Appendix S3.

**Diagnostic analysis of the carcass samples**
RNA was extracted from 700 µl of the AVL-swab elute using the QIAamp Viral RNA Mini Kit (Qiagen, Manchester, UK), following the manufacturer's instructions. The 2-step inactivation, shown to completely inactivate EBOV in blood samples (Smither et al. 2015) of the AVL-stored samples was completed with addition 560µl of 95% ethanol in a class III biological safety cabinet. Following inactivation, samples were handled on the bench employing standard laboratory safety precautions. The RT–qPCR were performed using the Light Cycler 480 Master Hydrolysis Probes (Roche, Mannhein, Germany) and SmartCycler instrument (Cepheid, CA, USA). All RT-qPCR assays were validated with an EBOV positive control (L-gene) and a negative extraction control.

At the beginning of the programme the RT-qPCR detection of EBOV was conducted at the CIRMF in Fransville, Gabon. From 2010 onwards the analysis was conducted in the RoC, at the National Public Health Laboratory (NPHL).

**Early sampling protocols**
Several carcass sampling and diagnostic methods have been used since the team first responded to a 2004 great ape Ebola mortality event. Our veterinary team received the first training in carcass sampling at the CIRMF in Fransville in 2004. The methodology was based on collection of a range of organ tissue samples preserved in RNAlater and 10% formalin for molecular and pathological analysis. The procedure was conducted using a full, closed hood Tyvek protective gown combining powered air purifying respirator (PAPR), multiple layers of nitrile gloves and sodium hypochlorite-based disinfection procedures. While this method ensures collection of high quality diagnostic samples that enable detailed mortality investigation, the method relies on extensive training in the collection of samples and biosafety. The carcasses were sampled by a highly qualified, trained veterinary team.
A simplified version of this protocol was cascaded to field research team in the Nouabale-Ndoki National Park 2005 onwards to enable faster response to carcasses for the collection of diagnostic samples. This method combined the same PPE and biosafety component with swab sampling and collection of muscle tissue from a single incision site on the carcass. The samples were preserved in RNAlater.
The sampling protocol introduced in 2017 can be found in the electronic supplement, Appendix 2.