Ubiquigent

## CASE STUDY: USP7 inhibitors

# DUB*profiler*–Cell

**Examining the target engagement and selectivity of covalent and non–covalent inhibitors of USP7 in vitro and in live cells**

## Background

The deubiquitinating enzyme USP7 (also known as HAUSP) regulates the activities of numerous proteins broadly characterized as tumour suppressors, DNA repair proteins, immune responders, viral proteins, and epigenetic modulators. Aberrant USP7 activity may promote oncogenesis and viral disease making it a compelling target for therapeutic intervention. Several drug discovery programs have identified inhibitors of USP7, with cellular proof of concept and anti-proliferative activity demonstrated in cancer models.

FT671 and FT827 are potent and selective USP7 inhibitors developed by FORMA Therapeutics. FT671 is a non-covalent inhibitor of USP7, while FT827 features a vinylsulfonamide moiety that covalently modifies the catalytic Cys223 of USP7 and therefore inhibits the enzyme in a covalent manner. Consistent with USP7 target engagement in cells, FT671 was shown to cause degradation of the USP7 substrate MDM2, leading to re-activation of p53 and inhibition of tumour growth in mice (Turnbull et al., 2017).

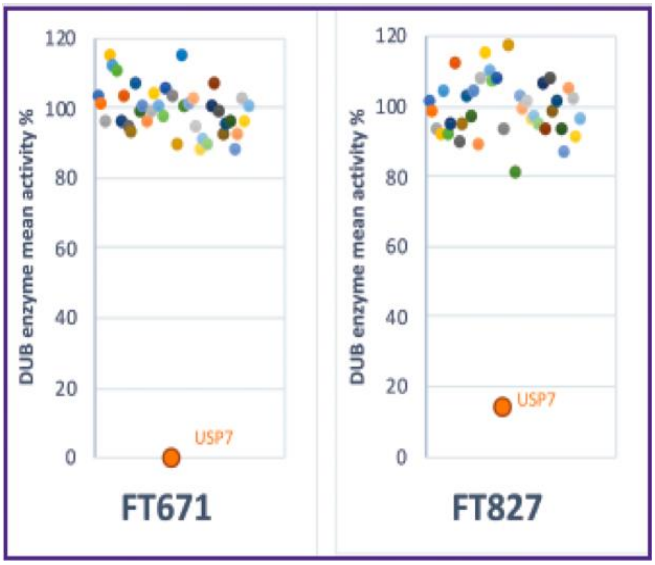


*Figure 1: DUBprofiler characterisation of USP7 inhibitor selectivity*

*An in vitro Ubiquitin-Rhodamine110 cleavage assay establishes the selectivity of the two USP7 inhibitors FT671 and FT827 (compounds kindly supplied by FORMA Therapeutics); each dot represents one of 45 DUB assays on the panel (data generated at Ubiquigent and published in Turnbull et al. Nature 550, 481-486, 2017).*

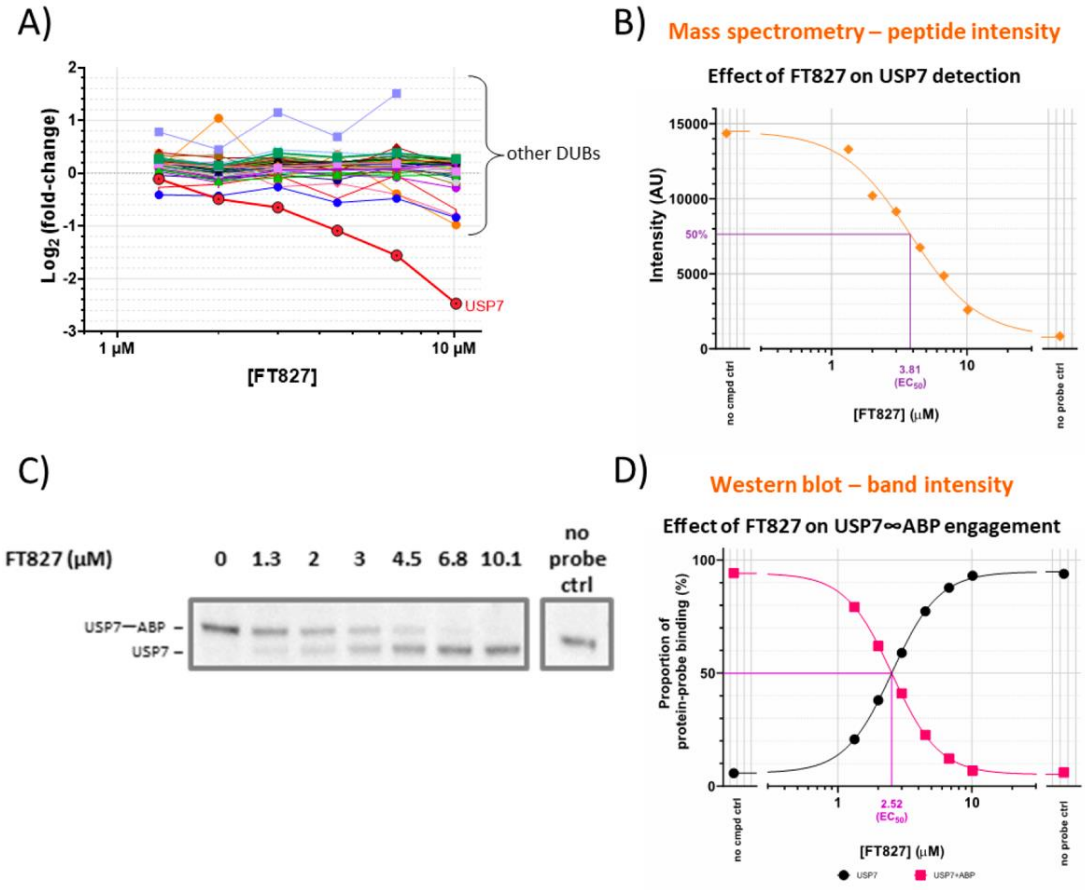


*Figure 2: DUBprofiler-Cell reports target engagement by covalent DUB inhibitors.*

*FT827, a covalent inhibitor of USP7, prevents the binding of the activity-based probe to USP7 in a dose-dependent and highly selective manner (A). Estimated $EC_{50}$ values based on MS data (B) correlate with data obtained by Western blot detection (C, D).*

## *In vitro* profiling of USP7 inhibitors in the DUBprofiler platform

DUB*profiler*™ is a deubiquitinase (DUB) enzyme compound profiling platform designed to quickly determine the selectivity and potency of DUB inhibitors. Ubiquigent™ screened FT671 and FT827 in our single point DUB*profiler in vitro* assay against the Ubiquitin-Rhodamine110 substrate and established the selectivity of both compounds for USP7.

## Evaluation of the USP7 inhibitors in the DUB*profiler*–Cell lysate–based assays (Western Blot and MS analysis)

Lysates were prepared from MCF7 cells and pre-incubated with a full concentration range of each compound, followed by the ABP. Parameters were optimised according to the covalent or non-covalent mechanism of action of each compound. Lysates were examined by Western blotting using a validated anti-USP7 antibody (Figure 2C, 3C). The DUBome of lysates for MS analysis were first enriched using the HA tag on the probe prior to MS analysis (Figure 2A, 3A). The data shown in Figure 2B vs 2D, and Figure 3B

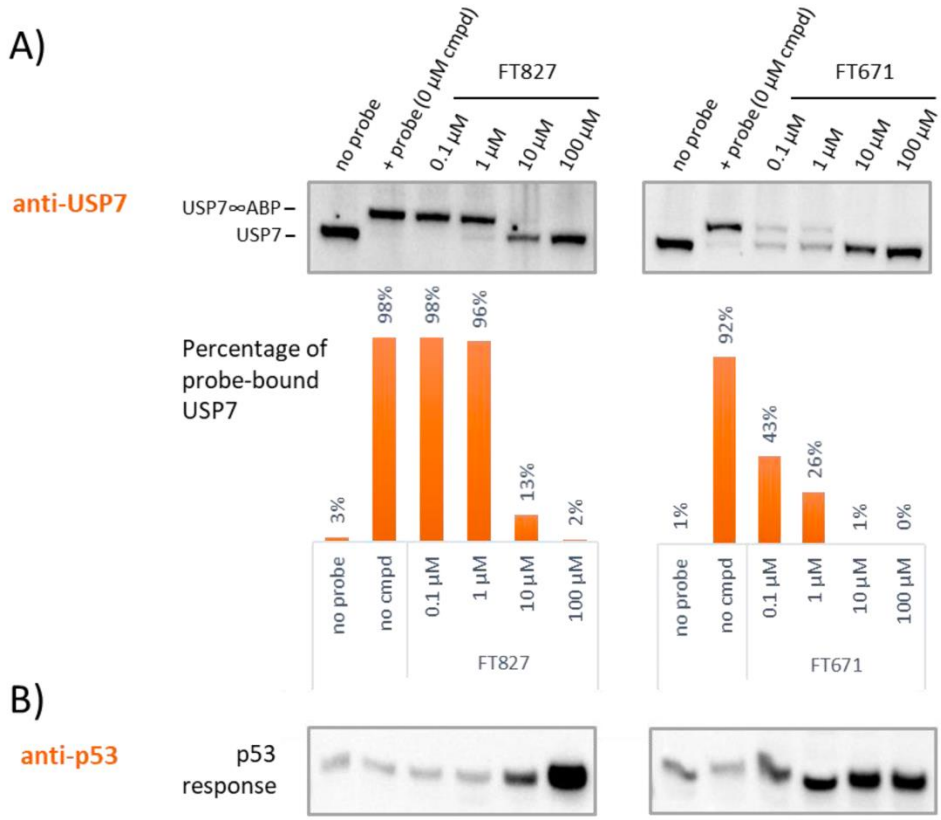


*Figure 4: Demonstration of compound target engagement and achievement of predicted pharmacodynamic endpoint in live cells, conducted in the same experiment.*

*Both USP7 inhibitors prevent probe binding in the DUBprofiler-Cell live cell assay (A); the bar graphs indicate the percentage of probe binding, which is reduced in a dose-dependent manner in lysates prepared from live cells treated with either compound. In addition, consistent with the predicted cellular outcome of inhibition of USP7, p53 levels are increased in a dose-dependent manner at concentrations of compound that reduce or prevent probe binding (B).*

vs 3D) demonstrates the level of agreement between the two assay formats – western blotting and MS – and highlights the added value of the MS readout in demonstrating selectivity of the compounds towards USP7 versus all other active DUBs in the chosen cell line.

## Evaluation of the USP7 inhibitors in the DUB*profiler*–Cell live cell assay

MCF7 cells were seeded and treated with various concentrations of FT827 or FT671 (or DMSO) for 6 hours. Cell pellets were collected, and lysates prepared from each sample; samples were then incubated with the activity probe and analysed by Western blotting (Figure 4).

The data demonstrates that the incubation of live cells with test compounds can prevent the binding of the ABP to the compounds' target DUB (Figure 4A) in a dose-dependent manner, as was previously demonstrated in lysates treated with compounds in vitro (Figures 2 and 3). The ability to do so, in a therapeutically relevant cell line, allows for target engagement to be correlated with biomarkers of response or phenotypic readouts, such as induction of p53 (Figure 4B). It also has the benefit of demonstrating cell permeability of the compounds of interest and allows the

compounds to engage with DUBs in their endogenous environment in the presence of relevant binding partners and in their natural subcellular location(s).

## Conclusion

The case study presented herein demonstrates the ability of the DUB*profiler*-Cell™ assay to report on the target engagement and selectivity of covalent and non-covalent inhibitors of USP7 in vitro and in live cells. As such, DUB*profiler*-Cell is a very powerful new addition to our DUB-focused Drug Discovery Platform.

## Contact us

**We look forward to discussing your DUB*profiler*–Cell project with you. Please email: services@ubiquigent.com**