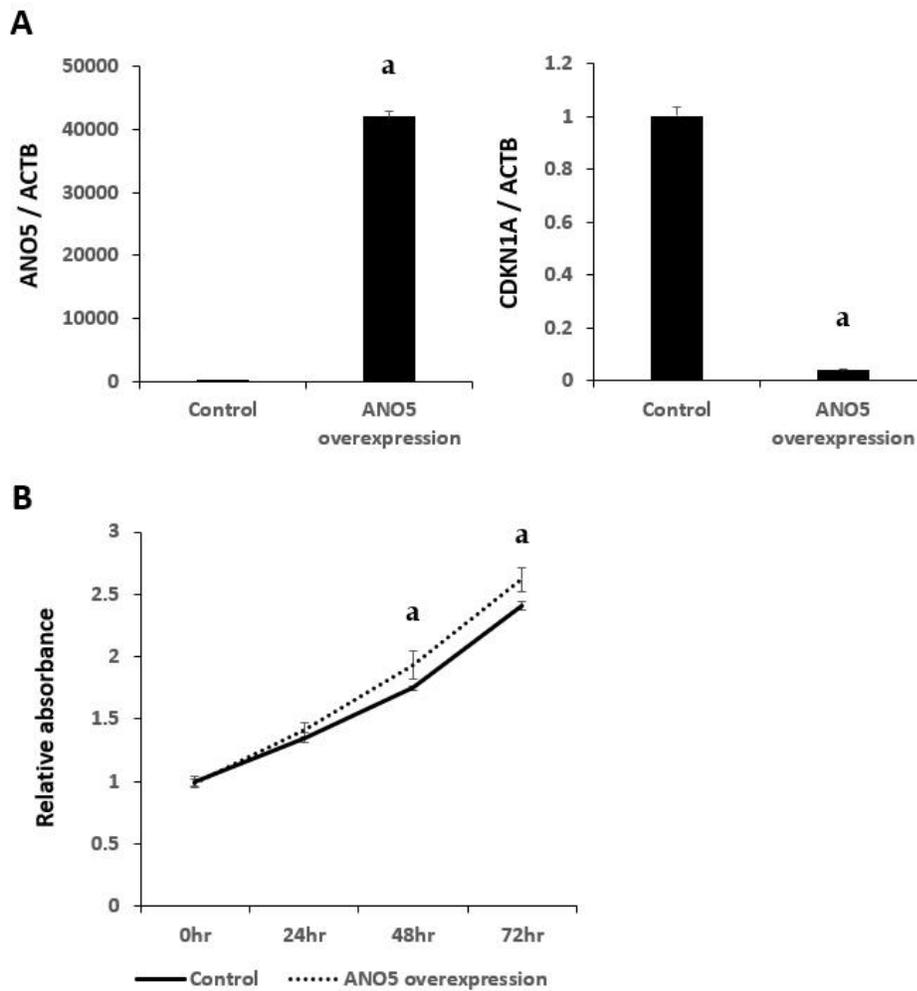
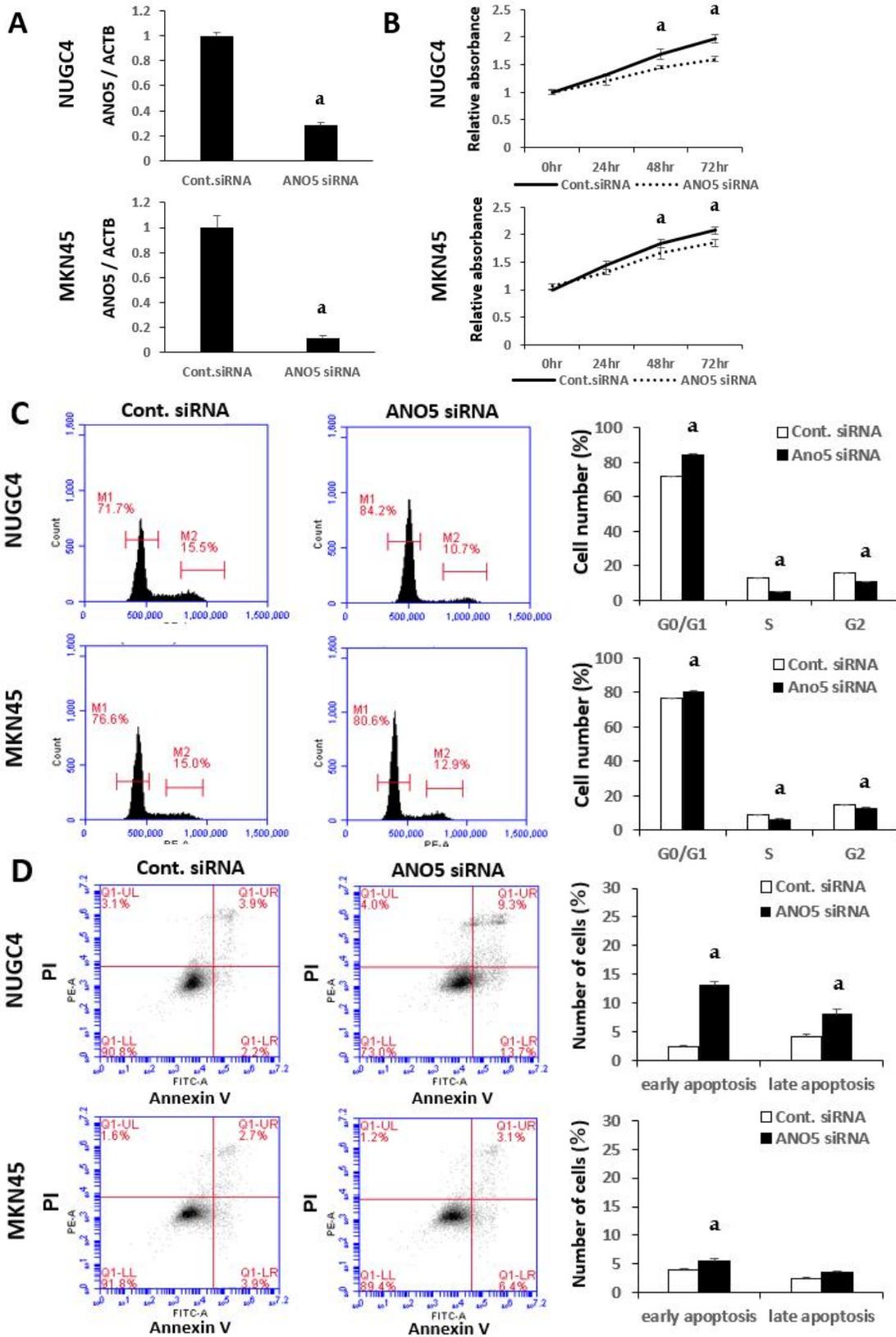


**Supplementary Figure 1 ANO5 expression of normal gastric tissues compared with GC tissues.** The expression levels of ANO5 mRNA were significantly higher in 12 GC tissue specimens than in the corresponding 12 non-cancerous tissue specimens in GC patients. Gene expression levels were normalized to the level of ACTB. <sup>a</sup> $P < 0.05$  (significantly different from GC tissue)

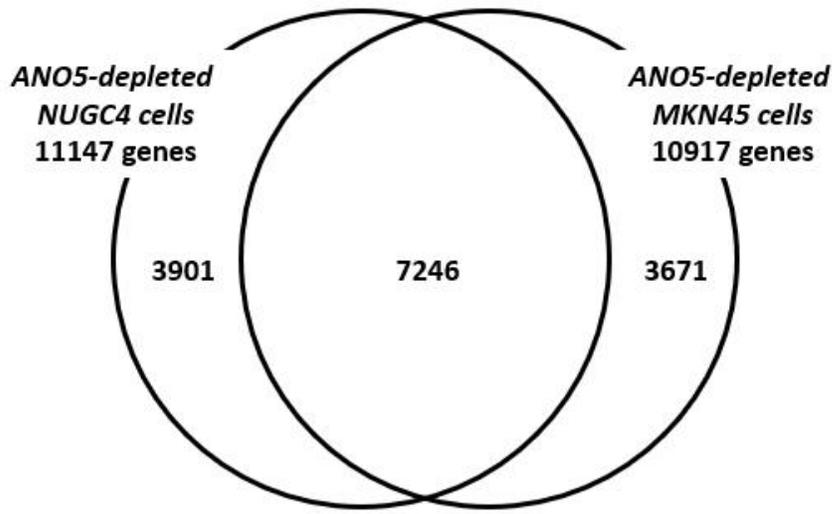


**Supplementary Figure 2 ANO5.** A: Expression of ANO5 and CDKN1A/p21 in MKN7 cell following ANO5 overexpression. The overexpression of ANO5 markedly increased ANO5 mRNA levels and decreased CDKN1A/p21 mRNA levels in MKN7 cell; B: Cell proliferation of MKN7 cell following ANO5 overexpression. The overexpression of ANO5 increased the relative absorbance of MKN7 cells. Mean  $\pm$  SEM.  $n = 3$ . <sup>a</sup> $P < 0.05$  (significantly different from control).

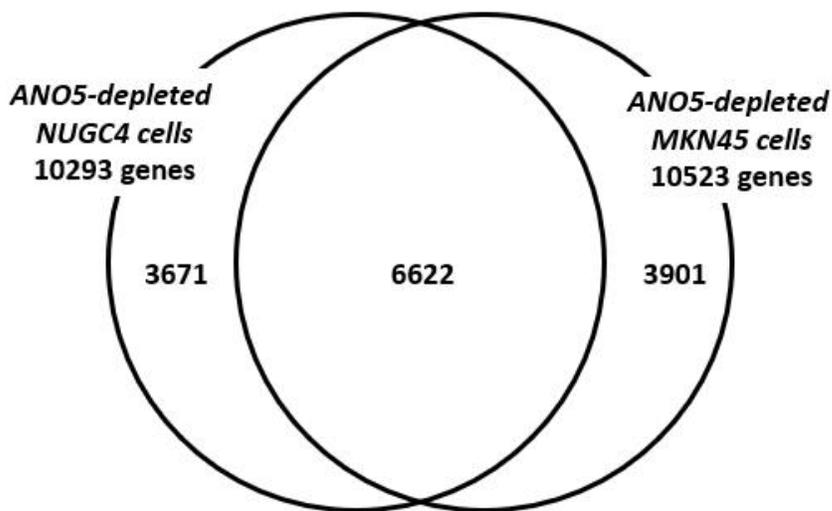


**Supplementary Figure 3 Effects on cell growth and survival in NUGC4 and MKN45 cells following ANO5 siRNA transfection (HSS137120).** A: ANO5 siRNA effectively reduced ANO5 mRNA levels in NUGC4 and MKN45 cells; B: The proliferative ability of NUGC4 and MKN45 cells was significantly suppressed following ANO5 siRNA down-regulation; C: The down-regulation of ANO5 increased the number of cells in the G<sub>0</sub>/G<sub>1</sub> phase in NUGC4 and MKN45 cells. Cells transfected with control or ANO5 siRNA were stained with propidium iodide (PI) and analyzed by flow cytometry; D: The down-regulation of ANO5 increased the early and late apoptotic cell proportions of NUGC4 and MKN45 cells. Control or ANO5 siRNA-transfected cells were stained with PI and annexin V and subjected to flow cytometry. Mean  $\pm$  SEM.  $n = 3$ . <sup>a</sup> $P < 0.05$  (significantly different from control siRNA).

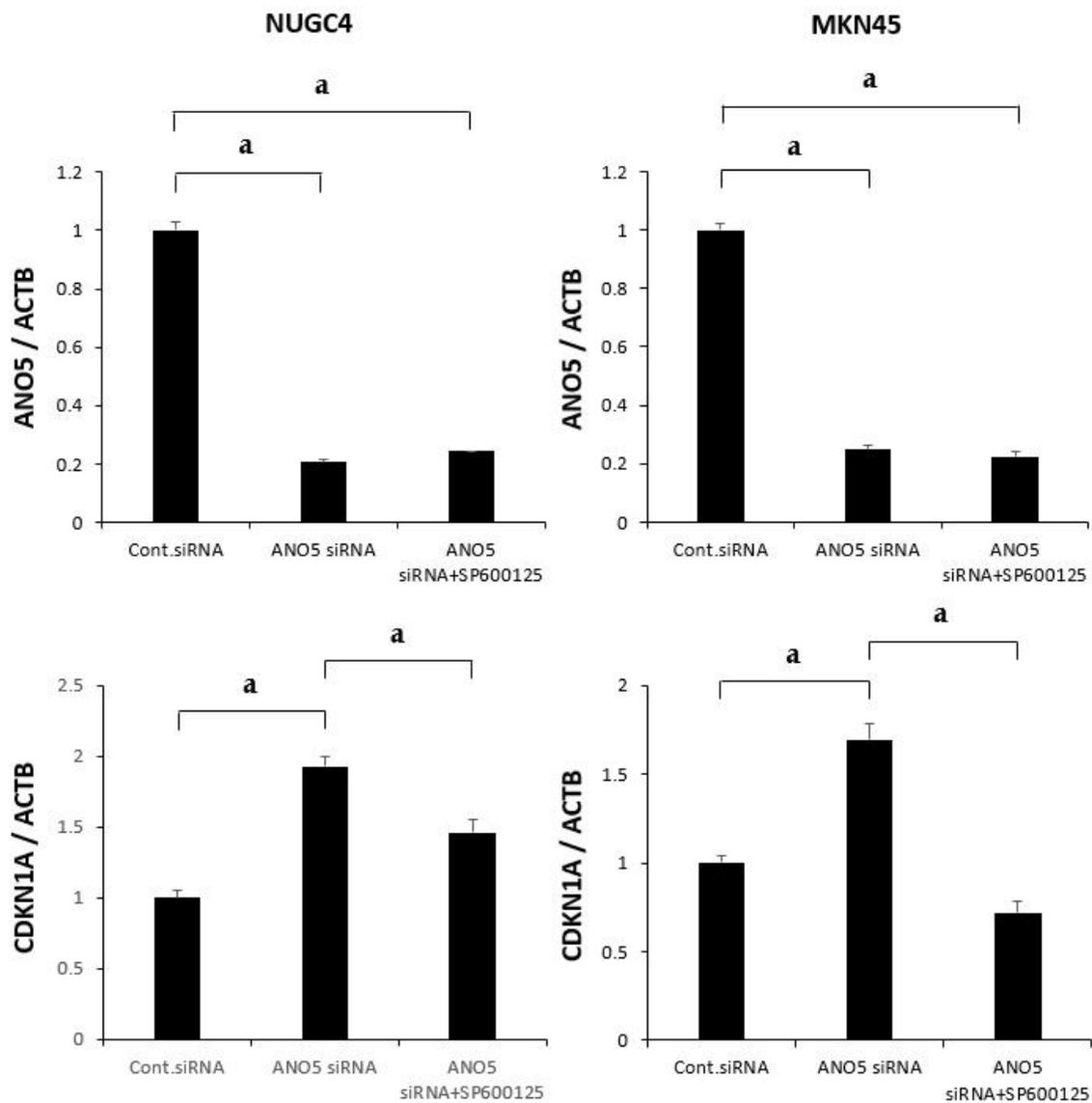
### Upregulated genes



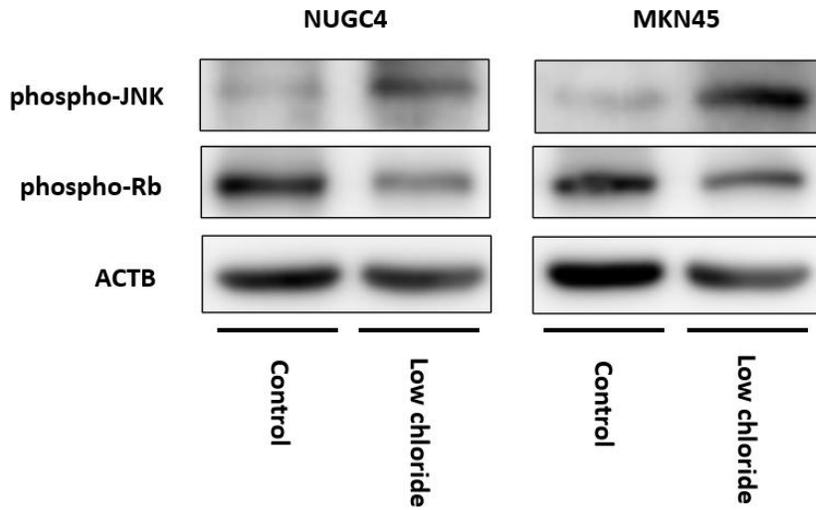
### Downregulated genes



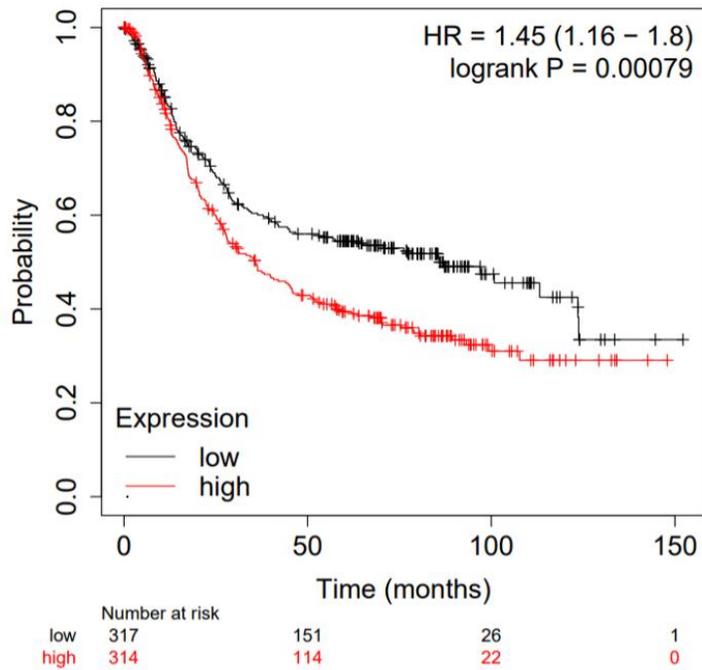
**Supplementary Figure 4 Gene expression changes in ANO5-depleted NUGC and MKN45 cells.** Among the 21440 genes, 7246 genes were upregulated and 6622 genes were downregulated in both cell lines, for a total of 13868 genes (64.7%) with identical expression direction in NUGC4 and MKN45 cells.



**Supplementary Figure 5 Expression of ANO5 and CDKN1A/p21 mRNA in NUGC4 and MKN45 cells treated with JNK inhibitors after silencing ANO5.** Treatment with JNK inhibitors made no difference in ANO5 mRNA expression. The increase of CDKN1A/p21 mRNA expression induced by ANO5 silencing in NUGC4 and MKN45 cells was suppressed by JNK inhibition. Mean  $\pm$  SEM.  $n = 3$ .  $^aP < 0.05$  (significantly different from control).



**Supplementary Figure 6 Expression of phosphorylated JNK protein and phosphorylated Rb protein cultured by low chloride medium.** Low chloride conditions increased phosphorylated JNK and reduced phosphorylated Rb in NUGC4 and MKN45 cells.



**Supplementary Figure 7 Overall survival analysis according to the expression level of ANO5 based on the Kaplan Meier plotter.** The data obtained from the Kaplan-Meier plotter database revealed a relationship between high ANO5 expression and a poor prognosis in GC.

**Supplementary Table 1 Gene expression changes in ANO5-depleted cells related to “Cell Cycle: G<sub>1</sub>/S Checkpoint Regulation”**

<b>Gene symbol</b>	<b>Gene Name</b>	<b>Gene ID</b>	<b>Fold change (NUGC4)</b>	<b>Fold change (MKN45)</b>
CCNE2	<i>Cyclin E2</i>	TC0800011148.h g.1	-4.760	-1.351
CDC25A	<i>Cell division cycle 25A</i>	TC0300011009.h g.1	-3.480	-1.410
CDK2	<i>Cyclin dependent kinase 2</i>	TC1200007819.h g.1	-53.840	-3.514
CDK4	<i>Cyclin dependent kinase 4</i>	TC1200010977.h g.1	-6.570	-1.514
CDK6	<i>Cyclin dependent kinase 6</i>	TC0700011785.h g.1	-17.080	-4.116
CDKN1A (p21 Cip1)	<i>Cyclin dependent kinase inhibitor 1A</i>	TC0600007847.h g.1	1.910	1.123
E2F1	<i>E2F transcription factor 1</i>	TC2000008894.h g.1	-7.920	-2.244
SMAD3	<i>SMAD family member 3</i>	TC1500007633.h g.1	2.780	1.591

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<i>SMAD4</i>	<i>SMAD</i>	TC1800009229.h	1.160	1.490
	<i>family</i>	g.1		
	<i>member 4</i>			
<i>SUV39H1</i>	<i>Suppressor</i>	TC0X00007200.h	-3.120	-1.427
	<i>of</i>	g.1		
	<i>variegation</i>			
	3-9			
	<i>homolog 1</i>			

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**Supplementary Table 2 Comparison of the gene expression changes of genes displayed in Table 1 following ANO5 silencing**

	Gene symbol	Fold change (NUGC4)	Fold change (MKN45)
Upregulated genes	PLCXD1	31.97	7.826
	NDRG4	29.88	5.712
	CYP3A5	24.81	17.91
	PLCXD1	23.44	7.158
	REG4	19.70	4.805
	CIDEB	17.86	6.290
	DHRS9	17.02	12.67
	CIDEC	16.73	11.36
	SPRR1A	16.66	32.80
	APOD	16.57	14.53
	SEMA7A	16.09	8.175
	C11orf86	15.57	4.234
	BNIP1	15.52	10.44
	SUSD2	15.25	18.53
	MAPRE3	14.99	6.450
	CYP1A1	14.69	12.48
	GOLT1A	13.79	3.468
	CNN2	13.74	12.32
	ANTXR2	13.54	2.978
	APOBEC1	12.19	9.778
Downregulated genes	CDK2	-53.84	-3.514
	DTL	-27.60	-2.035
	RABL3	-20.16	-3.979
	CKS1B	-19.34	-2.027
	LMNB1	-19.18	-1.871

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IFRD2	-18.61	-2.533
PLK1	-17.89	-2.159
GIN51	-17.87	-2.638
CDK6	-17.08	-1.133
XRCC2	-14.77	-2.800
POGLUT3	-14.47	-4.074
DSG2	-14.37	-3.478
NEMP1	-14.31	-2.655
CBX5	-14.20	-1.949
ANKRD52	-14.20	-3.881
ITGB1	-14.16	-2.574
H2BC14	-13.25	-2.059
SCAMP2	-13.05	-3.605
CMTM7	-13.02	-2.730
GIN54	-12.66	-1.743

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