Table S1: Amino acid changes to the HexA α-subunit to convert the dimer interface from α to β and to introduce the putative GM2A binding surface from β- onto the α- subunit

Residue position (α-numbering)	Change (α to β)	Reason
184	Ser (S) to Lys (K)	Generate β dimer Interface
209	Pro (P) to Gln (Q)	Generate β dimer Interface
228	Asn (N) to Ser (S)	Generate β dimer Interface
229	Pro deleted	Generate β dimer Interface
230	Val (V) to Leu (L)	Generate β dimer Interface
231	Thr (T) to Ser (S)	Generate β dimer Interface
429	Pro (P) to Gln (Q)	Generate β dimer Interface & GM2AP binding site
432	Lys (K) to Arg (R)	GM2AP binding site
433	Asp (D) to Lys (K)	GM2AP binding site
436	Ile (I) to Lys (K)	GM2AP binding site
466	Asn (N) to Ala (A)	Generate beta dimer Interface
491	Ser (S) to Arg (R)	GM2AP binding site

493	Leu (L) to Met (M)	GM2AP binding site
494	Thr (T) to Asp (D)	GM2AP binding site
495	Phe (F) to Asp (D)	GM2A binding site
498	Glu (E) to Asp (D)	GM2AP binding site
508	Leu (L) to Val (V)	Generate β dimer Interface
513	Gln (Q) to Ala (A)	Generate β dimer Interface
518	Asn (N) to Tyr (Y)	Generate β dimer Interface
519	Val (V) to Ala (A)	Generate β dimer Interface
521	Phe (F) to Tyr (Y)	Generate β dimer Interface
523	Glu (E) to Asn (N)	Generate β dimer Interface

Table S2: PCR Primers used to characterize exons 1 and 11 of HexA and Exon 1 of HexB in WT and HEKHexABKO lines (see **Fig. S2**).

Gene/Region	ID	Sequence	Product Size (bp)
HexAexon1	b-for	GGTCCTCCTGGGGTCGCA	470
	b-rev	CGAAAACAGGTCGGGAGACTAG	
HexAexon2	f-For	GAGGAGAAGAGGGGCACAAC	366
	f-Rev	CTGATGCTCGGCCTGGA	
HexAExon1	d-for	GCCTGGCAAGTCCTTTACCT	692
	d-rev	GGACAAGTCCGACTCACCTG	
HexAintron1	c-for	CCGCTGGAAACTCACTTCCT	446
	c-rev	GTGTTCAAGCTAGCCAGGGT	
HexAexon1	a-for	TCCTTTACCTCCCCGTAGGC	282
	a-rev	ACACCCTGCCCTTTCACTTC	
HexAexon1	e-for	GCTCCAGGCTTTGGTTTTCG	229
	e-rev	CACCTGTGAGGTAAGGACGG	
HexAexon11	g-for	TTGCTGCTGGGGAACAGA	425
	g-rev	GTGTAACCCTTCCACCAACTCTC	
HexBexon1	h-for	GTCATCTGACTCGGTGACTC	252
	h-rev	TTCGGGGTCATCTTCACCAAG	

Supplementary Information: Figures

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Figure S1. Codon optimized nucleotide sequence of the α-derived hybrid (μ) subunit of homodimeric HexM.

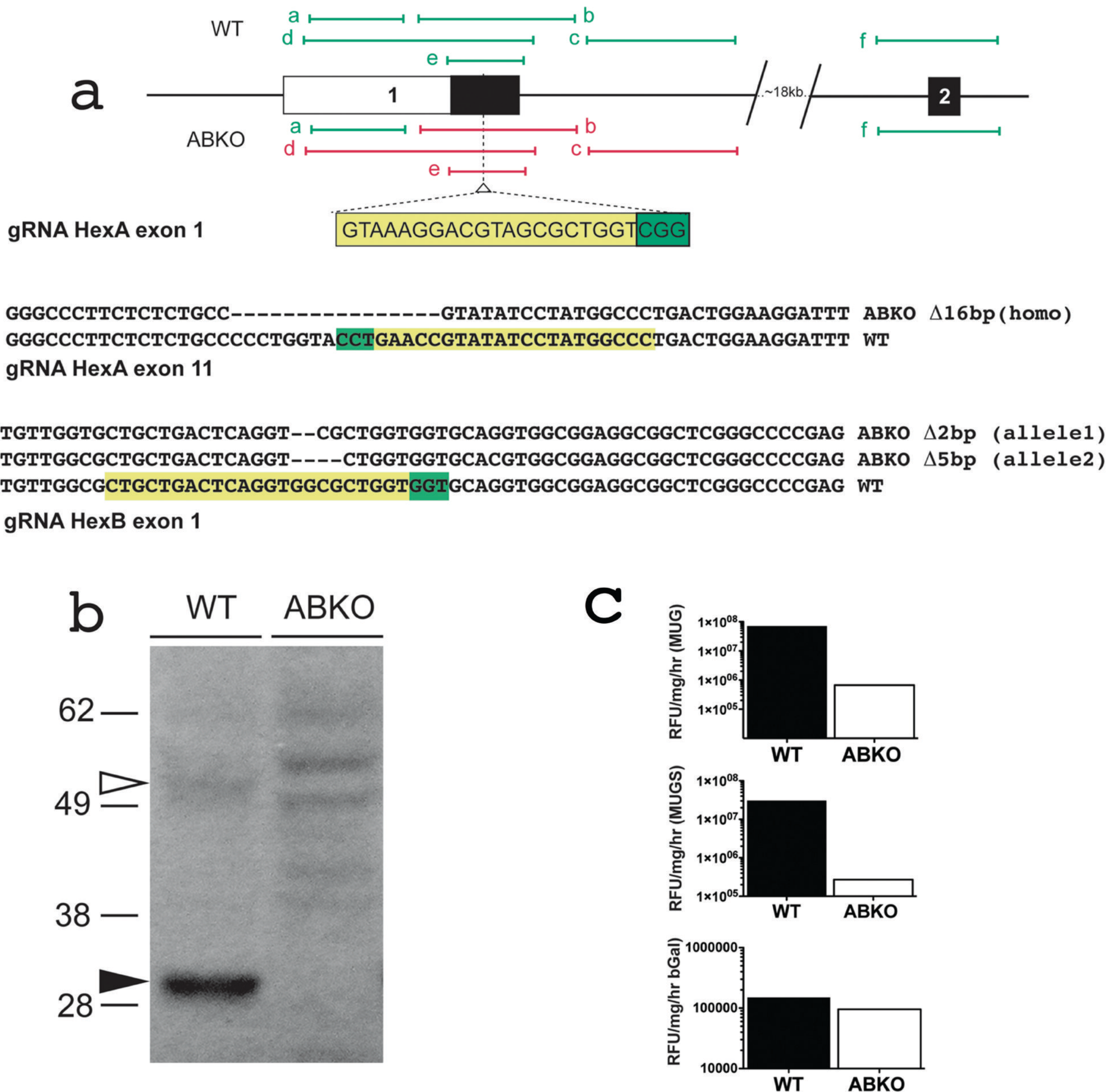

Figure S2. Characterization of HEKHexABKO cell line. (**a)** Highlighted in green and yellow are the three guide RNA sequences used to target exon1 and exon 11 of HexA and exon 1 of HexB in HEK293 cells. The upper schematic in panel **a** shows a scaled graphic representation of exons 1 and 2 (boxes), and the PCR primers (vertical bars) and their expected products (horizontal lines) used to characterize the large deletion in exon1 in the

ABKO line. Primers that resulted in products in the WT and ABKO lines are highlighted in green, those that only produced a product in the WT line are in red. Sequence of primers used to generate the PCR products are described in **Table S2** with the corresponding labels. Sequences surrounding the remaining guide RNAs in exon 11 of HexA and exon1 of HexB show the 16bp deletion that was found in exon 11 of both HexA alleles, and the 2bp or 5bp deletion found in exon1 of the two HexB alleles. Each of the deletions would result in a frame-shift followed by a prematurely terminated codon. (**b)** Comparison of the major α- and β- polypeptide levels in the WT HEK cells (20 μg) versus the HEXABKO line (ABKO, 40 μg). Western blot of lysates from WT and ABKO lines were probed with antibody against human Hex A (recognized bot the α- and β- polypeptides). Even though five times the amount of protein was loaded in the case of ABKO lysates, bands corresponding to the polypeptides from either of the HexA subunits were only observed in WT lysate. (**c**). Comparison of Hex activity levels (log scale, Y-axis) in WT versus ABKO line. Specific activity of HexA/B and bGal enzymes in WT and ABKO lines were determined using the fluorogenic substrates MUGS, MUG and MUbGal, respectively. Bgal activity levels in WT and ABKO lines are unchanged whereas MUGS and MUG activity in the ABKO line is reduced by more than 3 log orders in comparison to WT cells.