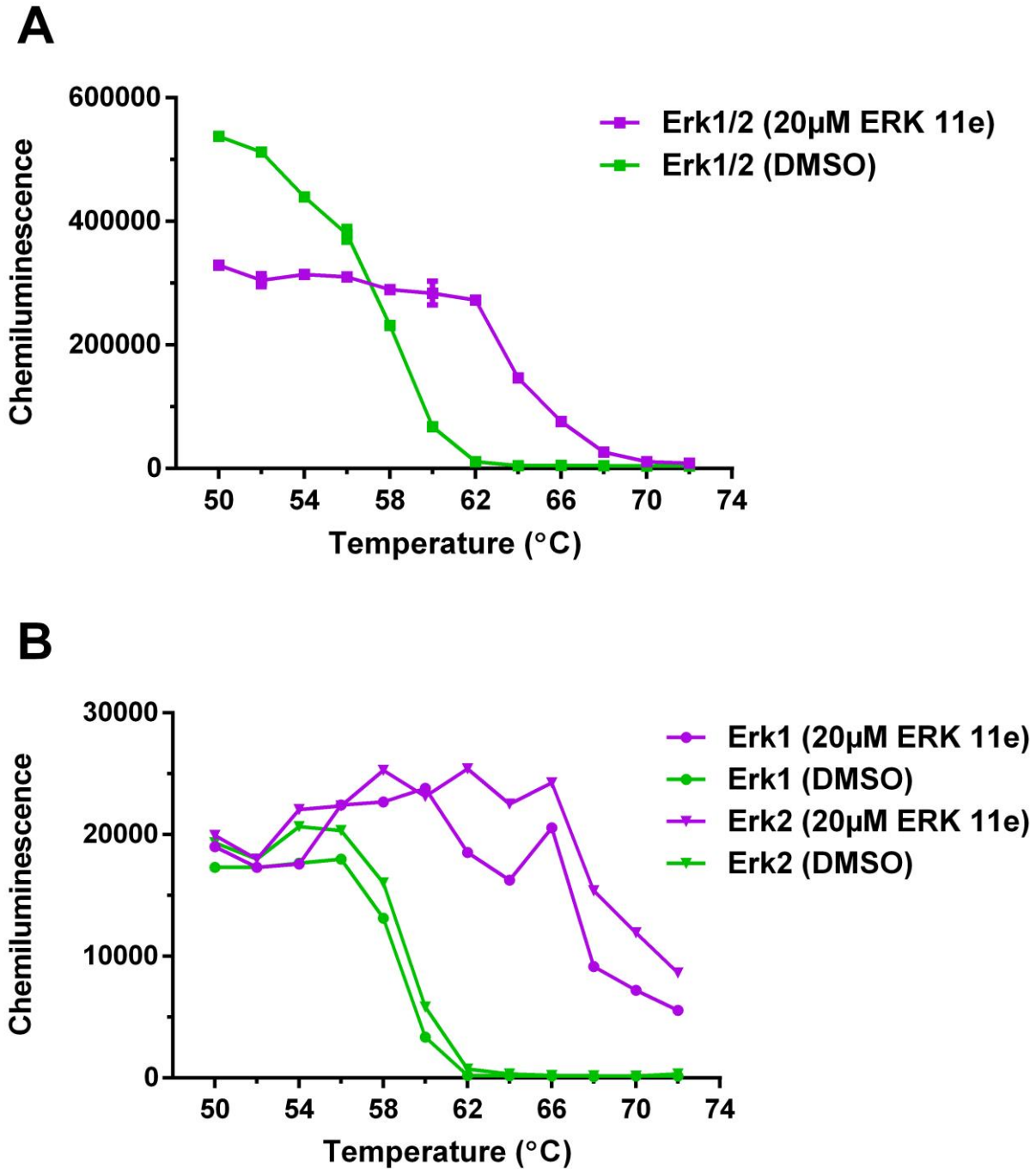
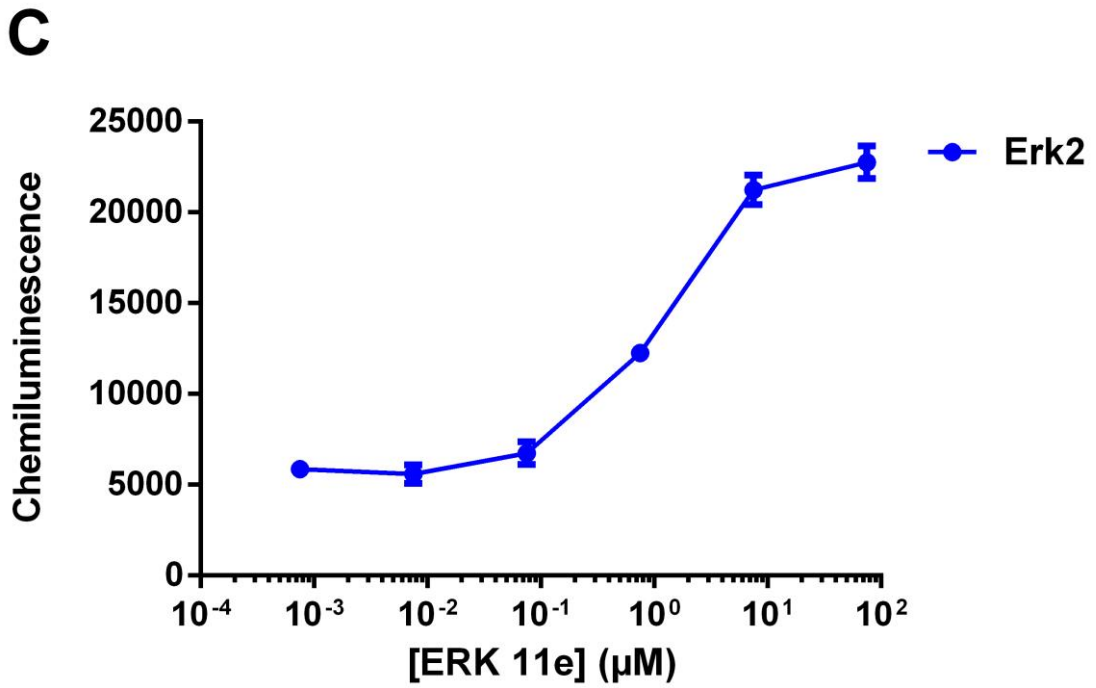

A
600000
400000
200000
0
Chemiluminescence
50
54
58
62
66
70
74
Temperature (°C)
Erk1/2 (20μM ERK 11e)
Erk1/2 (DMSO)
B
30000
20000
10000
0
Chemiluminescence
50
54
58
62
66
70
74
Temperature (°C)
Erk1 (20μM ERK 11e)
Erk1 (DMSO)
Erk2 (20μM ERK 11e)
Erk2 (DMSO)



In order to verify binding of ERK 11e to the ERK2 protein kinase (MAPK1) we investigated its ability to stabilize the intracellular kinase. This was achieved by treating K562 cells in both a CETSA melting curve and an $ITDRF_{CETSA}$ experiment. The CETSA melting curve was first analyzed with a SureFire™ kit (PerkinElmer, product number: TGRES500) directed towards Erk1 and Erk2 (the antibody pair does not distinguish between these isoforms). The results are illustrated in (A) and demonstrate that 20 µM ERK 11e (purple) thermo-stabilized either or possibly both of the isoforms in K562 cells resulting in ~7°C shift in T_{agg} compared to the control (green). However, the data also show a clear quenching effect of the ligand on the maximal chemiluminescence signal, which can be seen by comparing the two curves at the lower temperatures (*e.g.* 50°C), indicating that the compound binding affects the antibodies ability to recognize either or possibly both of the kinases. This quenching was not observed in the Western blot analysis of the same samples for either ERK1 nor ERK2 (B). As our primary interest was in the ERK2 isoform we also investigated to what extent ERK 11e affected the AlphaScreen signal using purified recombinant ERK2 and a complete compound-induced signal loss was confirmed (data not shown). It remains to be demonstrated whether this is a concern also for the Erk1 isoform. Given the dual specificity of the antibody pair in the SureFire™ kit we also wanted to pursue the Western blot based protocol for studies of ERK 11e binding to ERK2. The results from an $ITDRF_{CETSA}$ experiment conducted at 60°C (C) demonstrate that ERK 11e thermo-stabilized ERK2 in K562 cells with an inflection point giving 50% stabilization at 1.2±0.17 µM (given as the average ± standard deviation). We hence conclude that binding of ERK 11e can be monitored using both CETSA formats, but that the AlphaScreen based approach would ideally require the identification of another

antibody pair. As an alternative to changing antibodies, the user can attempt alternative buffer compositions, such as pH changes or mild denaturing conditions during the detection step, such that compound binding and thus quenching of the antibody recognition is prevented. A prerequisite for such an approach is that antibody recognition is still achieved, i.e. the antibodies tolerate and bind the target under these conditions. Should neither of these alternatives work, it is still possible to perform a screen; as discussed in the Limitations section any false negatives, defined as compounds that do bind to and stabilize the ERK2 kinase but that are lost in the screen because of substantial signal quenching, will be detected in a parallel counter-screening campaign in which the quenching effect is studied using cells or cell lysates at a temperature well below the apparent aggregation temperature, *e.g.* at 37°C.