

Supplementary Materials: Programmed Cell Death Alterations Mediated by Synthetic Indole Chalcone Resulted in Cell Cycle Arrest, DNA Damage, Apoptosis and Signaling Pathway Modulations in Breast Cancer Model

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1. The structure of compound was confirmed by ^1H , ^{13}C NMR, IR and HRMS spectroscopy:

^1H NMR (DMSO- d_6 , 600 MHz): 12.11 (s, 1H, NH), 7.88 (dd, 1H, J 15.4, 1.5 Hz, H-3), 7.71 (td, 1H, J 7.6, 1.8 Hz, H-6''), 7.66 (d, 1H, J 7.8 Hz, H-4'), 7.59 (tdd, 1H, J 8.1, 5.2, 1.8 Hz, H-4''), 7.35 – 7.32 (m, 3H, H-7'', H-5'', H-3''), 7.16 (td, 1H, J 7.5, 1.1 Hz, H-6'), 7.11 (td, 1H, J 7.7, 1.1 Hz, H-5'), 7.06 (dd, 1H, J 15.4, 2.2 Hz, H-2), 4.36 (t, 2H, J 6.5 Hz, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 1.82 (qt, 2H, J 7.4, 6.5 Hz, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 1.01 (t, 3H, J 7.4 Hz, $\text{OCH}_2\text{CH}_2\text{CH}_3$).

^{13}C NMR (DMSO- d_6 , 150 MHz): 187.3 (C, d, JCF 2.2 Hz, C-1), 159.9 (C, d, JCF 249.1 Hz, C-2''), 156.9 (C, C-2'), 136.9 (CH, C-3), 133.0 (CH, JCF 8.7 Hz, C-4''), 132.0 (C, C-7'a), 130.3 (CH, d, JCF 3.2 Hz, C-6''), 128.2 (C, d, JCF 14.4 Hz, C-1''), 125.0 (C, C-3'a), 124.7 (CH, d, JCF 3.2 Hz, C-5''), 121.8 (CH, C-5'), 121.2 (CH, C-6'), 118.4 (CH, C-4'), 116.4 (CH, d, JCF 23.0 Hz, C-3''), 115.7 (CH, d, JCF 5.5 Hz, C-2), 111.5 (CH, C-7'), 93.8 (C, C-3'), 73.0 (CH_2 , $\text{OCH}_2\text{CH}_2\text{CH}_3$), 22.1 (CH_2 , $\text{OCH}_2\text{CH}_2\text{CH}_3$), 10.0 (CH_3 , $\text{OCH}_2\text{CH}_2\text{CH}_3$).

IR (KBr) ν_{max} 1624, 1607, 1579, 1561, 1517, 1484, 1460, 1339, 1280, 1229, 1211, 1203, 1065 cm^{-1} .

HRMS: m/z $[\text{M}+\text{H}]^+$: 324.13989 for $\text{C}_{20}\text{H}_{19}\text{NO}_2\text{F}$ (calcd. 324.13943).

2. Densitometric analyses of Western blot

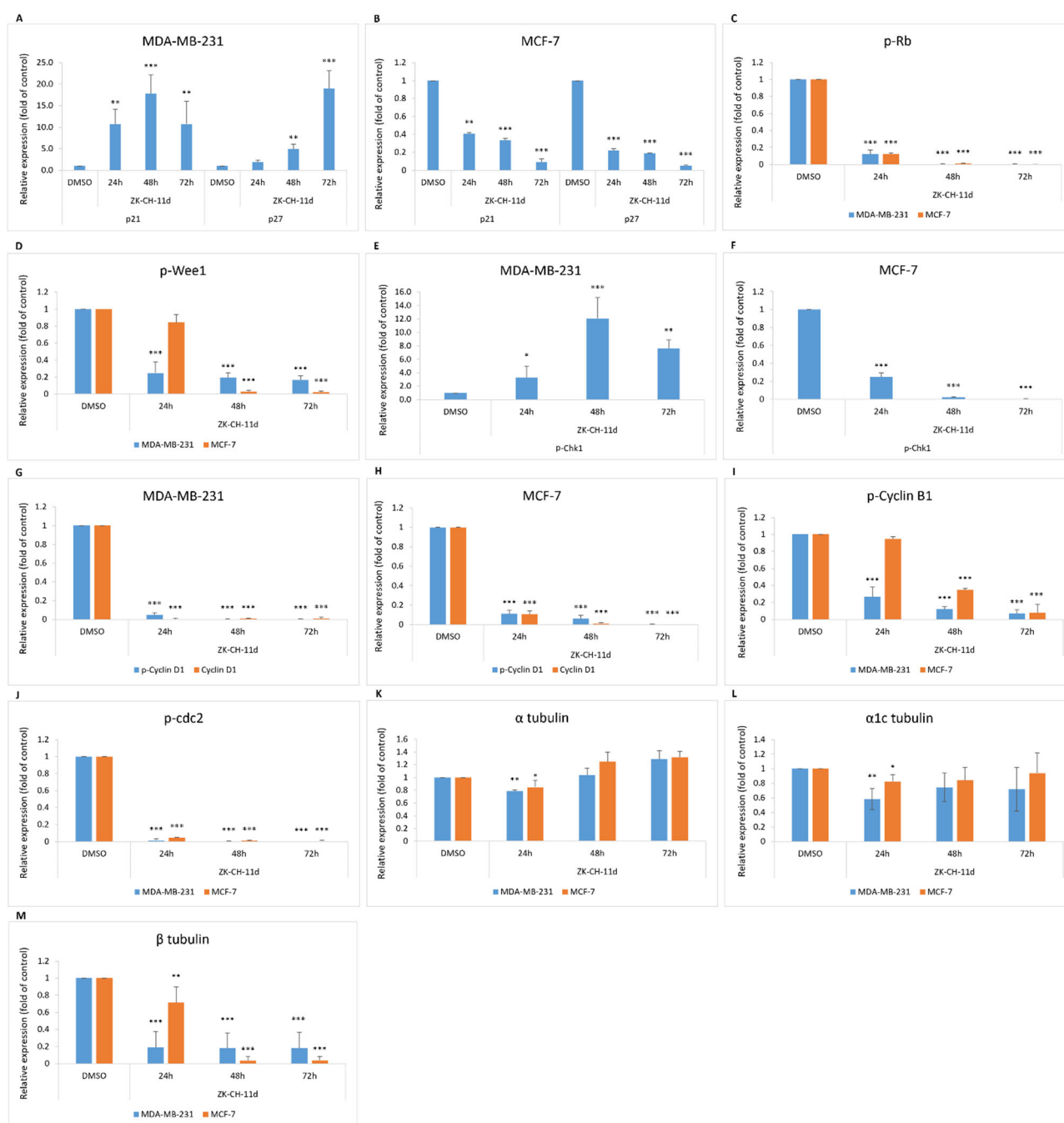


Figure S1. Densitometric analysis of western blot assay: relative expression of p21, p27 (A, B), p-Rb (C), p-Wee1 (D), p-Chk1 (E, F), p-Cyclin D1, Cyclin D1 (G, H), p-Cyclin B1 (I), p-cdc2 (J), α tubulin (K), α 1c tubulin (L) and β tubulin (M) in MDA-MB-231 and MCF-7 cells after ZK-CH-11d treatment. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle.

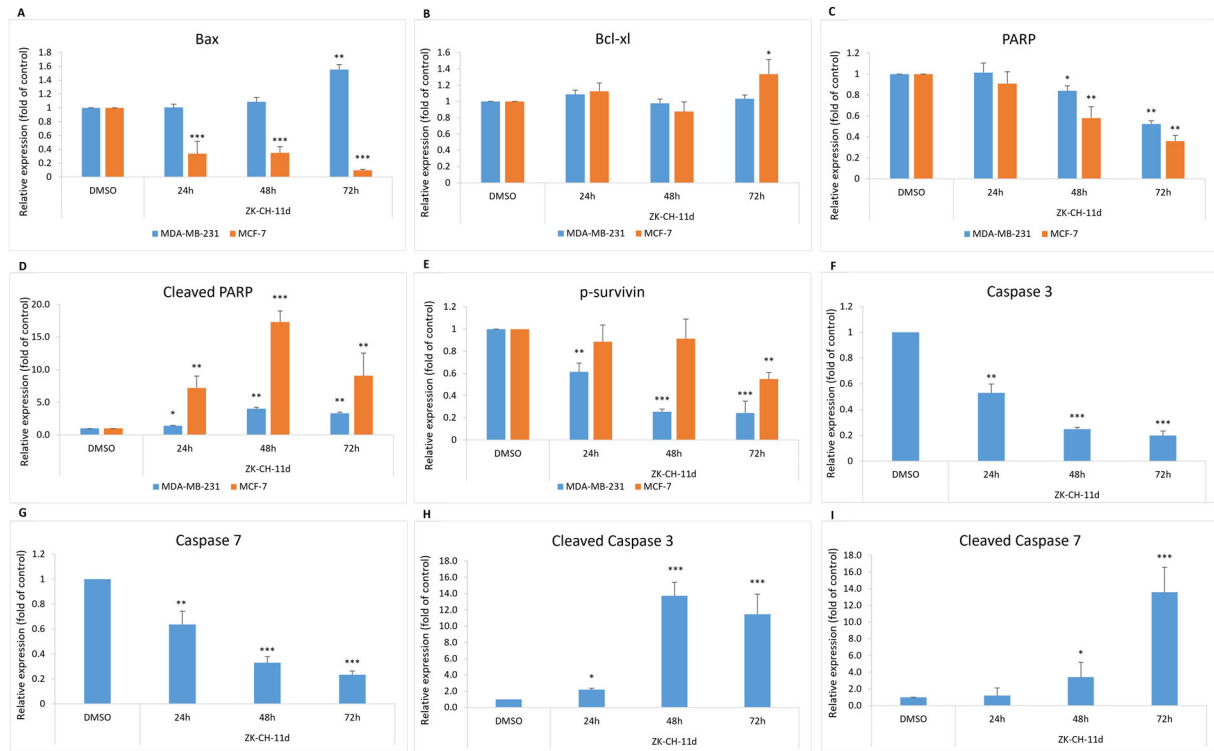


Figure S2. Densitometric analysis of western blot assay: relative expression of Bax (A), Bcl-xl (B), PARP (C), cleaved PARP (D), p-survivin (E), Caspase 3 (F) and Caspase 7 (G) and their cleaved forms (H, I) in MDA-MB-231 and MCF-7 cells after ZK-CH-11d treatment. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle.

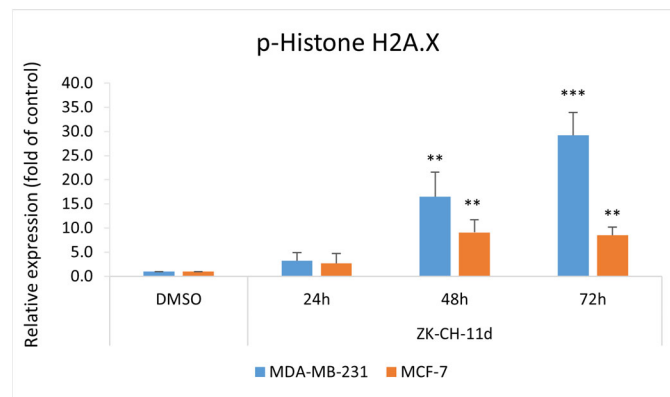


Figure S3. Densitometric analysis of western blot assay: relative expression of p-histone H2A.X in MDA-MB-231 and MCF-7 cells after ZK-CH-11d treatment. Statistical significance: ** $p < 0.01$, *** $p < 0.001$ vs. vehicle.

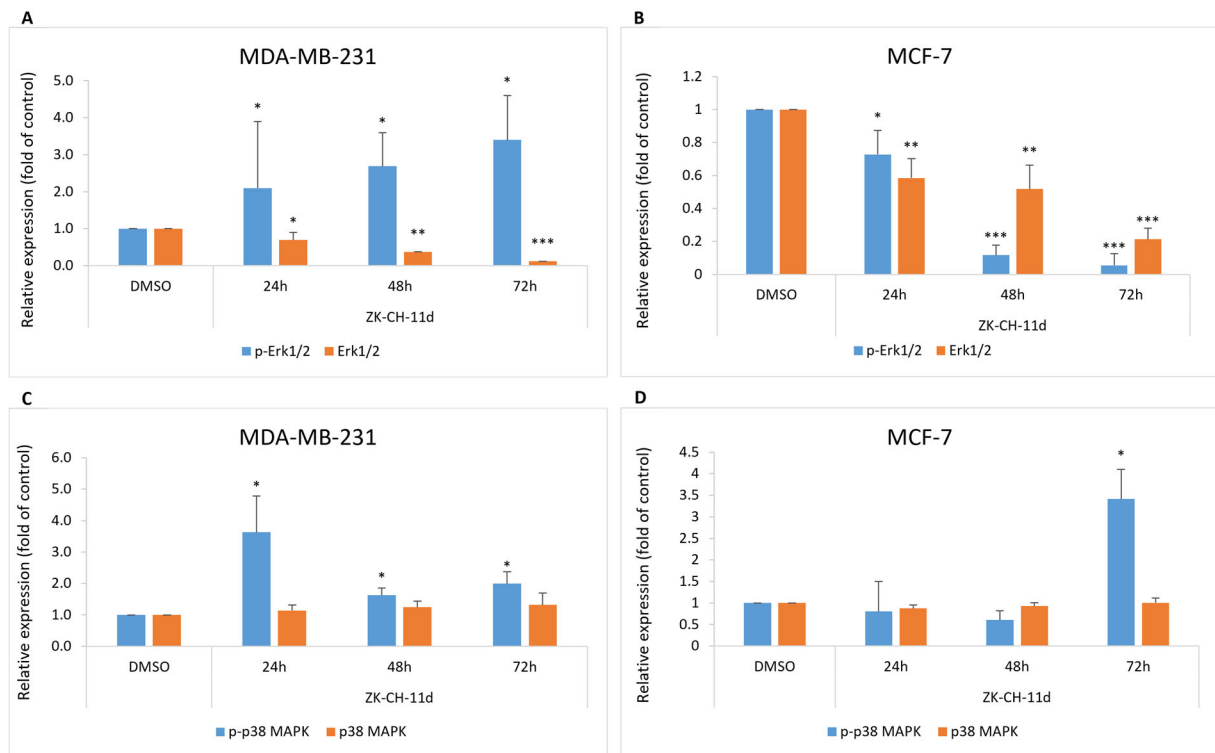


Figure S4. Densitometric analysis of western blot assay: relative expression of p-Erk1/2 and Erk1/2 (**A, B**) and p-p38 MAPK and p-38 MAPK (**C, D**) in MDA-MB-231 and MCF-7 cells after ZK-CH-11d treatment. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle.

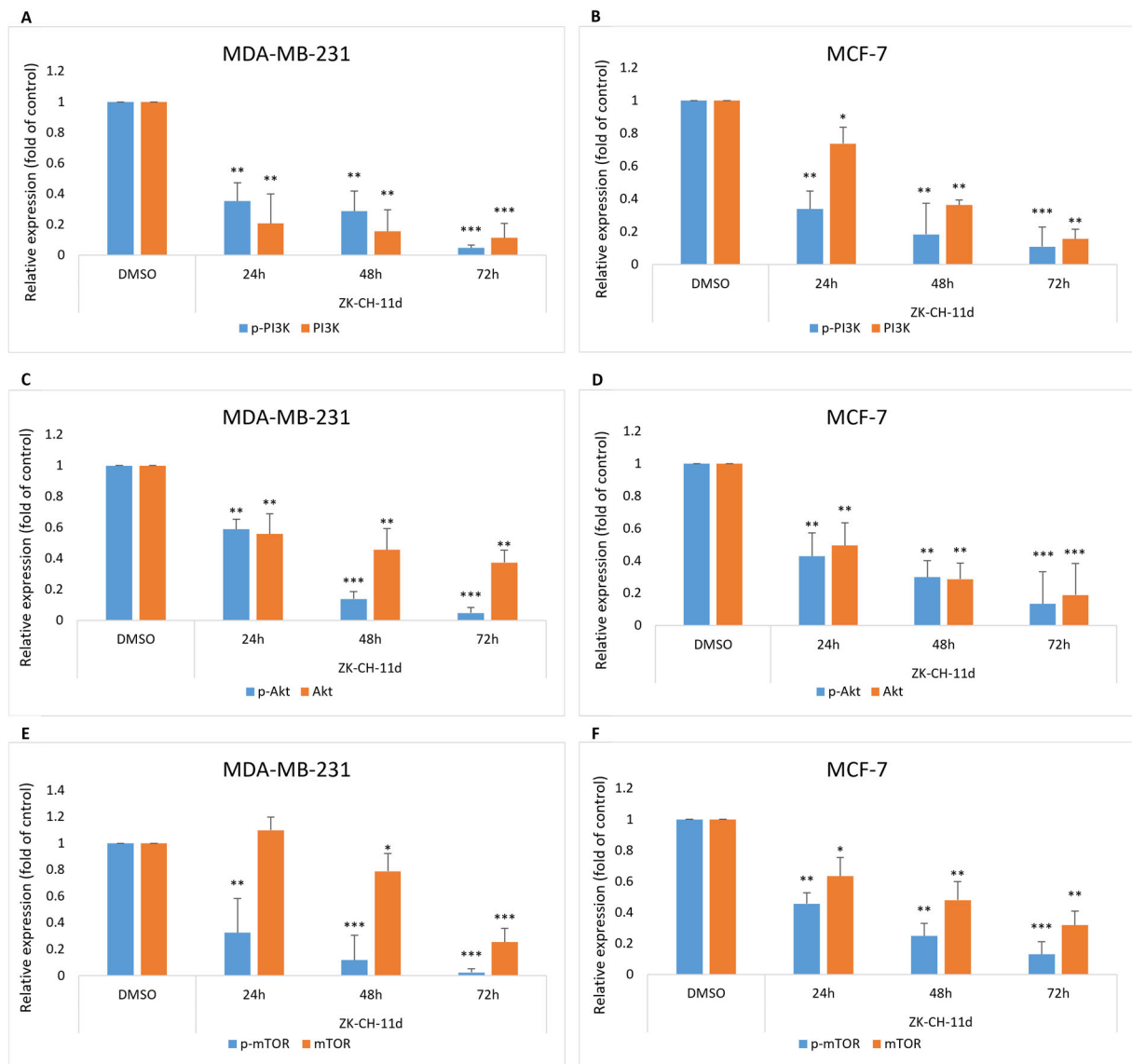


Figure S5. Densitometric analysis of western blot assay: relative expression of p-PI3K and PI3K (A, B), p-Akt and Akt (C, D) and p-mTOR and mTOR (E, F) in MDA-MB-231 and MCF-7 cells after ZK-CH-11d treatment. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle.

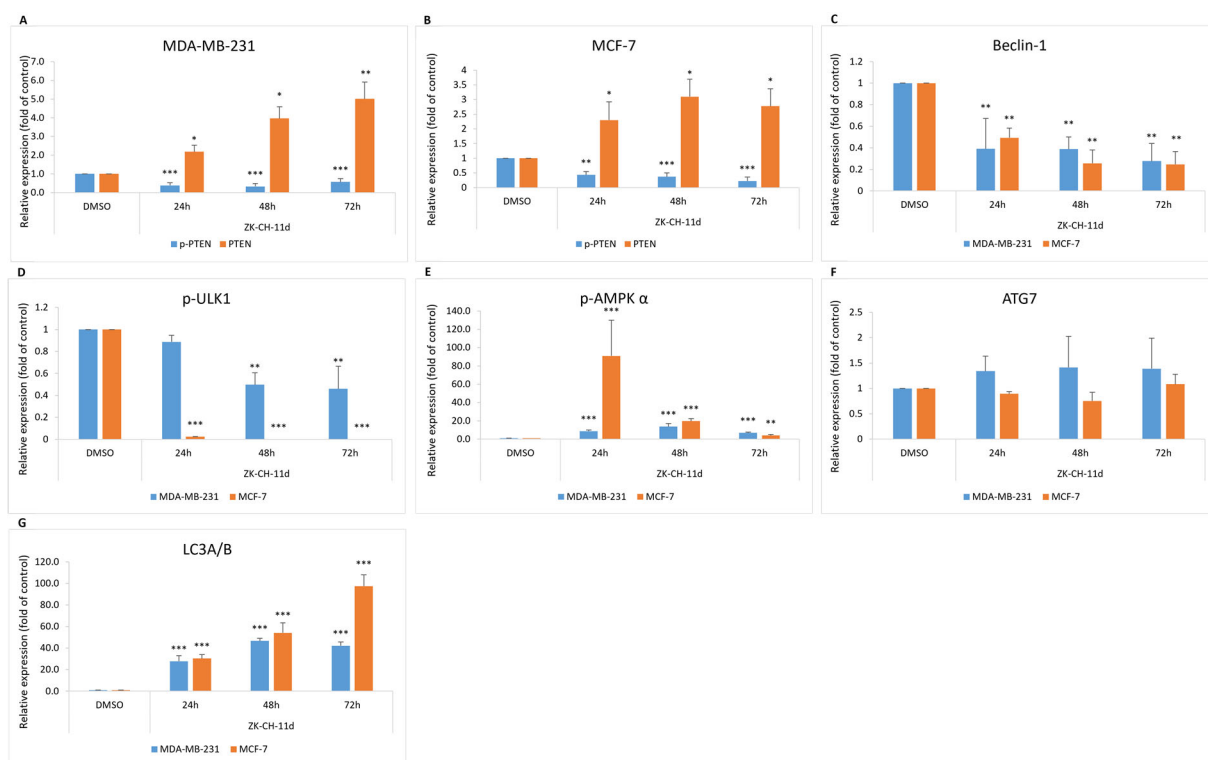


Figure S6. Densitometric analysis of western blot assay: relative expression of p-PTEN (A, B), PTEN (A, B), beclin-1 (C), p-ULK1 (D), p-AMPKα (E), ATG7 (F) and LC3A/B (G) in MDA-MB-231 and MCF-7 cells after ZK-CH-11d treatment. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle.