

## Descriptions of Additional Supplementary Files

### Supplementary Data 1

#### Source data underlying Fig. 4c.

Flow cytometric median fluorescence intensities (MFI) of ERK1/2 phosphorylation in HCT116 cells upon FLAG-positive delivery of the indicated SptP120 fused binders (E3\_5, K27, K55 and NS1). Data were analysed 10 minutes post-infection in the presence of bortezomib (BZB; 50 nM). Last column shows relative MFI of ERK1/2 phosphorylation calculated compared to the SptP120-E3\_5 control DARPin treated cells.

### Supplementary Data 2

#### Source data underlying Fig. 4d.

Flow cytometric median fluorescence intensities (MFI) of GSK3 $\beta$  phosphorylation in HCT116 cells upon FLAG-positive delivery of the indicated SptP120 fused binders (E3\_5, K27, K55 and NS1). Data were analysed 10 minutes post-infection in the presence of bortezomib (BZB; 50 nM). Last column shows relative MFI of GSK3 $\beta$  phosphorylation calculated compared to the SptP120-E3\_5 control DARPin treated cells.

### Supplementary Data 3

#### Source data underlying Fig. 4f.

Flow cytometric median fluorescence intensities (MFI) of ERK1/2 phosphorylation in HeLa Kyoto cells upon FLAG-positive delivery of the indicated SptP120 fused binders (E3\_5, K27, K55 and NS1) in the presence of bortezomib (BZB; 50 nM). Data were analysed after 10 minutes incubation in fresh medium with or without EGF induction (20ng) post-infection. Last column shows relative MFI of ERK1/2 phosphorylation calculated compared to the SptP120-E3\_5 control DARPin treated cells with EGF induction.

### Supplementary Data 4

#### Source data underlying Fig. 4g.

Flow cytometric median fluorescence intensities (MFI) of GSK3 $\beta$  phosphorylation in HeLa Kyoto cells upon FLAG-positive delivery of the indicated SptP120 fused binders (E3\_5, K27, K55 and NS1) in the presence of bortezomib (BZB; 50 nM). Data were analysed after 10 minutes incubation in fresh medium with or without EGF (20ng) post-infection. Last column shows relative MFI of GSK3 $\beta$  phosphorylation calculated compared to the SptP120-E3\_5 control DARPin treated cells with EGF induction.

|                                 | Cells/Single Cells/Live/FLAG+<br>Median (PE-A) | Relative MFI |
|---------------------------------|--|--------------|
| AC20180531_pERK_HCT116_E3_5+BZB | 2189   | 100          |
| AC20180531_pERK_HCT116_K27+BZB  | 1506   | 68.7985381   |
| AC20180531_pERK_HCT116_K55+BZB  | 1345   | 61.4435815   |
| AC20180531_pERK_HCT116_NS1+BZB  | 1335   | 60.9867519   |
| AC20180606_pERK_HCT116_E3_5+BZB | 2723   | 100          |
| AC20180606_pERK_HCT116_K27+BZB  | 2025   | 74.3665075   |
| AC20180606_pERK_HCT116_K55+BZB  | 1642   | 60.3011385   |
| AC20180606_pERK_HCT116_NS1+BZB  | 1731   | 63.5695924   |
| AC20180608_pERK_HCT116_E3_5+BZB | 2639   | 100          |
| AC20180608_pERK_HCT116_K27+BZB  | 1815   | 68.7760515   |
| AC20180608_pERK_HCT116_K55+BZB  | 1748   | 66.2372111   |
| AC20180608_pERK_HCT116_NS1+BZB  | 1953   | 74.005305    |
| AC20180629_pERK_HCT116_E3_5+BZB | 1899   | 100          |
| AC20180629_pERK_HCT116_K27+BZB  | 1368   | 72.0379147   |
| AC20180629_pERK_HCT116_K55+BZB  | 1141   | 60.0842549   |
| AC20180629_pERK_HCT116_NS1+BZB  | 1147   | 60.4002106   |
| AC20180706_pERK_HCT116_E3_5+BZB | 2103   | 100          |
| AC20180706_pERK_HCT116_K27+BZB  | 1320   | 62.767475    |
| AC20180706_pERK_HCT116_K55+BZB  | 1244   | 59.1535901   |
| AC20180706_pERK_HCT116_NS1+BZB  | 1397   | 66.4289111   |
| AC20180725_pERK_HCT116_E3_5+BZB | 2359   | 100          |
| AC20180725_pERK_HCT116_K27+BZB  | 1487   | 63.0351844   |
| AC20180725_pERK_HCT116_K55+BZB  | 1239   | 52.5222552   |
| AC20180725_pERK_HCT116_NS1+BZB  | 1302   | 55.1928783   |

### Supplementary Data 1

|                                   | Cells/Single Cells/Live/FLAG+<br>Median (Pacific Blue-A) | Relative MFI |
|-----------------------------------|--|--------------|
| AC20180531_pGSK3b_HCT116_E3_5+BZB | 8562   | 100          |
| AC20180531_pGSK3b_HCT116_K27+BZB  | 7333   | 85.64587713  |
| AC20180531_pGSK3b_HCT116_K55+BZB  | 6821   | 79.6659659   |
| AC20180531_pGSK3b_HCT116_NS1+BZB  | 6573   | 76.76944639  |
| AC20180606_pGSK3b_HCT116_E3_5+BZB | 11586  | 100          |
| AC20180606_pGSK3b_HCT116_K27+BZB  | 10483  | 90.47988952  |
| AC20180606_pGSK3b_HCT116_K55+BZB  | 9620   | 83.03124461  |
| AC20180606_pGSK3b_HCT116_NS1+BZB  | 9054   | 78.14603832  |
| AC20180608_pGSK3b_HCT116_E3_5+BZB | 10019  | 100          |
| AC20180608_pGSK3b_HCT116_K27+BZB  | 8486   | 84.69907176  |
| AC20180608_pGSK3b_HCT116_K55+BZB  | 8448   | 84.31979239  |
| AC20180608_pGSK3b_HCT116_NS1+BZB  | 8081   | 80.65675217  |
| AC20180629_pGSK3b_HCT116_E3_5+BZB | 8834   | 100          |
| AC20180629_pGSK3b_HCT116_K27+BZB  | 7284   | 82.4541544   |
| AC20180629_pGSK3b_HCT116_K55+BZB  | 7496   | 84.85397329  |
| AC20180629_pGSK3b_HCT116_NS1+BZB  | 6866   | 77.72243604  |
| AC20180706_pGSK3b_HCT116_E3_5+BZB | 10602  | 100          |
| AC20180706_pGSK3b_HCT116_K27+BZB  | 8448   | 79.68307866  |
| AC20180706_pGSK3b_HCT116_K55+BZB  | 7714   | 72.75985663  |
| AC20180706_pGSK3b_HCT116_NS1+BZB  | 7748   | 73.08055084  |
| AC20180725_pGSK3b_HCT116_E3_5+BZB | 10699  | 100          |
| AC20180725_pGSK3b_HCT116_K27+BZB  | 9555   | 89.30741191  |
| AC20180725_pGSK3b_HCT116_K55+BZB  | 8893   | 83.11991775  |
| AC20180725_pGSK3b_HCT116_NS1+BZB  | 8486   | 79.31582391  |

## Supplementary Data 2

|                                     | Cells/Single Cells/Live/FLAG+<br>Median (PE-A) | Relative MFI |
|-------------------------------------|--|--------------|
| AC20180629_pERK_HK_E3_5+BZB_noEGF   | 2270   | 43.1805212   |
| AC20180629_pERK_HK_E3_5+BZB_20ngEGF | 5257   | 100          |
| AC20180629_pERK_HK_K27+BZB_noEGF    | 2083   | 39.6233593   |
| AC20180629_pERK_HK_K27+BZB_20ngEGF  | 2495   | 47.4605288   |
| AC20180629_pERK_HK_K55+BZB_noEGF    | 1558   | 29.6366749   |
| AC20180629_pERK_HK_K55+BZB_20ngEGF  | 2849   | 54.1944075   |
| AC20180629_pERK_HK_NS1+BZB_noEGF    | 2270   | 43.1805212   |
| AC20180629_pERK_HK_NS1+BZB_20ngEGF  | 4343   | 82.613658    |
| AC20180706_pERK_HK_E3_5+BZB_noEGF   | 2828   | 47.5613858   |
| AC20180706_pERK_HK_E3_5+BZB_20ngEGF | 5946   | 100          |
| AC20180706_pERK_HK_K27+BZB_noEGF    | 2660   | 44.7359569   |
| AC20180706_pERK_HK_K27+BZB_20ngEGF  | 2759   | 46.4009418   |
| AC20180706_pERK_HK_K55+BZB_noEGF    | 2203   | 37.0501177   |
| AC20180706_pERK_HK_K55+BZB_20ngEGF  | 2853   | 47.9818365   |
| AC20180706_pERK_HK_NS1+BZB_noEGF    | 2734   | 45.9804911   |
| AC20180706_pERK_HK_NS1+BZB_20ngEGF  | 4026   | 67.7093845   |
| AC20180725_pERK_HK_E3_5+BZB_noEGF   | 1642   | 39.3199234   |
| AC20180725_pERK_HK_E3_5+BZB_20ngEGF | 4176   | 100          |
| AC20180725_pERK_HK_K27+BZB_noEGF    | 1646   | 39.4157088   |
| AC20180725_pERK_HK_K27+BZB_20ngEGF  | 1603   | 38.3860153   |
| AC20180725_pERK_HK_K55+BZB_noEGF    | 1532   | 36.6858238   |
| AC20180725_pERK_HK_K55+BZB_20ngEGF  | 2029   | 48.5871648   |
| AC20180725_pERK_HK_NS1+BZB_noEGF    | 1549   | 37.0929119   |
| AC20180725_pERK_HK_NS1+BZB_20ngEGF  | 2917   | 69.8515326   |

### Supplementary Data 3

|                                       | Cells/Single Cells/Live/FLAG+<br>Median (Pacific Blue-A) | Relative MFI |
|---------------------------------------|--|--------------|
| AC20180629_pGSK3b_HK_E3_5+BZB_noEGF   | 8697   | 77.3204125   |
| AC20180629_pGSK3b_HK_E3_5+BZB_20ngEGF | 11248  | 100          |
| AC20180629_pGSK3b_HK_K27+BZB_noEGF    | 8893   | 79.0629445   |
| AC20180629_pGSK3b_HK_K27+BZB_20ngEGF  | 9364   | 83.2503556   |
| AC20180629_pGSK3b_HK_K55+BZB_noEGF    | 8581   | 76.2891181   |
| AC20180629_pGSK3b_HK_K55+BZB_20ngEGF  | 8814   | 78.3605974   |
| AC20180629_pGSK3b_HK_NS1+BZB_noEGF    | 8736   | 77.6671408   |
| AC20180629_pGSK3b_HK_NS1+BZB_20ngEGF  | 10675  | 94.905761    |
| AC20180706_pGSK3b_HK_E3_5+BZB_noEGF   | 10179  | 81.2759502   |
| AC20180706_pGSK3b_HK_E3_5+BZB_20ngEGF | 12524  | 100          |
| AC20180706_pGSK3b_HK_K27+BZB_noEGF    | 9599   | 76.6448419   |
| AC20180706_pGSK3b_HK_K27+BZB_20ngEGF  | 10156  | 81.0923028   |
| AC20180706_pGSK3b_HK_K55+BZB_noEGF    | 9054   | 72.2931971   |
| AC20180706_pGSK3b_HK_K55+BZB_20ngEGF  | 10202  | 81.4595976   |
| AC20180706_pGSK3b_HK_NS1+BZB_noEGF    | 10699  | 85.4279783   |
| AC20180706_pGSK3b_HK_NS1+BZB_20ngEGF  | 12553  | 100.231555   |
| AC20180725_pGSK3b_HK_E3_5+BZB_noEGF   | 8336   | 83.0196196   |
| AC20180725_pGSK3b_HK_E3_5+BZB_20ngEGF | 10041  | 100          |
| AC20180725_pGSK3b_HK_K27+BZB_noEGF    | 7974   | 79.414401    |
| AC20180725_pGSK3b_HK_K27+BZB_20ngEGF  | 8373   | 83.3881088   |
| AC20180725_pGSK3b_HK_K55+BZB_noEGF    | 7992   | 79.593666    |
| AC20180725_pGSK3b_HK_K55+BZB_20ngEGF  | 8562   | 85.2703914   |
| AC20180725_pGSK3b_HK_NS1+BZB_noEGF    | 8373   | 83.3881088   |
| AC20180725_pGSK3b_HK_NS1+BZB_20ngEGF  | 9427   | 93.8850712   |

#### Supplementary Data 4