Ligand	Association rate constant (1/Ms)	Calculated quantity
0.1 ng CTA standard	13,066 ± 900	0.15 ng
1 ng CTA standard	24,753 ± 695	0.45 ng
10 ng CTA standard	61,976 ± 1,187	14 ng
100 ng CTA standard	83,668 ± 1,337	104 ng
No treatment sample	17,064 ± 821	0.22 ng
+ NECA sample	17,925 ± 960	0.24 ng
+ GA sample	7,524 ± 429	0.09 ng
Mock RNAi	15,373 ± 198	0.19 ng
Control RNAi	14,890 ± 601	0.18 ng
Hsp90 RNAi	3,489 ± 109	0.06 ng

TABLE S1. **Calculations of cytosolic CTA1.** Association rate constants were calculated from the SPR data presented in Figures 4C and 6C. The association rate constants for the CTA standards were plotted as a function of protein quantity, and the slope of the resulting standard curve was used to calculate the amount of CTA1 detected in the dislocation experiments.

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. **GA does not affect the activity of cytosolic CTA1.** CHO cells were transfected with a plasmid encoding a CTA1 subunit that is expressed directly in the cytosol. Intracellular cAMP levels were then calculated at either 2 hrs (n = 1) or 4 hrs (n = 2) post-transfection for cells chased in the absence or presence of 0.1 μM GA. Triplicate samples were used for each measurement.

FIGURE S2. **Proteasome inhibition does not affect the accumulation of cytosolic CTA1.** HeLa cells were pulse-labeled at 4°C for 30 min with 1 μg / ml of CT. The cells were then chased for 2 hr at 37°C in toxin-free medium that contained no additions, 100 μM ALLN, 100 μM MG132, 0.1 μM GA, 0.1 μM GA & 100 μM ALLN, or 0.1 μM GA & 100 μM MG132. Selective permeabilization of the plasma membrane with digitonin was used to partition cell extracts into separate organelle and cytosolic fractions. An SPR sensor slide coated with an anti-CTA antibody was used to detect the cytosolic pool of CTA1 from untreated (no treatment) or drug-treated cells. CTA standards were perfused over the sensor slide as positive controls. In the time frame of this 2 hr pulse-chase experiment, inhibition of the proteasome with ALLN or MG132 did not alter the level of cytosolic CTA1 in either untreated or GA-treated cells. The disruption of proteasome function was confirmed with an assay that detected an inhibition of bulk protein turnover in radiolabeled cells exposed to ALLN or MG132. One of two representative experiments is shown.

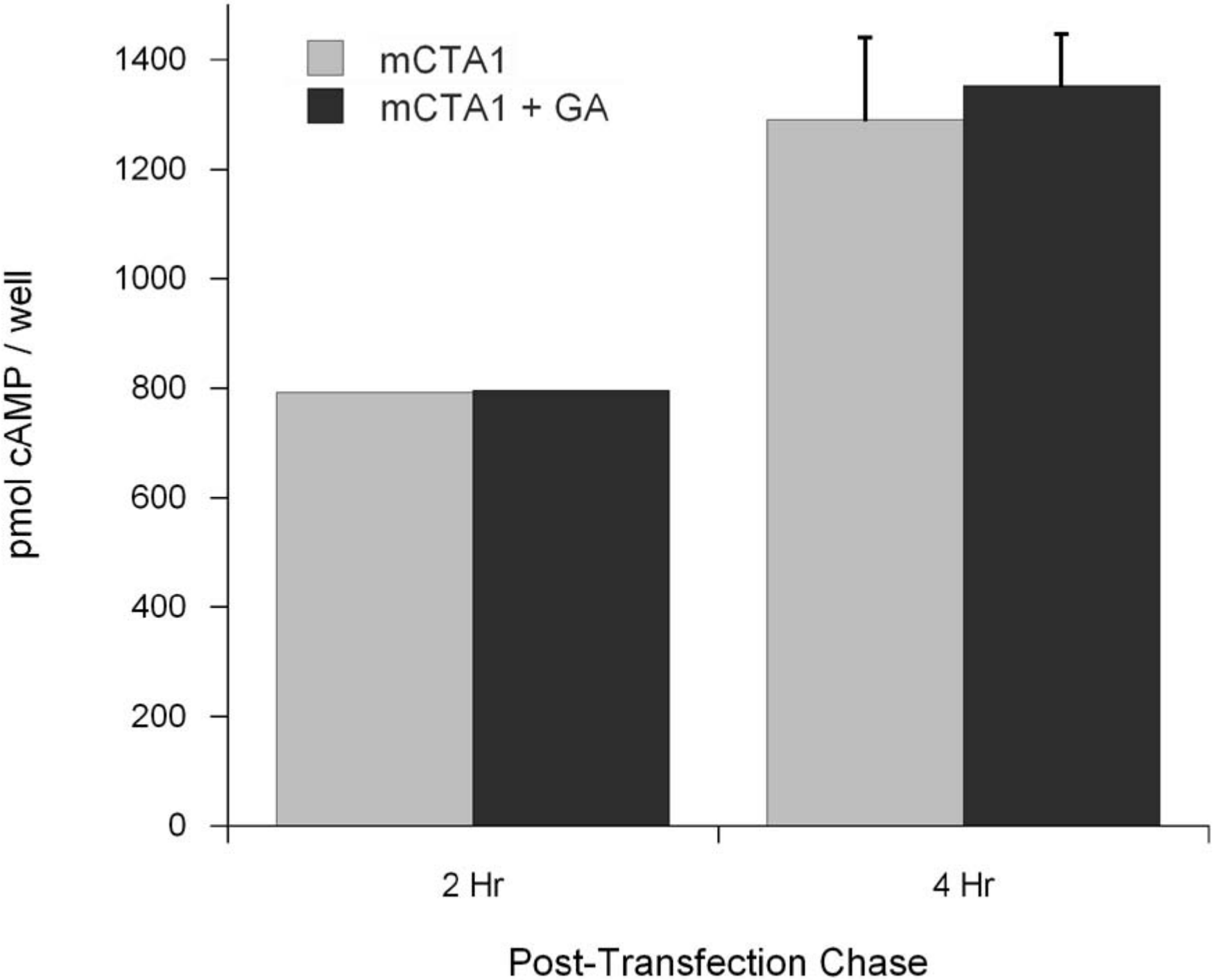
mCTA1
mCTA1 + GA
1400
1200
1000
800
600
400
200
0
pmol cAMP / well
2 Hr
4 Hr
Post-Transfection Chase

FIGURE S1

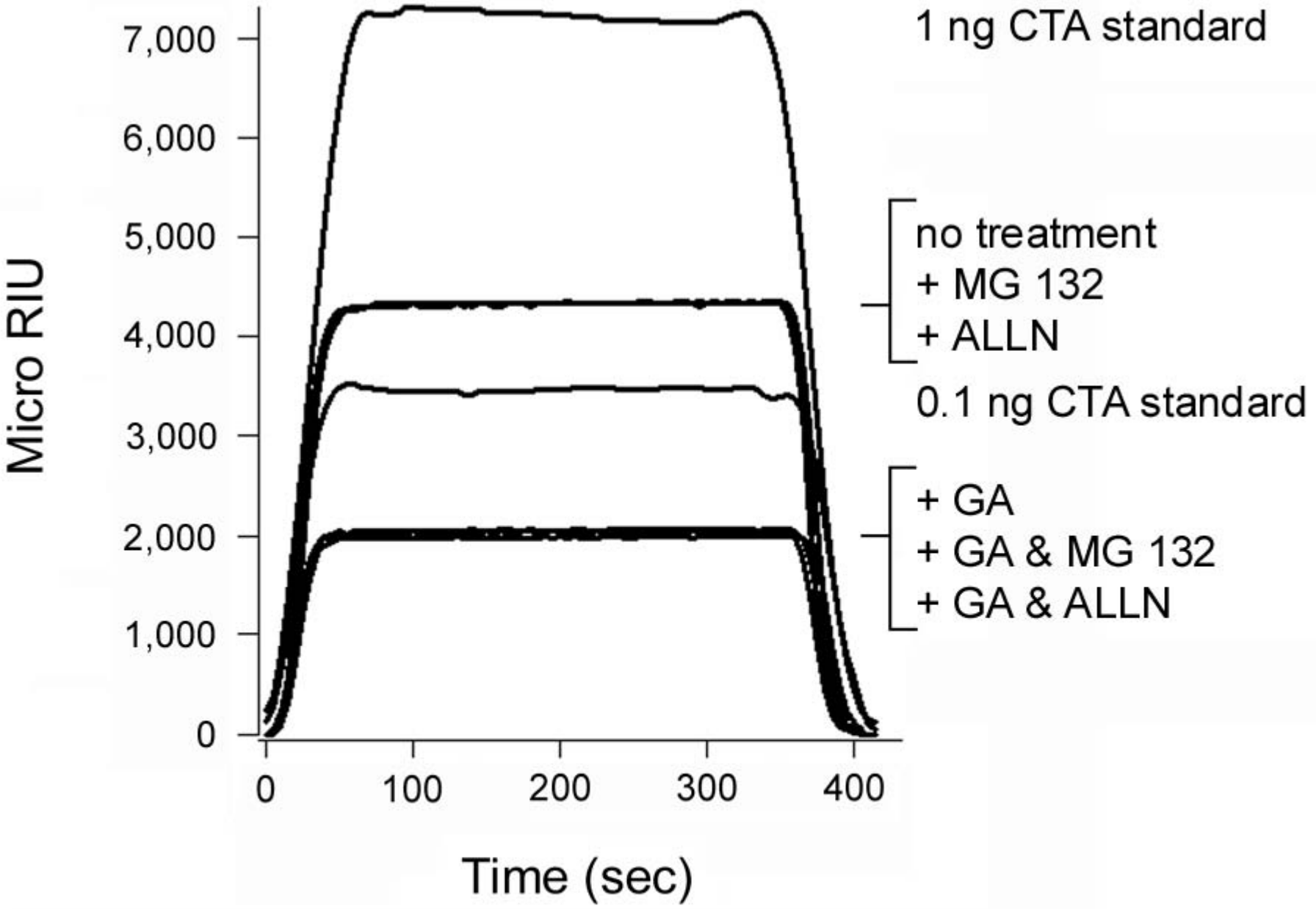
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
Micro RIU
0
100
200
300
400
Time (sec)
1 ng CTA standard
no treatment
+ MG 132
+ ALLN
0.1 ng CTA standard
+ GA
+ GA & MG 132
+ GA & ALLN

FIGURE S2