Supporting Information for

Cell-specific Retention and Action of Pheophorbide-based Photosensitizers in Human Lung Cancer Cells

Erin C. Tracy, Mary-Jo Bowman, Ravendra K. Pandey and Heinz Baumann

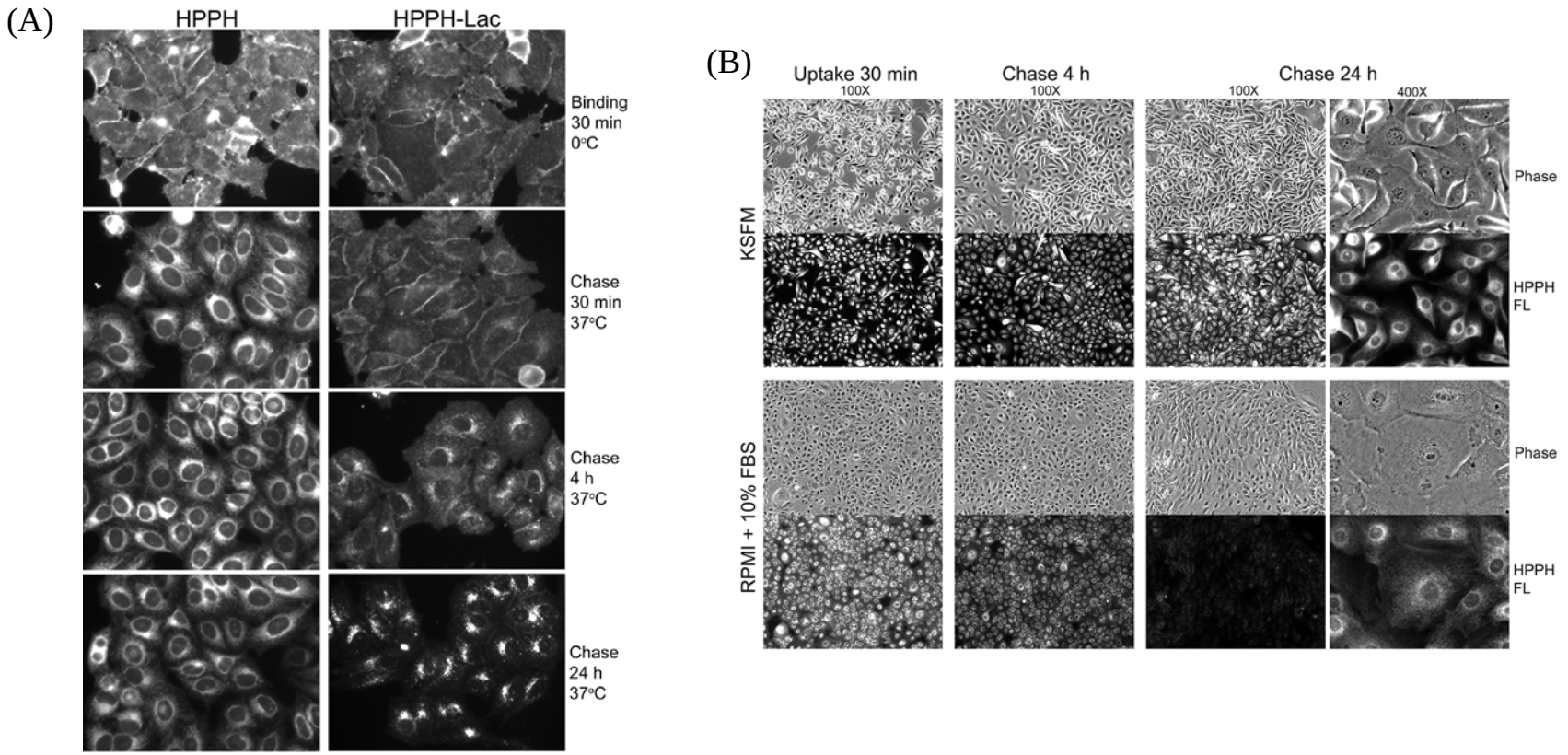

Figure S1. Cellular uptake of HPPH and HPPH-Lac by lung cells. (**A**) Time course of internalization was monitored by the cellular localization of PS fluorescence in TEC-1 cells. Binding, under suppression of endocytosis, was determined in cells incubated for 30 min in serum-free RPMI containing 3.2 μM HPPH on ice. Uptake and accumulation in intracellular compartments was evaluated after return of the cultures to fresh, PS-free medium and incubation at 37°C for 30 min, 4 h and 24 h. Following raising the culture temperature, plasma membrane-associated HPPH was internalized within 30 min with subcellular retention in mitochondria and ER. HPPH-Lac, more stably interacted with cell surface where it was still detectable after 30 min incubation at 37°C. Subsequent endocytosis and lysosomal accumulation required several hours of incubation. Only the fluorescent images of the cells, taken under identical conditions at 400X magnification, are shown. (**B**) Effect of serum on cellular morphology and retention of HPPH by primary N-ECs (L364, passage 3). ECs were grown to confluence in KSFM. The cells were then incubated for 30 min at 37°C in either KSFM or RPMI containing 3.2 μM HPPH. After recording the cell-associated PS-fluorescence, the incubation of the cells with fresh, PS-free medium was continued for 4 and 24 h. The cellular morphology and PS level was imaged by phase and fluorescent microscopy at 100X and 400X magnification. Serum-induced differentiation of EC is evident by gaining a squamous morphology. All images at 100X magnification were made by using identical camera settings. Detection of HPPH fluorescence at 400X magnification in cells after 24 h chase in serum-containing RPMI required a 6-times longer exposure.

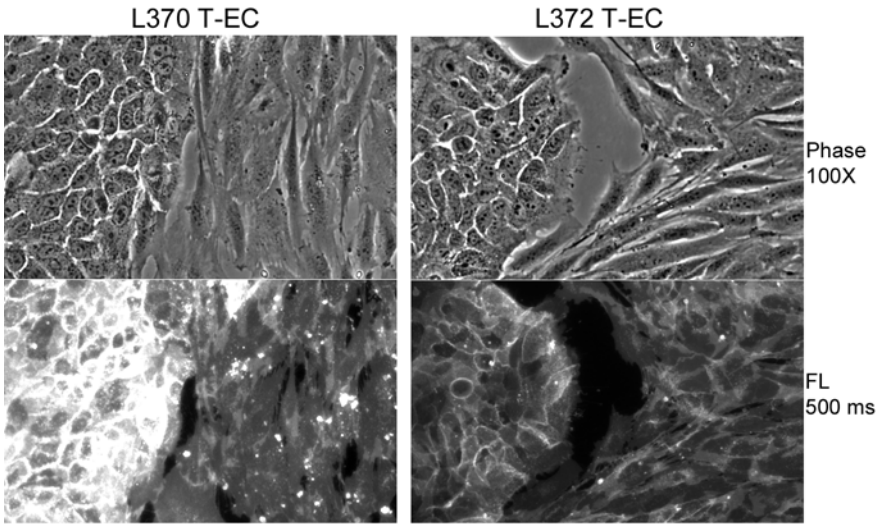

Figure S2. Enhanced binding of HPPH-Lac detected in one case of T-EC. Co-cultures of T-EC and stromal cells (L370 and L372) were treated for 30 min on ice with serum-free medium containing 3 μM HPPH-Lac. Cell-associated PS fluorescence was visualized by microscopy at 100X magnification. In each panel, the segregated cluster of T-EC is shown on the left and the surrounding Fb on the right half of the images.

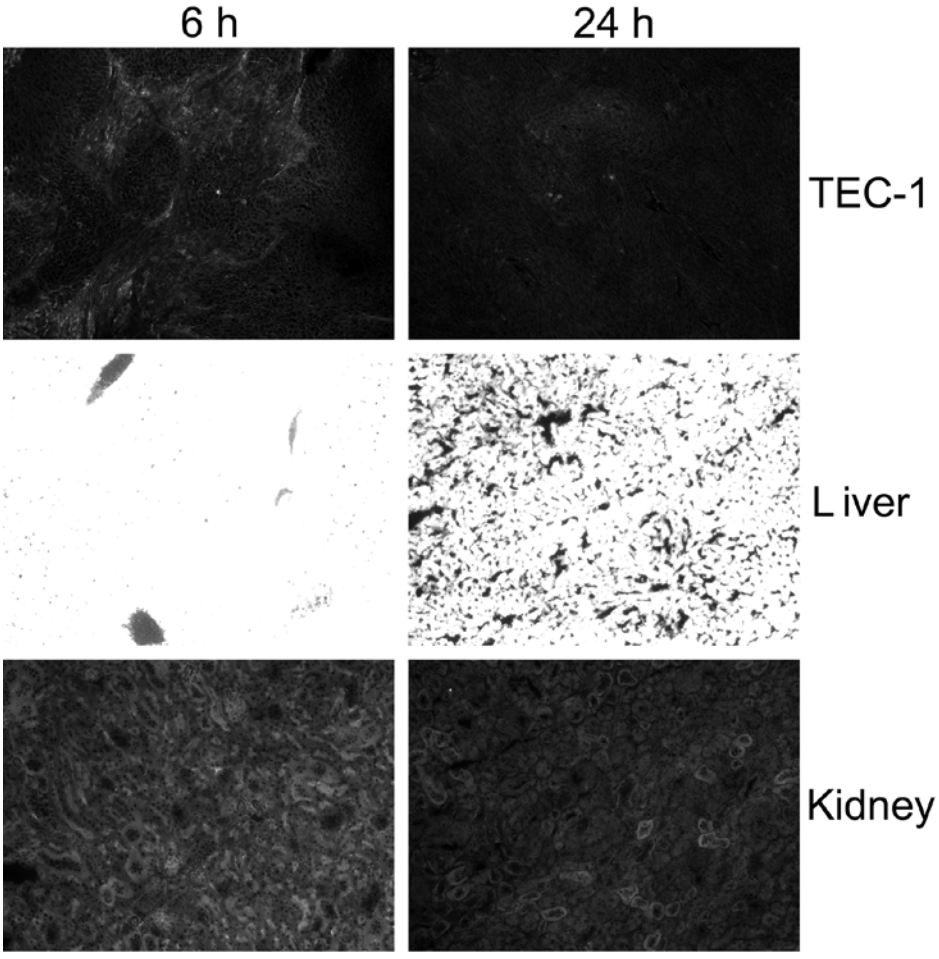

Figure S3. Hepatic sequestration of HPPH-Lac. TEC-1 xenograft-bearing SCID mice were tested for organ distribution of HPPH-Lac by using identical procedures as described for HPPH in Fig. 8. Mice were injected i.v. with HPPH-Lac (3 μmole/kg) and organs were collected after 6 and 24 h. The PS fluorescence in 5-μm cryosections of tumor, liver and kidney were imaged by microscopy.