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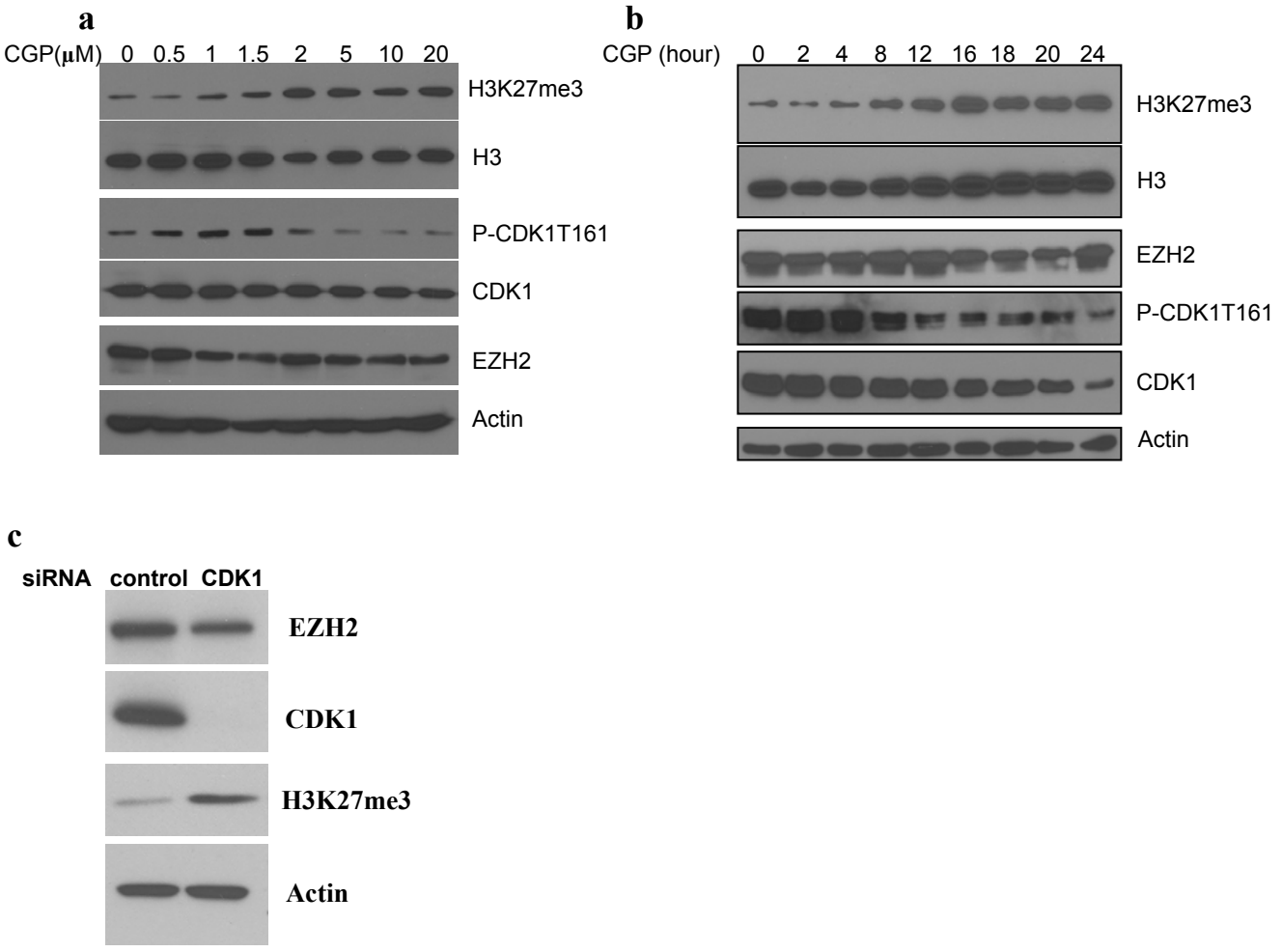

Figure S1 Inhibition of CDK1 enhances H3K27 trimethylation. (a)H3K27 trimethylation increased by the treatment of CGP in a dose depedent manner. Cells were treated with CGP for different concentrations, and H3K27 trimethylation was measured by Western blotting. **(b)** H3K27 trimethylation is dynamically regulated by CDK1. Cells were treated with 5 μM CGP for different times, and H3K27 trimethylation was measured by Western blotting. **(c)** Knock-down of CDK1 enhances H3K27 trimethylation. Western blotting analysis of H3K27 trimethylation in HeLa cells transfected with control or CDK1 SMARTpool siRNA (Dharmacon RNA Technologies) for 48 hrs.

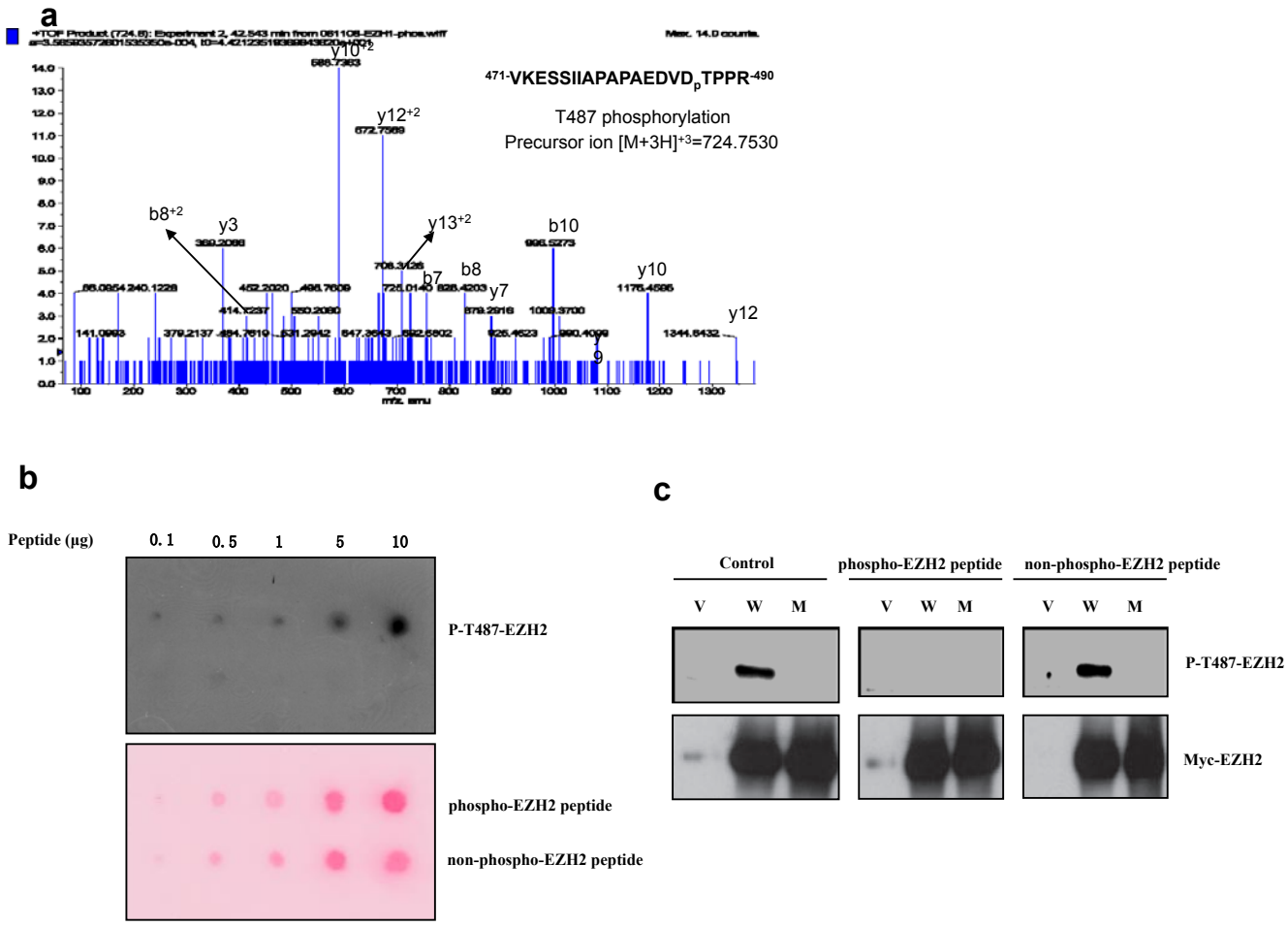

Figure S2 CDK1 phosphorylated EZH2 at Thr487 *in vivo*. **(a)**Lysates of Hela cells were immunoprecipitated with anti-EZH2 and analyzed by mass spectrometry analysis. **(b)** Dot blot assay of the EZH2 peptides containing phosphorylated or non-phosphorylated Thr487. Phospho- or non-phospho-peptides were immunoblotted with an phosphoT487-EZH2 antibody. **(c)** Lysates of 293 cells transfected with Myc-EZH2 or Myc-T487A-EZH2 mutant were immunoprecipitated with anti-Myc. Anti-phosphoT487-EZH2 sera were pre-incubated with peptide (50 mg/mL) at 37 oC for 30 min then immunoblotted. Membrane was stripped and re-immunoblotted with anti-myc antibody. V, Vector; W, WT-EZH2; M, T487A-EZH2 mutant.

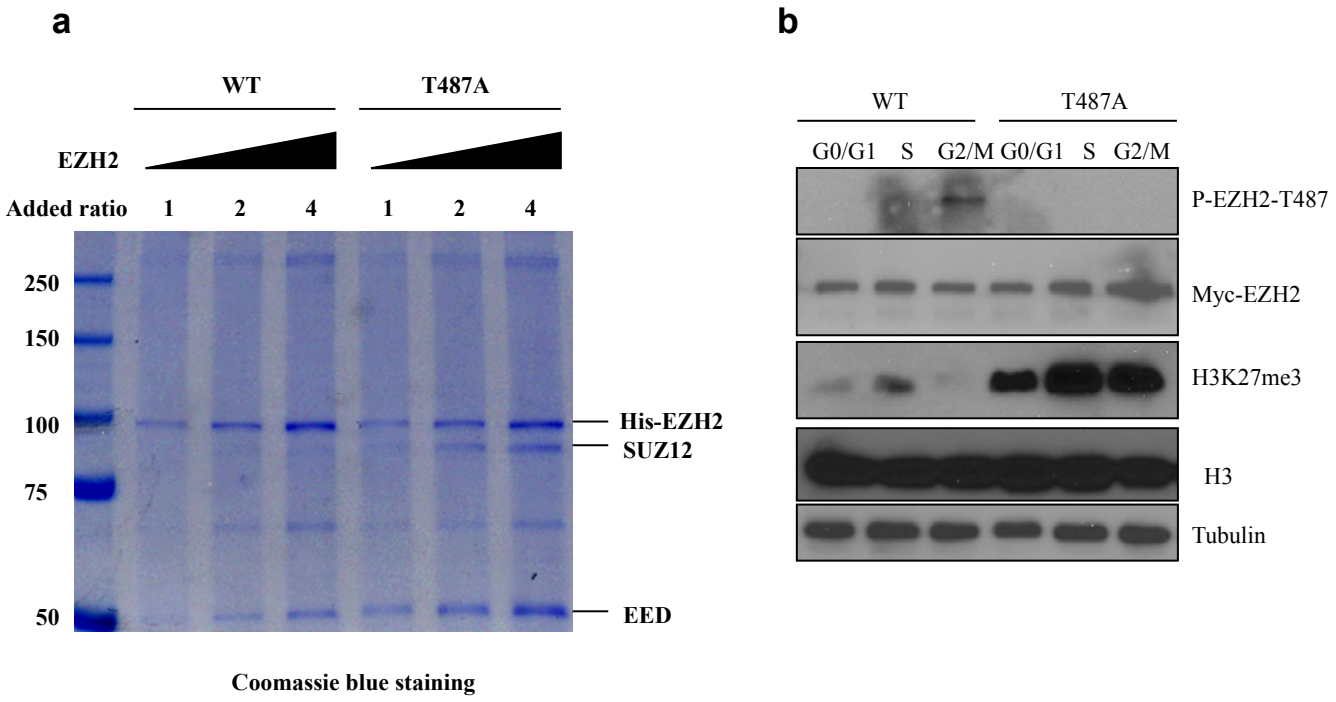

Figure S3 (a) Phosphorylation of EZH2 at Thr 487 disrupts binding of EZH3 with SUZ12 and EED. PRC2 complex were purified from MCF7 cell lines stably expressing Myc-His-tagged WTEZH2 or T487A-EZH2 mutant by using a combination of protein G-crosslinked Myc antibody and nickel column purification. PRC2 complex from WT-EZH2 or T487A-EZH2 mutant were titrated, 0.5, 1, and 2 μg proteins were loaded on SDS-PAGE gel, then stained by coomassie blue. Left side showed the molecular weight marker. Right side showed the names of subunit of the PRC2 complex. **(b)** Phosphorylation of EZH2 at Thr487 was regulated during cell cycle. MCF7 cell lines stably expressing WT-EZH2 or T487A-EZH2 mutant were treated either by serum starvation (to collect cells at G0/G1 phase) or by double thymidine blockage and release to collect cells at S and G2/M phases. Then cells lysates were immunoblotted with antibodies as shown.

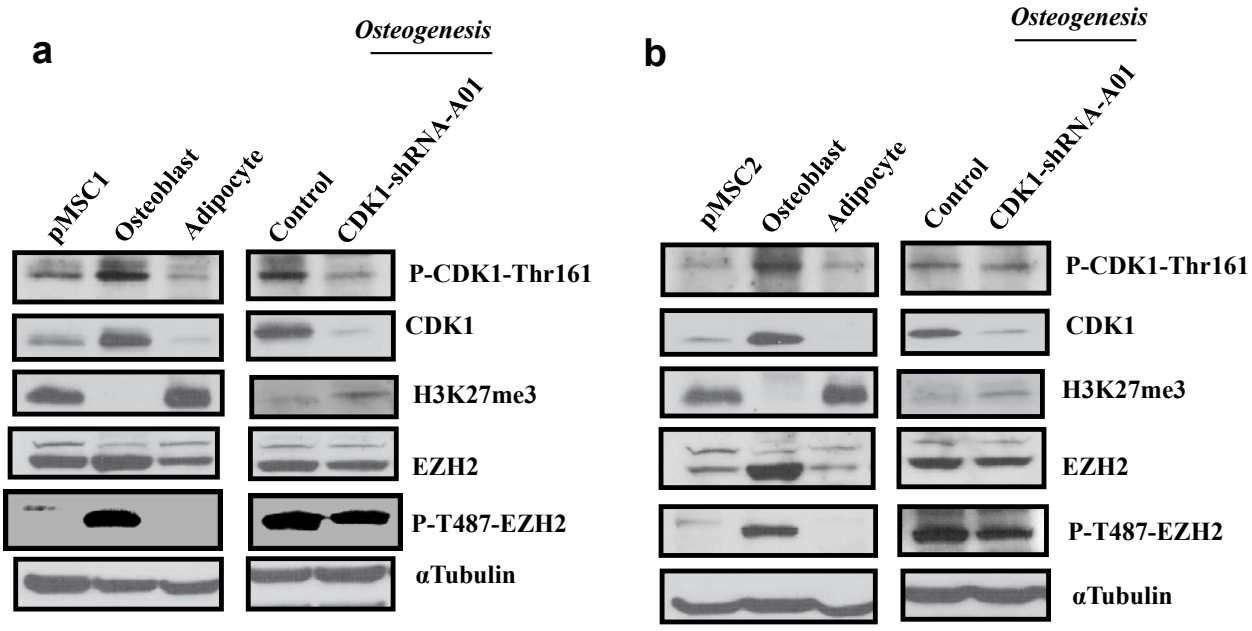

Figure S4 Osteoblast differentiation medium induces activation of CDK1 and phosphorylation of EZH2 in primary hMSC. **(a)** and **(b)** The primary hMSC (pMSC1 and pMSC2) were cultured in control medium or OM with or without CDK1 shRNA infection. Lysates were subjected to immunoblot analysis using antibodies as shown.

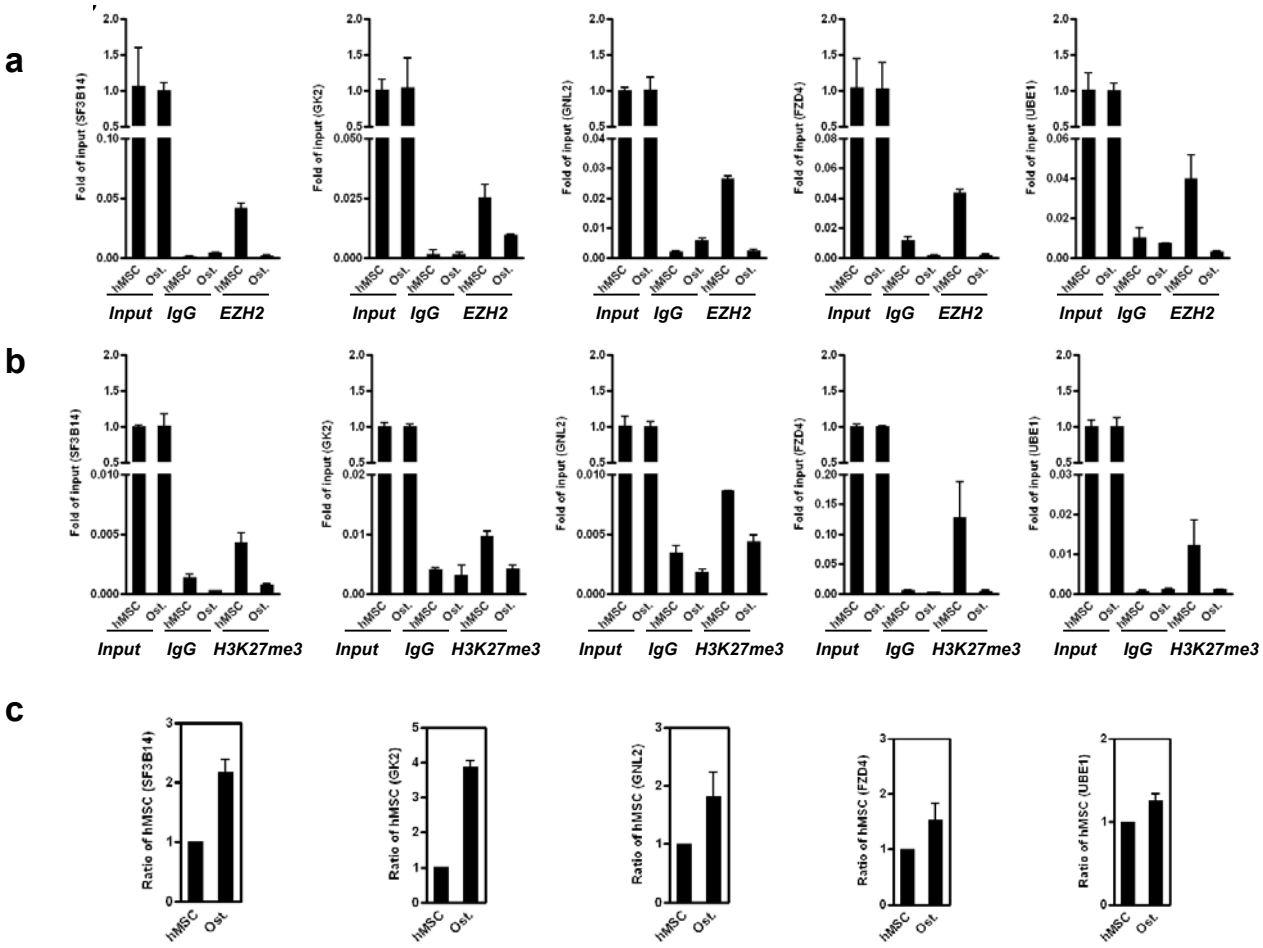

Figure S5 Validation of ChIP-on-chip results for five genes. These five genes were randomly selected from ChIP-on-chip results whose binding to EZH2 were lost after osteogenic differentiation. qChIP were done on the promoters of the five genes indicated by using (**a**)EZH2 antibody and (**b**) H3K27me3 antibody. **(c)** qRT-PCR detection of the expression of the five genes.

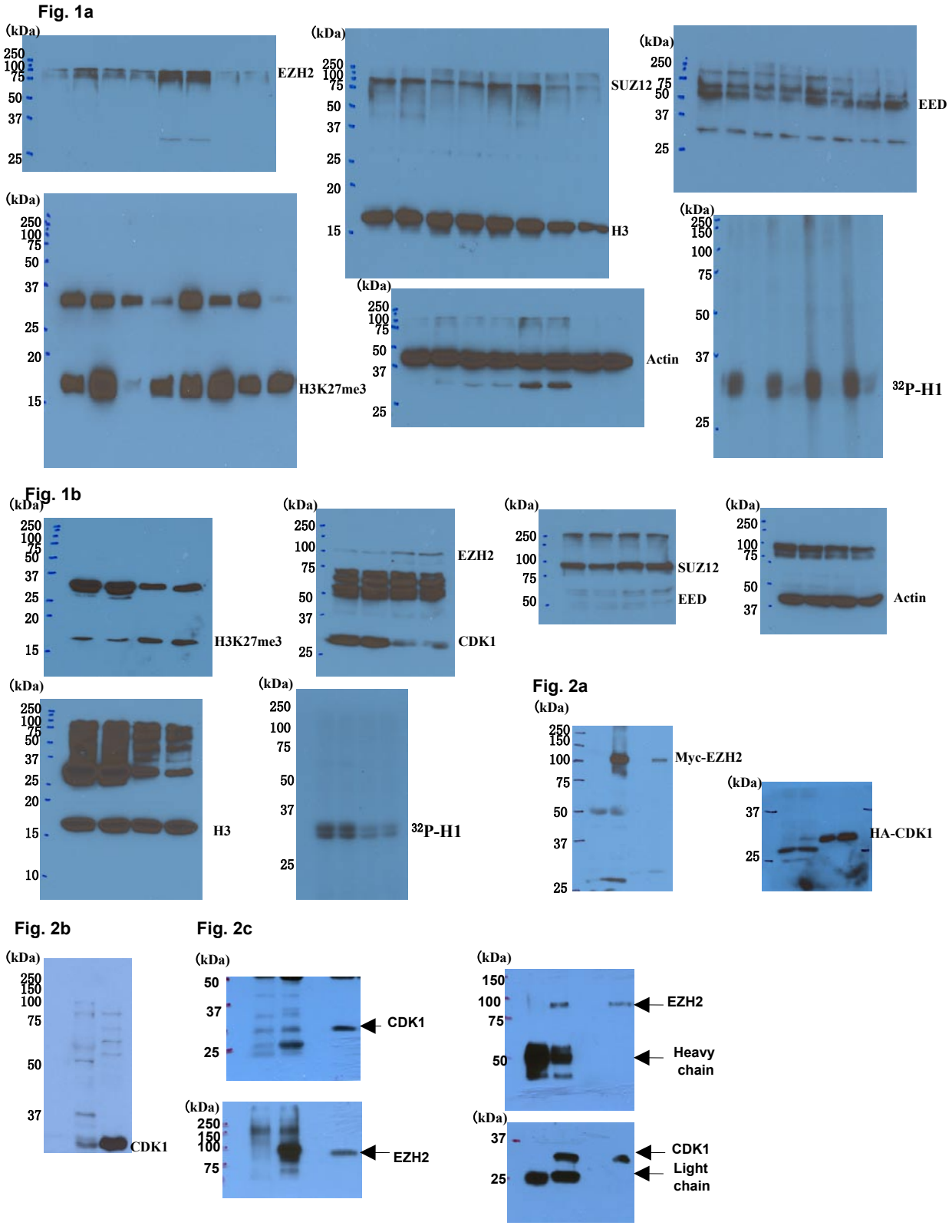

Figure S6 Full scans

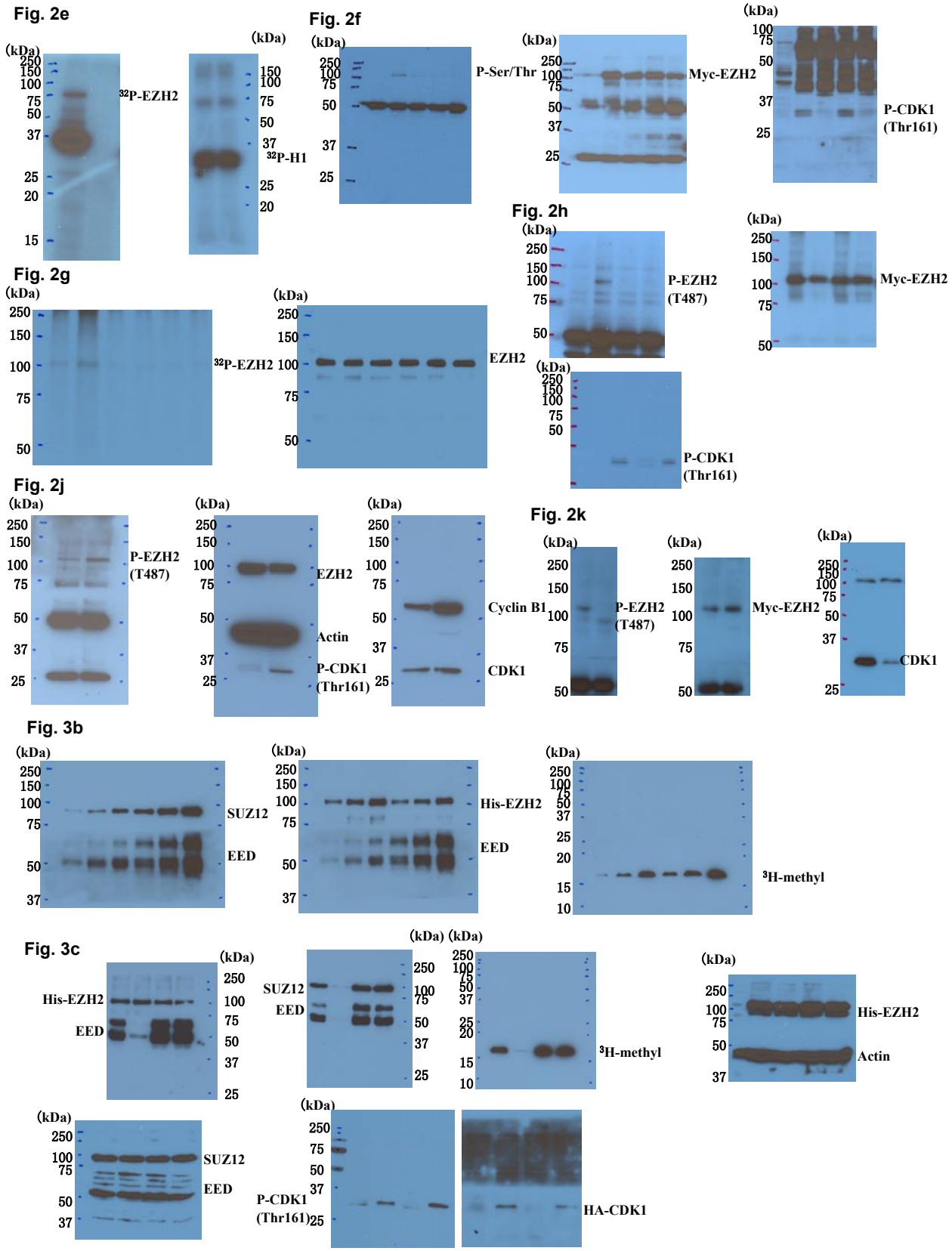

Figure S6 Full scans continued

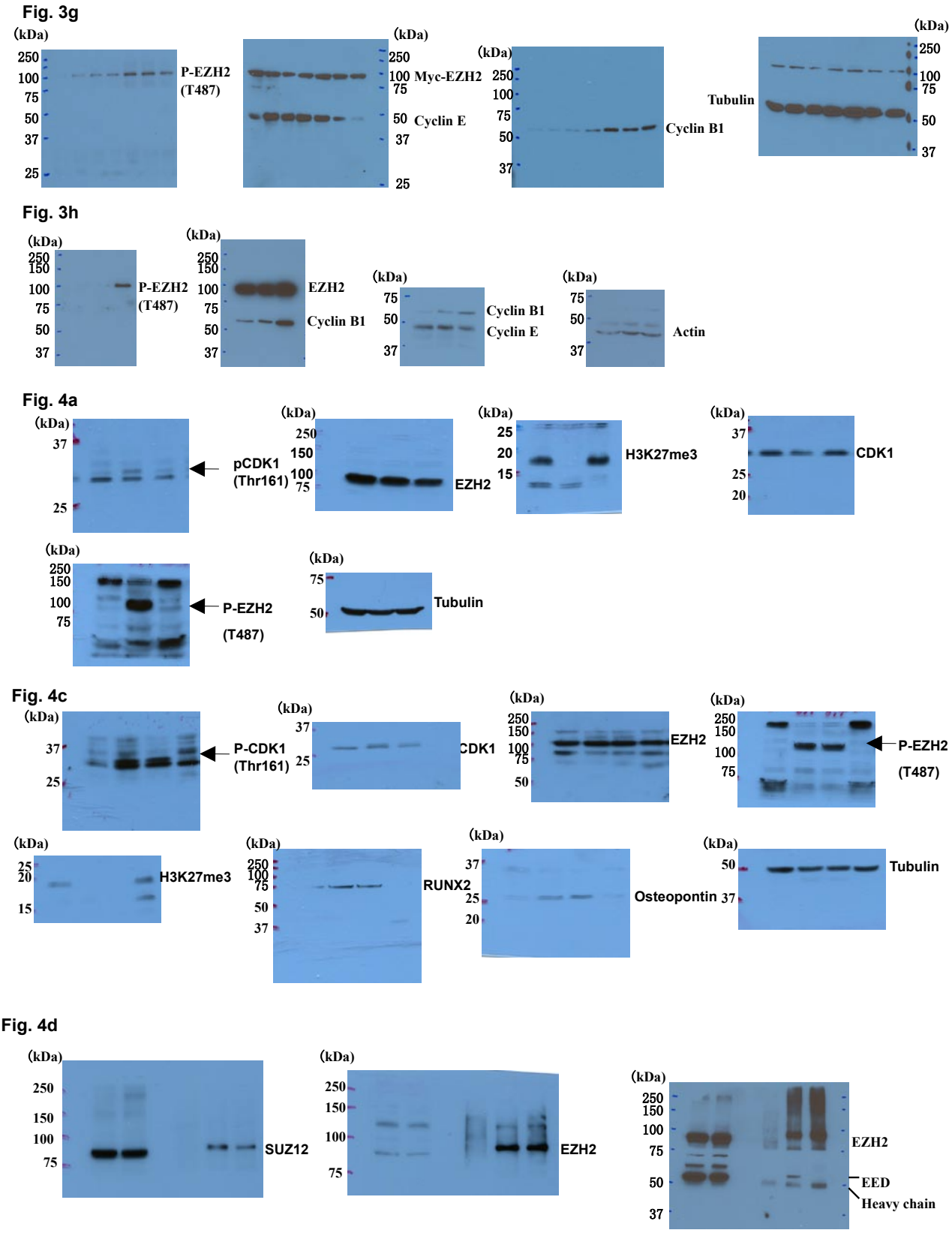

Figure S6 Full scans continued