

## Supplemental Material

### Acetylation-Primed SUMOylation Drives ROR $\beta$ Turnover via a p300-SIRT1 Regulatory Axis

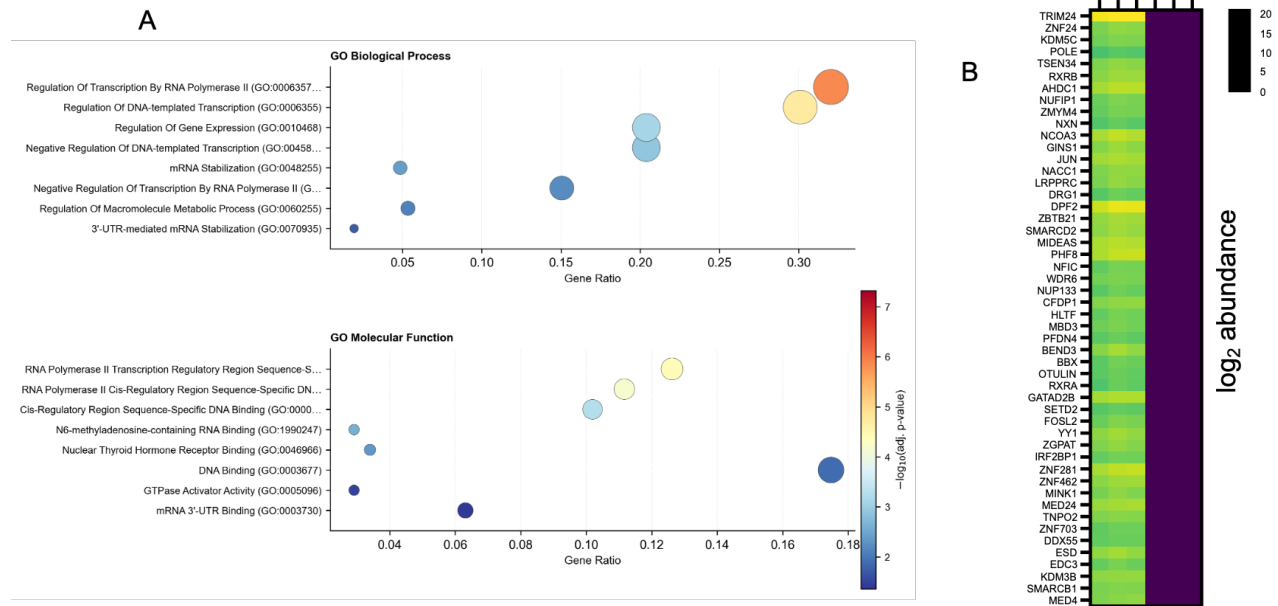
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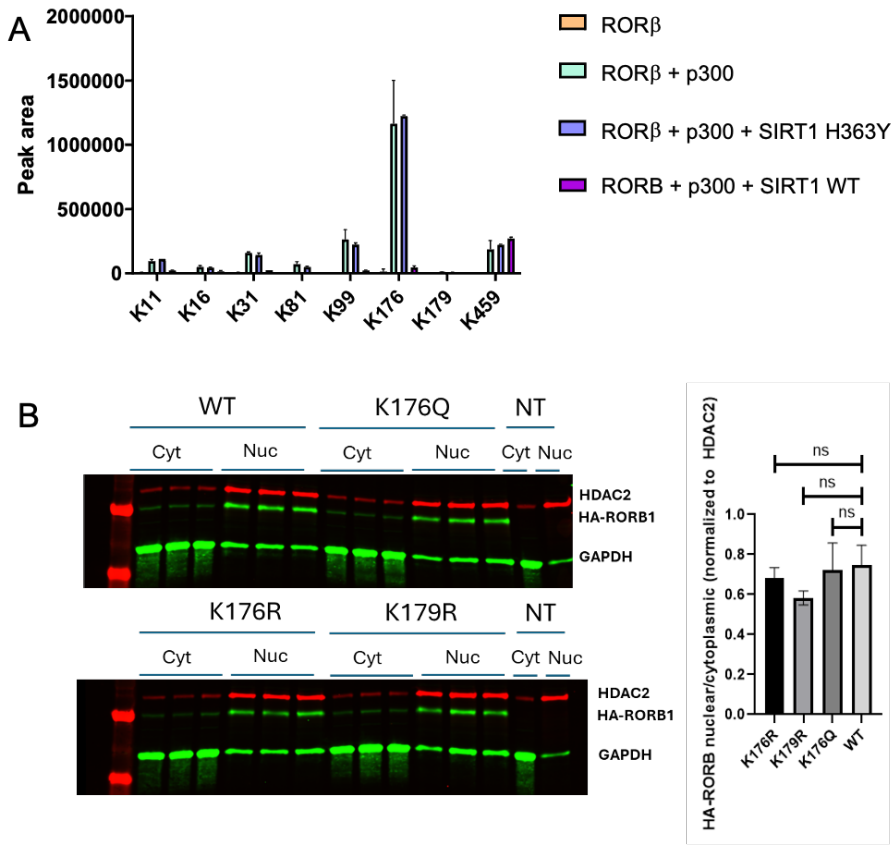
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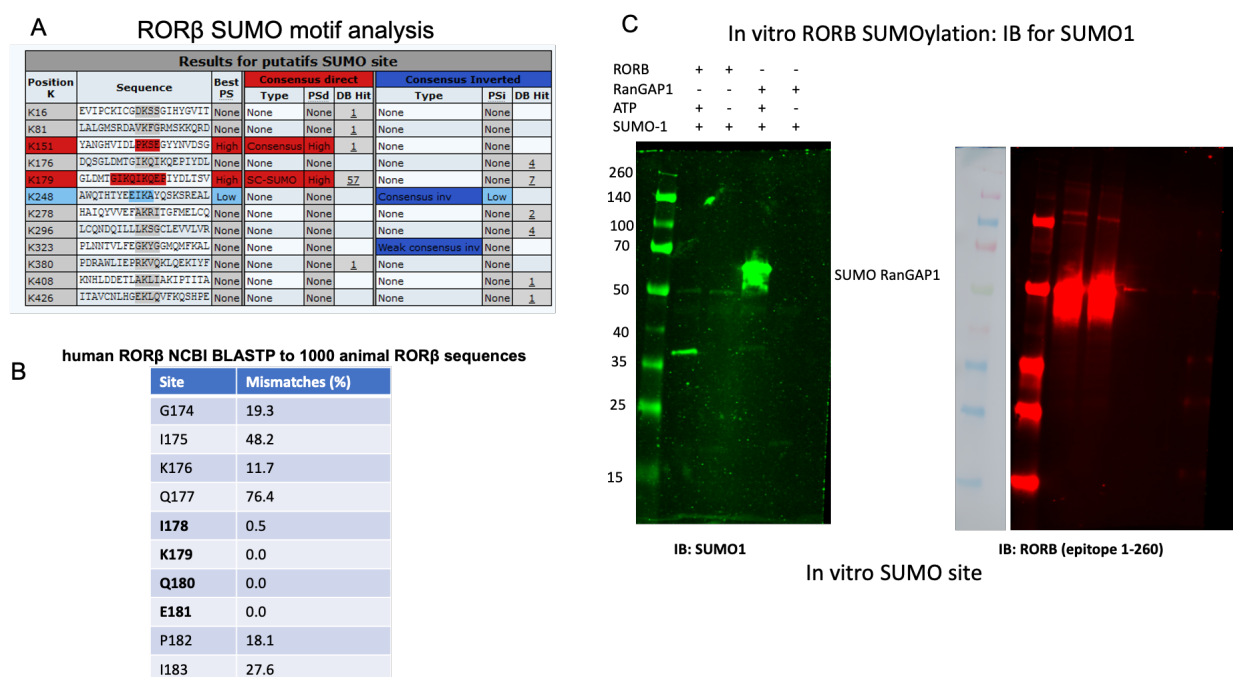
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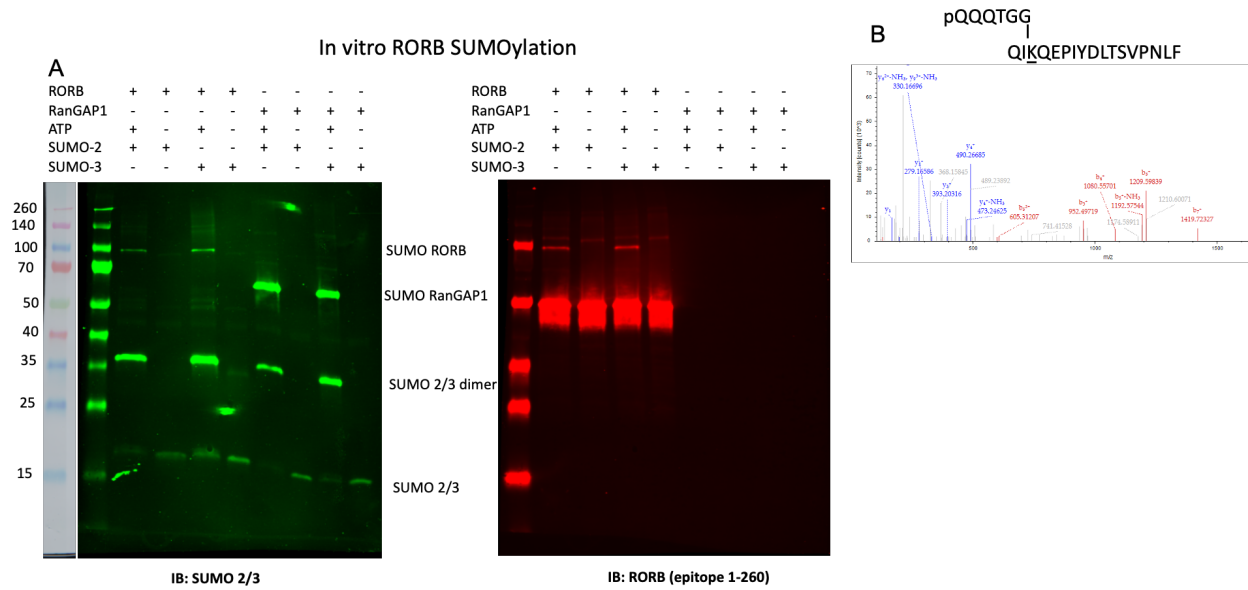
**Figure S1.** A. GO analysis of 206 ROR $\beta$  interactors found in common for two full repeated RIME experiments. The list of 206 proteins was taken from proteins found in both datasets with  $\log_2\text{FC} \geq 1.5$  and  $p\text{-value} < 0.05$ . GO analyses were performed with David Bioinformatics tools (DOI: 10.1093/bioinformatics/bth456). B. Heatmap of top 50 highest confidence ROR $\beta$  interactors sorted by p-value (with lowest p-values ranked highest). This corresponds to the experimental dataset shown in **Fig. 1A**.



**Figure S2.** A. RORB acetylation sites detected with trypsin/chymotrypsin digest and including conditions with the catalytically inactive SIRT1 H363Y mutant. Following IP-MS of ROR $\beta$  and after washing the beads the protease digest was performed on-bead. B. Subcellular fractionation of HEK293T transiently transfected cells shows no significant differences in nuclear/cytoplasmic partitioning. After nuclear/cytoplasmic partitioning samples were immunoblotted for HA and for the nuclear marker HDAC2 and cytoplasmic marker GAPDH.



**Figure S3.** A. SUMO motif analysis of ROR $\beta$  amino acid sequence was performed with Jassa webserver (DOI: 10.1093/bioinformatics/btv403). and shows a SUMO motif at K179 with 57 matches to known SUMO sites. B. Conservation analysis of select residues in the ROR $\beta$  hinge was performed by a multiple sequence alignment of 1000 animal ROR $\beta$  sequences using NCBI BLASTP. Conservation was assessed by the rate of mismatches at the residues shown. C. In vitro SUMOylation assay for ROR $\beta$  conjugation to SUMO1. Recombinant ROR $\beta$  was incubated with E1 and E2 ligases and SUMO1, +/- ATP required for SUMOylation. RanGAP1 served as a positive control. Samples of the reactions were immunoblotted for SUMO1 and ROR $\beta$ .



**Figure S4.** A. In vitro SUMOylation assay for ROR $\beta$  conjugation to SUMO2 and SUMO3. Recombinant ROR $\beta$  was incubated with E1 and E2 ligases and SUMO2 or SUMO3, +/- ATP required for SUMOylation. RanGAP1 served as a positive control. Blotting was done for SUMO2/3 or for ROR $\beta$ . B. Mass spectrometry MS2 assignment of K179-containing peptide with QQQTGG SUMO2 remnant. Spectra taken from reaction with SUMO2 after excising gel band at 100 kDa. The “p” on the N-terminal Q of the QQQTGG sequence represent pyro- modification of this residue, a common artifact in mass spectrometry of peptides with an N-terminal Q.