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Supplemental Information

Enhancing the Therapeutic Potential of Sulfamidase for the Treatment of Mucopolysaccharidosis IIIA

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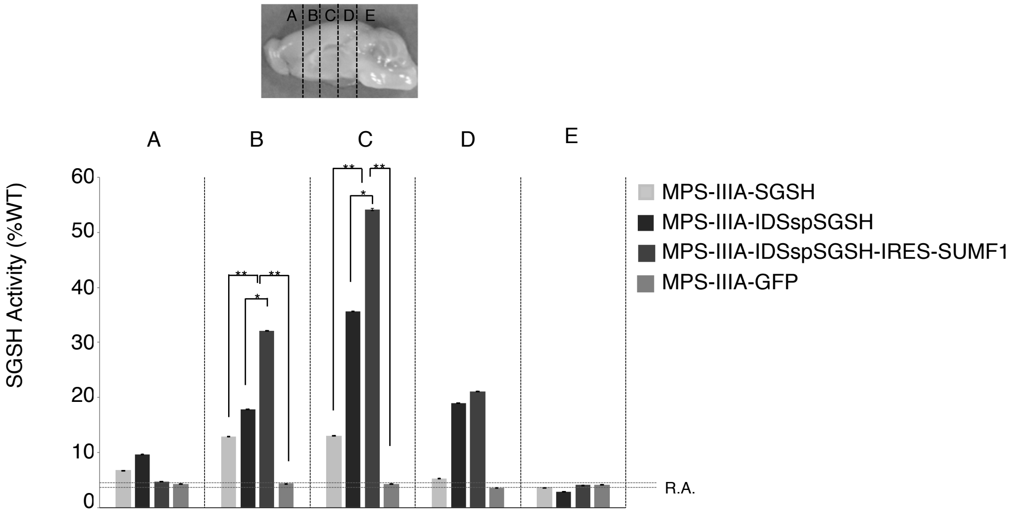
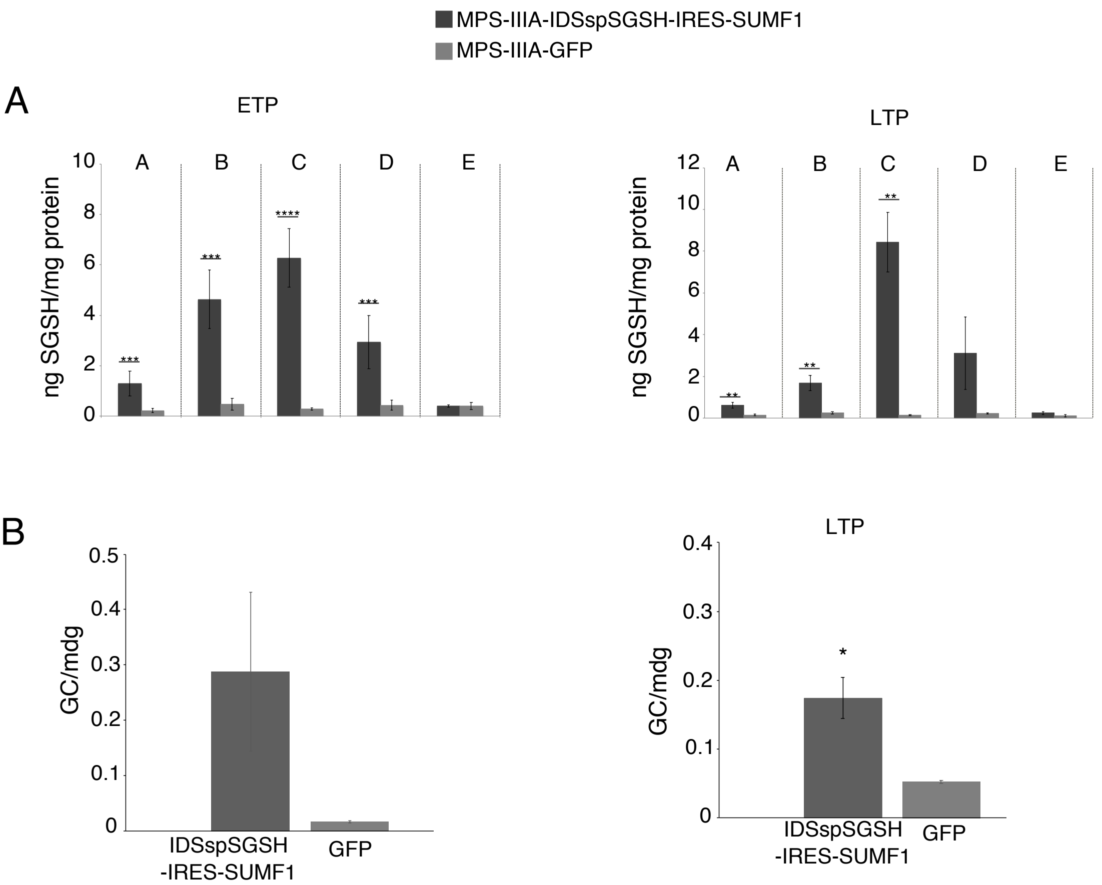
Supplementary Figure 1



Supplementary Figure 1. Sulfamidase activity in the brain of MPS-IIIA mice injected with AAV9 vectors carrying different SGSH expression cassettes

P60 MPS-IIIA mice were intra-CSF injected (via lateral ventricle administration: ICV) with 5.4×10^{12} GC/Kg of AAV9 encoding under the CMV promoter the following expression cassettes: *GFP*, *SGSH* WT, *IDSspSGSH*, or *IDSspSGSH*-IRES-*SUMF1*. The brain and the first region of the spinal cord of treated mice was divided in five slices (A-E) covering the main representative area of the CNS (A: olfactory bulb and prefrontal cortex, B: frontal cortex, lateral septum and basal ganglia regions, C: parietal cortex, hippocampus, striatum, thalamus, D: occipital cortex, pons, hippocampus; E: cerebellum, medulla oblongata, cervical region of spinal cord). One month after injection sulfamidase activity was measured in these areas and expressed as the percentage of the activity found in control GFP-treated WT mice. N = 3-4 animals per group. Data represent mean ± SEM. *P<0.05, **P<0.01 MPS-IIIA-IDSspSGSH-IRES-SUMF1VS MPS-IIIA-GFP, MPS-IIIA-IDSspSGSH-IRES-SUMF1 VS MPS-IIIA-IDSspSGSH, MPS-IIIA-IDSspSGSH-IRES-SUMF1 VS MPS-IIIA-SGSH. One-way ANOVA followed by Tukey's post hoc test.

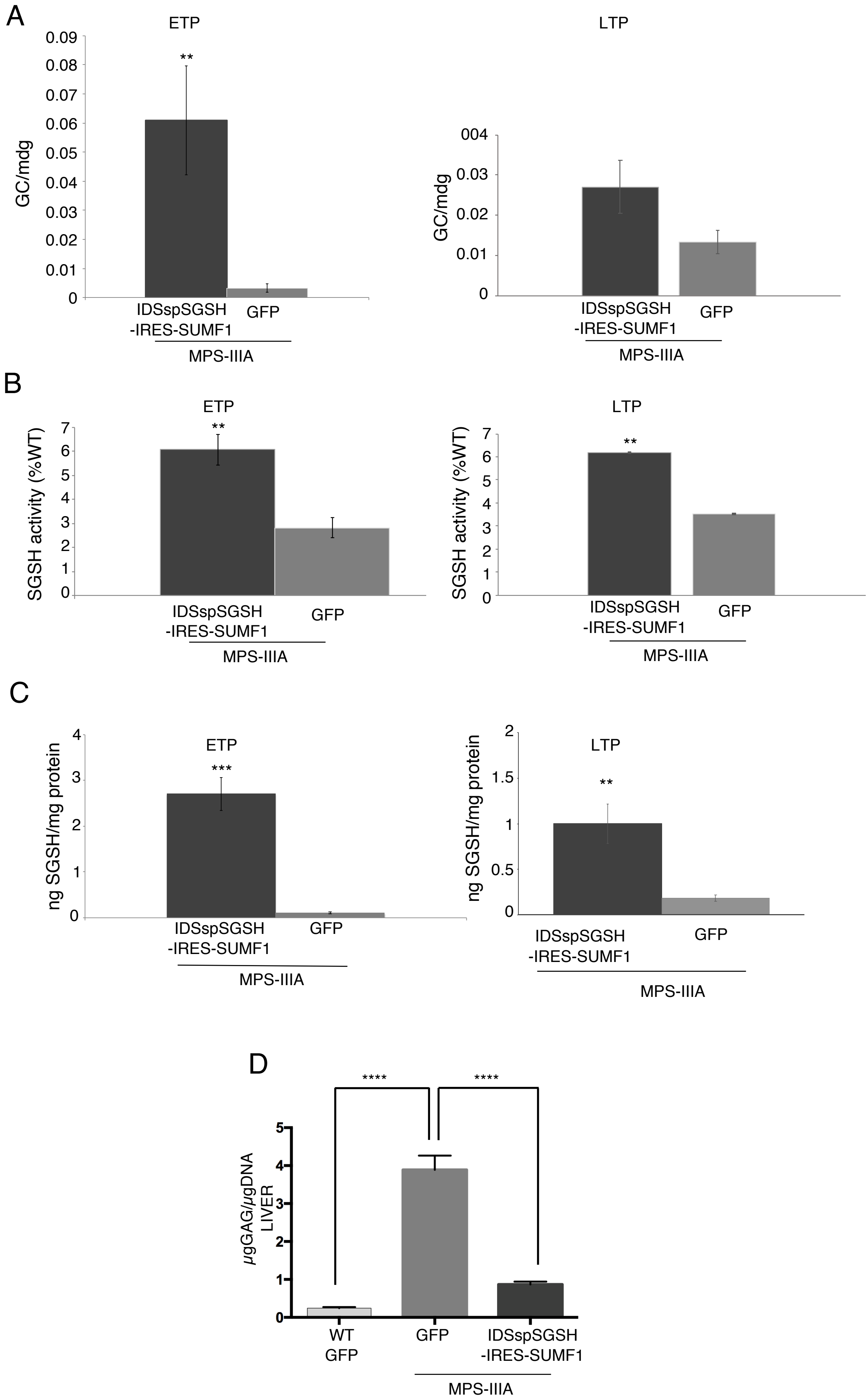
Supplementary Figure 2



Supplementary Figure 2. Sulfamidase protein and vector copy numbers quantitation in the brain of MPS-IIIA mice injected with AAV9 bearing the IDSspSGSH-IRES-SUMF1 transgene

(A) Sulfamidase protein was immuno-quantified by ELISA and expressed as ng of SGSH/mg protein in the five CNS slices (A-E; as described in the supplementary figure 1) of the indicated experimental groups of mice at ETP and LTP. Age-matched WT and MPS-IIIA mice ICV injected with AAV9 encoding for GFP were used as control. Data represent mean ± SEM. N= 5-7 animals for each group. **P<0.01, ***P<0.001, ****P<0.0001 VS MPS-IIIA-GFP. One-way ANOVA followed by Tukey's post hoc test. (B) Vector genome copy number (expressed as GC/mouse diploid genome; mdg) were measured in the whole brain samples from MPS-IIIA mice ICV injected with AAV9 encoding IDSsp*SGSH*-IRES-*SUMF1* and age-matched MPS-IIIA mice ICV injected with AAV9 encoding GFP at ETP and LTP. Data represent mean ± SEM. N= 5-7 animals for each group. *P<0.05, Student T-test.

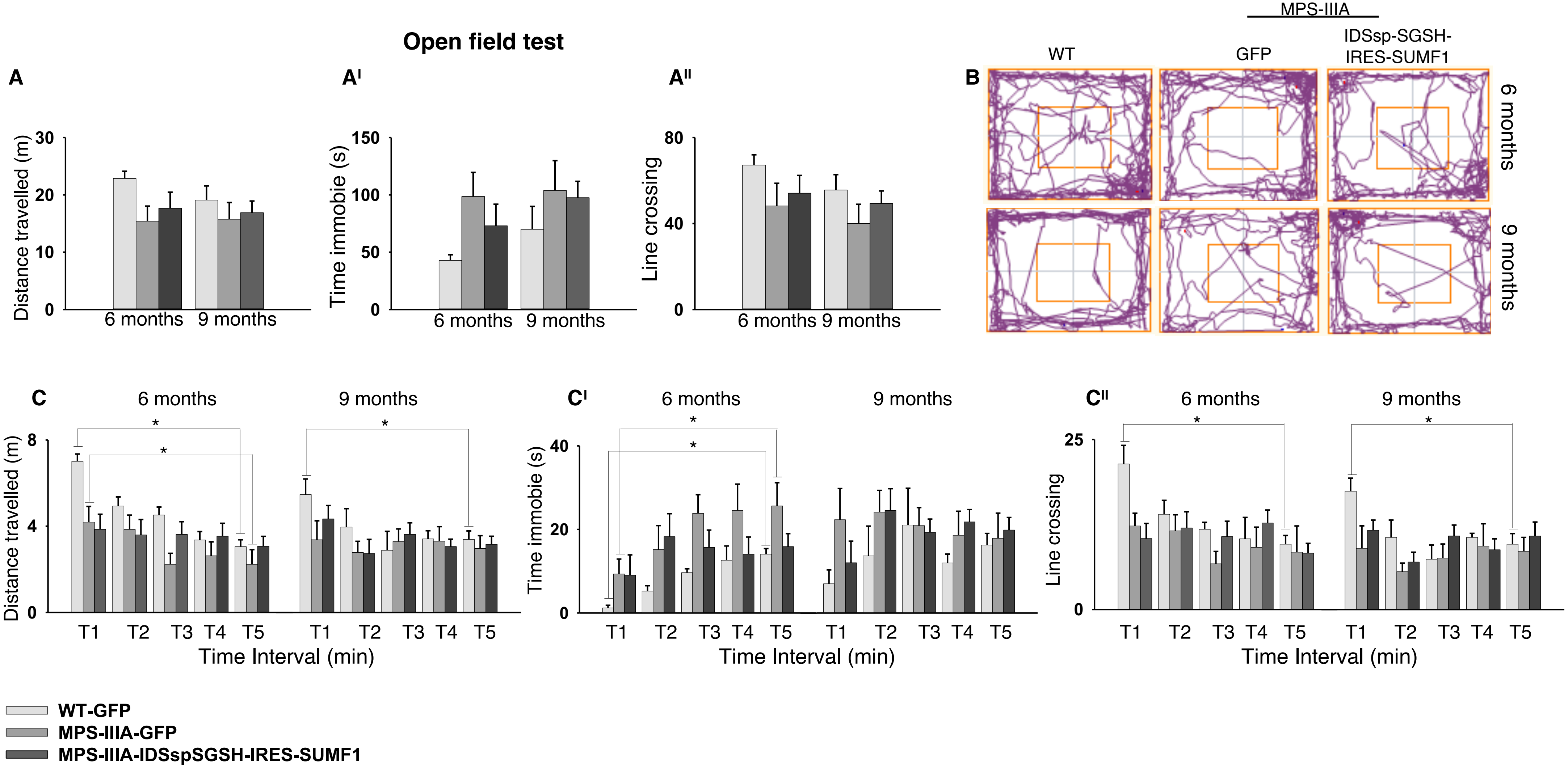
Supplementary Figure 3



Supplementary Figure 3. Liver transduction in MPS-IIIA mice injected with AAV9 bearing IDSspSGSH-IRES-SUMF1 transgene

(A-C) Vector genome copy number (expressed as GC/mouse diploid genome; mdg) (A), sulfamidase activity (expressed as the percentage of WT sulfamidase activity) (B) and ELISA immunoquantification of the sulfamidase protein (expressed as ng of SGSH/mg protein) (C) were measured in liver samples from MPS-IIIA mice ICV injected with AAV9 encoding IDSsp*SGSH*-IRES-*SUMF1* and age-matched WT and MPS-IIIA mice ICV injected with AAV9 encoding GFP at ETP and LTP. Data represent mean ± SEM. N= 6-8 animals for each group. **P<0.01, ***P<0.001. *vs* MPS-IIIA GFP-treated. Student T-test. (D) Quantitative analysis of GAG content (µgGAG/µgDNA) in liver samples collected at LTP in MPS-IIIA mice ICV injected with AAV9 encoding *IDSspSGSH*-IRES-*SUMF1*. Age-matched WT and MPS-IIIA mice ICV injected with AAV9 encoding GFP were used as controls. N = 6 animals per group. Data represent mean ± SEM; ****P<0.0001. One-way ANOVA followed by Tukey's post hoc test.

Supplementary Figure 4



Supplementary Figure 4. Assessment of exploratory activity in MPS-IIIA mice injected with AAV9 bearing IDSspSGSH-IRES-SUMF1 transgene.

MPS-IIIA mice and relative controls (WT) were tested at 6 and 9 months of age in the open field test. (A- AII) There were no significant differences between groups at any of the testing age in the total distance travelled (m) [Group (F2/16=1.16; p=0.22); Distance (F1/16=0.65; p=0.43); Group x Distance x Age (F2/16=0.45; p=0.64)] (A), total number of line crossings [Group (F2/16=1.94; p=0.17); Line crossing (F1/16=1,77; p=0.20); Group x Line crossing x Age (F2/16=0.24; p=0.78)] (AI) and total immobility time (sec) [Group (F2/16=1.46; p=0.25); Immobility time (F1/16=2.31; p=0.14); Group x Immobility time x Age (F2/16=0.12; p=0.88)] (AII). (C-CII) A deeper analysis of the results considering 1 min time intervals (T1-T5) evidenced that at 6 months of age MPS-IIIA mice, as compared to WT littermates, showed reduced distance travelled [Time interval (F4/64=17.9; p<0.0001); Time intervals x Group (F4/64=3.16; p=0.004), Age x Time intervals x Group (F4/64=2.6; p=0.01)] (C), increased immobility time [Time intervals (F4/64=2.4; p=0.05); Group x Age x Time interval (F8/64=2.04; p=0.05)] (CI) and reduced line crossing frequency [Time interval (F4/64=6.8; p=0.0001); Group x Time intervals (F4/64=2.66; p=0.01)] (CII) mainly present in the very first minute of the task; these behavioral defects, however, were not anymore detectable at 9 months of ageing probably due to a test-retest habituation effect observed in WT animals [distance, age x time interval (F8/64=3.73; p=0.0085); time immobile, age x time intervals (F4/64=1.61; p=0.05), line crossing, age x time intervals (F4/64=2.4; p=0.05)]. Representative track-plots of the trajectory in the open field (B). * p<0.05, Duncan post hoc analysis.

Supplementary Table 1

Pvalue of SGSH activity in CNS areas of wild type pigs

CNS regions	IDSsp-SGSH-IRES-SUMF1 vs IDSspSGSH	IDSsp-SGSH-IRES-SUMF1 vs SGSH-IRES-SUMF1	IDSsp-SGSH-IRES-SUMF1 vs SGSH	IDSsp-SGSH-IRES-SUMF1 vs PBS
Frontal cortex	ns	ns	*	**
Accumbens	**	**	****	****
Parietal ccortex	ns	**	***	****
Amigdala	ns	*	**	***
Hypotalamus	ns	**	*	**
N.caudatus	ns	*	*	***
Putamen	ns	**	*	***
Sub.Nigra	ns	*	*	**
Stria	ns	*	*	***
Hippocmpus	ns	**	**	***
Thalamus	*	***	**	****
C.callosum	*	****	****	****
Subcallosus	*	**	***	****
N.Pontis	ns	ns	ns	**
Culliculli	*	**	*	***
Occipital cortex	ns	**	**	***
Cerebellum	ns	*	*	***
Pons	**	*	**	****
Med. Ablong.	ns	*	ns	***
Spinal cord	ns	ns	*	***

P ≤0,05; ** P ≤0,01; *** P ≤0,001; **** P ≤0,001; ns: not significant.
No significant differences of SGSH activity in CNS regions among SGSH-IRES-SUMF1 VS SGSH experimental groups.