Supplemental methods

Cell lines. Clinical glioma specimens and normal brain tissue samples were collected in the Department of Pathology and Laboratory Medicine at UCLA (sample 157, 374, 339), MDA (sample 267, 28, 30, 11, 711), and OSU (sample 83, 528, 84, 57, 185A, 1016 and 2313), and processed to the research laboratories after de-identification of the samples, as described previously (1-4). The human fetal neural stem cell sample (16wf) was established at UCLA. NHAs line was purchased from Lonza.

Pearson's correlation analysis. Expression data of kinase-encoding genes and EZH2 was extracted from TCGA database. All the data was converted into Log_2 form and then Pearson r coefficient was calculated by using the following formula:

$$r_{x,y} = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i-\bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i-\bar{y})^2}}$$

In terms of the strength of relationship, the value of the r coefficient varied between 1 and -1. When the value of the r coefficient laid around 1 or -1, then it was considered to be a perfect degree of positive or negative association between the 2 variables. The statistical significance was calculated by using F-test. The Correlation analysis results could be found in Supplemental Table 1.

IP. Cells were lysised with 500 μL Lysis Buffer containing 1% protease inhibitor and phosphatase inhibitor. Picked 25μL magnetic beads and washed with 175 μL TBST. Then mixed all the cell lysis with pre-washed magnetic beads and adjusted the volume to 500 μL with Lysis Buffer and incubated with mixing under 4°C for 1 h to remove unspecific bindings. Collected the supernatant with magnetic stand and then incubated with the antibody and mixed under 4°C overnight. Then moved the antigen sample/antibody mixture to the tube and incubated with mixing under room temperature for 1 h. Collected the magnetic beads with magnetic stand and incubated with low-pH elution buffer at room temperature with mixing for 10 min. Magnetically separated the beads and saved the supernatant containing target protein. Normalized the pH with 15 μL of neutralization buffer for each 100 μL of elution buffer. IP samples were running electrophoresis with 4%-12% Bis-Tris protein gel.

Protein expression, purification and GST pull-down assay. GST-fusion constructs of EZH2 or GST proteins were transformed into *E. coli* BL21 (DE3) pLysS cells (Invitrogen) and grown to an A600 of 0.4. Following the induction with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 6 h at 32°C, the fusion proteins were purified using the standard methods (5). GST-EZH2 protein was from SignalChem (E396-30G-20). The bacterias were harvested and resuspended in STE buffer (150 mM NaCl, 10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 0.1 mg/mL lysozyme), and incubated on ice for 15 min. Sonication was followed by adding 100 μL of 1 M DTT and 1.4 ml of 10% Sarkosyl to 10 ml of cell suspension. The cell lysate was then incubated with 4 mL of 10% Triton X-100 for 30 min at room temperature, and

glutathione-sepharose beads were added and incubated for 4 h at 4°C. A complex of the GST-fusion proteins and the beads were obtained by centrifugation.

For GST pull-down assay, purified NEK2-WT and K37R mutant protein were incubated with GST fusion protein-coupled glutathione beads for 4 h at 4°C. The precipitates collected by centrifugation were resuspended in 2 × Laemmli sample buffer and subjected to SDS-PAGE. Immunoblotting analysis was performed with an anti-NEK2 antibody.

KINOMEscan screening and in vitro cell-free kinase binding assay. CMP3a compound was diluted into the appropriate concentration and performed by using the scanEDGE platform which included 97 kinase candidates. DMSO was used as a negative control to eliminate the unspecific background. The KINOMEscan screening assay and in vitro cell-free kinase binding assay were performed by DiscoverX Corporation. (https://www.discoverx.com/services/drug-discovery-development-services/kinase-profiling/kinomescan)

In vitro kinase phosphorylation assay. Recombinant human NEK2 and EZH2 proteins were purchased from Signalchem. NEK2 kinase activity was measured with a nonradioactive method using anti-phosphorylated serine/theorine antibody. Reactions with EZH2 (100 ng) was performed in a total volume of 20 μL with variable amounts of the recombinant kinase NEK2 (0 ng, 100 ng, 200 ng and 500 ng) with 100 μM ATP in kinase buffer (25 mM Tris, pH 7.5, 10 mM $MgCl_2$, 2 mM EGTA, 1 mM dithiothreitol, and 1 mM sodium orthovandate) at 30°C for 30 min. Reactions were stopped by addition of 10 mM EDTA and stored at -20°C until analysis by western blotting.

Lentivirus production and infection. Plasmid DNA was prepared by using a large-scale plasmid purification kit (QIAGEN). HEK293FT packaging cells were co-transfected with the pLKO.1 vector encoding the shRNA and the helper plasmids for virus production (psPAX2 and pMD2.G), using Trans-IT (Mirus). Before transduction, spheres were dissociated into single cells with Accutase. Cells were seeded in Laminin pre-treated 6 cm petri dishes with 4×10^5 cells per well in a final volume of 5 mL. Lentivirus contained medium was collected at 48 h and 72 h. Then Lenti-X Concentrator was added into the medium and mixed gently at 4°C for 72 h. Produced lentivirus was collected by ultracentrifugation of the HEK293T supernatant at 25,000 rpm. To assess transduction efficiency, cells on each plate were infected with lentivirus expressing green fluorescent protein (GFP) as a control. Transduction was deemed efficient if > 70% cells were GFP-expressing. The sequences of all the plasmid were listed in Supplemental Table 2. Lentivirus infection was performed as described previously (2, 4).

RNA isolation and qRT-PCR. RNA was isolated by using RNeasy mini kit (QIAGEN, 74104) according to the manufacturer's instructions. RNA concentration was determined using a Nanodrop 2000 (Thermo Fisher Scientific). cDNA was synthesized by using iScript reverse transcription supermix for RT-PCR (Bio-rad, 170-8841) according to the manufacturer's protocol. The reverse-transcribed cDNA

was analyzed by qRT-PCR, and GAPDH or 18S was used as an internal control. Each qRT-PCR included a 10 μL reaction mixture per well containing 2.5 μL cDNA, 0.5 μL forward primer (0.5 μM), 0.5 μL reverse primer (0.5 μM), 1.5 μL of DNase/ RNase-free distilled water, and 5 μL SYBR green reagent (QIAGEN). The following cycles were performed during DNA amplification: 94°C for 2 min, 40 cycles of 94°C (30 s), 60°C (30 s), and 72°C (40 s). All the primer sequences were listed in Supplemental Table 3.

IHC. IHC were performed as previously described (2-4). After tissue embedding and sectioning were performed, the sections were incubated with the indicated primary antibodies overnight at 4°C, followed by incubation with an HRP-conjugated secondary antibody for 1 h at room temperature. Signals were detected by using DAB substrate kit (Vector). Nuclei were stained with hematoxylin. Samples incubated without primary antibodies were used as negative controls.

ICC. ICC was performed as previously described (4). Neurospheres were dissociated into single cells and seeded onto coverslips coated with 0.5% Laminin (1×10^4 per well). After 24 h, the cells were fixed with 4% (wt/vol) PFA, blocked with 1% BSA containing 0.3% Triton-X, and treated with primary antibody at 4°C overnight. The cells were then incubated with Alexa Flour 488-conjugated secondary antibody or Alexa Flour 555-conjugated secondary antibody for 1 h at room temperature. The coverslips were mounted with mounting medium containing DAPI (Vecter). Identical filters, objectives, and acquisition parameters were used for each experiment.

Fluorescence quantification. S Fluorescence images were captured by using a confocal microscope (Olympus, Fluoview 1000) and were edited by using Adobe Photoshop CS6.0. Clone area and brain lobe size were measured (in pixels) for quantification by using the histogram function of Adobe Photoshop CS6.0.

PCR-mediated kinase mutagenesis. The PCR-mediated mutagenesis method was used to obtain the NEK2 mutant K37R (6). The primer pairs P1/P4 and P3/P2 were used to amplify two NEK2 fragments. The first PCR (PCR1) mixture was firstly incubated at 95°C for 5 min, followed by 95°C denaturing for 30 second, 60°C annealing for 30 second, and 72°C extension for 1 min. After 18 cycles, the PCR1 products were used as template in a second PCR (PCR2), primed by oligonucleotides P1 and P2 to carry out another 18 cycles of PCR with the same PCR conditions used in PCR1. Resulting PCR2 product was digested with BstBI/BamHI and cloned into corresponding sites of pCDH-EF1-MCS-IRES-Puro plasmid (System Biosciences). Obtained plasmid was designated as pCDH-mutNEK2. The absence of unwanted mutations in the inserts and vector-insert boundaries was verified by sequencing. The sequences of 3'UTR-shRNAs and primers used in this experiment were provided in Supplemental Table 2 and Supplemental Table 4, respectively.

General compound synthetic experiments. All solvents were reagent grade or HPLC grade and all starting materials were obtained from commercial sources and used without further purification. Purity of final compounds were assessed using a Shimadzu ultra-high throughput LC/MS system (SIL-20A, LC-20AD, LC-MS 2020,

Phenomenex® Onyx Monolithic C-18 Column) at variable wavelengths of 254 nM and 214 nM (Shimadzu PDA Detector, SPD-MN20A) and was > 95%, unless otherwise noted. The HPLC mobile phase consisted of a water-acetonitrile gradient buffered with 0.1% formic acid. 1H NMR spectra was recorded at 400 MHz and 13C spectra was recorded at 100 MHz, both completed on a Varian 400 MHz instrument (Model #4001S41ASP). Compound activity was determined with the EZ Reader II plate reader (PerkinElmer®, Walthman, USA). All compounds were purified using silicagel (0.035-0.070 mm, 60 Å) flash chromatography, unless otherwise noted. Microwave assisted reactions were completed in sealed vessels using a Biotage Initiator microwave synthesizer.

Synthesis of Compound 2 (CMP2). 4-chloropyridin-2-amine (7.425 g, 57.8 mmol) and 2-chloroacetaldehyde 50% w/w (8.80 mL, 69.3 mmol) were heated in 1-butanol (23.10 mL, 2.5 M) at 130°C for 12 h. The solvent was evaporated from the reaction and the title compound was purified via DCM/MeOH and isolated as an off-white solid, 1a (6.95 g, 79%). 1a (35.6 g, 233 mmol) was dissolved in acetic acid (292 mL). The reaction was stirred under ice and bromine (13.85 mL, 268 mmol) was added dropwise. The resulting precipitate was filtered off and neutralized to generate the free-base, brominated compound, 1b (35 g, 64.8%). 4-chloro-2-hydroxybenzoic acid (30 g, 174 mmol) was added to methanol (60 mL) and sulfuric acid (130 mmol, 6.94 mL). The reaction was stirred at reflux for 120 h. The reaction was neutralized with the addition of solid NaHCO3, diluted with water, and extracted with 6:1 ether/DCM. The organic was dried with $MgSO_4$ and condensed to generate the title compound, 1c (31.4 g, 97%). 1-(2-(trifluoromethyl) phenyl) ethanone (19.92 mL, 133 mmol) was dissolved in THF (400 mL). Following, sodium borohydride (7.37 g, 199 mmol) was added and the reaction was heated to 55°C for 48 h. The reaction was quenched with the addition of dilute HCl under ice. The organic was extracted with ethyl acetate, dried with $MgSO_4$, and isolated as a viscous oil, 1d (24 g, 95%). 1c (5 g, 26.8 mmol) and 1d (5.61 g, 29.5 mmol) were dissolved in DCM (80 mL) and cooled in an ice bath. Following, triphenylphosphine (10.18 g, 38.9 mmol) was added and DEAD (6.97 g, 38.9 mmol) was added dropwise. After the addition, the reaction was removed from the ice bath and stirred at room temperature for 12 h. The crude reaction was condensed and adsorbed onto silica and purified via flash chromatography using Hexanes/EtOAC. The title compound was isolated as white solid/crystals, 1e (6.12 g, 63.7%). 1e (6.122 g, 17.07 mmol) was dissolved in dioxane (100 mL) and degassed with argon. Following 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (6.50 g, 25.6 mmol), potassium acetate (5.02 g, 51.2 mmol), Pd2(Dba)3 (0.156 g, 0.171 mmol), and PCy3 (0.143 g, 0.512 mmol) was added to the reaction. The reaction was placed under an argon atmosphere and heated to 100 °C for 12 h. The crude reaction was added to water, extracted with ether, and washed with water for 5 times. The ether extract was collected, dried, and absorbed onto silica. The reaction was purified via flash chromatography using hexanes/EtOAc to generate the title compound as a yellow oil, 1f (7.47 g, 97%). 1b (0.615 g, 2.66 mmol) and 1f (1.196 g, 2.66 mmol) were added to 4:1 DMF/Water (26 mL). The reaction was degassed with argon and bis(triphenylphosphine)palladium(II) dichloride (0.093 g, 0.133 mmol) and sodium

carbonate (1.395 g, 13.28 mmol) was added. The reaction was placed under positive argon pressure, sealed, and heated to 70°C for 12 h. The reaction solvent was evaporated, diluted with water, and extracted with 2:1 EtOAc/ether. The organic was collected, dried, and absorbed onto silica and purified with flash chromatography using a hexanes/EtOAc gradient to isolate the title compound, 1g (0.746 g, 59.2%). 1g (1.515 g, 2.424 mmol) was dissolved in dioxane (24.24 mL) and degassed with argon. Following 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (0.923 g, 3.64 mmol), potassium acetate (0.714 g, 7.27 mmol), Pd2(Dba)3 (0.067g, 0.073 mmol), and PCy3 (0.061 g, 0.218 mmol) was added to the reaction. The reaction was placed under an argon atmosphere and heated to 85°C for 12 h. Afterwards, the reaction solvent was evaporated and the crude reaction was dissolved in EtAOc. The organic layer was washed with 1:1 brine/water for 3 times, collected, and dried with $MgSO_4$. The organic layer was condensed to generate 1h, which was used without further purification (1.042 g, 89%). 1h (0.100 g, 0.270 mmol) and 2-(4-iodo-1H-pyrazol-1-yl)-N,N-dimethylethanamine (0.071 mg, 0.268 mmol) were added to a 5 mL microwave vial. 4:1 DMF/Water (3 mL) was added to the vial and the reaction was degassed with argon. Following, sodium carbonate (0.065 g, 0.620 mmol), Pd2(Dba)3 (5.67 mg, 6.20 μmol), and PCy3 (5.20 mg, 0.019 mmol) were added to the vial. The reaction was sealed and heated under microwave irridation for 30 min at 120°C. The solvent was evaporated and the crude reaction was transferred to a silica loading column. The reaction was purified via flash chromatography using a DCM/MeOH gradient to isolate the desired compound 1i (0.031 mg, 26.2%). MeOH/NH3 (7.0 M, 5 mL) was used to dissolve 1i (0.031 mg, 0.054 mmol). The reaction was sealed and heated to 100°C for 96 h. The reaction was purified via flash chromatography using a DCM/MeOH gradient to isolate the desired compound 2 (0.016 mg, 53.5%).

Synthesis of Compound 3 (CMP3), Compound 3a (CMP3a) and Compound (CMP3b). Compounds 3, 3a, and 3b were synthesized in a similar fashion to that of CMP2 (Supplemental Figure 7A). Methyl 2-mercaptoacetate (4.31 mL, 47.1 mmol) was added to MeOH (94 mL) and cooled in an ice bath. Following, sodium hydride (3.39 g, 85 mmol) was slowly added and the reaction was stirred for 1 h at room temperature. The reaction was cooled to -10°C and ethyl propiolate (5.01 mL, 48.0 mmol) was slowly added. The reaction was stirred at 0°C for 1 h, room temperature for 1 h, and heated to 45°C for 3 h. Following, the reaction was heated for 12 h at 35°C. All reaction solvent was evaporated and the reaction was redissolved in water, neutralized to pH 7, and extracted 10 times with 3:1 chloroform/IPA. The organic extracts were combined, evaporated, and dissolved in EtOAc. The EtOAc/reaction mixture was filtered through a pad of silica and washed with EtOAc. The organic was collected and condensed to generate the title compound, 3c (3.66 g, 49.1%). 3d was synthesized according to the synthesis of 1e (89%). CMP3d (0.150 mg, 0.454 mmol) was dissolved in THF (5 mL) and cooled to -20°C. Following, trimethyl borate (0.103 mL, 0.908 mmol) was added. LDA (0.908 mL, 1.362 mmol) was added to the reaction dropwise over the course of 10 min. After about 30 min, all starting material was consumed and the boronic acid was generated. In a separate reaction vessel, 1b (0.454

mmol) was dissolved in 7:3 THF/Water and degassed with argon. Sodium carbonate (0.193 g, 1.816 mmol) and Pd2(dppf)Cl2 (8.30 mg, 0.011 mmol) was added and the reaction was heated to 65°C. The boronic acid was added dropwise over the course of 10 min and the reaction was heated a reflux for 2 h. The reaction was absorbed onto silica and purified via flash chromatography using a DCM/MeOH gradient. The desired compound was isolated and dried, 3e (0.067 mg, 30.7%).

A degassed (N2 bubbling) solution of the compound of intermediate 3e（721 mg, 1.5 mmol） in 1, 4-dioxane (15 mL) was added with bis(pinacolato)diboron (571 mg, 2.25 mmol), KOAc (736 mg, 7.5 mmol) Pd2(Dba)3 (41.2 mg, 0.045 mmol), and PCy3 (37.9 mg, 0.135 mmol). Then mixture was heated at 100°C for 2 h. The reaction mixture was filtered over a pad of celite and concentrated under vacuum to obtain the crude product 3f which was used in the next step without further purification.

A solution of crude 3f (735.4 mg, 1.5 mmol) and 3g（596.4 mg, 2.25 mmol）in DMF (12 mL) and water (3 mL) was added with Na_2CO_3 (795 mg, 7.5 mmol), Pd2(Dba)3 (41.2 mg, 0.045 mmol), and PCy3 (37.9 mg, 0.135 mmol). Then the reaction mixture was stirred at 120°C for 30 min under microwave irradiation. The reaction mixture was filtered over a pad of celite and concentrated under vacuum. The residue was purified with column chromatography (2-4% MeOH /DCM) to afford the product 3h (233 mg, 40% over two steps) as a yellow solid.

A solution of 3h (233 mg, 0.4 mmol) and ammonia solution (7M) in methanol (4 mL) was added with ammonia solution (1.5 mL, NH3 25-28%), then the reaction mixture was stirred at 80°C for 5 d. The solvent was removed under reduced pressure. The residue was purified with column chromatography (2-10% MeOH /DCM) to afford the product (439.6, 68%) as a yellow solid.

Preparative chiral HPLC separation of racemic 3i resulted in pure enantiomers 3a and 3b. (Chiralpak AD, EtOH/ACN/DEA=90/10/0.1 (V/V/V), 25ml/min) (Supplemental Figure 7, B and D).

CMP3a: 1H NMR (400 MHz, CDCl3) δ 8.13 (d, J = 7.1 Hz, 1H), 7.77 (d, J = 4.1 Hz, 2H), 7.61 (d, J = 9.8, 7.0 Hz, 4H), 7.51 (t, J = 7.3 Hz, 1H), 7.34 (t, J = 7.3 Hz, 1H), 7.16 (s, 1H), 7.03 (s, 1H), 6.94 – 6.83 (m, 1H), 6.66 (s, 1H), 5.79 (d, J = 5.8 Hz, 1H), 4.31 – 3.96 (m, 2H), 2.89 – 2.51 (m, 2H), 2.21 (s, 6H), 1.70 (d, J = 6.0 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 163.81, 154.64, 147.62, 140.54, 136.80, 134.63, 133.80, 133.30, 130.29, 128.46, 127.29, 126.50, 126.46 (q, J = 30.2 Hz), 126.11 (q, J = 5.7Hz), 124.38 (q, J = 272.1 Hz), 123.82, 120.43, 119.07, 115.41, 112.97, 112.43, 112.30, 76.26, 58.98, 50.62, 45.56), 24.89. 19F NMR (376 MHz, CDCl3) δ -58.31 (s). HRMS (ESI) m/z calcd for C28H28N6O2SF3 (M+H) + 569.1947, found 569.1922.

Computational modeling. Computational modeling studies were completed using AutoDock Vina, AutoDock Tools, and Discovery Studio 3.5. Using AutoDock Tools, kinase crystal structures were prepared as follows: 1) All hydrogens were added as 'Polar Only', 2) A grid box for the ATP binding site was created. Compounds to be computationally modeled were assigned appropriate rotatable bonds using AutoDock Tools. To computational model the compounds, AutoDock Vina was employed.

AutoDock Vina provides docking scores in terms of ΔG values. After the modeling study, kinase targets with high affinity ΔG values were visualized and analyzed with Discovery Studio 3.5.

NEK2 biochemical inhibition assay. Kinase activity was measured in a microfluidics assay that monitors the separation of a phosphorylated product from substrate. The assay was run using a 12-sipper chip on a Caliper EZ Reader II (PerkinElmer®, Walthman, USA) with separation buffer (100 mM HEPES, 10 mM EDTA, 0.015% Brij-35, 0.1% CR-3 [PerkinElmer®, Walthman, USA]). In 96-well polypropylene plates (Greiner, Frickenhausen, Germany) compound stocks (20 mM in DMSO) were diluted into kinase buffer (50 mM HEPES, 0.075% Brij-35, 0.1 % Tween 20, 2 mM DTT, 10 mM MgCl2, and 0.02% NaN3) in 12-point ½log dilutions (2 mM–6.32 nM). Afterwards, 1 µL was transferred into a 384-well polypropylene assay plate (Greiner, Frickenhausen, Germany). The NEK2 enzyme (Invitrogen™, Grand Island, USA) was diluted in kinase buffer to a concentration of 2 nM and 5 µL of the enzyme mixture was transferred to the assay plate. The inhibitors/NEK2 enzyme was incubated for 60 min with minor shaking. A substrate mix was prepared containing ATP (Ambresco®, Solon, USA) and 5FAM tagged NEK2 peptide (PerkinElmer®, Walthman, USA) dissolved in kinase buffer, and 5 µL of the substrate mix was added into the assay plate. Running concentrations were as follows: ATP (190 µM), peptide (1.5 µM), compound 12-point ½log dilutions (0.2 mM-0.632 nM). For positive control, no inhibitor was added. For negative control, no enzyme was added. For running control, quizartinib was utilized. The plate was run until 10-20% conversion based on the positive control wells. The following separation conditions were utilized: upstream voltage -500V, downstream voltage, -1900V, chip pressure -0.8. Percent inhibition was measured for each well comparing starting peptide to phosphorylated product peaks relative to the baseline. Dose response curves, spanning the IC50 dose, were generated in GraphPad Prisim 6 and fit to an exponential one-phase decay line and IC50 values were obtained from the half-life value of the curve. IC50 values were generated in duplicate and error was calculated from the standard deviation between values.

Supplemental references

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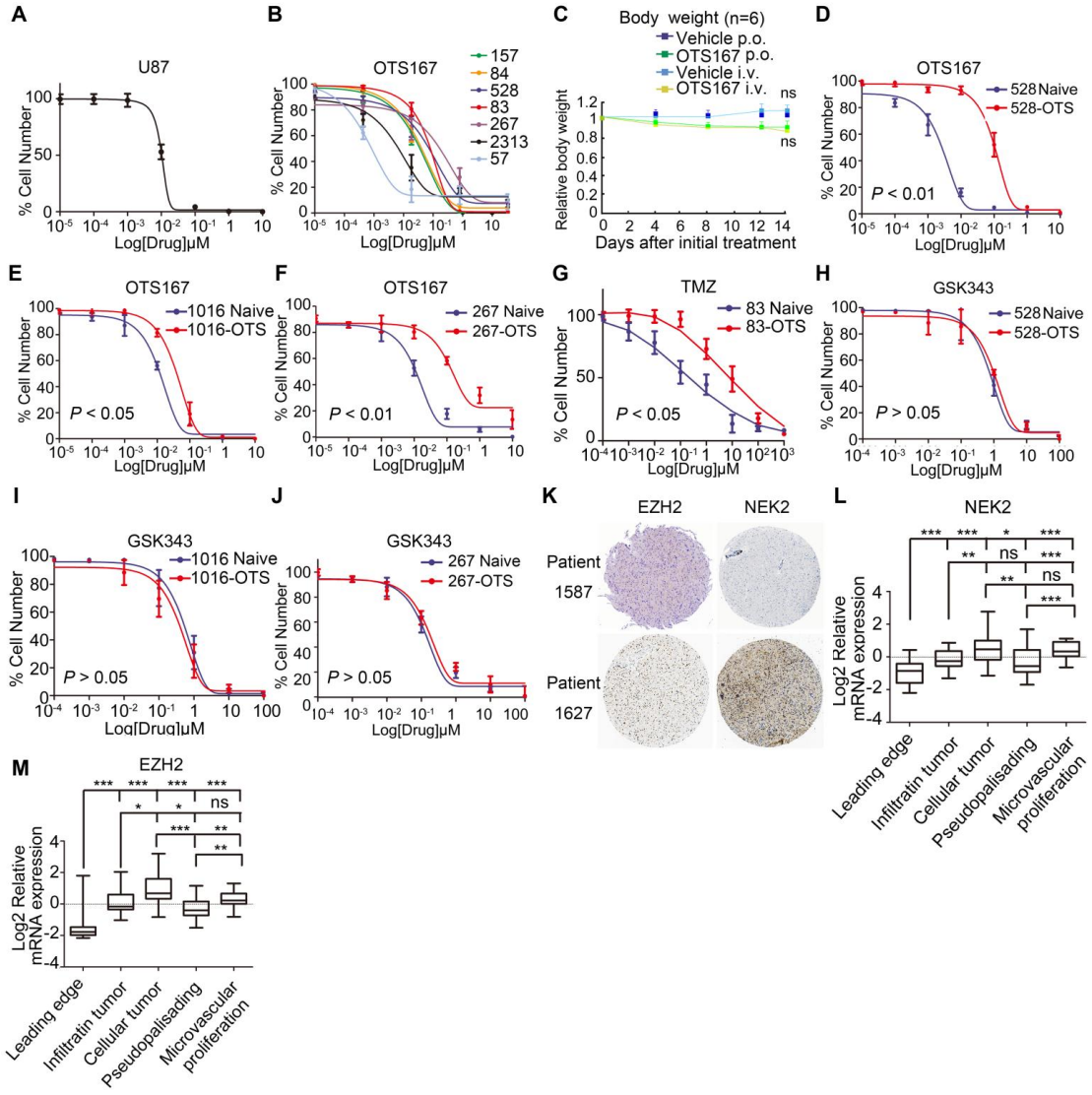
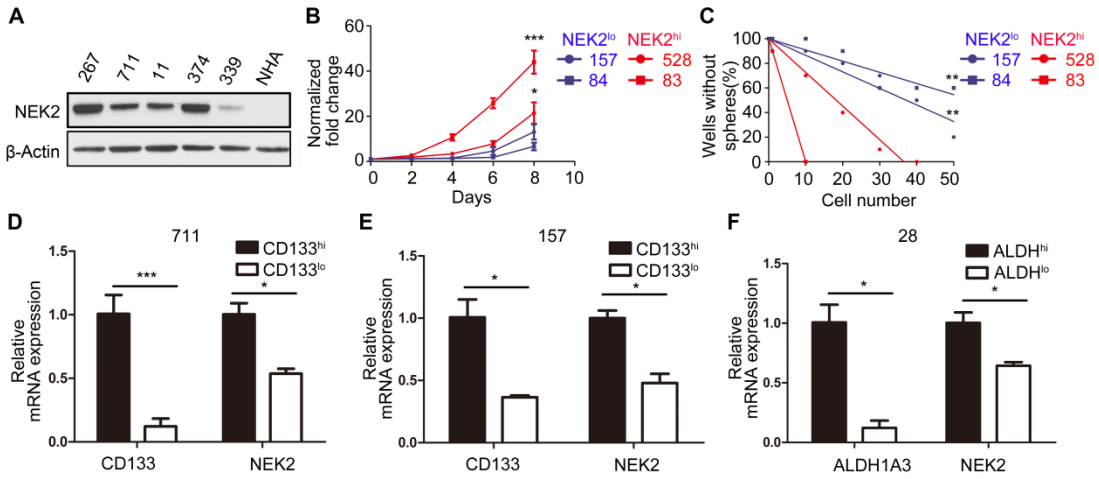
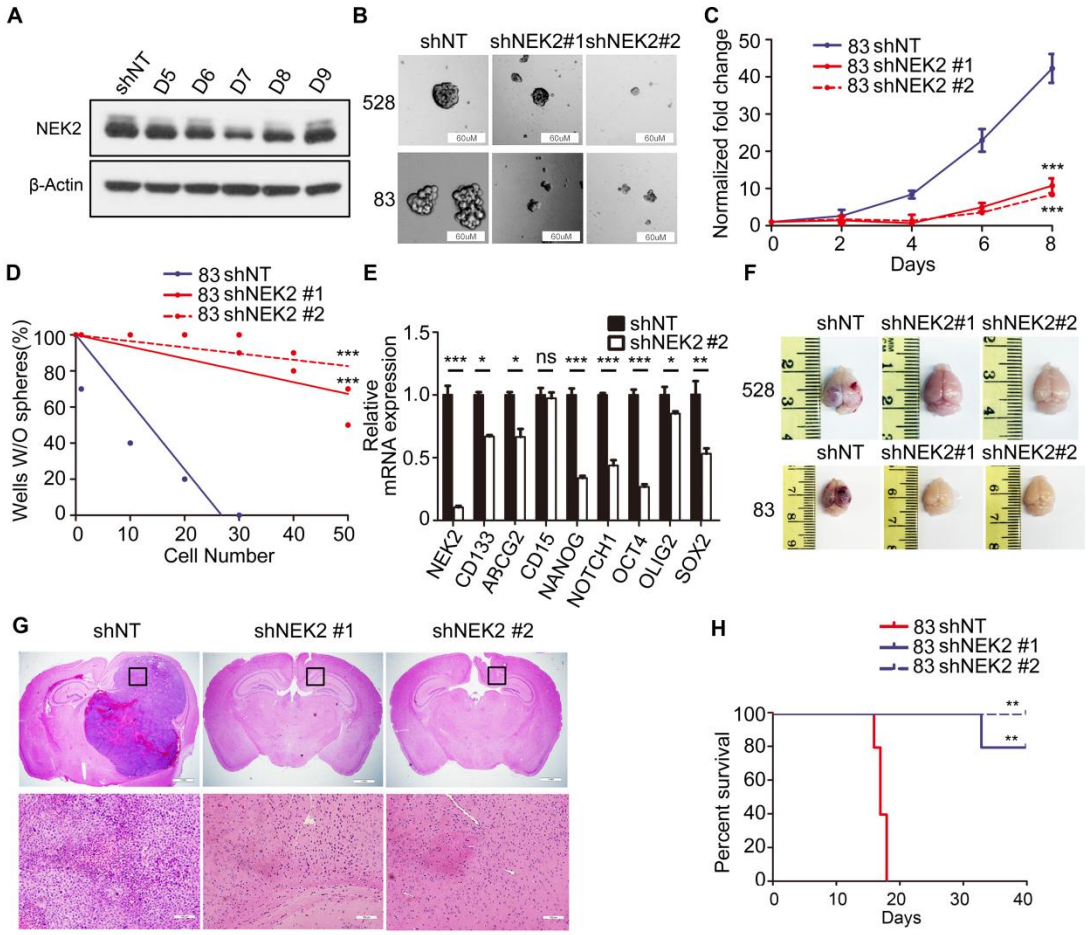
Supplemental figures



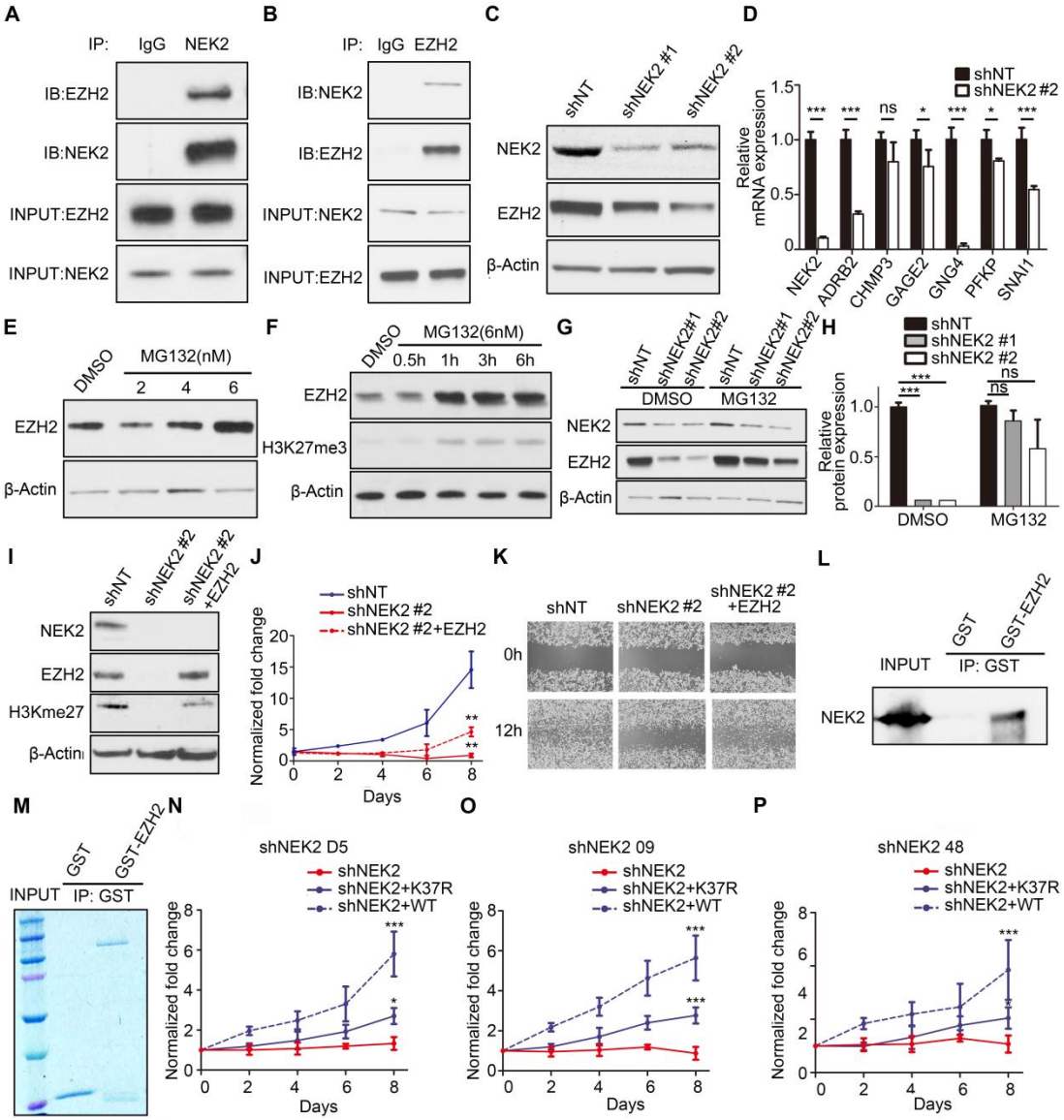
Supplemental Figure 1. OTS167-treated glioma spheres gain therapeutic resistance through EZH2-dependent mechanisms. (A) In vitro cell viability assay indicated that OTS167 inhibited U87 cell growth in a dose-dependent manner (*n* = 6). **(B)** In vitro cell viability assay for OTS167 in patient-derived glioma sphere cells (*n* = 6). **(C)** In vivo results showed no noticeable body weight changes due to OTS167 treatments in the U87 sub-Q xenograft mouse model. *P* > 0.05 (ns), *n* = 6, by 1-way ANOVA. **(D-F)** In vitro cell viability assays for OTS167 in naïve glioma spheres and OTS167-resistant glioma spheres (**D:** 528, **E:** 1016, **F:** 267, *n* = 6, by 1-way ANOVA). **(G)** In vitro cell viability assay for TMZ in 83 naïve cells and OTS-resistant cells. *P* < 0.05, *n* = 6, by 1-way ANOVA. **(H-J)** In vitro cell viability assays for GSK343 in naïve glioma spheres and OTS-resistant glioma spheres (**H:** 528, **I:** 1016, **J:** 267, *n* = 6, by 1-way ANOVA). **(K)** Representative IHC images of EZH2 (left panels) and NEK2 (right panels) from the Human Protein Atlas database. **(L and M)** Relative mRNA expression for NEK2 **(L)** and EZH2 **(M)** in different tumor regions from the Ivy GBM database. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *P* > 0.05 (ns), by 1-way ANOVA followed by Dennett's post-hoc test.



Supplemental Figure 2. NEK2 is functionally required for in vitro cell proliferation and clonogenicity ability in GSCs. (A) Western blotting for NEK2 expression in the indicated glioma spheres. **(B)** In vitro growth assay showed cell proliferation was enhanced in the NEK2^{hi} group glioma spheres (83 and 528) compared with the NEK2^{lo} group glioma spheres (84 and 157). **P* < 0.05, ****P* < 0.001, *n* = 6, by 1-way ANOVA. **(C)** In vitro clonogenicity assay indicated that clonogenicity was increased in the NEK2^{hi} group glioma spheres (83 and 528) compared with the NEK2^{lo} group glioma spheres (84 and 157). ***P* < 0.01, *n* = 10, by ELDA. **(D and E)** qRT-PCR for NEK2 mRNA expression in CD133^{hi} and CD133^{lo} cells derived from PN 711 glioma spheres **(D)** and PN 157 glioma spheres **(E)**. **P* < 0.05, ****P* < 0.001, *n* = 3, by 2-tailed *t*-test. **(F)** qRT-PCR of NEK2 mRNA expression in ALDH^{hi} and ALDH^{lo} cells derived from MES 28 glioma spheres. **P* < 0.05, *n* = 3, by 2-tailed *t*-test.

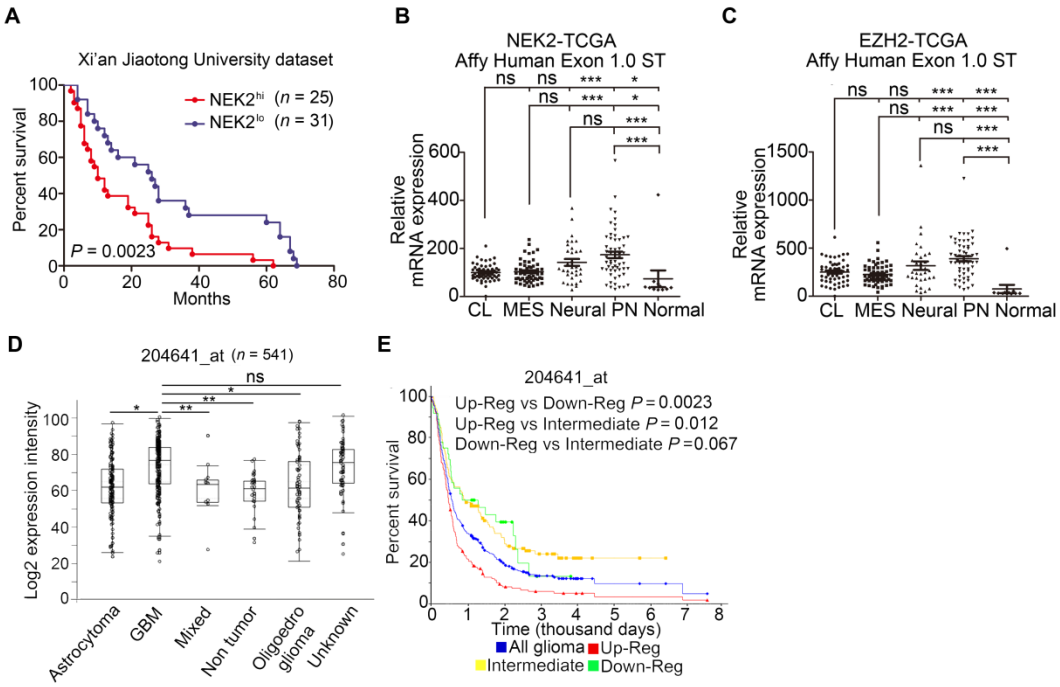


Supplemental Figure 3. The effects of NEK2 silencing on cell proliferation and self-renewal activity. (A) Western blotting for NEK2 in 83 glioma spheres transduced with shRNA against NEK2 (D5, D6, D7, D8 and D9) or shNT. **(B)** Images of 528 (upper panels) or 83 glioma spheres (lower panels) transduced with shNEK2 no. 1, no. 2 or shNT. **(C)** In vitro growth assay showing that shNEK2 inhibited cell proliferation of 83 glioma spheres. ***$P < 0.001$, $n = 6$, by 1-way ANOVA. **(D)** In vitro clonogenicity assay indicated that NEK2 silencing decreased the clonogenicity of 83 glioma spheres. ***$P < 0.001$, $n = 10$, by ELDA. **(E)** qRT-PCR for stemness markers expression in 711 glioma spheres transduced with shNEK2 no. 2 or shNT. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $P > 0.05$ (ns), $n = 3$, by 2-tailed t-test. **(F)** Representative images of mouse brains after the intracranial transplantation of 528 glioma spheres (upper panels) or 83 glioma spheres (lower panesl) transduced with shNEK2 no. 1, no. 2 or shNT. **(G)** Representative images of an H&E-stained mouse brain section after the intracranial transplantation of 83 glioma spheres transduced with shNEK2 no. 1, no. 2 or shNT. Scale bars: 1 mm (top) and 100 μm (bottom) **(H)** Kaplan-Meier analysis of nude mice harboring intracranial tumors derived from 83 glioma spheres transduced with a shNT control ($n = 6$), shNEK2 no. 1 ($n = 5$) or shNEK2 no. 2 ($n = 5$). **$P = 0.0026$, for shNT versus shNEK2 no. 1; **$P = 0.0023$, for shNT versus shNEK2 no. 2; both by log-rank test.

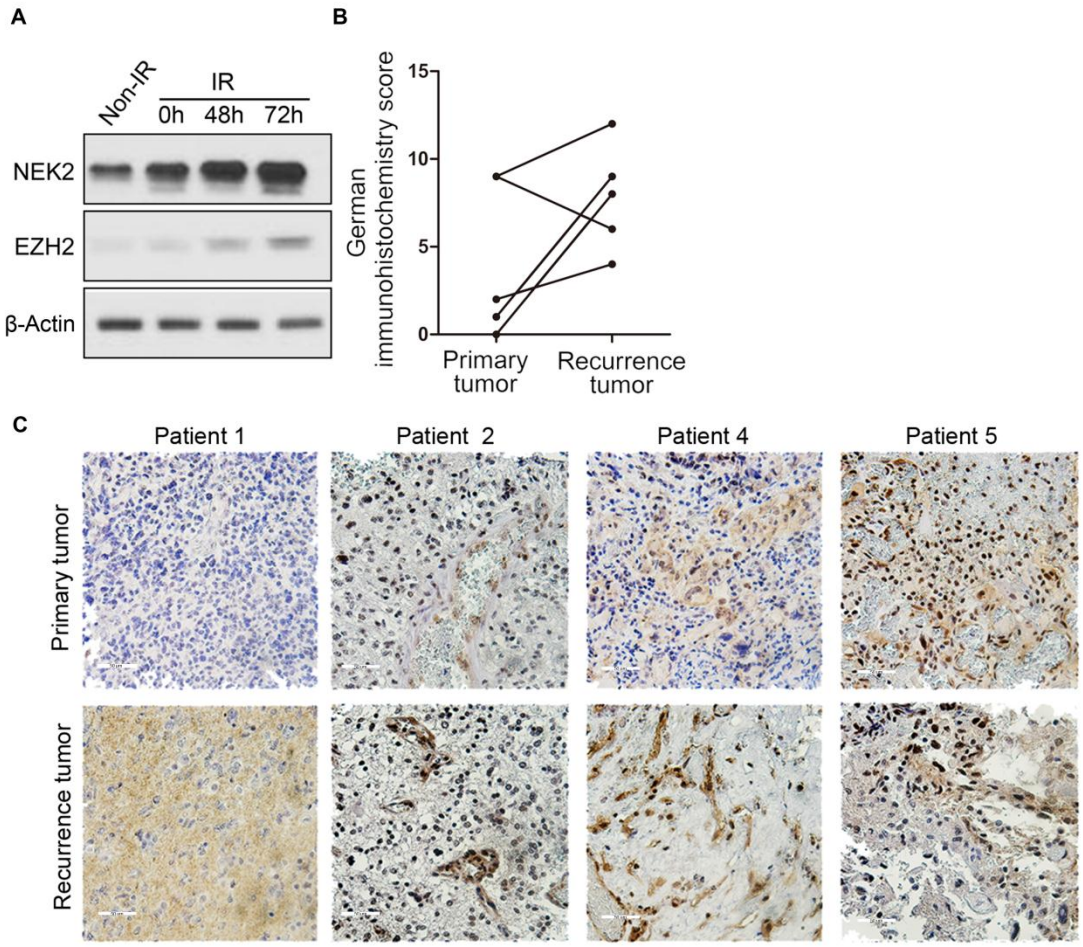


Supplemental Figure 4. NEK2 protects EZH2 from degradation by forming a complex. (A and B) Immunoblotting for IP using an anti-NEK2 antibody **(A)** or an anti-EZH2 antibody (**B**) in 83 glioma spheres. IgG served as a control. **(C)** Western blotting for NEK2 and EZH2 in 83 glioma spheres transduced with shNEK2 or shNT. **(D)** qRT-PCR for EZH2 downstream targets expression in 528 glioma spheres transduced with shNEK2 no. 2 or shNT. **P* < 0.05, ****P* < 0.001, *P* > 0.05 (ns), by 2-tailed *t*-test. **(E)** Western blotting for EZH2 in 528 glioma spheres treated with different doses of MG132 for 6 h. **(F)** Western blotting for EZH2 and H3K27me3 in 528 glioma spheres treated with MG132 (6 nM). **(G and H)** Western blotting for EZH2 in 83 glioma spheres transduced with shNEK2 no. 1, no. 2 or shNT after MG132 or DMSO treatment **(G)**. EZH2 protein was degraded faster after NEK2 silencing, and this effect could be reversed by MG132 **(H)**. ****P* < 0.001, *P* > 0.05 (ns), *n* = 3, by 1-way ANOVA followed by Dennett's post-hoc test. **(I)** Western blotting for NEK2, EZH2 and H3K27me3 in 267 glioma spheres transduced with shNT or shNEK2 no. 2 followed by transfection of an EZH2 overexpression vector. **(J)** In vitro cell growth assay indicated that NEK2 silencing decreased the cell growth of 267 glioma spheres, which could be partially rescued by EZH2 exogenous expression. ***P* < 0.01, *n* = 6, by 1-way ANOVA. **(K)** Wound-healing

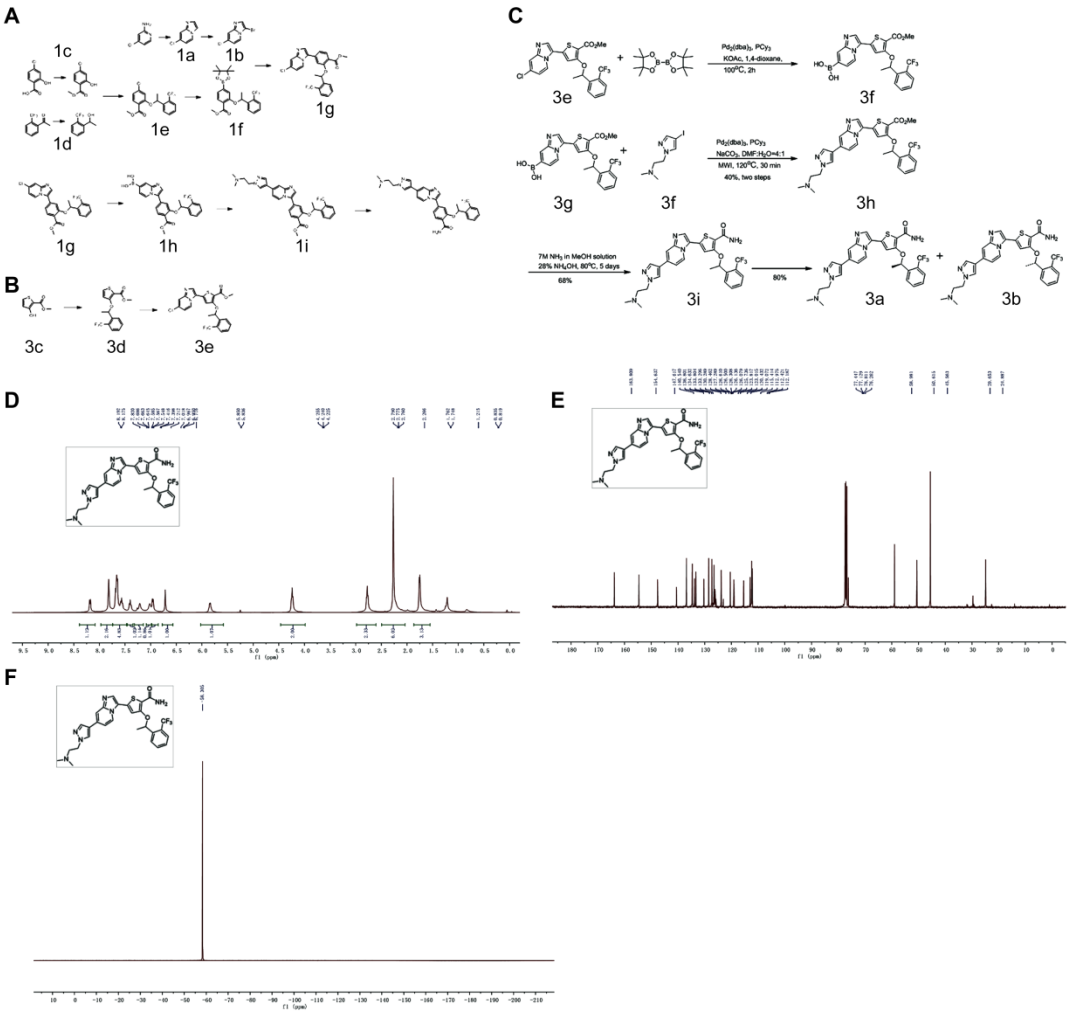
assay showed that the motility of 267 cells was decreased by NEK2 silencing, which could be rescued by the exogenous expression of EZH2. **(L and M)** The GBM-022 cell lysate and the GST fusion protein were incubated together with glutathione-agarose beads. The complexes recovered from the beads were resolved on SDS-PAGE and analyzed by western blotting **(L)**. Coomassie blue staining of GST and GST-EZH2 showed that similar amounts of each protein were used **(M)**. **(N-P)** In vitro cell growth assays indicated that NEK2 silencing via a 3' UTR-shNEK2 lentivirus decreased the cell proliferation of 267 glioma spheres and this effects could be partially rescued by NEK2-WT but not by K37R mutation overexpression. (**N:** shNEK2 D5, **O:** shNEK2 09, **P:** shNEK2 48, $*P < 0.05$, $***P < 0.001$, $n = 6$, by 1-way ANOVA).



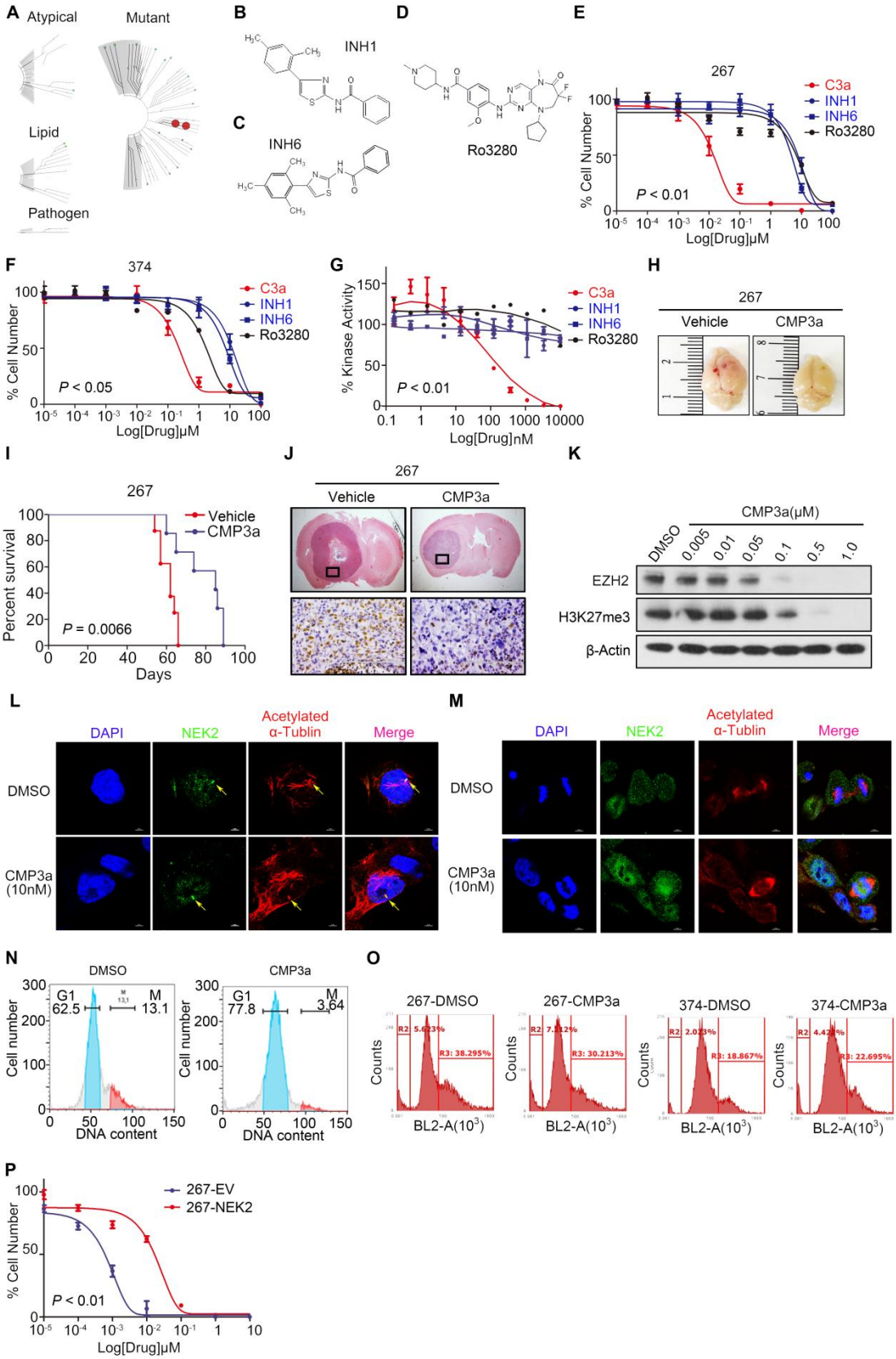
Supplemental Figure 5. NEK2 expression is related to poor prognosis in glioma patients. (A) Kaplan-Meier analysis was conducted to evaluate the correlation between NEK2 expression and the survival of 56 glioma patients from the Department of Neurosurgery, the First Affiliated Hospital of Xi'an Jiaotong University. $P = 0.0023$, by log-rank test. **(B and C)** An analysis from the TCGA database indicated that NEK2 **(B)** and EZH2 **(C)** were highly enriched in 4 subtypes of GBM tumors compared with normal brain tissue. $^{*}P < 0.05$, $^{***}P < 0.001$, $P > 0.05$ (ns), by 1-way ANOVA followed by Dennett's post-hoc test. **(D)** An analysis of the Rembrandt database indicated that NEK2 expression was elevated in GBM samples. $^{*}P < 0.05$, $^{**}P < 0.01$, $P > 0.05$ (ns), by 1-way ANOVA followed by Dennett's post-hoc test, probe set: 204641_s_at. **(E)** An analysis of the Rembrandt data indicated an inverted correlation between NEK2 expression and the post-surgical survival in glioma patients. $n = 120$ for NEK2-upregulated group, $n = 321$ for NEK2-intermediate group, $n = 100$ for NEK2-downregulated group (probe set: 204641_s_at). P values were determined by log-rank test.



Supplemental Figure 6. NEK2 expression is elevated in recurrent GBM. (A) Western blotting for NEK2 and EZH2 expression in 83 glioma spheres after radiation (12 Gy). **(B)** An analysis of NEK2 expression in primary untreated and post-radiation recurrent tumor sections from 5 matched GBM cases with the GIS. **(C)** Representative IHC image of NEK2-stained primary untreated and post-radiation recurrent tumor sections from matched GBM cases. Scale bars: 50 μm.

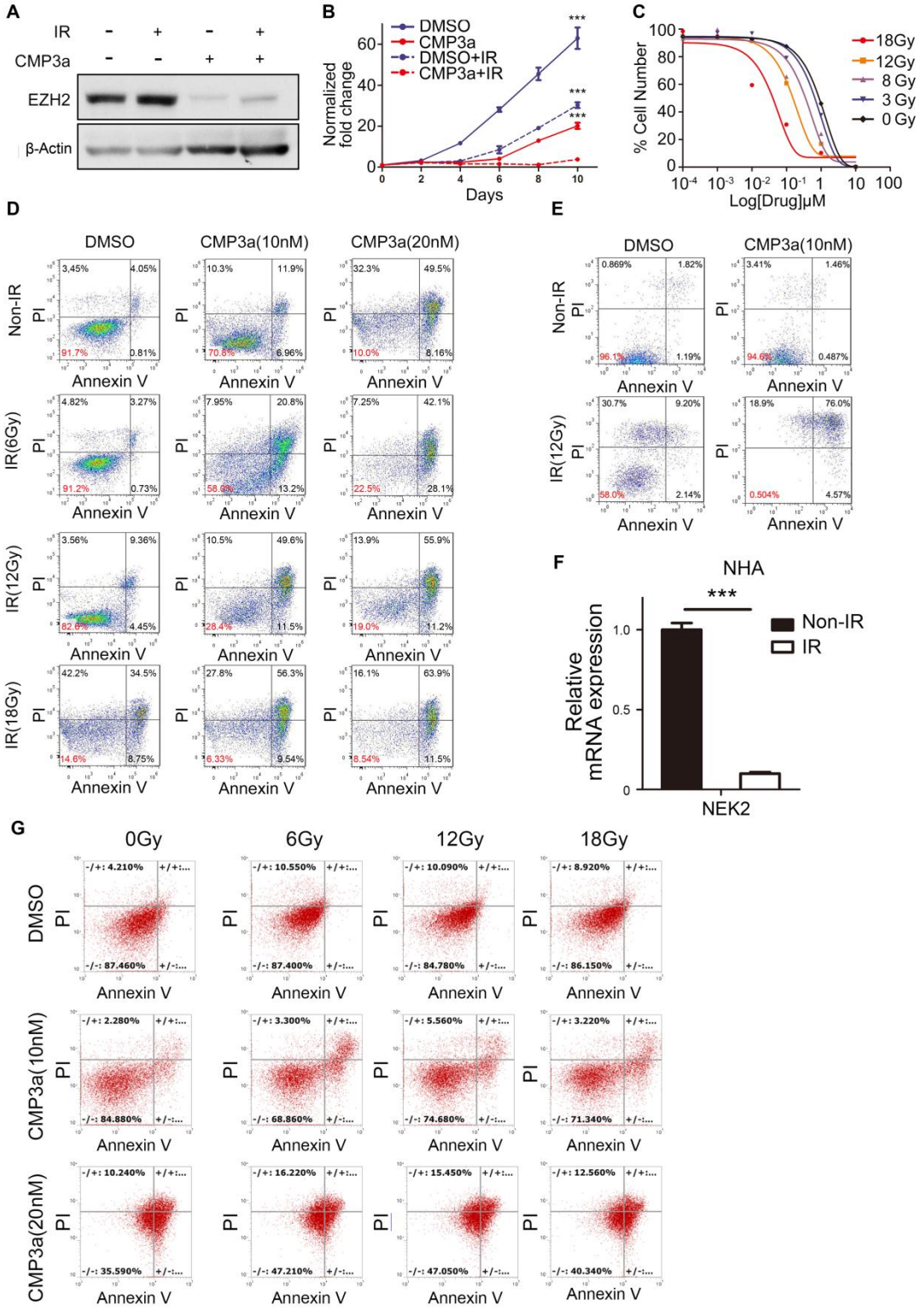


Supplemental Figure 7. Synthesis of the NEK2 inhibitor CMP3a. (A-C) General procedures for synthesis of Compound 2 **(A)**, Compound 3e **(B)** and Compound 3a **(C)**. **(D-F)** Purification of CMP3a by using 1H NMR spectra.



Supplemental Figure 8. Increased radio-sensitivity and decreased tumor growth in GBM induced by CMP3a treatment mainly depends on EZH2 inhibition. (A) CMP3a was screened at 15 nM against atypical, lipid, pathogen and mutant kinases using KINOMEscan. **(B-D)** The

chemical structure of INH1 **(B)**, INH6 **(C)** and Ro3280 **(D)**. **(E and F)** In vitro cell viability assays to compare CMP3a and the other 3 NEK2 inhibitors (INH1, INH6, and Ro3280) in 267 **(E)** and 374 glioma spheres **(F)**. *n* = 6, by 1-way ANOVA. **(G)** In vitro cell-free kinase binding assay indicated that CMP3a inhibited NEK2 kinase activity more efficiently than the other 3 inhibitors. $P < 0.01$, *n* = 3, by 1-way ANOVA. **(H)** Representative images of mouse brains transplanted with 267 glioma spheres followed by a continuous 10-day CMP3a or vehicle treatment via tail vein injection. **(I)** Kaplan-Meier analysis for mice survival after intracranial transplantation of 267 glioma spheres and followed by CMP3a (*n* = 5) or vehicle treatment (*n* = 6) mentioned above. $P = 0.0066$, by log-rank test. **(J)** H&E (upper panels) and EZH2 stained (lower panels) brain sections from mice intracranially transplanted with 267 glioma spheres and followed by CMP3a or vehicle treatment mentioned above. **(K)** Western blotting showed that EZH2 expression was decreased in 267 glioma spheres treated with CMP3a in a dose-dependent manner. **(L and M)** ICC for NEK2 location (**L**) and cell mitosis (**M**) in 267 glioma spheres treated with CMP3a or DMSO. Acetylated α-Tubulin was used to label the microtubes and centrosomes. Scale bars: 4 μm **(N)** Flow cytometry showed 267 glioma spheres not only expressed NEK2 during the division phase but also during other cell cycle phases. **(O)** Flow cytometry showed that inhibitory doses of CMP3a did not affect cell cycle progression in 267 (left panel) and 374 glioma spheres (right panel). **(P)** In vitro cell viability assay for CMP3a in 267 glioma spheres transduced with NEK2 overexpression vector (267-NEK2) or empty vector (267-EV). $P < 0.01$, *n* = 6, by 1-way ANOVA.



Supplemental Figure 9. CMP3a increases radio-sensitivity in GBM without affecting normal brain cells. (A) Western blotting for EZH2 expression in 528 glioma spheres pre-treated with CMP3a then followed with or without radiation (12 Gy). **(B)** In vitro growth assay showed combination of CMP3a with radiation (12 Gy) decreased the proliferation of 528 glioma spheres. ***P < 0.001, n = 6, by 1-way ANOVA. **(C)** In vitro cell viability assay for CMP3a combined with different doses of radiation in 528 glioma spheres. ***P < 0.05, n = 6, by 1-way ANOVA. **(D and E)**

Flow cytometry for apoptosis in 267 **(D)** or 528 glioma spheres **(E)** pre-treated with CMP3a (10 nM or 20 nM) for 8 h followed with or without radiation treatment. **(F)** qRT-PCR showed NEK2 expression was decreased after radiation in NHA cells. ***$P < 0.001$, $n = 3$, by 2-tailed *t*-test. **(G)** Flow cytometry for apoptosis showed that CMP3a did not significantly affect the radio-sensitivity of NHA cells.

Supplemental Table 1. Primers for qRT-PCR

Primer Name	Primer Sequence
NEK2-For	TCCCCACTGAAATGAACTTTCT
NEK2-Rev	CAGCTTGCTAAAGGAACGGA
EZH2-For	CCCTTCTCAGATTTCTTCCCA
EZH2-Rev	GGACTCAGAAGGCAGTGGAG
CD133-For	ACTCCCATAAAGCTGGACCCC
CD133-Rev	TCAATTTTGGATTCATATGCCTT
ALDH1A3-For	CACTTCTGTGTATTCGGCCA
ALDH1A3-Rev	TGGATCAACTGCTACAACGC
GAGE2-For	TGGCTATGAGCTTCAGGCTT
GAGE2-Rev	AAGAAGGGGAACCAGCAACT
STMN3-For	TGCTGTCGCTCATCTGCTCC
STMN3-Rev	TCACCTCCATGTCCCCGTACT
ADRB2-For	TCCTGGATCACATGCACAAT
ADRB2-Rev	GAGCACAAAGCCCTCAAGAC
RUNX3-For	GTCTGGTCCTCCAGCTTCTG
RUNX3-Rev	CTGTGTTCACCAACCCCAC
WNT-For	GGAGGAGGCTACGTTCACAA
WNT-Rev	TTTCTGCTACGCTGCTGCT
PFKP-For	TGGAGACACTCTCCCAGTCG
PFKP-Rev	GGGCCAAGGTGTACTTCATC
SNAI1-For	TCTGAGTGGGTCTGGAGGTG
SNAI1-Rev	CTCTAGGCCCTGGCTGCTAC
CHMP3-For	CCTTCTTGGCAGCATCTTTC
CHMP3-Rev	CTGTTTGGAAAGACCCAGGA
GNG4-For	CTGGCTTGGGAGATGCTAGT
GNG4-Rev	CTTCGCCGGGTTAGTGG
GAPDH-For	GAAGGTGAAGGTCGGAGTCA
GAPDH-Rev	TTGAGGTCAATGAAGGGGTC
NANOG-For	CCTGTGATTTGTGGGCCTG
NANOG-Rev	GACAGTCTC CGTGTGAGGCAT
ABCG2-For	TATAGCTCAGATCATTGTCACAGTC
ABCG2-Rev	GTTGGTCGTCAGGAAGAAGAG
CD90-For	ATACCAGCAGTTCACCCATCCAGT
CD90-Rev	ATTTGCTGGTGAAGTTGGTTCGGG
NOTCH1-For	GAGGCGTGGCAGACTATGC
NOTCH1-Rev	CTTGTACTCCGTCAGCGTGA
OLIG2-For	CGGCTTTCCTCTATTTTGGTT
OLIG2-Rev	GTTACACGGCAGACGCTACA
SOX2-For	GTCATTTGCTGTGGGTGATG
SOX2-Rev	AGAAAAACGAGGGAAATGGG
CD15-For	GAGGGTAGATTGGGGGAAAC

CD15-Rev	CACTGCTCGCTGCCTCTC
OCT4-For	CATCGGCCTGTGTATATCCC
OCT4-Rev	GAAGGAGAAGCTGGAGCAAA
18S-For	GGCCCTGTAATTGGAATGAGTC
18S-Rev	CCAAGATCCAACTACGAGCTT

Supplemental Table 2. Pearson's correlation analysis for expression of kinase-encoding genes and EZH2 in TCGA dataset

Gene Symbol	Pearson r	*P* value (F-test)
BUB1	0.813652863	0.000372914
TTK	0.798998032	0.000728295
BUB1B	0.780525856	0.251129674
CHEK1	0.774938753	0.001437243
MELK	0.771824874	4.96006E-05
CDC7	0.683756735	0.836274315
DBF4	0.680979906	0.475043096
AURKB	0.667802714	0.005957114
NEK2	0.666747659	0.022613461
DTYMK	0.665958284	0.002027106
PLK4	0.662109873	0.230828058
TK1	0.653724472	0.186399428
GSG2	0.61881433	0.818330182
CDK2	0.609936622	0.158068302
PLK1	0.5764022	1.09683E-08
PRKD3	0.569100227	5.39113E-12
CHEK2	0.556657762	0.004255159
WEE1	0.553802103	0.531622748
CDKN3	0.548444052	0.203151403
PRKDC	0.527883301	8.75569E-08
RAF1	0.519975547	1.70022E-10
STK38	0.511878045	0.000268247
RIOK1	0.503562115	1.97639E-12
MAPK7	0.501781021	7.19977E-11
SRPK1	0.487975588	0.000699981
EPHB3	0.464101242	0.52871992
MASTL	0.451118072	0.048713124
CASK	0.448588472	0.010741661
UCK2	0.446690021	1.33846E-05
PIP5K1A	0.442273897	2.51321E-16
PRPF4B	0.441285675	9.67711E-13
TRIB2	0.438862425	0.011378005
PDGFRA	0.429440279	9.33884E-12
UNK	0.425078377	2.98703E-09
CSNK1G3	0.415158385	3.2539E-25
PASK	0.41395258	1.38736E-08
TRIM28	0.41371804	5.35063E-05
RYK	0.405860265	2.44795E-13
CDK6	0.403253202	0.002395675
PTK7	0.402242324	1.33181E-10
EPHB2	0.39033135	0.193291235

CLK2	0.389904219	1.4991E-13
MAP3K1	0.386808424	0.667754239
NME1	0.386458204	3.43069E-12
PRKD1	0.386195063	0.078367697
CSNK1G1	0.385787206	1.49313E-22
PDIK1L	0.385016352	5.28284E-12
CSNK2A1	0.382336304	1.15834E-14
CDK4	0.373071907	1.16157E-08
TRRAP	0.371195734	2.28725E-05
NME4	0.367888597	8.3397E-10
HK2	0.356024018	0.000423532
CDK8	0.355109453	0.204295446
STK36	0.353702189	0.121920168
CSNK1E	0.325079803	0.56766375
UCK1	0.320449751	2.52333E-11
PRKD2	0.319559849	3.08739E-06
CSNK1A1	0.319339968	2.94554E-22
VRK1	0.316271189	0.001184504
YES1	0.307651685	1.60219E-09
BRD3	0.298233654	0.030664299
BRD2	0.297703569	7.19615E-09
MAST2	0.292200362	1.95075E-10
NME7	0.291636875	1.47475E-08
CDK3	0.290456768	1.4257E-17
CSNK1A1L	0.289205465	4.1162E-13
TRIO	0.287956978	0.642921299
AKT2	0.283573308	0.000415303
TRIB3	0.283364904	0.882257532
PI4K2B	0.282126536	0.000233343
ZAK	0.281712243	2.35981E-05
GSK3B	0.278689393	3.26679E-05
TLK2	0.276613644	7.64477E-20
PKMYT1	0.274308089	9.13742E-11
STK10	0.271735777	6.43333E-09
ICK	0.266802604	1.39703E-08
STK32B	0.266661847	0.053519469
MAP3K14	0.264733563	0.000197822
MAPKAPK5	0.262829722	5.45988E-25
STK35	0.26146121	2.99342E-32
IRAK1	0.258431259	1.79477E-16
PIK3C3	0.257667683	1.04693E-17
TYK2	0.257332354	2.44122E-08
IKBKB	0.248563454	8.12477E-31
ABL1	0.24016769	1.19037E-25

MAPK12	0.236584306	1.94063E-05
TRIM33	0.235653853	3.98063E-07
PANK3	0.235622501	6.59482E-09
PKN1	0.231609787	4.19513E-08
PAK2	0.230860251	5.26381E-17
PKN3	0.228705807	5.36042E-11
RIPK1	0.227736153	8.70851E-10
STK19	0.225905341	2.35949E-08
SNRK	0.22500347	4.46885E-06
AK2	0.223275017	1.80569E-08
AKT1	0.22067134	0.661128866
PSKH1	0.220119587	1.8947E-22
RPS6KB1	0.219884966	1.06225E-23
DYRK2	0.218199816	0.000797061
EIF2AK3	0.215324831	3.8984E-13
TGFBR1	0.214359775	6.87631E-05
LRRK2	0.213310069	0.001831722
NEK1	0.212420439	4.22447E-06
STK33	0.212124357	0.123744208
NEK9	0.211936699	6.79834E-12
DDR1	0.209896475	0.227678381
CLK3	0.209246147	1.88033E-18
PRPS1	0.20829367	7.87647E-06
GRK4	0.208169561	0.009381138
MAP3K4	0.207740108	6.13092E-07
ADCK4	0.206211748	7.69521E-08
MAPK8	0.205145594	1.10878E-21
FER	0.202481287	3.65199E-12
EPHB1	0.201320888	1.55571E-08
BRAF	0.194118788	1.535E-18
PHKB	0.192791341	2.37083E-16
IPMK	0.188924086	2.90754E-08
MAP2K2	0.187815653	4.57303E-06
MAPK14	0.183451204	1.14489E-14
MAPK14	0.183451204	1.14489E-14
STK17A	0.181772817	0.416876296
PRKX	0.180793074	0.118539915
CERK	0.180611197	2.26589E-14
ETNK2	0.179937597	0.099514066
EEF2K	0.178725477	1.08687E-10
NEK8	0.178133358	3.00327E-31
NEK4	0.177352915	9.06967E-07
MARK3	0.177227343	8.85626E-17
ROCK1	0.174532349	5.2313E-32

FLT4	0.173700963	2.47959E-39
UCKL1	0.173078742	1.38988E-10
HUNK	0.170295182	2.62976E-32
IRAK4	0.166605863	4.65445E-05
DGKI	0.163872352	0.008757007
VRK3	0.161296508	1.5761E-07
CLK1	0.157406854	2.04073E-06
ACVR2B	0.155360178	0.037595914
MAP4K4	0.153838977	8.90326E-05
RIOK2	0.15362183	3.39613E-17
STK25	0.152906953	2.18323E-08
FYN	0.149643471	0.013510716
SMG1	0.149621044	1.69469E-08
ADPGK	0.147743549	2.08229E-09
PRKY	0.147656649	0.000116889
DGKD	0.147540845	0.000360636
DAPK3	0.14692593	2.90717E-06
DGKH	0.145448155	2.68446E-30
TAF1	0.145222879	3.58444E-06
TP53RK	0.144654078	2.17216E-07
MAK	0.142891509	1.30267E-08
PRKAR2A	0.142886563	1.36285E-16
MYLK2	0.142317146	0.424980136
BMPR2	0.139334116	8.78184E-16
PRKAA2	0.137036403	2.54849E-09
FLT1	0.136416366	0.79378076
CDK7	0.133028711	3.15404E-22
GUCY2F	0.131025877	1.80576E-14
CDK10	0.128441943	3.58494E-25
DGUOK	0.12736807	3.30083E-10
ALPK3	0.127247384	8.32522E-23
MARK1	0.126503619	0.521297654
MRC2	0.122315094	0.004468323
TAOK1	0.120668064	0.860459354
OXSR1	0.120354743	5.6076E-18
TAF1L	0.119713873	4.11311E-15
ALPK2	0.118154514	9.71636E-15
CSNK1D	0.115093219	3.43577E-12
NAGK	0.109821445	3.15491E-07
ETNK1	0.105645609	2.35298E-15
TRPM7	0.102890977	8.56595E-10
PKN2	0.101133195	1.65277E-09
PIM1	0.094593089	5.81582E-06
NEK6	0.093185411	0.660313147

SRC	0.091256069	1.12809E-13
STK32A	0.090294343	0.069549301
ROR1	0.088463849	0.005632067
MAP4K5	0.086583902	0.002250612
EGFR	0.086543107	2.37707E-20
CSNK2A2	0.084829279	2.08526E-11
DYRK1A	0.08137654	1.32846E-32
STK16	0.080473978	0.002354246
ATR	0.079213156	1.72048E-20
FASTK	0.075801302	1.34097E-13
TAOK3	0.075108593	0.0002404
MAP2K5	0.073077308	6.00795E-09
PDK1	0.07276636	0.558415367
BRD4	0.071297395	0.263723723
MAP3K2	0.07108806	1.04789E-14
RIOK3	0.070343868	1.35635E-19
NPR2	0.07004866	1.19191E-07
NPR2	0.07004866	1.19191E-07
FGFR1	0.069462322	0.01559421
PANK4	0.068410291	2.31349E-13
TLK1	0.067048219	6.52092E-10
TAOK2	0.06543861	2.85926E-11
TAOK2	0.06543861	2.85926E-11
EPHB4	0.064912938	2.41987E-05
EPHA7	0.061129107	1.0537E-22
PHKG1	0.061054175	0.083921644
PFKFB3	0.057738179	0.01027914
ADCK1	0.056939356	2.94817E-10
IGF1R	0.056553116	0.000166325
BMPR1A	0.054565475	0.000394501
MAP4K3	0.052975717	5.43591E-16
DDR2	0.052831619	0.998392906
BCKDK	0.052288373	6.40336E-17
STK31	0.050441637	0.544075524
RPS6KA3	0.050338177	4.26709E-11
MAPK10	0.049117958	0.027818249
MAPK10	0.049117958	0.027818249
GAK	0.045747462	2.70654E-06
MKNK2	0.043921033	3.40727E-10
DCAKD	0.04248452	1.95897E-11
MAPKAPK2	0.041727936	7.59625E-14
DMPK	0.040876298	0.000604443
XYLB	0.039303503	2.40826E-17
DGKB	0.037285322	0.008679807

MYO3A	0.035656052	1.29588E-12
PFKFB4	0.035262374	0.00018283
GALK1	0.03410053	0.000432011
GCK	0.031418097	4.71121E-07
AAK1	0.025442569	1.86742E-34
STK11	0.022757969	1.49156E-12
RIPK2	0.022340795	1.65359E-11
MAP3K7	0.022225876	8.49116E-17
PIK3CA	0.021878446	5.41997E-08
PAK4	0.021092291	1.48093E-16
STK24	0.020735251	3.74213E-16
PRKRA	0.020371448	2.77846E-15
ERBB2	0.019955846	0.017846016
LATS1	0.017168053	3.99921E-19
MAP2K6	0.016271375	3.41615E-14
PRKAA1	0.015552092	0.000387366
IKBKE	0.014551673	1.32754E-09
EPHA2	0.012154707	0.001510595
CLK4	0.01106692	7.45527E-07
NTRK1	0.008215281	0.000766371
LATS2	0.007715943	0.224179135
MAPK6	0.006964976	7.1252E-07
DYRK3	0.006380736	0.826128465
JAK1	-2.01866E-05	8.78415E-07
KDR	-0.00535598	0.002012191
STK3	-0.005940717	5.77494E-15
PRKCA	-0.006420655	2.45185E-09
PANK1	-0.006673226	0.021749729
EIF2AK4	-0.008835083	3.51196E-10
CAMK2D	-0.013972	0.061917749
GK	-0.015572176	3.09793E-07
PANK2	-0.017126437	1.19285E-16
CDK5R1	-0.017545852	0.011617121
TIE1	-0.018977895	8.42494E-09
CHKA	-0.019576135	1.11373E-10
AKT3	-0.021675413	0.031202494
PAK7	-0.022033729	7.16173E-12
RIPK4	-0.024117756	0.865834635
MAP2K7	-0.024694648	0.000450857
MAP3K6	-0.024909422	2.88578E-05
STK17B	-0.027111596	0.32599964
ROCK2	-0.028662286	3.40006E-06
PGK2	-0.032369458	1.07289E-38
STK4	-0.032468497	9.8384E-22

CKM	-0.032523925	1.05412E-11
NME6	-0.032642999	3.32775E-14
KIT	-0.033541353	1.77373E-07
NEK11	-0.033824587	0.194042087
CKB	-0.035493422	0.037588013
NEK3	-0.035587344	1.16948E-05
INSR	-0.03689228	4.81547E-05
MARK4	-0.037031039	3.8989E-19
GALK2	-0.037502358	4.70191E-12
MAP3K12	-0.038610085	1.57064E-12
ABL2	-0.045136915	4.67162E-09
GNE	-0.046082936	1.7256E-05
MAP2K3	-0.046821092	3.09181E-09
STK38L	-0.047403019	3.62522E-10
RET	-0.04748168	0.032593894
RET	-0.04748168	0.032593894
LIMK2	-0.048097958	0.000714297
TEX14	-0.050231863	6.49713E-06
ERBB3	-0.050947219	0.212298245
FGR	-0.052888174	5.6452E-09
ADK	-0.053673355	2.60111E-05
RPS6KA6	-0.053937581	0.000391819
ITPKC	-0.054223824	0.048522897
FRK	-0.054275105	0.000601244
CIT	-0.056306611	0.048963577
SPHK1	-0.057886637	0.094408379
PIK3CG	-0.058521882	0.000224118
NTRK3	-0.061180024	0.881534038
TRIB1	-0.062700908	0.033232733
AURKC	-0.063167603	3.72466E-14
PSKH2	-0.063906127	1.4955E-24
DYRK1B	-0.064152413	1.81353E-13
DYRK4	-0.064250419	4.20036E-11
HIPK2	-0.064412027	0.365530139
HIPK1	-0.064451403	3.8951E-07
PAPSS1	-0.065808228	2.87922E-05
MAP3K13	-0.066003552	1.32596E-14
CSNK1G2	-0.066169127	8.48202E-06
PAK3	-0.06916	2.38614E-06
LTK	-0.069644406	0.197165822
HKDC1	-0.069855699	0.206491813
PRKG2	-0.071843173	1.45946E-12
FGFR4	-0.073533927	1.45351E-13
GSK3A	-0.074557001	1.86764E-14

ADCK2	-0.076370989	1.77046E-11
PIK3R4	-0.078184599	6.24575E-15
PFKFB1	-0.078367679	2.28325E-23
GRK6	-0.080560904	5.01286E-25
PHKA1	-0.080889029	0.015638074
PGK1	-0.081186884	0.005566183
ACVRL1	-0.085162466	6.55768E-09
ALPK1	-0.085995736	0.078192507
MAST1	-0.087253241	0.087204695
KSR1	-0.088211281	3.91637E-39
TTBK2	-0.088755692	5.53814E-31
RPS6KB2	-0.091222598	4.30343E-14
GK2	-0.097039336	6.23809E-46
PHKA2	-0.097472319	5.2896E-06
GRK5	-0.098043581	3.37094E-09
OBSCN	-0.098130972	2.57945E-22
PIP5K1C	-0.098738423	4.56037E-10
RIPK3	-0.098985939	0.920336768
HIPK3	-0.10035222	3.72816E-16
PTK2	-0.101383912	6.34659E-11
MET	-0.102515411	0.020120181
GRK7	-0.102761122	1.02421E-33
ULK1	-0.10401448	4.22188E-13
EPHA3	-0.104581322	0.763918746
ACVR1	-0.104801801	1.15075E-09
BLK	-0.105997419	0.005229059
HK3	-0.107762791	0.007560032
CDKL3	-0.107814769	0.000269942
SCYL1	-0.108016867	5.94672E-19
PKLR	-0.108062063	2.72213E-08
NPR1	-0.109642887	1.07036E-06
MST1R	-0.11185623	5.78532E-10
ROR2	-0.112717467	8.7777E-11
PLK3	-0.113062705	0.354895646
ACVR1B	-0.113401045	4.09685E-28
SPHK2	-0.113616118	2.33005E-10
CDK5	-0.113820982	0.000503129
MAPK1	-0.115266017	3.27136E-10
PRKCI	-0.116537488	7.33625E-06
HCK	-0.11698171	0.077770173
LCK	-0.117513854	0.655438992
AMHR2	-0.119657457	9.79111E-08
DGKA	-0.119711572	5.63305E-13
BCR	-0.123098652	0.000731136

DAPK1	-0.123672132	7.99353E-05
ZAP70	-0.124387695	2.98921E-08
LIMK1	-0.124737648	0.190111155
PDGFRB	-0.125541738	0.00159768
PMVK	-0.129038454	1.75161E-08
CDC42BPA	-0.130217419	1.20712E-07
SRMS	-0.130224467	1.15887E-37
PRKACA	-0.131480191	1.50766E-31
PCK2	-0.132143237	2.06433E-08
MARK2	-0.133991887	1.51089E-17
CSK	-0.135320516	9.92891E-14
NTRK2	-0.135965673	6.18172E-05
MOS	-0.136526563	2.45738E-26
DCK	-0.137834855	0.033677213
RNASEL	-0.138576946	8.01179E-17
CDK9	-0.141726619	7.56128E-19
MAPK11	-0.142544662	3.08525E-12
ATM	-0.144090159	3.83007E-14
BMP2K	-0.144543982	0.005253049
ITK	-0.146690208	0.036267382
EPHA1	-0.147972209	0.000236521
PTK6	-0.148722837	1.48139E-09
BTK	-0.14952627	0.666925205
PRKG1	-0.152155346	2.02099E-25
PRPS2	-0.152156139	0.440438877
MAP3K3	-0.152867656	3.91617E-12
PFKL	-0.154540718	3.75155E-10
LYN	-0.155207755	0.000133849
MAP4K1	-0.15810987	1.85629E-24
PDK3	-0.159054084	0.010147618
STK32C	-0.159909063	0.010028556
PIM3	-0.161968446	1.90614E-05
RPS6KL1	-0.162746708	2.33673E-27
HSPB8	-0.163237408	0.000251874
NRK	-0.166139499	2.13239E-28
TESK1	-0.166572274	6.03476E-16
MADD	-0.169524788	3.03473E-11
ILK	-0.170891794	0.001538953
MKNK1	-0.174054661	0.002062597
MUSK	-0.17698649	2.13783E-56
CHUK	-0.179767481	1.28513E-07
TGFBR2	-0.180079842	7.64094E-06
AK1	-0.182292608	0.009994212
SRPK2	-0.183669915	5.4585E-05

PRKCH	-0.186028461	0.000640701
INSRR	-0.18753243	1.03497E-57
TEC	-0.188891093	1.01023E-08
TXK	-0.190119172	5.11559E-25
MAPK13	-0.192147644	0.116737544
KHK	-0.19488793	2.67604E-05
PCK1	-0.198367726	0.000105125
CDC42BPB	-0.19927707	1.49418E-19
DGKQ	-0.199844876	1.402E-14
ERN1	-0.20259877	1.40754E-24
ADCK5	-0.203335653	2.22762E-18
MAPK4	-0.204503595	0.004398519
AXL	-0.205224031	0.001374848
TTN	-0.205477872	5.14565E-14
HIPK4	-0.206798601	1.1074E-24
GUCY2D	-0.210513233	2.36807E-50
MAP3K10	-0.211315304	0.000110121
PIP5KL1	-0.213956266	4.8128E-35
TK2	-0.218481173	3.9028E-18
MAP3K8	-0.221080907	0.000618391
MYO3B	-0.221643471	5.37102E-18
IRAK3	-0.223276606	0.272363637
ERBB4	-0.225992689	0.911231954
EPHA5	-0.227722892	0.038151392
NEK7	-0.227995696	1.49896E-09
FN3KRP	-0.229584819	8.48257E-14
PFKM	-0.234134034	0.014758728
RPS6KA1	-0.23723687	0.009212573
ITPKB	-0.242023525	0.509824484
TNIK	-0.242808534	0.660606106
PIK3CB	-0.242878204	4.0807E-05
PFKFB2	-0.242971291	0.005607367
EPHA6	-0.244717075	3.73384E-34
ROS1	-0.244879675	0.532429229
PDPK1	-0.247167366	9.06107E-08
JAK2	-0.24739505	5.5026E-06
BRDT	-0.247623283	3.14744E-43
FES	-0.248836143	0.125307794
FGFR3	-0.249131563	0.000135768
MAP3K9	-0.254893792	2.65535E-20
IRAK2	-0.255383776	1.46427E-26
MAPKAPK3	-0.256342475	1.12945E-06
PFKP	-0.263100528	0.002112393
BMX	-0.263167317	3.06916E-10

CAMK1D	-0.265412425	0.01507113
FUK	-0.268864314	1.20798E-10
AK3	-0.27101208	0.000970098
TBK1	-0.275617722	1.02467E-08
RFK	-0.277360438	0.000464031
MAPK9	-0.279395427	1.33505E-08
MAPK9	-0.279395427	1.33505E-08
DGKE	-0.280088504	0.000187146
MAP3K11	-0.28100638	8.34836E-12
DGKG	-0.287528433	0.007384676
SYK	-0.290829412	0.478160381
AK7	-0.292199659	0.051750369
TEK	-0.29309262	0.077078606
UHMK1	-0.294784836	6.79583E-11
ALK	-0.298445988	0.000796447
GUCY2C	-0.29988989	1.61589E-18
NLK	-0.30080548	1.58523E-07
CALM1	-0.301692175	0.008055101
LMTK3	-0.30241751	0.000753418
RPS6KA5	-0.305978921	0.218842567
MERTK	-0.307090989	0.793206813
DAPK2	-0.308626028	0.257688878
NME5	-0.311175314	0.042565873
TPK1	-0.315860221	0.06617183
SLK	-0.321132389	1.76427E-11
PIK3CD	-0.324604769	1.80507E-12
MVK	-0.324690142	1.18653E-12
CKMT2	-0.32591858	0.026688508
ITPK1	-0.329770128	1.2175E-05
TYRO3	-0.33193085	0.018201272
PRKCD	-0.335416144	0.000113704
MAP2K4	-0.33690692	0.031778825
KIAA1804	-0.337684594	7.49256E-07
CAMK1	-0.342876248	0.003199717
PLK2	-0.344707902	0.038331484
RPS6KC1	-0.347902954	8.33494E-14
ULK2	-0.349500666	0.739413251
PAK1	-0.355456888	1.23554E-05
PIM2	-0.355487319	4.95415E-06
EPHA4	-0.356300365	0.527350374
RPS6KA2	-0.358789243	0.000406795
COL4A3BP	-0.360669587	0.002160487
TNNI3K	-0.363826733	0.840252487
CDKL1	-0.365510032	2.5033E-06

CAMK2B	-0.372671432	7.07274E-06
PINK1	-0.372785104	4.51982E-12
ADRBK2	-0.375874216	8.27014E-07
MAP3K5	-0.382671892	0.38282448
PRKCG	-0.385842734	1.44308E-21
FGFR2	-0.392637841	0.931699905
CALM2	-0.39294682	0.484823722
CAMKK2	-0.393684365	8.19322E-09
PHKG2	-0.395884084	2.10656E-18
CAMK4	-0.395938993	0.000498921
JAK3	-0.397110664	1.14051E-16
PRKCQ	-0.400797884	0.860799032
ADRBK1	-0.403999433	3.20318E-31
MAST4	-0.407889636	3.10787E-08
STK39	-0.407928933	3.03079E-06
NME3	-0.413962681	9.40159E-06
KSR2	-0.417213008	0.44670023
CALM3	-0.418310121	5.47096E-10
PRKCE	-0.426359754	8.23951E-06
FLT3	-0.426457428	1.1127E-11
PDK4	-0.429324869	0.002765049
MYLK	-0.439609885	0.835850292
EPHA8	-0.441578661	1.49873E-19
AATK	-0.443742414	0.116150523
EPHA10	-0.450941253	2.60133E-09
ITPKA	-0.452571144	0.024748683
PDXK	-0.457745691	4.00942E-13
RBKS	-0.460128852	0.309715663
PIP5K1B	-0.470499978	0.009761461
PACSIN1	-0.472862823	3.26759E-10
GUK1	-0.477747624	0.001525999
PRKACB	-0.481607216	0.559154202
PDK2	-0.483365438	9.00339E-10
CAMK2G	-0.490083086	0.449483515
HK1	-0.492302317	4.58411E-16
MAP2K1	-0.495520039	2.78161E-12
SGK2	-0.496284742	0.008242437
MAP4K2	-0.506392723	3.05804E-23
TRPM6	-0.509112851	1.43348E-09
CDKL2	-0.510077342	0.101062307
ACVR1C	-0.516738947	0.080588663
UGP2	-0.517081751	5.15325E-14
CAMK1G	-0.51816292	0.709788672
PTK2B	-0.522582761	2.77327E-11

MAPK3	-0.523633671	5.16299E-11
PRKACG	-0.527668093	5.63346E-20
CAMK2A	-0.528009913	0.243932022
PXK	-0.533170352	0.057530762
PAK6	-0.53629798	1.69661E-06
STYK1	-0.53679069	4.1648E-06
CDKL5	-0.537907853	0.005055007
DGKZ	-0.5380749	2.59313E-07
RPS6KA4	-0.543240495	9.85334E-08
AK5	-0.548576916	2.95759E-10
CAMKK1	-0.561907486	0.00048974
CAMK2N1	-0.567419423	0.570133762
TESK2	-0.576532885	0.002995297
EPHB6	-0.577874927	0.905750476
MAST3	-0.583326764	0.069460782
MATK	-0.586922742	0.902000903
PNCK	-0.620301375	0.385697597

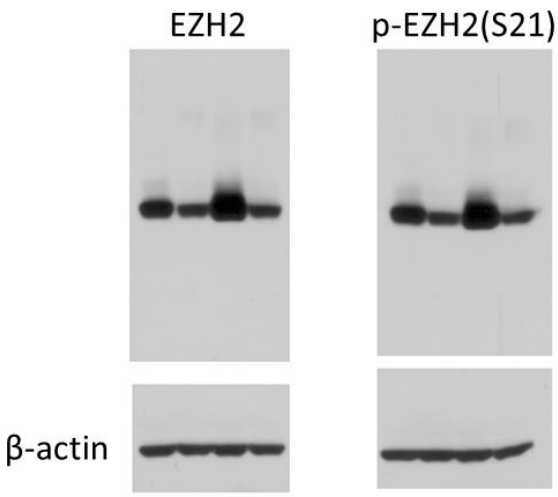
Supplemental Table 3. Sequences of NEK2 shRNAs

Name	Sequence
D5 (3'UTR)	CCGGGCCATGCCTTTCTGTATAGTACTCGAGTACTATACA GAAAGGCATGGCTTTTT
D6	CCGGCGTTCGTTACTATGATCGGATCTCGAGATCCGATCA TAGTAACGAACGTTTTT
D7	CCGGCGTTACTCTGATGAATTGAATCTCGAGATTCAATTC ATCAGAGTAACGTTTTT
D8	CCGGGCAGACGAGCAAAGAAGAAATCTCGAGATTTCTTC TTTGCTCGTCTGCTTTTT
D9	CCGGCCTGTATTGAGTGAGCTGAAACTCGAGTTTCAGCT CACTCAATACAGGTTTTT
09 (3'UTR)	CCGGGCCATGCCTTTCTGTATAGTACTCGAGTACTATACA GAAAGGCATGGCTTTTTG
48 (3'UTR)	CCGGGCCATGCCTTTCTGTATAGTACTCGAGTACTATACA GAAAGGCATGGCTTTTT

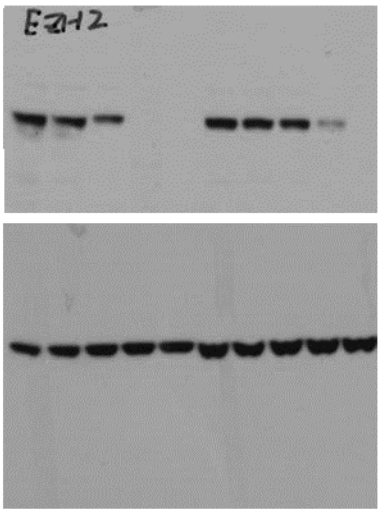
Supplemental Table 4. Primers for PCR-mediated mutagenesis

Name	Sequence
P1	AAAATTCGAACCATGCCTTCCCGGGCTGAGGACTATGA
P2	TATAGGATCCGCTAGCGCATGCCCAGGATCTGTCTG
P3	ATGGCAAGATATTAGTTTGGAGAGAACTTGACTATGGCTCC ATGACAGAA
P4	TTCTGTCATGGAGCCATAGTCAAGTTCTCTCCAAACTAATAT CTT

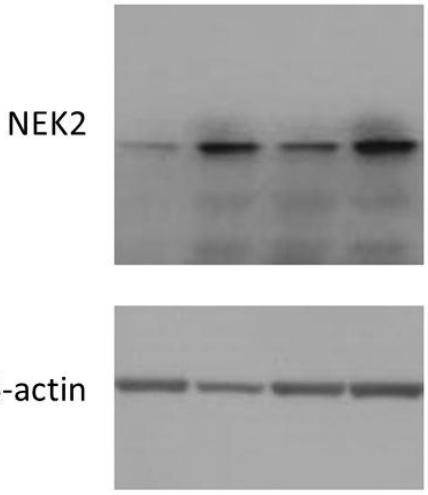
Full unedited gel for Figure 1F



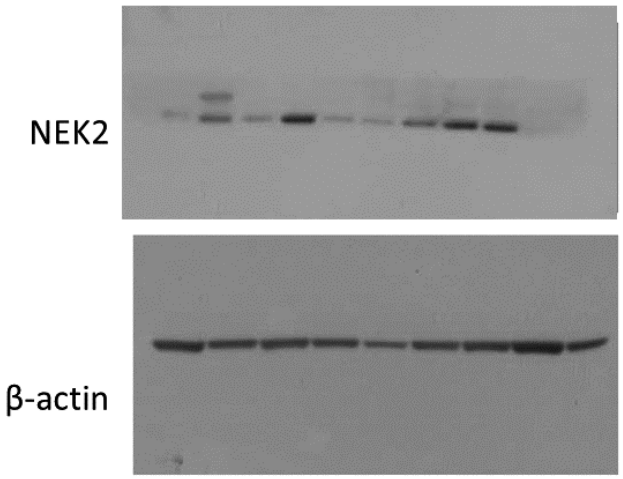
Full unedited gel for Figure 1G



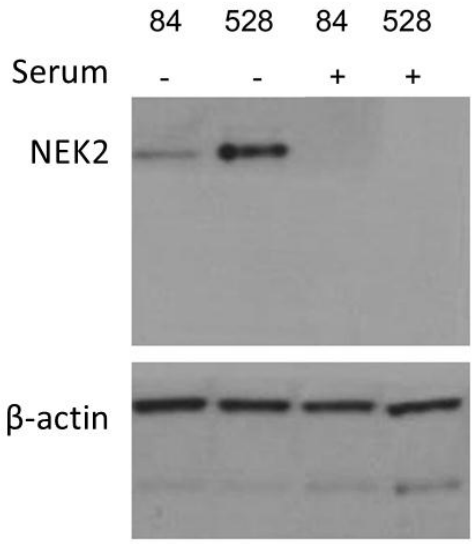
Full unedited gel for Figure 1J



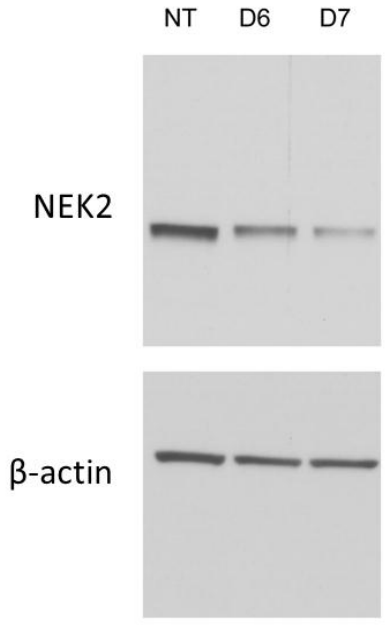
Full unedited gel for Figure 2A



Full unedited gel for Figure 2C

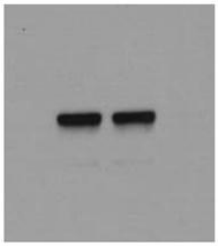


Full unedited gel for 3A

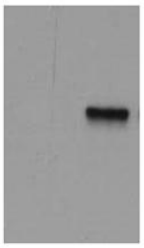


Full unedited gel for Figure 4A

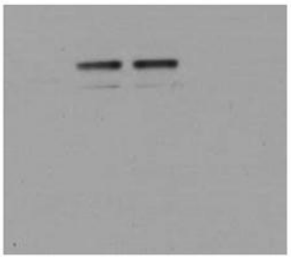
INPUT: EZH2



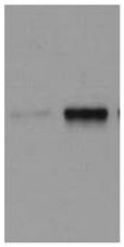
IB: EZH2



INPUT: NEK2

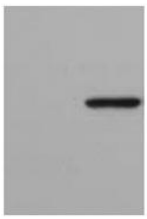


IB: NEK2

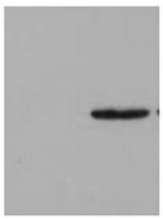


Full unedited gel for Figure 4B

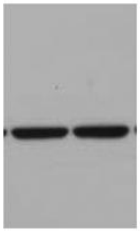
IB: NEK2



IB: EZH2



INPUT: NEK2



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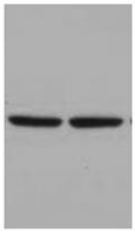
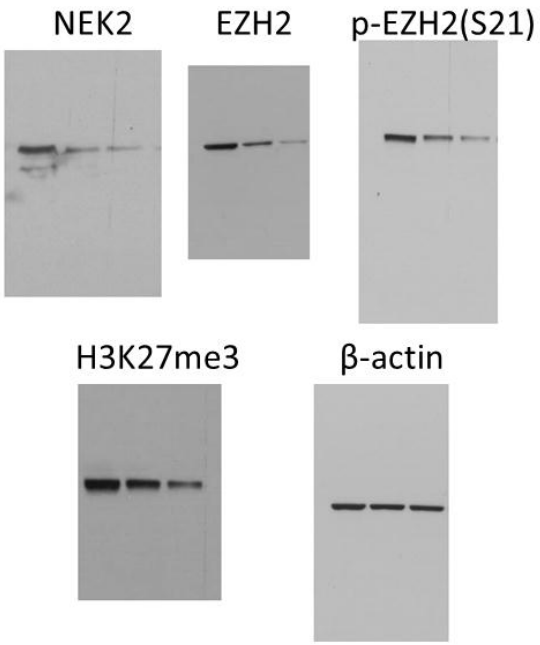
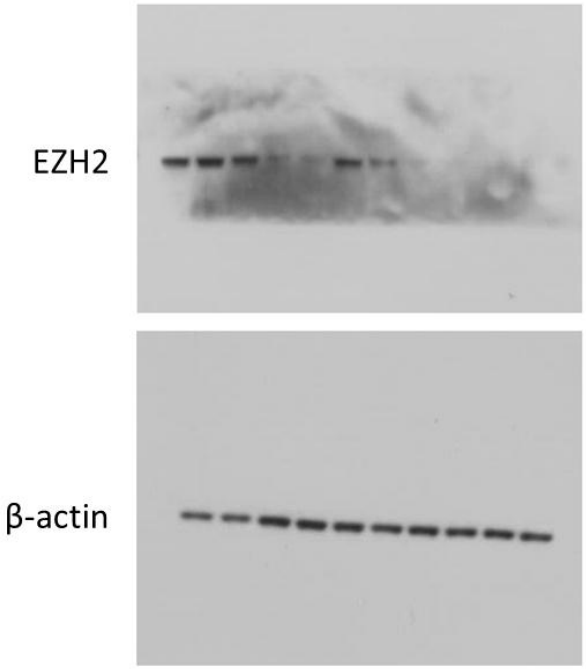


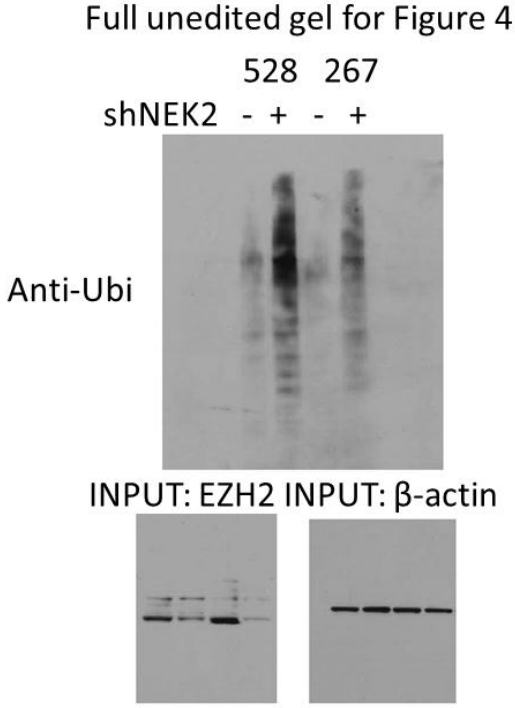
Full unedited gel for Figure 4D



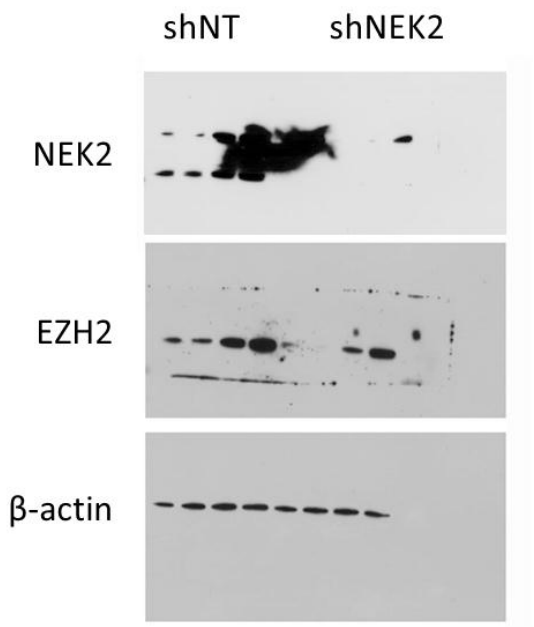
Full unedited gel for Figure 4F



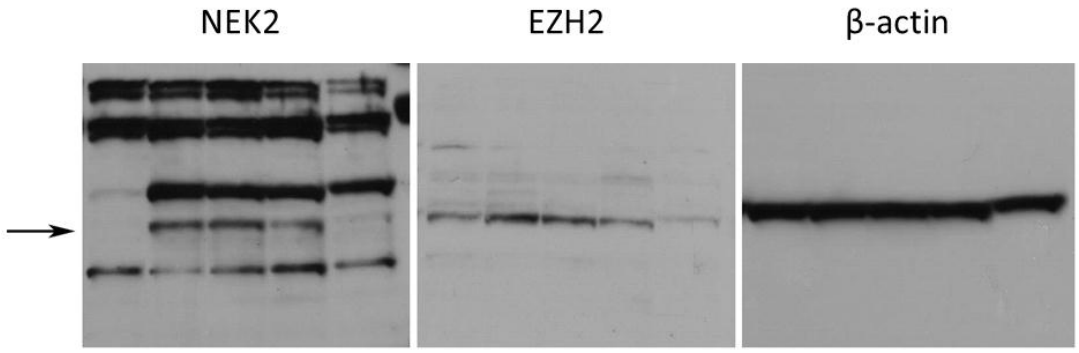
Full unedited gel for Figure 4H



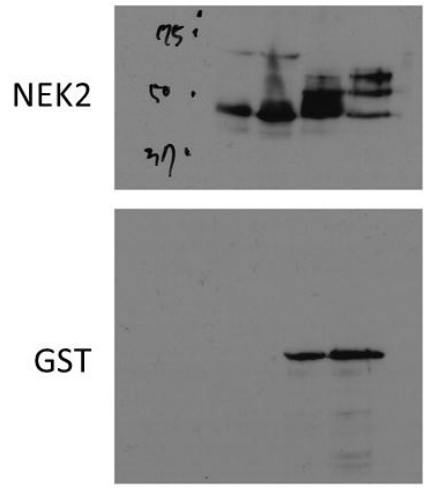
Full unedited gel for Figure 4I



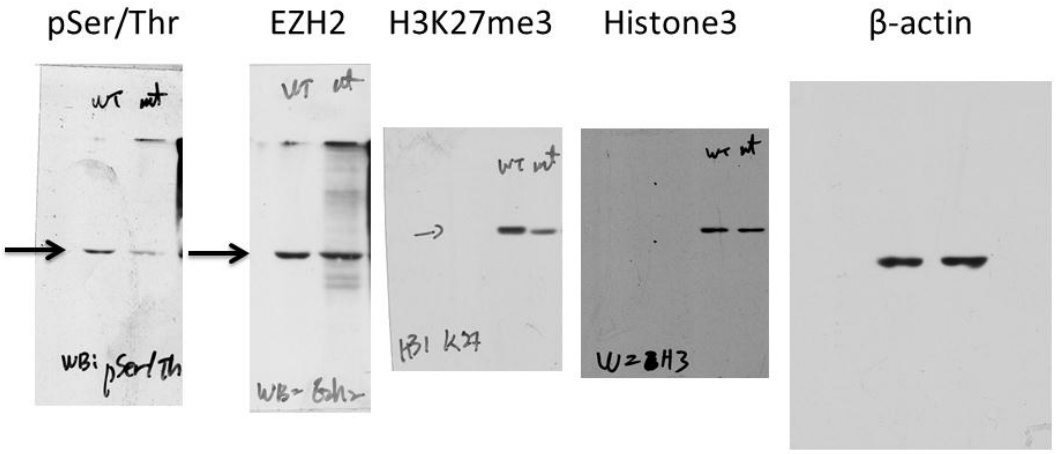
Full unedited gel for Figure 4K



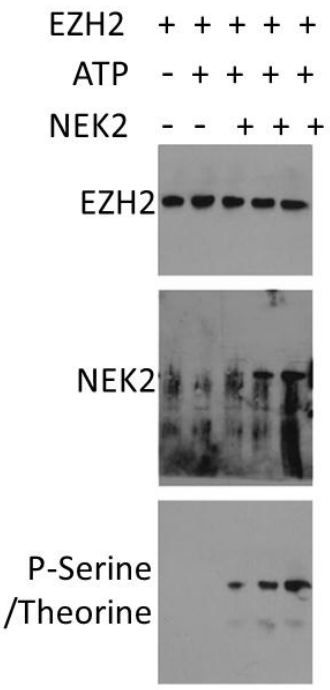
Full unedited gel for Figure 4L



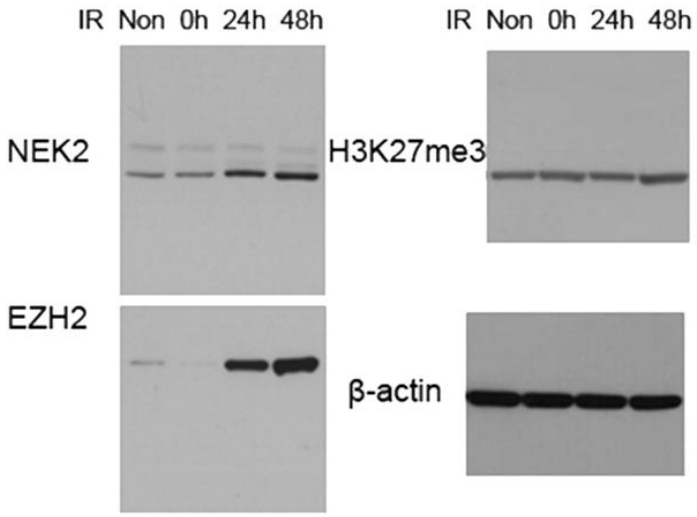
Full unedited gel for Figure 4M



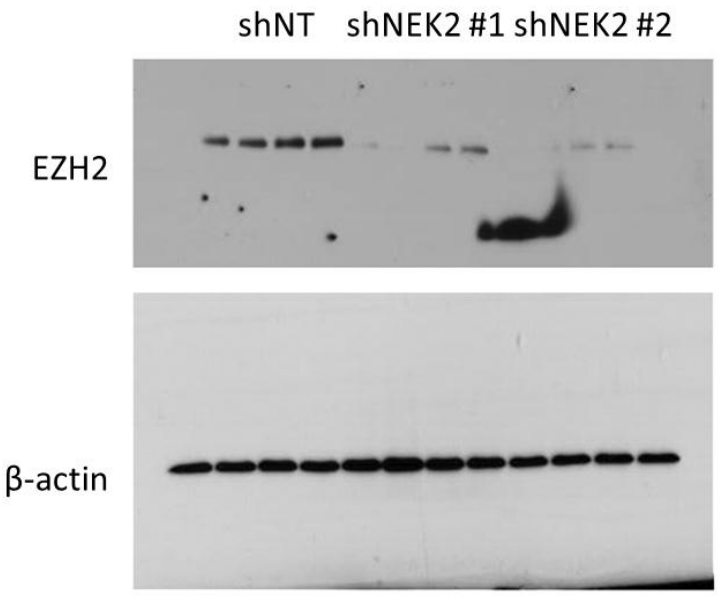
Full unedited gel for Figure 4N



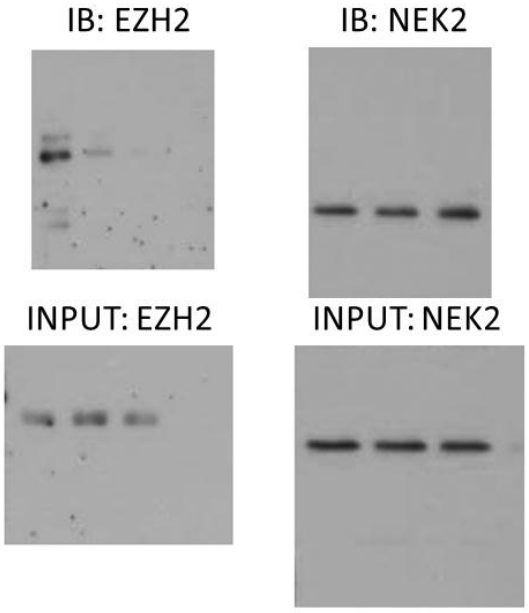
Full unedited gel for Figure 6D



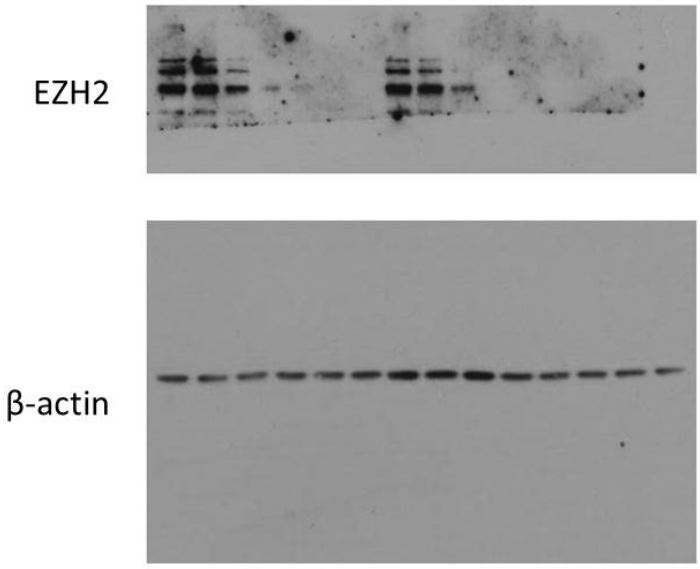
Full unedited gel for Figure 6F



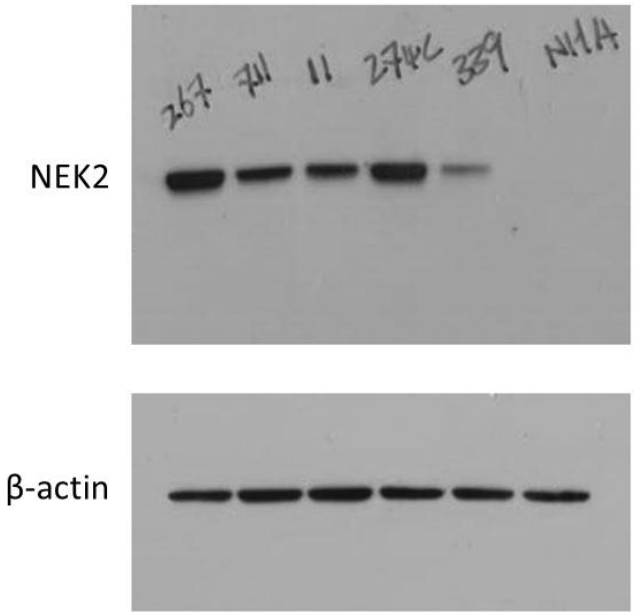
Full unedited gel for Figure 7H



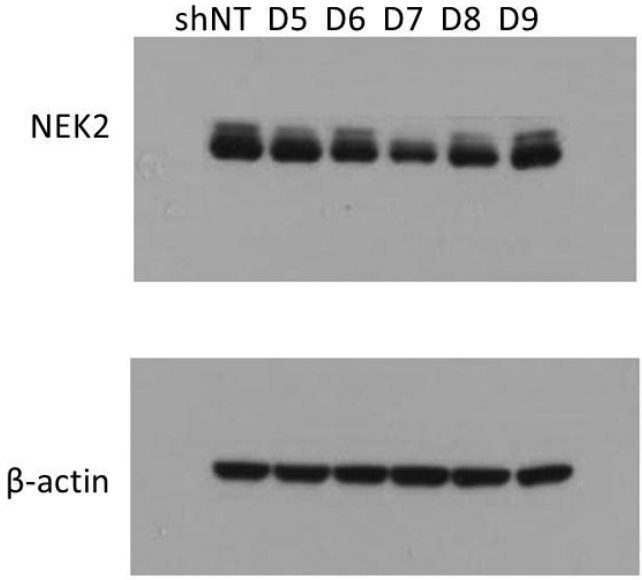
Full unedited gel for Figure 7I



Full unedited gel for Supplemental Figure 2A

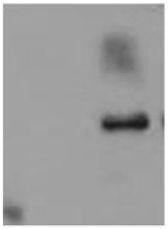


Full unedited gel for Supplemental Figure 3A

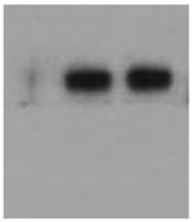


Full unedited gel for Supplemental Figure 4A

IB: EZH2



INPUT: EZH2



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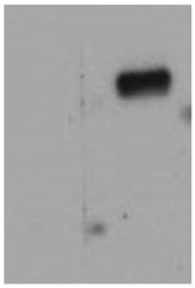

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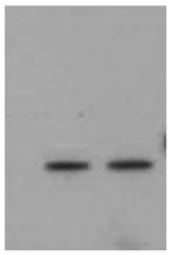
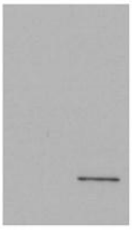


Full unedited gel for Supplemental Figure 4B

IB: NEK2



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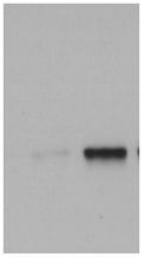

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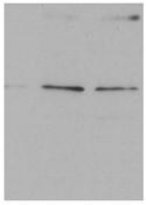

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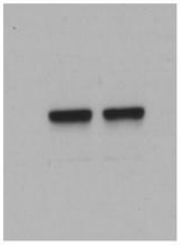
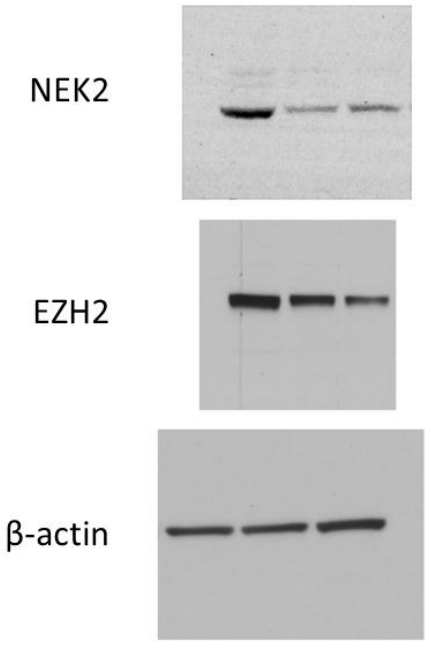
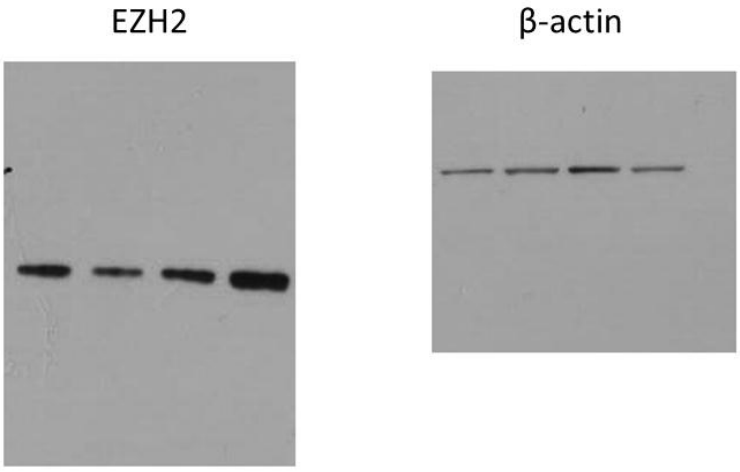


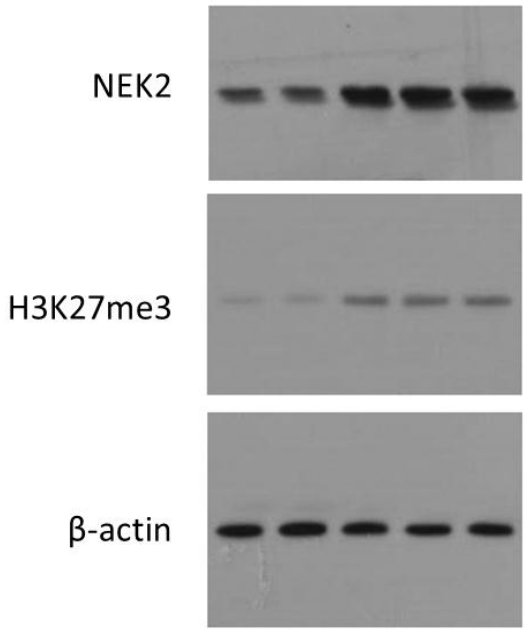
Full unedited gel for Supplemental Figure 4C



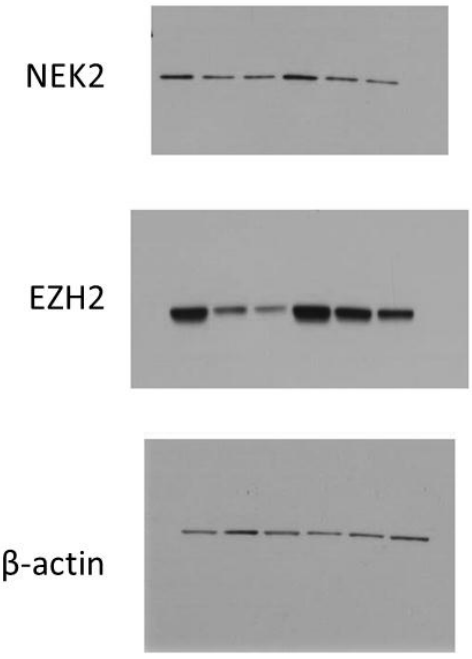
Full unedited gel for Supplemental Figure 4E



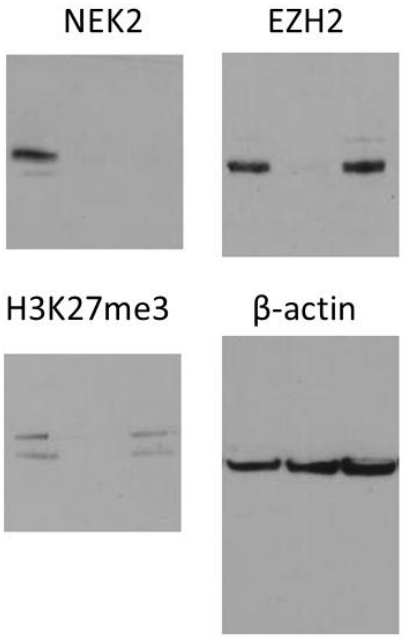
Full unedited gel for Supplemental Figure 4F



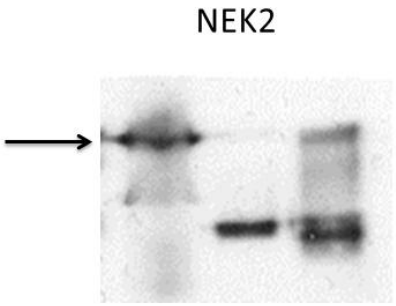
Full unedited gel for Supplemental Figure 4G



Full unedited gel for Supplemental Figure 4I

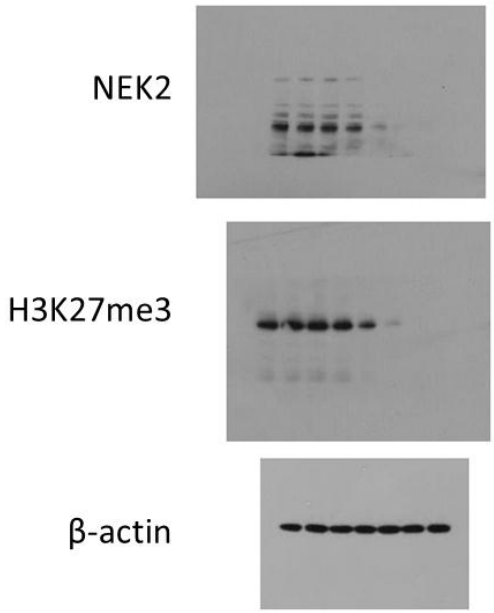


Full unedited gel for Supplemental Figure 4L



Full unedited gel for Supplemental Figure 6A

Full unedited gel for Supplemental Figure 8K



Full unedited gel for Supplemental Figure 9A