Supplementary figure S4. **Specificity of IMP1-zipcode RNA sequence interactions detected by TriFC.** Cells were transfected with plasmids expressing IMP1 fused to the C-terminal complementing portion of Venus (VenusC), the MS2 coat protein fused to the N-terminal complementing portion of Venus (VenusN), and reporter
mRNAs containing sequences as indicated. Fluorescence micrographs (right column) and differential interference contrast images (DIC, left column) of representative cells are shown. To confirm the specificity of the signal we expressed MS2-VenusN and IMP1-VenusC in the absence of reporter mRNA and could not detect complementation (A). When we removed the zipcode sequence from the reporter mRNA we could no longer detect fluorescence (B). The MS2 operator sequence alone was not sufficient for fluorescence complementation (C). When reporter mRNAs were expressed on separate plasmids, one RNA incorporating the MS2 operator sequence, the other incorporating the zipcode sequence, and co-expressed along with the MS2 and IMP1 fusion proteins, fluorescence was not observed (D). Therefore, both RNA elements had to be present on a single transcript for a signal to be detected. This indicates that the association of mRNAs bearing complementing portions of Venus fluorescent protein with similar complexes during processing, export or translation was not sufficient to give a response in the TriFC system. Scale bar, 20 μm.