Supplemental information

CRISPR/Cas9 delivery with one single adenoviral vector devoid of all viral genes

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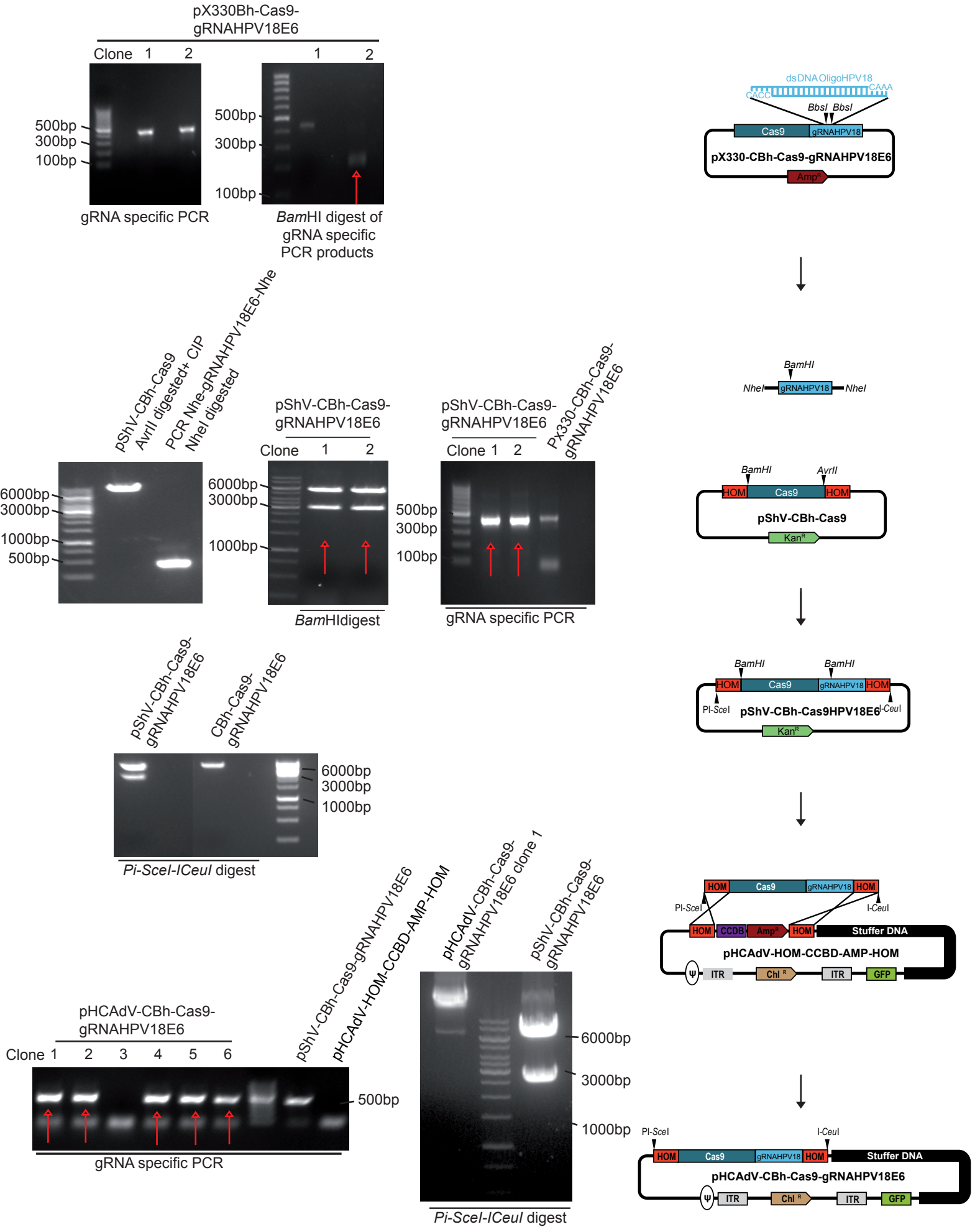
Supplementary Table 1: Sequences of gRNA oligonucleotides used in this study

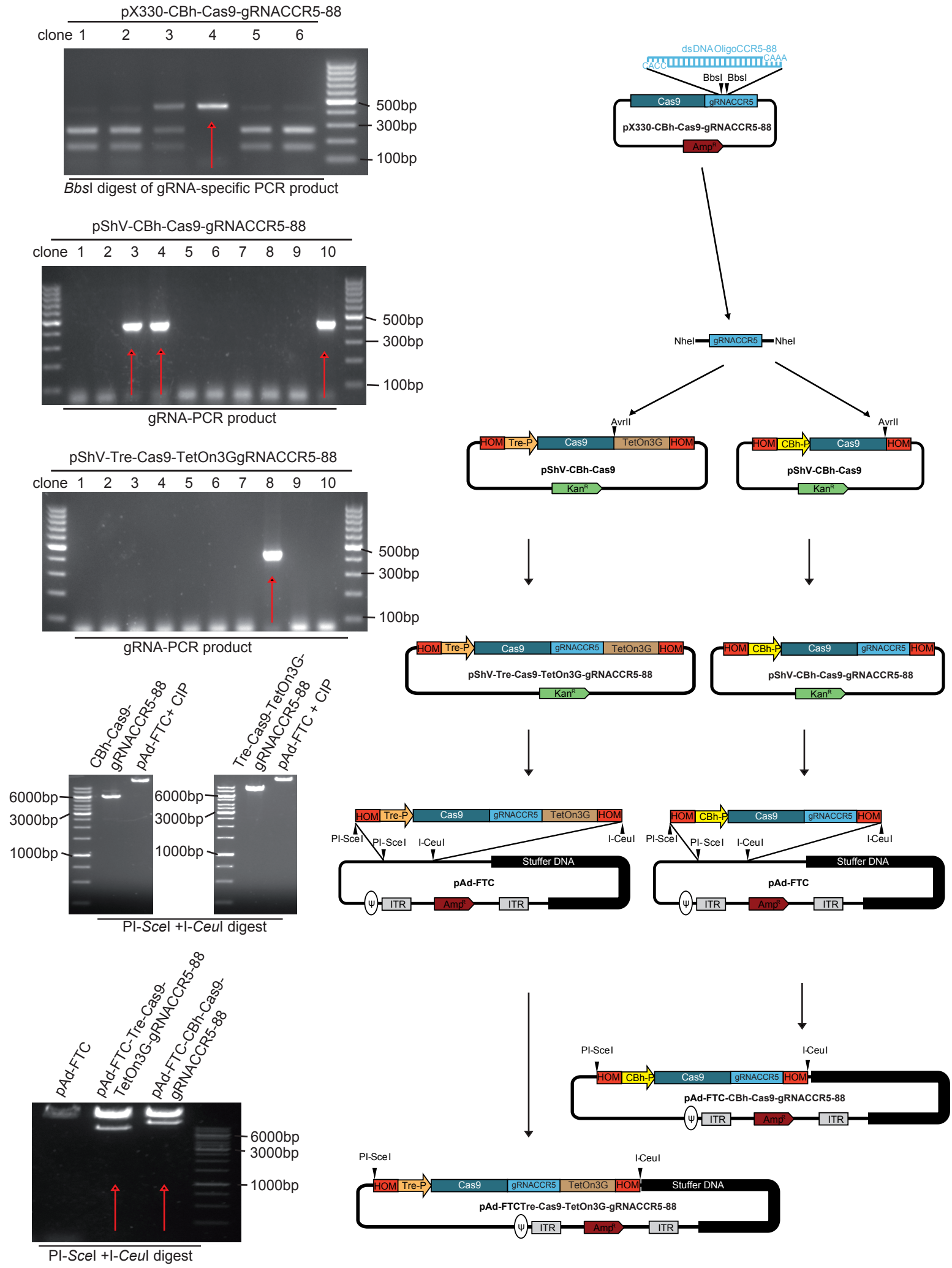
Oligo name		Sequence	
t1 (1)	5’	gGCGCTTTGAGGATCCAACA	3’
CCR5-88	5’	gTCACTATGCTGCCGCCCAGT	3’
Cr1 (2)	5’	CATTGGCTTTGATTTCCCTA	3’
Cr5 (2)	5’	CCAGTTGCCTAAGAACTGGT	3’

Supplementary Table 2: Primers used in this study

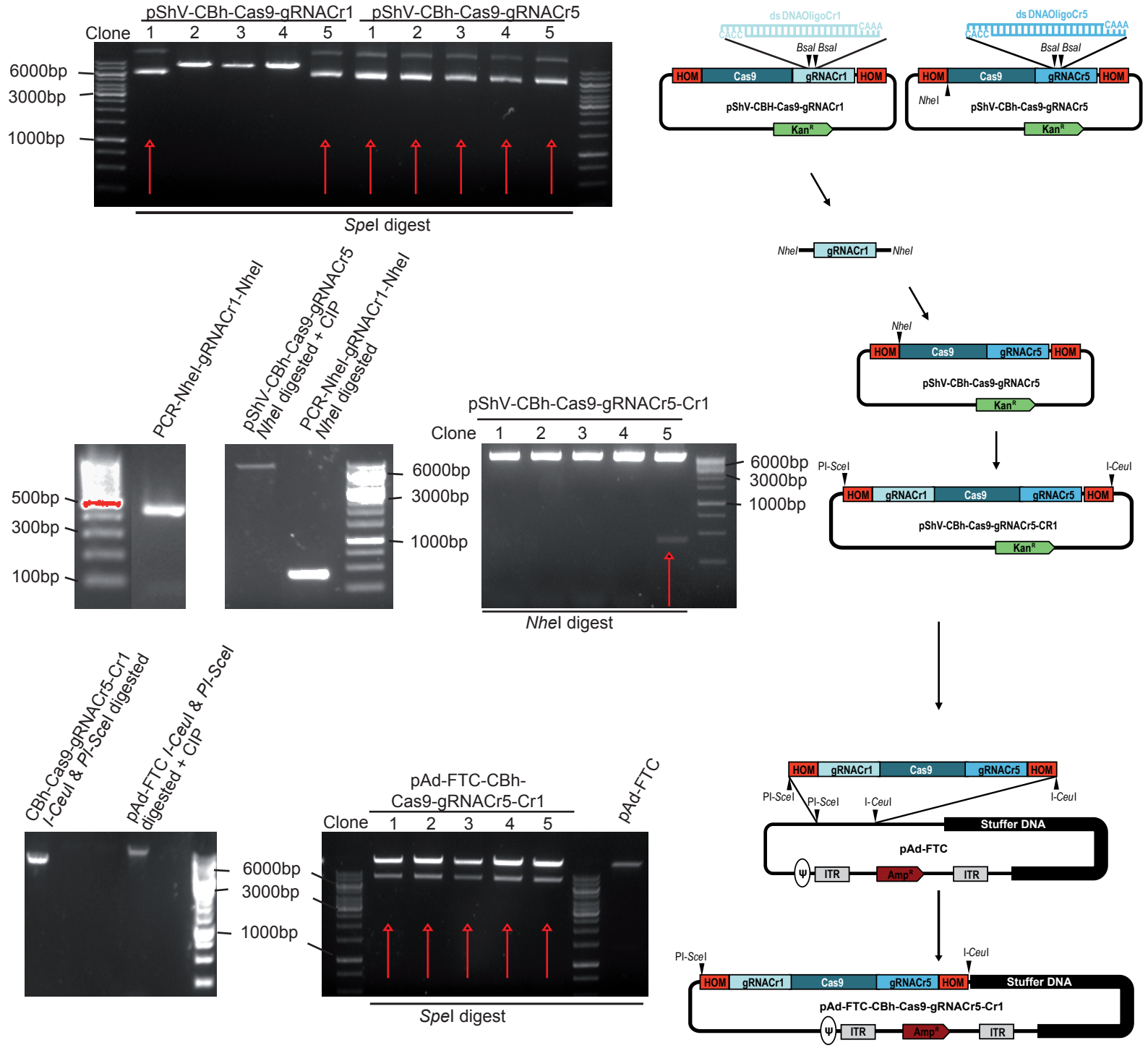
Primer name		Sequence	
Cas9_ fwd_AccI	5’	ATGCAACTGCAGTAGTCGAC	3’
Cas9_rev_XmaI	5’	ATCCCGGGCTCCCCAGCATGCCTGC	3’
Tet3G_PacI_fwd	5’	ATTAATTAACAGAGTAATTCATACAAAAGGACTCGC	3’
Tet3G_PacI_rev	5’	ATTAATTAATTGGTCGAGCTGGATACTTCC3’	3’
gRNA NheI fwd	5’	ATGCTAGCGAGGGCCTATTTCCCATGATTCC	3’
gRNA NheI rev	5’	ATGCTAGCCTGCAGAATTGGCGCACG	3’
HR sh fwd	5’	GGTGGACTCACAGGCCATTCTGCTTTTATTTGGTCAAC CTCAGTTCACAATAACTATAACGGTCCTAAGGTAG	3’
HR SH rev	5’	GAACTCTCCTTGAAGTGGGCAGATTACCCTTTGAATAA CGTTCTATCCCGTGCCATTTCATTACCTCTTTCTC	3’
gRNA BamHI fwd	5’	ACTGGGATCCGAGGGCCTATTTCCCATGATTCC	3’
gRNA BamHI rev	5’	ACGTGGATCCCTGCAGAATTGGCGCACG	3’
HPV18T7E1fwd	5’	CTTGCATAACTATATCCACTCCC	3’
HPV18E6_rev	5’	ATTCAACGGTTTCTGGCAC	3’
CCR5_fwd	5’	AGATGGATTATCAAGTGTCAAGTCC	3’
CCR5_rev3	5’	GATGACCATGACAAGCAGC	3’
CelI-CR1-F (2)	5’	GAGAGGTTATGTGGCTTTACCA	3’
CelI-CR5-R-(2)	5’	CTGCGTAGTGCCAAAACAAA	3’



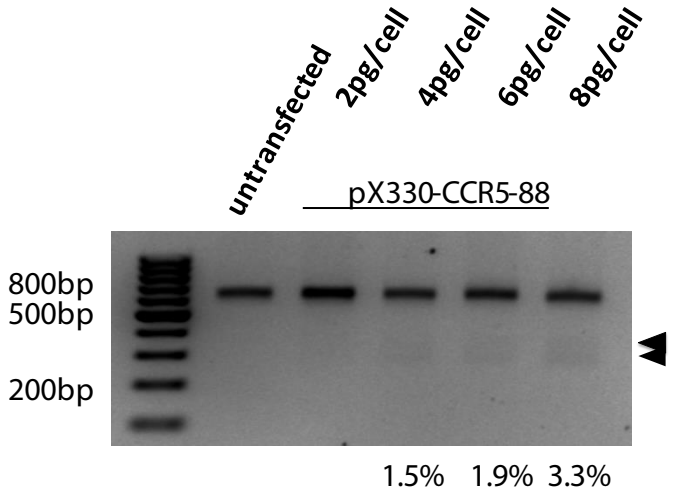
Supplementary Figure 1: Cloning of HCAdV-CBh-Cas9-gRNAHPV18E6. The cloning of the construct was performed as schematically shown on the right panel. Representative agarose gel pictures of each cloning step are depicted on the left. Positive clones are indicated by red arrows.



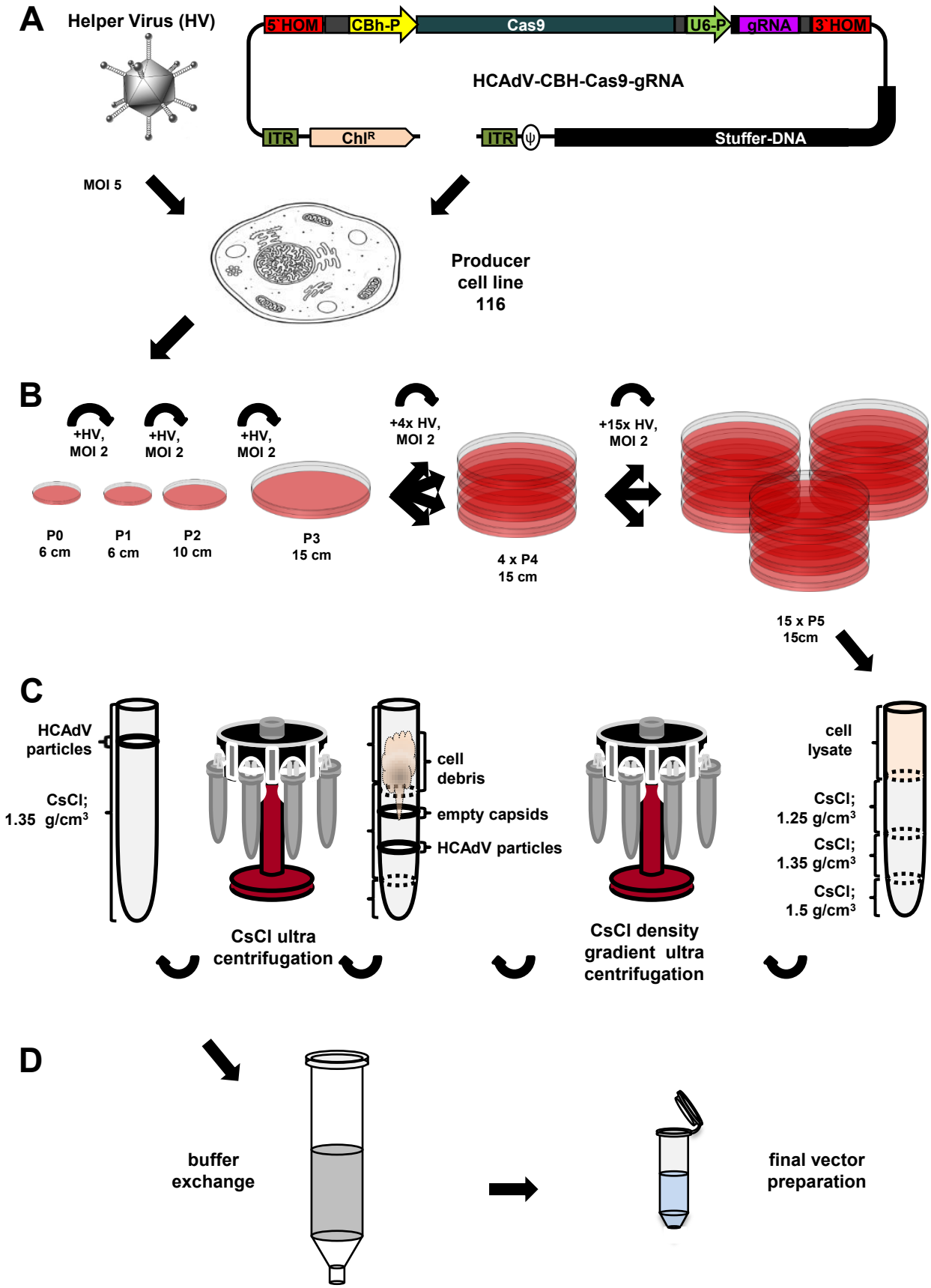
Supplementary Figure 2: Cloning of HCAdV-CBh-Cas9-gRNACCR5-88 and HCAdV-TRE-Cas9-TetOn3G-gRNACCR5-88: The cloning of these constructs was done as schematically shown on the right panel. Representative agarose gel pictures of each cloning step are depicted on the left. Positive clones are indicated by red arrows.



Supplementary Figure 3: Cloning of HCAdV-CBh-Cas9-gRNACr5-Cr1. The cloning of the construct was done as schematically shown on the right panel. Representative agarose gel pictures of each cloning step are depicted on the left. Positive clones are indicated by red arrows.



Supplementary Figure **4**: **Functionality tests after plasmid transfection of pX330-CCR5-88 in A549 cells.** Cells were transfected with increasing amounts of plasmid DNA(2-8pg/cell). Two days after transfection genomic DNA was extracted and subjected to T7E1 assay**.** For functional gRNAs cleavage products of 278bp and 360bp were expected. Mutat onrates of CRISPR Cas9 at the CCR5 locus are shown below the image.



Supplementary Figure 5: Accelerated medium scale HCAdV amplification and purification. (A) Transfection of linearized HCAdV genome containing customized CRSIPR/Cas9 expression units into the HCAdV producer cell line (116 cells) and subsequent infection with the HV AdNG163R-2. (B) Amplification of HCAdV by serial serial transfer of cell virus lysate to a new tissue culture dish and co-infecting with HV, lysate from 16x 15cm dishes was used for HCAdV purification. (C) HCAdV particles were purified by ultracentrifugation using CsCl gradients. (D) Buffer exchange of purified HCAdV particles. HCAdV, high-capacity adenoviral vector; ITR, adenovirus serotype 5 inverted terminal repeat; Ψ, packaging signal; HV, helper virus; MOI, multiplicity of infection; P, passage, CsCl, caesium chloride.

Supplementary information: Complete sequence of the Shuttle plasmid pShV-TRE-Cas9-TetOn3G-gRNA

LOCUS Exported 10895 bp ds-DNA circular SYN 23-JUL-2015
DEFINITION .
ACCESSION .
VERSION .
KEYWORDS Finished Plasmid Cloned pShV Cas9 TRE gRNA Bsa1 Spe1 scaffold
TET... SOURCE synthetic DNA construct
ORGANISM recombinant plasmid
REFERENCE 1 (bases 1 to 10895)
AUTHORS Theo
TITLE Direct Submission
JOURNAL Exported Donnerstag, 9. Jun 2016 from SnapGene 2.5.0
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Supplementary informaiton: Complete sequence of the Shuttle plasmid pShV-CBh-Cas9-gRNA

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  AUTHORS   Theo
  TITLE     Direct Submission
  JOURNAL   Exported Donnerstag, 9. Jun 2016 from SnapGene 2.5.0
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  TITLE     Direct Submission
  JOURNAL   Exported Donnerstag, 9. Jun 2016 from SnapGene 2.5.0
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COMMENT     This file is created by VectorDesigner
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36601 gtttacgtgg agactcgccc aggtgttttt ctcaggtgtt ttccgcgttc cgggtcaaag
36661 ttggcgtttt gattc//

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