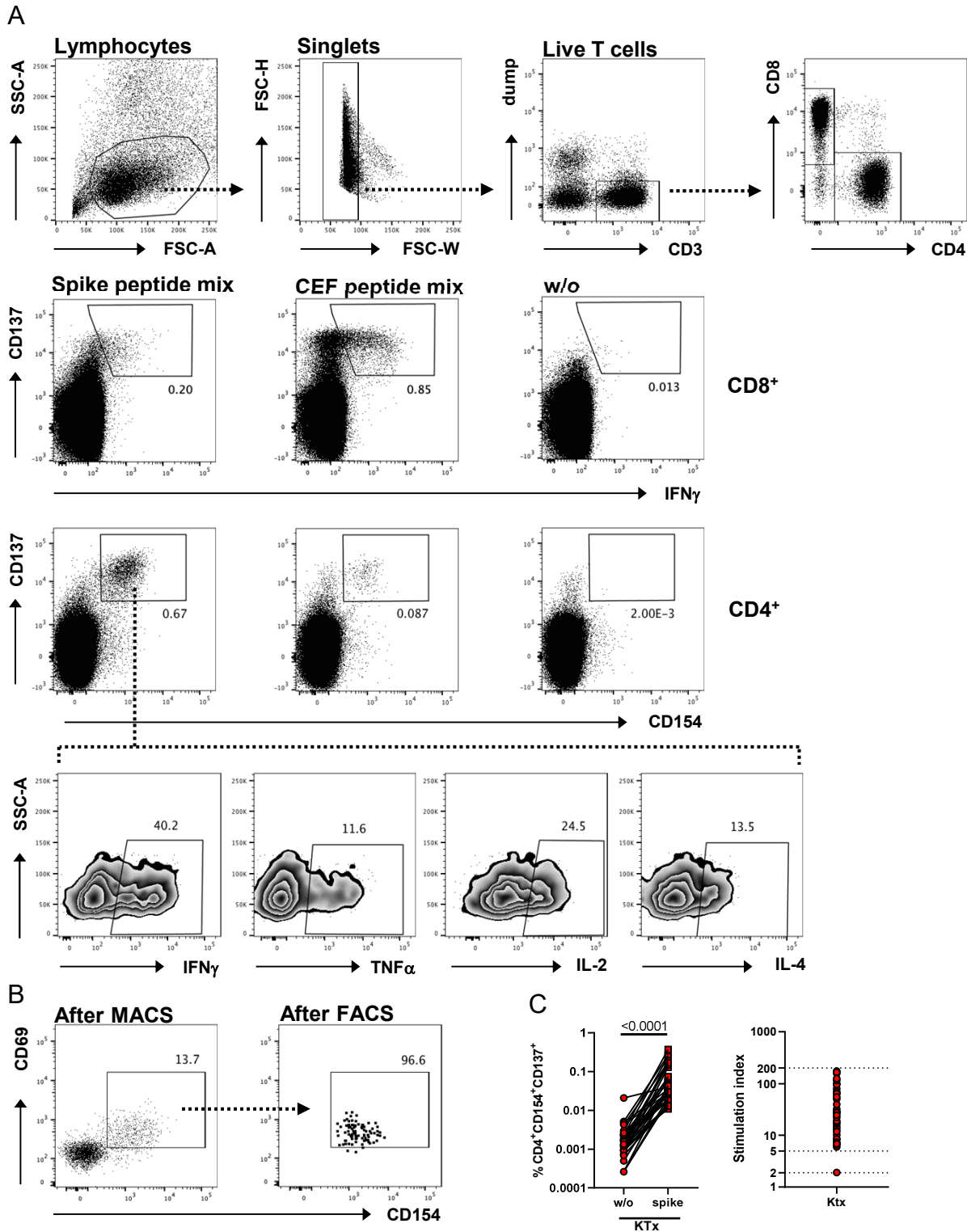


Impaired Humoral and Cellular Immunity after SARS-CoV2 BNT162b2 (Tozinameran) Prime-Boost Vaccination in Kidney Transplant Recipients

Sattler et al.

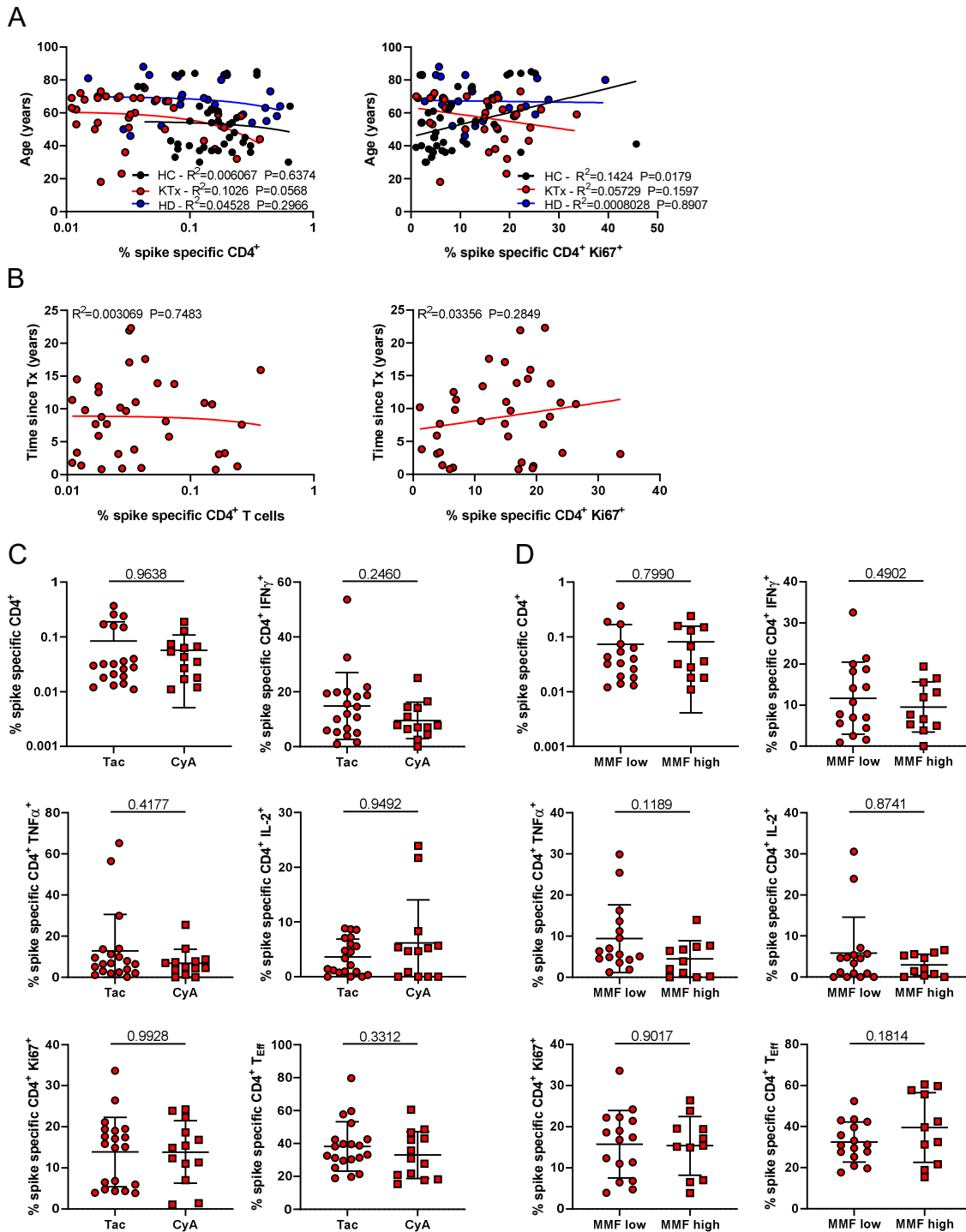
Supplemental Figures 1-5

Supplemental Figure 1, Sattler et al.



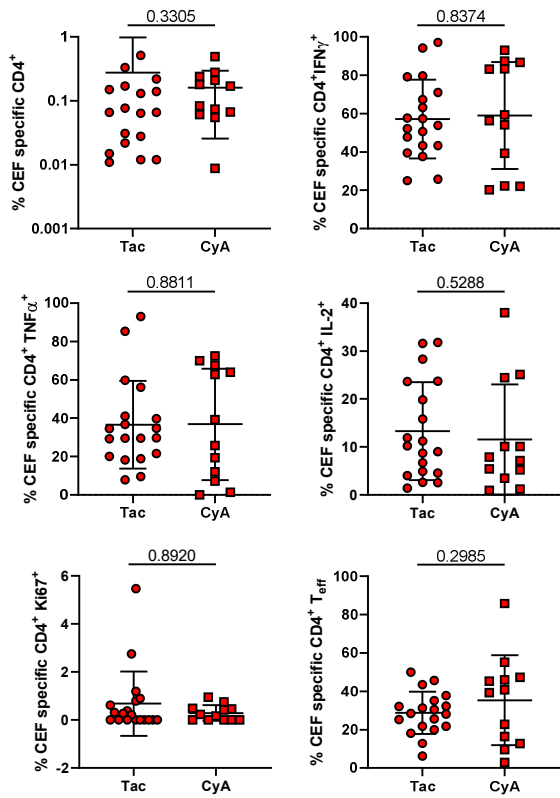
**Identification and enrichment of antigen-specific T cells.** (A) PBMC were stimulated or not with spike or CEF peptide mix for 16 h as indicated. Antigen-specific CD8<sup>+</sup> T cells were detected within live single CD14<sup>+</sup>CD19<sup>+</sup>CD3<sup>+</sup> (dump<sup>-</sup>) lymphocytes according to CD137 and IFN $\gamma$  co-expression. Live single CD14<sup>+</sup>CD19<sup>+</sup>CD3<sup>+</sup> specific CD4<sup>+</sup> Th cells were identified based on co-expression of CD154 and CD137. Specific CD4<sup>+</sup>CD154<sup>+</sup>CD137<sup>+</sup> Th cells were further analyzed for expression of IFN $\gamma$ , TNF $\alpha$ , IL-2 and/or IL-4. (B) Exemplary reanalysis dot plots depicting pre-enrichment of spike specific CD4<sup>+</sup> Th cells via MACS based on activation induced CD154 expression (left), followed by purification via FACS (right). (C) Frequencies of CD4<sup>+</sup>CD154<sup>+</sup>CD137<sup>+</sup> T cells in unstimulated vs. spike-stimulated samples from responding KTx patients with paired analysis (left) and corresponding stimulation indices (SI) with marks at the defined lower cut-off of 2 for a positive response and at 5 and 200 highlighting the SI range of all but one patient (right); if the percentage in the unstimulated control was 0, the minimum value within the patient cohort was used instead. n as in Fig. 2B.

Supplemental Figure 2, Sattler et al.



**Association of the vaccine specific CD4<sup>+</sup> T cell response with age and IS medication.** (A) Age of HC, KTx and HD patients was correlated with frequencies of spike-specific CD4<sup>+</sup> Th cells (left) or frequencies of proliferating Ki67<sup>+</sup> cells within the antigen-specific Th cell population (right) with n as in Fig. 2B. (B) Correlation of time since transplantation with frequencies of spike-specific CD4<sup>+</sup> Th cells (left) or frequencies of proliferating Ki67<sup>+</sup> cells within the antigen-specific Th cell population (right) in KTx patients with n as in Fig. 2B. (C and D) KTx patients were stratified according to immunosuppressive drug regimen. Frequencies of spike specific Th cells or those expressing IFN $\gamma$ , TNF $\alpha$ , IL-2, Ki67 or showing a CD45RO<sup>+</sup>CD62L<sup>-</sup> T<sub>Erf</sub> phenotype were compared in patients receiving Tacrolimus or Cyclosporine A based (C) or low vs. high MMF therapy (D) (Tac: n=20, CyA: n=13, respectively). Graphs show mean  $\pm$  SD.

Supplemental Figure 3, Sattler et al.



**Association of CEF-specific CD4<sup>+</sup> T cell responses with IS medication.** KTx patients were stratified according to immunosuppressive drug regimen. Frequencies of CEF specific Th cells or those expressing IFN $\gamma$ , TNF $\alpha$ , IL-2, Ki67 or showing a CD45RO<sup>+</sup>CD62L<sup>-</sup> T<sub>eff</sub> phenotype were compared in patients receiving Tacrolimus or Cyclosporine A based therapy (Tac: n=19, CyA: n=12, respectively). Graphs show mean  $\pm$  SD.

## Supplemental Figure 4, Sattler et al.

A

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_UV_RESPONSE_UP	128	-0,5641754	-2,1331058	0	1,74E-04	1,00E-04	2415 tags=37%, list=15%, signal=43%	
HALLMARK_INFLAMMATORY_RESPONSE	130	-0,53510743	-2,0387063	0	3,49E-04	4,00E-04	3152 tags=42%, list=20%, signal=51%	
HALLMARK_IL2_STATS_SIGNALING	173	-0,51259583	-2,0187755	0	2,33E-04	4,00E-04	2829 tags=42%, list=18%, signal=50%	
HALLMARK_HYPOXIA	154	-0,5184372	-2,0026288	0	2,13E-04	5,00E-04	2504 tags=38%, list=16%, signal=45%	
HALLMARK_MTORC1_SIGNALING	193	-0,49838114	-1,9945359	0	2,04E-04	6,00E-04	2922 tags=36%, list=18%, signal=44%	
HALLMARK_ALLOGRAFT_REJECTION	155	-0,5003024	-1,9453664	0	3,54E-04	0,0012	2184 tags=26%, list=14%, signal=30%	
HALLMARK_GLYCOLYSIS	155	-0,49042752	-1,9036313	0	5,27E-04	0,0021	3428 tags=41%, list=22%, signal=51%	
HALLMARK_IL6_JAK_STAT3_SIGNALING	63	-0,53159034	-1,7956237	2,15E-04	0,002151928	0,0096	1727 tags=33%, list=11%, signal=37%	
HALLMARK_KRAS_SIGNALING_DN	75	-0,5130576	-1,7953149	6,46E-04	0,001929919	0,0097	1002 tags=20%, list=6%, signal=21%	
HALLMARK_TNFA_SIGNALING_VIA_NFKB	169	-0,4499193	-1,770671	2,24E-04	0,002578845	0,0142	2525 tags=33%, list=16%, signal=38%	
HALLMARK_MYC_TARGETS_V2	58	-0,4802872	-1,6108648	0,005877231	0,014407734	0,084	2769 tags=34%, list=17%, signal=42%	
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	46	-0,49912915	-1,5893345	0,013463627	0,016247097	0,1021	3159 tags=39%, list=20%, signal=49%	
HALLMARK_INTERFERON_GAMMA_RESPONSE	176	-0,3968431	-1,5691535	6,83E-04	0,01840697	0,1236	3221 tags=30%, list=20%, signal=37%	
HALLMARK_ADIPOGENESIS	165	-0,39565256	-1,5510837	0,001363017	0,02058476	0,1473	1928 tags=23%, list=12%, signal=26%	
HALLMARK_COAGULATION	59	-0,46534306	-1,5489655	0,013098561	0,019737085	0,1509	1883 tags=29%, list=12%, signal=33%	
HALLMARK_ESTROGEN_RESPONSE_LATE	126	-0,40467334	-1,531774	0,002889531	0,021827238	0,1759	2437 tags=31%, list=15%, signal=36%	
HALLMARK_COMPLEMENT	134	-0,39254677	-1,4974877	0,00447828	0,028232267	0,2339	2885 tags=30%, list=18%, signal=36%	
HALLMARK_HEME_METABOLISM	157	-0,37600276	-1,4624645	0,005859815	0,036552105	0,3058	2821 tags=30%, list=18%, signal=36%	
HALLMARK_CHOLESTEROL_HOMEOSTASIS	66	-0,4293268	-1,4580432	0,026105717	0,036226396	0,317	2940 tags=32%, list=19%, signal=39%	
HALLMARK_APICAL_JUNCTION	119	-0,38424537	-1,4372096	0,010772404	0,04122356	0,3686	2884 tags=29%, list=18%, signal=36%	
HALLMARK_MYOGENESIS	105	-0,38624853	-1,421316	0,015333333	0,045172434	0,4122	2460 tags=25%, list=16%, signal=29%	
HALLMARK_PI3K_AKT_MTOR_SIGNALING	94	-0,3814115	-1,3787745	0,034290805	0,06129168	0,5295	3289 tags=37%, list=21%, signal=47%	
HALLMARK_ANDROGEN_RESPONSE	84	-0,38136986	-1,3548361	0,043639537	0,071430475	0,6026	2829 tags=26%, list=18%, signal=32%	
HALLMARK_APOPTOSIS	135	-0,34027776	-1,2972382	0,048965055	0,10887173	0,7705	3078 tags=35%, list=19%, signal=43%	
HALLMARK_SPERMATOGENESIS	74	-0,37096983	-1,2909025	0,08660534	0,1097702	0,7865	3055 tags=27%, list=19%, signal=33%	
HALLMARK_MYC_TARGETS_V1	200	-0,30730104	-1,2327498	0,06382979	0,1641945	0,9099	3387 tags=29%, list=21%, signal=36%	
HALLMARK_ESTROGEN_RESPONSE_EARLY	132	-0,3216158	-1,2223709	0,092887595	0,17019427	0,927	2914 tags=30%, list=18%, signal=36%	
HALLMARK_XENOBIOTIC_METABOLISM	121	-0,31138954	-1,1705942	0,15408456	0,23529126	0,976	2348 tags=25%, list=15%, signal=29%	
HALLMARK_P53_PATHWAY	169	-0,28477034	-1,1138054	0,20823902	0,32681426	0,996	1719 tags=17%, list=11%, signal=18%	
HALLMARK_KRAS_SIGNALING_UP	116	-0,2912415	-1,0899824	0,2715217	0,36367878	0,9979	1949 tags=20%, list=12%, signal=22%	
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	111	-0,28320986	-1,0562775	0,3199912	0,42700946	0,9996	2618 tags=22%, list=17%, signal=26%	
HALLMARK_G2M_CHECKPOINT	187	-0,26357728	-1,0458515	0,3312442	0,43807837	0,9997	3374 tags=27%, list=21%, signal=34%	
HALLMARK_E2F_TARGETS	192	-0,24550505	-0,977073	0,5110102	0,60369354	1	3311 tags=27%, list=21%, signal=33%	
HALLMARK_ANGIOGENESIS	15	-0,38621876	-0,9644302	0,50950414	0,6214266	1	1921 tags=27%, list=12%, signal=30%	
HALLMARK_INTERFERON_ALPHA_RESPONSE	93	-0,25187108	-0,9093883	0,6471905	0,76174366	1	397 tags=4%, list=3%, signal=4%	
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	94	-0,24942395	-0,90142405	0,67144114	0,7628438	1	2526 tags=20%, list=16%, signal=24%	
HALLMARK_PEROXISOME	81	-0,25073645	-0,88513416	0,7019462	0,78545314	1	2880 tags=23%, list=18%, signal=29%	
HALLMARK_WNT_BETA_CATENIN_SIGNALING	33	-0,29142243	-0,86507404	0,6753191	0,8135227	1	4172 tags=39%, list=26%, signal=53%	
HALLMARK_NOTCH_SIGNALING	23	-0,30740926	-0,84920996	0,68510276	0,8279177	1	1711 tags=17%, list=11%, signal=19%	
HALLMARK_APICAL_SURFACE	23	-0,3014496	-0,828369	0,7181818	0,8480437	1	4367 tags=35%, list=28%, signal=48%	

B

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_UV_RESPONSE_DN	97	0,42865124	1,5239751	0,00582878	0,07493693	0,2038	2270 tags=24%, list=14%, signal=28%	
HALLMARK_BILE_ACID_METABOLISM	71	0,35608056	1,2131244	0,14803958	0,5646358	0,9715	1132 tags=15%, list=7%, signal=17%	
HALLMARK_TGF_BETA_SIGNALING	47	0,3707881	1,1713269	0,2067803	0,50112796	0,9919	1416 tags=19%, list=9%, signal=21%	
HALLMARK_OXIDATIVE_PHOSPHORYLATION	198	0,26288894	1,0292147	0,3792065	0,8755382	0,9999	2654 tags=19%, list=17%, signal=23%	
HALLMARK_FATTY_ACID_METABOLISM	126	0,25521058	0,9454126	0,5820029	1	1	2510 tags=20%, list=16%, signal=23%	
HALLMARK_MITOTIC_SPINDLE	178	0,23568171	0,9080746	0,7056292	0,9987016	1	3859 tags=27%, list=24%, signal=35%	
HALLMARK_PROTEIN_SECRETION	89	0,25184608	0,88440883	0,70160544	0,92864144	1	2126 tags=18%, list=13%, signal=21%	
HALLMARK_DNA_REPAIR	144	0,23132612	0,8698067	0,7767025	0,85021406	1	2998 tags=22%, list=19%, signal=27%	
HALLMARK_HEDGEHOG_SIGNALING	19	0,28498533	0,73240435	0,83780235	0,9576085	1	2234 tags=21%, list=14%, signal=24%	

**Gene set enrichment analysis result for genes sorted by differential expression p-value and direction of regulation in KTx vs. HC.** Enrichments for genes (A) down- or (B) upregulated in KTx vs. healthy individuals are shown. ES: enrichment score, NES: normalized enrichment score, NOM p -val: raw p-value, FDR: false discovery rate, FWER: family-wise error rate.

## Supplemental Figure 5, Sattler et al.

A

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_GLYCOLYSIS	138	-0,48345116	-1,8287017	0	0,007433969	0,0054	1576 tags=28%, list=13%, signal=31%	
HALLMARK_UV_RESPONSE_UP	115	-0,47041073	-1,7274398	1,88E-04	0,017351985	0,0245	2675 tags=45%, list=23%, signal=58%	
HALLMARK_MTORC1_SIGNALING	187	-0,42718133	-1,6798857	0	0,020352745	0,0433	1981 tags=27%, list=17%, signal=32%	
HALLMARK_XENOBIOTIC_METABOLISM	108	-0,45810518	-1,6656884	1,89E-04	0,017777689	0,0497	1984 tags=32%, list=17%, signal=39%	
HALLMARK_MYC_TARGETS_V2	57	-0,49633196	-1,6382238	0,005408538	0,019057767	0,0663	3305 tags=58%, list=28%, signal=80%	
HALLMARK_APICAL_SURFACE	20	-0,6067829	-1,6073259	0,019530479	0,022692421	0,0939	1573 tags=30%, list=13%, signal=35%	
HALLMARK_WNT_BETA_CATENIN_SIGNALING	28	-0,55339086	-1,57638	0,017605634	0,02668883	0,1264	2715 tags=43%, list=23%, signal=56%	
HALLMARK_MYOGENESIS	71	-0,44039714	-1,5007114	0,015748031	0,04948565	0,2501	2166 tags=35%, list=18%, signal=43%	
HALLMARK_DNA_REPAIR	143	-0,38873306	-1,4767677	0,006228766	0,05560669	0,3049	1621 tags=22%, list=14%, signal=26%	
HALLMARK_ESTROGEN_RESPONSE_LATE	114	-0,3943081	-1,4511386	0,010638298	0,06404827	0,3726	2018 tags=29%, list=17%, signal=35%	
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	108	-0,37865734	-1,3821554	0,027573181	0,10618592	0,574	1587 tags=20%, list=14%, signal=23%	
HALLMARK_ADIPOGENESIS	155	-0,35083774	-1,3407478	0,02631579	0,13674612	0,6992	1896 tags=27%, list=16%, signal=32%	
HALLMARK_ALLOGRAFT_REJECTION	148	-0,33960602	-1,29957	0,04447795	0,1753115	0,8118	1390 tags=20%, list=12%, signal=22%	
HALLMARK_HYPOXIA	131	-0,3428638	-1,2901081	0,052820902	0,17509621	0,8347	1968 tags=27%, list=17%, signal=33%	
HALLMARK_CHOLESTEROL_HOMEOSTASIS	62	-0,38684174	-1,2877407	0,10009681	0,16603923	0,8396	1864 tags=26%, list=16%, signal=31%	
HALLMARK_PEROXISOME	78	-0,36796567	-1,2771143	0,09290445	0,16886579	0,8641	1229 tags=17%, list=10%, signal=18%	
HALLMARK_PI3K_AKT_MTOR_SIGNALING	91	-0,356438	-1,2645499	0,09163196	0,17515218	0,8885	2104 tags=27%, list=18%, signal=33%	
HALLMARK_TNFA_SIGNALING_VIA_NFKB	160	-0,32696941	-1,2596805	0,060977913	0,17198606	0,8984	2895 tags=38%, list=25%, signal=49%	
HALLMARK_IL2_STAT5_SIGNALING	164	-0,32278168	-1,2454358	0,066753685	0,18032515	0,9216	1478 tags=19%, list=13%, signal=21%	
HALLMARK_FATTY_ACID_METABOLISM	121	-0,33270532	-1,2335275	0,09382065	0,18653947	0,9352	2493 tags=26%, list=21%, signal=33%	
HALLMARK_KRAS_SIGNALING_DN	55	-0,35172927	-1,1464943	0,22848894	0,32001868	0,9943	2717 tags=38%, list=23%, signal=49%	
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	41	-0,36580947	-1,1314687	0,26830202	0,33564013	0,9969	2839 tags=37%, list=24%, signal=48%	
HALLMARK_INFLAMMATORY_RESPONSE	121	-0,28651872	-1,0637712	0,31603774	0,4764479	0,9999	1561 tags=16%, list=13%, signal=18%	
HALLMARK_ESTROGEN_RESPONSE_EARLY	113	-0,2890921	-1,0604345	0,3254371	0,46507168	0,9999	1992 tags=27%, list=17%, signal=32%	
HALLMARK_APICAL_JUNCTION	93	-0,28925905	-1,0374317	0,3748053	0,5045558	0,9999	2443 tags=28%, list=21%, signal=35%	
HALLMARK_APOPTOSIS	129	-0,27663374	-1,0339795	0,37471783	0,49388325	0,9999	2962 tags=36%, list=25%, signal=48%	
HALLMARK_HEME_METABOLISM	143	-0,26331586	-0,9976748	0,45519096	0,5696921	1	2275 tags=26%, list=19%, signal=32%	
HALLMARK_P53_PATHWAY	161	-0,2509777	-0,9701577	0,5198954	0,6242107	1	1233 tags=16%, list=11%, signal=17%	
HALLMARK_NOTCH_SIGNALING	19	-0,35768104	-0,9342219	0,5431927	0,70242625	1	2533 tags=42%, list=22%, signal=54%	
HALLMARK_OXIDATIVE_PHOSPHORYLATION	197	-0,23607048	-0,93288046	0,64928734	0,6828343	1	1730 tags=19%, list=15%, signal=22%	
HALLMARK_COMPLEMENT	121	-0,22164218	-0,8193923	0,87114954	0,93663484	1	2094 tags=22%, list=18%, signal=27%	
HALLMARK_MYC_TARGETS_V1	200	-0,20483164	-0,81089294	0,9442849	0,9229208	1	1244 tags=12%, list=11%, signal=13%	
HALLMARK_SPERMATOGENESIS	58	-0,2102307	-0,6947868	0,9622715	1	1	2638 tags=26%, list=22%, signal=33%	
HALLMARK_COAGULATION	46	-0,21791056	-0,6867504	0,94808745	0,97978604	1	2094 tags=22%, list=18%, signal=26%	

B

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_MITOTIC_SPINDLE	159	0,44528213	1,737718	0	0,010422074	0,0179	1598 tags=23%, list=14%, signal=27%	
HALLMARK_UV_RESPONSE_DN	87	0,39546713	1,4120328	0,029046517	0,16643237	0,437	2316 tags=32%, list=20%, signal=40%	
HALLMARK_PROTEIN_SECRETION	88	0,36356315	1,3011699	0,07064429	0,27900046	0,7696	1707 tags=24%, list=15%, signal=28%	
HALLMARK_KRAS_SIGNALING_UP	99	0,34570763	1,2596259	0,08398727	0,28699598	0,8692	1845 tags=21%, list=16%, signal=25%	
HALLMARK_E2F_TARGETS	182	0,30002373	1,1899107	0,0957782	0,3790868	0,969	2486 tags=27%, list=21%, signal=34%	
HALLMARK_G2M_CHECKPOINT	169	0,26614198	1,0466211	0,3258595	0,75955534	0,9997	2645 tags=27%, list=23%, signal=35%	
HALLMARK_ANDROGEN_RESPONSE	78	0,29347807	1,0280854	0,39508787	0,71913296	0,9999	2284 tags=28%, list=19%, signal=35%	
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	70	0,288305	0,98897487	0,47003156	0,7650182	1	373 tags=9%, list=3%, signal=9%	
HALLMARK_TGF_BETA_SIGNALING	42	0,3073257	0,9582165	0,521757	0,78557426	1	1187 tags=17%, list=10%, signal=18%	
HALLMARK_IL6_JAK_STAT3_SIGNALING	59	0,28399596	0,95088637	0,53798383	0,7305782	1	1103 tags=14%, list=9%, signal=15%	
HALLMARK_INTERFERON_ALPHA_RESPONSE	92	0,25108352	0,9030983	0,6659677	0,8030188	1	3298 tags=37%, list=28%, signal=51%	
HALLMARK_INTERFERON_GAMMA_RESPONSE	171	0,2293831	0,9016661	0,73079354	0,73971325	1	2977 tags=32%, list=25%, signal=42%	
HALLMARK_BILE_ACID_METABOLISM	61	0,24797216	0,83061695	0,79502714	0,83938235	1	1115 tags=13%, list=10%, signal=14%	

**Gene set enrichment analysis result for genes sorted by differential expression p-value and direction of regulation in HD vs HC.** Enrichments for genes (A) down- or (B) upregulated in HD vs. healthy individuals are shown. ES: enrichment score, NES: normalized enrichment score, NOM p -val: raw p-value, FDR: false discovery rate, FWER: family-wise error rate.