**Supplementary Figures**

**Fig. S1. Extended clinical annotation of primary canine lung cancer patients.** Cohort distribution of: (A) Affected breeds, (B) Age at diagnosis, (C) Primary tumor location distribution, (D) Sex, (E) Adenocarcinoma subtype, and (F) Treatment. Yr=years.

**Fig. S2. Somatic copy number plots derived from exome sequencing of five primary canine pulmonary adenocarcinomas (cPAC) and matched constitutional DNA.** Tumor copy number states determined by tCoNutT analysis of tumors and matched constitutional DNA from five cPAC cases is shown with each canine chromosome plotted on the x-axis (shown in alternating green and black) and log2 fold change shown on the y-axis.

**Fig. S3. Somatic mutation signatures identified by exome sequencing in primary canine lung cancers.** (A) The distribution of somatic single nucleotide mutation types in their trinucleotide context from tumor/normal exome sequencing of five cPAC cases. (B) The most common mutation signatures based on trinucleotide context and frequency of somatic single nucleotide mutations from tumor/normal exome sequencing of five cPAC cases. COSMIC Signature 1A (C>T substitutions at NpCpG trinucleotides that are associated with age) was present in four cases. COSMIC Signature 2 (C>T and C>G substitutions at TpCpN, associated with APOBEC cytidine deaminase activity) was present in two cases.

**Fig. S4. Comparison of HER2 mutant allele fractions measured using droplet digital PCR and targeted sequencing in tumor tissue.** *HER2*V659E was evaluated by ddPCR in tumor DNA samples in which these mutations were also detected using amplicon sequencing. We observed a high correlation between allele fractions measured using both techniques (Pearson’s r 0.976, p=0.0008).

**Fig. S5. HER2 expression in primary canine lung cancer based on quantitative real-time PCR analyses.** Box plots are shown for HER2 2^(-ΔΔCt) expression fold-change relative to the housekeeping gene HPRT (x-axis) in 49 tumors and 14 matched normal lung tissues comparing (A) Normal lung tissue versus tumor tissue, and (B) Normal lung tissue versus *HER2*-wild-type tumor tissue versus *HER2*-mutant tumor tissue.

**Fig. S6. HER2 cellular location and function in primary canine lung cancer.** (A) Canine papillary adenocarcinoma with intense, complete, circumferential membrane (white arrow) and lateral cytoplasmic membrane (black arrow) anti-HER2 antibody positive staining (brown) in a patient with wild-type HER2. (B) Canine papillary adenocarcinoma with moderate cytoplasmic (black arrow) anti-HER2 antibody positive staining (light brown) in a patient with wild-type HER2. x 40; bar 50 µm. (C) Anti-HER2 immunohistochemistry of a Grade 1 canine papillary adenocarcinoma wild type for HER2. x 20. (D) Segmentation mark-up of the tumor from adjacent normal lung. Tumor is identified by green, whereas red is area within tumor that contains no tissue, and yellow represents areas of non-tumor such as necrosis or tumor stroma. x20.

**Fig. S7. Canine primary lung cancer cell line sensitivity to lapatinib.** Four canine cell lines (three *HER2*WT and one*HER2*V659E) were treated with 14 lapatinib doses ranging from 100 μM to 5.5x10-2 nM for 72 hours with CellTiterGlo viability endpoints were measured and shown as percent survival relative to DMSO vehicle control.

**Fig. S8. Canine primary lung cancer cell line sensitivity to erlotinib.** Five canine cell lines (three *HER2*WT and two *HER2*V659E) and one human cell line BT474 (*HER2*amp) were treated with 10 erlotinib doses ranging from 5x10-8 to 50 μM for 72 hours with CellTiterGlo viability endpoints measured and shown as percent growth inhibition relative to DMSO vehicle control.

**Fig. S9. Canine primary lung cancer cell line sensitivity to trastuzumab.** Five canine cell lines (three *HER2*WT and two *HER2*V659E) and one human cell line BT474 (*HER2*amp) were treated with 10 trastuzumab doses ranging from 1x10-6 μg/ml to 1000 μg/ml for 72 hours with CellTiterGlo viability endpoints measured and shown as percent growth inhibition relative to PBS vehicle control. Relative survival was measured with respect of the sensitive cell line BT474.

**Supplementary Tables**

Table S1. Informatic tools utilized in primary canine lung cancer genomic analyses.

Table S2. Extended clinical and multiplatform annotation of primary canine lung cancer cases.

Table S3. Sequencing metrics for primary canine lung cancer exome analysis.

Table S4. Somatic coding SNVs identified by exome sequencing of primary canine lung cancers.

Table S5. Somatic coding CNVs identified by exome sequencing of primary canine lung cancers.

Table S6. Somatic coding SVs identified by exome sequencing of primary canine lung cancers.

Table S7. Canine genomic regions covered by custom amplicon panel.

Table S8. Sequencing metrics for primary canine lung cancer amplicon analysis.

Table S9. Somatic coding SNVs identified by panel sequencing of primary canine lung cancers.

Table S10. Germline SNPs in COSMIC Tier 1 cancer genes identified by exome and panel sequencing in primary canine lung cancers.

Table S11. Multi-platform validation of *HER2* mutations.

Table S12. Non-invasive detection of *HER2*V659E in the plasma of primary canine lung cancer patients.

Table S13. HER2 protein expression and quantification by immunohistochemistry.