**Supplementary Table S1. Genes selected for multiplex qRT-PCR array**

|  |  |  |
| --- | --- | --- |
| **Gene Symbol** | **Gene Name** | **Public RefSeq** |
| ARG1 | arginase 1 | NM\_000045.3 |
| **CCL5** | **chemokine (C-C motif) ligand 5** | **NM\_002985.2** |
| CD1A | CD1a molecule | NM\_001763.2 |
| CD4 | CD4 molecule | NM\_000616.4 |
| CD8A | CD8a molecule | NM\_001768.6 |
| **CD74** | **CD74 molecule, major histocompatibility complex, class II invariant chain** | **NM\_004355.3** |
| CD79A | CD79a molecule, immunoglobulin-associated alpha | NM\_001783.3 |
| CD83 | CD83 molecule | NM\_001251901.1 |
| **CD84** | **CD84 molecule** | **NM\_003874.3** |
| **CD163** | **CD163 molecule** | **NM\_203416.2** |
| CD274 | CD274 molecule (PD-L1) | NM\_014143.3 |
| **CXCL1** | **chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)** | **NM\_001511.2** |
| **CXCR4** | **chemokine (C-X-C motif) receptor 4** | **NM\_001008540.1** |
| EGR2 | early growth response 2 | NM\_000399.3 |
| FOXP3 | forkhead box P3 | NM\_014009.3 |
| **GNLY** | **granulysin** | **NM\_006433.3** |
| GZMB | granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) | NM\_004131.4 |
| **HLA-DRA** | **major histocompatibility complex, class II, DR alpha** | **NM\_019111.4** |
| ICOS | inducible T-cell co-stimulator | NM\_012092.3 |
| ICOSLG | inducible T-cell co-stimulator ligand | NM\_015259.4 |
| IDO1 | indoleamine 2,3-dioxygenase 1 | NM\_002164.5 |
| IFNA1 | interferon, alpha 1 | NM\_024013.1 |
| IFNG | interferon, gamma | NM\_000619.2 |
| IL1A | interleukin 1, alpha | NM\_000575.3 |
| IL1B | interleukin 1, beta | NM\_000576.2 |
| IL2 | interleukin 2 | NM\_000586.3 |
| IL4 | interleukin 4 | NM\_000589.3 |
| IL6 | interleukin 6 (interferon, beta 2) | NM\_000600.3 |
| IL6R | interleukin 6 receptor | NM\_000565.3 |
| IL10 | interleukin 10 | NM\_000572.2 |
| IL10RB | interleukin 10 receptor, beta | NM\_000628.3 |
| IL12A | interleukin 12A (natural killer cell stimulatory factor 1, p35) | NM\_000882.3 |
| IL13 | interleukin 13 | NM\_002188.2 |
| **IL15** | **interleukin 15** | **NM\_172175.2** |
| IL17A | interleukin 17A | NM\_002190.2 |
| IL17RC | interleukin 17 receptor C | NM\_032732.5 |
| IL18 | interleukin 18 (interferon-gamma-inducing factor) | NM\_001562.3 |
| IL21 | interleukin 21 | NM\_021803.3 |
| IL22 | interleukin 22 | NM\_020525.4 |
| IL22RA2 | interleukin 22 receptor, alpha 2 | NM\_181309.1 |
| IL23A | interleukin 23, alpha subunit p19 | NM\_016584.2 |
| IL23R | interleukin 23 receptor | NM\_144701.2 |
| **IL32** | **interleukin 32** | **NM\_004221.4** |
| ITGAL | integrin, alpha L, antigen CD11A (p180) | NM\_002209.2 |
| LAG3 | lymphocyte-activation gene 3 | NM\_002286.5 |
| **LYZ** | **lysozyme** | **NM\_000239.2** |
| **P2RX1** | **purinergic receptor P2X, ligand-gated ion channel, 1** | **NM\_002558.2** |
| PDCD1 | programmed cell death 1 (PD-1) | NM\_005018.2 |
| **PRF1** | **perforin 1 (pore forming protein)** | **NM\_005041.4** |
| PTGS2 | prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | NM\_000963.2 |
| PTPRC | protein tyrosine phosphatase, receptor type, C, CD45 | NM\_002838.4 |
| **PTPRCAP** | **protein tyrosine phosphatase, receptor type, C-associated protein** | **NM\_005608.2** |
| **S100A9** | **S100 calcium binding protein A9** | **NM\_002965.3** |
| SOCS1 | suppressor of cytokine signaling 1 | NM\_003745.1 |
| SOCS3 | suppressor of cytokine signaling 3 | NM\_003955.3 |
| TBX21 | T-box 21 | NM\_013351.1 |
| TGFB1 | transforming growth factor, beta 1 | NM\_000660.4 |
| TLR3 | toll-like receptor 3 | NM\_003265.2 |
| TLR4 | toll-like receptor 4 | NM\_138554.3 |
| TNF | tumor necrosis factor | NM\_000594.2 |
| VEGFA | vascular endothelial growth factor A | NM\_001025366.2 |

Genes in **COLOR** were chosen based on whole genome microarray results. Others were selected as candidate genes from the literature. Public RefSeq and approved gene symbols were obtained from HUGO Gene Nomenclature Committee website: <http://www.genenames.org/>. Genes used as expression controls (not listed) included GUSB, 18S, and ACTB.

**Supplementary Table S3.** **Functionally annotated gene categories from DAVID analysis of whole genome microarray results comparing PD-L1+ to PD-L1(-) melanomas.** Shown are functional categories with at least 10 distinct member genes recognized from microarray results, and a Benjamini-Hochberg adjusted p-value (FDR) <0.015.

|  |  |  |  |
| --- | --- | --- | --- |
| **Functionally-related gene categorya** | **No. genes in submitted list/total genes**  **in category (%)** | **p-valueb** | **Benjamini adjusted**  **p-value** |
| Heterodimer | 28/103 (27.2) | 8.95E-09 | 1.91E-06 |
| Antigen processing and presentation | 24/83 (28.9) | 1.52E-06 | 2.63E-04 |
| Positive regulation of immune system process | 46/238 (19.3) | 1.68E-07 | 2.96E-04 |
| Defense response | 89/615 (14.5) | 3.63E-07 | 4.27E-04 |
| Positive regulation of immune response | 32/145 (22.1) | 9.33E-07 | 8.21E-04 |
| Type I diabetes mellitus | 14/42 (33.3) | 8.60E-05 | 3.71E-03 |
| Allograft rejection | 13/36 (36.1) | 7.16E-05 | 4.12E-03 |
| Inflammatory response | 51/325 (15.7) | 1.87E-05 | 7.30E-03 |
| Hemopoietic or lymphoid organ development | 43/260 (16.5) | 2.68E-05 | 7.85E-03 |
| Hemopoiesis | 40/236 (16.9) | 3.06E-05 | 8.25E-03 |
| Leukocyte activation | 40/242 (16.5) | 5.43E-05 | 1.12E-02 |
| Cell activation | 45/287 (15.7) | 6.35E-05 | 1.23E-02 |

a Per DAVID web tool (<http://david.abcc.ncifcrf.gov/>) (Huang DW, Nat Protoc 2009; Huang DW Nucleic Acids Res 2009).

bDAVID adjustment of the Fisher exact test (hypergeometric distribution) p-value..

**Supplementary Table S5. Genes over-expressed in PD-L1+ vs. PD-L1(-) melanomas, assessed by qRT-PCR.** Shown are genes with ≥2 fold up-regulation in PD-L1+ specimens and p-value ≤ 0.1 when normalized to GUSB or PTPRC (CD45).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **GUSB** | | **PTPRC** | |
| **Gene Symbola** | **Protein Function** | **Fold-change** | **p-valueb** | **Fold-change** | **p-valueb** |
| CCL5 | RANTES, chemotactic for T cells | NA | NS | 2.6 | 0.038 |
| CD8A | Co-receptor for TCR, predominantly expressed on CTL | NA | NS | 2.4 | 0.062 |
| CD84 | Immunoglobulin receptor family that enhances  IFN-g secretion | 2.1 | 0.102 | NA | NS |
| CD163 | Scavenger receptor, marks monocytes and macrophages | 2.5 | 0.040 | NA | NS |
| CD274 | PD-L1, a ligand for PD-1, which is expressed on inflammatory cells and some cancers | 4.8 | 0.100 | 4.4 | 0.067 |
| CXCL1 | Chemokine ligand secreted by melanoma cells and expressed by macrophages, neutrophils, and epithelial cells | 8.2 | 0.030 | 8.3 | 0.046 |
| IFNG | Critical cytokine for adaptive and innate immunity, potent activator of macrophages | 9.2 | 0.005 | 11.3 | 0.087 |
| IL10 | Anti-inflammatory cytokine that down-regulates Th1 cytokines, MHC class II, and co-stimulatory molecules | 3.5 | 0.056 | 3.3 | 0.032 |
| IL10RB | Essential accessory chain for the IL-10 receptor complex | 2.0 | 0.026 | NA | NS |
| IL18 | Pro-inflammatory cytokine that contributes to IFN-g release | 2.4 | 0.088 | 2.3 | 0.128 |
| IL21 | Cytokine that supports activation of T, B, and NK cells | NA | NS | 4.1 | 0.092 |
| LAG3 | Immunoglobulin superfamily member involved in the regulation of T-cell tolerance | 10.7 | 0.010 | 10.7 | 0.042 |
| LYZ | Innate immune enzyme with antimicrobial properties | 2.3 | 0.069 | 2.1 | 0.006 |
| PDCD1 | PD-1, immune checkpoint receptor that negatively regulates T cell responses | 8.2 | 0.049 | 7.3 | 0.011 |
| PRF1 | Cytolytic protein found in granules of CTL and NK cells | 3.3 | 0.085 | 3.1 | 0.027 |
| TLR3 | Member of Toll-like receptor family of pattern recognition receptors of innate immune system | 5.1 | 0.047 | 4.6 | 0.026 |

a Obtained from HUGO Gene Nomenclature Committee website: <http://www.genenames.org/>.

bData were analyzed with Expression Suite Software (v. 1.0.4, Life Technologies) using the comparative Ct method (Ct), normalized to either GUSB or PTPRC (CD45). A 2-tailed, unpaired Student’s t-test was used to determine the statistical significance of fold-change values.

Abbreviations: CTL, cytotoxic T lymphocyte; NA, not applicable; NK, natural killer cell; NS, not significant.