Supporting Information

Identification of FDA-approved small molecules capable of disrupting the Calmodulin-Adenylyl Cyclase 8 interaction via direct binding to Calmodulin

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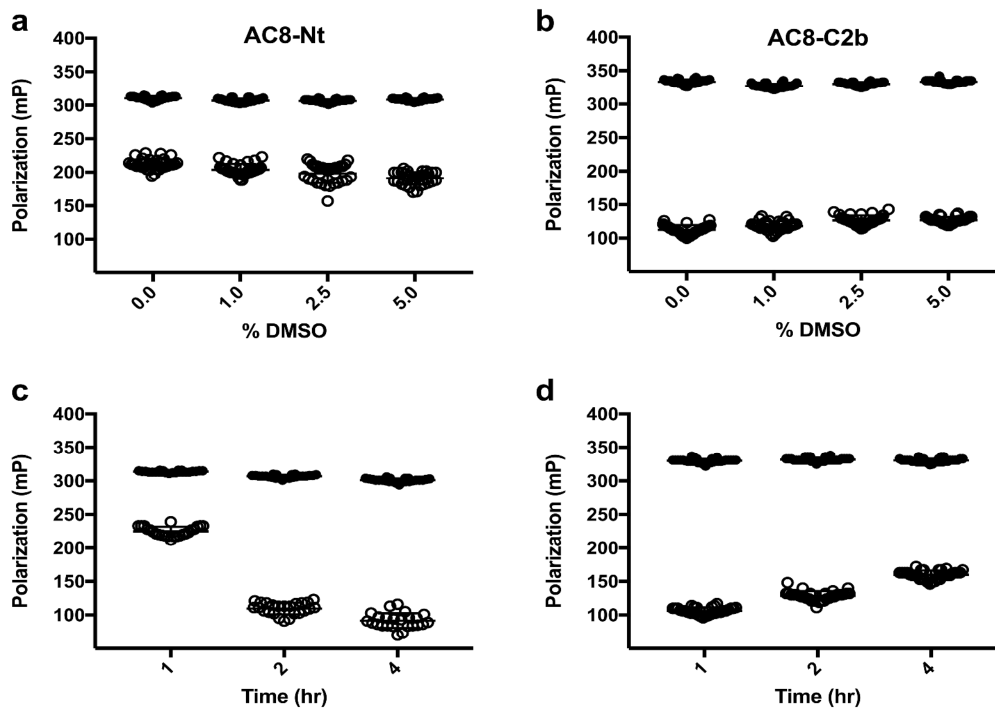
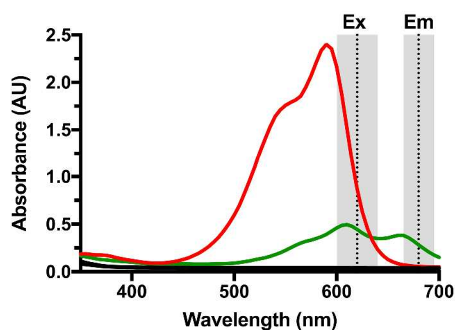
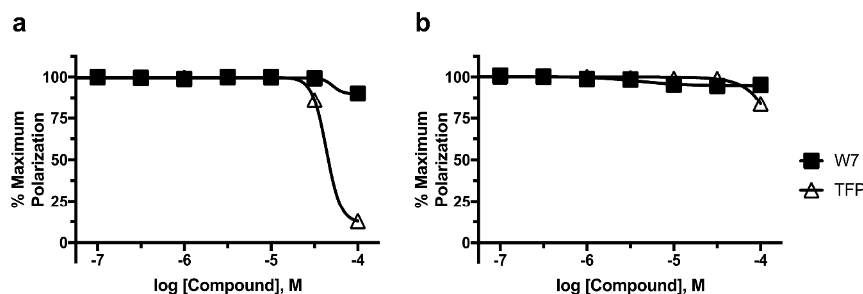
1. Supporting Figures



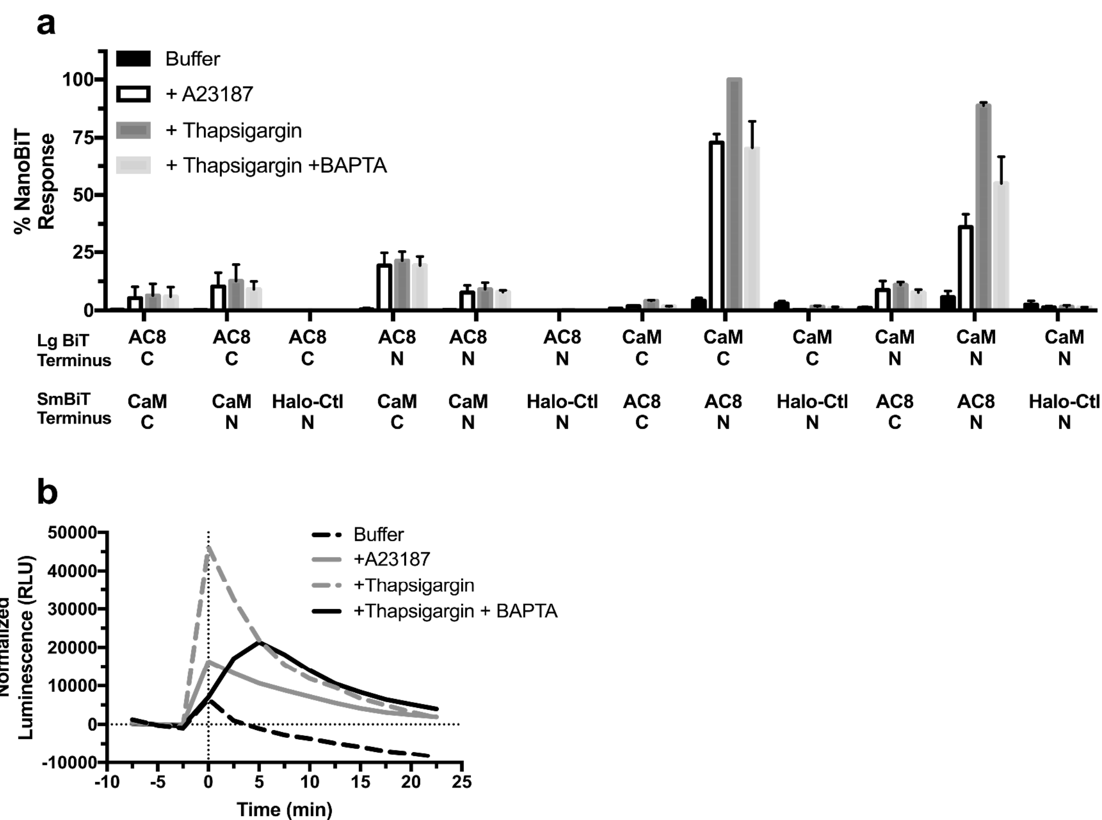
Supporting Figure 1. High through suitability and stability of CaM/AC8 peptide fluorescence polarization assay. (a) CaM FP assay tolerance to multiple concentrations of DMSO using 316 nM GST-CaM and 100 nM Cy5-labeled AC8-Nt (a) or AC8-C2b (b) in the absence (filled) and presence (empty) of 10 mM EGTA. FP assay stability over time for 100 nM Cy5-labeled AC8-Nt (c) or AC8-C2b (d) in the absence (filled) and presence (empty) of 10 mM EGTA. Data represent at least n=24 wells for each condition tested.



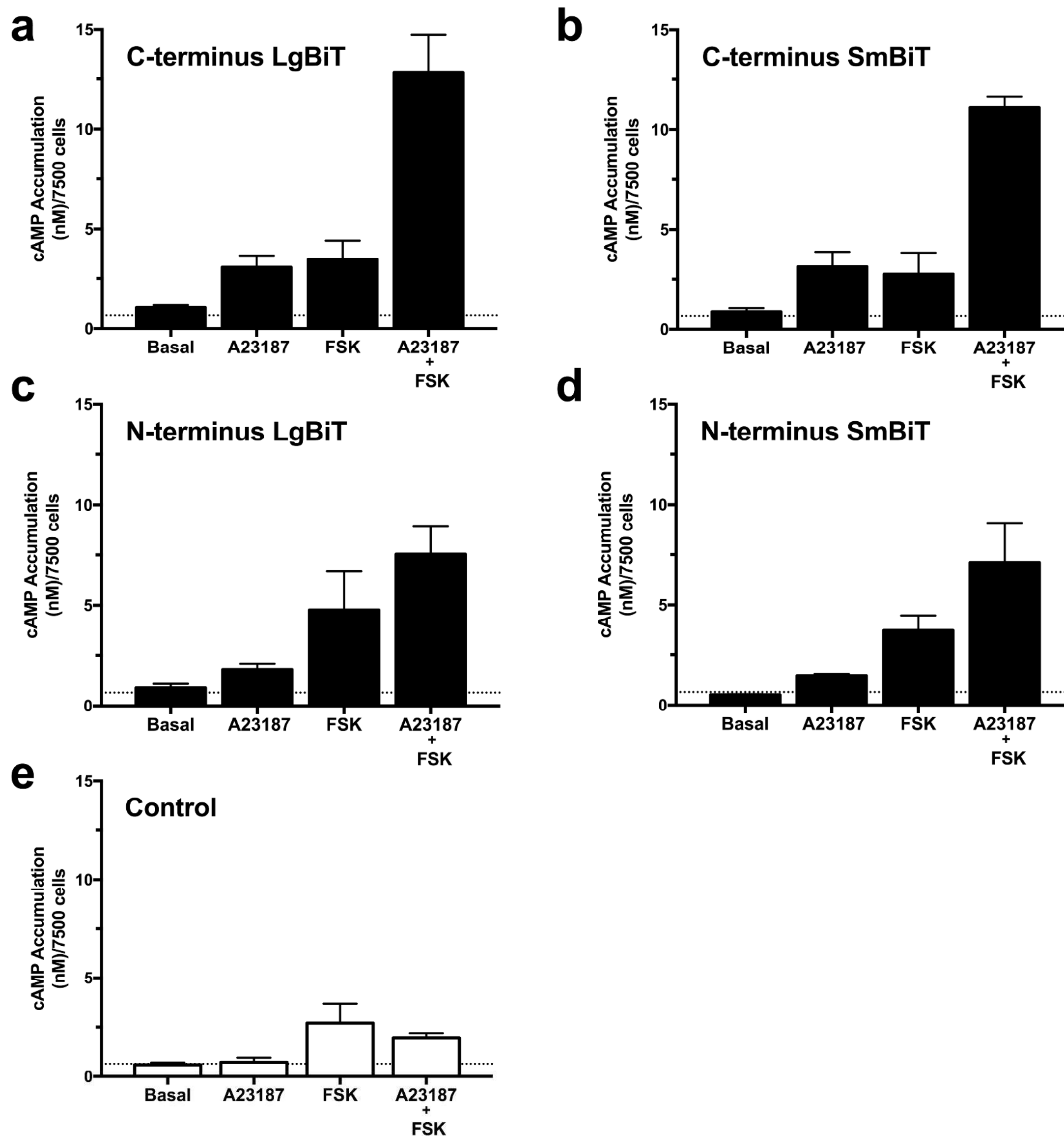
Supporting Figure 2. Identification of optically interfering primary screen hits. Absorbance scan from 350-700 nm of 100 μM hit compounds in assay buffer. Spectral regions used for Cy5 excitation and emission are highlighted in grey and labeled Ex and Em, respectiviely. Hits that did not result in appreciable absorption regions are shown in black and are indiscernible from buffer alone. Two hits, crystal violet (red) and mitoxantrone (green), showed strong absorbance in these spectral regions.



Supporting Figure 3. CaM inhibitors W7 and TFP are not potent inhibitors of CaM/AC8 peptide interactions. The prototypical CaM inhibitors W7 and TFP were tested for their ability against the CaM/AC8-Nt (a) and AC8-C2b (b) in concentration response. Only TFP at the highest concentration tested was able to produce an appreciable amount of inhibition, and only against AC8-Nt, not AC8-C2b.



Supporting Figure 4. Development of cell-based AC8/CaM NanoBiT assay
(a) AC8 and CaM were labeled with indicated NanoLuciferase BiT on the indicated terminus. Area under the curve analysis was performed for 20-25 minutes following treatment with A23187 or thapsigargin with and without BAPTA-AM. Data represent mean ± SEM of at least three independent experiments. SmBiT- Halo (Halo-Ctl) served as a control protein-protein interaction partner, as per manufacturer instructions. (b) Representative NanoBiT luminescence kinetic trace. Luminescence prior to addition of Ca^{2+}-mobilizing agents 1 μM A23187 or 1 μM Thapsigargin was normalized to zero and then monitored for 22.5 min.



Supporting Figure 5. Activity of NanoBiT tagged AC8 constructs in cells
To ensure that NanoBiT-tagged rAC8 retained catalytic activity, Ca^{2+}-stimulated cAMP formation was measured in HEK293T cells that were transiently transfected with rAC8 tagged with the indicated NanoBiT fragment and incubated with 0.5 mM IBMX (Basal), 10 μM A23187 (A23187), 100 nM forskolin (FSK), or 10 μM A23817 and 100 nM FSK combined. Data represent mean ± SEM from three independent experiments.

2. Supporting Tables

Supporting Table 1. Z'-factor of CaM/AC8 FP

AC8-Nt				AC8-C2b			
% DMSO	Z'	Time (hr)	Z'	% DMSO	Z'	Time (hr)	Z'
0	0.65	1	0.71	0	0.86	1	0.89
1	0.65	2	0.83	1	0.85	2	0.86
2.5	0.54	4	0.80	2.5	0.85	4	0.83
5	0.70			5	0.89		