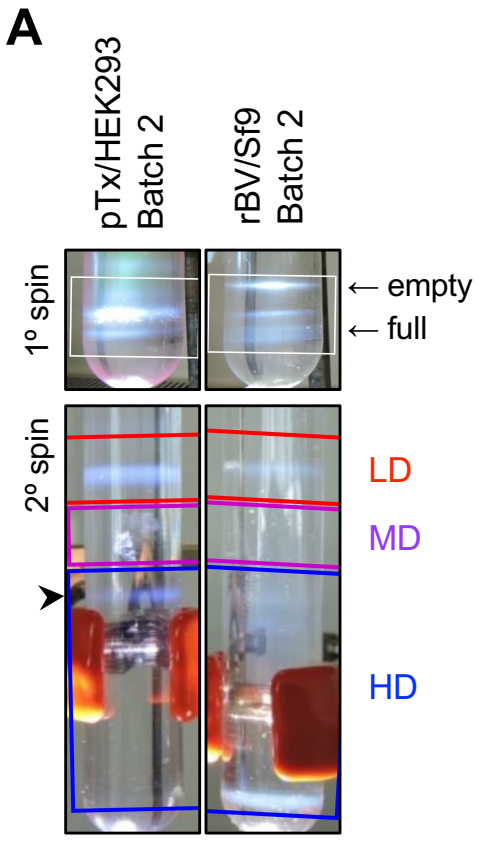
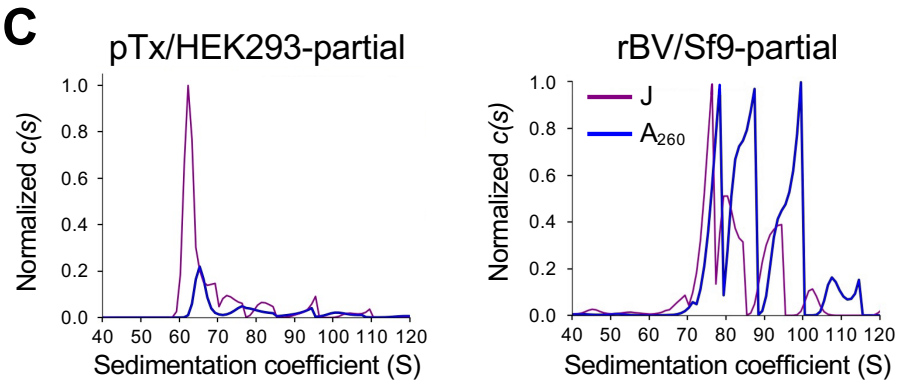
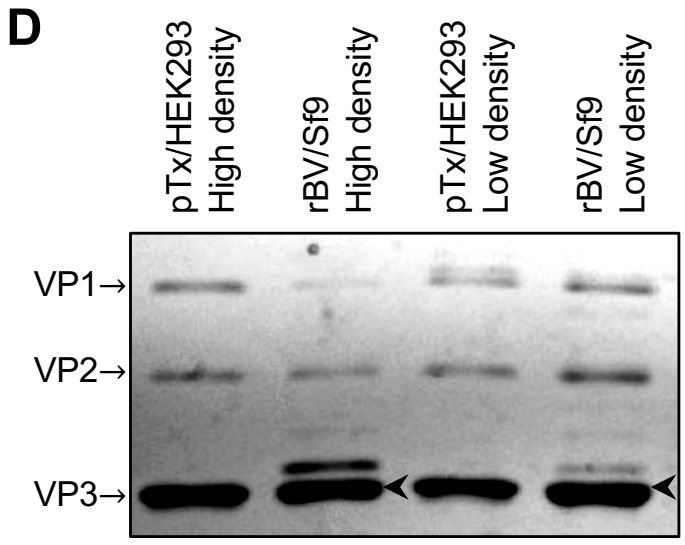


B

Fraction densities (g/cm3)

	pTx/HEK293	rBV/Sf9
Low density (empty)	1.288 to 1.33	1.283 to 1.329
Medium density (partial)	1.33 to 1.357	1.335 to 1.353
High density (Full + oversized)	1.372 to 1.612	1.366 to 1.56





Supplementary Figure 3. Purification of full, partial, and empty fractions by CsCl gradient ultracentrifugation. (A) Photographs of batch 2 pTx/HEK293- and rBV/Sf9-produced vectors subjected to CsCl density gradient ultracentrifugation. Pictures demonstrate the first (1º) and second (2º) spins to obtain empty (red box, LD), partial (violet box, MD), and full + oversized (blue box, HD) particle fractions reported in Figures 4-6. Following the second centrifugation, we observed among the high-density fractions, which consist of full and oversized particles, that the pTx/HEK293-produced vector demonstrated a clear delineated band (arrowhead), while the rBV/Sf9-produced vector showed a poorly defined high-density population. **(B)** Table to summarize the densities for the three fractions. **(C)** Analytical ultracentrifugation analyses of pTx/HEK293- and rBV/Sf9-produced vectors. Blue and purple signals respectively correspond to the sedimentation profile obtained by absorbance at 260 nm (A_{260}) and interference fringe shift (J). The x axis is the sedimentation coefficient (S). The y axis is the normalized distribution *c*(*s*). The fringe shift signal was used to determine the percentage of the different types of capsids. **(D)** Silver stained PAGE gel of isolated full and empty vector fractions. Protein bands attributed to VP1, VP2, and VP3 are annotated. The high- and low-density fractions of the pTx/HEK293- and rBV/Sf9-produced vectors displayed the approximate and expected 1:1:10 ratio of VP1:VP2:VP3 capsids.[1] Notably, the rBV/Sf9-produced vectors showed minor baculoviral cathepsin-cleaved VP1/2 bands.[2, 3] Silver staining of capsids for the partial fractions were similar to full and empty fractions (unpublished observations).

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3. Cecchini, S, Virag, T, and Kotin, RM (2011). Reproducible high yields of recombinant adeno-associated virus produced using invertebrate cells in 0.02- to 200-liter cultures. Hum Gene Ther 22: 1021-1030.