**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** | **14** | 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\* | - | **79** | **105** | µM | KD\_EO |
| KD(pERK:ORF45) | 0.8\* | - | **0.79** | **1** | µM | KD\_pEO |
| KD(MKK:ERK) | 4.0\* | - | **4** | **3.9** | µM | KD\_EK |
| KD(MKP:ERK) | - | - | 15 | **2** | µM | KD\_EP |
| KD(PP:RSK) | - | - | - | 0.055 | µM | KD\_RP2 |
| koff(ERK:RSK) | 10.0\* | **13** | **13** | **16** | min-1 | koff\_ER |
| koff(ORF45:RSK) | 0.05\* | **0.044** | **0.044** | **0.045** | min-1 | koff\_OR |
| koff(ERK:ORF45) | - | - | 559 | **736** | min-1 | koff\_EO |
| koff(pERK:ORF45) | - | 5.6 | **5.6** | **7** | min-1 | koff\_pEO |
| koff(MKK:ERK) | - | - | 19 | **20** | min-1 | koff\_EK |
| koff(MKP:ERK) | - | - | 326 | **32** | min-1 | koff\_EP |
| koff(PP:RSK) | - | - | - | 0.42 | min-1 | koff\_RP2 |
| kcat(MKK) | - | - | 0.52 | **0.79** | min-1 | kp\_E |
| kcat(ERK) | - | - | 10 | **5.7** | min-1 | kp\_R |
| kcat(bg) | - | - | - | 0.0088 | min-1 | kp\_K\_bg |
| kcat(EGF) | - | - | - | 0.33 | min-1 | kp\_K\_egf |
| kcat(MKP) | - | - | 19 | **3.5** | min-1 | kdp\_E |
| kcat(PP, RSK) | - | - | - | 21 | min-1 | kdp\_R |
| kcat(PP, MKK) | - | - | - | 0.1 | min-1 | kdp\_K |
| a | - | 0.087 | **0.087** | **0.1** | µM | a |
| d | - | 0.0027 | **0.0027** | **0.0024** | - | 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.