# CTOT 08 Study Design
# 24-month Multi-Center Observational Study
# Surveillance Biopsies at 2-6, 12 and 24 months

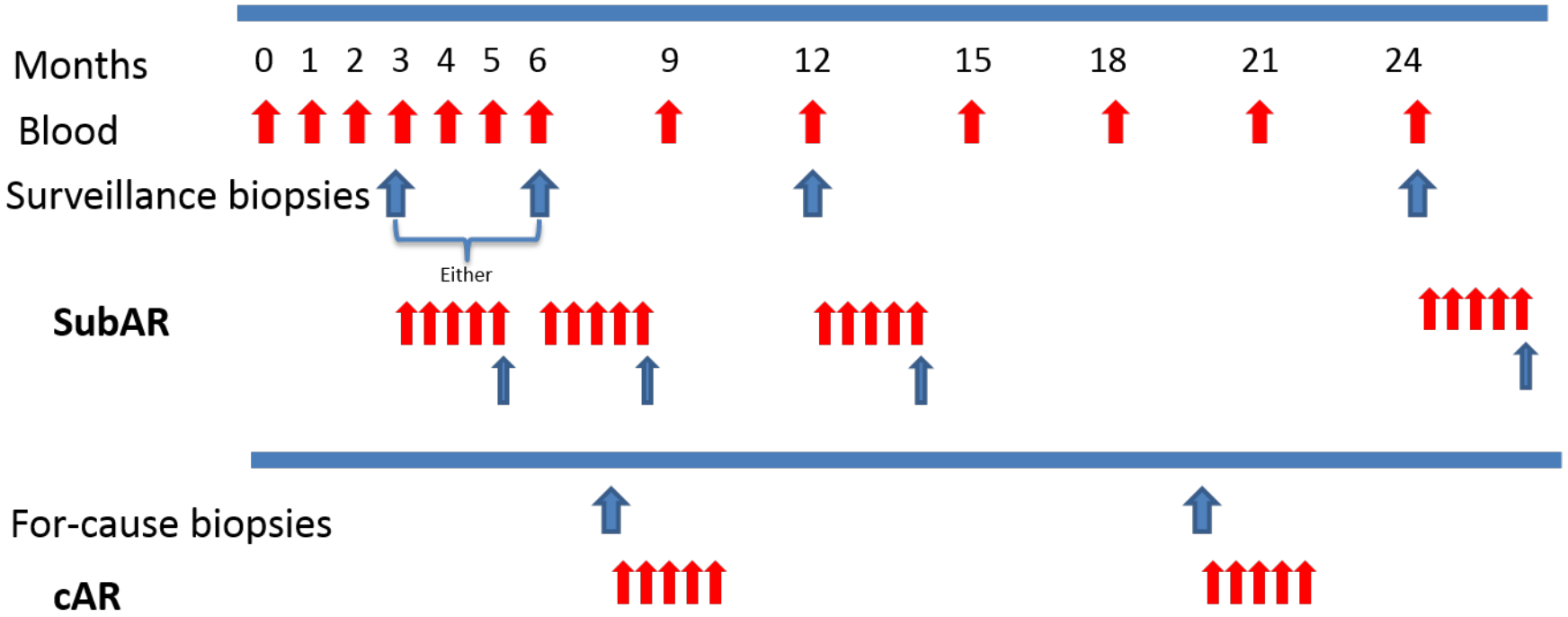



Figure S1

# ComBat Adjustment By Phenotype

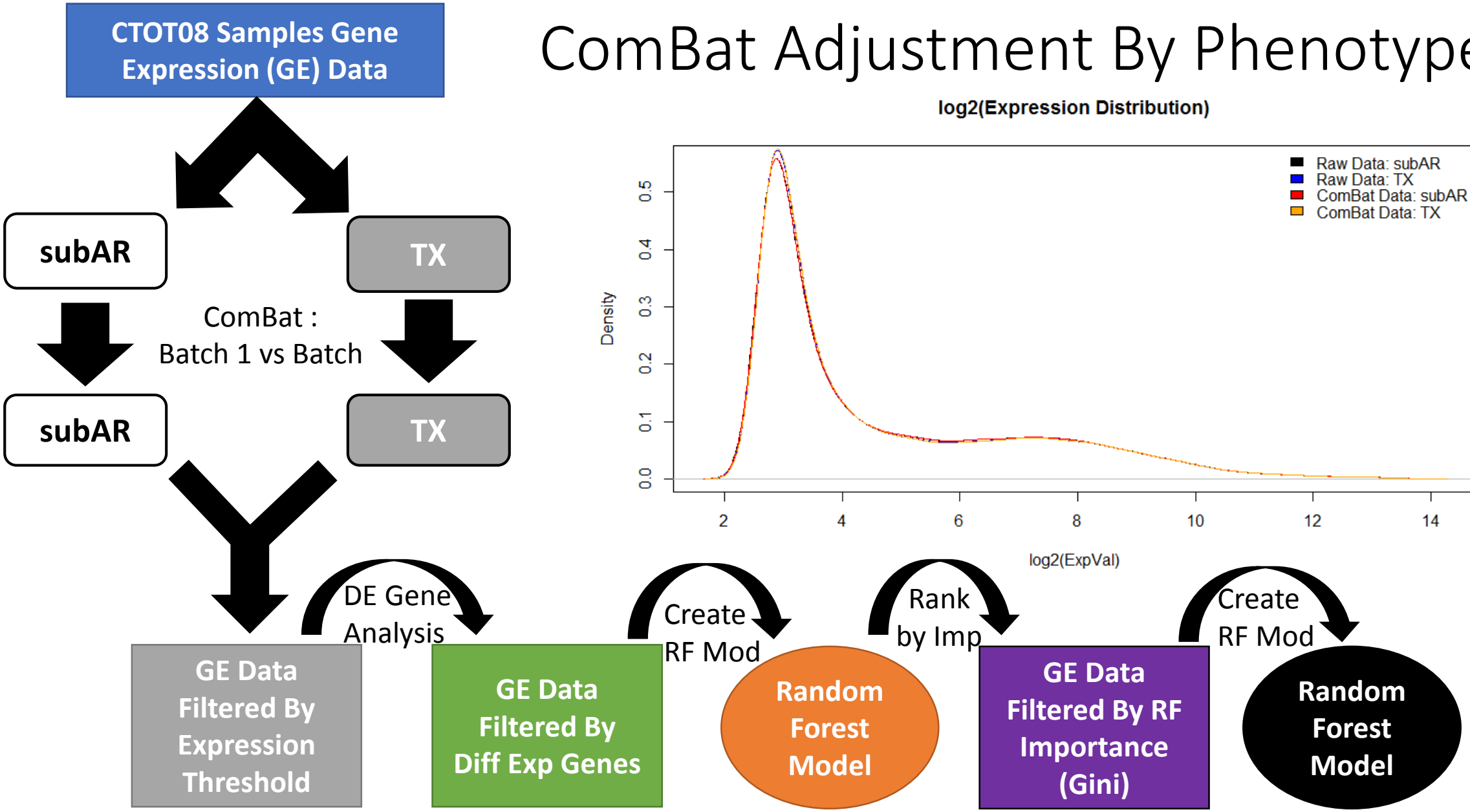



Figure S2

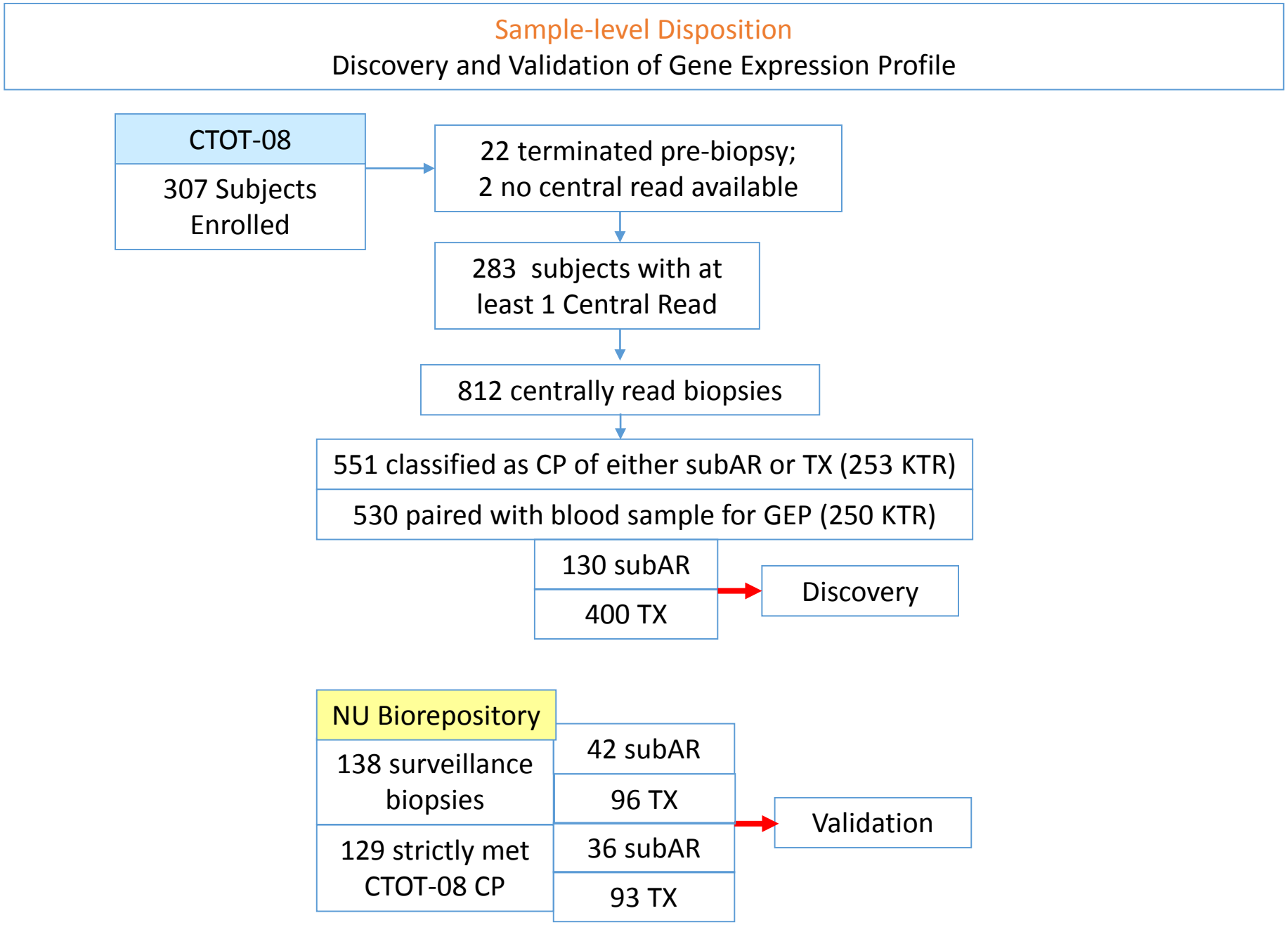

Sample-level Disposition
Discovery and Validation of Gene Expression Profile
CTOT-08
307 Subjects Enrolled
22 terminated pre-biopsy;
2 no central read available
283 subjects with at least 1 Central Read
812 centrally read biopsies
551 classified as CP of either subAR or TX (253 KTR)
530 paired with blood sample for GEP (250 KTR)
130 subAR
400 TX
Discovery
NU Biorepository
138 surveillance biopsies
42 subAR
96 TX
129 strictly met CTOT-08 CP
36 subAR
93 TX
Validation

Figure S3 A

Subject-level Disposition

Prevalence of Clinical Phenotype and Gene Expression Profile, and Impact on Month 24 Graft Outcome

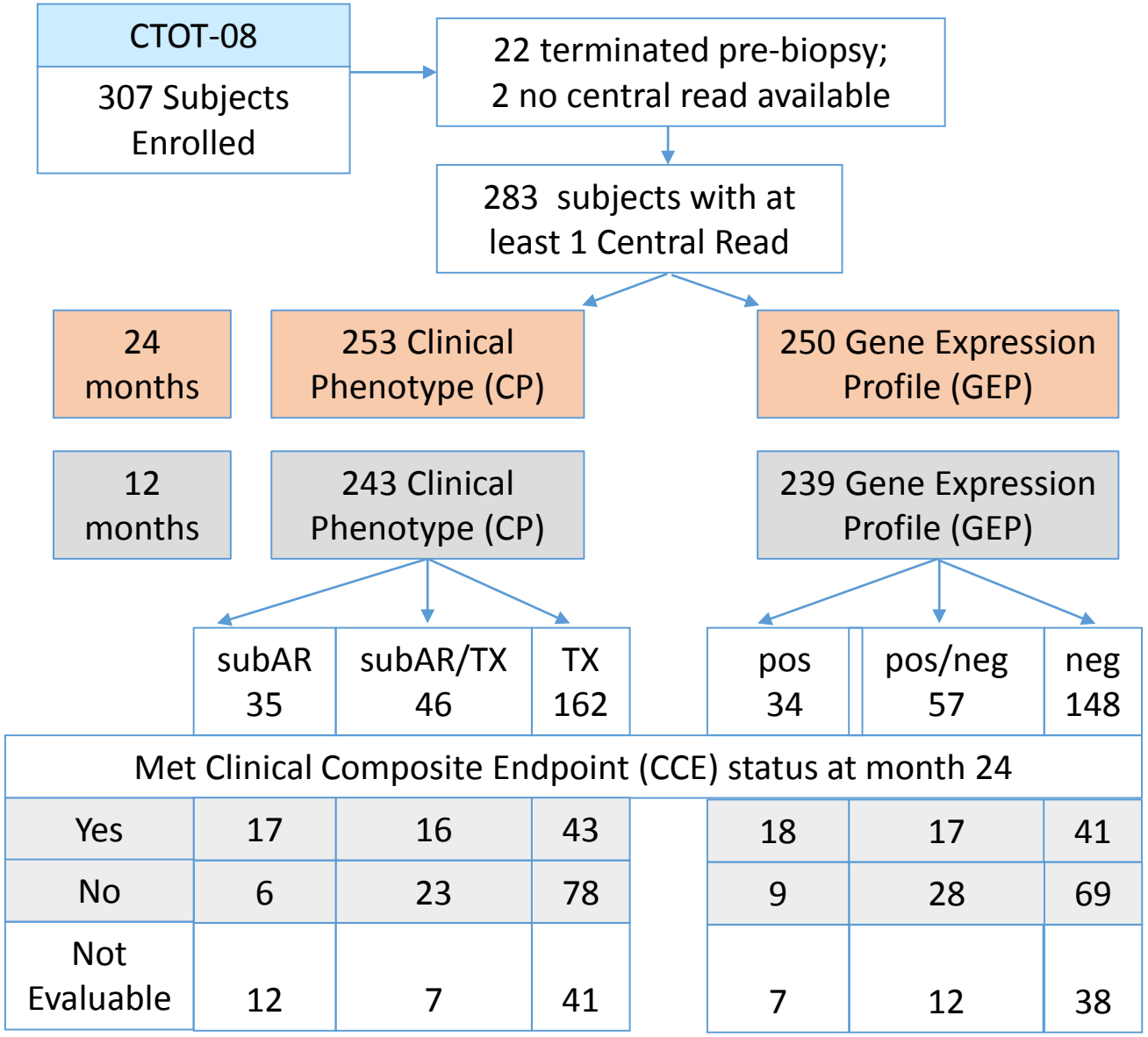
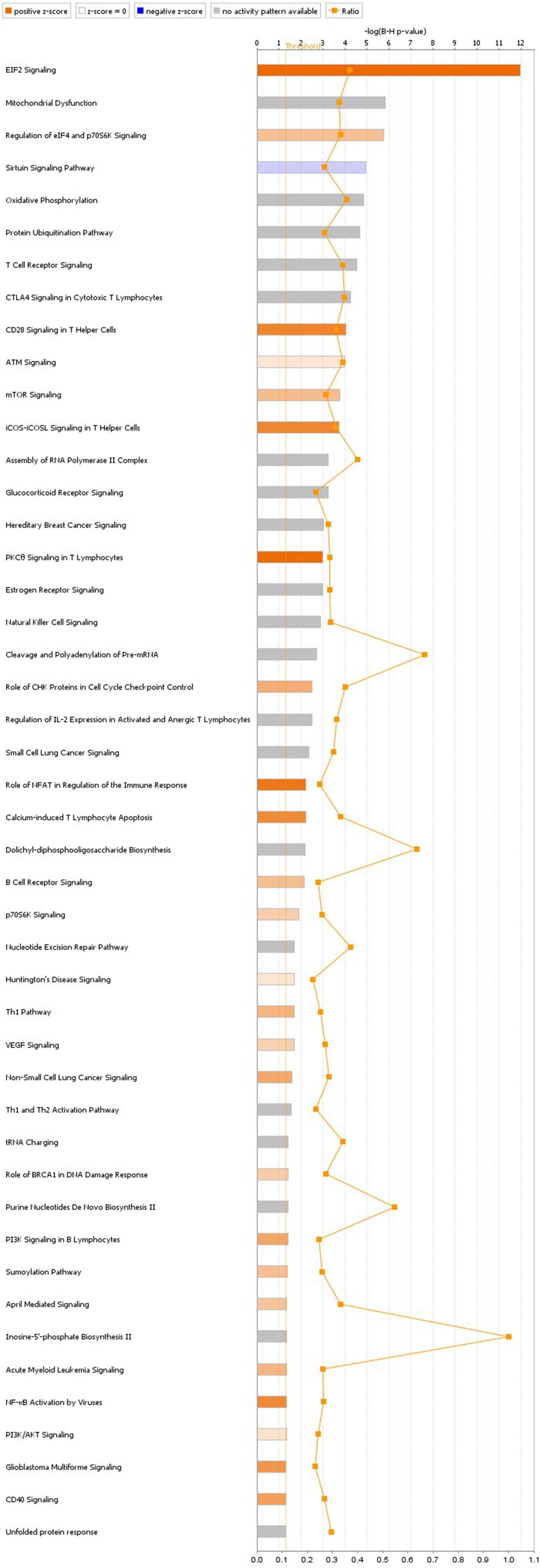



Figure S3 B


positive z-score
z-score = 0
negative z-score
no activity pattern available
Ratio
-log(B-H p-value)
0
1
2
3
4
5
6
7
8
9
10
11
12
Threshold
EIF2 Signaling
Mitochondrial Dysfunction
Regulation of eIF4 and p70S6K Signaling
Sirtuin Signaling Pathway
Oxidative Phosphorylation
Protein Ubiquitination Pathway
T Cell Receptor Signaling
CTLA4 Signaling in Cytotoxic T Lymphocytes
CD28 Signaling in T Helper Cells
ATM Signaling
mTOR Signaling
iCOS-iCOSL Signaling in T Helper Cells
Assembly of RNA Polymerase II Complex
Glucocorticoid Receptor Signaling
Hereditary Breast Cancer Signaling
PKCθ Signaling in T Lymphocytes
Estrogen Receptor Signaling
Natural Killer Cell Signaling
Cleavage and Polyadenylation of Pre-mRNA
Role of CHK Proteins in Cell Cycle Checkpoint Control
Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes
Small Cell Lung Cancer Signaling
Role of NFAT in Regulation of the Immune Response
Calcium-induced T Lymphocyte Apoptosis
Dolichyl-diphosphooligosaccharide Biosynthesis
B Cell Receptor Signaling
p70S6K Signaling
Nucleotide Excision Repair Pathway
Huntington's Disease Signaling
Th1 Pathway
VEGF Signaling
Non-Small Cell Lung Cancer Signaling
Th1 and Th2 Activation Pathway
tRNA Charging
Role of BRCA1 in DNA Damage Response
Purine Nucleotides De Novo Biosynthesis II
PI3K Signaling in B Lymphocytes
Sumoylation Pathway
April Mediated Signaling
Inosine-5'-phosphate Biosynthesis II
Acute Myeloid Leukemia Signaling
NF-κB Activation by Viruses
PI3K/AKT Signaling
Glioblastoma Multiforme Signaling
CD40 Signaling
Unfolded protein response
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
Ratio

CTOT08_subARvTX_exp5_fdr_0.1_deg FINAL Ingenuity - 2017-10-17 03:39 PM
NU_TSRI_subARvTX_limma_degenes_exp5_FDR_0.1 Ingenuity FINA

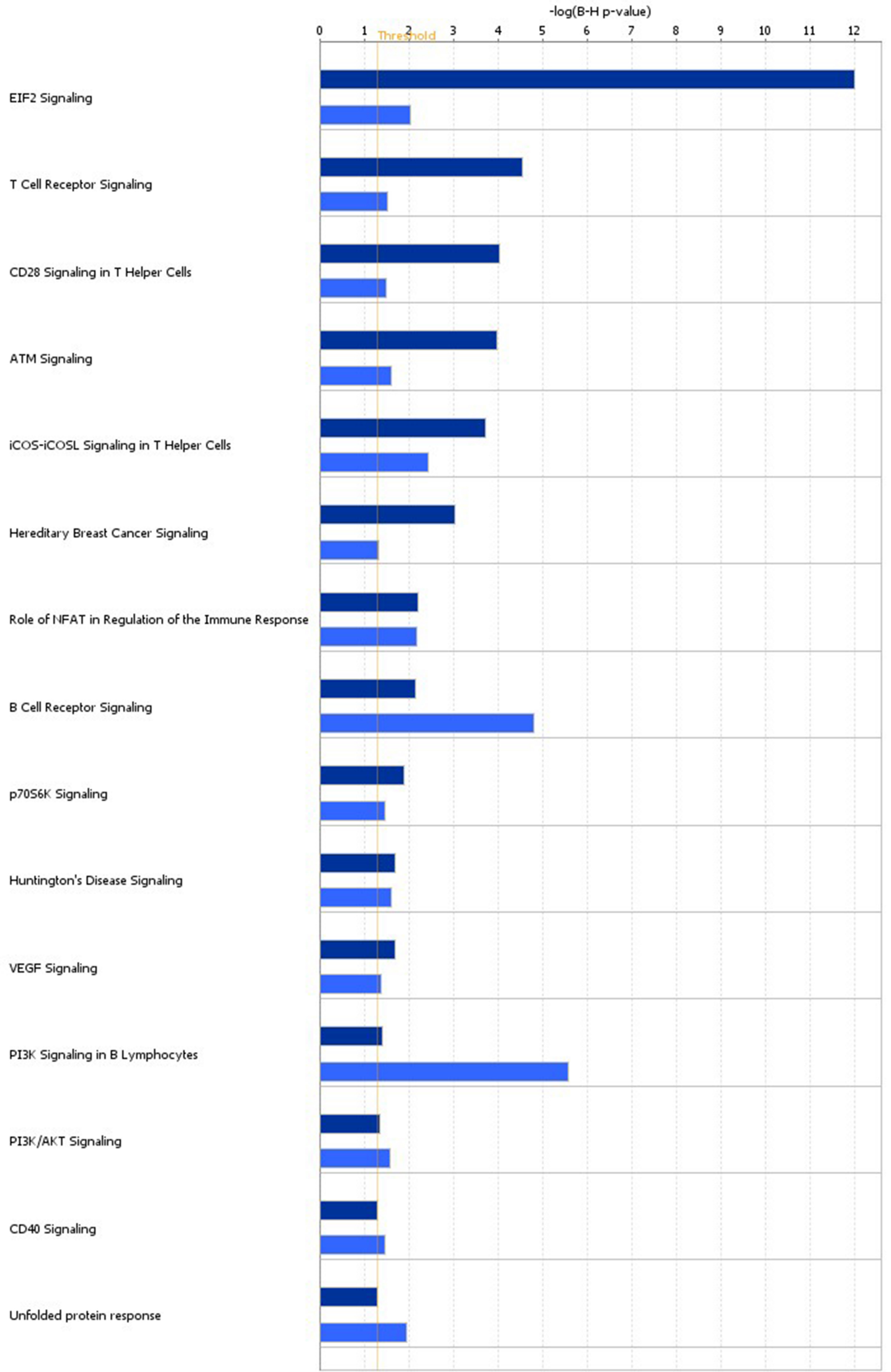


Table S1A

| NAME | ORIGINAL SIZE | AFTER RESTRICTING TO DATASET | STATUS |
|---|---|---|---|
| HALLMARK_TNFA_SIGNALING_VIA_NFKB | 200 | 34 | |
| HALLMARK_HYPOXIA | 200 | 25 | |
| HALLMARK_CHOLESTEROL_HOMEOSTASIS | 74 | 9 | |
| HALLMARK_MITOTIC_SPINDLE | 200 | 48 | |
| HALLMARK_WNT_BETA_CATENIN_SIGNALING | 42 | 6 | |
| HALLMARK_TGF_BETA_SIGNALING | 54 | 10 | |
| HALLMARK_IL6_JAK_STAT3_SIGNALING | 87 | 13 | |
| HALLMARK_DNA_REPAIR | 150 | 46 | |
| HALLMARK_G2M_CHECKPOINT | 200 | 53 | |
| HALLMARK_APOPTOSIS | 161 | 40 | |
| HALLMARK_NOTCH_SIGNALING | 32 | | Rejected! |
| HALLMARK_ADIPOGENESIS | 200 | 41 | |
| HALLMARK_ESTROGEN_RESPONSE_EARLY | 200 | 26 | |
| HALLMARK_ESTROGEN_RESPONSE_LATE | 200 | 28 | |
| HALLMARK_ANDROGEN_RESPONSE | 101 | 17 | |
| HALLMARK_MYOGENESIS | 200 | 15 | |
| HALLMARK_PROTEIN_SECRETION | 96 | 29 | |
| HALLMARK_INTERFERON_ALPHA_RESPONSE | 97 | 11 | |
| HALLMARK_INTERFERON_GAMMA_RESPONSE | 200 | 35 | |
| HALLMARK_APICAL_JUNCTION | 200 | 28 | |
| HALLMARK_APICAL_SURFACE | 44 | 6 | |
| HALLMARK_HEDGEHOG_SIGNALING | 36 | | Rejected! |
| HALLMARK_COMPLEMENT | 200 | 42 | |
| HALLMARK_UNFOLDED_PROTEIN_RESPONSE | 113 | 44 | |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 105 | 23 | |
| HALLMARK_MTORC1_SIGNALING | 200 | 42 | |
| HALLMARK_E2F_TARGETS | 200 | 53 | |
| HALLMARK_MYC_TARGETS_V1 | 200 | 90 | |

| | | | |
|---|---|---|---|
| HALLMARK_MYC_TARGETS_V2 | 58 | 27 | |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 200 | 19 | |
| HALLMARK_INFLAMMATORY_RESPONSE | 200 | 25 | |
| HALLMARK_XENOBIOTIC_METABOLISM | 200 | 38 | |
| HALLMARK_FATTY_ACID_METABOLISM | 158 | 32 | |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION | 200 | 78 | |
| HALLMARK_GLYCOLYSIS | 200 | 36 | |
| HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY | 49 | 8 | |
| HALLMARK_P53_PATHWAY | 200 | 30 | |
| HALLMARK_UV_RESPONSE_UP | 158 | 34 | |
| HALLMARK_UV_RESPONSE_DN | 144 | 16 | |
| HALLMARK_ANGIOGENESIS | 36 | | Rejected! |
| HALLMARK_HEME_METABOLISM | 200 | 32 | |
| HALLMARK_COAGULATION | 138 | 14 | |
| HALLMARK_IL2_STAT5_SIGNALING | 200 | 43 | |
| HALLMARK_BILE_ACID_METABOLISM | 112 | 16 | |
| HALLMARK_PEROXISOME | 104 | 27 | |
| HALLMARK_ALLOGRAFT_REJECTION | 200 | 60 | |
| HALLMARK_SPERMATOGENESIS | 135 | 17 | |
| HALLMARK_KRAS_SIGNALING_UP | 200 | 29 | |
| HALLMARK_KRAS_SIGNALING_DN | 200 | | Rejected! |
| HALLMARK_PANCREAS_BETA_CELLS | 40 | 5 | |

| NAME | SIZE | ES | NES | NOM p-val | FDR q-val |
|---|---|---|---|---|---|
| HALLMARK_ALLOGRAFT_REJECTION | 60 | 0.23833334 | 2.2235951 | 0.00193424 | 0.01883258 |
| HALLMARK_MYC_TARGETS_V2 | 27 | 0.27706343 | 1.6922538 | 0.02708333 | 0.17724957 |
| HALLMARK_E2F_TARGETS | 53 | 0.18842201 | 1.6042675 | 0.04868154 | 0.18714932 |
| HALLMARK_COMPLEMENT | 42 | 0.20145792 | 1.5660663 | 0.05068226 | 0.16818096 |
| HALLMARK_MYC_TARGETS_V1 | 90 | 0.1414142 | 1.5358555 | 0.06759443 | 0.15477161 |
| HALLMARK_WNT_BETA_CATENIN_SIGNALING | 6 | 0.340537 | 1.0449481 | 0.39285713 | 0.8247684 |
| HALLMARK_PANCREAS_BETA_CELLS | 5 | 0.35777417 | 0.9852894 | 0.45418328 | 0.8446085 |
| HALLMARK_INTERFERON_GAMMA_RESPONSE | 35 | 0.13671783 | 0.96372235 | 0.48643005 | 0.7880705 |
| HALLMARK_ESTROGEN_RESPONSE_LATE | 28 | 0.14549567 | 0.92958206 | 0.5346154 | 0.76739895 |
| HALLMARK_CHOLESTEROL_HOMEOSTASIS | 9 | 0.24811536 | 0.9167464 | 0.57938147 | 0.7158631 |
| HALLMARK_UNFOLDED_PROTEIN_RESPONSE | 44 | 0.10489515 | 0.8130562 | 0.71656686 | 0.8266552 |
| HALLMARK_SPERMATOGENESIS | 17 | 0.16332565 | 0.7919265 | 0.72888017 | 0.78835714 |
| HALLMARK_UV_RESPONSE_DN | 16 | 0.12705322 | 0.618169 | 0.93801653 | 0.92930704 |

| NAME | SIZE | ES | NES | NOM p-val | FDR q-val |
|---|---|---|---|---|---|
| HALLMARK_HEME_METABOLISM | 32 | -0.5254706 | -3.5342536 | 0 | 0 |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 19 | -0.49248 | -2.563297 | 0 | 0.00153571 |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION | 78 | -0.2403394 | -2.4869049 | 0 | 0.00226886 |
| HALLMARK_ADIPOGENESIS | 41 | -0.2994207 | -2.2813294 | 0.00210084 | 0.00504314 |
| HALLMARK_MYOGENESIS | 15 | -0.4827586 | -2.2637756 | 0.00187266 | 0.00479438 |
| HALLMARK_COAGULATION | 14 | -0.4854141 | -2.1370194 | 0.00200401 | 0.0121508 |
| HALLMARK_INTERFERON_ALPHA_RESPONSE | 11 | -0.5252989 | -2.135683 | 0 | 0.01041498 |
| HALLMARK_MITOTIC_SPINDLE | 48 | -0.2455179 | -2.0300305 | 0.00201613 | 0.017907 |
| HALLMARK_APICAL_JUNCTION | 28 | -0.3208161 | -1.9916805 | 0.0020202 | 0.01917527 |
| HALLMARK_HYPOXIA | 25 | -0.3088633 | -1.8169229 | 0.018 | 0.04775797 |
| HALLMARK_XENOBIOTIC_METABOLISM | 38 | -0.2456547 | -1.7982427 | 0.01953125 | 0.04759722 |
| HALLMARK_PROTEIN_SECRETION | 29 | -0.2778757 | -1.7744664 | 0.01176471 | 0.05081609 |
| HALLMARK_P53_PATHWAY | 30 | -0.2742574 | -1.7739929 | 0.01976285 | 0.04710695 |
| HALLMARK_APICAL_SURFACE | 6 | -0.5952849 | -1.7671478 | 0.00813008 | 0.04494856 |
| HALLMARK_KRAS_SIGNALING_UP | 29 | -0.2582395 | -1.6658285 | 0.0407767 | 0.07239236 |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 23 | -0.2882564 | -1.6344534 | 0.02658487 | 0.07768508 |
| HALLMARK_APOPTOSIS | 40 | -0.2100993 | -1.566538 | 0.05323194 | 0.10222071 |
| HALLMARK_IL2_STAT5_SIGNALING | 43 | -0.1942712 | -1.493282 | 0.06736842 | 0.13382521 |
| HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY | 8 | -0.4216907 | -1.4817903 | 0.07251909 | 0.13265704 |
| HALLMARK_ANDROGEN_RESPONSE | 17 | -0.2967466 | -1.453924 | 0.08811475 | 0.1419576 |
| HALLMARK_IL6_JAK_STAT3_SIGNALING | 13 | -0.3086011 | -1.3721074 | 0.13157895 | 0.18778306 |
| HALLMARK_UV_RESPONSE_UP | 34 | -0.1946464 | -1.3581616 | 0.11890838 | 0.18942033 |
| HALLMARK_TGF_BETA_SIGNALING | 10 | -0.3154098 | -1.223742 | 0.2423077 | 0.30521765 |
| HALLMARK_ESTROGEN_RESPONSE_EARLY | 26 | -0.1982658 | -1.1971172 | 0.24425887 | 0.32202977 |
| HALLMARK_FATTY_ACID_METABOLISM | 32 | -0.177964 | -1.1841006 | 0.24390244 | 0.3231822 |
| HALLMARK_TNFA_SIGNALING_VIA_NFKB | 34 | -0.1715136 | -1.1775097 | 0.23108384 | 0.3176512 |
| HALLMARK_DNA_REPAIR | 46 | -0.1302761 | -1.0168883 | 0.40292275 | 0.5131421 |
| HALLMARK_INFLAMMATORY_RESPONSE | 25 | -0.167117 | -0.9978296 | 0.4262295 | 0.5227221 |

| | | | | | |
|---|---|---|---|---|---|
| HALLMARK_BILE_ACID_METABOLISM | 16 | -0.1761662 | -0.8471114 | 0.65564203 | 0.7338694 |
| HALLMARK_GLYCOLYSIS | 36 | -0.1011905 | -0.7248176 | 0.81904763 | 0.89381605 |
| HALLMARK_MTORC1_SIGNALING | 42 | -0.0941494 | -0.7137597 | 0.84599155 | 0.87997425 |
| HALLMARK_G2M_CHECKPOINT | 53 | -0.0805165 | -0.6925765 | 0.8542094 | 0.8782975 |
| HALLMARK_PEROXISOME | 27 | -0.1061655 | -0.6640456 | 0.8852459 | 0.8833107 |

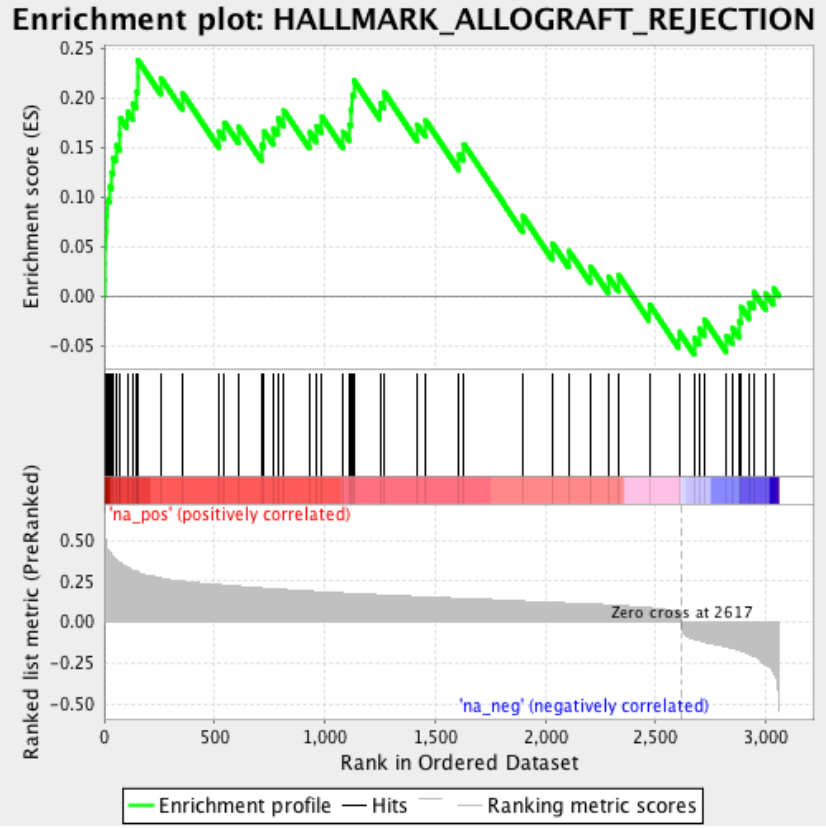

Enrichment plot: HALLMARK_ALLOGRAFT_REJECTION
Enrichment score (ES)
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
'na_pos' (positively correlated)
Ranked list metric (PreRanked)
0.50
0.25
0.00
-0.25
-0.50
Zero cross at 2617
'na_neg' (negatively correlated)
0
500
1,000
1,500
2,000
2,500
3,000
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S5A

Table S1B

| NAME | SIZE | ES | NES | NOM p-val | FDR q-val |
|---|---|---|---|---|---|
| HALLMARK_TNFA_SIGNALING_VIA_NFKB | 27 | 0.34630716 | 2.2195516 | 0 | 0.01537943 |
| HALLMARK_ALLOGRAFT_REJECTION | 15 | 0.41785946 | 1.9719326 | 0.00400802 | 0.05285456 |
| HALLMARK_INTERFERON_GAMMA_RESPONSE | 16 | 0.35805905 | 1.7035453 | 0.02729045 | 0.14941299 |
| HALLMARK_APOPTOSIS | 18 | 0.34367928 | 1.7028997 | 0.02414487 | 0.11271172 |
| HALLMARK_KRAS_SIGNALING_UP | 12 | 0.30834138 | 1.2789063 | 0.17450981 | 0.6062781 |
| HALLMARK_MITOTIC_SPINDLE | 26 | 0.20321079 | 1.2384405 | 0.203125 | 0.5871179 |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 15 | 0.24887171 | 1.176204 | 0.26252505 | 0.62166286 |
| HALLMARK_IL2_STAT5_SIGNALING | 11 | 0.27990893 | 1.1312063 | 0.30452675 | 0.62793404 |
| HALLMARK_UV_RESPONSE_UP | 12 | 0.2691257 | 1.1170832 | 0.3187251 | 0.5840298 |
| HALLMARK_PROTEIN_SECRETION | 12 | 0.2540984 | 1.066703 | 0.3608871 | 0.6123108 |
| HALLMARK_INFLAMMATORY_RESPONSE | 13 | 0.23321952 | 1.0066841 | 0.41614908 | 0.66099924 |
| HALLMARK_HYPOXIA | 16 | 0.1681994 | 0.8123587 | 0.6673307 | 0.98092985 |
| HALLMARK_G2M_CHECKPOINT | 14 | 0.17888205 | 0.79593503 | 0.7261663 | 0.9370209 |
| HALLMARK_MTORC1_SIGNALING | 21 | 0.14429314 | 0.78031844 | 0.734127 | 0.89682156 |
| HALLMARK_APICAL_JUNCTION | 13 | 0.1732997 | 0.7377354 | 0.7777778 | 0.9026552 |
| HALLMARK_ESTROGEN_RESPONSE_EARLY | 12 | 0.17221154 | 0.7161518 | 0.81670064 | 0.8738094 |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 13 | 0.13951592 | 0.6032765 | 0.9160305 | 0.93667006 |

| NAME | SIZE | ES | NES | NOM p-val | FDR q-val |
|---|---|---|---|---|---|
| HALLMARK_OXIDATIVE_PHOSPHORYLATION | 13 | -0.5716513 | -2.4835703 | 0 | 6.00E-04 |
| HALLMARK_MYC_TARGETS_V1 | 13 | -0.39434212 | -1.730875 | 0.03269231 | 0.10710335 |
| HALLMARK_ADIPOGENESIS | 16 | -0.27238628 | -1.3247694 | 0.13565892 | 0.4482592 |
| HALLMARK_HEME_METABOLISM | 15 | -0.27807865 | -1.3164601 | 0.13663366 | 0.3468159 |
| HALLMARK_XENOBIOTIC_METABOLISM | 13 | -0.26975048 | -1.1532564 | 0.31460676 | 0.49571335 |
| HALLMARK_P53_PATHWAY | 20 | -0.18901846 | -0.99214524 | 0.48197344 | 0.66992176 |
| HALLMARK_ESTROGEN_RESPONSE_LATE | 11 | -0.20239972 | -0.8159179 | 0.6912065 | 0.88119715 |
| HALLMARK_COMPLEMENT | 15 | -0.16569954 | -0.77436656 | 0.730916 | 0.8377188 |
| HALLMARK_ANDROGEN_RESPONSE | 15 | -0.16170213 | -0.7524818 | 0.7680312 | 0.7743956 |

| NAME | ORIGINAL SIZE | AFTER RESTRICTING TO DATASET | STATUS |
|---|---|---|---|
| HALLMARK_TNFA_SIGNALING_VIA_NFKB | 200 | 27 | |
| HALLMARK_HYPOXIA | 200 | 16 | |
| HALLMARK_CHOLESTEROL_HOMEOSTASIS | 74 | | Rejected! |
| HALLMARK_MITOTIC_SPINDLE | 200 | 26 | |
| HALLMARK_WNT_BETA_CATENIN_SIGNALING | 42 | | Rejected! |
| HALLMARK_TGF_BETA_SIGNALING | 54 | | Rejected! |
| HALLMARK_IL6_JAK_STAT3_SIGNALING | 87 | | Rejected! |
| HALLMARK_DNA_REPAIR | 150 | | Rejected! |
| HALLMARK_G2M_CHECKPOINT | 200 | 14 | |
| HALLMARK_APOPTOSIS | 161 | 18 | |
| HALLMARK_NOTCH_SIGNALING | 32 | | Rejected! |
| HALLMARK_ADIPOGENESIS | 200 | 16 | |
| HALLMARK_ESTROGEN_RESPONSE_EARLY | 200 | 12 | |
| HALLMARK_ESTROGEN_RESPONSE_LATE | 200 | 11 | |
| HALLMARK_ANDROGEN_RESPONSE | 101 | 15 | |
| HALLMARK_MYOGENESIS | 200 | | Rejected! |
| HALLMARK_PROTEIN_SECRETION | 96 | 12 | |
| HALLMARK_INTERFERON_ALPHA_RESPONSE | 97 | | Rejected! |
| HALLMARK_INTERFERON_GAMMA_RESPONSE | 200 | 16 | |
| HALLMARK_APICAL_JUNCTION | 200 | 13 | |
| HALLMARK_APICAL_SURFACE | 44 | | Rejected! |
| HALLMARK_HEDGEHOG_SIGNALING | 36 | | Rejected! |
| HALLMARK_COMPLEMENT | 200 | 15 | |
| HALLMARK_UNFOLDED_PROTEIN_RESPONSE | 113 | | Rejected! |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 105 | 15 | |
| HALLMARK_MTORC1_SIGNALING | 200 | 21 | |
| HALLMARK_E2F_TARGETS | 200 | | Rejected! |
| HALLMARK_MYC_TARGETS_V1 | 200 | 13 | |

| | | | |
|---|---|---|---|
| HALLMARK_MYC_TARGETS_V2 | 58 | | Rejected! |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 200 | 13 | |
| HALLMARK_INFLAMMATORY_RESPONSE | 200 | 13 | |
| HALLMARK_XENOBIOTIC_METABOLISM | 200 | 13 | |
| HALLMARK_FATTY_ACID_METABOLISM | 158 | | Rejected! |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION | 200 | 13 | |
| HALLMARK_GLYCOLYSIS | 200 | | Rejected! |
| HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY | 49 | | Rejected! |
| HALLMARK_P53_PATHWAY | 200 | 20 | |
| HALLMARK_UV_RESPONSE_UP | 158 | 12 | |
| HALLMARK_UV_RESPONSE_DN | 144 | | Rejected! |
| HALLMARK_ANGIOGENESIS | 36 | | Rejected! |
| HALLMARK_HEME_METABOLISM | 200 | 15 | |
| HALLMARK_COAGULATION | 138 | | Rejected! |
| HALLMARK_IL2_STAT5_SIGNALING | 200 | 11 | |
| HALLMARK_BILE_ACID_METABOLISM | 112 | | Rejected! |
| HALLMARK_PEROXISOME | 104 | | Rejected! |
| HALLMARK_ALLOGRAFT_REJECTION | 200 | 15 | |
| HALLMARK_SPERMATOGENESIS | 135 | | Rejected! |
| HALLMARK_KRAS_SIGNALING_UP | 200 | 12 | |
| HALLMARK_KRAS_SIGNALING_DN | 200 | | Rejected! |
| HALLMARK_PANCREAS_BETA_CELLS | 40 | | Rejected! |

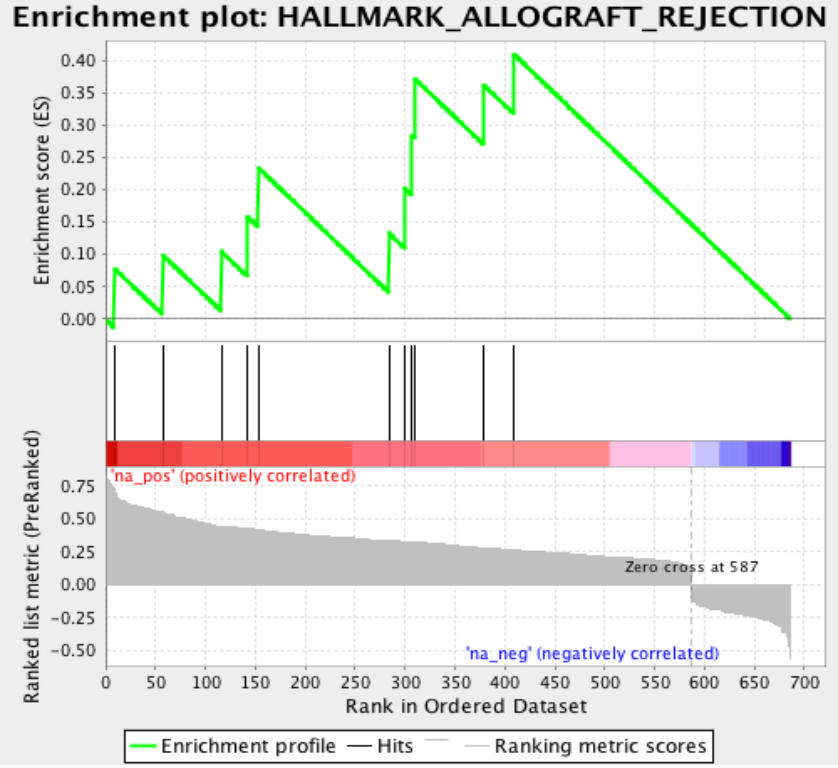

Enrichment plot: HALLMARK_ALLOGRAFT_REJECTION
Enrichment score (ES)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
'na_pos' (positively correlated)
Ranked list metric (PreRanked)
0.75
0.50
0.25
0.00
-0.25
-0.50
Zero cross at 587
'na_neg' (negatively correlated)
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S5B

# Figure S6

| | |
|---|---|
| AARSD1 | alanyl-tRNA synthetase domain containing 1 |
| AP2M1 | adaptor related protein complex 2 mu 1 subunit |
| ARHGDIB | Rho GDP dissociation inhibitor beta |
| ASB6 | ankyrin repeat and SOCS box containing 6 |
| BTD | biotinidase |
| C20orf27 | chromosome 20 open reading frame 27 |
| C9orf16 | chromosome 9 open reading frame 16 |
| CFL1 | cofilin 1 (down-regulated in subAR) |
| CIAO1 | cytosolic iron-sulfur assembly component 1 |
| CNDP2 | carnosine dipeptidase 2 |
| CXorf56 | chromosome X open reading frame 56 |
| DDX39B | DExD-box helicase 39B |
| EMP3 | epithelial membrane protein 3 |
| EXOC4 | exocyst complex component 4 |
| FAM103A1 | family with sequence similarity 103 member A1 |
| FCGR2B | Fc fragment of IgG receptor IIb (upregulated in subAR) |
| GNAI2 | G protein subunit alpha i2 (down-regulated in subAR) |
| HLA-J | major histocompatibility complex, class I, J (pseudogene) |
| HMGXB3 | HMG-box containing 3 |
| HSPB1 | heat shock protein family B (small) member 1 (down-regulated in subAR) |
| IFNAR1 | interferon alpha and beta receptor subunit 1 (up-regulated in subAR) |
| ILK | integrin linked kinase |
| KCMF1 | potassium channel modulatory factor 1 |
| KIAA0141 | KIAA0141 |
| KLHDC4 | kelch domain containing 4 |
| LOC101928595 | uncharacterized LOC101928595 |
| LRWD1 | leucine rich repeats and WD repeat domain containing 1 |
| MIB2 | mindbomb E3 ubiquitin protein ligase 2 |
| MYO19 | myosin XIX |
| MYO1C | myosin IC |
| MYPOP | Myb related transcription factor, partner of profilin |
| OS9 | OS9, endoplasmic reticulum lectin |
| PFN1 | profilin 1 |
| PKM | pyruvate kinase M1/2 (down-regulated in subAR |
| PKNOX1 | PBX/knotted 1 homeobox 1 |
| PTK2B | protein tyrosine kinase 2 beta (down-regulated in subAR) |
| RBBP9 | RB binding protein 9, serine hydrolase |
| RBM3 | RNA binding motif protein 3 |
| RBM5 | RNA binding motif protein 5 |
| RLIM | ring finger protein, LIM domain interacting |
| RPUSD3 | RNA pseudouridylate synthase domain containing 3 |
| RUSC1 | RUN and SH3 domain containing 1 |
| SARNP | SAP domain containing ribonucleoprotein |
| SH3BGRL3 | SH3 domain binding glutamate rich protein like 3 |
| SLC25A19 | solute carrier family 25 member 19 |
| SLC35D2 | solute carrier family 35 member D2 |
| SNX19 | sorting nexin 19 |
| SNX20 | sorting nexin 20 |
| STN1 | STN1, CST complex subunit |
| TMEM62 | transmembrane protein 62 |
| TPMT | thiopurine S-methyltransferase |
| TRAPPC1 | trafficking protein particle complex 1 |
| TTC9C | tetratricopeptide repeat domain 9C |
| TWF2 | twinfilin actin binding protein 2 |
| UCP2 | uncoupling protein 2 |
| UQCR11 | ubiquinol-cytochrome c reductase, complex III subunit XI |
| USP31 | ubiquitin specific peptidase 31 |

**Supplemental Table ST2A-D. Impact on the Clinical Phenotype (CP) on 24-month Transplant Outcome (2A) and Association between *dn*DSA and the CP (2B). Impact of the Gene Expression Profile (GEP) on 24-month Transplant Outcome (2C) and Association between *dn*DSA and the CP (2D).**

**2A. Association of Clinical Phenotypes (CP) with Composite Clinical Endpoint (CCE)**

| | TX only (no subAR) | | subAR only (No TX) | | subAR and TX | | ≥1 subAR (subAR only and subAR and TX) | | subAR only vs. TX only | | ≥1 subAR vs. TX only | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **n/N** | **%** | **n/N** | **%** | **n/N** | **%** | **n/N** | **%** | **OR (95% CI)*** | **p-value*** | **OR (95% CI)*** | **p-value*** |
| CCE | 43/121 | 35•5% | 17/23 | 73•9% | 16/39 | 41•0% | 33/62 | 53•2% | 5•1 (1•7, 16•9) | <0•001 | 2•1 (1•1, 4•0) | 0•027 |
| ≥ IFTA II | 10/121 | 8•3% | 5/23 | 21•7% | 5/39 | 12•8% | 10/62 | 16•1% | 3•1 (0•7, 11•3) | 0•07 | 2•1 (0•7, 6•1) | 0•13 |
| BPAR | 23/121 | 19•0% | 13/23 | 56•5% | 7/39 | 18•0% | 20/62 | 32•3% | 5•5 (1•9, 15•9) | <0•001 | 2•0 (0•9, 4•3) | 0•06 |
| eGFR | 17/121 | 14•1% | 5/23 | 21•7% | 6/39 | 15•4% | 11/62 | 17•7% | 1•7 (0•4, 5•6) | 0•35 | 1•3 (0•5, 3•2) | 0•52 |

* 95% exact confidence interval presented with p-value resulting from a Fisher's Exact Test.

**2B. Association between de novo Anti-HLA Antibody and de novo DSA development and the Clinical Phenotype**

| **Clinical Phenotype at any time post-tx** | **subAR only (N=33)** | **TX only (N=146)** | **p-value[1]** | **≥1 subAR (N=107)** | **TX only (N=146)** | **p-value[1]** |
|---|---|---|---|---|---|---|
| Anti-HLA Class 1 | 5 (15•15%) | 27 (18•49%) | 0•6509 | 15 (14•02%) | 27 (18•49%) | 0•3447 |
| Anti-HLA Class 2 | 8 (24•24%) | 33 (22•60%) | 0•8396 | 26 (24•30%) | 33 (22•60%) | 0•7526 |
| DSA Class 1 | 6 (18•18%) | 6 (4•11%) | 0•0103+ | 9 (8•41%) | 6 (4•11%) | 0•1523 |
| DSA Class 2 | 7 (21•21%) | 8 (5•48%) | 0•0084+ | 21 (19•63%) | 8 (5•48%) | 0•0005 |
| | | | | | | |
| **Clinical Phenotype within Year 1** | **subAR only (N=35)** | **TX only (N=162)** | **p-value[1]** | **≥1 subAR (N=81)** | **TX only (N=162)** | **p-value[1]** |
| Anti-HLA Class 1 | 4 (11•43%) | 30 (18•52%) | 0•3142 | 9 (11•11%) | 30 (18•52%) | 0•1381 |
| Anti-HLA Class 2 | 7 (20•00%) | 38 (23•46%) | 0•6587 | 18 (22•22%) | 38 (23•46%) | 0•8294 |
| DSA Class 1 | 6 (17•14%) | 6 (3•70%) | 0•0086+ | 9 (11•11%) | 6 (3•70%) | 0•0237 |
| DSA Class 2 | 7 (20•00%) | 11 (6•79%) | 0•0225+ | 16 (19•75%) | 11 (6•79%) | 0•0024 |

[1] p-value from Chi-square test except where + indicates use of Fisher's Exact test.

**2C. Association of Gene Expression Profile (GEP) with Composite Clinical Endpoint**

| | TX only (no subAR) | | subAR only (No TX) | | subAR and TX | | ≥1 subAR (subAR only and subAR and TX) | | subAR only vs. TX only | | ≥1 subAR vs. TX only | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **n/N** | **%** | **n/N** | **%** | **n/N** | **%** | **n/N** | **%** | **OR (95% CI)*** | **p-value*** | **OR (95% CI)*** | **p-value*** |
| CCE | 41/110 | 37•3% | 18/27 | 66•7% | 17/45 | 37•8% | 35/72 | 48•6% | 3•4 (1•3, 9•3) | 0•009 | 1•6 (0•8, 3•0) | 0•17 |
| ≥ IFTA II | 10/110 | 9•1% | 3/27 | 11•1% | 7/45 | 15•6% | 10/72 | 13•9% | 1•3 (0•2, 5•4) | 0•72 | 1•6 (0•6, 4•6) | 0•34 |
| BPAR | 24/110 | 21•8% | 14/27 | 51•9% | 5/45 | 11•1% | 19/72 | 26•4% | 3•9 (1•4, 10•2) | 0•003 | 1•3 (0•6, 2•7) | 0•48 |
| Delta eGFR | 15/110 | 13•6% | 6/27 | 22•2% | 7/45 | 15•6% | 13/72 | 18•1% | 1•8 (0•5, 5•7) | 0•37 | 1•4 (0•6, 3•4) | 0•53 |

* 95% exact confidence interval presented with p-value resulting from a Fisher's Exact Test.

**2D. Association between de novo Anti-HLA Antibody and de novo DSA development and the Gene Expression Profile (GEP)**

| **GEP at any time post-tx** | **subAR only (N=32)** | **TX only (N=134)** | **p-value[1]** | **≥1 subAR (N=116)** | **TX only (N=134)** | **p-value[1]** |
|---|---|---|---|---|---|---|
| Anti-HLA Class 1 | 5 (15•63%) | 27 (20•15%) | 0•5600 | 15 (12•93%) | 27 (20•15%) | 0•1279 |
| Anti-HLA Class 2 | 8 (25•00%) | 34 (25•37%) | 0•9652 | 25 (21•55%) | 34 (25•37%) | 0•4779 |

| | | | | | | |
|---|---|---|---|---|---|---|
| DSA Class 1 | 6 (18•75%) | 6 (4•48%) | 0•0128[+] | 9 (7•76%) | 6 (4•48%) | 0•2760 |
| DSA Class 2 | 6 (18•75%) | 9 (6•72%) | 0•0439[+] | 20 (17•24%) | 9 (6•72%) | 0•0096 |
| | | | | | | |
| **GEP within Year 1** | **subAR only (N=34)** | **TX only (N=148)** | **p-value[1]** | **≥1 subAR (N=91)** | **TX only (N=148)** | **p-value[1]** |
| Anti-HLA Class 1 | 3 (8•82%) | 29 (29•59%) | 0•1368 | 9 (9•89%) | 29 (29•59%) | 0•0463 |
| Anti-HLA Class 2 | 9 (26•47%) | 38 (25•68%) | 0•9239 | 17 (18•68%) | 38 (25•68%) | 0•2122 |
| DSA Class 1 | 5 (14•71%) | 6 (4•05%) | 0•0338[+] | 8 (8•79%) | 6 (4•05%) | 0•1299 |
| DSA Class 2 | 6 (17•65%) | 14 (9•46%) | 0•2195[+] | 12 (13•19%) | 14 (9•46%) | 0•3689 |
| [1] p-value from Chi-square test except where [+] indicates use of Fisher's Exact test. | | | | | | |

## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

**Figure SF1 – CTOT 08 study design**: Subjects had serial blood sampling (red arrows) coupled with periodic surveillance kidney biopsies (upper blue arrows). If subjects were diagnosed with subclinical acute rejection (subAR), they had more frequent blood sampling (lower red arrows) and a follow up biopsy 8 weeks later (skinny blue arrows). If subjects presented with renal dysfunction, they underwent "for cause" biopsies. Episodes of clinical acute rejection also had more frequent blood sampling for 8 weeks, but no follow up biopsy. All patients were scheduled for a biopsy at 24 months post-transplant as part of the clinical composite endpoint (CCE).

**Figure SF2: Workflow for biomarker discovery.** Peripheral blood collected in PAXGene tubes was processed in batches using correction and normalization parameters. Following ComBat (17) adjustment for batch effect using surrogate variable analysis (18), differential gene expression (DGE) analysis was performed (Linear Models for Microarray data – LIMMA) using False Discovery Rate (FDR) <0.05 (19, 20). To test for and validate biologic relevance of differential gene expression data, we performed comparative analyses of gene pathway mapping of the DGE in both cohorts using: 1) Ingenuity Pathway Analysis (21), 2) Database for Annotation, Visualization and Integrated Discovery (DAVID) (22), and 3) Gene Set Enrichment Analysis (GSEA) (23). DGE were then used to populate Random Forests models. Gini importance metrics were used to select the top model optimized for AUC. Bootstrap resampling (24) was used to test for overfitting of the final model.

**Figure SF3A** illustrates the *sample-level* Of 307 subjects enrolled in CTOT-08, 283 with stable renal function had centrally-read surveillance biopsies and serial clinical data, and 253/283 had

sufficient data to define the clinical phenotype of either subAR or Transplant eXcellent (TX) (i.e. no subAR) for each paired (surveillance biopsy and peripheral blood) sample used for biomarker discovery. During the 24-month observational period, these 253 subjects underwent 742 centrally-read biopsies; 191 were 'for cause' (associated with acute renal dysfunction) and were therefore not considered as surveillance biopsies, performed only in the setting of stable renal function. The remaining 551 were classified as having the clinical phenotypes of either subAR (n= 136 [24.7%]; 79% 'borderline changes', 21% $\geq$1A rejection) or TX (no rejection or other histologic findings; n=415 [75.3%]). 530 surveillance biopsies with available paired peripheral blood samples were used for biomarker discovery. Despite meeting the more general definition of either rejection or no rejection on a surveillance biopsy, the remaining 21 paired samples did not meet the strict criteria for either TX or subAR based on the pre-defined phenotype algorithm and were excluded. Of note, there were no instances of BK virus nephropathy among the 530 biopsies. In contrast to the CTOT-08 discovery cohort, patients contributing to the Northwestern University (NU) Biorepository did not undergo serial sampling. Instead, these paired samples, used for validation of the biomarker were obtained at the time of surveillance biopsies performed at the NU transplant center and represent single time points within 24 months following KT.

**Figure SF3B** illustrates the subject-level disposition for both the clinical phenotype and the gene expression profile (GEP) used to assess the impact of each on the clinical endpoints. Of 307 subjects, 283 with stable renal function had centrally-read surveillance biopsies and serial clinical data. At 24 months, 253/283 had sufficient data to determine the clinical phenotype of either subAR or TX according to the predetermined algorithm, and 250/283 had sufficient gene expression data to define a positive versus a negative molecular profile according to the predetermined test threshold (0.375). At 12 months post-transplant, 243 and 239 had sufficient data to define the clinical phenotype and gene expression profile respectively. The 12-month data were used to determine the association with each component of the CCE according to the

subject-level classification. Thirty-five, 162, and 46 subjects were respectively classified as clinical phenotypes of subAR only, TX only, and subAR or TX. Similarly, 34, 148, and 57 were classified respectively as positive only, negative only and positive or negative for the biomarker. We then determined that overall, 76 patients classified for the clinical phenotypes within these groups met the CCE, 107 did not, and 60 patients were not evaluable due to missing data required for all components of the CCE. Similarly, 76 patients classified according to the biomarker met the CCE and 106 did not have sufficient data to determine all 3 components. Associations in these patients, however, were determined for each individual component.

**Figure SF4** illustrates the Ingenuity Pathway Analysis results for the CTOT-08 and NU Biorepository (129/138) cohorts respectively. DGE data (LIMMA; FDR <0.01) from paired samples with the clinical phenotype of either subAR vs. TX, were subjected to molecular pathway mapping in both the discovery and validation cohorts using Ingenuity Pathway Analysis (IPA), of data from the 530 CTOT-08 paired samples that were used to populate the Random Forests models. We identified 46 significant canonical pathways (Benjamini-Hochberg corrected p-value <0.05), several linked to T and B-cell immunity. A bar graph of the 46 pathways ranked by their -log BH corrected p-values is shown in **Supplemental Figure SF4A**. IPA mapping of data from the 129/138 NU validation set identified 15 shared pathways with sets of shared genes that were also directionally validated (up or downregulated in both cohorts). The comparative bar graph (dark blue – discovery cohort; light blue – validation cohort) of the 15 shared pathways ranked by their -log BH corrected p-values is shown in **Supplemental Figure SF4B**.

**Table ST1A: Pre-ranked GSEA - CTOT-8 Differentially Expressed Genes**. Differential gene expression data, ranked based on fold-change, were tested against the Hallmark gene sets

(which represent specific well-defined biological states or processes and display most coherent expression) of GSEA. Among the positively enriched gene sets, the Allograft Rejection gene set is identified as the only significant candidate (q value <0.019), with 60 of its genes present in our list of CTOT differentially expressed genes.

**Figure SF5A: Hallmark Allograft Rejection – CTOT-08 GSEA Enrichment Plot** (n= 60, ES= 0.24, NES= 2.22, p-value= 0.002, q-value= 0.019). Gene set enrichment analysis (GSEA) for Allograft Rejection genes in the 530 paired sample CTOT-08 discovery cohort confirms that the Allograft Rejection gene set containing up-regulated differentially expressed genes is significantly enriched in subAR. The enrichment plot shows the distribution of genes in the Allograft Rejection gene set that are correlated with the subAR or TX clinical phenotypes.

**Table ST1B: Pre-ranked GSEA - NU Biorepository Differentially Expressed Genes.** Differential gene expression data, ranked based on fold-change, were tested against the Hallmark gene sets of GSEA. It identified TNFα-signaling and Allograft Rejection gene sets as top two positively enriched candidates.

**Figure SF5B: Hallmark Allograft Rejection – NU Biorepository GSEA Enrichment Plot:** (n=11, ES=0.41, NES= 1.64, p-value= 0.04, q-value= 0.35). Gene set enrichment analysis (GSEA) for Allograft Rejection genes in the 129 paired sample NU Biorepository validation cohort confirms that the Allograft Rejection gene set containing up-regulated differentially expressed genes is significantly enriched in subAR. The enrichment plot shows the distribution of genes in the Allograft Rejection gene set that are correlated with the subAR or TX phenotypes.

**Figure SF6 - Gene expression Profile Classifiers:** The top Random Forests model selected 61 probe sets that mapped to 57 genes. Of these 38 were up-regulated and 19 down-regulated. Only

7 genes linked to the top 10 Ingenuity immune/inflammatory pathways relevant to rejection. Of these, 2 were up-regulated 5/7 genes were significant at FDR <5%. Of interest, 38/57 (67%) genes were up-regulated for subAR vs. TX (19 down-regulated), and only 7/57 mapped to known allo-inflammatory pathways (Ingenuity) in both discovery and validation cohorts except for PKM and IFNAR1, who had FDR>5% in the validation cohort. Of the 7 genes that mapped to allo-inflammatory pathways, only 2/7 were up-regulated in subAR and 5 (except PKM and KFNAR1) were down-regulated.

**Table ST2A-D. Clinical validity:** We divided CTOT-08 subjects into 3 distinct groups of subjects who met the following criteria either within the first year or the study period (2 years) following KT: 1) subAR or positive biomarker only; 2) no subAR (TX) or negative biomarker only; and 3) $\geq$1 instance of subAR or a positive biomarker with at least 1 TX or negative biomarker. We assessed the clinical significance using either the composite clinical endpoint (CCE) or the gene expression profile (GEP) biomarker test result. This table shows the clinical significance of both the clinical phenotype (CP) and the gene expression profile (GEP) of subAR within the first 12 months on the composite clinical endpoint (CCE), as well as the association between the CP and GEP both within 12 and 24 months following transplantation and the development of *de novo* DSA (*dn*DSA) by the end of the study period (24 months).

Statistically significant differences (p-value <0.05) are highlighted: **A.** Impact of the Clinical Phenotype (CP) within the first 12 months on the clinical composite endpoint (CCE) (**ST2A**); **B.** Association between the CP within the first 12 months and at 24 months following KT on the development of *dn*DSA at 24 months (**ST2B**). **C.** Impact of the Gene Expression Profile (GEP) in the first 12 months following KT on the CCE (**ST2C**); **D.** Association between the GEP within the first 12 months and 24 months following KT on the development of *dn*DSA at 24 months (**ST2D**).