Supplemental Materials and Methods

RT-qPCR

1µg of RNA was reverse transcribed using Reverse Transcriptase (NEB; Frankfurt, Germany) with Random Hexamer primers. Synthesized cDNA was further diluted five-fold with water. Quantitative PCR was performed using 2X Sybergreen mix (Thermofisher Scientific; Darmstadt, Germany) and Bio-Rad (Munich, Germany) system. Relative amount of mRNA was calculated by ΔΔCt method against β-Actin. The list of primers used in this study is in Supplemental Table S4.

Western blotting

Cell line samples were harvested using 1mM EDTA in PBS, and lysed using RIPA buffer (1% NP-40, 0.1% SDS, 0.5% Sodium deoxycholate, 50mM Tris-Cl pH 7.5, 150mM NaCl and 1mM $MgCl_2$) containing phosphatase inhibitor (Sigma-Aldrich) and Protease inhibitor cocktail (Roche; Mannheim, Germany) for 20 minutes on ice. For sciatic nerve tissue samples, samples were further physically lysed using dounce homogenizer. Samples were centrifuged at 16,000g and 4°C for 20min. Concentration of the lysates was measured by BCA method, and boiled with Laemmli buffer for 10 minutes. Boiled lysates were fractionated by SDS-PAGE. Transferred membranes were blocked with 5% skim milk and 0.1% Tween-20 in PBS, and incubated with primary antibodies overnight at 4°C. After washing (PBS with 0.1% Tween-20, three times), membranes were incubated with secondary antibodies at room temperature for 1 hour. After washing, membranes were developed using ECL solution (Amarsham; Little Chalfont, UK). The list of primary antibodies is in Supplemental Table S2. For secondary antibodies, anti-mouse and anti-

rabbit peroxidase (Cell Signaling) and anti-guine-pig peroxidase (Jackson ImmunoResearch; West Grove, PA, USA) were used. For quantification of western blot data, ImageJ software was used.

Chromatin immunoprecipitation

After removing media and washing once with PBS, cells were fixed with 1% paraformaldehyde in PBS at room temperature for 15 minutes. Fixation was then quenched by adding 125mM Glycine. Harvested cells were resuspended in hypotonic buffer (20mM HEPES, pH 7.9, 2mM KCl and 1mM DTT) and incubated on ice for 20 minutes. Plasma membrane was lysed by adding 0.3% Triton X-100 and briefly vortexing. Samples were centrifuged at 4°C for 1 minute to spin down the nucleus. Supernatant was discarded and nucleus was washed once with hypotonic buffer. Nucleus was then lysed with RIPA buffer and transferred to TPX tube (Daigenode; Liege, Belgium) for sonication. Sonication was performed using bio-ruptor at high power with 30 sec rest/sonication setting for 40-50 cycles. After centrifugation at 4°C for 20 minutes, supernatants were transferred to a new tube and DNA concentration was measured. 5µg chromatin was used for HDAC5 ChIP (Santa Cruz sc-133225; Dallas, TX, USA). After overnight antibody incubation, 20 µl Protein A/G beads (Thermofisher) were added for 1 hour. After washing the beads (three times with RIPA, one time with RIPA containing 500mM NaCl), chromatin was eluted using elution buffer (100mM sodium carbonate, 400mM NaCl, and 1% SDS) at 30 °C for 2 hours with vigorous shaking. Eluted samples were de-crosslinked at 65 °C overnight with RNase, and then 1 hour with Proteinase K at 60 °C. DNA was recovered using DNA gel elution column (Qiagen, city state).

For experiments using mouse sciatic nerve, hypotonic buffer was omitted and around 0.5µg chromatin was used for H3 (Abcam ab1791) and H3-Acetyl (Millipore 06-559) ChIP. Tissues were coarsely chopped using scissor in PBS followed by fixation and quenching. Samples were then resuspended in 300 µl SDS buffer (50mM Tris-Cl pH 7.5, 10mM EDTA and 1% SDS). After sonication, lysates were diluted ten times in dilution buffer (20mM Tris-Cl pH 7.5, 167mM NaCl, 1.2mM EDTA, 1.1% Triton X-100 and 0.1% SDS).

For experiments using rat sciatic nerve, sciatic nerves from 5-6 P14 rat pups were collected and pooled for each experiment. Tissues were minced and digested with 1:1 mixture of Type II collagenase and trypsin for 20 minutes. Cell clumps were removed using cell strainer, and 20µl of F4/80 microbead (Miltenyi Biotec 130-110-443; Bergisch Gladbach, Germany) was added for 6 sciatic nerve tissues. The rest of the depletion process was carried out following the manufacture's guide. Downstream procedures are the same as above. The same antibody was used in immunofluorescence and ChIP experiments. The amount of serum added to lysate was determined by measuring the efficiency of titrated anti-Maf serum to immunoprecipitate over-expressed Flag-Maf in 293T cells compared to 3 µg of anti-Flag (Sigma) antibody. ChIP efficiency was calculated by normalizing Ct of antibody to that of input. Final data are presented by normalizing values of controls as 1. The list of primers used in this study is in Supplemental Table S5.

In vivo ubiquitination assay

Flag-Maf and HA-Ub constructs were transfected in 1:10 ratio. Cells were incubated with TAK165 48 hours after transfection. On the next day, 20 µM MG132 was treated for 4 hours before

harvesting. Cells were lysed in RIPA buffer, and 1mg of lysates were incubated with an anti-Flag antibody (Sigma-Aldrich F1804) overnight.

Chemicals

Recombinant Nrg1β (396-HB-050) was purchased from R&D systems (Minneapolis, MN, USA). Trametinib (MEK1/2 inhibitor; S2673), U0126 (MEK1/2 inhibitor; S1102), MK2206 (AKT inhibitor; S1078), Ruxolitinib (JAK inhibitor; S1378), Go6983 (PKC inhibitor; S2911), and TAK165 (ErbB2 inhibitor; S2216) were purchased from Selleckchem (Munich, Germany). MβCD Methyl-β-cyclodextrin (Cholesterol depleting reagent; C4555), MG132 (Proteasome inhibitor; SML1135), Chloroquine (Lysosome inhibitor; C6628), Cyclosporin A (PP2B inhibitor; 30024), U-73122 (PLCγ inhibitor; U6756), Xestospongin C (IP3R antagonist; X2628), STO-609 (CaM-KK inhibitor; S1318), and Ionomycin (Ca^{2+} inophore; I9657) were purchased from Sigma-Aldrich (Hamburg, Germany). Myr-AIP II (CaMKII inhibitor; 189482) was purchased from Millipore (Darmstadt, Germany).