

# Allosteric mitochondrial ClpP agonist ONC206 alters stress response, metabolic and epigenetic profiles to elicit anti-cancer efficacy in high-grade gliomas

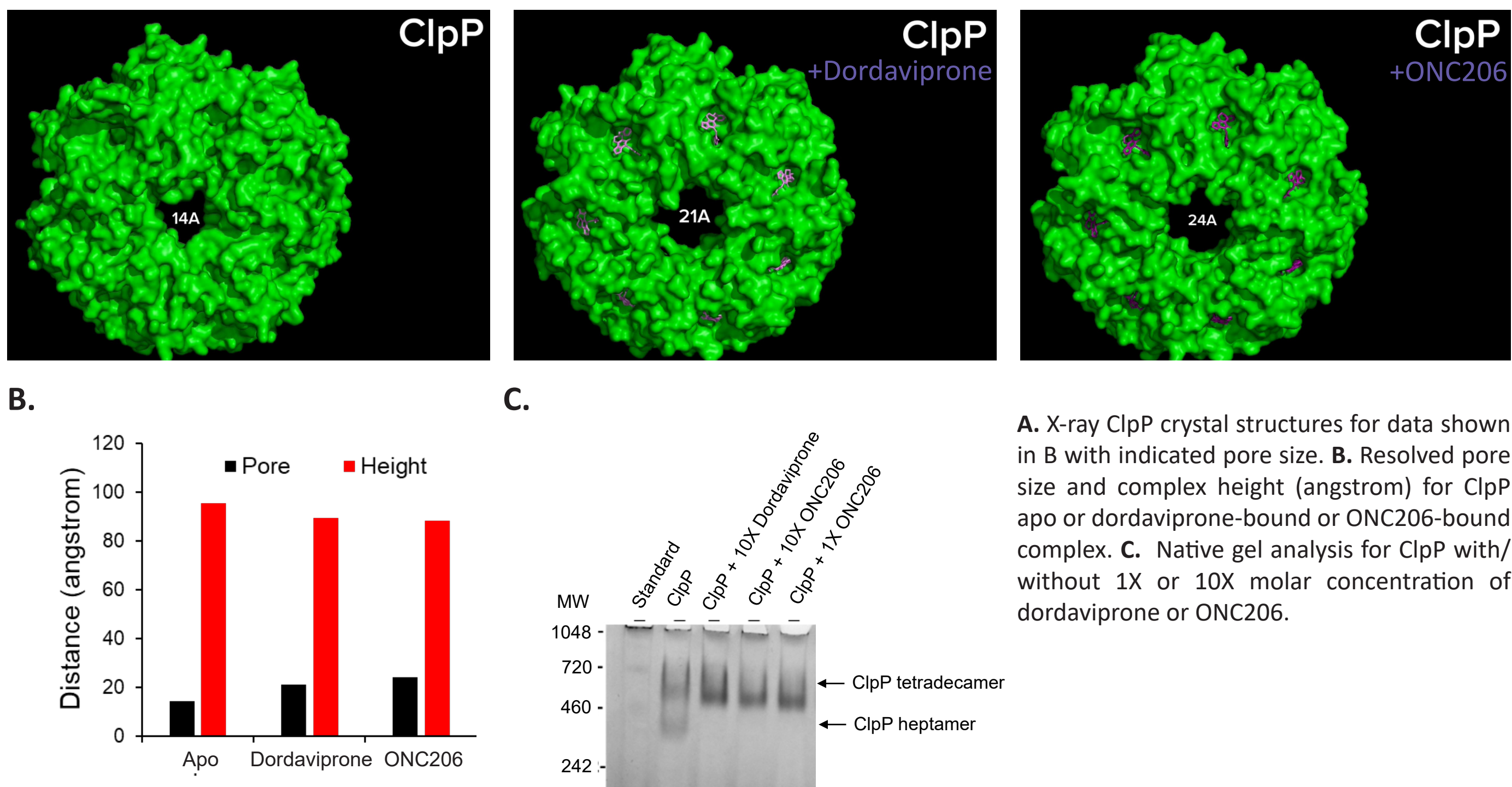
Varun V. Prabhu, Cristina Maranto, Andrew Lee, Scott Foster and Joshua E. Allen  
Chimerix, Inc.

## Introduction

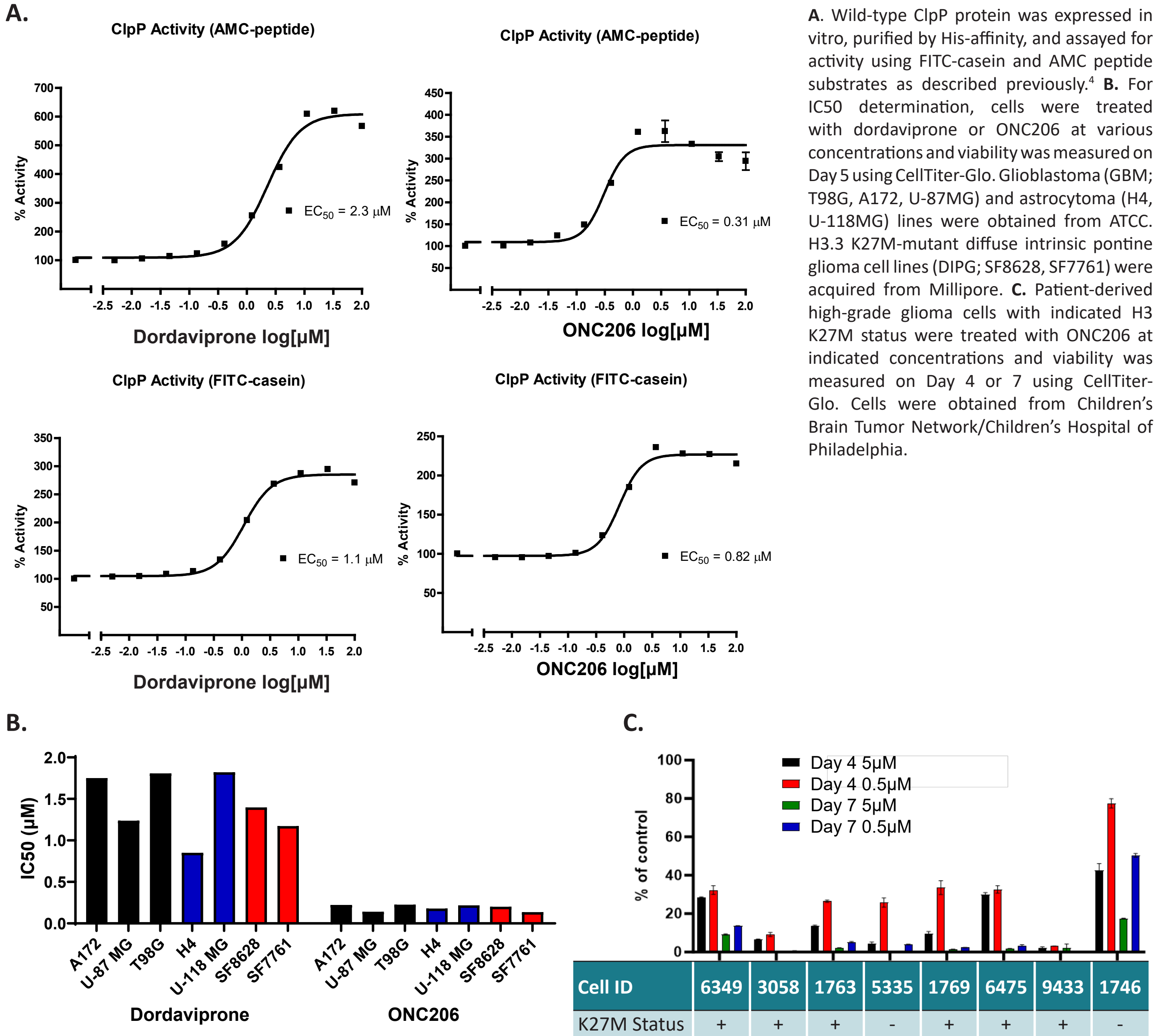
- Dordaviprone (ONC201), a first-in-class imipridone, is an oral, blood-brain barrier penetrating, selective small molecule antagonist of dopamine receptor D2 (DRD2) and agonist of the mitochondrial protease caseinolytic mitochondrial matrix peptidase proteolytic subunit (ClpP).<sup>1-5</sup>
- Dordaviprone has demonstrated tolerability and durable tumor regressions in patients with H3 K27M-mutant glioma.<sup>6</sup>
- ONC206, a chemical derivative of dordaviprone, is the second imipridone to be developed and is currently in Phase 1 clinical development.<sup>7</sup>
- Relative to dordaviprone, ONC206 has demonstrated differentiated DRD2 receptor pharmacology<sup>8</sup>, improved potency, enhanced absorption/tissue distribution, and preclinical anti-cancer activity in vitro and in vivo.<sup>9-13</sup>

## Results

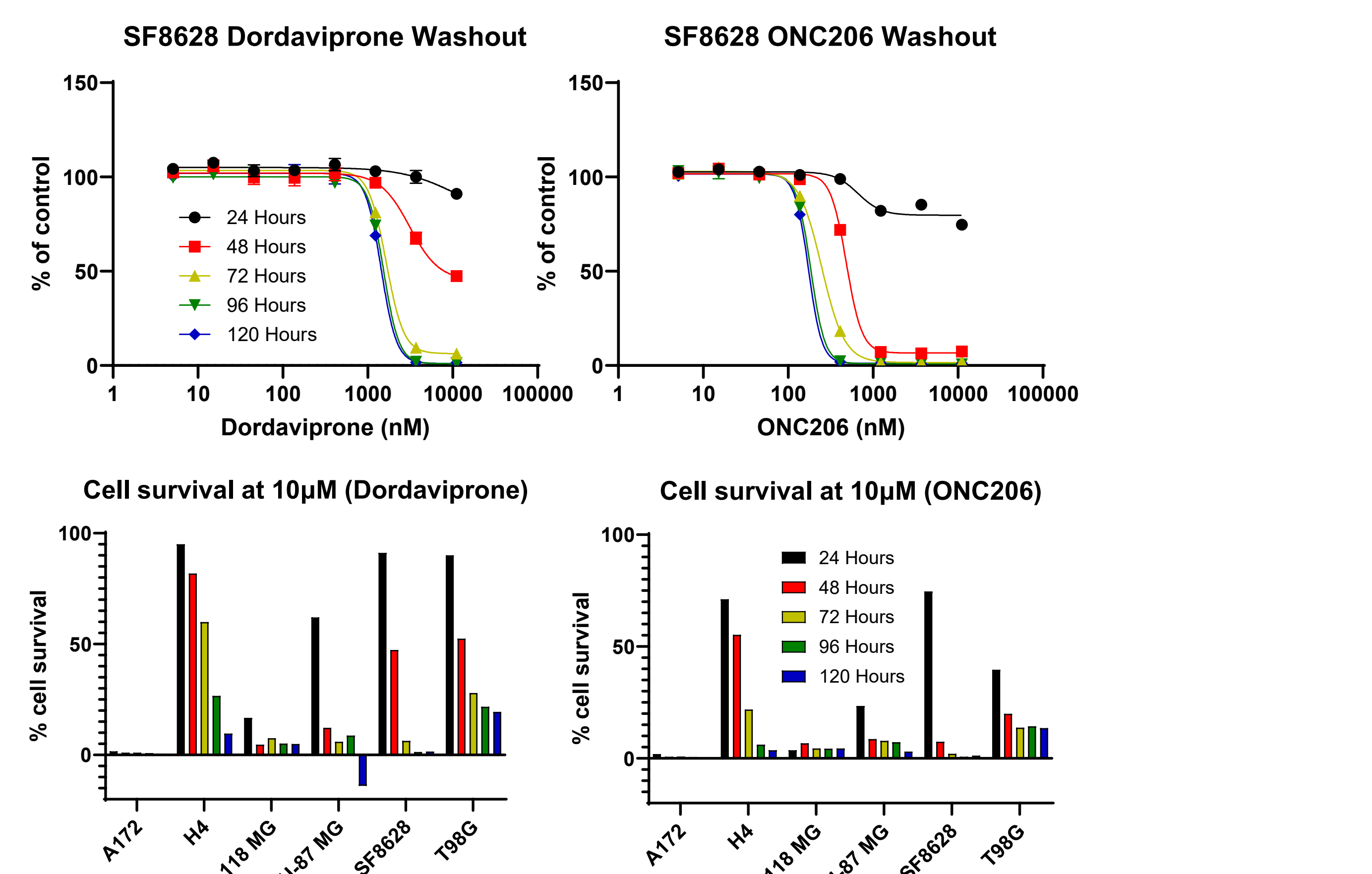
**Figure 1. Co-crystallization with ClpP revealed distinctions in the ONC206-ClpP resolved X-ray crystal structure relative to the dordaviprone-bound or apo complex**



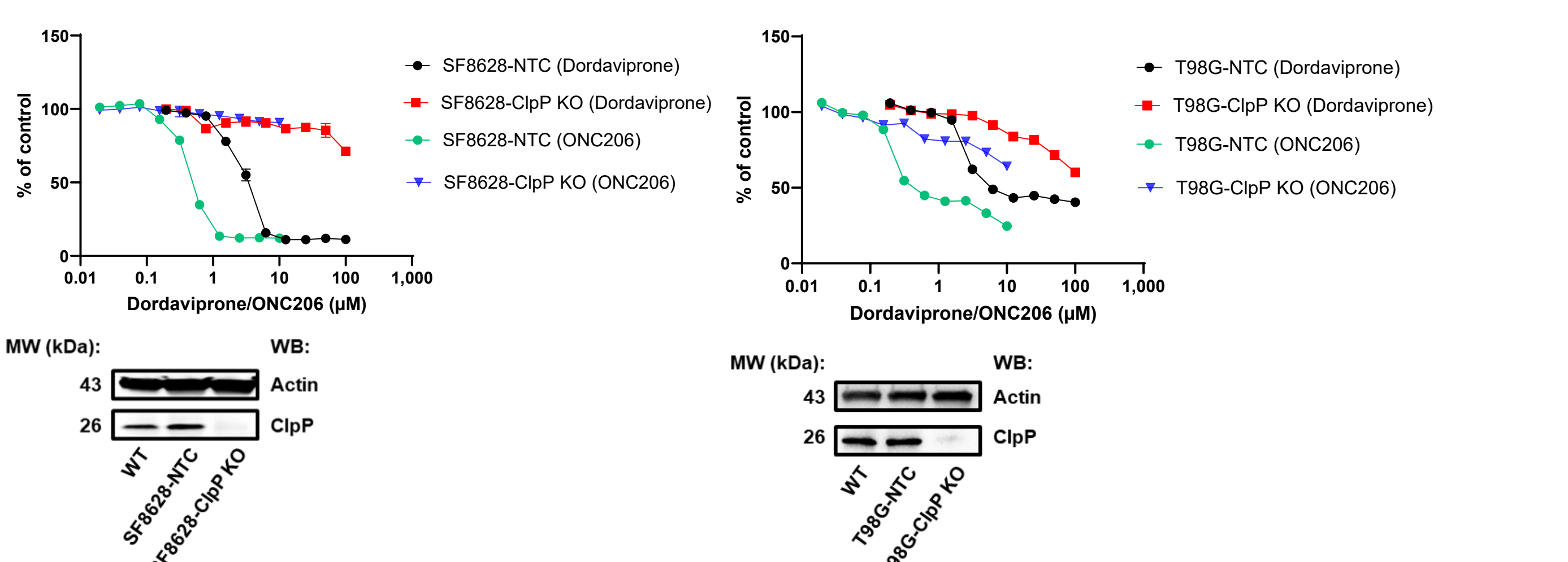
**Figure 2. ONC206 exhibits nanomolar potency in cell-free proteolysis assays and against glioma cell lines**



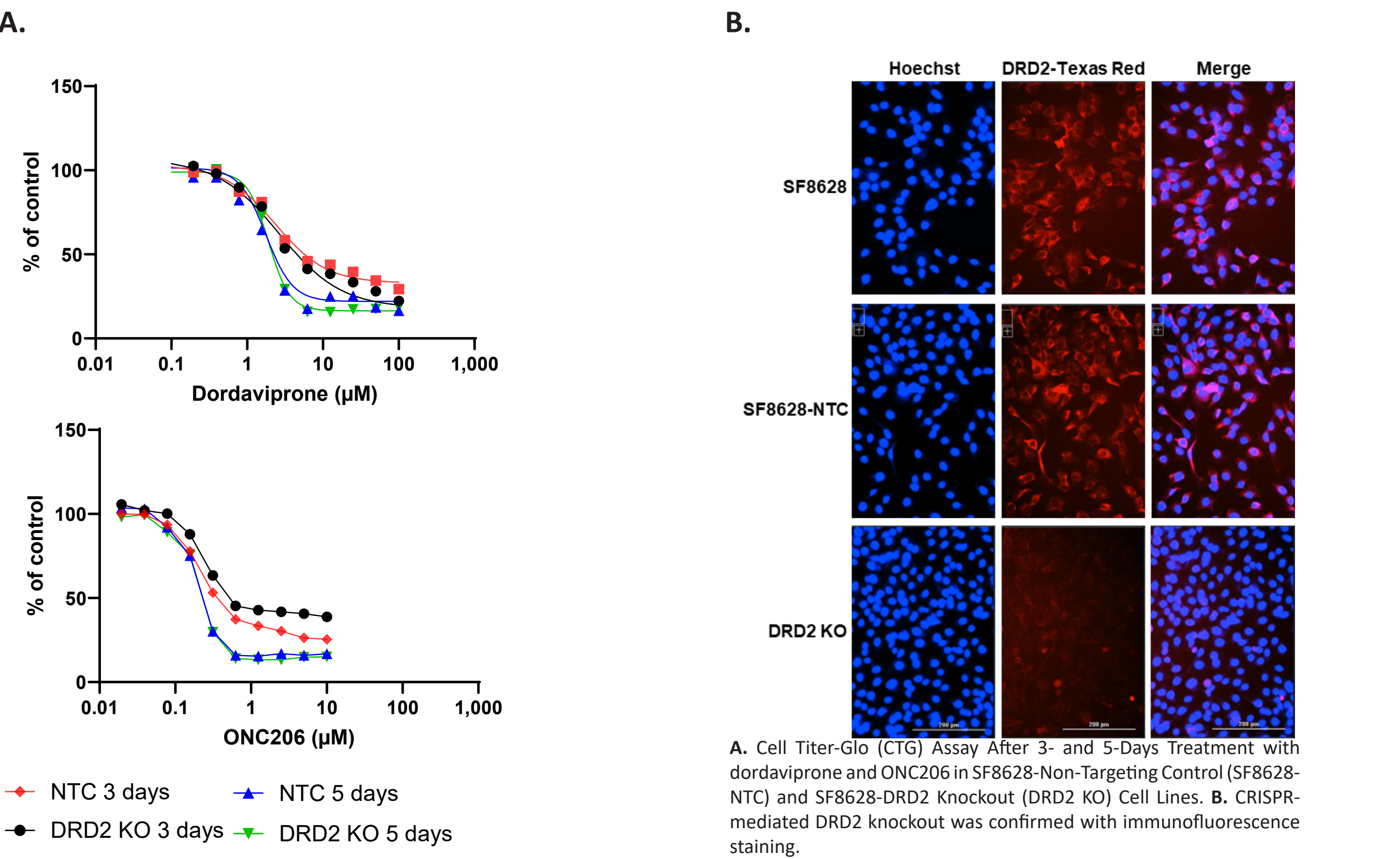
**Figure 3. Sustained ONC206 exposure needed to impair glioma cell viability**



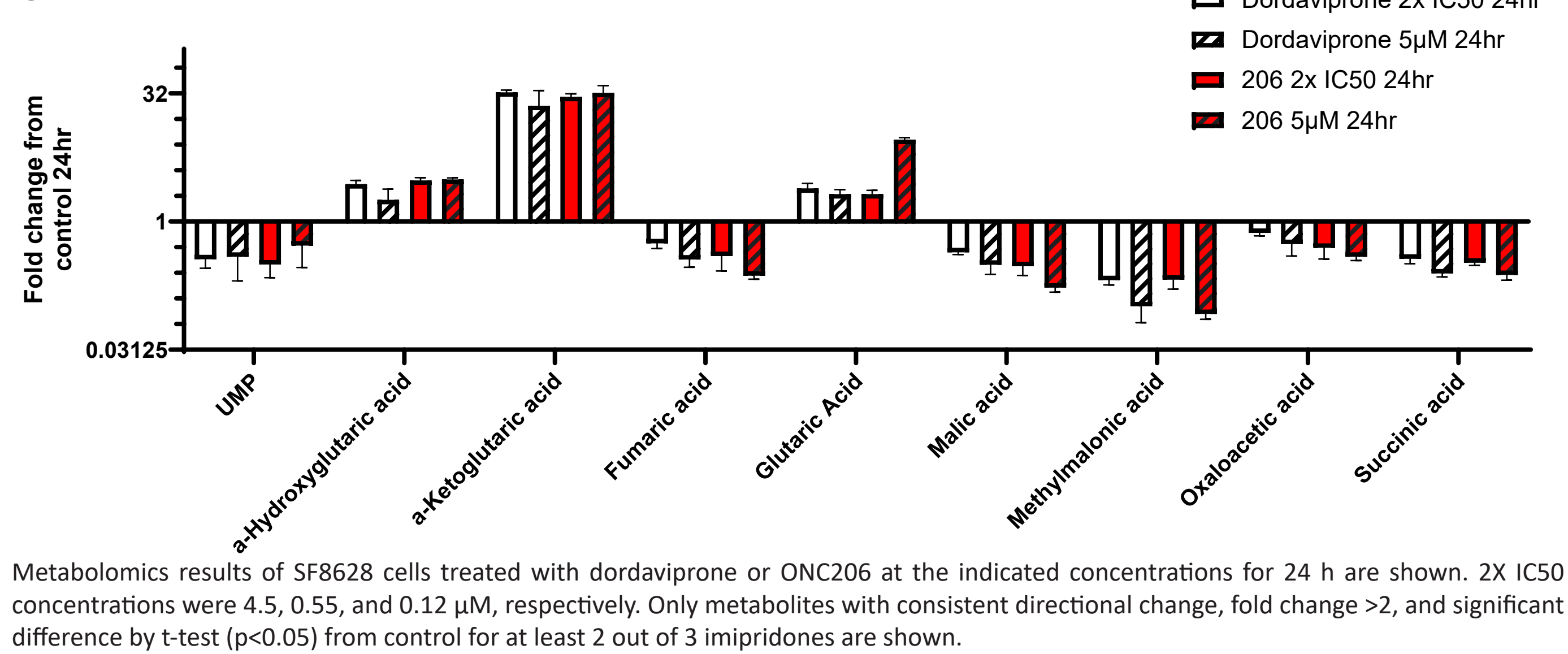
**Figure 4. ClpP knockout impairs dordaviprone and ONC206 efficacy in glioma cells in vitro**



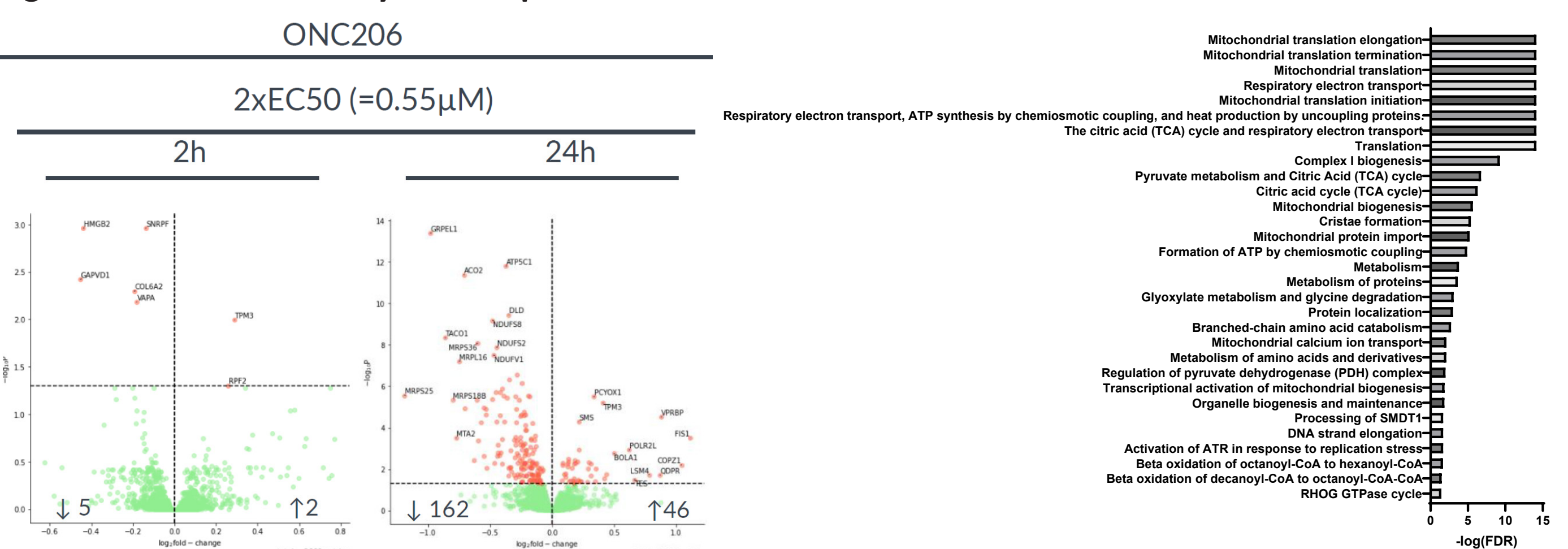
**Figure 5. DRD2 knockout does not impair dordaviprone and ONC206 efficacy in glioma cells in vitro**



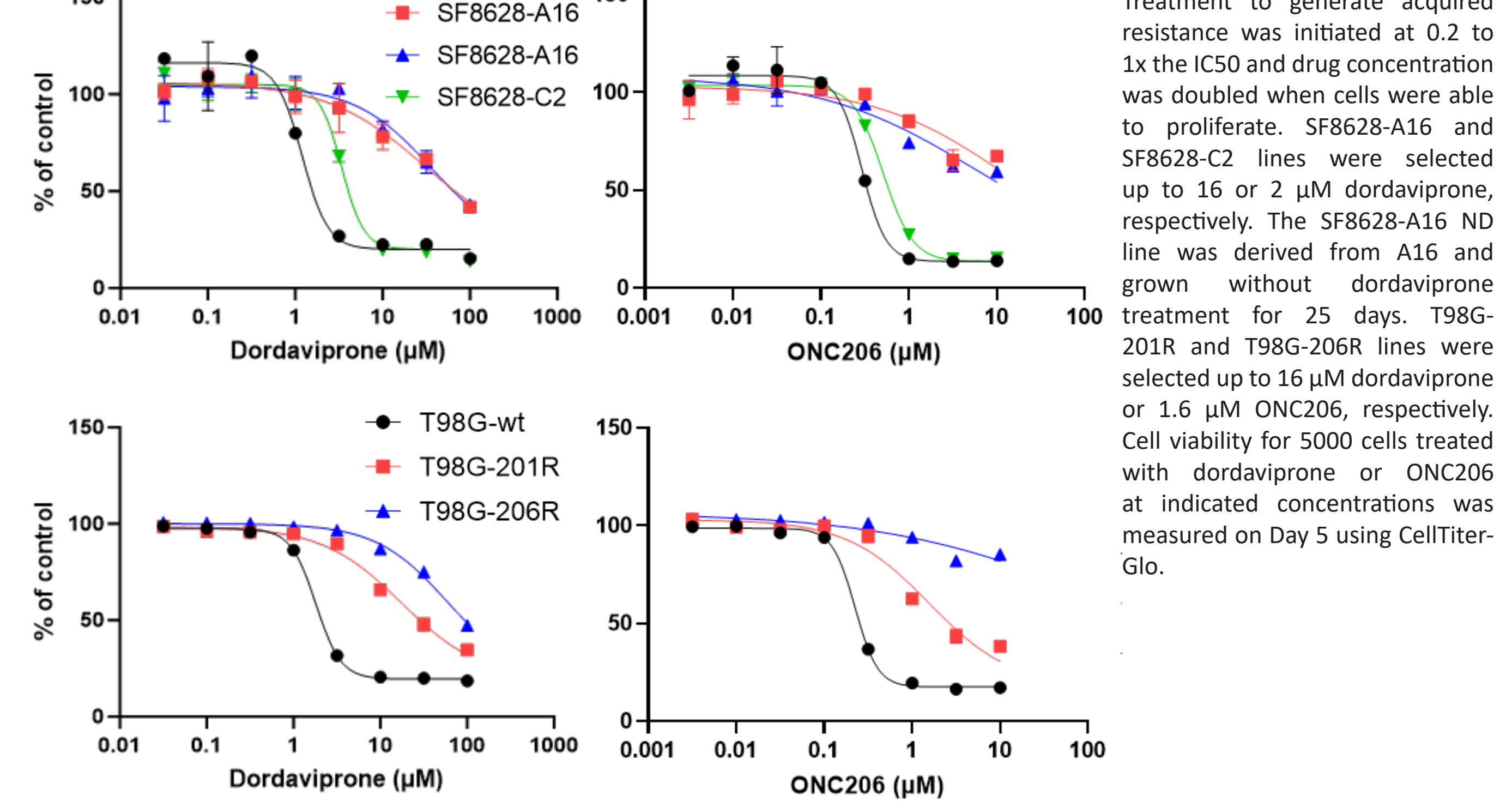
**Figure 6. ONC206 alters the mitochondrial metabolome**



**Figure 7. Proteomics analysis in response to ONC206 treatment in SF8628 cells<sup>14</sup>**

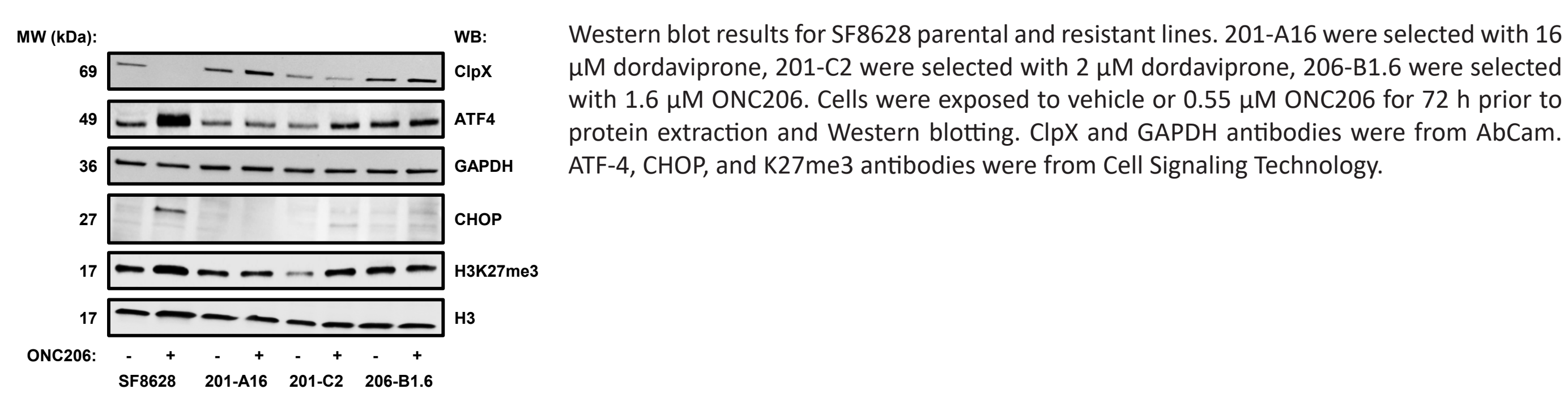


**Figure 8. Glioma cells with acquired resistance to dordaviprone showed cross-resistance to ONC206 in vitro**



## Results

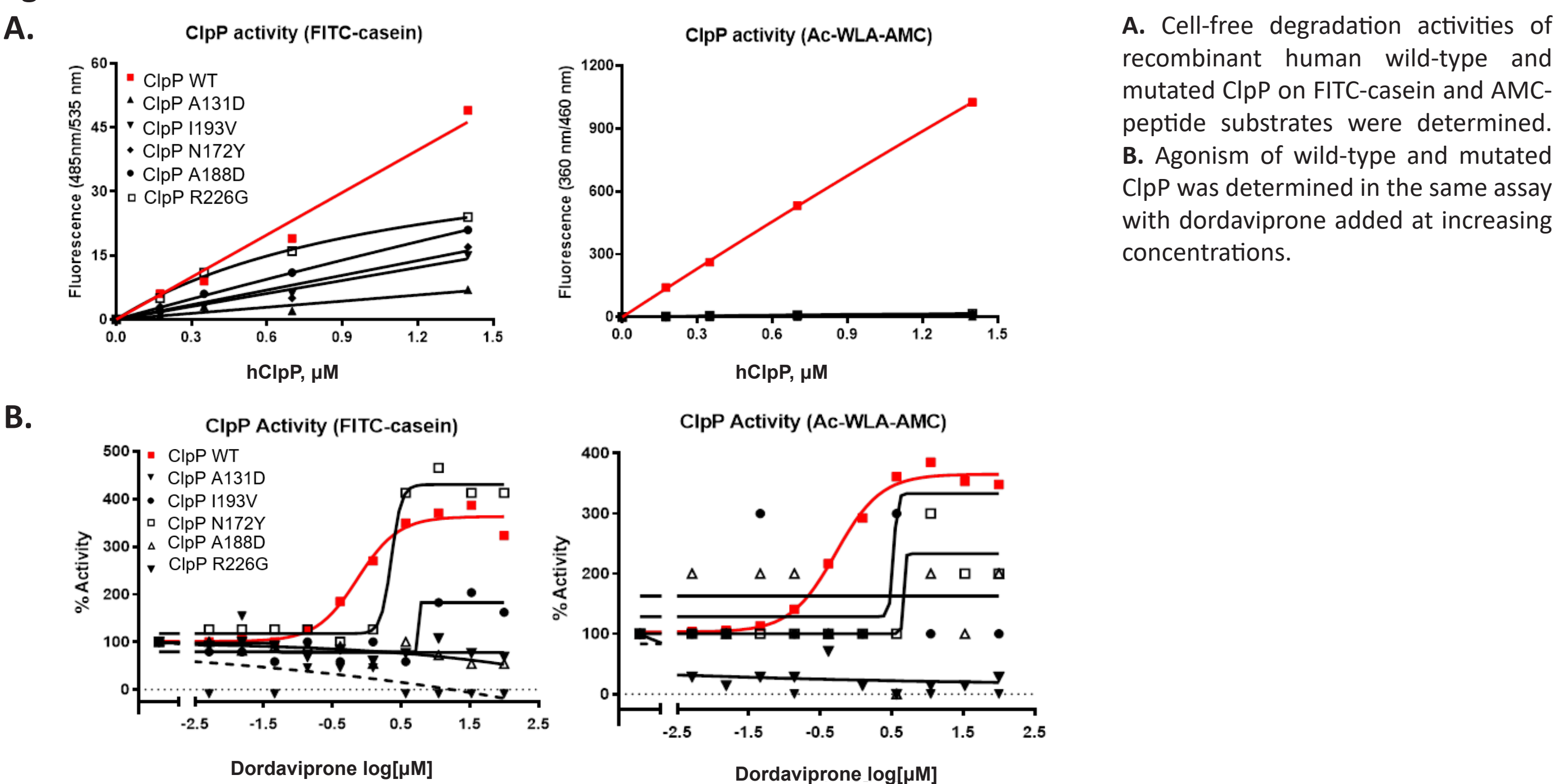
**Figure 9. ONC206 induces expression of ATF4, CHOP, and H3 K27me3 while reducing ClpX in parental, but not resistant cells**



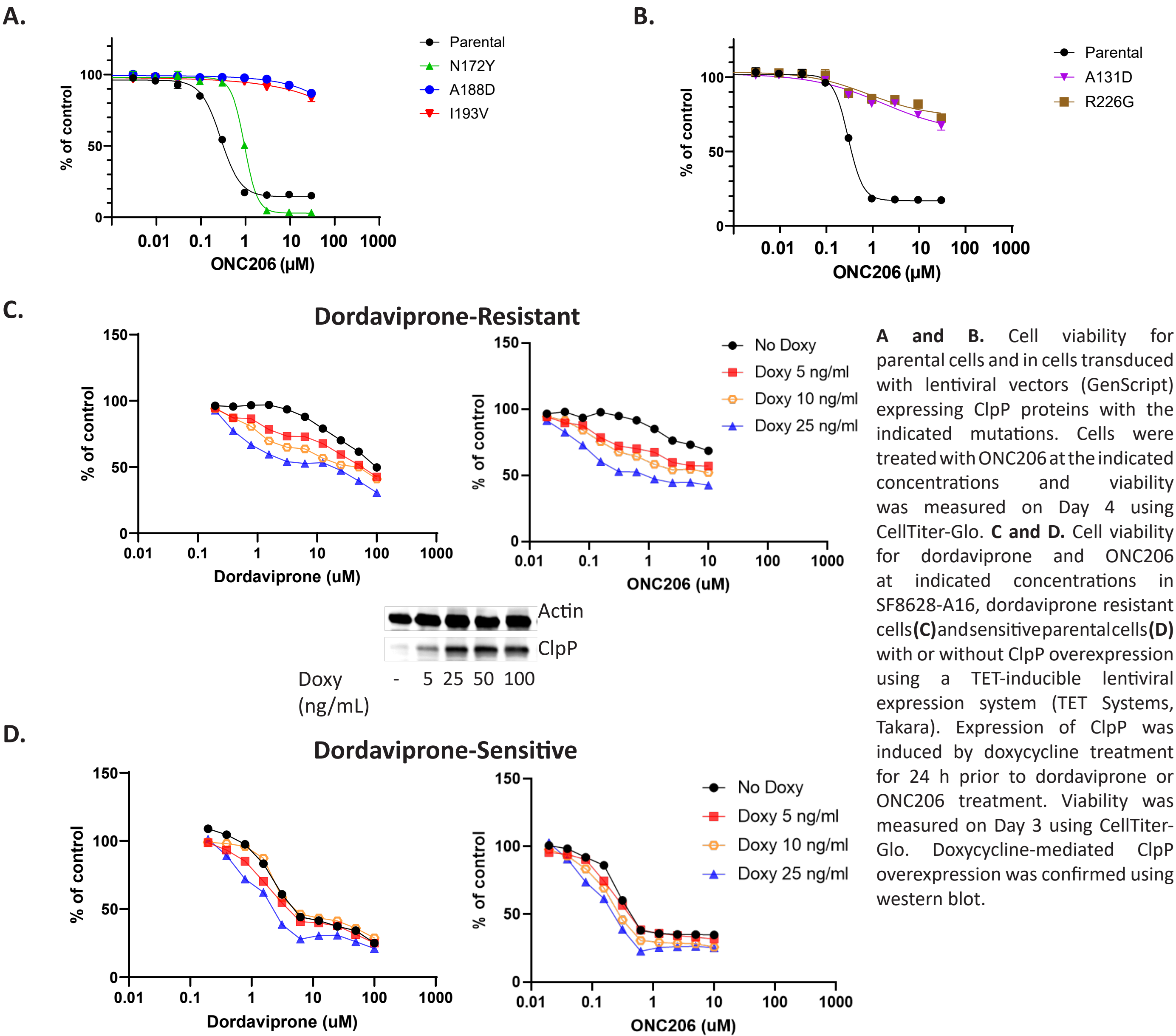
**Table 1. Positions of ClpP mutations identified in dordaviprone/ONC206 resistant glioma cells**

| Parental Line     | Name     | Selection (µM)       | ClpP Mutation(s) | Mutation Location       | Mutant Proportion |
|-------------------|----------|----------------------|------------------|-------------------------|-------------------|
| T98G, GBM         | 201-R    | Dordaviprone (16 µM) | A131D            | ClpP monomer interfaces | 17-20%            |
|                   |          |                      | R226G            | ClpX interface          | 12-21%            |
| T98G, GBM         | 206-R    | ONC206 (1.6 µM)      | R226G            | ClpX interface          | 43-54%            |
| SF8628, DIPG K27M | 201-A16  | Dordaviprone (16 µM) | I193V            | ClpX interface          | 50-67%            |
| SF8628, DIPG K27M | 201-C2   | Dordaviprone (2 µM)  | N172Y            | ClpP monomer interfaces | 22-33%            |
| SF8628, DIPG K27M | 206-B1.6 | ONC206 (1.6 µM)      | A188D            | ClpX interface          | ~50%              |

**Figure 10. ClpP with resistance mutations are catalytically impaired and reduce dordaviprone-mediated agonism**



**Figure 11. Expression of ClpP mutants is sufficient for imipridone resistance while over-expression of wild-type ClpP partially reverses resistance**



## Conclusions

- ONC206 exhibits nanomolar potency in glioma cell lines
- ClpP expression and agonism is key for the anti-cancer efficacy of ONC206 in vitro
- Acquired resistance to ONC206 in vitro is associated with alterations in ClpP
- ONC206 treatment is associated with alterations in metabolism, ISR, and histone methylation