**Supplemental Materials and Methods**

**Fluorescence in situ hybridization (FISH)**

FISH probes used for characterizing the *NUT*-fusion were as following: *NUT*: 5' centromeric probes, RP11-368L15 and RP11-1084A12 (biotin labeled, red) and 3’ telomeric probes, RP11-1H8 and RP11-64o3 (digoxigenin labeled, green); *BRD4*: 5’ centromeric probes, RP11-207i16 and RP11-3055m5 (biotin labeled, red), and 3' telomeric probes, RP11-319O10 and RP11-681D10 (digoxigenin labeled, green); *NSD3*: 5′ centromeric BAC clones CTD-2538P2 and RP11-957P17 (biotin labeled, red) and the 3′ telomeric BAC clones CTB-497A2 and RP11-90P5 (digoxigenin labeled, green); *BRD3*: 5’ telomeric probes, RP11-145E17 and RP11-92B21 (biotin labeled, red), and 3' centromeric probes, RP11-260C10 and RP11-153P4 (digoxigenin labeled, green); *ZNF532*: 3′ telomeric BAC probes RP11-350K6 and -1061A13 (digoxigenin labeled, green) and the 5′ centromeric probes RP11-351N16 and -722P5 (biotin labeled, red); *ZNF592*: 5’ centromeric probes, RP11-68P22 and RP11-957L15 (biotin labeled, red), and 3' telomeric probes, CH17-282L14 and RP11-1079K14 (digoxigenin labeled, green). 200 nuclei were counted in four different areas of each tumor. Eighty percent positive interpretable nuclei were defined as positive for a rearrangement.

**Next Generation Sequencing (Archer® FusionPlex®)**

An Anchored Multiplex PCR (AMP) assay was used for detection of targeted fusion transcripts using next generation sequencing (NGS) (1). Total nucleic acid was isolated from FFPE sections of UNC tumor surgically removed from a lung metastasis after histological review for tumor enrichment. The total nucleic acid was reverse transcribed with random hexamers, followed by second strand synthesis to create double-stranded complementary DNA (cDNA). The double-stranded cDNA was end-repaired, adenylated, and ligated with a half-functional adapter. Two hemi-nested PCR reactions using the Archer® FusionPlex® Solid Tumor Kit (ArcherDX, Boulder, CO) primers were performed to create a fully functional sequencing library that targets specific genes (exons) listed previously (2) and validated for clinical reporting, including amongst multiple other genes *BRD3* (exons 9-12), *BRD4* (exons 10, 11), and *NUTM1* (exon 3). Illumina NextSeq 2 x 150 base paired-end sequencing results were aligned to the hg19 human genome reference using bwa-mem (3). A laboratory-developed algorithm was used for fusion transcript detection and annotation (2). The integrity of the input nucleic acid and the technical performance of the assay were assessed with a qualitative reverse transcription qPCR assay and assessing the DNA/RNA content in the sequencing results. The assay is validated for samples showing 5% or higher tumor cellularity.

**Cell Culture**

293T and TC-797 (4) cell lines were cultured in monolayer in DMEM (Invitrogen) supplemented with 1x Penicillin Streptomycin (Hyclone, South Logan, UT), 1x Glutamax (Gibco), and 10% (v/v) Bovine Growth Serum (BGS) (Hyclone). The PER-403 (5) cell line was cultured in the above media except with 20% (v/v) Fetal Bovine Serum (FBS) (Hyclone) instead of BGS. 797TRex-FLAG-BRD4-NUT-HA and U2OSTRex-FLAG-BRD4-NUT were created as previously described (6) and were maintained in the above described conditions except with 10% (v/v) tetracycline-free FBS (Hyclone) instead of BGS. FLAG-BRD4-NUT-HA or FLAG-BRD4-NUT expression was induced by 1 µg/ml tetracycline treatment. Generation of and culture conditions of the 797-ZNF532-NBioTAP derivative is described below.

**RT-/RT-qPCR**

Nested RT-PCR was performed using total nucleic acid sample purified for the Archer® FusionPlex® as a template. Control template RNA was isolated from NC cell line TC-797 using RNeasy Mini Kit (Qiagen, Mansfield, MA) following the manufacturer’s instructions. cDNA was synthesized from both templates using Superscript IV reverse transcriptase (Invitrogen, Carlsbad, CA) and random hexamers. The Nested PCR was performed using Ex Taq DNA Polymerase (Takara Bio Inc., Japan). Primers used in the first-round of PCR were as following: ZNF592\_3551FF GAAACCCTGATTTGAGCCAGAC, NUT\_567R GTTGGTGGGAGAAAGGGAAGTG. The second-round PCR was performed using the following primers: ZNF592\_3576F CAAAGTGAAACCTCCGGGTG, NUT\_527R CGGCACTAGGTTTCATGCTC, ZNF592\_3564F GAGCCAGACGTCCAAAGTGAA, NUT\_527R2 CGGCACTAGGTTTCATGCTCA, ZNF592\_3571F ACGTCCAAAGTGAAACCTCCG, NUT\_526R GGCACTAGGTTTCATGCTCA.

RT-qPCR was performed to analyze an established 797-ZNF532-NBioTAP cell line as following: RNA was purified from TC-797 or 797-ZNF532-NBioTAP cells using Rneasy Mini Kit (Qiagen), and cDNA was synthesized using iScript reverse transcription supermix (Bio-Rad, Hercules, CA) qPCR was performed using iQ SYBR Green Supermix (Bio-Rad). Data shown are mean values ± standard deviations from three biological replicates. Statistical significance was determined by two-tailed Student’s t test with significance cutoff of p < 0.01.

**Antibodies**

Primary antibodies used for western blots were as follows: anti-ZNF592 (1:1000 dilution, A301-530A, Bethyl Laboratories, Montgomery, TX), anti-NUT c52B1 (1:1000 dilution, Cat #3625, Cell Signaling Technologies, Danvers, MA), anti-ZMYND8 (1:5000 dilution, A302-089A, Bethyl Laboratories), anti-Tubulin clone B-5-1-2 (1:10000 dilution, T6074, Sigma Aldrich, St Louis, MO), Peroxidase Anti-Peroxidase (PAP) Soluble Complex antibody produced in rabbit (1:1000 dilution, P1291, Sigma Aldrich). The secondary antibodies used are as follows: Anti-rabbit IgG HRP-linked Antibody (1:1000 dilution, #7074, Cell Signaling Technology), Polyclonal Anti-Mouse Immunoglobulin (1:20000 dilution, 0260, Dako/Agilent, Santa Clara, CA). The blots were visualized by Pierce ECL Plus Western blotting substrate (Thermo Fisher Scientific). Primary antibodies used for the immunofluorescence on FFPE were as follows: anti-NUT c52B1 (1:200 dilution, Cat #3625, Cell Signaling Technologies), anti-H3K27ac MABI0309 (1:200 dilution, Cat#39685, Active Motif, Carlsbad, CA). Antibodies used for immunofluorescence of cultured cells are as follows: anti-NUT c52B1 (1:1000 dilution, Cat #3625, Cell Signaling Technologies), anti-ZNF592 (1:1000 dilution, Bethyl Laboratories), anti-ZNF532 (1:1000 dilution, A303-329A, Bethyl Laboratories), anti-ZMYND8 (1:2000 dilution, A302-089A, Bethyl Laboratories), anti-HA clone 16B12 (1:10000 dilution, Cat # 901501, BioLegend, San Diego, CA). Secondary antibodies were anti-rabbit Alexa Fluor 488, anti-rabbit Alexa Fluor 594, anti-mouse Alexa Fluor 488 (Invitrogen).

**siRNA**

The following siRNA were purchased from Dharmacon (Lafayette, CO) and transfected at a final concentration of 50 nM: siZNF592-1 (cat. no. D020790-1) CCUCAAACCUCCAGGCAUA, siZNF592-2 (cat. no. D020790-3) GCGGAGAAAGACCACAUUA, siCTRL (control) ON-TARGET plus siRNA #1 (Dharmacon; cat no. D-001810-01-20). The following siRNA targeting the BioTAP tag was purchased from Integrated DNA Technologies (IDT) (Redwood City, CA) and transfected at final concentration of 10 nM: siBioTAP (cat. no. CD.Ri.22448.13.8) GUAGACGCGAAUUGUGAUAUACCTA (positive strand), UAGGUAUAUCACAAUUCGCGUCUACUU (negative strand). siCTRL (control), ON-TARGET plus siRNA #1 (Dharmacon; cat no. D-001810-01-20) was purchased from Dharmacon.

**Generation of N-BioTAP-ZNF532 797 cells using CRISPR/Cas9**

A pAAV vector was designed to insert the Blasti-P2A-N-BioTAP cassette into exon 4 of *ZNF532* as follows. A 5’-ZNF532-Blasti-P2A-N-BioTAP-3’ZNF532 DNA cassette (sequence available upon request) was synthesized as a gene block fragment (IDT) and introduced into the NheI/BamHI restriction sites of pAAV-MCS2 (Addgene, plasmid 46954) by Gibson assembly (New England Biolab, Ipswich, MA). The pAAV-nEFCas9 (Addgene, plasmid 87115) was used to express Cas9, and the pAAV-tagBFP U6-gRNA expression vector (gift from Tim Martin at Harvard Medical School) was used to express a gRNA targeting the start codon of *ZNF532* (GCTCAAATTAATGACCATGG). Recombinant AAV2 was packaged in 293T cells using pHelper and pRC2-mi342 plasmids (Clontech Laboratories, Mountain View, CA) as described (7). 3 days after transfection, cells were harvested and AAV2 was isolated using AAVpro Extraction Solution (Clontech). Two million 797 cells were infected with 30ul of each adeno-associated virus (AAV2). Following 1 week of culture post-AAV infection, cells were selected with 7.5ug/ml Blasticidin (Invitrogen). Surviving cells were re-plated and single-cell clones were isolated and expanded, and genotyping PCR and sequencing were performed to check for proper integration of the Blasti-P2A-N-BioTAP cassette in the *ZNF532* locus. Homozygosity of the Blasti-P2A-N-BioTAP cassette insertion was determined by absence of the wildtype 597bp and presence of the larger 1578bp PCR fragment (as in Fig. S3A), using the following PCR primers: (NgenZNF532\_(1S) 5'-TCAGAGTTTCGTAGTTACCGT-3' and NgenZNF532\_(1A) 5'-CCTCAATCTTCTCGTCGTCAT-3').

**Immunofluorescence and quantification of ZNF592/ZMYND8 foci**

797TRex-FLAG-BRD4-NUT-HA cells and U2OSTRex-FLAG-BRD4-NUT cells were treated for 36 hours with 1 µg/ml of tetracycline to induce FLAG-BRD4-NUT-HA or FLAG-BRD4-NUT expression. Immunofluorescence was performed as described (8). Image analysis was performed using ImageJ software. Nuclear boundaries were defined using the “Analyze Particles” function on DAPI stained images. Foci were identified and quantified by the “Find Maxima” function with a noise tolerance of 80 and “Measure” function. Forty cells were counted in each experiment, and statistical significance was determined by two-tailed Student’s t test with significance cutoff of p < 0.01.

**Imaging**

Images of all immunofluorescence and dual-color FISH (*BRD3-NUT* fusion, *NSD3* split-apart, *ZNF532* split-apart) were taken using a SPOT RT3 camera mounted on a Nikon Eclipse E600 microscope using SPOT 5.0 software. Image of Dual-color FISH fusion assay of *BRD4-NUT* was taken in the Center for Advanced Molecular Diagnostics at Brigham and Women’s Hospital.

**References**

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