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Title: Crucial role of reactive oxygen species (ROS) for the proapoptotic effects of indirubin derivatives in cutaneous SCC cells
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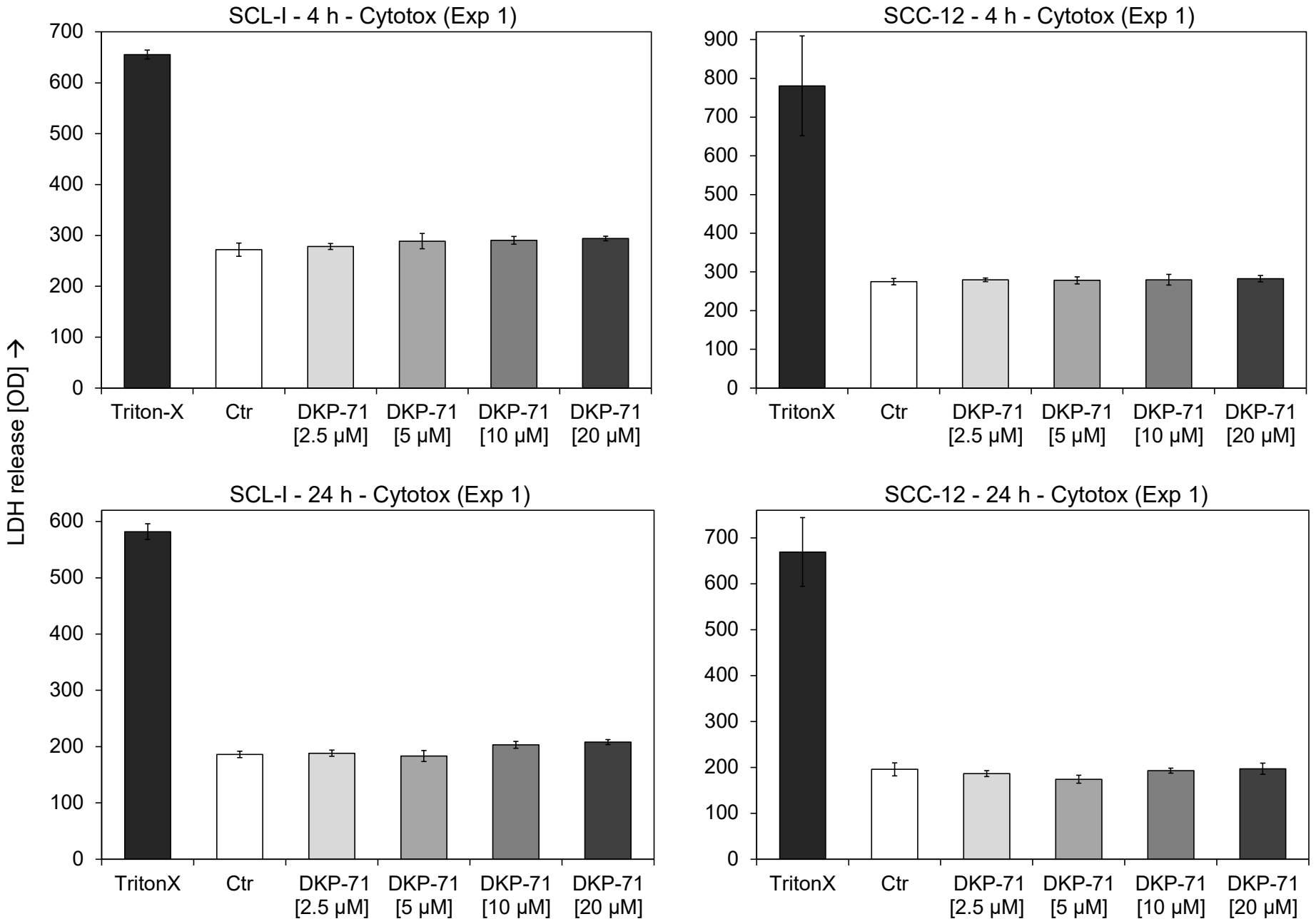
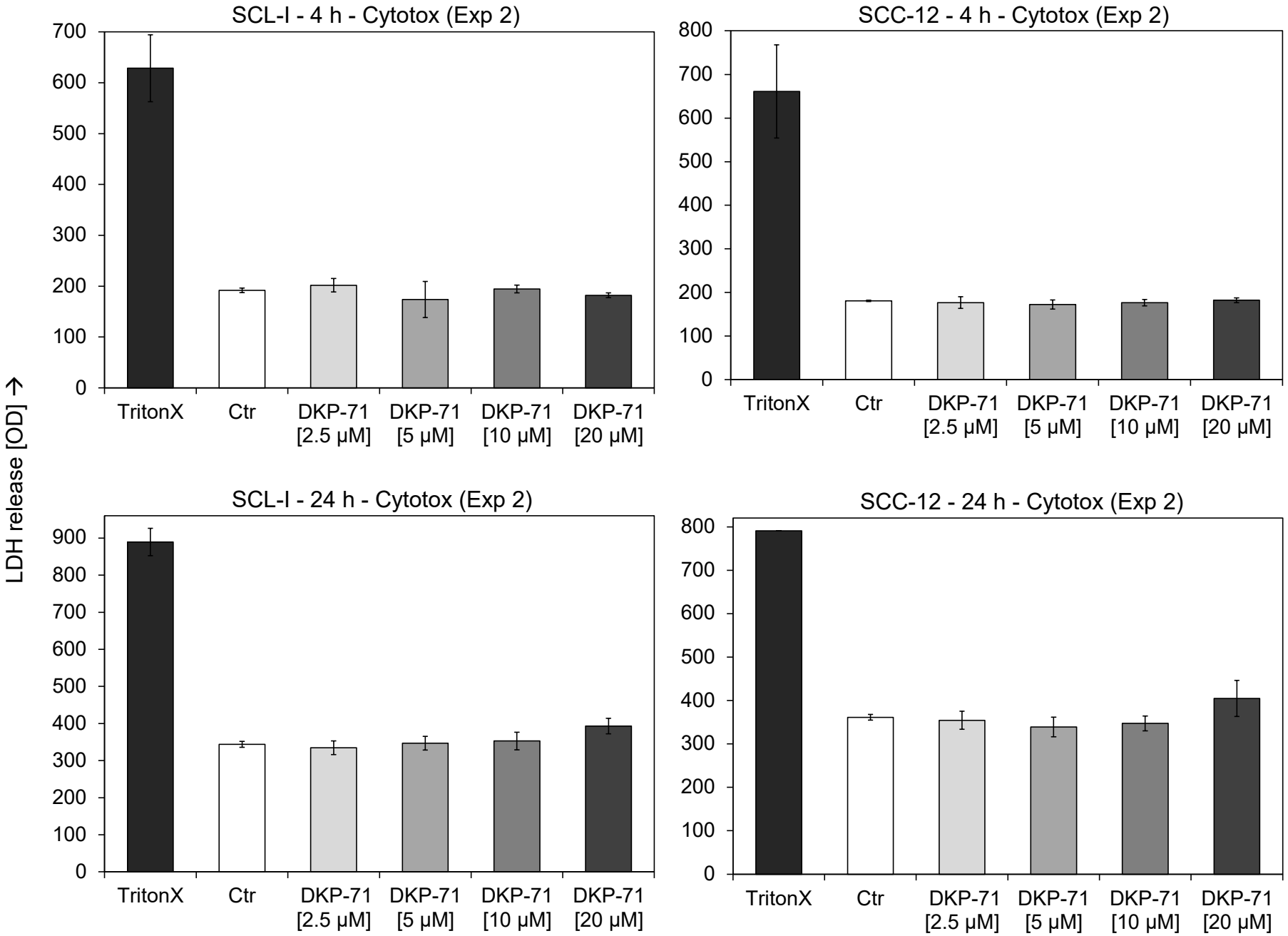
Supplementary figures

Suppl Figure S1a – Cytotoxicity in 4 cell lines (Exp 1)



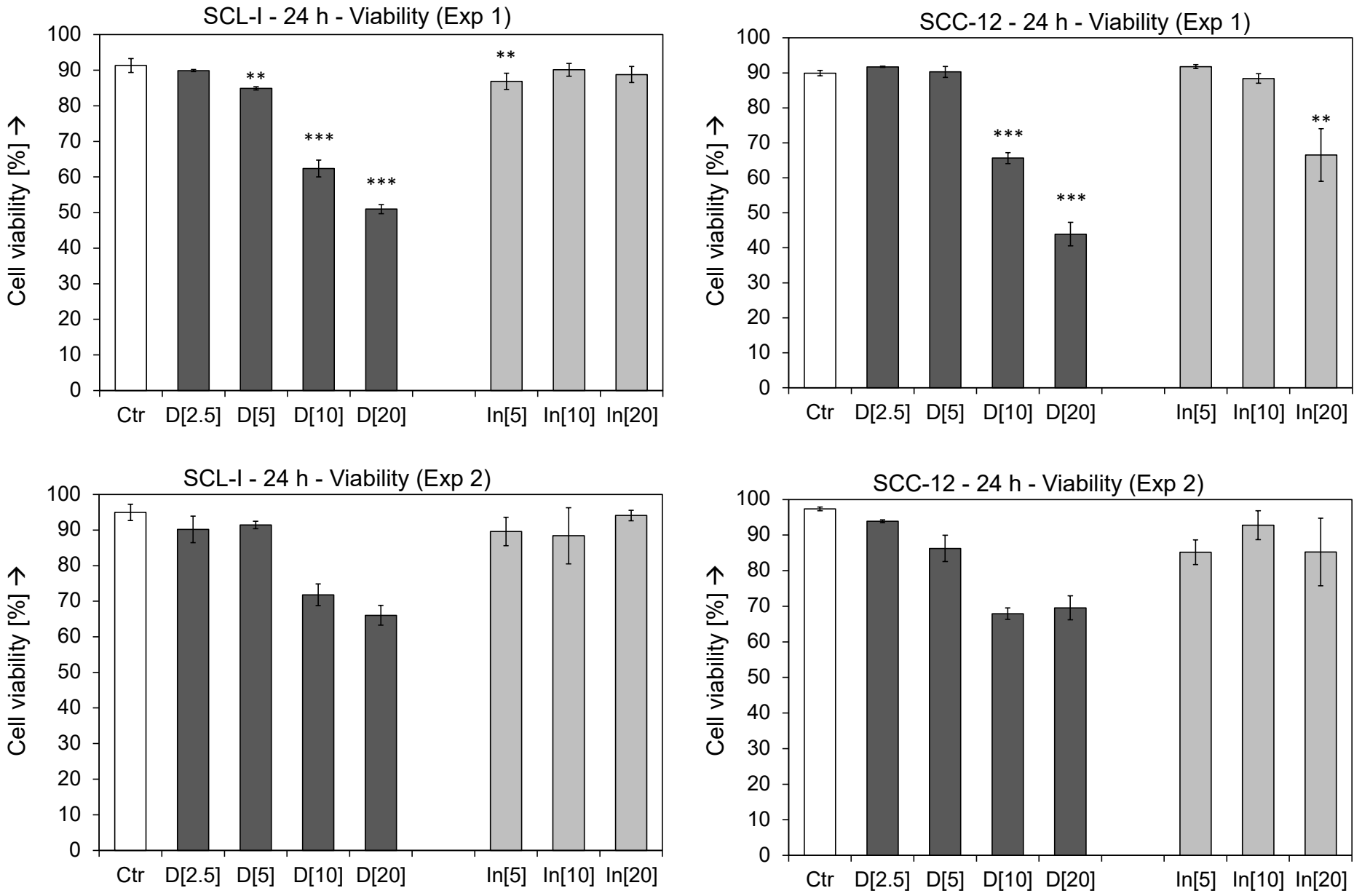
Cell lines SCL-I and SCC-12 were treated with increasing concentrations of DKP-071 (2.5, 5, 10, 20 µM). LDH release assays were performed at 4 h (above) and at 24 h (below). LDH values were compared to non-treated cells (Ctr) as well as to positive controls (cultures lysed with triton X). Experiment 1 of two independent experiments is shown, each one including triplicate values.

Suppl Figure S1b – Cytotoxicity in 4 cell lines (Exp 2)



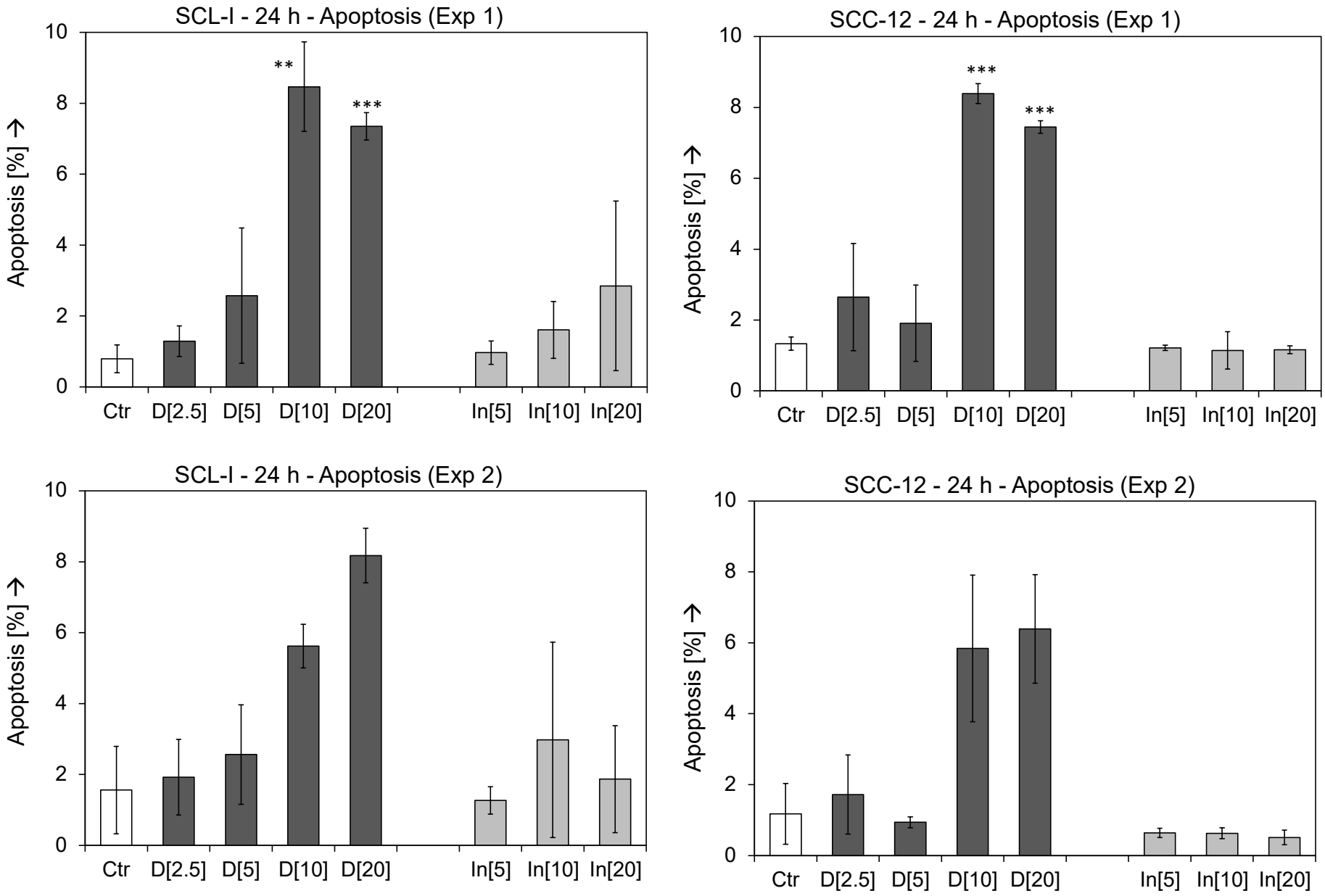
Cell lines SCL-I and SCC-12 were treated with increasing concentrations of DKP-071 (2.5, 5, 10, 20 µM). LDH release assays were performed at 4 h (above) and at 24 h (below). LDH values were compared to non-treated cells (Ctr) as well as to positive controls (cultures lysed with triton X). Experiment 2 of two independent experiments is shown, each one including triplicate values.

Suppl Figure S2a – Dose dependency for DKP-071 / Comparison with non substituted Indirubin (Cell viability)



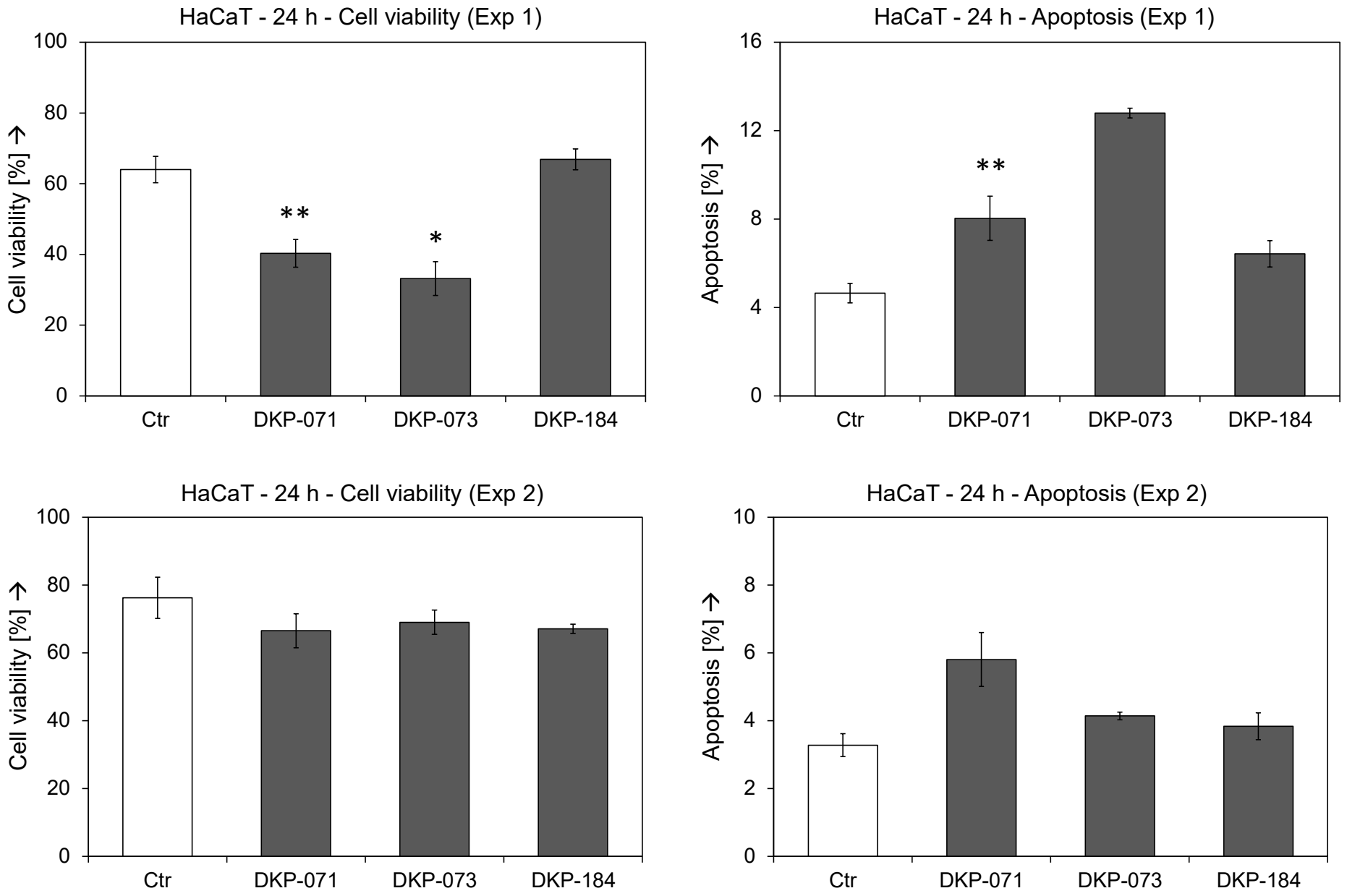
Cell lines SCL-I and SCC-12 were treated with 2.5, 5, 10 and 20 µM of DKP-071 respectively with 5, 10 and 20 µM of non-substituted Indirubin (In). The effects on cell viabilty (Calcein-AM staining) were determined at 24 h of treatment. Ctr, non-treated cells. Two independent experiments are shown for both cell lines (top / bottom), each one including triplicate values. Statistical significance was calculated for all 6 values of both experiments and is indicated for experiment 1 (**, $p < 0.01$; ***, $p < 0.001$).

Suppl Figure S2b – Dose dependency for DKP-071 / Comparison with non substituted Indirubin (Apoptosis)



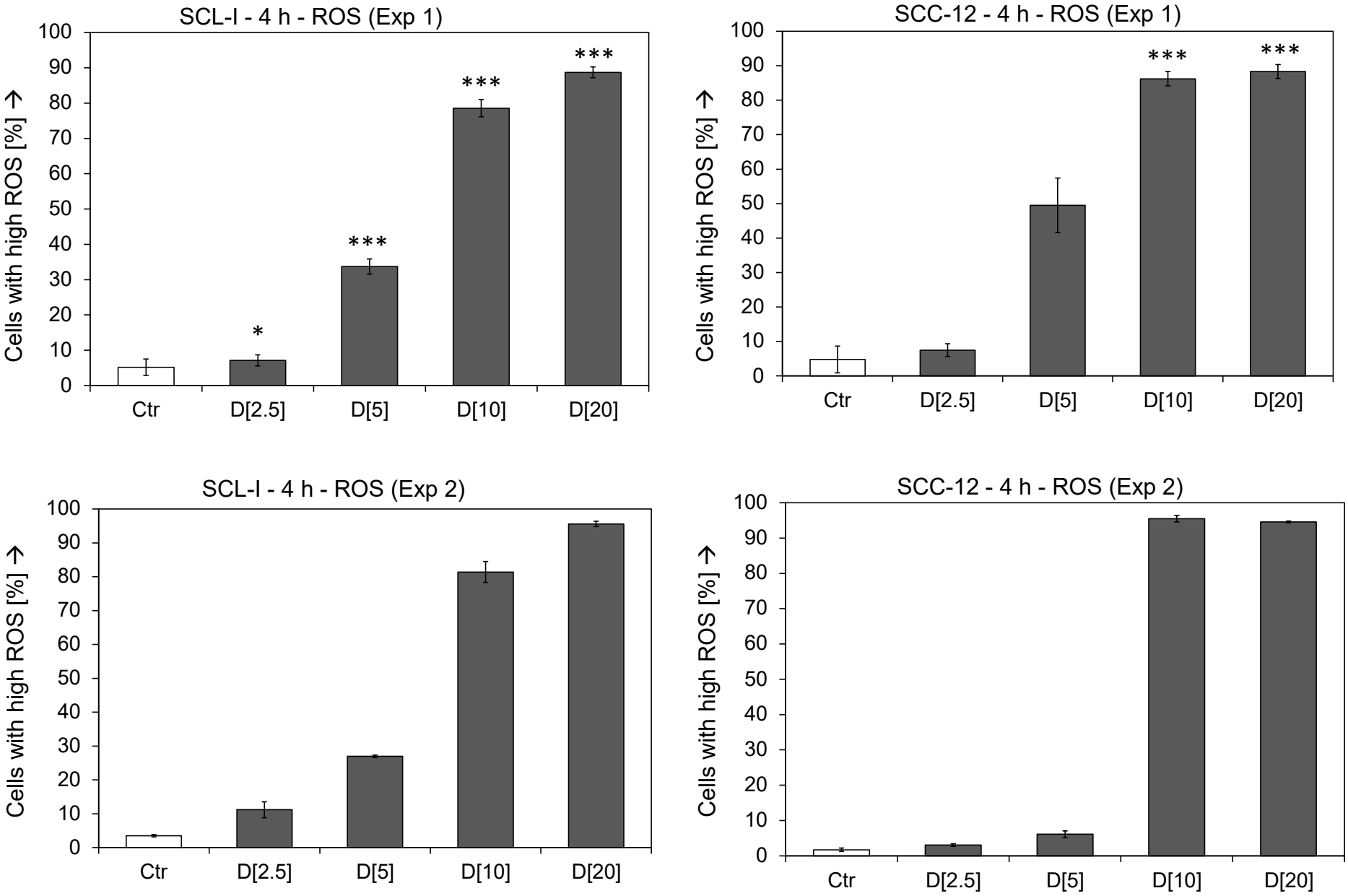
Cell lines SCL-I and SCC-12 were treated with 2.5, 5, 10 and 20 µM of DKP-071 respectively with 5, 10 and 20 µM of non-substituted Indirubin (In). The effects on apoptosis (propidium iodide staining) were determined at 24 h of treatment. Ctr, non-treated cells. Two independent experiments are shown for both cell lines (top / bottom), each one including triplicate values. Statistical significance was calculated for all 6 values of both experiments and is indicated for experiment 1 (**, $p < 0.01$; ***, $p < 0.001$).

Suppl Figire S3 – Apoptosis and cell viability in HaCaT cells in response to DKPs



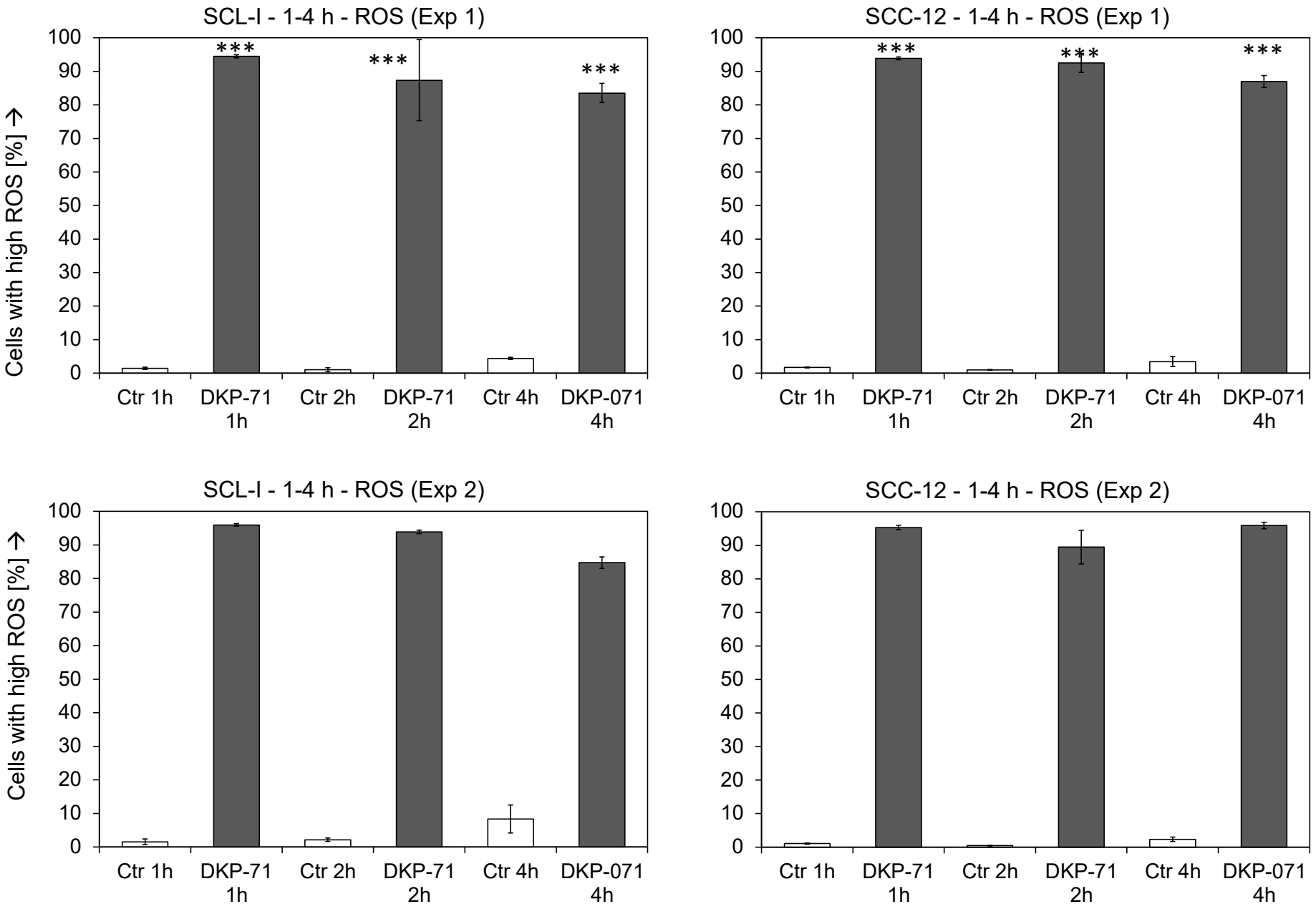
The effects of indirubin derivatives DKP-071, DKP-073 and DKP-184 (10 µM) on cell viability (left) and on apoptosis (right side) were determined in the immortalized keratinocyte cell line HaCaT. Two independent experiments are shown (top / bottom), each one including triplicate values. Statistical significance was calculated for all 6 values of both experiments and is indicated for experiment 1 (*, $p < 0.05$; **, $p < 0.01$).

Suppl Figure S4 – Dose dependency of ROS production for DKP-071



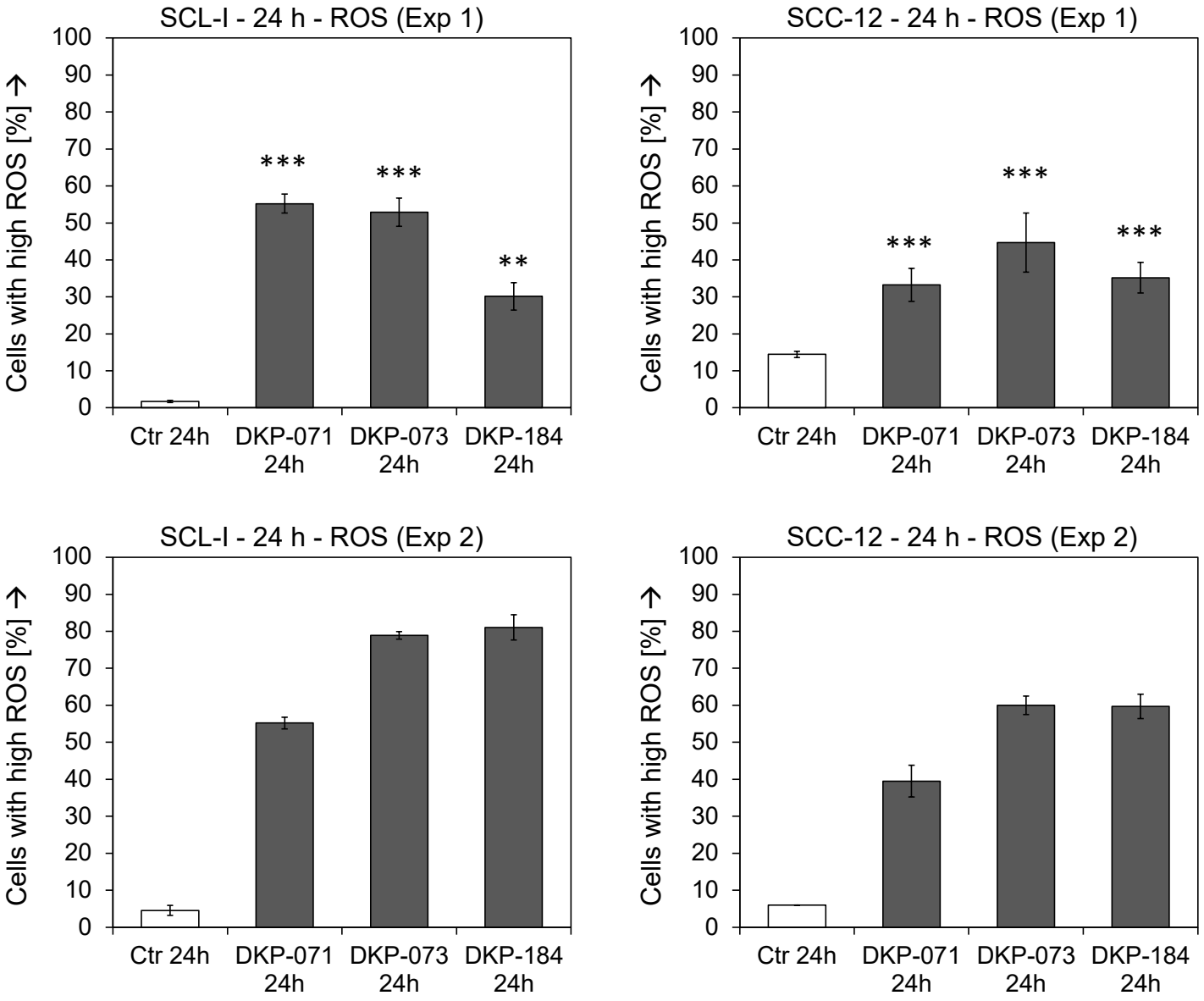
Cell lines SCL-I and SCC-12 were treated with 2.5, 5, 10 and 20 µM of DKP-071. The effects on ROS production were determined at 4 h of treatment (H_2DCF-DA staining). Ctr, non-treated cells. Two independent experiments are shown for both cell lines (top / bottom), each one including triplicate values. Statistical significance was calculated for all 6 values of both experiments and is indicated for experiment 1 (*, $p < 0.05$; ***, $p < 0.001$).

Suppl Figure S5a – Time dependency of ROS production for DKP-071 (1 – 4 h)



Time dependency of ROS production induced by DKP-071 (10 µM) was determined in SCL-I and SCC-12 at 1 h, 2 h and at 4 h of treatment (H_2DCF-DA staining). Two independent experiments are shown (top / bottom), each one including triplicate values. Statistical significance was calculated for all 6 values of both experiments and is indicated for experiment 1 (***, $p < 0.001$).

Suppl Figure S5b – Time dependency of ROS production for DKP-071 (24 h)



Effects of DKP-071, DKP-073 and DKP-184 (10 µM) on ROS production were determined in SCL-I and SCC-12 at 12 h of treatment (H_2DCF-DA staining). Two independent experiments are shown (top / bottom), each one including triplicate values. Statistical significance was calculated for all 6 values of both experiments and is indicated for experiment 1 (**, $p < 0.01$; ***, $p < 0.001$).