

Supplementary information

Ripening-induced chemical modifications of papaya pectin inhibit cancer cell proliferation

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Table S1. Papaya water-soluble polysaccharides linkage analysis (PP: papaya pectin from different harvesting days – 1, 2 3 and 4).

Monomer	1PP	2PP	Mol (%)	
			3PP	4PP
t-Rha	0.8 ± 0.2	0.6 ± 0.3	0.7 ± 0.2	0.5 ± 0.1
2-Rha	0.8 ± 0.2	1.6 ± 0.3	2.0 ± 0.2	1.7 ± 0.1
2,4-Rha	0.1 ± 0.1	0.7 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
t-Fuc	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2,4-Fuc	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
t-Araf	2.1 ± 0.1	1.1 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
t-Arap	0.9 ± 0.1	0.5 ± 0.2	0.8 ± 0.1	0.7 ± 0.1
2-Araf	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
3-Araf	0.7 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
5-Ara(f)	1.0 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
2,5-Ara(f)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3,5-Ara(f)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Ara(OAc)5	1.0 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
t-Xylp	0.7 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
2-Xyl	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
2,4-Xyl	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
4-Xyl	0.9 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
3,4-Xyl	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Xyl(OAc)5	0.3 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
t-Man	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4-Man	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
4,6-Man	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Man(OAc)6	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	1.0 ± 0.1
t-Gal	0.8 ± 0.1	1.0 ± 0.3	2.6 ± 0.6	1.8 ± 0.3
2-Gal	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
3-Gal	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
3,4-Gal	0.6 ± 0.2	0.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
4-Gal	1.0 ± 0.1	0.8 ± 0.2	1.1 ± 0.2	0.3 ± 0.1
2,4-Gal	0.3 ± 0.1	0.8 ± 0.2	0.3 ± 0.1	0.4 ± 0.2
3,6-Gal	4.4 ± 0.1	2.7 ± 0.3	2.7 ± 0.2	1.9 ± 0.3
4,6-Gal	0.4 ± 0.1	0.4 ± 0.1	1.1 ± 0.4	0.4 ± 0.2
6-Gal	4.6 ± 0.4	1.9 ± 0.3	2.1 ± 0.2	2.2 ± 0.2
2,4,6-Gal	0.1 ± 0.1	0.4 ± 0.3	0.2 ± 0.1	0.4 ± 0.2
Gal(OAc)6	1.2 ± 0.5	2.3 ± 0.6	1.2 ± 0.3	1.0 ± 0.2
t-GalA	1.9 ± 0.4	2.6 ± 0.5	7.9 ± 2.8	4.2 ± 0.2
4-GalA	60.7 ± 2.4	64.3 ± 2.1	58.5 ± 3.5	68.5 ± 2.0
3,4-GalA	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
4,6 GalA	6.4 ± 1.3	8.0 ± 1.1	5.5 ± 0.6	5.0 ± 1.1
2,4 GalA	2.6 ± 1.1	5.1 ± 2.1	2.8 ± 0.7	1.8 ± 0.5
t-Glc	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1
2-Glc	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
3-Glc	0.4 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
4-Glc	0.8 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
3,4-Glc	0.3 ± 0.1	0.3 ± 0.3	0.9 ± 0.2	0.7 ± 0.1
4,6-Glc	0.1 ± 0.1	0.8 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
Glc(OAc)6	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
t-GlcA	1.1 ± 0.3	1.8 ± 0.5	1.5 ± 0.3	1.8 ± 0.3

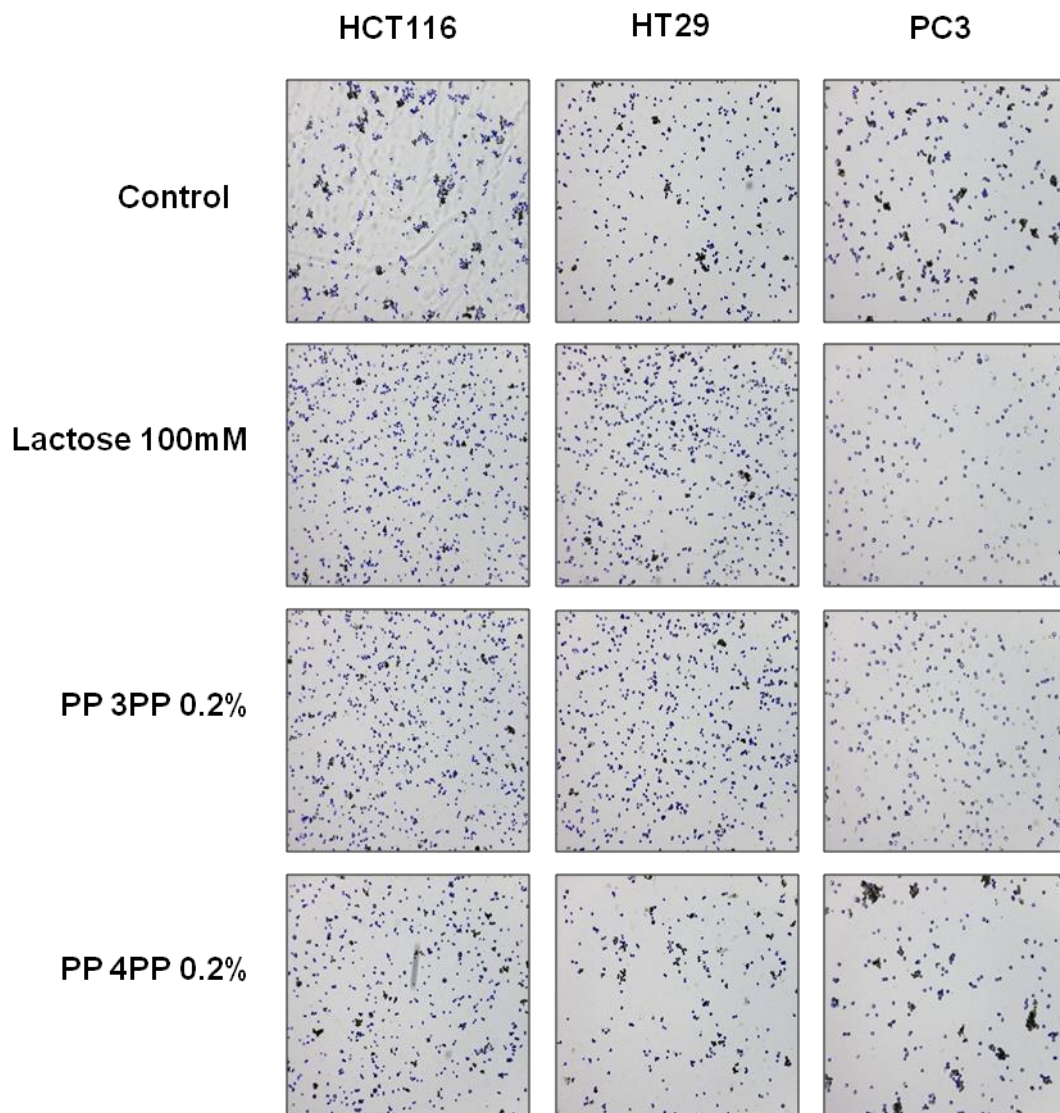


Figure S1. Homotypic aggregation. Cells were counted in an automatic cell counter and the blue circles were automatic generated by the machine software and is related to single or double cells.

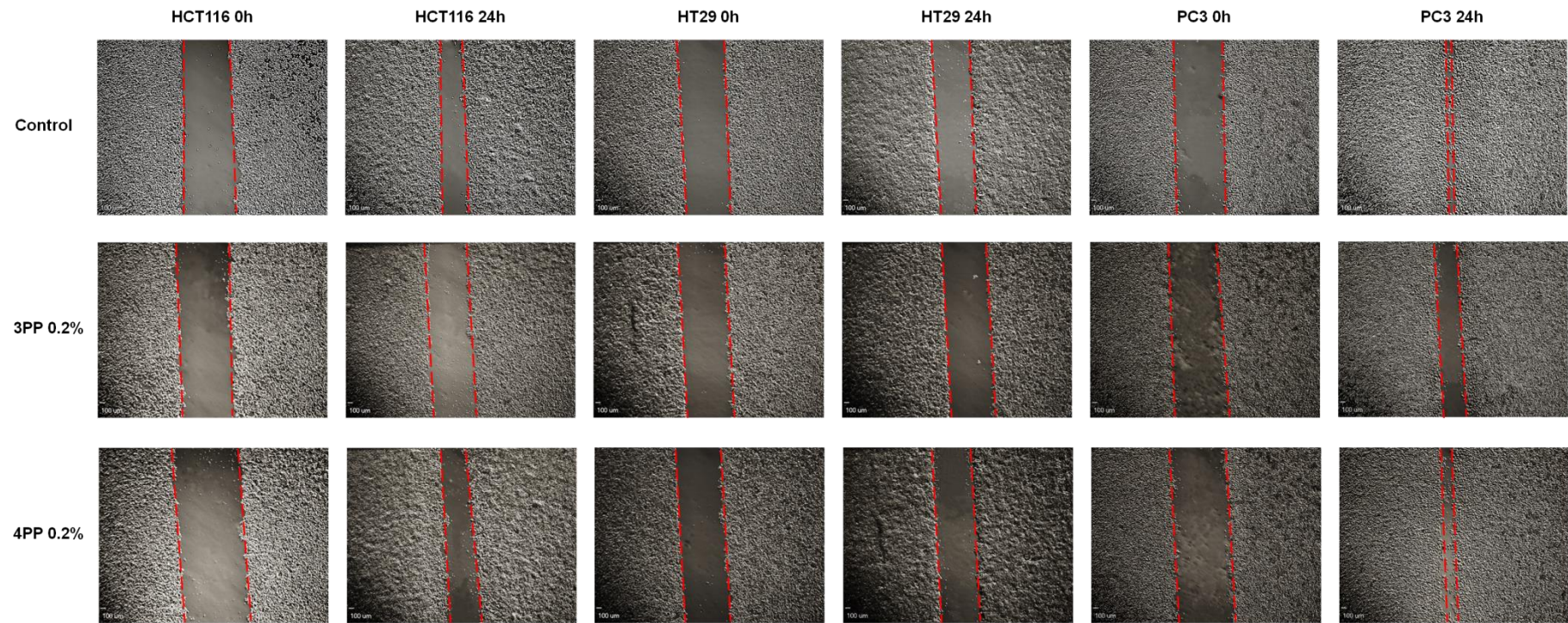


Figure S2. Migration assay based on wound healing assay. Photographs of cancer cell cultures in 0 h (after scratching) and after 24 h of treatment or not (control) indicating the wound closure. Scale bar: 100 μm.

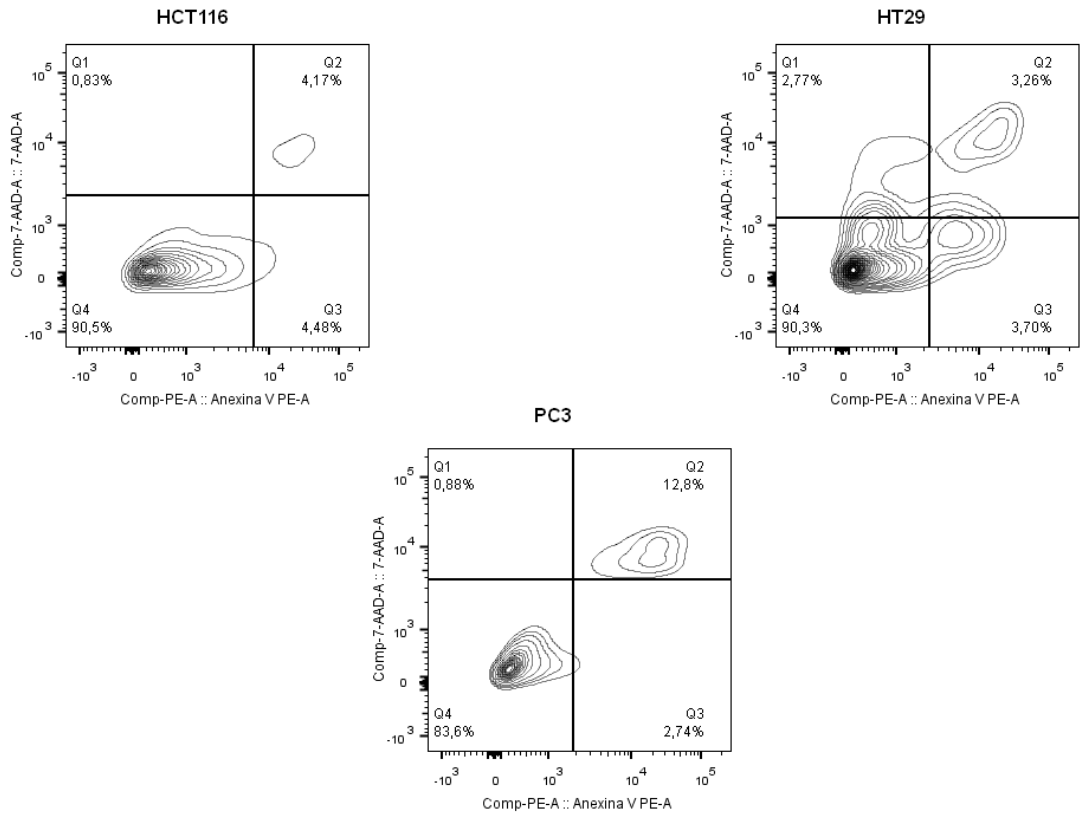


Figure S3. Effects of lactose in HCT116, HT29 and PC3 apoptosis by flow cytometry. Lactose did not induce cell death. Cells were treated with 100 mM of lactose for 24 h.

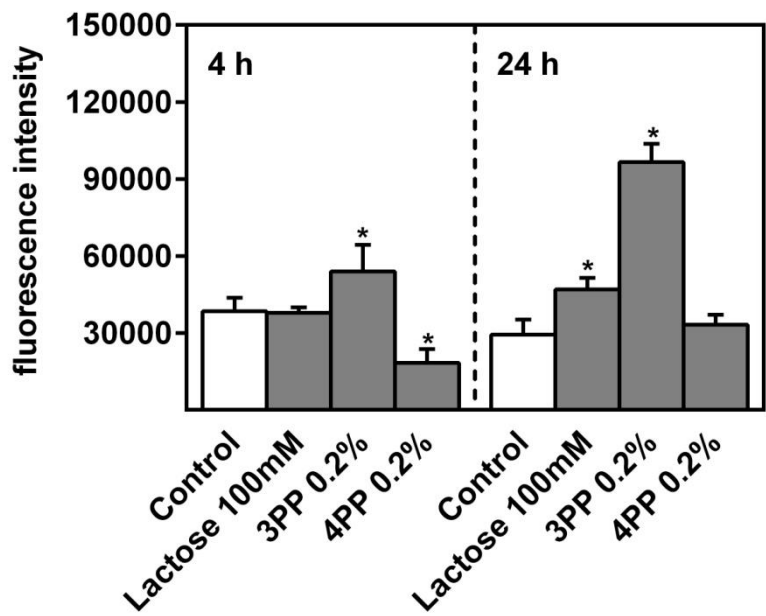


Figure S4. ROS detection on HCT116 cells measured by DCFDA. 3PP and not 4PP induced ROS accumulation. Cells were treated and after 4 h or 24 h cells were incubated with DCFDA for 45 min. Data was expressed as mean \pm SD of fluorescence ratios for two independent experiments. * $P < 0.05$ vs control, according to Dunnett's test. PP: papaya pectin (water-soluble fraction).

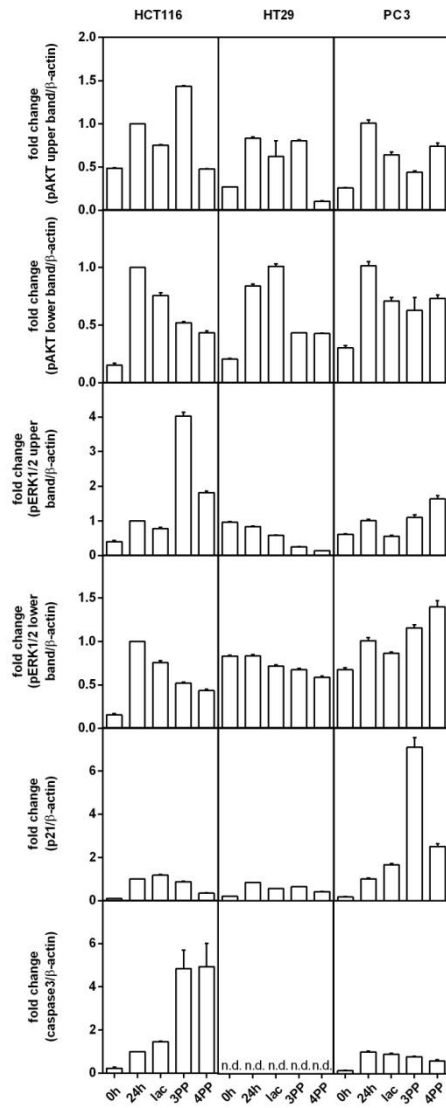


Figure S5. Relative density of bands from western blotting experiments. PP: papaya pectin (water-soluble fraction). Band intensities were separated in upper and lower bands for pAKT and pERK1/2 from two experiments.

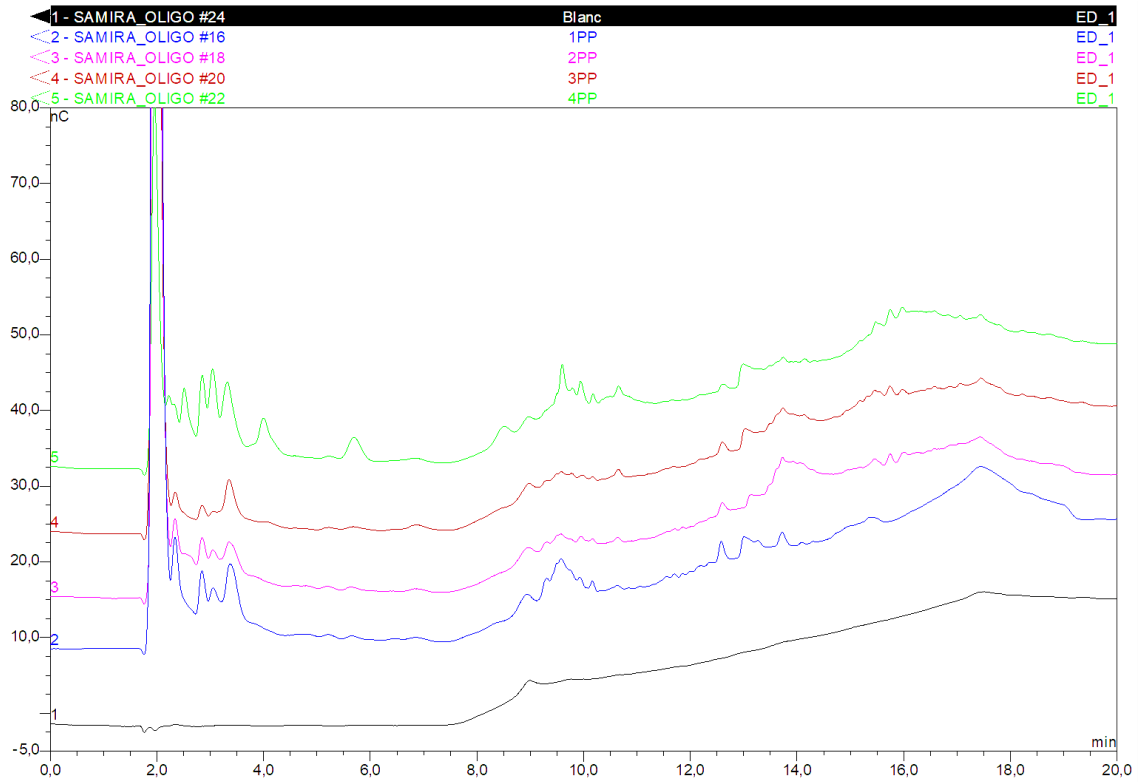


Figure S6. Qualitative analysis for oligosaccharide detection in papaya pectins. Black line: Blank. Blue line: 1PP. Purple line: 2PP. Red line: 3PP. Green line: 4PP.

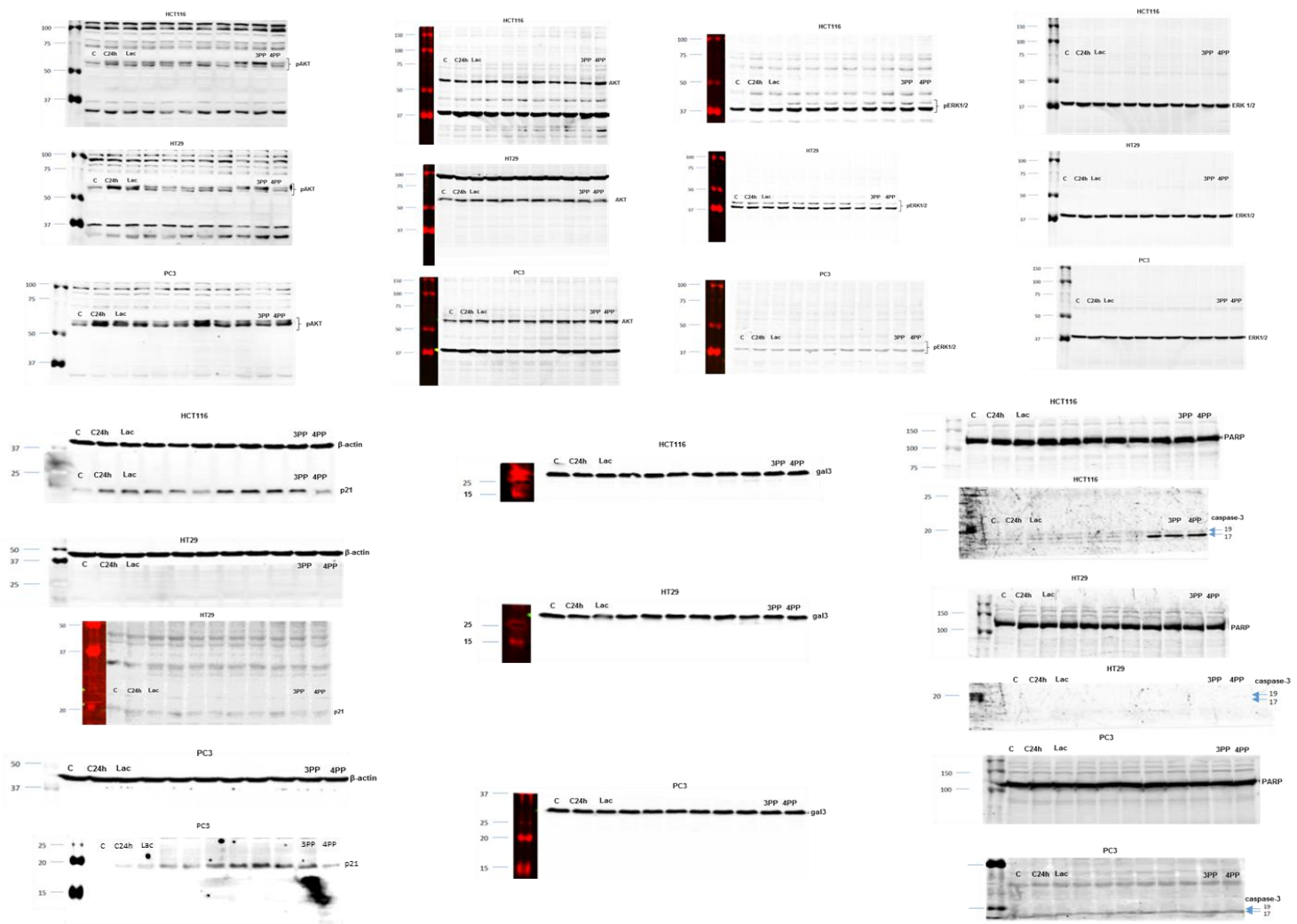


Figure S6. Representative western blotting from proteins analyzed in the article.