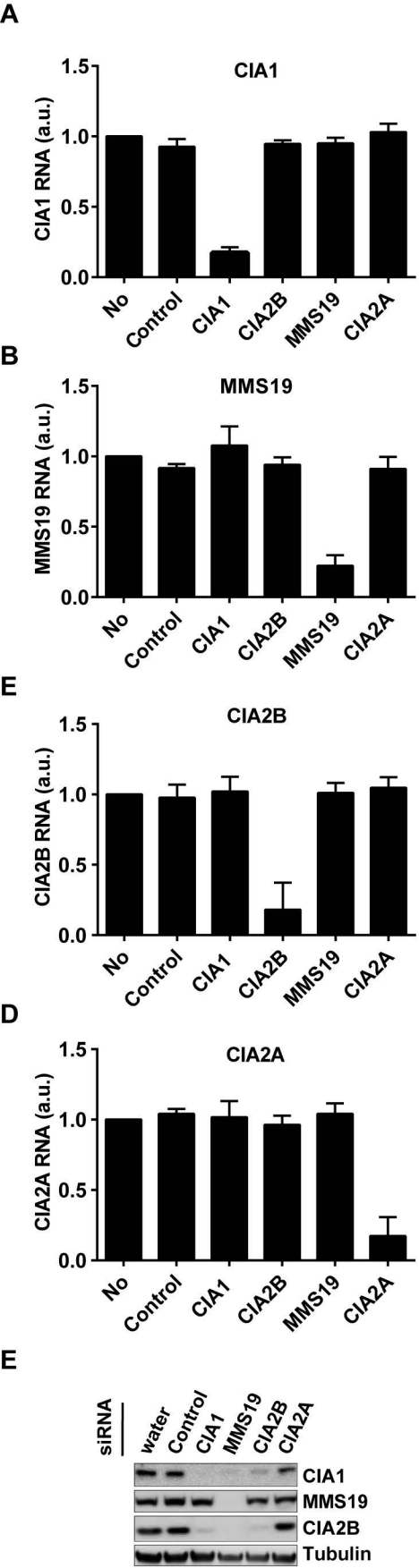


**Figure S1. RNAi-mediated depletion of individual CIA targeting factors decreases the cellular protein levels of other CIA targeting components.**
HEK 293T cells were transiently transfected either with siRNAs directed against the indicated CIA targeting factors or with control siRNA. Residual mRNA levels of CIA1 (A), CIA2B (B), MMS19 (C) and CIA2A (D) were determined, normalized to β-actin mRNA, and presented relative to mock-transfected cells (no siRNA). (E) Cell extracts were analyzed by immunoblotting for the steady-state protein levels of the indicated CIA targeting factors. Tubulin served as a loading control. Representative results of three independent experiments are shown.

Fig. S2, Upadhyay *et al*.

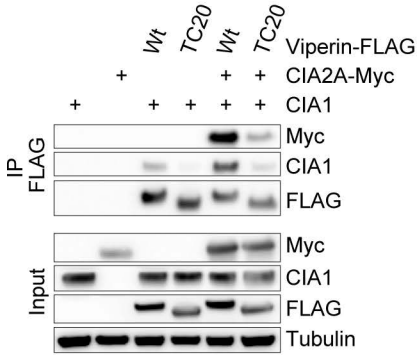


**Figure S2. CIA1 and CIA2A binding to viperin is inefficient when its C terminus is removed.** HEK293T cells were transiently transfected with plasmids encoding wild-type and TC20 viperin-FLAG, CIA1 and Myc-tagged CIA2A as indicated (+). After growth for one day, cell extracts were subjected to anti-FLAG immunoprecipitation (IP). Whole cell lysates (input) and immunoprecipitated proteins were analyzed by immunoblotting using antibodies as indicated. Tubulin served as loading control. The blots are representative of three independent experiments.

Fig. S3, Upadhyay et al.

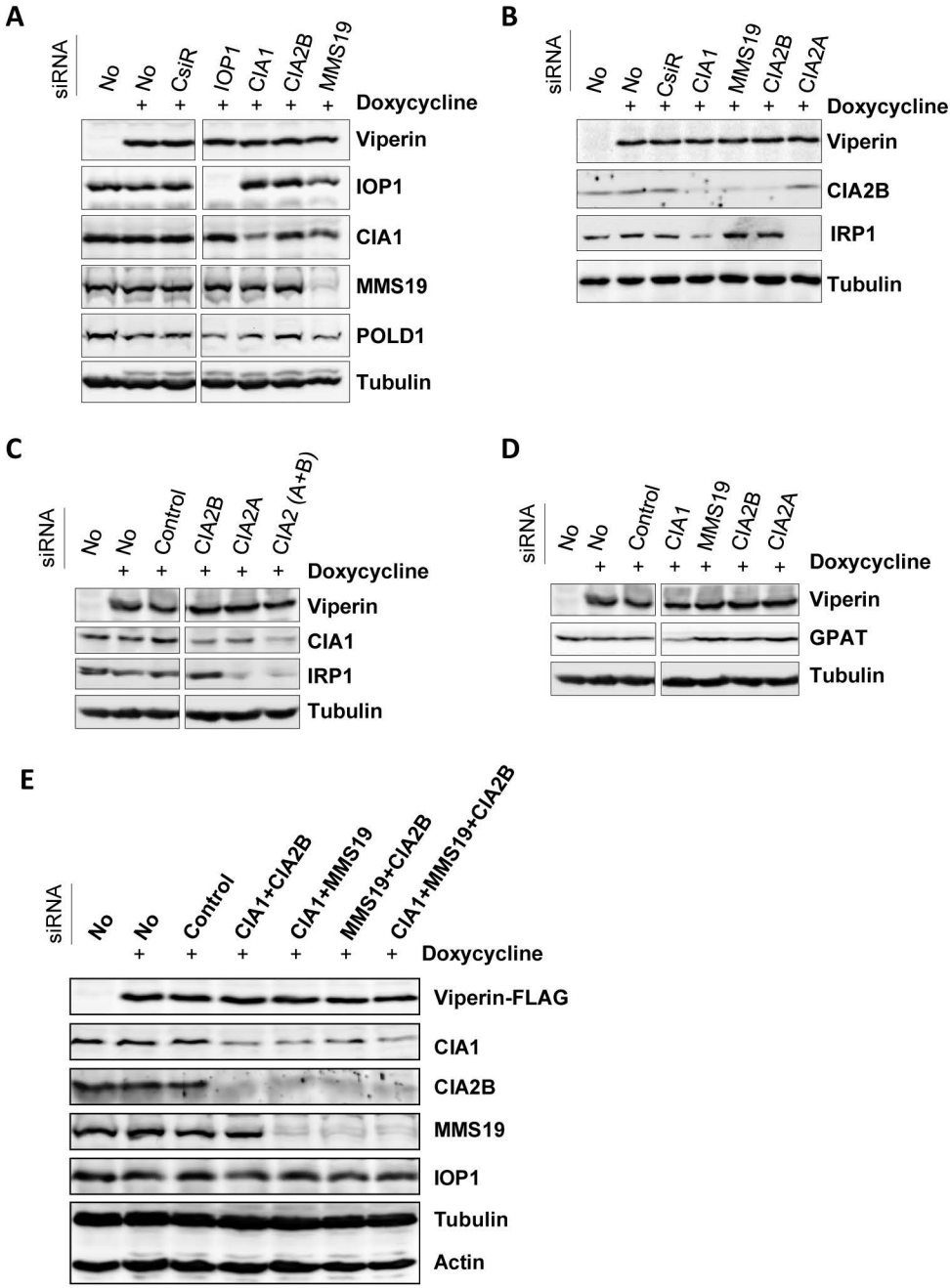


**Figure S3. Protein levels of CIA factors and Fe/S target proteins after RNAi-mediated CIA protein depletion.** HEK FLP-IN T Rex cells expressing FLAG-tagged viperin were depleted for the indicated CIA factors by two successive rounds of siRNA transfection at a three-day interval. During transfection round two, one sample aliquot was used for 55Fe radiolabeling (see Fig. 5). Another aliquot served for the determination of the steady state-protein levels of the indicated CIA factors or the Fe/S client proteins POLD1, IRP1 and GPAT. Tubulin and actin served as loading controls. Representative results from four independent experiments are shown. Note that the bands for viperin and tubulin in parts C and D were taken from the same immunostain, which was cropped differently for the two parts.