Figure S1

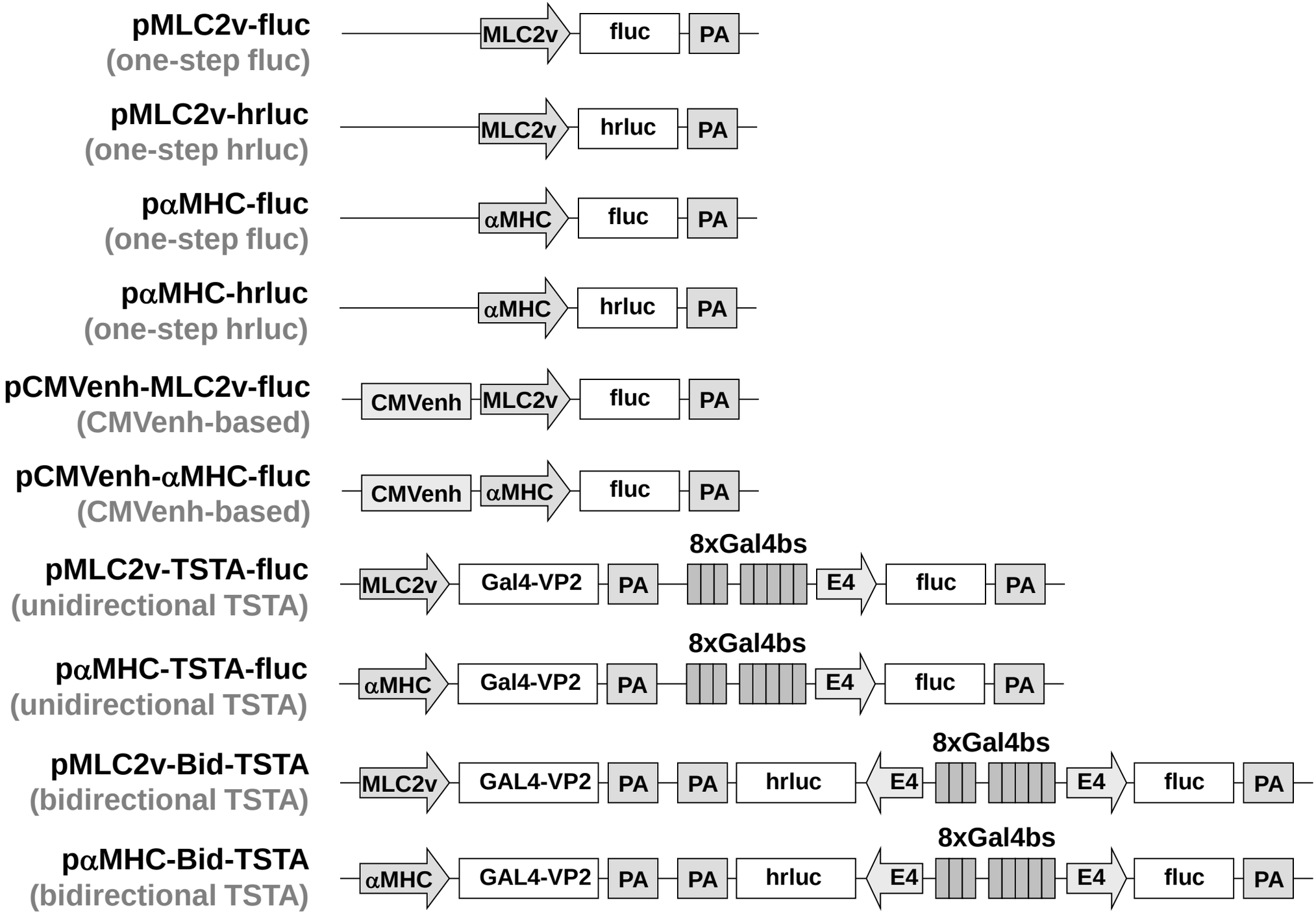
pMLC2v-fluc
(one-step fluc)
MLC2v
fluc
PA
pMLC2v-hrluc
(one-step hrluc)
MLC2v
hrluc
PA
pαMHC-fluc
(one-step fluc)
αMHC
fluc
PA
pαMHC-hrluc
(one-step hrluc)
αMHC
hrluc
PA
pCMVenh-MLC2v-fluc
(CMVenh-based)
CMVenh
MLC2v
fluc
PA
pCMVenh-αMHC-fluc
(CMVenh-based)
CMVenh
αMHC
fluc
PA
pMLC2v-TSTA-fluc
(unidirectional TSTA)
8xGal4bs
MLC2v
Gal4-VP2
PA
E4
fluc
PA
pαMHC-TSTA-fluc
(unidirectional TSTA)
8xGal4bs
αMHC
Gal4-VP2
PA
E4
fluc
PA
pMLC2v-Bid-TSTA
(bidirectional TSTA)
8xGal4bs
MLC2v
GAL4-VP2
PA
PA
hrluc
E4
E4
fluc
PA
pαMHC-Bid-TSTA
(bidirectional TSTA)
8xGal4bs
αMHC
GAL4-VP2
PA
PA
hrluc
E4
E4
fluc
PA

Figure S2

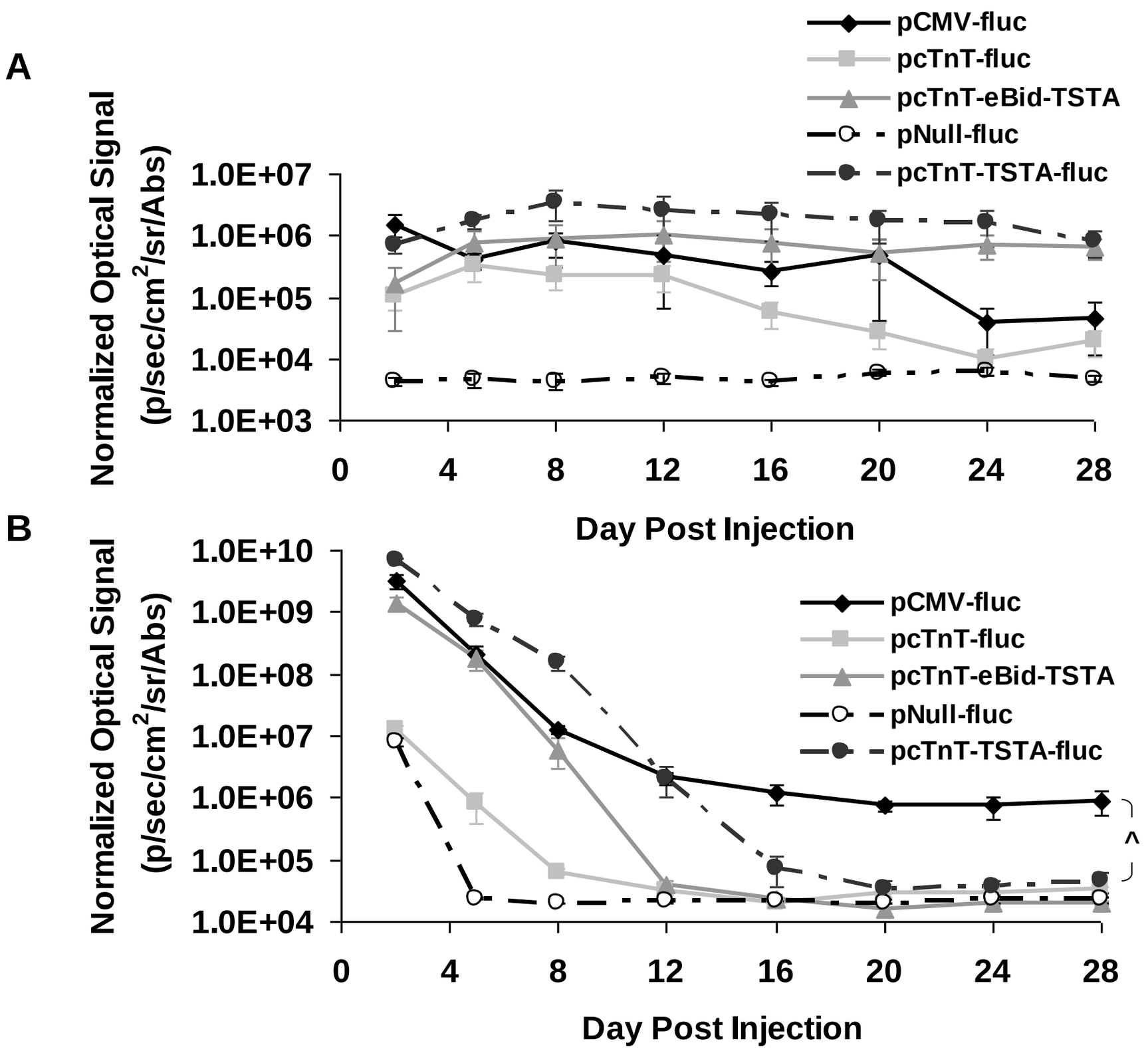
A
Normalized Optical Signal (p/sec/cm²/sr/Abs)
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
0
4
8
12
16
20
24
28
Day Post Injection
pCMV-fluc
pcTnT-fluc
pcTnT-eBid-TSTA
pNull-fluc
pcTnT-TSTA-fluc
B
Normalized Optical Signal (p/sec/cm²/sr/Abs)
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
0
4
8
12
16
20
24
28
Day Post Injection
pCMV-fluc
pcTnT-fluc
pcTnT-eBid-TSTA
pNull-fluc
pcTnT-TSTA-fluc

Figure S3

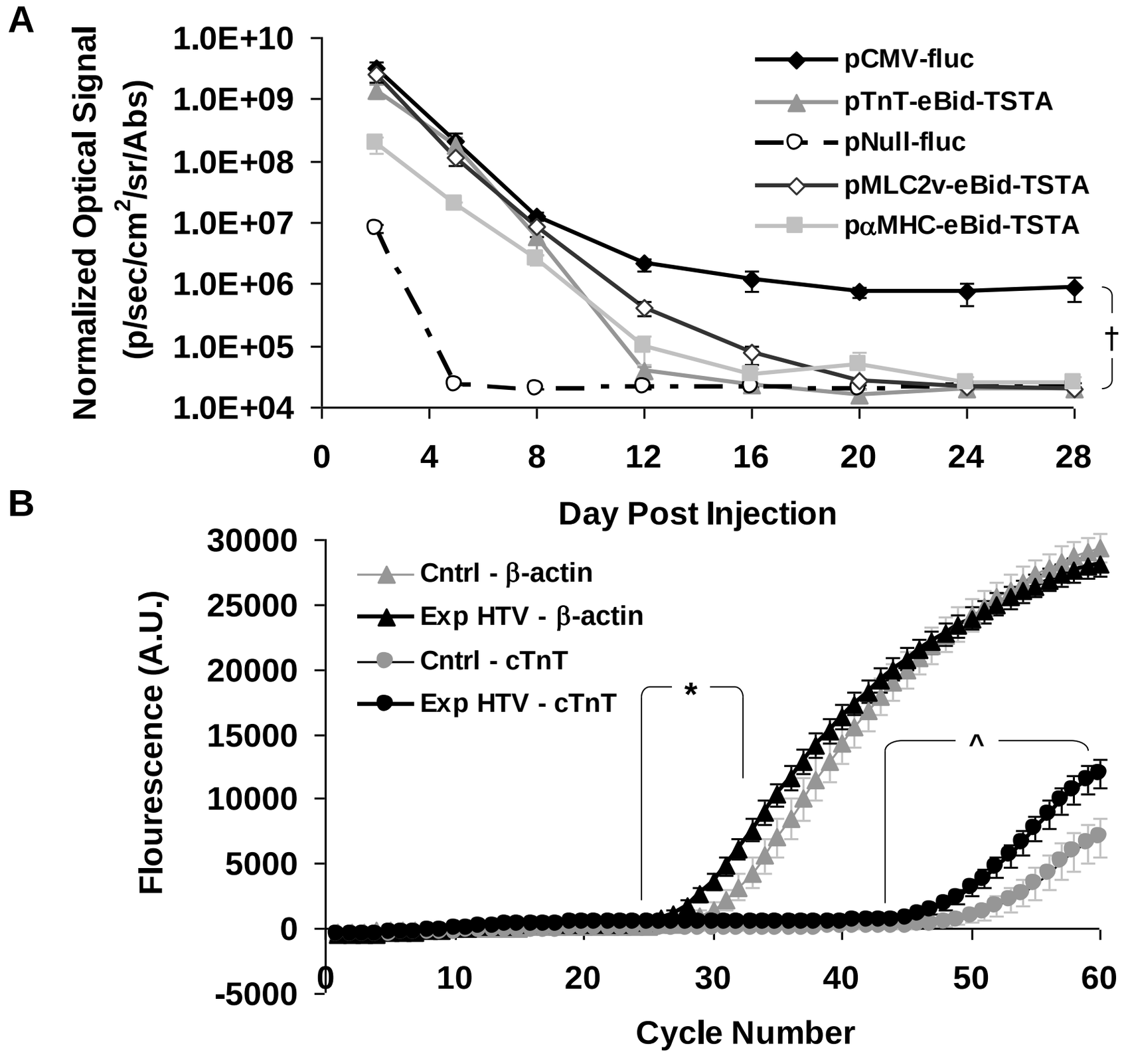
A
Normalized Optical Signal (p/sec/cm²/sr/Abs)
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
0
4
8
12
16
20
24
28
Day Post Injection
pCMV-fluc
pTnT-eBid-TSTA
pNull-fluc
pMLC2v-eBid-TSTA
pαMHC-eBid-TSTA
†
B
Flourescence (A.U.)
30000
25000
20000
15000
10000
5000
0
-5000
0
10
20
30
40
50
60
Cycle Number
Cntrl - β-actin
Exp HTV - β-actin
Cntrl - cTnT
Exp HTV - cTnT
*
^

Figure S4

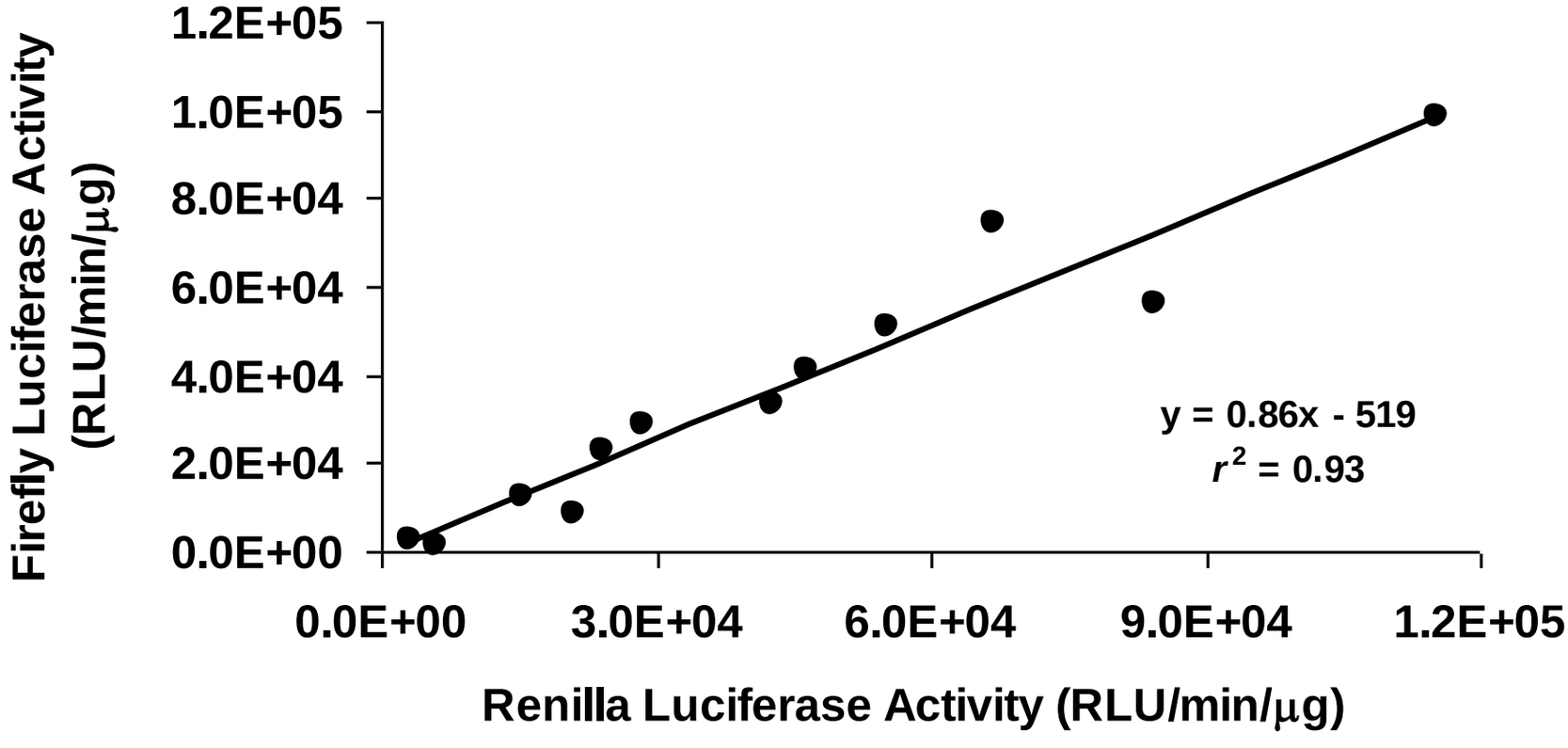
Firefly Luciferase Activity (RLU/min/μg)
1.2E+05
1.0E+05
8.0E+04
6.0E+04
4.0E+04
2.0E+04
0.0E+00
0.0E+00
3.0E+04
6.0E+04
9.0E+04
1.2E+05
Renilla Luciferase Activity (RLU/min/μg)
y = 0.86x - 519
r2 = 0.93

Table S1

Plasmid Vectors	FLuc Activity (% pCMV-fluc)	RLuc Activity (% pCMV-hrluc)
pNull-fluc	0.2 ± 0.0	0.0 ± 0.0
pCMV-fluc	100.0 ± 6.4	0.0 ± 0.0
pNull-hrluc	0.0 ± 0.0	1.2 ± 0.1
pCMV-hrluc	0.0 ± 0.0	100.0 ± 5.3
pMLC2v-fluc	0.2 ± 0.0	0.0 ± 0.0
pαMHC-fluc	4.6 ± 0.1	0.0 ± 0.0
pMLC2v-hrluc	0.0 ± 0.0	0.3 ± 0.0
pαMHC-hrluc	0.0 ± 0.0	3.6 ± 0.2
pCMVenh-MLC2v-fluc	28.4 ± 1.0	0.0 ± 0.0
pCMVenh-αMHC-fluc	48.3 ± 1.1	0.0 ± 0.0
pMLC2v-TSTA-fluc	14.8 ± 1.6*	0.0 ± 0.0
pαMHC-TSTA-fluc	125.4 ± 15.6*	0.0 ± 0.0
pMLC2v-Bid-TSTA	3.1 ± 0.3^	1.1 ± 0.1
pαMHC-Bid-TSTA	32.3 ± 0.5^	44.3 ± 1.0

Table S2

Plasmid Vectors	Cardiac Specificity Index
pNull-fluc	3.1 ± 0.3
pCMV-fluc	1.0 ± 0.2
pMLC2v-fluc	1.9 ± 0.4
pαMHC-fluc	7.2 ± 0.8
pCMVenh-MLC2v-fluc	0.6 ± 0.0
pCMVenh-αMHC-fluc	1.9 ± 0.2*
pMLC2v-TSTA-fluc	1.4 ± 0.3
pαMHC-TSTA-fluc	4.1 ± 0.8* ^
pMLC2v-Bid-TSTA	0.4 ± 0.1* †
pαMHC-Bid-TSTA	1.6 ± 0.2* †

Titles and Legends to Supplementary Figure and Tables

Figure S1 Schematics of experimental vectors containing either MLC2v or αMHC. Abbreviations: MLC2v, rat ventricular myosin light chain 2 promoter; fluc, firefly luciferase gene; PA, SV40 poly(A) tail; hrluc, synthetic Renilla luciferase gene; αMHC, rat alpha-myosin heavy chain promoter; CMVenh, cytomegalovirus enhancer; TSTA, two-step transcriptional amplification; Gal4-VP2, fusion gene combining yeast Gal4 and two tandem repeats of herpes simplex virus VP16; 8xGal4bs, 8 repeats of Gal4 binding sites; E4, adenovirus E4 minimal promoter; CMV, cytomegalovirus promoter.

Figure S2 *In vivo* bioluminescence assessment of pcTnT-TSTA-fluc following intramyocardial or hydrodynamic tail vein injection. Serial BLI was performed on mice that had undergone either intramyocardial co-injections of pcTnT-TSTA-fluc and pCMV-β-gal or mice that had received the same treatments except via HTV administration. **(a)** The average heart signal (dark gray circle, dashed line) following intramyocardial vector administration was normalized by transfection efficiency (β-gal expression), plotted for the post-operative days shown, and superimposed on **Figure 3b** for comparison with other animal groups receiving different plasmids. **(b)** The average normalized hepatic signal (dark gray circle, dashed line) following HTV vector injection is plotted in the same fashion and superimposed on **Figure 4b** for comparison with other animal groups. The error bars represent SEM for 5 animals. ^$P<0.0001$ between pCMV-fluc and pcTnT-TSTA-fluc (or any other group) between days 16 and 28.

Figure S3 Acute effects of hydrodynamic tail vein injection on hepatic gene expression. Mice received hydrodynamic tail vein co-injections of 1) pαMHC-eBid-TSTA + pCMV-β-gal, 2) pMLC2v-eBid-TSTA + pCMV-β-gal, or injection of PBS alone. BLI was used to assess longitudinal hepatic fluc expression in the first 2 mouse groups, whereas qRT-PCR was used in the last group to assess for hepatic cTnT mRNA and β-actin mRNA 48 hr after injection of PBS. **(a)** The average hepatic signal normalized by transfection efficiency (β-gal expression) is specifically plotted for mouse groups corresponding to

pαMHC-eBid-TSTA (light gray square) and pMLC2v-eBid-TSTA (empty diamond). The plots are superimposed on selected plots from **Figure 4b** for easier comparison. The error bars represent SEM for 5 animals. †P<0.0001 between pCMV-fluc and pαMHC-eBid-TSTA (or pMLC2v-eBid-TSTA) from days 20 to 28. **(b)** The average real-time fluorescence intensity for the cycle numbers indicated is plotted for both the cTnT mRNA (circle) and the β-actin mRNA (triangle) from either livers exposed to PBS (black) or livers from intact control mice (gray). The error bars represent SEM for 7 liver samples. *P<0.05 between "Cntrl - β-actin" and "Exp HTV - β-actin" for PCR cycles 26-33. ^P<0.05 between "Cntrl – cTnT" and "Exp HTV – cTnT" for PCR cycles 44-60.

Figure S4 ***Ex vivo*** **correlation between FLuc and RLuc/M185V enzyme activities.** Mice intramyocardially injected with pcTnT-eBid-TSTA were euthanized at various time points, with their heart homogenates assayed for FLuc and RLuc/M185V enzyme activities, which were normalized to total protein and plotted against each other.

Table S1 ***In vitro*** **fluc and hrluc expression of MLC2v- or αMHC-containing vectors.** HL-1 cells were separately co-transfected with each of the vectors listed and pCMV-β-gal, and assayed for FLuc, RLuc, and β-GAL activities 24 hr later. The FLuc and RLuc activities were normalized to total protein, corrected for transfection efficiency (β-GAL activity), and expressed as a percentage of pCMV-fluc and pCMV-hrluc, respectively. Data are presented as mean±SEM % for triplicate determinations. *P<0.02 compared to CMVenh-based vector of the same promoter; ^P<0.03 compared to unidirectional TSTA vector of the same promoter.

Table S2 Cardiac specificity index of experimental vectors containing either MLC2v or αMHC. Cardiac (HL-1) and non-cardiac cells (C2C12, Hepa1-6 and NIH3T3) were co-transfected with each of the vectors listed and pCMV-β-gal, and assayed 24 hr later for FLuc, RLuc, and β-GAL activities. The

FLuc activity was normalized to total protein, corrected for transfection efficiency (β-GAL activity), and used to calculate a cardiac-specificity index (CSI) for each vector. The CSI measures the cardiac specificity of a given vector relative to pCMV-fluc. Data are expressed as mean±SEM for triplicate determinations. *P<0.05 compared to one-step vector of the same promoter; ^P<0.05 compared to CMVenh-based vector of the same promoter; †P<0.04 compared to unidirectional TSTA vector of the same promoter.