

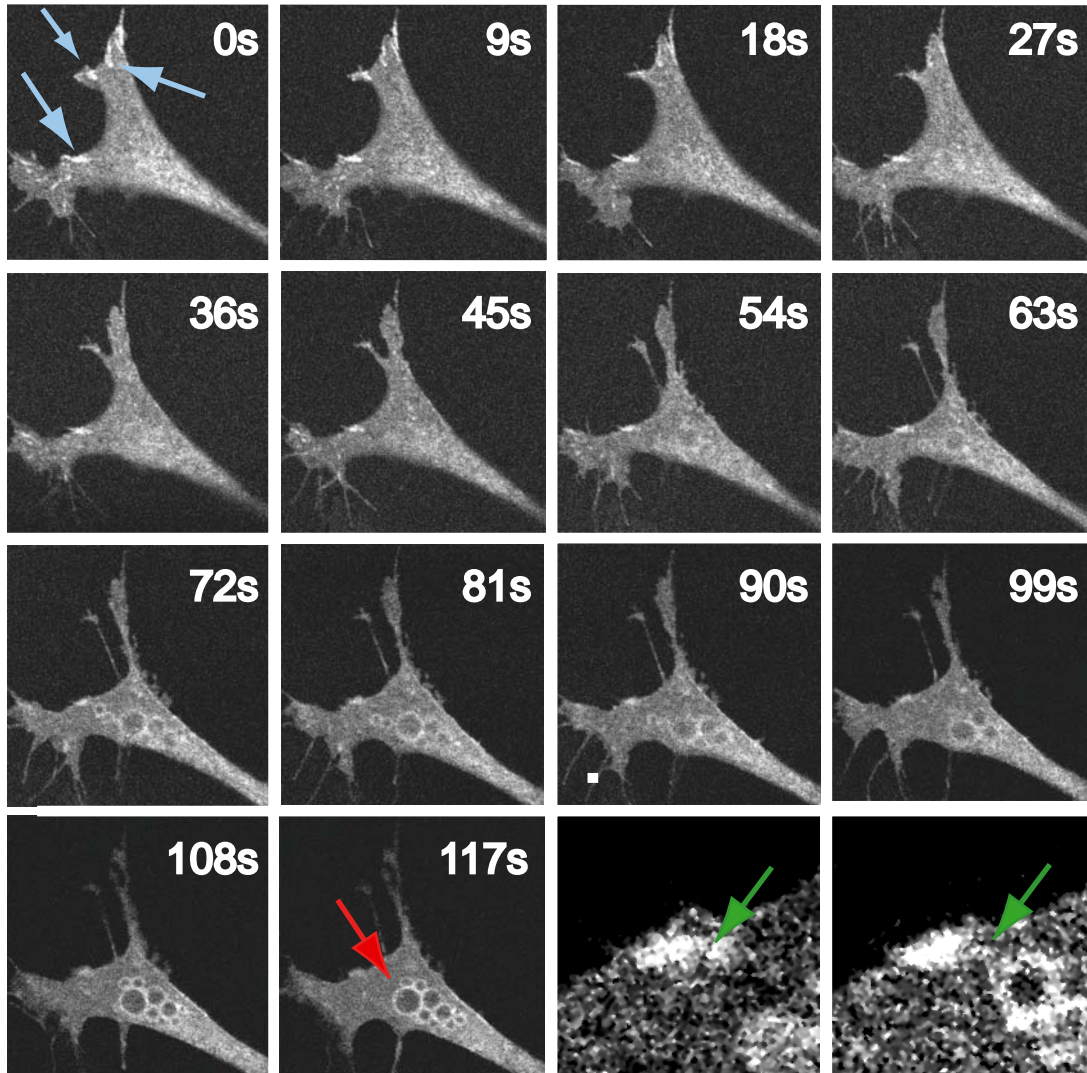
## Supplementary Data

**Supplementary Figure 1. Live imaging of the ts LA29 mutant of v-Src fused to GFP.** v-Src-GFP, enriched at linear structures (possible focal adhesions) at the end of a long cell process (blue arrows) appears on large vesicles (macropinosomes: red arrow) as the cell transforms at the permissive temperature. The final panels show close-ups at 62 seconds and 66 seconds where the putative focal adhesion appears to split and is ‘spun off’ onto the nearby vesicle (green arrows) Scale bar = 4µm. The stills are taken from a movie that can be viewed as Supplementary Movie 1.

**Supplementary Figure 2. Depletion of annexin 2 from Müller cells results in relocalisation of activated Src.** MIO cells were depleted of annexin 2 using siRNA for 4 days and then fixed and stained for annexin 2, Src-pY416 and F-actin. Following loss of annexin 2 cells become flattened, cortical actin and ruffles are lost, and there is enrichment of stress-fibres. Active Src (Src-pY416) relocalises from the cell ruffles (yellow arrow) to the ends of bundles of stress-fibres (blue arrows, coincident with focal adhesions).

**Supplementary Figure 3. Over-expression of an siRNA-hardened annexin 2 reverses the loss of function phenotype of annexin 2-depleted cells.** (A) R1LA29 cells were depleted of annexin 2 siRNA for 3 days and then GFP alone or (B), a hardened annexin 2-GFP construct was introduced into the cells by transient transfection. After 48 h the cells were grown at 35°C to induce v-Src mediated transformation. Cells were fixed and stained for Src-pY416 and actin. Green cells expressing hdx2-GFP or GFP alone were classified as transformed or non-transformed on the basis of cell shape, the distribution of F-actin and Src-pY416. (C) Close-up of a hdx2-GFP expressing cell to show that hdx2-GFP localises to the cell cortex and perinuclear domain in the same manner as the wild-type protein. (D) Histogram of pooled data from 3 independent experiments showing that following depletion of annexin 2 and reintroduction of either a hardened annexin 2 construct or GFP, there are significantly ( $p = 0.001$ ) fewer untransformed cells following transfection with the hardened annexin 2.

Sup. Fig.1



Sup. Fig. 2

