Bivalent molecular mimicry by ADP protects metal redox state and promotes coenzyme B_{12} repair

Harsha Gouda, Romila Mascarenhas, Markus Ruetz, Madeline Yaw and Ruma Banerjee*

Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109

*__Corresponding Author__: Ruma Banerjee, rbanerje@umich.edu

Table S1: AdoCbl loading onto human MCM from the ATR•AdoCbl•PPPi ternary product complex*

Metabolite	**Rate of transfer (k_{obs})**	**% Completion**
No metabolite	ND	ND
ATP	0.09 ± 0.01 min^{-1}	58-65 %
M-CoA	ND	ND
ATP and M-CoA	0.21 ± 0.01 min^{-1}	92-94 %

ND: not detectable, *The data represent the mean ± SD of 3 independent experiments.

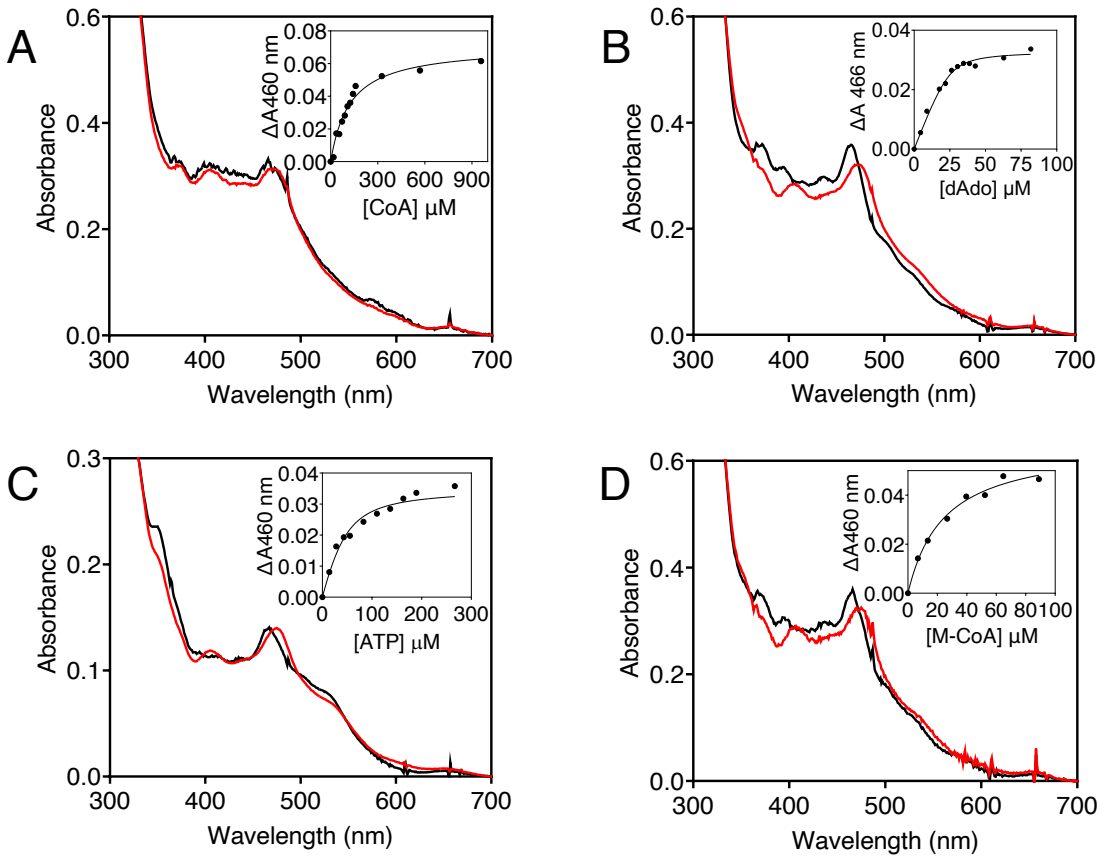

Figure S1: Determination of dissociation constants for binding of CoA (**A**), dAdo (**B**), ATP (**C**), and M-CoA (**D**) to the human MCM•cob(II)alamin complex. Varying concentrations of each metabolite were titrated into a solution of MCM•cob(II)alamin (40 μM cofactor concentration) in anaerobic buffer A at 20 ˚C. *Insets*. The difference in 460 nm absorbance was plotted versus metabolite concentration to obtain the respective K_d values. The spectra are representative of at least three independent experiments.

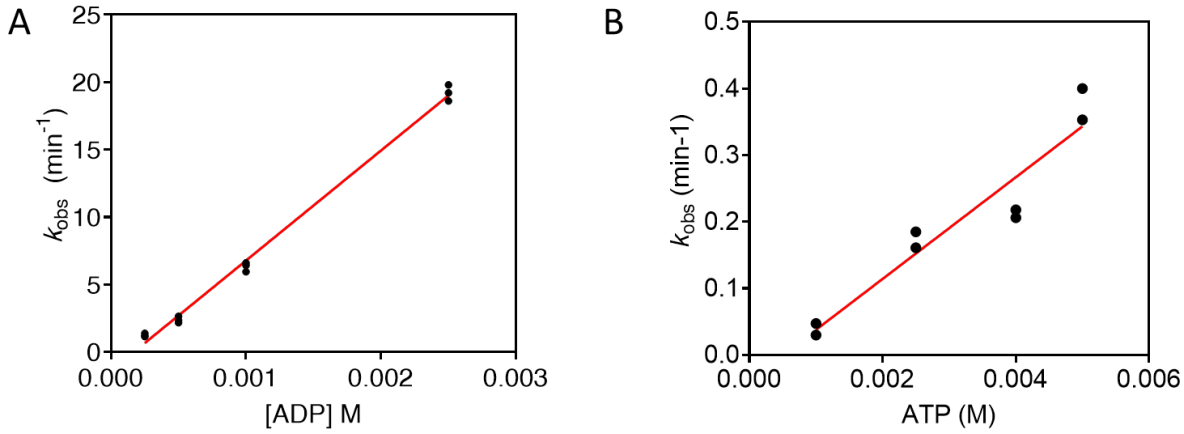

Figure S2: ADP binds more rapidly than ATP to the MCM•cob(II)alamin complex. Varying concentrations (0.25–2.5 mM) of ADP (**A**) or (1–5 mM) ATP (**B**) were rapidly mixed with 40 μM human MCM•cob(II)alamin (1:1) in anaerobic buffer A at 25˚C and the k_{obs} was plotted as a function of the respective nucleotide concentration. The data were obtained from two independent experiments.

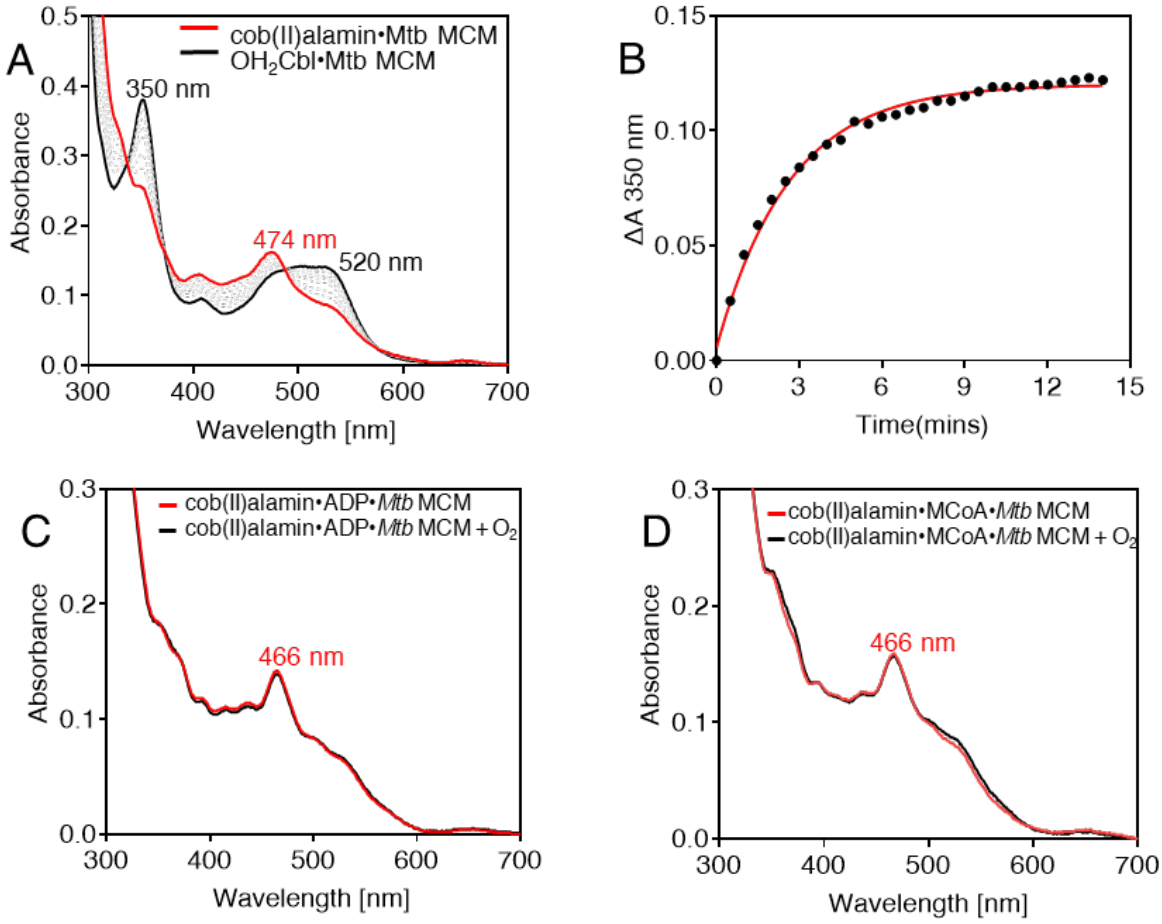

Figure S3: Metabolites prevent *Mtb* MCM•cob(II)alamin oxidation. (**A**) *Mtb* MCM•cob(II)alamin (20 μM in buffer A, *red* trace) oxidizes to H_2OCbl (*black* trace) in air. (**B**) *Mtb* MCM•cob(II)alamin oxidation was monitored at 350 nm and yielded a k_{obs} of 0.28 min^{-1} at 20˚C. (**C, D**) Binding of ADP (C) or M-CoA (D) to *Mtb* MCM•cob(II)alamin (*red*) prevents cofactor oxidation (*black*).

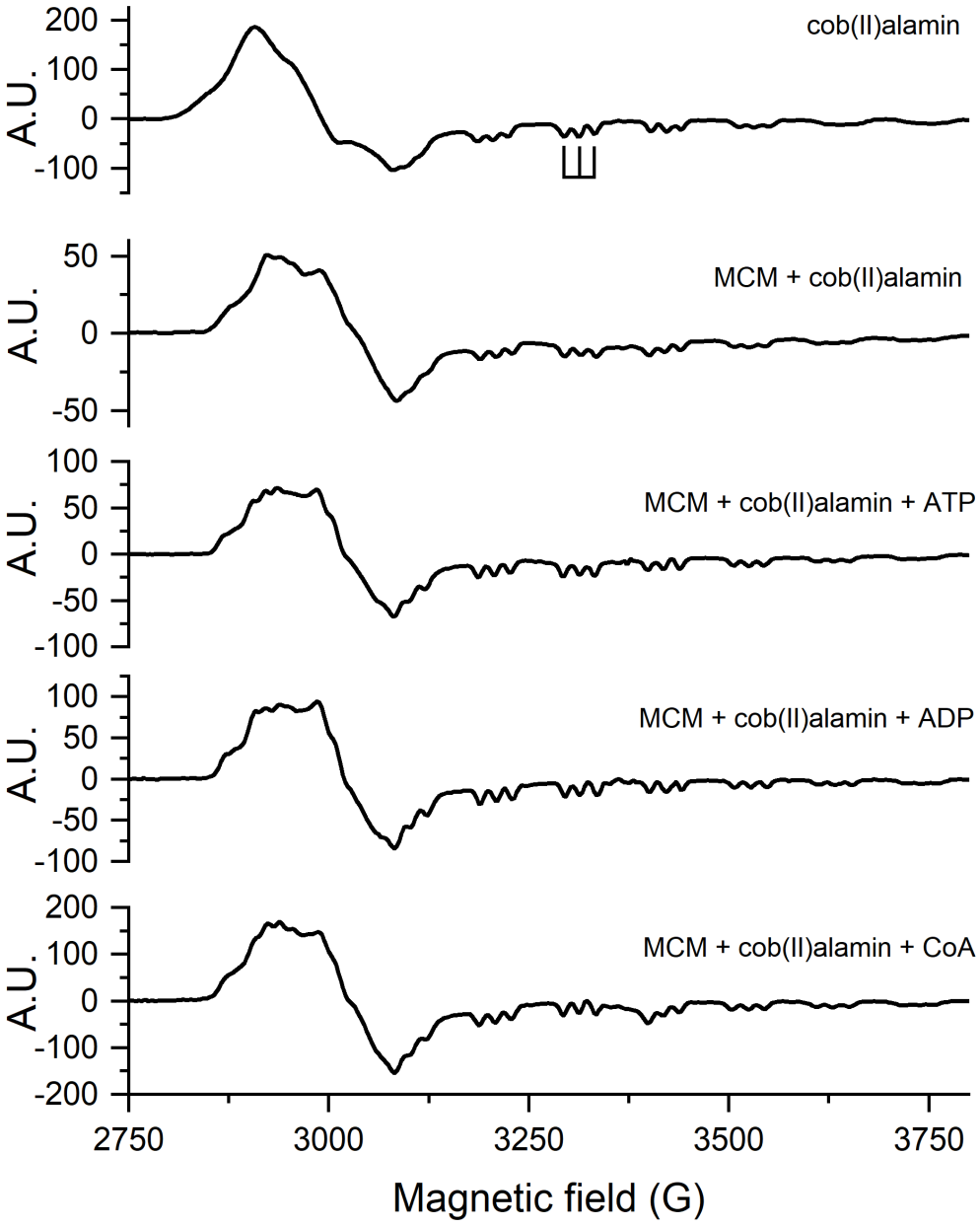

Figure S4: Cob(II)alamin remains 5-coordinate upon binding of metabolites to human MCM. EPR spectra of cob(II)alamin (100 μM) free or bound to human MCM (100 μM homodimer) in 50 mM HEPES, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, 10 % glycerol pH 7.5 buffer in the presence of the indicated metabolites (5 mM each)**.** Triplet superhyperfine structures (vertical lines) indicate coupling of a nitrogen ligand to the unpaired electron in cob(II)alamin and therefore, retention of the 5-coordinate state. The EPR spectra were obtained at 80K using the following parameters: 9.38 GHz microwave frequency, power 2 mW, modulation amplitude 10 G, modulation frequency 100 kHz, 3000 G sweep width centered at 3500 G, conversion time 164 ms, time constant 82 ms. A.U. = arbitrary units

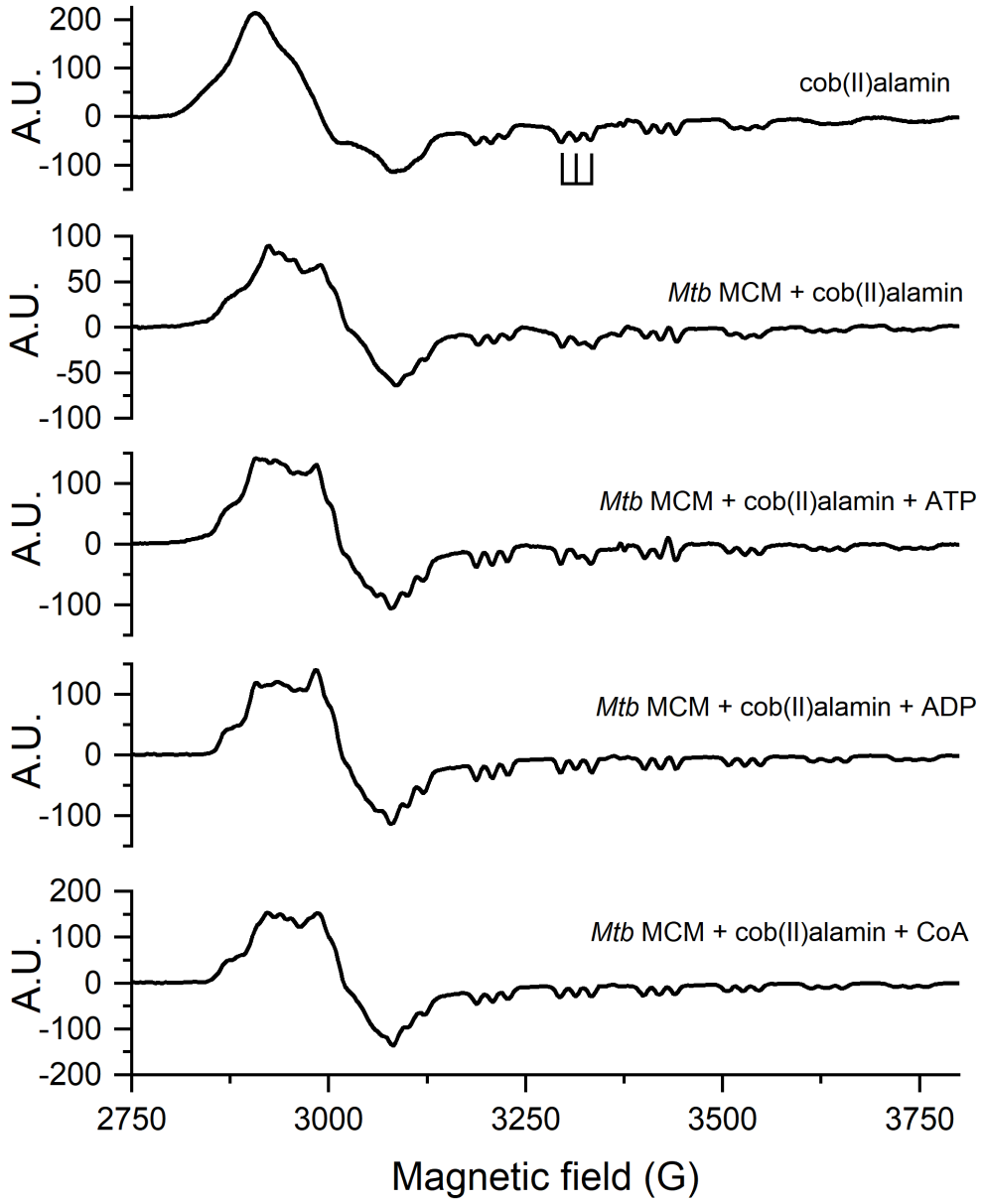

Figure S5: Cob(II)alamin remains 5-coordinate upon binding of metabolites to *Mtb* MCM. EPR spectra of cob(II)alamin (100 µM) free or bound to *Mtb* MCM (100 µM heterodimer) in 50 mM HEPES, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, 10 % glycerol pH 7.5 buffer in the presence of the indicated metabolites (5 mM each). Triplet superhyperfine structures are indicated by the vertical lines. The EPR spectra were obtained at 80K using the following parameters: 9.38 GHz microwave frequency, power 2 mW, modulation amplitude 10 G, modulation frequency 100 kHz, 3000 G sweep width centered at 3500 G, conversion time 164 ms, time constant 82 ms. A.U. = arbitrary units

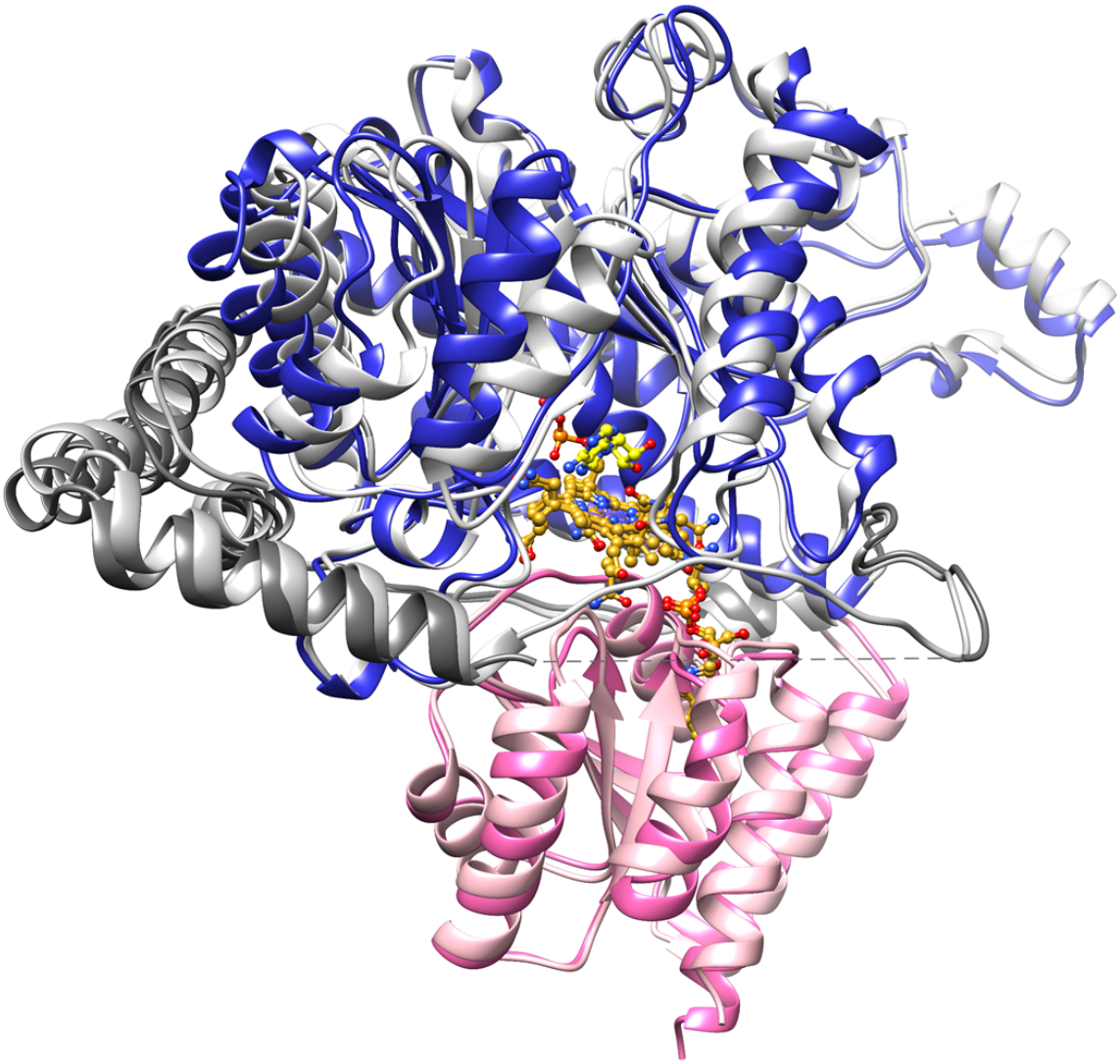

Figure S6: Overlay of human MCM•cob(II)alamin•ADP (*light shades*) and MCM•OH_2Cbl (*dark shades*). ADP induces closing-in of the substrate domain and ordering of the interconnecting belt (*light grey*). ADP thus protects cob(II)alamin from oxidation by O_2 and precludes H_2O binding in the upper axial ligand position to coordinate the resulting cob(III)alamin, which prefers a 6-c geometry. This figure is an enlarged view of Fig. 3A.

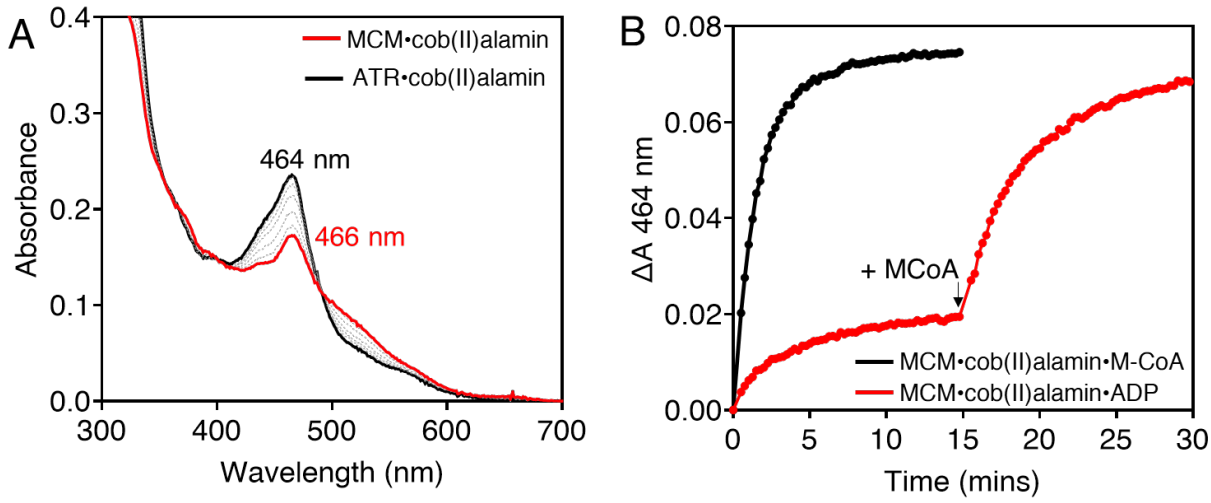

Figure S7. **CblA•GTP-catalyzed cob(II)alamin off-loading from human MCM to ATR**. (**A**) Transfer of cob(II)alamin from MCM (*red*) to ATR (*black*) is signaled by an increase in intensity at 464 nm. (**B**) M-CoA (*black*) promotes while ADP (red) limits cob(II)alamin off-loading from MCM. The inhibitory effect of ADP is reversed by M-CoA.