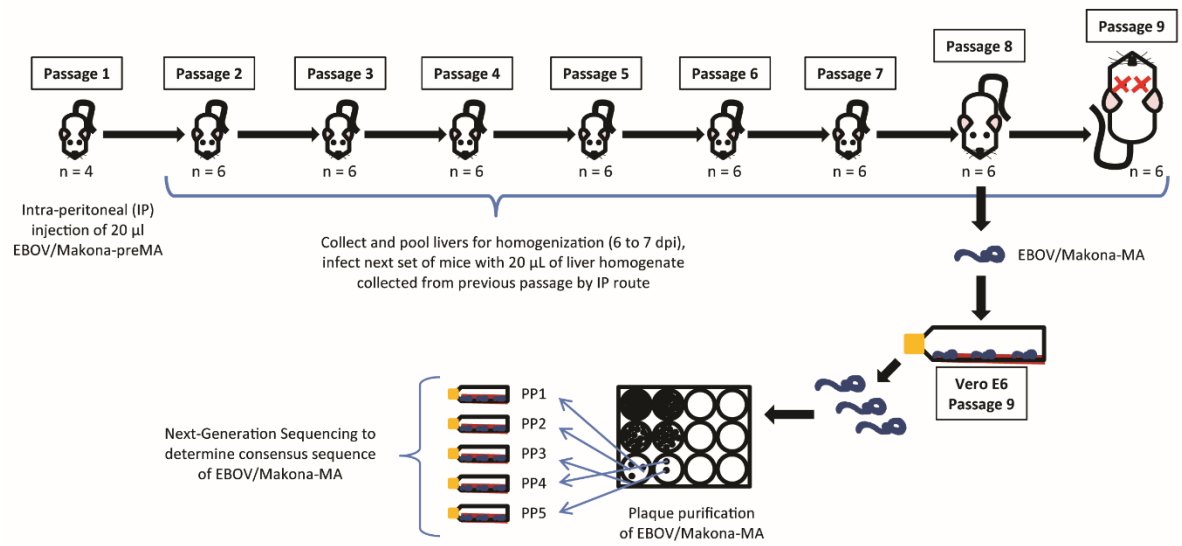
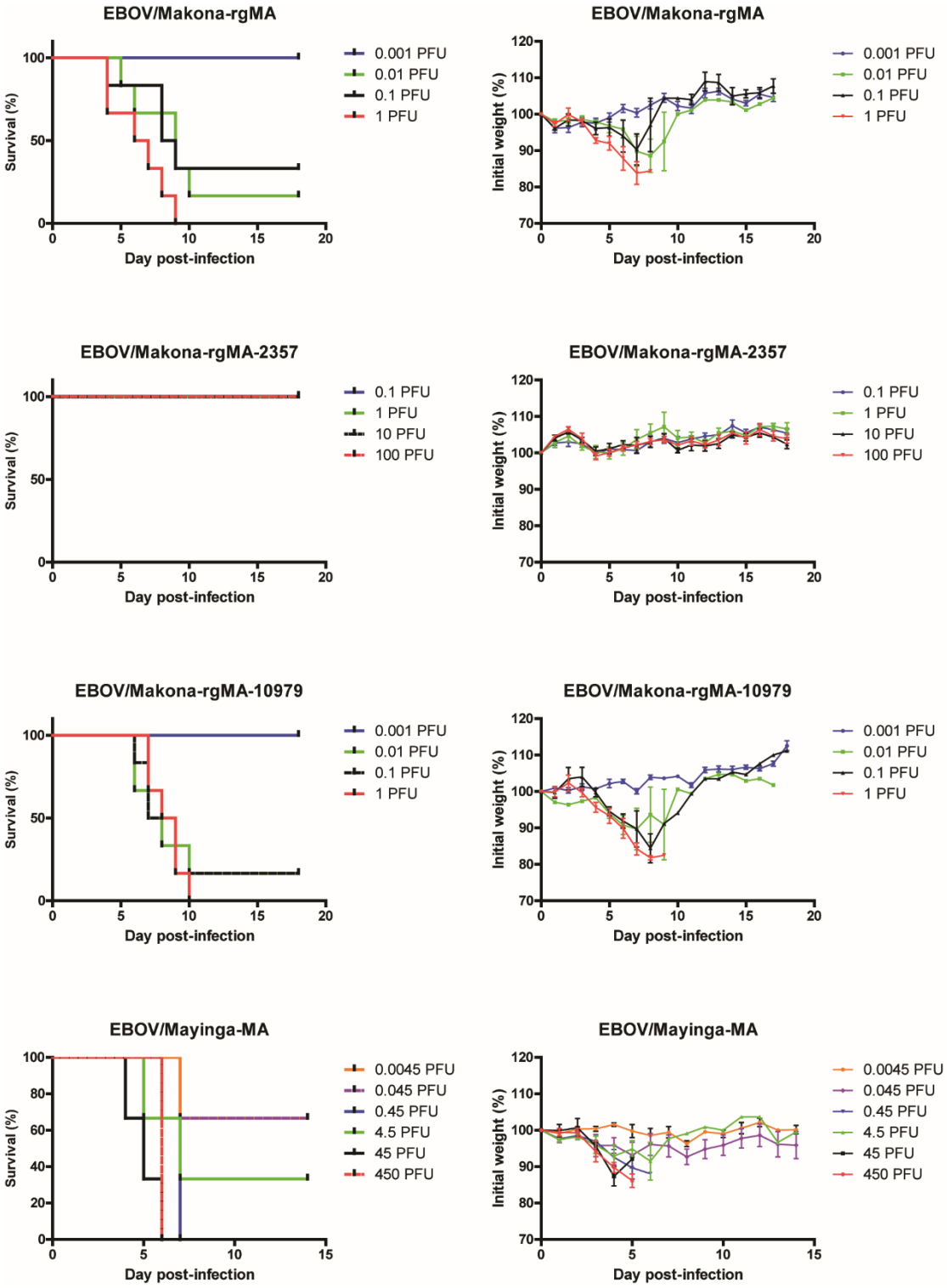



**Supplemental Figure S1.** Generation of mouse-adapted EBOV/Makona by serial passaging in suckling BALB/c mice. For passage 1, mice (*n* = 4, 3-to-4 days old) were infected by intra-peritoneal (IP) injection with 20 µl of EBOV/Makona-preMA. Livers were collected from the mice on day 6 or 7 post-infection (dpi) and were pooled, homogenized, and clarified of cell debris by centrifugation. For passage 2, liver homogenate that was collected from the previous passage was used to infect a new set of naïve suckling mice (*n* = 6, 3-to-4 days old). This process was repeated for a total of 7 passages in 3-to-4 day old suckling mice, while passage 8 was used to infect 7-to-8 day old suckling mice. The 9$^{th}$ passage in 14-day old mice resulted in uniform lethality. The passage 8 liver homogenate was used to grow a tissue culture stock using Vero E6 cells. The tissue culture stock of EBOV/Makona-MA was plaque purified by standard plaque assay, and five individual plaques were chosen for subsequent stock generation (PP1 to PP5). Viral RNA was extracted from each of these stocks, and viral genomes were sequenced by Next-generation Sequencing to determine the consensus sequence of EBOV/Makona-MA.




**Supplemental Figure S2.** Survival and weight loss curves for mouse-adapted Ebola viruses. Groups of BALB/c mice ($n$ = 3 to 6) were infected by intra-peritoneal injection with either EBOV/Makona-rgMA, EBOV/Makona-rgMA-2357, EBOV/Makona-rgMA-10979, or EBOV/Mayinga-MA. Ten-fold serial dilutions of viral doses were performed and administered to each group. Survival and weight loss were monitored for 14–18 days post-infection.

**Supplemental Table S1.** List of mutagenesis primers used to create Ebolavirus (EBOV)/Makona-preMA (mouse-adapted) and EBOV/Makona-rgMA. Nucleotides that are highlighted in red indicate the mutation site.

| Mutagenesis Primers | |
|---|---|
| C07-MA-678F | CGGACGGTTTCCTTCTCATGCTTTGTC |
| C07-MA-688R | GAAACCGTCCGCACTCTCTTGAAAATC |
| C07-MA-6226F | TGAGACCAGTTGGACTGAATCTCGAG |
| C07-MA-6236R | AACTGGTCTCAATTGATTTGTGGATGAC |
| C07-MA-6769F | TTGAACCAAGATTCACACCACAGTTTC |
| C07-MA-6779R | TCTTGGTTCAAGTTGGACGTAGGTCAA |
| C07-MA-10338F | AAAAAACCATGGCCAAAGCTACGGG |
| C07-MA-10348R | CATGGTTTTTTTCTCAGGTCTTGCTTGG |
| C07-MA-10489F | TGTGATTCACAAAGGAATGGCCCTATTGC |
| C07-MA-10499R | TGTGAATCACATCAAACTCAATACCAGC |
| C07-MA-14376F | GTTTACTCCAGTTAAAAACTTATCTCCG |
| C07-MA-14386R | CTGGAGTAAACCTGAGGTAACTGGATC |
| C07-MA-16170F | TTTCTGTTTACATAGGCGGTGCTGCA |
| C07-MA-16180R | GTAAACAGAAAAATGGGGATCAATACTC |
| C07-MA-2357F | AGATCAGGACAACATTCAAGAG |
| C07-MA-2357R | CTCTTGAATGTTGTCCTGATCT |
| C07-MA-10979F | ATGAACCGCATGAAGCCTGG |
| C07-MA-10979R | CCAGGCTTCATGCGGTTCAT |

**Supplementary Table S2.** Comparison of amino acid identities in the genes of human and mouse-adapted Ebola viruses (EBOV). EBOV/Mayinga is a human isolate of Ebola virus from 1976, a mouse-adapted version of the virus was generated, EBOV/Mayinga-MA, and the differences in amino acid sequences in each protein are shown (highlighted in blue). EBOV/Makona is a human isolate from 2014, EBOV/Makona-preMA contains a subset of mutations found in EBOV/Mayinga-MA, and was used as the starting virus for passaging in mice to generate the full mouse-adapted version of EBOV/Makona. EBOV/Makona-rgMA is the reverse genetics generated mouse-adapted EBOV/Makona, containing an amino acid change in NP (highlighted in red) and VP24 (highlighted in yellow), which differs from EBOV/Makona-preMA. Two variants of EBOV/Makona-rgMA were generated, EBOV/Makona-rgMA-2357 and EBOV/Makona-rgMA10979.

| Ebola Virus | NP | | VP35 | GP | | | VP30 | VP24 | | | L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EBOV/Mayinga | S72 | H630 | 12A | S65 | S246 | 544I | NCR nt 9563A | NCR | T50 | M212 | F934 | I1532 |
| EBOV/Mayinga-MA | G72 | H630 | 12V | P65 | P246 | 544T | NCR nt 9563G | NCR nt 10343+A | I50 | M212 | L934 | V1532 |
| EBOV/Makona | S72 | H630 | 12V | S65 | S246 | 544T | NCR nt 9563A | NCR | T50 | K212 | F934 | I1532 |
| EBOV/Makona-preMA | G72 | H630 | 12V | P65 | P246 | 544T | NCR nt 9563A | NCR nt 10343+A | I50 | K212 | L934 | V1532 |
| EBOV/Makona-rgMA | G72 | N630 | 12V | P65 | P246 | 544T | NCR nt 9563A | NCR nt 10343+A | I50 | M212 | L934 | V1532 |
| EBOV/Makona-rgMA-2357 | G72 | N630 | 12V | P65 | P246 | 544T | NCR nt 9563A | NCR nt 10343+A | I50 | K212 | L934 | V1532 |
| EBOV/Makona-rgMA-10979 | G72 | H630 | 12V | P65 | P246 | 544T | NCR nt 9563A | NCR nt 10343+A | I50 | M212 | L934 | V1532 |

**Supplemental Table S3.** Next-generation sequencing of EBOV/Makona-MA plaque picks 1 to 5. A virus stock of EBOV/Makona-MA passaged eight times in mice was generated in Vero E6 cells using liver homogenate that was collected from mice. This tissue culture stock of EBOV/Makona-MA was plaque purified by standard plaque assay, and five individual plaques (PP1 to PP5) were chosen and stocks of each plaque were grown up using Vero E6 cells. Viral RNA from each EBOV/Makona-MA-PP1 to PP5 stocks was extracted and next-generation sequencing was performed using the Illumina MiniSeq platform with library preparation using the Nextera DNA Flex Library Prep Kit. Viral sequences obtained were compared to a reference EBOV/Makona-Gueckedou-C07 sequence for assembly. The percent frequency of mutations in each plaque pick are shown.

| | Position | Mutation | Freq % | Amino Acid | Gene |
|---|---|---|---|---|---|
| EBOV/Makona-MA-PP1 | 683 | A → G | 100 | S72G | NP |
| | 2357 | C → A | 100 | H630N | NP |
| | 3012 | A → G | 100 | | NP/VP35 intergenic region |
| | 5684 | T → G | 100 | | VP40/GP intergenic region |
| | 6231 | T → C | 100 | S65P | GP |
| | 6774 | T → C | 100 | S246P | GP |
| | 8021 | A → G | 5.7 | I367V | GP |
| | 9849 | C → T | 100 | | VP30/VP24 intergenic region |
| | 10342 | $(A)_{6 \rightarrow 7}$ | 100 | | VP30/VP24 intergenic region |
| | 10493 | C → T | 100 | T50I | VP24 |
| | 10979 | A → T | 100 | K212M | VP24 |
| | 14380 | T → C | 100 | F934L | L |
| | 16174 | A → G | 100 | I1532V | L |
| | 18813 | T → C | 100 | | L /- intergenic region |
| EBOV/Makona-MA-PP2 | 683 | A → G | 100 | S72G | NP |
| | 1600 | G → A | 100 | M377I | NP |
| | 2357 | C → A | 100 | H630N | NP |
| | 6231 | T → C | 100 | S65P | GP |
| | 6774 | T → C | 100 | S246P | GP |
| | 7368 | T → A | 100 | L149Q | GP |
| | 10342 | $(A)_{6 \rightarrow 7}$ | 100 | | VP30/VP24 intergenic region |
| | 10493 | C → T | 100 | T50I | VP24 |
| | 10979 | A → T | 100 | K212M | VP24 |
| | 14380 | T → C | 100 | F934L | L |
| | 15301 | A → G | 9.2 | K1241E | L |
| | 16174 | A → G | 100 | I1532V | L |
| EBOV/Makona-MA-PP3 | 256 | C → T | 100 | | - / NP intergenic region |
| | 683 | A → G | 100 | S72G | NP |
| | 2357 | C → A | 100 | H630N | NP |
| | 4441 | C → T | 11.6 | | VP35/VP40 intergenic region |
| | 6231 | T → C | 100 | S65P | GP |
| | 6774 | T → C | 100 | S246P | GP |

| | | | | | |
|---|---|---|---|---|---|
| | 10243 | G → A | 100 | | VP30/VP24 intergenic region |
| | 10342 | $(A)_{6 \rightarrow 7}$ | 100 | | VP30/VP24 intergenic region |
| | 10493 | C → T | 100 | T50I | VP24 |
| | 10979 | A → T | 100 | K212M | VP24 |
| | 14380 | T → C | 100 | F934L | L |
| | 16174 | A → G | 100 | I1532V | L |
| EBOV/Makona-MA-PP4 | 683 | A → G | 100 | S72G | NP |
| | 2357 | C → A | 100 | H630N | NP |
| | 6231 | T → C | 100 | S65P | GP |
| | 6774 | T → C | 100 | S246P | GP |
| | 10342 | $(A)_{6 \rightarrow 7}$ | 100 | | VP30/VP24 intergenic region |
| | 10493 | C → T | 100 | T50I | VP24 |
| | 10979 | A → T | 100 | K212M | VP24 |
| | 14380 | T → C | 100 | F934L | L |
| | 16174 | A → G | 100 | I1532V | L |
| | 18761 | A → G | 100 | | L / - intergenic region |
| EBOV/Makona-MA-PP5 | 683 | A → G | 100 | S72G | NP |
| | 1967 | A → C | 15.1 | N500H | NP |
| | 2357 | C → A | 100 | H630N | NP |
| | 6231 | T → C | 100 | S65P | GP |
| | 6774 | T → C | 100 | S246P | GP |
| | 9694 | T → C | 100 | | VP30/VP24 intergenic region |
| | 10342 | $(A)_{6 \rightarrow 7}$ | 100 | | VP30/VP24 intergenic region |
| | 10493 | C → T | 100 | T50I | VP24 |
| | 10979 | A → T | 100 | K212M | VP24 |
| | 14380 | T → C | 100 | F934L | L |
| | 16174 | A → G | 100 | I1532V | L |

**Supplemental Table S4.** Serial passage of wild-type EBOV/Makona in suckling BALB/c mice. Groups of progressively older suckling BALB/c mice ($n$ = 4 to 6) were infected with WT EBOV/Makona by intraperitoneal injection. For each passage, livers were collected, pooled, and used to infect a new set of naïve mice for 11 passages. Corresponding viral loads were measured by RT-qPCR for EBOV L (mean Ct values shown).

| | Serial Passage of wild-type EBOV/Makona | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 |
| **Age of Mice (Balb/c)** | 3 day (n=4) | 3 day (n=6) | 3 – 4 day (n=6) | 3 – 4 day (n=6) | 3 – 4 day (n=6) | 3 – 4 day (n=6) | 3 – 4 day (n=6) | 7 - 8 day (n=6) | 14 day (n=6) | 14 day (n=6) | 14 day (n=6) |
| **Liver collection** | 6 dpi | 6 dpi | 6 dpi | 7 dpi | 7 dpi | 7 dpi | 7 dpi | 7 dpi | 7 dpi | 7 dpi | 7 dpi |
| **Mean Ct value** | 23.9 | 24.3 | 20.2 | 20.8 | 24.3 | 23.1 | 22.5 | 24.5 | 26.8 | 24.6 | 28.0 |
| **% mortality** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |