ATP13A3 is a major component of the enigmatic mammalian polyamine transport system

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ATP13A3 is involved in Polyamine Transport

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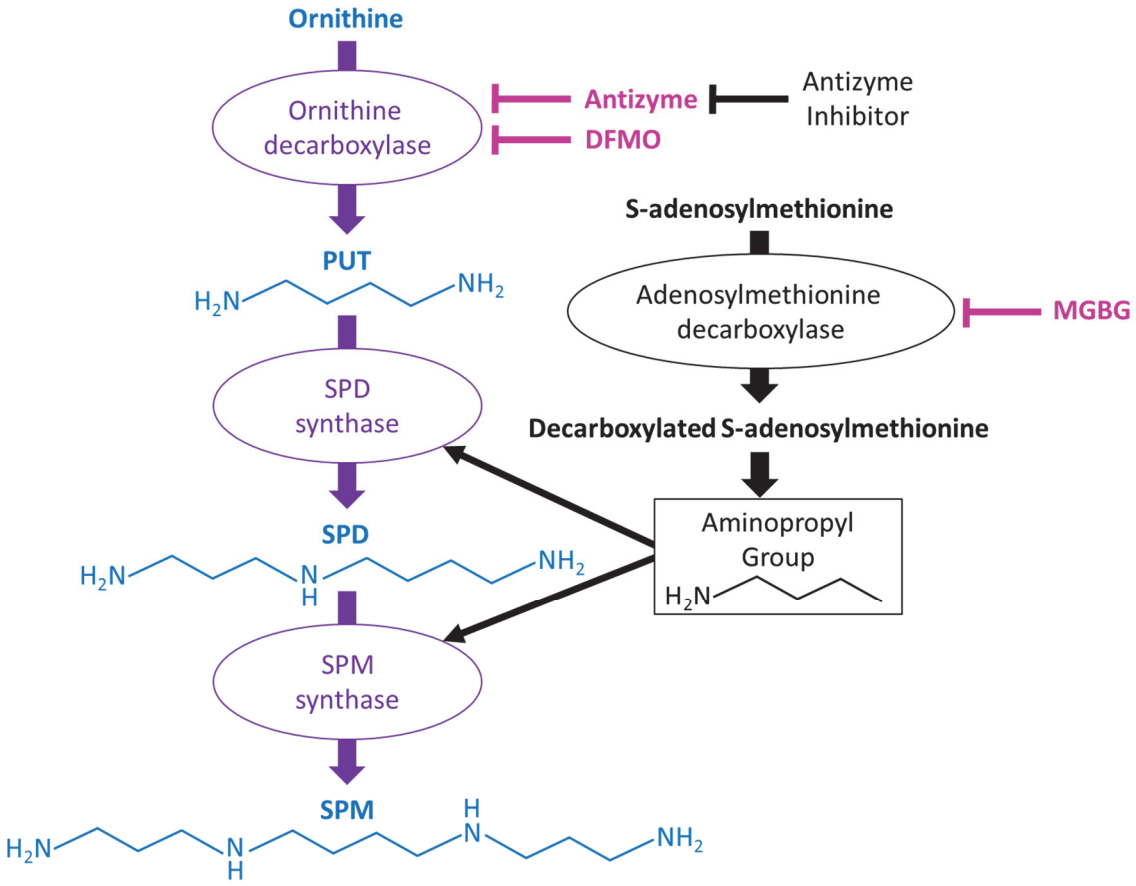

Figure S1. Polyamine biosynthesis pathway and its inhibitors. DFMO, difluoromethylornithine; **MGBG**, methylglyoxal bis-(guanyl hydrazone); **SPM**, spermine; **SPD**, spermidine; **PUT**, putrescine

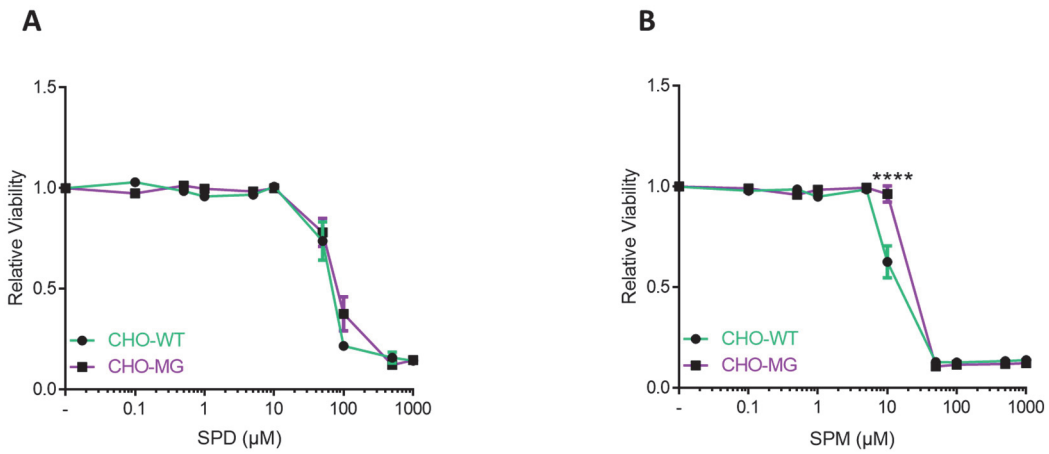

Figure S2. Effect of polyamines on the viability of CHO-MG cells. *A, B,* Cells were treated for 24 h with the indicated concentrations of only SPD *(A)* and SPM *(B)*. CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS) was used to assess cell viability and dose-response curves were plotted (n=3). Data represent mean ± SEM (*****P*<0.0001) generated with two-way ANOVA and Bonferroni *post hoc* corrections. **SPM**, spermine; **SPD**, spermidine.

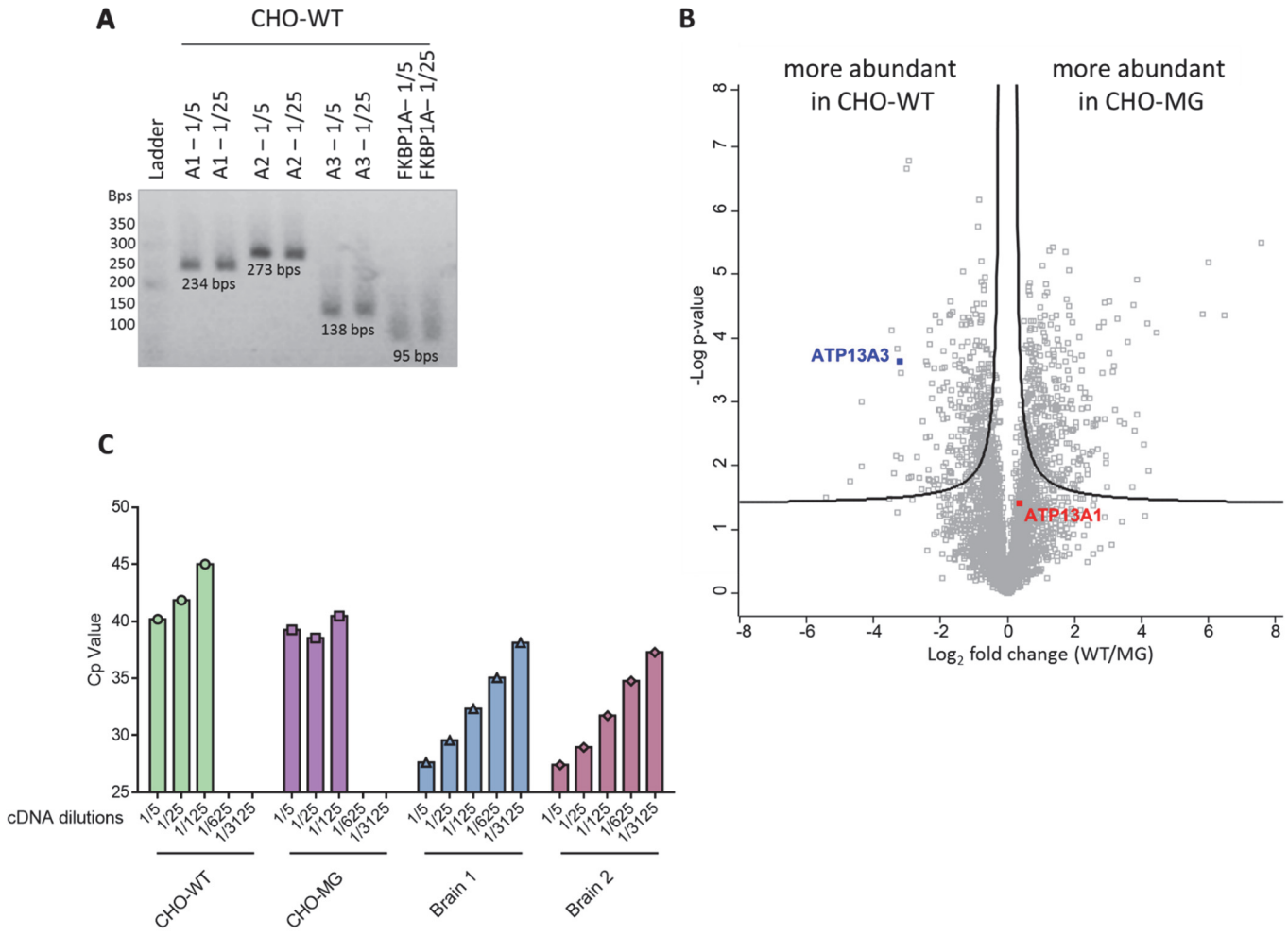

Figure S3. ATP13A3 is downregulated in CHO-MG cells. *A,* ATP13A1-3 mRNA levels were measured with qPCR using SYBR Green master mix and qPCR products from two cDNA dilutions (1/5, 1/25) were run on an agarose gel to check the specificity of the primers. *B,* Proteomic analysis was carried out on membrane fractions of CHO-WT and CHO-MG cells and detected proteins including ATP13A3 were plotted in a volcano plot (n=4). *C,* Cp values, demonstrating the ATP13A4 mRNA primer linearity in hamster brain tissues *versus* the CHO cells, are shown across the indicated cDNA dilutions. **A1-A3**, ATP13A1-3; **FKBPA1**, FKBP prolyl isomerase 1A

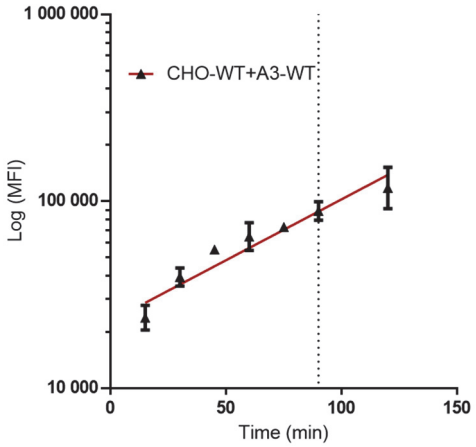

Figure S4. Uptake of BODIPY-PUT uptake falls within the linear phase. Cells were treated with 5 µM BODIPY-PUT at 37°C for the indicated times**.** Uptake was measured in terms of mean fluorescence intensity (MFI) up to 1 x 10^4 events (debris-free) per condition on the flow cytometer. Data represent mean ± SEM. The dashed line refers to the time point that was used for the treatment of BODIPY-polyamines in all flow cytometry experiments (n=2-4). **A3-WT**, overexpression of wild type ATP13A3; **A3-DN**, overexpression of ATP13A3 catalytically dead mutant D498N; **BODIPY**, boron dipyrromethene; **PUT**, putrescine

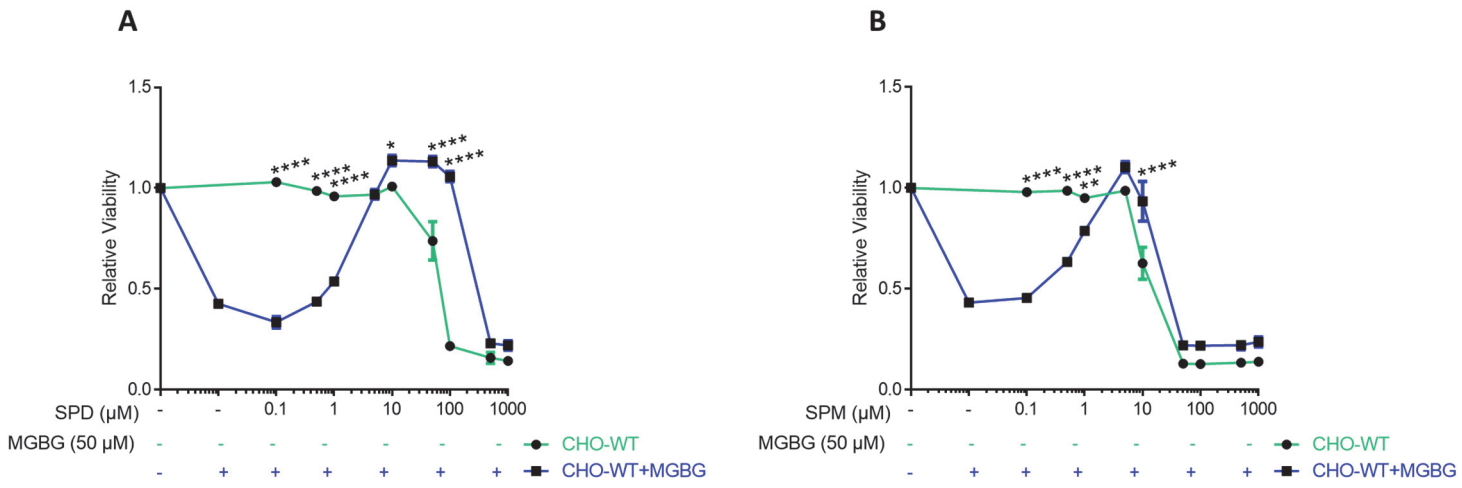

Figure S5. Competition of MGBG with SPD and SPM in CHO-WT cells. *A, B,* Cells were treated for 24 h with the indicated concentrations of SPD (n=3) *(A)* and SPM (n=3) *(B)* with or without 50 μM MGBG co-treatment. CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS) was used to assess cell viability and dose-response curves were plotted. Data represent mean ± SEM (**P*<0.05, ***P*<0.01, ****P*<0.001, and *****P* <0.0001) generated with two-way ANOVA and Bonferroni *post hoc* corrections. **MGBG**, methylglyoxal bis-(guanyl hydrazone); **SPM**, spermine; **SPD**, spermidine

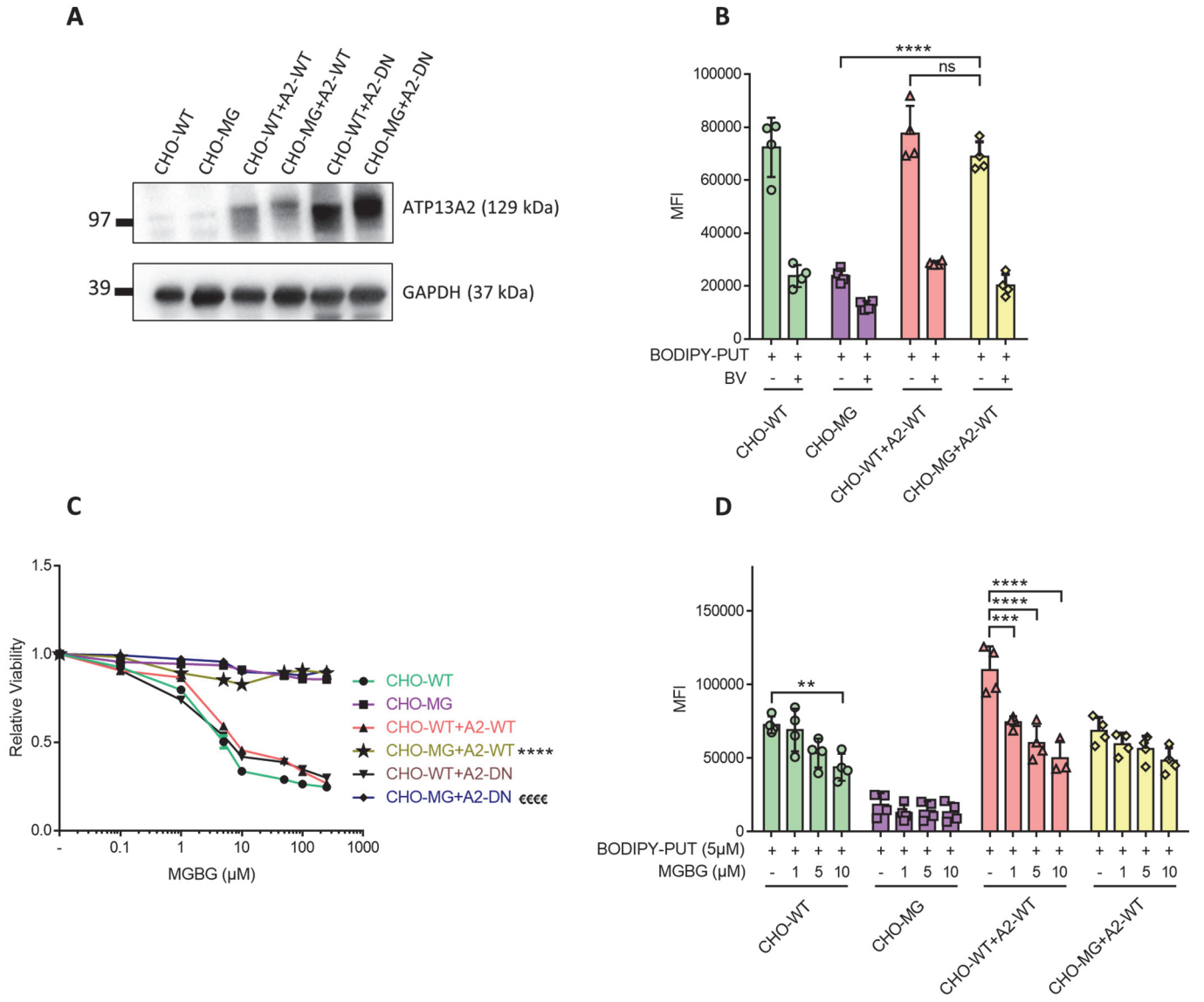

Figure S6. Expression of wild-type ATP13A2 restores polyamine uptake, but it does not affect MGBG resistance in CHO-MG cells. *A,* Stable cell lines were generated by lentiviral transduction to overexpress wild type (A2-WT) or a catalytically dead mutant (A2-DN) of ATP13A2. Expression of the viral vectors was verified by immunoblotting using ATP13A2 selective antibody, while the loading was monitored by house-keeping protein GAPDH selective antibody. *B, D,* Cells were treated with 5 μM BODIPY-PUT alone (B) or combined with MGBG (D) for 90 min at 37°C with or without 90 min of 1 mM BV pre-treatment to inhibit polyamine uptake. Uptake was measured in terms of mean fluorescence intensity (MFI) up to 1 x 10^4 events (debris-free) per condition on the flow cytometer (n=4). *C,* Cells were treated for 24 h with different doses of MGBG. Cell viability was assessed using CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS) and dose-response curves were plotted (n=3). Data represent or mean ± SD *(B, D)* and individual data points (representing replicates) are overlaid on bar graph plots, or mean ± SEM *(C)* (**P<0.01, ****/$^{€€€€}P$<0.0001, ns = not significant, **** *vs* CHO-WT+A2-WT, and $^{€€€€}$ *vs* CHO-WT+A2-DN). Analyses were performed using two-way ANOVA and Bonferroni post hoc corrections. **BODIPY**, boron dipyrromethene; **BV**, benzyl viologen; **GAPDH**, Glyceraldehyde 3-phosphate dehydrogenase; **MGBG**, methylglyoxal bis-(guanyl hydrazone); **PUT**, putrescine; **A2-WT**, overexpression of wild type ATP13A2; **A2-DN**, overexpression of ATP13A2 catalytically dead mutant D508N