

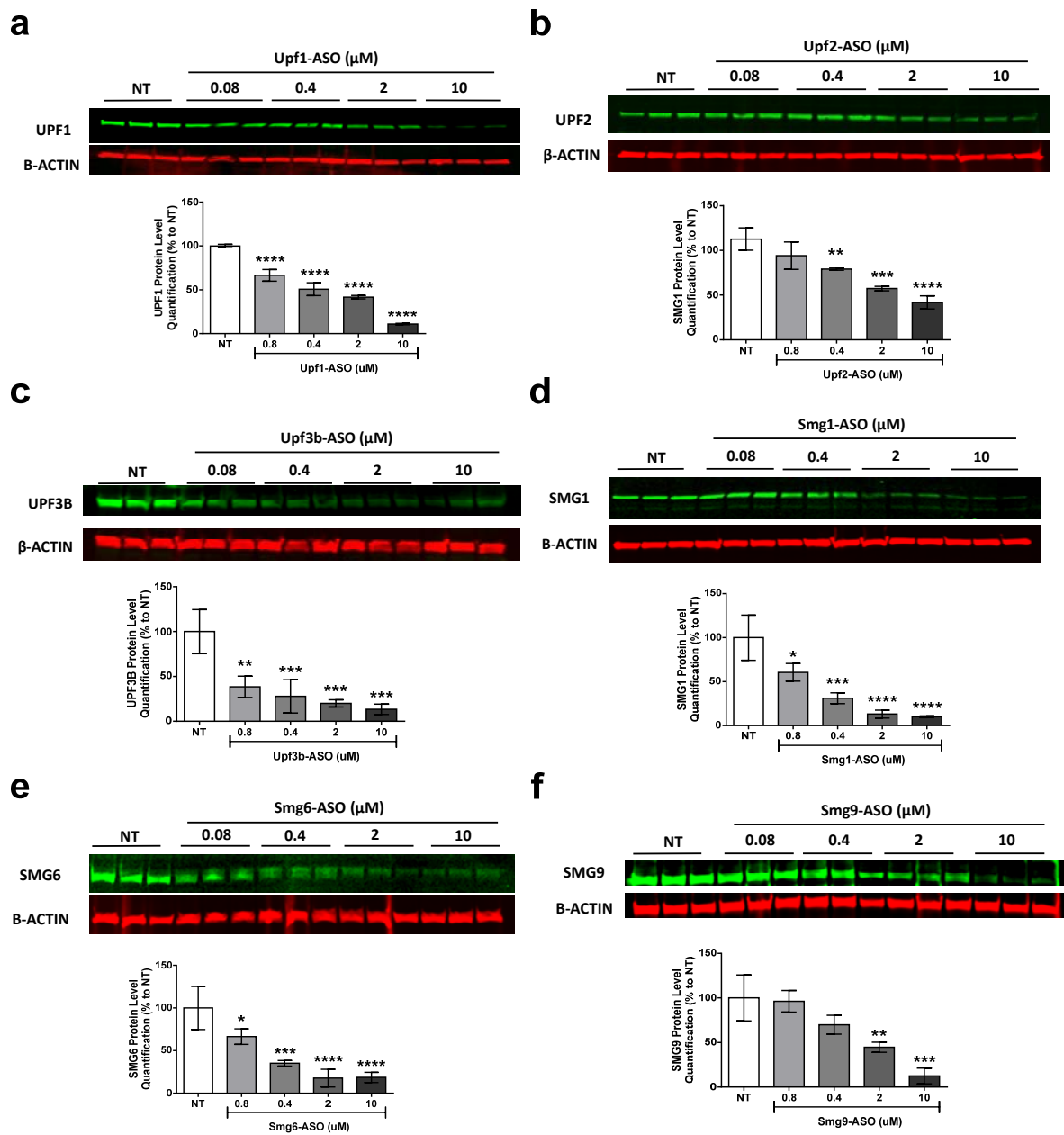


Fig. S1

Figure S1. ASO targeting NMD factors achieve protein level target reduction in a dose-dependent manner

Mouse MHT cells were treated with ASOs targeting mouse NMD factors by free uptake for 72 hrs at the indicated concentrations. NMD factor protein levels were evaluated by western blot with antibodies specific to each of the NMD factors. β -Actin was included as loading control. Western blot membranes were scanned using an Odyssey imaging system and quantified using Image Studio (LI-COR).

Quantification results are presented as means plus or minus standard errors (n=3). The protein level in NT samples was set as 1. **a** *Upf1*-ASO treated cells. **b** *Upf2*-ASO treated cells. **c** *Smg1*-ASO treated cells. **d** *Smg6*-ASO treated cells. **e** *Upf3b*-ASO treated cells. **f** *Smg9*-ASO treated cells. Statistical significance was determined using a one-way ANOVA and Dunnett's multiple comparison test in Prism. All groups were compared to NT group within each measurement. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

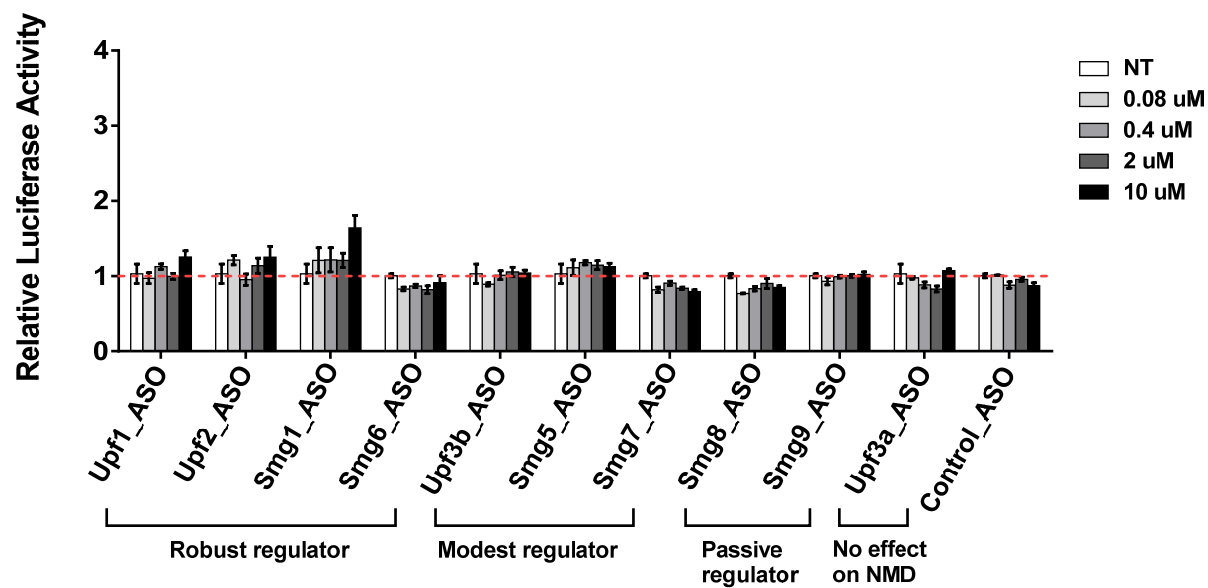
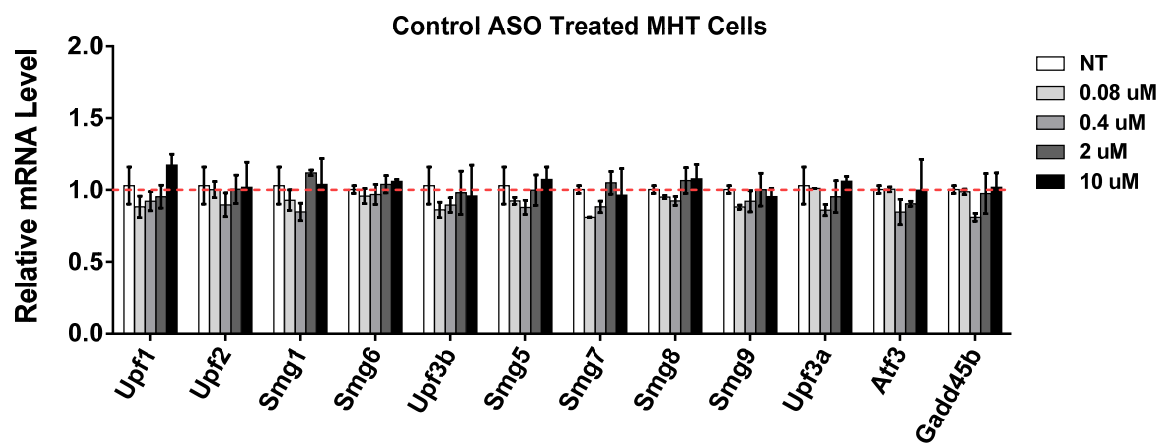


Fig. S2

Figure S2. ASOs targeting NMD factors do not affect wild-type β -*GLOBIN* luciferase reporter.

Relative luciferase activity is shown from the wild-type β -*GLOBIN* luciferase reporter cell line after ASO treatment. Cells were treated with ASOs targeting the mouse NMD factors *Upf1*, *Upf2*, *Smg1*, *Smg6*, *Upf3b*, *Smg5*, *Smg7*, *Smg8*, *Smg9*, or *Upf3a* by free uptake at the concentrations indicated for 72 hrs. Results are normalized and presented as in Figure 1b.

a



b

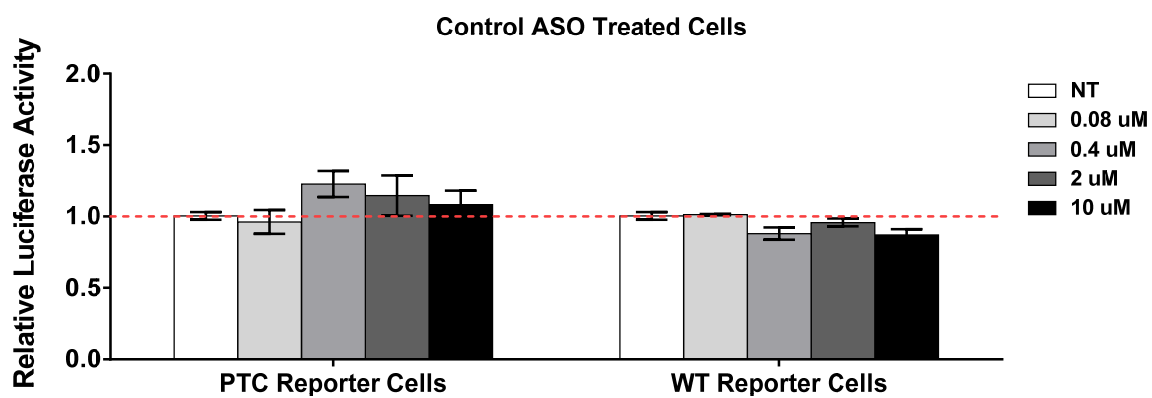


Fig. S3

Figure S3. Control ASO has no effect on the levels of the NMD factors and the NMD substrates.

Mouse MHT cells were treated with a control-ASO by free uptake for 72 hrs at the indicated concentrations. Results are normalized and presented as in Figure 1. **a** qPCR analysis of the mRNA levels of each NMD factor and/or NMD substrate. **b** Relative luciferase activity is shown from the PTC- or WT- β -*GLOBIN* luciferase reporter cell line.

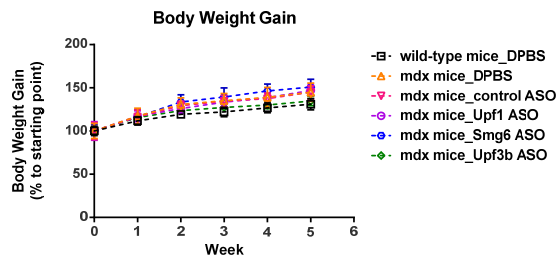
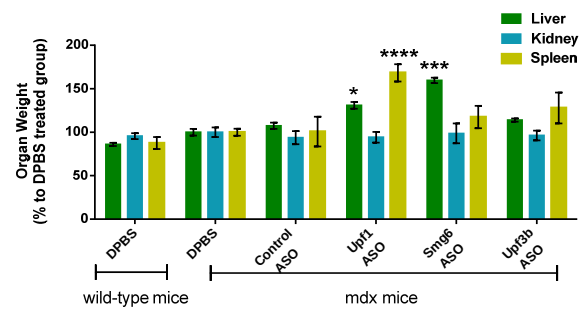
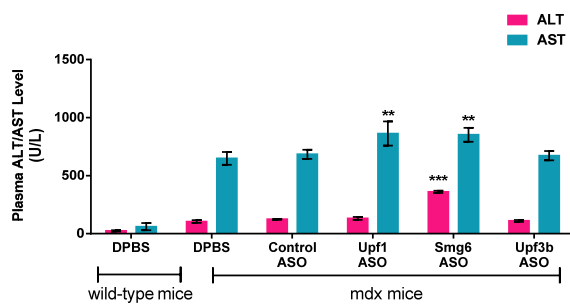
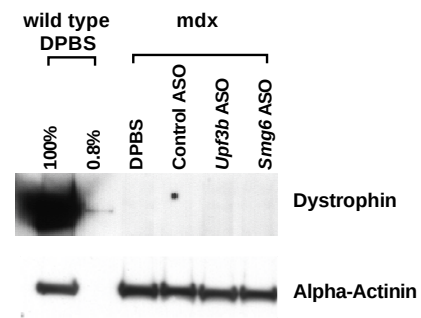
a**b****c****d**

Fig. S4

Figure S4. ASO-mediated Upf3b depletion is well tolerated in mdx mice.

Study was performed as described in Figure 4. Results are presented as means plus or minus standard errors. **a** Body weights measured once a week. **b** Liver, kidney, and spleen weights measured at necropsy. **c** Plasma ALT and AST levels measured by clinical analyzer at necropsy. **d** Dystrophin protein levels from TA tissue samples analyzed by western blot with a dystrophin-specific antibody. Alpha-actinin was included as a loading control. Statistical significance was determined using a one-way ANOVA and Dunnett's multiple comparison test in Prism. All groups were compared to DPBS treated mdx mouse group within each measurement. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

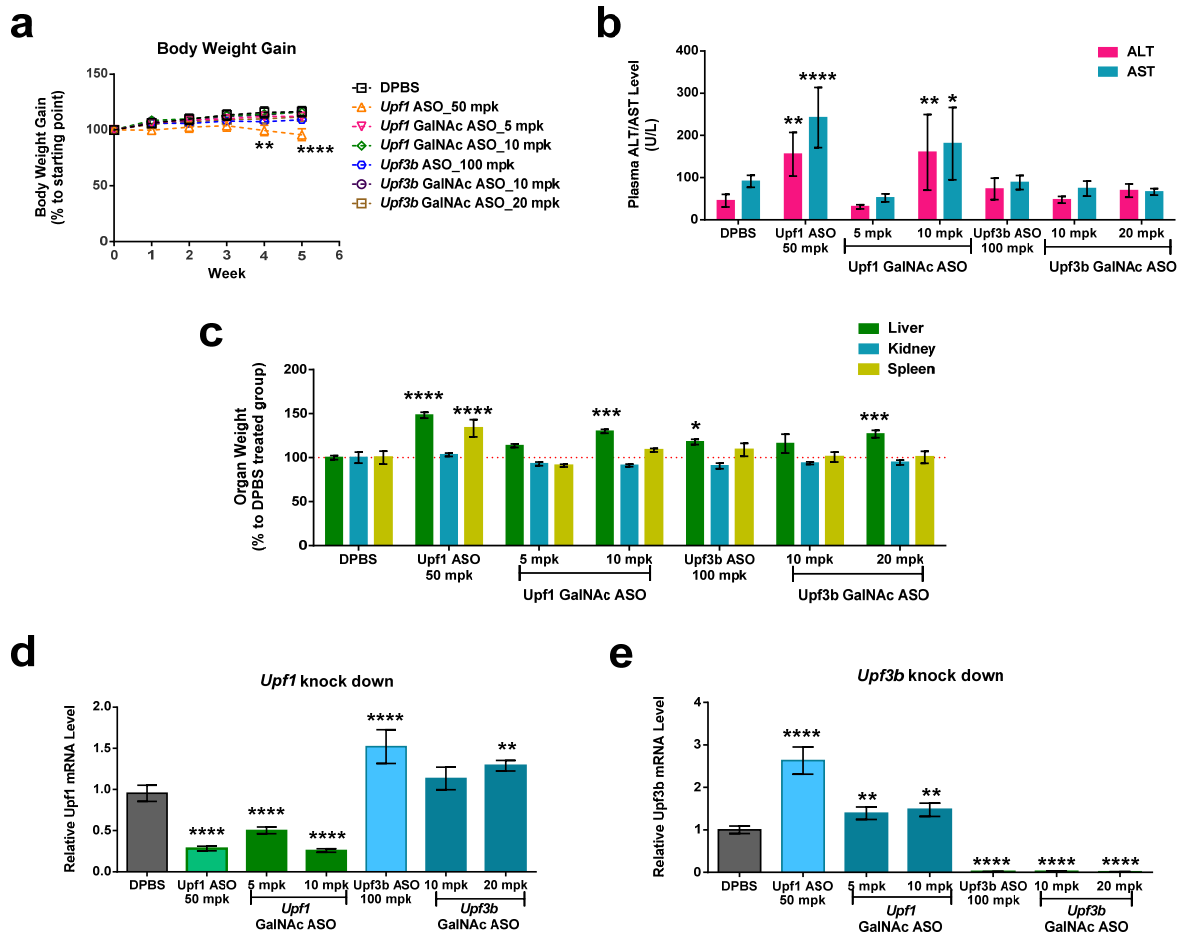


Fig. S5

Figure S5. GalNAc-ASOs significantly improves ASO efficacy and tolerability in the liver.

Mice (n=4) were treated with DPBS, *Upf1*-ASO (50 mg/kg/week), *Upf1*-GalNAc-ASO (5 or 10 mg/kg/week), *Upf3b*-ASO (100 mg/kg/week), or *Upf3b*-GalNAc-ASO (10 or 20 mg/kg/week) every 5 days with 6 total doses for 4.5 weeks. Necropsy was performed 48 hrs after the last dose of ASO. Results are presented as means plus or minus standard errors. **a** Body weights measured once a week. **b** Plasma ALT and AST levels measured by clinical analyzer at necropsy. **c** Liver, kidney, and spleen weights measured at necropsy. **d, e** mRNA was purified from mouse liver and *Upf1* (**d**) or *Upf3b* (**e**) mRNA levels were measured by qPCR. *Gapdh* was used as an endogenous control. *Upf1* or *Upf3b* mRNA level in DPBS treated animals was set as 1. Statistical significance was determined using either a two-way ANOVA (panel **a, b**, and **c**) or a one-way ANOVA (panel **d** and **e**) and Dunnett's multiple comparison test in Prism. All groups were compared to DPBS-treated mouse group. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

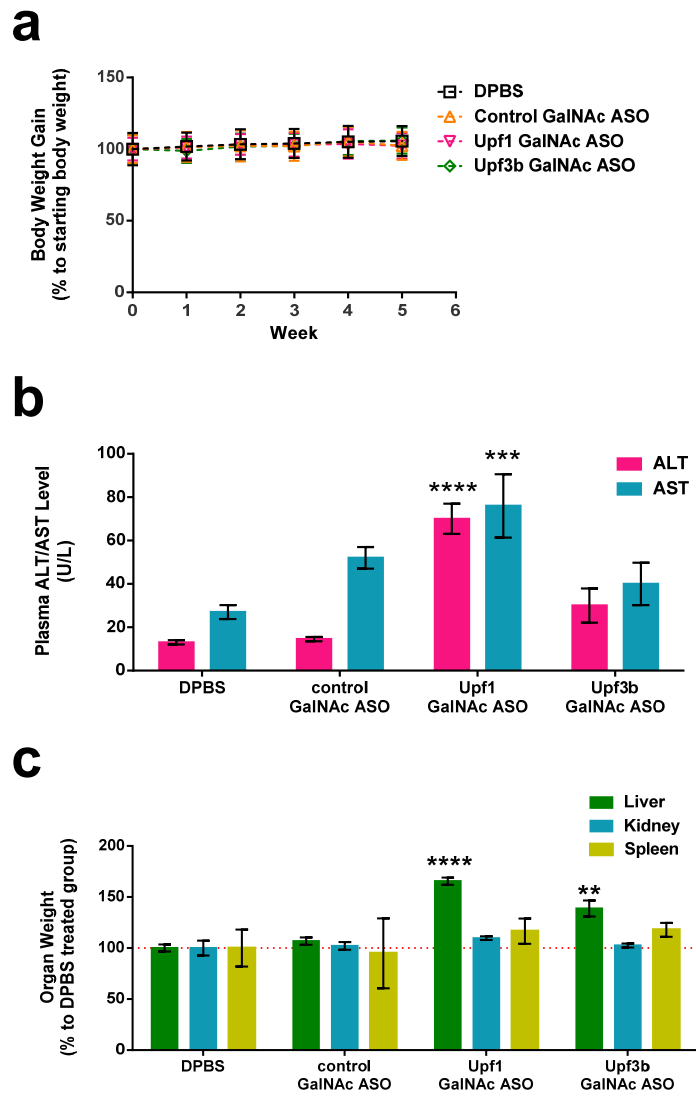


Fig. S6

Figure S6. An *Upf3b*-GalNAc-ASO, but not an *Upf1*-GalNAc-ASO, is well tolerated in hemophilia mice. Study was performed as described in Figure 5. Results are presented as means plus or minus standard errors. **a** Body weights measured once a week. **b** Plasma ALT and AST levels measured by clinical analyzer at necropsy. **c** Liver, kidney, and spleen weights measured at necropsy. Statistical significance was determined using a one-way ANOVA and Dunnett's multiple comparison test in Prism. All groups were compared to DPBS-treated mice. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

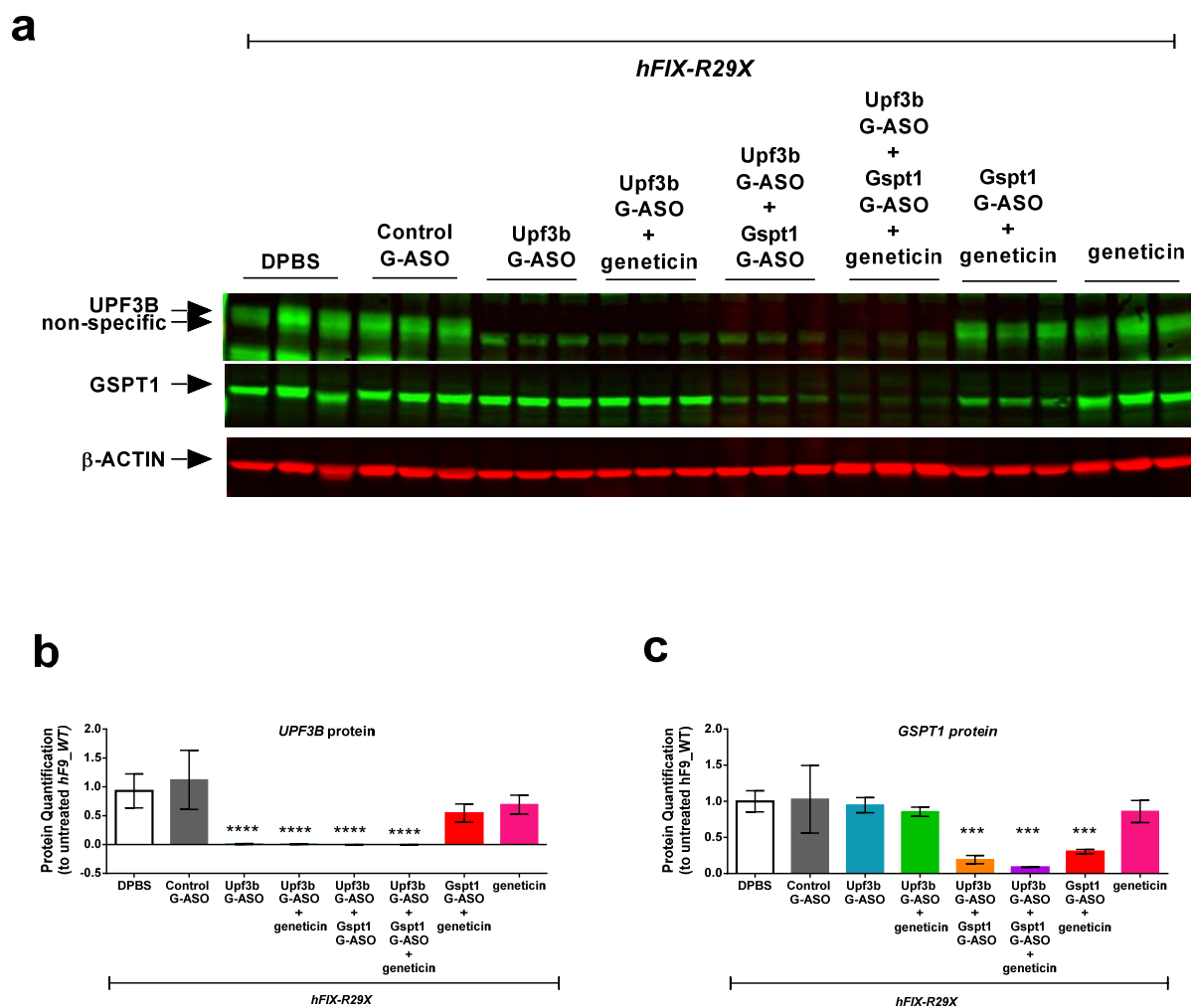


Fig. S7

Figure S7. ASO-mediated depletion of UPF3B and GSPT1 in Hemophilia mice.

Study was performed as described in Figure 7. Western blot analysis was performed on mouse liver samples. **a** Odyssey images of the western blot. **b** and **c** Image studio quantification of the western blot image in **a**. β -ACTIN protein levels were used as a loading control. Results are presented as means plus or minus standard errors. Statistical significance was determined using a one-way ANOVA and Dunnett's multiple comparison test in Prism. All groups were compared to DPBS-treated mice. *** $p < 0.001$; **** $p < 0.0001$.