## SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure S1. HACE1 favors melanoma cell migration.**

(A) Boyden chamber migration assay of 501MEL treated with control (siCtl) or two different HACE1 (siH1 and siH2) siRNA. (B) Quantification of 3 independent experiments. The results are expressed as the mean ± SD of the percentage of cells in control conditions. (C) Western blot analysis of 501MEL cells following siRNA transfection. (D) Migration assay of a short-term melanoma cell culture (C-13.08) transfected with control (siCtl) or HACE1 (siH1) siRNA. Representative images are shown. (E) Quantification of 3 independent experiments as in B. (F) Western Blot analysis of C-13.08 cells following siRNA treatment. (G) Boyden chamber experiments showing the migration ability of A375 cells transfected with control (siCtl) or two different HACE1 (siH2 and siH3) siRNA. (H) Quantification of 3 independent experiments as in B. (I) Western Blot analysis of A375 cells following siRNA treatment. (J) Boyden chamber experiments showing the migration abilityof A375 overexpressing HACE1. (K) Quantification of 3 independent experiments. The results are expressed as the mean ± SD of the percentage of cells in control conditions. (L) Western blot analysis of A375 overexpressing HACE1.

**Supplemental Figure S2. RAC1 is involved in melanoma cell migration and its GTP-bound form is targeted to the degradation pathway by HACE1.**

(A) Boyden chamber migration assay of 501MEL treated with control (siCtl) or RAC1 (siRAC) siRNA. (B) Western Blot analysis of 501MEL cells following siRNA treatment. (C) G-LISA RAC1 Activation assay of A375 transfected with control (siCtl) or HACE1 (siH1) siRNA, exposed or not to CNF toxin (Cytotoxic Necrotizing Factor). GTP-bound RAC1 level was evaluated by ELISA as recommended by the manufacturer; The results represent the mean± SD of the % of RAC1 activation (n=3). (D) Western Blot analysis of A375 cells following siRNA and/or CNF treatment.

**Supplemental Figure S3. HACE1 favors melanoma cell adhesion.**

(A) xCELLigence impedance assay with 501MEL cells transfected with control (siCtl) or HACE1 (siH1. siH2 and siH3) siRNA. The results represent the mean of cell adhesion index ± SD (n=3) in function of time. (B) xCELLigence impedance assay with A375 cells left untransfected or transfected with control (siCtl) or HACE1 (siH2) siRNA. The results represent the mean of cell adhesion index ± SD (n=3) in function of time.

**Supplemental Figure S4. HACE1 favors lung colonization and decreases survival of patients.**

(A, B C. and D) Melanoma cells were labelled with a fluorescent dye (CMFDA) before injection in mice. (A) A375 cells transfected with control (siCtl) or HACE1 (siH1) siRNA were injected in tail vein of nude mice. 30 min before euthanasia. mice were subjected to retro-orbital injection of lectin labelling blood vessels (Alex594-*Lycopersicon esculentum* lectin). Representative pictures showing pulmonary blood vessels (red) and cells (green) remaining in the lungs 24h after cell injection. (B) Representative pictures and quantification (cells per field, mean ± SD, n=9) of lung colonization by C-10.01 cells exposed or not to HACE1 (siH1) siRNA after 24h. (C) Representative pictures and quantification (cells per field, mean ± SD, n=9) of lung colonization by A375 melanoma cells transfected with control (siCtl) or HACE1 (siH2, siH3) siRNA. Mice were sacrificed 30min or 24h after cell injection and whole lungs were analyzed by fluorescence microscopy. (D) Representative pictures and quantification (cells per field, mean ± SD, n=9) of lung colonization by 501MEL (EV) and 501MEL overexpressing HACE1 (C29) after 24h. (E) Kaplan Meier analysis of the effect of HACE1 expression (median) on the survival of melanoma patients after the onset of metastasis. Data set GSE19234.Log-rank (Mantel-Cox) test.

**Supplemental Figure S5. HACE1 silencing inhibits ITGαV expression as well as ITGAV and ITGB1 promoter activity.**

(A) Immunofluorescence study of ITGαV expression in short-term melanoma cell culture (C-14.27) exposed to control (siCtl) or HACE1 (siH1) siRNA. (B) ITGAV promoter activity in 501MEL and C-09.10 exposed to control (siCtl) or HACE1 (siH1) siRNA. Quantification of 3 independent experiments. The results are mean ± SD of promoter activity in the control condition. (C. D) ITGB1 (C) and ITGAV (D) promoter activity in A375 cells exposed to control (siCtl) or HACE1 (siH1) siRNA. Quantification of 3 independent experiments. The results are mean± SD of promoter activity in control condition.

**Supplemental Figure S6. Quantification of secreted fibronectin by ELISA.**

Concentration of fibronectin measured in cell culture supernatants of A375 and 501MEL exposed to control (siCtl) or HACE1 (siH2, siH3) siRNA after 72h using ELISA (n=3).

**Supplemental Figure S7. Extracellular fibronectin inhibits migration of melanoma cells and ITGAV and ITGB1 expression.**

(A) Boyden chamber migration assay of 501MEL cells treated with control (siCtl) or Fibronectin (siFN1) siRNA. (B) Western blot analysis showing the effects of fibronectin silencing on ITGαV and fibronectin (FN) expression in 501MEL cells. (C) Boyden chamber migration assay of WM793 transfected with a control (siCtl) or Fibronectin (siFN1. siFN2) siRNA. (D)Western blot analysis showing the effects of fibronectin silencing on ITGαV. ITGβ1 and fibronectin (FN) expression in WM793 cells. (E) Western blot analysis of 501MEL cells transfected with control (siCtl) or HACE1 (siH1) siRNA or exposed to the conditioned medium of these with HACE1. ITGAV and HSP90 (loading control) antibodies.

**Supplemental Figure S8. Fibronectin interacts with HACE1 and undergoes K27 ubiquitination.**

(A) GST pull-down assay. Western blot analysis of recombinant, GST or GST-HACE1 with anti-HACE1 antibodies (upper panel). Recombinant GST or GST-HACE1 were incubated with cell lysate (CL) or conditioned medium (CM) from A375 melanoma cells. Bound proteins were probed with antibody to fibronectin (FN). Fibronectin detection in cell lysate (CL) or conditioned medium (Input). (B) Cellular lysates from C-10.01 melanoma cells were subjected to immunoprecipitation with antibody to fibronectin (FN-IP). Proteins were analyzed by western blot with anti-FN and anti-HACE1 antibodies. (C) COS cells were transfected with empty vector, or vectors encoding HA-tagged K27-, K48- and K63-ubiquitin. Total proteins (Input) and protein immunoprecipitated with non-relevant (IP-Myc) or fibronectin (IP-FN) antibodies were analysed by western blot with antibodies to fibronectin (FN) or to HA-tag (HA).

## SUPPLEMENTAL MATERIALS AND METHODS

### Luciferase assays

Melanoma cells were seeded in a 24-well plates, and transient transfections were conducted the following day using Lipofectamine and control plasmid or ITGAV(S706188) or ITGB1 (S710176) Light switch reporter gene from Active Motive. pCMVbGal plasmid was transfected to control the variability in transfection efficiency. 6h later, the medium was changed and the siRNA (siCtl or siH1) were transfected. Seventy-two hours later, cells were harvested in an appropriate lysis buffer and soluble extracts were assayed for luciferase and -galactosidase activities. Results represent the mean+/-SEM of 3 to 5 independent experiments done in triplicate.

## RAC1 activation assay

RAC1 activation kit was from Tebu (027BK128-S). A375 cells treated with a control (siCtl) or HACE1 (siH1) siRNA were exposed or not with CNF (10nM) for 24h. Cells were processed as recommended by the manufacturer.

## Human fibronectin ELISA kit

Human fibronectin was measured in cell culture supernatants using a kit purchased from abcam (ab108847) according to manufacturer's instruction.

## siRNAs

Si RNA to HACE1:
siH1 is a mix of 3 siRNA with the following sequences:
  sc-95301A:CCAAAUAUCUGCCAGAUAAtt
  sc-95301B:CAACAGAGAUCACUUCUAUtt
  sc-95301C:CGUCCAGCUUGUUACUGAAtt
siH1 was purchased from Santa cruz (sc-95031)
siH2 is a single siRNA purchases from qiagen (Hs_HACE1_4: SI00127694) with the following sequence TACGCTATATTGTATGAATTA
siH3 is a single siRNA purchased from qiagen (Hs_HACE1_5: SI03062227) with the following sequence CACTCGTTAGATGAATGGCTA

**Supplemental table S1.** List of the genes regulated by si HACE1 in 4 different melanoma cell cultures

| Entrez Gene Name | Gene symbol | Log Ratio | Log-Odds |
|---|---|---|---|
| HD domain containing 2 | HDDC2 | -3.779 | 7.347 |
| interleukin 11 | IL11 | -2.79 | 0.090 |
| proliferating cell nuclear antigen | PCNA | -2.427 | 3.227 |
| plasminogen activator. urokinase | PLAU | -2.002 | 1.203 |
| TNF receptor superfamily member 12A | TNFRSF12A | -1.891 | 3.629 |
| integrin subunit alpha V | ITGAV | -1.805 | 2.458 |
| FOS like 1. AP-1 transcription factor subunit | FOSL1 | -1.801 | 2.368 |
| protein disulfide isomerase family A member 4 | PDIA4 | -1.784 | 3.829 |
| DEAH-box helicase 33 | DHX33 | -1.778 | 3.920 |
| heat shock protein family A (Hsp70) member 5 | HSPA5 | -1.744 | 2.284 |
| cytohesin 3 | CYTH3 | -1.736 | 3.880 |
| aspartate beta-hydroxylase | ASPH | -1.729 | 4.056 |
| MYB proto-oncogene like 1 | MYBL1 | -1.725 | 1.362 |
| high mobility group AT-hook 2 | HMGA2 | -1.699 | 4.528 |
| HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1 | HACE1 | -1.665 | 4.854 |
| SH2 domain containing 5 | SH2D5 | -1.605 | 1.822 |
| mitogen-activated protein kinase 6 | MAPK6 | -1.587 | 3.655 |
| adenosine A2b receptor | ADORA2B | -1.551 | 1.115 |
| putative homeodomain transcription factor 2 | PHTF2 | -1.54 | 4.640 |
| twinfilin actin binding protein 1 | TWF1 | -1.512 | 3.547 |
| transmembrane protein 2 | TMEM2 | -1.459 | 3.663 |
| DNA polymerase epsilon 3. accessory subunit | POLE3 | -1.447 | 1.159 |
| RAB21. member RAS oncogene family | RAB21 | -1.431 | 2.227 |
| sosondowah ankyrin repeat domain family member C | SOWAHC | -1.409 | 2.755 |
| calmodulin 1 | CALM1 | -1.398 | 1.693 |
| sterol O-acyltransferase 1 | SOAT1 | -1.386 | 3.343 |
| pantothenate kinase 3 | PANK3 | -1.374 | 2.572 |
| metallothionein 1L (gene/pseudogene) | MT1L | -1.371 | 1.207 |
| TATA-box binding protein associated factor 13 | TAF13 | -1.37 | 0.541 |
| transmembrane protein 64 | TMEM64 | -1.37 | 3.629 |
| melanoregulin | MREG | -1.362 | 3.239 |
| metallothionein 1E | MT1E | -1.356 | 1.179 |
| integrin subunit beta 1 | ITGB1 | -1.351 | 2.952 |
| TIMELESS interacting protein | TIPIN | -1.342 | 0.818 |
| solute carrier family 25 member 24 | SLC25A24 | -1.326 | 2.811 |
| syndecan 4 | SDC4 | -1.315 | 0.874 |
| U2AF homology motif (UHM) kinase 1 | UHMK1 | -1.31 | 3.609 |
| killer cell lectin like receptor G1 | KLRG1 | -1.306 | 3.353 |
| homeodomain interacting protein kinase 1 | HIPK1 | -1.305 | 3.289 |
| spindle and kinetochore associated complex subunit 2 | SKA2 | -1.299 | 3.110 |
| metallothionein 1B | MT1B | -1.297 | 1.170 |
| transmembrane protein 245 | TMEM245 | -1.292 | 1.938 |
| pro-apoptotic WT1 regulator | PAWR | -1.29 | 2.023 |
| proline rich 11 | PRR11 | -1.282 | 0.982 |
| far upstream element binding protein 3 | FUBP3 | -1.267 | 3.357 |
| NUS1 dehydrodolichyl diphosphate synthase subunit | NUS1 | -1.25 | 2.119 |
| STAM binding protein like 1 | STAMBPL1 | -1.235 | 1.631 |
| exoribonuclease 1 | ERI1 | -1.232 | 1.470 |
| family with sequence similarity 60 member A | FAM60A | -1.221 | 2.159 |
| integrin subunit alpha 5 | ITGA5 | -1.219 | 0.978 |
| glutamate-ammonia ligase | GLUL | -1.21 | 2.916 |
| kinesin family member 23 | KIF23 | -1.209 | 0.857 |
| G3BP stress granule assembly factor 1 | G3BP1 | -1.2 | 0.861 |
| solute carrier family 39 member 10 | SLC39A10 | -1.2 | 2.689 |
| MOB kinase activator 1A | ENST00000396049 | -1.193 | 1.927 |
| metallothionein 2A | MT2A | -1.192 | 1.818 |
| SPOC domain containing 1 | SPOCD1 | -1.185 | 0.407 |
| metallothionein 1X | MT1X | -1.172 | 0.500 |
| beta-1.4-N-acetyl-galactosaminyltransferase 1 | B4GALNT1 | -1.153 | 0.171 |

| | | | |
|---|---|---|---|
| core 1 synthase. glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1 | C1GALT1 | -1.153 | 2.704 |
| syntaxin 3 | STX3 | -1.153 | 2.685 |
| cornichon family AMPA receptor auxiliary protein 1 | CNIH1 | -1.152 | 1.735 |
| ankyrin repeat and SOCS box containing 2 | ASB2 | -1.151 | 1.185 |
| solute carrier family 37 member 2 | SLC37A2 | -1.15 | 0.554 |
| ADAM metallopeptidase with thrombospondin type 1 motif 4 | ADAMTS4 | -1.145 | 2.295 |
| thyroid hormone receptor interactor 13 | TRIP13 | -1.145 | 0.793 |
| proline rich 3 | PRR3 | -1.138 | 0.988 |
| transgelin 2 | TAGLN2 | -1.137 | 0.071 |
| ubiquitin conjugating enzyme E2 S | UBE2S | -1.137 | 1.636 |
| melanoma cell adhesion molecule | MCAM | -1.129 | 0.046 |
| DEP domain containing 1 | DEPDC1 | -1.123 | 0.319 |
| MAF bZIP transcription factor F | MAFF | -1.09 | 1.757 |
| DBF4 zinc finger | DBF4 | -1.079 | 0.791 |
| myosin VA | MYO5A | -1.075 | 1.273 |
| CCR4-NOT transcription complex subunit 8 | CNOT8 | -1.074 | 2.619 |
| prolyl 3-hydroxylase 2 | P3H2 | -1.071 | 0.071 |
| ribosomal protein S6 kinase A4 | RPS6KA4 | -1.07 | 0.666 |
| phospholipase C beta 2 | PLCB2 | -1.069 | 0.894 |
| enolase 2 | ENO2 | -1.067 | 0.257 |
| 5'-nucleotidase ecto | NT5E | -1.063 | 1.450 |
| endothelin converting enzyme 1 | ECE1 | -1.055 | 0.197 |
| armadillo repeat containing 1 | ARMC1 | -1.052 | 1.456 |
| solute carrier family 4 member 7 | SLC4A7 | -1.051 | 2.670 |
| phospholipase A2 group XVI | PLA2G16 | -1.045 | 0.362 |
| NDC1 transmembrane nucleoporin | NDC1 | -1.033 | 1.236 |
| chloride voltage-gated channel 4 | CLCN4 | -1.026 | 0.058 |
| downstream neighbor of SON | DONSON | -1.021 | 0.228 |
| potassium channel tetramerization domain containing 20 | KCTD20 | -1.02 | 2.501 |
| dihydrolipoamide S-acetyltransferase | DLAT | -1.006 | 2.129 |
| lysine demethylase 5B | KDM5B | 0.833 | 0.293 |
| protein serine kinase H1 | PSKH1 | 1.005 | 0.755 |
| poly(ADP-ribose) polymerase family member 16 | PARP16 | 1.012 | 2.284 |
| branched chain keto acid dehydrogenase E1. alpha polypeptide | BCKDHA | 1.016 | 0.079 |
| cytochrome b reductase 1 | CYBRD1 | 1.019 | 0.579 |
| TP53 target 1 (non-protein coding) | TP53TG1 | 1.025 | 0.529 |
| yippee like 3 | YPEL3 | 1.025 | 1.137 |
| REV3 like. DNA directed polymerase zeta catalytic subunit | REV3L | 1.026 | 1.381 |
| serine/threonine kinase 10 | STK10 | 1.039 | 1.092 |
| phosphatidylcholine transfer protein | PCTP | 1.045 | 1.965 |
| interferon regulatory factor 7 | IRF7 | 1.048 | 2.592 |
| non-POU domain containing. octamer-binding | NONO | 1.049 | 1.390 |
| protocadherin beta 5 | PCDHB5 | 1.059 | 0.150 |
| frequently rearranged in advanced T-cell lymphomas 2 | FRAT2 | 1.068 | 0.378 |
| acyl-CoA oxidase 1 | ACOX1 | 1.077 | 2.301 |
| family with sequence similarity 214 member A | FAM214A | 1.077 | 0.342 |
| transmembrane protein 65 | TMEM65 | 1.08 | 0.568 |
| RAB40B. member RAS oncogene family | RAB40B | 1.087 | 1.899 |
| zinc finger BED-type containing 3 | ZBED3 | 1.092 | 2.695 |
| semaphorin 3D | SEMA3D | 1.099 | 0.905 |
| transmembrane protein 198 | TMEM198 | 1.103 | 1.257 |
| zinc finger and BTB domain containing 47 | ZBTB47 | 1.105 | 2.348 |
| zyg-11 related cell cycle regulator | ZER1 | 1.106 | 1.628 |
| IDNK. gluconokinase | IDNK | 1.127 | 0.064 |
| eukaryotic translation initiation factor 3 subunit I | EIF3I | 1.135 | 0.074 |
| LYR motif containing 9 | LYRM9 | 1.139 | 0.617 |
| calcium homeostasis endoplasmic reticulum protein | CHERP | 1.163 | 0.708 |
| NLR family member X1 | NLRX1 | 1.167 | 2.373 |
| drebrin like | DBNL | 1.168 | 0.521 |
| replication protein A1 | RPA1 | 1.18 | 0.664 |
| kringle containing transmembrane protein 1 | KREMEN1 | 1.184 | 1.718 |
| SATB homeobox 1 | SATB1 | 1.184 | 2.001 |
| Meis homeobox 2 | MEIS2 | 1.203 | 2.890 |
| chromosome 4 open reading frame 46 | C4orf46 | 1.206 | 0.842 |
| SAMM50 sorting and assembly machinery component | SAMM50 | 1.21 | 2.784 |
| phospholysine phosphohistidine inorganic pyrophosphate phosphatase | LHPP | 1.242 | 1.127 |

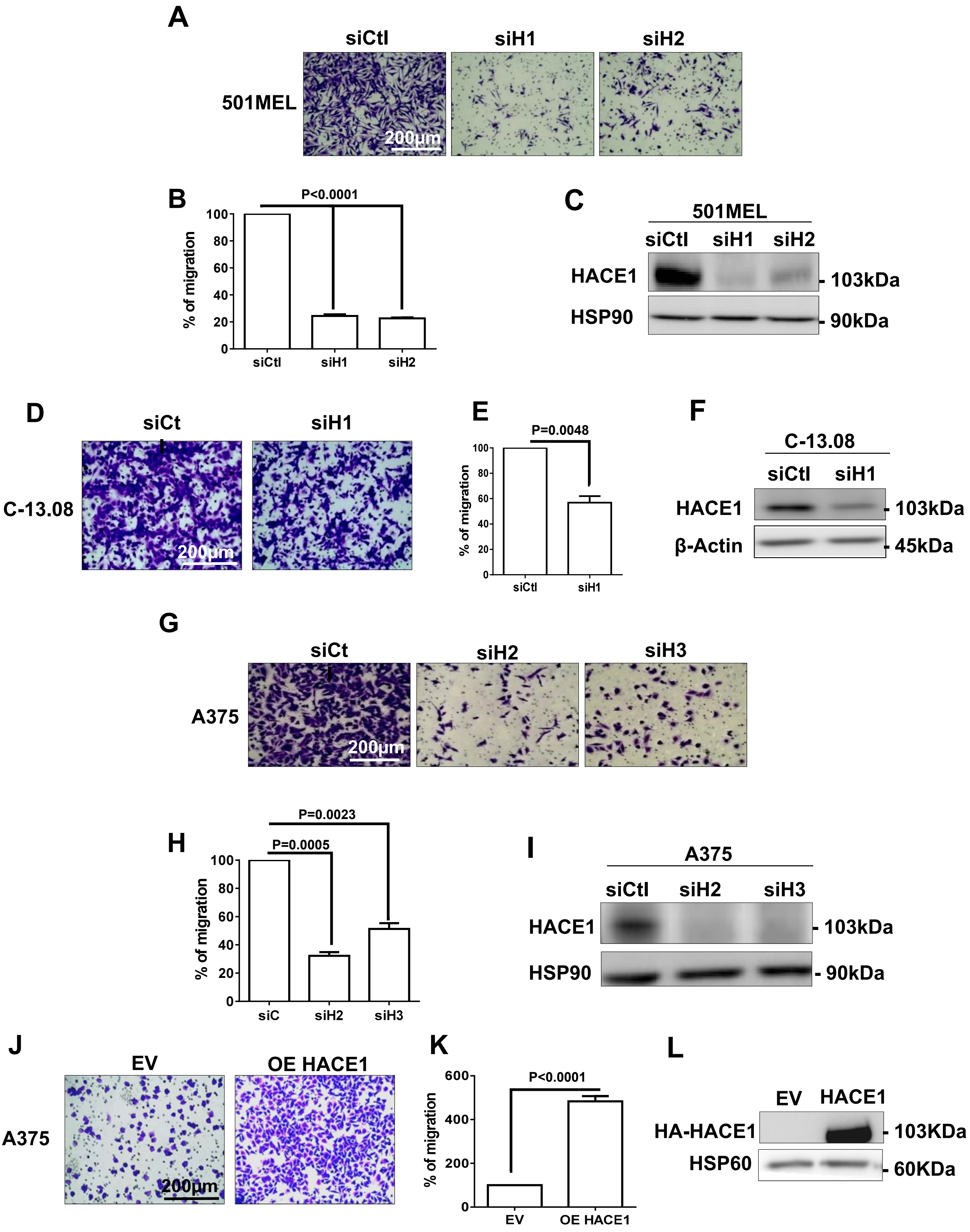
| | | | |
|---|---|---|---|
| GABA type A receptor-associated protein | GABARAP | 1.26 | 2.239 |
| mitochondrial intermediate peptidase | MIPEP | 1.266 | 2.441 |
| TatD DNase domain containing 3 | TATDN3 | 1.27 | 0.608 |
| MAX dimerization protein 4 | MXD4 | 1.278 | 0.487 |
| G protein-coupled receptor 137B | GPR137B | 1.288 | 1.828 |
| histone cluster 1. H4h | HIST1H4H | 1.288 | 1.216 |
| histone deacetylase 3 | HDAC3 | 1.303 | 3.229 |
| decorin | DCN | 1.307 | 1.154 |
| Dexi homolog | DEXI | 1.314 | 2.692 |
| proline rich nuclear receptor coactivator 1 | PNRC1 | 1.332 | 0.181 |
| nuclear protein 1. transcriptional regulator | NUPR1 | 1.337 | 3.242 |
| chromosome 4 open reading frame 3 | C4orf3 | 1.341 | 1.222 |
| histone cluster 1. H2ac | HIST1H2AC | 1.342 | 3.106 |
| HMG-box transcription factor 1 | HBP1 | 1.343 | 0.392 |
| dispatched RND transporter family member 2 | DISP2 | 1.346 | 3.048 |
| transmembrane protein 8A | TMEM8A | 1.346 | 2.823 |
| GATS protein like 3 | GATSL3 | 1.347 | 0.542 |
| hdc homolog. cell cycle regulator | HECA | 1.352 | 3.852 |
| ankyrin repeat family A member 2 | ANKRA2 | 1.358 | 1.202 |
| histone cluster 1. H2bk | HIST1H2BK | 1.401 | 3.370 |
| calcium binding and coiled-coil domain 1 | CALCOCO1 | 1.424 | 0.992 |
| N-sulfoglucosamine sulfohydrolase | SGSH | 1.425 | 3.451 |
| chromosome 6 open reading frame 120 | C6orf120 | 1.431 | 3.098 |
| ubiquinol-cytochrome c reductase. complex III subunit X | UQCR10 | 1.444 | 3.466 |
| kelch like family member 24 | KLHL24 | 1.451 | 0.460 |
| G protein subunit gamma 10 | GNG10 | 1.472 | 4.072 |
| solute carrier family 35 member F5 | SLC35F5 | 1.481 | 3.539 |
| phospholipid scramblase 4 | PLSCR4 | 1.491 | 2.841 |
| histone cluster 2. H2aa3 | ENST00000607355 | 1.514 | 0.951 |
| spermidine/spermine N1-acetyltransferase 1 | SAT1 | 1.544 | 1.356 |
| peptidylglycine alpha-amidating monooxygenase | PAM | 1.548 | 2.067 |
| transmembrane protein 134 | TMEM134 | 1.586 | 1.945 |
| splicing factor 3b subunit 5 | SF3B5 | 1.592 | 4.880 |
| ADP ribosylation factor like GTPase 6 interacting protein 1 | ARL6IP1 | 1.638 | 4.263 |
| dpy-19 like 1 (C. elegans) | DPY19L1 | 1.663 | 4.533 |
| claudin 12 | CLDN12 | 1.679 | 5.059 |
| phosphoinositide-3-kinase regulatory subunit 2 | PIK3R2 | 1.785 | 4.170 |
| leucine rich repeat and Ig domain containing 2 | LINGO2 | 1.909 | 0.209 |
| CDC42 small effector 2 | CDC42SE2 | 2.115 | 4.881 |
| neurocalcin delta | NCALD | 2.174 | 3.577 |

**Supplemental table S2.** Top 5 function annotations associated with genes regulated by HACE1 silencing in 4 different melanoma cell cultures. From Ingenuity Analysis Pathway. Qiagen®

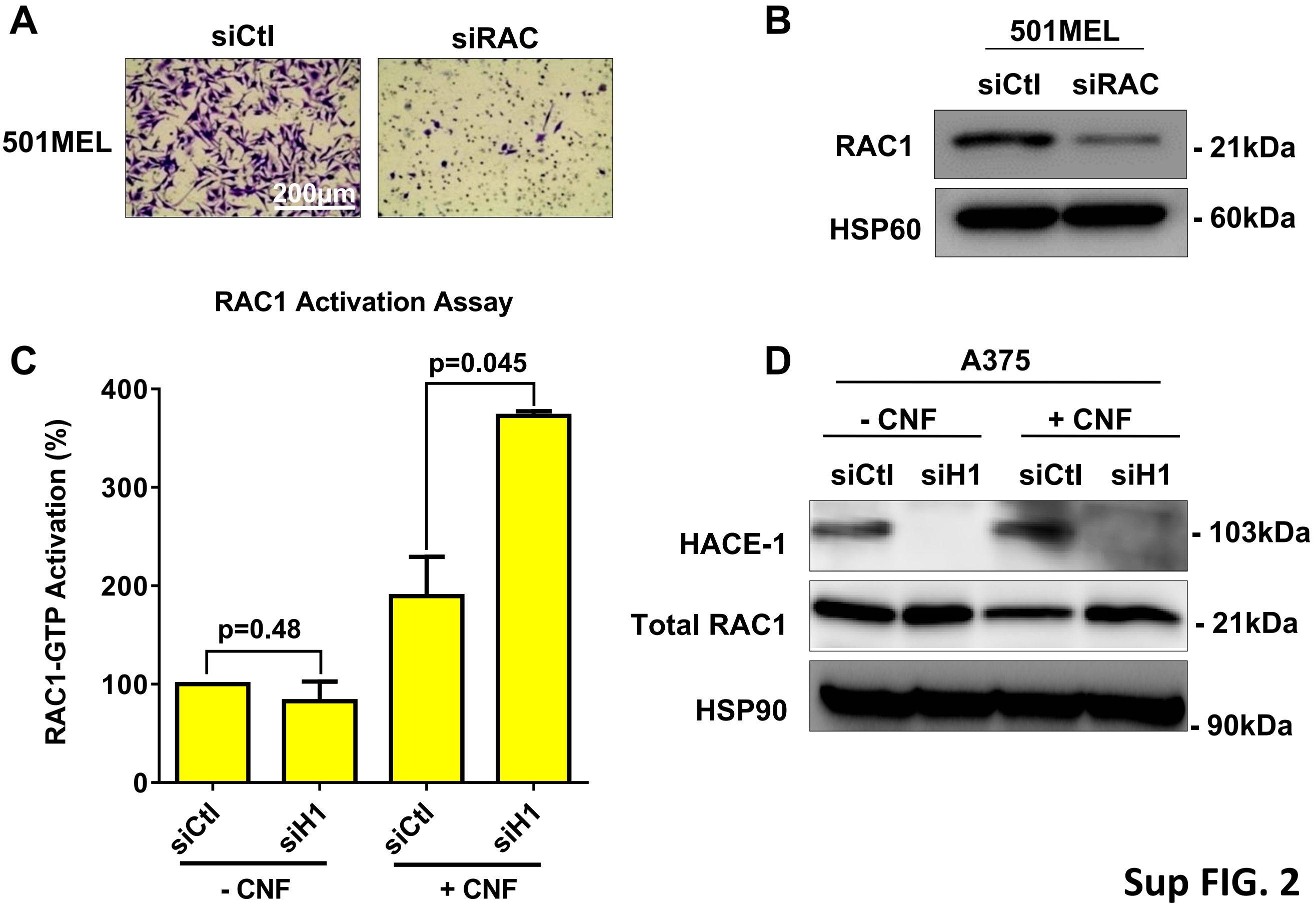
| Categories | Diseases or Functions Annotation | Activation z-score | p-Value |
|---|---|---|---|
| **Cellular Movement** | migration of cells | -3.52 | 2.00E$^{-02}$ |
| **Cellular Movement** | migration of tumor cell lines | -3.45 | 1.02E$^{-02}$ |
| **Cellular Movement** | cell movement | -3.33 | 1.87E$^{-02}$ |
| **Cellular Movement** | cell movement of tumor cell lines | -3.13 | 2.21E$^{-02}$ |
| **Cellular Growth and Proliferation** | proliferation of cells | -3.12 | 3.03E$^{-04}$ |

**Supplemental table S3.** Top 5 upstream regulators associated with genes regulated by HACE1 silencing in 4 different melanoma cell cultures. From Ingenuity Analysis Pathway. Qiagen®

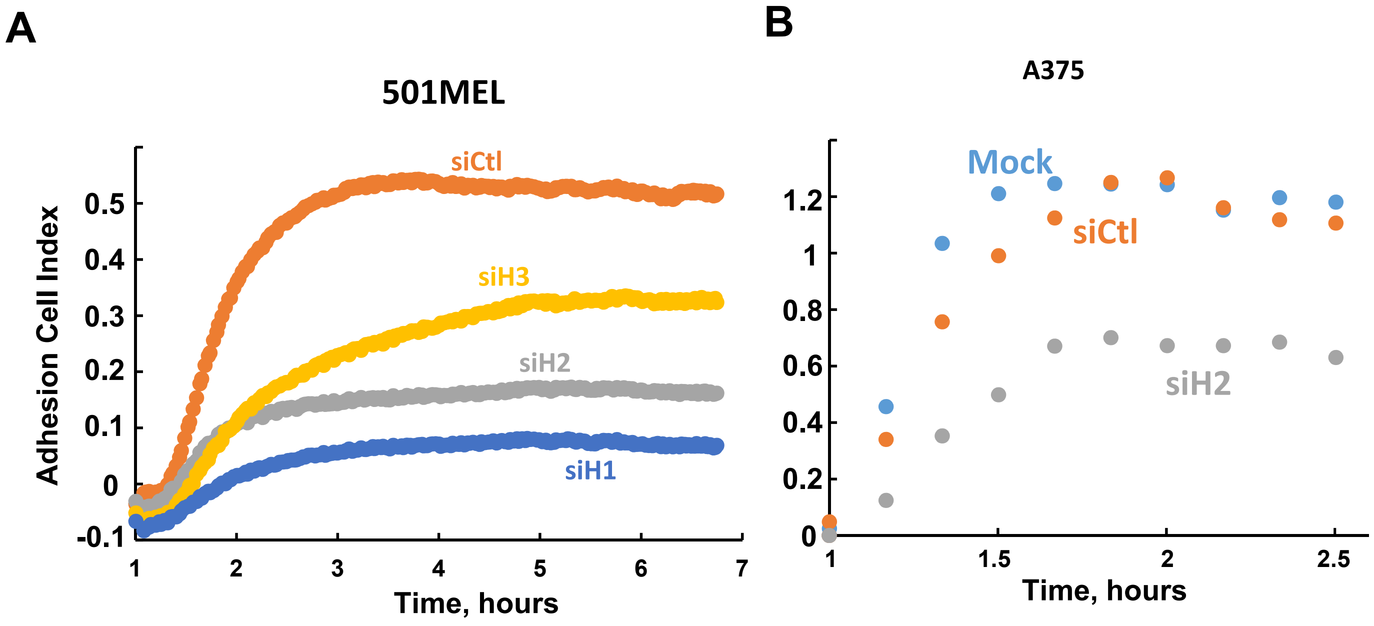
| Upstream Regulator | Molecule Type | Activation z-score | p-value of overlap |
|---|---|---|---|
| **F2** | peptidase | -3.09 | 1.42E-05 |
| **TGFB1** | growth factor | -2.88 | 1.21E-03 |
| **IL1A** | cytokine | -2.79 | 2.74E-05 |
| **FN1** | enzyme | -2.57 | 6.27E-04 |
| **SYVN1** | transporter | -2.45 | 4.08E-04 |


A
siCtl
siH1
siH2
501MEL
200µm
B
P<0.0001
% of migration
100
80
60
40
20
0
siCtl
siH1
siH2
C
501MEL
siCtl
siH1
siH2
HACE1
HSP90
103kDa
90kDa
D
siCtl
siH1
C-13.08
200µm
E
P=0.0048
% of migration
100
80
60
40
20
0
siCtl
siH1
F
C-13.08
siCtl
siH1
HACE1
β-Actin
103kDa
45kDa
G
siCtl
siH2
siH3
A375
200µm
H
P=0.0023
P=0.0005
% of migration
100
80
60
40
20
0
siC
siH2
siH3
I
A375
siCtl
siH2
siH3
HACE1
HSP90
103kDa
90kDa
J
EV
OE HACE1
A375
200µm
K
P<0.0001
% of migration
600
400
200
0
EV
OE HACE1
L
EV
HACE1
HA-HACE1
HSP60
103KDa
60KDa

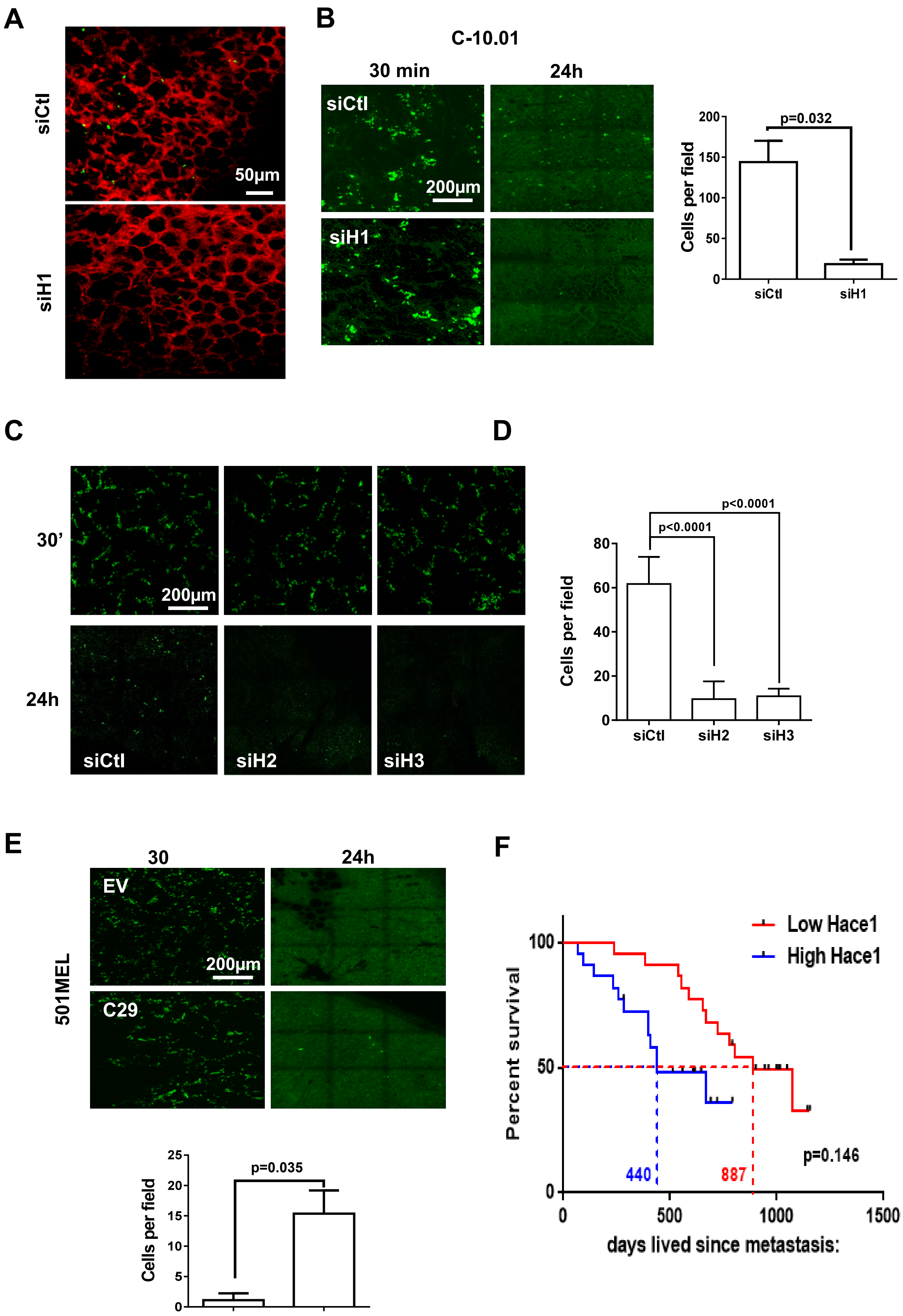
Sup FIG. 1


A
siCtl
siRAC
501MEL
200μm
B
501MEL
siCtl
siRAC
RAC1
- 21kDa
HSP60
- 60kDa
RAC1 Activation Assay
C
RAC1-GTP Activation (%)
400
300
200
100
0
p=0.48
p=0.045
siCtl
siH1
siCtl
siH1
- CNF
+ CNF
D
A375
- CNF
+ CNF
siCtl
siH1
siCtl
siH1
HACE-1
- 103kDa
Total RAC1
- 21kDa
HSP90
- 90kDa

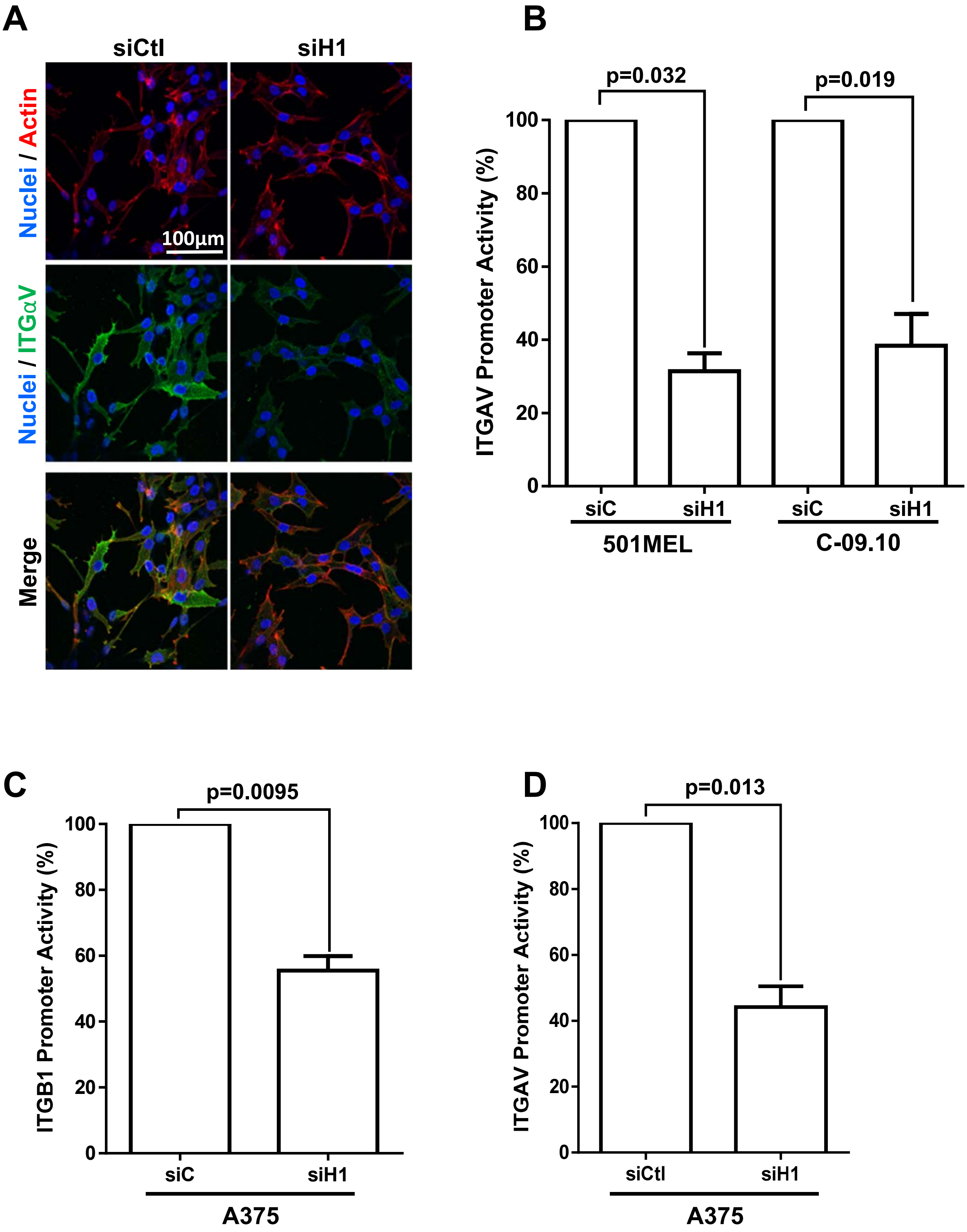
Sup FIG. 2


A
501MEL
Adhesion Cell Index
0.5
0.4
0.3
0.2
0.1
0
-0.1
1
2
3
4
5
6
7
Time, hours
siCtl
siH3
siH2
siH1
B
A375
Mock
siCtl
siH2
1.2
1
0.8
0.6
0.4
0.2
0
1
1.5
2
2.5
Time, hours

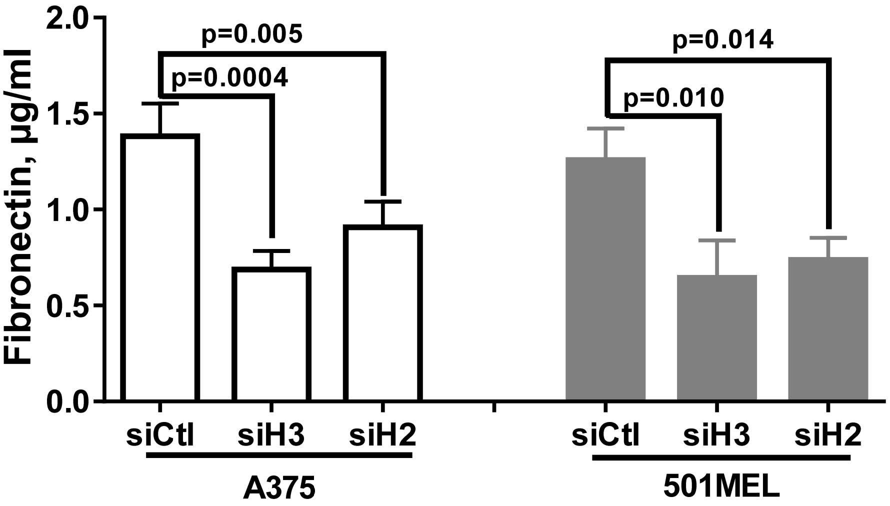
Sup FIG. 3


A
siCtl
50µm
siH1
B
C-10.01
30 min
24h
siCtl
200µm
siH1
Cells per field
200
150
100
50
0
p=0.032
siCtl
siH1
C
30’
200µm
24h
siCtl
siH2
siH3
D
Cells per field
80
60
40
20
0
p<0.0001
p<0.0001
siCtl
siH2
siH3
E
501MEL
30
24h
EV
200µm
C29
Cells per field
25
20
15
10
5
0
p=0.035
EV
C29
F
Low Hace1
High Hace1
Percent survival
100
50
0
440
887
p=0.146
0
500
1000
1500
days lived since metastasis:

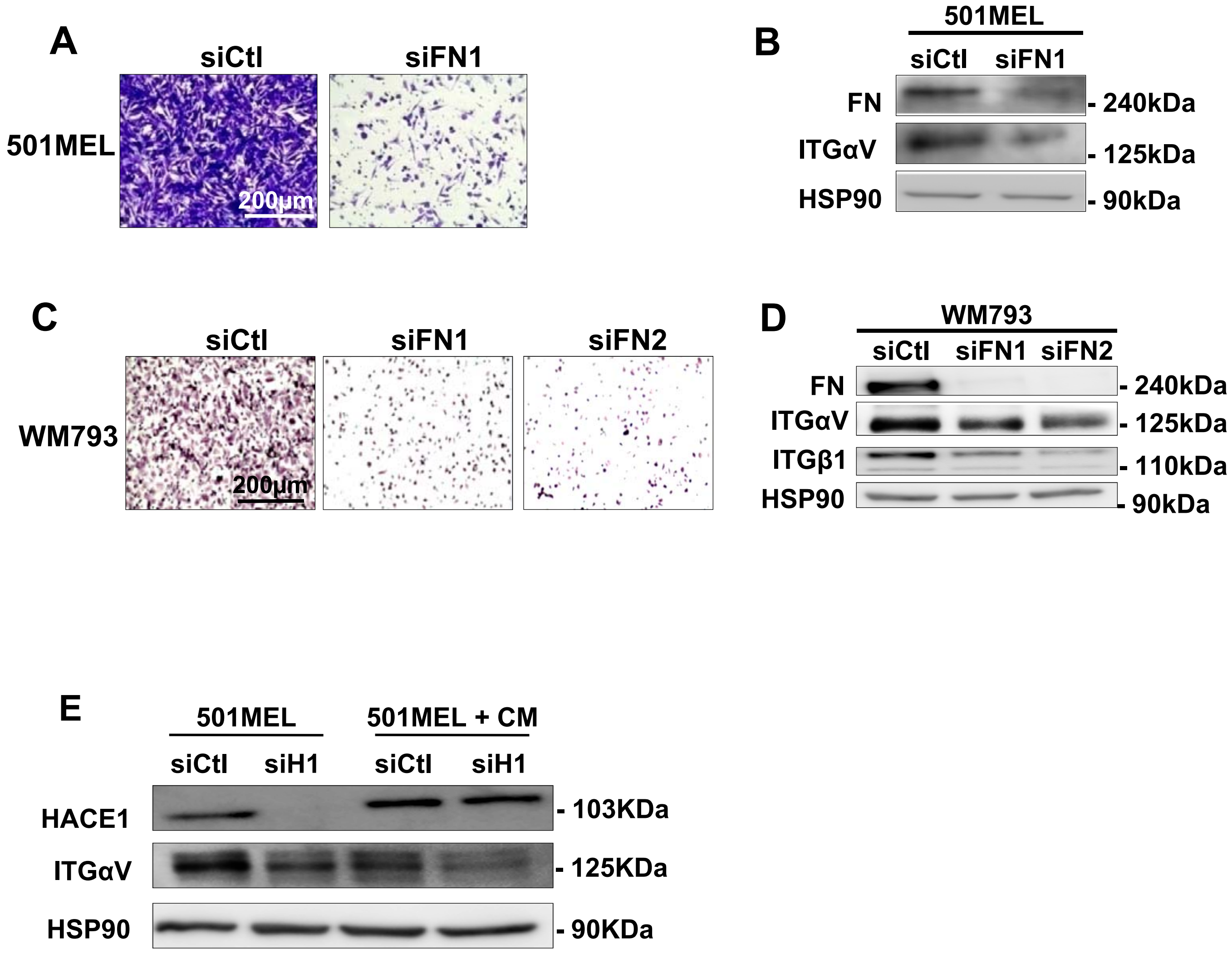
Sup FIG. 4


A
siCtl
siH1
Nuclei / Actin
100µm
Nuclei / ITGαV
Merge
B
p=0.032
p=0.019
ITGAV Promoter Activity (%)
100
80
60
40
20
0
siC
siH1
siC
siH1
501MEL
C-09.10
C
p=0.0095
ITGB1 Promoter Activity (%)
100
80
60
40
20
0
siC
siH1
A375
D
p=0.013
ITGAV Promoter Activity (%)
100
80
60
40
20
0
siCtl
siH1
A375

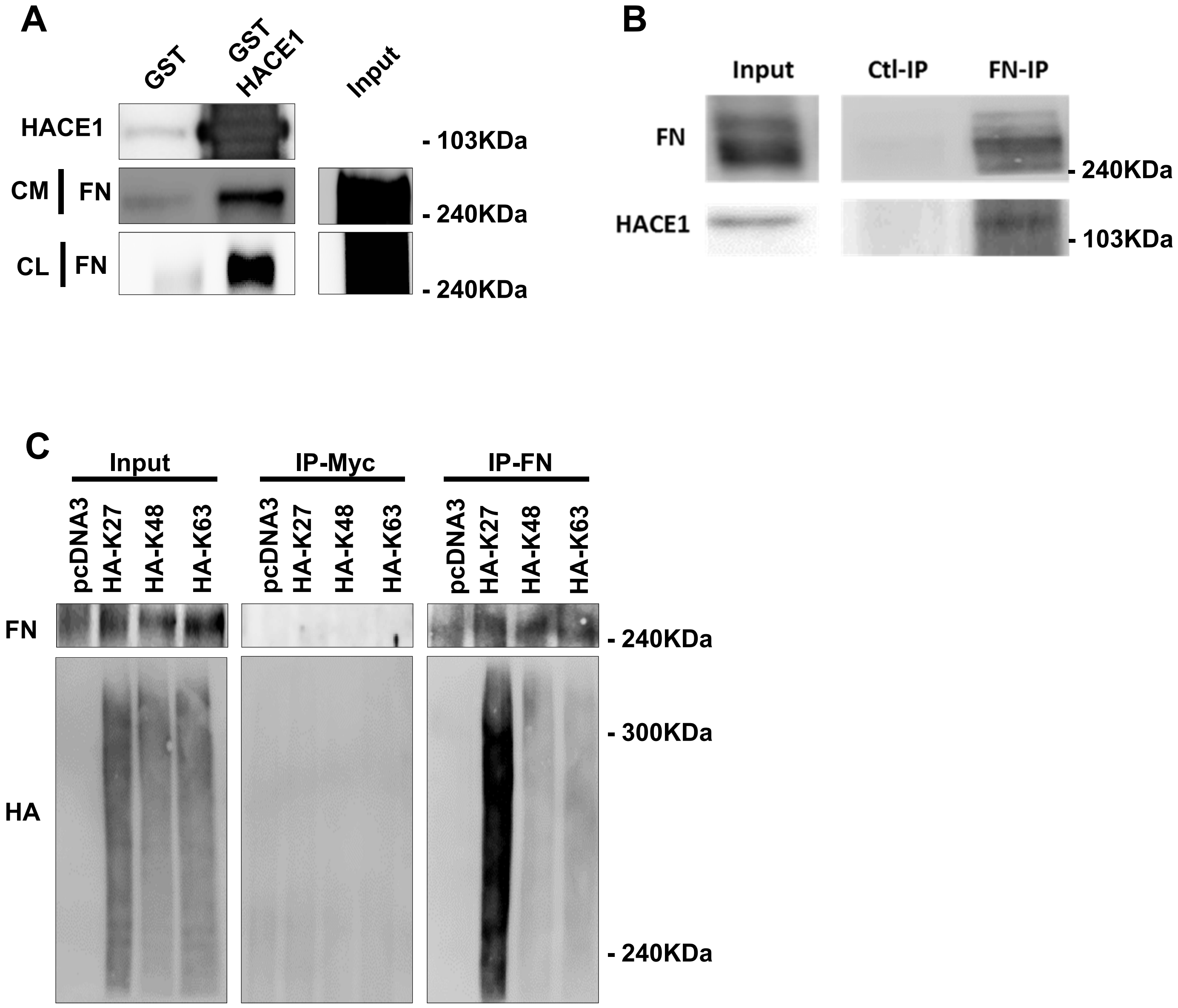
**Sup FIG. 5**


Fibronectin, µg/ml
2.0
1.5
1.0
0.5
0.0
p=0.005
p=0.0004
p=0.014
p=0.010
siCtl
siH3
siH2
A375
siCtl
siH3
siH2
501MEL

Sup FIG. 6


A
siCtl
siFN1
501MEL
200μm
B
501MEL
siCtl
siFN1
FN
ITGαV
HSP90
- 240kDa
- 125kDa
- 90kDa
C
siCtl
siFN1
siFN2
WM793
200μm
D
WM793
siCtl
siFN1
siFN2
FN
ITGαV
ITGβ1
HSP90
- 240kDa
- 125kDa
- 110kDa
- 90kDa
E
501MEL
501MEL + CM
siCtl
siH1
siCtl
siH1
HACE1
ITGαV
HSP90
- 103KDa
- 125KDa
- 90KDa

Sup FIG. 7


A
GST
GST HACE1
Input
HACE1
CM
FN
CL
FN
- 103KDa
- 240KDa
- 240KDa
B
Input
Ctl-IP
FN-IP
FN
HACE1
- 240KDa
- 103KDa
C
Input
IP-Myc
IP-FN
pcDNA3
HA-K27
HA-K48
HA-K63
pcDNA3
HA-K27
HA-K48
HA-K63
pcDNA3
HA-K27
HA-K48
HA-K63
FN
HA
- 240KDa
- 300KDa
- 240KDa

Sup FIG. 8