

## **Supplemental Materials:**

Supplemental Figure 1 - Phosphoproteomics workflow.

Supplemental Figure 2- Changes in phosphorylated proteins corresponding to genes present in the Kyoto Encyclopedia of Genes and Genomes (KEGG): A) ERBB signaling pathway (rno04012), B) JAK-STAT pathway signaling (rno04630).

Supplemental Table 1 - EGF vs Gefitinib phosphoproteome.

Supplemental Table 2 - Phosphoinositide pathway proteins changed by EGF.

Supplemental Table 3 - Transcription factors changed by EGF.

Supplemental Video - Intracellular calcium measured by Fluo-4 fluorescence.

**Supplemental Figure 1. Phosphoproteomics workflow.** A. Workflow for the sample preparation of total- and phospho-proteomics by LC-MS/MS and the subsequent bioinformatic analysis. B. TMT-isobaric quantification of phosphosites from three pairs of vehicle-treated (Ctr) and EGF-treated (EGF) samples.

**Supplemental Figure 2. Changes in phosphorylated proteins corresponding to genes present in the Kyoto Encyclopedia of Genes and Genomes (KEGG): A) ERBB signaling pathway (rno04012), B) JAK-STAT pathway signaling (rno04630).** Values for  $\text{Log}_2(\text{EGF}/\text{Control})$  are shown as individual blocks colored to show magnitude of change (red, increased; blue, decreased) as generated by Bioconductor Pathview (<https://bioconductor.org/packages/release/bioc/html/pathview.html>) run on R.

**Supplemental Video - Intracellular calcium measured by Fluo-4 fluorescence.** Freshly isolated rat IMCD segments were microdissected and loaded with calcium indicator dye, Fluo-4. The same tubule was

treated in order with vehicle, 0.1  $\mu$ M EGF, 100  $\mu$ M carbachol, and lastly with ionophore with a brief wash in between treatments.