**SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental Figure 1. Keratin staining of cervical lymph nodes from Ndrg2-deficient mice treated with 4-NQO.**

Representative IHC images showing no metastasis of SCC in the cervical lymph nodes of the mice.

**Supplemental Figure 2. Effect of 4-NQO treatment on an OSCC cell line was observed through the activation of the AKT signalling pathway and cell migration ability.**

(A) Activation of the AKT signalling pathway was observed by western blot analysis at each indicated time after treatment of SAS/OSCC cell line with 4-NQO.

(B) The cell migration ability was determined in SAS/OSCC cells using an in vitro scratch assay 12 hours after treatment with various amounts of 4-NQO. Black lines indicate the initial edge of the cells and red arrows indicate the migrated cells after the scratch was made.

(C) HaCaT cell lines expressing shRNA against NDRG2 or luciferase as a control were cultured in serum-free DMEM for 24 hours. The quiescent cells were treated with 4-NQO in serum-free medium and were subjected to Western bolt analysis of PI3K/AKT signaling pathway.

(D) Cell migration ability under four different conditions with/without expression of NDRG2 and with/without 4-NQO treatment in HaCaT cells was determined by in vitro scratch assays. Images show HaCaT cells under four different conditions on the glass slide; the black lines indicate the initial edge of the cells, while the red arrows indicate the migrated cells after the scratch was made. The bar graph illustrates the relative migration rates of HaCaT cells under the four different conditions indicated under the bar.

(E) The images show migrated HaCaT/shNDRG2 cells under the four different conditions indicated in the figure; these cells were stained with crystal violet in a cell invasion assay using Boyden chambers (magnification, ×100). The bar graph shows the number of migrated cells under the four different conditions. Star (\*) indicates statistical significance of p<0.05.

(F) Activation of the AKT signalling pathway in MEF cells from wild-type mice treated with 4-NQO was observed along with increased levels of phosphorylated AKT, GSK3β and S6 up to 20 minutes; however, the constitutive activation of the AKT signalling pathway was observed in MEF cells from Ndrg2-deficient mice before and after the treatment with 4-NQO.

(G) A cell migration assay was performed in HSC3/OSCC cells with or without NDRG2 expression and 4-NQO treatment. The activation of cell migration by 4-NQO was completely suppressed by forced NDRG2 expression, which can be observed in microscopy images (top) and according to the distance the cells migrated (bottom). Black lines indicate the initial edge of the cells and red arrows indicate the migrated cells after the scratch was made. Star (\*) indicates statistical significance at p<0.05.

(H) The activation of invasion by HSC3/OSCC cells after 4-NQO treatment was suppressed by forced NDRG2 expression, which was observed by staining the migrated cells (top) and the number of cells that migrated (bottom). Star (\*) indicates statistical significance at p<0.05.

**Supplemental Figure 3.** **Immunofluorescence staining of E-cadherin, Vimentin, and NDRG2 in OSCC cell lines for determining EMT progression by treatment with 4-NQO**

(A, B) Immunofluorescence staining was used to determine the expression of NDRG2 and E-cadherin **(A)**,and NDRG2 and Vimentin **(B)** before and after the treatment of SAS/OSCC cells, with or without NDRG2 expression, with 4-NQO. The nuclei were stained with DAPI (blue), and each protein was stained by each specific antibody-conjugated fluorescent dye; NDRG2 by Alexa flour 488 (green), E-cadherin by Alexa flour 623 (yellow), and Vimentin by Alexa flour 555 (red).

(C) Images show the morphology of HaCaT cells with or without NDRG2 expression before and 24 hours after the 4-NQO treatment.

(D) Reverse-transcription PCR (RT-PCR) analysis of EMT-related genes in 4-NQO-treated HaCaT.

(E) Quantitative RT-PCR analysis of EMT-related targets mRNA in HaCaT/shluc and HaCaT/shNDRG2. Star (\*) indicates statistical significance at p<0.05. The data represent at least three experiments performed in triplicate.

**Supplemental Figure 4. Activation of NF-κB signalling in SAS/OSCC cell line treated with 4-NQO.**

(A) Activation of NF-κB signalling was determined at the level of the nuclear transfer of p65, an NF-κB transcription factor, before and 2 hours after 4-NQO treatment under low (mock) or high NDRG2 expression conditions. The cytosolic fraction was confirmed by β-actin and the nuclear fraction was confirmed by histone H1.

(B**)** HaCaT cell lines expressing shRNA against NDRG2 or luciferase as a control were cultured in serum-free DMEM for 24　hours. The quiescent cells were treated with 4-NQO in serum-free medium and were subjected to Western bolt analysis of NF-B signaling pathway.

(C) Cytosolic and nuclear proteins were subjected to Western bolt analysis as indicated.

(D) Cells were transfected with pNF-κB-Luc and pRL-TK plasmid, stimulated with 4-NQO, and then subjected to NF-κB reporter assays. Star (\*) indicates statistical significance at p<0.05.

(E) Images show the morphology of SAS cells with or without BAY 11-7082 before and 24 hours after the 4-NQO treatment.

**Supplemental Figure 5.** **Hypothetical model for the molecular mechanism of EMT progression in OSCC cells.**

The scheme depicts EMT activation by 4-NQO in the development and metastasis of OSCC in the absence of NDRG2 expression due to the activation of NF-κB signalling after PI3K/AKT activation.