Supplementary Figures and Tables

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Fig. S2 Schematic of cross-correction and correlation of enzymatic activity in cell lysates and supernatants

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Fig.S4 β-hexosaminidase isoenzyme characterization

Supplementary Table 1. Percentage of enzymatic isoforms in untreated and LV-transduced murine and human cell types.

Figure S1

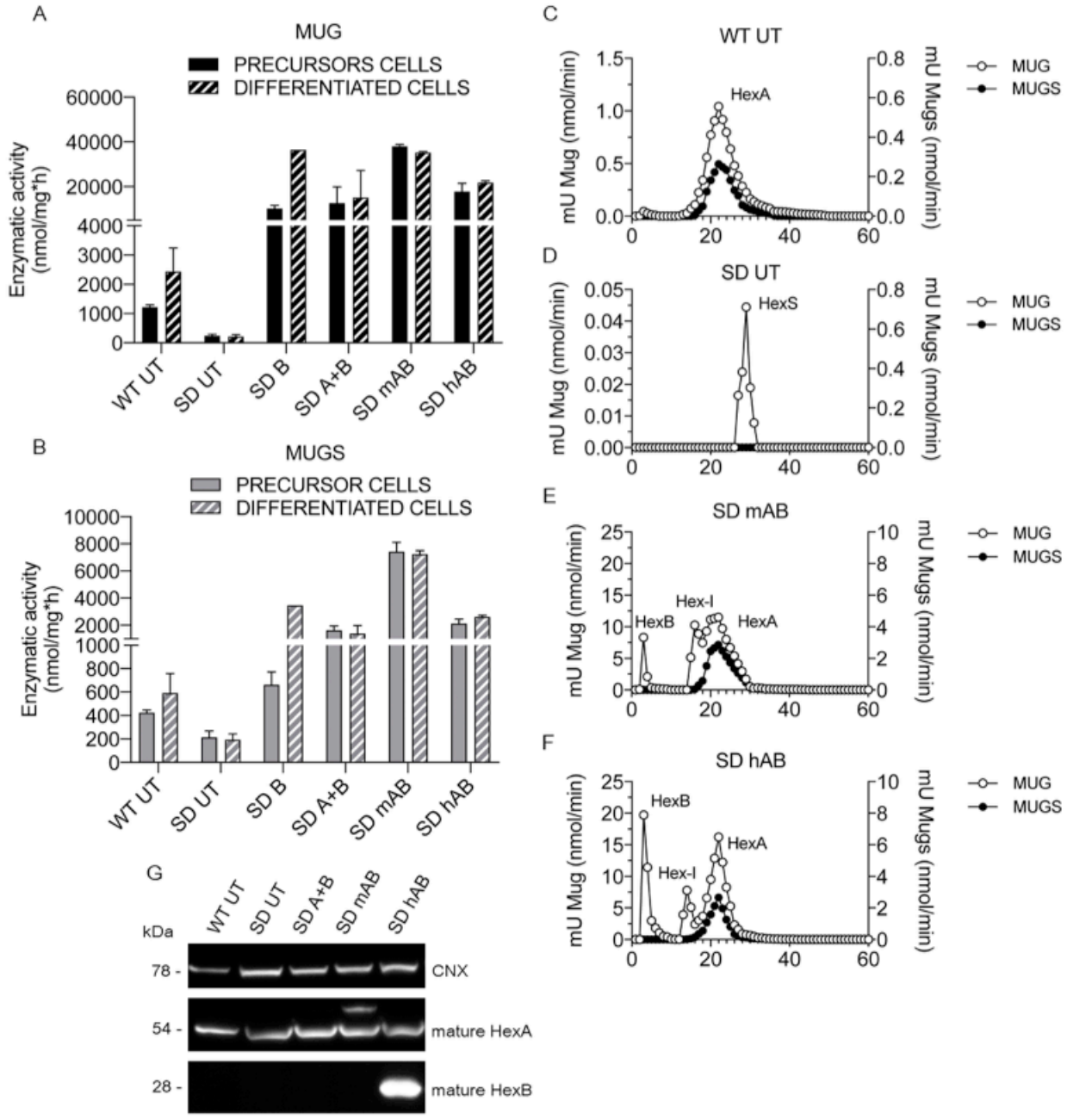

Fig. S1 Time-course analysis, enzymatic activity, and isoenzyme composition in UT and LV-transduced NPCs.

(A-B) MUG- and MUGS-related activity in WT UT, SD UT and SD precursors and differentiated cells. **(C-F)** Diethylaminoethyl Cellulose (DEAE) chromatography showing the presence of HexA, HexB, HexS and HexI isoforms in untreated (UT) (C, WT; D, SD) and LV-transduced SD differentiated neurons/glial cells (E, LV.mAB; F, LV.hAB). Enzymatic activity (expressed as nmol/min; mU MUG on the left and mU MUGS on the right) and fraction number (0.5 ml) are plotted on the y and x axis, respectively. **(G)** Western blot analysis on untreated (UT, WT and SD) and LV.transduced SD NPCs (mono- and bicistronic murine and human LVs) using antibodies recognizing the α- (murine and human) and β-chains (human). Calnexin (Cnx) was used as internal control. The 54 kDa band present in all the samples indicates the mature HexA protein. The 28 kDa bands indicating the mature HexB protein is present only in LV.hAB-transduced SD NPCs.

Figure S2

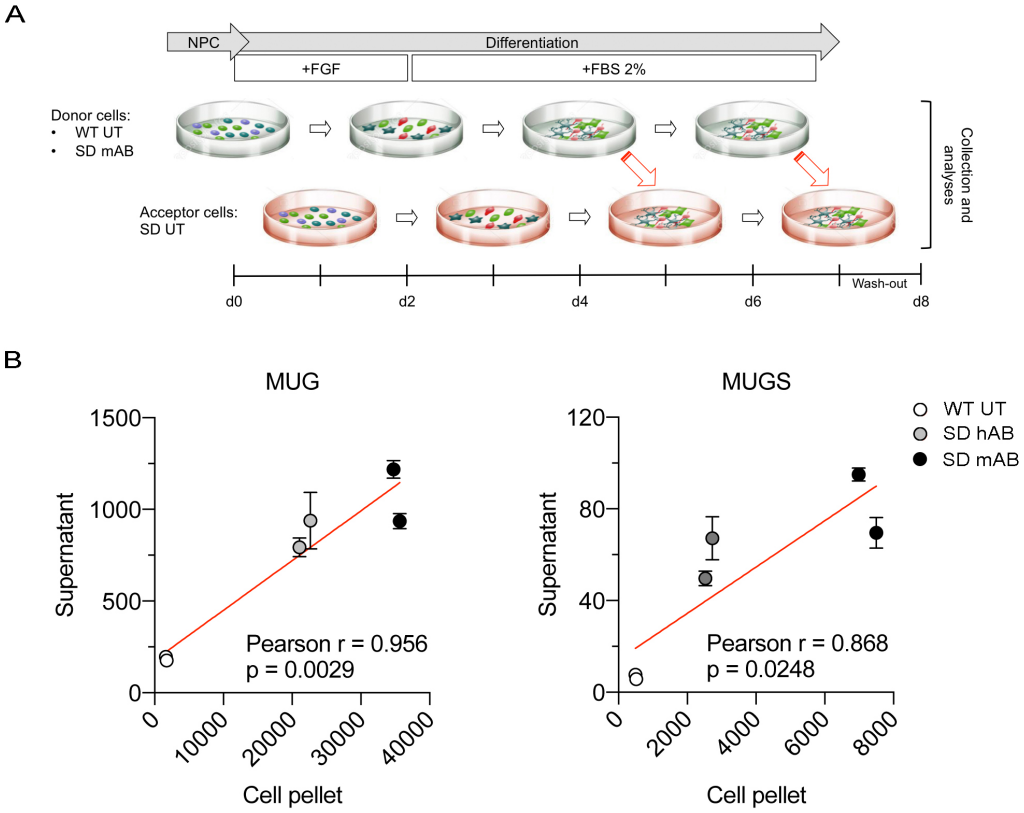

Fig. S2 Schematic of cross-correction and correlation of enzymatic activity in cell lysates and supernatants

(A) Schematic of cross-correction experiments. Untranduced (UT) WT and SD LV.mAB NPCs were differentiated and used as the source of secreted enzyme (*donor cells*). SD NPCs and progeny (*acceptor cells*) - at the same stage of differentiation as donor cells- were exposed to the donor supernatant. After a 24h-washout with fresh medium, cross-corrected cells were collected and analysed for intracellular HexA and HexB activity. **(B)** Correlation of enzymatic activity (MUG and MUGS, expressed as nmol/mg*hour) measured in the cell pellet (intracellular, on the x axis) and in the supernatant (extracellular, on the y axis) of WT UT and LV-transduced SD cells (LV.mAB and LV.hAB) n=3; Pearson r = 0.956, p = 0.0029 for MUG; Pearson r = 0.868, p = 0.0248 for MUGS).

Figure S3

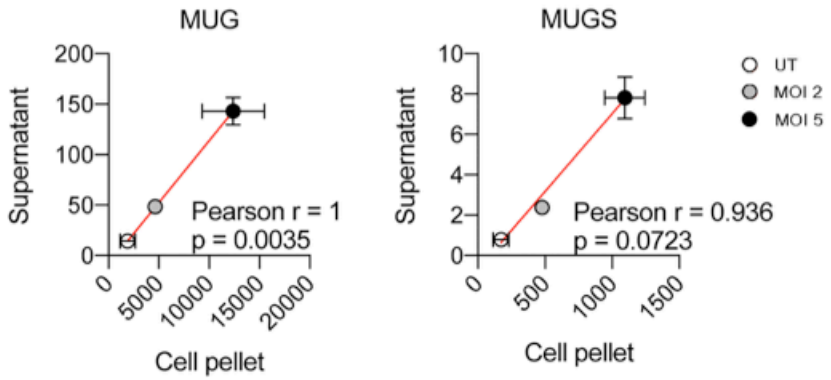

Fig. S3 Enzymatic activity in human iPSC-derived NPCs

Correlation of enzymatic activity (MUG and MUGS, expressed as nmol/mg*hour) measured in the pellet (intra-cellular, on the x axis) and in the supernatant (extra-cellular, on the y axis) of untransduced (UT) and LV.hAB-transduced human iPSC-derived NPCs (Multiplicity Of Infection: MOI2 and MOI5). n=3; Pearson r = 1, p = 0.0035, ** for MUG; Pearson r = 0.936, p = 0.0723 for MUGS).

Figure S4

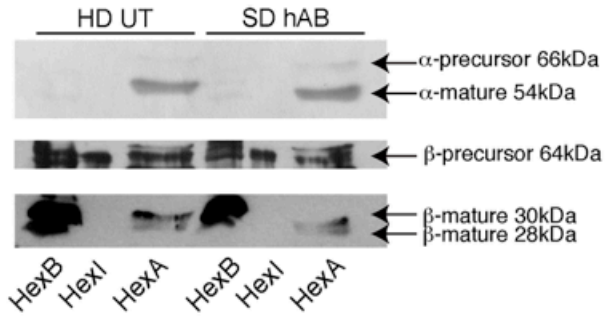

Fig.S4 β-hexosaminidase isoenzyme characterization

Western blotting using anti-α- and anti-β-subunit antibodies performed on Hex isoenzymes (HexB, Hex-I and HexA) from HD hFF and LV.hAB-transduced SD hFF (20 MOI) after DEAE-chromatography separation show the presence of precursor and mature α-subunit in the HexA fraction, precursor and mature β-subunit in HexB fraction; precursor of β-subunit in HexI fractions. The pattern of α- and β-subunit was comparable in Hex isoenzymes from HD UT and LV.hAB-transduced SD hFF (SD hAB).

Supplementary Table 1. Percentage of enzymatic isoforms in untreated and LV-transduced murine and human cell types. The Table shows the percentages of each peak of Hex activity. The percentage is calculated as the ratio of a given peak of activity to the total Hex activity recovered by DEAE-cellulose chromatography of each sample. The recovery of Hex activity by DEAE-cellulose chromatography is 95-98% in all experiments.

	% HexB	**% HexI**	**% HexA**	**% Hex S**
Murine NPCs				
WT UT	43	0	57	0
SD B	11	53	36	0
SD A+B	6	22	53	19
SD mAB	3	30	67	0
SD hAB	40	6	54	0
Murine differentiated cells				
WT UT	9	0	91	0
SD mAB	9	21	70	0
SD hAB	27	15	58	0
Murine HSPCs				
WT UT	16	21	63	0
SD B	3	37	60	0
SD A+B	24	39	37	0
SD mAB	3	2	95	0
SD hAB	18	8	74	0
Human iPSCs-derived NPCs (healthy donors)				
UT	47	5	48	0
hAB	43	15	42	0
Human HSPCs (CD34+) (healthy donors)				
UT	52	11	37	0
hAB	52	11	37	0
Human Fibroblasts				
Healthy donors (HD) UT	40	20	40	0
SD hAB	23	20	57	0