





	CAR-E		CAR-Em2		
	Unique	Total	Unique	Total	M.W (kD)
TREX complex					
THOC2	29	37	1	1	183
THOC5	13	16			79
THOC6	7	9	1	1	38
THOC3	5	5			39
THOC1	5	5			76
UAP56/URH49	13	15			49
Prp19 complex	and U2AF2				
XAB2	9	12	1	1	100
AQR	10	13	2	2	171
CDC5L	4	4	1	1	92
PRPF19	6	6			55
U2AF2	7	11			54
Cap binding pro	oteins				
ARS2	43	73	23	74	101
NCBP1	18	28	7	24	92
NCBP2	3	3	3	6	18
U1 snRNP					
SNRNP70	11	15	5	10	52
SNRPA	7	8			31
U2snRNP					
SF3B1	21	24	5	5	146
SF3A1	14	18	5	6	89
SF3B3	14	17	6	12	136
DDX46	14	14	4	4	117
SF3B2	9	9	6	8	100
SF3B4	3	3	1	2	44
U5 snRNP					
SNRNP200	47	56	4	4	245
PRPF8	46	49	2	3	274
EFTUD2	20	24			109
DDX23	15	21	1	1	96
PRPF6	13	13	1	1	107
SNRNP40	8	11			39

### Table S1 Proteins present in CAR-E and CAR-E<sub>m2</sub> mRNPs

U4/U6.U5 tri-sn	RNP				
SART1	16	18	4	4	90
USP39	6	6			65
SNRNP27	3	3			19
U4/U6 SNRNP		2			
PRPF4	8	8	1	1	58
PRPF3	7	7	1	1	78
Other Spliceoso	mal protein	S			
DHX15	38	66	10	12	91
RBM25	20	26	2	2	100
SR140	15	17			119
SRRM2	14	16	2	2	300
TFIP11	14	15			97
DDX5	13	14	4	5	69
PRPF40A	10	10	5	6	109
PRPF38B	9	11			64
ZCCHC8	9	9	1	2	79
CROP	8	10	2	4	51
PRPF4B	6	7			117
PUF60	5	5	7	11	60
RBM39	5	5	3	3	59
SNRPD2	3	3	1	1	14
RBM17	3	3			45
SNRPD1	3	3			13
SNRPB	2	2			25
ZRANB2			5	6	37
SR proteins					
SFRS1	9	11	А	А	28
SFRS7	5	5	-		20
SFRS4	4	5	1	1	57
SFRS2	4	7	1	1	25
SFRS6	4	, 6	1	1	40
TRA2B	5	6	-	-	34
SFRS15	4	5			126
SFRS3	3	5	1	1	19
Polyadenylation	ו				
PABPC1	10	12	3	3	71
CPSF1	9	9			161

PABPC4	8	8			71
CPSF7	7	7	1	1	52
CPSF6	6	6	1	1	59
PCF11	4	5			173
FIP1L1	4	4			67
Nuclear pore co	omplex				
NUP214	5	5	4	4	214
NUP88	5	5			84
EJC					
ACIN1	20	22	3	3	152
EIF4A3	4	4	1	1	47
Exosome					
SKIV2L2	31	38	4	7	118
DIS3	18	23	3	3	109
EXOSC10	13	13	4	4	101
EXOSC4	3	5	1	2	26
EXOSC2	4	4	1	1	33
SMN complex					
GEMIN5	4	4	3	4	169
DDX20	5	5			92
hnRNP proteins	5				
HNRNPL	12	14	5	6	64
HNRNPR	12	17	2	3	71
HNRNPUL1	11	15	4	8	96
SYNCRIP	9	10	3	3	70
HNRNPU	8	10	1	1	91
HNRNPK	8	9	3	3	51
HNRNPA2B1	7	7	5	7	37
HNRNPA3	5	5	2	3	40
HNRNPM	5	5			78
HNRNPA1	4	5	1	1	39
HNRNPH1	3	3	1	1	49
PTBP1			10	40	57
Transcription re	elated				
DHX9	37	52	16	45	141
RDBP	23	44	16	44	43
POLR2A	18	21			217

TCERG1	18	22	3	3	124
CCAR1	14	18			133
GTF2I	15	17	3	3	112
THRAP3	14	17	4	4	109
POLR2B	14	15			134
WHSC2	13	15	1	1	52
MTA2	13	15	1	1	75
COBRA1	11	17	5	15	66
HSPA8	11	13	6	11	71
SMARCA5	11	11	2	2	122
CHD4	11	11	1	1	218
KDM1	9	10	2	2	93
C21orf66	8	9			105
TOP2A	7	7	1	1	174
SMARCA4	6	7	6	6	185
GTF3C1	6	6			239
RBBP4	5	5	2	2	48
HDAC2	5	5			55
UHRF1	5	5	1	1	90
XRN2	4	5	1	1	109
IFI16	4	5			88
NONO	4	4			54
DIDO1	4	4	1	1	244
SMARCA1	4	4			123
LARP7	3	4			67
HDAC1	3	4	1	1	55
BCLAF1	3	4			106
POLR2E	3	3			25
SUPT16H			3	9	120
DNA repair rel	ated				
PRKDC	43	47	13	20	469
XRCC6	17	21			70
SMC4	14	16	3	3	147
XRCC5	12	15	1	1	83
PARP1	12	12	3	3	113
DDB1	8	9	1	1	127
RAD50	7	7	3	3	154
SMC3	6	6	4	4	142
RECQL	6	6	1	1	73
CHAF1A	5	5			107
DDB2	3	4			48

Others					
ACACA	106	185	53	123	266
ILF3	29	51	11	28	95
FTSJD2	28	42	6	8	95
ZC3H18	27	44	11	17	106
HDLBP	27	30	8	10	141
DNMT1	25	29	1	1	183
DHX36	22	26	3	4	115
IQGAP1	20	20	7	8	189
HELZ	19	20	6	7	219
RBBP6	14	16			202
MCM3	12	13	2	2	91
ILF2	11	17	2	3	43
DDX21	11	12	4	4	87
C17orf85	10	12	12	37	71
RBM15	10	10			107
ZFC3H1	10	10	1	1	226
STRBP	9	12	5	9	74
PHAX	9	12	5	5	44
ADAR	9	11	17	59	136
LARP1	9	11	2	2	124
IFIT1	9	10	4	7	55
KPNA2;LOC728	9	10	3	6	58
MCM5	9	9	1	1	82
DDX17	8	10	4	7	72
MCM7	8	9	2	2	81
KIAA1967	8	8	10	17	103
BAZ1A	7	9			179
TFRC	7	8	2	2	85
RBM26	7	8			114
NCL	7	7	5	8	77
UPF1	7	7	3	3	124
AGGF1	7	7	2	2	81
ACACB	7	7	1	1	277
KIAA1429	7	7			202
BUB3	6	10	1	2	37
MATR3	6	7	11	26	95
DDX1	6	6	2	2	82
MOV10	6	6	2	3	114
C22orf28	6	6	1	2	55
SMC1A	6	6	2	2	143
RBM7	6	6			31
CHAF1B	5	6			61

ELAVL1	5	6	5	9	36
ZC3H4	5	5	2	2	140
ADARB1	5	5	2	5	81
SMC2	5	5	2	2	136
AHNAK	5	5	1	1	63
WDR33	5	5			146
ZC3H13	5	5			197
KIAA0182	5	5			136
TNRC6B	4	6			194
CSDE1	4	5	2	2	89
LMO7	4	4	5	5	193
MTHFD1	4	4	4	6	102
HIST1H1C	4	4	2	4	21
ZFR	4	4	1	1	117
PLK1	4	4			68
DNAJC2	4	4	1	1	72
USP7	4	4	1	1	128
KHDRBS1	3	4			48
TNRC6A	3	4			210
DDX6	3	4			54
ZCCHC8	3	4	1	2	79
LUC7L2	3	3	4	6	47
KPNA1	3	3	2	3	60
HELLS	3	3	1	1	97
PDS5A	3	3	4	4	151
MEPCE	3	3			74
SERBP1	2	3	2	5	45
G3BP1	2	3	1	1	52
HSPA1A;HSPA1	2	2	4	4	70
DICER1	1	1	12	22	219
KHSRP			8	14	73
NOLC1	1	1	8	13	74
TCOF1	1	1	10	13	152
ROD1			5	13	60
IFIT2			3	4	55
HDGF2			3	4	74

#### **Supplementary Figure Legends**

## Figure S1. The tandem CAR-E promotes protein expression of Slu7 and DDX3x cDNAs.

(A) Schematic of CMV-Slu7 or CMV-DDX3x cDNA constructs indicating the position where the tandem CAR-E was inserted. The location of the HA tag is shown. (B, C) Western blot showing protein expression from Slu7 (B) or DDX3x (C) constructs containing or lacking the tandem CAR-E at 24 hr (Day 1) or 45 hr (Day 2) after transient transfection to HeLa cells. Co-transfected EGFP was used as a loading control. (D) FISH was used to determine the nucleocytoplasmic distribution of the indicated transcripts 30 hr after transient transfection of the respective constructs into HeLa cells. DAPI staining was used to identify the nucleus. Scale bar: 10 μm.

# Figure S2. RNAi of XAB2, CRNKL1 or U2AF2 blocks export of naturally intronless HSPB3 and IFNα1 mRNA.

(A, B) Schematic of CMV-HSPB3 or CMV- IFN $\alpha$ 1 constructs used for microinjection. The length of the 5'UTR, coding region, and 3'UTR are indicated. Vector sequences are shown as gray lines. BGH pA: bovine growth hormone polyA site. CMV-constructs encoding HSPB3 (C, E, G) or IFN $\alpha$ 1 (D, F, H) were microinjected into the nuclei of the indicated knockdown or negative control knockdown HeLa cells. Dextran 70 kD was used as an injection marker. Scale bar: 10µm. Note that the knockdown efficiencies of each factor are shown in Fig. 4 of the main paper.

### **Supplementary Table Legend**

The list of proteins detected by mass spectrometry in the tandem CAR-E and CAR- $E_{m2}$ RNPs is shown. Proteins were divided into categories based on their best-known functions. The number of total and unique peptides and molecular weight of the proteins are indicated.