Supplementary Materials and Methods

Transient Transfection by Nucleofection

Lung cell fibroblasts from wild type and annexin 1 knockout mice ($5x10^5$) were transfected with 2.5μg hEGFR, 2.5μg annexin1-GFP or 2.5μg Y21F Annexin1-GFP using Human Dermal Fibroblast Nucleofector™ Kit (Amaxa Biosystems) according to the manufacturers instructions.

Calcium Phosphate Transfection to create a stable cell line

HEp2 cells were transfected with annexin 1-GFP plasmid DNA using the calcium phosphate method. Normal growth medium was replaced 48 hours post transfection with full medium containing 0.5mg/ml G418 was to select for transfected cells. Cells were left for approximately 10-14 days and medium changed every 3-5 days. Colonies from single transfected cells were picked using trypsin to detach cells within an area defined using sterile cloning rings. Expression of annexin 1-GFP was determined using fluorescence microscopy and a cell line derived from a single clone was chosen for its uniform low expression of the plasmid.

Annexin 1 siRNA knockdown

Experiments were performed using control oligonucleotide 5'-AACAATGCACAGCGTCAACAG-3' that did not affect annexin 1 levels, or for annexin 1 knockdown with both of the following oligonucleotides together: 5'-AATCCATCCTGGATGTCGCT-3' and 5'-ATGGTTAAAGGTGTGGATGAA-3'.

Cloning and Site Directed Mutagenesis

Annexin 1 was isolated by PCR using the forward primer 5'-CAAGAAGCTCGAGATAAAGACACG-3', and the reverse primer 5-'CAAGGAGGATCCGCGTTTCCTCC-3', and Pfu turbo DNA polymerase (Stratagene). The PCR product was inserted into the Xho I and BamH I sites of the pGFP-N1 plasmid from Clontech in frame with the GFP coding sequence.

Y21F mutation of annexin 1 was performed using the Stratagene Quikchange™ mutagenesis kit using the forward primer 5'-GAAGAGCAGGAATTTGTTCAAACTGTGAAGTC-3' and the complementary reverse primer 5'-GACTTCACAGTTTGAACAAATTCCTGCTCTTC-3'. The point mutation required to change the sequence from tyrosine (TAT) to phenylalanine (TTT) is underlined.

EGF Degradation

Cells were incubated with ^{125}I-EGF (1ng/ml) for 10 min at 37°C, washed at 4°C and surface bound EGF stripped by acid washing twice (0.1M glycine, 0.9% NaCl pH3.0) at 4°C. Cells were then chased with serum free media at 37°C before lysis in 4°C 1% Tx100 for 10 min. The percentage degradation of EGF in the medium was determined as described (Futter et al, 1996) except using 20% TCA for 1 hour at 4°C.

EGFR degradation

Cells were incubated with EGF for up to 3 hours. Cells were lysed in PBS containing 2mM EDTA, 1%TX100 and protease inhibitor cocktail (Calbiochem). EGFR was immunoprecipitated with sheep anti-EGFR (Fitzgerald Industries International), rabbit

anti-goat IgG (Dako) and protein A sepharose. Immunoprecipitates were boiled in reducing sample buffer and analysed by western blotting with sheep anti-EGFR antibody and anti-sheep IgG conjugated to HRP (Jackson Laboratories). Bands were visualised using ECL (Pierce) and Intelligent Dark Box II (Fugi) and Image Reader LAS-1000 software and semi-quantitative analysis performed using Aida software.

Assessment of efficiency of annexin 1 knockdown

HeLa cells after incubation with siRNAs were lysed in reducing sample buffer, western blotted with anti-annexin 1 antibody and anti-tubulin antibody (as a loading control) and bands visualised as described for EGFR degradation.