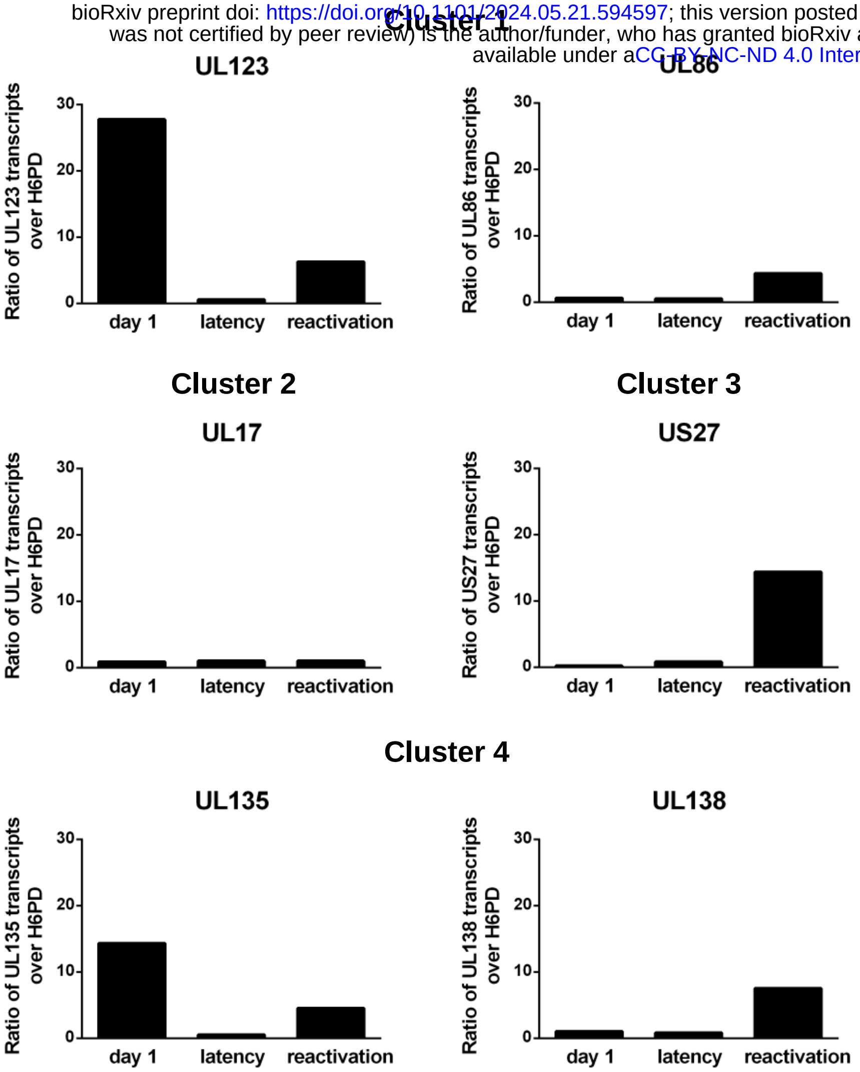
Supplementary Figure 1. RT-qPCR confirmation of select transcripts in WT infection. THP-1 cells were infected with WT HCMV (MOI = 2) and cultured in suspension cell dishes. Total RNA was collected at 1 dpi during the establishment of latency. At 5 dpi, cell cultures were divided and treated with TPA (reactivation) to trigger re-expression of viral genes or DMSO (latency) to maintain the latent infection. Total RNA was collected from suspension cells (latency) and from adherent cells (reactivation) at 7dpi. RT-qPCR was performed to quantify viral transcripts and confirm the patterns of viral gene expression observed in the RNA-Seq analysis. Viral transcripts from each gene expression cluster (this study) and three canonical kinetic gene classes (IE, E, L) were selected. Data are expressed as ratio of viral transcripts over the cellular transcript H6PD and represent a single biological replicate analyzed in triplicate.

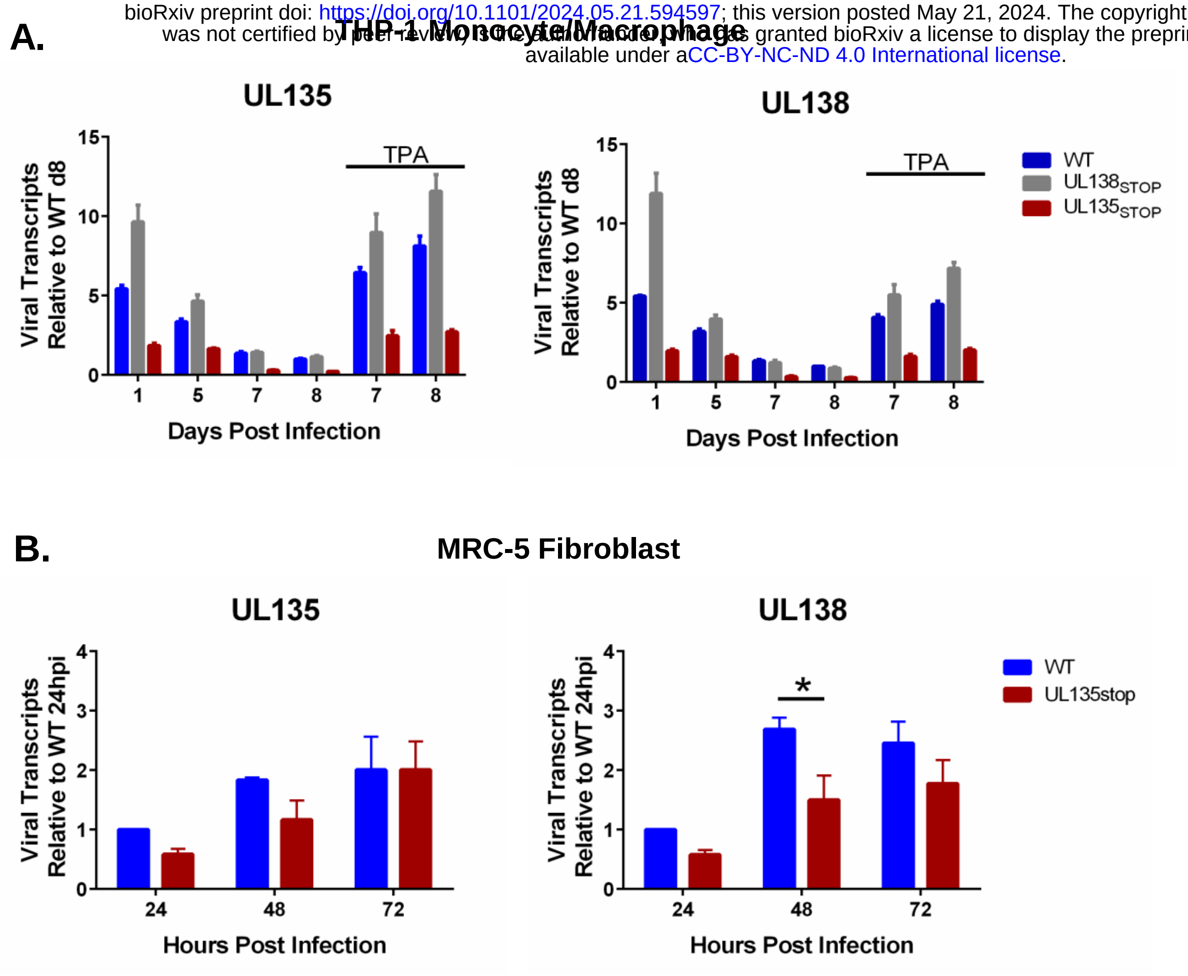
Supplementary Figure 2. Comparison of representative UL*b*' transcripts in hematopoietic versus replication-permissive cells. A) THP-1 cells were infected with WT, Δ*UL138*$_{\text{STOP}}$, or Δ*UL135*$_{\text{STOP}}$ HCMV (MOI = 2) and cultured in suspension cell dishes to establish latent infection. At 5 dpi, cells were treated with TPA to trigger re-expression of viral genes or with DMSO to maintain latent infection. Total RNA was isolated at the indicated time points and RT-qPCR was used to quantitate representative UL*b*' transcripts *UL135* and *UL138*. Data are shown as the ratio of each viral transcript over cellular H6PD and represent a single biological replicate analyzed in triplicate and used to confirm viral gene expression patterns observed in the RNA-Seq analysis. **B)** MRC-5 fibroblasts were infected with WT or Δ*UL135*$_{\text{STOP}}$ HCMV (MOI = 1) to establish replicative infection. Total RNA was collected at the indicated time points and RT-qPCR was used to quantify *UL135* and *UL138* transcripts. Data are expressed as fold change in viral transcripts over WT infection at 24 hours post infection (hpi). Error bars represent the SEM between three biological replicates analyzed in duplicate. Multiple t-tests (one per time point) were performed using the Holm-Sidak correction for multiple comparisons. Statistical significance where *, $P < 0.05$.

Supplementary Data Set 1. Simple Enrichment Analysis. Simple Enrichment Analysis (SEA) (*66*) was performed against the CIS-BP database of transcription factors (*67*) to identify transcription factor binding motifs that are significantly enriched in viral gene expression cluster 4 when compared to other viral gene expression clusters. The degree of significance for which each transcription factor is enriched in cluster 4 is expressed as a p-value. The percentage of cluster 4 HCMV genes associated with each predicted transcription factor binding site is shown (% HCMV c4 genes) as well as percent of HCMV genes from clusters 1, 2, and 3 (% HCMV c1, 2, 3, genes). Enrichment ratio represents the relative enrichment of each transcription factor binding site in cluster 4 versus clusters 1, 2, and 3. Figure quality images depicting the motif consensus sequences identified in cluster 4 genes were created using the WebLogo web-based application (*71*, *72*).

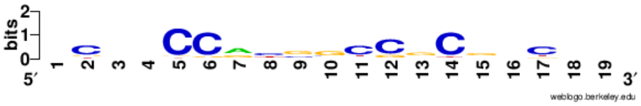
Supplementary Data Set 2. Differential Expression Analysis. Transcription factors corresponding to the significantly enriched transcription factor binding sites in cluster 4 were ranked by degree of differential expression at each time point dependent on the presence of pUL135 in our RNA-Seq analysis. Negative binomial modeling of gene expression with the *DESeq2* package from R (*68*) was used to determine differential expression. Log fold change (logFC) of gene expression for $UL135_{\mathrm{STOP}}$ virus over viruses expressing the UL135 protein (WT and $UL138_{\mathrm{STOP}}$ averaged) is shown. Statistical significance where *, $P < 0.05$; **, $P < 0.005$ and ***, $P < 0.0005$.

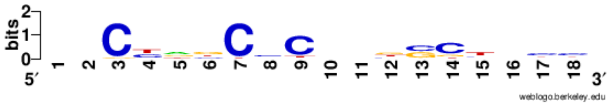
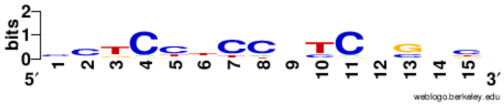
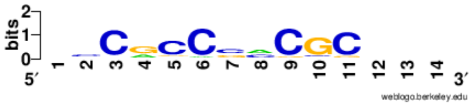


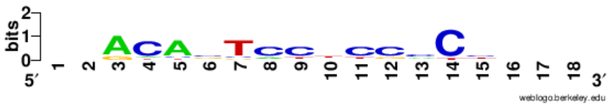
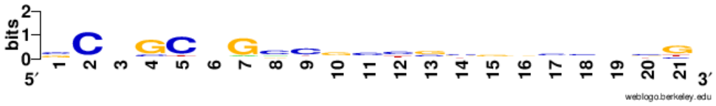
Supplementary Figure 1



Supplementary Figure 2

Motif Consensus	Gene Symbol	p-value	% HCMV c4 Genes	% HCMV c1, 2, 3 Genes	Enrich Ratio
	CXXC1	2.27 e $^{-4}$	58.3 %	8.6 %	6.73
	PPARG	3.05 e $^{-4}$	25 %	0 %	47.08
	TFAP2A	3.05 e $^{-4}$	25 %	0 %	47.08
	ZNF23	4.40 e $^{-4}$	58.3 %	9.2 %	6.28
	ZNF524	7.00 e $^{-4}$	75 %	20.4 %	3.68
	PLAG1	8.77 e $^{-4}$	75 %	21.1 %	3.57
	TFAP2C	1.17 e $^{-3}$	25 %	0.7 %	23.54
	ZNF100	1.37 e $^{-3}$	75 %	21.7 %	3.46
	IRF3	1.39 e $^{-3}$	50 %	7.9 %	6.34

Motif Consensus	Gene Symbol	p-value	% Cluster 4 Genes	% HCMV c1, 2, 3 Genes	Enrich Ratio
	IRF9	1.62 e^{-3}	41.7 %	5.3 %	7.85
	ZNF662	1.96 e^{-3}	50 %	8.6 %	5.88
	ZSCAN22	2.02 e^{-3}	33.3 %	2.6 %	11.77
	ESR1	2.80 e^{-3}	25 %	1.3 %	15.69
	ZNF429	3.45 e^{-3}	58.3 %	14.5 %	4.09
	EGR1	3.71 e^{-3}	75 %	24.3 %	3.10
	TIGD1	4.94 e^{-3}	16.7 %	0 %	35.31
	TBX19	4.94 e^{-3}	16.7 %	0 %	35.31
	ZNF594	4.94 e^{-3}	16.7 %	0 %	35.31

Motif Consensus	Gene Symbol	p-value	% Cluster 4 Genes	% HCMV c1, 2, 3 Genes	Enrich Ratio
	ZNF684	5.36 e $^{-3}$	25 %	2 %	11.77
	ZNF584	5.36 e $^{-3}$	25 %	2 %	11.77
	CDX1	5.59 e $^{-3}$	25 %	1.3 %	15.69
	EGR4	6.31 e $^{-3}$	50 %	11.2 %	4.58
	ZNF548	8.36 e $^{-3}$	33.3 %	3.9 %	8.41
	SOX12	8.41 e $^{-3}$	50 %	11.2 %	4.58
	EGR2	8.99 e $^{-3}$	75 %	28.3 %	2.67

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B	
Day 1 undifferentiated	TFAP2C	ENSG00000087510	-1.160202415	0.190043	-4.15709	6.1343708	0.0008556	0.963543	**
	PPARG	ENSG00000132170	0.370753256	5.218841	3.523475	0.000607	0.0062923	-1.20051	*
	CXXC1	ENSG00000154832	0.157189768	5.641158	1.239288	0.217696	0.49348942	-6.34767	
	ZNF524	ENSG00000171443	0.295048003	3.084672	0.908604	0.36541	0.64959332	-6.69994	
	ZNF100	ENSG00000197020	0.088718788	4.539584	0.52154	0.602968	0.81698032	-6.97596	
	PLAG1	ENSG00000181690	0.135495698	1.077953	0.49638	0.620549	0.8270811	-6.98876	
	IRF3	ENSG00000126456	-0.068330399	5.866038	-0.43323	0.665636	0.85339777	-7.01811	
	ZNF23	ENSG00000167377	-0.065414956	-2.07581	-0.14147	0.887738	0.95776564	-7.10203	
	TFAP2A	ENSG00000137203	-0.023972528	1.000728	-0.12839	0.898057	0.96195426	-7.1038	

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B
Day 3 undifferentiated	TFAP2C	ENSG00000087510	-0.798834005	0.190043	-2.86228	0.004977	0.12550995	-2.57596
	IRF3	ENSG00000126456	-0.198747266	5.866038	-1.26011	0.210115	0.77750388	-5.69694
	PPARG	ENSG00000132170	0.093190801	5.218841	0.885644	0.377609	0.88944352	-6.08861
	CXXC1	ENSG00000154832	-0.099681904	5.641158	-0.78589	0.433503	0.91307676	-6.17022
	TFAP2A	ENSG00000137203	0.115498875	1.000728	0.618592	0.537377	0.94725838	-6.28544
	ZNF524	ENSG00000171443	-0.197595561	3.084672	-0.6085	0.544026	0.94947014	-6.29152
	PLAG1	ENSG00000181690	-0.152047787	1.077953	-0.55702	0.57857	0.95464582	-6.32098
	ZNF100	ENSG00000197020	0.081226818	4.539584	0.477498	0.63389	0.96730839	-6.3614
	ZNF23	ENSG00000167377	0.216359789	-2.07581	0.467919	0.640705	0.96850953	-6.36585

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B
Day 5 undifferentiated	CXXC1	ENSG00000154832	-0.225505293	5.641158	-1.77789	0.077996	0.35376617	-4.63076
	PPARG	ENSG00000132170	0.16329105	5.218841	1.551846	0.123375	0.43551737	-4.98393
	PLAG1	ENSG00000181690	0.411142146	1.077953	1.506194	0.134688	0.45417076	-5.04969
	TFAP2A	ENSG00000137203	-0.247783589	1.000728	-1.32709	0.187041	0.52213634	-5.28939
	ZNF524	ENSG00000171443	-0.387980027	3.084672	-1.19479	0.234564	0.57186158	-5.44757
	IRF3	ENSG00000126456	-0.148654527	5.866038	-0.94251	0.347857	0.67176677	-5.70418
	TFAP2C	ENSG00000087510	-0.152089103	0.190043	-0.54495	0.586818	0.82580627	-5.98702
	ZNF23	ENSG00000167377	0.229986979	-2.07581	0.49739	0.619839	0.84332314	-6.0108
	ZNF100	ENSG00000197020	0.029735345	4.539584	0.174801	0.861535	0.95074931	-6.11493

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B
Day 5.5 undifferentiated	PPARG	ENSG00000132170	0.182528313	5.218841	1.734669	0.085409	0.60943602	-4.53355
	ZNF23	ENSG00000167377	-0.61479136	-2.07581	-1.3296	0.186212	0.76088461	-5.10337
	TFAP2C	ENSG00000087510	-0.365373922	0.190043	-1.30916	0.193021	0.76862837	-5.12827
	CXXC1	ENSG00000154832	-0.157928606	5.641158	-1.24511	0.215555	0.79077285	-5.20389
	PLAG1	ENSG00000181690	0.196650438	1.077953	0.720417	0.472692	0.91338257	-5.6831
	ZNF100	ENSG00000197020	0.098327204	4.539584	0.578023	0.564349	0.93868392	-5.76948
	TFAP2A	ENSG00000137203	-0.104598384	1.000728	-0.56021	0.576397	0.94173547	-5.77897
	IRF3	ENSG00000126456	-0.041989988	5.866038	-0.26623	0.790528	0.98121094	-5.89272
	ZNF524	ENSG00000171443	-0.065643849	3.084672	-0.20215	0.840147	0.98379825	-5.90679

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B
Day 6 undifferentiated	TFAP2A	ENSG00000137203	0.20669957	1.000728	1.107047	0.270524	0.92464593	-5.18008
	TFAP2C	ENSG00000087510	0.235149384	0.190043	0.842558	0.401179	0.95999793	-5.4112
	ZNF524	ENSG00000171443	0.183991866	3.084672	0.566605	0.572058	0.9773683	-5.58622
	PPARG	ENSG00000132170	0.053351166	5.218841	0.507026	0.613082	0.98007183	-5.61507
	ZNF23	ENSG00000167377	-0.183838918	-2.07581	-0.39759	0.691653	0.98577356	-5.65976
	PLAG1	ENSG00000181690	0.058973258	1.077953	0.216045	0.829326	0.99296086	-5.71009
	ZNF100	ENSG00000197020	-0.035209278	4.539584	-0.20698	0.836382	0.9940531	-5.71182
	IRF3	ENSG00000126456	0.028346522	5.866038	0.179724	0.857677	0.9977019	-5.71659
	CXXC1	ENSG00000154832	0.005258322	5.641158	0.041457	0.967002	0.99883043	-5.73042

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B
Day 8 undifferentiated	CXXC1	ENSG00000154832	0.284361045	5.641158	2.190365	0.030463	0.46870048	-3.61542
	TFAP2A	ENSG00000137203	0.293741114	1.000728	1.537056	0.126955	0.62803435	-4.68428
	TFAP2C	ENSG00000087510	0.418787284	0.190043	1.466046	0.145294	0.64438295	-4.77901
	ZNF23	ENSG00000167377	0.463869989	-2.07581	0.980142	0.32902	0.77719166	-5.31046
	ZNF524	ENSG00000171443	0.306699358	3.084672	0.922769	0.358009	0.79291867	-5.35956
	IRF3	ENSG00000126456	0.127334203	5.866038	0.78877	0.431827	0.83183543	-5.46288
	ZNF100	ENSG00000197020	-0.09500345	4.539584	-0.54564	0.58634	0.89476917	-5.60941
	PPARG	ENSG00000132170	-0.05424017	5.218841	-0.50362	0.615465	0.90637544	-5.62936
	PLAG1	ENSG00000181690	-0.020320356	1.077953	-0.07273	0.942143	0.98848408	-5.74187

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B
Day 5.5 differentiated	TFAP2C	ENSG00000087510	0.464213495	0.190043	1.663311	0.098902	0.71881807	-4.78323
	PLAG1	ENSG00000181690	-0.392897379	1.077953	-1.43936	0.152696	0.85336045	-5.10905
	TFAP2A	ENSG00000137203	-0.09065915	1.000728	-0.48555	0.628183	0.99991207	-5.97955
	PPARG	ENSG00000132170	0.044178342	5.218841	0.419851	0.675357	0.99991207	-6.00799
	IRF3	ENSG00000126456	-0.054938401	5.866038	-0.34832	0.728217	0.99991207	-6.03427
	ZNF524	ENSG00000171443	-0.110408863	3.084672	-0.34001	0.734457	0.99991207	-6.03701
	ZNF23	ENSG00000167377	-0.139931741	-2.07581	-0.30263	0.762705	0.99991207	-6.04851
	CXXC1	ENSG00000154832	0.027335438	5.641158	0.215513	0.829739	0.99991207	-6.07011
	ZNF100	ENSG00000197020	-0.018806493	4.539584	-0.11056	0.912157	0.99991207	-6.0865

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B
Day 6 differentiated	PPARG	ENSG00000132170	-0.308388064	5.218841	-2.93078	0.00406	0.08845639	-2.20072
	TFAP2A	ENSG00000137203	0.26509314	1.000728	1.419793	0.158303	0.53608525	-5.24294
	TFAP2C	ENSG00000087510	0.345174731	0.190043	1.236787	0.21862	0.59882606	-5.47478
	ZNF23	ENSG00000167377	-0.555554661	-2.07581	-1.20149	0.231965	0.60874713	-5.51592
	PLAG1	ENSG00000181690	-0.295316164	1.077953	-1.08187	0.281514	0.64704137	-5.6467
	CXXC1	ENSG00000154832	0.116318301	5.641158	0.917057	0.360982	0.70548616	-5.80486
	IRF3	ENSG00000126456	0.143897732	5.866038	0.912349	0.363444	0.70687041	-5.809
	ZNF524	ENSG00000171443	0.248932154	3.084672	0.76659	0.444855	0.75501121	-5.92678
	ZNF100	ENSG00000197020	0.084999029	4.539584	0.499673	0.618235	0.84770069	-6.08987

	gene symbol	ensembl	logFC UL135$_{STOP}$/avg(WT+UL138$_{STOP}$)	AvgExpr	t	p Value	adj p Value	B
Day 8 differentiated	PPARG	ENSG00000132170	-0.533937604	5.218841	-5.07431	1.4632583	0.00027186	5.018128 ***
	TFAP2A	ENSG00000137203	0.374334215	1.000728	2.004869	0.047264	0.3996679	-4.36901
	TFAP2C	ENSG00000087510	0.458430353	0.190043	1.64259	0.103128	0.56369915	-4.99464
	ZNF100	ENSG00000197020	-0.231246487	4.539584	-1.3594	0.176611	0.68461188	-5.40089
	PLAG1	ENSG00000181690	-0.369958488	1.077953	-1.35532	0.177902	0.68711741	-5.4062
	ZNF23	ENSG00000167377	-0.610993475	-2.07581	-1.32139	0.188926	0.70162407	-5.44978
	CXXC1	ENSG00000154832	0.133474273	5.641158	1.052315	0.294804	0.78697692	-5.75724
	IRF3	ENSG00000126456	0.114885831	5.866038	0.728406	0.467807	0.85997536	-6.03635
	ZNF524	ENSG00000171443	0.200066211	3.084672	0.616106	0.53901	0.88553754	-6.10965