SUPPLEMENTAL MATERIALS

SUPPLEMENTAL FIGURES S1-S3 and TABLE S1

LEGENDS TO SUPPLEMENTAL FIGURES S1-S3

Fig. S1. Detection of $\Delta\psi_m$ (DiOC6 and TMRM), mitochondrial mass (MTG) and activation of mTOR (pS6RP) in DN T cells in healthy subject (black histogram), SLE patient in remission (blue histogram) and SLE patient in flare (red histogram).

Fig. S2. Increased production of IL-4 in DN lupus T cells. A, Representative dot plot analysis of DN T cells by gating on $CD3^+/CD4^-/CD8^-$ (DN) T cells. B, Measurement of intracellular IL4, IL-17, and IFN-γ production in permeabilized $CD3^+$, $CD4^+$, $CD8^+$, and DN T cells.

Fig. S3. Functional hierarchy of metabolic biomarkers in SLE. MHP is caused by exposure to nitric oxide (NO). Synthesis of NO and maintenance of GSH in reduced form depend on the production of NADPH by the pentose phosphate pathway (PPP). MHP promotes mTOR activation which in turn controls the expression of the transcription factor FoxP3 and IL-4. Increased IL-4 production by DN T cells may account for diminished expression of FoxP3 by $CD25^+/CD4^+$ T cells.

Fig. S1

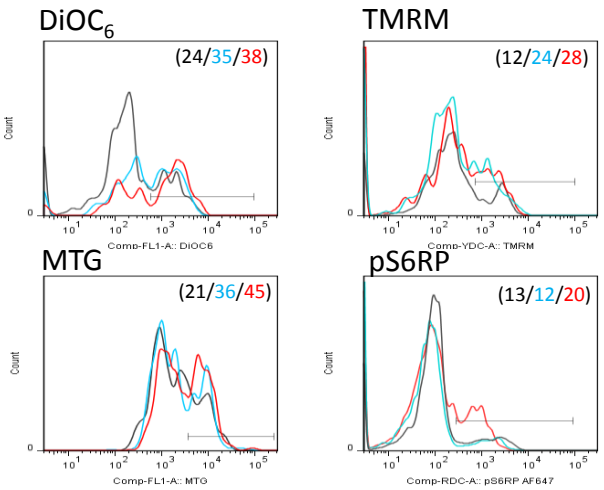
DiOC6
(24/35/38)
Count
0
Comp-FL1-A:: DiOC6
TMRM
(12/24/28)
Comp-YDC-A:: TMRM
MTG
(21/36/45)
Comp-FL1-A:: MTG
pS6RP
(13/12/20)
Comp-RDC-A:: pS6RP AF647

Fig. S2

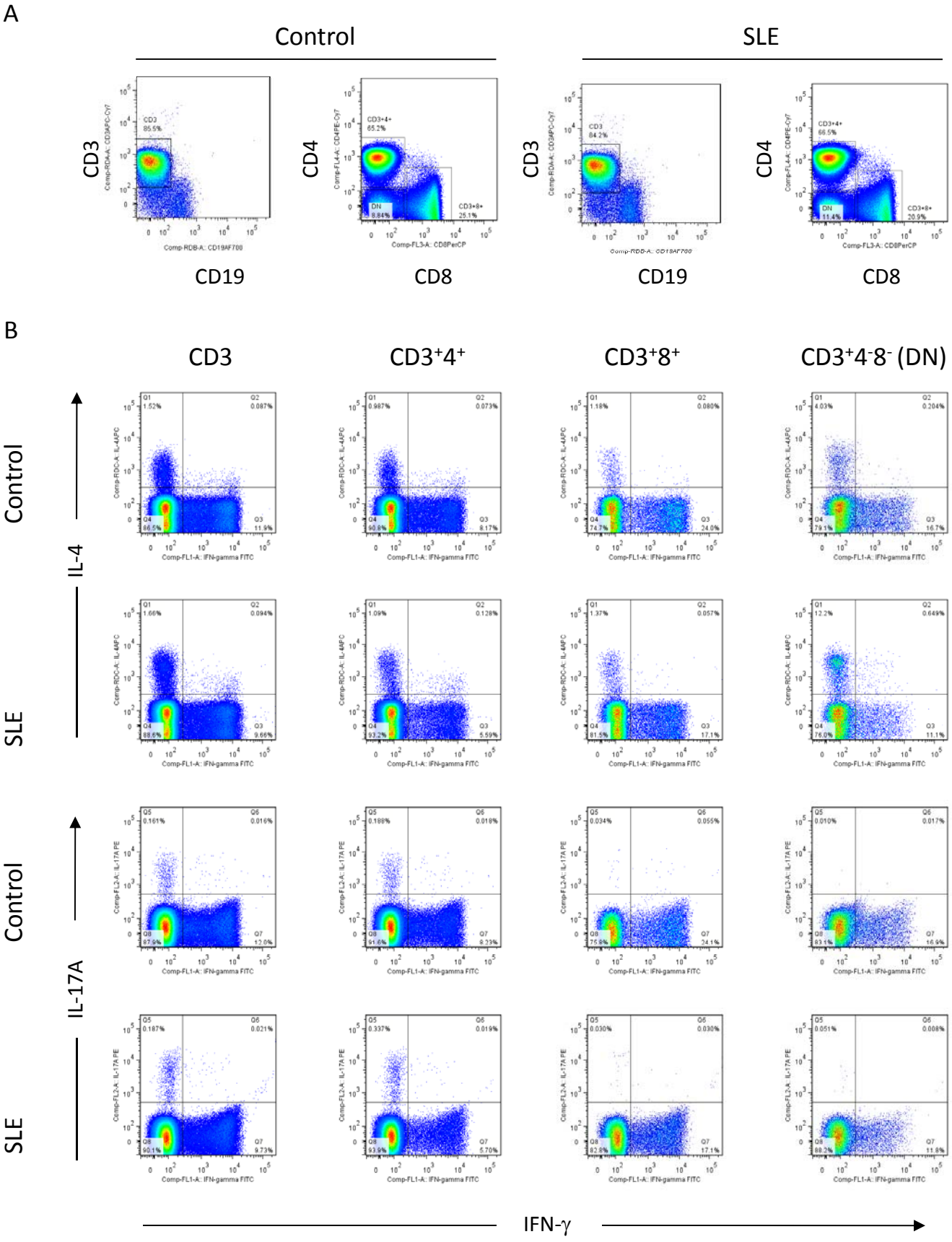
A
Control
SLE
CD3
CD4
CD19
CD8
B
CD3
CD3+4+
CD3+8+
CD3+4-8- (DN)
Control
SLE
IL-4
IL-17A
IFN-γ

Fig. S3

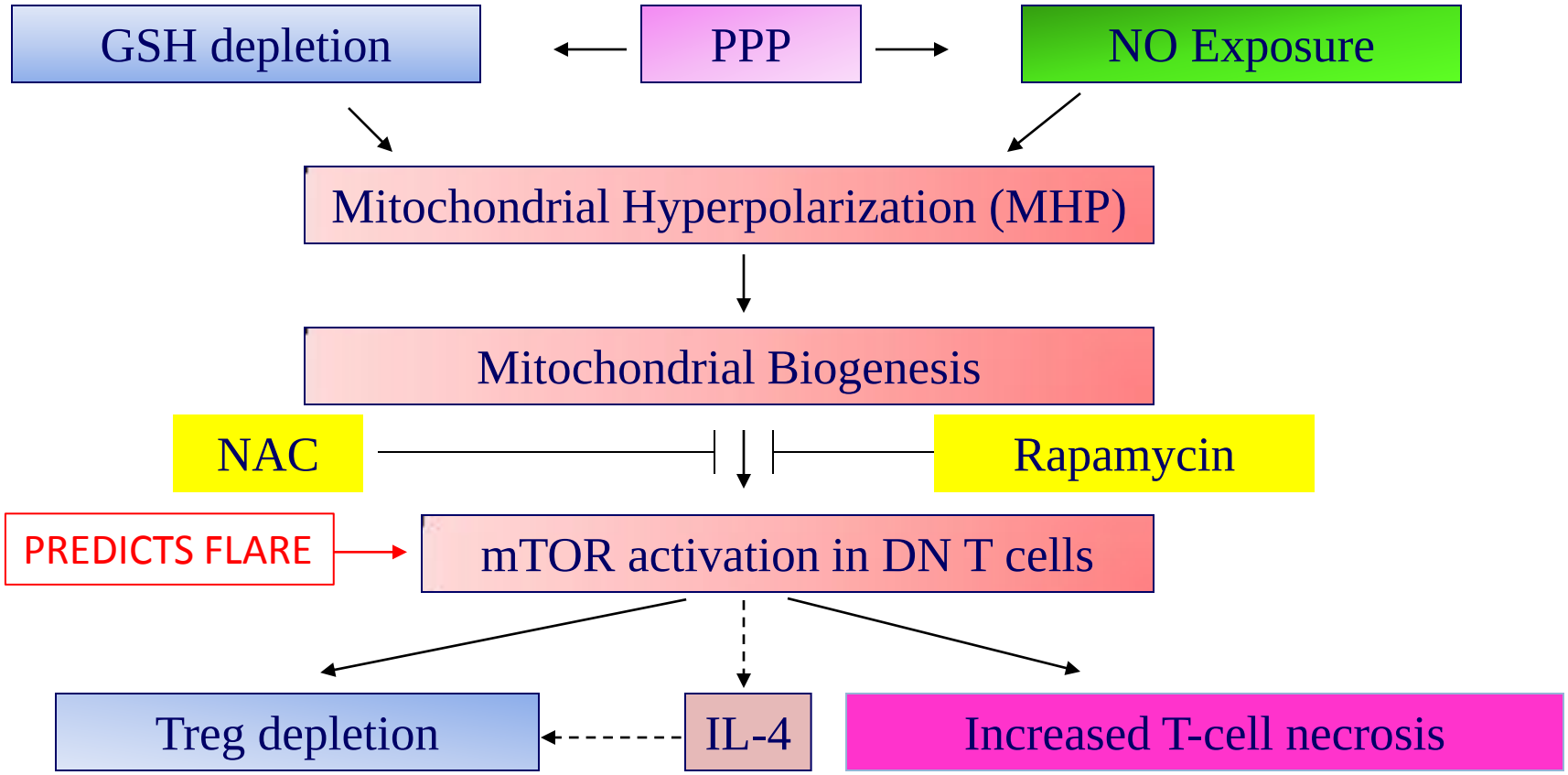
GSH depletion
PPP
NO Exposure
Mitochondrial Hyperpolarization (MHP)
Mitochondrial Biogenesis
NAC
Rapamycin
PREDICTS FLARE
mTOR activation in DN T cells
Treg depletion
IL-4
Increased T-cell necrosis

Table S1. Correlation of SLEDAI, BILAG and traditional biomarkers of disease activity, such as C3, C4, and anti-DNA, with 212 metabolic, cell surface, and gene expression biomarkers during 274 visits of 59 patients with SLE. Correlation r values were considered significant at p < 0.000236 when corrected for multiple comparisons (0.05/212). Metabolic biomarkers with correlation p values below 0.000236 relative to at least one of the traditional disease activity measures are shown and highlighted in yellow (SLEDAI, BILAG, C3, C4 or anti-DNA).

| | | Control | | | | | CD3/CD28 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SLEDAI | BILAG | C3 | C4 | dsDNA | SLEDAI | BILAG | C3 | C4 | dsDNA |
| SLEDAI | r | 1 | .450** | -.225** | -.237** | .237** | 1 | .450** | -.225** | -.237** | .237** |
| | p | | 1.44E-14 | 0.00037 | 0.00016 | 0.000389 | | 1.4435E-14 | 0.00036613 | 0.00016255 | 0.00038856 |
| BILAG | r | .450** | 1 | -0.09639 | 0.04342 | -0.08459 | .450** | 1 | -0.0963855 | 0.04341883 | -0.0845858 |
| | p | 1.44E-14 | | 0.13009 | 0.49611 | 0.211408 | 1.4435E-14 | | 0.13009224 | 0.49610747 | 0.21140763 |
| Fatigue | r | .259** | .526** | 0.04056 | 0.04804 | 0.100503 | .259** | .526** | 0.04055995 | 0.04804079 | 0.10050287 |
| | p | 3.47E-05 | 4.07E-19 | 0.53787 | 0.46552 | 0.148627 | 3.4696E-05 | 4.066E-19 | 0.53786505 | 0.46551945 | 0.14862713 |
| C3 | r | -.225** | -0.09639 | 1 | .695** | -0.02222 | -.225** | -0.0963855 | 1 | .695** | -0.0222175 |
| | p | 0.000366 | 0.130092 | | 1.4E-38 | 0.738626 | 0.00036613 | 0.13009224 | | 1.4413E-38 | 0.73862551 |
| C4 | r | -.237** | 0.043419 | .695** | 1 | -.218** | -.237** | 0.04341883 | .695** | 1 | -.218** |
| | p | 0.000163 | 0.496107 | 1.4E-38 | | 0.000928 | 0.00016255 | 0.49610747 | 1.4413E-38 | | 0.00092768 |
| dsDNA | r | .237** | -0.08459 | -0.02222 | -.218** | 1 | .237** | -0.0845858 | -0.0222175 | -.218** | 1 |
| | p | 0.000389 | 0.211408 | 0.73863 | 0.00093 | | 0.00038856 | 0.21140763 | 0.73862551 | 0.00092768 | |
| % of CD3+4+ | r | -0.09325 | 0.105709 | .200** | .268** | .130* | -0.0965875 | .126* | .174** | .205** | 0.10245647 |
| | p | 0.130719 | 0.086488 | 0.00125 | 1.3E-05 | 0.049432 | 0.11744767 | 0.0413111 | 0.00506413 | 0.00094877 | 0.1229235 |
| % of CD3+8+ | r | 0.068604 | -0.09723 | -.166** | -.234** | -0.11587 | 0.05188901 | -.163** | -.207** | -.235** | -0.0501914 |
| | p | 0.266699 | 0.115014 | 0.00754 | 0.00014 | 0.080835 | 0.40109807 | 0.00794062 | 0.00082107 | 0.00013994 | 0.45073937 |
| % necrotic CD4 | r | .260** | 0.083098 | -0.02779 | -0.0884 | 0.070758 | .154* | -0.0178143 | 0.02734735 | -0.0270895 | -0.012118 |
| | p | 2.11E-05 | 0.181625 | 0.65931 | 0.16027 | 0.291698 | 0.01278029 | 0.77496591 | 0.66445079 | 0.66742483 | 0.8568715 |
| % necrotic CD3+4-8- | r | .230** | -0.05089 | -0.08371 | -.154* | 0.01234 | .172** | -0.0468435 | -0.0317325 | -0.0605795 | -0.0277665 |
| | p | 0.000189 | 0.413877 | 0.18359 | 0.01413 | 0.85428 | 0.00543706 | 0.45198959 | 0.61470734 | 0.33625066 | 0.67936773 |
| % of DiOC6hi in CD3+4-8- | r | 0.081637 | -.163* | -.258** | -.295** | .380** | .264** | -0.1039187 | -.351** | -.317** | .333** |
| | p | 0.261558 | 0.024276 | 0.00034 | 3.7E-05 | 3.27E-07 | 0.00022229 | 0.15253502 | 7.3754E-07 | 8.6659E-06 | 9.0321E-06 |
| % of TMRMhi in CD3+4-8- | r | 0.094012 | -0.10268 | -0.11906 | -.316** | .410** | 0.11628759 | -0.0691745 | -.204** | -.229** | .223** |
| | p | 0.129805 | 0.097881 | 0.05761 | 2.6E-07 | 1.65E-10 | 0.06065039 | 0.26548554 | 0.00106337 | 0.00022951 | 0.00075958 |
| MFI of TMRM in CD3+4-8- | r | .212** | -0.02337 | -0.07175 | -.198** | .517** | 0.0803612 | -0.0827026 | -0.0593338 | -0.0946372 | .345** |
| | p | 0.00057 | 0.707079 | 0.25364 | 0.00152 | 9.18E-17 | 0.19561796 | 0.18286983 | 0.34534266 | 0.13176065 | 1.1421E-07 |
| % of MTGhi in CD3+4-8- | r | .254** | -0.04204 | -.131* | -.264** | .284** | .209** | -0.0821765 | -.179** | -.184** | .200** |
| | p | 3.38E-05 | 0.49887 | 0.03696 | 2E-05 | 1.52E-05 | 0.00067284 | 0.18567926 | 0.00420747 | 0.00316 | 0.00256797 |
| % of MTGhi in CD19 | r | 0.116557 | -0.06701 | -.201** | -.274** | 0.037589 | -0.0397807 | 0.00469161 | 0.01178075 | 0.00395278 | -.182** |
| | p | 0.060052 | 0.28075 | 0.00127 | 8.7E-06 | 0.574874 | 0.5222734 | 0.93987115 | 0.85149938 | 0.94991709 | 0.00617236 |
| MFI of MTG in CD3+4-8- | r | .254** | 0.019403 | -.142* | -.262** | .357** | .251** | -0.001955 | -0.1055879 | -0.1018803 | .194** |
| | p | 3.29E-05 | 0.755052 | 0.02315 | 2.3E-05 | 3.64E-08 | 4.0209E-05 | 0.97492432 | 0.09246684 | 0.10456147 | 0.00341415 |
| MFI of NAO in CD3+4-8- | r | 0.044737 | -0.04747 | -.148* | -.179** | .200** | 0.03484502 | 0.01258003 | -0.0387914 | -0.093939 | .303** |
| | p | 0.472607 | 0.445969 | 0.01804 | 0.0043 | 0.002658 | 0.57594216 | 0.84001248 | 0.53828007 | 0.13542141 | 3.9918E-06 |
| MFI of DCF-DA in CD3 | r | .163* | 0.100307 | -.249** | -.235** | .204** | 0.01815976 | 0.06602428 | -0.1013264 | -0.0526361 | 0.01785935 |
| | p | 0.010283 | 0.115111 | 8.8E-05 | 0.00022 | 0.002846 | 0.77553853 | 0.29939298 | 0.11515616 | 0.41401372 | 0.7950564 |
| MFI of DCF-DA in CD19 | r | 0.105885 | 0.036034 | -.233** | -.257** | 0.100221 | 0.04540808 | -0.0370292 | -0.1160126 | -.164* | 0.07248971 |
| | p | 0.096165 | 0.572219 | 0.00026 | 5.2E-05 | 0.144913 | 0.47566628 | 0.56085575 | 0.07103821 | 0.01053025 | 0.29114571 |
| MFI of DCF-DA in CD8 | r | .218** | 0.100364 | -.294** | -.239** | .300** | .130* | 0.03139489 | -.154* | -0.1106929 | .150* |
| | p | 0.000548 | 0.114905 | 3.3E-06 | 0.00017 | 8.44E-06 | 0.04001774 | 0.62199009 | 0.01604681 | 0.08508211 | 0.0278454 |
| % of FoxP3+CD25- in CD3 | r | -0.04377 | 0.020166 | -0.01088 | -0.0683 | -0.0366 | 0.08046113 | -0.0995183 | -.167** | -.253** | .207** |
| | p | 0.478887 | 0.744317 | 0.86221 | 0.27536 | 0.583314 | 0.19248974 | 0.10667605 | 0.00731545 | 4.204E-05 | 0.00172607 |
| % of FoxP3+CD25- in CD3+4+ | r | -0.00901 | 0.028463 | -0.04365 | -0.1118 | -0.05238 | .135* | -0.0899322 | -.243** | -.341** | 0.1174016 |
| | p | 0.88417 | 0.645251 | 0.48601 | 0.07348 | 0.432225 | 0.02831197 | 0.14504847 | 8.4393E-05 | 2.1333E-08 | 0.07753391 |
| % of FoxP3-CD25- in CD3+4+ | r | 0.013428 | 0.067212 | .142* | .244** | 0.039234 | 0.05935871 | -0.0857923 | -0.0758339 | -0.0827995 | .134* |
| | p | 0.828085 | 0.276544 | 0.02325 | 7.8E-05 | 0.556474 | 0.33668506 | 0.1645567 | 0.225693 | 0.18577593 | 0.04364552 |
| % of FoxP3-CD25+ in CD3+4+ | r | 0.075245 | -0.11603 | -.165** | -.242** | 0.056307 | 0.0147916 | .229** | 0.07282353 | .205** | -.215** |
| | p | 0.223028 | 0.059733 | 0.00814 | 9.2E-05 | 0.398483 | 0.81094453 | 0.00017064 | 0.24470608 | 0.00095395 | 0.00109917 |
| % of FoxP3-CD25- in CD19 | r | 0.096938 | -0.01014 | -0.00904 | -0.0998 | -0.05039 | .214** | -0.1065926 | -.277** | -.377** | .268** |
| | p | 0.116118 | 0.869762 | 0.88529 | 0.11045 | 0.449949 | 0.00046653 | 0.0838744 | 6.6652E-06 | 4.1433E-10 | 4.3483E-05 |
| % of FoxP3-CD25+ in CD19 | r | -0.09863 | 0.008408 | 0.01516 | 0.10442 | 0.05291 | -.215** | 0.10413914 | .281** | .378** | -.265** |
| | p | 0.109843 | 0.891852 | 0.80893 | 0.09483 | 0.427588 | 0.00043393 | 0.09129116 | 4.5902E-06 | 3.661E-10 | 5.1122E-05 |
| % of FoxP3+CD25+ in CD8 | r | -.217** | -0.08311 | 0.07263 | .127* | -0.11678 | -.319** | 0.03638883 | .218** | .257** | -.239** |
| | p | 0.000386 | 0.178192 | 0.24596 | 0.04182 | 0.079135 | 1.188E-07 | 0.55610419 | 0.00041799 | 3.0185E-05 | 0.00027314 |
| % of CD25+ in CD4+FoxP3+ | r | -0.08469 | 0.014801 | .142* | .150* | 0.055351 | -.229** | 0.09081513 | .244** | .281** | -.209** |
| | p | 0.170085 | 0.810822 | 0.02295 | 0.01635 | 0.406551 | 0.00017746 | 0.1411257 | 7.8408E-05 | 4.7198E-06 | 0.00150622 |