We would like to thank the reviewers for their careful and thoughtful review of our work. We have addressed the reviewers comments and hope that you will find that the alterations to the manuscript further support our conclusions. We believe these textual and graphical changes have significantly improved the manuscript. As such, we greatly appreciate your important contributions to our work.

Reviewer #2:

The study by Schnorenberg et al demonstrates that cathepsin B-cleavable peptide amphiphile mimicking BH3 death domain of BIM promptly enters the cytoplasm, localises to mitochondria, specifically binds Bcl-2 proteins and induces cell death. It extends the authors' previous reports on therapeutic peptide amphiphile delivery. The incorporation of the cleavable linker appears to overcome the apparent issues of reduced uptake and target binding, by liberating active peptides of interest from tagged hydrophobic tails through the endosomal cathepsin B activity. These findings imply the use of cathepsin-cleavable nanostructures as a promising deliverable peptide-based therapeutic approach.

While the study is interesting and the manuscript is well-written, I would bother the authors with a few comments on experimental design and data interpretation.

Point 1A: Could the authors please clarify the effect of cathepsin inhibitor CA-074Me on BIM_{A,Cath,K}PA_{2}?

As cathepsin B is an end/lysosomal protein, where one would see the BIM_{A,Cath,K}PA_{2} accumulation in these compartments, upon the drug treatment. If not, it should be at least comparable to the non-cleavable BIM_{A,K}PA_{2}. However, as the authors described in figure 5 and figure S5, CA-074Me diminishes PA uptake and causes their accumulation on cell surface. Is CA-074Me known to block endocytosis? Is there any alternative to this drug that can be used?

Response 1A: We agree with the reviewer on this point and have amended our text to reflect these thoughts. CA-074Me is not known to block endocytosis and is a well described inhibitor agent of cathepsin B, especially the intracellular type as it undergoes intracellular transition into the active CA-074 via esterases. Specifically, upon further inspection of the images in Figure 5 and Figure S5, as the reviewer points out, some of the fluorescently-tagged peptide resides not at the cell surface, as we originally described, but actually in what would be consistent with early endosomes. We and others have shown that PAs migrate to early endosomes and can quickly be recycled back to the cell surface (Missirlis, et al. Plos One, 2013). We have adjusted our description of these experiments to reflect these thoughts. In this regard, we cannot assume that BIM_{A,Cath,K}PA_{2} would mirror the localization of BIM_{A,K}PA_{2} because they still may have slightly different membrane penetration or trafficking, even given the similar lipid tail. However, as is pointed out by the reviewer, there is more overlap than what we had initially described and perhaps it would be more similar given different time constraints. We have adjusted the text to reflect these thoughts.

Point 1B: In Figure 1A, I am not convinced that BIM_{A,K}PA_{2} localises to cellular membrane as it appears to concentrate in distinct spots (organelles? Golgi perhaps?).
Response 1B: Overlaying the light microscopy images (first column of Figure 2a) we do not believe that the accumulation of BIM$_{A,K}$PA$_2$ is intracellular. However, as discussed above, we do agree with the reviewer that upon closer inspection, a small amount of BIM$_{A,K}$PA$_2$ does appear to enter the cell. This would also be consistent with our functional data (Figure 6). One thing also to remember is that these images are from live cells, and therefore what is captured is a z-plane image. We made sure not to fix and flatten the cells so as not to misinterpret non-specific membrane staining with intracellular staining.

Point 2: MitoTracker Red is a membrane potential dependent dye, which is not an ideal stain for mitochondria during apoptosis. It may explain why MitoTracker panels in imaging data appear diffuse. MitoTracker Green or other potential-independent dye should be used instead. The quality of microscopy images is relatively poor. It would be great if the authors can show images with greater signals and contrast.

Response 2: We agree with the reviewer that the MitoTracker used in these experiments, particularly as the cells undergo the early stages of apoptosis and have mitochondrial membrane disruption, causes a diffuse “blushing” of the cells in some cases, especially in areas where there is a large concentration of mitochondria. We believe that this, in part, was also because of the live imaging of the cells and their abundance of mitochondria making a perfectly clear z-plane difficult to capture and may also be due to the concentration of MitoTracker used. In our experience, using less was suboptimal in our setup but works well when cells are fixed. We use MitoTracker Red because our products were labelled with FITC. We have sharpened the confocal images throughout the manuscript in hopes that they will be now clearer.

Point 3: Having all necessary experimental set-ups, I wonder why the authors did not do time-lapse live imaging. It would immensely improve the paper by addressing the kinetics of uptakes, endo/lysosomal escape and apoptosis induction.

Response 3: We did in fact do live cell time-lapse imagining and have now included a series of images in a new figure, Figure S4, of the manuscript. Here we show that the cell becomes more diffusely green over time and this overlays with mitochondria in the cells and is associated with nuclear shrinking. Unfortunately, as the reviewer points out above, the MitoTracker Red appears rather concentrated around the nucleus and rather diffuse. However, we feel that this image helps to relate how the intracellular concentration of the BIM BH3 peptide changes over time, and as such placed it in the supplementary section of the manuscript.

Point 4: LysoTracker was mentioned in the Materials and Methods section (sub-heading 2.15), but I cannot seem to find the relevant data for it.

Response 4: Thank you for pointing this out. We have removed the mention of LysoTracker from Materials and Methods, as it was not used in the manuscript.