Supporting Information

Di Pilato et al. 10.1073/pnas.1424341112

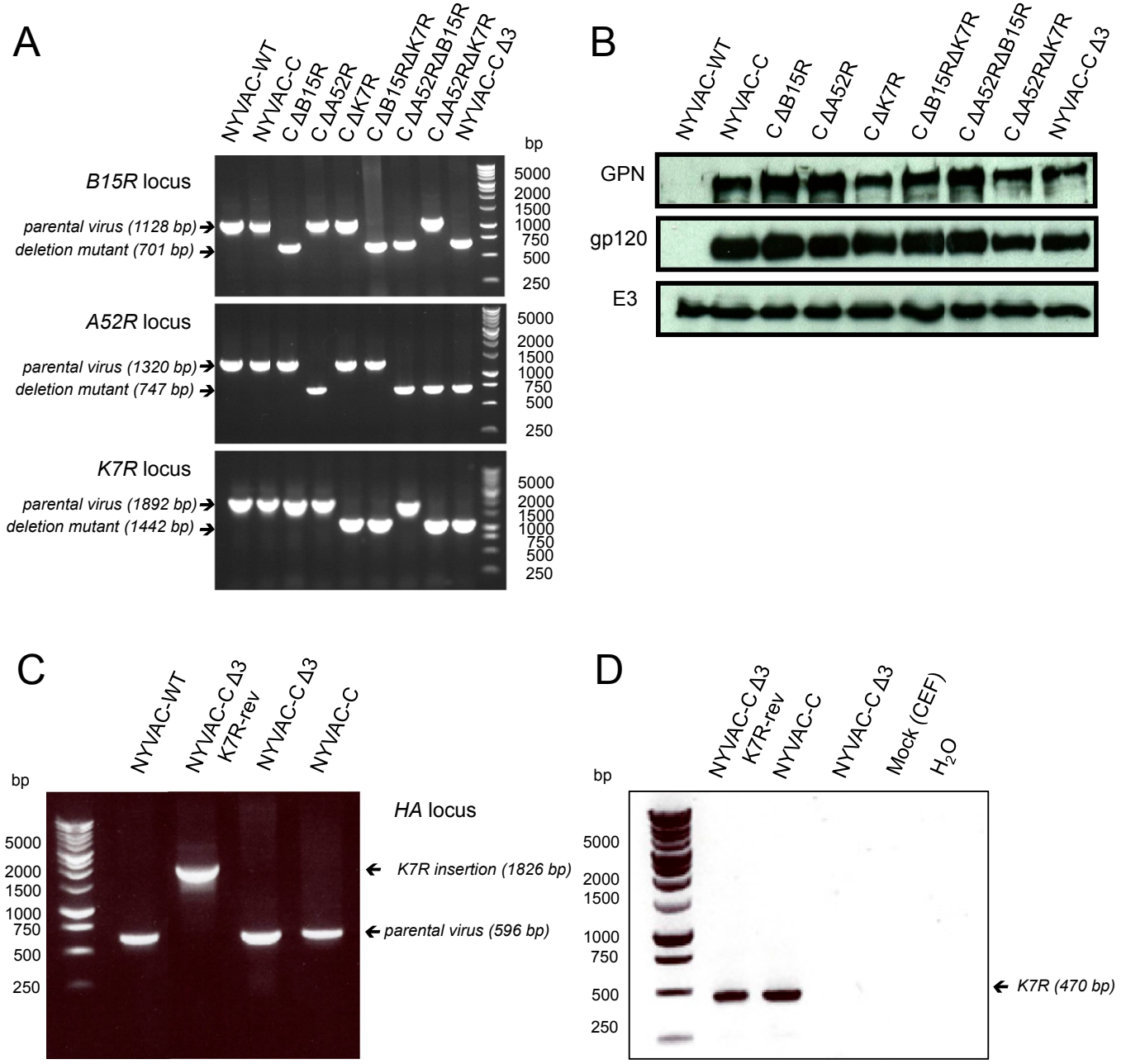

Fig. S1. Generation and characterization of NYVAC-C mutants. (*A*) PCR analysis to confirm *A52R*, *B15R*, and *K7R* gene deletion. Viral DNA was extracted from BSC-40 cells infected with the viruses as indicated (2 PFUs per cell, 24 h). (*B*) Western blot showing expression of HIV-1 antigens and E3 VACV protein in BSC-40 cells infected as in *A*. (*C*) PCR analysis to confirm *K7R* gene insertion. Viral DNA was extracted from BSC-40 cells infected with the viruses as indicated (1 PFU per cell, 24 h). (*D*) RT-PCR analysis to confirm *K7R* gene expression. RNA was extracted from CEF cells infected with the viruses as indicated (1 PFU per cell, 24 h).

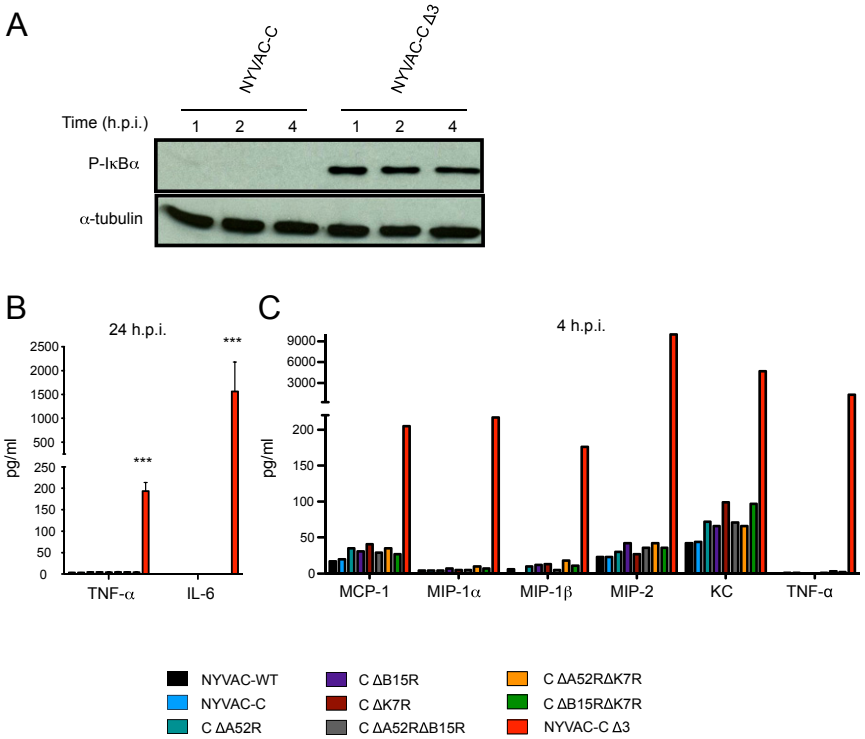

Fig. S2. Activation of NFκB pathway in THP-1 cells and cytokine/chemokine levels in supernatants of JJ74 macrophages and primary peritoneal macrophages after NYVAC infection. (*A*) Phosphorylated IkBα analyzed by Western blot in THP-1 human monocytes, differentiated into macrophages, infected with NYVAC-C or NYVAC-C Δ3 (5 PFUs per cell) for 1, 2, and 4 h. α-tubulin was used as the internal loading control. (*B*) Concentrations of cytokines TNF-α and IL-6 at 24 h post-infection, quantified by immunoassay in supernatants of J774 cells infected as indicated. Values show mean ± SEM of duplicates, representative of three independent experiments. (*C*) Concentration of MCP-1, MIP-1α, MIP-1β, MIP-2, KC, and TNF-α at 4 h postinfection in supernatants of primary peritoneal macrophages infected with indicated viruses. Data are representative of two independent experiments. ***$P < 0.001$.

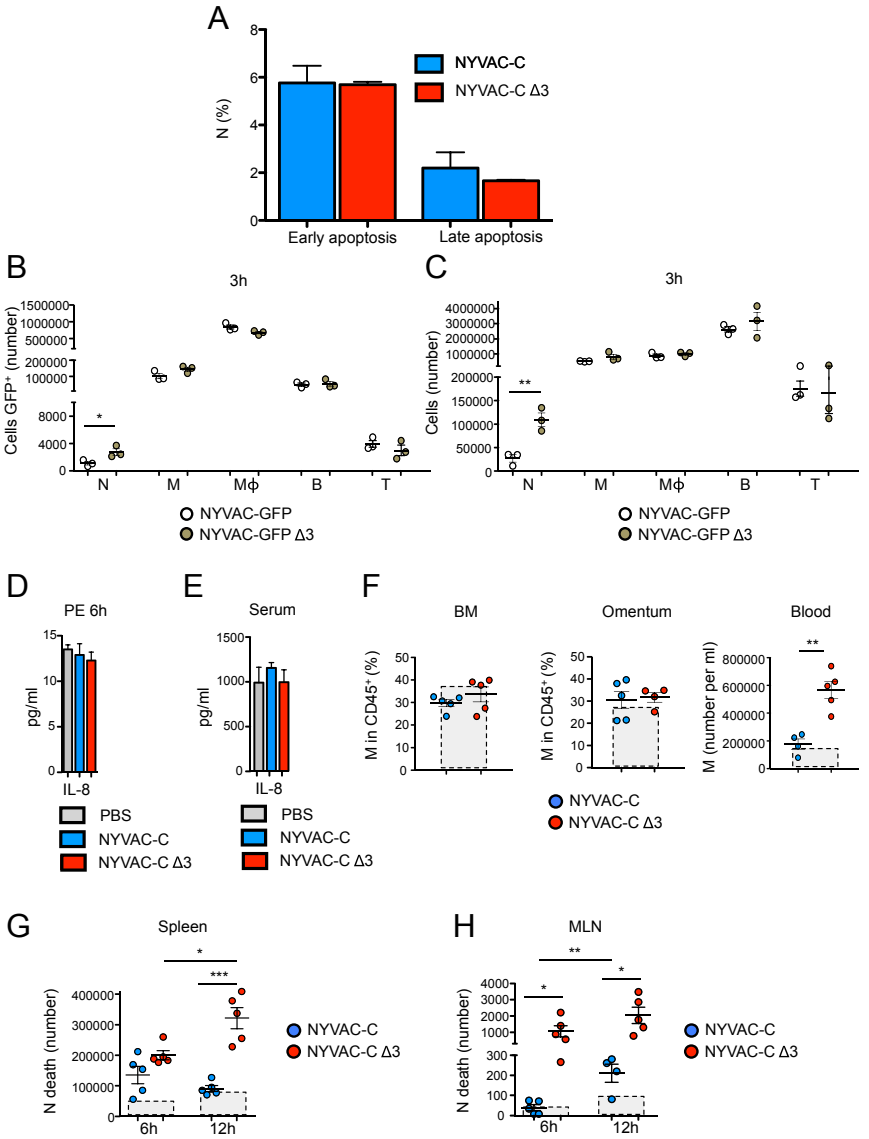

Fig. S3. In vitro and in vivo neutrophil cell death, PEC characterization after NYVAC-GFP or NYVAC-GFP Δ3 infection, and IL-8 secretion and monocyte trafficking after NYVAC-C or NYVAC-C Δ3 infection. (*A*) Percentages of early apoptotic (Annexin V^{+}·PI^{-}) and late apoptotic (Annexin V^{+}·PI^{+}) migrated neutrophils toward supernatants of NYVAC- or NYVAC-C Δ3-infected (4 h) J774 macrophages. Values show mean ± SEM of duplicates. (*B*) Absolute numbers of GFP^{+} neutrophils (N), monocytes (M), macrophages (Mϕ), B cells (B), and T cells (T) in the peritoneal cavity of NYVAC-GFP– or NYVAC-GFP Δ3-injected (10^{7} PFUs) mice at 3 h postinfection. Each point represents an individual mouse. (*C*) Absolute numbers of neutrophils (N), monocytes (M), macrophages (Mϕ), B cells (B), and T cells (T) in the peritoneal cavity of NYVAC-GFP– or NYVAC-GFP Δ3-injected (10^{7} PFUs) mice at 3 h postinfection. Each point represents an individual mouse. Shown are IL-8 levels at 6 h postinfection in peritoneal exudates (*D*) and serum (*E*) of PBS-, NYVAC-C–, or NYVAC-C Δ-3-injected mice ($n = 5$ per group). (*F*) Percentages of monocytes in BM and omentum, and total number of monocytes in blood at 6 h postinfection from NYVAC-C– or NYVAC-C Δ3-injected mice. Boxes with dashed lines indicate absolute numbers of cells in PBS-injected mice. Each point represents an individual mouse. Total numbers of neutrophil cell death in spleen (*G*) and in MLNs (*H*) at 6 and 12 h postinfection from NYVAC-C– or NYVAC-C Δ3-injected mice. Boxes with dashed lines indicate absolute numbers of cell deaths in PBS-injected mice. Each point represents an individual mouse. Graphs show mean ± SEM. Data are representative of two independent experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

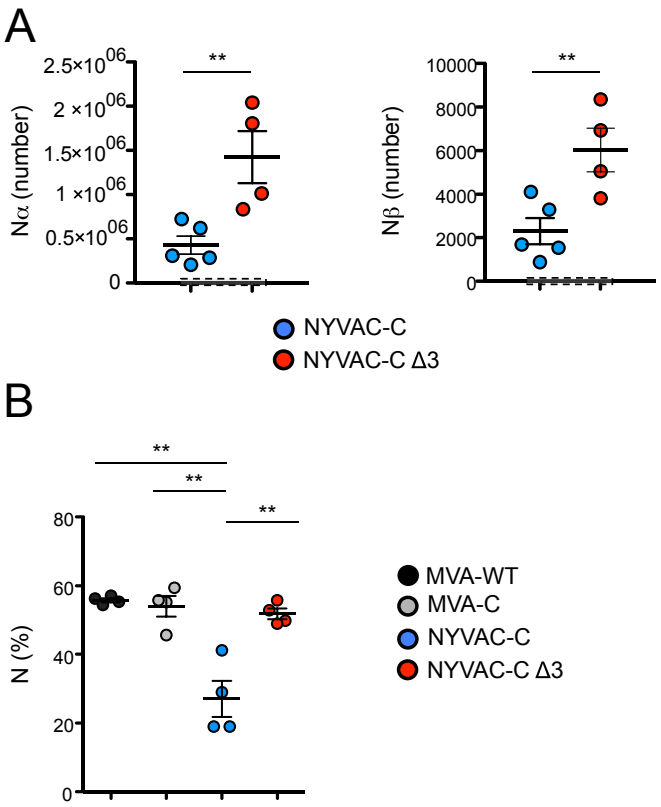

Fig. S4. Nα and Nβ neutrophils in NYVAC-peritoneal exudate-injected mice and percentages of PEC neutrophils in different poxvirus-infected mice. (*A*) Absolute numbers of peritoneal Nα and Nβ neutrophils in NYVAC-C– and NYVAC-C Δ3-peritoneal exudate-injected mice. Boxes with dashed lines indicate percentages in PBS-peritoneal exudate-injected mice. (*B*) Percentages of neutrophils at 6 h postinfection from MVA-WT– or MVA-C–, or NYVAC-C– or NYVAC-C Δ3-injected mice. Graphs show mean ± SEM; each point represents an individual mouse. Data are representative of two independent experiments. $^{**}P < 0.01$.

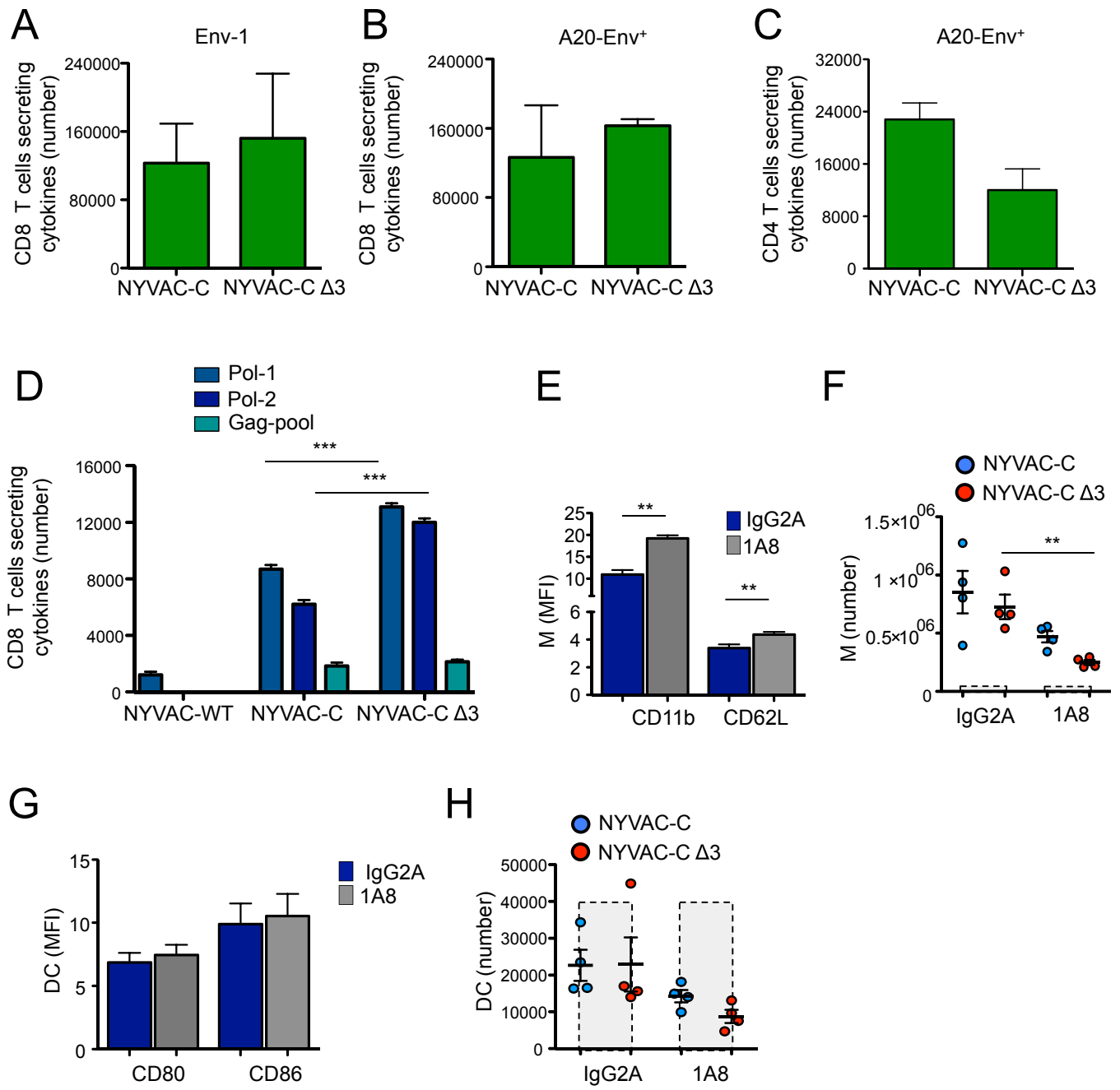

Fig. S5. Magnitude of the specific CD8 and CD4 T-cell response to HIV-1 antigens, and M or DC activation and absolute numbers in 1A8-pretreated/NYVAC-infected mice. Shown is the vaccine-induced HIV-1–specific T-cell response in mice ($n = 4$ per group) infected with 10^7 PFUs of NYVAC-C or NYVAC-C Δ3. The response was measured 11 d after the last i.p. immunization, after stimulation of splenocytes with Env-1 peptide (*A*) or with A20 Env$^+$ (*B* and *C*), or after the last intramuscular immunization and after stimulation of splenocytes with HIV-1 peptides/pools (*D*). Total value (magnitude) is the sum of percentages of CD8 or CD4 T cells per spleen that secrete IFN-γ and/or TNF-α and/or IL-2 and/or CD107a. Values show mean ± SEM of two independent experiments. Shown are the MFIs of indicated markers in monocytes (M) (*E*) and in DCs (*G*) at 6 h postinfection in NYVAC-injected and IgG2A- or 1A8-pretreated mice. Also shown are M (*F*) and DC (*H*) absolute numbers at 6 h postinfection in NYVAC-injected and IgG2A- or 1A8-pretreated mice. Boxes with dashed lines indicate numbers in PBS-injected mice. Graphs show mean ± SEM; each point represents an individual mouse. $^{**}P < 0.01$, $^{***}P < 0.001$.

Table S1. Primers used for the deletion of *A52R*, *B15R*, and *K7R* genes and for the insertion of *K7R* gene

Genes	Flanks of genes	Name of primers	Sequence of primers
A52R	Left	LFA52R-Apa	TATATTGGGCCCTACTACGATTAA
A52R	Left	LFA52R-Sph	CTTTATGCATGCGGTGATCACACA
A52R	Repeated left	LF′A52R-Eco	ATTAGAGAATTCTACGATTAACGA
A52R	Repeated left	LF′A52R-Cla	CTTTATATCGATGGTGATCACACA
A52R	Right	RFA52R-Cla	GGAAATATCGATGAAAGTTAAAAG
A52R	Right	RFA52R-Bam	TCTGCCGGATCCAATGTAGTAATG
B15R	Left	LFB15R-Kpn-F	TTTATAGGTACCTTCTTTGAGGACT
B15R	Left	LFB15R-Kpn-R	GTTGGCGGTACCCTTACCAATTGCA
B15R	Repeated left	LF′B15R-Aat	TTCTTTGACGTCTGTTTTCCTGAAG
B15R	Repeated left	LF′B15R-Xba	GTTGGCTCTAGACTTACCAATTGCA
B15R	Right	RFB15R-Kpn	CGTATGGGTACCTGAGTTGTACATC
B15R	Right	RFB15R-Bam	GTGTCGGGATCCGAATTAGCATATT
K7R	Left	LFK7R-Aat	CAAAGGACGTCATCATCATTTTTTCACC
K7R	Left	LFK7R-Xba	GCTCTAGAGACTATCTCACAAAAG
K7R	Repeated left	LF′K7R-Eco	CGGAATTCATCATCATTTTTTCACC
K7R	Repeated left	LF′K7R-Cla	CCATCGATGACTATCTCACAAAAG
K7R	Right	RFK7R-Cla	CCATCGATTCTAGAAAAAAAAATTGAATTG
K7R	Right	RFK7R-Bam	CGGGATCCAACAAGGGGTTGG
K7F		K7RinternalFwd-Bam	CGCGGATCCATGGCGACTAAATTAG
K7R		K7RinternalRev-Not	AAGGAAAAAAGCGGCCGCTCAATTCAATTTTTTTTC

Restriction enzyme cleavage sites are underlined.