Cell Reports, Volume *34*

Supplemental Information

Vesicular Glutamate Transporters (SLCA17 A6, 7, 8) Control Synaptic Phosphate Levels

Cyril Cheret, Marcelo Ganzella, Julia Preobraschenski, Reinhard Jahn, and Gudrun Ahnert-Hilger

Figure S1

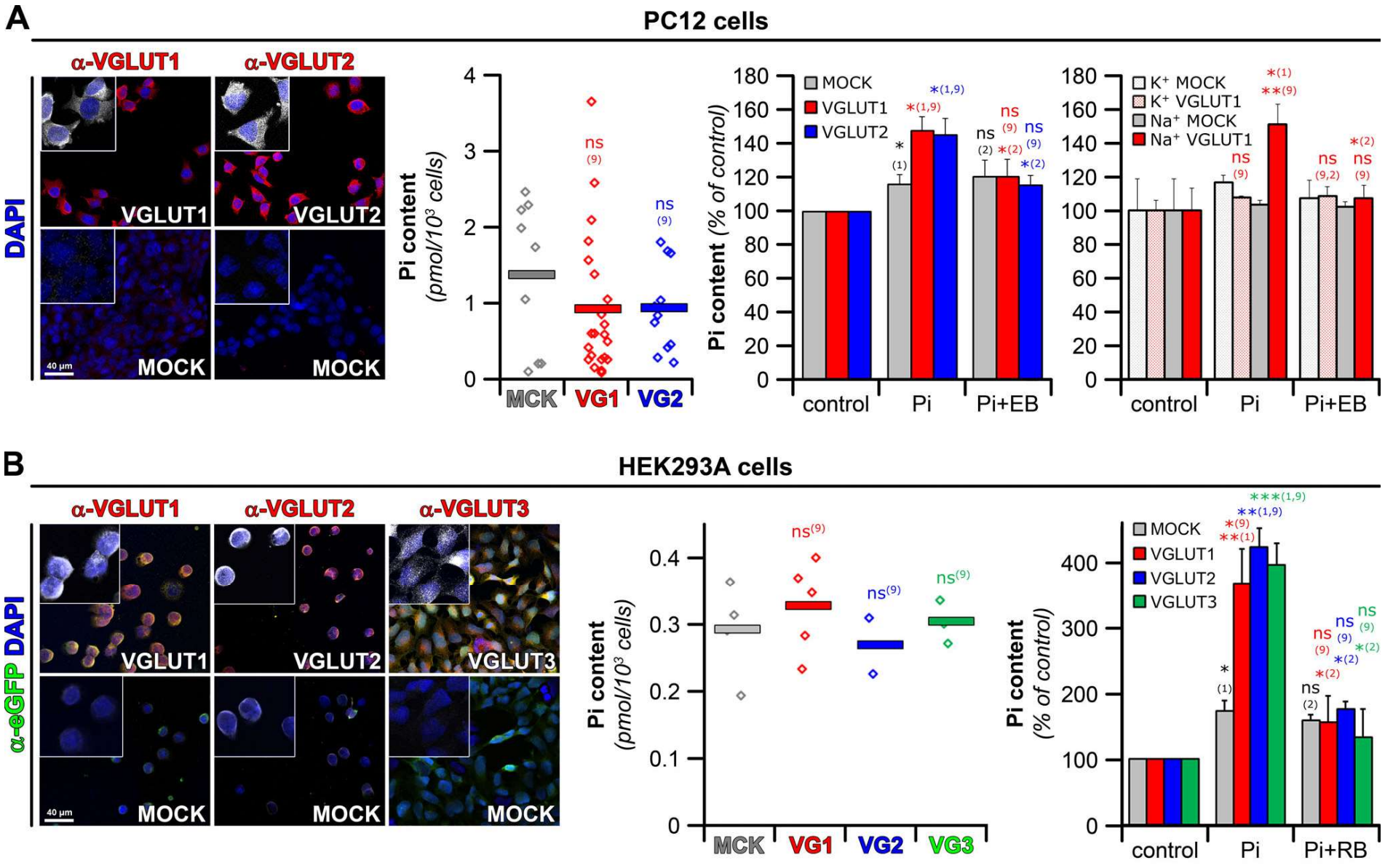
A
PC12 cells
α-VGLUT1
α-VGLUT2
DAPI
VGLUT1
VGLUT2
MOCK
MOCK
40 µm
Pi content (pmol/10³ cells)
MCK
VG1
VG2
Pi content (% of control)
MOCK
VGLUT1
VGLUT2
control
Pi
Pi+EB
K⁺ MOCK
K⁺ VGLUT1
Na⁺ MOCK
Na⁺ VGLUT1
B
HEK293A cells
α-VGLUT1
α-VGLUT2
α-VGLUT3
α-eGFP DAPI
VGLUT1
VGLUT2
VGLUT3
MOCK
MOCK
MOCK
40 µm
Pi content (pmol/10³ cells)
MCK
VG1
VG2
VG3
Pi content (% of control)
MOCK
VGLUT1
VGLUT2
VGLUT3
control
Pi
Pi+RB

Figure S1 *(refers to Fig. 1A)*: **Pi content in VGLUT-1, -2 and -3 transfected PC12 and HEK293A cell lines**

A,B) IHC (left) in PC12 (A) or HEK293A (B) cells indicate high expression of VGLUT-1, -2 or -3 when stably transfected with the respective IRES-eGFP constructs. Such VGLUTs-specific signals were absent in the MOCK clones expressing only eGFP. Inserts show 2 fold-increased magnification of cells labeled with DAPI (blue) and immunostained for the respective VGLUT variant (white).

A) PC12 cells were incubated in Pi-free media for 1 h followed by depolarization (10 min) and repolarization (30 min) in the absence of Pi. Pi uptake was performed by incubation for 15 min in uptake buffer $\pm$ 10 mM Pi + EB. Pi concentration (given as pmol Pi/10^3 cells) in controls (complete absence of Pi during starvation and incubation) were comparable between the different PC12 clones (left graph). Presence of either VGLUT variant increased Pi content upon Pi presentation (middle graph), which was abrogated by the VGLUT inhibitor EB (10 µM). Values were normalized to the controls (no Pi) presented in the left graph. Substituting Na^+ by K^+ (both 80 mM) abolished Pi accumulation.

B) Middle and Right: HEK293A cells were incubated in Pi-free media for 2 h. Pi uptake was performed by incubation for 15 min in uptake buffer ± 10 mM [Pi] ± RB. Pi concentrations (given as pmol Pi/10^3 cells) in the controls (complete absence of Pi during the whole experiment) were comparable between the different HEK293A lines (middle). A potent increase in cellular Pi levels (right) was observed when either VGLUT variant was expressed, which was abrogated in the presence of the VGLUT inhibitor RB (10 µM). Values are normalized to the controls (no Pi) given in the middle panel.

Data show mean values ± SEM from 2 to 9 independent experiments.

Significance towards: (1) No Pi; (2) Pi; (9) MOCK.

Figure S2

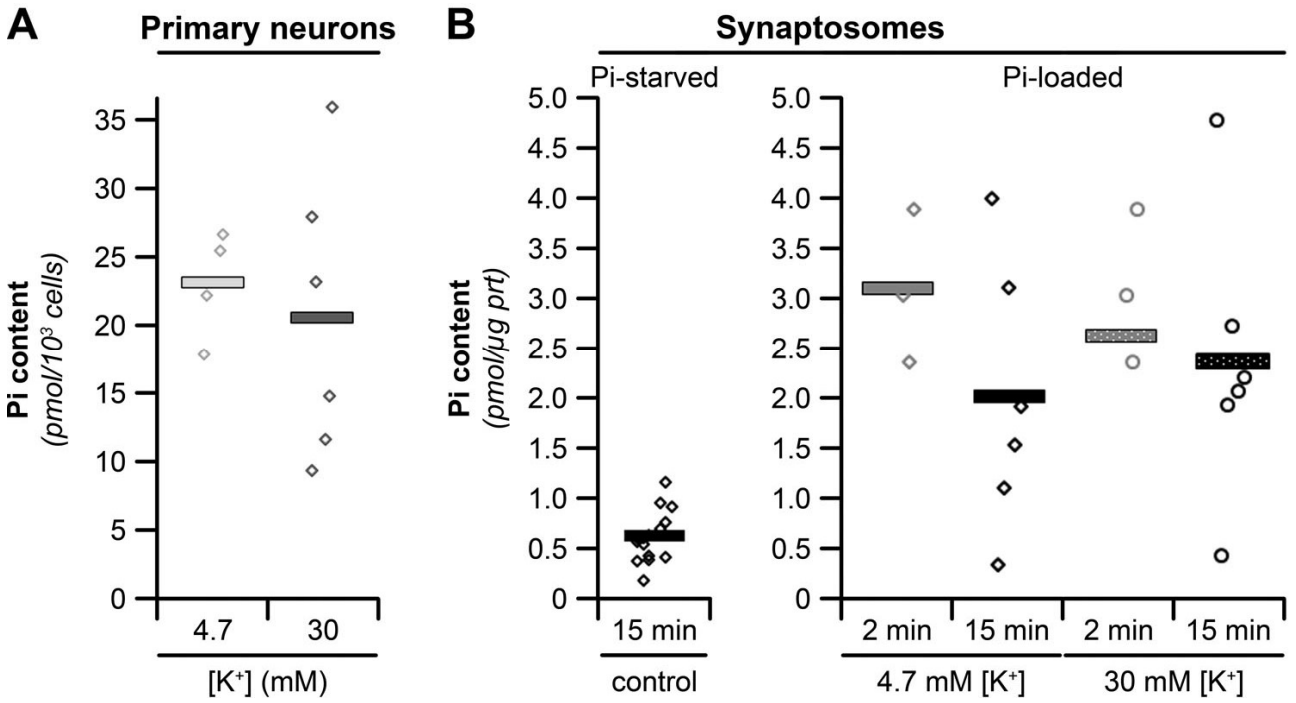

Figure S2 *(refers to Fig. 1B)*: **Pi content of neurons and synaptosomes**

A) Pi content of cultivated neurons under control conditions (cells were never exposed to Pi throughout the experiment, see Fig. 2) given as pmol Pi/10^3 cells from at least 4 different preparations (mean values ± SEM). The stimulation procedure did not change resting cytoplasmic Pi concentrations.

B) Pi content of adult rat synaptosomes depolarized/repolarized in the absence of Pi (left) or with 10 mM [Pi] (right) successively in KREBS 30 mM [K^+] then KREBS 4.7 mM [K^+], before being further incubated in uptake buffer without Pi for 2 to 15 min (Left: Pi-starved; controls from Fig. 1C left. Right: Pi-loaded; controls from Fig.1C right).

Pi content after Pi preloading and 2-15 min incubation in Pi-free buffer declined with time, indicating a synaptosomal Pi efflux. As expected, Pi preloading strongly increased Pi content compared to Pi starvation (left vs right, 2nd column).

Data show values from 3 to 22 independent experiments.

Pi concentrations from all synaptosomal preparations are given as pmol/µg protein.

Figure S3

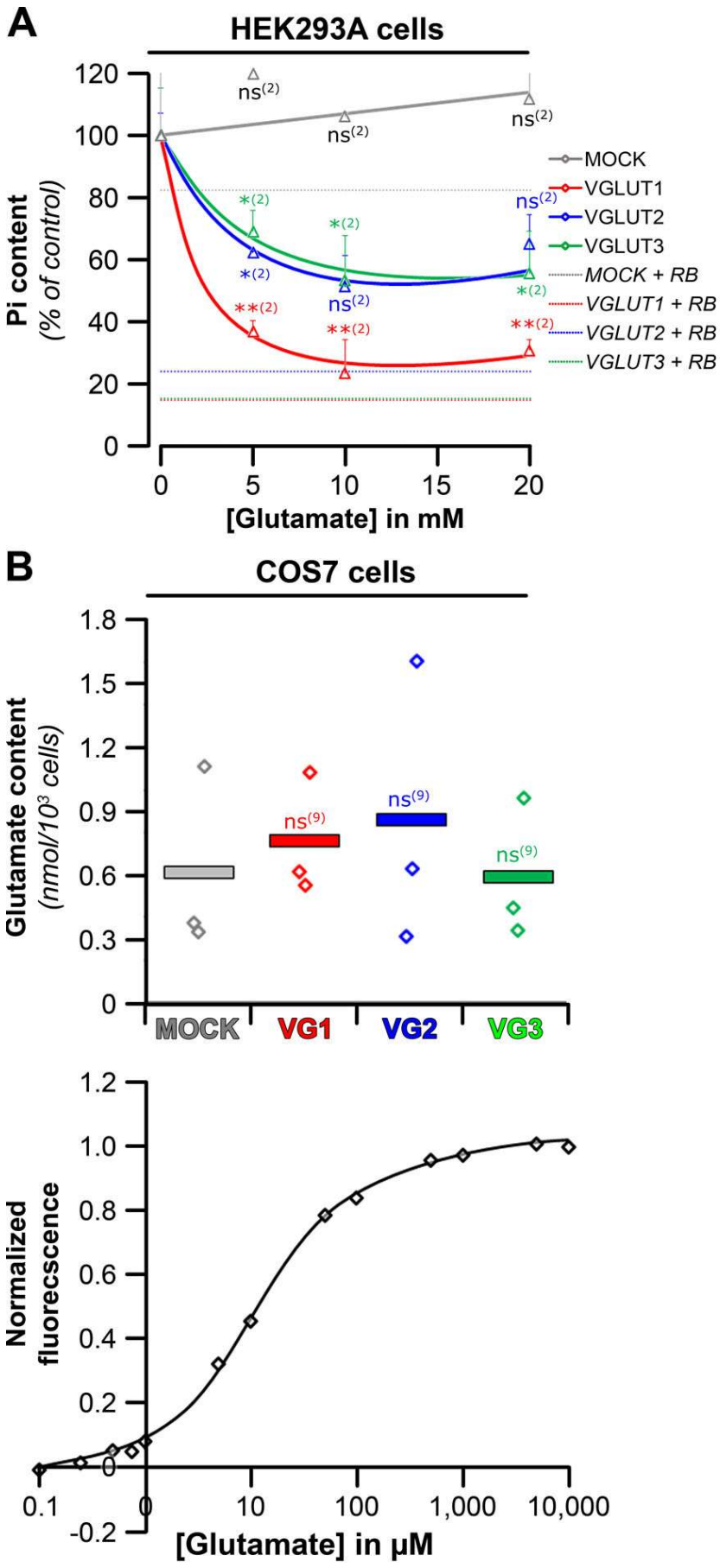
A
HEK293A cells
Pi content
(% of control)
MOCK
VGLUT1
VGLUT2
VGLUT3
MOCK + RB
VGLUT1 + RB
VGLUT2 + RB
VGLUT3 + RB
[Glutamate] in mM
B
COS7 cells
Glutamate content
(nmol/10³ cells)
MOCK
VG1
VG2
VG3
Normalized
fluorescence
[Glutamate] in µM

Figure S3 *(refers to Fig. 3)*: **Pi uptake and glutamate interference in cell lines expressing VGLUT variants**

A) Pi content of HEK293A-VGLUT1-3 or -MOCK cells exposed to 10 mM Pi in the presence of increasing concentrations of glutamate.
Data are expressed as % of control (10 mM [Pi], no [Glutamate] in uptake buffer). Dotted lines represent the condition Pi + RB and show the effect of complete VGLUT inhibition, for comparison. Though glutamate inhibited VGLUT-mediated Pi uptake as observed in COS7 cells and synaptosomes, VGLUT variants showed dissimilarities and Ki values for VGLUT1|2|3 were notably higher (3.41|3.39|4.64 mM, respectively) in the HEK293A clones than those observed in our other models.
Values represent the mean ± SEM from 1 (MOCK) to 4 experiments.
B) Glutamate content was measured in VGLUT-transfected COS7 cells after starving the cells in KREBS 4.7 mM [K+] buffer and uptake buffer, both glutamate- and Pi-free (control condition for Fig. 3A right).
The glutamate concentration curve obtained by the iGluSNfr method ranges from 10 µM to 10 mM [glutamate].
Values represent the mean ± SEM from at least 3 experiments.
Significance towards: (2) Pi; (9) MOCK.

Figure S4

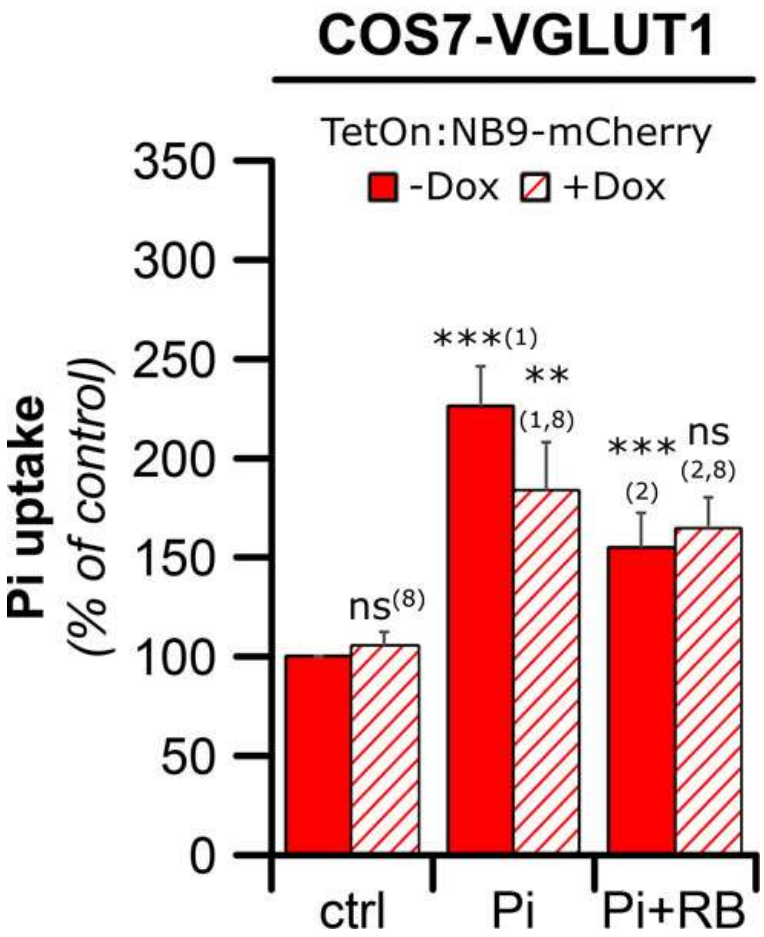

Figure S4 *(refers to Fig. 4)*: **Effect of NB9-2A-mCherry on Pi-uptake in COS7-VGLUT1 cells**

Doxycycline-induced expression of NB9 reduced Pi uptake to levels comparable to those observed in the presence of the VGLUT inhibitor RB in COS7-VGLUT1-TetOn:NB9-mCherry, preincubated in the absence (plain bars) or presence (striped bars) of 500 ng/mL [doxycycline]. Though this NB9-2A-mCherry construct showed a comparable NB9-specific Pi uptake inhibition as the reverse orientation construct (including non-cumulative NB9/RB inhibition), it appeared notably less efficient (compare to Fig. 4).
All values were normalized to control (no Dox, no Pi) and represent mean values ± SEM from 5 experiments.
Significance towards: (1) no Pi; (2) Pi; (8) no Dox.