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Supplemental Information

Overcoming Limitations

Inherent in Sulfamidase to Improve

Mucopolysaccharidosis IIIA Gene Therapy

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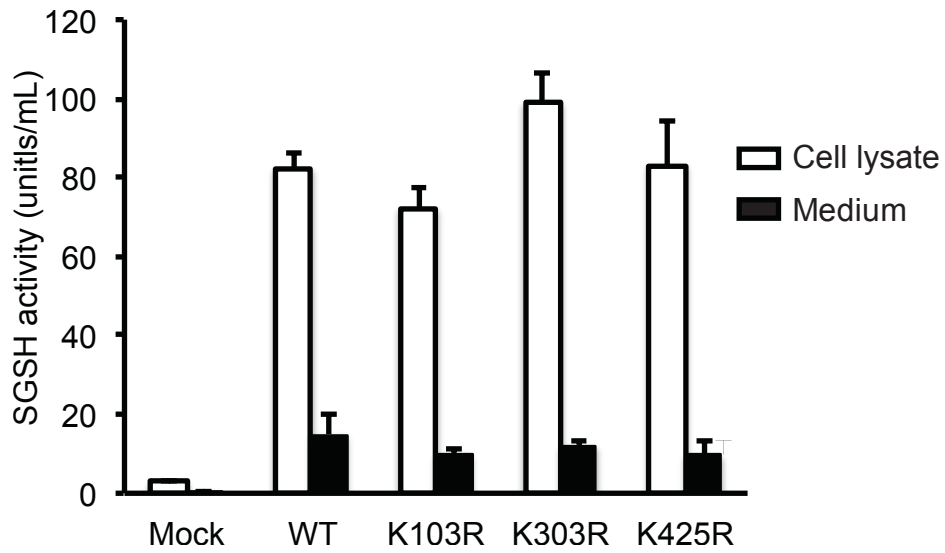


Fig. S1. Effects of ubiquitination site modifications on SGSH enzyme secretion. Three SGSH variants were generated by mutagenesis (K-to-R) to eliminate known ubiquitin binding sites at K103, K303 and K425 (Table S1). Vectors expressing wild type or SGSH variants were transfected into HEK 293 cells and cells and media collected and assayed for SGSH enzyme activity, n=3, data are mean \pm SD.

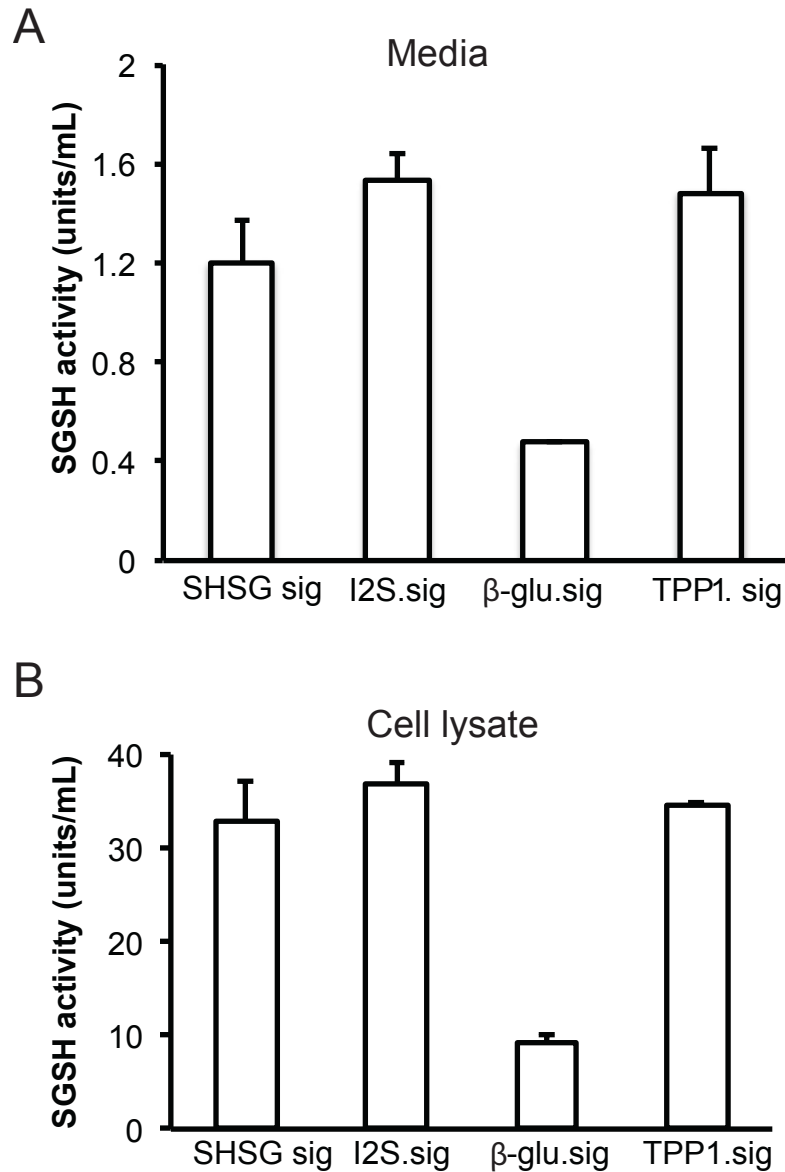


Fig. S2. Effects of different signal peptides on SGSH enzyme secretion. SGSH signal peptide was substituted with signal peptides from iduronate-2-sulphatase (I2S), β -Glu or TPP1. Vectors were transfected into HEK 293 cells and media (a) and cells (b) collected and assayed for SGSH enzyme activity, n=3, data are mean \pm SD.

Table S1. Primer sequences for the construction of the SGSH variants

M6P variants	Mutation site	Primer	
SGSHv1	N41Q	Primer 1	CTCGAGGCCACCATGAGCT
		Primer 2	CGATGGCGCTGTTCTGGTACG
		Primer 3	GCGTACCAGAACAGCGCCATC
		Primer 4	GACGGAGGTCCTTGTACCAG
SGSHv2	N142Q	Primer 1	CTCGAGGCCACCATGAGCT
		Primer 2	GACGGAGCCCTGCTCCTCC
		Primer 3	GGAGGAGCAGGGCTCCGTC
		Primer 4	GACGGAGGTCCTTGTACCAG
SGSHv3	N151Q	Primer 1	CTCGAGGCCACCATGAGCT
		Primer 2	GCTTAATTCTAGTGATCTGCCGCC
		Primer 3	GGCGGCAGATCACTAGAATTAAGC
		Primer 4	GACGGAGGTCCTTGTACCAG
SGSHv4	N264Q	Primer 1	CTCGAGGCCACCATGAGCT
		Primer 2	CCAGTGTGTCCTGCAGGACAC
		Primer 3	GTGTCCTGCAGGACACACTGG
		Primer 4	GACGGAGGTCCTTGTACCAG
SGSHv5	N413Q	Primer 1	CTCGAGGCCACCATGAGCT
		Primer 2	GTGGTGCCTGCAGGAGGTC
		Primer 3	GACCTCCTGCAGCGCACCCAC
		Primer 4	GACGGAGGTCCTTGTACCAG
Ubiquitin variants			
SGSH.Uv1	K103R	Primer 1	CTCGAGGCCACCATGAGCT
		Primer 2	GCTCCGCACCCGGTCAAGG
		Primer 3	CCTTCGACCGGGTGC GGAGC
		Primer 4	GACGGAGGTCCTTGTACCAG
SGSH.Uv2	K303R	Primer 1	CTCGAGGCCACCATGAGCT
		Primer 2	CCCAGCGGCGTGGGTGCTC
		Primer 3	CCCAGCGGCGTGGGTGCTC
		Primer 4	GACGGAGGTCCTTGTACCAG
SGSH.Uv3	K425R	Primer 1	CTCGAGGCCACCATGAGCT
		Primer 2	GACGGAGGTCCCGGTACCAGC