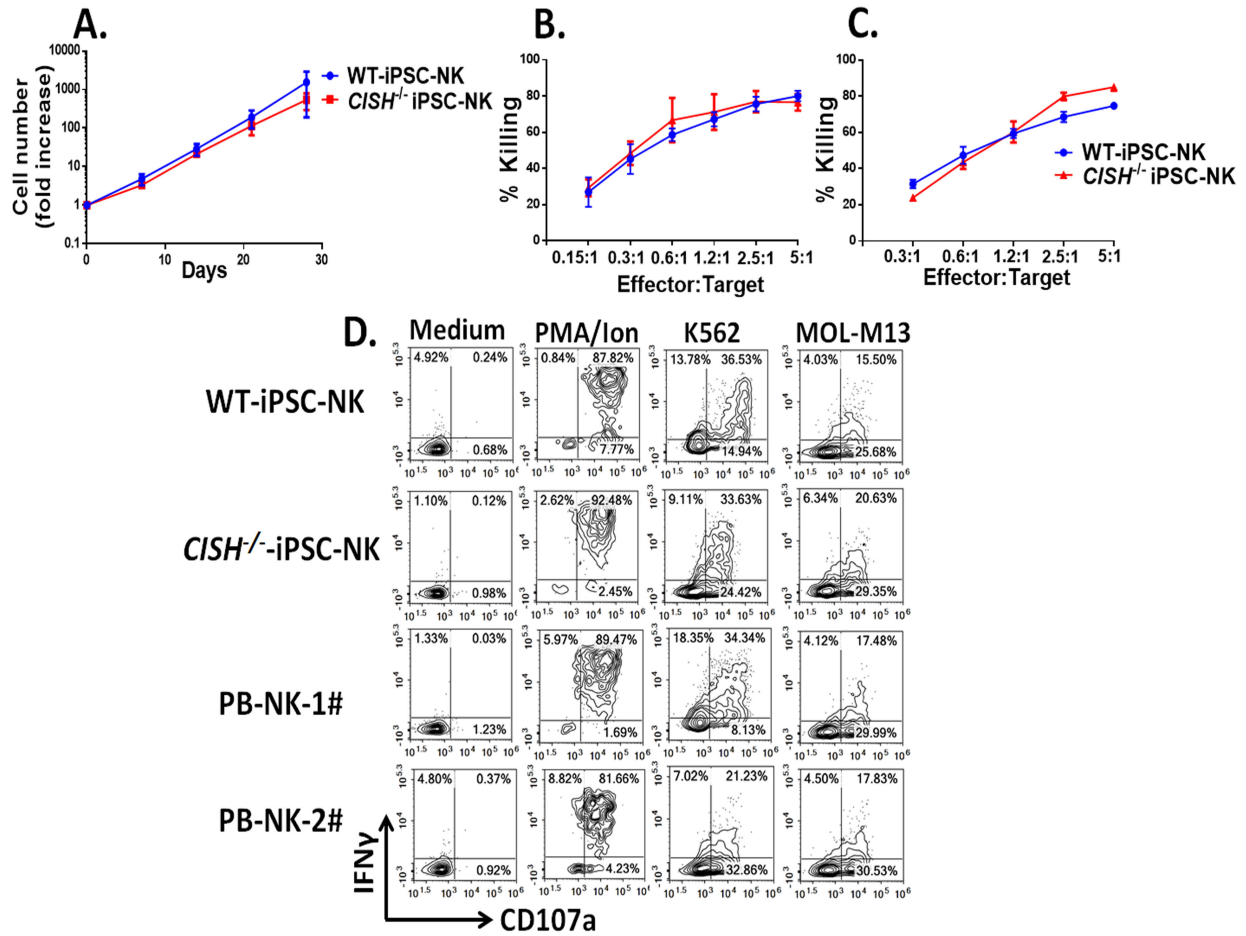
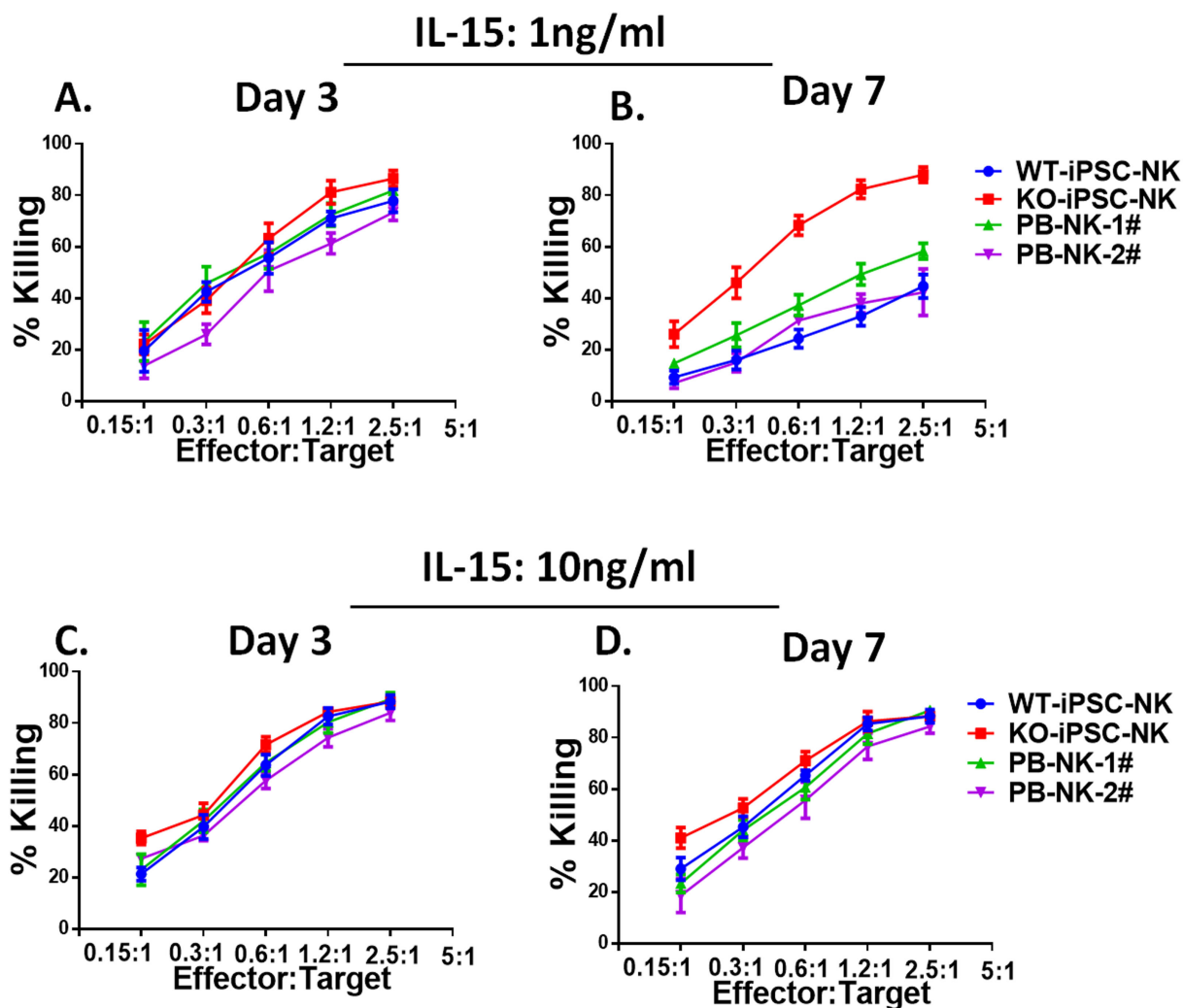


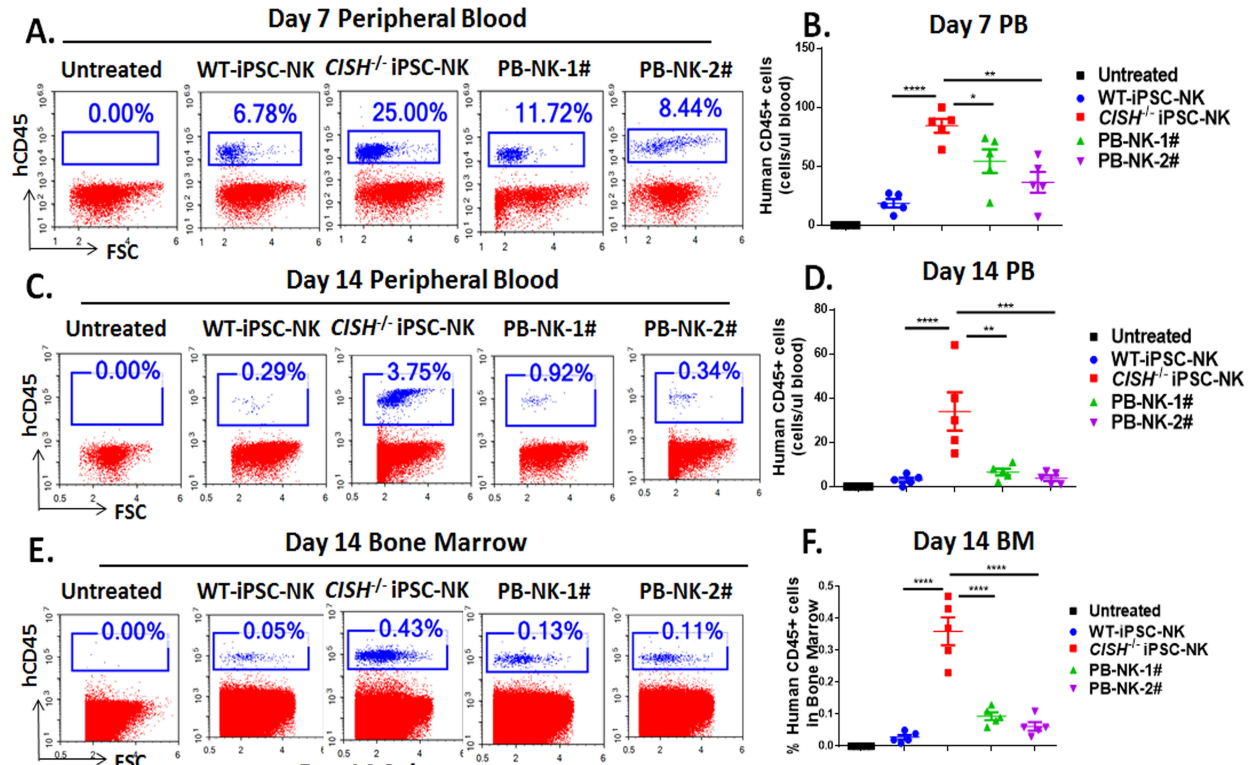
**Supplemental Figure S1. Related to Figure 1. Characterization of *CISH*<sup>-/-</sup> iPSC.** **A.** Immunoblot of CIS expression in iPSCs and hematopoietic progenitor cells (HPC). Undifferentiated iPSCs and day 6 embryoid bodies (EB) were treated with IL-2 (100 U/ml) for 24 hours. The whole day 6 EB (90% CD34<sup>+</sup> cells) contain HPCs. As expected, these undifferentiated iPSCs and HPCs do not express CIS. iPSC-NK cells treated with IL-2(100 U/ml) for 0 and 24 hours were used as negative and positive controls. GAPDH was used as loading control. **B.** Phase microscope images of WT-iPSC and *CISH*<sup>-/-</sup> iPSC cells. Scale bar: 400 μM. **C.** Staining of pluripotency markers TRA1-81 and SSEA-4 by flow cytometry. **D.** Karyotype of WT-iPSC and *CISH*<sup>-/-</sup> iPSC.



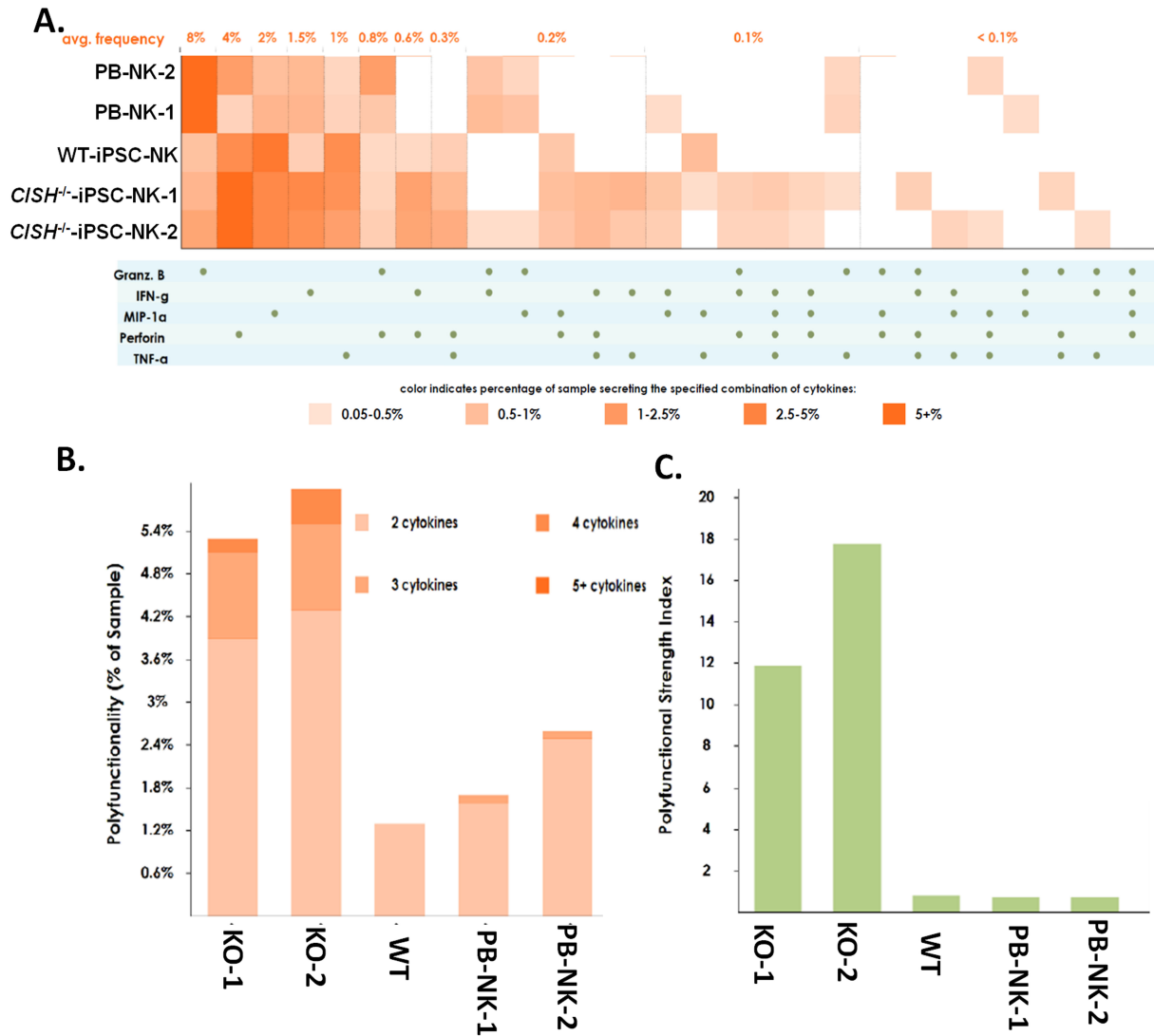
**Supplemental Figure S2. Related to Figure 3.** *CISH*<sup>-/-</sup> iPSC-NK cells and WT-iPSC-NK cells show similar expansion and killing activity when maintained with aAPC plus high cytokine concentration. **A.** *CISH*<sup>-/-</sup> iPSC-NK cells and WT-iPSC-NK cells were expanded with aAPC plus IL-2 (100 U/ml) as previously reported. Cell growth was counted weekly. **B-C.** After 3 weeks' expansion with aAPC plus IL-2, killing against K562 (**B**) and MOLM-13 (**C**) cells were evaluated using CellEvent™ Caspase-3/7 Green Flow Cytometry Assay after 4 hours' co-incubation of NK cells and target cells. **D.** WT-iPSC-NK cells, *CISH*<sup>-/-</sup> iPSC-NK-cells and PB-NK cells from 2 donors (PB-NK-1# and PB-NK-2#) were expanded with aAPC plus IL-2. After expansion, cells were left unstimulated or stimulated with a 1:1 ratio of target cells and stained for CD107a and IFN $\gamma$  4 hours later. Data at A-C were shown as mean  $\pm$  SD. Data at A-D were repeated in 3 separate experiments.



**Supplemental Figure S3. Related to Figure 3. *CISH*<sup>-/-</sup> iPSC-NK cells show better killing activity when maintained with low concentration of IL-15 in comparison with WT-iPSC-NK or PB-NK cells A-D.** WT-iPSC-NK cells, *CISH*<sup>-/-</sup> iPSC-NK-cells and PB-NK cells from 2 donors (PB-NK-1# and PB-NK-2#) were incubated with 1 ng/ml IL-15 (A, B) or 10 ng/ml IL-15 (C, D) for 3 days (A, C) or 7 days (B, D). Then killing against K562 were evaluated using CellEvent™ Caspase-3/7 Green Flow Cytometry Assay after 4 hours' co-incubation of NK cells and target cells (n=3). Data were shown as mean ± SD. Data and were repeated in 2 separate experiments.



**Supplemental Figure S4. Related to Figure 4. *CISH*<sup>-/-</sup> iPSC-NK cells have better in vivo persistence and bone marrow homing compared with WT-iPSC-NK and PB-NK cells.** NSG mice were untreated or dosed with one iv injection of  $10^7$  WT-iPSC-NK cells or *CISH*<sup>-/-</sup> iPSC-NK cells or PB-NK-1# or PB-NK-2# cells. NK cells were supported by every other day injections of IL-2. **A.** Representative flow cytometric plot of human CD45<sup>+</sup> cells in population from peripheral blood (PB) at day 7. **B.** Quantification of human CD45<sup>+</sup> cells in PB. **C, E:** Representative flow cytometric plot of human CD45<sup>+</sup> cells in population from PB (C) and Bone Marrow (BM) (E) at day 14. **D, F:** Quantification of human CD45<sup>+</sup> cells in PB (D) and BM (F). Each dot represents one recipient mouse (n=5). Median  $\pm$  SD is shown. Statistics: One way ANOVA, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001.



**Supplemental Figure S5. Related to Figure 5. *CISH*<sup>-/-</sup> iPSC-NK cells show improved single-cell polyfunctionality** **A.** *CISH*<sup>-/-</sup> iPSC-NK cells and WT iPSC-NK cells were incubated with a low concentration of IL-15 (1 ng/ml) overnight and then stimulated with IL-12 (10 ng/ml) and IL-18 (100 ng/ml). Cytokine production at the single cell level was measured and analyzed using the Isoplex 32-plex, immune cytokine response panel. 5 effector cytokines (Granzyme B, IFN $\gamma$ , MIP-1 $\alpha$ , Perforin, TNF $\alpha$ ) involved in cytotoxic function were shown. **B.** Quantification of percentage of cell population with polyfunctionality (secret >2 cytokines). **C.** Polyfunctionality was measured through a PSI, spanning a pre-specified panel of 32 key immunologically relevant molecules across major categories: homeostatic/proliferative, inflammatory, chemotactic, regulatory, and immune effector. PSI, polyfunctionality strength index.

**Supplemental Table 1. List of antibodies used for mass cytometry panel. Related to STAR method**

<b>Tag</b>	<b>Marker</b>	<b>Clone</b>	<b>Isotype</b>	<b>Vendor</b>	<b>Catalog number</b>
89Y	CD45	HI30	IgG1	Fluidigm	3089003B
Qdot655	CD19	SJ25-C1	Mouse IgG1 kappa	Invitrogen	Q10179
141Pr	KIR2DS4	JJC11.6	Mouse IgG1 kappa	Miltenyi	130-092-679
142Nd	CD57	HCD57	Other	Fluidigm	3142007B
143Nd	2B4	REA112	Recomb. human IgG1	Miltenyi	130-124-523
144Nd	CD38	REA572	Recomb. human IgG1	Miltenyi	130-122-307
PE	CD32	REA997	Recomb. human IgG1	Miltenyi	130-116-599
145Nd	PE	PE001	Mouse IgG1	Fluidigm	3145006B
146Nd	CD8	RPA-T8	Mouse IgG1	Fluidigm	3146001B
147Sm	KIR3DL2	DX31	Unknown	Mabtech	Custom
148Nd	CD14	RMO52	Mouse IgG2a	Fluidigm	3148010B
149Sm	Syk	4D10.2	Mouse IgG2a	Fluidigm	3149020B
150Nd	CD34	AC136	Mouse IgG2a	Miltenyi	130-108-040
151Eu	TIGIT	MBSA43	Mouse IgG1 kappa	Invitrogen	16-9500-82
152Sm	Siglec-7	194211	Mouse IgG1	R&D Systems	MAB11381
153Eu	TIM-3	F38-2E2	Unknown	Miltenyi	Custom
154Sm	NKG2C	REA205	Recomb. human IgG1	Miltenyi	130-122-278
155Gd	KIR2DL1/S1	11PB6	Mouse IgG1 kappa	Miltenyi	130-092-682
156Gd	LILRB1	GHI/75	IgG2b	Fluidigm	3156020B
158Gd	KIR2DL1	REA284	Recomb. human IgG1	Miltenyi	130-122-279
159Tb	CD2	RPA-2.10	Mouse IgG1 kappa	eBioscience	14-0029-82
161Dy	Ki-67	B56	Mouse IgG1	Fluidigm	3161007B
162Dy	CD27	L128	Mouse IgG1	Fluidigm	3162009B
163Dy	KIR2DL3	REA147	Recomb. human IgG1	Miltenyi	130-122-280

164Dy	CD161	HP-3G10	Mouse IgG1	Fluidigm	3164009B
165Ho	KSP37	TDA3	Mouse IgG1 kappa	Biolegend	346603
166Er	NKG2D	ON72	IgG1	Fluidigm	3166016B
167Er	KIR3DL1	DX9	IgG1	Fluidigm	3167013B
168Er	NKp30	AF29-4D12	Mouse IgG1 kappa	Miltenyi	130-092-554
169Tm	NKG2A	Z199	IgG2b	Fluidigm	3169013B
170Er	CD3	UCHT1	Mouse IgG1	Fluidigm	3170001B
171Yb	DNAM-1	DX11	IgG1	Fluidigm	3171013B
172Yb	KIR2DL2/S2/L3	GL183	Mouse IgG1	Beckman Coulter	IM1846
173Yb	Granzyme B	GB11	Mouse IgG1	Fluidigm	3173006B
174Yb	CD94	HP-3D9	IgG2a	Fluidigm	3174015B
175Lu	Perforin	B-D48	Mouse IgG1	Fluidigm	3175004B
176Yb	CD56	NCAM16.2	Mouse IgG2b	Fluidigm	3176008B
209Bi	CD16	3G8	IgG1	Fluidigm	3209002B

**Supplemental Table 2. Top 100 significant enriched Gene ontology (GO) pathways in *CISH*<sup>-/-</sup> iPSC-NK cells in comparison WT iPSC-NK cells. Related to Figure 5**

<b>GOID</b>	<b>Description</b>	<b>padj</b>
GO:0002521	leukocyte differentiation	3.62E-06
GO:0009617	response to bacterium	2.08E-05
GO:1902105	regulation of leukocyte differentiation	2.36E-05
GO:0002573	myeloid leukocyte differentiation	4.05E-05
GO:0002761	regulation of myeloid leukocyte differentiation	0.001906594
GO:0009306	protein secretion	0.001906594
GO:0001659	temperature homeostasis	0.002952161
GO:0035966	response to topologically incorrect protein	0.004100741
GO:0070663	regulation of leukocyte proliferation	0.004100741
GO:0002790	peptide secretion	0.004100741
GO:1902107	positive regulation of leukocyte differentiation	0.005910755
GO:0006986	response to unfolded protein	0.006383619
GO:0030098	lymphocyte differentiation	0.006383619
GO:0032944	regulation of mononuclear cell proliferation	0.006383619
GO:0030217	T cell differentiation	0.006383619
GO:0001819	positive regulation of cytokine production	0.006383619
GO:0002683	negative regulation of immune system process	0.006383619
GO:0002237	response to molecule of bacterial origin	0.006383619
GO:0050663	cytokine secretion	0.006430113
GO:0030968	endoplasmic reticulum unfolded protein response	0.006430113
GO:0035967	cellular response to topologically incorrect protein	0.006430113
GO:0051047	positive regulation of secretion	0.006430113
GO:0050714	positive regulation of protein secretion	0.006430113
GO:1904894	positive regulation of STAT cascade	0.006430113
GO:0050670	regulation of lymphocyte proliferation	0.006430113
GO:0032496	response to lipopolysaccharide	0.006430113
GO:1904892	regulation of STAT cascade	0.006430113
GO:0006614	SRP-dependent cotranslational protein targeting to membrane	0.006430113
GO:0051249	regulation of lymphocyte activation	0.007305719
GO:0050708	regulation of protein secretion	0.007305719
GO:0034620	cellular response to unfolded protein	0.007305719
GO:1903707	negative regulation of hemopoiesis	0.007305719
GO:0030517	negative regulation of axon extension	0.007564823
GO:1903708	positive regulation of hemopoiesis	0.008916126
GO:0031649	heat generation	0.008916126
GO:0050777	negative regulation of immune response	0.00990916
GO:0070661	leukocyte proliferation	0.00990916



GO:1903532	positive regulation of secretion by cell	0.010217165
GO:0002694	regulation of leukocyte activation	0.010217165
GO:0048871	multicellular organismal homeostasis	0.010217165
GO:0070972	protein localization to endoplasmic reticulum	0.010217165
GO:0032963	collagen metabolic process	0.010217165
GO:0007162	negative regulation of cell adhesion	0.010217165
GO:0051250	negative regulation of lymphocyte activation	0.010217165
GO:0002793	positive regulation of peptide secretion	0.010217165
GO:0046427	positive regulation of JAK-STAT cascade	0.010217165
GO:0006613	cotranslational protein targeting to membrane	0.010217165
GO:0046425	regulation of JAK-STAT cascade	0.010217165
GO:0098542	defense response to other organism	0.010217165
GO:0031650	regulation of heat generation	0.010217165
GO:0050715	positive regulation of cytokine secretion	0.010217165
GO:0030099	myeloid cell differentiation	0.010266201
GO:0050865	regulation of cell activation	0.01120007
GO:0097696	STAT cascade	0.01120007
GO:0042330	taxis	0.01120007
GO:1902106	negative regulation of leukocyte differentiation	0.012054418
GO:0048305	immunoglobulin secretion	0.012054418
GO:0050707	regulation of cytokine secretion	0.012054418
GO:0002292	T cell differentiation involved in immune response	0.012054418
GO:0045047	protein targeting to ER	0.012405075
GO:0048008	platelet-derived growth factor receptor signaling pathway	0.012405075
GO:0006935	chemotaxis	0.012405075
GO:0042110	T cell activation	0.012734567
GO:0072599	establishment of protein localization to endoplasmic reticulum	0.012734567
GO:0019731	antibacterial humoral response	0.013006017
GO:0030888	regulation of B cell proliferation	0.013410282
GO:0002695	negative regulation of leukocyte activation	0.013462366
GO:0007160	cell-matrix adhesion	0.013559666
GO:0048841	regulation of axon extension involved in axon guidance	0.013688204
GO:0045580	regulation of T cell differentiation	0.013859414
GO:0050856	regulation of T cell receptor signaling pathway	0.013859414
GO:0042113	B cell activation	0.013878471
GO:0030574	collagen catabolic process	0.013995604
GO:0007159	leukocyte cell-cell adhesion	0.014073056
GO:0051222	positive regulation of protein transport	0.014073056
GO:0002791	regulation of peptide secretion	0.014476373
GO:0050868	negative regulation of T cell activation	0.015199065
GO:0009435	NAD biosynthetic process	0.015199065

GO:0022407	regulation of cell-cell adhesion	0.016106186
GO:0051043	regulation of membrane protein ectodomain proteolysis	0.016661935
GO:0048514	blood vessel morphogenesis	0.017820436
GO:0002675	positive regulation of acute inflammatory response	0.017820436
GO:0010631	epithelial cell migration	0.017820436
GO:0007259	JAK-STAT cascade	0.017820436
GO:1904951	positive regulation of establishment of protein localization	0.017820436
GO:0036005	response to macrophage colony-stimulating factor	0.017820436
GO:0036006	cellular response to macrophage colony-stimulating factor stimulus	0.017820436
GO:0061844	antimicrobial humoral immune response mediated by antimicrobial peptide	0.018313008
GO:0001667	ameboidal-type cell migration	0.018313008
GO:0045655	regulation of monocyte differentiation	0.018313008
GO:0051023	regulation of immunoglobulin secretion	0.018313008
GO:0050863	regulation of T cell activation	0.019709414
GO:0002294	CD4-positive, alpha-beta T cell differentiation involved in immune response	0.020146934
GO:0030316	osteoclast differentiation	0.020227355
GO:1903706	regulation of hemopoiesis	0.020227355
GO:0050867	positive regulation of cell activation	0.020456786
GO:0090132	epithelium migration	0.020573919
GO:0045926	negative regulation of growth	0.021159138
GO:0031532	actin cytoskeleton reorganization	0.021221061
GO:0045619	regulation of lymphocyte differentiation	0.021221061