

Supplementary Materials for  
**Biomarkers from subcutaneous engineered tissues predict acute rejection of organ allografts**

Russell R. Urie *et al.*

Corresponding author: Lonnie D. Shea, [ldshea@umich.edu](mailto:ldshea@umich.edu)

*Sci. Adv.* **10**, eadk6178 (2024)  
DOI: 10.1126/sciadv.adk6178

**The PDF file includes:**

Figs. S1 to S14  
Tables S1 to S4  
Legends for data S1 to S7

**Other Supplementary Material for this manuscript includes the following:**

Data files S1 to S7

## SUPPLEMENTARY MATERIALS

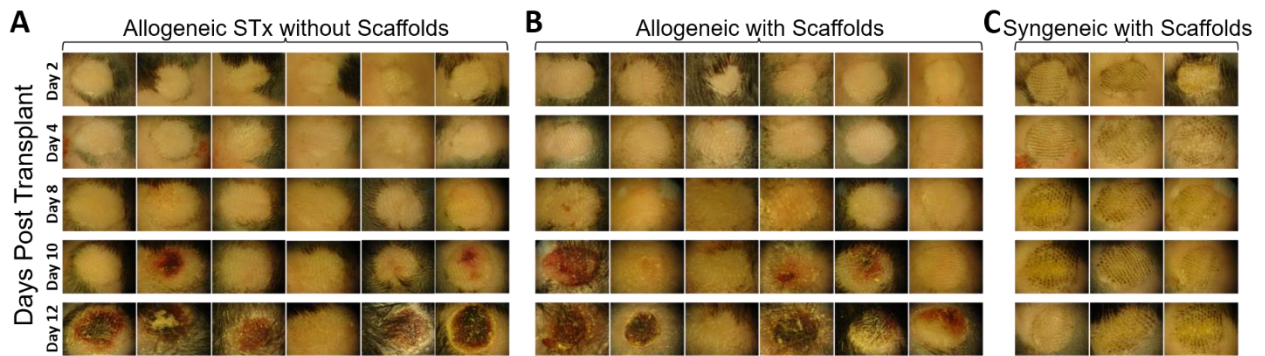


Fig. S1. Longitudinal skin transplant (STx) images for scoring grafts. (A-C) Representative skin graft images following adoptive C57Bl/6 (B6) T cell transfer to Rag2<sup>-/-</sup> graft recipients for (A) BALB/c (B/c) to B6 Rag2<sup>-/-</sup> allogeneic recipients without scaffold implants, (B) B/c to B6 Rag2<sup>-/-</sup> allogeneic recipients with subcutaneous scaffold implants, and (C) B6 to B6 Rag2<sup>-/-</sup> syngeneic recipients with subcutaneous scaffold implants. Transplant occurs on Day -28, and Day 0 is defined by syngeneic adoptive T cell transfer.

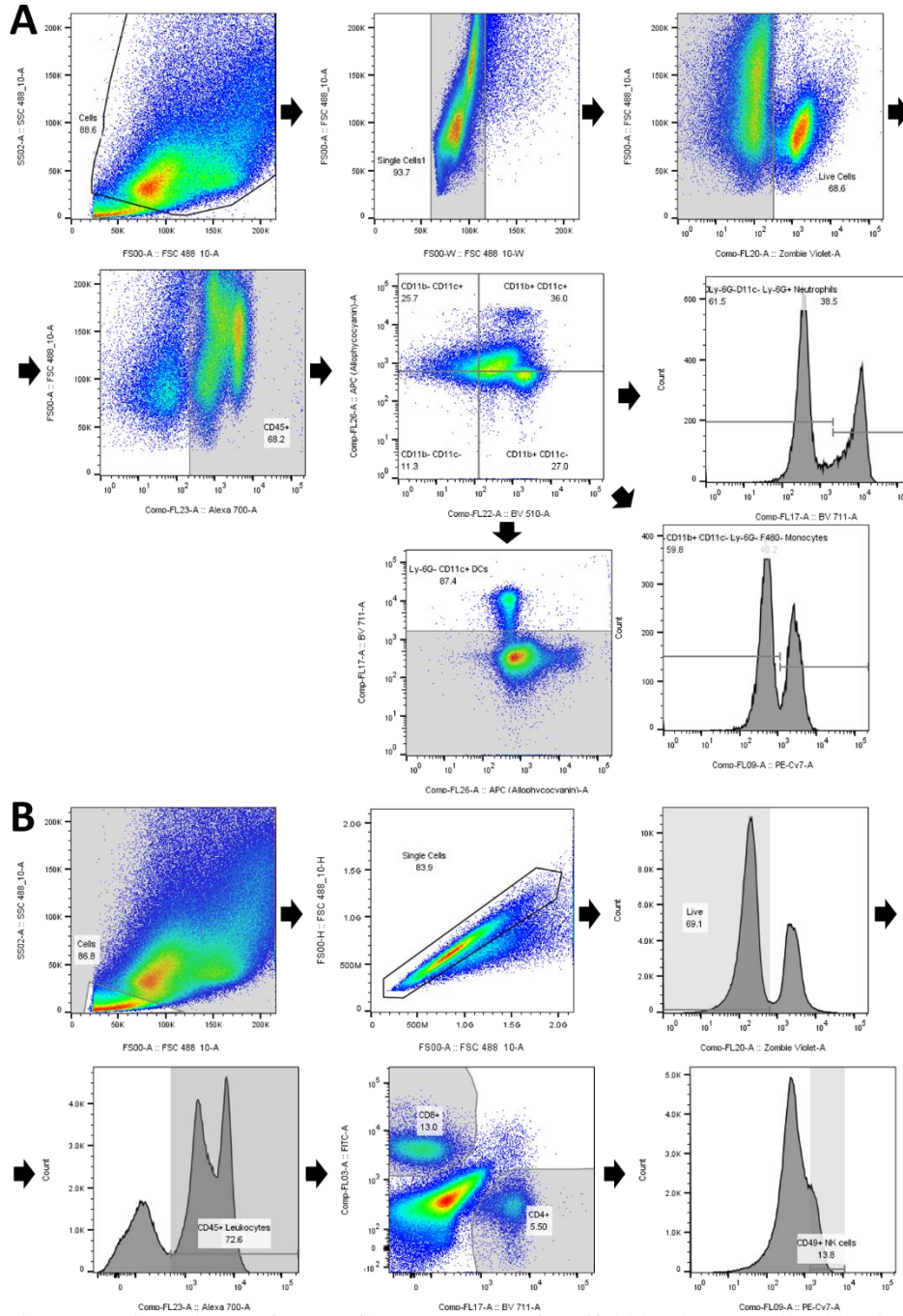


Fig. S2. Gating schemes for STx flow cytometry of scaffold implants, cardiac blood, and skin graft samples for (A) innate immune cells and (B) lymphocytes. Splenic cells shown, with these gates also applied to scaffold and skin transplant graft cells. Gates determined by compensation with single color controls and fluorescent-minus-one controls.

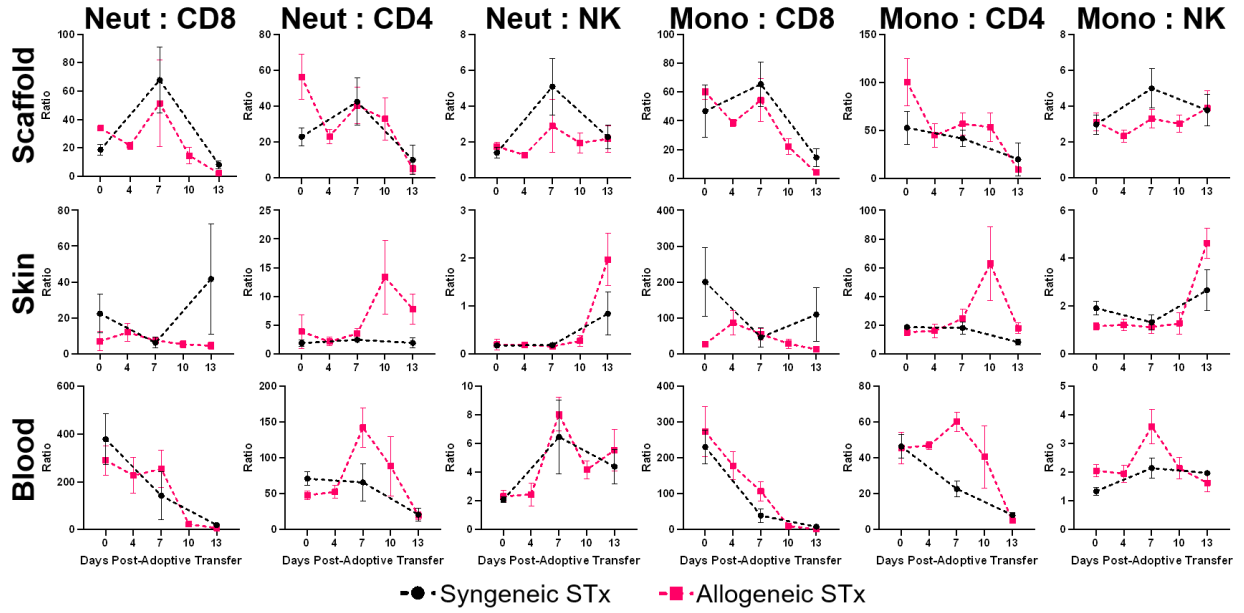


Fig. S3. Immune cell ratios at the scaffold implant (top row), skin graft (middle row), and in the blood (bottom row) during various timepoints after adoptive T cell transfer; mean  $\pm$  SEM with N=4 graft recipients. Neut = Neutrophil, CD8 = CD8<sup>+</sup> T cell, CD4 = CD4<sup>+</sup> T cell, NK = Natural Killer cell, Mono = Monocyte.

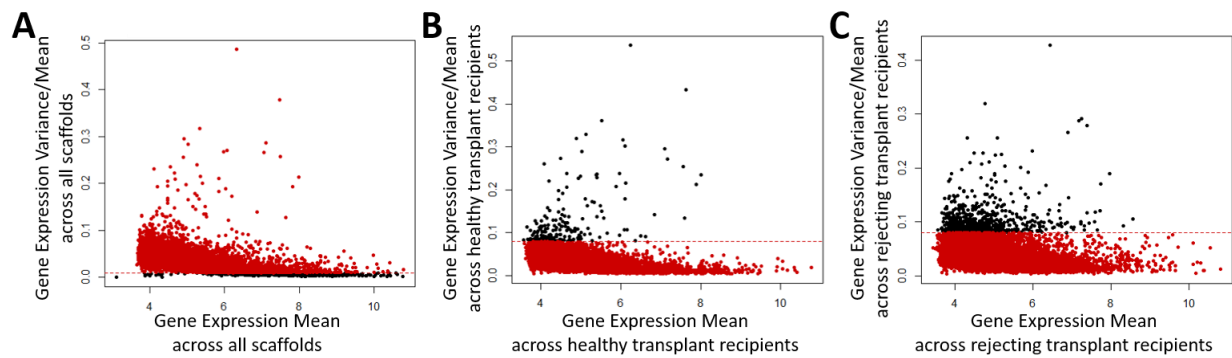


Fig. S4. Filtering of lowly expressed and uniformly expressed genes in STx scaffold RNA sequencing for gene biomarker candidate selection. (A-C) Mean and variance in expression of each non-zero gene at the scaffold for gene filtering. Points represent individual genes with non-zero scaffold expression. Dotted red line indicates variance/mean cutoff value chosen to filter genes for those with (A) moderate or high expression variance across all skin transplant recipients; (B) moderate to low expression variance/mean in healthy skin transplant recipients (i.e., syngeneic recipients across all timepoints and day 0 scaffolds from allogeneic recipients); and (C) rejecting skin transplant recipients (i.e., day 7 and day 13 scaffolds from allogeneic recipients). Red points indicate genes that pass filtering for subsequent analyses, while black points indicate genes excluded from subsequent analyses due to too low inter-group variance and/or too high intra-group variance.

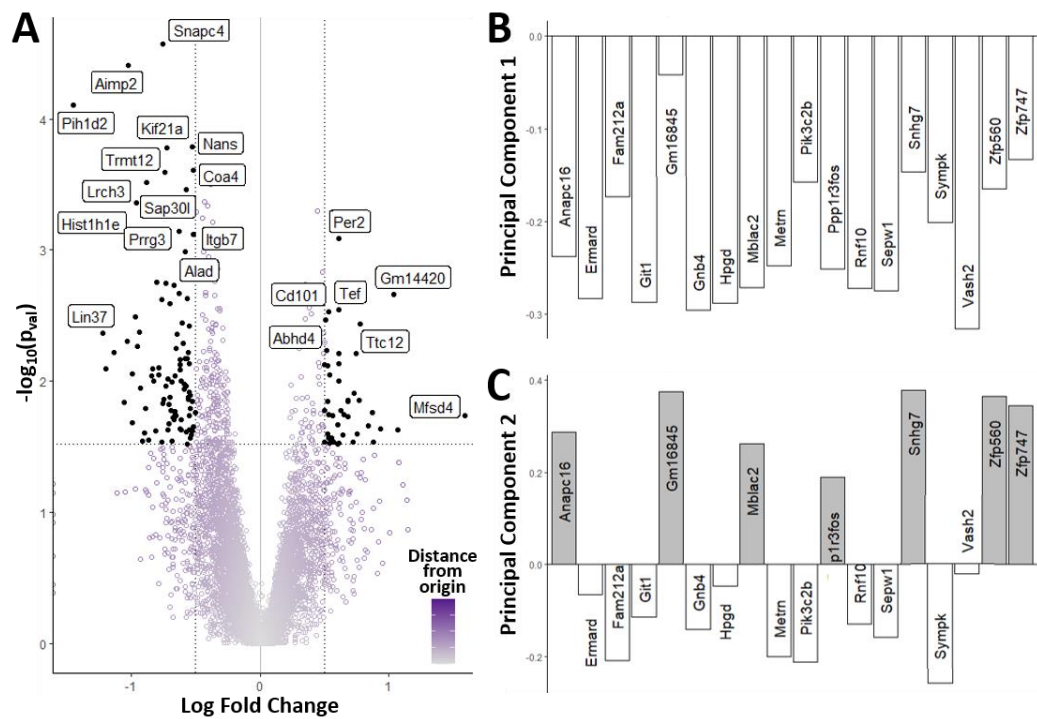


Fig. S5. Differentially expressed genes in STx rejection. **(A)** Volcano plot visualizing the differentially expressed genes between healthy STx recipients and acute cellular allograft rejection (ACAR) according to non-parametric t-test. **(B-C)** Loadings of the sparse 18-gene panel of STx ACAR for **(B)** principal component 1 and **(C)** principal component 2.

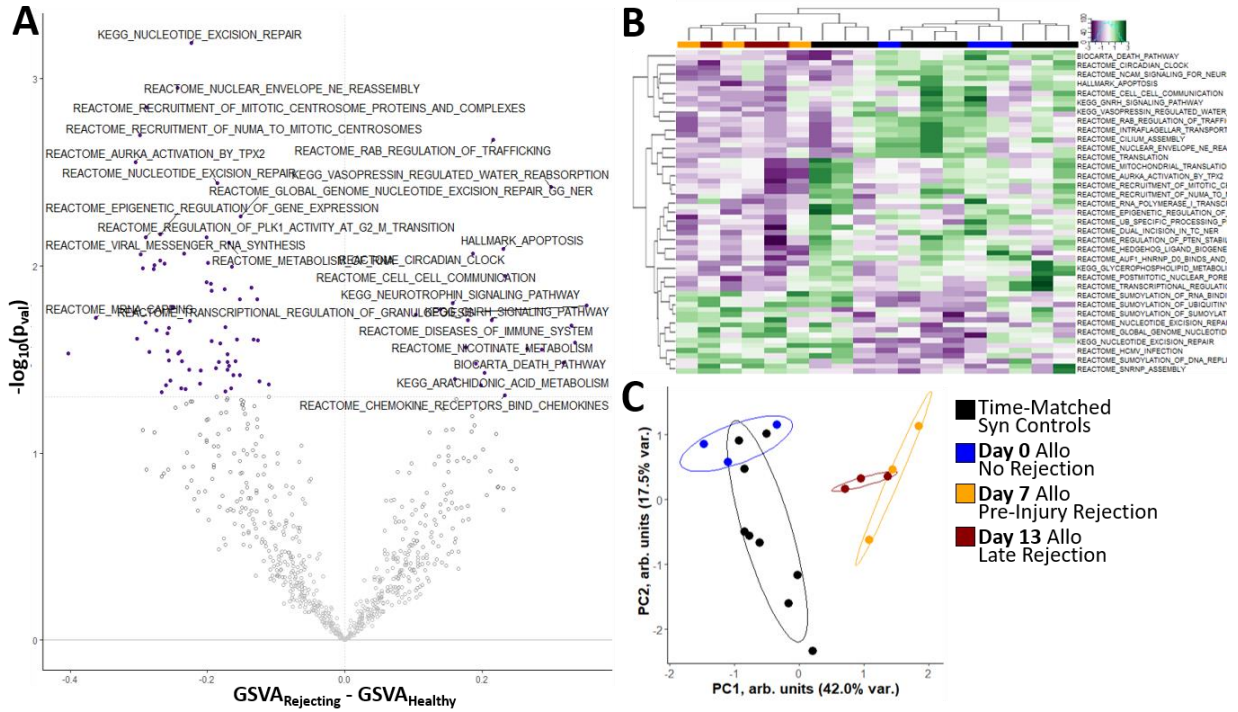


Fig. S6. Pathways distinguishing ACAR from healthy STx recipients. **(A)** Volcano plot visualizing the differentially enriched pathways between healthy STx recipients and ACAR according to Gene Set Variation Analysis (GSVA) score differentials. Shortened pathway names, see Supple. Table 1. **(B)** Clustered heatmap of elastic net-derived GSWA pathways as a sparse pathway set for distinguishing ACAR from healthy transplant recipients at the scaffold. Columns indicate individual scaffold samples. **(C)** Principal component clustering of scaffolds based on this sparse pathway panel. Points indicate individual scaffold samples. All ellipses=70% confidence interval (CI).

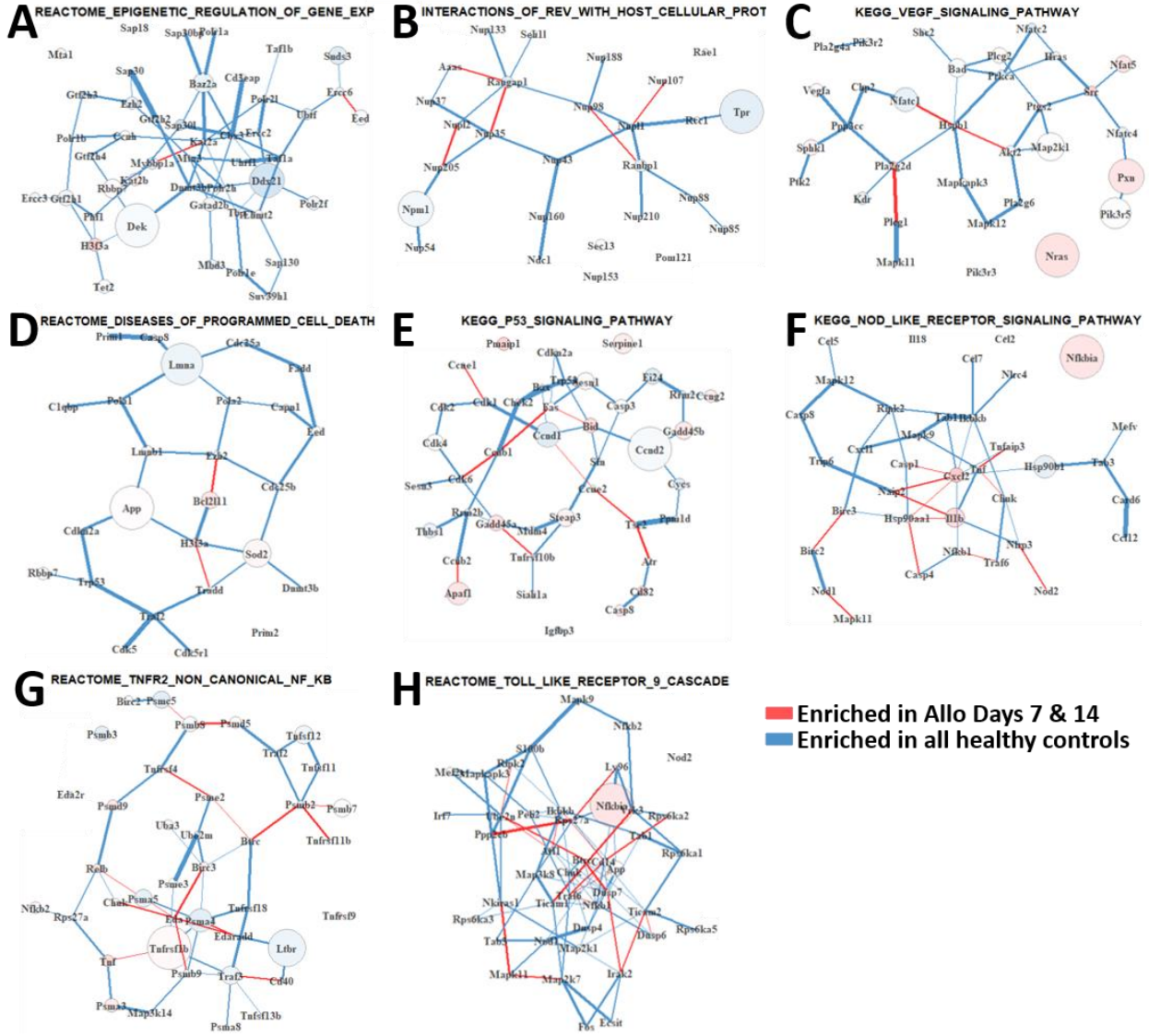


Fig. S7. Differential gene expression networks of the top differentially-enriched pathways at the scaffold during STx ACAR. (A) Epigenetic Regulation of Gene Expression, (B) Interactions of REV with Host Cellular Proteins, (C) VEGF Signaling Pathway, (D) Diseases of Programmed Cell Death, (E) P53 Signaling Pathway, (F) NOD-like Receptor Signaling Pathway, (G) TNFR2 Non-Canonical NFκB Pathway, and (H) Toll-Like Receptor 9 Cascade Pathway visualized by differential network analyses between ACAR and healthy STx recipient scaffolds. Gene expression displayed for pathway constituents where enrichment or depletion is defined as a differential connectivity p-value < 0.01.



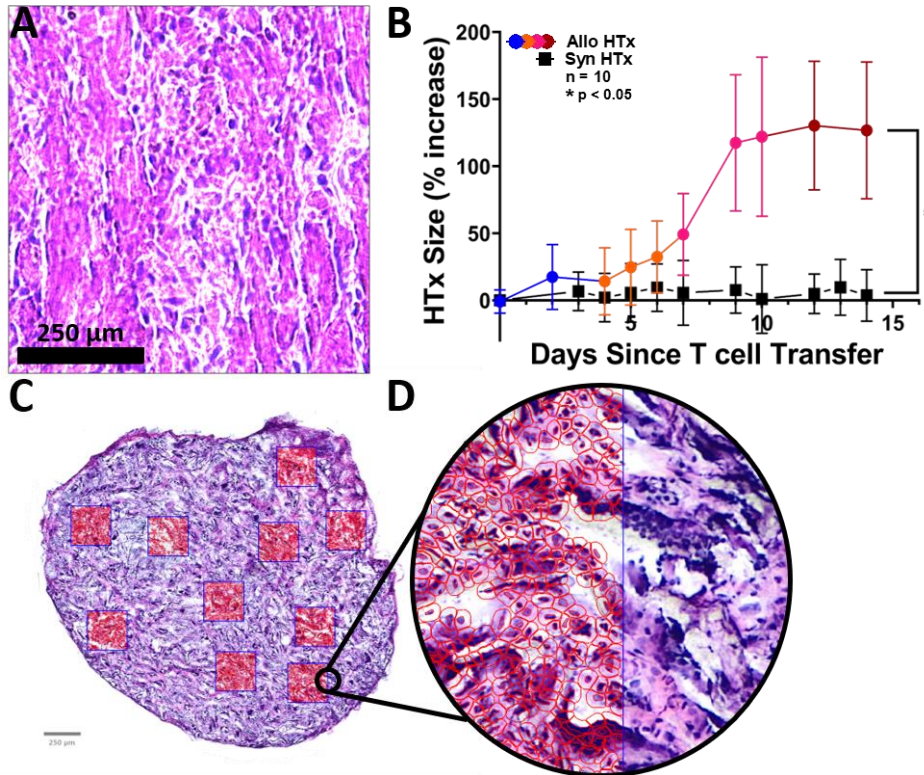


Fig. S8. Heart transplant (HTx) allograft edema during ACAR. (A) Cross-sectional image of allogeneic HTx graft before adoptive T cell transfer, stained by hematoxylin and eosin. Image representative of N=3 heart grafts from independent recipients. Scale bar = 250 $\mu$ m. (B) Swelling of the allografts as ACAR progresses after adoptive T cell transfer and syngeneic grafts absent of ACAR swelling; mean  $\pm$  SEM with N=10 HTx recipients per group. (C) Representative image of 14 $\mu$ m thick scaffold staining by hematoxylin and eosin, with 10 annotated areas in the QuPath software and counted cells. (D) Magnification of annotated area edge highlighting cell count derivation using QuPath software.

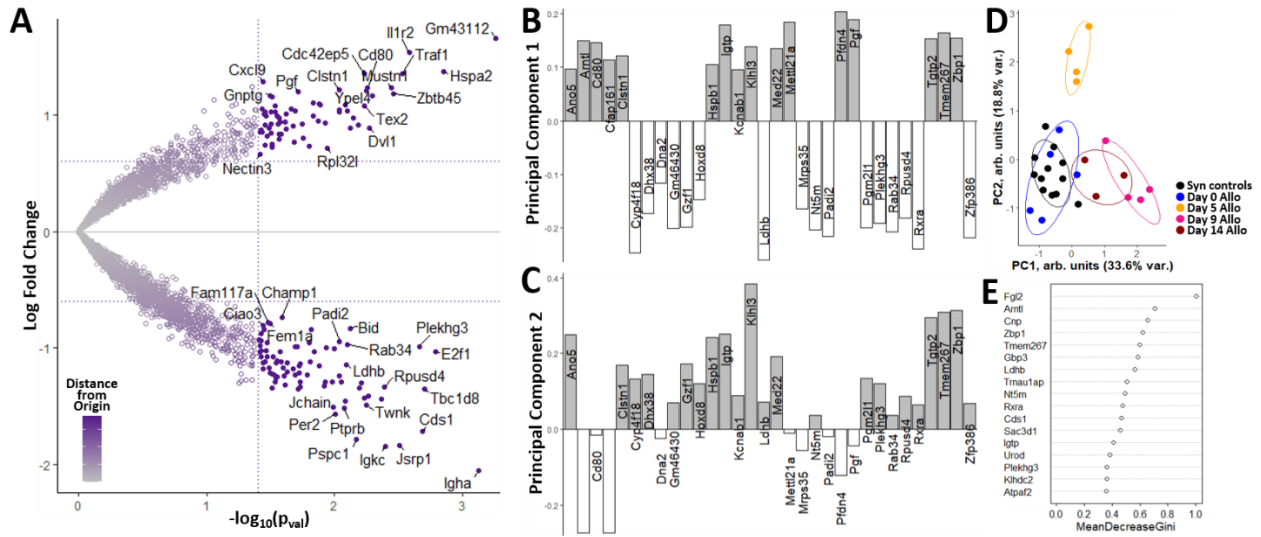


Fig. S9. Differentially expressed genes in HTx rejection. **(A)** Volcano plot visualizing the differentially expressed genes between healthy HTx recipients and ACAR according to non-parametric t-test. **(B, C)** Loadings of the sparse 17-gene panel of HTx ACAR for **(B)** principal component 1 and **(C)** principal component 2. **(D)** Principal component clustering of scaffolds based on this sparse HTx panel. Points indicate individual scaffold samples. All ellipses=70% CI. **(E)** Relative importance of each of the 17 panel genes in distinguishing ACAR from healthy HTx recipients in the unsupervised singular value decomposition (SVD) and supervised Random Forest (RF) scoring, as defined by Gini index (arbitrary units).

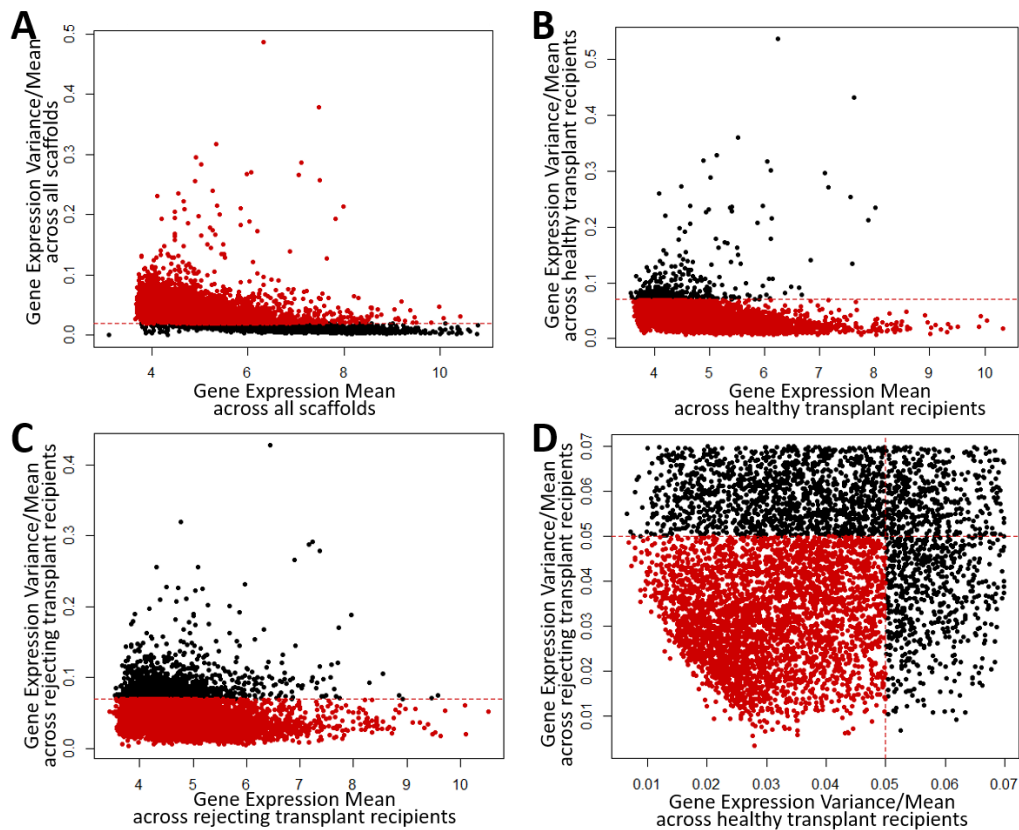


Fig. S10. Variance filtering of batch corrected STx and HTx genes for gene biomarker candidate selection. **(A-D)** Mean and variance in expression of each non-zero gene at the scaffold for gene filtering. Points represent individual genes with non-zero scaffold expression. Dotted red line indicates variance/mean cutoff value chosen to filter genes for those with **(A)** moderate or high expression variance across all skin transplant recipients and **(B)** moderate to low expression variance/mean in healthy skin transplant recipients (i.e., syngeneic recipients across all timepoints and day 0 scaffolds from allogeneic recipients) and **(C)** rejecting skin transplant recipients (i.e., day 7 and day 13 scaffolds from allogeneic recipients). **(D)** Further stringent filtering of intra-group variance in healthy and ACAR scaffolds. Red points indicate genes that pass filtering for subsequent analyses, while black points indicate genes excluded from subsequent analyses due to too low inter-group variance and/or too high intra-group variance.

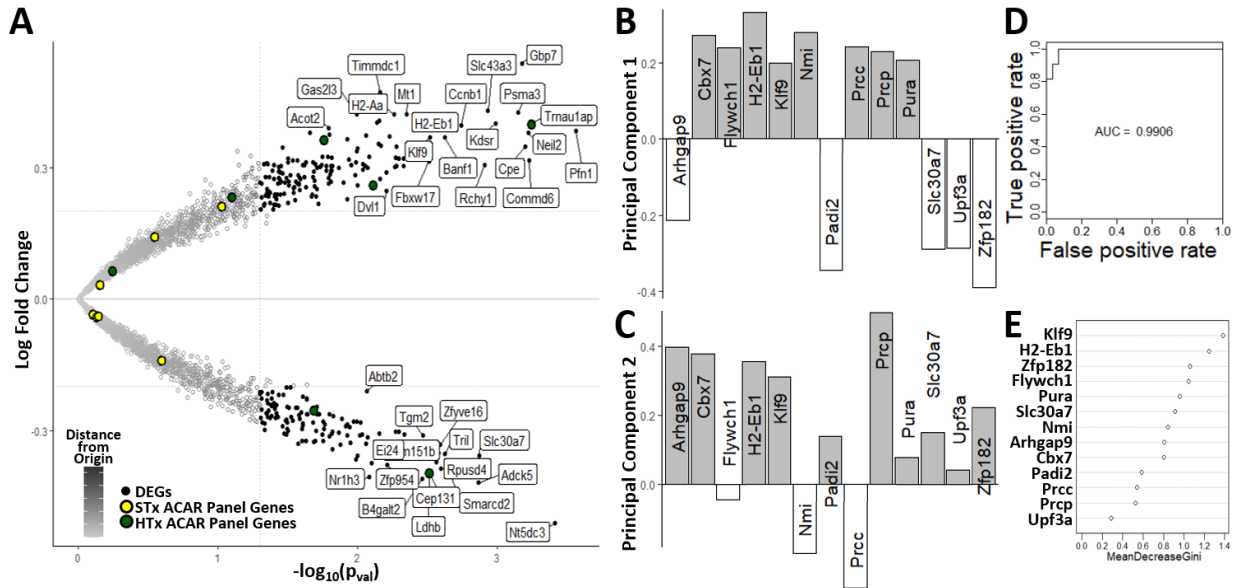


Fig. S11. Principal component loadings and analysis of the combined ACAR sparse gene expression panel. **(A)** Volcano plot visualizing the differentially expressed genes between healthy Tx recipients and ACAR according to non-parametric t-test. **(B, C)** Loadings of the 13-gene panel for **(B)** principal component 1 and **(C)** principal component 2. **(D)** Receiver-operator characteristic curve for unsupervised SVD and supervised RF scoring of samples based on 13-gene panel derived at the scaffold. **(E)** Relative importance of each of the 13 panel genes in distinguishing ACAR from healthy recipients in the unsupervised SVD and supervised RF scoring, as defined by Gini index (arbitrary units).

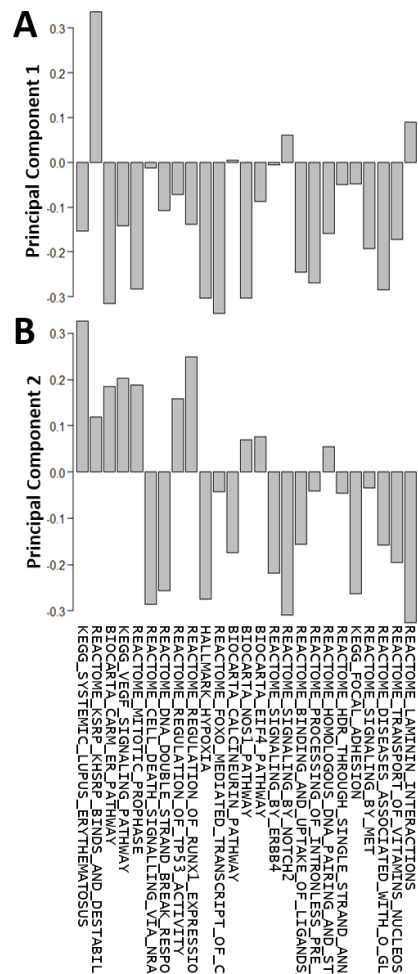


Fig. S12. Principal component loadings and analysis of combined ACAR pathways for (A) principal component 1 and (B) principal component 2. Shortened pathway names, see Supple. Table 3.

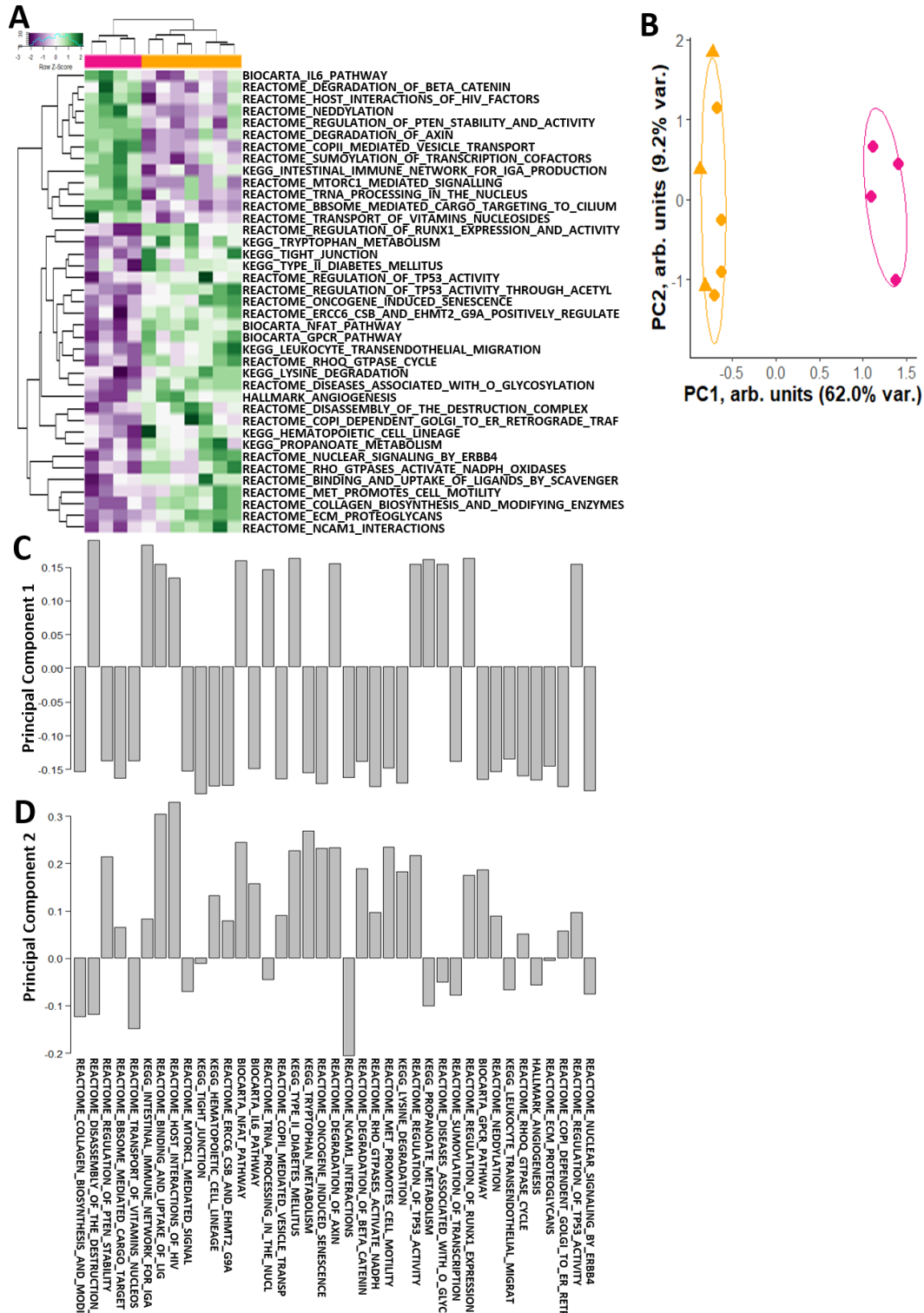


Fig. S13. Differential pathway enrichment between pre-injury and mid-injury ACAR. (A) Clustered heatmap of elastic net-derived GSVA pathways as a sparse pathway set for distinguishing pre-injury and mid-injury ACAR at the scaffold. Columns indicate individual scaffold samples. (B) Principal component clustering of scaffolds based on this sparse pathway set. Points indicate individual scaffold samples. All ellipses=70% CI. (C, D) Loadings of the sparse 17-gene panel of HTx ACAR for (C) principal component 1 and (D) principal component 2. Shortened pathway names, see Supple. Table 3.

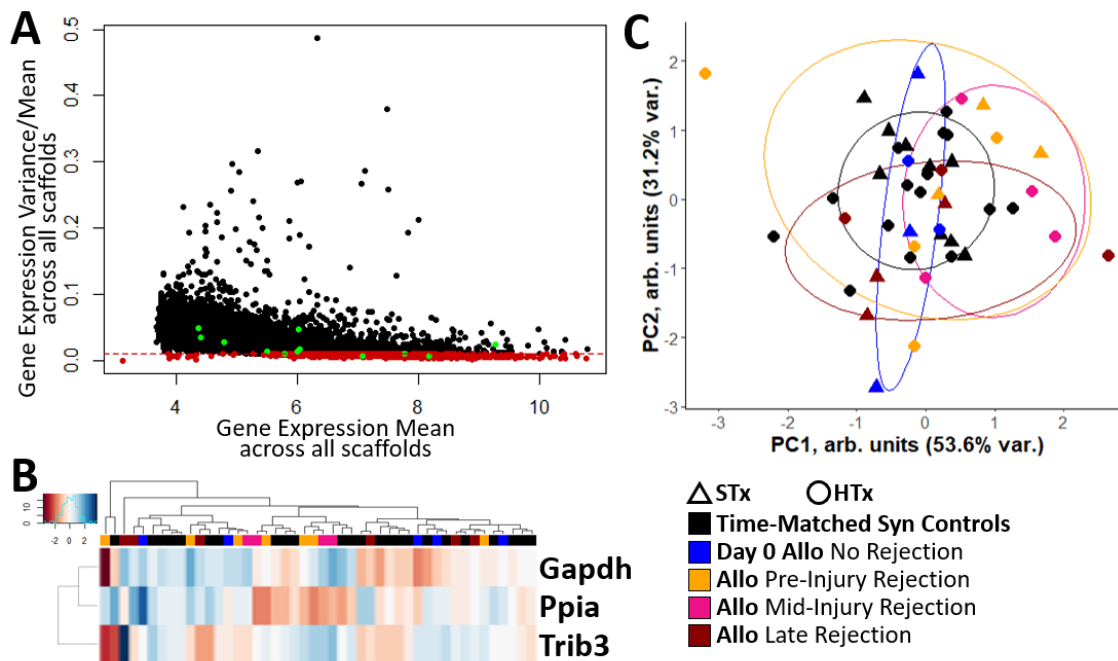


Fig. S14. Selecting housekeeping genes from combined ACAR for PCR controls. (A) Mean and variance in expression of each non-zero gene at the scaffold for gene filtering. Points represent individual genes with non-zero scaffold expression. Dotted red line indicates variance/mean cutoff value chosen to filter genes for those with very low variance across all transplant recipients. Red points indicate genes that pass filtering as potential housekeeping genes, while black points indicate genes excluded due to high variance. Green points indicate housekeeping genes employed in the OpenArray™ system. (B) Clustered heatmap and (C) principal component clustering indicating that *Gapdh*, *Ppia*, and *Trib3* can serve as adequate housekeeping genes for scaffold implants due in ACAR.

Table S1. Pathways and genes differentially represented at the scaffold during ACAR in STx.

GSEA Pathways	GSVA Elastic Net Pathways	ACAR DEGs	ACAR Panel	Serial ACAR Panel
FOXP3 Targets in Thymus Upregulated	Reactome Rab Regulation Of Trafficking	<i>Igfbp2</i>	<i>Hpgd</i>	<i>Tmem234</i>
IRF4 Targets in Activated Dendritic Cell	Reactome Tbc Rabgaps	<i>Dnajb2</i>	<i>Anapc16</i>	<i>Sfxn5</i>
Mature B Lymphocyte Upregulated	Kegg Vasopressin Regulated Water Reabsorption	<i>Slc4a3</i>	<i>Sympk</i>	<i>Zfp963</i>
Differentiating T Lymphocyte	Reactome Signaling By Gpcr	<i>Per2</i>	<i>Mblac2</i>	<i>Rcsd1</i>
Abnormal Lymphocyte Morphology	Kegg Neurotrophin Signaling Pathway	<i>Ptpn4</i>	<i>Sepw1</i>	<i>Vwf</i>
Bone Marrow Immature Neutrophil	Hallmark Apoptosis	<i>Mfsd4</i>	<i>Vash2</i>	<i>Gm19897</i>
Hallmark IL6 JAK STAT3 Signaling	Reactome Neutrophil Degranulation	<i>Aspm</i>	<i>Ermard</i>	
Hallmark Allograft Rejection	Kegg GnRH Signaling Pathway	<i>Brinp3</i>	<i>Shhg7</i>	
LPS vs LPS Low Monocyte Downregulated	Reactome Cell Cell Communication	<i>Pcp41l</i>	<i>Gm16845</i>	
Unstim vs FCGR1B Stim DC Upregulated	Reactome Ncam Signaling For Neurite Out Growth	<i>Ptprk</i>	<i>Ppp1r3fos</i>	
Tconv PLN Downregulated vs CD24Hi Tconv Thymus	Kegg ErbB Signaling Pathway	<i>Fig4</i>	<i>Metm</i>	
DC Upregulated vs Basophil	Reactome Circadian Clock	<i>Cirbp</i>	<i>Pik3c2b</i>	
Conventional vs Plasmacytoid DC Spleen Upregulated	Reactome Glutathione Conjugation	<i>Eid3</i>	<i>Fam212a</i>	
CD8 Neg DC Spleen vs CD8 Pos DC Spleen Upregulated	Reactome Transcriptional Regulation Of Granulopoiesis	<i>Rpl41</i>	<i>Zfp560</i>	
Monocyte Upregulated vs MDC	Reactome Diseases Of Immune System	<i>Sap30l</i>	<i>Zfp747</i>	
Unstim vs LPS Stim Macrophage Down	Reactome Nicotinate Metabolism	<i>Dhrs7b</i>	<i>Rnf10</i>	
Naive CD4 T cell vs Day5 IL4 Conv Treg Down	Reactome Sumoylation Of Sumoylation Proteins	<i>Camkk1</i>	<i>Gnb4</i>	
Wildtype vs MYD88KO Macrophage Down	Reactome Sumoylation Of Ubiquitylation Proteins	<i>Ccl2</i>	<i>Git1</i>	
Naive T cell vs Neutrophil Upregulated	Reactome Sumoylation Of RNA Binding Proteins	<i>Ska2</i>		
Naive CD4 T cell vs Neutrophil Upregulated	Reactome Viral Messenger RNA Synthesis	<i>Hoxb5</i>		
Naive CD4 T cell vs Monocyte Down	Reactome Transcriptional Regulation By Small RNAs	<i>Snord104</i>		
Naive vs 24hr in vitro Stim CD8 T cell Down	Reactome Postmitotic Nuclear Pore Complex Npc Reformation	<i>Cep131</i>		
Nonsuppressive T cell vs Activated Treg Upregulated	Kegg Nucleotide Excision Repair	<i>Acot2</i>		
Naive vs Memory CD8 T cell Upregulated	Reactome Global Genome Nucleotide Excision Repair Gg Ner	<i>Ifi2712a</i>		
Regulation of Inflammatory Response to Antigenic Stimulus	Reactome Nucleotide Excision Repair	<i>Trmt61a</i>		
Negative Regulation of MAP Kinase Activity	Reactome Dual Incision In Tc Ner	<i>Gng4</i>		
Myeloid Leukocyte Activation	Reactome Transcription Coupled Nucleotide Excision Repair Tc Ner	<i>Hist1h1e</i>		
Lymph Vessel Development	Reactome Nervous System Development	<i>Gm16907</i>		
Leukocyte Proliferation	Reactome Ub Specific Processing Proteases	<i>Ttc37</i>		
Granulocyte Activation	Reactome Pten Regulation	<i>Nt5dc2</i>		
Cell Activation involved in Immune Response	Reactome Regulation Of Pten Stability And Activity	<i>Bmp4</i>		
Alpha Beta T cell Proliferation	Reactome Signaling By Tgfb Family Members	<i>Dlgap5</i>		
Duodenal Immune Cells	Reactome Signaling By Tgf Beta Receptor Complex	<i>Abhd4</i>		
	Reactome Auf1 Hnrnp D0 Binds And Destabilizes mRNA	<i>Esco2</i>		
	Reactome Mapk6 Mapk4 Signaling	<i>Cldn10</i>		
	Reactome Formation Of The Early Elongation Complex	<i>Ropn11</i>		
	Reactome mRNA Capping	<i>Tnfrsf11b</i>		
	Reactome Epigenetic Regulation Of Gene Expression	<i>Trmt12</i>		
	Reactome RNA Polymerase I Transcription Initiation	<i>Fam203a</i>		
	Reactome RNA Polymerase I Transcription	<i>Tef</i>		
	Reactome RNA Polymerase I Promoter Escape	<i>Creld2</i>		
	Reactome RNA Polymerase I Transcription Termination	<i>Kif21a</i>		
	Reactome Aggrephagy	<i>Pus7l</i>		
	Reactome Selective Autophagy	<i>Kansl2</i>		
	Kegg Oxidative Phosphorylation	<i>Itgb7</i>		
	Reactome Mitochondrial Protein Import	<i>Mettl22</i>		



	Reactome Recruitment Of Numa To Mitotic Centrosomes	<i>Lrch3</i>		
	Reactome Recruitment Of Mitotic Centrosome Proteins And Complexes	<i>Zbtb20</i>		
	Reactome Aurka Activation By Tpx2	<i>Bace2</i>		
	Reactome Regulation Of Plk1 Activity At G2 M Transition	<i>U2af1</i>		
	Reactome Mitochondrial Translation	<i>Gabbr1</i>		
	Reactome Protein Folding	<i>H2.M2</i>		
	Reactome Translation	<i>Ptk7</i>		
	Reactome The Role Of Gtse1 In G2 M Progression After G2 Checkpoint	<i>Dus3l</i>		
	Reactome Metabolism Of RNA	<i>Fam98a</i>		
	Reactome Nuclear Envelope Ne Reassembly	<i>Arap3</i>		
	Reactome Hcmv Infection	<i>Vps37c</i>		
	Reactome Anchoring Of The Basal Body To The Plasma Membrane	<i>Slc25a28</i>		
	Reactome Organelle Biogenesis And Maintenance	<i>Gata3</i>		
	Reactome Cilium Assembly	<i>Thns1l</i>		
	Reactome Intraflagellar Transport	<i>Tubb4b</i>		
	Kegg Glycerophospholipid Metabolism	<i>Snape4</i>		
	Kegg Glycerolipid Metabolism	<i>Hspa5</i>		
		<i>Tnfrsf6</i>		
		<i>Dusp19</i>		
		<i>Aplnr</i>		
		<i>Foxs1</i>		
		<i>Gm14420</i>		
		<i>Slco4a1</i>		
		<i>Zbtb46</i>		
		<i>Med12l</i>		
		<i>Mnd1</i>		
		<i>Cd5l</i>		
		<i>Txnip</i>		
		<i>Cd101</i>		
		<i>Alpk1</i>		
		<i>Gipc2</i>		
		<i>Bach2os</i>		
		<i>Pnrc1</i>		
		<i>Nans</i>		
		<i>Alad</i>		
		<i>Pdzklip1</i>		
		<i>Btbd19</i>		
		<i>AU022252</i>		
		<i>Ppcs</i>		
		<i>Pla2g2d</i>		
		<i>Rps19.ps3</i>		
		<i>Abcb8</i>		
		<i>Fndc4</i>		
		<i>Tada2b</i>		
		<i>Uchl1</i>		
		<i>Brdt</i>		
		<i>Zfp605</i>		
		<i>Vsig10</i>		
		<i>Ephb4</i>		
		<i>Aimp2</i>		
		<i>Mtus2</i>		
		<i>Peg10</i>		
		<i>Ptn</i>		
		<i>Zfp862.ps</i>		
		<i>St3gal5</i>		

		<i>Mcm2</i>		
		<i>Gdf3</i>		
		<i>Gsg1</i>		
		<i>Klhl42</i>		
		<i>Hspbp1</i>		
		<i>Rps19</i>		
		<i>Tgfb1</i>		
		<i>Sertad3</i>		
		<i>Gm19897</i>		
		<i>Lin37</i>		
		<i>Cep89</i>		
		<i>Fah</i>		
		<i>Coa4</i>		
		<i>Spon1</i>		
		<i>Rabep2</i>		
		<i>Map2k7</i>		
		<i>Efnb2</i>		
		<i>Snx25</i>		
		<i>Tll1</i>		
		<i>Npy1r</i>		
		<i>Zfp961</i>		
		<i>Ces2g</i>		
		<i>Tango6</i>		
		<i>Arhgap42</i>		
		<i>Slc36a4</i>		
		<i>Ppan</i>		
		<i>Rnf26</i>		
		<i>Ttc12</i>		
		<i>Pih1d2</i>		
		<i>Rasgrf1</i>		
		<i>Rpgr</i>		
		<i>Usp11</i>		
		<i>Zfp449</i>		
		<i>Prrg3</i>		
		<i>Tceanc</i>		

Table S2. Pathways and genes differentially represented at the scaffold during ACAR in HTx.

GSVA Elastic Net Differentially Enriched Pathways	ACAR DEGs	ACAR Panel
Hallmark Angiogenesis	<i>Gstt1</i>	<i>Tmem267</i>
Reactome Bbosome Mediated Cargo Targeting To Cilium	<i>Rab34</i>	<i>Sac3d1</i>
Reactome Binding and Uptake Of Ligands By Scavenger Receptors	<i>Nr1h3</i>	<i>Cnp</i>
Reactome Collagen Biosynthesis and Modifying Enzymes	<i>Dbf4</i>	<i>Klhdc2</i>
Reactome Copi Dependent Golgi To Er Retrograde Traffic	<i>Tbc1d8</i>	<i>Igtp</i>
Reactome Copii Mediated Vesicle Transport	<i>Cyp4f18</i>	<i>Atpaf2</i>
Reactome Degradation of Axin	<i>Ruvbl2</i>	<i>Arntl</i>
Reactome Degradation of Beta Catenin by The Destruction Complex	<i>Bid</i>	<i>Cds1</i>
Reactome Disassembly of The Destruction Complex and Recruitment of Axin to The Membrane	<i>Pgf</i>	<i>Nt5m</i>
Reactome Diseases Associated with O Glycosylation of Proteins	<i>Prg4</i>	<i>Ldhb</i>
Reactome Ecm Proteoglycans	<i>Zfp715</i>	<i>Plekhg3</i>
Reactome Ercc6 Csb and Ehmt2 G9a Positively Regulate Rna Expression	<i>Adora2b</i>	<i>Gbp3</i>
Reactome Host Interactions of HIV Factors	<i>Qrs11</i>	<i>Zbp1</i>
Reactome Met Promotes Cell Motility	<i>Arg1</i>	<i>Trnau1ap</i>
Reactome Mtorc1 Mediated Signaling	<i>Ptprb</i>	<i>Rxra</i>
Reactome Ncam1 Interactions	<i>Jsrp1</i>	<i>Urod</i>
Reactome Neddylation	<i>Tcn2</i>	<i>Fgl2</i>
Reactome Nuclear Signaling By Erbb4	<i>Pspc1</i>	
Reactome Oncogene Induced Senescence	<i>Angpt1</i>	
Reactome Regulation of Pten Stability and Activity	<i>Wdyhv1</i>	
Reactome Regulation of Runx1 Expression and Activity	<i>Arap3</i>	
Reactome Regulation of Tp53 Activity	<i>Sac3d1</i>	
Reactome Regulation of Tp53 Activity Through Acetylation	<i>RNAseh2c</i>	
Reactome Rho Gtpases Activate NADPH Oxidases	<i>Twink</i>	
Reactome Rhoq Gtpase Cycle	<i>Gdf11</i>	
Reactome Sumoylation Of Transcription Cofactors	<i>Exosc7</i>	
Reactome Transport of Vitamins Nucleosides and Related Molecules	<i>Mettl21a</i>	
Reactome Trna Processing In The Nucleus	<i>Il1r2</i>	
Kegg Hematopoietic Cell Lineage	<i>Traf1</i>	
Kegg Intestinal Immune Network for Iga Production	<i>Egfl7</i>	
Kegg Leukocyte Transendothelial Migration	<i>Nmi</i>	
Kegg Lysine Degradation	<i>Slc43a3</i>	
Kegg Propanoate Metabolism	<i>Hoxd8</i>	
Kegg Tight Junction	<i>Map1a</i>	
Kegg Tryptophan Metabolism	<i>Ndufaf1</i>	
Kegg Type Ii Diabetes Mellitus	<i>Gzfl</i>	
Biocarta GPCR Pathway	<i>E2f1</i>	
Biocarta Il6 Pathway	<i>Zbp1</i>	
Biocarta Nfat Pathway	<i>Ptprd</i>	
	<i>Trnau1ap</i>	
	<i>Padi2</i>	
	<i>Abcb1b</i>	
	<i>Pank4</i>	
	<i>Dvl1</i>	
	<i>Trmt44</i>	
	<i>Cds1</i>	
	<i>Cxcl5</i>	
	<i>Ldhb</i>	
	<i>Zfp626</i>	
	<i>Gab1</i>	
	<i>Snx19</i>	
	<i>Rpusd4</i>	
	<i>Nt5m</i>	
	<i>Mansc1</i>	

	<i>Ypel4</i>	
	<i>Uaca</i>	
	<i>Plvap</i>	
	<i>Dna2</i>	
	<i>Trappc14</i>	
	<i>Gpr160</i>	
	<i>Ptger2</i>	
	<i>Dhx38</i>	
	<i>Tigar</i>	
	<i>Clstm1</i>	
	<i>Mrps35</i>	
	<i>Tex2</i>	
	<i>Mapk1ip1</i>	
	<i>Zbed3</i>	
	<i>Zfp386</i>	
	<i>Uggt2</i>	
	<i>Mustn1</i>	
	<i>Atpaf2</i>	
	<i>Trp53rkb</i>	
	<i>AY074887</i>	
	<i>Zfp553</i>	
	<i>C87436</i>	
	<i>Champ1</i>	
	<i>Ankrd16</i>	
	<i>Zbtb45</i>	
	<i>Bag5</i>	
	<i>Nlrc3</i>	
	<i>Mical3</i>	
	<i>Pfdn4</i>	
	<i>Plekhg3</i>	
	<i>Tnfrif6</i>	
	<i>Exoc6</i>	
	<i>Per2</i>	
	<i>Hspa2</i>	
	<i>Zfp954</i>	
	<i>Ganc</i>	
	<i>Cdc42ep5</i>	
	<i>Jchain</i>	
	<i>Sco1</i>	
	<i>Tut1</i>	
	<i>Plekhf1</i>	
	<i>Cd80</i>	
	<i>Igkc</i>	
	<i>Rpl23a.ps3</i>	
	<i>Gm17251</i>	
	<i>Igha</i>	
	<i>Rnf113a2</i>	
	<i>Mir6236</i>	
	<i>Gm43112</i>	
	<i>Rpl32l</i>	
	<i>Gm46430</i>	
	<i>Gm48887</i>	
	<i>Gm7926</i>	

Table S3. Pathways and genes differentially represented at the scaffold conserved during ACAR in both STx and HTx.

HTx and STx Differentially Enriched Pathways	Pre-Graft Injury Pathways	ACAR DEGs	ACAR Panel
Reactome Laminin Interactions	Biocarta Il6 Pathway	<i>Bcl6b</i>	<i>Klf9</i>
Reactome Transport of Vitamins Nucleosides and Related Molecules	Reactome Degradation of Beta Catenin	<i>Mcm3ap</i>	<i>H2-Eb1</i>
Reactome Diseases Associated with O Glycosylation of Proteins	Reactome Host Interactions of HIV Factors	<i>Col18a1</i>	<i>Pura</i>
Reactome Signaling by Met	Reactome Neddylation	<i>Gria3</i>	<i>Slc30a7</i>
Kegg Focal Adhesion	Reactome Regulation of Pten Stability and Activity	<i>Rab34</i>	<i>Prpc</i>
Reactome Hdr Through Single Strand Annealing Ssa	Reactome Degradation of Axin	<i>Napsa</i>	<i>Flywch1</i>
Reactome Homologous Dna Pairing and Strand Exchange	Reactome Copii Mediated Vesicle Transport	<i>Timmdc1</i>	<i>Cbx7</i>
Reactome Processing of Intronless Pre mRNAs	Reactome Sumoylation of Transcription Cofactors	<i>Map2k7</i>	<i>Prcc</i>
Reactome Binding and Uptake of Ligands by Scavenger Receptors	Kegg Intestinal Immune Network for Iga Production	<i>Tbc1d8</i>	<i>Nmi</i>
Reactome Signaling by Notch2	Reactome Mtorc1 Mediated Signaling	<i>Ralb</i>	<i>Arhgap9</i>
Reactome Signaling by Erbb4	Reactome Trna Processing In The Nucleus	<i>Matk</i>	<i>Zfp182</i>
Biocarta Eif4 Pathway	Reactome Bbsome Mediated Cargo Targeting to Cilium	<i>Ccl8</i>	<i>Upf3a</i>
Biocarta Nos1 Pathway	Reactome Transport of Vitamins Nucleosides	<i>Rnmt</i>	<i>Padi2</i>
Biocarta Calcineurin Pathway	Reactome Regulation of Runx1 Expression and Activity	<i>Kdsr</i>	
Reactome Foxo Mediated Transcription of Cell Cycle Genes	Kegg Tryptophan Metabolism	<i>Slc3a2</i>	
Hallmark Hypoxia	Kegg Tight Junction	<i>Zfp715</i>	
Reactome Regulation of Runx1 Expression and Activity	Kegg Type Ii Diabetes Mellitus	<i>Nelfb</i>	
Reactome Regulation of Tp53 Activity Through Acetylation	Reactome Regulation of Tp53 Activity	<i>Cacfd1</i>	
Reactome DNA Double Strand Break Response	Reactome Regulation of Tp53 Activity Through Acetylation	<i>Bola1</i>	
Reactome Cell Death Signaling Via Nrage Nrif and Nade	Reactome Oncogene Induced Senescence	<i>Eef1a2</i>	
Reactome Mitotic Prophase	Reactome Erc6 Csb and Ehmt2 G9a Positively Regulate Rna	<i>Med1</i>	
Kegg Vegf Signaling Pathway	Biocarta Nfat Pathway	<i>Pfn1</i>	
Biocarta Carm Er Pathway	Biocarta Gpcr Pathway	<i>Mknk2</i>	
Reactome Ksrp Khsrp Binds and Destabilizes mRNA	Kegg Leukocyte Transendothelial Migration	<i>Fkbp1b</i>	
Kegg Systemic Lupus Erythematosus	Reactome Rhoq Gtpase Cycle	<i>Mink1</i>	
	Kegg Lysine Degradation	<i>Acot2</i>	
	Reactome Diseases Associated with O Glycosylation	<i>Zfyve16</i>	
	Hallmark Angiogenesis	<i>Capn7</i>	
	Reactome Disassembly of The Destruction Complex	<i>Mrpl57</i>	
	Reactome Copi Dependent Golgi to Er Retrograde Traffic	<i>Angpt1</i>	
	Kegg Hematopoietic Cell Lineage	<i>Parvb</i>	
	Kegg Propanoate Metabolism	<i>Mroh1</i>	
	Reactome Nuclear Signaling By Erbb4	<i>Vapa</i>	
	Reactome Rho Gtpases Activate Nadph Oxidases	<i>Axin1</i>	
	Reactome Binding and Uptake of Ligands by Scavenger	<i>Slc25a46</i>	
	Reactome Met Promotes Cell Motility	<i>Tapbp</i>	
	Reactome Collagen Biosynthesis and Modifying Enzymes	<i>Impact</i>	
	Reactome Ecm Proteoglycans	<i>Cd74</i>	
	Reactome Ncam1 Interactions	<i>Banfl</i>	
		<i>Arl3</i>	
		<i>Cbr2</i>	
		<i>Mmp19</i>	
		<i>Prim1</i>	

		<i>Mettl21a</i>	
		<i>Slc39a10</i>	
		<i>Pou2f1</i>	
		<i>Abi2</i>	
		<i>Fubp3</i>	
		<i>Nmi</i>	
		<i>Hoxd8</i>	
		<i>Gabpb1</i>	
		<i>Bub1</i>	
		<i>Bcl2l11</i>	
		<i>E2f1</i>	
		<i>Hps3</i>	
		<i>Kcnab1</i>	
		<i>Col15a1</i>	
		<i>Tm2d1</i>	
		<i>Hmgcl</i>	
		<i>Trnaulap</i>	
		<i>Padi2</i>	
		<i>Lrrc47</i>	
		<i>Rchy1</i>	
		<i>Naaa</i>	
		<i>Bhlhe41</i>	
		<i>Erc2</i>	
		<i>Adm</i>	
		<i>Slc25a14</i>	
		<i>Eif4ebp1</i>	
		<i>Mt1</i>	
		<i>Pdgd</i>	
		<i>Trak1</i>	
		<i>Kank1</i>	
		<i>Klf9</i>	
		<i>Fam151b</i>	
		<i>Polr3b</i>	
		<i>Uaca</i>	
		<i>Edil3</i>	
		<i>Foxn2</i>	
		<i>Neil2</i>	
		<i>Lysmd3</i>	
		<i>Colec12</i>	
		<i>Fam13b</i>	
		<i>Ppfibp2</i>	
		<i>Upf3b</i>	
		<i>H2-Aa</i>	
		<i>H2-DMa</i>	
		<i>Fermt2</i>	
		<i>Vopp1</i>	
		<i>Fbxw17</i>	
		<i>Tgm2</i>	
		<i>Cpe</i>	
		<i>Arl5c</i>	
		<i>Txnip</i>	
		<i>Upf3a</i>	
		<i>BC028528</i>	
		<i>Isg20</i>	
		<i>Znfx1</i>	
		<i>Gsap</i>	
		<i>Flywch1</i>	
		<i>Gbp7</i>	
		<i>Zfp770</i>	

		<i>Ankib1</i>	
		<i>Ccnb1</i>	
		<i>Mapk1ip1</i>	
		<i>Ndufa7</i>	
		<i>Tril</i>	
		<i>Pura</i>	
		<i>Rtn4rl1</i>	
		<i>Camk2n1</i>	
		<i>Zfp319</i>	
		<i>Irgm1</i>	
		<i>Mcmbp</i>	
		<i>Pigm</i>	
		<i>Cbx7</i>	
		<i>Ifi205</i>	
		<i>Arfgap3</i>	
		<i>Rhob</i>	
		<i>Slc30a7</i>	
		<i>Zfp182</i>	
		<i>Cd47</i>	
		<i>Mrps14</i>	
		<i>C1qmf3</i>	
		<i>Psmc3</i>	
		<i>H2-Eb1</i>	
		<i>Prep</i>	
		<i>Ei24</i>	
		<i>Phactr2</i>	
		<i>Zfp780b</i>	
		<i>Zfp26</i>	
		<i>Chchd1</i>	
		<i>Hspbp1</i>	
		<i>Cd59b</i>	
		<i>Trem12</i>	
		<i>H2-Ab1</i>	
		<i>Snrnp40</i>	
		<i>Gas2l3</i>	
		<i>Commd6</i>	
		<i>Gm12359</i>	
		<i>Smarcd2</i>	
		<i>Zfp703</i>	
		<i>Rasgef1b</i>	
		<i>Gm4890</i>	
		<i>Gpr137b-ps</i>	
		<i>Rnf113a2</i>	
		<i>Mir6398</i>	

Table S4. Antibodies used for flow cytometry or T cell depletion.

Innate Immune Cell Panel			Lymphocyte Panel		
Antibody	Manufacturer	Clone	Antibody	Manufacturer	Clone
Zombie Violet Cell Viability	Biolegend #423113	---	Zombie Violet	Biolegend #423113	---
BV510 anti-mouse CD11b	Biolegend #101263	M1/70	BV510 CD19	Biolegend #115546	6D5
BV711 anti-mouse Ly-6G	Biolegend #127643	1A8	BV711 CD4	Biolegend #100447	GK1.5
FITC anti-mouse Ly-6C	Biolegend #128006	HK1.4	FITC CD8a	Biolegend #100706	53-6.7
PE-Cy7 anti-mouse F4/80	Biolegend #123114	BM8	PE-Cy7 CD49b	Biolegend #108922	DX5
APC anti-mouse CD11c	Biolegend #117310	N418	---	---	---
AF700 CD45	Biolegend #103128	30-F11	AF700 CD45	Biolegend #103128	30-F11

### Auxiliary Supplementary Materials

- Data file S1. Tabular data for figures and supplemental figures, including readme.  
Data used to generate main plots and figures, including a readme and data of supporting figures as well.
- Data file S2. Flow cytometry Flowjo software analyses of STx samples.  
Flow cytometry data, compensation, gating, and population selections for subsequent analyses.
- Data file S3. Raw RNA-seq counts from STx scaffold samples.  
Sequencing raw gene expression from scaffold samples collected from skin transplant recipients.
- Data file S4. Normalized RNA-seq counts from STx scaffold samples.  
Sequencing normalized counts per million gene expression from scaffold samples collected from skin transplant recipients.
- Data file S5. Raw RNA-seq counts from HTx scaffold samples.  
Sequencing raw gene expression from scaffold samples collected from heart transplant recipients.
- Data file S6. Normalized RNA-seq counts from HTx scaffold samples.  
Sequencing normalized counts per million gene expression from scaffold samples collected from heart transplant recipients.
- Data file S7. R script of code for sequencing analyses.  
R language script file of sequencing analyses, as detailed in the Methods, including elastic net regression and Gene Set Variation Analysis.