Supplementary figure legends

Supplementary Figure 1. RFP-XIAP functions as a caspase inhibitor

(**A**) MECs transiently transfected with monomeric RFP or RFP-XIAP were detached from ECM for 4- or 8-h and the percentage of cells containing apoptotic nuclei was scored. For the transfected cells, only red cells were counted. Note that RFP-XIAP suppresses anoikis.

(**B,C**) MECs were co-transfected with a YFP-tagged caspase-3 sensor and either RFP (top panels) or RFP-XIAP (lower panels). After 4-h treatment with or without 10 μM staurosporine (STS), cells were fixed and stained with DAPI and the percentage of co-transfected cells with nuclear localised GFP was counted. Asterix indicates nuclear localised GFP. Note that RFP-XIAP suppresses STS-induced caspase activity. Data in these and subsequent experiments represent the average of 3 independent experiments +/- S.E.M.

Supplementary Figure 2. Myc-XIAP (but not Myc-Survivin) also induces MOMP

(**A**) MECs transiently expressing Myc-XIAP or Myc-Survivin were scored for cytochrome c release.

(**B**) Immunoblot analysis of MECs transiently expressing Myc-XIAP or Myc-Survivin.

Supplementary Figure 3. Caspase binding is not required for XIAP to induce MOMP, but is required for XIAP to prevent apoptosis downstream of MOMP

(**A**) MECs expressing the wild type or caspase-binding mutant (D148A/W310A) form of RFP-XIAP were immunostained for cytochrome c and the percentage of transfected cells with released cytochrome c quantified after 18 hr and 42 hr. Note that the RFP-XIAP caspase-binding mutant induces cytochrome c release to similar levels as the wild type protein.

(**B**) The level of apoptosis in cells transfected cells was scored by examining nuclear morphology. The RFP-XIAP caspase-binding mutant fails to prevent apoptosis downstream of MOMP, as indicated by a significant (* indicates $p < 0.05$) increase in apoptotic cells at 42 hr.

(**C**) A representative image of a D148A/W310A expressing cell with an apoptotic nucleus is shown (arrow).

Supplementary Figure 4. Cells transiently expressing XIAP fail to form colonies

MECs transiently transfected with RFP (upper panels) or RFP-XIAP (lower panels) were cultured for 5-days following transfection. Cells were fixed and immunostained for

cytochrome c. Representative images are shown. In the top panels, an RFP-expressing colony has formed. In the bottom panels, the arrow indicates a non-apoptotic RFP-XIAP-expressing cell that has released cytochrome c. Note that in longer-term experiments, we were unable to obtain RFP-XIAP-expressing colonies of cells (not shown).

Supplementary Figure 5. RFP-XIAP expression abrogates proliferation

To assess the affect of RFP-XIAP expression on proliferation, cultures of MECS or MEFs were transfected with RFP-XIAP or control RFP and then treated with EdU to assess DNA replication. Proliferation levels were determined by counting the percentage of transfected cells that were EdU positive.

(A) RFP-XIAP expression significantly reduced levels of proliferation in MECs compared with untransfected or RFP transfected control (*** indicates $p < 0.01$).

(B) In MEF cells RFP-XIAP had the same effect of reducing proliferation compared with controls.

(C) Representative images for proliferating MECs and MEFs. Arrowheads indicate transfected cells that are EdU positive, which are only present in the panels of control RFP transfected cells. Arrows indicate transfected cells that are not EdU positive. The few RFP-XIAP expressing cells that were EdU positive had not released cytochrome c (data not shown).

Supplementary Figure 6. Cells transiently expressing RFP-XIAP have lost mitochondrial membrane potential

To determine whether XIAP-induced cytochrome c release led to the subsequent loss of mitochondrial membrane potential, rhodamine 123 was added to cultures of MECs or MEFs that were expressing RFP-XIAP or control RFP. Live cells were then imaged to examine the distribution of rhodamine 123, which is only taken up into mitochondria that retain membrane potential.

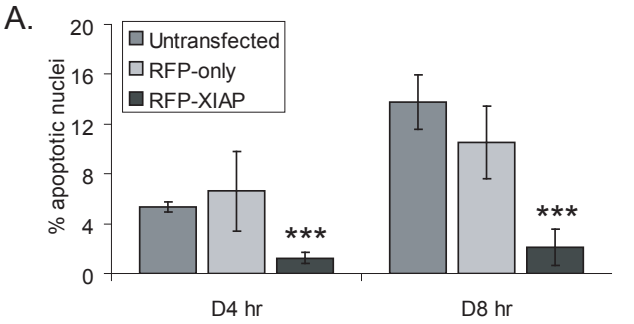
(A, B) Representative images of MECs and MEFs treated with Rhodamine 123. Rhodamine 123 has a punctuate (mitochondrial) distribution in untransfected or RFP-expressing control cells. In contrast, rhodamine 123 is absent from the mitochondria of RFP-XIAP-expressing cells (arrows) indicating that these cells have lost mitochondrial membrane potential.

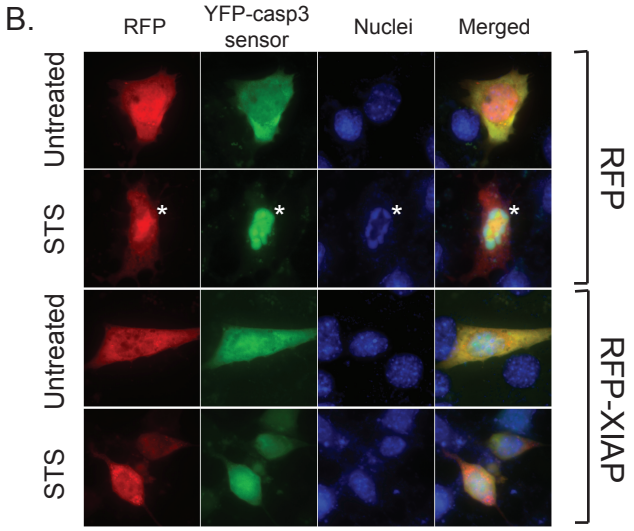
Supplementary Figure 7. RFP-XIAP induces cytochrome c release in human mammary epithelial cells

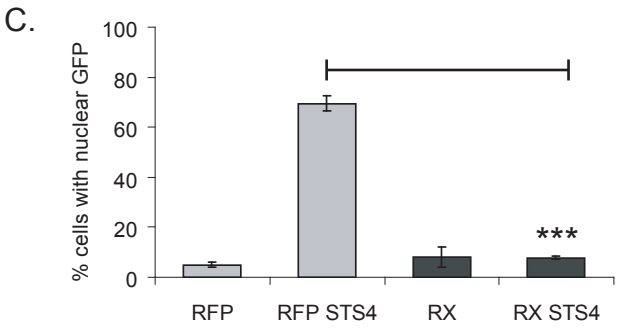
Mouse MECs and human mammary epithelial cells (MCF10A) were transiently transfected with RFP-XIAP or control RFP and the percentage of transfected cells that had released cytochrome c release scored. RFP-XIAP induced cytochrome c release to similar levels in both cell lines. Thus, in addition to mouse MECs, exogenous XIAP induced MOMP in human MECs.

Supplementary Figure 8. RFP-XIAP localisation

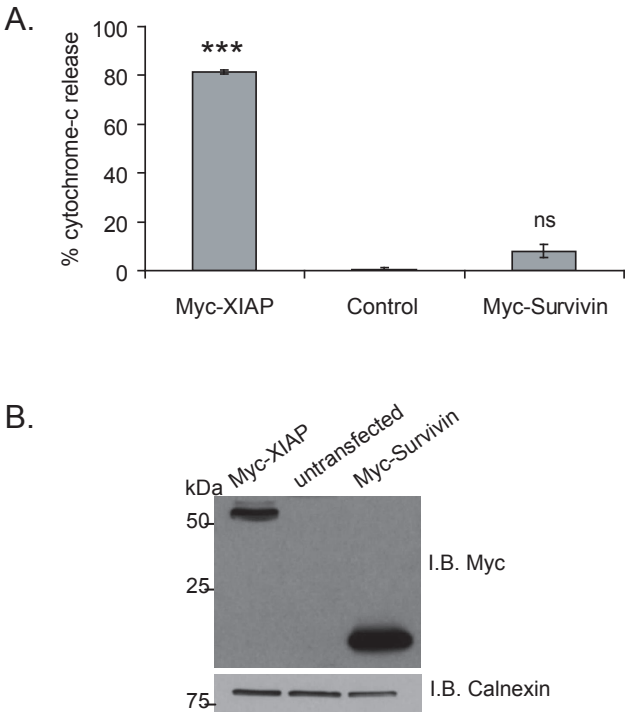
MECs transiently expressing RFP-XIAP were immunostained for markers of different membrane compartments; calnexin (ER), GM130 (Golgi) and LAMP1 (endosomes). To visualise the membrane associated RFP-XIAP, the cytosolic portion of the protein was removed by permeabilizing cells with 50 mg/ml digitonin prior to fixation. Arrows show RFP-XIAP localisation.







Owens/Streuli Fig S1



Owens/Streuli Fig S2

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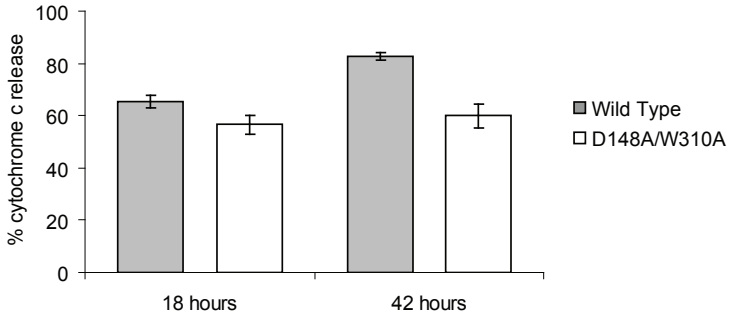

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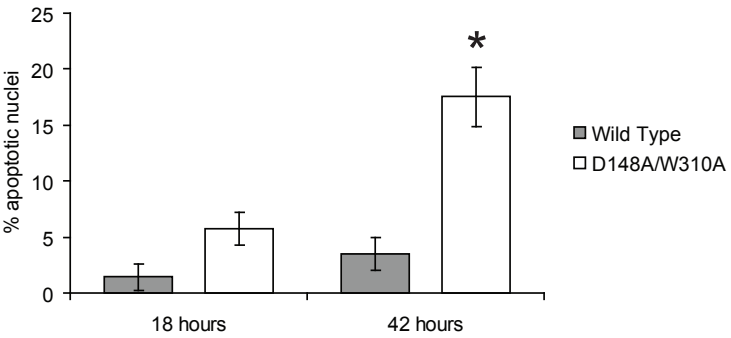

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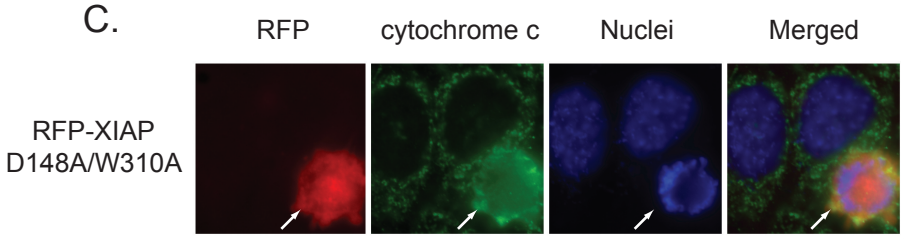
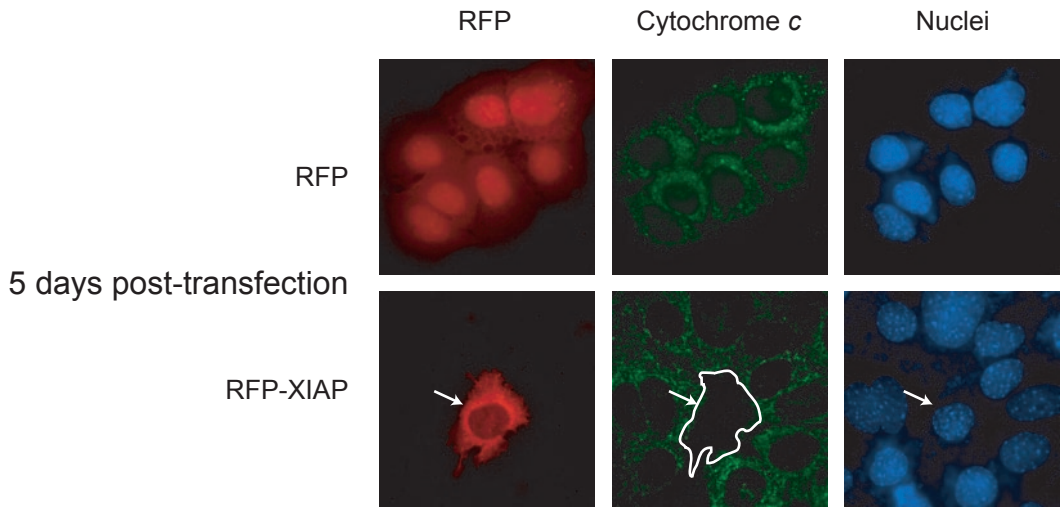


Owens/Streuli Fig S3



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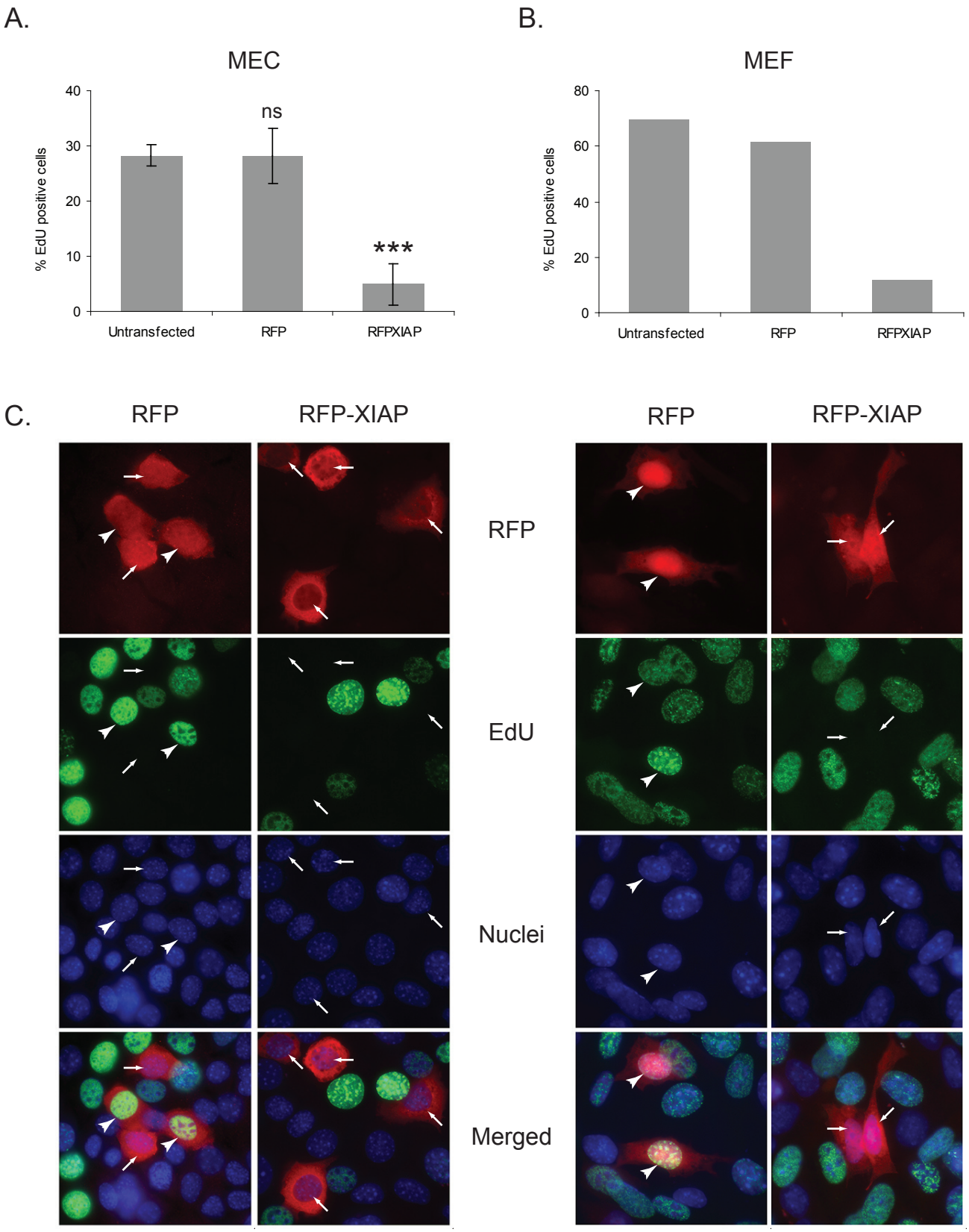
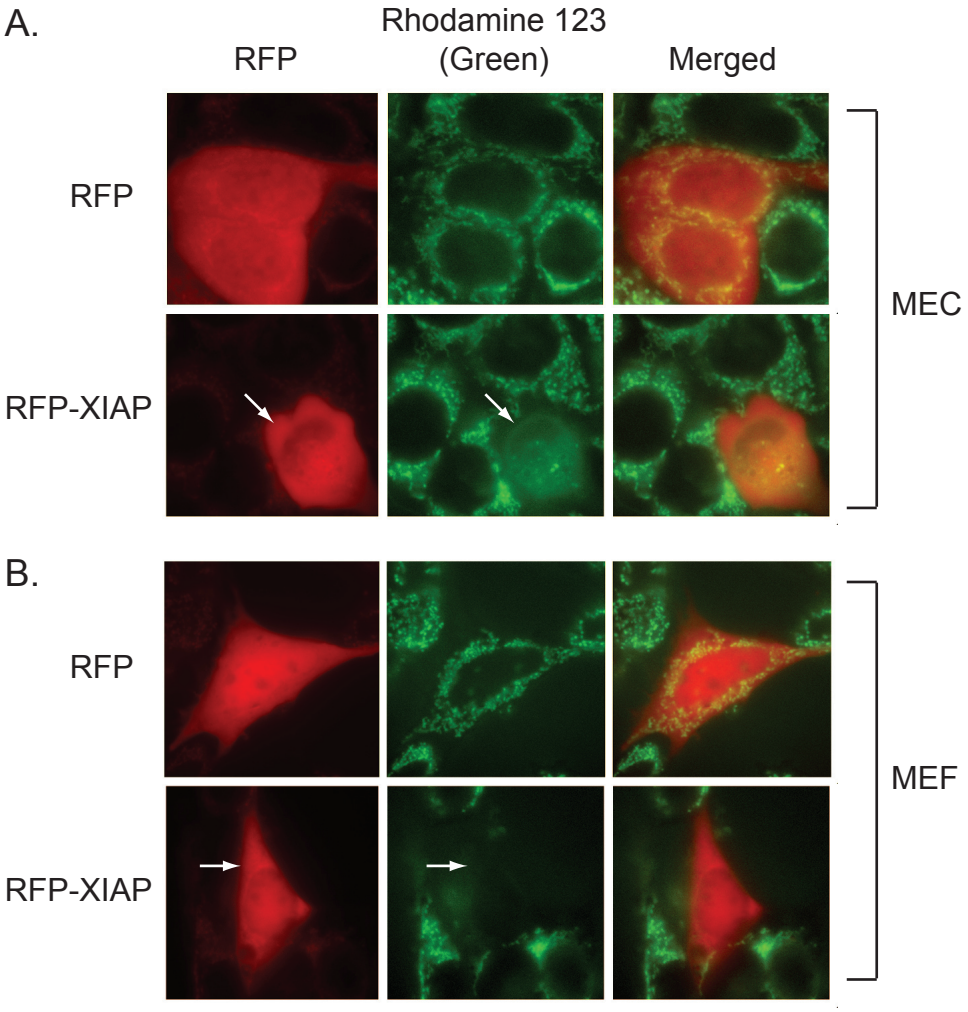
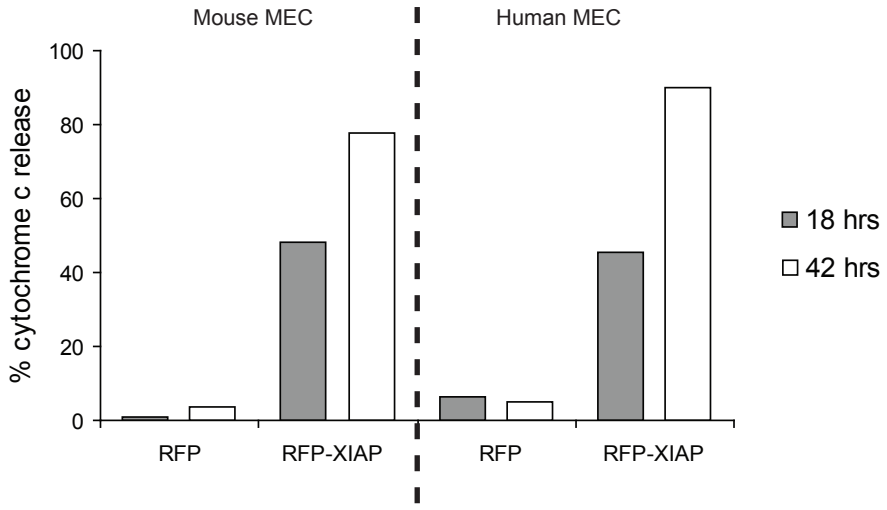


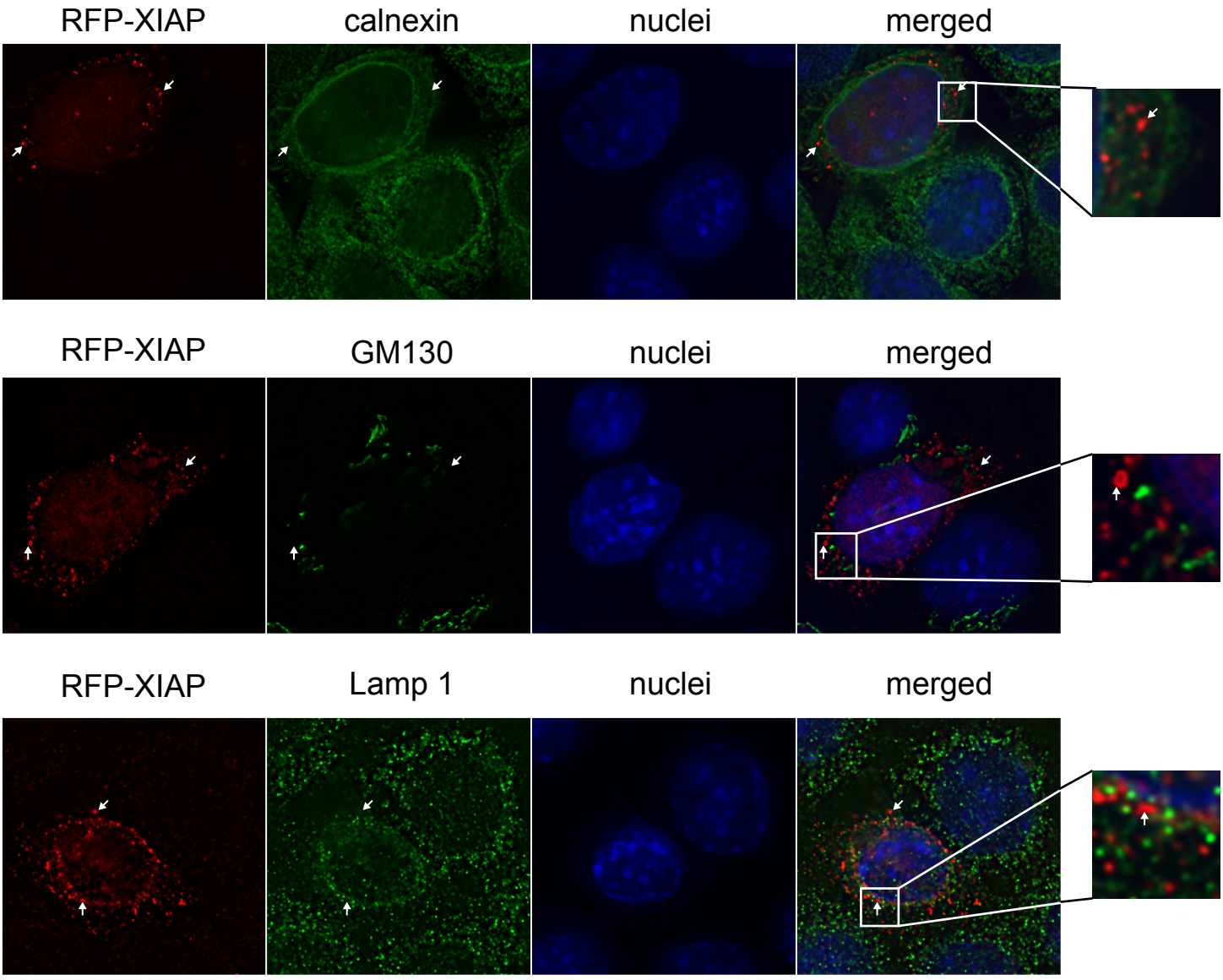
Owens/Streuli Fig S5



Owens/Streuli Fig S6



Owens/Streuli Fig S7



Owens/Streuli Fig S8