# Epigenetic dysregulation of naïve CD4+ T-cell activation genes in childhood food allergy

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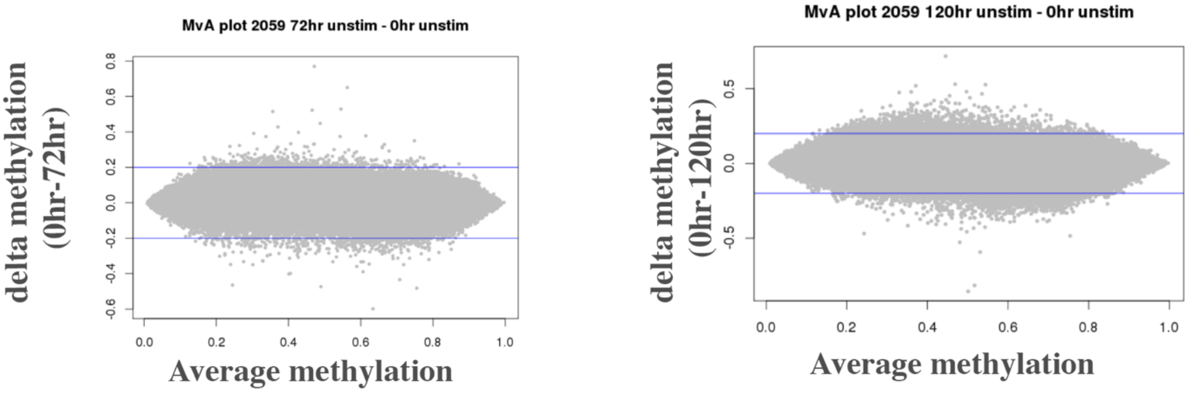
## Supplementary Table 1 - Clinical characteristics of study participants

| | **Controls (n=21)** | **Cases (n=45)** | |
|---|---|---|---|
| | | *transient egg allergy (n=31)* | *persistent egg allergy (n=13)* |
| **Demographics** | | | |
| SEIFA quintile (median) | 3 | 3 | 3 |
| Gender: males (n,%) | 9 (43) | 15 (48) | 6(46) |
| Caucasian (n,%) | 17 (81) | 21 (68) | 11 (85) |
| Family History FA (n,%) | 0(0) | 7(23) | 1 (8) |
| Has siblings (n,%) | 13 (62) | 12 (39) | 7 (54) |
| Attended day care (n,%) | 8 (38) | 9 (29) | 3 (23) |
| Median age egg introduction (month) | 5.5 | 6 | 5.5 |
| Pet ownership (n,%) | 10 (47) | 12 (39) | 7 (53) |
| **Clinical characteristics** | | | |
| Egg Allergy (12m) (n,%) | 0 | 26 (100) | 18 (100) |
| Egg Allergy (followup) (n,%) | NA | 0 (0) | 18(100) |
| Average SPT egg white (12m) (mm) | 0.2 | 3.9 | 5.3 |
| Average SPT egg white Followup (mm) | NA | 0.75 | 3.2 |
| Average sIgE egg white (kU/mL) | 0.09 | 1.1 | 10.4 |
| Average sIgE egg white Followup (kU/mL) | NA | 0.44 | 7.9 |
| Peanut Allergy (12m) | 0 (0) | 0 (0) | 0 (0) |
| Peanut Allergy (followup) | 0 (0) | 0 (0) | 0 (0) |
| Eczema diagnosis at 12m | 7 (33) | 13 (42) | 7 (53) |

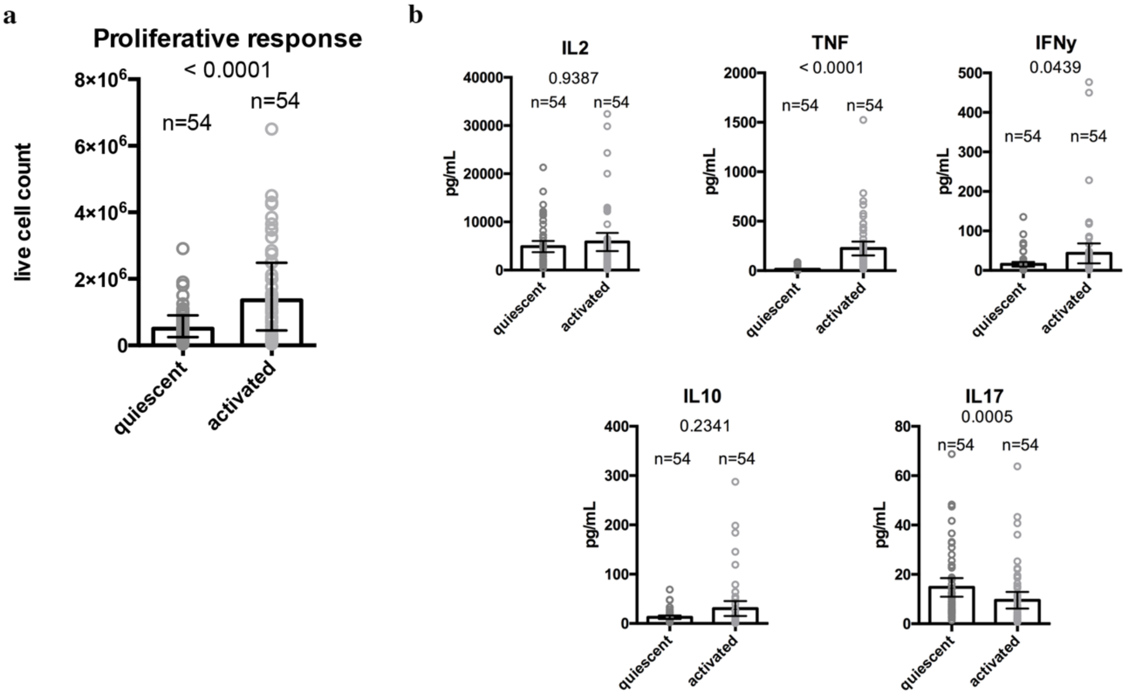
*SEIFA: socioeconomic index for areas; sIgE: specific Immunoglobulin E; SPT: skin prick test*



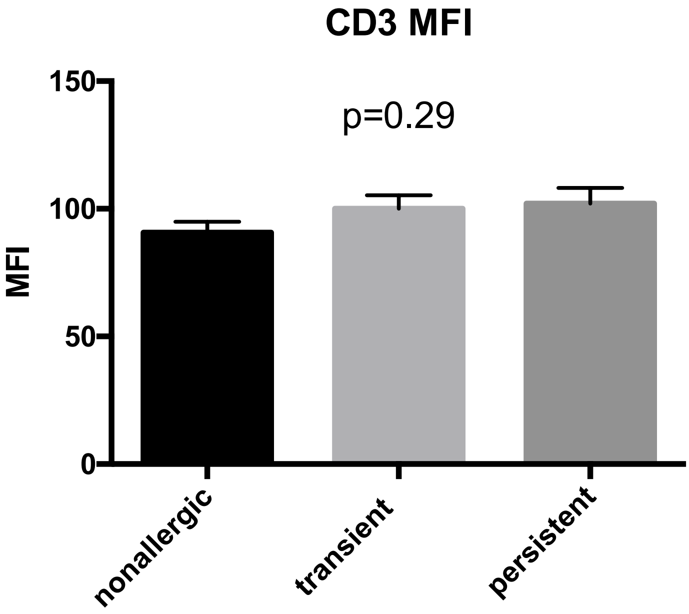
**Supplementary Figure 1. Time course of culture-induced DNA methylation changes.** Genome-wide DNA methylation data from naïve CD4+ T-cells isolated from two healthy infant donors. Methylation profiles were generated using the IlluminaHumanMethylation 450k array with 0hr, 72hr and 120 hr time points. MvA plots show the fold change (x-axis) as a function of average methylation (y-axis) representative data from one infant. Left panel is the change in DNA methylation between 0hr and 72hr unstimulated naive T cells, and right panel is 0 hr and 120 hr unstimulated naïve T cells. Blue lines are drawn at fold change +/- 20%. A substantially higher number of data points exhibited a fold change of more than 20% after resting in culture for 120 hours compared with 72 hours.

**Supplementary Table 2- Test statistics for enrichment of TCR activation-associated differentially methylated CpG at histone modifications in naïve T-cells determined by ChIP-seq.** ChIP-seq data were downloaded from the ENCODE consortium and signal peaks were tested for overlapping segments using Forbes coefficient.

| #Rank | Track title | Similarity to query track | P-value | Overlap between query and reference track (bps) | Genome coverage of track (bps) | Nr. of reference track elements |
|---|---|---|---|---|---|---|
| 1 | E038-H3K4me1.broadPeak | 6.36 | 0.004 | 452 | 394351178 | 185925 |
| 2 | E038-H3K4me3.broadPeak | 4.12 | 0.004 | 297 | 399524440 | 338126 |
| 3 | E038-H3K27ac.broadPeak | 3.08 | 0.004 | 298 | 536236329 | 425959 |
| 4 | E038-H3K36me3.broadPeak | 1.87 | 0.004 | 187 | 555781954 | 254336 |
| 5 | E038-H3K9me3.broadPeak | 0.58 | 0.669 | 82 | 778291280 | 509626 |
| 6 | E038-H3K27me3.broadPeak | 0.67 | 1.00 | 83 | 683853862 | 413197 |



**Supplementary Figure 2. Function responses to anti-CD3/CD28 stimulation at 12-months.** Following 72 hours of stimulation, activated and quiescent cells were harvested and counted using an automated cell counter. **(a)** Proliferative response is presented as absolute cell counts. **(b)** Cell supernatants were assayed for a range of cytokines using multiplexed bead arrays. Activation of T-cell induced significant induction of TNF and IFNy. IL4 and IL6 produciton was below detection limits (data not shown). All data are presented as median with interquartile range, groups compared using the Mann Whitney test for matched samples. Exact P values are shown.



**Supplementary Figure 3. – Surface expression of CD3 on naïve T-cell collected at baseline.** Graph shows the mean fluorescence intensity (MFI) for each group. Data is presented as mean with standard error. Groups were compared using a one-way ANOVA.

## Supplementary Table 3- differentially expressed and methylated genes in quiescent naïve T cells

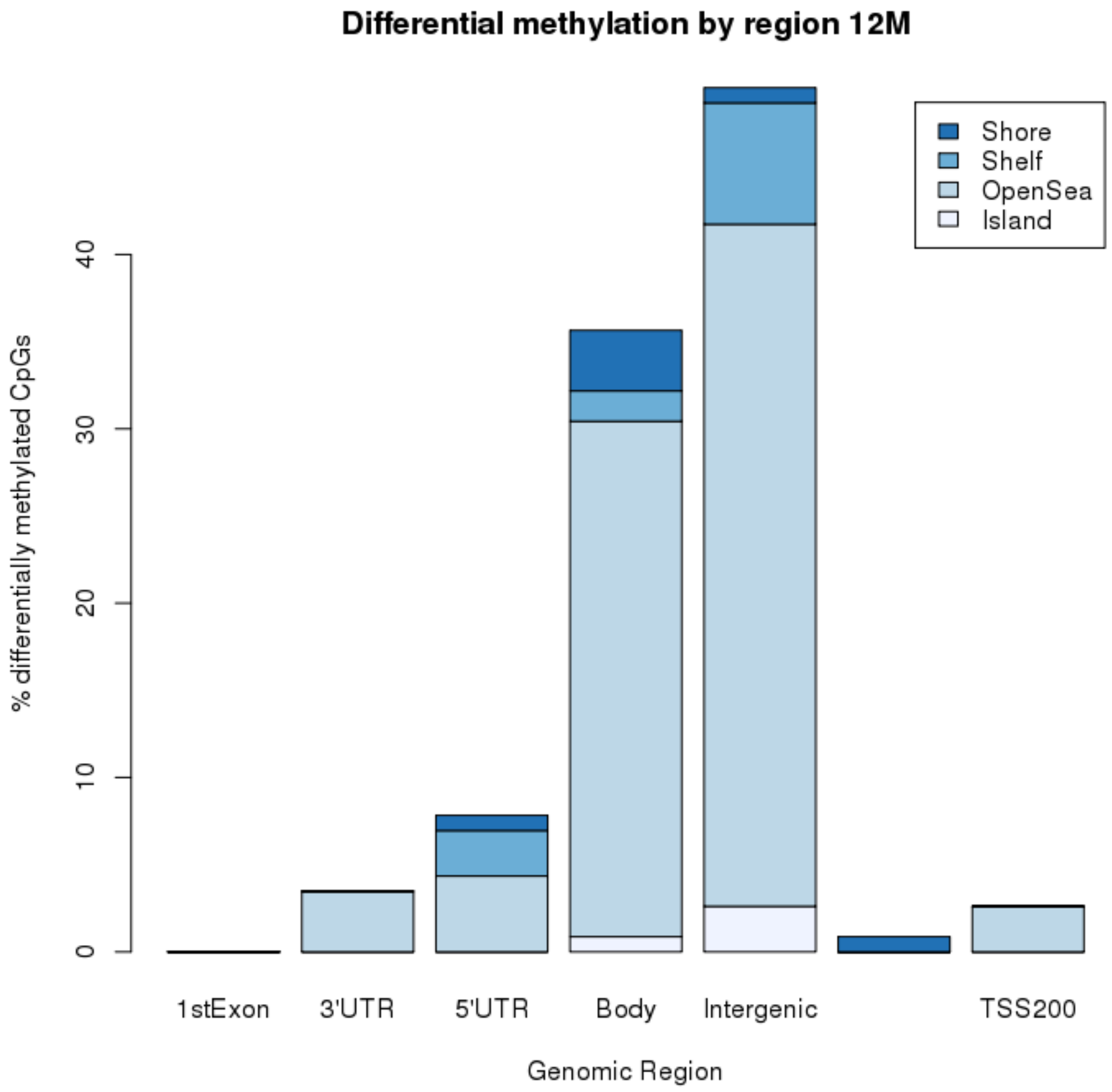
### Differentially expressed genes

| Ensmbl ID | symbol | biotype | description | logFC | AveExpr | t | P.Value | adj.P.Val |
|---|---|---|---|---|---|---|---|---|
| ENSG00000080854 | IGSF9B | protein_coding | immunoglobulin superfamily, member 9B | 0.72 | 4.20 | 4.98 | 0.00 | 0.04 |
| ENSG00000112167 | SAYSD1 | protein_coding | SAYSVFN motif domain containing 1 | 0.31 | 4.12 | 4.94 | 0.00 | 0.04 |

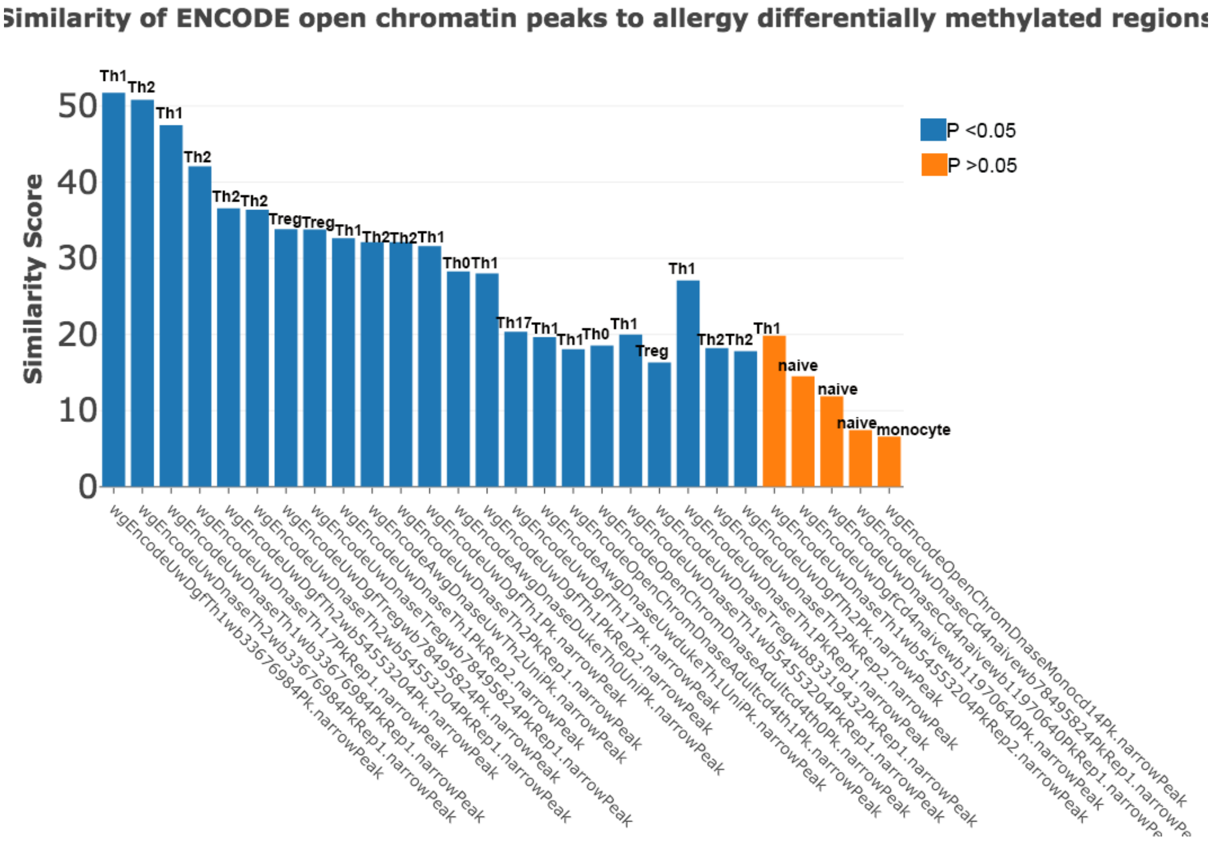
### Differentially methylated CpG

| Probe ID | symbol | chr | position | deltabeta | AveMeth | t | P.Value | adj.P.Val |
|---|---|---|---|---|---|---|---|---|
| cg26475005 | KIAA1217 | chr10 | 24649480 | -0.03 | -0.61 | -6.24 | 0.00 | 0.01 |
| cg25316898 | C18orf21 | chr18 | 33552869 | 0.01 | -3.28 | 5.71 | 0.00 | 0.04 |

*Ave.Expr = average expression level, Adj.P.Val = false discovery rate corrected P value, chr = chromosome, deltabeta = absolute difference in methylation (case – control), logFC = log fold change (cases – control), pos = position.*



**Supplementary Figure 4 – Overlap of differentially methylated CpG with genomic regions.** Bar chart shows the percentage of food allergy associated differentially methylated regions stratified by genomic feature.



**Supplementary Figure 5 – Enrichment test of allergy dmrs compared to effector, naïve and monocyte open chromatin regions.** Publicly available digital genomic footprint and DNAse I peaks for differentiated effector CD4 T-cells (Th1, Th2, Th17, Treg), naïve T-cells and monocytes were downloaded from the ENCODE consortium data repository. A statistical test was performed comparing overlaps of allergy-dmrs to ENCODE DNAse1 and footprint peaks using the Forbes coefficient, and the datasets were ranked in order of descending similarity to allergy dmrs (y-axis). Significantly enriched open chromatin peaks are coloured blue, and p-values were determine using monte carlo randomization strategy. Exact data track names are shown on the x-axis. Allergy-dmrs were more enriched in regions of accessible chromatin that define effector T-cells rather than naïve T-cells. Data from monocytes was included as a negative control.

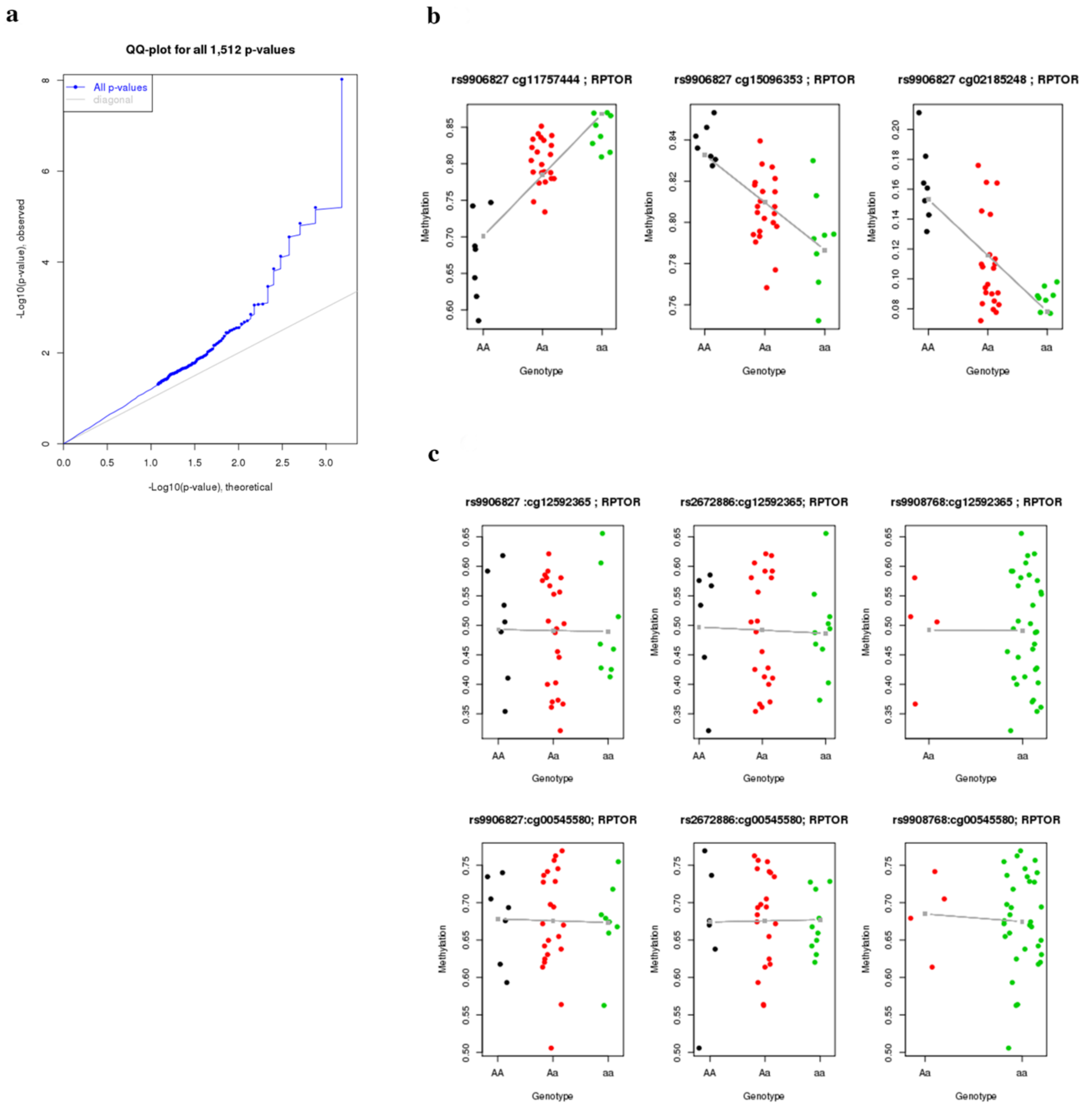
**Supplementary Table 4 – Association analysis of candidate SNPs with food allergy.** SNPs showing weak evidence for an association with food allergy are highlighted. Analysis is based on n=42 under a logistic model adjusted for ancestry.

| CHR | SNP | BP | GENE | A1 | NMISS | OR | STAT | P |
|---|---|---|---|---|---|---|---|---|
| 1 | rs4240895 | 9713386 | PIK3CD | T | 42 | 1.47 | 0.73 | 0.464 |
| 1 | rs57784284 | 9716125 | PIK3CD | A | 41 | 1.58 | 0.71 | 0.476 |
| 1 | rs35478578 | 66321311 | PDE4B | A | 42 | 0.77 | -0.33 | 0.745 |
| 1 | rs17451451 | 66388153 | PDE4B | G | 42 | 0.47 | -0.97 | 0.332 |
| 1 | rs2310752 | 66392405 | PDE4B | A | 42 | 0.97 | -0.05 | 0.962 |
| 1 | rs12096433 | 66430203 | PDE4B | A | 41 | 0.35 | -0.84 | 0.399 |
| 1 | rs2503215 | 66490013 | PDE4B | A | 42 | 0.63 | -0.60 | 0.545 |
| 1 | rs74969060 | 66490092 | PDE4B | G | 41 | 0.61 | -0.63 | 0.526 |
| 1 | rs7530150 | 66513674 | PDE4B | A | 41 | 0.54 | -0.65 | 0.515 |
| 1 | rs76165263 | 66587178 | PDE4B | A | 41 | NA | NA | NA |
| 1 | rs11208807 | 66634921 | PDE4B | T | 42 | 0.53 | -1.10 | 0.271 |
| 1 | rs6689595 | 66776137 | PDE4B | C | 40 | 0.34 | -1.64 | 0.102 |
| 1 | rs61796507 | 66837253 | PDE4B | T | 41 | 0.17 | -1.54 | 0.123 |
| 1 | rs6667969 | 66849760 | PDE4B | T | 42 | 2.14 | 1.39 | 0.164 |
| 1 | rs57569414 | 154380419 | IL6R | A | 42 | 0.69 | -0.44 | 0.657 |
| 1 | rs4537545 | 154418879 | IL6R | T | 42 | 0.68 | -0.68 | 0.497 |
| 2 | rs13401241 | 25518470 | DNMT3A | C | 42 | 0.93 | -0.13 | 0.893 |
| 2 | rs6711622 | 25531350 | DNMT3A | A | 42 | 0.93 | -0.15 | 0.883 |
| 2 | rs7575625 | 25548902 | DNMT3A | G | 42 | 0.66 | -0.78 | 0.436 |
| 2 | rs10203724 | 102688158 | IL1R1 | A | 42 | 1.15 | 0.18 | 0.855 |
| 2 | rs12712126 | 102696472 | IL1R1 | C | 41 | 2.40 | 1.51 | 0.132 |
| 2 | rs13029804 | 102723017 | IL1R1 | C | 42 | 0.61 | -0.79 | 0.427 |
| 2 | rs7580384 | 102760718 | IL1R1 | A | 42 | 1.99 | 1.18 | 0.240 |
| 2 | rs1420106 | 103035044 | IL189RAP* | T | 41 | 1.63 | 0.73 | 0.464 |
| 2 | rs2058659 | 103054556 | IL189RAP* | T | 42 | 1.14 | 0.22 | 0.826 |
| 2 | rs11465702 | 103057892 | IL189RAP* | G | 42 | 1.80 | 0.74 | 0.457 |
| 2 | rs10166061 | 238906511 | UBEF2-SLCY | C | 41 | 1.08 | 0.09 | 0.926 |
| 2 | rs4663826 | 238908120 | UBEF2-SLCY | T | 42 | 1.13 | 0.15 | 0.877 |
| 3 | rs73228187 | 111308568 | CD96 | G | 42 | 1.10 | 0.12 | 0.904 |
| 3 | rs17281304 | 111323019 | CD96 | T | 42 | 2.93 | 1.04 | 0.300 |
| 3 | rs73230114 | 111328598 | CD96 | A | 42 | 0.74 | -0.40 | 0.690 |
| 3 | rs9868266 | 111338169 | CD96 | T | 42 | 1.07 | 0.08 | 0.934 |
| 11 | rs80226633 | 44588256 | CD82 | C | 41 | NA | NA | NA |
| 5 | rs803139 | 132131024 | IL4 | A | 41 | 1.53 | -0.29 | 0.444 |
| 6 | rs6937545 | 32418031 | HLADQB1 | A | 42 | 0.8 | -0.291 | 0.771 |
| 7 | rs6946141 | 24925834 | OSBPL3 | G | 42 | 0.56 | -1.15 | 0.250 |

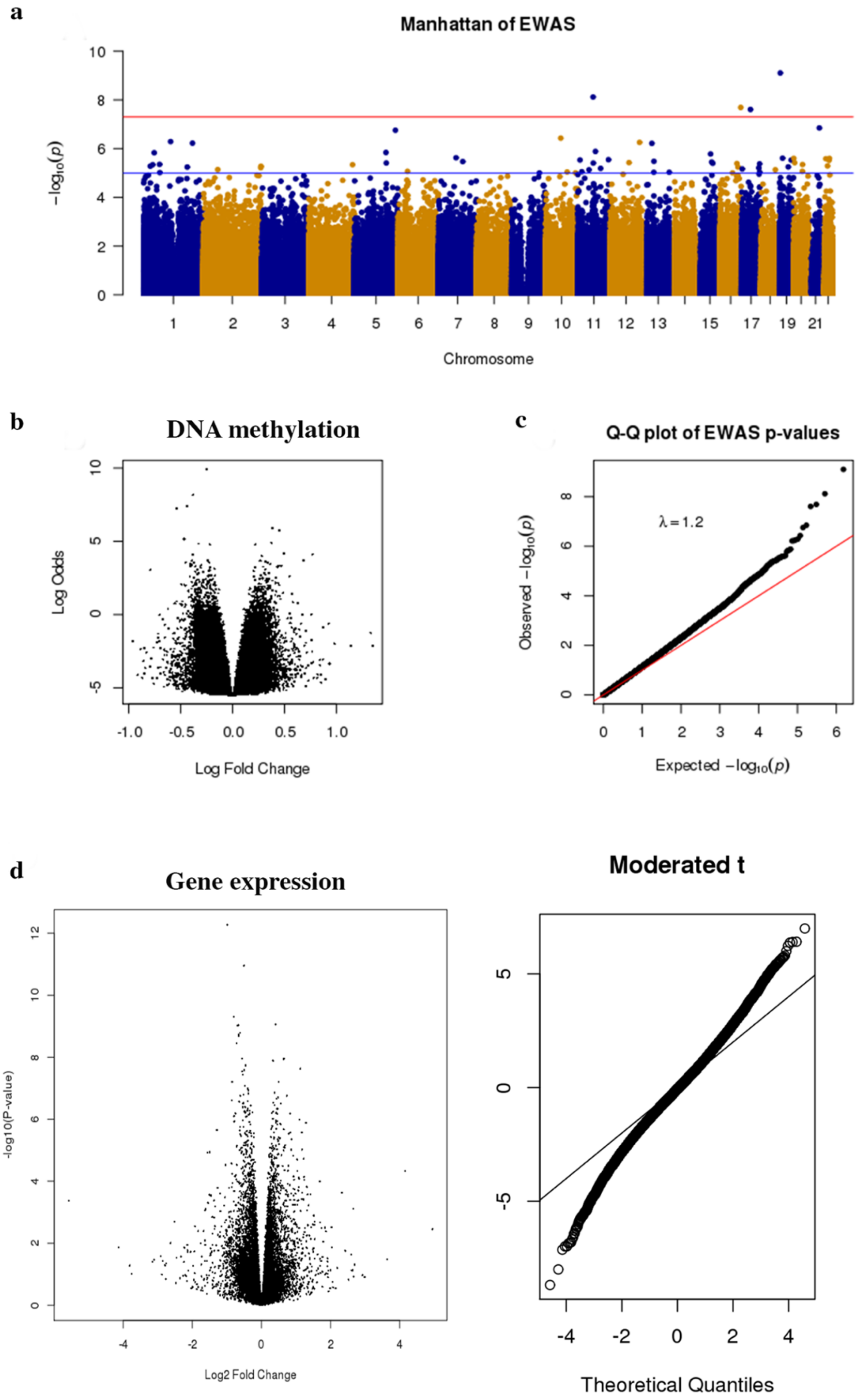
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 11 | rs11038069 | 44605335 | CD82 | A | 42 | 1.04 | 0.08 | 0.939 |
| 11 | rs3781753 | 44630049 | CD82 | A | 41 | 0.99 | -0.02 | 0.980 |
| 11 | rs80053099 | 44642255 | CD82 | C | 42 | 2.61 | 1.36 | 0.173 |
| 13 | rs55708275 | 41174224 | FOXO1 | A | 42 | 0.75 | -0.44 | 0.662 |
| 13 | rs1986649 | 41178824 | FOXO1 | T | 42 | 0.52 | -0.84 | 0.403 |
| 13 | rs4429172 | 41189143 | FOXO1 | A | 42 | 0.58 | -1.00 | 0.319 |
| 13 | rs7335520 | 41196407 | FOXO1 | T | 42 | 0.56 | -1.07 | 0.287 |
| 13 | rs56204202 | 41234397 | FOXO1 | C | 41 | 0.87 | -0.18 | 0.856 |
| 17 | rs4889856 | 78513632 | RPTOR | C | 41 | 1.82 | 1.12 | 0.261 |
| 17 | rs11651818 | 78530300 | RPTOR | C | 42 | 0.25 | -1.66 | 0.096 |
| 17 | rs12942701 | 78544604 | RPTOR | A | 41 | 0.60 | -0.57 | 0.565 |
| 17 | rs7226296 | 78547657 | RPTOR | G | 41 | 0.71 | -0.62 | 0.534 |
| 17 | rs7215496 | 78547753 | RPTOR | T | 42 | 1.91 | 0.94 | 0.346 |
| 17 | rs12951309 | 78558286 | RPTOR | A | 42 | 1.41 | 0.46 | 0.646 |
| 17 | rs7225476 | 78561603 | RPTOR | G | 42 | 0.37 | -1.56 | 0.120 |
| 17 | rs201324621 | 78601169 | RPTOR | A | 42 | 1.41 | 0.46 | 0.646 |
| 17 | rs8072592 | 78615361 | RPTOR | G | 42 | 0.42 | -1.24 | 0.215 |
| 17 | rs9906827 | 78665405 | RPTOR | T | 42 | 3.33 | 1.81 | 0.070 |
| 17 | rs1564868 | 78685176 | RPTOR | G | 42 | 0.66 | -0.73 | 0.464 |
| 17 | rs9915378 | 78708418 | RPTOR | A | 40 | 0.62 | -0.85 | 0.395 |
| 17 | rs6565479 | 78712423 | RPTOR | A | 42 | 1.08 | 0.13 | 0.896 |
| 17 | rs72852717 | 78741954 | RPTOR | A | 39 | NA | NA | NA |
| 17 | rs7225755 | 78761708 | RPTOR | C | 42 | 0.82 | -0.38 | 0.707 |
| 17 | rs72852775 | 78761726 | RPTOR | G | 42 | 0.27 | -1.22 | 0.224 |
| 17 | rs2672886 | 78781651 | RPTOR | C | 41 | 4.20 | 1.99 | 0.047 |
| 17 | rs734338 | 78782340 | RPTOR | A | 42 | 1.66 | 1.05 | 0.295 |
| 17 | rs73351957 | 78829069 | RPTOR | A | 42 | 4.00 | 0.93 | 0.351 |
| 17 | rs9912092 | 78841693 | RPTOR | G | 42 | 0.71 | -0.52 | 0.605 |
| 17 | rs2289764 | 78865546 | RPTOR | C | 42 | 1.21 | 0.30 | 0.763 |
| 17 | rs9908768 | 78902775 | RPTOR | A | 41 | 9.20 | 1.68 | 0.093 |
| 18 | rs12964682 | 60786227 | BLC2 | G | 42 | 0.66 | -0.79 | 0.431 |
| 18 | rs956572 | 60820571 | BLC2 | A | 42 | 0.85 | -0.28 | 0.780 |
| 18 | rs7230970 | 60821076 | BLC2 | C | 41 | 0.70 | -0.54 | 0.587 |
| 18 | rs72943023 | 60841614 | BLC2 | G | 42 | 0.75 | -0.40 | 0.693 |
| 18 | rs4941187 | 60849216 | BLC2 | C | 42 | 1.49 | 0.78 | 0.435 |
| 18 | rs12457700 | 60860246 | BLC2 | T | 42 | 1.49 | 0.78 | 0.435 |
| 18 | rs11152372 | 60860930 | BLC2 | G | 42 | 1.33 | 0.56 | 0.577 |
| 18 | rs17070848 | 60905500 | BLC2 | T | 40 | 0.23 | -1.41 | 0.159 |
| 18 | rs7240326 | 60917351 | BLC2 | T | 41 | 1.37 | 0.68 | 0.494 |
| 18 | rs2849382 | 60976193 | BLC2 | C | 42 | 0.38 | -1.39 | 0.164 |
| 18 | rs949037 | 60979013 | BLC2 | C | 42 | 1.33 | 0.51 | 0.611 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 18 | rs76945431 | 60989414 | BLC2 | T | 42 | NA | NA | NA |
| 19 | rs373658 | 3143320 | GNA15 | T | 41 | 1.03 | 0.06 | 0.951 |
| 19 | rs675975 | 3144508 | GNA15 | A | 42 | 0.88 | -0.25 | 0.803 |
| 19 | rs35047169 | 3154978 | GNA15 | A | 42 | 0.44 | -0.64 | 0.523 |
| 20 | rs2424904 | 31352316 | DNMT3B | C | 42 | 1.25 | 0.39 | 0.695 |
| 20 | rs242542 | 31403426 | DNMT3B | G | 42 | 1.91 | 0.72 | 0.472 |
| 22 | rs7290469 | 22150982 | MAPK1 | A | 42 | 1.33 | 0.52 | 0.600 |
| 22 | rs2070505 | 22162257 | MAPK1 | C | 42 | 1.13 | 0.21 | 0.833 |
| 22 | rs58212445 | 22209190 | MAPK1 | C | 41 | 1.03 | 0.02 | 0.982 |
| 22 | rs55762115 | 22224598 | MAPK1 | G | 39 | 1.83 | 0.98 | 0.329 |

*A1: minor allele, A2: major allele, F_A: frequency of minor allele in cases, F_U: frequency of minor allele in controls, BP: base position.*



**Supplementary Figure 6 – QQplots for QTL association analysis.** (a) show the distribution of p-values for pairwise comparisons of SNPs with corresponding CpG methylation levels at *RPTOR*. (b) An example of significant *RPTOR* meQTL associations. (c) Boxplots for SNP: *RPTOR* pairs restricted to the food allergy-associated dmps in *RPTOR* (cg12592365, cg00545580)



**Supplementary Figure 7 – Diagnostic plots of genome-wide data modelling at baseline in activated cells.** (a) Manhattan of epigenome-wide association (DNA methylation data). (b) Volcano plot of effect sizes. (c) qqplot of pvalue distribution with genomic inflation factor. (d) Volcanoplot and qqplot of moderated t statistic for RNAseq analysis.