Validation data for H-151

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Version 22F07-MM

H-151 is a potent, irreversible and selective small molecule inhibitor of stimulator of interferon (IFN) genes (STING). Its activity has been tested using the human monocytic THP1-Dual™ cells and the murine macrophage RAW-Lucia™ ISG cells which endogenously express STING. These cells stably express an interferon regulatory factor (IRF)-inducible secreted Lucia luciferase reporter gene. Stimulation of these cells with STING agonists, such as 2'3'-cGAMP, leads to a significant IRF response which is inhibited in a dose-dependent manner by H-151 (Figure 1). Importantly, the inhibitory effect of H-151 appears to be STING-specific as it does not affect other signaling pathways such as the RIG-I signaling pathway (Figures 2 & 4).
Moreover, H-151 effectively blocks STING activity induced with fluorinated or bisphosphorothioate STING analogs such as 3'3'-cGAMP Fluorinated or 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) (Figures 2 & 3). Notably, H-151 is effective against all the STING variants tested, including constitutively active disease-associated mutants such as S154 (N154S) and M155 (V155M; Figure 3). Furthermore, H-151 inhibits the STING-NF-κB pathway in a dose-dependent manner (Figure 4), as confirmed in THP1-Dual™ cells, which also express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene.

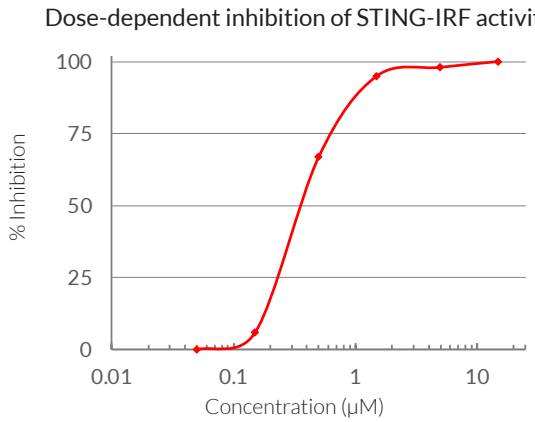

Figure 1: Effect of H-151 on the IRF response of THP1-Dual™ cells to 2'3'-cGAMP. THP1-Dual™ cells were incubated with 20 µg/ml of 2'3'-cGAMP and increasing concentrations of H-151. After overnight incubation, the IRF response was assessed by determining Lucia luciferase activity in the supernatant using the detection reagent QUANTI-Luc™. Data are shown as percentage (%) inhibition.

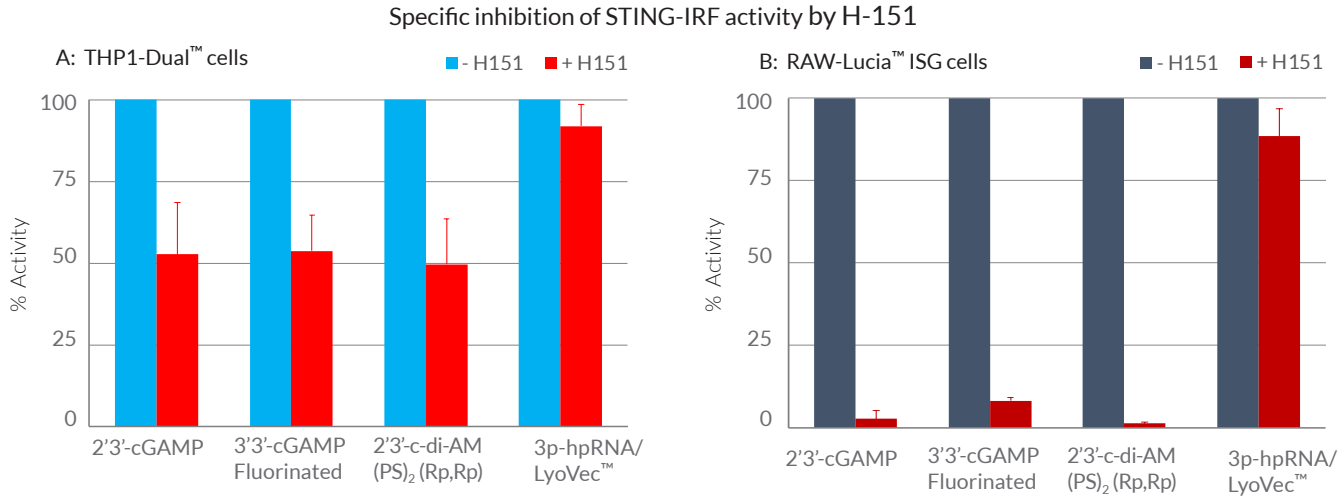

Figure 2. H-151 specifically inhibits STING activity. A: THP1-Dual™ cells and **B:** RAW-Lucia™ ISG cells were incubated with 20 µg/ml of 2'3'-cGAMP, 7.5 µg/ml of 3'3'-cGAMP Fluorinated, 7.5 µg/ml of 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) or 1 µg/ml of 3p-hpRNA/LyoVec™, in the presence or absence of H-151 at 130 ng/ml (0.47 µM). The RIG-I ligand 3p-hpRNA was delivered into the cytoplasm using the cationic lipid transfection reagent, LyoVec™. After overnight incubation, the IRF response was assessed by determining Lucia luciferase activity in the supernatant using the detection reagent QUANTI-Luc™. Data are shown as percentage (%) activity.

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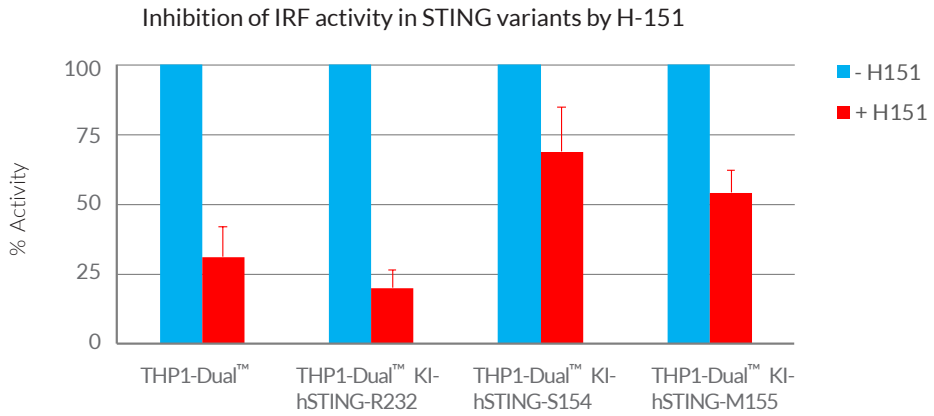

Figure 3: H-151 inhibits the activity of multiple human STING variants. THP1-Dual™ and THP1-Dual™ KI-STING cells were incubated in the presence or absence of 400 ng/ml of H-151. THP1-Dual™ and THP1-Dual™ KI-STING-R232 cells were further incubated with 7.5 µg/ml of 3'3'-cGAMP Fluorinated while THP1-Dual™ KI-STING-S154 and THP1-Dual™ KI-STING-M155 cells were not incubated with a STING ligand as they constitutively express activated STING. After overnight incubation, the IRF response was assessed by monitoring Lucia luciferase activity using the detection reagent QUANTI-Luc™. Data are shown as percentage (%) activity.

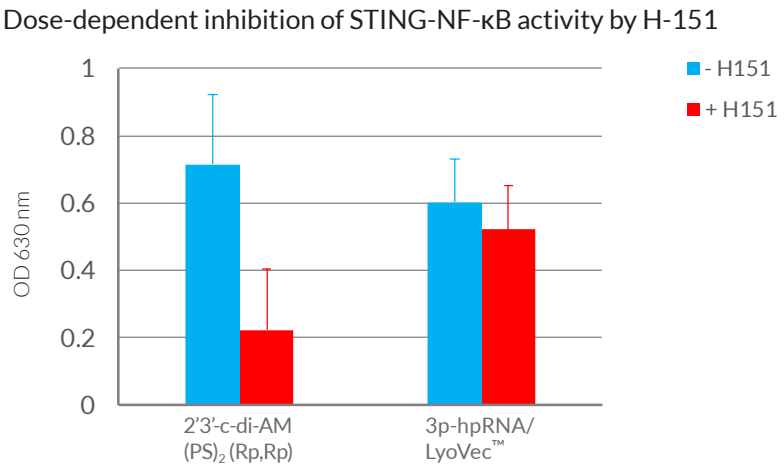

Figure 4: Effect of H-151 on the NF-κB response of THP1-Dual™ cells to 2'3'-c-di-AM(PS)$_2$ (Rp,Rp). THP1-Dual™ cells were incubated with 7.5 µg/ml of 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) or 1 µg/ml of 3p-hpRNA/LyoVec™, in the presence or absence of H-151 at 130 ng/ml (0.47 µM). The RIG-I ligand 3p-hpRNA was delivered into the cytoplasm using the cationic lipid transfection reagent, LyoVec™. After a 24h incubation, NF-kB activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 630 nm.