

Figure S1A, B, C

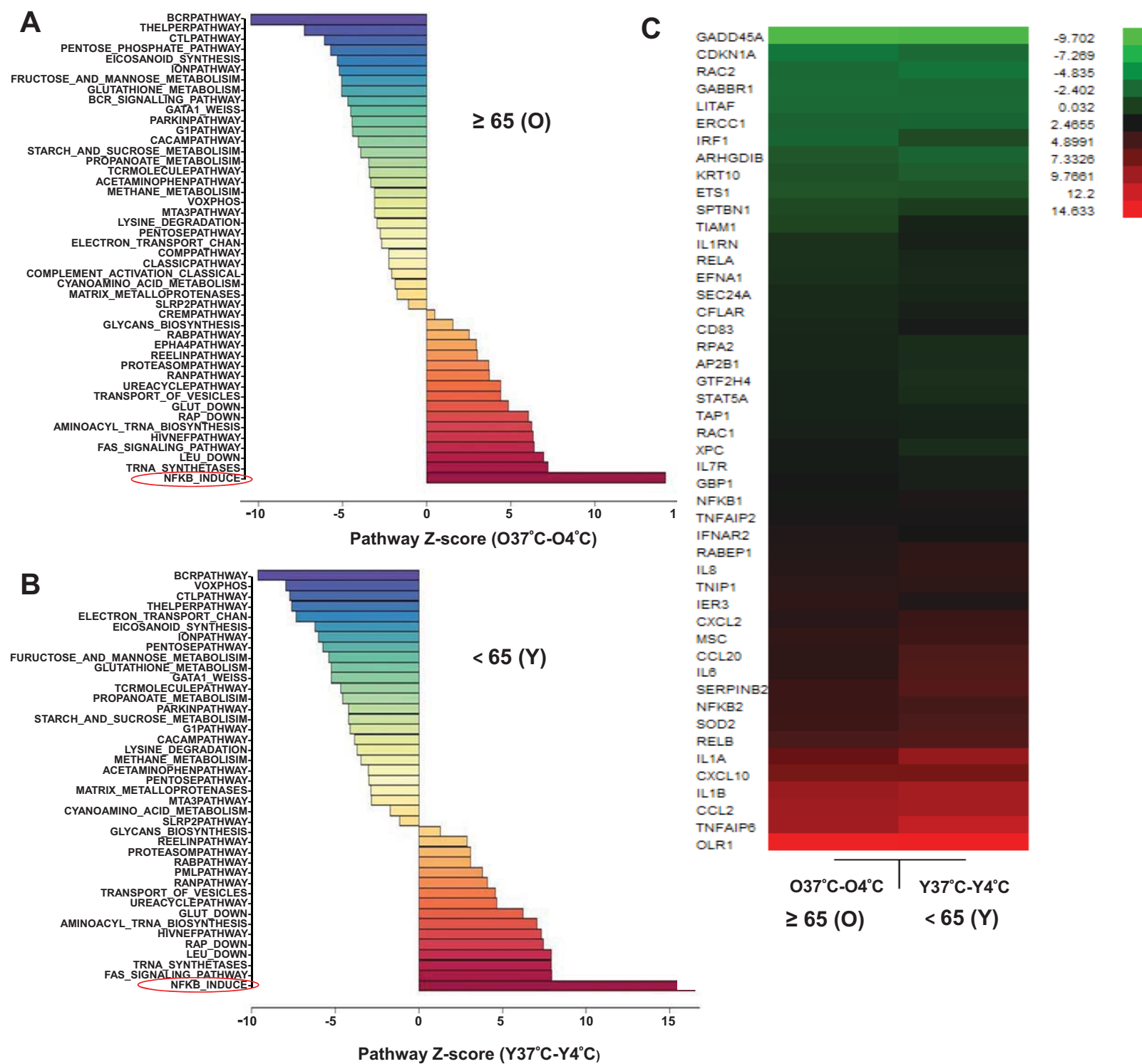


Figure S1D

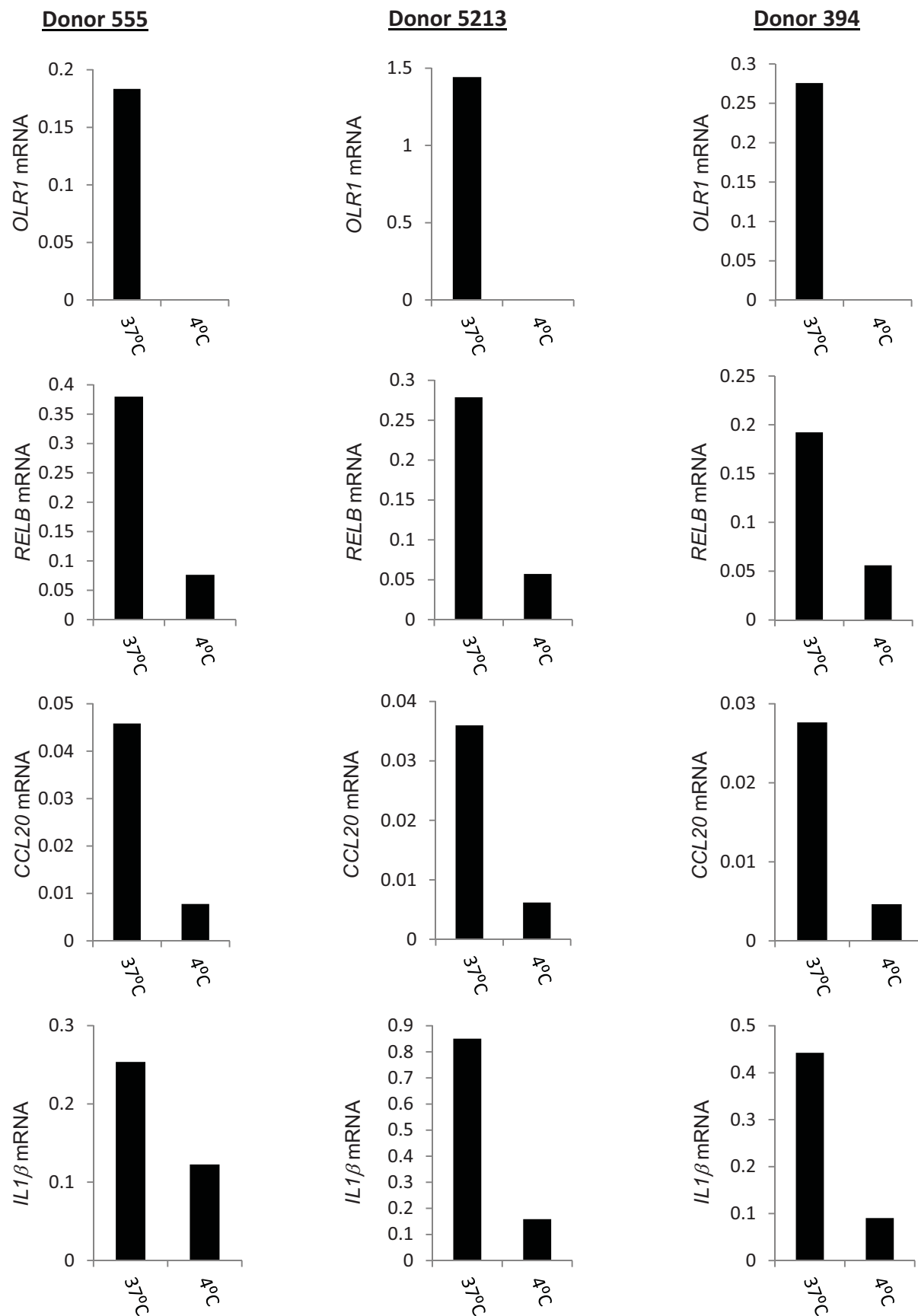


Figure S1. Gene expression changes in CD4⁺ T lymphocytes cultured at 37°C for 4h. 10x10⁶ CD4⁺ T cells from 23 individuals (age range 29-82) (Table S1C) were either kept at 4°C during and after purification, or cultured at 37°C for 4h. Total RNA prepared from these cells was analyzed by microarrays as described in the Methods section. Average gene expression profiles were compared between samples maintained at 4°C to those incubated at 37°C. Pathways maximally up- or down-regulated by 37°C incubation were determined by Parametric Analysis of Gene Set Enrichment (PAGE, Kim and Volsky 2005) in A) subjects aged 65 years or older (n=8) and B) subjects younger than 65 years (n=15). C) Genes within the NF-κB-induced pathway whose expression was significantly changed (p -value ≤ 0.05 , Z-ratio ≥ 1.5) by *ex vivo* culture. Averaged data from subjects ≥ 65 yrs and subjects less than 65 years are shown in the left and right columns, respectively. D) CD4⁺ T cells from three individuals were cultured at 37°C or maintained at 4°C. RNA from these cells were assayed by quantitative RT-PCR for the indicated genes using the AB 7500 Real Time PCR System (Applied Biosystems). Primer sequences are shown in Table S4. Expression values after normalization to GAPDH are shown on the Y axis.

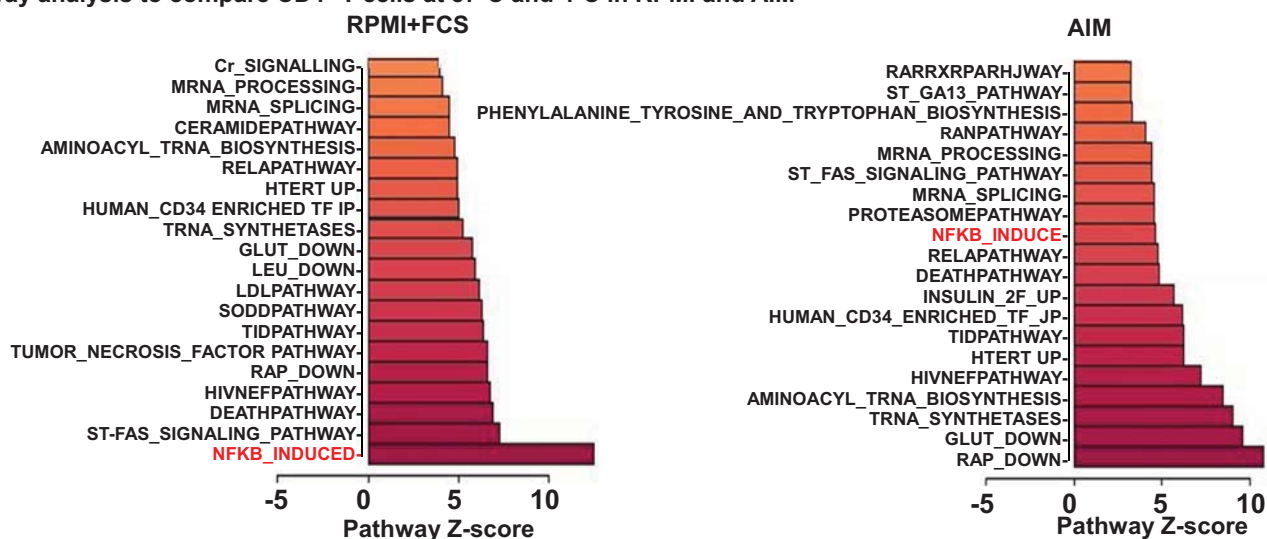
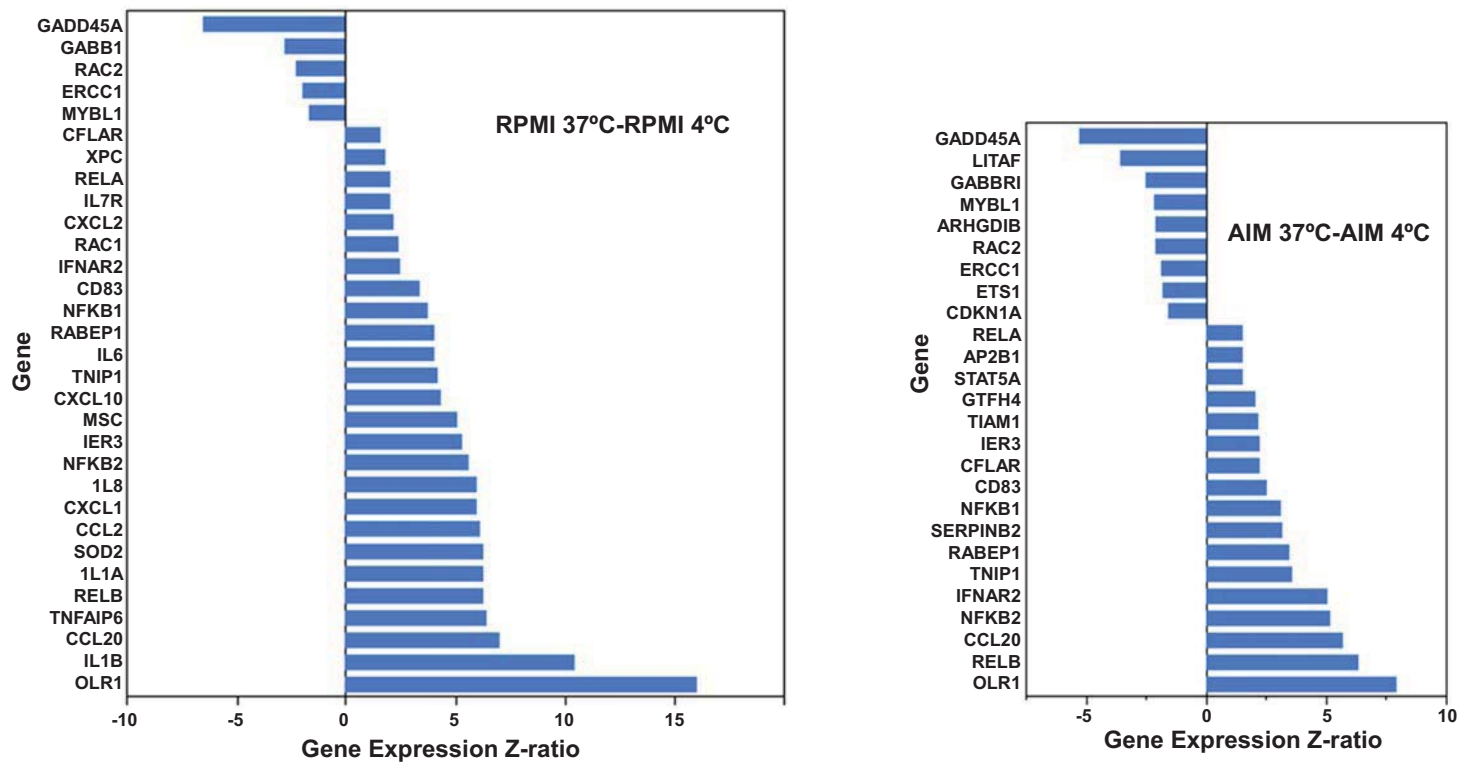
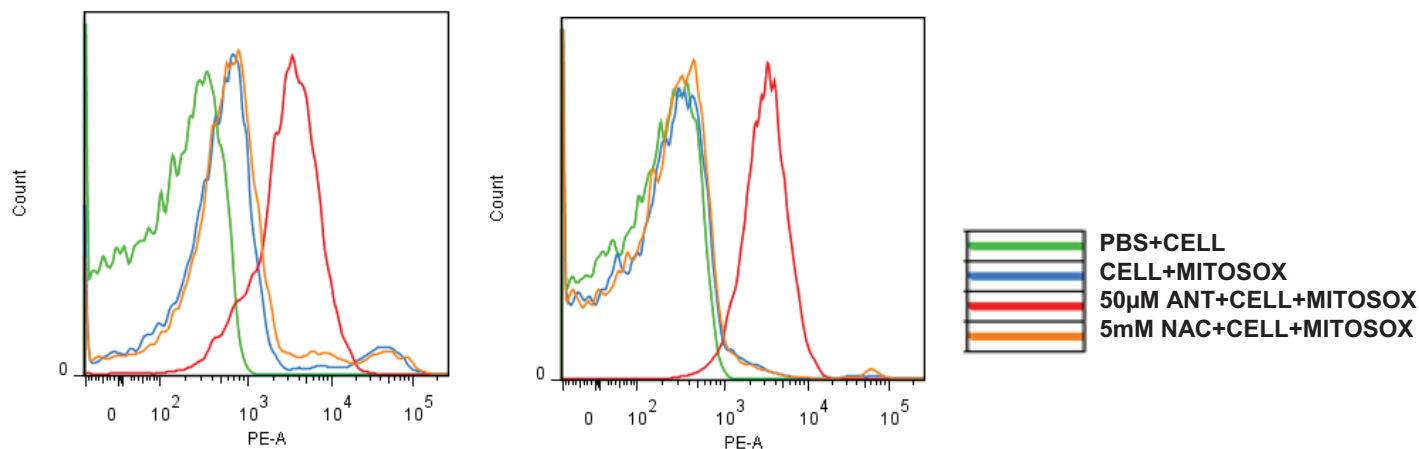
A Pathway analysis to compare CD4⁺ T cells at 37°C and 4°C in RPMI and AIM**B** Significant NF-κB induced pathway genes expression

Figure S2 Gene expression analysis in CD4⁺ T Cells cultured in different media. CD4⁺ T cells from 8 individuals (age range 30-75) (Table S1D) were cultured in RPMI medium containing 10% fetal calf serum (labeled RPMI) or synthetic medium (labeled AIM, catalog # 12055091 Life Technologies) lacking FCS for 4h at 37°C. Total RNA prepared from these cells was analyzed by microarrays as described in the Methods section. Average gene expression profiles were compared between cells cultured in either medium to cells maintained at 4°C. A) Up-regulated pathways determined by PAGE in RPMI medium (left panel) or AIM (right panel) are shown. B) Genes within the NF-κB-induced pathway whose expression was significantly changed (p -value ≤ 0.05 , Z-ratio ≥ 1.5) in either culture condition.

Figure S3

A NAC pretreatment at 4°C

NAC pretreatment at 37°C



B

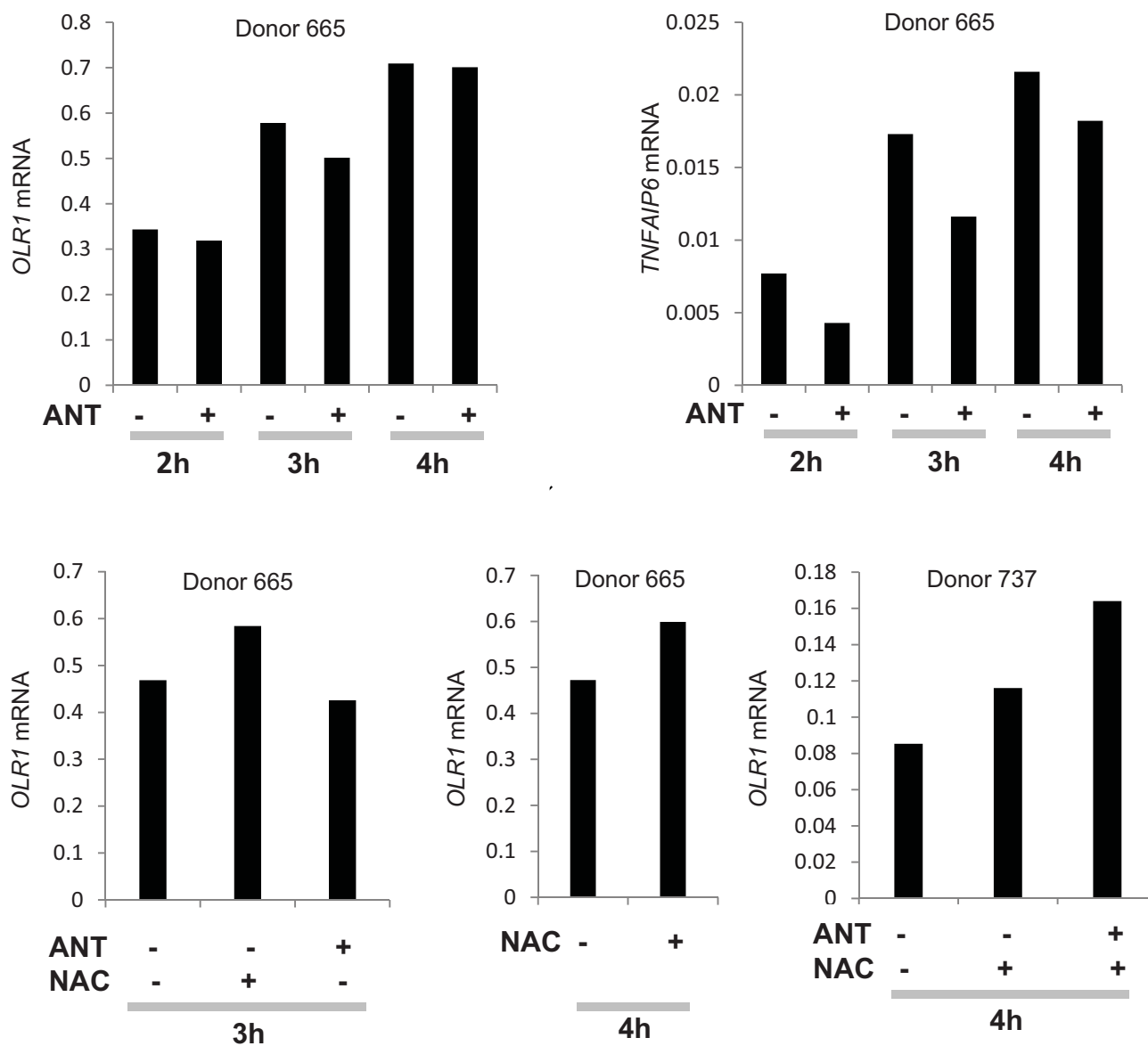


Figure S3. Role of mitochondrial oxidative stress in regulating gene expression program in CD4⁺ T cells cultured *ex vivo*. A) Representative flow cytometric profiles of CD4⁺ T cells incubated at 37°C for 4h. Cells were treated with 50 μM antimycin A (Ant) to induce mitochondrial ROS, 5mM N-acetyl cysteine (NAC) to quench cellular ROS or left untreated (PBS and mitoxox only) for the duration of culture. Cells were labeled with MitoSOX Red mitochondrial superoxide indicator (5μM, Invitrogen) according to the manufacturer's recommendation and analyzed using a BD FACS Canto II cytometer. B) Quantitative RT-PCR analysis of gene expression in cells treated as indicated. Experiments were carried out with cells from two donors. Test gene expression was normalized to GAPDH for each comparison.

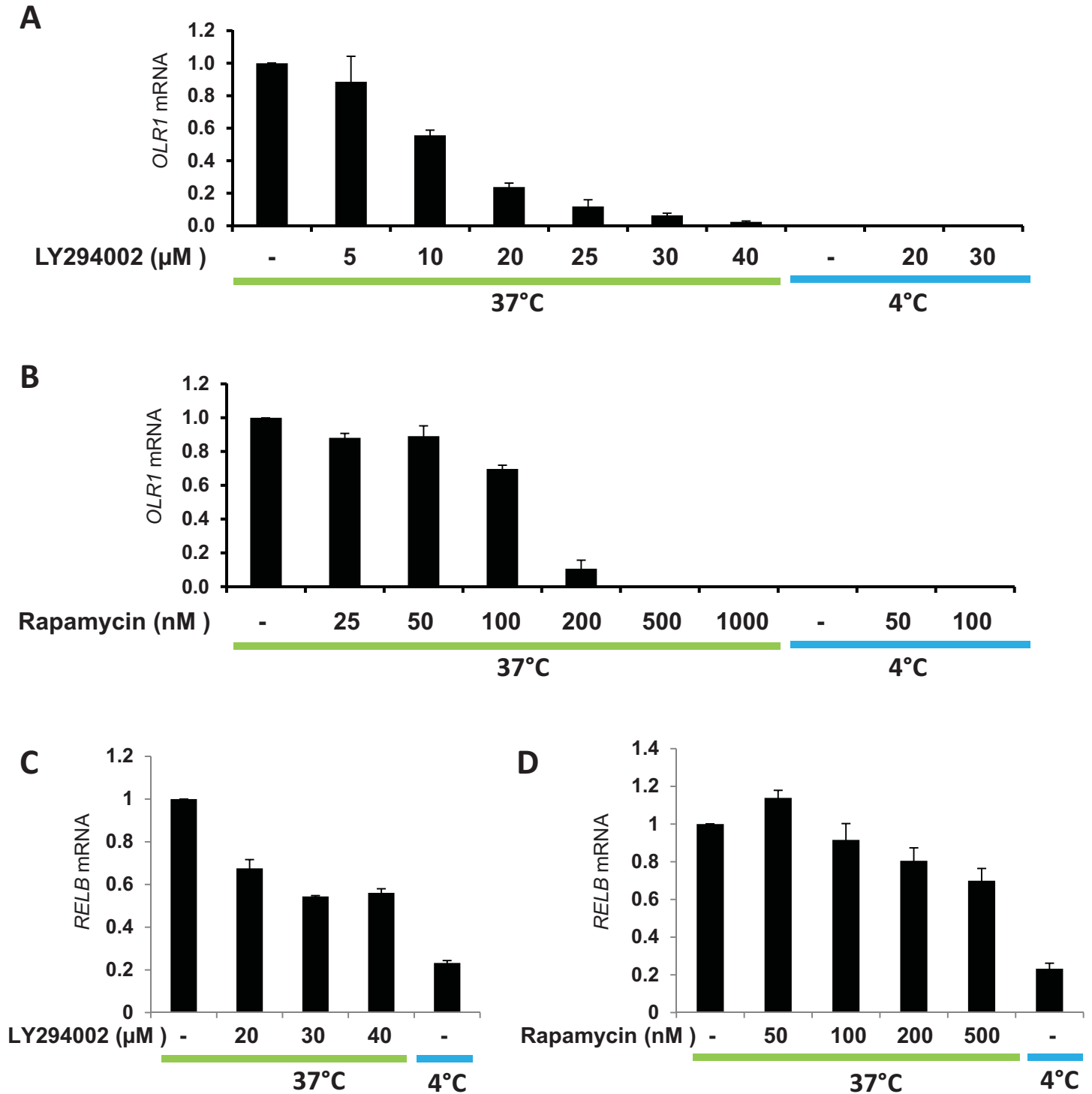


Figure S4. Effect of LY294002 (PI3 kinase inhibitor) and rapamycin (mTOR inhibitor) on gene expression induced by *ex vivo* culture of CD4⁺ T Cells. RNA isolated from CD4⁺ T cells that were kept at 4°C or incubated at 37°C in RPMI medium for 4h in the presence of various concentrations of LY294002 or rapamycin as indicated was analyzed by quantitative RT-PCR for expression of *OLR1* or *RELB* genes. The dose range of LY294002 and rapamycin did not reduce cell viability over the time course of the experiment. Error bars reflect the standard error of the mean (\pm SEM). Test gene expression was normalized to GAPDH for each comparison.

Table S1A. Group 1 Donors' Sex and Age

| Donor # | Sex | Age | |
|---------|-----|-----|-----|
| ND29 | M | 25 | <65 |
| ND60 | F | 29 | |
| ND33 | M | 30 | |
| BL10 | M | 30 | |
| ND55 | M | 40 | |
| ND57 | F | 40 | |
| ND58 | F | 41 | |
| ND53 | M | 43 | |
| ND32 | M | 52 | |
| ND36 | M | 52 | |
| ND59 | M | 52 | |
| BL01 | F | 55 | |
| BL08 | F | 55 | |
| BL21 | M | 55 | |
| BL11 | F | 57 | |
| BL04 | M | 60 | |
| BL12 | M | 60 | |
| BL19 | F | 61 | |
| BL20 | F | 62 | |
| BL18 | M | 63 | |
| BL03 | F | 68 | |
| BL16 | F | 70 | |
| BL17 | M | 70 | |
| ND31 | F | 71 | |
| BL06 | F | 71 | |
| BL22 | M | 72 | |
| BL07 | M | 73 | |
| BL09 | M | 75 | |
| BL13 | F | 75 | |
| BL05 | M | 77 | |
| BL02 | F | 81 | |
| | | | ≥65 |

Table S1B. Group 2 Donors' Sex and Age

| Donor # | Sex | Age | |
|---------|-----|-----|-----|
| ND703 | M | 26 | <35 |
| ND691 | M | 28 | |
| ND340 | M | 30 | |
| ND554 | M | 33 | |
| BL60 | F | 35 | |
| BL75 | F | 66 | ≥65 |
| BL61 | M | 66 | |
| BL52 | F | 68 | |
| BL73 | F | 68 | |
| BL57 | M | 70 | |
| BL76 | M | 71 | |
| BL56 | M | 72 | |
| BL58 | M | 73 | |
| BL67 | M | 73 | |
| BL68 | M | 73 | |
| BL70 | M | 74 | |
| BL63 | M | 75 | |
| BL59 | M | 75 | |
| BL78 | F | 76 | |
| BL66 | M | 76 | |
| BL49 | M | 77 | |
| BL64 | M | 83 | |
| BL74 | M | 83 | |

Table S1C. Donors' Sex and Age
(CD4⁺ T cells were incubated for 4h at 37°C and 4°C)

| Donor # | Sex | Age | Degree |
|---------|-----|-----|--------|
| ND60 | F | 29 | 4 |
| ND60 | F | 29 | 37 |
| BL10 | M | 30 | 4 |
| BL10 | M | 30 | 37 |
| BL60 | F | 35 | 4 |
| BL60 | F | 35 | 37 |
| ND55 | M | 40 | 4 |
| ND55 | M | 40 | 37 |
| BL25 | M | 44 | 4 |
| BL25 | M | 44 | 37 |
| BL35 | F | 49 | 4 |
| BL35 | F | 49 | 37 |
| BL44 | M | 44 | 4 |
| BL44 | M | 44 | 37 |
| BL27 | M | 52 | 4 |
| BL27 | M | 52 | 37 |
| BL28 | M | 56 | 4 |
| BL28 | M | 56 | 37 |
| BL33 | M | 54 | 4 |
| BL33 | M | 54 | 37 |
| BL53 | F | 53 | 4 |
| BL53 | F | 53 | 37 |
| BL24 | M | 63 | 4 |
| BL24 | M | 63 | 37 |
| BL30 | M | 67 | 4 |
| BL30 | M | 67 | 37 |
| BL32 | F | 62 | 4 |
| BL32 | F | 62 | 37 |
| BL38 | M | 61 | 4 |
| BL38 | M | 61 | 37 |
| BL51 | F | 66 | 4 |
| BL51 | F | 66 | 37 |
| BL36 | M | 74 | 4 |
| BL36 | M | 74 | 37 |
| BL45 | M | 76 | 4 |
| BL45 | M | 76 | 37 |
| BL50 | F | 73 | 4 |
| BL50 | F | 73 | 37 |
| BL56 | M | 72 | 4 |
| BL56 | M | 72 | 37 |
| BL39 | M | 79 | 4 |
| BL39 | M | 79 | 37 |
| BL2 | F | 81 | 4 |
| BL2 | F | 81 | 37 |
| BL54 | M | 82 | 4 |
| BL54 | M | 82 | 37 |

Table S1C. Donors' Sex and Age
(CD4⁺ T cells were incubated in RPMI or AIM V medium
for 4h at 37°C and 4°C)

| Donor # | Sex | Age | Degree | Medium |
|---------|-----|-----|--------|--------|
| ND672 | M | 30 | 4 | RPMI |
| ND672 | M | 30 | 4 | AIM |
| ND672 | M | 30 | 37 | RPMI |
| ND672 | M | 30 | 37 | AIM |
| ND561 | M | 36 | 4 | RPMI |
| ND561 | M | 36 | 4 | AIM |
| ND561 | M | 36 | 37 | RPMI |
| ND561 | M | 36 | 37 | AIM |
| ND651 | M | 25 | 4 | RPMI |
| ND651 | M | 25 | 4 | AIM |
| ND651 | M | 25 | 37 | RPMI |
| ND651 | M | 25 | 37 | AIM |
| ND656 | M | 32 | 4 | RPMI |
| ND656 | M | 32 | 4 | AIM |
| ND656 | M | 32 | 37 | RPMI |
| ND656 | M | 32 | 37 | AIM |
| ND229 | F | 34 | 4 | RPMI |
| ND229 | F | 34 | 4 | AIM |
| ND229 | F | 34 | 37 | RPMI |
| ND229 | F | 34 | 37 | AIM |
| BL61 | M | 66 | 4 | RPMI |
| BL61 | M | 66 | 4 | AIM |
| BL61 | M | 66 | 37 | RPMI |
| BL61 | M | 66 | 37 | AIM |
| BL62 | F | 64 | 4 | RPMI |
| BL62 | F | 64 | 4 | AIM |
| BL62 | F | 64 | 37 | RPMI |
| BL62 | F | 64 | 37 | AIM |
| BL63 | M | 75 | 4 | RPMI |
| BL63 | M | 75 | 4 | AIM |
| BL63 | M | 75 | 37 | RPMI |
| BL63 | M | 75 | 37 | AIM |

RPMI 1640 with serum (10% FBS)
AIM V Serum Free Media (no serum)

Table S2. Significant genes in untreated cells (0h).

| Gene Symbol | Genbank Accession Number | 0O-0Y (Z-ratio) | 0O-0Y (p) | 0O-0Y (fold change) |
|-------------|--------------------------------|--------------------|--------------|------------------------|
| CD9 | NM_001769.2 | 6.53 | 0.0020 | 1.45 |
| IL1B | NM_000576.2 | 6.46 | 0.0391 | 1.62 |
| C15orf48 | NM_032413.2 | 5.88 | 0.0196 | 1.28 |
| LILRA3 | NM_006865.2 | 5.63 | 0.0270 | 1.51 |
| OLR1 | NM_002543.2 | 5.51 | 0.0426 | 1.17 |
| ENG | NM_000118.1 | 4.96 | 0.0233 | 1.45 |
| CLEC5A | NM_013252.2 | 4.86 | 0.0260 | 1.35 |
| TMEM51 | NM_018022.1 | 4.84 | 0.0262 | 1.36 |
| CD14 | NM_000591.1 | 4.47 | 0.0439 | 1.31 |
| CST7 | NM_003650.2 | 4.41 | 0.0497 | 1.42 |
| PLEK | NM_002664.1 | 4.39 | 0.0221 | 1.31 |
| PTPNS1 | NM_080792.1 | 4.36 | 0.0219 | 1.25 |
| CXCL16 | NM_022059.1 | 4.20 | 0.0198 | 1.20 |
| FPR1 | NM_002029.3 | 4.12 | 0.0203 | 1.29 |
| TNFAIP2 | NM_006291.2 | 4.07 | 0.0461 | 1.40 |
| ZNF218 | NM_173485.2 | 4.03 | 0.0078 | 1.32 |
| IGSF6 | NM_005849.1 | 4.03 | 0.0465 | 1.23 |
| FGR | NM_005248.1 | 3.93 | 0.0493 | 1.34 |
| LYN | NM_002350.1 | 3.82 | 0.0279 | 1.21 |
| SLC16A3 | NM_004207.1 | 3.74 | 0.0045 | 1.33 |
| WARS | NM_213646.1 | 3.73 | 0.0258 | 1.32 |
| VASH1 | NM_014909.2 | 3.66 | 0.0200 | 1.36 |
| SRXN1 | NM_080725.1 | 3.60 | 0.0416 | 1.36 |
| SLC43A2 | NM_152346.1 | 3.52 | 0.0217 | 1.22 |
| CTSZ | NM_001336.2 | 3.48 | 0.0492 | 1.31 |
| PLAUR | NM_001005376.1 | 3.48 | 0.0094 | 1.30 |
| MARCKSL1 | NM_023009.4 | 3.44 | 0.0273 | 1.21 |
| PEA15 | NM_003768.2 | 3.28 | 0.0176 | 1.12 |
| CENTA2 | NM_018404.1 | 3.27 | 0.0378 | 1.22 |
| PFC | NM_002621.1 | 3.23 | 0.0002 | 1.29 |
| RAB31 | NM_006868.2 | 3.18 | 0.0444 | 1.22 |
| CSTB | NM_000100.2 | 3.14 | 0.0253 | 1.17 |
| HLA-DRB4 | NM_021983.4 | 3.07 | 0.0329 | 1.16 |
| C15orf39 | NM_015492.3 | 3.02 | 0.0313 | 1.29 |
| CYP27A1 | NM_000784.2 | 2.99 | 0.0398 | 1.24 |
| CEBPA | NM_004364.2 | 2.85 | 0.0308 | 1.29 |
| ZDHHC7 | NM_017740.1 | 2.80 | 0.0422 | 1.21 |
| UBL5 | NM_024292.2 | 2.78 | 0.0355 | 1.18 |
| TAP1 | NM_000593.5 | 2.75 | 0.0446 | 1.13 |
| P2RX7 | NM_002562.4 | 2.69 | 0.0434 | 1.27 |
| GRINA | NM_000837.1 | 2.68 | 0.0371 | 1.25 |
| CTSB | NM_001908.3 | 2.64 | 0.0246 | 1.20 |

| | | | | |
|-----------|----------------|------|--------|------|
| OR5P3 | NM_153445.1 | 2.61 | 0.0313 | 1.28 |
| GSTO1 | NM_004832.1 | 2.60 | 0.0499 | 1.15 |
| FGF14 | NM_175929.1 | 2.59 | 0.0403 | 1.25 |
| ATP6V1F | NM_004231.2 | 2.55 | 0.0195 | 1.20 |
| LGMN | NM_005606.5 | 2.51 | 0.0276 | 1.21 |
| ZMYND15 | NM_032265.1 | 2.48 | 0.0283 | 1.25 |
| STARD8 | NM_014725.2 | 2.42 | 0.0240 | 1.28 |
| IFNGR2 | NM_005534.2 | 2.42 | 0.0489 | 1.14 |
| ABI3 | NM_016428.2 | 2.32 | 0.0293 | 1.28 |
| LITAF | NM_004862.2 | 2.30 | 0.0188 | 1.07 |
| TRIM21 | NM_003141.3 | 2.27 | 0.0386 | 1.22 |
| NEUROG3 | NM_020999.2 | 2.20 | 0.0048 | 1.23 |
| CMIP | NM_030629.1 | 2.20 | 0.0119 | 1.11 |
| LAIR2 | NM_002288.3 | 2.17 | 0.0467 | 1.27 |
| DNCL1 | NM_003746.1 | 2.17 | 0.0259 | 1.10 |
| TAPBP | NM_172208.1 | 2.16 | 0.0270 | 1.19 |
| RHOB | NM_004040.2 | 2.15 | 0.0463 | 1.20 |
| PSMA7 | NM_152255.1 | 2.15 | 0.0424 | 1.19 |
| SH2D2A | NM_003975.2 | 2.15 | 0.0443 | 1.10 |
| SHKBP1 | NM_138392.1 | 2.13 | 0.0340 | 1.23 |
| D15Wsu75e | NM_015704.1 | 2.11 | 0.0114 | 1.20 |
| CENTA1 | NM_006869.1 | 2.10 | 0.0385 | 1.25 |
| PAPPA2 | NM_020318.1 | 2.08 | 0.0323 | 1.23 |
| TICAM1 | NM_014261.1 | 2.07 | 0.0281 | 1.15 |
| CD58 | NM_001779.1 | 2.05 | 0.0274 | 1.14 |
| RHOC | NM_175744.3 | 2.03 | 0.0135 | 1.18 |
| C9orf89 | NM_032310.2 | 2.01 | 0.0455 | 1.17 |
| PRKCD | NM_212539.1 | 2.01 | 0.0143 | 1.13 |
| C9orf19 | NM_022343.2 | 2.01 | 0.0264 | 1.09 |
| LCE1F | NM_178354.1 | 1.98 | 0.0024 | 1.22 |
| MYO1C | NM_033375.3 | 1.94 | 0.0445 | 1.25 |
| TPST2 | NM_001008566.1 | 1.92 | 0.0014 | 1.22 |
| MTA2 | NM_004739.2 | 1.91 | 0.0253 | 1.15 |
| SAT | NM_002970.1 | 1.90 | 0.0468 | 1.02 |
| CDC42EP2 | NM_006779.2 | 1.89 | 0.0107 | 1.22 |
| NT5DC2 | NM_022908.1 | 1.86 | 0.0040 | 1.22 |
| MRO | NM_031939.2 | 1.86 | 0.0030 | 1.19 |
| TMEM16G | NM_001001891.1 | 1.85 | 0.0175 | 1.22 |
| LOC94431 | NM_145237.1 | 1.84 | 0.0067 | 1.20 |
| CYP4A11 | NM_000778.2 | 1.84 | 0.0126 | 1.20 |
| PGD | NM_002631.2 | 1.84 | 0.0394 | 1.14 |
| TNIP3 | NM_024873.2 | 1.83 | 0.0068 | 1.23 |
| SPTAN1 | NM_003127.1 | 1.82 | 0.0398 | 1.07 |
| SERF1B | NM_022978.1 | 1.80 | 0.0426 | 1.15 |
| ZYX | NM_003461.4 | 1.80 | 0.0462 | 1.09 |
| PSMA5 | NM_002790.2 | 1.79 | 0.0494 | 1.08 |
| RNASE1 | NM_198232.1 | 1.77 | 0.0273 | 1.21 |
| TA-NFKBH | NM_139239.1 | 1.77 | 0.0003 | 1.20 |
| IGFBP4 | NM_001552.2 | 1.75 | 0.0481 | 1.22 |
| C1QA | NM_015991.1 | 1.70 | 0.0435 | 1.22 |
| HMBOX1 | NM_024567.2 | 1.70 | 0.0414 | 1.18 |
| RLN2 | NM_005059.2 | 1.69 | 0.0402 | 1.22 |

| | | | | |
|-----------|----------------|-------|--------|-------|
| CLTB | NM_007097.2 | 1.69 | 0.0033 | 1.11 |
| PTPN1 | NM_002827.2 | 1.66 | 0.0223 | 1.08 |
| LTBP1 | NM_206943.1 | 1.65 | 0.0128 | 1.19 |
| PSMD4 | NM_002810.1 | 1.65 | 0.0266 | 1.10 |
| OR2D3 | NM_001004684.1 | 1.61 | 0.0060 | 1.18 |
| MTHFD1L | NM_015440.3 | 1.61 | 0.0142 | 1.18 |
| ARPC5L | NM_030978.1 | 1.61 | 0.0260 | 1.07 |
| SP8 | NM_198956.1 | 1.60 | 0.0231 | 1.17 |
| SORBS2 | NM_003603.4 | 1.59 | 0.0338 | 1.20 |
| LGALS12 | NM_033101.2 | 1.58 | 0.0278 | 1.18 |
| DBNL | NM_014063.5 | 1.58 | 0.0328 | 1.06 |
| SIGLEC9 | NM_014441.1 | 1.57 | 0.0146 | 1.20 |
| TRPV5 | NM_019841.3 | 1.57 | 0.0036 | 1.18 |
| DXS9879E | NM_006014.2 | 1.56 | 0.0332 | 1.12 |
| RPA3 | NM_002947.3 | 1.56 | 0.0408 | 1.09 |
| PSORS1C2 | NM_014069.1 | 1.55 | 0.0149 | 1.18 |
| DGCR8 | NM_022720.5 | 1.55 | 0.0070 | 1.17 |
| GTPBP2 | NM_019096.3 | 1.54 | 0.0002 | 1.19 |
| PRY | NM_004676.2 | 1.54 | 0.0058 | 1.18 |
| CD2BP2 | NM_006110.1 | 1.54 | 0.0219 | 1.13 |
| BRI3 | NM_015379.3 | 1.54 | 0.0477 | 1.09 |
| SR-A1 | NM_021228.1 | 1.53 | 0.0364 | 1.20 |
| PVRL2 | NM_002856.1 | 1.53 | 0.0174 | 1.18 |
| C19orf30 | NM_174947.2 | 1.51 | 0.0178 | 1.16 |
| CBX7 | NM_175709.2 | 1.50 | 0.0317 | 1.09 |
| ZNF75A | NM_153028.1 | -1.51 | 0.0446 | -1.05 |
| DNAJB14 | NM_001031723.1 | -1.52 | 0.0097 | -1.07 |
| CHD6 | NM_032221.3 | -1.53 | 0.0452 | -1.06 |
| B3GALT2 | NM_003783.2 | -1.54 | 0.0189 | -1.04 |
| JMY | NM_152405.1 | -1.54 | 0.0158 | -1.07 |
| PDHX | NM_003477.1 | -1.55 | 0.0356 | -1.08 |
| C1orf123 | NM_017887.1 | -1.55 | 0.0349 | -1.10 |
| NIN | NM_020921.2 | -1.56 | 0.0457 | -1.08 |
| GTF2IRD2B | NM_001003795.1 | -1.57 | 0.0382 | -1.05 |
| SNTB1 | NM_021021.2 | -1.57 | 0.0451 | -1.06 |
| C13orf10 | NM_022118.3 | -1.57 | 0.0173 | -1.11 |
| ZFP1 | NM_153688.1 | -1.58 | 0.0195 | -1.04 |
| ADK | NM_001123.2 | -1.58 | 0.0220 | -1.06 |
| ZNF600 | NM_198457.1 | -1.58 | 0.0443 | -1.06 |
| ZF | NM_021212.1 | -1.58 | 0.0389 | -1.09 |
| PRKRIP1 | NM_024653.1 | -1.58 | 0.0161 | -1.10 |
| TCEAL8 | NM_153333.2 | -1.59 | 0.0078 | -1.07 |
| SPHAR | NM_006542.2 | -1.60 | 0.0289 | -1.04 |
| MOBK1B | NM_018221.1 | -1.60 | 0.0042 | -1.04 |
| BET1L | NM_016526.3 | -1.60 | 0.0234 | -1.15 |
| ZNF652 | NM_014897.1 | -1.61 | 0.0095 | -1.05 |
| C20orf7 | NM_199052.1 | -1.61 | 0.0316 | -1.09 |
| TUBGCP5 | NM_052903.2 | -1.62 | 0.0087 | -1.07 |
| USP15 | NM_006313.1 | -1.62 | 0.0288 | -1.07 |
| AHCTF1 | NM_015446.3 | -1.63 | 0.0286 | -1.11 |
| C11orf46 | NM_152316.1 | -1.63 | 0.0365 | -1.11 |
| SMARCA3 | NM_003071.2 | -1.65 | 0.0152 | -1.07 |

| | | | | |
|-----------|----------------|-------|--------|-------|
| USP33 | NM_201626.1 | -1.65 | 0.0389 | -1.12 |
| SETD6 | NM_024860.1 | -1.66 | 0.0020 | -1.07 |
| NUCKS1 | NM_022731.2 | -1.66 | 0.0084 | -1.08 |
| FAM3C | NM_014888.1 | -1.68 | 0.0253 | -1.06 |
| NIPBL | NM_015384.3 | -1.69 | 0.0374 | -1.09 |
| C13orf24 | NM_006346.2 | -1.71 | 0.0143 | -1.07 |
| CCR9 | NM_031200.1 | -1.71 | 0.0010 | -1.08 |
| HSU79303 | NM_013301.1 | -1.72 | 0.0211 | -1.08 |
| POMT1 | NM_007171.2 | -1.72 | 0.0137 | -1.08 |
| C20orf108 | NM_080821.1 | -1.72 | 0.0332 | -1.10 |
| ZNF42 | NM_198055.1 | -1.72 | 0.0307 | -1.11 |
| PPOX | NM_000309.2 | -1.75 | 0.0061 | -1.09 |
| PPA2 | NM_176869.1 | -1.75 | 0.0332 | -1.11 |
| CRSP7 | NM_004831.3 | -1.75 | 0.0398 | -1.13 |
| SNX25 | NM_031953.2 | -1.76 | 0.0335 | -1.09 |
| RAB11FIP2 | NM_014904.1 | -1.76 | 0.0417 | -1.09 |
| KIAA0195 | NM_014738.2 | -1.76 | 0.0478 | -1.14 |
| ZNF518 | NM_014803.2 | -1.77 | 0.0381 | -1.10 |
| FLJ30596 | NM_153013.2 | -1.78 | 0.0032 | -1.06 |
| FLJ22555 | NM_024520.1 | -1.78 | 0.0342 | -1.13 |
| NR1D2 | NM_005126.2 | -1.79 | 0.0398 | -1.09 |
| POLR3B | NM_018082.3 | -1.80 | 0.0091 | -1.09 |
| KIAA1324L | NM_152748.2 | -1.80 | 0.0323 | -1.09 |
| ZNF181 | NM_001029997.1 | -1.81 | 0.0426 | -1.08 |
| ARG2 | NM_001172.3 | -1.81 | 0.0069 | -1.08 |
| C10orf35 | NM_145306.1 | -1.82 | 0.0053 | -1.09 |
| RUNX2 | NM_004348.3 | -1.82 | 0.0469 | -1.10 |
| TAF1L | NM_153809.1 | -1.85 | 0.0436 | -1.07 |
| AMMECR1 | NM_001025580.1 | -1.85 | 0.0076 | -1.11 |
| ALS2CR13 | NM_173511.2 | -1.85 | 0.0476 | -1.18 |
| TUBGCP3 | NM_006322.3 | -1.86 | 0.0407 | -1.08 |
| GOPC | NM_020399.2 | -1.86 | 0.0446 | -1.10 |
| INPP5E | NM_019892.3 | -1.86 | 0.0414 | -1.14 |
| NT5E | NM_002526.1 | -1.87 | 0.0054 | -1.07 |
| C1orf86 | NM_182533.1 | -1.87 | 0.0073 | -1.09 |
| PIGN | NM_012327.3 | -1.87 | 0.0364 | -1.10 |
| RPS6KA5 | NM_004755.2 | -1.87 | 0.0163 | -1.12 |
| ITM2B | NM_021999.2 | -1.87 | 0.0459 | -1.26 |
| NCKAP1 | NM_013436.3 | -1.88 | 0.0409 | -1.13 |
| GUF1 | NM_021927.1 | -1.89 | 0.0178 | -1.09 |
| CEP57 | NM_014679.3 | -1.89 | 0.0138 | -1.10 |
| INPP5A | NM_005539.2 | -1.90 | 0.0219 | -1.09 |
| C10orf38 | NM_001010924.1 | -1.90 | 0.0118 | -1.10 |
| C1QDC1 | NM_023925.3 | -1.91 | 0.0211 | -1.10 |
| SLC35B3 | NM_015948.2 | -1.91 | 0.0382 | -1.10 |
| ACAD8 | NM_014384.1 | -1.92 | 0.0489 | -1.09 |
| KLHL3 | NM_017415.1 | -1.94 | 0.0193 | -1.12 |
| CARD8 | NM_014959.1 | -1.95 | 0.0233 | -1.14 |
| MGC3207 | NM_032285.1 | -1.95 | 0.0238 | -1.15 |
| RNF138 | NM_016271.3 | -1.97 | 0.0323 | -1.11 |
| ZBTB20 | NM_015642.2 | -2.00 | 0.0176 | -1.11 |
| ZNF439 | NM_152262.1 | -2.02 | 0.0002 | -1.09 |

| | | | | |
|-----------|----------------|-------|--------|-------|
| SLC16A10 | NM_018593.3 | -2.02 | 0.0478 | -1.11 |
| IDI1 | NM_004508.2 | -2.03 | 0.0170 | -1.13 |
| SMAP | NM_014267.3 | -2.04 | 0.0313 | -1.09 |
| ZNF684 | NM_152373.2 | -2.05 | 0.0329 | -1.09 |
| HOP | NM_139212.2 | -2.05 | 0.0380 | -1.13 |
| C8orf52 | NM_017864.2 | -2.06 | 0.0218 | -1.14 |
| GCN5L2 | NM_021078.1 | -2.06 | 0.0479 | -1.16 |
| CAMK2G | NM_172169.1 | -2.07 | 0.0397 | -1.11 |
| CARD11 | NM_032415.2 | -2.07 | 0.0316 | -1.18 |
| ZNF154 | NM_003444.1 | -2.10 | 0.0239 | -1.11 |
| ZNF644 | NM_016620.2 | -2.11 | 0.0144 | -1.11 |
| ZNRF2 | NM_147128.3 | -2.13 | 0.0346 | -1.11 |
| MTX3 | NM_001010891.1 | -2.13 | 0.0364 | -1.13 |
| FKBP11 | NM_016594.1 | -2.13 | 0.0293 | -1.20 |
| ST13 | NM_003932.3 | -2.14 | 0.0411 | -1.16 |
| CNAP1 | NM_014865.2 | -2.15 | 0.0025 | -1.12 |
| GOLGA8A | NM_181077.2 | -2.16 | 0.0182 | -1.11 |
| CROP | NM_006107.2 | -2.17 | 0.0497 | -1.14 |
| MFGE8 | NM_005928.1 | -2.18 | 0.0303 | -1.18 |
| WDR26 | NM_025160.4 | -2.26 | 0.0485 | -1.19 |
| PLCL1 | NM_006226.1 | -2.28 | 0.0036 | -1.11 |
| PPIA | NM_203430.1 | -2.28 | 0.0239 | -1.14 |
| NARG1L | NM_018527.2 | -2.35 | 0.0244 | -1.15 |
| EVA1 | NM_144765.1 | -2.37 | 0.0069 | -1.13 |
| OFD1 | NM_003611.1 | -2.38 | 0.0219 | -1.18 |
| PNN | NM_002687.2 | -2.41 | 0.0158 | -1.23 |
| SESN1 | NM_014454.1 | -2.42 | 0.0225 | -1.21 |
| BACH2 | NM_021813.1 | -2.44 | 0.0433 | -1.17 |
| FLJ34443 | NM_175918.2 | -2.44 | 0.0138 | -1.19 |
| EIF2AK4 | NM_001013703.2 | -2.44 | 0.0409 | -1.22 |
| CRSP3 | NM_004830.2 | -2.46 | 0.0451 | -1.16 |
| IRS2 | NM_003749.2 | -2.49 | 0.0103 | -1.19 |
| MAP4K5 | NM_006575.3 | -2.50 | 0.0284 | -1.18 |
| C14orf106 | NM_018353.3 | -2.51 | 0.0379 | -1.21 |
| TTC14 | NM_133462.1 | -2.53 | 0.0144 | -1.22 |
| IL11RA | NM_147162.1 | -2.54 | 0.0141 | -1.19 |
| LOC159090 | NM_145284.3 | -2.56 | 0.0211 | -1.15 |
| EIIs1 | NM_152793.1 | -2.57 | 0.0245 | -1.19 |
| ITGA4 | NM_000885.4 | -2.59 | 0.0440 | -1.21 |
| FAM63A | NM_018379.2 | -2.60 | 0.0324 | -1.29 |
| LASS6 | NM_203463.1 | -2.62 | 0.0079 | -1.20 |
| FBXL3 | NM_012158.1 | -2.63 | 0.0319 | -1.21 |
| CCNL1 | NM_020307.2 | -2.64 | 0.0426 | -1.17 |
| HMGB1 | NM_002128.3 | -2.69 | 0.0340 | -1.13 |
| YPEL2 | NM_001005404.3 | -2.82 | 0.0035 | -1.21 |
| ABCB1 | NM_000927.3 | -2.84 | 0.0246 | -1.22 |
| LRMP | NM_006152.2 | -2.84 | 0.0104 | -1.23 |
| ADI1 | NM_018269.1 | -3.02 | 0.0017 | -1.29 |
| LMOD3 | NM_198271.2 | -3.11 | 0.0252 | -1.35 |
| AKR1C3 | NM_003739.4 | -3.14 | 0.0071 | -1.28 |
| ITM2A | NM_004867.3 | -3.14 | 0.0138 | -1.29 |
| SMAD5 | NM_001001419.1 | -3.18 | 0.0178 | -1.25 |

| | | | | |
|---------|-------------|-------|--------|-------|
| LYCAT | NM_182551.3 | -3.23 | 0.0182 | -1.26 |
| RPL7 | NM_000971.3 | -3.23 | 0.0273 | -1.32 |
| TDRD1 | NM_198795.1 | -3.27 | 0.0128 | -1.28 |
| PABPC3 | NM_030979.2 | -3.33 | 0.0126 | -1.22 |
| GPR15 | NM_005290.1 | -3.35 | 0.0012 | -1.27 |
| RPLP1 | NM_001003.2 | -3.37 | 0.0280 | -1.35 |
| ADCY7 | NM_001114.2 | -3.48 | 0.0448 | -1.28 |
| BTBD15 | NM_014155.2 | -3.50 | 0.0429 | -1.26 |
| ZDHHC11 | NM_024786.1 | -3.67 | 0.0423 | -1.36 |
| HBA1 | NM_000558.3 | -3.70 | 0.0379 | -1.45 |
| LRAP | NM_022350.1 | -4.35 | 0.0386 | -1.42 |
| HBB | NM_000518.4 | -4.37 | 0.0235 | -1.60 |
| SH3YL1 | NM_015677.1 | -4.46 | 0.0281 | -1.38 |
| LRRN3 | NM_018334.3 | -8.97 | 0.0000 | -2.06 |

Table S2. Significant genes in untreated cells (0h). Comparison of younger (Y, less than 65 years) and older (O, 65 years or older) cohorts identified with Z-ratios greater than 1.5 and *p*-values less than 0.05. Gene Z-scores within each group were compared between Y and O groups to obtain a Z-ratio indicative of differential expression between the groups. Differentially regulated genes with Z-ratio absolute values greater than 1.5 and *p*-values less than 0.05 are shown in the table arranged by descending Z-ratios. Fold changes, *p*-value and Genebank accession number are as indicated. Positive Z-ratios correspond to genes expressed at higher levels in O compared to Y.

Table S3. Z-ratio of NF- κ B-induced pathway genes in first and second group.

| Gene Symbol | 0O-0Y (group 1) Z-ratio | 0O-0Y (group 2) Z-ratio |
|--------------------|------------------------------------|------------------------------------|
| IL1B | 6.464 | 7.276 |
| CXCL10 | 5.538 | 2.826 |
| IL6 | 4.788 | 2.209 |
| IL1A | 4.293 | 3.370 |
| TNFAIP2 | 4.068 | 3.569 |
| CCL2 | 4.043 | 5.635 |
| CXCL2 | 3.463 | 2.143 |
| TNF | 3.383 | 1.796 |
| CD83 | 2.710 | 2.709 |
| IL8 | 2.550 | 4.534 |
| NFKB2 | 2.388 | 2.507 |
| MSC | 2.308 | 3.806 |
| CDKN1A | 2.202 | 2.132 |
| SOD2 | 2.110 | 2.083 |
| CCL5 | 1.898 | 5.136 |
| RELA | 1.727 | 1.738 |
| NFKB1 | 1.724 | 2.463 |
| TNFAIP6 | 1.627 | 3.855 |
| RELB | 1.545 | 2.735 |
| SPTBN1 | -2.001 | -3.146 |

Table S4. Primer sequences used for real-time quantitative PCR

| Gene | Forward (F) and Reverse (R) Primers |
|-------------------------------|---|
| <i>GAPDH</i> | (F) 5'- TCC ATG ACA ACT TTG GTA TCG TG -3' (R) 5'- ACA GTC TTC TGG GTG GCA GTG -3' |
| <i>OLR1</i> | (F) 5'- GAA ATC CAA AGA GCA AAT GGA -3' (R) 5'- TTA AAT GAG CCC GAG GAA AA -3' |
| <i>RELB</i> | (F) 5'- GCC GGC AGC ATC CTT -3' (R) 5'- GAC TCG GTG AGG CCA GTC -3' |
| <i>CCL20</i> | (F) 5'- GCG CAA ATC CAA AAC AGA CT -3' (R) 5'- ACC TCC AAC CCC AGC AA -3' |
| <i>IL1 β</i> | (F) 5'- CAA AAT ACC TGT GGC CTT GG -3' (R) 5'- TGA AGA CAA ATC GCT TTT CCA -3' |
| <i>TNFAIP6</i> | (F) 5'- GGA TTT GGA AAA ACT GGC ATT -3' (R) 5'- TTT GGG AAG CCT GGA GAT TT -3' |