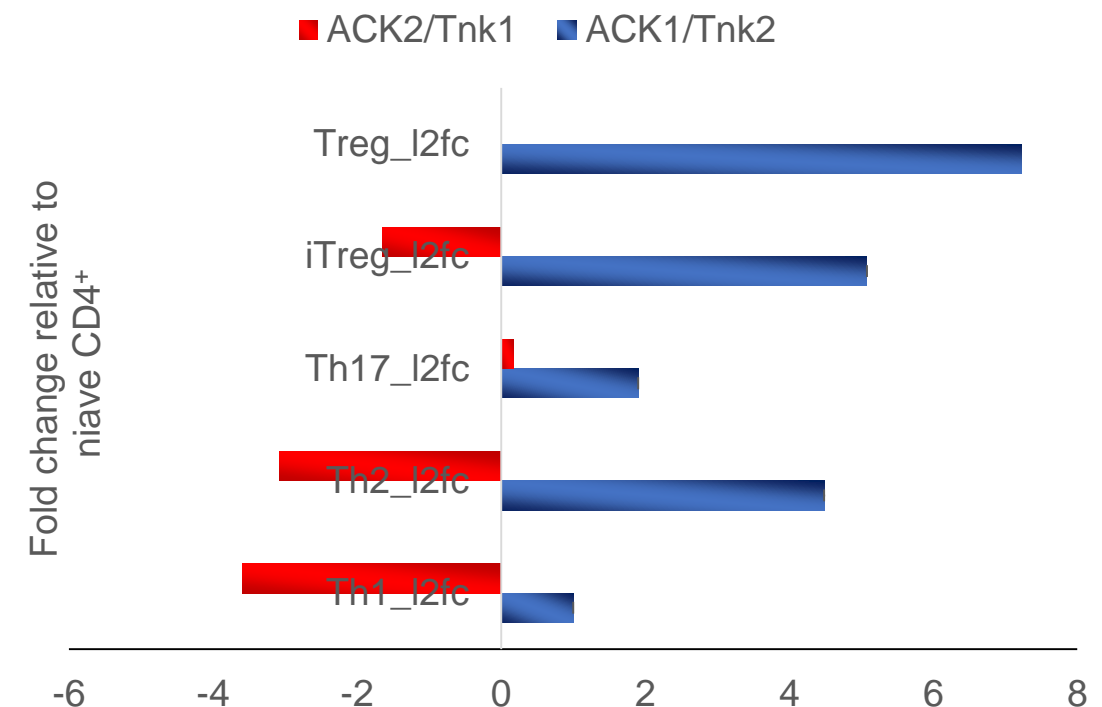
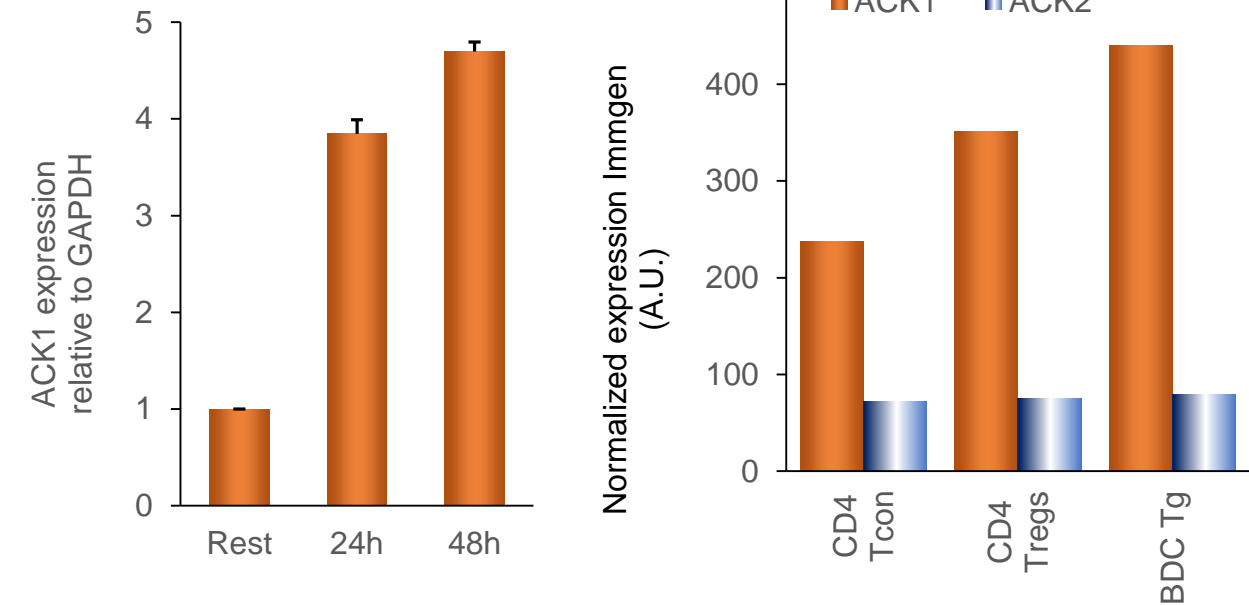


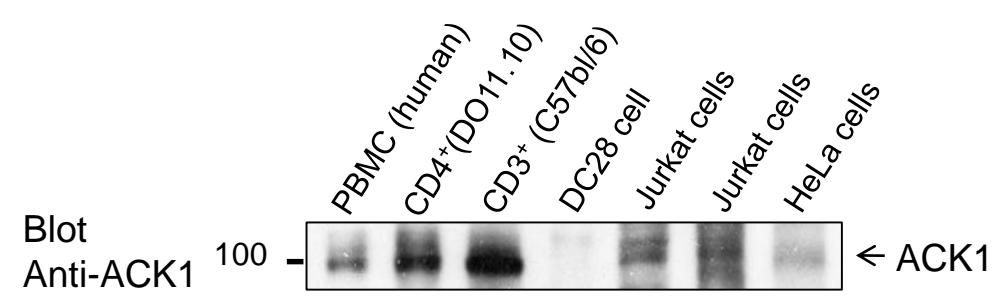
Supplementary Fig S1. ACK1 and ACK2 expression in T-cells (A) Left panel, quantitative RT-PCR measurement of ACK1 expression in CD4⁺ primary T-cells upon anti-CD3 stimulation in primary T-cells. Middle panel, ACK1 (*red*) and ACK2 (*silver*) expression from Immgen array expression database (CD4 T conventional (Tcon), T Regulatory (Treg) and transgenic pancreatic-infiltrating T-cells (BDC)). Right panel, RNA-seq data from expression array (<http://www.th-express.org/#browse>), showing fold change in RNA level as compared to naïve CD4⁺ cells ([40](#)). (B) Western blot showing ACK1 protein expression in various cell types. (C) Sequence alignment of human and mouse ACK1 (Tnk2) proteins. (D) Antibody specificity controls for confocal microscopy experiments shown in figure 3.

Supplementary Figure S1

A



B



C

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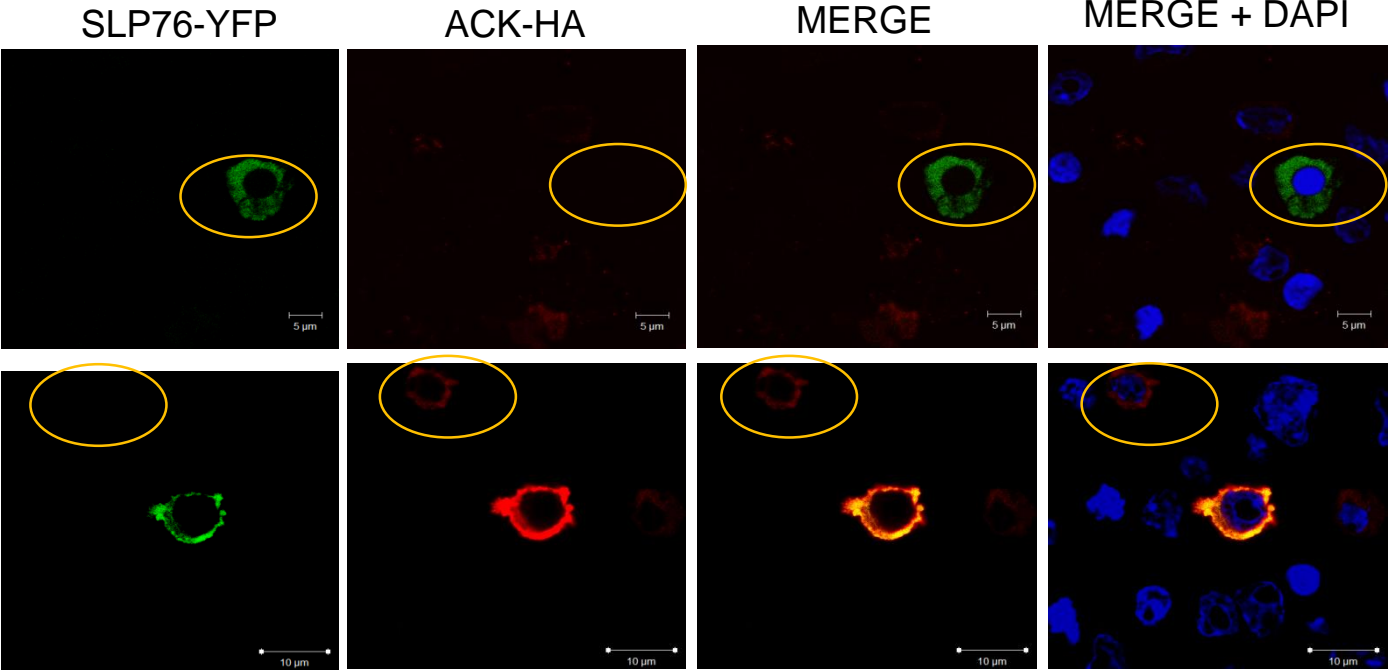
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ACK1_HUMAN (1) MQPEEGTGWLLELLSEVQLQQYFLRLRDDLNITRLSHFEYVKNEDELEKIGMRPGQRRLEAVKRRKALCKRKSWSMSKVFSGKRLEAEFP PHHSQSTFRK
Consensus (1) MQPEEGTGWLLELLSEVQLQQYFLRLRDDLNITRLSHFEYVKNEDELEKIGMRPGQRRLEAVKRRKALCKRKSWSMSKVFSGKRLEAEFP HSQSTFRK
101 200
ACK1_MOUSE (101) PSPTPCSLP GEGT LQSLTCLIGEKDLRLLLEKLDGSGFVRRGEWDAPAGKTVSVAVKLCPDVLVLSQPEAMDDFIREVNHSLDHRNLIRLYGVVLTLP
ACK1_HUMAN (101) TSPAPGPGA GEGP LQSLTCLIGEKDLRLLLEKLDGSGFVRRGEWDAPAGKTVSVAVKLCPDVLVLSQPEAMDDFIREVNHSLDHRNLIRLYGVVLTLP
Consensus (101) SP PG GEG LQSLTCLIGEKDLRLLLEKLDGSGFVRRGEWDAPAGKTVSVAVKLCPDVLVLSQPEAMDDFIREVNHSLDHRNLIRLYGVVLT P
201 300
ACK1_MOUSE (201) MKMVTTELAPLGSLLDRLRKHQGHFLLGTLSTRYAVQVAEGMAYLESKRFIHRDLAARNLLLATRDVLKIGDFGLMRALPQNDHYVMQEHRKVPFAWCAPE
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Consensus (201) MKMVTTELAPLGSLLDRLRKHQGHFLLGTLSTRYAVQVAEGMAYLESKRFIHRDLAARNLLLATRDVLKIGDFGLMRALPQNDHYVMQEHRKVPFAWCAPE
301 400
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Consensus (301) SLKTRTFSHASDTWMFGVTLWEMFTYGQEPWIGLNGSQILHKIDKEGERLPRPEDCPQDIYNVMVQCWAHKPEDRPTFVALRDFLLEAQPTDMRALQDFE
401 500
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ACK1_HUMAN (401) EPDKLHIQMNVDVITVIEGRAENYWWRGQNTRTLTCVGFPRNVVTSVAGLSAQDISQPLQNSFIHTGHGSDSDPRHCWGF PDRIDELYLGNPMDPPDLLSVE
Consensus (401) EPDKLHIQMNVDVITVIEGRAENYWWRGQNTRTLTCVGFPRNVVTSVAGLSAQDISQPLQNSFIHTGHGSDSDPRHCWGF PDRIDELYLGNPMDPPDLLSVE
501 600
ACK1_MOUSE (501) LSTSRPTQHLGRVVKREPPPRPPQPAIFTQKT TYDPVSED P DPLSSDFKRLGLRKPALPRGLWLAKPSARVPGTKADRS SGC EVTLIDFGEEPVPVTPRPC
ACK1_HUMAN (501) LSTSRPQHLGGVKKP----- TYDPVSED Q DPLSSDFKRLGLRKPALPRGLWLAKPSARVPGTKASR SGC EVTLIDFGEEPVPVTPRPC
Consensus (501) LSTSRP QHLG VKK TYDPVSED DPLSSDFKRLGLRKPALPRGLWLAKPSARVPGTKA R SGA EVTLIDFGEEPVPV RPC
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ACK1_MOUSE (601) APSLAQLAMDACSLLDKTPPQSPTRALPRPLHPTPVVDWDARPLPPPPAYDDVAQDEDDFEVCSINSTLVGAGVPAGPSQGTNYAFVPEQAQMPPALED
ACK1_HUMAN (586) APSLAQLAMDACSLLDETPPQSPTRALPRPLHPTPVVDWDARPLPPPPAYDDVAQDEDDFEVCSINSTLVGAGVPAGPSQGTNYAFVPEQARPPPPLED
Consensus (601) APSLAQLAMDACSLLD TPPQSPTRALPRPLHPTPVVDWDARPLPPPPAYDDVAQDEDDFEVCSINSTLVGAGLPAGPSQG TNYAFVPEQA PP LED
701 800
ACK1_MOUSE (701) NLFLLPPQGGGKPPSSVQTAEIFQALQQEEMRQLQVPTGQLT PSP PGGDDKPQVPPRVPIPPRPTRPV LSPAPSGEEETS WPGPASPPRVPPREPLS
ACK1_HUMAN (686) NLFLLPPQGGGKPPSSAQTAEIFQALQQEEMRQLQVPTGQLT PSP PGGDDKPQVPPRVPIPPRPTRPV LSPAPSGEEETS WPGPASPPRVPPREPLS
Consensus (701) NLFLLPPQGGGKPPSS QTAEIFQALQQEEMRQLQ P G PSPSGDDKPQVPPRVPIPPRPTRP V LSPAPSGEEETS WPGPASPPRVPPREPLS
801 900
ACK1_MOUSE (801) PQGSRTPSPLVPPGSSPLPRLSSSPGKTMPTTQSFASDPKYATPQVIQAPGPRAGPCILPIVRDGGKVSSTHYLLPERP YLERYQRFLREAQSPEEP
ACK1_HUMAN (786) PQGSRTPSPLVPPGSSPLPRLSSSPGKTMPTTQSFASDPKYATPQVIQAPGPRAGPCILPIVRDGGKVSSTHYLLPERP YLERYQRFLREAQSPEEP
Consensus (801) PQGSRTPSPLVPPGSSPLP RLSSSPGKTMPTTQSFASDPKYATPQVIQAPGPRAGPCILPIVRDGGKVSSTHYLLPERP YLERYQRFLREAQSPEEP
901 1000
ACK1_MOUSE (901) AALPVEPLLPPPSTPAPAAPTATVRPMPQAAPDPKANFSTNNSNPGARPPSLRAAARLPQRGCPGDGQEAARPADKIQMLQAMVHGVTTTEECQALQSHS
ACK1_HUMAN (886) TPLPVEPLLPPPSTPAPAAPTATVRPMPQAALDPKANFSTNNSNPGARPPPPRAAARLPQRGCPGDGPEACRPADKIQMA--MVHGVTTTEECQALQCHG
Consensus (901) LPVP LLPPPSTPAPAAPTATVRPMPQAA DPKANFSTNNSNPGARPP RA ARLPQRGCPGDG EAARPADKIQM MVHGVTTTEECQALQ H
1001 1055
ACK1_MOUSE (1001) WSVQRAAQYLKVEQLFGLGLRPRVECHKVLEMFDWNLEQAGCHLLGSCGPAHKKR
ACK1_HUMAN (984) WSVQRAAQYLKVEQLFGLGLRPRVECHKVLEMFDWNLEQAGCHLLGSGWCPAHKKR
Consensus (1001) WSVQRAAQYLKVEQLFGLGLRPR ECHKVLEMFDWNLEQAGCHLLGS GPAHKKR

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D

Controls for antibody specificity: single transfected cells

Transfection



Untransfected but stimulated with mouse OKT3 antibody (5 mins) Jurkat cells

