

8 Appendix A

8.1 Affymetrix QC report for Bioconductor analysis

Affymetrix arrays Quality Control (QC) report reported by Dr. Lefebvre using Bioconductor.

Array Index	Array Name
1	m2_1_2cRNA.CEL
2	m3_1_0_AB2.2_1st_bio_rep.CEL
3	m3_1_3cRNA_AB2.2.CEL
4	m3_2_5cRNA.CEL
5	m3_2_0_AB2.2_BA271_2h_1st_bio_rep.CEL
6	m3_2_0_cRNA_AB2.2_0ai.CEL
7	m3_3_0_AB2.2_BA271_4h_1st_bio_rep.CEL
8	m3_3_8cRNA.CEL
9	m3_3_9cRNA.CEL
10	m3_4_0_AB2.2_2nd_bio_rep.CEL
11	m3_4_2cRNA.CEL
12	m3_4_3cRNA.CEL
13	m3_5_0_AB2.2_BA271_2h_2nd_bio_rep.CEL
14	m3_5_M_cRNA_AB2.2_0ai.CEL
15	m3_5_N_cRNA_AB2.2_0ai.CEL
16	m3_6_0_AB2.2_BA271_4h_2nd_bio_rep.CEL
17	m3_6_P_cRNA_AB2.2_0ai.CEL
18	m3_6_R_cRNA_AB2.2_0ai.CEL
19	m3_7_0_AB2.2_3rd_bio_rep.CEL
20	m3_7_3cRNA.CEL
21	m3_7_AcRNA.CEL
22	m3_8_0_AB2.2_BA271_2h_3rd_bio_rep.CEL
23	m3_8_3cRNA.CEL
24	m3_8_CcRNA.CEL
25	m3_9_0_AB2.2_BA271_4h_3rd_bio_rep.CEL
26	m3_9_3cRNA.CEL
27	m3_9_EcRNA.CEL

Mon Dec 18 16:50:05 2006

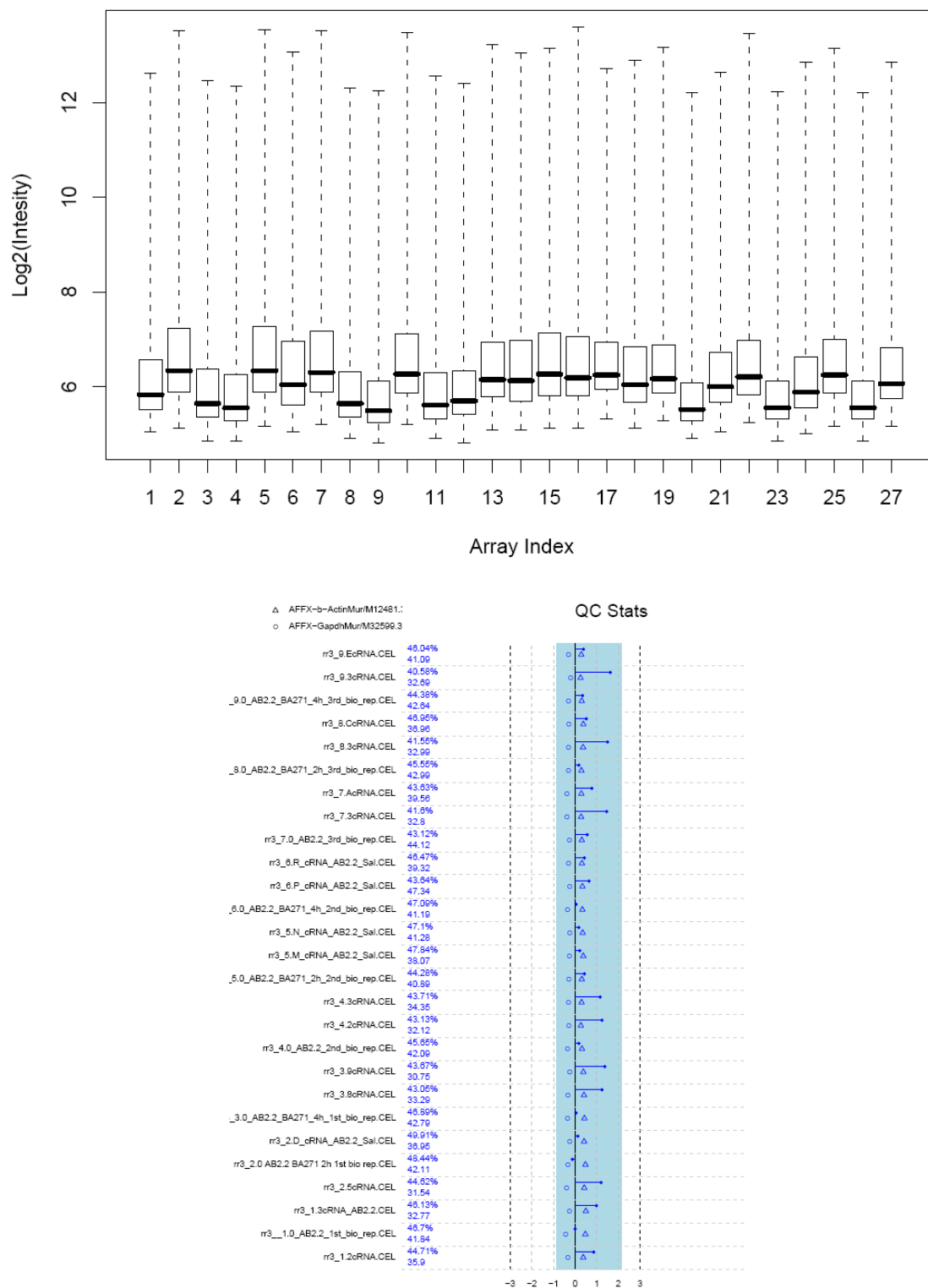


Figure 8.1 Affymetrix QC report

8.2 Gene Ontology analysis of the top GeneSpring annotated genes

The p-value is determined by:

- Number of entities in the entity list with the particular GO term and its children;
- The number of entities with the GO term in the experiment;
- The total number of entities in the entity list;
- The total number of entities in the experiment.

GeneSpring analysis of genes differentially expressed at 4 hours infection of murine AB2.2 ES cells are reported here divided by cellular component

8.2.1 GO description for cellular component of the top genes deriving from GeneSpring analysis

Table 8.1 GeneSpring data analyzed for GO term Cellular component

Filter on 1.5 fold change 4-0h (89 genes, 58 annotated) selected with GO:5575:cellular_component with p value< 0.05					
Category	Genes in Category	% of Genes in Category	Genes in List in Category	% of Genes in List in Category	p-Value
GO:5623: cell	14323	62.74	43	74.14	0.0457
GO:42598: vesicular fraction	136	0.596	3	5.172	0.00502
GO:5792: microsome	136	0.596	3	5.172	0.00502
GO:5622: intracellular	9415	41.24	37	63.79	0.00044
GO:5634: nucleus	4517	19.79	24	41.38	0.00013
GO:5783: endoplasmic reticulum	601	2.633	7	12.07	0.0008
GO:5789: endoplasmic reticulum membrane	71	0.311	2	3.448	0.0141
GO:5798: Golgi vesicle	3	0.0131	1	1.724	0.0076
GO:43229: intracellular organelle	8064	35.33	35	60.34	8.80E-05
GO:43231: intracellular membrane-bound organelle	7056	30.91	34	58.62	1.15E-05
GO:42175: nuclear envelope-endoplasmic reticulum network	74	0.324	2	3.448	0.0152
GO:43226: organelle	8064	35.33	35	60.34	8.80E-05
GO:43227: membrane-bound organelle	7056	30.91	34	58.62	1.15E-05

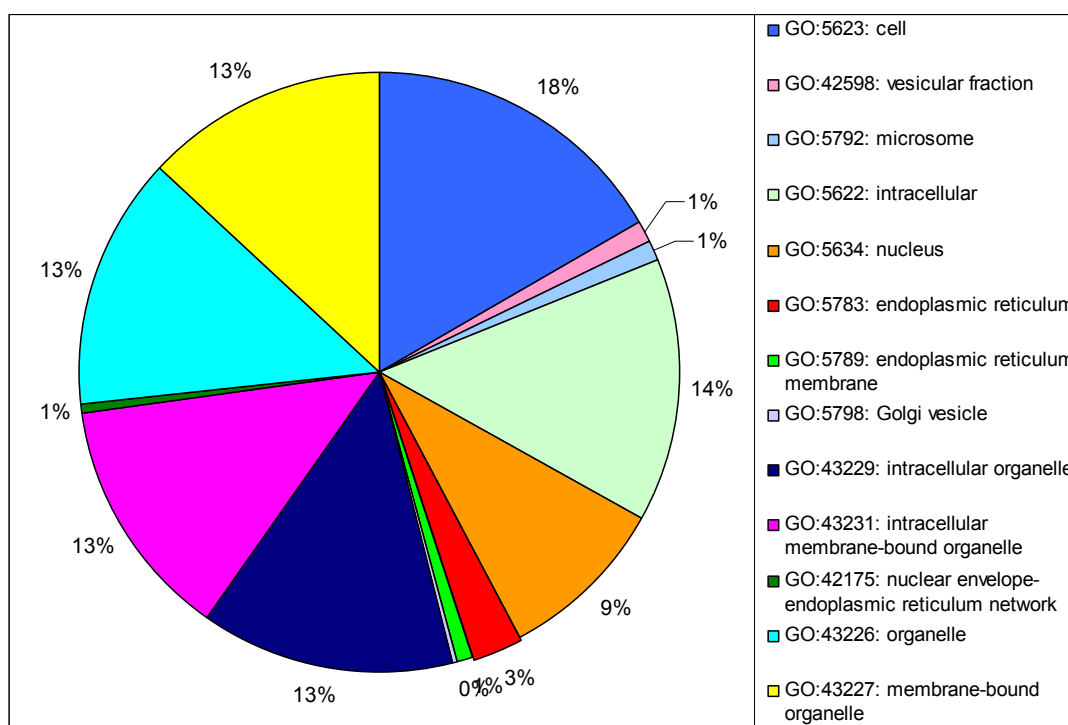


Figure 8.2 Pie chart of the GeneSpring data analyzed for GO term Cellular components

8.2.2 GO description for biological process of the top genes deriving from GeneSpring analysis

Table 8.2 GeneSpring pear-comparison data analyzed for GO term Biological Process

Filter on 1.5 Fold Change 4-0h (89genes, 59 annotated) selected with GO:8150: Biological_process					
Category	Genes in Category	% of Genes in Category	Genes in List in Category	% of Genes in List in Category	p-Value
GO:35112: genitalia morphogenesis	14	0.0613	1	1.724	0.035
GO:30539: male genitalia morphogenesis	14	0.0613	1	1.724	0.035
GO:19222: regulation of metabolism	1639	7.18	11	18.97	0.0025
GO:9892: negative regulation of metabolism	209	0.916	4	6.897	0.00197
GO:31324: negative regulation of cellular metabolism	172	0.753	4	6.897	0.000962
GO:45934: negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	161	0.705	4	6.897	0.000752
GO:16481: negative regulation of transcription	157	0.688	4	6.897	0.000684
GO:45892: negative regulation of transcription, DNA-dependent	97	0.425	2	3.448	0.0253
GO:122: negative regulation of transcription from RNA polymerase II promoter	83	0.364	2	3.448	0.0189
GO:31323: regulation of cellular metabolism	1582	6.93	11	18.97	0.00189
GO:19219: regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	1469	6.435	10	17.24	0.00362
GO:45449: regulation of transcription	1464	6.413	10	17.24	0.00353
GO:6355: regulation of transcription, DNA-dependent	1350	5.914	9	15.52	0.00667
GO:398: nuclear mRNA splicing, via spliceosome	98	0.429	2	3.448	0.0258

GO:8380: RNA splicing	110	0.482	2	3.448	0.0319
GO:375: RNA splicing, via transesterification reactions	98	0.429	2	3.448	0.0258
GO:377: RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	98	0.429	2	3.448	0.0258
GO:6091: generation of precursor metabolites and energy	688	3.014	5	8.621	0.0303
GO:6118: electron transport	457	2.002	5	8.621	0.00603
GO:6350: transcription	1643	7.197	10	17.24	0.0079
GO:6351: transcription, DNA-dependent	1431	6.269	9	15.52	0.00962
GO:8632: apoptotic program	58	0.254	3	5.172	0.000435
GO:6919: caspase activation	33	0.145	3	5.172	8.04E-05
GO:43026: regulation of caspase activation	13	0.0569	3	5.172	4.37E-06
GO:43154: negative regulation of caspase activation	11	0.0482	3	5.172	2.53E-06
GO:1719: inhibition of caspase activation	11	0.0482	3	5.172	2.53E-06
GO:42981: regulation of apoptosis	229	1.003	3	5.172	0.0205
GO:43066: negative regulation of apoptosis	68	0.298	3	5.172	0.000694
GO:6916: anti-apoptosis	57	0.25	3	5.172	0.000413
GO:43067: regulation of programmed cell death	231	1.012	3	5.172	0.021
GO:43069: negative regulation of programmed cell death	68	0.298	3	5.172	0.000694
GO:50791: regulation of physiological process	2582	11.31	17	29.31	0.000159
GO:43118: negative regulation of physiological process	479	2.098	7	12.07	0.000204
GO:51243: negative regulation of cellular physiological process	445	1.949	7	12.07	0.00013
GO:51244: regulation of cellular physiological process	2460	10.78	17	29.31	8.75E-05
GO:41: transition metal ion transport	61	0.267	2	3.448	0.0105
GO:6829: zinc ion transport	12	0.0526	2	3.448	0.000412
GO:8333: endosome to lysosome transport	4	0.0175	1	1.724	0.0101
GO:6892: post-Golgi transport	11	0.0482	1	1.724	0.0276
GO:6893: Golgi to plasma membrane transport	4	0.0175	1	1.724	0.0101
GO:7034: vacuolar transport	14	0.0613	1	1.724	0.035
GO:7041: lysosomal transport	4	0.0175	1	1.724	0.0101
GO:7033: vacuole organization and biogenesis	19	0.0832	1	1.724	0.0472
GO:7040: lysosome organization and biogenesis	9	0.0394	1	1.724	0.0226
GO:50794: regulation of cellular process	2550	11.17	17	29.31	0.000137
GO:48523: negative regulation of cellular process	485	2.125	7	12.07	0.000221
GO:50789: regulation of biological process	2849	12.48	17	29.31	0.000517
GO:43085: positive regulation of enzyme activity	85	0.372	3	5.172	0.00133
GO:51345: positive regulation of hydrolase activity	33	0.145	3	5.172	8.04E-05
GO:43280: positive regulation of caspase activity	33	0.145	3	5.172	8.04E-05
GO:48519: negative regulation of biological process	559	2.449	7	12.07	0.000519
GO:50790: regulation of enzyme activity	143	0.626	3	5.172	0.00577
GO:51336: regulation of hydrolase activity	38	0.166	3	5.172	0.000123
GO:43281: regulation of caspase activity	33	0.145	3	5.172	8.04E-05
GO:50896: response to stimulus	1556	6.816	11	18.97	0.00166
GO:6950: response to stress	823	3.605	11	18.97	6.05E-06
GO:6986: response to unfolded protein	16	0.0701	4	6.897	6.67E-08
GO:9408: response to heat	50	0.219	4	6.897	7.92E-06
GO:9628: response to abiotic stimulus	363	1.59	9	15.52	3.16E-07
GO:9266: response to temperature stimulus	53	0.232	4	6.897	1.00E-05
GO:42221: response to chemical stimulus	251	1.1	5	8.621	0.00044

8.3 Genes analyzed by RT-PCR deriving from ASCA analysis

Table 8.3 Table Gene resulting from ASCA analysis which expression was confirmed by RT-PCR

cluster	Gene Symbol	Gene Title	Pathway	go biological process term
3	Bfar	bifunctional apoptosis regulator	---	apoptosis /// anti-apoptosis
3	Rhod	ras homolog gene family, member D	---	small GTPase mediated signal transduction
8; 8	Apaf1	apoptotic peptidase activating factor 1	Apoptosis	neural tube closure /// proteolysis /// apoptosis /// caspase activation /// defense response /// multicellular organismal development
9; 9	Socs3	suppressor of cytokine signaling 3		regulation of cell growth /// regulation of protein amino acid phosphorylation /// negative regulation of signal transduction /// negative regulation of insulin receptor signaling pathway
7; 5; 5	Lamp2	lysosomal-associated membrane protein 2	-	tRNA aminoacylation for protein translation
7; 5	Fst	folliculin	TGF_Beta_Signaling_Pathway	

9 Appendix B

9.1 Flow cytometric characterization of murine ES cells AB2.2, esDCs and BMDCs testing the expression of myeloid surface markers

In order to highlight the difference between the original murine ES cell line AB2.2 and the derived esDC we decided to test myeloid surface markers. We were able to highlight the expression of these markers when the cells were dislocated using a non enzymatic buffer. May be these buffer is been to mild and dislocate only those cells at the edge of the cells clusters which usually are the most differentiated one or our ES cell line has hematopoietic progenitor characteristic.

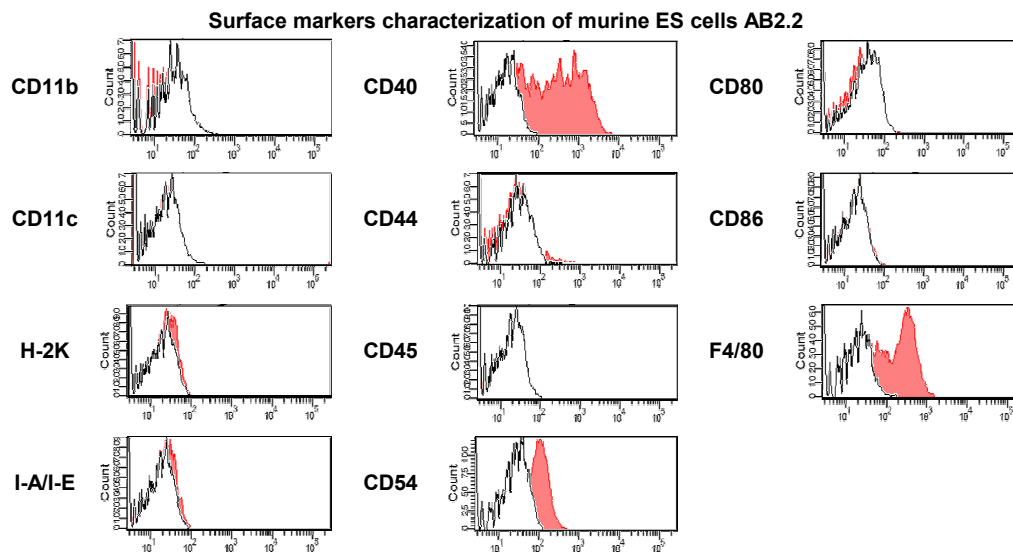


Figure 9.1 Flow cytometry characterization of murine AB2.2 ES cells

Murine ES cells were also stained for surface markers used to define esDC. Here are reported histograms representing a few target surface markers, red peaks, which expression was compared to their relative isotype control antibody, white peaks.

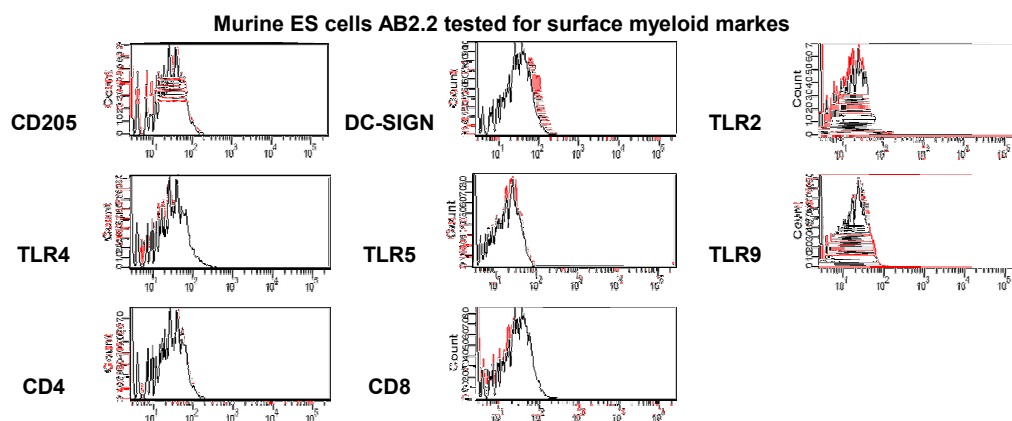


Figure 9.2 Murine ES cells characterization for myeloid markers

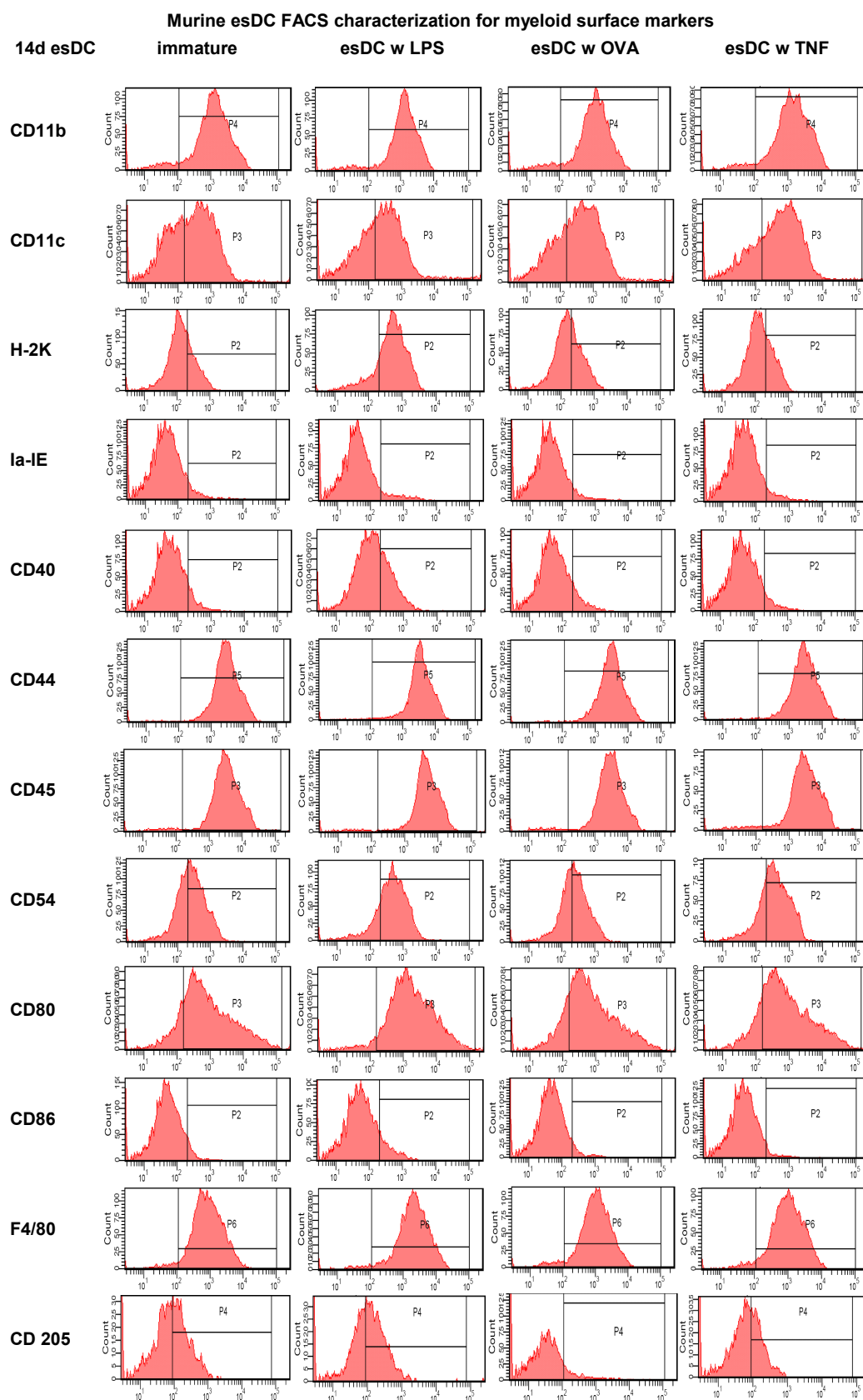
Figure 9.3 Flow cytometric characterization of immature and mature esDCs

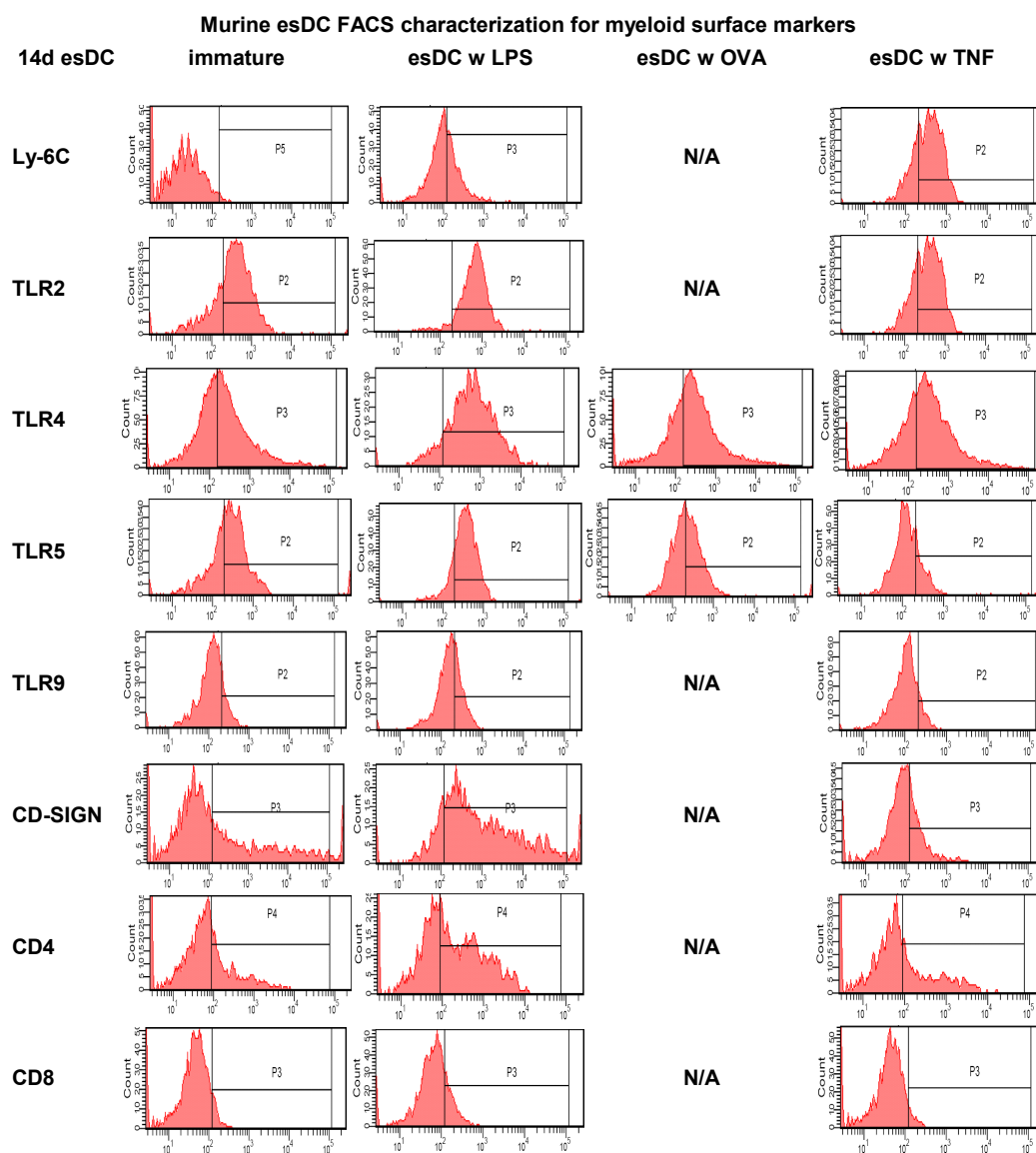
Figure 9.4 Flow cytometric characterization of immature and mature esDCs, number 2

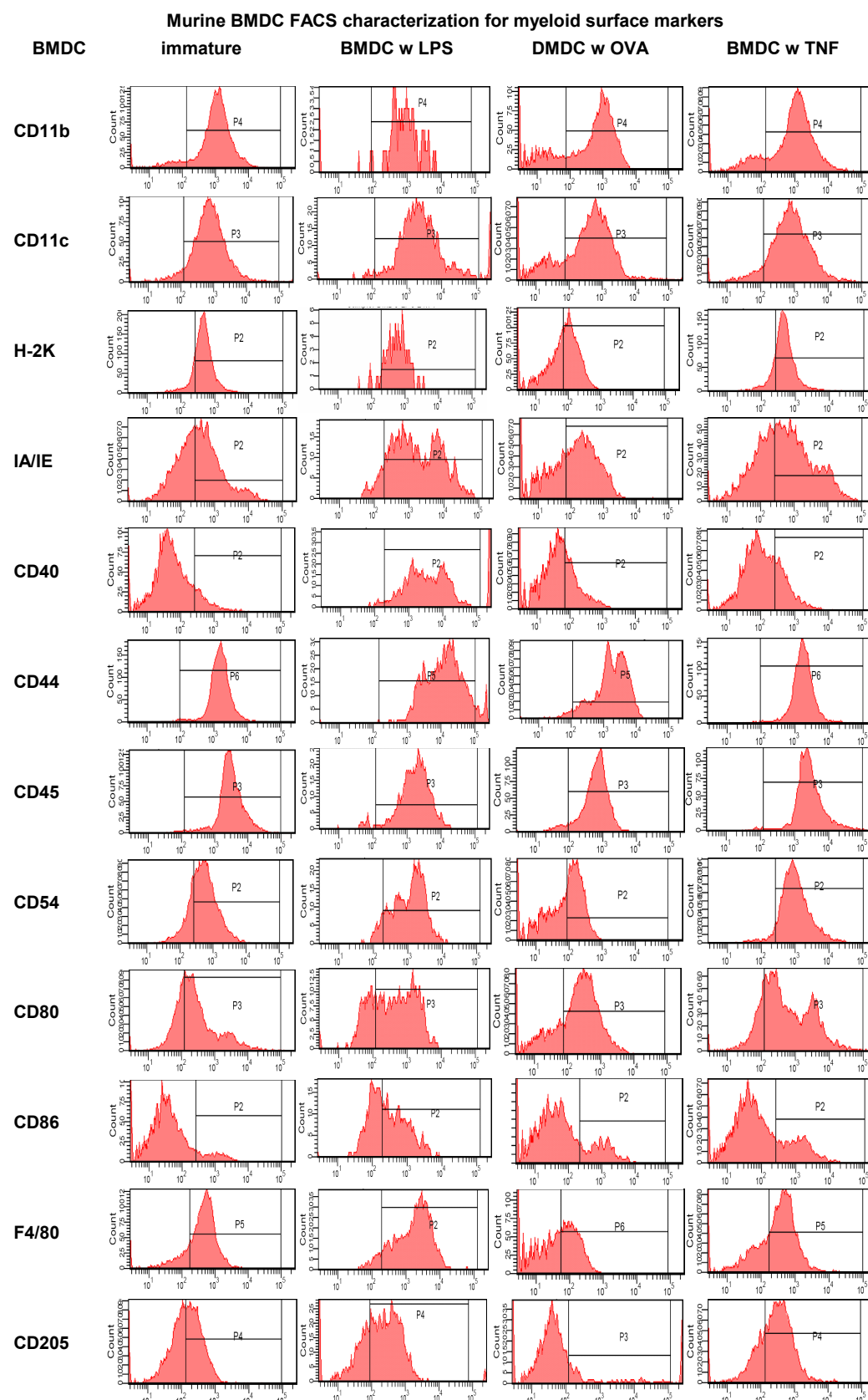
Figure 9.5 Flow cytometric characterization of BMDCs immature and mature

Figure 9.6 Flow cytometric characterization of immature and mature BMDCs, number 2