Supplemental Figure Legends

Supplemental Figure 1. HK normalization corrects block age effect on RNA recovery. Plotted are the log2 geometric means of 758 gene probes from 301 needle core kidney biopsies by age of block. The yield of RNA without housekeeping normalization shows a steady decline. Normalization using 12 housekeeping (HK) genes per the standard normalization procedure corrects this effect. (Pearson correlation coefficient, r).

Supplemental Figure 2. AMR pathways are not affected by tubulitis in CAMR while TCMR pathways correlate with tubulitis. A) AMR pathway scores do not correlate with tubulitis (Banff t) in CAMR. B) TCMR pathways correlate with tubulitis. TCMR pathway scores show a strong correlation with the extent of tubulitis whether in CAMR or non-CAMR. (p values by logistic regression).

Supplemental Figure 3. AMR pathway scores not correlated with glomerular inflammation (Banff g scores). A) AMR pathway scores are not correlated with glomerulitis (Banff g) in CAMR. B) Pan-Endothelium pathway scores are not correlated with transplant glomerulopathy scores (Banff cg). (p values by logistic regression, red; Mann-Whitney, blue).

Supplemental Figure 4: AMR pathway scores are not correlated with endarteritis (v score) and glomerular basement membrane duplication (cg score) in CAMR. A) AMR pathway scores are not correlated with endarteritis (v score) in TCMR or CAMR. B) TCMR pathway scores correlate with the presence of endarteritis in TCMR and Mixed rejection. (p values by logistic regression, red; Mann-Whitney, blue).

Supplemental Figure 5: Differential Gene Expression in DSA$^+$ Positive and DSA$^-$ CAMR. Four endothelial genes are differentially expressed. Six genes were differentially expressed in DSA$^+$ CAMR related to NK cells, endothelium and lymphocyte chemotaxis/angiogenesis. One gene was elevated in DSA neg CAMR, the hepatocyte growth factor receptor (MET) typically expressed in epithelial cells, including proximal tubular epithelium.

Supplemental Figure 6. M2 Macrophage Subset is associated with graft failure. Macrophage gene sets associated with M2 but not M1 macrophages are elevated in biopsies with TCMR or CAMR that fail within 3 years. Genes in the panels are indicated. M2 but not M1 macrophage scores were correlated with outcome in both TCMR and CAMR (with or without TCMR). (p values, two tailed t test).

Supplemental Figure 7. Differential gene expression in CAMR is associated with graft failure 3 years after biopsy (indication biopsies). Several macrophage associated genes are elevated in grafts that failed (green highlight).

Supplemental Figure 8. Differential gene expression in TCMR is associated with graft failure 3 years after biopsy (indication biopsies). Several macrophage associated genes are elevated in grafts that failed (green highlight).

Supplemental Figure 9. Prediction of CAMR declines progressively with later onset. A and B) AMR pathway scores in samples with a diagnosis of NER, BS or TCMR decrease with later onset of CAMR. (p values by logistic regression, red; Mann-Whitney, blue).

Supplemental Figure 10. Random Forest Outcome Classification in CAMR/Mixed. Representative RF classification of outcome in CAMR/Mixed cases using the top 24 variables selected from all variables combined (clinical, pathology, pathway/cell types and individual genes (n=953) (see Supplemental Figure 12 A-D). All types of variables contributed. The variables are ranked by relative importance to the correct RF classification and color-coded by type. All variables are higher in the Fail group except KT2, Glomerular Endothelium*, FCER1A, PDGFRB, IFIT1, CD207, and SLC4A1.* Seventy random samples were used to generate 2000 different trees and then classify the samples which were not used to generate each tree (the Out Of Bag samples, OOB). Details are given in the Methods section.

Supplemental Figure 11. Random Forest Outcome Classification in BS-TCMR. Representative RF classification of outcome in BS-TCMR cases using the top 33 variables selected from all variables combined (clinical, pathology, pathway/cell types and individual genes (n=953) (see Supplemental Figure 13 A-D). All types of variables contributed except the clinical/laboratory variables. The variables are ranked by relative importance to the correct RF classification and color-coded by type. All variables are higher in Fail group, except Banff i.

Thirty-five random samples were used to generate 2000 different trees and then classify the samples which were not used to generate each tree (the Out Of Bag samples, OOB). Details are given in the Methods section.

Supplemental Figure 12. Random Forest Outcome Classification in CAMR/Mixed Using Each Category of Variables. Top variables are listed from A) Clinical and

Laboratory Variables B) Pathology C) Pathway/Cell type Scores D) Individual Transcripts.

Supplemental Figure 13. Random Forest Outcome Classification in BS-TCMR Using Each Category of Variables. Top variables are listed from A) Clinical and Laboratory Variables B) Pathology C) Pathway/Cell Type Scores and D) Individual Transcripts.

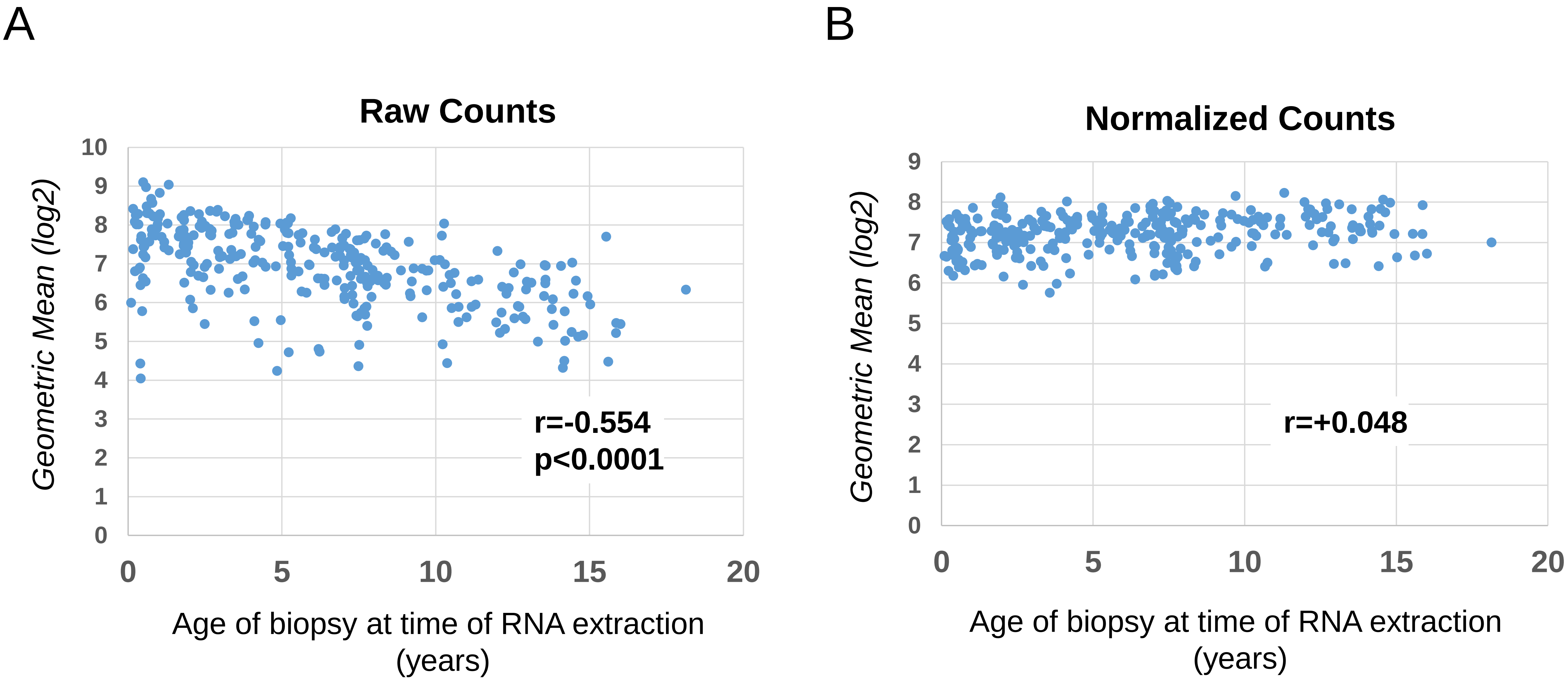
Supplemental Table 1. Aggregate Banff scores and other pathology features by diagnostic category.

Supplemental Table 2. HOT Panel Pathway Gene Sets. Includes NanoString Advanced Analysis Pathways and cell types, published transplant gene sets (Microarray, nCounter) and custom gene sets and cell types (Colvin et al).

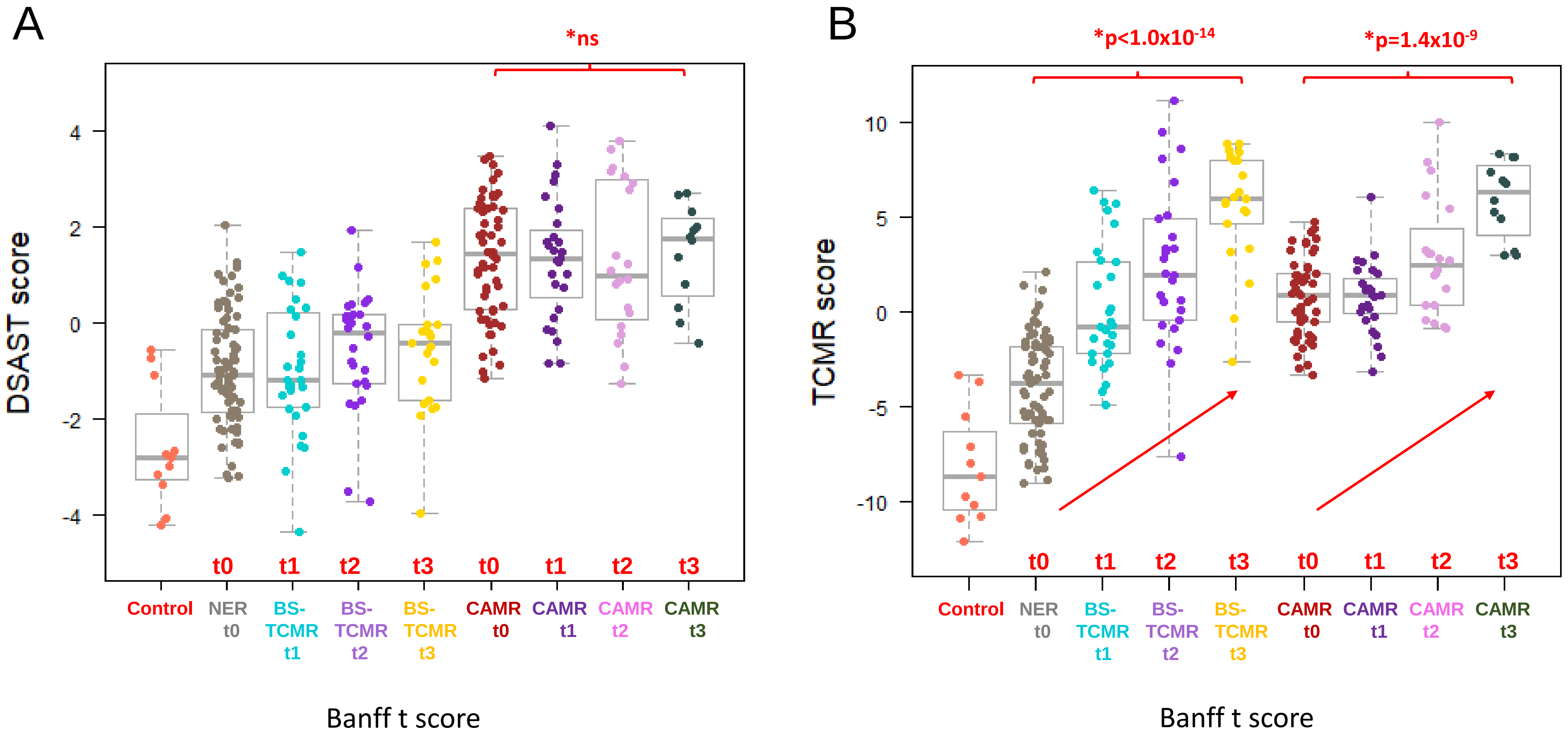
Supplemental Table 3. Random Forest % Correct Classification of 3-Year Graft Survival by Variable Type.

Supplemental Table 4. Univariate analysis of all variables: No CAMR vs PreCAMR within 5 years.

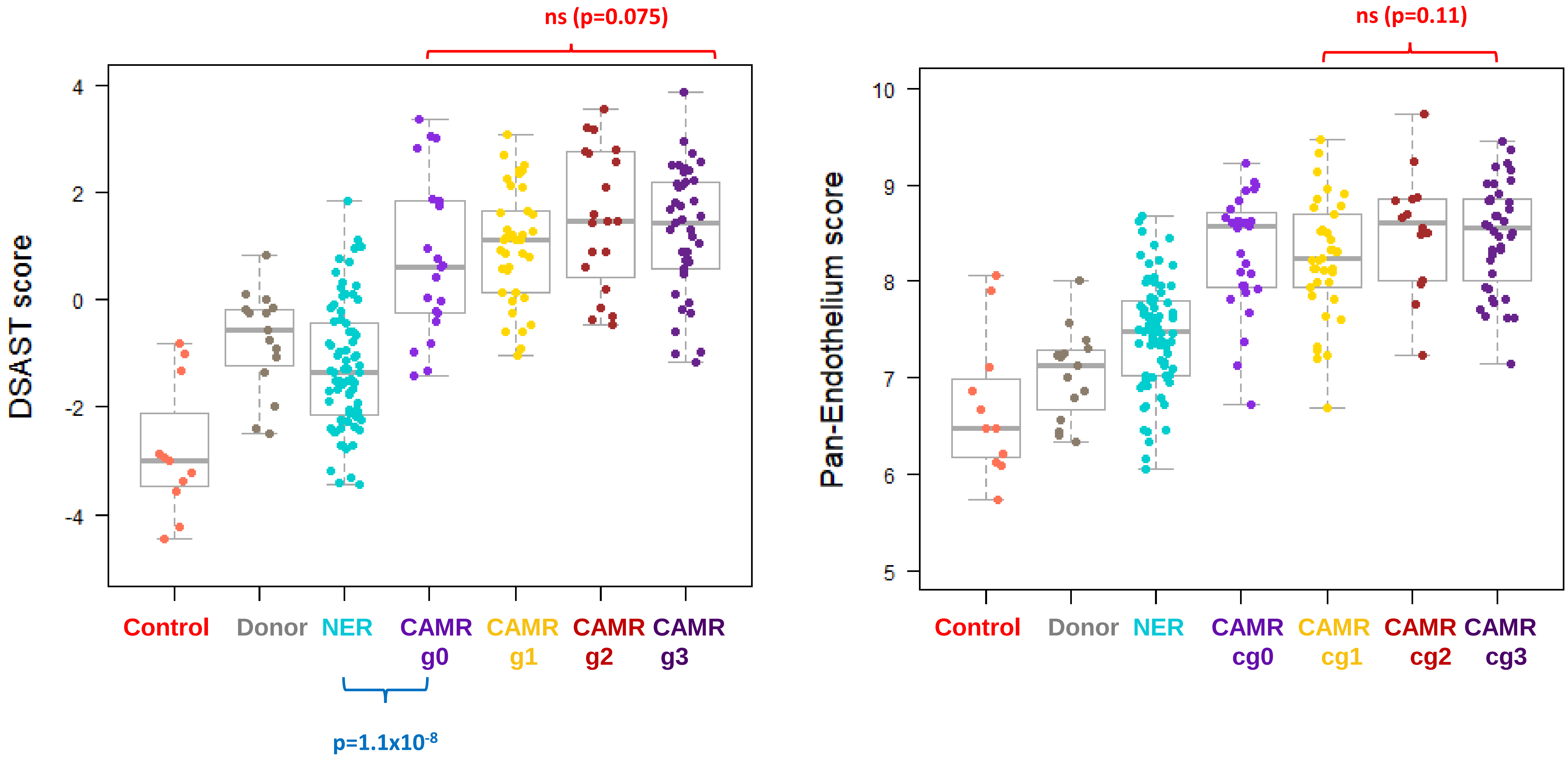
Supplemental Figure 1



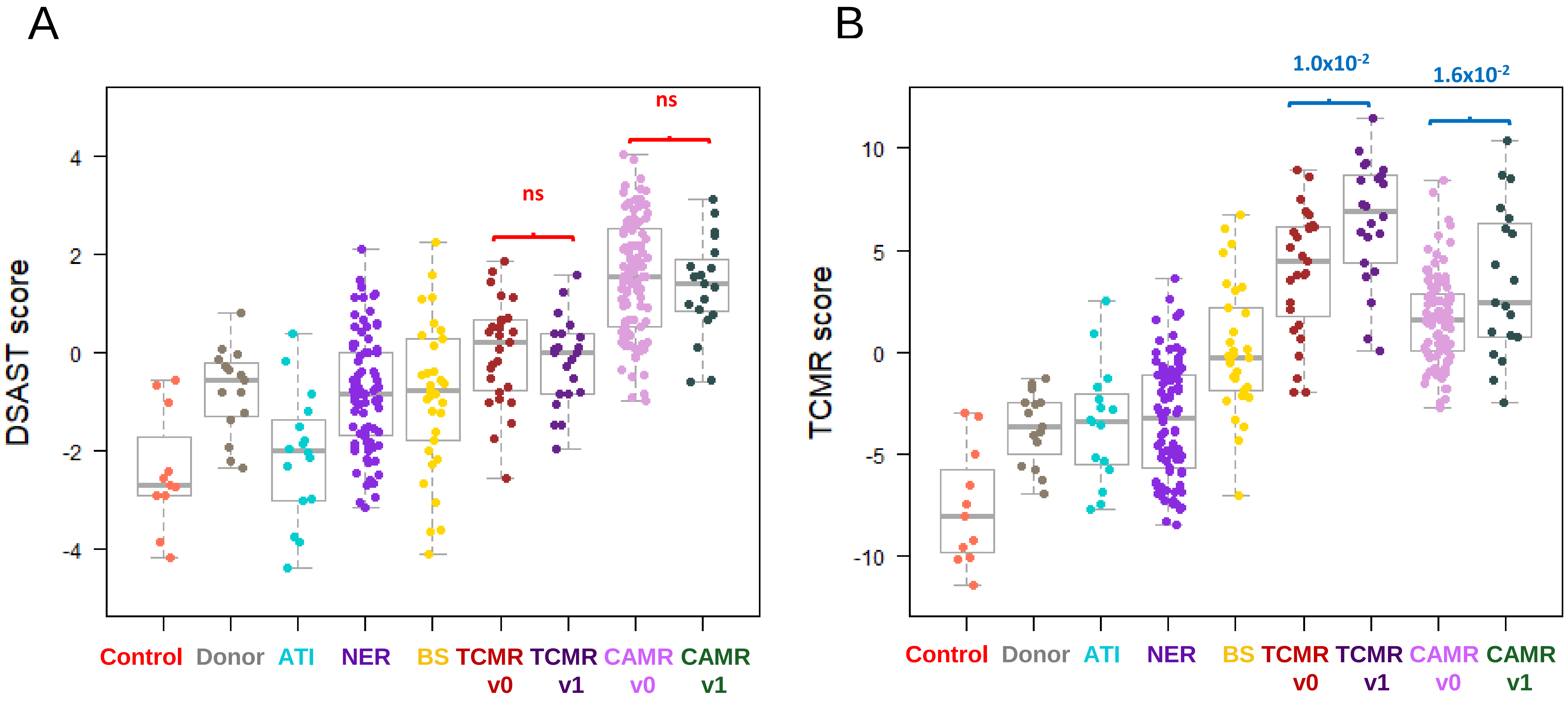
Supplemental Figure 2



Supplemental Figure 3



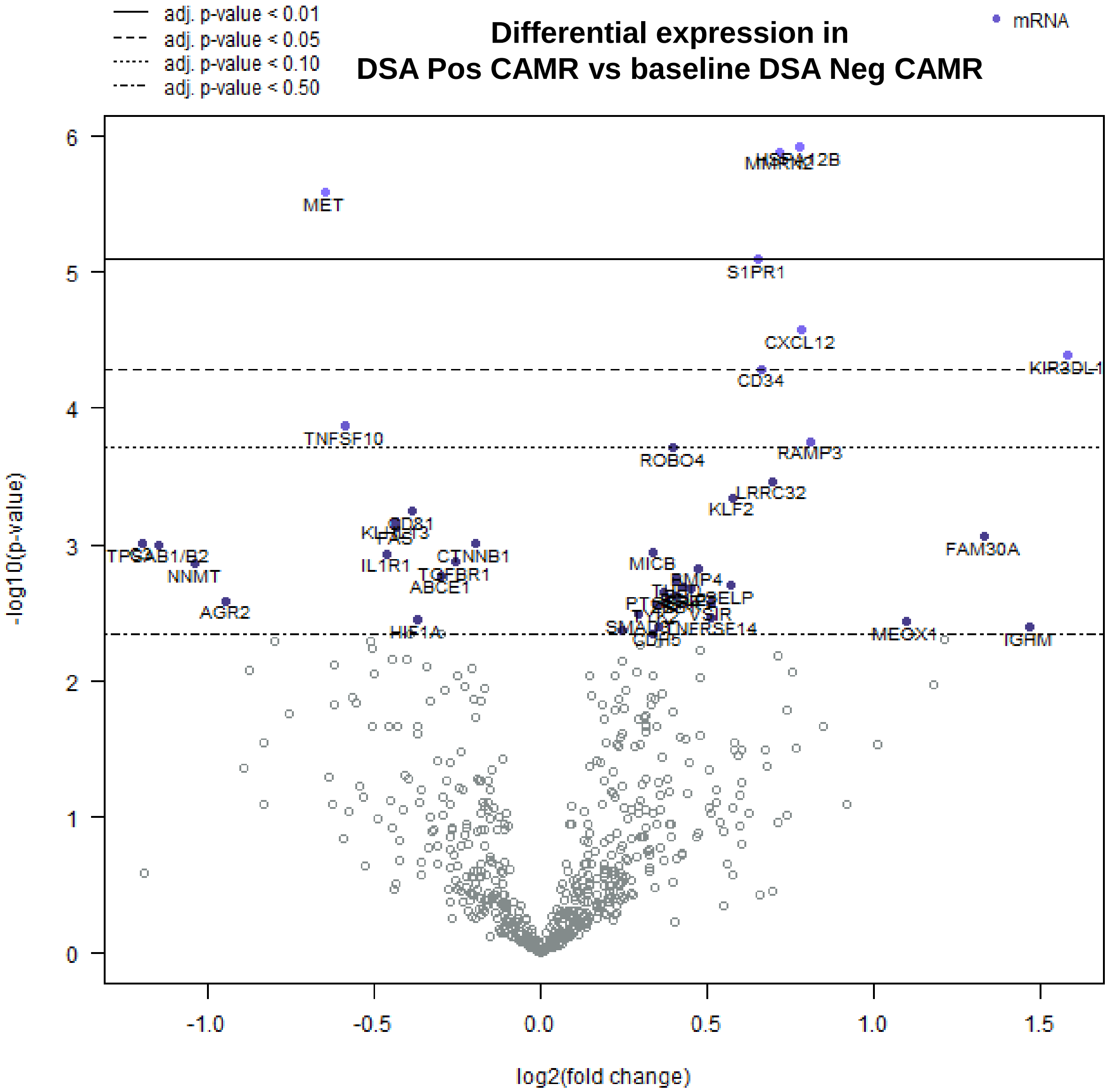
Supplemental Figure 4



Supplemental Figure 5

Higher in DSA Neg

Gene	Log2 fold change	std error (log2)	BY.p.value
MET	-0.647	0.135	0.00393



Higher in DSA Pos

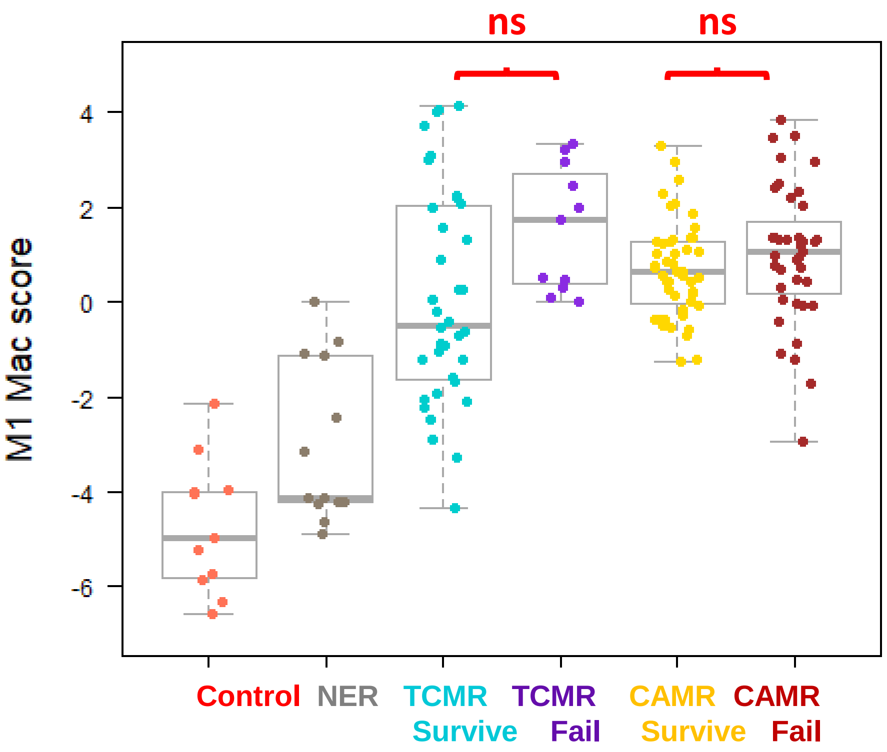
Gene	Log2 fold change	std error (log2)	BY.p.value
KIR3DL1	1.58	0.377	0.0305
CXCL12	0.781	0.183	0.024
HSPA12B	0.777	0.156	0.00292
MMRN2	0.719	0.145	0.00292
CD34	0.662	0.161	0.0333
S1PR1	0.652	0.143	0.00912

Endothelial Genes

KIR3DL1 – NK cells
CXCL12 - chemotactic LC, angiogenesis

Supplemental Figure 6

A

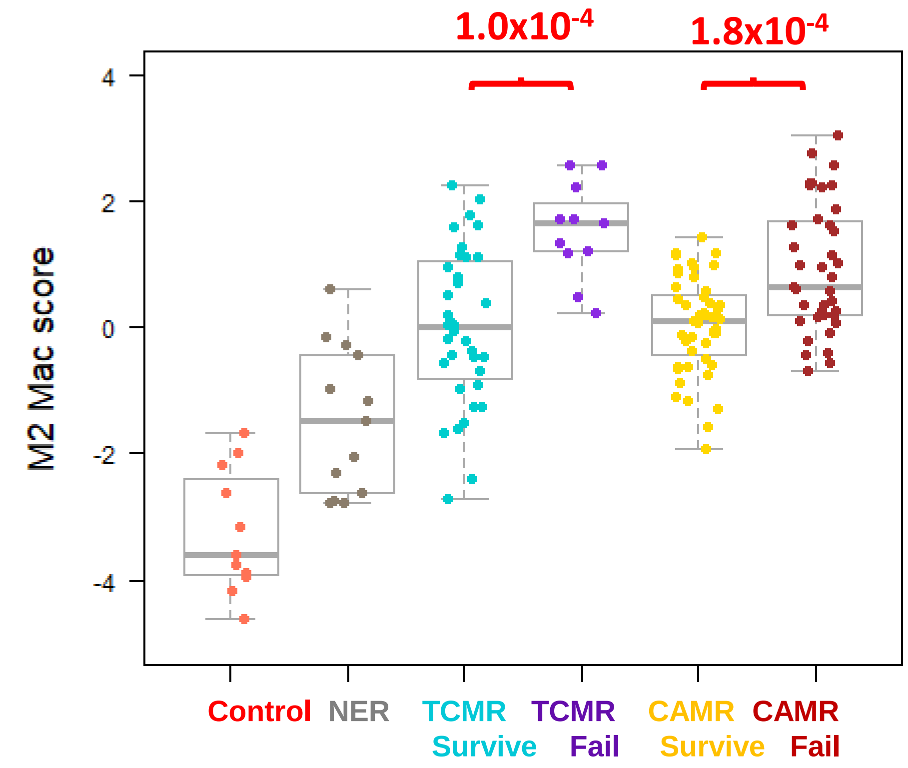


M1 Gene panel

M1

IL21R
CCL19
CCR7
CD38
CD40
CXCL10
CXCL13
CXCL9
IDO1
IL12
IL6
LAG3
LILRB2
NOD1
PLA1A
SOCS1
TLR7
TNF
TNFAIP6

B

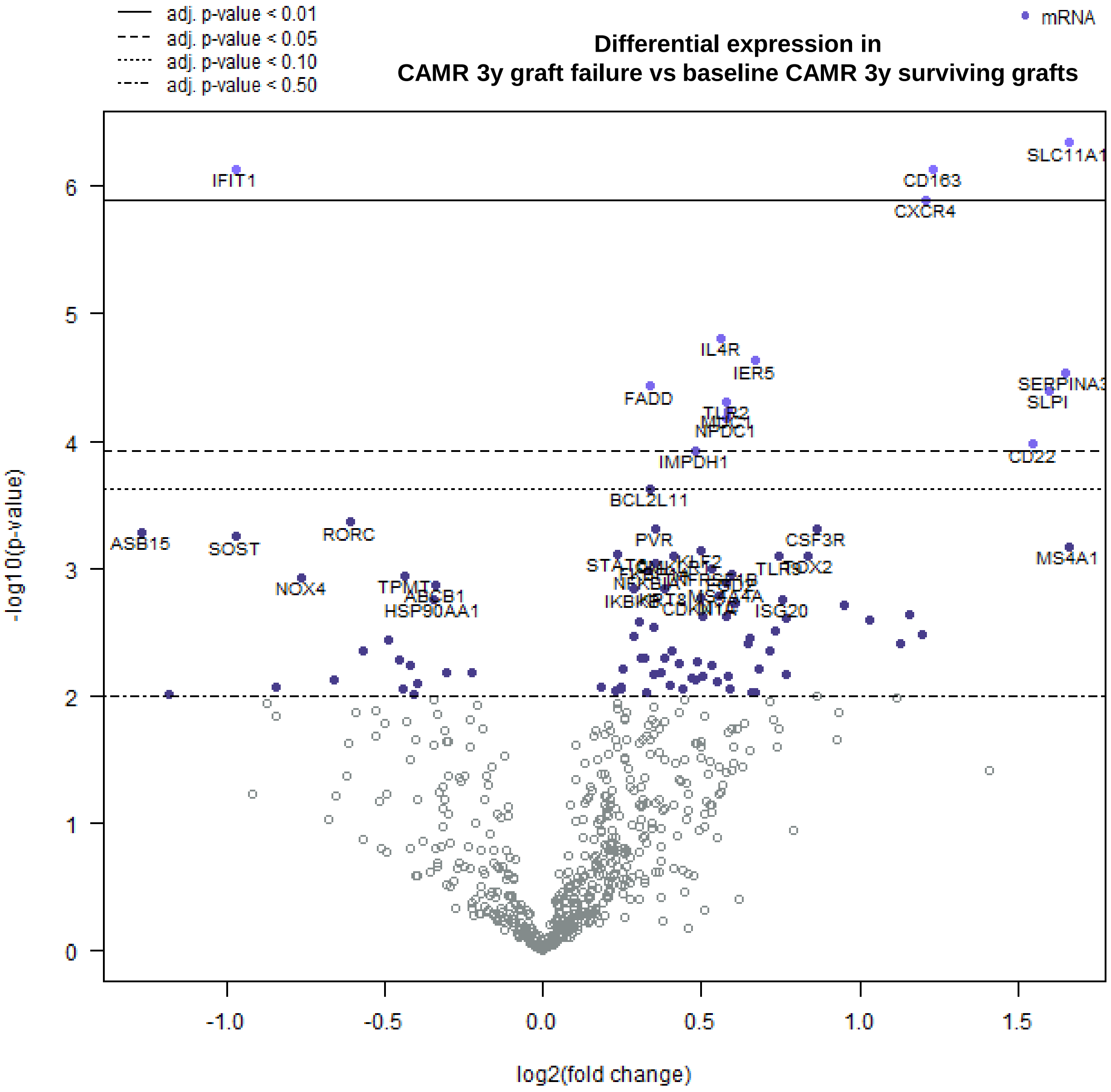


M2 Gene panel

M2

ALOX15
ARG1
CCL13
CCL18
CD163
CD68
CD209
IL10
MS4A6A
PDCD1LG2
TGFB1

Supplemental Figure 7



CAMR
3 year graft failure

Gene	Log2 fold change	std error (log2)	BY.p.value
SLC11A1	1.66	0.315	0.00112
SERPINA3	1.65	0.382	0.0191
SLPI	1.6	0.378	0.0207
CD22	1.55	0.389	0.0375
CD163	1.23	0.239	0.00112
CXCR4	1.21	0.24	0.00145
IER5	0.673	0.154	0.0178
MUC1	0.584	0.141	0.0243
NPDC1	0.58	0.141	0.0256
TLR2	0.576	0.138	0.0224
IL4R	0.559	0.125	0.0143
IMPDH1	0.479	0.121	0.0389
FADD	0.335	0.0788	0.0207

🟩 Macrophage

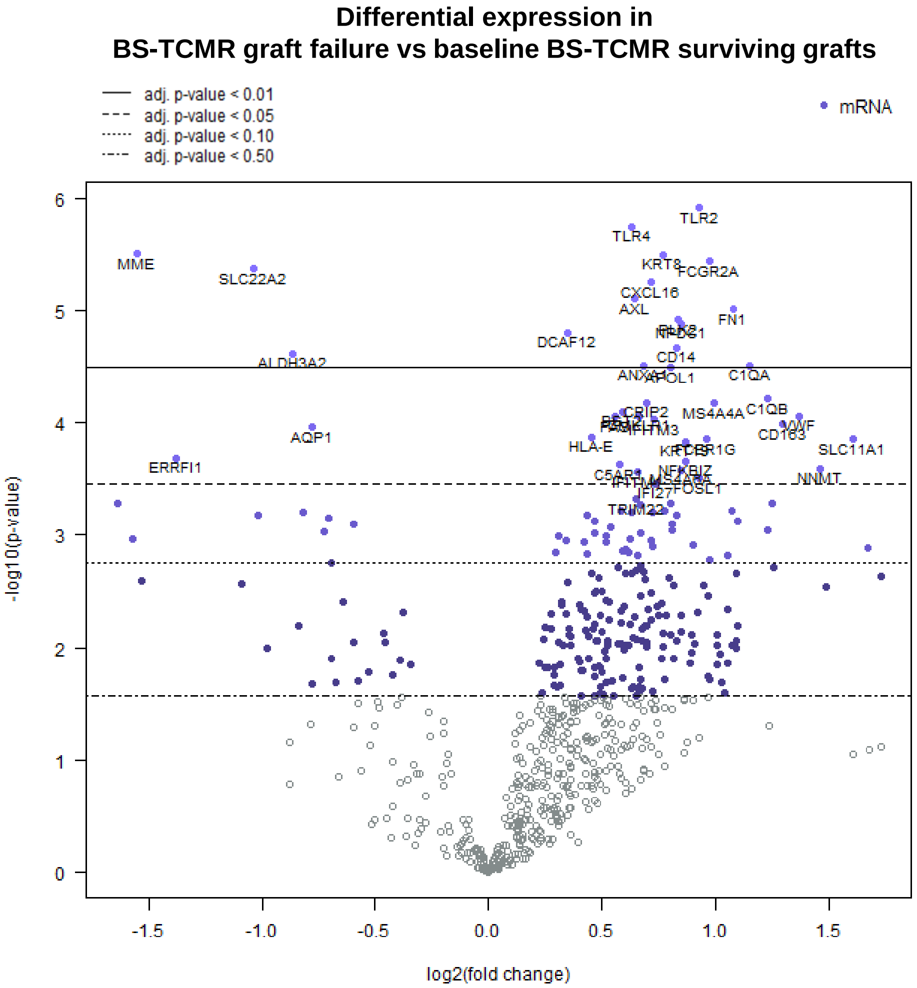
CAMR excluding CAMR+TCMR

Supplemental Figure 8

BS-TCMR Survival

Gene	Log2 fold change	std error (log2)	BY.p.value
MME	-1.55	0.321	3.27E-03
ERRFI1	-1.38	0.363	2.92E-02
SLC22A2	-1.04	0.217	3.27E-03
ALDH3A2	-0.867	0.199	7.84E-03
AQP1	-0.777	0.196	1.85E-02
DCAF12	0.35	0.0784	5.95E-03
HLA-E	0.454	0.116	2.14E-02
FAS	0.56	0.139	1.68E-02
C5AR1	0.581	0.154	3.17E-02
BST2	0.594	0.146	1.68E-02
TLR4	0.633	0.127	3.27E-03
AXL	0.646	0.14	4.50E-03

Tubular Epithelium



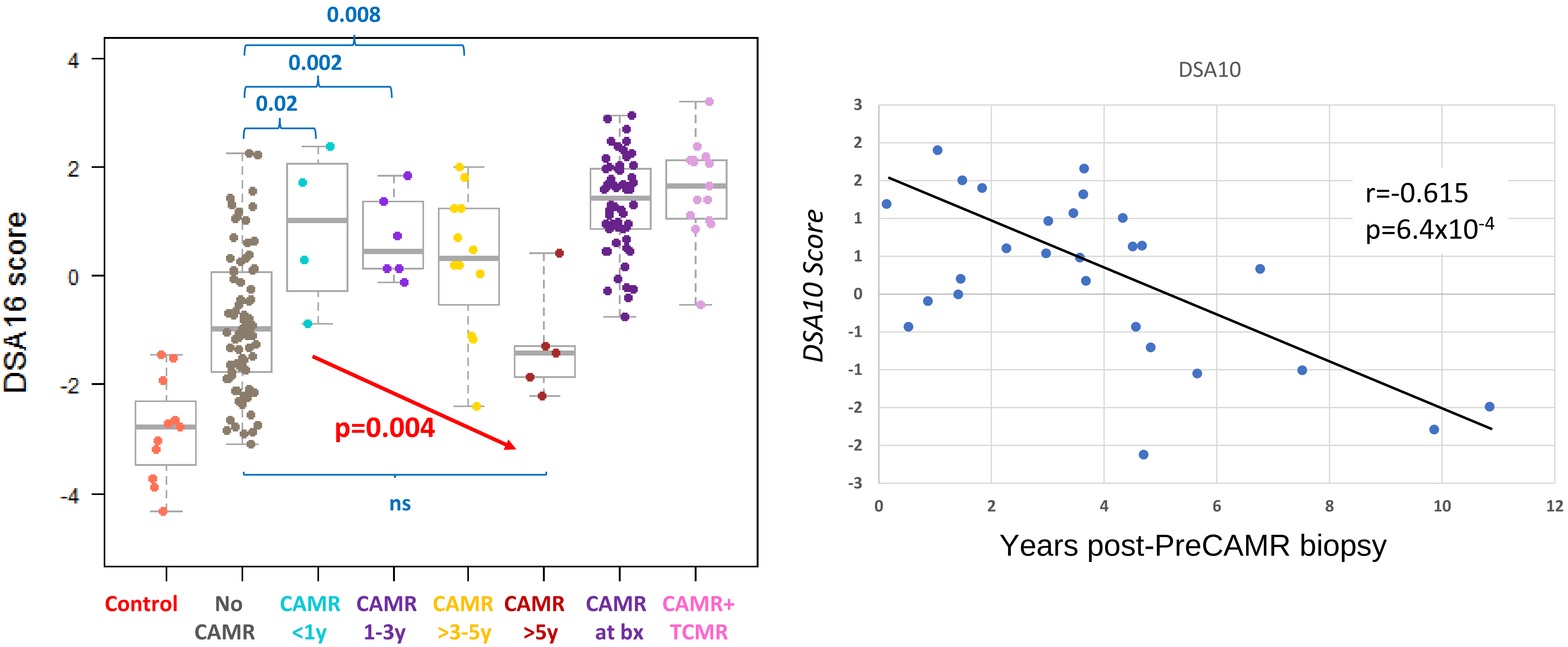
BS-TCMR Fail

Gene	Log2 fold change	std error (log2)	BY.p.value
SLC11A1	1.61	0.411	2.14E-02
NNMT	1.46	0.391	3.32E-02
VWF	1.37	0.341	1.68E-02
CD163	1.3	0.327	1.79E-02
C1QB	1.23	0.299	1.53E-02
C1QA	1.15	0.268	8.64E-03
FN1	1.08	0.237	4.89E-03
MS4A4A	0.996	0.243	1.53E-02
FCGR2A	0.972	0.202	3.27E-03
FCER1G	0.961	0.246	2.14E-02
TLR2	0.93	0.184	3.27E-03

Macrophage

Endothelium

Supplemental Figure 9



Supplemental Figure 10

Random Forest Outcome Classification

CAMR/Mixed

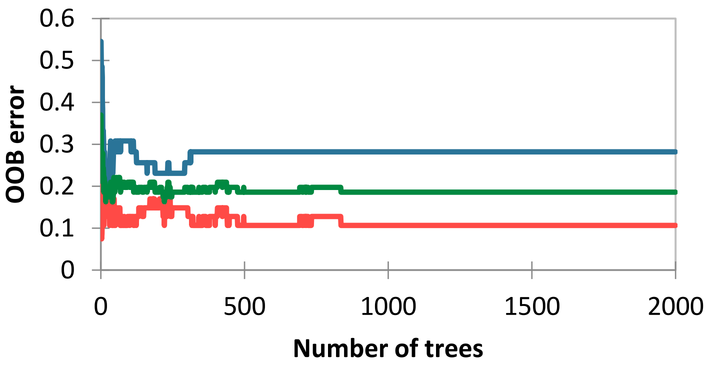
Confusion matrix (OOB sample):

	Outcome Observed			
RF Classification	**Survive**	**Fail**	**Total**	**% Correct with RF Classification**
Survive	**42**	**11**	**53**	**79%**
Fail	**5**	**28**	**33**	**85%**
Total	**47**	**39**	**86**	
% Correct without RF	**55%**	**45%**		

Chi2 6.4x10-9

Forest type: Classification
Method: Bagging
Sampling method: Random with replacement
Sample sizes: 60
Required number of trees in the forest: 2000
Number of trees built: 2000
Seed (random numbers): 1757329514

OOB error evolution



Top 30 Variables by Importance

Variables	Overall
SLC11A1	**36.879**
Cr at biopsy	**16.944**
% Globally Sclerotic	**16.150**
CD207	**13.359**
CD163	**11.187**
Glomerular Endothelium	**11.115**
TLR2	**10.249**
IFIT1	**9.740**
CD274	**9.533**
NFKBIA	**9.323**
FCER1A	**9.271**
TNFRSF1B	**8.700**
ANKRD22	**8.606**
Complement Inhibition	**7.464**
CDKN1A	**7.188**
% C4d	**7.135**
MAPK13	**6.574**
PDGFRB	**6.273**
SERPINE1	**6.243**
SLC4A1	**6.073**
% i-IFTA	**5.847**
KT2	**5.369**
MRC1	**5.268**
ah	**5.176**
HLA-C	**4.365**
IL4R	**3.354**
i	**3.238**
ASB15	**2.617**
Years post transplant	**2.597**
Macrophages	**2.428**

Clinical/Lab
Pathway/Cell
Pathology
Gene

*All higher in Fail group except KT2, Glomerular endothelium, *FCER1A, ASB15, PDGFRB, IFIT1, CD207,SLC4A1*

Supplemental Figure 11

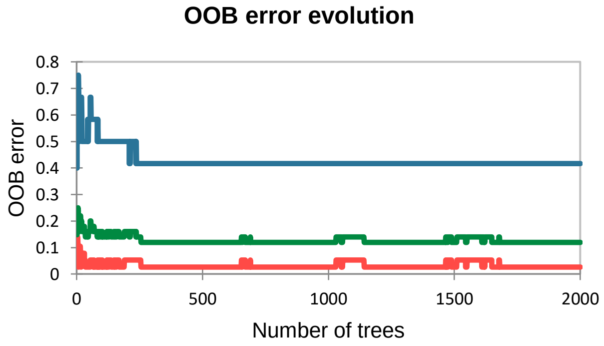
Random Forest Outcome Classification

BS-TCMR

Confusion matrix
(OOB sample):

	Outcome Observed			
RF Classification	**Survive**	**Fail**	**Total**	**% correct with RF Classification**
Survive	**37**	**5**	**42**	**88%**
Fail	**1**	**7**	**8**	**88%**
Total	**38**	**12**	**50**	
% Correct without RF	**76%**	**24%**		
			Chi2	**2.7x10-5**

Forest type: Classification
Method: Bagging
Sampling method: Random with replacement
Sample sizes: 35
Required number of trees in the forest: 2000
Number of trees built: 2000
Seed (random numbers): 671507506



Top 39 Variables by Importance

Variables	Overall
🟧 Cytosolic DNA Sensing	12.491
🟧 Extracellular Matrix Organization	11.032
ALAS1	10.312
IFTA	10.246
TLR4	9.294
IFI6	8.973
🟧 Endothelium	8.932
CRIP2	8.635
CXCL16	8.605
CD14	8.211
🟧 Pan-BM	8.193
🟧 IRRAT	7.922
🟩 Years post transplantation	7.884
🟨 g	7.342
KRT8	6.930
🟧 M2 Macrophage	6.809
🟧 AKI	6.553
FAS	6.441
🟧 Fibrosis	6.035
🟧 Neutrophil degranulation	5.404
🟨 mi (ptc+g)	5.357
C1QA	5.345
NOS3	4.795
MS4A7	4.770
🟨 i	4.721
FCER1G	4.473
PLAUR	4.307
IRITD3	4.269
RHOJ	4.230
TLR5	4.211
DCAF12	3.061
COL3A1	2.836
🟨 ptc	2.781
🟧 Progression GoCAR	2.609
VWF	2.437
🟧 *mTOR*	2.356
MS4A6A	1.833
ITGAM	1.639
CCL21	0.835

🟩 **Clinical/Lab**
🟧 **Pathway/Cell**
🟨 **Pathology**
Gene

*All variables higher in Fail group, except i

Supplemental Figure 12A

CAMR/Mixed
Clinical and Lab Variables

Confusion matrix (OOB sample):

from \ to	G1 CAMR Survive	G2 CAMR Fail	Total	% correct
G1 CAMR Survive	37	7	44	84.1
G2 CAMR Fail	10	28	38	73.7
Total	47	35	82	79.3

Variables	G1 CAMR Survive	G2 CAMR Fail	Overall
Cr at biopsy	62.480	70.302	80.164
Years post transplant	16.100	17.155	21.156
Age (yrs)	17.352	5.888	14.690
Gender	-2.184	10.105	7.200
# DSAs	-2.673	7.488	3.741
DSA	4.265	-0.492	2.282
Max MFI	-8.820	8.232	1.462
DSA Class	-2.293	-1.891	-2.838

Total Correct 79%

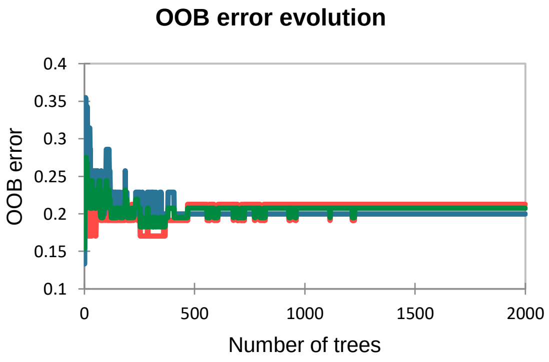
Forest type: Classification
Method: Bagging

Sampling method: Random with replacement
Sample sizes: 70

Required number of trees in the forest: 2000

Number of trees built: 2000

Seed (random numbers): 692640582



Omit 4 Cr>10

Supplemental Figure 12B

CAMR/Mixed Pathology Variables

Confusion matrix (OOB sample):

from \ to	G1 CAMR Survive I	G2 CAMR Fail I	Total	% correct
G1 CAMR Survive	36	14	50	72.0
G2 CAMR Fail	11	25	36	69.4
Total	47	39	86	70.9

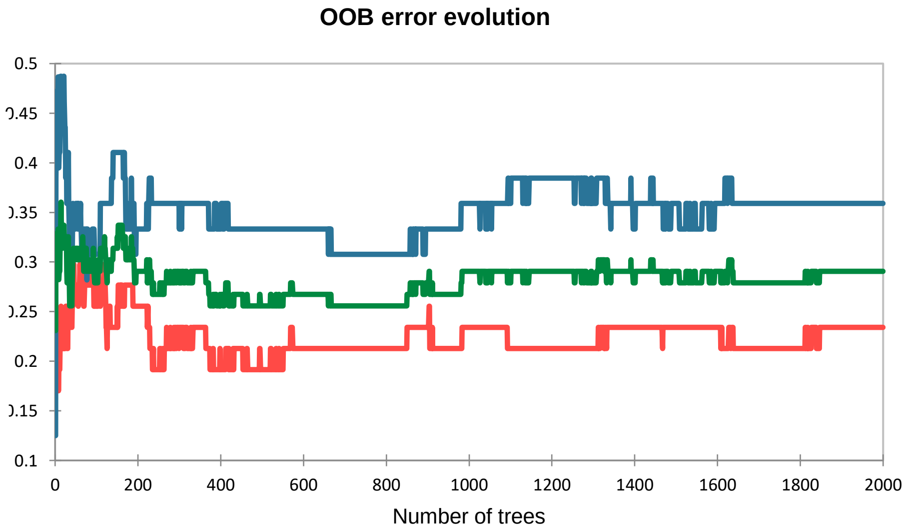
Total Correct 71%

Variables	G1 CAMR Survive	G2 CAMR Fail	Overall
%C4d	31.386	20.355	33.667
ah	17.586	18.760	24.310
i+t	13.034	13.561	17.326
i	8.461	9.734	12.081
C4d	12.066	1.597	10.369
% iIFTA	4.307	3.004	4.938
ci	-1.055	5.626	2.894
% fibrosis	2.351	1.782	2.856
% Globally Sclerotic	2.456	0.769	2.193
cv	-2.647	5.301	1.831
t	-1.718	3.313	1.282
v	-3.270	2.887	-0.044
cg	-3.386	1.863	-1.150
ct	-1.743	-0.036	-1.267
mi (ptc+g)	-7.455	5.160	-1.456
g	-3.818	1.038	-2.514
% tubular atrophy	-4.025	-2.417	-4.662
ptc	-5.832	-1.233	-4.777

Forest type: Classification
Method: Bagging

Sampling method: Random with replacement
Sample sizes: 70
Required number of trees in the forest: 2000
Number of trees built: 2000

Seed (random numbers): 1110815619



Supplemental Figure 12C

CAMR/Mixed
Pathway/Cell Type Scores

Confusion matrix (OOB sample):

from \ to	G1 CAMR Survive I	G2 CAMR Fail I	Total	% correct
G1 CAMR Survive I	30	16	46	65.2
G2 CAMR Fail I	17	23	40	57.5
Total	47	39	86	61.6

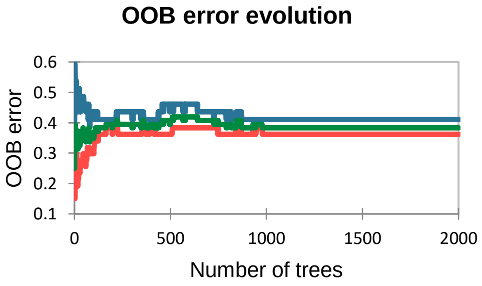
Total Correct 62%

Forest type: Classification
Method: Bagging

Sampling method: Random with replacement
Sample sizes: 70

Required number of trees in the forest: 2000
Number of trees built: 2000

Seed (random numbers): 1362447273



Top 20

Variables	G1 CAMR Survive I	G2 CAMR Fail I	Overall
Complement Inhibition	17.871	16.179	21.304
KT2	9.924	9.046	12.058
Macrophages.vs.CD45	10.032	7.278	10.878
GBM	9.067	8.975	10.715
CD4.vs.CD45	7.846	7.330	9.322
Glomerular Endothelium	6.905	5.856	8.139
eGFR later	7.273	3.213	7.947
M2 Mac	7.714	3.842	7.728
AMAT1	5.305	5.309	7.558
MS1	3.466	6.983	7.368
Tubule	5.902	4.069	6.448
AKI	6.359	1.741	5.945
Rho GTPase signaling	3.227	4.872	5.486
Neutrophil degranulation	4.479	3.736	5.481
TGF-beta Signaling	3.290	2.439	4.261
KT1	0.330	5.362	4.235
Macrophages	3.176	3.496	4.132
M2 Mac10	5.323	-0.933	3.833
AMR	-0.030	4.803	3.613
IRRAT	3.084	0.357	3.454

Supplemental Figure 12D

CAMR/Mixed
Individual transcript counts

Confusion matrix (OOB sample):

from \ to	G1 CAMR Survive I	G2 CAMR Fail I	Total	% correct
G1 CAMR Survive I	39	11	50	78.0
G2 CAMR Fail I	8	28	36	77.8
Total	47	39	86	77.9

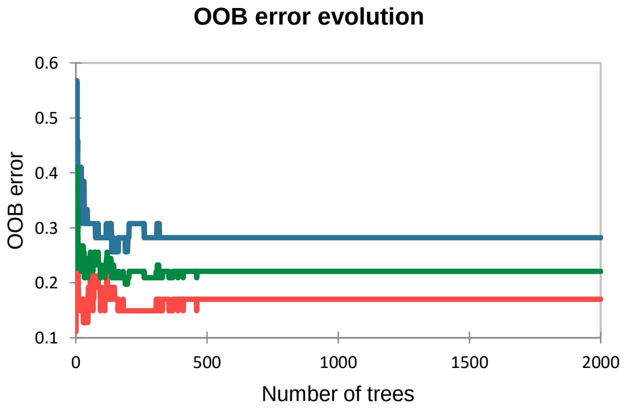
Total Correct 78%

Forest type: Classification
Method: Bagging

Sampling method: Random with replacement
Sample sizes: 70

Required number of trees in the forest: 2000
Number of trees built: 2000

Seed (random numbers): 22568641



Top 25

Variables	G1 CAMR Survive I	G2 CAMR Fail I	Overall
SLC11A1	33.067	32.116	36.304
TLR2	9.610	10.232	11.251
CD274	-0.705	8.357	7.888
FCER1A	2.633	7.506	7.815
TNFRSF1B	7.231	5.093	7.308
CD163	6.628	5.328	7.082
NFKBIA	6.355	4.907	7.021
IFIT1	6.017	5.214	6.646
ANKRD22	3.034	6.481	6.369
SERPINE1	4.906	4.660	5.572
S100A9	4.038	4.490	5.168
SLC4A1	3.422	4.156	4.821
CD207	4.201	4.011	4.653
MALL	4.086	2.775	4.611
MRC1	3.506	3.015	3.950
MS4A4A	1.680	4.189	3.804
BRWD1	5.198	-1.134	3.749
CMKLR1	2.203	3.403	3.526
MAPK13	2.961	2.419	3.365
GBP5	-0.401	3.417	3.299
GIMAP5	1.655	2.393	3.245
TGIF1	3.214	2.391	3.208
CDH13	0.209	3.443	3.075
HYAL2	2.811	1.735	2.960
SLC12A3	3.036	1.070	2.887

Supplemental Figure 13A

BS-TCMR
Clin/Lab

Confusion matrix (OOB sample):

from \ to	F1 TCMR-BS Survive	F2 TCMR-BS Fail	Total	% correct
F1 TCMR-BS Survive	31	12	43	72.1
F2 TCMR-BS Fail	7	0	7	0.0
Total	38	12	50	62.0

Variables	F1 TCMR-BS Survive	F2 TCMR-BS Fail	Overall
DSA Present at biopsy 1-26-22	15.793	-3.273	12.383
# DSAs	2.131	-1.918	0.693
Years post tx (Calc)	3.479	-8.562	-2.162
Gender	-3.506	0.859	-2.482
Cr at time of bx	-4.201	-0.179	-2.953

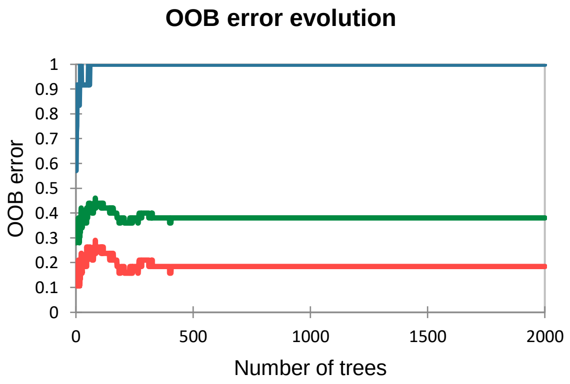
Total Correct 62%

Forest type: Classification
Method: Bagging

Sampling method: Random with replacement
Sample sizes: 35

Required number of trees in the forest: 2000
Number of trees built: 2000

Seed (random numbers): 1975305146



Supplemental Figure 13B

BS-TCMR Pathology

Confusion matrix (OOB sample):

from \ to	F1 TCMR-BS Survive	F2 TCMR-BS Fail	Total	% correct
F1 TCMR-BS Survive	33	10	43	76.7
F2 TCMR-BS Fail	5	2	7	28.6
Total	38	12	50	70.0

Variables	F1 TCMR-BS Survive	F2 TCMR-BS Fail	Overall
ptc	13.726	11.985	15.959
mi (ptc+g)	13.430	12.893	15.145
% fibrosis	11.399	9.040	12.930
g	11.801	5.629	10.765
C4d	9.980	4.232	8.763
% tubular atrophy	8.682	-3.054	5.962
i	11.635	-7.480	5.174
ct	4.102	-2.642	3.181
v	4.194	-2.874	2.525
t	4.979	-6.633	0.664

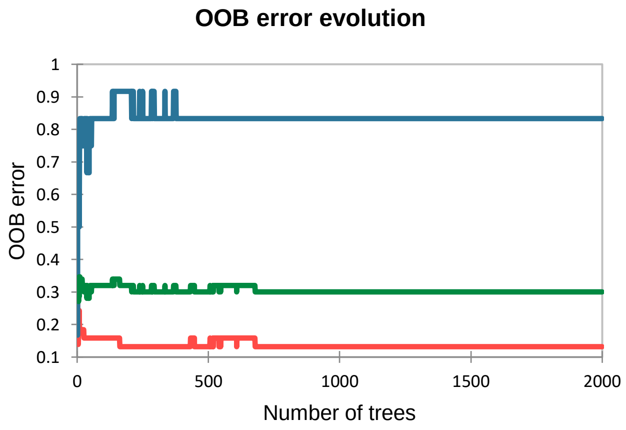
Total Correct 70%

Forest type: Classification
Method: Bagging

Sampling method: Random with replacement
Sample sizes: 35

Required number of trees in the forest: 2000
Number of trees built: 2000

Seed (random numbers): 678733136



Supplemental Figure 13C

Confusion matrix (OOB sample):

from \ to	F1 TCMR-BS Survive	F2 TCMR-BS Fail	Total	% correct
F1 TCMR-BS Survive	34	5	39	87.2
F2 TCMR-BS Fail	4	7	11	63.6
Total	38	12	50	82.0

BS-TCMR
Pathways/Cell Types

Variables	F1 TCMR-BS Survive	F2 TCMR-BS Fail	Overall
Extracellular Matrix Organization	17.438	9.663	16.878
Pan-BM	15.828	7.391	15.633
IRRAT	15.482	2.602	14.722
IFTA	7.710	6.094	8.830
Neutrophil degranulation	8.865	5.423	8.812
Endothelium	8.174	6.327	8.343
Macrophages	7.558	5.609	7.965
Cytosolic DNA Sensing	7.933	0.965	7.864
M2 Mac	6.785	4.650	7.081
IRITD5	5.690	4.292	6.033
Fibrosis	5.961	3.899	6.012
Complement Components	3.944	4.607	5.262
IRITD3	5.365	3.982	5.034
Complement Inhibition	3.676	3.558	4.853
AKI	6.453	1.416	4.679
KT1	2.625	4.132	4.187
NK	4.117	1.214	4.169
CAMR NHP	4.809	2.553	4.075
Progression GoCAR	3.953	1.820	3.583
ABMR-RATs	6.802	-2.633	3.347
M2 Mac11	2.933	1.967	3.057
Exhausted CD8	2.576	0.633	2.693
QCMAT	2.593	1.224	2.556
ENDAT	2.481	0.900	2.514
IL6 Signaling	2.310	0.426	2.482

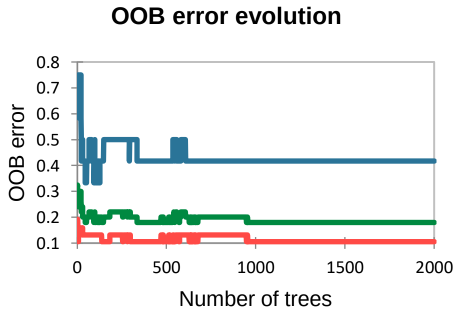
Total Correct 82%

Forest type: Classification
Method: Bagging

Sampling method: Random with replacement
Sample sizes: 35

Required number of trees in the forest: 2000
Number of trees built: 2000

Seed (random numbers): 651298721



Supplemental Figure 13D

BS-TCMR
Individual Transcripts

Confusion matrix (OOB sample):

from \ to	F1 TCMR-BS Survive	F2 TCMR-BS Fail	Total	% correct
F1 TCMR-BS Survive	33	9	42	78.6
F2 TCMR-BS Fail	5	3	8	37.5
Total	38	12	50	72.0

Total Correct 72%

Variables	F1 TCMR-BS Survive	F2 TCMR-BS Fail	Overall
FCGR2A	9.846	10.431	10.845
IFI6	8.048	7.693	8.259
KRT8	7.152	6.795	7.431
COL4A1	5.453	7.716	7.148
TLR4	5.963	5.227	6.251
FN1	4.919	6.165	5.992
CXCL16	4.644	5.303	5.358
CD14	4.288	4.896	5.024
CRIP2	4.401	4.181	4.415
ALAS1	3.857	4.356	4.253
ITGAX	4.025	2.548	4.099
FAS	3.725	3.889	4.091
MS4A7	3.199	4.178	3.932
COL1A1	3.042	3.654	3.831
C1QA	3.300	3.729	3.780
CD163	2.471	3.865	3.574
ITGB6	2.646	3.165	3.410
VWF	3.255	3.183	3.385
FCER1G	2.890	3.241	3.327
DCAF12	3.322	2.849	3.312
LCN2	3.278	3.129	3.288
TNF	3.022	1.029	3.243
RHOJ	2.865	2.872	3.028
ISG15	2.549	2.521	2.831
MS4A4A	2.051	2.790	2.736
AXL	2.460	2.447	2.620
PDCD1LG2	2.694	1.665	2.561
COL3A1	2.116	2.970	2.558
PLAUR	2.358	2.394	2.548
NOS3	2.371	2.351	2.514

Forest type: Classification
Method: Bagging

Sampling method: Random with replacement
Sample sizes: 35

Required number of trees in the forest: 2000
Number of trees built: 2000

Seed (random numbers): 1908038177