SUPPLEMENTARY METHODS

Sample isolation, RNA extraction, and microarray experiments, discovery cohort - University of Chicago

Blood samples were obtained by venous phlebotomy from each subject. PBMCs were then obtained by Ficoll density centrifugation. After washing, the PBMCs were suspended on TRIzol (Invitrogen) and RNA extracted following the manufacturer's protocol. RNA yield and quality was evaluated using NanoDrop at 260 nm and the 2100 Bioanalyzer (Agilent Technologies) and specimens were stored at -70^{o}C for later use. Labeling reactions for microarray experiments were performed using a GeneChip WT cDNA Synthesis and Amplification Kit (Affymetrix). In brief, a ribosomal RNA reduction step was performed on 1 μg of total RNA followed by cDNA synthesis with random hexamers tagged with a T7 promoter sequence. The double-stranded cDNA product was then used as a template for T7 RNA polymerase amplification to produce copies of antisense cRNA. Random hexamers were used to prime reverse transcription of the cRNA from the first cycle to produce single-stranded DNA in the sense orientation using dUTP incorporation to increase the reproducibility of the fragmentation. The single-stranded DNA was then treated with a combination of uracil DNA glycosylase and apurinic/apyrimidinic endonuclease 1 to break the DNA strand. In turn, these strands were biotinylated by use of terminal deoxynucleotidyl transferase (TdT) with the Affymetrix proprietary DNA Labeling Reagent. Labeling efficiency was evaluated using the gel-shift assay. The labeled single stranded DNA was hybridized using GeneChip Human 1.0 exon ST arrays (Affymetrix) and scanned using the Affymetrix GeneChip Scanner 3000, following the manufacturer's protocol. Data were processed using dChip software (*37*). Briefly, after whole array quintile normalization the exon intensities were summarized into gene expression levels by mapping exon probe sets into U133_Plus_2 consensus or exemplar sequences based on Affymetrix annotation U133 Plus Vs Hu Ex (03/09).

Sample isolation, RNA extraction, and microarray experiments, replication cohort - University of Pittsburgh

Peripheral blood was collected in a cell preparation tube, followed by centrifugation to isolate PBMCs. These cells were suspended in QIAzol (Qiagen) and stored at -80 ^{o}C. Total RNA was extracted and purified using the miRNeasy Mini Kit (Qiagen) and QIAcube device (Qiagen), following the manufacturer's protocols. After extraction, total RNA yield and quality were evaluated using NanoDrop at 260 nm and the 2100 Bioanalyzer (Agilent Technologies). Labeling reactions were performed using Agilent Quick Amp labeling kit, one-color (Agilent Technologies). Briefly, an initial cDNA strand was synthesized using 400 ng of total RNA and an oligo(*42*)24 primer containing T7 RNA polymerase. This cDNA was then used as a template to generate Cy3-labeled cRNA by a reverse transcriptase enzyme. The cRNA was fragmented, hybridized to Whole Human Genome Oligo Microarray, 4 x 44K (G4112F, Agilent Technologies), and scanned using an Agilent Microarray Scanner. For array readout, Agilent Feature Extraction software version 10.7 was used (*53*). To normalize the gProcessed signal, cyclic-LOESS was performed using the bioconductor package as described previously (*38*). The average of the gene expression signal was used in the case of replicated probes for the same gene with different expression values.

qRT-PCR in discovery and replication cohorts

Quantitative reverse transcription (qRT)-PCR was performed on patient's samples from the discovery (*n*=43) and replication (*n*=74) cohorts. To confirm the performance and prognostic significance of candidate genes in a more clinically feasible platform, a custom, multi-sample, high-throughput, qRT-PCR assay was designed based on the Wafergen 5K SmartChip (5184 wells). qRT-PCR was performed for

every subject's sample, except for 2 patients from the discovery cohort and 1 subject from the replication cohort owing to insufficient amounts of RNA. SmartChip wells were preloaded with PCR primers for the genes *CD28, ICOS, LCK, ITK* and *ACTB*. Forward and reverse primers for these genes were designed using the Universal ProbeLibrary System (Roche) based on the Primer3 software program. Each chip was set to have 46 samples, one positive control (universal cDNA) and one negative control (NTC) with the primers in four replicates printed for each one of the samples by the manufacturing group. All primers preloaded on SmartChips were verified using human universal reference total RNA in conjunction with a two-step qPCR assay. The starting total RNA concentration was 150 ng per sample to generate cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) following the manufacturer's protocol. The cDNA (100 pg) was combined with LightCycler SYBR I green master mix (Roche) and dispensed in each well using the customized IDEX/Innovadyne Nanodrop system. SmartChip thermocycler conditions for the PCR were: Three minute heat activation at 95^{o}C followed by 40 cycles of PCR denaturing at 95^{o}C for 35 seconds and annealing/extending at 60^{o}C for 65 seconds, PCR was then followed by a melting curve with 0.4^{o}C resolution. qRT-PCR was not performed in two IPF subjects from the microarray discovery cohort and in one IPF subject from the microarray replication cohort because RNA samples were exhausted for these subjects.

Significance Analyses of Microarrays (SAM)

To test the association between PBMC microarray gene expression and TFS in IPF patients from the discovery cohort, we used SAM with censored outcome data (*34*). In our case, we used censored TFS data $y_j = (t_j,\Delta_j)$, where t_j is time to outcome and $\Delta_j = 1$, if the observation is death or transplant (whichever happens first) or $\Delta_j = 0$, if the patients were still alive when they were lost to follow-up or at

the conclusion of the study. SAM computes a modified Cox score test statistic for each gene, measuring the strength of the relationship between gene expression differences and TFS in two groups. In genes with a positive Cox score, higher gene expression correlated with shorter TFS time whereas lower gene expression correlated with longer TFS time. In genes with a negative Cox score, higher gene expression correlated with longer TFS time whereas lower gene expression correlated with shorter TFS time. SAM performs permutation analysis of the TFS data to assess statistical significance and account for multiple testing by controlling the false discovery rate (FDR) (*35*). For univariate gene selection we chose genes with an FDR below 5% and a Cox score above or equal to (≥) 2.5 and below or equal to (≤) -2.5.

Flow cytometry experiments, replication cohort

Flow cytometry studies were performed in IPF patients (*n*=72) from the replication cohort using methods that have previously detailed and illustrated (*11, 33*). In brief, freshly isolated PBMC were stained with anti-human CD4-allophycocyanin and anti-human CD28-FITC monoclonal antibodies (mAb). Individual aliquots of these cells were also stained with mAb against other cell surface epitopes of interest (ICOS and CD3ε in *n*=20 IPF patients). ITK and LCK (*n*=16) were evaluated among identical preparations that were and fixed and permeabilized, using reagents supplied in a kit (Cytofix/Cytoperm, BD Bioscience), prior to incubation with the respective mAb having specificities for these intracellular molecules. mAb, including isotype control antibodies, were purchased from BD Bioscience. Flow cytometry characterizations were performed on >10,000 live cells using a BD FACSCalibur (BD Bioscience). Gates for quantitative analyses were set using control fluorochrome-positive and -negative PBMCs, including isotype controls.

Unless otherwise denoted, data are delineated as percentages of cells within the respective autologous $CD4^+CD28^+$ and $CD4^+CD28^{null}$ subpopulations (see example in Fig. S2) expressing the phenotypic characteristic(s) of interest (i.e., ICOS, ITK, and LCK). Cell surface expression of CD3ε was determined as mean fluorescent intensity (MFI). Because absolute values of MFI can vary somewhat from day to day, even using the same flow cytometer and despite appropriate calibration, cell surface CD3ε expression is depicted as a ratio of autologous, concurrent $CD4^+CD28^{null}/CD4^+CD28^+$ MFI values.

Gene set analysis (GSA)

We collected gene set information using a molecular signature database (MSigDB) of annotated canonical pathways (*36*) including 1452 curated gene sets from the following databases: BioCarta (*37*), CORUM MIPS (*38*), KEGG (*39*), Pathway Interaction Database (*40*), Reactome (*41*), Sigma-Aldrich (*42*), Signaling Gateway (*43*), Science Signaling (*44*) and SuperArray (*45*). Gene sets of the MSigDB were included in the analysis if they contained at least 20 but not more than 200 genes; this cutoff was selected as a good balance for gene sets to reduce the multiple-testing issue and to avoid testing overly narrow or too large canonical pathways. To evaluate the association between gene sets and TFS in IPF patients from the discovery and replication cohorts, we used the GSA method with censored outcome data (*46*). In our case, we used censored TFS data. GSA calculates a Cox score test statistic for each gene similar to SAM and then uses a Maxmean summary statistic; this is the mean of the positive or negative part of gene scores in the gene set, whichever is large in absolute values. A gene set with a negative Maxmean score was one in which lower expression of most genes in the gene set correlated with shorter TFS time and a gene set with a positive Maxmean score was one in which higher expression of most genes in the gene set correlated with shorter TFS time. GSA also performed permutation analysis to control the FDR for each identified gene set. We applied a threshold at FDR=5% and the gene set rank

was determined by their either positive or negative Maxmean score (whichever was larger in absolute values).

SUPPLEMENTARY FIGURES

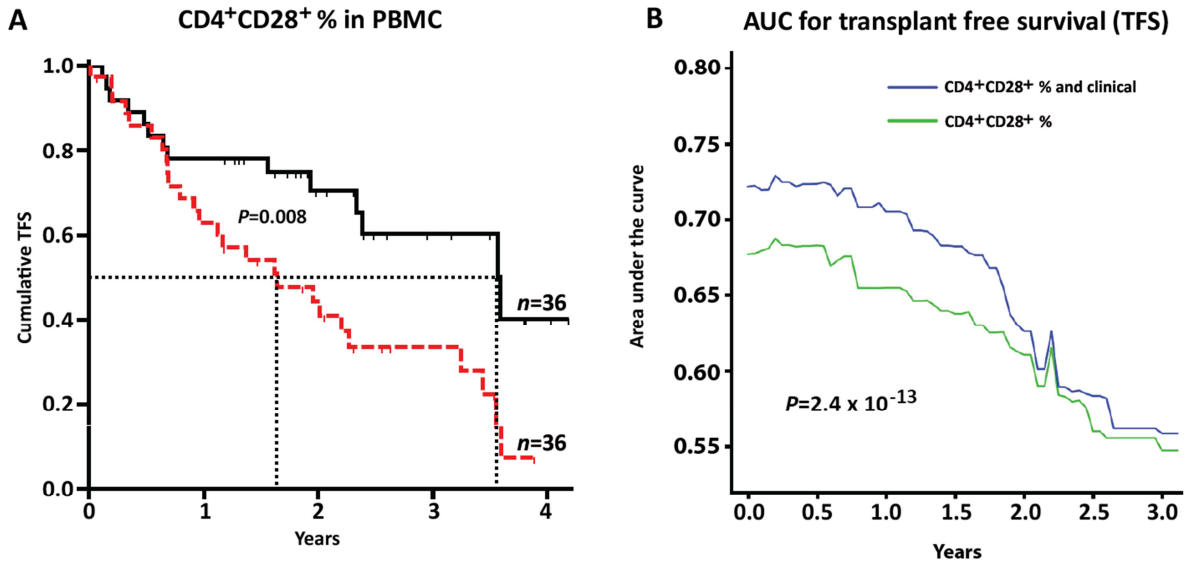

Fig. S1. $CD4^+CD28^+$ T cells predict TFS. (**A**) TFS analysis in the replication cohort (*n*=72) with percentages of $CD4^+CD28^+$ T cells in PBMC. In the Kaplan-Meier plot, the red lines are patients with percentages of $CD4^+CD28^+$ T cells below the median value; the black lines are patients whose percentages of $CD4^+CD28^+$ T cells were above the median value. *P*-value was determined by the Log-rank test. (**B**) Area under the curve (AUC) of time-dependent ROC analysis for TFS based on models including the percentage of $CD4^+CD28^+$ T cells in PBMC alone or in combination with clinical variables consisting of age, gender, and FVC%. *P*-value was determined by the Wilcoxon signed rank test

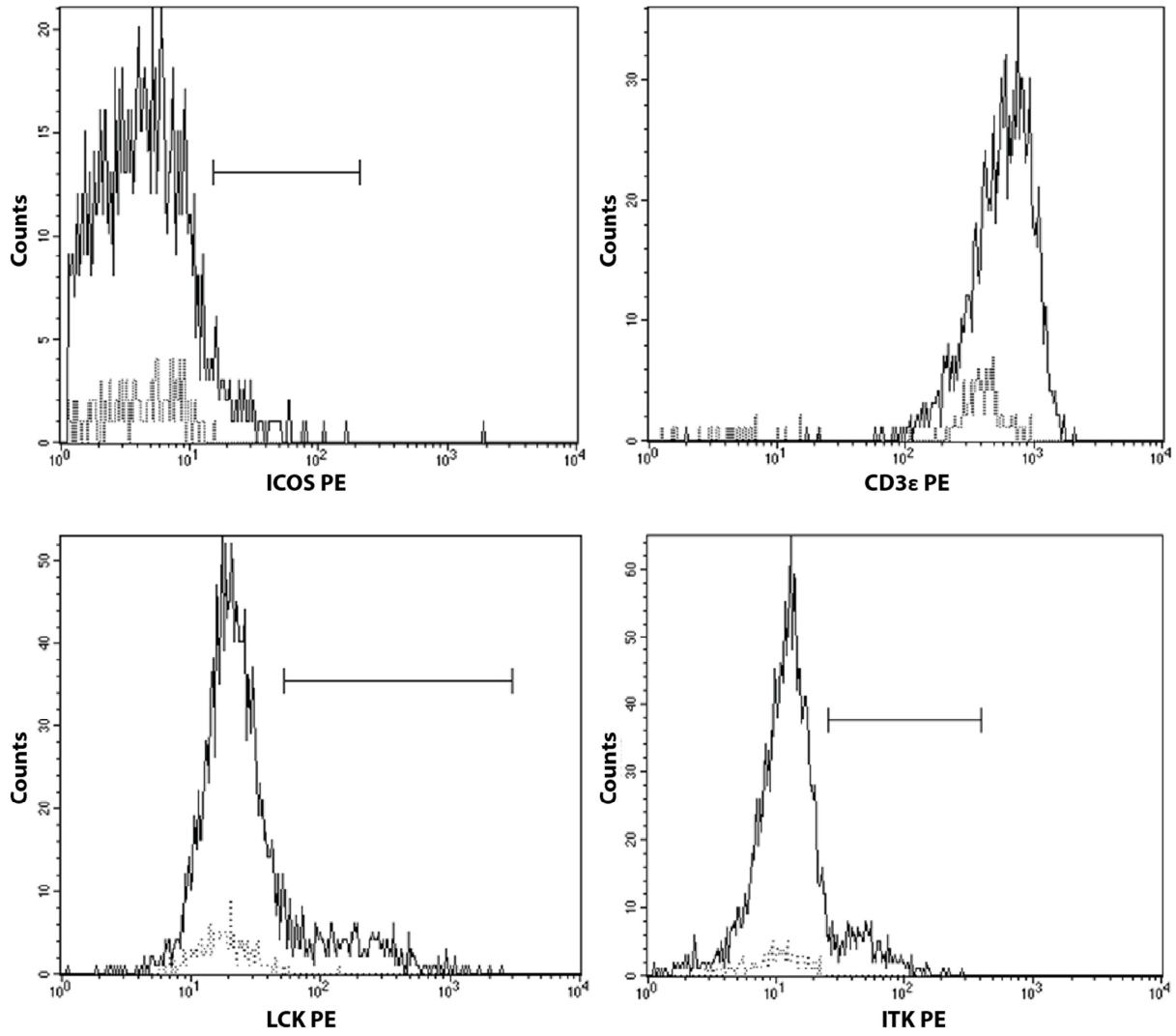

Fig. S2. $CD4^{+}CD28^{null}$ T cells have decreased protein expression of T cell markers. Expressions of ICOS, the T cell receptor complex protein CD3e, and tyrosine kinases LCK and ITK among paired autologous $CD4^{+}CD28^{+}$ and $CD4^{+}CD28^{null}$ T cells are illustrated here in one (*n*=1) replication cohort IPF patient. The horizontal bars denote those cells that express the various proteins (these gates were determined by isotype-negative and -positive controls). $CD4^{+}CD28^{+}$ cells are illustrated by solid dark lines, whereas $CD4^{+}CD28^{null}$ cells are depicted by faint interrupted lines.

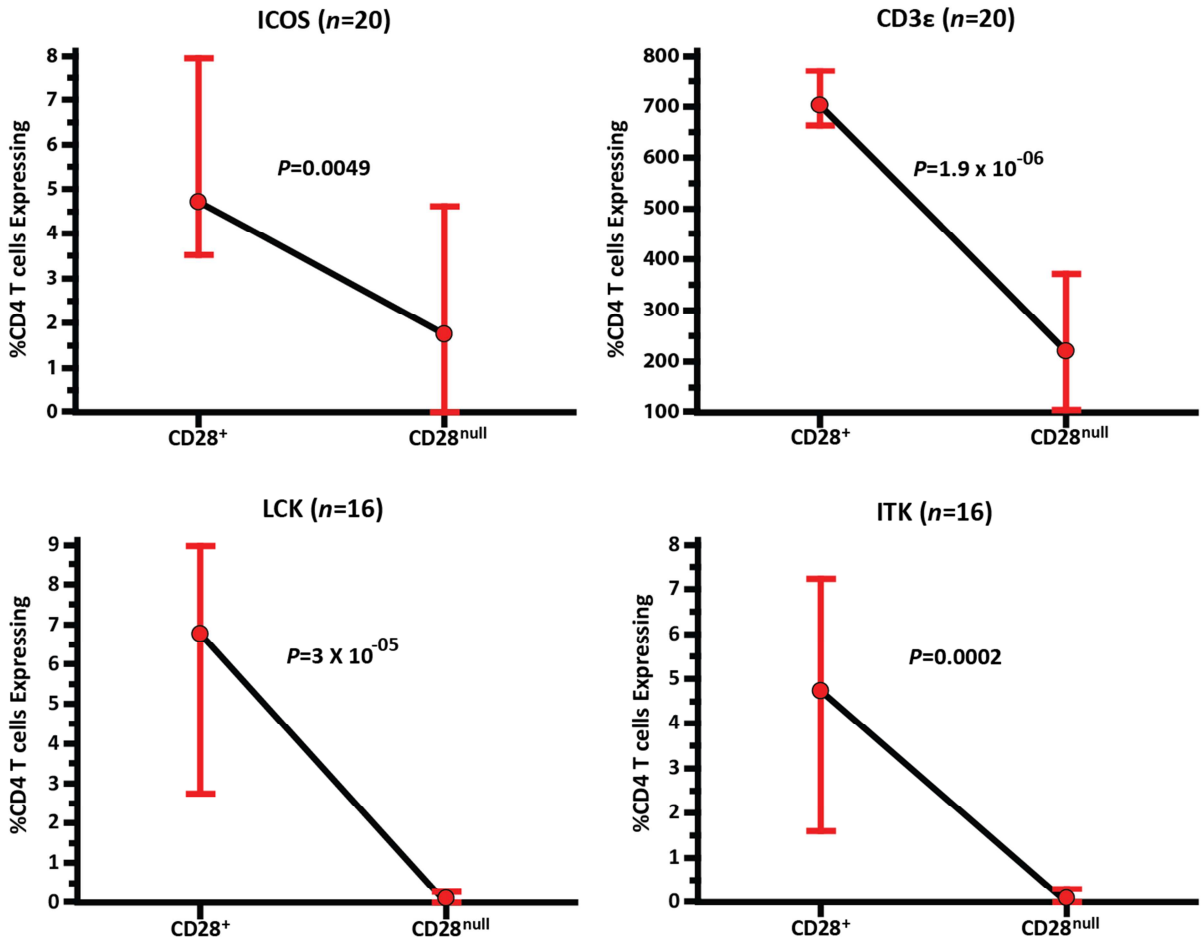

Fig. S3. $CD4^{+}CD28^{null}$ cells have decreased protein expression of selected T cell markers. Expressions of ICOS, the T cell receptor complex protein CD3e, and tyrosine kinases LCK and ITK among paired autologous $CD4^{+}CD28^{+}$ and $CD4^{+}CD28^{null}$ T cells in IPF patients from the replication cohort. Data are medians ±95% CI for a median, with black lines connecting the paired samples. *N* represents the number of subjects with paired samples. *P*-values determined with a Wilcoxon test for paired samples

SUPPLEMENTARY TABLES

Table S1. Clinicopathological characteristics of the IPF patients in the two cohorts. *P*-values were calculated using the Fisher's exact test (a Freeman-Halton extension was used for race) except for age and pulmonary function tests where an unpaired, two tailed, t-test was used. P-values for differences in transplant free survival were calculated with the Log-rank test. FVC%: Forced vital capacity percent predicted, DLCO%: Carbon monoxide diffusing capacity percent predicted. FEV1% Forced expiratory volume in 1 second percent predicted. HRCT, high resolution computed tomography. UIP, usual interstitial pneumonia.

Characteristic	Discovery cohort (*n*=45)	Replication cohort (*n*=75)	P-value
Age (yr)[‡]			
Mean ± SD	66.9 ± 8.1	68.9 ± 8.1	0.21
Range	43.0 – 84.0	50.0 – 84.0	
Gender, *n* (%)			
Males	40 (88.9)	52 (69.3)	0.02
Females	5 (11.1)	23 (30.7)	
Race, *n* (%)			<0.01
Caucasian	37 (82.2)	73 (97.4)	
Black	3 (6.7)	1 (1.3)	
Hispanic	5 (11.1)	0 (0)	
Oriental	0 (0)	1 (1.3)	
Smoking status, *n* (%)			0.84
Ever smoker	27 (60)	47 (62.7)	
Never smoker	18 (40)	28 (33.3)	
Pulmonary function tests (mean ± SD)			
FVC (%)	62 ± 14	65 ± 16	0.20
DLCO (%) [§]	44 ± 17	49 ± 18	0.13
FEV1 (%)	75 ± 16	76 ± 17	0.46
Diagnostic strategy, *n* (%)			0.11
Clinical + HRCT+ UIP Proven	24 (53.3)	52 (69.3)	
Clinical + HRCT	21 (46.7)	23 (30.7)	
Immunosuppressive therapy, *n* (%) [¶]			0.13
No	43 (95.6)	64 (85.3)	
yes	2 (4.4)	11 (14.7)	
Lung transplants – *n* (%)			0.02
No	43 (95.6)	60 (80)	
Yes	2 (4.4)	15 (20)	

‡ Age at the time of blood draw.

§ Four subjects in the microarray replication cohort did not have DLCO% values available within 3 months of blood draw.

¶ Immunosuppressive therapy was defined as the use of prednisone, azathioprine, or a combination of both at the time of blood draw. Two subjects in the discovery and six subjects in the replication cohort were on prednisone alone. Two subjects were on azathioprine alone and three subjects were in a combination of prednisone and azathioprine in the replication cohort at blood draw.

Table S2. Significant gene sets associated with TFS in the discovery cohort. Gene-set significance was defined as FDR<5% and the gene-set rank was determined by their either positive or negative Maxmean score (whichever was larger in absolute values). A negative gene-set was one in which lower expression of most genes in the gene set correlated with higher risk (shorter TFS). A positive gene-set was one in which higher expression of most genes in the gene set correlated with higher risk (shorter TFS). Gene-set names and description are obtained from the molecular signature database (MSigDB).

Rank	Standard gene-set name	Gene-set description	Maxmean score
1	BIOCARTA_CTLA4_PATHWAY	The co-stimulatory signal during T-cell activation	-1.91
2	REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX	Genes involved in degradation of the extracellular matrix	1.75
3	REACTOME_GENERATION_OF_SECOND_MESSENGER_MOLECULES	Genes involved in generation of second messenger molecules	-1.38
4	REACTOME_RNA_POL_I_TRANSCRIPTION_TERMINATION	Genes involved in RNA polymerase I transcription termination	-1.20
5	KEGG_ALLOGRAFT_REJECTION	Allograft rejection	-1.11
6	REACTOME_CYTOSOLIC_TRNA_AMINOACYLATION	Genes involved in cytosolic tRNA aminoacylation	-1.08
7	KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	Aminoacyl-tRNA biosynthesis	-1.06
8	REACTOME_DOWNSTREAM_TCR_SIGNALING	Genes involved in downstream TCR signaling	-1.04
9	REACTOME_TRNA_AMINOACYLATION	Genes involved in tRNA aminoacylation	-1.00
10	KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	Intestinal immune network for IgA production	-0.93
11	REACTOME_TCR_SIGNALING	Genes involved in TCR signaling	-0.87
12	KEGG_RNA_DEGRADATION	RNA degradation	-0.86
13	REACTOME_COSTIMULATION_BY_THE_CD28_FAMILY	Genes involved in costimulation by the CD28 family	-0.81
14	REACTOME_CD28_DEPENDENT_PI3K_AKT_SIGNALING	Genes involved in CD28 dependent PI3K/Akt signaling	-0.79
15	KEGG_BLADDER_CANCER	Bladder cancer	0.70
16	PID_TCR_PATHWAY	TCR signaling in naïve CD4+ T cells	-0.61
17	KEGG_GLYCEROPHOSPHOLIPID_METABOLISM	Glycerophospholipid metabolism	0.40
18	REACTOME_GLYCEROPHOSPHOLIPID_BIOSYNTHESIS	Genes involved in glycerophospholipid biosynthesis	0.38

Table S3. Clinicopathological characteristics of the IPF patients in the two major clusters of the replication cohort. *P*-values were calculated using the Fisher's exact test except for age and pulmonary function tests where an unpaired, two tailed, *t*-test was used. *P*-values for differences in transplant free survival were calculated with the Log-rank test. FVC%, forced vital capacity, percent predicted, DLCO%, carbon monoxide diffusing capacity, percent predicted. FEV1%, forced expiratory volume in 1 second, percent predicted. HRCT, high-resolution computed tomography. UIP, usual interstitial pneumonia.

Characteristics	Cluster 1 (*n*=45)	Cluster 2 (*n*=30)	*P*-value[†]
Age (yr)[‡]			
Mean ± SD	67.9 (± 7.3)	70.5 (± 9.2)	0.18
Range	56 – 82	50 – 84	
Gender, *n* (%)			
Males	28 (62.2%)	24 (80%)	0.12
Females	17 (37.8%)	6 (20%)	
Race, *n* (%)			0.51
Caucasian	43 (95.6%)	30 (100%)	
Others	2 (4.4%)	0	
Smoking status, *n* (%)			0.46
Ever smoker	30 (63%)	17 (62.1%)	
Never smoker	15 (37%)	13 (37.9%)	
Pulmonary function tests (mean ± SD)			
FVC%	65% (± 16)	66% (± 18)	0.88
DLCO%[§]	49% (± 15)	48% (± 22)	0.76
FEV1%	76% (± 16)	77% (± 18)	0.65
Diagnostic strategy, *n* (%)			0.2
Clinical + HRCT+ UIP Proven	34 (75.6%)	18 (60%)	
Clinical + HRCT	11 (24.4%)	12 (40%)	
Immunosuppressive therapy – *n* (%)[¶]			0.1
No	41 (91.1%)	23 (76.7%)	
yes	4 (8.9%)	7 (23.3%)	

‡ Age at the time of blood draw.

§ Three subjects in cluster 1 and one subject in cluster 2 did not have DLCO% values available within 3 months of blood draw.

¶ Immunosuppressive therapy was defined as the use of prednisone, azathioprine or a combination of both at the time of blood draw. Two subjects in cluster 1 and four subjects in cluster 2 were on prednisone alone. Two subjects in cluster 1 were on azathioprine alone and three subjects in cluster 2 were in a combination of prednisone and azathioprine, at the time of the blood draw.

Table S4. Median TFS times and CIs. Patient groups from Fig. 2B, Fig. 4A, and fig. S1A were compared. NA, not applicable, indicates when the median TFS time was not reached or the 95% confidence level could not be calculated owing to values out of the range of the time of observation (Range: 0 to 4.18 years).

Figure panel – outcome subgroups	**Median TFS (years)**	**95% Lower confidence level**	**95% Upper confidence level**
Fig.2B – Cluster 1	3.44	2.27	NA
Fig. 2B – Cluster 2	1.62	0.92	NA
Fig. 4A – High *CD28*	3.44	2.34	NA
Fig. 4A – Low *CD28*	1.12	0.69	3.24
Fig. 4A – High *ICOS*	NA	2.39	NA
Fig. 4A – Low *ICOS*	0.92	0.65	1.96
Fig. 4A – High *ITK*	3.44	2.2	NA
Fig. 4A – Low *ITK*	1.17	0.69	3.55
Fig. 4A – High *LCK*	2.39	2.2	NA
Fig. 4A – Low *LCK*	1.17	0.7	3.59
fig. S1A – High $CD4^{+}CD28^{+}$ %	3.59	2.39	NA
fig. S1A – Low $CD4^{+}CD28^{null}$ %	1.64	0.96	3.44

Table S5. Significant gene sets associated with TFS in the replication cohort. Gene-set significance is defined as a FDR<5% and the gene-set rank is determined by their either positive or negative Maxmean score (whichever is larger in absolute values). A negative gene-set is one in which lower expression of most genes in the gene set correlates with higher risk (i.e., shorter transplant free survival). A positive gene-set is one in which higher expression of most genes in the gene set correlates with higher risk (i.e., shorter transplant free survival). Gene-set names and description are obtained from the molecular signature database (MSigDB).

Rank	Standard gene-set name	Gene-set description	Maxmean score
1	BIOCARTA_CTLA4_PATHWAY	The co-stimulatory signal during T-cell activation	-1.24
2	BIOCARTA_NKT_PATHWAY	Selective expression of chemokine receptors during T-cell polarization	-1.16
3	REACTOME_GENERATION_OF_SECOND_MESSENGER_MOLECULES	Genes involved in generation of second messenger molecules	-1.03
4	REACTOME_SHC1_EVENTS_IN_ERBB4_SIGNALING	Genes involved in SHC1 events in ERBB4 signaling	0.87
5	BIOCARTA_CSK_PATHWAY	Activation of Csk by cAMP-dependent protein kinase inhibits signaling through the T cell receptor	-0.86
6	ST_T_CELL_SIGNAL_TRANSDUCTION	T cell signal transduction	-0.83
7	REACTOME_NEF_MEDIATES_DOWN_MODULATION_OF_CELL_SURFACE_RECEPTORS_BY_RECRUITING_THEM_TO_CLATHRIN_ADAPTERS	Genes involved in Nef-mediates down modulation of cell surface receptors by recruiting them to clathrin adapters	-0.80
8	REACTOME_SPHINGOLIPID_DE_NOVO_BIOSYNTHESIS	Genes involved in sphingolipid de novo biosynthesis	0.77
9	PID_ALK1PATHWAY	ALK1 signaling events	0.73
10	KEGG_DORSO_VENTRAL_AXIS_FORMATION	Dorso-ventral axis formation	0.70
11	KEGG_BASAL_CELL_CARCINOMA	Basal cell carcinoma	-0.68
12	REACTOME_SIGNALLING_TO_RAS	Genes involved in signalling to RAS	0.67
13	REACTOME_ACTIVATED_NOTCH1_TRANSMITS_SIGNAL_TO_THE_NUCLEUS	Genes involved in activated NOTCH1 transmits signal to the nucleus	0.65
14	PID_INTEGRIN_CS_PATHWAY	Integrin family cell surface interactions	-0.62
15	REACTOME_TCR_SIGNALING	Genes involved in TCR signaling	-0.62
16	REACTOME_NETRIN1_SIGNALING	Genes involved in netrin-1 signaling	-0.61
17	REACTOME_SIGNALING_TO_ERKS	Genes involved in signaling to ERKs	0.60
18	PID_HNF3APATHWAY	FOXA1 transcription factor network	0.58
19	KEGG_PENTOSE_PHOSPHATE_PATHWAY	Pentose phosphate pathway	0.56
20	PID_SYNDECAN_1_PATHWAY	Syndecan-1-mediated signaling events	-0.56
21	PID_MAPKTRKPATHWAY	Trk receptor signaling mediated by the MAPK pathway	0.56
22	KEGG_BLADDER_CANCER	Bladder cancer	0.53
23	PID_LIS1PATHWAY	Lissencephaly gene (LIS1) in neuronal migration and development	0.47
24	REACTOME_SPHINGOLIPID_METABOLISM	Genes involved in sphingolipid metabolism	0.46
25	PID_TRKRPATHWAY	Neurotrophic factor-mediated Trk receptor signaling	0.44
26	PID_NETRIN_PATHWAY	Netrin-mediated signaling events	0.43
27	KEGG_HEMATOPOIETIC_CELL_LINEAGE	Hematopoietic cell lineage	-0.43
28	REACTOME_DAG_AND_IP3_SIGNALING	Genes involved in DAG and IP3 signaling	-0.40
29	REACTOME_GOLGI_ASSOCIATED_VESICLE_BIOGENESIS	Genes involved in Golgi associated vesicle biogenesis	0.37
30	REACTOME_TRANS_GOLGI_NETWORK_VESICLE_BUDDING	Genes involved in trans-Golgi network vesicle budding	0.33
31	PID_IL1PATHWAY	IL1-mediated signaling events	0.32

Table S6. Multivariate Cox proportional hazard model including *CD28* and clinical variables. All covariates in this model are continuous except for gender.

Covariate	Beta (b) coefficient	Standard error	*P*-value	Exp(b)	95% CI of Exp(b)
ΔCT CD28	0.6266	0.1828	0.0006	1.8713	1.3103 to 2.6725
Age	0.02796	0.02058	0.1743	1.0284	0.9879 to 1.0705
Gender	0.6473	0.4098	0.1142	1.9105	0.8592 to 4.2479
FVC% predicted	-0.03638	0.01226	0.0030	0.9643	0.9415 to 0.9876

Table S7. Multivariate Cox proportional hazard model including *ICOS* and clinical variables. All covariates in this model are continuous except for gender.

Covariate	Beta (b) coefficient	Standard error	*P*-value	Exp(b)	95% CI of Exp(b)
ΔCT ICOS	0.5338	0.1904	0.0050	1.7055	1.1765 to 2.4722
Age	0.02792	0.02127	0.1894	1.0283	0.9865 to 1.0719
Gender	0.6237	0.4124	0.1305	1.8658	0.8348 to 4.1702
FVC% predicted	-0.03411	0.01211	0.0049	0.9665	0.9439 to 0.9896

Table S8. Multivariate Cox proportional hazard model including *LCK* and clinical variables. All covariates in this model are continuous expect for gender.

Covariate	Beta (b) coefficient	Standard error	*P*-value	Exp(b)	95% CI of Exp(b)
ΔCT LCK	0.4069	0.2057	0.0479	1.5022	1.0058 to 2.2435
Age	0.03943	0.02032	0.0523	1.0402	0.9998 to 1.0823
Gender	0.6719	0.4128	0.1036	1.9580	0.8754 to 4.3792
FVC% predicted	-0.03283	0.01178	0.0053	0.9677	0.9457 to 0.9902

Table S9. Multivariate Cox proportional hazard model including *ITK* and clinical variables. All covariates in this model are continuous except for gender.

Covariate	Beta (b) coefficient	Standard error	*P*-value	Exp(b)	95% CI of Exp(b)
ΔCT ITK	0.4864	0.2041	0.0172	1.6264	1.0923 to 2.4216
Age	0.03556	0.02085	0.0881	1.0362	0.9949 to 1.0792
Gender	0.5983	0.4184	0.1527	1.8190	0.8045 to 4.1129
FVC% predicted	-0.03273	0.01178	0.0054	0.9678	0.9458 to 0.9903

Table S10. AUCs and SEs for TFS. The Cox models evaluated for TFS are summarized as follows: (I) Genomic and clinical (ΔCt of *CD28*, *ICOS*, *LCK*, *ITK*, age, gender, FVC%); (II) Genomic (ΔCt of *CD28*, *ICOS*, *LCK* and *ITK*); (III) Clinical (Age, Gender and FVC%); (IV) $CD4^+CD28^+$ % (percentage of $CD4^+CD28^+$ T cells in PBMC); and (V) $CD4^+CD28^+$ % and clinical (percentage of $CD4^+CD28^+$ T cells in PBMC, age, gender and FVC%). All pairwise comparisons between models were statistically significant (P<0.0001, Wilcoxon signed rank test) with the exception of the comparison between model II versus model V (P=0.44, Wilcoxon signed rank test)

Years	Model I mean AUC	Model I SE	Model II mean AUC	Model II SE	Model III mean AUC	Model III SE	Model IV mean AUC	Model IV SE	Model V mean AUC	Model V SE
0.00	0.779	0.047	0.756	0.037	0.712	0.049	0.677	0.042	0.722	0.041
0.05	0.780	0.046	0.758	0.036	0.712	0.049	0.678	0.042	0.722	0.040
0.10	0.779	0.046	0.756	0.036	0.709	0.047	0.679	0.043	0.719	0.039
0.15	0.779	0.046	0.758	0.036	0.710	0.047	0.681	0.043	0.720	0.039
0.20‡	**0.785**	**0.048**	**0.766**	**0.039**	**0.709**	**0.043**	**0.688**	**0.042**	**0.729**	**0.038**
0.25	0.760	0.044	0.756	0.036	0.704	0.046	0.683	0.044	0.724	0.042
0.30	0.760	0.044	0.756	0.036	0.704	0.046	0.683	0.044	0.724	0.042
0.35	0.759	0.044	0.740	0.034	0.706	0.046	0.682	0.045	0.722	0.043
0.40	0.761	0.044	0.730	0.035	0.706	0.046	0.682	0.045	0.723	0.043
0.45	0.761	0.044	0.730	0.035	0.706	0.046	0.682	0.045	0.723	0.043
0.50	0.763	0.044	0.734	0.036	0.706	0.046	0.683	0.045	0.723	0.043
0.55	0.764	0.043	0.736	0.036	0.707	0.045	0.682	0.045	0.724	0.043
0.60	0.763	0.043	0.731	0.036	0.710	0.046	0.669	0.041	0.723	0.042
0.65	0.746	0.046	0.724	0.037	0.697	0.048	0.673	0.041	0.716	0.042
0.70	0.747	0.045	0.692	0.033	0.682	0.043	0.676	0.042	0.720	0.043
0.75	0.747	0.045	0.692	0.033	0.682	0.043	0.676	0.042	0.720	0.043
0.80	0.742	0.043	0.690	0.031	0.685	0.044	0.655	0.038	0.708	0.040
0.85	0.742	0.043	0.690	0.031	0.685	0.044	0.655	0.038	0.708	0.040
0.90	0.742	0.043	0.690	0.031	0.685	0.044	0.655	0.038	0.708	0.040
0.95	0.746	0.044	0.690	0.031	0.688	0.045	0.655	0.038	0.711	0.040
1.00	0.727	0.045	0.693	0.030	0.670	0.045	0.655	0.038	0.705	0.040
1.05	0.727	0.045	0.693	0.030	0.670	0.045	0.655	0.038	0.705	0.040
1.10	0.727	0.045	0.693	0.030	0.670	0.045	0.655	0.038	0.705	0.040
1.15	0.727	0.045	0.690	0.032	0.671	0.045	0.653	0.038	0.703	0.041
1.20	0.726	0.044	0.692	0.034	0.663	0.042	0.647	0.035	0.693	0.038
1.25	0.726	0.044	0.692	0.034	0.663	0.042	0.647	0.035	0.693	0.038
1.30	0.725	0.044	0.693	0.034	0.663	0.042	0.647	0.035	0.692	0.038
1.35	0.722	0.043	0.690	0.032	0.647	0.036	0.644	0.032	0.689	0.036
1.40	0.719	0.042	0.678	0.036	0.643	0.035	0.640	0.032	0.683	0.036
1.45	0.719	0.042	0.678	0.036	0.643	0.035	0.640	0.032	0.683	0.036

1.50	0.718	0.042	0.678	0.036	0.638	0.036	0.638	0.033	0.682	0.036
1.55	0.718	0.042	0.678	0.036	0.638	0.036	0.638	0.033	0.682	0.036
1.60	0.702	0.038	0.678	0.036	0.631	0.033	0.639	0.033	0.678	0.033
1.65	0.704	0.038	0.673	0.037	0.630	0.034	0.630	0.036	0.676	0.034
1.70	0.704	0.038	0.673	0.037	0.630	0.034	0.630	0.036	0.676	0.034
1.75	0.699	0.037	0.669	0.035	0.622	0.031	0.625	0.036	0.668	0.033
1.80	0.699	0.037	0.669	0.035	0.622	0.031	0.625	0.036	0.668	0.033
1.85	0.691	0.034	0.660	0.032	0.608	0.032	0.626	0.038	0.655	0.036
1.90	0.684	0.037	0.660	0.032	0.607	0.032	0.616	0.035	0.638	0.038
1.95	0.683	0.037	0.658	0.033	0.604	0.033	0.613	0.036	0.631	0.040
2.00	0.672	0.040	0.644	0.031	0.598	0.035	0.611	0.035	0.626	0.038
2.05	0.672	0.040	0.644	0.031	0.598	0.035	0.611	0.035	0.626	0.038
2.10	0.665	0.042	0.632	0.034	0.589	0.036	0.590	0.035	0.601	0.037
2.15	0.665	0.042	0.632	0.034	0.589	0.036	0.590	0.035	0.601	0.037
2.20	0.690	0.047	0.657	0.044	0.614	0.042	0.615	0.041	0.626	0.046
2.25	0.652	0.045	0.616	0.036	0.583	0.037	0.584	0.036	0.590	0.039
2.30	0.651	0.045	0.617	0.036	0.583	0.038	0.583	0.036	0.589	0.039
2.35	0.645	0.042	0.596	0.032	0.579	0.035	0.580	0.033	0.586	0.036
2.40	0.646	0.042	0.595	0.032	0.580	0.035	0.581	0.033	0.587	0.036
2.45	0.625	0.044	0.570	0.028	0.577	0.035	0.576	0.034	0.585	0.037
2.50	0.620	0.045	0.558	0.028	0.569	0.036	0.560	0.033	0.584	0.037
2.55	0.620	0.045	0.558	0.028	0.569	0.036	0.560	0.033	0.584	0.037
2.60	0.611	0.041	0.531	0.015	0.574	0.038	0.555	0.030	0.582	0.036
2.65	0.603	0.036	0.532	0.016	0.542	0.026	0.555	0.030	0.562	0.026
2.70	0.603	0.036	0.532	0.016	0.542	0.026	0.555	0.030	0.562	0.026
2.75	0.603	0.036	0.532	0.016	0.542	0.026	0.555	0.030	0.562	0.026
2.80	0.603	0.036	0.532	0.016	0.542	0.026	0.555	0.030	0.562	0.026
2.85	0.603	0.036	0.532	0.016	0.542	0.026	0.555	0.030	0.562	0.026
2.90	0.603	0.036	0.532	0.016	0.542	0.026	0.555	0.030	0.562	0.026
2.95	0.603	0.036	0.532	0.016	0.542	0.026	0.555	0.030	0.562	0.026
3.00	0.598	0.036	0.532	0.016	0.541	0.026	0.547	0.026	0.558	0.026
3.05	0.598	0.036	0.532	0.016	0.541	0.026	0.547	0.026	0.558	0.026
3.10	0.598	0.036	0.532	0.016	0.541	0.026	0.547	0.026	0.558	0.026
3.15	0.598	0.036	0.532	0.016	0.541	0.026	0.547	0.026	0.558	0.026
3.20	0.590	0.033	0.530	0.014	0.539	0.026	0.542	0.026	0.552	0.026
3.25	0.581	0.034	0.526	0.015	0.539	0.026	0.532	0.025	0.550	0.027
3.30	0.581	0.034	0.526	0.015	0.539	0.026	0.532	0.025	0.550	0.027
3.35	0.581	0.034	0.526	0.015	0.539	0.026	0.532	0.025	0.550	0.027
3.40	0.581	0.034	0.526	0.015	0.539	0.026	0.532	0.025	0.550	0.027

3.45	0.557	0.030	0.525	0.015	0.514	0.011	0.507	0.005	0.525	0.015
3.50	0.557	0.030	0.525	0.015	0.514	0.011	0.507	0.005	0.525	0.015

‡ The highest AUC for all tested Cox proportional hazard models was seen at 0.2 years or 2.4 months

Table S11. Multivariate Cox proportional hazard model including the percentage of $CD4^+CD28^+$ T cells and clinical variables. All covariates in this model are continuous except for gender. $CD4^+CD28^+$ T cell %: percentage of $CD4^+CD28^+$ T cells in the PBMCs.

Covariate	**Beta (b) coefficient**	**Standard error**	***P*-value**	**Exp(b)**	**95% CI of Exp(b)**
$CD4^+CD28^+$ T cell %	-0.03886	0.01593	0.0147	0.9619	0.9325 to 0.9922
Age	0.03390	0.02099	0.1063	1.0345	0.9930 to 1.0777
Gender	0.7761	0.4096	0.0581	2.1729	0.9776 to 4.8297
FVC% predicted	-0.02652	0.01248	0.0336	0.9738	0.9504 to 0.9978