Figure S1

A

CCAP- or Burs-KZip*  
HindII  
CCAP/Burs  
EcoRI  
KZip*  
PstI  
SV40  
Mini white

B

CCAP-Syn21-KZip*"p10  
HindII  
CCAP promoter  
EcoRI  
syn21-KZip*  
p10  
XbaI  
Mini white

CCAP-4XKZip*  
HindII  
CCAP promoter  
EcoRI  
KZip*-(T2A-KZip*)3  
XbaI  
Mini white

CCAP-KZip*  
HindII  
CCAP promoter  
EcoRI  
KZip*  
XbaI  
Mini white

CCAP-KZip*"::HRD  
HindII  
CCAP promoter  
EcoRI  
KZip*:Hairy Repressor Domain  
XbaI  
Mini white

CCAP-KZip*"::3XHA  
HindII  
CCAP promoter  
EcoRI  
KZip*:3XHA  
XbaI  
Mini white

C

1  2  3  4  5  6
Control (No KZip*)  Syn21-KZip*"p10  4XKZip*  KZip*  KZip*"::HRD  KZip*"::3XHA

CCAP Split-GAL4

D

<table>
<thead>
<tr>
<th>Construct</th>
<th>Average # of Cells Labeled</th>
<th># of Preps</th>
<th>Average Intensity per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No KZip*)</td>
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<td>9</td>
<td>2.3</td>
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<tr>
<td>Syn21-KZip*&quot;p10</td>
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<td>8</td>
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<tr>
<td>4XKZip*</td>
<td>20</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>KZip*</td>
<td>18</td>
<td>6</td>
<td>1.0</td>
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<tr>
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<td>7</td>
<td>1.1</td>
</tr>
<tr>
<td>KZip*&quot;::3XHA</td>
<td>23</td>
<td>6</td>
<td>1.3</td>
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Figure S1:

(A) Schematic of the KZip^+ constructs designed to be expressed under the control of the CCAP or Burs gene promoters and used in the experiments shown in Fig. 2A-B.

(B) Schematic of modified KZip^+ constructs created to optimize the efficacy of the Killer Zipper technology.

(C) Representative confocal projection views of adult CNS wholemounts expressing a UAS-2XEGFP reporter (black) driven in CCAP-expressing neurons by the elav-VP16AD∩CCAP-GAL4DBD Split GAL4 driver. Each preparation (except for the control, lane 1), additionally expresses the indicated KZip^+ construct in CCAP-expressing neurons. KZip^+ activity substantially attenuates and/or eliminates reporter expression in all cases with the clearest repression seen with use of translational enhancers (2). All KZip^+ constructs containing a C-terminal fusion, including the T2A peptide (3), the Hairy transcriptional repressor domain (5) or the 3XHA tag (6) showed reduced repression compared to the original, untagged KZip^+ construct (4).

(D) Quantification of the results for the KZip^+ constructs. For each construct tested in (B), confocal Z-stacks of the indicated number of preparations (# of preps) were scored as described in Materials and Methods for the total number of EGFP-labeled CCAP neurons and for the average intensity of labeling per cell (1, lowest level of labeling; 3, highest). The KZip^+-p10 construct eliminates reporter expression in approximately 75% of cells and those cells that do show expression are labeled at the lowest level.