Supplementary Information
for

Combinatorial therapy using RNAi and curcumin nano-architectures regress tumors in breast and colon cancer models

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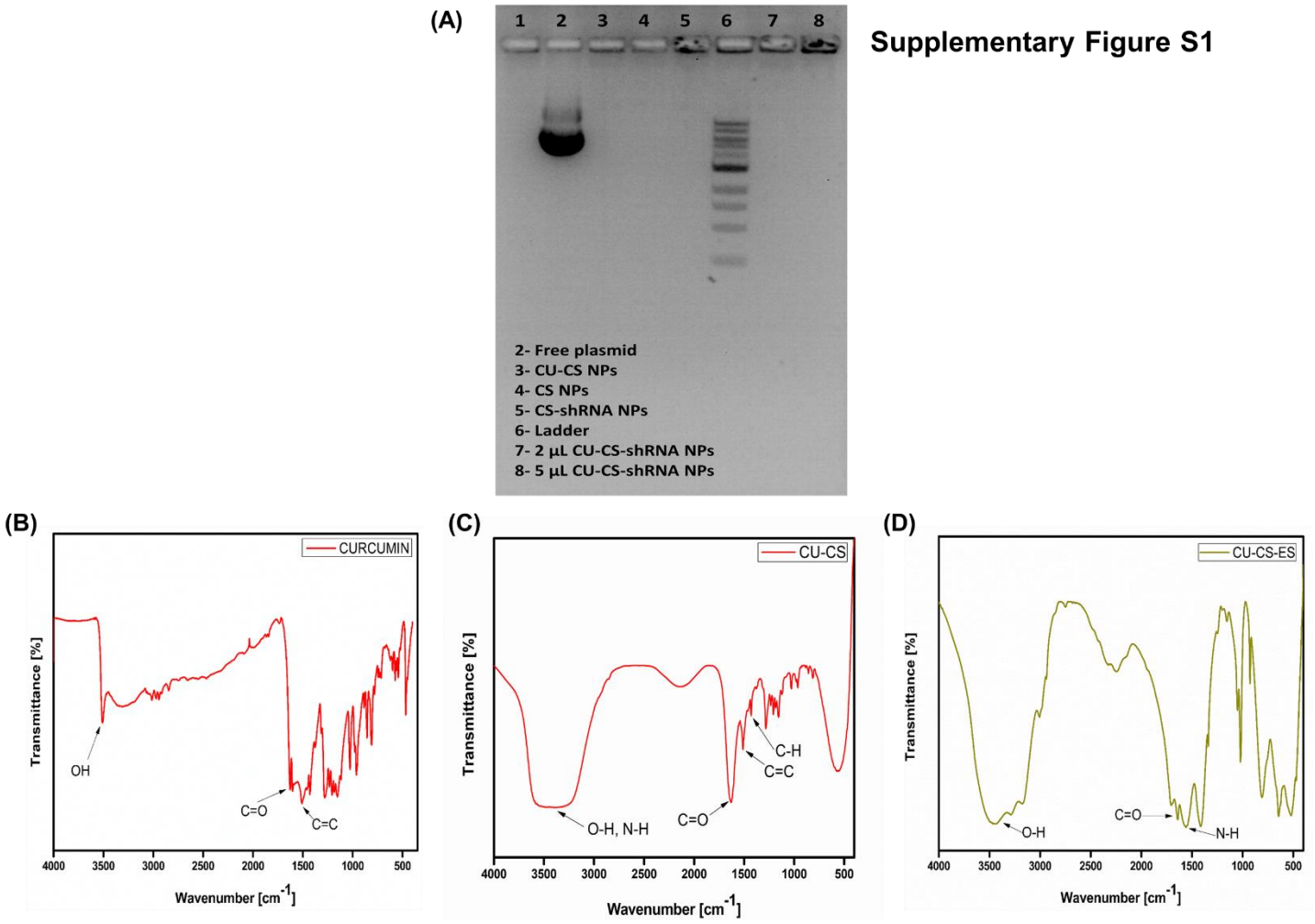
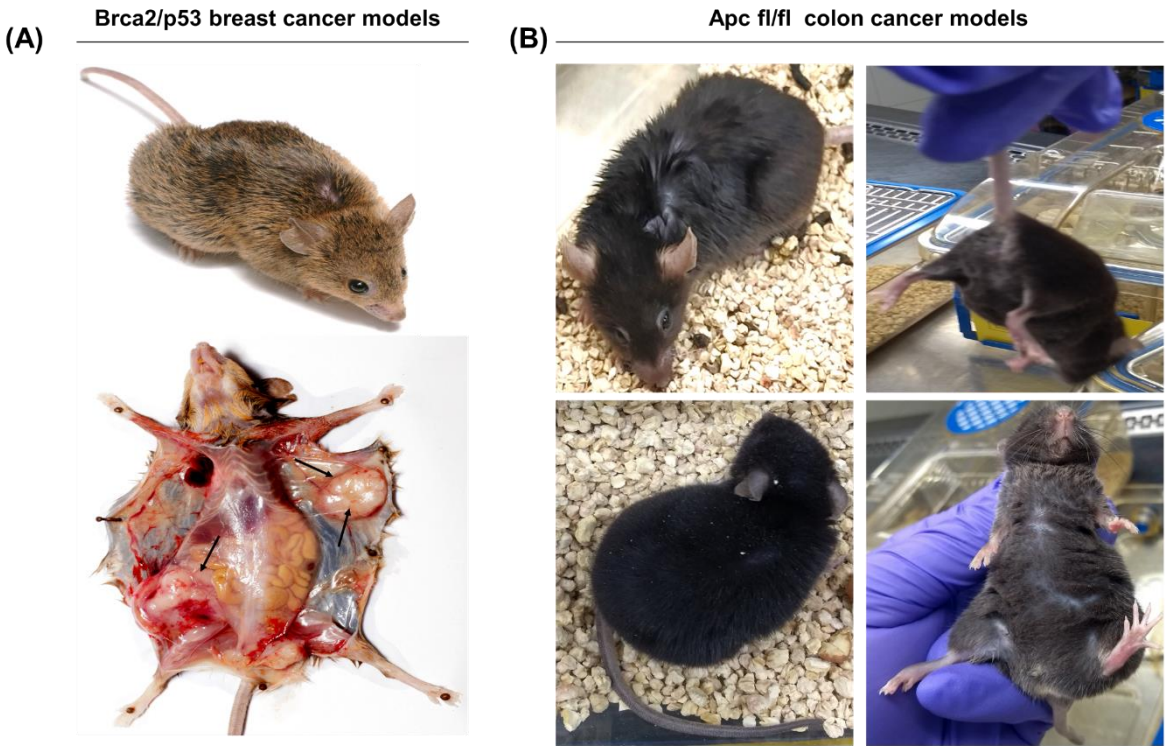
Supplementary Figure S1



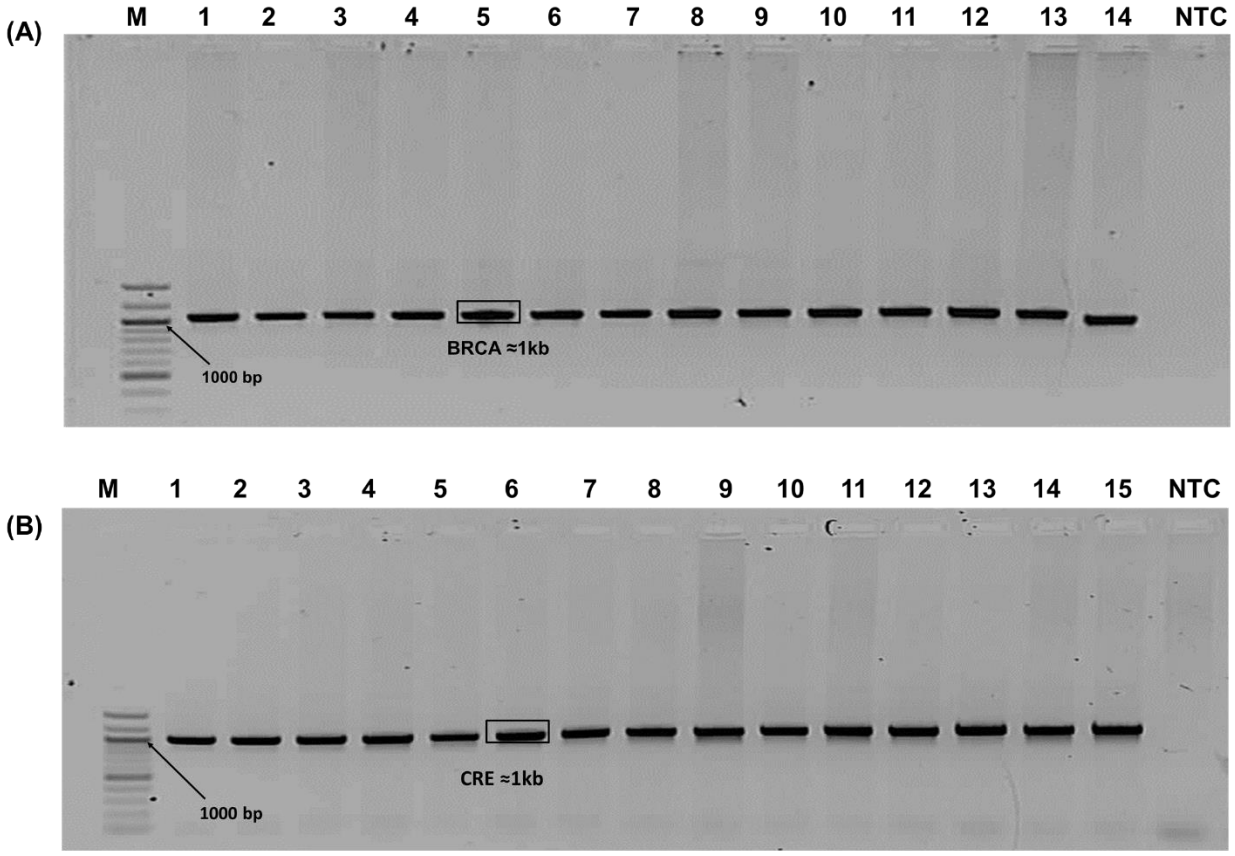
Supplementary Figure S1: Characterization of Nanoparticles (A) Gel retardation assay of bound NPs with shRNA and their respective controls. As free plasmid DNA will move faster in the gel, and the bound plasmid will get stuck in the wells. FTIR spectrum of **(B)** Bare CU, **(C)** CU coated with CS (CU-CS), **(D)** CU coated with CS and ES (CU-CS-ES)

Supplementary Figure S2



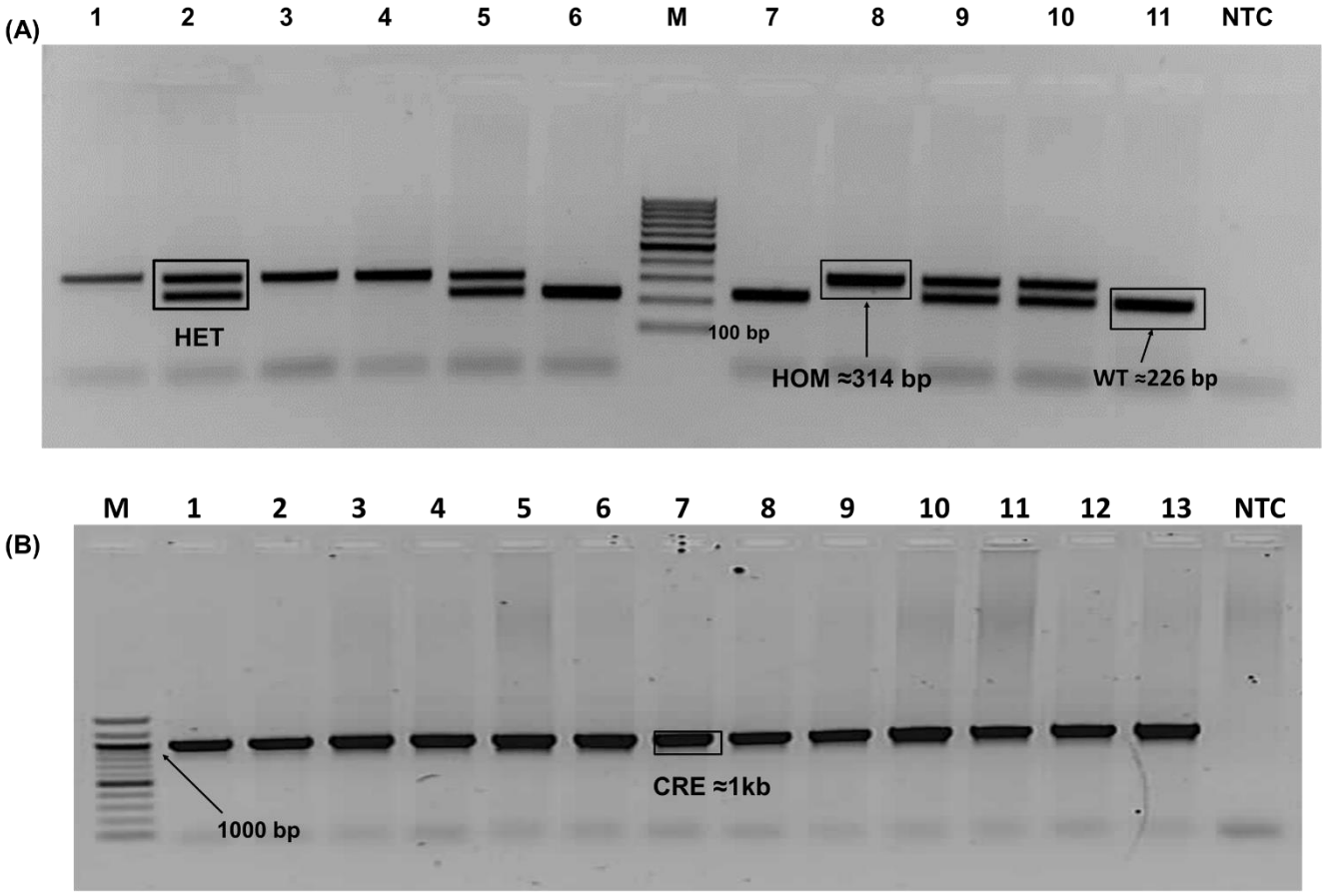
Supplementary Figure S2: Two different murine models used in the study. (A) Brca2/p53 breast cancer models, where the conditional Brca2/p53 knockout are controlled under Blg-cre transgene. This model develops tumors on any of the five pairs of mammary after 6-15 months of birth. (B) Apc fl/fl conditional knockout colon cancer models develops the crypt progenitor phenotype in the intestine upon induction with β-naphthoflavone, and tamoxifen, demonstrating a progressive knockout, causing mortality of the animal within 8-10 days. These mice show knockout phenotypes like piloerection, enlarged abdomen and rectal bleeding.

Supplementary Figure S3



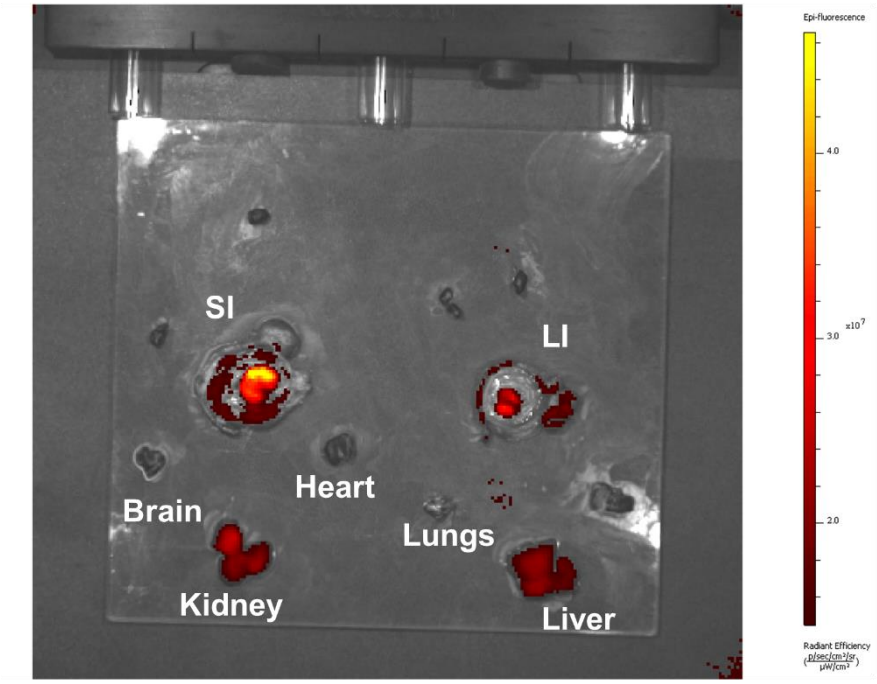
Supplementary Figure S3: PCR products representing genotyping Brca2/p53 mutant models. M represents DNA ladder, NTC represents non template control (without DNA) **(A)** Lanes 1-14 denotes different mice tail samples of Brca2 gene which is approximately 1000 bp. **(B)** Lanes 1-15 denotes different mice tail samples PCR products of Cre gene in Brca2/p53 mutant mice which is approximately 1000 bp.

Supplementary Figure S4



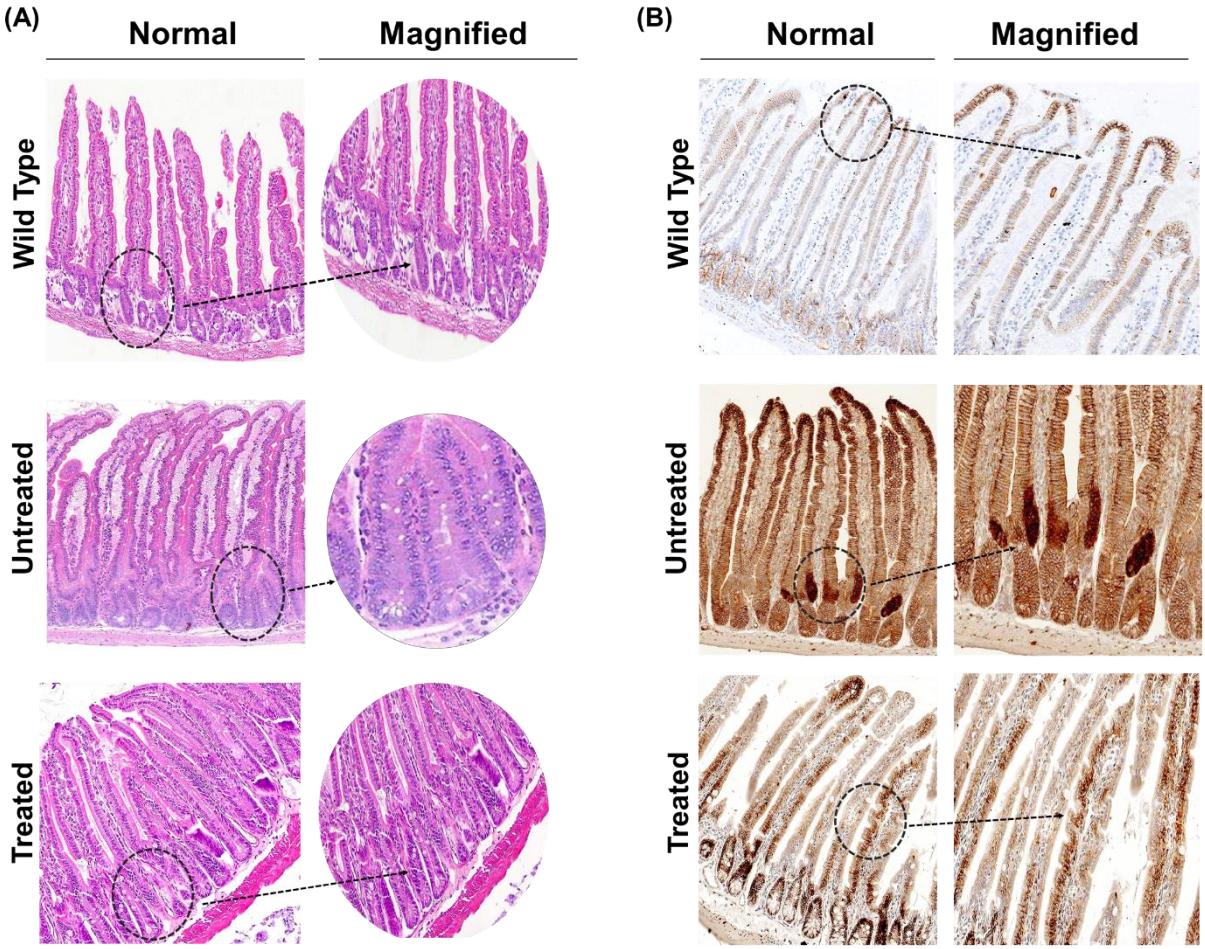
Supplementary Figure S4: PCR products representing genotyping of AhCre-ErT Apcfl/fl colon cancer models. M represents DNA ladder, NTC represents non template control (without DNA) **(A)** Lanes 1-6, 7-11 denotes different mice tail samples. Single band indicates homozygous (HOM) APC flox mice (~314 bp) whereas double band represents heterozygous (HET) APC flox mice and WT represents wild type like mice (~226 bp), **(B)** Lanes 1-13 denotes different mice tail samples PCR products of Cre gene in Apc knockout mice which is approximately 1000 bp.

Supplementary Figure S5



Supplementary Figure S5: Biodistribution of the CU-CS-Ephb4 shRNA NPs conjugated with cyanine 7.5nhs ester far red dye in C57/ BALBc healthy mice after 24 hrs of treatment. Treatment with the bio drug resulted in the localization of the CU-CS-Ephb4 shRNA NPs in the regions of small intestine (SI), large intestine (LI), liver and kidney after 24 hrs of treatment.

Supplementary Figure S6



Supplementary Figure S6: (A) Hematoxylin and Eosin staining of Wild-type, untreated, and treated cohort samples with Ephb4 shRNA bound to CU-CS-ES nanocomposite complex. Treatment cohort exhibited marked difference as compared to untreated sections, where the gut morphology convalesces to a non-Wnt wild type like state. **(B)** Immunohistochemical Analysis of β-catenin in the gut sample, Wild-type, untreated, and treatment cohort with Ephb4 shRNA bound to CU-CS-ES nanocomposite complex. Marked circles denotes that Apc deficient mice without treatment has massive translocation of β-catenin in the nucleus, as compared to the treated sections

Table S1. Primers used for genotyping of mouse models. Forward and Reverse primers used for genotyping ApC $^{fl/fl}$ and Brca2 mice during the breeding program

PCR	**Primer Sequences (5' to 3')**	**Expected Products**
APC $^{fl/fl}$	APC (F) = GTTCTGTATCATGGAAAGATAGGTGGTC APC (R) = CACTCAAAACGCTTTTGAGGGTTGATTC	HOM = 314bp WT = 226bp
Brca2	Brca2 (F) = TTCTTGCTGGTTTTTGTTTTC Brca2 (R) = GCTAAATTTAATTGTTTTACAGCC	Brca2= ~1000bp
CRE	CRE (F) = TGACCGTACACCAAAATTTG CRE (R) = ATTGCCCCTGTTTCACTATC	CRE = ~1000bp

Table S2: Zeta potential of formulations (CU-CS Nanoparticles) from batch 1 to 5

Sr. No.	**Formulation**		**Batch Name**	**Zeta Potential (mV)**	**PDI**
	CU % (w/v)	CS % (w/v)			
1.	1.0	-	1CU	-24.78	0.247
2.	1.0	1.0	1CU-1CS	+51.52	0.172
3.	-	1.0	1CS	+29.77	0.218
4.	1.0	2.0	1CU-2CS	+55.51	0.297
5.	-	2.0	2CS	+33.54	0.282

Table S3. FTIR peaks of pure CU, CS, ES, CU-CS and CU-CS-ES

Samples	**FTIR peaks (cm^{-1})**	**Corresponding structure**
Pure CU	3500	O–H stretching
	1628	C=O stretching
	1509	C=C stretching
	1425	C–H bending
	1280	C–O aromatic stretching
	1026	C–O–C stretching
Pure CS	3450	C–H and N–H stretching
	2961 and 2921	C–H stretching
	1655	C=O stretching
	1590	N–H bending
	1092	C–O stretching
Pure ES	3100 to 3500	O–H stretching
	2998 and 2954	C–H stretching
	1730	C=O stretching
	1450	C–H bending
	1150	C–O–C stretching
CU-CS	3200 to 3600	O–H and N–H stretching
	1635	C=O stretching
	1512	C=C stretching
	1430	C–H bending
	1280	C–O aromatic stretching
	1150 and 1026	C–O–C stretching
CU-CS-ES	3460	O–H stretching
	3285	N–H stretching
	1640 and 1705	C=O stretching
	1560	N–H bending
	1412	C–H bending
	1020 and 1050	C–O–C stretching