**Table S2. Starting and fitted parameters of the computational model**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| parameter | measured | SPR fit^ | in vitro fit^ | in cell fit^ | unit | abbreviation |
| KD(ERK:RSK) | 2.5\* | - | **2.4** | **2.6** | µM | KD\_ER |
| KD(ERK:pRSK) | 12.5\*\* | - | **12.2** | **13.1** | µM | KD\_EpR |
| KD(ORF45:RSK) | 0.0012\* | - | **0.0012** | **0.0012** | µM | KD\_OR |
| KD(ORF45:pRSK) | 0.0012\* | - | **0.0012** | **0.0012** | µM | KD\_OpR |
| KD(ERK:ORF45) | 19.9\* | - | **79.4** | **101.5** | µM | KD\_EO |
| KD(pERK:ORF45) | 0.8\* | - | **0.79** | **0.98** | µM | KD\_pEO |
| KD(MKK:ERK) | 4.0\* | - | **4.1** | **3.9** | µM | KD\_EK |
| KD(MKP:ERK) | - | - | 4.6 | **4.7** | µM | KD\_EP |
| KD(PP:RSK) | - | - | - | 0.23 | µM | KD\_RP2 |
| koff(ERK:RSK) | 10.0\* | **13.3** | **13.5** | **16.2** | min-1 | koff\_ER |
| koff(ORF45:RSK) | 0.05\* | **0.044** | **0.044** | **0.043** | min-1 | koff\_OR |
| koff(ERK:ORF45) | - | - | 570.3 | **756.8** | min-1 | koff\_EO |
| koff(pERK:ORF45) | - | 5.6 | **5.7** | **7.3** | min-1 | koff\_pEO |
| koff(MKK:ERK) | - | - | 20.0 | **18.8** | min-1 | koff\_EK |
| koff(MKP:ERK) | - | - | 34.7 | **34.7** | min-1 | koff\_EP |
| koff(PP:RSK) | - | - | - | 0.92 | min-1 | koff\_RP2 |
| kcat(MKK) | - | - | 0.52 | **0.56** | min-1 | kp\_E |
| kcat(ERK) | - | - | 10.5 | **10.2** | min-1 | kp\_R |
| kcat(bg) | - | - | - | 0.0073 | min-1 | kp\_K\_bg |
| kcat(EGF) | - | - | - | 0.48 | min-1 | kp\_K\_egf |
| kcat(MKP) | - | - | 6.8 | **6.8** | min-1 | kdp\_E |
| kcat(PP, RSK) | - | - | - | 4.1 | min-1 | kdp\_R |
| kcat(PP, MKK) | - | - | - | 0.076 | min-1 | kdp\_K |
| a | - | 0.087 | **0.087** | **0.10** | µM | a |
| d | - | 0.0027 | **0.0026** | **0.0025** | - | d |

**\***This study (KD for MKK:ERK binding was measured by competitive FP assay; koff for ERK:RSK and ORF45:RSK was determined using the SPR BioEvaluation software, GE Healtcare)

\*\* RSK has a phosphoswitch region next to the MAPK docking motif and the binding affinity weakens (~ 5-fold) if this ERK regulated site is phosphorylated (Gógl et al., 2018)⁠

**^** Parameters in normal or bold type were free or constrained, respectively.