SUPPORTING INFORMATION

Elucidating the molecular interactions of chemokine CCL2 orthologs with flavonoid baicalin

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Running Title: Interaction of baicalin with CCL2 chemokines

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Table S1: List of primers used for site directed mutagenesis of mCCL2-P8A and hCCL2-P8A mutant proteins.

Name of Mutant	Primer Sequence
mCCL2-P8A	**Fwd: 5'-** GATGCAGTTAACGCCGCACTCACCTGCTGC **-3'** **Rev: 5'-** GCAGCAGGTGAGTGCGGCGTTAACTGCATC **-3'**
hCCL2-P8A	**Fwd: 5'-** GCAATCAATGCCGCAGTCACCTGCTG **-3'** **Rev: 5'-** CAGCAGGTGACTGCGGCATTGATTGC **-3'**

Table S2: Total amount of protein obtained for mCCL2-P8A and hCCL2-P8A after each step of purification.

Purification steps	**mCCL2-P8A**
After 1st Ni-NTA column chromatography	65 ± 5
After Dialysis	50 ± 5
After ion-exchange chromatography	25 ± 3
After reverse Ni NTA chromatography	10± 2
Total mCCL2-P8A protein obtained after final purification	**8± 2 mg/L**

Purification steps	**hCCL2-P8A**
After 1st Ni-NTA column chromatography	60 ± 5
After Dialysis	45 ± 5
After ion-exchange chromatography	18 ± 3
After reverse Ni NTA chromatography	8 ± 2
Total hCCL2-P8A protein obtained after final purification	**6 ± 2 mg/L**

Table S3: Percentage of secondary structural elements in CCL2 wild type and monomeric proteins (for both human and murine orthologs) as estimated using DICHROWEB-K2D software.

Protein	Helix (%)	Sheet (%)	Coil (%)
mCCL2-WT	**17.3**	**14.6**	**68.1**
hCCL2-WT	**17.2**	**14.9**	**67.9**
mCCL2-P8A	**17.9**	**14.6**	**67.5**
hCCL2-P8A	**17.8**	**14.8**	**67.4**

Figure S1: Multiple sequence alignment of CCL2 chemokine from rodent and primate families. The sequence alignment was generated from CLUSTAL W software. The secondary structural elements are shown on the top of the sequences and the presence of βc-stand at C terminal, which is specifically observed in murine family is represented in cyan color.

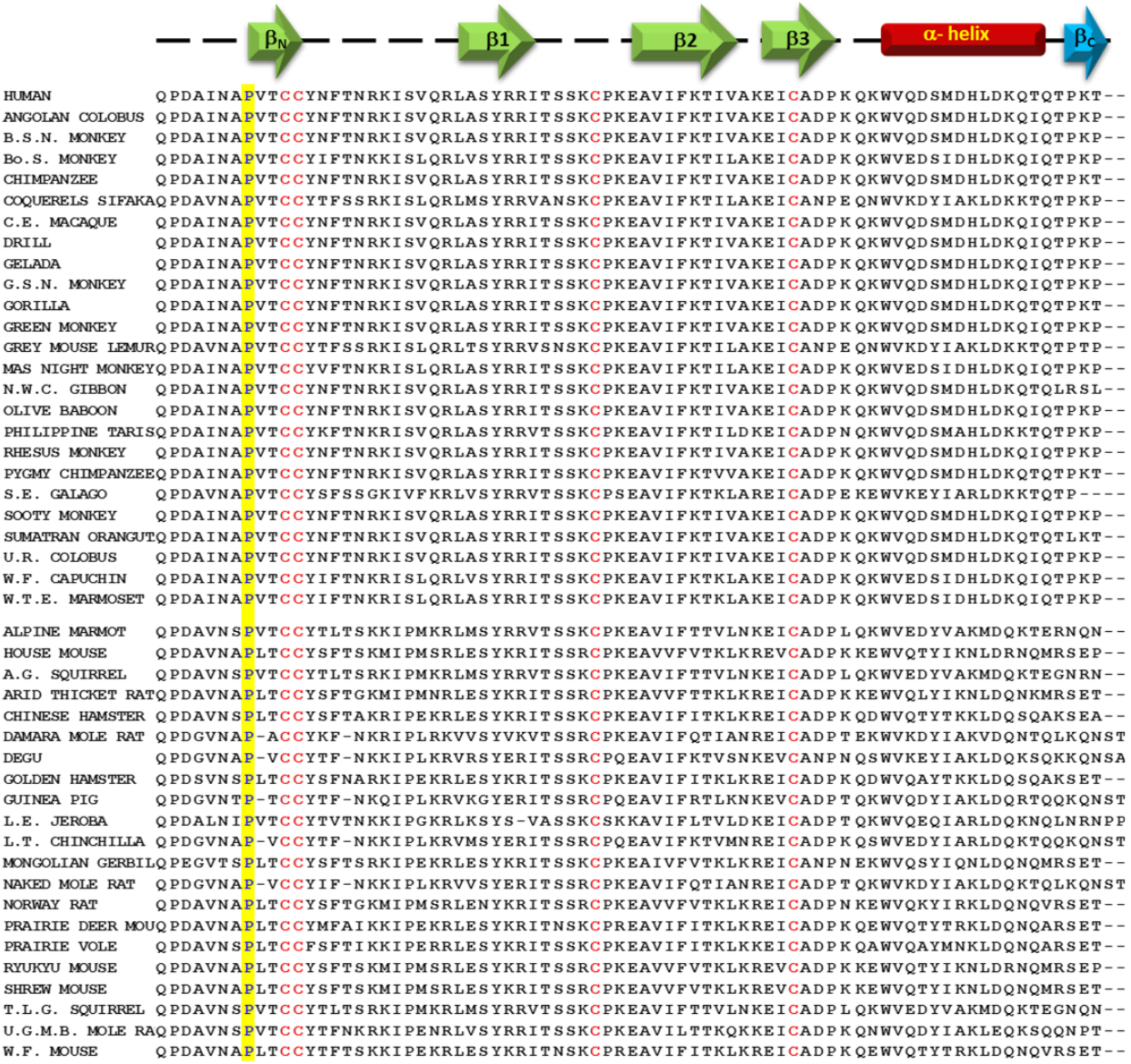

Figure S2: (A) Site directed mutagenesis; Lane M - DNA marker; Lane 2- mCCL2-P8A mutant gene; Lane 3- hCCL2-P8A mutant gene. **(B)** Protein overexpression profile: Lane M- Marker (Bovine serum albumin (BSA)-66 kDa and hen egg lysozyme (HEL)-14 kDa); Lane 2- uninduced sample of mCCL2-P8A protein; Lane 3- induced sample of mCCL2-P8A protein. **(C)** Ni-NTA affinity chromatography profile: Lane M- marker; Lane 2 – supernatant obtained after lysis, Lane 3 – flow through; Lane 4 and 5 - wash I (20 mM imidazole + 20 mM Tris + 500 mM NaCl), wash II (50 mM imidazole + 20 mM Tris + 500 mM NaCl); and Lane 6-9 - Elution fractions (400 mM imidazole + 20 mM Tris + 500 mM NaCl) having purified fusion mCCL2-P8A protein. **(D)** TEV digestion profile: Lane M – marker; Lane 2 - undigested mCCL2- protein; Lane 3 - digested mCCL2-P8A protein containing the left over undigested protein, thioredoxin tag, and mCCL2-P8A protein. **(E)** Ion-exchange chromatography profile for tag (TRX) separation: Lane M-marker; Lane 2- supernatant (collected after TEV digestion); Lane 3- flow through; Lane 4-5 - wash I (50 mM NaCl + 20 mM Tris), wash II (100 mM NaCl + 20 mM Tris); Lane 6-8- Elution fractions (500 mM NaCl + 20 mM Tris) having purified mCCL2-P8A protein. **(F)** Reverse Ni-NTA affinity chromatography profile: Lane M – Marker; Lane 2- pure mCCL2-P8A protein (MW-9 kDa).

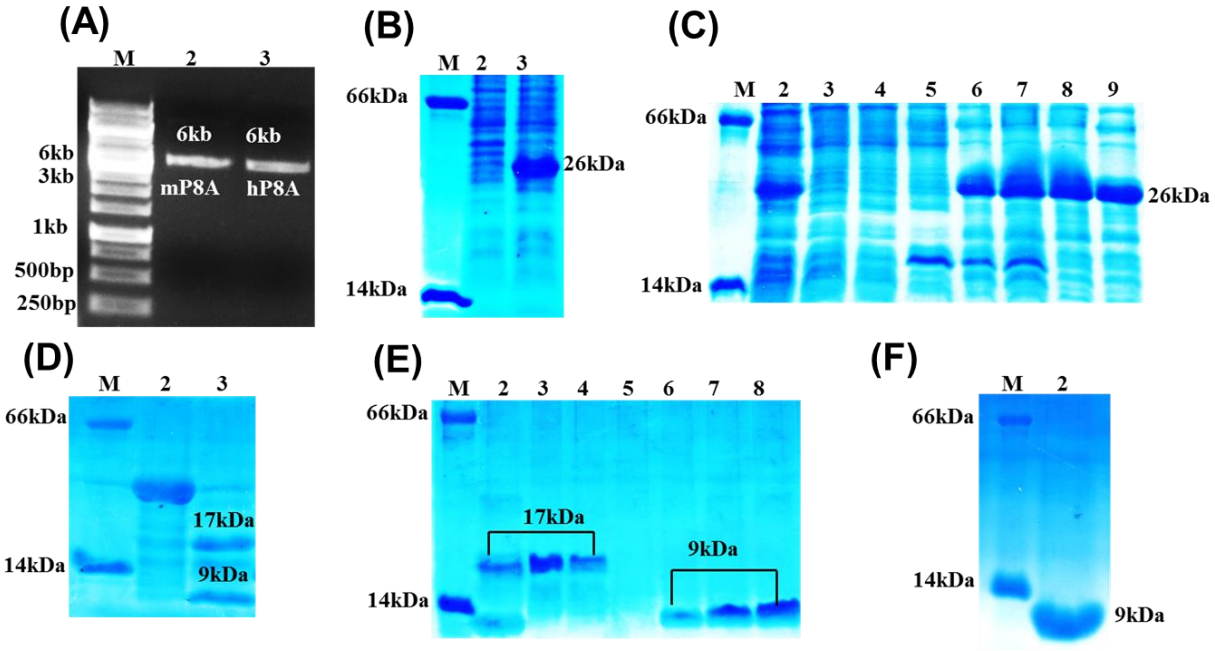

Figure S3: (A) Protein overexpression profile for hCCL2-P8A mutant: Lane M- Marker (Bovine serum albumin (BSA) – 66 kDa and hen egg lysozyme (HEL)-14 kDa); Lane 2- uninduced sample of hCCL2-P8A protein; Lane 3- induced sample of hCCL2-P8A protein. **(B)** Ni-NTA affinity chromatography profile: Lane M- marker; Lane 2 - supernatant obtained after lysis, Lane 3 - flow through; Lane 4 and 5 - wash I (20 mM imidazole + 20 mM Tris + 500 mM NaCl), wash II (50 mM imidazole + 20 mM Tris + 500 mM NaCl); and Lane 6-9 - Elution fractions (400 mM imidazole + 20 mM Tris + 500 mM NaCl) having purified fusion hCCL2-P8A protein. **(C)** TEV digestion profile: Lane M - marker; Lane 2 - undigested monomeric protein; Lane 3 - digested hCCL2-P8A protein containing the left over undigested protein, thioredoxin tag, and hCCL2-P8A protein. **(D)** Ion-exchange chromatography profile for tag (TRX) separation: Lane M- marker; Lane 2- supernatant (collected after TEV digestion); Lane 3- flow through; Lane 4-5 - wash I (50 mM NaCl + 20 mM Tris), wash II (100 mM NaCl + 20 mM Tris); Lane 6-8 - Elution fractions (500 mM NaCl + 20 mM Tris) having purified hCCL2-P8A protein. **(E)** Reverse Ni-NTA affinity chromatography profile: Lane M - Marker; Lane 2 – pure hCCL2-P8A protein (MW-9 kDa).

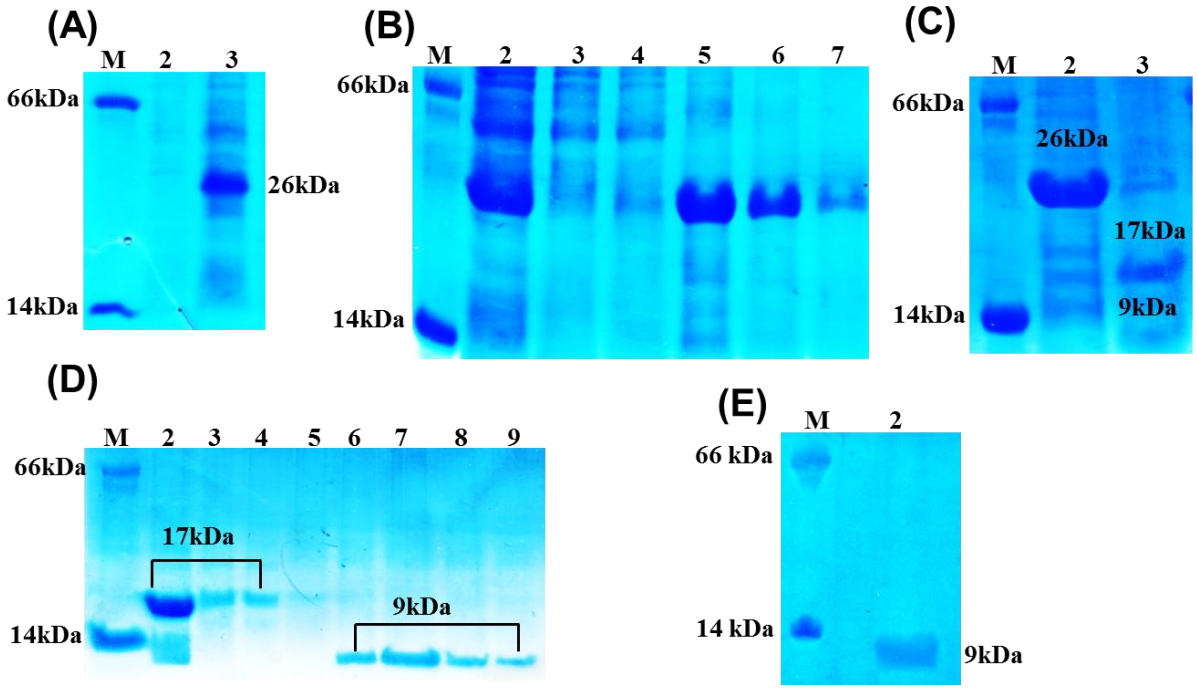

Figure S4: Overlay of tryptophan fluorescence of WT (mCCL2 and hCCL2) and monomeric (mCCL2-P8A and hCCL2-P8A) CCL2 orthologs.

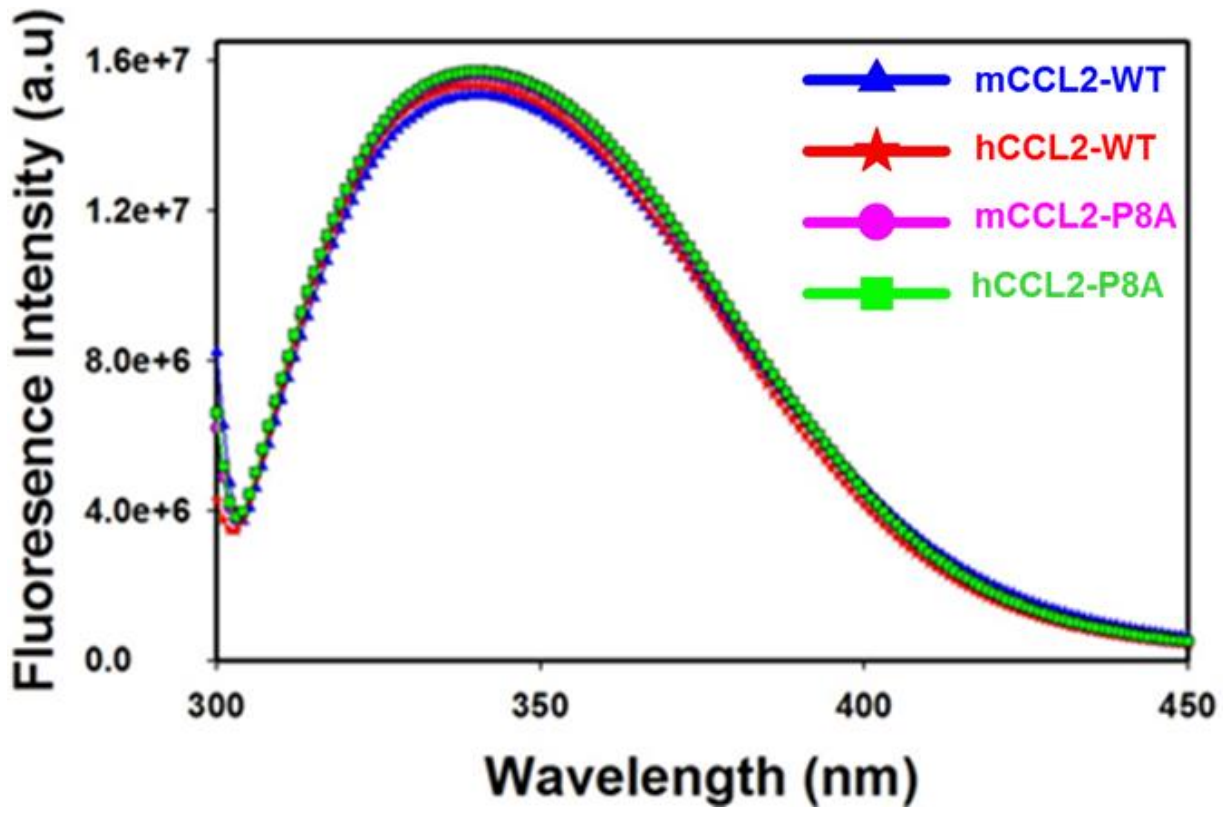

Figure S5: Overlay of tryptophan fluorescence lifetime decay profile of WT (mCCL2 and hCCL2) and monomeric (mCCL2-P8A and hCCL2-P8A) CCL2 orthologs.

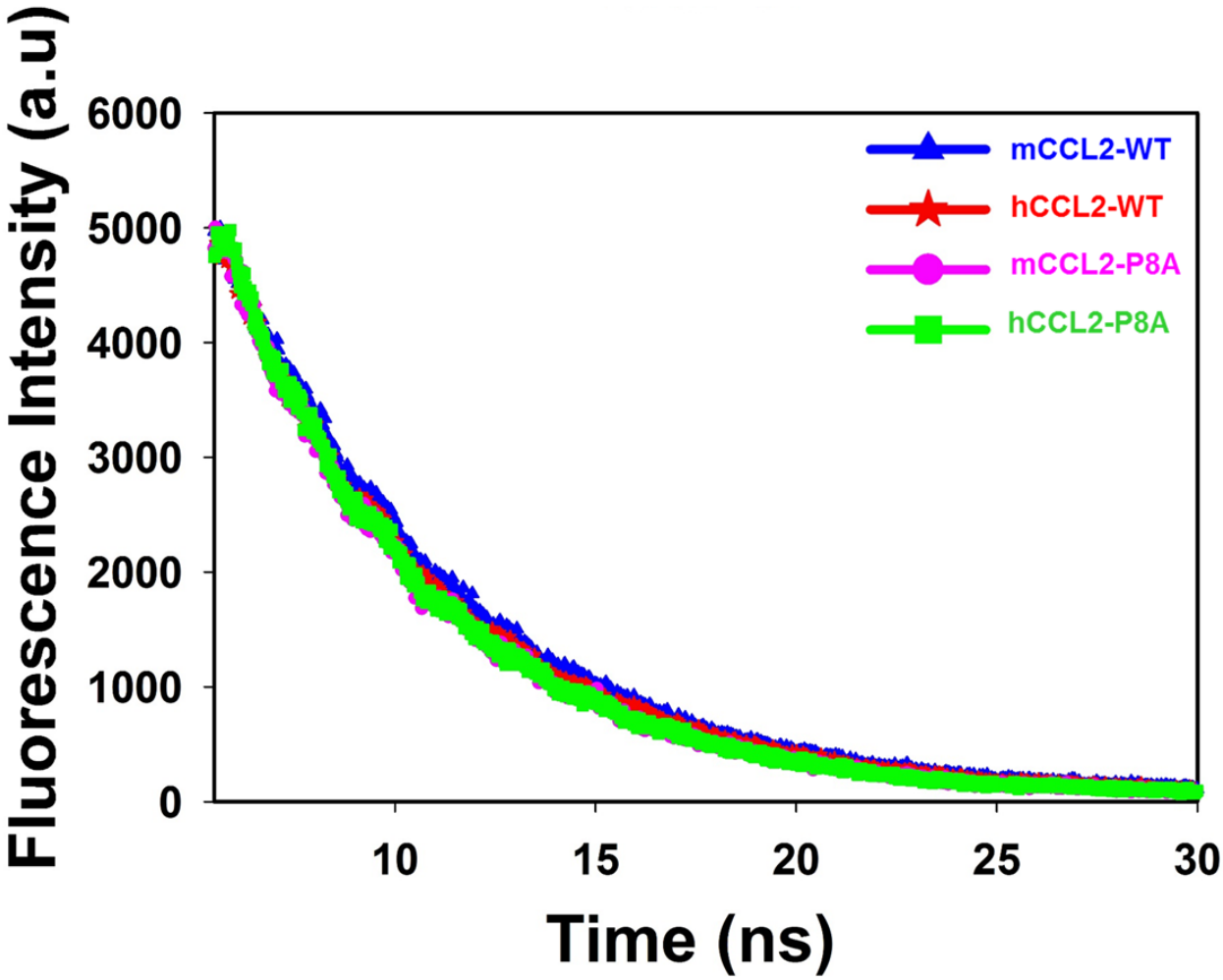

Figure S6: ^{15}N-HSQC overlay of mCCL2-P8A at 500 μM (blue) and 50 μM (red) concentration.