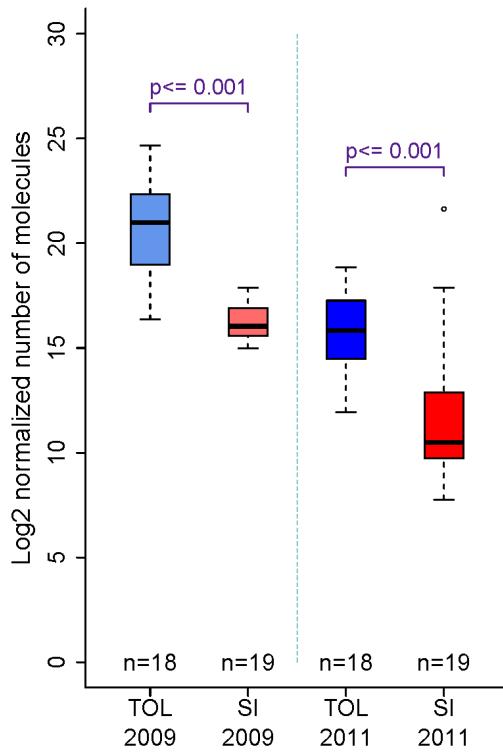
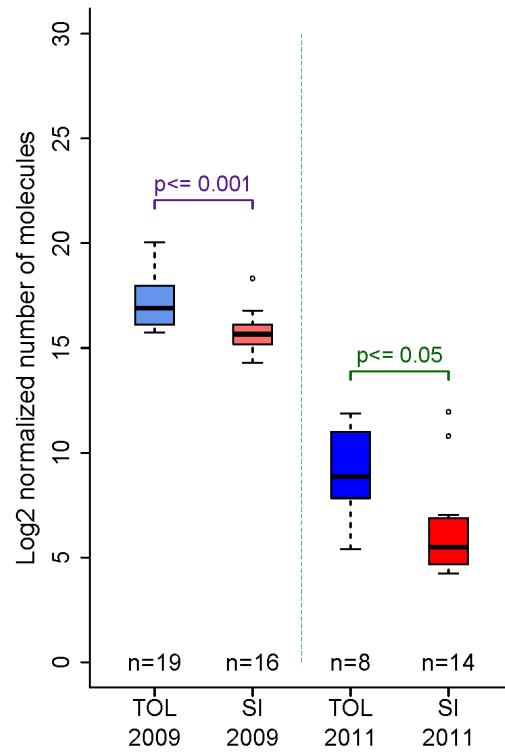


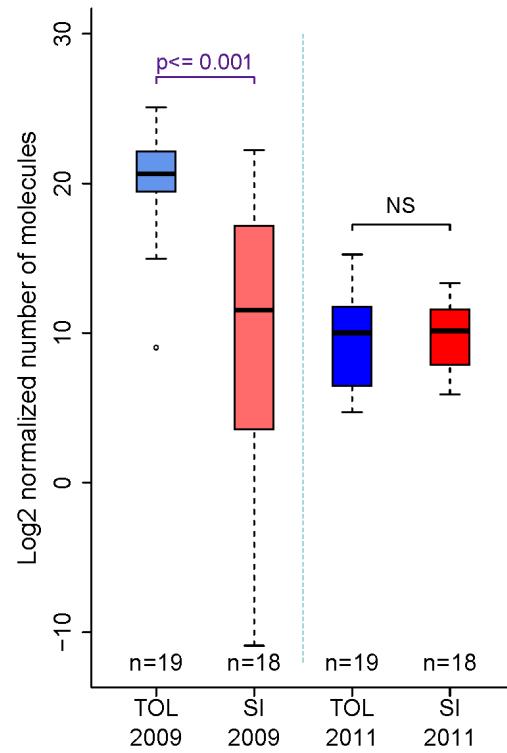
IGKV1D-13



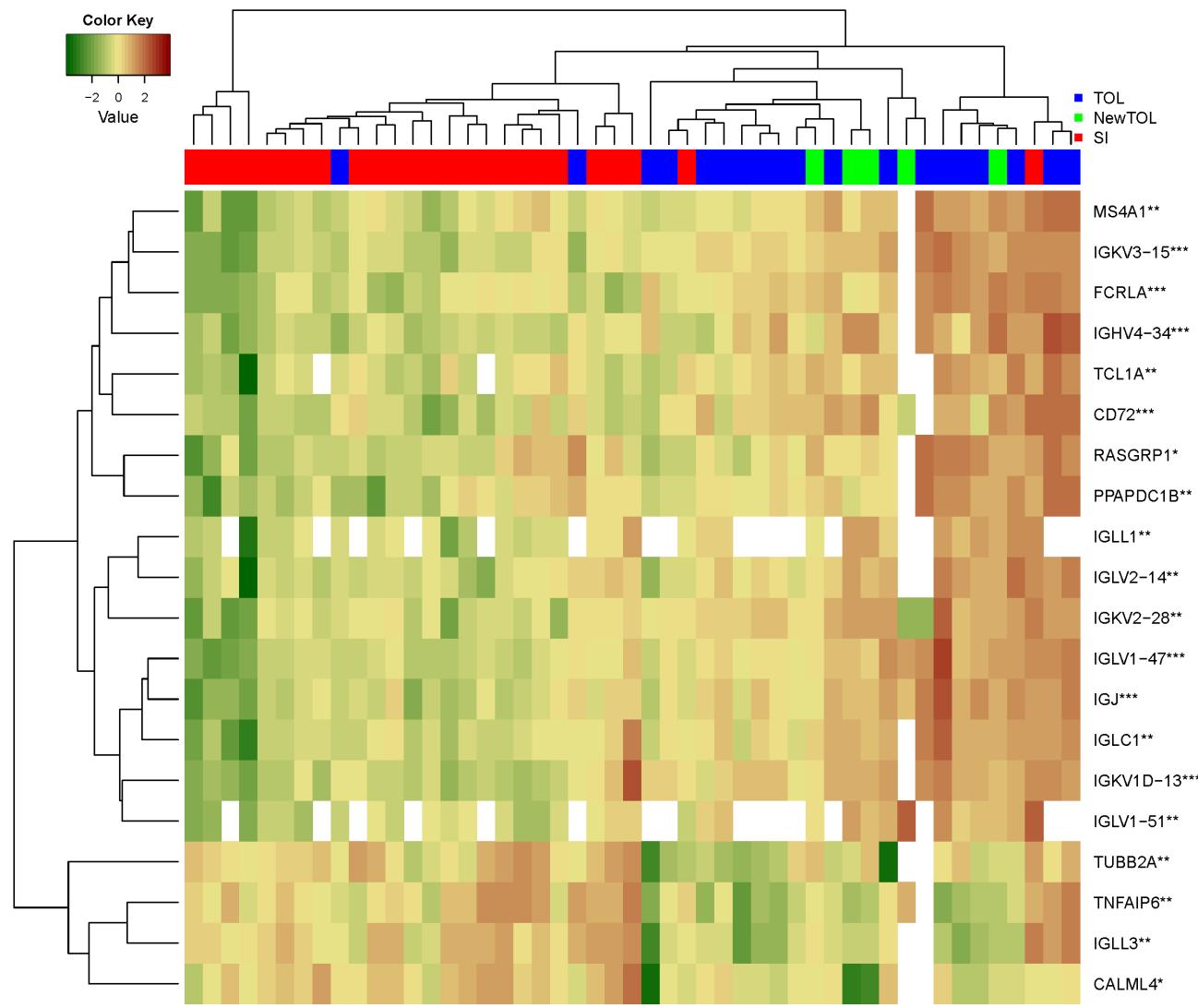
IGLL-1



IGKV4-1



Supplemental Figure S1: Technical reproducibility of Sequenom analysis. The same RNA samples were tested in 2009 and 2011 using Sequenom technology. Fresh reagents were used in 2011, but the same 226 gene multiplex structure was maintained. The difference between TOL and SI samples is clearly reproducible for both IGKV1D-13 and IGLL-1. However, the absolute values are not maintained. https://www.itntrialshare.org/FACTOR_figS1.url



Supplemental Figure 2: Hierarchical clustering using Euclidean distance measure showing the top 20 genes differentially expressed between tolerant, new tolerant and standard immunosuppression participants by t-test with False Discovery Rate (FDR) correction. (* = p-value < 0.05, ** = p-value < 0.005, *** = p-value < 0.001). White or blank cells show values not available due to assay limitations. Fifteen of the 20 genes are B cell specific, the exceptions being RASGRP1, PPAPDC1B, TUBB2A, TNFAIP6, and CALML4.

https://www.itntrialshare.org/FACTOR_figS2.url

Supplemental Table 1

ITN507, ITN013, ITN010, & ITN036 participant specimens analyzed for gene expression, flow cytometry, and BAFF levels analysis

ITN507

| Participant | Gene Expression | Flow Cytometry | BAFF Levels |
|---------------------|------------------------|-----------------------|--------------------|
| <i>Tolerant</i> | | | |
| FACTOR_551339 | G | | B |
| FACTOR_841311 | G | F | B |
| FACTOR_150776 | G | F | B |
| FACTOR_944735 | | F | B |
| FACTOR_191757 | | F | B |
| FACTOR_274363 | | F | B |
| FACTOR_712246 | G | | B |
| FACTOR_919105 | G | | B |
| FACTOR_681387 | G | F | B |
| FACTOR_948987 | G | F | B |
| FACTOR_987079 | G | F | B |
| FACTOR_808379 | G | F | B |
| FACTOR_701789 | G | F | |
| FACTOR_402941 | G | F | |
| FACTOR_579210 | | F | |
| FACTOR_911411 | G | F | B |
| FACTOR_909034 | G | F | B |
| FACTOR_231375 | G | F | B |
| FACTOR_679149 | G | F | |
| FACTOR_157168 | | F | |
| FACTOR_118518 | G | F | |
| FACTOR_238870 | | | B |
| FACTOR_974351 | G | F | B |
| FACTOR_208304 | G | F | B |
| FACTOR_541582 | G | F | B |
| <i>New Tolerant</i> | | | |
| FACTOR_882447 | G | F | B |
| FACTOR_833683 | G | F | B |
| FACTOR_465810 | G | F | B |
| FACTOR_645626 | G | F | B |
| FACTOR_243751 | G | F | B |
| FACTOR_377176 | | F | B |
| FACTOR_690732 | | | B |

ITN507

| Participant | Gene Expression | Flow Cytometry | BAFF Levels |
|-----------------------------------|-----------------|----------------|-------------|
| <u>Standard Immunosuppression</u> | | | |
| FACTOR_662711 | G | F | B |
| FACTOR_553478 | G | | B |
| FACTOR_583976 | G | F | B |
| FACTOR_143335 | G | F | B |
| FACTOR_532410 | | F | B |
| FACTOR_743013 | G | | B |
| FACTOR_445323 | G | F | B |
| FACTOR_908887 | G | | |
| FACTOR_505880 | G | F | |
| FACTOR_671619 | G | F | |
| FACTOR_249795 | G | F | |
| FACTOR_212280 | | F | |
| FACTOR_762897 | G | F | B |
| FACTOR_193782 | G | F | |
| FACTOR_109622 | | F | B |
| FACTOR_213935 | | F | |
| FACTOR_278451 | G | F | B |
| FACTOR_245217 | G | | B |
| FACTOR_418828 | | F | B |
| FACTOR_405881 | G | F | B |
| FACTOR_700990 | G | F | |
| FACTOR_982795 | | F | |
| FACTOR_936912 | | F | |
| FACTOR_930968 | G | F | |
| FACTOR_930960 | | F | |
| FACTOR_850120 | G | | |
| FACTOR_439639 | G | F | |
| FACTOR_486391 | G | | |
| FACTOR_553535 | G | | |
| FACTOR_884046 | G | F | B |
| FACTOR_573541 | G | | B |
| FACTOR_853380 | G | F | |
| FACTOR_205722 | | F | B |
| FACTOR_432603 | G | F | B |

ITN013

| Participant | Gene Expression |
|--|-----------------|
| <i>Sirolimus Monotherapy</i> | |
| ITN013ST_877664 | G |
| ITN013ST_597519 | G |
| ITN013ST_187319 | G |
| ITN013ST_371019 | G |
| ITN013ST_307165 | G |
| ITN013ST_805828 | G |
| ITN013ST_561464 | G |
| <i>Standard Immunosuppression Multiagent</i> | |
| ITN013ST_145787 | G |
| ITN013ST_492821 | G |

ITN010 & ITN036

| Participant | Gene Expression |
|---|-----------------|
| <i>Tolerant</i> | |
| ITN036ST_968123 | G |
| ITN036ST_456317 | G |
| ITN036ST_464831 | G |
| ITN010ST_14804 | G |
| ITN010ST_564051 | G |
| ITN010ST_687298 | G |
| ITN010ST_93464 | G |
| <i>Return to Standard Immunosuppression</i> | |
| ITN036ST_264166 | G |

Assay Combination Legend:

- (G) Gene Expression
- (F) Flow Cytometry Analysis
- (B) BAFF Levels

Supplemental Table 2

232 genes of interest tested by gene expression (including six housekeeping genes)

| Gene No. | Multiplex Gene ID | | | | | | | | | | | |
|----------|-------------------|----------|----------|------------|-----------|-------------|------------|----------|----------|----------|----------|----------|
| | W1 | W2 | W3 | W4 | W5 | W6 | W7 | W8 | W9 | W10 | W11 | W12 |
| 1 | PLOD3 | IGLL1 | AKR1C3 | MRPS31 | IGHV4-34 | TSPAN5 | EIF5AP1 | IGLV1-47 | TBC1D5 | IL23A | PLDN | TUBB3 |
| 2 | SMAD5 | BCL2L1 | CD55 | IGF2BP2 | BLNK | KV105_HUMAN | MAFG | SWAP70 | HLA-C | ZNF331 | RAD21 | IGKV3-20 |
| 3 | TGM2 | IGLV1-51 | BCL11A | MGC33894 | GAS7 | RBPMS | TBB3_HUMAN | FCRLA | TAOK2 | FAM129C | PLAUR | TNF |
| 4 | BMP7_HUMAN | IL10 | BLM | MATK | FOXO1 | IGKC | RAP2A | CYP1B1 | RAD52 | IGHG2 | KLRC1 | TRDC |
| 5 | LOXL2 | TGFBR1 | BACH2 | MSL-1 | IL8 | STAP1 | PHCA | IGKV1-27 | SHMT2 | BIRC3 | PCDHGC5 | GPBAR1 |
| 6 | LOXL1 | TGFB2 | BBX | IKZF3 | CXXC5 | IGKV2-28 | STRBP | EIF5B | IGKV2-24 | DHX9 | HLA-C_2 | VPREB3 |
| 7 | PLOD1 | TGFB1 | BTLA | IL-1 | NFATC4 | SPTB | PML | PPAPDC1B | HLA-DOB | DDHD2 | F3 | |
| 8 | SMAD6 | HAVCR2 | BTLA_2 | MLTK_HUMAN | ARID5B | LRRFIP1 | SDC4 | MS4A1 | SLC12A8 | IGKV1-12 | WDR67 | |
| 9 | PDGFA | IL6 | BCL11A_2 | MIB1 | FZD4 | AFF3 | RELB | RASGRP1 | TCL1A | RAD50 | IGHD | |
| 10 | EDN1 | CD3E | FNBP2 | CD38 | UPP1 | SFRP2 | RHOH | IGKV3-15 | ROCK1 | Klrk1 | PPP3CA | |
| 11 | SMAD7 | TGFBR3 | CXCL3 | RPL14 | KCNJ2 | USP34 | SLAMF7 | GPR114 | DEFA4 | CALML4 | ICAM1 | |
| 12 | CTGF | PECAM1 | EBI2 | NFAT5 | PDE4B | PPAPDC1B_2 | IGLL3 | KIAA0746 | SLC2A3 | DYHC | IGHM | |
| 13 | LOX | TGFB3 | CDK6 | MEF2C | PTCH1 | CD72 | SESTD | SON | TSPAN3 | KLRB1 | YWHAE | |
| 14 | FGF2 | IFNG | CXCL4 | PALM2 | KLRC2 | CXCL1 | RPS6 | C9orf45 | TRA2A | IRF-4 | UBC | |
| 15 | HGF | TBX21 | BMP6 | NBPF16 | ZBTB10 | GFOD2 | RAB38 | IGKV4-1 | USP10 | ESPN | OR13C4 | |
| 16 | SPG3A | TGFBR2 | CD79B | NFKB2 | CUGBP1 | CXCL2 | SMURF1 | IGLC1 | IGLV2-14 | PPP1R12A | NR4A3 | |
| 17 | SMAD3 | HAVCR1 | KLRD1 | P2RX5 | RAB5C | TPD52 | TUBB2A | ADARB1 | CD160 | MS4A1_2 | ZNF295 | |
| 18 | PLOD2 | CD40 | CXorf38 | SPEN | IGKV1D-13 | CEP350 | TSPAN13 | ATRX | GNA11 | SF3B1 | IGHA1 | |
| 19 | SMAD1 | SPIB | EIF2S1 | ZNF267 | JAK1 | NKG2F_HUMAN | SH2D1B | IGJ | TUBB4 | RAB2A | POLR3H | |
| 20 | SMAD2 | IL2RA | FEZ1 | PTGDR | SYNPO2L | CCL20 | TNFAIP6 | SAMD9L | BACH2_2 | KLRC3 | IGKV1-33 | |

Housekeeping genes: GAPDH, UBC, HPTR1, TBP, B2M, YWHAZ

Supplemental Table 3

Intersection of statistically significant genes comparing SI vs. TOL in the current experiment and Newell KA et al., J Clin Invest. 2010

| Gene | Number of SI in current exp. | Number of TOL in current exp. | p-value in current exp. | Number of SI in Newell et al. 2010 | Number of TOL in Newell et al. 2010 | p-value in Newell et al. 2010 |
|-------------|------------------------------|-------------------------------|-------------------------|------------------------------------|-------------------------------------|-------------------------------|
| ADARB1 | 24 | 19 | 0.0127 | 23 | 19 | 0.0125 |
| ARID5B | 24 | 19 | 0.0013 | 23 | 18 | 0.0109 |
| BACH2_2 | 24 | 19 | 0.0310 | 24 | 18 | 0.0129 |
| BLNK | 24 | 19 | 0.0079 | 23 | 19 | 0.0193 |
| EBI2 | 24 | 19 | 0.0160 | 24 | 18 | 0.0087 |
| FCRLA | 24 | 19 | <0.001 | 22 | 18 | <0.001 |
| FOXO1 | 24 | 19 | 0.0041 | 24 | 19 | 0.0097 |
| IGHA1 | 24 | 18 | 0.0173 | 24 | 19 | 0.0018 |
| IGHD | 24 | 19 | 0.0211 | 20 | 19 | 0.0051 |
| IGHV4-34 | 24 | 19 | <0.001 | 24 | 19 | <0.001 |
| IGJ | 24 | 19 | <0.001 | 24 | 19 | <0.001 |
| IGKC | 24 | 19 | 0.0216 | 24 | 19 | <0.001 |
| IGKV1-27 | 23 | 19 | 0.0106 | 24 | 19 | 0.0023 |
| IGKV1D-13 | 24 | 19 | <0.001 | 19 | 18 | <0.001 |
| IGKV2-28 | 24 | 19 | <0.001 | 11 | 16 | 0.0181 |
| IGKV3-15 | 24 | 19 | <0.001 | 11 | 17 | 0.0219 |
| IGKV3-20 | 24 | 18 | 0.0025 | 24 | 19 | <0.001 |
| IGLC1 | 24 | 19 | <0.001 | 24 | 16 | 0.0014 |
| IGLL1 | 19 | 8 | 0.0011 | 16 | 19 | <0.001 |
| IGLV1-47 | 24 | 19 | <0.001 | 24 | 19 | 0.0136 |
| KIAA0746 | 24 | 19 | 0.0056 | 21 | 18 | 0.0102 |
| KV105_HUMAN | 24 | 19 | 0.0116 | 24 | 19 | <0.001 |
| MS4A1 | 24 | 19 | 0.0013 | 24 | 19 | <0.001 |
| MS4A1_2 | 24 | 18 | 0.0133 | 23 | 18 | <0.001 |
| PLAUR | 24 | 18 | 0.0121 | 24 | 19 | 0.0321 |
| PPAPDC1B | 24 | 19 | <0.001 | 22 | 19 | 0.0014 |
| PPAPDC1B_2 | 24 | 18 | 0.0141 | 19 | 17 | 0.0102 |
| PTCH1 | 24 | 19 | 0.0199 | 23 | 19 | 0.0269 |
| STAP1 | 24 | 18 | 0.0030 | 20 | 18 | 0.0042 |
| SWAP70 | 24 | 19 | 0.0043 | 24 | 19 | 0.0095 |