

SGK1 affects RAN/RANBP1/RANGAP1 via SP1 to play a critical role in pre-miRNA nuclear export: a hypothesis for a new route of epigenomic regulation.

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Supplementary file- Figure Legend

Suppl. File 1 qRT-PCR evaluation for exogenous SGK1, RANBP1, RANGAP1 expression, and SGK1 and RANBP1 silencing. Graphs represent the fold increase values +/- SD of the either transduced/transfected or silenced genes, except for SGK1 silencing which values are presented as percentage of fold expression +/- SD. Statistical significance has been calculated as detailed in the Methods section. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Suppl. File 2 Western blot analysis of HUH7 stably overexpressing EGFP-SGK1 or EGFP in presence or not of transiently silencing for SP1 (shSP1); western blot of RANBP1-silenced or scrambled HUH7 with or without 12.5 μ M SI113 treatment for 72 h; western blot of HUH7 cells stably silenced SGK1 or scrambled. Cell extracts were loaded on SDS-polyacrylamide gel electrophoresis for immunoblotting using SP1 antibody, RANBP1 antibody, SGK1 antibody and GAPDH antibody.

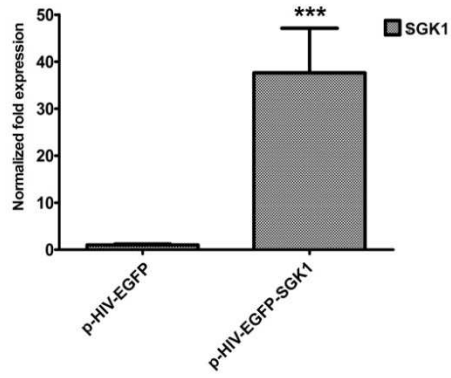
Suppl. File 3 Quantitative RT-PCR analysis of mature microRNAs in the cytoplasm of HUH7 cells stably over-expressing EGFP-SGK1 or EGFP, with or without transiently silenced RANBP1 (shRANBP1). Statistical significance has been calculated as detailed in the Methods section. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Suppl. File 4 Cell viability analysis by The Countess™ automated cell counter in HUH7-GFP and HUH7-RANBP1-GFP (top panel) and HUH7-GFP, HUH7-RANBP1-GFP, HUH7-RANGAP1 and HUH7-RANBP1/RANGAP1 (bottom panel) cell lines 72 h after treatment with either SI113 at the indicated concentrations or vehicle alone. Results are reported as means of three independent experiments, each conducted in triplicate, and expressed as the percentage of viable control cells treated with DMSO alone (vehicle). The fluorescence microscopy for RANBP1-GFP expression is shown at the top of the panel.

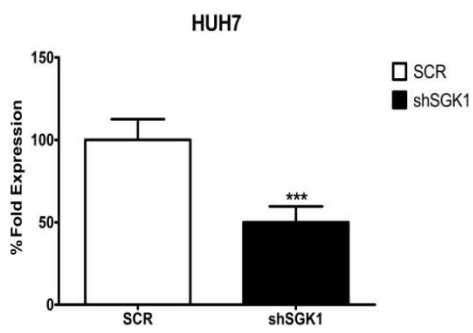
Suppl. File 5 Representative immunofluorescence analysis of HUH-EGFP cells (top panel), no differences was recordable with the scrambled control cells, reported in Fig 6d and e. Samples were incubated with a conformational Active-RAN monoclonal antibody, or with an anti-RANBP1 goat polyclonal antibody or an anti-RANGAP1 monoclonal antibody and 4',6-diamidino-2-phenylindole (0.05% $\mu\text{g/ml}$). Histograms representing the quantification of immunofluorescence showed in Fig. 6d and 6e (bottom panel). The values are plotted as relative IF unit expressed as percentage of RAN-GTP/RAN total ratio (left), RANBP1 (middle) and RANGAP1 (right).

Suppl. File 1

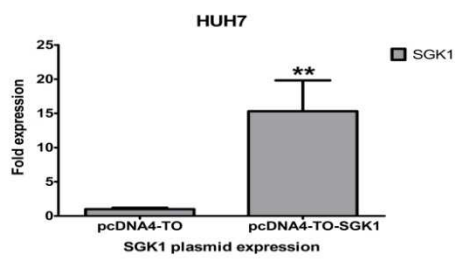
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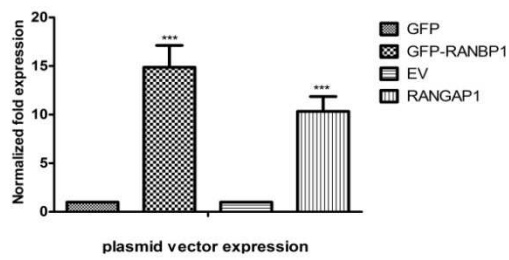
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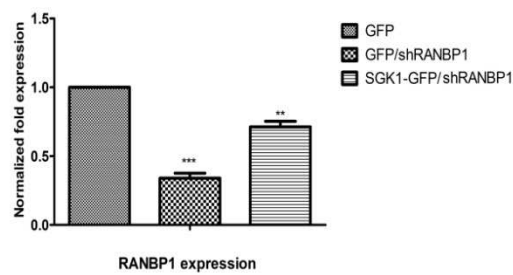
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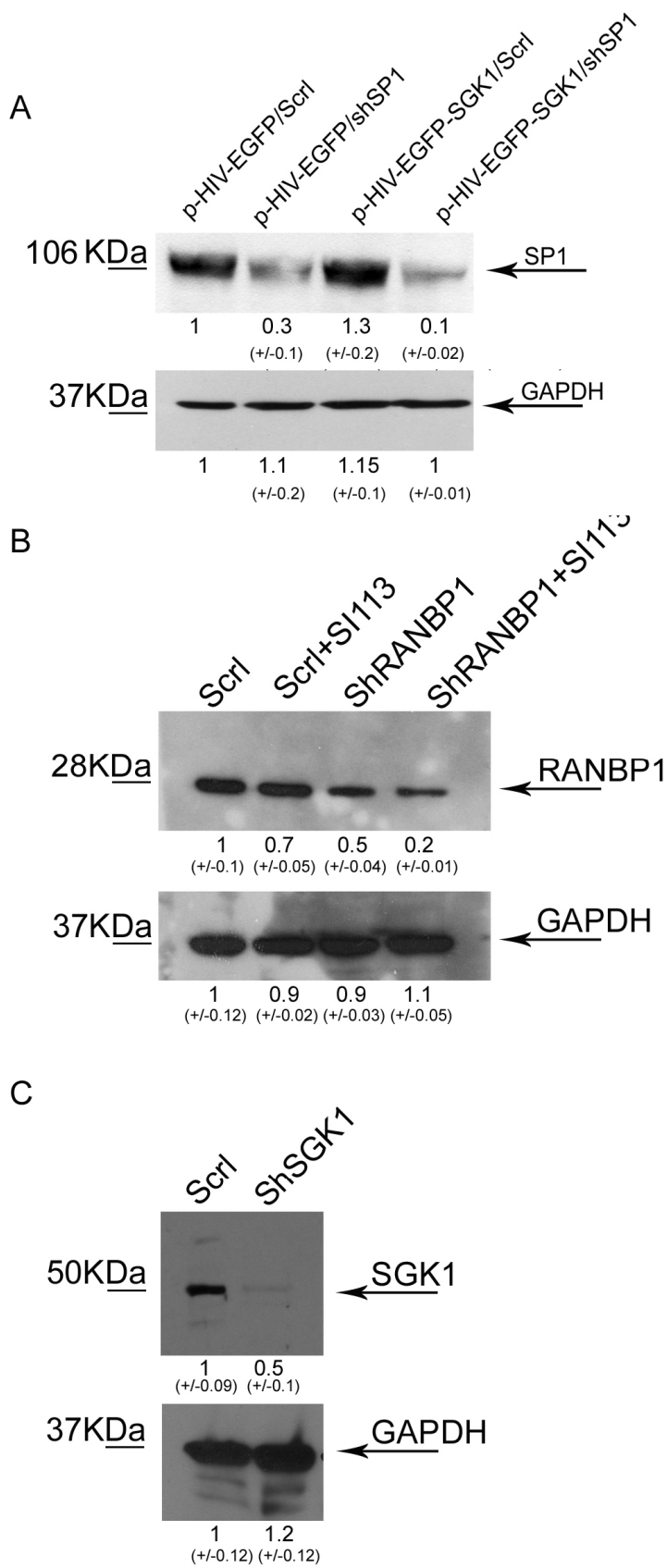


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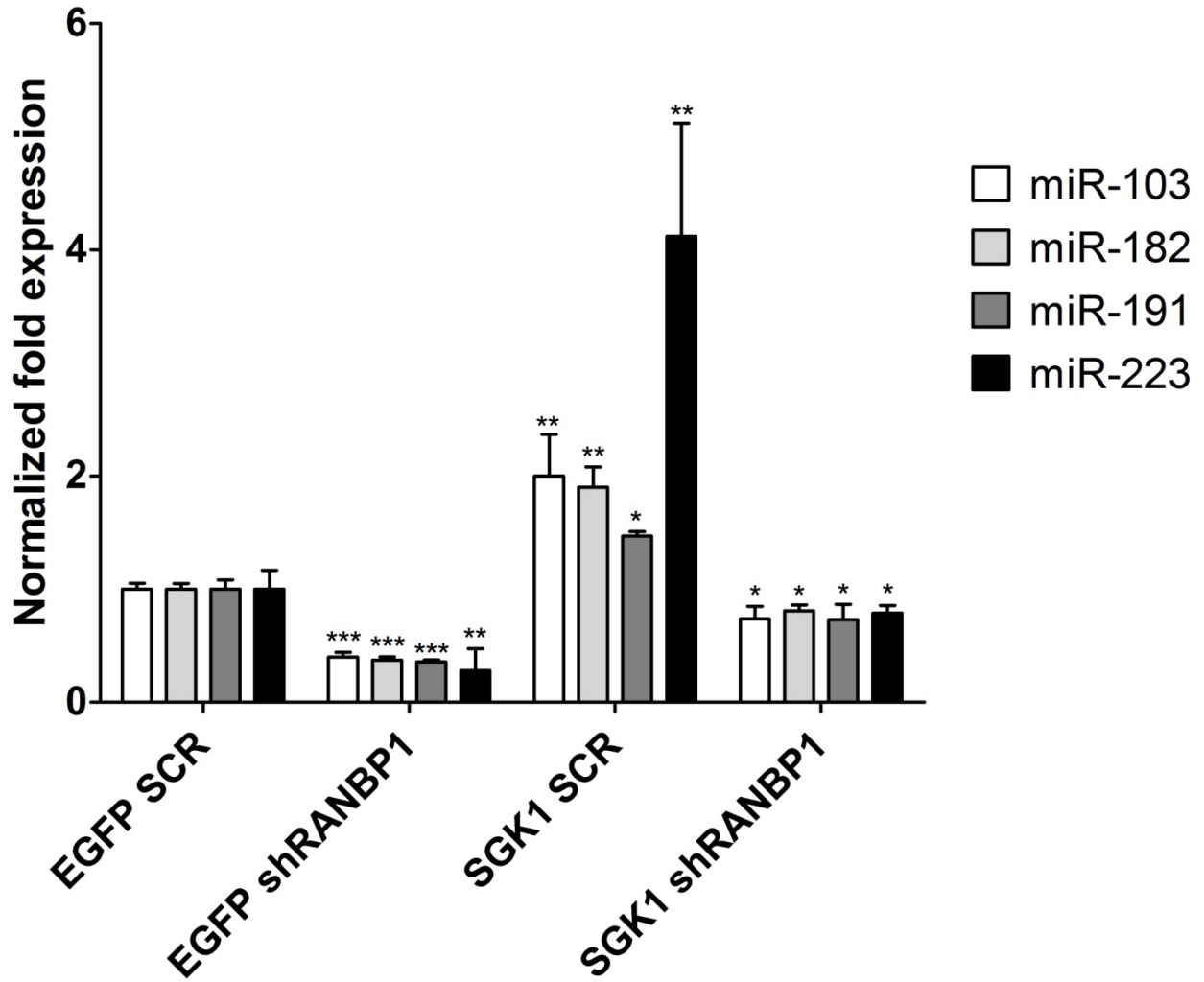


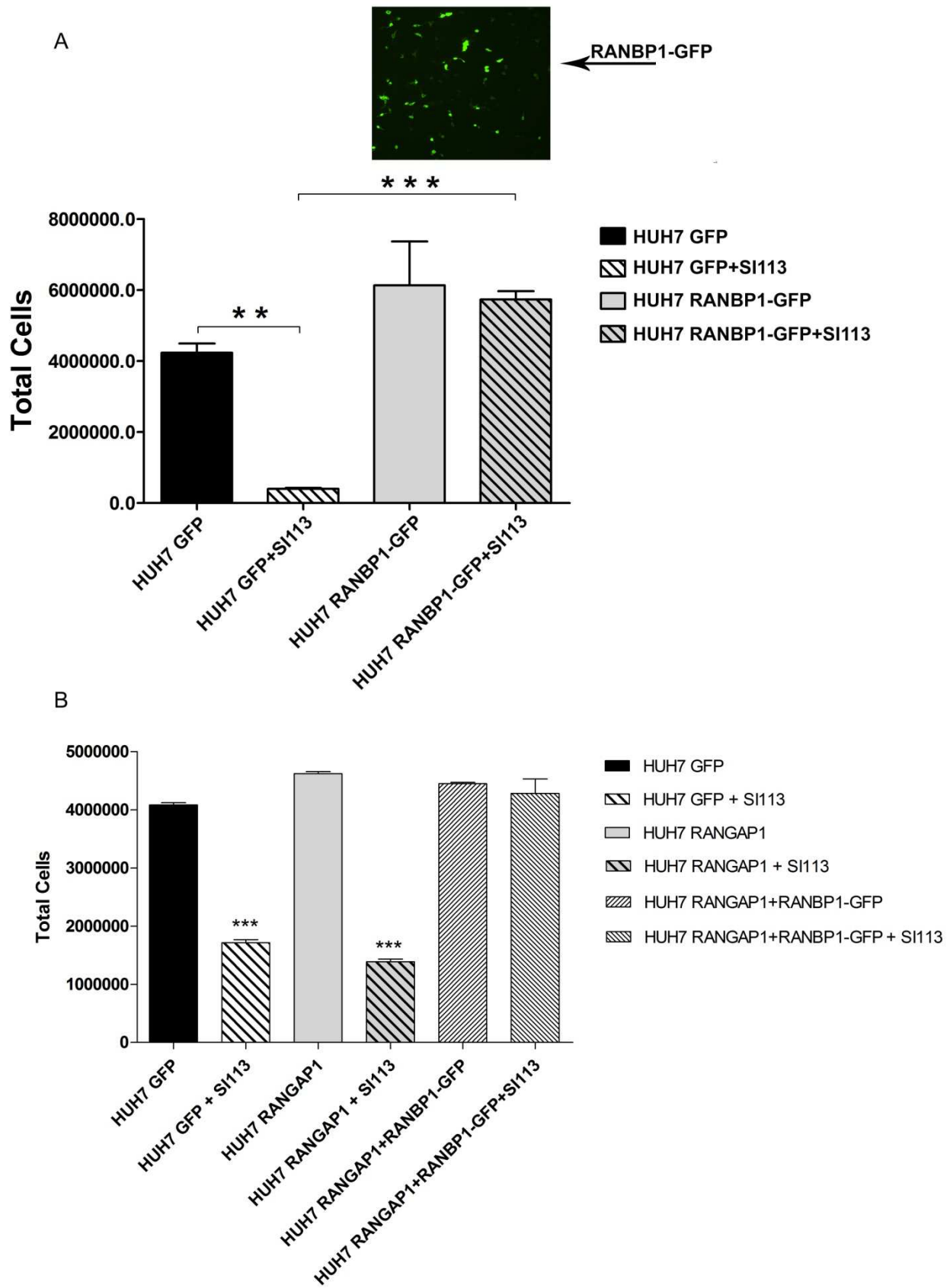
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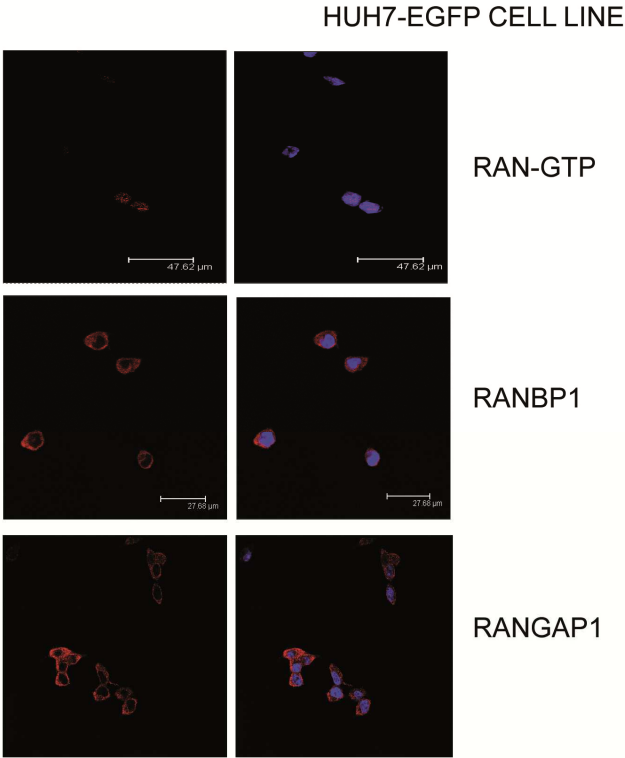


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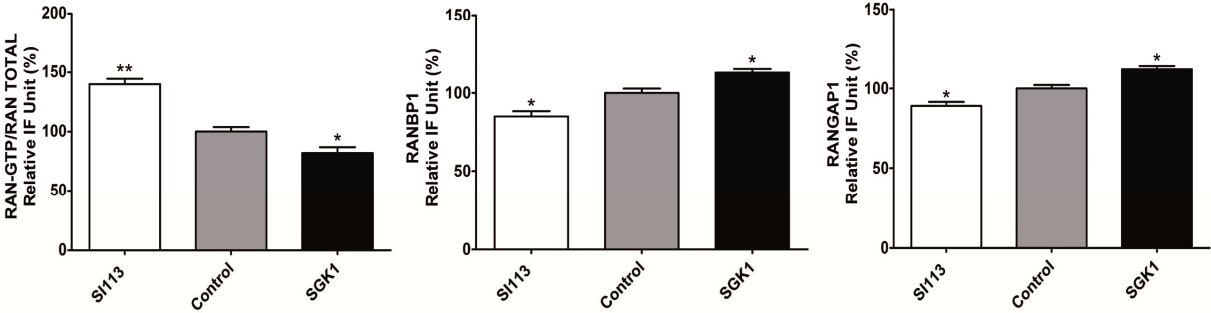




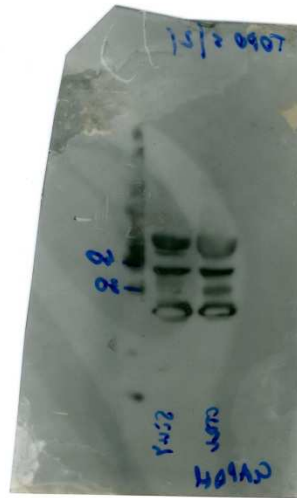
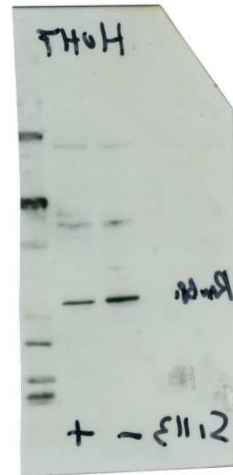
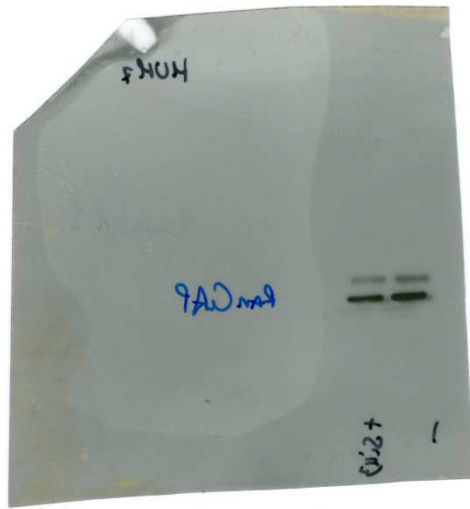
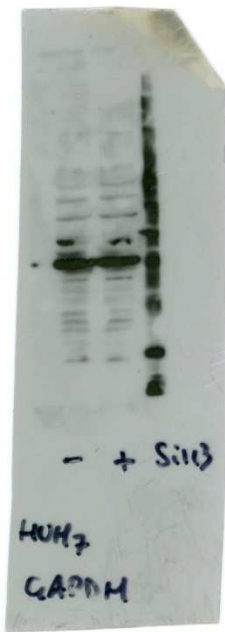
A



B



Raw gels Figure 5



Raw gels SF 2

