

Additional file 1

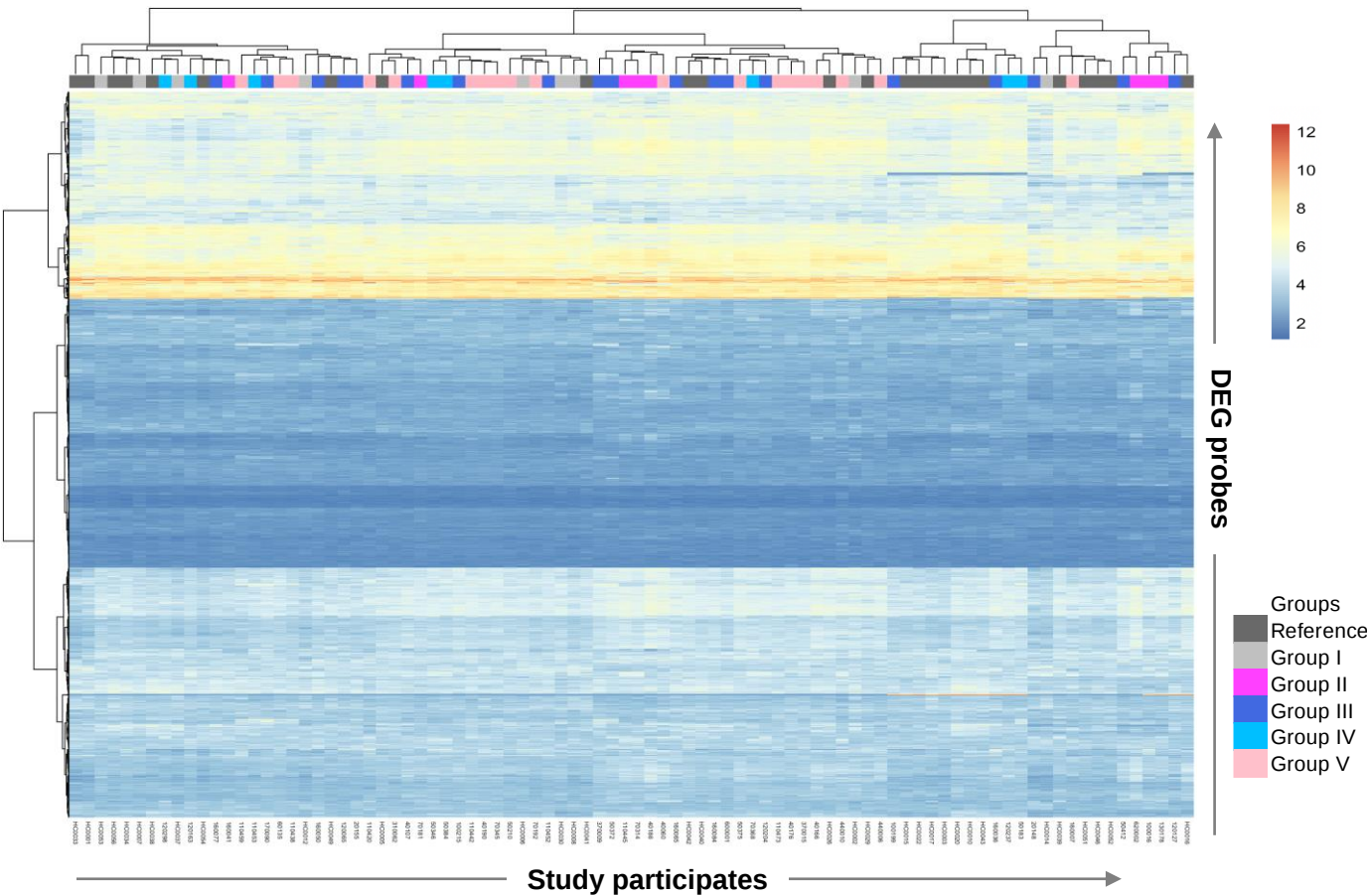


Figure S1. Hierarchical clustering of expression values among 88 study participants. Hierarchical clustering analysis was performed using the top 40% highly variable DEG probes (2,634 of 6,586) (right y axis, list of DEG available in supplemental material 2) representing the top 40% highly variable unique DEG (929 of 2,322) to predict the relatedness of the study groups (colored bars on top), including Reference Group (healthy nonsubstance use, n = 25, dark grey bars), Group I (healthy using a combination of alcohol, marijuana and tobacco, n = 9, light grey bars), Group II (virally unsuppressed nonsubstance-using YWH, n = 8, pink bars), Group III (virally suppressed nonsubstance-using YWH, n = 19, dark blue bars), Group IV (virally suppressed YWH using marijuana, n = 8, light blue bars), and Group V (virally suppressed YWH using marijuana plus tobacco, n = 19, light pink bars). Heatmap scales between 2 to 12 log₂ gene expression values.

A.

T Cell Exhaustion Signaling (#2)					Role of BRCA1 in DNA Damage (#3)					Mitotic Roles of Polo-Like Kinase (#4)					Death Receptor Signaling (#5)					PI3K/AKT Signaling (#6)				
DEG	Group				DEG	Group				DEG	Group				DEG	Group				DEG	Group			
	II	III	IV	V		II	III	IV	V		II	III	IV	V		II	III	IV	V		II	III	IV	V
PIK3CB					SLC19A1					SMC3					MAP2K7					PPP2R1B				
PIK3C2A					ARID2					PPP2R1B					MAP4K4					PIK3CB				
JAK1					POU2F1					PPP2R2A					TNFRSF10A					PTEN				
IFNAR2					SMARCC2					PPP2R2B					APAF1					SYNJ2				
ACVR1B ^a					BRD7					WEE1					BID					FOXO3				
BMPR2					IFNG					CCNB1					CFLAR					JAK1				
PDK1					STAT1					CCNB2					NAIP					AKT2				
PPP2R1B					ATF1					CDC7					NFKB2 ^d					FOXO1				
LAG3					ATR					CDC20					FADD					GAB2 ^f				
CD274					BARD1					CDK1					TNFRSF10B					PPP2R2B				
IFNG					BRIP1					FBXO5					XIAP					RAP2A				
PPP2R2B					FANCL					PRC1					SPTAN1					ITGA4				
RAP2A					MSH2					SLK					ACTB					ITGB1				
STAT1					NBN					PPP2R5C					MAPK8					JAK2				
CTLA4					RAD50					ANAPC4					FAS					MAP3K8				
EOMES					RAD51					ANAPC10					PARP9					NRAS				
HLA-DPA1					RFC3					HSP90AA1					CASP3					RPS6KB1				
JAK2					MSH6					KIF11					CASP7					EIF4E				
MAPK9					ACTB					PLK4					PARP12					HSP90AA1				
NRAS					TP53					PPP2R1A					PARP14					ILK				
PRDM1					E2F7					PTTG1					ROCK1					INPP5F				
BATF					E2F8					TGFB1					TBK1					NFKBIB ^g				
CD28 ^b					FANCA										ACTA2					PPP2R5C				
PPP2R5C					FANCF										BIRC2					KRAS				
KRAS					FANCM										CYCS					TP53				
PPP2R2A					HLTF										FASLG					PPP2R2A				
MAPK8					MRE11										NFKBIB					PTGS2				
PTPN11					RBBP8 ^c										PARP2 ^e					YWHAZ				

- a. Additional Pathway #2 genes uniquely down-regulated in Group II [not shown]: AKT2, BCL6, FOXO1, FOXP1, IL6R, MAPK1, MGAT5, NFAT5 and PIK3CG.
- b. Additional Pathway #2 genes uniquely upregulated in Group II [not shown]: HLA-DQA1, IL10RA, IL12RB1, IRF9, NFATC2, PDCD1, PIK3R3, PPP2R1A, PRKCQ, RAP1B, RRAS, RRAS2, SMAD3, STAT2, STAT4, TBX21, TGFB1 and TGFB3.
- c. Additional Pathway #3 genes uniquely upregulated in Group II [not shown]: RFC5 and SMARCE1.
- d. Additional Pathway #5 genes uniquely down-regulated in Group II [not shown]: TNFRSF21 and TNFRSF25.
- e. Addition Pathway #5 genes uniquely upregulated in Group II [not shown]: PARP3, PARP11 and PARP15.
- f. Additional Pathway #6 genes uniquely down-regulated in Group II [not shown]: LIMS1, MAPK1, MDM2, NFKB2, PDPK1, PIK3CG, RAF1 and THEM4.
- g. Additional Pathway #6 genes uniquely upregulated in Group II [not shown]: PIK3R3, PPP2R1A, RAP1B, RRAS, RRAS2, SOS1 and YWHAQ.

B.

NF-κB Signaling (#7)					PI3K Signaling in B Lymphocytes (#8)					Role of Pattern Recognition Receptors ^a (#10)					Ovarian Cancer Signaling (#11)					Apelin Pancreas Signaling (#12)				
DEG	Group				DEG	Group				DEG	Group				DEG	Group				DEG	Group			
	II	III	IV	V		II	III	IV	V		II	III	IV	V		II	III	IV	V		II	III	IV	V
IGF1R					CAMK2G					PIK3CB					APC					PRKAA1				
MAP2K7					PIK3CB					SYK					PIK3CB					PIK3CB				
MAP4K4					PTEN					PIK3C2A					PTEN					PIK3C2A				
PELI1					SYK					C5AR1					TCF4					PRKAG2				
PIK3CB					ITPR1					IL1A					PIK3C2A					PRKAR1A				
FGFR1					ATF6					LTB					ARRB1					NFKB2				
PIK3C2A					IRS2					MAPK1					CD44					PIK3CG				
IL1R1					CBL					NFKB2 ^f					PRKAG2					MAPK9				
IL1R2					FOXO3					CCL5					PRKAR1A					PRKACB				
BMPR2					AKT2					IFNG					AKT2					PIK3R3				
AKT2 ^b					BCL10					IL15					MAPK1					PRKAB2				
FADD					CD40 ^d					C1QB					MMP9 ^h					MAPK8				
RAP2A					RAP2A					DDX58					FZD3					PDE3B				
AZI2					ATF1					IRF1					FZD2									
MALT1					ATF3					MAPK9					RAP2A									
MAP3K8					MALT1					OAS1					FZD1									
NRAS					NRAS					OAS2					MSH2									
PRKACB					PDIA3					OAS3					NRAS									
TBK1					PIK3AP1					PRKCH					PRKACB									
TNFSF13B					ATF5					TNFSF13B					RAD51									
UBE2N					CAMK2D					EIF2AK2					RPS6KB1									
BMPR1A					CD81					EIF2S1					CDKN2A									
EIF2AK2 ^c					FYN ^e					FASLG					PIK3R3									
PDGFRB					ATF6B					IRF3					RAP1B ⁱ									
KRAS					BLK					IRF7 ^g					KRAS									
LTBR					CD79A					C1QA					TP53									
MAPK8					KRAS					CLEC7A					MSH6									
TNFAIP3					DAPP1					MAPK8					PTGS2									

- a: Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses
- b: Additional Pathway #7 genes uniquely down-regulated in Group II [not shown]: BCL10, CD40, CREBBP, CSNK2A1, EP300, IGF2R, IL1A, MAP3K3, NFKB2, PIK3CG, PRKCZ, RAF1 and TLR6.
- c: Additional Pathway #7 genes uniquely upregulated in Group II [not shown]: FGFR2, LCK, NFKBIB, PIK3R3, PRKCQ, RAP1B, RRAS, RRAS2, TAB3, TGFB3 and TLR3.
- d: Additional Pathway #8 genes uniquely down-regulated in Group II [not shown]: IL4R, MAPK1, NFAT5, NFKB2, PDPK1, PIK3CG, PPP3CA, PRKCZ, PTPRC, RAC1 and RAF1.
- e: Additional Pathway #8 genes uniquely upregulated in Group II [not shown]: NFATC2, NFKBIB, PLEKHA3, PPP3CB, RAP1B, RRAS and RRAS2.
- f: Additional Pathway #10 genes uniquely down-regulated in Group II [not shown]: PIK3CG, PRKCZ, TLR6 and TNFSF14.
- g: Additional Pathway #10 genes uniquely upregulated in Group II [not shown]: PIK3R3, PRKCQ, TGFB1 and TLR3.
- h: Additional Pathway #11 genes uniquely down-regulated in Group II [not shown]: PGF, PIK3CG and RAF1.
- i: Additional genes Pathway #11 genes uniquely upregulated in Group II [not shown]: RRAS and RRAS2.

Figure S2. DEG in perturbed pathways. A. DEG in 5 pathways perturbed in both study Groups II and V. **B.** DEG in pathways perturbed uniquely in Group III [2 pathways, NF-κB Signaling (Pathway #7) and PI3K Signaling in B Lymphocytes (Pathway #8)] and Group V (Pathways #10, #11 and #12). Red-filled space: up-regulated gene; Green-filled space: down-regulated gene. Blue box: DEG specific to Group IV; Brown box: DEG specific to Group V.