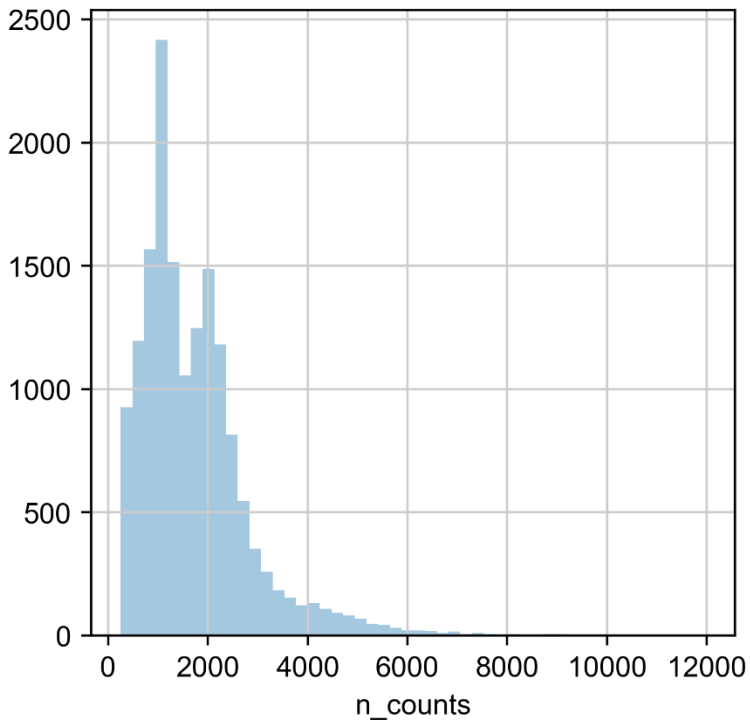
Droplet-based single-cell RNA-seq

Single-cell libraries were generated using the Chromium Controller and Single Cell 3' Library & Gel Bead Kit v2 (10x Genomics) according to the manufacturer's protocol. Briefly, an aliquot of patient PBMCs was stained with DAPI for discrimination between live and dead cells, and a maximum of 100,000 live, doublet-excluded cells were sorted into 1.5 ml tubes. Cells were pelleted by centrifuging for 5 min at 4 °C at 300 x g and resuspended in PBS with 0.04% BSA. Up to 17,000 cells suspended in reverse transcription reagents, along with gel beads, were segregated into aqueous nanoliter-scale gel bead-in-emulsions (GEMs). The GEMs were then reverse-transcribed in a C1000 Thermal Cycler (Bio-Rad) programmed at 53 °C for 45 min, 85 °C for 5 min, and hold at 4 °C. After reverse transcription, single-cell droplets were broken, and the single-strand cDNA was isolated and cleaned with Cleanup Mix containing Dynabeads MyOne SILANE (Thermo Fisher Scientific). cDNA was then amplified with a C1000 Thermal Cycler programed at 98 °C for 3 min, 10 cycles of (98 °C for 15 s, 67 °C for 20 s, 72 °C for 1 min), 72 °C for 1 min, and hold at 4 °C. Subsequently, the amplified cDNA was fragmented, end-repaired, A-tailed, and index adaptor ligated, with cleanup in-between steps using SPRIselect Reagent Kit (Beckman Coulter). Post-ligation product was amplified with a T1000 Thermal Cycler programed at 98 °C for 45 s, 10 cycles of (98 °C for 20 s, 54 °C for 30 s, 72 °C for 20 s), 72 °C for 1 min, and hold at 4 °C. The sequencing-ready library was cleaned up with SPRIselect and sequenced by the Biomedical Sequencing Facility at CeMM using the Illumina HiSeq 3000/4000 platform and the 75 bp paired-end configuration.

Preprocessing and analysis of single-cell RNA-seq data

Preprocessing of the single-cell RNA-seq data was performed using Cell Ranger version 2.0.0 (10x Genomics). Raw sequencing files were demultiplexed using the Cell Ranger command 'mkfastq'. Each sample was aligned to the human reference genome assembly 'refdata-cellranger-GRCh38-1.2.0' using the Cell Ranger command 'count', and all samples were aggregated using the Cell Ranger command 'aggr' without depth normalization. Raw expression data were then loaded into R version 3.4.0 and analyzed using the Seurat package version 2.0.1 with the parameters suggested by the developers[54]. Specifically, single-cell profiles with less than 200 detected genes (indicative of no cell in the droplet), more than 3,000 detected genes (indicative of cell duplicates), or more than 15% of UMIs stemming from mitochondrial genes were discarded. Read counts were normalized dividing by the total UMI count in each cell, multiplied by a factor of 10,000, and log transformed. The number of UMIs per cell and the percent of mitochondrial reads per cell were then regressed out using Seurat's standard analysis pipeline.

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Counts after norm: Counts range from  327.5  to  997.2
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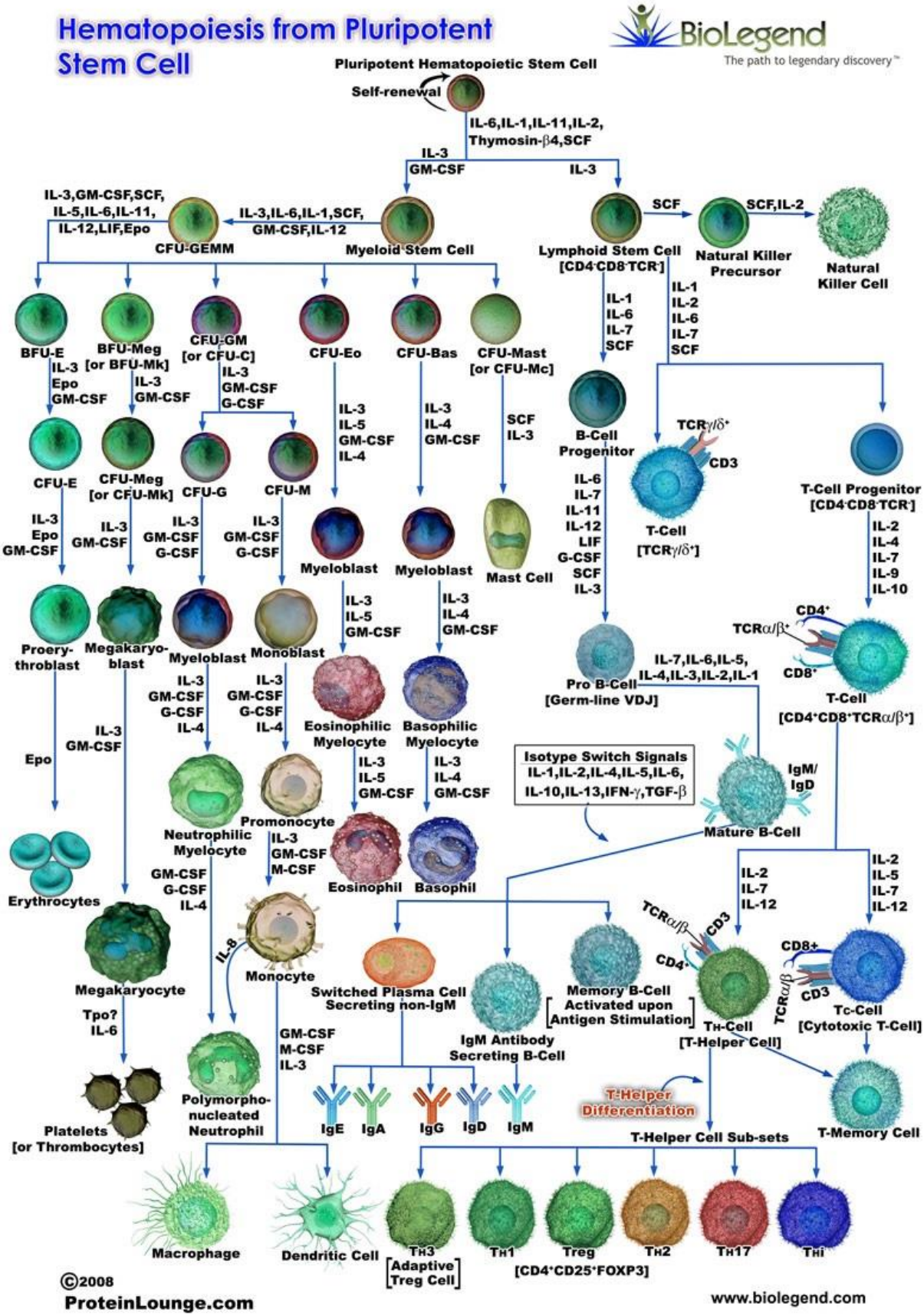
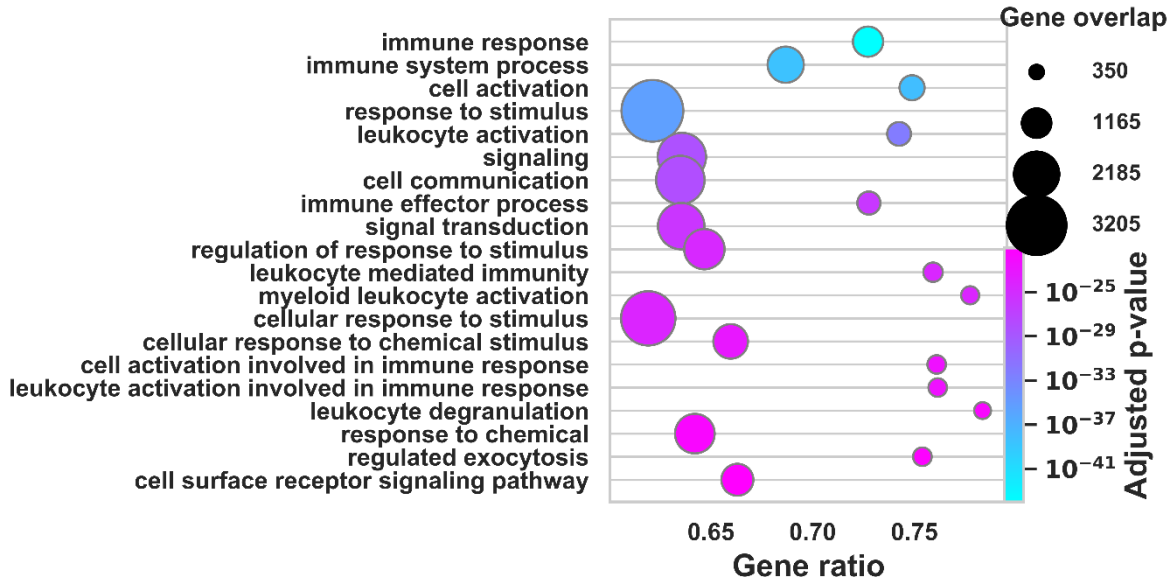
Hematopoiesis from Pluripotent Stem Cell



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T cell:                {'CD28', 'CD27', 'CD3D', 'CD3E', 'CD3G'}
CD4+ T cell:           {'IL2RA', 'CD4', 'FOXP3', 'CTLA4'}
CD8+ T cell:           {'CD8B', 'CD4', 'GZMK', 'CD8A'}
Regulatory T cell:     44
B cell:                {'MS4A1', 'BLNK', 'CD19', 'CD37', 'CD79B', 'CD79A'}
Macrophage:            {'CSF1R', 'CD14', 'CD163', 'CD68', 'FCGR3A'}
Natural killer cell:   {'NCAM1', 'CCL3', 'KLRC1', 'GZMB', 'KLRK1',
'FCGR3A', 'KLRD1', 'KLRB1', 'CD160', 'CD247', 'FCGR3B', 'NKG7', 'GNLY',
'KLRF1'}
Naive T cell:          {'CD27', 'CD8B', 'CCR7', 'CD8A'}
Naive CD8+ T cell:     87
Memory T cell:         83
Naive CD4+ T cell:     32

0           Memory T cell
1           Memory T cell
2           Memory T cell
3           Memory T cell
4           Memory T cell
5           Memory T cell
6       Naive CD4+ T cell
7     Natural killer cell
8               Macrophage
9                   B cell
10          Memory T cell

Memory T cell = Effector CD8+ Memory T cell
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