

Scheme S1

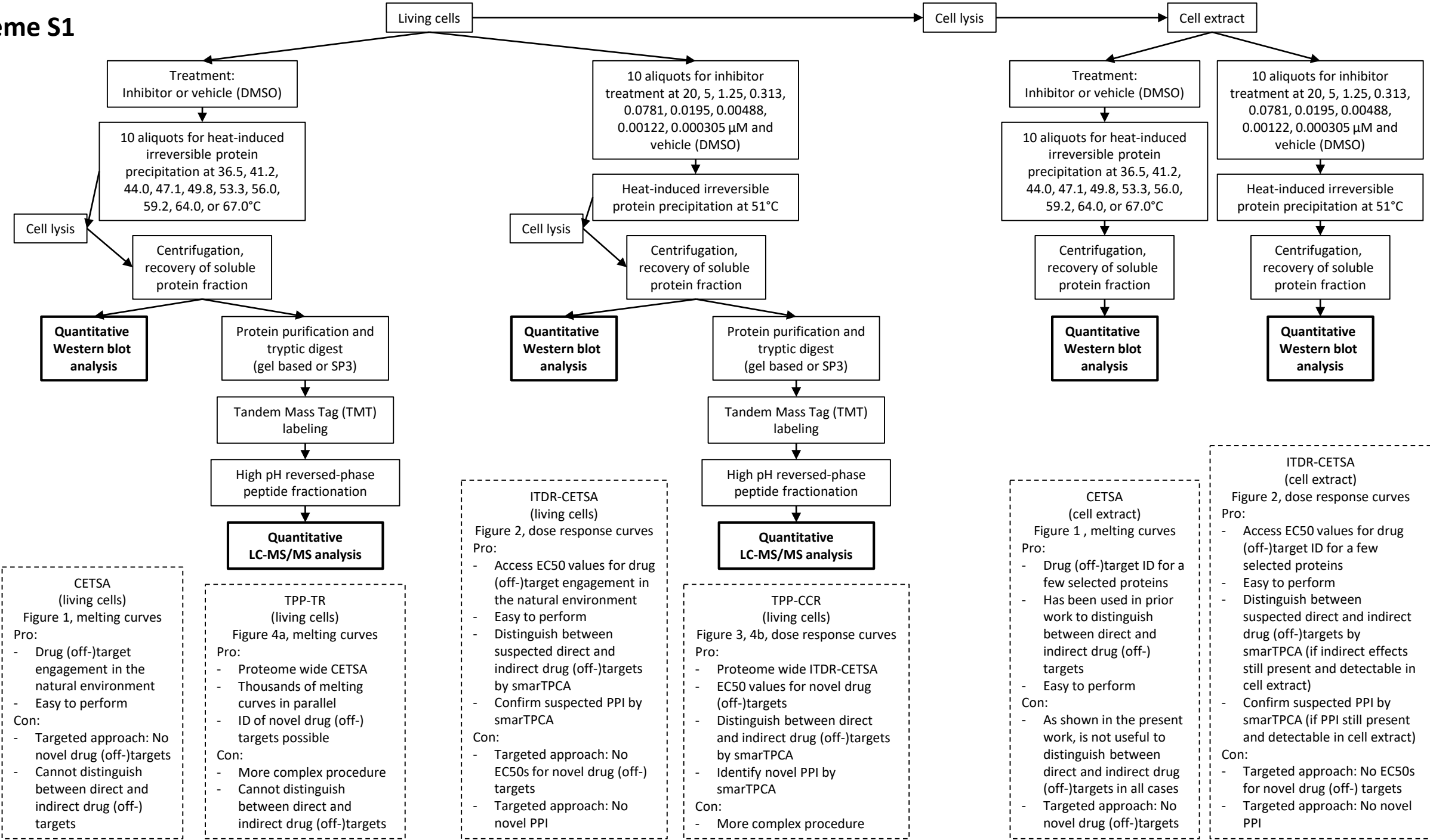


Figure S1

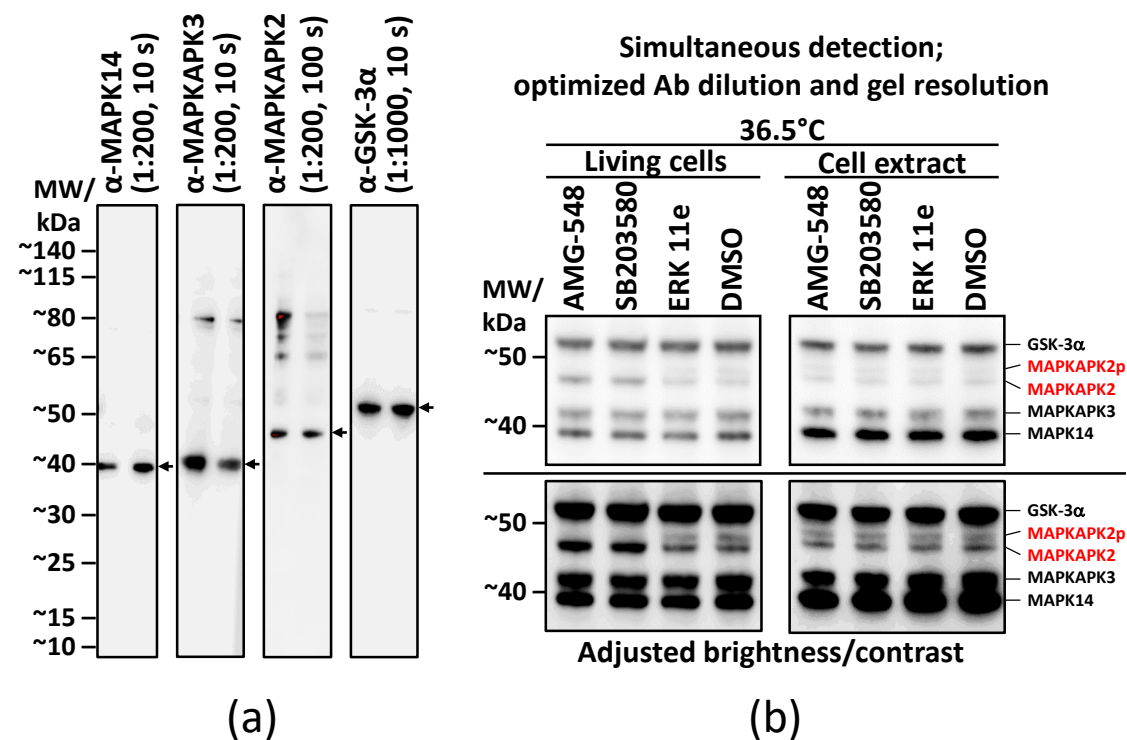


Figure S1. Immunoblot detection of MAPK14, MAPKAPK3, MAPKAPK2, and GSK-3 α . **(a)** Separate detection under non-optimized SDS-PAGE and WB conditions (duplicates) from untreated HL-60 cell extract (5 μ g total protein per gel lane). The dilution of the primary antibody and the exposure times are denoted in parenthesis above the respective lanes. The specific signals are marked by arrows. No unspecific signals potentially disturbing the simultaneous detection of the proteins from one gel lane are detected below about 60 kDa. **(b)** Simultaneous detection of the proteins from one gel lane under optimized SDS-PAGE (voltage, running time, cooling) and WB conditions (α -MAPK14 (1:2500), α -MAPKAPK3 (1:1300), α -MAPKAPK2 (1:50), and α -GSK-3 α (1:1000)). Samples from the lowest CETSA temperature treatment for all inhibitors in living cells and cell extract were analyzed and the resulting immunoblot signals are shown without (top panels) and with adjusted brightness and contrast (bottom panels) for a more detailed presentation of the signals for MAPKAPK2 and MAPKAPK2p.

Figure S2

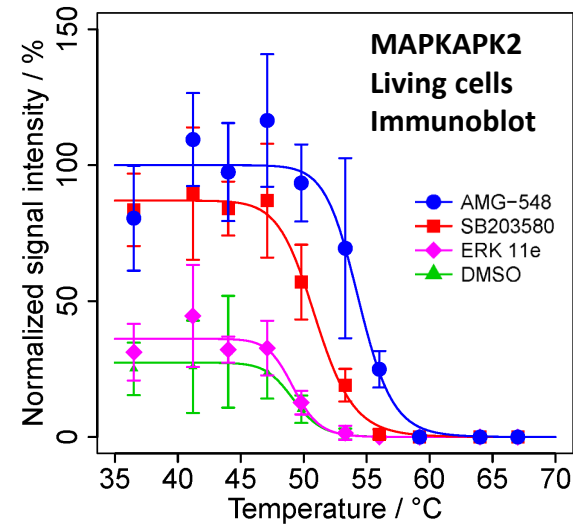
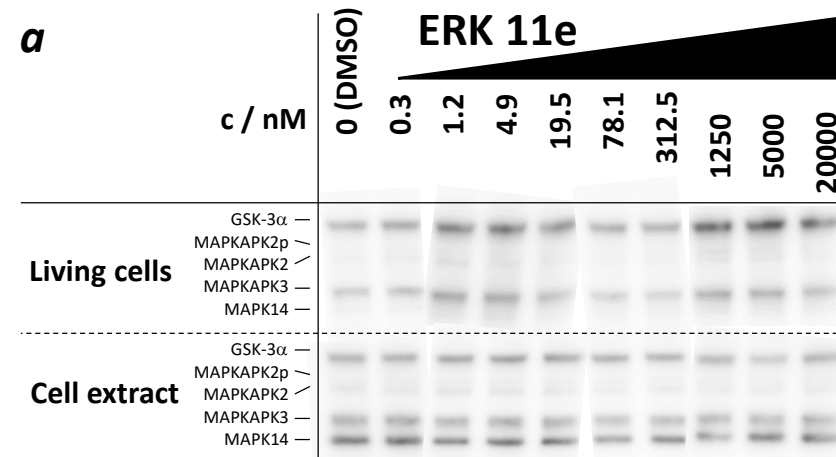


Figure S2. Melting curves of MAPKAPK2 from living cell CETSA experiments without relative (inhibitor treatment) signal normalization.

Figure S3



(see Figure S3b for figure caption)

Figure S3

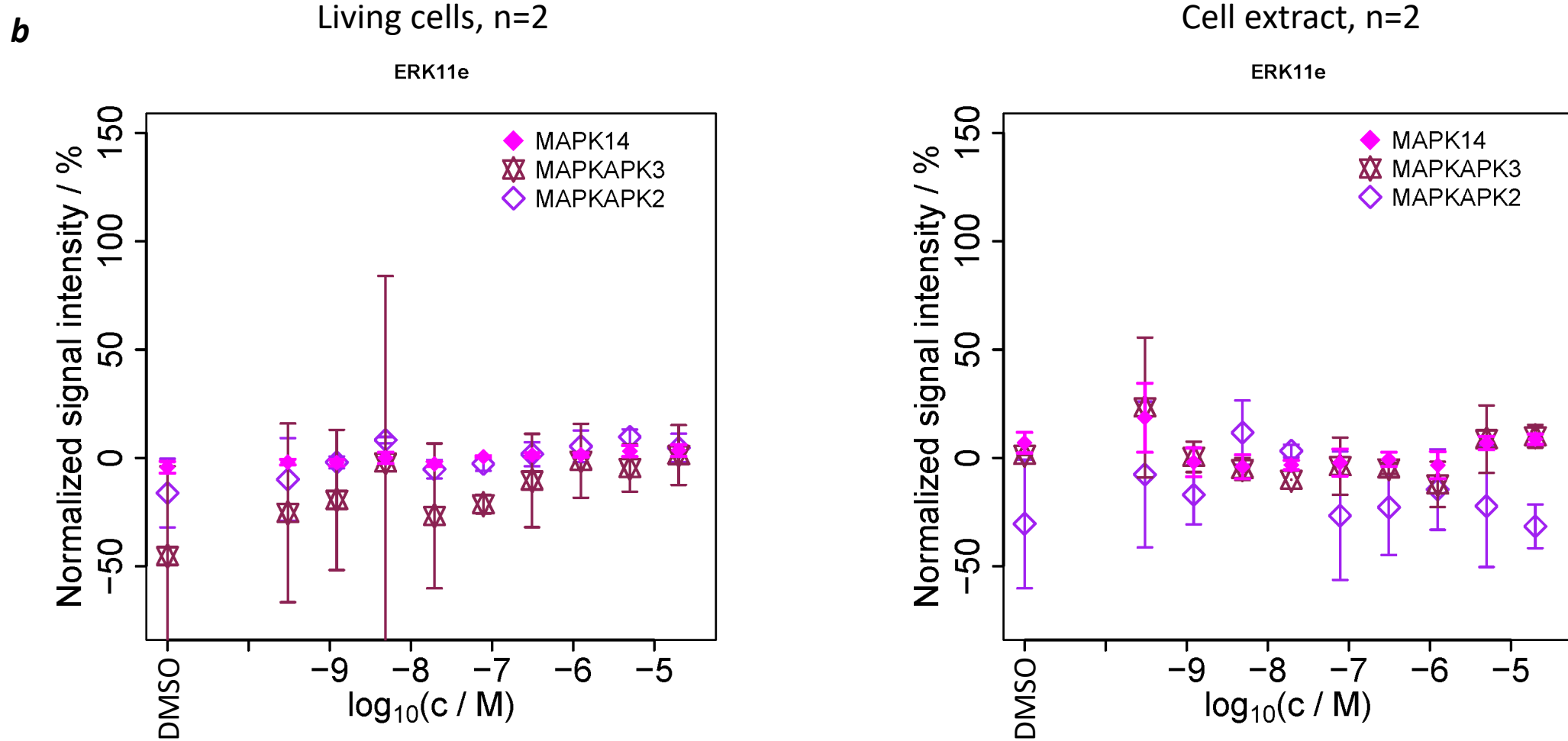


Figure S3. Supplementary data to Figure 2 of the main text showing the absence of dose responses for the control compound ERK 11e in ITDR-CETSA experiments at a constant treatment temperature of 51°C. **(a)** Immunoblots for one of the replicates of ITDR-CETSA experiments for living cells and cell extract, respectively. **(b)** Integrated, normalized signal intensities from the immunoblots. Datapoints and error bars represent means over the replicates +/- one standard deviation. For MAPKAPK2p, no meaningful data could be obtained due to low immunoblot signal intensity.

Figure S4

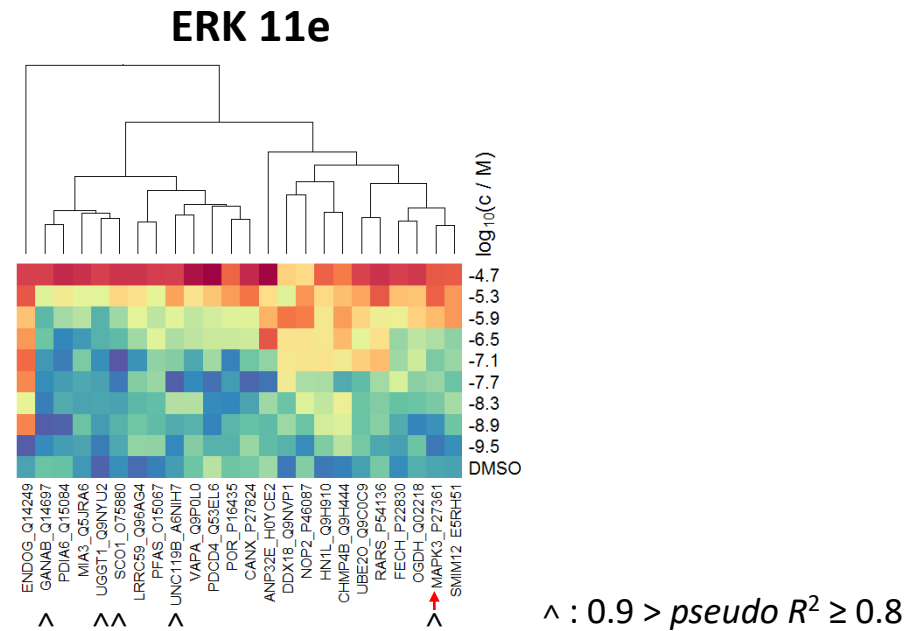


Figure S4. Supplementary data to Figure 3 of the main text showing the heat map after cluster analysis of the filtered (acceptable fit of dose-response curves for stabilized proteins, see experimental section for filter criteria) dose responses (mean over replicates) derived from TPP-CCR experiments in living cells for ERK 11e.

Figure S5

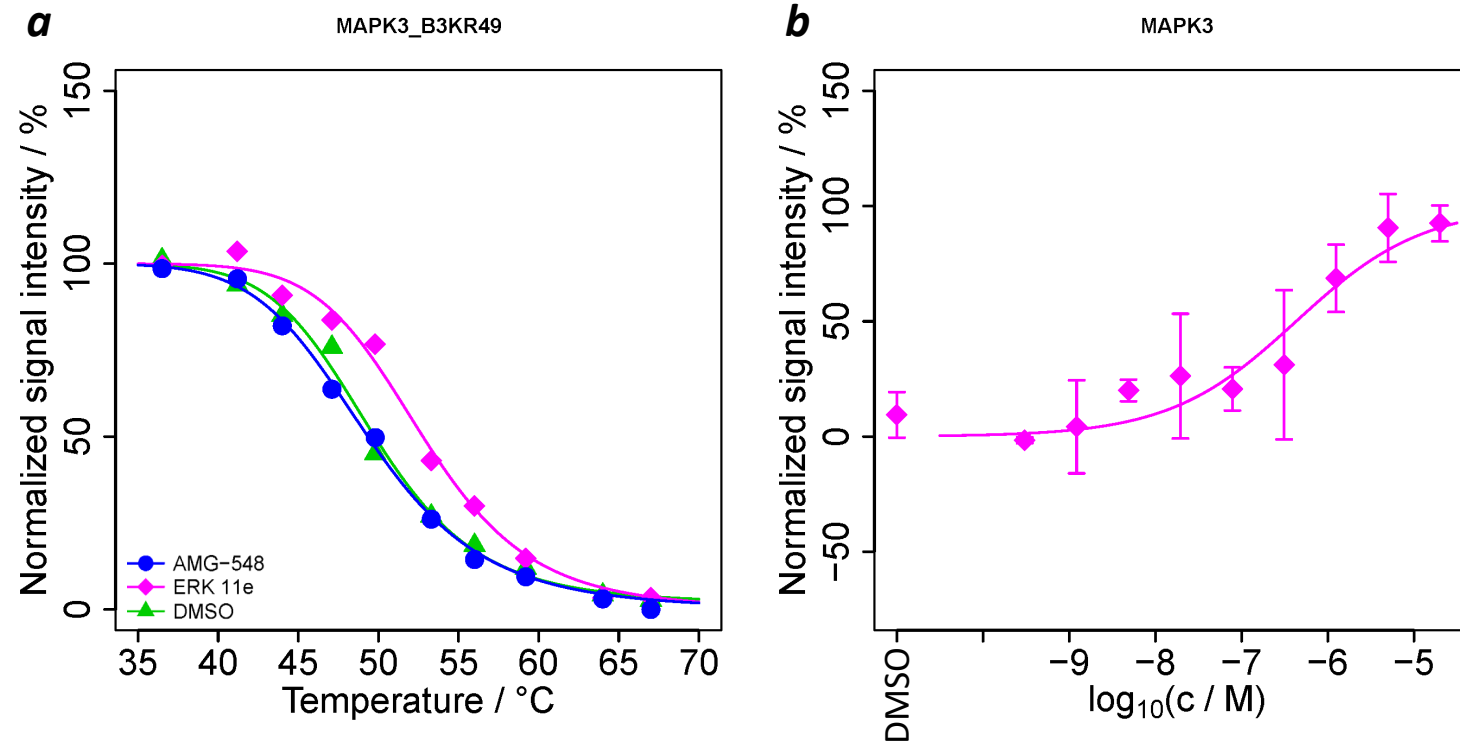


Figure S5. Melting curves (*a*) and dose response curves (*b*) for MAPK3 from the TPP-TR and TPP-CCR experiments. A melting curve for MAPK3 upon SB203580 treatment could not be determined due to missing reporter intensities.

Figure S6

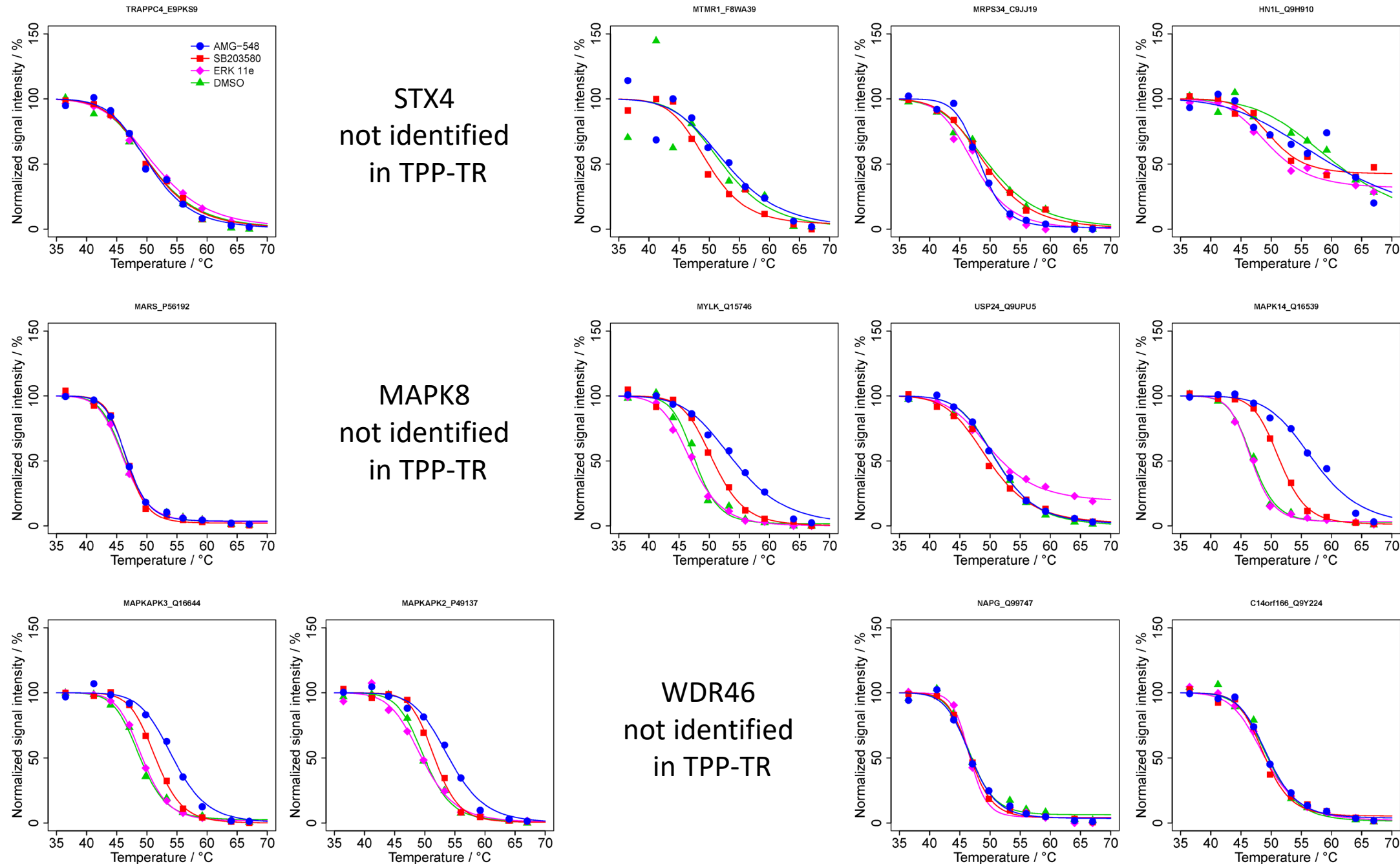


Figure S6: Melting curves from a single set of TPP-TR experiments of the proteins with acceptable stabilizing dose-response behavior in the TPP-CCR experiments shown in Figure 3 of the main text.