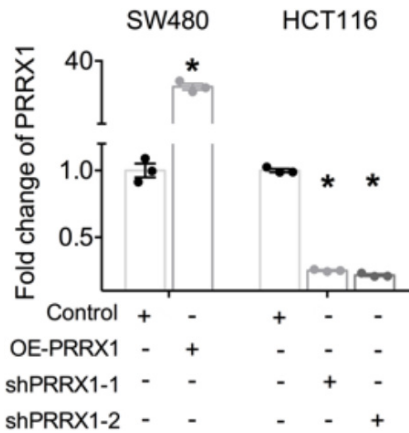**Figure S1** Kaplan-Meier survival analysis in paraffin CRC samples from GEO dataset (related to *Figure 1*). (A-C) Kaplan-Meier survival analysis on data of the clinical CRC patients from GEO GSE 103479 dataset. CRC, colorectal cancer; GEO, Gene Expression Omnibus.

Figure S2 qRT-PCR assay for the transcriptional level of PRRX1 (related to *Figure 2*). *, P<0.05 *vs.* control. OE, overexpression; sh, short hairpin; qRT-PCR, quantitative reverse transcription polymerase chain reaction; PRRX1, paired related homeobox 1.
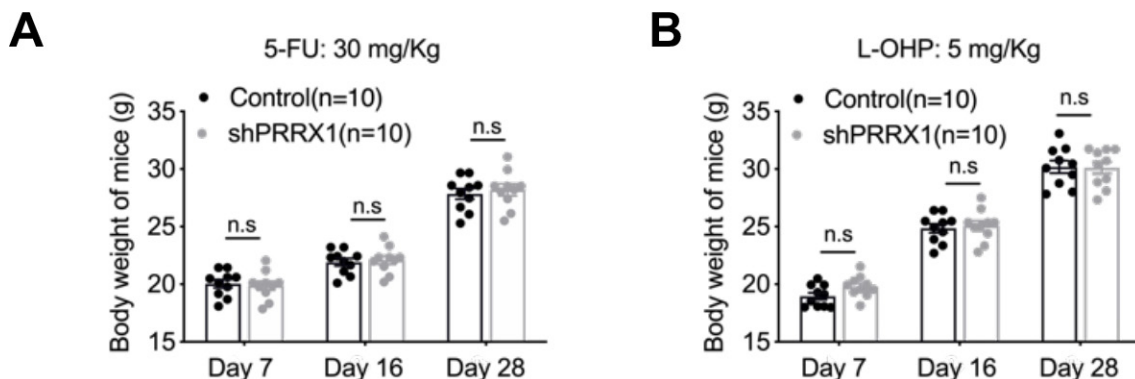
Figure S3 The alteration of body weight in mice undergoing chemotherapy (related to *Figure 5*). n.s., not significant; PRRX1, paired related homeobox 1; 5-FU, 5-fluorouracil; L-OHP, oxaliplatin.
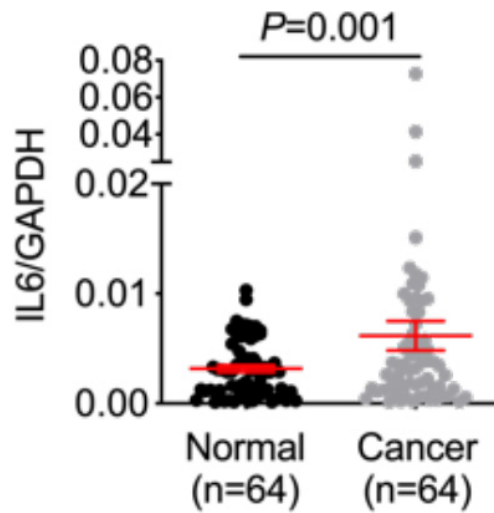
**Figure S4** Paired Student's *t*-test was used to analyze the difference in IL-6 transcription between fresh CRC and adjacent normal tissues from Zhujiang Hospital (related to *Figure 6*). The data were normalized to GAPDH and are expressed as the means ± SEMs. CRC, colorectal cancer; SEMs, SEM, standard error of the mean; PRRX1, paired related homeobox 1; IL-6, interleukin 6; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
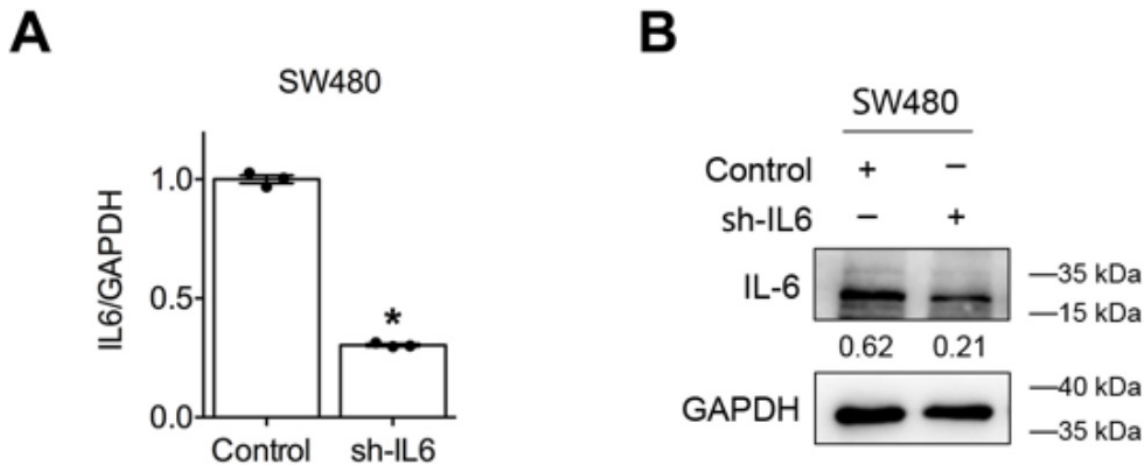
Figure S5 The efficiency of vector with IL-6 targeting shRNA (related to *Figure 7*). (A) qRT-PCR assay for the expression IL-6; (B) Western blot assay for the expression IL-6. *, P<0.05 *vs.* control. shRNA, short hairpin RNA; qRT-PCR, quantitative reverse transcription polymerase chain reaction. PRRX1, paired related homeobox 1; IL-6, interleukin 6; sh, short hairpin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
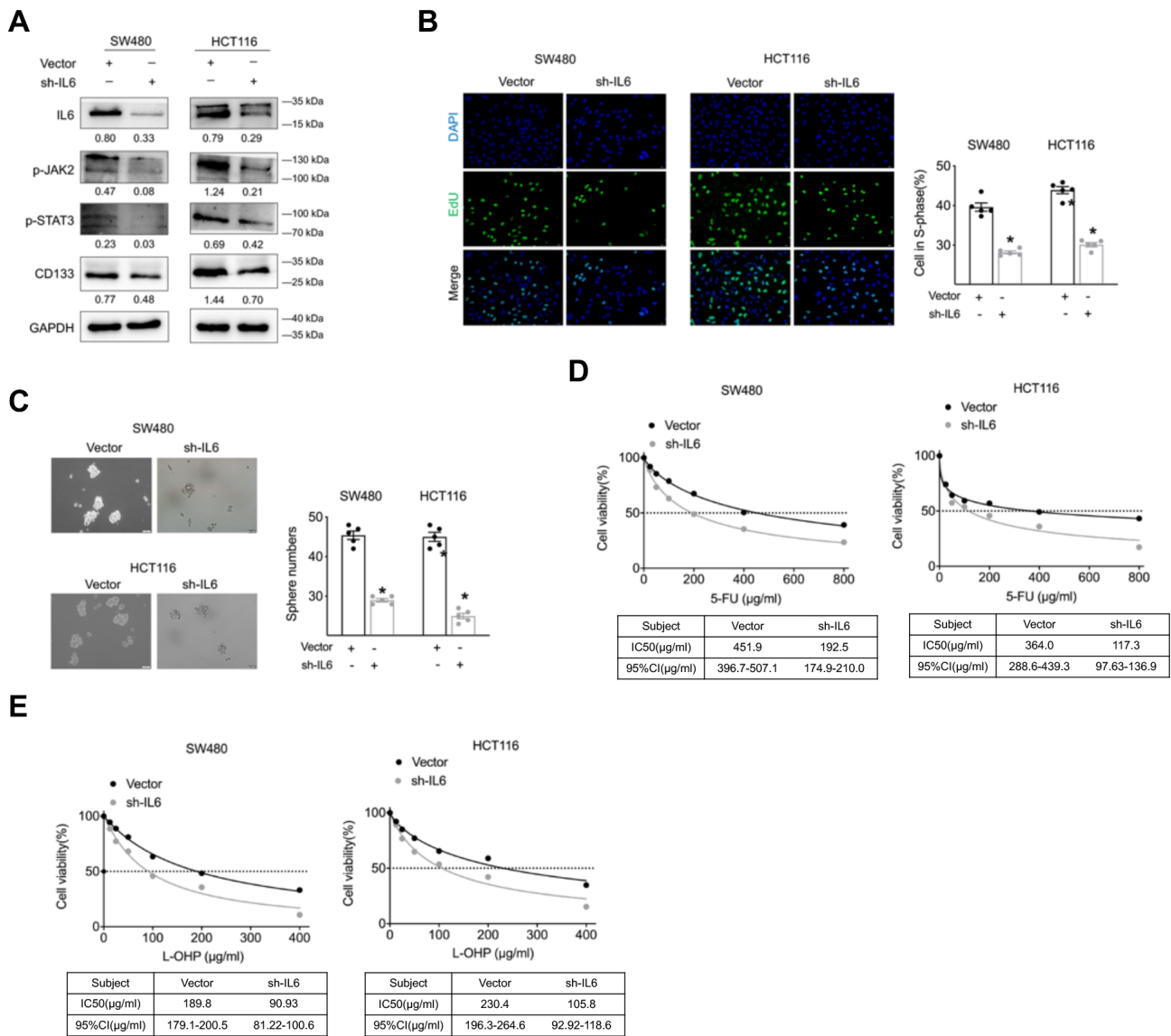
Figure S6 The role of IL-6 in regulating the activation of JAK/STAT signaling, cell proliferation and stemness, as well as sensitivity to chemotherapy (related to *Figure 7*). (A) Representative Western blots for CD133 and JAK2/STAT3 signaling-associated signatures. The values under the membrane represent the expression of genes normalized to the expression of the reference gene GAPDH. (B) Representation of EdU incorporation assay (magnification, ×400). Bars in the right panel reflect the means ± SEMs. (C) Representation of sphere formation assay (scale bar, 100 μm), bars in the right panel reflect the means ± SEMs. (D,E) CCK-8 assay for investigating the half maximal inhibitory concentration of CRC cells to 5-FU and L-OHP. *, P<0.05 *vs.* the control. PRRX1, paired related homeobox 1; CI, confidence interval; CRC, colorectal cancer; IL-6, interleukin 6; sh, short hairpin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; EdU, 5-ethynyl-2'-deoxyuridine; CCK-8, Cell Counting Kit-8; SEM, standard error of the mean; 5-FU, 5-fluorouracil; L-OHP, oxaliplatin.
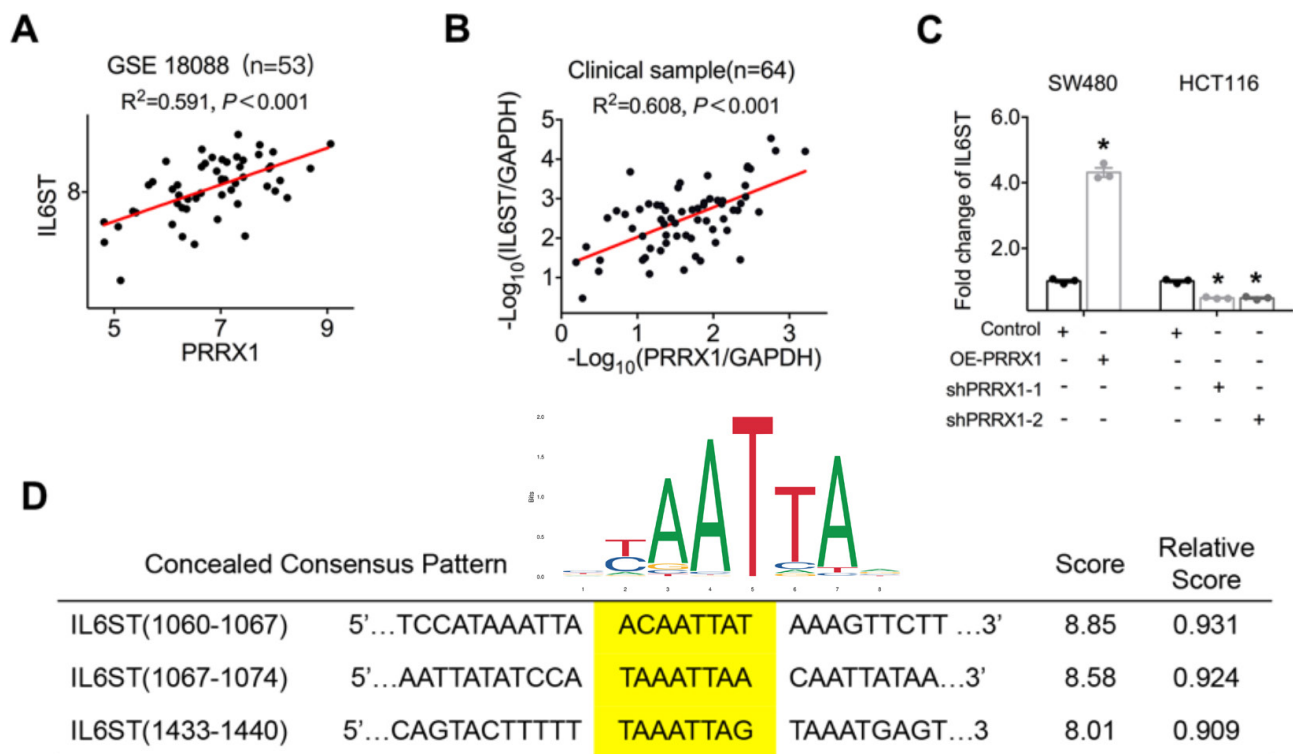
Figure S7 PRRX1 directly promotes the transcription of IL6ST (related to *Figure 6*). (A) Pearson correlation analysis on the data of clinical CRC samples from GEO GES18088 dataset. (B) Pearson correlation analysis on the data of clinical CRC samples from Zhujiang Hospital. (C) qRT-PCR assay for the expression IL6ST. (D) The prediction of bind site for IL6ST by JASPAR software. *, P<0.05 *vs.* control. CRC, colorectal cancer; GEO, Gene Expression Omnibus; qRT-PCR, quantitative reverse transcription polymerase chain reaction. PRRX1, paired related homeobox 1; IL6ST, interleukin 6 cytokine family signal transducer; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; OE, overexpression; sh, short hairpin.

Table S1 Gene set enrichment analysis of samples from GEO GSE18088 dataset

Name of gene set	Size	NES	P value	q-value
JAK STAT signaling pathway	148	1.83749	0.000	0.077
Hypertrophic cardiomyopathy HCM	82	1.81116	0.000	0.058
Cell adhesion molecules CAMs	122	1.80709	0.000	0.039
Focal adhesion	186	1.78739	0.002	0.039
Leukocyte transendothelial migration	106	1.78090	0.000	0.033
RIG I like receptor signaling pathway	63	1.77430	0.004	0.031
Calcium signaling pathway	170	1.76983	0.000	0.028
Chemokine signaling pathway	169	1.76926	0.000	0.025
MAPK signaling pathway	254	1.75771	0.000	0.026
Prion diseases	35	1.75535	0.002	0.024
ECM receptor interaction	81	1.75135	0.000	0.023
Pathways in cancer	311	1.74608	0.000	0.023
Regulation of actin cytoskeleton	195	1.74049	0.002	0.023
Cytokine receptor interaction	239	1.73913	0.000	0.022
Toll like receptor signaling pathway	97	1.73328	0.002	0.022
Fc gamma R mediated phagocytosis	84	1.72004	0.004	0.024
NOD like receptor signaling pathway	49	1.71504	0.002	0.024
Glycosphingolipid biosynthesis ganglio-series	15	1.70025	0.004	0.028
Small cell lung cancer	82	1.69420	0.002	0.029
Viral myocarditis	67	1.69094	0.002	0.029
Lysosome	115	1.68457	0.023	0.030
Complement and coagulation cascades	66	1.68214	0.002	0.030
Apoptosis	81	1.67350	0.002	0.032
Intestinal immune network for IgA production	44	1.65789	0.000	0.036
Dilated cardiomyopathy	89	1.65029	0.016	0.038
Antigen processing and presentation	82	1.65024	0.015	0.037
Arrhythmogenic right ventricular cardiomyopathy ARVC	73	1.64171	0.022	0.039
Hematopoietic cell lineage	83	1.63995	0.006	0.039
Natural killer cell mediated cytotoxicity	128	1.63928	0.016	0.038
T cell receptor signaling pathway	105	1.63763	0.006	0.037
Epithelial cell signaling in *Helicobacter pylori infection*	61	1.62962	0.008	0.039
Leishmania infection	62	1.62594	0.000	0.039
B cell receptor signaling pathway	70	1.62251	0.019	0.039
Neuroactive ligand receptor interaction	251	1.62157	0.002	0.039
Pantothenate and CoA biosynthesis	16	1.61854	0.002	0.039
Glycosaminoglycan degradation	21	1.61384	0.023	0.040
VEGF signaling pathway	70	1.60776	0.012	0.041
Neurotrophin signaling pathway	121	1.58459	0.021	0.051
Autoimmune thyroid disease	49	1.57942	0.047	0.053
Asthma	27	1.57567	0.014	0.053
Bladder cancer	38	1.55232	0.008	0.066
Pathogenic *Escherichia coli* infection	43	1.54329	0.014	0.070
Tryptophan metabolism	37	1.52738	0.015	0.080
Fc epsilon RI signaling pathway	75	1.51519	0.027	0.086
Adipocytokine signaling pathway	66	1.50714	0.025	0.089
Melanogenesis	98	1.49995	0.032	0.094
Axon guidance	125	1.49690	0.016	0.094
Renal cell carcinoma	65	1.49431	0.038	0.094
Cytosolic DNA sensing pathway	51	1.49374	0.040	0.093
TGF beta signaling pathway	82	1.49250	0.028	0.092
Type I diabetes mellitus	40	1.49079	0.046	0.091
Melanoma	71	1.47508	0.042	0.100
Prostate cancer	87	1.46074	0.034	0.106
Type II diabetes mellitus	43	1.45929	0.033	0.105
Renin angiotensin system	15	1.40800	0.048	0.138
WNT signaling pathway	143	1.39181	0.043	0.143

GEO, Gene Expression Omnibus; NES, normalized enrichment score; GSE, gene set enrichment; MAPK, mitogen-activated protein kinase; ECM, extracellular matrix; CoA, coenzyme A; VEGF, vascular endothelial growth factor; TGF, transforming growth factor.

Table S2 Correlation of subject characteristics and PRRX1 expression among 64 CRC patients

Characteristics	Total, n	PRRX1		P value[1]
		Negative, n (%)	Positive, n (%)	
Sex				0.777
Female	17	9 (52.9)	8 (47.1)	
Male	47	23 (48.9)	24 (51.1)	
Age at diagnosis, years				0.719
≤50 years	9	5 (55.6)	4 (44.4)	
>50 years	55	27 (49.1)	28 (50.9)	
Tumor location				0.017
Right colon	21	15 (71.4)	6 (28.6)	
Left colon	43	17 (39.5)	26 (60.5)	
T classification				0.031
Tis+T0	0	0 (0.0)	0 (0.0)	
T1+T2	44	26 (59.1)	18 (40.9)	
T3+T4	20	6 (30.0)	14 (70.0)	
N classification				0.042
N0	38	23 (60.5)	15 (39.5)	
N1+N2	26	9 (34.6)	17 (65.4)	
M classification				0.039
M0	49	28 (57.1)	21 (42.9)	
M1	15	4 (26.7)	11 (73.3)	
Stage				0.006
S1+S2	41	26 (63.4)	15 (36.6)	
S3+S4	22	6 (27.3)	16 (72.7)	

[1], Pearson χ^2 test of independence between covariables and PRRX1 expression. CRC, colorectal cancer; PRRX1, paired related homeobox 1.

Table S3 Correlation of subject characteristics and PRRX1 expression among 101 CRC patients

Characteristics	Total, n	PRRX1		P value[1]
		Negative, n (%)	Positive, n (%)	
Sex				0.354
Female	41	14 (34.1)	27 (65.9)	
Male	60	26 (43.3)	34 (56.7)	
Age at diagnosis				0.958
≤50 years	23	9 (39.1)	14 (60.9)	
>50 years	78	31 (39.7)	47 (60.3)	
Stage				0.001
S1+S2	31	20 (64.5)	11 (35.5)	
S3+S4	70	20 (28.6)	50 (71.4)	
T classification				0.376
Tis+T0	0	0 (0.0)	0 (0.0)	
T1+T2	11	3 (27.3)	8 (72.7)	
T3+T4	90	37 (41.1)	53 (58.9)	
N classification				0.005
N0	41	23 (56.1)	18 (43.9)	
N1+N2	60	17 (28.3)	43 (71.7)	
M classification				0.018
M0	89	39 (43.8)	50 (56.2)	
M1	12	1 (8.3)	11 (91.7)	

[1], Pearson χ^2 test of independence between covariables and PRRX1 expression. CRC, colorectal cancer; PRRX1, paired related homeobox 1.

Table S4 Gene set enrichment analysis in PRRX1-high expression group

Gene symbol	Rank in gene list	Rank metric score	Running ES
IL24	12	0.4923172	0.05360
IL6	14	0.471102238	0.10541
CSF2RB	149	0.319922715	0.13408
IL13RA2	151	0.319191635	0.16916
SOCS3	206	0.299733847	0.19952
AKT3	314	0.265616506	0.22352
OSMR	319	0.264418781	0.25243
IL7R	356	0.253123224	0.27854
OSM	613	0.19913733	0.28794
IL10RA	736	0.178937823	0.30168
STAT2	965	0.152587026	0.30733
SOCS2	981	0.15103285	0.32322
CSF3R	1,154	0.13463223	0.32963
JAK2	1,237	0.129329011	0.33986
IL15	1,262	0.127606675	0.35273
PIK3R5	1,271	0.126796067	0.36630
STAT4	1,319	0.12271452	0.37751
SOCS5	1,329	0.121858232	0.39048
IL6ST	1,346	0.120822825	0.40300
IL2RG	1,465	0.113219693	0.40969
CSF2RA	1,479	0.112283133	0.42142
IL2RB	1,490	0.111915424	0.43325
STAT1	1,502	0.111375235	0.44497
LEPR	1,612	0.10532885	0.45123
PIK3CG	1,645	0.103253968	0.46103
IL10	1,736	0.098016962	0.46742
IL11	1,822	0.093419947	0.47355
IL21R	1,903	0.089635096	0.47951
CBLB	1,934	0.088591591	0.48779
IL2RA	1,984	0.086666621	0.49494
SPRED1	2,062	0.084273487	0.50045
IL12RB1	2,147	0.081286743	0.50529
CSF2	2,234	0.078384355	0.50972
PIAS3	2,285	0.076921284	0.51574
LIF	2,364	0.074292228	0.52011
IFNGR1	2,380	0.073843352	0.52750
IL15RA	2,412	0.073004693	0.53402
IFNAR2	2,499	0.070736475	0.53760
SOCS1	2,502	0.070651777	0.54528
STAT5A	2,644	0.066800371	0.54574
JAK3	2,666	0.066377006	0.55202
IL4R	2,689	0.065770023	0.55819
CTF1	2,705	0.065501392	0.56466
PIK3CD	2,708	0.065418154	0.57177
STAM2	2,955	0.0602763	0.56638
AKT2	2,976	0.059717916	0.57197
GHR	3,017	0.058942169	0.57651
IL3RA	3,132	0.056912579	0.57720

ES, enrichment score.