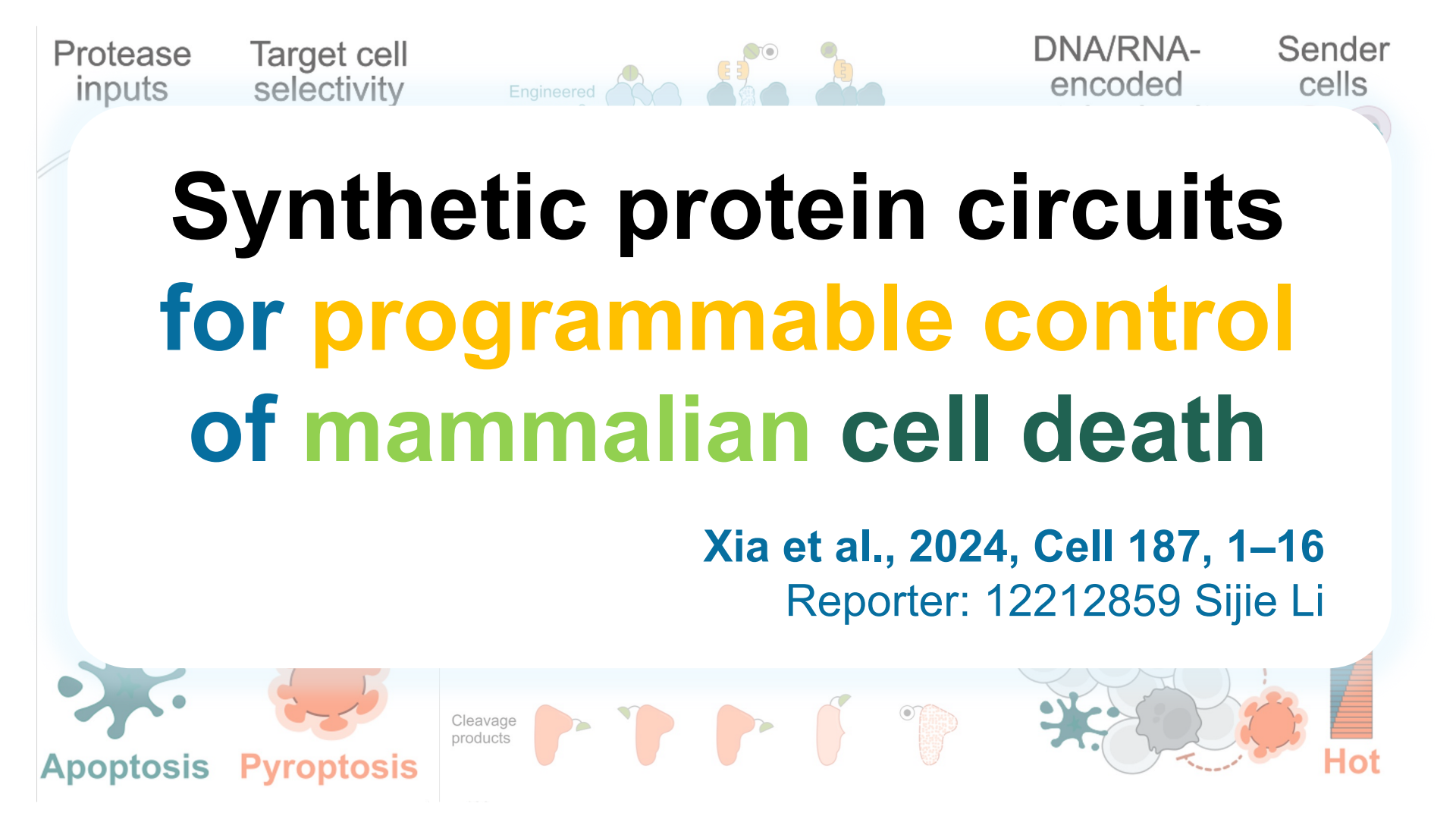
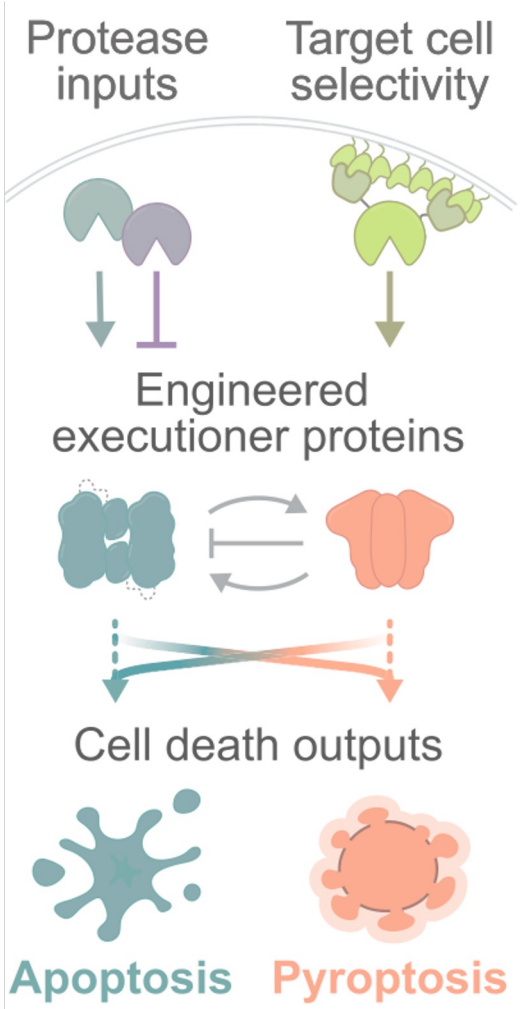
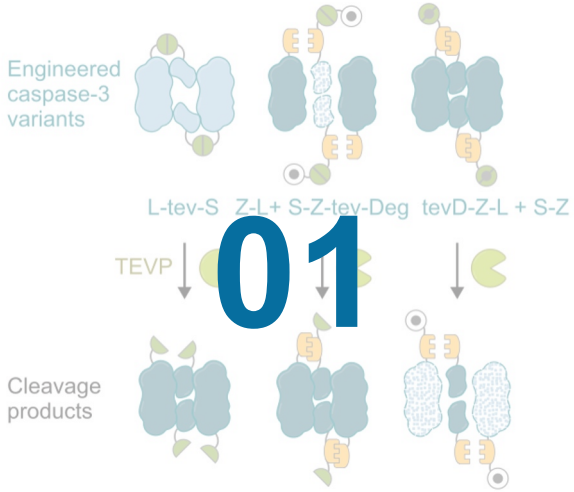


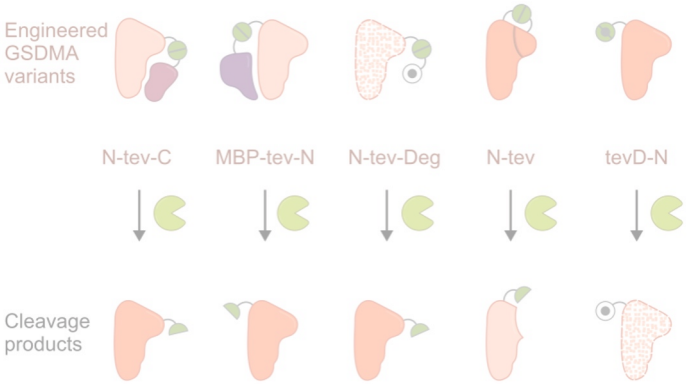
Synthetic protein circuits for programmable control of mammalian cell death

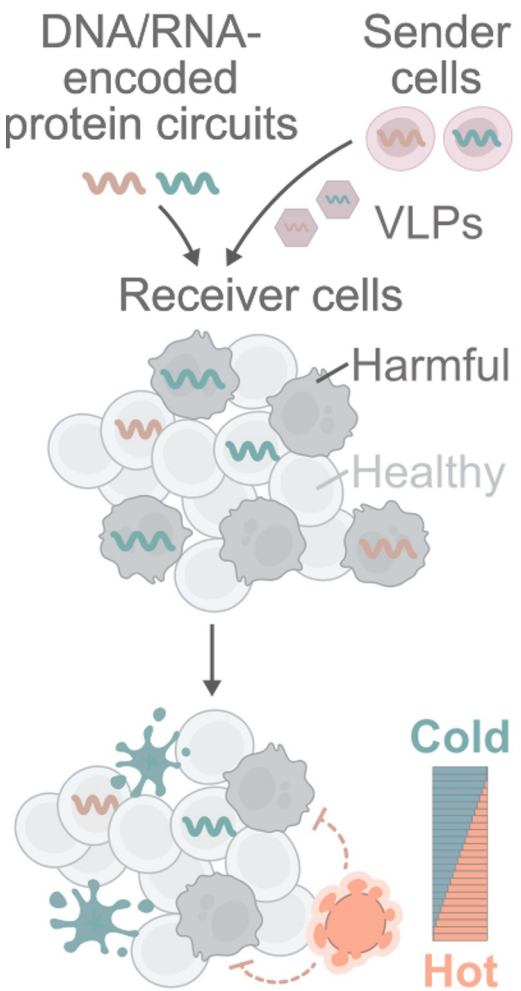
Xia et al., 2024, Cell 187, 1–16
Reporter: 12212859 Sijie Li


Protease inputs
Target cell selectivity
Engineered executioner proteins
Cell death outputs
Apoptosis
Pyroptosis

01 PAST


Engineered caspase-3 variants
L-tev-S
Z-L+ S-Z-tev-Deg
tevD-Z-L + S-Z
TEVP
Cleavage products

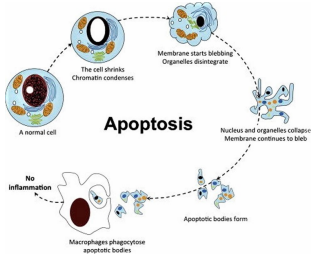

Engineered GSDMA variants
N-tev-C
MBP-tev-N
N-tev-Deg
N-tev
tevD-N
Cleavage products


DNA/RNA-encoded protein circuits
Sender cells
VLPs
Receiver cells
Harmful
Healthy
Cold
Hot

Cell Death?

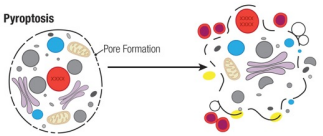
Apoptosis

- Triggered by **caspase-3** and intrinsic signals
- **Cell shrinkage**, membrane blebbing, nuclear fragmentation
- **No leakage** of intracellular contents
- Suitable for eliminating **senescent** or **excess** cells



Pyroptosis

- Triggered by **inflammatory caspases** (e.g. caspase-1) or **activated GSDMs**
- Causes **membrane pore formation**, cell swelling, lysis
- Releases **IL-1β, IL-18**, **ATP**, and **DAMPs**
- Useful for **anti-tumor immunity** or **pathogen clearance**
- Needs careful control to avoid **inflammatory storms**

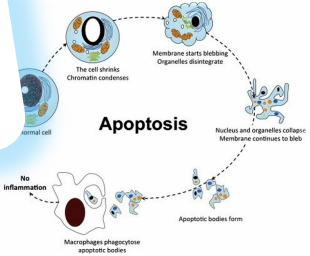


Cell Death—main Pathways are uncovered

Apoptosis

- Triggered by **caspase-3**
- **Cell shrinkage**
- **No leakage** of i
- Suitable for elim **excess** cells

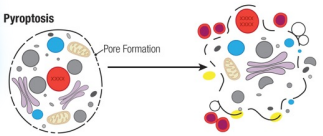
COLD: Immunologically silent
→ avoids inflammation



Pyroptosis

- Triggered by **inflammatory caspases**
  or **activated GSDM**
- Causes **me**
- Releases **IL**
- Useful for **a** **immunity** or **pathogen clearance**
- Needs careful control to avoid **inflammatory storms**

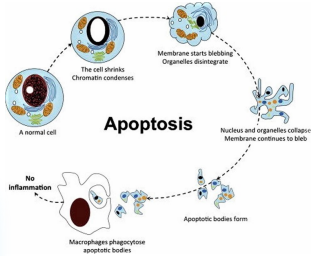
HOT: Immunologically active
→ inflammatory storms



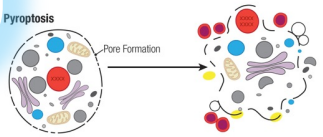
Cell Death?

Apoptosis

- Suitable for eliminating **senescent** or **excess** cells



Expect to carry out the correct death modes under corresponding situation.
BUT, **reality do not always work in this way**…SO?



Pyroptosis

- Useful for **anti-tumor immunity** or **pathogen clearance**

We want CELL DEATH

Before, To eliminate **senescent**, **cancerous**, or **infected** cells, we use…

✅ Cytotoxic Drugs

Induce apoptosis (mostly); poor specificity

✅ CAR-T cells

Deliver granzymes → Induce either apoptosis or pyroptosis

✅ Chemogenetics / Optogenetics

Conditional expression or dimerization of apoptotic proteins

We want SPECIFIC Cell Death

Before, To eliminate **senescent**, **cancerous**, or **infected** cells, we use…

❌ Cytotoxic Drugs

Induce apoptosis (mostly); poor specificity

✅ CAR-T cells

Deliver granzymes → Induce either apoptosis or pyroptosis

❌ Chemogenetics / Optogenetics

Conditional expression or dimerization of apoptotic proteins
→ limit spatial targeting!

We want SPECIFIC Cell Death in SPECIFIC MODE

Before, To eliminate **senescent**, **cancerous**, or **infected** cells, we use…

✗ Cytotoxic Drugs

Induce apoptosis (mostly); poor specificity

✗ CAR-T cells

Deliver granzymes → Induce

✗ Chemogenetics / Op

Conditional expression or dim
→ limit spatial targeting!

Synthetic Protein Circuits

Require New Method to achieve Programmable Control of Cell Death!!!

Why Synthetic Protein Circuits?

gene circuits

- operate at the transcriptional level, relying on promoter activity, mRNA synthesis, and protein translation.

VS

protein circuits

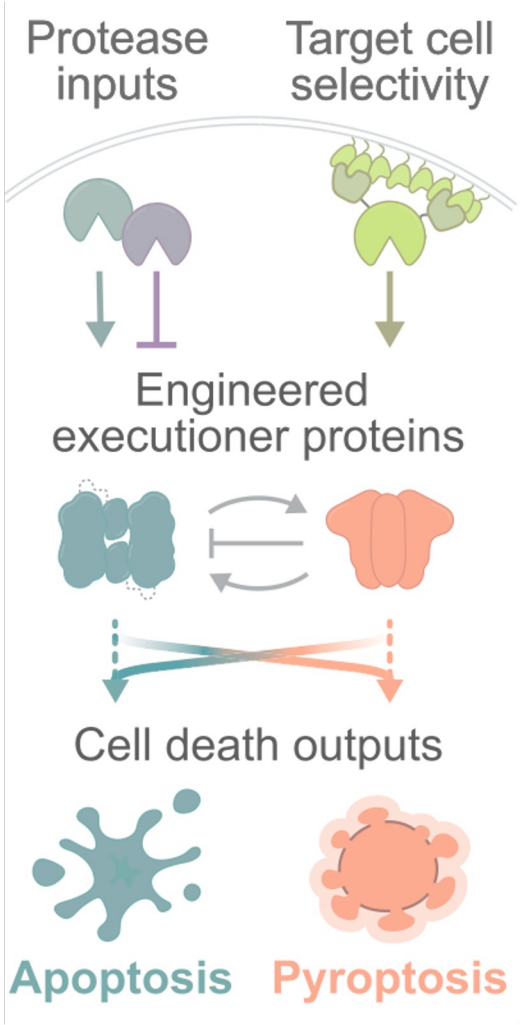
- function **post-translationally**, offering **faster** responses and finer **temporal control**
- **more orthogonal** to endogenous gene expression systems, reducing crosstalk and enhancing modularity

(*Gao et al., 2018, Science*)

Foundation of Synthetic Protein Circuits

- utilize **engineered proteases** (e.g., TEVP) to cleave specific sites on functional domains (e.g., caspase or gasdermin) → **activating or inhibiting** cellular processes (Gao et al., Science 2018)
- Protein degradation tags (**degrons**), **caging domains**, and **heterodimerizing motifs** (e.g., leucine zippers) are combined to control the **stability**, **localization**, or **activity** of key proteins (Iwamoto et al., ChemBio 2010)

✅ **logical operations**, **signal integration**, and **programmable response behaviors** within living cells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Cell death outputs
Apoptosis
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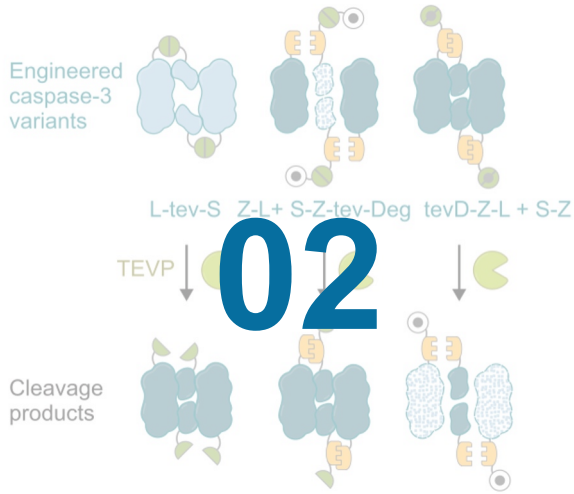
Engineered caspase-3 variants
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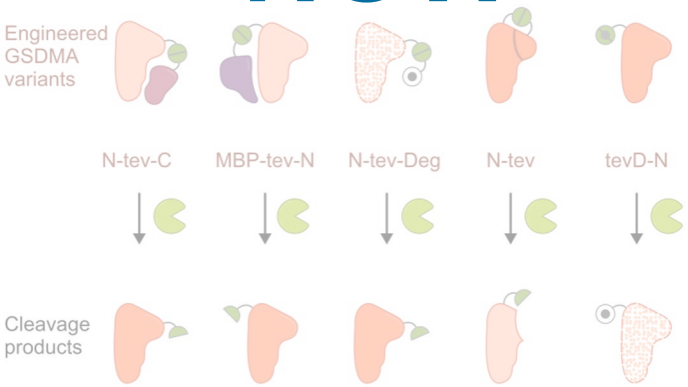
tevD-Z-L + S-Z
TEVP
Cleavage products

02 NOW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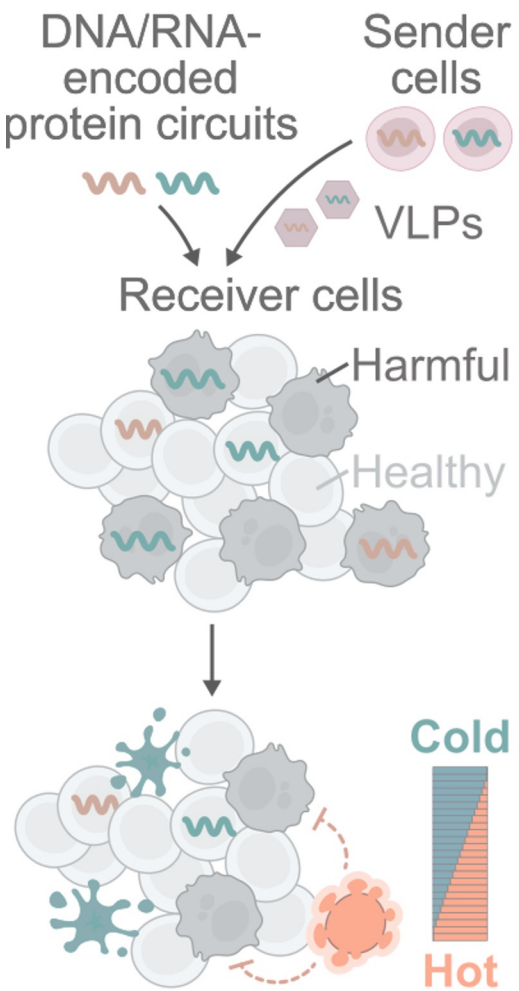
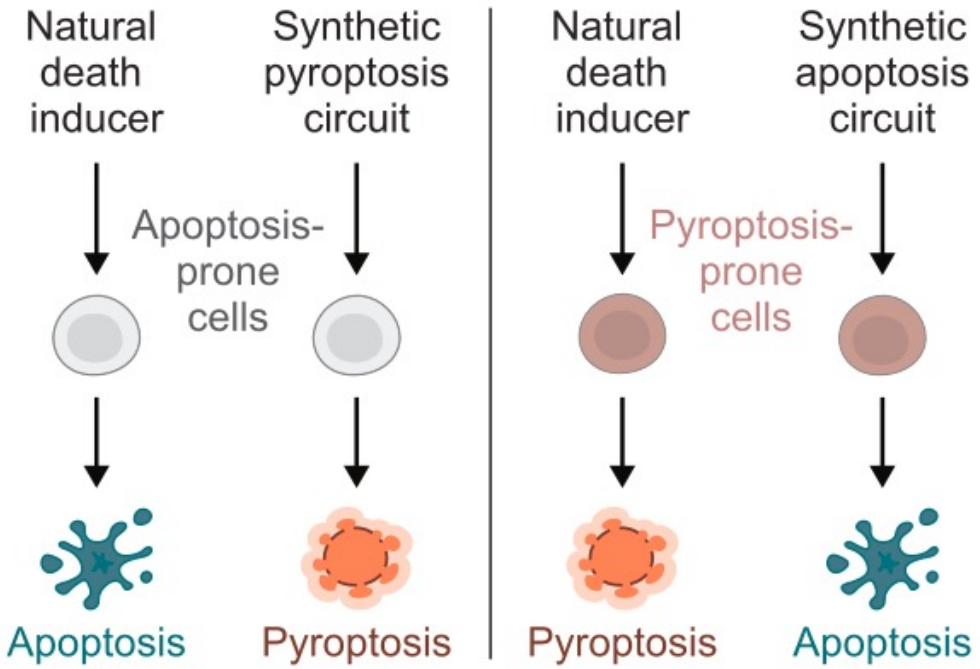
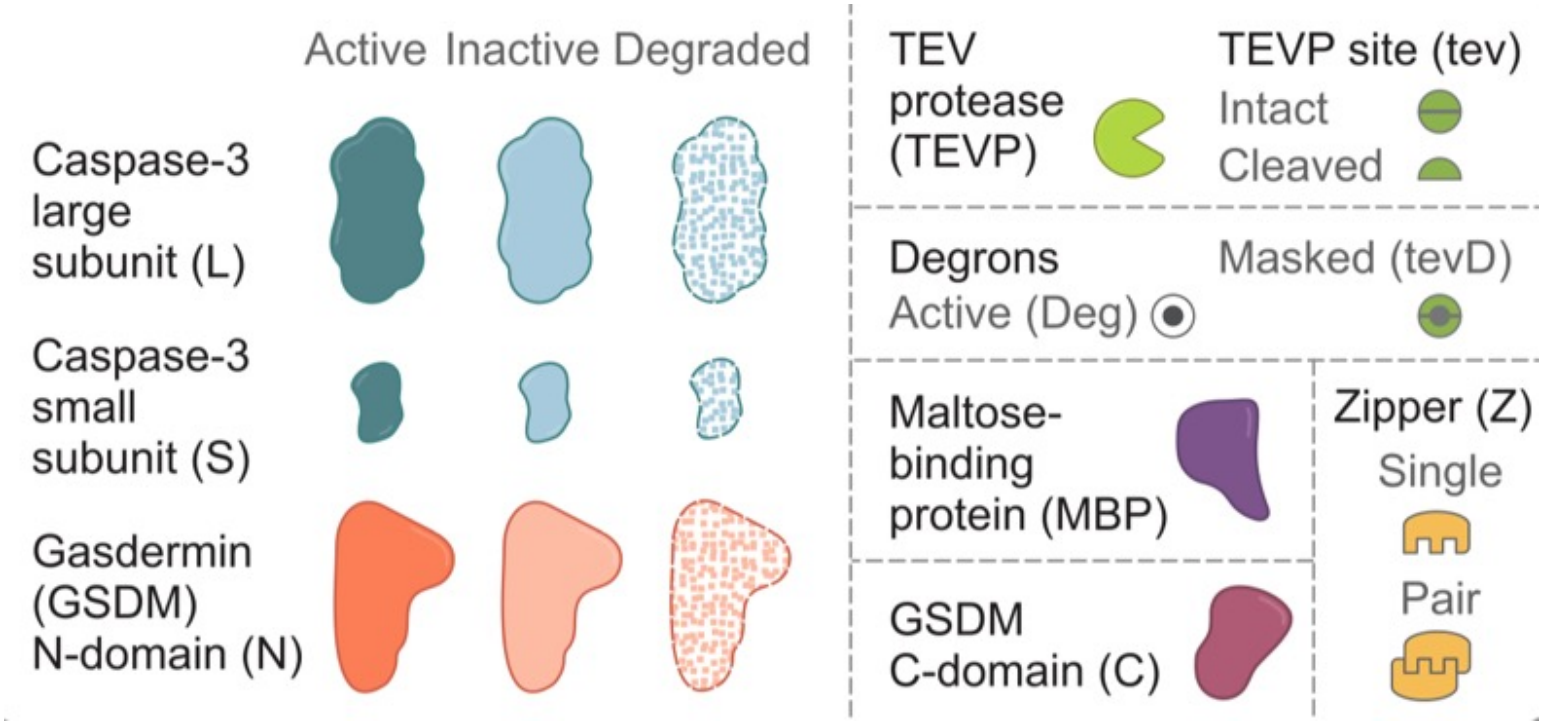

DNA/RNA-encoded protein circuits
Sender cells
VLPs
Receiver cells
Harmful
Healthy
Cold
Hot

GOAL: Programmable Control of Cell Death

- Control Over Cell Death Modes
- Selective Cell Elimination
- Adaptability to Delivery Methods
- Mimicry of Natural Processes



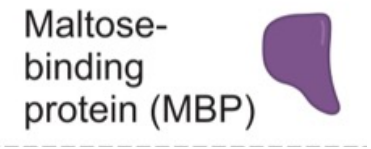
Building Blocks



Building Blocks

TEV Protease (TEVP): A viral protease used to cleave specific engineered sites (TEVP sites) in the circuit, enabling precise activation or deactivation of components like caspase-3 or GSDM.
TEVP Site (tev): The site (a range of sequence) that TEVP acted on.

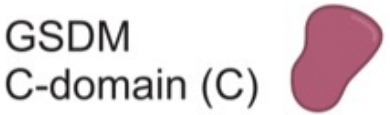
Maltose-Binding Protein (MBP): Used as a bulky tag to sterically block activity (e.g., caging the N-domain of GSDM). Removal by TEVP cleavage restores function.



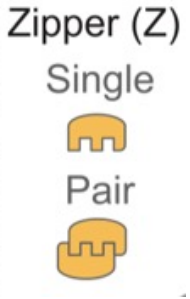
Building Blocks

Degrons (Deg): Tags that promote protein degradation via proteasomal pathways, reducing the activity of the tagged protein. It can be masked by caging with TEVP sites, requiring cleavage to become functional.

GSDM C-Terminal Domain (C): Auto-inhibitory, it binds the N-terminal domain in intact gasdermin proteins, preventing pore formation and pyroptosis.



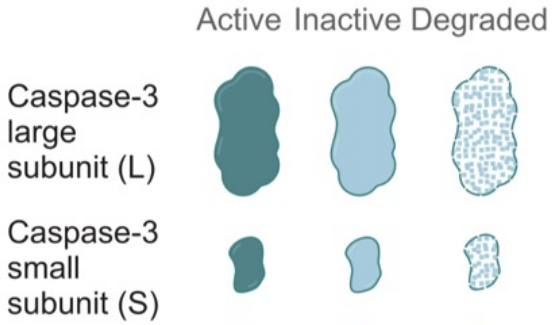
Leucine Zippers (Z): Designed as “single” or “paired” motifs for heterodimerization, these facilitate proximity interactions or artificial linkages in the circuits, such as stabilizing proteins or masking functional domains.



Apoptosis Modules

Mechanism of Apoptosis

1. Activation
2. caspase-3 proteolytic cleavage → separating its pro-domain, large subunit (L), and small subunit (S)
3. active caspase-3 forms a dimer (non-covalent interactions) between the large and small subunits → cleaves various downstream substrates, such as poly(ADP-ribose) polymerase (PARP) and lamin proteins
4. programmed cell death



* This pathway is conserved across the caspase family (so not only for caspase-3), with similar mechanisms observed in other caspase-driven apoptosis processes.

Apoptosis Modules

Mechanism of Apoptosis

1. Activation
2. **caspase-3 proteolytic cleavage** → separating its pro-domain, large subunit (L), and small subunit (S)
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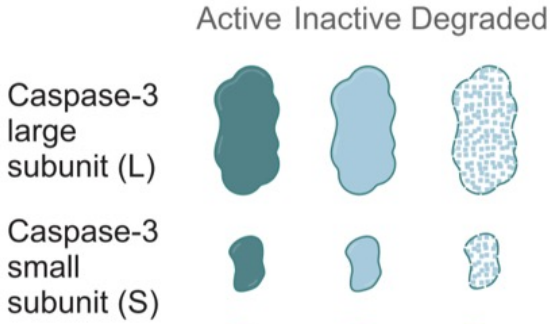
Regulate it!

Modulate its stability



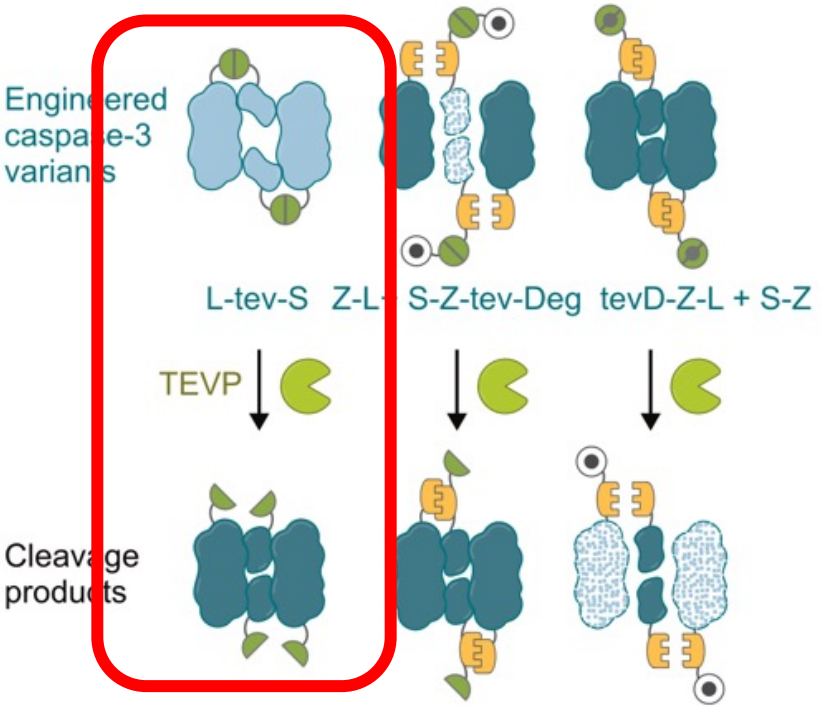
* This pathway is conserved across the caspase family (so not only for caspase-3), with similar mechanisms observed in other caspase-driven apoptosis processes.

Apoptosis Modules

L-tev-S

apoptosis induction

- caspase-3 engineered with a TEVP cleavage site between its large and small subunits
- TEVP cleavage → subunits separated → activate the dimer

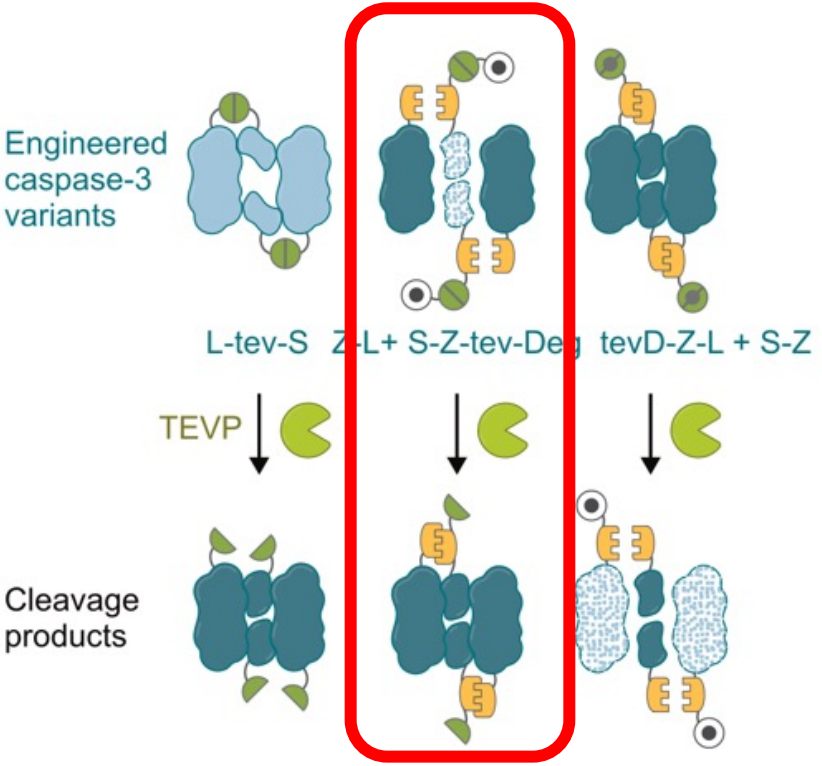


Apoptosis Modules

Z-L and S-tev-Deg

apoptosis induction

- active degron fused to the small subunit → suppresses caspase-3 activity
- TEVP cleavage removes the degron → leucine zippers facilitate dimerization

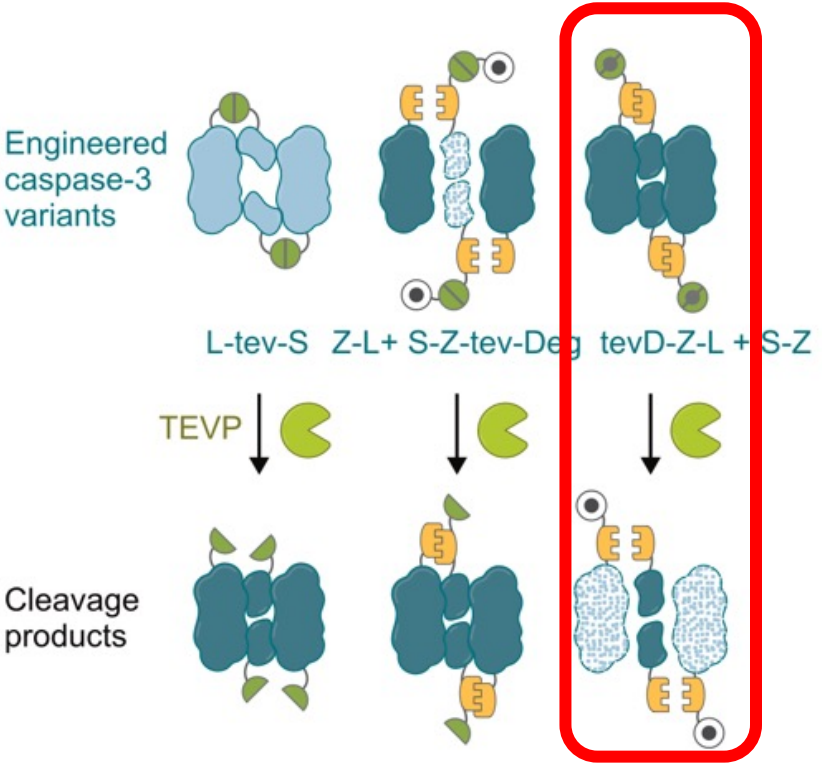


Apoptosis Modules

tevD-Z-L + S-Z

apoptosis inhibition

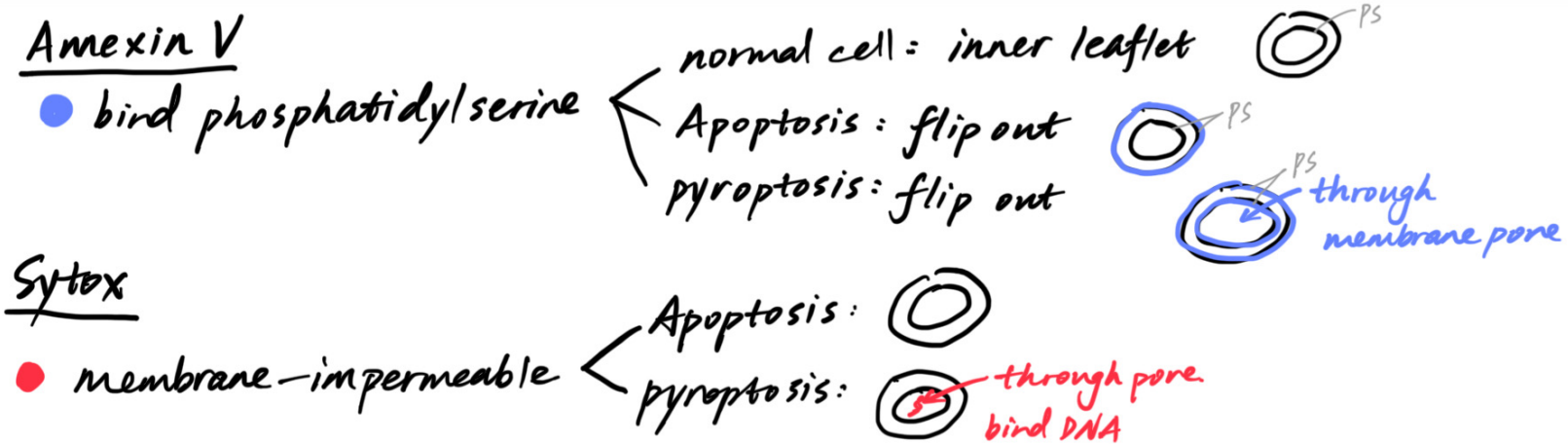
- caged degron fused to the large subunit → masking its activity
- TEVP cleavage exposes the degron → caspase-3 degrade



Apoptosis Modules Validation

Death mode characterization

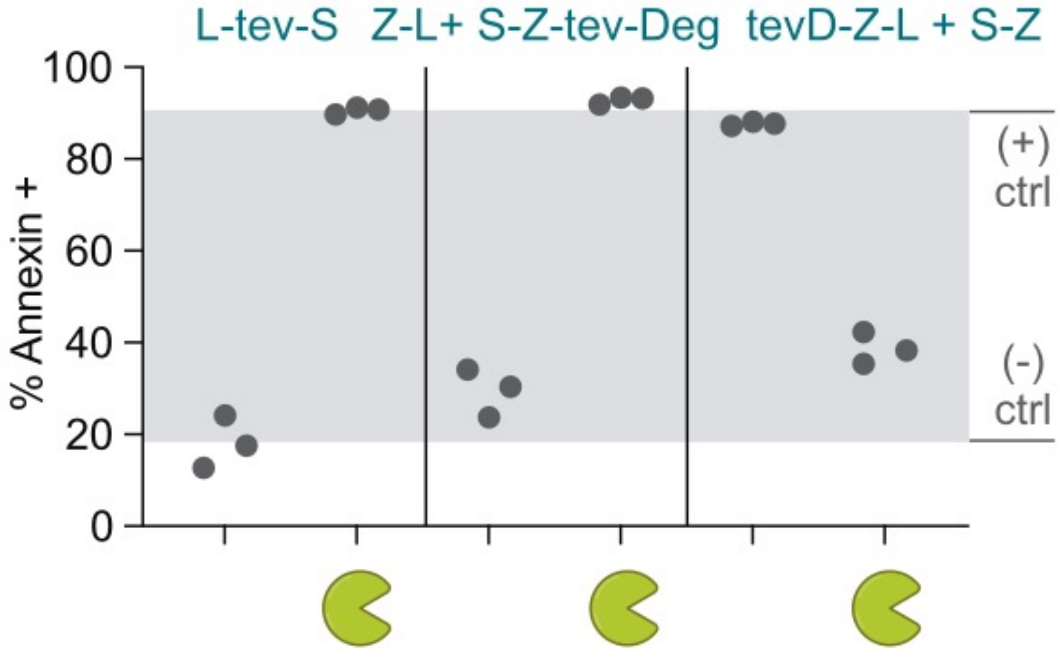
Annexin and Sytox staining



In brief, **Annexin indicating apoptotic and pyroptotic** states and **Sytox specifically marking pyroptosis**

Apoptosis Modules Validation

- **Annexin: apoptosis and pyroptosis**
- **Sytox: pyroptosis**



Pyroptosis Modules

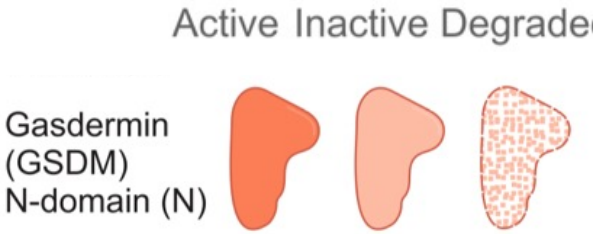
Mechanism of Pyroptosis

1. Activation
2. activated caspase-1/-4/-5 cleave GSDMA → generate:

Regulate it!

Modulate its stability

- **N-terminal domain (active)** → inserts into membrane, forms pores
  - pores release **IL-1β**, **IL-18**, and **DAMPs** → trigger inflammation
  - ion imbalance → **osmotic swelling** → **membrane rupture**
- **C-terminal domain (inactive)** → remains in cytosol, no pore formation

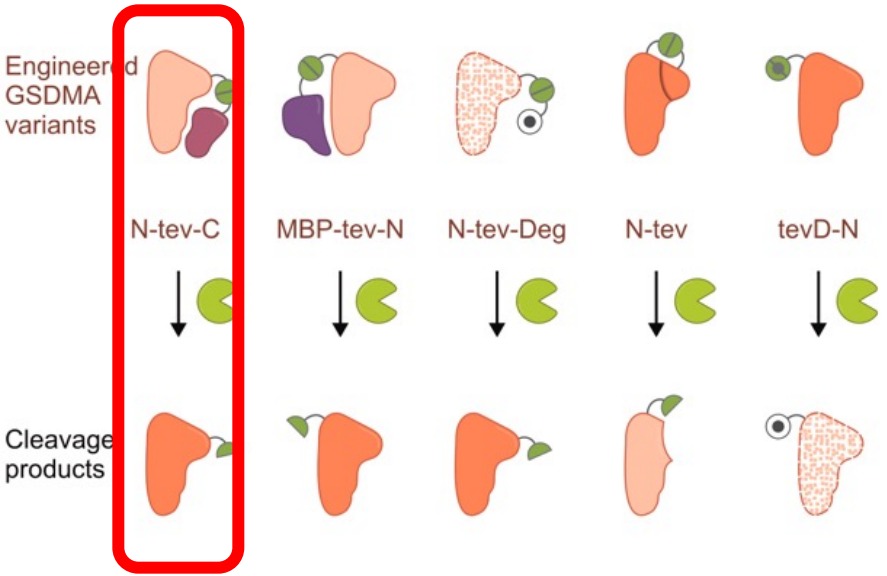


Pyroptosis Modules

N-tev-C

pyroptosis induction

- gasdermin protein contains a TEVP cleavage site between its N-terminal (pore-forming) and C-terminal (auto-inhibitory) domains
- TEVP cleavage
  → activates pyroptosis

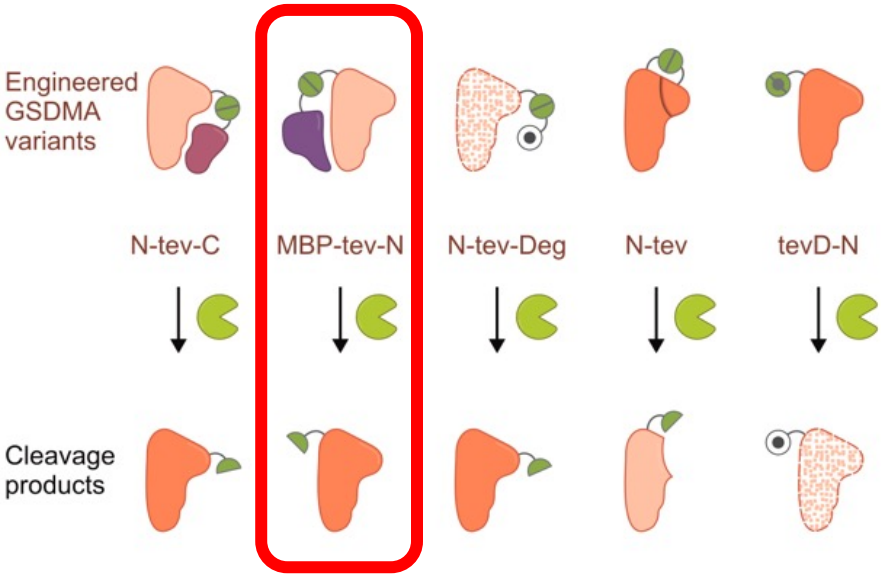


Pyroptosis Modules

MBP-tev-N

pyroptosis induction

- maltose-binding protein fused to the N-terminal domain → block its pore-forming activity
- TEVP cleavage removes MBP → activating pyroptosis

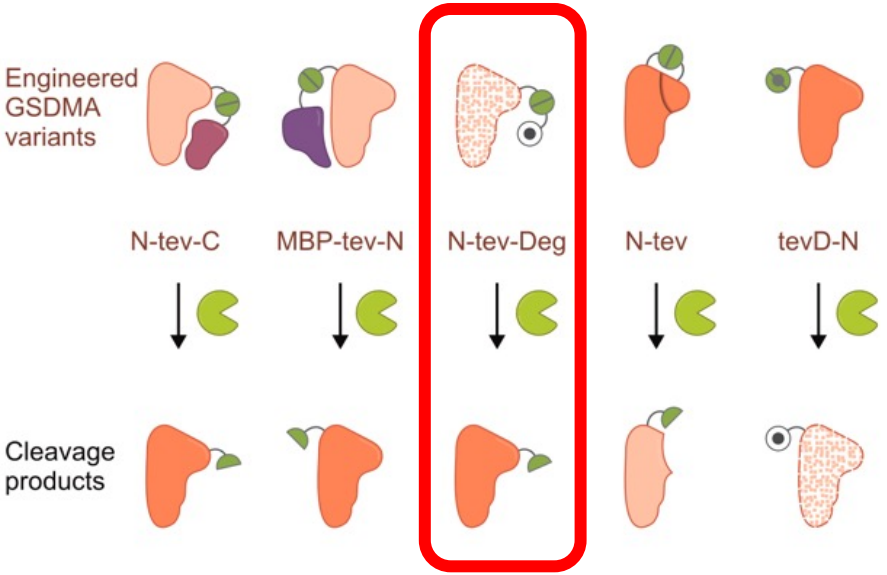


Pyroptosis Modules

N-tev-Deg

pyroptosis induction

- N-domain fused to a degron → suppressing its activity
- TEVP cleavage removes the degron → activating the pyroptosis pathway



Pyroptosis Modules

N-tev

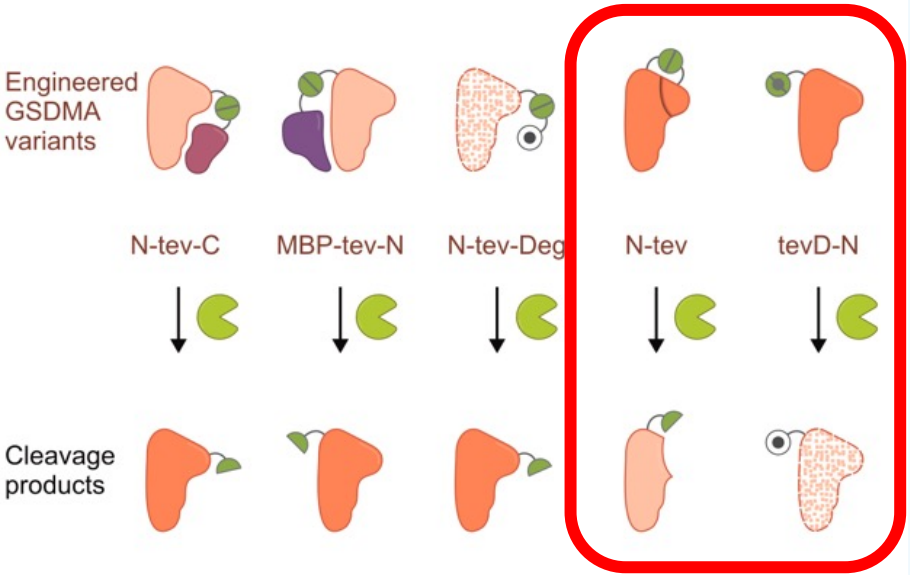
pyroptosis inhibition

- N-terminal domain with a TEVP cleavage site for direct activation

tevD-N

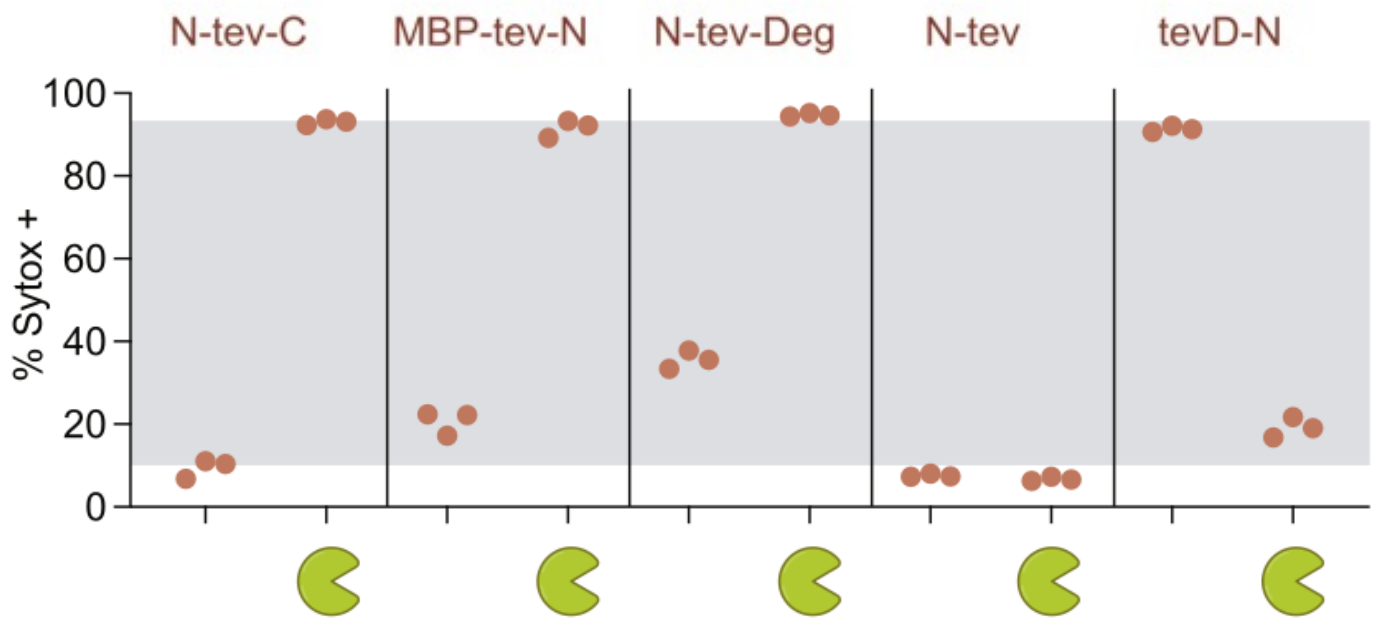
pyroptosis inhibition

- caged degron masks the activity of the N-domain
- TEVP cleavage → degron exposed → marking the protein for degradation



Pyroptosis Modules Validation

- **Annexin: apoptosis and pyroptosis**
- **Sytox: pyroptosis**

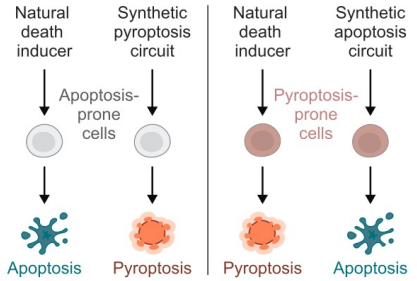


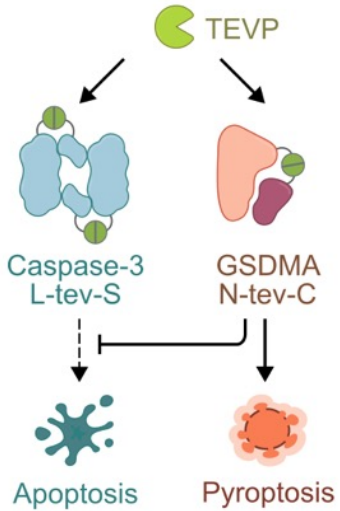
What's Next?

- ✓ Apoptosis Module
- ✓ Pyroptosis Module

"Synostosis circuits"

Add A few elements to fullfill…

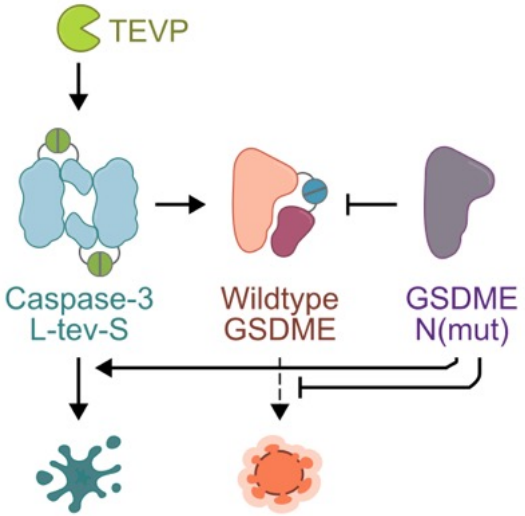




Driving pyroptosis in apoptosis-prone cells

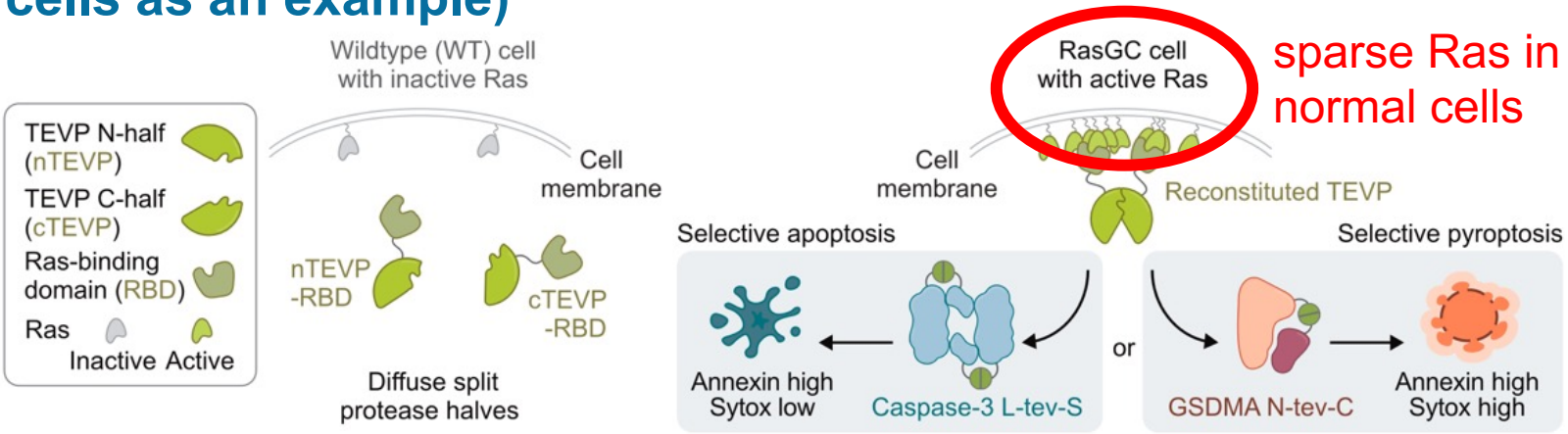
Driving apoptosis in pyroptosis-prone cells

GSDMEN(mut) is GSEME with its N domian mutant so that it can inhibit its WTcounterpart



What's Next?--Specificity

Synostosis circuits selectively eliminate target cells (cancer cells as an example)



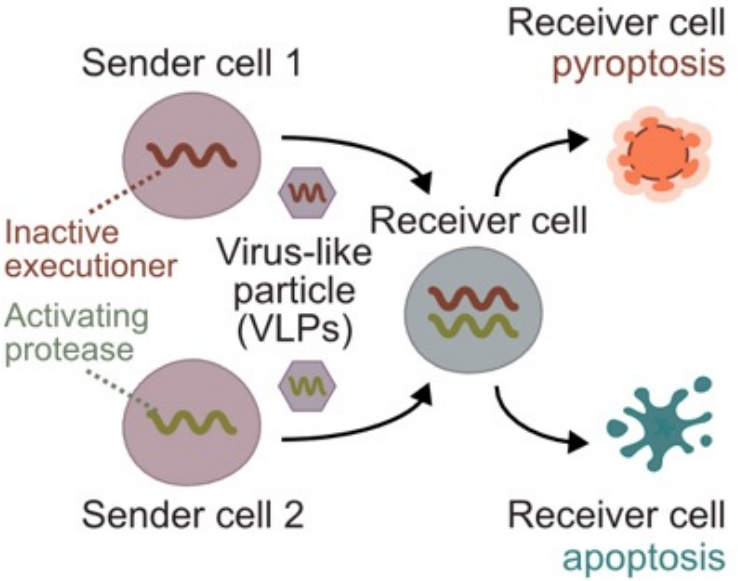
- Ras sensor with split TEVP fused to Ras-binding domains
- cancer cells have active **Ras clustering** at the membrane → brings the TEVP halves together → **reconstituting** active TEVP → activates downstream circuits → **selective** apoptosis or pyroptosis

What's Next?--Applicability

Synostosis circuits support intercellular operations

Split-Sender System

- **Split pyroptosis circuit** into an inactive executioner (e.g., GSDMA N-terminal domain) and an activating protease (e.g., TEVP) → Prevent self-destruction of sender cells
- Each component packaged into separate VLPs
- Receiver cells take up both components from the VLPs → reconstitute the circuit → undergo pyroptosis or apoptosis



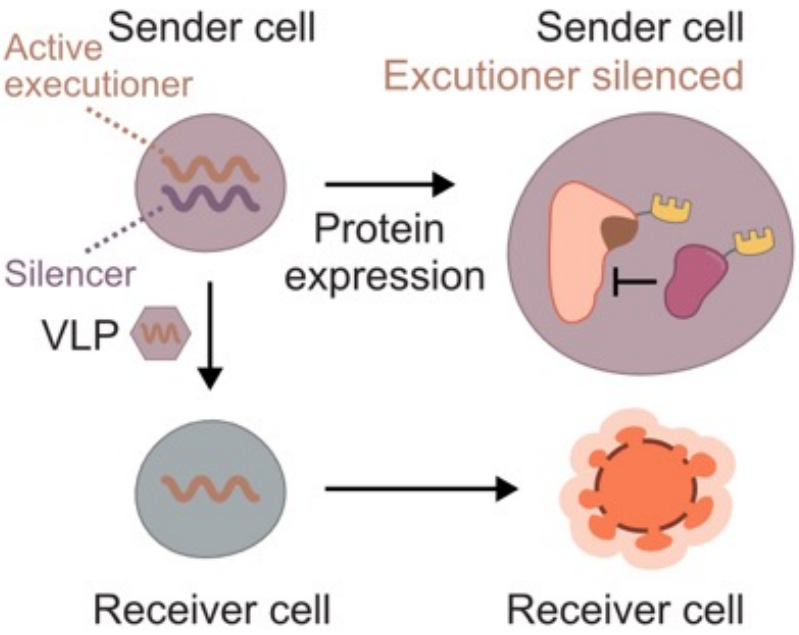
What's Next?--Applicability

Synostosis circuits support intercellular operations

Blocking Synpoptosis Activity in Senders

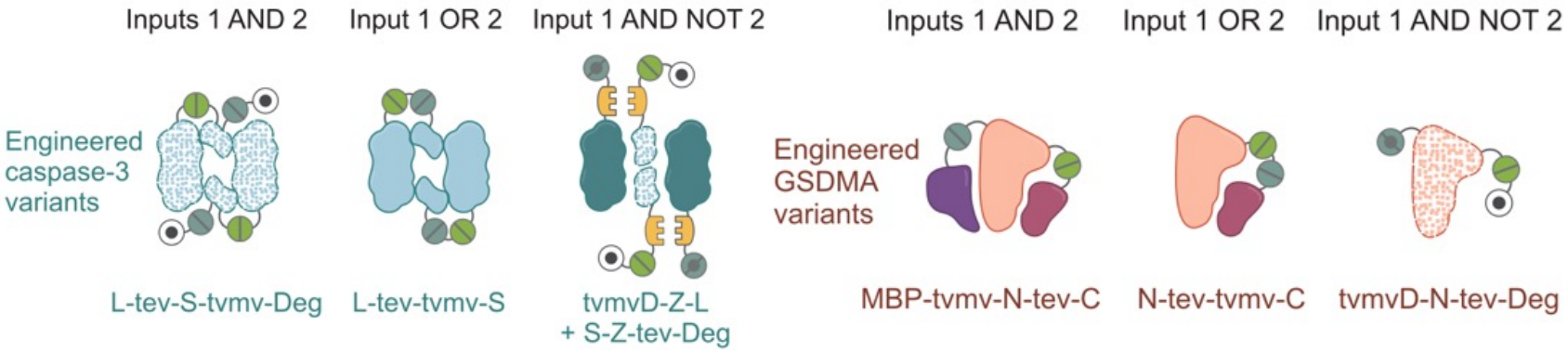
a more compact system

- sender cells **express silencer** domains → inhibit executioner activity → protects the senders
- functional VLPs still trigger cell death in receivers

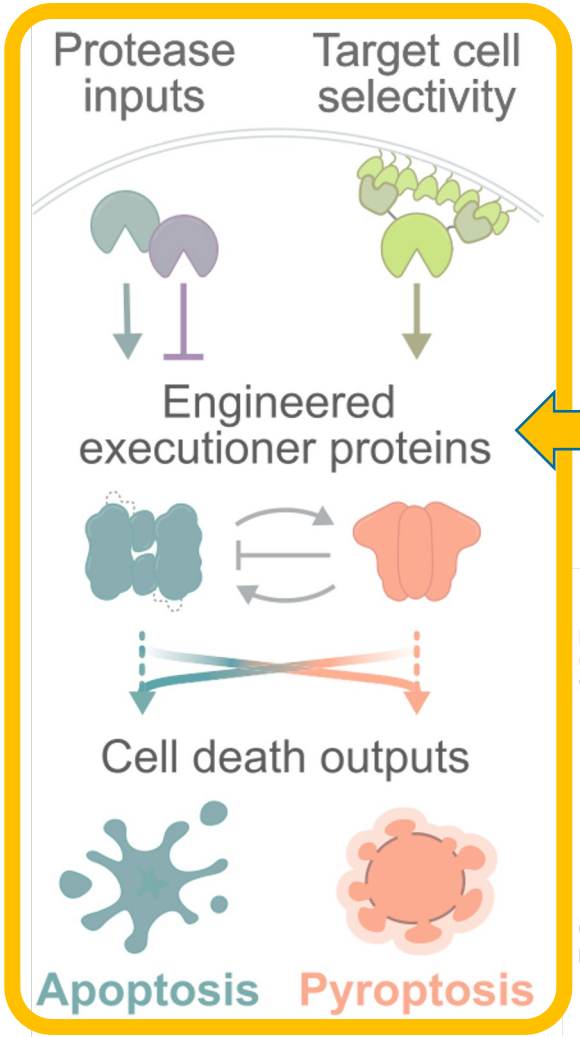


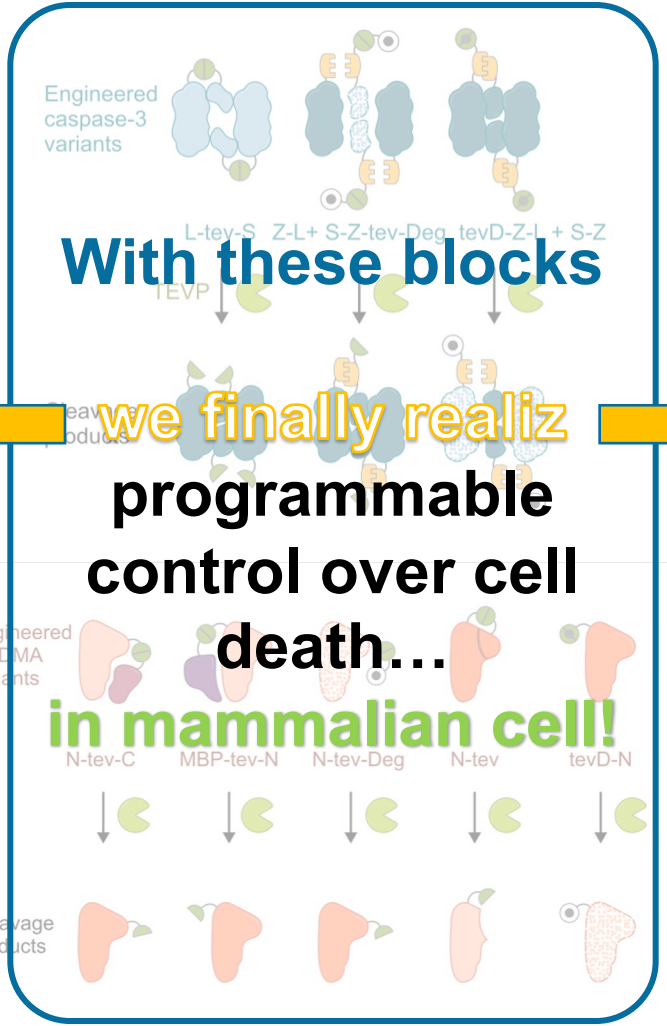
What's Next?--Sophistication

Synostosis circuits perform combinatorial computation!!



With these simple **boolean logic gates**, more complex implemants can be done (just by combination🤔), facilitating the death process to be more **adaptive**.



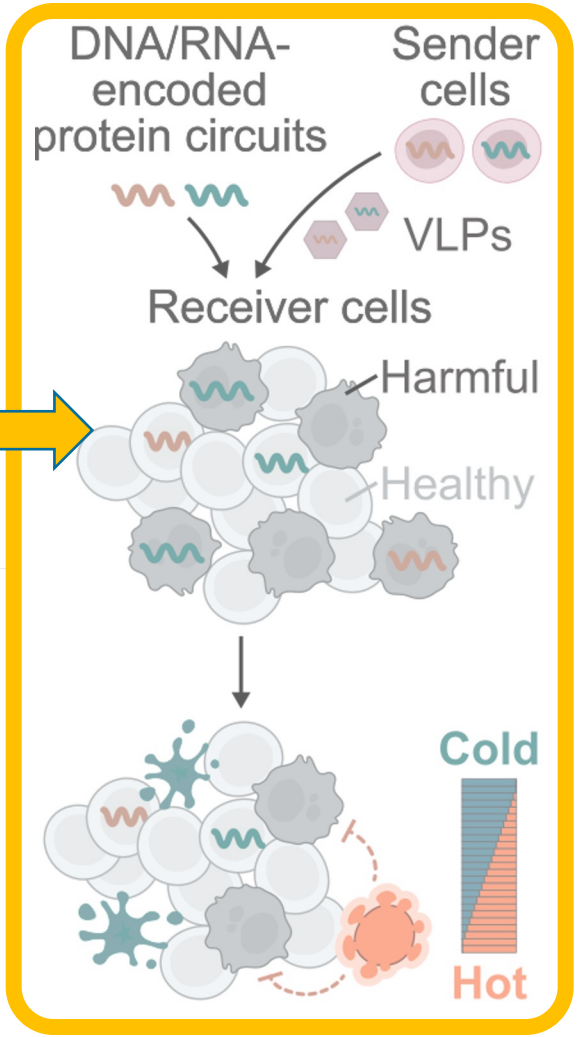


With these blocks

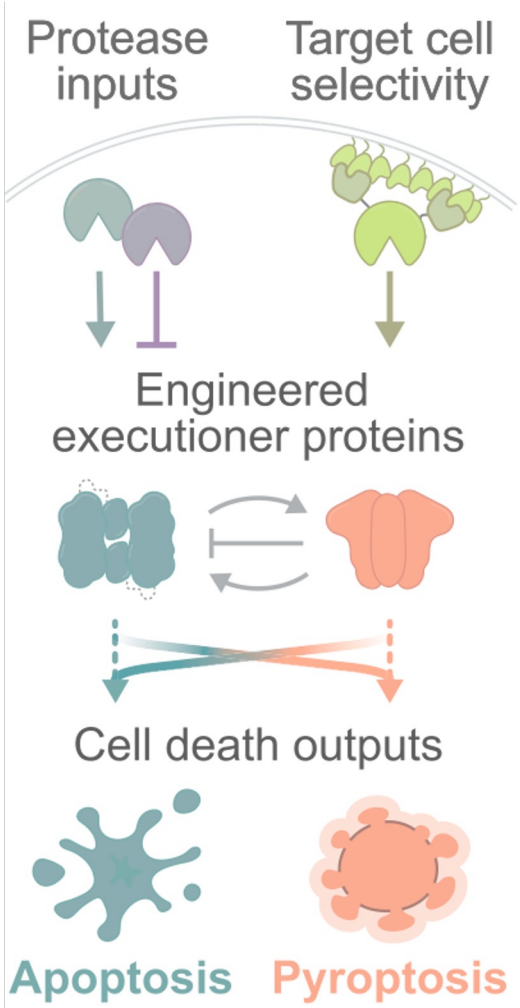
we finally realiz

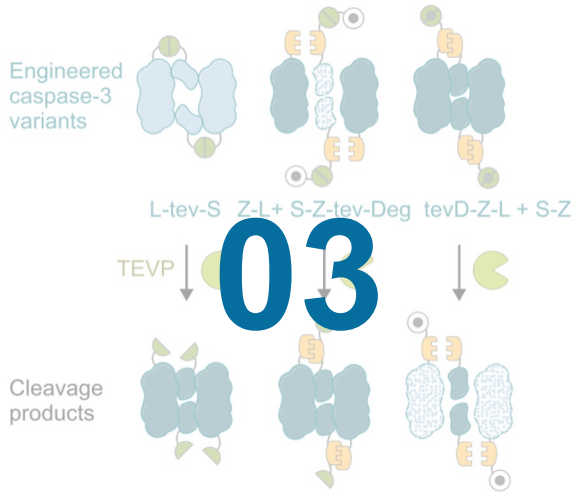
programmable control over cell death…

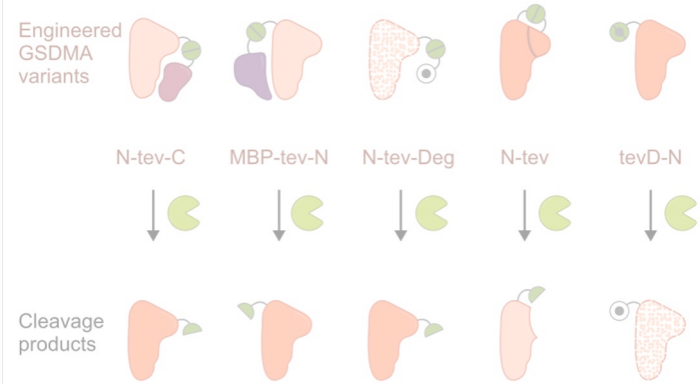
in mammalian cell!

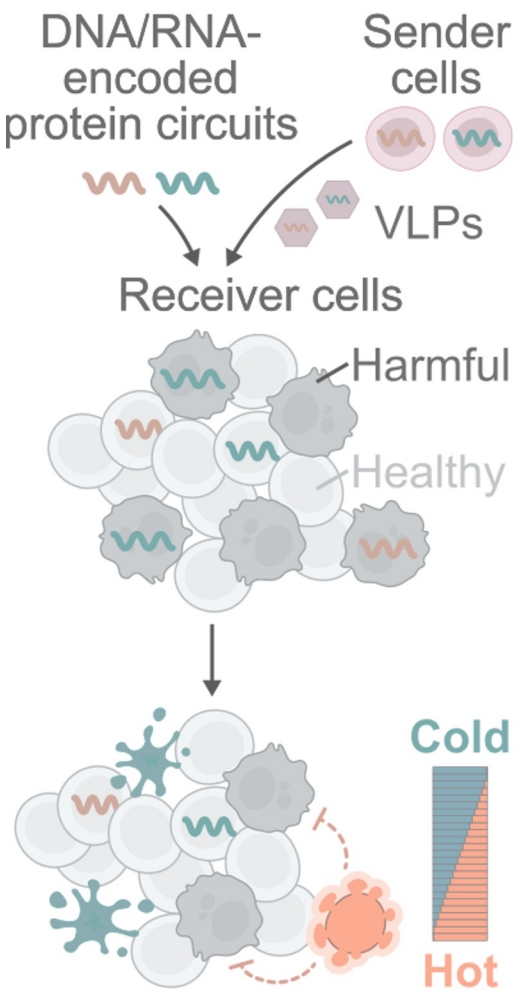


03 FUTURE


Protease inputs
Target cell selectivity
Engineered executioner proteins
Cell death outputs
Apoptosis
Pyroptosis

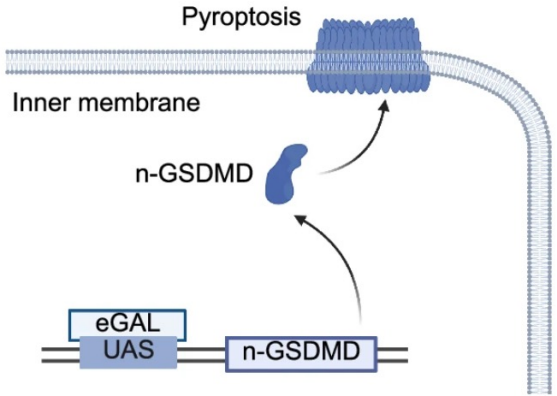

Engineered caspase-3 variants
L-tev-S
Z-L+ S-Z-tev-Deg
tevD-Z-L + S-Z
TEVP
Cleavage products


Engineered GSDMA variants
N-tev-C
MBP-tev-N
N-tev-Deg
N-tev
tevD-N
Cleavage products


DNA/RNA-encoded protein circuits
Sender cells
VLPs
Receiver cells
Harmful
Healthy
Cold
Hot

Cited by…

- It is a relatively “fresh” article!
- So far, it has been cited by 12 articles (from scopus)
- And most of them are used as a background introduction / achievement (Qiu et al., 2025; Yu et al., 2025; Bai et al., 2025; Luca et al., 2025)



A conceptual extension

“encoded PRCIS to express the N-terminal domain of gasdermin D (n-GSDMD), which is known to form membrane pores that facilitate pyroptosis” (Liu et al., 2025)

Cited by…

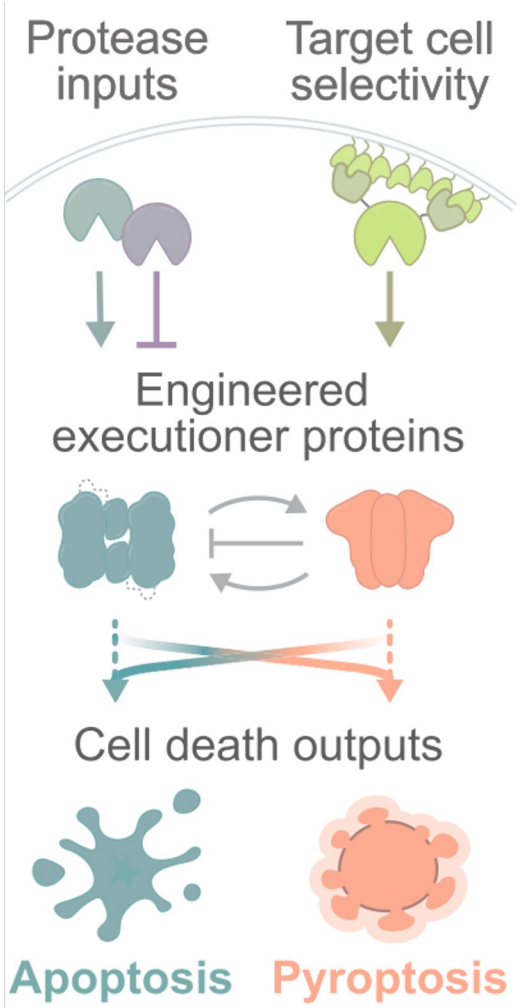
- It is a relatively “fresh” article!
- So far, it has been cited by 12 articles (from scopus)
- …and possible application (Orehek et al.,2025)

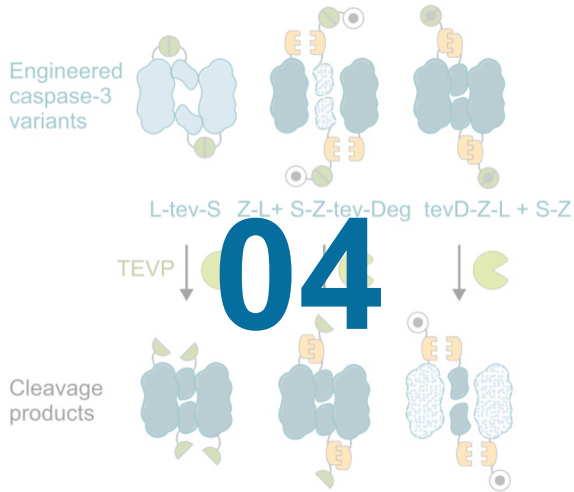
Design Inspirations for Inflammatory Skin Disorders

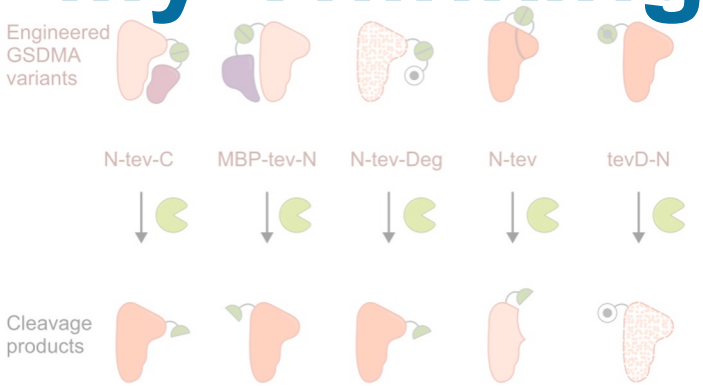
“This technology can also serve as a targeted therapeutic approach to modulate the functions of pathogenic cells in **inflammatory skin diseases**”
“a synthetic circuit tailored for **pathogenic KCs** can be designed to **respond to mechanical stress** signals mediated by piezo channels”
(Yin et al., 2025)
A **different circumstance** aside from cell death.

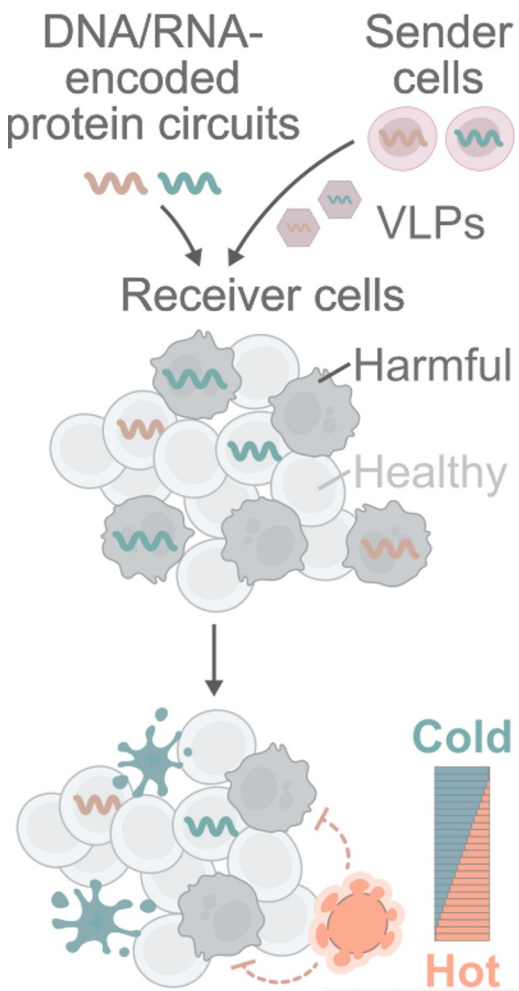
04

My Thinking









Potential improvement

From my perspective (some are noted in the Discussion too), this technique can further improve by overcome the following main points.

- **Limited to Apoptosis and Pyroptosis**: Other forms of cell death, such as necrosis or autophagy, are not directly addressed by the circuits, which limits their applicability to broader cellular contexts.
- **Inability to Function in Non-Mammalian Systems**: While the ultimate goal is to benefit human health, extending the circuit's applicability to common model organisms such as *Drosophila* or *C. elegans* would greatly enhance experimental flexibility and accelerate biological discovery.
- **Unknown Single-Cell Dynamics of Synostosis Circuit**: Exploring how individual cells respond to circuit activation could uncover cell-to-cell variability and reveal intermediate or hybrid death phenotypes. With the growing accessibility of single-cell technologies, I believe this direction holds great promise!

Potential improvement

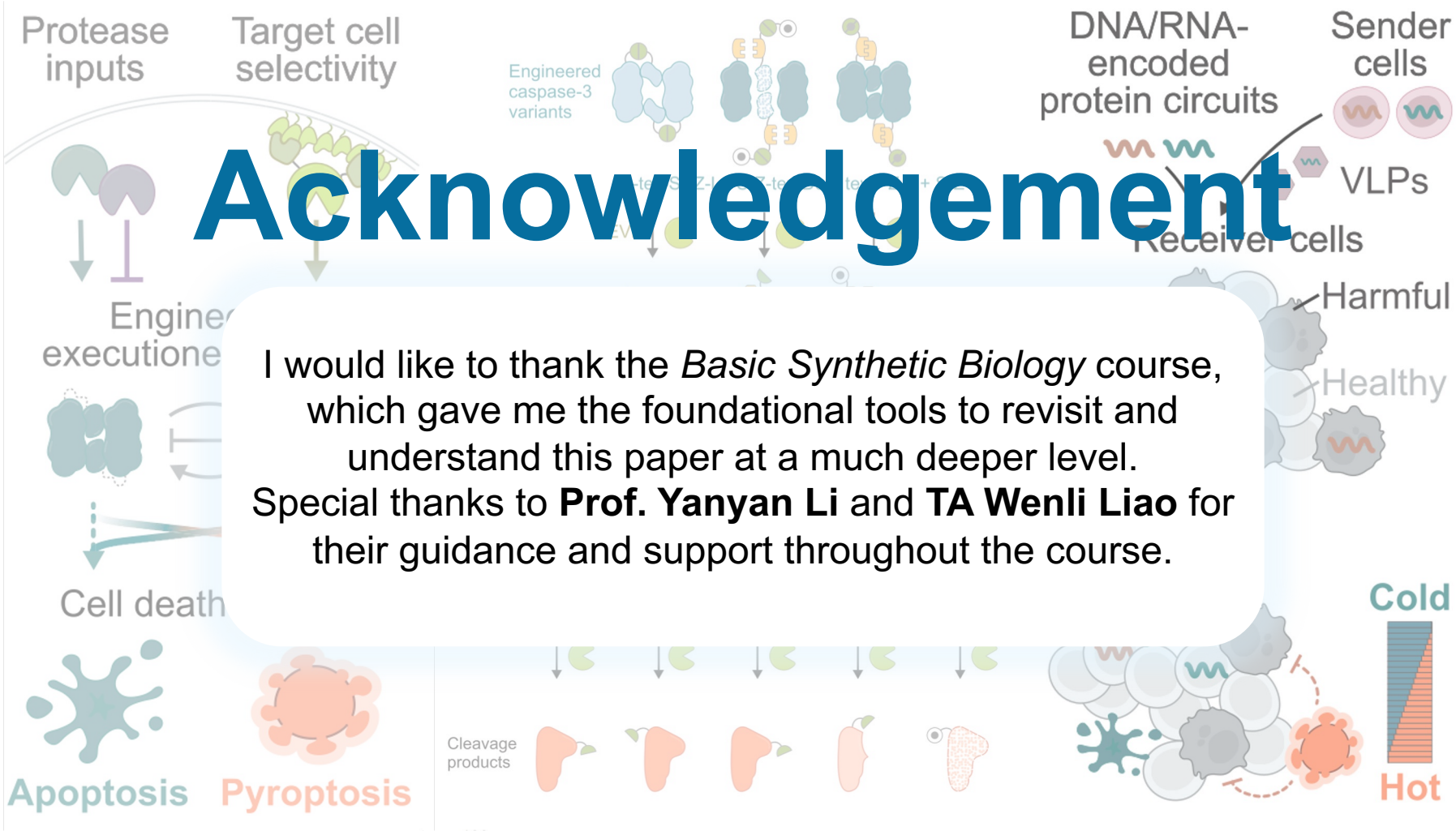
Of course, there are many points can be further improved (e.g. delivery optimization, advanced logic computation, orthogonality enhancement, etc.)

And I think, the following two directions can be starting points:

- Currently, the activity of synpoptosis circuits is **concentration-dependent** → **deepen understanding of cellular dynamics** to optimize concentration thresholds. This could involve **theoretical modeling.**
- Development of mechanisms to **actively control intracellular concentration levels in real time**. This could include engineering synthetic feedback loops, precise drug delivery systems, or leveraging molecular scaffolds to localize and stabilize circuit components. Such advancements would enable finer control over cell death activation, enhancing specificity and efficiency in therapeutic applications.

Inspirations

- What really amazed me is how **editable** biological systems are!!!!
- At first it all looked super complex—but in the end, it's **just basic biology, rearranged**. Simple cleavage, degradation, dimerization... all wired together like logic blocks.
- The hard part isn't doing it—it's **designing it, especially the fundamental block**. Choosing the right tools, the right targets, and knowing how they'll behave inside a living cell… That takes deep understanding of both **biological mechanisms** and **cellular dynamics,** what I'll focus on as I learn more about synthetic biology.



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