Supplemental Figure Legends

**Supplemental Figure S1: Dose-dependent phosphorylation of the sst\textsubscript{2A} receptor.** HEK293 cells stably expressing the rat sst\textsubscript{2A} receptor were exposed to SS-14 in concentrations ranging from $10^{-9}$ to $10^{-4}$ M for 5 min. The levels of phosphorylated sst\textsubscript{2A} receptors (*upper panel*) and total sst\textsubscript{2A} receptors (*lower panel*) were then determined by Western blot analysis. The positions of the molecular mass markers are indicated on the *left* (in kDa).

**Supplemental Figure S2: Time-dependent phosphorylation of the sst\textsubscript{2A} receptor.** HEK293 cells stably expressing the rat sst\textsubscript{2A} receptor were exposed to 1 µM SS-14 for 0, 1, 2, 3, 5, 10, 20 min. The levels of phosphorylated sst\textsubscript{2A} receptors (*upper panel*) and total sst\textsubscript{2A} receptors (*lower panel*) were then determined by Western blot analysis. The positions of the molecular mass markers are indicated on the *left* (in kDa).

**Supplemental Figure S3: Lack of effect of RKIP levels on sst2A phosphorylation.** A-C, HEK293 cells stably expressing sst\textsubscript{2A} were transfected siRNA targeted to RKIP or non-silencing siRNA control (CTL) for 72 h and then exposed to 1 µM SS-14, 100 nM PMA or 1 µM SS-14 plus 100 nM PMA for 5 min. D-F, HEK293 cells stably expressing sst\textsubscript{2A} were transfected with empty vector (MOCK) or RKIP for two days and then exposed to 1 µM SS-14, 100 nM PMA or 1 µM SS-14 plus 100 nM PMA for 5 min. Equal protein levels were used for visualization of total and phosphorylated receptor levels and GRKs by Western blot analyses as described in "Materials and Methods". sst\textsubscript{2A} phosphorylation was quantified and expressed as percentage of the maximal phosphorylation in stimulated control cells. Data correspond to the mean ± SEM from at least four independent experiments performed in duplicate. The results were analyzed by two-tailed Student’s paired *t*-test. The positions of molecular mass markers are indicated on the *left* (in kDa).
Supplemental Figure S4: SOM230 inhibits octreotide induced sst<sub>2A</sub> phosphorylation. HEK293 cells stably expressing the rat sst<sub>2A</sub> receptor were exposed to SOM230 in concentrations ranging from 0 to 10<sup>-5</sup> M for 3 min prior the addition of 10<sup>-6</sup> M of octreotide for 5 min. The levels of phosphorylated sst<sub>2A</sub> receptors (upper panel) and total sst<sub>2A</sub> receptors (lower panel) were then determined by Western blot analysis. Note that SOM230 reduced the level octreotide-induced sst<sub>2A</sub> phosphorylation in a dose-dependent manner. The positions of the molecular mass markers are indicated on the left (in kDa).

Supplemental Figure S5: Octreotide and SOM230 stimulate ERK phosphorylation. HEK293 cells stably expressing the rat sst<sub>2A</sub> receptor were exposed to 1 µM octreotide or to 10 µM SOM230 for 0, 5, 10, 20, 30 min. The levels of phosphorylated ERK (upper panel) and total ERK (lower panel) were then determined by Western blot analysis. Shown is one of six independent experiments performed in duplicate. The positions of the molecular mass markers are indicated on the left (in kDa).

Supplemental Figure S6: Inhibition of G<sub>αi</sub> activity prohibits somatostatin-induced ERK activation. HEK293 cells stably expressing the rat sst<sub>2A</sub> receptor were either not pretreated (CTL) or pretreated with 300 nM pertussis toxin (PTX) for 18 h before exposure to 1 µM SS-14 for 0, 2, 5, 10 or 15 min. The levels of phosphorylated ERK (upper panel) and total ERK (lower panel) were then determined by Western blot analysis. The positions of the molecular mass markers are indicated on the left (in kDa).
log [SS-14] M

<table>
<thead>
<tr>
<th>-9</th>
<th>-8</th>
<th>-7</th>
<th>-6</th>
<th>-5</th>
<th>-4</th>
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psst2A

sst2A
CTL | PTX
---|---
0  | 0  | min SS-14
2  | 2  | pERK 42/44 kDa
5  | 5  | ERK 42/44 kDa
10 | 10 |
15 | 15 |