uL3 mediated nucleolar stress pathway as a new mechanism of action of antiproliferative G-quadruplex TBA derivatives in colon cancer cells

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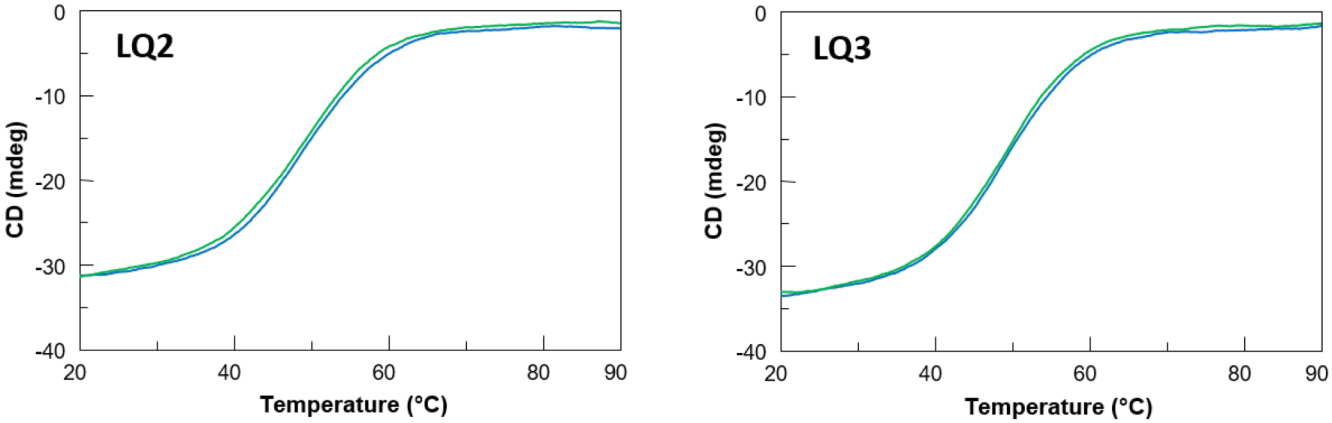


Figure S1. CD heating and cooling experiments. CD melting (blue) and annealing (green) curves registered as a function of temperature of the modified quadruplexes LQ2 and LQ3 at 294 nm. CD data were recorded in a 0.1 cm pathlength cuvette with a scan rate of 30°C/h at 50μM ODN strand concentration in K^+ buffer (10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl, pH 7.0).

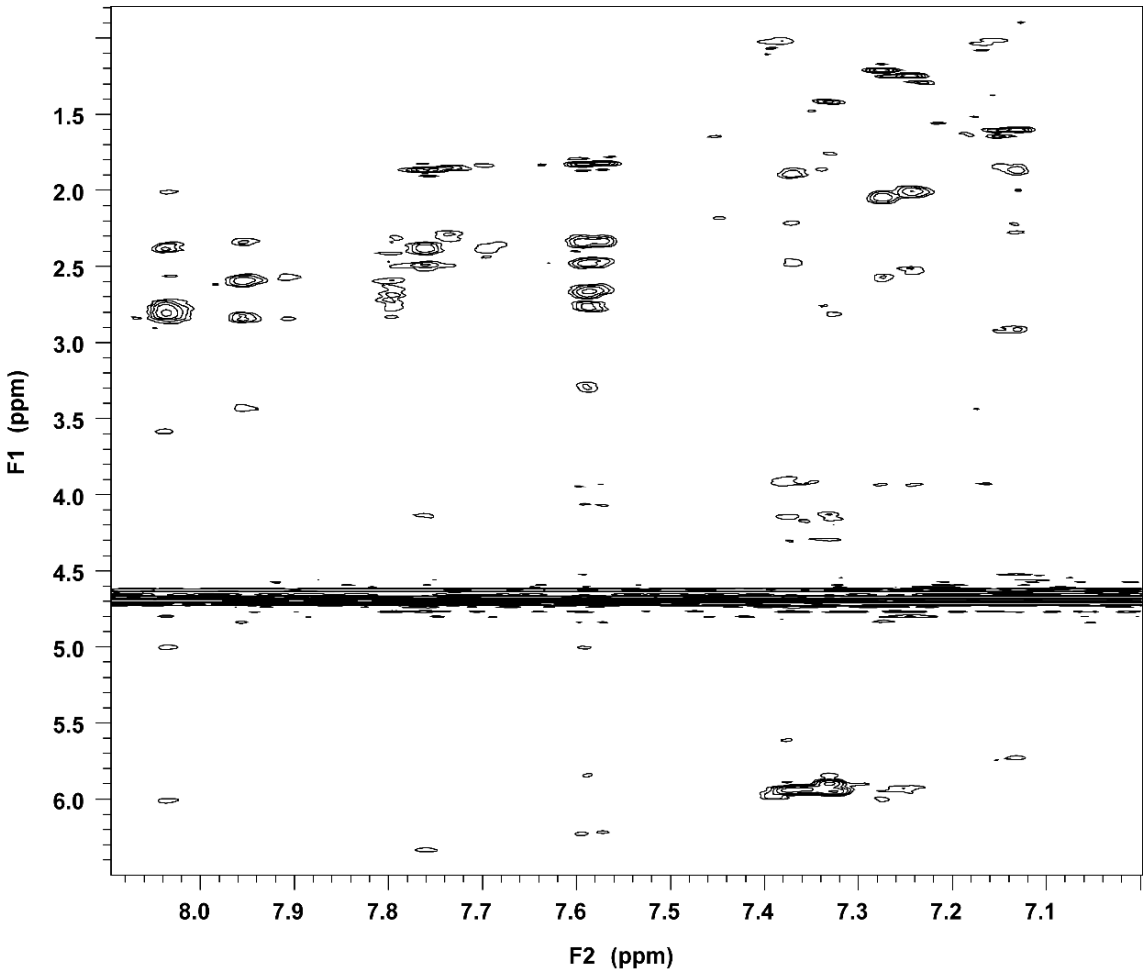


Figure S2. Two-dimensional NMR experiments. Expanded 2D NOESY spectrum of LQ2 (500 MHz; 25°C; strand concentration 3 mM; 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA, pH 7.0 in H2O/D2O 9:1; total volume 0.6 ml; mixing time 180 ms) correlating aromatic base protons and sugar protons.

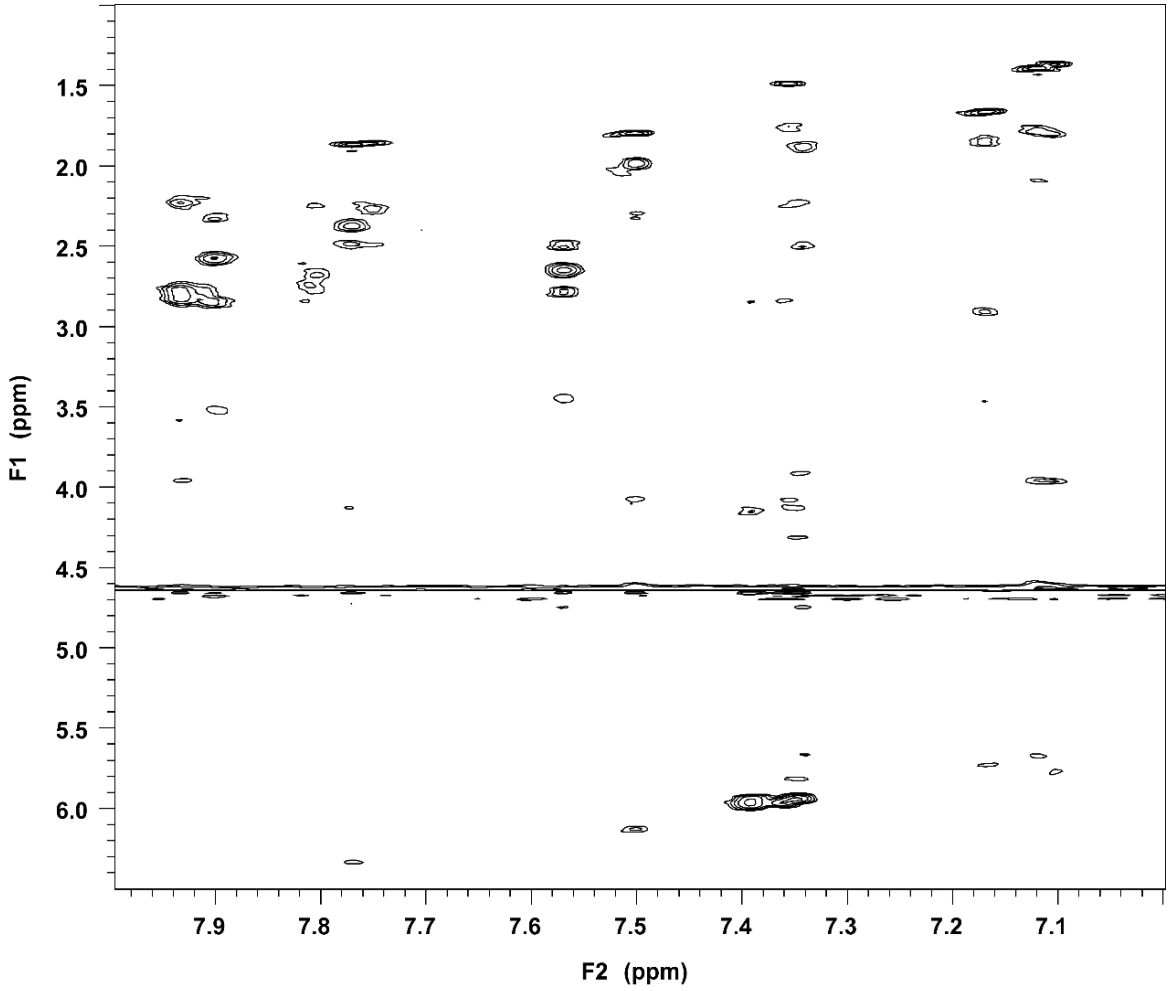


Figure S3. Two-dimensional NMR experiments. Expanded 2D NOESY spectrum of LQ3 (500 MHz; 25°C; strand concentration 3 mM; 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA, pH 7.0 in H_2O/D_2O 9:1; total volume 0.6 ml; mixing time 180 ms) correlating aromatic base protons and sugar protons.

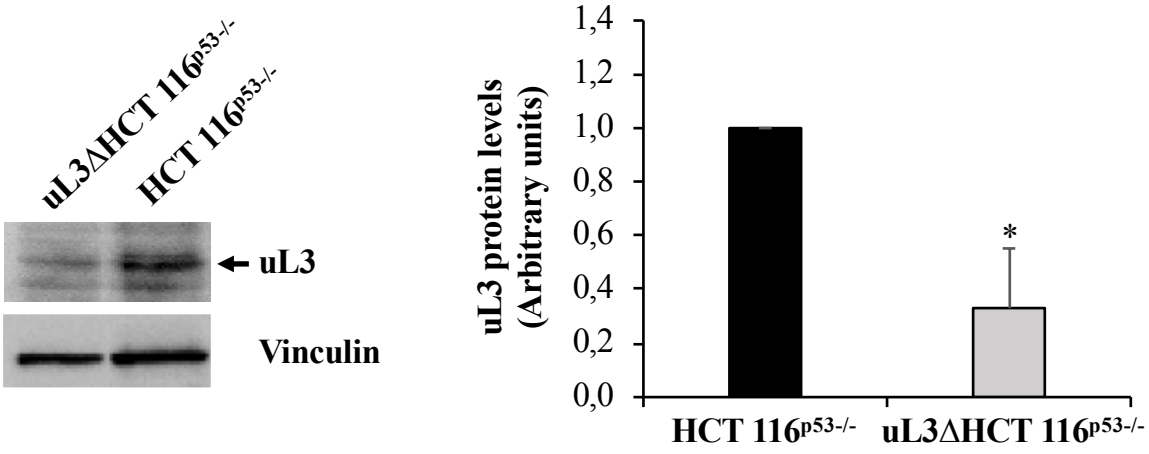


Figure S4. Representative western blotting analysis of uL3 in HCT 116$^{p53-/-}$ and uL3ΔHCT 116$^{p53-/-}$ cells. Protein extracts were analyzed by western blotting with the indicated antibodies. Vinculin was used as loading controls. Full-length blot is presented in Figure S8. Quantification of signals is shown. Bars represent the mean of triplicate experiments; error bars represent the standard deviation. *$p < 0.05$ vs. HCT 116$^{p53-/-}$ cells set at 1.

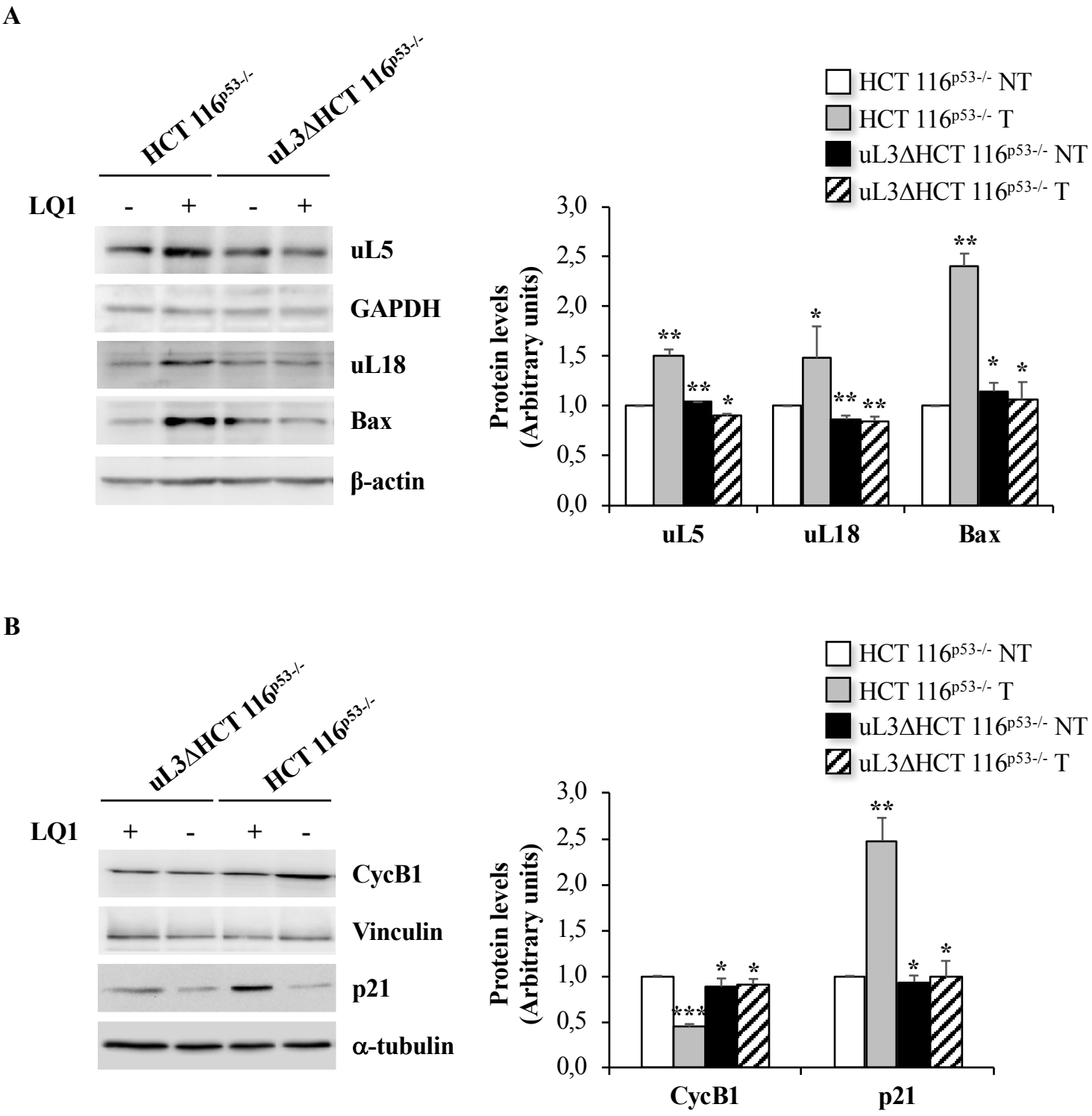


Figure S5. LQ1 treatment modulates the expression of nucleolar stress, cell cycle and apoptosis related genes. Representative western blot analysis of uL5, uL18 and Bax (**A**), CycB1 and p21 (**B**). HCT 116$^{p53-/-}$ and uL3ΔHCT 116$^{p53-/-}$ cells were treated with 10 μM of LQ1 for 48h. After treatment, protein extracts from the samples were analyzed by western blotting with antibodies against indicated proteins. GAPDH, β-actin, Vinculin and α-tubulin ware used as loading controls. Full-length blot is presented in Figures S9 and S10. Quantification of signals is shown. Bars represent the mean of triplicate experiments; error bars represent the standard deviation. *P<0.05, **P<0.01, ***P<0.001 vs. HCT 116$^{p53-/-}$ cells set at 1. NT: untreated; T: LQ1 treated.

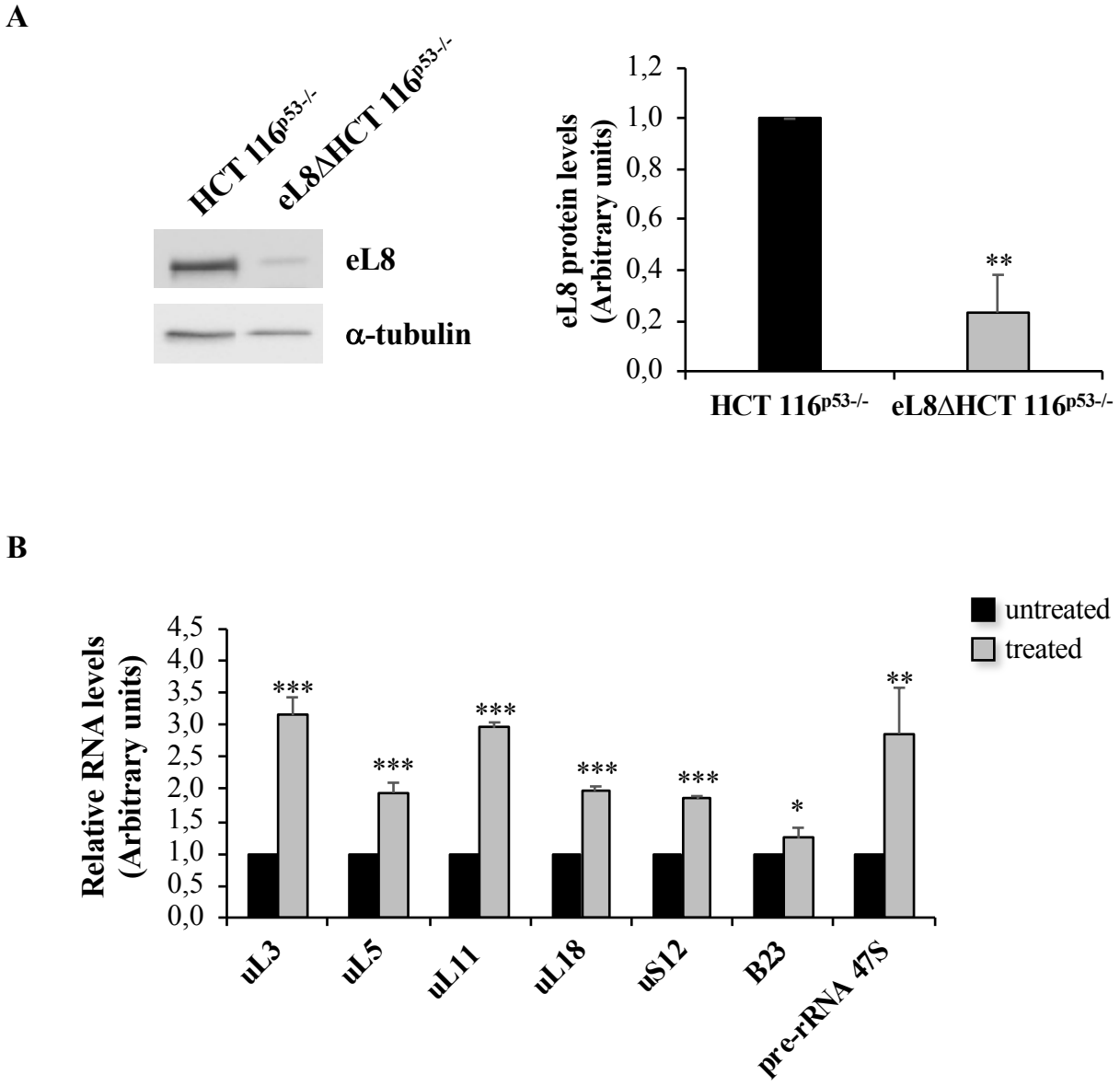
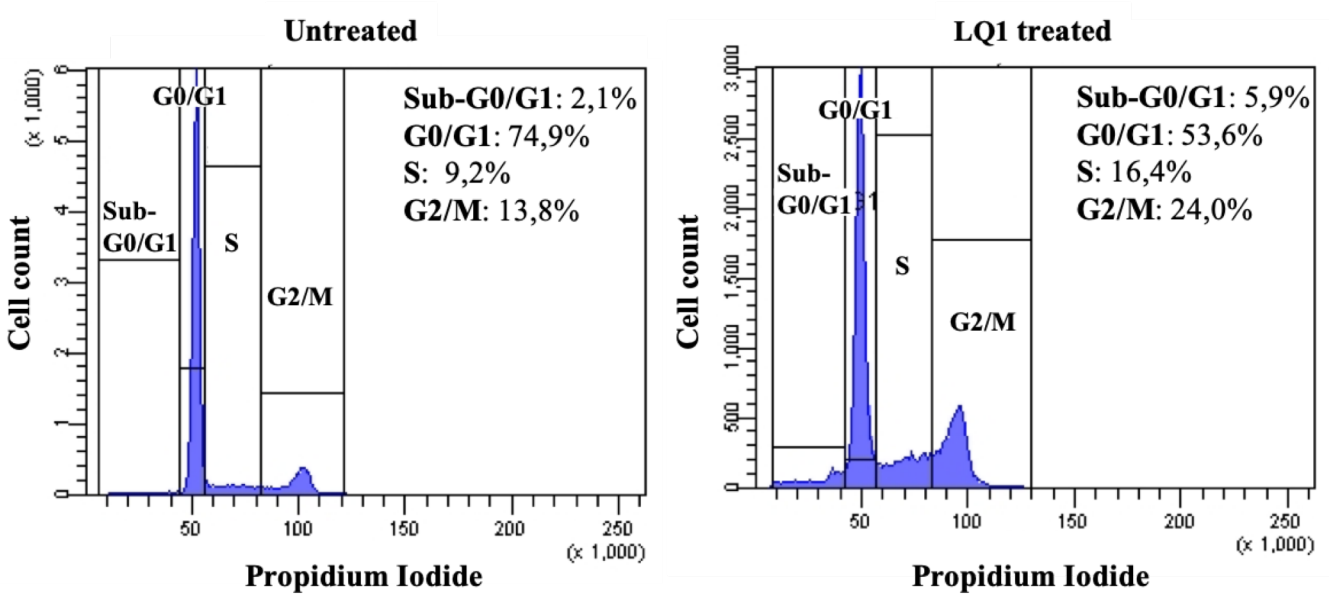


Figure S6. LQ1 treatment induces nucleolar stress in eL8ΔHCT 116$^{p53-/-}$ cells. (**A**) Representative western blot analysis of eL8 in HCT 116$^{p53-/-}$ and eL8ΔHCT 116$^{p53-/-}$ cells. Protein extracts were analyzed by western blotting with the indicated antibodies. **α**-tubulin was used as loading controls. Full-length blot is presented in Figure S11. Quantification of signals is shown. Bars represent the mean of triplicate experiments; error bars represent the standard deviation. **p < 0.01 vs. HCT 116$^{p53-/-}$ cells set at 1. (**B**) Total RNA from eL8ΔHCT 116$^{p53-/-}$ cells, untreated or treated with 10 μM of LQ1 for 48 h, was subjected to RT-qPCR with primers specific for indicated genes (Table 2). Quantification of signals is shown. Bars represent the mean of triplicate experiments; error bars represent the standard deviation. *P<0.05, **P<0.01, ***P<0.001 vs. untreated cells set at 1.

A



B

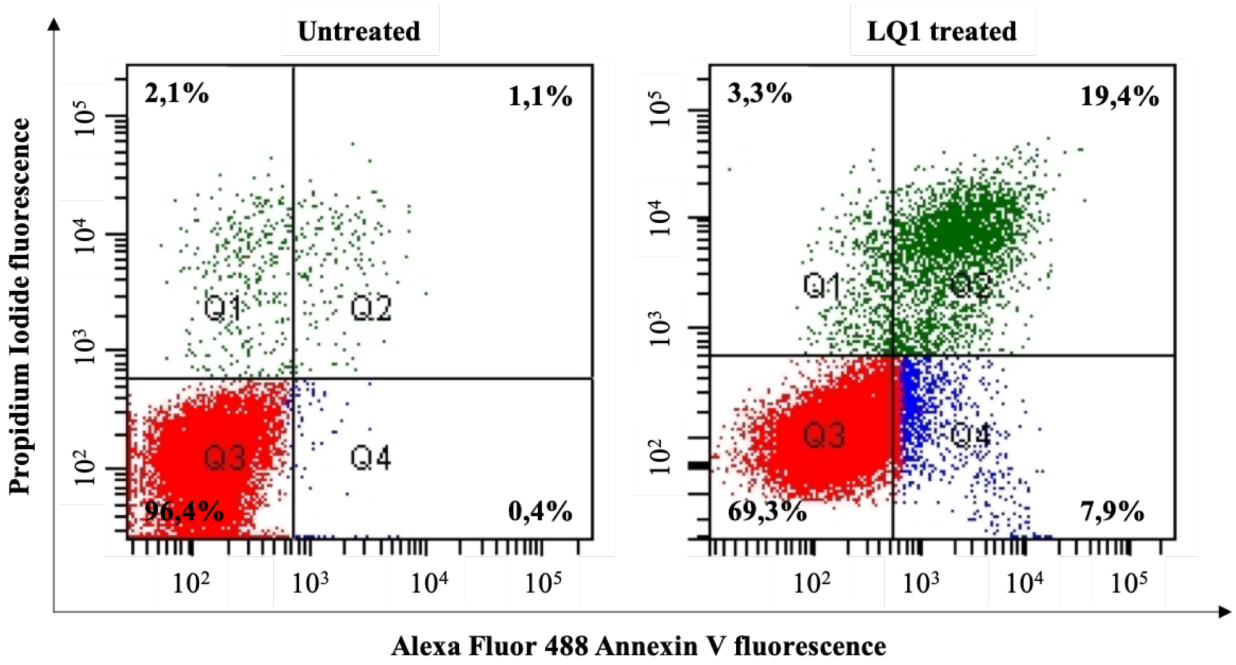


Figure S7. LQ1 treatment causes cell cycle arrest and apoptosis in eL8ΔHCT 116$^{p53-/-}$ cells. (**A**) Cells were incubated with 10 μM of LQ1 for 48 h and the cell cycle distribution was evaluated using PI staining and flow cytometry analysis. (**B**) Cell death was assessed by FACS analysis of Annexin V and PI staining. Representative dot plots are shown.

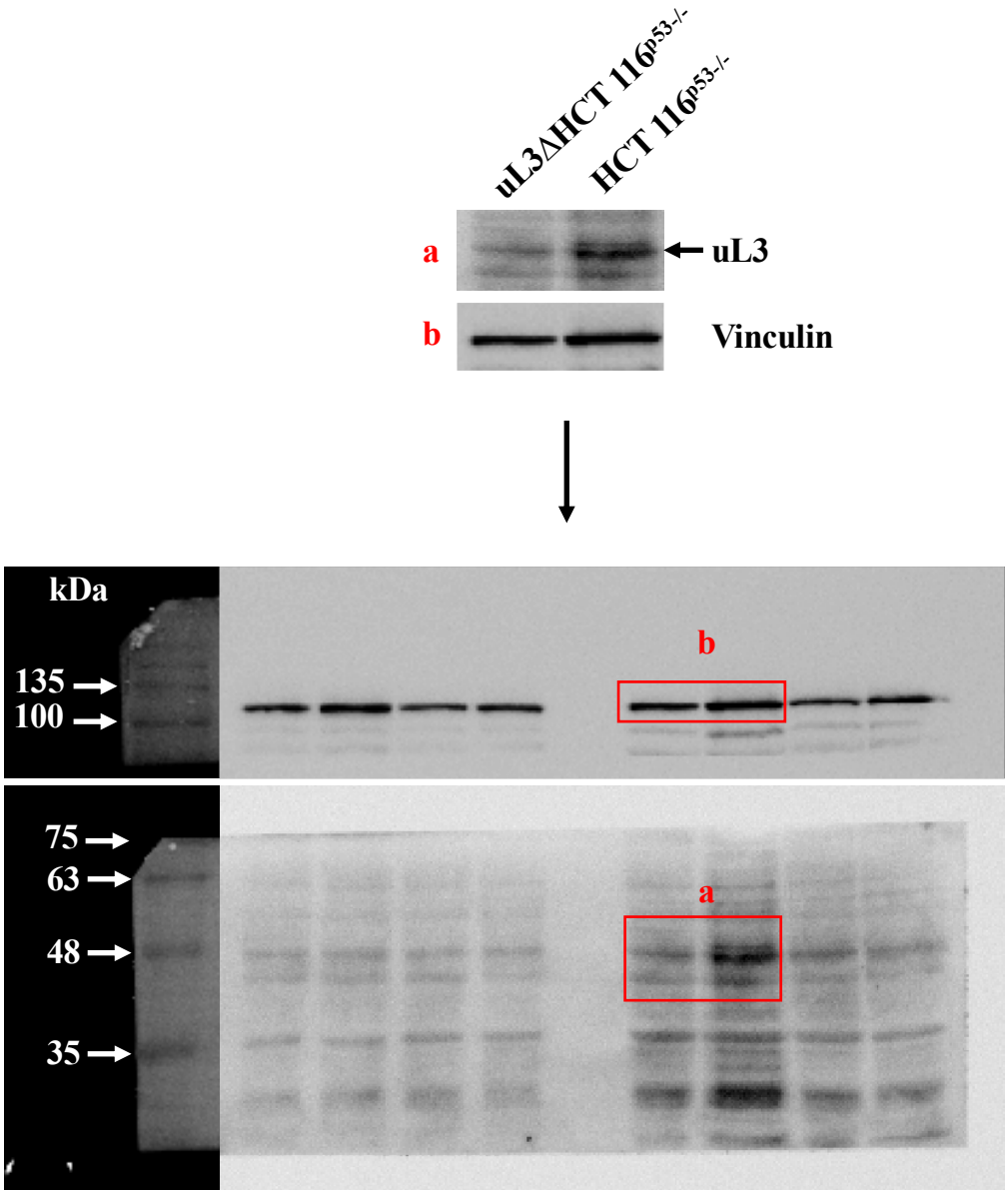


Figure S8. Full-length blots of western blotting showed in Figure S4.

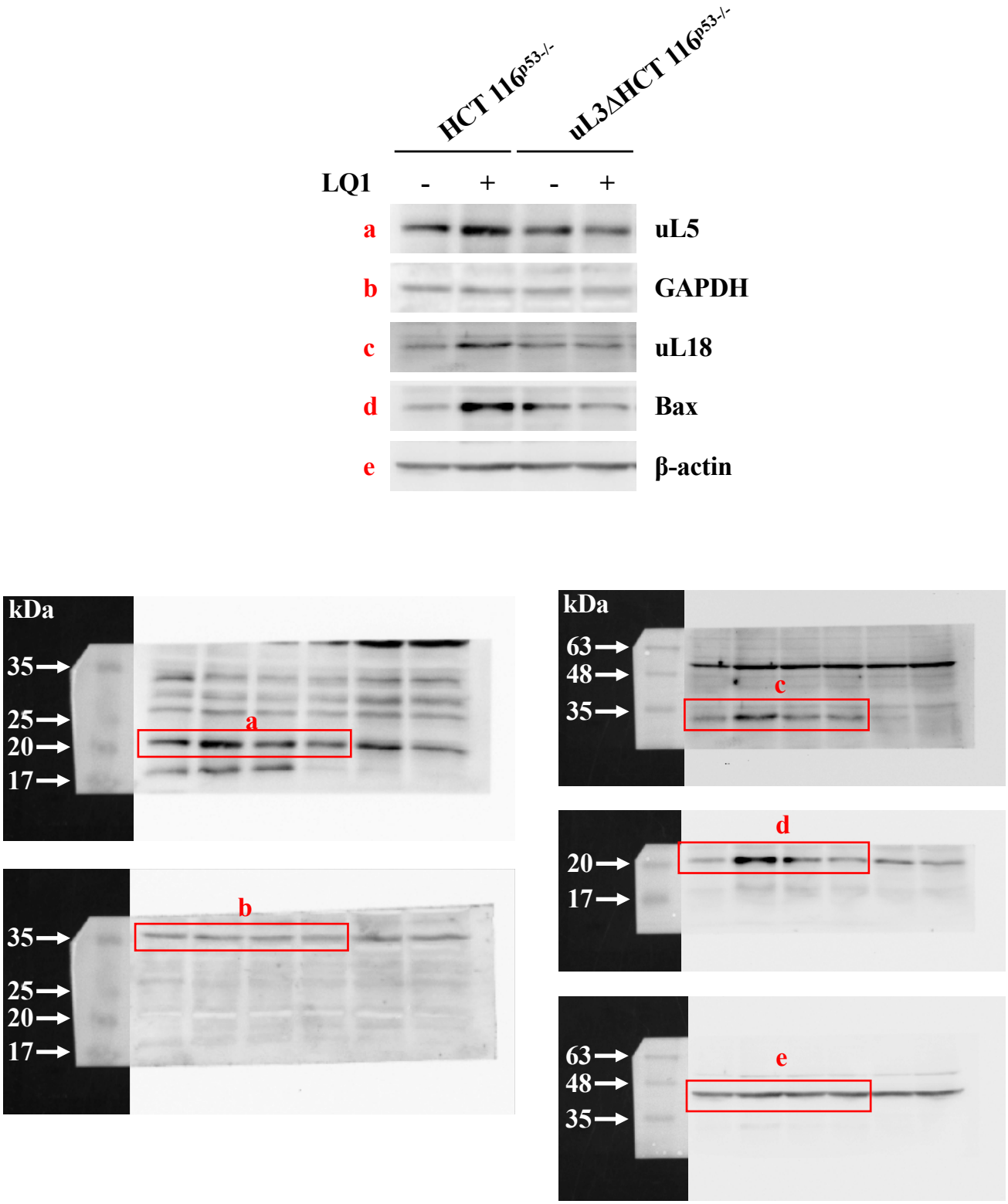


Figure S9. Full-length blots of western blotting showed in Figure S5A.

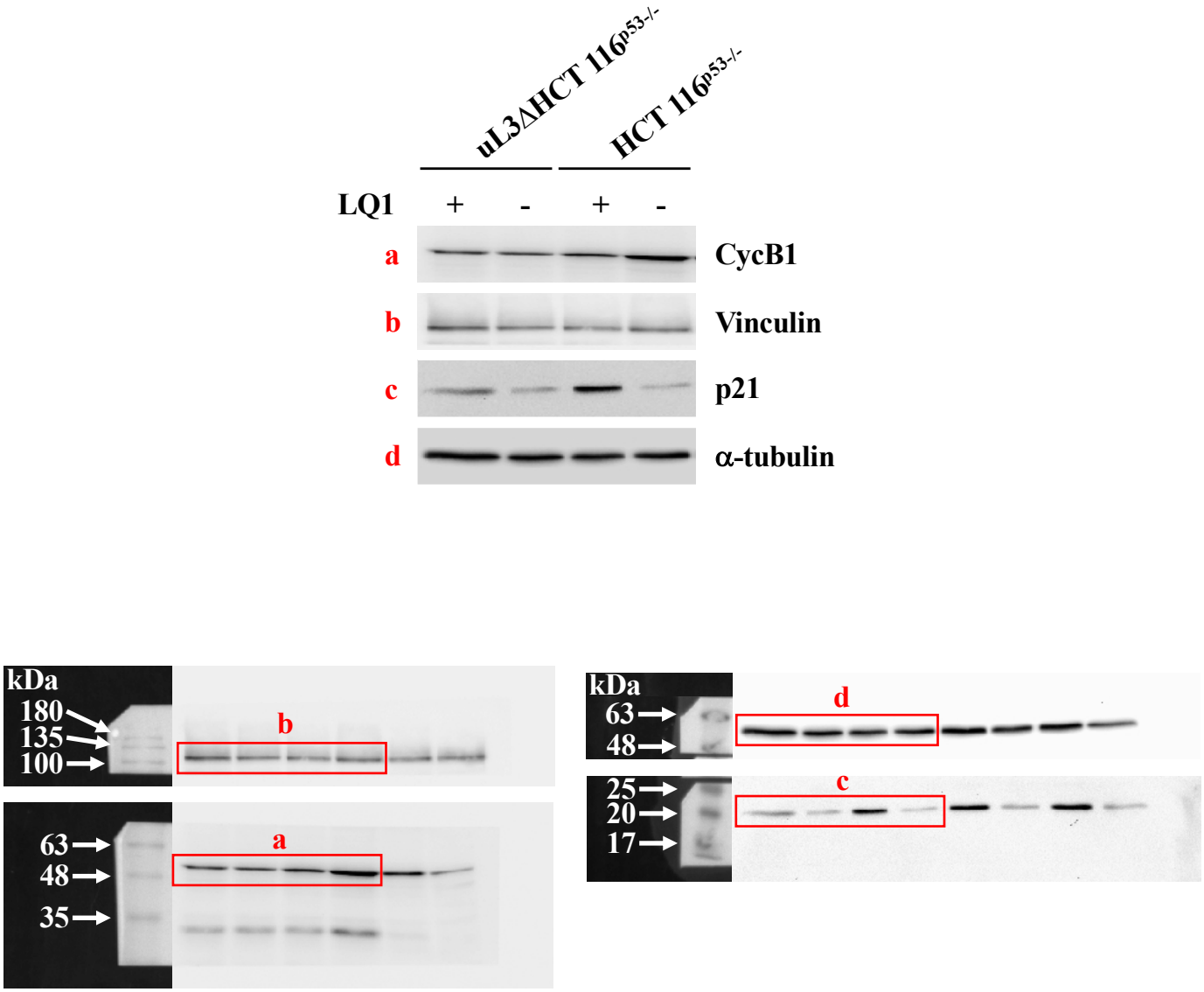


Figure S10. Full-length blots of western blotting showed in Figure S5B.

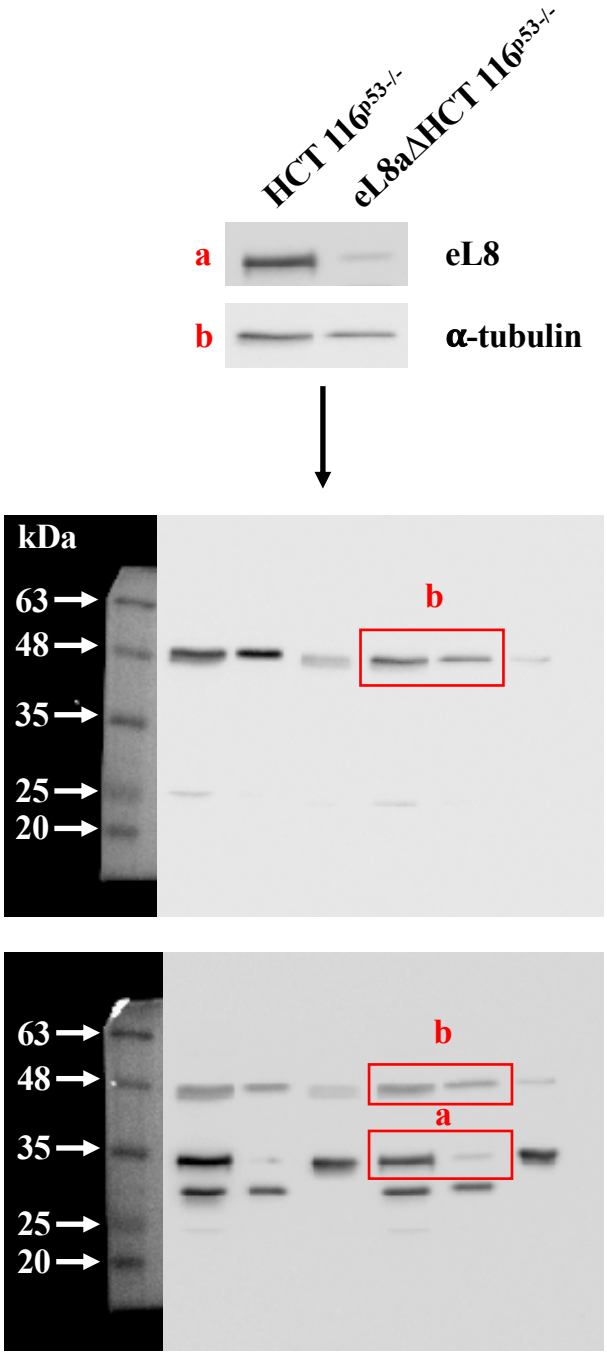


Figure S11. Full-length blots of western blotting showed in Figure S6A.

LQ2 5'-$G_{L1}G_{L2}T_{D3}T_{L4}G_{L5}G_{L6}T_{L7}G_{L8}T_{L9}G_{L10}G_{L11}T_{D12}T_{L13}G_{L14}G_{L15}$-3'							
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3
$\underline{G}_{L1}$	7.32	5.95	2.82	4.89	4.30	4.16	-
G_{L2}	8.04	6.00	2.40/2.82	5.00	4.21	4.17	-
T_{D3}	7.60	6.23	2.34/2.49	4.84	4.24	4.07	1.83
T_{L4}	7.25	5.93	2.02/2.53	4.79	4.22	3.93	1.26
$\underline{G}_{L5}$	7.33	5.88	2.78/3.30	4.77	4.52	4.06	-
G_{L6}	7.59	5.84	2.49/2.68	5.01	N.D.	4.08/3.95	-
T_{L7}	7.76	6.34	2.38/2.51	4.75	4.31	4.15	1.86
G_{L8}	7.37	5.61	1.90/2.22	4.57	4.30	3.90	-
T_{L9}	7.14	5.74	1.87/2.28	4.53	4.20	N.D.	1.60
$\underline{G}_{L10}$	7.36	5.94	2.82/3.58	4.80	4.16	N.D.	-
G_{L11}	8.04	6.03	2.38/2.78	5.03	N.D.	4.14	-
T_{D12}	7.57	6.22	2.34/2.49	4.84	4.24	4.07/3.93	1.82
T_{L13}	7.28	6.00	2.06/2.58	4.80	4.20	3.95	1.21
$\underline{G}_{L14}$	7.37	5.94	2.84/3.43	4.84	N.D.	N.D.	-
G_{L15}	7.95	6.05	2.35/2.61	4.80	4.29	4.16	-

LQ3 5'-$G_{L1}G_{L2}T_{L3}T_{D4}G_{L5}G_{L6}T_{L7}G_{L8}T_{L9}G_{L10}G_{L11}T_{L12}T_{D13}G_{L14}G_{L15}$-3'							
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3
$\underline{G}_{L1}$	7.36	5.97	2.84	4.88	N.D.	N.D.	-
G_{L2}	7.81	5.85	2.25/2.68	4.97	4.61	4.29	-
T_{L3}	7.36	5.85	2.10/2.24	4.86	N.D.	N.D.	1.49
T_{D4}	7.10	5.78	1.82/2.78	5.00	4.33	N.D.	1.36
$\underline{G}_{L5}$	7.35	5.94	2.80/3.45	4.78	4.30	N.D.	-
G_{L6}	7.57	5.82	2.50/2.66	5.00	4.36	4.24	-
T_{L7}	7.77	6.34	2.38/2.50	4.75	4.31	4.14	1.86
G_{L8}	7.34	5.68	1.88/2.23	4.69	N.D.	N.D.	-
T_{L9}	7.17	5.74	1.84/2.29	4.53	N.D.	3.65	1.66
$\underline{G}_{L10}$	7.39	5.94	2.85/3.60	4.80	4.29	N.D.	-
G_{L11}	7.93	5.80	2.23/2.80	5.00	4.25	4.13	-
T_{L12}	7.50	6.13	2.00/2.31	4.62	4.26	4.08	1.80
T_{D13}	7.12	5.68	1.78/2.08	4.61	4.28	N.D.	1.39
$\underline{G}_{L14}$	7.39	5.96	2.85/3.52	4.80	4.33	4.18	-
G_{L15}	7.90	6.02	2.34/2.59	4.69	4.12	N.D.	-

Table S1. Chemical shift values. Proton chemical shifts assignment for G-quadruplex formed by ODNs LQ2 and LQ3 (500 MHz, T = 25°C) in 10 mM KH2PO4/K2HPO4, 70 mM KCl and 0.2 mM EDTA (pH 7.0). N.D. = not determined. Residues adopting the syn glycosidic conformation are underlined.