Antigen-Specific Modulation of Capsid Immunogenicity with Tolerogenic Nanoparticles Results in Successful AAV Vector Readministration

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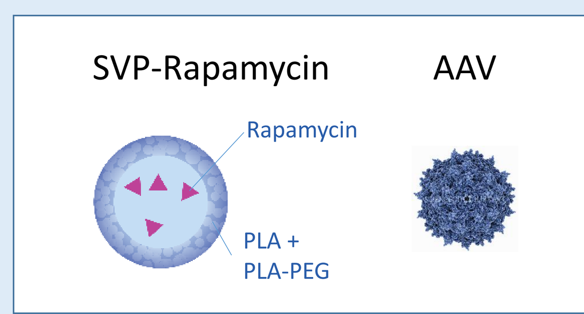
Abstract

Gene transfer approaches based on the adeno-associated virus (AAV) vector platform have shown great promise both in preclinical animal models and in the clinic. However, there may be circumstances where re-treatment may become necessary, particularly for systemic applications in pediatric patients. In addition, it would be desirable to be able to boost expression in patients who have inadequate expression levels. Currently vector re-administration is limited by the formation of neutralizing antibodies to AAV, which mediate vector clearance and inhibit efficacy. Additionally, CD8 immunity against the AAV capsid or the transgene can reduce or eliminate expression from transduced cells. We have recently developed a novel strategy to induce immune tolerance to protein biotherapeutic drugs based on the co-administration of biodegradable nanoparticles containing rapamycin (SVP-Rapamycin; Kishimoto et al., Nature Nanotech, 2016). SVP-Rapamycin has been shown to mitigate the formation of anti-drug antibodies against a pegylated uricase enzyme in a Phase 1b trial. Here we demonstrate that SVP-Rapamycin added on to AAV8-based gene therapy inhibits the formation of anti-AAV8 antibodies and enables successful vector re-dosing in both mice and nonhuman primates. The effect of SVP-Rapamycin was antigen-specific, as treated mice showed normal immune responses to subsequent challenge with unrelated antigens. In addition, co-administration of AAV with SVP-Rapamycin inhibited CD8+ T cell infiltrates in the liver and inhibited the ex vivo recall responses of CD8+ T cells.
In conclusion, co-administration of SVP-Rapamycin with AAV vector mitigates immunogenicity, enabling AAV vector re-administration.



Key Findings

- SVP-Rapamycin co-administration with AAV8 vectors can completely inhibit the formation of anti-AAV8 antibodies (Figure 1A), enabling successful production of human Factor IX transgene derived from vector re-administration (Figure 1B). SVP-Rapamycin treatment has no effect on the transduction efficiency of the first vector administration (Figure 1C), but significantly increases transduction efficiency of the second vector administration (Figure 1D), correlating with the inhibition of anti-AAV8 antibody response.
- While the total frequency of CD19+ B cells in not affected (Figure 2A), the frequency of B cells secreting AAV capsid-specific IgG and IgM antibodies (Figure 2B) and germinal center B cells (Figure 2C) are significantly reduced by the co-administration of SVP
- SVP-Rapamaycin inhibits CD8+ T cell responses specific to the capsid (Figure 3A) in a "double hit" experimental model in which the capsid is administered as both a protein (vector) and an expressed product (transgene). SVP-Rapamycin also reduces the number of total splenic T cells and interferon-γ producing T cells following vector administration in this model.
- SVP-rapamycin also inhibits CD8 T cell infiltrates in the liver following AAV8 vector administration (Figure 4).
- The effect of SVP-Rapamycin appears antigen-specific to the co-administered AAV vector (Figure 5).
- At the level of spleen and LN, administration of SVPs does not negatively affect the frequency of Tregs, while it does decrease the CD4+ and CD8+ T cell count (data not shown). In lymph nodes, a significant increase in Treg frequency is observed in animals treated with SVP-Rapamycin (data not shown). Importantly, depletion of Tregs with anti-CD25 antibody treatment results in loss of efficacy of SVPs (Figure 6).
- The ability of SVP-Rapamycin to inhibit anti-AAV8 IgG, IgM, and neutralizing antibody responses has been translated to nonhuman primates (Figure 7). The inhibition of anti-AAV8 antibodies correlated with successful production of therapeutic levels of human Factor IX protein following vector re-administration. No changes in body weight, liver enzyme levels, or vector biodistribution were observed (not shown).

Conclusions

- SVP-Rapamycin can be used to inhibit humoral immune responses to AAV vectors in mice and enable productive re-administration of AAV vectors
- The approach is also efficacious in controlling AAV-specific CD8+ T cell responses
- The effect of SVP-Rapamycin appears to be antigen-specific
- Translation to NHPs confirmed the efficacy of the approach
- SVP-Rapamycin are potentially a powerful strategy for targeted modulation of AAV immunogenicity

Figure 1. Inhibition of anti-AAV8 antibodies by SVP-Rapamycin enables vector re-administration

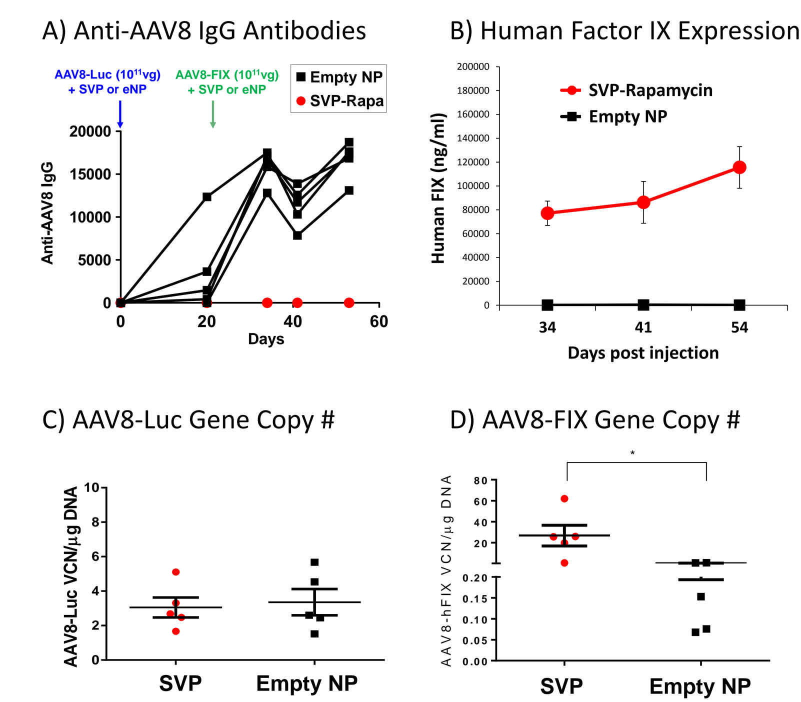


Figure 1. Co-administration of SVP-Rapamycin with AAV8 gene therapy vectors prevents the formation of anti-AAV8 antibodies and enables successful vector readministration. C57Bl/6 mice (n = 5/group) were treated with $4x10^{12}$ vg/kg AAV8-Luciferase on day 0 and with $4x10^{12}$ vg/kg AAV8-human factor IX (AAV8-FIX) on day 21. On both days 0 and 21, mice were concomitantly administered SVP-Rapamycin or empty nanoparticles. **A).** Anti-AAV8 IgG antibody levels determined by ELISA. **B).** Serum levels of human FIX determined by ELISA. **C) and D).** Vector copy numbers for AAV8-Luc (C) and AAV8-FIX (D) determined by quantitative PCR.

Figure 2. SVP-Rapamycin treatment reduces capsid-specific B cells and germinal center formation but not total B cells

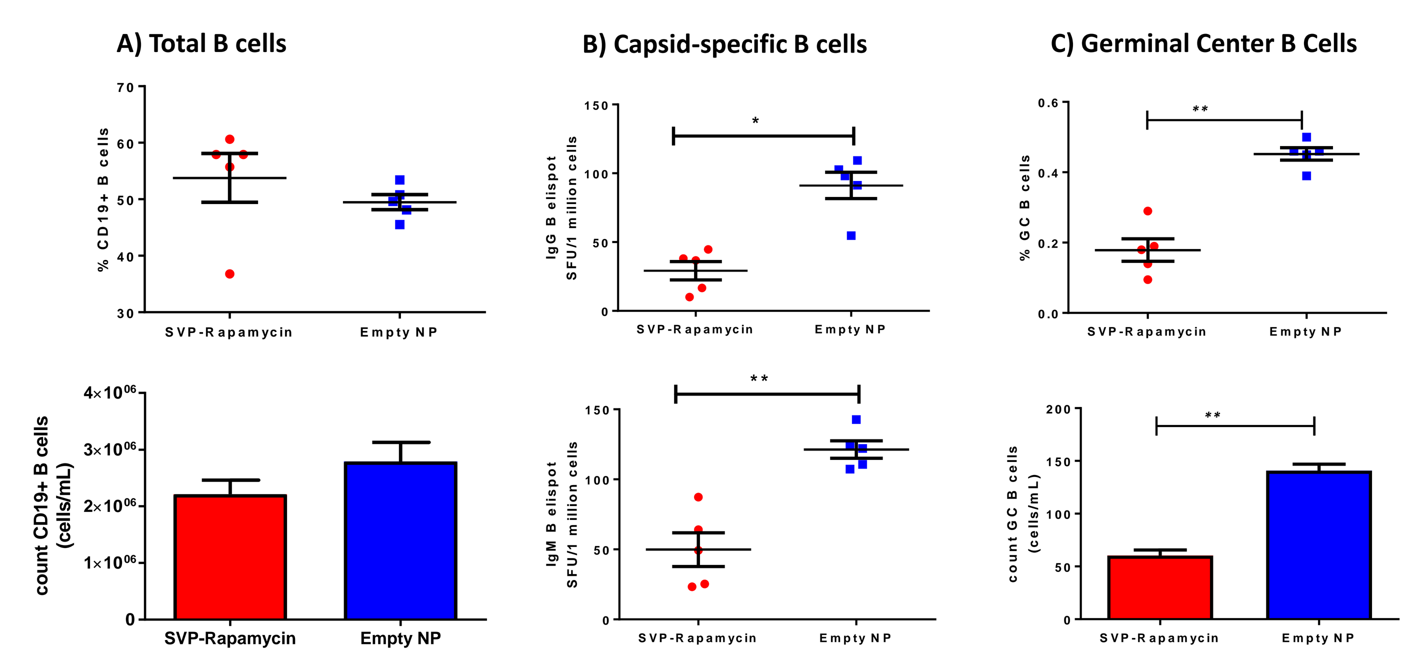


Figure 2. SVP-Rapamycin treatment reduces capsid-specific B cells and germinal center formation but not total B cells. C57Bl/6 mice (n = 5/group) were administered AAV8 vector encoding the VP-1 capsid antigen (AAV8-VP-1) concomitantly with SVP-Rapamycin (red) or empty nanoparticles (blue). **A).** SVP-Rapamycin treatment does not affect total B cells. Total CD19+ B cells were evaluated by flow cytometry. **B).** SVP-Rapamycin treatment inhibits the formation of capsid-specific B cells. Capsid-specific B cells were enumerated by ELISpot. The number of anti-capsid IgG spot forming units (SFU) are shown per 10^6 splenocytes. **C).** SVP-Rapamycin treatment inhibits germinal center B cells. The percentage and number of B220+, GL-7+, CD95+ germinal center B cells in the spleen were assessed by flow cytometry.

Figure 3. SVP-Rapamycin treatment inhibits capsid-specific T cell responses

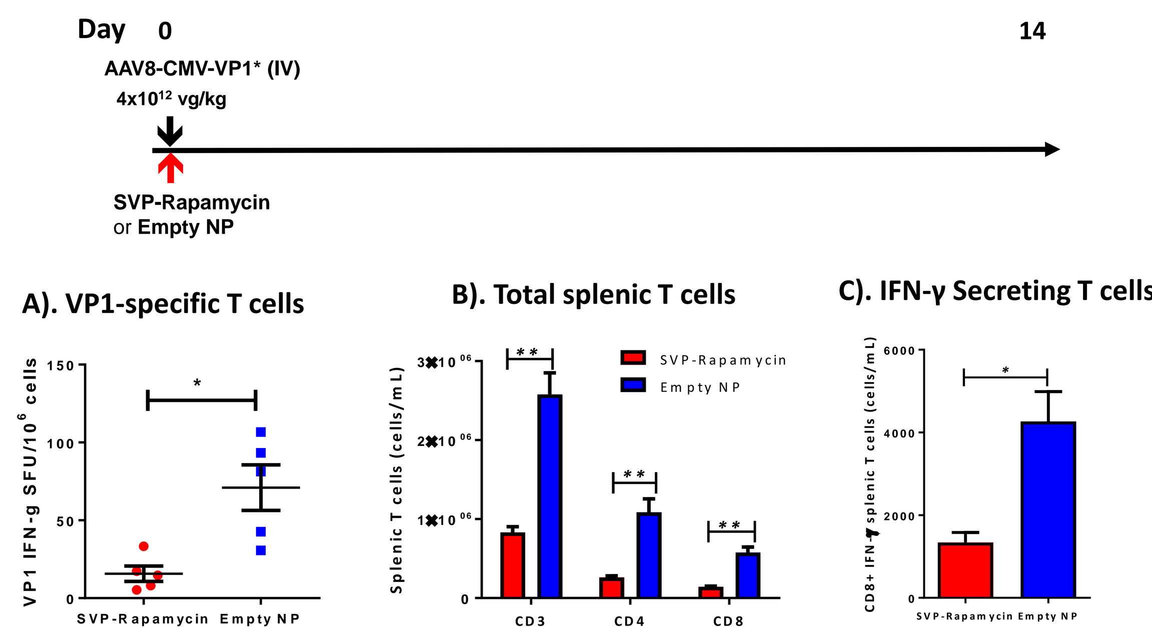


Figure 3. SVP-Rapamycin treatment inhibits capsid-specific T cell responses. Capsid-specific T cells responses were evaluated in a 'double hit' model in which the capsid is administered as both a protein (vector) and expressed product (transgene). C57Bl/6 mice (n = 5/group) were administered AAV8 vector encoding the VP-1 capsid antigen (AAV8-VP-1) with SVP-Rapamycin (red) or empty nanoparticles (blue). **A).** SVP-Rapamycin treatment inhibits the formation of capsid-specific T cells. Splenocytes were restimulated with VP-1. VP-1-specific T cells secreting interferon-γ (IFN-γ) were enumerated by ELISpot. The number of spot forming units are shown per 10^6 splenocytes. **B).** SVP-Rapamycin treatment reduces total splenic T cells in response to AAV vector administration. Total T cells were evaluated by flow cytometry. **C).** SVP-Rapamycin treatment inhibits IFN-γ-producing T cells. The number of IFN-γ-producing splenic T cells were evaluated by flow cytometry.

Figure 4. SVP-Rapamycin treatment inhibits CD8 T cell infiltrates in the liver.

	Day 0	Day 21
Untreated	PBS	PBS
Control empty NP	AAV-Luc + eNP	AAV-Luc + eNP
SVP-Rapamycin	AAV-Luc + SVP	AAV-Luc + SVP

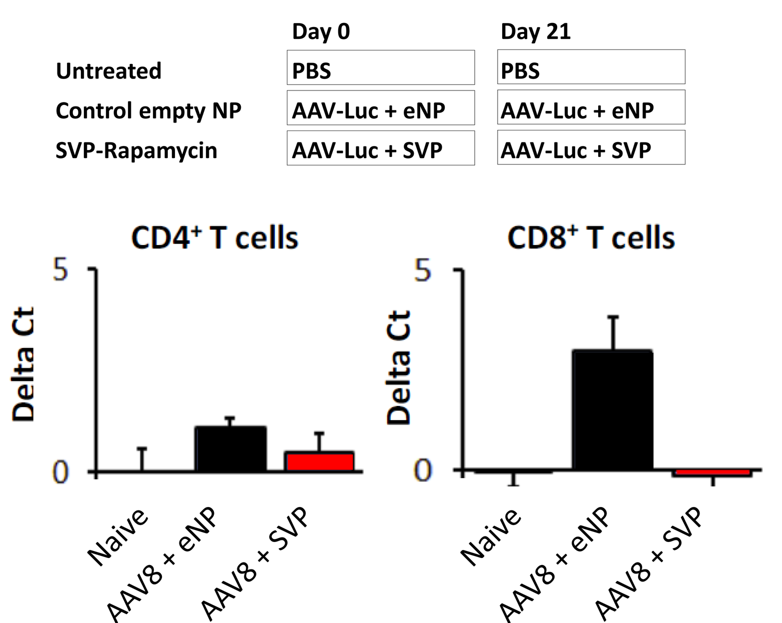


Figure 4. SVP-Rapamycin treatment inhibits CD8 T cell infiltrates in the liver. C57Bl/6 mice (n = 5/group) were administered AAV8-Luciferase (AAV8-Luc) with SVP-Rapamycin (SVP) or empty nanoparticles (eNP) on days 0 and 21. Negative control animals received phosphate-buffered saline (PBS) only. Total CD4 (left panel) and CD8 (right panel) T cells in the liver were enumerated by quantitative PCR.

Figure 5. Effects of SVP-Rapamycin are specific to the co-administered antigen.

Prime: AAV8-Luc + SVP-Rapamycin or Empty NP

Challenge: 1) AAV8-hFIX vector or 2) AAV5-hFIX vector or 3) hFIX protein in cFA

Day 0 — 20 — 56

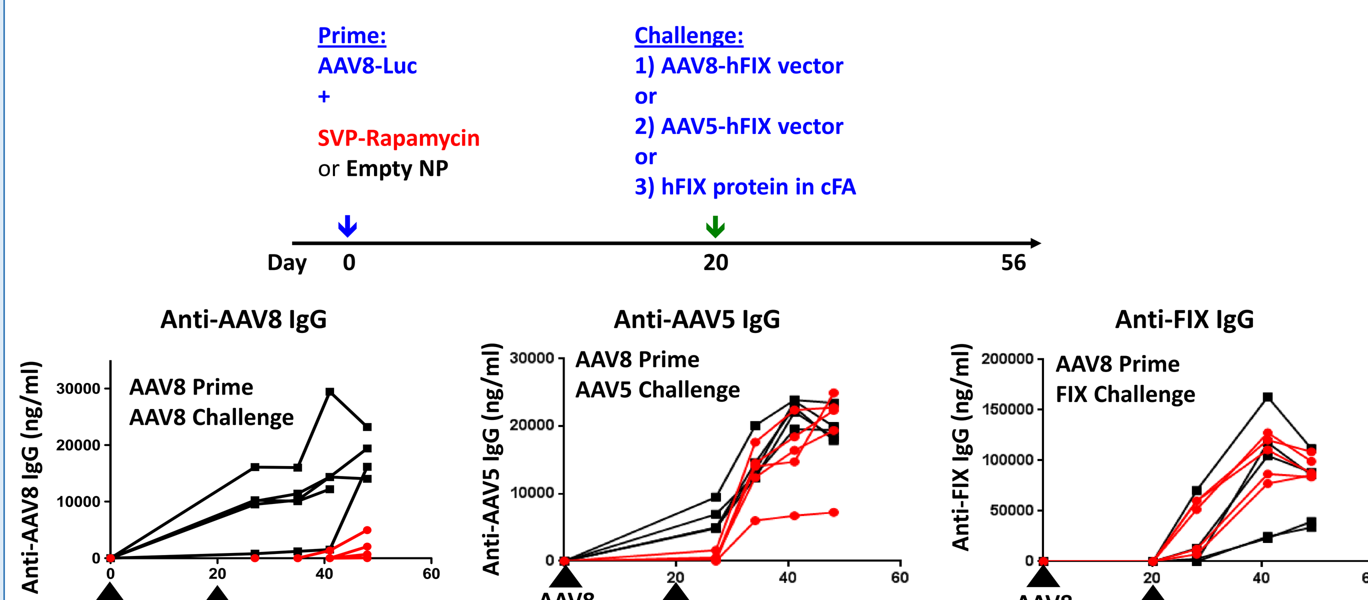


Figure 5. Effects of SVP-Rapamycin are specific to the co-administered antigen. C57Bl/6 mice (n = 5/group) were administered AAV8-Luciferase (AAV8-Luc) with SVP-Rapamycin (red symbols) or empty nanoparticles (black symbols) on day 0. On day 21, mice were challenged with either AAV8-Luc (left panel), AAV5-Luc (middle panel), or FIX protein emulsified in complete Freund's adjuvant (CFA) in the absence of SVP-Rapamycin. Antibodies to AAV8 (left), AAV5 (middle) and FIX protein (Right) were assessed by ELISA.

Figure 6. Effects of SVP-Rapamycin are reversed by depletion of CD25+ CD4 T cells.

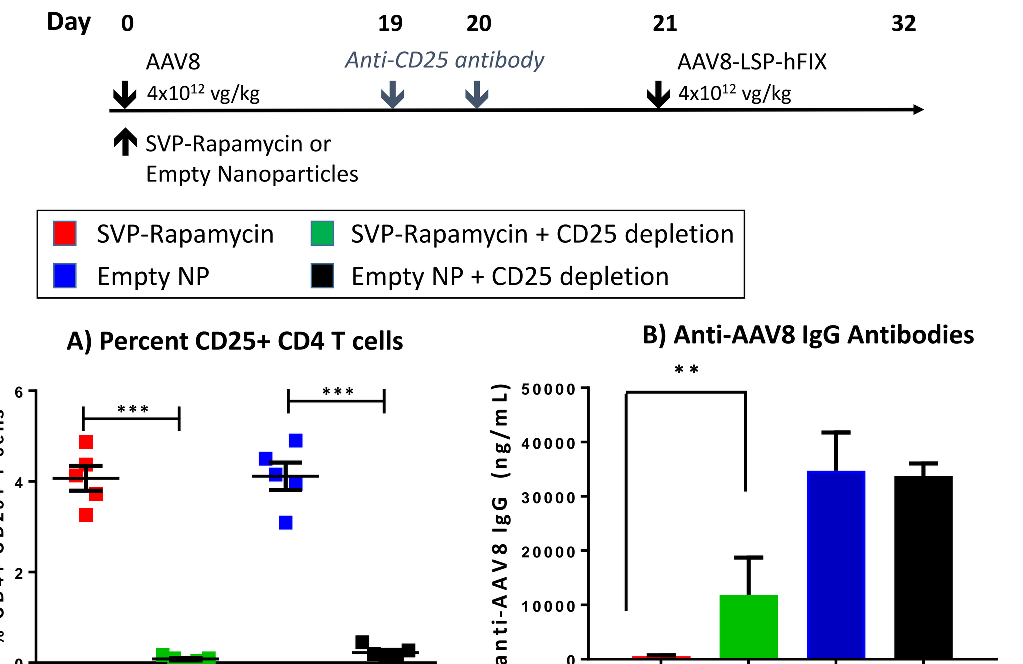


Figure 6. Effects of SVP-Rapamycin are reversed by depletion of CD25+ CD4 T cells. C57Bl/6 mice (n = 5/group) were administered AAV8 vector ($4x10^{12}$ vg/kg) with SVP-Rapamycin or empty nanoparticles on day 0. On days 19 and 20, indicated groups were received a depleting anti-CD25 monoclonal antibody. On day 21, mice were challenged with AAV8-FIX in the absence of SVP-Rapamycin. **A).** Anti-CD25 antibody treatment results in depletion of CD25+ CD4 T cells. Sentinel mice were sacrificed on day 21 and the percentage of CD25+ CD4 T cells in the spleen were analyzed by flow cytometry. **B).** Anti-AAV8 antibody titers were assessed on day 32 by ELISA.

Figure 7. SVP-Rapamycin inhibits anti-AAV8 antibody responses and enables successful vector re-administration in nonhuman primates

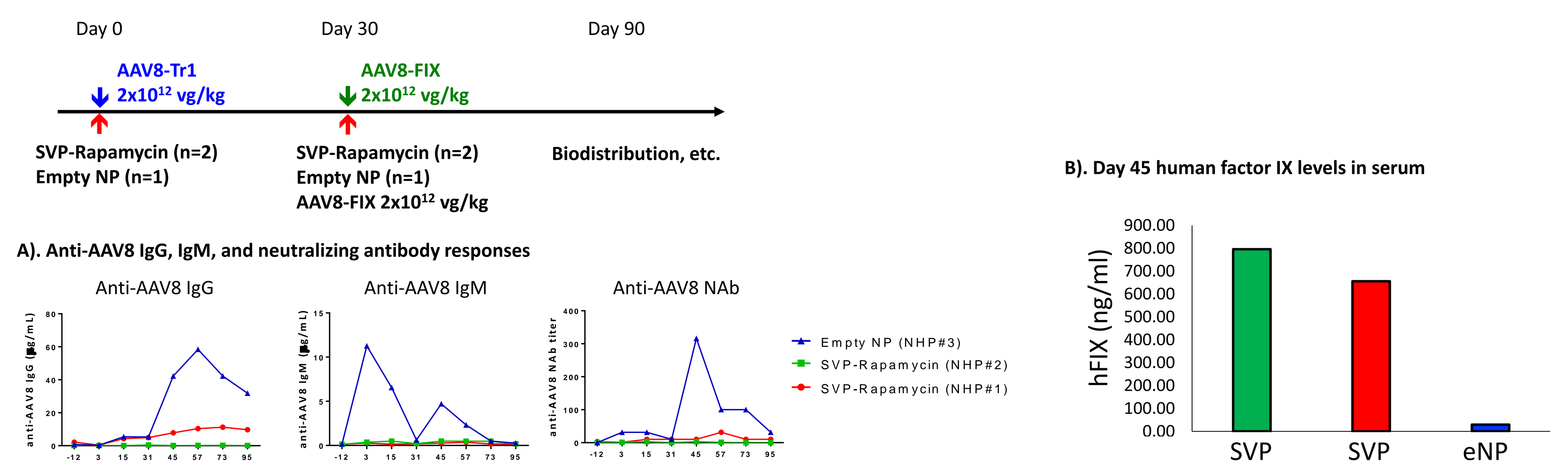


Figure 7. SVP-Rapamycin inhibits anti-AAV8 antibody responses and enables successful vector re-administration in nonhuman primates (NHP). NHP (Macaca fascicularis) were administered $2x10^{12}$ vg/kg AAV8 vector encoding transgene 1 (AAV8-Tr1) with SVP-Rapamycin (n = 2) or empty nanoparticles (n = 1) on day 0. On day 30, NHPs were administered $2x10^{12}$ vg/kg AAV8-human factor IX (AAV8-FIX) together with nanoparticles, as described above. **A).** SVP-Rapamycin treatment inhibits the formation of anti-AAV8 antibodies. Anti-AAV8 IgG (left panel) and IgM (middle panel) antibodies were assessed by ELISA. Anti-AAV8 neutralizing antibodies (right panel) were evaluated by a cell-based neutralizing antibody assay. **B).** SVP-Rapamycin treatment enables expression of human Factor IX following second vector administration. Serum levels of human factor IX were assessed on day 45 by ELISA.