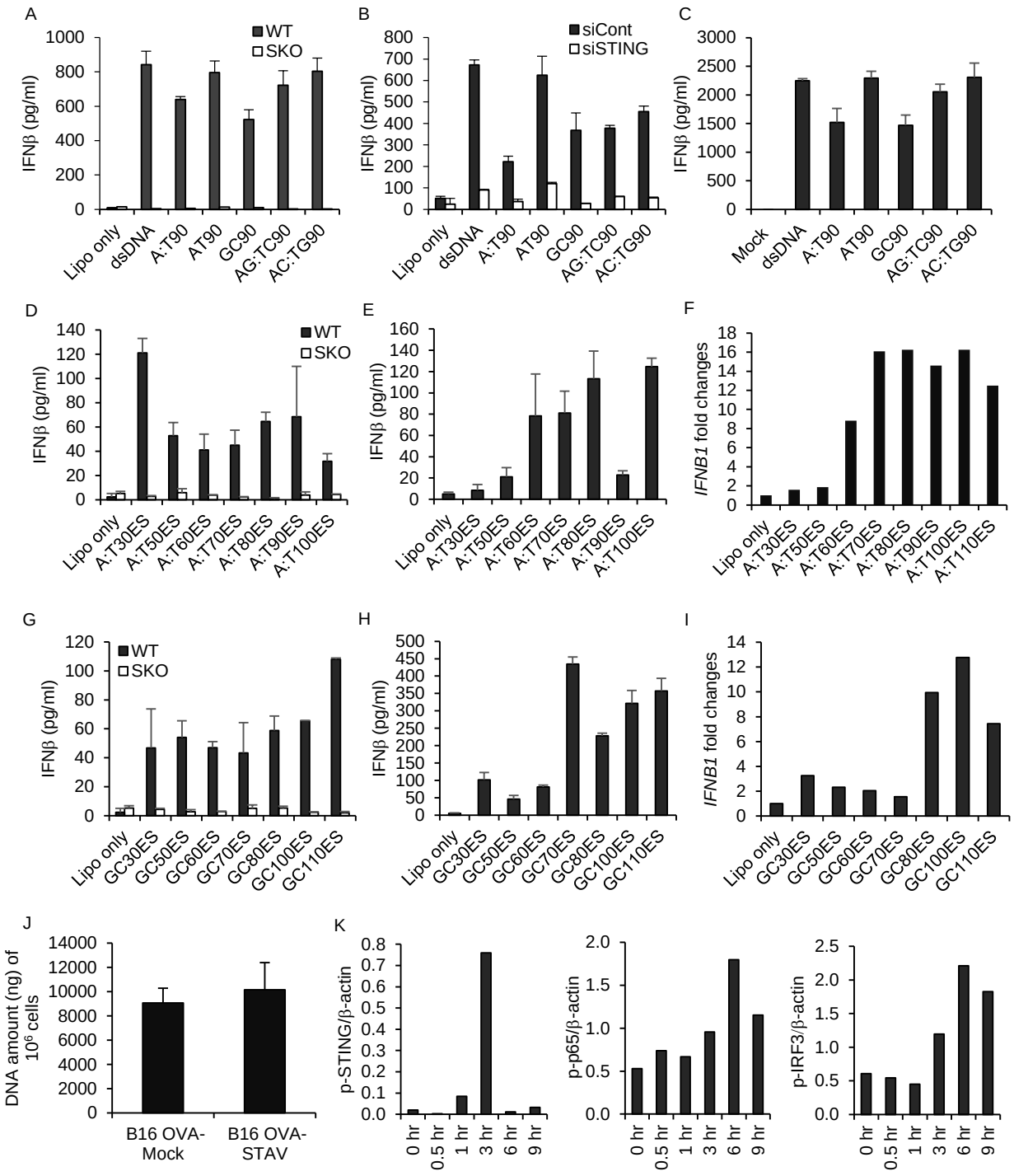

Figure S1, related to Figure 1. Various STING ligands with different sequences and length (related to Figure 1). (A-C) IFNβ ELISA assay in MEFs (A), hTERT (B) or human Macrophages (C) transfected with various STING ligands of different sequences. (D, E) IFNβ ELISA assay in MEFs (D), hTERT (E) transfected with different length of AT rich-STING ligands. (F) qRT-PCR analysis of *IFNB1* in human macrophages transfected with different length of AT rich-STING ligands. (G, H) IFNβ ELISA assay in MEFs (G), hTERT (H) transfected with different length of GC rich-STING ligands. (I) qRT-PCR analysis of *IFNB1* in human macrophages transfected with different length of GC rich-STING ligands. (J) Evaluation of total DNA amount in B16 OVA cells transfected with 3 μg of STAVs. (K) Quantitative densitometry of the protein expression of Western Blot in Figure 1D. Error bars indicate mean ± SD.

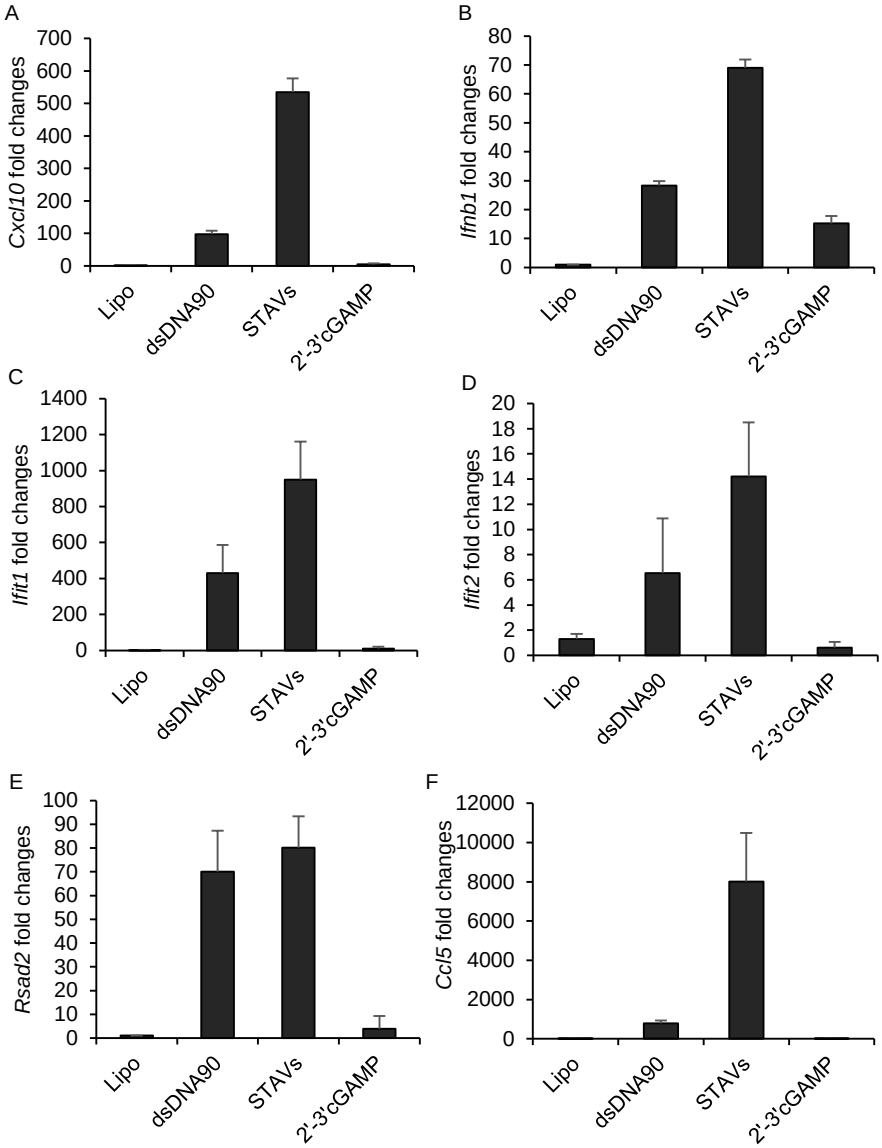

Figure S2, related to Figure 1. qPCR analysis of gene expression in B16-OVA cells induced by various DNA stimuli. (A-F) B16-OVA cells were transfected with 3 μg/ml dsDNA90, STAVs or 2'-3'cGAMP for 3 hr. Total RNA was extracted and analyzed by qRT-PCR for stimulation of a panel of innate immune responsive genes. qRT-PCR of *Cxcl10* (A), *Ifnb1* (B), *Ifit1* (C), *Ifit2* (D), *Rsad2* (E), *Ccl5* (F). Error bars indicate mean ± SD.

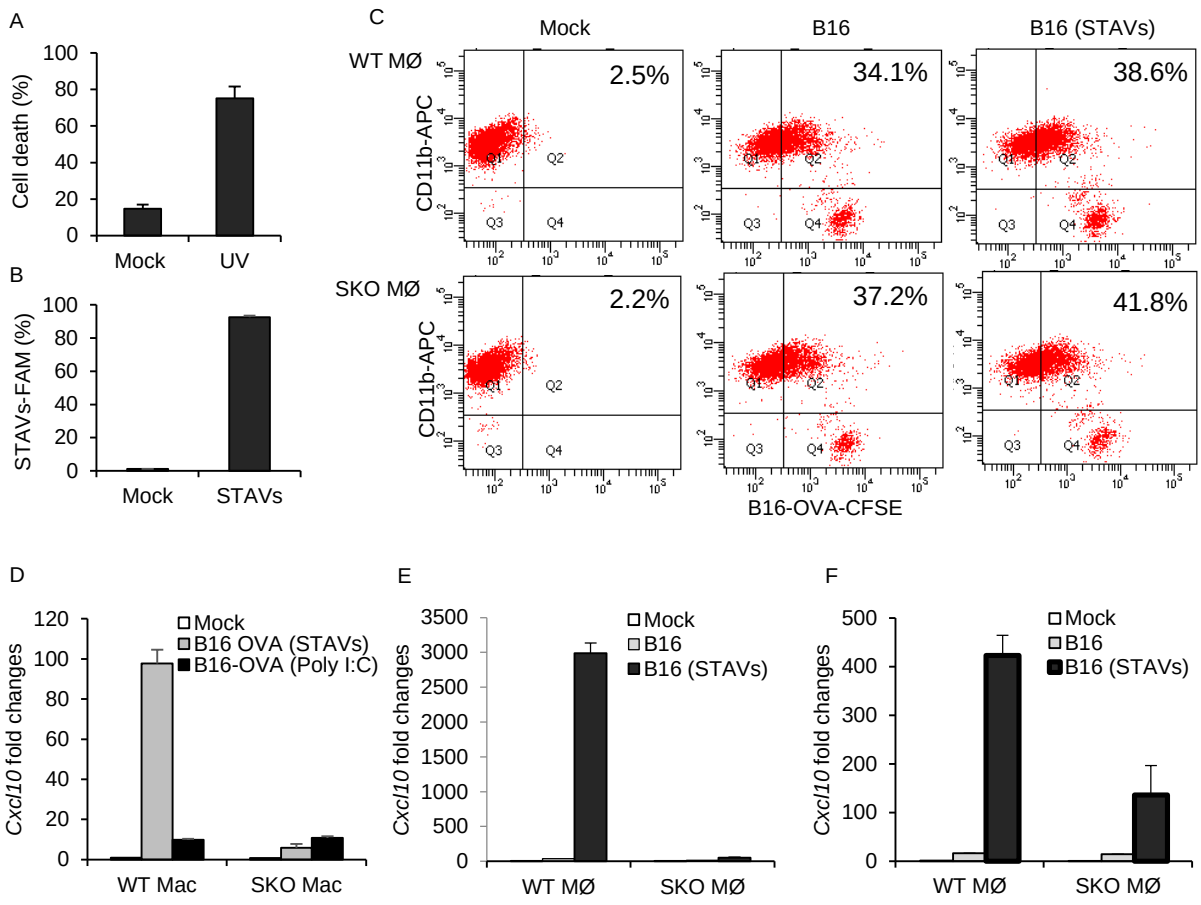

Figure S3, related to Figure 1. Activation of macrophages and dendritic cells following phagocytosis of B16 cells. (A) Percentage of cell death of UV treated B16 cells stained with propidium iodide and annexin V staining by flow cytometry. (B) Flow cytometry analysis for residual FITC-labelled STAVs in B16 cells at 24 hr after UV irradiation. (C) Percentage of phagocytosis by WT or SKO macrophages (CD11b-APC) following cellular engulfment of CFSE labelled B16 cells (FITC) with or without STAVs. (D) qRT-PCR analysis of *Cxcl10* in WT and SKO MØ following engulfment of UV-irradiated B16 cells containing STAVs or Poly I:C. (E, F) qRT-PCR analysis of *Cxcl10* in WT and SKO MØ following engulfment of Cisplatin (50 μM) (E) or hydrogen peroxide (H_2O_2, 10 mM) (F) treated B16 cells containing STAVs. Error bars indicate mean ± SD.

Table S1, related to Figure 1. Fold changes of gene array analysis in B16 cells transfected with STAVs.

Gene_Symbol	STAVs vs Mock
Ccl5	80.0
Ifit1	66.1
Ifih1	49.9
Cxcl10	34.5
Mx2	21.3
Gbp7	20.3
Rsad2	19.0
Isg15	17.9
Gbp3	14.1
Gm20559	13.4
Parp14	12.4
Ddx58	11.2
1700007K13Rik	9.1
Trp53inp1	8.7
Klhl38	8.0
Oasl1	7.7
Tmem140	7.6
Gbp2	7.1
Mx1	6.8
Usp18	6.8
Parp9	6.3
NA	6.3
Oasl2	6.1
Btg2	5.9
Plk2	5.8
Apol9b	5.7
Cmpk2	5.6
Irgm1	5.5
Csf1	5.0
9230114K14Rik	4.9
Ddx60	4.7
NA	4.7
Tapbp	4.6
Irgm2	4.4
Samd9l	4.4
Herc6	4.4
NA	4.4
Apobec1	4.3
Sesn2	4.2
Ifitm3	4.1
Tnfrsf10b	4.1
Stat2	4.0
Rhbdf2	3.9
Nfkbie	3.9
Trim21	3.8
NA	3.7
Znfx1	3.7
Xaf1	3.6

Gene expression fold changes of illumina array shown in Figure 1B. Fold change analysis was performed between two groups and differentially expressed genes were selected based on threshold of Fold change ≥ 3 from a comparison between untransfected and STAVs transfected B16 cells.

Table S2, related to Figure 2. Fold changes of gene array analysis in WT or SKO macrophages following phagocytosis of UV-irradiated B16 cells.

Gene Symbol	WT MØ (293)	WT MØ (293-STAVs)	SKO MØ (293)	SKO MØ (293-STAVs)
Gm14446	2.7	86.4	1.1	1.0
Cmpk2	5.2	80.0	1.7	1.5
Cxcl10	3.1	64.1	1.4	1.4
NA	4.4	56.3	16.3	10.7
Gm4951	3.1	51.8	1.0	1.1
NA	1.1	51.1	34.6	32.5
Ccl12	2.2	45.4	2.6	1.6
Rsad2	4.2	44.8	1.3	1.2
Ifi205	3.9	43.7	1.1	1.0
Cd69	1.8	42.0	1.1	1.2
Ifit1	3.5	40.2	1.2	0.9
Iigp1	1.4	35.4	0.5	0.6
Igtp	2.8	33.6	1.8	1.5
Usp18	3.4	31.4	1.5	1.0
Pyhin1	0.9	31.2	0.4	0.3
Mx1	1.7	29.3	0.6	0.6
Mx2	2.1	27.7	0.7	0.7
Gbp5	1.3	27.2	0.9	0.7
NA	1.7	24.6	1.1	0.9
Pydc3	4.0	24.2	1.0	0.7
Oasl1	1.9	24.0	1.2	1.2
Tnfsf10	1.5	23.2	0.9	0.8
Serpinb2	23.0	23.2	19.5	19.0
Trim30c	1.1	20.5	0.9	0.7
NA	3.6	20.1	2.7	2.4
Gbp3	1.6	19.4	1.1	1.2
NA	10.1	19.1	9.4	9.6
Pydc4	0.8	18.9	0.3	0.3
NA	9.5	18.9	2.7	6.0
Ifit2	1.7	18.2	1.2	1.0
NA	14.5	18.1	6.1	6.1
NA	14.5	18.1	6.1	6.1
Gm12250	0.9	17.4	0.5	0.5
Irf7	1.4	16.9	1.0	0.8
NA	1.4	16.3	0.9	0.9
NA	1.1	16.2	0.9	0.6
NA	5.6	15.9	3.4	1.6
Isg15	1.9	15.7	1.0	0.7
Phf11b	1.1	15.7	0.9	0.9
Ccl2	5.6	14.9	5.4	5.9
NA	4.7	14.6	14.0	20.7
Ddx60	0.9	14.5	1.1	0.9
Zbp1	1.7	13.8	1.1	1.1
NA	1.1	13.5	0.5	0.5
Cd274	3.1	13.1	2.5	3.6

Gene expression fold changes of illumina array shown in Figure 2B. Fold change analysis was performed between two groups and differentially expressed genes were selected based on threshold of Fold change ≥ 13 from a comparison between untreated WT macrophages (MØ) and WT MØ and SKO MØ following engulfment of irradiated 293 cells with/without STAVs.

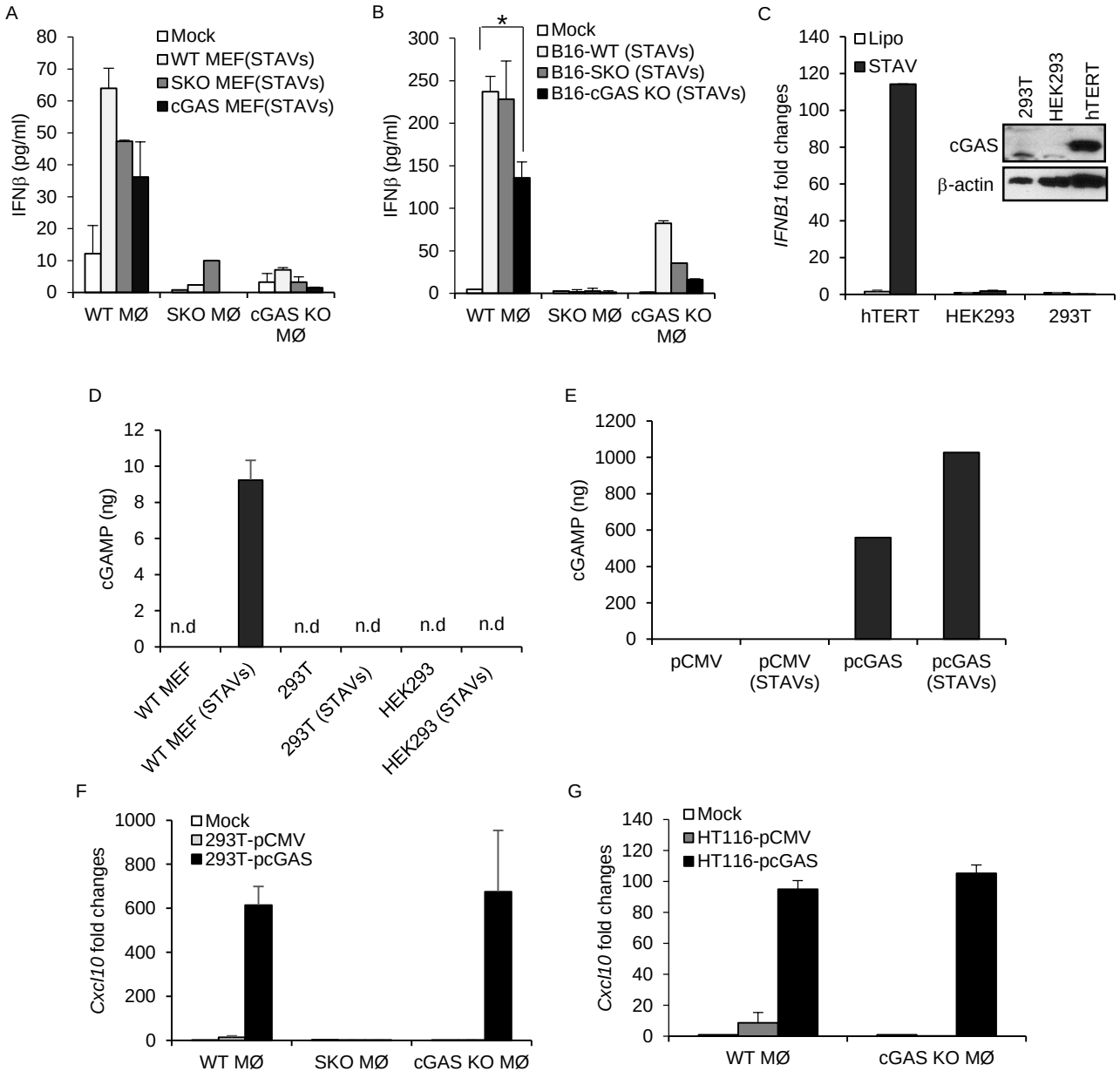

Figure S4, related to Figure 3 and 4. Macrophage activation following phagocytosis of STING or cGAS deficient cells. (A) ELISA analysis of IFNβ in WT, SKO and cGASKO macrophages following engulfment of UV irradiated WT, SKO and cGAS KO MEFs with STAVs. (B) ELISA analysis of IFNβ in WT, SKO and cGASKO macrophages following engulfment of UV irradiated B16 OVA (B16-WT), B16 OVA-SKO (B16-SKO) and B16 OVA-cGAS KO (B16-cGAS KO) cells with STAVs. B16 OVA-SKO and B16 OVA-cGAS KO cells were generated by CRISPR technique. (C) qRT-PCR analysis of *IFNB1* and western blot analysis of cGAS expression in human cell lines such as hTERT, HEK293, and 293T cells transfected with STAVs. Data is representative of at lease three independent experiments. Error bars indicate s.d. *; $p<0.05$, Student's t-test. (D) Measurement of cGAMP levels by a hybrid mass spectrometer in 293T cells. The 293T cells were reconstituted with pcGAS or pCMV as control vector. n.d.: not detected. (E) Measurement of cGAMP levels by a hybrid mass spectrometer in 293T with/without STAVs. (F) qRT-PCR analysis of *Cxcl10* in WT, SKO, and cGAS KO macrophages following engulfment of UV-irradiated 293T cells reconstituted with/without pcGAS vector. (G) qRT-PCR analysis of *Cxcl10* in WT, SKO, and cGAS KO macrophages following engulfment of UV-irradiated HT116 cells reconstituted with/without pcGAS vector. Error bars indicate mean ± SD.

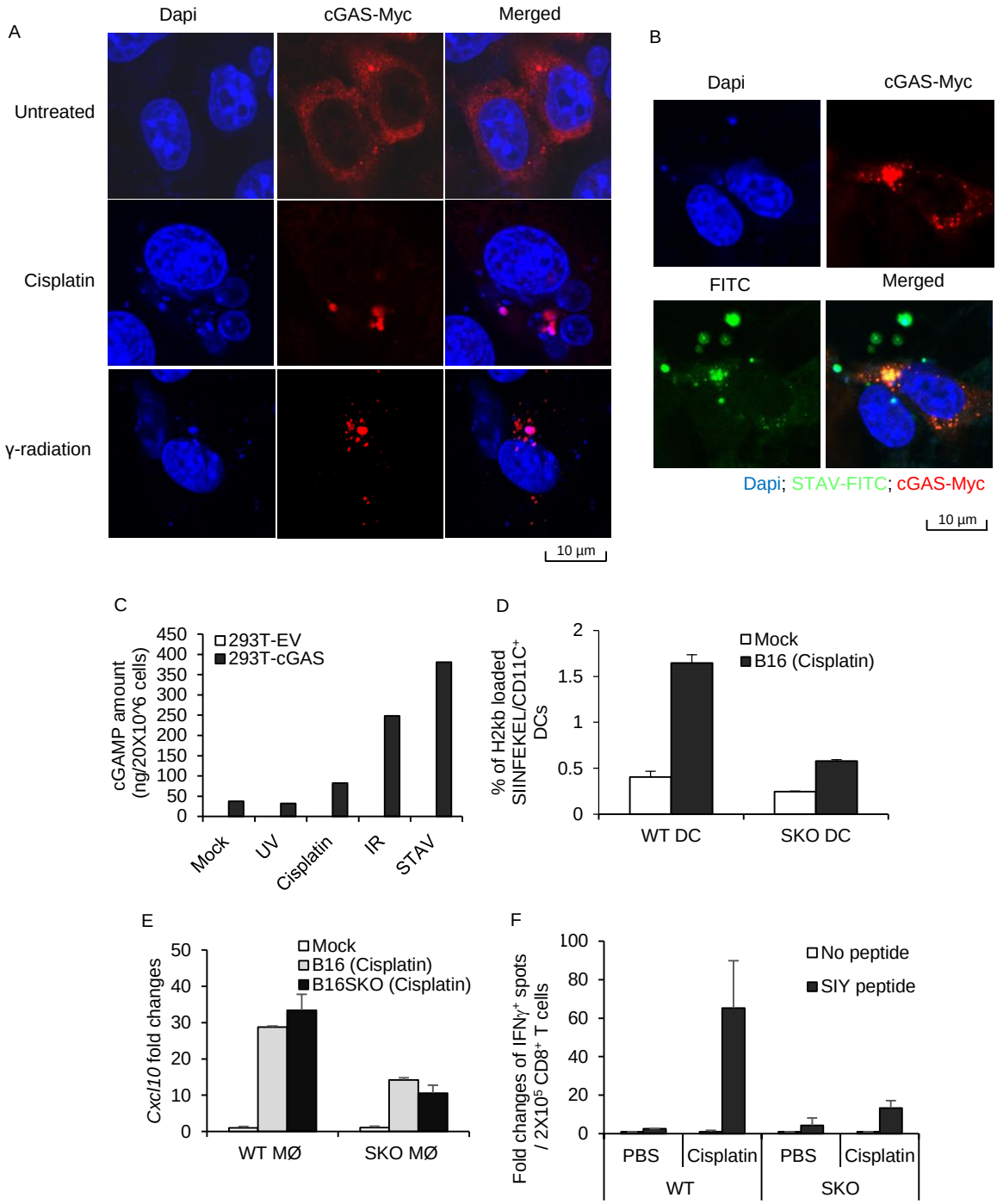

Figure S5, related to Figure 4. cGAS activation by cytosolic DNA. (A) Fluorescent Microscopy analysis of B16-OVA cells treated with Cisplatin (10 μM) for 48 hr or γ-radiation (10 Gy) for 6 days. B16 cells were transfected with cGAS-Myc 24 hr prior to staining and stained with anti-c-Myc antibody (red) and DAPI for nuclear counter staining (blue). Merged images show colocalization of cytosolic micronulear DNA and cGAS (purple); bar size, 10 μm. (B) Fluorescent Microscopy analysis of B16-OVA cells co-transfected with cGAS-Myc (red) and STAV-FITC (green). DAPI is used for nuclear counter staining (blue). Merged images show colocalization of STAV DNA and cGAS (orange); bar size, 10 μm. (C) Mass-Spectrometry analysis of cGAMP production in 293T stable cell lines expressing cGAS treated with UV (120 mJ/cm), Cisplatin (50 μM), IR (100 Gy), and STAVs (3 μg/ml) for 24 hr. 293T-EV: control vector, 293T-cGAS: cGAS expressing retroviral vector. (D) H2kb loaded SIINFEKL on CD8α$^+$CD11C$^+$ dendritic cells following phagocytosis of cisplatin-treated B16 cells. (E) qRT-PCR analysis of *Cxcl10* in WT and SKO macrophages following phagocytosis of cisplatin treated B16 cells. (F) IFNγ ELISPOT assay in CD8$^+$ T cells from WT, SKO and cGASKO mice treated with cisplatin. The mice were subcutaneously injected with B16-SIY cells on the flank. 50 μg of cisplatin was injected intraperitoneally once a week three times. CD8$^+$ T cell priming was evaluated by IFNγ ELISPOT. Error bars indicate mean ± SD.

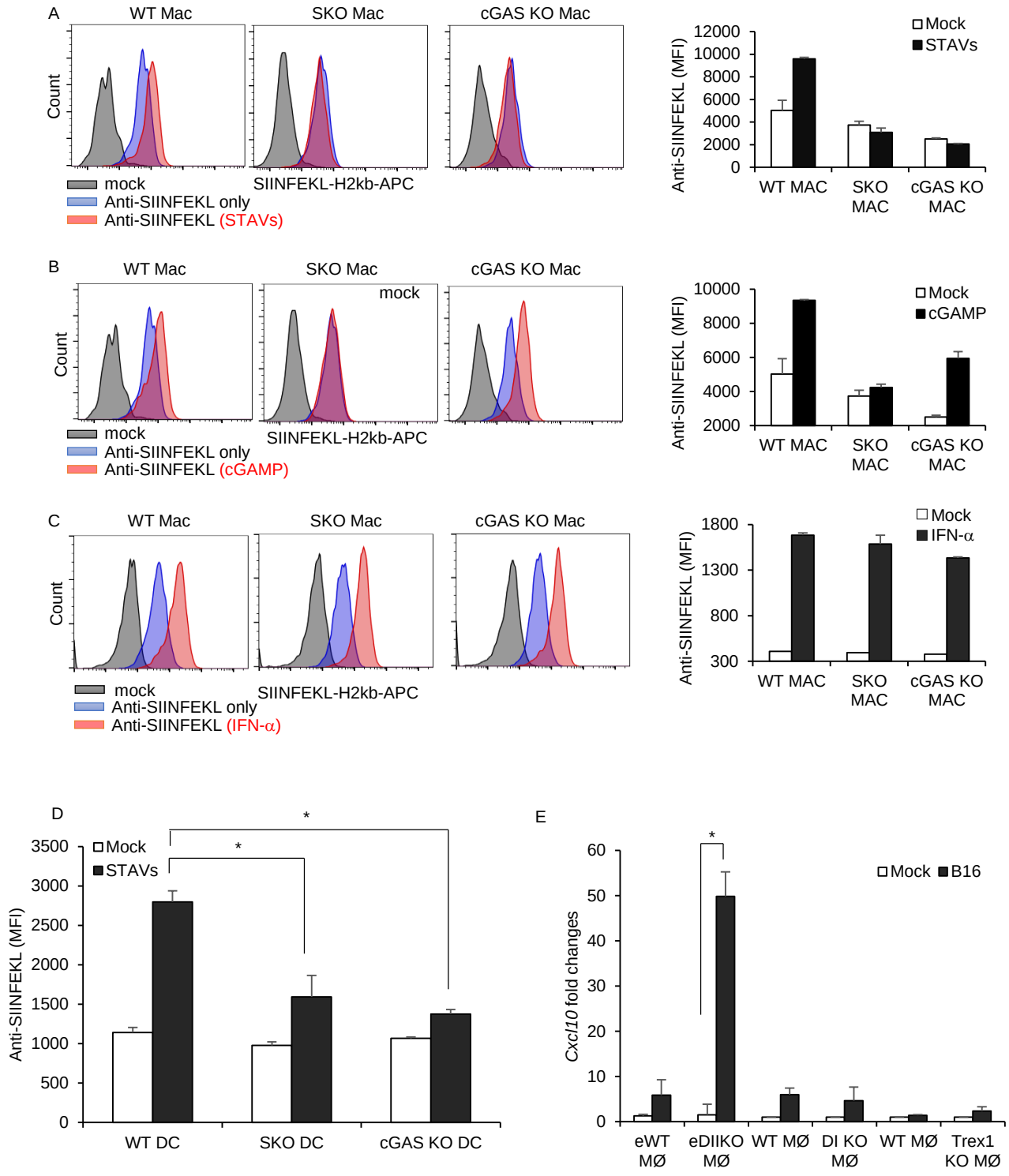

Figure S6, related to Figure 6. Activation of antigen presenting cells by STAVs. (A-C) WT, SKO, cGAS KO macrophages were transfected with 3 μg/ml of STAVs (A) or cGAMP (B) and IFN-α (C) treatment for 24 hr followed by pulsing with or without SIINFEKL. Antigen presentation was evaluated by flow cytometry analysis using antibody reacting with OVA peptide SIINFEKL bound to H-2Kb of MHC class I. (D) H2kb loaded SIINFEKL on $CD8\alpha^{+}CD11C^{+}$ dendritic cells transfected with 3 μg/ml of STAVs for 24 hr using same antibody as (A-C). (E) qRT-PCR analysis of *Cxcl10* in WT, DNase II KO, DNase I KO, Trex1 KO macrophages following phagocytosis of irradiated B16-OVA. DI KO; DNase I KO, eWT; WT embryo, eDII KO; DNase II KO embryo. Error bars indicate mean ± SD. *; $p<0.05$, Student's t-test.

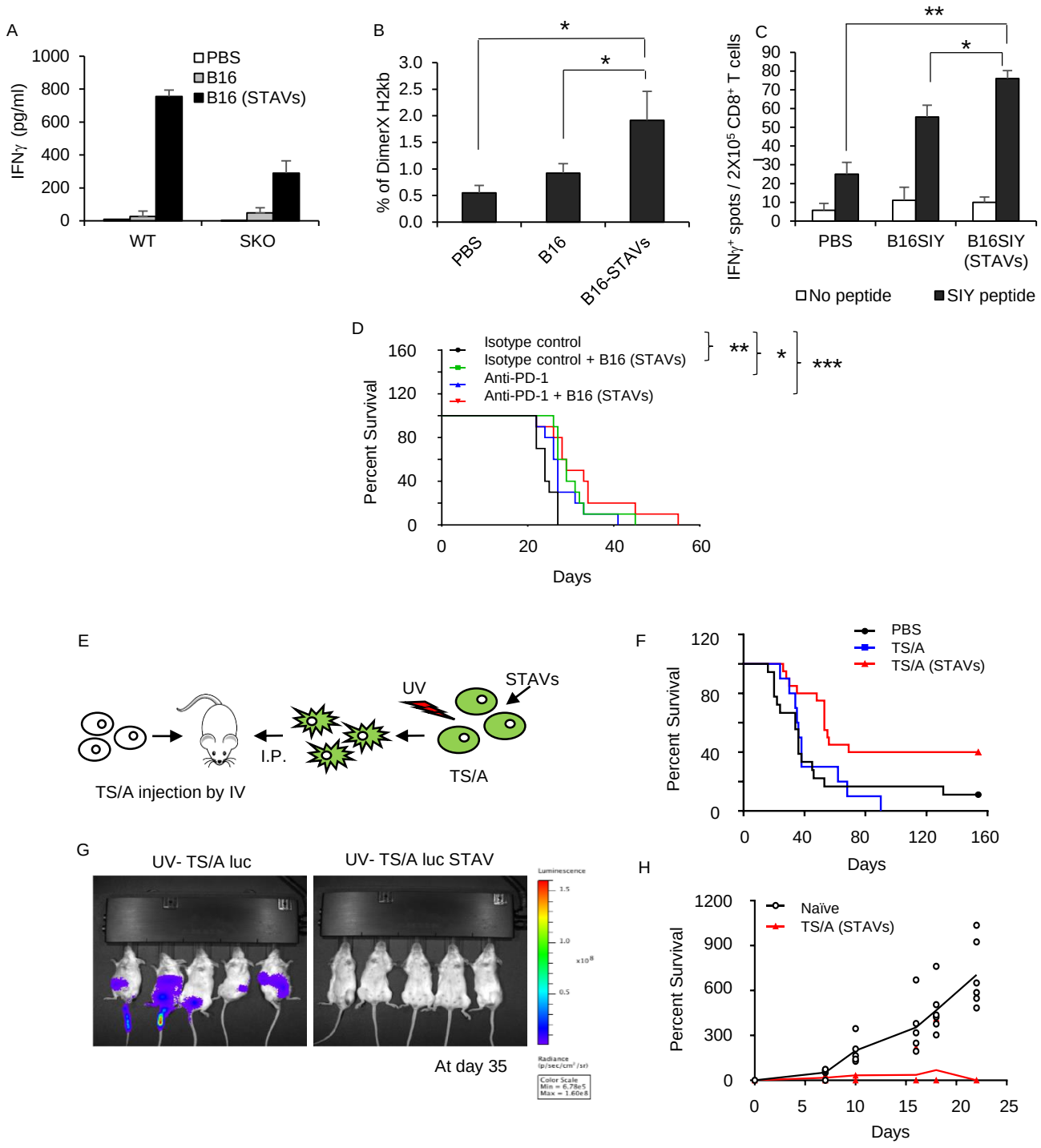
A
IFNγ (pg/ml)
1000
800
600
400
200
0
PBS
B16
B16 (STAVs)
WT
SKO
B
% of DimerX H2kb
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
PBS
B16
B16-STAVs
C
IFNγ+ spots / 2X10^5 CD8+ T cells
90
80
70
60
50
40
30
20
10
0
**
*
PBS
B16SIY
B16SIY (STAVs)
No peptide
SIY peptide
D
Percent Survival
160
120
80
40
0
0
20
40
60
Days
Isotype control
Isotype control + B16 (STAVs)
Anti-PD-1
Anti-PD-1 + B16 (STAVs)
**
*
***
E
UV
STAVs
I.P.
TS/A
TS/A injection by IV
F
Percent Survival
120
80
40
0
0
40
80
120
160
Days
PBS
TS/A
TS/A (STAVs)
G
UV- TS/A luc
UV- TS/A luc STAV
At day 35
Luminescence
1.5
1.0
0.5
H
Percent Survival
1200
900
600
300
0
0
5
10
15
20
25
Days
Naïve
TS/A (STAVs)

Figure S7, related to Figure 7. Protection of tumor metastasis by dead cell immunization and checkpoint inhibitor and CD8+ T cell priming. (A) IFNγ measurement in splenocytes from WT and SKO at 7 days after the second immunization. B16 OVA cells were transfected by STAVs for 3 hr and irradiated by UV (120 mJ/cm). After 24 hr, WT and SKO were i.p. injected with the irradiated B16 cells with/without STAV, twice every week. (B) Frequency of SIY specific $CD8^+$ T cells in the splenocytes isolated from WT mice immunized with UV irradiated-B16 SIY cells containing STAVs. (C) IFNγ ELISPOT analysis in $CD8^+$ T cells isolated from same mice as (B). (D) C57/BL6 WT mice were I.V. injected with B16 OVA cells ($5X10^4$ cells/mouse). On day 1, 3, 7, and 14, the mice were I.P injected by UV irradiated B16 OVA cells ($1X10^6$ cells/mouse) with STAVs. On same days, mice were injected with 100 μg either Isotype control or anti-PD-1. Survival rates control (n=10), B16 (STAVs) (n=10), anti-PD-1 (n=10), and combination with anti-PD-1 and B16 (STAVs) (n=10). Armenian Hamster IgG was used as control. p=0.0002. p values are based on Logrank test, with $p<0.05$ considered statistically significant. *: $p<0.05$, **: $p<0.005$, ***: $p<0.0005$. (E) Schematic representation of post-vaccination for TS/A-luc cells mediated lung metastasis. Balb/C mice were I.V. injected with TS/A-luc cells ($1X10^5$ cells/mouse). On day 1, 3, and 14, the mice were I.P injected by UV irradiated TS/A-luc cells ($4X10^6$ cells/mouse) with or without STAVs. (F) Survival rates of PBS, TS/A-luc, and TS/A-luc (STAVs). TS/A-luc: post-vaccinated group with UV irradiated TS/A-luc cells, TS/A-luc (STAVs): UV irradiated TS/A-luc cells with STAVs (TS/A vs TS/A (STAVs): p=0.008, n=20/group). p values are based on Logrank test, with $p<0.05$ considered statistically significant. (G) TS/A-luc or TS/A-luc (STAVs) mice were anesthetized and injected with a luciferin substrate i.p., and the luciferase activity was detected on day 35 using IVIS. (H) The immunized mice with TS/A-luc (STAVs) were re-challenged with TS/A-luc cells $1X10^5$ cells/mouse) on the flank (n=6). Naïve mice were used as controls (n=6). Tumor size was measured at the indicated time points. (Naïve mice vs TS/A (STAVs): p=0.03, Student's t-test). Error bars indicate mean ± SD.

Table S3. related to STAR Methods. Synthetic DNA Sequences.

polyA90	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
polyT90	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
polyAT90	ATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATAT
polyAG90	AGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
polyAC90	ACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACACAC
polyTG90	GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
polyTC90	CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
polyGC90	GCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC
polyA30ES	A*A*A*AAAAAAAAAAAAAAAAAAAAAAAA*A*A*A
polyT30ES	T*T*T*TTTTTTTTTTTTTTTTTTTTTTTT*T*T*T
polyA50ES	A*A*A*AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA*A*A*A
polyT50ES	T*T*T*TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT*T*T*T
polyA60ES	A*A*A*AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA*A*A*A
polyT60ES	T*T*T*TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT*T*T*T
polyA70ES	A*A*A*AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA*A*A*A
polyT70ES	T*T*T*TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT*T*T*T
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polyGC50ES	G*C*G*CGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG*C*G*C
polyGC60ES	G*C*G*CGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG*C*G*C
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dsDNA90-S	TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACATACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACA
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