

Supplementary Materials for

Brain-targeted enzyme-loaded nanoparticles: A breach through the blood-brain barrier for enzyme replacement therapy in Krabbe disease

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Confocal Imaging

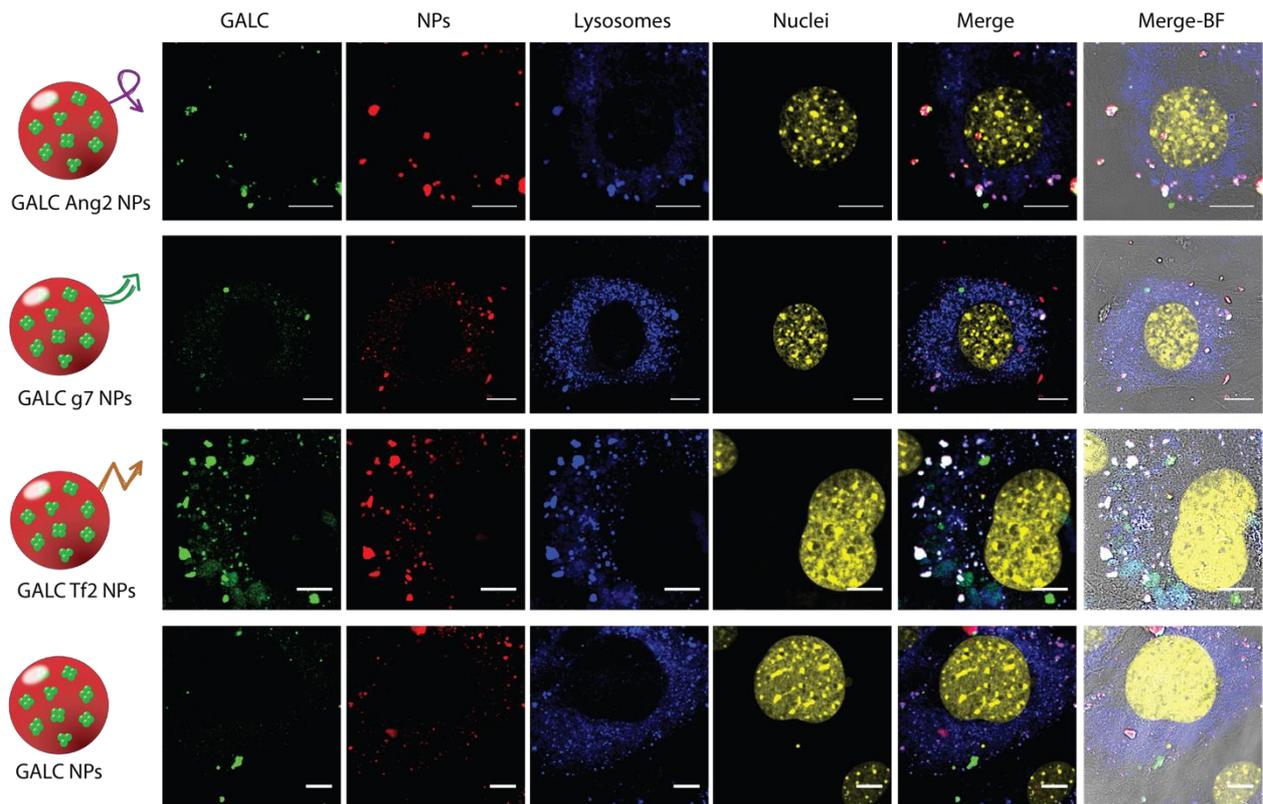


Fig. S1. Intracellular localization of targeted GALC CLEA NPs in WT cells. Confocal imaging. Representative confocal images of WT fibroblasts treated with fluorescently labelled GALC Ang2 NPs, GALC g7 NPs, GALC Tf2 NPs or GALC NPs. From the left to the right column: GALC (green, stained with Atto-488), NPs (red, stained with Atto-633), lysosomes (blue, stained with LysoTracker Red DND-99), nuclei (yellow, stained with DAPI), superimposition of GALC, NPs, lysosomes and nuclei fluorescence and superimposition of all channels with brightfield image. Scale bars: 10 μ m.

Confocal Imaging

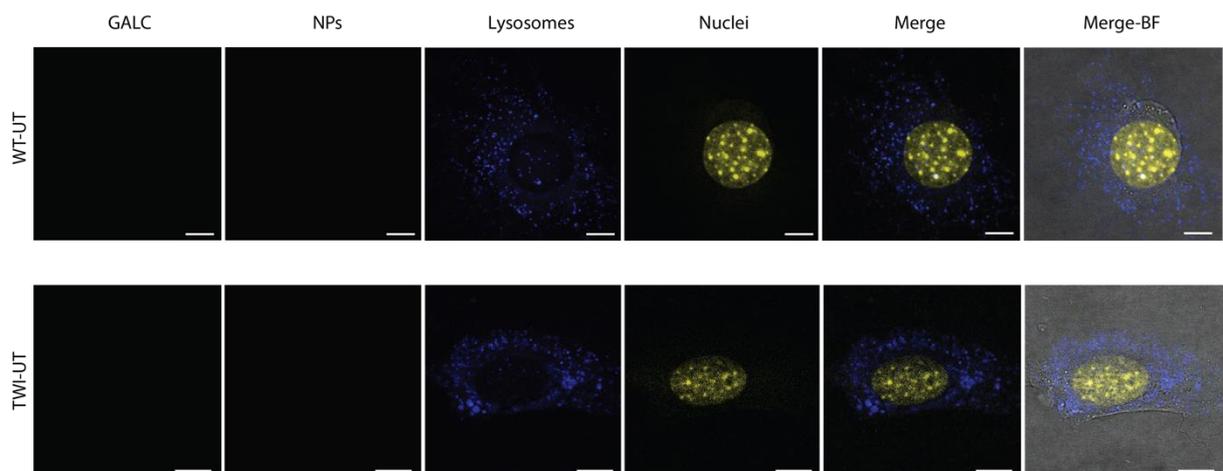


Fig. S2. Untreated WT and TWI fibroblasts. Confocal imaging. Representative confocal images of untreated WT and TWI fibroblasts. From the left to the right column: green channel (used for GALC in the treated cells, stained with Atto-488), red channel (used for NPs in the treated cells, stained with Atto-633), lysosomes (blue, stained with LysoTracker Red DND-99), nuclei (yellow, stained with DAPI), superimposition of green channel, red channel, lysosomes and nuclei fluorescence and superimposition of all channels with brightfield image. Scale bars: 10 μ m.

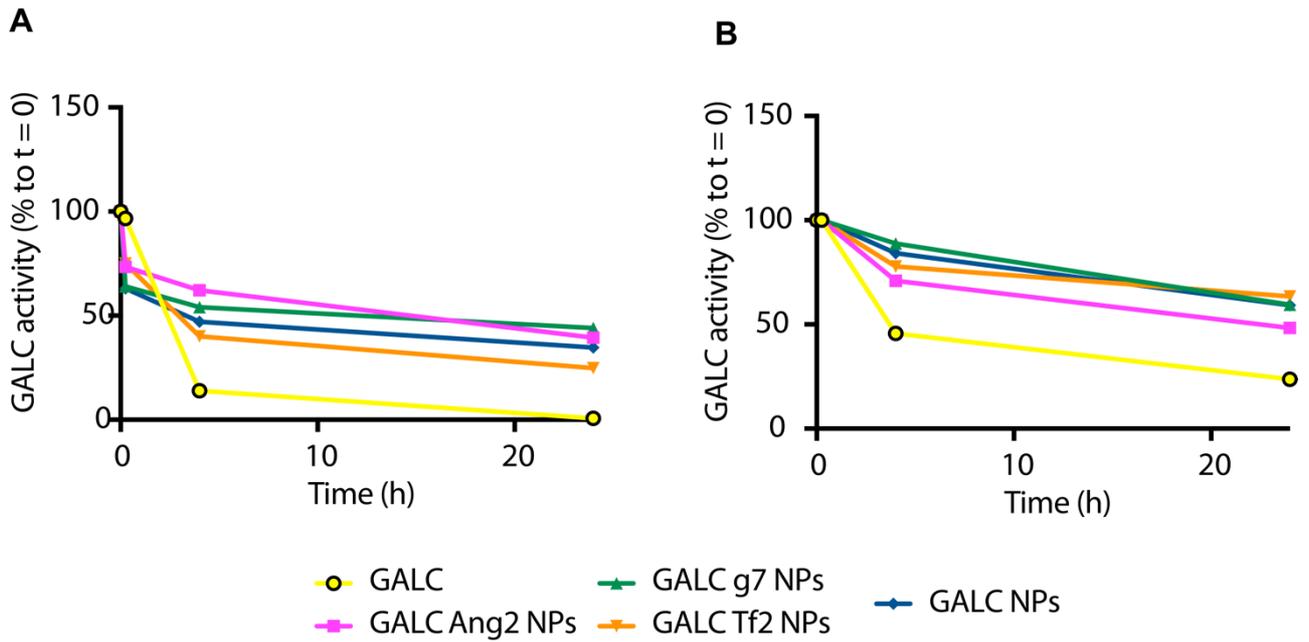


Fig. S3. GALC-loaded NPs activity over time in the presence and absence of serum proteins. (A) GALC activity over time upon incubation of free GALC, GALC Ang2 NPs, GALC g7 NPs, GALC Tf2 NPs and GALC NPs in medium containing 50% fetal bovine serum at 37°C and at final GALC concentration 100 U/ml. (B) GALC activity over time upon incubation of free GALC, GALC Ang2 NPs, GALC g7 NPs, GALC Tf2 NPs and GALC NPs in serum protein-free medium (PBS) at 37°C and at final GALC concentration 100 U/ml. Activity is expressed as percentage respect to the activity same sample (free GALC or GALC-loaded NPs) at the same concentration in PBS prior incubation.