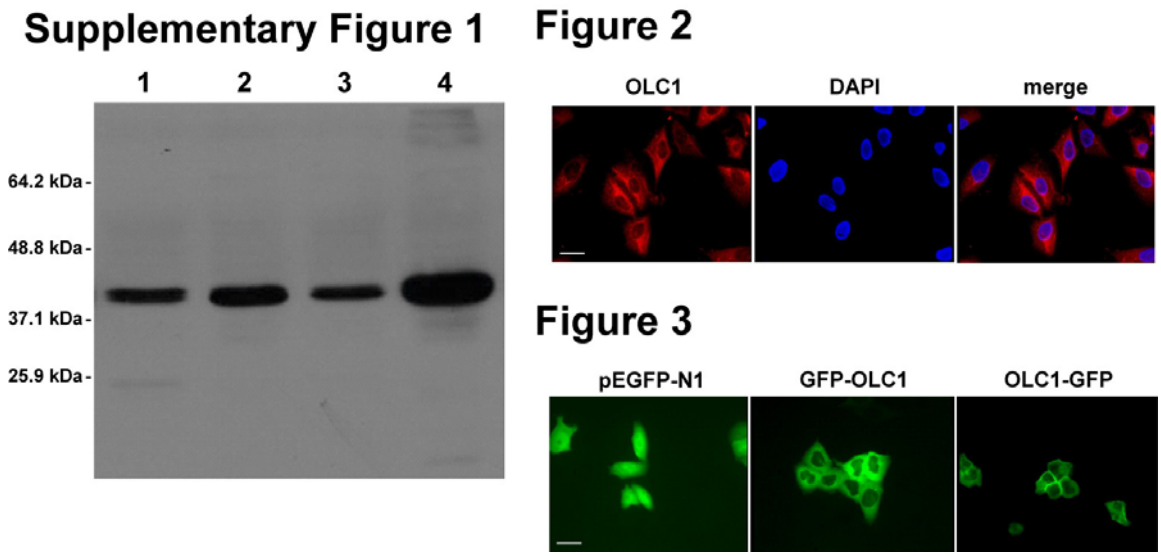


Supplementary Figure Legends

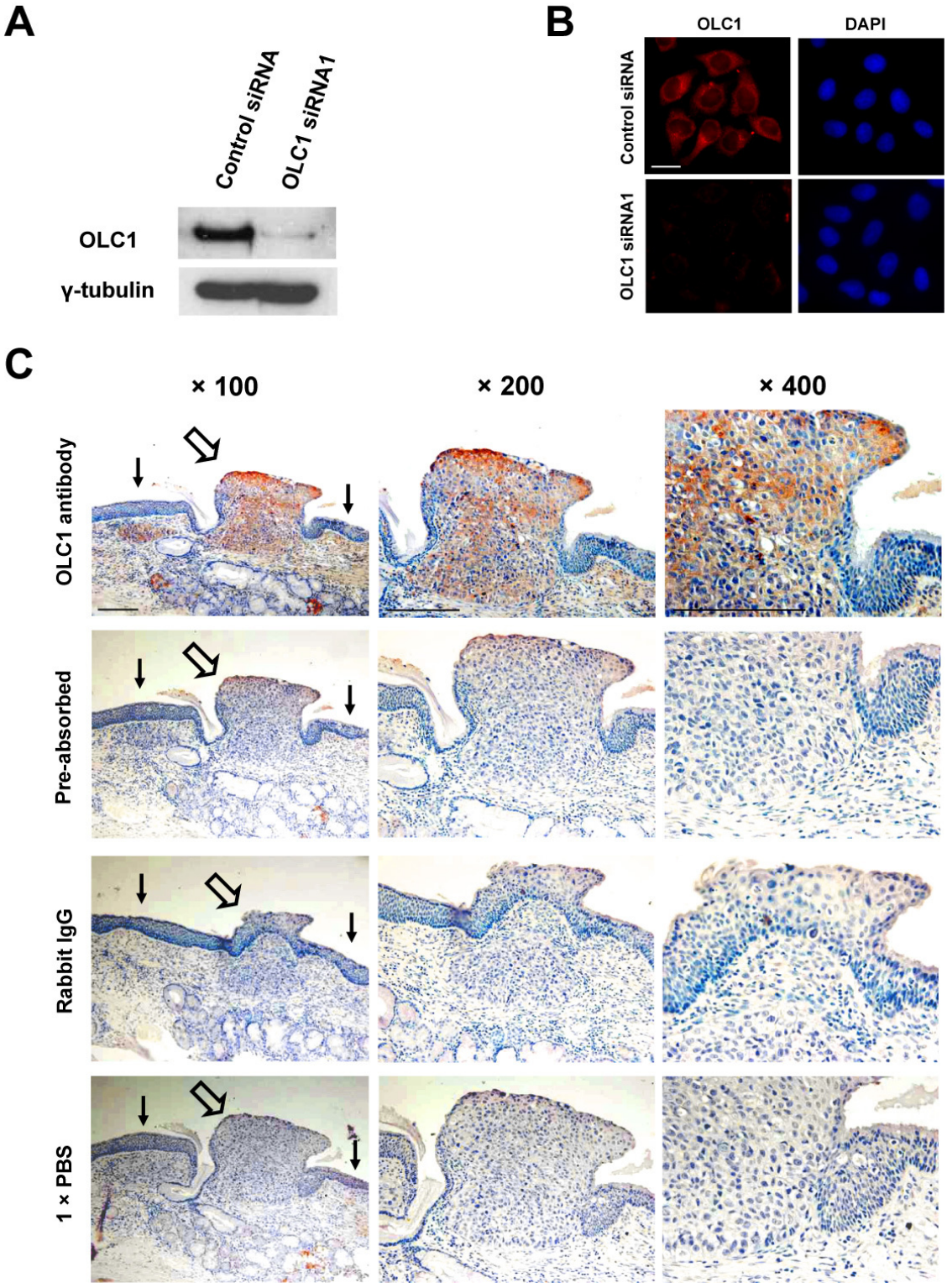
Supplementary Figure 1. Immunoblotting analysis of OLC1 protein. A rabbit polyclonal anti–OLC1 antibody was used at a dilution of 1:1000. Lane 1: HeLa cell lysates; lane 2: H1299 cells; lane 3: H520 cells; lane 4: H520 cells harvested 24 hours after transfection with OLC1 DNA. Each lane contains 50 μg of total protein.

Supplementary Figure 2. Immunofluorescence staining of OLC1. Immunofluorescence staining of H520 cells was performed using the rabbit polyclonal anti–OLC1 antibody (1:100) and tetramethylrhodamine isothiocyanate-labeled secondary anti–rabbit IgG antibody (1:100, Santa Cruz). Cell preparation and staining procedures were conducted according to standard protocols. 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) was used for nuclear staining. Cells were visualized using a fluorescence microscope. Scale bar=20 μm.

Supplementary Figure 3. Green fluorescent protein (GFP) fusion localization of OLC1. OLC1 was subcloned into the pEGFP-N1 and pEGFP-C3 vectors (Clontech, Mountain

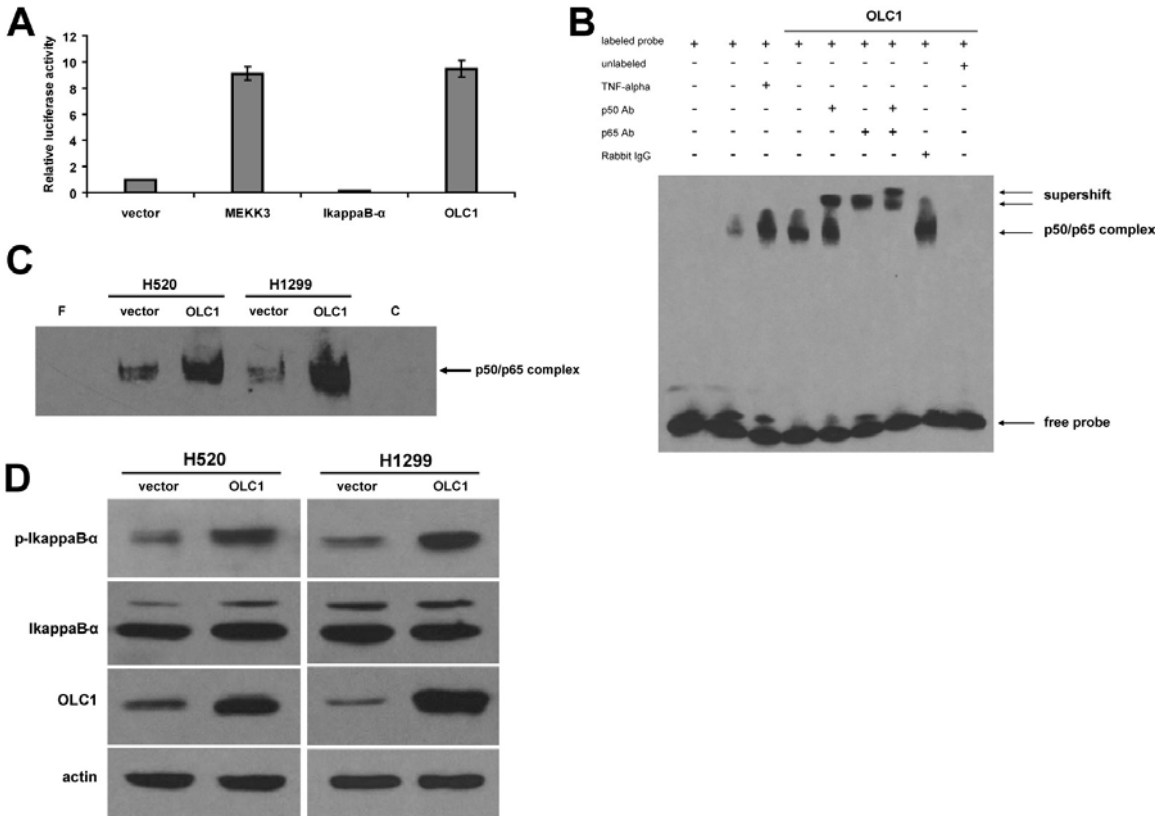
View, CA) to create OLC1-GFP and GFP-OLC1. OLC1-GFP, GFP-OLC1, and the pEGFP-N1 parent vectors were transfected into H520 cells. Twenty four hours after transfection, GFP fluorescence was visualized using a fluorescence microscope. Scale bar=20 μm.

Supplementary Figure 4



Supplementary Figure 4. The specificity of the rabbit polyclonal anti–OLC1 antibody. **A**) OLC1 siRNA1 induced OLC1 protein knockdown in H520 cells. Cells were harvested 48 hours after siRNAs transfection and subjected to immunoblotting as in Supplementary Figure 1. γ-tubulin was used as a control for protein loading and transfer. **B**) Immunofluorescence staining of OLC1. Immunofluorescence staining was performed as Supplementary Figure 2. Cells were fixed 36–48 hours after siRNAs transfection. Scale bar=20 μm. **C**) Serial sections from a lung squamous cell carcinoma tissue sample. Immunohistochemical staining of OLC1 was performed with antibody pre-absorbed with antigen, nonimmune rabbit IgG globulin, and phosphate-buffered saline (1 × PBS) as controls. **Open arrows** indicate early invasive carcinoma and **black arrows** indicate the nearby normal bronchial epithelia. Figures with ×100, ×200 and ×400 amplification are shown. Scale bars=100 μm.

Supplementary Figure 5

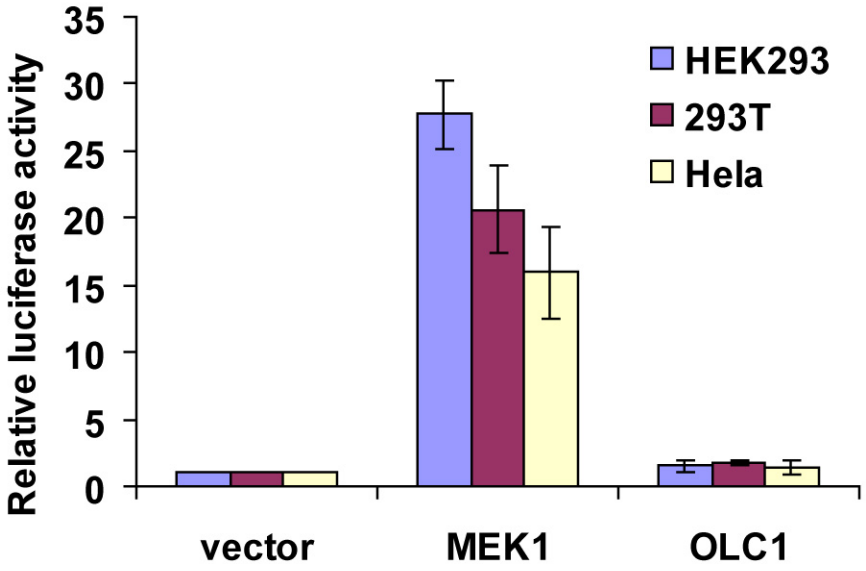


Supplementary Figure 5. Effect of OLC1 overexpression on NF-kappaB. **A**) Reporter assay based signaling pathway screening in HeLa cells. Briefly, 1.5×10^6 Hela cells were seeded onto a 96-well-plate. Twenty hours later, the cells were cotransfected with 62.5 ng of candidate expression vector, 62.5 ng pNF-κB-luc luciferase reporter vector (Stratagene), and 6.25 ng phRL (Renilla luciferase reporter vector, as an internal control, Promega) using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Twenty-four hours after transfection, the cells were harvested in passive lysis buffer (Promega), and the activities of firefly and Renilla luciferase were quantified using the dual luciferase reporter assay system (Promega) according to the manufacturer's instructions. Cells that were transfected with expression vectors for the known NF-kappaB

modulators, MEKK3 and IkappaB-α, served as stimulating and inhibiting controls, respectively. Mean values and 95% confidence intervals (error bars) of reporter activity (fluorescence intensity relative to empty vector transfected cells, set as 1.0) are shown from three independent experiments. **B)** Electrophoretic mobility shift and supershift assays. We performed these assays using nuclear protein extracts prepared with the NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce). The NF-kappaB double-stranded oligonucleotides were annealed using standard procedures. The protein-DNA binding reaction was performed using the LightShift Chemiluminescent EMSA Kit (Pierce): nuclear protein (10 μg) in 2.5% glycerol, 1 μg of poly dI-dC, 50 mM KCl, and 5 mM $MgCl_2$ were incubated on ice for 10 minutes and then at room temperature for 20 minutes. Electrophoresis was done at room temperature for 1.5 hours at 100V, and the probes were transferred from the gel to nylon membranes (Millipore, Bedford, MA). The biotin labeled DNA was detected with the streptavidin-conjugated horseradish peroxidase, according to the manufacturer's instructions. For supershift assays, the following antibodies were used: rabbit polyclonal anti–p50 and rabbit polyclonal anti–p65 (Santa Cruz Biotechnology, Santa Cruz, CA). All lanes contain the labeled NF-kappaB probe. **Arrows** indicate the complexes supershifted by anti–p50, –p65 antibodies, or the NF-kappaB complex. Cells that were treated with TNF-α at 10 ng/mL for 6 hours were used as a positive control and those nuclear protein extracts that were incubated with normal rabbit IgG were used as a negative control for the p50 and p65 antibodies. A representative dataset of three independent experiments is shown. **C**) Electrophoretic mobility shift assay using H520, H1299, and MBE cells. The **arrow** indicates the NF-kappaB complex. A representative dataset of three independent experiments is shown.

D) Effect of OLC1 overexpression on phosphorylation of IkappaB-α. H1299 and H520 cells that were transfected with OLC1 DNA were harvested 24 hours after transfection and subjected to immunoblotting using rabbit polyclonal anti–IkappaBα (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA), rabbit polyclonal anti–phospho-IkappaBα (Ser32) (1:1000, Cell Signaling, Danvers, MA), rabbit polyclonal anti–OLC1 (1:1000) prepared above, and mouse monoclonal anti–β-actin (1:5000, Sigma-Aldrich). Actin was used as a control for protein loading and transfer. A representative blot of three independent experiments is shown.

Supplementary Figure 6



Supplementary Figure 6. Reporter assay analysis of the MAPK pathway activating function of OLC1. The experiments were performed as the NF-kappaB reporter assay described in Supplementary Figure 5. The pcDB-OLC1 plasmid was transfected together with pFA2-Elk1 (Stratagene), which contains the GAL4-Elk1-fusion gene. A plasmid

containing the GAL4 binding sequence upstream of the luciferase gene, pFR-Luc (Stratagene), was used as the reporter. Cells that were also transfected with a vector expressing MEK1, a known MAPK modulator, were used as a positive control. The assays were performed in HEK293, 293T, and HeLa cells. Mean fluorescence intensity and 95% confidence intervals (error bars) are shown from three independent experiments.