Supplementary Materials and Methods:

SILAC labeling and EGF/TKI treatment of cells

PC9 and H1975 cell lines were cultured at 37°C, 5% CO_2 for at least five passages in RPMI medium 1640 (Pierce) containing L-arginine and L-lysine (light), 13C6-Arginine and D4-Lysine (medium), or 13C615N4-Arginine and 13C615N2-Lysine (heavy) (Cambridge Isotope laboratories) with 10% dialyzed fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin.

After complete labeling, the cells were expanded to 15 cm dishes, and serum-starved for 16 h prior to the experiment. The three states of cells were untreated (light), those stimulated with EGF (100 ng/mL) for 3 min (medium), or treated with erlotinib (100 nM) for 1 h before EGF stimulation (heavy). Following treatment, cells were quick-chilled and washed with cold phosphate buffered saline (PBS) before cell lysis. For mass spectrometry, cells were lysed in urea lysis buffer as described in Materials and Methods. For immunoprecipitation, cells were lysed in NP-40 lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.4, 1% NP-40, and 1 mM EDTA) supplemented with protease and phosphatase inhibitor cocktails (Roche).

Mass spectrometry

Sample preparation: Cells were prepared as described above. Protein concentrations were determined by the modified Lowry method (BioRad). Equal amounts of protein lysates of each SILAC state were mixed together to constitute 30 mg pooled lysate. The combined lysate was reduced with 4.5 mM dithriothreitol (Sigma Aldrich), alkylated with 10 mM iodoacetamide (Sigma Aldrich), and subsequently digested with trypsin (Worthington) at 37˚C overnight. The digest was then acidified with TFA (final concentration of 1%) and peptides were purified using a solid phase extraction C18 column (Supelco, Bellefonte, PA), lyophilized, and stored at -80˚C.

Phosphotyrosine enrichment: Phosphotyrosine peptides were enriched prior to mass spectrometry analyses using a PhosphoScan Kit (p-Tyr-100, Cell Signaling). The lyophilized peptides were dissolved in immunoaffinity purification (IAP) buffer (50 mM MOPS, pH 7.2, 10 mM sodium phosphate, 50 mM NaCl) and incubated with 40 µL of immobilized anti-phosphotyrosine antibody for 1 h at 4°C. The antibody beads were centrifuged for 1 min at 1500 x g, and the supernatant was separated and saved. The antibody-bound beads were washed at 4^0C three times with 1 mL of IAP buffer and twice with water by inverting tube 5 times per wash. The phosphotyrosine-containing peptides were eluted with 55 µL of 0.15% TFA by gently tapping the bottom of the tube and standing at room temperature for 10 min.

Capillary RPLC-MS/MS analyses: Enriched phosphopeptides were analyzed on an LTQ Orbitrap XL ETD (Thermo Scientific) mass spectrometer interfaced with a dual nano pump (Eksigent) and an Agilent 1100 microwell plate autosampler. Phosphopeptides were loaded onto a trap column (75 µm x 2 cm, Magic C18AQ 5 µm, 100Å, Michrom Bioresources), separated on an analytical column (75 µm x 15 cm, Magic C18AQ, 5 µm, 100Å, Michrom Bioresources,) at 300 nL/min flow rate with a running time of 75 min. The MS data was acquired at a resolution of 60,000 at m/z 400 and MS/MS data was acquired on an ion trap or HCD. For each cycle of data-dependent analysis the 6 or 10 most abundant precursors were selected for MS/MS analysis with normalized collision energy of 35%. Multistage activation mode was enabled with neutral loss masses of 32.66, 48.99, and 97.97. Selected ions for fragmentation were excluded dynamically for 90 sec.

Data Analysis: Peptides and proteins were identified and quantified using the Maxquant software package (version 1.3.0.5) with the Andromeda search engine (1). MS/MS spectra were searched against the Uniprot human protein database and quantification was performed using default parameters for the 3-state SILAC in MaxQuant. The parameters used for data analysis include trypsin

as a protease with two missed cleavages allowed. Carbamidomethyl cysteine was specified as a fixed modification. Phosphorylation at serine, threonine and tyrosine, deamidation of asparagine and glutamine, oxidation of methionine, and protein N-terminal acetylation were specified as variable modifications. The precursor mass tolerance was set to 7 ppm and fragment mass tolerance to 0.5 Da for ITMS and 20 ppm for FTMS. The false discovery rate was calculated using a decoy database and a 1% cut-off was applied.

Transfection and infection procedures: Transfection of various cell lines for transient expression of proteins was carried out using Fugene X-tremeGENE 9 DNA transfection reagent following the manufacturer's recommendations. Briefly, cells at 80 to 90% confluence in a 10 cm dish were transfected with 5 to 10 μg of target plasmid. After 24 h, the medium containing transfection reagent was removed and the cells were grown in fresh growth medium for 48 h before preparing cell lysates for immunoblot analysis.

Lentivirus was produced by transfection of subconfluent HEK293T cells in a 6 cm dish with various plasmids using X-tremeGENE 9 DNA transfection reagent. 24 h post-transfection, medium was replaced with DMEM supplemented with 30% FBS and medium containing lentivirus particles was collected at 48 and 72 h after transfection. The virus-containing medium was filtered through a 0.45 μM filter (Millipore) and stored as 1 mL aliquots at -80°C. For stably expressing cells, 1-1.5 x 10^6 target cells were plated in 6 cm plate and transduced after 24 h with 1 ml lentivirus in presence of 8 μg/ml polybrene. The next day, transfection medium was replaced with fresh growth medium and cells were allowed to grow for another 48 h before transferring to 10 cm dish and adding antibiotics for selection and expansion.

Real-time PCR assays for copy number variation analyses

To assay for loss of heterozygosity in lung tumors, Mig6 copy number was compared to germ line copy number in the same mouse (tail DNA of newborn pup). Real-time PCR was conducted using an ABI Prism 7900HT Sequence detection system (Applied Biosystems). Multiplex reactions (25 □l) containing FAM-MGB Mig6 copy number assay (Applied Biosystems: Mm00117871_cn) and VIC-TAMRA labeled Transferrin Receptor (Tfrc) copy number reference assay (Applied Biosystems) were performed in triplicate using 96 well plates with approximately 10 ng template and 1x Universal Master Mix (Applied Biosystems-without Amp Erase UNG). Determination of copy number was performed using the standard curve method of absolute quantification and ABI SDS v2.1 software (User Bulletin #2, Applied Biosystems) with normalization to Tfrc as an internal reference for copy number. We used mouse genomic control DNA carrying two copies each of Mig6 and Tfrc for our standard curves. Copy number analyses are based on the premise that all controls and unknowns carry two copies of Tfrc; thus, the ratio of Mig6 to Tfrc can be used to assign Mig6 copy number.

Real-time PCR assays for expression analyses

Depending on the experiment, real-time PCR was conducted in 96 well plates using either an ABI Prism 7900HT Sequence detection system or 7500 Real-time PCR system, or 384 well-plates and a ViiA7 Real-time PCR system (Applied Biosystems). Singleplex reactions (10 □l or 20 □l) containing FAM-MGB expression assay for endogenous *Mig6* (Mm00505292_m1), *EGFR* (Mm00433023_m1), surfactant associated protein (Mm00488144_m1), transgenic *EGFR* (Hs01076078_m1) or *rtTA* (custom assay from Varmus Lab) were performed using 1 to 30 ng template and 1x Universal Master Mix (Applied Biosystems-without Amp Erase UNG). *GAPDH* endogenous control assay (VIC-MGB, primer limited) singleplex reactions were run in parallel and relative expression determined using the standard curve method of absolute quantification and ABI software specific for the real-time system used (User Bulletin #2, Applied Biosystems) using control cDNA from a single mouse wild-type for

Mig6 and expressing the human *EGFR* transgene under control of the *CC10* (U398: $EGFR^{+}$, $CC10^{+}$, $Mig6^{+/+}$) promoter.

Immunohistochemistry staining: Immunohistochemistry of 4 μm thick serial sections of mouse lung tissues was performed using standard methods and described elsewhere (Politi, Genes and Dev, 2013). Tissue sections were probed with anti-$EGFR^{L858R}$, anti-pERK, anti-Ki67, anti-p19ARF, and anti-TTF1 antibodies following the instructions of the manufacturer. Briefly, the tissue sections were deparaffinized in xylene and the antigens were heat retrieved (>95^{0}C for 10 minutes) in 1mM EDTA. The tissue sections were blocked with 3% H_2O_2 and goat serum prior to incubating with primary antibodies overnight at 4ºC. Tissue sections were washed and then incubated with rabbit/mouse secondary antibodies (Dako) and the antigens were visualized by 3,39-diaminobenzidine (Vector Laboratories).

Immunofluorescence staining: Cells were grown on glass-bottom 24-well plates, serum starved for 18 h, and then stimulated with EGF 100 ng/ml for 10, 30 and 60 minutes. EGF stimulation was terminated with a cold PBS wash followed by fixation in 4% paraformaldehyde. For immunofluorescence, cells were probed with tEGFR mAb (BD Biosciences), $EGFR^{L858R}$ antibody (nanoTools), EEA1 (Abcam), and LAMP1 (Cell Signaling Technology) primary antibodies and Alexa Fluor 488 and 568-conjugated respective secondary antibodies (Invitrogen).

Pathological characterization of tissue sections of mouse tumors: In order to characterize the histopathology of EGFR mutant mice in different Mig6 backgrounds, five haematoxylin-eosin stained sections of formalin-fixed, paraffin-embedded lungs per animal from each genotype were examined. Animals were derived from three independent lines. Semi-quantitative assessment of the neoplastic changes ranging from normal epithelium, preneoplastic (AAH and bronchiolization of alveoli) changes to adenomas and adenocarcinoma formation was performed on over 100 animals. The

extent, location and cytomorphology of macrophages and other inflammatory cells were noted. Intrapulmonary airways were assessed for Clara cell atypia, hyperplasia and dysplasia and alveolar compartment for Type 2 (T2) cells alterations from hyperplasia, atypia to dysplasia and AAH.

Image Analysis Methods:

Semi-Quantitative assessment of $EGFR^{L858R}$ immunolabeling: $EGFR^{L858R}$ immunolabeling was quantified using the Aperio Image Analysis Toolbox Software Membrane Analysis algorithm. For membrane analysis, all lung tissue in each section, excluding tissue folds, was manually segmented using drawing tools in the Aperio ImageScope software. This manual segmentation was used to define the region of interest for image analysis and to measure the total analysis area in each section. The Membrane Analysis was used to identify cells with cytoplasmic membrane immunolabeling and semi-quantitatively score each cell as strong positive (3^+), moderate positive (2^+), weak positive (1^+) or negative (0) based on the intensity of DAB labeling. Scores were set based on thresholds for the measured intensity of DAB chromogen in each cell on a 0 (complete saturation/ black) to 255 (no labeling) scale. Software parameters, including cell size, shape, and thresholding parameters, were optimized for cell identification and for immunolabeling scoring prior to image analysis. Optimization was based on subjective assessments of algorithm performance by the operator on a subset of tissue specimens. Only cells identified as having $\geq$ 50% of membrane labeling were included in the final results. This cut-off value was chosen because, based on subjective review of mark-up images following the analysis, it provided the best balance of specificity and sensitivity of cell identification. Analysis output includes percent and absolute numbers of cells with strong (3^+), moderate (2^+), weak (1^+) and no (0) immunolabeling. For each sample, a Membrane, or L858R, Score was calculated. This score is a modification of the H-score (Histopathology Score) algorithm that has been previously used to quantify immunolabeling in multiple cancer types including lung and breast[1-5]. The H-score is a derived variable that accounts for both the percent of immunolabeled cells and the labeling intensity in each cell by weighing the percent of cells with different amounts of

labeling (3^+, 2^+, 1^+) based on their labeling intensity. These scoring systems assume that there is a consistent distribution of hyperplastic and neoplastic cells throughout the tissue and there is consistent labeling throughout the tissue section.

H-Score = ((Percent 3^+ cells)*3) + ((Percent 2^+ cells)*2) + ((Percent 1^+ cells)*1)

Since we were not able to quantify all unlabeled cells, we modified the H-score by calculating the density of cells per millimeter squared of lung tissue area with 3^+, 2^+, or 1^+ labeling (for each individual specimen, area is a constant).

Membrane (L858R) Score = ((absolute 3^+ cells/mm^2)*3) + ((absolute 2^+ cells/mm^2)*2) + ((absolute 1^+/mm^2)*1).

Quantification of TTF-1 immunolabeled type II pneumocytes: Quantification of TTF-1 immunolabeling was performed using the Aperio Image Analysis Toolbox Nuclear Analysis algorithm. Similar to the Membrane Analysis, all lung tissue in each section was manually segmented using drawing tools in the Aperio ImageScope software. The Nuclear Analysis identifies immunolabeled nuclei based on their labeling intensity, size, and shape. These software parameters, including minimum and maximum nuclear size, nuclear shape (roundness, compactness, and elongation), immunolabeling intensity, and nuclear thresholding and segmentation parameters were optimized to detect nuclei in all specimens. Nuclear Analysis outputs reported include number of immunopositive nuclei, analysis area (mm^2), and number of immunopositive nuclei per mm^2.

All analyses were performed and results were collated blinded to individual animals' genotypes. Mark-up images of each specimen were reviewed to assess for accurate image segmentation following the analysis as a standard quality control procedure. After the analysis, data was tabulated according to genotype. Results were rounded to the nearest thousandths.

Supplementary Figure Legends

Supplementary Figure S1. Serial MRI imaging and lung histopathology analyses show rapid tumor progression of mutant EGFR-driven tumorigenesis in Mig6$^{-/-}$ background.

Percent change in tumor burden as determined by MRI imaging of (A) *EGFR^{L858R} Line 57* mice and (B) *EGFR^{Del} Line 11* mice shows early tumor development in *EGFR^{L858R} Mig6$^{-/-}$* and *EGFR^{Del} Mig6$^{-/-}$* mice. (C) Table showing summary of lung pathology of doxycycline inducible mutant EGFR transgenic mice in *Mig6$^{+/+}$*, *Mig6$^{+/-}$*, or *Mig6$^{-/-}$* background. (D) Representative photomicrographs of *CC10^{rtTA}/EGFR^{L858R}/Mig6$^{+/+}$* mouse lungs (176 days on doxycycline). Inset A). Two distinct adenomas in a background of moderately congested lung parenchyma with unremarkable bronchioles (BL) and vessels (Ve) of mouse line 56. Open arrow points to the smaller adenoma nodule (Haematoxylin and eosin stain, original magnification x20). Inset B) At a higher power there is a sharp demarcation between the smaller adenoma (Tu) and adjacent lung parenchyma that is infiltrated by macrophages (m; haematoxylin and eosin stain, original magnification x200). Inset C) Characteristic nuclear immunoreactivity for TTF1 in a wild type Mig6 mouse line 57 lung. An adenoma (Tu) shows a high density of nuclei as most cells are positive. The adjacent lung parenchyma illustrates the distribution of immunoreactive type2 alveolar cells (T2), while macrophages (m) are negative (Immunoperoxidase stain, original magnification x200). Tu = tumor; Ve = vessel; BL = brochiolus; m = macrophage; T2 = type 2 pneumocyte; Lu = bronchiolar lumen

(E) Representative photomicrographs of Mig6 heterozygous and knock-out mouse lungs. Inset A). A cross section of a whole lung from *CC10^{rtTA}/EGFR^{L858R}/Mig6$^{+/-}$* mouse line 56 (81 days on doxycycline) with multiple adenomas and adenocarcinomas (solid arrows; hematoxylin and eosin stain, original magnification x10). Inset B). A higher power view of an adenocarcinoma with glandular features from the same animal (hematoxylin and eosin stain, original magnification x200). Inset C). Adenocarcinoma from a *CC10^{rtTA}/EGFR^{Del}/Mig6$^{-/-}$* mouse (22 days on doxycycline) with sheet like growth of histologically malignant cells (hematoxylin and eosin stain, original magnification x200). Inset D). The same lungs also show marked Clara cell hyperplasia and

transformation at bronchioloalveolar junctions (open arrow) in addition to the growth and malignant transformation of alveolar parenchyma (open arrowhead and inset C; hematoxylin and eosin stain, original magnification x50). Inset E). Both abnormalities are strongly immunoreactive for TTF1. In addition type2 pneumocytes and bronchiolar lining of were positive in these animals. (*CC10^{rtTA}/EGFR^{L858R}/Mig6$^{-/-}$* mouse line 56; immunoperoxidase stain, original magnification x200). BL= bronchiolus; Lu = bronchiolar lumen; T2 = type2 pneumocyte).

Supplementary Figure S2 (A) Representative photomicrographs of *CC10^{rtTA}/EGFR^{L858R}/Mig6$^{+/-}$* mouse lungs at early stages of doxycycline treatment show variability of tumor progression. A cross section of a whole lung from *CC10^{rtTA}/EGFR^{L858R}/Mig6$^{+/-}$* mice (line 56) stained with H&E shows the presence of distinct alveolar structure indicative of normal lung tissue (inset A and B) as well as adenomas (inset C) and adenocarcinomas (inset D). B. Tumor formation in *CC10^{rtTA}/EGFR^{L858R}/Mig6$^{-/-}$* mice is associated with no significant change in senescence and apoptosis. Immunohistochemical staining of tumor-bearing sections from the lungs of *EGFR^{L858R}/Mig6$^{+/+}$*, *EGFR^{L858R}/Mig6$^{+/-}$*, and *EGFR^{L858R}/Mig6$^{-/-}$* mice at 9 days of doxycycline treatment with senescence specific marker p19ARF (A-C) shows no significant change in p19ARF expression in tumor or normal lung areas of these mice. TUNEL assay also shows no significant apoptotic cells in the mouse lung tissue sections from these mice (E-G). D and H are positive control for p19ARF and TUNEL assay, respectively. (Scale bar: 100 μm).

Supplementary Figure S3. Rapid progression of tumorigenesis 7 days after doxycycline induction of mutant EGFR in *Mig6$^{-/-}$* mice

(A-B) Immunohistochemistry of lung tissue sections (line 56 - A, line 57 - B) from *CC10^{rtTA}/EGFR^{L858R}/Mig6$^{+/+}$*, *CC10^{rtTA}/EGFR^{L858R}/Mig6$^{+/-}$*, and *CC10^{rtTA}/EGFR^{L858R}/Mig6$^{-/-}$* littermates after 7 days of doxycycline induction with H & E (A-C), Ttf1 (D-F), EGFR^{L858R} (G-I) show significantly increased tumor burden in *Mig6$^{-/-}$* mice. (Scale bar: 200 μm).

Supplementary Figure S4. No loss of heterozygosity (LOH) in lung tumors of Mig6$^{+/-}$ mice. Real-time qPCR assays for *Mig6* copy number variation were performed on tail and lung tumor DNA of each mouse (except for #14 and #15 where tail DNA was no longer available). DNA samples 1-6 were from *CCSP^{rTTA}/EGFR^{mut}/Mig6$^{+/+}$* mice, and DNA samples 7-15 were from *CCSP^{rTTA}/EGFR^{mut}/Mig6$^{+/-}$* mice. Tail DNA samples from various *Mig6$^{+/+}$*, *Mig6$^{+/-}$*, and *Mig6$^{-/-}$* mice were used as controls for the copy number assay. Determination of copy number was performed using the standard curve method of absolute quantification and normalized to Tfrc, an internal reference for copy number. No LOH was detected in lung tumor DNA from *Mig6$^{+/-}$* mice. Values are mean ± SD from three independent experiments.

Supplementary Figure S5. QPCR analysis of mRNA expression in lung tumor samples from *Mig6$^{+/+}$*, *Mig6$^{+/-}$*, and *Mig6$^{-/-}$* mice. RNA was isolated from lung tumor tissue samples from *Mig6$^{+/+}$*, *Mig6$^{+/-}$*, and *Mig6$^{-/-}$* mice treated with doxycycline for human *EGFR* transgene expression and euthanized at survival end-point. The expression of endogenous *Mig6* (A), *Egfr* (B), transgenic human *EGFR* (C), *rtTA* (D), and *Sftpc* (E), was determined by quantitative RT-PCR with GAPDH mRNA as endogenous control. Results were analyzed as described "Materials and Method" section. Values are mean ± SD from 2-3 experiments except very few cases where experiment was performed only once.

Supplementary Figure S6. QPCR analysis of mRNA expression in lung samples from *Mig6$^{+/+}$*, *Mig6$^{+/-}$*, and *Mig6$^{-/-}$* mice at early stages of doxycycline treatment. (A-E) RNA was isolated from lung tissue samples from *Mig6$^{+/+}$*, *Mig6$^{+/-}$*, and *Mig6$^{-/-}$* mice treated with doxycycline for 9 or 14 days. The expression of *Mig6* (A), mouse *Egfr* (B), transgenic human *EGFR* (C), *rtTA* (D), and *Sftpc* (E) was determined by real-time quantitative PCR. GAPDH mRNA was used as endogenous control and the relative expression was determined using the standard curve method of absolute quantification and ABI software. Values are mean ± SD from 2 to 3 experiments. Note, some normal lung samples in the 9 day group have the *CC10^{rtTA}* transgene, but not the mutant EGFR transgene accounting for the rtTA expression.

Supplementary Figure S7. MS and MS/MS spectra of Y394/395 containing tryptic peptide identified in MIG6 (gene symbol ERRFI1). (Upper panel) MS/MS spectrum of the MIG6 peptide obtained from the three-state SILAC experiment in PC9 cells showing phosphorylation at Y394. Inset shows MS spectrum of this peptide with EGF (M)/Serum starved (L) SILAC ratio of 1.2 and Erlotinib + EGF (H)/ EGF(M) SILAC ratio of 0.2, indicating significant dephosphorylation of Y394 upon erlotinib treatment of PC9 cells. (Lower panel) MS/MS spectrum of the same peptide identified from H1975 cells that harbor $EGFR^{L858R/T790M}$ shows that both Y394 and Y395 sites are phosphorylated. Inset shows the MS spectrum with M/L SIALC ratio of 0.97 and H/M SILAC ratio of 1.36, demonstrating there is no significant dephosphorylation of these sites upon erlotinib treatment of these TKI-resistant cells.

Supplementary Figure S8. Specificity of monoclonal antibodies made against wild type and mutant EGFRs. HEK293T cells stably transfected with control (pcDNA), $EGFR^{WT}$, $EGFR^{L858R}$, $EGFR^{DelE746\text{-}A750}$ plasmids were used to prepare lysates. Cell lysates were immunoblotted with Del EGFR ($EGFR^{Del\ E746\text{-}A750}$), corresponding WT EGFR ($EGFR^{E746}$), L858R EGFR ($EGFR^{L858R}$), and corresponding WT EGFR ($EGFR^{L858}$) specific monoclonal antibodies along with RhoGDI specific antibodies for loading control (upper panel). The WT EGFR and L858R EGFR transfected HEK293T lysates were immunoprecipitated with anti-$EGFR^{L858}$, anti-$EGFR^{L858R}$ monoclonal antibodies, or mouse IgG control antibodies as indicated, and immunoblotted with a different EGFR specific antibody that detects both wild type and mutant EGFRs (bottom panel).

Supplementary Figure S9. Co-localization of (A-B) MIG6 and EEA1, a marker of early endosomes with both WT EGFR and $EGFR^{L858R}$ and (C-D) LAMP1, a late endosomal marker with WT EGFR, but not $EGFR^{L858R}$. HBECs expressing WT EGFR or $EGFR^{L858R}$ were serum starved overnight and stimulated with EGF for the indicated time-periods. A. Either WT EGFR (upper panel-green) or $EGFR^{L858R}$ (lower panel-green) co-localizes with MIG6 (red) at both 10 min and 1 h of EGF stimulation. B. WT EGFR (green) co-localizes with EEA (red), a marker of early

endosomes at 10 and 30 min of EGF stimulation (upper panel). $EGFR^{L858R}$ (green) co-localizes with EEA1 (red) both at 10 and 60 min of EGF stimulation, indicating mutant EGFRs are internalized into early endosomes similar to the WT EGFR. $EGFR^{L858R}$ remains on the surface of many cells following EGF stimulation for 60 min. HBECs expressing WT EGFR or $EGFR^{L858R}$ were serum starved as described above and stimulated with EGF for 2 h followed by immunofluorescence staining with lysosomal marker LAMP1 and WT EGFR ($EGFR^{L858}$) (C) or $EGFR^{L858R}$ (D) specific antibodies. WT EGFR (green) co-localizes with LAMP1 (red) indicating lysosomal trafficking of WT EGFR. However, co-localization of LAMP1 with $EGFR^{L858R}$ (green) is not significant at this time-point following EGF stimulation.

Reference:

1.Cox J, Neuhauser N, Michalski A, Scheltema RA, Olsen JV, Mann M. Andromeda: a peptide search engine integrated into the MaxQuant environment. J Proteome Res. 2011;10:1794-805.