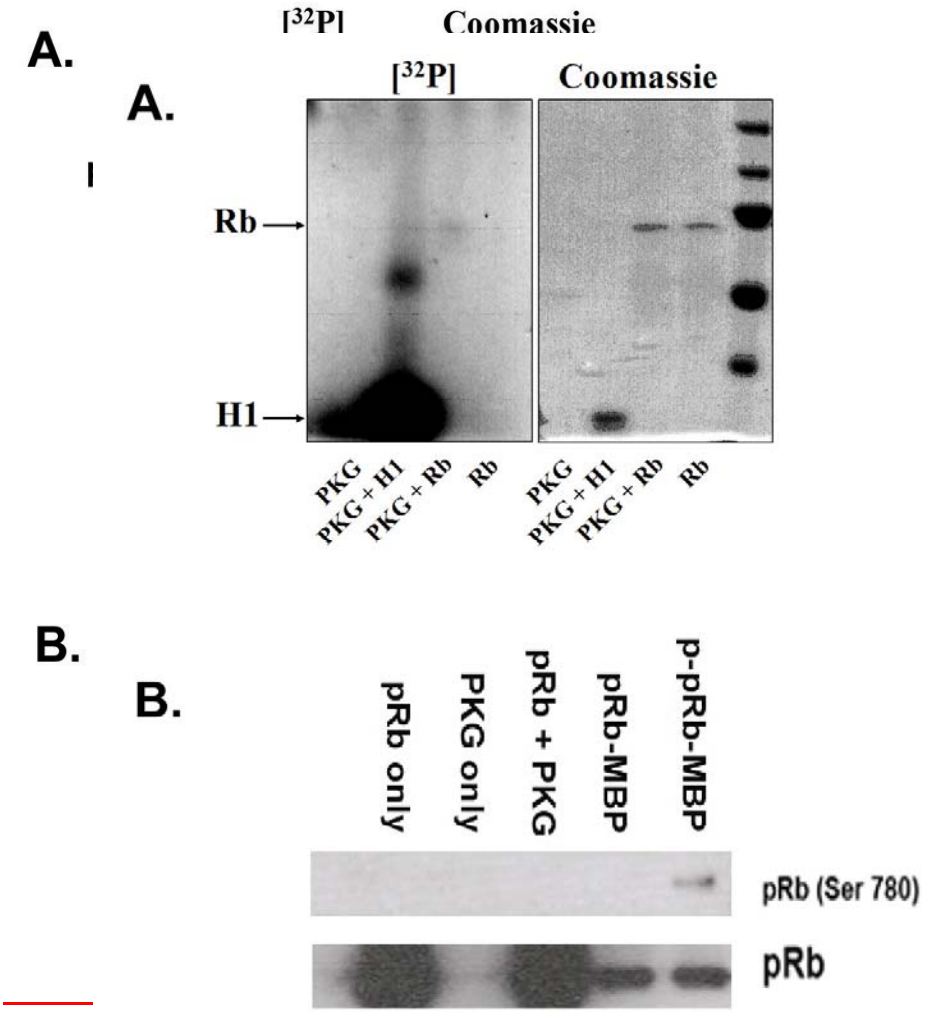
SI Fig. 1. pRb is a weak substrate for PKG *in vitro*. (A) *In vitro* kinase assay. FLAG epitope tagged PKG1a was expressed in HEK293T cells and immunopurified using anti-FLAG-M2 agarose. The pure enzyme was incubated alone, with histone H1 (2.5 μg positive control), with cRb fusion protein (2.2 μg), in kinase buffer containing 10 μM cGMP and 10 μCi [^{32}P]ATP. Rb incubated in reaction buffer without PKG is shown as a negative control. Reactions were at 37^{o}C for 90 min. Reactions were stopped by boiling in PAGE buffer and then the whole reaction was separated on 10% gels and then subjected to autoradiography. (B) Kinase assay reactions were labeled with anti-pRb (Ser780) antibody with non-phosphorylated pRb-MBP and phosphorylated pRb-MBP as controls.

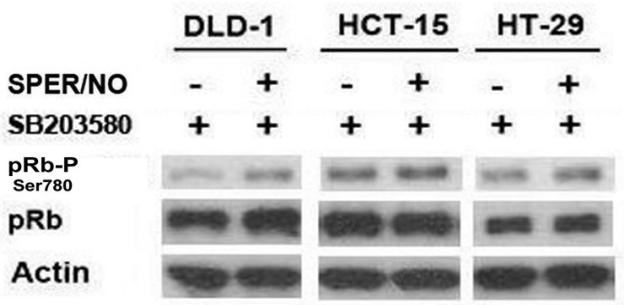
SI Fig. 2. The MAPK/p38 kinase pathway is not involved in nitric oxide mediated pRb hyperphosphorylation. Three human colon cancer cell lines were either pre-treated with vehicle or SB203580 (10 μM), an MAPK/p38 phosphorylation inhibitor, then exposed to SPER/NO (50 uM). This inhibitor was not capable of inhibiting the induction of pRb phosphorylation by SPER/NO.

SI Fig. 3. pRb is degraded in colon cancer cells exposed to high levels of SPER/NO. (A) HT-29 cells were treated with indicated concentrations of SPER/NO for 4 hours. As NO concentrations exceed 100 μM, both pRb and its phosphorylation form decrease. p53 phosphorylation levels continue to increase, consistent with previously published results (Hofseth et al., PNAS, 2003; Thomas DD et al., PNAS, 2004). (B) HT-29 cells were co-cultured with different ratios of stimulated ANA-1 cells. pRb and its phosphorylation form decrease at 1:10 when NO concentrations is above 50 μM. (C) pRb and phosphorylated pRb level of TK-6 (a lymphoblastoid cell line) and HCT-116 cells (another human colon cancer cell line), decrease after incubation with 0.5 mM SPER/NO.

Supporting Information Figure 6



Supporting Information Figure 7



Supporting Information Figure 8