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Supplemental Information

IL-15 blockade and rapamycin rescue multifactorial loss of factor VIII from AAV-transduced hepatocytes in hemophilia A mice

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Supplementary Table S1: Summary of results:

Fig.	Summary of Experiment Results	Treatment Groups	FVIII activity levels at EOE (% normal)	Inhibitors at EOE (n/n alive)
S1	Success of liver-directed AAV8-hFVIII gene therapy is dependent on the strain of hemophilia A mice.	Hemophilia A mice (F8e16-/-) on C57BL6/129	13% (0-33%) at week 16	0/8
		Hemophilia A mice (F8e16-/-) on BALB/c	0% at week 16	18/18
S2	Transient immune suppression using rapamycin and Flt3L delays anti-hFVIII/inhibitor formation.	Vector only	0% at week 12	7/7
		Rapamycin + Flt3L + rhFVIII 3x/wk i.v. for 4 wks	<1% (0-3%) at week 12	7/8
S3	Prolonged immune suppression using rapamycin prevents inhibitor formation.	Vector only	0% at week 15	3/3
		Rapamycin 3x/wk i.v. for 11 wks	9% (0-51%) at week 15	0/6
		Rapamycin + Flt3L 3x/wk i.v. for 11 wks	3% (0-14%) at week 15	0/6
S4	Immune suppression with rapamycin for 8 wks prevents inhibitor formation but does not preserve hFVIII levels.	Vector only	0% at week 16	5/5
		Rapamycin 2x/wk i.v. for 2 wks	0% at week 16	3/6
		Rapamycin 2x/wk i.v. for 4 wks	0% at week 16	2/4
		Rapamycin 2x/wk i.v. for 6 wks	0% at week 16	3/6
		Rapamycin 2x/wk i.v. for 8 wks	1% (0-2%) at week 16	0/6
1, S6	Sustained systemic hFVIII expression after transient rapamycin administration combined with anti-IL-15 therapy. The rapamycin-based regimens prevent formation of antibodies to AAV capsid, allowing for vector readministration.	Vector only	0% at week 16	7/7
		Rapamycin 2x/wk i.v. for 8 wks	<1% (0-2%) at week 16	0/7
		Rapamycin + anti-IL-15 mAb 2x/wk i.v. for 8 wks	8% (0-24%) at week 16	0/9
2, 3, 5, 8, S8, S9, S13, S14, S19, S20	Anti-CD122 has broad and lasting anti-inflammatory effects and is effective in preserving hFVIII expression. $CD8^+$ T cell depletion after 8-week rapamycin regimen partially prevents loss of hFVIII expression. IL-15 blockade has no effect on transcription of mRNA or gene copy numbers. Vector copy number and hFVIII mRNA transcript levels correlated with each other, but neither correlated well with loss of hFVIII expression. In rapamycin-treated mice, phospho-eIF2α correlated positively with IFN-γ and negatively with hFVIII activity, suggesting cellular stress and inflammation are interlinked but can be disrupted with IL-15 blockade.	Vector only	0% at week 16	5/5
		Rapamycin 2x/wk i.v. for 8 wks	5% (0-15%) at week 16	0/21
		Rapamycin + anti-IL-15 mAb 2x/wk i.v. for 8 wks	9% (0-24%) at week 16	0/18
		Rapamycin + anti-CD122 mAb 2x/wk i.v. for 8 wks	15% (0-51%) at week 16	0/9
		Rapamycin 2x/wk i.v. for 8 wks, then anti-CD8 mAb 2x/wk i.v. for 8 wks	4% (0-11%) at week 16	0/8
4, S10, S11, S12	Flow cytometry at 3 wks after gene transfer (while immune suppressed) showed rapamycin ablates splenic Tfh, Tfr, and GC B cells and hepatic $CD8^+$ Teff. Hepatic NK cells are decreased with anti-IL-15 mAb and ablated with anti-CD122 mAb, while both mAbs similarly reduce hepatic $CD8^+$ Trm cells.	Vector only	-	-
		Rapamycin 2x/wk i.v. for 3 wks		
		Rapamycin + anti-IL-15 mAb 2x/wk i.v. for 3 wks		
		Rapamycin + anti-CD122 mAb 2x/wk i.v. for 3 wks		
6, 7, S15, S16, S17, S18	smFISH of hepatocytes shows a decline in gene copy numbers/cell rather than genome-containing cells. Western blots show induction of ER stress.	Vector only	0% at week 14	8/8
		Rapamycin 2x/wk i.v. for 14 wks	32% (6-61%) at week 14	0/7

Supplementary Figures:

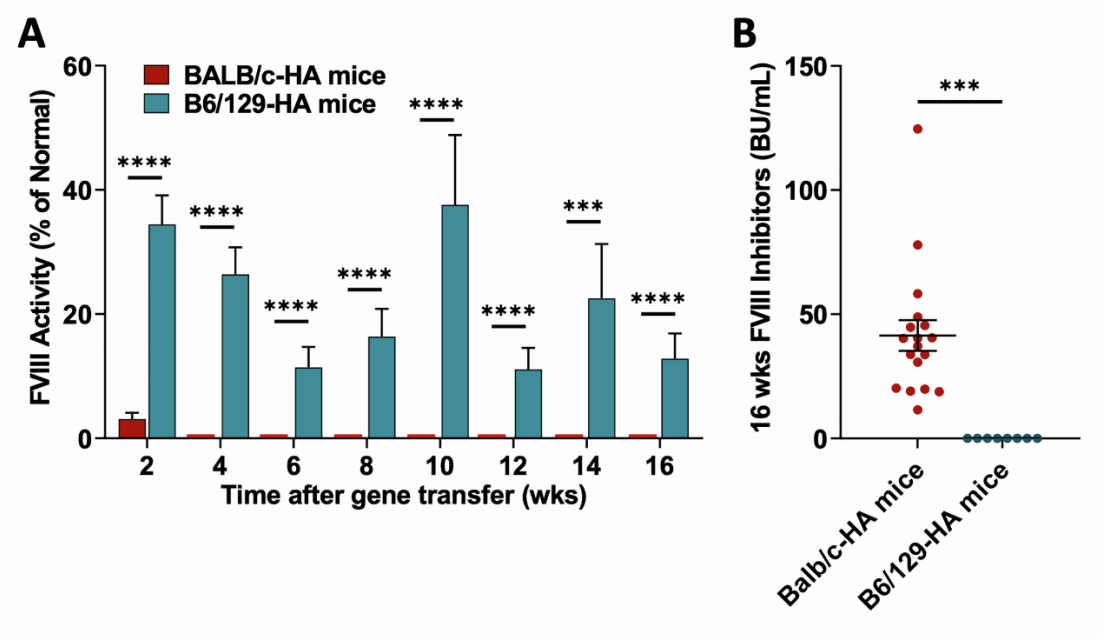

Fig. S1. Success of liver-directed AAV8-hFVIII gene therapy is dependent on the strain of hemophilia A mice. Hemophilia A mice (F8e16$^{-/-}$) on C57BL6/129 (n=8) or BALB/c (n=18) genetic backgrounds received hepatic gene transfer with AAV8-hFVIII vector (codon-optimized) at a dose of 1x10^{11} vg/mouse. **A.** FVIII activity in plasma as a function of time after gene transfer (averages ±SD, **** indicates *P*<0.0001). **B.** Inhibitor titers (BU/ml) at week 16 shown for individual mice and as averages ±SEM. Significance was determined with multiple unpaired t tests, corrected for multiple comparisons using Holm-Šídák post hoc test. *** *P*<0.001, **** *P*<0.0001.

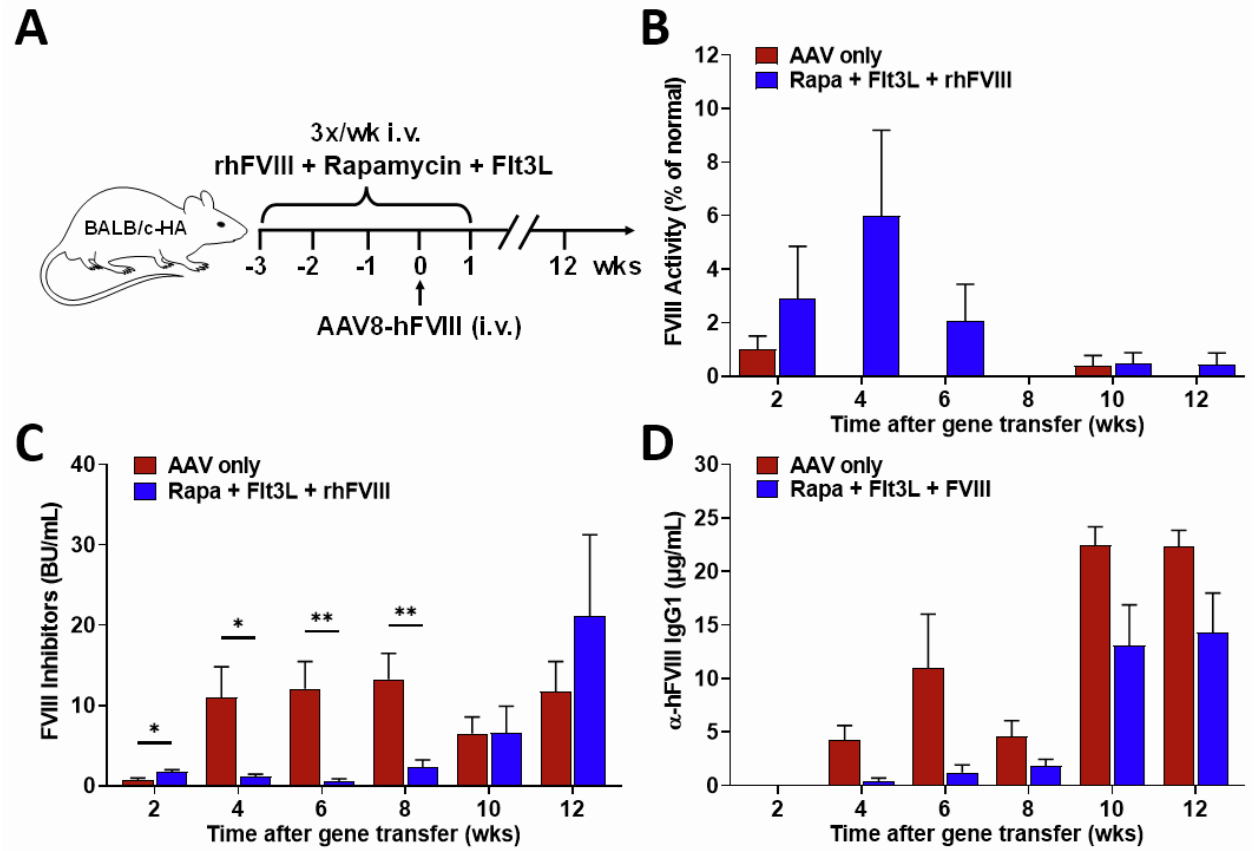

Fig. S2. Delay in inhibitor formation by transient immune suppression using rapamycin and flt3L. A. Experimental outline: Hemophilia A mice (BALB/c F8e16-/-) received hepatic gene transfer with AAV8-hFVIII vector (codon-optimized) at a dose of $1x10^{11}$ vg/mouse (n=7-8 per experimental group). Animals were additionally treated three times per week, three weeks prior to and one week after gene transfer with rapamycin combined with flt3L and recombinant human FVIII ("Rapa + Flt3L + rhFVIII") or no immune modulation ("AAV only"). **B.** FVIII activity in plasma as a function of time after gene transfer (averages ±SEM). **C.** Inhibitory antibody titers against hFVIII as a function of time after gene transfer (averages ±SEM). **D.** IgG1 antibodies against hFVIII as a function of time after gene transfer (averages ±SEM). Significance was determined with multiple unpaired t tests, corrected for multiple comparisons using Holm-Šídák post hoc test. * $P<0.05$, ** $P<0.01$.

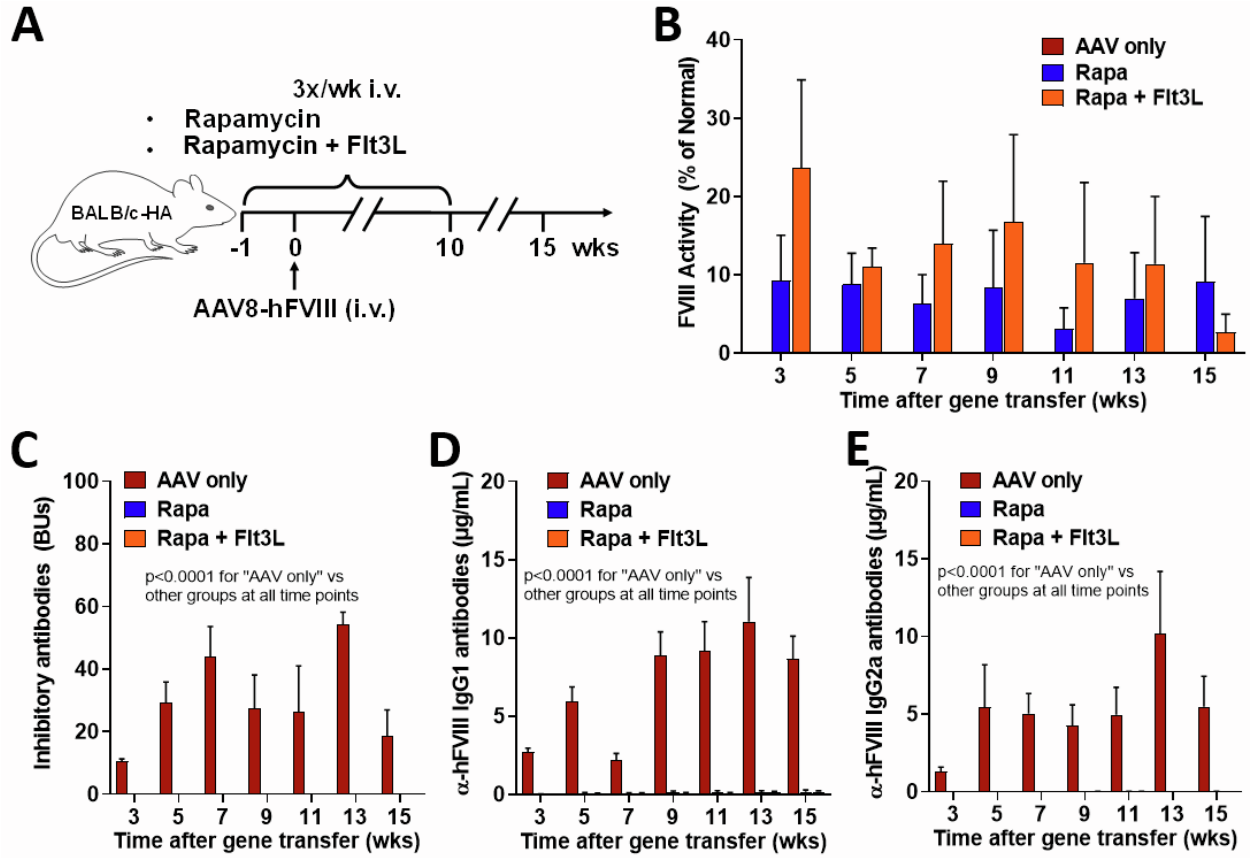

Fig. S3. Prevention of inhibitor formation against FVIII by prolonged immune suppression. A. Experimental outline: Hemophilia A mice (BALB/c F8e16-/-) received hepatic gene transfer with AAV8-hFVIII vector (codon-optimized) at a dose of $1x10^{11}$ vg/mouse (n=4-6 per experimental group). Animals were additionally treated three times per week one week prior to and ten weeks after gene transfer, with rapamycin ("Rapa"), rapamycin combined with flt3L ("Rapa + Flt3L"), or no immune modulation ("AAV only"). **B.** FVIII activity in plasma as a function of time after gene transfer (averages ±SEM). **C.** Inhibitory antibody titers against hFVIII as a function of time after gene transfer (averages ±SEM). **D.** IgG1 antibodies against hFVIII as a function of time after gene transfer (averages ±SEM). **E.** IgG2a antibodies against hFVIII as a function of time after gene transfer (averages ±SEM). Significance was determined by one-way ANOVA for each time point and corrected for multiple comparisons using Tukey post hoc test.

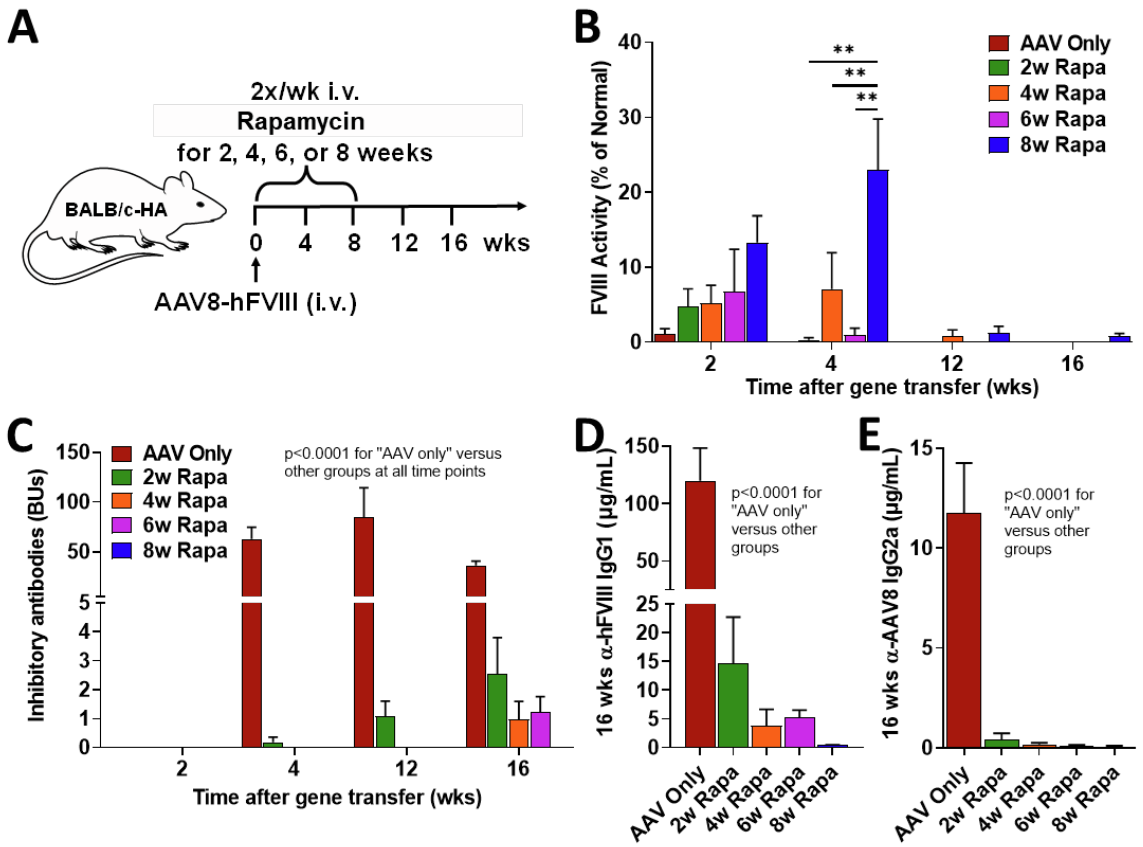

Fig. S4. Eight weeks of rapamycin administration is sufficient to prevent inhibitor formation against FVIII but does not result in sustained FVIII activity. A. Experimental outline: Hemophilia A mice (BALB/c F8e16-/-) received hepatic gene transfer with AAV8-hFVIII vector (codon-optimized) at a dose of $2x10^{11}$ vg/mouse (n=6 per experimental group). Animals were additionally treated twice per week for either 2, 4, 6, or 8 weeks after gene transfer, with rapamycin ("2w, 4w, 6w, 8w Rapa") or no immune modulation ("AAV only"). **B.** FVIII activity in plasma as a function of time after gene transfer (averages ±SEM). **C.** Inhibitory antibody titers against hFVIII as a function of time after gene transfer (averages ±SEM). **D.** IgG1 antibodies against hFVIII as a function of time after gene transfer (averages ±SEM). **E.** IgG2a antibodies against hFVIII as a function of time after gene transfer (averages ±SEM). Significance was determined by one-way ANOVA for each time point and corrected for multiple comparisons using Tukey post hoc test (B, C). Significance was determined with multiple unpaired t tests, corrected for multiple comparisons using Holm-Šídák post hoc test (D, E). ** $P<0.01$.

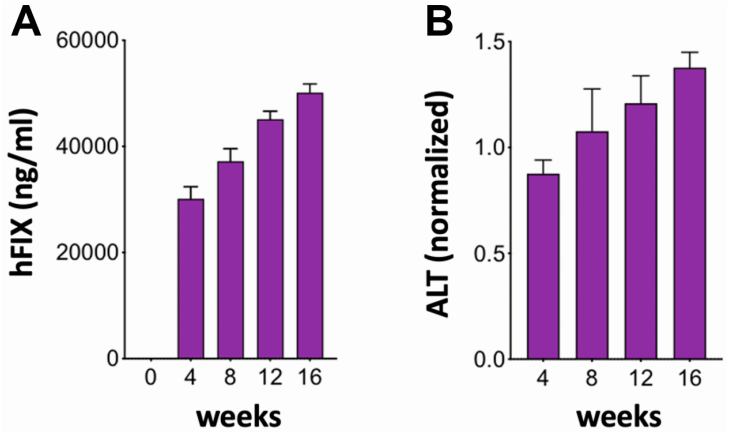

Fig. S5. Hepatic AAV8-hFIX gene transfer in hemophilia A mice. A. Plasma levels of hFIX as a function of time after gene transfer in BALB/c F8e16-/- mice ($2x10^{11}$ vg/mouse given by intravenous injection; n=7). **B.** Liver enzyme (alanine aminotransferase, ALT) levels in plasma normalized to average pre-treatment levels.

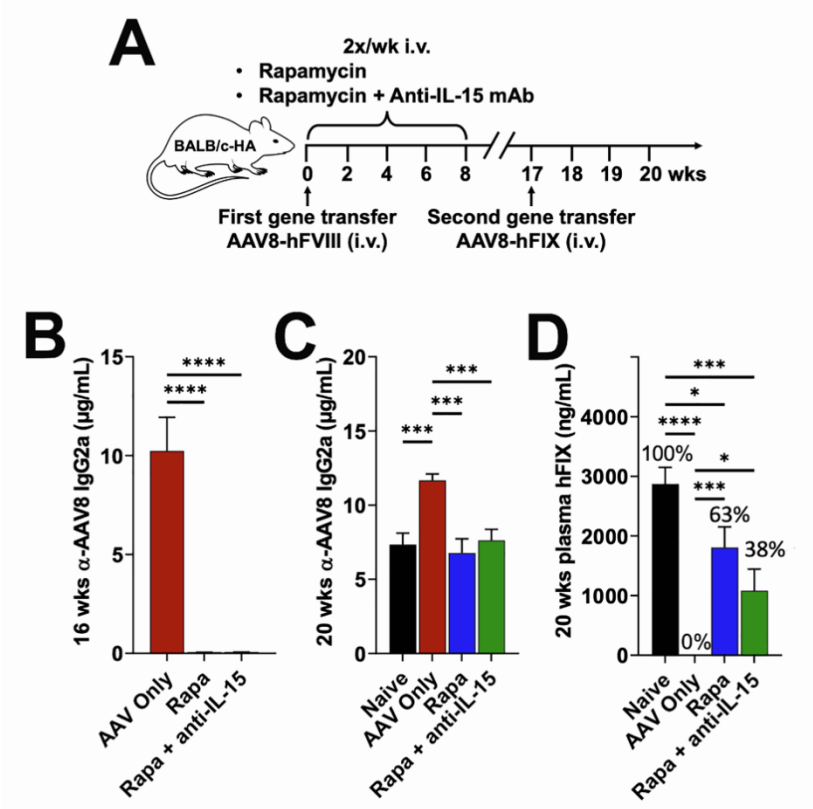

Fig. S6. Successful vector re-administration after initial immune suppression with rapamycin. A. Experimental outline: Hemophilia A mice (BALB/c F8e16$^{-/-}$) received hepatic gene transfer with AAV8-hFVIII vector (codon-optimized) at a dose of $2x10^{11}$ vg/mouse (n=7-9 per experimental group). Animals were additionally treated twice per week with rapamycin ("Rapa") or rapamycin combined with anti-IL-15 ("Rapa + anti-IL-15") for the first 8 weeks after gene transfer, starting the day of vector administration. Control mice received vector but no immune modulation ("AAV

only"). Naïve mice served as positive control for AAV8-hFIX gene transfer (identical dose given 17 weeks after initial vector administration). **B.** IgG2a antibody titers against AAV8 capsid at week 16. **C.** IgG2a antibody titers against AAV8 capsid at week 20. **D.** Plasma levels of hFIX at week 20 (i.e. 3 weeks after AAV8-hFIX administration). Also shown is percent hFIX expression relative to average levels in naïve control mice after AAV8-hFIX administration (100%, no first vector administration). Significance was determined by multiple unpaired t tests, corrected for multiple comparisons using Holm-Šídák post hoc test (B-D). * *P*<0.05, *** *P*<0.001, **** *P*<0.0001.

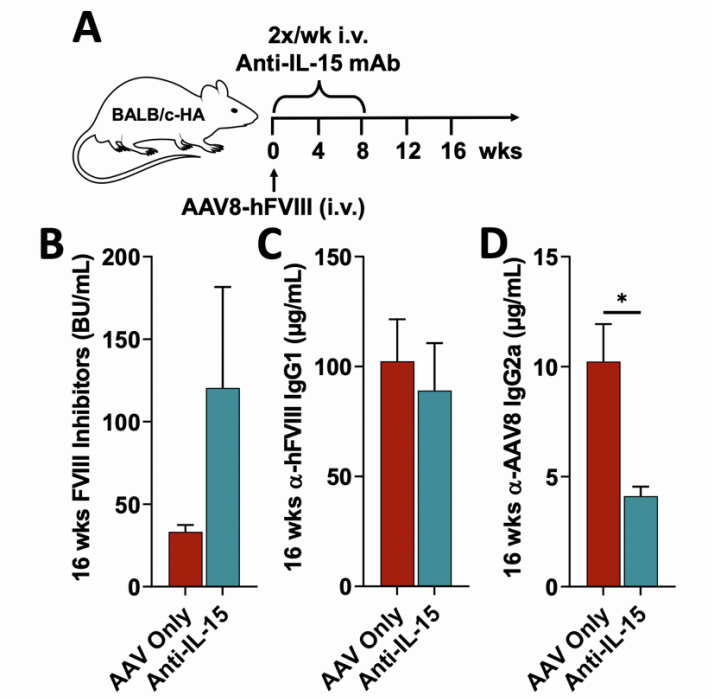

Fig. S7. IL-15 blockade by itself does not prevent antibody formation against FVIII or AAV8 capsid. A. Experimental outline: Hemophilia A mice (BALB/c F8e16-/-) received hepatic gene transfer with AAV8-hFVIII vector (codon-optimized) at a dose of $2x10^{11}$ vg/mouse (n=5-7 per experimental group). Animals were additionally treated twice per week with anti-IL-15 ("Anti-IL-15") for the first 8 weeks after gene transfer or received no immune modulation ("AAV only"). **B.** Inhibitory antibody titers against hFVIII at week 16 (averages ±SEM). **C.** IgG1 antibody titers against hFVIII at week 16 (averages ±SEM). **D.** IgG2a antibody titers against AAV8 capsid at week 16 (averages ±SEM). Significance was determined with multiple unpaired t tests, corrected for multiple comparisons using Holm-Šídák post hoc test. * *P*<0.05.

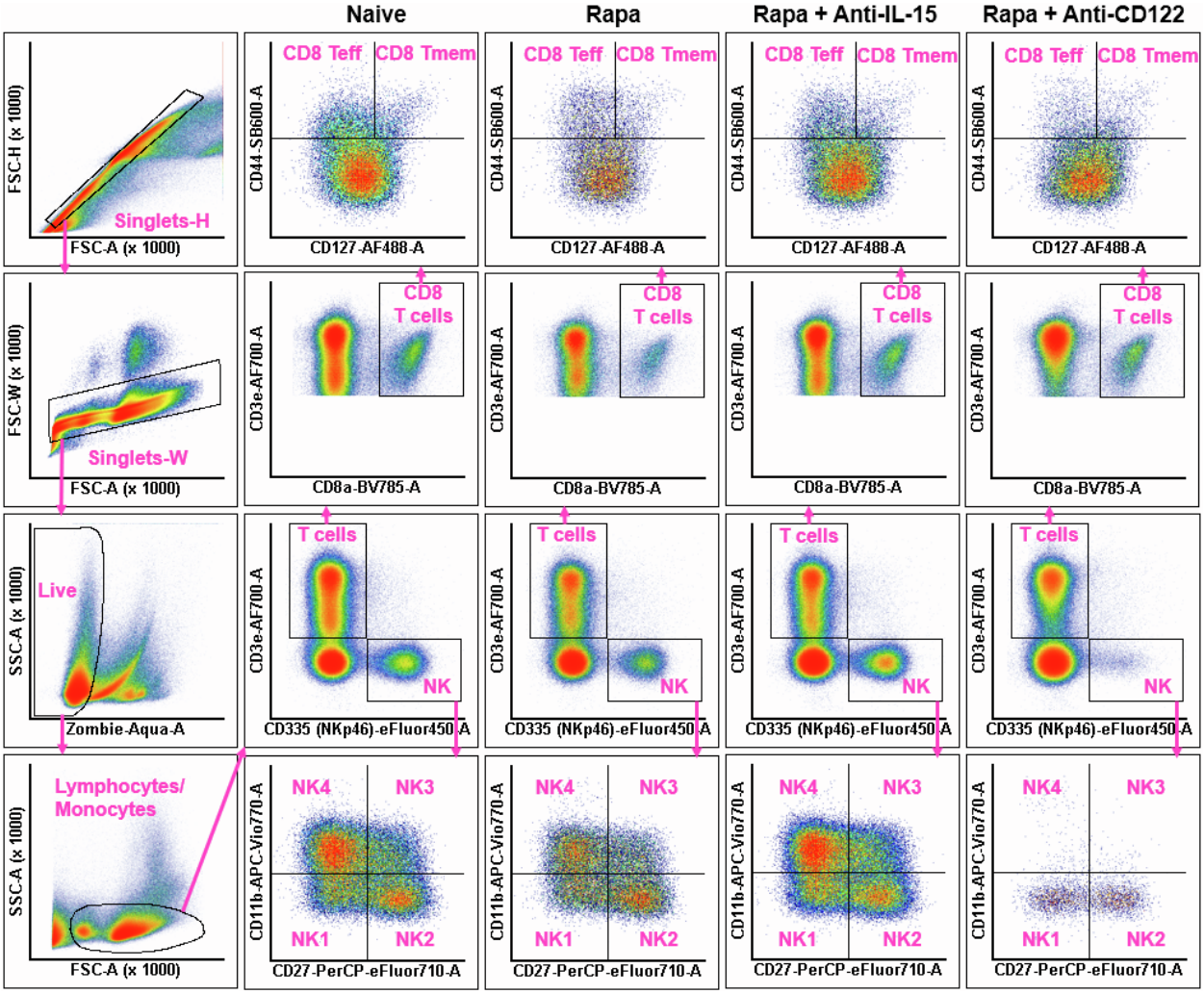

Fig. S8. Flow cytometry gating strategy for hepatic CD8⁺ T cells and NK cells (week 16). Hemophilia A mice (BALB/c F8e16$^{-/-}$) received hepatic gene transfer with AAV8-hFVIII vector at a dose of 2x10^{11} vg/mouse. Animals were additionally treated twice per week with rapamycin ("Rapa") or rapamycin combined with anti-IL-15 ("Rapa + Anti-IL-15") or with anti-CD122 ("Rapa + Anti-CD122") for the first 8 weeks after gene transfer or received no immune modulation ("AAV only"). Livers were collected 16 weeks after gene transfer. Isolation using microbeads positively separated the F4/80⁺ fraction from the fraction used for NK and CD8⁺ T cell analysis by flow cytometry. NK cells (NKp46⁺CD3ε⁻) were further classified based on increasing maturity: NK1 (CD11b^{low}CD27^{low}) < NK2 (CD11b^{low}CD27^{high}) < NK3 (CD11b^{high}CD27^{high}) < NK4 (CD11b^{high}CD27^{low}). CD8⁺ T cells (CD3ε⁺CD8α⁺) were further classified by subtype: CD8⁺ effector T cells (CD8 T_{eff}; CD44⁺CD127⁻) or CD8⁺ memory T cells (CD8 T_{mem}; CD44⁺CD127⁺).

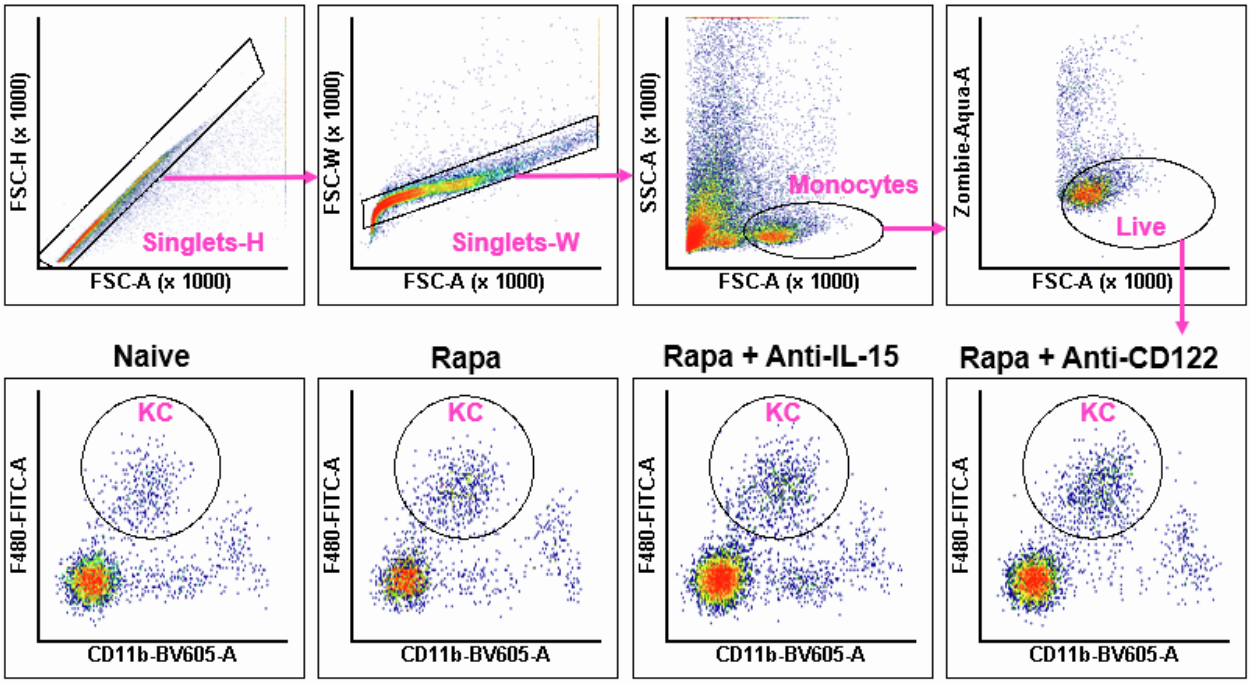

Fig. S9. Flow cytometry gating strategy for Kupffer cells (week 16). Hemophilia A mice (BALB/c F8e16$^{-/-}$) received hepatic gene transfer with AAV8-hFVIII vector at a dose of 2x10^{11} vg/mouse. Animals were additionally treated twice per week with rapamycin ("Rapa") or rapamycin combined with anti-IL-15 ("Rapa + Anti-IL-15") or with anti-CD122 ("Rapa + Anti-CD122") for the first 8 weeks after gene transfer or received no immune modulation ("AAV only"). Livers were collected 16 weeks after gene transfer. Isolation using microbeads positively separated the F4/80^{+} fraction for flow cytometry analysis of Kupffer cells (KC; F4/80^{+}CD11b^{mid}).

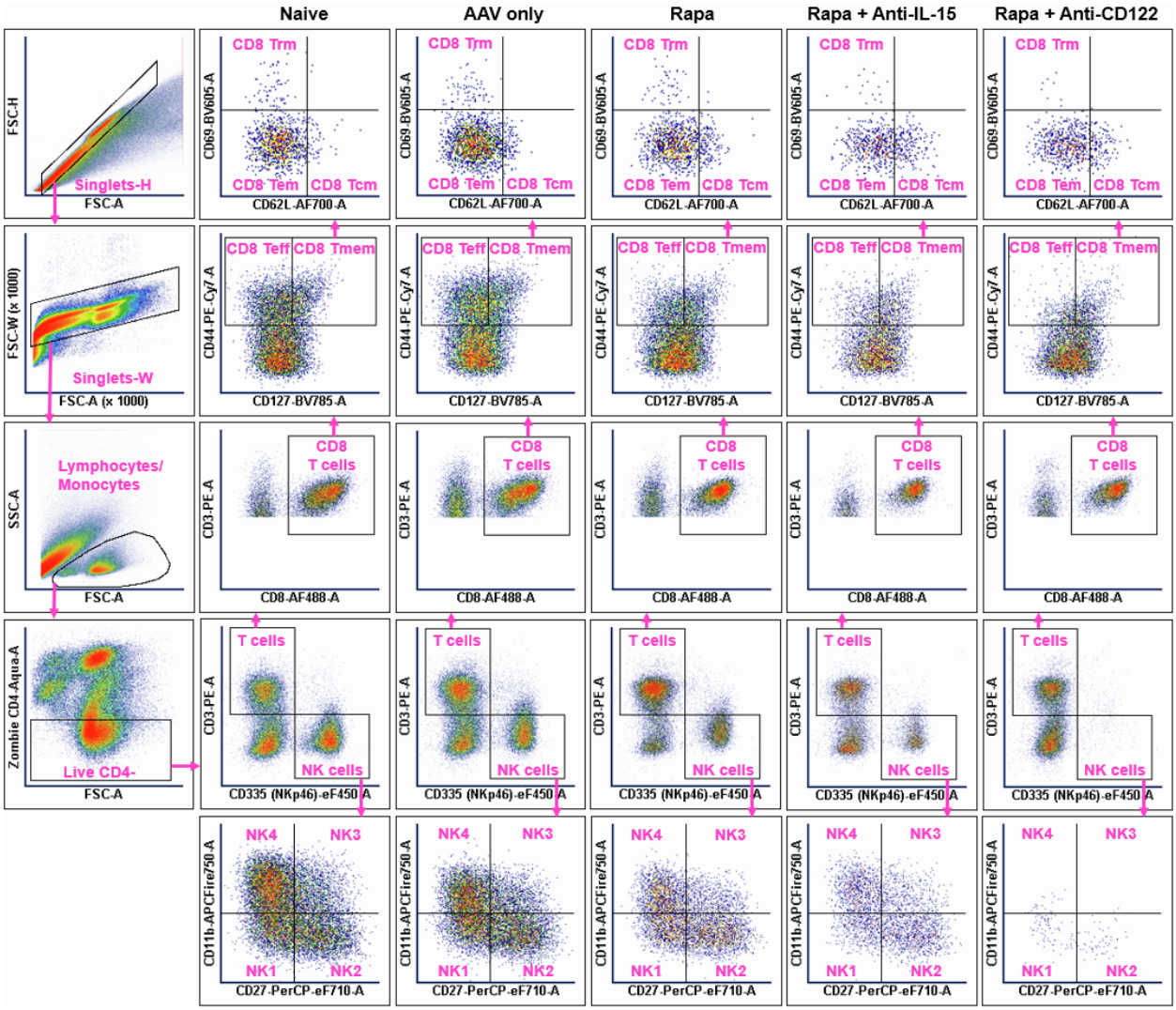

Fig. S10. Flow cytometry gating strategy for hepatic CD8⁺ T cells and NK cells (week 3). Hemophilia A mice (BALB/c F8e16$^{-/-}$) were either untreated ("Naïve") or received hepatic gene transfer with AAV8-hFVIII vector at a dose of 2x10^{11} vg/mouse. Animals receiving vector were either additionally treated twice per week with rapamycin ("Rapa"), or rapamycin combined with anti-IL-15 ("Rapa + Anti-IL-15") or anti-CD122 ("Rapa + Anti-CD122"), or received no immune modulation ("AAV only"). Livers were collected 3 weeks after gene transfer and cells were isolated for NK and CD8⁺ T cell analysis by flow cytometry. NK cells (NKp46⁺CD3ε⁻) were further classified based on increasing maturity: NK1 (CD11b^{low}CD27^{low}) < NK2 (CD11b^{low}CD27^{high}) < NK3 (CD11b^{high}CD27^{high}) < NK4 (CD11b^{high}CD27^{low}). CD8⁺ T cells (CD3ε⁺CD8α⁺) were further classified by subtype: CD8⁺ effector T cells (CD8 T_{eff}; CD44⁺CD127⁻), CD8⁺ effector memory T cells (CD8 T_{em}; CD44⁺CD127⁺CD62L⁻CD69⁻), CD8⁺ resident memory T cells (CD8 T_{rm}; CD44⁺CD127⁺CD62L⁻CD69⁺), or CD8⁺ central memory T cells (CD8 T_{cm}; CD44⁺CD127⁺CD62L⁺CD69⁻).

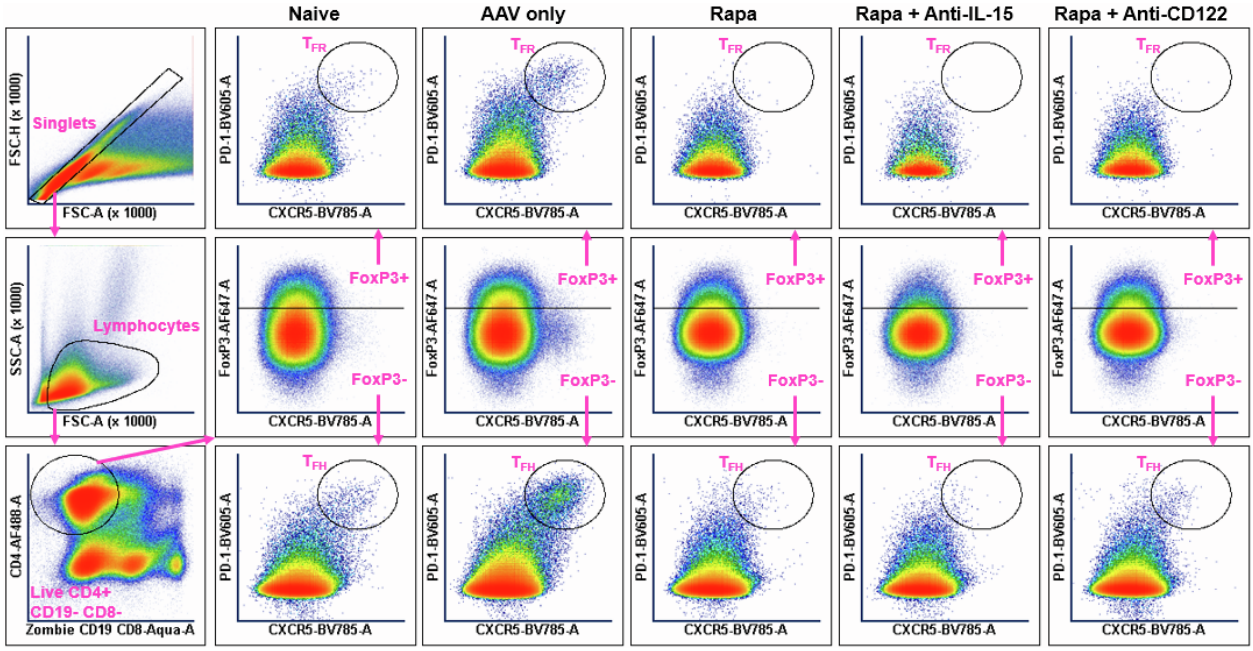

Fig. S11. Flow cytometry gating strategy for splenic CD4$^+$ T cells (week 3). Hemophilia A mice (BALB/c F8e16$^{-/-}$) were either untreated ("Naïve") or received hepatic gene transfer with AAV8-hFVIII vector at a dose of 2x10^{11} vg/mouse. Animals receiving vector were either additionally treated twice per week with rapamycin ("Rapa"), or rapamycin combined with anti-IL-15 ("Rapa + Anti-IL-15") or anti-CD122 ("Rapa + Anti-CD122"), or received no immune modulation ("AAV only"). Spleens were collected 3 weeks after gene transfer and analysis by flow cytometry. Isolation using microbeads positively separated the CD19$^+$ fraction from the fraction used for CD4$^+$ T cell analysis by flow cytometry. CD4$^+$ T cells (CD4$^+$CD19$^-$CD8$^-$) were further classified by subtype: follicular regulatory T cells (T_{fr}; PD1^{high}CXCR5$^+$FoxP3$^+$) or follicular helper T cells (T_{fh}; PD1^{high}CXCR5$^+$FoxP3$^-$).

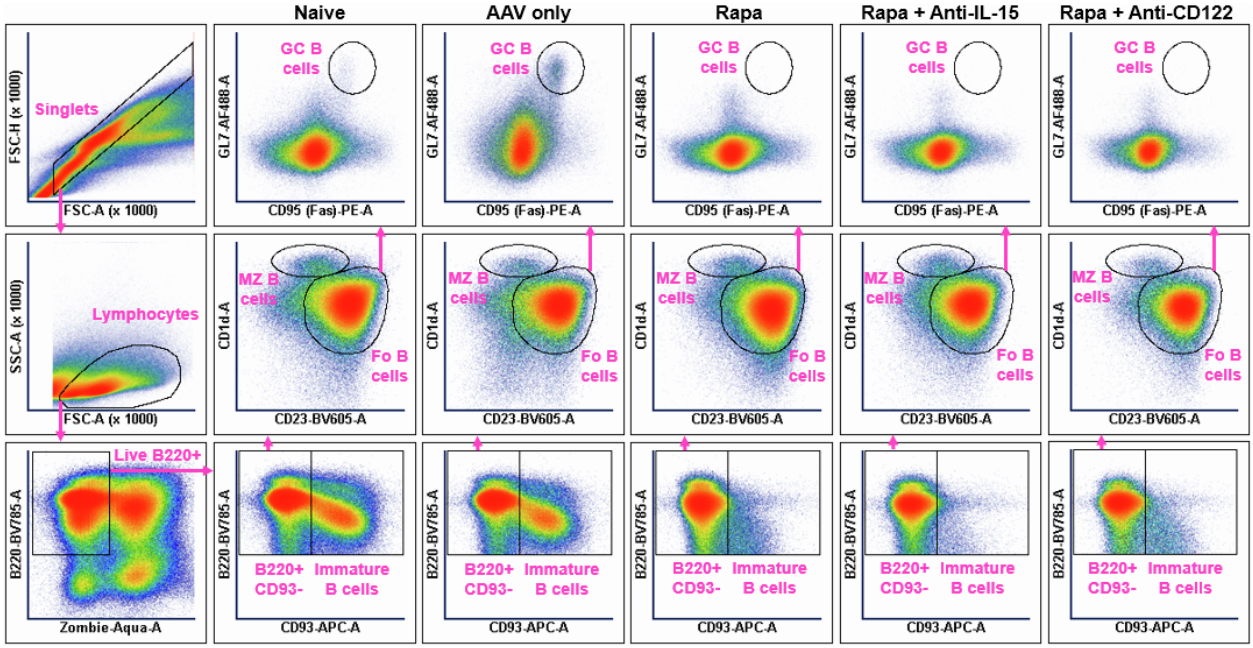

Fig. S12. Flow cytometry gating strategy for splenic B cells (week 3). Hemophilia A mice (BALB/c F8e16$^{-/-}$) were either untreated ("Naïve") or received hepatic gene transfer with AAV8-hFVIII vector at a dose of 2x10^{11} vg/mouse. Animals receiving vector were either additionally treated twice per week with rapamycin ("Rapa"), or rapamycin combined with anti-IL-15 ("Rapa + Anti-IL-15") or anti-CD122 ("Rapa + Anti-CD122"), or received no immune modulation ("AAV only"). Spleens were collected 3 weeks after gene transfer and analysis by flow cytometry. Isolation using microbeads positively separated the CD19^{+} fraction for B cell analysis by flow cytometry. Mature B cells (B220^{+}CD93^{-}) were further classified by subtype: marginal zone B cells (MZ B cells; CD1d^{high}CD23^{low}), follicular B cells (Fo B cells; CD1d^{low}CD23^{high}), or germinal center B cells (GC B cells; CD1d^{low}CD23^{high}CD95^{+}GL7^{high}).

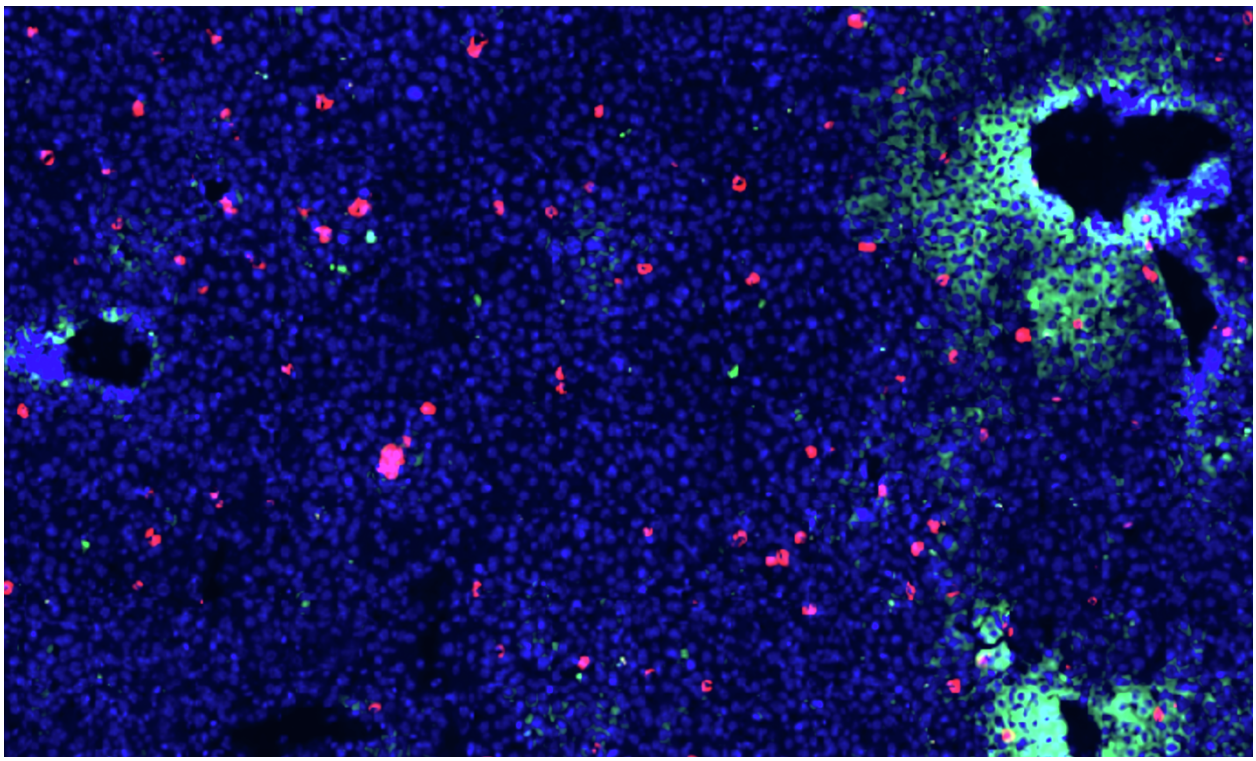

Fig. S13. Example of CD8^{+} T cell infiltration in the liver of a hemophilia A mouse 16 weeks after AAV8-hFVIII gene transfer. This BALB/c F8e16$^{-/-}$ mouse is identical to the animal shown in Fig. 5D of the main manuscript. It had received hepatic gene transfer with AAV8-hFVIII vector at a dose of 2x10^{11} vg. Shown are immunofluorescent stains for hFVIII (green) and CD8 (red). Blue stain is DAPI. Original magnification: 200x.

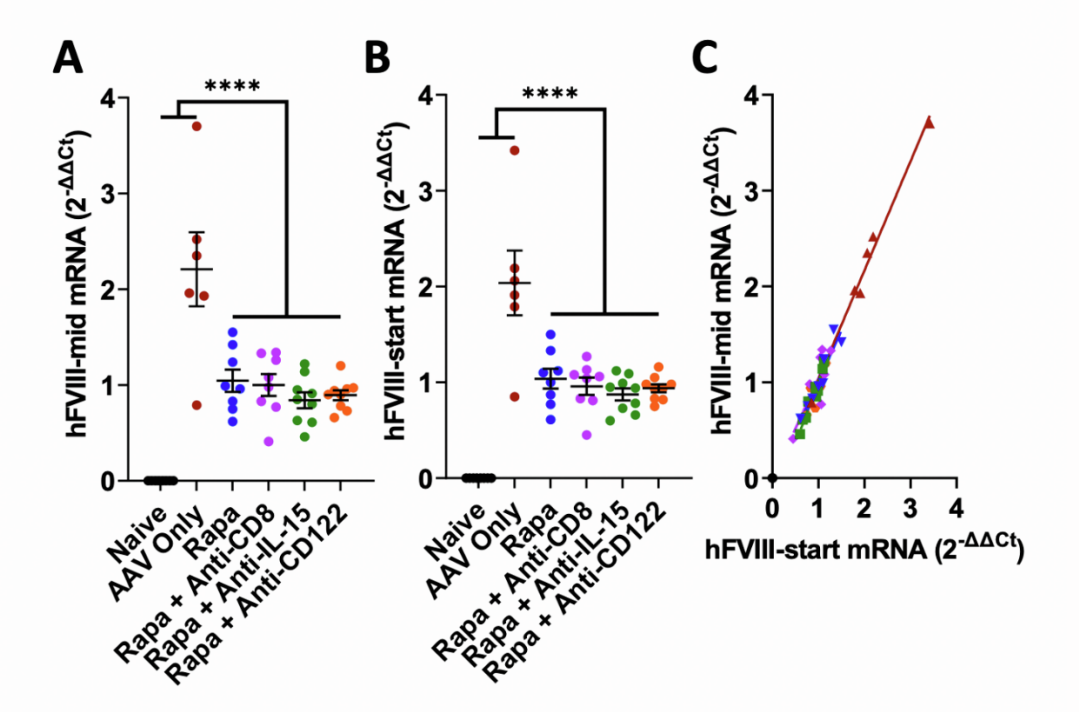

Fig. S14. F8 transgene mRNA levels quantitated using two different primer pairs. A. Liver F8 transgene (targeting transgene middle) mRNA levels (normalized to endogenous β-actin mRNA levels using standard $2^{-\Delta\Delta Ct}$ methods) relative to average of "Rapa" treated mice (averages ±SEM; week 16). **B.** Liver F8 transgene (targeting transgene start) mRNA levels (normalized to endogenous β-actin mRNA levels using standard $2^{-\Delta\Delta Ct}$ methods) relative to average of "Rapa" treated mice (averages ±SEM; week 16). **C.** Simple linear regression line drawn to show correlation between measurement of mRNA levels by targeting transgene middle ("hFVIII-mid and start ("hFVIII-start mRNA"). Significance was determined with multiple unpaired t tests, corrected for multiple comparisons using Holm-Šídák post hoc test. **** $P<0.05$.

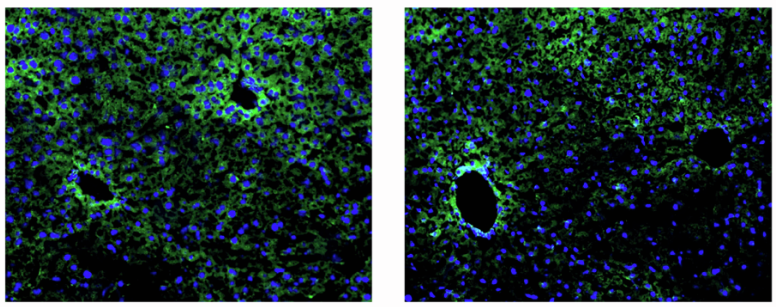

Fig. S15. hFVIII expression (green) in liver of a HA-BALB/c mouse 14 weeks after AAV8-hFVIII gene transfer. This animal had initial hFVIII activity >200% of normal (week 4) that declined to ~6% by week 14 despite continuous immune suppression with rapamycin. Blue stain: DAPI. Original magnification: 200x.

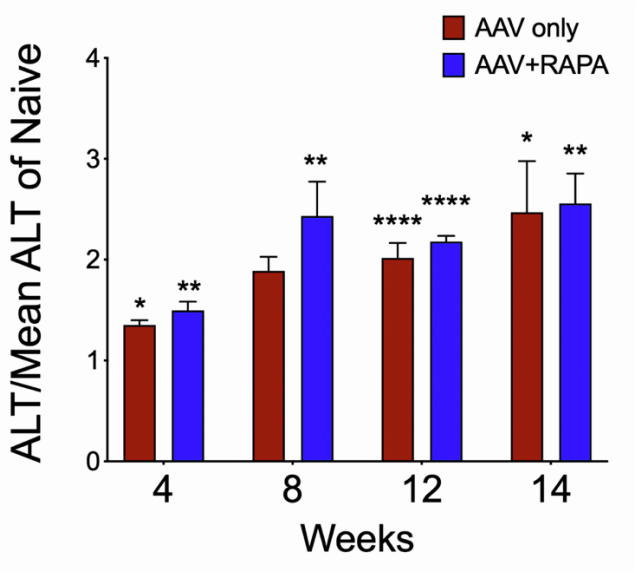

Fig. S16. ALT (alanine aminotransferase) levels normalized to those in naïve HA-BALB/c mice as a function of time after AAV8-hFVIII gene transfer without ("AAV only") or with rapamycin administration (twice per week for the duration of the experiment; "rapa"). Statistical significance is indicated for comparison to naïve animals (no gene transfer or immune suppression).

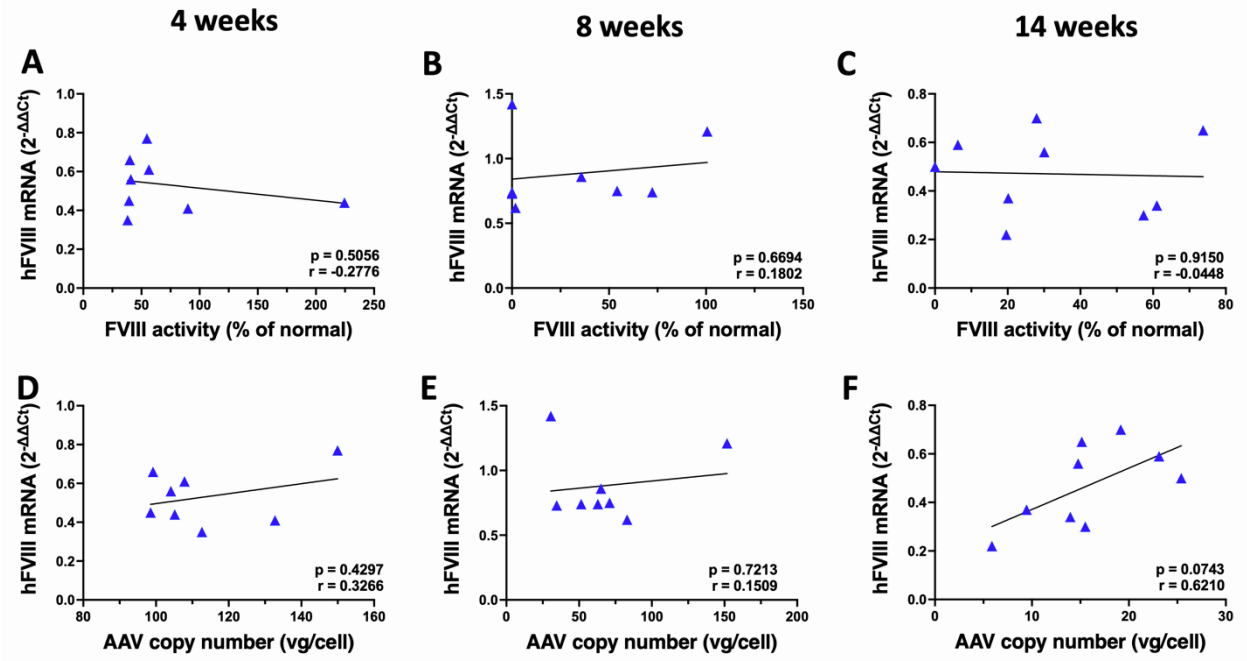

Fig. S17. Hepatic F8 mRNA vs FVIII activity in plasma (**A-C**) or vector copy number (**D-F**) at different time points (4, 8, and 14 weeks) after AAV8-hFVIII gene transfer to HA-BALB/c mice. Mice received rapamycin twice per week for the duration of the experiment. No correlation between hepatic mRNA and systemic FVIII expression was observed. Vector copy numbers and mRNA levels did not correlate for weeks 4 and 8 but trended toward a correlation by week 14.

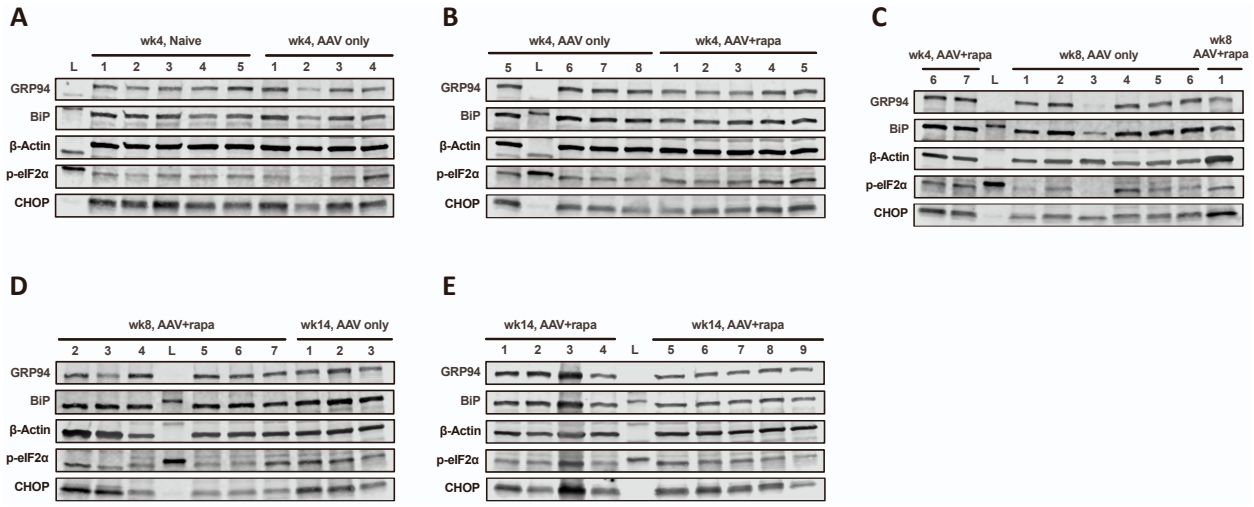

Fig. S18. Western blots for cellular stress markers GRP94, BiP, and CHOP, as well as β-actin, phosphorylated and total eIF2α. Protein extracts were obtained from livers of HA-BALB/c mice 16 weeks after AAV8-hFVIII gene transfer.

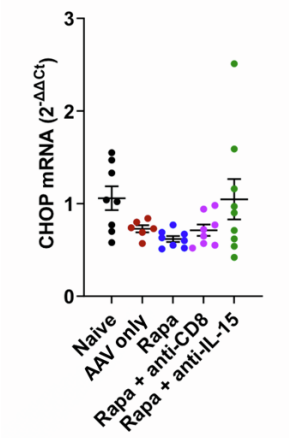

Fig. S19. Hepatic CHOP mRNA expression 16 weeks after AAV8-hFVIII gene transfer to HA-BALB/c mice. Treatment groups are indicated. Each symbol represents an individual animal.

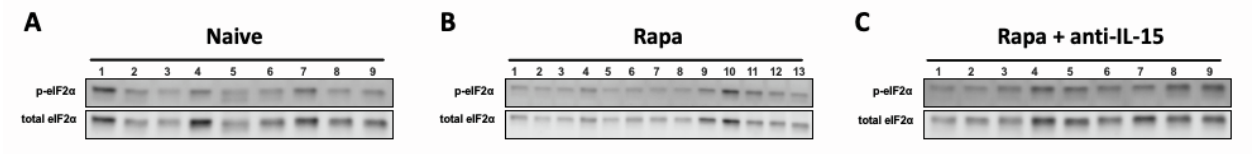

Fig. S20. Western blots for phosphorylated and total eIF2α. Protein extracts were obtained from livers of HA-BALB/c mice 16 weeks after AAV8-hFVIII gene transfer. Western blots were probed with antibodies that are specific for the phosphorylated form of murine eIF2α or detect total murine eIF2α.

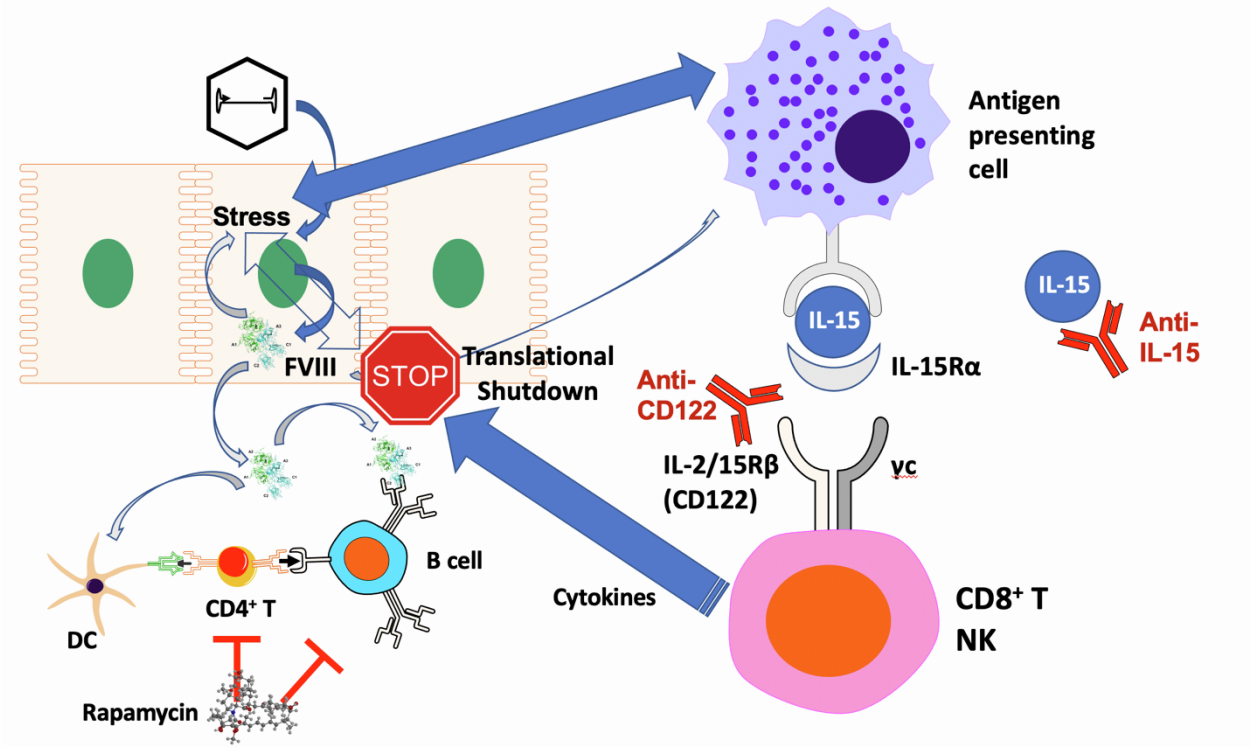

Fig. S21. Model for interaction between FVIII expression and the immune system, leading to shutdown of transgene expression in AAV-transduced hepatocytes, and targeting of IL-15 signaling to preserve FVIII protein production.

Supplementary Methods:

Supplementary Table S2: Immunophenotyping:

Cell Type	Abbreviation	Phenotype
Double negative NK cells	NK1	$NKp46^{+}CD3^{-}CD11b^{lo}CD27^{lo}$
$CD11b^{lo}$ NK cells	NK2	$NKp46^{+}CD3^{-}CD11b^{lo}CD27^{hi}$
Double positive NK cells	NK3	$NKp46^{+}CD3^{-}CD11b^{hi}CD27^{hi}$
$CD27^{lo}$ NK cells	NK4	$NKp46^{+}CD3^{-}CD11b^{hi}CD27^{lo}$
$CD8^{+}$ effector T cells	T_{eff}	$CD3^{+}CD8^{+}CD127^{-}CD44^{+}$
$CD8^{+}$ memory T cells	T_{mem}	$CD3^{+}CD8^{+}CD127^{+}CD44^{+}$
$CD8^{+}$ effector memory T cells	T_{em}	$CD3^{+}CD8^{+}CD127^{+}CD44^{+}CD62L^{-}CD69^{-}$
$CD8^{+}$ resident memory T cells	T_{rm}	$CD3^{+}CD8^{+}CD127^{+}CD44^{+}CD62L^{-}CD69^{+}$
$CD8^{+}$ central memory T cells	T_{cm}	$CD3^{+}CD8^{+}CD44^{+}CD127^{+}CD62L^{+}CD69^{-}$
T follicular helper cells	T_{fh}	$CD4^{+}PD1^{hi}CXCR5^{+}FoxP3^{-}$
T follicular regulatory cells	T_{fr}	$CD4^{+}PD1^{hi}CXCR5^{+}FoxP3^{+}$
Immature B cells	ImmB	$B220^{+}CD93^{+}$
Marginal zone B cells	MZB	$B220^{+}CD93^{-}CD1d^{hi}CD23^{lo}$
Follicular B cells	FoB	$B220^{+}CD93^{-}CD1d^{lo}CD23^{hi}$
Germinal center B cells	GCB	$B220^{+}CD93^{-}CD1d^{lo}CD23^{hi}CD95^{+}GL7^{+}$
Kupffer cells	KC	$F4/80^{+}CD11b^{mid}$

Note that maturation of mouse NK cells follows a 4-stage developmental program (*Blood* **113 (22):** 5488-5496, 2009). Further, CD27 distinguished between two subsets of mature NK cells with distinct responsiveness and migratory capacity (*J Immunol* **176 (3):** 1517-1524, 2006).

Supplementary Table S3: Antibodies used for flow cytometry:

Hepatic NK cell/$CD8^{+}$ T cell panel

Antigen	Clone	Fluorochrome	Company
Nkp46	29A1.4	eFluor450	Thermo Fisher Scientific
Zombie Aqua Fixable Viability Kit			BioLegend
CD44	IM7	SB600	Thermo Fisher Scientific
CD8α	53-6.7	BV785	BioLegend
CD127	SB/199	AF488	BioLegend
CD27	LG.7F9	PerCP-eFluor710	Thermo Fisher Scientific
NKG2D	CX5	PE	BioLegend
CD62L	MEL-14	PE-Cy7	BioLegend
CD3	17A2	AF700	BioLegend
CD11b	REA592	APC-Vio770	Miltenyi Biotec

Kupffer cell panel

Antigen	Clone	Fluorochrome	Company
CD11b	M1/70	BV605	BioLegend
Zombie Aqua Fixable Viability Kit			BioLegend
F4/80	BM8	FITC	BioLegend
CD206	C068C2	PE	BioLegend
iNOS	CXNFT	AF700	Thermo Fisher Scientific

Splenic $CD4^+$ T cells panel

Antigen	Clone	Fluorochrome	Company
Zombie Aqua Fixable Viability Kit			BioLegend
CD19	6D5	BV510	BioLegend
CD8	53-6.7	BV510	BioLegend
PD-1	29F.1A12	BV605	BioLegend
CXCR5	L138D7	BV785	BioLegend
FoxP3	MF-14	AF647	BioLegend

Splenic B cells panel

Antigen	Clone	Fluorochrome	Company
CD1d	1B1	BV421	BioLegend
Zombie Aqua Fixable Viability Kit			BioLegend
CD23	B3B4	BV605	BD Biosciences
B220	RA3-6B2	BV785	BioLegend
GL7	GL7	AF488	BioLegend
CD95	SA367H8	PE	BioLegend
CD93	AA4.1	APC	BioLegend

FISH probes:

Probe C1 (AAV-co-hF8-transgene (1058881-C1) detecting *hF8* mRNA):
accactgacctgggacagtgaatgatccccctgatctgcggcctcgacggtatCGATGCCACCATGCAGATCGAGCTGTCTA
CCTGCTTCTTCCTGTGCCTGCTGCGGTTCTGCTTCAGCGCCACCCGGCGGTACTACCTGGGCGCCGTGGA
ACTGAGCTGGGACTACATGCAGAGCGACCTGGGGGAGCTGCCCGTGGACGCCAGATTCCCCCCAAGAG
TGCCCAAGAGCTTCCCCTTCAACACCTCCGTGGTGTACAAGAAAACCCTGTTCGTCGAGTTCACCGACCA
CCTGTTCAATATCGCCAAGCCCAGACCCCCCTGGATGGGCCTGCTGGGCCCTACAATCCAGGCCGAGGT
GTACGACACCGTGGTCATCACCCTGAAGAACATGGCCAGCCACCCCGTGTCCCTGCACGCCGTGGGCGT
GTCCTACTGGAAGGCCTCTGAGGGCGCCGAGTACGACGACCAGACCAGCCAGCGCGAGAAAGAGGAC
GACAAAGTCTTTCCTGGCGGCAGCCATACCTACGTGTGGCAGGTCCTGAAAGAAAACGGCCCTATGGCC
TCCGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCACGTGGACCTGGTCAAGGACCTGAACAGCGGC
CTGATCGGCGCCCTGCTCGTGTGTAGAGAGGGCAGCCTCGCCAAAGAGAAAACCCAGACCCTGCACAA
GTTCATCCTGCTGTTCGCCGTGTTCGACGAGGGCAAGAGCTGGCACAGCGAGACAAAGAACAGCCTGA

TGCAGGACCGGGACGCCGCCTCTGCCAGAGCCTGGCCTAAGATGCACACCGTGAACGGCTACGTGAAC
AGAAGCCTGCCCGGACTGATCGGCTGCCACCGGAAGTCCGTGTACTGGCACGTGATCGGCATGGGCAC
CACCCCCGAGGTGCACAGCATCTTTCTGGAAGGCCACACCTTCCTCGTGCGGAACCACAGACAGGCCAG
CCTGGAAAT

Probe C2 (AAV-co-hF8-transgene-O1-sense (1058891-C2) detecting vector genome):
TGCAGATGCTCGCCGATCAGACATTCCACCCGCCAGATGCCGGCCTTGCTGGGCAGCATTTCCACTGTCT
CGAACACGCCGGGGTACAGGTTGTACAGGGCCATCTTGTACTCTTCTTTCTTCCGCACTGTGAACACGTG
GCCGCTGAAGTGGATGCTGTGGATGTTCTCGTTGCTGCCCATGCTCAGCAGATACCACCGGATTCTCTGA
TCCTGGGCCATGACCAGGCCGGGCAGGGTGTCCATGATGTAGCCGTTGATGGCGTGGAACCGATAGTT
CTCTTTGAAGGTAGGATCTTCCATCTGGATGTTGCAGGGGGCTCTGCAGTTTCTTTCCATGTTCTCGGTG
AAGTACCAGCTCTTTGTCTCATCGAAGATGGTGAAGAACAGGGCAAATTCCTGCACTGTGACCTGCCGG
CCGTGGGCGGGGTTCAGGGTGTTGGTGTGGCAGACGAGCAGAGGTCCAATCAGGCCAGAGTGCACGT
CCTTTTCCAGGTCCACATCGGAGAAGTAGGCCCAGGCCTTGCAGTCGAACTCGTCCTTTGTGGGGGCCA
TGTGGTGCTGCACCTTCCAGAAGTAGGTCTTAGTCTCGTTGGGCTTCACGAAGTTCTTCCGGGGTTCGGC
GCCCTGCCGCTGGTCCTCTTCGTAGCTGATCAGGCTGCTGTAGAAGCTGTAGGGTCTGGAGGCCTGGTT
TCTGAAGGTGACCATGATGTTATCTTCCACCTCGGCTCTAATGTAAGGTCCCAGCAGTCCCAGGTGCTCG
TTCAGCTCGCCCCGATACAGGGGCTGGGTGAAGCTGCCGTCGGTGAACTCCTGGAACACCACTTTCTTG
AACTGGGGCACGCTGCCGCTCTGGGCTCTGTTCCGCAGCACGTGGGGGCTGCTGCTCATGCCGTAGTCC
CACAGTCTTTCCACGGCGGCAATGAAGTAGTGCCGGGTTTTCTTCTGGAAGGACCGGGGGCTCTGGTTC
TCGTCCTCGTCGTAGATGTCGAAATCCTCTTTCTTCATCTCGACGCTGATGGTGTCGTCGTAATCAATCTC
TTCCTGGTCGGACTGCAGGGTGGTCCGGGTGATCTCTCTCTGGTGCCGCTTCAGCACGGGGGGGTTCTG
GCTGAAGCTTCTGGGCTCGATGGCGTTGTTCTTGCTCAGCAGGTAGGCGCTGATGTCCTCATAGCTGTCC
TCGTAGTAGTCGCCGGTGTTCTTGTCGCAGCTGGACACCTTCAGCAGGGCGGTCATGCCCCGGTTCCGG
AAGTCGCTGTTGTGGCAGCCCAGAATCCACAGGCCGGGGTTTTCCATGCTCATGAACACGGTTTCGCCG
GAGAAGGGGAACAGGGTCAGGGTATCCTCGTACACCATCTTGTGCTTGAAGGTGTAGCCGCTGAAGAA
CACGCTCAGGAAGTCGGTCTGGGCGCCGATGCTCAGGATGTACCAGTAGGCCACCTCGTGCAGGCACA
CGCTCAGCTGCAGGCTGTCGAACACGTAGCCATTGATGGAGTGCATGATGTTGCTGGCCTGGAACTCGG
GATCTTCCAGCTGCACGCCGGCAGGGTTGGGCAGGAACCGCTGGATATTCTCGGTCAGATACCAGGACC
GATTCTCATCGAACACGCTGAACAGGATCACGTTCCGCTTGTCGCTCATGATCTGGTTGCCCCGCTGGTC
CACGCTTTCTTTGTAGCAGATCAGCAGAGGGCCGATCAGCCCGGAGGCCAGGTCCCGTTCCATGTTCAC
GAAGCTGC