SUPPLEMENTARY FIGURE LEGEND

Figure S1. Twist1 upregulates Bmi1 and aurora kinase A expression, and co-amplification of *BMI1* and *AURKA* in $CD44^+$ HNSCC cells. (A) Western blot of Twist1, Bmi1 and aurora kinase A in FaDu cells transfected with an empty vector (EV) or a Twist1-expressing vector. (B) Western blot of Twist1, Bmi1 and aurora kinase A in OECM-1 cells transfected with a shRNA against a scrambled sequence (sh-scr) or Twist1 (sh-Twist1). β-actin was a loading control for western blot. (C) Left, representative result of flow cytometry for sorting the $CD44^+$ cells in OECM-1 cells. Right, relative mRNA levels of *BMI1* and *AURKA* in $CD44^+$ versus $CD44^-$ OECM-1 cells. (D) Left, representative result of flow cytometry for sorting the $CD44^+$ cells in a primary HNSCC culture. Right, relative mRNA levels of *BMI1* and *AURKA* in $CD44^+$ versus $CD44^-$ cells in a primary HNSCC culture. All experiments were triplicated. Data represents mean ± S.E.M. $^*P < 0.05$, $^{**}P < 0.01$.

Figure S2. Akt pathway and let-7i repression are two independent mechanisms induced by Bmi1 to regulate aurora kinase A expression. (A) Western blot of aurora kinase A in OECM-1 cells transfected with miR-15b or a control sequence. β-actin was a loading control. (B) Quantitative RT-PCR for examining the relative let-7i expression in FaDu-CDH, FaDu-Bmi1 treated with a vehicle control (DMSO) or different doses of LY294002. All experiments were triplicated. Data represents mean ± S.E.M. $^{***}P < 0.001$. (C) Western blot of Bmi1, total Akt, phosphorylated Akt (serine 473), total GSK-3β, phosphorylated GSK-3β (serine 9), β-catenin, and aurora kinase A in SAS cells receiving a shRNA against Bmi1 (SAS-sh-Bmi1) or a scrambled sequence (SAS-sh-scr). β-actin was a loading control. (D) Quantitative RT-PCR for examining the relative let-7i level in SAS-sh-Bmi1-1 vs. SAS-sh-scr (n=3). Data represents mean ± S.E.M. $^{**}P < 0.01$.

Figure S3. Suppression of Bmi1 or aurora kinase A reduces centrosomal amplification, aneuploidy, reduces p53 serine 315 phosphorylation and stabilizes p53 in OECM-1 cells. (A) Representative results of γ-tubulin immunoflouresent staining in OECM-1 cells transfected with a control sequence for shRNA experiment (OECM1-sh-scr), a shRNA against *BMI1* (OECM1-sh-Bmi1) or *AURKA* (OECM1-sh-AURKA). The green signals represent γ-tubulin staining, whereas the blue signals represent nuclear DNA staining by DAPI. The arrows indicate the locations of centrosomes. HPF, high power field. Scale bar = 50μm in the first to third column, and 25μm in the fourth column. (B) Quantification of centrosome

analysis in OECM1-sh-scr vs. OECM1-sh-Bmi1 (left), and OECM1-sh-scr vs. OECM1-sh-AURKA (right). The results were presented as percentage of cells with 1, 2, or >2 centrosomes (100 cells were counted for each clone). The experiments were triplicated. Data represents mean ± S.E.M. $**P < 0.01$, $*** P < 0.001$. (C) Upper: representative pictures of karyotype analysis in OECM-1 cells transfected with a control sequence for shRNA experiment (OECM1-sh-scr) or a shRNA against *BMI1* (OECM1-sh-Bmi1). The number of chromosomes was shown in each panel. Lower: quantification of the percentage of cells with aneuploidy. (D) Upper: representative pictures of karyotype analysis in OECM-1 cells transfected with a control sequence for shRNA experiment (OECM1-sh-scr) or a shRNA against *AURKA* (OECM1-sh-AURKA). The number of chromosomes was shown in each panel. Lower: quantification of the percentage of cells with aneuploidy. For each experiment, 30 cells were counted to determine the percentage of aneuploidy. The experiments were triplicated in each clone. Data represents mean ± S.E.M. $*P < 0.05$. (E) Upper: western blot of Bmi1, aurora kinase A, and p53 in OECM-1 cells receiving shRNA against *BMI1* (OECM1-sh-Bmi1) or a scrambled sequenced (OECM1-sh-scr). Lower: western blot for detecting p53 serine 315 phosphorylation in the above cells treated with a proteasome inhibitor MG132. β-actin was a loading control. (F) Upper: western blot of Bmi1, aurora kinase A, and p53 in OECM-1 cells receiving shRNA against *AURKA* (OECM1-sh-AURKA) or a scrambled sequenced (OECM1-sh-scr). Lower: western blot for detecting p53 serine 315 phosphorylation in the above cells treated with a proteasome inhibitor MG132.

Figure S4. Knockdown of aurora kinase A represses EMT, and overexpression of aurora kinase A is sufficient to induce EMT. (A) Western blot of Bmi1, aurora kinase A, epithelial markers (E-cadherin and γ-catenin) and mesenchymal markers (N-cadherin and vimentin) in OECM-1 cells transfected with a control sequence for shRNA experiment (OECM1-sh-scr) or a shRNA against *AURKA* (OECM1-sh-AURKA). β-actin was a loading control. (B) Immunofluorescent staining of E-cadherin (red) in OECM1-sh-scr and two OECM1-sh-AURKA clones. Blue: nuclear DNA staining by DAPI. Scale bar = 20μm. (C) Upper: representative photos of wound healing assay after 0 or 48 hours. Lower: fold change of migrated cells in wound healing assay. The experiments were triplicated. Data represents mean ± S.E.M. $**P < 0.01$. (D) Upper: representative pictures of transwell invasion assay. Lower: fold change of invaded cells in transwell invasion assay. The experiments were triplicated. Data represents mean ± S.E.M. $*P < 0.05$. (E) Western blot of Bmi1, aurora kinase A, epithelial markers (E-cadherin and γ-catenin) and mesenchymal markers (N-cadherin and vimentin) in FaDu cells transfected with a control vector (FaDu-CDH) or an aurora kinase A expressing vector (FaDu-AURKA). β-actin was a loading control. (F) Immunofluorescent staining of E-cadherin (red) in FaDu-CDH and FaDu-AURKA. Blue: nuclear DNA

staining by DAPI. Scale bar = 20μm. (G) Upper : representative photos of wound healing assay after 0 or 18 hours. Lower: fold change of migrated cells in wound healing assay. The experiments were triplicated. Data represents mean ± S.E.M. *$P < 0.05$. (H) Upper: representative pictures of transwell invasion assay. Lower: fold change of invaded cells in transwell invasion assay. The experiments were triplicated. Data represents mean ± S.E.M. *$P < 0.05$.

Figure S5. The influence of aurora kinase A on the expression of different EMT regulators. (A) Western blot of Bmi1, aurora kinase A and different EMT regulators (Slug, Zeb1, SIP1, Twist1) in FaDu cells transfected with a control vector (FaDu-CDH), a Bmi1-expressing vector and a control sequence for shRNA experiment (FaDu-Bmi1-sh-scr), or a Bmi1-expressing vector and a shRNA against *AURKA* (FaDu-Bmi1-sh-AURKA). β-actin was a loading control. (B) Western blot of Bmi1, aurora kinase A and different EMT regulators (Snail, Slug, Zeb1, SIP1, Twist1) in OECM-1 cells transfected with a control sequence for shRNA experiment (OECM1-sh-scr) or a shRNA against *AURKA* (OECM1-sh-AURKA). β-actin was a loading control. (C) Western blot of aurora kinase A and different EMT regulators (Snail, Slug, Zeb1, SIP1, Twist1) in FaDu cells transfected with a control vector (FaDu-CDH) or a aurora kinase A-expressing (FaDu-AURKA). β-actin was a loading control. (D) Relative expression level of *AURKA* and *SNAI1* in FaDu-CDH vs. FaDu-AURKA (n=3). Data represents mean ± S.E.M. **$P < 0.01$.

Figure S6. Aurora kinase A increases GSK-3β phosphorylation and interacts with GSK-3β in HNSCC cell lines. (A) Western blot of Bmi1, aurora kinase A, total GSK-3β, and serine 9-phosphorylated GSK-3β in OECM-1 cells transfected with a control sequence for shRNA experiment (OECM1-sh-scr) or a shRNA against *AURKA* (OECM1-sh-AURKA). (B)-(E) Co-immunoprecipitation assays in HNSCC cell lines. An anti-aurora kinase A (upper) or an anti-GSK-3β (lower) antibody was used to pull down endogenous proteins from OECM-1 (B), FaDu (C), SAS (D) or CAL-27 (E). The arrows indicate the pull-downed GSK-3β or aurora kinase A. An IgG was used as a control for immunoprecipitation.