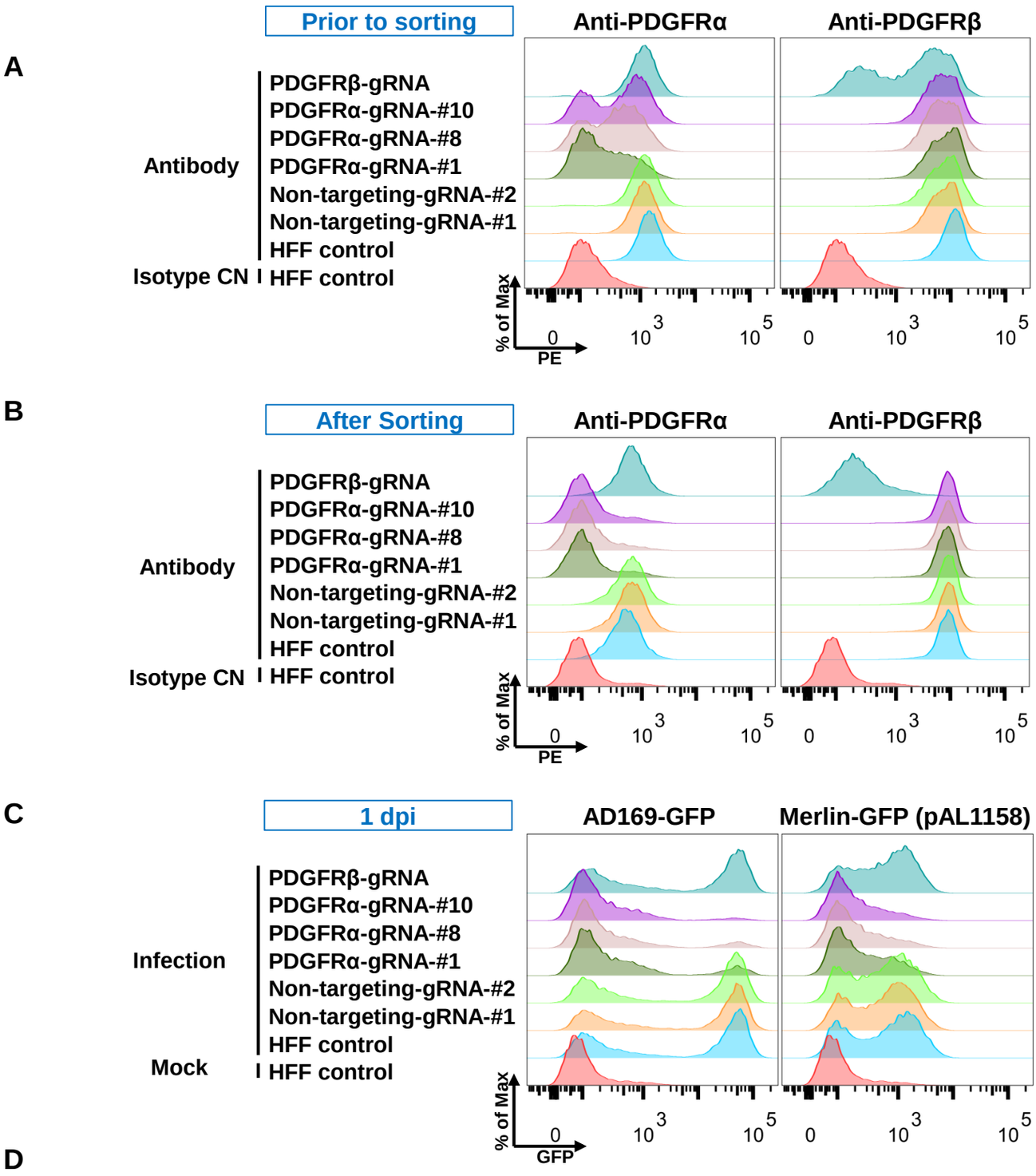

Fig. S1. PDGFRα, but not PDGFRβ, is required for trimer-only HCMV infection. (A) HFF cells were transduced with lentiviruses expressing Cas9 and non-targeting gRNAs, or gRNAs specific for PDGFRα or PDGFRβ. 14 days after transduction, cell surface staining of PDGFRα and PDGFRβ was measured prior to sorting by FACS. (B) PDGFRα-low or PDGFRβ-low populations were sorted by FACS from HFF cells expressing gRNAs against PDGFRα or PDGFRβ as shown above, and surface staining was performed to confirm the purity of sorted cells. (C) PDGFRα-low, PDGFRβ-low and control cells were infected by AD169-GFP or Merlin-GFP (pAL1158) at multiplicity of 3 FFU/cell, and GFP expressed from the viral genome was monitored by flow cytometry to determine the percentage of infected cells at 1 dpi. (D) AD169-GFP and Merlin-GFP yields from cells infected in panel C were quantified at 5 dpi. Mean ± SD is presented for three biological replicates assayed in triplicate.

www.pnas.org/cgi/doi/10.1073/pnas.1806305115

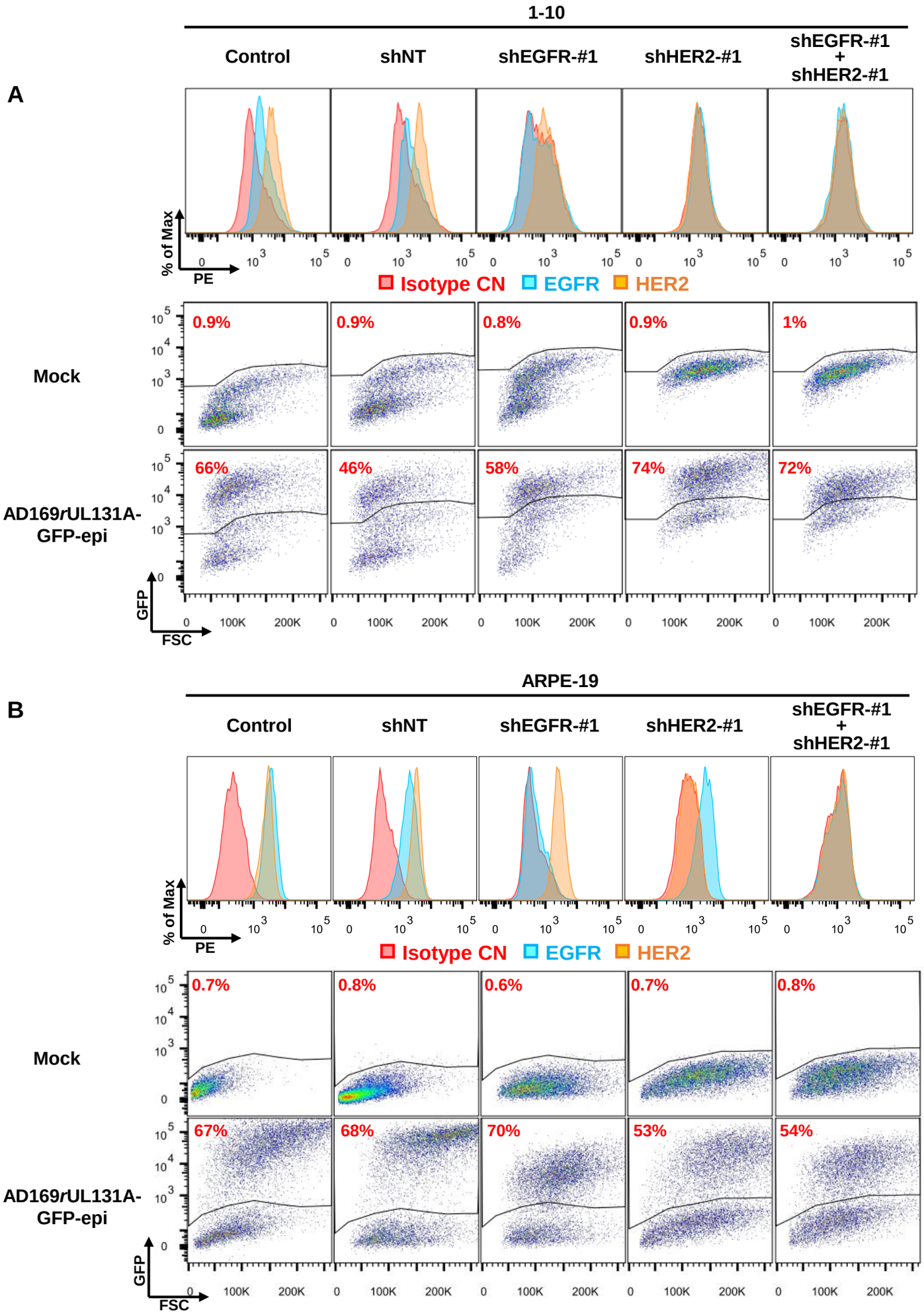

Fig. S2. EGFR and HER2 are not required for AD169 derivatives containing the pentameric complex to enter PDGFRα-KO fibroblasts or ARPE-19 epithelial cells. PDGFRα-KO cells (clone 1-10) (A) and ARPE-19 cells (B) were transduced with lentiviruses expressing shRNAs targeting EGFR, HER2 or both. Top panels: Cell surface staining of EGFR and HER2 was performed to confirm the knockdown. Bottom panels: Cells were infected with AD169*r*UL131A-GFP-epi at a multiplicity of 3 FFU/cell, and GFP expression was measured at 24 hpi to assay the percentage of infected cells. The percentage of IE1-positive cells is labeled in red. Control: cells without lentiviral transduction; shNT: non-targeting shRNA.

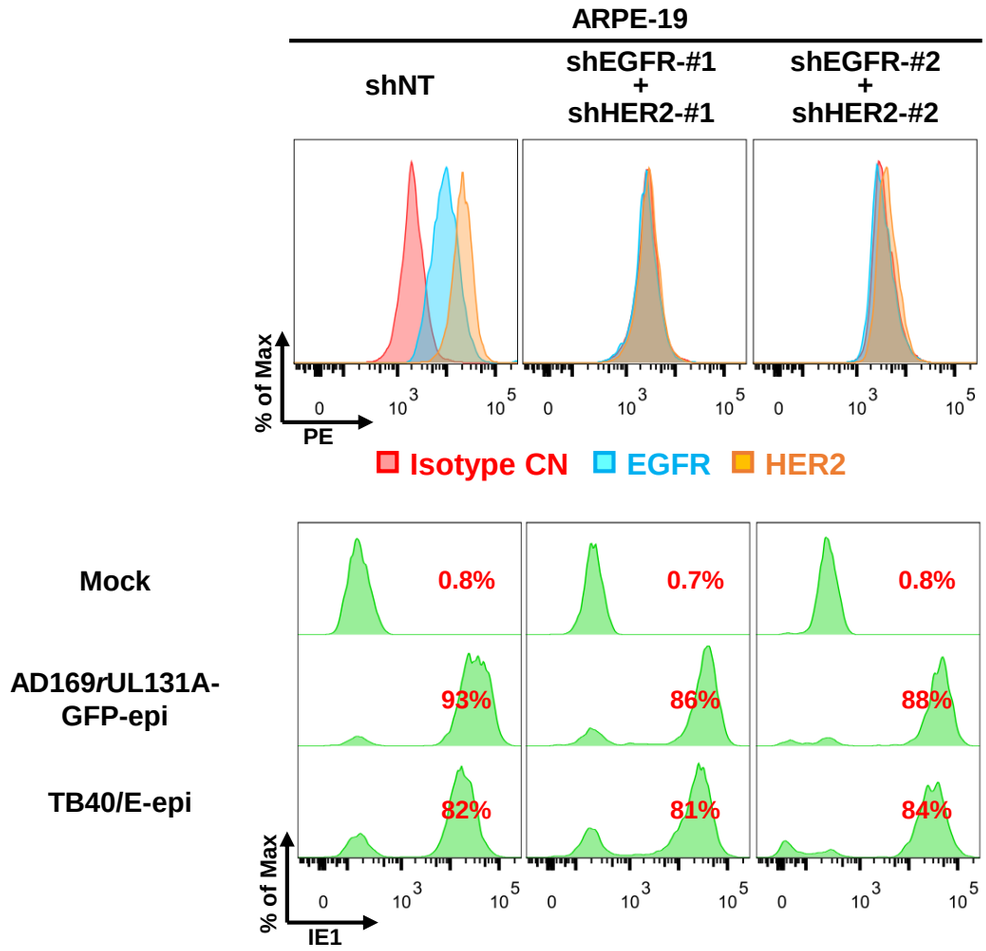

Fig. S3. EGFR and HER2 are not required for TB40/E to enter ARPE-19 epithelial cells. ARPE-19 cells were transduced with lentiviruses expressing shRNAs targeting both EGFR and HER2. shRNAs labeled #1 correspond to those used in the experiment reported in figure S2, and shRNAs labeled #2 correspond to the additional set. Top panels: Cell surface staining of EGFR and HER2 was performed to confirm the knockdown. Bottom panels: Cells were infected with AD169*r*UL131A-GFP-epi or TB40/E-epi at a multiplicity of 3 FFU/cell, and IE1 expression was measured at 24 hpi to assay the percentage of infected cells. The percentage of IE1-positive cells is labeled in red. Control: ARPE-19 cells without lentiviral transduction; shNT: nontargeting shRNA.

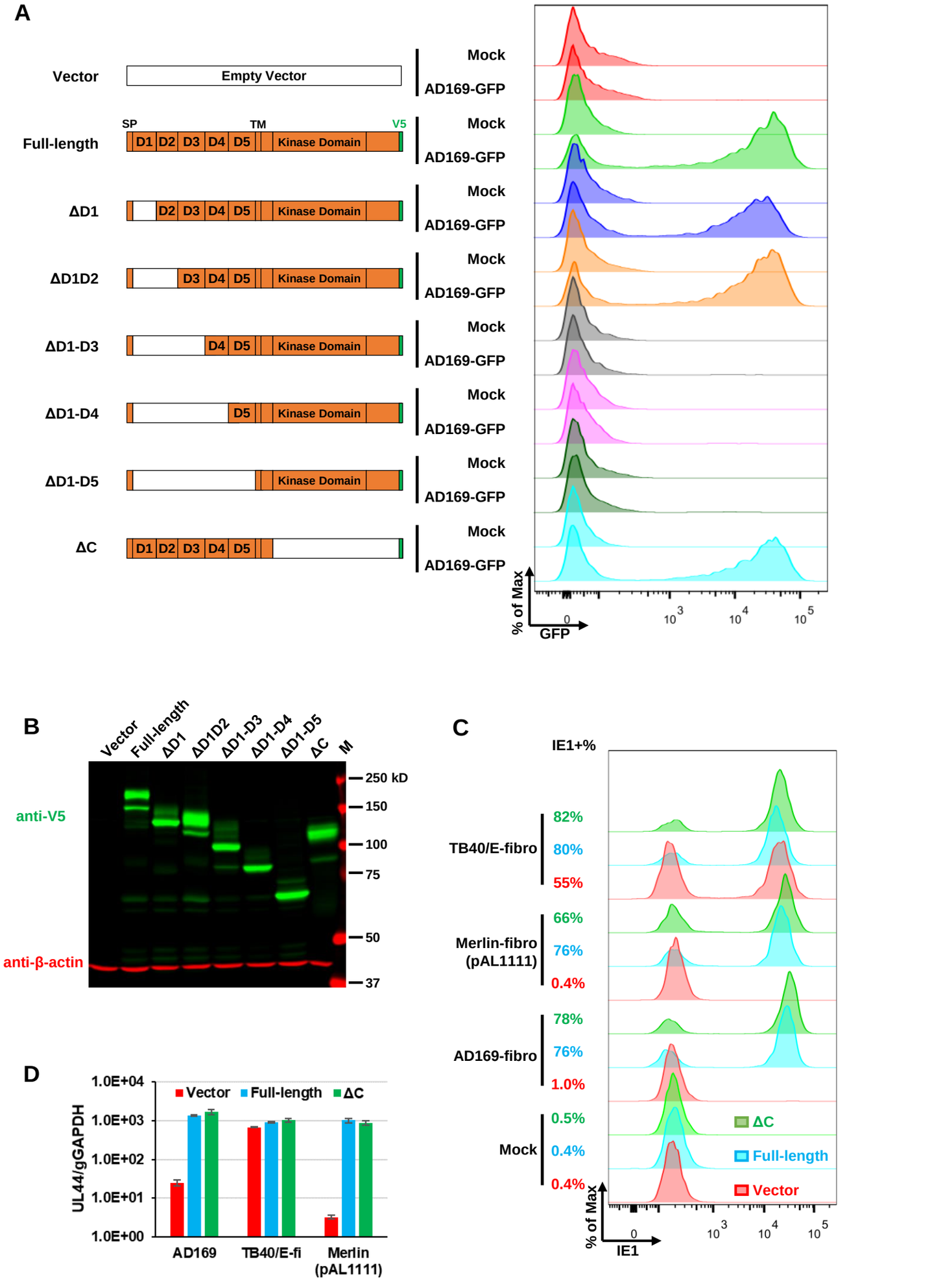

Fig. S4. IgG-like domain 3, but not the kinase domain, is required for trimer-only HCMV to enter PDGFRα-expressing ARPE-19 cells. (A) ARPE-19 cells were transduced with lentiviruses expressing V5-tagged, full-length or deleted variants of PDGFRα. Transduced ARPE-19 cells were infected with AD169-GFP at a multiplicity of 3 FFU/cell, and GFP-positive cells were quantified at 24 hpi by flow cytometry. (B) Expression of V5-tagged full length and truncated PDGFRα variants in ARPE-19 was confirmed by western blot. M: protein marker. (C) Transduced ARPE-19 cells were infected with AD169-fibro, Merlin-fibro (pAL1111) or TB40/E-fibro at a multiplicity of 3 FFU/cell, and IE1-positive cells were quantified at 24 hpi by flow cytometry. (D) Viral genomes were quantified in cells infected in (C) at 4 dpi by qPCR using primers specific for the viral UL44 coding region and the cellular GAPDH locus. Mean ± SD is presented for three biological replicates assayed in triplicate.

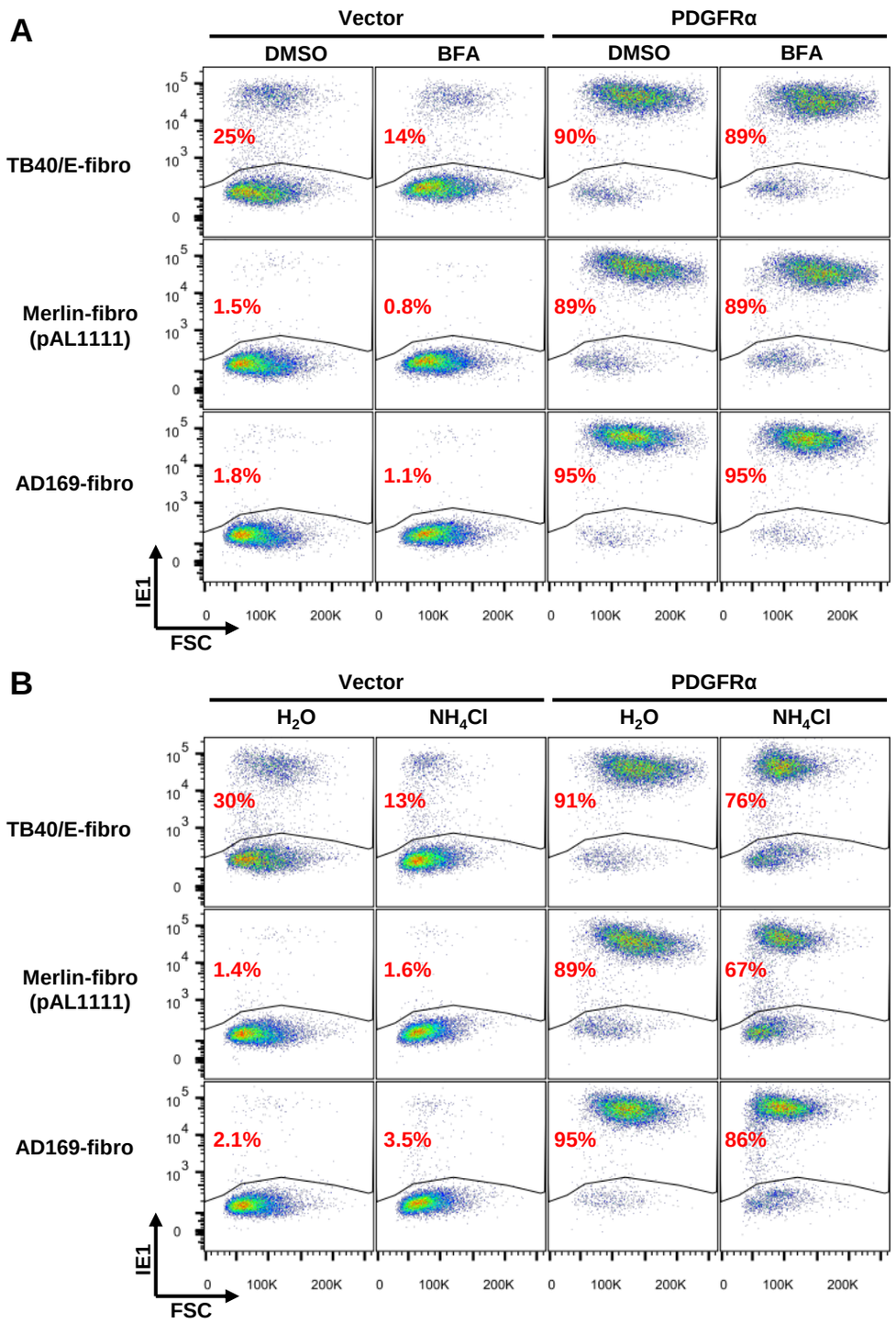

Fig. S5. Entry of trimer-only virus into PDGFRα-expressing ARPE-19 cells is pH independent. ARPE-19 cells transduced with lentivirus expressing PDGFRα or empty vector were pre-treated with 40 nM bafilomycin A1 (BFA) (A) or 40 μM NH_4Cl (B) for 1 h prior to infection with TB40/E-fibro, Merlin-fibro, or AD169-fibro at a multiplicity of 3 FFU/cell in the presence of the inhibitor for 1 h at 37℃. Infected cells were cultured with inhibitors and IE1 expression was assayed at 20 hpi to measure the percentage of infected cells. The percentage of IE1-positive cells is labeled in red.

```
CLUSTAL O(1.2.4) multiple sequence alignment


AA91-130          ------------------------------------------------------------  0
D3-AA202-306      PFNVYALKATSELDLEMEALKTVYKSGETIVVTCAVFNNEVVDLQWTYPGEVKGKGITML  60


AA91-130          -------------------SAAHTGLYTCYYNHTQTEENELEGRHIYIYVPDPDVAFVP  40
D3-AA202-306      EEIKVPSIKLVYTLTVPEATVKDSGDYECAARQATREVKEMKKVT--------------  105
                                     :. .:* * *  .::  * :*::
```

Fig. S6. Amino acid sequence alignment between PDGFRα amino acid 91-130 and IgG-like domain 3 (amino acid 202-306).

Table S1

GeCKO-1st PCR primer	Sequence
v2Adaptor_F	AATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCG
v2Adaptor_R	TCTACTATTCTTTCCCCTGCACTGTtgtgggcgatgtgcgctctg

GeCKO-2nd PCR Primer	Illumina flowcell attachment sequence & Illumina sequencing primer sequence	Barcode	Priming site	Length	Purification	Complete Primer Sequence
F01	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CGATGT	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATGTtcttgtggaaaggacgaaacaccg
F02	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TGACCA	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACCAtcttgtggaaaggacgaaacaccg
F03	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ACAGTG	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACAGTGtcttgtggaaaggacgaaacaccg
F04	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GCCAAT	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCAATtcttgtggaaaggacgaaacaccg
F05	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CAGATC	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGATCtcttgtggaaaggacgaaacaccg
F06	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CTTGTA	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGTAtcttgtggaaaggacgaaacaccg
F07	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATCACG	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCACGtcttgtggaaaggacgaaacaccg
F08	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TTAGGC	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTTAGGCtcttgtggaaaggacgaaacaccg
F09	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ACTTGA	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACTTGAtcttgtggaaaggacgaaacaccg
F10	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GATCAG	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCAGtcttgtggaaaggacgaaacaccg
F11	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TAGCTT	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCTTtcttgtggaaaggacgaaacaccg
F12	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GGCTAC	tcttgtggaaaggacgaaacaccg	88	PAGE	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCTACtcttgtggaaaggacgaaacaccg
R01	CAAGCAGAAGACGGCATACGAGAT		TCTACTATTCTTTCCCCTGCACTGT	49	PAGE	CAAGCAGAAGACGGCATACGAGATTCTACTATTCTTTCCCCTGCACTGT

Primer Name	Primer Sequence (gRNA)	Source
PDGFRa_gRNA_1_Fwd	CACCGGACCTTCAATGGACTTACCC	GeCKOv2 library
PDGFRa_gRNA_1_Rev	AAACGGGTAAGTCCATTGAAGGTCC	
PDGFRa_gRNA_8_Fwd	CACCGCTAAGACCAGGAACGCCGGA	Designed by Desktop Genetics
PDGFRa_gRNA_8_Rev	AAACTCCGGCGTTCCTGGTCTTAGC	
PDGFRa_gRNA_10_Fwd	CACCGCGTTTCCCCTGACTTATACA	Designed by Desktop Genetics
PDGFRa_gRNA_10_Rev	AAACTGTATAAGTCAGGGGAAACGC	
PDGFRb_gRNA_11_Fwd	CACCGCATCGCACCCGGAAGCCGCA	Designed by Desktop Genetics
PDGFRb_gRNA_11_Rev	AAACTGCGGCTTCCGGGTGCGATGC	
non-targeting_gRNA_Fwd	CACCGATCGTTTCCGCTTAACGGCG	GeCKOv2 library
non-targeting_gRNA_Rev	AAACCGCCGTTAAGCGGAAACGATC	
non-targeting_gRNA_2_Fwd	CACCGCGGGGAAATTTTACCGACGA	GeCKOv2 library
non-targeting_gRNA_2_Rev	AAACTCGTCGGTAAAATTTCCGCGC	

		Note for Deletion Variant
PDGFRa_gRNA1_Q5_Fwd	ggacctatccTGGAGAAGTGAAAGGCAAAG	
PDGFRa_gRNA1_Q5_Rev	actgcaaatcAACCACCTCATTGTTAAAAAC	
Ra-FL-V5_Fwd	attcgcGGATCCACCATGGGGACTTCCCATCCGG	
Ra-SP-115-V5_Fwd	attcgcGGATCCACCATGGGGACTTCCCATCCGGCGTTCCTGGTCTTAGGCTGTCTTCTCACAGGGCTGAGCCTAATCCTCTGCAGGCACATTTACATCTATGTGCCAGAC	**ΔD1 (ΔAA24-114)**
Ra-SP-202-V5_Fwd	attcgcGGATCCACCATGGGGACTTCCCATCCGGCGTTCCTGGTCTTAGGCTGTCTTCTCACAGGGCTGAGCCTAATCCTCTGCCCATTTAATGTTTATGCTTTAAAAGCAACATCAG	**ΔD1D2 (ΔAA24-201)**
Ra-SP-319-V5_Fwd	attcgcGGATCCACCATGGGGACTTCCCATCCGGCGTTCCTGGTCTTAGGCTGTCTTCTCACAGGGCTGAGCCTAATCCTCTGCCCCACCTTCAGCCAGTTGGA	**ΔD1-D3 (ΔAA24-318)**
Ra-SP-414-V5_Fwd	attcgcGGATCCACCATGGGGACTTCCCATCCGGCGTTCCTGGTCTTAGGCTGTCTTCTCACAGGGCTGAGCCTAATCCTCTGCCCTTCATCCATTCTGGACTTGGTC	**ΔD1-D4 (ΔAA24-413)**
Ra-SP-520-V5_Fwd	attcgcGGATCCACCATGGGGACTTCCCATCCGGCGTTCCTGGTCTTAGGCTGTCTTCTCACAGGGCTGAGCCTAATCCTCTGCACCCTGCGTTCTGAACTCACG	**ΔD1-D5 (ΔAA24-519)**
Ra-1-592-V5_Rev	atcgtaACGCGTCGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCGCCACCTCCATCTCTTGGAAACTCCCATCTTG	**ΔC (ΔAA593-1089)**
Ra-FL-V5-STOP_Rev	atcgtaACGCGTTTACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCGCCACCCAGGAAGCTGTCTTCCACCAGG	
Ra-FL_Rev	atcgtaACGCGTTTACAGGAAGCTGTCTTCCACCAGG	

		Adapted from Sigma Mission shRNA:
shNT_Fwd	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTTG	SHC002
shNT_Rev	AATTCAAAAACAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTG	
shEGFR_1_Fwd	CCGGGCCACAAAGCAGTGAATTTATCTCGAGATAAATTCACTGCTTTGTGGCTTTTTG	TRCN0000295969
shEGFR_1_Rev	AATTCAAAAAGCCACAAAGCAGTGAATTTATCTCGAGATAAATTCACTGCTTTGTGGC	
shEGFR_2_Fwd	CCGGGCTGGATGATAGACGCAGATACTCGAGTATCTGCGTCTATCATCCAGCTTTTTG	TRCN0000298822
shEGFR_2_Rev	AATTCAAAAAGCTGGATGATAGACGCAGATACTCGAGTATCTGCGTCTATCATCCAGC	
shHER2_1_Fwd	CCGGTGTCAGTATCCAGGCTTTGTACTCGAGTACAAAGCCTGGATACTGACATTTTTG	TRCN0000039878
shHER2_1_Rev	AATTCAAAAATGTCAGTATCCAGGCTTTGTACTCGAGTACAAAGCCTGGATACTGACA	
shHER2_2_Fwd	CCGGGAATATGTGAACCAGCCAGATCTCGAGATCTGGCTGGTTCACATATTCTTTTTG	TRCN0000039882
shHER2_2_Rev	AATTCAAAAAGAATATGTGAACCAGCCAGATCTCGAGATCTGGCTGGTTCACATATTC	

UL44_F	GTGCGCGCCCGATTTCAATATG	for qPCR
UL44_R	GCTTTCGCGCACAATGTCTTGG	for qPCR
gGAPDH_F	CCCCACACACATGCACTTACC	for qPCR
gGAPDH_R	CCTAGTCCCAGGGCTTTGATT	for qPCR