# Azurin interaction with the lipid raft components ganglioside GM-1 and caveolin-1 increases membrane fluidity and sensitivity to anti-cancer drugs

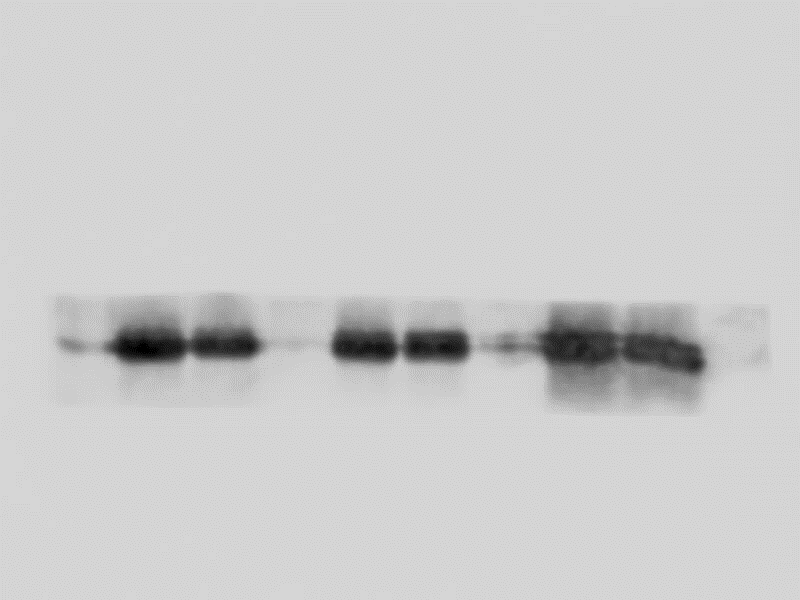
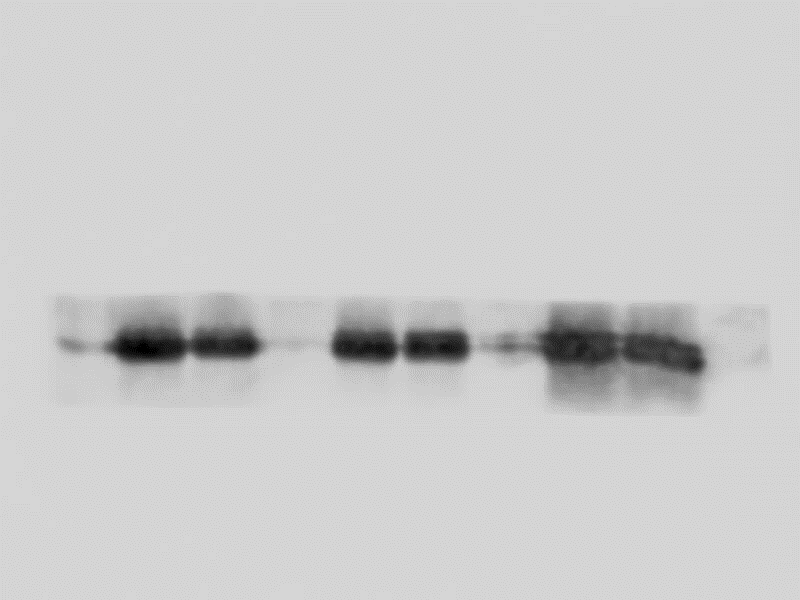
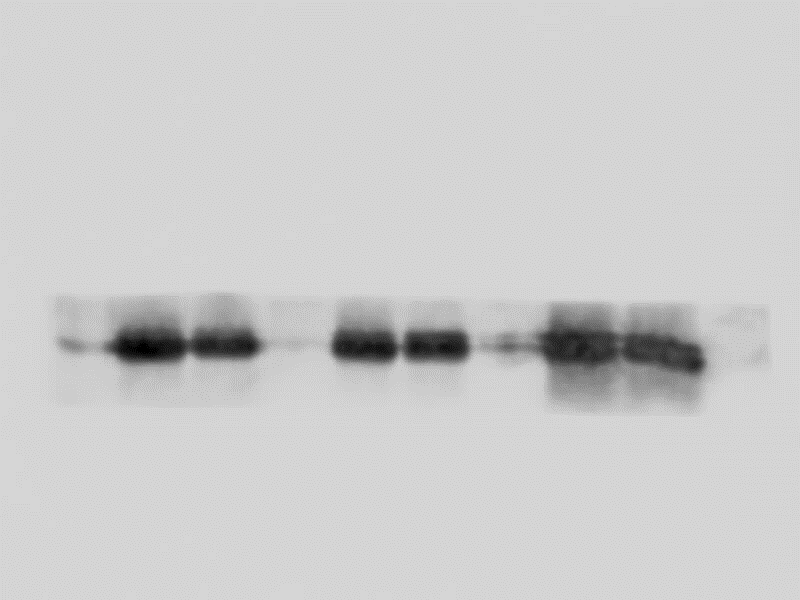
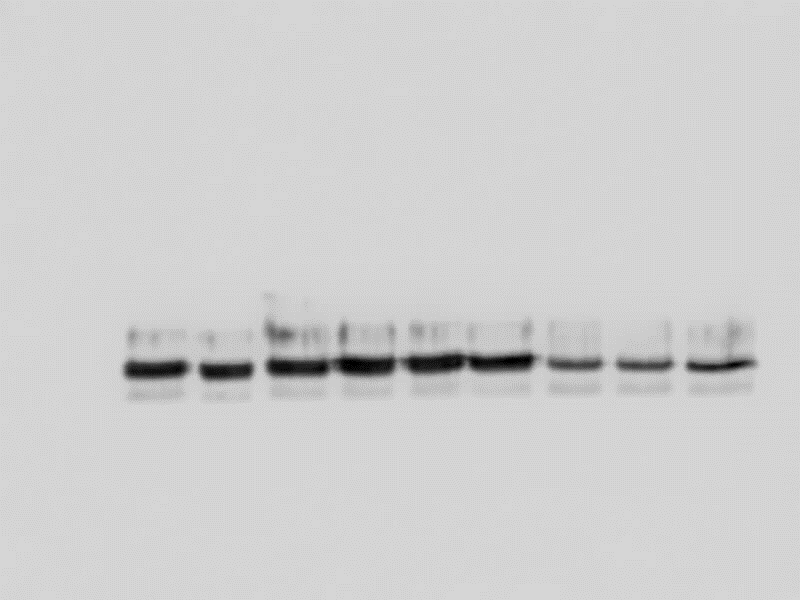
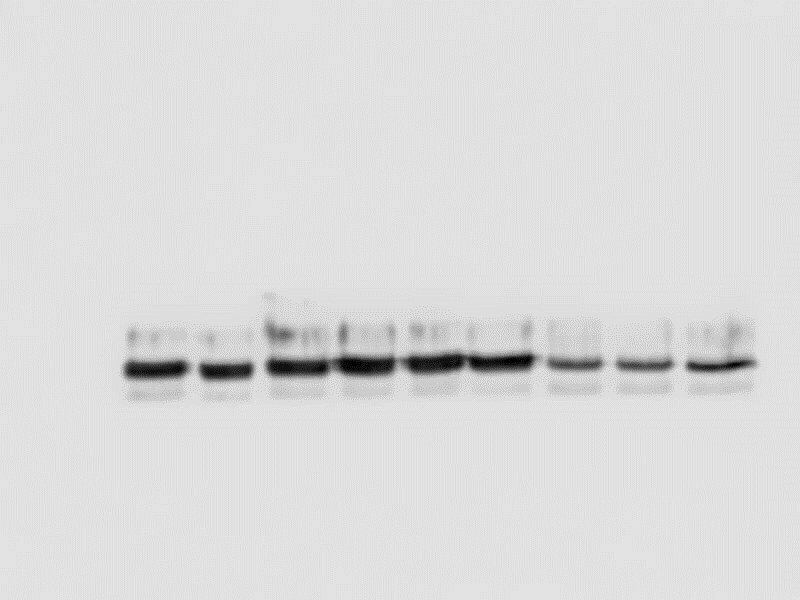
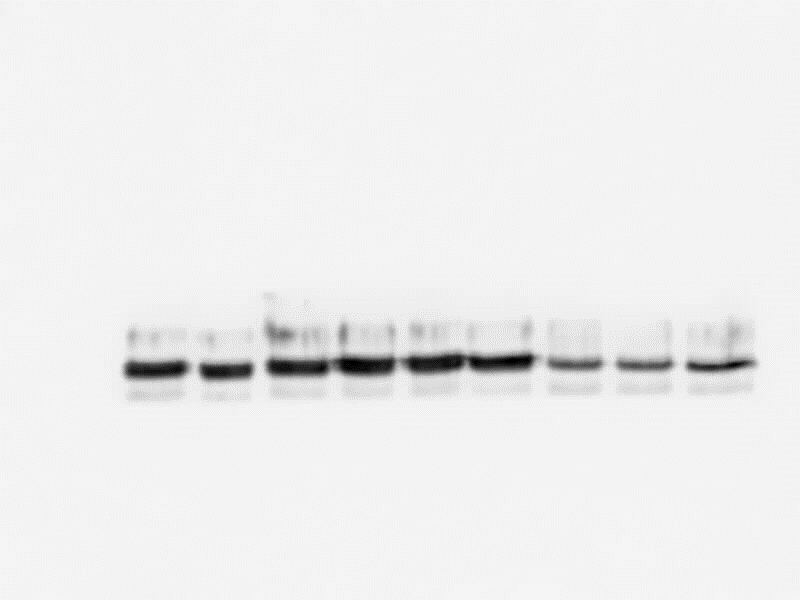
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**HeLa**

Azurin

F114A

100µM

**MCF-7**

Untreated

WT

100µM

**WB: Caveolin-1**

**WB: GAPDH**

F114A

100µM

Untreated

WT

100µM

F114A

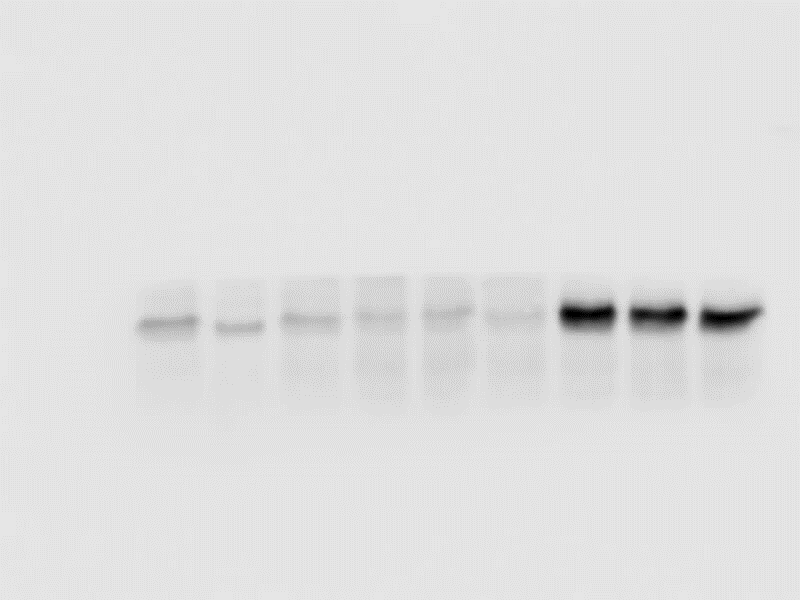
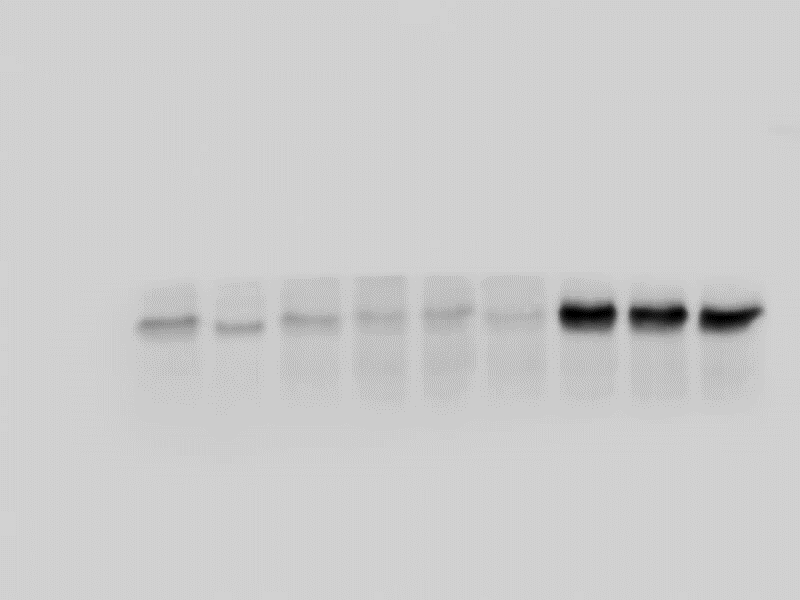
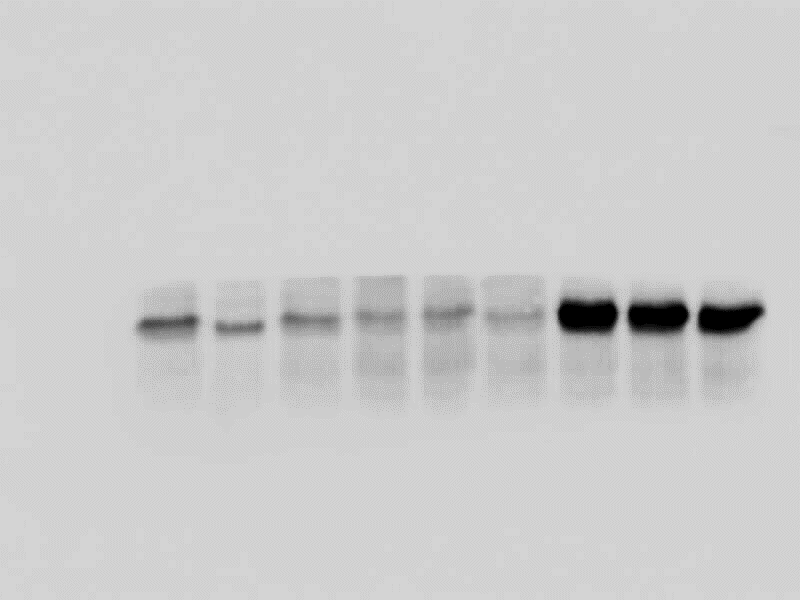
100µM

Untreated

WT

100µM

**HT-29**



**WB: Azurin**

Supplementary Figure 1

Supplementary Figure 1: Entry of azurin WT and F114A mutant in MCF-7 and HeLa cells. Cells were exposed to 50-100µM of both proteins for 48h, after which cells were lysed and protein entry inside cells was determined by Western blot (top panel). Endogenous levels of caveolin-1 in each cell line (middle panel).

A)



B)



Supplementary Figure 2: Fluorescence spectra of the donors Atto390-WT (A) or Atto390-F114A (B) acquired with 390 nm excitation and measured over the emission wavelength range of 400 to 470 nm, since no acceptor (FITC-labeled CSD peptide) emits there. The FITC-labeled CSD peptide was titrated from 0 to 14μM. Fluorescence measurements were carried out with a SLMAminco 8100 Series 2 spectrofluorimeter (Rochester) with double excitation and emission monochromators (MC-400), in a right angle geometry. The light source was a 450-W Xe arc lamp and the reference a Rhodamine B quantum counter solution. Quartz cuvettes (1×1cm) from Hellma Analytics were used.

Supplementary information: Full-lenght Western blot images. Images are referred to the same conditions and Figure number as indicated in the main text.

**MCF-7**

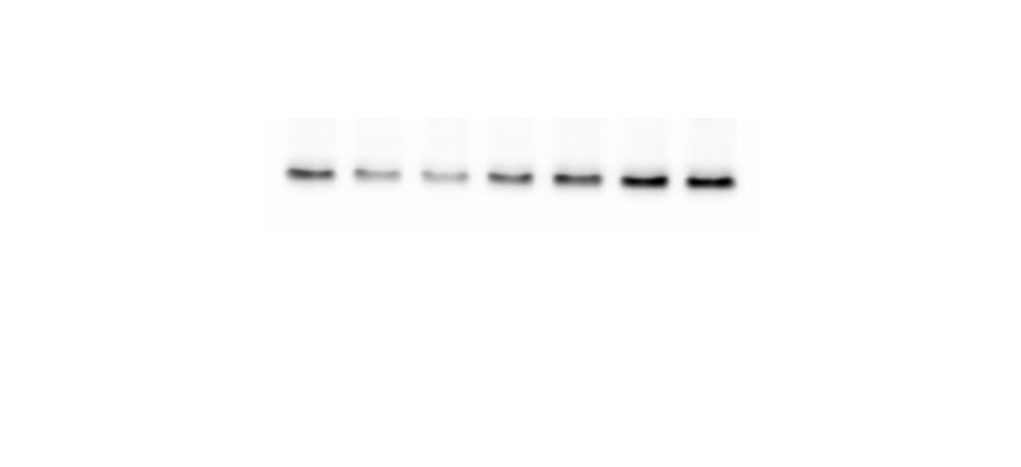
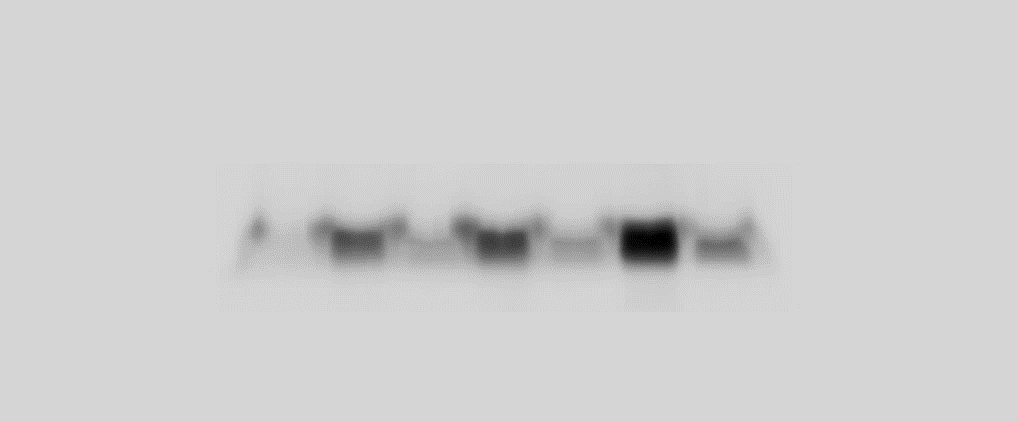


Figure 1C

**WB: Azurin**

**(15 kDa)**

**WB: GAPDH**

**(37 kDa)**

F114A

100µM

WT

100µM

F114A

50µM

WT

50µM

Untreated

Untreated

**2 hours**

**WB: Azurin**

**(15 kDa)**

Azurin

**30 min**

**WB: GAPDH**

**(37 kDa)**

F114A

25µM

WT

25µM

WT

50µM

F114A

50µM

WT

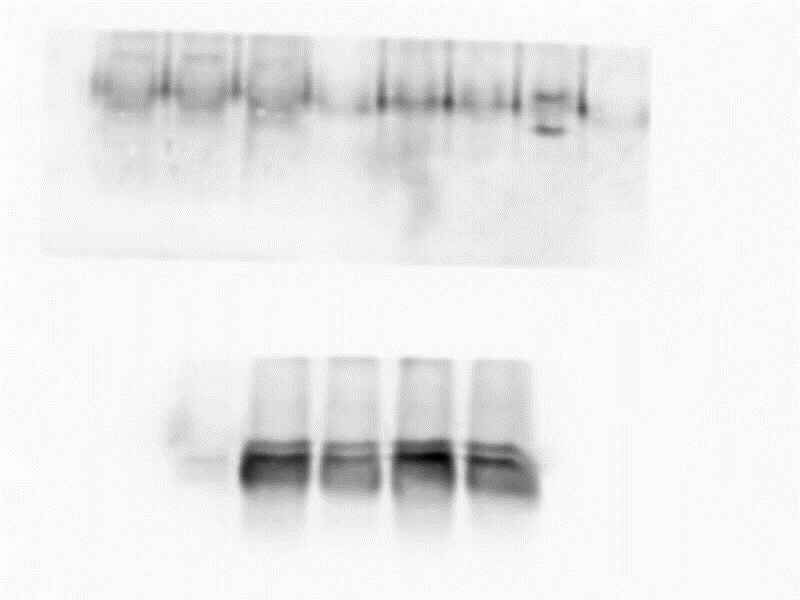
100µM

F114A

100µM

**HeLa**

**30 min**



F114A

100µM

**WB: Azurin**

**(15 kDa)**

Azurin

WT

100µM

F114A

50µM

WT

50µM

Untreated

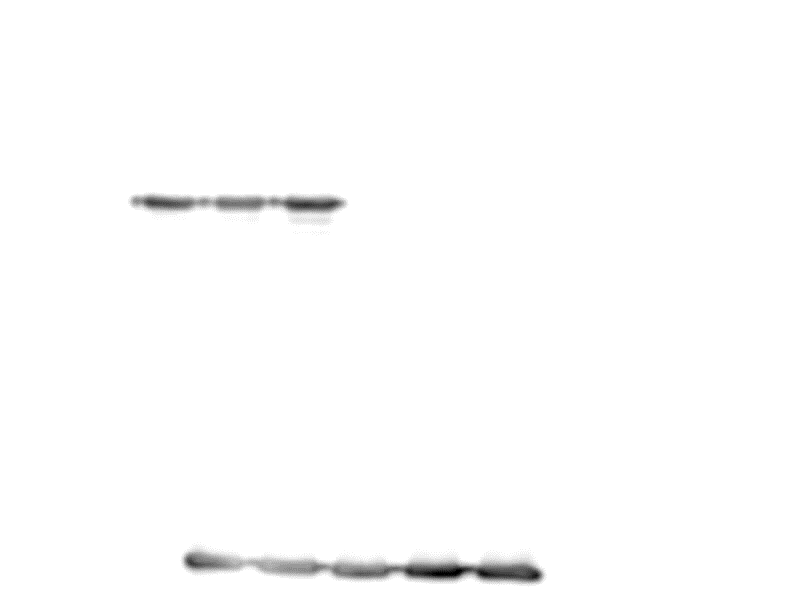


Figure 1C

**WB: Azurin**

**(15 kDa)**

**WB: GAPDH**

**(37 kDa)**

**WB: GAPDH**

**(37 kDa)**

**2 hours**



Figure 1C

**WB: GAPDH**

**(37 kDa)**

**WB: Azurin**

**(15 kDa)**

**2 hours**

**HT-29**

Untreated

WT

50µM

F114A

50µM

WT

100µM

F114A

100µM

**WB: GAPDH**

**(37 kDa)**

Azurin

**30 min**

**WB: Azurin**

**(15 kDa)**

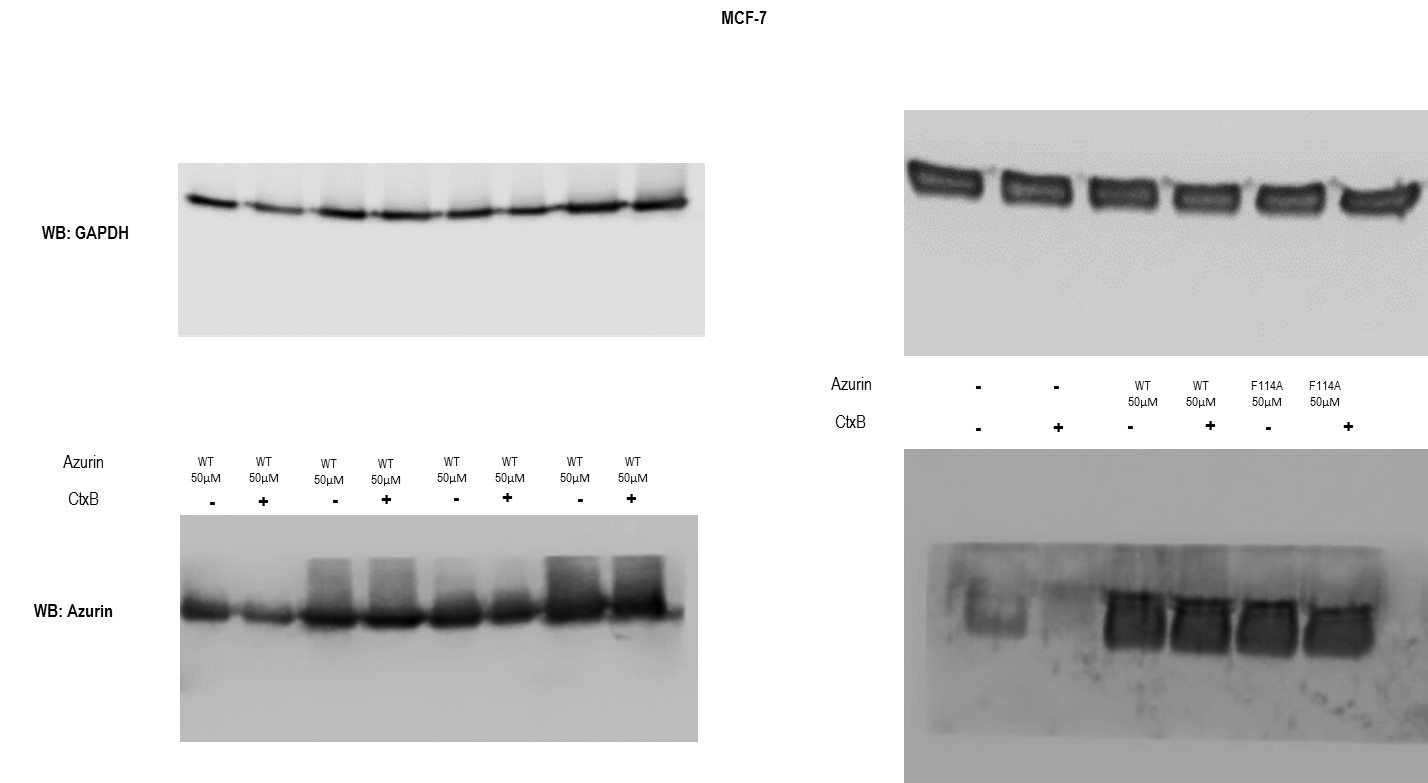
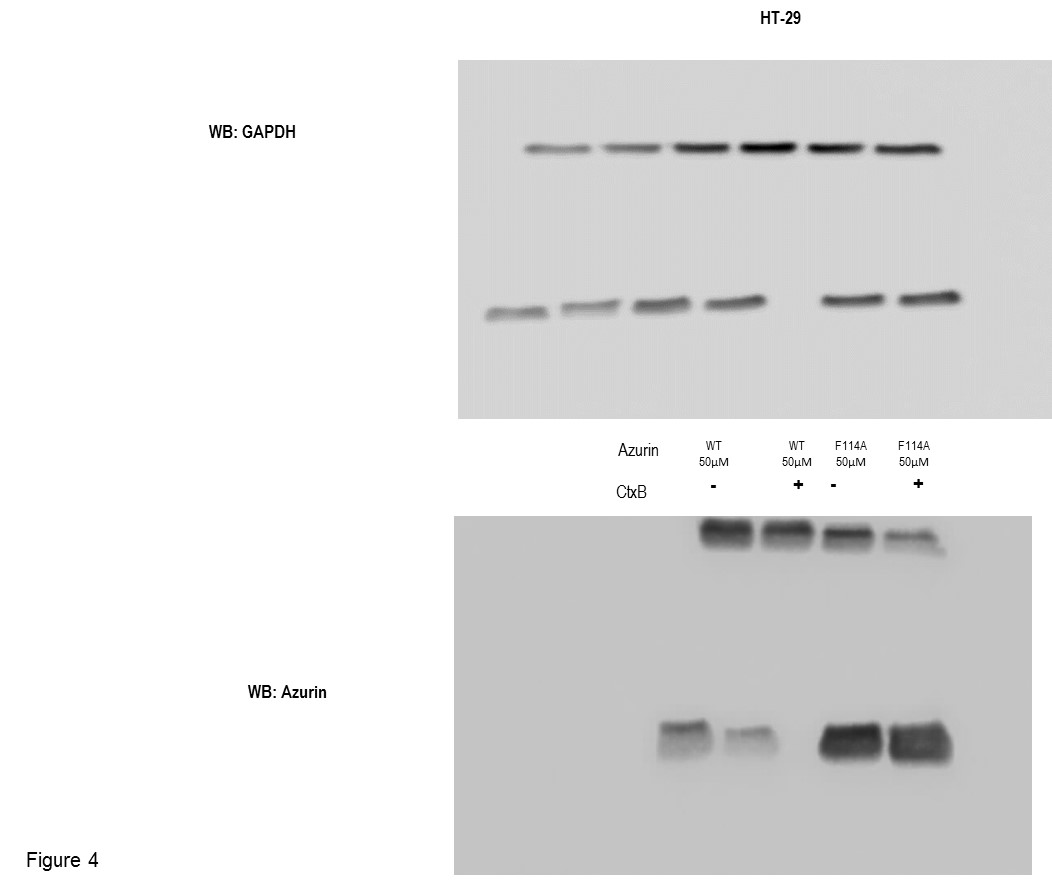


Figure 2A

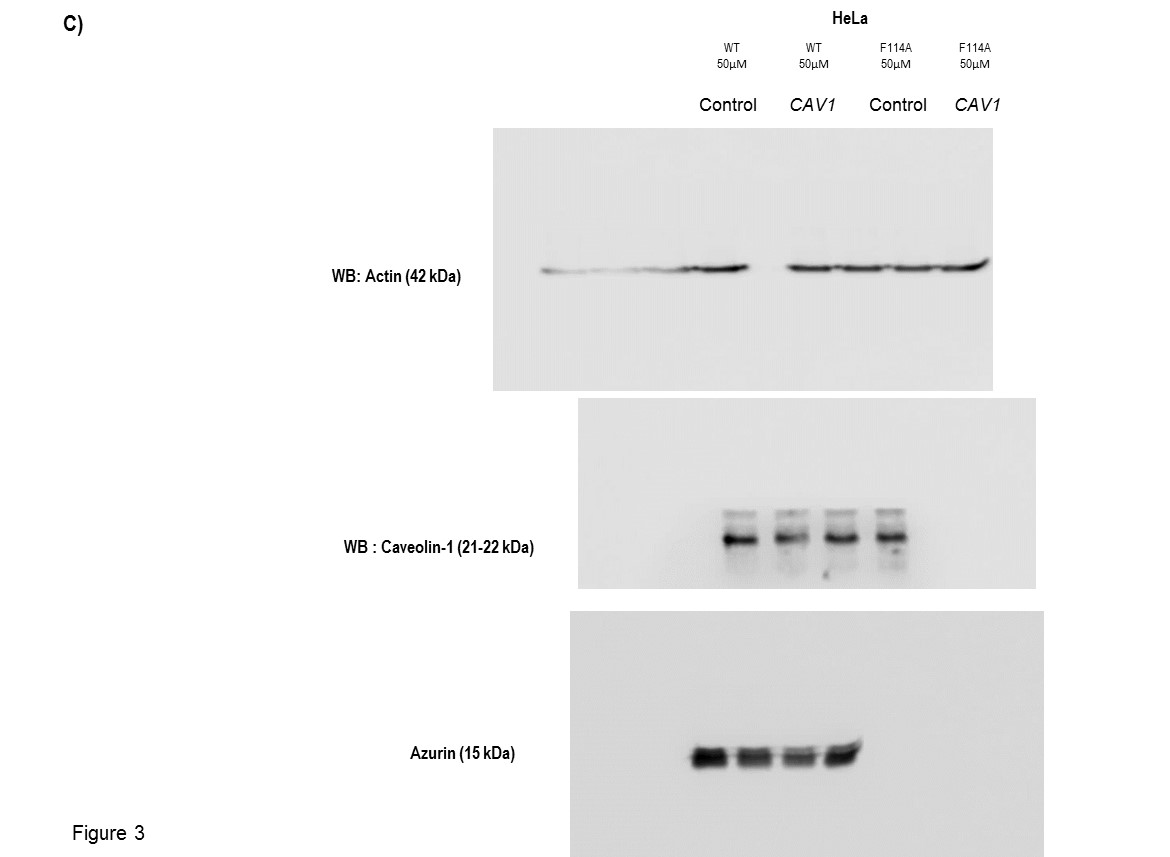
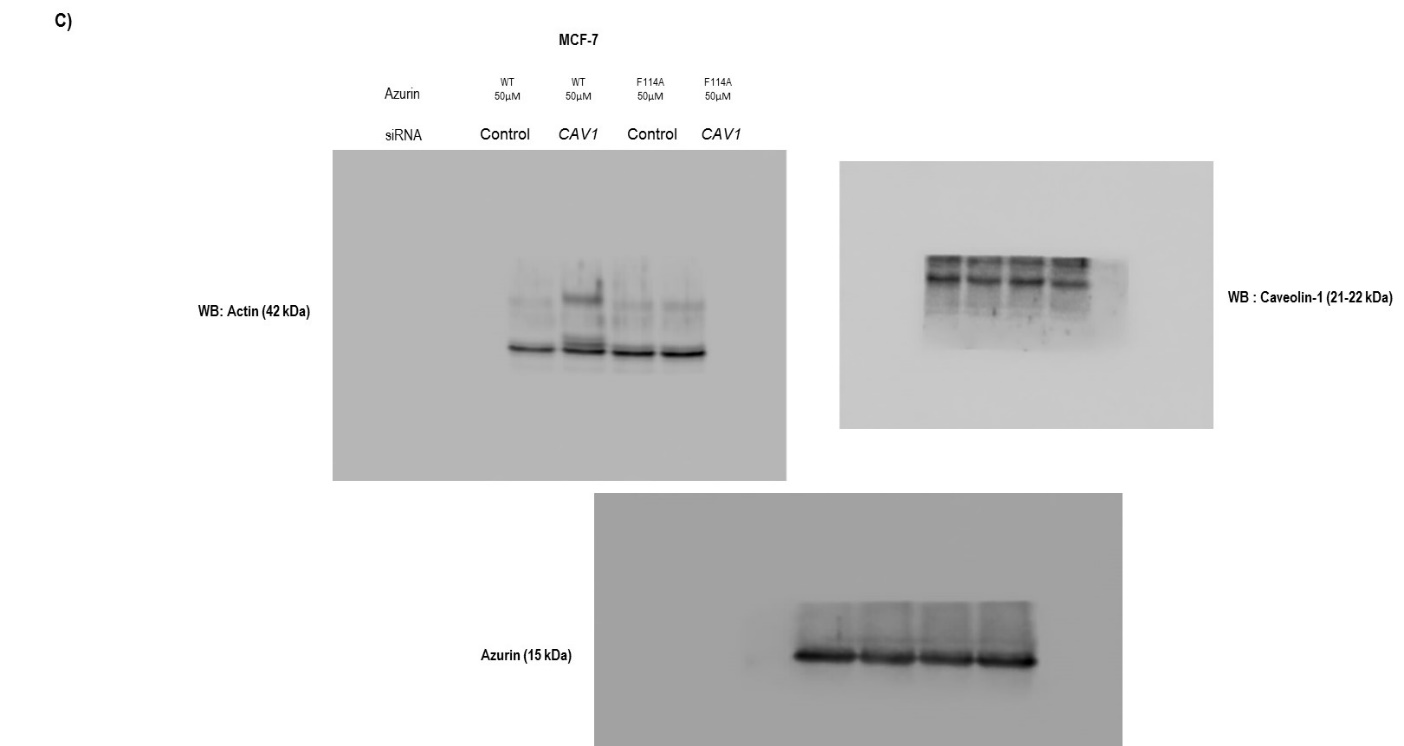
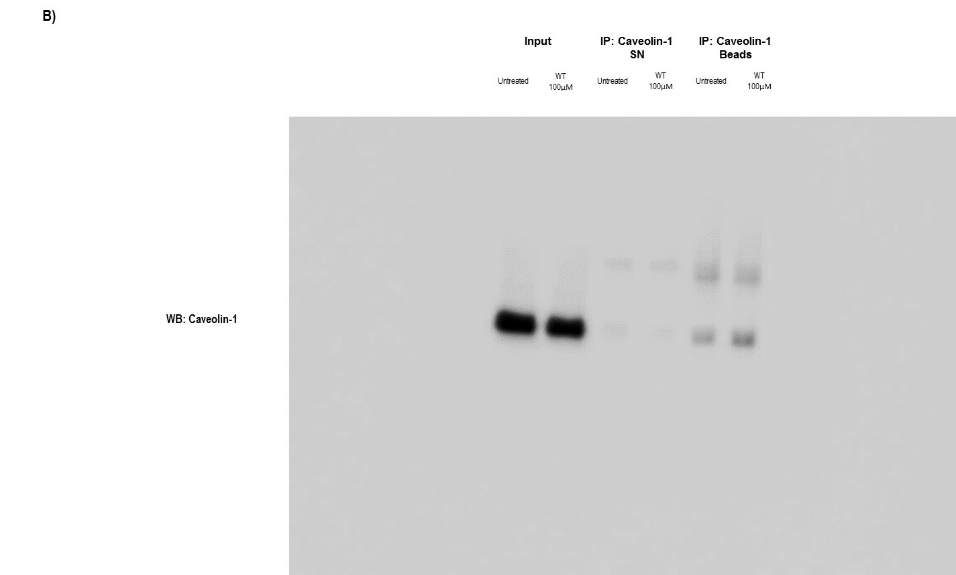
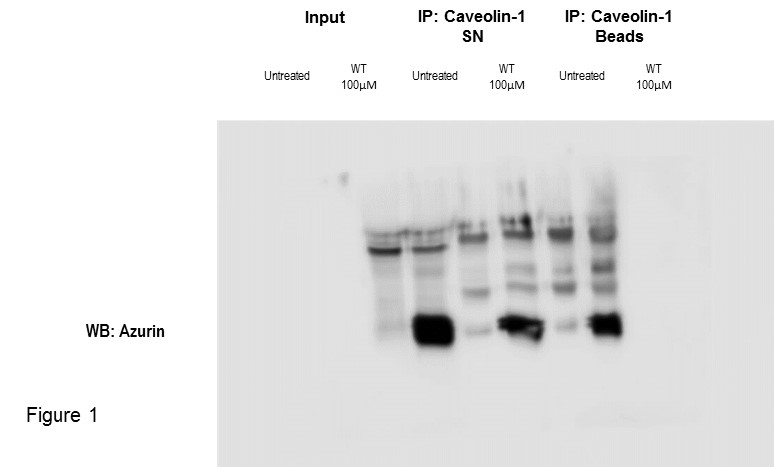


Figure 3A





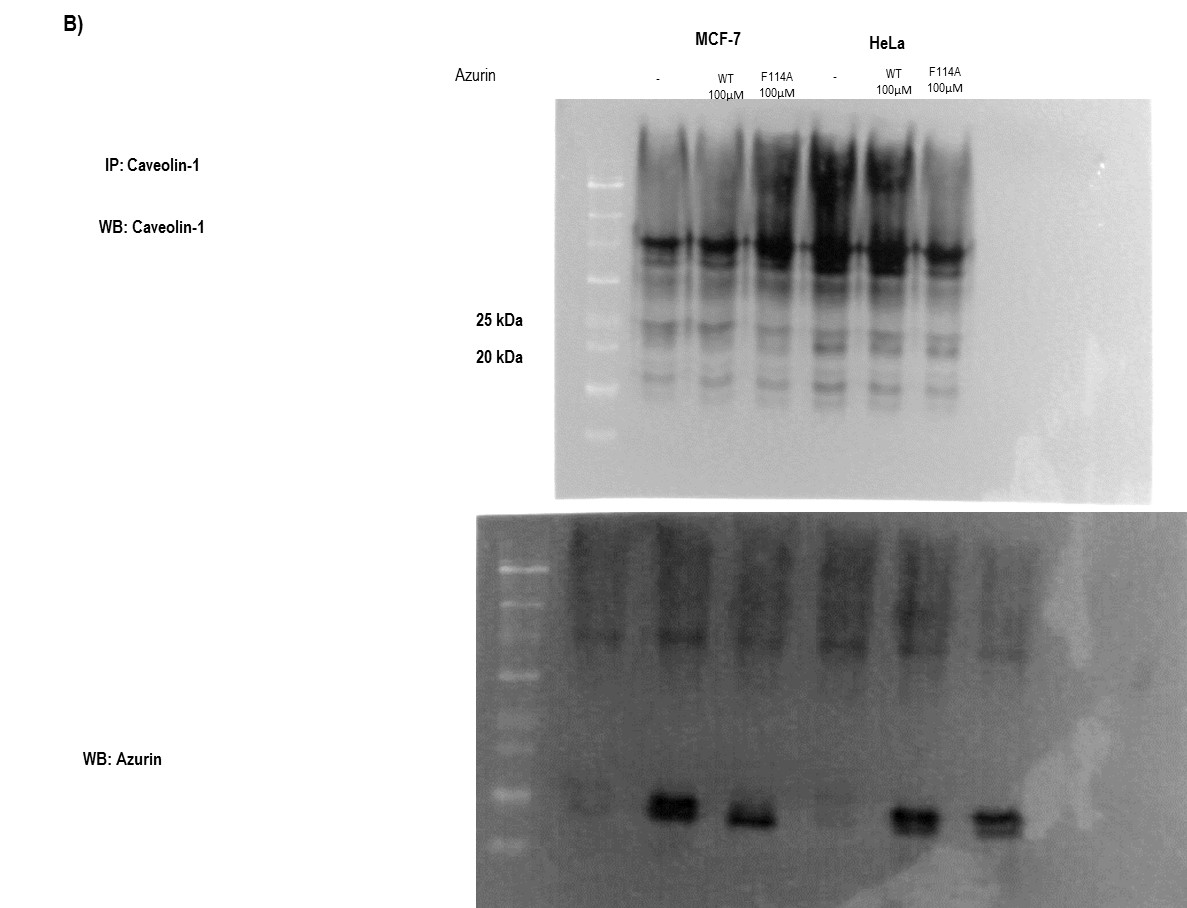


Figure 3C

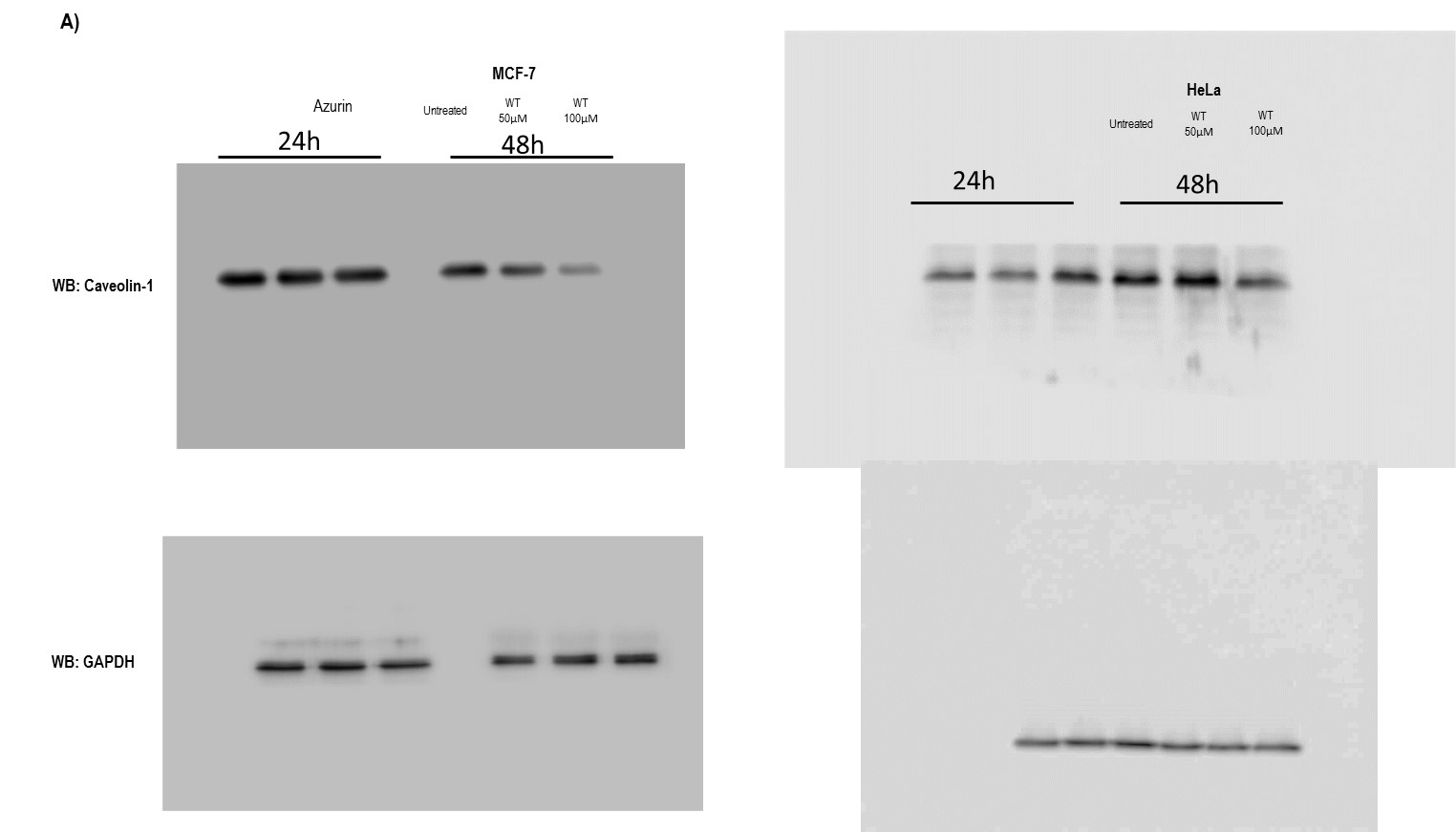


Figure 3D