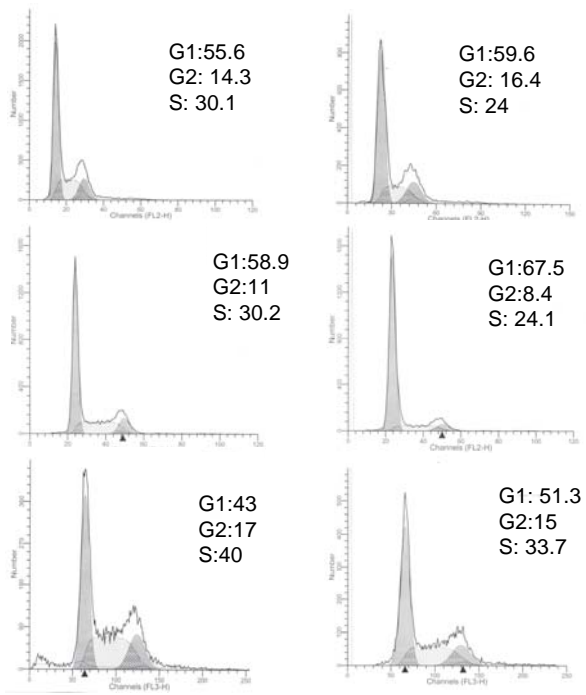


# S1A

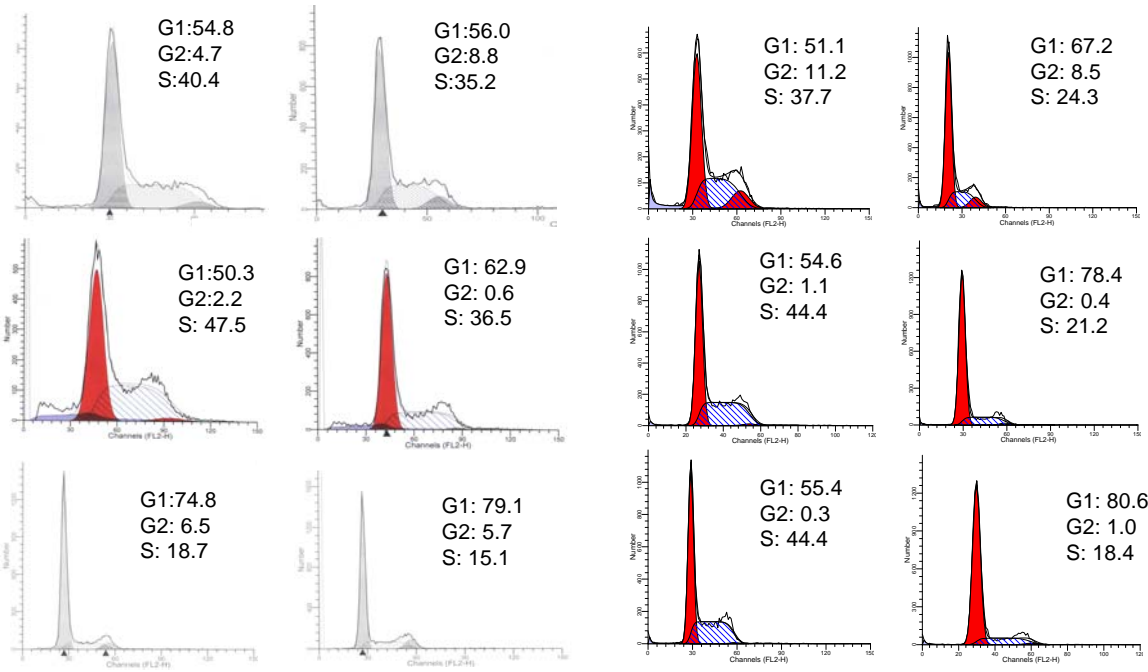


Ubch7

NS

48h

# S1B



UbchH7

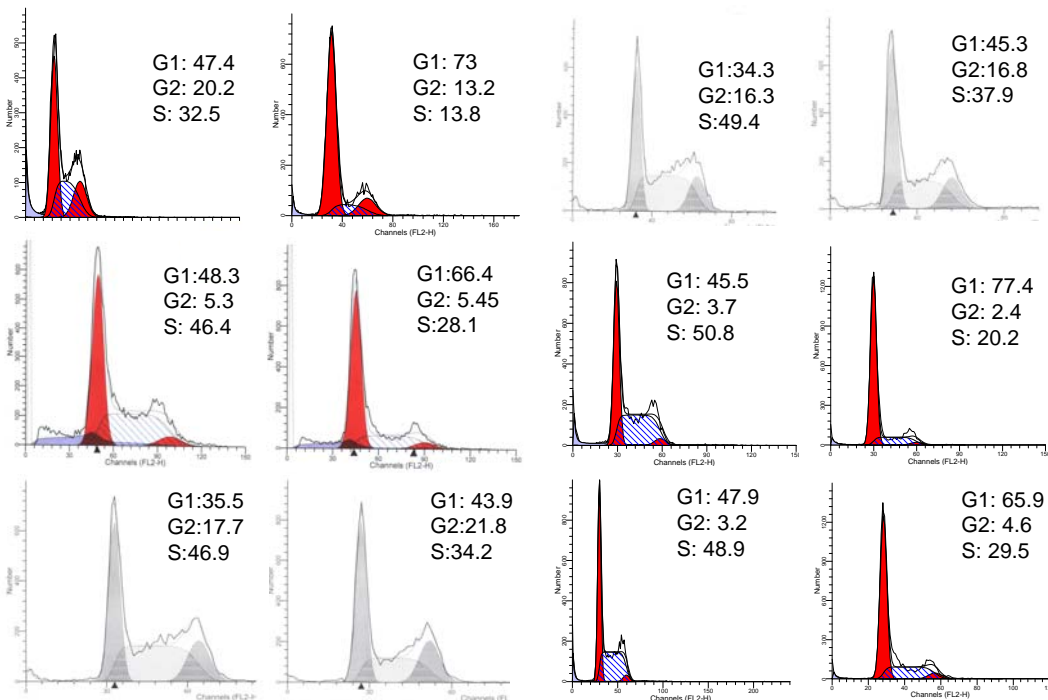
NS

UbchH7

NS

72h

# S1C



UbchH7

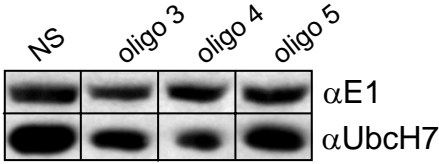
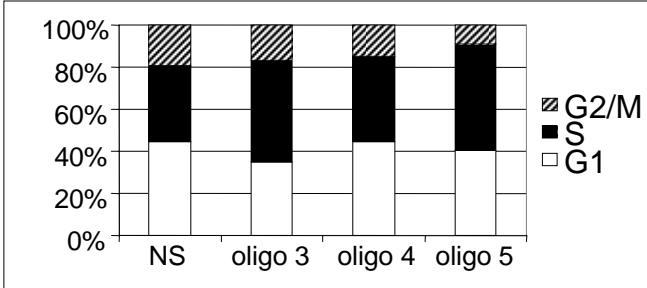
NS

UbchH7

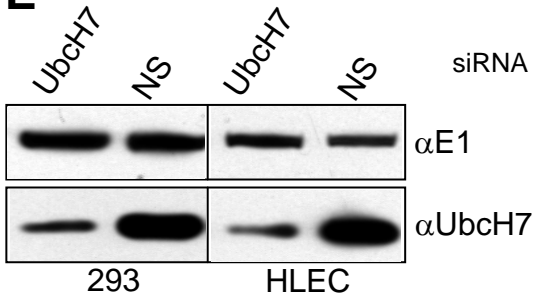
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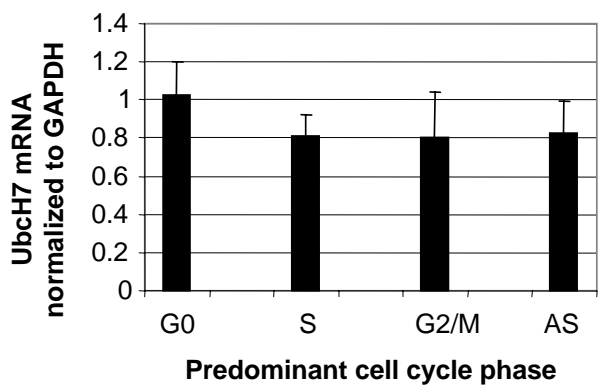
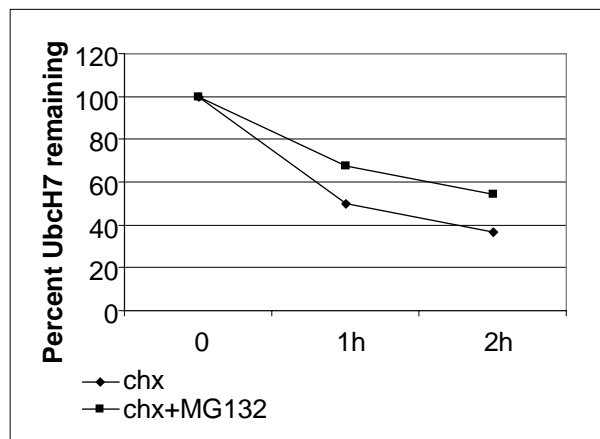
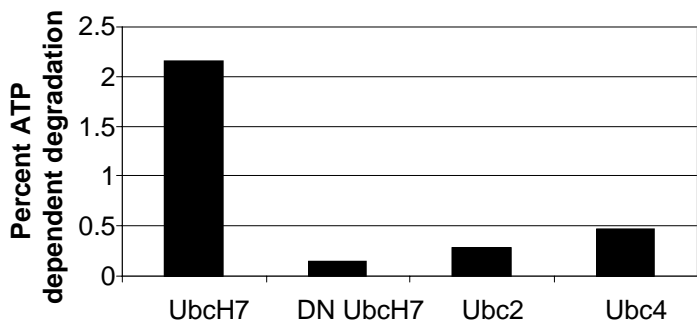
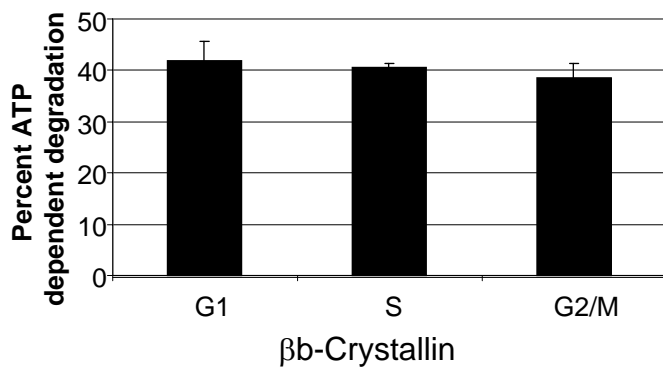
96h

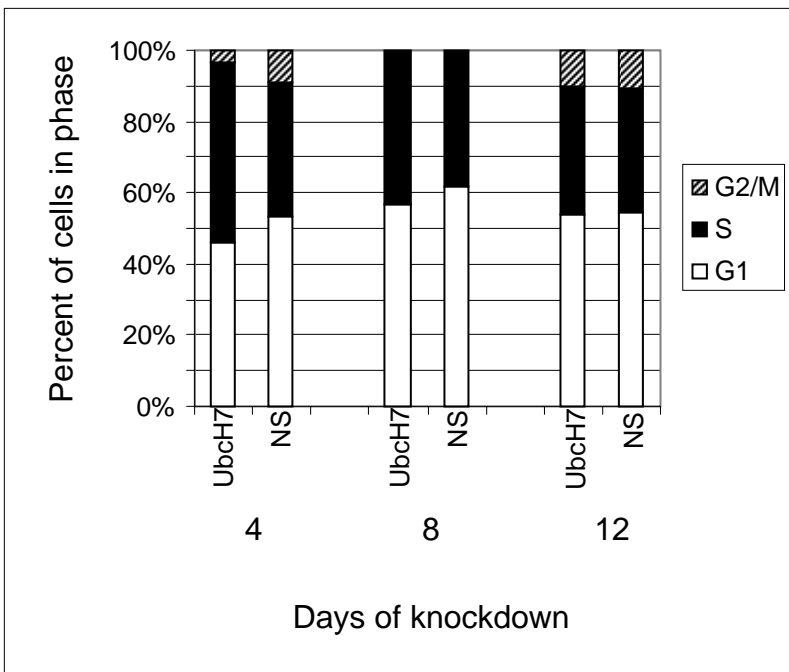
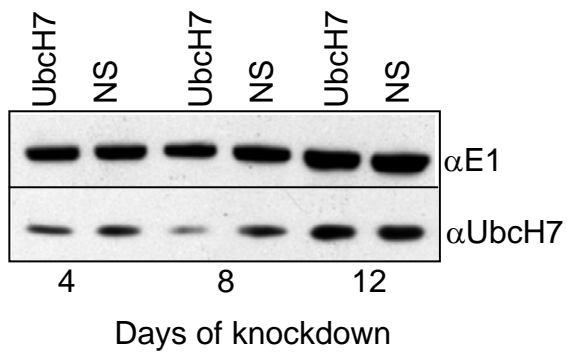
# S1D



# S1E



**S2A****B****C****D**

**S3**

S1 **(A-C)**. Modfit histogram analysis of data summarized in Fig. 2B. All Ubch7 depletions are on the left, corresponding NS siRNA from the same experiment on the right. In some experiments 7-AAD (FL-3) was used instead of PI (FL-2) for cell cycle analysis. **(A)** Histograms for 48 h knockdown. **(B)** 72 h **(C)** 96 h. **(D)** Knockdown of Ubch7 with three different siRNAs results in S phase increase. Upper panel: summary of FACS analysis. Lower panel: immunoblot showing remaining Ubch7 expression. **(E)** Immunoblot analysis of Ubch7 expression for experiment shown in Fig. 2C.

S2 **(A)** Quantitative RT-PCR of Ubch7 from HLE cells synchronized by contact inhibition. mRNA was isolated from cells immediately after release from contact inhibition (cells in G0/G1), 18h after release (S phase) 32h after release (G2/M) and 40h (asynchronous). The amount of Ubch7 mRNA compared to GAPDH immediately after release was set to 1 and the ratio of Ubch7 to GAPDH at other time points were compared to this value. **(B)** HeLa cells were treated with cycloheximide in the presence or absence of MG132 for 1 or 2 hours as indicated. Levels of Ubch7 protein were determined by immunoblot and compared to the level in untreated cells. **(C)** Degradation of E2s. <sup>125</sup>I labeled E2s were added to reticulocyte lysates supplemented with Ubc4 and ATP. Degradation was performed as in (4C). **(D)** Cells synchronized using HU as in Fig. 1A, were lysed and the resulting extracts were used for degradation of  $\beta$ -B crystallin in the presence of Ubc4, ATP and ubiquitin.

S3 Long term knockdown of Ubch7 results in S phase delay. Upper panel: immunoblot analysis of Ubch7 knockdown. Lower panel: cell cycle profile.