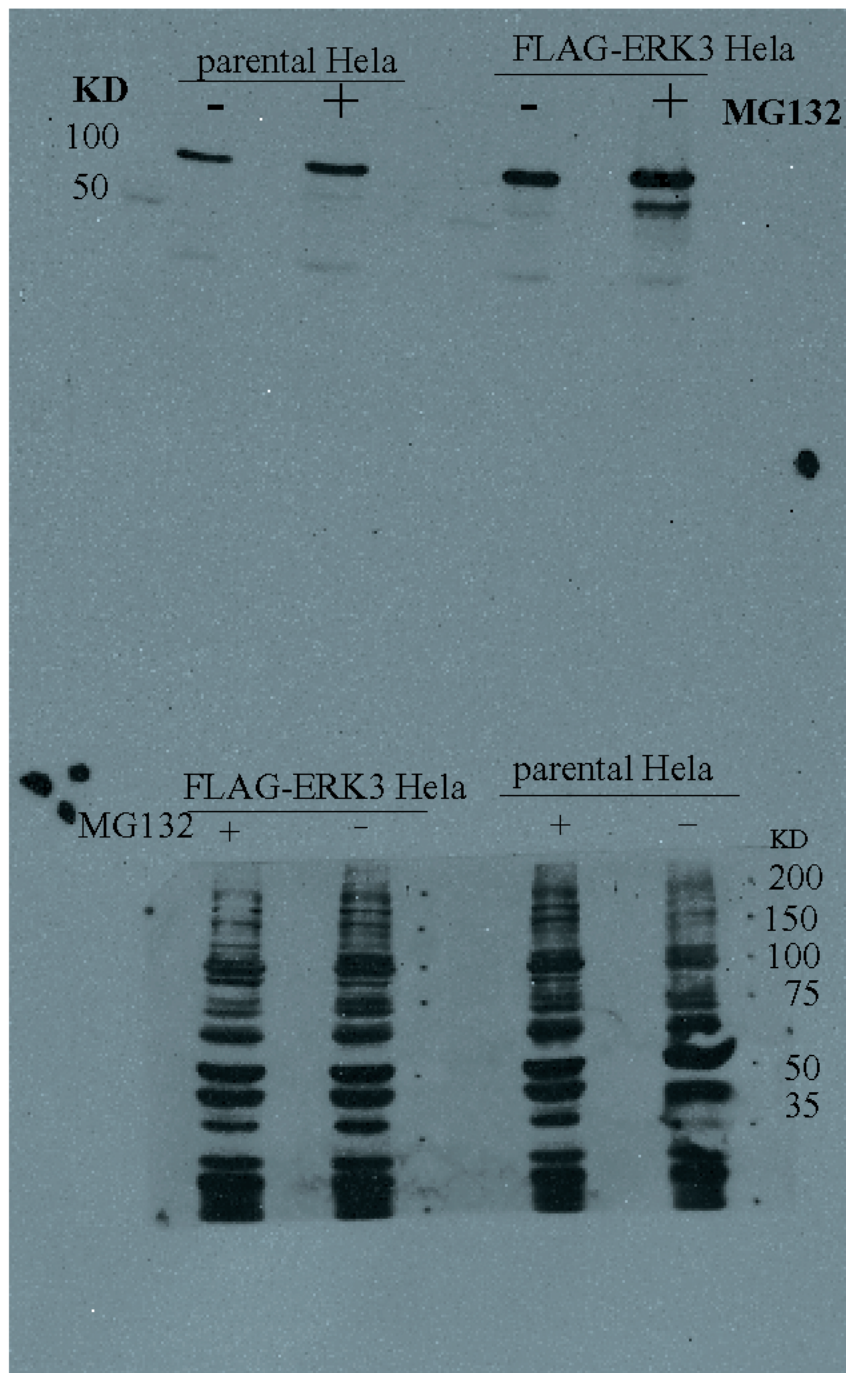


## Supplemental Figure 1



Scan of the film for immunoblot of crude extracts (50ug of protein/lane) from the parental or FLAG-ERK3 cell lines incubated in media supplemented with MG132 at a final concentration of 10uM. Primary antibody is the rabbit C-terminal (at 1;1000).

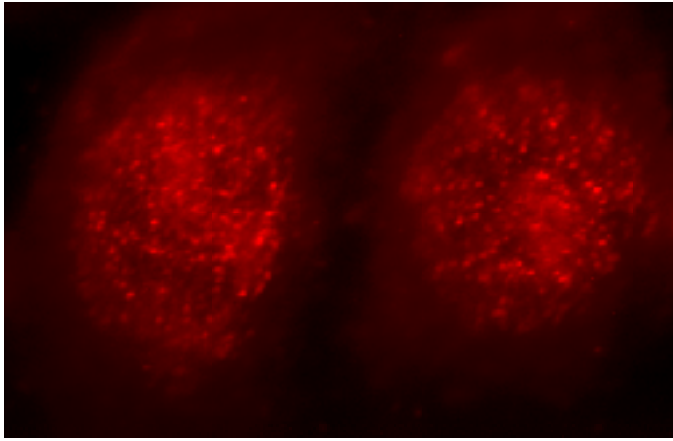
Scan of the film for immunoblot of crude extracts (50ug of protein/lane) from the parental or FLAG-ERK3 cell lines incubated in media supplemented with MG132 at a final concentration of 10uM. Primary antibody is the sc-156 N-terminal ERK3 antibody used at a 1;1000 dilution

\* Note that these blots were exposed on the same piece of film.

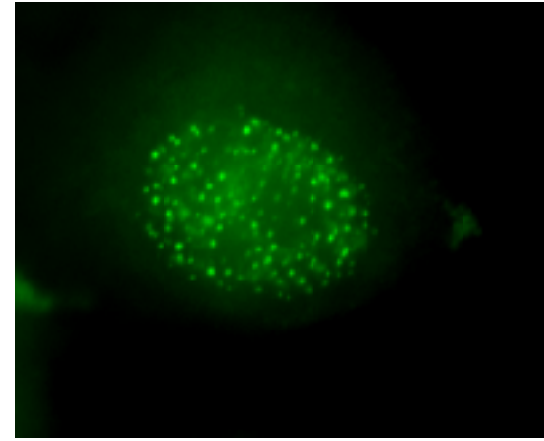
# Subcellular localization of FLAG-ERK3 and GFP-ERK3 in HeLa cells

**HeLa FLAG ERK3 cell line**

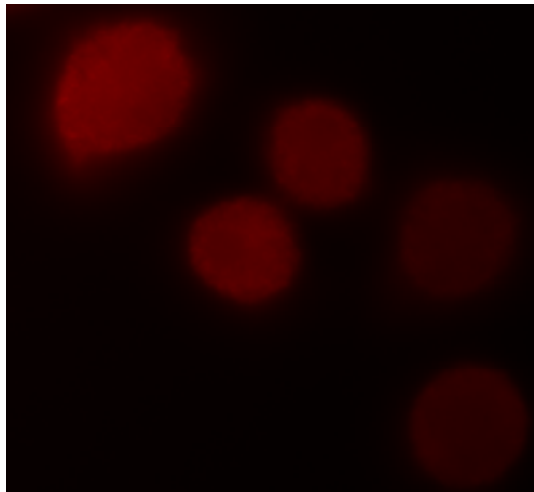
Cy3-FLAG at 1;200 dilution



**Nuclear GFP-ERK3  
in a HeLa cell**

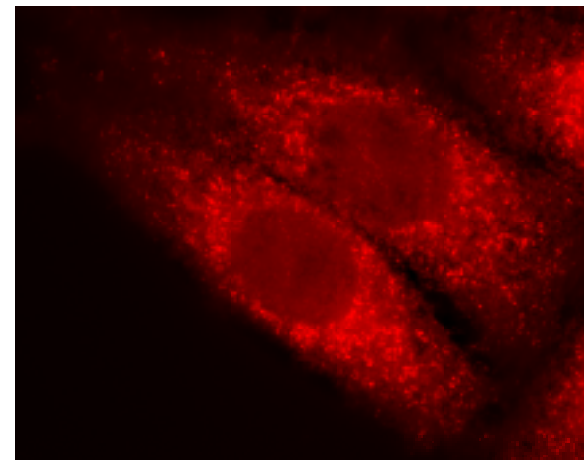


**Parental HeLa cell line**

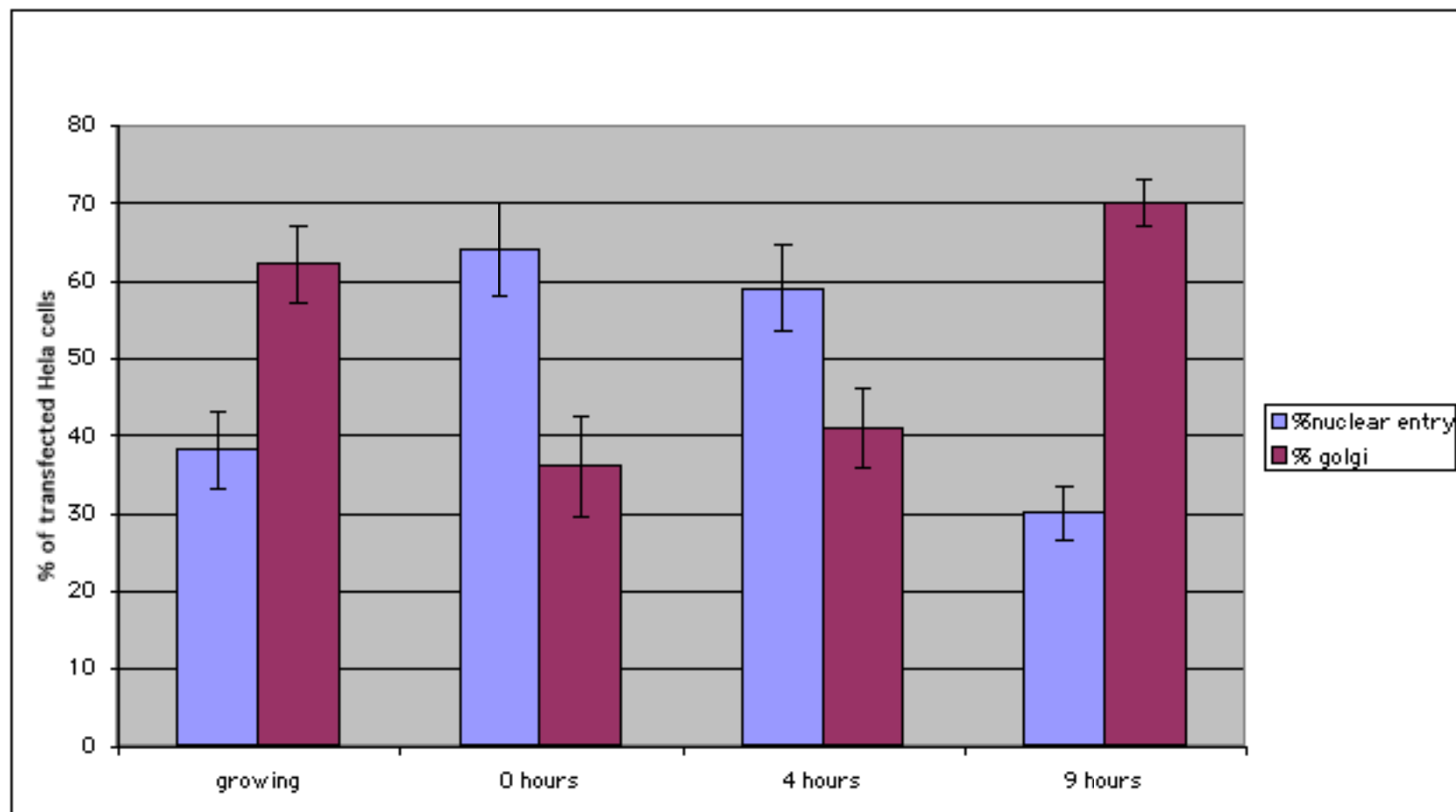


Cy3-FLAG at 1;200 dilution

**HeLa FLAG ERK3 cell line**



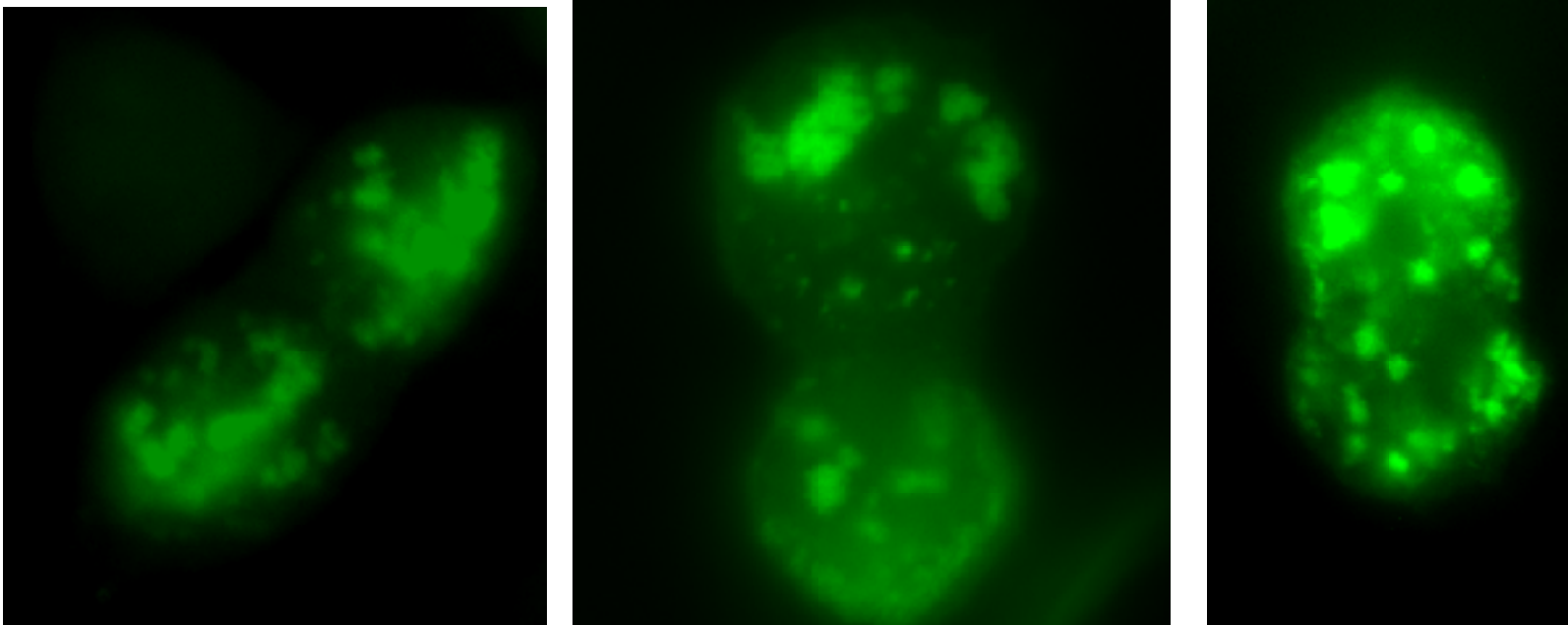
Cy3-FLAG at 1;200 dilution



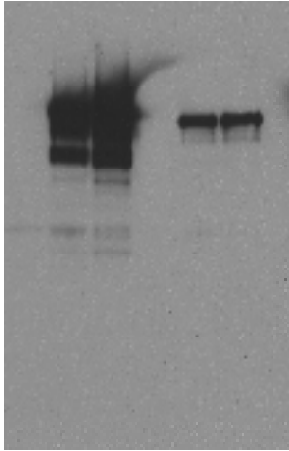
<b>G1</b>	<b>50</b>	<b>35</b>	<b>25</b>	<b>68</b>
<b>S</b>	<b>28</b>	<b>27</b>	<b>9</b>	<b>12</b>
<b>G2M</b>	<b>21</b>	<b>38</b>	<b>66</b>	<b>20</b>

## Supplemental Figure 4

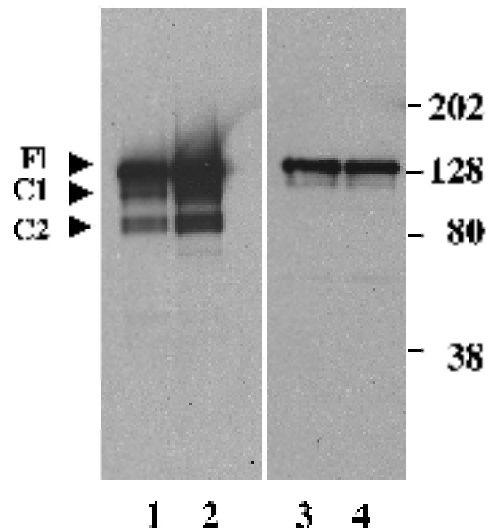
**Subcellular localization of GFP-ERK3 FL in HeLa cells undergoing telophase**



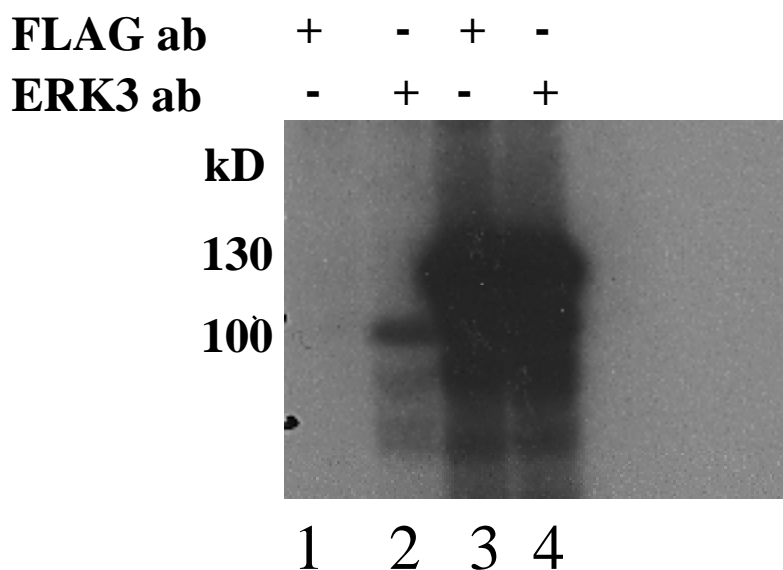
**Original Photoshop scan of film used to make figure 6C**



**Figure 6C was made using the Microsoft Power Point application program. This figure was trimmed so that lanes 1 and 2 would be closer to lanes 3 and 4.**



## GFP-ERK3 FL is a functional protein kinase



In this experiment, we tested whether the GFP-ERK3 FL protein was a functional protein kinase in a coupled immunoprecipitation autophosphorylation assay (1). 200 ug of crude cell extract was used in each immunoprecipitation together with the indicated antibody.

The immunoprecipitates in lanes 3 and 4 were prepared from 293 cells transfected with the plasmid GFP-ERK3 FL. The control immunoprecipitates are in lanes 1 and 2.

The GFP-ERK3 FL protein is aminoterminally tagged with a FLAG tag, while the endogeneous protein does not contain this epitope. Thus the FLAG antibody does not immunoprecipitate the endogeneous ERK3 protein in untransfected cells (lane 1). In contrast, the FLAG antibody can immunoprecipitate the GFP-ERK3 FL protein (lane 3) expressed in transfected cells. Finally, note that the carboxy-terminal ERK3 antisera can immunoprecipitate both the endogeneous ERK3 protein (lane 2) and the GFP-ERK3 FL protein (lane 4).

1. Sanchez, I. et. al, Nature 372: 794-770 (1993)