

Recent Progress with FKBP-Derived Destabilizing Domains

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Supplementary Data

Materials and Methods

Cell Culture, Transfections and Transductions

The NIH3T3 cell line was cultured in DMEM supplemented with 10% heat-inactivated donor bovine serum (Invitrogen), 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. The HeLa cell line was cultured with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin.

The ΦNX ecotropic and amphotropic packaging cell lines were transfected using standard Lipofectamine 2000 protocols. Viral supernatants were harvested 48 h post-transfection, filtered and concentrated 10-fold using an Amicon Ultra centrifugal filter device (Millipore, 100-kDa cut-off). NIH3T3 (ecotropic virus) or HeLa (amphotropic virus) cells were incubated with the concentrated retroviral supernatants supplemented with 4 µg/mL polybrene for 4 h at 37 °C. Cells were washed once with PBS and cultured in growth media for 24-36 hrs to allow for viral integration.

Flow Cytometry

Twenty-four hours prior to analysis, transduced NIH3T3 cells were plated at 1×10^5 cells per well of a 12-well plate and treated as described. Cells were trypsinized and resuspended in 200 µL PBS. Cells were analyzed at the Stanford Shared FACS Facility using FlasherII with 10,000 events represented.

Internal DD Linker Sequences

Short Linkers: N-term GSGGGSEF (8 aa); C-term GSGSEF (6 aa)

Medium Linkers: N-term GSGGGSEFGGGGS (13aa); C-term GSGSEFGGGSGGGGS (14 aa)

Long Linkers: N-term GSGGGSEFGGGSGGGGS (18 aa); C-term GSGSEFGGGSGGGSGGGGS (18 aa)

Cell Cycle Analysis

Media from HeLa cells cultured at subconfluence was removed and reserved. Cells were harvested using trypsin-EDTA for 15-20 min at 37 °C, and the trypsin was quenched with the reserved media. Cells were harvested as such so as not to lose any of the less adherent mitotic cells, and to provide a single cell suspension for flow cytometry analysis. Cells were then harvested by centrifugation at 1000 rpm for 4 min in a Beckman-Coulter Allegra 6R swinging bucket centrifuge. Cells were washed once with PBS, and then resuspended in 100 µL PBS in a 1.5-mL microfuge tube. Cells were fixed by adding 900 µL ice-cold 70% ethanol dropwise while vortexing slowly (speed ~4). Note that the final concentration of ethanol should not be

greater than 80%. Fixed cells were stored at -20°C for at least 30 min, up to one month. Fixed cells were washed once with PBS and resuspended in 500 μL PI staining solution (10 $\mu\text{g}/\text{mL}$ propidium iodide, 50 $\mu\text{g}/\text{mL}$ RNase A, 2 mM EDTA in PBS). Cells were transferred to clear 12 \times 75 mm polystyrene tubes (Falcon #352054) and incubated for 30 min at 37°C . Cells were then analyzed by flow cytometry on FACScalibur, with no less than 30,000 events represented. Data were analyzed using FlowJo software (Tree Star, Stanford, CA) and the cell cycle synchronization wizard in ModFit LT software (Verity Software House, Topsham, ME).

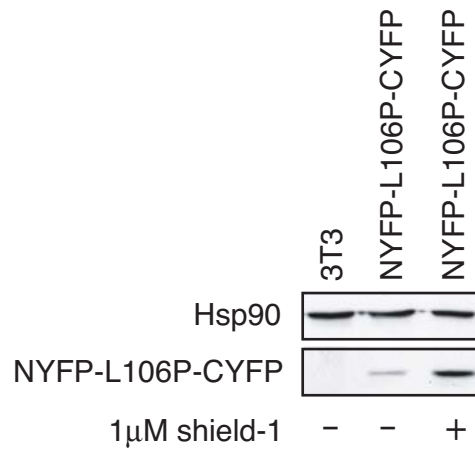


Figure S1: NIH3T3 cells stably expressing NYFP/L106P/CYFP were either mock-treated or treated with 1 μM Shield-1 for 24 h. Immunoblotting was performed with anti-YFP and anti-Hsp90 antibodies.

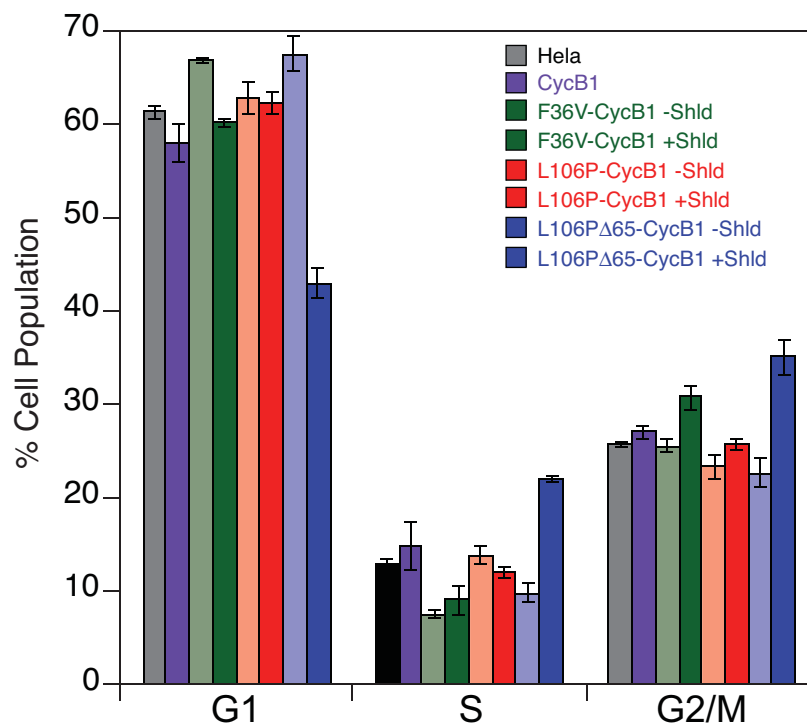


Figure S2: HeLa cells expressing cyclin B1 constructs were released from double thymidine block, treated with Shield-1 or vehicle for 12 hours, and analyzed for DNA content by flow cytometry. Cell cycle phase percentages were determined using ModFit. Experiment performed in triplicate (\pm s.d.).