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Supplemental information

CMTM6 shapes antitumor T cell response through modulating protein expression of CD58 and PD-L1

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Figure S1

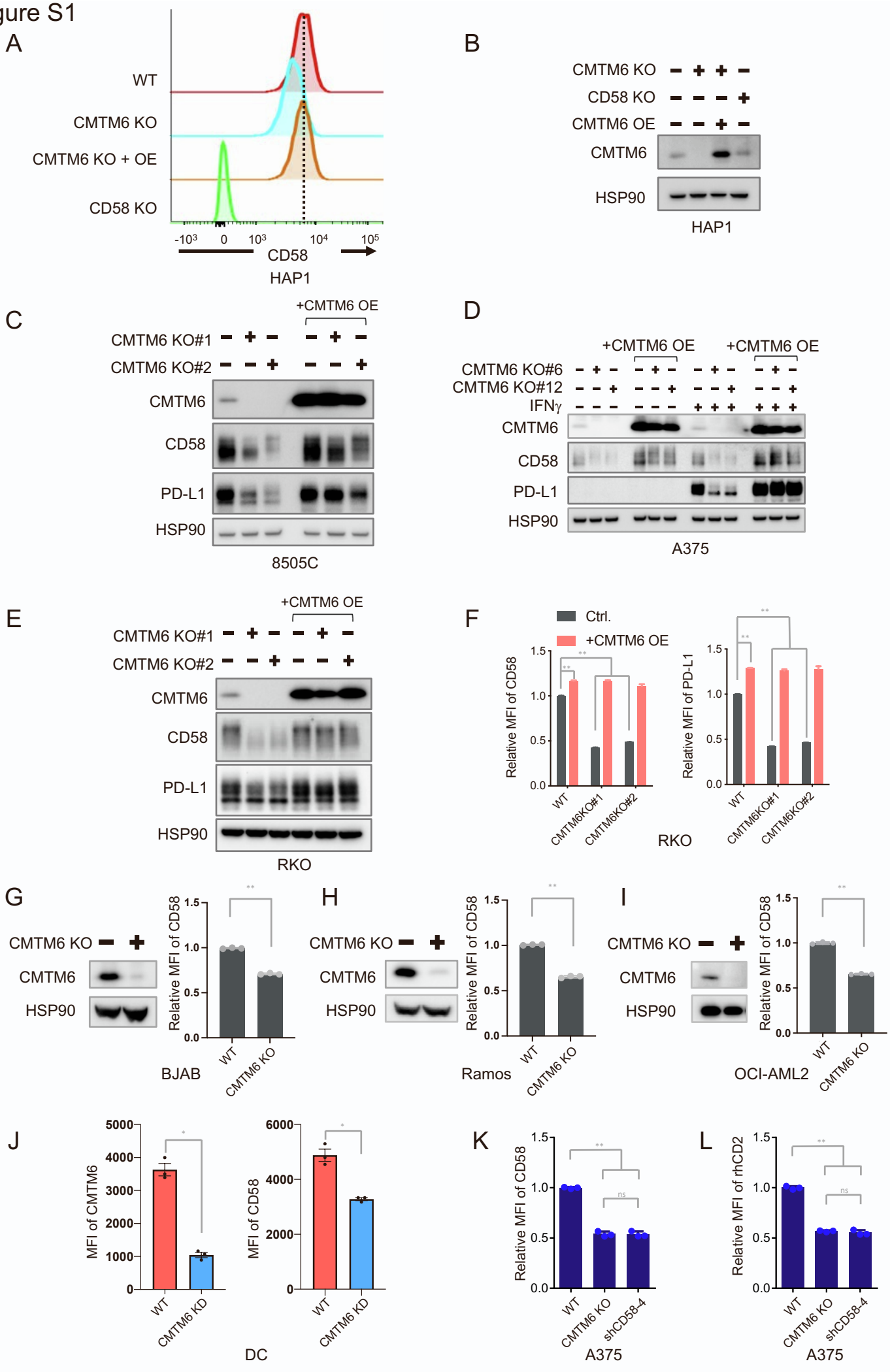
A
WT
CMTM6 KO
CMTM6 KO + OE
CD58 KO
-10³ 0 10³ 10⁴ 10⁵
CD58
HAP1
B
CMTM6 KO
CD58 KO
CMTM6 OE
CMTM6
HSP90
HAP1
C
+CMTM6 OE
CMTM6 KO#1
CMTM6 KO#2
CMTM6
CD58
PD-L1
HSP90
8505C
D
+CMTM6 OE
+CMTM6 OE
CMTM6 KO#6
CMTM6 KO#12
IFNγ
CMTM6
CD58
PD-L1
HSP90
A375
E
+CMTM6 OE
CMTM6 KO#1
CMTM6 KO#2
CMTM6
CD58
PD-L1
HSP90
RKO
F
Ctrl.
+CMTM6 OE
Relative MFI of CD58
Relative MFI of PD-L1
WT
CMTM6KO#1
CMTM6KO#2
RKO
G
CMTM6 KO
CMTM6
HSP90
Relative MFI of CD58
WT
CMTM6 KO
BJAB
H
CMTM6 KO
CMTM6
HSP90
Relative MFI of CD58
WT
CMTM6 KO
Ramos
I
CMTM6 KO
CMTM6
HSP90
Relative MFI of CD58
WT
CMTM6 KO
OCI-AML2
J
MFI of CMTM6
MFI of CD58
WT
CMTM6 KD
DC
K
Relative MFI of CD58
WT
CMTM6 KO
shCD58-4
A375
L
Relative MFI of rhCD2
WT
CMTM6 KO
shCD58-4
A375

Figure S1. CMTM6 loss in HAP1 cells leads to reduced CD58 expression, Related to Figure 1

(A) Flow cytometry analysis of CD58 expression in wild-type (WT), CMTM6-knockout (CMTM6 KO), CMTM6-reconstituted (CMTM6 KO + OE) and CD58-knockout (CD58 KO) HAP1 cells.

(B) Western blot analysis of CMTM6 expression in wild-type (WT), CMTM6-knockout (CMTM6 KO), CMTM6-reconstituted (CMTM6 OE) and CD58-knockout (CD58 KO) HAP1 cells. HSP90 served as a control.

(C-E) Western blot analysis of CMTM6, CD58, and PD-L1 in wild-type (WT), CMTM6-knockout (CMTM6 KO), CMTM6-overexpressing (WT+CMTM6 OE), and CMTM6-reconstituted (CMTM6 KO+CMTM6 OE) 8505C cells (C), A375 cells with or without IFNγ exposure (D), and RKO cells (E). HSP90 served as a control.

(F) Flow cytometry analysis of CD58 and PD-L1 expression in wild-type (WT), CMTM6-knockout (CMTM6 KO), CMTM6-overexpressing (WT+CMTM6 OE), and CMTM6-reconstituted (CMTM6 KO+CMTM6 OE) RKO cells. Data represent the mean ± standard deviation of triplicates and were analyzed using a two-way ANOVA test (with Tukey's multiple comparisons test). A p-value greater than 0.05 indicates non-significance (ns), while a p-value less than 0.0001 is denoted as **.

(G-I) Western blot analysis of CMTM6 and flow cytometry analysis of CD58 expression in wild-type (WT) and CMTM6-knockout (CMTM6 KO) B-cell lymphoma cells – BJAB (G), Ramos (H) and acute myeloid leukemia cells - OCI-AML2 (I). HSP90 served as a control.

(J) Flow cytometry analysis of CMTM6 and CD58 expression in wild-type (WT) and CMTM6-knockdown (CMTM6 KD) primary dendritic cells that were generated from human peripheral blood-derived progenitors.

(K) Flow cytometry analysis of CD58 expression in wild-type (WT), CMTM6-knockout (CMTM6 KO), and CD58-knockdown (shCD58-4) A375 cells.

(L) Flow cytometry analysis of rhCD2 binding in wild-type (WT), CMTM6-knockout (CMTM6 KO), and CD58-knockdown (shCD58-4) A375 cells described in (K).

The data are presented as mean ± standard deviation (SD) of triplicates and were analyzed using unpaired two-tailed student's t-test (G-J) or one-way ANOVA (with Tukey's multiple comparisons test) (K-L). The statistical significance levels are indicated as follows: ns (not significant; $p \geq 0.05$), * ($p < 0.05$), and ** ($p < 0.0001$).