SUPPORTING INFORMATION

Adding Fingers To An Engineered Zinc Finger Nuclease Can Reduce Activity

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Sequences of oligonucleotides used in EMSA studies

All sequences written 5' to 3' with ZFP binding site shown in **bold**. The complementary strand of all annealed double-stranded constructs consisted of 15 thymidine residues followed by the reverse complement of the 5' to 3' strand

Target site for GZF1 3-finger protein:
biotin-GCC **GAA GAT GGT** GCG

Target site for GZF1 6-finger protein:
biotin-GCC **GAA GAT GGT GAA GAT GGT** GCG

Target site for GZF3 3-finger protein:
biotin-GCC **AGG GAT AAC** GCG

Target site for GZF3 6-finger protein:
biotin-GCC **AGG GAT AAC AGG GAT AAC** GCG

Target site for Zif268 protein:
biotin-GCC **GCG TGG GCG T**GC C

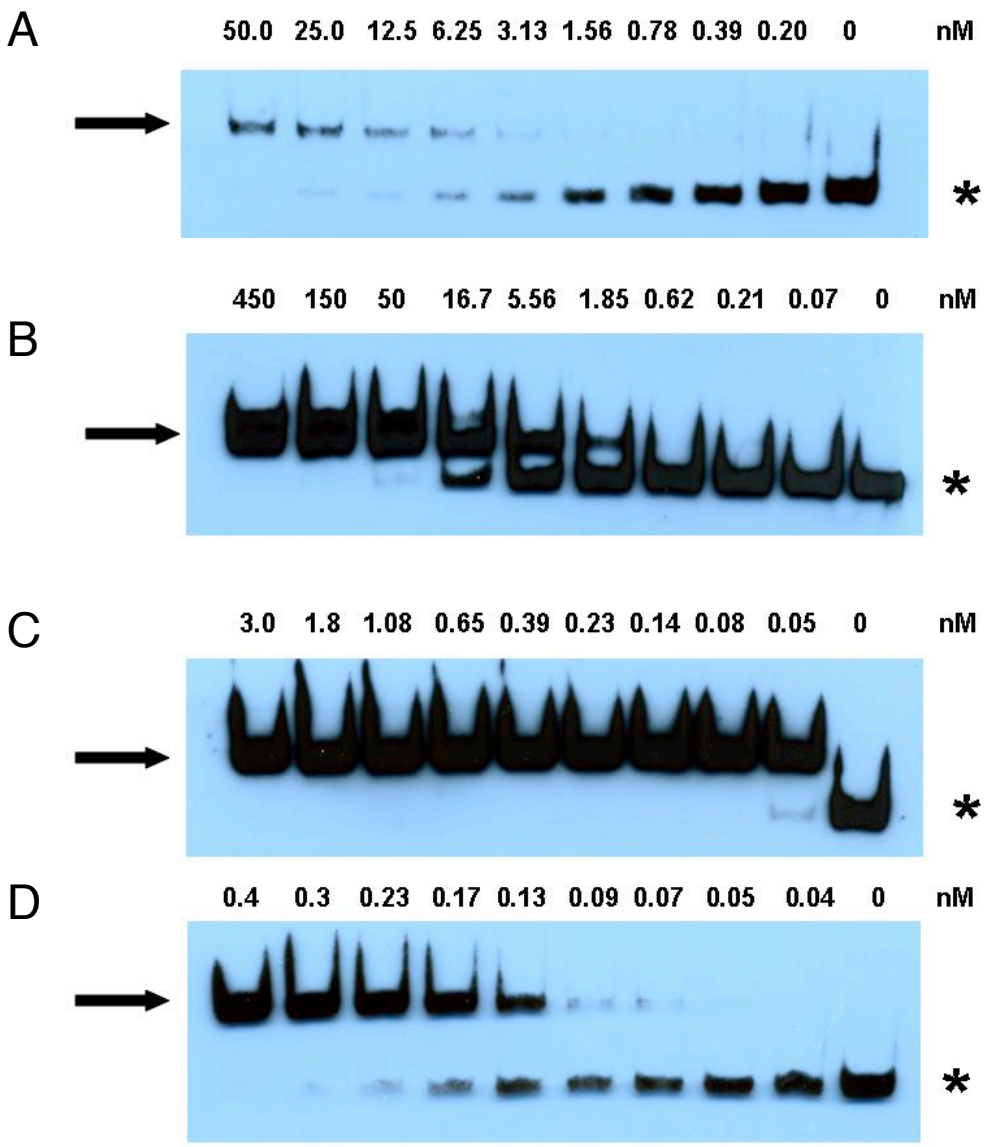

Figure S-1. Electromobility shift assay (EMSA) of engineered zinc finger proteins. Exemplary data are shown for (A) GZF3[3] on its specific target (Target site for GZF3 3-finger protein), (B) GZF3[3] on its non-specific target (Target site for GZF1 6-finger protein), (C) GZF3[6] on its specific target (Target site for GZF3 6-finger protein), and (D) GZF3[6] on its half-site target (Target site for GZF3 3-finger protein). The concentrations of the protein are indicated. Arrows, bound probe; asterisks, free probe.

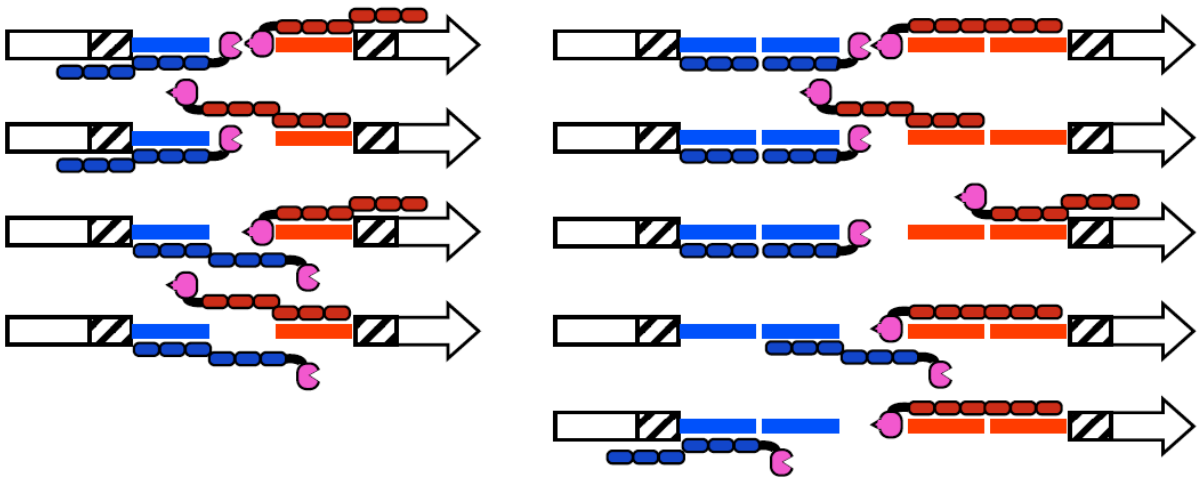

Figure S-2. Potential models that describe how the binding of subsets of fingers at the target site used in this study could reduce nuclease activity. Only the top binding scheme in each column would produce an active nuclease. Reduced activity could also be caused by the subsets of fingers facilitating binding to off-target sites, thus reducing the concentration of nuclease at the target site (not shown).