Cell death inhibits cancer at multiple stages, ranging from transformation to metastasis. As such, cell death must be inhibited to allow cancer to develop. Importantly, cell death sensitivity also dictates how well anti-cancer therapies work. Our research focuses upon mitochondrial regulation of cell death and inflammation. Mitochondria are key cellular organelles that power life but are also essential for the major form of regulated cell death called apoptosis. We aim to understand how mitochondria control cell death and define how this process is deregulated in cancer. Our findings will be used to improve and develop new ways to selectively kill cancer cells.

Mitochondria, cell death and cancer
Apoptosis requires caspase protease activation leading to widespread substrate cleavage and rapid cell death. During apoptosis, mitochondrial outer membrane permeabilisation (MOMP) occurs, a crucial event that is required for caspase activation. Following MOMP, mitochondrial intermembrane space proteins, such as cytochrome c, are released into the cytoplasm where they cause caspase activation and apoptosis. Given its key role in controlling cell survival, mitochondrial outer membrane integrity is highly regulated, largely through interactions between pro- and anti-apoptotic Bcl-2 proteins. Cancer cells often inhibit apoptosis by preventing MOMP, often through upregulation of anti-apoptotic Bcl-2 proteins. Importantly, this can be exploited therapeutically and newly developed anti-cancer therapies target these apoptotic blocks.

Mito-priming - a method to enforce Bcl-2 addiction
Many methods to trigger mitochondrial apoptosis suffer from engaging this process in a slow, asynchronous manner that is often accompanied with unwanted, off-target effects. Driven by a desire to circumvent these issues, we developed a method called ‘mito-priming’. In this method, pro- and anti-apoptotic Bcl-2 proteins are expressed at equal levels. Cells in this primed state are massively sensitive to drugs that target Bcl-2 proteins called BH3 mimetics, rapidly undergoing cell death within a matter of minutes following BH3 mimetic addition (Fig. 1A and 2).

Applying mito-priming to define the potency and selectivity of Bcl-2 targeting drugs
Directly targeting Bcl-2 proteins to trigger or sensitise cancer cell death represents an exciting and effective new way to treat cancer. For example, the recently clinically approved BH3 mimetic compound, venetoclax exploits the Bcl-2 addicted state of chronic lymphocytic leukaemia (17p deleted CLL), triggering remarkable therapeutic responses. The Bcl-2 protein family is comprised of various different members (e.g. Bcl-2, BCL-xL and MCL-1) that can be targeted to varying degrees by different BH3 mimetics. Importantly, failure to neutralise specific Bcl-2 proteins represents a means of cancer cell resistance to BH3 mimetic therapy. We have surveyed a range of BH3 mimetic compounds using our mito-priming method to define the selectivity and potency of different Bcl-2 family members. Moreover, in combination with CRISPR-Cas9 genome editing to delete BAX and BAK (proteins essential for MOMP), we have applied mito-priming to address whether Bcl-2 targeting compounds kill in an on-target (MOMP) dependent manner. In sum, mito-priming provides a highly useful way to screen for specific and potent, new Bcl-2 targeting drugs.

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Figure 1
Mito-priming as a method to enforce Bcl-2 addiction. Cells express equimolar amounts of a pro-apoptotic BH3-only protein (in this case GFP-BID) and anti-apoptotic Bcl-2 protein (in this case BCL-xL) making use of a 2A-linker sequence. Co-expression of these two proteins renders cells highly sensitive to BH3-mimetic addition (ABT), which induces MOMP and mitochondrial apoptosis.

Figure 2
Mito-priming enables rapid and synchronous mitochondrial apoptosis. SVEC murine endothelial cells co-expressing GFP-BID and BCL-xL were treated with ABT-737. Cell viability was measured by live-cell imaging and exclusion of the green dye, SYTOX Green. Left: Viable cells at the point of BH3 mimetic addition (ABT) treatment. Right: Cells at 8 hours following BH3 mimetic addition, at which point most have died (SYTOX Green positive).