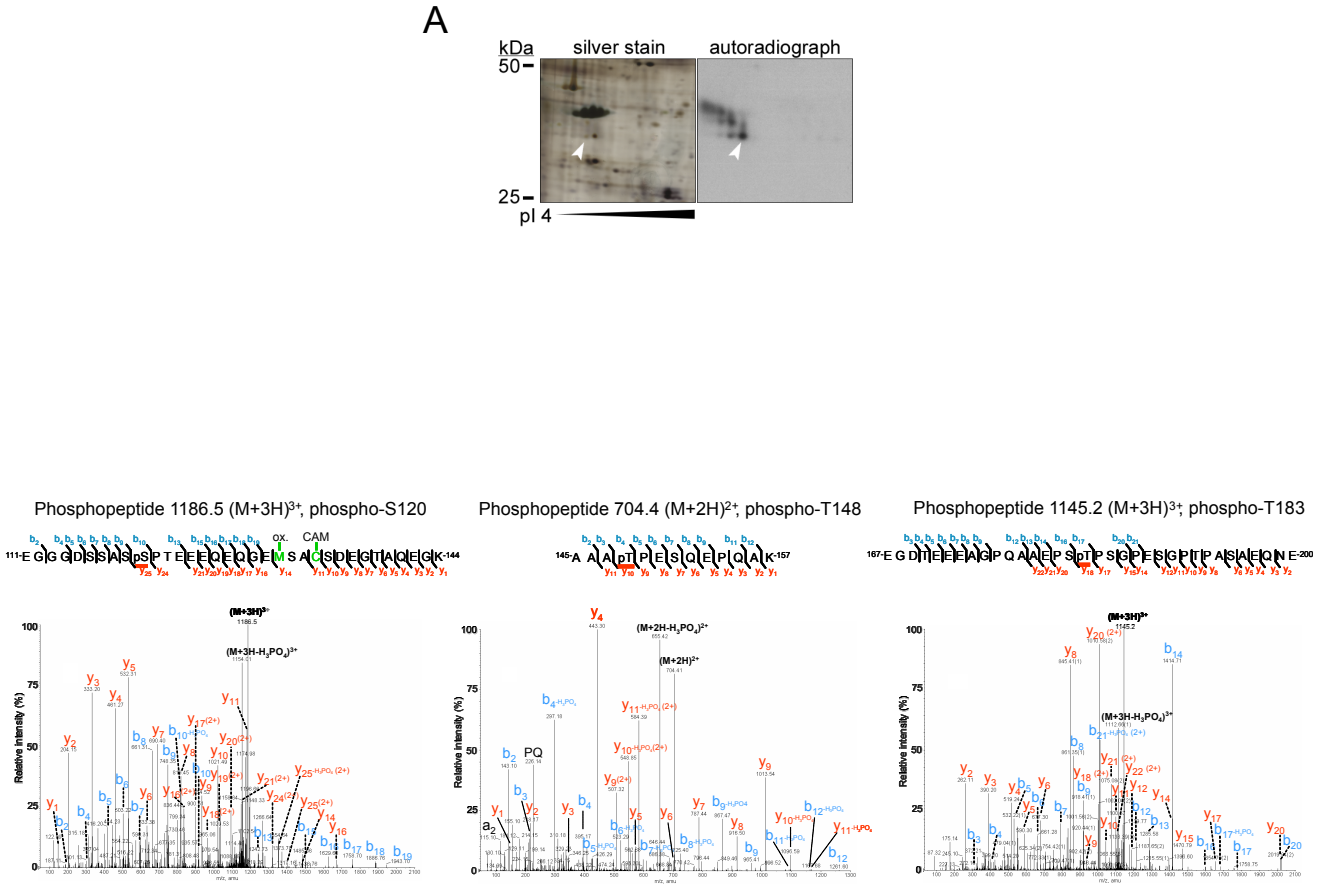
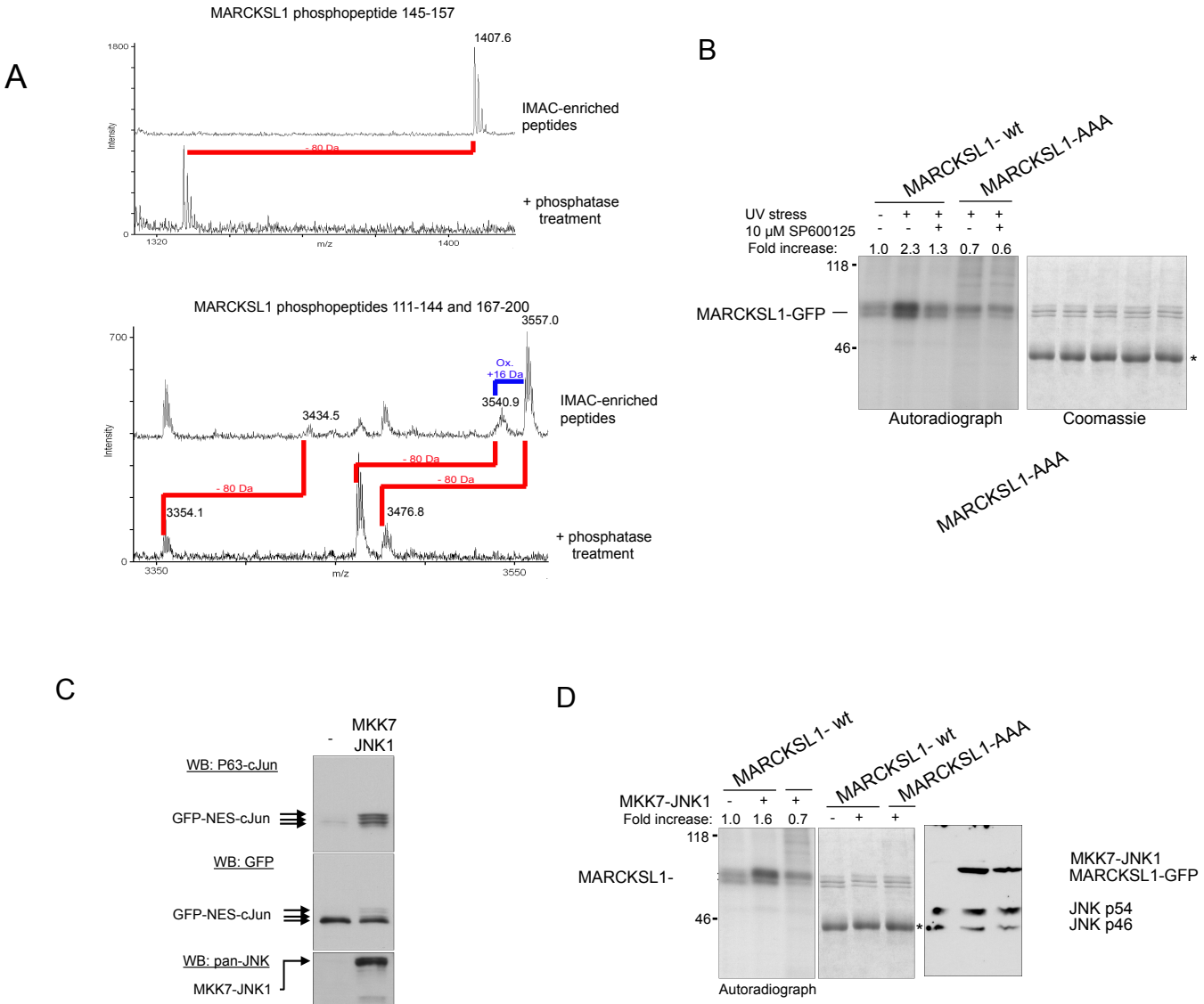
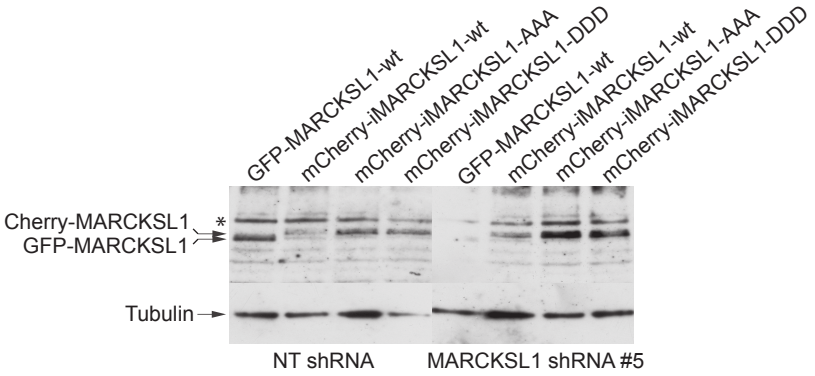


Supplementary 1a. A. Mouse brain lysate was phosphorylated by recombinant JNK and separated by 2DE. A strongly phosphorylated spot was detected by autoradioagraphy and the corresponding protein visualized by silver staining. **B.** CIC fragment ion spectra for phosphopeptides (S120, T148, T183) were obtained for JNK1-phosphorylated MARCKSL1, showing a characteristic neurtral ion loss of 98Da (corresponding to H3P04). Idendtified b-ions (blue) y-ions (red) covering the N- and C- termini are shown.



Supplementary Figure 1b. (A) MALDI-TOF MS spectra of JNK-phosphorylated MARCKLS1 phosphopeptides enriched by IMAC treated with or without alkalinephosphatase revealed a characteristic 80 Da shift corresponding to MARCKSL1 phosphorylated peptide. **(B)** To test whether JNK phosphorylates MARCKSL1 in intact cells, ATP pools were labeled with ^{32}P and cells stimulated with UV (80J/m^2) to activate cellular JNK. UV-induced a 2.3 fold increase in MARCKSL1-GFP phosphorylation, that was prevented by pre-incubation with the JNK inhibitor SP600125 (10µM). There was no net phosphorylation of MARCKSL1-AAA-GFP indicating that no additional JNK phosphorylation sites existed. (* heavy chain of GFP IgG). **(C)** Wesern blots of MEF cells expressing GFP-NES-cJun and MKK7-JNK1 show functionality of dominant active MKK7-JNK1. **(D)** Metabolic labeling of ATP pools was carried out as in A. Expression of GFP-MKK7-JNK1 increased phosphorylation of MARCKLS1-GFP, not MARCKSL1-AAA-GFP.



Supplementary Figure 2. MARCKSL1 mutants that were insensitive to shRNA #5 (iMARCKSL1) were generated and tested for insensitivity to shRNA. While MARCKSL1-WT was efficiently silenced by shRNA #5, the iMARCKSL1 variants were not. Non-specific band (*).

Predictive Modeling suggests that the JNK phosphorylation sites are surface accessible.

A

Sequence alignment of MARCKS and MARCKSL1

30.6% identity in 173 residues overlap

```
MARCKS         76 SAATPAAAEKDEAAAATEPGAGAADKEAAEAEPAEPSSPAAEAEGASASSTSSPKAEDGA
MARCKSL1       19 AGASPAKANGQENGHVRSNG-DLTPKGEGESPPVNGTDEAAGATGDAIEPAPPSQEAEAK
                     * ** *   *        *      *   *  *       ** * *

MARCKS        136 APSPSSETPKKKKKRFSFKKSFKLSGFSFKKSKKESGEGAEAEGATAEGAKDEAAAAAGG
MARCKSL1       78 GEVAPKETPKKKKK-FSFKKPFKLSGLSFKRNRKEGGGDSSASSPTEEEQEQGEMSACSD
                  ******** ***** ***** ***    ** *    *   * *         *

MARCKS        196 EGAAAPGEQAGGAGAEGAAGGEPREAEAAEPEQPEQPEQPAAEEPQAEEQSEA
MARCKSL1      137 EGTAQEGKAAATPESQEPQAKGAEASAASKEGDTEEEAGPQAAEPSTPSGPES
                  ** *  *  *                    *      *    * * **      *
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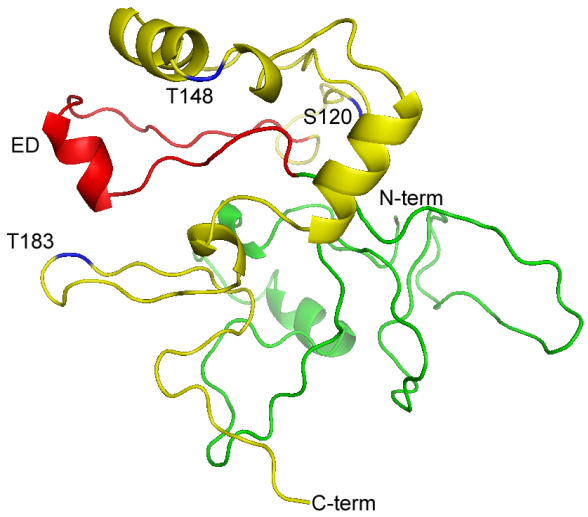
B



Supplementary figure 3. JNk phosphorylation sites on MARCKSL1 surround the effector domain and are not conserved in MARCKS. (A) A sequence alignment of mouse MARCKSL1 and its homolog MARCKS performed using the SIM alignment tool from Expasy. The homologous effector domain (ED, bold) and JNK phosphorylation sites (red) are highlighted. For comparison, PKC phosphorylation sites are marked green. (B) Predictive modeling indicates that JNK phosphorylation sites on MARCKSL1 are surface accessible. Overall sequence homology between MARCKS and MARCKSL1 is 30.6% (37, 46, 52) while the shared effector domain (ED) through which they bind actin is 87% identical. Interestingly, the JNK phosphorylation sites on MARCKSL1 are not conserved in MARCKS, suggesting that JNK may phosphorylate MARCKSL1 exclusively. To visualize the JNK phosphorylation sites on MARCKSL1 within a 3D framework, we used Rosetta modeling software (11) as a first approximation to obtain structural insights, as no crystal structure exists. The Rosetta model indicates a highly flexible protein with JNK phosphorylation sites (blue) surrounding the ED and on the surface of MARCKSL1, rendering them accessible for phosphorylation. We propose that phosphorylation of MARCKSL1 induces a conformational change that unshields the "lysine-rich" ED. This could change MARCKSL1 from a protein that can merely bind F-actin to one that bundles/crosslinks F-actin. ED unshielding may result from charge repulsion among the juxtaposed JNK phosphorylation sites T183 and T148.