

Supporting Information for Targeting STING oligomerization with small-molecule inhibitors

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A**BB-CI-Yne**

sp|Q3TBT3|STING_MOUSE (100%), 42,831.5 Da
 sp|Q3TBT3|STING_MOUSE Stimulator of interferon genes protein OS=Mus musculus OX=10090 GN=Tmem173
 6 exclusive unique peptides, 7 exclusive unique spectra, 7 total spectra, 89/378 amino acids (24% coverage)

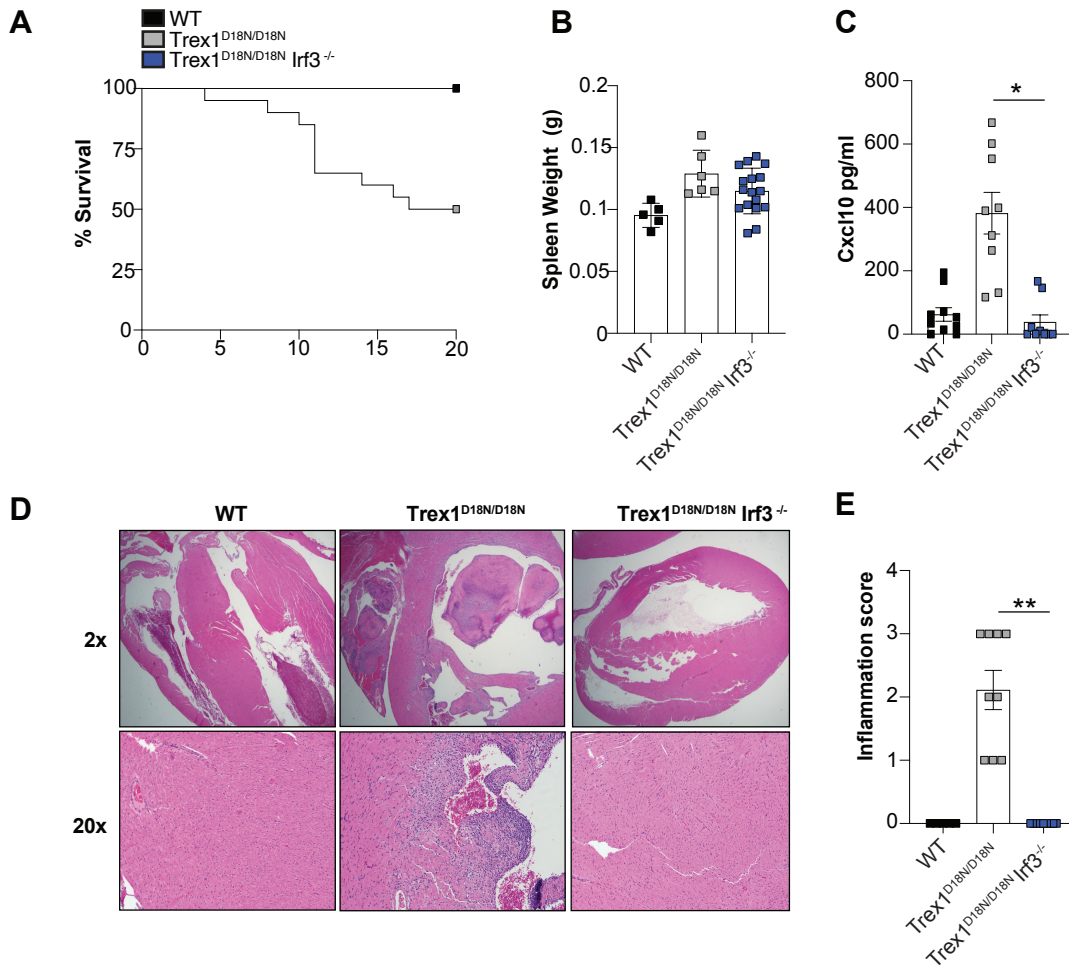
MPYSNLHPAI	PRPRGHRSKY	VALIFLVLASL	MILWVAKDPP	NHTLKYLALH
LASHELGLLL	KNLCCLAEEL	CHVOSRYOGS	YWKAVRACLG	CPIHCMAMIL
LSSYFYFLON	TADIYLSWMF	GLLVLYKSLS	MLLGLOSLTP	AEVSAVCEEK
KLNVAHGLAW	SYIIGYLRLLI	LPGLQARIRM	FNOLHNNMLS	GAGSRRLYIL
FPLDCGVPDN	LSVVDPNIRF	RDMLPOONID	RAGIKNRVYS	NSVYEILENG
OPAGVCILEY	ATPLOTLFAM	SODAKAGFSR	EDRLEOAKLF	CRTLEEILED
VPESRNNCRLL	IYVOEPTDGN	SFSLSOEVLRL	HIRQEEKEEV	TMNAPMTSVA
PPPSVLSQEP	RLLSGMDQP	LPLRTDLI		

B**BB-CI-Yne + BB-CI**

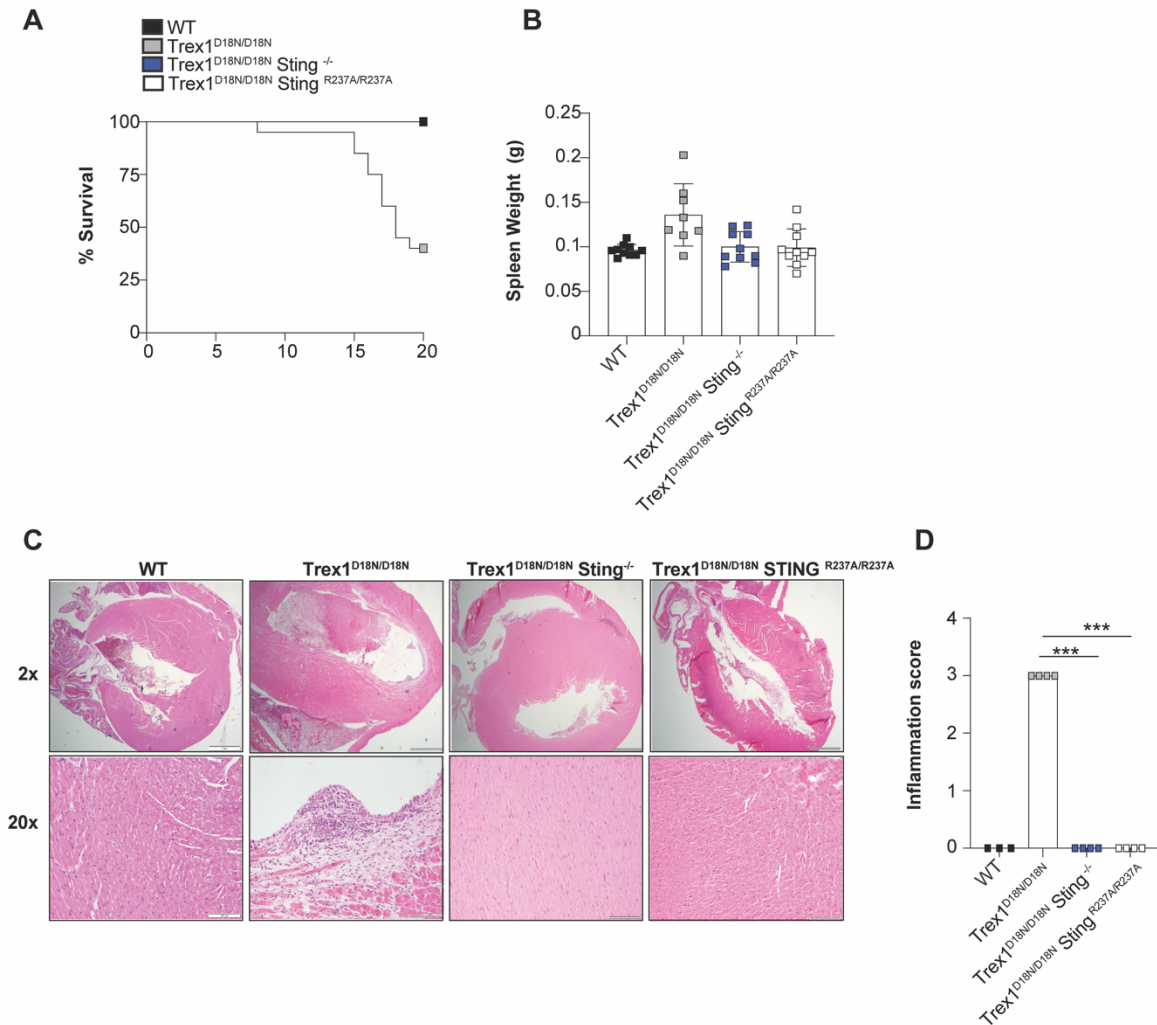
sp|Q3TBT3|STING_MOUSE (100%), 42,831.5 Da
 sp|Q3TBT3|STING_MOUSE Stimulator of interferon genes protein OS=Mus musculus OX=10090 GN=Tmem173
 1 exclusive unique peptides, 1 exclusive unique spectra, 1 total spectra, 10/378 amino acids (3% coverage)

MPYSNLHPAI	PRPRGHRSKY	VALIFLVLASL	MILWVAKDPP	NHTLKYLALH
LASHELGLLL	KNLCCLAEEL	CHVOSRYOGS	YWKAVRACLG	CPIHCMAMIL
LSSYFYFLON	TADIYLSWMF	GLLVLYKSLS	MLLGLOSLTP	AEVSAVCEEK
KLNVAHGLAW	SYIIGYLRLLI	LPGLQARIRM	FNOLHNNMLS	GAGSRRLYIL
FPLDCGVPDN	LSVVDPNIRF	RDMLPOONID	RAGIKNRVYS	NSVYEILENG
OPAGVCILEY	ATPLOTLFAM	SODAKAGFSR	EDRLEOAKLF	CRTLEEILED
VPESRNNCRLL	IYVOEPTDGN	SFSLSOEVLRL	HIRQEEKEEV	TMNAPMTSVA
PPPSVLSQEP	RLLSGMDQP	LPLRTDLI		

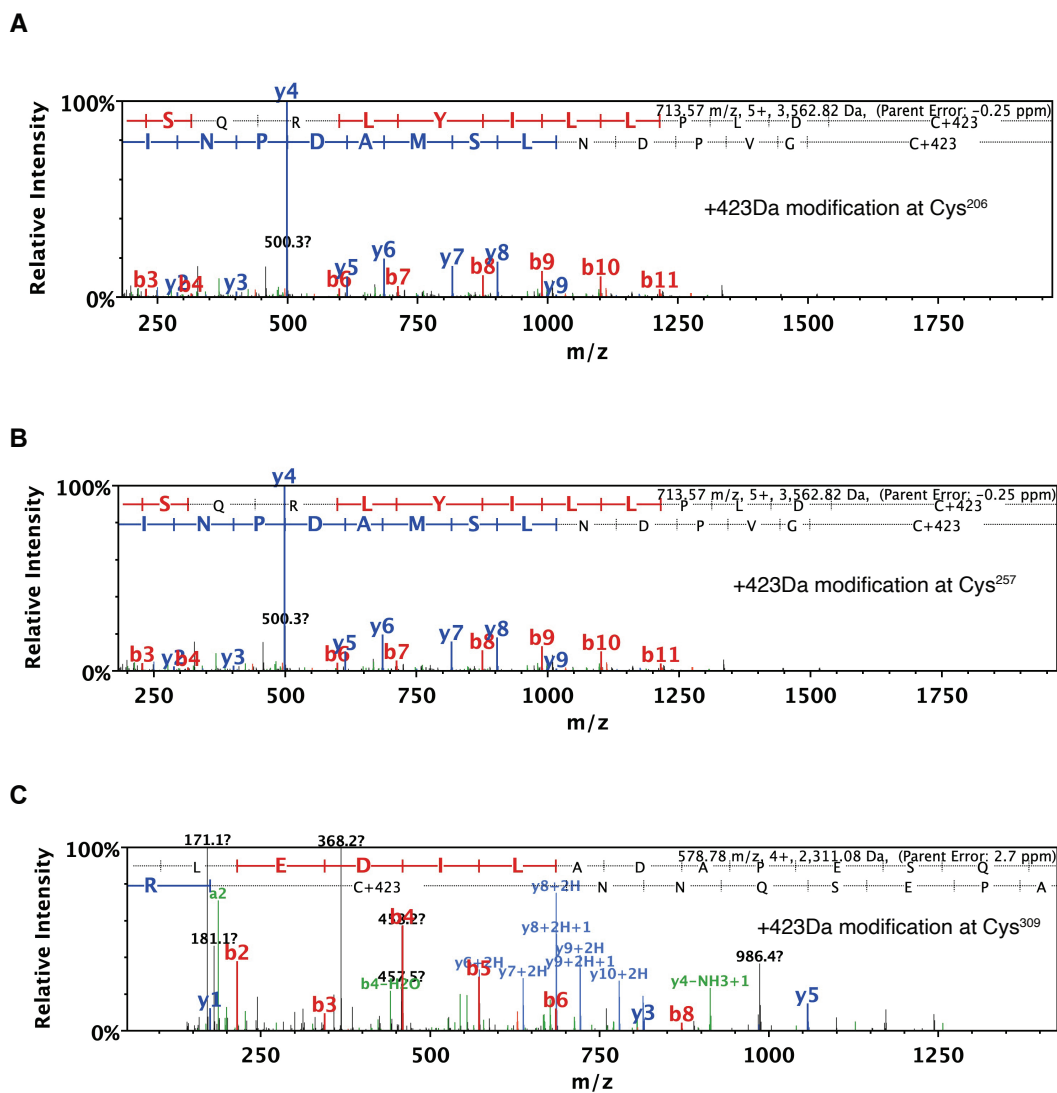
Supplementary Figure 1. BB-CI-amidine binds to STING. (A–B) Sequence coverage of STING from analysis of peptides identified in streptavidin bead pull-downs from clicked lysates of BMDMs treated with BB-CI-Yne (A) or BMDMs co-treated with BB-CI-amidine and BB-CI-Yne (B) (related to Figure 4). Representative of 3 independent replicates.



Supplementary Figure 2. IRF3 deficiency protects against experimental AGS in *Trex1^{D18N/D18N}* mice. (A) Survival analysis of WT (n=20), *Trex1^{D18N/D18N}* (n=20), *Trex1^{D18N/D18N}/Irf3^{-/-}* mice (n=20). (B) Spleen weights of WT (n=5), *Trex1^{D18N/D18N}* (n=6), *Trex1^{D18N/D18N}/Irf3^{-/-}* mice (n=17) (C) ELISA analysis of serum Cxcl10 in WT (n=10), *Trex1^{D18N/D18N}* (n=9), *Trex1^{D18N/D18N}/Irf3^{-/-}* mice (n=9). (D–E) Representative H&E staining of tissue sections from hearts (D) and pathology evaluation of heart sections (E) from WT (n=5) and *Trex1^{D18N/D18N}* (n=9) and *Trex1^{D18N/D18N}/Irf3^{-/-}* mice (n=9). *, $P < 0.05$, **, $P < 0.01$ by two-way ANOVA. Error bars show means \pm SEM.



Supplementary Figure 3. STING deficiency and cGAMP binding blockade protect against experimental AGS in *Trex1*^{D18N/D18N} mice. (A) Survival analysis of WT (n=10), *Trex1*^{D18N/D18N} (n=20) and *Trex1*^{D18N/D18N}/*STING*^{-/-} (n=10) and *Trex1*^{D18N/D18N}/*STING*^{R237A/R237A} (n=10) mice. (B) Spleen weights of WT (n=10), *Trex1*^{D18N/D18N} (n=8), *Trex1*^{D18N/D18N}/*STING*^{-/-} (n=10) and *Trex1*^{D18N/D18N}/*STING*^{R237A/R237A} (n=10) mice. (C–D) Representative H&E staining (C) and pathology scoring of tissue sections from hearts (D) of WT (n=3), *Trex1*^{D18N/D18N} (n=4) and *Trex1*^{D18N/D18N}/*STING*^{-/-} (n=4) and *Trex1*^{D18N/D18N}/*STING*^{R237A/R237A} (n=4) mice., ***, *P*<0.001. two-way ANOVA. Error bars show means ± SEM.



Supplementary Figure 4. BB-Cl-amidine modifies Cys²⁰⁶, Cys²⁵⁷ and Cys³⁰⁹ in STING. (A–C) Representative mass spectrometry spectra of STING modified by BB-Cl-amidine AT Cys²⁰⁶ (A), Cys²⁵⁷ (B) and Cys³⁰⁹ (C) identified in tryptic digests from recombinant STING (10 μ g) incubated with BB-Cl-amidine (10 μ M) for 1 h at 37 °C.