Supplementary Materials

## Calcium Regulates HCC Proliferation as well as EGFR Recycling/Degradation and Could Be a New Therapeutic Target in HCC

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**Figure S1.** Western blot analysis of EGFR pathway activation in HepG2, HUH-7, HUH-6, and Hep3B cell lines.



**Figure S2.** Western blot analysis of HepG2, HUH-7, HUH-6, and Hep3B starved cell lines treated with GEF IC50 or AZ IC50 (as indicated in Table 1) (DMSO as control) for 3 h before stimulation with 100 ng/mL of EGF for 30 min.



В.



**Figure S3. A** and **B**: Western blot panels of HepG2, HUH-7, HUH-6, and Hep3B starved cell lines stimulated with 100 ng/mL of EGF for 30 min before and during treatment with GEF or AZ IC50 (as indicated in Table 1) (DMSO as control). Treatments were performed for 30 min, 3 h and 6 h.





**Figure S4.** Starved HUH-7 cells (T0) were left untreated (/) (0% FBS as CTR) or treated with 100 ng/mL EGF, 2 mM EDTA, 0.5% DMSO, or combined compounds (as indicated in the figures). The cell signaling cascade was analyzed by western blot after 6 h and 24 h.



**Figure S5.** HUH-7 cells treated with EDTA or EGTA for 24 h with or without EGF were analyzed by western blot.



В.



**Figure S6. A** and **B**: Starved HUH-7 and HUH-6 cells (T0) were left untreated (as CTR) or treated with 2 mM EDTA or 10  $\mu$ M BAPTA\_AM with or without 100 ng/mL EGF. The cell signaling cascade was analyzed by western blot after 6 h and 24 h.







**Figure S7. A** and **B**: Starved HUH-7, HUH-6, HepG2, and Hep3B cells (T0) were left untreated (as CTR) or treated with 10  $\mu$ M BAPTA\_AM. After 30 min, 40  $\mu$ M MG132 were added for a further 30 min. 100 ng/mL EGF were added for a total time of 6 h before cells harvesting.



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