

Fig. S1: Analysis for GAPDH activity. The glycolytic activity of commercial human GAPDH, purified recombinant human GAPDH (GST-GAPDH) and its variants; C152G, C156G and C247G was monitored by the formation of NADH at 340 nm. The reactions were performed at 25°C at the indicated times.

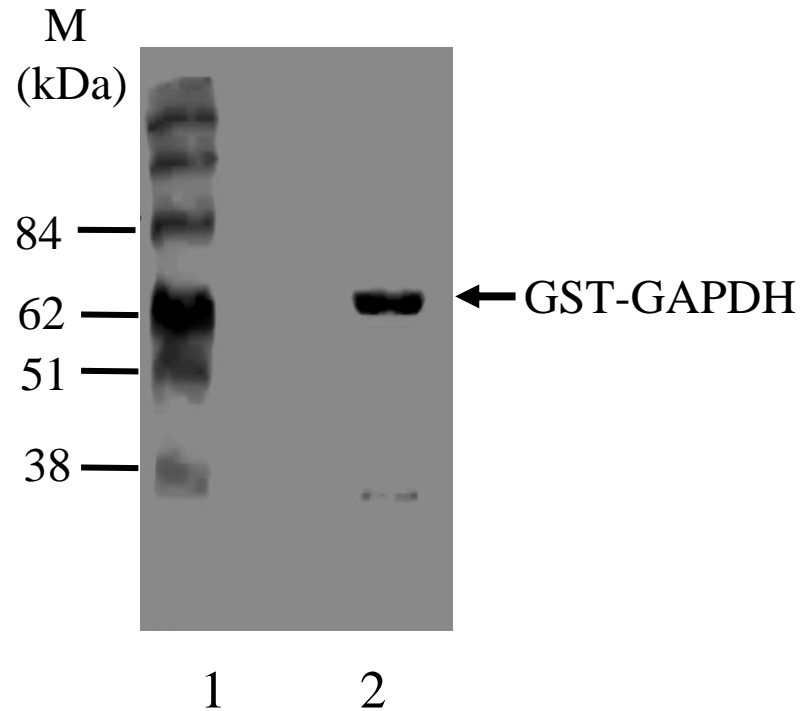


Fig. S2: Expression of recombinant human GAPDH as GST fusion. GST-GAPDH present in total extracts derived from strain BW528 carrying the plasmid pGST-GAPDH was purified on a GST affinity column and analyzed on a SDS-PAGE gel stained with Coomassie blue. Lane 2 contained 200 ng of the affinity-purified recombinant GST-GAPDH fusion protein. Lane 1, molecular weight standards (kDa).

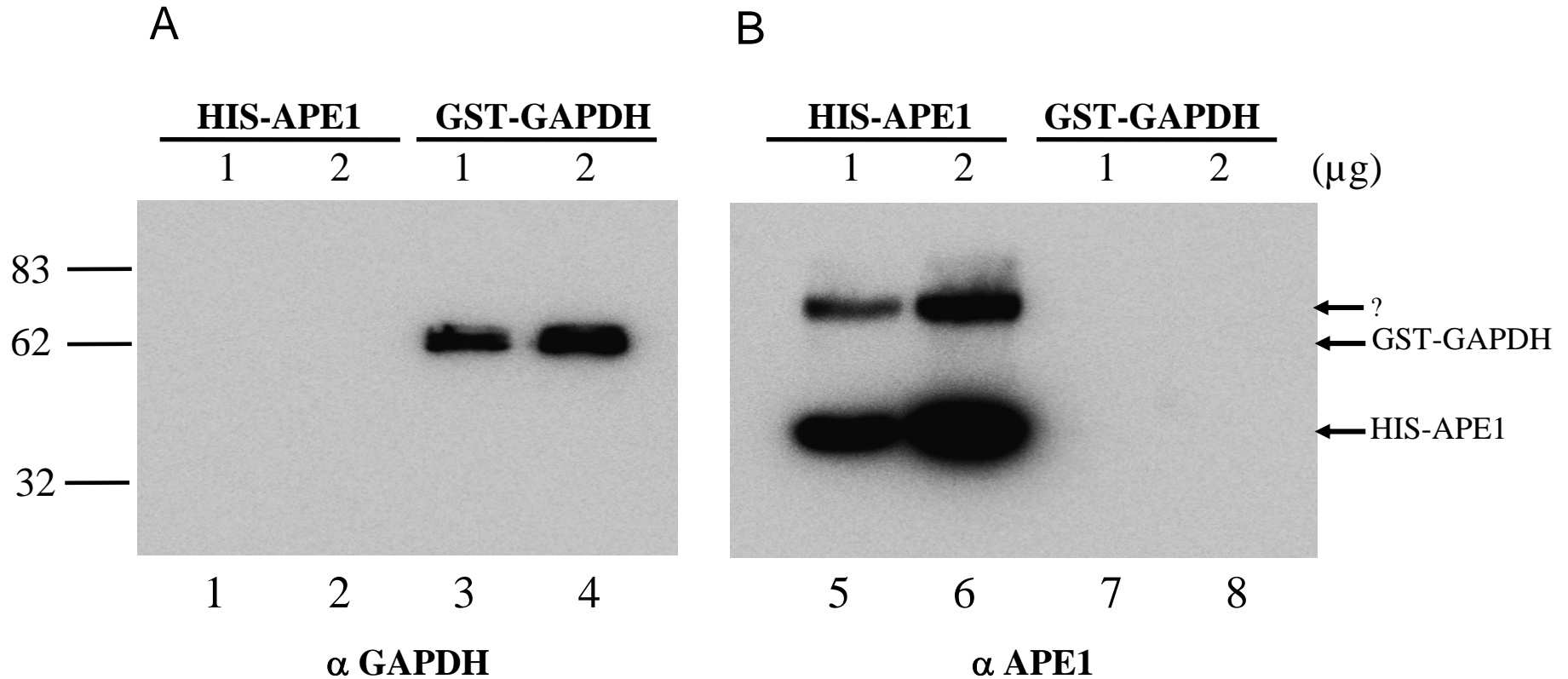


Fig. S3: No cross-reactivity of anti-GAPDH towards APE1 and vice versa. (A) and (B) are duplicated blots with the indicated amounts of purified HIS-APE1 (lanes 1, 2, 5 and 6) and purified GST-GAPDH (lanes 3, 4, 7 and 8), which were probed with anti-GAPDH and anti-APE1, respectively. The upper arrow indicated by ? in (B) is a possible dimeric form of HIS-APE1.

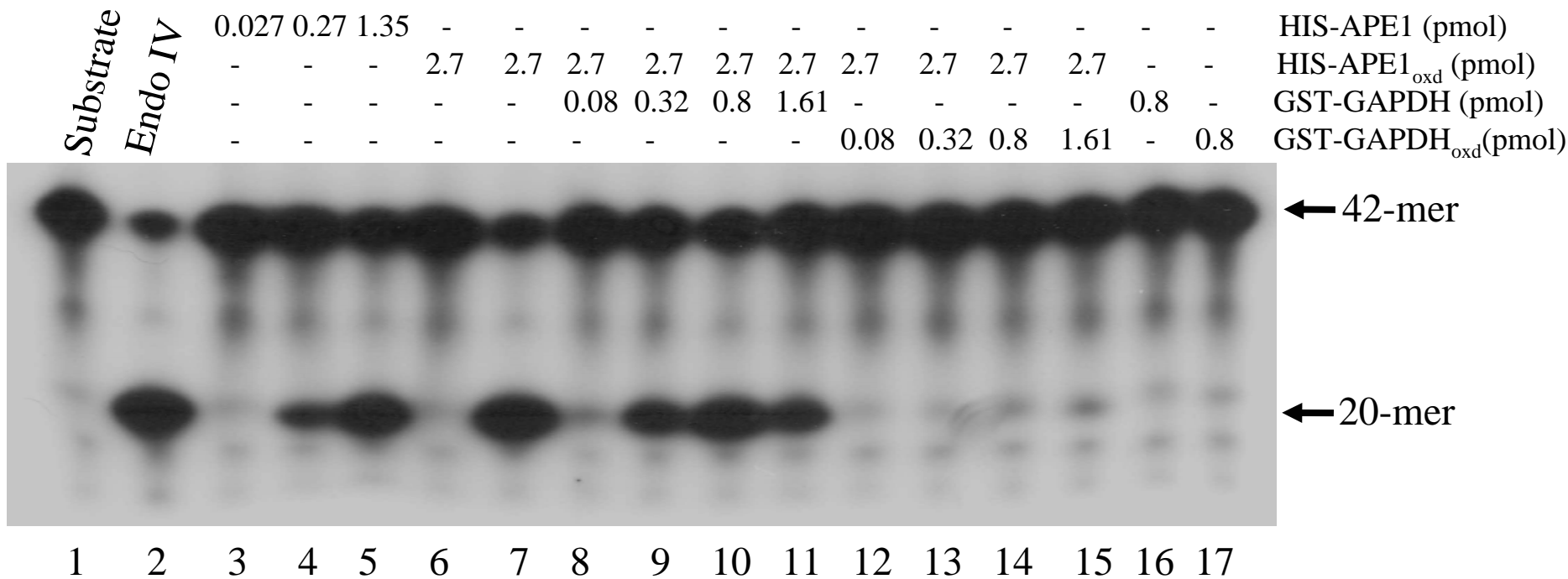


Fig. S4: H₂O₂-inactivated GAPDH is unable to reactivate oxidized APE1. The reactivation of oxidized APE1 was analyzed by AP endonuclease assay in the presence of either reduced (lanes 8-11) or oxidized (lanes 12-15) GAPDH. Both oxidized APE1 and GAPDH were prepared by chemical treatment with H₂O₂.

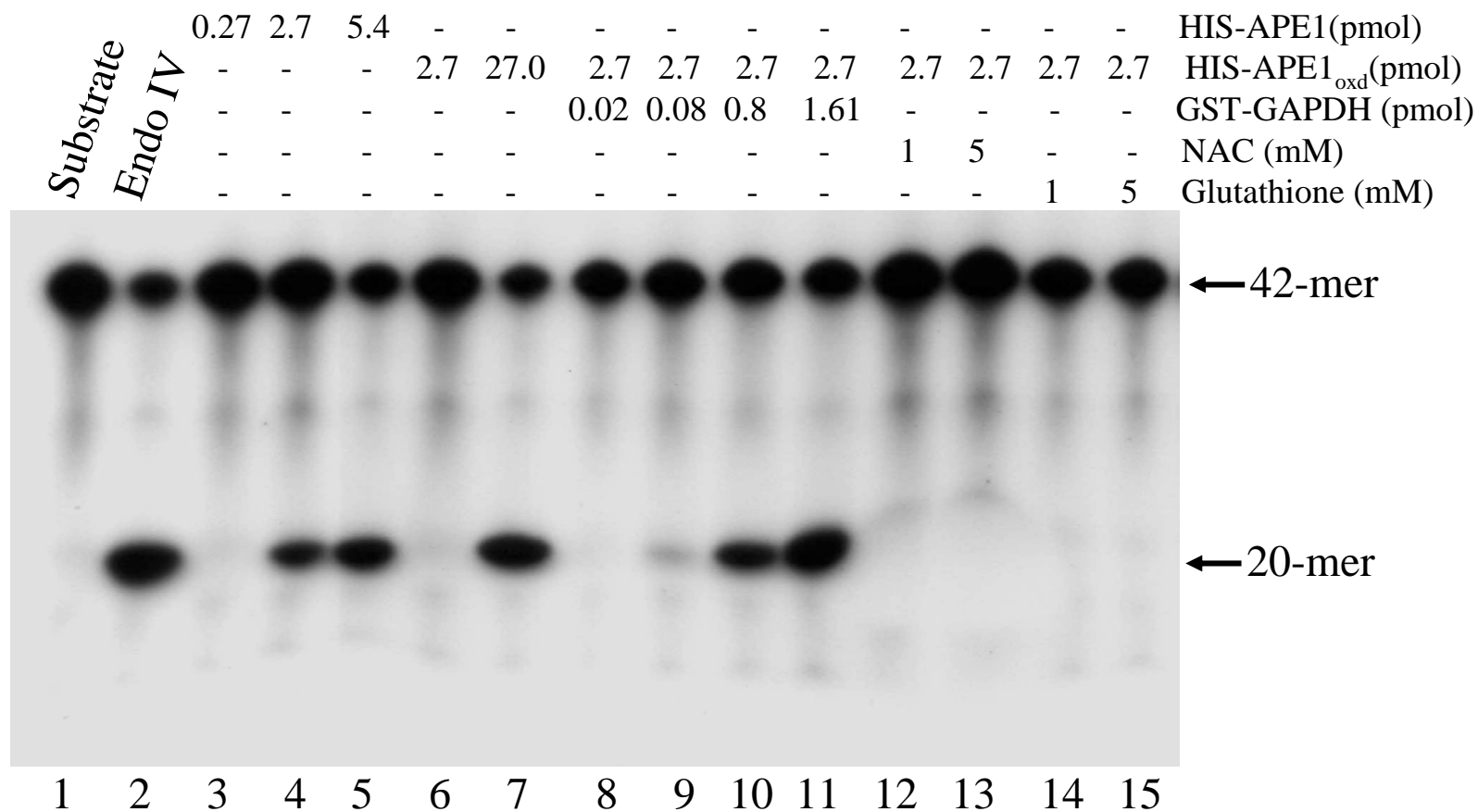


Fig. S5: GAPDH, but not NAC or glutathione, reactivates the AP endonuclease activity of oxidized APE1. Fixed amount of HIS-APE1_{oxd} was incubated with the indicated concentrations of either NAC (lanes 12 and 13) or glutathione (lanes 14 and 15) for 5 min at RT and assayed for AP endonuclease activity. Controls (lanes 1-7) are as in panel A, lanes 8-11, fixed amount of HIS-APE1_{oxd} incubated with increasing concentrations of purified GST-GAPDH.

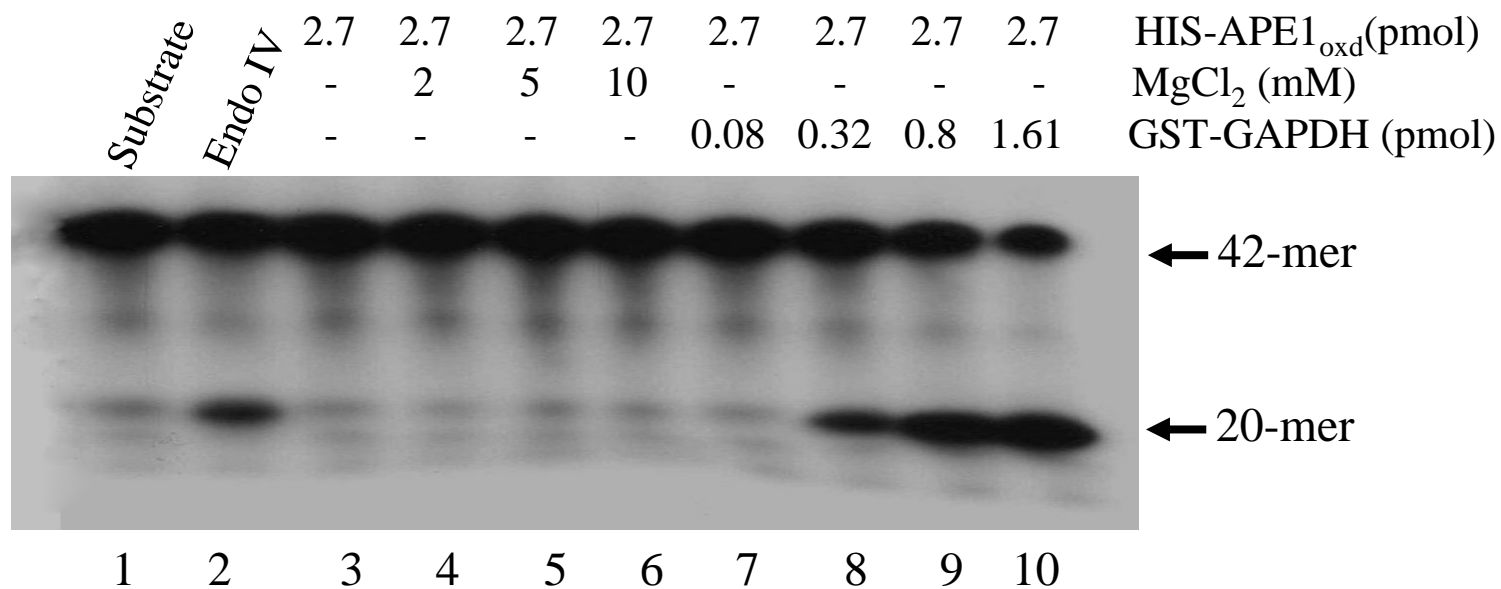


Fig. S6: Oxidized APE1 is not reactivated by Mg²⁺. HIS-APE1 was subjected to H₂O₂-oxidation and the enzyme activity was analyzed by AP endonuclease assay in the presence of increasing concentrations of either MgCl₂ (lanes 4-6) or GST-GAPDH (7-10).

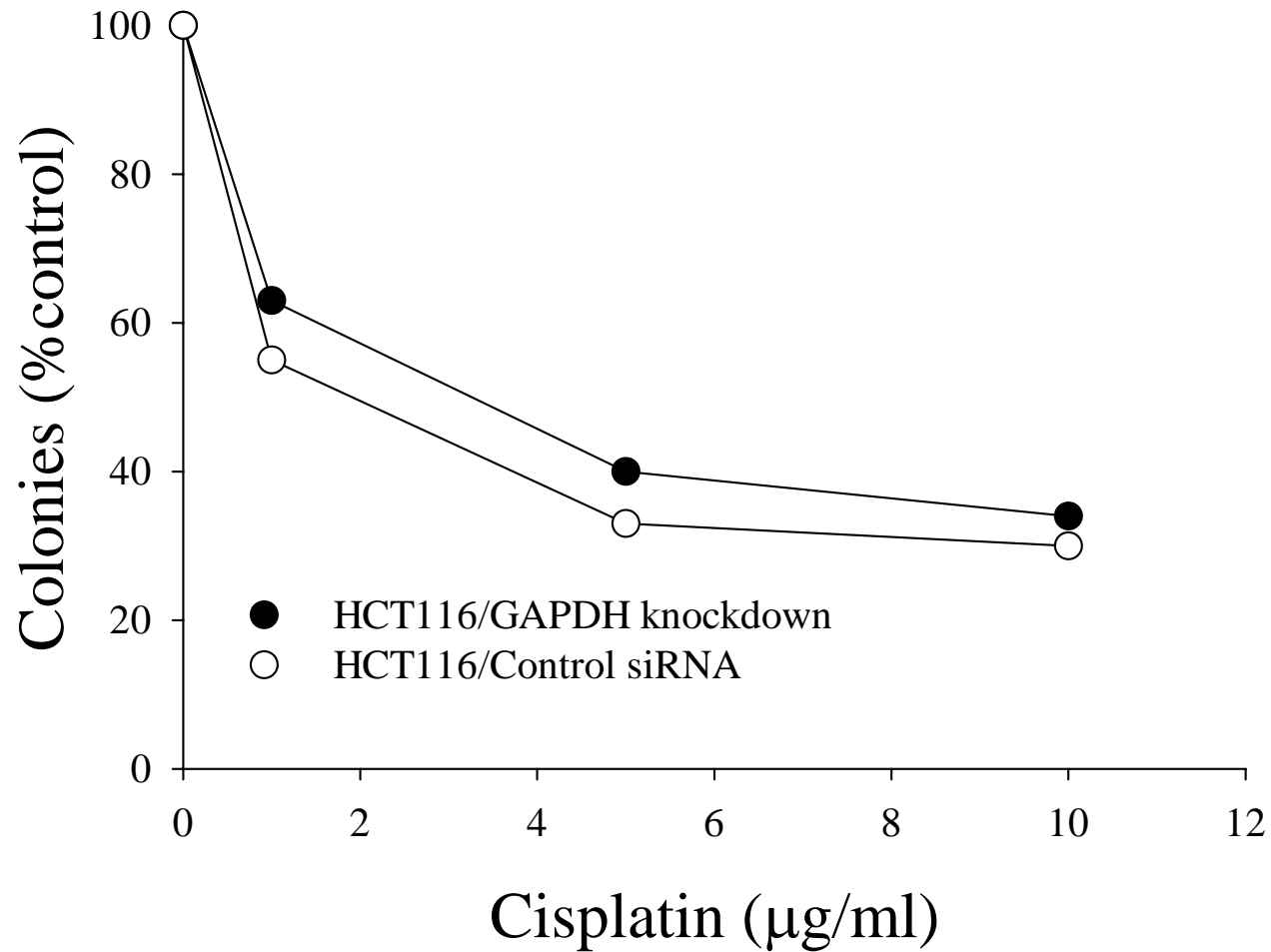


Fig. S7: GAPDH knockdown cells are not sensitive to cisplatin. Cells were treated for 1 hr with cisplatin in FBS free media and then plated to score for the surviving fractions by colony assay.