

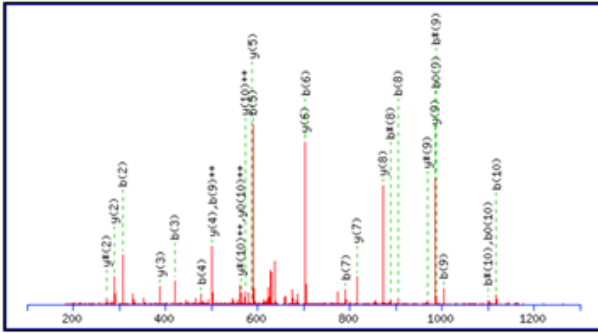
## Supplemental Figures and Legends:

A

Protein sequence coverage: 31%

Matched peptides shown in **bold red**.

1	MSSILPFTTP	VVKRLLGWKK	<b>SAGSGGGAGG</b>	<b>GEQNGQEEKW</b>	<b>CEKAVKSLVK</b>
51	KLKKTGRLDE	LEKAITTQNC	NTKCVTIPST	CSEIWGLSTA	NTVDQWDTTG
101	LYSFSEQTRS	LDGRLQVSHR	KGLPHVIYCR	LWRWPDLSH	HELK <b>AIENCE</b>
151	<b>YAFNLKKDEV</b>	<b>CVNPHYQQRV</b>	<b>ETPVLPPVLV</b>	<b>PRHTEILTEL</b>	PPLDDYTHSI
201	PENTNFPAGI	EPQSNYIPET	PPPGYISEDG	ETSDQQLNQS	MDTGSPAELS
251	PTLSPVNH	LDLQPVTYSE	PAFWCSIAYY	ELNQR <b>VGETF</b>	<b>HASQPSLTVD</b>
301	<b>GFTDPSNSER</b>	<b>FCLGLLSNVN</b>	RNATVEMTRR	HIGRGVRLYY	IGGEVFAECL
351	SDSAIFVQSP	NCNQRYG <b>WHP</b>	<b>ATVCKIPPGC</b>	NLKIFNNQEF	AALLAQSVNQ
401	GFEAVYQLTR	MCTIRMSFVK	GWGAEYRRQT	VTSTPCWIEL	HLNGPLQLWD
451	<b>KVLTQMGSPS</b>	<b>VRCSSMS</b>			



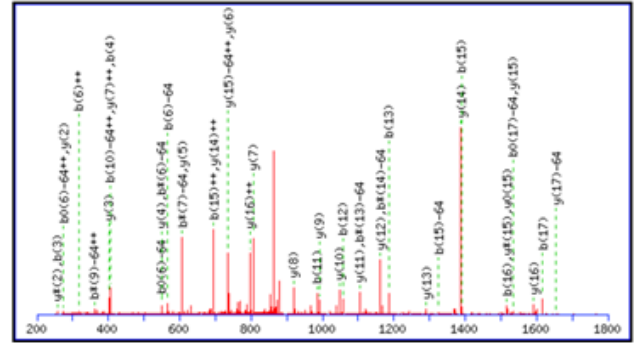
Monoisotopic mass of neutral peptide  $M_r(\text{calc})$ : 1291.6707  
 Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)  
 Ions Score: 67 Expect: 3.4e-005  
 Matches : 28/86 fragment ions using 42 most intense peaks ([help](#))

B

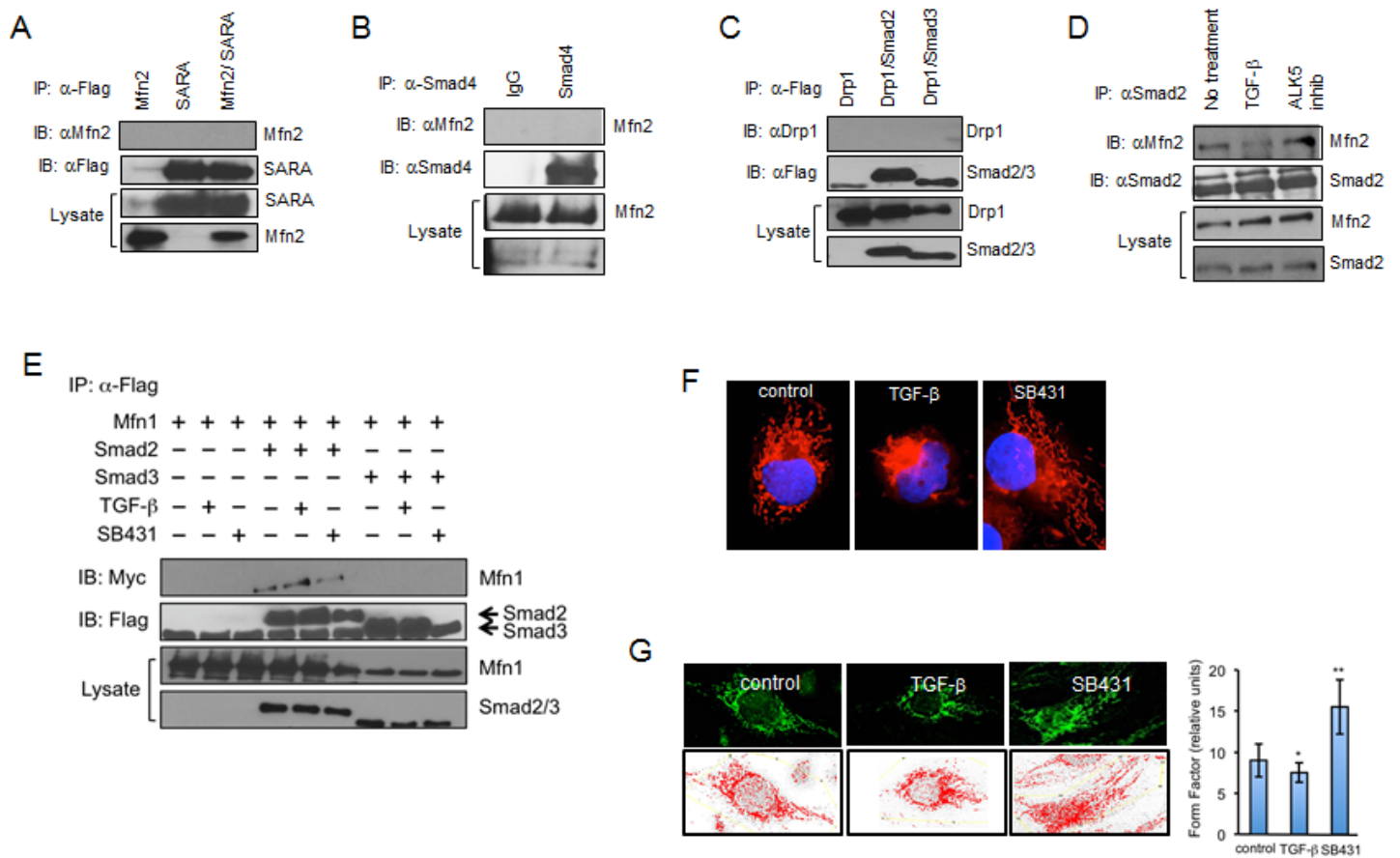
Protein sequence coverage: 7%

Matched peptides shown in **bold red**.

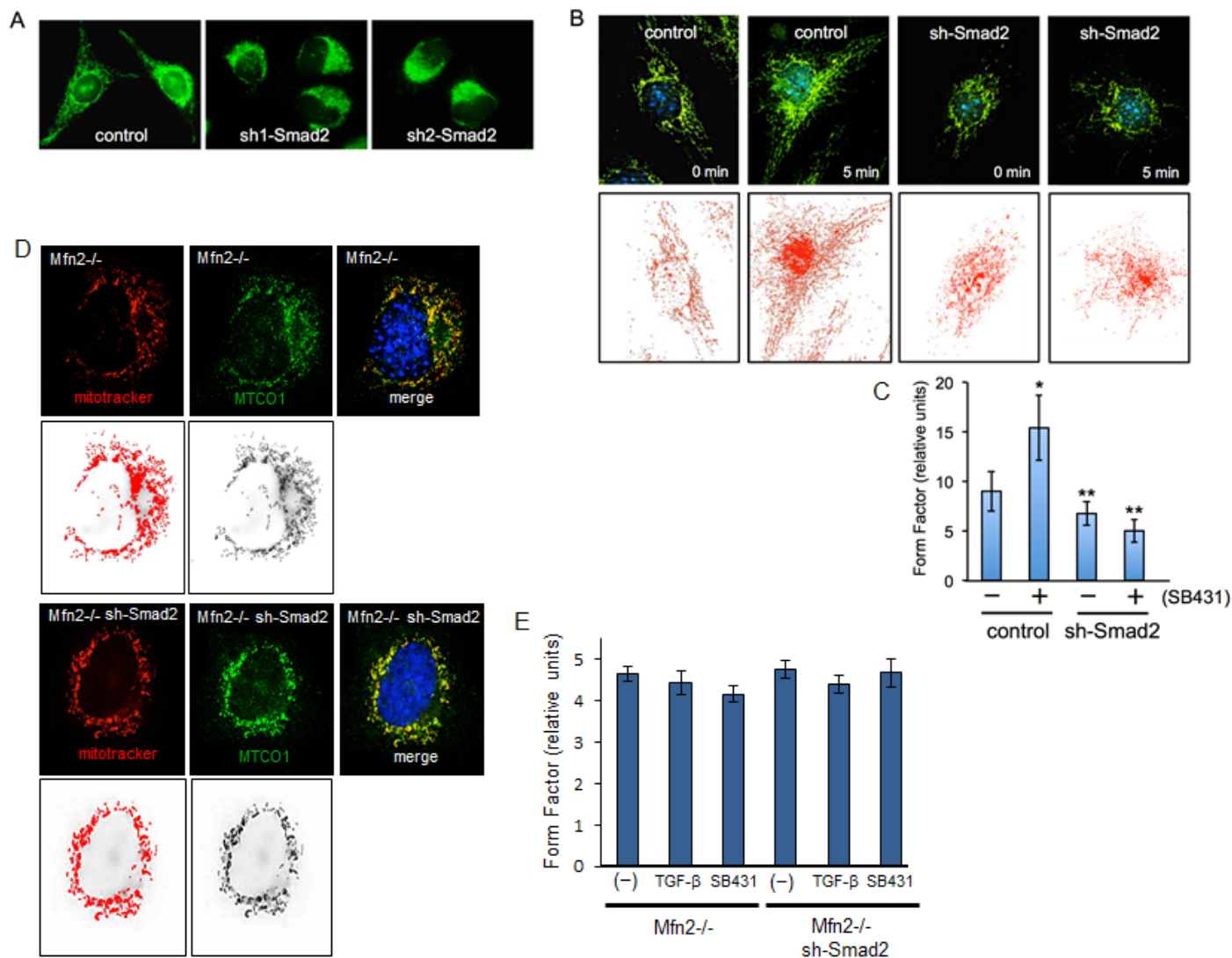
1	MSLLFSRCNS	IVTVKDKKRH	MAEVNASPLK	HFVTAKKKIN	GIFEQLGAYI
51	QESASFLEDT	HRNTELDPVT	TEEQVLVDKVG	YLSKVRGISE	VLARRHMKVA
101	FFGRTSNGKS	TVINAMLWOK	VLPSTGIGHTT	NCFLRVGGTD	GHEAFLLTEG
151	SEEKKSVKTV	NQLAHLHQD	EQLHAGSMVS	VMWPNKCPKL	LKDDLVLMS
201	PGIDVTTLED	SWIDKFCLDA	DVFLVANSE	STLMQTEKQF	FHKVSRLSR
251	PNIFILNRRW	DASASEPEYM	EEVRRQHMER	CTSLFDVDELG	VVDRAQAGDR
301	IFFVSAKEVL	SARVQ <b>KAGGM</b>	<b>PEGGGALAE</b>	<b>FQVRMFEFQN</b>	<b>FERQFECCIS</b>
351	QSAVKTKFEQ	HTVRAKQIAE	AVRLIMDSLH	IAAQEQRYVC	LEMREERQDR
401	LRFIDKQLEL	LAQDYKLRIK	<b>QITEEVERQV</b>	STAMAEIIRR	LSVLVDEYQM
451	DFHPSVVLK	VYKNEHLRHI	<b>EEGLGRNSD</b>	RCSTAIASSL	QTMQQDMIDG
501	LKPLLPVSMR	NQIDMLVPRQ	CFSLSYDLNC	DKLCADFQED	IEFHFSLGWT
551	MLVNRFLGPK	NSRRALLGYS	DQVQRPLPLT	PANPSMPPLP	QSSLTQEELM
601	VSMVTGLASL	TSRTSMGILV	VGGVVKAVG	WRLIALSFGL	YGLLYYERL
651	TWTTKAKERA	FKRQFVEYAS	EKLQLIISYT	GSNCSHQVQQ	ELSGTFAHLC
701	QQVDITR <b>DLN</b>	<b>EQEIAAMNKK</b>	VEALDSLQSR	AKLLRNKAGW	LDSELNMFTH
751	QYLQPSR				



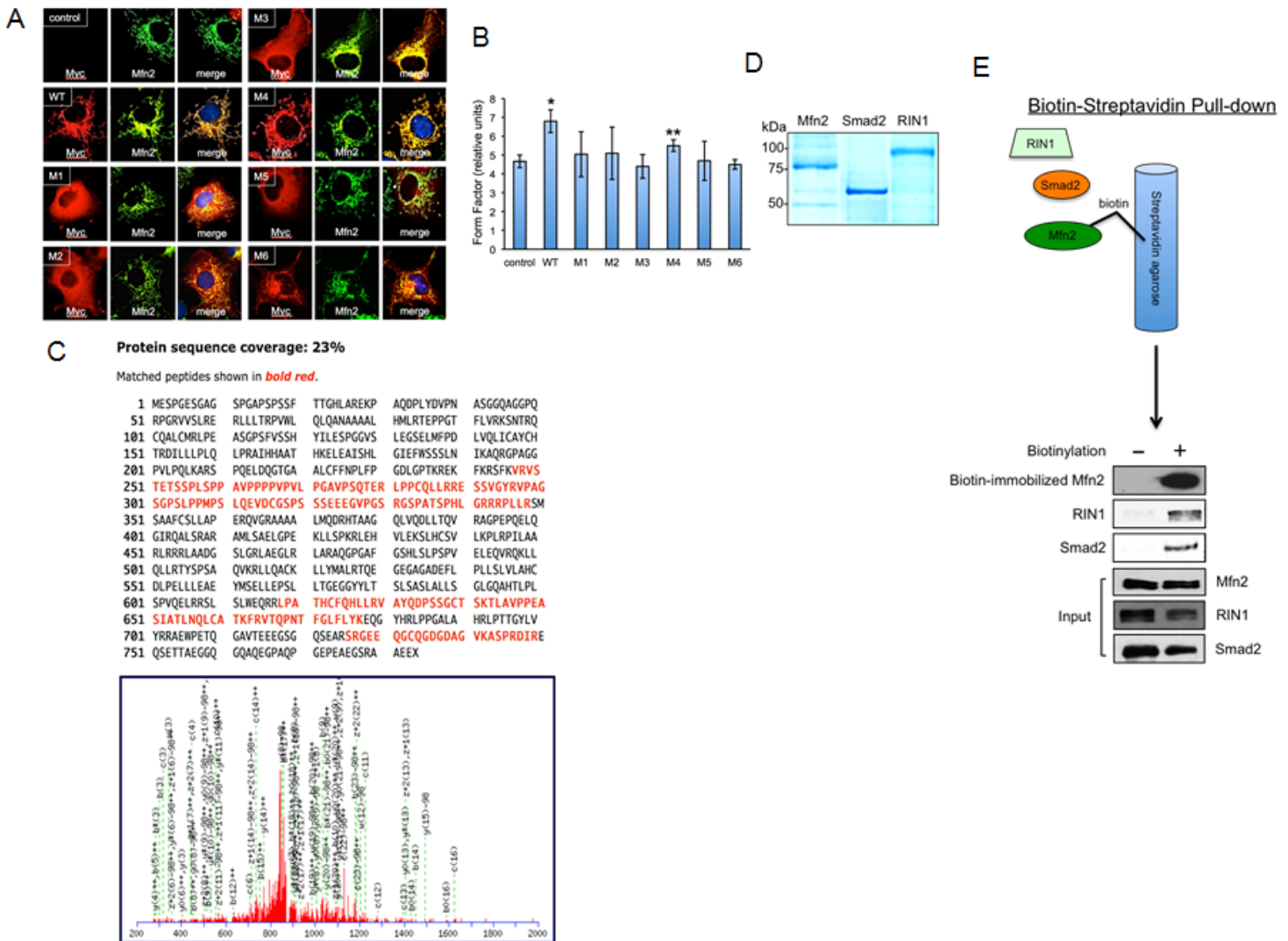
**Figure S1. Identification of new Smad2 binding partners by Mass spectrometry/proteomics, related to Figure 1.** Mass spectrometry peptide fragment identification of Smad2 and Mfn2. Immunoprecipitation of Flag-tagged Smad2 in COS-7 cells was subjected to nano LC-MS/MS and proteomics analyses. The mass spectrometry chromatogram peaks and internal sequence of the protein that matched peptide fragments for (A) Smad2 and (B) Mfn2 are shown in red.



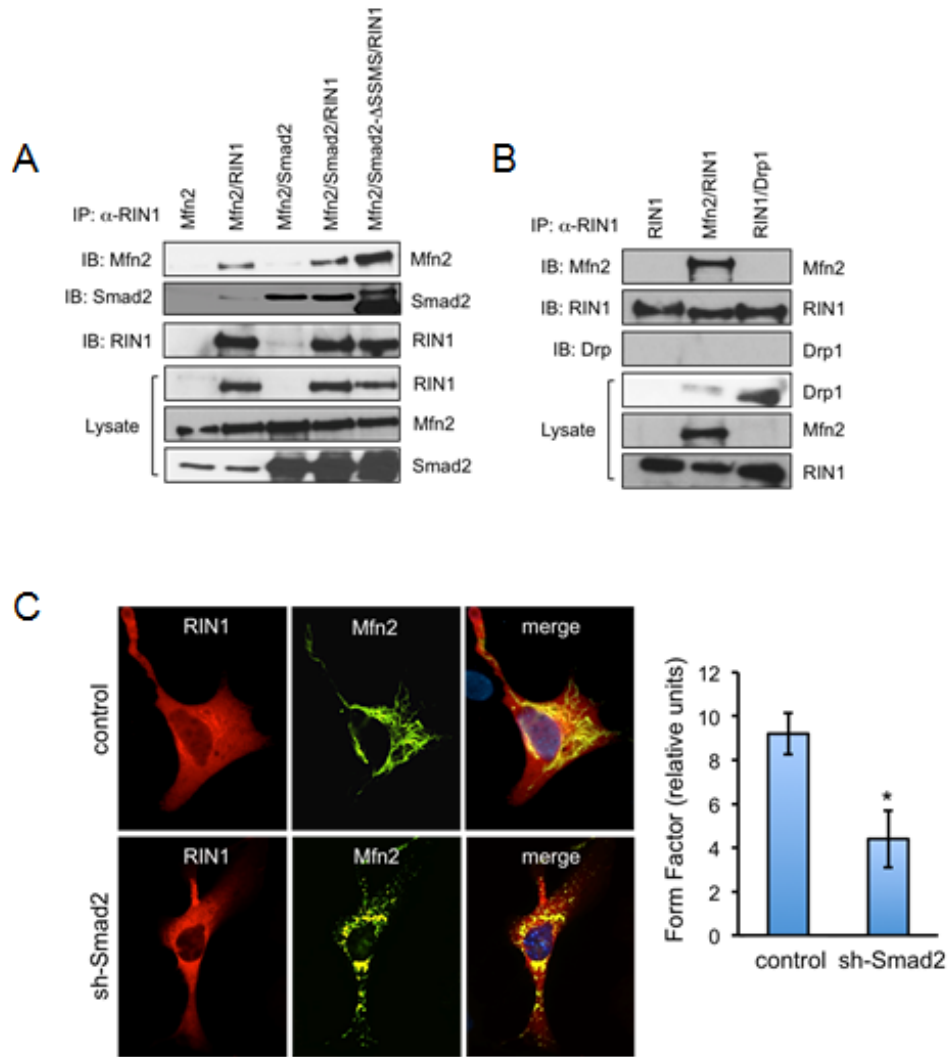
**Figure S2. Biochemical analysis of Mfn2/Smad2 interaction, related to Figure 1 and Figure 2.** (A) COS-7 expressing Myc-Mfn2, Flag-SARA, and Myc-Mfn2 plus Flag-SARA were immunoprecipitated with Flag antibody. (B) COS-7 cells were immunoprecipitated with IgG control or Smad4 antibody. (C) COS-7 cells expressing Drp1, Drp1 and Flag-Smad2, Drp1 and Flag-Smad3 were immunoprecipitated with Flag antibody. (D) COS-7 cells were serum deprived for 3 h prior to SB431542 (30  $\mu$ M) or TGF- $\beta$ 1 (200 pM) treatment for 30 min, then immunoprecipitated with Smad2 antibody. (E) COS-7 cells expressing Myc-Mfn1, Myc-Mfn1 and Flag-Smad2, or Myc-Mfn1 and Flag-Smad3 were subjected to serum deprivation for 3 h prior to SB431542 (30  $\mu$ M) or TGF- $\beta$ 1 (200 pM) treatment for 30 min. (F) COS-7 cells subjected to TGF- $\beta$ 1 (200 pM) or SB431542 (30  $\mu$ M) treatment along with mitotracker (red) for 15 min prior to fixation. (G) MEECs treated with TGF- $\beta$ 1 (200 pM) or SB431542 (30  $\mu$ M) for 5 min were fixed, stained for MTCO1, then quantified. Data shown are representative of 25 cells each from three independent experiments. Error bars represent SEM and T-test shows relative to control: \* $p=0.002$ , \*\* $p<0.001$ .



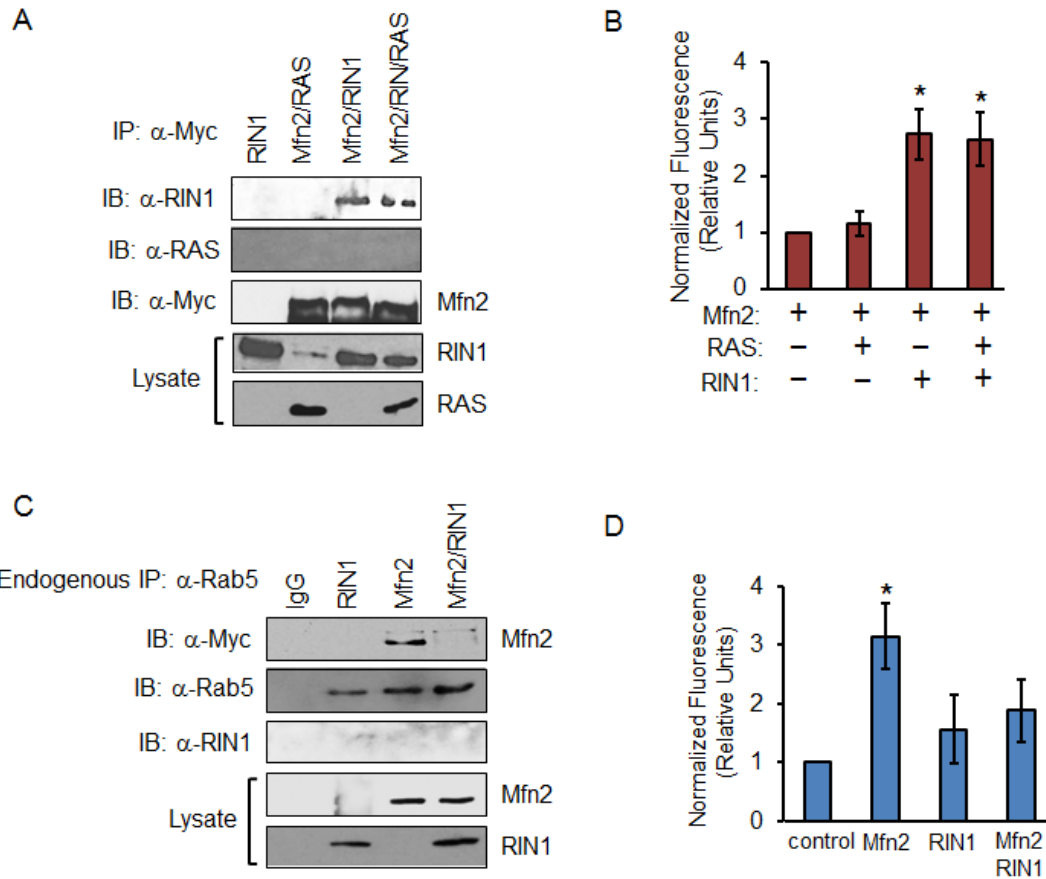
**Figure S3. Smad2-mediated mitochondrial fusion, related to Figure 2 and Figure 3.** (A) Mfn2 staining of control and Smad2-depleted HeLa cells using two shRNA sequences (sh1-Smad2 and sh2-Smad2). (B) Control and sh-Smad2 MEECs were treated with or without SB431542 (30  $\mu$ M) for 5 min prior to fixation and MTCO1 staining. (C) Quantification based on 30 cells. (D) Mfn2<sup>-/-</sup> MEFs transfected with control or sh-Smad2 were treated with TGF- $\beta$ 1 (200 pM) or SB431542 (30  $\mu$ M) for 10 min, then stained with mitotracker (red) for 15 min prior to fixation and secondary staining for MTCO1 (green). (E) Form factor graph quantification based on 20 cells for each group. Data shown are representative of three independent experiments. Error bars represent SEM and T-test shows relative to control: \* $p$ <0.01, \*\* $p$ <0.05.



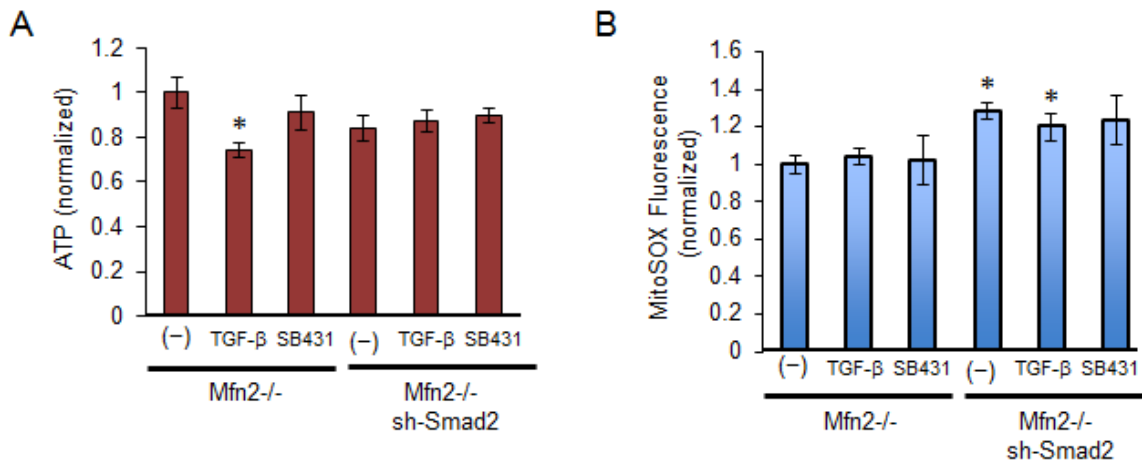
**Figure S4. Structural determinants of the Mfn2/Smad2 interaction, related to Figure 3.** (A) COS-7 cells transfected with control (vector), WT or mutants (M1-M6) were fixed, co-immunostained for Myc (red) and Mfn2 (green). (B) Graph represents averaged form factor based on at least 20 cells and representative of three independent experiments. Error bars represent SD and T-test shows relative to control: \* $p=0.0006$ , \*\* $p=0.03$ . (C) Mass spectrometry/peptidomic identification of RIN1. Chromatogram peaks and peptide sequences obtained from Smad2 immunoprecipitate are shown in red. (D) Coomassie staining of 0.5  $\mu$ g His-tag purified Mfn2, Smad2 and RIN1. (E) Purified RIN1 and Smad2 were incubated with biotinylated Mfn2 immobilized to streptavidin agarose. As negative control, RIN1, Smad2 and non-biotinylated Mfn2 were incubated in streptavidin agarose.



**Figure S5. Smad2 requirement in RIN1/Mfn2 interaction, related to Figure 5 and 6.** (A) COS-7 cells expressing Myc-Mfn2, Myc-Mfn2 and RIN1, Myc-Mfn2 and Flag-Smad2-WT, Myc-Mfn2, RIN1, and Flag-Smad2-WT together, or Myc-Mfn2, RIN1, and Flag-Smad2 $\Delta$ cyto and immunoprecipitated with RIN1 antibody. (B) COS-7 cells expressing RIN1, Myc-Mfn2 and RIN1, or RIN1 and Drp1 were immunoprecipitated with RIN1 antibody. (C) A549 control and sh-Smad2 stables were transfected with RIN1 and Mfn2, fixed and co-immunostained for RIN1 (red) and Mfn2 (green). Graph represents averaged form factor quantification of at least 20 cells. Error bars represent SEM and T-test shows relative to control: \* $p < 0.01$ .



**Figure S6. Characterization of Ras and Rab5 binding to Mfn2, related to Figure 6.** (A) COS-7 cells expressing RIN1, Myc-Mfn2 and RAS, Myc Mfn2 and RIN1, Myc-Mfn2, RIN1 and RAS together were immunoprecipitated with Myc antibody. (B) Graph shows changes in MANT-GTP fluorescence from Myc-Mfn2 bound RIN1 and RAS immunocomplexes. Each error bar represents the SEM of a representative data set collected out of three independent experiments normalized to Mfn2 alone control (\* $p < 0.0007$ , \*\* $p < 0.01$ ). (C) COS7 cells expressing RIN1, Mfn2, Mfn2 and RIN1 together were immunoprecipitated with IgG or Rab5 antibody. (D) Graph shows MANT-GTP fluorescence from isolated Myc-Mfn2 bound RIN1 and Rab5 immunocomplexes. COS-7 cells expressing vector only, Mfn2 alone, RIN1 alone, Mfn2 and RIN1 together were immunoprecipitated with IgG and endogenous Rab5 antibody. Each error bar represents the SEM of a representative data set collected out of three independent experiments normalized to control (\* $p < 0.01$ ).



**Figure S7: Requirement of Smad2-based Mfn2 activation in mitochondrial function, related to Figure 7.** (A) Graph represents ATP levels in control and sh-Smad2 Mfn2<sup>-/-</sup> MEFs treated AIK5 inhibitor SB431542 (30 μM) or TGF-β1 (200 pM) for 30 min. Error bars represent SEM and T-test shows relative to control no treatment: \*p<0.05. (B) Graph shows mitochondrial superoxide in control and sh-Smad2 Mfn2<sup>-/-</sup> MEFs treated AIK5 inhibitor SB431542 (30 μM) or TGF-β1 (200 pM) for 30 min. Error bars represent SEM and T-test shows relative to control no treatment: \*p<0.008.