**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\* | - | **2.5** | **2.7** | µM | KD\_ER |
| KD(ERK:pRSK) | 12.5\*\* | - | **13** | **13** | µM | KD\_EpR |
| KD(ORF45:RSK) | 0.0012\* | - | **0.0012** | **0.0012** | µM | KD\_OR |
| KD(ORF45:pRSK) | 0.0012\* | - | **0.0012** | **0.0011** | µM | KD\_OpR |
| KD(ERK:ORF45) | 19.9\* | - | **78** | **99.9** | µM | KD\_EO |
| KD(pERK:ORF45) | 0.8\* | - | **0.8** | **0.99** | µM | KD\_pEO |
| KD(MKK:ERK) | 4.0\* | - | **4.1** | **4.1** | µM | KD\_EK |
| KD(MKP:ERK) | - | - | 1.2 | **1.2** | µM | KD\_EP |
| KD(PP:RSK) | - | - | - | 0.23 | µM | KD\_RP2 |
| koff(ERK:RSK) | 10.0\* | **13** | **13** | **15** | min-1 | koff\_ER |
| koff(ORF45:RSK) | 0.05\* | **0.044** | **0.041** | **0.04** | min-1 | koff\_OR |
| koff(ERK:ORF45) | - | - | 550 | **748** | min-1 | koff\_EO |
| koff(pERK:ORF45) | - | 5.6 | **5.6** | **7.4** | min-1 | koff\_pEO |
| koff(MKK:ERK) | - | - | 14 | **14** | min-1 | koff\_EK |
| koff(MKP:ERK) | - | - | 7.2 | **6.8** | min-1 | koff\_EP |
| koff(PP:RSK) | - | - | - | 0.72 | min-1 | koff\_RP2 |
| kcat(MKK) | - | - | 1.4 | **1.4** | min-1 | kp\_E |
| kcat(ERK) | - | - | 3.2 | **3.2** | min-1 | kp\_R |
| kcat(bg) | - | - | - | 0.013 | min-1 | kp\_K\_bg |
| kcat(EGF) | - | - | - | 0.22 | min-1 | kp\_K\_egf |
| kcat(MKP) | - | - | 2.5 | **2.7** | min-1 | kdp\_E |
| kcat(PP, RSK) | - | - | - | 4.6 | min-1 | kdp\_R |
| kcat(PP, MKK) | - | - | - | 0.23 | min-1 | kdp\_K |
| a | - | 0.087 | **0.087** | **0.1** | 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.