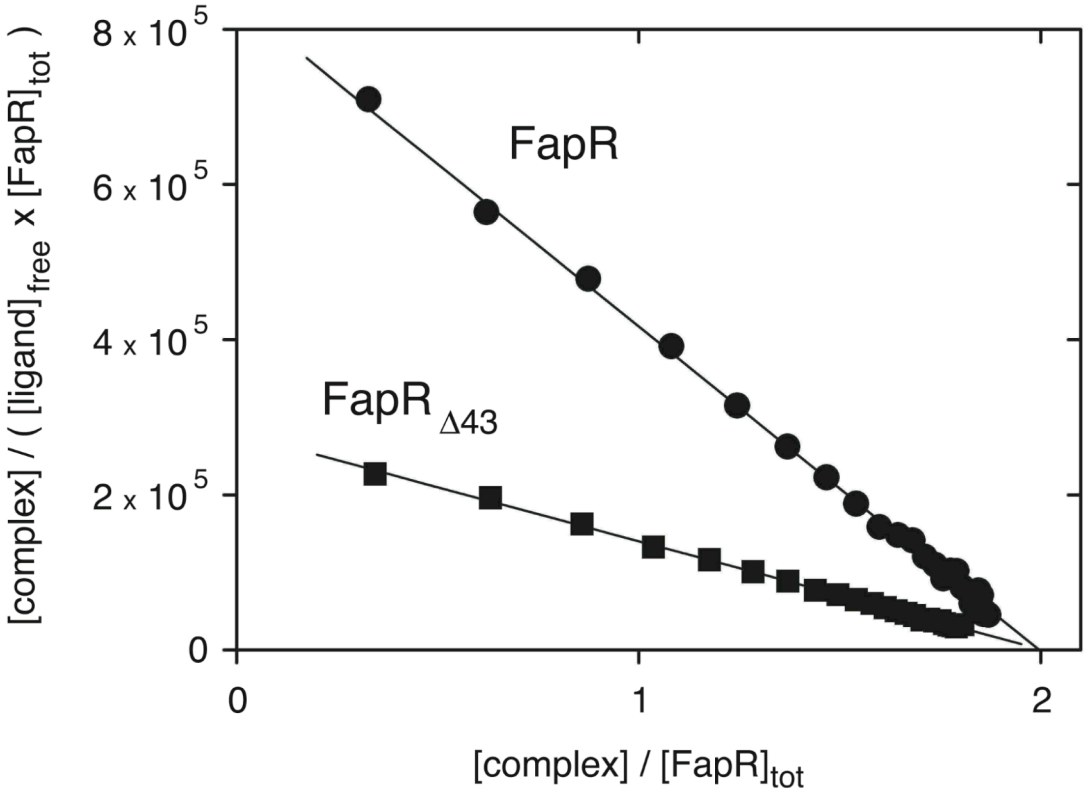

Figure S1. Scatchard plot of malonyl-CoA binding to full-length FapR and $FapR_{\Delta 43}$ produced from the isothermal titration calorimetry (ITC) data presented in Figure 3A of the main manuscript. For each protein, the ratio of bound to free malonyl-CoA varies linearly with bound malonyl-CoA as the total concentration of inducer increases in the calorimeter cell with every injection. The straight lines indicate the presence of two independent malonyl-CoA-binding sites with identical association constants.

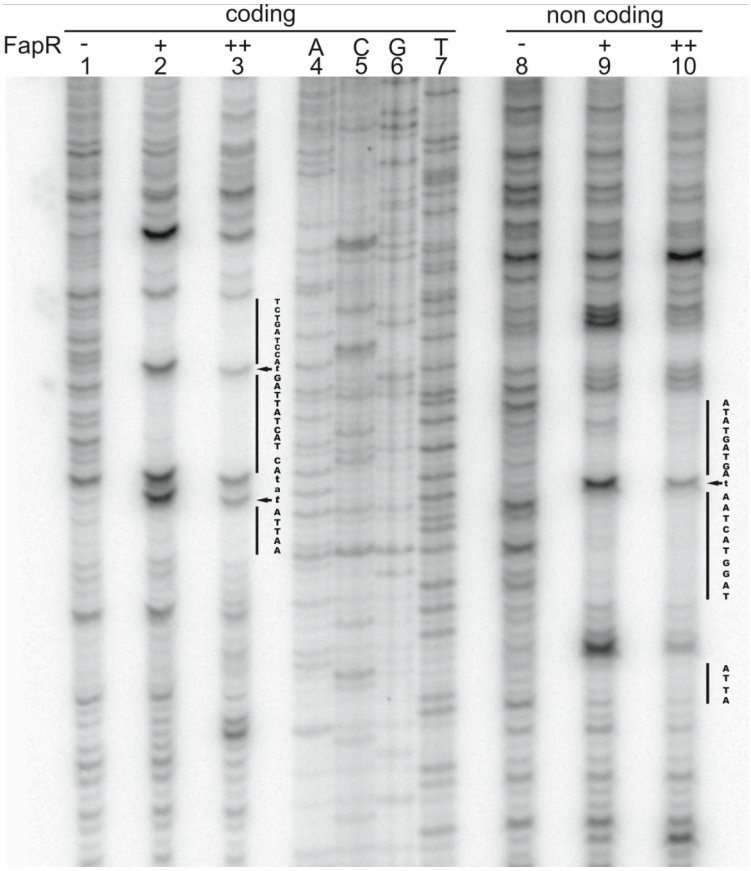

Figure S2. DNAse I footprinting assay of the *fapR* promoter region. DNAse I footprinting of FapR protein on both strands of a 193 bp DNA fragment containing the *fapR* promoter (see Materials and Methods). Sequencing reactions were performed on the same DNA fragment labeled at the coding strand (lanes 4 to 7). Lanes 1 to 3 and 8 to 10 show the DNAse I digestion products of P*fapR* DNA in the presence of 6.9 µM (+), 69 µM (++) or in the absence of FapR (-). Lines mark the protected regions in each strand. Capital letters denote protected bases. Arrows indicate hypersensitive bonds.

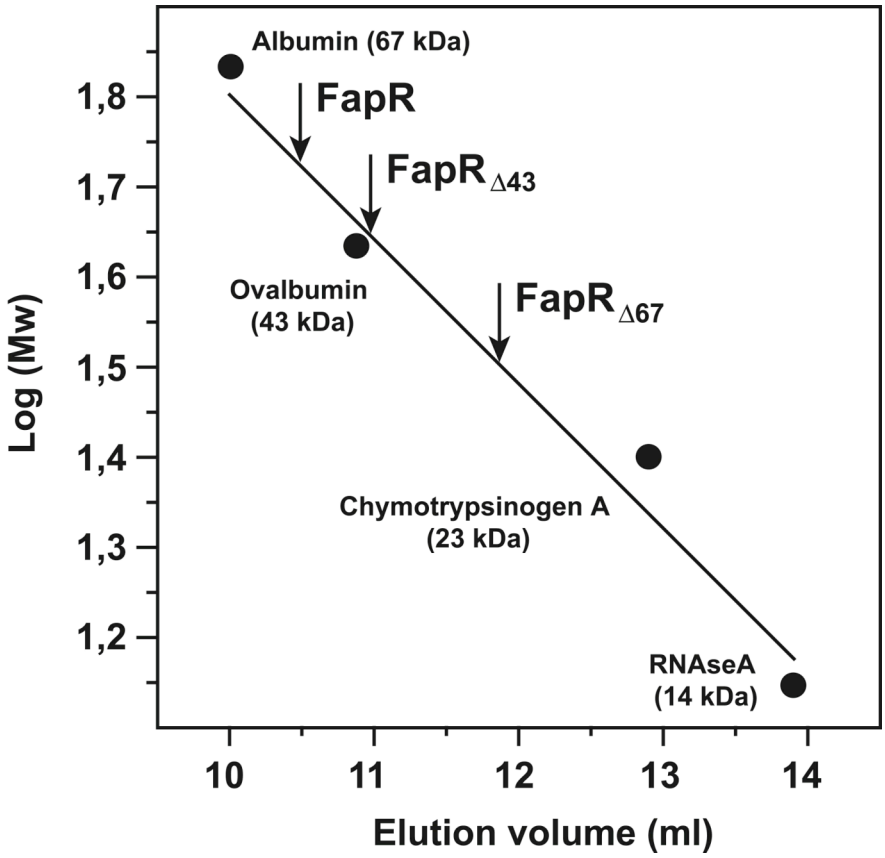

Figure S3. FapR is a homodimer in solution, as indicated by gel filtration chromatography. The theoretical and experimental molecular weights are respectively 45.6 kDa and 53.7 kDa for full-length FapR, 35.4 kDa and 44.7 kDa for $FapR_{\Delta 43}$, and 29.6 kDa and 32.4 kDa for $FapR_{\Delta 67}$. Gel filtration was performed using a calibrated Superdex-75 column (Amersham) equilibrated in 50 mM Tris-HCl pH 7.5 and 150 mM NaCl at 1 ml/min.

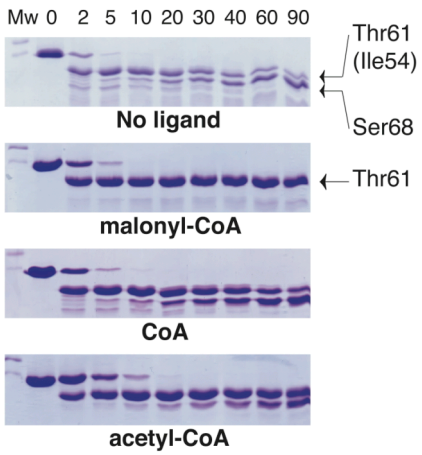

Figure S4. Trypsin cleavage of $FapR_{\Delta 43}$ pre-incubated with different ligands (the N-terminal residues of selected proteolytic fragments are indicated). Malonyl-CoA binding protects specifically $FapR_{\Delta 43}$ (and full-length FapR, data not shown) from proteolysis at Lys67 in loop A, in agreement with the structural results. This protection effect is not attained (i.e. loop A remains flexible) when the repressor is incubated with related analogues of malonyl-CoA such as CoA, acetyl-CoA (shown above), propionyl-CoA, butiryl-CoA or succinyl-CoA (not shown).

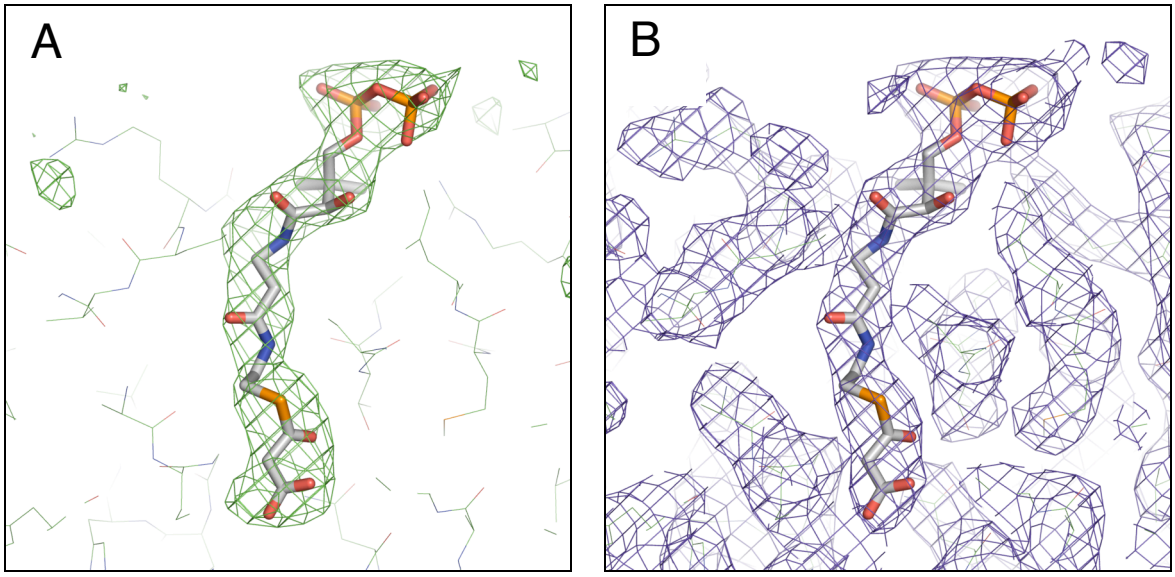

Figure S5. Malonyl-CoA (except for its 3'P-nucleoside moiety) is clearly defined in the electron density maps. **(A)** Initial difference Fourier (Fo-Fc) synthesis (contoured at 3σ) used to build the bound ligand. **(B)** Final (2mFo-DFc) electron density map (contoured at 1σ).