1 Array design description

A brief description of the array design, feature location, information on the cDNA collection and the spotting protocols can be found on the producer website (http://www.microarray.org/sfgf/jsp/home.jsp).

Protocols for the post processing procedure can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2 Experiment description

2.1 Experimental design

2.1.1 Laboratory, authors, contact

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2.1.2 Type of experiment

Comparison of mice T-lymphocytes after allogeneic bone marrow transplantation (BMT) with CD40-CD40L costimulation blockade (group TOL) and without the blockade (group CTRL).

2.1.3 Experiment factors

The expression of genes were investigated and compared between the two groups TOL vs. CTRL.

2.1.4 Hybridizations

Six hybridizations (3 replicates for each group).

2.1.5 Reference

Universal Mouse reference RNA (Stratagene®) was used as hybridization reference.

2.1.6 Quality control

To test for reproducibility of sample processing, RNA of three specimen was reprocessed twice.

2.2 Samples used, extract preparation and labeling

2.2.1 Bio-source properties

Organism: *Mus musculus*

2.2.2 Biomaterial manipulations, hybridization and labeling protocol

Blood samples were harvested three weeks after BMT and T-lymphocytes were enriched with FACS.

Protocols for RNA extraction, RNA labeling, hybridization and washing of microarrays can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2.3 Hybridization procedures and parameters

Sample	Array	Sample	Array
TOL 1	MMK 023	CTRL 1	MMK 022
TOL 2	MMK 020	CTRL 2	MMK 021
TOL 3	MMK 033	CTRL 3	MMK 030

The hybridization protocol can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2.4 Measurement data and specification of data processing

2.4.1 Raw data description

Scan hardware: GenePix Personal 4100 A
Scan software: GenePix Pro 4.1

Raw data images can be found in the data section of our website (http://www.meduniwien.ac.at/nephrogene/).

Array	Laser power		PMT Gain		Lines Averaged	Background Subtraction	Scan region
	635 nm	532 nm	635 nm	532 nm			
MMK 020	2.83	3.48	740	570	1	LocalFeature	116,139,2052,6778
MMK 021	3.28	3.43	740	590	3	LocalFeature	92,248,2108,6685
MMK 022	2.8	3.42	740	570	1	LocalFeature	116,418,2053,6577
MMK 023	3.11	3.44	740	570	1	LocalFeature	116,403,2069,6623
MMK 030	2.94	3.46	600	510	1	LocalFeature	162,275,2071,6705
MMK 033	2.94	3.44	600	510	1	LocalFeature	121,372,2103,6729

2.4.2 Image analysis and quantitation

Image griding and calculation of spot intensity was performed with GenePix Pro 4.1 software.

2.4.3 Normalized and summarized data

Normalization:
Normalization was done through the default computed normalization by SMD (see http://genome-www5.stanford.edu/help/results_normalization.shtml). For data retrieval the $\log_2$ (R/G normalized ratio [median]) was used.

Cluster analysis:
TIGR Multi Experiment Viewer 3.0.3 (http://www.tm4.org/mev.html) was used for hierarchical clustering of genes and experiments respectively.

Linkage rule: Complete linkage
Distance measure: Euclidean distance

Significance analysis:
Genes showing a 2-fold change in expression between the two groups, TOL and CTRL, respectively, and a $p<0.001$ (unpaired t-test) were considered as statistical differentially regulated. No adjustment for multiple testing was performed.