Supplementary Documents:

Supplementary Figure S1. EGF stimulation induces EGFR phosphorylation in SK-N-BE cells. A)SK-N-BE cells were cultured in serum-free medium for 12 h. Cells were then treated with EGF at 1, 5, 10, 50 and 100 ng/mL for 10 minutes. Whole cell lysates were used to detect phospho-EGFR and total EGFR by immunoblotting with specific antibodies. B) Western blot data shows that at concentration of 5 μM, U0126 can inhibit EGF-mediated upregulation of p-ERK. Serum starved SK-N-BE cells were pretreated with U0126 at the indicated concentration for 3 h and EGF treatment was carried out to induce p-ERK for 12 h. Expression of total ERK was not changed and used as loading control. C) AG1478 at concentration of 5 μM can inhibit phosphorylation of EGFR. Serum starved SK-N-BE cells were pretreated with AG1478 and then subjected to EGF treatments for 12 h. Western blot experiments were performed to see the expression of p-EGFR. Total EGFR was used as loading control.

Supplementary Figure S2. siRNA-mediated knockdown of Sp1 reduces endogenous expression of *MYCN*. A, SK-N-BE cells were transiently transfected with control siRNA and two siRNAs (si #1 and si #2) against *Sp1*. Seventy-two hours after transfection, total RNA was used to check the mRNA expression of *Sp1* and *MYCN* by RT-PCR (left). Whole cell lysates were used for immunoblotting with specific antibodies against Sp1 and MYCN (right). GAPDH and actin were used for internal loading controls. The data represent the three independent experiments. B, *MYCN* core promoter (CP) luciferase construct was co-transfected with control siRNA and siRNA raised against *Sp1* in SK-N-BE cells. At 72 hours after transfection, firefly luciferase assays were performed to determine relative promoter activities. C, SK-N-BE cells were transfected with control siRNA and siRNA against *Sp1*. Forty-eight hours after transfection, cells were transferred to 96-well dishes (1000 cells/well). WST-8 assays were performed on the indicated days to determine cell proliferation. This experiment was performed on three independent occasions.

Supplementary Figure S3. Phosphorylation of Sp1 is important for its transcriptional efficiency to induce *MYCN*. A, Sp1 was phosphorylated following EGF treatment. SK-N-BE cells were cultured in serum-free medium with or without CIAP (50 unit/mL) and U0126 (5 μM) for 12 h, followed by treatment with EGF (20 ng/mL). At 12 hours after EGF treatment, whole cell lysates were collected and used for immunoblotting with specific antibodies. B, Mithramycin-A effectively inhibits EGF-mediated *MYCN* induction. SK-N-BE cells were cultured in serum-free medium with or without Mithramycin-A at different doses for 12 h. Cells were then treated with EGF for 6 h, and RT-PCR was performed to detect endogenous expression of *MYCN*. Expression of *GAPDH* was used as an internal loading control. C, Dephosphorylating agent inhibits *MYCN* induction upon EGF treatment. SK-N-BE cells were cultured in serum-free medium with or without EGF (20 ng/mL) for 16 h. During the starvation, CIAP was added at 0, 10 and 15 h corresponding to the CIAP treatment of 12, 6 and 1 h. Total RNA isolated from the cells was subjected to RT-PCR. D, Dephosphorylating agent inhibits Sp1 recruitment to the *MYCN* promoter. A similar experiment was performed as in panel C, and cross-linked chromatin was isolated from the cells and used to pull down with specific Sp1 antibody or with normal rabbit serum (NRS). Pulled-down chromatin was then used for amplification by PCR using a primer set targeting *MYCN* core promoter region (-221, +21).

Supplementary Figure S4. NLRR1-stably expression in cells leads to oncogenic characteristics. A, Colony formation assays. SK-N-BE cells were transiently transfected with mock- and NLRR1-expressing plasmids. G418-resistant colonies were stained (top panel) and counted as the relative number of colonies between the mock- and NLRR1-transfected cells (bottom panel). B, RT-PCR assays. Total RNA was extracted from three mock- and NLRR1-stably expressing SH-SY5Y cell clones and subjected to RT-PCR. mRNA expression of *NLRR1* and *NMYC* was checked by PCR using specific primer sets. C, NLRR1-stably expressing clones proliferate faster than the mock clones. Mock- and NLRR1-expressing SH-SY5Y clones were subjected to WST-8 assays to check proliferation on the indicated days. The data represent three independent experiments and plotted as mean ± SD.

Supplementary Materials and Methods:

Colony Formation Assays. SK-N-BE cells cultured in six-well culture dishes were transiently transfected with 500 ng expression plasmid encoding MYC-NLRR1 or empty plasmid (pcDNA3.1Myc-His). Forty-eight hours after transfection, cells were cultured in medium supplemented with G418 (1000 μg/ml). Every 3 days, medium was replaced with fresh medium containing G418. After 2 weeks of selection, the viable colonies were stained with Giemsa's solution. Stained colonies were scored.

RNA Interference. To knock down Sp1 in the cells, siRNA targeting Sp1 was transfected in SK-N-BE cells with control siRNA using Lipofectamine RNAiMAX (Invitrogen). Seventy-two hours after transfection, total RNA and whole cell lysates were prepared. Two siRNAs (Si#1 and Si#2) targeting Sp1, named On-TARGETplus Duplex (Catalog number J-026959-05 and J-026959-06), along with control siRNA, were purchased from Dharmacon.

Antibodies for Immunoblotting. Whole cell lysates were prepared as mentioned in the main text. Membranes were incubated with monoclonal anti-phospho EGFR (1H12; Cell Signaling, Beverly, MA), anti-EGFR (Rockland, Gilbertsville, PA), anti-Sp1 (DAM1718081, Upsate/Millipore) and anti-E2F1 (KH95, Santa Cruz Biotechnology) overnight at 4 °C. Other procedures and antibodies used for immunoblotting are described in the main text.

Reagents. Mithramycin-A, a specific inhibitor of Sp1, was purchased from Sigma Chemicals Co. Calf intestinal alkaline phosphatase (CIAP) was purchased from Takara Bio Inc.