

Identification of cancer-associated missense mutations in *hace1* that impair cell growth control and Rac1 ubiquitylation

Author list

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Supplementary information

Supplementary Figure S1: Control of HA-HACE1 mutant expression

(a) Expression of HA-tagged HACE1 WT and mutants in MCF-12A cell lines was controlled by immunoblots anti-HA (IB: HA). (b) Impact of expression of HA-HACE1 WT and M114V, G175S, G175C and A204T mutants on Rac protein level in MCF-12A cell lines. Proteins were detected by immunoblotting anti-HA and anti-Rac. Graph shows quantification of Rac signals normalized to GAPDH and expressed as fold changes to vector. (c) Expression of HA-tagged HACE1 WT and mutants in MCF7 cell lines was controlled by immunoblots anti-HA. (a-c) Immunoblot anti-GAPDH on total cell lysates was performed as loading control.

Supplementary Figure S2: Efficiency of Rac1 ubiquitylation by HACE1 G175N

Immunoblots showing levels of ubiquitylation of Rac1 Q61L catalyzed by HACE1 WT, G175S and G175N mutants. Precipitated His₆-tagged ubiquitylated proteins (HisP) were immunoblotted anti-HA to reveal Rac1 Q61L ubiquitylation profiles. Immunoblots anti-HA and anti-myc on total cell lysates show protein expression and anti-actin is used as loading control. Each value corresponds to mean of three independent measurements analyzed by unpaired t-test with * $p \leq 0.05$ or non-significant (ns).

Supplementary Figure S3: Ability of HACE1 V140A to control cell proliferation

(a) Graphs show kinetics of cell proliferation. MCF-12A cell lines expressing HA-HACE1 wild-type or V140A. Each value corresponds to mean values of two independent experiments with technical quadruplicate analyzed by one-way ANOVA Dunnett's multiple comparison test with *** $p \leq 0.0003$, * $p \leq 0.015$ or non-significant (ns).

(b) Immunoblot anti-HA show HACE1 WT and V140A expression.

Supplementary Figure S4: Rac1 association with HACE1 mutants

Association of myc-Rac1 Q61L with HA-tagged HACE1 wild-type (WT) and mutants was assessed by immunoprecipitation anti-myc (IP: myc) followed by immunoblots anti-HA (IB: HA) in HEK293 cells. Immunoblot anti-myc (IB: myc) shows levels of immunoprecipitated Rac1. Immunoblots on total lysates (Total) show expression of constructs.

Supplementary Figure S5: Binding of HACE1 domains to active Rac1

Association of HACE1 domains described in the panel with either Flag-Rac1 Q61L (active) or Flag-Rac1 T17N (inactive) assessed by immunoprecipitation anti-Flag (IP: Flag) followed by immunoblots anti-HA (IB: HA). Cells were co-transfected with expression vectors of Flag-Rac1 Q61L or T17N and various HA-tagged HACE1 constructs. Immunoblots anti-Flag (IB: Flag) show levels of immunoprecipitated Rac1. Immunoblots on total lysate show expression of constructs.

Supplementary Figure S6: Cancer-associated mutations in ANK domain

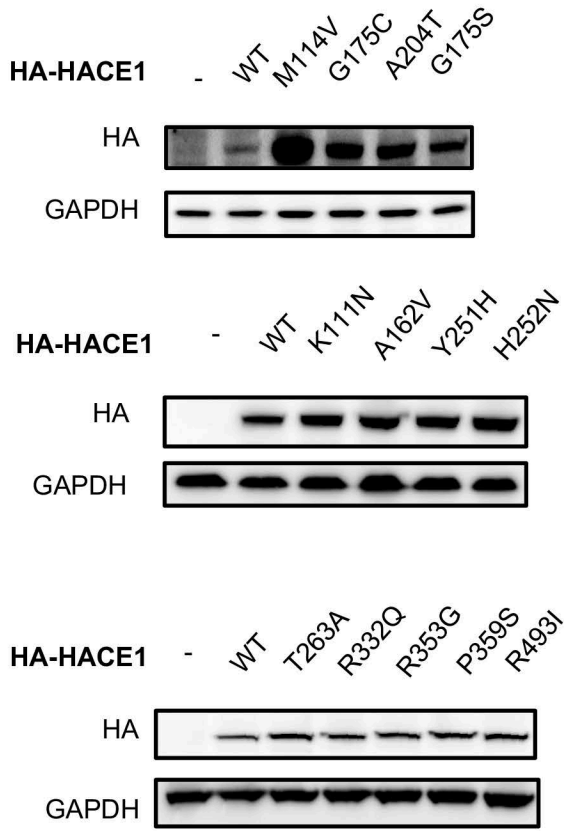
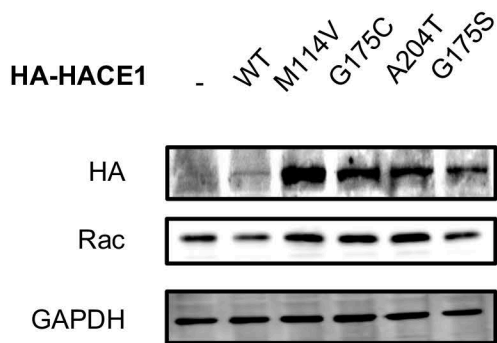
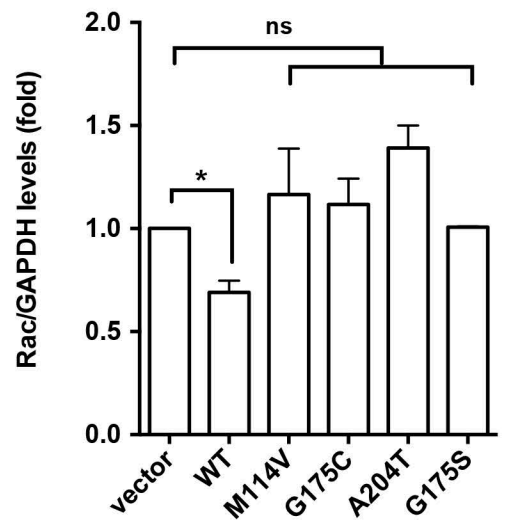
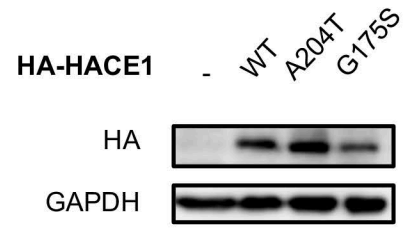
Histogram of repartition of cancer-associated missense mutations in each ankyrin repeat 1 to 7. Analysis conducted on data sets from the COSMIC version 79.

Supplementary Table 1: Cancer-associated somatic mutations in HACE1 1-545

Mutation (Amino acid)	Mutation (DNA)	Domain of HACE1	Primary tissue/ Histopathological type of cancer	References
K111N	G333T	ANK	Large intestine/carcinoma	19
M114V	A340G	ANK	Ovary/carcinoma	19,28
A162V	C485T	ANK	Ovary/carcinoma	19,28
G175C	G523T	ANK	Lung/carcinoma	19
G175S	G523A	ANK	Skin/carcinoma	19,29
A204T	G610A	ANK	Large intestine/carcinoma	19
Y251H	T751C	ANK	Large intestine/carcinoma	19
H252N	C754A	ANK	Ovary/carcinoma	19,28
T263A	A787G	MID	Large intestine/carcinoma	19,30
R332Q	G995A	MID	Large intestine/carcinoma	19,31
R353G	A1057G	MID	Large intestine/carcinoma	19,30
P359S	C1075T	MID	Pancreas/carcinoma	19,32
R493I	G1478T	MID	Large intestine/carcinoma	19,30

Supplementary Table 2: Sequence of the primers used to generate HACE1 mutants

Mutation Amino acid	Mutation DNA	Forward primers (5'-3' sequence)	Reverse primers (5'-3' sequence)
HACE K111N	G333T	tggcagcaagaaatgggcaga ataaatgtatgagtaaattattaga	tctaataattactcatacatttattctgcc catttctgtctgcca
HACE1 M114V	A340G	ctaataattactcacacatttctct gccatttctgtctgcc	ggcagcaagaaatgggcagaagaa atgtgtgagtaaattattag
HACE1 V140A	T419C	acattggctggctgcaaatgggc ggacag	catgttgctgccagaacaatcacaa gacgacagtga
HACE1 V140L	G418T	acagcaatacattggctggctttg aatgggcgga	tccgccattcaaagccagccaatgt attgctgt
HACE1 A162V	C485T	gttgatgttgaggatgtcatgggg cagacagca	tgctgtctgccccatgacatcctcaaca tcaac
HACE1 Q173A	C517G, A518C	cgcttctgtaccggtcgcgaggc aacatgcag	tgcattgtcctgcggaacggtcaca agacg
HACE1 N174A	A520G, A521C	tgctgtctgtgaccggcctggca ggcaacatgc	gcatgttgctgccaggccggtcaca agacgaca
HACE1 G175C	G523T	gttgctgccagaactgtcacaa gacgacag	ctgtctgtgtgacagttctggcaggc aac
HACE1 G175N	G522A, G523A	tgactgtctgtgattgttctgg caggcaacatg	catgttgctgccagaacaatcacaa gacgacagtga
HACE1 G175S	G523A	ctgtctgtgtgactgttctggcag gcaac	gttgctgccagaacagtcacaagac gacag
HACE1 A204T	G610A	caggagcaactccattgtacttta cttgacatcatgg	ccatgactgcaagtaagtaaatgg agttgctcctg
HACE1 Y251H	T751C	aagcctcgggtgatgtgaattaat acttcacaagtctctc	gagagactgtgaagtattaattcaac atcaccggaggctt
HACE1 H252N	C754A	gaaaaagcctcgggtatattgaa taatacttcacaagtctctc	gagagactgtgaagtattaattcaata taaccggaggcttttc
HACE1 T263A	G787A	ttcagactattattcaaatggcac agaatgaagacctccgag	ctcggaggcttctcattctgtgccattga ataatagtctgaaa
HACE1 R332Q	G995A	gggaggatggaccaatttga gacgtgacaaaac	gtttgtcacgtcttcaaattgtccatc ctccc
HACE1 R353G	A1057G	aatgggaataaaaactccaggaa gccagggttcaagc	gctgaacacctggcttctggagtttta ttccatt
HACE1 P359S	C1075T	gtgccaaagcaattccagactctt gaacacctggcttctt	aagaagccagggttcaagagtctgg aattgcttggcac
HACE1 R493I	G1478T	tctgcaaacatgatgaagttttaa atgctttgtaataataatcccaa attatattgac	gtcaaataaatttgggatttataaac aaagcattttaaacttcatcatgttgc aga

a**b****c**

myc-HACE1	-	WT	G175S	G175N
HA-Rac1Q61L	+	+	+	+
His-ub	+	+	+	+

