**SV-BR-1-GM, a Clinically Effective GM-CSF-Secreting Breast Cancer Cell Line, Expresses an Immune Signature and Directly Activates CD4+ T Lymphocytes**

**Supplementary Data Sheet 2:**

**SUPPLEMENTARY MATERIALS AND METHODS**

Quantitative RT-PCR for validation of gene expression

**Table S1. Quantitative RT-PCR TaqMan® reagents.** Validation of gene expression on a subset of samples by quantitative RT-PCR was conducted on an ABI 7900HT real-time PCR instrument at the University of Minnesota Genomics Center using the commercially available TaqMan® assays (Thermo Fisher Scientific, Waltham, MA) listed.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene Symbols | Assay Identifiers | Amplicon Lengths | Probes exon spanning? | RefSeqs (NCBI) |
| CUL1 | Hs01117001\_m1 | 113 | Yes | NM\_003592.2 |
| HLA-DRA | Hs00219578\_m1 | 129 | Yes | NM\_019111.4 |
| HLA-DRB3 | Hs02339733\_m1 | 74 | Yes | NM\_022555.3 |
| HLA-DMA | Hs00185435\_m1 | 100 | Yes | NM\_006120.3 |
| HLA-DMB | Hs00157943\_m1 | 148 | Yes | NM\_002118.4 |
| CD74 | Hs00269961\_m1 | 102 | Yes | NM\_001025158.2 NM\_001025159.2  NM\_004355.3 |

**Table S2. Samples for quantitative RT-PCR.** Validation of gene expression on a subset of samples by quantitative RT-PCR was conducted at the University of Minnesota Genomics Center (MN) using the samples listed. RIN, RNA Integrity Number; OD, Optical Density.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Samples | RIN | [RNA] [ng/l] | OD 260/280 | OD 260/230 |
| MCB cryo | 7.5 | 587.1 | 1.96 | 1.97 |
| CP Lot IV cryo | 6.9 | 935.1 | 2.06 | 1.9 |
| CP Lot VIII cryo | 10 | 108.1 | 1.84 | 2.23 |
| CP Lot VIII cryo | 9.9 | 107.7 | 1.89 | 1.91 |
| CP Lot IV culture | 9.9 | 514.4 | 2 | 1.91 |
| CP Lot VIII culture | 10 | 71.24 | 1.8 | 2.41 |

Gene expression by nCounter

**Table S3. nCounter CodeSets.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene Symbol (NCBI)** | **CodeSet Sequence** | **Isoforms Hit By Probe / Comments** | **Isoforms Not Hit By Probe** |
| **ADA** | TCCAAGAAGACCATGATCTCAATAGTCAGTTACTGATGCTCCTGAACCCTATGTGTCCATTTCTGCACACACGTATACCTCGGCATGGCCGCGTCACTTC | NM\_001322050.1;NM\_001322051.1;NM\_000022.3;NR\_136160.1 |  |
| **ADGRE5** | GAACCTGCATTCCAAGAAGCAAGCCGAACTGGAGGAGATATATGAAAGCAGCATCCGTGGTGTCCAACTCAGACGCCTCTCTGCCGTCAACTCCATCTTT | NM\_001784.4;NM\_078481.3;NM\_001025160.2;XM\_017027547.1;XM\_011528451.2 |  |
| **ADRM1** | AAGTGGACCTGGCCAGTGTGCTGACGCCGGAGATAATGGCTCCCATCCTCGCCAACGCGGATGTCCAGGAGCGCCTGCTTCCCTACTTGCCATCTGGGGA | NM\_001281438.1;XM\_017027610.1;NM\_001281437.1;XM\_017027607.1;XM\_005260257.1;XM\_017027608.1;NM\_007002.3;XM\_011528503.1;XM\_017027605.1;XM\_017027602.1;XM\_017027609.1;XM\_017027603.1;NM\_175573.2;XM\_017027604.1;XM\_017027606.1 |  |
| **APTX** | CCCAAAATGCAGGTTTACAAAGATGAGCAGGTGGTGGTGATAAAGGATAAATACCCAAAGGCCCGTTACCATTGGCTGGTCTTACCGTGGACCTCCATTT | XM\_017014833.1;XM\_011517939.2;XM\_017014834.1;XR\_929276.2;XM\_006716791.3;NR\_036576.1;NM\_001195248.1;XR\_929277.2;NM\_001195254.1;XM\_017014837.1;XR\_001746325.1;NM\_175073.2;NR\_036578.1;XM\_017014838.1;XM\_006716792.2;XR\_001746324.1;NM\_175069.2;XM\_017014835.1;XM\_017014832.1;XM\_017014831.1;XM\_017014836.1;NR\_036579.1;NM\_001195251.1;XM\_011517938.1;XR\_001746326.1;NR\_036577.1;NM\_001195250.1;NM\_001195249.1;NM\_001195252.1 | XR\_929279.1;XR\_428423.2 |
| **B2M** | CGGGCATTCCTGAAGCTGACAGCATTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCTATCCA | NM\_004048.2;XM\_005254549.3 |  |
| **CAV1** | AACCGCGACCCTAAACACCTCAACGATGACGTGGTCAAGATTGACTTTGAAGATGTGATTGCAGAACCAGAAGGGACACACAGTTTTGACGGCATTTGGA | NM\_001172897.1;NM\_001172896.1;NM\_001172895.1;NM\_001753.4 |  |
| **CD58** | GTGCTTGAGTCTCTTCCATCTCCCACACTAACTTGTGCATTGACTAATGGAAGCATTGAAGTCCAATGCATGATACCAGAGCATTACAACAGCCATCGAG | NM\_001779.2;XM\_017002869.1;NM\_001144822.1;NR\_026665.1 |  |
| **CD74** | TTCAGCCCCCAGCCCCTCCCCCATCTCCCACCCTGTACCTCATCCCATGAGACCCTGGTGCCTGGCTCTTTCGTCACCCTTGGACAAGACAAACCAAGTC | NM\_001025159.2;NM\_004355.3;XM\_017010089.1;XM\_017010090.1;NM\_001025158.2 |  |
| **CD83** | CTGTTCTTGAAGCAGTAGCCTAACACACTCCAAGATATGGACACACGGGAGCCGCTGGCAGAAGGGACTTCACGAAGTGTTGCATGGATGTTTTAGCCAT | NM\_001040280.1;NM\_001251901.1;NM\_004233.3 |  |
| **CSF2** | AGATGAGGCTGGCCAAGCCGGGGAGCTGCTCTCTCATGAAACAAGAGCTAGAAACTCAGGATGGTCATCTTGGAGGGACCAAGGGGTGGGCCACAGCCAT | NM\_000758.3 |  |
| **CXCL16** | CCATGGGTTCAGGAATTGATGAGCTGTCTTGATCTCAAAGAATGTGGACATGCTTACTCGGGGATTGTGGCCCACCAGAAGCATTTACTTCCTACCAGCC | NM\_001100812.1;NM\_022059.3 |  |
| **CXCL8** | ACAGCAGAGCACACAAGCTTCTAGGACAAGAGCCAGGAAGAAACCACCGGAAGGAACCATCTCACTGTGTGTAAACATGACTTCCAAGCTGGCCGTGGCT | NM\_000584.3 |  |
| **DGUOK** | TTGTAAAGAATCTGTAACCAATACCATGAAGTTCAGGCTGTGATCTGGGCTCCCTGACTTTCTGAAGCTAGAAAAATGTTGTGTCTCCCAACCACCTTTC | NR\_134896.1;NM\_080916.2;NR\_134898.1;NM\_001318863.1;NM\_080918.2;NM\_001318859.1;NR\_134894.1;NR\_134897.1;NM\_001318861.1;XM\_011532647.2;XR\_244926.3;NM\_001318860.1;NR\_134893.1;NR\_134895.1;XR\_001738656.1;NM\_001318862.1 |  |
| **GNG5** | ACGACCCACCGACCCACGAATCGGCCCGGCCGTCGCGTGCACCATGTCTGGCTCCTCCAGCGTCGCCGCTATGAAGAAAGTGGTTCAACAGCTCCGGCTG | NM\_005274.2 |  |
| **HLA-A** | AGGAGGGGCCGGAGTATTGGGACCAGGAGACACGGAATGTGAAGGCCCAGTCACAGACTGACCGAGTGGACCTGGGGACCCTGCGCGGCTACTACAACCA | Targets HLA-A\*11:01 | NM\_002116.7;NM\_001242758.1;XR\_430999.1;XM\_005275331.2;XM\_017030288.1 |
| **HLA-A** | AGGAGGGGCCGGAGTATTGGGACGAGGAGACAGGGAAAGTGAAGGCCCACTCACAGACTGACCGAGAGAACCTGCGGATCGCGCTCCGCTACTACAACCA | Targets HLA-A\*24:02 | NM\_002116.7;NM\_001242758.1;XR\_430999.1;XM\_005275331.2;XM\_017030288.1 |
| **HLA-B** | AGCCTGCGGAACCTGCGCGGCTACTACAACCAGAGCGAGGCCGGGTCTCACATCATCCAGAGGATGTATGGCTGCGACCTGGGGCCCGACGGGCGCCTCC | Targets HLA-B\*35:08; also targets HLA-A/C/E RefSeq transcripts at >91%. | XR\_926175.1;XM\_011514557.1;NM\_005514.7 |
| **HLA-B** | AGCCTGCGGAACCTGCGCGGCTACTACAACCAGAGCGAGGCCGGGTCTCACACTTGGCAGACGATGTATGGCTGCGACCTGGGGCCGGACGGGCGCCTCC | Targets HLA-B\*55:01; also targets HLA-A/C RefSeq transcripts at >90%. | XR\_926175.1;XM\_011514557.1;NM\_005514.7 |
| **HLA-DMA** | TTATTTGACAAAGAGTTCTGCGAGTGGATGATCCAGCAAATAGGGCCAAAACTTGATGGGAAAATCCCGGTGTCCAGAGGGTTTCCTATCGCTGAAGTGT | NM\_006120.3 |  |
| **HLA-DMB** | CCCGTGAGCTGGAAGGAACAGATTTAATATCTAGGGGCTGGGTATCCCCACATCACTCATTTGGGGGGTCAAGGGACCCGGGCAATATAGTATTCTGCTC | NM\_002118.4 |  |
| **HLA-DRA** | GGCCAACATAGCTGTGGACAAAGCCAACCTGGAAATCATGACAAAGCGCTCCAACTATACTCCGATCACCAATGTACCTCCAGAGGTAACTGTGCTCACG | NM\_019111.4 |  |
| **HLA-DRB1** | CCTATAACTTGGAATGTGGGTGGAGGGGTTCATAGTTCTCCCTGAGTGAGACTTGCCTGCTTCTCTGGCCCCTGGTCCTGTCCTGTTCTCCAGCATGGTG | NM\_001243965.1;NM\_002124.3; also targets HLA-DRB5 (XM\_011514562) at 95%. | XM\_011547738.2 |
| **HLA-DRB3** | TTGGAGCTGCGTAAGTCTGAGTGTCATTTCTTCAATGGGACGGAGCGGGTGCGGTACCTGGACAGATACTTCCATAACCAGGAGGAGTTCCTGCGCTTCG | Targets HLA-DRB3\*01:01; also targets HLA-DRB1 RefSeq transcripts at >90%. | NM\_022555.3 |
| **HLA-DRB3** | TTGGAGCTGCTTAAGTCTGAGTGTCATTTCTTCAATGGGACGGAGCGGGTGCGGTTCCTGGAGAGACACTTCCATAACCAGGAGGAGTACGCGCGCTTCG | Targets HLA-DRB3\*02:02; also targets HLA-DRB1 RefSeq transcripts at >90%. | NM\_022555.3 |
| **HLA-DRB6** | TTCCAGGCAGTTACGGAACTGGGGCGGCCTGTCGCAGAGAACTGGAACAGCCAGAAGGGCATCCCGGAGGAGAAGCGGGACAAGATGGACGACTACTGCA | NR\_001298.1 |  |
| **HLA-F** | TGCGCTCCTGGACCGCGGCGGACACCGTGGCTCAGATCACCCAGCGCTTCTATGAGGCAGAGGAATATGCAGAGGAGTTCAGGACCTACCTGGAGGGCGA | XR\_001743376.1;XR\_001743374.1;XM\_017010814.1;XM\_017010815.1;NM\_001098478.1;XM\_011514564.1;NM\_018950.2;XM\_017010811.1;XM\_017010813.1;NM\_001098479.1;XM\_017010810.1;XR\_001743373.1;XM\_017010812.1 |  |
| **ICAM3** | AGCGTCCAGCTGCGAGTCCTGTATGGTCCCAAAATTGACCGAGCCACATGCCCCCAGCACTTGAAATGGAAAGATAAAACGAGACACGTCCTGCAGTGCC | NM\_001320608.1;NM\_001320606.1;NM\_001320605.1;NM\_002162.4 |  |
| **IL10** | AAGGATCAGCTGGACAACTTGTTGTTAAAGGAGTCCTTGCTGGAGGACTTTAAGGGTTACCTGGGTTGCCAAGCCTTGTCTGAGATGATCCAGTTTTACC | NM\_000572.2 |  |
| **IL15** | AGGGTGATAGTCAAATTATGTATTGGTGGGGCTGGGTACCAATGCTGCAGGTCAACAGCTATGCTGGTAGGCTCCTGCCAGTGTGGAACCACTGACTACT | NM\_172175.2;NM\_000585.4;NR\_037840.2 |  |
| **IL18** | GACAGTCAGCAAGGAATTGTCTCCCAGTGCATTTTGCCCTCCTGGCTGCCAACTCTGGCTGCTAAAGCGGCTGCCACCTGCTGCAGTCTACACAGCTTCG | XM\_011542805.1;NM\_001562.3;XM\_011542806.2;NM\_001243211.1 |  |
| **IL6** | GGCACTGGCAGAAAACAACCTGAACCTTCCAAAGATGGCTGAAAAAGATGGATGCTTCCAATCTGGATTCAATGAGGAGACTTGCCTGGTGAAAATCATC | XM\_011515390.2;NM\_001318095.1;XM\_005249745.4;NM\_000600.4 |  |
| **KITLG** | AGGACTCTATTTTAAGGACTGCGGGACTTGGGTCTCATTTAGAACTTGCAGCTGATGTTGGAAGAGAAAGCACGTGTCTCAGACTGCATGTACCATTTGC | NM\_003994.5;NM\_000899.4 |  |
| **PSMA4** | GTACATTGGCTGGGATAAGCACTATGGCTTTCAGCTCTATCAGAGTGACCCTAGTGGAAATTACGGGGGATGGAAGGCCACATGCATTGGAAATAATAGC | NM\_001330676.1;NM\_001102668.2;NM\_001330673.1;NM\_002789.5;NM\_001102667.2;NM\_001330675.1 |  |
| **RPL38** | TCACAGCCCGACGAAAGGATGCCAAATCTGTCAAGATCAAGAAAAATAAGGACAACGTGAAGTTTAAAGTTCGATGCAGCAGATACCTTTACACCCTGGT | NM\_001035258.1;NM\_000999.3 |  |
| **TMEM14C** | AGGTTCTACCACTCTGGAAAATTCATGCCTGCAGGTTTAATTGCAGGTGCCAGTTTGCTGATGGTCGCCAAAGTTGGAGTTAGTATGTTCAACAGACCCC | NM\_001165258.1;NM\_016462.3 |  |
| **TNFSF14** | GGCGTGTCAGCCCTGCTCCAGACACCTTGGGCATGGAGGAGAGTGTCGTACGGCCCTCAGTGTTTGTGGTGGATGGACAGACCGACATCCCATTCACGAG | XM\_017027418.1;XM\_017027417.1;NM\_003807.4;XR\_001753777.1;XR\_936212.2;NM\_172014.3 |  |
| **UBE3C** | TAGAGAGTAGATGTTCAAGAAAGAGTGGTGGAGCACCCTGGCTTTTCTATTTCGTTTTAACTGTTGGCGAAAATTATTTGGGGGCCCTCTCTGAGGAAGG | XR\_927552.2;NM\_014671.2;XM\_017012818.1;XM\_005249564.4 |  |