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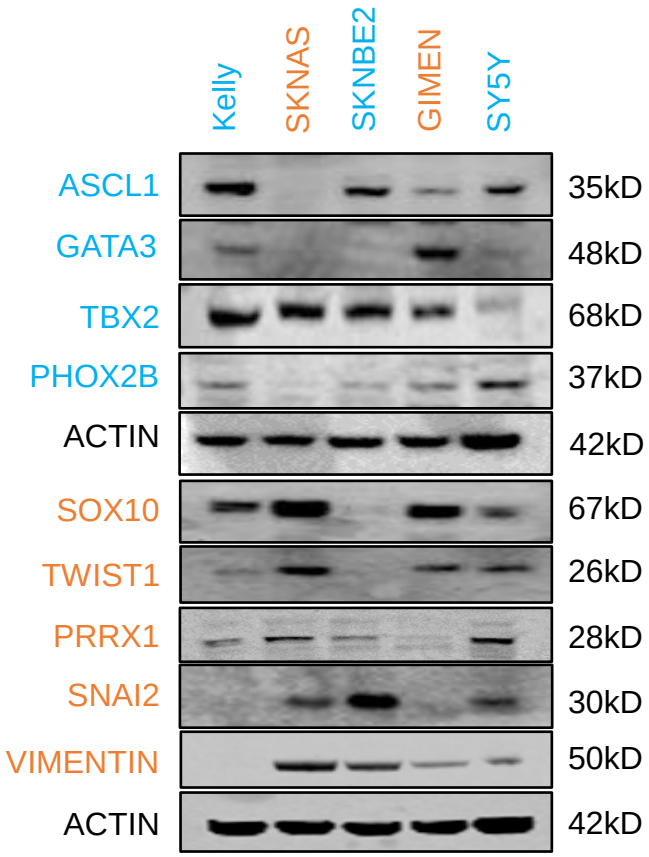
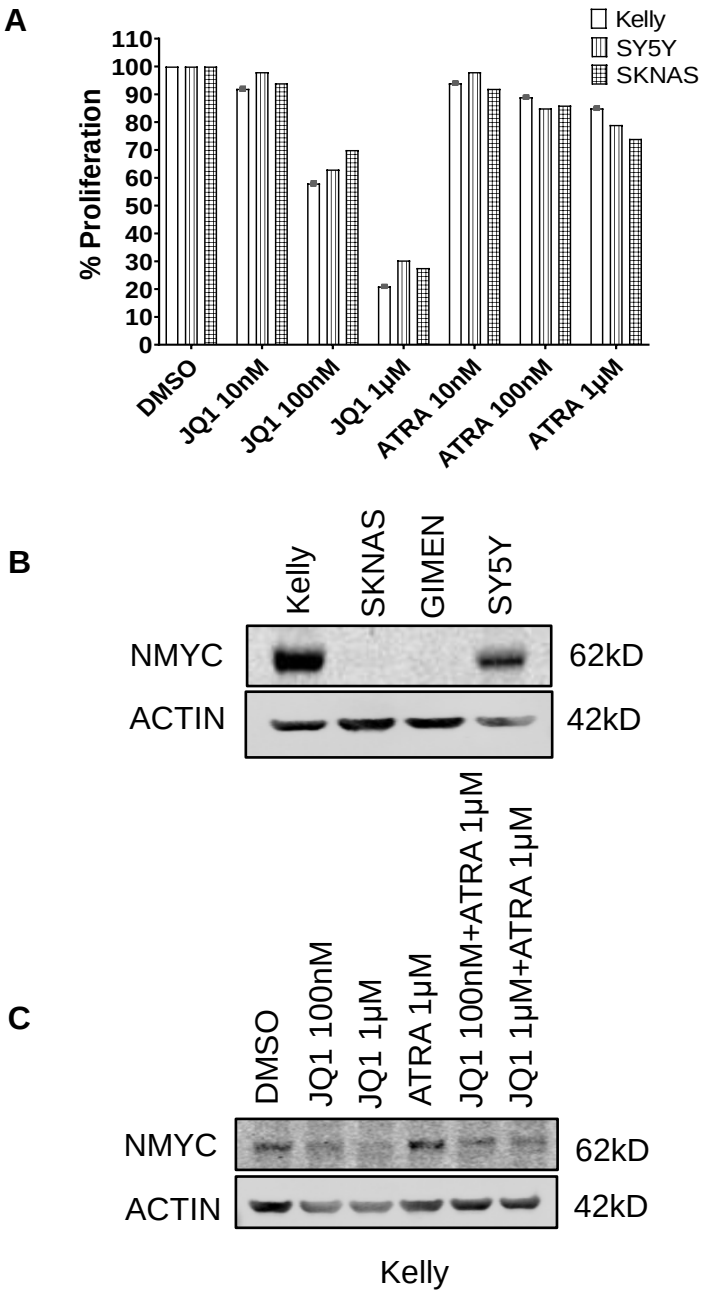
Supplemental information

Therapeutically targeting oncogenic CRCs facilitates induced differentiation of NB by RA and the BET bromodomain inhibitor

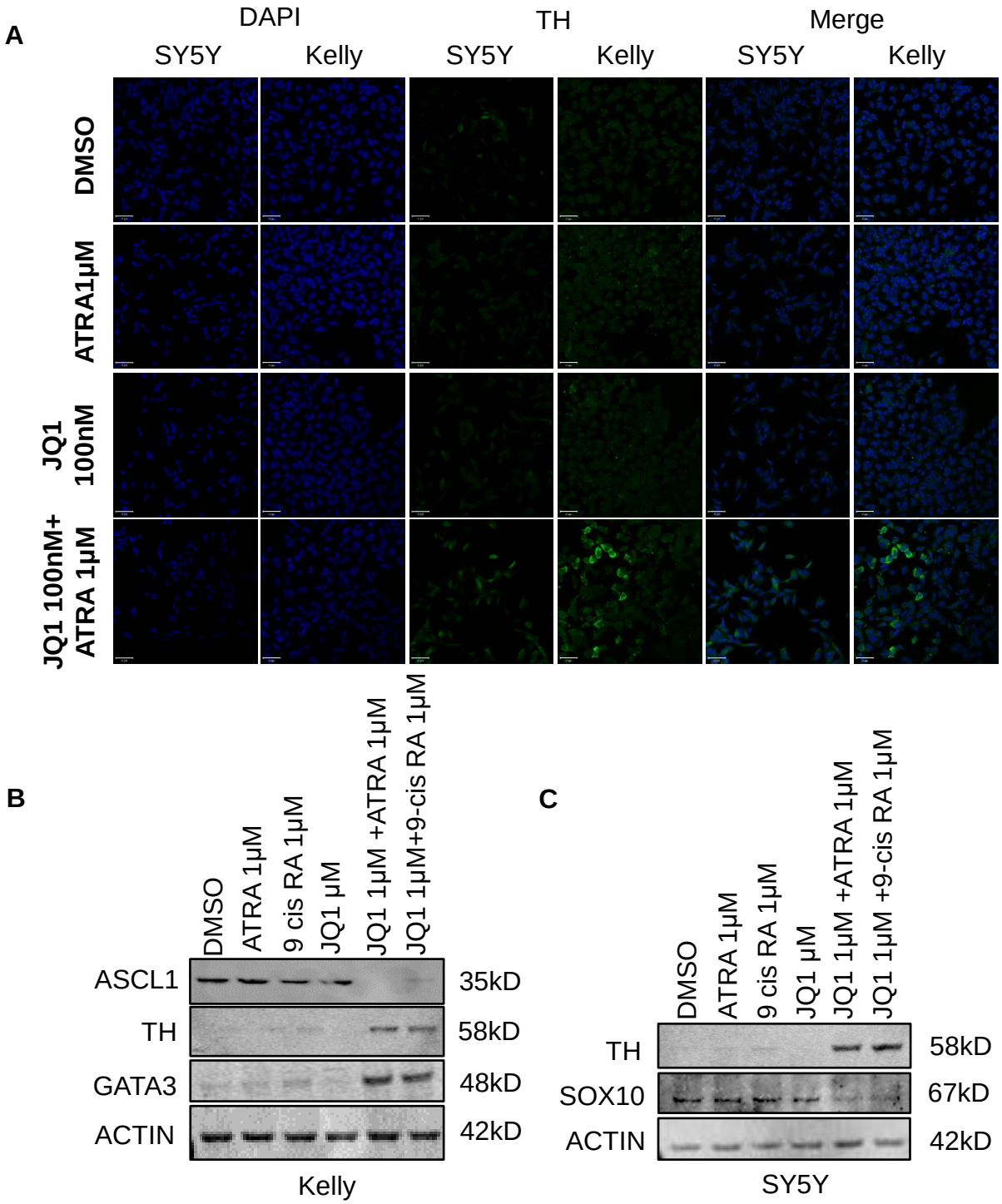
Satyanarayana Alleboina, Nour Aljouda, Mellessa Miller, and Kevin W. Freeman



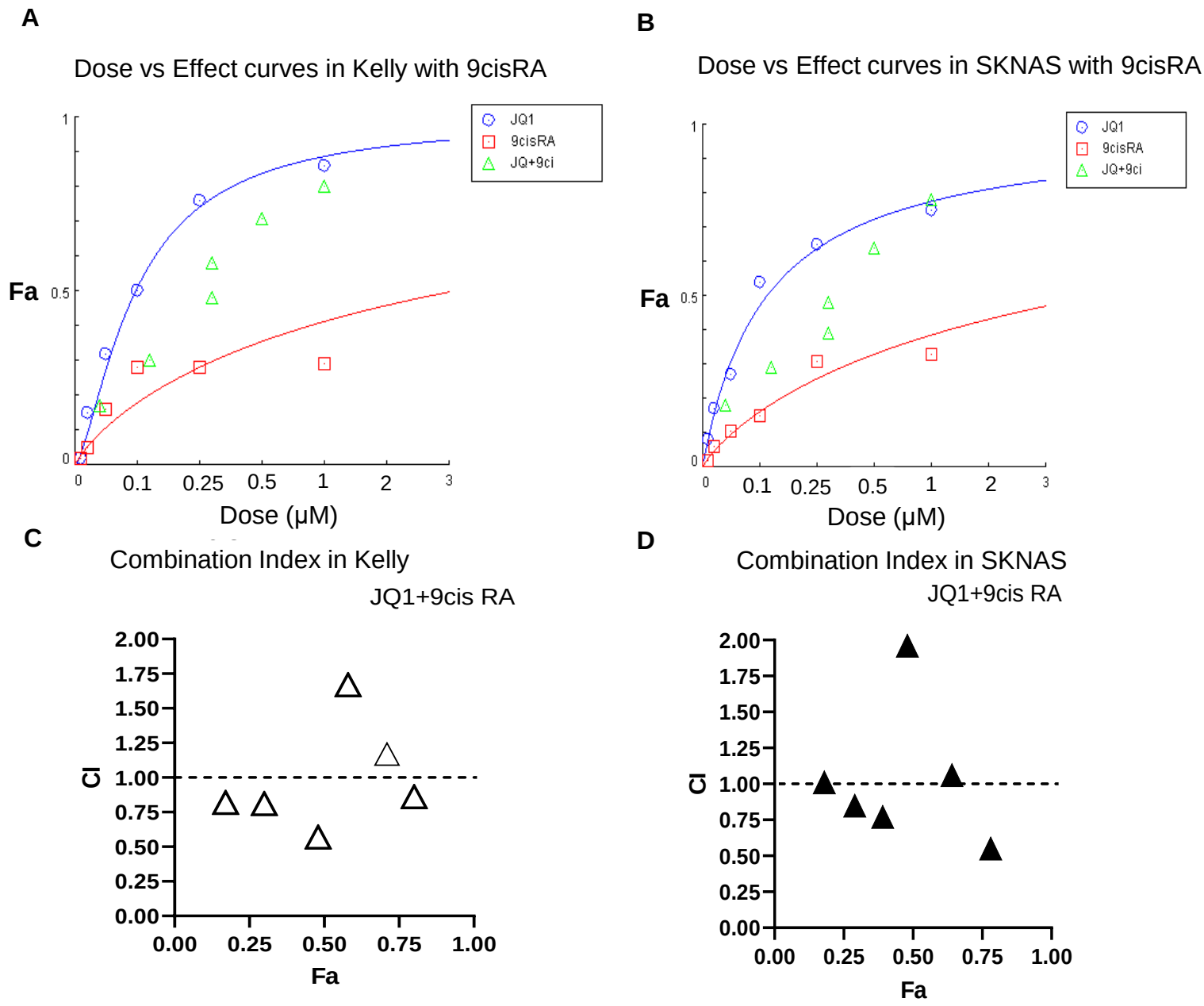
Supplemental Figure 1. Comparison of basal core regulatory transcription factor expression in NB cell lines: Western blot analysis of protein expression of core regulatory transcription factors ASCL1, GATA3, TBX2 and PHOX2B that drive adrenergic identity and core regulatory transcription factors SOX10, TWIST1, PRRX1, SNAI2 that drive mesenchymal identity including the mesenchymal marker vimentin in the NB cell lines Kelly, SKNAS, SKNBE2, GIMEN and SY5Y cells.



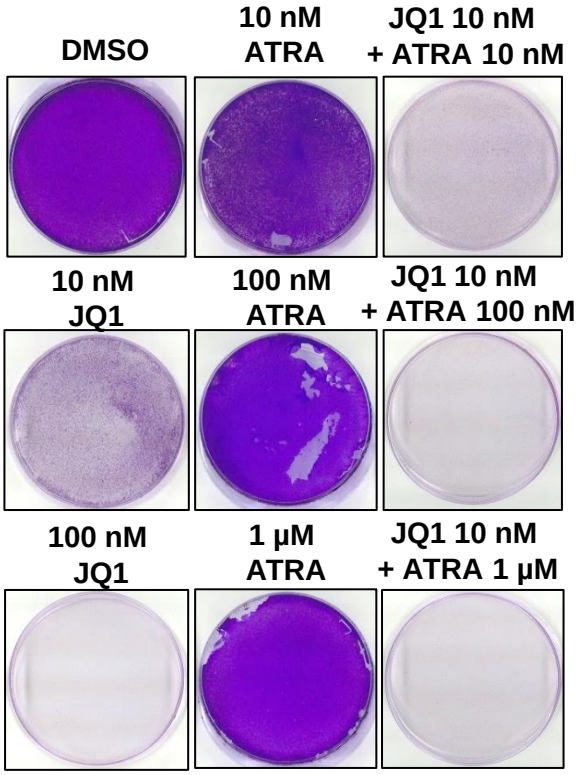
Supplemental Figure 2. Effect of ATRA and JQ1 combinations on NB cell proliferation. (A) Drug Titrations from 10nM to 10µM concentrations were analyzed by CyQuant assay to optimize effective combinations for checking the expression of stemness and differentiation markers. Representative data of 10, 100, 1µM JQ1 and ATRA on NB cells. (B) Representative western blot showing a basal protein expression of MYCN between NB cells Kelly, SKNAS, GIMEN and SY5Y. (C) Protein expression of MYCN in Kelly cells treated with ATRA and JQ1 combinations.



Supplemental Figure 3. Comparison of all trans retinoic acid vs 9 cis retinoic acid in NB cell differentiation and loss of stemness. (A) Analysis of TH expression by Immune fluorescence staining in Kelly and SY5Y cells treated with JQ1 and ATRA combinations after four days. Representative images were obtained by confocal microscopy, with a 50μM scale bar. (B) Representative western blots of protein expression of ASCL1, TH and GATA3 in Kelly cells under the treatment of all trans retinoic acid vs 9 cis retinoic acid in combination with JQ1. (C) protein expression of TH and SOX10 in SY5Y cells treated with all trans retinoic acid vs 9 cis retinoic acid in combination with JQ1.



Supplemental Figure 4. Effect of all trans retinoic acid vs 9 cis retinoic acid treatments on dose vs response and combination index in Kelly and SKNAS cells. (A) Effect of 9 cis retinoic acid independently and in combination with JQ1 on dose vs response in Kelly cells. (B) effect of 9 cis retinoic acid independently and combination with JQ1 on dose vs response in SKNAS cells. (C) Treatment effect of 9 cis retinoic acid with JQ1 on combination index in Kelly cells. (D) Treatment effect of 9 cis retinoic acid with JQ1 on combination index in SKNAS cells. The effect of dose vs response on NBCL was analyzed by Compusyn software according to Chau and Talalay method.



Supplemental Figure 5. Effect of ATRA and JQ1 combinations on NB cell proliferation and differentiation. (A) Representative images of 10-day duration treatment effect of ATRA, JQ1 and combinations on proliferation of SKNAS cells analyzed by crystal violet staining.