A

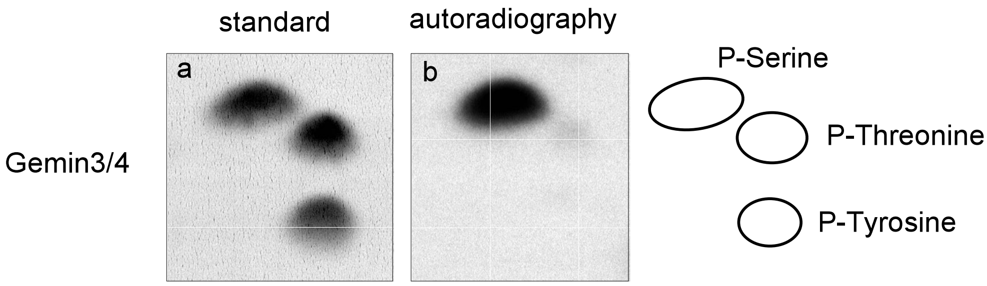

Fig. A. Phosphorylation of a 100kDa-component of the SMN-complex.
Phospho-amino acid analysis of the 100kDa-protein. The [32P]-phosphate labelled proteins from Fig. 1A were transferred to a PVDF-membrane, excised and hydrolysed in HCl. The amino acids were mixed with a phospho-amino acids standard and were separated by 2D-TLC. Autoradiography (panel b) was compared with the position of ninhydrin stained standard phospho-amino acids (left panel, a). Asterisks denote partially hydrolysed protein.

B

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                   10        20        30        40        50        60        70        80
                    |         |    *    |*        |         |         |         |         |
human     MAMSSGGSGGGVPEQEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDICETSGKP--KTTPKRKPAKKN
mouse     MAMGSGG-AGS--EQEDTVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDICETPDKP--KGTARRKPAKKN
rat       MAMGSGGGAGS--EQEDTVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDMCETSDKP--KGTARRKPAKKN
dog       ---------GGLPEPEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDISEASDKP--KSTPKRKPAKKN
cat       MAM---GGGSGVPEQEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDISEASDKP--KGTPKRKPAKKN
cow       M----GGGGGGFPEPEDSVLFRRGTGESDDSDVWDDTALIKAYDKAVASFKHALKNGDISEASEKP--KGTPKRKSAK-N
chicken   MAG--------------RVLFRRGAGQSDDSDMWDDTALIKAYDKAVASFKNALKNGDCSEPSDKQEQRAGVKRKNSKKN
X.leavis  MAGLEDGG---------EVLFRRGAGQSDDSDIWDDTALIKAYDKAVSSFKRALKNEDCTIGAETEEKNPRTKRKNNKKN
D.rerio   MANGA-----------EDVVFCRGTGQSDDSDIWDDTALIKAYDKAVASFKNALKGED--GATPQENDNPGKKRKNNKKN

                   90       100       110       120       130       140       150       160
                    |         |         |         |         |         |         |         |
human     KSQK-KNTAASLQQWKVGDKCSAIWSEDGCI--YPATIASIDFKRETCV-VVYTGYGNREEQNLSDLLSPICEVANNIEQ
mouse     KSQK-KNATTPLKQWKVGDKCSAVWSEDGCI--YPATITSIDFKRETCV-VVYTGYGNREEQNLSDLLSPTCEVANSTEQ
rat       KNQK-KNATAPLKQWKAGDKCSAVWSEDGCV--YPATITSVDLKRETCV-VVYTGYGNKEEQNLSDLLSPTCEVANNTEQ
dog       KSQK-KNATTALKQWKVGDKCSAVWSEDGCI--YPATIASIDFKRETCV-VVYTGYGNREEQNVSDLLSPACEVANNVEQ
cat       KSQK-KNTTAALKQWKVGDKCSAIWSEDGCV--YPATIASIDFKRETCV-VVYTGYGNREEQNVSDLLSPASAVDNNVEQ
cow       KSQR-KNTTSPSKQWKVGDNCCAIWSEDGCI--YPATIASIDFKRETCV-VVYTGYGNREEQNLSDLLSPTSEVAN-IEQ
chicken   RNRN-KSNAVPLKQWKVGDSCNAVWSEDGNV--YPATIASINLKRGTCV-VTYTGYGNKEEQNLADLLPPASDETNENE-
X.leavis  RSRK-KCNAAPLKKWRIGDTCNAVWSEDGNI--YSATISSIDAKRRTCI-VVYSGYGNSEEHSLADLRFPDTSEAESDQR
D.rerio   KSRK-RCNAAPDKE--VGDSCYAFWSEDGNL--YTATITSVDQEKGTCV-VFYTDYGNEEEQNLSDLLTEPPDMDEDALK

                  170       180       190       200       210       220       230       240
                    |         |         |         |         |         |         |         |
human     NAQENENESQVSTDESENS-RSPGNKSDNIKPKSAP----WNSFLPPPPPMPGPRLGPGKPGLKFNGPPPPPPPPPPHLL
mouse     NTQENE--SQVSTDDSEHSSRSLRSKAHS-KSKAAP----WTSFLPPPPPMPGSGLGPGKPGLKFNGPPPPPPLPPPPFL
rat       NTQENE--SQVSTDDSEHSSRSLRSKAHS-KSKAAP----WTSFLPPPPPVPGAGLGPGKPGLRFSGPPPPPP-PPPPFL
dog       DTQENENESQISTDESENSSRSPGNKPNNIKSKAAP----WNSFLPPPPPMSGSGLGPGKPGVKFSGPPPPPPPP--HFL
cat       NAQENENESQISTDESENSCRSPGNKSNNVKSKATS----WNSFLPPPPPMPGAGLGPAKPGLKFNGPPPPPPPPP-HFL
cow       NAQENENESQISTDESENSSRSPLNKPNNIRSRAAP----WNSFLPPPPHMPRSGLGPGKSGLNFSGPPPPPPPPP-HFL
chicken   --------TPYSTDESEKSSQSHHNENNCTKARFSPKNLR----FPIPPTP--PGL--GRHGSKFRTLPP--------FL
X.leavis  DQEQEINGDEHSTDESDRSSRSHQSKD--PQNRETPKSSQWNGQFPPVPPPFMPGF--GRHGEKLDQAHP--------FL
D.rerio   TANVKETESSTEESDRSFTPQKSGHAKHKSKSNFPMGPPSWFPSFPPGPPPPPPHFK------KMDGRRGEGPGPS---F

                  250       260       270       280       290       300
                    |         |         |         |         |         |
human     SCWLPPFPSGPPIIPPPPPICPDSLDDADALGSMLISWYMSGYHTGYYMGFRQNQKEGR-CSHSL-N
mouse     PCWMPPFPSGPPIIPPPPPISPDCLDDTDALGSMLISWYMSGYHTGYYMGFRQNKKEGK-CSHT--N
rat       PCWMPPFPSGPPIIPPPPPISPDCLDDTDALGSMLISWYMSGYHTGYYMGFRQNKKEGKKCSHT--N
dog       SCWLPPFPSGPPIIPPPPPICPDSLDDADALGSMLISWYMSGYHTGYYMGFKQNQKEGR-CSH-F-N
cat       SCWLPPFPSGPPIIPPPPPICPDSLDDADALGSMLISWYMSGYHTGYYMGFKQNQKEGR-CSH-F-N
cow       SRWLPPFPAGPPMIPPPPPICPDSLDDADALGSMLISWYMSGYHTGYYMGFKQSQKEGR-YSH-F-N
chicken   SCWPPPFPAGPPLIPPPPPMGPDSPEDDEALGSMLIAWYMSGYHTGYYLGLKQSRMEAALEREAYLK
X.leavis  SGWPPPFLPGPPMIPPPPPMSPDACEDDEALGSMLISWYMSGYHTGYYLGLKQGRMESSIGKPPHQK
D.rerio   PGWPPMIPLGPPMIPPPPPMSPDFGEDDEALGSMLISWYMSGYHTGYYMGLRQGRKEAAASKKSHRK
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Fig. B. The Phospho-serines in the QSDDSD-motif of SMN are located in a region conserved among higher eucaryotes.
Sequence-alignment of SMN from different species. The consensus motif of SMN is boxed. Phosphorylated serines in human SMN are indicated by an asterisk.

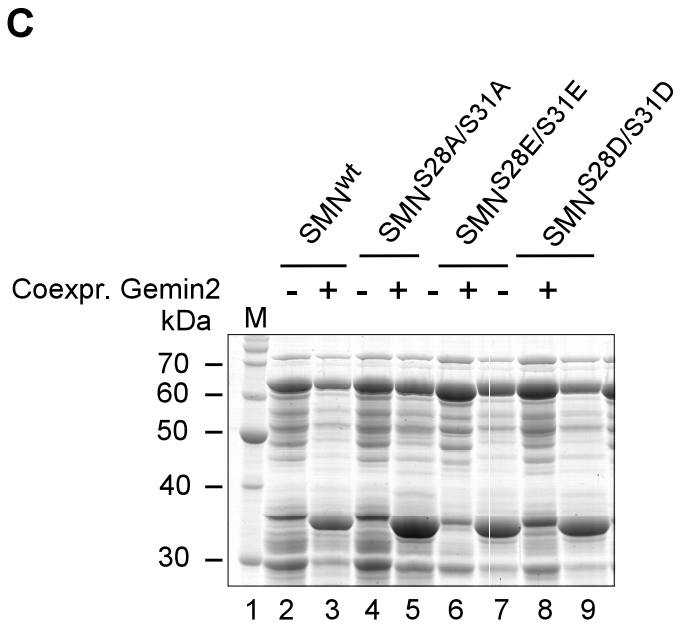

Fig. C. Phosphorylation of serine 28 and 31 in SMN does not affect binding to Gemin2 *in vitro.*
Full length human SMN and mutated forms thereof were subcloned into the pGEX 5X-1 vector and cotransfected in *E.coli BL21 DE3* with Gemin2, subcloned in pET28a-vector. Protein induction was carried out in superbroth medium, containing ampicilin and kanamycin, with 1mM IPTG for 6h at 16°C. Bacteria were harvested by centrifugation and resuspended in extraction buffer (300mM NaCl, 50mM Tris/HCl pH 7.5, 1mM DTT, 5mM EDTA, 5mM EGTA, 0,1% Igepal and protease inhibitors Leupeptin, PepstatinA, Aprotenin (10µg/ml each) and AEBSF (0.1mM). GST-SMN fusion proteins were purified with glutathion-sepharose beads as described by the manufactorer. Lanes 2, 4, 6 and 8 show expression of SMN without Gemin2, lanes 3, 5, 7 and 9 show coexpression of SMN and Gemin2.

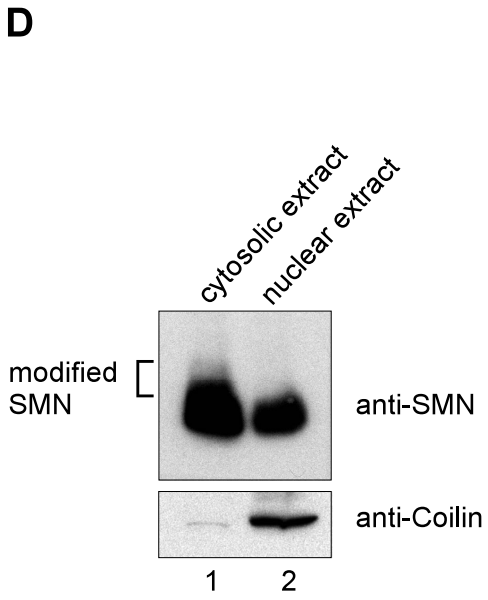

Fig. D. Compartment-specific phosphorylation of SMN.
Cytoplasmic (lane 1) and nuclear HeLa extracts (lane 2) were separated by SDS-PAGE and analysed by western blotting using 7B10 monoclonal SMN-antibody (upper panel). Extracts were blotted in parallel with anti-Coilin antibody (clone 56, BD Transduction Laboratories, lower panel) to monitor the purity of the obtained extracts.

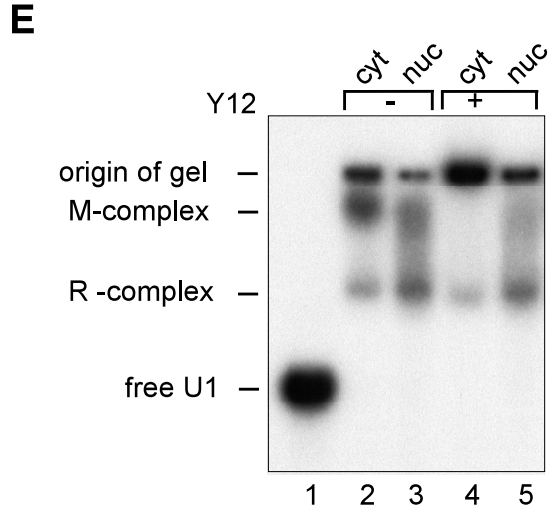

Fig. E. HeLa extract from the cytosol, but not from the nucleus is active in U snRNP assembly.
[32P]-labelled U1 snRNA was incubated with either HeLa cytosolic (lane 2) or nuclear (lane 3) extract. Assembly was analysed by native gel electrophoresis. Lane 4 shows incubation of cytosolic, lane 5 of nuclear assembly reaction with Y12 anti Sm-antibody.

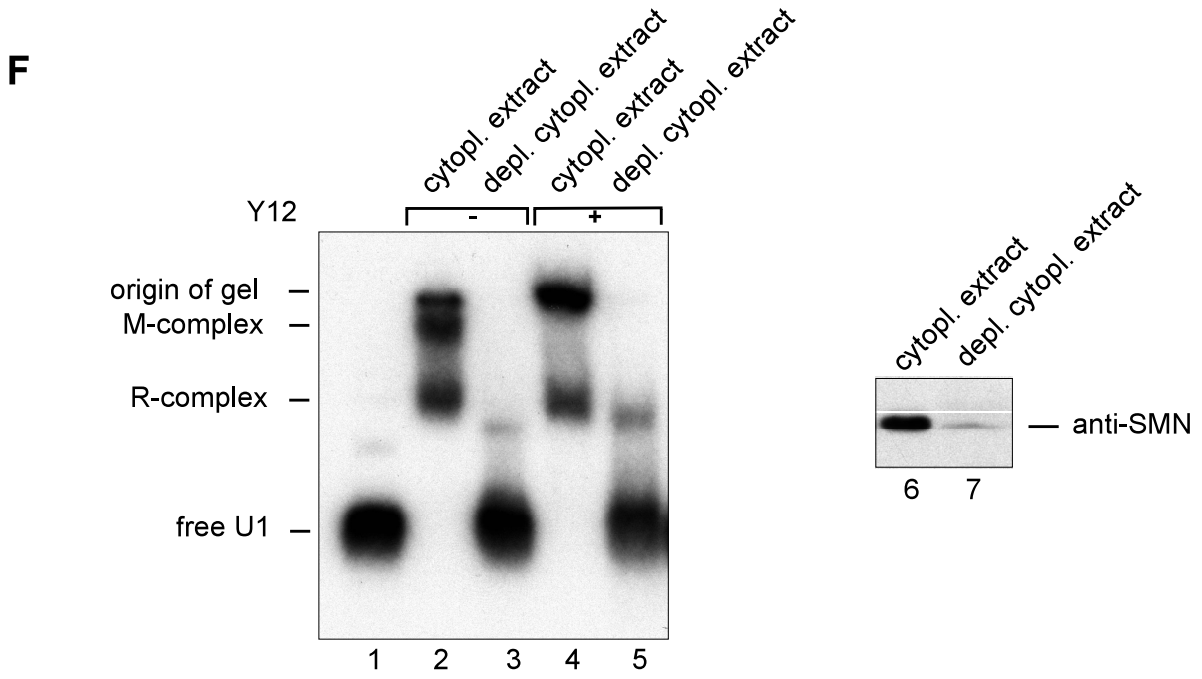

Fig. F. Cytosolic HeLa extract, depleted from SMN is inactive in U snRNP assembly.
[32P]-labelled U1 snRNA was incubated with same amounts of either HeLa cytosolic (lane 2) or SMN depleted HeLa cytosolic (lane 3) extract. Assembly was analysed by native gel electrophoresis. Lane 4 and 5 show incubation of both assembly reaction with Y12 anti Sm-antibody. Right panel shows western blot analysis with monospecific anti-SMN antibody of cytosolic HeLa extract (lane 6) and SMN depleted cytosolic extract.