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Supplemental Material to:

Valerie Edmond, Galina Merdzhanova, Stephanie Gout, Elisabeth Brambilla, Sylvie Gazzeri and Beatrice Eymin

A new function of the splicing factor SRSF2 in the control of E2F1-mediated cell cycle progression in neuroendocrine lung tumors

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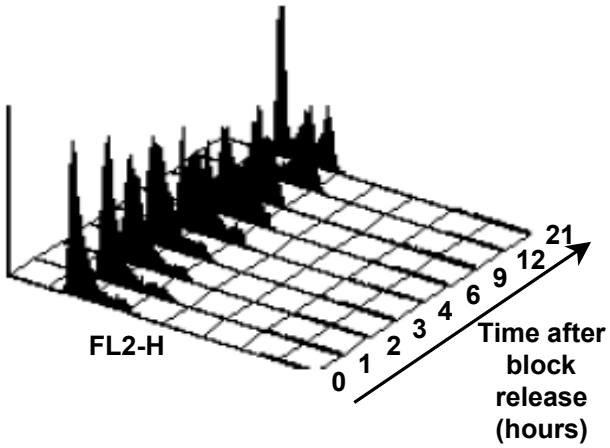
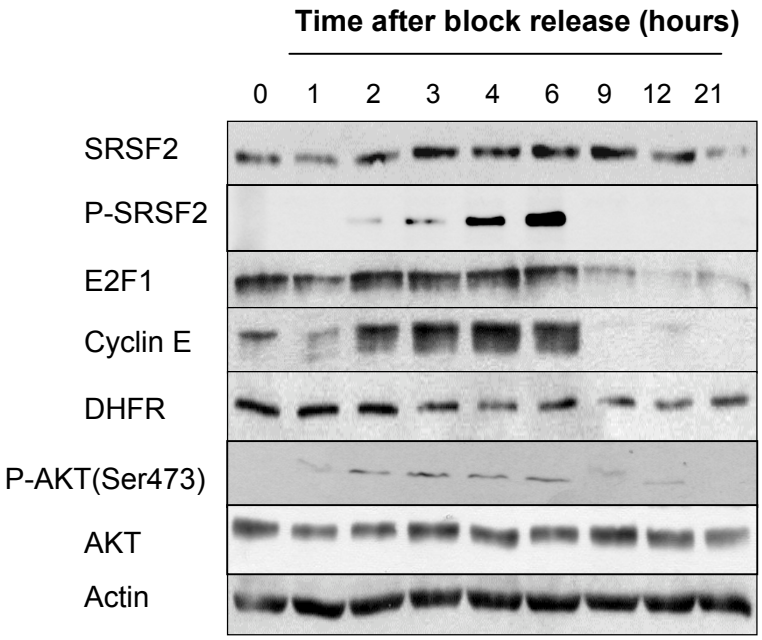
Edmond et al Suppl Figure 1

A



B



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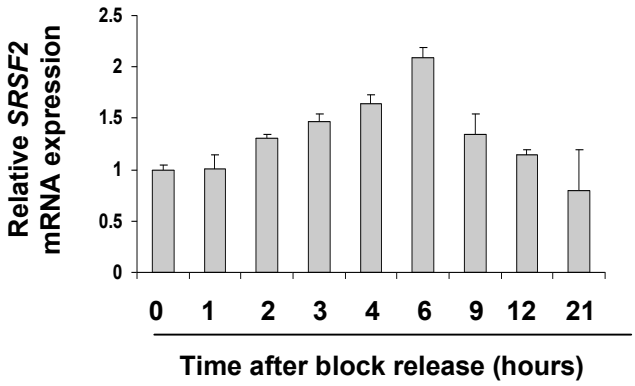
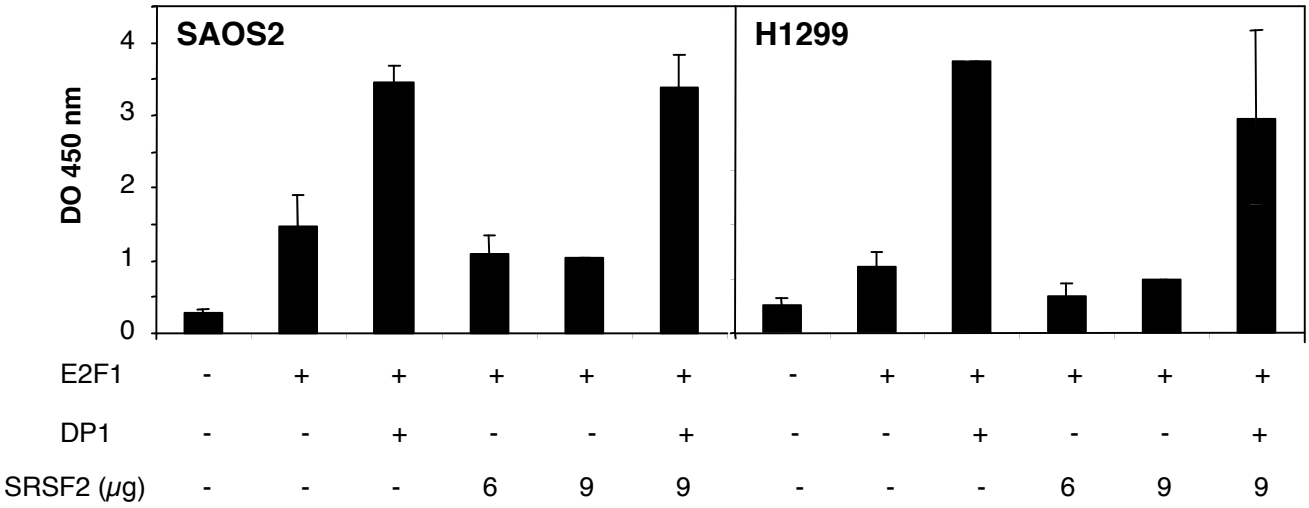


Supplementary Figure 2



Supplementary Figures

Supplementary Figure 1: SRSF2 is a cell-cycle regulated protein

U-2 OS cells were synchronized by a double block of thymidine as described in the Materials and Methods section, then released in thymidine free complete medium and harvested at the indicated time points. **(A)** Cell cycle distribution was assessed by flow cytometry after propidium iodide staining. **(B)** Western blot analysis of SRSF2, E2F1, cyclin E and DHFR proteins was performed at the indicated times after block release. Actin was used as a loading control. **(C)** *SRSF2* transcripts were quantified by RT-QPCR at different times following block release as indicated. Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) was used as a reference. Relative gene expression was calculated for each sample, as the ratio of *SRSF2* copy number (target gene) to *gapdh* mRNA copy number multiplied by 100, thus normalizing *SRSF2* mRNA expression. The ratio obtained at time 0 (block release) was arbitrarily assigned a value of 1.

Supplementary Figure 2: SRSF2 overexpression does not increase E2F1 DNA-binding activity.

SAOS-2 and H1299 cells were co-transfected for 48 h with 3 μg pCMV-E2F1 in the presence or absence of 9 μg pCMV-DP1 and increasing amounts of pcDNA3-SRSF2 (6 and 9 μg). Nuclear extracts obtained from Nuclear Extraction Kit (Panomics) were used in an ELISA assay for the analysis of E2F1 DNA-binding activity according to the manufacturer's protocol.

Supplementary Table 1: Correlation between P-SRSF2 and Cyclin E protein levels in NE lung tumors.

	P-SRSF2 -		P-SRSF2 +		P
	Cyclin E -	Cyclin E +	Cyclin E -	Cyclin E +	
Number of tumors	6	0	10	11	0.0083

P-SRSF2 -: tumors without P-SRSF2 overexpression (score ≤100); P-SRSF2 +: tumors overexpressing P-SRSF2 (score >100).
Cyclin E -: tumors without cyclin E overexpression (score <40); cyclin E +: tumors overexpressing cyclin E (score ≥40).
P: Chi-2 test.