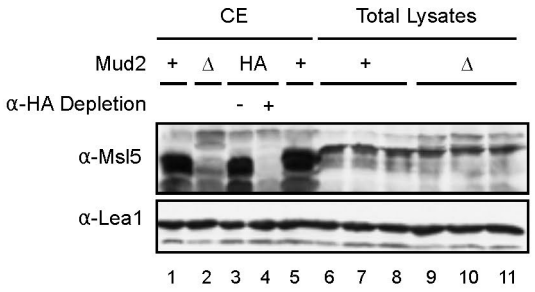
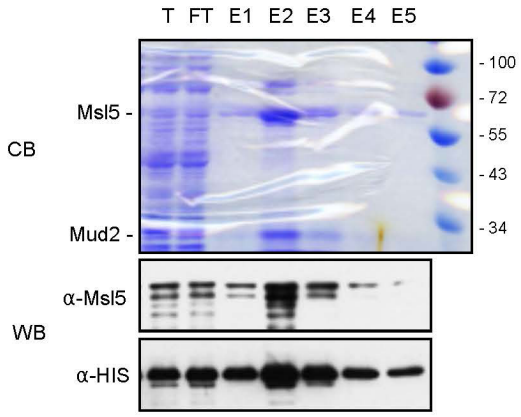
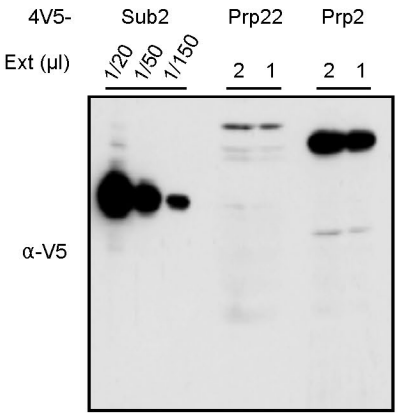


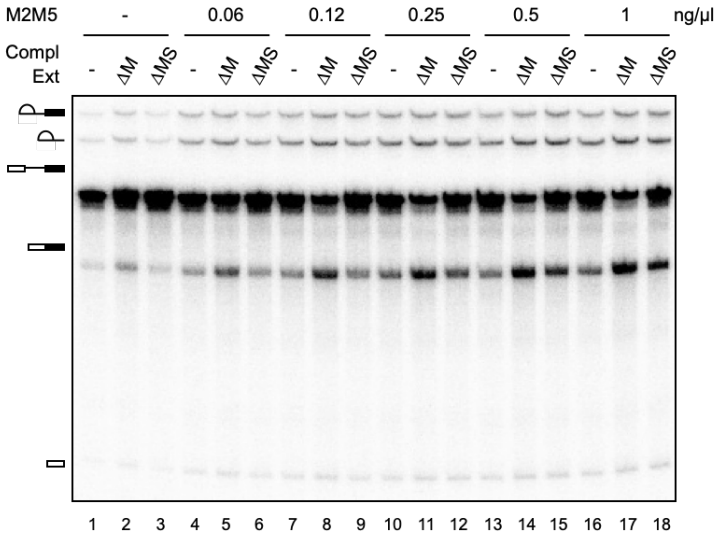
Supplemental_Figure_S1. Depletion of Mud2 *in vivo* does not affect Msl5 levels. Western blot of splicing extracts (lanes 1-5) and total cell lysates (lanes 6-11) probed with anti-Msl5 and anti-Lea1 antibodies. Lanes 1 and 5-8: wild-type Mud2; lanes 2 and 9-11: Mud2 depletion; lane 3: Mud2-HA; lane 4: Mud2-HA depleted with anti-HA antibody.



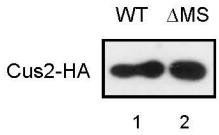
Supplemental_Figure_S2. Purification of Msl5 and His-tagged Mud2 co-expressed in *E. coli* by fractionation on NTA-Agarose column. Top panel: SDS-PAGE of eluted fractions stained with Coomassie bule. Middle panel: WB probed with anti-Msl5 antibody. Bottom panel: WB probed with anti-HIS-tag antibody. T, total; FT, flow-through fraction; E1-E5, eluted fractions 1-5; CB, Coomassie blue; WB, Western blot.



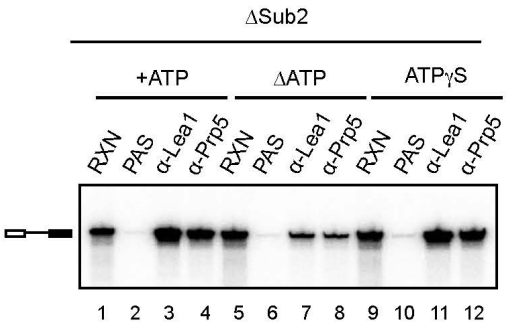
Supplemental_Figure_S3. Levels of Sub2 are more than 50-fold higher in splicing extracts than Prp2 or Prp22. Western blot of Sub2-4V5, Prp22-4V5 and Prp2-4V5 extracts loaded with indicated volumes and probed with anti-V5 antibody. Ext, splicing extracts.



Supplemental_Figure_S4. Splicing complementation of ΔMS extracts. Splicing was performed at 25˚C for 30 min in ΔMS extracts without (lanes 1, 4, 7, 10, 13 and 16) or with the addition of 2 µg/µl ΔM (lanes 2, 5, 8, 11, 14 and 17) or ΔMS (lanes 3, 6, 9, 12, 15 and 18) extracts, together with various amounts of M2M5 proteins. M2M5, recombinant Mud2-Msl5 dimer; Compl Ext, complementing extract; ΔM, MUD2 deletion; ΔMS, MUD2, SUB2-double deletion.



Supplemental_Figure_S5. Cus2 levels in splicing extracts are not affected by *MUD2* deletion. Western blotting of Cus2-HA in wild-type and ΔMS extracts probed with anti-HA antibody following immunoprecipitation with anti-HA antibody. WT, wild-type; ΔMS, *MUD2*, *SUB2*-double deletion.



Supplemental_Figure_S6. ATP is required for stable formation of the FIC in ΔSub2 extracts. Splicing was performed at 25˚C for 15 min with U257G mutant pre-mRNA in ΔSub2 extracts pre-incubated with 10 mM glucose (lanes 5-8), or in the presence of 2 mM ATP (lanes 1-4) or 2 mM ATPγS (lanes 9-12), followed by precipitation with anti-Lea1 or anti-Prp5 antibody. RXN, 1/10 of splicing reaction mixture; PAS, protein A-Sepharose.