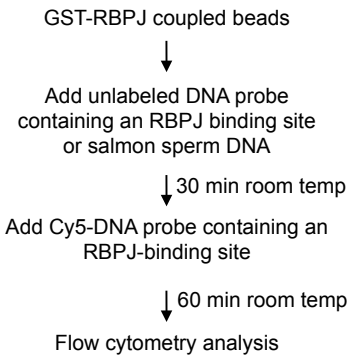
## Supplemental Materials

## Supplemental Figures



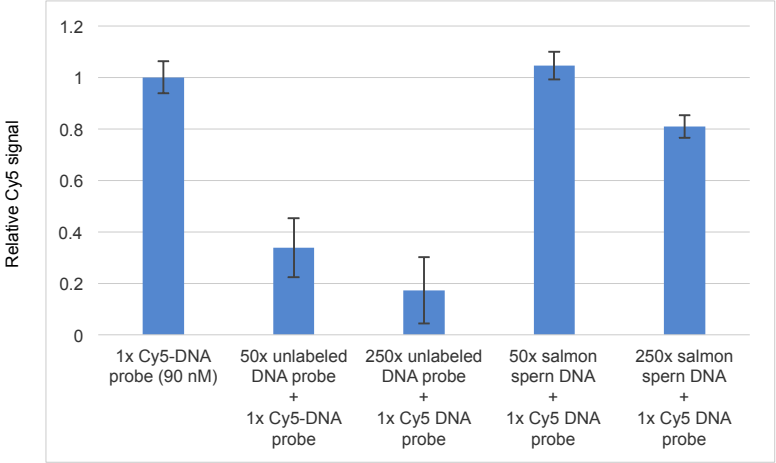




**Figure S1. Comparison of DNA with or without RBPJ binding motifs in competing the interactions of RBPJ and the Cy5 DNA probe containing an RBPJ binding motif.** GST-RBPJ coupled beads were incubated with unlabeled DNA with an RBPJ binding motif or with salmon sperm DNA for 30 minutes at room temperature. The Cy5-labeled DNA probe with an RBPJ binding motif was then added to 90 nM (1x), the reactions were incubated for 60 min at room temperature before flow cytometry analysis. The unlabeled DNA probe with an RBPJ binding motif competed more efficiently than salmon sperm DNA for RBPJ-Cy5 DNA probe interactions. Shown is mean +/-SEM, n=4.

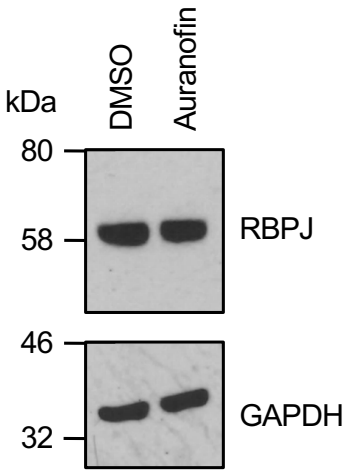



**Figure S2. Auranofin treatment does not alter RBPJ protein levels.** Western blot analysis showing the RBPJ protein levels in 293T cells with or without 2.5 μM auranofin treatment for 24 hours.

**Table S1.** Raw data for Figure 3B. Shown are firefly luciferase activities (top) and renilla luciferase activities (bottom). 293T cells were transfected with 4xRBPJ-firefly luc and TK-renilla luc. Notch-On cells had NICD, and Notch-Off cells contained the empty vector. 48-hr post transfection, cells were treated with varying amounts of auranofin for 24 hours, and luciferase activities were determined.

| 4xRBPJ-Fluc | Notch Off | | | Notch ON | | |
|---|---|---|---|---|---|---|
| auranofin (uM) | n1 | n2 | n3 | n1 | n2 | n3 |
| DMSO | 93488 | 91344 | 120514 | 492118 | 462068 | 459822 |
| 1 | 110964 | 99623 | 89956 | 300263 | 506446 | 325391 |
| 2 | 51628 | 46576 | 72924 | 104389 | 259154 | 256557 |
| 4 | 99046 | 132567 | 100626 | 141629 | 275057 | 270991 |
| 6 | 105792 | 50476 | 22119 | 30036 | 18645 | 43223 |
| 8 | 4680 | 4924 | 5308 | 6731 | 6119 | 32202 |
| 10 | 3100 | 3661 | 4045 | 7625 | 6229 | 6158 |

| TK-Rluc | Notch Off | | | Notch ON | | |
|---|---|---|---|---|---|---|
| auranofin (uM) | n1 | n2 | n3 | n1 | n2 | n3 |
| DMSO | 30839 | 28059 | 30073 | 5012 | 3913 | 4163 |
| 1 | 31299 | 31362 | 24578 | 3197 | 4053 | 3648 |
| 2 | 10407 | 10801 | 16141 | 2142 | 2729 | 2848 |
| 4 | 10533 | 8136 | 8529 | 1534 | 1725 | 2773 |
| 6 | 4050 | 2101 | 1217 | 1177 | 1635 | 1832 |
| 8 | 1678 | 957 | 872 | 961 | 968 | 1184 |
| 10 | 1792 | 1692 | 1253 | 831 | 1522 | 1874 |

| Table S2: Primers used in qPCR | | |
|---|---|---|
| Name of primer set | Primer name | Sequence (5' to 3') |
| Primers used for ChIP-qPCR | | |
| hHES1 | hHES1_ChIP_F | CCT CCC ATT GGC TGA AAG T |
| | hHES1_ChIP_R | CGG ATC CTG TGT GAT CCC TA |
| hHEY1 | hHey1_pro_F3 | CCGCATGAATGGAGAAGAAT |
| | hHey1_pro_R3 | AGGCTCCGATTACAGGTTCA |
| 4xCSLbs | 4xCSL-LUC_for | AAC AGT ACC GGA ATG CCA AG |
| | 4xCSL-LUC_rev | TGG TTT GTC CAA ACT CAT CAA |
| mHes1 | mHes1_for | TCCTCCCATTGGCTGAAAGTT |
| | mHes1_rev | ATATCTGGGACTGCACGCGAAC |
| mZfp334 | mZfp334_for | AAACGGAAACGCATCCATAG |
| | mZfp334_rev | CAACTCCCTGTGGGAAATGT |
| mNaprt1 | mNaprt1_for | GCACCAAAGGCAAAACAACT |
| | mNaprt1_rev | CTCTAGGGGGCTCTCAGCTT |
| mNanog | mNanog_for | GGAGAATAGGGGGTGGGTAG |
| | mNanog_rev | CAGCCTTCCCACAGAAAGAG |
| mActB | mActB_for | GACCAGGCCGTATATGGAGA |
| | mActB_rev | AGCAGTCTGCAAAGCAGTGA |
| primers used for RT-qPCR | | |
| hHEY1 | hHES1_RT_F | GCT GTT GCC CTG GAG TTG |
| | hHES1_RT_R | ACG CTT TGC CTC TGG TTA AA |
| hHES1 | hHey1_RT_F | TCA ACA CGA CAC CGG ATA AA |
| | hHey1_RT_R | TCA GCT GGC TCA GAC TTT CA |
| hTCERG1 | hTCERG1_RT_F | ATC CTA ATA TGC CGC CAA TG |
| | hTCERG1_RT_R | GAG GCA TGA AAG GTG GTC TC |
| hATCB | hACTb_RT_F | TCG TGC GTG ACA TTA AGG AG |
| | hACTb_RT_R | ATG CCA GGG TAC ATG GTG GT |