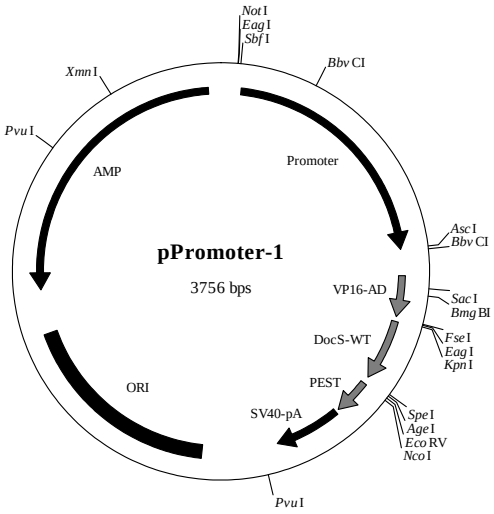
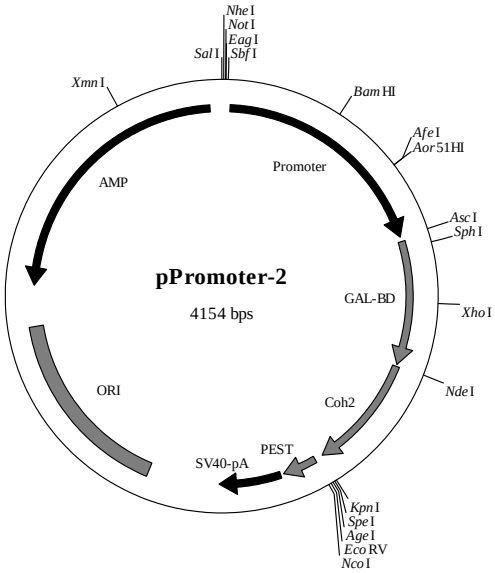
Supplementary Information

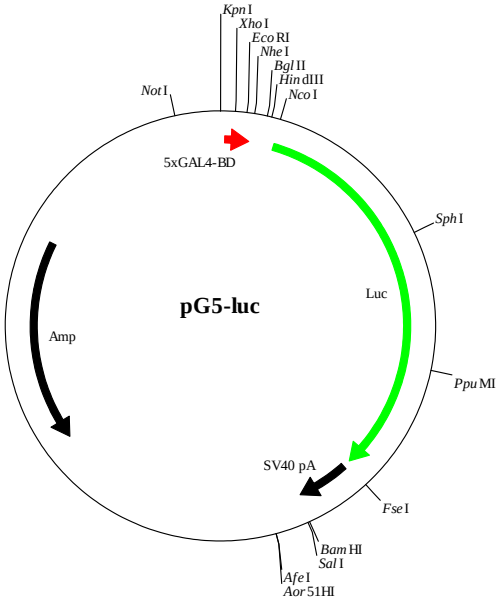
Plasmid maps

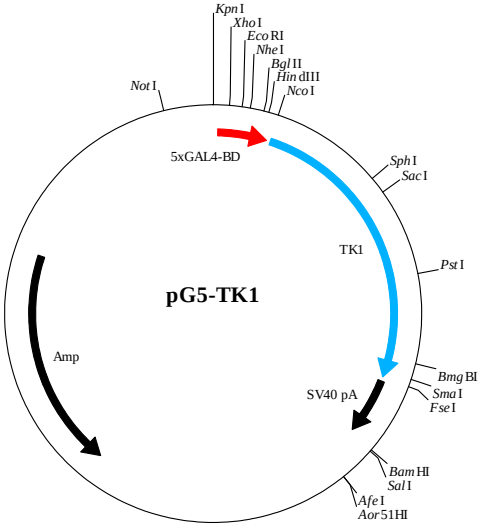
Input plasmids



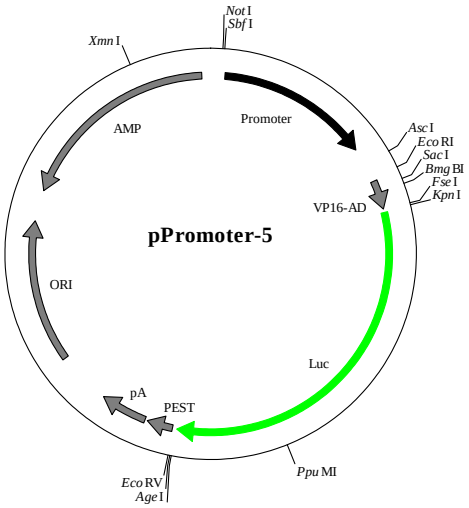


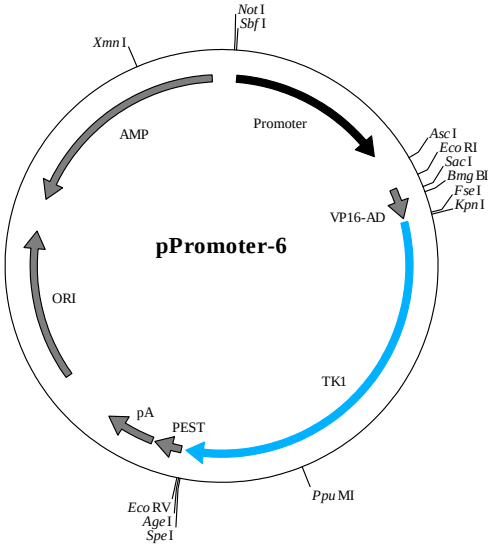
Output plasmids



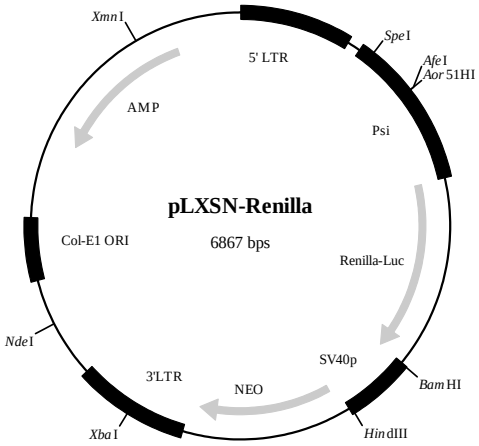


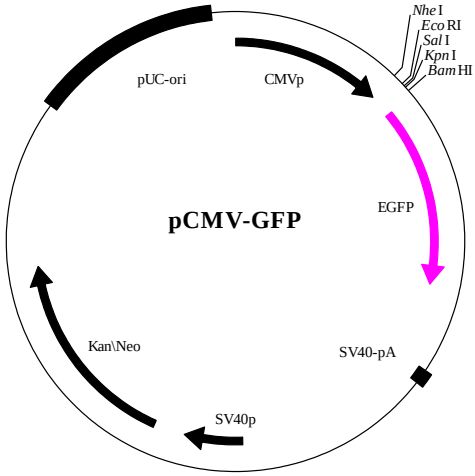
Auxiliary plasmids





Additional plasmids





Plasmid composition for luciferase and cytotoxicity assays

Sample	Plasmid 1	Plasmid 2	Plasmid 3	Plasmid 4	pGEMtEasy
1	pCycD-5			pLXSN-Renilla	1ug
2	pCXCL1-5			pLXSN-Renilla	1ug
3	pSSX1-5			pLXSN-Renilla	1ug
4	pH2A1-5			pLXSN-Renilla	1ug
5	pCycD-1	pCycD-2	pG5*luc*	pLXSN-Renilla	
6	pCycD-1	pCXCL1-2	pG5*luc*	pLXSN-Renilla	
7	pCycD-1	pSSX1-2	pG5*luc*	pLXSN-Renilla	
8	pCycD-1	pH2A1-2	pG5*luc*	pLXSN-Renilla	
9	pCXCL1-1	pCycD-2	pG5*luc*	pLXSN-Renilla	
10	pCXCL1-1	pCXCL1-2	pG5*luc*	pLXSN-Renilla	
11	pCXCL1-1	pSSX1-2	pG5*luc*	pLXSN-Renilla	
12	pCXCL1-1	pH2A1-2	pG5*luc*	pLXSN-Renilla	
13	pSSX1-1	pCycD-2	pG5*luc*	pLXSN-Renilla	
14	pSSX1-1	pCXCL1-2	pG5*luc*	pLXSN-Renilla	
15	pSSX1-1	pSSX1-2	pG5*luc*	pLXSN-Renilla	
16	pSSX1-1	pH2A1-2	pG5*luc*	pLXSN-Renilla	
17	pH2A1-1	pCycD-2	pG5*luc*	pLXSN-Renilla	
18	pH2A1-1	pCXCL1-2	pG5*luc*	pLXSN-Renilla	
19	pH2A1-1	pSSX1-2	pG5*luc*	pLXSN-Renilla	
20	pH2A1-1	pH2A1-2	pG5*luc*	pLXSN-Renilla	
21			pG5*luc*	pLXSN-Renilla	1ug
22				pCMV-GFP	1.8ug

Table S1. Plasmid composition used for each luciferase assay sample in WI38 and HCT116 cells. Each plasmid was used at a concentration of 0.5µg/sample, unless otherwise indicated.

Sample	Plasmid 1	Plasmid 2	Plasmid 3	Plasmid 4	pGEMtEasy
1	pCXCL1-5			pLXSN-Renilla	1ug
2	pSSX1-5			pLXSN-Renilla	1ug
3	pH2A1-5			pLXSN-Renilla	1ug
4	pCXCL1-1	pCXCL1-2	pG5*luc*	pLXSN-Renilla	
5	pCXCL1-1	pSSX1-2	pG5*luc*	pLXSN-Renilla	
6	pCXCL1-1	pH2A1-2	pG5*luc*	pLXSN-Renilla	
7	pSSX1-1	pCXCL1-2	pG5*luc*	pLXSN-Renilla	
8	pSSX1-1	pSSX1-2	pG5*luc*	pLXSN-Renilla	
9	pSSX1-1	pH2A1-2	pG5*luc*	pLXSN-Renilla	
10	pH2A1-1	pCXCL1-2	pG5*luc*	pLXSN-Renilla	
11	pH2A1-1	pSSX1-2	pG5*luc*	pLXSN-Renilla	
12	pH2A1-1	pH2A1-2	pG5*luc*	pLXSN-Renilla	
13			pG5*luc*	pLXSN-Renilla	1ug
14				pCMV-GFP	1.8ug

Table S2. Plasmid composition comprising each luciferase assay sample in HEK-293T cells. Each plasmid was used at a concentration of 0.5µg/sample, unless otherwise indicated. For experiments with DocS mutants, the same plasmid compositions were used, but wild type DocS in plasmid pPROMOTER-1 was replaced with the relevant mutant DocS derivative.

Sample	Plasmid 1	Plasmid 2	Plasmid 3	Plasmid 4	pGEMtEasy
1	pCycD-6			pCMV-GFP	1ug
2	pCXCL1-6			pCMV-GFP	1ug
3	pSSX1-6			pCMV-GFP	1ug
4	pH2A1-6			pCMV-GFP	1ug
5	pCycD-1	pCycD-2	pG5-TK1	pCMV-GFP	
6	pCycD-1	pCXCL1-2	pG5-TK1	pCMV-GFP	
7	pCycD-1	pSSX1-2	pG5-TK1	pCMV-GFP	
8	pCycD-1	pH2A1-2	pG5-TK1	pCMV-GFP	
9	pCXCL1-1	pCycD-2	pG5-TK1	pCMV-GFP	
10	pCXCL1-1	pCXCL1-2	pG5-TK1	pCMV-GFP	
11	pCXCL1-1	pSSX1-2	pG5-TK1	pCMV-GFP	
12	pCXCL1-1	pH2A1-2	pG5-TK1	pCMV-GFP	
13	pSSX1-1	pCycD-2	pG5-TK1	pCMV-GFP	
14	pSSX1-1	pCXCL1-2	pG5-TK1	pCMV-GFP	
15	pSSX1-1	pSSX1-2	pG5-TK1	pCMV-GFP	
16	pSSX1-1	pH2A1-2	pG5-TK1	pCMV-GFP	
17	pH2A1-1	pCycD-2	pG5-TK1	pCMV-GFP	
18	pH2A1-1	pCXCL1-2	pG5-TK1	pCMV-GFP	
19	pH2A1-1	pSSX1-2	pG5-TK1	pCMV-GFP	
20	pH2A1-1	pH2A1-2	pG5-TK1	pCMV-GFP	
21			pG5-TK1	pCMV-GFP	1ug
22	pH2A1-1	pH2A1-2	pG5*luc*	pCMV-GFP	1ug

Table S3. Plasmid composition comprising each TK1 cytotoxicity-assay sample in WI38 cells. Plasmids 1-3 were each used at a concentration of 0.5µg/sample; pCMV-GFP was used at a concentration of 0.2µg/sample, and pGEM-t-Easy was added at the indicated concentrations.

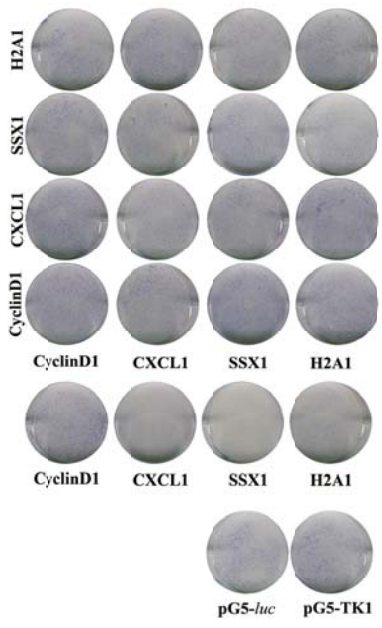
Sample	Plasmid 1	Plasmid 2	Plasmid 3	Plasmid 4	pGEMtEasy
1	pCXCL1-6			pCMV-GFP	1ug
2	pSSX1-6			pCMV-GFP	1ug
3	pH2A1-6			pCMV-GFP	1ug
4	pCXCL1-1	pCXCL1-2	pG5-TK1	pCMV-GFP	
5	pCXCL1-1	pSSX1-2	pG5-TK1	pCMV-GFP	
6	pCXCL1-1	pH2A1-2	pG5-TK1	pCMV-GFP	
7	pSSX1-1	pCXCL1-2	pG5-TK1	pCMV-GFP	
8	pSSX1-1	pSSX1-2	pG5-TK1	pCMV-GFP	
9	pSSX1-1	pH2A1-2	pG5-TK1	pCMV-GFP	
10	pH2A1-1	pCXCL1-2	pG5-TK1	pCMV-GFP	
11	pH2A1-1	pSSX1-2	pG5-TK1	pCMV-GFP	
12	pH2A1-1	pH2A1-2	pG5-TK1	pCMV-GFP	
13			pG5-TK1	pCMV-GFP	1ug
14	pH2A1-1	pH2A1-2	pG5*luc*	pCMV-GFP	1ug

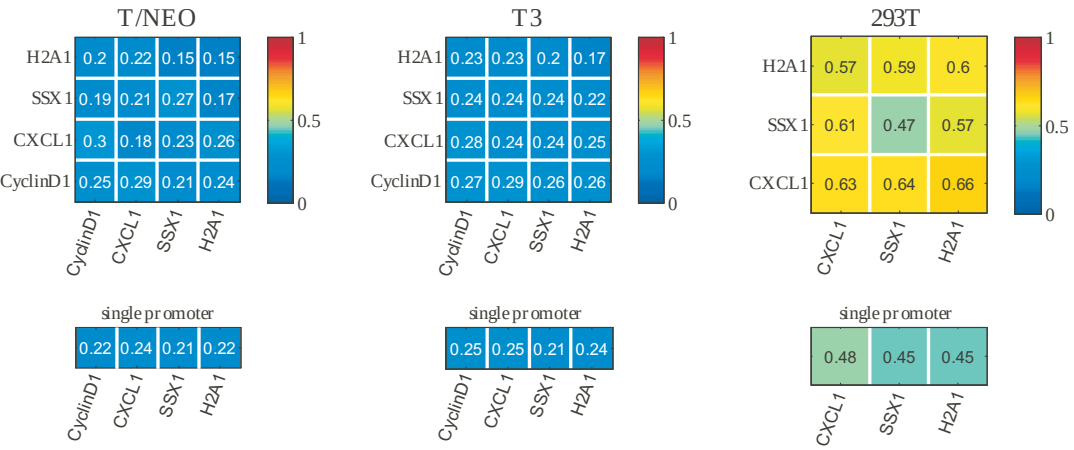
Table S4. Plasmid composition comprising each TK1 cytotoxicity-assay sample in HEK-293T cells. Plasmids 1-3 were each used at a concentration of 0.5µg/sample; pCMV-GFP was used at a concentration of 0.2µg/sample, and pGEM-t-Easy was added at the indicated concentrations.

Supplementary Figures

Figure S1: The TK1 killer gene output of the integrator, as determined by final cell density visualized with crystal violet staining in T/NEO cells. Middle set: Viability assay for the single promoters. Bottom row: Negative controls with luciferase or TK1 output expressed under a GAL4 promoter in the presence of GNC. The densities of all T/Neo samples were very low. Nevertheless, growth of these cells under DPI treatment was comparable to that in the presence of the pG5-luc negative control sample indicating that the inhibition in T/Neo cell growth was caused by transfection cytotoxicity (causing a significant growth arrest even before GNC treatment).

Figure S2: Calibration of transfection efficiency in cell viability assays based on measurements of total GFP immediately before BVDU/GCV treatment. Total GFP in each sample of T/Neo, T3 and HEK-293T cells were comparable, indicating similar transfection efficiency. The STDEV for WI38 cells (T/NEO and T3) are ~50%, and for HEK-293T are ~10%.


H2A1
SSX1
CXCL1
CyclinD1
CyclinD1
CXCL1
SSX1
H2A1
CyclinD1
CXCL1
SSX1
H2A1
pG5-luc
pG5-TK1


T/NEO
H2A1 0.2 0.22 0.15 0.15
SSX1 0.19 0.21 0.27 0.17
CXCL1 0.3 0.18 0.23 0.26
CyclinD1 0.25 0.29 0.21 0.24
CyclinD1 CXCL1 SSX1 H2A1
1
0.5
0
T3
H2A1 0.23 0.23 0.2 0.17
SSX1 0.24 0.24 0.24 0.22
CXCL1 0.28 0.24 0.24 0.25
CyclinD1 0.27 0.29 0.26 0.26
CyclinD1 CXCL1 SSX1 H2A1
1
0.5
0
293T
H2A1 0.57 0.59 0.6
SSX1 0.61 0.47 0.57
CXCL1 0.63 0.64 0.66
CXCL1 SSX1 H2A1
1
0.5
0
single promoter
0.22 0.24 0.21 0.22
CyclinD1 CXCL1 SSX1 H2A1
single promoter
0.25 0.25 0.21 0.24
CyclinD1 CXCL1 SSX1 H2A1
single promoter
0.48 0.45 0.45
CXCL1 SSX1 H2A1