**Supporting Information**

**SI Materials and Methods**

**Small interfering RNA (siRNA) design and construction:** The Rab family genes consist of a network of protein that has a major contribution for antigen cross-presentation starting from enocytosis to the formation of MHC-1/peptide complex. The proteins of this family have been found to involve in exocytotic pathways (Rab 3C), MHC-I endocytic trafficking (Rab 22A) and effect on phagosome (Rab 10 and 27A) as previously described ([Zou *et al.*, 2009](#_ENREF_3)). Hence, two pair of siRNA against these four bovine Rab genes were designed through online tool of siDirect version 2.0 (<http://sidirect2.rnai.jp/>) with a representative mRNA sequence for each target gene ([Naito & Ui-Tei, 2012](#_ENREF_1)). Another online tool (<http://biotools.nubic.northwestern.edu/OligoCalc.html>) was used to check the self-complementarity of siRNA. These siRNA pairs for each gene were optimized to select most efficient one.

**siRNA synthesis, transfection and Q-RT-PCR:** The 18-25-nt-long interfering RNA against screened Rab genes were synthesized from GenePharma (Shanghai, China). The genes in TaDCs were silenced by siRNAs upon transfection as recommended by manufacturer. The transfection with a pair of siRNA (3C and 10; 22A and 27A) for simultaneous genes silencing was performed according to the previously used protocol ([Rückert *et al.*, 2010](#_ENREF_2)). Transfection reagents were used as negative control After 6 h of transfection, culture medium was replaced with fresh medium containing OVA to precede the endocytosis and antigen presentation assay as already described (Subheading: preparation of antigen presenting cells).

**Table S1:** Synthesized and optimized siRNAs for silencing of Rab 3C, 10, 22A and 27A.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **Rab genes** | **Sequences type** | **Sense (5` to 3`)** | **Target region** |
| 1 | 3C | Target | CTCAAATCAAAACATACTCGTGG | 508-530 |
| Guide | ACGAGUAUGUUUUGAUUUGAG |
| Passenger | CAAAUCAAAACAUACUCGUGG |
| 2 | 10 | Target | TGCCTTCAACACCACCTTTATTT | 590-612 |
| Guide | AUAAAGGUGGUGUUGAAGGCA |
| Passenger | CCUUCAACACCACCUUUAUUU |
| 3 | 22A | Target | CAGTACCAAAATGAACTACATAA | 398-420 |
| Guide | AUGUAGUUCAUUUUGGUACUG |
| Passenger | GUACCAAAAUGAACUACAUAA |
| 4 | 27A | Target | AGCAGAGTTTTCTCAATGTCAGA | 427-449 |
| Guide | UGACAUUGAGAAAACUCUGCU |
| Passenger | CAGAGUUUUCUCAAUGUCAGA |

In target sequences there are 21nt target + 2nt overhang

The Q-RT-PCR was performed according to already mentioned protocol after transfection of TaDCs with two different genes siRNA to find out the silencing of Rab genes and their effects on T lymphocytes proliferation (Fig. S1). The change in T lymphocytes proliferation upon Rab genes silenced were calculated by following formula.

Role of Rab gene = 100 x ($Proliferation before- proliferation after$)/proliferation before

There found 36.29% (Rab 3C and 10 silenced) and 22.85% (Rab 22A and 27A silenced) decreased proliferation of CD8+ cells upon knockdown at low (10-20) passages while no such change was found in the proliferation of CD4+ cells induced by TaDC-APC. Our findings have indication for the role of Rab genes in antigen cross-presentation. There was no complete inhibition of CD8+ cell proliferation that might be low transfection efficacy and involvement of other Rab genes in continuously dividing TaDCs.

**References**

**Naito, Y. and Ui-Tei, K.** (2012). siRNA design software for a target gene-specific RNA interference. *Frontiers in Genetics,* **3**, 102.

**Rückert, F., Samm, N., Lehner, A.-K., Saeger, H.-D., Grützmann, R. and Pilarsky, C.** (2010). Simultaneous gene silencing of Bcl-2, XIAP and Survivin re-sensitizes pancreatic cancer cells towards apoptosis. *BMC Cancer,* **10**, 379.

**Zou, L., Zhou, J., Zhang, J., Li, J., Liu, N., Chai, L., Li, N., Liu, T., Li, L. and Xie, Z.** (2009). The GTPase Rab3b/3c-positive recycling vesicles are involved in cross-presentation in dendritic cells. *Proceedings of the National Academy of Sciences,* **106**, 15801-15806.

**Figures Legend**

**Fig. S1:** The expression of Rab (3C and 10) and (22A and 27A) were simultaneously knockdown using siRNA in TaDCs at low (10-20) passages. Genes silencing were confirmed by Q-RP-PCR after 24 h post-transfection (A). Endocytosis of OVA-FITC in TaDC was measured after 24 h post-incubation (B). The proliferation rate of CD8+ cells decreased 36.29 (C) and 22.85% (D) upon silencing of Rab (3C/10 and 22A/27A), respectively.

