Validation data for G3-YSD

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Version 24F14-MM

G3-YSD (G3-ended Y-form Short DNA) is a potent agonist for the cytosolic DNA sensor cGAS. This 26-mer DNA palindromic sequence derived from HIV-1 contains unpaired guanosines trimers (G3) in Y-form dsDNA-ssDNA junctions. These guanosine-overhangs have been identified as minimal recognition motifs for cGAS. The activity of this ligand has been tested using THP1-DualTM cells which express multiple cytosolic DNA sensors (CDSs) and two inducible reporter genes (interferon regulatory factor (IRF)-inducible secreted Lucia luciferase and NF- κ B-inducible SEAP). Stimulation of these cells with the CDS ligands, G3-YSD and VACV-70, complexed to LyoVecTM leads to a significant IRF response (**Figure 1**). Of note, this response is stronger when using G3-YSD compared to VACV-70. As expected, intracellular delivery of G3-YSD Control does not induce an IRF response in these cells (**Figure 1**). Importantly, the IRF response to intracellular delivery of G3-YSD is strictly cGAS-dependent (**Figure 2**).



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