Appendix for

CANCER-ASSOCIATED MUTATIONS IN *VAV1* TRIGGER VARIEGATED SIGNALING OUTPUTS AND T CELL LYMPHOMAGENESIS

by

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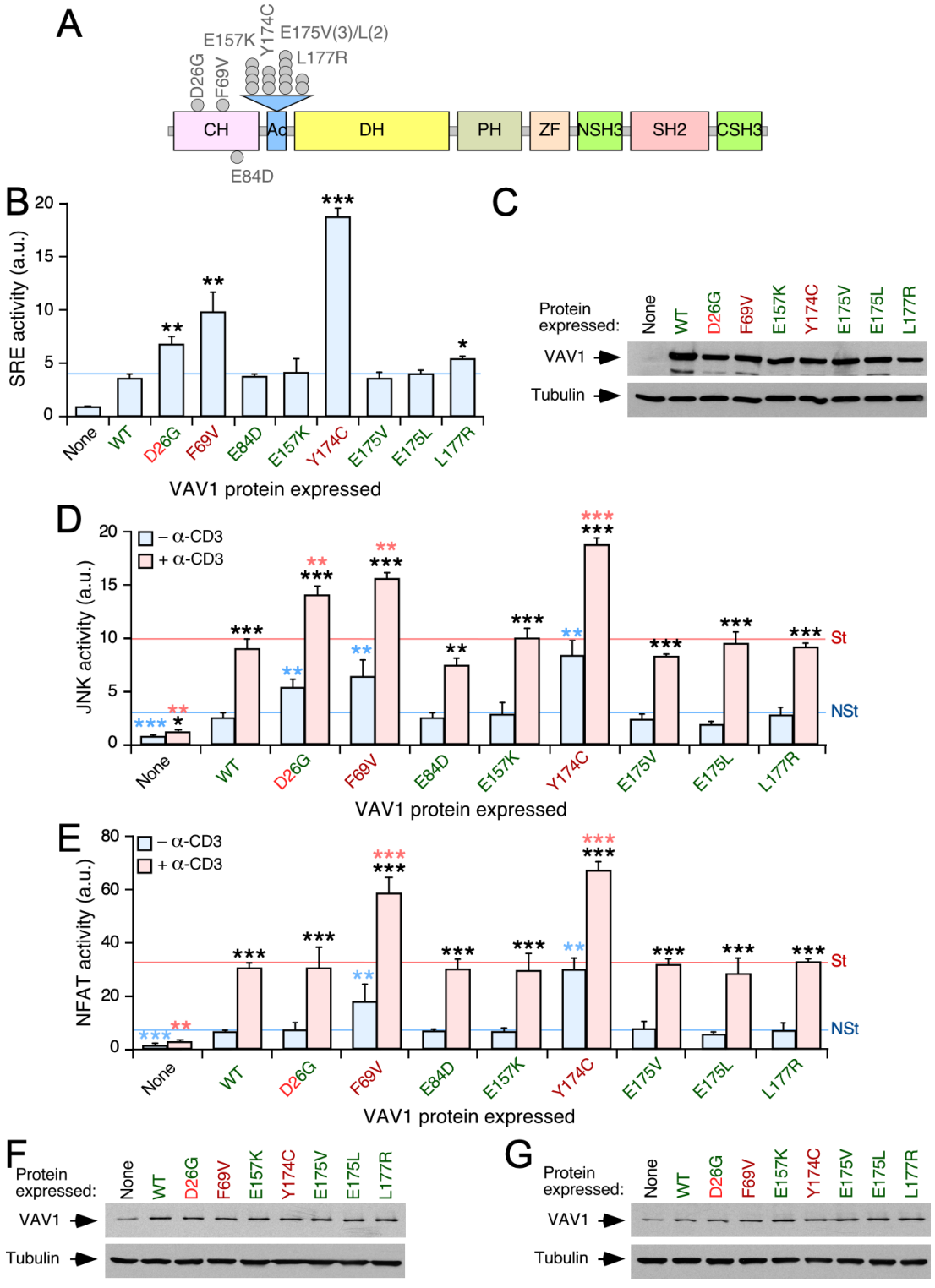
Appendix Figures S1 to S9 and legends

Appendix Table S1



APPENDIX FIGURE S1. Effect of *VAV1* mutations located in the CH and acidic domains in the canonical pathways of the protein

(A) Localization of the *VAV1* missense mutations found in PTCL (top) and NSCLC (bottom) that have been tested in the assays shown in this figure. Each circle depicts one patient (same notation will be used in the rest of figures of this file).

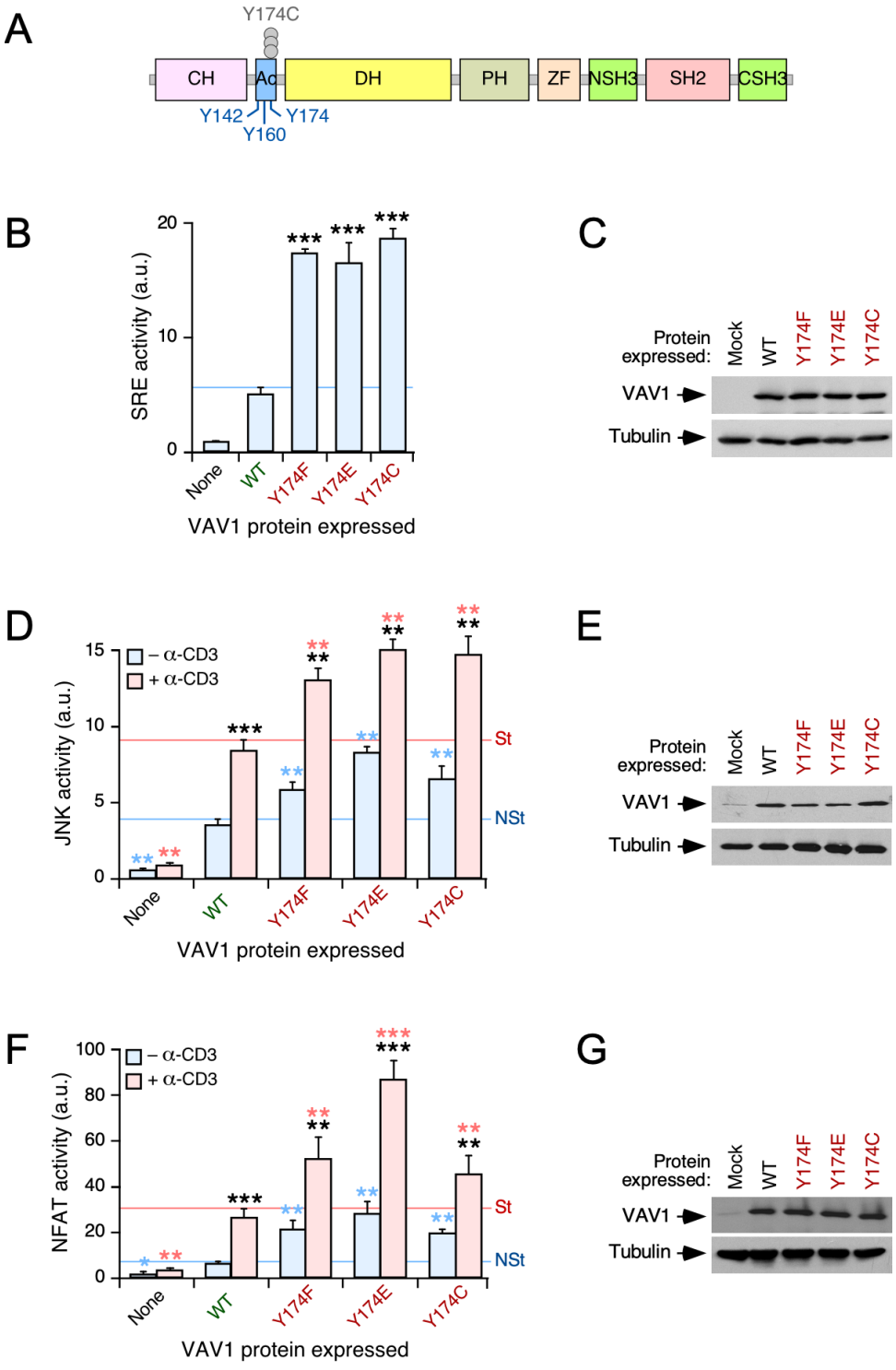
(B) SRE promoter activity of COS cells expressing the indicated Vav1 mutant proteins (bottom). Mutations have been labeled as in **Figure 1C** to indicate the functional subclass (this labelling will be used in the rest of figures shown in this Supplemental Information file). *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ using the Mann–Whitney U test. n = 3 independent experiments, each performed in triplicate.

(C) Representative immunoblot showing the abundance of the indicated VAV1 proteins in the transfected cells used in panel B. Endogenous tubulin α has been used as loading control.

(D and E) Activation of JNK (D) and NFAT (E) by the indicated ectopically expressed Vav1 proteins in Jurkat cells under nonstimulation (blue bars) and anti-CD3-stimulation (red bars) conditions. NSt, nonstimulated; St, stimulated. *P* values are given relative to nonstimulated (blue asterisks) and stimulated (red asterisks) cells expressing $Vav1^{WT}$ in the same cell line. We also included *P* values for the values exhibited by each Vav1 mutant protein relative to those obtained in nonstimulated condition (black asterisks). We have not included statistics in mock-transfected cells (none) for the sake of simplicity. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test). n = 3 independent experiments, each performed in triplicate.

(F and G) Representative immunoblots showing the abundance of the ectopic Vav1 proteins in the transfected cells used in panels D (F) and E (G), respectively. Endogenous tubulin α has been used as loading control in all the cases.

In B, D and E, values are shown as means ± SEM from three independent experiments (each performed in triplicate).



APPENDIX FIGURE S2. Functional impact of the mutations in the regulatory Y[174] VAV1 phosphosite in the canonical pathways of the protein

(A) Localization of the *VAV1* missense mutations found in PTCL that have been tested in the assays shown in this figure. The regulatory phosphosites of Vav1 are indicated at the bottom in blue.

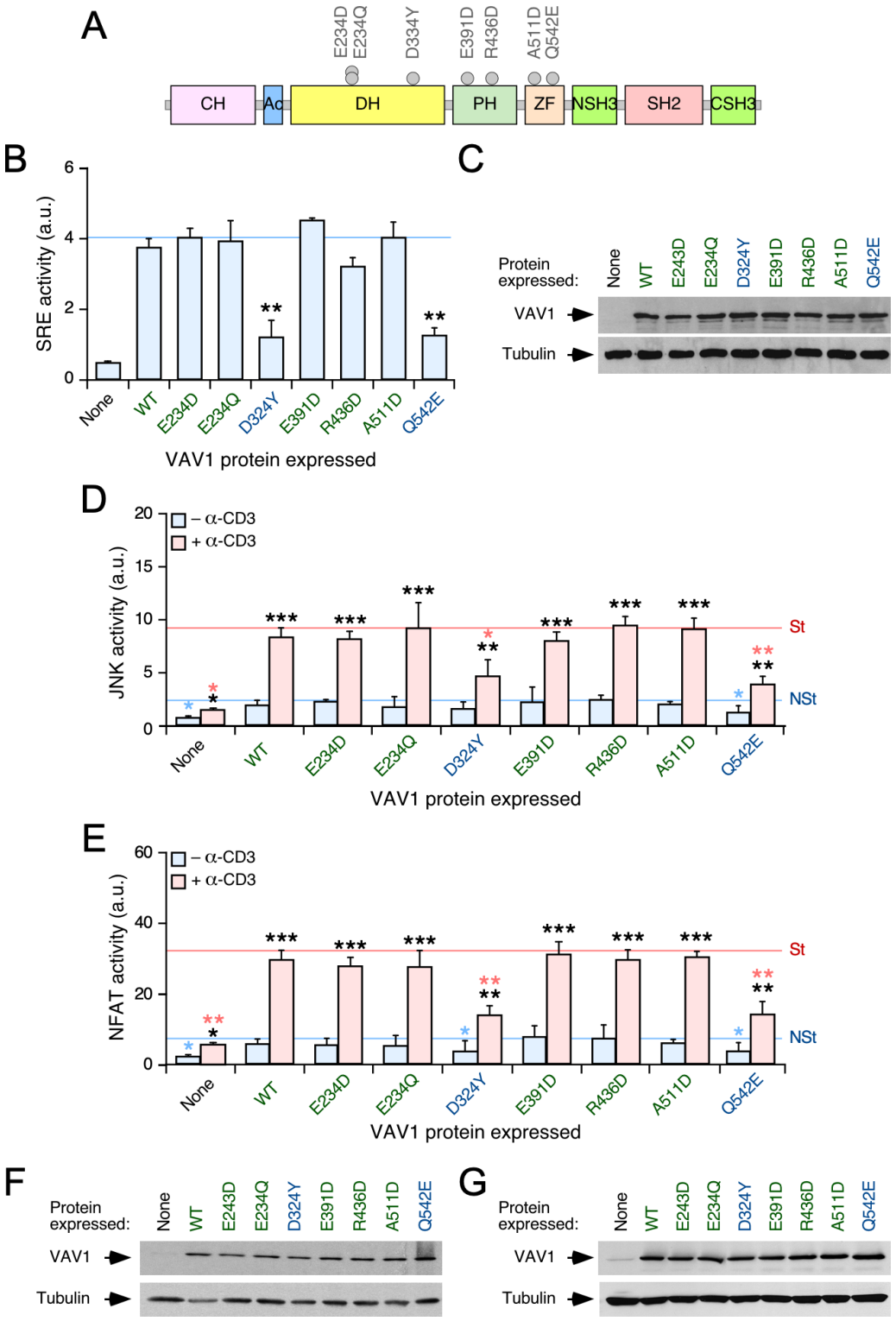
(B) Levels of stimulation of SRE triggered by the indicated VAV1 proteins in COS cells. ***, $P \leq 0.001$ using the Mann-Whitney U test of indicated experimental values compared to $Vav1^{WT}$-expressing cells (n = 3 independent experiments, each performed in triplicate).

(C) Representative immunoblot showing the abundance of the ectopic Vav1 proteins and endogenous tubulin α in the experiment shown in B.

(D and F) Activation of JNK (D) and NFAT (F) by the indicated Vav1 mutants in Jurkat cells under nonstimulation (blue bars) and anti-CD3-stimulation (red bars) conditions. Data and *P* values are depicted as in **Appendix Figures S1D** and **S1E**. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test).

(E and G) Representative immunoblots showing the abundance of the ectopic Vav1 proteins in the transfected cells used in D (E) and F (G), respectively. Endogenous tubulin α has been used as loading control in all the cases.

In B, D and F, values are shown as means ± SEM from three independent experiments (each performed in triplicate).



APPENDIX FIGURE S3. Effect of the lung-tumor detected mutations located in the VAV1 catalytic core (DH-PH-ZF) in the canonical pathways of the protein

(A) Localization of the *VAV1* missense mutations found in NSCLC that have been tested in the assays shown in this figure.

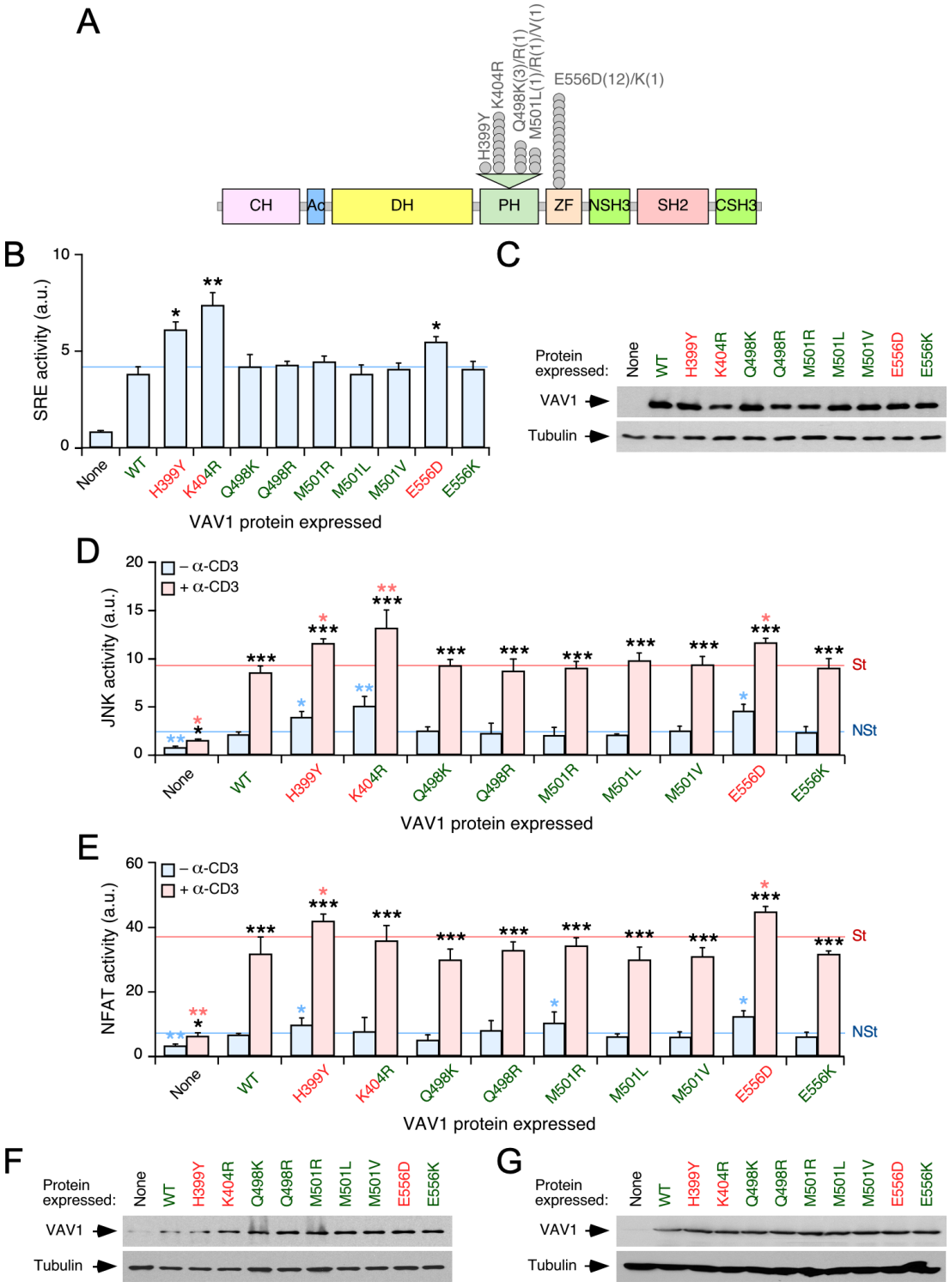
(B) SRE promoter activity of COS cells expressing the indicated Vav1 mutant proteins (bottom). Data and *P* values are depicted as in **Appendix Figure S1B**. **, $P \leq 0.01$. Statistical values were obtained using the Mann-Whitney U test.

(C) Representative immunoblot showing the abundance of the indicated Vav1 proteins in the transfected cells used in B. Endogenous tubulin α has been used as loading control.

(D and **E)** JNK (D) and NFAT (E) activity elicited by the indicated Vav1 proteins in Jurkat cells under nonstimulation (blue bars) and anti-CD3-stimulation (red bars) conditions. Data and *P* values are depicted as in **Appendix Figure S1D** and **S1E** (*n* =3 independent experiments, each performed in triplicate). *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test).

(F and **G)** Representative immunoblots showing the abundance of the ectopic Vav1 proteins and the endogenous tubulin α in the experiments used in D (F) and E (G), respectively.

In B, D and E, values are shown as means ± SEM from three independent experiments (each performed in triplicate).



APPENDIX FIGURE S4. Effect of *VAV1* mutations found in the catalytic core of the protein in the canonical pathways of the protein

(A) Localization of the *VAV1* missense mutations found in PTCL that target the PH and ZF domains of the protein.

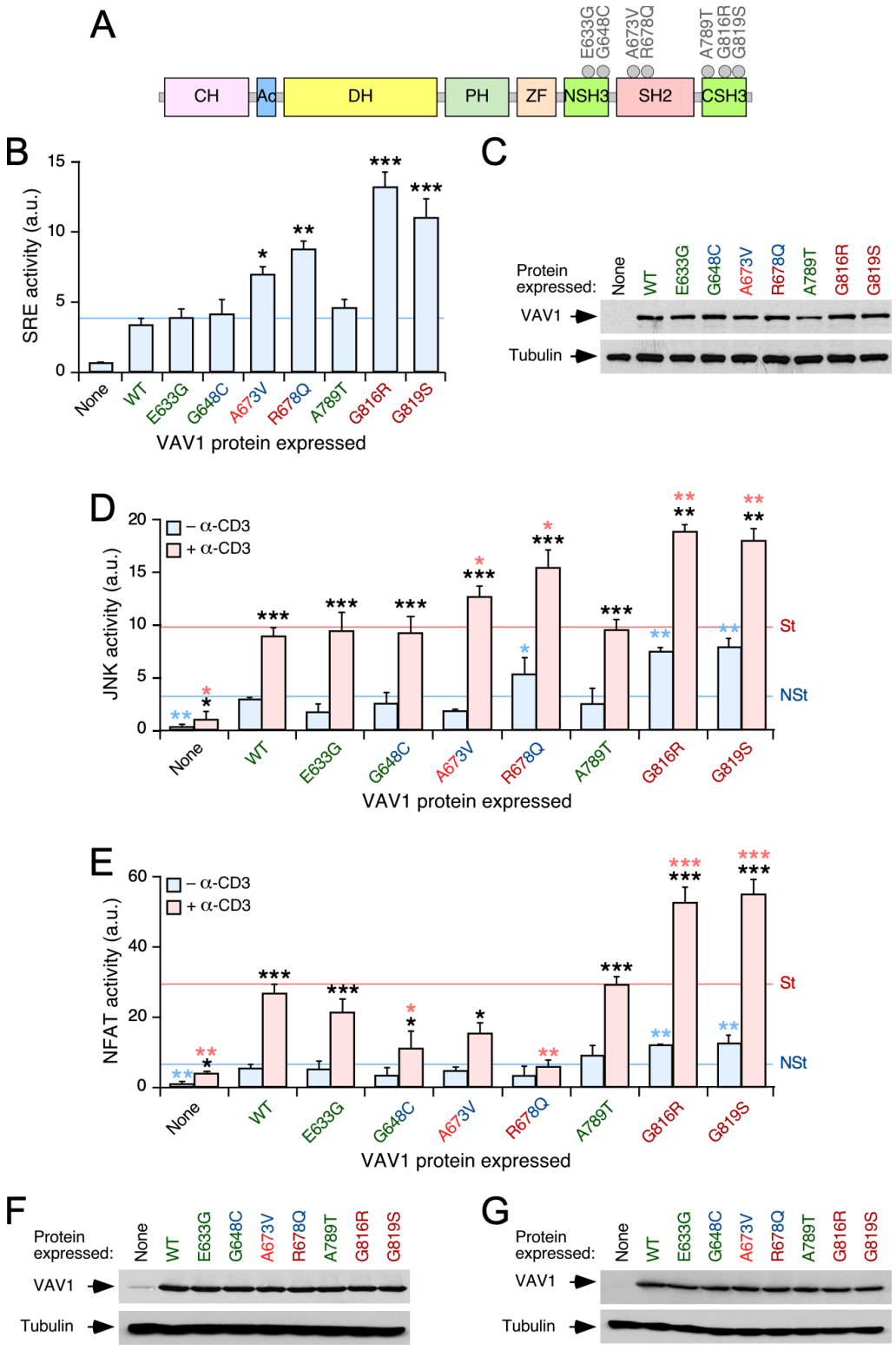
(B) SRE promoter activity of COS cells expressing the indicated VAV1 mutant proteins. Data and *P* values are depicted as in **Appendix Figure S1B**. *, $P \leq 0.05$; **, $P \leq 0.01$ (Mann–Whitney U test).

(C) Representative immunoblot showing the abundance of the indicated Vav1 proteins in the transfected cells used in B. Endogenous tubulin α has been used as loading control.

(D and **E)** JNK (D) and NFAT (E) activity showed by indicated Vav1 proteins in nonstimulated (blue bars) and anti-CD3-stimulated (red bars) Jurkat cells. Data and *P* values are depicted as in Appendix **Figures S1D** and **S1E**. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test).

(F and **G)** Representative immunoblots showing the abundance of the ectopic Vav1 proteins in the transfected cells used in D (F) and E (G), respectively. Endogenous tubulin α has been used as loading control in all the cases.

In B, D and E, values are shown as means ± SEM from three independent experiments (each performed in triplicate).



APPENDIX FIGURE S5. Functional impact of *VAV1* gene mutations located in the NSH3, SH2 and CSH3 in the canonical activities of the protein

(A) Localization of the mutations targeting the VAV1 SH3-SH2-SH3 cassette found in lung tumor patients.

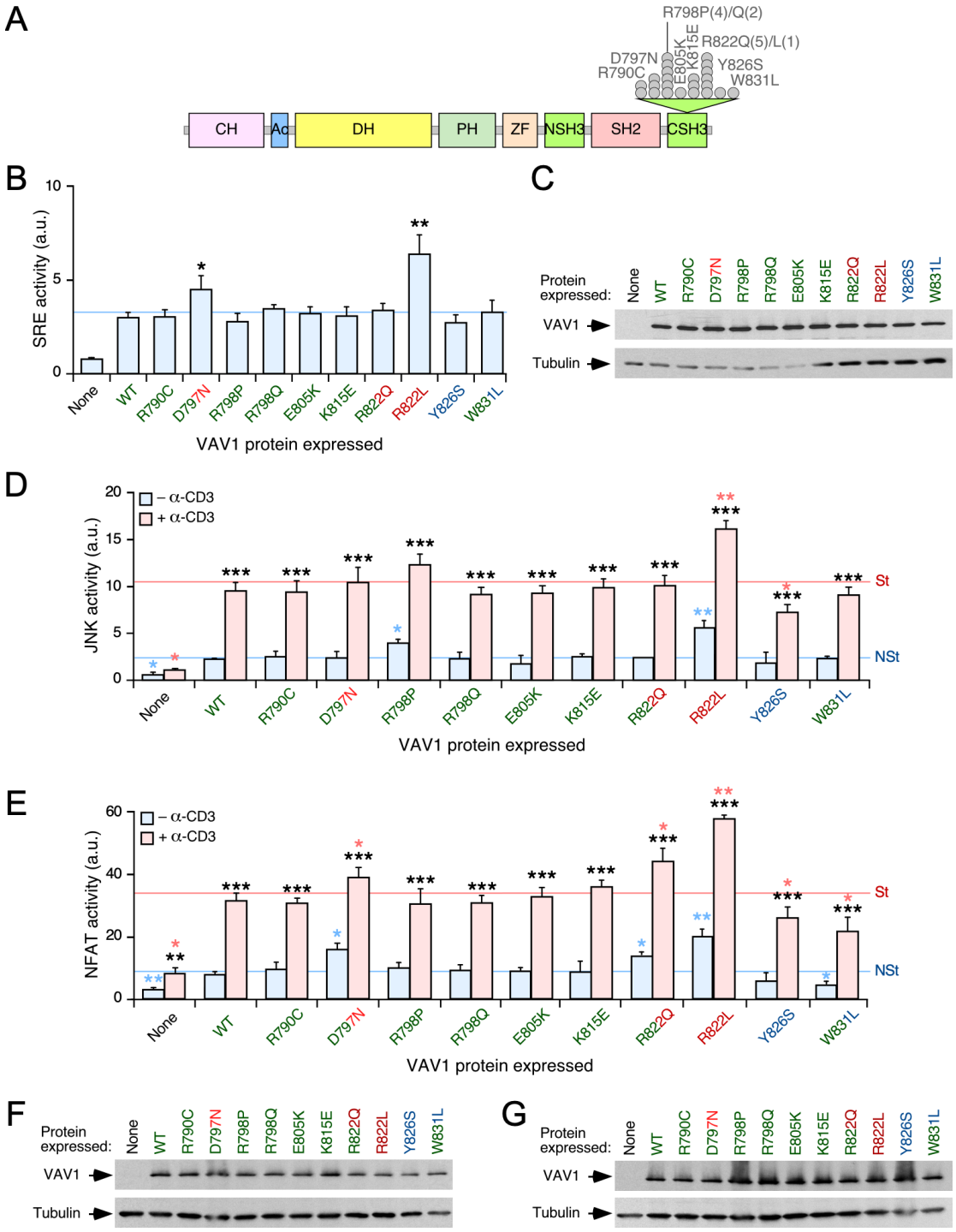
(B) SRE promoter activity elicited by Vav1 mutant proteins in COS cells. Data and *P* values are depicted as in **Appendix Figure S1B**. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test).

(C) Representative immunoblot showing the abundance of the indicated Vav1 proteins in the transfected cells used in B. Endogenous tubulin α has been used as loading control.

(D and E) Activation of JNK (D) and NFAT (E) by the indicated Vav1 proteins in Jurkat cells under nonstimulation (blue bars) and anti-CD3-stimulation (red bars) conditions. Data and *P* values are depicted as in **Appendix Figures S1D** and **S1E**. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test).

(F and G) Representative immunoblots showing the abundance of the ectopic Vav1 proteins in the transfected cells used in D (F) and E (G), respectively. Endogenous tubulin α has been used as loading control.

In B, D and E, values are shown as means ± SEM from three independent experiments (each performed in triplicate).



APPENDIX FIGURE S6. Functional characterization of *VAV1* mutations found in the CSH3 domain in PTCL patients

(A) Localization of the mutations.

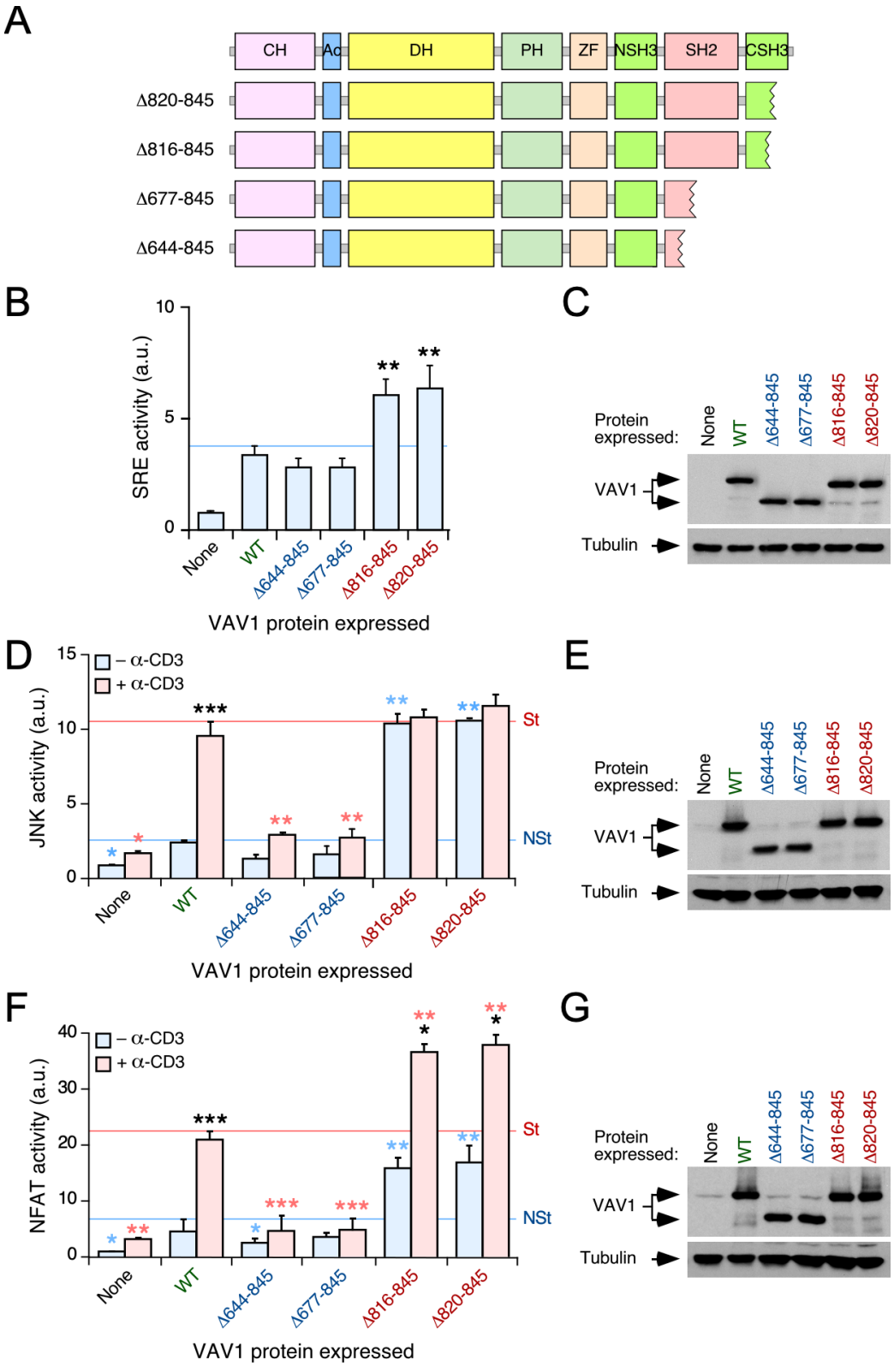
(B) SRE promoter activity of COS cells expressing the indicated Vav1 mutant proteins. *, $P \leq 0.05$; **, $P \leq 0.01$ (Mann-Whitney U test).

(C) Representative immunoblot showing the abundance of the indicated Vav1 proteins in the transfected cells used in B. Endogenous tubulin α has been used as loading control.

(D and **E)** Activation of JNK (D) and NFAT (F) by the indicated Vav1 proteins in nonstimulated (blue bars) and anti-CD3-stimulated (red bars) Jurkat cells. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test).

(F and **G)** Representative immunoblots showing the abundance of the ectopic Vav1 proteins and endogenous tubulin α (loading control) in the transfected cells used in D (F) and E (G), respectively.

In B, D and E, values are shown as means ± SEM from three independent experiments (each performed in triplicate).



APPENDIX FIGURE S7. Effect of *VAV1* gene truncations in the canonical pathways of the protein

(A) Depiction of the inframe stop mutations identified in the C-terminal domains of VAV1 in NSCLC.

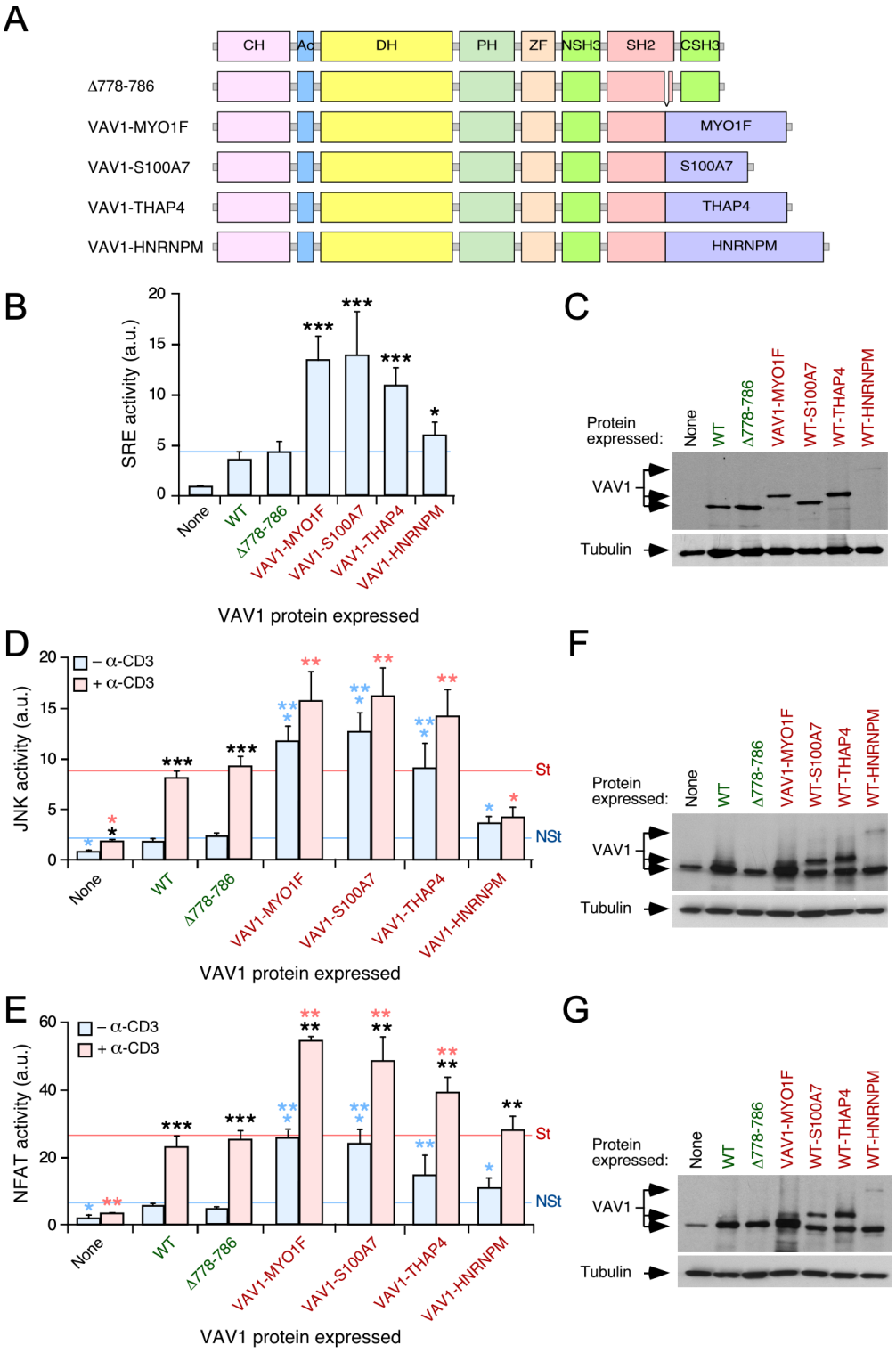
(B) SRE promoter activity triggered by VAV1 mutant proteins in COS cells. *, $P \leq 0.05$; **, $P \leq 0.01$ (Mann-Whitney U test).

(C) Representative immunoblot showing the abundance of the indicated Vav1 proteins and endogenous tubulin α in the transfected cells used in B.

(D and F) Activation of JNK (D) and NFAT (E) by the indicated Vav1 proteins in Jurkat cells under nonstimulation (blue bars) and anti-CD3-stimulation (red bars) conditions. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test).

(E and G) Representative immunoblots showing the abundance of the ectopic Vav1 proteins in the transfected cells used in D (E) and F (G), respectively. Endogenous tubulin α has been used as loading control.

In B, D and F, values are shown as means ± SEM from three independent experiments (each performed in triplicate).



APPENDIX FIGURE S8. Functional impact of internal focal deletions and *VAV1* fusion genes found in PTCLs

(A) Depiction of the Vav1 mutant proteins used in these experiments.

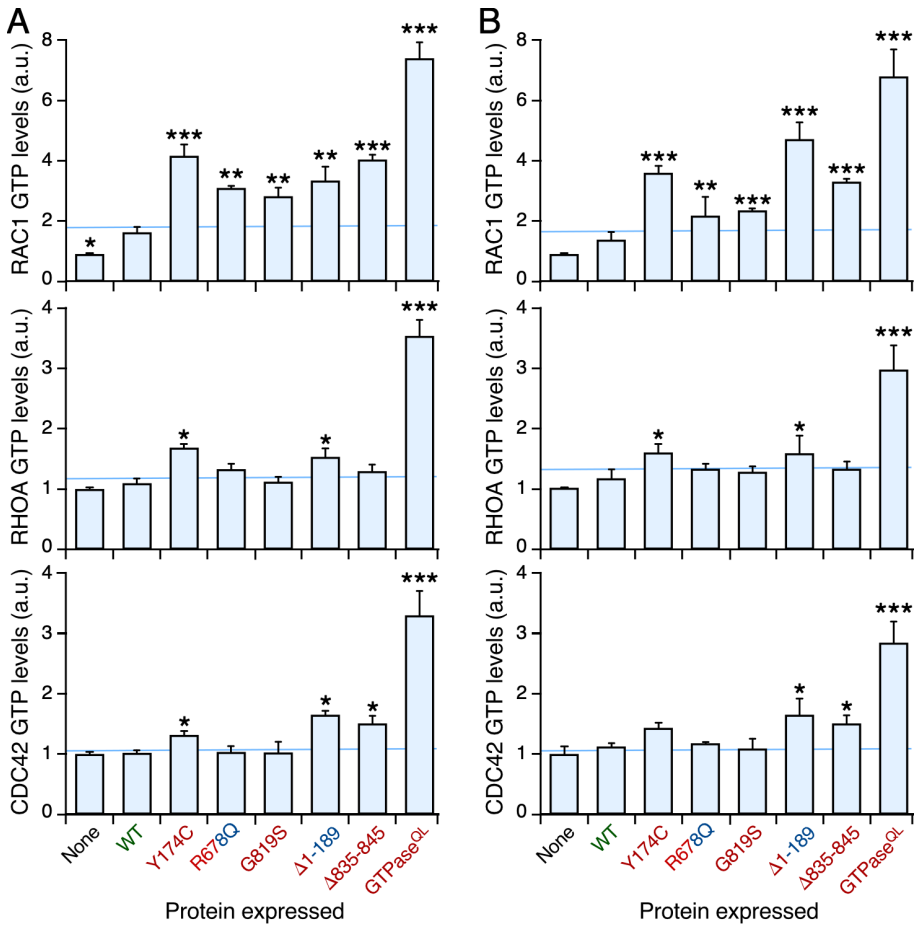
(B) Levels of stimulation of SRE elicited by the indicated Vav1 proteins in COS cells. *, $P \leq 0.05$, ***, $P \leq 0.001$ (Mann-Whitney U test).

(C) Representative immunoblot showing the abundance of the ectopic Vav1 proteins and endogenous tubulin α in the experiment shown in B.

(D and E) Activation of JNK (D) and NFAT (F) by ectopically expressed Vav1 proteins in Jurkat cells under nonstimulation (blue bars) and anti-CD3-stimulation (red bars) conditions. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Mann-Whitney U test).

(F and G) Representative immunoblots showing the abundance of the ectopic Vav1 proteins in the transfected cells used in D (F) and E (G), respectively. Endogenous tubulin α has been used as loading control in all the cases.

In B, D and E, values are shown as means ± SEM from three independent experiments (each performed in triplicate).



APPENDIX FIGURE S9. Functional impact of indicated proteins in the activation of RHO family proteins

(A and B) Levels of GTP-bound RAC1 (A,B; upper panels), RHOA (A,B; middle panels) and CDC42 (A,B; bottom panels) in COS1 (A) and Jurkat (B) cells transiently transfected with the indicated proteins (bottom). Data are shown as means ± SEM from three independent experiments (each performed in duplicate). As positive control, we used the active versions ($GTPase^{QL}$) of the indicated GTPases as well as the $Vav1^{\Delta 1\text{-}189}$ protein. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ using the Mann-Whitney U test.

APPENDIX TABLE S1. List of primers used in this study. Fw, forward; Rv, reverse

Mouse *Vav1* (NM_011691)	
Primer	**Sequence**
E26G_Fw	5'-AGCCACCGTGTGACCTGGGGGGGGGCCCAGGTGTGT-3'
E26G_Rv	5'-ACACACCTGGGCCCCCCCCCAGGTCACACGGTGGCT-3'
F69V_Fw	5'-CGGCCCCAGATGTCCCAGGTCCTTTGTCTTAAGAACATT-3'
F69V_Rv	5'-AATGTTCTTAAGATAAAGGACCTGGGACATCTGGGGCCG-3'
E84D_Fw	5'-ACCTTCCTGTCTACTTGCTGTGACAAGTTCGGCCTCAAGCGCAGT-3'
E84D_Rv	5'-ACTGCGCTTGAGGCCGAACTTGTCACAGCAAGTAGACAGGAAGGT-3'
E157K_Fw	5'-GACACCGCAGAGGAAGACAAGGACCTTTATGACTGCGTG-3'
E157K_Rv	5'-CACGCAGTCATAAAGGTCCTTGTCTTCCTCTGCGGTGTC-3'
Y174C_Fw	5'-GAGGCAGAGGGGGACGAGATCTGCGAGGACCTAATGCGCTTGGAG-3'
Y174C_Rv	5'-CTCCAAGCGCATTAGGTCCTCGCAGATCTCGTCCCCCTCTGCCTC-3'
E175V_Fw	5'-GCAGAGGGGGACGAGATCTACGTGGACCTAATGCGCTTGGAGTCG-3'
E175V_Rv	5'-CGACTCCAAGCGCATTAGGTCCACGTAGATCTCGTCCCCCTCTGC-3'
E175L_Fw	5'-GCAGAGGGGGACGAGATCTACTTGGACCTAATGCGCTTGGAGTCG-3'
E175L_Rv	5'-CGACTCCAAGCGCATTAGGTCCAAGTAGATCTCGTCCCCCTCTGC-3'
L177R_Fw	5'-GACGAGATCTACGAGGACCGAATGCGCTTGGAGTCGGTG-3'
L177R_Rv	5'-CACCGACTCCAAGCGCATTCGGTCCTCGTAGATCTCGTC-3'
E201A_Fw	5'-CGCTGCTGCTGCCTGCGGGCGATCCAGCAGACGGAGGAG-3'
E201A_Rv	5'-CTCCTCCGTCTGCTGGATCGCCCGCAGGCAGCAGCAGCG-3'
E234D_Fw	5'-GAGACCATCTTTGTCAACATTGACGAGCTGTTCTCTGTGCATACC-3'
E234D_Rv	5'-GGTATGCACAGAGAACAGCTCGTCAATGTTGACAAAGATGGTCTC-3'
E234Q_Fw	5'-GAGACCATCTTTGTCAACATTCAGGAGCTGTTCTCTGTGCATACC-3'
E234Q_Rv	5'-GGTATGCACAGAGAACAGCTCCTGAATGTTGACAAAGATGGTCTC-3'
D324Y_Fw	5'-GGCCGATTCACCCTACGGTATCTGCTGATGGTACCTATG-3'
D324Y_Rv	5'-CATAGGTACCATGCAGATACCGTAGGGTGAATCGGCC-3'
H337Y_Fw	5'-ATGCAGCGGGTGCTGAAGTACTACCTCCTTCTCCAGGAGCTAGTG-3'
H337Y_Rv	5'-CACTAGCTGGAGAAGGAGGTAGTACTTCAGCACCCGCTGCAT-3'
E391D_Fw	5'-AACTTTCAGCTGTCCATTGACAACCTGGACCAGTCTCTG-3'
E391D_Rv	5'-CAGAGACTCGGTCCAGGTTGTCAATGGACAGCTGAAAGTT-3'
N399Y_Fw	5'-CTGGACCAGTCTCTGGCTTACTATGGCCGGCCCAAGATT-3'
N399Y_Rv	5'-AATCTTGGGCCGGCCATAGTAAGCCAGAGACTGGTCCAG-3'
K404R_Fw	5'-GCTAACTATGGCCGGCCCAGGATTGACGGTGAGCTCAAG-3'
K404R_Rv	5'-CTTGAGCTCACCGTCAATCCTGGGCCGGCCATAGTTAGC-3'
R436D_Fw	5'-TCACTGCTCATCTGTAAAAGCCGCGGGGACTCTTACGAC-3'
R436D_Rv	5'-GTCGTAAGAGTCCCCGCGGCTTTTACAGATGAGCAGTGA-3'
Q498K_Fw	5'-CTGAAGAAGAAGTGGATGGAAAAGTTCGAAATGGCCATCTCCAAC-3'
Q498K_Rv	5'-GTTGGAGATGGCCATTTCGAACTTTTCCATCCACTTCTTCTTCAG-3'
Q498R_Fw	5'-CTGAAGAAGAAGTGGATGGAACGGTTCGAAATGGCCATCTCCAAC-3'
Q498R_Rv	5'-GTTGGAGATGGCCATTTCGAACCGTTCCATCCACTTCTTCTTCAG-3'
M501R_Fw	5'-GTGGATGGAACAGTTCGAACGGGCCATCTCCAACATTTACC-3'
M501R_Rv	5'-GGTAAATGTTGGAGATGGCCCGTTCGAACTGTTCCATCCAC-3'
M501L_Fw	5'-GTGGATGGAACAGTTCGAATTGGCCATCTCCAACATTTACC-3'
M501L_Rv	5'-GGTAAATGTTGGAGATGGCCAATTCGAACTGTTCCATCCAC-3'
M501V_Fw	5'-GTGGATGGAACAGTTCGAAGTGGCCATCTCCAACATTTACC-3'
M501V_Rv	5'-GGTAAATGTTGGAGATGGCCACTTCGAACTGTTCCATCCAC-3'
A511D_Fw	5'-TACATTTACCCAGAGAATGAAACAGCCAATGGGCATGAT-3'
A511D_Rv	5'-ATCATGCCCATTGGCTGTTTCATTCTCTGGGTAAATGTA-3'
Q542E_Fw	5'-CTCAGAGGCACATTCTACGAGGGATATCGCTGTTACAGG-3'
Q542E_Rv	5'-CCTGTAACAGCGATATCCCTCGTAGAATGTGCCTCTGAG-3'

E556D_Fw	5’-GCCGGGCACCTGCACACAAGGATTGTCTGGGGAGAGTGCCTCC-3’
Mouse *Vav1* (NM_011691)	
E556D_Rv	5’-GGAGGCACTCTCCCCAGACAATCCTTGTGTGCAGGTGCCCGGC-3’
E556K_Fw	5’-GCCGGGCACCTGCACACAAGAAGTGTCTGGGGAGAGTGCCTCC-3’
E556K_Rv	5’-GGAGGCACTCTCCCCAGACACTTCTTGTGTGCAGGTGCCCGGC-3’
E633G_Fw	5’-GAGCTCACTAAGGCAGAGGCTGGGCACAACTGGTGGGAGGGAAGG-3’
E633G_Rv	5’-CCTTCCCTCCCACCAGTTGTGCCCAGCCTCTGCCTTAGTGAGCTC-3’
G648C_Fw	5’-GCTACAAATGAAGTCTGCTGGTTTCCCTGTAAC-3’
G648C_Rv	5’-GTTACAGGGAAACCAGCAGACTTCATTTGTAGC-3’
A673V_Fw	5’-CAGAGGGTGAAAGATACAGTGGAGTTCGCCATCAGCATT-3’
A673V_Rv	5’-AATGCTGATGGCGAACTCCACTGTATCTTTCACCCTCTG-3’
R678Q_Fw	5’-TATGCGGGCCCTATGGAACAAGCAGGCGCTGAGGGCATC-3’
R678Q_Rv	5’-GATGCCCTCAGCGCCTGCTTGTTCCATAGGGCCCGCATA-3’
A789T_Fw	5’-TATTTTGGCACTGCCAAAACCCGCTACGACTTCTGTGCC-3’
A789T_Rv	5’-GGCACAGAAGTCGTAGCGGGTTTTGGCAGTGCCAAAATA-3’
R790C_Fw	5’-GGCACTGCCAAAGCCTGCTACGACTTCTGTGCC-3’
R790C_Rv	5’-GGCACAGAAGTCGTAGCAGGCTTTGGCAGTGCC-3’
D797N_Fw	5’-CGCTACGACTTCTGTGCCCGGGAAACGTCGGAACTGTCCCTTAAG-3’
D797N_Rv	5’-CTTAAGGGACAGTTCCGACGTTTCCCGGGCACAGAAGTCGTAGCG-3’
R798P_Fw	5’-GACTTCTGTGCCCGGGACCCGTCGGAACTGTCCCTTAAG-3’
R798P_Rv	5’-CTTAAGGGACAGTTCCGACGGGTCCCGGGCACAGAAGTC-3’
R798Q_Fw	5’-GACTTCTGTGCCCGGGACCAGTCGGAACTGTCCCTTAAG-3’
R798Q_Rv	5’-CTTAAGGGACAGTTCCGACTGGTCCCGGGCACAGAAGTC-3’
E805K_Fw	5’-AGGTCGGAACTGTCCCTTAAGAAGGGTGATATCATCAAGATCCTC-3’
E805K_Rv	5’-GAGGATCTTGATGATATCACCCTTCTTAAGGGACAGTTCCGACCT-3’
K815E_Fw	5’-CATCAAGATCCTCAATAAGGAGGGACAGCAAGGCTGGTGGC-3’
K815E_Rv	5’-GCCACCAGCCTTGCTGTCCCTCCTTATTGAGGATCTTGATG-3’
G816R_Fw	5’-TGGCGTGGGGAGATCTACTGACGGATCGGCTGGTTCCCT-3’
G816R_Rv	5’-AGGGAACCAGCCGATCCGTCAGTAGATCTCCCCACGCCA-3’
G819S_Fw	5’-GAGATCTACGGCCGGATCAGCTGGTTCCCTTCTAACTAT-3’
G819S_Rv	5’-ATAGTTAGAAGGGAACCAGCTATCCGGCCGTAGATCT-3’
R822Q_Fw	5’-GGACAGCAAGGCTGGTGGCAAGGGGAGATCTACGGCCGG-3’
R822Q_Rv	5’-CCGGCCGTAGATCTCCCCTTGCCACCAGCCTTGCTGTCC-3’
R822L_Fw	5’-GGACAGCAAGGCTGGTGGCTTGGGGAGATCTACGGCCGG-3’
R822L_Rv	5’-CCGGCCGTAGATCTCCCCAAGCCACCAGCCTTGCTGTCC-3’
Y826S_Fw	5’-GGCTGGTGGCGTGGGGAGATCTCCGGCCGGATCGGCTGGTTCCCT-3’
Y826S_Rv	5’-AGGGAACCAGCCGATCCGGCCGGAGATCTCCCCACGCCACCAGCC-3’
W831L_Fw	5’-GAGATCTACGGCCGGATCGGCTTGTTCCCTTCTAACTATGTGGAG-3’
W831L_Rv	5’-CTCCACATAGTTAGAAGGGAACAAGCCGATCCGGCCGTAGATCTCT-3’
Δ644_Fw	5’-GAGGGAAGGAATACTGCTTAAAATGAAGTCGGCTGGTTT-3’
Δ644_Rv	5’-AAACCAGCCGACTTCATTTTAAGCAGTATTCCTTCCCTC-3’
Δ677_Fw	5’-TGGTATGCGGGCCCTATGTAACGAGCAGGCGCTGAGGGC-3’
Δ677_Rv	5’-GCCCTCAGCGCCTGCTCGTTACATAGGGCCCGCATACCA-3’
Δ816_Fw	5’-TGGCGTGGGGAGATCTACTGACGGATCGGCTGGTTCCCT-3’
Δ816_Rv	5’-AGGGAACCAGCCGATTCAGCCGTAGATCTCCCCACGCCA-3’
Δ820_Fw	5’-ATCTACGGCCGGATCGGCTGATTCCCTTCTAACTATGTG-3’
Δ820_Rv	5’-CACATAGTTAGAAGGGAATCAGCCGATCCGGCCGTAGAT-3’

Human *VAV1 (NM_005428)*	
Primer	**Sequence**
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D26G_Rv	5’-ACAACACCTGAGCCCCCCCCCAGGTCACGCGGTGGCT-3’
H399Y_Fw	5’-CTGGACCAGTCTCTGGCTTACTATGGCCGGCCCAAGATC-3’

H399Y_Rv	5’-GATCTTGGGCCGGCCATAGTAAGCCAGAGACTGGTCCAG-3’