Supplemental Methods

Mass spectrometry

LC-MS-MS

Peptides were fractionated using a reverse phase BEH C18 column (1.7 μm, 75 μm x 150 mm, Waters) on a nanoUPLC Acquity system (Waters) using buffer A (2% acetonitrile, 0.1% formic acid) and buffer B (98% acetonitrile, 0.1% formic acid). Analytes were eluted over a 30 min linear gradient of 0-60% of solvent B with 300 nl/min flow rate. The nanoUPLC instrument was coupled to a 4000QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer (AB SCIEX, Framingham, MA). Eluted peptides were ionized in positive mode using a fused silica PicoTip emitter (New Objective, Woburn, MA) with a spray voltage of 2300 V, curtain gas of 13, nebulizer gas of 13, interface heater temperature of 180°C, unit resolution for Q1 and Q3 for MRM mode, and at low resolution in enhanced product ion scan. The method for the 4000QTRAP mass spectrometer includes a MIDAS algorithm for dependent Enhanced Product Ion scans (MS/MS), which are triggered when the MRM signal exceeds a threshold (typically 50 counts/s). Precursor ions were dynamically excluded for one minute after two occurrences.

Database search

MS/MS fragment ion data were searched against a human database using the Mascot algorithm (Matrix Sciences, London, UK) in ProteinPilot 3.0 software (AB SCIEX, Framingham, MA) with a mass tolerance for precursor ions of 0.05 Da, fragment ion tolerance 0.6 Da, no missed cleavage, carboxymethylation as a fixed modification for cysteines, and oxidation for methionine as variable modifications. In order to match experimental data to the translational start of AIB1-Δ4 the database search was performed using semi-trypsin pattern of fragmentation, where tryptic specificity was assigned at C-terminus and the N-terminus was allowed to be a non-tryptic cleavage and additional variable modifications such as N-terminal peptide acetylation and de-methionation.

Immunofluorescence for AIB1 KO MEFs

$2x10^6$ AIB1 KO MEFs were transiently transfected with 4 μg FLAG AIB1-Δ4 alone and either 2, 4, or 6 μg of p300-HA or AIB1 using the MEF 2 Nucleofector kit (Amaxa, Lonza) and plated on glass coverslips after transfection. 24 hours later cells were fixed, permeabolized, stained, mounted, and quantified as CHO cells in experimental methods. p300 (1:500, Abcam) and anti-rabbit AlexaFluor594 (1:1000, Invitrogen) were used to stain for p300-HA. Stained AIB1 KO MEFs were analyzed on Olympus Fluoview-FV300 Laser Scanning Confocal System in the Microscopy and Imaging Shared Resource at Georgetown.

ChIP in T47D A1-2 cells

T47D A1-2 cells were transfected with either FLAG AIB1-Δ4 or FLAG empty vector. 24 hours later cells were stimulated with 10nM R5020 for 1 hour and lysates were processed as described above. To examine the binding of AIB1-Δ4 to the MMTV promoter endpoint PCR was used with the following conditions 96°C 4 minutes followed by 30 cycles of 94°C 1 min, 60°C 1 min, 72°C 1 min. Primer sequences in the MMTV promoter were MMTV s: 5'-CGGTTCCCAGGGCTTAAGTAAGTT-3' and MMTV as: 5'- GGATGGCGAACAGACACAAACACA-3'.