Supplementary Material

Enzyme Replacement Therapy Clears Gb3 Deposits from a Podocyte Cell Culture Model of Fabry Disease but Fails to Restore Altered Cellular Signaling

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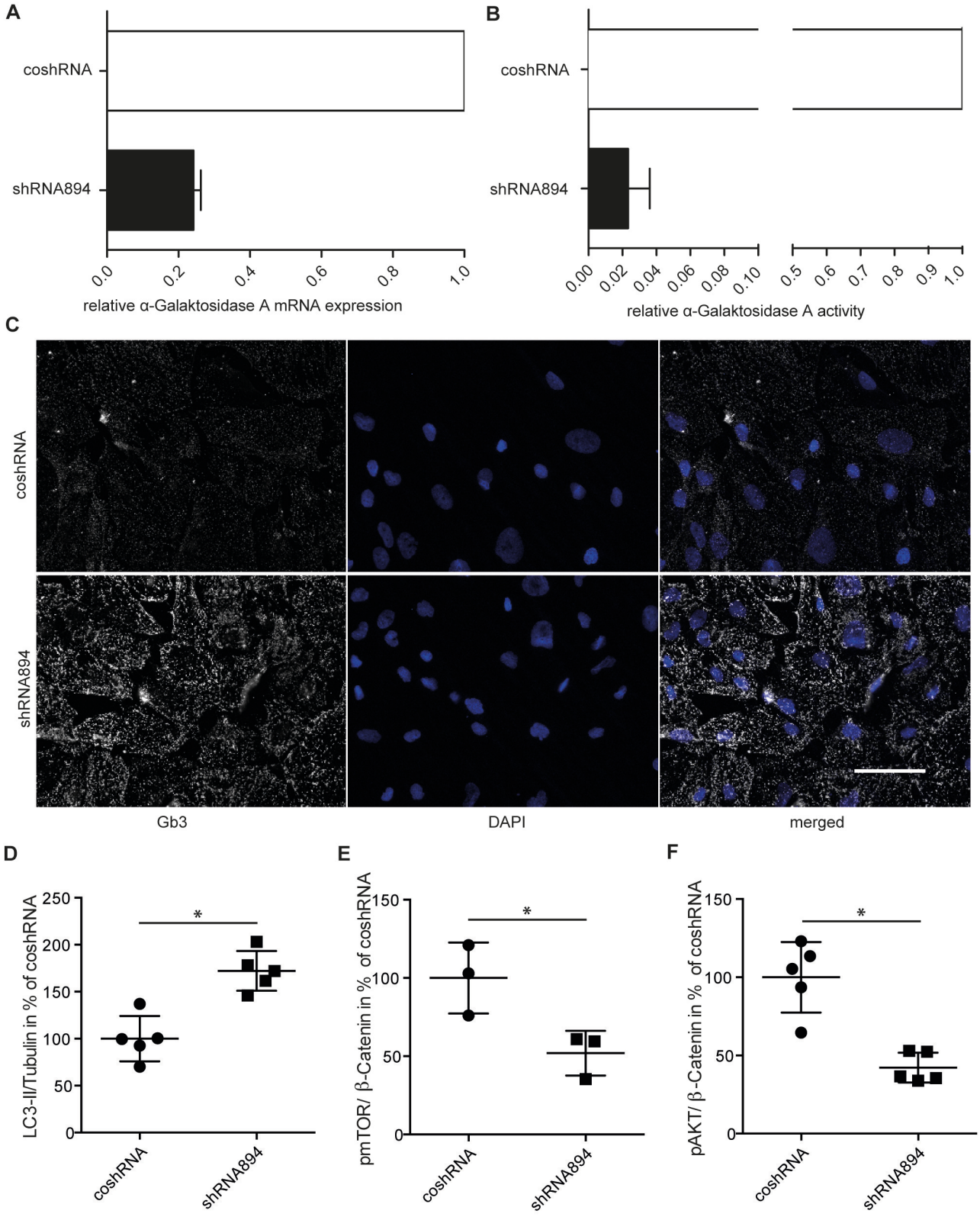
A
coshRNA
shRNA894
0.0
0.2
0.4
0.6
0.8
1.0
relative α-Galaktosidase A mRNA expression
B
coshRNA
shRNA894
0.00
0.02
0.04
0.06
0.08
0.10
0.5
0.6
0.7
0.8
0.9
1.0
relative α-Galaktosidase A activity
C
coshRNA
shRNA894
Gb3
DAPI
merged
D
LC3-II/Tubulin in % of coshRNA
250
200
150
100
50
0
*
coshRNA
shRNA894
E
pmTOR/ β-Catenin in % of coshRNA
150
100
50
0
*
coshRNA
shRNA894
F
pAKT/ β-Catenin in % of coshRNA
150
100
50
0
*
coshRNA
shRNA894

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Supplementary Figure S1. Baseline characteristics of control and α-Gal A-deficient podocytes. (A) RT-qPCR of α-Gal A expression, normalized against *ACTB* expression in control (coshRNA - set as 100%) and α-Gal A-deficient podocytes (shRNA894) (mean ± SD, n=5). (B) α-Gal A activity of α-Gal A-deficient podocytes (shRNA894) depicts a decrease in enzyme activity compared to control samples (mean ± SD, n=5). (C) Immunofluorescence of Gb3 (gray), counterstained with DAPI (blue) of control and Fabry podocytes (scale bar 50 µm). (D) Densitometric quantification of LC3-II/ß-tubulin immunoblot. Band intensity of LC3-II was normalized to β-tubulin band intensity (mean ± SD, n=5, Student's T-test, *=p<0,05). (E) Densitometric quantification of phospho-mTOR/ß-Catenin immunoblots. Band intensity of phospho-mTOR was normalized to β-Catenin band intensity (mean ± SD, n=3, Student's T-test *=p<0,05). (F) Densitometric quantification of phospho-AKT/ß-Catenin immunoblots. Band intensity of phospho-AKT was normalized to β-Catenin band intensity (mean ± SD, n=5, Student's T-test *=p<0,05).