Suppl.3

A

<table>
<thead>
<tr>
<th>GST-PHnTSS</th>
<th>+</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>ephrin-B1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>stimulation</td>
<td>Fc</td>
<td>EphB2-Fc</td>
</tr>
</tbody>
</table>

IB:anti-ephrin-B1

37 -

pull glutathione sepharose

IB:anti-ephrin-B1

50 -

37 -

Total lysate

IB: anti-GST

50 -

75 -

Total lysate

B

IP

NRS

ephrin-B1

IB: anti-ephrin-B1

50 -

37 -

KD

IB: STEF PHn-TSS (anti-flag)

50 -

37 -

total lysate
Supplementary information 3

(A) The association of Tiam1 with ephrin-B1 is increased by the stimulation of EphB2-Fc. GST-tagged PHnTSS fragment of Tiam1 and ephrin-B1 were transiently expressed in 293T cells. After stimulation of transfected cell with preclustered EphB2-Fc (4 μg/ml) for 15 min, cells were lysed for affinity precipitation of GST-PHnTSS with glutathione sepharose. Co-precipitants were separated by SDS-PAGE, and immunoblotted with anti-ephrin-B1. Expressions of ephrin-B1 and PHnTSS fragment of Tiam1 are shown at the bottom.

(B) Ephrin-B1 associates with PHnTSS domain of STEF. 293T cells were transfected with plasmids encoding ephrin-B1 and flag-tagged PHnTSS domain of STEF. Cell lysate were immunoprecipitated with anti ephrin-B1 (C18) antibody or normal rabbit serum (NRS) as indicated, and then, immunoblotted with anti-flag antibody. Co-precipitated PHnTSS of STEF is indicated by an arrowhead.