

SUPPLEMENTAL DATA

Supplemental Figure Legends

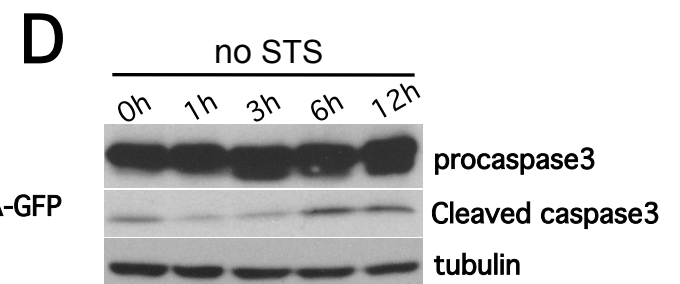
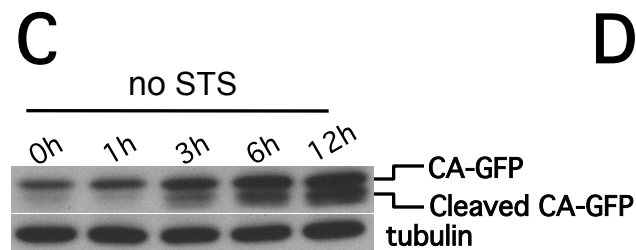
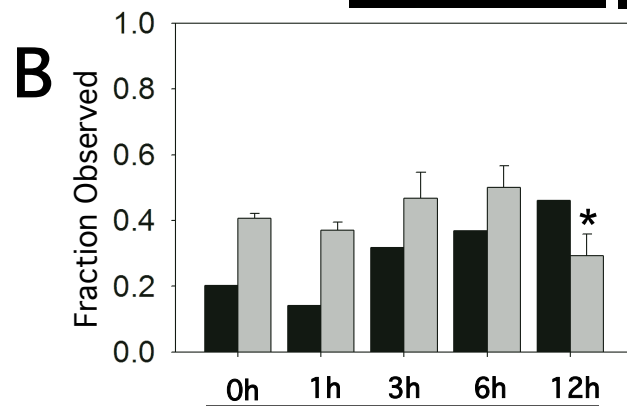
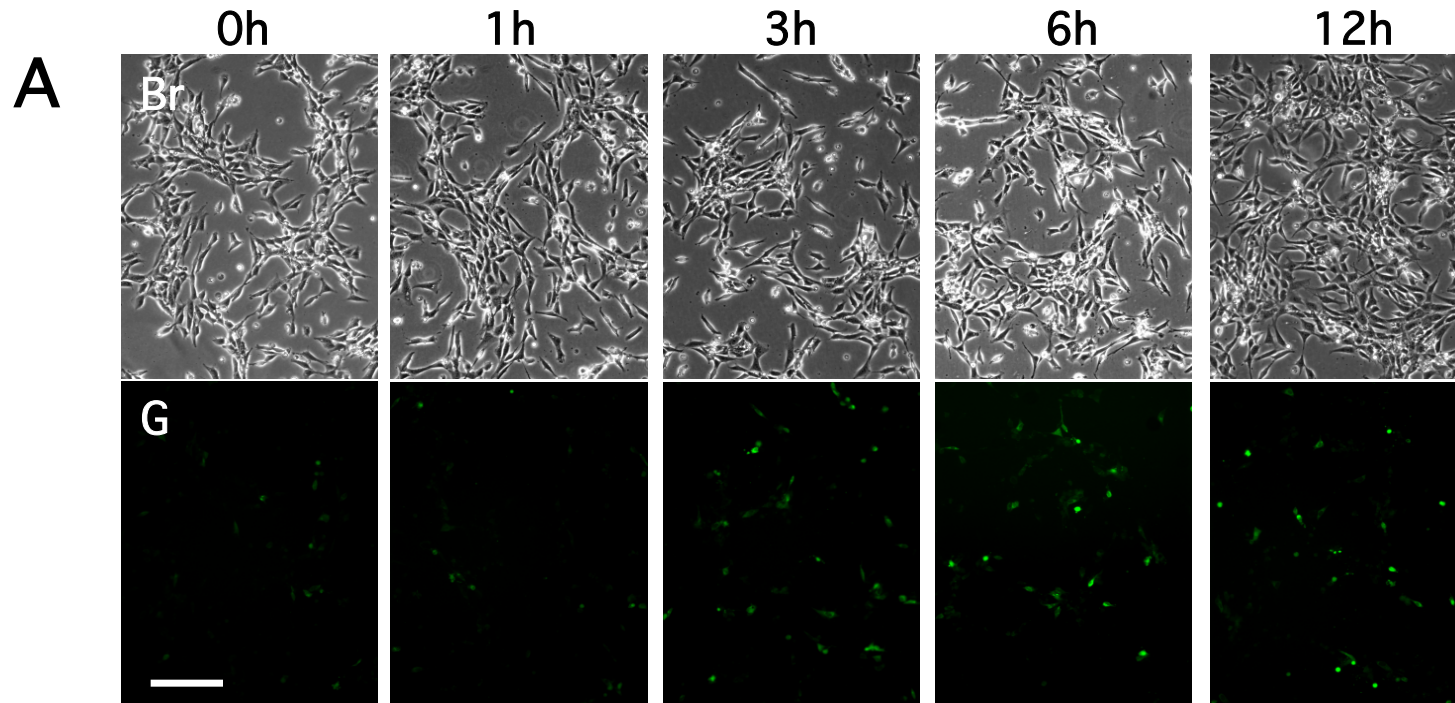
Figure S1. Fluorescent properties of CA-GFP in untreated mammalian cells. (A) Fluorescent response of CA-GFP-transfected NIH 3T3 cells at identical times to those shown in Fig. 3. Scale bar represents 50 μm . Br; Brightfield. G; GFP fluorescence. (B) Fraction of fluorescent cells (gray bars) and fraction of cleaved CA-GFP (black bars). *significance level; $p < 0.05$ relative to 0 time point. (C) CA-GFP cleavage without induction of apoptosis is observed as a function of time by immunoblotting with an anti-GFP antibody. (D) The presence of cleaved caspase-3 was observed without induction of apoptosis by immunoblotting with an anti-caspase-3 antibody. Tubulin was probed as a loading control. (E) Quantification of CA-GFP and tubulin protein expression levels as a function of time based on the immunoblots in C of this figure (0 μM STS) and Figure 3B (1 μM STS). The concentration of tubulin remained constant, however there was some increase in the total level of CA-GFP in the cell due to expression from the strong Cytomegalovirus promoter, which has been shown to function even during apoptosis. Some aspect of our manipulation, potentially something as routine as removal from the incubator, leads to induction of low levels of caspases. We observe an increase in active (cleaved) caspase-3 that is paralleled by cleavage of CA-GFP. Thus if we were able to diminish this background, we may be able to observe an even higher Green/Red ratio.

Figure S2. Time-lapse imaging of NIH 3T3 cells expressing CA-GFP and mLumin without staurosporine (STS) treatment. Time-lapse images of NIH 3T3 cells co-expressing CA-GFP and mLumin were recorded at the times identical to those shown in Fig. 4 after the addition of DMSO as the control buffer. DIC; differential interference contrast images. G; Green channel monitoring CA-GFP fluorescence. R; Red channel, monitoring mLumin fluorescence. Scale bar represents 25 μm .

Movie S1. Time-lapse movie of apoptosis in NIH 3T3 cells expressing CA-GFP and mLumin. Cells were monitored on DIC, and Green (CA-GFP) and Red (mLumin) channels for 7 hours after the addition of staurosporine (STS) for the increase in green fluorescence as the cells die by apoptosis.

Movie S2. Time-lapse movie of NIH3T3 cells expressing CA-GFP and mLumin. Cells were monitored on DIC, Green (CA-GFP) and Red (mLumin) channels for 7 hours after the addition of DMSO.

Fig. S1



E

no STS		0 Hours	1 Hour	3 Hours	6 Hours	12 Hours	Average
1 uM STS	Full-length CAGFP	1380	1820	1283	768	590	NA
	Cleaved CAGFP	412	1482	2006	2152	2265	NA
	CAGFP total	1792	3302	3290	2919	2855	NA
	Tubulin	2704	2975	2815	2672	2375	2708±221
0 uM STS	Full-length CAGFP	1675	1725	2195	2222	1819	NA
	Cleaved CAGFP	433	373	1108	1483	1968	NA
	CAGFP total	2108	2097	3303	3706	3787	NA
	Tubulin	2530	2523	2420	2409	2766	2530±143

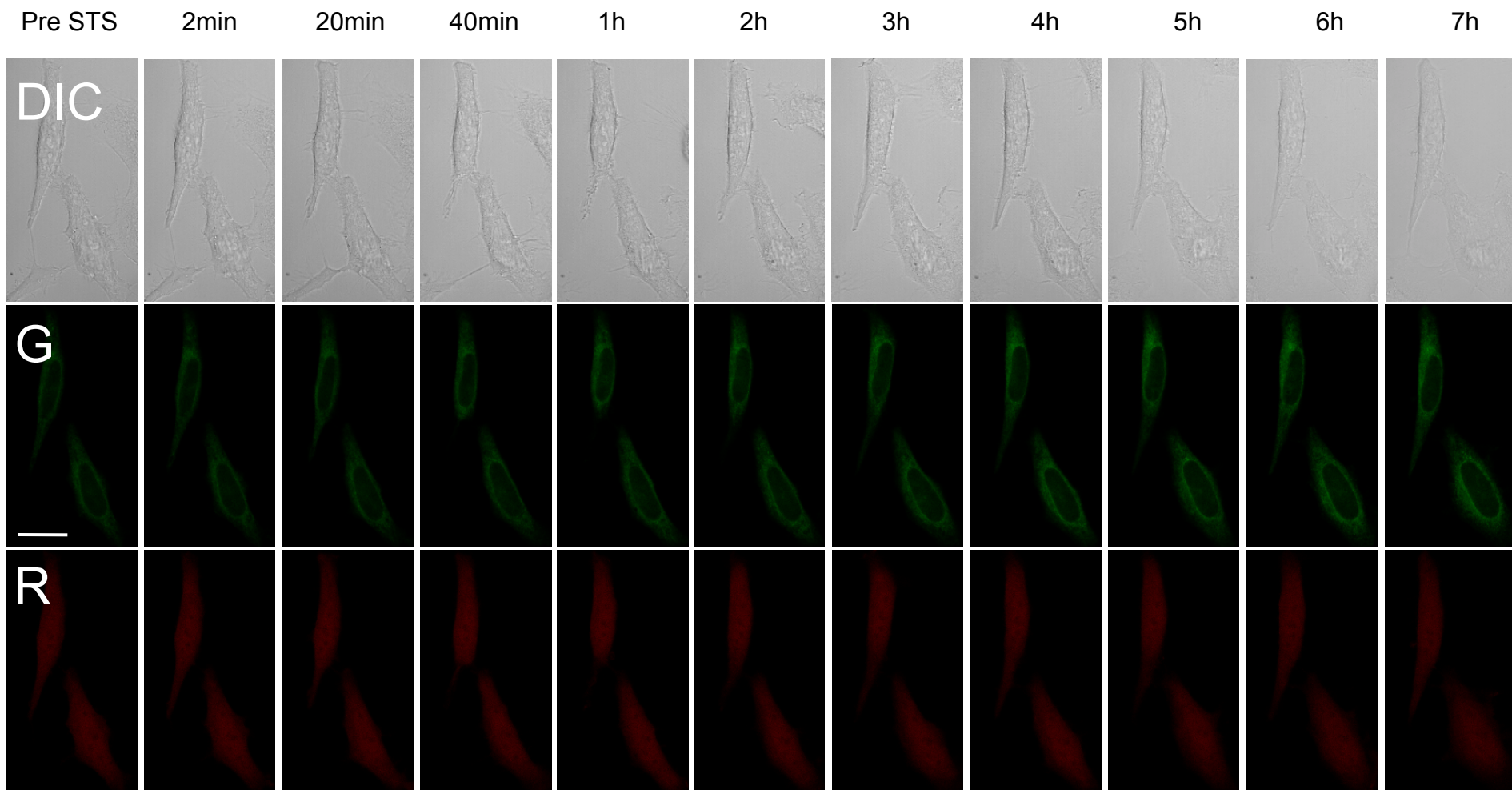


Fig. S2