SUPPLEMENTARY INFORMATION

Membrane permeabilization is mediated by distinct epitopes in mouse and human orthologs of the necroptosis effector, MLKL

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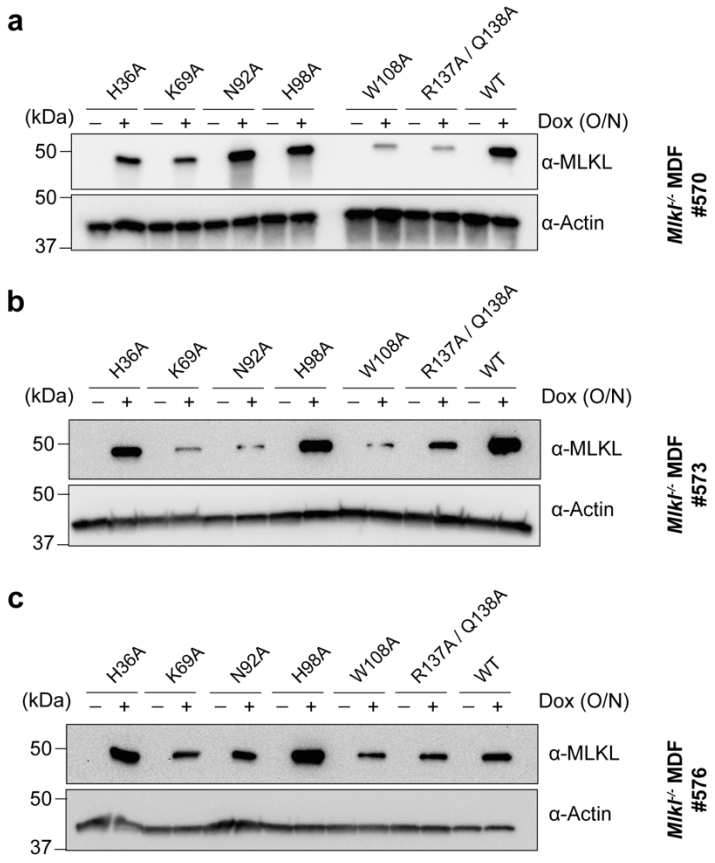
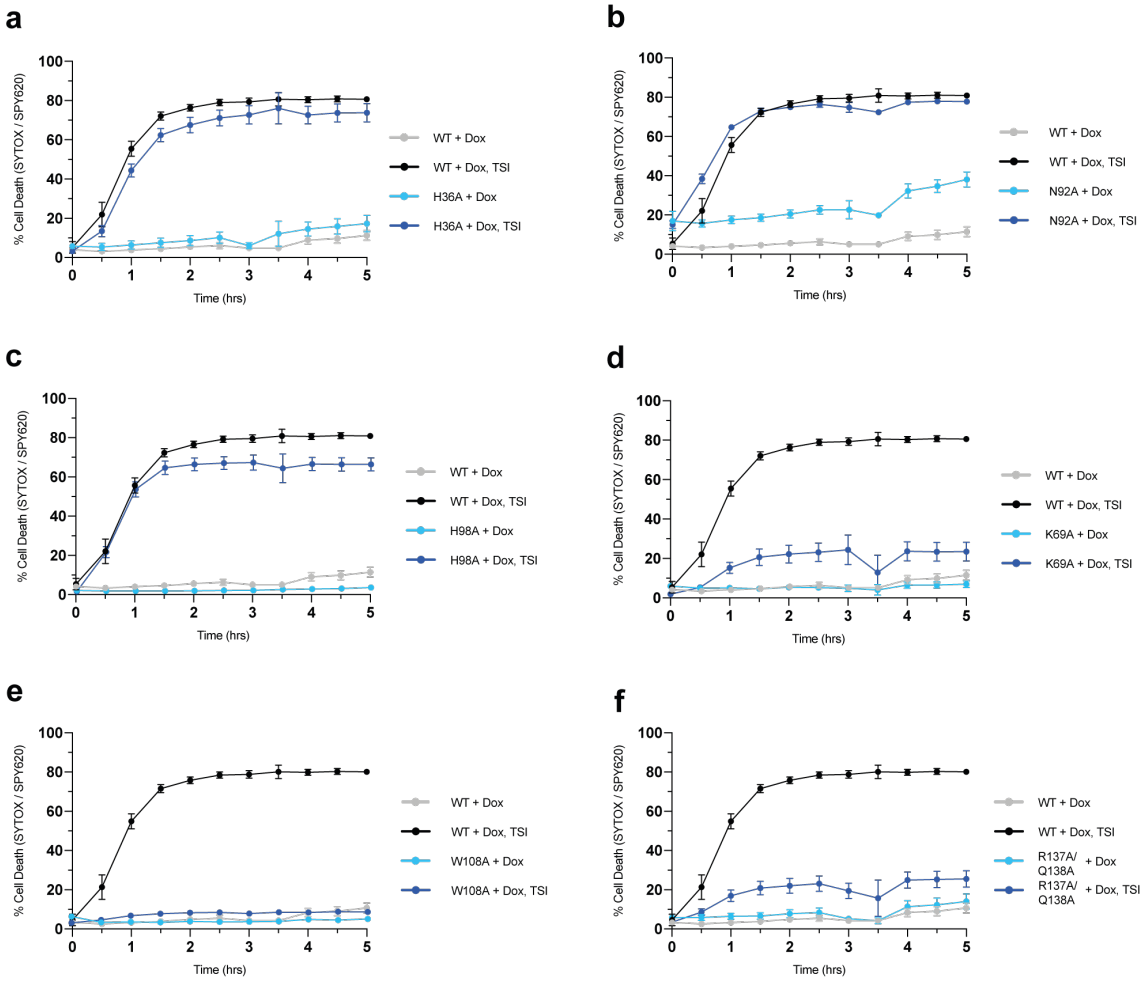
SUPPLEMENTARY FIGURES

Supplementary Figure 1 | Expression of wild-type and mutant mouse MLKL in *Mlkl*$^{-/-}$ MDF cells. Following doxycycline (Dox) induction, whole cell-lysates were fractioned by SDS-PAGE and probed by immunoblot for MLKL with anti-actin as a loading control. Immunoblots are representative of $n = 2$ independent experiments.



Supplementary Figure 2 | Raw IncuCyte data of cell death mediated by wild-type and mutant mouse MLKL. a-f) To establish the contribution of each lipid-binding residue in cellular necroptosis signaling, full-length wild-type (WT) and mutant mouse MLKL were stably introduced into *Mlkl*$^{-/-}$ MDF cells. Following doxycycline (Dox) treatment to induce expression, percent cell death was quantified using IncuCyte S3 live cell imaging in the presence or absence of the necroptotic stimulus, TNF and Smac-mimetic Compound A and pan-caspase inhibitor, IDN-6556, (TSI) for 5 h, by determining the number SYTOX Green-positive cells (dead cells) relative to the number of SPY620-positive cells (total cell confluency). The percent cell death of wild-type mouse MLKL is shown in each plot as a reference. Data represent mean ± SEM from three biologically independent *Mlkl*$^{-/-}$ MDF cell lines (n = 6 to 9).



SUPPLEMENTARY TABLES

Supplementary Table 1 | Summary of wild-type and mutant mouse MLKL properties

MLKL mutation	Location of mutation	Lipid-binding (Impact on liposome permeabilization)	Necroptotic signalling function
mMLKL WT			+
mMLKL Y15A/E16A	α1 helix	N/D	-[1]
mMLKL C18A/C24A/C28A	α1/2 helix	N/D	-[1]
mMLKL K22A/R30A	α2 helix and preceding loop	N/D	-[1]
mMLKL H36A	α2 helix	Yes (compromised)	+[†]
mMLKL R63A/D65A	α3 helix	N/D	-[1]
mMLKL K69A	α3 helix	Yes (compromised)	Reduced[†]
mMLKL E70A/N72A	α3 helix	N/D	-[1]
mMLKL E76A/K77A	α3 helix	N/D	-[1]
mMLKL K80A/K81A	α3-α4 loop	N/D	-[1]
mMLKL N92A	α3-α4 loop	Yes (comparable to WT)	+[†]
mMLKL H98A	α4 helix	Yes (compromised)	+[†]
mMLKL H98A/E99A	α4 helix	N/D	-[1]
mMLKL E102A/K103A	α4 helix	N/D	-[1]
mMLKL R105A/D106A	α4 helix	N/D	-[1, 2]
mMLKL W108A	α4 helix	Yes (compromised)	-[†]
mMLKL E109A/E110A	α4 helix	N/D	-[1, 2]
mMLKL $LLLL^{112\text{-}115}AAAA$	α4 helix	N/D	-[1]
mMLKL R137A/Q138A	First brace helix	Yes (compromised)	-[†]

N/D = Not determined; - = loss-of-function; [†] This study

Supplementary Table 2 | Summary of wild-type and mutant human MLKL properties

MLKL mutation	Location of mutation	Lipid- or IP6-interactor	Impact on liposome permeabilization	Necroptotic signalling function
hMLKL WT				+
hMLKL E2A/N3A	α1 helix	Lipid	Compromised	-[3]
hMLKL K5A	α1 helix	Lipid	Compromised	-[3]
hMLKL H15A	α1 helix	IP6	N/D	N/D[4]
hMLKL K16A/R17A	α1 helix	Lipid	Compromised	Reduced[3, 5]
hMLKL E19A	α1 helix	IP6	N/D	N/D[6]
hMLKL K22Q/K25Q	α2 helix and preceding loop	Lipid	Compromised	-[7]
hMLKL R29E/R30E	α2 helix	Lipid	Compromised	-[7]
hMLKL L36A	α2 helix	IP6	N/D	N/D[6]
hMLKL K50A/K51A	α2-α3 loop	Lipid	Compromised	-[3]
hMLKL K78A	α3 helix	IP6	N/D	N/D[6]
hMLKL D107A/E111A	α4 helix	Lipid	Compromised	-[5, 8]
hMLKL L114A	α4 helix	Lipid	Compromised	-[8]
hMLKL L116A	α4 helix	IP6	N/D	N/D[6]
hMLKL E119A	α4 helix	IP6	N/D	N/D[6]
hMLKL R152A	First brace helix	IP6	N/D	N/D[6]

N/D = Not determined; - = loss-of-function

Supplementary Table 3 | Primers used in this study

Primer name	Sequence
mMLKL Bam 5′ fwd*	5′-CGCGGATCCatggataaattgggacagatcatc-3′
mMLKL 158 stop EcoRI rev	5′-CGCGAATTCAgctaatttgcaactgcatcaggataac-3′
mMLKL 464 stop EcoRI rev	5′-CGGAATTCttacaccttcttgtccgtggattc-3′

*Restriction sites underlined

Supplementary Table 4 | Plasma membrane-like lipid mix for liposomes

Lipid	Proportion of plasma membrane-like mix	Source
Phosphatidylethanolamine (POPE)	20%	Avanti Polar Lipids (Alabaster, AL, USA)
Phosphatidylcholine (POPC)	40%	
Phosphatidylinositol (PI)	10%	
Phosphatidylserine (DOPS)	20%	
Phosphatidylglycerol (POPG)	10%	

SUPPLEMENTARY REFERENCES

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