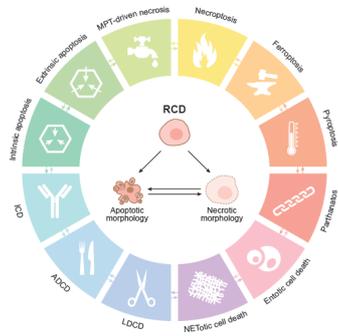
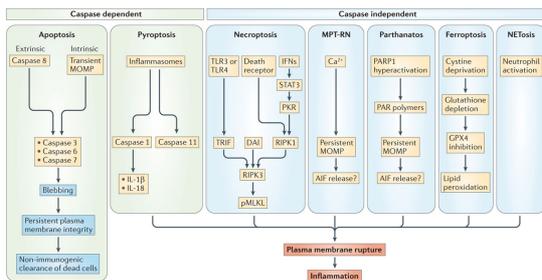


MCBO Lecture: Cell Death

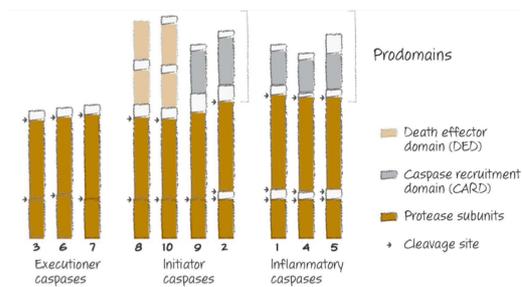


Different roads to cell death



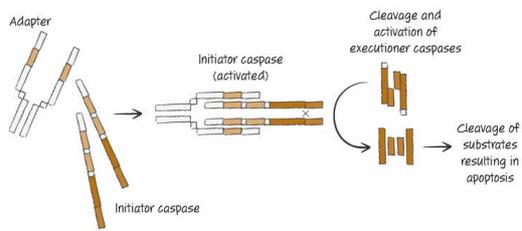
A. Linkermann et al. Nature Reviews Immunology 14, 759-767 (2014)

Caspases show conserved structural features



Caspases: cytein-dependent aspartate-directed proteases

Activation of caspases follows conserved rules

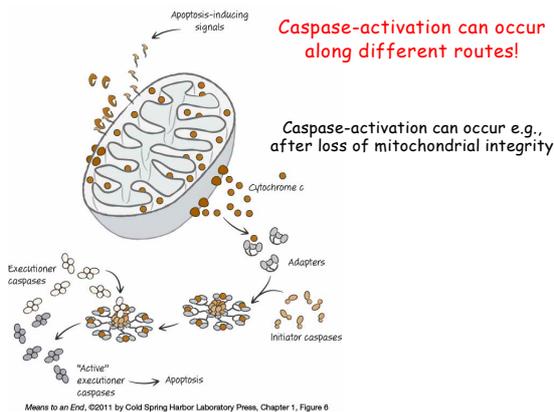


Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 1, Figure 4

Caspases: cytein-dependent aspartate-directed proteases

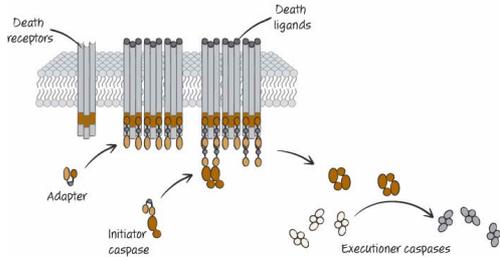
Caspase-activation can occur along different routes!

Caspase-activation can occur e.g., after loss of mitochondrial integrity



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Caspase activation can occur e.g., in response to receptor/ligand interaction



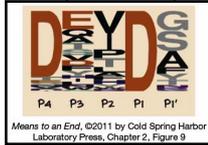
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 1, Figure 7

Different caspases prefer different peptide sequences, at least in vitro

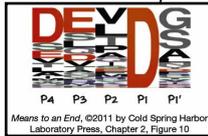
- Caspase-1 WEHD
- Caspase-2 VDQQD
- Caspase-3 DELD
- Caspase-4 LEVD
- Caspase-5 (W/L) EHD
- Caspase-6 (T/V) QVD
- Caspase-7 DEVD
- Caspase-8 LETD
- Caspase-9 LEHD

Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 2, Figure 7

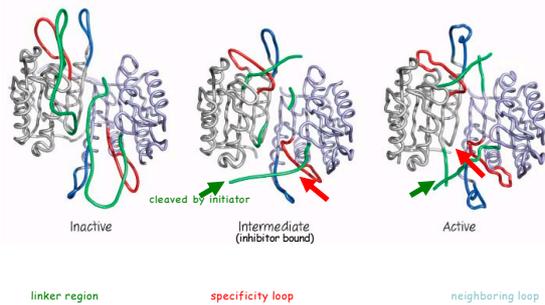
Peptide library



Proteom analysis

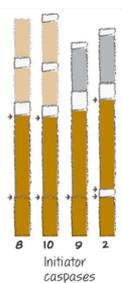


Effector caspases are present as inactive dimers, activated by cleavage between their small & large subunit



Effector caspase activation is mediated by apical caspases (8/9/10)

- Initiator caspases
 - Not activated by cleavage
 - Are present as inactive monomers
 - Need to dimerize for activation
 - Autoprocessing stabilizes dimer



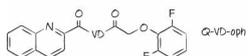
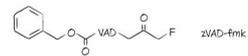
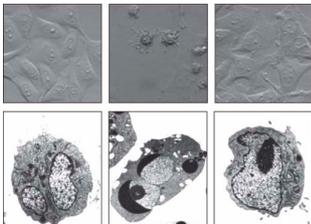
Death by a 1000 cuts?

- Chromatin condensation: (C3/C7)
 - Acinus: p-histones, assists CAD function
 - Loss of function -> death proceeds
- Nuclear integrity: (C6)
 - Lamins: breakdown & regeneration of nuclear envelope
 - Loss of function -> death proceeds
- Electron transport
 - NDUFS1 - complex I of e-transport chain
 - ATP loss, generation of superoxide, OH⁻ radicals

Death by a 1000 cuts?

- Phosphatidylserine (PI) exposure
 - Xkr8/Ced8 (lipid scramblase)
 - Downregulated in tumor cell lines
 - Caspase-3 substrate
- Membrane blebbing: (C3&7)
 - Gelsolin,
 - p21 activated kinase (PAK)
 - ROCK-1 kinase
 - Actin remodelling, cleavage removes inhibitory domains,
 - Inhibition blocks blebbing but not death

Inhibition of caspases blocks morphological changes, but, most of the times, not cell death

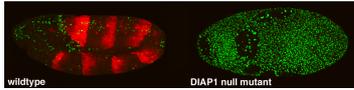


Control Apoptosis Caspases inhibited
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 3, Figure 13

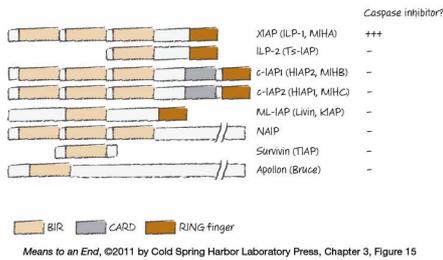
If caspases are so dangerous, can they be controlled, once activated?

- Viral caspase inhibitors
 - Baculovirus (insect virus)
 - IAP (Inhibitor of apoptosis protein)
 - P35 (substrate & irreversible inhibitor)
 - Cowpox virus
 - Serpins (serine protease inhibitors, crmA)

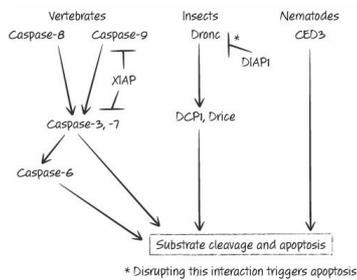
- Drosophila
 - Dronc - dIAP1



The IAPs in mice & men

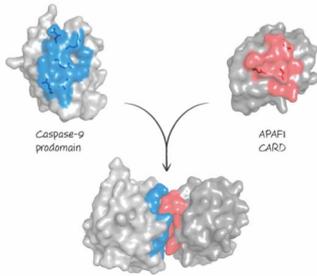


.....the story so far.....



.....but, how get adapters activated?

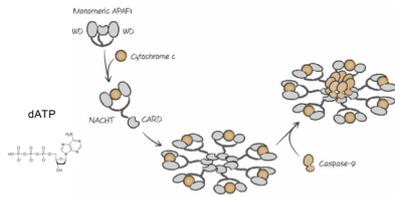
Mitochondrial apoptosis Apoptotic protease activating factor 1



Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 4, Figure 2

The „Apoptosome“

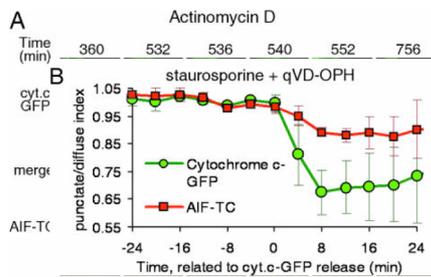
Apoptotic protease activating factor (APAF1)



NATP
CITTA
MET-E
TP1

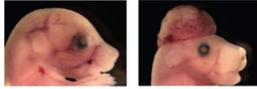
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 4, Figure 4

Release of cytochrome C is fast and complete



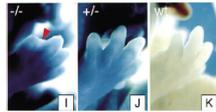
Consequences of „Apoptosome“ loss

Cytochrome C K72A knock-in



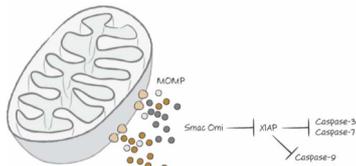
Hao et al, Cell, 2005

APAF-1 gene trap



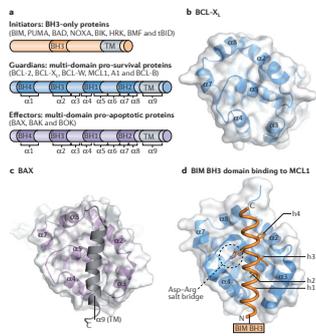
F. Ceccconi et al, Cell 1998

In vertebrate cells, mitochondrial outer membrane permeabilization (MOMP) promotes release of IAP antagonists

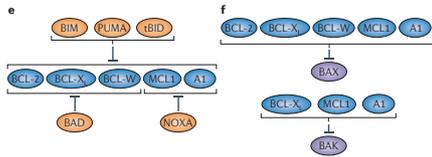


Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 4, Figure 7

MOMP is controlled by the Bcl2 family

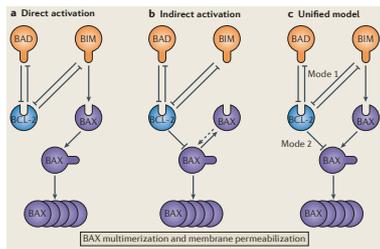


MOMP is controlled by the Bcl2 family



Peter Czabotar et al. Nat. Rev. Mol. Biol. 2013

Possible modes of Bax/Bak activation



Peter Czabotar et al. Nat. Rev. Mol. Biol. 2013

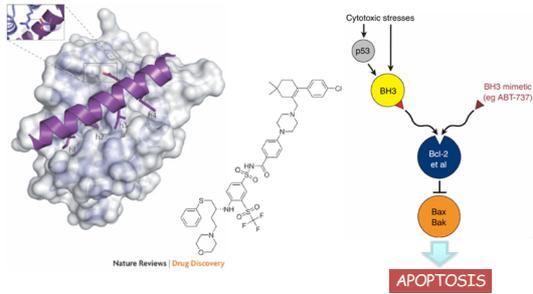
Most Anticancer Therapies Involve BH3-only proteins

- IR & DNA-damaging drugs -> Puma, Noxa
- RTK inhibitors -> Bim, Bmf, Bad
- HDACi -> Noxa, Bmf
- Proteasome blocker -> Noxa, Bim
- mAbs -> ADCC, loss of Bcl2/Mcl1 expression,



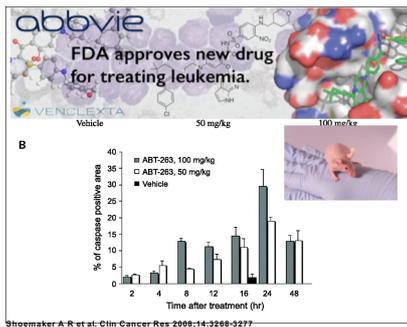
- DRUG-RESISTANCE PHENOTYPES
 - Cellular detoxification systems (GSH, MDR)
 - Amplification of survival molecules (Her2, ER)
 - Mutations in RTK binding pocket (Bcr/abl)
 - Overexpression of pro-survival genes (Bcl2, Mcl1, Bcl-x)
 - Loss/silencing of tumor suppressor genes (ARF, p53, PTEN)
 - Loss/silencing of pro-death gene/function (e.g. Puma, Bim)

Overcoming conventional drug-resistance by mimicking BH3-only protein function



Adams & Cory, Oncogene 2007

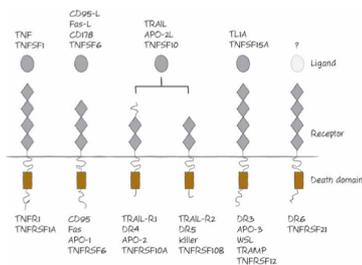
Histologic analysis of caspase-3 activation in H146 xenograft tumors treated with ABT-263.



©2008 by American Association for Cancer Research

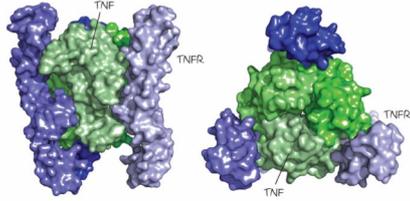
AACR Clinical Cancer Research

The other road to cell death, i.e. the extrinsic apoptosis pathway



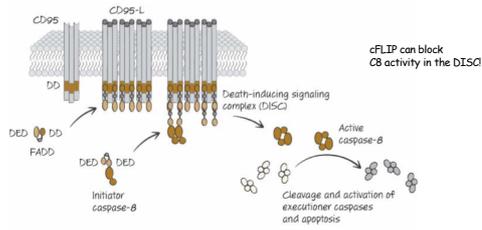
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 6, Figure 1

TNF/TNF-R family proteins act as trimers or multiples of trimers



Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 6, Figure 2

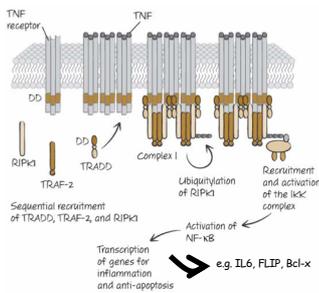
CD95L-mediated DISC formation leads to caspase-8 → caspase-3/7 activation



Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 6, Figure 6

DISC: death-inducing signalling complex

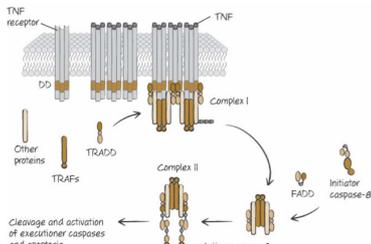
TNF can signal more than just cell death
NF-κB activation via Complex I



Kinase domain of RIPK1 not critical here!

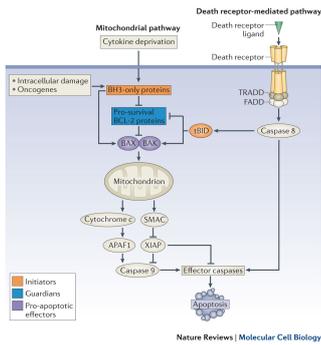
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 6, Figure 9

TNF-mediated apoptosis requires endocytosis and formation of complex II



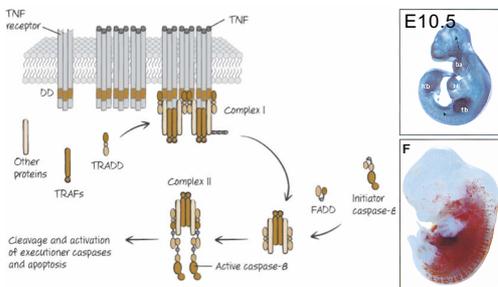
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 6, Figure 10

Extrinsic and intrinsic pathway can be linked via the BH3-only protein Bid



Nature Reviews | Molecular Cell Biology

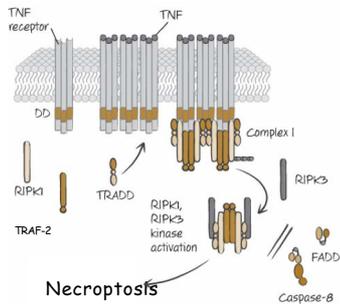
Necrosis can be triggered by death receptors



Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 6, Figure 10

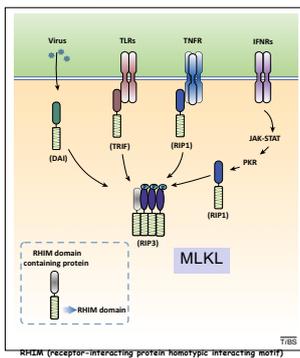
Varvelomsev, Immunity, 1998

Necrosis can be triggered by death receptors

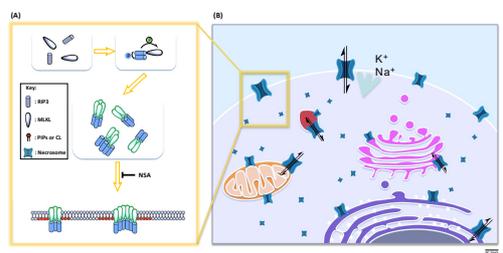


Means to an End. ©2011 by Cold Spring Harbor Laboratory Press, Chapter 8, Figure 6

Different immune response signalling pathways can trigger necroptosis



MLKL-Lipid interactions drive pore formation

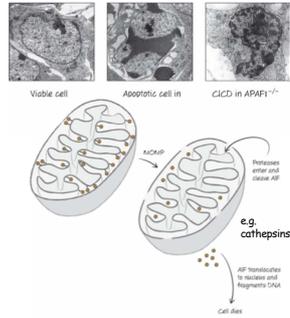


- Phosphatidylinositol phosphate lipids (PIP) -> interaction critical for killing and membrane targeting
- Cardiolipin (CL) - inner mitochondrial membrane -> exposed during necrosis -> role unclear

Trends in Biochemical Sciences December 2014, Vol. 39, No. 12

Caspase-independent cell death (CICD) after MOMP

- Less efficient
 - Distinct morphology
 - Necrosis-like
- Some cells can recover from MOMP if caspases are blocked
 - Neurons, also in cell culture
 - miMOMP

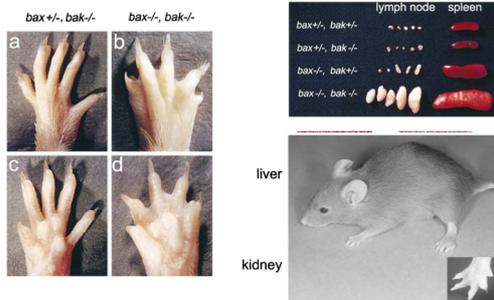


Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 4, Figure 9

Mechanisms of CICD

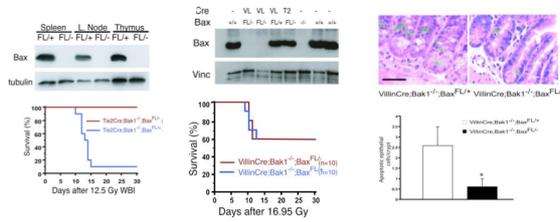
- Global mode
 - Mitochondrial catastrophe
 - Loss of OM integrity, dilution of intermembrane space proteins
 - Mitochondrial dysfunction -> no more ATP production, lipid biogenesis, ROS scavenging.....
- Selective mode
 - Release of pro-death proteins from IMS
 - EndoG endonuclease
 - Can cleave DNA between nucleosomes
 - AIF, essential gene involved in electron transport
 - Tethered to inner membrane
 - released upon loss of membrane integrity and protease processing,
 - nuclear translocation - chromatolysis

No MOMP, no developmental cell death in vertebrates?



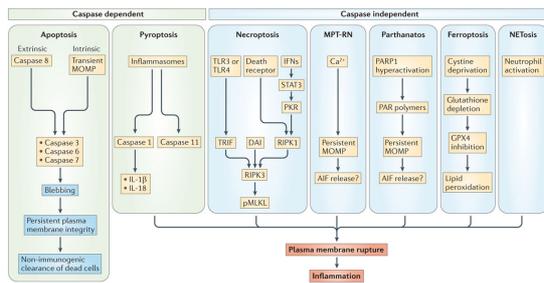
Lindsten Mol. Cell 2000

Cell type dependent differences in cell death modalities



Kirsch et al. Scienc

Different roads to cell death



A. Linkermann et al. Nature Reviews Immunology 14, 759-767 (2014)



Mitochondrial permeabilization engages NF-κB-dependent anti-tumour activity under caspase deficiency

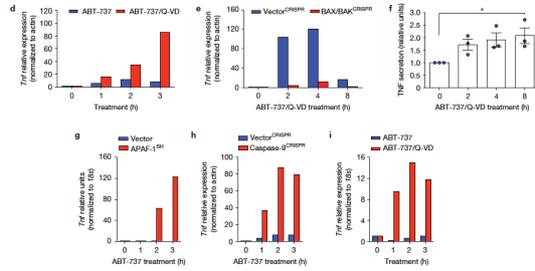
Evangelos Giampazolias^{1,2}, Barbara Zunino^{1,10}, Sandeep Dhayade^{1,10}, Florian Bock^{1,2}, Catherine Cloix^{1,2}, Kai Cao^{1,2}, Alba Roca^{1,2}, Jonathan Lopez^{1,2,11}, Gabriel Ichim^{1,2,11}, Emma Proksa¹, Camilla Rubio-Patiño¹, Loic Fort¹, Nader Yatim¹, Emma Woodham¹, Susana Orozco¹, Lucia Taraborrelli¹, Nieves Peltzer¹, Daniele Lecis¹, Laura Machesky¹, Henning Walczak¹, Matthew L. Albert^{1,6}, Simon Milling¹, Andrew Oberst¹, Jean-Ehrland Ricci¹, Kevin M. Ryan¹, Karen Blyth¹ and Stephen W. G. Tait^{1,10}

1116

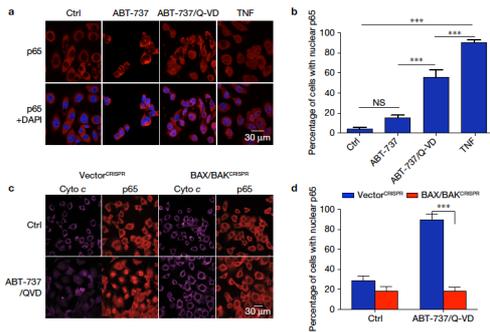
NATURE CELL BIOLOGY VOLUME 19 | NUMBER 9 | SEPTEMBER 2017

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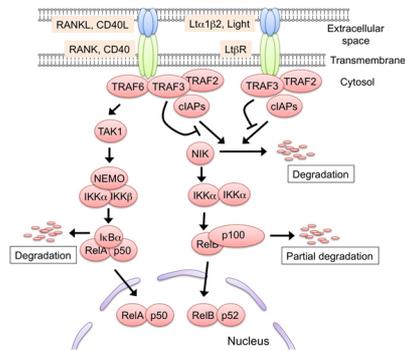
MOMP induces TNF production in the absence of caspase activity



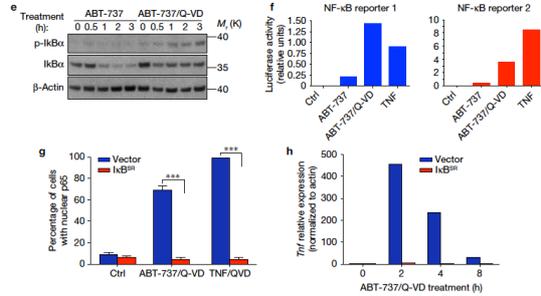
MOMP activates NF-κB signalling



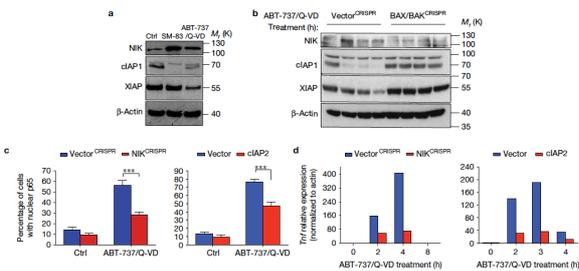
MOMP activates NF-κB signalling



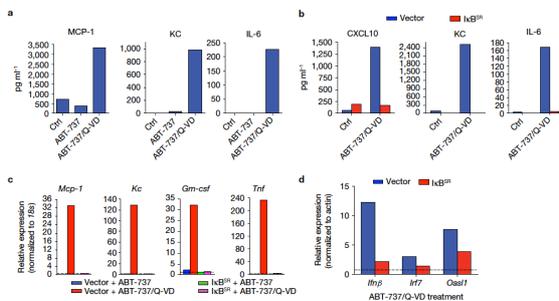
MOMP activates NF- κ B signalling

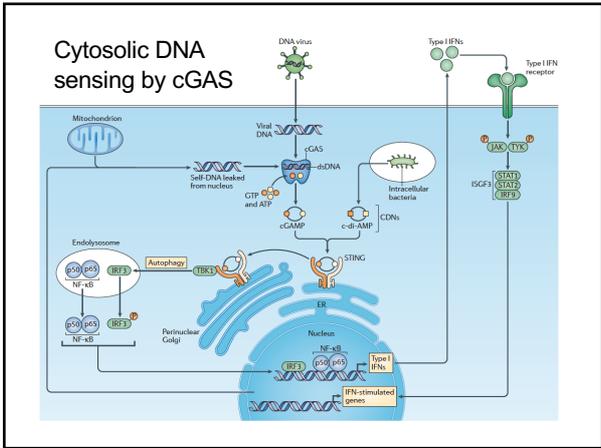


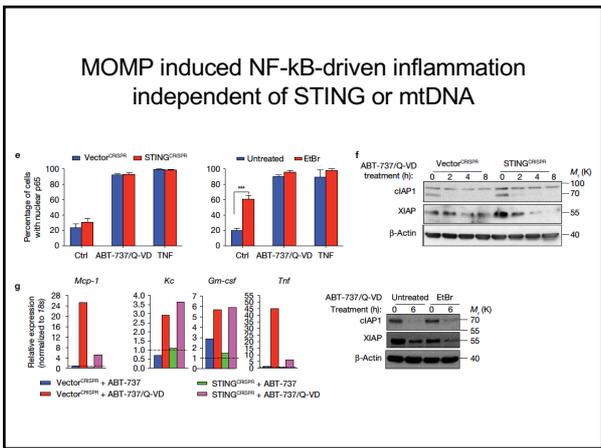
MOMP activates NF- κ B through IAP down-regulation and NIK activation

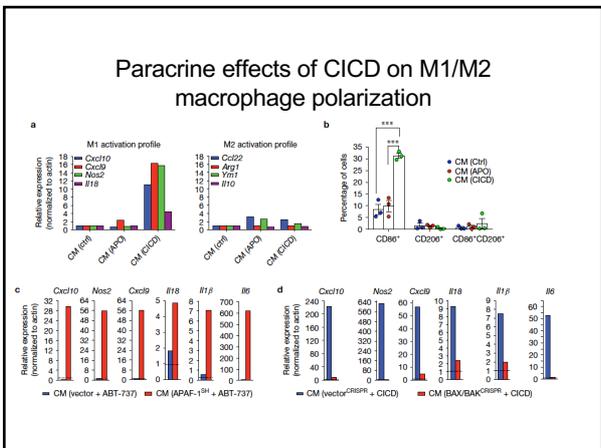


MOMP induces TNF-mediated inflammation in the absence of caspases



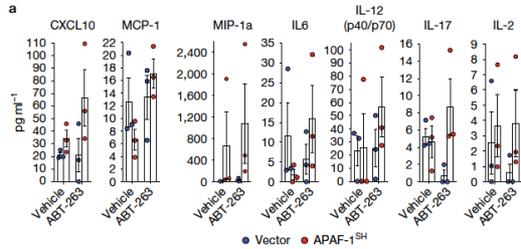




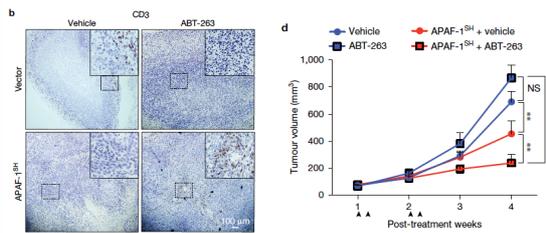


CICD is tumor suppressive, apoptosis is not

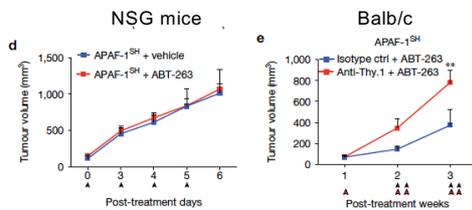
CT26 cells GFP-tBid t2A-BCL2 -> xenotransplant



CICD is tumor suppressive, apoptosis is not



CICD activates anti-tumor immunity



In RCC, caspase 7 expression is inversely correlated with survival, opposite for Nf-kB

