**Supplementary Tables**

**Table S1.** List of lung cancer cell lines and histopathological type used to determine the status of the *PARD3*.

**Table S2.** List of primers used for cloning, PCR and Sanger sequencing of *PARD3.*

**Table S3.** List of single nucleotide polymorphisms (SNPs) and variants of unknown biological significance identified in lung cancer cell lines and lung primary tumors tested.

**Table S4.** Clinical and pathological parameters of the NSCLC patients and tumors included in the *PARD3* gene alterations screening.

**Table S5.** List of genes selected from those genes that fulfilled the criteria: i) adjusted p<0.05; ii) at least 1.5-fold induction or repression in the wild type- H157tr-wtPAR3 (dox+) relative to H157tr (dox+).

**Supplementary Figure Legends**

**Figure S1.** *PARD3*-inactivation in the H157 lung cancer cell line. (A) 2% agarose gel with the PCR products of various exons of *PARD3* shows the absence of amplification of exons from 6 to 18, indicating the presence of a homozygous intragenic deletion. Appropriate wild type controls (C+) are also included. (B) Western blot of PAR3 in the indicated lung cancer cell lines. Some PAR3 isoforms could be detected and are indicated with their respective molecular weight. TUBULIN, total protein-loading control. (C) Upper panel, schematic representation of the structure of *PARD3*. Grey arrows indicate those exons included in the multiplex ligation-dependent probe amplification (MLPA) test. Middle panel, capillary gel electrophoresis images depicting the same large deletion in the H157 cell line. Red arrows indicate deleted exons. Lower panel, capillary gel electrophoresis images depicting the MLPA assay for *PARD3* in a normal DNA control.

**Figure S2.**Ratio charts of the multiplex ligation-dependent probe amplification (MLPA) depicting a large intragenic deletion (from exon 4 to 23) in one of the LSCCs but not a normal DNA. The presence of LOH is shown in the analysis of the D10S1780 microsatellite marker. No corresponding mRNA was available for this tumor.

**Figure S3.** Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) to determine the presence of promoter hypermethylation of *PARD3*. (A) Upper panel indicates the location of the probes to test for deletions (grey) and promoter hypermethylation (purple) at *PARD3*. Lower panel depicts the capillary gel electrophoresis images of two cell lines with (right) or without (left) methylation of CpGs. (B) Bisulfite genomic sequencing analyses of the promoter region of *PARD3*. Eight single clones are represented for each sample. Black and white squares represent methylated and unmethylated CpGs, respectively, and each vertical bar illustrates a single CpG. (C) Western blot of PAR3 in the indicated lung cancer cell lines. TUBULIN is also included as a total protein-loading control.

**Figure S4.** Relative abundance of the *PARD3* transcripts. (A) Schematic representation of the *PARD3* locus. The alternative exons are indicated in red. (B) Relative abundance of the alternative transcripts after sequencing 80 clones. Only those variants that are reported in pubic databases have been included in the graph.

**Figure S5.** (A) Western blot showing ectopic and transient expression of the indicated PAR3 proteins in the H157 cell line, immunoblotted with HA or PAR3 antibodies. The p.D41\_R74del and the p.D41-E689del consist of shorter proteins. TUBULIN, protein-loading control. (B) Left panel, immunofluorescence with the anti-PAR3 antibody in the indicated H157-derived cells, before (Dox-) and after (Dox+) induction of PAR3 expression with doxycycline (1 ng/µl; 24 h). Nuclei were stained with DAPI. Scale bar, 50 µm. Right panel, mRNA levels of *PARD3*, relative to the control *ACTB*, in the indicated H157-derived cells. Bars correspond to standard deviations from three replicates. (C) Cell colony formation assay of the indicated cells. In contrast to the control and mutant cells, restitution of wtPAR3 significantly reduces colony formation in H157 cells. At the bottom, quantification of the assay. (D) MTT assays to measure cell proliferation. The lines represent the absorbance measured at the indicated time relative to the absorbance measured at day 1 (E) Immunofluorescence with the anti-PAR3 and phalloidin antibodies in the indicated H157tr-wtPAR3 cells. Nuclei were stained with DAPI. Representative fluorescent images are shown.

**Figure S6**. (A) Immunofluorescence with the anti-PAR3 antibody in the indicated T98G-derived cells, after (Dox+) induction of PAR3 expression with doxycycline (1 ng/µl, 24h). Scale bar, 50 µm. Nuclei were stained with DAPI. Representative fluorescent images are shown. (B) Upper panel, western blot depicts the ectopic expression of the indicated mutants and of PAR3 and of the wtPAR3 under a tet-repressor-controlled vector in the presence (+) or absence (-) of doxycycline (1 ng/µl; 24 h). TUBULIN, protein-loading control. Lower panel, mRNA levels of *PARD3,* relative to the control *ACTB*, in the indicated T98G-derived cells, after induction of *PARD3* expression with doxycycline (1 ng/µl, 24h). Bars correspond to standard deviations of three replicates. (C) Immunofluorescence with the anti-PAR3 antibody in the indicated T98G glioma-derived cells after induction of PAR3 expression with doxycycline (1 ng/µl; 24 h). Nuclei were stained with DAPI. (D) Quantification of the percentage of protrusions relative to the total number of cells. (E) Western blots depicting an increase in the levels of PAR3 in the T98Gtr-pD41\_R74del but not in the T98Gtr-wtPAR3 cells, following treatment with either the proteasome inhibitor, MG132, or with the neutral cysteine proteases and proteasome inhibitor, ALLN, for the indicated hours. PAR3 expression was induced with dox (1 ng/µl, 24h).

**Figure S7.** (A) Western blot depicts the endogenous PAR3 protein in the indicated lung cancer cells and the ectopic expression of PAR3 in the H157tr-pD41\_R74del cells (dox, 1 ng/µl; 24 h). (B) Western blot, depicting expression levels of pY-STAT3 and of STAT3, in the H157tr and H157tr-wtPAR3 cells (dox, 1 ng/µl) at the indicated times after re-addition of calcium, after the calcium switch.

**Figure S8.** Immunostaining of PAR3 in lung primary tumors and head and neck squamous cell carcinomas (HNSCCs). (A) Immunostaining of PAR3 in lung tumors carrying the indicated mutations. Negative (-), low (+), moderate (++) and strong (+++) levels of immunostaining (original magnification, 200x). (B) Above, heterogeneous immunostaining of PAR3 in a HNSCC (upper panel) and in preneoplastic lesions (middle and lower panel). Below, distribution of PAR3 immunostaining categories among the two different types of preneoplastic lesions. (C) Distribution of PAR3 immunostaining categories for each indicated patient’s clinical parameters. Only tumors with available data are included. Asterisks denote statistical significance (\*p < 0.05; \*\*p < 0.01).