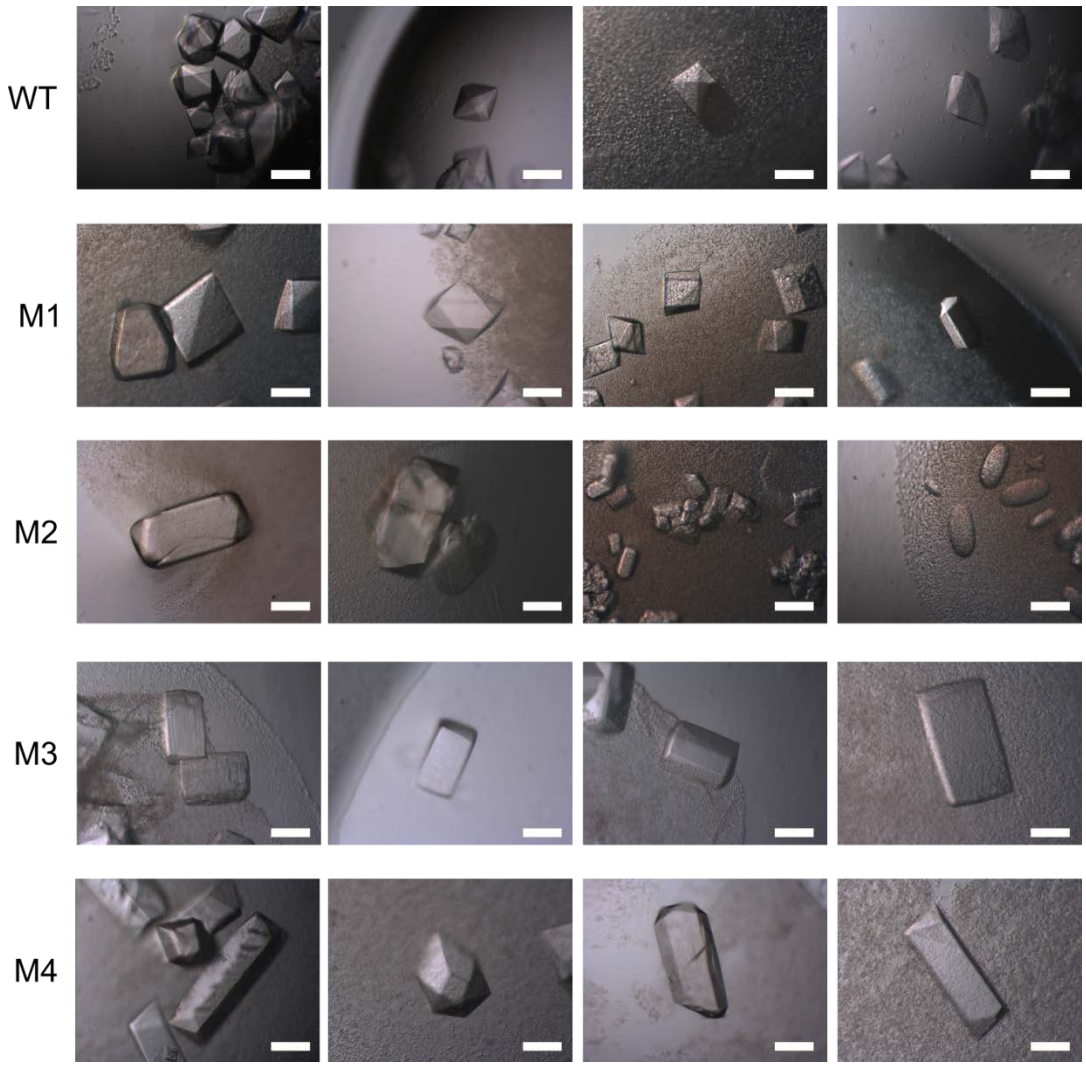

Figure S1. Morphology of the WT and M1-M4 P4-P6 crystals. Related to Figure 1.

The images were taken using bright-field microscopy. The scale bars are 100 μm.

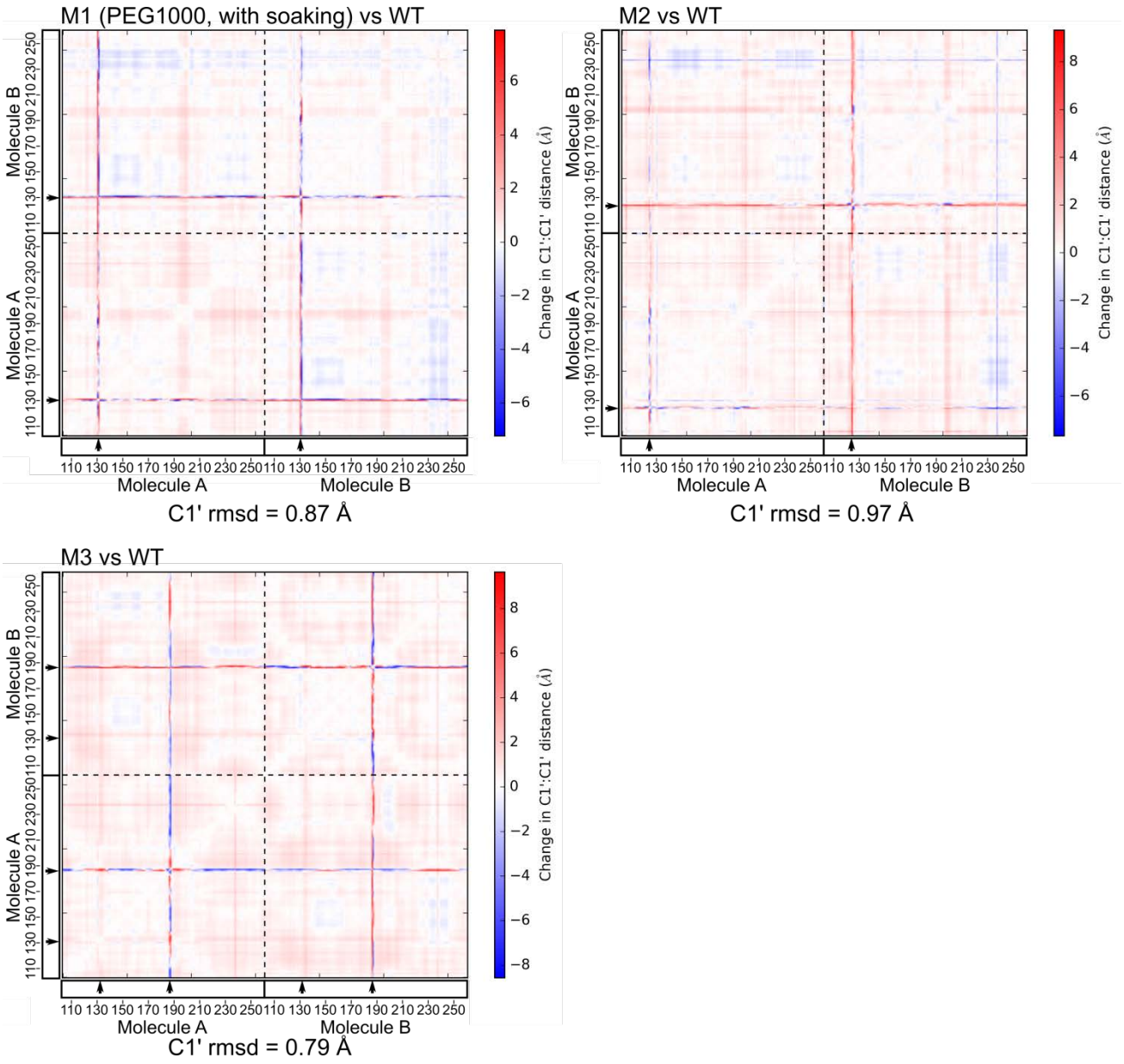

Figure S2. Difference distance matrix plots between M1-M3 mutant structures and WT P4-P6 (PDB ID 1GID). Related to Figures 2-4.

Both molecules A and B in the asymmetric unit are shown, separated by dashed lines. Mutated sites are marked with arrows. Calculated r.m.s. deviations for all C1' atoms in the asymmetric unit are shown below the plots.

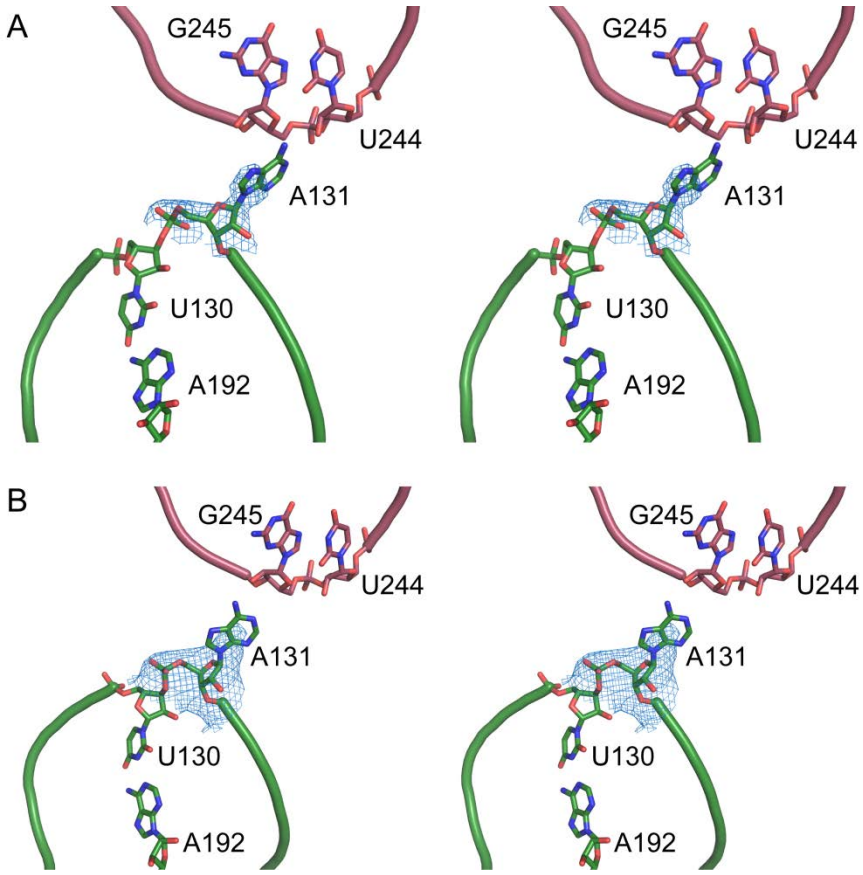

Figure S3. Lattice interactions in M1 mutant crystals grown under MPD conditions and without cryoprotection. Related to Figure 2.

(A) Stereo diagram of the P5a region of molecule A (green) showing U130-A192 base pair and proximity of A131 to molecule B (red). (B) The P5a region of molecule B (green) undergoes a similar local structural rearrangement, allowing A131 to contact molecule A (red). The structure was determined at 3.14 Å resolution. Blue mesh shows 2Fo-Fc maps contoured at 0.6σ.

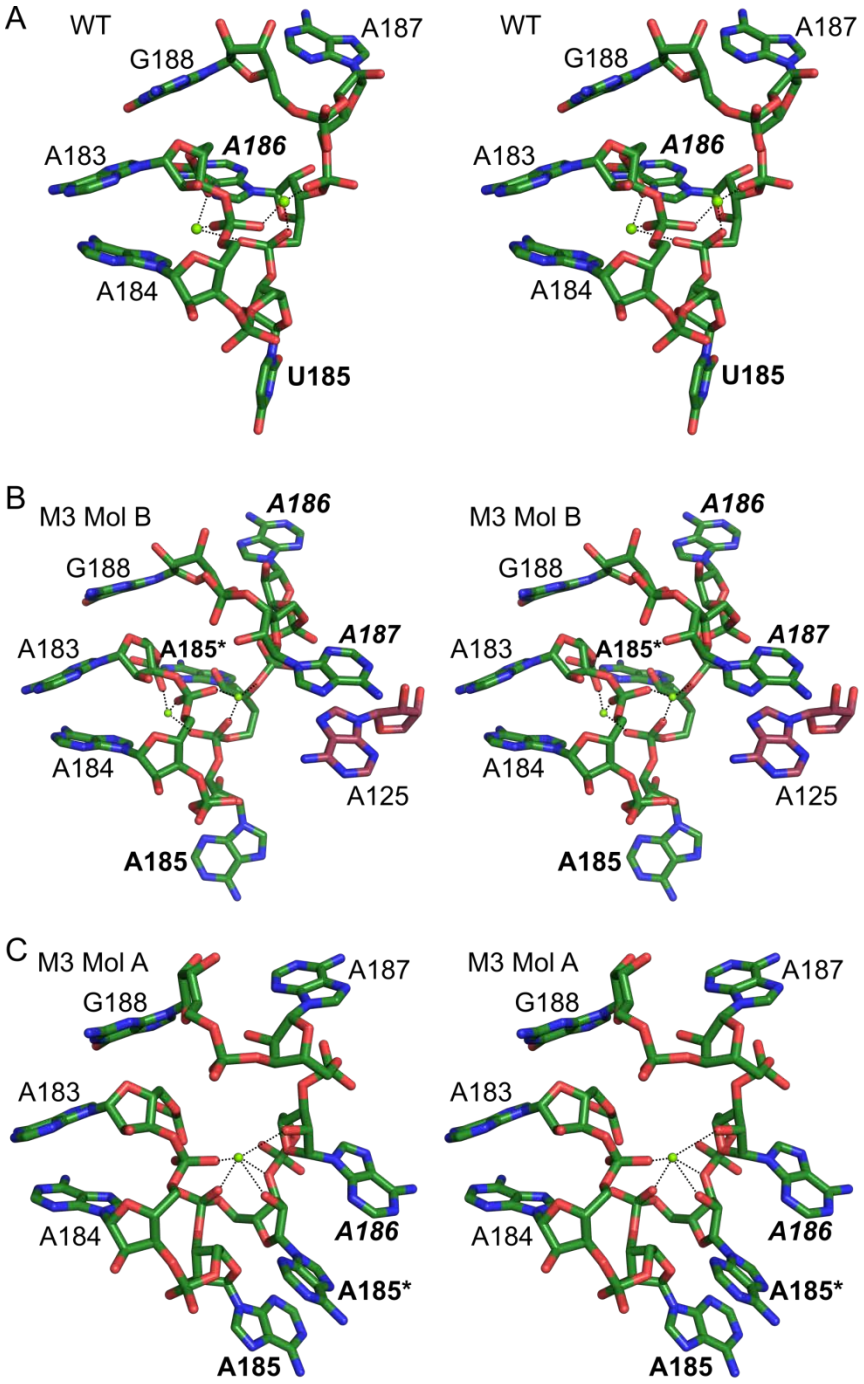

Figure S4. The M3 mutations induce differential structural changes in two unique molecules in the crystal. Related to Figure 4.

Stereo structural diagrams of (A) WT P4-P6, (B,C) M3 molecules B and A in the A-rich bulge region. Mg^{2+} ions bound to the RNA backbones are shown as green balls. Inner-sphere coordination of Mg^{2+} is indicated as dotted lines. Whereas two Mg^{2+} ions are bound to the WT and M3 molecule B, only one Mg^{2+} is observed in M3 molecule A. The structures of WT molecules A and B are almost identical, thereby only one is shown.

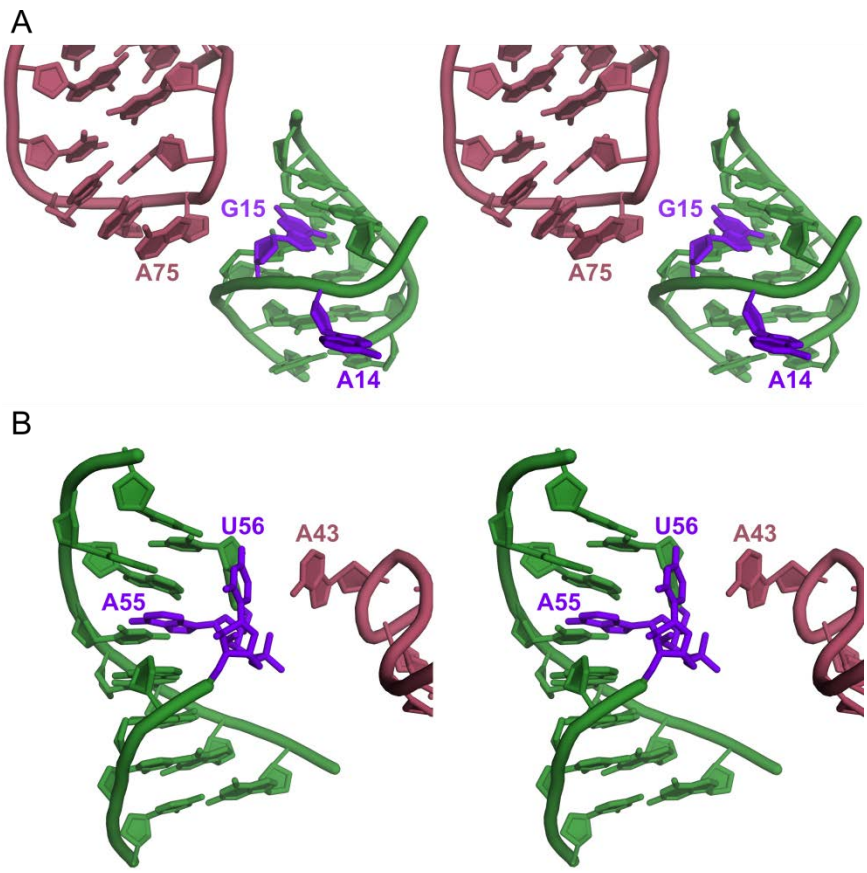

Figure S5. Two RNA crystals to which the bulge walking strategy may be applied to establish new lattice contacts. Related to Figure 2.

(A) In the SAM riboswitch structure (PDB code 2GIS) (Montange and Batey, 2006), A14 is bulged out and is not involved in any lattice contact. Mutating A14 to G should allow this residue to take the position of G15. Residue 15, either kept to be a G or mutated to an A to reinforce the new conformation, can then bulge out and forge a new lattice contact by stacking to A75 of a neighboring molecule. (B) In the structure of the Group II intron domains 5+6 (PDB code 1KXK) (Zhang and Doudna, 2002), the base of single-nucleotide bulge U56 does not make lattice contacts. A mutation of U56 to an A would allow it to fold in the helix while letting residue 55 to flip out and stack with A43 of a neighboring molecule. Residue 55 may be kept to the original A or mutated to a G. In both panels, the proposed bulge swap residues are highlighted in purple and the neighboring symmetry-related molecules are in green and red.