figure S1

Α		E	acc no	Unique peptides	Mascot percolator score	p-value	gene name	Enrichment factor Jmjd6-IPs/control
170 130 110 70 55	Jmjd6	control	Q6NYC1	16	932	0.00000	Jumonji domain containing protein 6	269.45
	01 02 03	04 05 06	Q9NQ29	1	127	0.00188	Putative RNA-binding protein Luc7-like 1	28.74
	MIE		Q9Y383	5	283	0.00009	Putative RNA-binding protein Luc7-like 2	7.59
			Q14498	2	104	0.00006	RNA-binding protein 39	5.25
			Q05519	1	25	0.00231	Serine/arginine-rich splicing factor 11	4.73
45 —			O95232	2	130	0.00009	Luc7-like protein 3	4.02
35 —	·	hand hand have	Q9UHX1	4	131	0.00396	Poly(U)-binding-splicing factor PUF60	2.46

Figure S1: Immunoprecipitation experiments of endogenous Jmjd6 from HeLa cells. (**A**) Western blot stained with anti-Jmjd6 antibody (sc28348, Santa Cruz) showing the final beads fractions of three independent anti-Jmjd6 pulldown experiments (01-03) and three independent corresponding control (Merck Millipore) experiments (04-06). (**B**) Anti-Jmjd6 and control samples have been analysed by label-free LC-MS/MS analysis and abbundance of co-immunoprecipitated proteins has been analysed. Table indicates enrichment factor of Jmjd6 and co-immunoprecipitated SR-proteins in anti-Jmjd6 samples in comparison to control samples. Mass spectrometry analyses summarise the protein identifications of the samples 01-06 shown in (A).

figure S2



Figure S2: Rescue experiments after Jmjd6 knockdown in HeLa cells. siRNA-mediated knockdown of endogenous Jmjd6 increases splicing activity (**A**) **+ (Fig. 9**). A subsequent transient expression of wildtype Jmjd6 in the Jmjd6 knock down cells reduces splicing activity in the double-reporter splicing assay. A similar effect was observed for an enzymatically inactive Jmjd6AxA variant (**B**). Nucleotide sequence of the pcDNA3-Jmjd6 and pcDNA3-Jm-jd6AxA constructs has been changed in order to avoid binding by siRNA1. Nucleotide changes did not result in a change in amino acid sequence (see material & methods). Jmjd6 levels were detected in immuno blots (**C**).

figure S3

A

SRSF11 (245-394): rrhsrsrsrsrrrtpsssrhrrsrsrsrsrksksprrrsksprrrshskergrrsrstsktrdkkkedkekkrsktppksystarrsrsasrerrrrrsrsgtrspkkprspkrklsrspsprrhkkekkkdkdkersrderers

Luc7like3 (292-432): RARDRERRKRSRSRSRHSSRTSDRRCSRSRDHKRSRSRERRRSRSRDRRRSRSHDRSERKHRSRSRDRRSKSRDRKSYKHRSKSRDREQDRKSKEKEKRGSDDKKSSVKSGSREKQSEDTNTESKESDTKNEVNGTSEDIKSEGDTQSN

U2AF65 (21-70): ENRHRKRSHSRSRSRDRKRRSRSRDRRNRDQRSASRDRRRRSKPLTRGA

Acinus S'(447-510): P**RSRSRSRDRRR**KE**R**AK**S**KEKK**S**EKKEKAQEEPPAKLLDDLF**R**KTKAAPCIYWLPLTD**S**QIVQK

В

SRSF1 (198-247): rspsygrsrsrsrsrsrsrsrsrsysprrsrgsprysprhsrsrsrt

Figure S3: Jmjd6 interacts with RS-domains of several SR-like proteins. Amino acid sequences of the Jmjd6-interacting domains of SRSF11, Luc7like3, U2AF65 and Acinus S' as validated in co-immunoprecipitation (co-IP) experiments (**A**). The RS-domain of SRSF1 is not bound by Jmjd6 in these co-IP experiments (**B**).