Supplementary Appendix for

Early acute anti-HLA antibody-negative microvascular rejection of kidney transplants is associated with preformed IgG antibodies against diverse glomerular endothelial cell antigens

Table of Contents

SUPPLEMENTARY MATERIALS AND METHODS

Ethics

The multicenter retrospective study was approved by the French Ministry of Research (CCTIRS# 14031bis, validated 10th April 2014) and by the Ethics Committee "*Ile de France II*" of Necker Hospital (IRB registration#: 1072, validated 24th March 2014). Each patient included in the present study was asked to provide written informed consent prior to enrollment in the study.

Central histological reading of renal allograft biopsies

Clinically indicated biopsy specimens were fixed with formalin, acetic acid, and alcohol and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin, Masson's trichrome, periodic acid–Schiff reagent, and Jones stain for a light microscopy evaluation. Immunohistochemical staining for C4d was systematically performed (rabbit anti-human monoclonal anti-C4d; 1/200 dilution; CliniSciences). Renal allograft biopsies from patients with AMVR but no anti-HLA DSAs and patients with both AMR and anti-HLA DSAs were classified using the updated Banff classification[1, 2] by two pathologists (MR and JPDVH) who were blinded to the patient groups.

Donor-specific anti-HLA antibodies

The presence of circulating anti-HLA-A, -B, -Cw, -DR, -DQ, and -DP DSAs was retrospectively and centrally performed by AC using single-antigen flow bead assays (One Lambda, Canoga Park, CA) on the Luminex® platform[3]. HLA typing of donors and recipients was performed using DNA typing (Innolipa HLA Typing Kit; Innogenetics). Beads showing a normalized MFI>500 were considered positive.

Anti-MICA, anti-AT1R and anti-ETAR antibody assessments

The presence of anti-MICA antibodies was retrospectively and centrally performed by ACG using flow bead assays (One Lambda, Canoga Park, CA) on the Luminex® platform.

Anti-AT1R and anti-ETAR Abs were measured with dedicated sandwich ELISAs (CellTrend GmbH, Luckenwalde, Germany, distributed by One Lambda) strictly according to the manufacturer's recommendations. Briefly, a 1:100 serum dilution was added in duplicate to

each well of the microplate and incubated at 4°C for 2 h. After performing the washing steps, the plates were incubated with the horseradish-peroxidase-labeled goat anti-human IgG used for detection for 1 h, washed, substrate was added, the plate was incubated and then the reaction was terminated. A standard curve allowed the optical density signal to be translated into a concentration expressed in units/mL of serum.

Assessment of natural antibodies

NAb levels were assessed using two separate methods described in a previous study[4]. Briefly, IgGs purified from the patient sera were tested for their reactivity to UV-induced apoptotic Jurkat cells using flow cytometry on a BD LSR Fortessa instrument (BD Biosciences). All samples were diluted 1:2 and assessed using the same instrument settings within the same experiment. As a second method, an ELISA was used to detect NAbs reacting to the oxidized lipid epitope MDA. MDA-modified BSA was generated as previously reported[4] and used to coat high-binding 96-well plates (Corning, Kennebunk, ME). A time-resolved fluorometry-based DELFIA was used as a read-out. Briefly, a biotinylated anti-human IgG secondary antibody was used followed by europium-labelled streptavidin for detection. Serum-purified IgGs were tested at a dilution of 1:10 in this assay.

Non-HLA antibody detection

Sera were tested against a panel of 62 non-HLA antigens in two single-antigen flow bead assays provided by One Lambda Inc. (Canoga Park, CA). One kit contained 57 antigens, and the other one gathered the 5 collagen-bearing beads, as the wash buffer was different for the two assays. Non-HLA antigens bound to microbeads were incubated with patient serum samples (20 μL serum of with 5 μL of beads). After washing, the bead-bound antibodies were detected with an anti-IgG PE-labeled secondary antibody (LS-AB2, One Lambda) and read on a Luminex 200 instrument (Luminex Corporation, TX). Results are presented as MFIs adjusted for non-specific binding using the following formula: *MFI adjusted*=*MFI* (*target beads*)−*MFI* (*negative control beads*). Positive values for each individual non-HLA antigen were calculated based on the mean MFI of the control group. Samples with an MFI less than the mean+3 SD were classified as negative and samples with an MFI greater than the mean+3 SD were classified as positive.

Endothelial cell crossmatching

Sera were tested with a custom EC crossmatch assay adapted from a previous study[5] using banked primary macrovascular ECs that were prospectively isolated and stored (DIVAT Sample Biocollection, French Health Ministry project number 02G55), as well as CiGEnCs[6]. Cells were activated by adding inflammatory cytokines (100 U/mL each of TNF-α and IFN-γ, purchased from R&D Systems) to the medium, followed by an incubation for 48 h to mimic an inflammatory state. After washes with PBS, cells were trypsinized and washed before an incubation with a 1:4 dilution of patient sera in PBS containing 0.05% BSA for 30 minutes. After two additional washes, cells were incubated with an Alexa Fluor® 488-conjugated anti-human IgG antibody (AffiniPure $F(ab')_2$ fragment donkey anti-human IgG (H+L), Interchim) for 20 minutes. Fluorescence was measured using flow cytometry (FACS LSR II®, BD Biosciences), and geometric MFIs were calculated using the FlowJo® software program. Pooled and individual serum samples from healthy volunteers without detectable anti-HLA antibodies (Etablissement Français du Sang, Nantes) were used as negative controls. A cut-off of a 2-fold increase in the geometric mean value from patients' sera compared with the negative control was established to define reactive sera.

RNA sequencing

Total RNAs were isolated from the CiGEnCs and from banked primary macrovascular ECs obtained from 5 donors using an RNeasy Kit (Qiagen), including a DNase treatment step. RNA quality was assessed using RNA Screen Tape 6000 Pico LabChips with a Tape Station (Agilent Technologies), and the RNA concentration was measured with spectrophotometry using Xpose (Trinean). RNAseq libraries were prepared from 2 µg of total RNA using a TruSeq Stranded mRNA LT Sample Prep Kit (Illumina) as recommended by the manufacturer. Half of the oriented cDNAs produced from the poly-A+ fraction was amplified using PCR (9 or 10 cycles). The RNAseq libraries were sequenced on an Illumina HiSeq2500 platform (paired-end sequencing, 130x130 bases, high-throughput mode). On average, 84 million paired-end reads per library sample were produced with a minimum of 47 million reads obtained for each sample. The RNAseq data are deposited at European Bioinformatics Institute (Annotare; https://www.ebi.ac.uk/arrayexpress/) under registration number E-MTAB-7003.

Protein array

A ProtoArray™ Human Protein Microarray v5.1 (Life Technologies, Foster City, CA) containing more than 9,000 protein features were used to profile circulating antibodies in 30 day 0 serum samples, including 20 samples from KTRs with early AMVR without anti-HLA DSAs and 10 samples from KTRs who remained stable over the first year after transplant (used as controls). Single samples were profiled at a 1:500 dilution, and a pairwise analysis between the two groups (Group 1 vs. Group 2) was performed to identify the potential group specificity of the immunogenic antigens. Established protocols (http://www.invitrogen.com) were used for sample preparation and data acquisition[7, 8]. The data analysis software ProtoArray Prospector 5.2 was used to analyze the signal intensities of fixation.

Quantitative PCR

The mRNAs were isolated using RNeasy Mini Kit (Qiagen, Courtaboeuf, France) and cDNAs were synthesized using a mixture containing RNAse inhibitors, a dNTP mixture, random hexamers, an $MgCl_2$ solution and MultiScribe reverse transcriptase (all from Thermo Fisher). Quantitative PCR reactions were assembled with TaqMan 2x Fast Universal PCR Master Mix (Thermo Fisher) and TaqMan primers and probes, and analyzed on a Viaa7 real-time system using QuantStudio real-time PCR software (Thermo Fisher). Primers and probes for *VEGFR2* (Hs00911700_m1), *ICAM2* (Hs00609563_m1), *PECAM1* (Hs01065279_m1), *VE-cadherin* (Hs00901465_m1), *CIITA* (Hs00172106_m1), *B2M* (Hs00187842_m1), *MBP* (Hs00921945_m1), *BMPR1A* (Hs00831730_s1), *EPHB6* (Hs01071144_m1), *LMOD1* (Hs00201704_m1) and *HPRT1* (Hs02800695_m1) were obtained from Thermo Fisher. For *GAPDH*, the following primers and probes were used: sense: 5’ CCACATCGCTCAGACACCAT 3’, antisense: 5’ TGACCAGGCGCCCAATA 3’, and probe: 5’ FAM-AGTCAACGGATTTGGTC-MGB 3’. Gene expression levels were normalized to GAPDH. For the *18S* RNA, the following primers and probes were used: sense: 5' GCCCGAAGCGTTTACTTTGA 3’, antisense: 5' TCCATTATTCCTAGCTGCGGTATC 3’, and probe: 5’ FAM-AAAGCAGGCCCGAGCCGCC-TAMRA 3’.

Flow cytometry

For endothelial cell phenotyping, cells were stained with PE-conjugated anti-VEGFR2, VioBright-515-conjugated anti-PECAM1, PE-Vio770-conjugated ICAM2, APC-conjugated anti-VE-cadherin, VioBlue-conjugated anti-HLA ABC (all from Miltenyi Biotec) and BV605-conjugated anti-HLA DR (Ozyme) antibodies and analyzed using an LSR Fortessa flow cytometer (BD Biosciences, San Jose, CA) with post-acquisition analysis using Kaluza software (Kaluza 2.1, Beckman Coulter).

Immunofluorescence staining

Confluent cells on gelatin-coated glass coverslips were fixed with 4% formaldehyde and permeabilized with a solution containing 0.1% Triton X-100 and 3% BSA (Thermo Fisher). Cells were then incubated with the following fluorescent dye-conjugated primary antibodies for 12 h at 4°C: VioBright-515-conjugated anti-PECAM1 and APC-conjugated anti-VE-cadherin (Miltenyi Biotec). For the experiments shown in **Supplementary Figure 2**, cells were incubated with the unconjugated primary antibodies against HLA-A,B,C or HLA-DP,DR,DQ (Ozyme). Unconjugated primary antibody binding was detected using an Alexa Fluor 647-conjugated anti-mouse IgG secondary antibody (Ozyme). Cells were then stained with DAPI and coverslips were mounted using Fluomont™ (Sigma-Aldrich) and examined using a Zeiss confocal microscope (Zeiss Confocal LSM 700). Zen900 software was used to generate representative images and ImageJ software (Java) was used to analyze the data.

Western blot

Cells were lysed in RIPA buffer containing protease and phosphatase inhibitors (PIC cocktail, Sigma-Aldrich). Cell lysates (20 µg) were resolved by SDS-PAGE (12%), and proteins were transferred to PVDF membranes (Amersham, Little Chalfont, UK) using a Trans-Blot SD semi-dry electrophoretic transfer cell (Bio-Rad, Marne-la-Coquette, France). Immunoblotting was performed using the following primary antibodies at dilution 1:1000: anti-LMOD1, anti-MBP (clone 2H9), anti-BMPR1A (clone 4B7B2) from Thermo Fisher Scientific (Rockford, IL, USA) and anti-GAPDH (sc-32233) from Santa Cruz Biotechnology (Dallas, TX, USA). Antibody-bound proteins were revealed using appropriate peroxidase-conjugated secondary antibodies and detected using an enhanced chemiluminescence (ECL) kit (Amersham) and luminescent image analyzer LAS-4000 (Fujifilm, Tokyo, Japan).

Statistical analysis

Protein array data were analyzed using ProtoArray™ Prospector software (Life Technologies). A mean increase in the signal intensity greater than 2 and a P value less than 0.05 were considered significant. The normalized average signal of fixation was used for the heat map representation of the protein array data.

For RNA sequencing data, FASTQ files were mapped to the ENSEMBL [Human (GRCh38/hg38)] reference sequence using "Hisat2" and counted with "featureCounts" from the "Subread" R package. Read count normalizations and group comparisons were performed using three independent and complementary methods, namely, Deseq2, edgeR, and LimmaVoom, and the results of each analysis were compared and grouped. The results were then filtered at a P value<0.05 and a fold change of 1.2. The average linkage clustering analysis was implemented in the Cluster 3.0 program and Java Tree View 1.1.6r4 software.

Clustering analyses were performed with hierarchical clustering using the Spearman correlation similarity measure and average linkage algorithm. Heat maps were created with the R package ctc: Cluster and Tree Conversion (http://www.r-project.org/) and images were created using Java Treeview software[9] to obtain a general overview of the data in terms of the within-array distributions of signals and the between-sample variability.

The R packages "res.pca" and "fviz_pca_ind" were used to process the matched data from the protein array and RNAseq and to perform a PCA.

The overall scoring included the frequency of responses in the patients with AMVR compared with the stable patient group and included the relative strength of reactivity observed, as previously described[10].

SUPPLEMENTARY DISCUSSION

In this study, we assessed the presence of unknown AECAs in sera from KTRs. These unknown AECAs specifically target microvascular endothelial antigens. We performed an integrated analysis combining the serological responses of patients with AMVR and stable KTRs with the microvascular EC-specific mRNA expression profiles to identify antigens of interest. The top identified antigens were recognized by greater than 30% of patients with AMVR. The antigen with the highest score in patients with AMVR was ZG16B. This antigen was immunogenic in 90.9% of patients with AMVR compared with 33.3% of stable KTRs. Interestingly, ZG16B is a protein identified in urinary exosomes[11]. Exosomes originate as internal vesicles of multivesicular bodies and are released into the extracellular environment after fusion with the plasma membrane. Urinary exosomes, which contain proteins, lipids and RNAs, are produced by podocytes and potentially ECs in glomeruli. The production of some autoantibodies (such as anti-perlecan) that contribute to rejection in organ transplant recipients was recently shown to be triggered by exosome-like vesicles[12].

The second highest antigen was leiomodin-1 (LMOD1). It was immunogenic in 68% of patients with AMVR, with twice the cut-off intensity compared with 25% of stable KTRs. Intriguingly, autoantibodies targeting LMOD1 were recently reported to be more abundantly detected in the sera from patients with nodding syndrome, an autoimmune epileptic disorder, than in unaffected controls. Thus, the authors showed that anti-LMOD1 antibodies are directly neurotoxic in an *in vitro* setting[13]. The potential deleterious effects of anti-LMOD1 antibodies on microvascular ECs could participate in microvascular lesions but remains to be assessed in the kidney transplant context.

We identified three other interesting antigens, namely, myelin basic protein (MBP), transglutaminase 2 (TGM2) and pleckstrin homology domain-containing adapter protein (PLEKHA1), which are all associated with the development of autoantibodies in autoimmune diseases. Anti-MBP Abs are deleterious in patients with multiple sclerosis, whereas anti-PLEKHA1 Abs contribute to type 1 diabetes and anti-TGM2 Abs are involved in celiac disease. In patients with multiple sclerosis, an autoimmune neurodegenerative disease leading to the destruction of the myelin sheath, the B cell-mediated contribution is important[14]. Thus, autoantibodies targeting MBP have been proposed as biomarkers for determining the clinical prognosis[15]. Moreover, anti-MBP Abs have also been detected in a murine model of

multiple sclerosis[16]. In patients with type 1 diabetes (TD1), the genes in the HLA region constitute the most important genetic risk, whereas other non-HLA genes also contribute to the development of autoantibodies. Sharma and colleagues[17] recently discovered that the PLEAKHA1 region presents a single nucleotide polymorphism (SNP) that is strongly correlated with T1D. In celiac disease, a long-term autoimmune disorder primarily affecting the small intestine, IgA antibodies targeting the endomysium are autoantigens that play a major role in the pathogenesis of the disease. Interestingly, Dieterich and colleagues identified tissue TGM2 as the endomysial autoantigen[18].

In conclusion, in the present study, we developed a homemade endothelial crossmatch assay and identified a common IgG response in sera from patients with AMVR that is specifically directed against constitutively expressed antigens of microvascular glomerular cells. Protein arrays and RNAseq were used to identify 857 antigenic targets of these AECAs. The development of an ELISA for the routine testing of each of these AECAs is not a conceivable solution, and thus *in vitro* cell-based assays are needed to assess the presence of AECAs. Finally, several of these AECAs are already known to function as autoantibodies involved in autoimmune disorders, suggesting a potential direct effect of AECAs on microvascular injury.

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Supplementary Table 1: Baseline characteristics of 38 patients with early AMVR in the absence of anti-HLA DSAs and 10 KTRs who remained stable during the first year after transplant

Variables	Patients with AMVR without anti-HLA DSAs, N=38	Stable patients, N=10	P
Recipient characteristics			
Male, n (%)	25 (65.8)	9 (90.0)	0.18
Age at transplantation, mean±SD, yrs	43.0±14.3	50.3±15.7	0.24
Cause of end-stage renal disease, n (%)			
Glomerulonephritis	10 (26.3)	1 (10.0)	0.41
Diabetes	6 (15.8)	0 (0.0)	0.32
Cystic/hereditary/congenital	7 (18.4)	4 (40.0)	0.21
Secondary glomerulonephritis	3 (7.9)	1 (10.0)	1.00
Hypertension	2 (5.3)	0 (0.0)	1.00
Interstitial nephritis	3 (7.9)	1 (10.0)	0.28
Miscellaneous conditions	2 (5.38)	1 (10.0)	0.41
Neoplasm	0 (0.0)	0 (0.0)	1.00
Etiology uncertain	5 (13.2)	2 (20.0)	0.63
Duration of dialysis before transplantation, mean±SD, yrs	3.9±4.4	4.2±3.8	0.59
Previous transplantation, n (%)	11 (28.9)	1 (10.0)	0.42
Transplant variables			
Donor age, mean±SD, yrs	50.4±12.6	50.7±19.9	0.81
Deceased donor, n (%)	28 (73.7)	6 (60.0)	0.45
Male donor, n (%)	17 (44.7)	7 (70.0)	0.29
Cold ischemia time[a], mean±SD, hrs	19.1±7.0	21.7±8.6	0.28
Preformed anti-HLA abs with an MFI>500, n (%)	19 (50.0)	3 (30.0)	0.66
Delayed graft function, n (%)	18 (47.3)	3 (30.0)	0.48
Number of post-transplant hemodialysis session, mean±SD	2.5±4.2	0.0±0.0	0.03
Immunosuppressive protocol			
Induction therapy, n (%)	38 (100.0)	10 (100.0)	1.00
Basiliximab/Thymoglobuline®, n (%)	33 (86.8)/5 (13.2)	9 (90.0)/1 (10.0)	1.00
Calcineurin inhibitor-based therapy, n (%)	37 (97.4)	9 (90.0)	1.00
Cyclosporine/Tacrolimus, n (%)	11 (28.9)/26 (68.4)	1 (10.0)/8 (80.0)	0.34
Purine synthesis inhibitor, n (%)	37 (93.9)	5 (50.0)	0.0002
mTOR inhibitor, n (%)	0 (0.0)	6 (60.0)	<0.0001
Steroid, n (%)	37 (97.4)	10 (100.0)	1.00

[a] in recipients of transplants from deceased donors only

Supplementary Table 2: Antigens that are more immunogenic in patients with AMVR than in stable KTRs (P<0.05)

Protein Locus	Percentage		Mean Value		P Value	Description
	Stable Group	AMVR Group	Stable Group	AMVR Group		
BC001135.1	8.33%	45.46%	1396.80531	1723.21153	0.01173958	transient receptor potential cation channel, subfamily M, member 8 (TRPM8)
BC001755.1	25%	68.18%	8417.42279	16045.3644	0.01309345	leiomodin-1
BC002758.1	8.33%	50%	1356.8921	2030.83847	0.00614931	adenosine deaminase, tRNA-specific 1 (ADAT1)
BC002955.1	41.67%	77.27%	2757.81706	3461.10623	0.03870862	Ubiquitin-specific peptidase 2 (USP2)
BC003398.1	8.33%	45.46%	1906.91261	3120.22664	0.01173958	MOB1, Mps one binder kinase activator-like 1B (yeast) (MOBK1B)
BC007102.1	8.33%	36.36%	1001.27233	1246.26502	0.0380784	cell differentiation protein RCD1 homolog
BC008435.1	41.67%	81.82%	2013.91388	3387.37465	0.01839011	peroxiredoxin 3 (PRDX3)
BC011600.1	33.33%	95.46%	35082.1649	42801.6293	5.89E-05	cDNA clone IMAGE:3050953, **** WARNING: chimeric clone ****
BC011781.2	58.33%	95.46%	7076.73332	4436.9737	0.00766284	chromosome 9 open reading frame 37 (C9orf37)
BC012381.1	16.67%	77.27%	1701.26378	2337.33378	0.01161727	neuropilin and tolloid-like protein 2
BC014020.1	58.33%	95.46%	12946.5971	13466.3071	0.00766284	BAI1-associated protein 2 (BAIAP2)
BC014394.1	25%	63.64%	4787.05277	7693.77217	0.02508746	A.T hook DNA-binding motif-containing protein 1
BC014667.1	16.67%	72.73%	56985.0598	67019.5845	0.00109945	immunoglobulin heavy constant gamma 1 (G1m marker) (IGHG1)
BC014975.1	25%	59.09%	1364.98021	1944.52892	0.04457771	family with sequence similarity 136, member A (FAM136A)
BC014991.1	33.33%	86.36%	55973.8119	65219.8809	0.00165721	N-methylpurine-DNA glycosylase (MPG)
BC016381.1	16.67%	72.73%	55040.7019	64792.9392	0.00109945	immunoglobulin heavy constant mu (IGHM)
BC017968.1	8.33%	59.09%	1267.5451	1901.91689	0.01116585	solute carrier family 16, member 10 (aromatic amino acid transporter) (SLC16A10)
BC019337.1	33.33%	81.82%	51446.6321	56255.4887	0.00484443	immunoglobulin heavy constant gamma 1 (G1m marker) (IGHG1)
BC022362.1	33.33%	86.36%	53126.5594	61125.3437	0.00165721	cDNA clone MGC:23888 IMAGE:4704496, complete cds
BC023144.1	16.67%	54.55%	2513.12309	7976.44762	0.02087528	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5
BC025314.1	8.33%	77.27%	52856.3307	63201.3546	3.33E-05	immunoglobulin heavy constant gamma 1 (G1m marker) (IGHG1)
BC026038.1	16.67%	72.73%	54642.5423	64325.87	0.00109945	Ig gamma-1 chain C region
BC026070.2	16.67%	50%	1010.45279	2885.35209	0.03689584	tubby like protein 2 (TULP2)
BC030814.1	8.33%	90.91%	26297.2838	32756.25	3.66E-07	immunoglobulin kappa variable 1-5 (IGKV1-5)

BC032372.1	8.33%	36.36%	659.660811	1569.44368	0.0380784	Ral GEF with PH domain and SH3 binding motif 1 (RALGPS1)
BC032416.1	8.33%	36.36%	910.095187	1948.35423	0.0380784	serine/arginine repetitive matrix 2 (SRRM2)
BC032451.1	16.67%	77.27%	47974.5559	56698.1655	0.00041408	cDNA clone MGC:40426 IMAGE:5178085, complete cds
BC033178.1	16.67%	72.73%	54148.5147	63741.6112	0.00109945	immunoglobulin heavy constant gamma 3 (G3m marker) (IGHG3)
BC033689.1	25%	77.27%	1991.98142	2690.84563	0.01161727	MARVEL domain containing 2 (MARVELD2)
BC033708.1	33.33%	77.27%	8233.78203	5686.0683	0.01161727	Ral GEF with PH domain and SH3 binding motif 1 (RALGPS1)
BC033766.1	8.33%	36.36%	3128.99802	4104.96661	0.0380784	NADH dehydrogenase (ubiquinone) flavoprotein 3, 10kDa (NDUFV3)
BC036184.1	16.67%	50%	2042.66279	2597.21201	0.03689584	tropomodulin-2
BC036767.1	25%	63.64%	2701.26286	3426.03477	0.02508746	RIB43A domain with coiled-coils 1 (RIBC1)
BC037854.1	8.33%	36.36%	624.348659	1294.67744	0.0380784	dynein, cytoplasmic 1, intermediate chain 1 (DYNC1I1)
BC039895.1	16.67%	54.55%	4798.84974	8691.82814	0.02087528	breast cancer anti-estrogen resistance 3 (BCAR3)
BC042193.1	16.67%	50%	1707.73531	2311.19213	0.03689584	G patch domain containing 2 (GPATCH2)
BC047536.1	16.67%	54.55%	28924.8231	35195.0396	0.02087528	sciellin (SCEL)
BC048299.1	41.67%	77.27%	5120.44732	8973.86743	0.03870862	spermatogenesis-associated, serine-rich 2 (SPATS2)
BC051733.1	33.33%	72.73%	2679.48847	3934.83874	0.02416484	leucine zipper protein 1, mRNA (cDNA clone MGC:51018 IMAGE:4838475), complete cds
BC053984.1	33.33%	77.27%	55974.4539	60117.9603	0.01161727	immunoglobulin heavy variable 4-31 (IGHV4-31)
BC054893.1	8.33%	72.73%	8848.6302	10373.7716	1.00E-04	immunoglobulin lambda variable 2-14 (IGLV2-14)
BC056508.1	8.33%	36.36%	915.224054	1208.37966	0.0380784	variable charge, Y-linked 1B (VCY)
BC059405.1	33.33%	68.18%	3334.35655	3249.13179	0.04507746	transducin-like enhancer protein 4
BC059947.1	33.33%	77.27%	6843.28379	9489.84091	0.01161727	Chondrosarcoma-associated gene 1 (CSAG1)
BC062336.1	66.67%	95.46%	53450.5972	57808.4919	0.02955665	immunoglobulin heavy constant gamma 1 (G1m marker), mRNA (cDNA clone MGC:71315 IMAGE:6300554), complete cds
BC062732.1	16.67%	72.73%	53095.6339	62502.2843	0.00109945	Ig kappa chain C region
BC066642.1	16.67%	72.73%	49214.3539	57543.3265	0.00109945	immunoglobulin heavy constant gamma 1 (G1m marker), mRNA (cDNA clone MGC:71306 IMAGE:5451018), complete cds
BC067091.1	33.33%	72.73%	51378.4987	54052.6298	0.02416484	immunoglobulin heavy constant gamma 1 (G1m marker), mRNA (cDNA clone MGC:71316 IMAGE:6301214), complete cds
BC067226.1	16.67%	68.18%	24404.4005	27781.4327	0.0025987	immunoglobulin kappa constant, mRNA (cDNA clone MGC:72070 IMAGE:30349629), complete cds

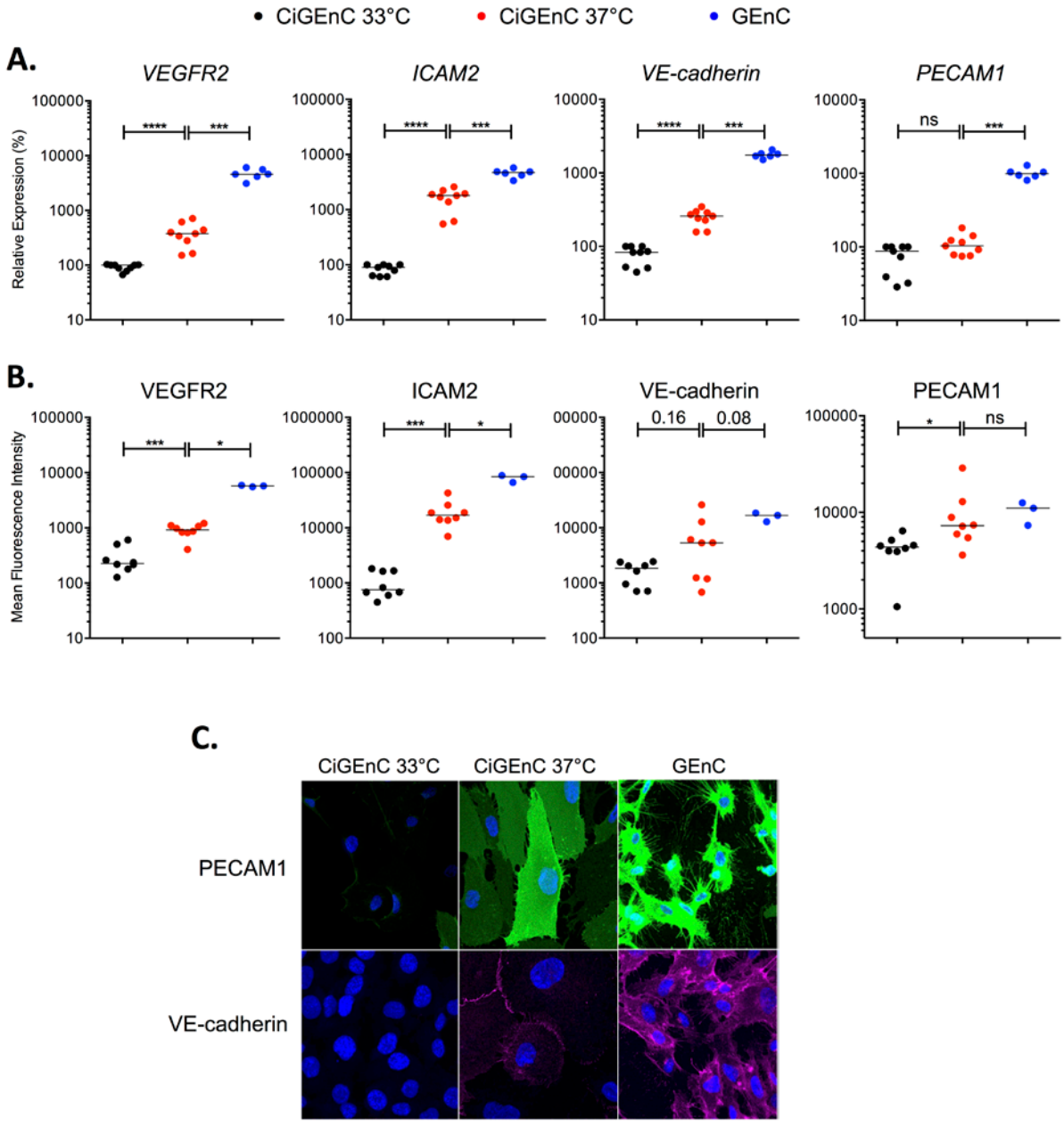
BC069020.1	8.33%	81.82%	25327.3334	31315.2871	9.52E-06	immunoglobulin heavy constant gamma 1 (G1m marker), mRNA (cDNA clone MGC:78608 IMAGE:6214622), complete cds
BC070361.1	8.33%	50%	34591.1579	38146.3129	0.00614931	immunoglobulin kappa constant, mRNA (cDNA clone MGC:88369 IMAGE:30352586), complete cds
BC072419.1	66.67%	95.46%	34694.5369	36192.2431	0.02955665	Ig gamma-1 chain C region
BC073782.1	16.67%	72.73%	53097.2245	62296.6046	0.00109945	cDNA clone MGC:88796 IMAGE:6295732, complete cds
BC073793.1	33.33%	77.27%	13061.0668	13660.5893	0.01161727	cDNA clone MGC:88813 IMAGE:6302307, complete cds
BC073937.1	33.33%	81.82%	7218.87476	8315.32892	0.00484443	immunoglobulin kappa constant, mRNA (cDNA clone MGC:90448 IMAGE:5226105), complete cds
BC078670.1	33.33%	72.73%	31705.0886	35052.314	0.02416484	immunoglobulin heavy constant gamma 1 (G1m marker), mRNA (cDNA clone MGC:88797 IMAGE:6295788), complete cds
BC092518.1	58.33%	90.91%	51483.6881	56383.912	0.03124079	Ig gamma-1 chain C region
BC095489.1	16.67%	54.55%	17284.7314	18949.645	0.02087528	immunoglobulin kappa constant, mRNA (cDNA clone MGC:111575 IMAGE:30328747), complete cds
BC096272.2	33.33%	77.27%	10256.9597	17416.5038	0.01161727	HIV-1 Rev binding protein, mRNA (cDNA clone MGC:116938 IMAGE:40006445), complete cds
BC099907.1	25%	77.27%	55249.1599	62741.8482	0.00265472	general transcription factor II-I
IGFBP6_Recombinant	33.33%	68.18%	4122.08981	4804.45222	0.04507746	IGFBP6 recombinant human protein
NM_000593.5	8.33%	45.46%	528.271488	1105.95158	0.01173958	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (TAP1)
NM_001025100.1	25%	63.64%	1665.66335	3239.32664	0.02508746	myelin basic protein
NM_001032293.1	16.67%	54.55%	17717.1465	30063.6859	0.02087528	zinc finger protein 207 (ZNF207), transcript variant 2
NM_001312.2	33.33%	68.18%	6439.58585	9350.98666	0.04507746	cysteine-rich protein 2 (CRIP2)
NM_001860.1	8.33%	36.36%	773.47464	1055.78263	0.0380784	solute carrier family 31 (copper transporters), member 2 (SLC31A2)
NM_001983.1	8.33%	40.91%	1162.27207	2030.45446	0.02152257	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence) (ERCC1), transcript variant 2
NM_002103.3	41.67%	77.27%	3556.46742	4017.64711	0.03870862	glycogen synthase 1 (muscle) (GYS1)
NM_002625.1	8.33%	36.36%	1547.23624	6489.90305	0.0380784	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1

NM_002638.1	16.67%	50%	1369.97487	1913.06652	0.03689584	peptidase inhibitor 3, skin-derived (SKALP) (PI3)
NM_002904.4	58.33%	95.46%	30676.7318	34898.2601	0.00766284	RD RNA binding protein (RDBP)
NM_002945.2	33.33%	72.73%	27987.4399	31306.8207	0.02416484	replication protein A1, 70 kDa (RPA1)
NM_004202.1	25%	59.09%	1265.71994	1948.08053	0.04457771	thymosin beta-4, Y-chromosomal
NM_004302	33.33%	77.27%	63759.8595	68998.1365	0.01161727	activin R1b recombinant human protein
NM_004329	16.67%	72.73%	48666.1222	57290.137	0.00109945	BMPR1A recombinant human protein
NM_004450.1	8.33%	36.36%	1378.07195	2440.3009	0.0380784	enhancer of rudimentary homolog (*Drosophila*) (ERH)
NM_004566.1	16.67%	54.55%	2267.29416	7240.49416	0.02087528	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3)
NM_004987.3	8.33%	45.46%	67541.4438	70292.9132	0.01173958	LIM and senescent cell antigen-like-containing domain protein 1
NM_005510.2	50%	86.36%	11890.6894	12612.3704	0.02564103	dom-3 homolog Z (*C. elegans*) (DOM3Z)
NM_006413.2	8.33%	36.36%	1657.80522	2085.72063	0.0380784	ribonuclease P protein subunit p30
NM_006790.1	8.33%	40.91%	9282.31567	11029.2684	0.02152257	myotilin (MYOT)
NM_006792.2	8.33%	45.46%	885.325688	4555.077	0.01173958	mortality factor 4 (MORF4), mRNA
NM_007099.1	8.33%	40.91%	1408.56951	1826.0404	0.02152257	acid phosphatase 1, soluble (ACP1), transcript variant 2
NM_007162.1	8.33%	40.91%	1569.44844	2517.03843	0.02152257	transcription factor EB (TFEB)
NM_013975.1	8.33%	45.46%	2660.28757	4735.6742	0.01173958	ligase III, DNA, ATP-dependent (LIG3), nuclear gene encoding mitochondrial protein, transcript variant alpha
NM_014049.3	8.33%	40.91%	1748.76616	2188.59181	0.02152257	acyl-coenzyme A dehydrogenase family, member 9 (ACAD9)
NM_014268.1	8.33%	45.46%	771.745138	1670.59557	0.01173958	microtubule-associated protein, RP/EB family, member 2 (MAPRE2)
NM_014481.2	25%	77.27%	50704.738	59300.2919	0.00265472	APEX nuclease (apurinic/apyrimidinic endonuclease) 2 (APEX2), nuclear gene encoding mitochondrial protein
NM_014923.2	8.33%	54.55%	1356.24474	2979.84535	0.00307465	fibronectin type III domain containing 3A (FNDC3A), transcript variant 2
NM_016564.1	25%	63.64%	2048.59549	3091.0582	0.02508746	cell cycle exit and neuronal differentiation 1 (CEND1)
NM_017451.1	33.33%	81.82%	9050.21611	9367.48708	0.00484443	BAI1-associated protein 2 (BAIAP2), transcript variant 2
NM_017706.2	8.33%	36.36%	1153.99859	1653.13306	0.0380784	WD repeat-containing protein 55
NM_017735.3	16.67%	54.55%	746.01178	804.122035	0.02087528	tetratricopeptide repeat protein 27
NM_018047.1	25%	59.09%	4851.00548	4819.61219	0.04457771	pre-mRNA-splicing factor RBM22
NM_020992.2	66.67%	95.46%	3536.37724	3557.85099	0.02955665	PDZ and LIM domain 1 (elfin) (PDLIM1)
NM_022977.1	8.33%	50%	639.918285	1299.29725	0.00614931	acyl-CoA synthetase long-chain family member 4 (ACSL4), transcript variant 2

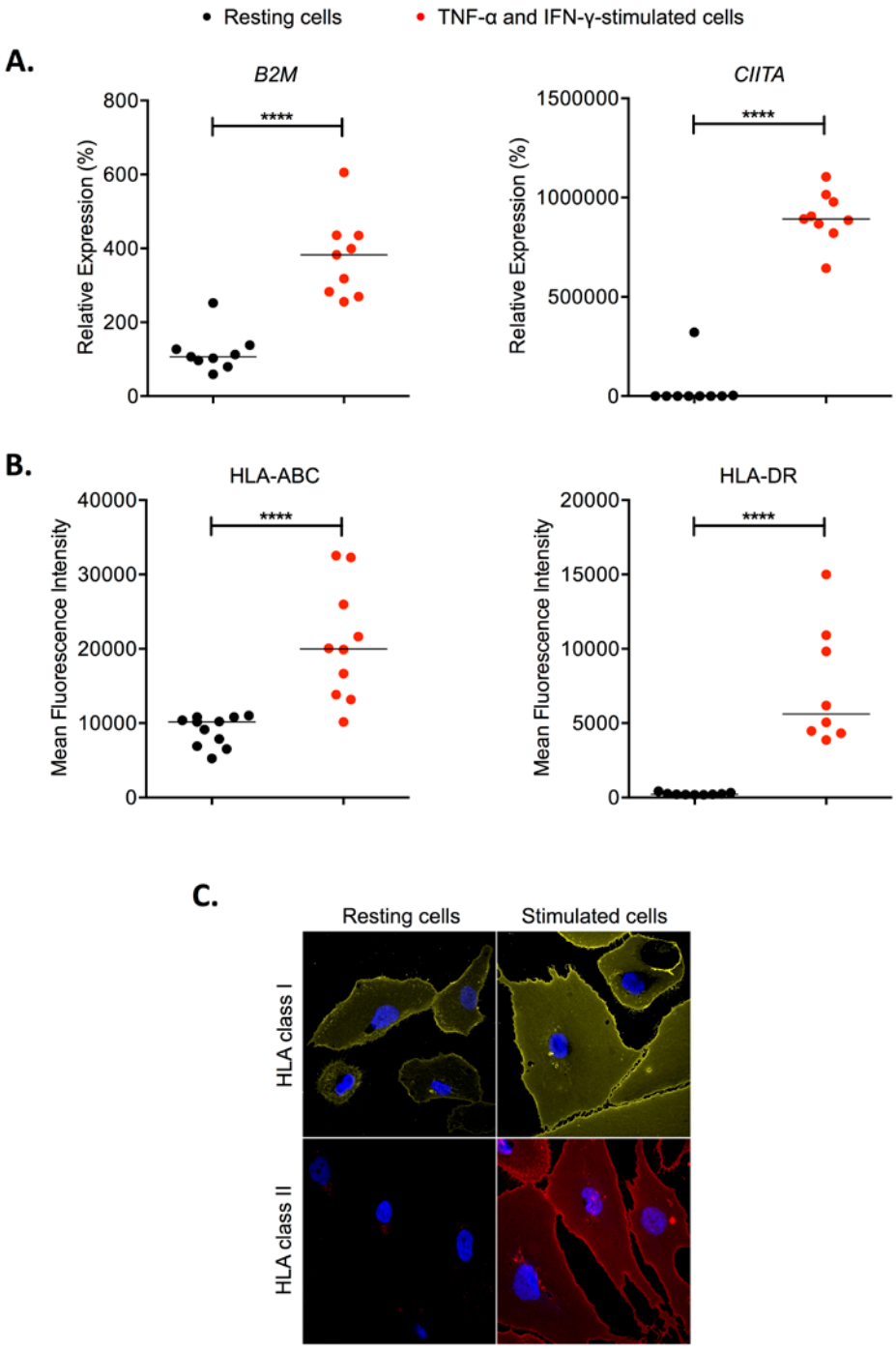
NM_031469.1	8.33%	40.91%	733.26459	1623.4096	0.02152257	SH3 domain binding glutamic acid-rich protein like 2 (SH3BGRL2)
NM_032975.2	8.33%	40.91%	1654.98553	2778.24497	0.02152257	dystrobrevin, alpha (DTNA), transcript variant 2, mRNA
NM_053005.2	8.33%	54.55%	2815.60617	4209.51162	0.00307465	HCCA2 protein (HCCA2)
NM_078630.1	66.67%	95.46%	2858.25416	3313.10931	0.02955665	male-specific lethal 3-like 1 (*Drosophila*) (MSL3L1), transcript variant 2
NM_080548.1	8.33%	36.36%	3210.77672	5727.81597	0.0380784	tyrosine-protein phosphatase non-receptor type 6
NM_130807.1	16.67%	54.55%	3780.46781	5514.13132	0.02087528	MOB1, Mps one binder kinase activator-like 2A (yeast) (MOBKL2A)
NM_144578.1	8.33%	40.91%	743.908335	1394.74235	0.02152257	chromosome 14 open reading frame 32 (C14orf32)
NM_145061.1	25%	59.09%	1315.65955	1739.6403	0.04457771	chromosome 13 open reading frame 3 (C13orf3)
NM_145252.1	33.33%	90.91%	5727.20399	7926.82147	0.00041774	similar to common salivary protein 1 (LOC124220)
NM_145716.2	8.33%	36.36%	3698.55666	9002.01724	0.0380784	single stranded DNA binding protein 3 (SSBP3), transcript variant 1
NM_173191.2	8.33%	59.09%	244.098103	811.032206	0.04457771	Kv channel interacting protein 2 (KCNIP2), transcript variant 2
NM_173468.2	8.33%	40.91%	1216.85016	1574.34065	0.02152257	MOB1, Mps one binder kinase activator-like 1A (yeast) (MOBKL1A)
NM_175907.3	16.67%	72.73%	62610.5486	73700.5011	0.00109945	zinc binding alcohol dehydrogenase, domain containing 2 (ZADH2)
NM_177973.1	16.67%	54.55%	3737.01917	2078.94662	0.02087528	sulfotransferase family, cytosolic, 2B, member 1 (SULT2B1), transcript variant 2
NM_178044.1	8.33%	40.91%	637.761772	889.252908	0.02152257	GIY-YIG domain containing 2 (GIYD2), transcript variant 2
NM_178553.2	8.33%	36.36%	12927.2234	19337.4665	0.0380784	uncharacterized protein C2orf53
NM_199129.1	16.67%	50%	909.687517	1262.53088	0.03689584	transmembrane protein 189
NP_000205.1	16.67%	72.73%	51919.1052	61117.9457	0.00109945	JAG1/JAGL1/CD339 protein
NP_000408.1	16.67%	68.18%	56117.1426	64188.1274	0.0025987	IL2Ra/CD25 protein
NP_000582.1	16.67%	72.73%	49196.9142	57782.0829	0.00109945	CD14 protein
NP_000868.1	16.67%	72.73%	51466.8807	63242.0236	0.00109945	IL1R1/CD121a protein
NP_001018016.1	16.67%	72.73%	53737.3637	63261.689	0.00109945	mucin-1/MUC-1 protein (Fc tag)
NP_001108225.1	16.67%	63.64%	57181.0678	64209.546	0.0055972	endoglin/CD105/ENG protein
NP_001183.2	16.67%	68.18%	33528.8887	41882.1955	0.0025987	TNFRSF17/BCMA/CD269 protein
NP_001775.2	16.67%	68.18%	56511.0379	64611.5322	0.0025987	CD97 protein
NP_001954.2	16.67%	68.18%	53789.7786	65004.6823	0.0025987	EGF/epidermal growth factor protein

NP_002167.1	16.67%	72.73%	48630.2868	57263.4877	0.00109945	interferon beta/IFN-beta/IFNB protein
NP_002174.1	16.67%	72.73%	57180.8465	67162.1144	0.00109945	IL3RA/CD123 protein
NP_003833.3	16.67%	63.64%	48845.1411	55552.307	0.0055972	TNFRSF10B/TRAILR2/CD262 protein
NP_004084.1	16.67%	63.64%	67075.0351	73317.2316	0.0055972	ephrin B2/EFNB2 protein
NP_004834.1	16.67%	72.73%	51263.7699	60347.4354	0.00109945	IL27Ra/TCCR/WSX1 protein
NP_006262.1	25%	86.36%	49594.7141	58104.2789	0.00029126	S100A1 protein
NP_054862.1	16.67%	68.18%	51758.1762	58956.1544	0.0025987	PD-L1 protein
NP_061947.1	16.67%	68.18%	58248.5808	65031.6355	0.0025987	DLL4 protein
NP_068576.1	16.67%	72.73%	52394.6963	61677.3535	0.00109945	ACE2/ACEH protein
NP_079515.2	8.33%	63.64%	51984.9227	62947.9699	0.0006473	PD-L2/B7-DC/CD273 protein
P01566	16.67%	72.73%	55034.8723	64785.6783	0.00109945	interferon alpha 10/IFNA10 protein
P01567	25%	81.82%	56287.6763	66801.0376	0.00097424	interferon alpha 7/IFNA7 protein
PV3835	8.33%	50%	256.498926	748.716233	0.00614931	MLCK protein (MLCK)
XM_376764.2	8.33%	36.36%	780.890847	1308.4105	0.0380784	paraneoplastic antigen MA2 (PNMA2)

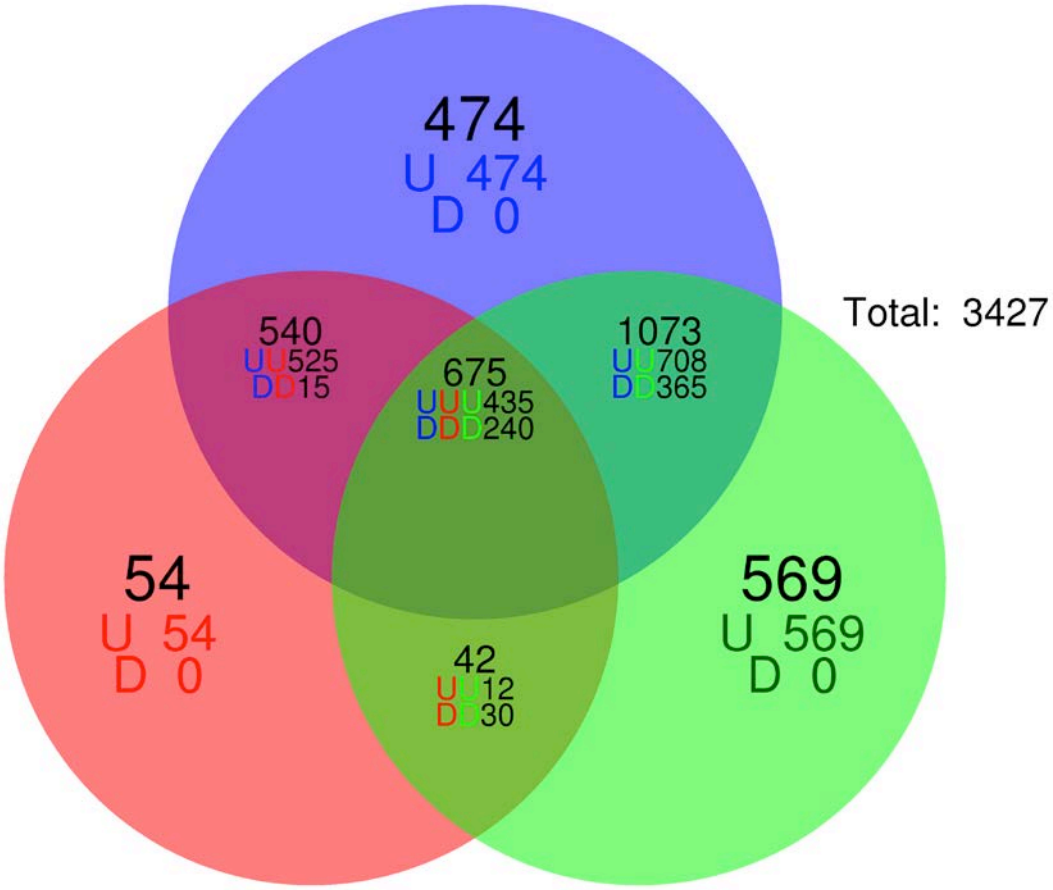
Supplementary Figure 1: CiGEnCs acquire an endothelial phenotype after 7 days of culture at 37°C. The CiGEnC phenotype was analyzed before (33°C) and after differentiation (37°C for 7 days) and compared to primary human glomerular microvascular ECs (GEnC) (Cell Systems, ACBRI 128, Kirkland, WA, USA). (***A.***) Quantitative PCR analysis of *VEGFR2*, *ICAM2*, *PECAM1* and *VE-cadherin*. N=6-9, ***P<0.001 and ****P<0.0001, Mann-Whitney test (***B.***) FACS analysis of VEGFR2, PECAM1, ICAM2, and VE-cadherin. N=3-8, *P<0.05 and ***P<0.001, Mann-Whitney test. (***C.***) Immunofluorescence staining for PECAM1 and VE-cadherin.



Supplementary Figure 2: Cytokine stimulation increases HLA expression in CiGEnCs. After 7 days of differentiation, CiGEnCs were challenged with TNF-α (100 IU/mL, Miltenyi Biotec) and IFN-γ (100 IU/mL, Miltenyi Biotec) and harvested after 24 h for RT-qPCR analysis and 48 h for both FACS and immunofluorescence analyses. (***A.***) Quantitative PCR analysis of *CIITA* (class II, major histocompatibility complex, transactivator) and *B2M* (β2-microglobulin). ****P≤0.0001, Mann-Whitney test (***B.***) FACS analysis of HLA-ABC and HLA-DR (***C.***) Immunofluorescence staining for HLA class I and HLA class II.



Supplementary Figure 3: Venn diagram illustrating the number of differentially expressed genes between microvascular ECs and macrovascular ECs determined using three statistical methods. The DEseq2 method identified 2762 genes (blue), the edgeR method identified 1311 genes (red) and the LimmaVoom method identified 2359 genes (green). Most of the genes were identified by two methods (U=upregulated genes and D=downregulated genes).



Supplementary Figure 4: Validation of antigen expression in micro and macrovascular ECs. (***A.***) CiGEnCs were analyzed after culture in differentiation media (37°C for 7 days) and compared to primary cultures of human macrovascular ECs (HAECs). Quantitative PCR analysis of *MBP*, *BMPR1A*, *EPHB6* and *LMOD1*. Gene expression levels were normalized to *GAPDH*, *HPRT1* and *18S*. P values were determined using the Mann-Whitney test (***B.***) Western blot analyses of MBP, BMPR1A, EPHB6, LMOD1 and GAPDH. (***C.***) Immunofluorescence staining for MBP, BMPR1a, EPHB6 and LMOD1.