Supporting Information

The post-synaptic scaffolding protein Tamalin regulates ligand-mediated trafficking of metabotropic glutamate receptors*

*Running title: Role of Tamalin in mGluR trafficking

Saurabh Pandey[1*], Namrata Ramsakha[1*], Rohan Sharma[1], Ravinder Gulia[1], Prachi Ojha[1], Wei Lu[2] and Samarjit Bhattacharyya[1,3]

[1] Department of Biological Sciences, Indian Institute of Science Education and Research Mohali, Mohali, Knowledge city, Sector – 81, SAS Nagar, PO: 140306, Punjab, India.

[2] Synapse and Neural Circuit Research Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA.

[3] To whom correspondence should be addressed: samarjit@iisermohali.ac.in, samarjitb@gmail.com

* These authors contributed equally to this work

S. Pandey's present address: Synapse and Neural Circuit Research Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA.

Content: Figures S1–S5

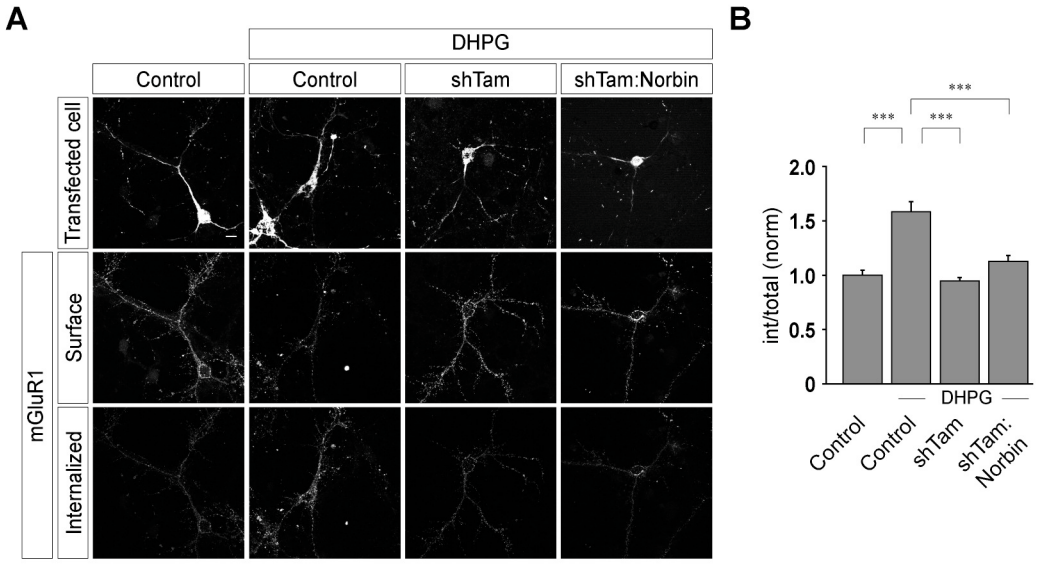
A
DHPG
Control
Control
shTam
shTam:Norbin
mGluR1
Transfected cell
Surface
Internalized
B
***
***
***
int/total (norm)
2.0
1.5
1.0
0.5
0
DHPG
Control
Control
shTam
shTam: Norbin

Figure S1

Figure S1. Replacement of the endogenous Tamalin with Norbin does not rescue the ligand-mediated endocytosis of mGluR1. (A) Representative images of surface and internalized myc-mGluR1, 30 min post 100 µM R,S-DHPG application in control cells, shTam expressing cells, shTam and wild-type Norbin expressing cells. (B) Quantitation of 100 µM R,S-DHPG induced endocytosis of myc-mGluR1 suggested that, knockdown of the endogenous Tamalin led to the inhibition of the endocytosis of myc-mGluR1 and replacement of the endogenous Tamalin with full-length Norbin did not rescue the normal endocytosis of the receptor (n values: control = 22, control + DHPG = 23, shTam + DHPG = 25, shTam:Norbin + DHPG = 16). All the results represented as means ± SEM. Scale bar = 10 µm. *** indicates $p < 0.001$.

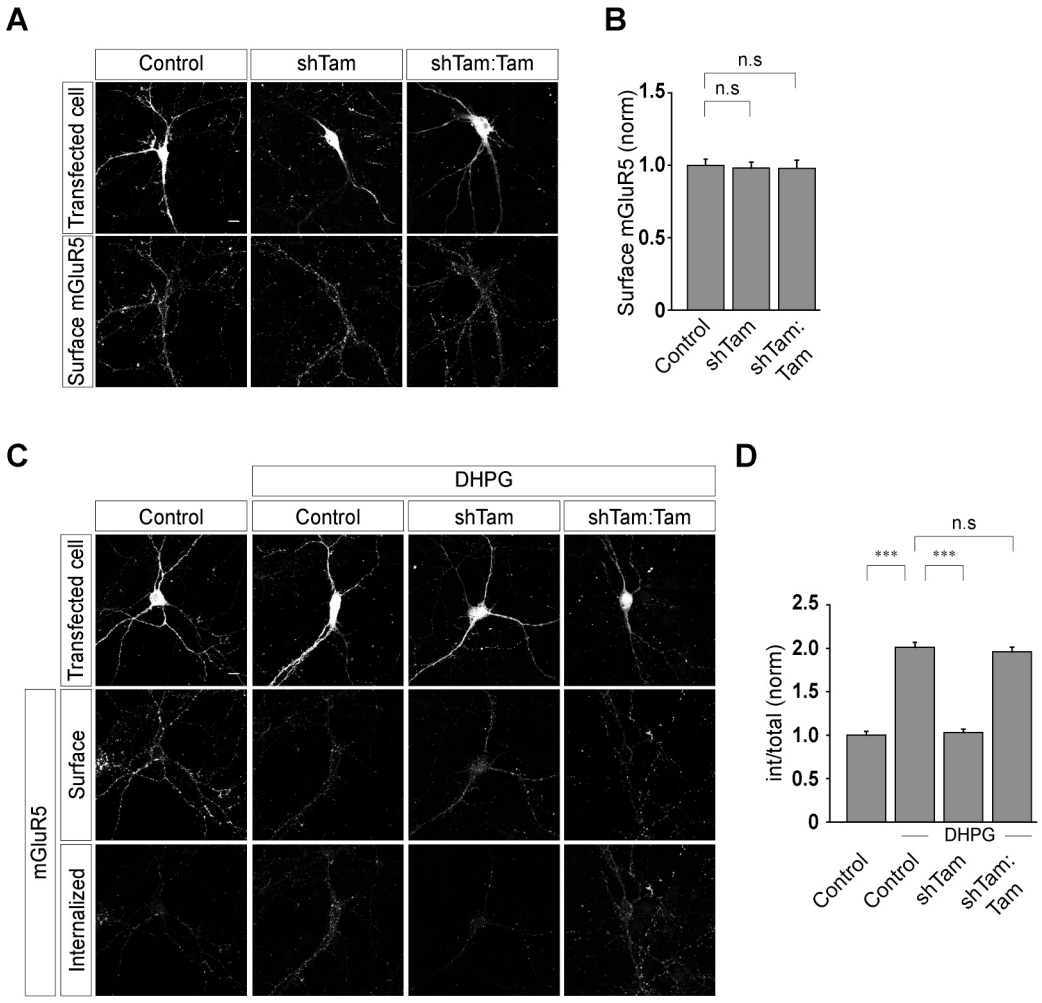
A
Control
shTam
shTam:Tam
Transfected cell
Surface mGluR5
B
n.s
n.s
Surface mGluR5 (norm)
1.5
1.0
0.5
0
Control
shTam
shTam:
Tam
C
DHPG
Control
Control
shTam
shTam:Tam
mGluR5
Transfected cell
Surface
Internalized
D
n.s
***
***
int/total (norm)
2.5
2.0
1.5
1.0
0.5
0
DHPG
Control
Control
shTam
shTam:
Tam

Figure S2

Figure S2. Knockdown of the endogenous Tamalin leads to the inhibition in the ligand-mediated endocytosis of mGluR5. (A) Representative images showing surface expression of myc-mGluR5 in control cells, shTam and shTam:Tam expressing cells. (B) Quantitation of myc-mGluR5 surface expression suggested that knockdown of the endogenous Tamalin and expression of the Tamalin replacement construct had no effect on the surface localization of myc-mGluR5 (n values: control = 31, shTam = 33, shTam:Tam = 30). (C) Representative images showing inhibition in the R,S-DHPG-mediated myc-mGluR5 endocytosis upon knockdown of the endogenous Tamalin and rescue of the endocytosis on expression of the Tamalin replacement construct. (D) Quantitation also suggested that knockdown of the endogenous Tamalin led to the inhibition in the ligand-mediated endocytosis of myc-mGluR5 which was rescued by the expression of the Tamalin replacement construct (n values: control = 31, control + DHPG = 32, shTam + DHPG = 32, shTam:Tam + DHPG = 32). All the results represented as means ± SEM. Scale bar = 10 µm. *** indicates $p < 0.001$ and n.s indicates $p > 0.05$.

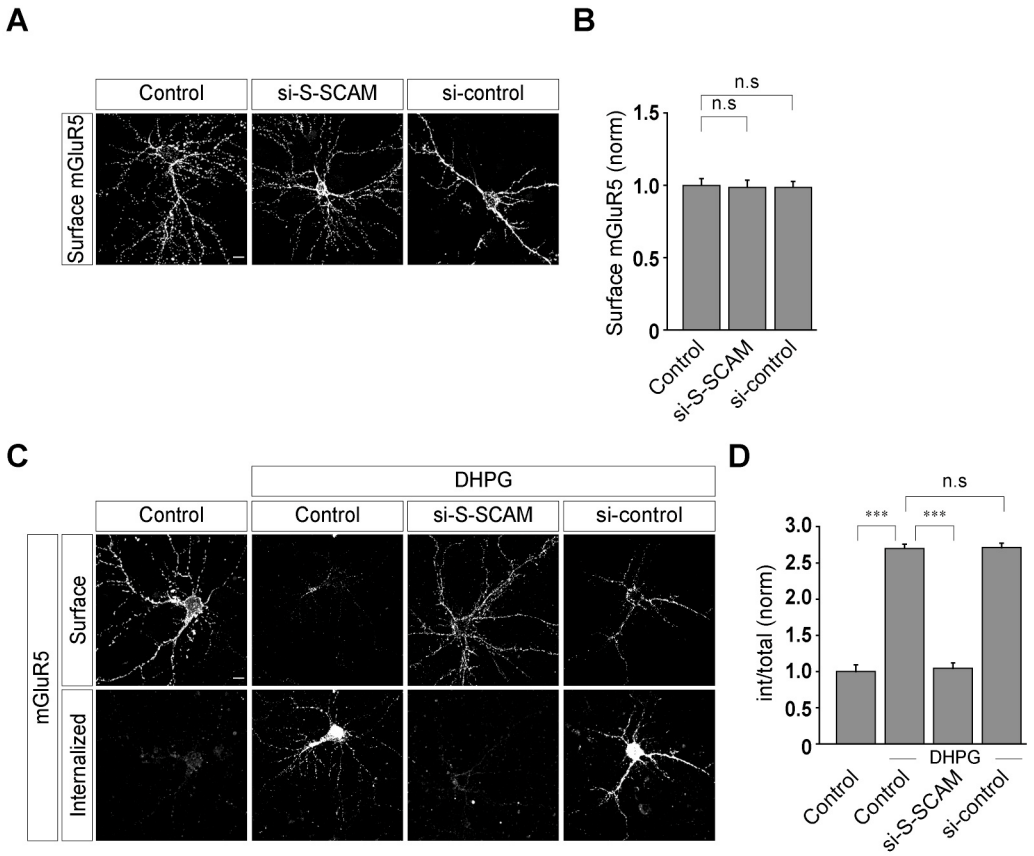
A
Control
si-S-SCAM
si-control
Surface mGluR5
B
n.s
n.s
Surface mGluR5 (norm)
1.5
1.0
0.5
0
Control
si-S-SCAM
si-control
C
DHPG
Control
Control
si-S-SCAM
si-control
mGluR5
Surface
Internalized
D
n.s
***
***
int/total (norm)
3.0
2.5
2.0
1.5
1.0
0.5
0
DHPG
Control
Control
si-S-SCAM
si-control

Figure S3

Figure S3. S-SCAM plays critical role in the ligand-mediated internalization of mGluR5. (A, B) Representative cells (A) and quantitation (B) suggested that acute knockdown of the endogenous S-SCAM had no effect on the surface expression of myc-mGluR5 (n values: control = 21, si-S-SCAM = 21, si-control = 21). (C, D) Representative images (C) and quantitation of the endocytosis index (D) suggested that knockdown of the endogenous S-SCAM led to the inhibition in the R,S-DHPG-mediated internalization of myc-mGluR5, whereas, in control cells and si-control transfected cells the receptor endocytosed normally (n values: control = 21, control + DHPG = 21, si-S-SCAM + DHPG = 20, si-control + DHPG = 20). All the results represented as means ± SEM. Scale bar = 10 μm. *** indicates $p < 0.001$ and n.s indicates $p > 0.05$.

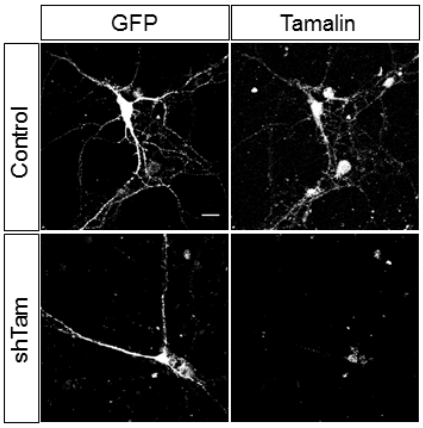

Figure S4

Figure S4: Knockdown of the endogenous Tamalin in primary hippocampal neurons. Representative images showing knockdown of the endogenous Tamalin by shTam in primary hippocampal neurons. Scale bar = 10 µm.

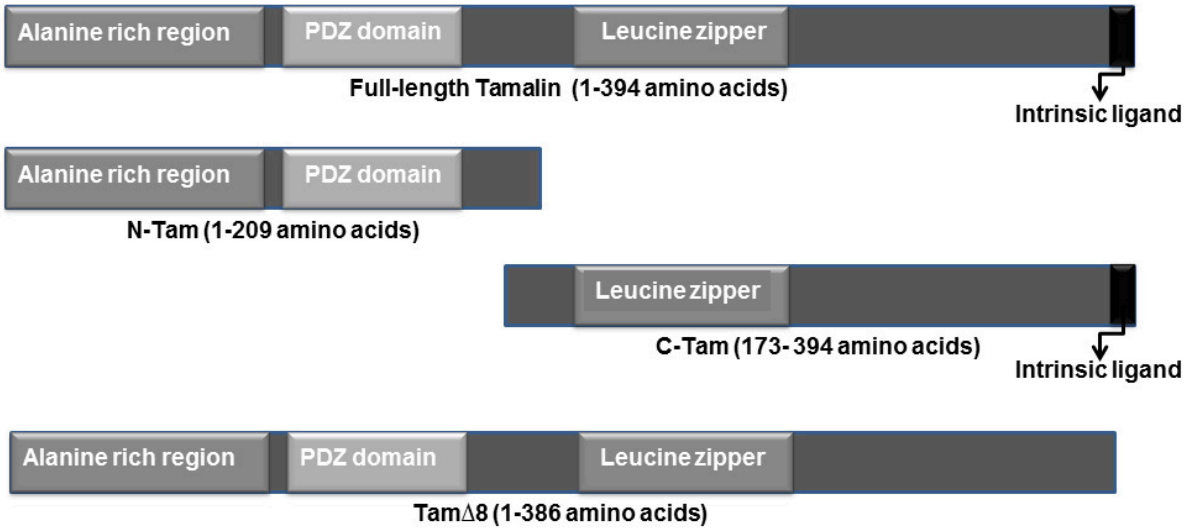
Alanine rich region
PDZ domain
Leucine zipper
Full-length Tamalin (1-394 amino acids)
Intrinsic ligand
Alanine rich region
PDZ domain
N-Tam (1-209 amino acids)
Leucine zipper
C-Tam (173- 394 amino acids)
Intrinsic ligand
Alanine rich region
PDZ domain
Leucine zipper
TamΔ8 (1-386 amino acids)

Figure S5

Figure S5. Schematic of various Tamalin constructs.