Plasmid construction:

PCS2-NLS-GAAZFP-FokI-RR was obtained from Addgene. The ZFN gene containing NLS was PCR amplified with primers "fokIRR forward NcoI" and "fokI reverse SalI site" for cloning into a pET24d vector to attach a C-terminal hexahistidine-tag. Site-specific mutation Y471→TAG was conducted with a Quikchange mutagenesis kit (Stratagene). We modified the pSSA luciferase reporter plasmid (obtained from Dr. Segal) to insert a 24 base-pairs stretch (5'- GAAGGTGTG ACTAGT CACACCTTC - 3') with two recognition zinc-finger sites pointing toward each other. A pGL3 control plasmid was used as the template for the PCR and amplified with primers Rvp3f and SSA-RR-SpeI-RR-r. This DNA and pSSA1-3 backbone plasmid were digested with BglII and EcoRI and ligated to construct the pSSA-GAA-GAA plasmid. This plasmid was then used in both *in vitro* and *in vivo*.

***In vitro* DSB assay:**

85 nM (final concentration) of wild type ZFN, caged ZFNY471ONBY, and the DNA substrate were mixed in 200 μL PCR tubes and irradiated with a UV transilluminator (UVP LLC) at 365 nm for 0, 1, 2, 5 or 10 minutes (365 nm), followed by addition of $MgCl_2$ and 0.1 mM DTT (final concentration) to start the reaction. The reaction was then incubated at 37 °C for exactly 1 hour. Reactions were stopped by addition of SDS-containing sample loading buffer, frozen, and stored in a –20 °C freezer before agarose gel analysis. The gels were stained with ethidium bromide and scanned on a Typhoon FLA 7000 phosphorimager (GE Healthcare).

Primer list:

PET24d NLS ZFN cloning:

fokIRR forward NcoI : 5'- TCT GCC ACC ATG GCT CCA AAG AAG AAG -3'

fokI reverse SalI site: 5'- GCA CTC GTC GAC AAA GTT TAT CTC GCC GTT ATT AAA TTT C -3'

Quikchange primer to introduce TAG at position Y471 (FokI annotation):

fokIY471anti: 5'- CAG ATT ATA ACC TCC GCT CTA AGC TTT AGT ATC CAC G -3'

FokIY471sense: 5'- CGT GGA TAC TAA AGC TTA GAG CGG AGG TTA TAA TCT G -3'

Quikchange primer to convert modified FokI dimer interface to wild-type:

FokI RR toWT short antisense: 5'- GAC ATA TCG TTG CAT TTC ATC TGC TTG GCC AAT TGG CAG-3'

FokI RR toWT short sense: 5'-CTG CCA ATT GGC CAA GCA GAT GAA ATG CAA CGA TAT GTC-3'

Luciferase reporter cloning:

SSA-RR-SpeI-RR-r 5'- CTC GAA TTC CCA CAC CTT CAC TAG TGA AGG TGT GCT CAC ATA GGA CCT CTC ACA CAC AG -3'

Rvp3f primer 5'- CTA GCA AAA TAG GCT GTC CC -3'