# Supplementary material

| | | | |
|---|---|---|---|
| **APMV-1 (avirulent)** | 111 | G-G-**R**-Q-G-**R**-L | 117 |
| **APMV-3 (wisconsin)** | 96 | P-**R**-P-S-G-**R**-L | 102 |
| **APMV-3 (Netherlands)** | 101 | A-**R**-P-**R**-G-**R**-L | 107 |
| **APMV-1 (virulent)** | 111 | G-**R**-**R**-Q-**K**-**R**-F | 117 |

**Figure S1:** F-Prot. cleavage site motif in APMVs: Basic amino acids (R=arginine, K=lysine) are in bold, mentioned numbers indicate amino acids position in the F protein [17].

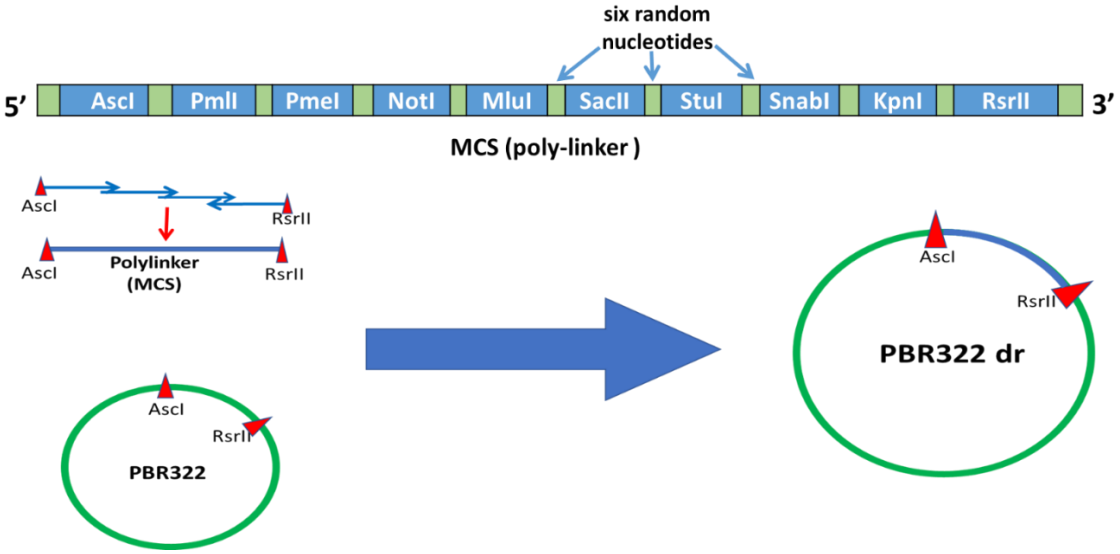


**Figure S2:** Preparation of the cloning plasmid vector PBR322/dr with the polylinker: A 116 nucleotides poly-linker was designed to have the same restriction enzyme sites order used to fragment the whole genome of APM3 strain Wisconsin (restriction enzyme sites were separated by random six-nucleotides). The poly-linker was created by conventional PCR using three forward and one reverse overlapping primers. The resulting gene fragment was cloned into the modified plasmid vector PBR322/dr.

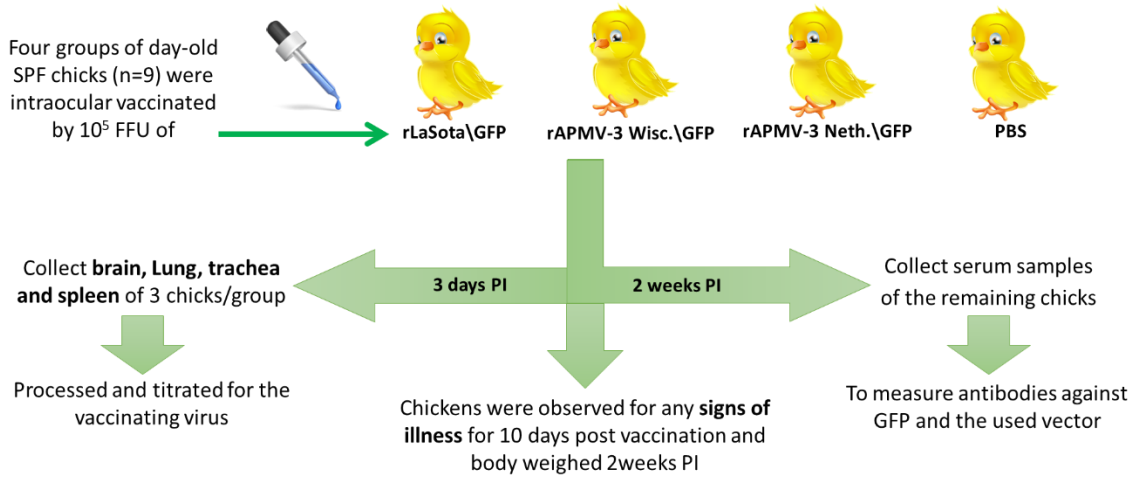


**Figure S3:** Schematic diagram of the in-vivo experiment for evaluation of rAPMV-3 Wisc.\GFP, rAPMV-3 Neth.\GFP and rLasota\GFP as vaccine vectors in chickens.

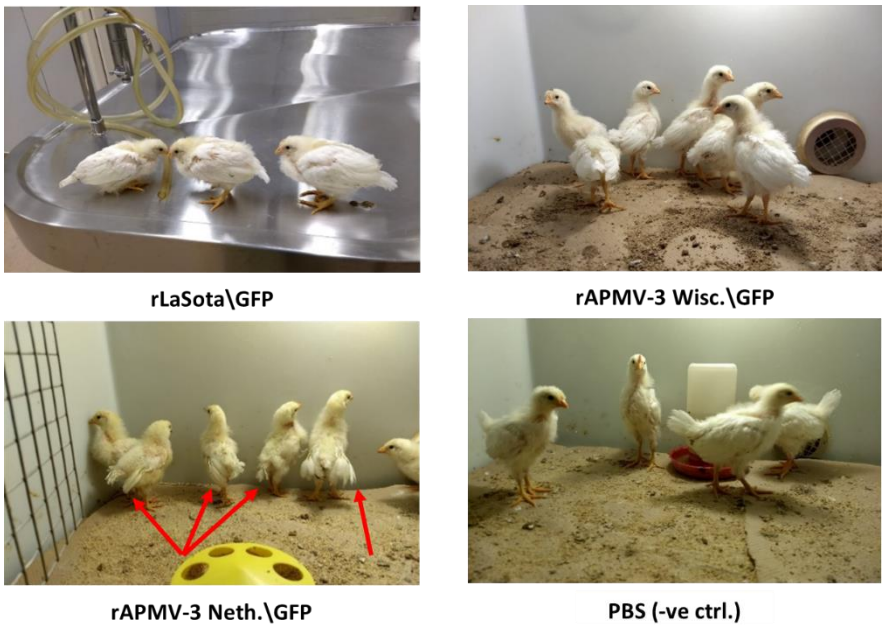


**Figure S4:** Pathological changes observed in vaccinated chicks (two weeks post immunization): Chicks vaccinated by rAPMV-3 Neth.\GFP are showing ruffled-abnormal feathering and stunted growth. Chicks vaccinated by rLaSota\GFP and rAPMV-3 Wisc.\GFP are showing normal body size with no signs of illness. Negative control group was mock vaccinated by PBS .

**Table S1:** Primers used for creation of cDNA sub-genomic fragments

| Subgenomic Fragment | Primer Direction | Primers (5′-3′) | Fragment size (Kbp) |
|---|---|---|---|
| **I** | Forward | ATTCGGCGCGCCTAATACGACTCACTATAGGGactaaacagaaagttaataagtgtttg | 1.6 Kbp |
| | Reverse | gtctgcAcGtgggattgggtgctgatgg | |
| **II** | Forward | caatcccaCgTgcagacaggccaccgcctcc | 1.5 Kbp |
| | Reverse | gcaatagTtTaaaCaagagagctagatgggttgtg | |
| **III** | Forward | tctcttGtttAaActattgctttataaaaaacc | 1.4 Kbp |
| | Reverse | ggtaagGCggcCgcagtgagtgatttatttcctg | |
| **IV** | Forward | ctcactgcGgccGCcttaccactagtaacaaattac | 2.1 Kbp |
| | Reverse | gggtgtacGCgtgtgtctcaacatgggactgc | |
| **V** | Forward | agacacacGCgtacaccctagtttctagtaaaacc | 2.1 Kbp |
| | Reverse | ttatctCcgCggcaaaggtgcaataatcaattc | |
| **VI** | Forward | cctttgccGcgGagataactaagcatttaag | 2.3 Kbp |
| | Reverse | actcttaggcctgctacagctgcag | |
| **VII** | Forward | tgtagcaggcctaagagtcgcatc | 1 Kbp |
| | Reverse | gcattatacgtaactatttgctc | |
| **VIII** | Forward | aatagttacgtataatgcaacc | 2 Kbp |
| | Reverse | gattacggtaccaaactgagtgtc | |
| **IX** | Forward | cagtttggtaccgtaatcgtcaatac | 2.3 Kbp |
| | Reverse | ggtcCGGACCGCGAGGAGGTGGAGATGCCATGCCGACCCactaaacaaaaagttata taaatggtttaattaac$^{+}$ | |

**Table S2:** Nucleotide changes involved in pAPMV-3 Wisc.FLC

A

| Subgenomic Fragment | | Nucleotid number | Original nucleotide | Replacement nucleotide | Original sequence | New sequence | Introduced RE sites |
|---|---|---|---|---|---|---|---|
| I | | Before (1) | GGCGCGCCTAATACGACTCACTATAGGG | | | | AscI + T7 promotor |
| | | 1590 | A | C | caagcg 1588-1593 | caCgTg | PmlI site |
| II | | 1592 | C | T | | | |
| | | 3074 | A | G | attttatc 3074-3081 | GtttAaAc | PmeI site |
| | | 3078 | T | A | | | |
| III | | 3080 | T | A | | | |
| | | 4391 | C | G | gccgccag 4389-4396 | gcGgccGC | NotI site |
| | | 4395 | A | G | | | |
| IV | | 4396 | G | C | | | |
| | | 6517 | C | G | accagt 6515-6520 | acGCgt | MluI site |
| V | | 6518 | A | C | | | |
| | | 8577 | A | G | ccacgt 8575-8580 | ccGcgG | SacII site |
| VI | VId | 8580 | T | G | | | |
| | VIc | | --- | --- | aggcct 10896 -10901 | aggcct | StuI site |
| | VIb | | --- | --- | tacgta 11916 -11921 | tacgta | SnabI site |
| | VIa | | --- | --- | ggtacc 13925 -13930 | ggtacc | KpnI site |
| | | After (16182) | GGGTCGGCATGGCATCTCCACCTCCTCGCGGTCCG | | | | HDV Ribozyme + RsrII |

B

| Deleted RE site | Original sequence | Corrected sequence | Changed nucleotide |
|---|---|---|---|
| **SacII** | ccgcgg 6386-6391 | TcgAgg | 6358 |
| | | | 6361 |
| **RsrII** | cggaccg 1291-1297 | cCgaTcg | 1264 |
| | | | 1267 |
| **RsrII** | cggaccg 2682-2688 | TggCccg | 2682 |
| | | | 2685 |