Photochemical Control of DNA Decoy Function Enables Regulation of NF-κB Activity

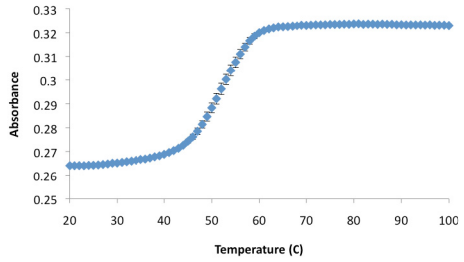
Supporting Information

Jeane M. Govan[1], Mark O. Lively[2], and Alexander Deiters[1,*]

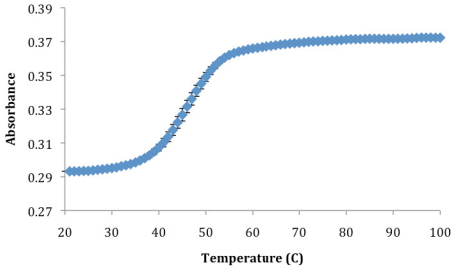
[1]North Carolina State University, Department of Chemistry, Raleigh, NC 27695, and [2]Wake Forest University School of Medicine, Center for Structural Biology, Winston-Salem, NC 27157
alex_deiters@ncsu.edu

Supporting Figures

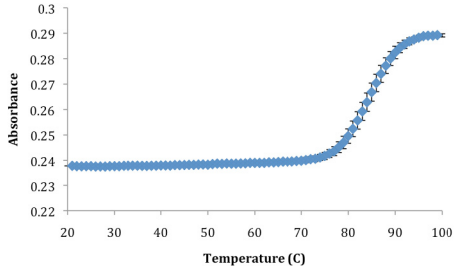
D0



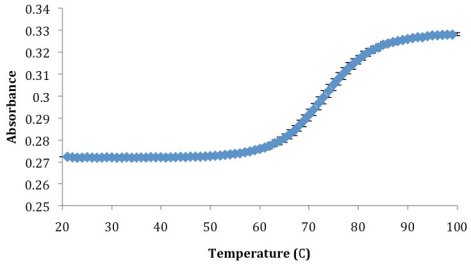
D1



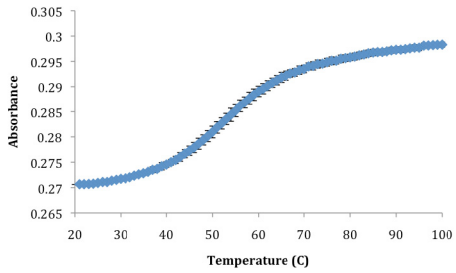
D2



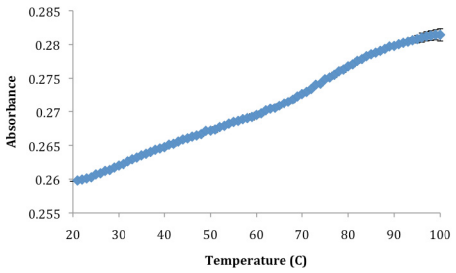
D5



D3 – UV

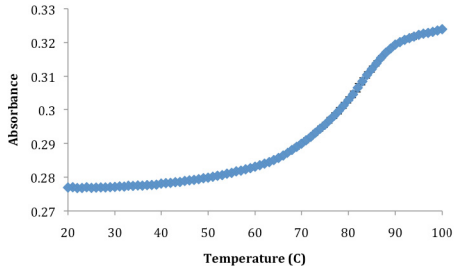


D6 – UV

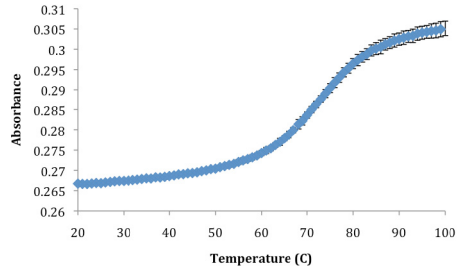


Continued on next page…

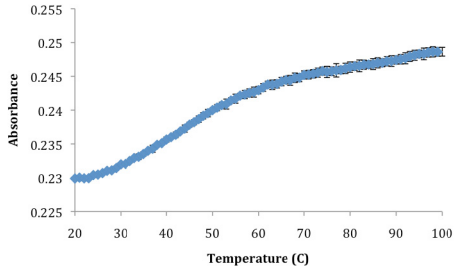
D3 + UV



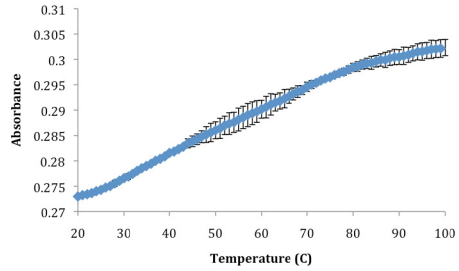
D6 + UV



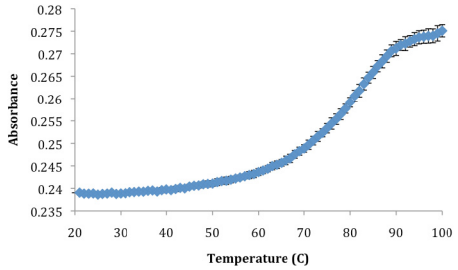
D4 – UV



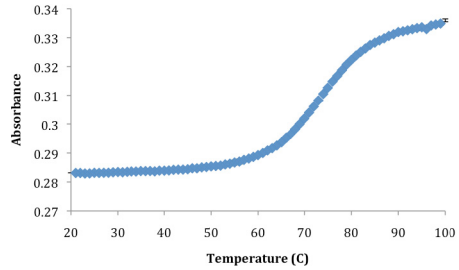
D7 – UV



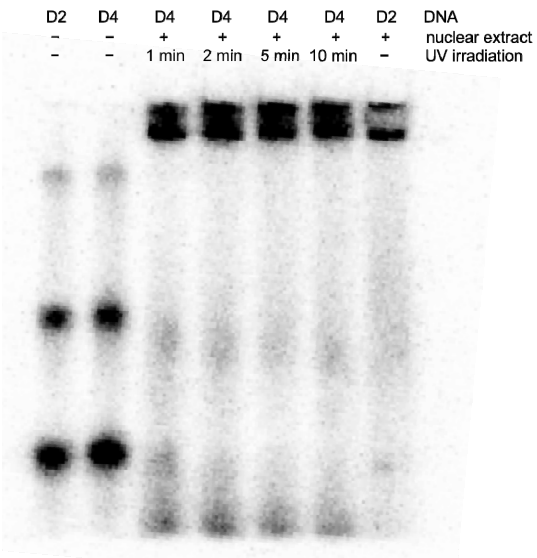
D4 + UV



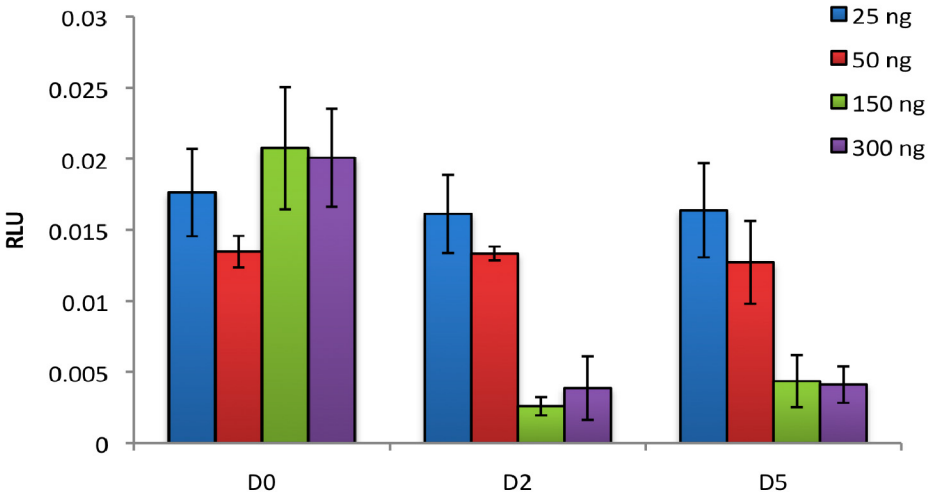
D7 + UV



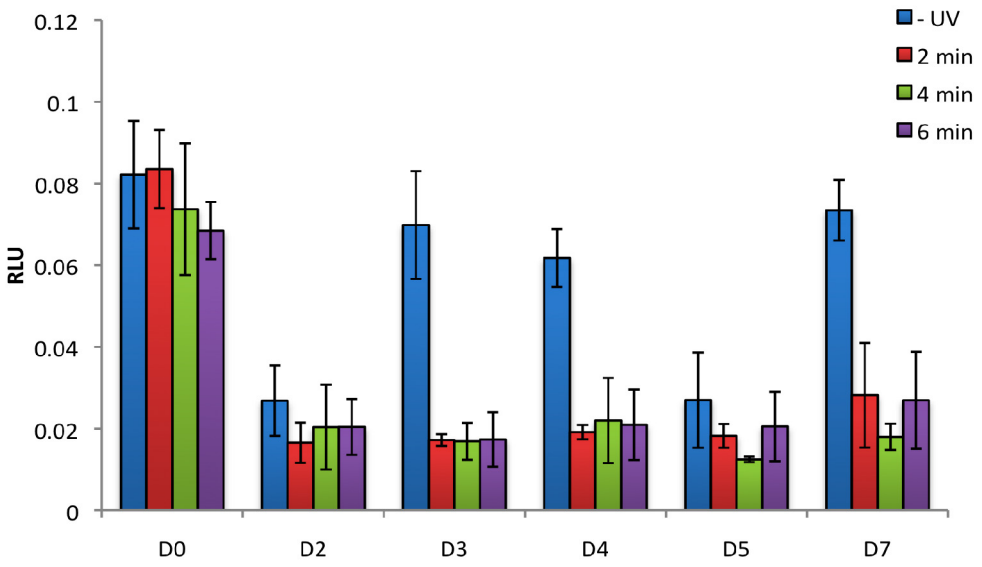
Supporting Figure S1. Melting curves of NF-κB DNA decoys D0-D7. DNA Decoys (1 μM) were incubated in 150 mM NaCl, 50 mM NaH_2PO_4, pH 7.4 buffer. The samples were protected from light or irradiated at 365 nm with an UV trasilluminator for 10 min.



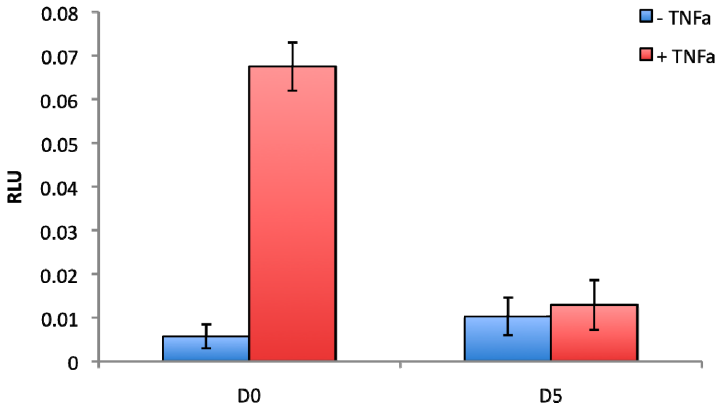
Supporting Figure S2. Irradiation time course of caged hairpin D4. Nuclear extracts were isolated from NF-κB/SEAP HEK 293 cells and caged decoys were irradiated for 1, 2, 5, and 10 min (365 nm, 25 W) and incubated with nuclear extracts at room temperature for 20 min. Samples were analyzed on a 16 % Native PAGE gel and imaged with a Typhoon 7000 phosphoroimager.



Supporting Figure S3. Optimization of transfected hairpin and dumbbell decoy in NF-κB/SEAP HEK 293 cells. NF-κB/SEAP HEK293 cells were transfected with caged and non-caged DNA decoys using X-tremeGENE. TNFα was added after 4 hours, and a SEAP assay was conducted after 24 hours using a Phospha Light Systems kit (Applied Biosystems). Cell viability was assayed using a Cell Titer Glo assay (Promega), and the SEAP signal was normalized to Cell Titer Glo signal. All experiments were performed in triplicate and error bars represent standard deviations.



Supporting Figure S4. UV irradiation time course of photochemical activation of NF-κB induced SEAP expression. NF-κB/SEAP HEK293 cells were transfected with caged and non-caged DNA decoys using X-tremeGENE. Cells were either irradiated for 2, 4, or 6 min (365 nm, 25 W) or kept in the dark. TNFα was added after 4 hours, and a SEAP assay was conducted after 24 hours using a Phospha Light Systems kit (Applied Biosystems). Cell viability was assayed using a Cell Titer Glo assay (Promega), and the SEAP signal was normalized to Cell Titer Glo signal. All experiments were performed in triplicate and error bars represent standard deviations.



Supporting Figure S5. TNFα induction of SEAP expression with DNA decoys. NF-κB/SEAP HEK293 cells were transfected with scramble and dumbbell DNA decoys using X-tremeGENE. SEAP expression was either not induced or induced with TNFα after 4 hours, and a SEAP assay was conducted after 24 hours using a Phospha Light Systems kit (Applied Biosystems). Cell viability was assayed using a Cell Titer Glo assay (Promega), and the SEAP signal was normalized to Cell Titer Glo signal. All experiments were performed in triplicate and error bars represent standard deviations.