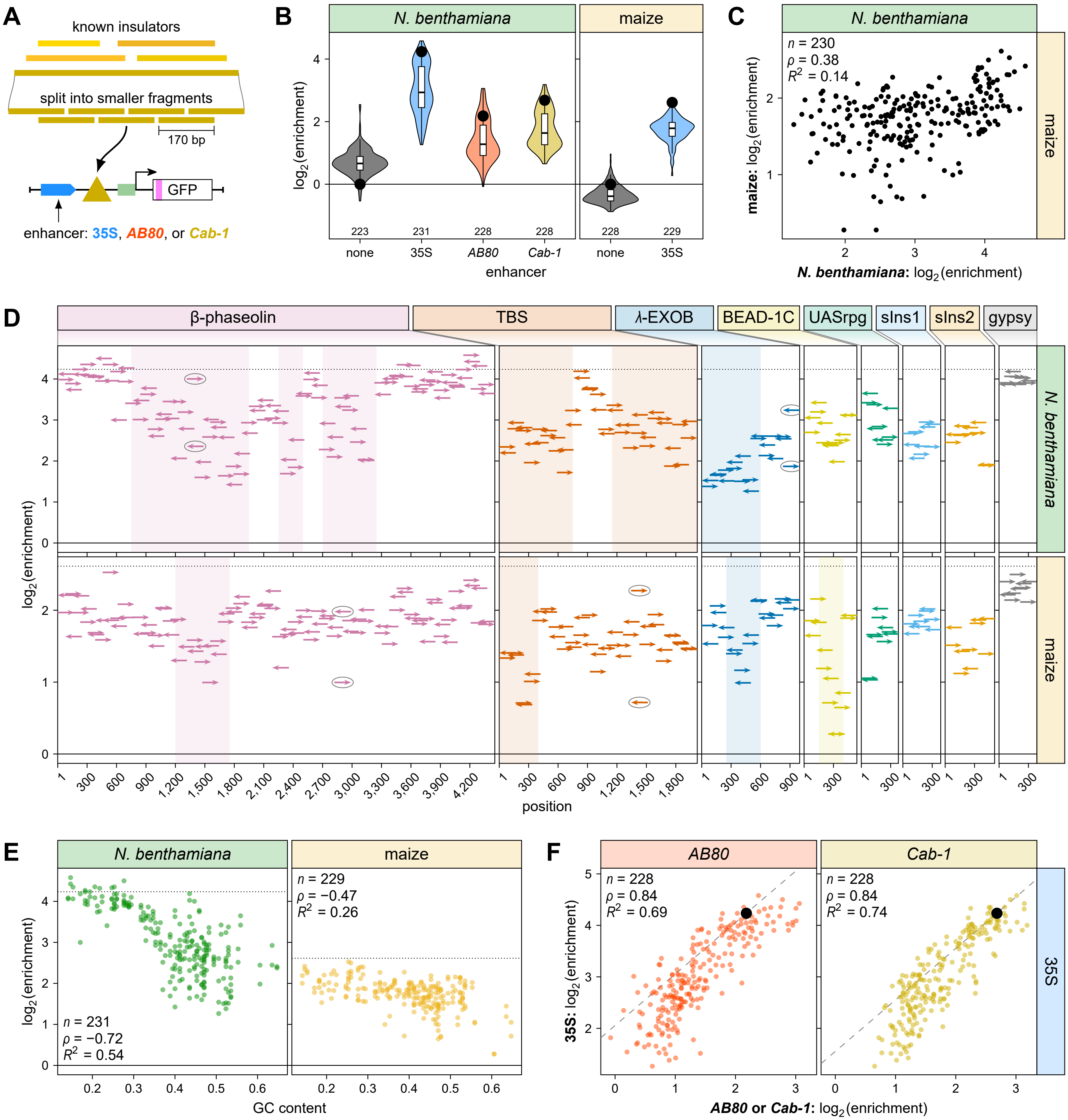


A
known insulators
split into smaller fragments
170 bp
GFP
enhancer: 35S, AB80, or Cab-1
B
N. benthamiana
maize
log2(enrichment)
none
35S
AB80
Cab-1
enhancer
C
n = 230
ρ = 0.38
R2 = 0.14
maize: log2(enrichment)
N. benthamiana: log2(enrichment)
D
β-phaseolin
TBS
λ-EXOB
BEAD-1C
UASrpg
sIns1
sIns2
gypsy
position
E
n = 231
ρ = −0.72
R2 = 0.54
n = 229
ρ = −0.47
R2 = 0.26
GC content
F
AB80
Cab-1
35S
n = 228
ρ = 0.84
R2 = 0.69
n = 228
ρ = 0.84
R2 = 0.74
35S: log2(enrichment)
AB80 or Cab-1: log2(enrichment)

Figure 1. Short fragments exhibit enhancer-blocking insulator activity. **A)** Known insulators were split into partially overlapping 170-bp fragments. The insulator fragments were cloned in the forward or reverse orientation between a 35S, *AB80*, or *Cab-1* enhancer and a 35S minimal promoter (green rectangle) driving the expression of a barcoded GFP reporter gene. Constructs without an enhancer (none) but with insulator fragments were also created. **B)** All insulator fragment constructs were pooled and subjected to Plant STARR-seq in *N. benthamiana* leaves (*N. benthamiana*) and maize protoplasts (maize). Reporter mRNA enrichment was normalized to a control construct without an enhancer or insulator (noEnh; log2 set to 0). The enrichment of a control construct without an insulator is indicated as a black dot. Violin plots represent the kernel density distribution and the box plots inside represent the median (center line), upper and lower quartiles, and 1.5× interquartile range (whiskers) for all corresponding constructs. Numbers at the bottom of each violin indicate the number of samples in each group. **C)** Correlation between the enrichment of insulator fragments in constructs with the 35S enhancer in *N. benthamiana* leaves and maize protoplasts. **D)** Enrichment of constructs with insulator fragments cloned between the 35S enhancer and minimal promoter. The position along the full-length insulator and the orientation (arrow pointing right, fwd; arrow pointing left, rev) of the fragments is indicated by arrows. Clusters of active fragments are shown as shaded areas. Insulators with highly orientation-dependent activity are circled. **E)** Correlation between insulator fragment enrichment and GC content for constructs with the 35S enhancer. **F)** Correlation between insulator fragment enrichment in *N. benthamiana* leaves in constructs with the indicated enhancers. The dashed line represents a y = x line fitted through the point corresponding to a control construct without an insulator (black dot). Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated in (**C**), (**E**), and (**F**). The dotted line in (**D**) and (**E**) represents the enrichment of a control construct without an insulator.

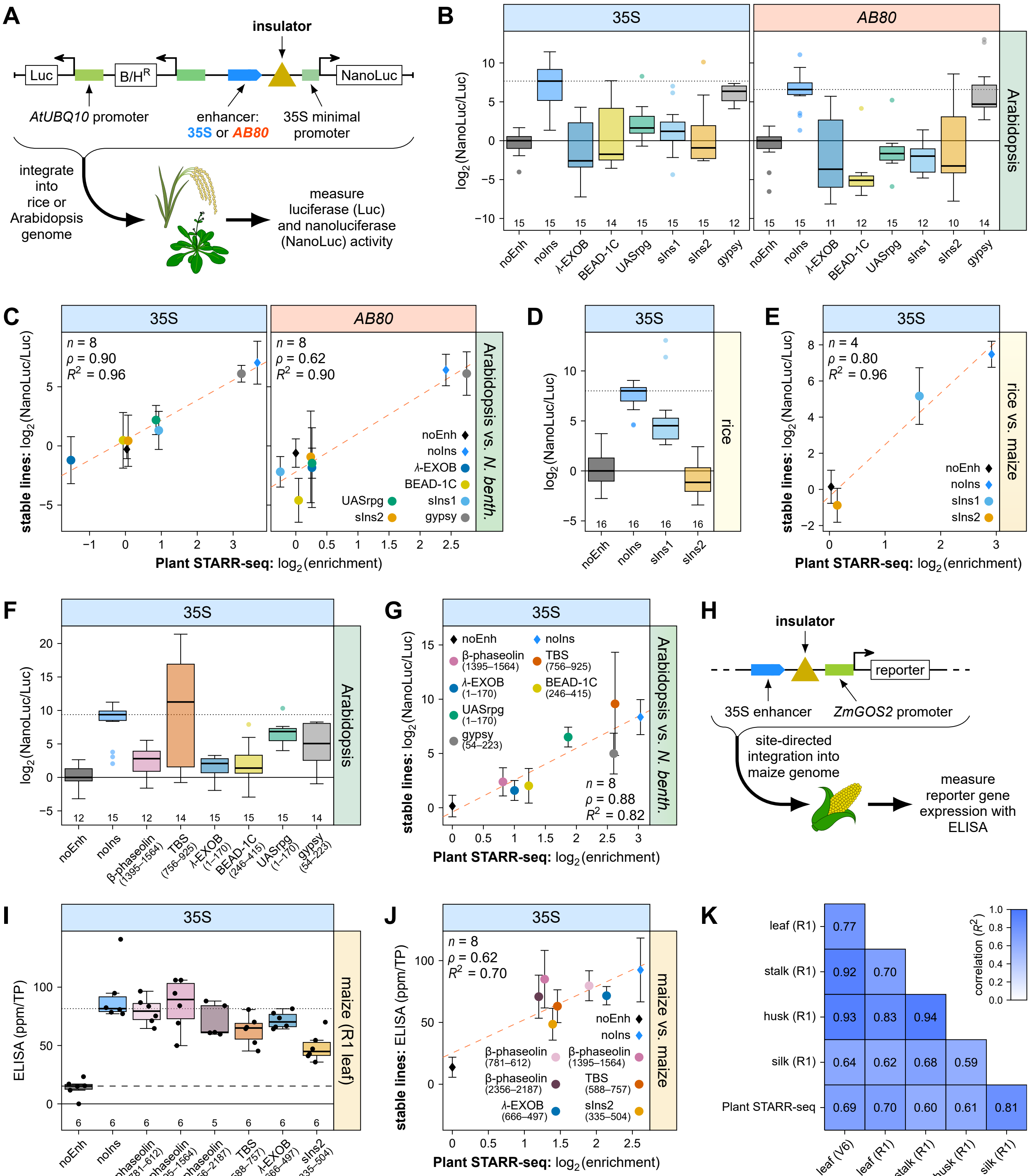

A
insulator
Luc
B/H^R
NanoLuc
AtUBQ10 promoter
enhancer: 35S or AB80
35S minimal promoter
integrate into rice or Arabidopsis genome
measure luciferase (Luc) and nanoluciferase (NanoLuc) activity
B
35S
AB80
Arabidopsis
log2(NanoLuc/Luc)
noEnh
noIns
λ-EXOB
BEAD-1C
UASrpg
sIns1
sIns2
gypsy
C
35S
AB80
n = 8
ρ = 0.90
R2 = 0.96
n = 8
ρ = 0.62
R2 = 0.90
Arabidopsis vs. N. benth.
stable lines: log2(NanoLuc/Luc)
Plant STARR-seq: log2(enrichment)
D
35S
rice
E
35S
n = 4
ρ = 0.80
R2 = 0.96
rice vs. maize
F
35S
Arabidopsis
β-phaseolin (1395–1564)
TBS (756–925)
λ-EXOB (1–170)
BEAD-1C (246–415)
UASrpg (1–170)
gypsy (54–223)
G
35S
n = 8
ρ = 0.88
R2 = 0.82
Arabidopsis vs. N. benth.
H
insulator
reporter
35S enhancer
ZmGOS2 promoter
site-directed integration into maize genome
measure reporter gene expression with ELISA
I
35S
maize (R1 leaf)
ELISA (ppm/TP)
β-phaseolin (781–612)
β-phaseolin (1395–1564)
β-phaseolin (2356–2187)
TBS (588–757)
λ-EXOB (666–497)
sIns2 (335–504)
J
35S
n = 8
ρ = 0.62
R2 = 0.70
maize vs. maize
stable lines: ELISA (ppm/TP)
K
correlation (R2)
leaf (R1)
stalk (R1)
husk (R1)
silk (R1)
Plant STARR-seq
leaf (V6)
0.77
0.92
0.70
0.93
0.83
0.94
0.64
0.62
0.68
0.59
0.69
0.70
0.60
0.61
0.81

Figure 2. Insulators are active in stable transgenic lines in Arabidopsis, rice, and maize. **A)** Transgenic Arabidopsis and rice lines were generated with T-DNAs harboring a constitutively expressed luciferase (Luc) gene and a nanoluciferase (NanoLuc) gene under control of a 35S minimal promoter coupled to the 35S or *AB80* enhancer (as indicated above the plots) with insulator candidates inserted between the enhancer and promoter. Nanoluciferase activity was measured in at least 4 plants from these lines and normalized to the activity of luciferase. The NanoLuc/Luc ratio was normalized to a control construct without an enhancer or insulator (noEnh; log2 set to 0). **B, C)** The activity of full-length insulators was measured in Arabidopsis lines (**B**) and compared to the corresponding results from Plant STARR-seq in *N. benthamiana* leaves (**C**). **D, E)** The activity of synthetic full-length insulators was measured in rice lines (**D**) and compared to the corresponding results from Plant STARR-seq in maize protoplasts (**E**). **F, G)** The activity of insulator fragments was measured in Arabidopsis lines (**F**) and compared to the corresponding results from Plant STARR-seq in *N. benthamiana* leaves (**G**). **H)** For transgenic maize lines, a reporter gene driven by the constitutive, moderate-strength *ZmGOS2* promoter and an upstream 35S enhancer was created and insulator fragments were inserted between the enhancer and promoter. The reporter gene cassette was inserted in the maize genome by site-directed integration and the expression of the reporter gene was measured in various tissues/developmental stages by ELISA. **I, J)** The activity of insulator fragments was measured in R1 leaves of transgenic maize lines (**I**) and compared to the corresponding results from Plant STARR-seq in maize protoplasts (**J**). **K)** Correlation (Pearson's R^2) between the expression of all tested constructs across different tissues and developmental stages. The correlation with Plant STARR-seq results from maize protoplasts is also shown. Box plots in (**B**), (**D**), (**F**), and (**I**) represent the median (center line), upper and lower quartiles (box limits), 1.5× interquartile range (whiskers), and outliers (points) for all corresponding samples from two to three independent replicates. Numbers at the bottom of each box plot indicate the number of samples in each group. For groups with less than 10 samples, individual data points are shown as black dots. In (**C**), (**E**), (**G**), and (**J**), the dashed line represents a linear regression line and error bars represent the 95% confidence interval. Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated. The dotted line in (**B**), (**D**), (**F**) and (**I**) represents the median enrichment of a control construct without an insulator, and the dashed line in (**I**) represents the median enrichment of a control construct without an insulator and without the 35S enhancer.

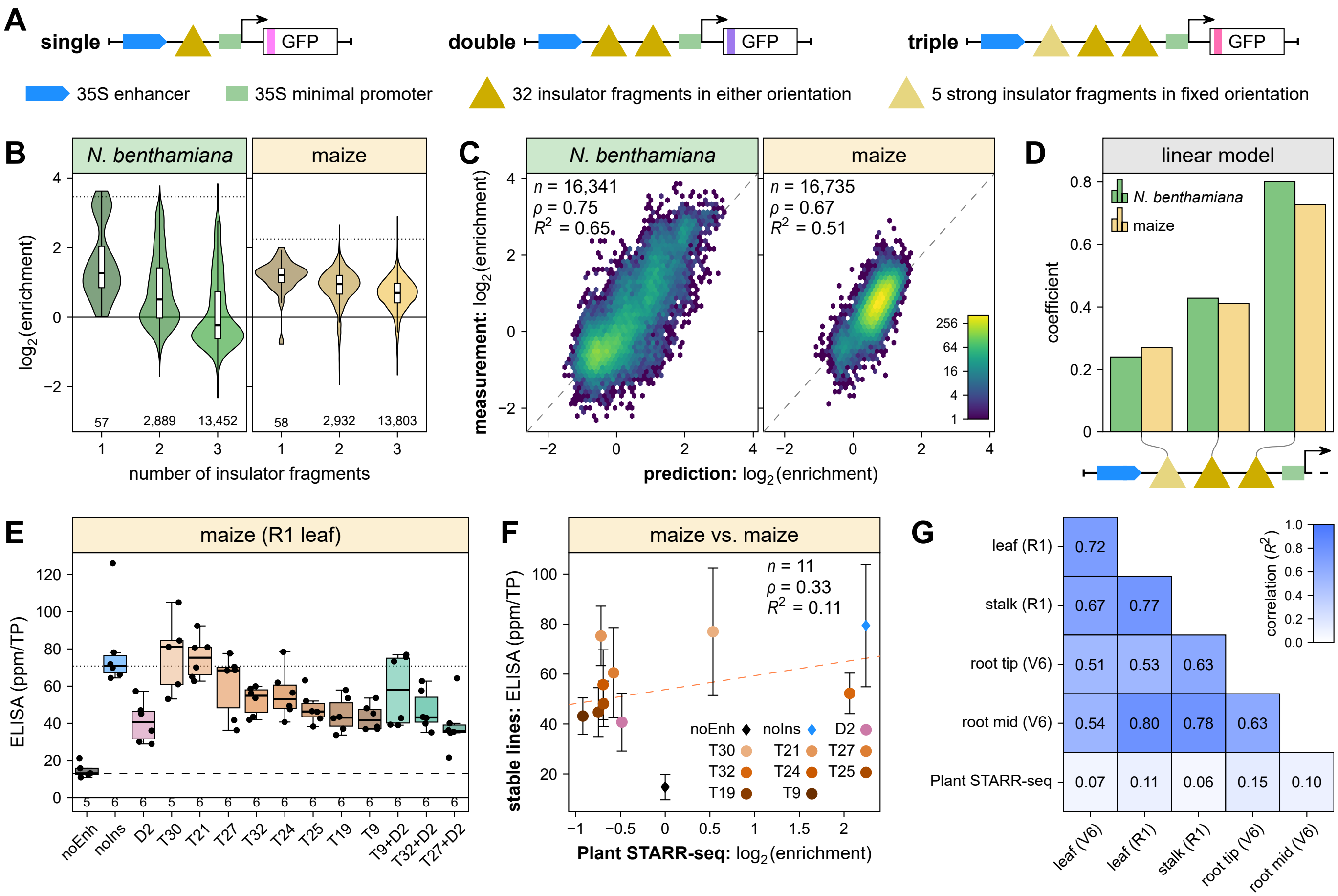


Figure 3. Insulator fragments can be stacked to create very strong enhancer-blocking insulators. **A)** One, two, or three 170-bp fragments of known insulators were cloned between a 35S enhancer and a 35S minimal promoter driving the expression of a barcoded GFP reporter gene. **B)** All insulator constructs were pooled and subjected to Plant STARR-seq in *N. benthamiana* leaves (*N. benthamiana*) and maize protoplasts (maize). Reporter mRNA enrichment was normalized to a control construct without an enhancer or insulator (log2 set to 0). Violin plots are as defined in Figure 1B. **C)** A linear model was trained to predict the enrichment of stacked insulator constructs based on the activity of individual insulator fragments and their position within the construct. The correlation between the model's prediction (prediction) and experimentally determined enrichment values (measurement) is shown as a hexbin plot (color represents the count of points in each hexagon). Pearson's R^2, Spearman's ρ, and number (n) of fragments are indicated. **D)** Coefficients assigned by the linear model to insulator fragments in the indicated positions of the stacked constructs. **E, F)** The activity of insulator fragment combinations in constructs as in Figure 2H was measured in R1 leaves of transgenic maize lines (**E**) and compared to the corresponding results from Plant STARR-seq in maize protoplasts (**F**). Box plots are as defined in Figure 2. The enrichment of a control construct without an insulator (noIns) is indicated as a dotted line. In (**F**), the dashed line represents a linear regression line and error bars represent the 95% confidence interval. Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated. **G)** Correlation (Pearson's R^2) between the expression of all tested constructs across different tissues and developmental stages. The correlation with Plant STARR-seq results from maize protoplasts is also shown. The dotted line in (**B**) and (**E**) represents the enrichment of a control construct without an insulator, and the dashed line in (**E**) represents the enrichment of a control construct without an insulator and without the 35S enhancer.

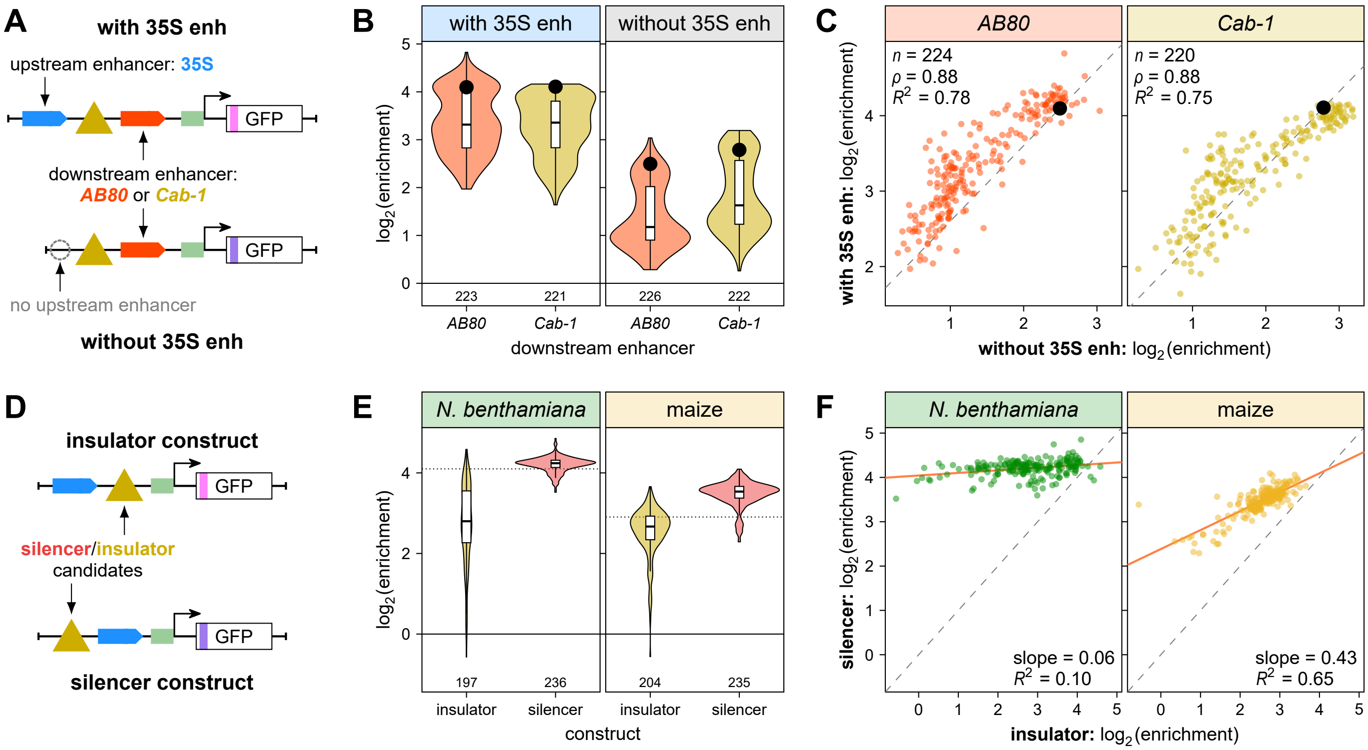


Figure 4. Insulators exhibit silencer activity in some contexts. **A)** Insulator fragments (yellow triangle) were cloned upstream of a *AB80* or *Cab-1* enhancer and a 35S minimal promoter (green rectangle) driving the expression of a barcoded GFP reporter gene. Half of the constructs also harbored a 35S enhancer upstream of the insulator fragments (with 35S enh) while the other half lacked an upstream enhancer (without 35S enh). **B)** All constructs were pooled and subjected to Plant STARR-seq in *N. benthamiana* leaves. Reporter mRNA enrichment was normalized to a control construct without an enhancer or insulator (noEnh; log2 set to 0). The enrichment of a control construct without an insulator is indicated as a black dot. **C)** Correlation between insulator fragment activity in constructs with or without the upstream 35S enhancer. The dashed line represents a y = x line fitted through the point corresponding to a control construct without an insulator (black dot). **D)** Insulator fragments (yellow triangle) were cloned in between (insulator construct) or upstream of (silencer construct) a 35S enhancer (blue arrow) and a 35S minimal promoter (green rectangle) driving the expression of a barcoded GFP reporter gene. **E)** All constructs were pooled and subjected to Plant STARR-seq in *N. benthamiana* leaves (*N. benthamiana*) or maize protoplasts (maize). Reporter mRNA enrichment was normalized to a control construct without an enhancer or insulator (noEnh; log2 set to 0). The enrichment of a control construct without an insulator is indicated as a dotted line. **F)** Comparison of the enrichment of insulator fragments in insulator or silencer constructs. A linear regression line is shown as a solid line and its slope and goodness-of-fit (R^2) is indicated. Violin plots in (**B**) and (**E**) are as defined in Figure 1B.

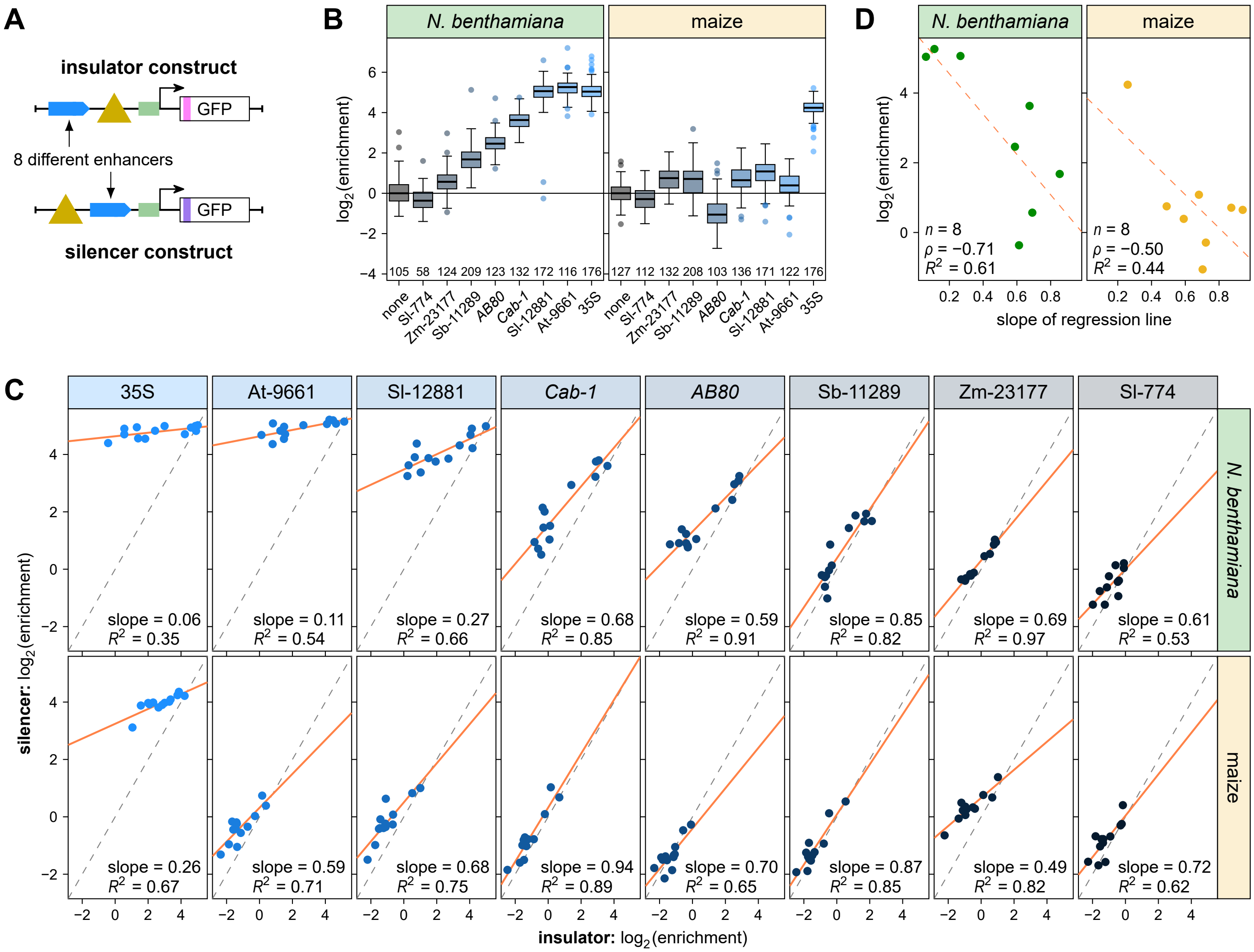
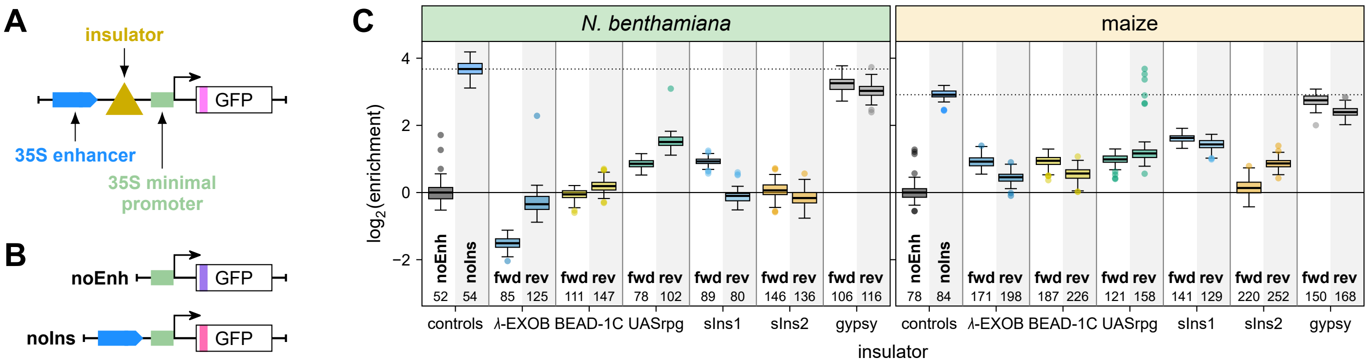
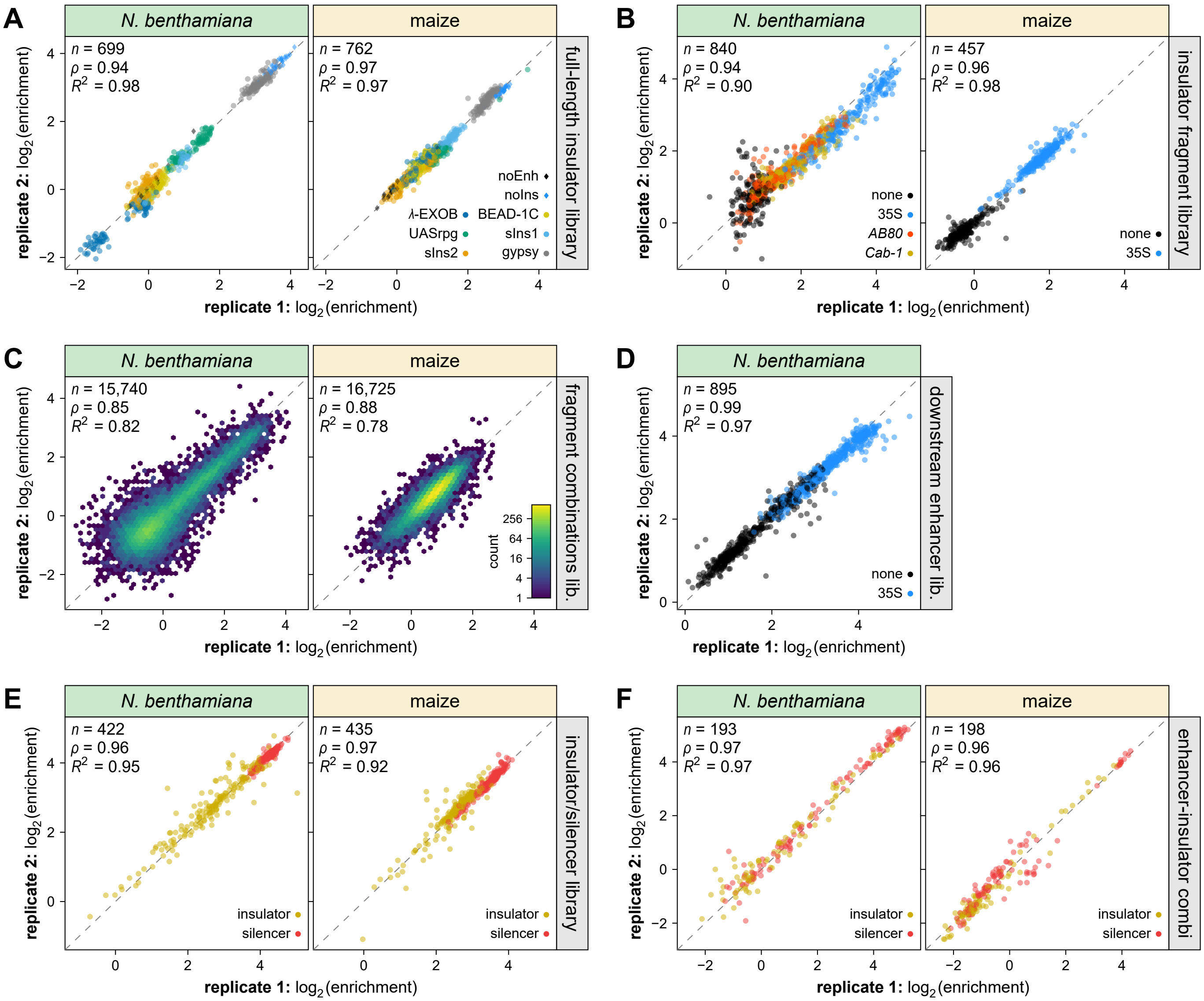


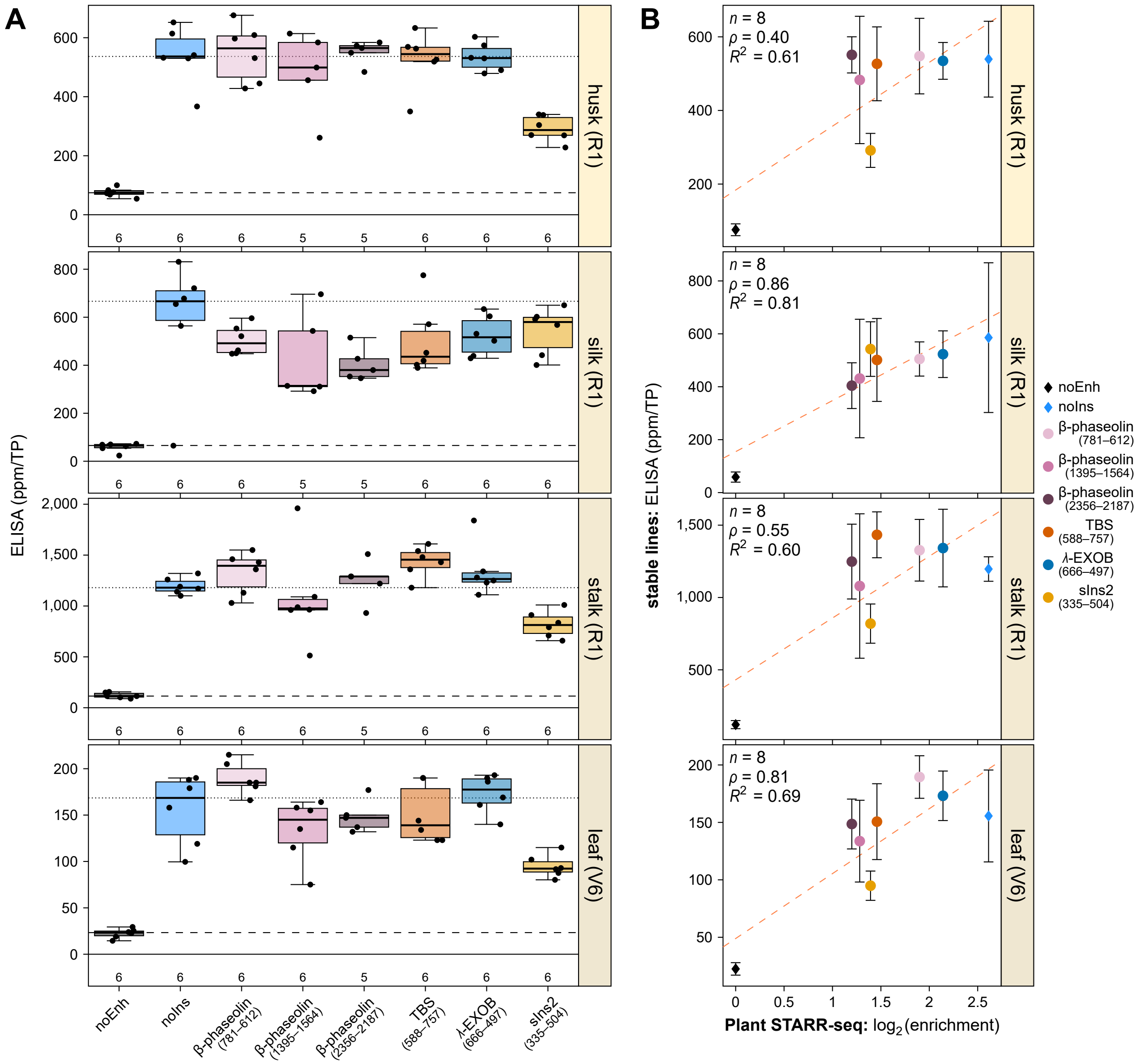
Figure 5. Silencer activity depends on enhancer strength. **A)** Selected insulators and insulator fragments (yellow triangle) were cloned in between (insulator construct) or upstream of (silencer construct) an enhancer and a 35S minimal promoter (green rectangle) driving the expression of a barcoded GFP reporter gene. Eight different enhancers were used to build these constructs. All constructs were pooled and subjected to Plant STARR-seq in *N. benthamiana* leaves (*N. benthamiana*) or maize protoplasts (maize). **B)** Strength of the eight enhancers in constructs without an insulator. Reporter mRNA enrichment was normalized to a control construct without an enhancer (none; log2 set to 0). Box plots represent the median (center line), upper and lower quartiles, and 1.5× interquartile range (whiskers) for all corresponding barcodes from two independent replicates. Numbers at the bottom of the plot indicate the number of samples in each group. **C)** Comparison of the enrichment of insulators and insulator fragments in insulator or silencer constructs. A linear regression line is shown as a solid line and its slope and goodness-of-fit (R^2) is indicated. **D)** Correlation between the slope of the regression lines from (**C**) and the strength of the corresponding enhancer from (**B**). Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated. A linear regression line is shown as a dashed line.



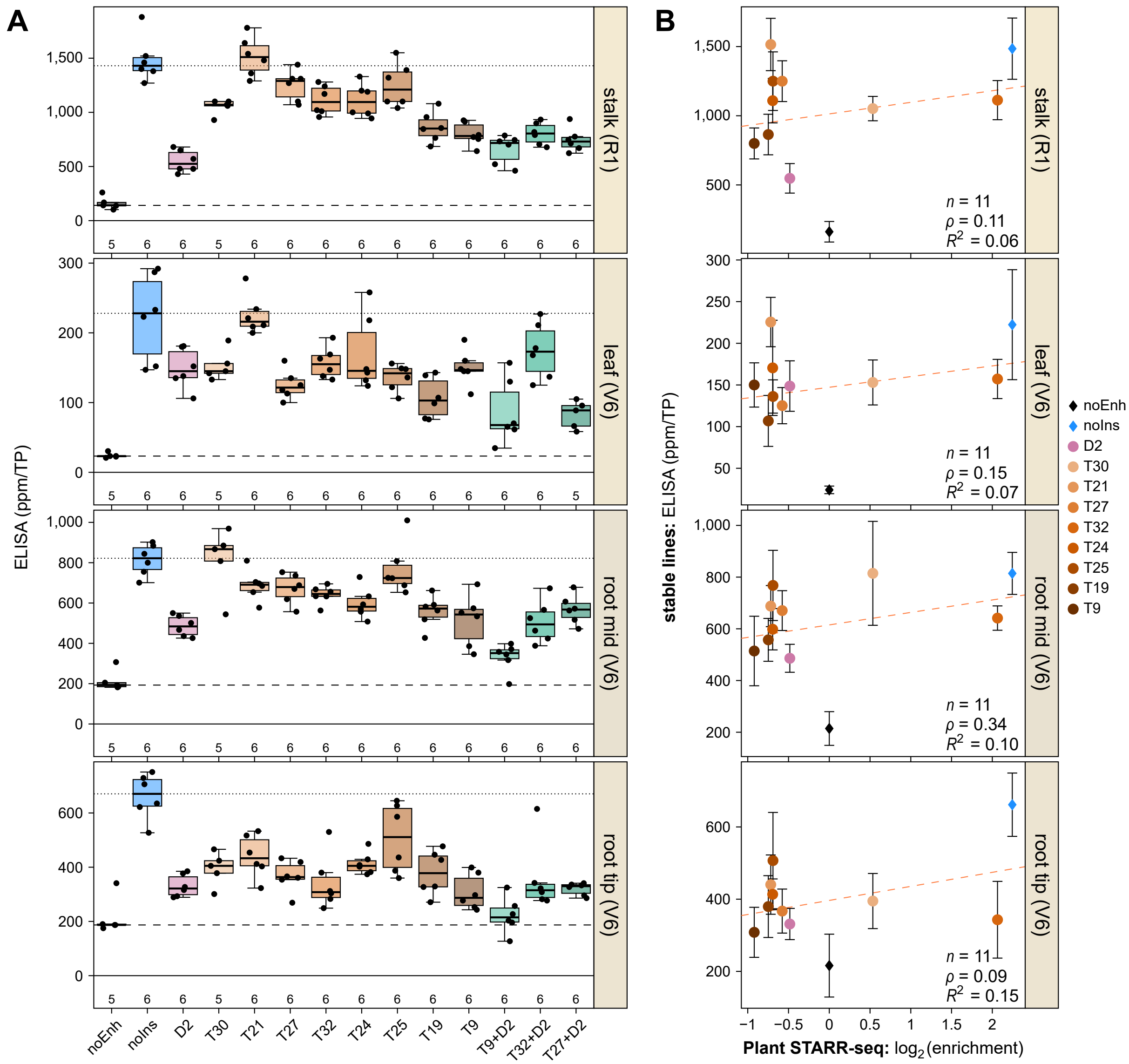
Supplementary Figure S1. Plant STARR-seq detects activity of enhancer-blocking insulators (Supports Figure 1). **A)** Full-length insulators were cloned in the forward (fwd) or reverse (rev) orientation between a 35S enhancer and a 35S minimal promoter driving the expression of a barcoded GFP reporter gene. **B)** In all experiments, control constructs as in (**A**) but without an insulator (noIns) or without an insulator and without an enhancer (noEnh) were added to the library. **C)** All insulator constructs were pooled and subjected to Plant STARR-seq in *N. benthamiana* leaves (*N. benthamiana*) and maize protoplasts (maize). Reporter mRNA enrichment was normalized to a control construct without an enhancer or insulator (noEnh; log2 set to 0). Box plots represent the median (center line), upper and lower quartiles, and 1.5× interquartile range (whiskers) for all corresponding barcodes from two independent replicates. Numbers at the bottom of the plot indicate the number of samples in each group. The enrichment of a control construct without an insulator (noIns) is indicated as a dotted line.



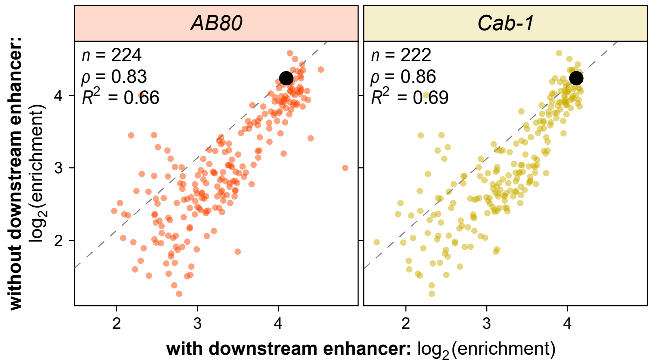
Supplementary Figure S2. Plant STARR-seq yields highly reproducible results (Supports all figures). **A–F)** Correlation between biological replicates of Plant STARR-seq for the full-length insulator library used in Supplementary Figure S1 (**A**), the insulator fragment library used in Figure 1 (**B**), the insulator fragment combination library used in Figure 3 (**C**), the downstream enhancer library (**D**) and the insulator/silencer library (**E**) used in Figure 4, and the enhancer-insulator combination library used in Figure 5 (**F**). Experiments were performed in *N. benthamiana* leaves (*N. benthamiana*) or maize protoplasts (maize) as indicated. Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated. The color in the hexbin plots in (**C**) represents the count of points in each hexagon.



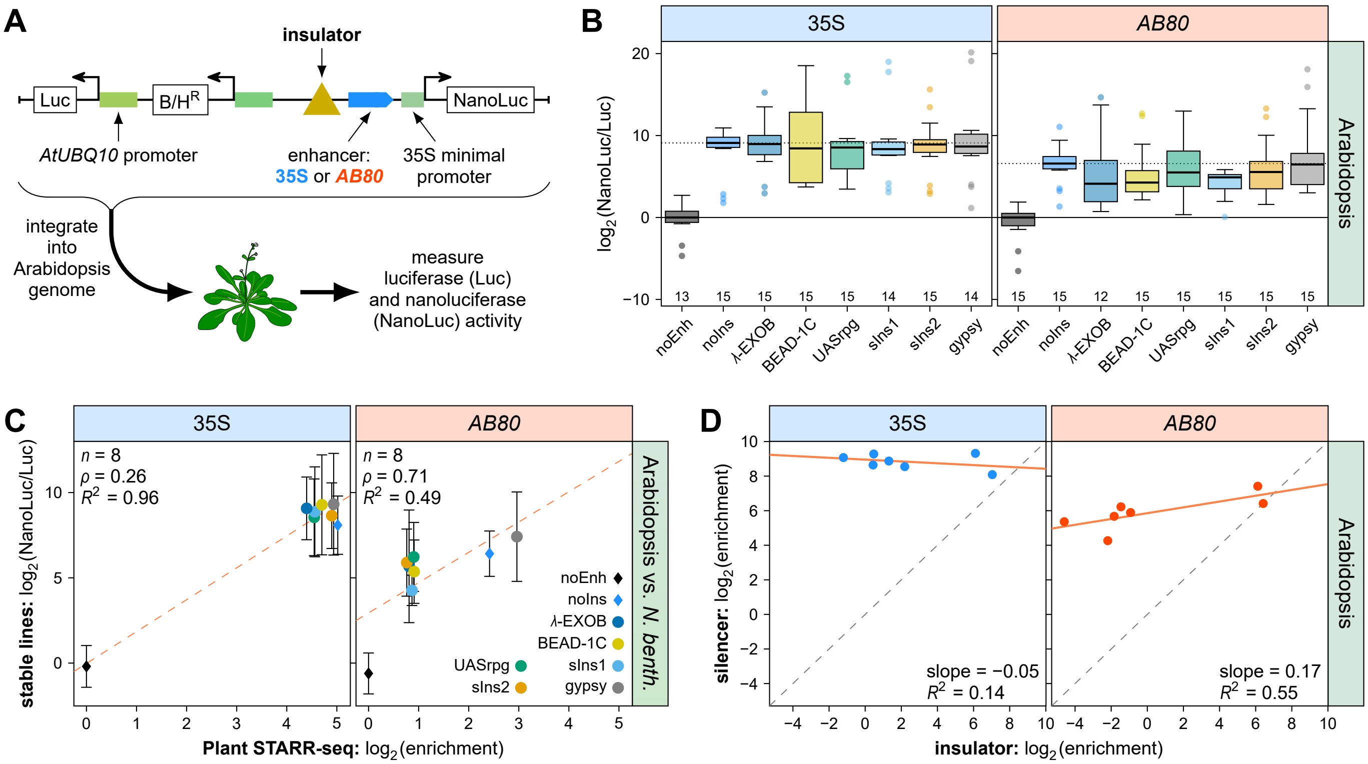
Supplementary Figure S3. Activity of insulator fragments in different maize tissues (Supports Figure 2). **A, B)** Transgenic maize lines were created using constructs as in Figure 2H. The activity of insulator fragments was measured in the indicated tissues (**A**) and compared to the corresponding results from Plant STARR-seq in maize protoplasts (**B**). Box plots in (**A**) represent the median (center line), upper and lower quartiles (box limits), 1.5× interquartile range (whiskers), and outliers (points) for all corresponding samples from two to three independent replicates. Numbers at the bottom of each box plot indicate the number of samples in each group. For groups with less than 10 samples, individual data points are shown as black dots. The dotted and dashed lines in (**A**) represent the median enrichment of control constructs without an insulator or without an enhancer, respectively. In (**B**), the dashed line represents a linear regression line and error bars represent the 95% confidence interval. Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated.



Supplementary Figure S4. Activity of insulator fragment combinations in different maize tissues (Supports Figure 3). **A, B)** Transgenic maize lines were created using insulator fragment combinations in constructs as in Figure 2H. The activity of insulator fragments was measured in the indicated tissues (**A**) and compared to the corresponding results from Plant STARR-seq in maize protoplasts (**B**). In (**A**), box plots are as defined in Supplementary Figure S3, and the dotted and dashed lines represent the median enrichment of control constructs without an insulator or without an enhancer, respectively. In (**C**), the dashed line represents a linear regression line and error bars represent the 95% confidence interval. Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated.



Supplementary Figure S5. Enhancers downstream of insulator fragments slightly reduce their activity (Supports Figure 4). Correlation between the activity of insulator fragments cloned between a 35S enhancer and a 35S minimal promoter with or without an additional *AB80* or *Cab-1* enhancer inserted between the insulator fragment and 35S minimal promoter. The dashed line represents a y = x line fitted through the point corresponding to a control construct without an insulator (black dot). Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated.



Supplementary Figure S6. Enhancer-dependent silencer activity in stable transgenic plants (Supports Figure 5). **A)** Transgenic Arabidopsis lines were generated with T-DNAs harboring a constitutively expressed luciferase (Luc) gene and a nanoluciferase (NanoLuc) gene under control of a 35S minimal promoter coupled to the 35S or *AB80* enhancer (as indicated above the plots) with insulator candidates inserted upstream of the enhancer. Nanoluciferase activity was measured in at least 4 plants from these lines and normalized to the activity of luciferase. The NanoLuc/Luc ratio was normalized to a control construct without an enhancer or insulator (noEnh; log2 set to 0). **B, C)** The activity of full-length insulators was measured in Arabidopsis lines (**B**) and compared to the corresponding results from Plant STARR-seq in *N. benthamiana* leaves (**C**). Box plots in (**B**) are as defined in Supplementary Figure S3 and the dotted line indicates the median activity of a control construct without an insulator. In (**C**), the dashed line represents a linear regression line and error bars represent the 95% confidence interval. Pearson's R^2, Spearman's ρ, and number (n) of constructs are indicated. **D)** Comparison of the mean NanoLuc/Luc ratio of full-length insulators in insulator (Figure 2B) or silencer constructs (**B**). A linear regression line is shown as a solid line and its slope and goodness-of-fit (R^2) is indicated.

Supplementary Table S1. Insulator fragments used in fragment combination library. Positions are numbered by increasing distance from the minimal promoter (position 1 is the fragment closest to the promoter, position 3 the most distal one).

insulator	start	stop	orientation	insulator activity in *N. benthamiana*
		fragments for position 1 and 2		
β-phaseolin	230	399	fwd and rev	bottom 25% (both orientations)
β-phaseolin	383	552	fwd and rev	bottom 25% (both orientations)
β-phaseolin	1148	1317	fwd and rev	top 25% (rev orientation)
β-phaseolin	1317	1486	fwd and rev	top 25% (rev orientation)
β-phaseolin	1395	1564	fwd and rev	top 25% (both orienations)
β-phaseolin	1633	1802	fwd and rev	top 25% (fwd orientation)
β-phaseolin	1712	1881	fwd and rev	top 25% (both orienations)
β-phaseolin	1791	1960	fwd and rev	top 25% (both orienations)
β-phaseolin	2266	2435	fwd and rev	top 25% (both orienations)
β-phaseolin	2345	2514	fwd and rev	top 25% (fwd orientation)
β-phaseolin	2741	2910	fwd and rev	top 25% (fwd orientation)
β-phaseolin	3058	3227	fwd and rev	top 25% (both orienations)
β-phaseolin	3454	3623	fwd and rev	bottom 25% (both orientations)
TBS	252	421	fwd and rev	top 25% (both orienations)
TBS	588	757	fwd and rev	top 25% (both orienations)
TBS	756	925	fwd and rev	bottom 25% (both orientations)
TBS	1681	1850	fwd and rev	top 25% (both orienations)
TBS	1765	1934	fwd and rev	top 25% (rev orientation)
λ-EXOB	1	170	fwd and rev	top 25% (both orienations)
λ-EXOB	83	252	fwd and rev	top 25% (both orienations)
λ-EXOB	166	335	fwd and rev	top 25% (both orienations)
λ-EXOB	249	418	fwd and rev	top 25% (both orienations)
λ-EXOB	332	501	fwd and rev	top 25% (both orienations)
λ-EXOB	415	584	fwd and rev	top 25% (both orienations)
λ-EXOB	663	832	fwd and rev	top 25% (rev orientation)
λ-EXOB	829	998	fwd and rev	top 25% (fwd orientation)
BEAD-1C	246	415	fwd and rev	top 25% (both orienations)
UASrpg	157	326	fwd and rev	top 25% (fwd orientation)
sIns1	54	223	fwd and rev	top 25% (rev orientation)
sIns2	335	504	fwd and rev	top 25% (both orienations)
gypsy	1	170	fwd and rev	bottom 25% (both orientations)
gypsy	54	223	fwd and rev	bottom 25% (both orientations)
		fragments for position 3		
β-phaseolin	1633	1802	fwd	top 5%
β-phaseolin	1712	1881	rev	top 5%
λ-EXOB	1	170	fwd	top 5%
λ-EXOB	332	501	fwd	top 5%
λ-EXOB	415	584	rev	top 5%

Supplementary Table S2. Insulator fragment combinations tested in stable transgenic maize lines. Positions are numbered by increasing distance from the minimal promoter (position 1 is the fragment closest to the promoter, position 3 the most distal one).

name	fragments	position 3	position 2	position 1	insulator activity in maize
D2	2		β-phaseolin 1148-1317, fwd	sIns2 335-504, fwd	strong
T30	3	β-phaseolin 1633-1802, fwd	λ-EXOB 663-832, fwd	β-phaseolin 1564-1395, rev	intermediate
T21	3	β-phaseolin 1881-1712, rev	λ-EXOB 663-832, fwd	λ-EXOB 170-1, rev	weak
T27	3	λ-EXOB 1-170, fwd	λ-EXOB 584-415, rev	TBS 925-756, rev	strong
T32	3	λ-EXOB 584-415, rev	λ-EXOB 832-663, rev	β-phaseolin 1960-1791, rev	strong
T24	3	λ-EXOB 584-415, rev	β-phaseolin 3227-3058, rev	λ-EXOB 170-1, rev	strong
T25	3	λ-EXOB 1-170, fwd	β-phaseolin 1802-1633, rev	β-phaseolin 1317-1148, rev	strong
T19	3	λ-EXOB 1-170, fwd	β-phaseolin 1148-1317, fwd	sIns2 335-504, fwd	strong
T9	3	λ-EXOB 584-415, rev	TBS 1765-1934, fwd	sIns2 335-504, fwd	strong

Supplementary Table S3. Insulators and insulator fragments used in the enhancer-insulator combination library.

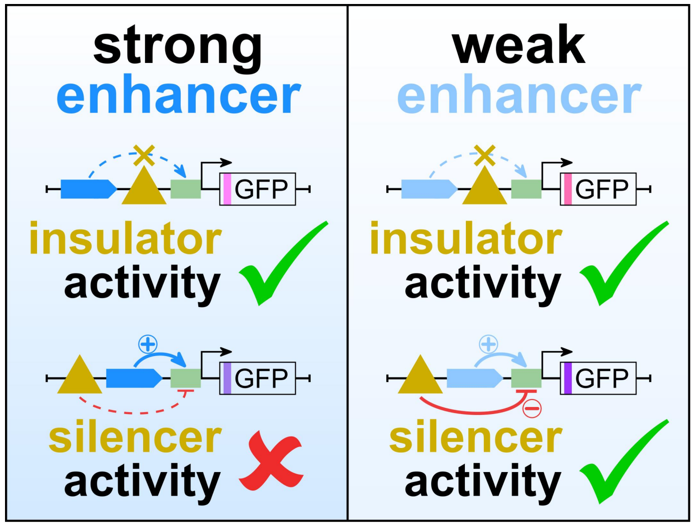
type	insulator	start	stop	orientation
full-length insulator	λ-EXOB	1	998	fwd
full-length insulator	BEAD-1C	1	538	fwd
full-length insulator	UASrpg	1	378	fwd
full-length insulator	sIns1	1	386	fwd
full-length insulator	sIns2	1	504	fwd
full-length insulator	gypsy	1	386	fwd
insulator fragment	β-phaseolin	1395	1564	fwd
insulator fragment	TBS	756	925	fwd
insulator fragment	λ-EXOB	1	170	fwd
insulator fragment	BEAD-1C	246	415	fwd
insulator fragment	UASrpg	1	170	fwd
insulator fragment	gypsy	54	223	fwd

Supplementary Table S4. Full-length insulator sequences.

insulator	sequence
β-phaseolin	1 CATAAGAAAT ATGAAAATCG TTATGAACTT TATTATTTGT TAAACGTTTT CATAACCGCA TAAAATTTTA TAAAGTCCCG 81 TCTATCTTTA ATATGTAGTC TAACATTTTC ATATTGAAAT ATATAATTTA CTTAATTTTA GTGTTGGTAG AAAGCATAAT 161 GATTTATTCT TGTTCATATA AATGTTTAAT ATAAACAAAC TCTTTACCTT AAGAAGGATT TCCCATTTTA TATTTTAAAA 241 ATATATTTAT CAAATATTTT TCAACCACGT AAATCTCATA ATAATAAGTT GTTTCAAAAG TAATAAAATT TAACTCCATA 321 ATTTTTTTAT TTGACTGATC TTAAAGCAAC ACCCAGTGAC ACAACTAGTA ATTTTTTTCT TTGAATAAAA AAATCCAATT 401 ATCATTGTAT TTTTTTTATA CAATGAAAAT TTCACCAAAC AATGATTTGT GGTATTTCTG AAGCAAGTCA TGTTAATGCA 481 AAATTCTATA ATTCACATTT GACACTACGG AAGTGACTGA AAATCTGTTT TTACATGCGA GACACATCAA TTTTTAATTC 561 TAAAGTAATT TTAATAATAG TTACTATATT CAAGATTTGA TATATCAAAT ACTCAATATT ACTTCTAAAA AATTAATTAG 641 ATATAATTAA AAAATTACTT TTTTAATTTT AAGTTTAATT GTTGGATTTG TGACTATTGA TTTATTATTC TACTATGTTT 721 AAACTGTTTT ATAGATAGTT TAAAGTAAAT ATAAGTATTG TAGAGTGTTA CCGTAAACTA TAAGATTTAT GTTGGACTAA 801 TTTTATGTTC TTCATTTGCA ATATTTTAAT ATATTTGTTG TTGGTTTACC TTTCTTGGTA TGTAAGTCCG TAACCAGAAT 881 TACTGTGGGT TGCCATGGCA CTCTGTAGTC TTTTGGTTCG TGCATGGATG CTTGCGCAAG AAAAAGACAT AGAACAAAGA 961 AAAAAGACAA AACAGAGAGA GAAAACGCAA TCACACAACC AACTCAAATT AGTCACTGGC TGATCAAGAT CGCCGCGTCC 1041 ATGTATGTCT AAATGCCATG CAAAGCAACA CGTGCTTAAC ATGCACTTTA AATGGCTCAC CCATCTCAAC CCACACACAA 1121 ACACATTGTC TTTTTCTTCA TCATCACCAC AACCACCTGT ATATATTCAT TCTCTTCCGC CACCTCAATT TCTTCACTTC 1201 AACACACGTC AACCTGCATA TGCGTGTCAT CCCATGCCCA AATCTCCATG CATGTTCCAA CCACCTTCTC TCTTATATAA 1281 TACCTATAAA TACCCCTAAT ATCACTCACT TCTTTCATCA TCCATCCATC CAGAGTACTA CTACTCTACT ACTATAATAC 1361 CCCAACCCAA CTCATATTCA ATACTACTCT ACTATGATGA GAGCAAGGGT TCCACTCCTG TTGCTGGGAA TTCTTTTCCT 1441 GGCATCACTT TCTGCCTCAT TTGCCACTTC ACTCCGGGAG GAGGAAGAGA GCCAAGATAA CCCCTTCTAC TTCAACTCTG 1521 ACAACTCCTG GAACACTCTA TTCAAAAACC AATATGGTCA CATTCGTGTC CTCCAGAGGT TCGACCAACA ATCCAAACGA 1601 CTTCAGAATC TTGAAGACTA CCGTCTTGTG GAGTTCAGGT CCAAACCCGA AACCCTCCTT CTTCCTCAGC AGGCTGATGC 1681 TGAGTTACTC CTAGTTGTCC GTAGTGGTAA GTAATTGCTA CTGGTATCAC TTGTTTCTTC TTGCAGAAAT AATGGTAATG 1761 AGTTTTTTTA TAATTTCAGG GAGCGCCATA CTCGTCTTGG TGAAACCTGA TGATCGCAGA GAGTACTTCT TCCTTACGCA 1841 AGGCGATAAC CCGATATTCT CTGATAACCA GAAAATCCCT GCAGGAACCA TTTTCTATTT GGTTAACCCT GACCCCAAAG 1921 AGGATCTCAG AATAATCCAA CTCGCCATGC CCGTTAACAA CCCTCAGATT CATGTACTGC CTTTTGTAAT ACCAAACTAA 2001 TTTTTTTGTT ATTTTAACTT GCAATTTCTC TCCAAATGTG ATGATAAATG TTTGTCCTGT AGGAATTTTT CCTATCTAGC 2081 ACAGAAGCCC AACAATCCTA CTTGCAAGAG TTCAGCAAGC ATATTCTAGA GGCCTCCTTC AATGTAAGAA AGAAAACAGC 2161 ATCTAACTAC ATATTTGCGT CATCTAACTA CATATTTTCG TTGCCATTTA GCTAGTACTT TGTCTAAATG TCACACTTGT 2241 TGAATTTGTT GAATGATATC ATTATATATG TTTGCATGAT TTTTATAGAG CAAATTCGAG GAGATCAACA GGGTTCTGTT 2321 TGAAGAGGAG GGACAGCAAG AGGGAGTGAT TGTGAACATT GATTCTGAAC AGATTGAGGA ACTGAGCAAA CATGCAAAAT 2401 CTAGTTCAAG GAAATCCCAT TCCAAACAAG ATAACACAAT TGGAAACGAA TTTGGAAACC TGACTGAGAG GACCGATAAC 2481 TCCTTGAATG TGTTAATCAG TTCTATAGAG ATGAAAGAGG TAAATACAAA GAAAAAACAT ATAGACAAAC TTAGCAATTG 2561 AGTTCTATTA TTCACTGTCG TCTTGGTTAG AAAATCTTAG TATTGAGAAT ATAATTAAAT AATGGTTTTT TTTGTTAACA 2641 AATTTAGGGA GCTCTTTTTG TGCCACACTA CTATTCTAAG GCCATTGTTA TACTAGTGGT TAATGAAGGA GAAGCACATG 2721 TTGAACTTGT TGGCCCAAAA GGAAATAAGG AAACCTTGGA ATTTGAGAGC TACAGAGCTG AGCTTTCTAA AGACGATGTA 2801 TTTGTAATCC CAGCAGCATA TCCAGTTGCC ATCAAGGCTA CCTCCAACGT GAATTTCACT GGTTTCGGTA TCAATGCTAA 2881 TAACAACAAT AGGAACCTCC TTGCAGGTAT ATATATTTAT TATATATGAC CATGAATTTG AATATAGGGT TGTTGATGGG 2961 ATTTTTTATT TATAATTGGT AATGCGTGAT TGTGATTGAA AATATGAAGG TAAGACGGAC AATGTCATAA GCAGCATCGG 3041 TAGAGCTCTG GACGGTAAAG ACGTGTTGGG GCTTACGTTC TCTGGGTCTG GTGAAGAAGT TATGAAGCTG ATCAACAAGC 3121 AGAGTGGATC GTACTTTGTG GATGGACACC ATCACCAACA GGAACAGCAA AAGGGAAGTC ACCAACAGGA ACAGCAAAAG 3201 GGAAGAAAGG GTGCATTTGT GTACTGAATA AGTATGAACT AAAATGCATG TATGGTGTAA GAGCTCATGG AGAGCATGGA 3281 AATATGTATC AGACCATGTA ACACTATAAT AACTGAGCTC CATCTCACTT CTTCTATGAA TAAACAAAGG ATGTTATGAT 3361 ATATTAACAC TATATGCACC TTACATAGTA ATACATTAAT ATTTAATACT TTTTATTTTA ACTTTTTAGT TTAAAATATT 3441 ATTATATTAT TAACTTTTTA GTTTAAAATA TTTATATTAT TATAAAGAGA AATAAACAAA GGATGTTATG ATATATTAAC 3521 ACTATATGTA CCTTACATAG TAATATATTA ATATTTAATA CTTTTTATTT TAACTTTTTA ATTTAAAATA TTATTATAAA 3601 TGATGCTTGT GTTTTATGTG TTGGCATGCT TGTATTTTAT GTGTTGACTT TCTGTGTGAA GGTAATGTGA TATGGTTAGC 3681 TGGTGGTAAC AATTGTGTTT TATGTGTTGG CTTTCTGTGA AGCTAATTTG ATATGGTTAG CTGATGGGAA CAAAATATTA 3761 AAGGAAGCTA ATTTGATATG GTTAGCTGAT AGTAACAAAA TATCAAAATA AATTTCTTCT TACTTTAATA AATTATATGA 3841 ATTGTGACGG ATTATATGGA ATGTATAGGA CAAAATCTTT AATAAATTAC ATGAATTGTG ACGGATTATG GAATGGAATG 3921 TAGCAAATAG GACAAAACAA ATGTTTGTAA GAACCAAGAG ATCCTAACCA TGTATAGGCT AACCATATAT AGGCTTAGGC 4001 CAAAAACAAA CCTAAACTCT TAAACTTTGA TTTATTATAA AAAAAAAATG ATTATTTAAT ATATAGGCTT AGGCCAAAAA 4081 CAAACCTAAA CTCTTAAACT TTGATTTATT ATAAAAAAAA AATGATTATT TAACACCATG CACCTTACAC CCTACTAAAT 4161 TTTAATATTT TAATTATTTT TAATTTTAAA AATTATTTTA ATATTTTACA TTATTTTCAC TAAAAACATT TGTTTTTTTT 4241 ATTATAAATA TTAATATTTT ATTATAGATA TAAAGTGAAG CAATATTGTT TGAAAATATC ATTACCCGTT ACAAAATATT 4321 GTACAACTAG CTATAAAAAA GCAAACCACA AGGAAACAGA AGACTTTCAC TTTGAAAAGG GGTGCCTGCT AAGACCGTAA 4401 TTTTGCTTCA GATAAAAAAC CATCACAACT CAATAGGTAC TCACAAAACT ACTT

insulator	sequence
TBS	1 TTCCTAACAC CTGGAGAACC TTTTATGTAC TTCACAACCC TCAATGCTGC TTCCAGGTGT GACTGTTTGG GTTGCTGCAT 81 GAATTGACTC AGCACCTGCA CTGCAAAGCT GATATCTGGC CTTGTGATTG TCAAGTAGAG AAGCTTTCCC ACCAGTCTCT 161 GATAGGATCC CACATCCTCT AACTCTGCAT CATCAGTCTT TCCTAAGTGC TTGTCATATT CTACAGTAGT CAGCCTTTGG 241 TTCTGTTCCA TTGGGGTTGA CACTGGTCTG CAGCCTCCCA GACCAACACC TGATATCAGT TCCAGTGCAT ACTTCCTCTG 321 GTTCAGTAGG ATTCCCTTTT CTGATCTCAG CACCTCAATG CCTAAGAAGT ATTTTAGTTC TCCCAAATCT TTCATCTTGA 401 AATGCTGATG CAGGGTTGCC TTTGCTTCTG AAATCAAAAC ATTGCTGCTG CCTGTTATTA ACAGATCATC CACATAAATC 481 AGGATTATGA CAAGGTCAGT CCCCTCTCTT TTGGTAAACA AGGAGTGATC ATAAGCACTT TGCATAAAAC CAGCCTGCAT 561 AAGGACAGTG GTAAGCTTGA TGTTCCACTG CCTTGATGCT TGCTTTAAAC CATAGAGGGA TTCAACAGCC TGCACACTTT 641 GGACTCCCCT TGGCTGTGAA AACCCTGAGG CAGAGACATA TAAACTTCTT CCATGAGGTC ACCTTGTAGA AAAGCATTGT 721 TGACATCCAT CTGGAAAAGG AACCAGCCCT TGGAAGCAGC AACAGATATG ACAGCTTTTA CAGTGACCAT TTTGGCCACT 801 GGAGAAAAAG TTTCATGGTA GTCAAGGCCT TCTTGCTGAG TGTATCCCTT GGCCACTAGC CTTGCCTTAA ACCTGTCAAC 881 TTCACCATTA GCTTTGTATT TAATTTTGTA CACCCATTTG GACCCTATAG GCTGTTTACC AGGGGGTAAA GGGACAATCT 961 CCCAGGTGTT ATTATCCTCA AGAGCCTGTA TCTCAAGGGA CATGGCCTCC ATCCATTTCT CATCTTGAGC TGCTTCTTTG 1041 AAGGATTTAG GTTCAGTATA AGTGGAGAAA GCACTCAAAT AAGCTTGATA GTGAACAGGC AAGTGATCAT AGGAAACATA 1121 GTTGGCTATA GGGTATGGAA CATCCCTAGA GCCTTTGTTC AGTGTCACAA AGTCCTTGAG CCAGATGGGA GGACCTGCAT 1201 TGCGTTTAGG TCTAGTGTGC AGGTTTGCTG GAACTAGAGA GGGATCAGCT ACAGCAGTAT GGTGCTCAAA TTCAGCATTT 1281 GCTAGGTCAG GCTCAGCTGA CTCAACTGAC TCTGCAGGAG CATGCAGGTG GCCTGAAGGT GCAGCATCAG CTGAAGTGAT 1361 ATGATTTTGG GTAGAATGAG TCCCCAAAGT GATATCCTCA TTGGCCTCTA CAATGTCAGG AATAAGAACT GGAGTATCAT 1441 CATCATCAGT ATGAAAGCTG GAAGGAAAAA TATCATTATA CACTAGCTGC AAAGCTGTAT CTTCAGAGCT AAGTGAACCT 1521 GCTCGAGTGA ACATATCAGG TTCATGGGAG ATGGACTCTT TGAAAGGGAA CTGAAACTCT CTGAAAACTA CATCCCTGCT 1601 CACTATGATC ACCTTATTAT CCAAGTCATA CAACCTGTAA CCCTTTTGAG TCTCAGAATA ACCAATGAAG ATGGTTCGCT 1681 TGGCTCTAGG TGCAAGTTTG TCACCTTTGG GCAGTGTTGC TGCAAAAGCA AGACACCCAA ACACTCTCAA GTGATCAAGC 1761 TTGGCATGTT TCTGATAGAG TAGTTCATAT GGACATTTGC CTTGTAAGAT TGGAGTAGGG AGTCTATTGA TCAGGTATAC 1841 AACAGTCTTG ACACAGTCTC CCCAAAACCT GGTAGGTACA CCACTCTGAA ACTTAAGTGC CCTTGCCATC TCAAGGATGT 1921 GTCTGTGCTT TCTCTCCACA ACACCATTCT GTTGTGGTGT GTAGGGACAG CTACTTTGAT GAACAATCCC AAGAGAGGCC 2001 AGCAACTCAT TACAACTT
λ-EXOB	1 AATTCAAACA GGGTTCTGGC GTCGTTCTCG TACTGTTTTC CCCAGGCCAG TGCTTTAGCG TTAACTTCCG GAGCCACACC 81 GGTGCAAACC TCAGCAAGCA GGGTGTGGAA GTAGGACATT TTCATGTCAG GCCACTTCTT TCCGGAGCGG GGTTTTGCTA 161 TCACGTTGTG AACTTCTGAA GCGGTGATGA CGCCGAGCCG TAATTTGTGC CACGCATCAT CCCCCTGTTC GACAGCTCTC 241 ACATCGATCC CGGTACGCTG CAGGATAATG TCCGGTGTCA TGCTGCCACC TTCTGCTCTG CGGCTTTCTG TTTCAGGAAT 321 CCAAGAGCTT TTACTGCTTC GGCCTGTGTC AGTTCTGACG ATGCACGAAT GTCGCGGCGA AATATCTGGG AACAGAGCGG 401 CAATAAGTCG TCATCCCATG TTTTATCCAG GGCGATCAGC AGAGTGTTAA TCTCCTGCAT GGTTTCATCG TTAACCGGAG 481 TGATGTCGCG TTCCGGCTGA CGTTCTGCAG TGTATGCAGT ATTTTCGACA ATGCGCTCGG CTTCATCCTT GTCATAGATA 561 CCAGCAAATC CGAAGGCCAG ACGGGCACAC TGAATCATGG CTTTATGACG TAACATCCGT TTGGGATGCG ACTGCCACGG 641 CCCCGTGATT TCTCTGCCTT CGCGAGTTTT GAATGGTTCG CGGCGGCATT CATCCATCCA TTCGGTAACG CAGATCGGAT 721 GATTACGGTC CTTGCGGTAA ATCCGGCATG TACAGGATTC ATTGTCCTGC TCAAAGTCCA TGCCATCAAA CTGCTGGTTT 801 TCATTGATGA TGCGGGACCA GCCATCAACG CCCACCACCG GAACGATGCC ATTCTGCTTA TCAGGAAAGG CGTAAATTTC 881 TTTCGTCCAC GGATTAAGGC CGTACTGGTT GGCAACGATC AGTAATGCGA TGAACTGCGC ATCGCTGGCA TCACCTTTAA 961 ATGCCGTCTG GCGAAGAGTG GTGATCAGTT CCTGTGGG
BEAD-1C	1 TTCAGTAATA CGGGTAGCTG GGACATGCCA TATTTGGAAC ACATTTATAC TAAAAAAGTA TTCATTGTTT ATCTGAAATT 81 CAAATTCCAC TGGGCATCCT GTGTTTTATC TGGCAATGCT AGGCATGCAG AATACCAAAA GTAAGCACCA GGCAGGCCAG 161 AGTCCCACCA TGAGCATCTT CAGGGCCCCT GGATGTGGAA GAGGGATGTT GAGGGCCCAG GGGCTGCCTT GCCGGTGCAT 241 TGGCTGCCCA GGCCTGCACT GCCGCCTGCC GGCAGGGGTC CAGTCCACGA GTCCCAGCTC CCTGCTGGCG GAAGTCCATT 321 TCAGAGCTTC CGGTTCTCCC AAGTCCAAGG ATTATGCTCA CTCCCCACCC ACAGTCTCTT AGTGTCTGTC CCTGCTCTAA 401 AGATGTTGTC TGGGCTTGAT ATTAATATGA GAGCTGACTG TTCCCTTCCT GATCTAGACC ATAACCATCT TCAAGTTAAA 481 TTGCTCCTCC TCTTCTAACT GCCCAACCTC ACCCACGTCT GACCATACCC AAGCACAG
UASrpg	1 AGCTTGCCTC GTCCCGCCGG GTCACCCGGC CAGCGACATG GAGGCCCAGA ATACCCTCCT TGACAGTCTT GACGTGCGCA 81 GCTCAGGGGC ATGATGTGAC TGTCGCCCGT ACATTTAGCC CATACATCCC CATGTATAAT CATTTGCATC CATACATTTT 161 GATGGCCGCA CGGCGCGAAC GAAAAATTAC GGCTCCTCGC TGCAGACCTG CGAGCAGGGA AACGCTCCCC TCACAGACGC 241 GTTGAATTCT CCCCACGGCG CGCCCCTGTA GAGAAATATA AAAGGTTAGG ATTTGCCACT GAGGTTCTTC TTTCATATAC 321 TTCCTTTTAA AATCTTGCTA GGATACAGTT CTCACATCAC ATCCGAACAT AAACAAAA
gypsy	1 CACGTAATAA GTGTGCGTTG AATTTATTCG CAAAAACATT GCATATTTTC GGCAAAGTAA AATTTTGTTG CATACCTTAT 81 CAAAAAATAA GTGCTGCATA CTTTTTAGAG AAACCAAATA ATTTTTTATT GCATACCCGT TTTTAATAAA ATACATTGCA 161 TACCCTCTTT TAATAAAAAA TATTGCATAC TTTGACGAAA CAAATTTTCG TTGCATACCC AATAAAAGAT TATTATATTG 241 CATACCCGTT TTTAATAAAA TACATTGCAT ACCCTCTTTT AATAAAAAAT ATTGCATACG TTGACGAAAC AAATTTTCGT 321 TGCATACCCA ATAAAAGATT ATTATATTGC ATACCTTTTC TTGCCATACC ATTTAGCCGA TCAATT

insulator	sequence
sIns1	1 AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG 81 TTCGATGTAA CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG 161 GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC TCTTCCTTTT TCAATATTAT 241 TGAAGCATTT ATCAGGGTTA TTGTCTCATG AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC 321 GCGCACATTT CCCCGAAAAG TGCCACCTGA CGTCCAACAT ATGGCACCGG AGGCTTTCGT CTTCAC
sIns2	1 GCTGAACGCA AAGCTGATCA CTCAGCGGAA GTTCGACAAT CTCACTAAGG CTGAGAGGGG CGGACTGAGC GAACTGGACA 81 AAGCAGGATT CATTAAACGG CAACTTGTGG AGACTCGGCA GATTACTAAA CATGTCGCCC AAATCCTTGA CTCACGCATG 161 AATACCAAGT ACGACGAAAA CGACAAACTT ATCCGCGAGG TGAAGGTGAT TACCCTGAAG TCCAAGCTGG TCAGCGATTT 241 CAGAAAGGAC TTTCAATTCT ACAAAGTGCG GGAGATCAAT AACTATCATC ATGCTCATGA CGCATATCTG AATGCCGTGG 321 TGGGAACCGC CCTGATCAAG AAGTACCCAG CACTGGAAAG CGAGTTCGTG TACGGAGACT ACAAGGTCTA CGACGTGCGC 401 AAGATGATTG CCAAATCTGA GCAGGAGATC GGAAAGGCCA CCGCAAAGTA CTTCTTCTAC AGCAACATCA TGAATTTCTT 481 CAAGACCGAA ATCACCCTTG CAAA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