OMTN, Volume *35*

Supplemental information

Identification of a histone deacetylase inhibitor as a therapeutic candidate for congenital central hypoventilation syndrome

Chiara Africano, Tiziana Bachetti, Paolo Uva, Gabriel Pitollat, Genny Del Zotto, Francesca Giacopelli, Giada Recchi, Nicolas Lenfant, Amélia Madani, Nathan Beckouche, Muriel Thoby-Brisson, and Isabella Ceccherini

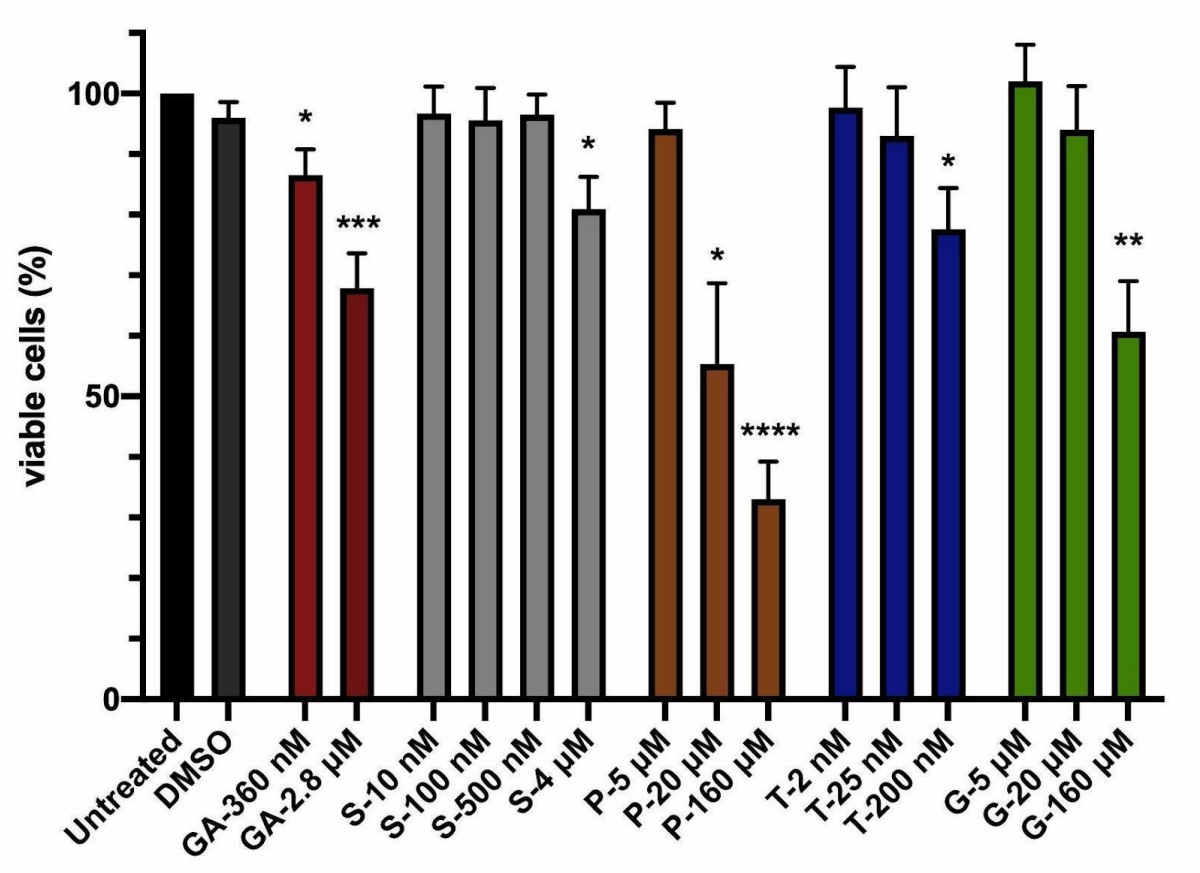

Figure S1. MTT assay to evaluate the *in vitro* toxicity of compounds and doses used for treatments.

Values are given as mean and SD obtained from 3 independent MTT assay experiments testing two doses used for subcellular localization experiments and a dose eight times the highest concentration. Molecules tested for 48 hours on Hela cells are Geldanamycin (GA), SAHA (S), Parthenolide (P), Trichostatin-a (T), and Guggulsterone (G). Student t-test assay confirmed that the GA-360 nM and the P-20 µM conditions significantly reduced cell viability compared to cells treated with DMSO, the commonly used drug solvent ($p<0.05$). However, all other treatments maintain good cell survival at experimental doses. Among toxic concentrations, GA-2.8 µM, P-160 µM and G-160 µM statistically reduced cell viability by 40%, 70% and 40% respectively. Instead, cells treated with the highest dose of S-4 µM still maintain a good cell viability, confirming the safety of the drug we chose. (Unpaired t-test * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$)

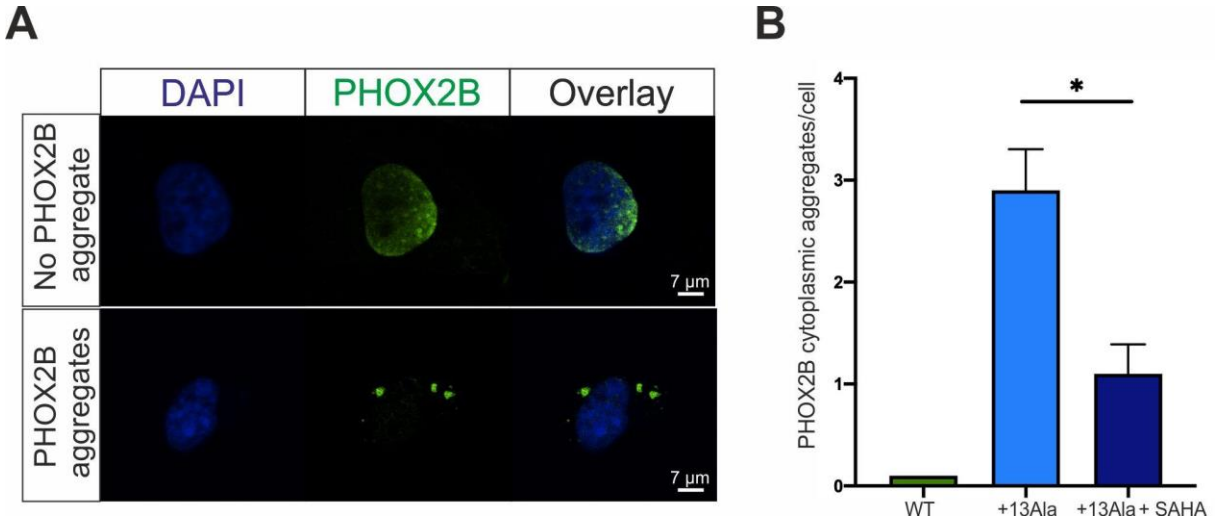

Figure S2. Analysis of the effect of SAHA on aggregation.

COS-7 cells were transfected with plasmids pcDNA3.1-PHOX2B-WT, pcDNA3.1-PHOX2B+7Ala and pcDNA3.1-PHOX2B+13Ala and treated with SAHA 10 nM. After 24 hours, cells were washed with PBS 1X, fixed 15 min in PFA 4%, and permeabilized 20 min with 0.1% TritonX100 in PBS, blocked 45 min in 0.1% Tween20 2% BSA in PBS and incubated 1 hour at room temperature with a specific PHOX2B primary antibody (1:500), followed by 30 minutes with the anti-mouse secondary antibody A488 (1:1000). The Slide was covered with ProLongTM antifade reagent (Thermo Fischer Scientific, USA) and DAPI (Roche, Swiss) for nuclei staining. PHOX2B cytosolic aggregates were analysed using a confocal microscope Leica SP5 (63X magnification) while the ImageJ Aggrecount (Klickstein et al., 2020) was used to quantify protein aggregates present in the cytoplasm per cell. (A) A representative image of cell with no PHOX2B aggregates is reported on the top. A representative image of cell with PHOX2B aggregates is reported on the bottom. COS-7 nuclei are represented in blue (DAPI), PHOX2B protein in green. Overlaps are illustrated on the right. (B) The histogram shows that in the WT condition there are no cytosolic aggregates, with aggregation becoming evident in the present of a mutant protein. Interestingly, PHOX2B+13Ala treated with SAHA shows a statistically significant decrease in the number of cytosolic aggregates per cell. Values are expressed as the mean ± SEM (Paired t- test * $p<0.05$).

Klickstein JA, Mukkavalli S, Raman M. (2020) AggreCount: an unbiased image analysis tool for identifying and quantifying cellular aggregates in a spatially defined manner. J Biol Chem. ***295****:17672-17683.*

Table S1: Pairwise differential gene expression between WT and +13Ala PHOX2B transfected cells, either 17AAG treated or untreated. (see the Supplementary Table S1.xls file)