Supplementary Figure Legends

Supplementary Figure 1. Knockdown of caspase 3 promotes resistance to apoptotic insults. The knockdown of caspase 3 promotes cellular survival in proapoptotic stimuli. Cells infected with a lentiviral vector expressing a control shRNA or an shRNA to caspase 3 were treated with the drugs (A) Docetaxol or (B) TRAIL (plus 100ng/ml IFNγ) or the toxin staurosporine (C) were assessed for cell survival after 24 hours by flow cytometry. The results shown represent the mean and standard error of three different determinations.

Supplementary Figure 2. Talin cleavage is a general event following ECM attachment. (A) Cleavage of talin in NB7 neuroblastoma cells expressing caspase 8 (+ caspase 8) or deficient in caspase 8 (-caspase 8) when plated on vitronectin (2μg/ml) or collagen (5μg/ml) substrates. Cells were then lysed in RIPA while in suspension (0) or ten (10’) or thirty (30’) minutes following substrate adhesion, proteins resolved by PAGE, and immunoblotting for talin performed. The amino-terminal FERM fragment is indicated by the arrow. (b) Cleavage of talin was also assessed in two caspase 8-expressing neuroblastoma cell lines (NB5 and NB16) treated with an shRNA to caspase 8 (+) or empty vector (-). After 48h, cells were allowed to attach to collagen substrate for 30 minutes, lysed, and the presence of talin assessed as described above.
Supplementary Figure 3. Identification of a calpain-like activity within the focal adhesions of Neuroblastoma Casp-8-expressing cells (NB7C8 and NB5 cells). Focal adhesions were prepared by differential extraction of cell components. Cytosolic fractions were first isolated by extraction with Triton X-100 detergent for 30 minutes. The remaining stable focal adhesions were isolated using RIPA (lacking deoxycholate), and debris (including any remaining nuclei) eliminated by centrifugation. Fractions were checked for their relative content of HSP90 and for actin by immunoblotting to monitor relative purity. Fractions from cells deficient in caspase 8 (-) or expressing caspase 8 (+) were incubated with activity–based probes including DCG-04 (for calpains/calpain-like activities) and AB19-BTMX (specific for caspases/ selective for caspase 8)(no caspase activity was detected in these experiments). The open arrowhead indicates significant accumulation of probe incorporation within the focal adhesion fraction of Casp-8-expressing NB7 cells (NB7C8). To determine if this was a general observation, NB5 cells expressing caspase 8 were infected with a lentiviral construct encoding shRNA to caspase 8, and selected for puromycin resistance (creating the NB5shC8 cell line). These cells were then allowed to attach to collagen for 30 minutes, and cytosolic and focal adhesion fractions isolated as described, fractions probed with DCG-04-bodipy-TMR, resolved by 1D-PAGE and visualized on a flat-bed fluorescence scanner.

Supplementary Figure 4. Calpain is activated during cell adhesion. (A) Talin cleavage is sensitive to calpeptin; NB7 cells expressing caspase 8 were plated on fibronectin substrate (2μg/ml) for times as indicated, in the presence of calpeptin (20μg/ml) or diluent. RIPA lysates were generated, resolved by PAGE and immunoblot analysis performed for talin. (B) Cleavage of the endogenous inhibitor calpastatin occurs within focal adhesions. Neuroblastoma deficient in caspase 8 (- Caspase 8) or expressing caspase 8 (+ Caspase 8) were plated on fibronectin substrate (2μg/ml) for times as indicated, focal adhesion fractions isolated as described above,
and the fractions resolved by PAGE and transferred to PVDF membranes for immunoblot analysis. The fractions were probed to assess cleavage of the calpain substrates talin and calpastatin; fragments are indicated by the arrows. The cleavage of calpastatin, a calpain inhibitor, is an indicator of calpain activation within the FA fractions.

**Supplementary Figure 5. Localization of Caspase 8 to the periphery of cells lacking or expressing FAK.** (A) A549 Cells expressing (Control shRNA) or lacking FAK (FAK shRNA) were allowed to attach to fibronectin substrates and spread for thirty minutes. Spreading cells were identified in low power fields (left panels) by using phalloidin to stain actin ruffles in the periphery (Actin), while caspase 8 distribution was determined by staining with anti-caspase 8 primary and Alexa 565 secondary antibody (red channel). Somewhat fewer FAK deficient A549 cells spread on fibronectin relative to FAK expressing cells. The merge shows the relative distributions of caspase 8 and actin filaments within the two A549 cell populations. (B) Mouse embryo fibroblasts lacking FAK (FAK -/-) or reconstituted for FAK expression (FAK-/-, +FAK) were allowed to attach to the fibronectin substrates (2mg/ml) for 30-60 minutes. Images of representative, reconstituted cells are shown as they spread (early and later in the process). A spread cell (in which selective distribution is mostly lost) caspase 8 is also shown for comparison. Note that FAK -/- cells can spread to some degree (dotted outline is the cell periphery or a well spread cell after 30 min), but disruption of caspase 8 localization to the periphery is observed.
Barbero et al., Supplementary Figure 1

- **Taxol**
  - Control shRNA
  - Casp-3 ShRNA

- **TRAIL**
  - Control shRNA
  - Casp-3 ShRNA

- **Staurosporine**
  - Control shRNA
  - Casp-3 ShRNA
Barbero et al., Supplementary Figure 4

**A**

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Talin

Actin

**B**

Isolated FA Fraction

- **Caspase 8**  + **Caspase 8**

30 60 30 60

Talin

Calpastatin

Actin

C8