

An alternatively spliced STING isoform localizes in the cytoplasmic membrane and directly senses extracellular cGAMP

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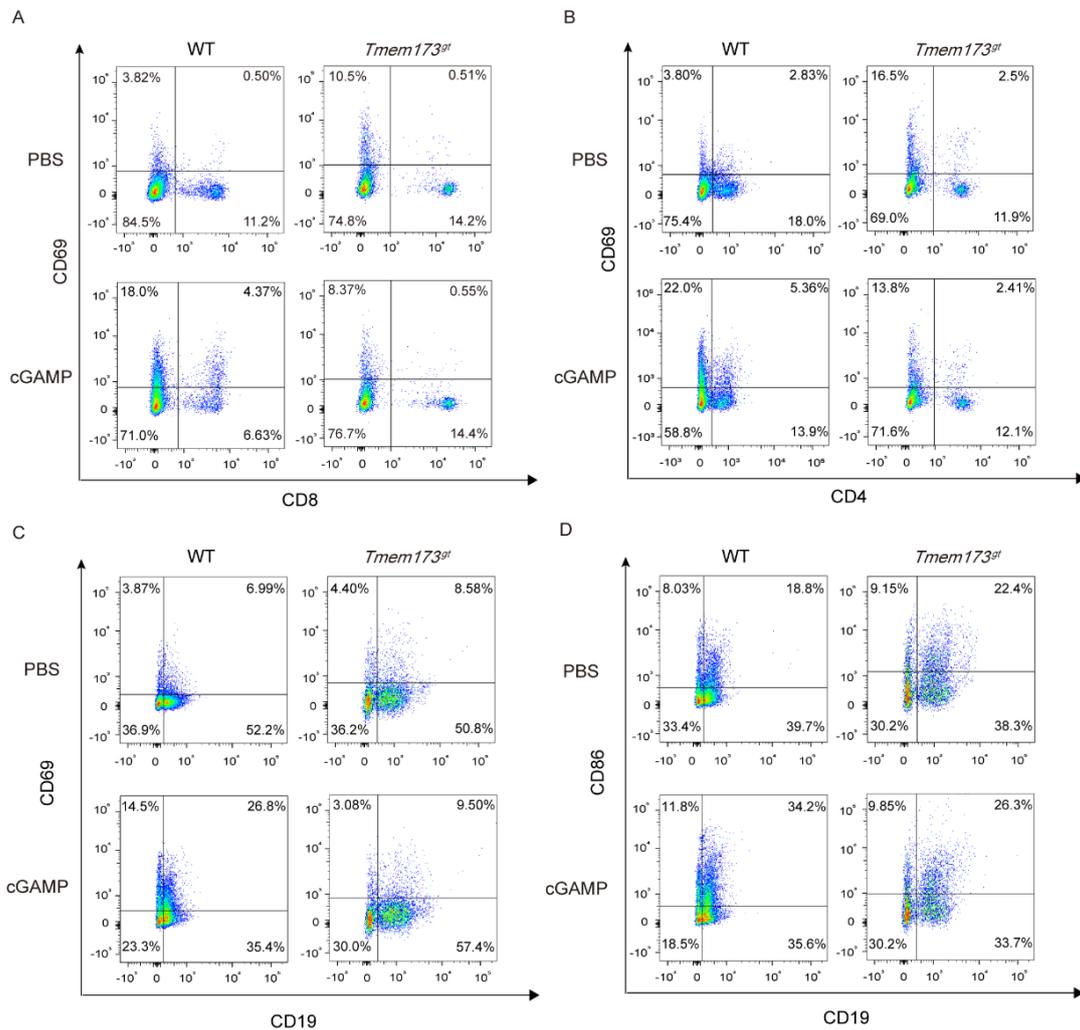
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Supplemental Data:

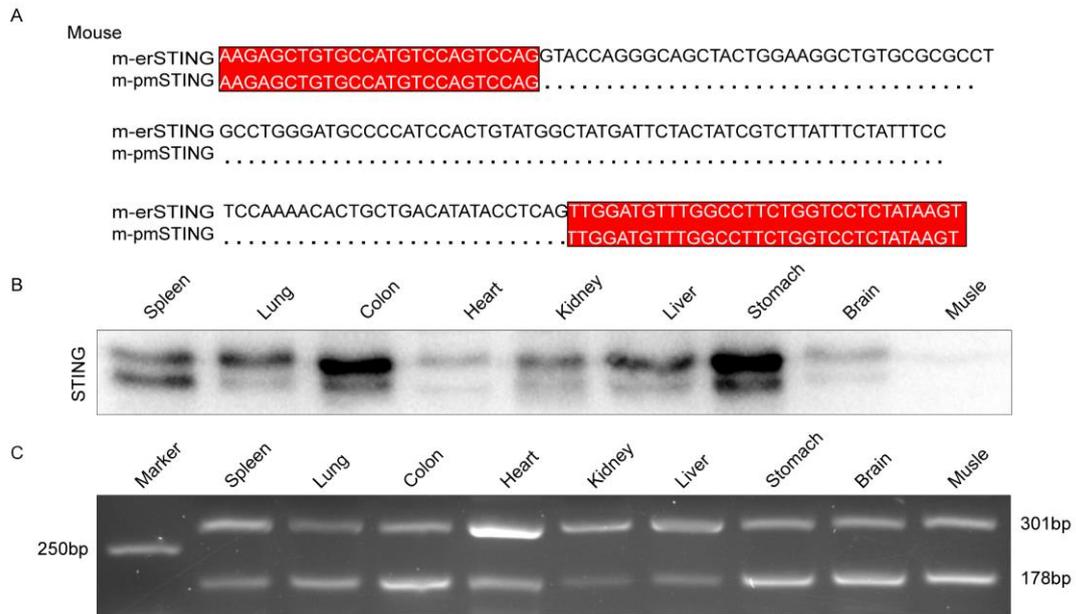
Supplementary table 1. The sequence of primers and siRNAs used in this study.

Primer or siRNA	Sequence (5'→3')
Mouse Tmem173 forward	GCTGTGCCATGTCCAGTC
Mouse Tmem173 reverse	CAACCGCAAGTACCCAAT
Human TMEM173 forward	TCTCCTCGTCATCATCCAG
Human TMEM173 reserve	AGGAGGATG TTCAGTGCC
negative control	UUCUCCGAACGUGUCACGUTT ACGUGACACGUUCGGAGAATT
si-cGAS-mus-660	GGCCGAGACGGUGAAUAAATT UUUAUUCACCGUCUCGGCCTT
si-cGAS-mus-892	GGAAAUCCGCUGAGUCAUUTT AAUGACUCAGCGGAUUUCCTT
si-cGAS-mus-1032	CCCUGCUGUAACACUUCUUTT AAGAAGUGUUACAGCAGGGTT
si-SLC19A1-1	CCAAGGAGCAGGUGACUAATT UUAGUCACCUGCUCCUUGGTT
si-SLC19A1-2	UAAGUGAACUGGUGGAAAATT UUUCCACCAGUUCACUUATT
si-SLC19A1-3	CCAGGAAACUAGAUCGCAUTT AUGCGAUCUAGUUCCUGGTT

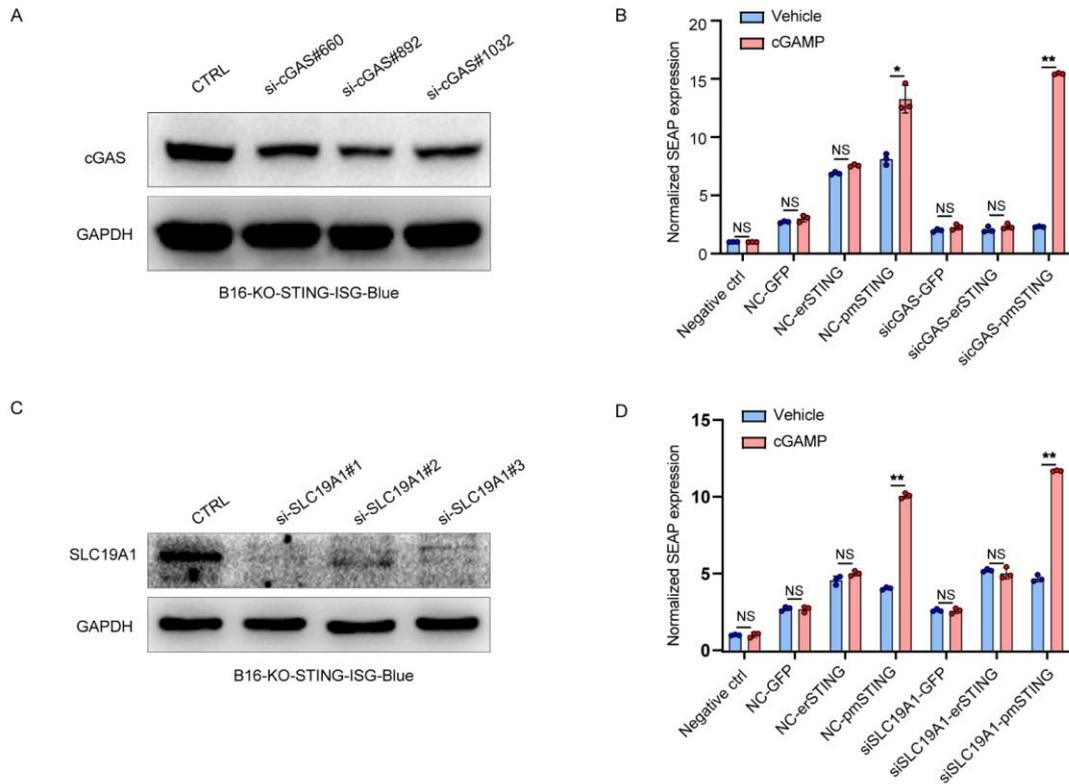
Supplemental Figures



Supplemental Figure 1. Extracellular cGAMP activates T and B lymphocytes. Related to Figure 1. (A) The expression of CD69 was detected by flowcytometry in *WT* and *Tmem173^{gt}* CD4⁺ T cells treated with vehicle or cGAMP. (B) The expression of CD69 was detected by flowcytometry in *WT* and *Tmem173^{gt}* CD8⁺ T cells treated with vehicle or cGAMP. (C) The expression of CD69 was detected by flowcytometry in *WT* and *Tmem173^{gt}* B cells treated with vehicle or cGAMP. (D) The expression of CD86 was detected by flowcytometry in *WT* and *Tmem173^{gt}* B cells treated with vehicle or cGAMP.



Supplemental Figure 2. An alternatively spliced isoform of mouse STING. Related to Figure 3. (A) cDNA sequence of both STING isoforms in mice splenocytes. (B) Both STING isoforms have been detected by Western Blot in different tissues of mice. (C) Both STING isoforms have been validated by PCR in different tissues of mice.



Supplemental Figure 3. Mouse pmSTING directly senses extracellular cGAMP and promotes IFN production. Related to Figure 4. (A) The expression of cGAS in B16^{Tmem173-/-} cells transfected with three different siRNAs targeting cGAS was detected by western blot. (B) B16^{Tmem173-/-} cells stably expressing alkaline phosphatase to monitor interferon activity were transfected with erSTING-FLAG or pmSTING-FLAG after knocking down cGAS. The alkaline phosphatase was detected in these cells after treated with vehicle or cGAMP (n=3). ** represents $p < 0.01$; NS means not significant, by 2-tailed, paired Student's *t* test. (C) The expression of SLC19A1 in B16^{Tmem173-/-} cells transfected with three different siRNAs targeting SLC19A1 was detected by western blot. (D) B16^{Tmem173-/-} cells stably expressing alkaline phosphatase to monitor interferon activity were transfected with erSTING-FLAG or pmSTING-FLAG after knocking down SLC19A1. The alkaline phosphatase was detected in these cells after treated with vehicle or cGAMP (n=3). * represents $p < 0.05$, ** represents $p < 0.01$, NS means not significant, by 2-tailed, paired Student's *t* test.

Human

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h-erSTING CGTAGCGCCGGGCCTTCTCCTCGTCATCATCCAGAGCAGCCAGTGTCCGGGAGGCAGA
h-pmSTING CGTAGCGCCGGGCCTTCTCCTCGTCATCATCCAGAGCAGCCAGTGTCCGGGAGGCAGA

h-erSTING ACATGCCCACTCCAGCCTGCATCCATCCATCCCCTGTCCCAGGGGTACAGGGGCCAGA
h-pmSTING AC.....

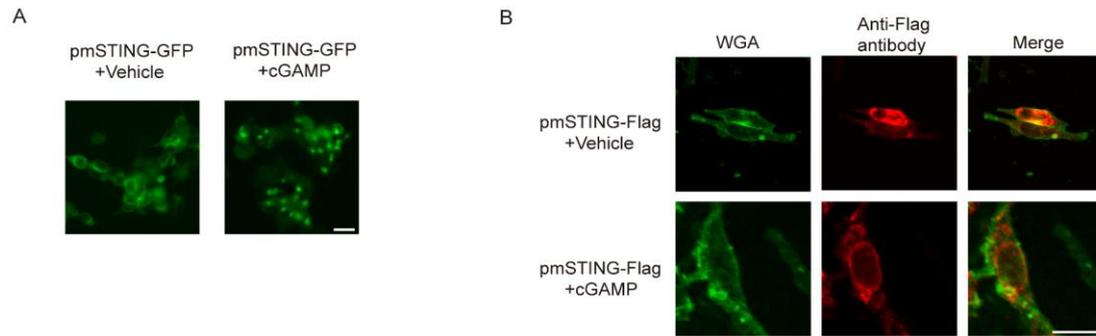
h-erSTING AGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCCTTTGGGGGCTAGGAGAGCCAC
h-pmSTING .....

h-erSTING CAGAGCACACTCTCCGGTACCTGGTGCTCCACCTAGCCTCCCTGCAGCTGGGACTGCTGT
h-pmSTING .....

h-erSTING TAAACGGGGTCTGCAGCCTGGCTGAGGAGCTGCGCCACATCCACTCCAGGTACCGGGGCA
h-pmSTING .....STACCGGGGCA

h-erSTING GCTACTGGAGGACTGTGCGGGCCTGCCTGGGCTGCCCTCCGCCGTGGGGCCCTGTTGC
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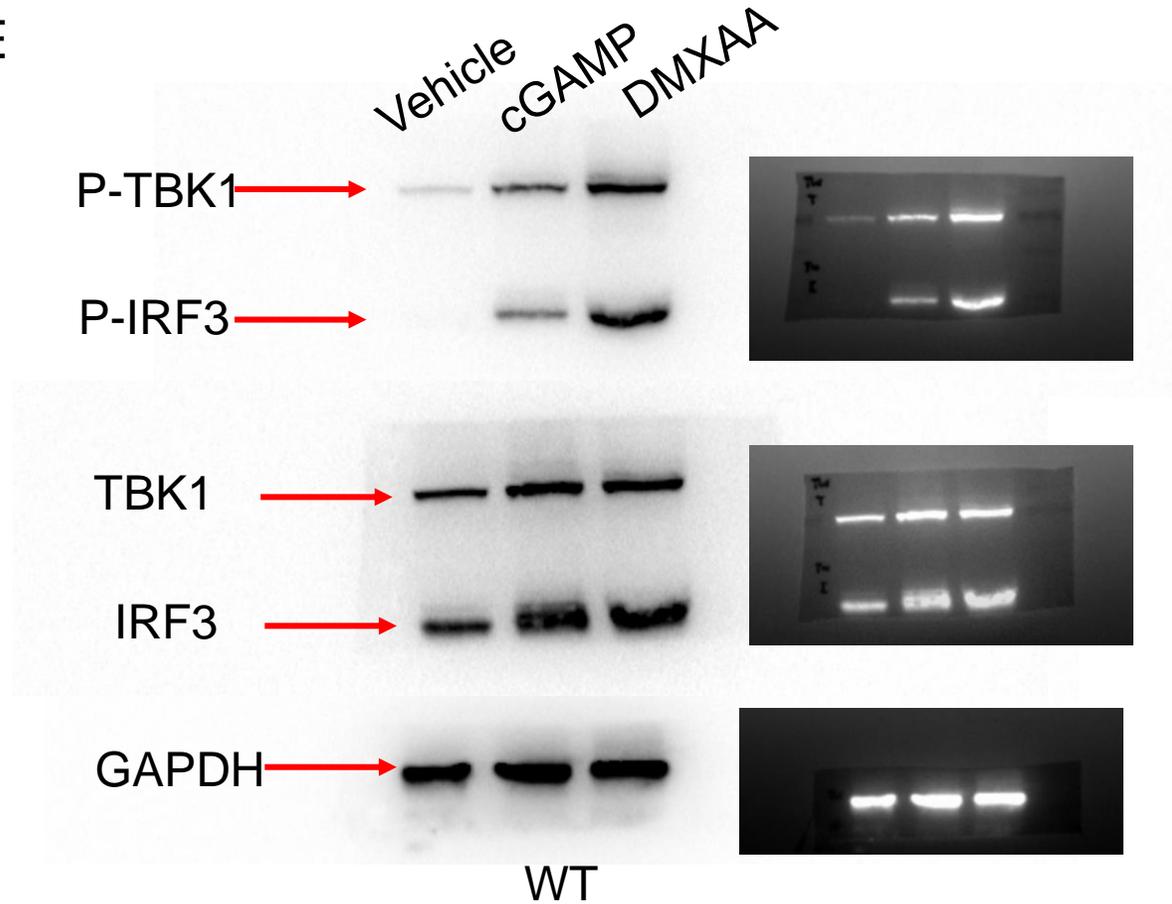
Supplemental Figure 4. The cDNA sequence of both human STING isoforms in PBMCs. Related to figure 6.



Supplemental Figure 5. Mouse pmSTING translocates from plasma membrane into the cytoplasm upon stimulating by the extracellular cGAMP. (A) The distribution of pmSTING-EGFP in 293T cells with or without extracellular cGAMP treatment using confocal microscopy. Scale bars: 50 μm . (B) B16^{Tmem173^{-/-}} cells were transfected with pmSTING-FLAG and treated with or without cGAMP. An antibody against FLAG was used to detect the distribution of pmSTING-FLAG in B16^{Tmem173^{-/-}} cells using confocal microscopy. Scale bars: 20 μm .

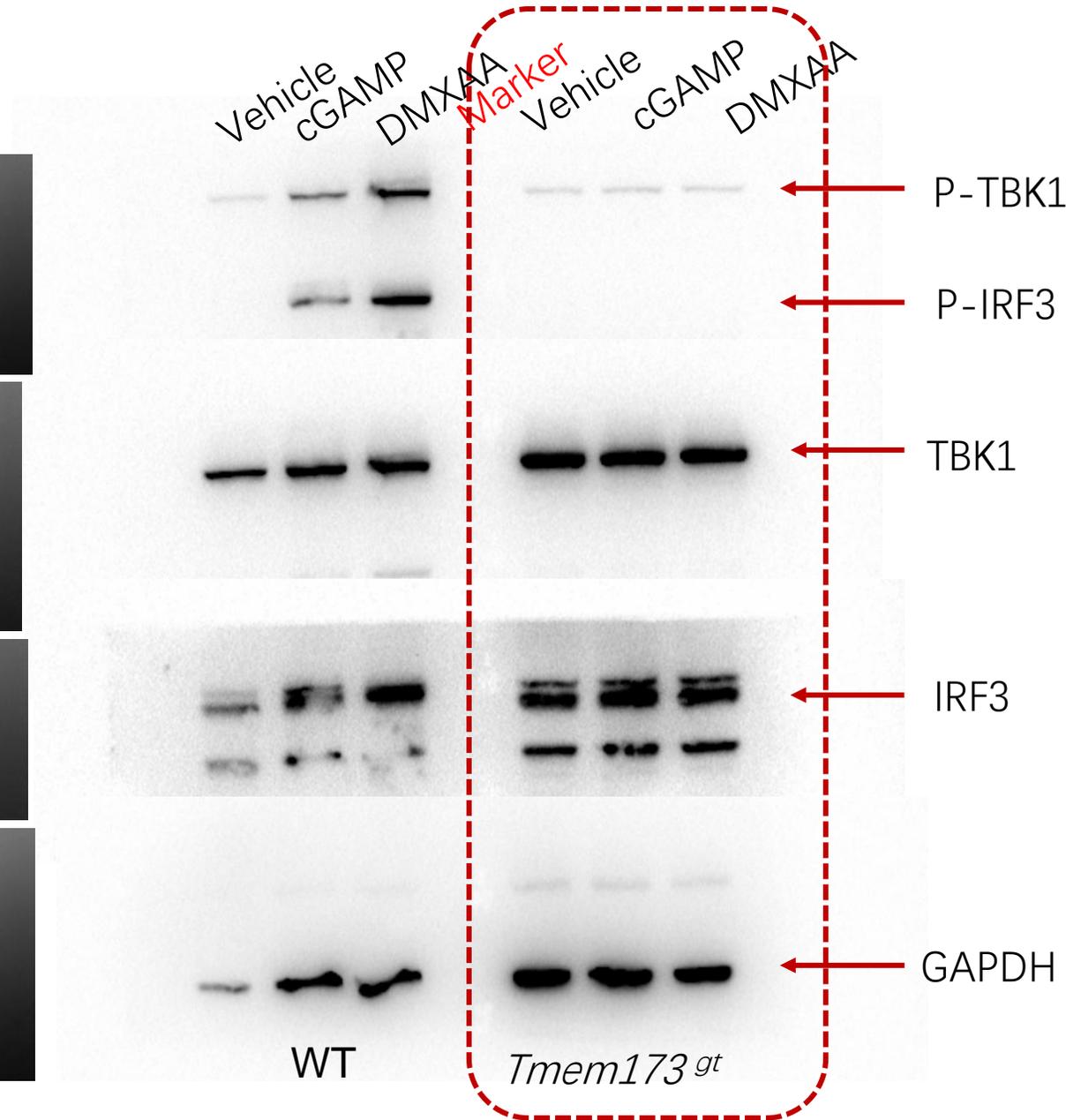
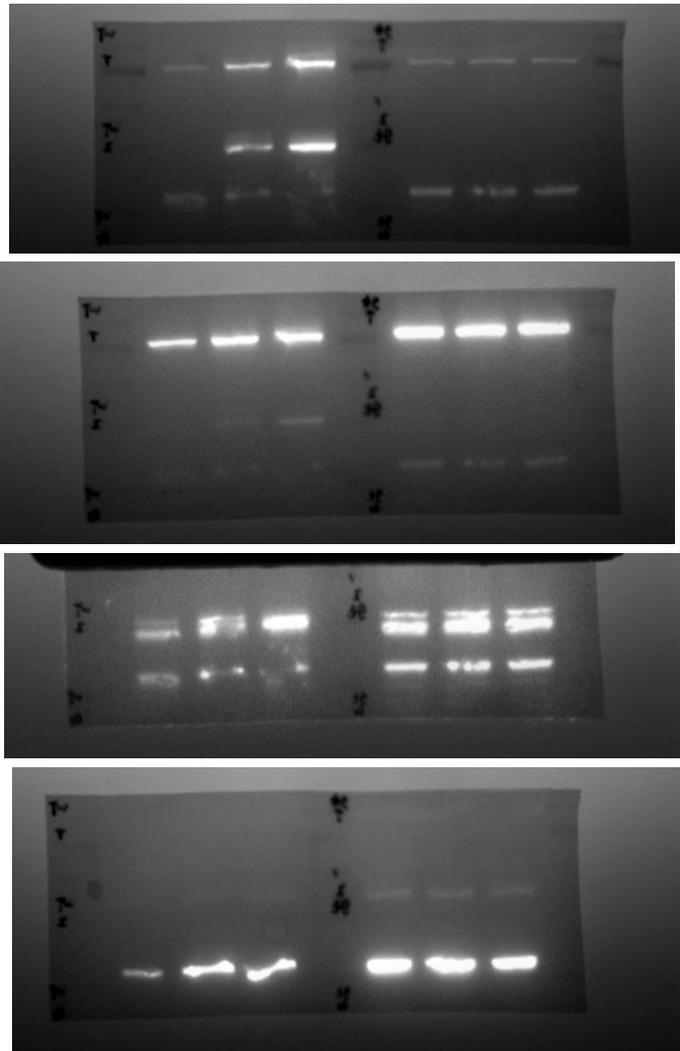
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Figure 1E



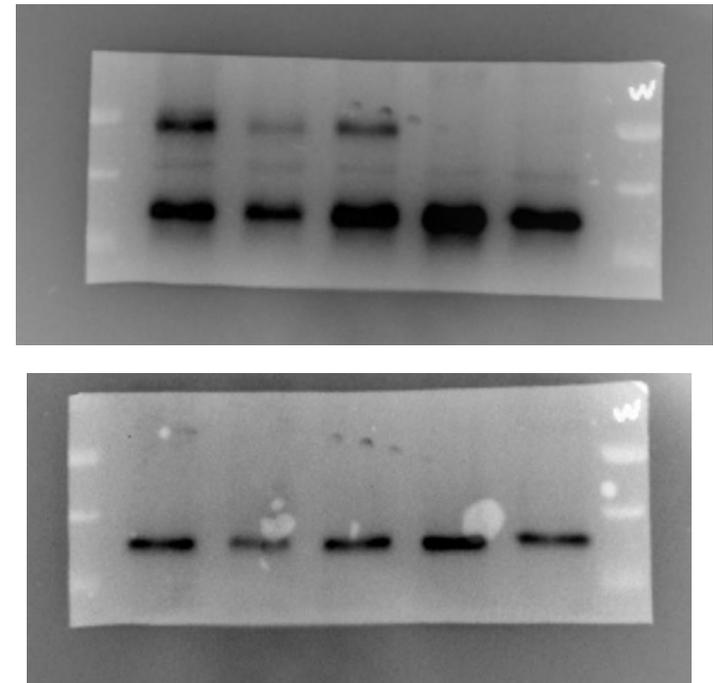
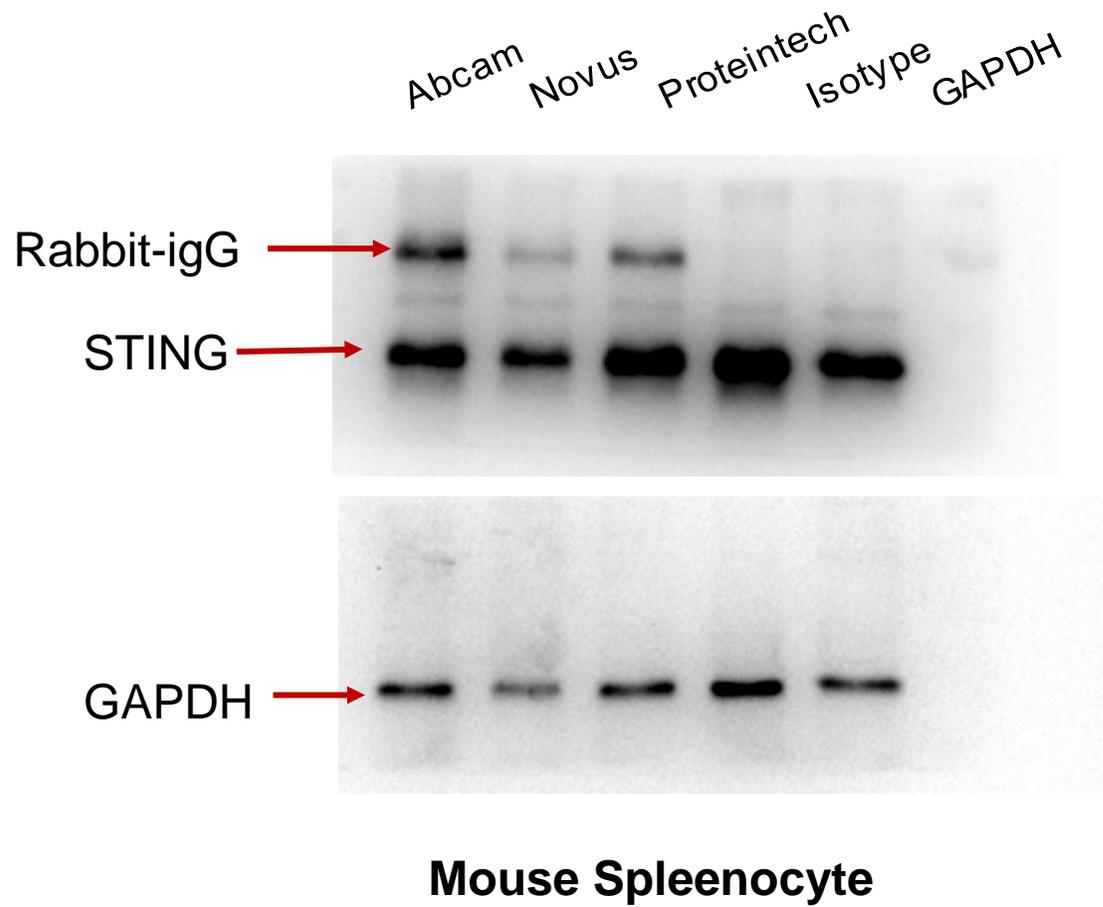
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Figure 1E



Full unedited gel for Figure 2

Figure 2B



Full unedited gel for Figure 3

Figure 3B

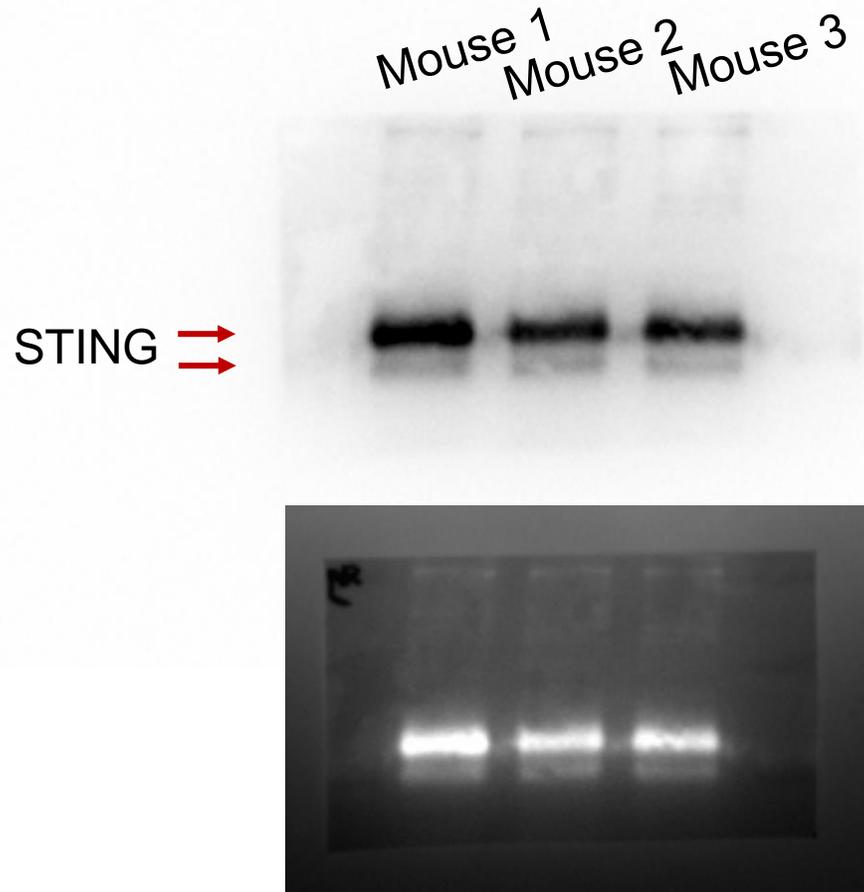
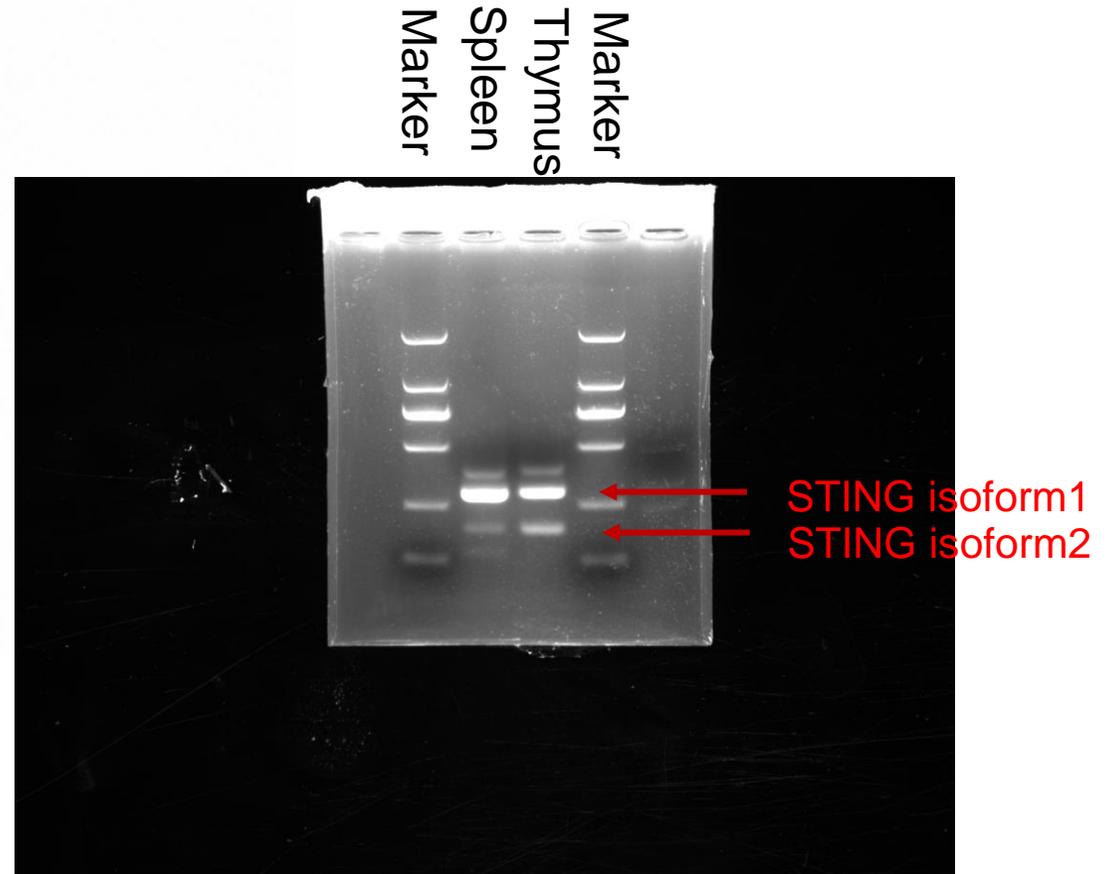
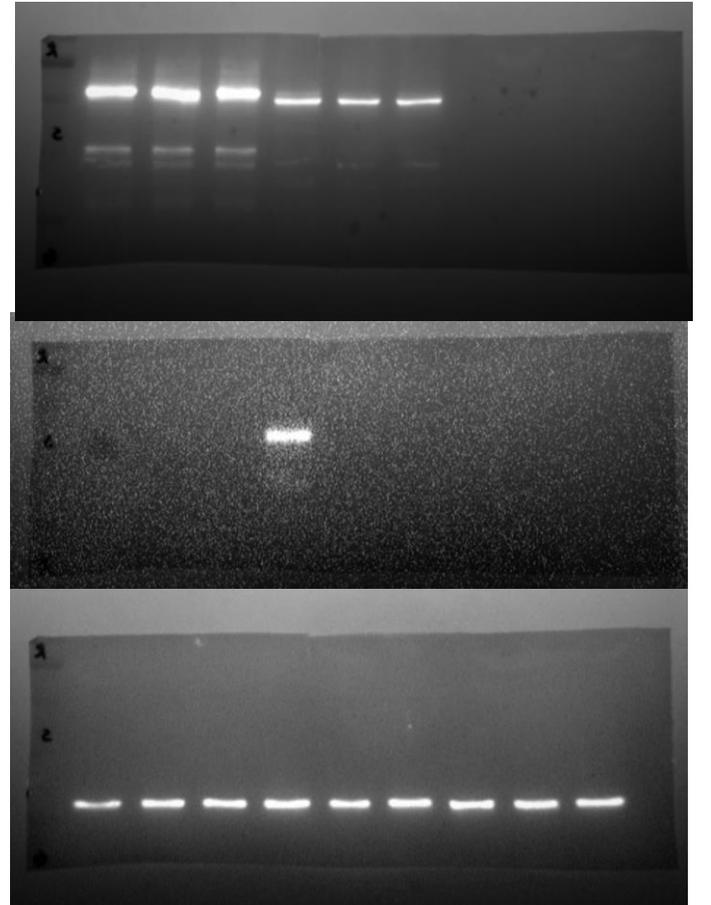
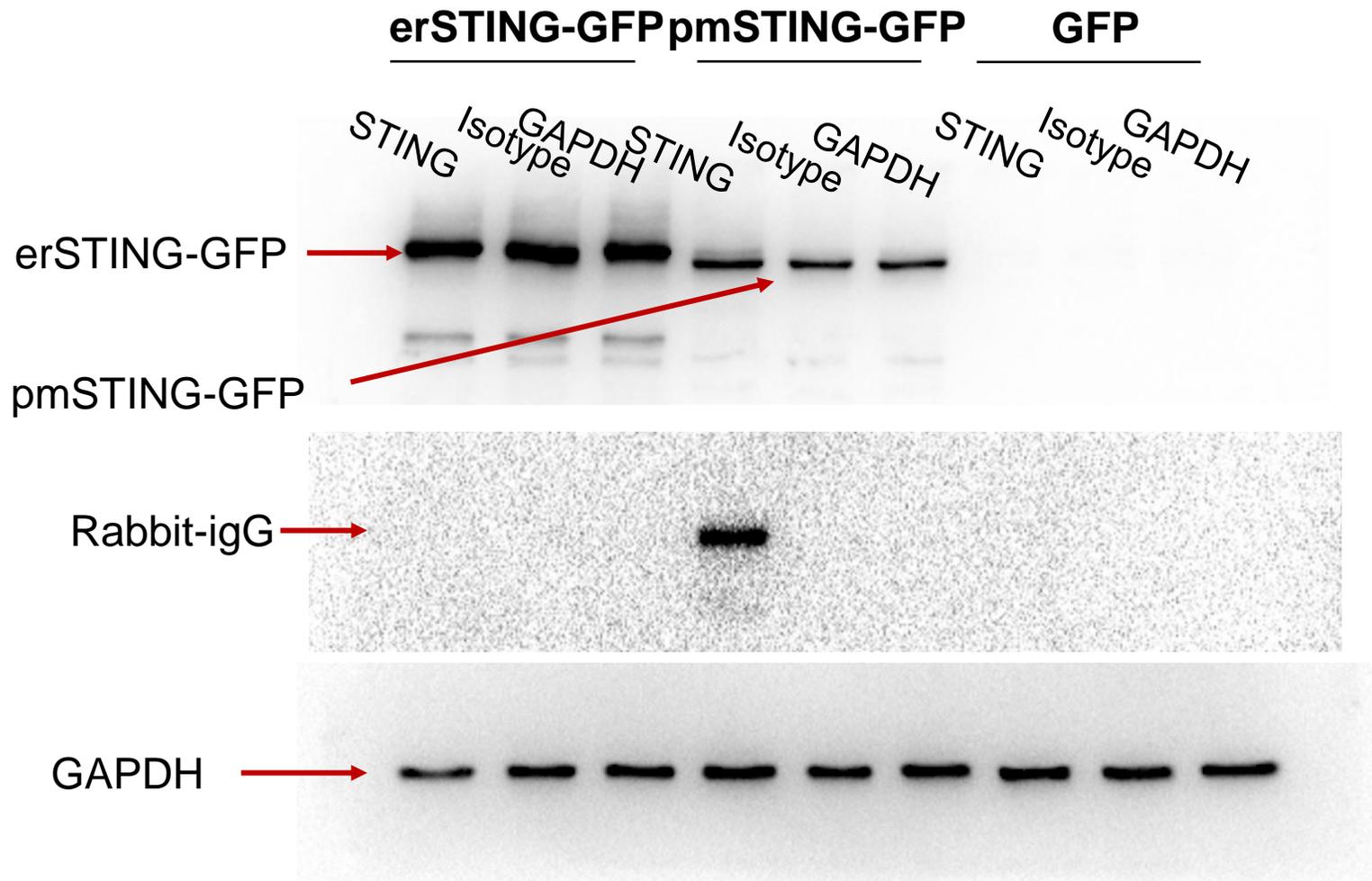


Figure 3C



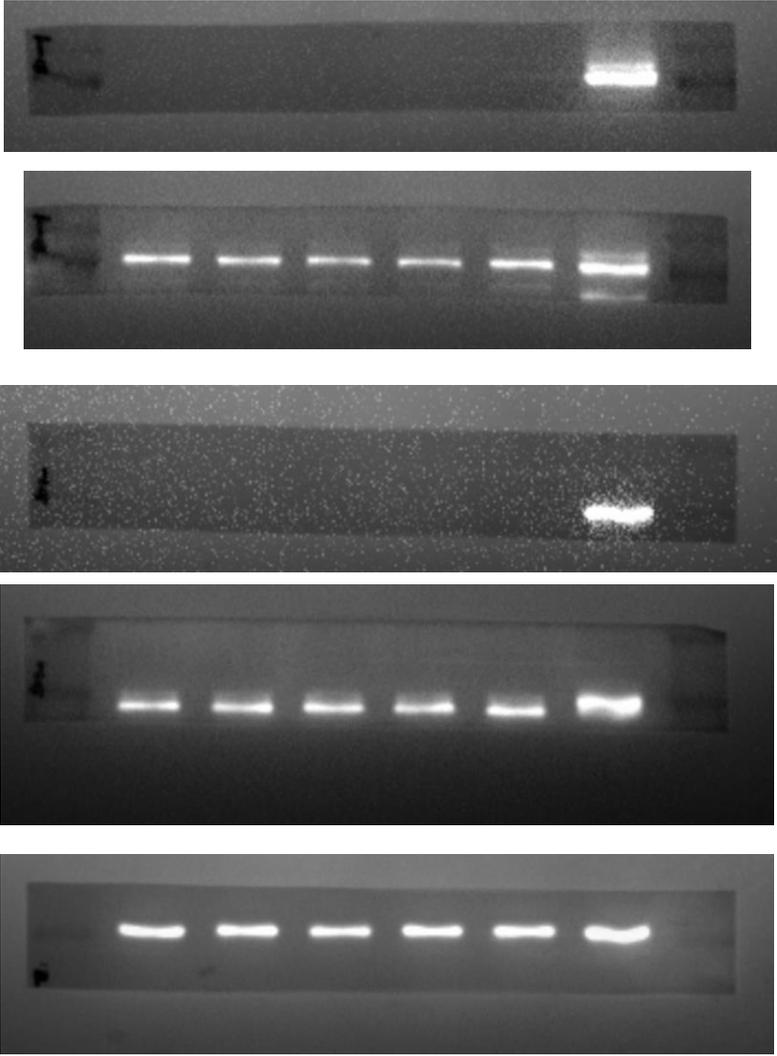
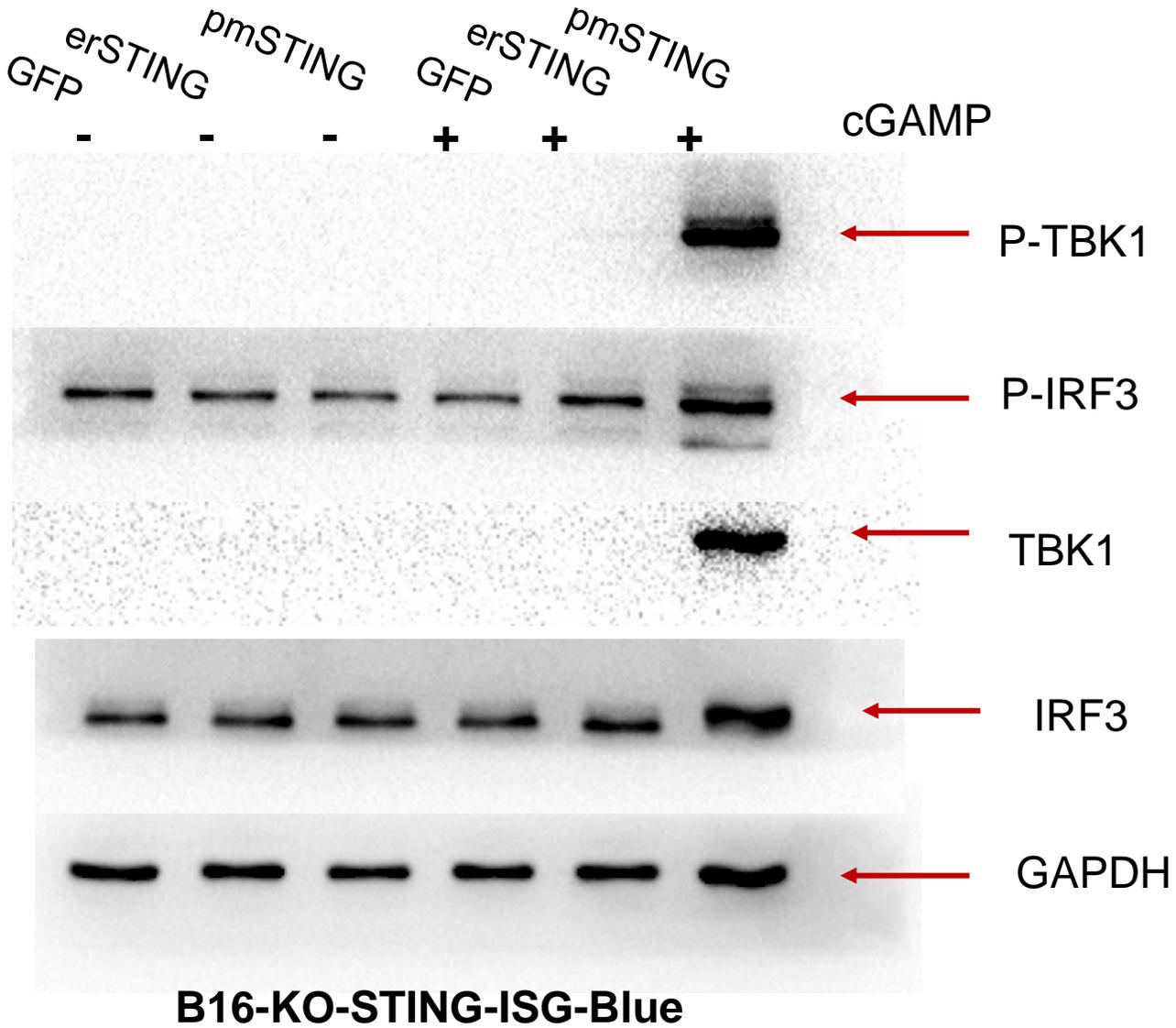
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Figure 3E



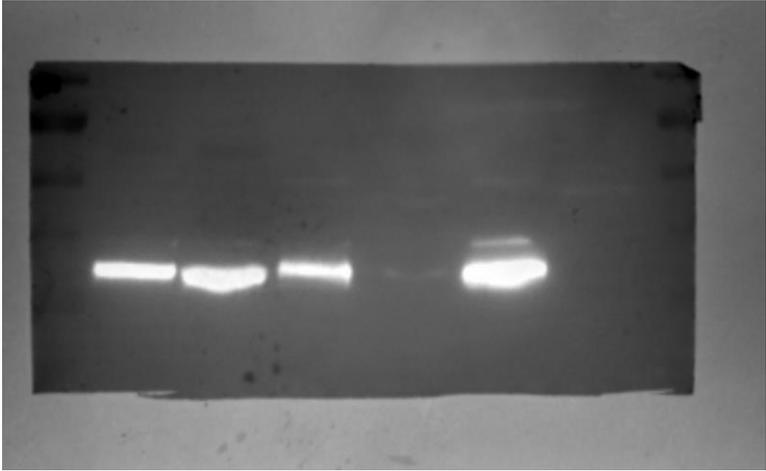
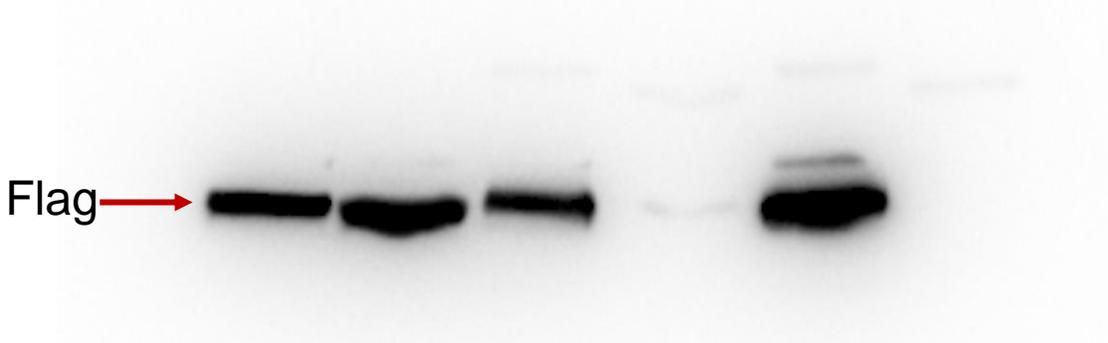
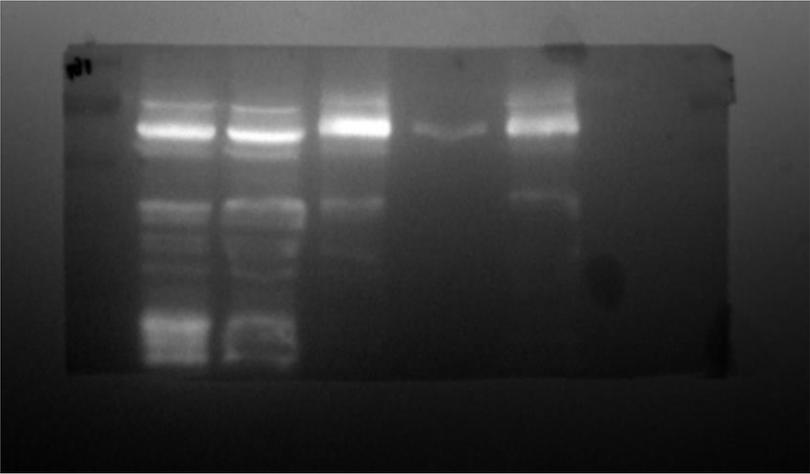
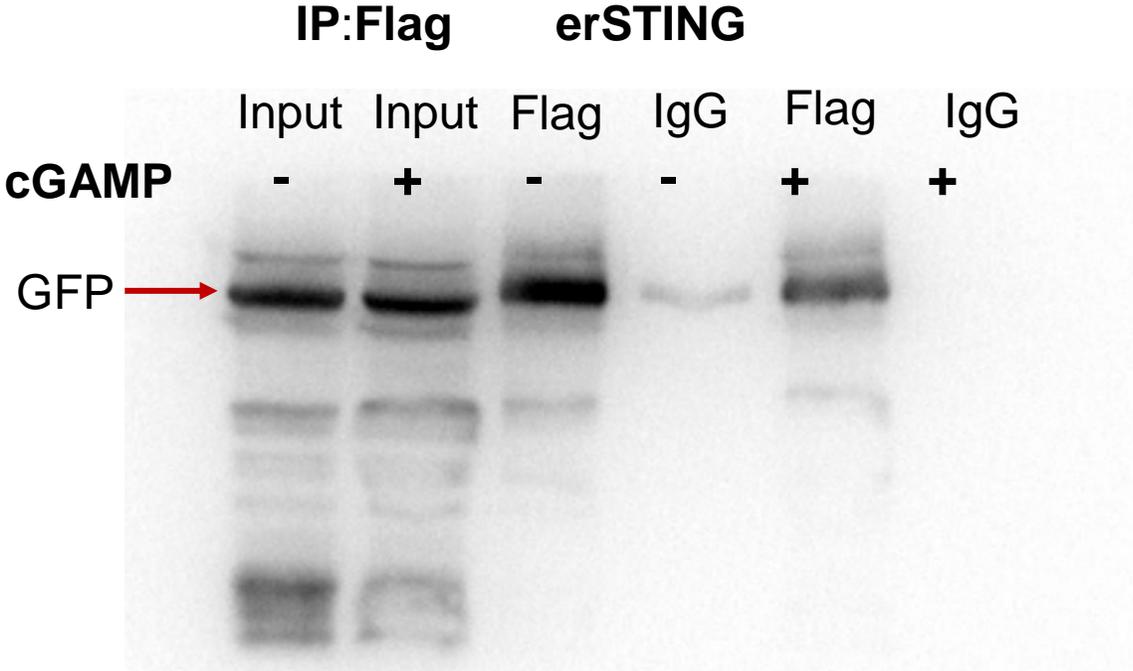
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Figure 4B



Full unedited gel for Figure 4

Figure 4C



B16-KO-STING-ISG-Blue

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Figure 4C

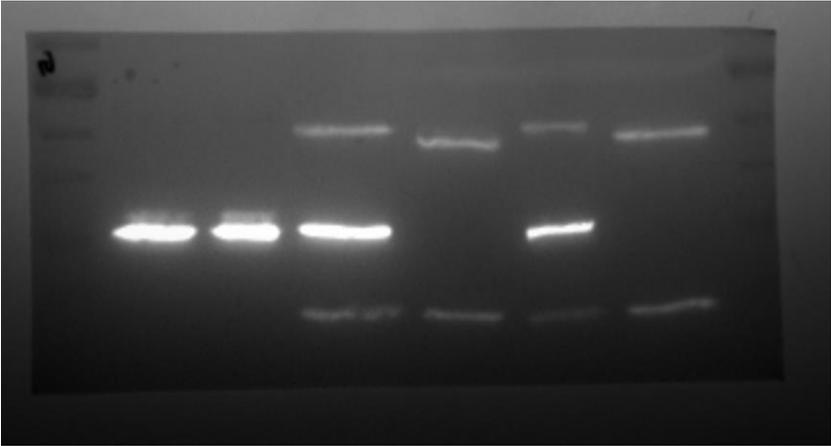
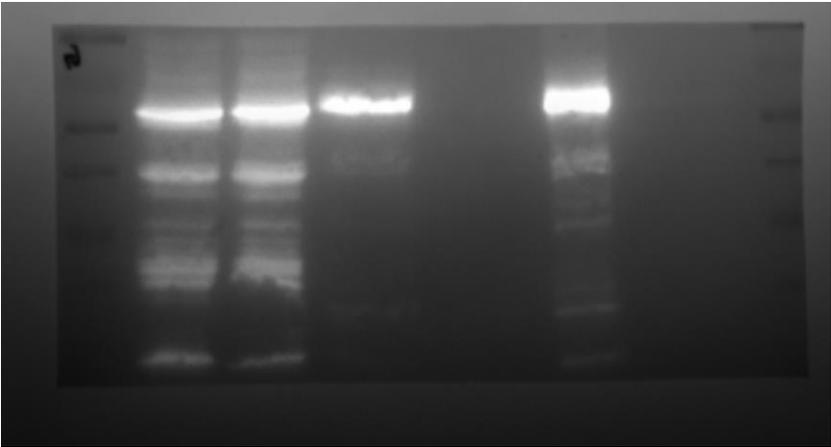
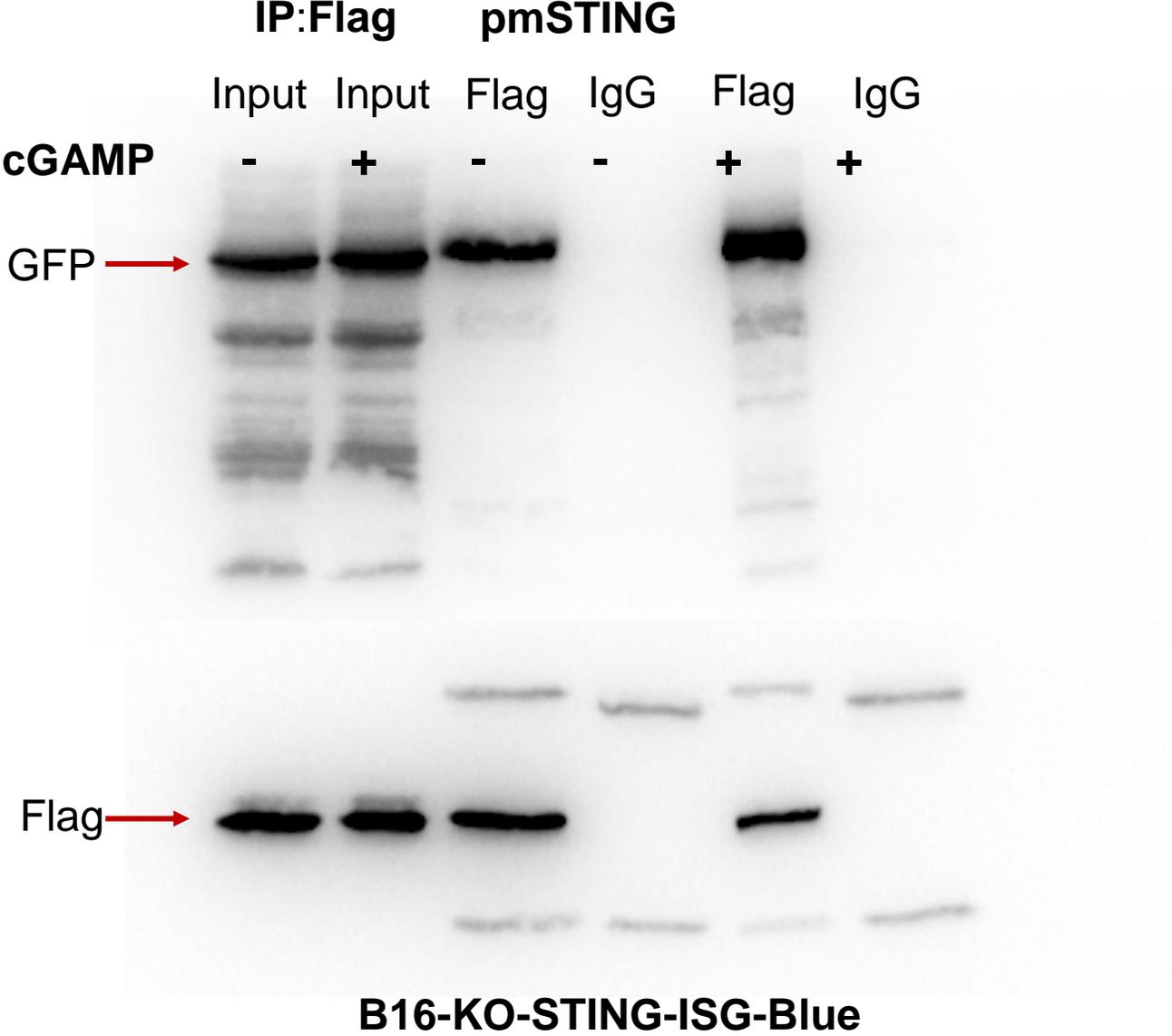
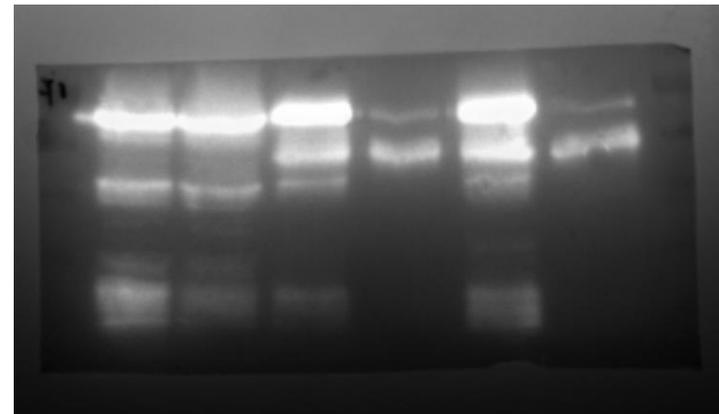
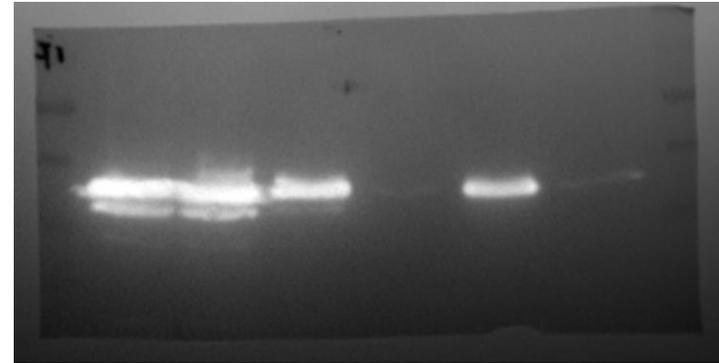
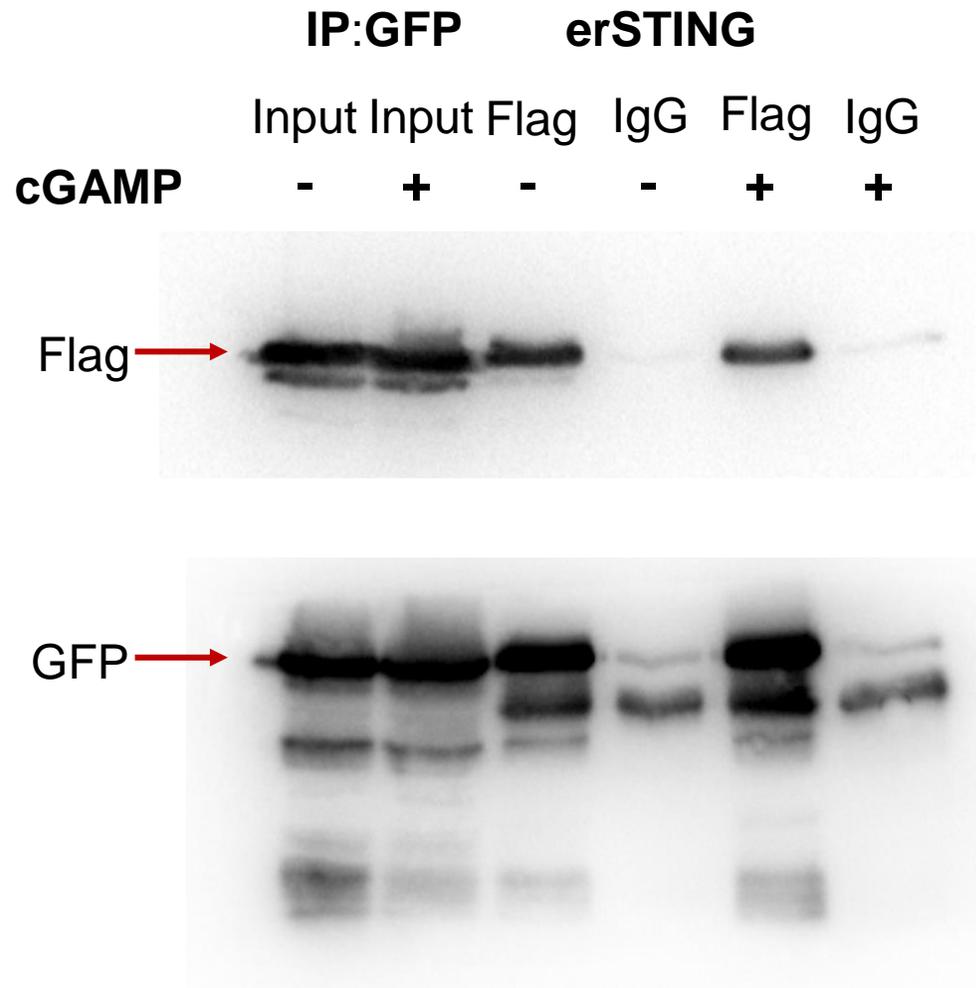


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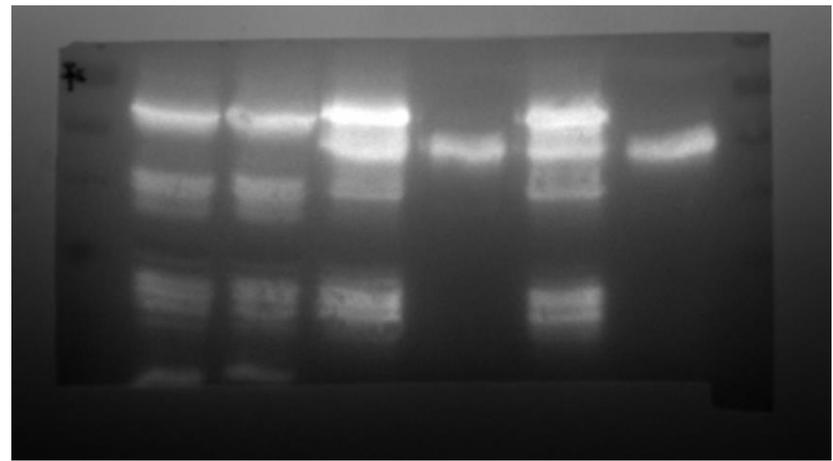
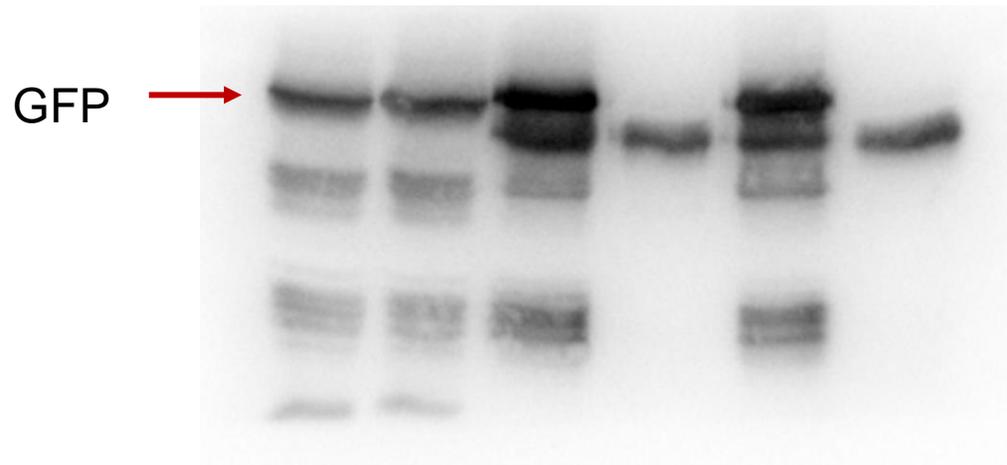
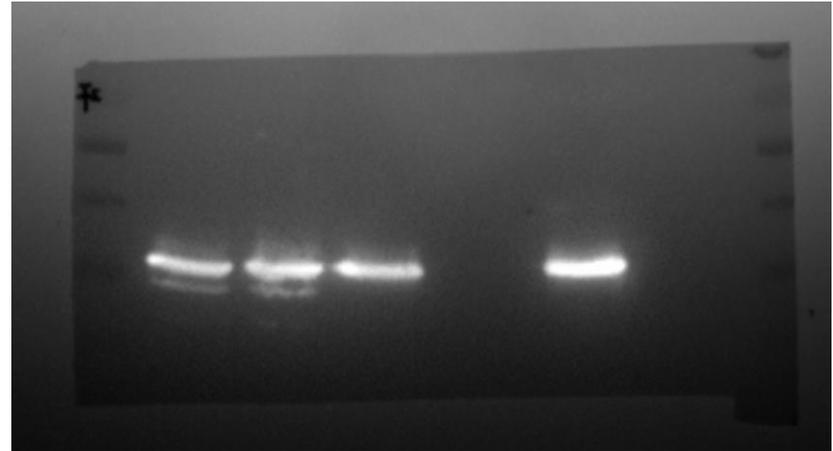
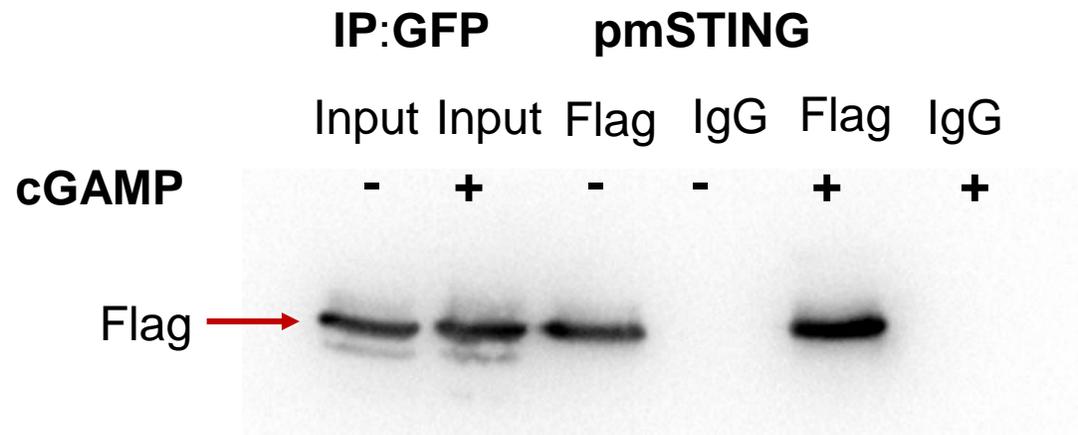
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Figure 4D

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B16-KO-STING-ISG-Blue

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Figure 4E

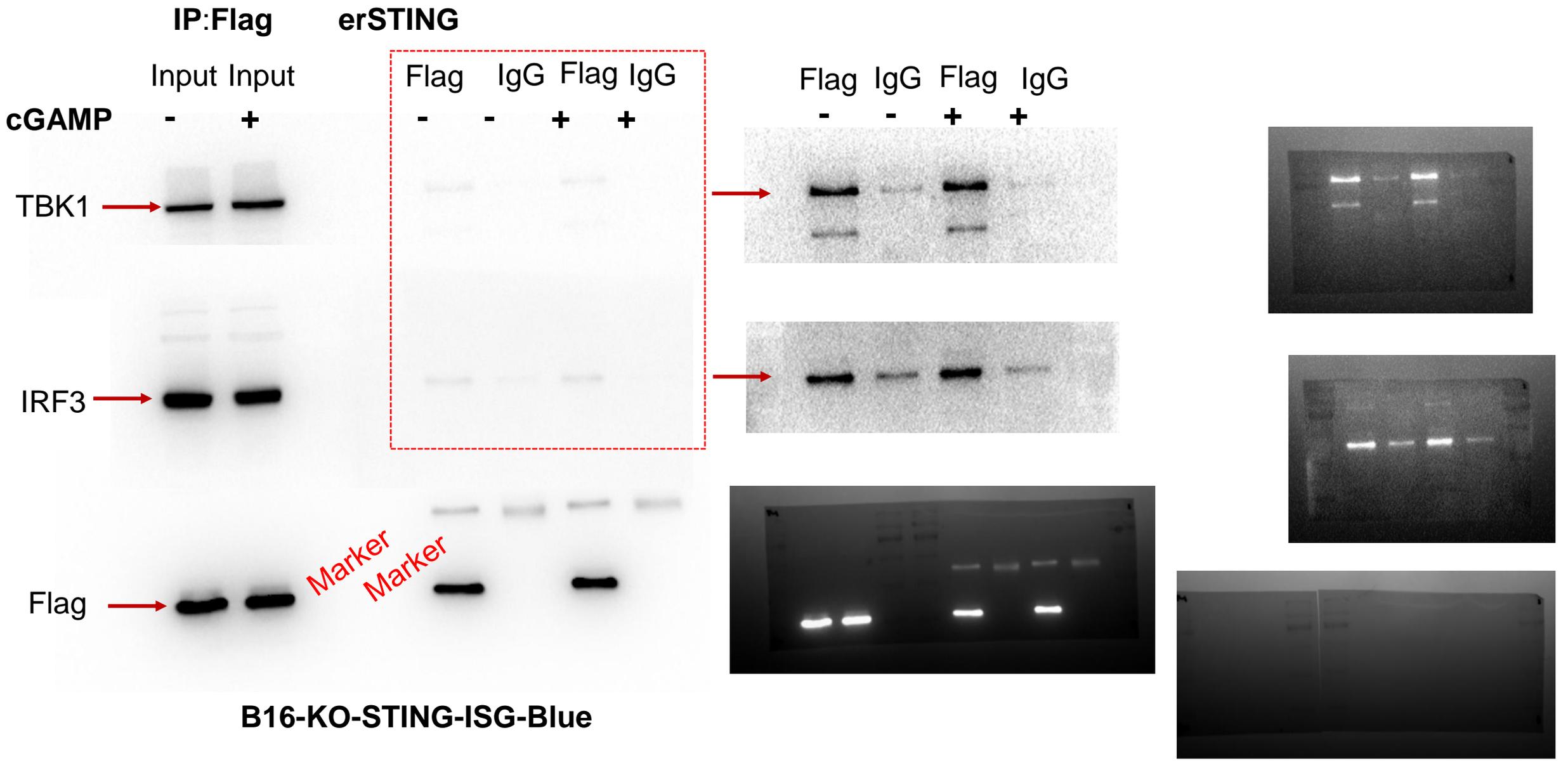
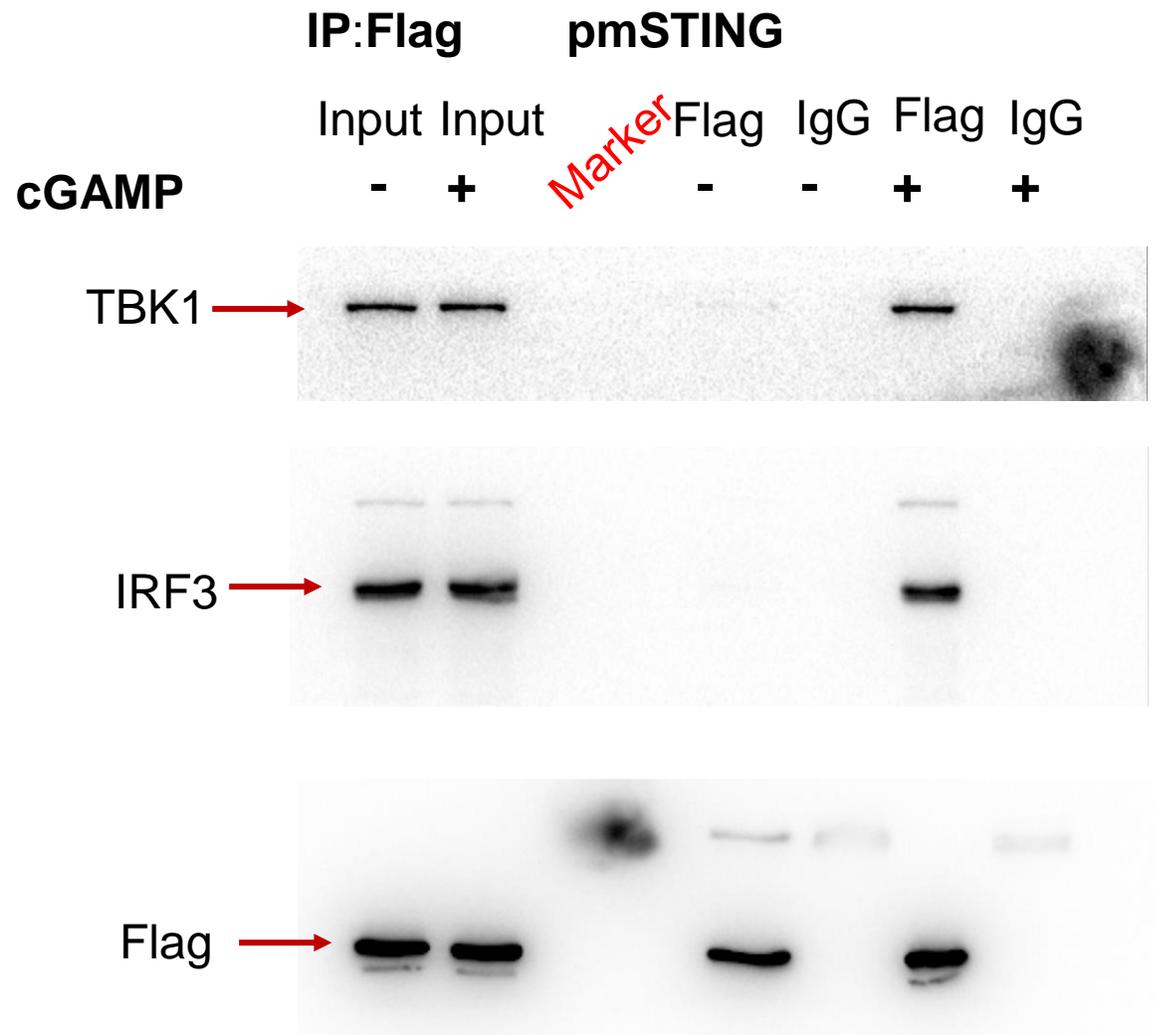
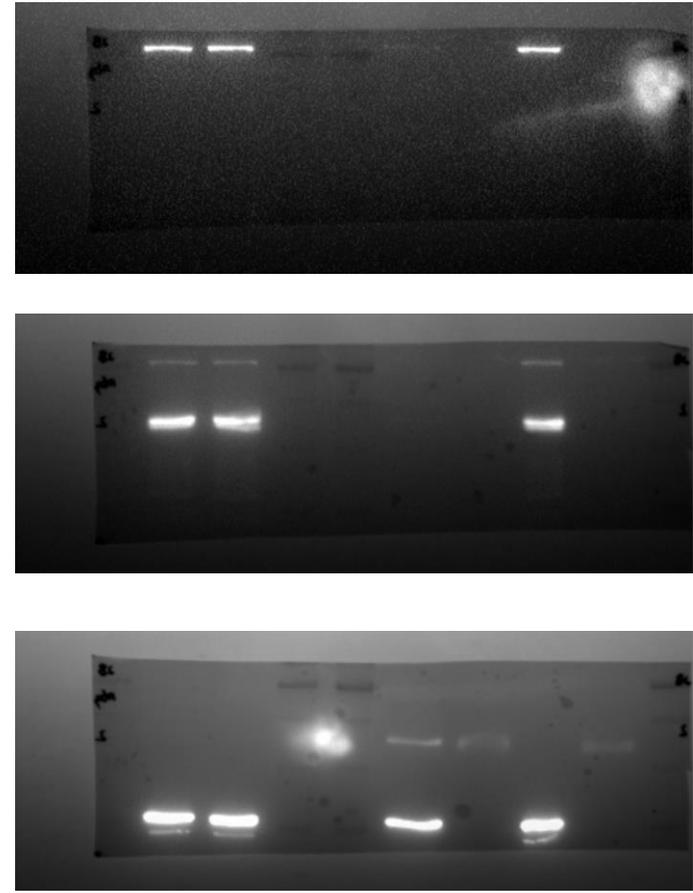


Figure 4E

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B16-KO-STING-ISG-Blue



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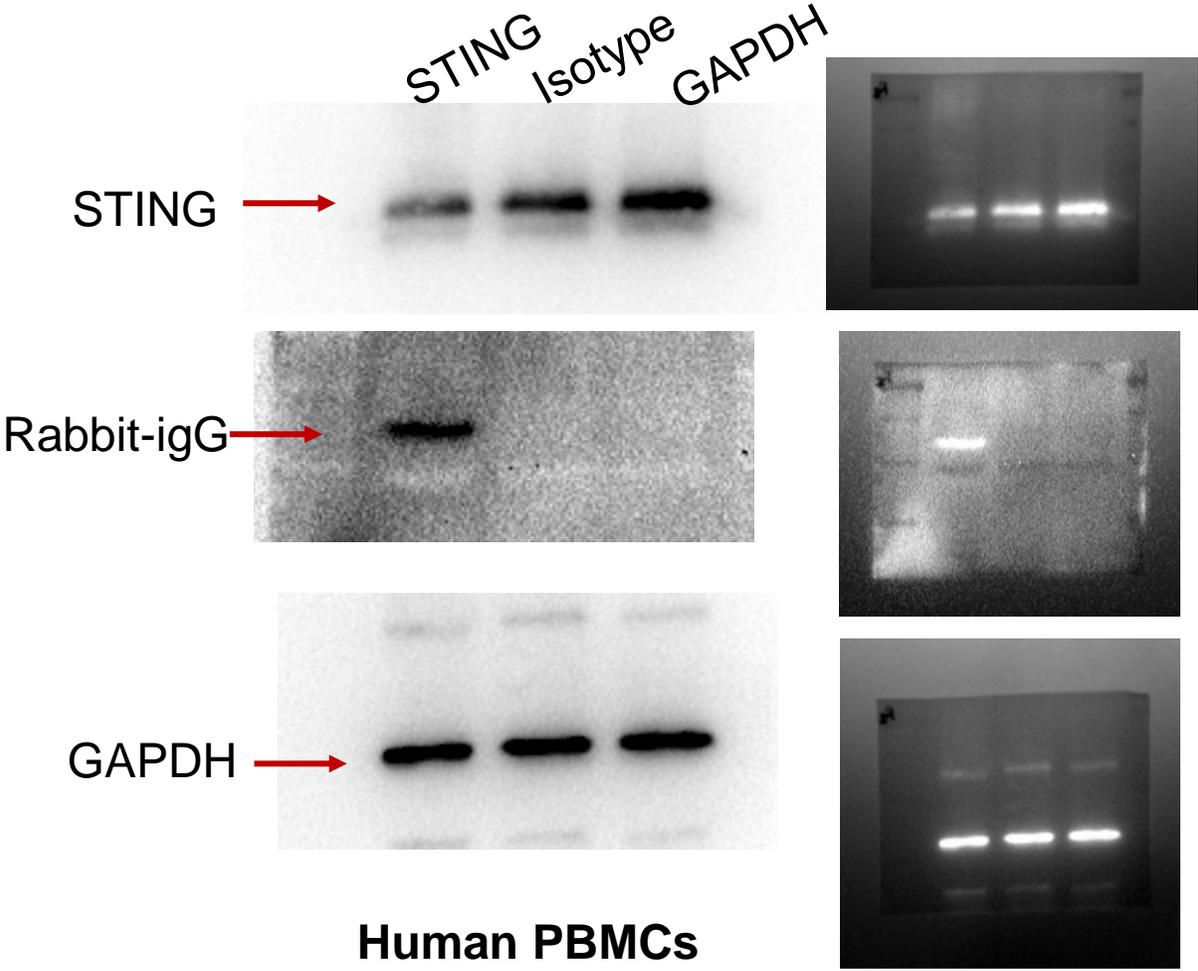


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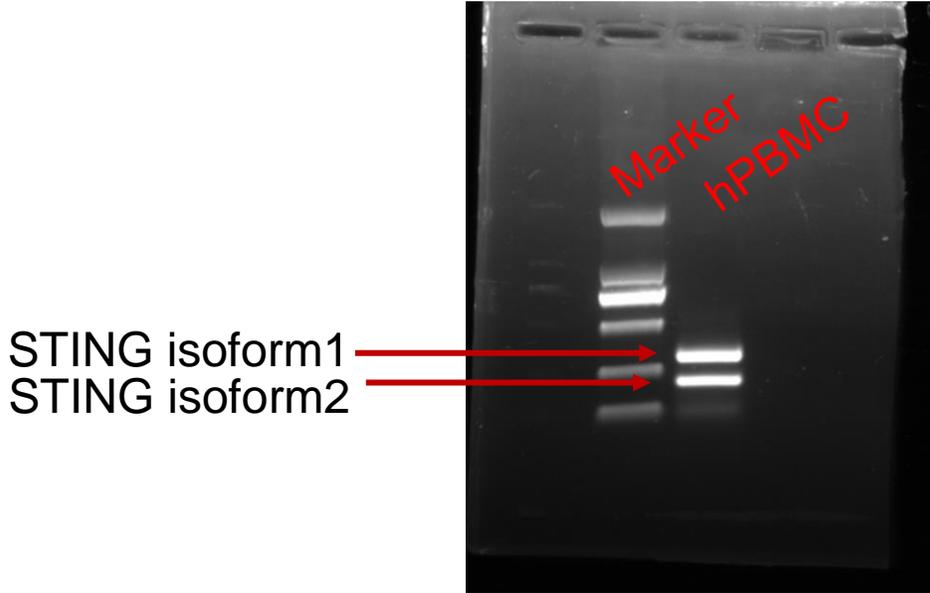
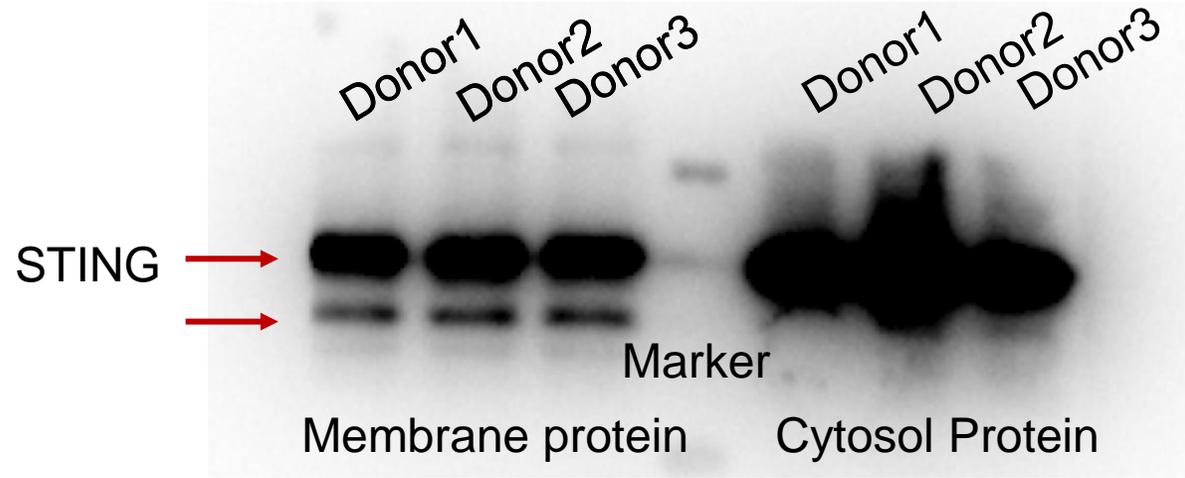
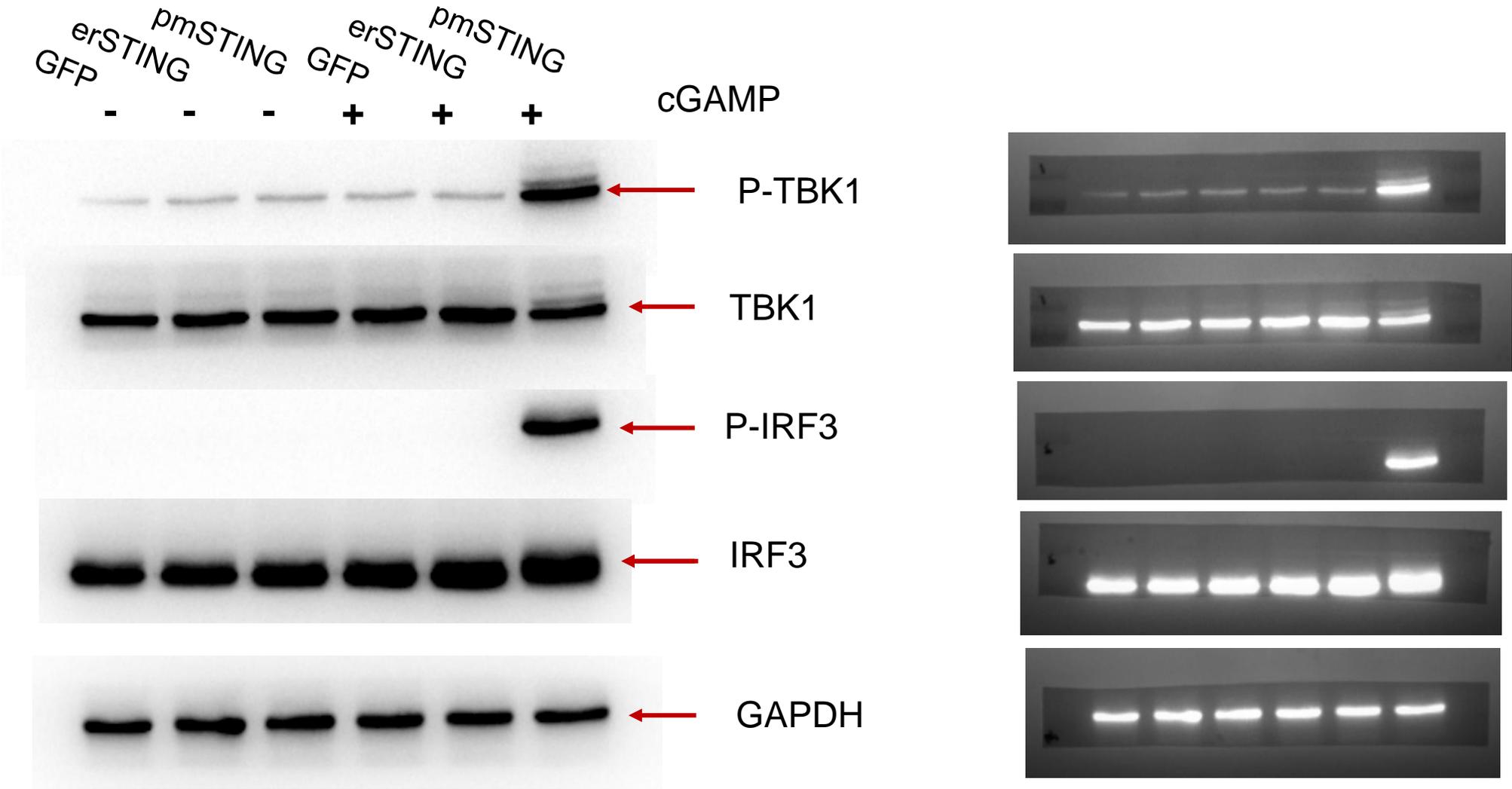


Figure 5G

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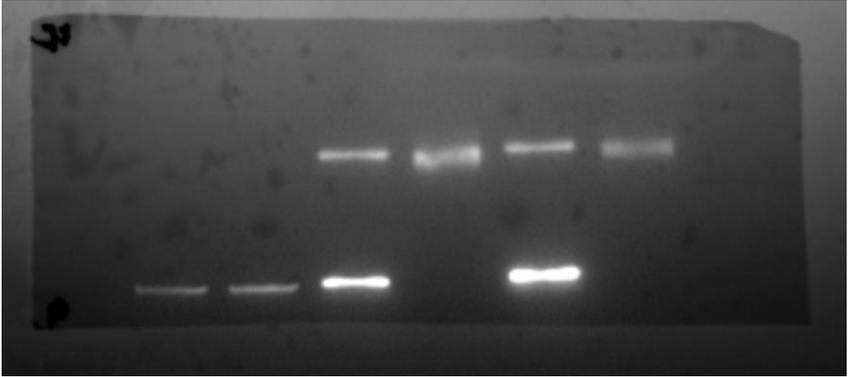
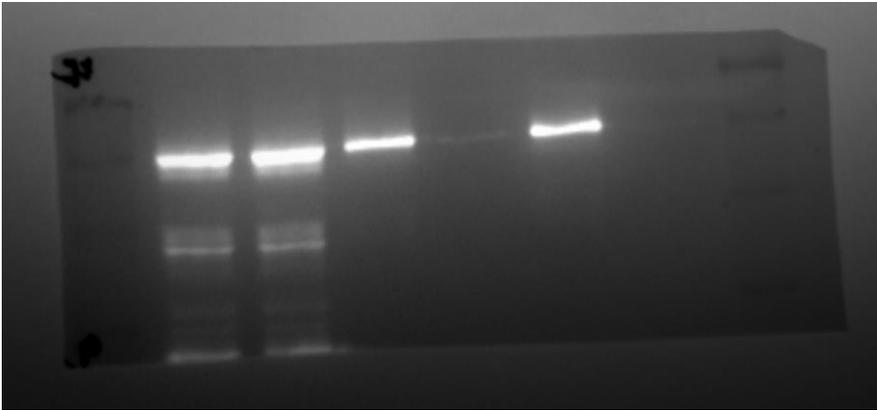
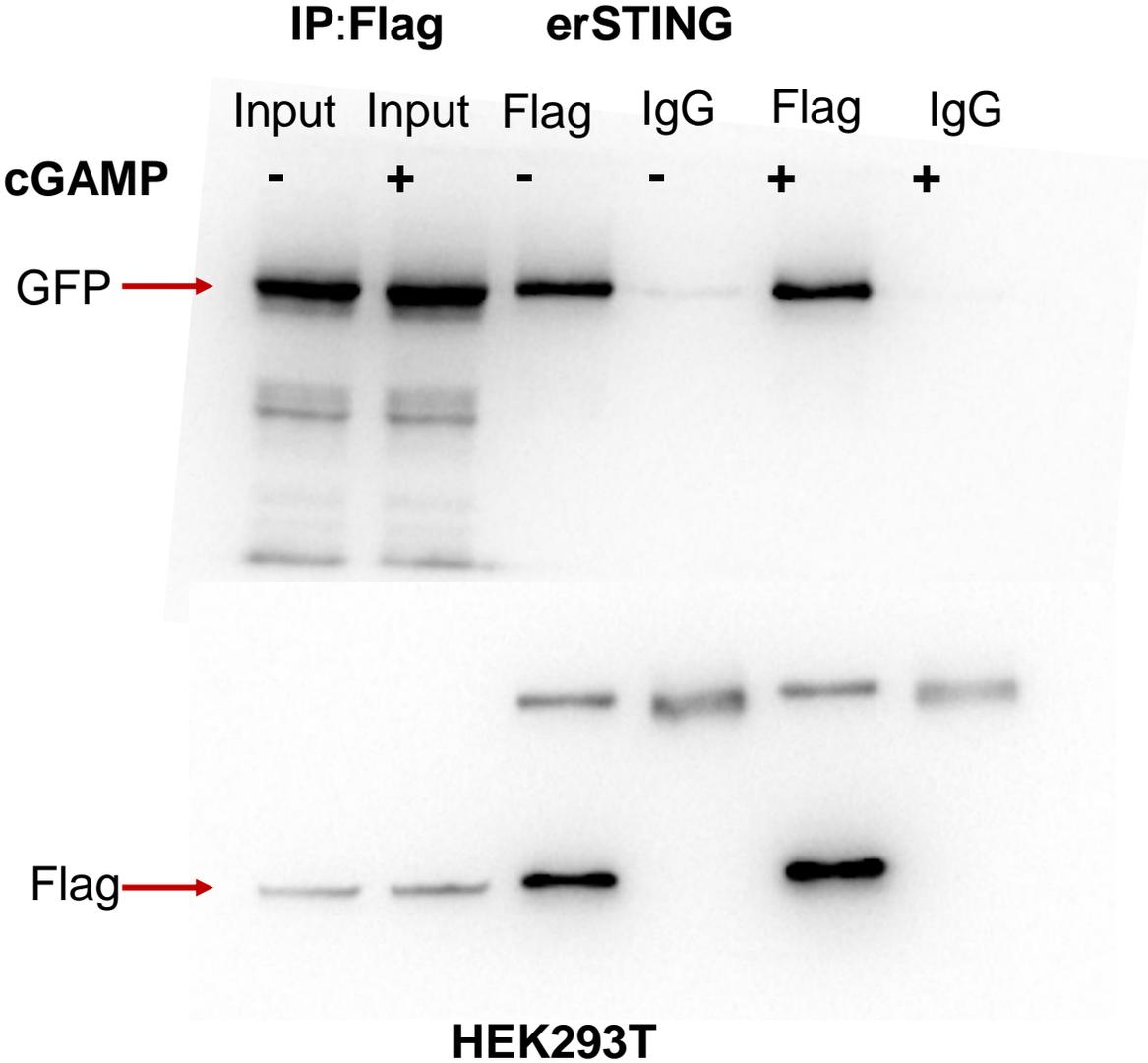
Figure 6B



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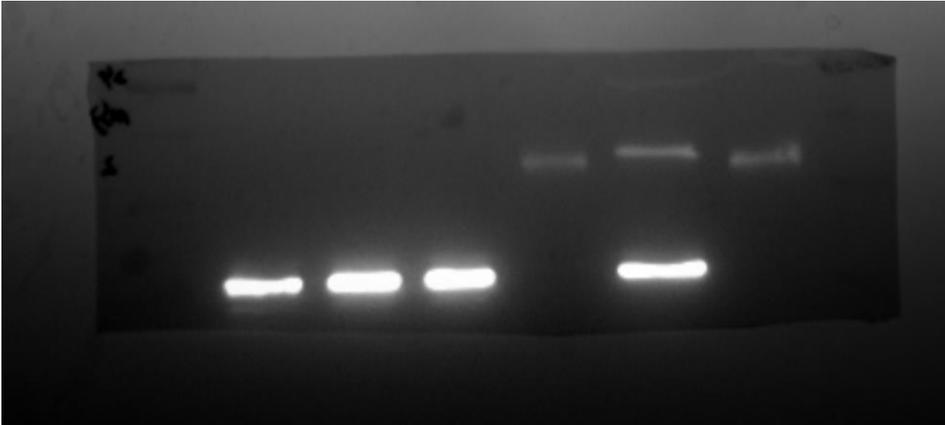
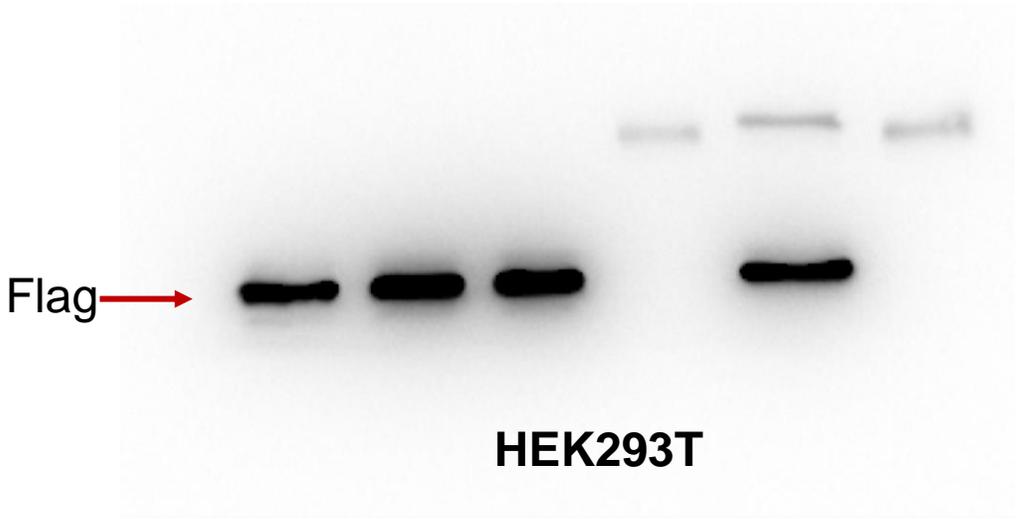
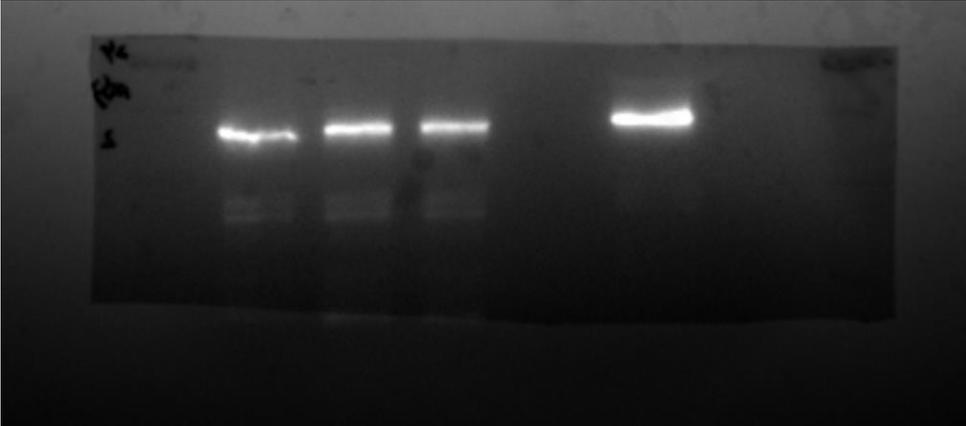
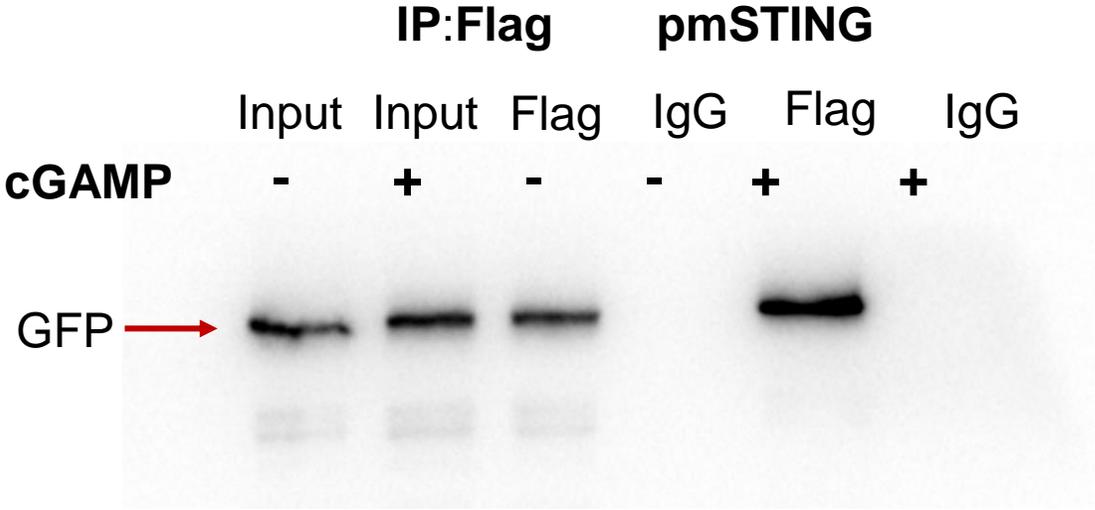
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Figure 6C



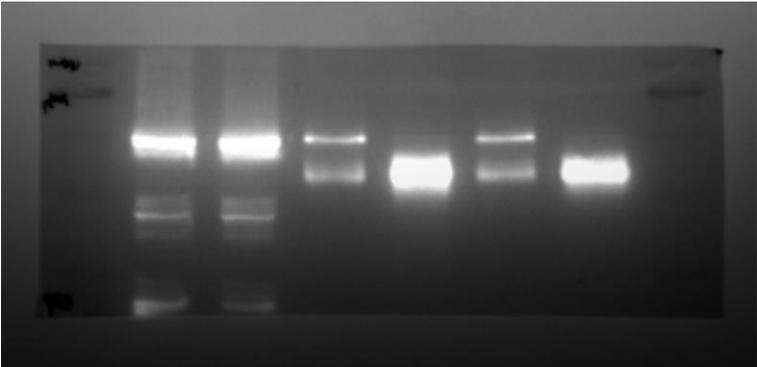
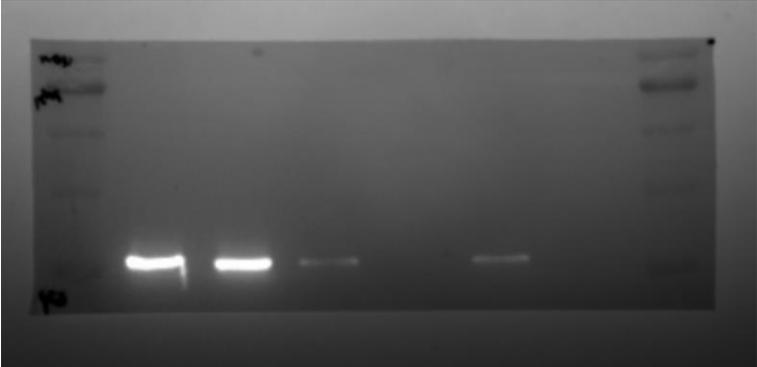
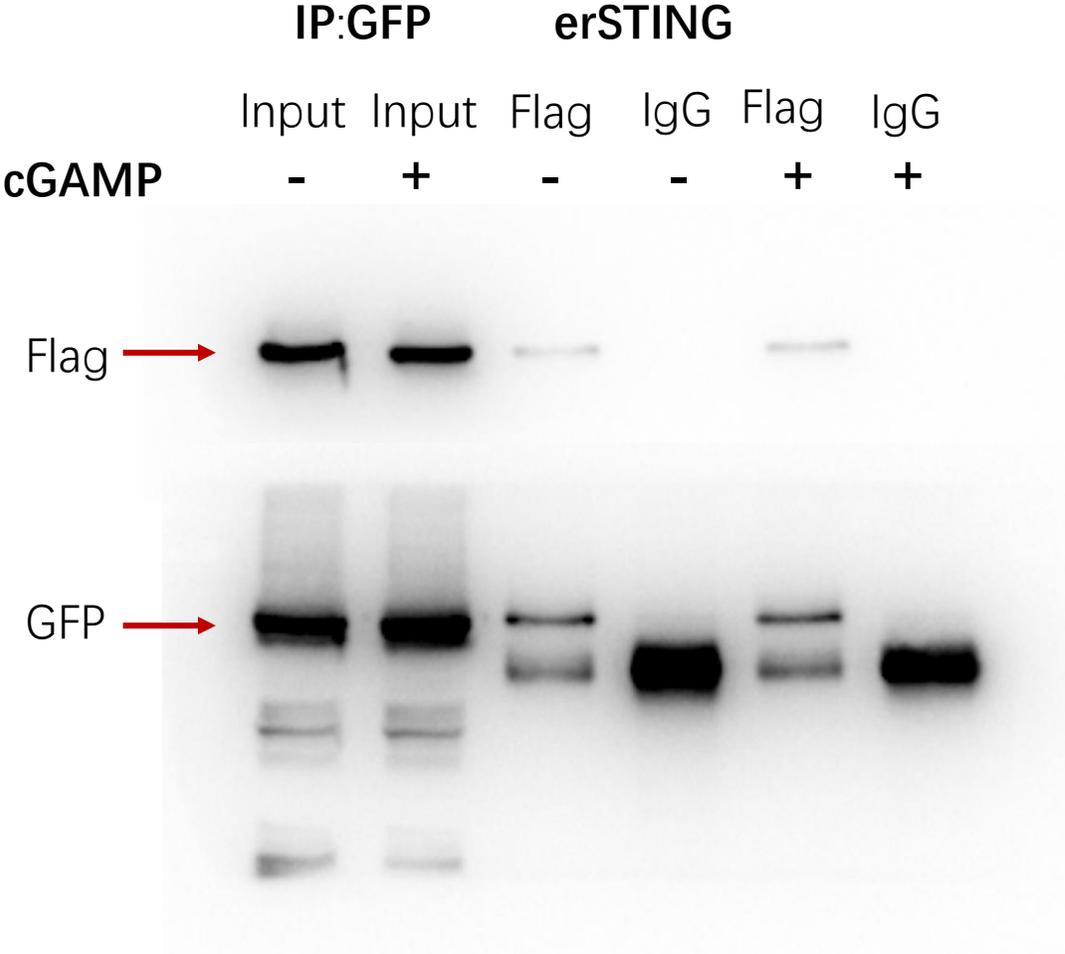
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Figure 6C



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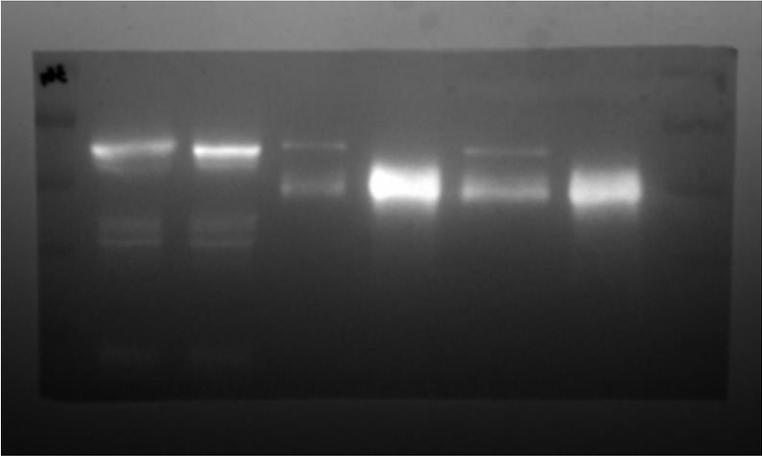
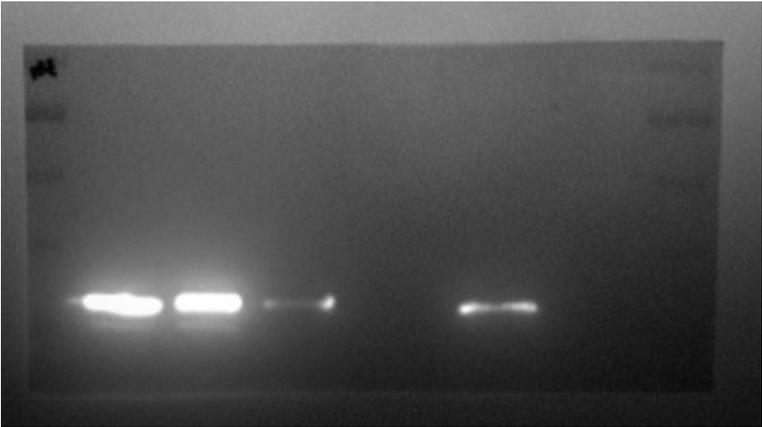
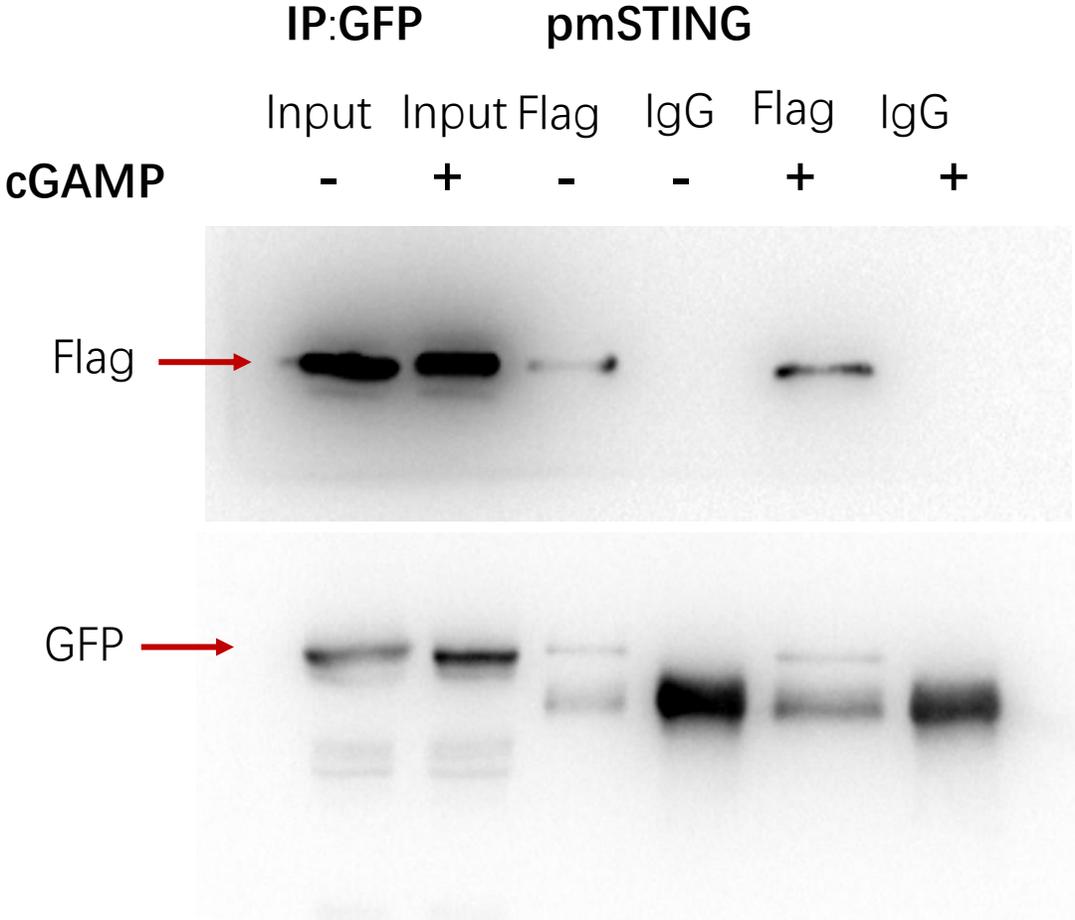
Figure 6D



HEK293T

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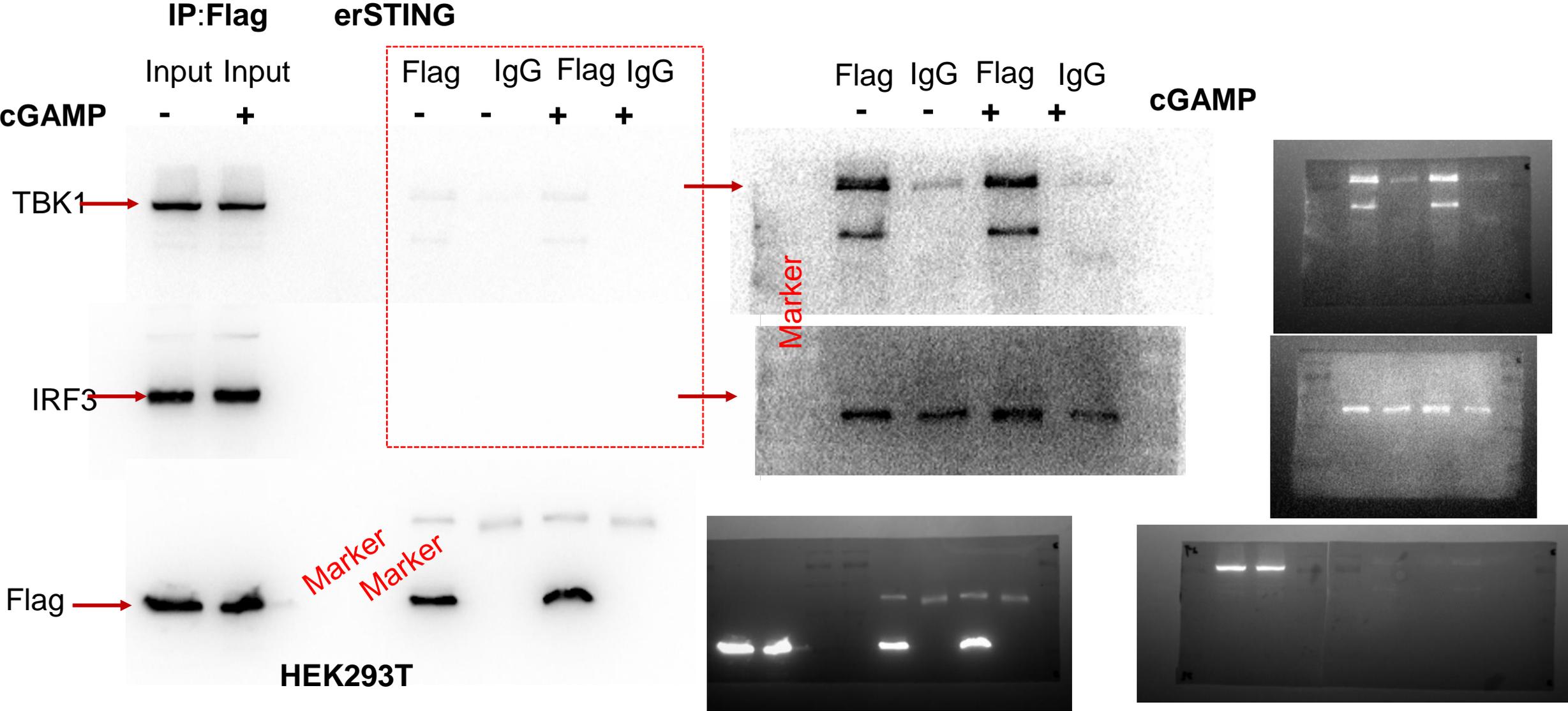
Figure 6D



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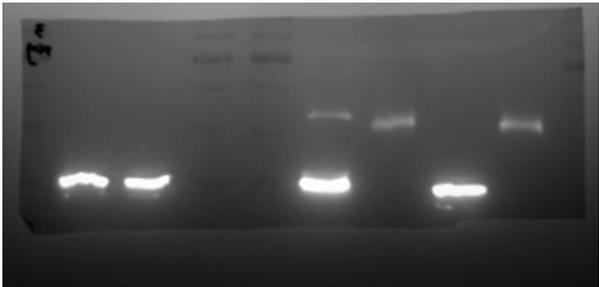
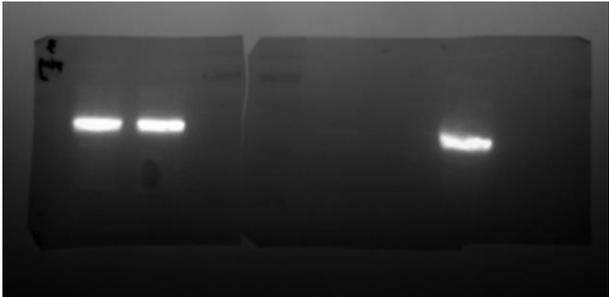
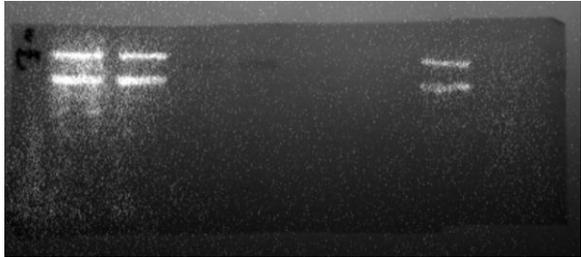
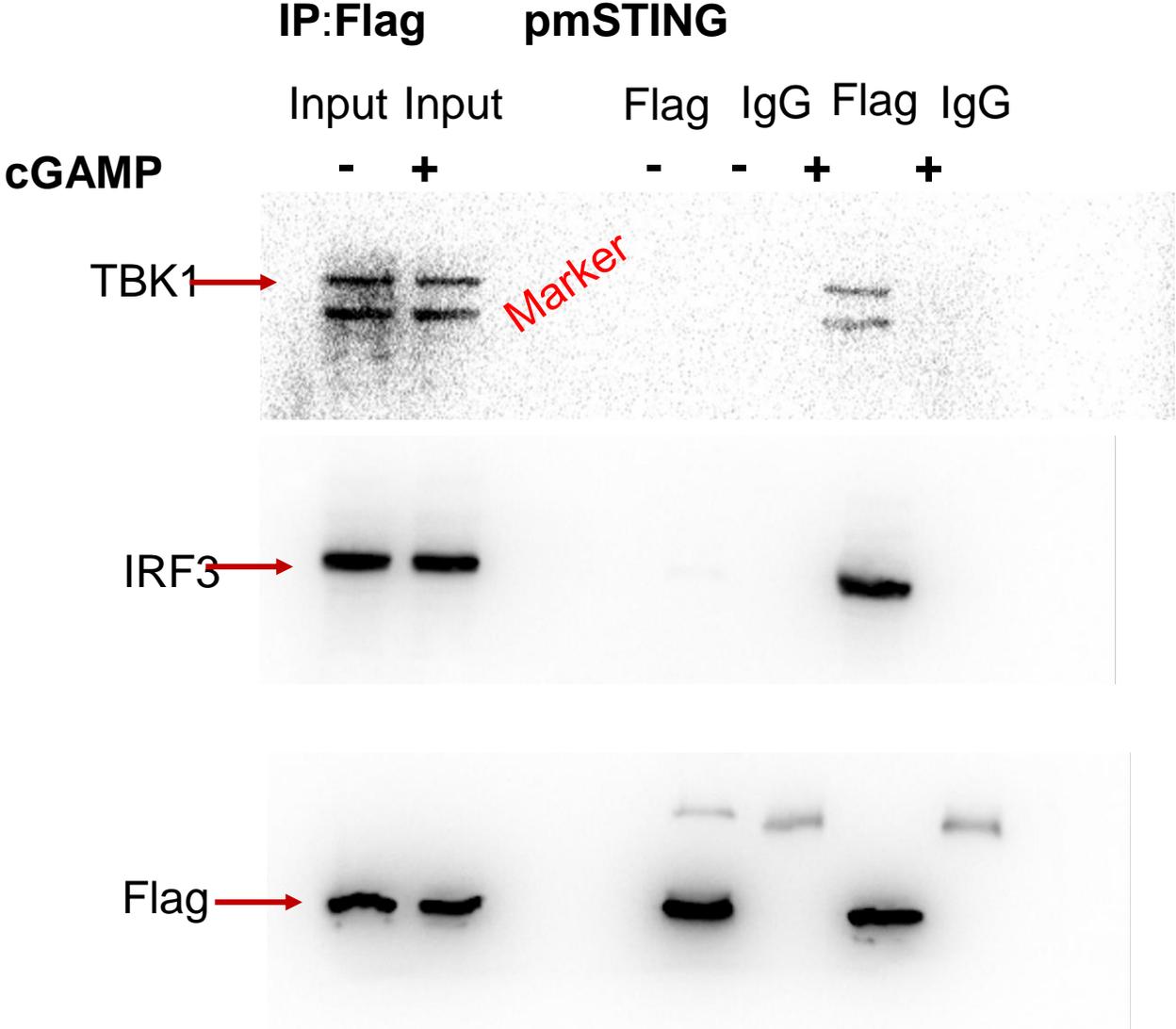
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Figure 6E



Full unedited gel for Figure 6

Figure 6E



HEK293T

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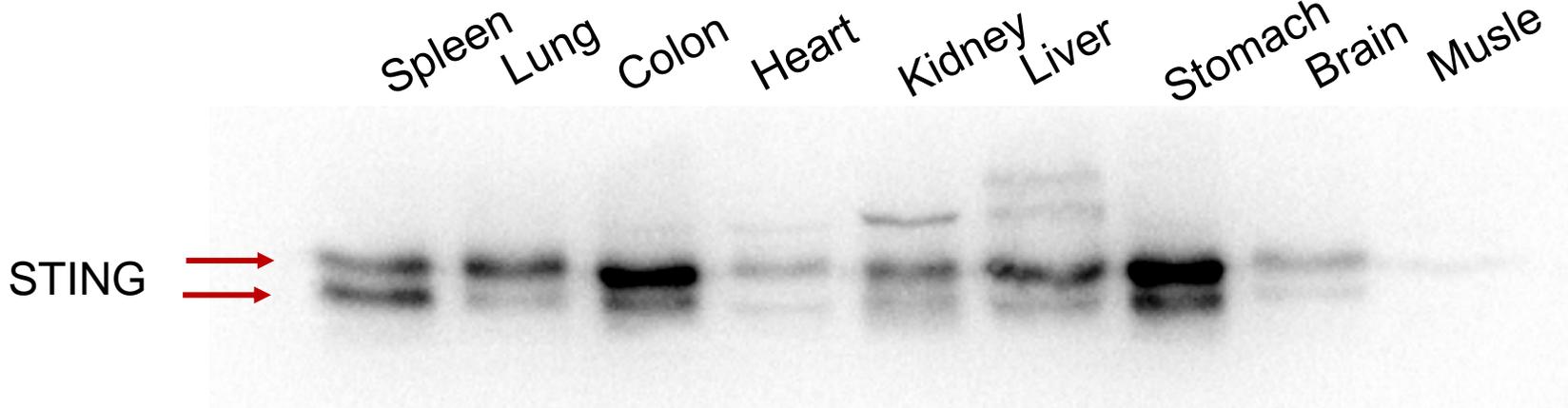


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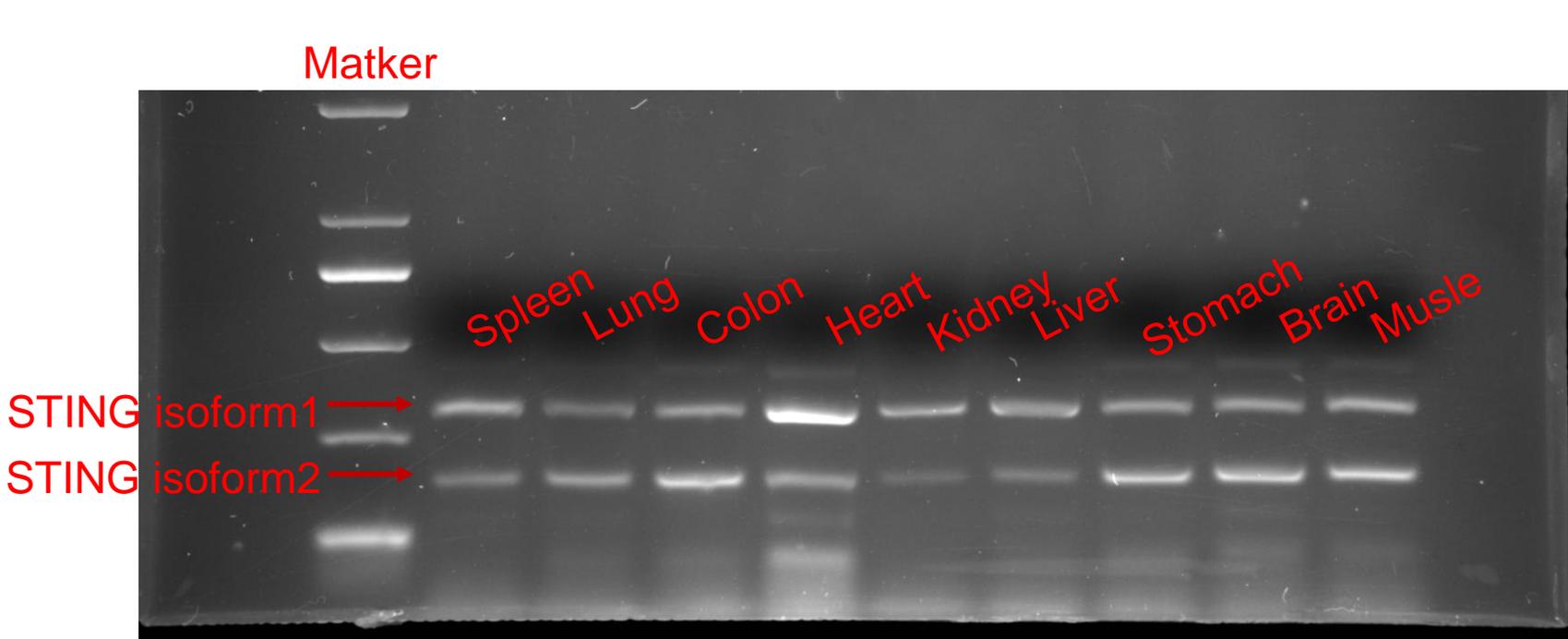


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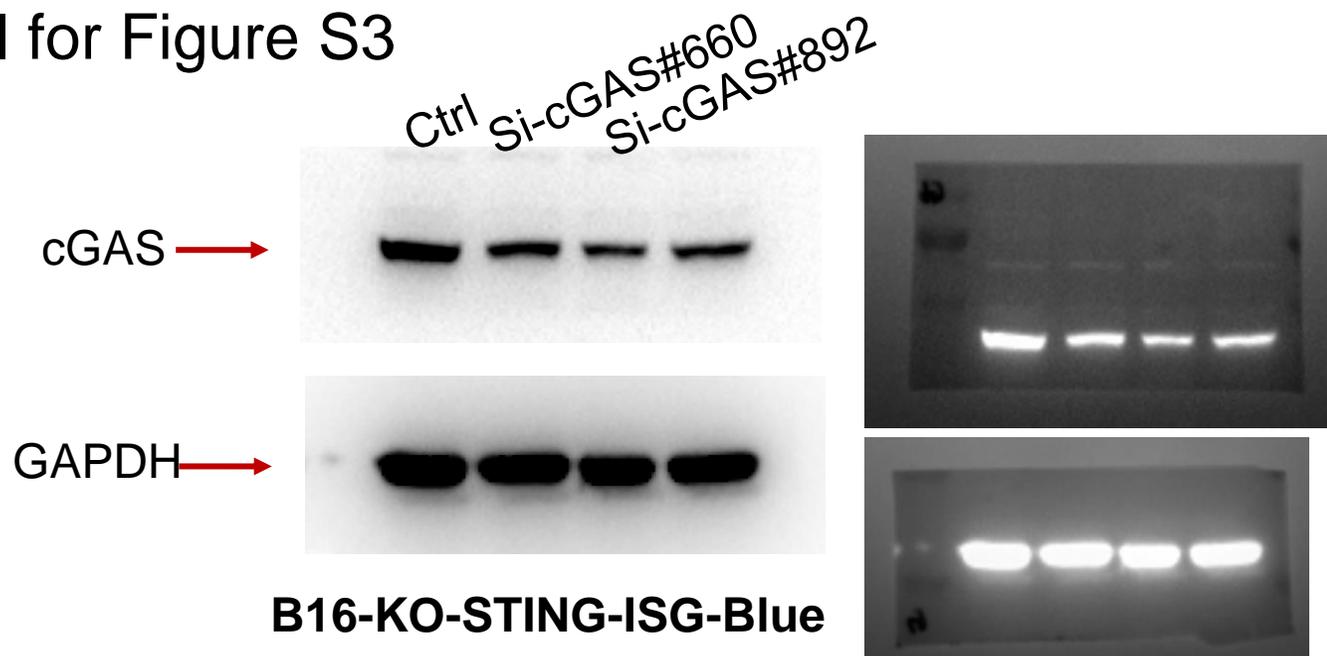


Figure S3 B

