Materials and Methods:

Culturing A431 cells:

A431 cells (from ATCC CRL-1555) were cultured in Dulbecco’s modified eagle’s medium (DMEM from Gibco) containing 10% (v/v) fetal calf serum (FCS), penicillin and streptomycin at 37º C with an atmosphere containing 5% CO₂.

Preparation of whole A431 cell samples for PINEM experiments:

A431 cells grown on 100 x 20 mm Corning plates were incubated on ice for 20 minutes and then washed twice with ice-cold PBS. The cells were then scraped from the plate manually using a cell scraper. No trypsin was used to detach the cells from the plate to avoid digesting the EGFR on the cell surface. Scraped cells were washed with ice-cold PBS and spun down at 1500x g for 5 minutes at 4º C. Finally, the cells were re-suspended in 1 ml ice-cold H₂O.

For the cells treated with EGF or aprotinin, an excess of either EGF (~20 µg, Thermo Fisher Scientific PHG0313) or aprotinin from bovine lung (Sigma Aldrich A6106-1MG) was added to 10 ml of DMEM which was then added to one (100 x20 mm) plate. The cells were incubated for 1 minute at 37º C and then 20 minutes on ice to allow ligand binding but not EGFR internalization. The cells were washed twice with ice cold PBS to remove the unbound EGF and then spun down and resuspended as described above for the non-treated cells.

For PINEM experiments, a silicon oxide TEM membrane grid (SiMPore Inc., 20 nm thick) was cleaned with an argon plasma for 2 minutes. A droplet (3-4 µl) of cells, either with or without EGF or aprotinin, was then applied onto the grid. The sample was allowed to dry
near ice and was then loaded into the microscope. Sample preparation and measurement were performed on the same day with the time between cell scraping and PINEM measurements being between 45-60 minutes.

**Preparation of A431 membrane vesicles for PINEM measurements:**

Membrane vesicles were isolated from A431 cells as mentioned in reference 29 (main text). In short, A431 cells were incubated with PBS containing 2 mM EGTA and scraped subsequently from the plate without trypsin to avoid digestion of EGFR on the cell surface. Subsequently, the cells were washed once with PBS to remove EGTA (5 minutes at 1000x g). Thereafter, the cells were lysed by passing them 10 times through a syringe and spun down at 1000x g to remove unbroken cells and cell debris. The supernatant was then spun at 100,000x g to harvest the membrane vesicles. The isolated vesicles were resuspended in HEPES buffer pH=7.4 with protease inhibitors and kept at -20 °C in small aliquots to avoid repeated thaw/freezing cycles.

Incubation of membrane vesicles with EGF was performed as detailed in reference 29 (main text). A431 vesicles were added on quantifoil grids (3-4 µl) and then blotted (blotting time= 4 seconds, force =6) and subsequently plunge frozen in liquid ethane/propane mixture. During PINEM measurements the samples were kept cold using liquid nitrogen.

**UEM and PINEM methodologies:**

The experiments described in this report were performed using the second generation ultrafast electron microscope (UEM2) located in the Physical Biology Center for Ultrafast Science and Technology at Caltech. The electron microscope of UEM2 functions at 200 KV and is equipped with a Gatan Imaging Filter and a 4 megapixel Ultrascan 1000 CCD. A fiber oscillator/amplifier laser system is also mounted on the microscope. The laser produces 1,038-
nm light in femtosecond pulses. The 1,038-nm fundamental pulse is frequency doubled (519 nm) and quadrupled (259 nm) for the pump and probe pulse generation, respectively. The timing of the pump and electron pulses was optimized (time zero determined) using silver nanowires. Then, PINEM images are obtained by setting the energy filter slit width to 10 eV and sequentially stepping the spectrum offset by 1-eV increments until only the gain region is selected (i.e., no part of the ZLP or loss regions contribute to the energy-filtered images). The repetition rate of the laser used in the experiments described here is 1 MHz. PINEM images were acquired with a pump fluence of 2.5 mJ/cm² (50 mW) with a full width at half maximum (FWHM) of 50 µm. The PINEM images reported here were recorded for (40-50 seconds). To measure the time profile of the photon-electron coupling on the cell surface (Figure 2 C & D), a total exposure time for these multiple images was around 5 minutes. No sample damage was detected in either UEM or PINEM modes during this time period.
Figure S1: A) Bright field image with the electron pulse confined to a cell on the grid with the corresponding electron energy spectrum. B) Bright field image with the electron pulse confined to a cell-free area on the grid and the electron energy spectrum of that area.
**Figure S2:** Additional UEM (left) and PINEM images (middle and right) of A431 cell without EGF. Electron-photon coupling is manifest by the bright ring around the cell periphery. Red arrows highlight the evanescent fields on the cell edge. Scale bar is 5 µm.
Figure S3: Sample orientation dependency. PINEM images of un-tilted cell (middle) or tilted by either -30° (left) or +30° (right) alters PINEM images, indicating a dependence of the electron-photon coupling on the orientation of the cell surface with respect to the beam. Blue line indicates the axis of rotation. Total electron dose used to record all three images was 0.3 e/A². Changes in sample height caused by tilting are expected to be only microns, perturbing time zero by only tens of femtoseconds (not significant).
**Figure S4:** Polarization dependency. Left) UEM image of the edge of an A431 cell. Middle & right) PINEM images of the same area with the polarization of the pump pulse parallel (middle) or perpendicular (right) to the cell surface. The blue dotted arrows indicate the polarization of the pump pulse; red arrows point to regions of strong electron-photon coupling on the cell surface.
Figure S5: Additional UEM (left) and PINEM images (middle and right) of A431 cells incubated with EGF, showing there is little (if any) photon-electron coupling. Scale bar is 5 µm.
Figure S6: Electron energy spectra with EGF (dotted blue) and aprotinin (red). Electron energy gain equivalent to one photon quantum is indicated (2.4 eV).