Supporting Information

Hydroxyl Groups on Annular Ring-B Dictate the Affinities of Flavonol-CCL2 Chemokine Binding Interactions

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Table S1: Summary of all the possible bonds of mCCL2/hCCL2-kampherol (KP) complexes as obtained from molecular docking. HPI and H-bond represent the hydrophobic interaction, and hydrogen bond respectively.

mCCL2	**Kaempferol (KP)**	**Type of Interaction & Distance**	**hCCL2**	**Kaempferol (KP)**	**Type of Interaction & Distance**
C11 (O)	O4H	H-Bond (2.4 Å)	C11 (O)	O4H	H-Bond (2.9 Å)
Y13 (N)	O2	H-Bond (3.1 Å)	Y13 (HH)	O2	H-Bond (3.3 Å)
Y13 (NH)	O2	H-Bond (2.4 Å)	Y13 (HH)	O3H	H-Bond (3.2 Å)
Y13 (NH)	O3H	H-Bond (3.2 Å)	N14 (Oδ1)	O3H	H-Bond (2.1 Å)
Y13 (NH)	O3	H-Bond (2.4 Å)	N14 (Oδ1)	O3	H-Bond (2.6 Å)
Y13 (NH)	C3	HPI (3.0 Å)	N14 (Cβ)	C6’	HPI (3.9 Å)
S14 (Hγ)	O3H	H-Bond (2.8 Å)	T16 (Oγ1)	O6H	H-Bond (2.2 Å)
S14 (Oγ)	O3	H-Bond (3.0 Å)	T16 (Oγ1)	O6	H-Bond (3.1 Å)
S14 (N)	O3	H-Bond (2.9 Å)	T16 (Hγ)	O6H	H-Bond (2.6 Å)
S14 (C)	C5’	HPI (3.5 Å)	T16 (N)	O6H	H-Bond (3.2 Å)
S14 (O)	O3H	H-Bond (3.0 Å)	T16 (N)	C5’	HPI (3.2 Å)
T16 (N)	C4’	HPI (3.5 Å)	T16 (Cα)	C5’	HPI (3.5 Å)
T16 (N)	O6	H-Bond (3.2 Å)	E50 (O)	O1	H-Bond (3.3 Å)
T16 (NH)	O6	H-Bond (3.2 Å)	E50 (Oη1)	O5	H-Bond (2.8 Å)
T16 (N)	C5’	HPI (2.9 Å)	E50 (Oη1)	O5H	H-Bond (2.2 Å)
E50 (Oη1)	O5H	H-Bond (2.4 Å)	E50 (Cβ)	C8	HPI (3.6 Å)
E50 (Cβ)	C8	HPI (3.8 Å)	C52 (NH)	O1	H-Bond (2.3 Å)
C52 (NH)	O1	H-Bond (2.4 Å)	C52 (N)	O1	H-Bond (3.0 Å)
C52 (N)	O1	H-Bond (3.5 Å)	C52 (Cβ)	C2	HPI (3.6 Å)
C52 (Cβ)	C6’	HPI (3.5 Å)	C52 (Cβ)	C1’	HPI (3.9 Å)

Table S2: Summary of all the possible bonds of mCCL2/hCCL2-quercitin (QT) complexes as obtained from molecular docking. HPI and H-bond represent the hydrophobic interaction, and hydrogen bond respectively.

mCCL2	**Quercetin (QT)**	**Type of Interaction & Distance**	**hCCL2**	**Quercetin (QT)**	**Type of Interaction & Distance**
C11 (O)	O4H	H-Bond (1.7 Å)	C11 (O)	O4H	H-Bond (2.2 Å)
C11 (NH)	O4H	H-Bond (3.5Å)	Y13 (HH)	O2	H-Bond (3.2 Å)
Y13 (NH)	O2	H-Bond (2.9 Å)	Y13 (HH)	O3H	H-Bond (3.7 Å)
Y13 (NH)	O3H	H-Bond (1.7 Å)	N14 (Oδ1)	O3H	H-Bond (2.1 Å)
Y13 (NH)	O3	H-Bond (2.2 Å)	N14 (Oδ1)	O3	H-Bond (2.8 Å)
Y13 (NH)	C3	HPI (3.3 Å)	T16 (Oγ1)	O6H	H-Bond (2.1 Å)
S14 (N)	O3	H-Bond (3.2 Å)	T16 (Oγ1)	O6	H-Bond (3.1 Å)
S14 (NH)	O3	H-Bond (2.2 Å)	T16 (Hγ)	O6H	H-Bond (2.5 Å)
S14 (NH)	O3H	H-Bond (2.2 Å)	T16 (N)	O6H	H-Bond (3.5 Å)
S14 (Hγ)	C6’	HPI (3.8 Å)	T16 (N)	C5’	HPI (3.3 Å)
S14 (Oγ)	O3	H-Bond (3.5 Å)	T16 (Cα)	C5’	HPI (3.5 Å)
T16 (N)	C4’	HPI (3.5 Å)	T16 (Cβ)	C4’	HPI (3.7 Å)
T16 (N)	O6	H-Bond (3.1 Å)	E50 (O)	O1	H-Bond (3.2 Å)
T16 (NH)	O6	H-Bond (3.1 Å)	E50 (Oη1)	O5	H-Bond (2.9 Å)
T16 (N)	C5’	HPI (3.2 Å)	E50 (Oη1)	O5H	H-Bond (2.3 Å)
E50 (Oη1)	O5H	H-Bond (2.1 Å)	E50 (Cβ)	C8	HPI (3.6 Å)
E50 (Cβ)	C2	HPI (3.6 Å)	I51 (CγH)	O7H	H-Bond (3.4 Å)
V51 (CγH)	O7H	H-Bond (2.8 Å)	C52 (NH)	O1	H-Bond (2.3 Å)
C52 (NH)	O1	H-Bond (2.6 Å)	C52 (N)	O1	H-Bond (3.1 Å)
C52 (Cβ)	C6’	HPI (3.2 Å)	C52 (Cβ)	C6’	HPI (3.7 Å)
C52 (C)	C5’	HPI (3.8 Å)	C52 (C)	C5’	HPI (3.9 Å)

Table S3: Summary of all the possible bonds of mCCL2/hCCL2-myricetin (MT) complexes as obtained from molecular docking. HPI and H-bond represent the hydrophobic interaction, and hydrogen bond respectively.

mCCL2	**Myricetin (MT)**	**Type of Interaction & Distance**	**hCCL2**	**Myricetin (MT)**	**Type of Interaction & Distance**
C11 (O)	O4H	H-Bond (2.0 Å)	C11 (O)	O4H	H-Bond (1.9 Å)
C11 (O)	O2	H-Bond (3.4 Å)	C11 (O)	O2	H-Bond (2.6 Å)
C12 (Cβ)	C7	HPI (3.9 Å)	C12 (Cα)	C4	HPI (3.9 Å)
Y13 (NH)	O3H	H-Bond (3.3 Å)	Y13 (HH)	O2	H-Bond (3.0 Å)
Y13 (NH)	O3	H-Bond (3.4 Å)	Y13 (HH)	O3H	H-Bond (3.2 Å)
Y13 (NH)	C3	HPI (3.8 Å)	N14 (Oδ1)	O3H	H-Bond (1.7 Å)
S14 (Oγ)	O8H	H-Bond (1.8 Å)	N14 (Oδ1)	O3	H-Bond (2.5 Å)
S14 (Hγ)	O8	H-Bond (3.1 Å)	N14 (Cβ)	C6’	HPI (3.2 Å)
S14 (C)	C5’	HPI (3.9 Å)	F15 (NH)	O8H	H-Bond (3.3 Å)
F15 (N)	O8H	H-Bond (2.8 Å)	T16 (N)	O7H	H-Bond (3.3 Å)
F15 (NH)	O8H	H-Bond (3.2 Å)	T16 (N)	O6	H-Bond (2.7 Å)
T16 (N)	O8	H-Bond (3.1 Å)	T16 (N)	O6H	H-Bond (2.6 Å)
T16 (NH)	O8	H-Bond (2.9 Å)	T16 (NH)	O6H	H-Bond (2.0 Å)
T16 (NH)	O6H	H-Bond (3.4 Å)	T16 (NH)	O7	H-Bond (2.5 Å)
E50 (Oη1)	O5H	H-Bond (1.7 Å)	E50 (O)	O1	H-Bond (3.8 Å)
E50 (Oη1)	O5	H-Bond (2.6 Å)	E50 (Oη1)	O5	H-Bond (2.7 Å)
E50 (Cβ)	C8	HPI (3.8 Å)	E50 (Oη1)	O5H	H-Bond (2.3 Å)
E50 (O)	O1	H-Bond (2.7 Å)	E50 (Cβ)	C8	HPI (3.9 Å)
V51 (CγH)	O7H	H-Bond (3.2 Å)	I51 (Cγ2)	O7H	HPI (3.9 Å)
C52 (NH)	O1	H-Bond (2.4 Å)	C52 (NH)	O1	H-Bond (3.3 Å)
C52 (N)	O1	H-Bond (3.3 Å)	C52 (Cβ)	C2’	HPI (3.9 Å)
C52 (Cβ)	C6’	HPI (3.7 Å)	C52 (O)	O7H	H-Bond (2.0 Å)

Figure S1: Fluorescence quenching patterns of mCCL2-WT **(A)**, mCCL2-P8A **(B)**, hCCL2-WT **(C)** and hCCL2-P8A **(D)** upon addition of buffer (10 µl and 20 µl). A protein concentration of 25 µM has been used for all the buffer titration experiments.

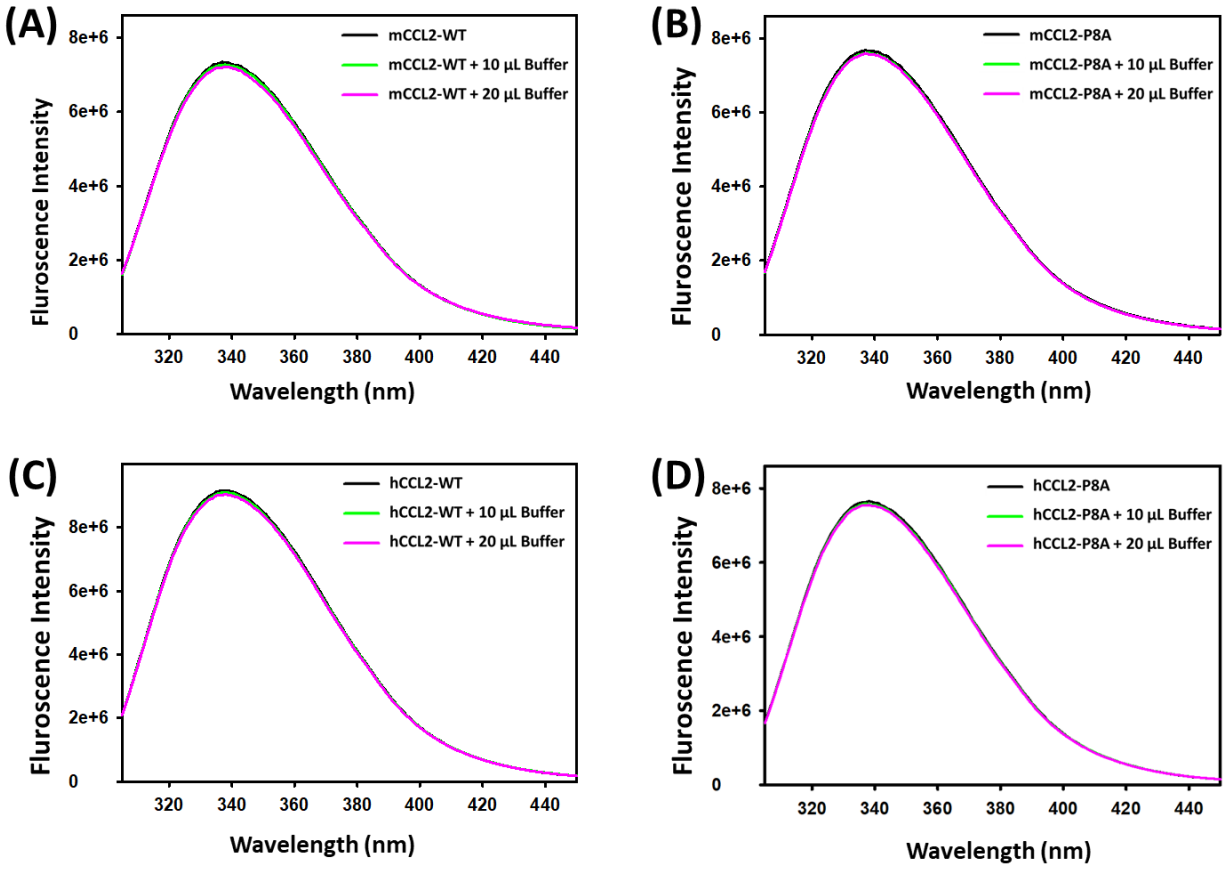

Figure S2: Florescence quenching pattern of CCL2 orthologs: Stern–Volmer plots of mCCL2-WT, mCCL2-P8A, hCCL2-WT and hCCL2-P8A complexed with kaempferol (KP) **(A-D)**; quercetin (QT) **(E-H)**; and myricetin (MT) **(I-L)** respectively.

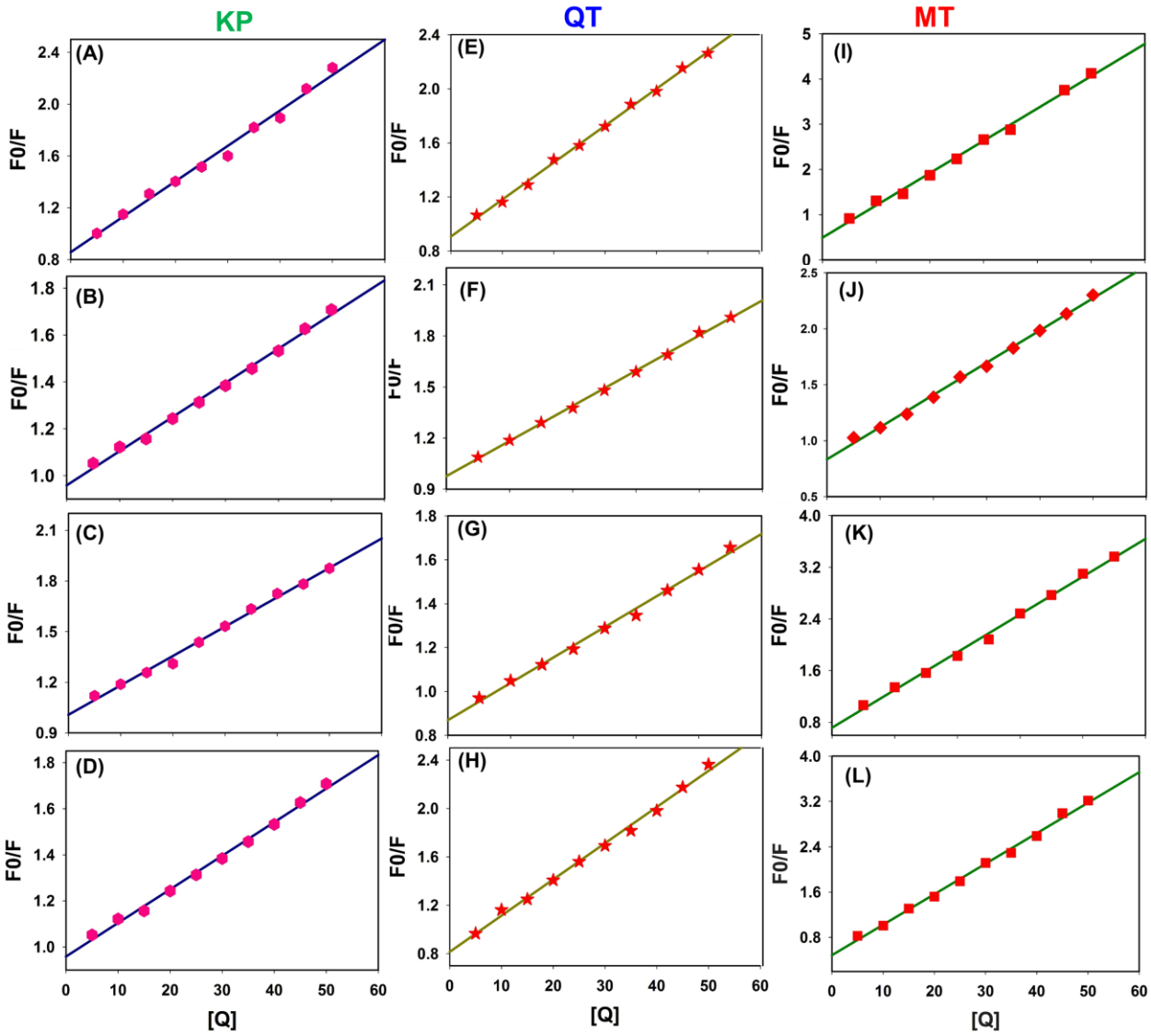

Figure S3: Double-log plots of mCCL2-WT, mCCL2-P8A, hCCL2-WT and hCCL2-P8A complexed with kaempferol (KP) **(A-D)**; quercetin (QT) **(E-H)**; and myricetin (MT) **(I-L)** respectively.

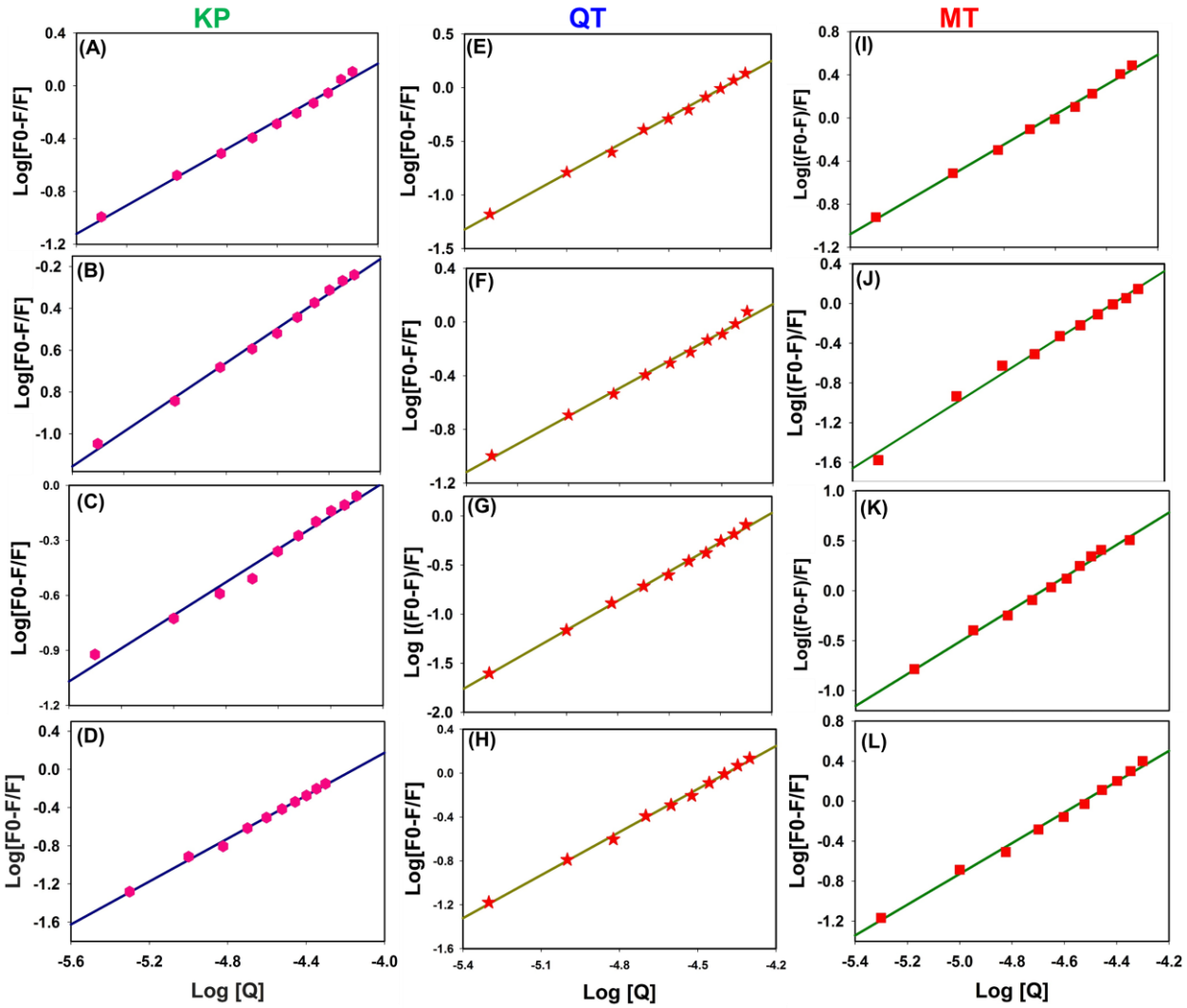

Figure S4: Representative plots showing the **(A)** Non-linear regression analysis, and **(B)** scatchard plot analysis of myricetin (MT) interaction with mCCL2-P8A. The obtained Kd value and number of binding sites (n) are 0.37 ± 0.04 μM and 1.2 ± 0.2 respectively.

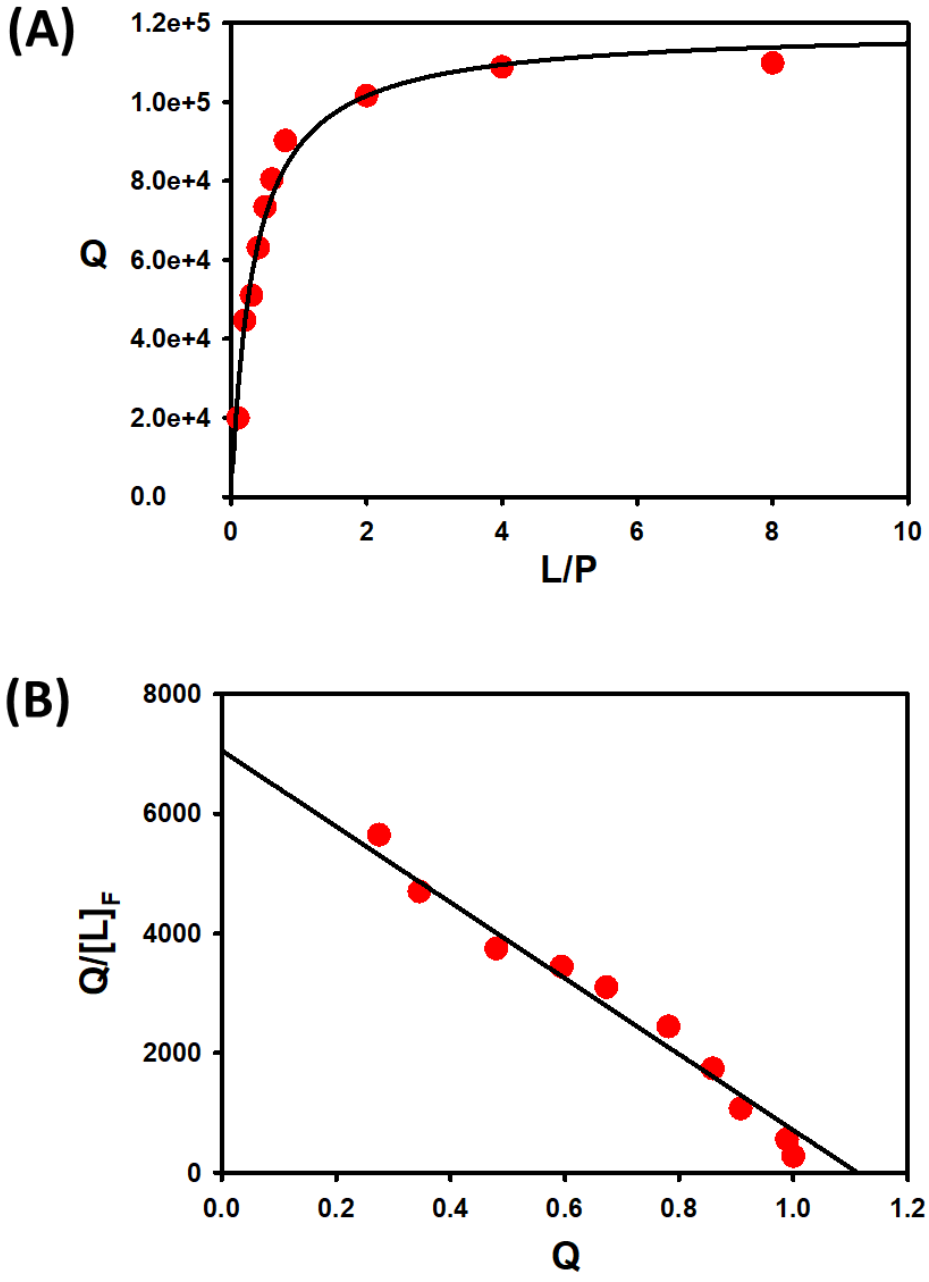

Figure S5: Coulomb- Short Range (Coul-SR) electrostatics of human and murine monomeric CCL2-flavonol (KP/QT/MT)-complex: hCCL2-Kaempferol **(A)**; hCCL2-Myricetin **(B)**; hCCL2-Quercetin **(C)**; mCCL2-Kaempferol **(D)**; mCCL2-Myricetin **(E)**; and mCCL2-Quercetin **(F)** respectively. The red line represents the average Coul-SR interaction energy value.