Supplementary Figure 1

FFN200 accumulates in cultured dopamine neurons.

(a) Representative images of FFN200-labeled dopamine ventral midbrain neurons obtained from TH-GFP mice, displaying different levels of FFN200 accumulation following incubation with 10 μM FFN200 for 30 min at 37°C. (b) Scatter plot of FFN200 intensity values at GFP-positive dopamine neurons, presented in ascending order in a logarithmic scale. Neurons were considered FFN200-positive when their fluorescence intensity was greater than two standard deviations above the mean FFN200 intensity of GFP-negative cells (the threshold is depicted as a dotted line; 80 cells from six dishes, four independent cultures). An example of an FFN200-negative neuron is shown in the top TH-GFP/FFN200 image pair in panel (a), and the following two image pairs show FFN200-positive neurons.
Supplementary Figure 2

Effect of dTBZ on dopamine release as a function of incubation time.

Single pulse-evoked dopamine release was measured by cyclic voltammetry in the dorsal striatum of control and 5 μM dTBZ-treated slices (n=3–4 mice with 1–2 slices averaged per mouse). Released dopamine was normalized for each condition to the average current of five prepulses applied before time point zero, which marks the beginning of dTBZ perfusion (in dTBZ-treated slices).
Supplementary Figure 3

Effect of 0 mM Ca\(^{2+}\) and TTX on FFN200 release in the dorsal striatum.

(a) Background intensity presented as mean percentage of \(F_i \pm \text{SEM}\) for slices stimulated in the presence of 2.4 mM Ca\(^{2+}\), in the absence of Ca\(^{2+}\) (0 mM Ca\(^{2+}\)) or in 2.4 mM Ca\(^{2+}\) with 1 µM TTX (2.4 mM Ca\(^{2+}\) + TTX; \(n=6\) for each condition in both panels, where \(n\) is number of mice, with 1–2 slices averaged per mouse). (b) Scatter plot of the percentage of destaining puncta in each independent experiment including mean ± SEM (\(*\ast p < 0.01\) by one-way ANOVA with Bonferroni's multiple comparison test).
Comparison of FFN200 and FFN102 release in the dorsal striatum.

(a) Background intensity presented as mean percentage of $F_i \pm$ SEM for both probes (n=9 and 8 slices from different mice for FFN200 and FFN102, respectively, for all panels in this figure). (b) Scatter plot of the percentage of FFN200 and FFN102 destaining puncta in response to 15 Hz stimuli in each independent experiment including mean $\pm$ SEM (n.s- not significantly different, $p=0.1269$, two-tailed unpaired t test). As a control, the percentage of FFN102 puncta selected as “destainers” in unstimulated slices was 5.1 $\pm$ 1.6% (n=5; not depicted). (c) FFN200 and FFN102 puncta intensity (background- and baseline rundown-corrected) over time, normalized to $\Delta F$, the fluorescence change between the last point of the baseline (100%) and the average of the last three data points of stimulation (0%) $\pm$ SEM, to facilitate comparison between FFN200 and FFN102 curves (* $p < 0.05$, two-tailed unpaired t test; $p=0.0370$). (d) Cumulative distribution of $t_{1/2}$ of destaining for FFN200 and FFN102 (147 and 175 puncta from nine and eight slices from different mice for FFN200 and FFN102, respectively).
**Supplementary Figure 5**

**Effects of short washout times on FFN200 release in the dorsal striatum.**

(a) Scatter plot of the percentage of destaining puncta in each independent experiment, including mean ± SEM, for typical 45 min washout (45’ washout) and shorter 25 min washout (25’ washout) (n=6 for both conditions in both panels, where n is number of mice, with 1–2 slices averaged per mouse; n.s- not significantly different, p=0.4771 by two-tailed unpaired t test). (b) Puncta fluorescence intensity (background- and baseline rundown-corrected) over time presented as mean percentage of F₀ ± SEM.