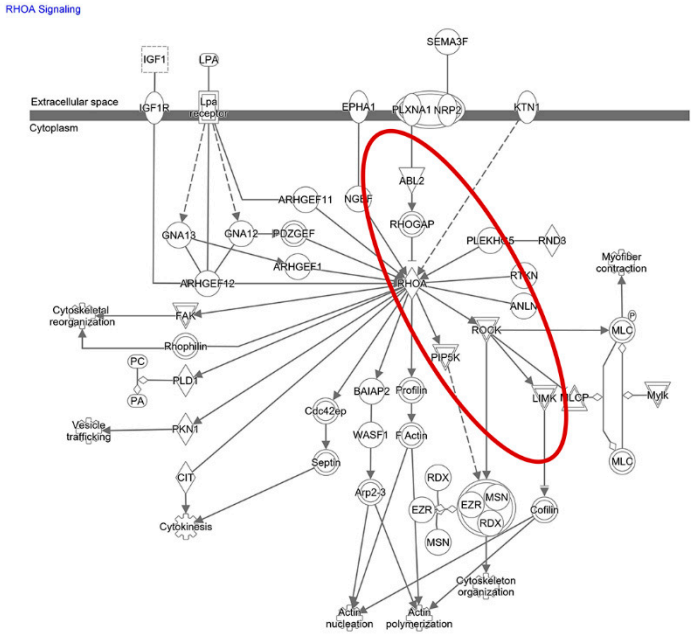
Legends to supplementary figures

Figure S1. Proteins represented both in proteome and phosphoproteome analyses. The Venn diagrams, drawn using InteractiVenn tool, show the number of proteins identified by phosphoproteome analysis (p-proteins), proteome analysis (proteins), and in common between the two datasets, in: (a) Patient 53 (53); (b) Patient 53's father (53F); (c) Patient 53's mother (53M); (d) Patient 67 (67); (e) Patient 67's father (67F); (f) Patient 67's mother (67M); (g) 281 young healthy control (281 yHC); (h) 283 young healthy control (283 yHC); (i) 519 adult healthy control (519 aHC); (j) 550 adult healthy control (550 aHC).


RHOA Signaling
SEMA3F
IGF1
LPA
Extracellular space
Cytoplasm
IGF1R
Lpa receptor
EPHA1
PLXNA1 NRP2
KTN1
ABL2
ARHGEF11
NGEF
RHOGAP
GNA13
GNA12
PDZGEF
PLEKHG5
RND3
Myofiber contraction
ARHGEF1
ARHGEF12
RHOA
RTKN
ANLN
Cytoskeletal reorganization
FAK
ROCK
MLC
Rhophilin
PIP5K
PC
PLD1
PA
BAIAP2
Profilin
LIMK
TLCP
Mylk
Vesicle trafficking
PKN1
Cdc42ep
WASF1
F Actin
Septin
MLC
CIT
Arp2-3
RDX
EZR
MSN
RDX
Cofilin
MSN
Cytokinasis
Cytoskeleton organization
Actin nucleation
Actin polymerization
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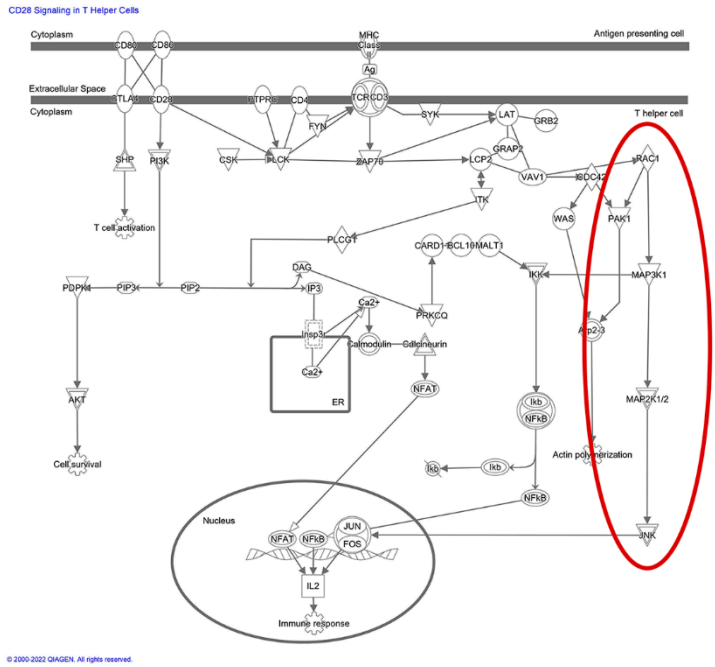
(a)


CD28 Signaling in T Helper Cells
Cytoplasm
MHC Class II
Antigen presenting cell
CD80
CD86
Ag
Extracellular Space
Cytoplasm
PTPRC
CD4
TCR CD3
FYN
SYK
LAT
GRB2
T helper cell
SHP
PI3K
CSK
PLCK
ZAP70
LCP2
GRAP2
VAV1
CDC42
RAC1
ITK
WAS
PAK1
T cell activation
PLCG1
CARD11 BCL10 MALT1
IKK
MAP3K1
DAG
PDPK1
PIP3
PIP2
IP3
Ca2+
PRKCQ
Arp2-3
Insp3r
Calmodulin
Calcineurin
Ca2+
ER
NFAT
AKT
MAP2K1/2
Ikb
NFkB
Cell survival
Actin polymerization
Ikb
Ikb
NFkB
Nucleus
NFAT
NFkB
JUN
FOS
JNK
IL2
Immune response
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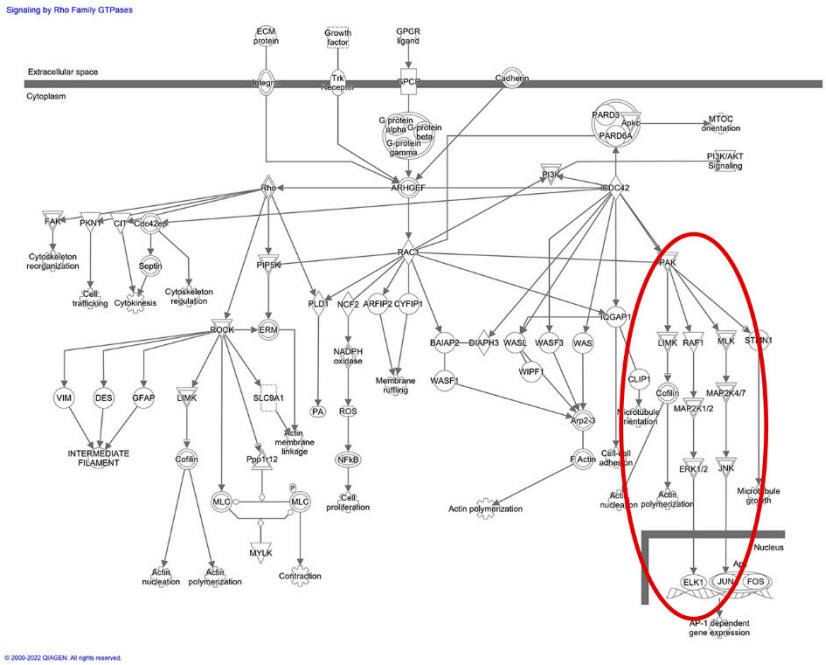
(b)


Signaling by Rho Family GTPases
ECM protein
Growth factor
GPCR ligand
Extracellular space
Cytoplasm
Integrin
Trk Receptor
GPCR
Cadherin
G protein alpha
G protein beta
G protein gamma
PARD3
PARD6A
aPKC
MTOC orientation
PI3K/AKT Signaling
PI3K
ARHGEF
Rho
CDC42
FAK
PKN1
CIT
Cdc42ep3
Cytoskeleton reorganization
Septin
Cell trafficking
Cytokinesis
Cytoskeleton regulation
PIP5K
RAC1
PAK
PLD1
NCF2
ARFIP2
CYFIP1
IQGAP1
ROCK
ERM
NADPH oxidase
BAIAP2
DIAPH3
WASL
WASF3
WAS
LIMK
RAF1
MLK
STMN1
Membrane ruffling
WASF1
WIPF1
CLIP1
Cofilin
MAP2K4/7
MAP2K1/2
VIM
DES
GFAP
LIMK
SLC9A1
PA
ROS
Actin membrane linkage
Arp2-3
Microtubule orientation
INTERMEDIATE FILAMENT
Cofilin
Ppp1r12
NFkB
F Actin
Cell-cell adhesion
ERK1/2
JNK
MLC
Cell proliferation
Actin polymerization
Actin nucleation
Microtubule growth
MYLK
Contraction
Actin nucleation
Actin polymerization
Nucleus
ELK1
JUN
FOS
AP-1 dependent gene expression
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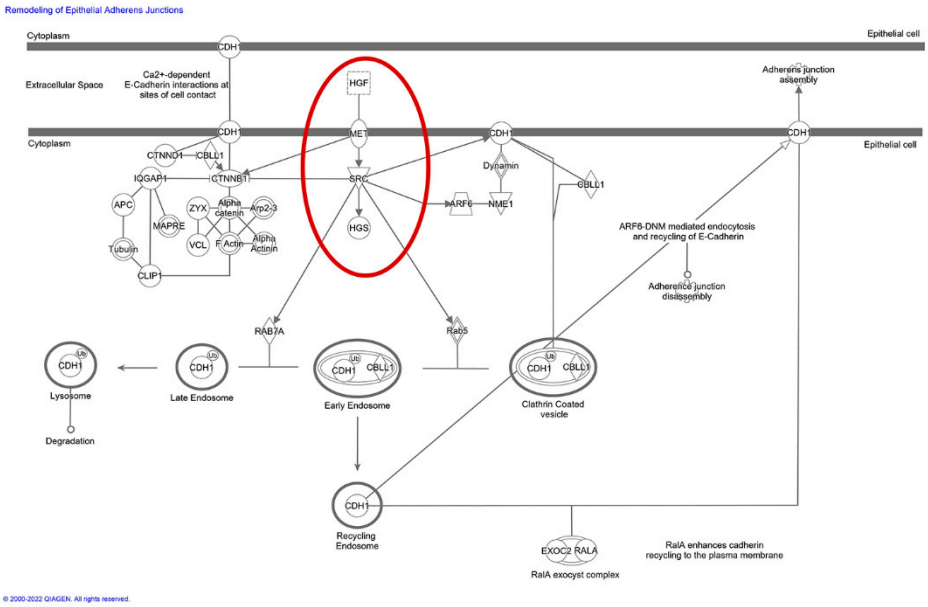
(c)


Remodeling of Epithelial Adherens Junctions
Cytoplasm
Epithelial cell
CDH1
Extracellular Space
Ca2+-dependent E-Cadherin interactions at sites of cell contact
HGF
Adherens junction assembly
CDH1
MET
CDH1
CDH1
Cytoplasm
Epithelial cell
CTNND1
CBLL1
Dynamin
IQGAP1
CTNNB1
SRC
CBLL1
APC
ZYX
Alpha catenin
Arp2-3
ARF6
NME1
MAPRE
HGS
ARF6-DNM mediated endocytosis and recycling of E-Cadherin
Tubulin
VCL
F Actin
Alpha Actinin
CLIP1
Adherence junction disassembly
RAB7A
Rab5
Ub
CDH1
Lysosome
Degradation
Late Endosome
Early Endosome
CBLL1
Clathrin Coated vesicle
Recycling Endosome
EXOC2 RALA
RalA exocyst complex
RalA enhances cadherin recycling to the plasma membrane
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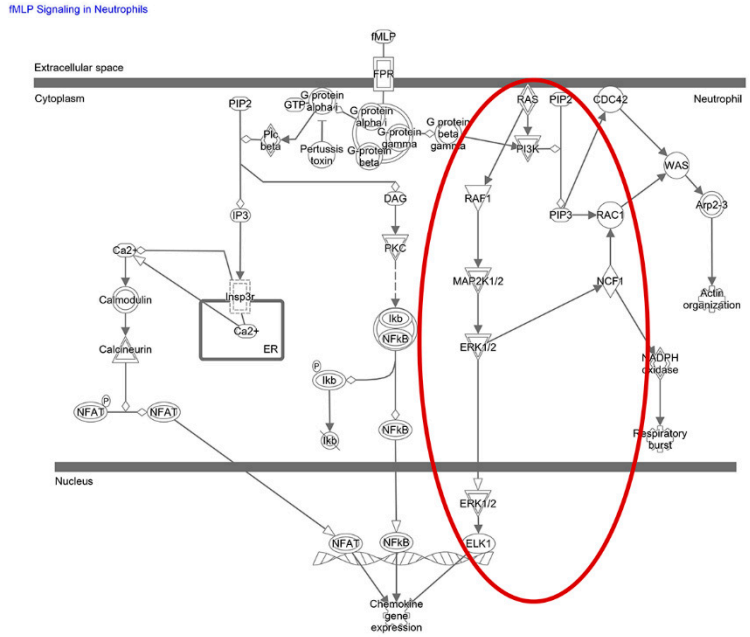
(d)


fMLP Signaling in Neutrophils
fMLP
FPR
Extracellular space
Cytoplasm
Neutrophil
PIP2
G protein GTP alpha i
G protein alpha i
G protein gamma
G protein beta
G protein beta gamma
Plc beta
Pertussis toxin
RAS
PIP2
CDC42
PI3K
WAS
Arp2-3
DAG
IP3
RAF1
PIP3
RAC1
PKC
Ca2+
MAP2K1/2
NCF1
Actin organization
Calmodulin
Insp3r
Ca2+
ER
Ikb
NFkB
ERK1/2
NADPH oxidase
Calcineurin
P
Ikb
NFAT1
NFAT
Ikb
NFkB
Respiratory burst
Nucleus
ERK1/2
NFAT
NFkB
ELK1
Chemokine gene expression
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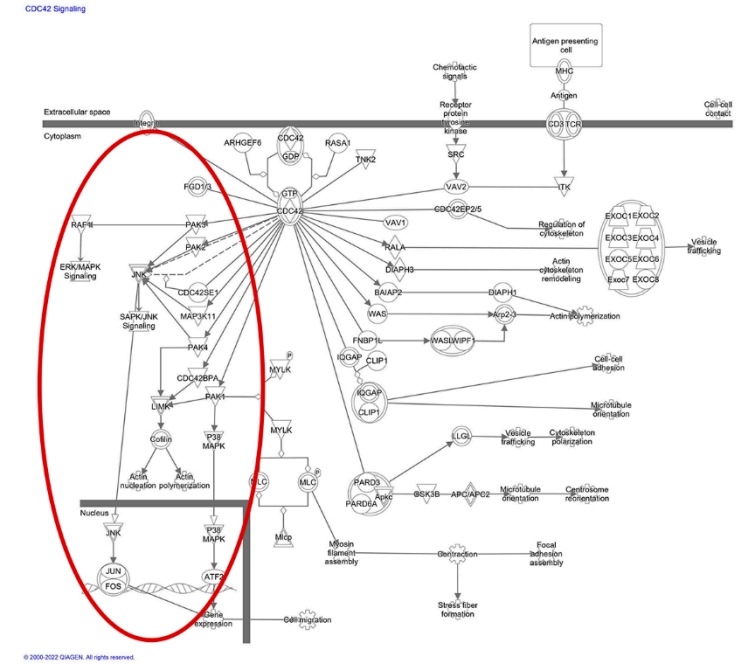
(e)


CDC42 Signaling
Antigen presenting cell
MHC
Antigen
Chemotactic signals
Receptor protein tyrosine kinase
CD3TCR
Cell-cell contact
Extracellular space
Cytoplasm
ARHGEF6
CDC42
RASA1
GDP
TNK2
SRC
FGD1/3
GTP
VAV2
ITK
CDC42
CDC42EP2/5
VAV1
Regulation of cytoskeleton
EXOC1EXOC2
EXOC3EXOC4
EXOC5EXOC6
Exoc7 EXOC8
Vesicle trafficking
RALA
DIAPH3
Actin cytoskeleton remodeling
RAF1
PAK3
PAK2
ERK/MAPK Signaling
JNK
CDC42SE1
BAIAP2
DIAPH1
SAPK/JNK Signaling
MAP3K11
WAS
Arp2-3
Actin polymerization
PAK4
FNBP1L
WASLVIPF1
IQGAP
CLIP1
P
MYLK
Cell-cell adhesion
CDC42BPA
IQGAP
CLIP1
PAK1
Microtubule orientation
LIMK1
MYLK
LLGL
Vesicle trafficking
Cytoskeleton polarization
Cofilin
P38 MAPK
Actin nucleation
Actin polymerization
CLC
MLC
PARD3
PARD6A
aPKC
GSK3B
APC/APC2
Microtubule orientation
Centrosome reorientation
Nucleus
JNK
P38 MAPK
MLCP
Myosin filament assembly
Contraction
Focal adhesion assembly
JUN
FOS
ATF2
Stress fiber formation
Gene expression
Cell migration
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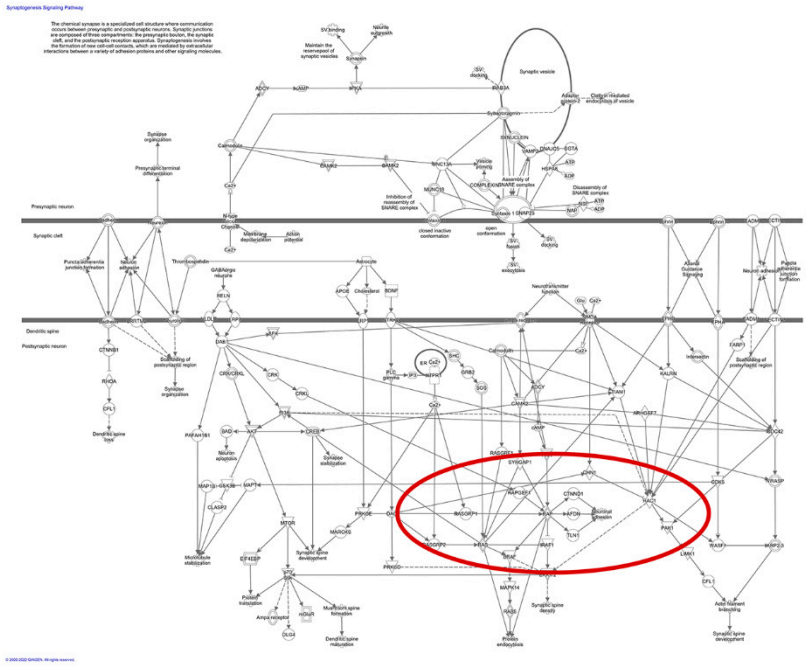
(f)



(g)

Figure S2. Main activated pathways highlighted by IPA enrichment analysis. Analysis of changed phosphoproteins/total protein datasets by IPA software identified the following seven canonical pathways significantly changed (Fisher's right-tailed exact test p-value < 0.05 and z-score ≥ 2 or ≤ −2) in patients 53 and 67 vs. young healthy controls: (a) RHOA Signaling; (b) CD28 Signaling in T Helper Cells; (c) Signaling by Rho Family GTPases; (d) Remodeling of Epithelial Adherens Junctions; (e) fMLP Signaling in Neutrophils; (f) CDC42 Signaling; (g) Synaptogenesis Signaling Pathway. In both patient's parents (53F-53M, 67F-67M) vs. age-matched controls, the same pathways were unchanged or only slightly activated. The pathways' branches connected to RAS signaling are circled in red.

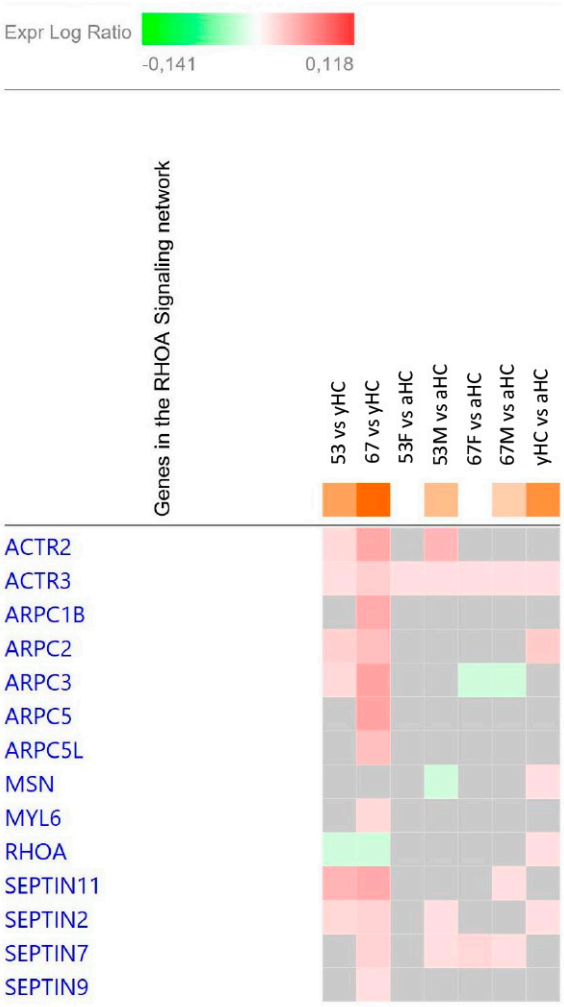


Figure S3. RHOA Signaling gene heatmap. The heatmap generated by IPA software shows the hypo-phosphorylation (in green) or hyper- phosphorylation (in red) of proteins belonging to RHOA signaling, based on log ratio data from LC-MS/MS analysis. Even if RHOA is hypo-phosphorylated in both patients (53 and 67) compared to young heathy controls (yHC), IPA software predicts the activation (orange rectangles) of RHOA Signaling in patients. In both patients' mothers (53M and 67M) vs. adult controls (aHC), the same pathway is only slightly activated, while RHOA Signaling activation is not predicted in the fathers (53F and 67F).