

Supplementary Materials for

Heat shock protein–based therapy as a potential candidate for treating the sphingolipidoses

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The PDF file includes:

- Fig. S1. rHSP70 uptake in NPC patient fibroblasts and lymphocytes.
 - Fig. S2. rHSP70 in *Npc1*^{-/-} mouse model pilot study and manual behavior quantification.
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 - Fig. S8. Plasma and thyroid gland distribution of ¹²⁵I-rHSP70.
- Legends for movies S1 to S4

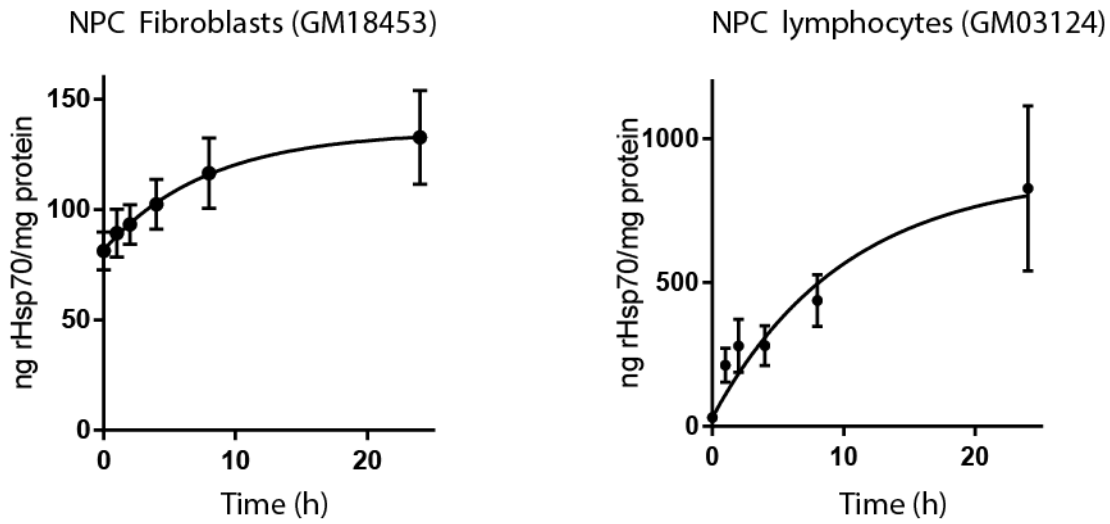
Other Supplementary Material for this manuscript includes the following:

(available at

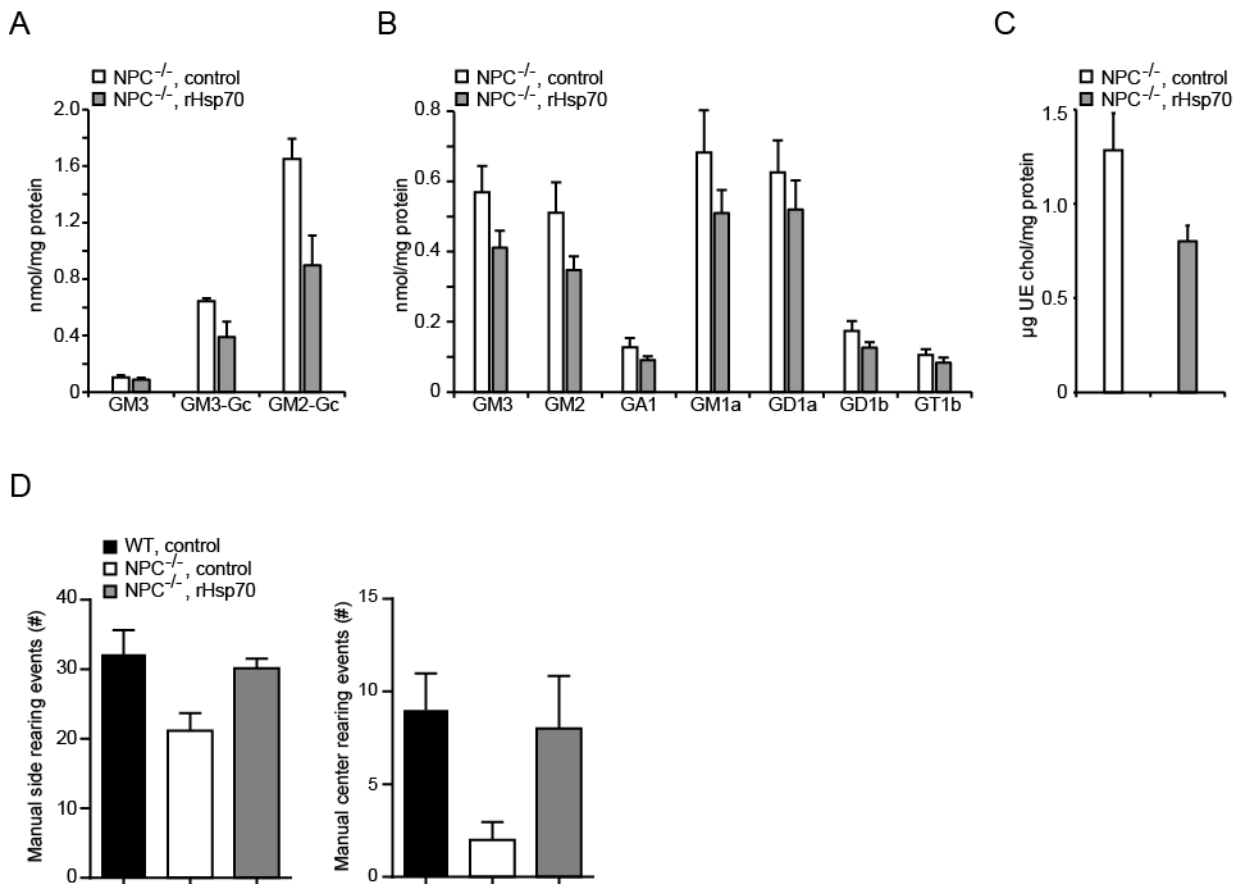
www.sciencetranslationalmedicine.org/cgi/content/full/8/355/355ra118/DC1)

- Movie S1 (.avi format). Uptake and effect of rHSP70 on the endolysosomal system in NPD primary patient fibroblasts.
- Movie S2 (.avi format). Endolysosomal dynamics in NPD primary patient fibroblasts.
- Movie S3 (.mp4 format). Video of gait analysis of PBS-treated control *Npc1*^{-/-} mice.

Movie S4 (.mp4 format). Video of gait analysis of rHSP70-treated *Npc1*^{-/-} mice.



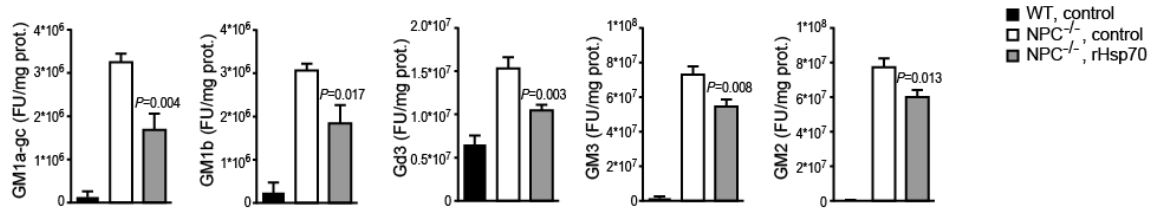
Supplementary Figure 1: rHSP70 uptake in NPC patient fibroblasts and lymphocytes. Quantification of rHsp70 uptake in NPC patient fibroblasts and lymphocytes measured by ELISA. Cells were administered 300nM rHsp70 and analysed for their amount of uptake at different time points up to 24h as indicated. Data points and association curves (non-linear regression, assuming one-phase association) represent average +/- stdev. N=5.



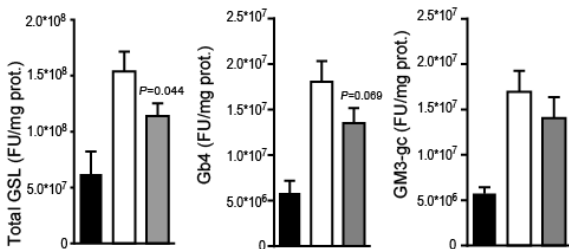
Supplementary Figure 2. rHSP70 in *Npc1*^{-/-} mouse model pilot study and manual behavior quantification.
a, b) Quantification of glycosphingolipid species extracted from livers (a) and brains (b) of control- (NPC^{-/-}, ctrl) or rHsp70-treated (NPC^{-/-} +rHsp70) NPC^{-/-} mice at P54, **c)** Quantification of unesterified cholesterol (UE Chol) extracted from livers of control- (NPC^{-/-}, ctrl) or rHsp70-treated (NPC^{-/-} +rHsp70) NPC^{-/-} mice at P54, **d)** Manual scoring of rearing behavior in wildtype (WT), control- (NPC^{-/-}, ctrl) or rHsp70-treated (NPC^{-/-} +rHsp70) NPC^{-/-} mice at the end of week 7. Mice were treated with 3mg/kg rHsp70, i.p., 3x/week from P21 to P53, , n=3 for panels a, b and c, n=6 for panel d, values represent means + S.D.

a

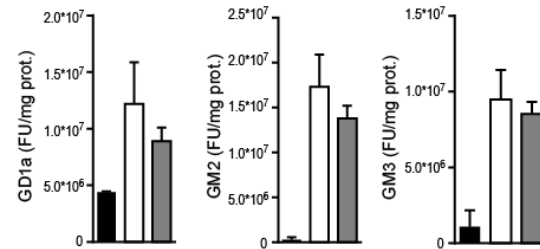
Cerebrum



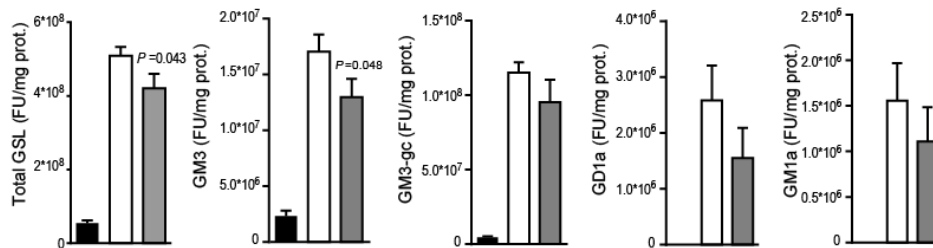
Kidney



Cerebellum

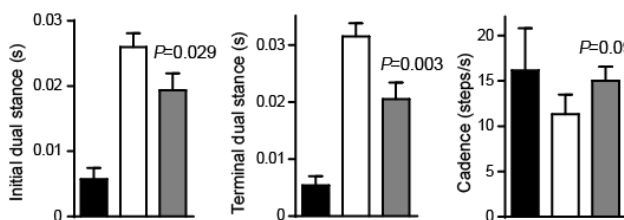


Liver



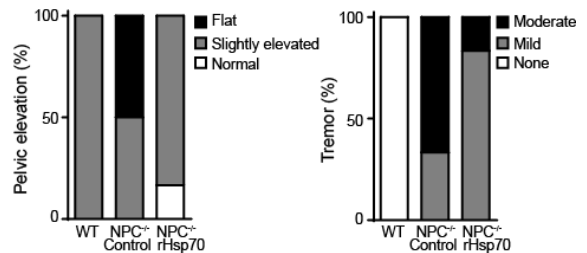
b

Gait analysis

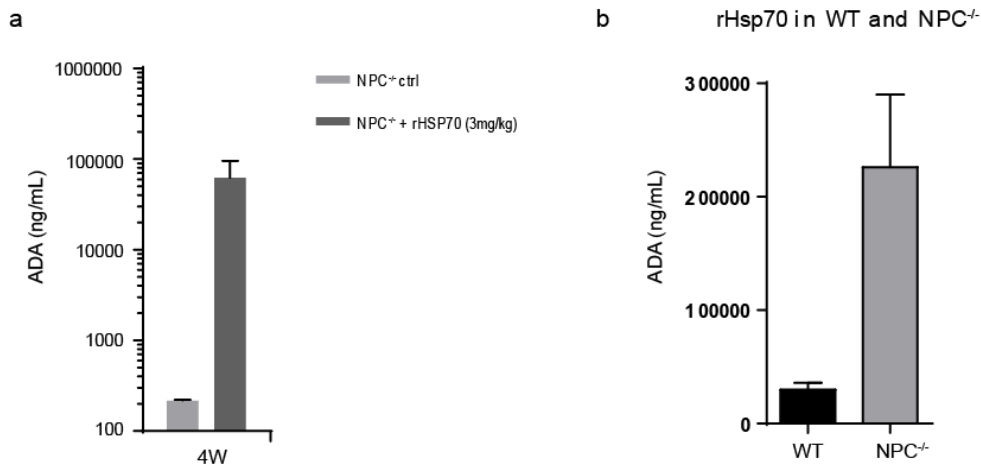


c

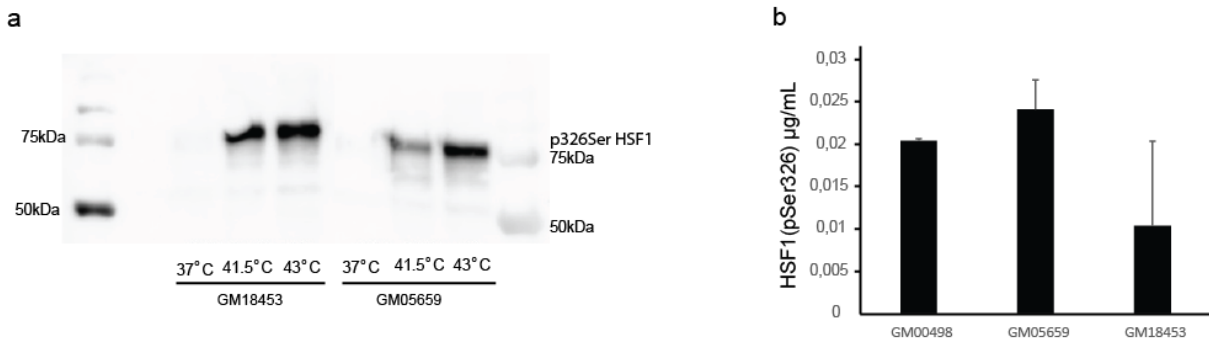
SHIRPA

Supplementary Figure 3. GSL and behavioral analyses of rHSP70-treated *Npc1*^{-/-} mice.

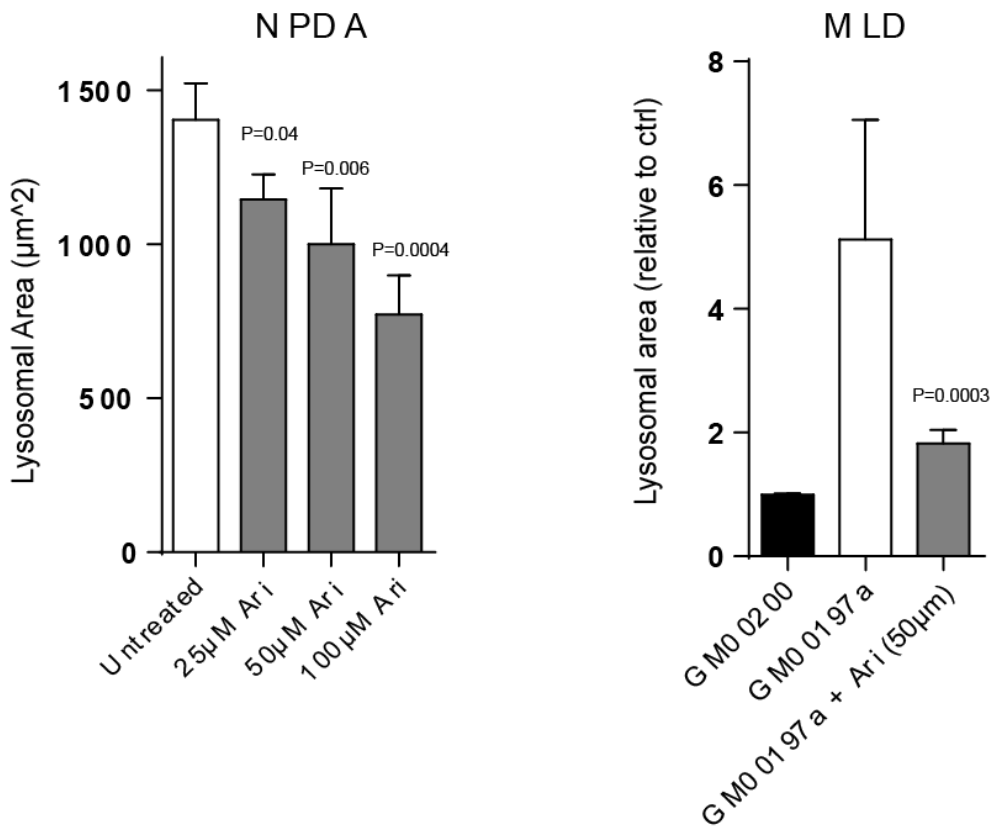
a) Quantification of glycosphingolipid species extracted from cerebrum, cerebellum, kidney and liver of wildtype (WT), control- (*NPC*^{-/-}, ctrl) or rHsp70-treated (*NPC*^{-/-} +rHsp70) *NPC*^{-/-} mice at P54. Mice were treated with 3mg/kg rHsp70, I.P., 3x/week from P21 to P53, n=5 for WT and ctrl, n=6 for rHsp70 treated, values represent means + SEM. **b)** Quantification of automated gait analysis (Noldus Catwalk XT system) of wildtype (WT, control), control- (*NPC*^{-/-}, ctrl) or rHsp70-treated (*NPC*^{-/-}, rHsp70) *NPC*^{-/-} mice at P49-52. Mice were treated with 3mg/kg rHsp70, I.P., 3x/week from P21, n=3 for WT, n=5 for Ctrl and n=6 for rHsp70-treated, values represent means + SEM. **c)** SHIRPA analysis of behavioral and neurological manifestations of disease in wildtype (WT, control), control- (*NPC*^{-/-}, ctrl)- or rHsp70-treated (*NPC*^{-/-}, rHsp70) *NPC*^{-/-} mice at week 7. Mice were treated with 3mg/kg rHsp70, I.P., 3x/week from P21, n=10 for all groups.



Supplementary Figure 4. Antidrug antibody responses against rHSP70. a) ADA response in *Npc1*^{-/-} mice treated with 3 mg/kg rHsp70 or sham (ctrl) 3x/week *i.p.* for 4 weeks, n=6 pr group, **b)** ADA response in wildtype BALB/c mice or *Npc1*^{-/-} mice administered 3mg/kg rHsp70 for 4 weeks, n=5 pr group

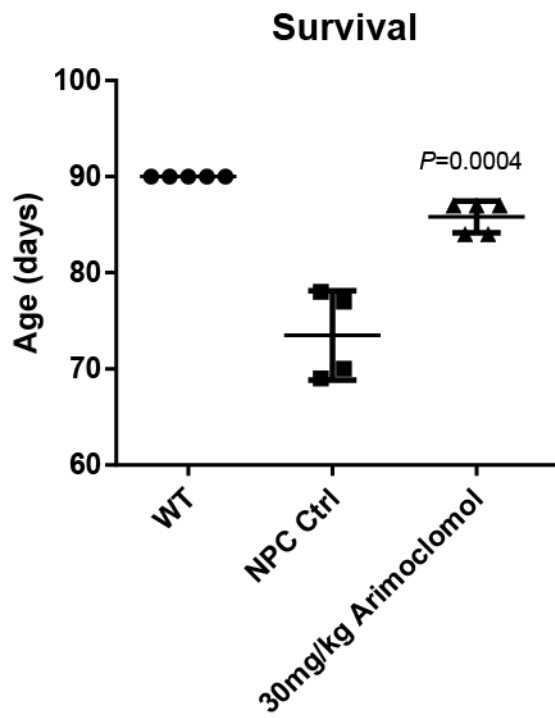


Supplementary Figure 5. In vitro analysis of activated HSF1 (pSer³²⁶ HSF1). **a)** Western Blot analysis of activation status of HSF-1 in wildtype (GM05659) and NPC1 patient fibroblasts (GM18453) under normal temperature and various levels of heat stress using a monoclonal rabbit anti HSF1 S326 phospho-antibody (ab76076); **b)** ELISA analysis of the activation status of HSF-1 (pSer326 HSF1) in wildtype (GM00498 and GM05659) or NPC1 patient fibroblasts (GM18453) under normal growth conditions.

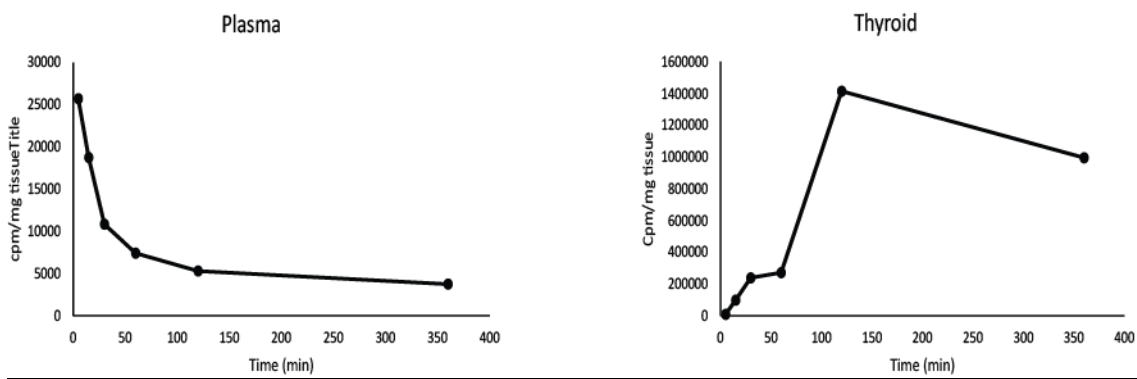


Supplementary Figure 6: Effect of arimoclomol on lysosomal accumulation in NPDA and MLD.

Quantification of lysosomal area of confocal cross sections of primary patient fibroblasts from NPDA or MLD, either sham-treated (Open/white Bars) or treated for 48h with the indicated concentrations of arimoclomol (Grey bars). GM00200 is an unaffected sibling (control) to the patient GM00197a. Values represent means + SEM for a minimum of three independent experiments. A minimum of 100 cells was analyzed for each experiment.



Supplementary Figure 7: Arimoclomol increases survival of *Npc1*^{-/-} mice. Analysis of survival in wildtype (WT), control (*Npc1*^{-/-}, ctrl)- or arimoclomol-treated (*Npc1*^{-/-}, 30mg/kg arimoclomol) *Npc1*^{-/-} mice at week 7. *Npc1*^{-/-} mice were treated with 30mg/kg arimoclomol daily in drinking water or sham (water) as control from three weeks of age, N=5 pr group.



Supplementary Figure 8: Plasma and thyroid gland distribution of ^{125}I -rHsp70. Time dependence on distribution of ^{125}I labelled rHsp70 after I.V. injection in the plasma and thyroid gland of healthy mice, n=3 pr timepoint.

Supplementary Movie 1: Uptake and effect of rHSP70 on the endolysosomal system in NPD primary patient fibroblasts. Alexa-Fluor 488 labelled rHSP70 (green) was administered to primary NPD cells labelled with lysotracker Red (red) and timelapsed over 16h on a confocal microscope (Zeiss LSM LIVE).

Supplementary Movie 2: Endolysosomal dynamics in NPD primary patient fibroblasts. Alexa-Fluor 488 labelled rHSP70 (green) was administered to primary NPD cells labelled with lysotracker Red (red) and timelapsed over 3min on a confocal microscope (Zeiss LSM LIVE).

Supplementary Movie 3. Video of gait analysis of PBS-treated control *Npc1*^{-/-} mice. Mice were sham treated with PBS, I.P., 3x/week from P21 to P53. Video captured as part of gait analysis at P49.

Supplementary Movie 4. Video of gait analysis of rHsp70-treated *Npc1*^{-/-} mice. Mice were treated with 3mg/kg rHSP70, I.P., 3x/week from P21 to P53. Video captured as part of gait analysis at P49

Online Supplementary methods:

Manual rearing:

Mice were placed in a large 'open field' cage box for 5 minutes. A side rear was recorded when mice reared on hind legs using the side of the cage as support. A centre rear was recorded when mice reared on hind legs un-aided. Rearings that were uncontrolled/the mouse fell over were discounted.