**Neutral sphingomyelinase (SMPD3) deficiency disrupts the Golgi secretory pathway and causes growth inhibition**

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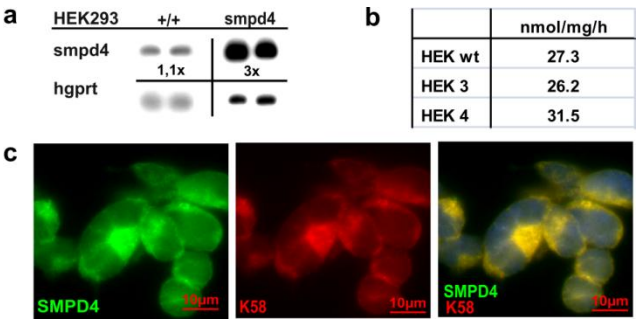
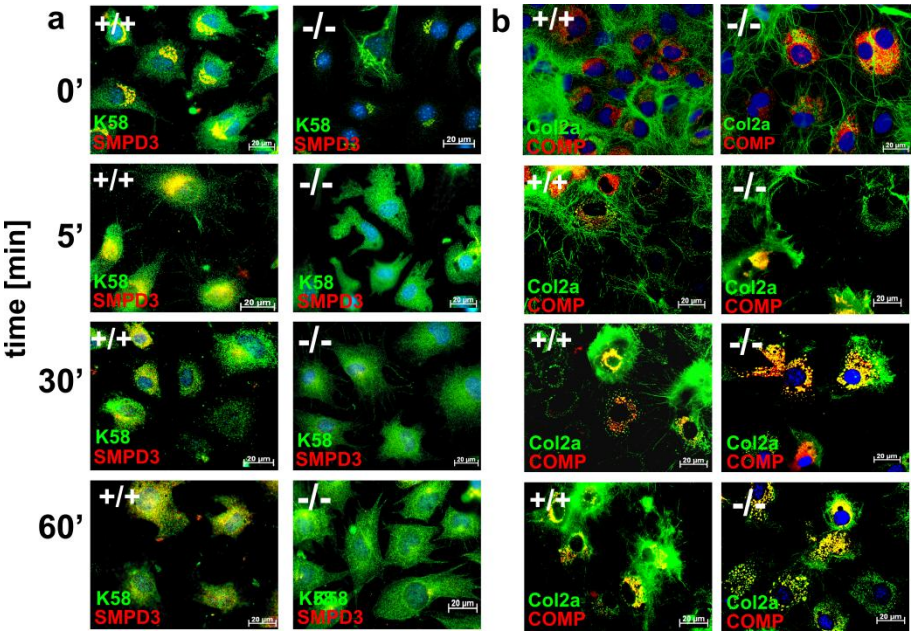
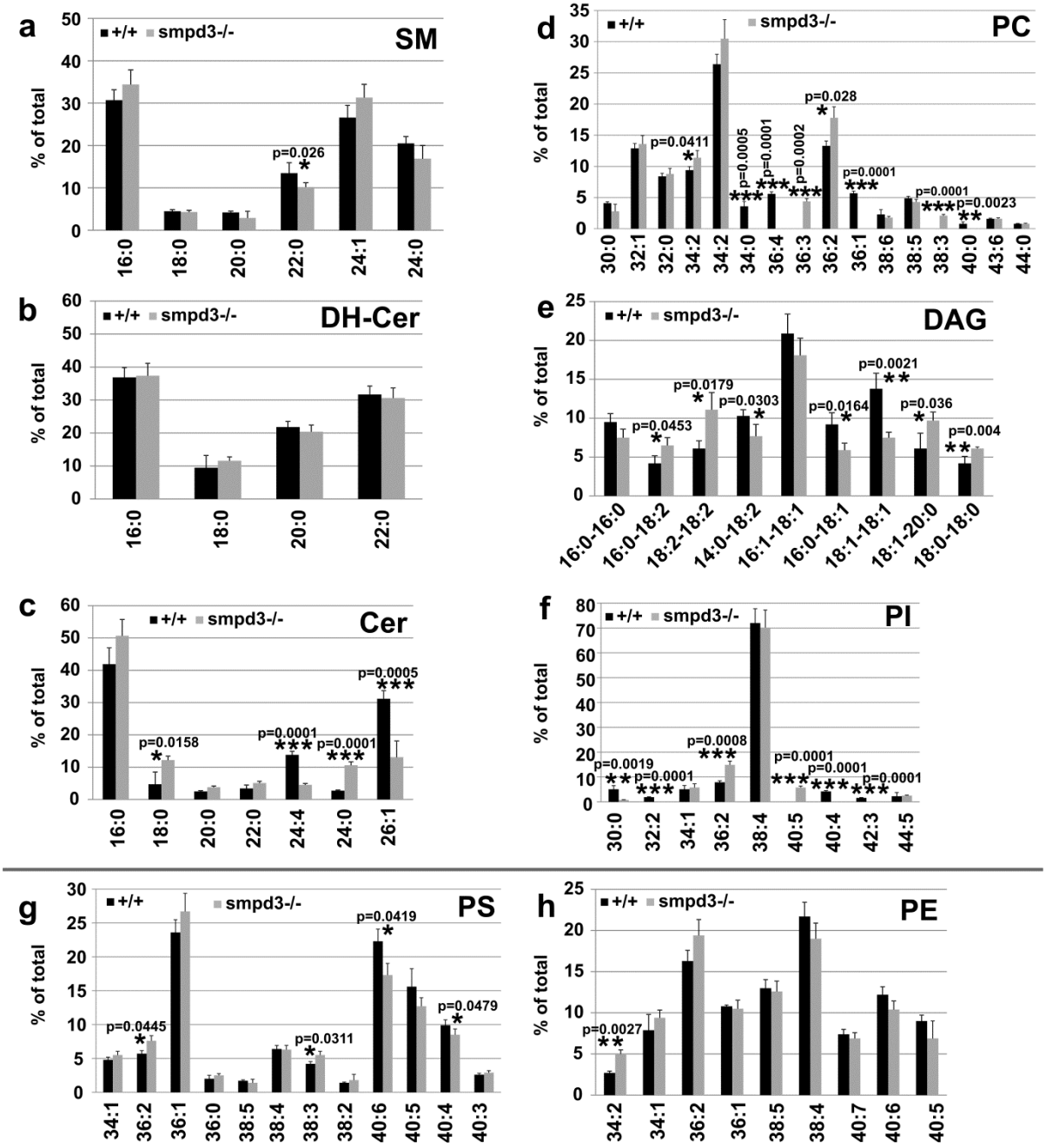
SMPD2, SMPD3 and SMPD5 are members of a superfamily of $Mg^{2+}$- dependent phosphohydrolases, clustered in the neighbor-joining dendrogram [1]. The DNA sequence of SMPD4 [2], however, shared neither sequence homology nor active site motifs of the catalytic domain matched by the superfamily profile. We studied the role of SMPD4 in HEK293 cells, stably transfected with a *smpd4-egfp* fusion construct and confirmed the subcellular topography of SMPD4-EGFP predominantly in the GC [2]. However, sensitive nSMase assays [3] in cell lysates of these cell lines revealed no increase of nSMase activity beyond that of *wt* HEK-cells, **Supplementary Figure S1b**. Our experiments preclude the bona fide SMPD4 as an nSMase and pointed to SMPD3 as the dominant mammalian nSMase.




**Supplementary Figure 1.** Enzymatic activity and immunohistochemistry of stably expressing *smpd4-egfp* HEK cells preclude SMPD4 as a neutral sphingomyelinase. (a) qRT-PCR of cRNA of wt and *smpd4-egfp* transfected HEK293 cells, (b) neutral sphingomyelinase activity was not increased in overexpressing *smpd4-egfp* transfected HEK cells, clones HEK 3 and 4. (c) Fluorescence images of *smpd4-egfp* overexpressing HEK cells indicated SMPD4-EGFP localization in the Golgi complex (green). Merged images indicated colocalization with Golgi marker K58 (red) in the Golgi complex.




**Supplementary Figure 2.** Time resolved disintegration of Golgi complex in wt and *smpd3-/-* chondrocytes on exposure to brefeldin. **(**a) Fluorescence images of p10 wt and *smpd3-/-* primary chondrocytes, grown in culture for six days on cover slips treated with 5µg BFA/ml for 5, 30 and 60 minutes and double stained with: SMPD3- (red) and K58-antibodies (green), **(**b) Col2a- (green) and COMP-antibodies (red).




**Supplementary Figure 3.** Phospholipidome of wt and *smpd3-/-* chondrocytes**. (**a-h) HPTLC-separation of SPM, Cer and PL classes of total lipid extract of wt and *smpd3-/-* chondrocytes. Quantitative MS/MS-analysis of sphingomyelin (SM) (a), dihydroceramide (DHCer) (b), ceramide (Cer) (c), phosphatidylcholine (PC) (d), diacylglycerol (DAG) (e), phosphatidylserine (PS) (f), phosphatidylinositol (PI) (g) and phosphatidylethanolamine (PE) (h). N=3

**Supplementary table1.**

Primers for real time-PCR

| | | | |
|---|---|---|---|
| smpd1 s | 5'-cctttatcaaccttaaccctggctaccgag-3' | elov4 as | 5'-ctgatggcgtaggcga-3' |
| smpd1 as | 5'-cgaagcttttgcctcaggtagataagcagc-3 | elov5 s | 5'-gtatggctgggaccaa-3' |
| smpd2 s | 5'-ctctgaaaaccactacaggctgtgaccctc-3 | elov5 as | 5'-catatcggattctcccgc-3' |
| smpd2 as | 5'-ctgggtctccgtttcccttgtcagaacgtg-3' | elov6 s | 5'-acccgaactaggtgac-3' |
| smpd3 s | 5'-ctggctgatttccgaaaatctacctcctcg-3' | elov6 as | 5'-cgggagactcggaaac-3' |
| smpd3 as | 5'-gtcaggccggacagctgggtgataaaactg-3' | elov7 s | 5'-gggaacattccatgcc-3' |
| sms1 s | 5'-atgctaacgctcacctacctatttatcaa-3' | elov7 as | 5'-gcaacctctgaccctt-3' |
| sms1 as | 5'-tagtcggcagagctgttatgtgtcgtttac-3' | bmp1 s | 5'-gtgtgggggccaagtgcgggcagatgtgaa-3' |
| sms2 s | 5'-acttctggtggtatcacttggtctgctggc-3' | bmp1 as | 5'-tcacttcctgctgtggagtgtgtcctggaa-3' |
| sms2 as | 5'-gctcaggtagacttctcattatcctccccg-3' | bmp2 s | 5'-caagccaaacacaaacagcggaagcgcctc-3' |
| degs s | 5'-gcttttagacccctgttc-3' | bmp2 as | 5'-tgctaacgacacccgcagccctccacaacc-3' |
| degs as | 5'-gtagtgcgggaggtca-3' | bmp4 s | 5'-cacctcatcacacgactactggacaccaga-3' |
| kinase s | 5'-tcatgtccggtgatgg-3' | bmp4 as | 5'-tttaccgtgagtcaagtcacccctgtgttg-3' |
| kinase as | 5'-ggcttgctaggcgaaa-3' | comp s | 5'-gagcagacgtactggc-3' |
| lyase s | 5'-atgtctgctaaggggt-3' | comp as | 5'-gggagaagcagaagaca-3' |
| lyase as | 5'-cggggtccgtagtataa-3' | Col2a s | 5'-ccacttcagctatggc-3' |
| spt s | 5'-tatgccacgtcgatgt-3' | Col2a as | 5'-cggtactcgatgacgg-3' |
| spt as | 5'-actcaatgatcggggt-3' | sox9 s | 5'-ctacccgcccatcacccgctcgcaatacga-3' |
| kdsr s | 5'-gacaagctactgcaggcgaagaaagacatt-3' | sox9 as | 5'-ctgtgtgtagactggttgttcccagtgctg-3' |
| kdsr as | 5'-cacagtgacgtacacattgtacggcttcac-3' | cerS1 s | 5'-agtgggcacttgtcgtacccgacggttgca-3' |
| a cer s | 5'-gcatgaattattggctcaaaaggcaccagc-3' | cerS1 as | 5'-caacggcagccacactcatccaccaccatg-3' |
| a cer as | 5'-gaatcctgtcaacatgcccacatatccaac-3' | cerS2 s | 5'-acgcgggatggaagaacacctgcaacaacc-3' |
| igf1 s | 5'-cacctcttctacctggcgctctgcttgctc-3' | cerS2 as | 5'-ttagctaggagccggctctttgctcctgcc-3' |
| igf1 as | 5'-tctgagtcttgggcatgtcagtgtggcgct-3' | cerS3 s | 5'-ccctgttcttcatcttcaccgtcgtcttct-3' |
| elov1 s | 5'-ccaaagctaccctctg-3' | cerS3 as | 5'-ctaacggccatgctgaccattggcaatgag-3' |
| elov1 as | 5'-actcgaaccatccgaag-3' | cerS4 s | 5'-cacgtctctgtgatgactccatcaagaact-3' |
| elov2 s | 5'-ctcctactacggcctg-3' | cerS4 as | 5'-gcgggccattggtaactgctactctaggcc-3' |
| elov2 as | 5'-ccgtcatgccattagc-3' | cerS5 s | 5'-ttggcgcagcttttatagttttcgtctagc-3' |
| elov3 s | 5'-gacttcgagacgtttcag-3' | cerS5 as | 5'-gcagttggcaccattgctagagctgctgcc-3' |
| elov3 as | 5'-cacggtttgcttgagg-3' | sec23 s | 5'-agcacagtggcggaagtcaggataccaggac-3' |
| elov4 s | 5'-ggattggaatcaagtggg-3' | sec23 as | 5'-tgcaagtttcttcaaatgatccatgaacac-3' |

## References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