*SUPPLEMENTARY FIGURES*

# Phenotypic and transcriptomic characterization of canine myeloid-derived suppressor cells

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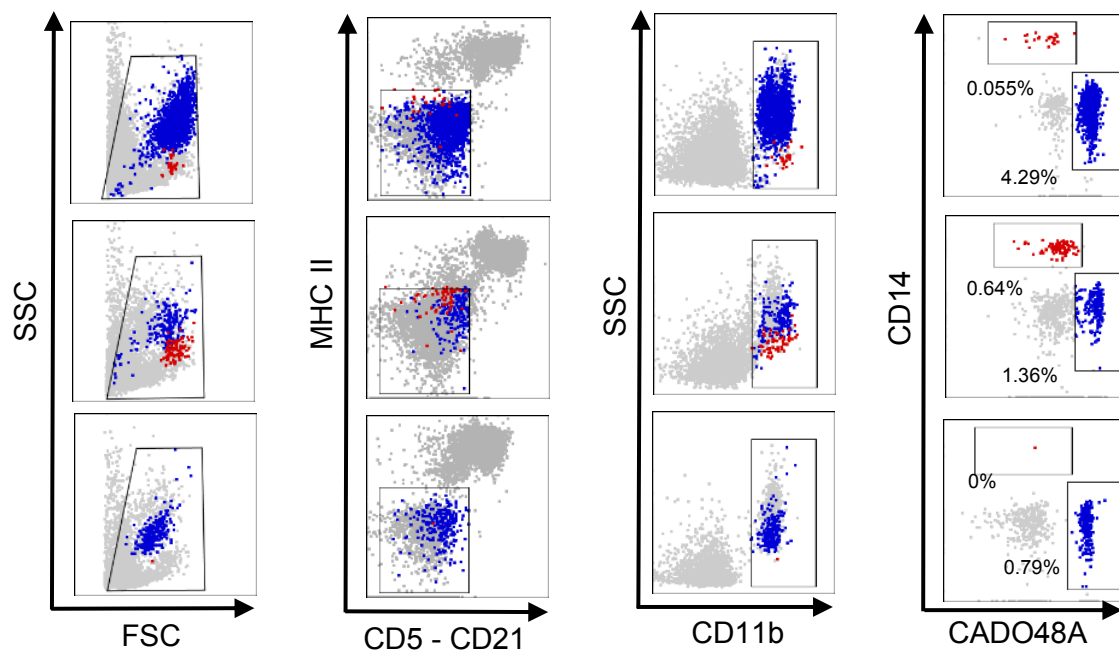
[¶]These authors contributed equally to this work

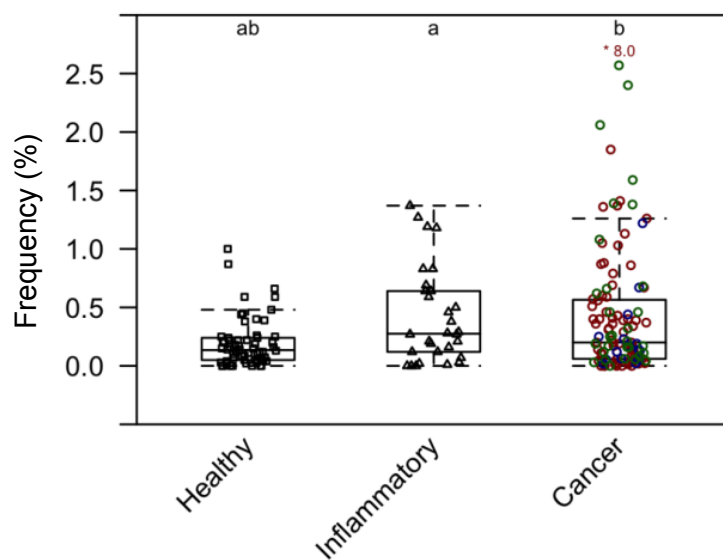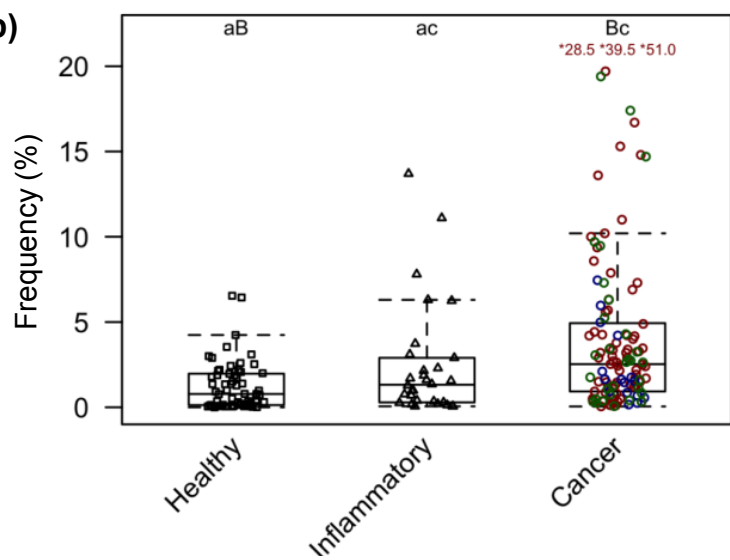[ξ]Current address: Barts Cancer Institute, Queen Mary University of London, London, UK

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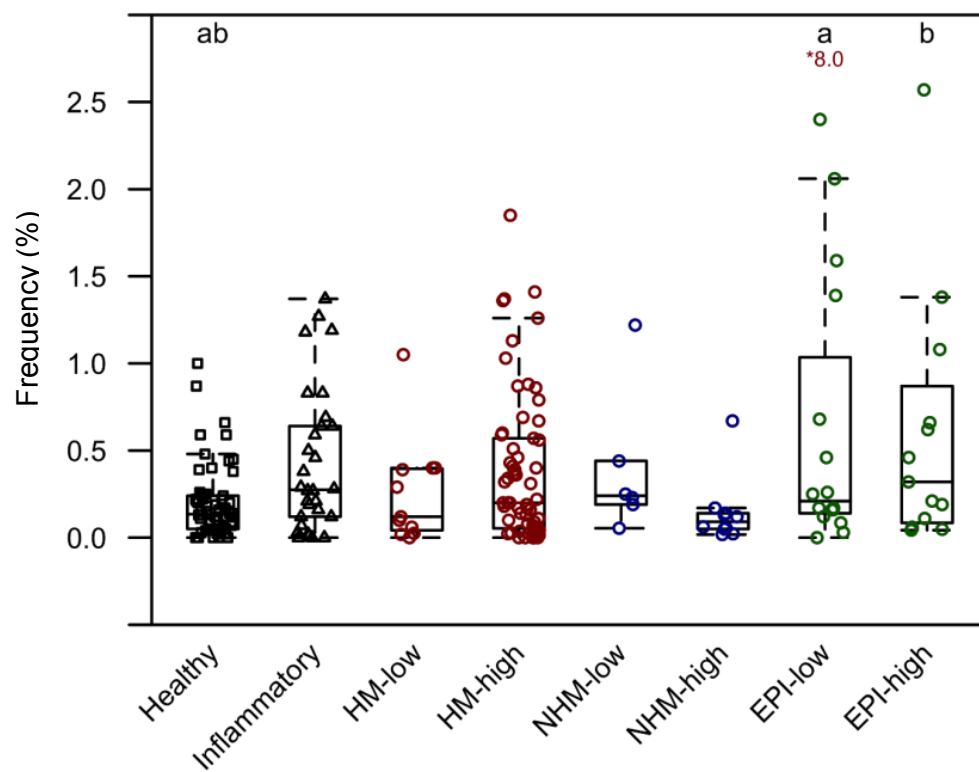
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a)

b)

d)

**Supplementary Figure S1. Frequencies of putative MDSCs differ in tumor-bearing dogs compared to healthy and inflammatory control dogs**

a) Parental gating of M-MDSCs (red dots) and PMN-MDSCs (blue dots) for the cascaded gating strategy used to identify both subsets in canine PBMCs. b) and c) Box-and-whisker plots depicting frequency of PMN-MDSCs (b) and M-MDSCs (c) in peripheral blood of canine healthy (square) and inflammatory (triangle) controls as well as tumor-bearing (circle) dogs. Frequencies are expressed as a percentage of total PBMCs. A capital letter denotes that the two groups are significantly different with $p < 0.001$, while a lowercase letter denotes a significant difference with $p < 0.05$. The red numbers represent dogs with frequencies off scale. The box represents quartiles, while the whisker-ends indicate the 5th and 95th percentile. Each dot represents a single dog, and the color indicates tumor histotype for tumor-bearing dogs (red: HM (hematopoietic mesenchymal), blue: NHM (non-hematopoietic mesenchymal), green: EPI (epithelial)). PMN-MDSCs: Healthy – 0.780% [0.133, 1.948], Inflammatory – 1.325% [0.305, 2.75]), Cancer – 2.53% [0.93, 4.935]), M-MDSCs: Healthy – 0.135% [0.05, 0.235], Inflammatory – 0.275% [0.12, 0.64], Cancer – 0.2% [0.06, 0.565]. d) Box-and-whisker plots depicting the frequency of PMN-MDSCs in peripheral blood of canine healthy and inflammatory controls as well as tumor-bearing dogs, grouped by cancer subtype (HM: hematopoietic mesenchymal, NHM: non-hematopoietic mesenchymal, EPI: epithelial) and burden (low vs high). Frequencies are expressed as a percentage of total PBMCs. A lowercase letter denotes a significant difference with $p < 0.01$. The box represents quartiles, while the whisker-ends indicate the 5th and 95th percentile. Each dot represents a single dog.

a) C PMN/H PMN
b) C MONO/H MONO
c) PMN-MDSC/H PMN
d) M-MDSC/H MONO
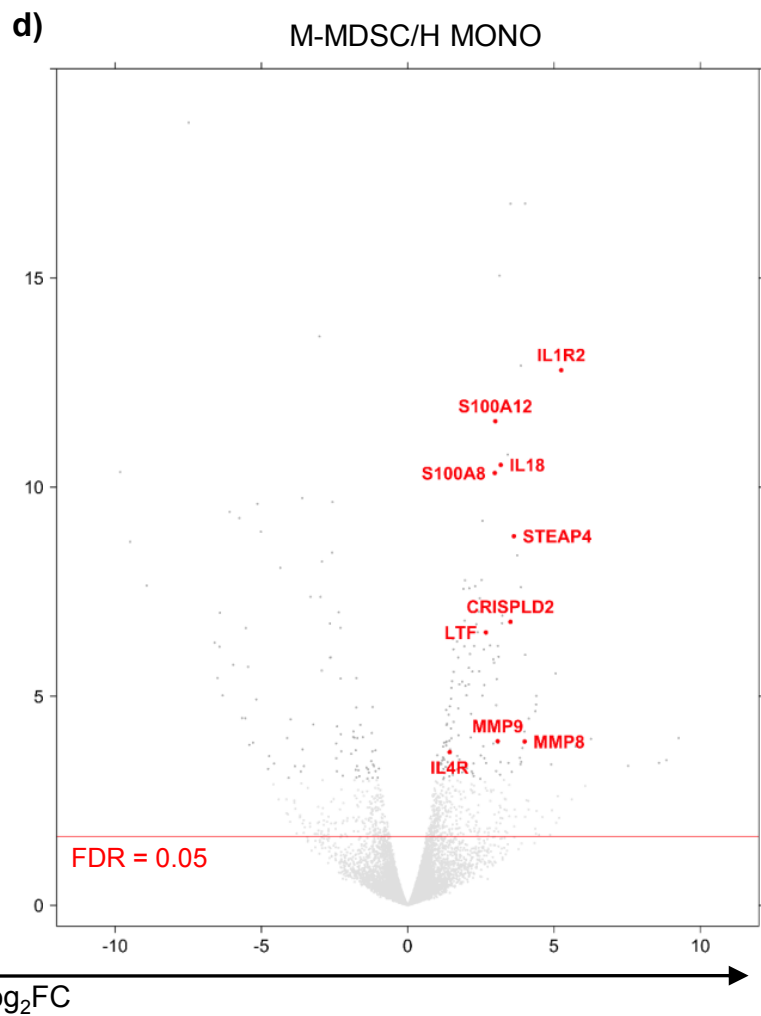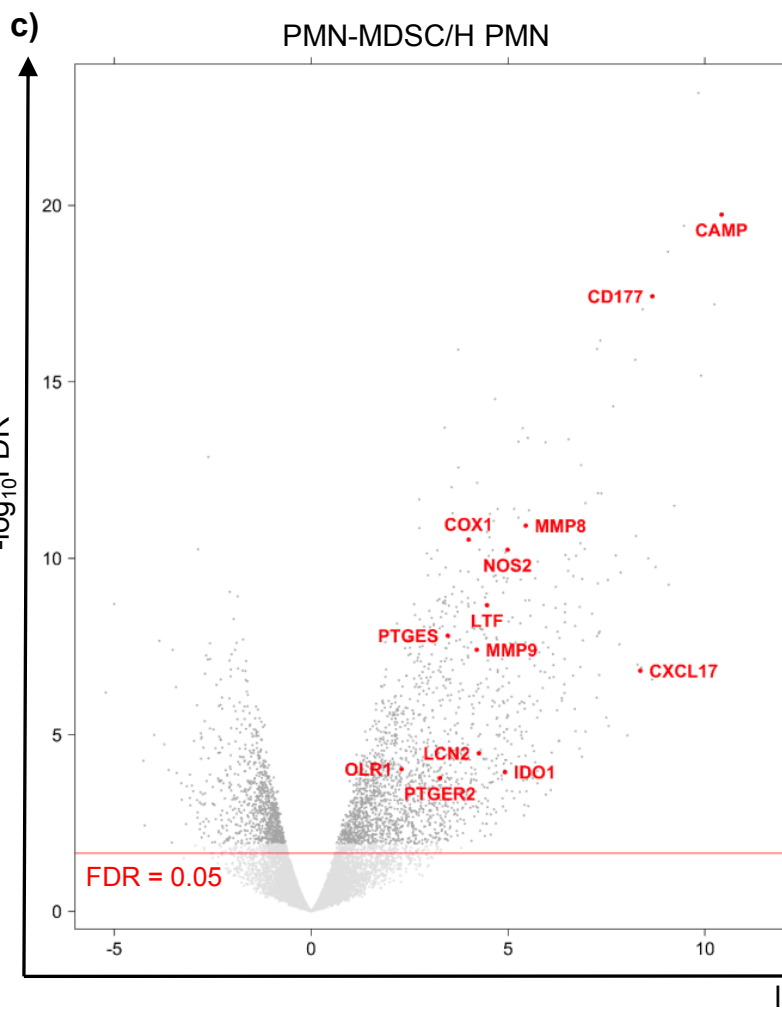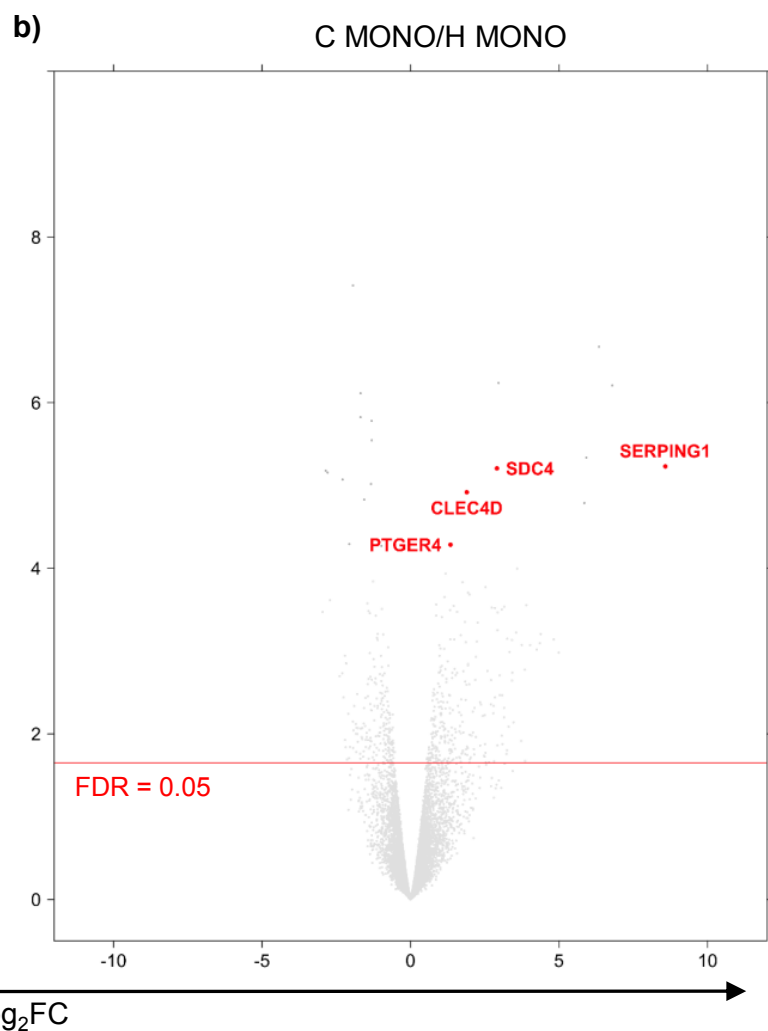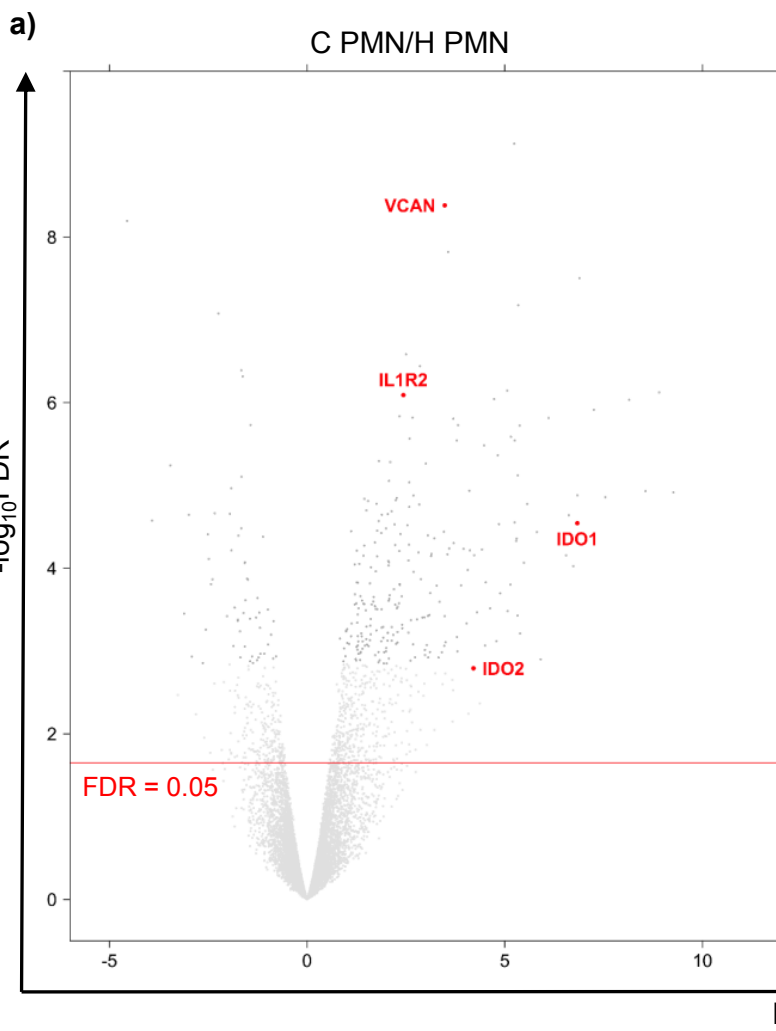
**Supplementary Figure S2. Canine MDSC subsets and PMNs are characterized by distinct transcriptomic signatures**

a-d) Volcano plots depicting differentially expressed genes in the indicated cell populations: C PMNs compared to H PMNs (a), C MONOs compared to H MONOs (b), PMN-MDSCs compared to H PMNs (c), and putative M-MDSCs compared to H MONOs (d). The red line indicates a significant FDR. Each dot represents one gene, and genes previously associated with MDSC function or novel but strongly upregulated and downregulated genes are labeled in red.
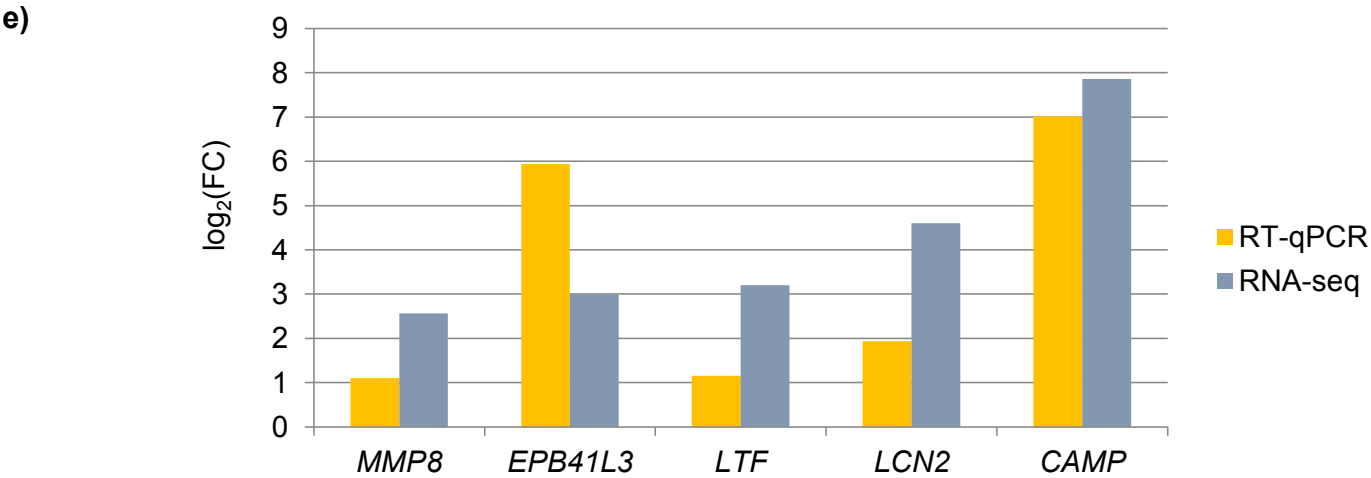
**a)**



**b)**
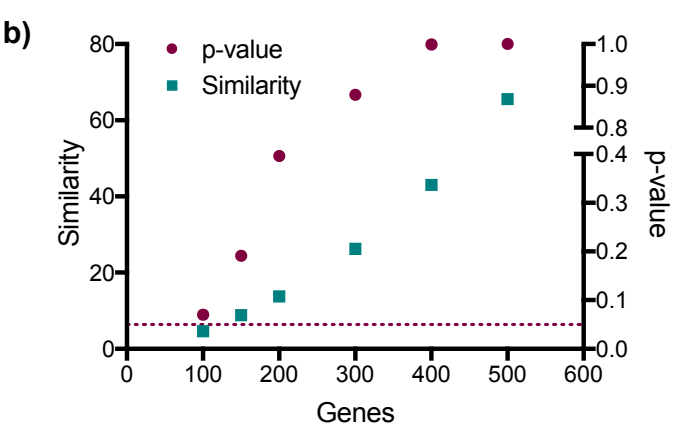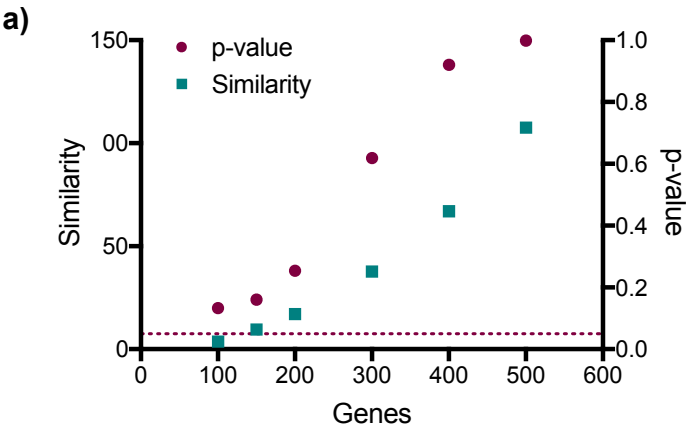


**c)**

| Shared Upregulated Genes In Human and Canine PMN-MDSCs (relative to C PMN) | | | |
|---|---|---|---|
| CTSG | TCN1 | S100A10 | CD86 |
| MPO | AKR1A1 | STOM | RPS17 |
| CAMP | RGS10 | GPI | RPS4X |
| ELANE | ABCA13 | PRDX1 | RPS5 |
| GNG11 | CYFIP1 | CD74 | RCC2 |
| PTGER2 | LTF | XPNPEP1 | RPSA |
| PPBP | EPB41L3 | LGALS1 | RPL15 |
| BPI | MMD | RPL3 | RPS12 |
| SLC2A5 | METAP2 | AHNAK | RPL5 |
| GP9 | MMP8 | PPA1 | RPL35 |
| LCN2 | FGD2 | F13A1 | PRNP |

**d)**

| Ingenuity Canonical Pathways | -log(p-value) | Ratio (x10$^{-2}$) | Molecules |
|---|---|---|---|
| EIF2 Signaling | 9.31 | 4.07 | RPL15, RPL35, RPL3, RPL5, RPS17, RPS5, RPS12, RPS4X, RPSA |
| Phagosome Maturation | 2.48 | 2.03 | MPO, PRDX1, CTSG |
| TREM1 Signaling | 2.00 | 2.67 | MPO, CD86 |
| Colorectal Cancer Metastasis Signaling | 1.87 | 1.21 | GNG11, MMP8, PTGER2 |
| HIF1α Signaling | 1.63 | 1.69 | SLC2A5, MMP8 |
| Gαi Signaling | 1.61 | 1.67 | GNG11, RGS10 |

**e)**

**Supplementary Figure S3. Cross-species analysis highlights genes differentially expressed in PMN-MDSCs shared by multiple mammalian taxa**

a) and b) Similarity score (square) and p-value (circle) are graphed using the top 100–500 differentially expressed genes in human and murine (a) and canine and murine (b) PMN-MDSCs relative to C PMNs. Dashed line indicates p = 0.05. c) Table listing the 44 shared upregulated genes in human and canine PMN-MDSCs shown in Figure 3b, listed in order of decreasing FC in the canine data, starting at CTSG down from left to right. d) Pathway enrichment analysis performed in IPA on the gene list in (c). Ratio indicates the proportion of genes found in our dataset relative to total number of genes in that pathway in IPA. e) Validation of RNA-Seq results of the five interesting genes upregulated in canine PMN-MDSCs compared to canine C PMNs using RT-qPCR. FC data are based on the mean result of four biological replicates.