

**Figure 1 – Supplement 1:   
Generation of a traceable version of the PSAM in an expression cassette with enhanced fluorescence**(A) Schematic of the PSAM (PSAM L141F,Y115F:5HT3HC) and EGFP co-expression plasmid. Top row shows 4 EGFP+ transfected and ten EGFP- PC12 cells. The bottom row shows [Ca2+]i increases in the transduced cells to the selective agonist PSEM89s. [Ca2+]i measured by Fura2 340:380 fluorescence ratio - F2R.  
(B) [Ca2+]i responses to PSEM89s in 4 EGFP+ and 5 EGFP- PC12 cells.  
(C) The maximum Ca2+ transient evoked by PSEM89s is sensitive to the length of C-terminal tag on PSAM.   
18 amino acids remain at the C-terminal end of PSAM when the 2A peptide is downstream of PSAM. This C-terminal residue reduced the maximum PSEM89s-evoked response. There was no difference between the original PSAM-IRES-EGFP plasmid and the HA tagged expression cassette EGFP-2A-PSAMHA (p<0.0001 Kruskal Wallis test with Bonferroni’s multiple comparison NS p>0.05, \*\*p<0.01, \*\*\*\*p<0.0001).  
(D) The C-terminal HA tag does not interfere with the concentration response relationship (p=0.6384 sum-of-squares F test). EC50: PSAM-IRES-EGFP - 3.37±0.04 vs EGFP-2A-PSAMHA - 3.86±0.09 µM  
(E) The upstream EGFP-P2A sequence produces a significantly brighter fluorescent signal than the IRES linker (Mann-Whitney test p<0.0001).   
Calcium transients are shown as mean ±SEM and fluorescence data as median with interquartile intervals. N equals the number of cells analysed from at least three replicated experiments.