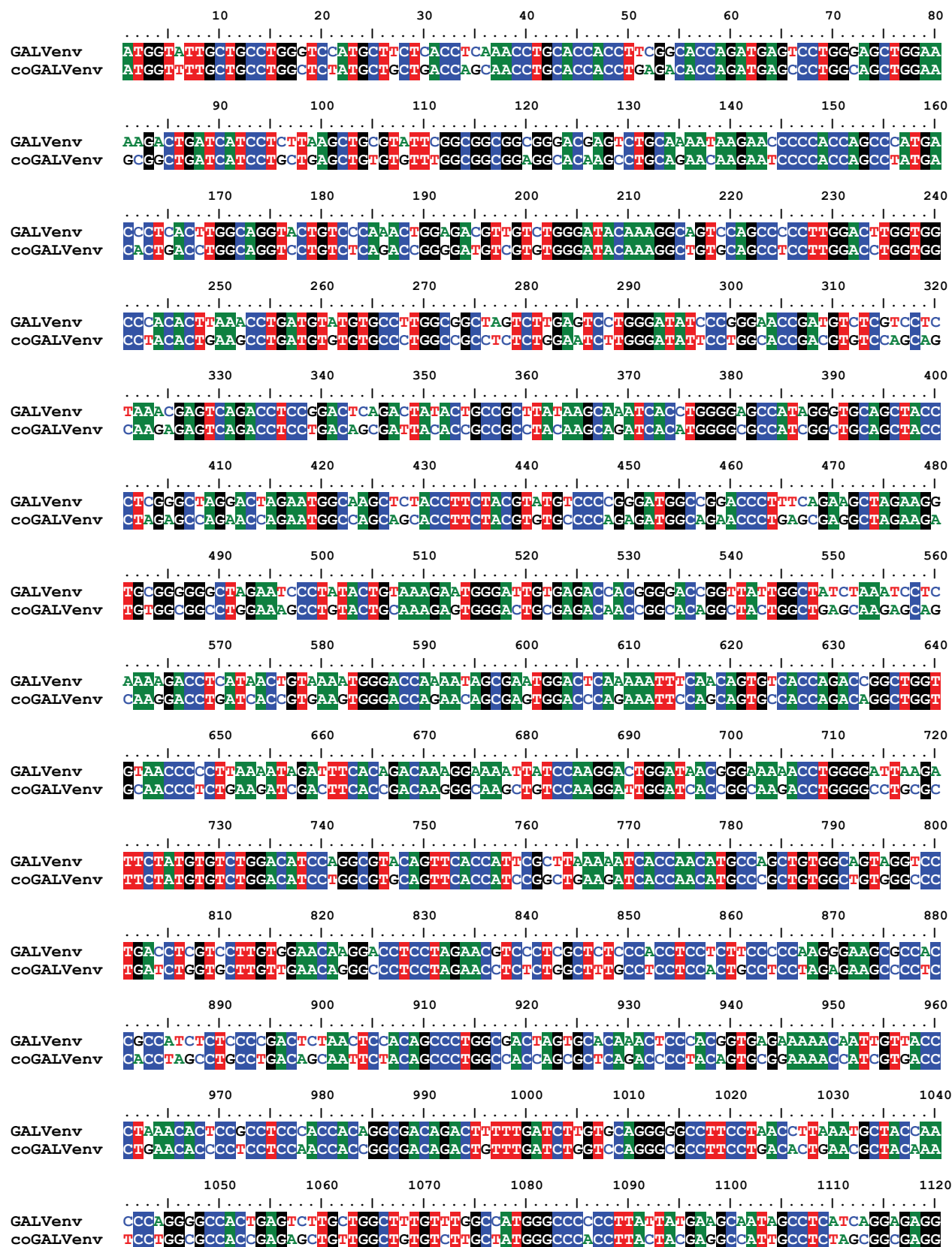
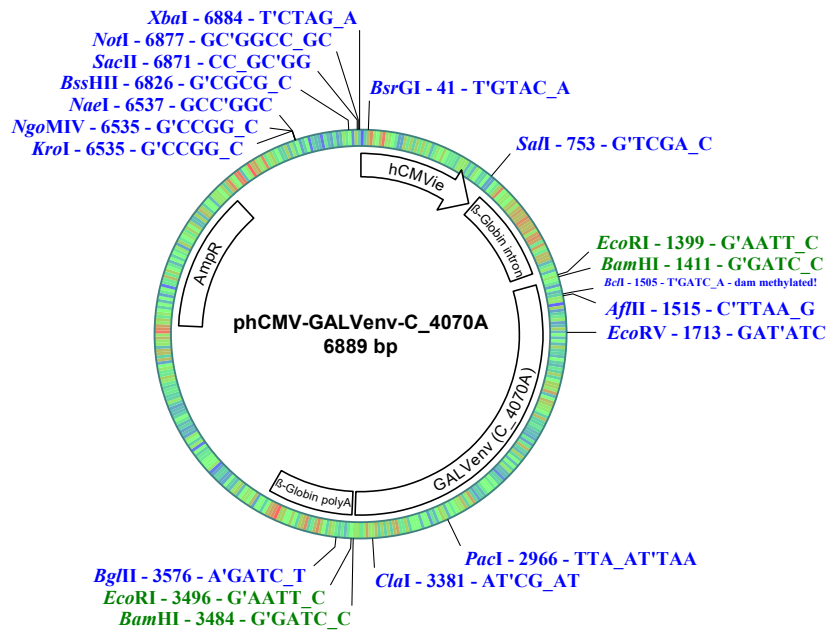


Figure S1: Comparison of the complete original and the optimized sequences of GALVenv.



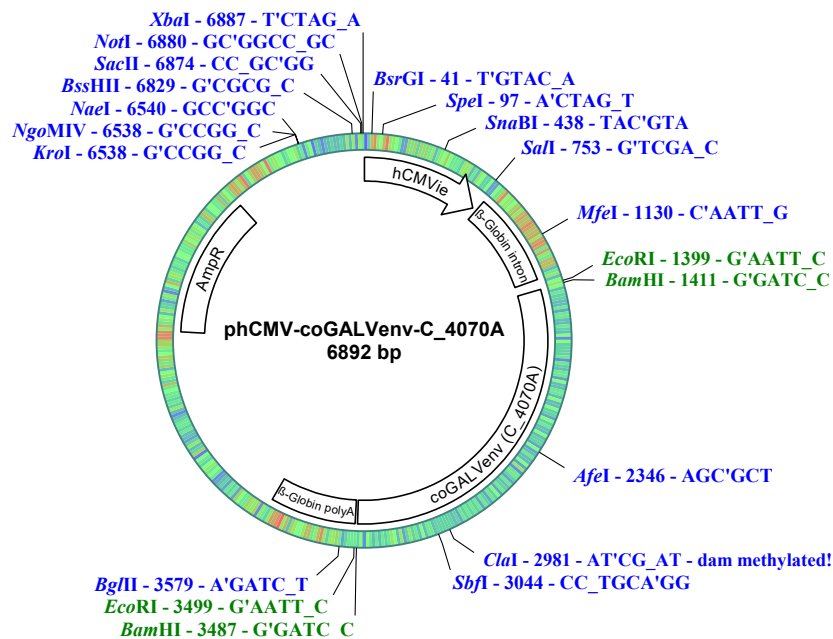
	1130	1140	1150	1160	1170	1180	1190	1200
GALVenv	TGGCTATCTCACCACCTTGACCGGTGCGGTGGGGACCCAGGAAAGCTACCCCTCACTGAGGTCTCAGGACACGGG							
coGALVenv	TGGCTATAGCACCACCTGGAATAGATGCAGTGGGGACCCAGGAAAGCTACCCCTGACAGAACTTCTGGCACGGC							
	1210	1220	1230	1240	1250	1260	1270	1280
GALVenv	TTGTGCATAGGAAGGTGCCCTTTACCCATCAGCATCTCTGCAATCAGACCCCTATCCATCAATTCCTCCGGAGACCATCA							
coGALVenv	CTGTGCATCGGCAAGGTGCCCTTTACACACCAGCACCTCTGCAATCAGACCCCTGAGCATCAAAGCAGCGGGCAGCCACCA							
	1290	1300	1310	1320	1330	1340	1350	1360
GALVenv	GTATCTGCTCCCTCTCAACCATAGCTGGTGGGCTTGCAGCACTGGCCTCACCCTTGCCCTCTCCACCTCAGTTTCTTAATC							
coGALVenv	GTACTGCTGCGCTAGCAATCATTCTTGGTGGGCTTGCAGCACCGGCTGACACCTTGCTCTGAGCACCAGCGTGTCAACC							
	1370	1380	1390	1400	1410	1420	1430	1440
GALVenv	AGACTAGAGATTCTGTATCCAGGTCCAGCTGATTCCTCGCATCTATTACTATCCTGAAGAAGTTTGTTTACAGGCCTAT							
coGALVenv	AGACAGGGACTTCTGATCCAAGTGCAGCTGATCCAGGATCTACTACTACCCAGGAAGTGTGTCTCCAGGCCTAC							
	1450	1460	1470	1480	1490	1500	1510	1520
GALVenv	GACAAATTCACCCAGGACTAAAGAGAGGCTGTCTCACTTACCCTAGCTGTTTTACTGCGGTGGGAATCACGGCGGG							
coGALVenv	GACAAATTCACCCTCGGACCAAGCGGGAAGCGGTGTCTCTGACACTGECTGTCTGTCTGGACTGGGAATCACAGCCGG							
	1530	1540	1550	1560	1570	1580	1590	1600
GALVenv	AATAGGTACTGGTTCAACTGCCCTTAAATTAAGGACCTATAGACCTCCAGCAAGGCCTGACAAGCCTCCAGATCGCCATAG							
coGALVenv	AATCGGAACAGGCAGCACTGCCCTCACTCAAGGGCCCTATCGATCTTCAGCAGGGACTGACCTCTCTCAGATTGCCATCG							
	1610	1620	1630	1640	1650	1660	1670	1680
GALVenv	ATGCTGACCTCCGGGCCCTCCAAAGACTCAGTTCAGCAAGTTAGAGGACTCACTGACTTCCCTGTCCGAGGTAGTGTCTCAA							
coGALVenv	ACGCCGATCTGAGAGCCCTGCAGGTAGCGTCTCAAGCTGGGAAGTAGCCTGACAGCAGCTGAGCGAGTCTGCTGTCAG							
	1690	1700	1710	1720	1730	1740	1750	1760
GALVenv	AATAGCAGAGGCCTTGACTTGCTGTTTCTAAAAAGAAGTGGCCTCTGTGCGGCCCTAAAGGAAGAGTGCTGTTTTTACAT							
coGALVenv	AATAGCAGAGGCCTTGACTTGCTGTTTCTGAAAGAAGCGGACTTTCGCCCGCTCTGAAAGAGGAATCTGTGTTTTACAT							
	1770	1780	1790	1800	1810	1820	1830	1840
GALVenv	AGACCACTCAGCTGCAGTACGGGACTCCATGAAAAAACTCAAAGAAAACTGGATAAAAGACAGTTAGAGCGCCAGAAAA							
coGALVenv	CGACCACTCTGGCGCCCTCGGGACAGCATGAAGAAGCTCAAAGCAAGCTGGACAAGCGCCAGCTGGAAGACAGAAGT							
	1850	1860	1870	1880	1890	1900	1910	1920
GALVenv	GCCAAACTGGTATGAAGGATGGTTCAATACCTCCCCCTGGTTTACCACCTTAATCTCCACCATCATGGGACCTCTAATA							
coGALVenv	CTCAGAAATGGTACGAAGCCTGGTTCAACACAAAGCCCCCTGGTTACCACACTCATCAGCACCATCATGGGCCCTCTGATC							
	1930	1940	1950	1960	1970	1980	1990	2000
GALVenv	GTACTCTTACTGATCTTACTCTTTGGACCTTGCATTCTCAATTCGATTAGTTCAATTTGTIAAAGACAGCATCTCAGTAGT							
coGALVenv	GTGCTGCTGTGATCCTCTCTGTTTGGCCCTGCATCTCTAAACAGACTGGTGCAGTTCTGTAAAGGACAGAAATCAGCGTCTGT							
	2010	2020	2030	2040	2050	2060		
GALVenv	CCAGGCTTTACTCTCTGACTCAACATAACACCAGCTAAACCCTAGAGTACGAGCCATGA							
coGALVenv	GCAGGCCCTGTGCTGACACAGCAATATCACCAGCTGAAGCCTCTGGAATACGAGCCCTGA							

### phCMV-GALVenv-C<sub>4070A</sub>:



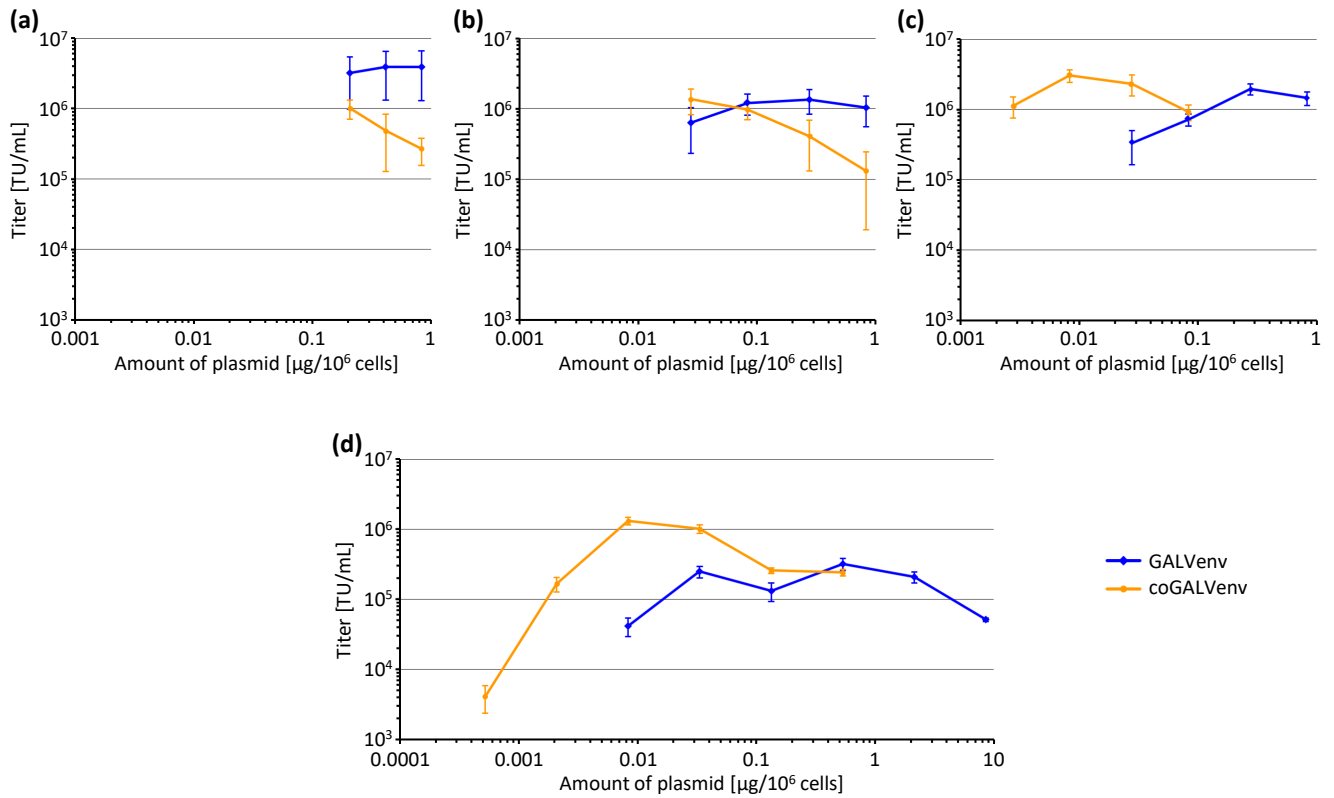
BamHI NcoI  
 1401 ATTCCTCGACGGAT**CCGCC**ATGGTATTGCTGCCTGGGTCCATGCTTCTCA  
   M V L L P G S M L L T

### phCMV-coGALVenv-C<sub>4070A</sub>:



BamHI NcoI  
 1401 ATTCCTCGACGGAT**CCGCCACC**ATGGTTTTTCTGCCTGGCTCTATGCTGC  
   M V L L P G S M L L

**Figure S2: Plasmid maps of GALVenv and coGALVenv.** Size differs by 3 bp because of shown differences in Kozak sequence (bold). hCMVie: human CMV immediate early promoter; C<sub>4070A</sub>: contains C-terminus of amphotropic MLV strain 4070A; AmpR: ampicillin resistance.



**Figure S3:** Dose-finding of coGALVenv plasmid amount needed for efficient packaging of lentiviral vectors. **(a)** Starting with conventional amounts of GALVenv and coGALVenv expressing plasmids, the codon-optimized coGALVenv plasmid resulted in lower titers than the non-optimized GALVenv. **(b)** Titer analysis of a larger range of plasmid amounts, the optimum of coGALVenv plasmid amount is still not visible. **(c)** Titer analysis with further reduced amounts of coGALVenv plasmid. The expression strength of the coGALVenv plasmid is more than a log step stronger than the expression strength of the non-optimized GALVenv. **(d)** Vector production at an even further extended range of plasmid amounts, ranging from 0.5 ng to 0.5  $\mu\text{g}$  of coGALVenv expressing plasmid per  $10^6$  293T producer cells and 8 ng to 8  $\mu\text{g}$  of GALVenv expressing plasmid per  $10^6$  293T producer cells (data of Fig. S3d also shown as Fig. 1b in main text). For a to d:  $n = 3$