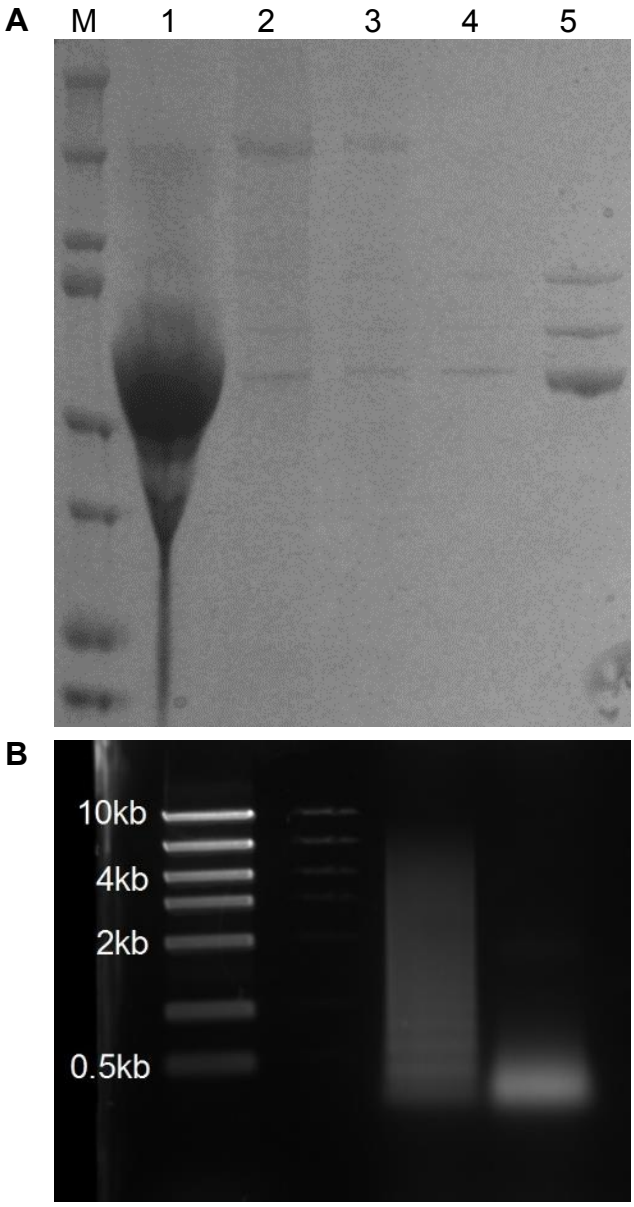

Figure S1. (A) A representative preparation of AAV-FVIII was purified from culture media only through anion-exchange and fractions were loaded onto an SDS-PAGE gel and stained with Coomassie Blue dye for analysis. Lanes were loaded as follows: M) molecular weight marker; 1) concentrated culture media; 2) pooled SEC eluate; 3) diafiltered and concentrated SEC eluate; 4) pooled anion-exchange fractions; 5) concentrated anion-exchange fractions diafiltered into PBS. (B) Agarose gel showing that benzonase nuclease is active in cell culture media concentrated by TFF. Lane 1: DNA marker (also slightly visible in Lane 2 due to overload; Lane 3: DNA isolated from concentrated media; Lane 4: highly digested DNA isolated from concentrated media after incubation for one hour with 25 U/mL benzonase nuclease. Three-fold less DNA was purified from treated culture media than from untreated culture media.

Supplementary Methods and Materials: To test the activity of benzonase nuclease in culture media concentrated by TFF, a sample of concentrated culture media was incubated with 25 U/mL of benzonase at 37°C for one hour. DNA from treated and untreated samples was then isolated using a Qiagen PCR Purification kit, and the concentration of each sample was quantitated by A260. The treated sample of concentrated media contained three-fold less DNA than the untreated sample. Equal volumes of DNA were loaded onto a 1% agarose gel, which was subsequently stained with Gel Red dye and imaged on a Bio-Rad Chemidoc.