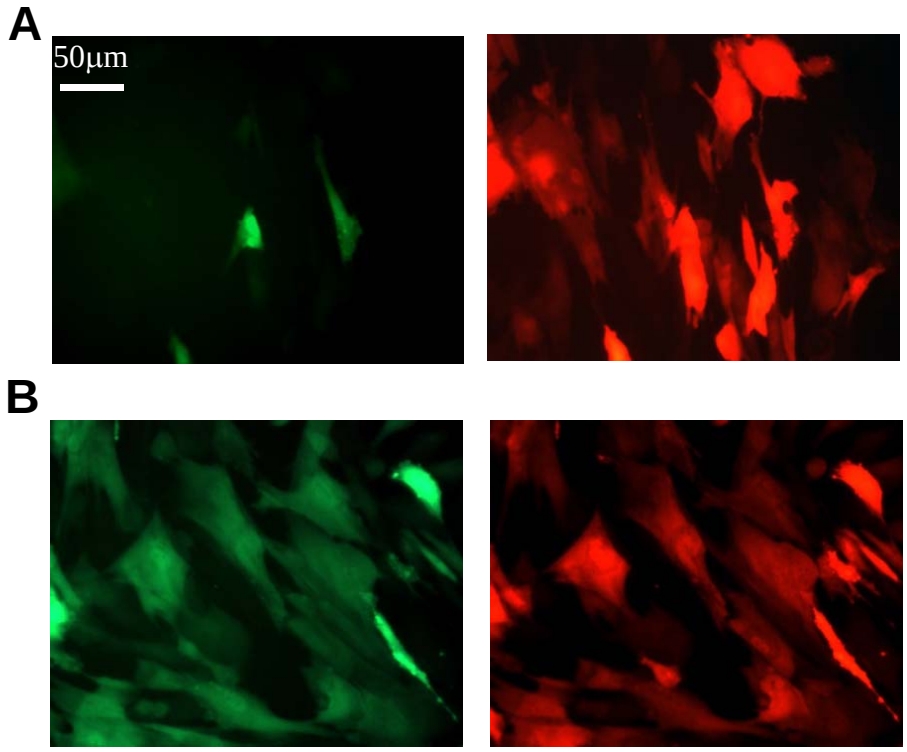
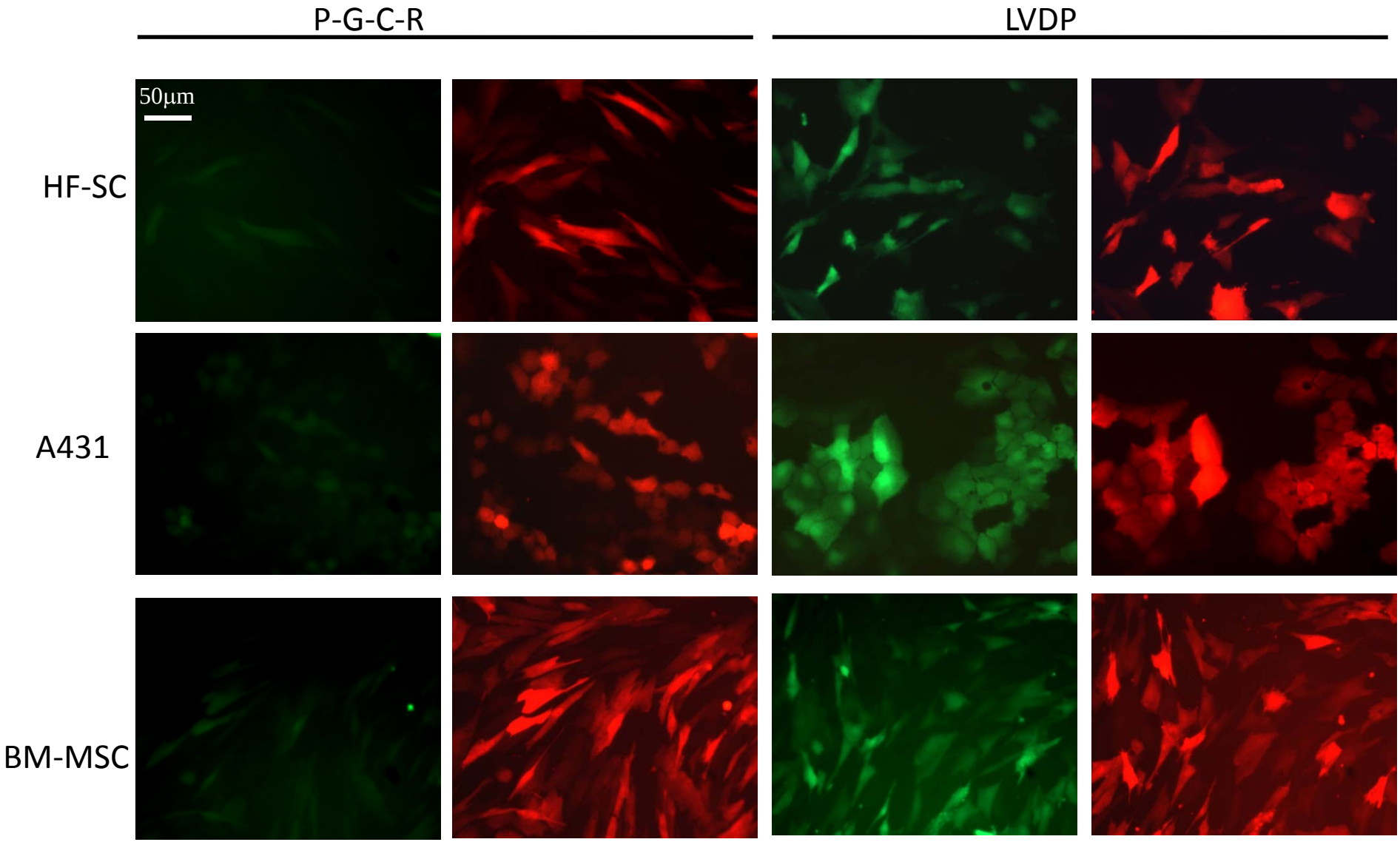
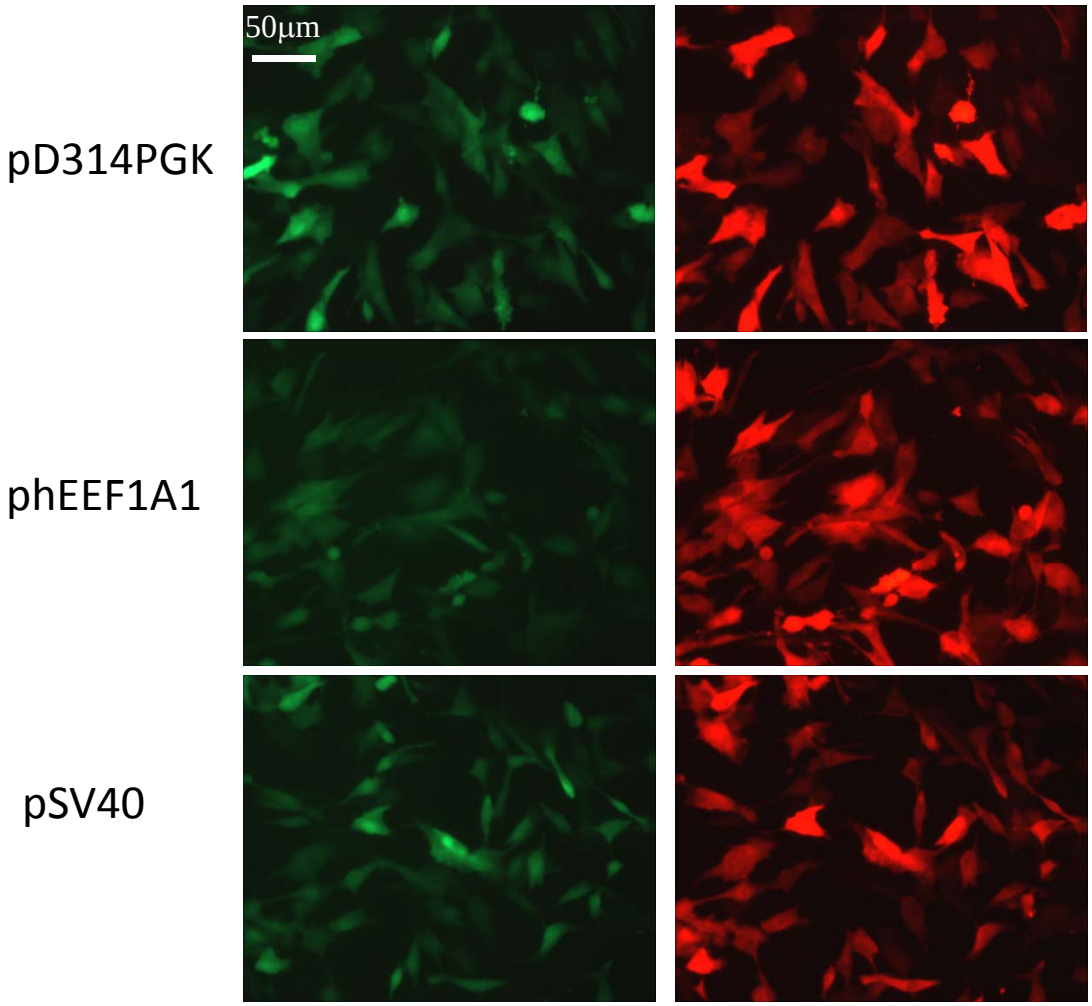
# Fig. S1



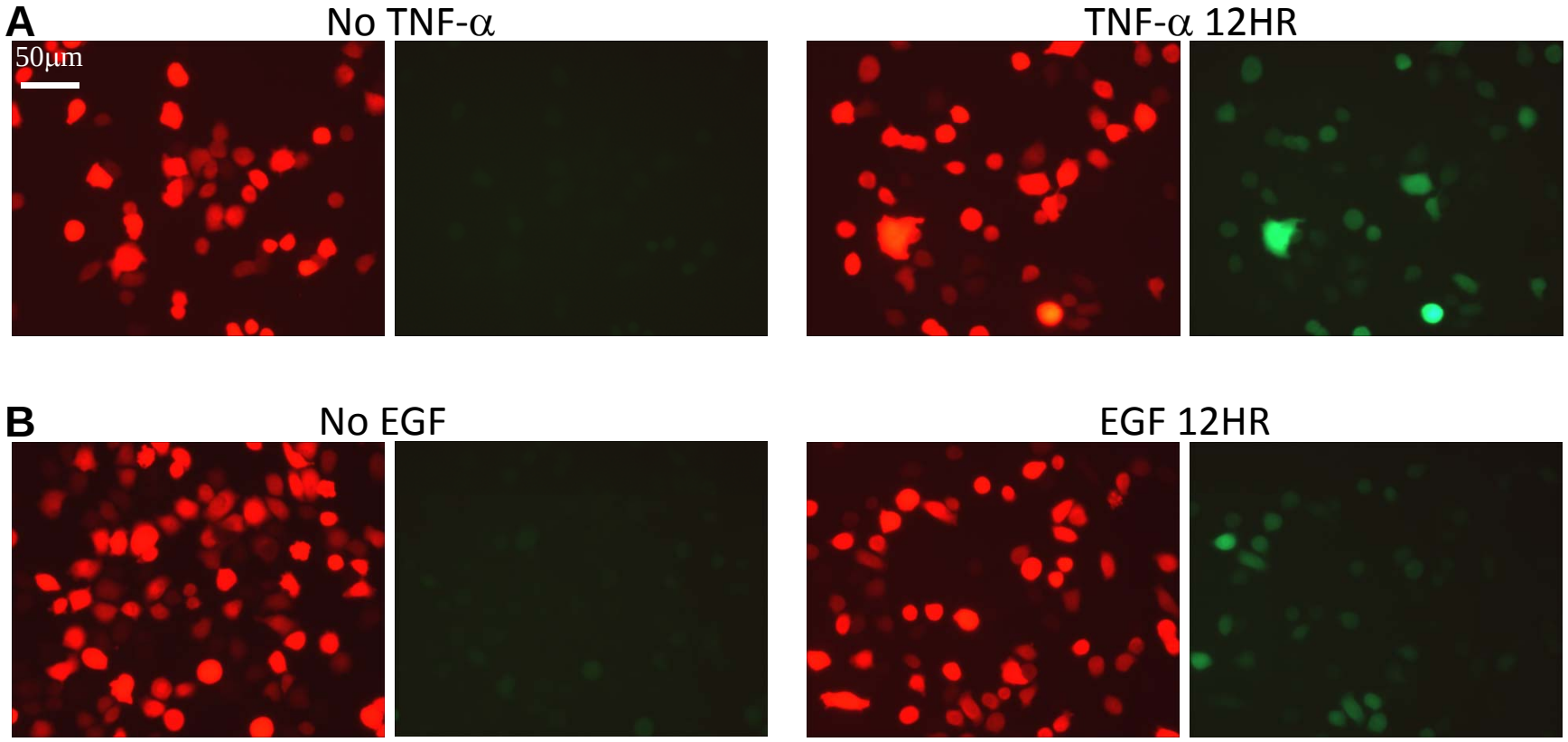
# Fig.S2



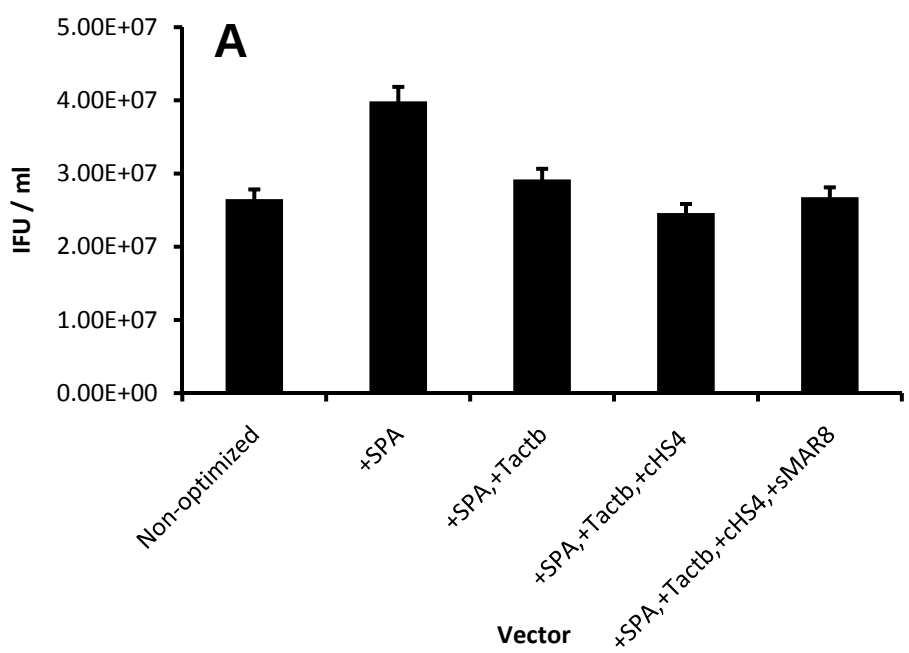
# Fig.S3



# Fig.S4



# Fig.S5


A
IFU / ml
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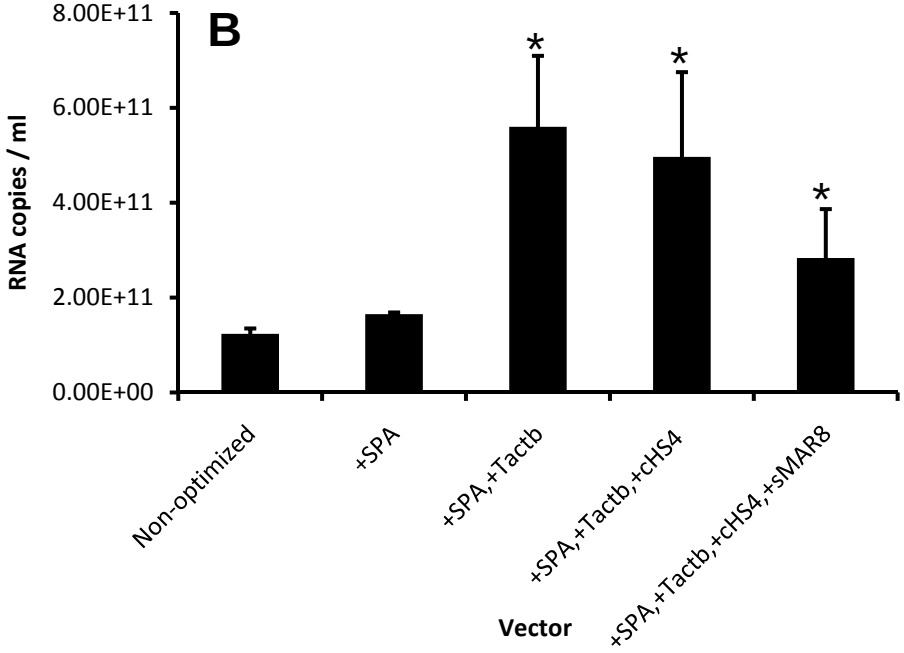
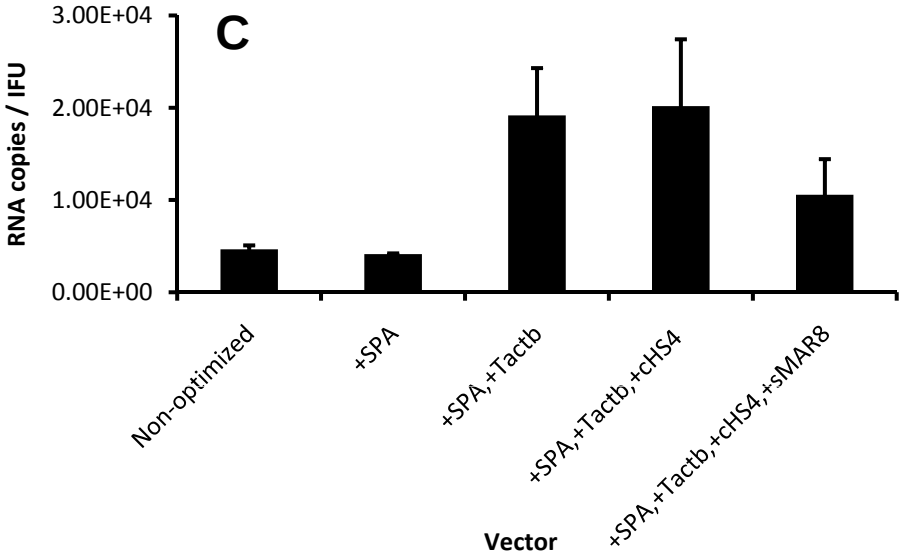
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
Non-optimized
+SPA
+SPA,+Tactb
+SPA,+Tactb,+cHS4
+SPA,+Tactb,+cHS4,+sMAR8
Vector


B
RNA copies / ml
8.00E+11
6.00E+11
4.00E+11
2.00E+11
0.00E+00
*
*
*
Non-optimized
+SPA
+SPA,+Tactb
+SPA,+Tactb,+cHS4
+SPA,+Tactb,+cHS4,+sMAR8
Vector


C
RNA copies / IFU
3.00E+04
2.00E+04
1.00E+04
0.00E+00
Non-optimized
+SPA
+SPA,+Tactb
+SPA,+Tactb,+cHS4
+SPA,+Tactb,+cHS4,+sMAR8
Vector

| Purpose | Vector # | Parental vector | Insert | Cloning sites | Primers/Oligos | Source of inserts | Remarks |
|---|---|---|---|---|---|---|---|
| Parental vectors | **1** | pCSCG | pA and multiple cloning site (MCS) | BamHI, XhoI | **pAMCSF**:TCGAAGAATTCACCGCGGAATCGATAACCGGTAGGCGCGCCAG GATCCACTCGAGAATGACGGCAATAAAAAGACAGAATAAAACCCACGGG TGTTGGGTCGTTTGTTCATAAACGCGGA; **pAMCSR**:GATCTCCGCGTTTATGAACAAACGACCCAACACCCGTGGGTTTT ATTCTGTCTTTTTATTGCCGTCATTCTCGAGTGGATCCTGGCGCGCCTACC GGTTATCGATTCCGCGGTGAATTCT | oligos | the oligoes were anealed and ligated into pCSCG to replace CMV-GFP segment in the original vector. After ligation, multiple cloning sites (EcoRI, SacII, ClaI, AgeI, ASCI, BamHI, XhoI) and the core sequence of Herplex Simplex Virus (HSV) thymidine kinase polyA were introduced. |
| | **2** | 1 | CMV_GFP | BamHI, XhoI | | subclone from pCSCG | subclone |
| | **3** | 2 | DsRed2 | NheI, XhoI | NheI_DsRed2F:AACAACGCTAGCTCGCCACCATGGCCTCCTCC; XhoI_DsRed2R:TGTTGTCTCGAGGGGGGAGGTGTGGGAGGTTT | PCR from pDsRed2-1 | DsRed2 was amplified with PCR and cloned into vector 2 to replace GFP |
| | **4** | 3 | ZsG | BamHI, AgeI | BamHI_ZsGR:TGGAGGGGATCCTCTAGAAGTTAGGGCAAGGCGGA; AgeI_ZsGF:ACAACAACCGGTATCCGCCACCATGGCCCAGT | PCR from pSIREN-RetroQ-ZsG | ZsG was amplified with PCR from pSIREN-RetroQ-ZsG vector and cloned into vector 3. Note that a cloning site XbaI was created upstream of BamHI |
| | **5** | 4 | phPGK | ClaI, AgeI | **ClaI_hPGKF**:ACAACAATCGATAATTCCACGGGGTTGGGGTT; **AgeI_hPGKR**:TGTTGTACCGGTTACAGCTGGGGAGAGAGGTC | PCR from hgDNA | phPGK was amplified from hgDNA |
| Clone polyAs downstream of vector #5 | **6** | 5 | hGHpA | XbaI, BamHI | **XbaI_hGHpAF**: ACAACATCTAGAGCAGTGCCGCTCTGTGGAGG; **BamHI_hGHpAR**: TGTTGTGGATCCGGCATGGCCAGGTAGCCTAT | PCR from hgDNA | hGHpA was amplified from hgDNA |
| | **7** | 5 | rGBpA | XbaI, BamHI | **XbaI_rGBpAF**:ACATGTTCTAGAGGGCAACGTGCTGGTTGTTG; **BamHI_rGBpAR**:TGTTGTGGATCCAGAGAAGAGGGACAGCTATG | PCR from pC4S1-FM4-FCS-hGH | rGBpA was amplified from vector pC4S1-FM4-FCS-hGH |
| | **8** | 5 | SPA | XbaI, BamHI | **XbaI_SPAF**:CTAGCAATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGG TTTTTTGTGTGTCTAGAG; **BamHI_SPAR**:GATCCTCTAGACACACAAAAAACCAACACACAGATCTAAT GAAAATAAAGATCTTTTATTG | oligos | the oligoes of SPA were anealed and ligated into vector 5. Note that after ligation the original XbaI was lost and a new XbaI was created downstream of SPA for cloning other sequences. |
| Clone terminators/pause sites downstream of SPA using vector #8 | **9** | 8 | S | XbaI, BamHI | **BamHI_SF**: ACAACAGGATCCAGGCGTAAATTGTAAGCGTT; **XbaI_SR**:TGTTGTGGATCCCCCTGTAGCGGCGCATTAAG | PCR from pEGFP-1 | the spacer sequence was amplified by PCR from vector pEGFP-1 |
| | **10** | 8 | CoTC | XbaI, BamHI | **XbaI_CoTCF**:ACAACATCTAGAATGTCCCTTATGGTGCTTCT; **BamHI_CoTCR**:ACAACAGGATCCGTGTTAATTAATTGTTGCCTTTGCTTCT CAT | PCR from hgDNA | the CoTC sequence was amplified by PCR from hgDNA. Note a PacI site was created downstream of CoTC for cloning other sequences. |
| | **11** | 8 | $T_{actb}$ | XbaI, BamHI | **XbaI_$T_{actb}$F**:ACAACATCTAGAGGTGGGGCAGTGGGGGCCAA; **BamHI_$T_{actb}$R**:ACAACAGGATCCACATTAATTAACTGACAGCCACGATCCC ATA | PCR from hgDNA | the CoTC sequence was amplified by PCR from hgDNA. Note a PacI site was created downstream of $T_{actb}$ for cloning other sequences. |
| | **12** | 8 | MAZ4 | XbaI, BamHI | **XbaI_MAZ4F**:CTAGAGCCTTGGGGGAGGGGGAGGCCAGAAGGCCTTGGG GGAGGGGGAGGCCAGAAGGCCTTGGGGGAGGGGGAGGCCAGAAGGCCT TGGGGGAGGGGGAGGCCAGAATTAATTAATGTG; **BamHI_MAZ4R**:GATCCACATTAATTAATTCTGGCCTCCCCCTCCCCCAAG GCCTTCTGGCCTCCCCCTCCCCCAAGGCCTTCTGGCCTCCCCCTCCCCCA AGGCCTTCTGGCCTCCCCCTCCCCCAAGGCT | oligos | the oligoes for MAZ4 were anealed and ligated into vector 8. Note a PacI site was created downstream of MAZ4 for cloning other sequences. |
| | **13** | 8 | $T_{msa}$ | XbaI, BamHI | **XbaI_$T_{msa}$F**:ACAACATCTAGACTCCCCTCGTGCCCCACACT; **BamHI_$T_{msa}$R**:ACAACAGGATCCACATTAATTAACCTCTCAAAAACAGAGC TCACT | PCR from mouse gDNA | the CoTC sequence was amplified by PCR from mouse gDNA.Note a PacI site was created downstream of $T_{msa}$ for cloning other sequences. |
| Clone insulators downstream of Tactb using vector #11 | **14** | 11 | sMAR4 | PacI, BamHI | **PacI_sMAR4F**:GATCCACATTAATTAATTCTAAATATATTAGAAATTAAAGA TTCTAAATATATTAGAAATTAAAGATTCTAAATATATTAGAAATTAAAGAT TCTAAATATATTAGAAATTAAAGAAT; **BamHI_sMAR4R**:TCTTTAATTTCTAATATATTTAGAATCTTTAATTTCTAAT ATATTTAGAATCTTTAATTTCTAATATATTTAGAATCTTTAATTTCTAATAT ATTTAGAATTAATTAATGTG | oligos | the oligos of sMAR4 were anealed and ligated into vector 11. Note that after ligation the original PacI was lost and a new PacI was created downstream of sMAR4 for cloning other sequences. |
| | **15** | 14 | sMAR8 | PacI, BamHI | **PacI_sMAR4F**:GATCCACATTAATTAATTCTAAATATATTAGAAATTAAAGA TTCTAAATATATTAGAAATTAAAGATTCTAAATATATTAGAAATTAAAGAT TCTAAATATATTAGAAATTAAAGAAT; **BamHI_sMAR4R**:TCTTTAATTTCTAATATATTTAGAATCTTTAATTTCTAAT ATATTTAGAATCTTTAATTTCTAATATATTTAGAATCTTTAATTTCTAATAT ATTTAGAATTAATTAATGTG | oligos | similar to cloing vector 14, another copy of sMAR4 was cloned. |
| | **16** | 11 | cHS4 | PacI, BamHI | **PacI_cHS4F**:ACAACATTAATTAACCTTCCGCGGGAGCTCACGG; **BamHI_cHS4R**:TGTTGTGGATCCGTGACGCACTGAACAGGTTG | PCR from chicken gDNA | cHS4 sequence was amplified from chicken gDNA |
| | **17** | 15 | cHS4 | PacI, BamHI | **PacI_cHS4F**:ACAACATTAATTAACCTTCCGCGGGAGCTCACGG; **BamHI_cHS4R**:TGTTGTGGATCCGTGACGCACTGAACAGGTTG | PCR from chicken gDNA | cHS4 sequence was amplified from chicken gDNA |
| | **18** | pCSCG | New MCS | BamHI, XhoI | **NpAMCSF**:TCGAAGAATTCACTCGAGAATGACGGCAATAAAAAGACAGAA TAAAACCCACGGGTGTTGGGTCGTTTGTTCATAAACGCGGAAGCGCTACG GTCCGAGTTAACA; **NpAMCSR**:GATCTGTTAACTCGGACCGTAGCGCTTCCGCGTTTATGAACAA ACGACCCAACACCCGTGGGTTTTATTCTGTCTTTTTATTGCCGTCATTCTC GAGTGAATTCT | oligos | After ligation, original BamHI and XhoI were. Note new MCS (AfeI, RsrII,XhoI, EcoRI, HpaI) and the same TKpA as in vector 1 were introduced. |
| | **19** | 18 | M9 | AfeI, RsrII | **AfeI_M9F**:ATTTCCATATAGAACAATGTAAATTCTTTCCTAGAGTTAAGCAG GGCTTTTTCATAAGCTGAAGCGCTACG; **RsrII_M9R**:GACCGTAGCGCTTCAGCTTATGAAAAAGCCCTGCTTAACTCT AGGAAAGAATTTACATTGTTCTATATGGAAAT | oligos | oligoes for M9 were anealed and ligated into vector 18 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Clone insulators downstream of TKpA | **20** | 18 | m3'HS1 | AfeI, RsrII | **AfeI_m3'HS1F**: TCATTTCTCTAATGATCCTGTTGCATACCAGTAGGGGGCAGAAGTGTTCC ACTGATTTCCGCCCTCCTCTCCAGCGCTACG; **RsrII_m3'HS1R**:GACCGTAGCGCTGGAGAGGAGGGCGGAAATCAGTGGAA CACTTCTGCCCCCTACTGGTATGCAACAGGATCATTAGAGAAATGA | oligos | oligoes for m3'HS1 were anealed and ligated into vector 18 |
| | **21** | 18 | H19HS2 | AfeI, RsrII | **RsrII_H19HS2F**:ACAACACGGTCCGCCAGCCAGTGTGGCTCACTA; **AfeI_H19HS2R**:ACAACAAGCGCTGTCTGCCGAGCAATATGTAG | PCR from mouse gDNA | insulator H19HS2 was amplified from mouse gDNA |
| | **22** | 19 | DsRed2_CMV_cHS4_Tactb _SPA_ZsG_phPGK | EcoRI, XhoI | | subclone from vector 16 | The insert DsRed2_CMV_cHS4_Tactb_SPA_ZsG_phPGK was cut from vectro 16 and subcloned into vector 19 |
| | **23** | 20 | DsRed2_CMV_cHS4_Tactb _SPA_ZsG_phPGK | EcoRI, XhoI | | subclone from vector 16 | The insert DsRed2_CMV_cHS4_Tactb_SPA_ZsG_phPGK was cut from vectro 16 and subcloned into vector 20 |
| | **24** | 21 | DsRed2_CMV_cHS4_Tactb _SPA_ZsG_phPGK | EcoRI, XhoI | | subclone from vector 16 | The insert DsRed2_CMV_cHS4_Tactb_SPA_ZsG_phPGK was cut from vectro 16 and subcloned into vector 21 |
| | **25** | pCSCG | sMAR4 | BamHI, XhoI | **XhoI_sMAR4F**:TCGAGTGTGGATCCTTCTAAATATATTAGAAATTAAAGAT TCTAAATATATTAGAAATTAAAGATTCTAAATATATTAGAAATTAAAGATT CTAAATATATTAGAAATTAAAGAT; **BamHI_sMAR4R**:GATCATCTTTAATTTCTAATATATTTAGAATCTTTAATTT CTAATATATTTAGAATCTTTAATTTCTAATATATTTAGAATCTTTAATTTCT AATATATTTAGAAGGATCCACAC | oligos | The oligoes for sMAR4 were anealed and ligated into pCSCG to replace CMV-GFP segment in the original vector. After ligation, the original BamHI was lost but a new BamHI was created upstream of sMAR4 |
| | **26** | 25 | sMAR4 | BamHI, XhoI | | same oligos as used in vector 25 | similar to cloing vector 25, another copy of sMAR4 was cloned. |
| | **27** | 26 | New MCS | BamHI, XhoI | | same oligos as used in vector 1 | similar to cloing vector 1, HSV TKpA together with mutiple cloning sites was cloned. |
| | **28** | 27 | DsRed2_CMV_cHS4_Tactb _SPA_ZsG_phPGK | EcoRI, XhoI | | subclone from vector 16 | The insert DsRed2_CMV_cHS4_Tactb_SPA_ZsG_phPGK was cut from vectro 16 and subcloned into vector 27 |
| Use EGFP to replace ZsGreen | **29** | 28 | SPA-EGFP | **AgeI, XbaI** | **AgeI_EGFP_SPAF**:ACAACACTCTAGACACACAAAAAACCAACACACAGAT CTAATGAAAATAAAGATCTTTTATTGCTACCGCTTTACTTGTACAGCTC; **XbaI_EGFP_SPAR**:ACAACACTCTAGACCGCTTTACTTGTACAGCTC | PCR from pEGFP-1 | EGFP was amplified from pEGFP-1 with primer containing SPA and cloned into vector 29 to replace ZsG-SPA |
| Clone promoters in place of PGK | **30** | 27 | DsRed2_CMV_cHS4_Tactb _SPA_EGFP | XhoI, AgeI | | subclone from vector 29 | The insert DsRed2_CMV_cHS4_Tactb_SPA_EGFP was cut from vector 29 and subcloned into vector 27 |
| | **31** | 30 | phEEF1A1 | EcoRI, ClaI | **EcoRI_hEEF1A1F:**ACAACAGAATTCGGAGAAGAGCATGCGTGAGG **ClaI_hEEF1A1R**:ACAACAATCGATAGGGGTAGTTTTCACGACAC | PCR from hgDNA | phEEF1A1 was amplified from hgDNA |
| | **32** | 30 | phD314PGK | **ClaI, AgeI** | **ClaI_hD314PGKF:**ACAACAATCGATTGGGCTGTGGCCAATAGCGG**;** **AgeI_hPGKR:**TGTTGTACCGGTTACAGCTGGGGAGAGAGGTC | PCR from hgDNA | phD314PGK was amplified from hgDNA |
| | **33** | 30 | pSV40 | EcoRI, AgeI | **EcoRI_pSV40F**: ACAACAGAATTCCTGAGGCGGAAAGAACCAGC; **AgeI_pSV40R**:TGTTGTACCGGTCGATCCTCATCCTGTCTCTT | PCR from pd2EGFP-1 | pSV40 was amplified from pEGFP-1 |
| | **34** | 30 | pACTA2 | ClaI, AgeI | **ClaI_ACTA2F**: ACAACAATCGATAACAGCTGGTCATGGCTGTA; **AgeI_ACTA2R**: TGTTGTACCGGTGCATGAACCCAGCCAAATCC | PCR from hgDNA | pACTA2 was amplified from hgDNA |
| use d2EGFP to replace EGFP | **35** | 30 | SPA-d2EGFP | AgeI, XbaI | **AgeI_d2EGFP**:ACAACAACCGGTGGTCGCCACCATGGTGAGCA; **XbaI_SPA_d2EGFP**:ACAACATCTAGACACACAAAAAACCAACACACAGAT CTAATGAAAATAAAGATCTTTTATTGCTAGAGCGCATCTACACATTGATC | PCR from pd2EGFP-1 | SPA-d2EGFP was amplified from pd2EGFP-1 with primer containing SPA and cloned into vector 30 to replace EGFP-SPA |
| Clone response elements | **36** | 35 | RE-NFKB | ClaI, AgeI | | subclone from pRE-NFKB_CMVmini_d2EGFP | RE-NFKB together with $CMV_{mini}$ was cut from vector pRE-NFKB_$CMV_{mini}$_d2EGFP and cloned into vector 35 |
| | **37** | 35 | RE-AP1 | ClaI, AgeI | | subclone from pRE-AP1_CMVmini_d2EGFP | RE-NFKB together with $CMV_{mini}$ was cut from vector pRE-AP1_$CMV_{mini}$_d2EGFP and cloned into vector 35 |

Note: all genomic DNA used was obtained from EMD (Gibbstown, NJ)