Supplemental Information

Primer Sequences for RT-PCR.

Oligonucleotides were all purchased from *IDT* (*IDT*, Coraville, IA). Primer pairs used for RT-PCR were:

Human Snail:

Fwd, 5'-TAACTACAGCGAGCTGCAGGAC-3'; Rev, 5'-GTGGCTTCGGATGTGCATCTTG-3'

Mouse Snail:

Fwd, 5'-TTCACCTTCCAGCAGCCCTAC -3'; Rev, 5'-TGCAGTGGGAGCAGGAGAATG-3'

Human Lats2:

Fwd, 5'-TCTGTGACTGGTGGAGTGTTG-3'; Rev, 5'-CTTCTGCTCCTGAAGGCTTTG-3'

Mouse Lats2:

Fwd, 5'-CTGCTGCCCAGTAAGTCTGAG-3'; Rev, 5'-GCACCGATGCCTAGAGTCTTG-3'

Human GAPDH:

Fwd, 5'-CGACCACTTTGTCAAGCTCA-3'; Rev, 5'-AGGGGTCTACATGGCAACTG-3'

Mouse GAPDH:

Fwd, 5'-CCTTCATTGACCTCAACTAC -3'; Rev, 5'-GGAAGGCCATGCCAGTGAGC -3'

Quantitative RT-PCR

Total RNA was produced from cells using RNEasy spin Kits (Invitrogen). Single-stranded cDNA was synthesized from 2 μg of total RNA with Superscript III (Invitrogen) and random hexamers. PCR amplification was performed with about 1 ng cDNA and specific primer pairs for Snail1 and Lats2, respectively. The amplification was done for 25 cycles (95° for 45 sec, 55° 45

sec, 72° for 50 sec). cDNA synthesis was monitored by PCR with GAPDH-primers for 20 cycles.

siRNA and shRNAi

pLKO.1-puro-Lats2 shRNA#1 targeted 5'- CCGTCGATTACTTCACTTGAA-3'

pLKO.1-puro-Lats2 shRNA#2 targeted 5'- CTACTCGCCATACGCCTTTAA-3'

Smartpool siRNA reagents (Dharmacon) were:

scrambled control (D-001210-02), Snail (M-010847-00) and GSK-3β (M-003010-03).

Human Lats2 siRNA, from Qiagen, targeted 5'-CAGGACCTTCACTGCATTAAA-3'

pLKO.1-puro-Snail1 shRNA#1 targeted 5'- GCAGGACTCTAATCCAGAGTT -3'

pLKO.1-puro-Snail1 shRNA#2 targeted 5'- GCTTTGAGCTACAGGACAAA-3'

RNAi transfection, shRNA lentivirus production and infection

100 pM siRNA were transfected into 2×10^5 cells with Lipofectamine 2000 (Invitrogen), according to manufacturers instructions. Seventy-two hours post-transfection, cells were tested for *In cellulo* bioluminescence assays as described (Naik and Piwnica-Worms, 2007) or harvested in RIPA buffer for Western blotting. Production of lentiviruses and retroviruses, and their infection of target cells were as described (Stewart et al., 2003). Following infection HT1080, HCT116 cells were selected with 2 μg/ml puromycin for 3-4 days; MDCK cells were selected with 4 μg/ml puromycin; and MCF10A cells selected with 1.5 μg/ml puromycin. MDA-MB-231 cells were selected with 2 μg/ml puromycin.

Subcellular Fractionation

Cytoplasmic and nuclear fractions were prepared by resuspending cells in lysis buffer A (10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% NP40, 1mM DTT, and 1.0 mM phenylmethylsulfonyl fluoride with Complete protein inhibitors). Nuclei were collected by centrifugation (10,000 g) for 10 min at 4°C, and washed 3 times in buffer A. Nuclei were lysed by shaking vigorously in buffer B (20 mM HEPES (pH 7.9), 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 1 mM phenylmethylsulfonyl fluoride) at 4°C for 20 min, and the resulting nuclear extracts were cleared by microcentrifugation (10,000 g) at 4°C for 10 min. All volumes were kept constant starting with 10^6 cells/100 µl of hypotonic buffer. Fraction purity was determined by Western blot for the cytoplasmic marker β-tubulin and the nuclear marker Lamin A/C.

Confocal Microscopy

Cells were grown on laminin-coated coverslips in 24-well plates, transfected with Snail1-GFP constructs, fixed in 4% PFA, and incubated with DAPI for DNA staining. After 3 washes with PBS, coverslips were then mounted. Cells were visualized using a Zeiss LSM510 confocal microscope.

Mass Spectrometry Analysis

HEK293 cells were transfected with p3xFLAG-Snail. After 48 hours transfection, cells were treated with 0.2 µg/ml Nocodazole for 24 hours. Cells were collected and lysed (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100), and Snail-Flag proteins immunoprecipitated with anti-Flag M2 affinity gel (Sigma) and eluted with 0.1 M glycine HCl, pH3.5. Proteins were precipitated using the 2D protein clean up kit (GE Healthcare) according to

the manufacturer's instructions. Bovine serum albumin (100 ng) was added to each sample as an internal standard. The pellets were dissolved in 9 M urea (20 – 40 µL) and reduced with 5 mM TCEP at pH 8.0 at room temperature for 30 min. The reduced proteins were alkylated with 10 mM iodoacetamide (Bio-Rad) in the dark at room temperature for 30 min. The reaction was quenched with 5 mM dithiothreitol (DTT) at room temperature for 10 min. The reduced and alkylated proteins were digested with endoproteinase Lys-C (1 µg) (Roche) at 37 °C for ~ 18h). The samples were diluted to a concentration of 2 M urea with water and then digested with trypsin (4 µg, Sigma, Cat. No. T6567) at 37 °C for ~ 18 h. Peptides were acidified with formic acid (5.5 ml, Sigma) and extracted 6 times with NuTip porous graphite carbon wedge tips (Glygen). The peptides were eluted with 60% acetonitrile (Burdick & Jackson) in 0.1% formic acid into 1.5 ml autosampler vials (Sun-SRI, Cat. No. 200 046). Peptide samples were dried and dissolved in 25 µl of aqueous acetonitrile/formic acid (1%/1%). The peptide digests were evaluated for quality using MALDI-TOF/TOF mass spectrometry, prior to nano-LC-MS analysis. For MALDI-TOF/TOF analysis, the peptide samples (0.5 µl) were mixed with an equal volume of matrix solution (α-cyano-hydroxycinnamic acid, Agilent Technologies) prior to spotting onto a stainless steel target.

The peptide mixture was initially analyzed using nano-LC-MS on a hybrid mass spectrometer (linear quadrupole ion-trap coupled to an Orbitrap LTQ-Orbitrap XL, Thermo-Fisher) in 'data-dependent' mode. This method of spectral acquisition is primarily to acquire survey scans (MS1) to identify signals that are consistent with the masses of phosphorylated peptides. The nano-LC systems consisted of a nanoflow HPLC (NanoLC-Ultra™) and a cHiPLC Nanoflex™. The LC column was a Nano-cHiPLC column (ChromXP C18-CL, 3 µm particle size, 120 Å pore size, Eksigent). The LC system was interfaced to the mass spectrometer with a

nanospray source (PicoView PV550; New Objective). The column was equilibrated in 98% solvent A (aqueous 1% formic acid) and 2% solvent B (acetonitrile containing 1% formic acid). The samples (5 µL) were injected from the LC-system autosampler at a flow rate of 1.5 µL/min followed by segmented linear gradient elution (250 nL/min) with solvent B: isocratic, 0-5 min; 2% B to 35% B, 5-120 min; 25% to 80%, 120-135 min; isocratic, 135-145 min; 80% to 2%, 145-155 min; and isocratic at 2% B, 155-170 min.

The survey scans (*m/z* 350-2000) (MS1) were acquired at high resolution (60,000 at *m/z* = 400) in the Orbitrap and the tandem MS spectra (MS2) were acquired in the linear ion trap at low resolution, both in profile mode. The maximum injection times for the MS1 scan in the Orbitrap and the LTQ were 50 ms and 100 ms, respectively. The automatic gain control targets for the Orbitrap and the LTQ were 2×10^5 and 3×10^4, respectively. The MS1 scans were followed by six MS2 events in the linear ion trap with collision activation in the ion trap (parent threshold = 1000; isolation width = 2.0 Da; normalized collision energy = 30%; activation Q = 0.250; activation time = 30 ms). Dynamic exclusion was used to remove selected precursor ions (-0.25/+1.5 Da) for 90 s after MS2 acquisition. A repeat count of 1, a repeat duration of 45 s, and a maximum exclusion list size of 500 was used. The following ion source parameters were used: capillary temperature 200 ºC, source voltage 2.7 kV, source current 100 µA, and the tube lens at 79 V. The data were acquired using *X*calibur, version 2.0.7 (Thermofisher).

The MS2 spectra were analyzed by searching the Uniprot database with a human taxonomy filter (downloaded on 11FEB2010) and manual interpretation. The accurate masses of the phosphopeptides and fragment ions were calculated using the Molecular Weight Calculator, version 6.45 (http://ncrr.pnl.gov/software/). For database searches, the LC-MS files were processed using MASCOT Distiller (Matrix Science, version 2.3.0.0) with the settings

previously described (Nittis et al., Mol Cell Proteomics, 2010). The resulting MS2 centroided files were used for database searching with MASCOT, version 2.1.6, and the following parameters: enzyme, trypsin; MS tolerance = 10 ppm, MS/MS tolerance = 0.8 Da with a fixed carbamidomethylation of Cys residues and the following variable modifications: Methionine, oxidation; and pyro-glu (N-term); Maximum Missed Cleavages = 5; and 1+, 2+ and 3+ charge states.

DATABASE SEARCHING-- Tandem mass spectra were extracted by BioWorks version 2.0. Charge state deconvolution and deisotoping were not performed. All MS/MS samples were analyzed using Mascot (Matrix Science, London, UK; version Mascot). Mascot was set up to search the uniprot_human_20100305 database (95621 entries) assuming the digestion enzyme trypsin. Mascot was searched with a fragment ion mass tolerance of 1.00 Da and a parent ion tolerance of 15 PPM. Iodoacetamide derivative of cysteine was specified in Mascot as a fixed modification. Oxidation of methionine and phosphorylation of tyrosine were specified in Mascot as variable modifications.

CRITERIA FOR PROTEIN IDENTIFICATION-- Scaffold (version Scaffold_2_06_02, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 0.0% probability as specified by the Peptide Prophet algorithm (Keller et al. 2002). Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 1 identified peptide. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii et al. 2003). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

Cell Proliferation Analysis and Flow Cytometry

2 x 10^4 cells per well were seeded in triplicate 24-well plates in 500 µl of growth medium on day 0. Cells were trypsinized, resuspended in a total volume of 500 µl of medium, and counted with a hemacytometer at intervals shown on figure legends. For flow cytometry analysis, cells were harvested by trypsinization and collected by centrifugation, and washed once with PBS and fixed in 5 ml of 70% ethanol for 2 hours at 4°C. Cells were washed once with PBS/1% BSA and then incubated with 1 ml of PBS/1% BSA containing 30 µg/ml propidium iodide (PI) and 0.25 mg/ml RNase A for 30 min at room temperature. Cells were analyzed for DNA content by flow cytometry using a FACS Calibur (BD Biosciences). The data were analyzed using CellQuest Analysis software (BD Biosciences).

Cell Migration Assays

Wound-healing assays were done as described in (Pratt et al. 2005). Cells were grown to confluence, starved overnight, and scratch wounded using a P200 pipette tip. Cells were allowed to recover for 30 min before recording migration rates. The total area of the wound covered by migrating cells 12 h after wounding was measured. Multiple wounds per plate were assessed to obtain statistical measurements.

Pull-down assays

ZZ-tagged human Snail1 proteins were expressed in *E. coli* BL21 at 16ºC O/N in 2YT medium using 0.5 mM IPTG. Bacteria were lysed in 50 mM Tris-HCl pH (7. 5), 500 mM NaCl, 2 mM $MgCl_2$ and 0.5 mM PMSF and the lysates cleared at 100,000 g. From these lysates, equal amounts of the expressed proteins were immobilized on 50 µl of IgG sepharose (GE Healthcare). The immobilized proteins were incubated for 3 h with 0.4 ml of a HeLa cell extract in 50 mM

Tris-HCl pH 7.5, 100 mM NaCl and 10 mM $MgCl_2$. After four washes with 500 µl binding buffer, bound proteins were eluted with 1.5 mM $MgCl_2$, precipitated with 95% isopropanol and analyzed by SDS-PAGE followed by Coumassie Blue staining or Western blotting.

Collagen Invasion assay

To analyze 3D invasion of MDA-231 cells, the method was modified from [1]. 10^5 cells were embedded in 20 µl of type I collagen gel (2.2 mg/ml) isolated from rat tail (BD Biosciences, Bedford, MA). After gelling, the plug was embedded in a cell-free, 500 µl collagen gel (2.2 mg/ml) cultured within a 24-well plate. After allowing the surrounding collagen matrix to gel (1 h at 37°C), and add 0.5 ml of culture medium on the top of the gel and further cultured for 2 days. Invasion distance from the inner collagen plug into the outer collagen gel was quantified.

Fish maintenance

Zebrafish were maintained at 28ºC under standard conditions. The embryos were staged as described in (Kimmel et al., 1995). AB and Tup-Lof wild-type strains were used in all experiments.

Morpholino oligonucleotide and mRNA injections, In situ hybridization

All morpholino antisense oligonucleotides were obtained from Gene Tools LLC and used as described (Nasevicius and Ekker, 2000). The *snail1b* and *lats2* morpholinos used in this study were previously described (Blanco et al., 2007; Chen et al., 2009). 2ng/embryo *snail1b*MO and 5ng/embryo *lats2*MO were injected in each case; the standard control morpholino was used for control injections. All oligonucleotides were injected into the yolk of one- or two-cell embryos

using a pressure injector (PicoSpritzer). For mRNA synthesis pCS2+ or pCS2+NLS MT vectors containing the different versions of snail1b were linealized by NotI and mRNA synthesized with SP6 RNA polymerase (Ambion mMessage mMachine system). 250pg/embryo of each mRNA was injected both for overexpression and rescue experiments. Phenotypic analyses involved at least 50 embryos for each specific marker studied under each experimental condition. See suppl. Table I for a detailed analysis and quantification of phenotypes. In situ hybridization of whole-mount zebrafish embryos for *hgg1* and *notail* was performed as previously described (Acloque et al., 2008).

Immunoblotting

Cells were lysed in RIPA buffer (20 mM Tris-HCl buffer (pH 8.0) containing 100 mM NaCl, 0.5% Triton X-100, 0.1% SDS, 10 mM NaF, 1 mM Na3VO4, 0.15 units/ml aprotinin, 20 μM leupeptin, and 20 μM pepstatin, and 1 mM phenylmethylsulfonyl fluoride (PMSF)). 50 μg whole cell lysate as determined by BCA analysis (Pierce, Rockford, IL) were loaded per lane for Western blot analysis. Antibodies were diluted in 2% BSA in 50 mM Tris_HCl (pH 8.0), 0.15 M NaCl, and 0.02% Tween-20 (TBST), and membranes were washed 4 times in TBST after application of both primary and secondary antibody. Bound primary antibodies were detected with horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit (Jackson ImmunoResearch, West Grove, PA) and visualized by using the ECL reagent (Pierce, Rockford, IL).

Immunoprecipitations

Cells were lysed in 50mMTris-HCl (pH 8.0), 0.1MNaCl, 5 mM EDTA, 0.5% Nonidet P-40, and 1 mM DTT (MCLB) supplemented with 1 μM microcystin-LR and protease inhibitors (1 mM

PMSF, 0.15 units/ml aprotinin, 20 μM leupeptin, and 20 μM pepstatin). Lysates were clarified by centrifugation at 16,000 g for 10 minutes. Cell lysates were precleared with protein A agarose for 1 h at 4 °C, then incubated with primary and Agarose-conjugated secondary antibodies for 2 h at 4 °C each. Beads were washed 4 times in MCLB, and bound proteins eluted by boiling in SDS/PAGE sample buffer.

***In vitro* Kinase Assays**

The kinase assays were performed as previously described (Kang et al., 2008) with minor modifications. Briefly, GST-Snail1 was produced in bacteria and purified with Glutathione Sepharose 4B (GE Healthcare Life Sciences). Flag tagged Lats2 or Lats2-KD was purified from HEK293 cells transfected with pcDNA-Flag-Lats2 or Lats2-KD. Kinase reactions were carried out in a reaction buffer consisting of 50 mM Tris-HCl, pH 7.4, 1 mM DTT, 10 mM MgCl2, 500 μM γ^{32}P-ATP, 2 μg of soluble GST-Snail1, 100 ng of Flag-Lats2. Reactions were incubated at 30°C for 1.5 h and then resolved by SDS-PAGE followed by Western blotting or autoradiography.

K. Zhang et al., Supplemental Table 1

A

Injection	Axial mesendoderm migration		
	Gain-of-function (GOF)	WT	Loss-of-function (LOF)
MOC		**86%** (36/42)	14% (6/42)
Lats2 MO		28% (17/61)	**72%** (44/61)
NLS-myc mRNA	5% (2/37)	**84%** (31/37)	11% (4/37)

WT snail1b mRNA	**60%** (57/95)	28% (27/95)	12% (11/95)
NLS-T196E-*snail1b*	**59%** (45/77)	28% (22/77)	13% (10/77)

B

Injection	rescue	no rescue
WT snail1b + *snail1b* MO	81% (48/59)	
NLS-T196E-*snail1b* + *snail1b* MO	64% (32/50)	
WT snail1b + *lats2* MO	66% (71/108)	
NLS-T196E-*snail1b* + *lats2* MO	62% (50/80)	

Supplemental Figure Legends

Supplemental Figure 1.

(A) Western blot of Snail1 expression in control HEK293 cells or HEK293.Snail1-CBG clone 8. (B) Morphology (phase contrast) of parental HEK293 and HEK293.Snail1-CBG clone 8. (C) Immunofluorescence analysis for subcellular distribution of Snail1-CBG in parental HEK293 cells (upper panel) and HEK293.Snail1-CBG clone 8. Nuclei were identified by DAPI stain. (D) RT-PCR analysis of mRNA levels following depletion of Lats2 by siRNA. (E) Western blot analysis for the indicated proteins in the same cells as in (D). (F) Relative bioluminescence photon values (F) for HEK293.Snail1-CBG clone 8, following indicated manipulation. SiLuc transfected clone 8 bioluminescence is arbitrarily set to equal 1. (G) Western blot for the indicated proteins in cell extracts following shRNAi depletion of Lats2 in clone 8 cells (2 different shRNAi). The siRNA and shRNAi used are distinct from those present in the Qiagen library. Relative levels of Snail1-CBG are indicated. (H) Lats2 or kinase inactive (K765R) Lats2 were transfected into clone 8, and Western blot for the indicated proteins performed. Relative levels of Snail1-CBG are indicated.

Supplemental Figure 2.

(A) Collision-induced fragmentation spectrum of phosphorylated peptide THTGEKPFSCPHCSR, encompassing residues 201-215 of Snail1. The mass agreement (within 10 ppm) between the observed (*m/z* 627.587) and theoretical value for the parent ion, the verified charge state, and the intense singlet at *m/z* 595.2, which is consistent with loss of phosphoric acid from the triply protonated parent ($[M+3H-H_3PO_4]^{3+}$ and consistent with the indicated

phosphopeptide (Fig. 3E). A series of y ($\mathbf{y_3}$, $\mathbf{y_5}$, $\mathbf{y_5^{2+}}$, $\mathbf{y_6}$, $\mathbf{y_7}$, $\mathbf{y_8}$, $\mathbf{y_9}$, $\mathbf{y_9^{2+}}$, $\mathbf{y_{10}^{2+}}$, $\mathbf{y_{12}^{2+}}$, and $\mathbf{y_{13}^{2+}}$ at *m/z* 422.4, 656.3, 328.7, 816.0, 903.4, 1050.3, 1147.4, 574.3, 638.4, 731.3, and 821.8, respectively) and b ions ($\mathbf{b_2}$, $\mathbf{b_3}$, $\mathbf{b_4}$, and $\mathbf{b_{10}}$ at *m/z* 239.2, 420.3, 477.3, and 1225.4, respectively) were observed that were assigned to the amino acid sequence. We concluded that Ser-203 was phosphorylated from i) a $\mathbf{y_{13}^{2+}}$ ion (doubly-charged fragment ion at *m/z* 821.8 with a phosphate moiety), ii) a $\mathbf{y_{12}^{2+}}$ ion (an unmodified fragment ion *m/z* 731.3, iii) a $\mathbf{b_3}$ ion (phosphorylated fragment ion *m/z* 420.3), and iv) a $\mathbf{b_2}$ ion (*m/z* 239.2). (B) LC-MS analysis and database search revealed a phosphopeptide (^{201}THTGEKPFSCPHCSR^{215}). Shown is the site-discriminating ions used to localize the phosphorylated residues in ^{201}THTGEKPFSCPHCSR^{215} peptide. The maximum ion intensity for each ion in the CID spectrum (Fig. S2A) is shown in parentheses (%). (C) HCT116 cells were treated with nocodazole (noco), to activate Lats2, or the proteasome inhibitor MG132. Cell extracts were then treated with λ-phosphatase (λPPase) (+) or untreated (-) and Western blot for the indicated proteins performed. (D) Snail1 or Snail1.T203A protein half-life in transfected HEK293 cells was determined by Western blot in cells pretreated with cycloheximide (CHX).

Supplemental Figure 3.

(B) Mapping of interaction between Snail1 and Lats2. HEK293 cell were co-transfected with Flag-Lats2 (lanes 1,3,4,5) or Lats2.K765R (lane 6) and WT Snail1 or indicated Snail1 mutants. Lats2 was immunoprecipitated (anti-Flag) and bound products Western blotted for the presence of Lats2 and Snail1 (upper panels). Lower panels are a Western blot of input controls (5% of total cell lysate). (B) Indicated deletion mutants of Flag-Snail1 and wt myc-Lats2 were co-

transfected into HEK293 cells. Snail1 was immunoprecipitated (anti-Flag) and bound products Western blotted for the presence of Lats2 (anti-Myc) and Snail1 (anti-Flag) (upper panels). The lower panels are the input controls (5% of total cell lysate).

Supplemental Figure 4.

(A) WT (+/+) or Lats2 null (-/-) MEFs were untreated, treated with nocodazole (noco), or the proteasome inhibitor MG132 and Western blot with the indicated antibodies performed. (B) WT (+/+) or Lats2 null (-/-) MEFs were infected with an empty retrovirus (-) or an H-RasV12 expressing retrovirus (+) and Western blot with the indicated antibodies performed. (C) Association of WT Snail1 and various T203 mutants (T203A and T203E) with nuclear membrane importins as determined by pull-down assays with added GST-Snail1, GST-Snail1-T203A, GST-Snail1-T203E as described in methods. Bound importins were detected by Western blot.

Supplemental Figure 5.

(A) Morphology of MCF10A cells transfected with WT Snail1 or various Snail1 mutants, as indicated. (B) Western blot analysis for the indicated proteins in lysates from the same set of cells as in (A).

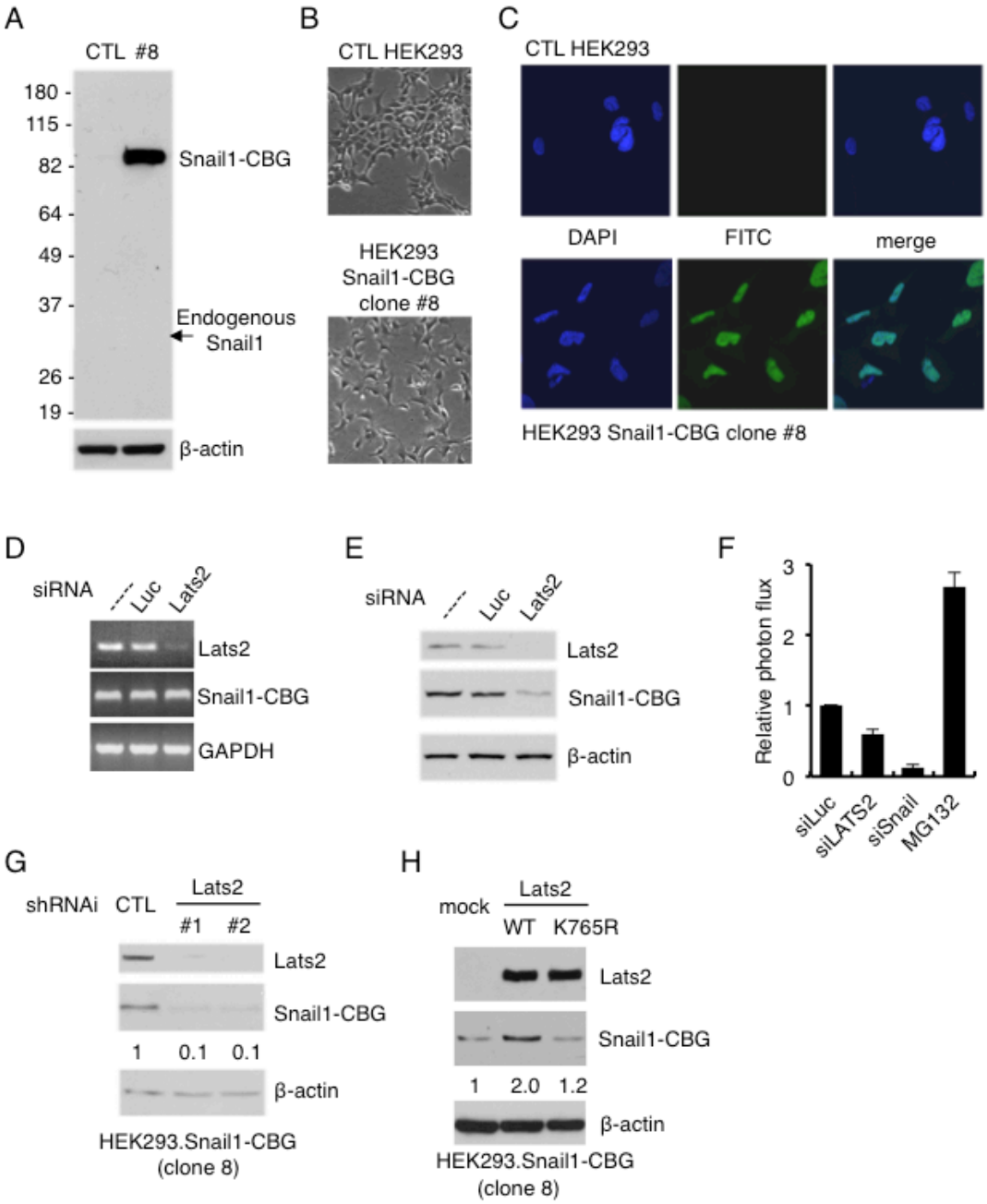
Supplemental Figure 6.

MCF10A cells transfected with WT Snail1, Snail1.T203A, or mock transfected were used in all experiments. (A) Cell proliferation curves. (B) Cell cycle DNA profile analysis. (C, D) Cell death response curves to TNFα (C) or serum withdrawal (D). (E) Scratch wound assay for cell motility, as measured by percent wound closure after 16 hours.

Supplemental Figure 7.

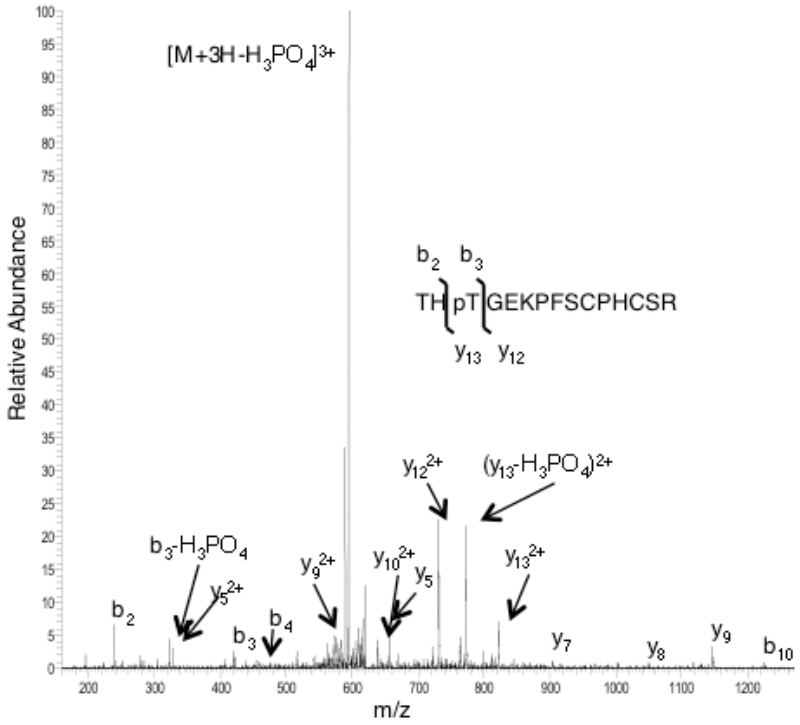
(A) MDA-MB-231 breast cancer cells were infected with lentiviruses expressing luciferase shRNA (CTL), Snail1 shRNA, Lats2 shRNA, or Lats1 shRNA and Western blot performed with the indicated antibodies. (B) Five mouse E7.5 embryos (1-5) were dissected from the mother of a $Lats2^{+/-}$ x $Lats2^{+/-}$ cross, and lysed. Western blots for the indicated proteins were performed. Equal amounts of protein were loaded per lane. (C) RT-PCR analysis of dissected Lats2-/- and Lats2+/- E7.5 embryos for Snail1 and Lats2. GAPDH was used as a control for total mRNA present in each sample.

K. Zhang et al., Supplemental Figure 1



K. Zhang et al., Supplemental Figure 2

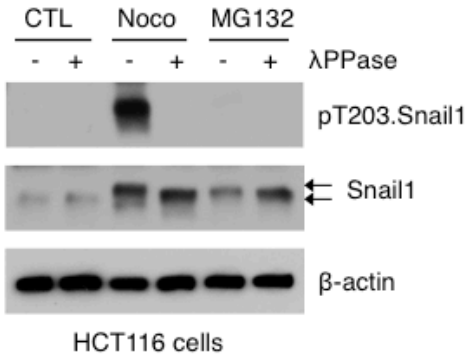
A



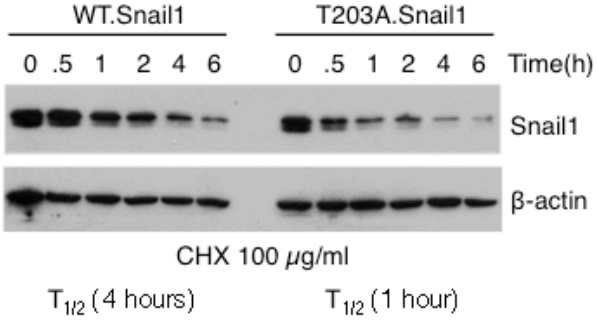
B

	Phospho-y ion	y ion	Phospho-b ion	b ion
pTHTGEKPFSCPHCSR		y14 (--)		
THpTGEKPFSCPHSCR	y13 (7.17)	y12 (22.44)	b3 (2.47)	b2 (6.50)
THTGEKPFpSCPHSCR	y7 (--)	y6 (0.28)	b9 (0.37)	b8 (--)
THTGEKPFSCPHpSCR	y2 (--)	y1 (--)	b14 (--)	b13 (--)

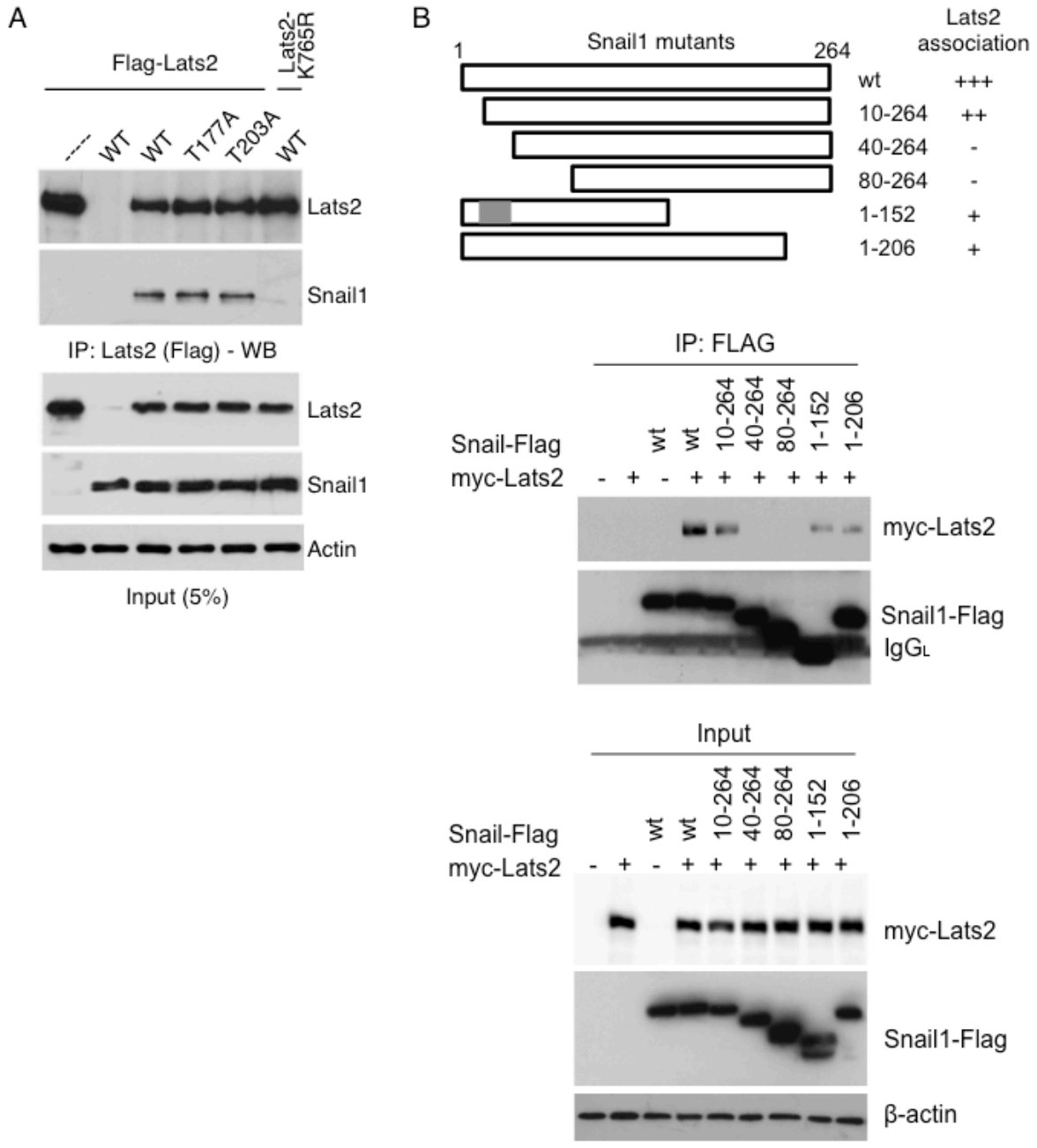
C



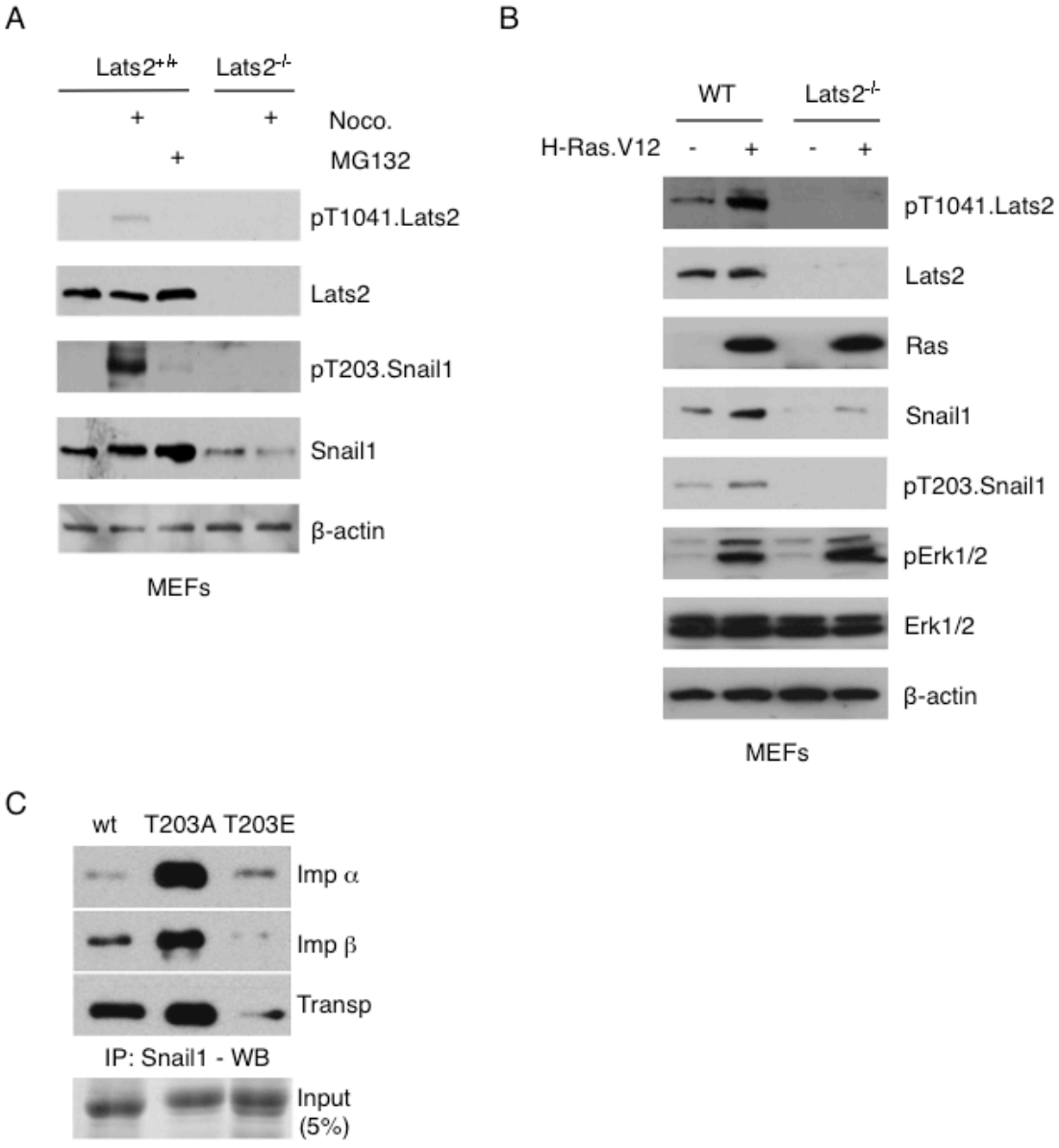
D



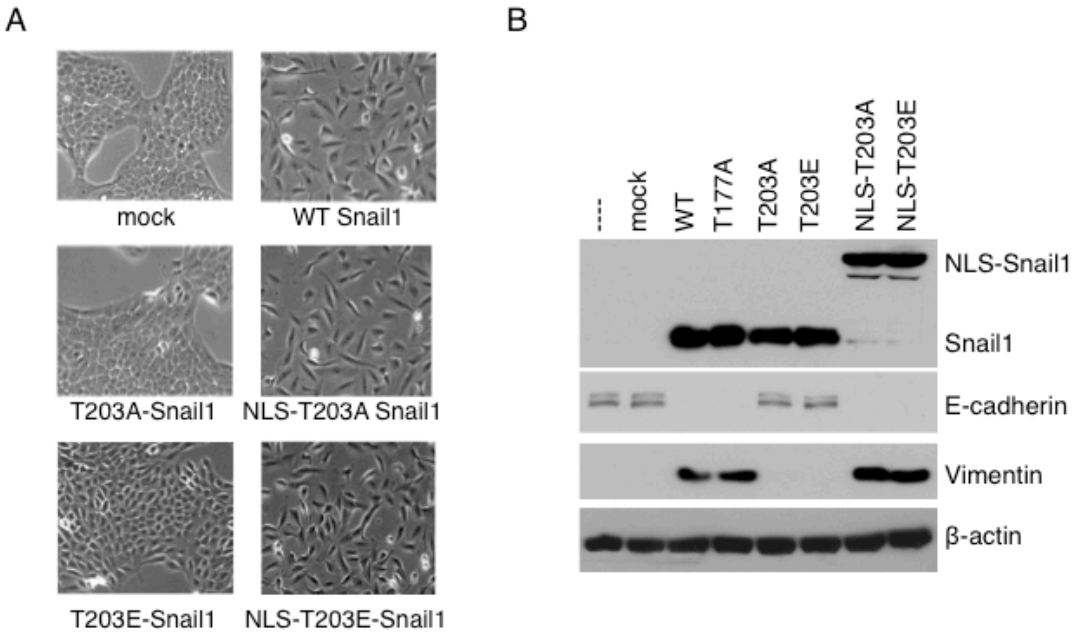
K. Zhang et al., Supplemental Figure 3



K. Zhang et al., Supplemental Figure 4

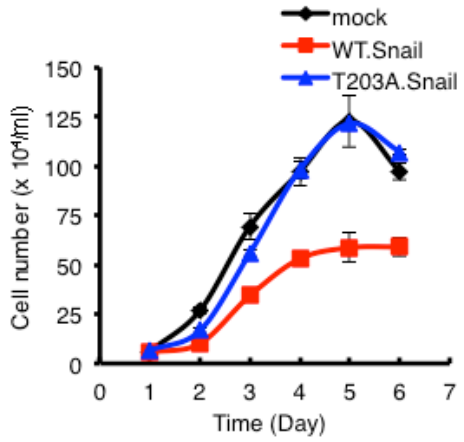


K. Zhang et al., Supplemental Figure 5

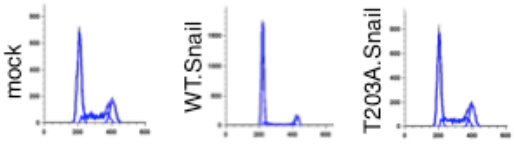


K. Zhang et al., Supplemental Figure 6

A



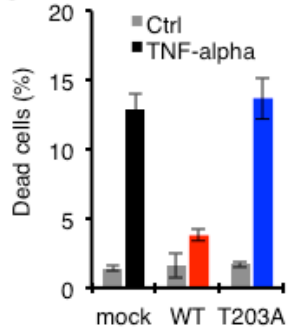
B



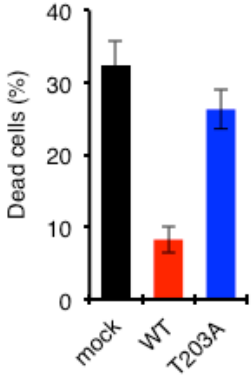
Cell Cycle Phase

MCF10A	G0/G1 (%)	S (%)	G2/M (%)
mock	48.6 ± 2.7	31.8 ± 0.6	18.4 ± 1.7
WT.Snail	66.1 ± 3.3	16.7 ± 1.4	13.0 ± 2.3
T203A.Snail	50.1 ± 3.2	30.1 ± 2.3	19.1 ± 2.4

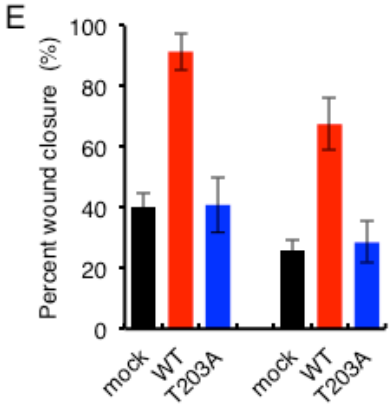
C



D



E



K. Zhang et al., Supplemental Figure 7