Supplement Table 1. List of primer sequences used for RT-PCR analysis in this study. F: Forward primer. R: reverse primer.

Genes	Primer sequences
Hsp90-alpha	F：5’ - TGGACAGCAAACATGGAGAG -3’ R：5’ - AGACAGGAGCGCAGTTTCAT -3’
Hsp90-beta	F：5’ - AGTTGGAATTCAGGGCATTG -3’ R：5’ - TTTCTCGGGAGATGTTCAGG -3’
Hsp90-B1 / Gp96	F：5’ - AAGGAGAAGAACCTGCTGCATG -3’ R：5’ - TGGGCTCCTCAACAGTTTCAGT-3’
Hsp70-A5 / Grp78	F：5’ - CGCCTGCAAGTCGGAAATT -3’ R：5’ - TCTTTGTTTGCCCACCTCCA -3’
HSP60	F：5’ - TAAAAGGAAAAGGTGACAAGG -3’ R：5’ - GGGCATCTGTAACTCTGTCTT -3’
GANAB	F：5’ - CGGCAATATGCTAGTCTCAC -3’ R：5’ - TGTCATTCCAGACAAAGAGG -3’
HYOU1	F：5’ - AAGGCTCACTTCAACCTGGA -3’ R：5’ - TCTCCTCTTCCTCCTGGACA -3’
Actin	F：5’- GTGGGGCGCCCCAGGCACCA -3’ R：5’- CTCCTTAATGTCACGCACGATTTC -3’

Supplement Table 2. Sequence design of shRNAs used in this study. For each gene, a 15-18 sense and antisense complementary hairpin oligonucleotide was generated against a specific mRNA sequence of each gene. This hairpin oligonucleotide included two restriction enzyme cleavage sites corresponding to the cleavage sites used to digest the pTOPO-U6 vector. To ensure the specificity of the constructs, all shRNAs sequences have been confirmed by BLAST database to have no homology to any part of the human genome. The nucleotide sequences in lowercase letters indicate the spacer for the hairpin folding area.

Gene	siRNA clone	siRNA sequence
Hsp60	Sh-1	5’- GGCACTACCACTGCTACT gaagcttg AGTAGCAGTGGTAGTGCC -3’
Hsp60	Sh-2	5’- GCCTATGTTCTGTTG gaagcttg CAACAGAACATAGGC -3’
GANAB	Sh-1	5’- CAAAAGACCCAGCTGAGG gaagcttg CCTCAGCTGGGTCTTTTG -3’
GANAB	Sh-2	5’- GACCCCATCACTCTC gaagcttg GAGAGTGATGGGGTC -3’
Gp96	Sh-1	5’- GGATGAATACTGTATTCAGGCC gaagcttg GGCCTGAATACAGTATTC -3’
Gp96	Sh-2	5’- GGATTATGGGTCACAGGA gaagcttg TCCTGTGACCCATAATCC-3’
Grp78	Sh-1	5’- GGTTAATGATGCTGAGAA gaagcttg TTCTCAGCATCATTAACC -3’
Grp78	Sh-2	5’- AGGTATTTTATAGAT gaagcttg ATCTATAAAATACCT -3’

Supplement Table 3. Prediction of functional network pathways associated with the HNC invasion phenotype. The 52 frequently proteins identified in invasion proteomes were imported into MetaCore™ algorithm analysis. Seven regulatory pathways were found to be significantly associated with invasiveness ($p<10^{-23}$).

Biological Process	Genes in total 52 objects	*p*-Value
Regulation of apoptosis	19	3.42e-40
Actin cytoskeleton organization and biogenesis	13	7.33e-30
Mechanism in double-strand break repair	14	1.93e-27
Cellular response to stress	14	1.93e-27
Branching morphogenesis of a tube	14	1.93e-27
Pathway in mitochondrial ornithine transport	13	2.28e-26
Regulation of cell cycle	12	3.08e-23

Figure legends

Supplement Figure 1. Invasion ability of different oral cancer cell sublines. Five cell lines were used: BM1, BM2, OECM1, Fadu, ad Detroit. Transwell invasion assay was used to determine cell invasion ability in HNC cells. Total of 10^5 parental cells and their invasive cell sublines (parental, first, second, third, and forth generations) were seeded onto the upper chamber of a 24-well Transwell plate coated with Matrigel following incubation at 37°C. The number of cells invading the lower chamber was determined after 24 hours. Data are expressed as fold change relative to that of the parental cells.

Supplement Figure 2. Comparative proteomic analysis of invasion phenotype in head neck cancer cells. (A) Comparative proteomics between parental and invasion subline cells. After the fractionation of subcellular proteins (cytosolic, membrane, and nuclear fractions), the proteomes of parental (Pt) and invasion (Iv) sublines of Fadu and Detroit cells were analyzed by gradient gel electrophoresis, as described under Materials and Methods. Arrows and numbers indicate the proteins identified in the experiments. (B) Functional distribution of the 52 proteins differentially expressed in invasive sublines that were identified by the proteomic method.

Supplement Figure 3. Examination of differential expressed genes in highly invasive HNC cells, as determined by RT-quantitative PCR method similarly as previously described. The expressions of 7 genes in five parental (Pt) and invasive (Iv) subline cells were analyzed. Actin gene expression was used as an internal control for each gene. ΔCt was the difference in the Ct values derived from the specific gene being assayed and control, while $\Delta\Delta$Ct represented the difference between the paired samples, as calculated by the formula $\Delta\Delta Ct = \Delta Ct$ of parental cells - ΔCt of subline cells. The relative fold change of a specific gene expression in a invasion subline cells compared to the parental cells was expressed as $2^{\Delta\Delta Ct}$.

Supplement Figure 4. Efficacy and specificity of chaperon protein knockdown by shRNA. In each experiment, approximately 5×10^5 cells were seeded in a 10-mm plate and transfected with either the shRNA or the vector plasmid in OECM1 cells. Cells were further cultured for two days and the cellular proteins were extracted to determine gene expression by western blot analysis. The expression of actin was determined as an internal control. (A) For each gene, two plasmid clones of shRNA (sh-1, sh-2) were designed. Differential effectiveness of the suppression of specific gene expression for the two constructs of shRNA was shown. (B) The specificity of the shRNA constructs was examined by western blot analysis for the four proteins Gp96, Grp78, Hsp60, and GANAB. 60-Sh: Hsp60-Sh2. GA-Sh: GANAB-Sh2, 96-Sh: Gp96-Sh1, 78-Sh: Grp78-Sh2.

Supplement Figure 5. Alteration of cell growth in Fadu cells after knockdown of gene expression by specific shRNAs. In each experiment, 5×10^5 cells were seeded in a 10-mm plate and transfected with either the shRNA (Hsp60-sh, GANA-sh, Gp96-sh, or Grp78-sh) or the vector plasmid. After that, cells were cultured for up to 5 days. Cell numbers were determined daily for each sample. Each experiment was performed in triplicate.

Supplement Figure 6. Alteration of cell invasion in Fadu cells after knockdown of gene expression by specific shRNAs. In each experiment, 5×10^5 cells were seeded in a 10-mm plate and transfected with either the shRNA (Hsp60-sh, GANA-sh, Gp96-sh, or Grp78-sh) or the vector plasmid. Cells were then seeded into the upper chamber of the Transwell in 1% FBS medium. The lower chamber contained complete culture medium, which included 10% FBS to trap cell invade through Matrigel-coated membrane. The invasive ability was determined in 2 days by counting the cells present in the lower chamber. Each experiment was performed in triplicate.