# 6687334

Statistical differences between groups were evaluated with the one-way ANOVA, followed by the Tukey’s multiple comparison test using GraphPad Prism version 5.04 for Windows (GraphPad Software, La Jolla, CA). Statistical differences were confirmed with Bonferroni’s comparison of selected pairs of columns and Student’s unpaired t test (two-tailed). Mean ± SD is shown. A value of P ≤ 0.05 was considered significant.

# 6687308

Patch-clamp data were acquired with PatchMaster software and exported to IGOR Pro (Wavemetrics). Current amplitudes were extracted from IGOR Pro and transferred to Excel (MS Office 2007), where all values for mean, standard deviation, and standard error of the mean were calculated. In some cases p-values based on unequal variances t test (unpaired, 2-tail) were calculated (Excel, TTEST function). Comparison of time course data was performed using one-way ANOVA with Tukey’s multiple comparison test. Significance level was set at 0.05.

# 6686905

In order to assess P2 modulation for reward incentive cue (prior to the flankers), an independent samples t-test was conducted. This analysis revealed significant P2 modulation differences between the two reward types (t(15) = 3.373, p = 0.004, d = 0.842), whereby P2 amplitude was greater when the participant was incentivized by picture reward then when they were incentivized by point reward (see Fig. 2c).

In order to investigate whether changing outcome expectation had an effect on P2 amplitudes following reward class incentive, an independent samples t-test was conducted comparing P2 amplitudes (150–200 ms) for picture and point reward incentives (see Fig. 3d). This analysis revealed the ERP amplitudes for the incentives were essentially the same (t(19) = 0.423, p = 0.677, d = 0.099), consistent with the hypothesis that uncertainty removed the information from the incentive screen as the earliest faithful predictor of future reward.

The second ANOVA for picture rewards (Fig. 3a2) also revealed a significant difference between levels of Reward Magnitude (F(1.708, 32.456) = 7.293, p = 0.004, η2 = 0.277). Again, t-test failed to reveal any significant difference between the top and bottom pictures (t (19) = 0.069, p = 0.945, d = 0.017). Surprisingly, this analysis did reveal that ERP amplitudes differed between null trial feedback and top picture (t(19) = 2.877, p = 0.010, d = 0.673) and bottom picture (t(19) = 3.236, p = 0.004, d = 0.748), however these effects were inverse to the point reward outcomes: the amplitude for null feedback was greater than that of both rewarding picture feedbacks. Based on the morphology of these reward generated ERPs it appears that point rewards evoked a Rew-P while rewarding pictures again evoked a N2. Null outcomes were nearly identical between conditions (Fig. 3c3). Similar to the results seen in Experiment 1, there was no significant correlation between P2 and Rew-P amplitudes for affective rewards (r = 0.404, p = 0.077) but there was a significant relationship for conditioned rewards (r = 0.485, p = 0.030).

# 6686882

(D, F, H, J, and L) Quantitative data of the number, area, and integrated density of synapsin (D) and SV2 (F) and number and area of RibP P0 (H) RibP S6 (J) and rRNA 5.8S (L) clusters per axonal length. Bars represent the mean ± SEM. Statistical significance by unpaired Student’s t test.

(N) Quantitative data of the number of RibP L10a clusters per axonal length. Bars represent the mean ± SEM of at least 2 independent experiments. Statistical significance by unpaired Student’s t test.

(B) Quantitative data of the levels of p-4E-BP1. Bars represent the mean ± SEM of 4 independent experiments. Bars represent the mean ± SEM. Statistical significance by unpaired Student’s t test is shown.

(D) Quantitative data of the levels of intra-axonal nascent peptides. Bars represent the mean ± SEM. Statistical significance by unpaired Student’s t test.

(C and D) Quantitative data of the puncta number of synapsin (C) and RibP L10a (D) clusters per axonal length. Bars represent the mean ± SEM. Statistical significance by unpaired Student’s t test.

Graphs and statistical analysis were performed in GraphPad Prism 6 software. Statistical significance was assessed by parametric tests. Unpaired two-tailed Student’s t test was performed for the comparison of two groups, while analysis of variance (ANOVA), followed by Dunnett’s or Tukey’s post hoc, used for comparisons between multiple groups. A p < 0.05 value was considered statistically significant.

# 6686859

Statistical analysis was using one-way ANOVA to evaluate differences of means between EGCG, EGC, and EC treatment groups in 1L-6 and 1L-8 ELISA, and densitometric analysis of proteins and zymograms. Due to large variability in protein expression profiles for signaling pathway analysis, data was normalized to positive control (IL-1β) before Student’s t-test with Bonferroni correction was performed. All tests assumed normal distribution where α = 0.05 was considered significant.

# 6686683

Unless otherwise stated, the data are presented as mean ± SEM inN = 4–5 mice/group. Data analysis: (A and B) independent samples one-way ANOVA followed by a post hoc Tukey’s HSD test; (C and D) two-tailed Student’s t test; and (E, repeated measures, and F, independent samples) two-way ANOVA followed by post hoc Sidak test; \*p < 0.05; \*\*p < 0.01, and \*\*\*p < 0.001.

In (B)–(D) and (G)–(I): WT (black bars) and Ager null (green bars) mice. Data are presented as mean ± SEM in N = 4–6 mice/group. Data analysis: (A, E, and F) one-way ANOVA with post hoc Tukey’s HSD test; (B and G) two-tailed Student’s t test; (C, D, H, and I) two-way ANOVA followed by a post hoc Sidak test or Tukey’s test, as appropriate; \*p < 0.05; \*\*p < 0.01, and \*\*\*p < 0.001.

In (F)–(K), the mean ± SEM is reported in N = 4–5 mice/group. In (A)–(K), Agerflox/flox Cre (−) shown as red and Agerflox/flox Cre (+) shown as green. Data analysis: (A–G, repeated measures, and J and K, independent samples) two-way ANOVA followed by a post hoc Bonferroni test; (H and I) two-tailed Student’s t test; \*p < 0.05; \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

(A–M) Source of donor iBAT: Agerflox/flox Cre (−) shown as red and Agerflox/flox Cre (+) shown as green. Data analysis: (B, G, and H, repeated measures, and C, independent samples) two-way ANOVA followed by a post hoc Bonferroni test; (D and F–H, area under the curve, and I–M) two-tailed Student’s t test. Where group mean variances were statistically different (p < 0.05), data were analyzed post hoc using the non-parametric Mann-Whitney U test; \*p < 0.05; \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

(A–M) Source of donor iWAT: Agerflox/flox Cre (−) shown as red and Agerflox/flox Cre (+) shown as green. Data analysis: (B, G, and H, repeated measures, and C, independent samples) two-way ANOVA followed by a post hoc Bonferroni test; (D–H, area under the curve, and I–M) two-tailed Student’s t test. Where group mean variances were statistically different (p < 0.05), data were analyzed post hoc using the non-parametric Mann-Whitney U test; \*p < 0.05; \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

In (A)–(E), WT (black) and Ager null (green). Data analysis: (A–D, repeated measures) two-way ANOVA followed by post hoc Bonferroni test; (E and H) two-tailed Student’s t test; (F, G, and I–K) one-way ANOVA followed by a post hoc Tukey’s HSD test; \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001.

The Figure Legends indicate all of the subject numbers/replicates per study; the mean ± standard error of the mean (SEM) is reported. The of details the specific statistical analyses based on the conditions and comparisons are listed in each figure legend and employed methods such as Student’s t test or ANOVA followed by the appropriate and indicated post hoc tests. In the event that variances were statistically different and thus not meeting the requirement of normal distribution, a non-parametric Mann-Whitney U test was employed. P values of < 0.05 were determined a priori as the threshold for statistical significance. Data analysis was performed using GraphPad Prism Software Version 8.02 (GraphPad, San Diego, CA).

# 6686201

(C) Luciferase activity was measured in response to DMSO, 5μm or 10μm Ro 08–2750 treated CTGF-promoter luciferase, renilar and YAP-WT co-transfected 293AD cells. Error bars represent SD; \*\* p<0.01; \*\*\* p<0.001 by two-tailed student’s t-test.

(A) Cell numbers were counted in DMSO, 5μm or 10μm Ro 08–2750 treated PANC1 and MDA-MB231 cells every 24 hrs for 72 hrs. Experiment was repeated independently three times. Error bars represent SD; \* P<0.05, \*\* P < 0.01 by two-tailed student’s t-test.

(B) Representative images of colony formation assay for DMSO or 10μm Ro 08–2750 treated PANC1 and MDA-MB231 cells for three weeks (Left panel). Quantifications of colony formation assay from three independent experiments (Right panel). Error bars represent SD; \*\* P < 0.01 by two-tailed student’s t-test.

(C) Representative images of wound healing assay for DMSO or 10μm Ro 08–2750 treated PANC1 and MDA-MB231 cells for 24 hrs (Left panel). Quantifications of wound closure estimation from three independent experiments (Right panel). Error bars represent SD; \*P < 0.05, \*\* P < 0.01 by two-tailed student’s t-test.

(C) mRNA expression (qRT-PCR) of YAP target genes CYR61, ADMTS1 and ANKRD1 in PANC1 cells; CYR61 and ANKRD1 in MDA-MB231 cells that treated with DMSO or 10μm Ro 08–2750. GAPDH was used as an internal control. Three independent experiments were performed. Error bars represent SD; \* p<0.05; \*\* p<0.01 by two-tailed student’s t-test.

(B) Cell numbers were counted in DMSO or 10μm Ro 08–2750 treated MDA-MB231 cells for 24 hrs or 48 hrs. Three independent experiments were performed. Error bars represent SD; \* P<0.05 by two-tailed student’s t-test.

(C) Representative images of wound healing assay for DMSO or 10μm Ro 08–2750 treated MDA-MB231 cells for 24 hrs (Left panel). Quantifications of wound closure estimation from three independent experiments (Right panel). Error bars represent SD; \*P < 0.05 by two-tailed student’s t-test.

(B) mRNA expression (qRT-PCR) of YAP target genes CTGF and CYR61 in PANC1 cells; CTGF, CYR61 and ANKRD1 in MDA-MB231 cells that treated without or with 200 ng/mL b-NGF treatment for 30 minutes. Three independent experiments were performed. GAPDH was used as an internal control. Error bars represent SD; \*\* p<0.01 by two-tailed student’s t-test.

(C) Cell numbers were counted in PANC1 or MDA-MB231 cells under the condition of control, 200 ng/mL b-NGF or 200 ng/mL b-NGF together with 1μm verteporfin treatment every 24hrs for 72 hrs. Three independent experiments were performed. Error bars represent SD; \*\* p<0.01 by two-tailed student’s t-test.

(D) Cell numbers were counted in PANC1 or MDA-MB-231 cells under the condition of siControl or siYAP together with 200 ng/mL b-NGF every 24hrs for 72 hrs. Insert: Immunoblot analyses were performed with anti-YAP or anti-GAPDH antibodies. The samples are lysates from siControl or siYAP transfected PANC1 or MDA-MB231 cells. GAPDH was used as loading control. Three independent experiments were performed. Error bars represent SD; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001 by two-tailed student’s t-test.

(E) Representative images of wound healing assay for DMSO or 200 ng/mL b-NGF treated PANC1 or MDA-MB231 cells in serum-free medium for 24 hours (Left panel). Quantifications of wound closure estimation from three independent experiments (Right panel). Error bars represent SD; \*\* P < 0.01 by two-tailed student’s t-test.

(B) Cell numbers were counted in siCon or siNTRK1 transfected PANC1 or MDA-MB231 cells every 24hrs for 72 hrs. Three independent experiments were performed. Error bars represent SD; \* p<0.05 by two-tailed student’s t-test.

(C) Quantifications of colony formation assay for siCon or siNTRK1 transfected PANC1 or MDA-MB231 cells for three weeks. Three independent experiments were performed. Error bars represent SD; \*\* p<0.01 by two-tailed student’s t-test.

(D) Representative images of 24 hrs wound healing assay for siCon or siNTRK1 transfected PANC1 or MDA-MB231 cells (Left panel). Quantifications of wound closure estimation from three independent experiments (Right panel). Error bars represent SD; \*\* P < 0.01 by two-tailed student’s t-test.

(C) Representative primary tumour images of shCon-or shNTRK1-transduced PANC1 or MDA-MB-231 cells subcutaneously injected into SCID mice (upper panel). Quantifications of primary tumour weight were measured after 6 weeks subcutaneous injection of shCon or shNTRK1-transduced PANC1 or MDA-MB-231 cells in SCID mice. n=5 mice per group; error bars represent SD; \*\*\* p<0.001 by two-tailed student’s t-test.

# 6686135

In vivo targeting of 125I-labeled targeted FTL-5X to ICAM-1. (a) Biodistribution of 125I-labeled FTL-5X conjugated to anti-ICAM and IgG2b isotype control in naïve and LPS-treated mice at 30 min. Tissue uptake is indicated as mean ± SEM (n = 3). (b) Localization ratio of selected organs. Significant differences determined by t test with Bonferroni correction to account for multiple comparisons (\*p < 0.05).

Statistical analysis was performed using Student’s t test with Bonferroni correction. Differences were deemed statistically significant at p < 0.05.

# 6686109

1Student t-test or chi-square test.

Descriptive statistical analysis was used to describe participants’ demographic and clinical data as mean±standard deviation. The Shapiro-Wilk test was used to explore the normality of all variables. The significance of differences in the baseline measurements between groups was tested by the Student t-test or the chi-square test. The mean changes in navicular height, the CSA of the AbdH muscle, and AbdH muscle activity between groups at baseline and at 4 weeks post-intervention were compared using the Student t-test. Statistical significance was set at p-value <0.05 for all tests.

# 6686008

Effects of K242R and AWAAA mutations on recruitment of various telomere-related factors to telomeres. a–d Dot-blot ChIP assays from asynchronous cell cultures to monitor binding of a Stn1, b Tpz1, c Trt1TERT, and d Rad26ATRIP at telomeres. Plots show mean values plus/minus SEM and distribution of individual data points from at least four independent experiments. Raw data values and statistical analysis of ChIP assays by two-tailed Student’s t-test are shown in Supplementary Data 1. Expression levels of myc-tagged proteins used in ChIP assays were monitored by western blot analysis. Anti-Cdc2 blots served as loading control. Molecular weight (kDa) of size markers are indicated

Characterization of strains expressing Tpz1-Stn1 fusion protein. a Schematic representation of the Tpz1-Stn1 fusion construct. b, d Southern blot analysis of telomeres for indicated strains. All samples, except for stn1∆ and stn1∆ tpz1-stn1-E132R, were prepared from strains that were extensively restreaked (>150 generations) on YES plates. For stn1∆ and stn1∆ tpz1-stn1-E132R strains in b, hybridization signals for telomeric repeats are completely lost due to chromosome circularization. c Pulsed-field gel analysis of telomeric NotI-fragments. e, f Quantitative dot-blot ChIP assays to monitor telomere localization for e Stn1 and f Ten1. Protein expression levels for indicated myc-tagged proteins were monitored by western blot, with Cdc2 as a loading control. Molecular weight (kDa) of size markers are indicated. Plots show mean values plus/minus SEM and distribution of individual data points from at least six independent experiments. Raw data values and statistical analysis of ChIP assays by two-tailed Student’s t-test are shown in Supplementary Data 1

Effects of Tpz1-Stn1 K242R and AWAAA mutations on recruitment of various telomere-related factors to telomeres. a, b, d–f Dot-blot ChIP assays from asynchronous cell cultures to monitor binding of a Ten1, b Po11 (DNA Polα), d Rad11 (RPA), e Rad26ATRIP, and f Trt1TERT at telomeres. Expression levels of myc- or FLAG-tagged proteins used in ChIP assays were monitored by western blot analysis. Anti-Cdc2 blots served as loading control. Molecular weight (kDa) of size markers are indicated. Plots show mean values plus/minus SEM and distribution of individual data points from at least three independent experiments. Raw data values and statistical analysis of ChIP assays by two-tailed Student’s t-test are shown in Supplementary Data 1. c Interaction of Stn1 and Po11 (DNA Polα), detected by co-IP analysis

Effects of TEL-patch mutants in tpz1-AWAAA mutant. a Sequence alignment of the TEL-patch region of TPP1 from human and mouse and Tpz1 from four Schizosaccharomyces species. Identical residues conserved among four or greater species are marked black, while amino acid residues that maintain similar chemical properties are marked gray. TEL-patch residues in Tpz1 mutated in this study and potentially equivalent sites in human TPP1 are also indicated. b, c Southern blot analysis to test effect of bK75A or cT78A in AWAAA mutants with or without Tpz1-Stn1 fusion on telomere length. All genomic DNA samples were prepared from cells that have been extensively restreaked (>150 generations) on YES plates. While strains used in Southern blot analysis did not carry an epitope tag, corresponding myc-tagged versions of wild-type and mutants (with or without Stn1 fusion) were used in western blot and co-IP experiments, and results indicated that none of the mutant combinations greatly affected protein expression or disrupted interaction of Tpz1 or Tpz1-Stn1 to Poz1, Pot1 or Ccq1 (Supplementary Fig 5b and 8). d, e Dot-blot ChIP assays from asynchronous cell cultures to monitor binding of d Ten1 and e Trt1TERT at telomeres. Expression levels of myc-tagged proteins used in ChIP assays were monitored by western blot analysis. Anti-Cdc2 blots served as loading control. Molecular weight (kDa) of size markers are indicated. Plots show mean values plus/minus SEM and distribution of individual data points from at least six independent experiments. Raw data values and statistical analysis of ChIP assays by two-tailed Student’s t-test are shown in Supplementary Data 1

Exponentially growing cells were crosslinked with formaldehyde by addition of 1/10 volume of fixation solution (11% formaldehyde, 100 mM NaCl, 1 mM EDTA pH 8.0, 0.5 mM EGTA pH 8.0, 50 mM Tris-HCl pH 8.0) for 20 min at room temperature, incubated additional 5 min after addition of Glycine at final concentration of 125 mM, washed 3× with TBS (20 mM Tris-HCl pH 7.6, 150 mM NaCl), pelleted, and frozen in liquid nitrogen28,61. Cells were lysed with FastPrep (MP Biomedicals) in lysis buffer (50 mM Hepes-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, Roche complete protease inhibitor cocktail) with glass beads, and processed for ChIP using monoclonal anti-myc (9B11) or anti-FLAG (M2 F1804) antibody. ChIP samples were analyzed with dot-blot with 32P labeled telomeric DNA probe, scanned with Amersham Typhoon, and quantified with NIH ImageJ software. For sub-telomere ChIP assays, primers indicated in Supplementary Fig 2a and Supplementary Table 4 are used in quantitative PCR with Bio-Rad CFX Connect Real-Time PCR detection system. ChIP sample values were normalized to Input samples and plotted as % precipitated DNA. For cell-cycle-ChIP assays, cdc25-22 cells were grown in YES liquid culture overnight at 25 °C, shifted to 36 °C for 3 h to arrest cells in G2/M-phase, and synchronously released into cell cycle by shifting back to 25 °C, and samples were collected every 20 min to be processed for ChIP assays28,61. Raw data values and statistical analysis of ChIP data by two-tailed Student’s t-test are shown in Supplementary Data 1.

Statistical analysis and individual raw data values for ChIP experiments are provided as a Microsoft Excel file in Supplementary Data 1. Graphs for these data values, plotted as either bar graphs with mean values plus/minus standard error of mean (SEM) or individual data points are also included in Supplementary Data 1. Pairwise two-tailed student’s t-test and one-way ANOVA analysis were performed with Excel. Statistically significant values (p < 0.05) are marked with red letters. We utilized multiple independently derived strains in most cases, and multiple independently grown and processed cultures to ensure that all conclusions are fully supported and reproducible. Replicates in experiments were defined as independently cultured and processed samples. For Southern blot, co-IP and western blot analysis, completely independent experiments were repeated at least twice and in many cases more to ensure that all results are reproducible even when only one representative blot is shown for a given experiment.

# 6685981

a HPLC chromatograms at 280 nm of oats bran extracts. b The MS spectra of peak avenanthramide A, avenanthramide B, avenanthramide C in oats bran extracts were obtained on ion-trap MS. c Dose-dependent activity of AVNs in HCT116 and DLD1 on indicated conditions as assessed by MTT assay. (n = 3, mean ± SD). d EdU assay showed proliferation rate of HCT116 and DLD1 cells treated with AVNs. e Columns showed mean values of three experiments and expressed as mean ± SD. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. P-values were calculated by a Student’s t-test. All experiments have been replicated three independent times

a Representative images of mitochondrial morphology in CRC cells treated with AVNs for 24 h visualized by labeled with MitoTracker Red. Scale bars represent 6 μm (left panel). Quantification of mitochondrial morphology in CRC cells (right panel). b Representative TEM images of HCT116 and DLD1 cells fixed after treated with AVNs for 24 h. Arrowheads represents the respective Mitochondria. Scale bars represent 2 μm. c Oxygen consumption rate (OCR) of HCT116 and DLD1 cells were measured in the presence of AVNs for 24 h (n = 3). d ATP production was determined in both HCT116 and DLD1 cells with the indicated concentrations of AVNs treatment. e HCT116 and DLD1 cells treated with the indicated concentrations of AVNs for 24 h, and ECAR was measured after consecutive injections of glucose (10 mM), oligomycin (1 μM), and 2-DG (50 mM) (n = 3). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. P-values were calculated by a Student’s t-test. All experiments have been replicated three independent times

a Western blot showing ND2, ND5, CYTB, COX2, and ATP6 in AVNs-treated CRC cells. b Intracellular ROS levels in HCT116 and DLD1 treated with indicated concentrations of AVNs were measured by flow cytometry. c The GSH and GSSG were measured respectively, and the ratio of GSH/GSSG was calculated in HCT116 and DLD1 cells treated with AVNs for 24 h. (n = 3, mean ± SD). d JC-1 dye was used to detect effects of AVNs treatment on MMP levels of HCT116 and DLD1 cells by flow cytometry. e The MMP levels derived from (D) was indicated as the ratio of red/green fluorescence. f Western blot was used to measure the expressions of cytochrome c in HCT116 and DLD1 cells treated with indicated concentrations of AVNs. Tim23 and GAPDH served as mitochondrial marker and cytosolic marker respectively. g The expression of cleaved-caspase 3 in both HCT116 and DLD1 cells following the treatment with indicated concentrations of AVNs for 24 h was measured and normalized by GAPDH. h Flow cytometry analysis depicts the effects of AVNs on apoptotic CRC cells stained with Annexin V/PI staining. HCT116 and DLD1 cells were pretreated with 10 mM NAC for 2 h and cultured with AVNs for 24 h. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. P-values were calculated by a Student’s t-test. All experiments have been replicated three independent times

a HCT116 and DLD1 cells treated with AVNs of 50 μg/ml for 24 h and stained with an anti-DDX3 antibody, MitoTracker, and DAPI. Scale bars represent 6 μm. b HCT116 and DLD1 cells treated with indicated concentration of AVNs and the expression of DDX3 was performed by western blot in cytosolic (C) and mitochondrial (mito) fractions of cell lysates. Tim23 and GAPDH served as mitochondrial marker and cytosolic marker respectively. c The effect of AVNs treatment on the mRNA expression levels of DDX3 was analyzed. (n = 3, mean ± SD). d HCT116 and DLD1 cells treated with the indicated concentrations of AVNs in the presence of 20 μM CHX for 0, 1, 3 h, respectively. DDX3 expression was detected by western blot. e DLD1 cells were treated with 50 μg/ml AVNs for 24 h with or without 20 μM MG132 or 30 μM chloroquine. The protein level of DDX3 was detected by western blot (Left panel). DLD1 cells were treated with AVNs for 24 h, followed by the addition of 20 μM MG132 for an additional 6 h. Cell extracts were subjected to immunoprecipitation with anti-DDX3 antibody. The conjugates were detected with anti-ubiquitin and anti-DDX3 antibody (Right panel). f The DDX3 overexpression cell lines were evaluated by western blot. After 24 h of treatment of 50 μg/ml AVNs, cell viability was measured by MTT assay. g–h HCT116 and DLD1 cells treated with 50 μg/ml AVNs after transfected with GFP or GFP-DDX3 (n = 3, mean ± SD). g The level of ROS was measured by flow cytometry. h CRC cell apoptosis induced by AVNs was assessed via Annexin V/PI method by using flow cytometry. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. P-values were calculated by a Student’s t-test. All experiments have been replicated three independent times

a SDS-PAGE of His-DDX3 recombinant proteins after purification by nickel affinity column chromatography. b Schematic demonstration of the injection and recovery of DDX3-bound ingredients using SPR biosensor. c HPLC/MS was carried out to identify DDX3-bound ingredients. The upper is HPLC chromatograms at 280 nm of DDX3-bound ingredients. The lower is the MS spectra of DDX3-bound ingredients. d The binding mode of AVN A with DDX3 based on molecular modeling experiments. A detailed view showed that AVN A (cyan) formed hydrogen bond (yellow dotted line) with key amino acid residues. e SPR assay was carried out to determine the binding affinity of DDX3 to AVN A. f Malachite green assay for ATP hydrolysis showing effects of 50 μg/ml AVNs or 30 μM AVN A on ATPase activity of DDX3. (n = 3, mean ± SD). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. P-values were calculated by a Student’s t-test. All experiments have been replicated three independent times

a Schematic representation of the truncation mutants of human DDX3. NTD, N-terminal regulatory domain; Helicase, Helicase domain; CTD, C-terminal regulatory domain. b Three truncation mutants of DDX3 were purified and determined by SDS-PAGE. c SPR assay was used to evaluate the binding affinities of three truncation mutants of DDX3 to AVN A. d Mutations K255A, R287A, S290A, and R294A in the ATP-binding pocket of DDX3 were performed by site-directed mutagenesis. DLD1 cells were transfected with wild-type DDX3 or the four site mutants, and then treated with 50 μg/ml AVNs or 30 μM AVN A, after 24 h cell viabilities were detected by MTT assay (n = 3, mean ± SD). e The expression of NDUFS2 and UQCRC1 in wild-type or the 4 DDX3 site mutants were determined by western blot. f The levels of ROS in wild-type or the four site mutants of DDX3 treated with 30 μM AVN A were measured by flow cytometry. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. P-values were calculated by a Student’s t-test. All experiments have been replicated three independent times

a BALB/c nude mice with DLD1 xenograft treated with vehicle, AVNs and AVN A via oral administration for three weeks. The tumor weight of mice from each group was measured. (n = 6, mean ± SD). b The tumor volume from each group was monitored. (n = 6, mean ± SD). c The relative body weight was evaluated during the treatment. (n = 6, mean ± SD). d The GSH/GSSG ratio in lysates of the tumors tissue (upper) and peripheral blood (lower) in mice administered by AVNs and AVN A were determined. e Immunohistochemical stained with antibodies against Caspase3 and DDX3 in tumor sections obtained from all vehicle, AVNs and AVN A treated groups. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. P-values were calculated by a Student’s t-test. All experiments have been replicated of three independent times. f The mice livers and kidneys from the same experiment as (A) were histopathologically evaluated. Scale bars: the black scale bar is 0.5 cm (left) and the white scale bar is 100 mm (right)

# 6685976

The experiments in this paper were done in vitro at the single-cell level. We collected data from hundreds of Piezo1 flicker events from several cells across multiple biological replicates to ensure reproducibility. Sample sizes are indicated in corresponding figure legends. OriginPro 2018 (OriginLab Corporation) was used for statistical analysis and generating plots. P values and statistical tests used are indicated in figure legends. A two-sample t-test was used where data were modeled by a normal distribution and the nonparametric Kolmogorov–Smirnov test was used in the case of non-normal distributions.

# 6685970

Loss-of-function mutation of Bcl11b biases the OR class choice of OSNs. a Distributions of class I (blue) and class II OSNs (magenta) and ISH with RNA probes for Bcl11b and a dorsal marker, Acsm4, in consecutive coronal sections of the MOE at P30. b Combination of IHC for Bcl11b (green) and ISH for OR genes (magenta). Arrowheads and arrow indicate co-labeled and not co-labeled OSNs, respectively. c Bar graphs showing the percentages of Bcl11b-positive cells that are labeled with each OR probe (n = 3 animals. The quantification data are summarized in Supplementary Data 1). d Microarray analysis of the expression of OR genes in the wild type and Bcl11b−/− MOE. A heat-map representation was obtained by hierarchical clustering using 36 OR gene probe sets (blue: class I genes; magenta: class II genes). Each row refers to independent preparations (n = 5 control mice, 6 Bcl11b−/− mice). Color scale indicates the log2 value of the signal intensity of OR gene normalized to the internal control, GAPDH (Supplementary Data 2). e ISH with mixed RNA probes for the eight class I and the eight class II genes in coronal sections of the wild type and Bcl11b−/− MOE at P0. f Quantification of the number of OSNs expressing class I or class II genes per section (control: black circles, Bcl11b−/−: open circles). Bar represents the mean values ± s.e.m. The quantification data and number of animals analyzed are summarized in Supplementary Data 1. g Bar graphs showing the percentage of change in the number of cells expressing each OR gene in Bcl11b−/− versus wild type mice (serial sections throughout the MOE at 100 μm interval were analyzed). The quantification data and number of animals analyzed are summarized in Supplementary Data 1. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.005, \*\*\*\*p < 0.001 (two-tailed t-test, n = at least 3, as the correction for multiple testing, p-values were adjusted based on false-discovery rate using the Benjamini–Hochberg (BH) method). D, dorsal; V, ventral; M, medial; L, lateral. Scale bars, 100 μm in (a) and (e); 10 μm in (b)

Bcl11b negatively regulates the expression of class I OR genes. a Genetic strategy of the gain-of-Bcl11b-function analysis in OSNs. Robust expression of Bcl11b throughout the MOE of the gain-of-function mutant mice was confirmed by IHC (Supplementary Fig. 5a). b ISH with mixed RNA probes for the four class I and dorsal class II genes in coronal sections of the MOE of the control and gain-of-function mutant mice. Scale bar, 100 μm. c Quantification of the number of OSNs expressing either class I or class II genes per section. Bars represent the mean values ± s.e.m. of the control (gray) and gain-of-function mutant mice (class I in blue, class II in magenta), respectively: Class I genes: 33.8 ± 1.60 in control and 3.78 ± 1.82 in mutant mice, p = 0.000243, two-tailed t-test, n = 3 independent experiments; Class II genes: 25.6 ± 1.57 in control and 26.4 ± 3.15 in mutant mice, p = 0.838, two-tailed t-test, n = 3 independent experiments. \*\*\*\*p < 0.001, NS, not significant. Quantification data and statistical details are summarized in Supplementary Data 1. d The log2-fold change values of OR gene expression analyzed by RNA-seq are arranged according to their relative positions along the chromosomes. Class I genes (blue), atypical class I genes (purple), and class II genes (magenta). Increased- and decreased OR genes (p < 0.05) are represented by filled circles. FPKMs in the control and gain-of-function mutant MOEs are summarized in Supplementary Data 3. e Merged representation of the bee-swarm and box-plots of RNA-seq FPKM values for class I (n = 128) and class II genes (n = 968) of the control (gray) and gain-of-function mutant (class I genes in blue; class II genes in magenta) mice

OSN-specific depletion and overexpression of Bcl11b alters behavioral responses to aversive odorants. a A video frame of the behavioral test, in which a male mouse is exposed to a filter paper impregnated with a particular aversive odor. Centre of a filter paper and the opposite side to a filter paper were determined to ‘0’ (black dot) and ‘1’ (dotted line), respectively. The white dot indicates center of the mouse body excluding tail. b, c Raster plots representing occupancy of each animal in two areas (magenta or blue) during the 10-min test period (x-axis) of Bcl11b cKO mice (n = 12 animals for 2MBA, n = 7 for TMT, n = 13 for DW) and control (n = 14 for 2MBA, n = 11 for TMT, n = 13 for DW) (b) and Bcl11b gain-of-function mutant (n = 16 for 2MBA, n = 4 for TMT, n = 8–9 for DW) and control (n = 12 for 2MBA, n = 4 for TMT, n = 9 for DW) (c). The two-color representation corresponds to color discrimination in (a). The graph of time bins is presented in Supplementary Fig. 9. d Representative trajectory plots of mouse positioning during the 10-min test period to 2MBA (black: control; blue: Bcl11b cKO) and TMT (black: control; magenta: Bcl11b cKO). Dotted circles indicate that mice tried to escape from the cage by climbing walls. e, f Aversion index of control (black) and Bcl11b cKO (blue: 2MBA; magenta: TMT; gray: DW) mice during the first 1 min (e) and 10-min (f) of test period. Each bar indicates merged representation of the bee-swarm and box-plots. \*p < 0.05 (two-tailed t-test). g Representative trajectory plots of mouse positioning during the 10-min trials to 2 MBA (black for control; cyan for Bcl11b gain-of-function mutant) and TMT (black for control; orange for Bcl11b gain-of-function mutant). g Aversion index of control (black) and Bcl11b gain-of-function mutant (cyan: 2MBA; orange: TMT; gray: DW) mice during the first 1-min (h) and 10-min (i) of test period. Each bar indicates merged representation of the bee-swarm and box-plots. \*p < 0.05, \*\*\*p < 0.005 (two-tailed t-test). All behavioral analysis data are summarized in Supplementary Data 1

# 6685917

Data are presented as mean ± SEM. Statistical significance was analyzed by two-tailed Student’s t test: \*p < 0.05, \*\*\*p < 0.001. Scale bars, 20 μm (B, D, and F) and 50 μm (G, H, and K). See also Figure S1.

(A) Illustrative western blot of MBP abundance (upper panel) showing that primary rat differentiating oligodendrocytes treated for 48–72 hr with HAMI3379, but not with BayCysLT2, in the presence of 0.20 nM triiodothyronine (T3) expressed higher MBP levels compared with untreated cells. Quantitative analysis of MBP-immunoreactive band corrected by β-actin from seven independent experiments (lower panel) showed that treatment with HAMI3379 significantly increased MBP expression in primary rat oligodendrocytes. Data are presented as mean + SEM. Statistical significance was analyzed by two-tailed Student’s t test, \*p < 0.05.

(F and G) Sholl analyses of oligodendrocyte morphology. Treatment with HAMI3379 increased branching in oligodendrocytes from GPR17+/−, but not from GPR17−/−, mice (F). Analysis of process complexity (G) revealed that GPR17+/− mouse oligodendrocytes treated with HAMI3379 displayed increased elaboration compared with oligodendrocytes cultured alone. No effect of HAMI3379 was observed in oligodendrocytes from GPR17−/− mice (n = 22 cells/condition). Data are presented as mean + SD of three independent experiments. Statistical significance was analyzed by unpaired two-tailed Student’s t test, \*p < 0.05.

(I) Quantification of normalized MBP+/O4+ ratios showed that HAMI3379 treatment efficiently increased the amount of MBP-positive human oligodendrocytes (mean + SEM; n = 3 independent experiments; two-tailed t test, \*p < 0.05, \*\*p < 0.01).

Data points were fitted to both three-parameter (fixed Hill slope) and four-parameter nonlinear regression isotherms using Prism 6.05 (GraphPad Software). Quantification of buffer corrected DMR signals was performed by calculation of the maximum response or the area under the curve (AUC) between at least 0 and 1200 s. Calcium responses were analyzed using the maximal peak fluorescence within 30 s after agonist addition. All data presented are mean +/± SEM. Statistical analyses were performed using two-tailed Student’s t test. P value significance thresholds were \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001.

# 6684934

All images were analyzed using the software ImageJ (2006 version). The data were analyzed using the software Graphpad Prism 7.0 (t-test/one-way ANOVA). P < 0.05 was considered statistically significant.

# 6684932

Results are reported as the mean ± standard deviation. Significance was tested by T-Test or ANOVA with post-hoc tests using GraphPad 3.0 software (San Diego, CA).

# 6684930

Heatmap showing the alterations in the mRNA expression of m6A-related genes in the TCGA and GEO datasets. The red color indicates upregulated expression; the green color indicates downregulated expression; the black color indicates no significant changes, and the white color indicates that the related gene is absent in the datasets. The data were statistically analyzed by Student’s t test (unpaired, two-tailed). A. mRNA expression patterns of m6A-related genes in COAD. B. mRNA expression patterns of m6A-related genes in READ.

# 6684922

The values for the experiments were presented as the means ± s.e.m. (standard error of the mean) or percent. Significant differences were determined with a 1-way ANOVA, except for Figure 3 which was analyzed using the paired t-test. GraphPad Prism 7 (GraphPad Software, Inc., San Diego, USA) was used as graphical and statistical tool. A p-value < 0.05 was considered as statistically significant (ns: not significant, \*: P < 0.05, \*\*: P < 0.01, \*\*\*: P < 0.001).

# 6684916

All the experiments were conducted in triplicate. All statistical analyses and Kaplan-Meier survival analysis were performed in GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA). The correlation between miR-206 expression and FZD7 levels in glioma tissues was evaluated by Pearson’s correlation analysis. Two-tailed Student’s t test was performed for other comparisons, and the results are expressed as the mean ± standard deviation. Data with P < 0.05 were considered statistically significant.

# 6684912

All the values were expressed as mean ± S.D. from at least three independent experiments. The difference between two groups was analyzed by the parametric unpaired Student’s t-test. To analyze the difference among three or more groups, one-way ANOVA analysis was implemented. The statistical analysis was performed by GraphPad Prism 6 and p values less than 0.05 are considered significant.