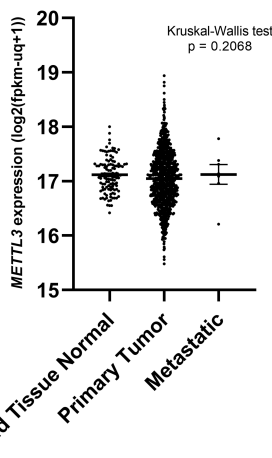
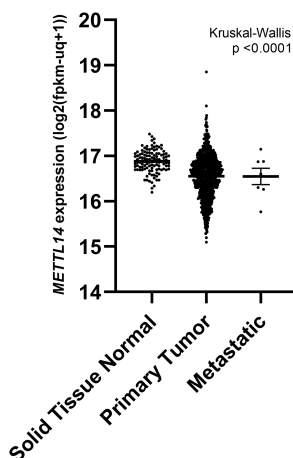
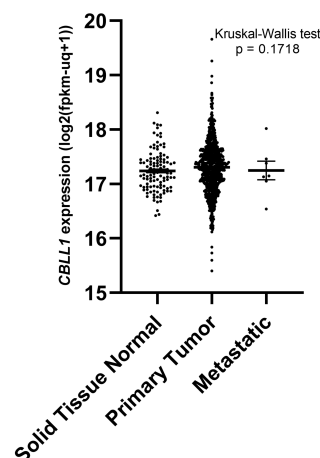
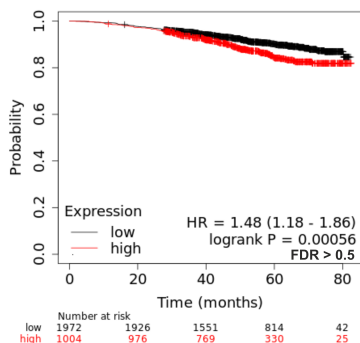
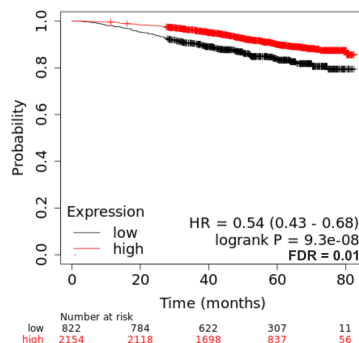
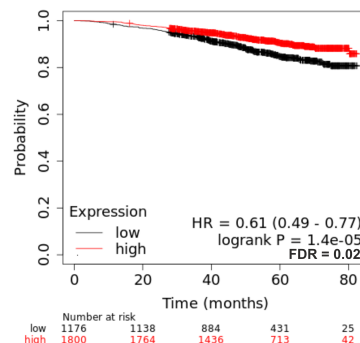


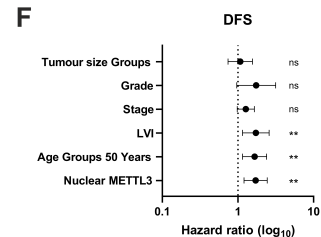
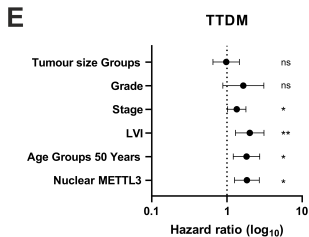
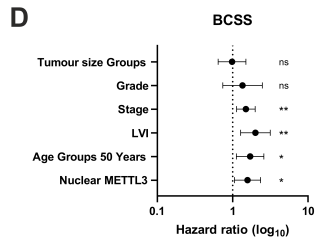
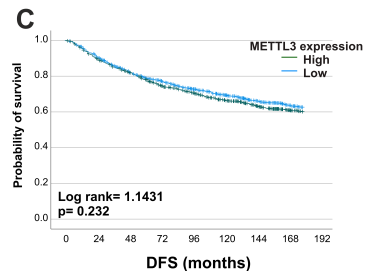
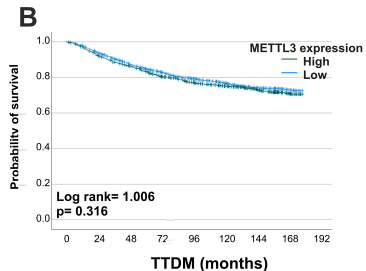
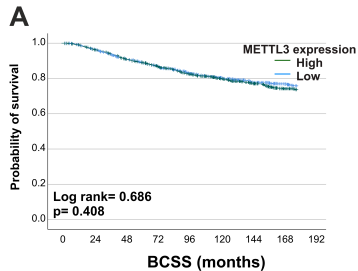
A

	Amplification	Deep Deletion	Missense mutation	Truncating mutation	No genetic alteration
METTL3	7 (0.7%)	2 (0.2%)	2 (0.2%)	1 (0.1%)	984 (98.8%)
METTL14	5 (0.5%)	0 (0%)	3 (0.3%)	1 (0.1%)	987 (99.1%)
CBLL1	6 (0.6%)	1 (0.1%)	1 (0.1%)	0 (0%)	988 (99.2%)

B**METTL3 expression****C****METTL14 expression****D****CBLL1 expression****E****METTL3****F****METTL14****G****CBLL1**

Supplementary Figure 1 Mutations and RNA expression of *METTL3*, *METTL14* and *CBLL1* in breast cancer

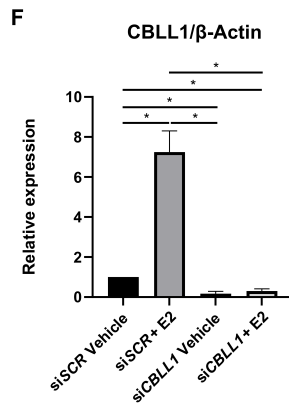
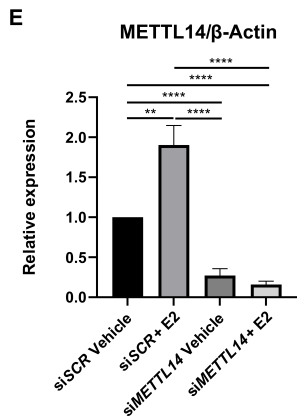
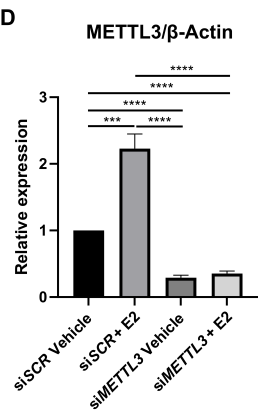
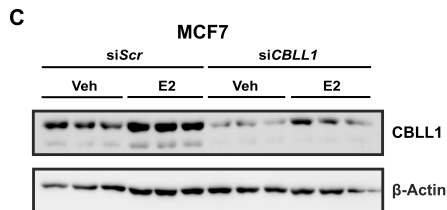
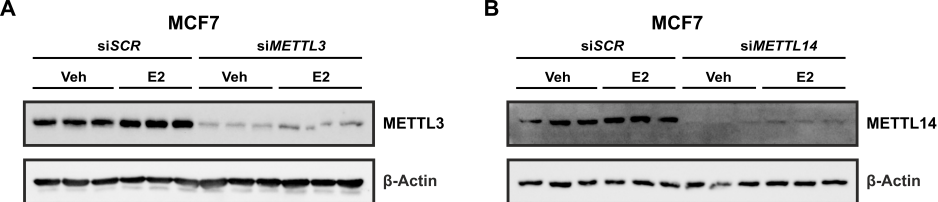
(A) Mutations in *METTL3*, *METTL14* and *CBLL1* in the Breast Invasive Carcinoma cohort (TCGA, PanCancer Atlas, n=996). Comparison of tumour and normal breast tissue expression of (B) *METTL3*, (C) *METTL14* and (D) *CBLL1* (TCGA GDC Xena, n=1284). KM plots showing the correlation of (E) *METTL3*, (F) *METTL14* and (G) *CBLL1* expression with overall survival (KM plotter, n=2976).



Supplementary Figure 2 METTL3 expression correlated with survival and prognosis in breast cancer patients

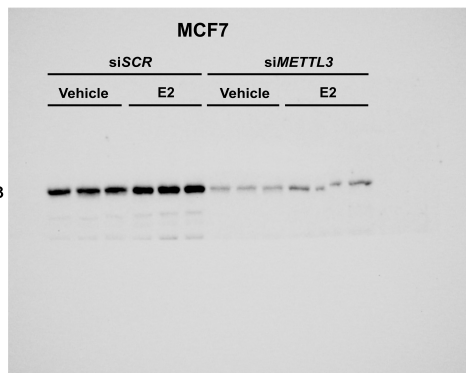
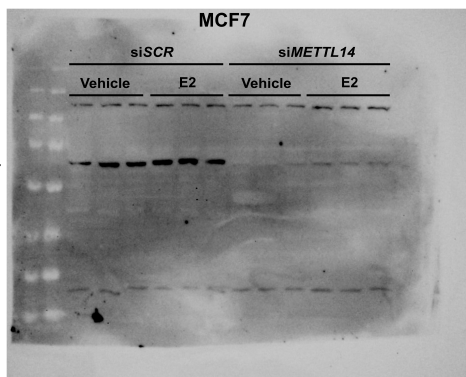
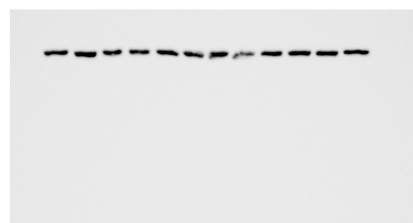
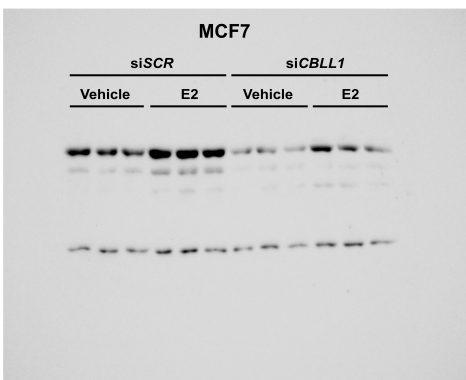
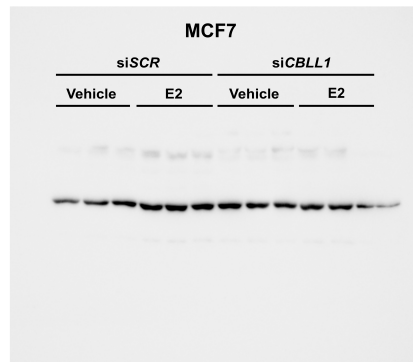
IHC staining of BCa patient samples was assessed by H-score, divided by median into high and low expression groups and correlated with breast cancer-specific survival (BCSS), time to distant metastasis (TTDM) and disease-free survival (DFS). METTL3 staining in all BCa patients, low: ≤ 105 , high: ≥ 106 . (A) Correlation of METTL3 staining and BCSS (n=2115, events at 15 years, low: 262/1210, high: 211/905). (B) Correlation of METTL3 staining and TTDM (n=2115, events at 15 years, low: 304/1210, high: 246/905). (C) Correlation of METTL3 staining and DFS (n=2115, events at 15 years, low: 410/1210, high: 332/905). Correlated using Kaplan Meier estimate and analysed by log-rank test.

Combined multivariate Cox regression analysis of METTL3 nuclear expression, tumour size, tumour grade, tumour stage, LVI and patient age with 15-year (D) BCSS (n=326), (E) TTDM (n=328) and (F) DFS (n=328) in TNBC patient samples. * = $p \leq 0.05$, ** = $p \leq 0.01$, ns = not significant.

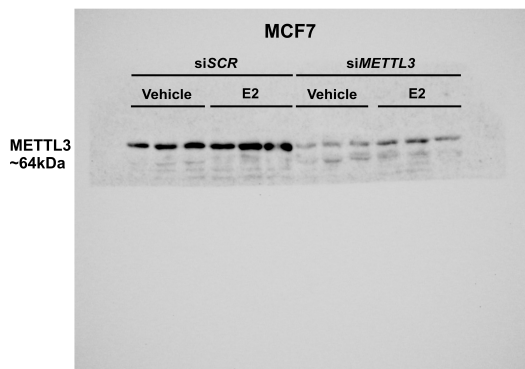
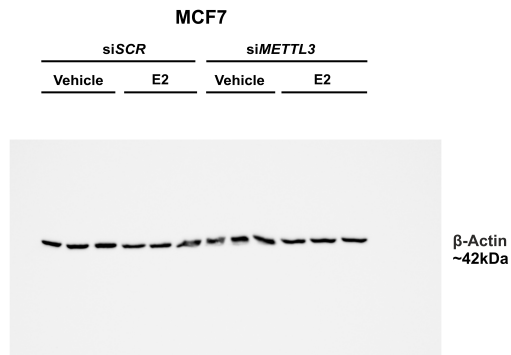
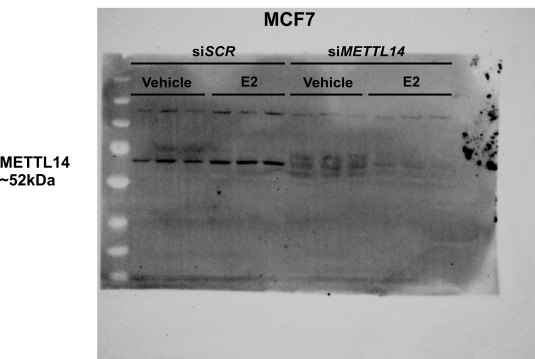
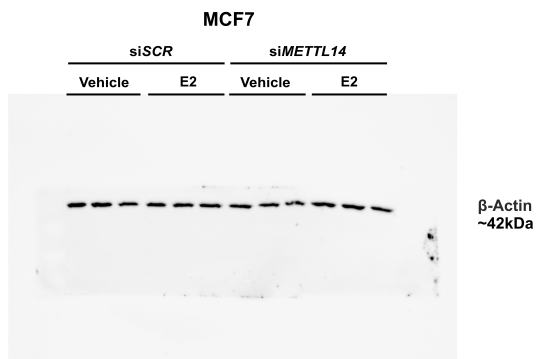
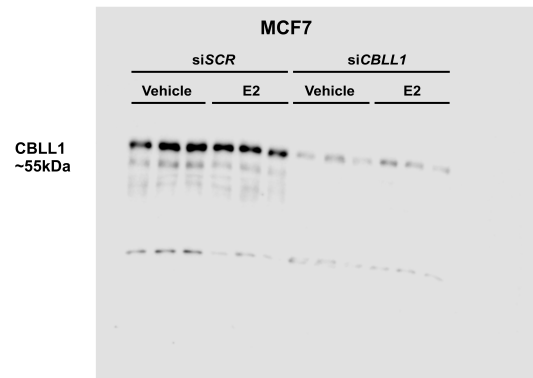
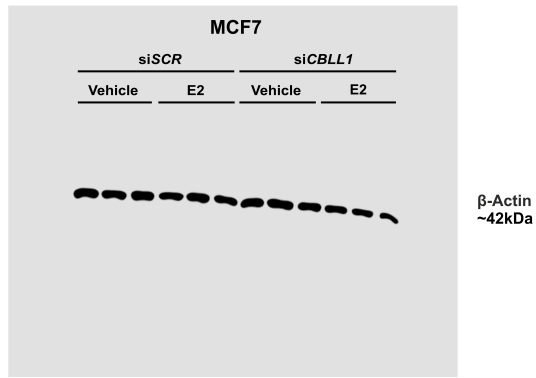


Supplementary Figure 3 Representative images of METTL3, METTL14 and CBLL1 expression.

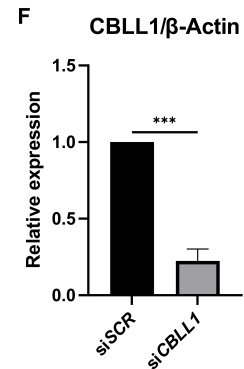
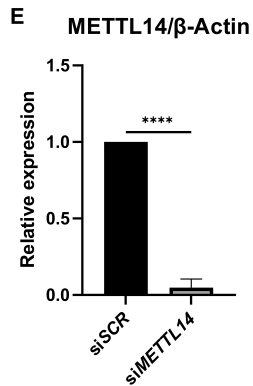
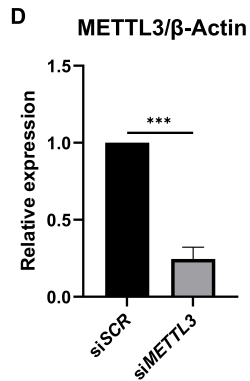
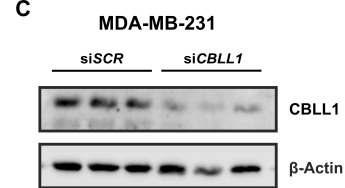
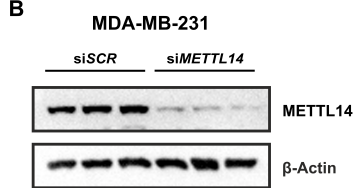
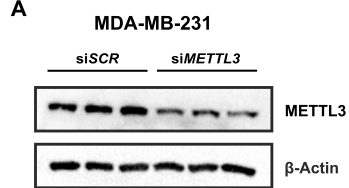
(A) Expression of METTL3 and β -Actin in MCF7 cells treated with siSCR or si*METTL3* and with DMSO vehicle or E2 (n=3); (B) expression of METTL14 and β -Actin in MCF7 cells treated with siSCR or si*METTL14* and with DMSO vehicle or E2 (n=3); (C) expression of CBLL1 and β -Actin in MCF7 cells treated with siSCR or si*CBLL1* and with DMSO vehicle or E2 (n=3). (D) Quantification of METTL3 protein expression in MCF7 cells following METTL3 siRNA-mediated knockdown treated with DMSO vehicle or E2 (n=6); (E) quantification of METTL14 protein expression in MCF7 cells following METTL14 siRNA-mediated knockdown treated with DMSO vehicle or E2 (n=6); (F) quantification of CBLL1 protein expression in MCF7 cells following CBLL1 siRNA-mediated knockdown treated with DMSO vehicle or E2 (n=6). Quantification based on two independent experiments of n=3 (shown in Supplementary Figures 4 & 5). * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$ by paired ANOVA.

AMETTL3
~64kDa**B** β -Actin
~42kDa**C**METTL14
~52kDa**D** β -Actin
~42kDa**E**CBLL1
~55kDa**F** β -Actin
~42kDa

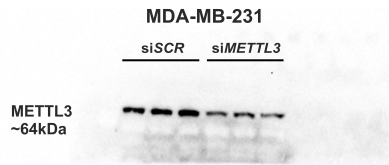
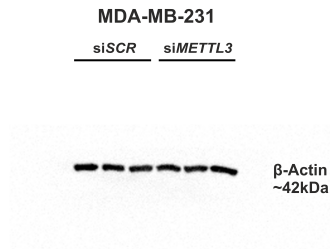
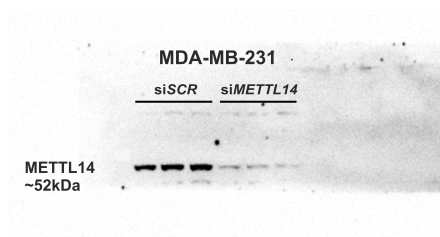
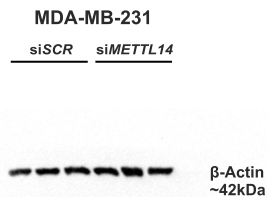
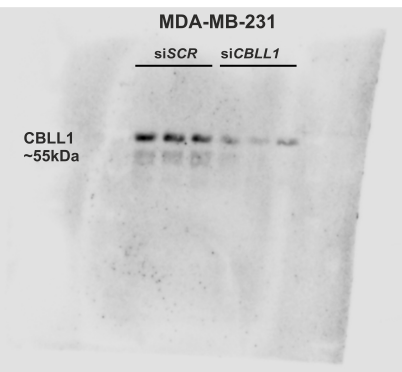
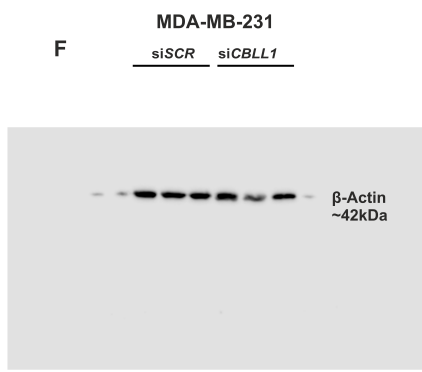
Supplementary Figure 4. Full uncropped annotated western blot images for first independent experiment (all n=3). Expression of METTL3 (A) and β -Actin (B) in MCF7 cells treated with siSCR or si*METTL3* and with DMSO or E2; expression of METTL14 (C) and β -Actin (D) in MCF7 cells treated with siSCR or si*METTL14* and with DMSO or E2; expression of CBLL1 (E) and β -Actin (F) in MCF7 cells treated with siSCR or si*CBLL1* and with DMSO or E2.

A**B****C****D****E****F**

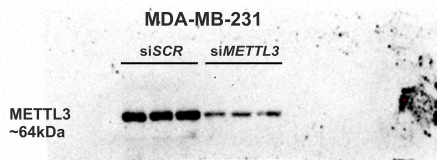
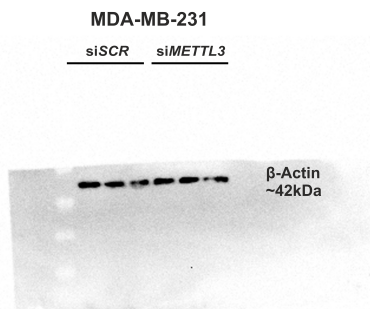
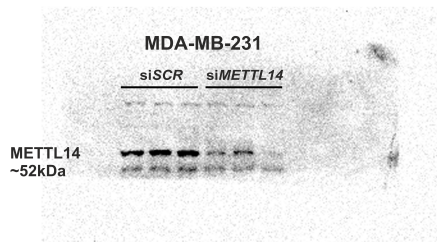
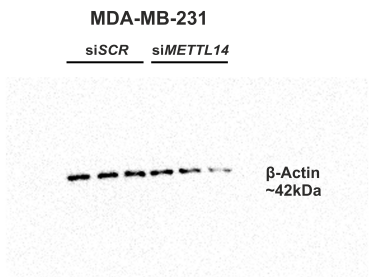
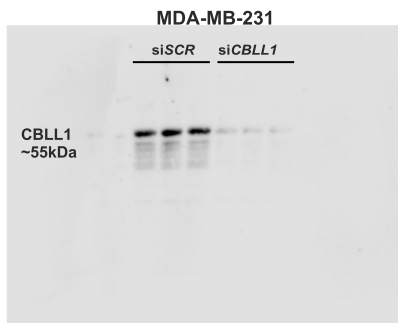
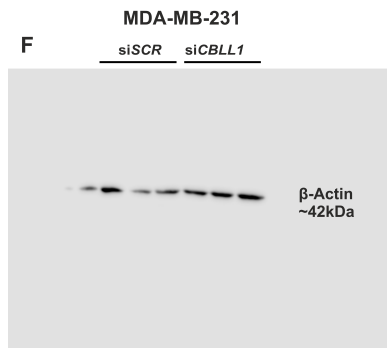
Supplementary Figure 5. Full uncropped annotated western blot images for second independent experiment (all n=3). Expression of (A) METTL3 and (B) β -Actin in MCF7 cells treated with siSCR or si*METTL3* and with DMSO or E2; expression of (C) METTL14 and (D) β -Actin in MCF7 cells treated with siSCR or si*METTL14* and with DMSO or E2; expression of (E) CBLL1 and (F) β -Actin in MCF7 cells treated with siSCR or si*CBLL1* and with DMSO or E2.



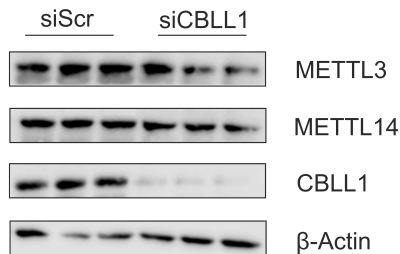
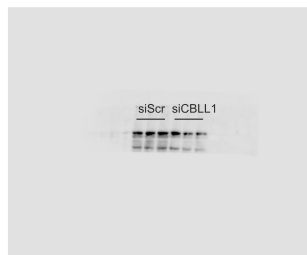
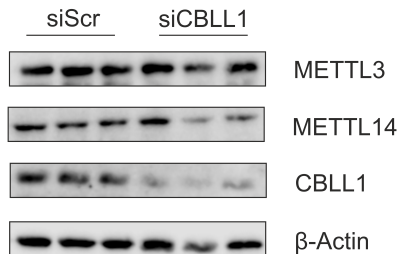
Supplementary Figure 6. Representative images of METTL3, METTL14 and CBLL1 expression. (A) Expression of METTL3 and β -Actin in MDA-MB-231 treated with siSCR or si*METTL3* (n=3); (B) expression of METTL14 and β -Actin in MDA-MB-231 treated with siSCR or si*METTL14* (n=3); (C) expression of CBLL1 and β -Actin in MDA-MB-231 treated with siSCR or si*CBLL1* (n=3). (D) Quantification of METTL3 protein expression in MDA-MB-231 following METTL3 siRNA-mediated knockdown (n=6); (E) quantification of METTL14 protein expression in MDA-MB-231 following METTL14 siRNA-mediated knockdown (n=6); (F) quantification of CBLL1 protein expression in MDA-MB-231 following CBLL1 siRNA-mediated knockdown (n=6). Quantification based on two independent experiments of n=3 (shown in Supplementary Figures 7 and 8). * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$ by paired t-test.

A**B****C****D****E****F**

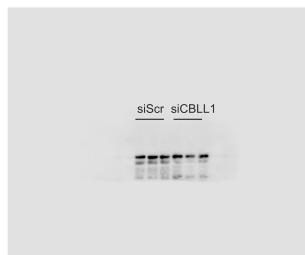
Supplementary Figure 7. Full uncropped annotated western blot images for first independent experiment (all n=3). Expression of (A) METTL3 and (B) β -Actin in MDA-MB-231 treated with siSCR or si*METTL3*; expression of (C) METTL14 and (D) β -Actin in MDA-MB-231 treated with siSCR or si*METTL14*; expression of (E) CBLL1 and (F) β -Actin in MDA-MB-231 treated with siSCR or si*CBLL1*.

A**B****C****D****E****F**

Supplementary Figure 8. Full uncropped annotated western blot images for second independent experiment (all n=3). Expression of (A) METTL3 and (B) β -Actin in MDA-MB-231 treated with siSCR or si*METTL3*; expression of (C) METTL14 and (D) β -Actin in MDA-MB-231 treated with siSCR or si*METTL14*; expression of (E) CBLL1 and (F) β -Actin in MDA-MB-231 treated with siSCR or si*CBLL1*.

A**B**

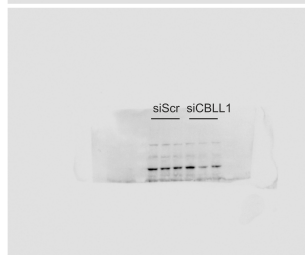
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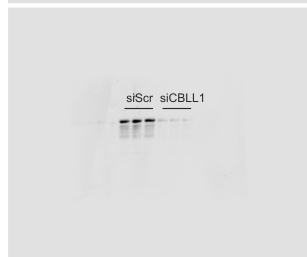
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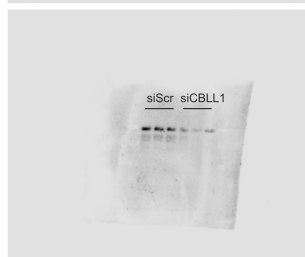
METTL14



METTL14



CBLL1



CBLL1

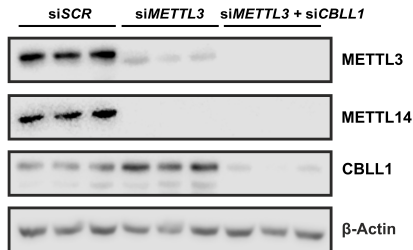
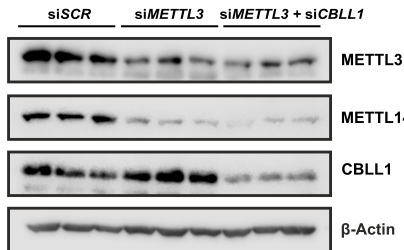
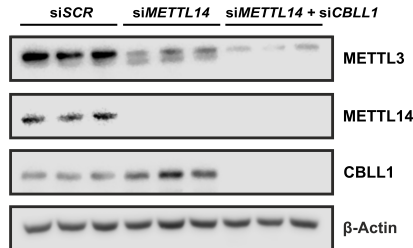
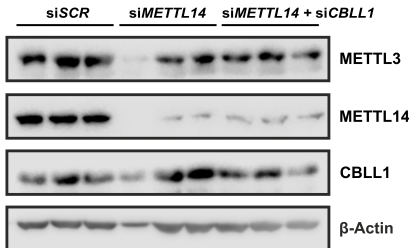


β-Actin

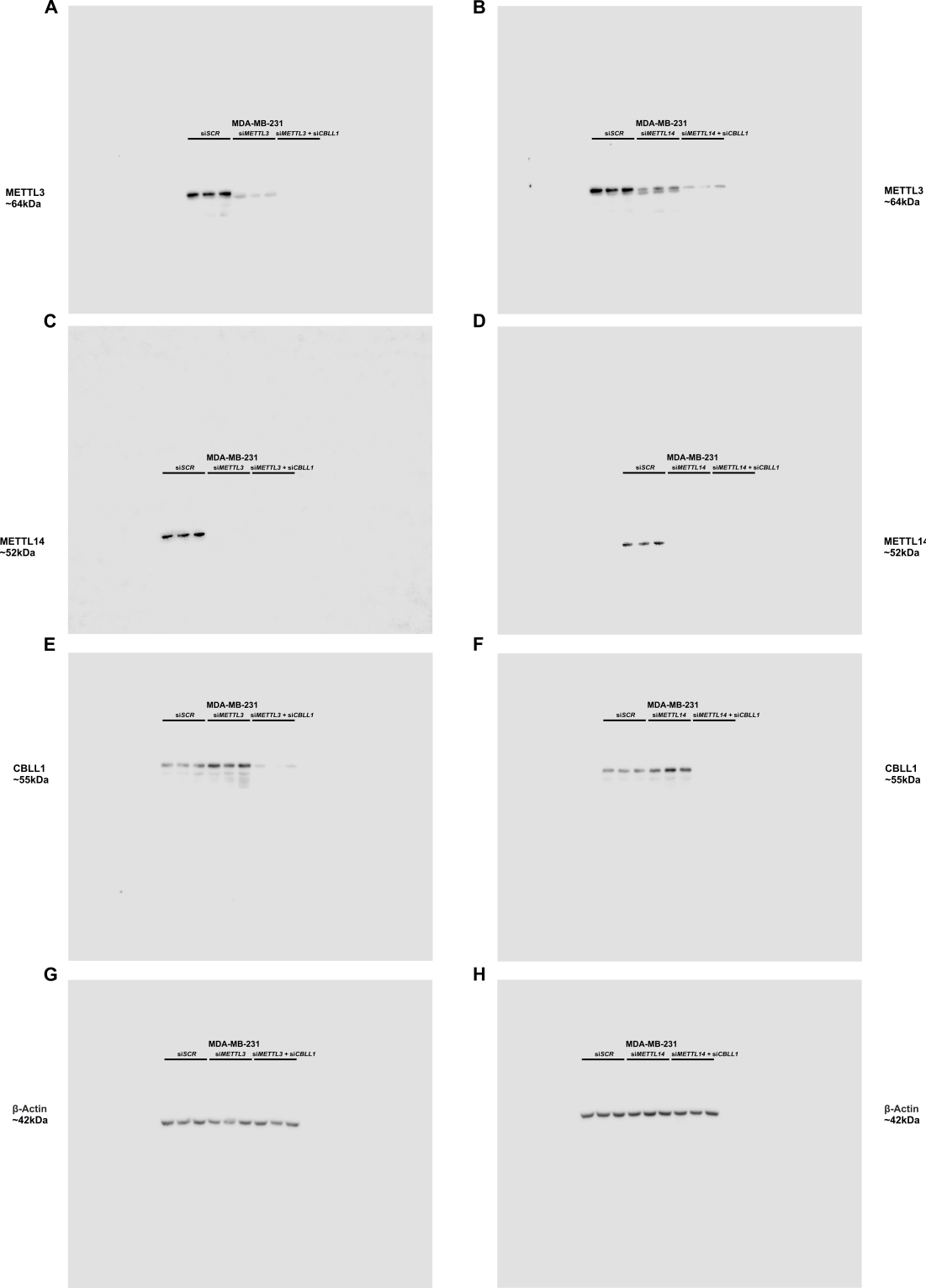


β-Actin

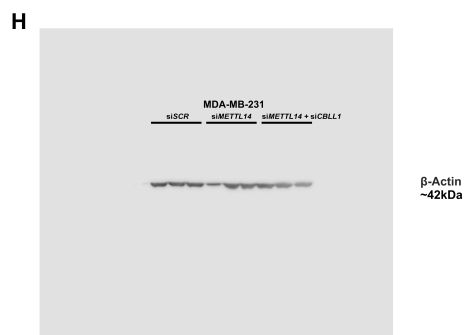
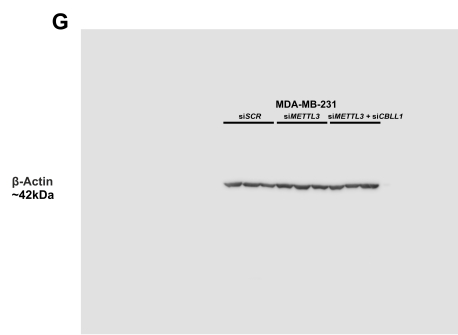
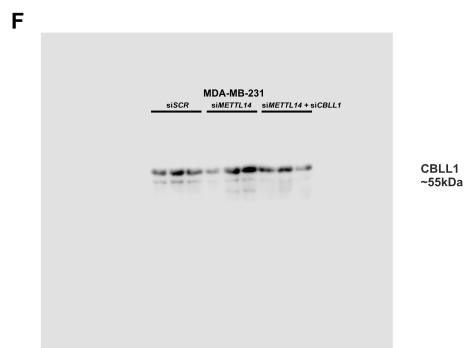
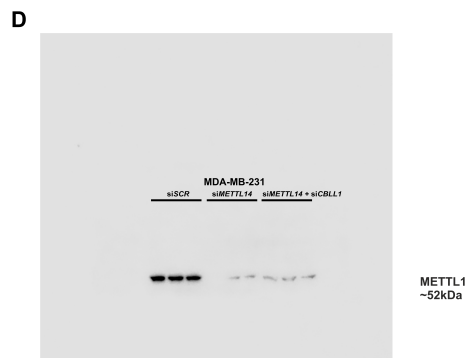
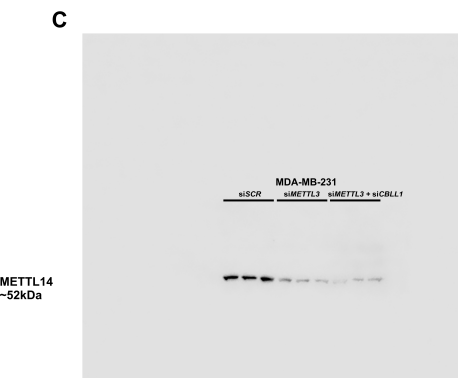
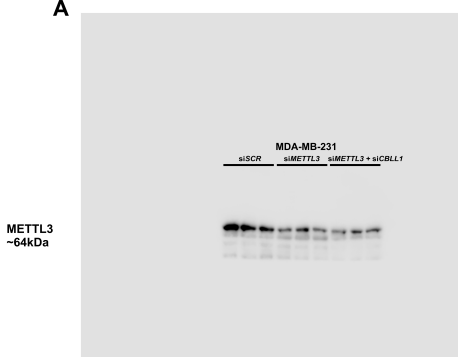
Supplementary Figure 9. Annotated western blots for the expression of METTL3, METTL14, CBLL1 and β -Actin in siRNA-mediated CBLL1 knockdown in MDA-MB-231 with full uncropped annotated western blot images below. (A) First independent experiment and (B) second independent experiment.

A**MDA-MB-231****B****MDA-MB-231****C****MDA-MB-231****D****MDA-MB-231**

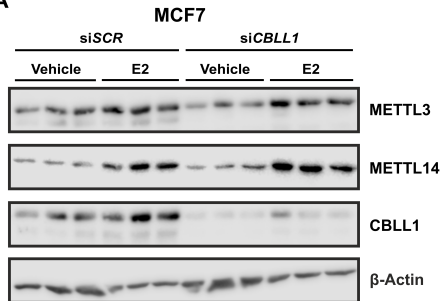
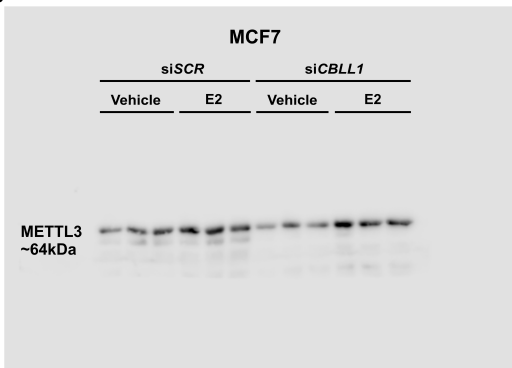
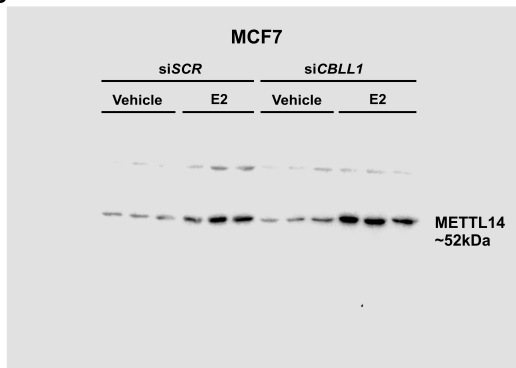
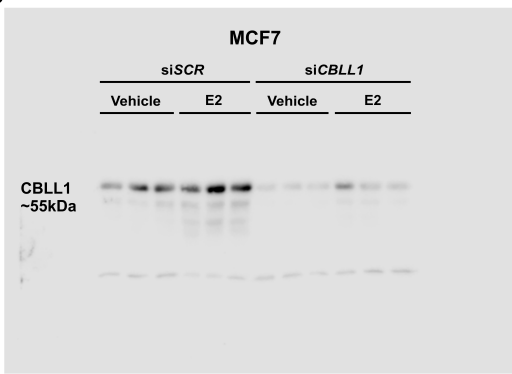
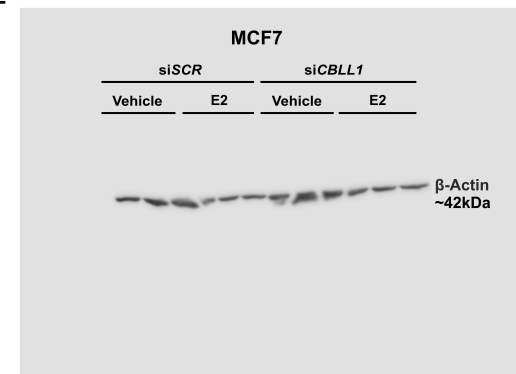
Supplementary Figure 10. Expression of METTL3, METTL14, CBLL1 and β -Actin in MDA-MB-231 treated with either siSCR, si*METTL3*, or a combination of si*METTL3* and si*CBLL1* (n=3) (A) experiment 1 and (B) experiment 2; expression of METTL3, METTL14, CBLL1 and β -actin in MDA-MB-231 treated with either siSCR, si*METTL14*, or a combination of si*METTL14* and si*CBLL1* (n=3) (C) experiment 1 and (D) experiment 2. Full uncropped western blot images shown in Supplementary Figure 11 and 12.



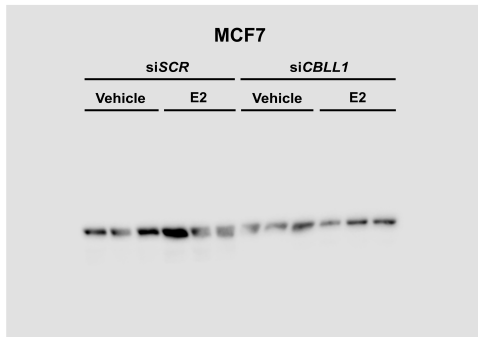
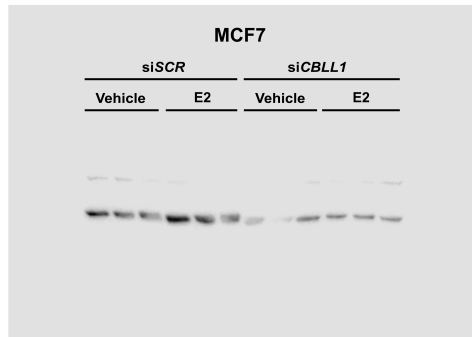
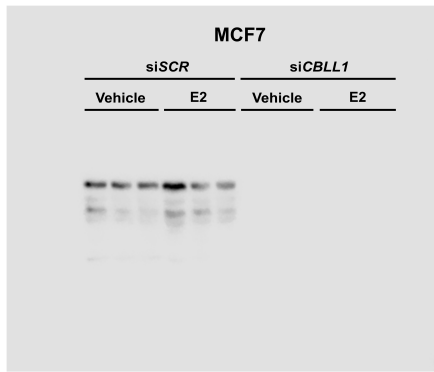
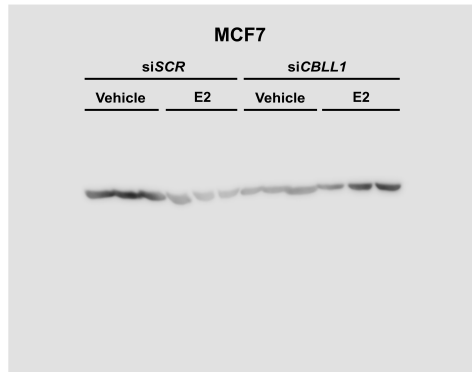
Supplementary Figure 11. Full uncropped annotated western blot images for first independent experiment (all n=3). Expression of (A) METTL3, (C) METTL14, (E) CBLL1 and (G) β -Actin in MDA-MB-231 treated with either siSCR, si*METTL3*, or a combination of si*METTL3* and si*CBLL1* (n=3). Expression of (B) METTL3, (D) METTL14, (F) CBLL1 and (H) β -Actin in MDA-MB-231 treated with either siSCR, si*METTL14*, or a combination of si*METTL14* and si*CBLL1* (n=3).



Supplementary Figure 12. Full uncropped annotated western blot images for second independent experiment (all n=3). Expression of (A) METTL3, (C) METTL14, (E) CBLL1 and (G) β -Actin in MDA-MB-231 treated with either siSCR, si*METTL3*, or a combination of si*METTL3* and si*CBLL1* (n=3). Expression of (B) METTL3, (D) METTL14, (F) CBLL1 and (H) β -Actin in MDA-MB-231 treated with either siSCR, si*METTL14*, or a combination of si*METTL14* and si*CBLL1* (n=3).

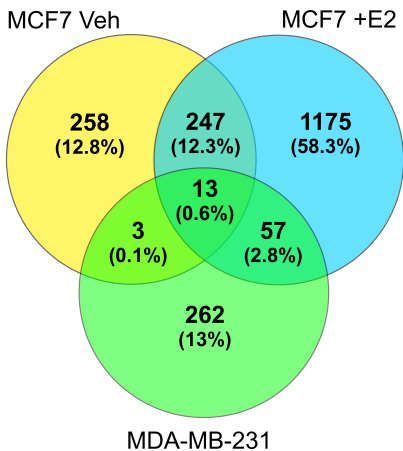
A**B****C****D****E**

Supplementary Figure 13. (A) Expression of METTL3, METTL14, CBLL1 and β -Actin in MCF7 treated with siSCR or siCBLL1 and with DMSO or E2 (n=3). Full uncropped annotated western blot images for first independent experiment (all n=3); expression of (B) METTL3, (C) METTL14, (D) CBLL1 and (E) β -Actin in MCF7 cells treated with siSCR or siCBLL1 and with DMSO or E2.

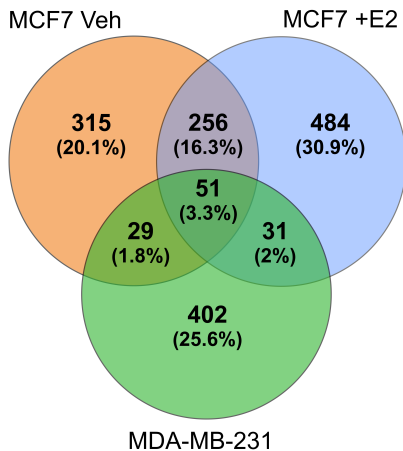
AMETTL3
~64kDa**B**METTL14
~52kDa**C**CBLL1
~55kDa**D** β -Actin
~42kDa

Supplementary Figure 14. Full uncropped annotated western blot images for second independent experiment (all n=3); expression of (A) METTL3, (B) METTL14, (C) CBLL1 and (D) β -Actin in MCF7 treated with siSCR or si*CBLL1* and with DMSO or E2.

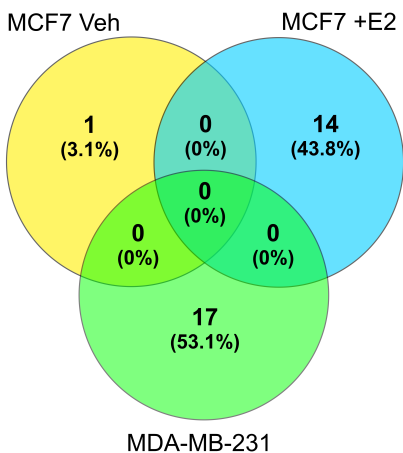
A Up-regulated following siMETTL3



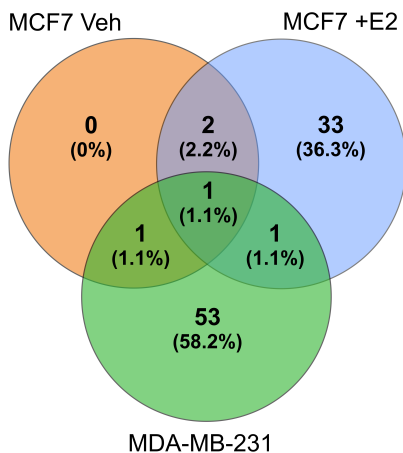
B Down-regulated following siMETTL3



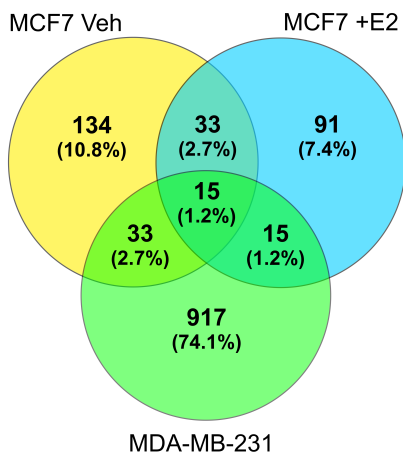
C Up-regulated following siMETTL14



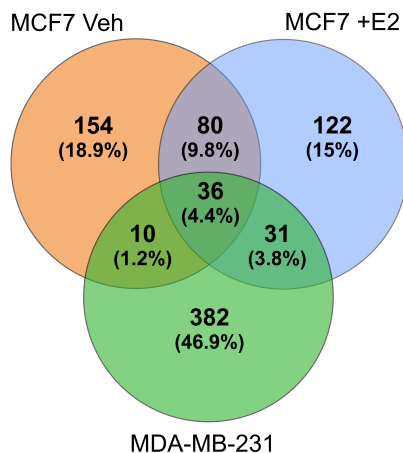
D Down-regulated following siMETTL14



E Up-regulated following siCBLL1



F Down-regulated following siCBLL1

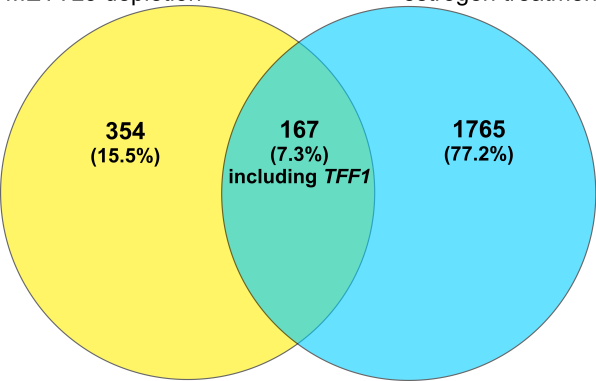


Supplementary Figure 15 Summary of differentially expressed genes in breast cancer cell lines in response to siRNA-mediated METTL3, METTL14 and CBLL1 knockdown

Summary of overlap in DEGs in MCF7 +/-E2, and MDA-MB-231. (A) Upregulated expression following METTL3 depletion, (B) downregulated expression following METTL3 depletion, (C) upregulated expression following METTL14 depletion, (D) downregulated expression following METTL14 depletion, (E) upregulated expression following CBLL1 depletion, (F) downregulated expression following CBLL1 depletion. Significant differential gene expression= $FC \pm 1.5$ and adjusted p-value < 0.05.

Genes upregulated by
METTL3 depletion

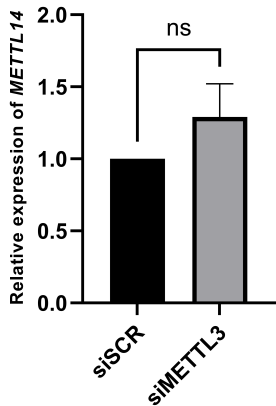
Genes upregulated by
estrogen treatment



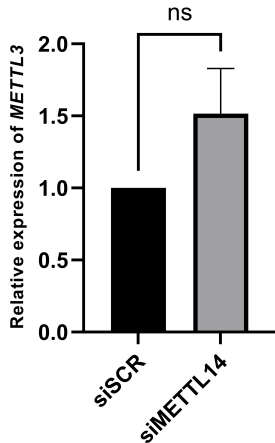
Supplementary Figure 16 METTL3 regulation of estrogen-induced genes in MCF7

Venn diagram of genes significantly upregulated by METTL3 depletion (increased in si*METTL3* compared to siSCR) compared with genes significantly up regulated by estrogen (increased in siSCR +E2 compared to siSCR Vehicle). Significant differential gene expression= $FC \pm 1.5$ and adjusted p-value < 0.05.

A Expression of *METTTL14*/ β -Actin
(n=6)



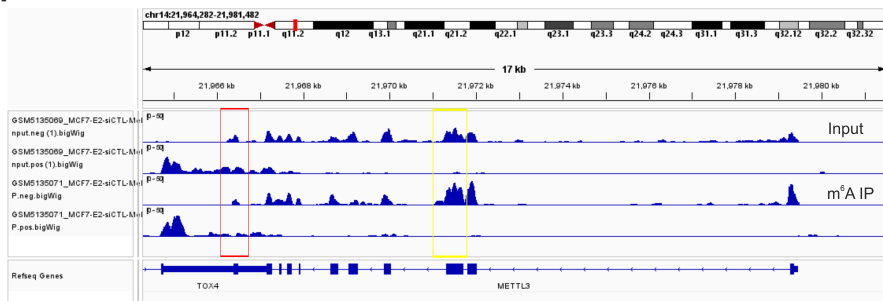
B Expression of *METTTL3*/ β -Actin
(n=6)



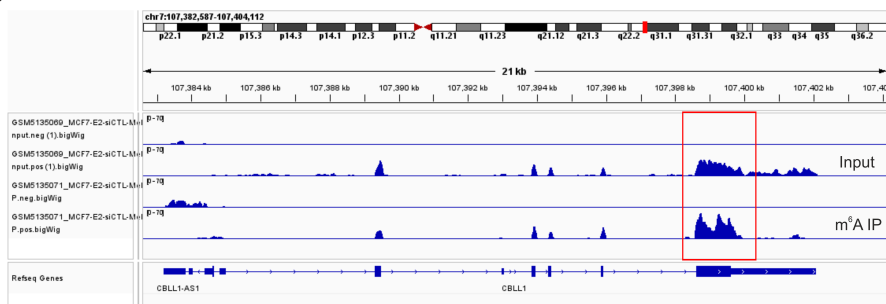
Supplementary Figure 17 Depletion of one catalytic heterodimer protein does not alter the transcript expression of the other MTC protein in MDA-MB-231

qRT-PCR of *METTL3* and *METTL14* expression following siRNA-mediated depletion of *METTL3* and *METTL14*. (A) *METTL14* expression following *METTL3* depletion and (B) *METTL3* expression following *METTL14* depletion in MDA-MB-231. Expression relative to β -Actin. * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.001$, **** = $p < 0.0001$, ns = not significant.

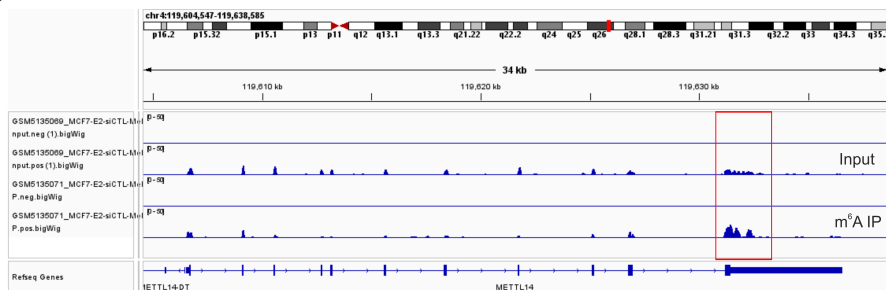
A



B

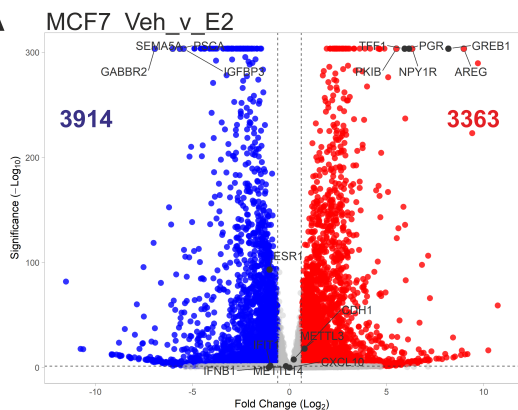
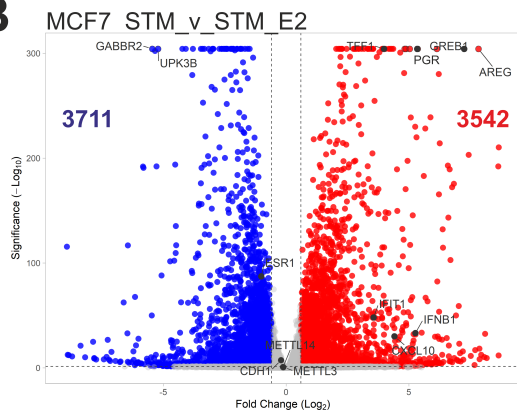
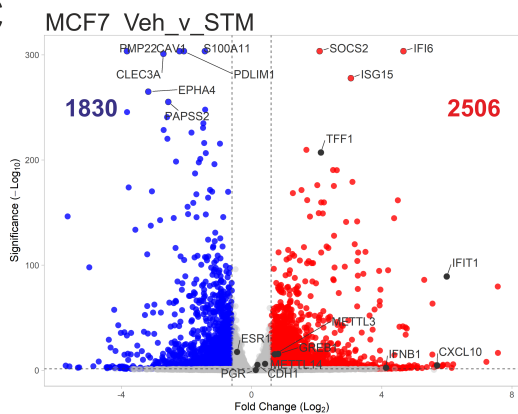
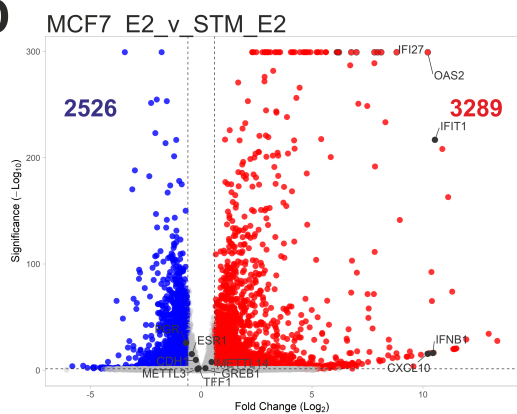
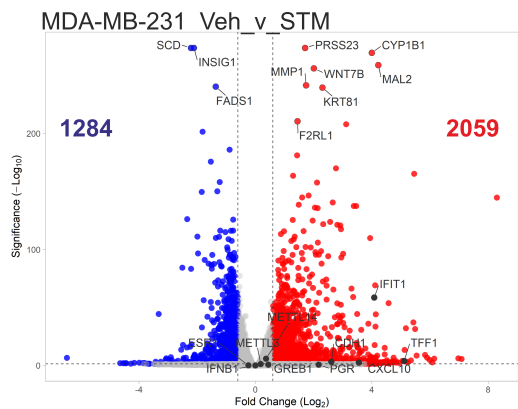


C



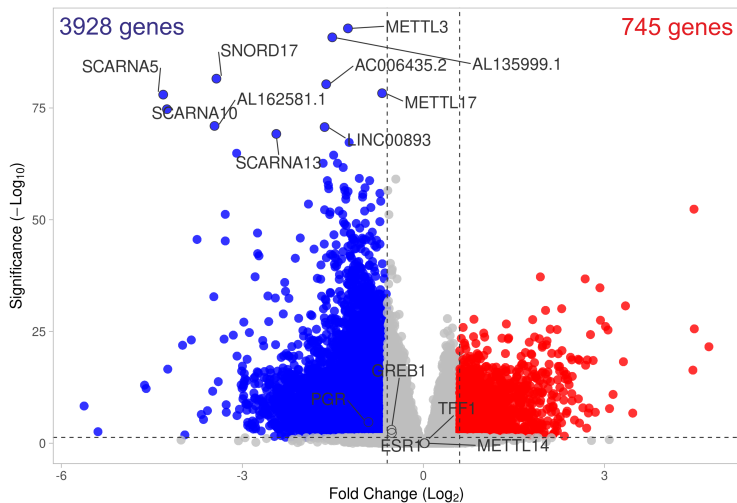
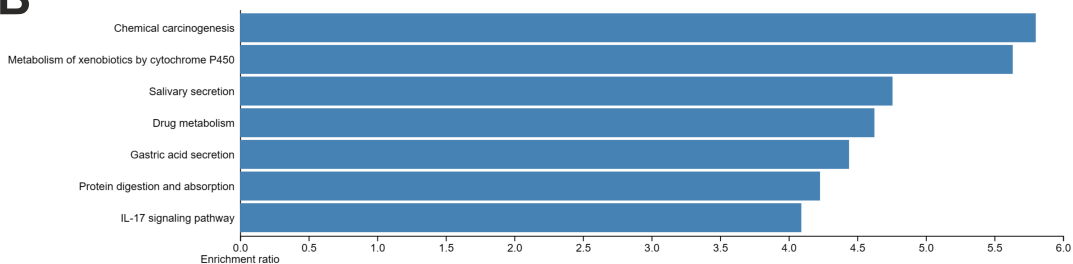
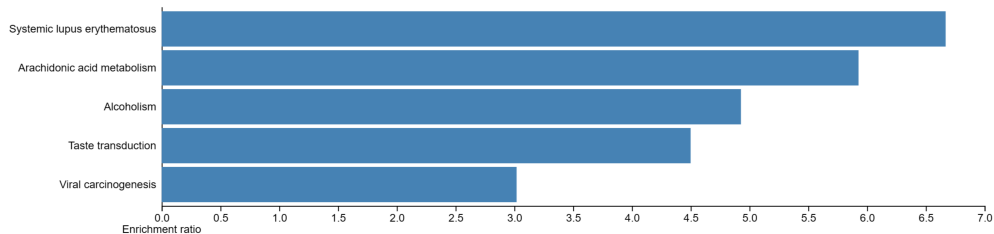
Supplementary Figure 18 Regulation of the m⁶A methylation complex components by m⁶A methylation.

Evidence of the regulation of (A) *METTL3*, (B) *CBLL1* and (C) *METTL14* by m⁶A methylation in MCF7, MeRIP-Seq data obtained from GEO series GSE143441. Peaks in second panel (m⁶A enriched) compared to first panel (no enrichment control) show area of m⁶A enrichment within gene. Red box highlights the last exon of each gene and a yellow box highlights the third exon of *METTL3*.

A**B****C****D****E**

Supplementary Figure 19 Differential gene expression with METTL3 inhibition in cell lines MCF7 and MDA-MB-231

RNA-seq reveals significantly differentially expressed genes with METTL3 inhibition. Volcano plot representing the Log2 fold change of differentially expressed genes upon estrogen treatment and METTL3 inhibition in cell line MCF7; Veh vs E2 (A), STM2457 vs STM2457 E2 (B), Veh vs STM2457 (C), and E2 vs STM2457 E2 (D). Volcano plot representing the Log2 fold change of differentially expressed genes upon METTL3 inhibition in cell line MDA-MB-231; Veh vs STM2457 (E). Genes were considered significant with a fold change (FC) of ≥ 1.5 or ≤ -1.5 and a FDR < 0.05.

A**B****C**

Supplementary Figure 20 Comparison of high and low *METTL3* expression in BCa tumour specimens

Differential gene expression between low and high *METTL3* expression in BCa tumour specimens utilising TCGA data (n=1078). (A) Volcano plot displays DEGs between Q1 and Q4 of *METTL3* expression ($\log_2FC$ of $\geq \pm 1$, $-\log_{10} padj > 1.3$). Enriched KEGG pathways were determined for the significant DEGs that were increased when *METTL3* expression was (B) low (Q1) and (C) high (Q4) (FDR < 0.05).

Supplementary Table 1. Correlations of METTL3, METTL14 and CBLL1 expression with expression of other proteins implicated in breast cancer assessed via IHC in the Nottingham BCa cohort. Statistical p values determined by two-tailed Spearman's correlation and $p \leq 0.05$ highlighted in bold.

Protein		METTL3	METTL14	CBLL1
METTL3	Correlation coefficient		0.748	0.353
	p value		p <0.0001	p <0.0001
	N		652	539
METTL14	Correlation coefficient	0.748		0.361
	p value	p <0.0001		p <0.0001
	N	652		683
CBLL1	Correlation coefficient	0.353	0.361	
	p value	p <0.0001	p <0.0001	
	N	539	683	
CMYC (Cytoplasmic)	Correlation coefficient	0.338	0.33	0.249
	p value	p <0.0001	p <0.0001	p <0.0001
	N	670	753	616
AR	Correlation coefficient	0.283	0.264	0.309
	p value	p <0.0001	p <0.0001	p <0.0001
	N	757	879	687
E-cadherin	Correlation coefficient	0.168	0.199	0.186
	p value	p <0.0001	p <0.0001	p <0.0001
	N	788	910	711
ER alpha	Correlation coefficient	0.299	0.305	0.192
	p value	p <0.0001	p <0.0001	p <0.0001
	N	812	944	745
FOXA1	Correlation coefficient	0.193	0.192	0.241
	p value	p <0.0001	p <0.0001	p <0.0001
	N	546	627	506
BRCA1 (Nuclear)	Correlation coefficient	0.19	0.258	0.169
	p value	p <0.0001	p <0.0001	p <0.0001
	N	673	782	616
BRCA1 (Cytoplasmic)	Correlation coefficient	0.022	0.072	-0.081
	p value	p 0.561	p 0.044	p 0.043
	N	671	780	615
BRCA2 (Nuclear)	Correlation coefficient	-0.002	-0.059	-0.083
	p value	p 0.959	p 0.143	p 0.062
	N	523	607	503
BRCA2 (Cytoplasmic)	Correlation coefficient	0.186	0.198	0.142
	p value	p <0.0001	p <0.0001	p 0.001
	N	524	608	504

Supplementary Table 2. Alternative splicing analysis following RNA-Seq of MCF7 and MDA-MB-231 cells subjected to siRNA-mediated knockdown of METTL3, METTL14 or CBLL1. Differentially spliced transcript and gene numbers are detailed for each alternative splicing event type. (A) Number of significant alternative splicing events compared between MCF7 treated with either siSCR or si*METTL3* and with Veh or E2 (n=3), and between MDA-MB-231 treated with either siSCR or si*METTL3* (n=3). (B) Number of significant alternative splicing events compared between MCF7 cells treated with either siSCR or si*METTL14* and with Veh or E2 (n=3), and between MDA-MB-231 treated with either siSCR or si*METTL14* (n=3). (C) Number of significant alternative splicing events compared between MCF7 cells treated with either siSCR or si*CBLL1* and with Veh or E2 (n=3), and between MDA-MB-231 cells treated with either siSCR or si*CBLL1* (n=3). Significant alternative splice event= dPSI $\geq$ 5% and FDR < 0.05. SE = skipped exon; A5SS = alternative 5' splice site; A3SS = alternative 3' splice site; MXE = mutually exclusive exon; RI = retained intron.

	MCF7				MDA-MB-231
	siSCR Veh vs siSCR +E2	siMETTL3 Veh vs siMETTL3 +E2	siSCR Veh vs siMETTL3 Veh	siSCR +E2 vs siMETTL3 +E2	siSCR vs siMETTL3
SE transcripts	2954	2213	1723	2896	1751
SE genes	1957	1517	1239	1897	1264
A5SS transcripts	605	484	377	736	306
A5SS genes	533	420	338	611	270
A3SS transcripts	622	419	379	602	242
A3SS genes	517	369	325	515	221
MXE transcripts	2702	701	542	3429	600
MXE genes	1620	464	372	1920	414
RI transcripts	514	290	302	623	269
RI genes	457	270	282	533	251

B

	MCF7				MDA-MB-231
	siSCR Veh vs siSCR +E2	siMETTL14 Veh vs siMETTL14 +E2	siSCR Veh vs siMETTL14 Veh	siSCR +E2 vs siMETTL14 +E2	siSCR vs siMETTL14
SE transcripts	2222	2459	1218	1457	1486
SE genes	1505	1667	920	1090	1091
A5SS transcripts	488	504	321	350	304
A5SS genes	423	429	279	315	271
A3SS transcripts	387	429	266	358	233
A3SS genes	340	378	246	321	205
MXE transcripts	707	717	430	614	535
MXE genes	455	472	292	398	403
RI transcripts	256	265	170	179	128
RI genes	246	252	167	170	126

C

	MCF7				MDA-MB-231
	siSCR Veh vs siSCR +E2	siCBLL1 Veh vs siCBLL1 +E2	siSCR Veh vs siCBLL1 Veh	siSCR +E2 vs siCBLL1 +E2	siSCR vs siCBLL1
SE transcripts	2213	3090	1199	1435	2822
SE genes	1467	1850	913	1044	1871
A5SS transcripts	479	507	238	280	577
A5SS genes	414	427	218	250	495
A3SS transcripts	323	470	276	271	489
A3SS genes	285	397	246	237	421
MXE transcripts	914	1033	537	555	761
MXE genes	546	640	370	386	512
RI transcripts	256	386	238	126	820
RI genes	236	344	218	123	694

Supplementary Table 3. Alternative splicing analysis following STM2457 treatment of MCF7 and MDA-MB-231. Differentially spliced transcript and gene numbers are detailed for each alternative splicing event type. (A) Number of significant alternative splicing events compared between MCF7 cells treated with either Vehicle or STM2457 and with Vehicle or E2 (n=3), and between MDA-MB-231 cells treated with either Vehicle or STM2457 (n=3). Significant alternative splice event= dPSI $\geq$ 5% and FDR < 0.05. SE = skipped exon; A5SS = alternative 5' splice site; A3SS = alternative 3' splice site; MXE = mutually exclusive exon; RI = retained intron.

A

	MCF7				MDA-MB-231
	Veh vs +E2	STM2457 Veh vs STM2457 +E2	Veh vs STM2457 Veh	+E2 vs STM2457 +E2	Veh vs STM2457
SE transcripts	3797	5854	3477	5054	2858
SE genes	2249	3033	2055	2748	1718
A5SS transcripts	696	920	714	865	466
A5SS genes	595	751	592	684	421
A3SS transcripts	694	968	722	844	422
A3SS genes	555	754	573	649	373
MXE transcripts	1258	1704	1168	1653	844
MXE genes	734	938	625	853	527
RI transcripts	700	1017	913	1034	459
RI genes	573	825	744	793	405