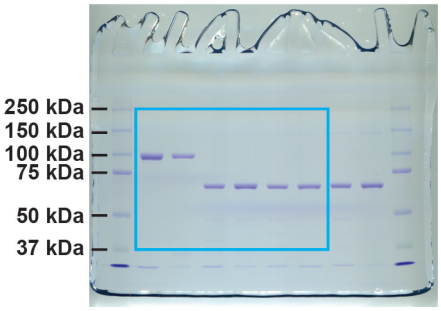
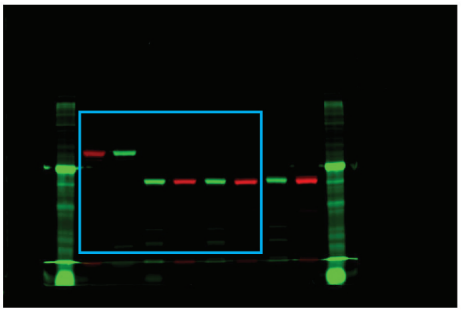
# SUPPLEMENTARY INFORMATION

doi:10.1038/nature20562

Extended Data Fig 2a left

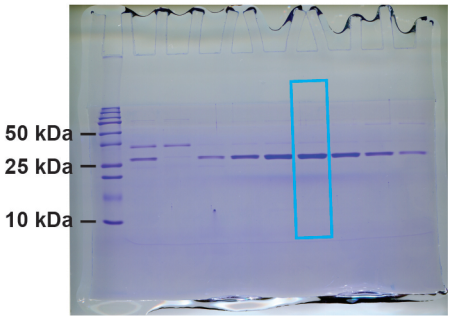



Coomassie stained



Fluorescent

Extended Data Fig 2a right

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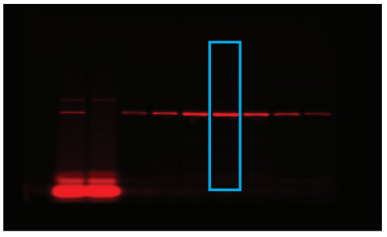
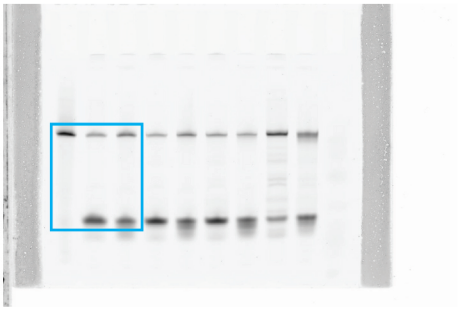


Fluorescent

Extended Data Fig 5a



**Supplementary Note 1:** The initial single molecule-imaging publications with MMR components utilized DNA curtain technology with a λ phage-based DNA substrate that ***did not contain a mismatch*** (Gorman et al., *Mol. Cell* 28:359 2007; Gorman et al. *Nat. Struct. Mol. Biol.* 17:932 2010). The *S.cerevisae* MMR components ScMsh2-ScMsh6 and ScMlh1-ScPms1 were visualized with quantum dot (~20 nm) labeled antibodies (~10 nm) that together appear significantly larger than the structure of eukaryotic MSH2-MSH6 (10 nm x 13 nm x 6 nm). Because these studies did not contain a mismatch it is hard to determine the significance of ScMsh2-ScMsh6 and ScMlh1-ScPms1 DNA binding events for MMR. A third single molecule imaging paper from this group utilized a DNA substrate containing three tandem mismatched nucleotides (Gorman et al., *Proc. Natl. Acad. Sci. USA* 109:E3074 2012), which is an unusual substrate for normal MMR and in some cases may be refractory to repair (see: Sawitzke et al., *Methods Enzymol* 421:171 2007). These studies reported an ScMsh2-ScMsh6/ScMlh1-ScPms1 complex near the mismatch that appeared stationary in the absence of ATP. Those results contrast the present single molecule-imaging studies and a recent cross-linked crystal structure of EcMutS with the N-terminal ATP-binding domain of EcMutL (Groothiuzen et al., *eLife* 4:e06744 2015), as well as several bulk biochemical analysis (Acharya et al., *Mol. Cell* 12:233 2003; Mendillo et al., *J. Biol. Chem.* 280:22245 2005; Mazur et al., *Mol. Cell* 22: 39 2006) that have demonstrated an ATP-dependent interaction between MSH and MLH/PMS components.