Supplementary Information

Bromodomain and extra-terminal protein inhibitors modulate natural killer cell function and differentiation

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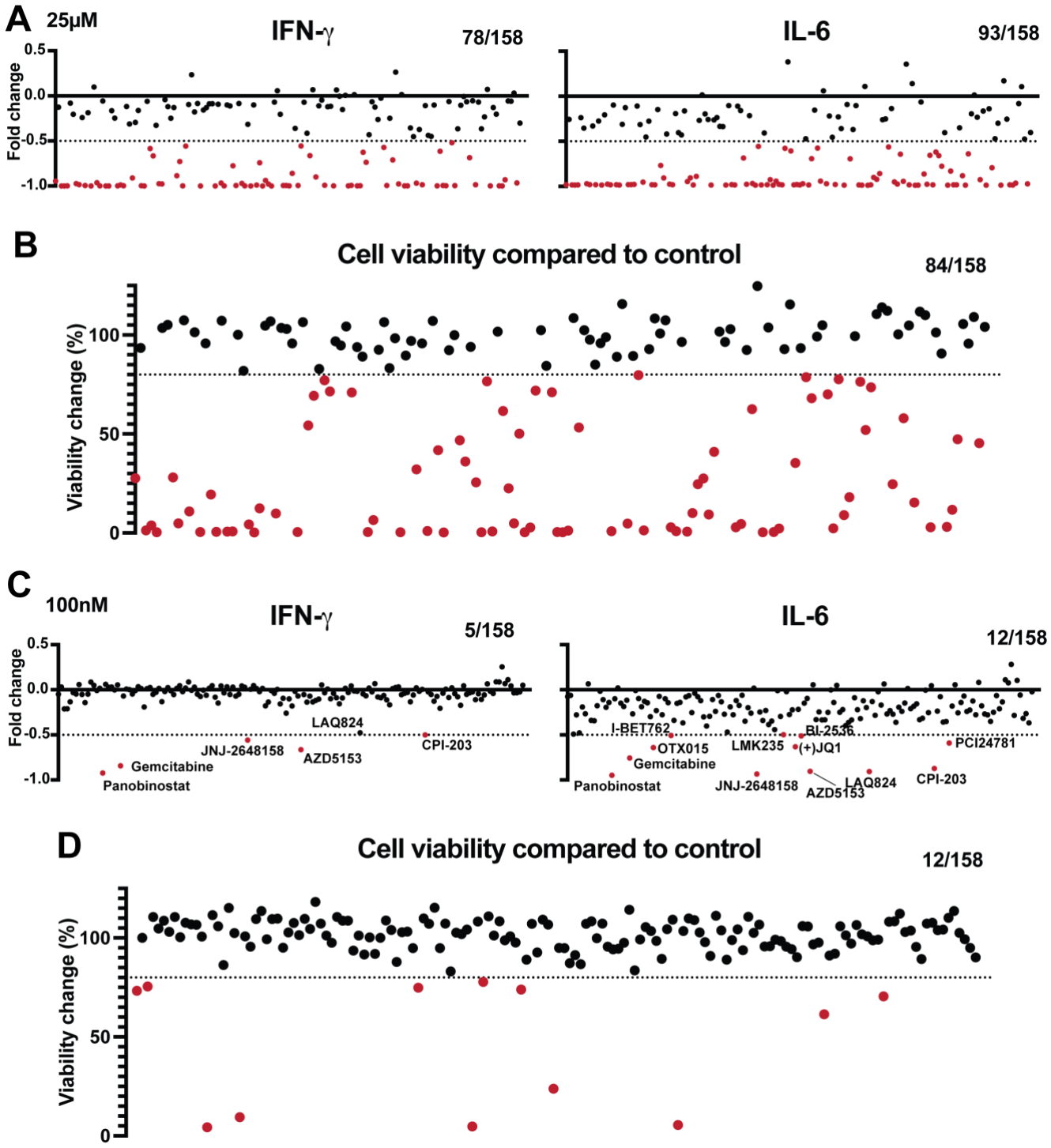
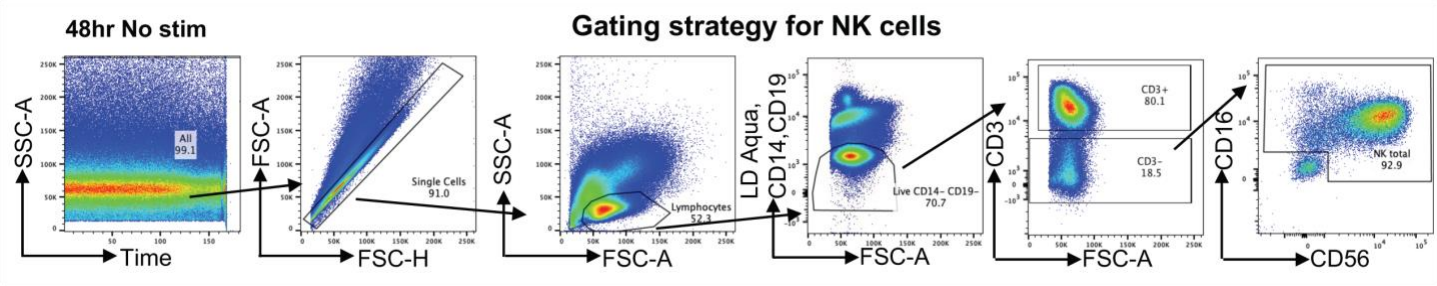
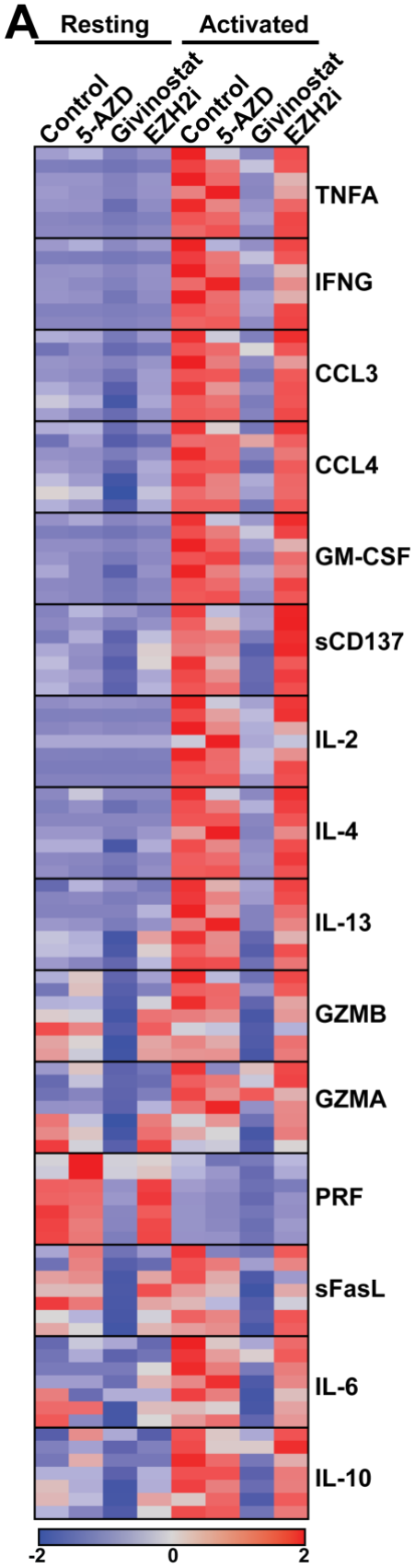


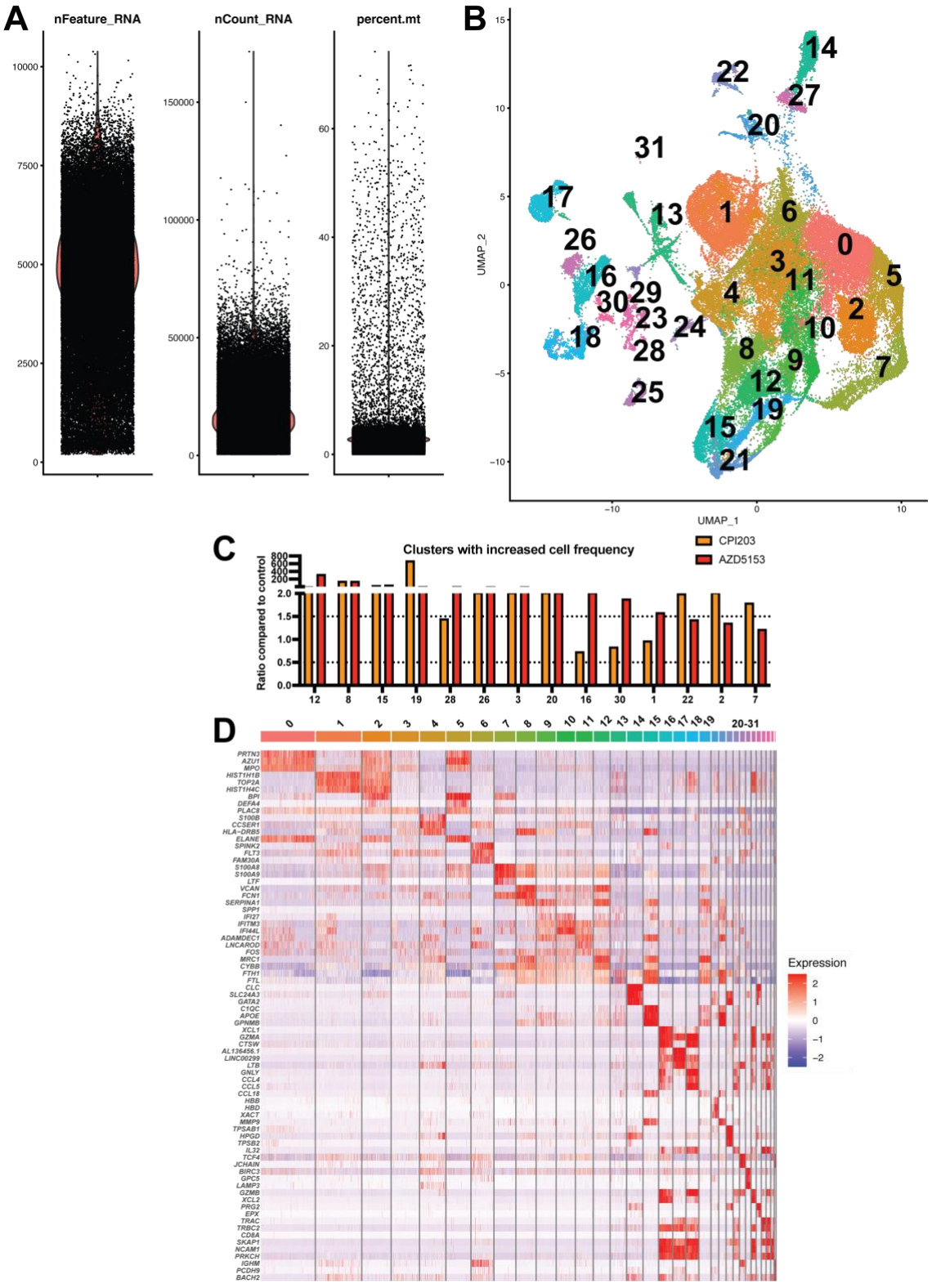
Supplemental Figure 1. Small-molecule drug screen identifies key epigenetic regulators of NK cell IFN-g and IL-6 production and cell viability. (A) Dot plot of fold-change of supernatant IFN-g and IL-6 levels for each individual drug (dots) compared to control treated NK-92 cells at 25μM drug concentration. Dots colored red have ≤0.5 fold change. **(B)** Dot plot of NK-92 cell viability when treated with individual drugs compared to control treatment at 25μM drug treatment concentration. K-means clustering utilized to group the drugs by protein pattern into four clusters (c1-c4). **(C)** Dot plot of fold-change of supernatant IFN-g and IL-6 for each individual drug (dots) compared to control treated NK-92 cells at 25μM drug concentration. Dots colored red have ≤0.5 fold change. Individual drugs labeled. **(D)** Dot plot of NK-92 cell viability when treated with individual drugs compared to control treatment at 100nM drug treatment concentration. The number of drugs that reached threshold out of the 158 total tested are shown in graph corners.



Supplemental Figure 2. Flow cytometry gating strategy for NK cells. Serial gates were set as indicated to identify live, CD14-CD19-CD3- NK cells expressing CD56 and/or CD16 within the lymphocyte-gated single cell population of PBMCs.



Supplemental Figure 3. Givinostat treatment reduces primary human NK cell proinflammatory cytokine activation. (A) Heatmap of normalized z-scores of cytokine and cytolytic molecule concentrations in the cell culture supernatant of resting or cytokine (IL-12 + IL-15) stimulated human primary NK cells (n=8) treated with 5-azacytidine, Givinostat or EZH2i compared with control.



Supplemental Figure 4. Single-cell RNA-seq of bone marrow hematopoietic cells cultured to promote NK cell fate. (A) Violin plots of the distributions of number of RNA transcripts (nFeature_RNA), number of unique molecular identifiers (UMIs; nCount RNA) and percentage of mitochondrial RNA (percent.mt) for each cell. **(B)** UMAP plot showing graph-based clusters identified from Seurat based on the transcriptomic signatures of each cell population. **(C)** Bar graph of the ratio of the frequencies of cells in each cluster compared to control for BETi (CPI203 or AZD5153) treated groups. Only clusters with increased or decreased ratios compared to control group are shown. **(D)** Heatmap showing the top genes upregulated in each cluster.

A

AZD5153 vs. Control

Upregulated

GeneID	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
XCL1	3.70E-177	1.454390343	0.339	0.721	1.13E-172
TNFSF10	3.94E-137	1.286184806	0.928	0.965	1.21E-132
RAB27B	4.24E-150	1.149112684	0.446	0.707	1.30E-145
ZNF683	2.23E-257	1.143403393	0.109	0.523	6.83E-253
BGLAP	4.19E-86	1.129435673	0.093	0.283	1.28E-81
XCL2	1.89E-118	1.091394836	0.339	0.651	5.79E-114
HOXA9	1.34E-17	0.99945663	0.323	0.386	4.11E-13
SELL	1.36E-109	0.998640493	0.298	0.574	4.17E-105
CCND2	5.62E-69	0.980266928	0.961	0.955	1.72E-64
IFI30	7.48E-13	0.953066938	0.511	0.597	2.29E-08

Down-regulated

GeneID	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
AL136456.1	6.51E-240	-3.638333007	0.566	0.09	2.00E-235
GNLY	1.71E-233	-2.505021367	0.761	0.267	5.25E-229
IL1R1	2.74E-139	-1.758054968	0.416	0.086	8.40E-135
ADAM12	7.87E-138	-1.502166937	0.352	0.028	2.41E-133
TRBC1	1.22E-217	-1.347833426	0.665	0.198	3.73E-213
CCDC26	1.21E-116	-1.323174809	0.363	0.068	3.72E-112
AHR	3.05E-96	-1.26167262	0.875	0.77	9.36E-92
AFF3	3.67E-48	-1.230935891	0.412	0.24	1.13E-43
CYP1B1	7.72E-53	-1.229161535	0.374	0.182	2.37E-48
NTRK2	3.00E-115	-1.178542064	0.297	0.018	9.20E-111

CPI-203 vs. Control

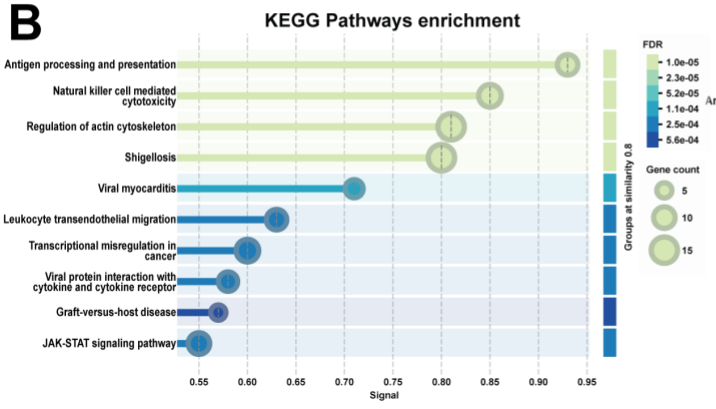
Upregulated

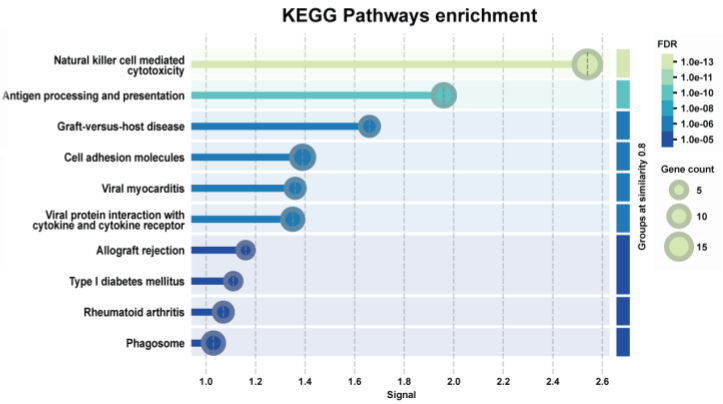
GeneID	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
CCL3	3.75E-57	1.028901256	0.45	0.684	1.15E-52
KLRC1	8.49E-78	1.020223679	0.696	0.806	2.60E-73
XCL2	3.40E-83	1.002348087	0.339	0.65	1.04E-78
CCL4L2	1.47E-14	0.995999697	0.192	0.297	4.50E-10
XCL1	3.77E-83	0.985860081	0.339	0.651	1.15E-78
CCL4	7.33E-35	0.939453106	0.511	0.71	2.25E-30
GIMAP7	1.09E-90	0.878290299	0.557	0.802	3.33E-86
GZMB	2.00E-50	0.868549631	0.66	0.856	6.14E-46
KIR2DL4	1.32E-43	0.754337498	0.422	0.586	4.03E-39
DUSP5	2.70E-61	0.747045823	0.484	0.677	8.29E-57

Down-regulated

GeneID	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
AL136456.1	2.66E-53	-1.940302861	0.566	0.404	8.14E-49
IL1R1	1.33E-72	-1.584313583	0.416	0.153	4.07E-68
CYP1B1	1.42E-44	-1.3985069	0.374	0.163	4.35E-40
ADAM12	4.11E-78	-1.336980748	0.352	0.062	1.26E-73
CCDC26	3.69E-79	-1.266689478	0.363	0.071	1.13E-74
NTRK2	4.30E-76	-1.177569845	0.297	0.026	1.32E-71
AHR	8.80E-67	-1.173712465	0.875	0.776	2.70E-62
AFF3	5.46E-44	-1.056246121	0.412	0.193	1.67E-39
ALDH1A3	1.82E-68	-0.930958603	0.263	0.018	5.58E-64
ENPP1	6.36E-57	-0.915951952	0.363	0.119	1.95E-52

B





Supplemental Figure 5. Differential gene expression of NK cell populations from scRNA-seq. (A) Top upregulated and downregulated genes comparing BETi-treated and control NK cells. Average log2 fold change (avg_log2FC) and percentage of cells in BETi (pct.1) or control (pct.2) with expression of each gene shown. Raw and adjusted p values also shown. Differential gene expression was performed using the nonparametric Wilcoxon rank sum test in Seurat. **(B)** Analysis of enriched KEGG pathways of the upregulated genes in the BETi-treated NK cells compared to control identified enriched pathways associated with NK cell cytotoxicity and antigen processing and presentation. String (String-db.org) was used to perform gene set enrichment pathway analysis including pathways from KEGG database[1,2].

References

1 Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* **44**, D457-462, doi:10.1093/nar/gkv1070 (2016).

2 Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* **28**, 27-30, doi:10.1093/nar/28.1.27 (2000).