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Supplementary Fig. S1. STING-independent induction of CXCL10 and IFN- β in a series of human melanoma cell lines in response to stimulation with polyI:C. Human melanoma cell lines were stimulated with polyI:C for 24 h. (A) CXCL10 and (B) IFN- β levels in cell culture supernatants were measured using ELISA and reported as mean \pm SD of three biological replicates. Statistical significance was determined by unpaired t-test (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).



Supplementary Fig. S2. Activation of STING signaling results in enhanced immunogenicity of MART-1 pulsed WM39 melanoma cells. (A) TIL 19 and (B) TIL 123 (both HLA-A2) were co-cultured with MART-1 pulsed WM39 cells for 24 h with or without 2'3'-cGAMP. IFN- γ (top), CXCL10 (middle) and IFN- β (bottom) levels in co-culture supernatants were measured using ELISA and reported as mean \pm SD and statistical significance was determined by the student's t-test. *, p < 0.05, **, p < 0.01.







С



D



Supplementary Fig. S3. STING-dependent and STING-independent induction of CXCL10 and IFN- β in WM3629 cell line. (A, B) WM3629 cells were stimulated with and without 2'3'cGAMP for 24 h. CXCL10 and IFN- β levels in cell culture supernatants were measured using ELISA. (C, D) WM3629 cells were stimulated with and without polyI:C for 24 h. CXCL10 and IFN- β levels in cell culture supernatants were measured using ELISA. Data represent mean \pm SD of 2-3 biological replicates.



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MART-1 pulsed WM39



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WM39 + w6/32



Supplementary Fig. S4. Stimulation with the STING agonist alone does not result in any major cytotoxic effect on melanoma cells. Lysis percentage of (A) WM39, (B) MART-1 pulsed WM39 and (C) WM39+w6/32 target cells in the ⁵¹Cr release cytotoxicity assay incubated with or without 2'3'-cGAMP in the absence of TIL. Data represent the mean \pm SD of 3-4 biological replicates. Statistical significance was determined by the student's t-test. *, p < 0.05.



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Supplementary Fig. S5. Blockade of IFNAR inhibits agonist-induced upregulation of MHC

class I (HLA-A.B.C.). (A) Representative histograms of HLA-A.B.C expression on WM39 cells stimulated with 2'3'-cGAMP in the presence or absence of α -IFNAR. (B) Mean fluorescence intensity (MFI) of HLA-A.B.C on indicated cells. Data are mean \pm SD of three biological replicates. Statistical significance was determined by unpaired t-test (***, p < 0.001).



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Supplementary Fig. S6. Knockdown of STING in melanoma cells does affect induction of CXCL10 and IFN- β in response to stimulation with polyI:C. WM39, sh-control and sh-STING cells were stimulated with polyI:C for 24 h. (A) CXCL10 and (B) IFN- β levels in cell culture supernatants were measured using ELISA. Data are presented as mean \pm SD of three biological replicates. P-values were calculated by one-way ANOVA (**P < 0.01, ***P < 0.001, ****P < 0.0001).