**SUPPLEMENTARY MATERIAL**

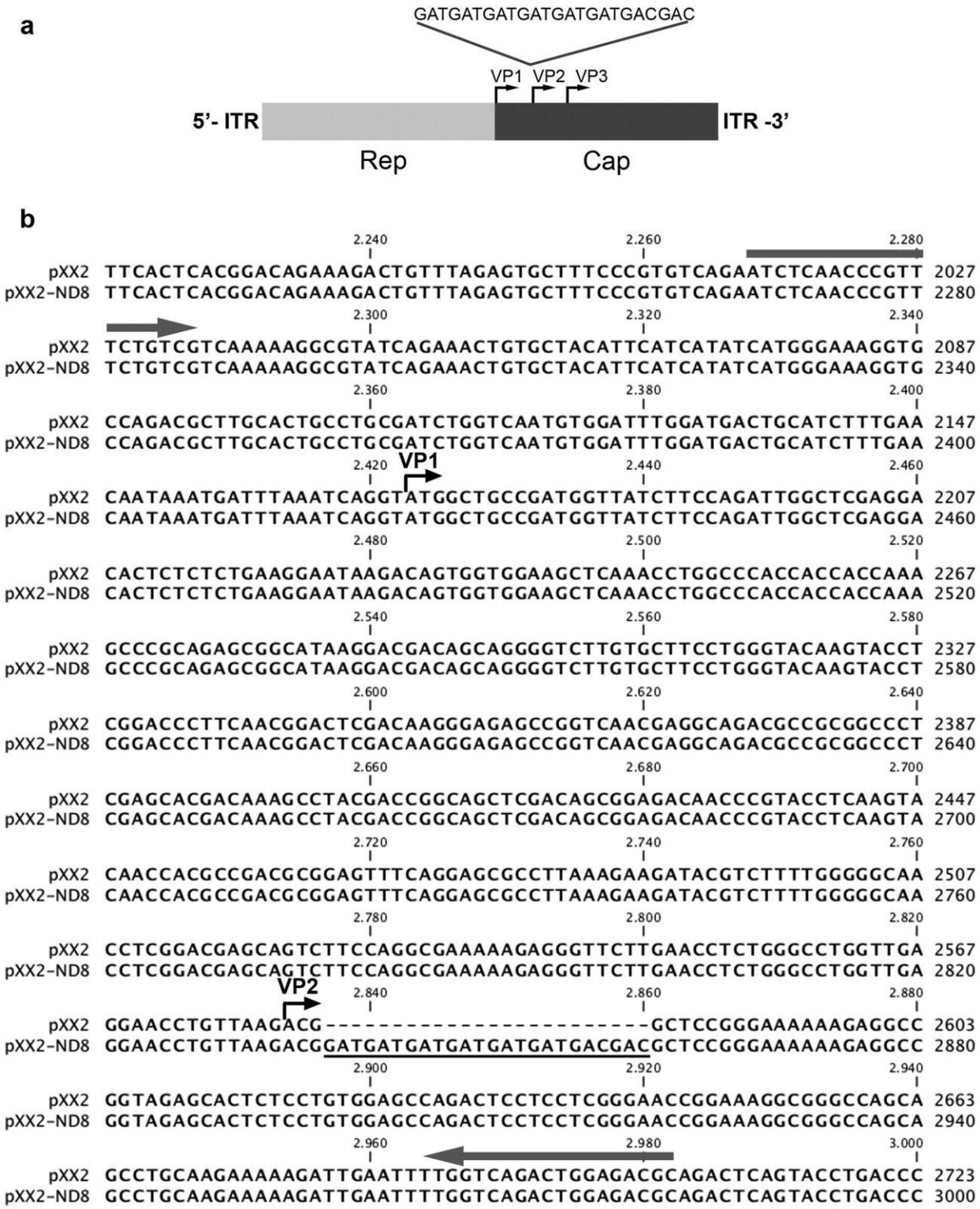
**Construction of pXX2-D8 plasmid**

To produce the AAV2 vector with the bone-targeting peptide, a sequence encoding eight aspartic amino acids (D8) was inserted immediately after the initial codon of VP2 protein (T138) in the packing plasmid pXX2 (Supplemental FigureS1). Site-directed mutagenesis was carried out with the primer 5′-gaggaacctgttaagacgGATGATGATGATGATGATGACGACgctccgggaaaaaagagg-3′ and its complementary sequence (sequence encoding the bone-targeting region capitalized). QuikChange® Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) was used according to manufacturer instructions. Resultant clones were evaluated by PCR using the primers pXX2-ND8-4F 5′-ATCTCAACCCGTTTCTGTCG-3′ and pXX2-ND8-4R 5′-GCGTCTCCAGTCTGACCAA-3′, which amplify a 691-bp or 715-bp PCR-fragment for the unmodified or modified pXX2 plasmid, respectively. The plasmid with the D8 insertion (pXX2-D8) was sequenced to ensure presence of the bone-targeting sequence without fortuitous mutations.

**AAV2 production**

AAV2 vectors were produced by calcium phosphate-mediated co-transfection of pAAV2-CBA-GALNS (or pSUB201 for wild type [WT] virus), pXX2 (or pXX2-D8) and pXX680 plasmids (Gene Therapy Center, University of North Carolina at Chapel Hill). pAAV2-CBA-GALNS plasmid carried the cytomegalovirus enhancer/ß-actin promoter (CBA) driving the expression of the human GALNS cDNA (GenBank accession number NM_000512.4). HEK293 cells (ATCC CRL-1573) were seeded on 15-cm plates with complete culture media Dulbecco's Modified medium (DMEM, Gibco, Carlsbad, CA). Media was supplemented with fetal bovine serum 15%, penicillin 100 U/mL and streptomycin 100 U/mL and transfected with 18:18:54 µg ratio (a 1:1:1 molar ratio) of pAAV, pXX2 (or pXX2-D8) and pXX680 plasmids, respectively. Forty-eight hours after transfection, cells were harvested, resuspended in lysis buffer (0.15 M NaCl, 50 mM Tris-HCl pH 8.5), and lysed by three freeze/thaw cycles. Solution was clarified by centrifugation at 1000g for 20 min at 4ºC. AAV vectors were purified by iodixanol gradient (Sigma-Aldrich, Saint Louis, MO) and Heparin-agarose type I affinity-chromatography as described previously (30).

AAV vectors were quantified by spectrophotometry based on the extinction coefficient of the AAV2 capsid proteins and genome (28). Physical titers were measured by using an AAV2 titration ELISA kit (Progen, Heidelberg, Germany), under manufacturer's instructions.



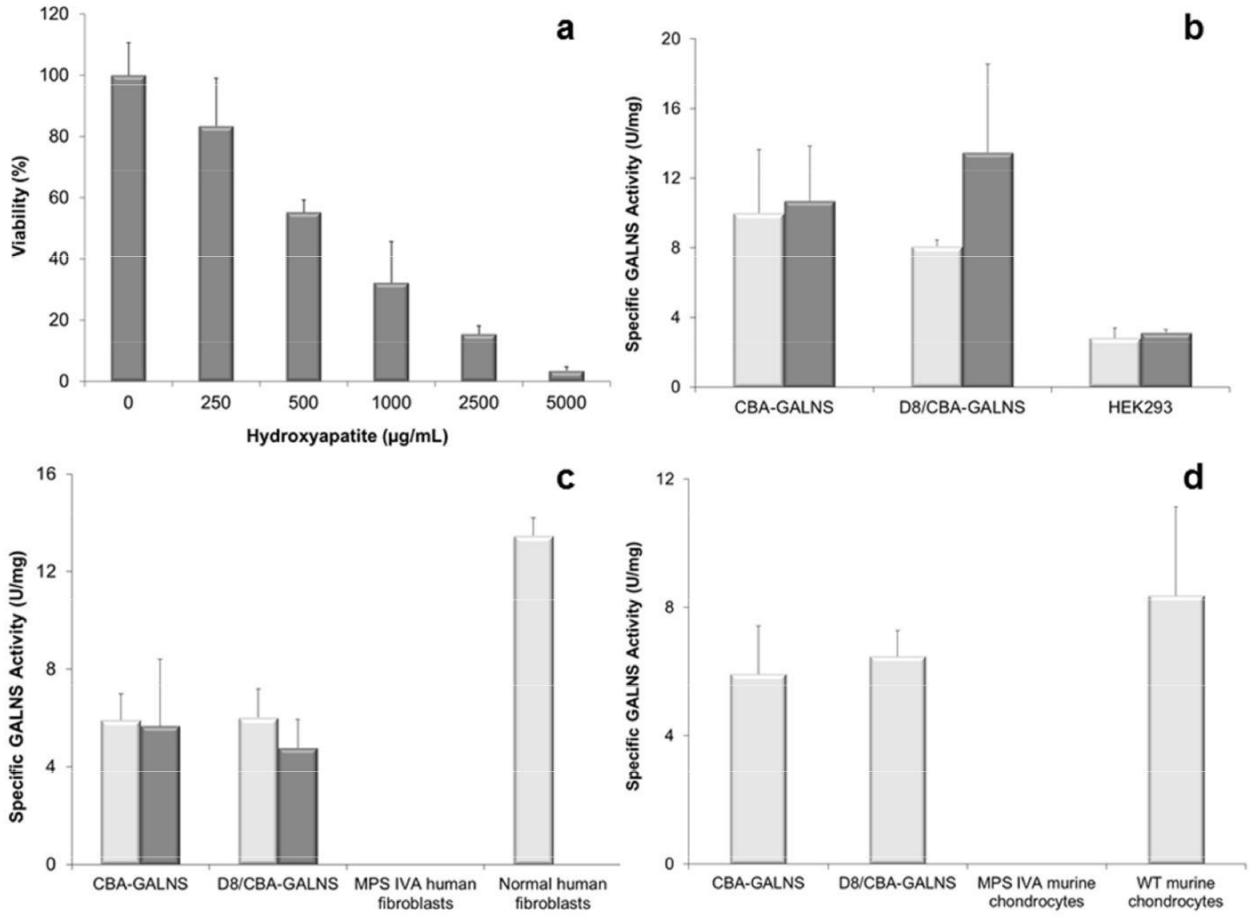

**Supplemental Figure S1. Insertion of a sequence encoding for an aspartic acid octapeptide (D8) AAV capsid.** (a) A sequence encoding for an aspartic acid octapeptide was inserted after the initial codon of the VP2 protein in pXX2 packing plasmid. (b) Sequencing of modified packing plasmid showed the precise insertion of D8 coding sequence without introducing fortuitous mutations. The bent arrows show the initial codons of VP1 and VP2 capsid proteins. Gray arrows show position of the pXX2-ND8-4F and pXX2-ND8-4R primers, which amplify a 691-bp or 715-bp PCR-fragment for the unmodified or modified pXX2 plasmid, respectively. ITR: Inverted Terminal Repeat.

**Hydroxyapatite cytotoxicity assay**

HA toxicity was evaluated in HEK293 cells before *in vitro* transduction experiments. Twenty-four hours before the experiment, 1 x $10^5$ cells/well were seeded in 24-well plates. Culture media was changed with media containing 250, 500, 1,000, 2,500, or 5,000 µg/mL of HA powder (Sigma-Aldrich). A 1% Triton-X-100 solution was used as a positive control, while complete culture media was used as negative control. After 24 hours of incubation, cells were washed with PBS and trypsinized. Live-dead cells were counted in a light microscope, using trypan-blue reagent.

***IN VITRO* transduction experiments**

1 x $10^5$ HEK293 cells, human MPS IVA fibroblasts or murine MPS IVA knock-out (*Galns*$^{-/-}$) chondrocytes were seeded in 12-well plates and transduced with 1 x $10^{10}$ vg (1 x $10^5$ vg/cell) of CBA-GALNS or D8/CBA-GALNS. Cells were harvested 4 days post-transduction and resuspended in 100 µL of 1% sodium deoxycholate. Because of the HA cytotoxicity, the effect of HA in vector transduction was evaluated. HA binding was assayed, with 1 x $10^{10}$ vg of CBA-GALNS or D8/CBA-GALNS vectors and 1,000 µg/mL HA in a final volume of 100 µL. Further, after 1 hour incubation, the HA-vector mixture was added to the cells cultured in 1 mL of media, resulting in 10 times dilution of the HA concentration. Cells were harvested 48 hours post-transduction and lysed by using 1% sodium deoxycholate. All experiments were done in triplicate.




**Supplemental Figure S2. (a) Hydroxyapatite cytotoxicity assay**. 1.0 x $10^5$ HEK293 cells were cultured with 250, 500, 1,000, 2,500 or 5,000 µg/mL HA, and cell viability was monitored 24 hours after incubation. Results are reported as a percentage of live cells after 24 hours compared with the initial cell population before incubation. **(b-d) *IN VITRO* transduction efficiency**. 1.0 x $10^5$ cells were transduced with 1.0 x $10^{10}$ vg of CBA-GALNS or D8/CBA-GALNS vectors. GALNS activity was measured in the cell

lysate after 48 hours in (b) HEK293 cells, (c) MPS IVA human fibroblasts and (d) MPS IVA murine chondrocytes. PBS was used as negative control of transduction. Normal human fibroblasts and WT murine chondrocytes were used as positive controls. HEK293 cells and human fibroblasts were cultured with (white bars) or without HA (filled bars). Error bars represent SDs from different samples (n=3).

**Vector genome biodistribution**

*Total vector genome distribution compared to liver*

Twenty-four hours after injection, vector DNA was detected mostly in bone marrow of mice treated with CBA-GALNS and D8/CBA-GALNS vectors (p=0.001). Vector genome copies per diploid cell in bone marrow were on average 4.8±3.8-fold and 11.0±6.3-fold higher than those in liver of CBA-GALNS and D8/CBA-GALNS treated mice, respectively (Supplemental Figure S3a). In bone and brain, the copy number of vector genomes was comparable to that found in the liver for both CBA-GALNS and D8/CBA-GALNS treated mice.

At 48 hours, vector genome copies per diploid cell of D8/CBA-GALNS were 10 ± 9.7-fold higher in bone than those in liver (Supplemental Figure S3a). In brain and bone marrow the copy number of vector genomes 48 hours after injection was comparable to that found in the liver for both CBA-GALNS and D8/CBA-GALNS treated mice (Supplemental Figure S3a).

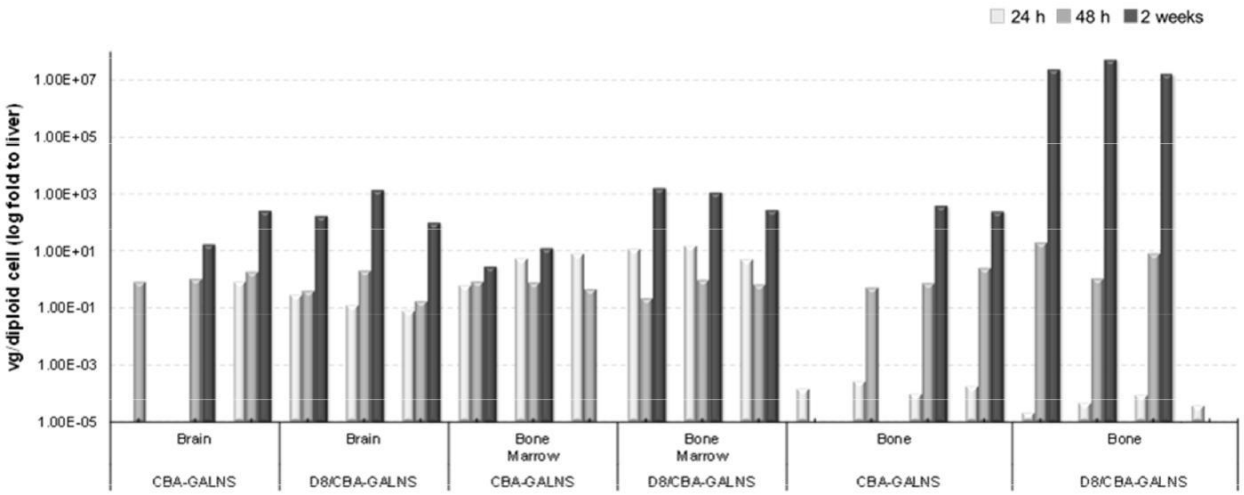
Two weeks after injection, vector DNA was increased in both bone marrow and bone. Vector genome copies in bone marrow D8/CBA-GALNS were 1,025±689 times higher than that in liver. In addition, vector DNA detected in bone when compared to liver was $31 \times 10^6 \pm 19 \times 10^6$ times higher in D8/CBA-GALNS, and 322±105 times higher in CBA-GALNS. A clear difference was noticed when values of two-weeks in bone are compared to those of 24 or 48 hours after injection (Supplemental Figure S3a).

*Total vector genome distribution compared to 24 hours post injection.*

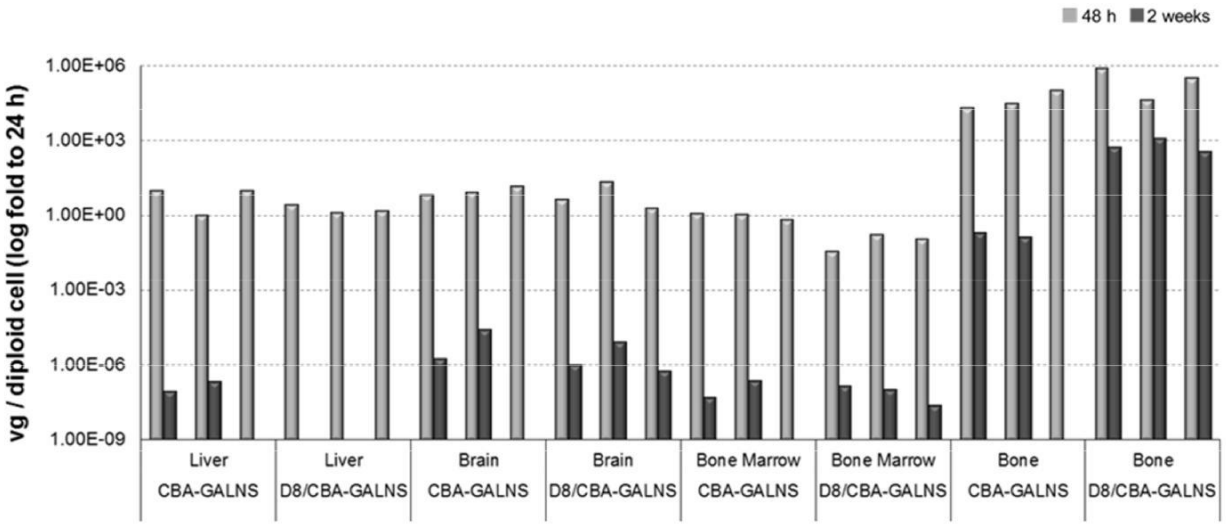
At 48 hours post injection, there was a 6.7±4.9-, 9.8±4.3-, and 49,000±43,000-fold increments of vg copies in the liver, brain, and bone, respectively, in CBA-GALNS treated mice, compared with those results at 24 hours. There was no change in the viral genome copies in bone marrow at 24 and 48 hours (Supplemental Figure S3b). The values of vg copies of D8/CBA-GALNS treated mice were 1.8±0.7-, 9.6±11.2-, and 0.1±0.06- fold compared to those observed at 24 hours in liver, brain and bone marrow, respectively. At 48 hours, there were more vg copies in bone of mice treated with D8/CBA-GALNS than at 24 hours (5,000 to 100,000 fold increase) (Supplemental Figure S3b).

After two weeks, vector genome copies were markedly reduced from 24-hour levels in all studied tissues, except in bone from mice treated with D8/CBA-GALNS (661.9±411.8 fold) (Supplemental Figure S3b).

**a.**


24 h
48 h
2 weeks
vg/diploid cell (log fold to liver)
1.00E+07
1.00E+05
1.00E+03
1.00E+01
1.00E-01
1.00E-03
1.00E-05
Brain
CBA-GALNS
Brain
D8/CBA-GALNS
Bone Marrow
CBA-GALNS
Bone Marrow
D8/CBA-GALNS
Bone
CBA-GALNS
Bone
D8/CBA-GALNS

**b.**


48 h
2 weeks
vg / diploid cell (log fold to 24 h)
1.00E+06
1.00E+03
1.00E+00
1.00E-03
1.00E-06
1.00E-09
Liver
CBA-GALNS
Liver
D8/CBA-GALNS
Brain
CBA-GALNS
Brain
D8/CBA-GALNS
Bone Marrow
CBA-GALNS
Bone Marrow
D8/CBA-GALNS
Bone
CBA-GALNS
Bone
D8/CBA-GALNS

**Supplemental Figure S3. Vector genome biodistribution.** 1.5 x $10^{11}$ vg of CBA-GALNS or D8/CBA-GALNS vectors were infused intravenously into 7- to 8-weeks-old MPS IVA *Galns*$^{-/-}$ mice. Vector genome was quantified in selected tissues after 24 hours, 48 hours and 2 weeks. Individual values are reported as the ratio of vg/diploid cell to liver (a), and to 24-hour levels (b). Vector genome was not detected in mice infused with PBS. All data n=3 except, n=6 for 48 hours (CBA-GALNS and D8/CBA-GALNS) and n=2 for 2-weeks (CBA-GALNS).