Supplemental Figure S1. Western blot analysis to detect CPA3 protein from canine MCT and human MC lysate (HMCL). Canine muscle tissue was used as a negative control tissue. The tissues were homogenized and probed with the rabbit anti-human CPA3 antibody. The antibody was able to recognize a band of approximately 75 kDa in size from both canine and human MC lysates while no bands were detected in muscle tissue.
Supplemental Figures S2-S6. CPA3 immunoreactivity in selected canine round cell tumors, skin, dog. No CPA3 positive neoplastic cells were observed in cutaneous lymphoma (S2), histiocytoma (S3) or plasmacytoma (S4). Furthermore, no immunoreactivity was detected in oral plasma cell tumor (S5) or in the splenic follicular lymphoma (S6). Few cells outside the tumor mass stain positive in the splenic sample (S6, arrows), but similar cells were positive also with the c-kit antibody and the negative rabbit IgG polyclonal isotype control stain.
Supplemental Figure S7. The unspecific binding of the primary antibody was evaluated using rabbit IgG, polyclonal isotype control, skin, dog. The micrographs show representative positive (a) and negative (b) staining of a high-grade MCT from a female flat-coated retriever. MCs (arrows) in the MCT were stained positively with the rabbit anti-human CPA3 antibody (a), while no MCs were stained using the rabbit IgG, polyclonal isotype control (b). Unspecific intraluminal and intimal staining was observed in the blood vessels (arrowheads) using both the CPA3 antibody and the rabbit IgG, polyclonal isotype control.
Supplemental Table S1. Non-neoplastic tissues for CPA analysis.

| Breed                          | Age (years) | Sex | Weight (kg) | Tissues analyzed |
|-------------------------------|-------------|-----|-------------|-----------------|
| Dog 1 Rottweiler              | 6           | M   | 41          | Skin            |
| Dog 2 Smooth fox terrier      | 4           | F   | 7.3         | Skin            |
| Dog 3* American Cocker Spaniel| 8           | F   | 9.5         | Skin            |
| Dog 4 Finnish Lapphund        | 7           | F,N | 15          | Skin            |
| Dog 5 Spanish Water Dog       | 8           | M,N | 29          | Skin            |
| Dog 6 Labrador retreiver      | 1           | F   | 17          | Sp, Li, SI      |
| Dog 7 French water dog        | 7           | F   | 17          | Sp, Li, SI      |
| Dog 8 Miniature Schnauzer     | 0.2         | F   | 3.8         | Sp, Li, SI      |
| Dog 9 Greyhound               | 2           | F   | 26          | Sp, Li, SI      |
| Dog 10 Bordercollie           | 1           | M   | 21          | Sp, Li          |
| Dog 11 Boxer                  | 1           | F   | 23          | SI              |

Non-neoplastic canine MCs were evaluated for their CPA tissue expression by immunohistochemistry from cutaneous, hepatic, splenic and small intestinal tissues. Sample marked with an asterix denotes for a biopsy sample, other samples were obtained from autopsies. F, female; Li, Liver; M, male; N, neutered; SI, small intestine; Sp, Spleen.
**Supplemental Table S2.** Patient data of dogs with cutaneous low grade or high grade MCTs

|                | Low grade (n=53) | High grade (n=10) |
|----------------|------------------|-------------------|
|                | Gender (n=51)    | Location (n=53)   |
|                |                  |                   |
| Age            | 7.8 y (3 m – 13 y) | 10.9 y (6 – 13.1 y) |
| Weight         | 27.8 kg (6.6–51.1 kg) | 19.4 kg (8.3–37.6 kg) |
| Sex Male       |                  |                   |
|                | 16 (31)          | 1 (10)            |
|                | 6 (12)           | 4 (40)            |
|                | 12 (24)          | 3 (30)            |
|                | 17 (33)          | 2 (20)            |
| Total*         | 51 (100)         | 10 (100)          |
| Location       |                  |                   |
| Limb           | 14 (29)          | 4 (40)            |
| Flank          | 10 (20)          | 1 (10)            |
| Head/neck      | 11 (22)          | 3 (30)            |
| Abdominal skin | 7 (14)           | 1 (10)            |
| Tail/perineal skin | 7 (14)    | 1 (10)            |
| Total†         | 49 (100)         | 10 (100)          |

*Gender was not recorded for 2 and †location for 4 low grade MCTs.
### Supplemental Table S3. Breeds of the sample population.

| Breed                                | n  | %    |
|--------------------------------------|----|------|
| Golden Retriever                     | 6  | 10.9 |
| Boxer                                | 5  | 9.1  |
| Labrador Retriever                   | 5  | 9.1  |
| Boston Terrier                       | 3  | 5.5  |
| Dogo Argentino                       | 3  | 5.5  |
| Mixed breed                          | 3  | 5.5  |
| Australian Terrier                   | 2  | 3.6  |
| Irish Terrier                        | 2  | 3.6  |
| Fox Terrier                          | 2  | 3.6  |
| Nova Scotia Duck Tolling Retriever   | 2  | 3.6  |
| French Bulldog                       | 2  | 3.6  |
| German Shepherd                      | 2  | 3.6  |
| Flat-coated Retriever                | 2  | 3.6  |
| Staffordshire Bullterrier            | 2  | 3.6  |
| American Cocker Spaniel              | 1  | 1.8  |
| Bernese Mountain Dog                 | 1  | 1.8  |
| Brasilian Terrier                    | 1  | 1.8  |
| Doberman                              | 1  | 1.8  |
| Spanish Water Dog                    | 1  | 1.8  |
| Jack Russell Terrier                 | 1  | 1.8  |
| Miniature Pinscher                   | 1  | 1.8  |
| Pug                                  | 1  | 1.8  |
| Parson Russell Terrier               | 1  | 1.8  |
| Petit Brabancon                      | 1  | 1.8  |
| Rhodesian Ridgeback                  | 1  | 1.8  |
| Shetland Sheepdog                    | 1  | 1.8  |
| Finnish Lapphund                     | 1  | 1.8  |
| Whippet                              | 1  | 1.8  |
| **Total**                            | 55 | 100  |

63 MCT samples from 56 dogs were included in this study. The breed of one of the dogs was unknown.
Supplemental Data 1. Nucleotide alignment of human and canine CPA3 sequences generated with the BLAST tool.

Query: Homo sapiens carboxypeptidase A3 (CPA3), mRNA
Query ID: NM_001870.4 Length: 1762

Sbjct: Canis lupus familiaris carboxypeptidase A3 (CPA3), mRNA
Sequence ID: XM_038571158.1 Length: 1693
Range 1: 64 to 679

Score: 1903 bits (1030), Expect: 0.0
Identities: 1442/1639 (88%), Gaps: 36/1639 (2%), Strand: Plus/Plus

Query 1     CAAAGAAGAACCATGAGGCTCATCCTGCCTGTGGGTTTGATTGCTACCACTCTTGCAATT  60
Sbjct  64 CAAAGAAGAACCATGTTGGTTCATCCTGCCTGTGGGTCTGATCGCTACCACCGGCAATT  123

Query 61    GCTCCTGTCGCCCTTGAAGGAGGAGAAGGTATTCCGCGTGAAGCCCCAGGATGAAAAACAA  120
Sbjct  124 GCTCCTGTCGCCCTTGAAGGAGGAGAAGGTATTCCGCGTGAAGCCCCAGGATGAAAAACAA  183

Query 181   ACCCACCACGTTAGCTGCTATATGAGTGGTGGATTTCCGAGTTAGTGAAAAGGAATCCCAG  180
Sbjct  244 ACCCACCACGTTAGCTGCTATATGAGTGGTGGATTTCCGAGTTAGTGAAAAGGAATCCCAG  243

Query 241   GCCATCCAGTCTGCCTTGGATCAAAATAAAATGCACTATGAAATCTTGATTCATGATCTA  240
Sbjct  304 GCCATCCAGTCTGCCTTGGATCAAAATAAAATGCACTATGAAATCTTGATTCATGATCTA  303

Query 301   CAAGAAGAAGATTGAGAAACAGTTTGATGTTAAAGAAGATATCCCAGGCAGGCACAGCTAC  360
Sbjct  364 CAAGAAGAAGATTGAGAAACAGTTTGATGTTAAAGAAGATATCCCAGGCAGGCACAGCTAC  363

Query 361   GCACGAGAATGGGTCTCCCCAGCATCTGCCAGTGGTTTGTCTATCGCACTGACCTCAACAGGAATTTTAATGCT  360
Sbjct  424 GCACGAGAATGGGTCTCCCCAGCATCTGCCAGTGGTTTGTCTATCGCACTGACCTCAACAGGAATTTTAATGCT  423

Query 421   CGTTCCAAGAACCAAAACTCCAAATGCATTGGCACTGACCTCAACAGGAACTTTAATGCT  480
Sbjct  484 CGTTCCAAGAACCAAAACTCCAAATGCATTGGCACTGACCTCAACAGGAACTTTAATGCT  483

Query 481   CGTTCCAAGAACCAAAACTCCAAATGCATTGGCACTGACCTCAACAGGAACTTTAATGCT  543
Sbjct  544 CGTTCCAAGAACCAAAACTCCAAATGCATTGGCACTGACCTCAACAGGAACTTTAATGCT  546

Query 541   GCACGAGAATGGGTCTCCCCAGCATCTGCCAGTGGTTTGTCTATCGCACTGACCTCAACAGGAATTTTAATGCT  540
Sbjct  604 GCACGAGAATGGGTCTCCCCAGCATCTGCCAGTGGTTTGTCTATCGCACTGACCTCAACAGGAATTTTAATGCT  603

Query 601   TGTTGAGAAGAAAAATAGTTAGCAAAACTCTTGGACCGAATGAGATTATTATTATACCTTCTCC  600
Sbjct  664 TGTTGAGAAGAAAAATAGTTAGCAAAACTCTTGGACCGAATGAGATTATTATTATACCTTCTCC  663

Query 661   GTGTTCACCTGTGTGATGATATTTTGTGTCTATCGCACTGACCTCAACAGGAATTTTAATGCT  659
Sbjct  724 GTATTCACAATGTGTGATGATATTTTGTGTCTATCGCACTGACCTCAACAGGAATTTTAATGCT  723

Query 721   CGTTCCAAGAACCAAAACTCCAAATGCATTGGCACTGACCTCAACAGGAACTTTAATGCT  780
Sbjct  784 CGTTCCAAGAACCAAAACTCCAAATGCATTGGCACTGACCTCAACAGGAACTTTAATGCT  783

Query 781   TCATGGAATCTCATCCTAAACCAATGACCCATACTGCGAGAATAACTATCGGGCCTCTGCA  840
**Supplemental Data 2.** Amino acid alignment of human and canine CPA3 sequences generated with the BLAST tool\(^1\). CPA3 antibody target epitope is denoted with red.

**Query:** mast cell carboxypeptidase A preproprotein [Homo sapiens] Query ID: NP_001861.2 Length: 417

**Sbjct:** mast cell carboxypeptidase A [Canis lupus familiaris] Sequence ID: XP_038427086.1 Length: 417 Range: 1 to 417

Score:782 bits(2019), Expect:0.0, Method:Compositional matrix adjust., Identities:366/417(88%), Positives:392/417(94%), Gaps:0/417(0%)

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**Query 1**

```
MRLILPVGLIATLAIAPVRDFREKVFRKVQDEKQADIIKDLAKTNELDFWYPGATHHV  60
```

**Sbjct 1**

```
MWFILPVGLIATLAIAPVRDFREKVFRKVQDEKQANIIKDLAKTNQLDFWYPDATHHV  60
```

**Query 61**

```
AANMMDVFRSVRSQAIQALDQNNMHEILILHQEIKEQFDVKEDIPGRHGYAKYN  120
```

**Sbjct 61**

```
TANMTDQVSREKESSIQALDQNNMHEILILHQEIKEQFDVKEDIPGRHGYAKYN  120
```

**Query 121**

```
NWEKIVAWTEKMMDKYPEMVSRIKIGSTVEDNFLYVKIFKEKnERRRHAIFDCGIHAREW  180
```

**Sbjct 121**

```
NWDKIVAWTEKMMDKYPEMVSRIKIGSTVEDNFLYVKIFKEKnERRRHAIFDCGIHAREW  180
```

**Query 181**

```
QNSKCIGTDLNRNFNASWNS+PCA+YRGPKPESEKETKVATFIRSLNKIY  300
```

**Sbjct 181**

```
QNSKCIGTDLNRNFNASWNS+PCA+YRGPKPESEKETKVATFIRSLNKIY  300
```

**Query 301**

```
ITFHSYQMLLFPPYGYTSKLPNHEDLAIVKAVIGTDVLS4TRYETRYIYGPESIYIPISG  360
```

**Sbjct 301**

```
ITFHSYQMLLFPPYGYTSKLPNHEDLAIVKAVIGTDVLS4TRYETRYIYGPESIYIPISG  360
```

**Query 361**

```
SSLDAYDLGKHTFAPFAELRDKGFGFLPESKRIKPTCREMTLMAVKFIAYIKHTK  417
```

**Sbjct 361**

```
SSLDAYDLGKHTFAPFAELRDKGFGFLPESKRIKPTCREMTLMAVKFIAYIKHTK  417
```

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**Reference:**

1. Madden T. The BLAST Sequence Analysis Tool. 2003 Aug 13