excessive macrophage scavenging and disposal of insulin-secretory vesicles. These findings also raise some key questions. The C57BL/6 mice are well studied models of diet-induced obesity and insulin resistance. Feeding C57BL/6 mice a diet rich in high fat for even one year produces hyper-insulinemia because islets are able to increase their mass and compensate by increasing insulin production (Youm et al., 2011). Despite chronic obesity, the mice do not show overt β cell failure, and they fail to progress to the insulin-dependent T2DM that typically happens in humans. Thus, whether macrophages can deplete insulin in the models of diet-induced obesity where islets can produce high insulin to compensate for insulin resistance cannot yet be fully established from the current animal models. Moreover, macrophages can signal in response to insulin, exacerbating an obesity-induced inflammatory state and insulin resistance (Bu et al., 2018) The discovery of insulin transcripts in the intra-islets macrophages raises questions about the role of insulin synthesis versus uptake in macrophages. Perhaps there are distinct subsets of insulin-disposing macrophages in islets, but the phenotype and identity of such cells remains obscure at this time. Also, given that currently there are no known islet-macrophage-specific Cre-drivers, it is not possible to specifically target these cells to study their role in islet development and T2DM. Nonetheless, the current study by Ying et al. (2018) succeeds in that it identifies peri-islet and intra-islet macrophages and reveals that macrophages have an insulin hunger with pathological consequences.

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Defying Death: The (W)hole Truth about the Fate of GSDMD Pores

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Pyroptosis is an inflammatory cell death response initiated by supramolecular organizing centers known as inflammasomes. In a recent issue of *Science*, Rühl et al. (2018) challenge the paradigm that inflammasome signaling necessitates pyroptosis by demonstrating that ESCRTIII-dependent membrane repair can delay or prevent gasdermin D-mediated cell death.

Inflammasome signaling connects the recognition of intracellular perturbations to activation of the inflammatory caspases. A major function of inflammasome signaling and subsequent caspase activation is the cleavage of pro-form interleukin (IL)-1 family cytokines such as IL-1β and IL-18 into their bioactive forms in the cytosol. These cytokines are secreted in an unconventional manner, as they lack N-terminal signal sequences necessary for entry into the vesicle-mediated biosynthetic pathway. Downstream of inflammasome activation, the lytic program of cell death, pyroptosis, has long

been a favored mechanism of bioactive IL-1 family release into the extracellular space where these cytokines can signal through their respective receptors. In addition to cleaving IL-1 family cytokines, active inflammatory caspases cleave the latent cytosolic protein gasdermin D (GSDMD) (Broz and Dixit, 2016). Cleavage of GSDMD releases the pore-forming N-terminal fragment from the autoinhibitory C-terminal fragment, allowing the N terminus of GSDMD to oligomerize and perforate the plasma membrane (Kovacs and Miao, 2017). These pores mediate IL-1 secretion and cell lysis. In a recent issue of *Science*, Rühl et al. reveal that a calcium-dependent membrane repair response, mediated by ESCRTIII components, antagonizes the execution phase of pyroptosis (Rühl et al., 2018). This ESCRTIII-mediated repair likely maintains membrane integrity by removal of GSDMD pores and thus prevents ultimate osmotic lysis of highly perforated cells during pyroptosis.

One mechanism of inflammasome activation occurs upon recognition of bacterial lipopolysaccharide (LPS) in the cytosol. The CARD domain of caspase-11 binds LPS leading to the activation of its latent enzymatic activity (Broz and Dixit, 2016). Active caspase-11 then cleaves GSDMD resulting in plasma membrane perforation (Broz and Dixit, 2016; Kovacs and Miao, 2017). Previous work illustrated that caspase-11-dependent potassium efflux occurs prior to assembly of the NLRP3 inflammasome. NLRP3 activation by intracellular LPS requires activation of caspase-11 and GSDMD (Broz and Dixit, 2016). Based on these combined data, it is likely that GSDMD pores mediate potassium efflux upstream of NLRP3- and caspase-1-dependent IL-1β cleavage. As premature rupture of the plasma membrane would result in the release of primarily pro-form IL-1 family members, the skewed presence of cleaved IL-1β in culture supernatants invokes a model whereby GSDMD is not instantaneously lytic. These findings implied the existence of mechanisms that prevent cell lysis during the critical time of NLRP3 inflammasome mediated cleavage of IL-1β.

Rühl et al. (2018) found that in addition to potassium efflux, cells flux calcium in a GSDMD- and caspase-11-dependent manner in response to intracellular delivery of bacterial LPS using primary and immortalized bone-marrow-derived macrophages. Careful chelation of calcium through co-treatment of fast acting BAPTA-AM and a drug export inhibitor was sufficient to increase cell lysis after LPS transfection in macrophages. These results suggested that calcium-dependent processes antagonize cell death after caspase-11 activation. Calcium flux is an evolutionarily conserved trigger for plasma membrane repair through exocytosis of vesicles such as lysosomes, mobilization of annexins, and recruitment of ESCRT machinery to sites of membrane injury (Cooper and McNeil, 2015). ESCRTIII machinery has been implicated in the membrane repair response to light induced injury, bacterial pore-forming toxins, and the host-derived MLKL pore (Cooper and McNeil, 2015; Gong et al., 2017). Consistent with data on other membrane damaging agents, the authors demonstrated recruitment of the ESCRT protein CHMP4 to the plasma membrane after expression of the pore forming N-terminal fragment of GSDMD. This recruitment was impaired in the presence of calcium chelators, reinforcing a role for GSDMD-mediated calcium flux in triggering repair of these pores. In the necroptosis pathway, which is a caspase-independent process of lytic cell death, the pores formed by MLKL mediate membrane permeabilization and phosphatidylserine (PS) externalization prior to full rupture of the plasma membrane (Gong et al., 2017). Rühl et al. (2018) also observed membrane permeabilization and PS externalization prior to cell lysis through monitoring calcium flux with Fluo-8, incorporation of the normally membrane impermeable DNA intercalating dye propidium iodide, and surface staining with the PS binding protein annexin V.

To provide further evidence that ESRCTIII machinery negatively regulates the pyroptotic cell fate, the authors characterized cells that overexpress the ESCRT-associated protein VPS4A or a dominant-negative VPS4A with the single amino acid change E228Q. One expectation would be that wild-type VPS4A provides a survival benefit to the cell by increasing reparative capacity, whereas the dominant-negative variant will inhibit membrane repair responses (Cooper and McNeil, 2015). By directly comparing these two altered cell states after LPS transfection and caspase-11 activation, the authors report an increase in membrane permeability and cell lysis in VPS4A E228Q cells. This increased permeability and cell lysis proved independent of the NLPR3 inflammasome. Because deficiency in ESCRTIII machinery initiates spontaneous necroptotic cell death (Gong et al., 2017), the authors performed RNAi experiments in the context of RIPK3-deficient immortalized macrophages that are genetically unable to undergo necroptosis. Reduced expression of ESCRTIII proteins as a result of siRNA transfection also resulted in increased cell lysis after activation of caspase-11.

Intriguingly, ESCRTIII-dependent membrane repair negatively regulated cell lysis and inflammasome-mediated cytokine release during *Salmonella* typhimurium infection. Synthetic caspase-1 activation by a chemically dimerizable caspase-1 variant in HEK293T cells revealed similar cellular responses. These results suggest that GSDMD-dependent membrane permeability is a conserved signal among the inflammasome pathways that recruits ESCRTIII machinery to the plasma membrane for the purpose of delaying or preventing lysis (Figure 1).

There are two implications of these findings. First, this study adds GSDMD pores to the list of membrane disruptions that can be repaired by the ESCRTIII machinery. The common use of ESCRTIII in membrane repair processes is explained by the common use of calcium fluxes as a signal that initiates these events. A major question that arises from these studies is whether other macrophage proteins regulate GSDMD pore residence in the plasma membrane. Whereas ESCRTIII controls the removal of these pores, a host protein that promotes GSDMD pore insertion in the membrane is yet to be defined. The process of membrane insertion and removal may emerge as a new regulatory step in the path to pyroptosis. The second implication of these findings relates to several recent studies demonstrating that pyroptosis is not a necessary consequence of inflammasome signaling (Wolf et al., 2016; Zanoni et al., 2016). Indeed, macrophages, dendritic cells, and neutrophils can utilize inflammasome-

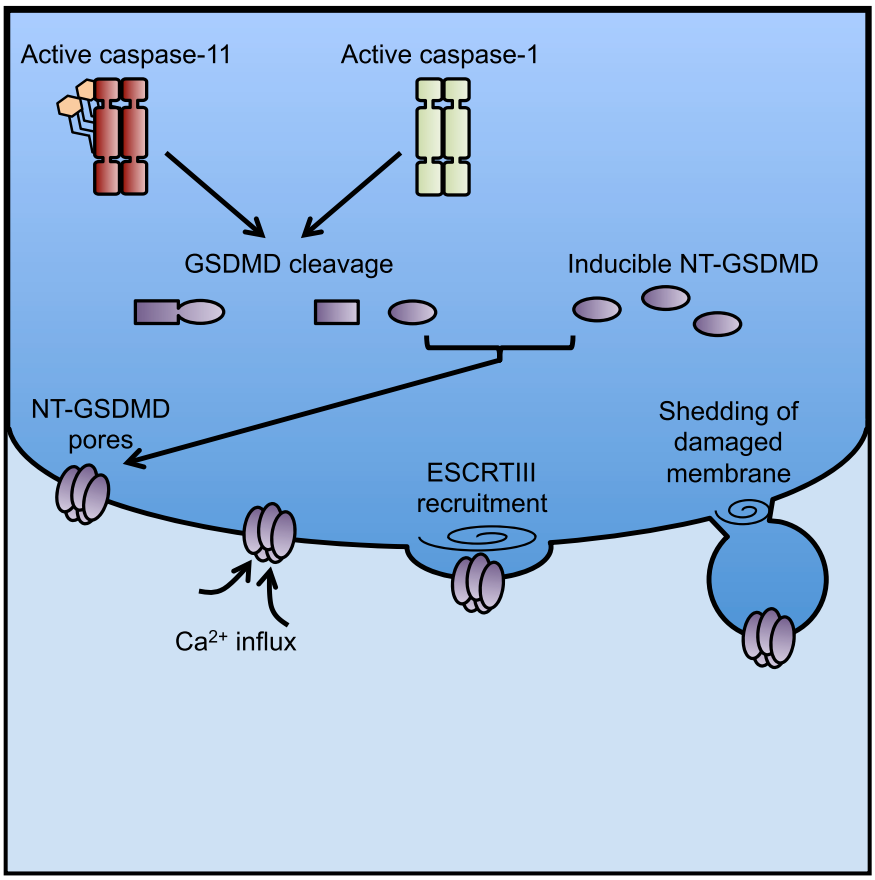

Figure 1. GSDMD Pores Activate ESCRTIII-Dependent Membrane Repair
Active inflammatory caspases cleave GSDMD into N and C-terminal fragments. The N-terminal fragment (NT-GSDMD) binds the inner leaflet of the plasma membrane and oligomerizes into a large pore. NT-GSDMD expression allows for the flux of calcium (Ca^{2+}) ions likely from the hypercalcemic extracellular space into the hypocalcemic cytosol. Ca^{2+} flux serves as a signal to recruit ESCRTIII machinery to the site of membrane damage. ESCRTIII filament oligomerization allows for shedding of damaged membrane through bleb formation.

dependent processes to activate GSDMD pore formation and remain viable (Evavold et al., 2018; Heilig et al., 2018). Under these conditions, the pores formed at the plasma membrane do not promote pyroptosis, but rather promote the secretion of IL-1 through these pores. The ability of living cells to add IL-1 to the repertoire of cytokines they secrete is notable, because this cytokine has been considered to only be released upon cell lysis. Consequently, cells that secrete cytokines from the cytosol and vesicle-mediated secretory pathway are considered to have achieved a hyperactive state, which coincides with an enhanced ability to stimulate adaptive immunity (Zanoni et al., 2016). The mechanisms that permit GSDMD to either promote pyroptosis or cell hyperactivation are unclear, but hyperactive cells display evidence of lower amounts of GSDMD pores than pyroptotic cells (Evavold et al., 2018).

One hypothesis based on the findings by Rühl et al. (2018) is that hyperactive cells are able to survive GSDMD pores at the plasma membrane through rapid removal by ESCRTIII membrane shedding. A prediction based on this hypothesis would be that inhibition of membrane repair proteins, such as the ESCRTIII machinery, or chelation of calcium would convert hyperactive cells into pyroptotic cells due to increased plasma membrane occupancy of lytic GSDMD pores leading to osmotic lysis. Moreover, if ESCRTIII-mediated membrane repair is a common response to host-derived GSDMD pores, one would expect that cell-free culture supernatants would contain GSDMD, as has been reported (Liu et al., 2016). According to the known role of ESCRTIII proteins at the plasma membrane in the budding of viral particles and extracellular vesicles (Cooper and McNeil, 2015), this pool of GSDMD is likely associated with shed plasma membrane.

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