SUPPLEMENTAL MATERIALS

SUPPLEMENTAL FIGURE LEGENDS

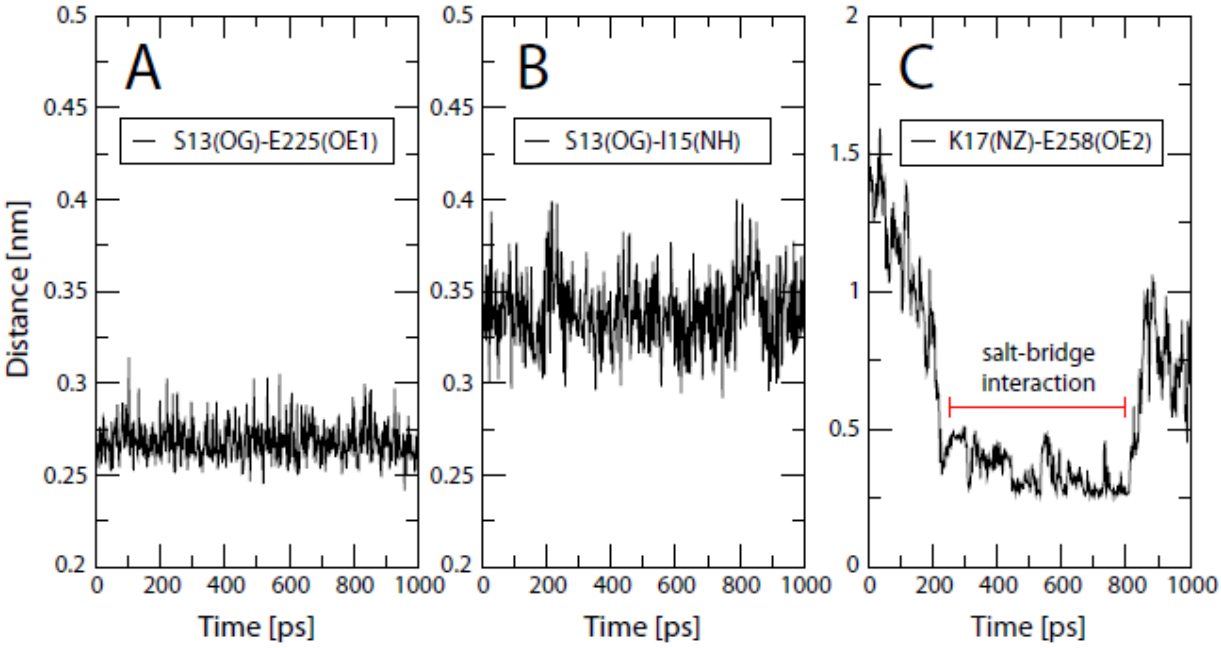
SUPPLEMENTAL FIGURE 1. Molecular dynamics simulations of MACFp1-derived peptides: distances between some interacting residues along MD trajectories. A and B. Distances between the hydroxyl group of Ser13 and two interacting atoms in the WT complex (A: the backbone nitrogen of Ile17, B: the closest oxygen of the carboxylate group of Glu225 on the EB1 protein). C. Distance between the sidechain nitrogen of Thr17Lys mutant and the carboxylate group of Glu258 on EB1 protein, which can form transient salt-bridge interactions.

SUPPLEMENTAL FIGURE 2. Effects of phosphorylation on the EB1-MACFp1 interaction. Representative peptide array (top) of phosphorylated variants of a MACFp1 derived peptide (Figure 5). All phosphoserine (pS) and phosphothreonine (pT) variants were synthesized on a cellulose membrane and subsequently probed for EB1 binding. The sequence list (bottom) displays all the phosphorylated MACFp1 variants tested. “+” and “-” signs on the left side of the sequences indicate binding or not binding, respectively.

SUPPLEMENTAL FIGURE 3. MALS analysis of EB1-TrxIpl1-p12 and EB1-TrxMACF-GCN4. MALS experiments of equimolar mixtures of EB1 and TrxIpl1-p12 (continuous line) or TrxMACF-GCN4 (discontinuous line). Molecular mass determination (horizontal lines located below the maximum of each peak) yielded values of 100 kDa for EB1-TrxMACF-GCN4 (discontinuous line) and 82 kDa for EB1-TrxIpl1-p12 (continuous line).

SUPPLEMENTAL FIGURE 4. Validation of the FP-assay for HTS. A. FP values of the positive (5 µM EB1 + 1.5 µM FC-MACFp1, corresponding to the bound state) and negative (1.5 µM FC-MCFp1 FC-MACFp1 alone, corresponding to the free –or completely displaced-state) controls at the concentrations used for the FP-assay, determined in 384-wells microtiter plates at 30ºC. From the mean (solid lines) and standard deviation values (dashed lines) of these controls, the Z´factor statistic parameter (22) is determined. B. Binding isotherms obtained by fluorescence polarization at 30ºC. FC-MACFp1 was titrated into a solution containing EB1, which was supplemented with 0% (triangles), 5% (circles) or 10% (diamonds) DMSO. Data points (symbols) were fitted (solid line) by using the ‘one-set of sites’ binding model, following Equation 7, as described in Experimental Procedures.

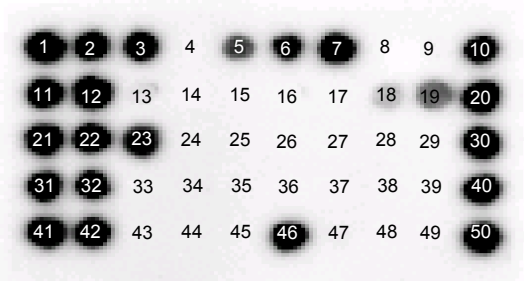
Supplemental Figure 1



Supplemental Figure 2

MACF2: ^{2}RAG**S**RP**ST**AKP**S**KIP**T**PQRK**S**PA**S**KLD^{28}

5 8 9 13 17 22 25



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-	RAGSRPSTAKP**S**KIPTPQRKSPASKLD	4	
+	RAGSRPSTAKPSKIPTPQRK**S**PASKLD	5	
+	RAGSRPSTAKPSKIPTPQRKSPA**S**KLD	6	
+	RAG**S**RPSTAKPSKIPTPQRKSPASKLD	7	
-	RAGSRP**S**TAKP**S**KIPTPQRKSPASKLD	8	
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+	RAGSRPSTAKPSKIPTPQRKSPASKLD	11	
+	RAGSRPSTAKPSKIPTPQRKSPASKLD	12	
-	RAGSRP**S**TAKPSKIPTPQRKSPA**S**KLD	13	
-	RAGSRPSTAKP**S**KIPTPQRK**S**PASKLD	14	
-	RAGSRPSTAKP**S**KIPTPQRKSPA**S**KLD	15	
-	RAGSRPSTAKPSKIPTPQRK**S**PA**S**KLD	16	
-	RAG**S**RPSTAKP**S**KIPTPQRKSPASKLD	17	
+	RAG**S**RPSTAKPSKIPTPQRK**S**PASKLD	18	
+	RAG**S**RPSTAKPSKIPTPQRKSPA**S**KLD	19	
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+	RAGSRPSTAKPSKIPTPQRKSPASKLD	21	
+	RAGSRPSTAKPSKIPTPQRKSPASKLD	22	
+	RAG**S**RP**S**TAKPSKIPTPQRKSPASKLD	23	
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-	RAGSRP**S**TAKPSKIPTPQRK**S**PA**S**KLD	25	
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-	RAGSRP**S**TAKP**S**KIPTPQRK**S**PASKLD	27	
-	RAG**S**RPSTAKPSKIPTPQRK**S**PA**S**KLD	28	
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-	RAG**S**RPSTAKP**S**KIPTPQRK**S**PASKLD	33	
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-	RAG**S**RP**S**TAKPSKIPTPQRK**S**PASKLD	35	
-	RAG**S**RP**S**TAKP**S**KIPTPQRKSPASKLD	36	
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+	RAGSRPSTAKPSKIPTPQRKSPASKLD	40	
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+	RAGSRPSTAKPSKIPTPQRKSPASKLD	42	
-	RAG**S**RP**S**TAKP**S**KIPTPQRKSPASKLD	43	
-	RAG**S**RP**S**TAKP**S**KIPTPQRK**S**PASKLD	44	
-	RAG**S**RP**S**TAKP**S**KIPTPQRK**S**PA**S**KLD	45	
+	RAGSRPS**T**AKPSKIPTPQRKSPASKLD	46	
-	RAGSRPSTAKPSKIP**T**PQRKSPASKLD	47	
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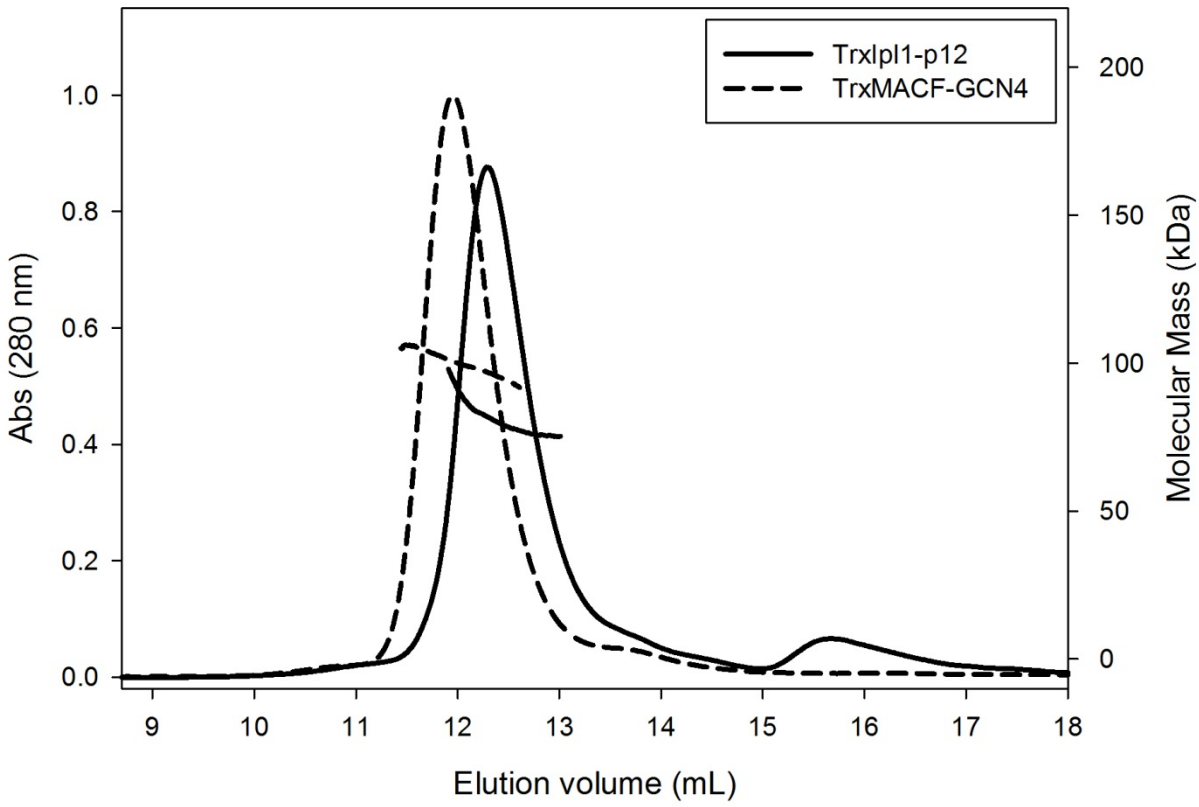
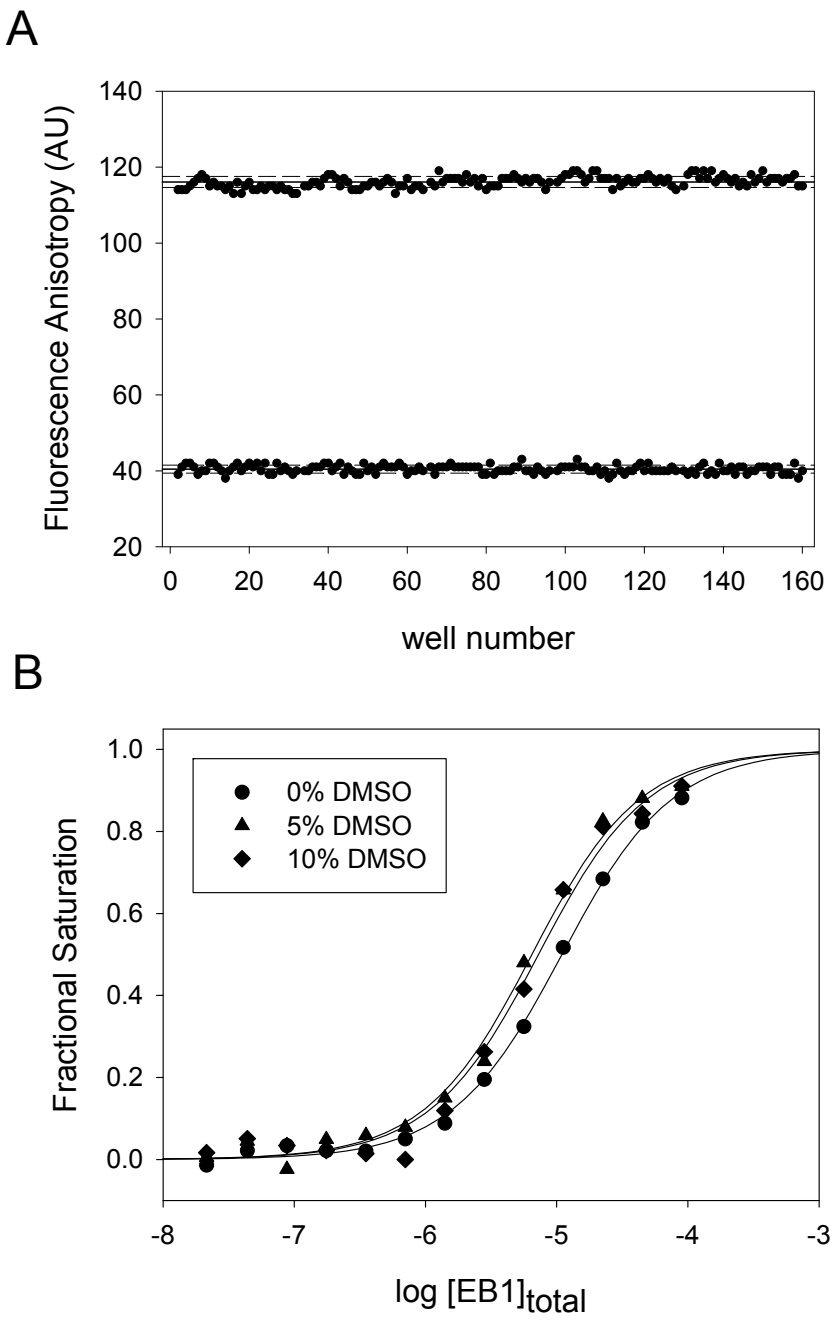
Supplemental Figure 3



Supplemental Figure 4