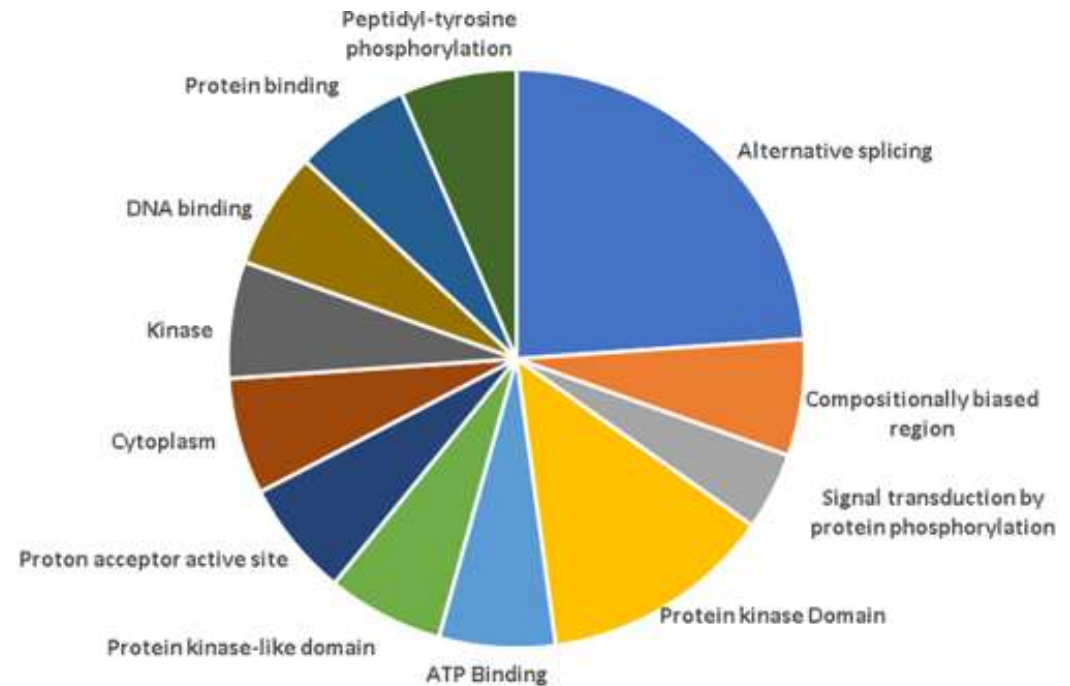


Figure S3

### E. LRIG1+/ALDH- Top miRNAs

Name	p-Value	Ratio
hsa-miR-5001-5p	0.0029	2.41
hsa-miR-193b-3p	0.007301	2.03
hsa-miR-1290	0.007807	3.22
hsa-miR-208a-3p	0.008697	3.06
hsa-miR-510-3p	0.00965	2.47
hsa-miR-365b-5p	0.010913	2.37
hsa-miR-663a	0.01348	3.70
hsa-miR-1297	0.014174	2.19
hsa-miR-205-5p	0.014201	4.33
hsa-miR-3614-5p	0.015049	3.18



### F. ALDH+/LRIG1- Top miRNAs

Name	p-Value	Ratio
hsa-miR-148a-3p	0.007872	1.64
hsa-miR-3928-3p	0.009459	1.12
hsa-miR-194-5p	0.012492	1.89
hsa-miR-375	0.021317	1.61
hsa-miR-22-3p	0.056906	1.69
hsa-miR-192-5p	0.062684	1.56
hsa-miR-185-5p	0.064618	1.32
hsa-miR-19a-3p	0.068956	1.96
hsa-miR-140-5p	0.071816	1.75
hsa-miR-27b-3p	0.078661	1.37

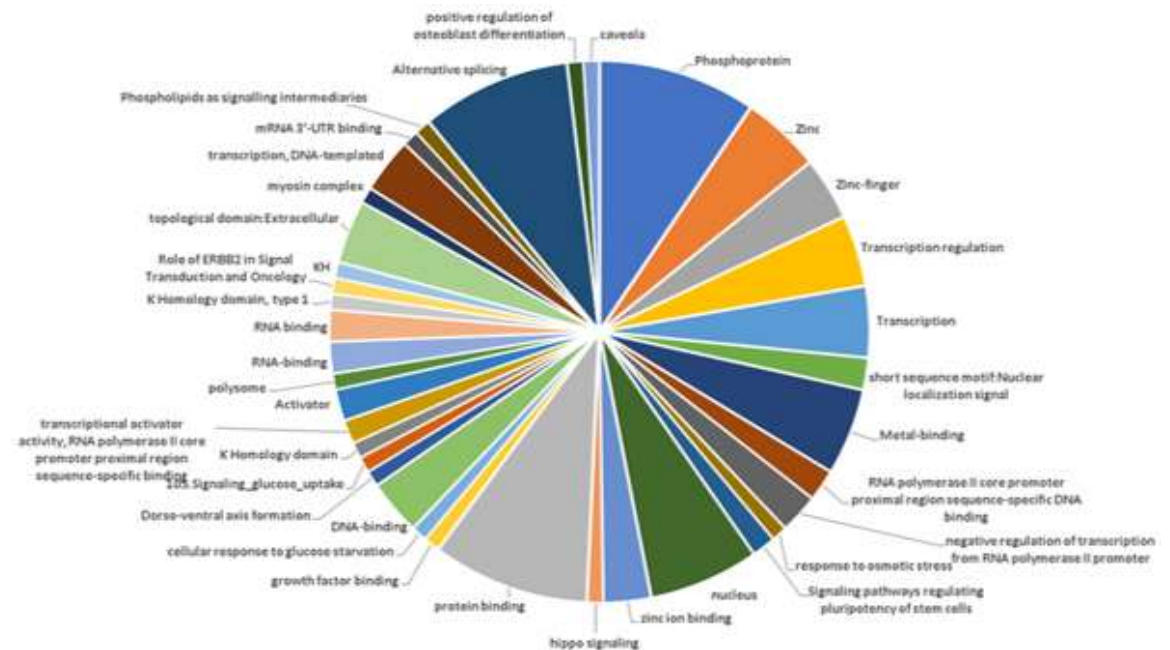
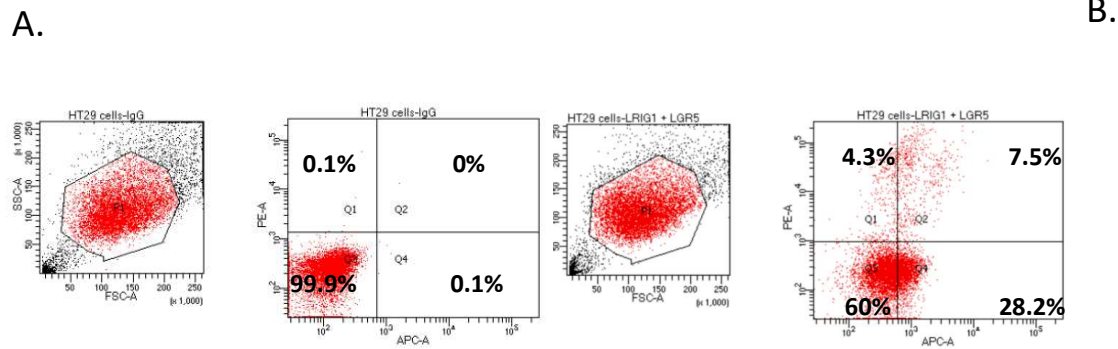
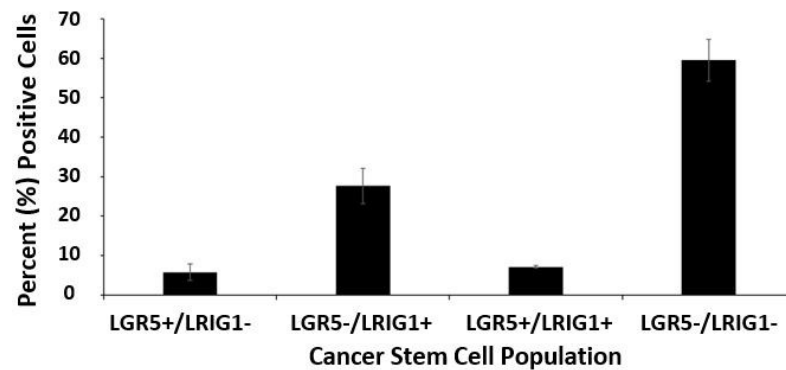


Figure S3

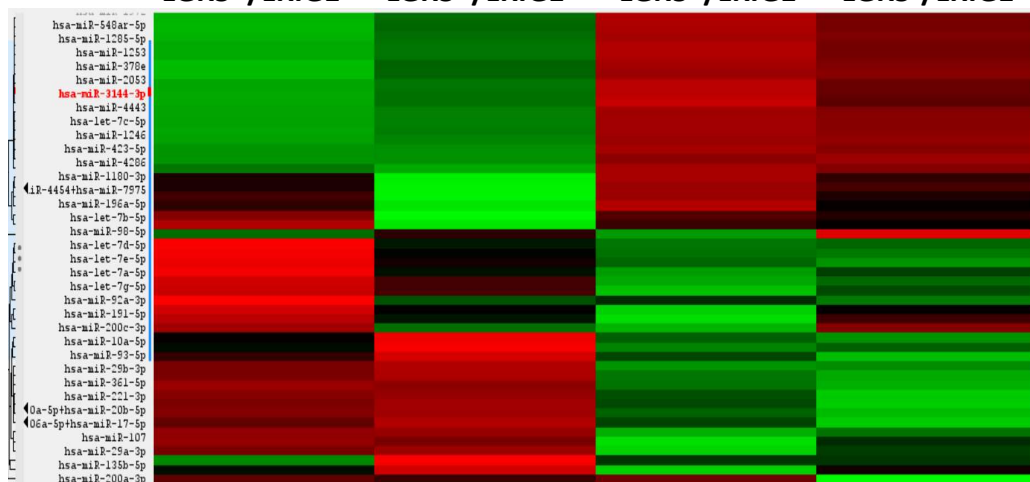


**B.**



**C.**

LGR5+/LRIG1- LGR5+/LRIG1+ LGR5-/LRIG1- LGR5-/LRIG1+



**D.**

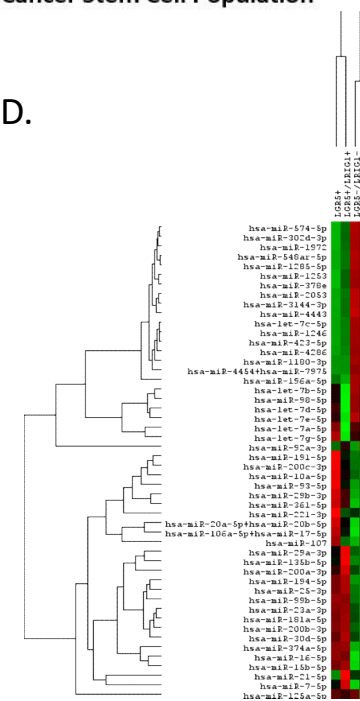
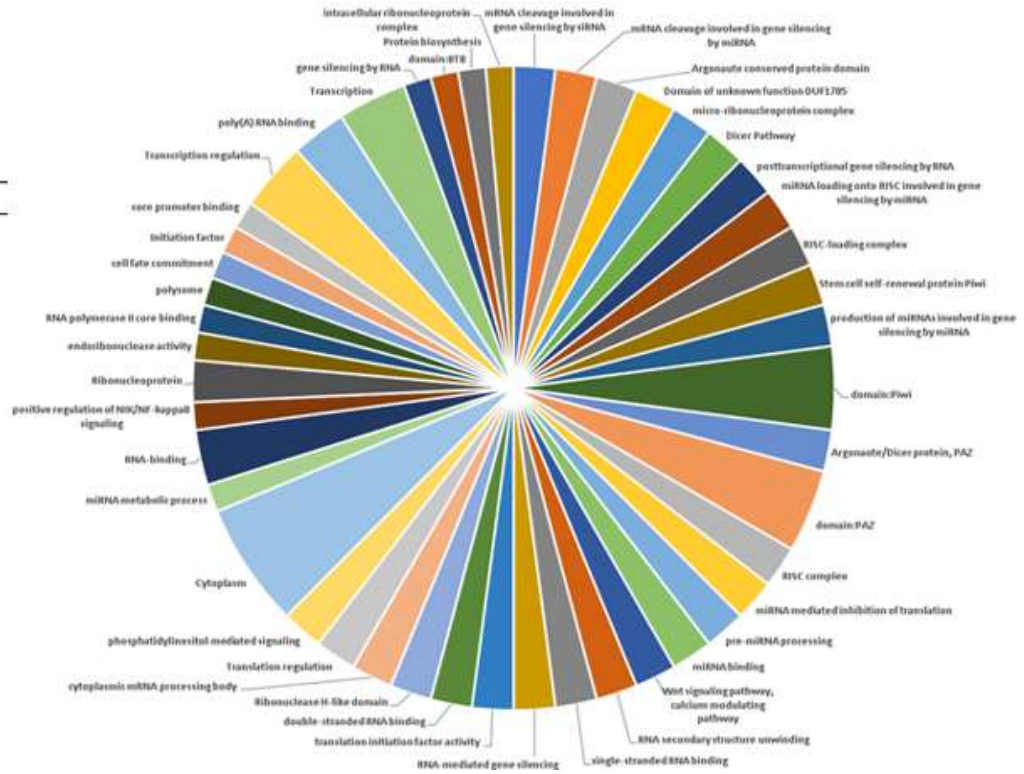


Figure S4

**E.**

**LGR5+/LRIG1- Top miRNAs**

Name	p-Value	Ratio
hsa-miR-455-3p	0.000516	5.626198
hsa-miR-424-5p	0.001786	7.473064
hsa-miR-454-3p	0.00185	3.375876
hsa-miR-544a	0.001903	9.050518
hsa-miR-935	0.001946	9.763292
hsa-miR-874-5p	0.002336	11.21283
hsa-miR-219a-2-3p	0.002448	13.2283
hsa-miR-218-5p	0.002587	9.866657
hsa-miR-532-5p	0.002709	6.301194
hsa-miR-1972	0.002842	24.14922



**F.**

**LRIG1+/LGR5- Top miRNAs**

Name	p-Value	Ratio
hsa-miR-200b-3p	0.006559	2.129456
hsa-miR-194-5p	0.010642	2.207357
hsa-miR-23a-3p	0.032224	1.784871
hsa-miR-29a-3p	0.034529	1.311528
hsa-miR-10a-5p	0.06363	2.184755
hsa-miR-30d-5p	0.071142	1.691372
hsa-miR-15b-5p	0.092976	1.587857
hsa-miR-374a-5p	0.107158	2.051671
hsa-miR-191-5p	0.120353	1.675972
hsa-miR-361-5p	0.128485	1.191644

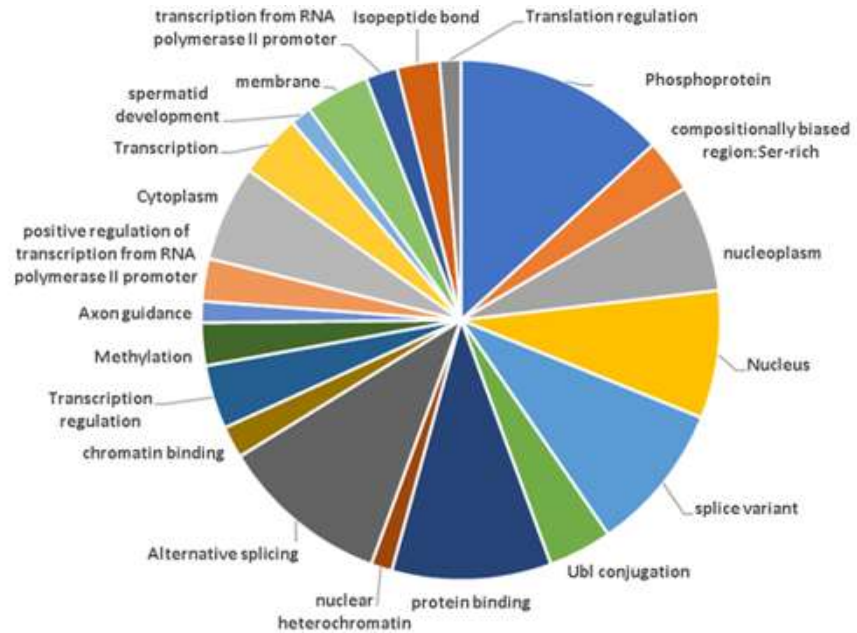
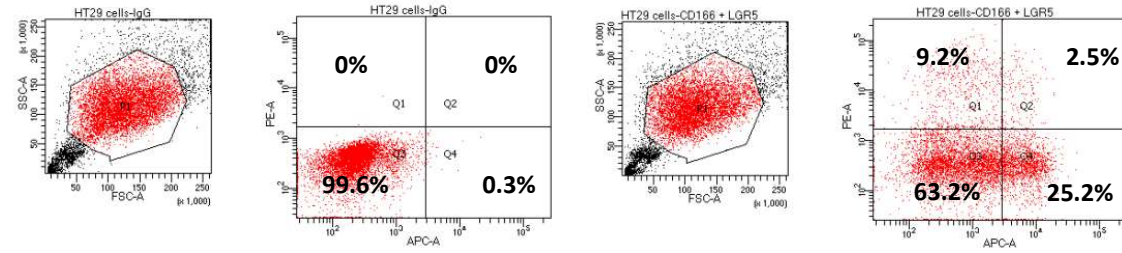
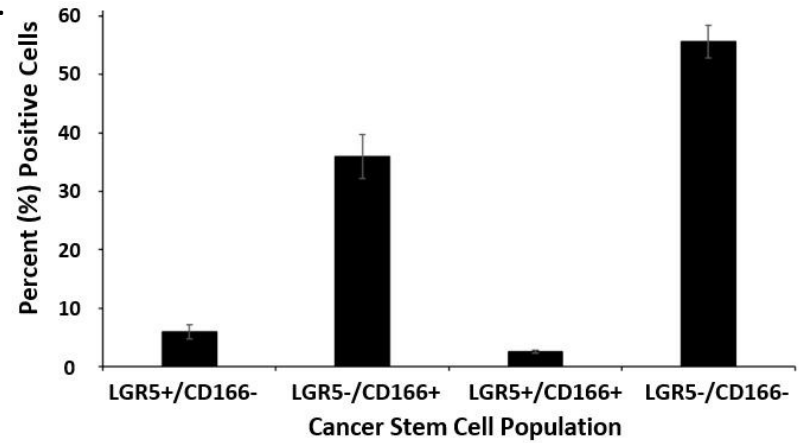


Figure S4

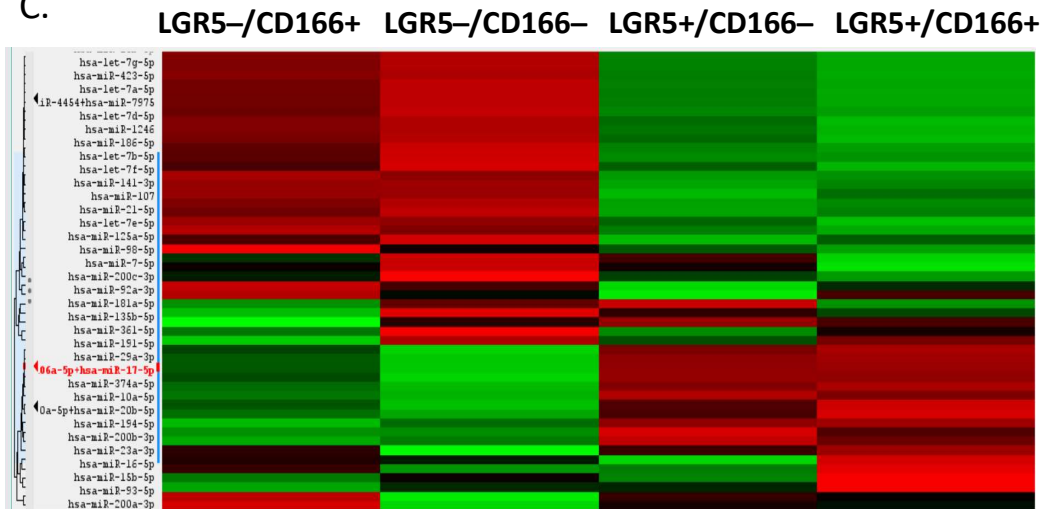
A.



B.



C.



D.

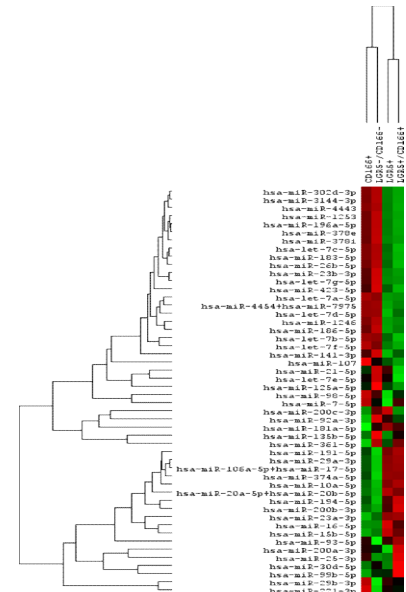
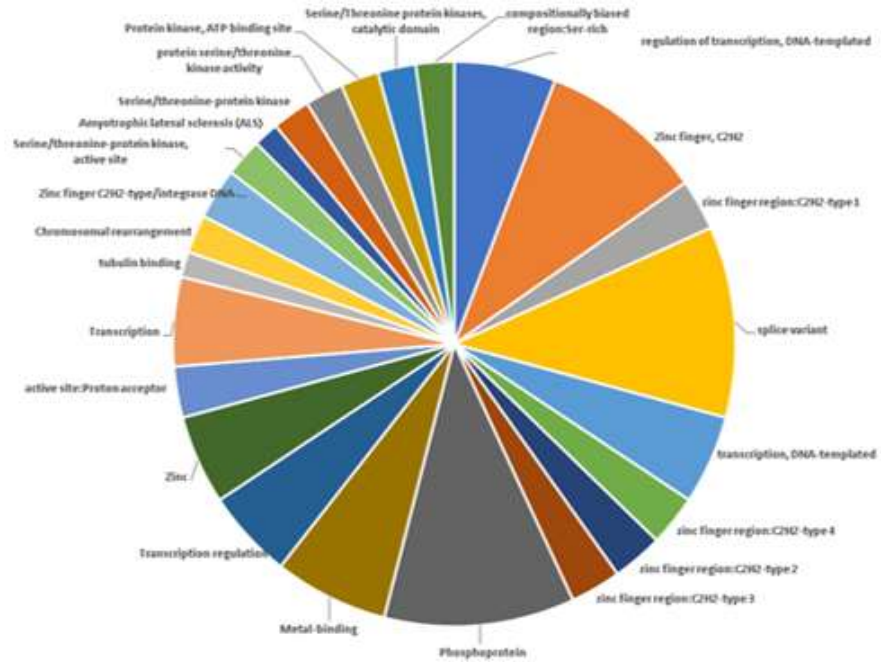


Figure S5

### E. LGR5+/CD166- Top miRNAs

Name	p-Value	Ratio
hsa-miR-4454*	0.002637	8.333
hsa-miR-664b-5p	0.005766	6.667
hsa-miR-196a-5p	0.005853	1.370
hsa-miR-3918	0.010346	5.556
hsa-miR-1204	0.013975	10.000
hsa-miR-376a-2-5p	0.014634	6.250
hsa-miR-362-3p	0.01532	9.091
hsa-miR-142-5p	0.015663	5.556
hsa-miR-1908-5p	0.017958	10.000
hsa-miR-142-3p	0.019367	3.226

\*hsa-miR-4454+hsa-miR-7975



### F. CD166+/LGR5- Top miRNAs

Name	p-Value	Ratio
hsa-miR-15b-5p	0.034841	2.06
hsa-miR-16-5p	0.055473	2.04
hsa-miR-29a-3p	0.143658	1.41
hsa-miR-194-5p	0.165687	1.44
hsa-miR-20a-5p*	0.174565	1.48
hsa-miR-1283	0.212371	3.81
hsa-miR-181a-5p	0.213746	1.46
hsa-miR-23a-3p	0.25129	1.37
hsa-miR-106a-5p**	0.26257	1.17
hsa-let-7i-5p	0.267539	11.00

\*hsa-miR-20a-5p+hsa-miR-20b-5p, \*\*hsa-miR-106a-5p+hsa-miR-17-5p

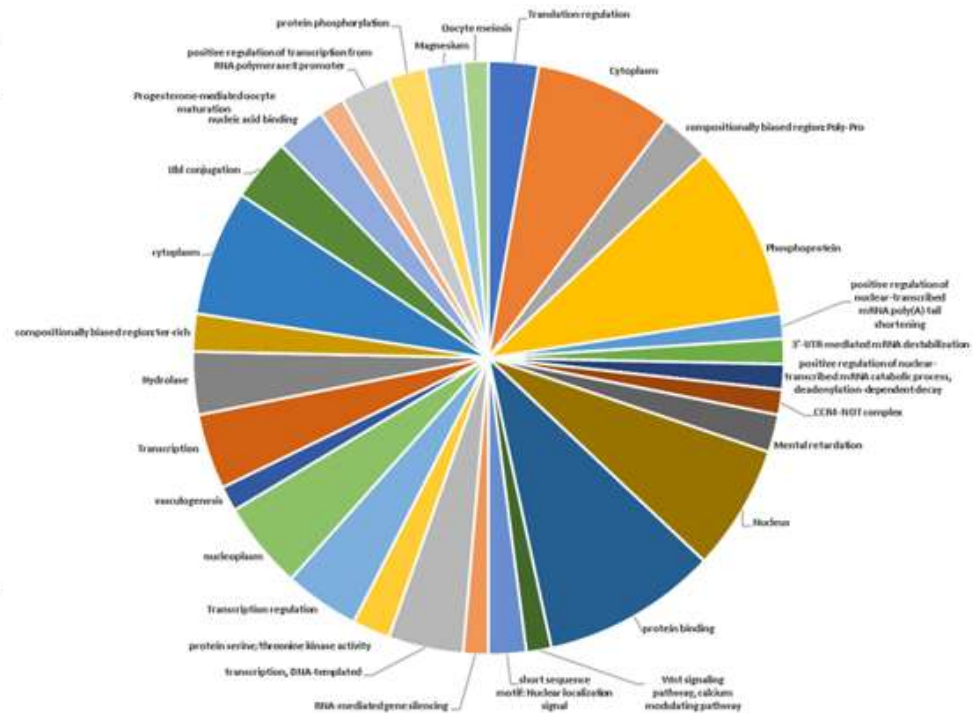


Figure S5