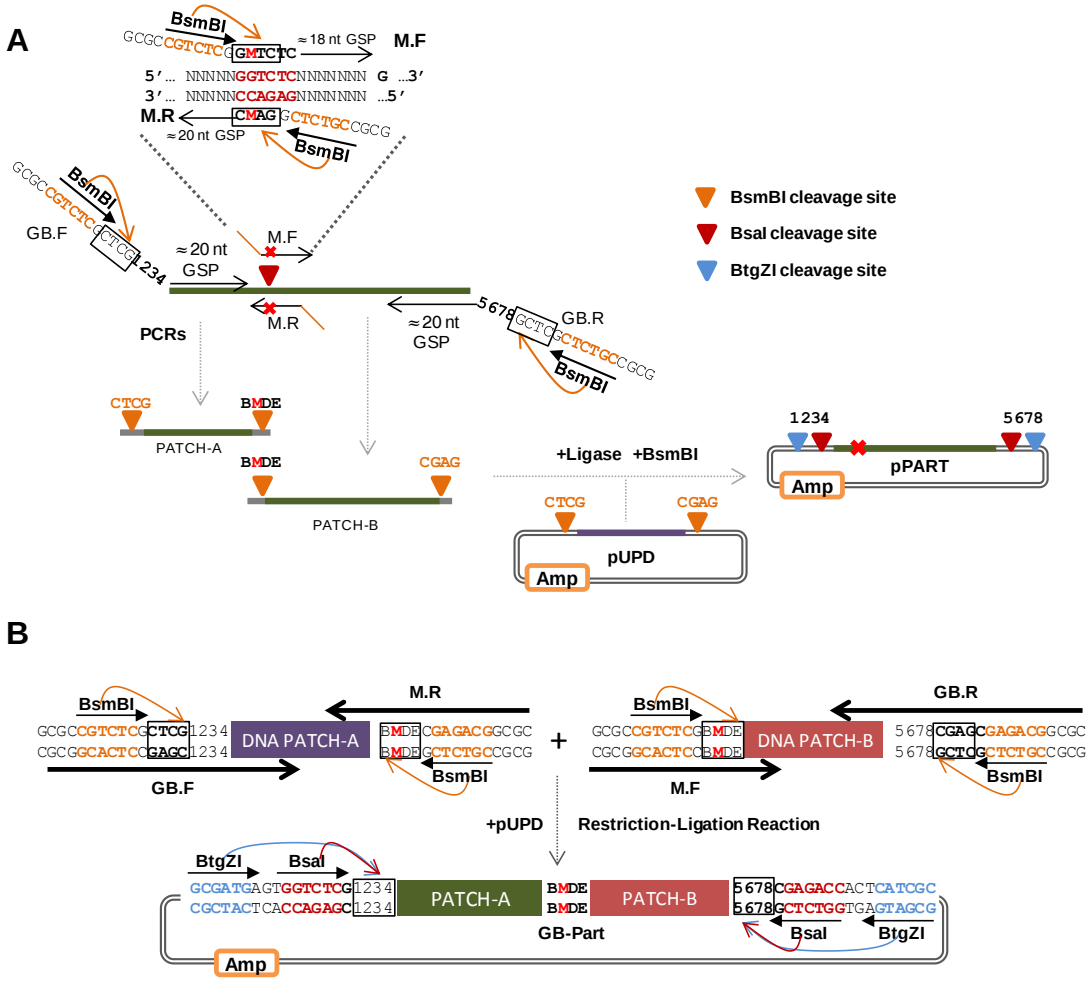
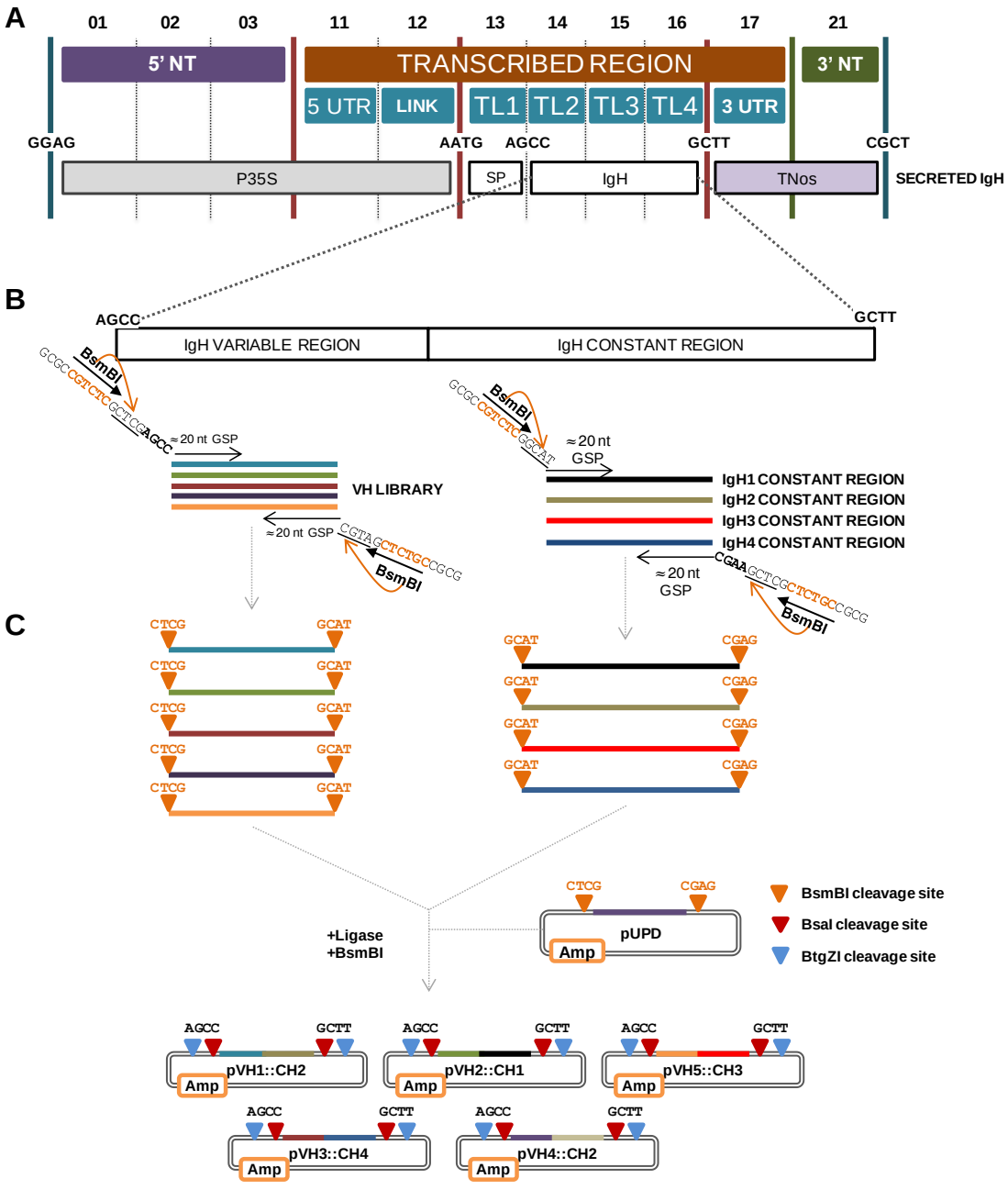
Supplementary Figure 1



Supplementary Figure 1. GB Domestication with the removal of internal restriction sites. (A) Internal Type IIs recognition sites (exemplified here with the GGTCTC BsaI recognition site) are mutagenized during domestication following a standard procedure. In addition to the GB.F and GB.R primers described in Figure 3, two other primers (M.F and M.R) are required here, which incorporate the flanking BsmBI overhangs and the single nucleotide change (C>M). Each oligo pair is used to amplify a GBpatch by PCR, and the resulting fragments are assembled together in a BsmBI restriction-ligation reaction into pUPD. The resulting GBpart is free of internal recognition sites and can be released from pUPD using BsaI or BtgZI. (B) A detailed view of the BsmBI restriction-ligation reaction that follows the amplification of both GBpatches. The BsmBI recognition sequences are shown in orange in the DNA sequence and are marked with orange triangles in the schemes; BsaI and BtgZI are labeled in red and blue, respectively; the enzymes cutting sites are boxed. Single nucleotide mismatches are depicted in red in the DNA sequence or are labeled with a cross in the oligo scheme.

Supplementary Figure 2



Supplementary Figure 2. Combinatorial antibody engineering using the GBpatch assembly level. A combinatorial fusion between a library of antibody variable regions and a collection of constant immunoglobulin domains can be obtained using the GBpatch functionality. A non standard boundary (GCAT) is required here to ensure seamless assemblies within the VH-IgH linker region, therefore avoiding the presence of undesired amino-acids in the final antibody sequence. (A) Diagram showing the position of the non standard junction. (B) Generation of GBpatches by PCR. (B) Combinatorial assembly of GBpatches to generate a new collection of the (14-16) standard GBparts ready for multipartite assembly.

Supplementary Figure 3

A

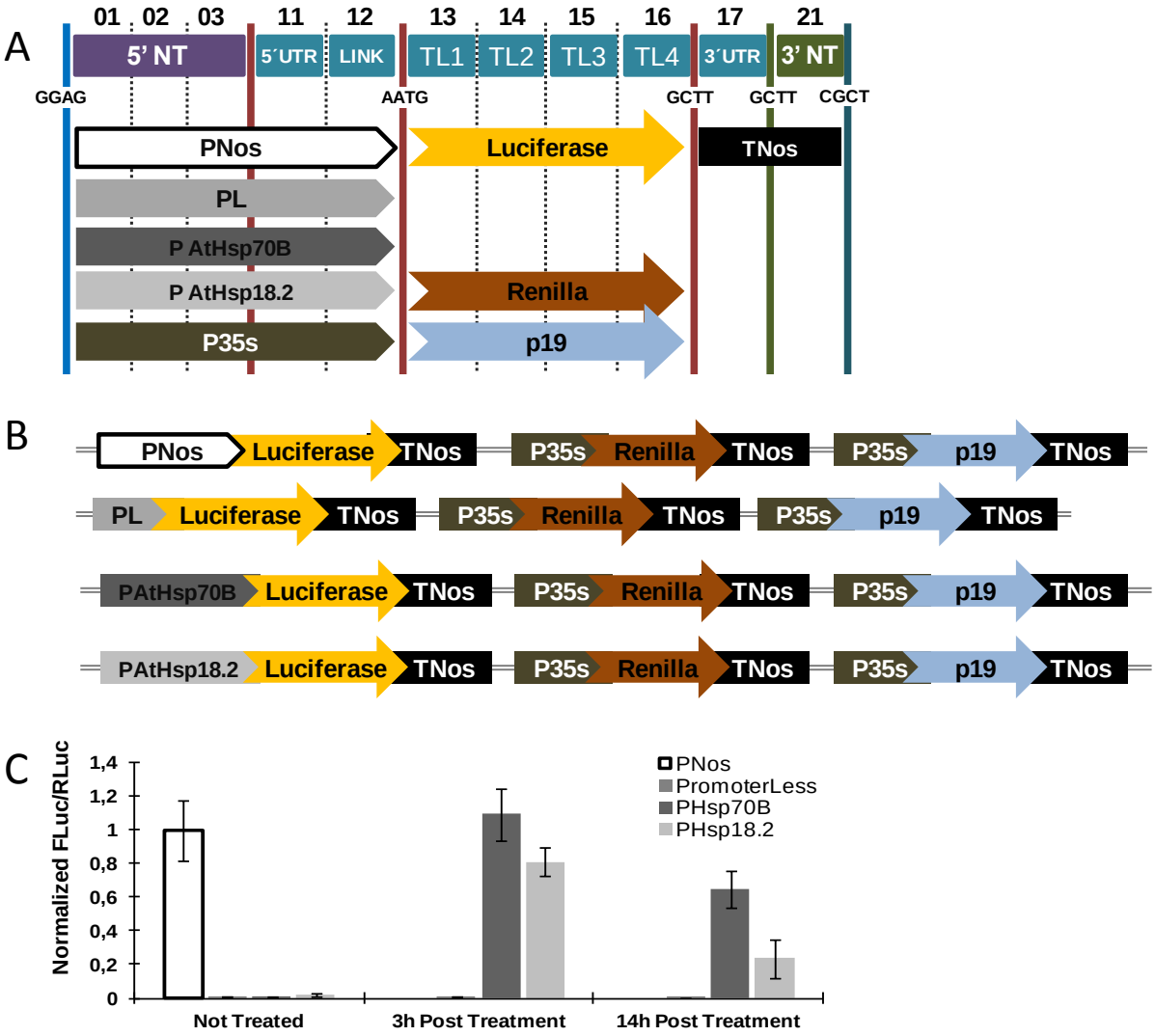
	TAtHSP18.2	TAtUBQ3	TAtAct2	T35s	TSlTctp	TSlMtb	TNos	TAtBch1
P2x35s	40.38 2.08		23.52 3.01	49.86 9.07			15.03 1.44	
P35s	39.68 3.66	22.36 1.64	18.66 2.50	40.41 3.43	32.11 2.03	24.98 2.62	13.57 1.80	11.96 0.87
PAtUbq10	32.27 2.91			18.27 4.28		15.79 3.04	11.92 1.58	
PSlMTb	10.23 1.09	8.11 0.72	4.83 0.54	6.90 0.83	5.71 0.30	6.94 0.95	3.79 0.52	2.82 0.50
PAtUbq3	14.06 1.25	8.00 1.54		5.76 1.27			3.41 0.85	
PSlUbc	4.92 0.49			2.07 0.39			1.89 0.31	3.82 1.05
PAtAct2	4.36 0.55			2.41 0.10		1.00 0.09	1.80 0.27	
PNosΩ	2.39 0.33		2.25 0.41	1.60 0.23			1.25 0.14	
PNos	2.61 0.54	1.70 0.13	1.60 0.31	1.52 0.23	1.38 0.22	1.12 0.21	1.00 0.16	0.77 0.18
PSlTctp	0.89 0.16			0.94 0.10			0.47 0.01	

B

	TAtHSP18.2	TAtUBQ3	TAtAct2	T35s	TSlTctp	TSlMtb	TNos	TAtBch1
P2x35s	39.20 8.94	25.62 3.14	24.08 5.20	22.92 4.10	20.77 3.86	16.89 3.55	15.03 1.44	11.56 2.92
P35s	35.40 8.70	23.13 3.53	21.75 5.10	20.70 4.15	18.76 3.88	15.26 3.49	13.57 1.80	10.44 2.81
PAtUbq10	31.10 7.64	20.32 3.11	19.11 4.48	18.18 3.65	16.48 3.41	13.40 3.07	11.92 1.58	9.17 2.47
PSlMTb	9.90 2.46	6.47 1.01	6.08 1.44	5.79 1.18	5.24 1.10	4.27 0.99	3.79 0.52	2.92 0.79
PAtUbq3	8.90 2.88	5.82 1.52	5.47 1.72	5.20 1.52	4.72 1.39	3.84 1.19	3.41 0.85	2.62 0.90
PSlUbc	4.92 1.31	3.22 0.59	3.03 0.77	2.88 0.65	2.61 0.60	2.12 0.53	1.89 0.31	1.45 0.42
PAtActUTR	4.69 1.20	3.06 0.52	2.88 0.71	2.74 0.59	2.48 0.55	2.02 0.49	1.80 0.27	1.38 0.39
PNosΩ	3.26 0.76	2.13 0.29	2.00 0.45	1.91 0.36	1.73 0.33	1.41 0.30	1.25 0.14	0.96 0.25
PNos	2.61 0.54	1.70 0.13	1.60 0.31	1.52 0.23	1.38 0.22	1.12 0.21	1.00 0.16	0.77 0.18
PSlTctp	1.23 0.26	0.80 0.07	0.75 0.15	0.72 0.11	0.65 0.11	0.53 0.10	0.47 0.01	0.36 0.09

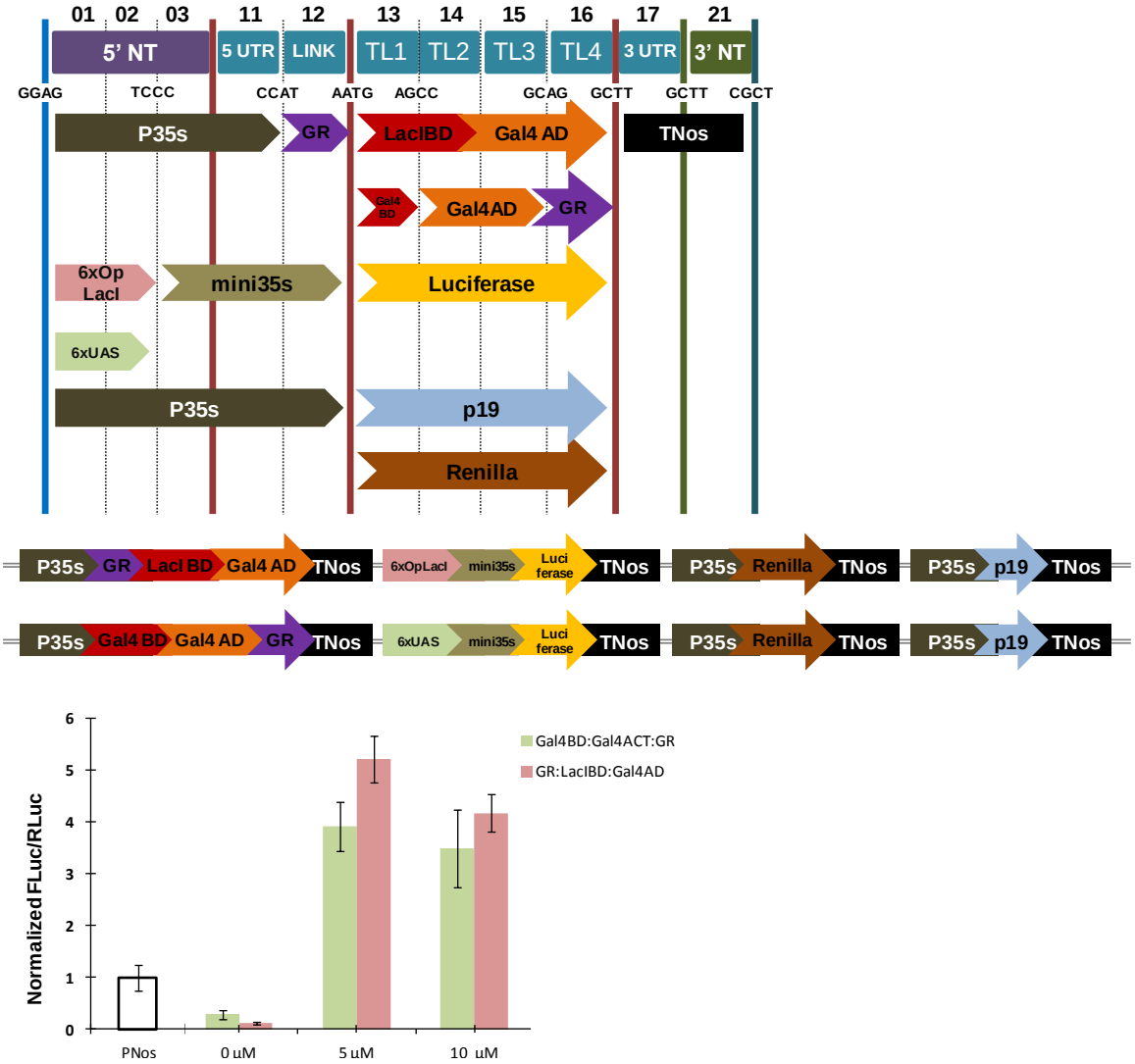
Supplementary Figure 3. Theoretical and experimental transcriptional activity of different promoter/terminator combinations. (A) ETA of 62 experimental (01-12)_(17-21) combinations. The SD of at least three replicates is indicated. (B) The TTA of all the possible combinations in the grid. The SD propagated from the ETA measures is also indicated. Colors indicate activity ranges from high (purple) to low (yellow).

Supplementary Figure 4



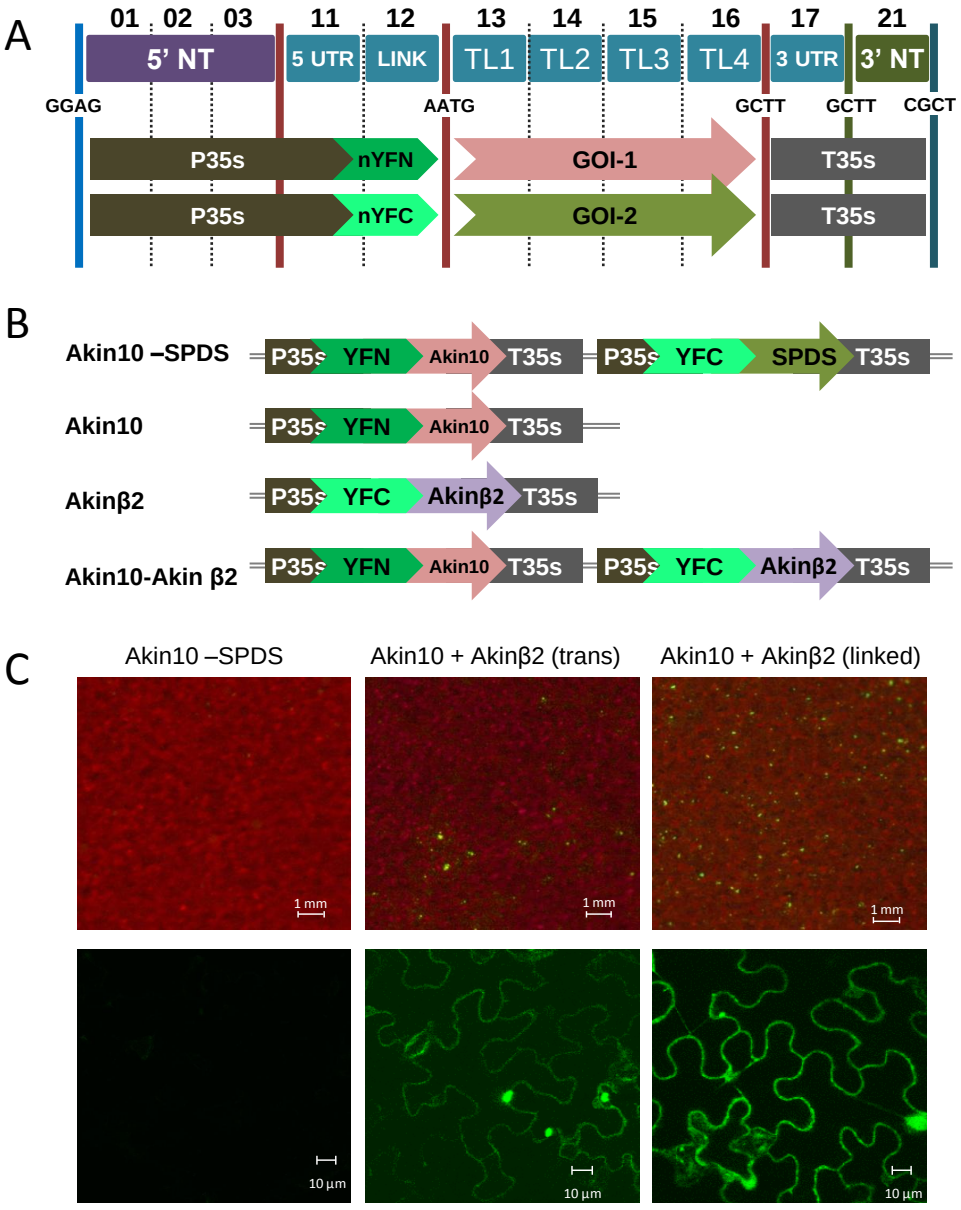
Suplementary Figure 4. Functional characterization of heat-shock promoters (A) GBparts used to build the heat shock transcriptional units (TUs). PNos indicates the nopaline syntase promoter; TNos is the nopaline syntase terminator; PL indicates a promoter-less construct; PHSP70B and PHSP18.2 are the promoter regions of the Arabidopsis AtHSP70B and AtHSP18.2 genes; P35s is the CaMV 35s promoter; p19 is the TBSV silencing suppressor. (B) Structure of the heat shock-regulated constructs. Multigene constructs were built by assembling the promoter of interest with the reporter luciferase and the TNos terminator in pDGBα1; the resulting unit was then combined in pDGBΩ1 with the previously assembled units 35s:Renilla:TNos-35s:p19:TNos. (C) Effect of heat shock treatment (2 h x 37ºC) on the heat shock promoters (HSPs) Activity. The ratios between HSP-driven Firefly (FLuc) and 35S-driven Renilla (RLuc) luciferase activities were normalized using a Nopaline Syntase construct. A promoter-less construct was also set as a negative control. Error bars represent the SD of at least three replicates.

Supplementary Figure 5



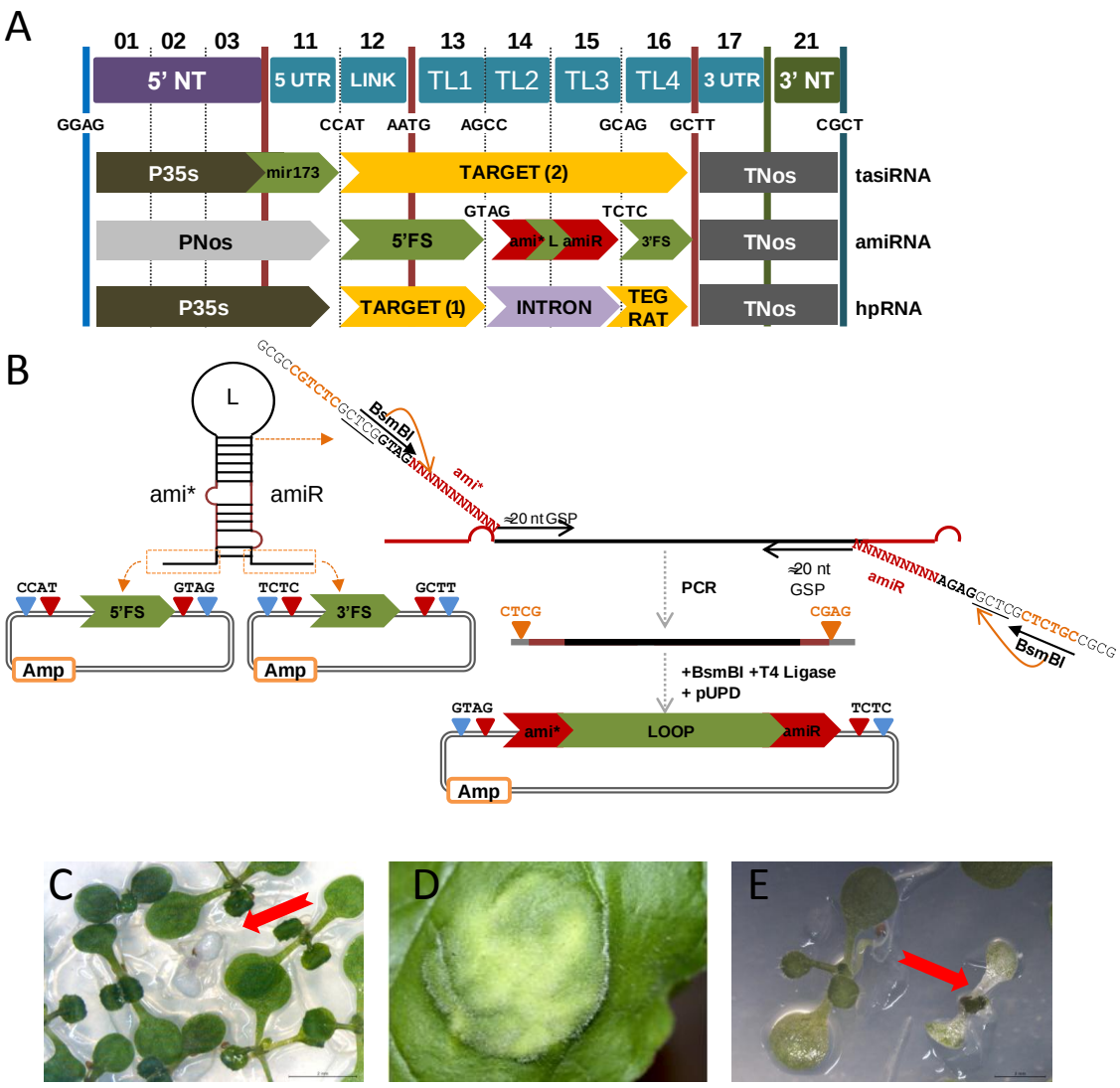
Supplementary Figure 5. Functional characterization of transactivation constructs. (A) GBparts used to build the dexamethasone-regulated constructs. P35s is the CaMV 35s promoter; GR is the rat glucocorticoid receptor; BD is the binding domain; AD is the activation domain; TNos is the nopaline synthase terminator; 6xOpLacI are six closely spaced *lac* operators; mini35s is the minimal CaMV 35s promoter; 6xUAS is an upstream activation sequence to which Gal4 binds; p19 is the TBSV silencing suppressor. **(B)** Structure of the dexamethasone regulated constructs. Multigene constructs were built by assembling the chimeric transcription factors into pDGBα1 and the operated promoter with the luciferase gene into pDGBα2. Both TUs were combined into pDGBΩ1. The resulting construct was later combined with the Renilla/P19 reference module into pDGBα1. **(C)** Effect of glucocorticoid treatment on the activity of the operated promoters. Firefly (FLuc) and Renilla (RLuc) Luciferase activities were measured after 24 h of treatment with different concentrations of dexamethasone. Ratios were normalized to the reference construct driven by the Nopalyne Syntase Promotor. Error bars represent the SD of at least three replicates.

Supplementary Figure 6



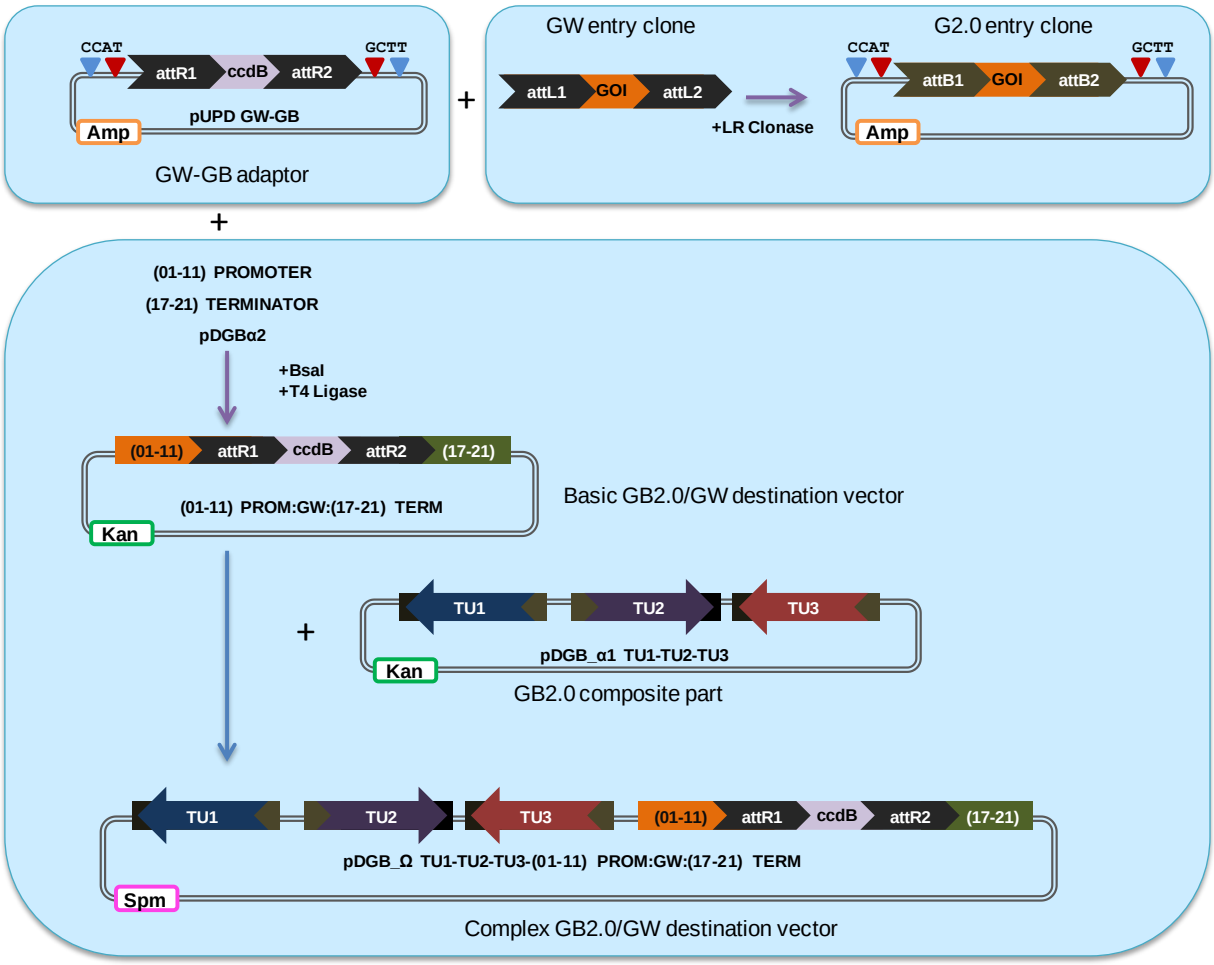
Supplementary Figure 6. Frequently Used Structures (FUS) for the protein-protein interaction analysis. (A) Grammar of the FUS used in Bifluorescent Complementation. (B) The constructs used in the evaluation of the linked and unlinked co-transformation analysis. (C) The BIFC analysis of two negative interaction partners in a linked co-transformation (Akin10-SPDS), two positive interaction partners co-transformed in trans (Akin10 + Akinβ2) and the same two interaction partners assayed in a linked co-transformation (Akin10-Akinβ2). Upper images were taken with a fluorescence-coupled binocular lens. Lower panels are the confocal micrographies.

Supplementary Figure 7



Supplementary Figure 7. The Frequently Used Structures (FUS) for endogenous gene silencing. (A) Grammar of the FUS used in the construction of transcriptional units (TUs) for gene silencing: hpRNA, amiRNA, tasiRNA. (B) Construction of the non standard GB parts for amiRNA. Flanking elements 5'FS and 3'FS are cloned as non standard (12-13) and (16) GBparts, respectively. The ami* and amiR sequences are included in the primers used to build the custom central GBpart (a dedicated 14-15 GBpart). (C) Functional assays of the phytoene deaturase (PDS) tasiRNA constructs in *A.thaliana.* The seeds transformed with the 35s:mir173:PDS:Tnos construct were plated in MS; transformed plants exhibited the albino phenotype (indicated with a red arrow). (D) The dual construct 35s:mir173:PDS*:Tnos-PNos:mir173:Tnos transiently transformed into *N.benthamiana* yielded a bleaching effect on the infiltrated area recorded at 7 d.p.i. (F) *A.thaliana* seeds transformed with 35s:PDSamiRNA:Tnos were plated in MS; transformed plants exhibited a slight albino phenotype (indicated with a red arrow), whereas negative seedlings showed normal growth. P35s-mir173 is the CaMV promoter fused to the mir173 target site; TARGET(1) is a fragment of the gene to be silenced; PNos is the Nopaline syntase promoter and P35s is the CaMV promoter. TARGET(2) and TEGRAT are representative fragments of a target gene in the inverted orientation. 5'FS and 3'FS are the 5' and 3' flanking sequences of the miRNA precursor, respectively; L refers to the loop of the amiRNA structure; amiR and ami* are the complementary target gene sequences.

Supplementary Figure 8



Supplementary Figure 8. Adapting Gateway (GW) technology to GB2.0 (A) The GW-GB adaptor is a GBSpart (e.g., a (12-16) GBSpart) with a GW cassette flanked by the artrR1 and attR2 sites. (B) The adapter vector can be used as destination plasmids for the GW entry clones, therefore translating GW entry clones into GB2.0 entry clones. (C) Alternatively, the GW-GB adapter can be used as an ordinary GBSpart to build increasingly complex GW destination vectors, where GW entry clones can be LR-cloned.