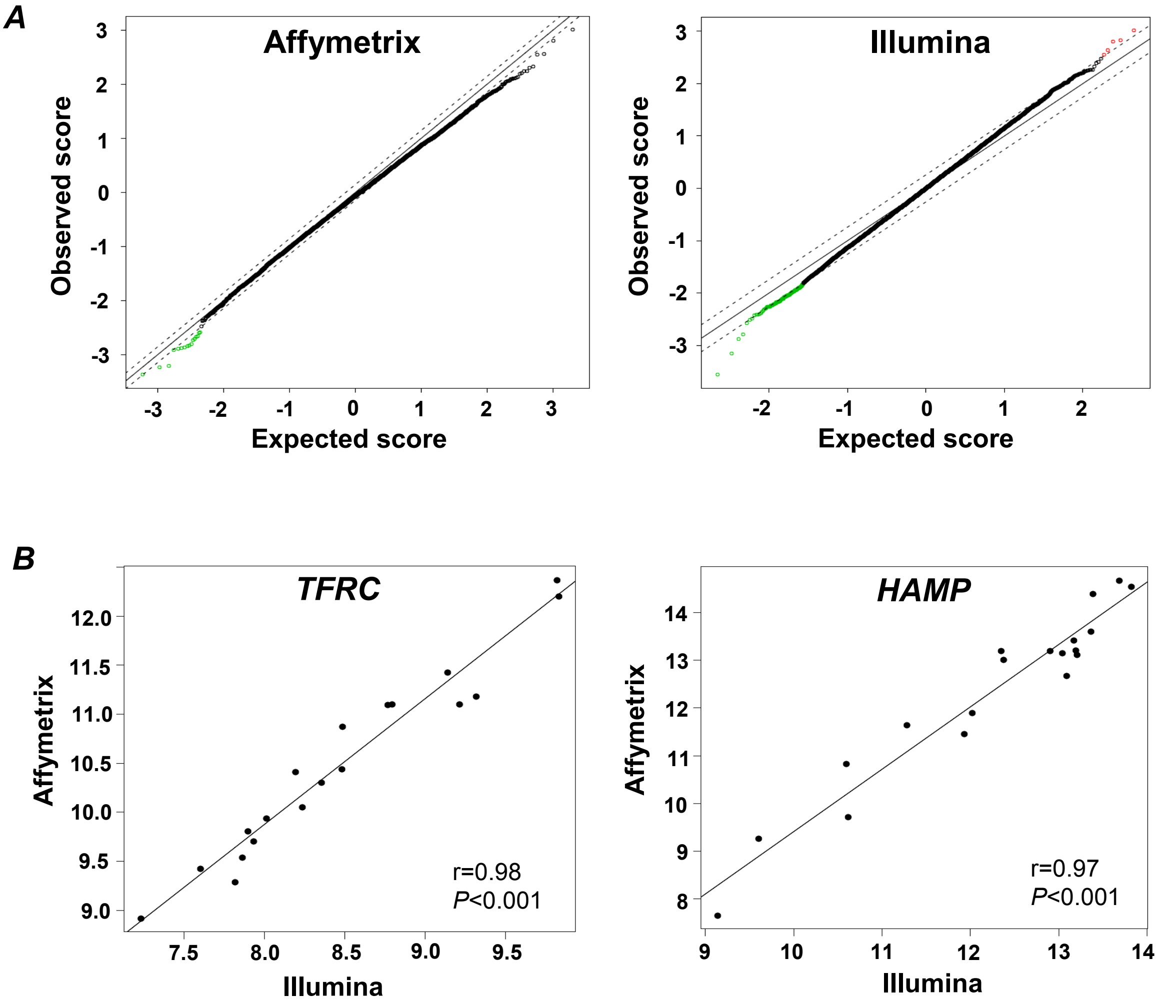
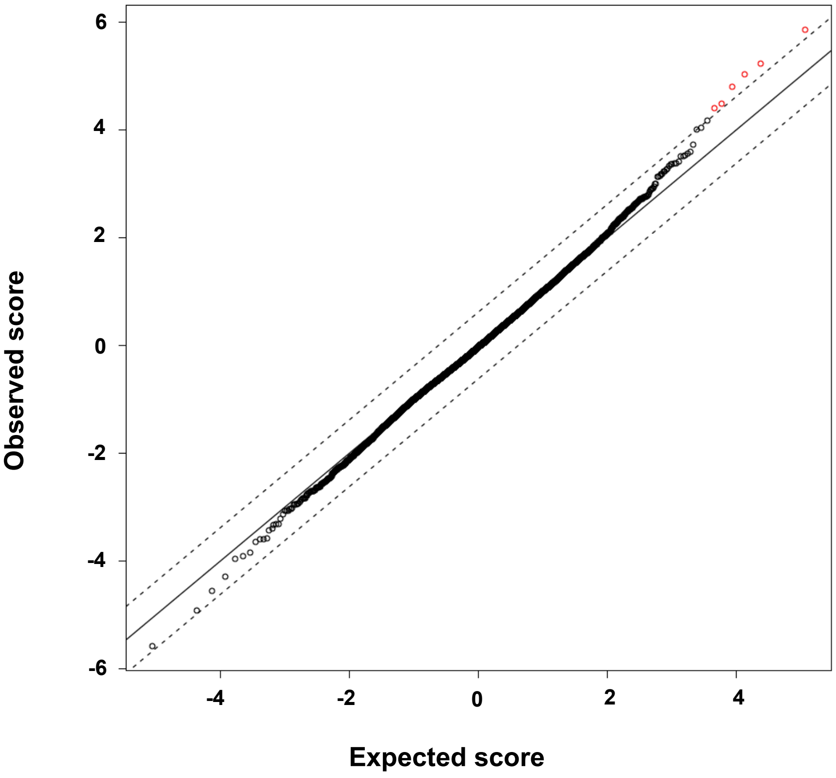
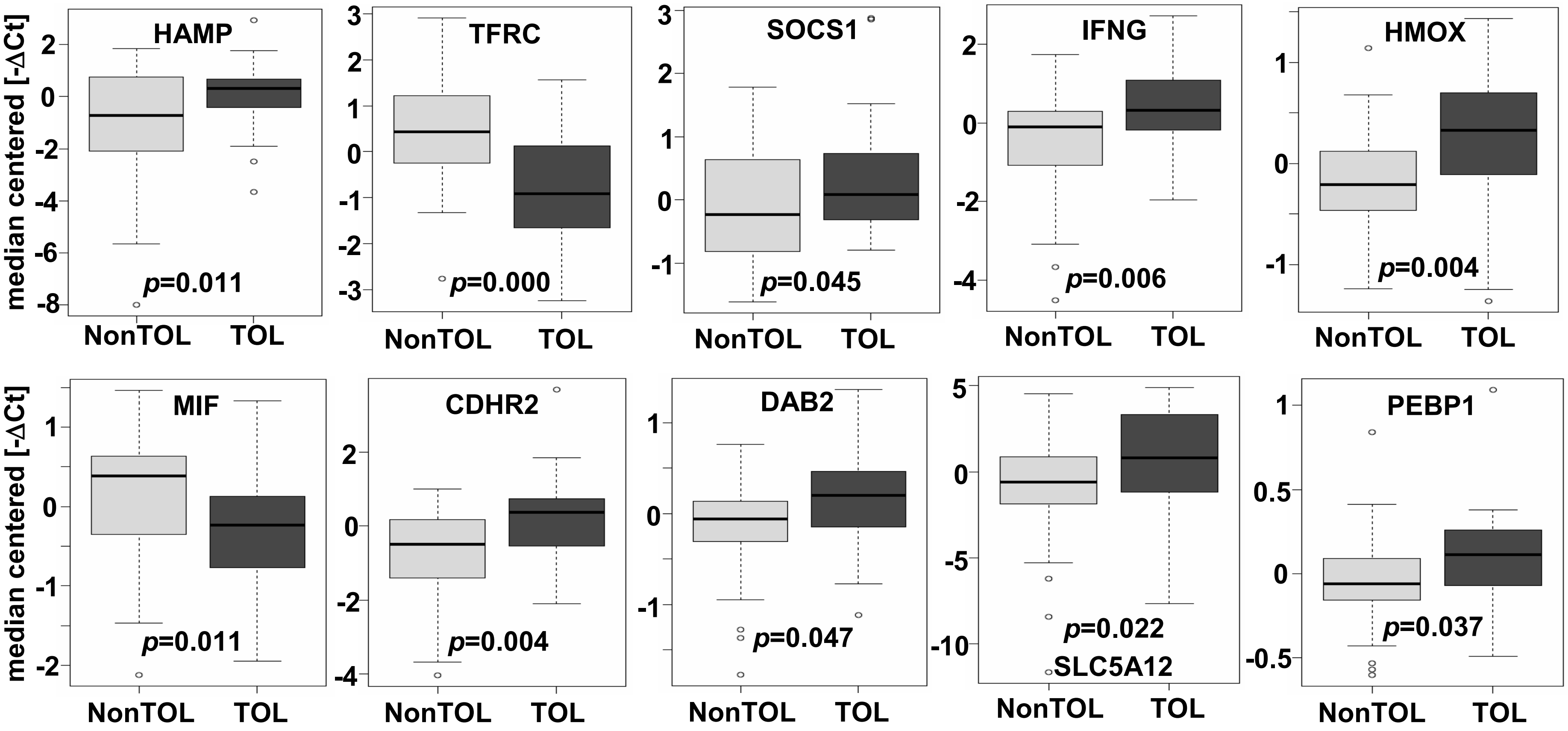


Supplementary Figure 1: Interplatform comparison of Affymetrix and Illumina microarray results of intra-graft gene expression. (*A*) QQ plots show the probability distributions of the differential gene expression values between TOL and Non-TOL samples analyzed on the Illumina (left panel) and Affymetrix (right panel) microarray platforms. The solid line y=x indicates the line where the observed expression differences are identical to the expected differences (random normal distribution). Each point corresponds to a probe set. Probe sets in green or red that deviated from the linear relationship to the theoretical t-statistic correspond to genes differentially expressed between the two conditions with FDR <25% (Affymetrix 16 probes, Illumina 176 probes). (*B*) Correlation between the expression levels (log2 fluorescent signal) of *HAMP* and *TFRC* as assessed by Illumina and Affymetrix microarrays.

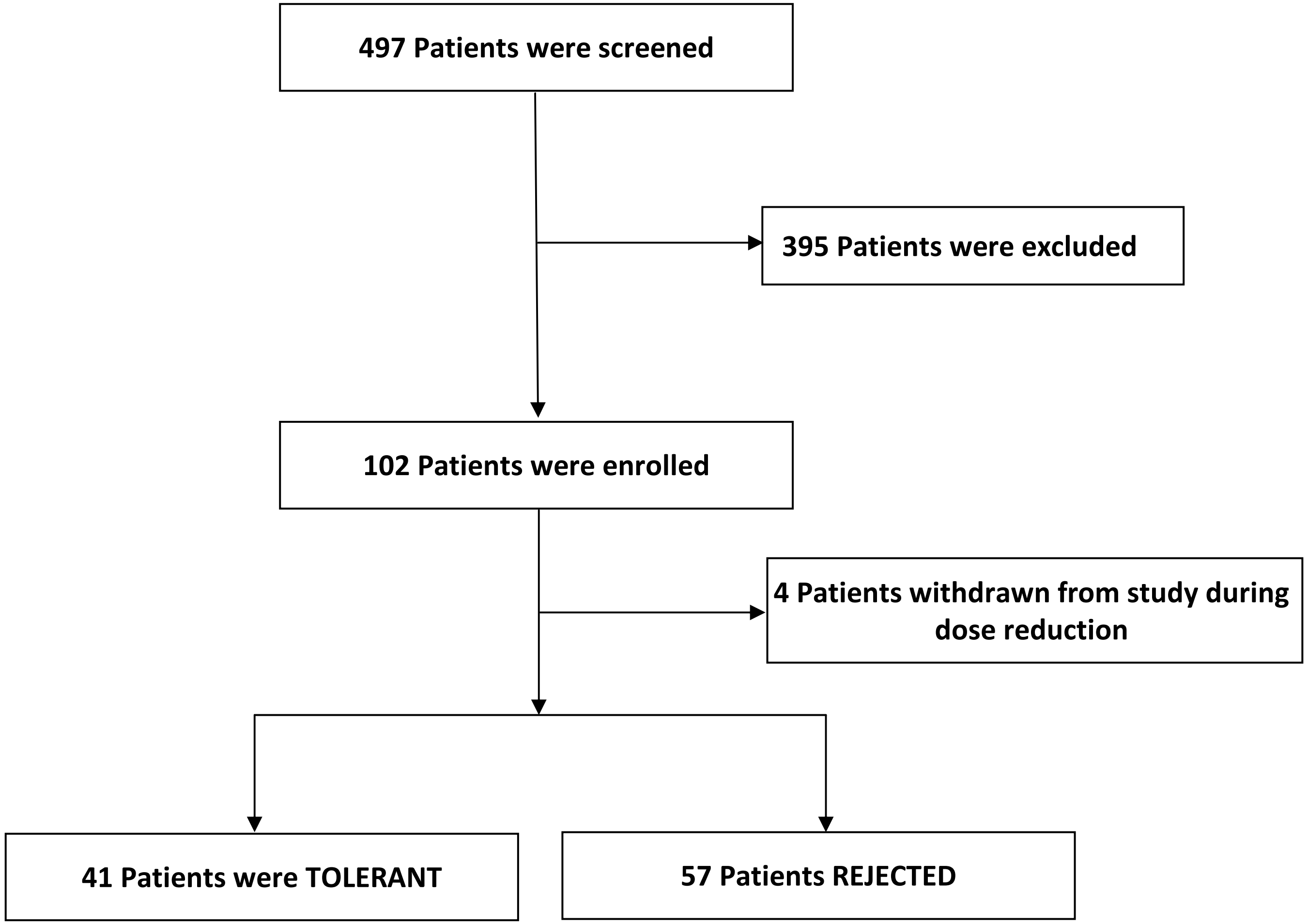


Accession number	Gene symbol	Gene name	Fold Change	FDR
NM_000900	MGP	matrix Gla protein	2,51	0,00
AW007532	IGFBP5	insulin-like growth factor binding protein 5	1,55	0,00
AU146646	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	1,59	0,00
NM_000609	CXCL12	chemokine (C-X-C motif) ligand 12	1,30	16,58
AI799915	DDR2	discoidin domain receptor tyrosine kinase 2	1,37	16,58
AA778095	NA	NA	1,34	16,58

Supplementary Figure 2: Paired comparison of intra-graft Affymetrix microarray transcriptional profiles from 5 TOL recipients before initiating drug minimization and 12 months after complete drug withdrawal. The QQ plot in the upper panel show the probability distribution of paired differential gene expression values. The solid line y=x indicates the line where the observed expression differences are identical to the expected differences (random normal distribution). Probe sets exhibiting differential expression should deviate from the linear relationship to the theoretical t-statistic (6 probe sets in red, FDR<25%). The lower panel displays the name and symbol of the 6 genes with differential gene expression between the two time points analyzed. Abbreviations: FDR (false discovery rate).



Supplementary Figure 3: Paired comparison of the 10 most differentially expressed genes as measured by qPCR intra-graft gene expression in TOL and NonTOL recipients before initiating drug minimization. All patients were enrolled at Hospital Clinic Barcelona only. Data is expressed as change in negative median centered delta Ct (-ΔCt). Significance is given as *p*-value of comparisons between NonTOL and TOL recipients. Box plots display medians for each category (center line) interquartile range (box upper and lower boundaries) and minimum and maximum (whiskers). Outlier values are depicted by circles.



Supplementary Figure 4: Patient enrolment

Supplementary Table 1: Histopathology findings in liver biopsies obtained during the course of the study*

Histological Evaluation	Baseline		Rejection	12-month post-weaning
	Non-tolerant	Tolerant		
Number of patients	57	41	51	39
Number of complete portal tracts	7 (1-34)	7 (1-22)	9 (2-22)	7 (2-18)
Number of central veins	5 (0-19)	4 (0-18)	7 (0-13)	5 (0-13)
Lobular inflammation	0 (0-2)	0 (0-2)	1 (0-2)	1 (0-2)
0 No 1 Sinusoidal cells and/or mild focal necrosis 2 Moderate, multiple necro-inflammatory foci 3 Marked, confluent or bridging necrosis				
Central perivenulitis (with or without endothelitis)	0 (0-2)	0 (0-2)	0 (0-3)	0 (0-2)
0 No 1 Patchy, focal perivenular inflammation 2 Perivenulitis is most of the central veins 3 Marked (confluent or bridging hepatocellular necrosis)				
Portal inflammation	1 (0-2)	1 (0-2)	2 (1-3)	1 (0-2)
0 No 1 Mild (small groups of inflammatory cells) 2 Moderate (>50% of the portal tracts, expansive) 3 Marked				
Interfase hepatitis	0 (0-2)	0 (0-2)	1 (0-3)	0 (0-2)
0 No 1 Mild 2 Moderate 3 Severe				
Bile duct lesions	0 (0-1)	0 (0-1)	1 (0-3)	0 (0-2)
0 No 1 Minimal (intraepithelial inflammatory cells or abnormal colangiocytes) 2 Moderate (epithelial lesions in most portal tracts, no destruction) 3 Marked (destructive lesions of the bile ducts)				
Bile duct loss	0 (0-1)	0	0 (0-1)	0 (0-1)
0 No 1 < 50% 2 ≥ 50%				
Portal vein branches	0 (0-2)	0 (0-2)	0 (0-2)	0 (0-2)
0 Present in all portal tracts 1 Absent in a minority of portal tracts 2 Absent in most of the portal tracts				
Portal vein endothelitis	0 (0-1)	0 (0-1)	1 (0-3)	0 (0-2)
0 No 1 Mild, in the minority of portal veins 2 Mild, in most of the portal veins 3 Marked				
Portal fibrosis	0 (0-2)	0 (0-2)	1 (0-3)	0 (0-2)
0 No 1 Minimal (minority of portal tracts) 2 Moderate (most of the portal tracts, periportal expansion) 3 Marked (bridging fibrosis) 4 Cirrhosis				
Perisinusoidal fibrosis	0 (0-1)	0 (0-2)	0 (0-2)	0 (0-2)
0 No 1 Focal patchy 2 Prominent				

* Values are median (range)

Supplementary Table 2: List of samples collected and tests performed on enrolled recipients.

Num	Patient group [A]	Center [B]	Liver tissue Illumina microarrays [C]		Affymetrix microarrays [C]			Liver tissue qPCR [C]		Serum hepcidin measurements [D]	
			1st	2nd	Liver Tissue 1st	Liver Tissue 2nd	PBMC 1st	1st	2nd	1st	2nd
1	TOL	B	●		●		●	●		●	●
2	TOL	B	●		●			●		●	●
3	TOL	B	●	●			●		●	●	●
4	TOL	B	●		●		●	●	●	●	
5	TOL	B	●				●	●		●	●
6	TOL	B	●				●	●	●	●	●
7	TOL	B	●				●	●		●	
8	TOL	B	●				●	●			●
9	TOL	B	●	●	●	●	●	●	●	●	●
10	TOL	B	●		●		●	●		●	●
11	TOL	B	●	●			●	●	●	●	●
12	TOL	R						●		●	
13	TOL	R						●			
14	TOL	R						●			
15	TOL	R						●		●	
16	TOL	R						●			
17	TOL	R						●		●	
18	TOL	B	●				●	●		●	●
19	TOL	B	●				●	●	●		●
20	TOL	B	●				●	●	●	●	●
21	TOL	R						●		●	
22	TOL	R						●		●	
23	TOL	R						●		●	
24	TOL	R						●		●	
25	TOL	R						●			
26	TOL	R						●			
27	TOL	B	●		●	●	●	●		●	●
28	TOL	B	●		●	●	●	●	●	●	●
29	TOL	B	●		●	●	●		●		
30	TOL	B	●		●	●	●	●	●	●	
31	TOL	B	●				●	●	●	●	
32	TOL	B	●				●	●	●	●	
33	TOL	B					●	●	●		
34	Non-TOL	B	●	●	●			●		●	●
35	Non-TOL	B	●	●			●	●		●	●

36	Non-TOL	B	●	●	●		●	●		●	●
37	Non-TOL	B	●	●	●			●		●	●
38	Non-TOL	B	●	●	●		●	●		●	●
39	Non-TOL	B	●	●	●		●	●		●	●
40	Non-TOL	B	●	●	●		●	●		●	●
41	Non-TOL	B	●	●	●		●	●		●	●
42	Non-TOL	B	●	●			●	●		●	●
43	Non-TOL	B	●	●	●		●	●			
44	Non-TOL	B	●	●	●		●	●		●	●
45	Non-TOL	B	●	●			●	●		●	●
46	Non-TOL	B	●	●	●		●	●		●	●
47	Non-TOL	B	●	●						●	●
48	Non-TOL	B	●	●			●	●		●	●
49	Non-TOL	B	●	●			●	●		●	●
50	Non-TOL	R						●			
51	Non-TOL	R						●		●	●
52	Non-TOL	R						●			●
53	Non-TOL	R						●		●	●
54	Non-TOL	L						●		●	
55	Non-TOL	L						●		●	
56	Non-TOL	B					●	●			
57	Non-TOL	B					●	●		●	●
58	Non-TOL	B	●	●			●	●		●	●
59	Non-TOL	B	●				●	●		●	●
60	Non-TOL	B	●				●	●		●	●
61	Non-TOL	B	●				●	●		●	●
62	Non-TOL	R						●		●	
63	Non-TOL	R						●		●	
64	Non-TOL	R						●			
65	Non-TOL	R						●		●	
66	Non-TOL	R						●		●	
67	Non-TOL	B					●	●		●	●
68	Non-TOL	B	●				●			●	●
69	Non-TOL	B	●				●	●		●	●
70	Non-TOL	B	●				●	●		●	
71	Non-TOL	B					●	●			
72	Non-TOL	B	●				●			●	●
73	Non-TOL	B	●				●	●			
74	Non-TOL	B					●	●			
75	Non-TOL	B					●	●			
76	HEPC	B	●								
77	HEPC	B	●								
78	HEPC	B	●								
79	HEPC	B	●								
80	HEPC	B	●								
81	HEPC	B	●								
82	HEPC	B	●								

83	HEPC	B	●								
84	HEPC	B	●								
85	HEPC	B	●								
86	HEPC	B	●								
87	HEPC	B	●								
88	REJ	B	●								
89	REJ	B	●								
90	REJ	B	●								
91	REJ	B	●								
92	REJ	B	●								
93	REJ	B	●								
94	REJ	B	●								
95	REJ	B	●								
96	REJ	B	●								
97	CONT-Tx	B	●					●			
98	CONT-Tx	B	●					●			
99	CONT-Tx	B	●					●			
100	CONT-Tx	B	●					●			
101	CONT-Tx	B	●					●			
102	CONT-Tx	B	●					●			
103	CONT-Tx	B	●					●			
104	CONT-Tx	B	●					●			
105	CONT	B	●					●			
106	CONT	B	●					●			
107	CONT	B	●					●			
108	CONT	B	●					●			
109	CONT	B	●					●			
110	CONT	B	●					●			
111	CONT	B	●					●			
112	CONT	B	●					●			
113	CONT	B	●					●			
114	CONT	B	●					●			

[A] Patient groups: TOL (tolerant), Non-TOL (non-tolerant), HEPC (liver recipients with recurrent hepatitis C), REJ (liver recipients with acute cellular rejection), CONT-Tx (liver recipients with normal liver histology 1 year after transplantation), CONT (non-transplanted patients undergoing surgery for colorectal liver metastases).

[B] Center: B=Hospital Clinic Barcelona, R=University “Tor Vergata” Rome, L=University Hospitals Leuven.

[C] 1st denotes samples collected before initiation of drug minimization and 2nd[nd] denotes samples collected at the time of rejection (Non-TOL) or 12 months after complete drug withdrawal (TOL). Peripheral blood mononuclear cells (PBMCs) were collected before initiation of drug minimization.

[D] 1st denotes samples collected before initiation of drug minimization and 2nd[nd] denotes samples collected 12 months after rejection (Non-TOL) or 12 months after complete drug withdrawal (TOL).

Supplementary Table 3: Genes differentially expressed at the time of liver allograft rejection [A]

Gene symbol	FDR	Fold-change TOL *versus* Non-TOL	Name
DOCK2	0.000	-1.465	Dedicator of cytokinesis 2
OBFC2A	0.000	-1.254	Oligonucleotide/oligosaccharide-binding fold containing 2A
CCL3L1	0.000	-1.095	Chemokine (C-C motif) ligand 3-like 1
CD72	0.000	-1.589	CD72 molecule
TAP2	0.000	-1.506	Transporter 2, ATP-binding cassette, sub-family B (
IL18BP	0.000	-2.038	Interleukin 18 binding protein
LILRB3	0.000	-1.234	Leukocyte immunoglobulin-like receptor, subfamily B
CXCL9	0.000	-3.505	Homo sapiens chemokine (C-X-C motif) ligand 9
CXCL10	0.000	-2.592	Chemokine (C-X-C motif) ligand 10
HS3ST2	0.000	-1.089	Heparan sulfate 3-O-sulfotransferase 2
WARS	0.000	-1.658	Tryptophanyl-tRNA synthetase
PLEK	0.000	-1.620	Pleckstrin
ARHGAP9	0.000	-1.521	Rho GTPase activating protein 9 (
PYHIN1	0.000	-1.079	Pyrin and HIN domain family, member 1
UBE2C	0.000	-1.518	Ubiquitin-conjugating enzyme E2C
HMMR	0.000	-1.549	Hyaluronan-mediated motility receptor
APOBEC3B	0.000	-1.069	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B
OLR1	0.000	-1.148	Oxidized low density lipoprotein (lectin-like) receptor 1
HLA-F	0.000	-1.781	Major histocompatibility complex, class I, F
HLA-DRA	0.000	-1.735	Major histocompatibility complex, class II, DR alpha
PTTG1	0.000	-1.749	Pituitary tumor-transforming 1
CD8A	0.000	-2.671	CD8a molecule (CD8A), transcript variant 2, mRNA.
SLC1A3	0.000	-1.512	Solute carrier family 1 member 3
IGSF6	0.000	-1.368	Immunoglobulin superfamily, member 6
IFNG	0.000	-1.174	Interferon, gamma (IFNG)
IRF8	0.000	-1.364	Interferon regulatory factor 8
ITGB2	0.000	-1.532	Integrin, beta 2
IL32	0.000	-1.846	Interleukin 32
ABHD4	0.000	1.009	Abhydrolase domain containing 4
STAT1	0.000	-1.836	Signal transducer and activator of transcription 1,
LAIR2	0.000	-1.296	Leukocyte-associated immunoglobulin-like receptor 2
CD2	0.000	-1.676	CD2 molecule
CTLA4	0.000	-1.450	Cytotoxic T-lymphocyte-associated protein 4
GBP1	0.000	-1.670	Guanylate binding protein 1, interferon-inducible,
STAT1	0.000	-1.999	Signal transducer and activator of transcription 1,.
EZH2	0.000	-1.269	Enhancer of zeste homolog 2
FABP5	0.000	-1.838	Fatty acid binding protein 5
CD83	0.000	-1.470	CD83 molecule
RAD51AP1	0.000	-1.312	Homo sapiens RAD51 associated protein 1
HMMR	0.000	-1.130	Homo sapiens hyaluronan-mediated motility receptor
CCL4L2	0.000	-1.424	Homo sapiens chemokine (C-C motif) ligand 4-like 2
CXCR4	0.000	-1.287	Chemokine (C-X-C motif) receptor 4
TAP1	0.000	-1.752	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
SRGN	0.000	-1.607	Serglycin
CD52	0.000	-1.551	CD52 molecule
CD300LF	0.000	-1.315	CD300 molecule-like family member f
FAM113B	0.000	-1.425	Family with sequence similarity 113, member B
FCGR1B	0.000	-1.370	Fc fragment of IgG, high affinity Ib, receptor (CD64)
APOL3	0.000	-1.758	Apolipoprotein L, 3 (APOL3), transcript variant beta/a,
TLR8	0.000	-1.366	Toll-like receptor 8

[A] Table shows the 50 most significant targets. Illumina microarrays were employed to analyze sequential samples collected before initiation of drug minimization and at the time of graft rejection. Abbreviations: FDR (false discovery rate).

Supplementary Table 4: Results of qPCR gene expression experiments on liver tissue samples from TOL and Non-TOL recipients [A]

Gene	*P*-value	Fold-change	Name	Inclusion criteria
TFRC	0.000024	-2.56	transferrin receptor (p90, CD71)	Microarray
HAMP	0.010525	2.063	hepcidin antimicrobial peptide	Microarray
HMOX1	0.004377	1.454	heme oxigenase1	Literature search
DAB2	0.047391	1.197	disabled homolog 2	Microarray
MIF	0.010830	-1.54	macrophage migration inhibitory factor	Microarray
PEBP1	0.037147	1.129	phosphatidylethanolamine binding protein 1	Microarray
SLC5A12	0.022330	2.657	solute carrier family 5 (sodium/glucose)	Microarray
CDHR2	0.003954	1.815	cadherin-related family member 2	Microarray
IFNG	0.006279	1.338	interferon, gamma	Literature search
SOCS1	0.044445	1.244	suppressor of cytokine signaling 1	Literature search
TFR2	ns	1.096	transferrin receptor 2	Literature search
FTH1	ns	1.045	ferritin, heavy polypeptide 1	Literature search
MCOLN1	ns	1.165	mucolipin 1	Microarray
HFE	ns	1.229	hemochromatosis	Literature search
HFE2	ns	1.341	hemochromatosis type 2 (juvenile)	Literature search
BMP2	ns	1.066	bone morphogenetic protein 2	Literature search
BMP4	ns	1.034	bone morphogenetic protein 4	Literature search
SMAD4	ns	1.088	SMAD family member 4	Literature search
CP	ns	-1.015	ceruloplasmin (ferroxidase)	Literature search
ACSL1	ns	-1.244	acyl-CoA synthetase long-chain family member 1	Microarray
UNG	ns	-1.074	uracil-DNA glycosylase	Microarray
TUBA4A	ns	-1.185	tubulin, alpha 4a	Microarray
COG5	ns	-1.021	component of oligomeric golgi complex 5	Microarray
ABAT	ns	-1.113	4-aminobutyrate aminotransferase	Microarray
DTWD2	ns	1.052	DTW domain containing 2	Microarray
POF1B	ns	-1.092	premature ovarian failure, 1B	Microarray
TIPARP	ns	1.032	TCDD-inducible poly(ADP-ribose) polymerase	Microarray
RBM23	ns	-1.011	RNA binding motif protein 23	Microarray
TTC3	ns	1.181	tetratricopeptide repeat domain 3	Microarray
FABP4	ns	1.343	fatty acid binding protein 4	Microarray
VNN3	ns	1.357	vanin 3	Microarray
ADORA3	ns	1.449	adenosine A3 receptor	Microarray
TAF15	ns	1.133	TATA box binding protein (TBP)-associated factor	Microarray
TP53I3	ns	1.607	tumor protein p53 inducible protein 3	Microarray
SAGE1	ns	1.314	sarcoma antigen 1	Microarray
DPP4	ns	-1.109	dipeptidyl-peptidase 4	Microarray
MYO19	ns	1.041	myosin XIX	Microarray
SH2D1B	ns	1.133	SH2 domain containing 1B	Microarray
TBX21	ns	1.290	T-box 21	Literature search
IRF1	ns	1.239	interferon regulatory factor 1	Literature search
IRF3	ns	1.142	interferon regulatory factor 3	Literature search
STAT1	ns	1.036	signal transducer and activator of transcription 1	Literature search
TNF	ns	1.092	tumor necrosis factor (TNF superfamily, member 2)	Literature search
TNFAIP3	ns	1.250	tumor necrosis factor, alpha-induced protein 3	Literature search
IL6	ns	1.382	interleukin 6	Literature search
IL10	ns	2.473	interleukin 10	Literature search
GATA3	ns	1.128	GATA binding protein 3	Literature search
IL1A	ns	5.704	interleukin 1, alpha	Literature search
IL1B	ns	-1.414	interleukin 1, beta	Literature search
IL18	ns	1.120	interleukin 18 (interferon-gamma-inducing factor)	Literature search
IL18BP	ns	1.381	interleukin 18 binding protein	Literature search
IL32	ns	-1.532	interleukin 32	Literature search
CCL3	ns	1.247	chemokine (C-C motif) ligand 3	Literature search
CXCR3	ns	1.216	chemokine (C-X-C motif) receptor 3	Literature search
CXCR7	ns	1.183	chemokine (C-X-C motif) receptor 7	Literature search
CXCL9	ns	-1.120	chemokine (C-X-C motif) ligand 9	Literature search

CXCL10	ns	1.211	chemokine (C-X-C motif) ligand 10	Literature search
CXCL11	ns	1.246	chemokine (C-X-C motif) ligand 11	Literature search
CCL21	ns	1.365	chemokine (C-C motif) ligand 21	Literature search
CD3D	ns	1.036	CD3d molecule, delta (CD3-TCR complex)	Literature search
CD8A	ns	-1.088	CD8a molecule	Literature search
MS4A1	ns	-1.043	membrane-spanning 4/CD20 B-cell differentiation	Literature search
CD37	ns	1.236	CD37 molecule	Literature search
FOXP3	ns	-1.354	forkhead box P3	Literature search
TGFB1	ns	1.311	transforming growth factor, beta 1	Literature search
CD274	ns	1.343	CD274 molecule	Literature search
FCER2	ns	-1.317	Fc fragment of IgE, low affinity II, receptor for (CD23)	Literature search
HLA-E	ns	1.096	major histocompatibility complex, class I, E	Literature search
HLA-G	ns	-3.054	major histocompatibility complex, class I, G	Literature search
MICA	ns	1.279	MHC class I polypeptide-related sequence A	Literature search
MICB	ns	1.080	MHC class I polypeptide-related sequence B	Literature search
TLR4	ns	1.006	toll-like receptor 4	Literature search
TLR8	ns	-1.042	toll-like receptor 8	Literature search
MYD88	ns	1.005	myeloid differentiation primary response gene (88)	Literature search
C3	ns	1.075	complement component 3	Literature search
NOS2	ns	-1.026	nitric oxide synthase 2, inducible	Literature search
PDCD1	ns	1.430	programmed cell death 1	Literature search
PDCD1LG2	ns	1.191	programmed cell death 1 ligand 2	Literature search
BCL2	ns	1.061	B-cell CLL/lymphoma 2	Literature search
FAS	ns	-2.210	Fas (TNF receptor superfamily, member 6)	Literature search
FASLG	ns	1.104	Fas ligand (TNF superfamily, member 6)	Literature search
GZMB	ns	-1.150	granzyme B (granzyme 2)	Literature search
PRF1	ns	1.328	perforin 1 (pore forming protein)	Literature search
SRGN	ns	1.106	serglycin	Literature search
FKBP1A	ns	1.119	FK506 binding protein 1A, 12kDa	Microarray
RORC	ns	1.091	RAR-related orphan receptor C	Literature search
NCAM1	ns	1.056	neural cell adhesion molecule 1	Microarray
SLAMF7	ns	1.225	SLAM family member 7	Microarray
IL2RB	ns	1.150	interleukin 2 receptor, beta	Microarray
IL12B	ns	1.005	interleukin 12B	Microarray
KLRF1	ns	1.125	killer cell lectin-like receptor subfamily F, member 1	Microarray
KLRD1	ns	1.078	killer cell lectin-like receptor subfamily D, member 1	Microarray
KLRK1	ns	1.006	killer cell lectin-like receptor subfamily K, member 1	Microarray
KLRC1	ns	3.213	killer cell lectin-like receptor subfamily C, member 1	Microarray
NCR1	ns	1.227	natural cytotoxicity triggering receptor 1	Microarray
AP1S2	ns	1.114	adaptor-related protein complex 1, sigma 2	Microarray
UBD	ns	-1.428	ubiquitin D	Microarray
PSMB9	ns	-1.012	proteasome (prosome, macropain) subunit 9	Microarray
SMARCD3	ns	3.496	SWI/SNF related, matrix associated	Microarray
BNC2	ns	1.005	basonuclin 2	Microarray
CX3CR1	ns	1.033	chemokine (C-X3-C motif) receptor 1	Microarray
LINGO2	ns	1.029	leucine rich repeat and Ig domain containing 2	Microarray
IGFBP7	ns	-1.135	insulin-like growth factor binding protein 7	Microarray
COL13A1	ns	1.139	collagen, type XIII, alpha 1	Microarray
HPRT1	NA	NA	hypoxanthine phosphoribosyltransferase 1	House-keeping
GAPDH	NA	NA	glyceraldehyde 3-phosphate dehydrogenase	House-keeping
18s rRNA	NA	NA	18S ribosomal RNA	House-keeping

[A] Table shows qPCR expression experiments conducted on liver tissue samples collected before initiation of drug minimization in recipients enrolled in Hospital Clinic Barcelona only. Abbreviations: qPCR (real-time PCR), NA (not applicable).

Supplementary Table 5: Association between tolerance-related qPCR gene expression markers and clinical parameters [A].

	***P*-value**	**Adjusted *P*-value [B]**
TFRC	<0.001	<0.001
CDHR2	0.004	0.027
HMOX1	0.004	0.031
IFNG	0.006	0.003
HAMP	0.011	0.053
MIF	0.011	0.029
SLC5A12	0.022	0.136
PEBP1	0.037	0.061
DAB2	0.047	0.038
SOCS1	0.044	0.032
ADORA3	0.067	0.064
TTC3	0.077	0.181

[A] Adjustment for clinical parameters was conducted on the qPCR expression data generated employing all available liver tissue samples.

[B] *P*-value adjusted for time since transplantation, recipient gender, recipient age at transplantation, and type of immunosuppressive regimen at enrolment.

Supplementary Table 6: Multivariable analysis of predictors associated with the development of operational tolerance [A]

Clinical parameter	Adjusted odds ratio [A]	*P*-value	95% confidence interval
Time from transplantation (per year increase)	1.348	0.003	1.034-1.762
Liver tissue transcriptional biomarkers	48.953	0.000	6.983-343.170

[A] Table shows the results of a logistic regression multivariable analysis incorporating recipient age, recipient gender, time since transplantation and class (tolerant/non-tolerant) prediction on the basis of liver tissue transcriptional biomarkers.

[A] Odds ratio of being tolerant over non-tolerant

Supplementary Table 7: Influence of serum ferritin and clinical parameters on the development of operational tolerance [A]

Clinical parameter	Adjusted odds ratio [B]	*P*-value	95% confidence interval
Time since transplantation (per year increase)	1.302	<0.001	1.132-1.497
Serum ferritin (per 10 ng/mL)	1.041	0.034	1.004-1.079

[A] Table shows the results of a logistic regression multivariable analysis incorporating serum ferrin levels, recipient age, recipient gender, and time since transplantation.

[B] Odds ratio of being tolerant over non-tolerant

Supplementary Table 8: Differences in hematological and iron-related parameters between tolerant and non-tolerant recipients [A].

	TOL	**Non-TOL**	***P-value***
Hemoglobin (g/L) [B]	147.8 (122-170)	139.4 (104-176)	*NS*
Hematocrit (%) [B]	43.5 (35-51)	41.3 (32-52)	*NS*
MCV (fL) [B]	92.9 (85-109)	92.7 (82-103)	*NS*
MCHC (g/L) [B]	335.7 (295-370)	339 (273.7-469.7)	*NS*
Serum Iron (mg/dL) [B]	106 (30-244)	84 (32-160)	*NS*
Serum ferritin (mg/dL) [B]	185.5 (26-864)	73.5 (3-304)	0.0026
Transferrin saturation (%) [B]	27.3 (17.8-59.4)	24.5 (3-56.7)	*NS*
sTR [B]	1.28 (0.7-2.15)	1.52 (0.8-2.58)	*NS*
IDA [C]	0	2 (7%)	*NS*
HFE polymorphism	3 H63D/n, 1 C282T/n	4 H63D/n 1 H63D/H63D	*NS*
C-reactive protein (mg/dL) [B]	0.47 (0.01-2.04)	0.65 (0.02-3.9)	*NS*

[A] Abbreviations: MCV (mean corpuscular volume), MCHC (mean corpuscular haemoglobin concentration), sTR (soluble transferring receptor), IDA (iron deficiency anemia), *NS* (not significant).

[B] Mean (range)

[C] Iron defiency anemia was defined as serum ferritin ≤12 mg/dL and haemoglobin <12 g/L in women and < 13 g/L in men. Data are expressed as number of patients (%).

Supplementary Table 9: Quantification of lymphocyte subsets by immunofluorescence in liver biopsies obtained before immunosuppression drug withdrawal [A].

	Phenotype	**N**	**Mean (SEM)**
CD4+/CD8+ ratio	TOL	15	1.4071 (0.103)
	Non-TOL	13	1.5705 (0.160)
CD4+FoxP3+/CD4+ ratio	TOL	15	0.0342 (0.005)
	Non-TOL	13	0.0390 (0.004)
Treg/Teff	TOL	15	0.0193 (0.002)
	Non-TOL	13	0.0231 (0.003)
Portal T_{eff} density (per mm^2)	TOL	15	2292.0 (180.337)
	Non-TOL	13	1941.0 (209.739)
Portal CD8 density (per mm^2)	TOL	15	988.20 (94.341)
	Non-TOL	13	814.50 (111.827)
Portal CD4 density (per mm^2)	TOL	15	1303.8 (105.166)
	Non-TOL	13	1126.5 (118.820)
Portal T_{reg} density (per mm^2)	TOL	15	43.64 (6.581)
	Non-TOL	13	45.40 (7.709)
Area/portal fields (μm^2)	TOL	15	18135.6 (2333.79)
	Non-TOL	13	23021.9 (4765.030)

[A] Abbreviations: Treg (CD4+FOXP3+ lymphocytes), Teff (CD4+FOXP3- and CD8+FOXP3- lymphocytes)

Supplementary Table 10: List of genes assessed in PBMCs employing Fluidigm qPCR gene expression

Gene Symbol	Name	Gene Symbol	Name
ABCB1	ATP-binding cassette, sub-family B member 1	*KLRB1*	killer cell lectin-like receptor subfamily B, member 1
AHNAK	AHNAK nucleoprotein	*KLRC4*	killer cell lectin-like receptor subfamily C, member 4
ALG8	asparagine-linked glycosylation 8	*KLRD1*	killer cell lectin-like receptor subfamily D, member 1
AMMECR1	Alport syndrome chromosomal region gene 1	*KLRF1*	killer cell lectin-like receptor subfamily F, member 1
ANKRD12	ankyrin repeat domain 12	*LAIR2*	leukocyte-associated immunoglobulin-like receptor 2
ARFGEF2	ADP-ribosylation factor guanine nucleotide-exchange factor 2	*LSM12*	LSM12 homolog (S. cerevisiae)
B2M	beta-2-microglobulin	*LUC7L3*	LUC7-like 3 (S. cerevisiae)
BAT2L2	HLA-B associated transcript 2-like 2	*MAN1A1*	mannosidase, alpha, class 1A, member 1
BNC2	basonuclin 2	*NAAA*	N-acylethanolamine acid amidase
BZRAP1	benzodiazapine receptor associated protein 1	*NAA15*	N(alpha)-acetyltransferase 15, NatA auxiliary subunit
C6orf62	chromosome 6 open reading frame 62	*NCALD*	neurocalcin delta
C9orf103	chromosome 9 open reading frame 103	*NCAM1*	neural cell adhesion molecule 1 / CD56
CD9	CD9 molecule, tetraspanin-29	*NCR1*	natural cytotoxicity triggering receptor 1
CDC42SE1	CDC42 small effector 1	*NIPBL*	Nipped-B homolog (Drosophila)
CD160	CD160 molecule, Natural killer cell receptor BY55	*NKG7*	natural killer cell group 7 sequence
CD244	CD244 molecule, natural killer cell receptor 2B4	*OSBPL5*	oxysterol binding protein-like 5
CLIC3	chloride intracellular channel 3	*PDE4B*	phosphodiesterase 4B, cAMP-specific
CNOT7	CCR4-NOT transcription complex, subunit 7	*PDGFRB*	platelet-derived growth factor receptor, beta polypeptide
CTBP2	C-terminal binding protein 2	*PION*	pigeon homolog (Drosophila)
CTSZ	cathepsin Z	*PTCH1*	patched 1
CX3CR1	chemokine (C-X3-C motif) receptor 1	*PTGDR*	prostaglandin D2 receptor (DP)
DAB2	disabled homolog 2 (Drosophila)	*PPIG*	peptidylprolyl isomerase G (cyclophilin G)
DENND1B	DENN/MADD domain containing 1B	*PSMD14*	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14
DIS3	DIS3 mitotic control homolog (S. cerevisiae)	*RASGEF1A*	RasGEF domain family, member 1A
DUSP2	dual specificity phosphatase 2	*RGS3*	regulator of G-protein signaling 3
ERBB2	erythroblastic leukemia viral oncogene homolog 2	*RPL27A*	ribosomal protein L27a
EPS8	epidermal growth factor receptor pathway substrate 8	*RPL31*	ribosomal protein L31
ERGIC2	ERGIC and golgi 2	*RPL37A*	ribosomal protein L37a
FANCG	Fanconi anemia, complementation group G	*RPL38*	ribosomal protein L38
FEM1C	fem-1 homolog c (C. elegans)	*RPS11*	ribosomal protein S11
FEZ1	fasciculation and elongation protein zeta 1 (zygin I)	*RSBN1L*	round spermatid basic protein 1-like
FLJ14213	proline rich 5 like	*SAMHD1*	SAM domain and HD domain 1
GEMIN7	gem (nuclear organelle) associated protein 7	*SCRIB*	scribbled homolog (Drosophila)
GFOD1	glucose-fructose oxidoreductase domain containing 1	*SH2D1B*	SH2 domain containing 1B
GNG2	guanine nucleotide binding protein (G protein), gamma 2	*SIRPB1*	signal-regulatory protein beta 1
GNPTAB	N-acetylglucosamine-1-phosphate transferase, alpha and beta subunits	*SKI*	v-ski sarcoma viral oncogene homolog (avian)
GP9	glycoprotein IX (platelet)	*SLAMF7*	SLAM family member 7
GZMB	granzyme B	*SLC16A7*	solute carrier family 16, member 7
HBA1;HBA2	hemoglobin, alpha 1;hemoglobin, alpha 2	*TCL1A*	T-cell leukemia/lymphoma 1A
HBG1;HBG2	hemoglobin, gamma A;hemoglobin, gamma G	*TRA@*	T cell receptor alpha locus
HIGD2A	HIG1 hypoxia inducible domain family, member 2A	*TRD@*	T cell receptor delta locus
HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	*UBD*	ubiquitin D
HPRT1	hypoxanthine phosphoribosyltransferase 1	*WDR67*	WD repeat domain 67
IL2RB	interleukin 2 receptor, beta	*YWHAZ*	tyrosine 3-monooxygenase zeta polypeptide
IL8	interleukin 8	*ZMIZ2*	zinc finger, MIZ-type containing 2
ITGA2B	integrin, alpha 2b (CD41)	*ZNF267*	zinc finger protein 267
KDM1B	lysine (K)-specific demethylase 1B	*ZNF295*	zinc finger protein 295
KIAA0100	KIAA0100	*18S*	Eukaryotic 18S rRNA

Supplementary Table 11: Genes differentially expressed in PBMCs between operationally tolerant and non-tolerant recipients before start of drug minimization (FDR<5%, fold change ± 1.32)

Probeset	Gene Symbol	Accession Number	GeneName	Fold change (Tolerant vs Non-tolerant)
211654_x_at	HLA-DQB1	M17565	major histocompatibility complex. class II. DQ beta 1	1.820
205249_at	EGR2	NM_000399	early growth response 2	1.767
210690_at	KLRC4	U96845	killer cell lectin-like receptor subfamily C. member 4	1.742
207723_s_at	KLRC3	NM_002261	killer cell lectin-like receptor subfamily C. member 3	1.639
1560172_at	INTS10	AK092931	integrator complex subunit 10	1.631
210286_s_at	SLC4A7	AF053755	solute carrier family 4. sodium bicarbonate cotransporter. member 7	1.627
234165_at	PTGDR	AK026202	prostaglandin D2 receptor (DP)	1.617
204794_at	DUSP2	NM_004418	dual specificity phosphatase 2	1.613
208937_s_at	ID1	D13889	inhibitor of DNA binding 1. dominant negative helix-loop-helix protein	1.585
201681_s_at	DLG5	AB011155	discs. large homolog 5 (Drosophila)	1.563
205239_at	AREG	NM_001657	amphiregulin	1.552
230967_s_at	USP7	BF433061	ubiquitin specific peptidase 7 (herpes virus-associated)	1.549
213524_s_at	G0S2	NM_015714	G0/G1switch 2	1.536
219630_at	PDZK1IP1	NM_005764	PDZK1 interacting protein 1	1.536
207724_s_at	SPAST	NM_014946	spastin	1.535
206115_at	EGR3	NM_004430	early growth response 3	1.520
231996_at	N4BP2	AB037834	NEDD4 binding protein 2	1.514
207072_at	IL18RAP	NM_003853	interleukin 18 receptor accessory protein	1.511
227803_at	ENPP5	AA609053	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative function)	1.494
206370_at	PIK3CG	NM_002649	phosphoinositide-3-kinase. catalytic. gamma polypeptide	1.487
1553176_at	SH2D1B	AF403479	SH2 domain containing 1B	1.460
220646_s_at	KLRF1	NM_016523	killer cell lectin-like receptor subfamily F. member 1	1.460
211597_s_at	HOPX	AB059408	HOP homeobox	1.453
238478_at	BNC2	H97386	basonuclin 2	1.451
216834_at	RGS1	S59049	regulator of G-protein signaling 1	1.447
37145_at	GNLY	M85276	granulysin	1.444
207793_s_at	EPB41	NM_004437	erythrocyte membrane protein band 4.1 (elliptocytosis 1. RH-linked)	1.440
207246_at	ZFY	NM_003411	zinc finger protein. Y-linked	1.440
227394_at	NCAM1	W94001	neural cell adhesion molecule 1	1.437
1559979_at	SYF2	BC015824	SYF2 homolog. RNA splicing factor (S. cerevisiae)	1.427
223946_at	MED23	AL136776	mediator complex subunit 23	1.423
232027_at	SYNE1	AL049548	spectrin repeat containing. nuclear envelope 1	1.421
1560014_s_at	PDXDC1	AI133523	pyridoxal-dependent decarboxylase domain containing 1	1.420
206366_x_at	XCL1	U23772	chemokine (C motif) ligand 1	1.418
1557257_at	BCL10	AA994334	B-cell CLL/lymphoma 10	1.417
1563498_s_at	SLC25A45	AK090434	solute carrier family 25. member 45	1.413
212070_at	GPR56	AL554008	G protein-coupled receptor 56	1.408

224231_at	PRO0471	AF111846	hypothetical LOC28994	1.407
202887_s_at	DDIT4	NM_019058	DNA-damage-inducible transcript 4	1.406
215581_s_at	MCM3AP	AK022303	minichromosome maintenance complex component 3 associated protein	1.405
226809_at	LOC100216479	AW188087	hypothetical LOC100216479	1.403
1558697_a_at	KIAA0430	BI600341	KIAA0430	1.401
1558859_at	LOC222159	AK027340	hypothetical protein LOC222159	1.397
229614_at	ZNF320	AI277652	zinc finger protein 320	1.397
212509_s_at	MXRA7	BF968134	matrix-remodelling associated 7	1.394
228789_at	MTMR6	AI290971	myotubularin related protein 6	1.389
238438_at	CNOT6L	R67226	CCR4-NOT transcription complex. subunit 6-like	1.380
218638_s_at	SPON2	NM_012445	spondin 2. extracellular matrix protein	1.377
205291_at	IL2RB	NM_000878	interleukin 2 receptor. beta	1.375
1554549_a_at	WDR20	BC030654	WD repeat domain 20	1.371
203921_at	CHST2	NM_004267	carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2	1.371
230120_s_at	PLGLB2	AI088455	plasminogen-like B2	1.371
207840_at	CD160	NM_007053	CD160 molecule	1.366
242463_x_at	ZNF600	AI620827	zinc finger protein 600	1.364
241495_at	CCNL1	AI675298	cyclin L1	1.359
205883_at	ZBTB16	NM_006006	zinc finger and BTB domain containing 16	1.356
227798_at	SMAD1	AU146891	SMAD family member 1	1.356
202644_s_at	TNFAIP3	NM_006290	tumor necrosis factor. alpha-induced protein 3	1.356
232568_at	MGC24103	AU145658	hypothetical MGC24103	1.353
227473_at	CTTN	N45140	cortactin	1.350
1555963_x_at	B3GNT7	CA503291	UDP-GlcNAc:betaGal beta-1.3-N-acetylglucosaminyltransferase 7	1.350
203562_at	FEZ1	NM_005103	fasciculation and elongation protein zeta 1 (zygin I)	1.343
235333_at	B4GALT6	BG503479	UDP-Gal:betaGlcNAc beta 1.4- galactosyltransferase. polypeptide 6	1.342
236316_at	FAM3C	AW015417	family with sequence similarity 3. member C	1.340
204013_s_at	LCMT2	NM_014793	leucine carboxyl methyltransferase 2	1.340
208122_x_at	KIR2DS3	NM_012313	killer cell immunoglobulin-like receptor. two domains. short cytoplasmic tail. 3	1.338
211685_s_at	NCALD	AF251061	neurocalcin delta	1.337
210148_at	HIPK3	AF305239	homeodomain interacting protein kinase 3	1.330
1553698_a_at	C1orf96	NM_145257	chromosome 1 open reading frame 96	1.325
209795_at	CD69	L07555	CD69 molecule	1.324
203502_at	BPGM	NM_001724	2.3-bisphosphoglycerate mutase	1.323
239384_at	FLJ44342	R18746	hypothetical LOC645460	1.323
217591_at	SKIL	BF725121	SKI-like oncogene	1.323
222139_at	KIAA1466	AI765383	KIAA1466 gene	1.322
1554309_at	EIF4G3	BC030578	eukaryotic translation initiation factor 4 gamma. 3	1.322
240413_at	PYHIN1	AI827431	pyrin and HIN domain family. member 1	1.320
1563502_at	ZDHHC2	AL833080	zinc finger. DHHC-type containing 2	-1.320
243764_at	VSIG1	AW085312	V-set and immunoglobulin domain containing 1	-1.321
204995_at	CDK5R1	AL567411	cyclin-dependent kinase 5. regulatory subunit 1 (p35)	-1.322
215505_s_at	STRN3	AF243424	striatin. calmodulin binding protein 3	-1.323
221916_at	NEFL	BF055311	neurofilament. light polypeptide	-1.325
226065_at	PRICKLE1	N98595	prickle homolog 1 (Drosophila)	-1.325
229420_at	RPL23A	AI557425	ribosomal protein L23a	-1.329
218232_at	C1QA	NM_015991	complement component 1. q subcomponent. A chain	-1.330
235306_at	GIMAP8	AI611648	GTPase. IMAP family member 8	-1.332
228170_at	OLIG1	AL355743	oligodendrocyte transcription factor 1	-1.338

214052_x_at	BAT2L2	AW301305	HLA-B associated transcript 2-like 2	-1.344
1559882_at	SAMHD1	AF147427	SAM domain and HD domain 1	-1.345
206343_s_at	NRG1	NM_013959	neuregulin 1	-1.347
227074_at	LOC100131564	AA524669	hypothetical protein LOC100131564	-1.347
226322_at	TMTC1	BF109231	transmembrane and tetratricopeptide repeat containing 1	-1.348
221960_s_at	RAB2A	AI189609	RAB2A. member RAS oncogene family	-1.349
243106_at	CLEC12A	AA916861	C-type lectin domain family 12. member A	-1.352
210172_at	SF1	D26121	splicing factor 1	-1.360
221288_at	GPR22	NM_005295	G protein-coupled receptor 22	-1.374
232315_at	ZNF880	AU149712	zinc finger protein 880	-1.377
1560112_at	WDFY2	AK054833	WD repeat and FYVE domain containing 2	-1.384
242197_x_at	CD36	W95035	CD36 molecule (thrombospondin receptor)	-1.388
205758_at	CD8A	AW006735	CD8a molecule	-1.390
224567_x_at	MALAT1	BG534952	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)	-1.393
1555878_at	RPS24	AK094613	ribosomal protein S24	-1.397
216563_at	ANKRD12	X80821	ankyrin repeat domain 12	-1.407
236808_at	FGFR1OP2	AA947475	FGFR1 oncogene partner 2	-1.408
240159_at	SLC15A2	AA836116	solute carrier family 15 (H+/peptide transporter). member 2	-1.409
203096_s_at	RAPGEF2	BF439282	Rap guanine nucleotide exchange factor (GEF) 2	-1.410
1566040_at	LOC100288656	BM263698	similar to hCG2003116	-1.418
222027_at	NUCKS1	AW515443	nuclear casein kinase and cyclin-dependent kinase substrate 1	-1.418
239891_x_at	RAB12	AA001052	RAB12. member RAS oncogene family	-1.419
239487_at	FAM98A	AI743261	family with sequence similarity 98. member A	-1.419
230557_at	XRRA1	AW513519	X-ray radiation resistance associated 1	-1.421
234849_at	TRA@	AE000659	T cell receptor alpha locus	-1.424
1554544_a_at	MBP	L18865	myelin basic protein	-1.426
223620_at	GPR34	AF039686	G protein-coupled receptor 34	-1.431
207826_s_at	ID3	NM_002167	inhibitor of DNA binding 3. dominant negative helix-loop-helix protein	-1.432
208920_at	SRI	AV752215	sorcin	-1.452
229028_s_at	ARL17B	W73694	ADP-ribosylation factor-like 17B	-1.509
237625_s_at	IGKC	BG548679	immunoglobulin kappa constant	-1.556
231647_s_at	FCRL5	AW241983	Fc receptor-like 5	-1.557
1562338_at	MARCH1	BQ718095	membrane-associated ring finger (C3HC4) 1	-1.601
238149_at	ZNF818P	AI651641	zinc finger protein 818 (pseudogene)	-1.602
238465_at	C5orf35	AI265933	chromosome 5 open reading frame 35	-1.610
202203_s_at	AMFR	NM_001144	autocrine motility factor receptor	-1.692
206765_at	KCNJ2	AF153820	potassium inwardly-rectifying channel. subfamily J. member 2	-2.150
239412_at	IRF5	BF223643	interferon regulatory factor 5	-2.428

Supplementary Table 12: Gene sets enriched in the tolerance-related transcriptional profile at baseline

Enriched in tolerant recipients	***p*-value**	***q*-value**	**Genes with highest enrichment scores**	**Gene set database**
Natural Killer (CD56) cell lineage	0.000	0.000	*KLRC3, NCAM1, CD160, BNC2, KIR3DL2, LAIR2, SH2D1B, KIR3DL1, KLRF1, CLIC3, AKR1C3, FEZ1, PDGFRB, GZMB, IL2RB*	Haematlas
Graft versus host disease	0.000	0.000	*KIR3DL1, KIR3DL2, KIR3DL3, KIR2DL1, GZMB, KIR2DL5A, KLRD1*	KEGG
Antigen processing and presentation	0.000	0.000	*KLRC4, KLRC3, KIR3DL2, KIR3DL1, KIR2DS3, KIR2DS4, KIR2DL5A, KIR2DL1, KIR2DS1, KLRD1*	KEGG
Natural Killer cell mediated cytotoxicity	0.000	0.001	*KLRC3, KIR3DL2, SH2D1B, KIR3DL1, KIR2DL2, GZMB, KIR2DL1, TNF, KLRD1, KIR2DL3*	Biocarta
VIP pathway	0.000	0.001	*EGR3, EGR2, PRKAR1B, NFKBIA, PRKAR2B, NFATC2, PPP3CC*	KEGG
DARPP32 events	0.000	0.006	*PDE4D, PRKAR2B, PRKAR2B, PPP3R1, PDE4B, PPP3C.*	Biocarta
Immunoregulatory interactions	0.000	0.013	*CD160, KIR3DL2, KIR3DL1, KIR2DL2, KIR2DS2, KIR2DL3, CD226, KIR2DL1, ICAM4*	Reactome
Differentiation pathway in PC12 cells	0.000	0.015	*EGR3, EGR2, EGR1, JUN*	Reactome
Muscle cell differentiation	0.000	0.018	*EREG, IGFBP3, GLMN, NOTCH1, SYNE1, BOC, ACTA1*	C2
Muscle contraction	0.000	0.084	*ACTA2, NEB, MYL4, MYL9, TNNC1, MYLK, ITGB5, DES*	Gene Ontology
NTHI pathway	0.002	0.082	*IL8, TNF, IL1B, NFKBIA, TGFBR1, DUSP1, CHUK, MAP2K6*	Biocarta
Enriched in non-tolerant recipients				
Monocyte (CD14) cell lineage	0.000	0.105	*CXCL10, NRG1, SASH1, CTSZ, C1QA, FUCA1, EDG3, ASAHL.*	Haematlas

Supplementary Table 13: List of genes differentially expressed between TOL and Non-TOL PBMC samples at baseline

Gene Symbol	**Fold change**	***p*-value**
SH2D1B	1.70	0.004
CLIC3	1.73	0.006
PSMD14	1.79	0.006
NCAM1	1.49	0.010
IL2RB	1.31	0.012
PDGFRB	1.62	0.013
GZMB	1.31	0.019
NCR1	1.47	0.023
GNG2	1.15	0.031
KLRF1	1.58	0.031
KLRC4	1.31	0.037