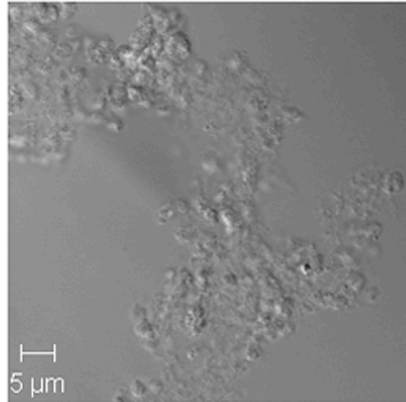


Supplemental Material:

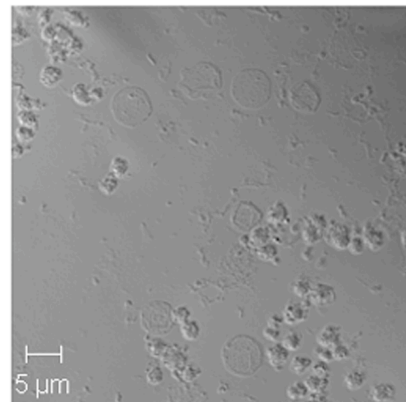
Figure 1:

DIC Images of Human Platelets

A

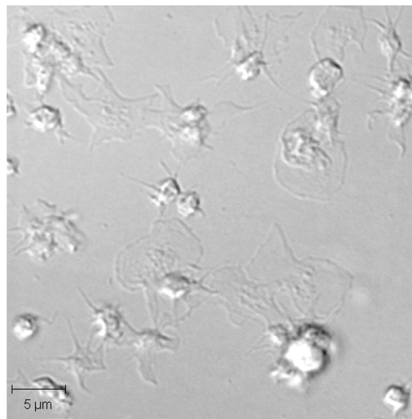


PBS

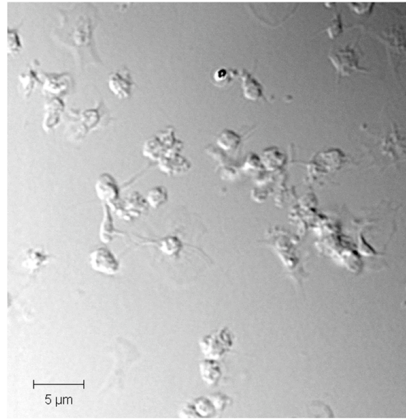


Reserpine

B



PBS



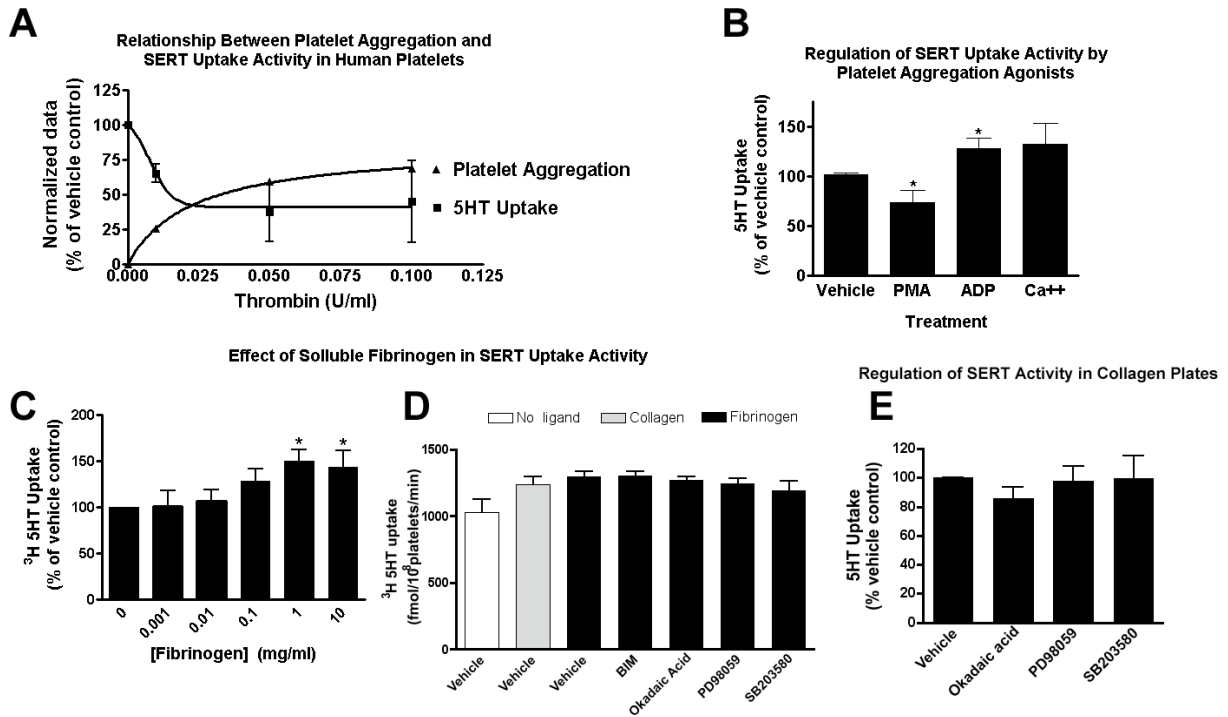
Citalopram

Reserpine- and citalopram- treated platelets have normal morphology but altered spreading. Confocal imaging (DIC) of human platelets seeded on fibrinogen-coated coverslips. We treated platelets with 10nM reserpine (30 min), 4.5uM citalopram (10min) or PBS before seeding onto fibrinogen-coated glass coverslips. Platelets were then incubated activated with 20μM ADP and fixed.

Table 1: Focal-adhesion proteins identified in proteomics experiments (N=3) that were found to interact specifically with either the amino- or carboxyl-terminus of SERT (or both), relative to GST samples.

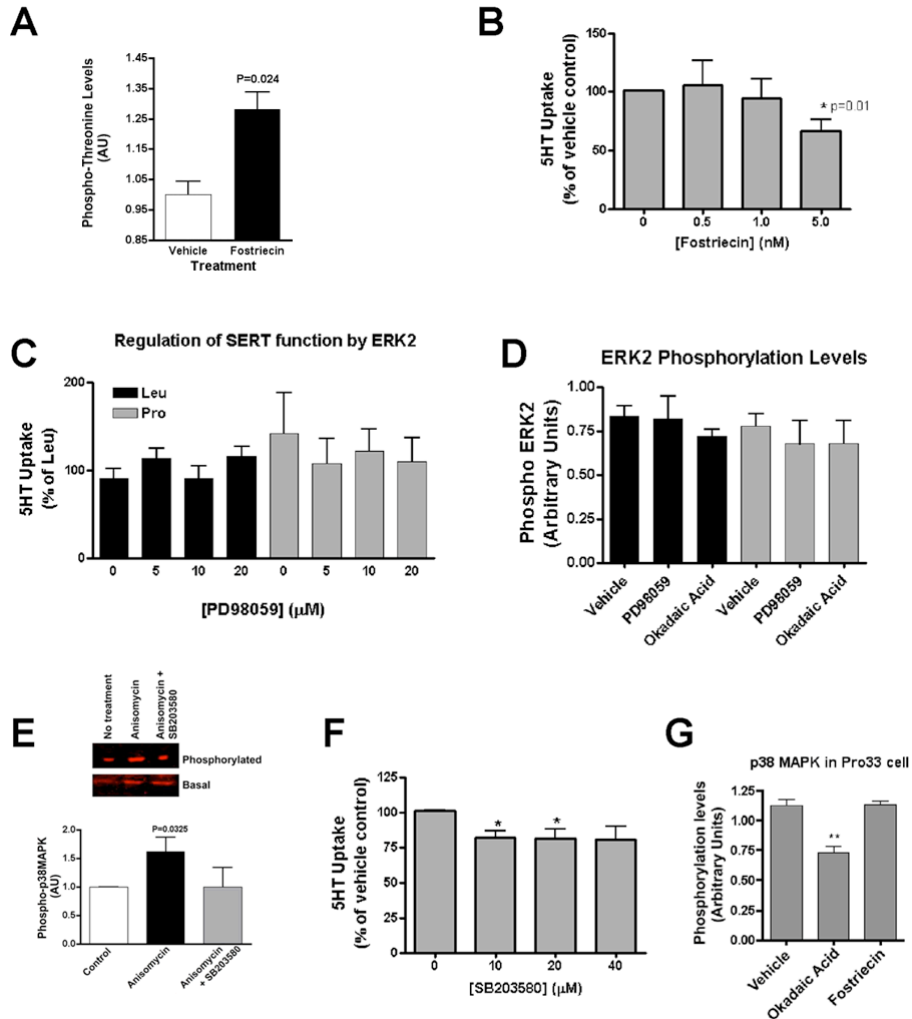
Protein	Accession #	Coverage	Peptide
Integrin alpha-IIb precursor	gi 124951	1.6%	-.VAIVVGAPR.-
			-.VYLFLQPR.-
Integrin beta 3 precursor	gi 4557677	1.5%	-.AKWDTANNPLYK.-
Fibrinogen	gi 11761629	5.0%	-.LKNSLFEYQK.-
			-.M#KPVPDLVPGNFK.-
			-.VQHIQLLQK.-
Actin	gi 6980848	3.4%	-.SPILGYWK.-
	gi 28336	10.9%	-.AVFPSIVGRPR.-
			-.HQGVM#VGM#GQK.-
			-.HQGVM#VGMGQK.-
	gi 113292	2.4%	-.AVFPSLVGR.-
gi 399003	3.0%	-.GYSFTTTAER.-	
Serotonin transporter	gi 4322274	4.0%	-.LTQSMAILR.-
	gi 112300	2.6%	-.SITPETPTEIPC*GDIR.-
	gi 6981554	2.0%	-.DREDC*QENGLVQK.-
Talin	gi 14916725	1.1%	-.KLLSAAKILADATAK.-
			-.KNKMDESK.-
			-.SVAQR.-

Figure 2:



SERT transport activity is altered by platelet aggregation agonists. A. Thrombin inhibits 5-HT uptake in a concentration-dependent manner. As platelet aggregation increases, there is a decrease in SERT transport activity. B. Platelets treated with α -PMA demonstrate decreased 5-HT uptake, whereas Ca⁺⁺ and ADP induce an increase in SERT uptake activity. C. High concentrations of soluble fibrinogen effects SERT transport activity. No effect was detected at 100 μ g/ml (concentration used to coat plates for immobilized assays). D. Platelets exposed to soluble collagen or fibrinogen (100 μ g/ml) did not exhibit enhanced SERT uptake. Kinase/phosphatase inhibitors had no effect in SERT uptake activity when platelets were exposed to soluble fibrinogen. E. Kinase and phosphatase inhibitors have no effect on 5-HT uptake performed on collagen-plated platelets.

Figure 3:



The enhanced SERT transport activity observed in Pro33 cells can be eliminated by inhibition of PP2A and p38MAPK but not ERK. A. Inhibition of basal Thr phosphorylation levels by incubation of HEK293 cells with 1nM of fostriecin for 10 min at 37°C. B. Concentration-curve of fostriecin in Pro33 cells. C. Concentration-curve of PD98059. No significant differences were found when ERK was inhibited in Leu33 or Pro33 cells. D. Basal ERK phosphorylation levels are not altered in Leu33 or Pro33 cells. E. p38MAPK phosphorylation levels are enhanced by incubating cells with anisomycin

and inhibited by SB203580. F. Concentration curve of SB203580. G. Fostriecin treatment of Pro33 cells does not alter basal p38 MAPK levels.

Methods:

Seeded platelet uptake assay. We used a modified Krebs-Ringer HEPES (KRH) buffer containing 130 mM NaCl, 1.3 mM KCl, 2.2 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 1.8 g/liter glucose, 10 mM HEPES, pH 7.4. Platelets were incubated at 37 °C in KRH buffer (0.2 ml/well) containing 100 μM pargyline, 100 μM L-ascorbic acid, and/or 1.0 mM tropolone (Sigma), with or without modifiers (as described in the results section). After 10-min incubation with 20nM [³H]5-HT at 37 °C, buffer was aspirated, and the cells were washed three times with ice-cold KRH buffer. Cells were solubilized with 0.5 ml of Microscint 20 (PerkinElmer Life Sciences), and tritium-labeled monoamine accumulation was quantitated using a TopCount plate scintillation counter (PerkinElmer Life Sciences). Specific 5-HT uptake was determined by subtracting the amount of [³H]5-HT, 1 μM fluoxetine.

Immunoprecipitations. Detergent extracts were prepared by washing 10⁸ platelets with PBS and lysis by either 1% Triton X-100 or RIPA buffer (for high stringency conditions) for 1 h at 4 °C. For SERT immunoprecipitations, 100μg of protein extract was precleared with 5μg of normal rabbit serum and 30 μl of 50% protein A-Sepharose bead slurry (Amersham Biosciences AB, Uppsala, Sweden) for 1 h at 4 °C. The precleared lysate was then incubated with either 1 μg of normal rabbit IgG or 1 μg of affinity-purified rabbit anti-SERT antibody (purified from either

no. 48 or no. 50 antisera) for 1 h at 4 °C followed by a 1-h incubation with 30 µl of a 50% protein A-Sepharose bead slurry at RT. Beads were then washed three times with PBS, and immunoprecipitated proteins were eluted in Laemmli buffer. Proteins were analyzed by SDS-PAGE (10%), electroblotted to polyvinylidene difluoride membranes (Amersham Biosciences), and probed with antibodies against talin, vinculin, Hic-5, actin, integrin α IIb or integrin β 3. Immunoreactive bands were detected by enhanced chemiluminescence (ECL; GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Multiple film (CL-X Posure™, Pierce) exposures were obtained to ensure data capture in the linear range of film.

In cell western blot. After incubation with the appropriate inhibitors, assay buffer was removed, and cells were immediately fixed with fresh 4% paraformaldehyde (Sigma) in PBS for 20 min at room temperature and washed four times with 1x PBS containing 0.1% Triton X-100 (Sigma) (5 min per wash) prior to 90min of blocking with Odyssey blocking buffer (Li-Cor Biosciences, Lincoln, NE). Primary antibodies were added in Odyssey blocking buffer at a 1:250 dilution and plates were incubated overnight at 4°C. Cells were then washed four times with 1x PBS containing 0.1% Tween 20 (Sigma) for 5 min. Bound antibody was detected with goat anti-rabbit IRDye® 680 secondary antibody (avoiding exposure to light; 1:200 in Odyssey Blocking Buffer, Molecular Probes, Inc., Eugene, OR) for 1h at RT. After four washes with 1x PBS, 0.1% Tween 20, the plate was scanned, and the captured image of the signal was processed and quantified with the Odyssey™ Infrared Imaging System (Li-Cor Biosciences).

Phosphorylated protein levels were assessed by normalizing for each specific MAPK total levels. Each experiment was conducted in duplicates and data is presented as means \pm SEM of three or more experiments.